# ANTIBODY-DRUG CONJUGATES COMPRISING GLP1 PEPTIDOMIMETICS AND USES THEREOF

## CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/077,983, filed September 14, 2020, the disclosure of which is herein incorporated by reference in its entirety.

## SEQUENCE LISTING

[0002] 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 September 8, 2021, is named 250298_000250_SL.txt and is 6,072 bytes in size.

## FIELD OF THE DISCLOSURE

[0003] The present disclosure relates to protein-drug conjugates (e.g., antibody-drug conjugates), pharmaceutical compositions, and methods of treating disease therewith. Also provided are peptidomimetic payloads and linker-payloads and methods of making same. More specifically, the present disclosure relates to protein-drug conjugates (e.g., antibody-drug conjugates) comprising GLP1 peptidomimetics and methods of treating GLP1R-associated conditions therewith.

## BACKGROUND OF THE DISCLOSURE

[0004] Diabetes is a chronic disease of abnormal glucose metabolism. 425 million people are estimated to be living with diabetes worldwide. Global diabetes drugs include insulin, DPP-4 inhibitors, glucagon-like peptide 1 receptor (GLP1R) agonists, but most patients do not achieve combined treatment goal to manage hyperglycaemia and cardiovascular risk factors.

[0005] Glucagon-Like Peptide 1 Receptor (GLP1R) is the receptor for glucagon-like peptide 1 (GLP1) and is expressed in the pancreatic beta cells. GLP1R is also expressed in the brain where it functions in the control of appetite, memory and learning. GLP1R is a member of the secretin family (Class B) of G protein-coupled receptors (GPCRs). Upon binding of its ligand, GLP1, GLP1R initiates a downstream signaling cascade through G$\alpha$s G-proteins that raises intracellular cyclic AMP (cAMP) levels, which leads to the transcriptional regulation of genes (Donnelly 2011). Activation of GLP1R results in increased insulin synthesis and release of insulin.

[0006] GLP1R and GLP1 are highly validated targets for obesity and type 2 diabetes. Marketed GLP1R agonists increase insulin secretion, thereby lowering blood glucose levels, but they require weekly or more frequent administration.

[0007] Accordingly, there is a need in the art for GLP1R agonists with longer duration and better safety. In certain embodiments, the present disclosure meets the needs and provides other advantages.

[0008] The foregoing discussion is presented solely to provide a better understanding of the nature of the problems confronting the art and should not be construed in any way as an admission as to prior art nor should the citation of any reference herein be construed as an admission that such reference constitutes "prior art" to the instant application.

## SUMMARY OF THE DISCLOSURE

[0009] Various non-limiting aspects and embodiments of the disclosure are described below.

[00010] In one aspect, compound having a structure of Formula (A):

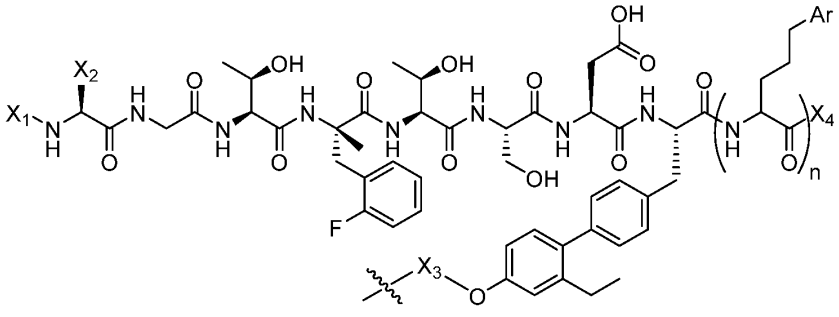
$$BA\text{-}(L\text{-}P)_m \quad (A),$$

wherein:

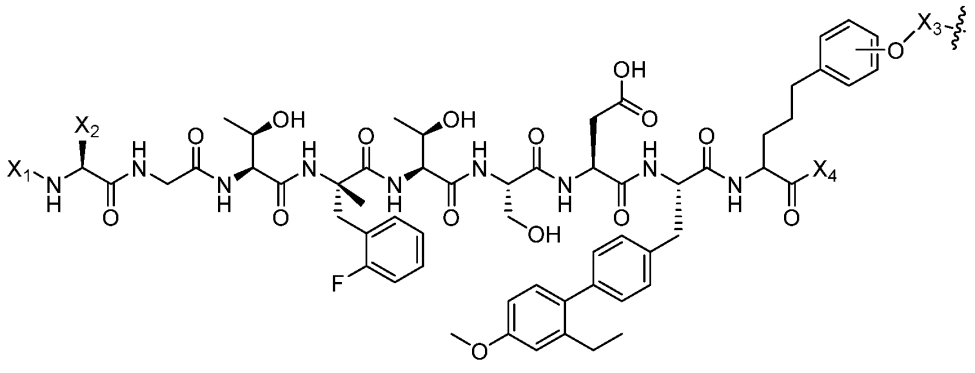
BA is an antibody or an antigen-binding fragment thereof;

L is a non-cleavable linker;

P is a payload having the structure selected from the group consisting of:

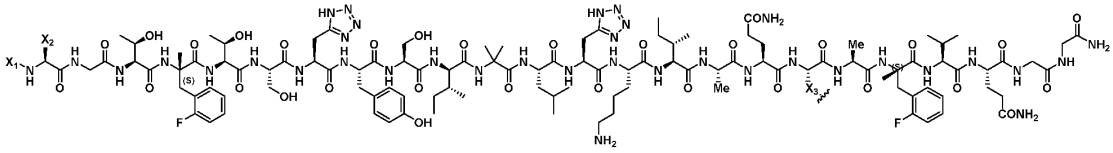


(P-IA),



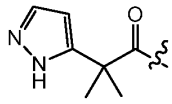
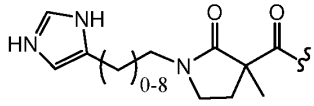
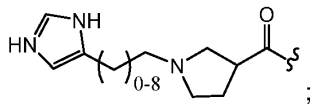
(P-IIA),

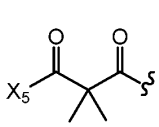
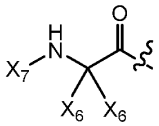
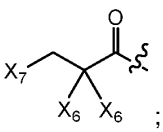
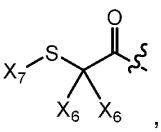
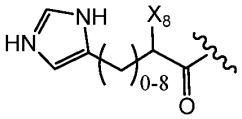
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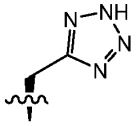
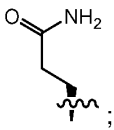


(P-IIIA),

wherein —§– is the point of attachment of the payload to L;

$X_1$ is selected from H;  ;  ;  ;

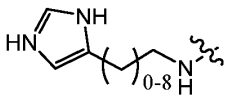
 ;  ;  ;  , and  ;

$X_2$ is selected from  and  ;

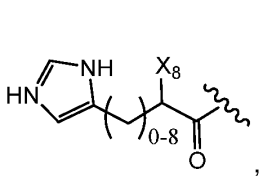
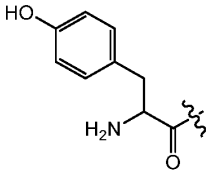
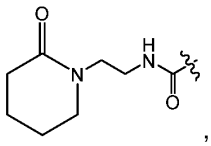
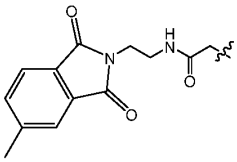
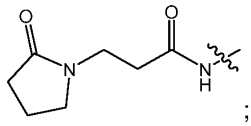
$X_3$ is selected from a bond, $–(CH_2)_{2-6}–NH–$, $–(CH_2)_{2-6}–Tr–$, and $–(CH_2)_{2-6}–Tr–(CH_2)_{1-6}–NH$, where Tr is a triazole moiety;

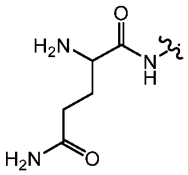
n is 0 or 1;

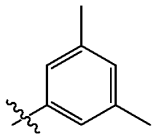
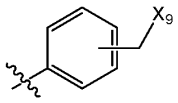
$X_4$ is selected from $-NH_2$, -OH and -N(H)(phenyl);

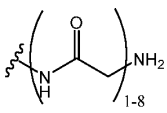
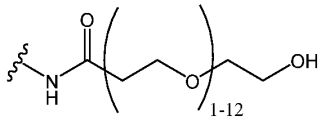
$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  ,  ,  ,  ,  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ;

Ar is selected from  and  ;

$X_9$ is selected from $-NH_2$,  , and  , and

m is an integer from 1 to 4

or a pharmaceutically acceptable salt thereof.

[00011] In another aspect, provided herein is a compound having a structure of Formula (I):

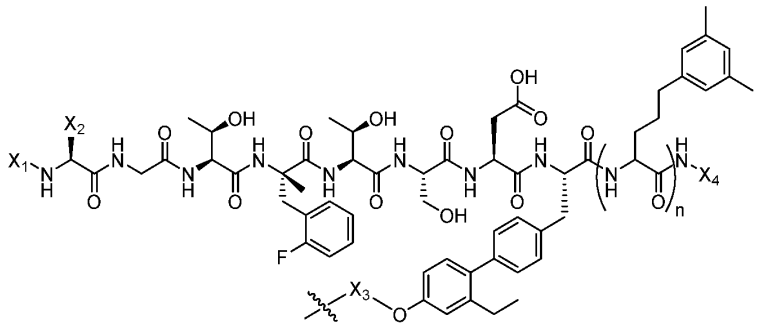
BA-L-P (I),

wherein:

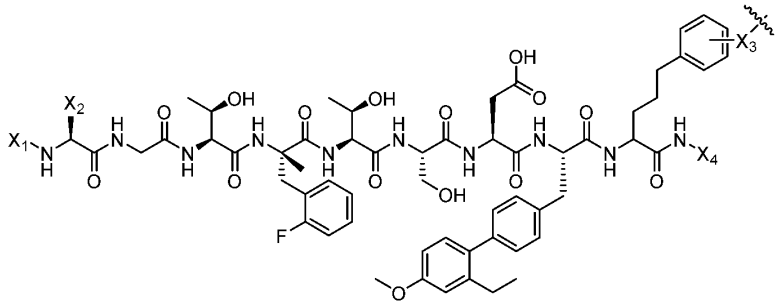
BA is an antibody or an antigen-binding fragment thereof;

L is a non-cleavable linker;

P is a payload having the structure selected from the group consisting of:

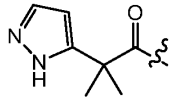
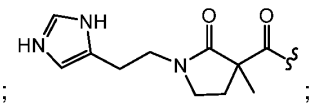
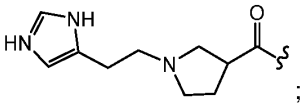


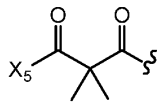
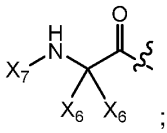
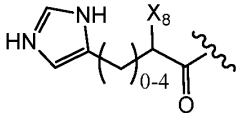
(P-I) and

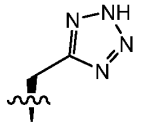
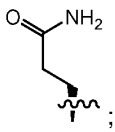


(P-II),

wherein —§– is the point of attachment of the payload P to L;

$X_1$ is selected from H;  ;  ;  ;

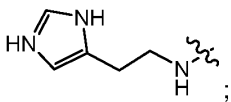
 ;  ; and  ;

$X_2$ is selected from  and  ;

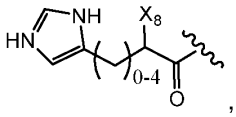
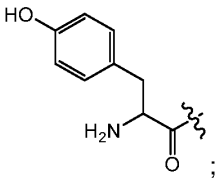
$X_3$ is selected from $–(CH_2)_{2-6}–NH–$ and $–(CH_2)_{2-6}–Tr–$, where Tr is a triazole moiety;

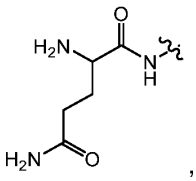
n is 0 or 1;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  , and  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ,

or a pharmaceutically acceptable salt thereof.

[00012] In some embodiments of the compound described herein, BA is a Glucagon-like peptide-1 receptor (GLP1R)-targeting antibody or an antigen-binding fragment thereof. In some embodiments, the GLP1R-targeting antibody is a GLP1R agonist antibody. In some embodiments, the GLP1R-targeting antibody is 5A10, 9A10, AB9433-I, h38C2, PA5-111834, NLS1205, MAB2814, EPR21819, or glutazumab.

[00013] In some embodiments, the linker L is attached to one or both heavy chains of the BA. In some embodiments, the linker L is attached to one or both heavy chain variable domains of the BA. In some embodiments, the linker L is attached to one or both light chains of the BA. In some embodiments, the linker L is attached to one or both light chain variable domains of the BA.

[00014] In some embodiments, the linker L is attached to BA via a glutamine residue. In some embodiments, the glutamine residue is introduced to the N-terminus of one or both heavy chains of the BA. In some embodiments, the glutamine residue is introduced to the N-terminus of one or both light chains of the BA. In some embodiments, the glutamine residue is naturally present in a CH2 or CH3 domain of the BA. In some embodiments, the glutamine residue is introduced to the BA by modifying one or more amino acids. In some embodiments, the glutamine residue is Q295 or N297Q.

[00015] In some embodiments, the linker L is attached to BA via a lysine residue.

[00016] In some embodiments, the antibody or antigen-binding fragment thereof is aglycosylated. In some embodiments, the antibody or antigen-binding fragment thereof is deglycosylated. In some embodiments, the antigen-binding fragment is an Fab fragment.

[00017] In one embodiment, m is 1. In one embodiment, m is an integer from 2 to 4. In one embodiment, m is 2.

[00018] In some embodiments, more than one L-P is attached to the BA. In some embodiments, two L-Ps are attached to the BA.

[00019] In some embodiments, the linker L has the structure of formula (L'):

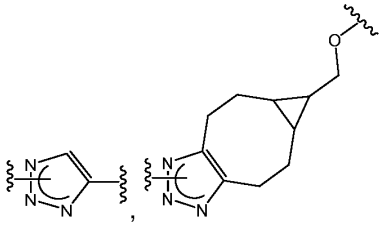
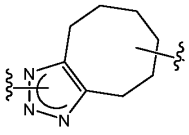
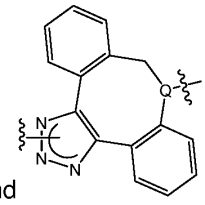
-La-Y-Lp- (L'),

wherein La is a first linker covalently attached to the BA;

Y is a group comprising a triazole, and

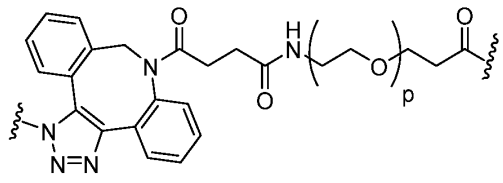
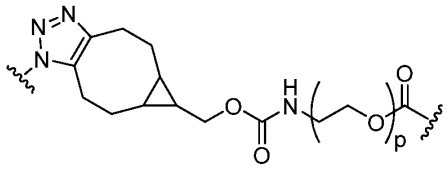
Lp is absent or a second linker covalently attached to the P, wherein when Lp is absent, Y is also absent.

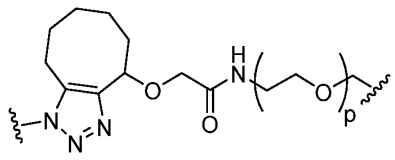
[00020] In some embodiments, Y has a structure selected from the group consisting of:

 , ,  , and  , wherein Q is C or N.

[00021] In some embodiments, Lp comprises a polyethylene glycol (PEG) segment having 1 to 36 $-CH_2CH_2O-$ (EG) units. In some embodiments, the PEG segment comprises between 2 and 30 EG units. In some embodiments, the PEG segment comprises between 4 and 24 EG units. In some embodiments, the PEG segment comprises 4 EG units, or 8 EG units, or 12 EG units, or 24 EG units. In some embodiments, the PEG segment comprises 4 EG units. In some embodiments, the PEG segment comprises 8 EG units.

[00022] In some embodiments, Y-Lp has a structure selected from the group consisting of:

 ;  ;

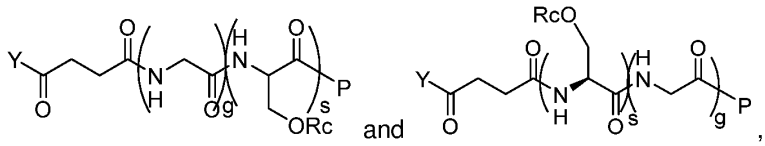
 , or a triazole regioisomer thereof,

wherein p is an integer from 1 to 36.

[00023] In some embodiments, the Lp comprises one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline and combinations thereof. In some embodiments, the Lp comprises 1 to 10 glycines. In some embodiments, the Lp comprises 1 to 6 serines. In some embodiments, the Lp comprises 1 to 10 glycines and 1 to 6 serines. In some embodiments, the Lp comprises 4 glycines and 1 serine. In some embodiments, the Lp is selected from the group consisting of Gly-Gly-Gly-Gly-Ser ($G_4S$) (SEQ ID NO: 1), Ser-Gly-Gly-Gly-Gly ($SG_4$) (SEQ ID NO: 2), and Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser ($G_4S$-$G_4S$) (SEQ ID NO: 3).

[00024] In some embodiments, the Lp comprises a combination of a PEG segment having 1 to 36 EG units and one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline and combinations thereof. In some embodiments, the serine residue comprises a carbohydrate group. In some embodiments, the serine residue comprises a glucose group.

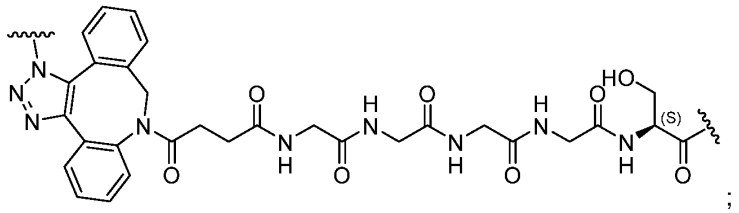
[00025] In some embodiments, Lp has a structure selected from the group consisting of:

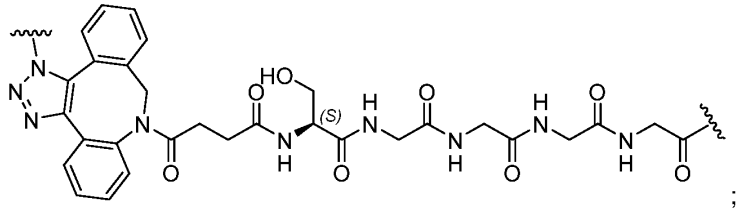

and

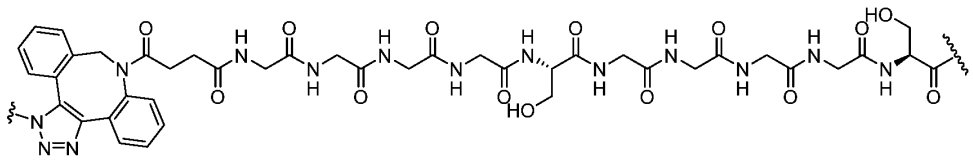
,

wherein Y is the group comprising a triazole and P is the payload, and wherein Rc is selected from H and glucose, g is an integer from 1 to 10 and s is an integer from 0 to 4.

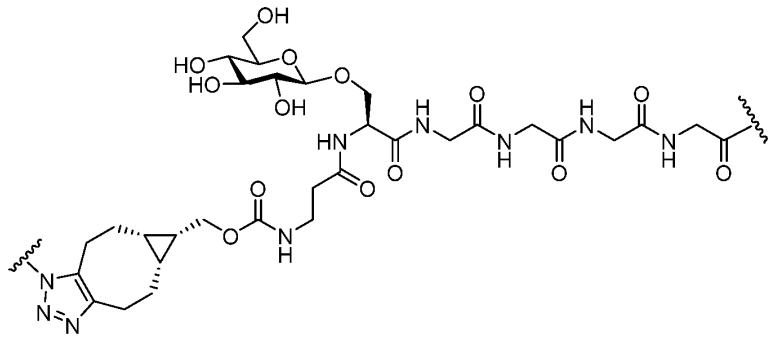
[00026] In some embodiments, Y-Lp has a structure selected from the group consisting of:


;

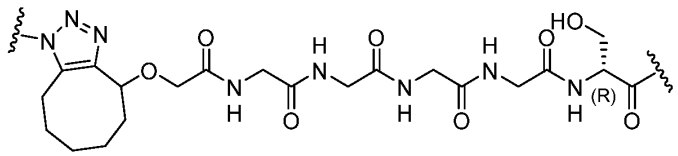

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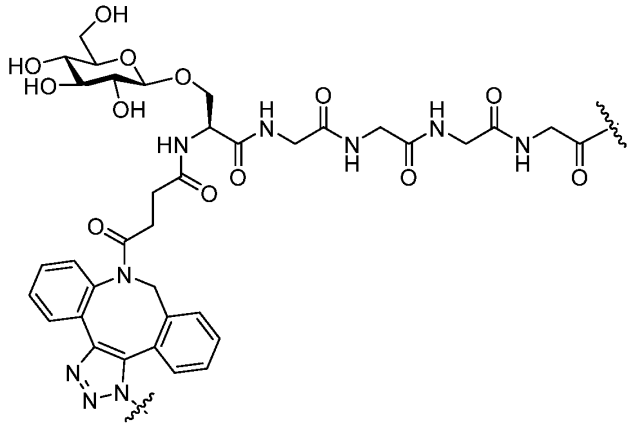
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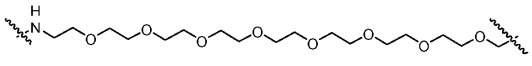


; and

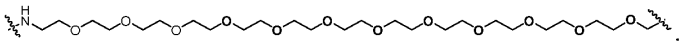


, or a triazole regioisomer thereof.

[00027] In some embodiments, La comprises a polyethylene glycol (PEG) segment having 1 to 36 -$CH_2CH_2O$- (EG) units. In some embodiments, the PEG segment comprises 4 EG units, or 8 EG units, or 12 EG units, or 24 EG units. In some embodiments, the PEG segment comprises 8 EG units. In some embodiments, La has a structure selected from the group consisting of



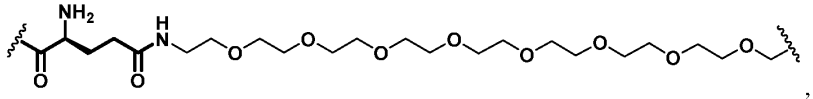
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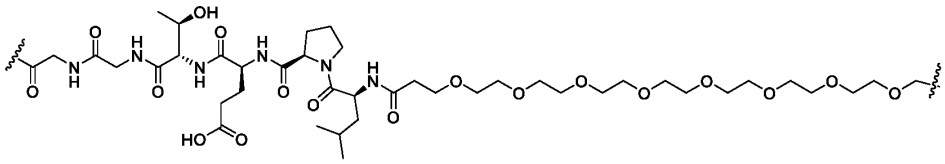
.

[00028] In some embodiments, La comprises one or more amino acids selected from glycine, threonine, serine, glutamine, glutamic acid, alanine, valine, leucine, and proline and combinations thereof. In some embodiments, La comprises 1 to 10 glycines and 1 to 6 serines. In some embodiments, La comprises 4 glycines and 1 serine. In some embodiments, La is selected from the group consisting of Gly-Gly-Gly-Gly-Ser ($G_4S$), Ser-Gly-Gly-Gly-Gly ($SG_4$), Gly-Gly-Ser-Gly-Gly-Ser-Gly-Gly ($G_2S$-$G_2S$-$G_2$), and Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly ($G_4S$-$G_4$).

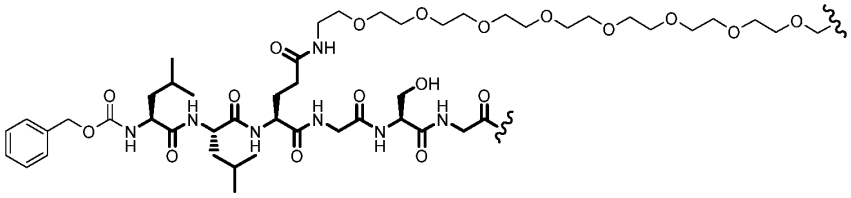
[00029] In some embodiments, La comprises a combination of a PEG segment having 1 to 36 EG units and one or more amino acids selected from glycine, threonine, serine, glutamine, glutamic acid, alanine, valine, leucine, and proline and combinations thereof. In some embodiments, La is selected from the group consisting of:



,

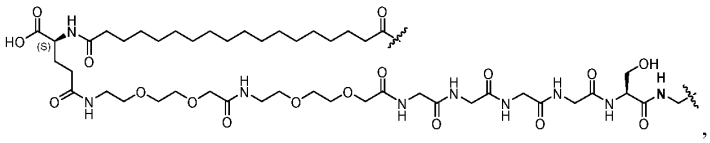


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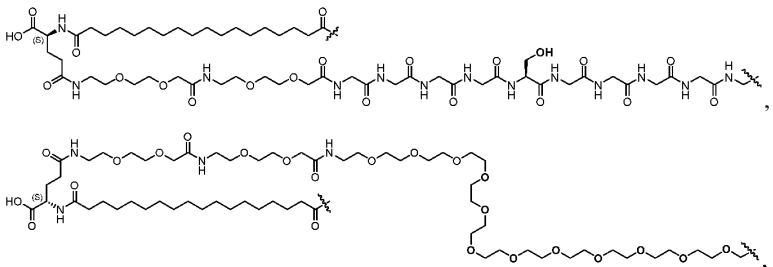


.

[00030] In some embodiments, La comprises a $-(CH_2)_{2\text{-}24}$- chain. In some embodiments, In some embodiments, La comprises a combination of a $-(CH_2)_{2\text{-}24}$- chain, a PEG segment having 1 to 36 EG units and one or more amino acids selected from glycine, threonine, serine, glutamine, glutamic acid, alanine, valine, leucine, and proline and combinations thereof. La is selected from the group consisting of:

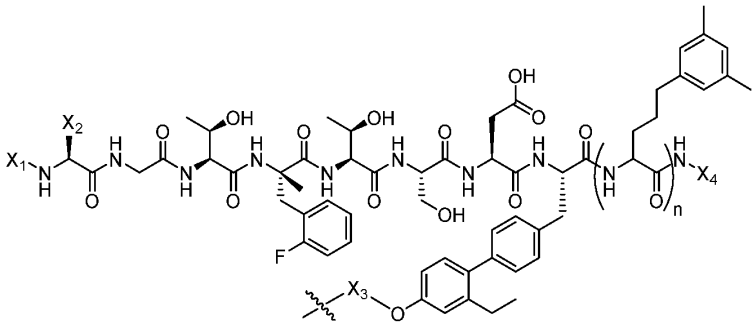


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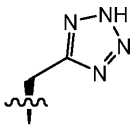


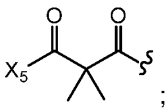
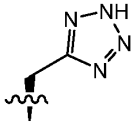
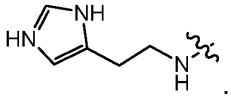
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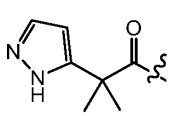
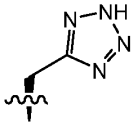
[00031] In various embodiments of the compound described herein, P has the structure

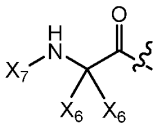
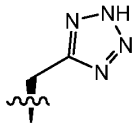


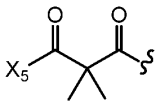
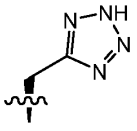
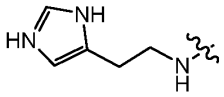
(P-I).

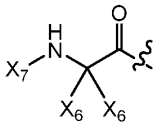
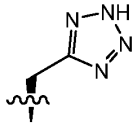
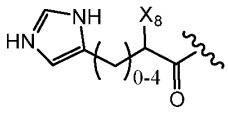
[00032] In some embodiments, $X_1$ is H; $X_2$ is  ; $X_3$ is selected from $–(CH_2)_{2-6}–$ NH– and $–(CH_2)_{2-6}–Tr–$, where Tr is a triazole moiety; n is 1, and $X_4$ is H.

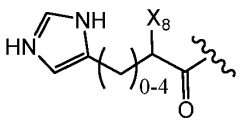
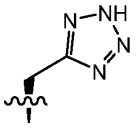
[00033] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2-6}–NH–$ ; n is 1; $X_4$ is H, and $X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  .

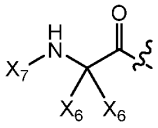
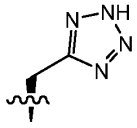
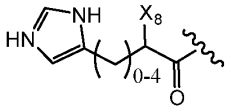
[00034] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2-6}–$ NH–; n is 1, and $X_4$ is H.

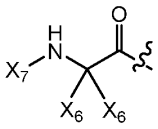
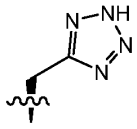
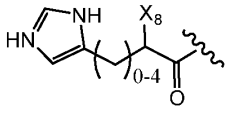
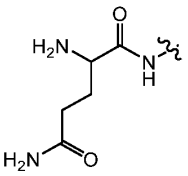
[00035] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$ ; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and $-CH_2OH$, and $X_7$ is H.

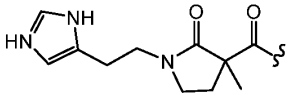
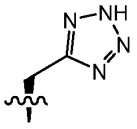
[00036] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–Tr–$  , where Tr is a triazole moiety; n is 1; $X_4$ is H, and $X_5$ is .

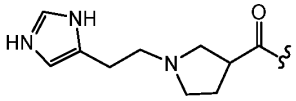
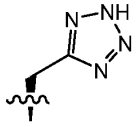
[00037] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$ ; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and $-CH_3$; $X_7$ is  , and $X_8$ is $-NH_2$.

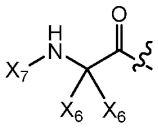
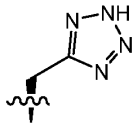
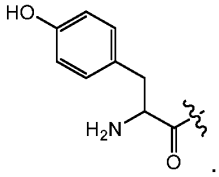
[00038] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H, and $X_8$ is H.

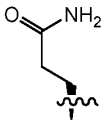
[00039] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$  ; n is 1; $X_4$ is H; $X_6$ is H at each occurrence; $X_7$ is , and $X_8$ is H.

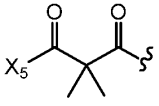
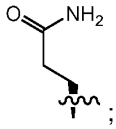
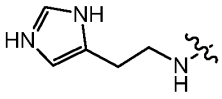
[00040] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$ ; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and $-CH_3$; $X_7$ is  , and $X_8$ is  .

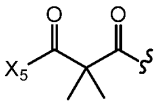
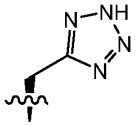
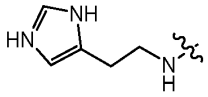
[00041] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1, and $X_4$ is H.

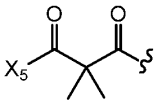
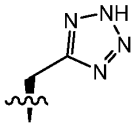
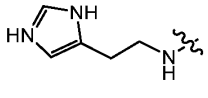
[00042] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1, and $X_4$ is H.

[00043] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$ ; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and $-CH_3$, and $X_7$ is  .

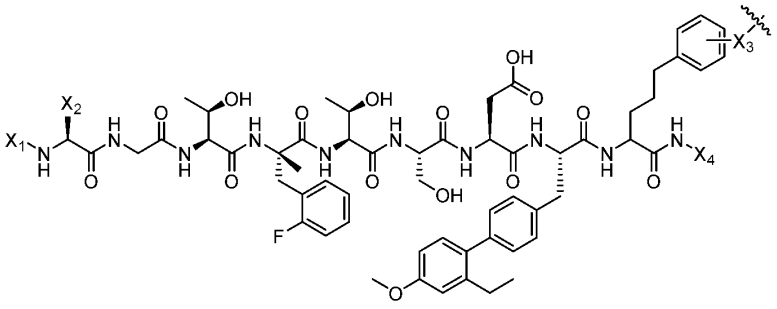
[00044] In some embodiments, $X_1$ is H; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1, and $X_4$ is H.

[00045] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1; $X_4$ is H, and $X_5$ is  .

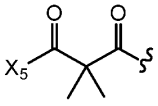
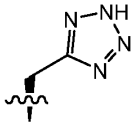
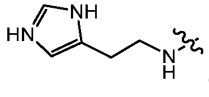
[00046] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$ ; n is 0; $X_4$ is phenyl, and $X_5$ is  .

[00047] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$ ; n is 1; $X_4$ is phenyl, and $X_5$ is  .

[00048] In various embodiments of the compound described herein, P has the structure

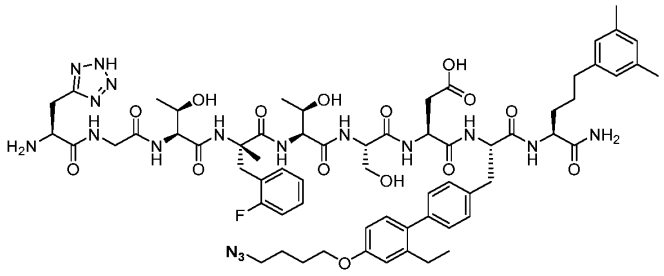


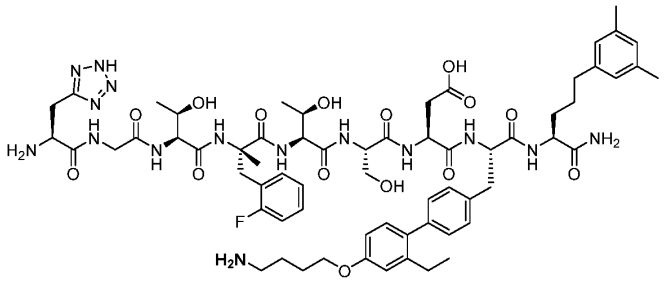
(P-II).

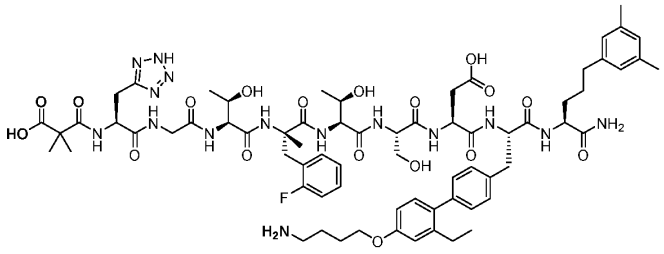
[00049] In some embodiments, $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$ ;$X_4$ is H, and $X_5$ is  .

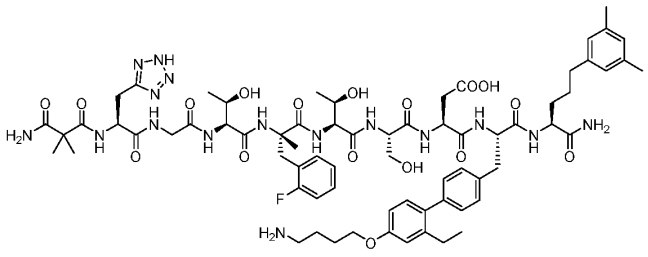
[00050] In various embodiments of the compound described herein, P has the structure selected from the group consisting of:

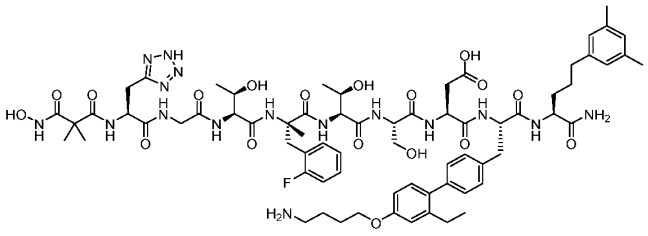
Structure

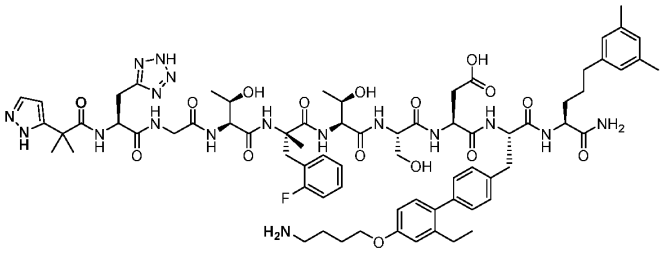

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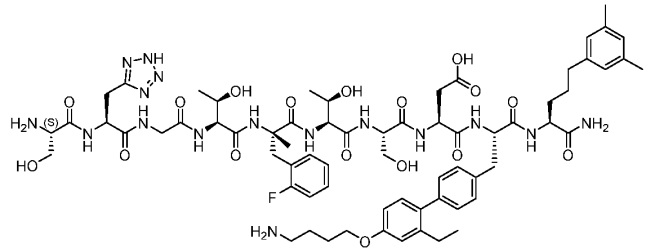

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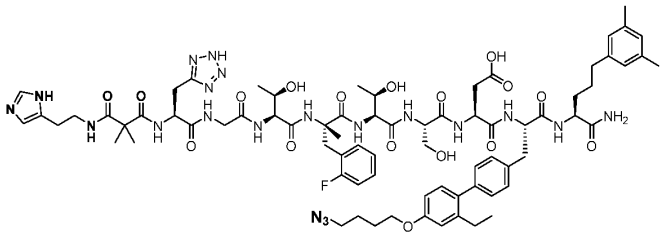

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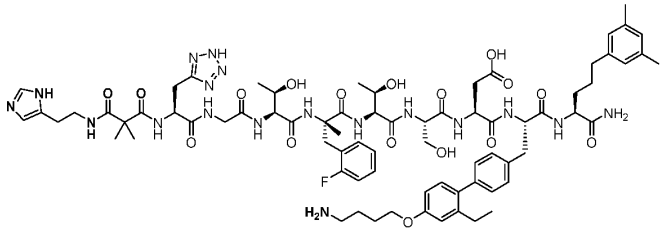

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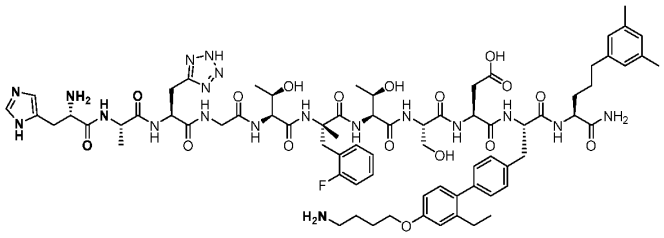

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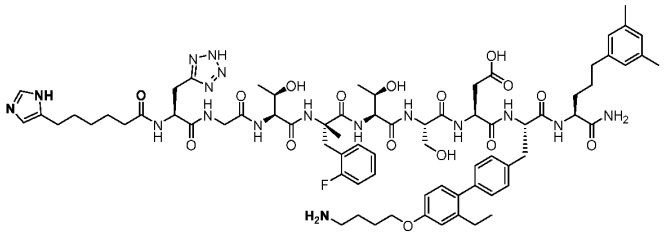

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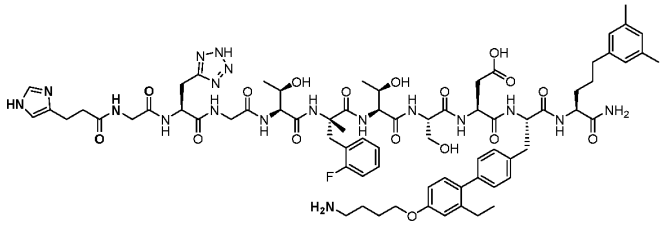

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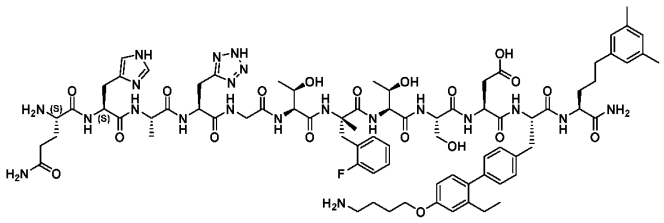

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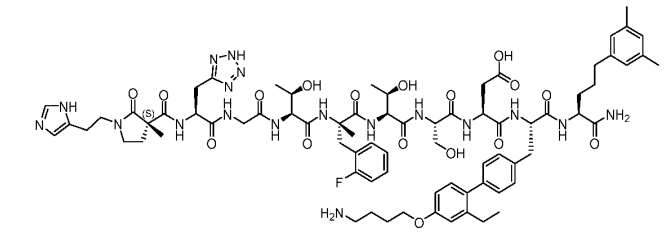

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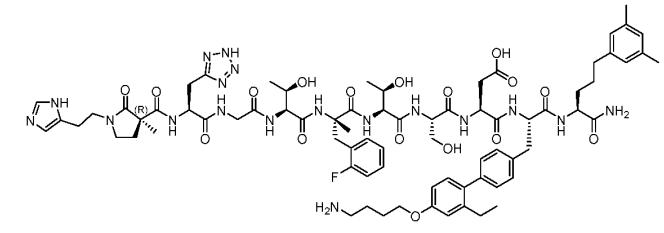

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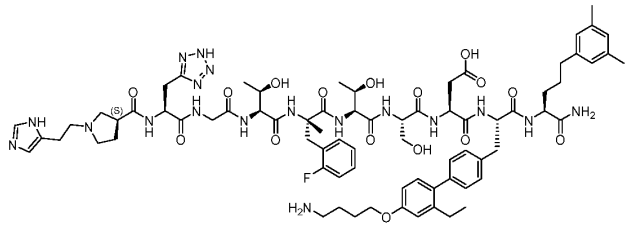

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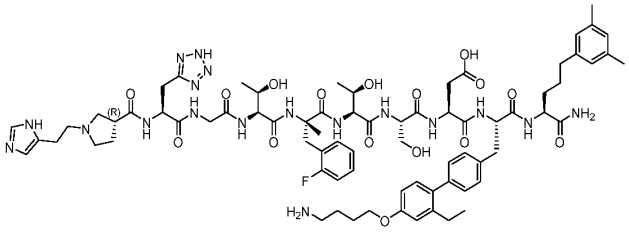

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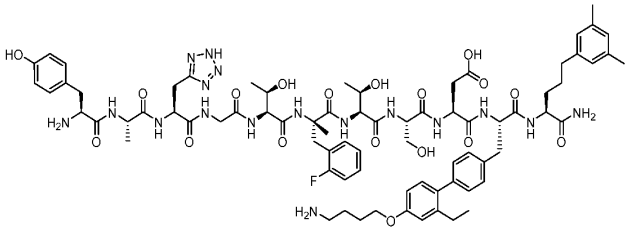

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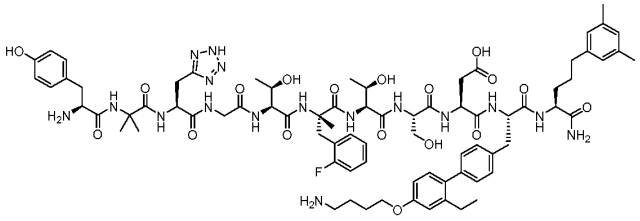

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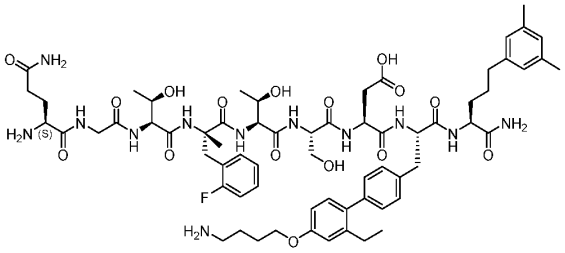

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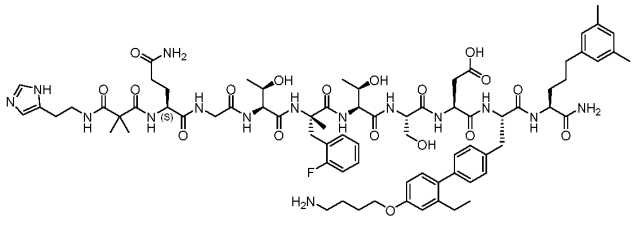

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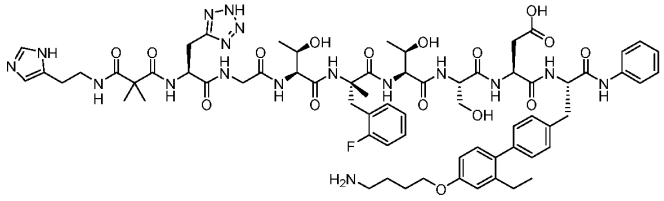

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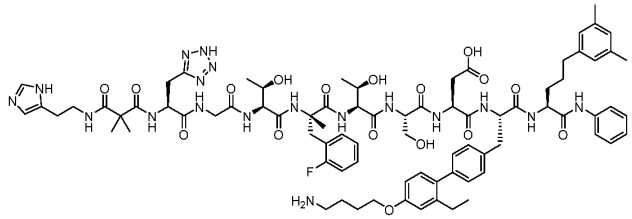

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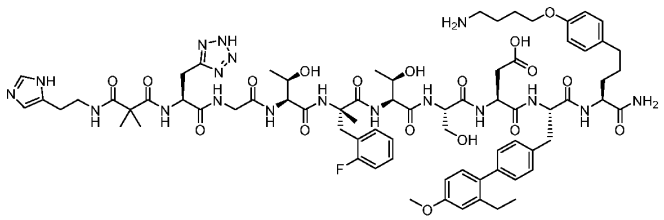

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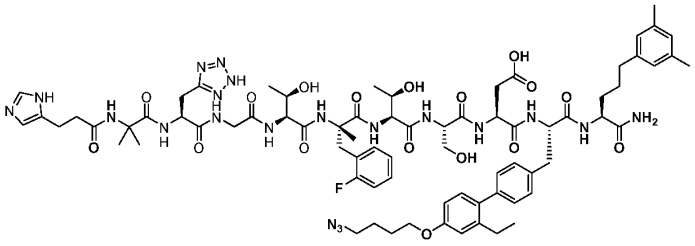

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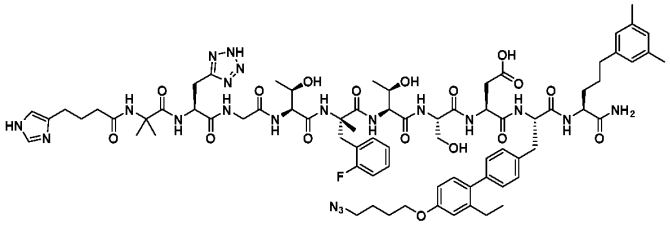

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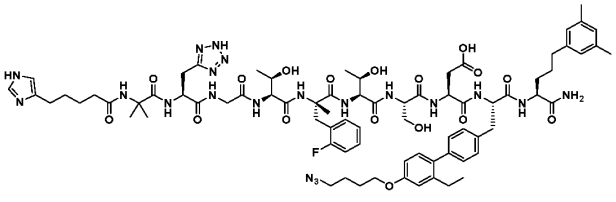

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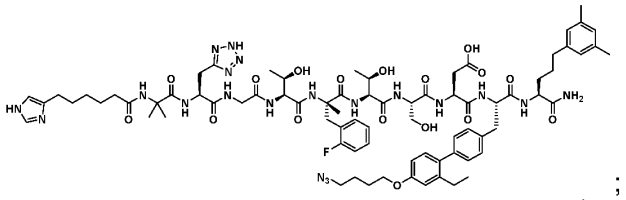

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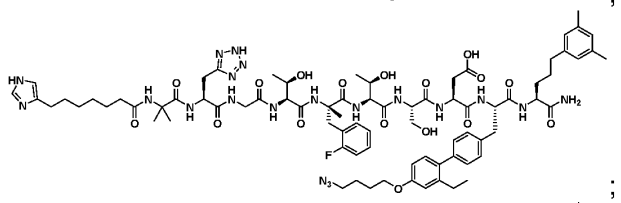

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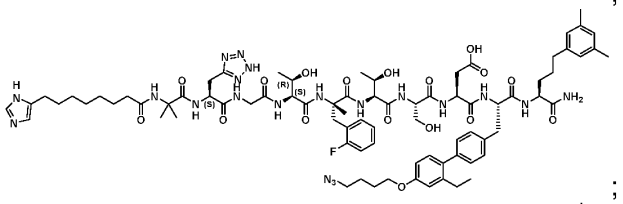

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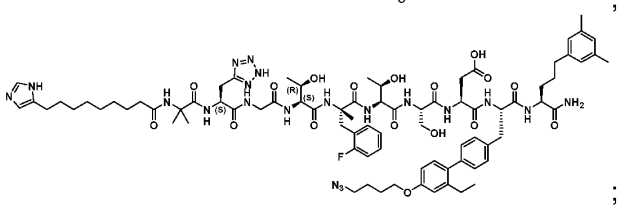

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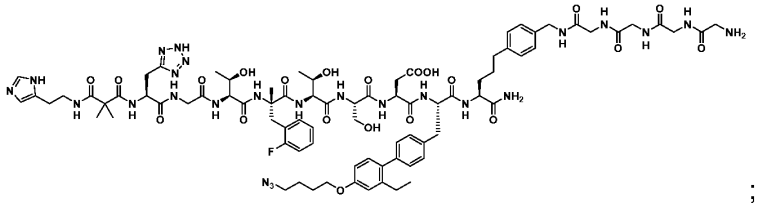

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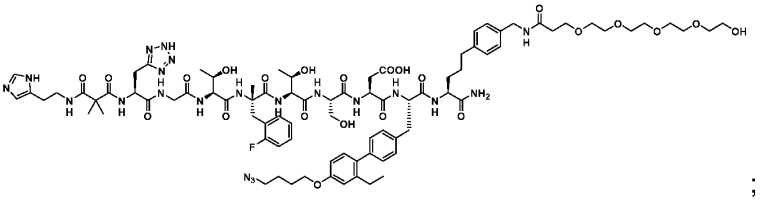

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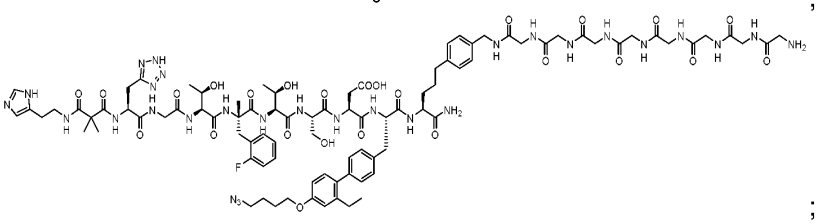

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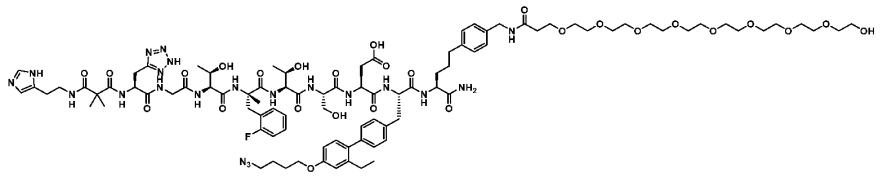

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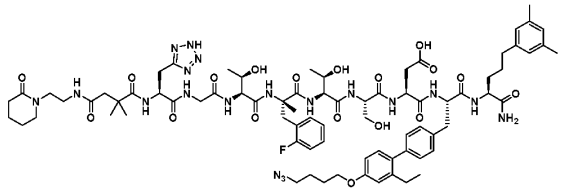

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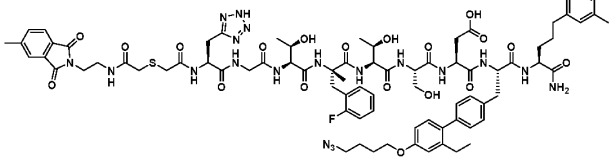

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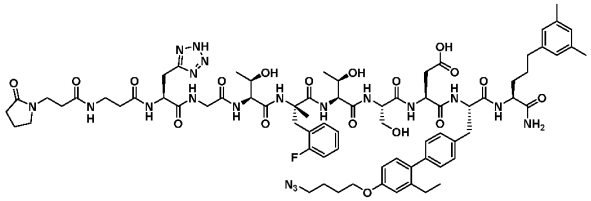

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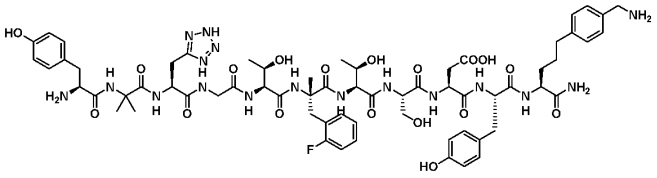

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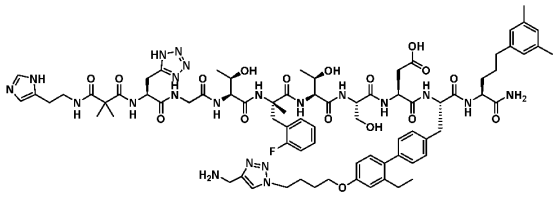

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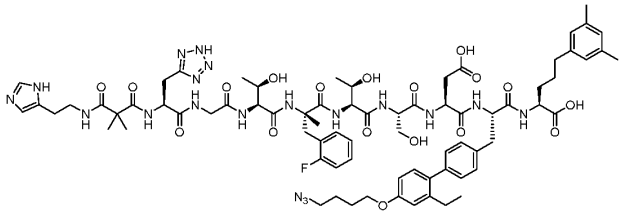

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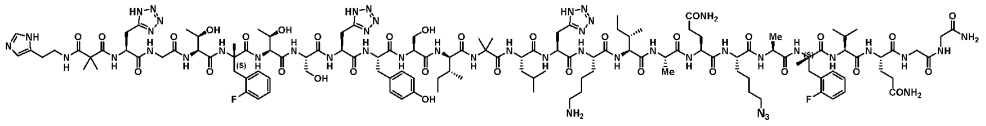

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, and



[00051] In various embodiments of the compound described herein, the compound has a half life of longer than 7 days in plasma.

[00052] In various embodiments of the compound described herein, the compound does not bind to G protein-coupled receptors (GPCRs) other than GLP1R.

[00053] In another aspect, provided herein is a pharmaceutical composition comprising the compound of any of the embodiments described herein.

[00054] In another aspect, provided herein is a pharmaceutical dosage form comprising the compound of any of the embodiments described herein.

[00055] In another aspect, provided herein is a method of selectively targeting GLP1R on a surface of a cell with the compound of any of the embodiments described herein. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is a pancreatic cell, a brain cell, a heart cell, a vascular tissue cell, a kidney cell, an adipose tissue cell, a liver cell, or a muscle cell.

[00056] In another aspect, provided herein is a method of enhancing GLP1R activity in an individual in need thereof comprising administering to the individual an effective amount of the compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein.

[00057] In another aspect, provided herein is a method of lowering blood glucose levels in an individual in need thereof comprising administering to the individual an effective amount of the compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein.

[00058] In another aspect, provided herein is a method of lowering body weight in an individual in need thereof comprising administering to the individual an effective amount of the compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein.

[00059] In another aspect, provided herein is a method of treating a GLP1R-associated condition in an individual in need thereof comprising administering to the individual an effective amount of the compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein. In some embodiments, the GLP1R-associated

condition is type II diabetes, obesity, liver disease, coronary artery disease, or kidney disease. In some embodiments, the GLP1R-associated condition is type II diabetes and/or obesity. In some embodiments,

[00060] In various embodiments of any of the method described herein, the compound, the composition, or the dosage form of the present disclosure is administered subcutaneously, intravenously, intradermally, intraperitoneally, or intramuscularly.In another aspect, provided herein is a method of producing the compound descrbed herein having a structure of Formula (A):

$$BA\text{-}(L\text{-}P)_m \quad (A),$$

the method comprising the steps of:

a) contacting, in the presence of a transglutaminase, the BA comprising at least m glutamine residues Gln with at least m equivalents of compound L-P, and

b) isolating the produced compound of Formula (A)

[00061] In another aspect, provided herein is a method of producing the compound described herein having a structure of Formula (I):

$$BA\text{-}L\text{-}P \quad (I),$$

the method comprising the steps of:

a) contacting, in the presence of a transglutaminase, the BA comprising at least one glutamine residue Gln with a compound L-P, and

b) isolating the produced compound of Formula (I).

[00062] In another aspect, provided herein is a method of producing the compound of claim 1 having a structure of Formula (A):

$$BA\text{-}(L\text{-}P)_m \quad (A),$$

wherein the linker L has has the structure of formula (L'):

$$\text{-La-Y-Lp-} \quad (L'),$$

wherein La is a first linker covalently attached to the BA;

Y is a group comprising a triazole, and

Lp is a second linker covalently attached to the P,

the method comprising the steps of:

a) contacting, in the presence of a transglutaminase, the BA comprising at least m glutamine residues Gln with the first linker La comprising an azide or an alkyne moiety;

b) contacting the product of step a) with at least m equivalents of compound Lp-P, wherein the second linker Lp comprises an azide or an alkyne moiety, wherein La and Lp are capable of reacting to produce a triazole, and

c) isolating the produced compound of Formula (A).

[00063] In another aspect, provided herein is a method of producing the compound described herein having a structure of Formula (I):

BA-L-P (I),

wherein the linker L has has the structure of formula (L'):

-La-Y-Lp- (L'),

wherein La is a first linker covalently attached to the BA;

Y is a group comprising a triazole, and

Lp is a second linker covalently attached to the P,

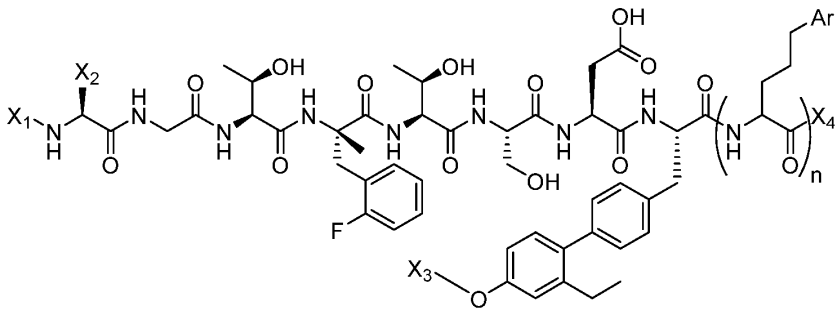
the method comprising the steps of:

a) contacting, in the presence of a transglutaminase, the BA comprising at least one glutamine residue Gln with the first linker La comprising an azide or an alkyne moiety;

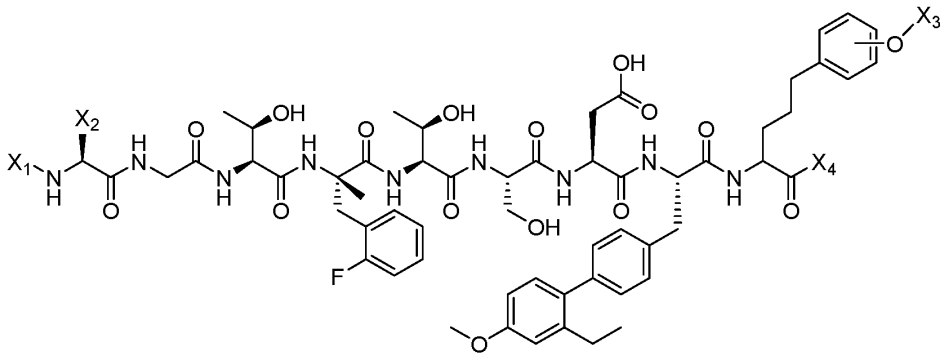
b) contacting the product of step a) with a compound Lp-P, wherein the second linker Lp comprises an azide or an alkyne moiety, wherein La and Lp are capable of reacting to produce a triazole, and

c) isolating the produced compound of Formula (I).

[00064] In another aspect, provided herein is a compound having a structure selected from the group consisting of Formula (P-IB), Formula (P-IIB), and Formula (P-IIIB):

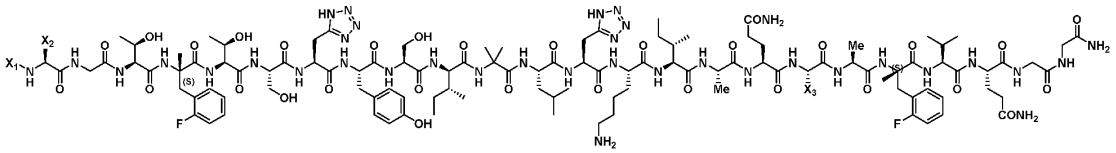


(P-IB),

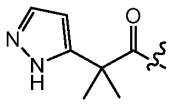
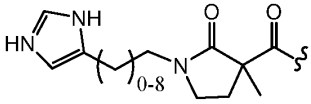
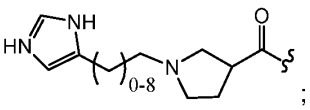


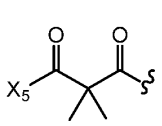
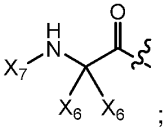
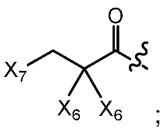
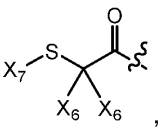
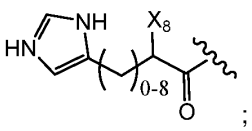
(P-IIB),

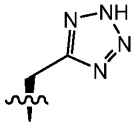
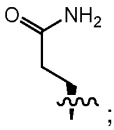
and



(P-IIIB), wherein:

$X_1$ is selected from H;  ;  ;  ;

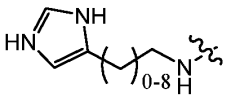
 ;  ;  ;  , and  ;

$X_2$ is selected from  and  ;

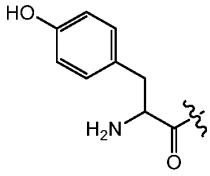
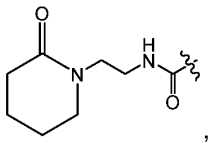
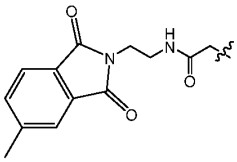
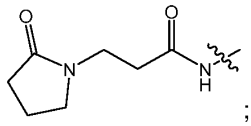
$X_3$ is selected from $-CH_3$, $-(CH_2)_{2-6}-NH_2$, $-(CH_2)_{2-6}-N_3$, and $-(CH_2)_{2-6}-Tr-(CH_2)_{1-6}-NH_2$, where Tr is a triazole moiety;

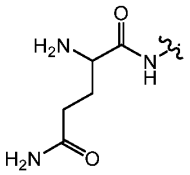
n is 0 or 1;

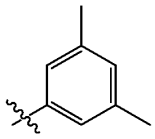
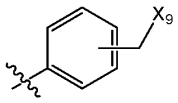
$X_4$ is selected from $-NH_2$, -OH and -N(H)(phenyl);

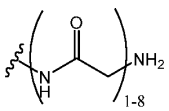
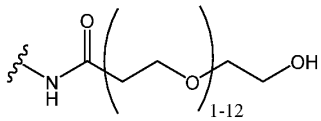
$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  ,  ,  ,  ,  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ;

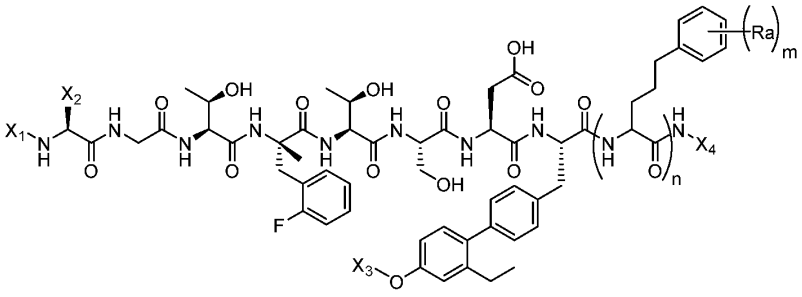
Ar is selected from  and  ;

$X_9$ is selected from $-NH_2$,  , and  , and

m is an integer from 1 to 4

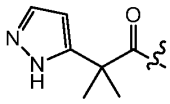
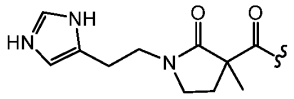
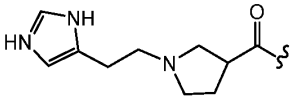
or a pharmaceutically acceptable salt thereof.

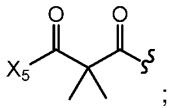
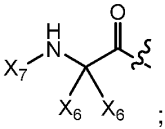
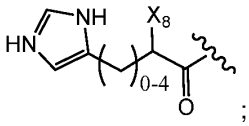
[00065] In another aspect, provided herein is a compound having a structure of Formula (II):

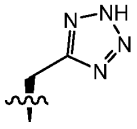
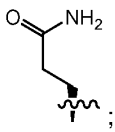


(II),

wherein:

$X_1$ is selected from H;  ;  ;  ;

 ;  ; and  ;

$X_2$ is selected from  and  ;

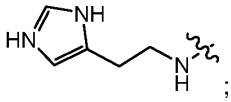
$X_3$ is selected from $–(CH_2)_{2\text{-}6}–NH_2$, $–(CH_2)_{2\text{-}6}–N_3$, and $-CH_3$, with the proviso that when $X_3$ is $-CH_3$, n is 1 and Ra in at least one occurrence is selected from $–(CH_2)_{2\text{-}6}–NH_2$ and $–(CH_2)_{2\text{-}6}–N_3$;

n is 0 or 1;

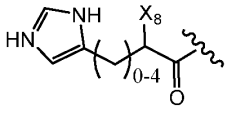
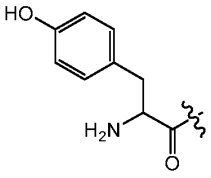
m is an integer from 0 to 3;

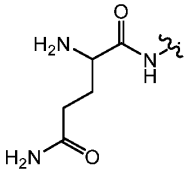
Ra is independently at each occurrence selected from $-CH_3$, $–(CH_2)_{2\text{-}6}–NH_2$, and $–(CH_2)_{2\text{-}6}–N_3$;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

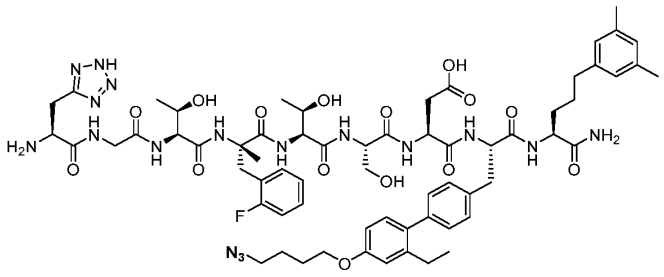
$X_7$ is selected from H,  , and  ;

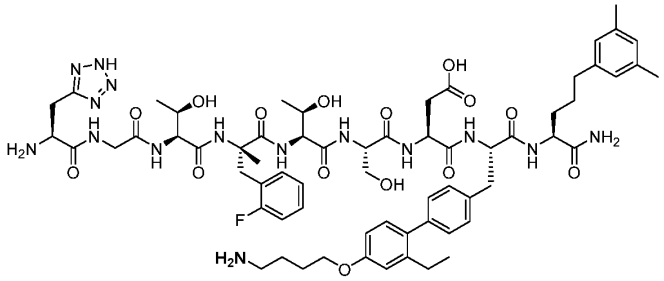
$X_8$ is selected from H, -OH, $-NH_2$, and  ,

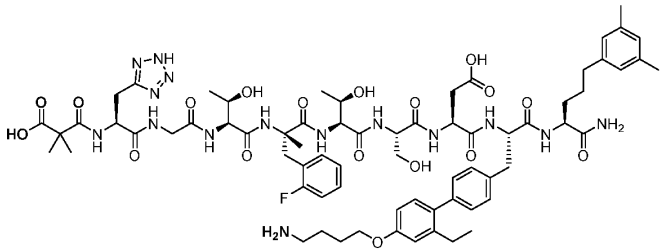
and pharmaceutically acceptable salts thereof.

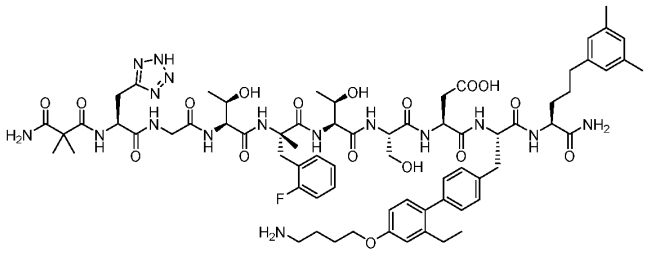
[00066] In some embodiments, P has the structure selected from the group consisting of:

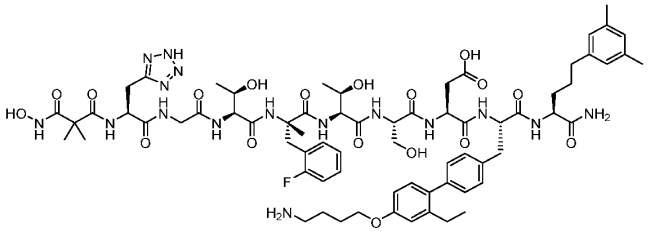
Structure

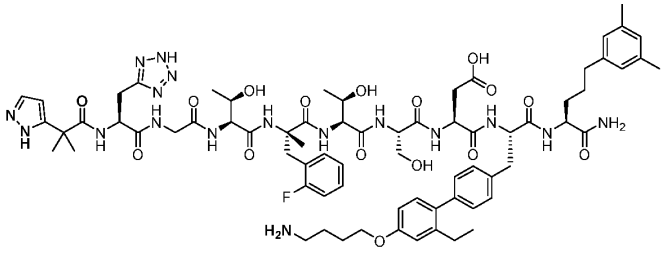

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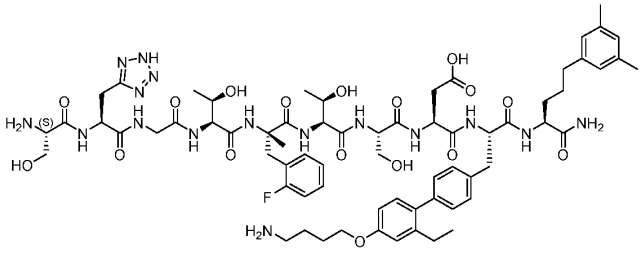

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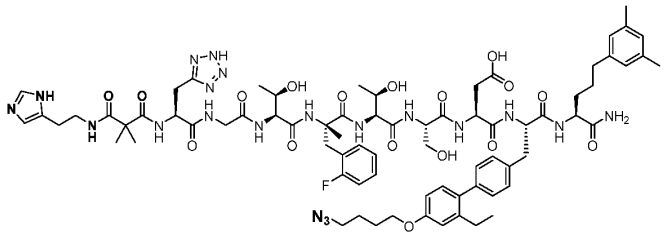

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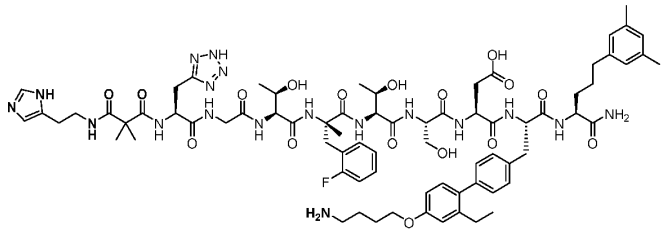

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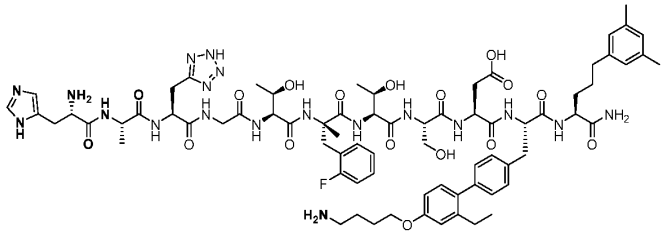

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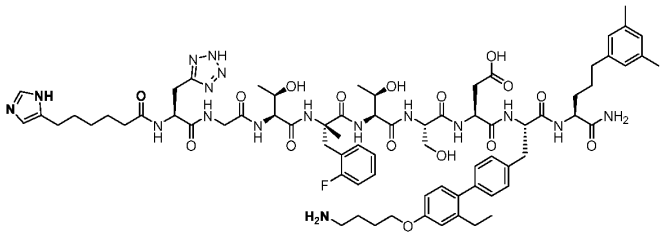

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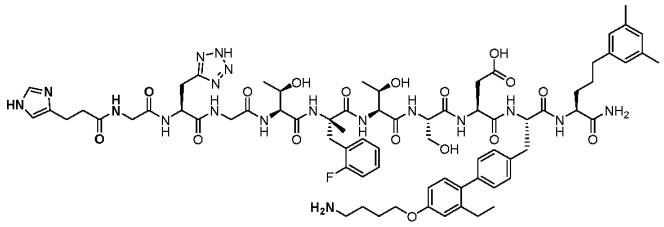

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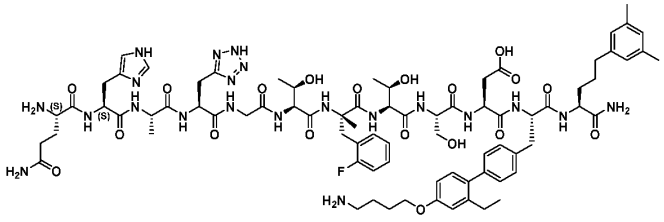

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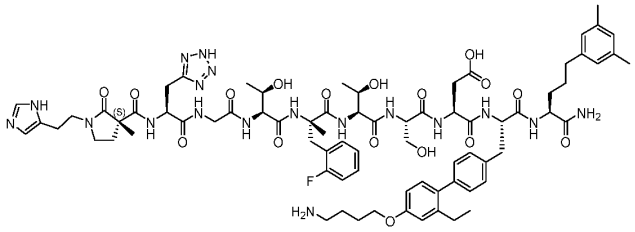

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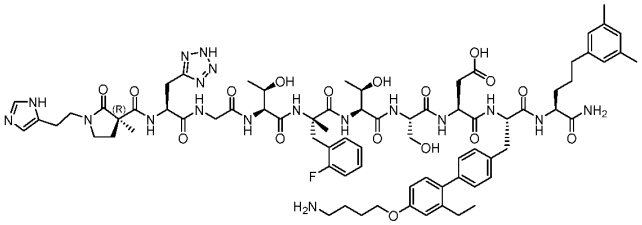

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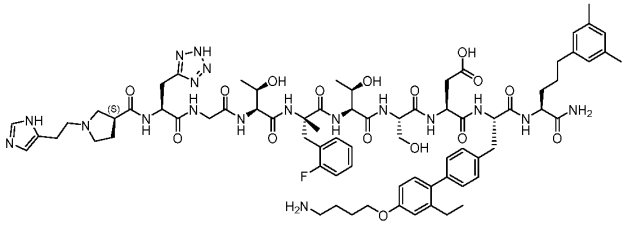

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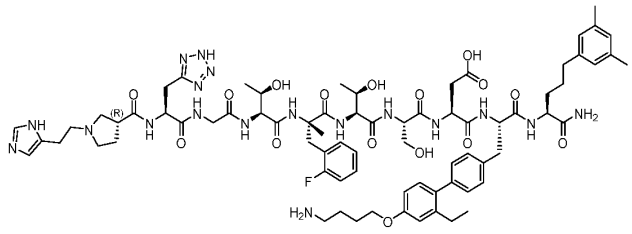

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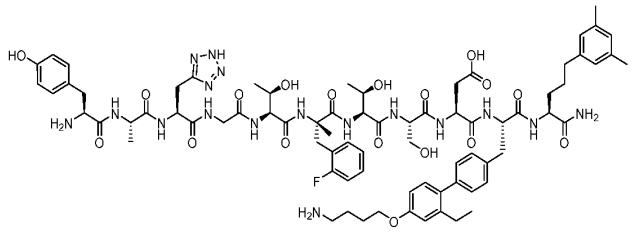

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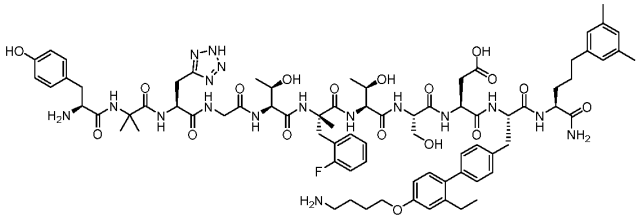

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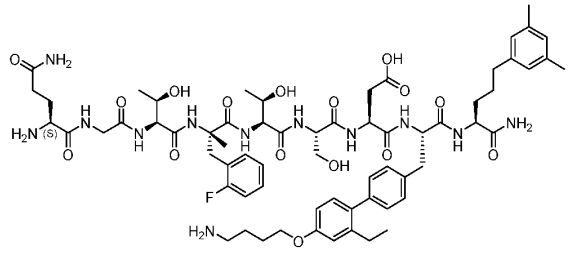

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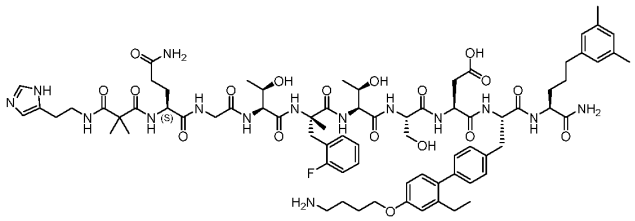

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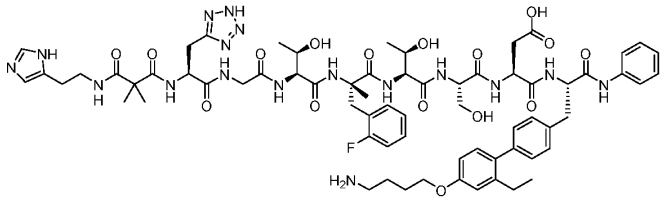

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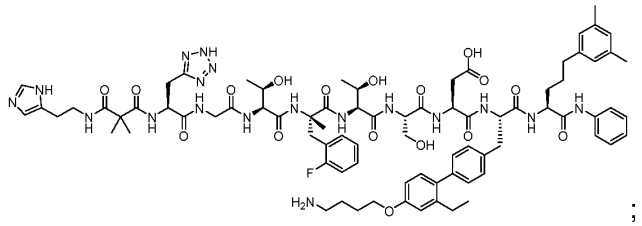

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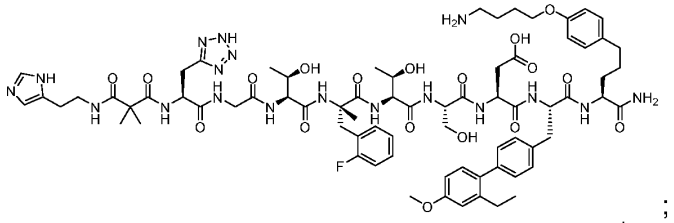

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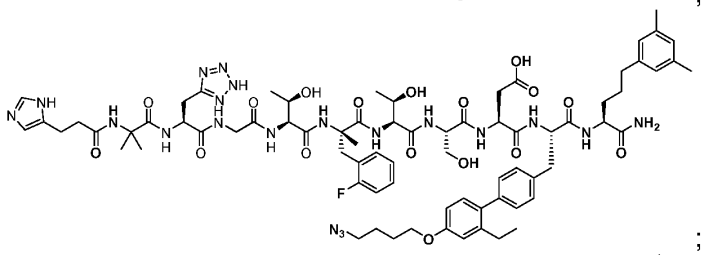

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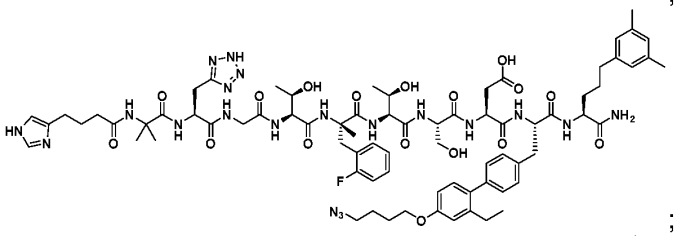

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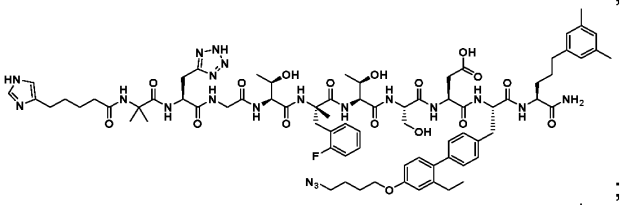

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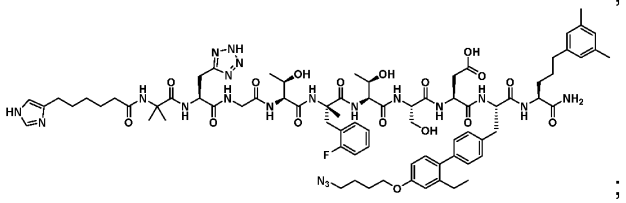

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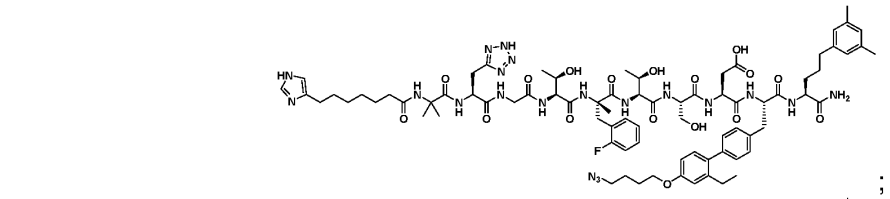

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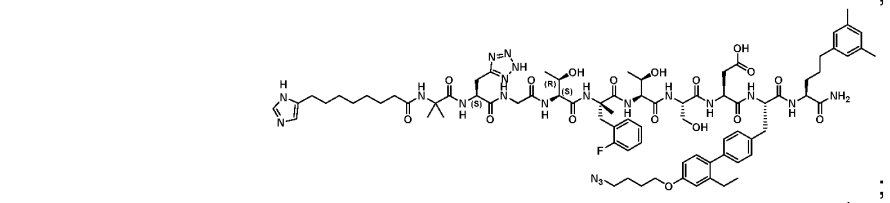

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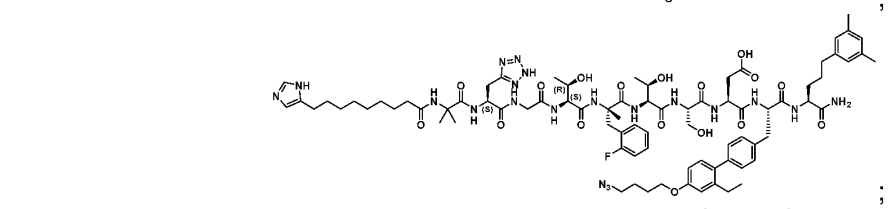

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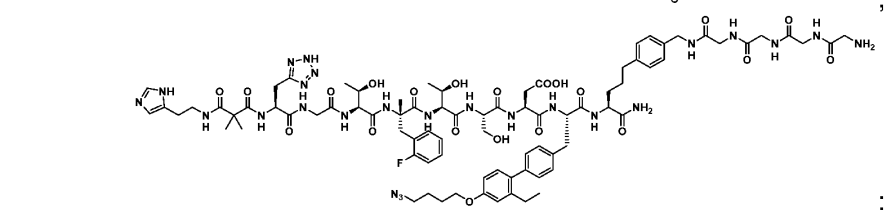

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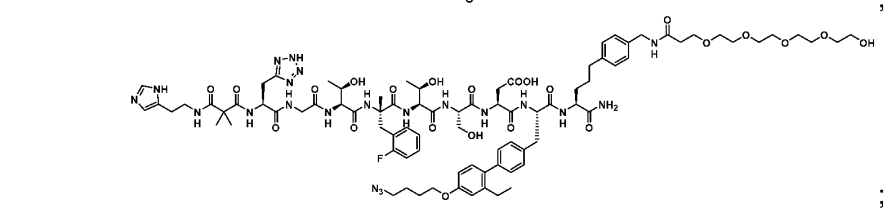

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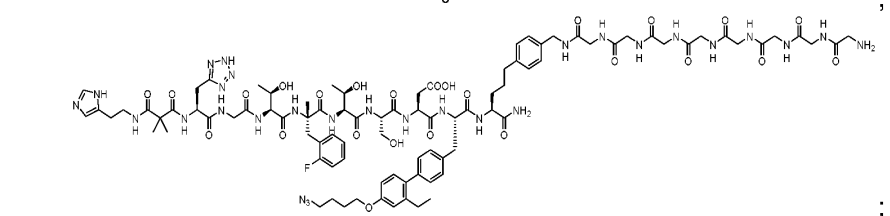

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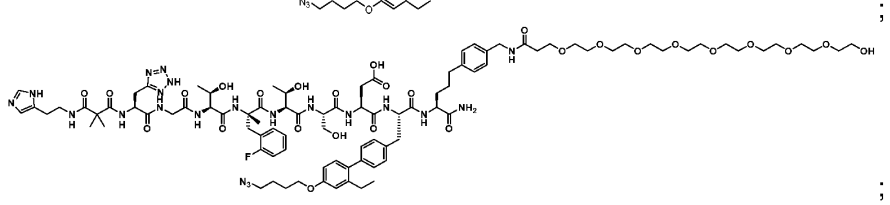

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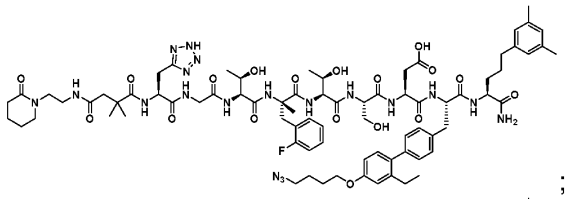

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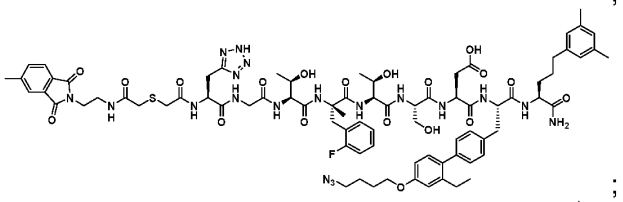

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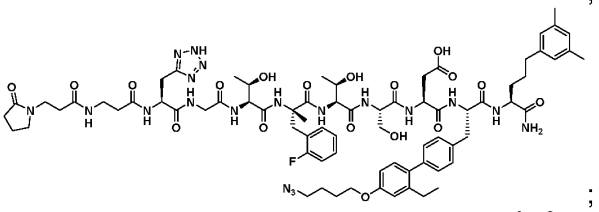

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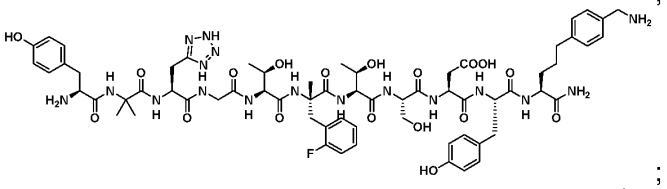

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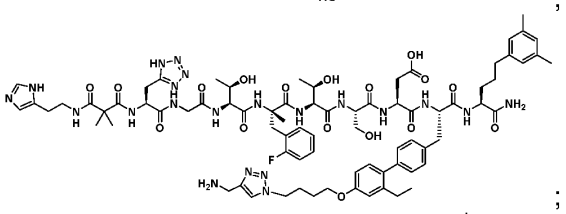

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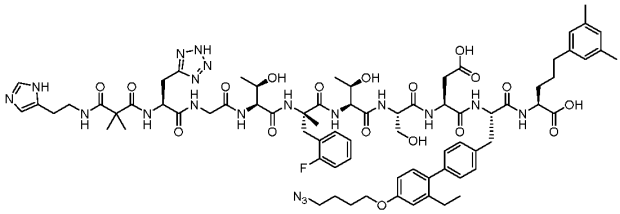

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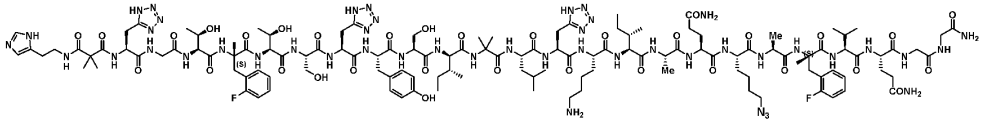

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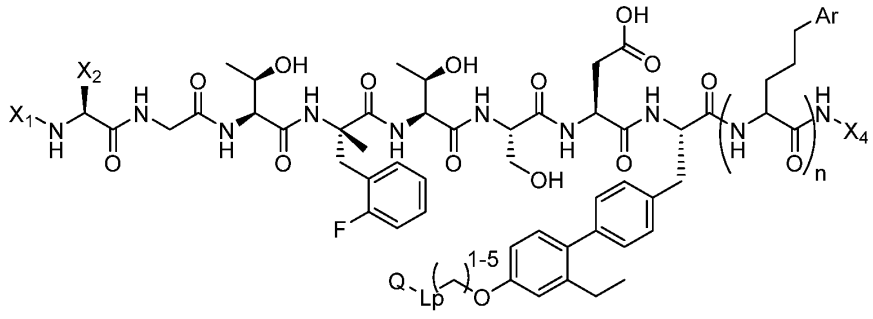

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, and

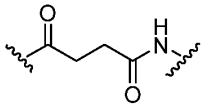

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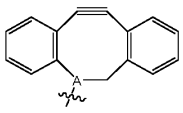
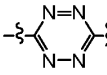
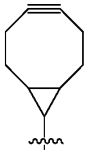
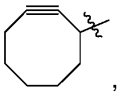
[00067] In another aspect, provided herein is a compound having a structure of Formula (C):

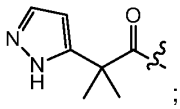
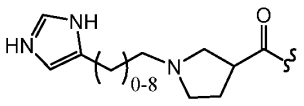


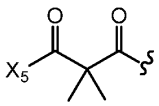
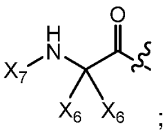
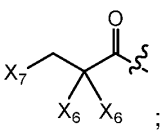
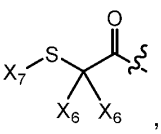
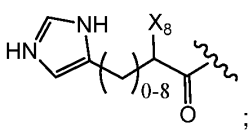
(C),

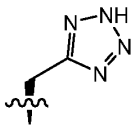
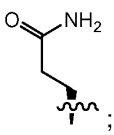
wherein:

$L_p$ is absent or a linker comprising one or more of  ; a carbamate group; a cyclodextrin; a polyethylene glycol (PEG) segment having 1 to 36 $-CH_2CH_2O-$ (EG) units; a $-(CH_2)_{2-24}-$ chain; a triazole; one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline, and combinations thereof;

Q is a moiety selected from $-NH_2$, $-N_3$, ,  ;  ;  ; and  , where A is C or N;

$X_1$ is selected from H;  ;  ;  ;

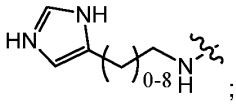
 ;  ;  ;  , and  ;

$X_2$ is selected from  and  ;

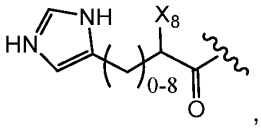
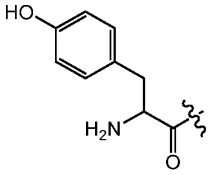
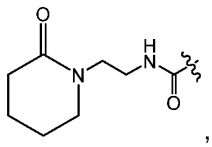
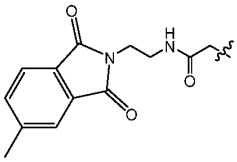
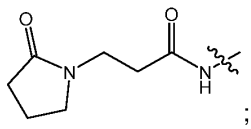
$X_3$ is selected from -$CH_3$, –$(CH_2)_{2-6}$–$NH_2$, –$(CH_2)_{2-6}$–$N_3$, and –$(CH_2)_{2-6}$–Tr–$(CH_2)_{1-6}$–$NH_2$, where Tr is a triazole moiety;

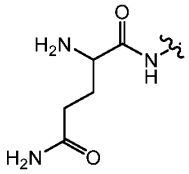
n is 0 or 1;

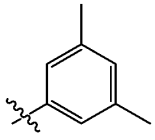
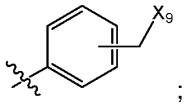
$X_4$ is selected from -$NH_2$, -OH and -N(H)(phenyl);

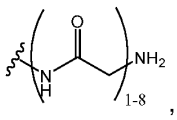
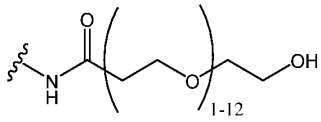
$X_5$ is selected from -OH, -$NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, -$CH_3$, and -$CH_2OH$;

$X_7$ is selected from H,  ,  ,  ,  ,  ;

$X_8$ is selected from H, -OH, -$NH_2$, and  ;

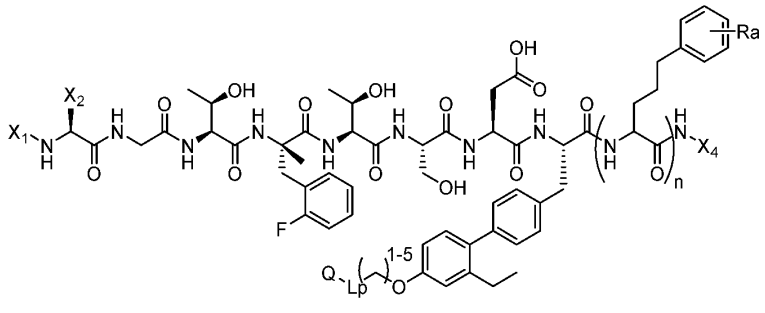
Ar is selected from  and  ;

$X_9$ is selected from -$NH_2$,  , and  , and

m is an integer from 1 to 4

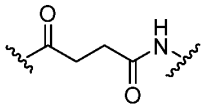
or a pharmaceutically acceptable salt thereof

[00068] In another aspect, provided herein is a compound having a structure of Formula (III):

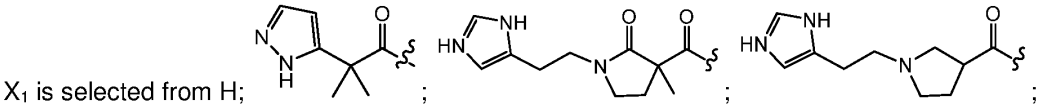


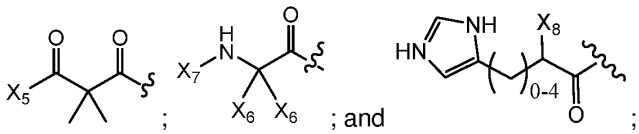
(III),

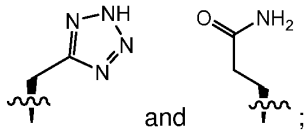
wherein:

$L_p$ is absent or a linker comprising one or more of  ; a carbamate group; a cyclodextrin; a polyethylene glycol (PEG) segment having 1 to 36 -$CH_2CH_2O$- (EG) units; one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline, and combinations thereof;

Q is a moiety selected from -$N_3$, , where A is C or N;

$X_1$ is selected from H;  ;

 ; and ;

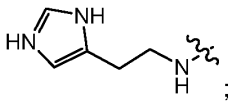
$X_2$ is selected from  and ;

$X_3$ is selected from –$(CH_2)_{2-6}$–$NH_2$, –$(CH_2)_{2-6}$–$N_3$, and -$CH_3$, with the proviso that when $X_3$ is -$CH_3$, n is 1 and Ra in at least one occurrence is selected from –$(CH_2)_{2-6}$–$NH_2$ and –$(CH_2)_{2-6}$–$N_3$;

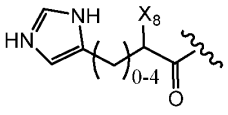
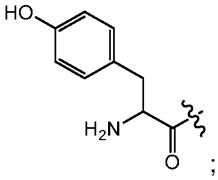
n is 0 or 1;

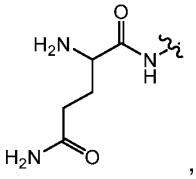
Ra is independently at each occurrence selected from H, -$CH_3$, –$(CH_2)_{2-6}$–$NH_2$, and –$(CH_2)_{2-6}$–$N_3$;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, -$NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, -$CH_3$, and -$CH_2OH$;

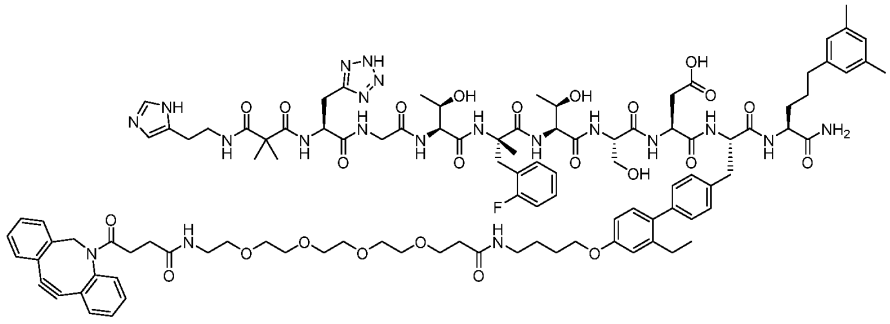
$X_7$ is selected from H,  , and  ;

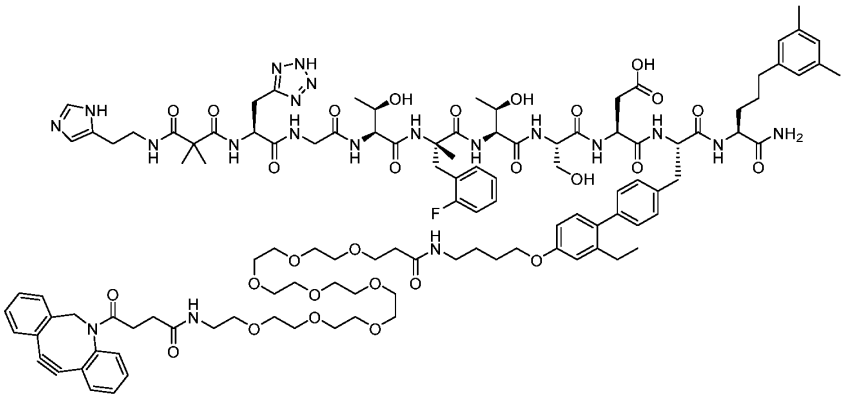
$X_8$ is selected from H, -OH, -$NH_2$, and  ,

and pharmaceutically acceptable salts thereof.

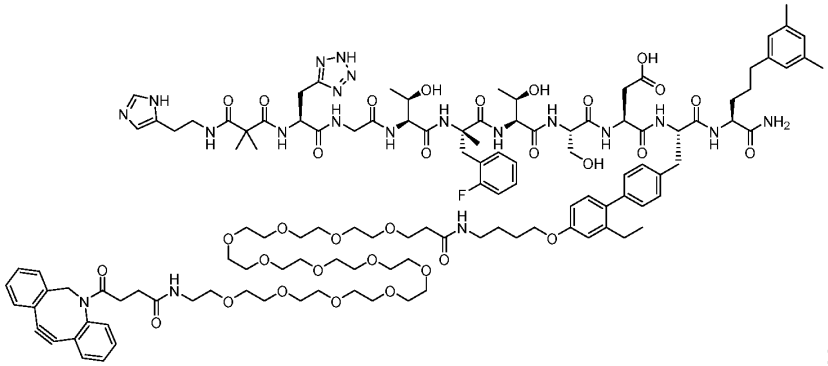
[00069] In some embodiments, The compound described above has a structure selected from the group consisting of:

**Structure**

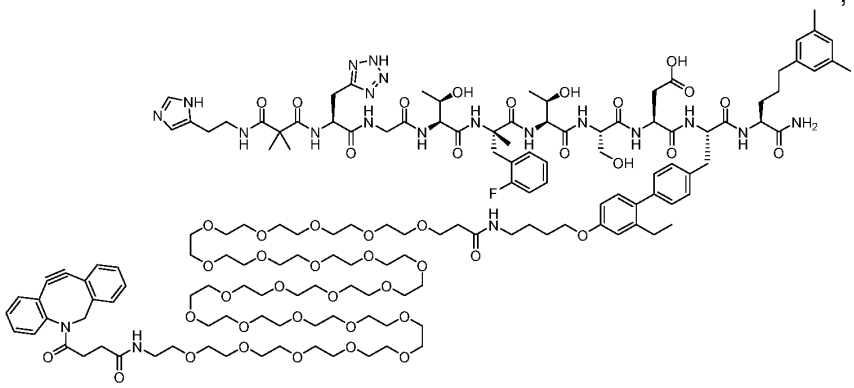
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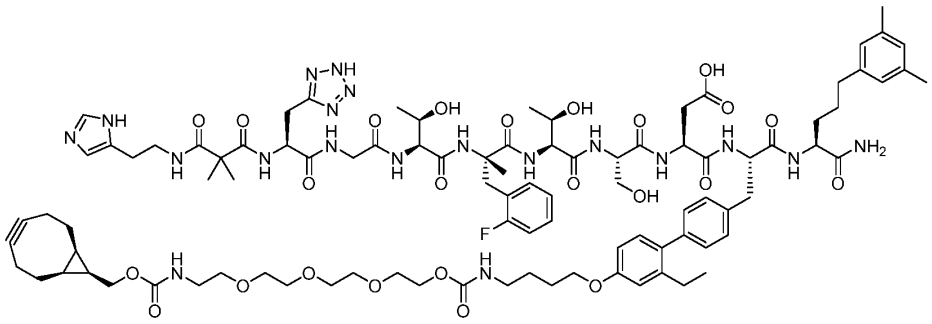
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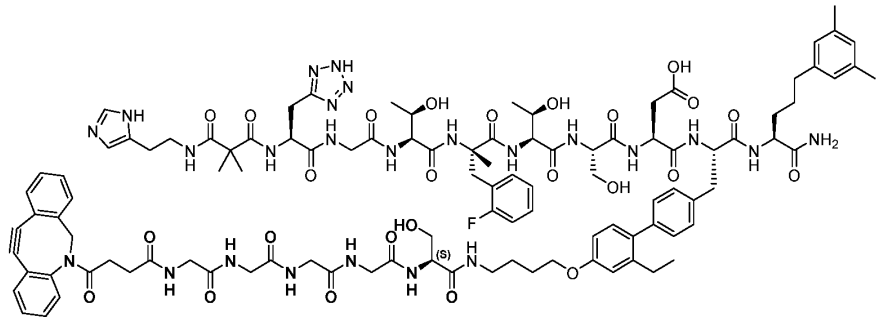
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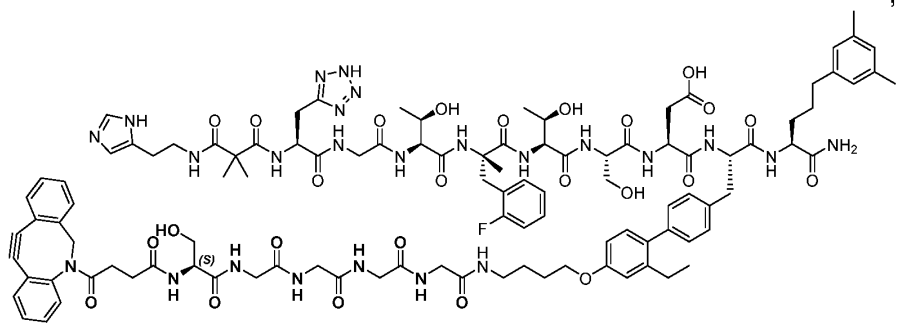
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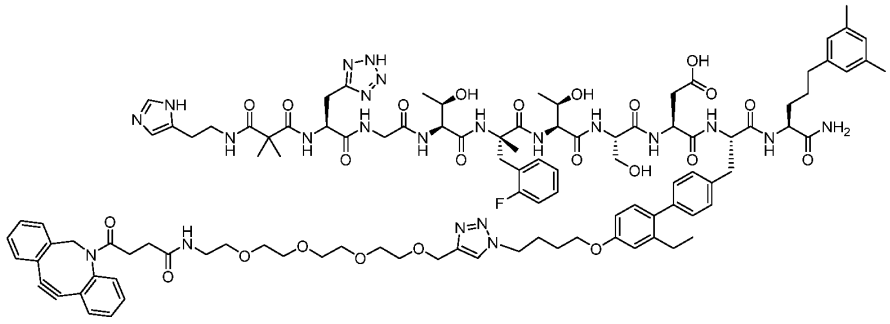
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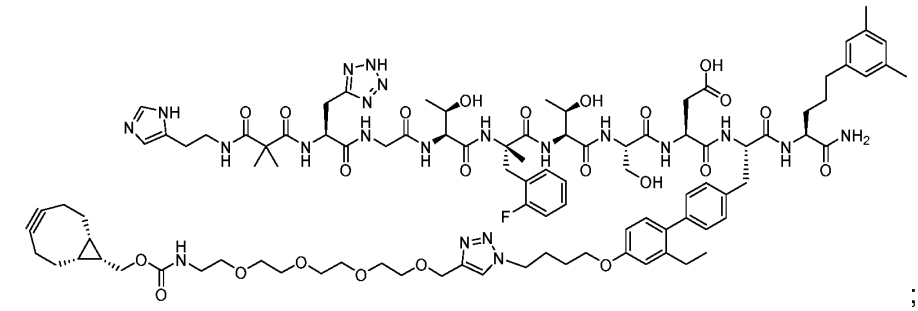
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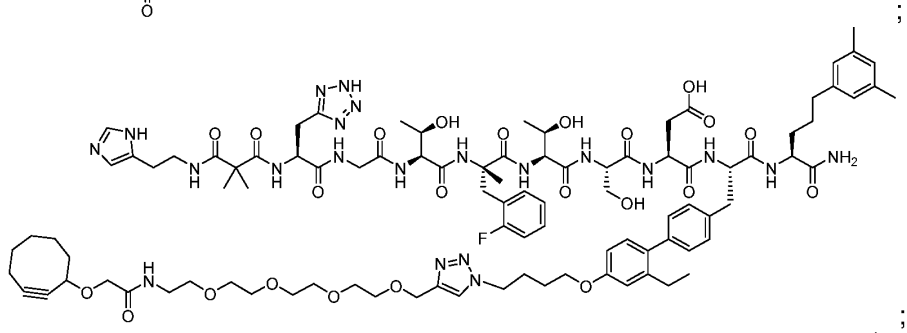
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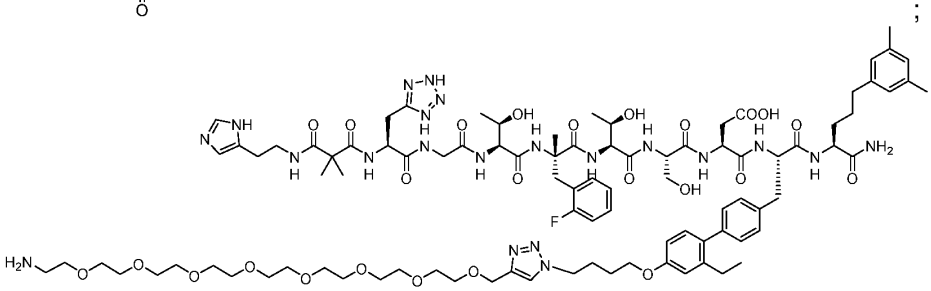
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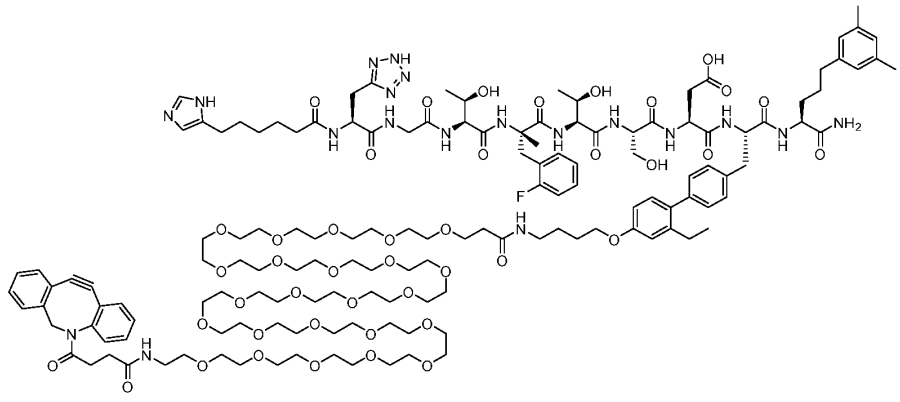
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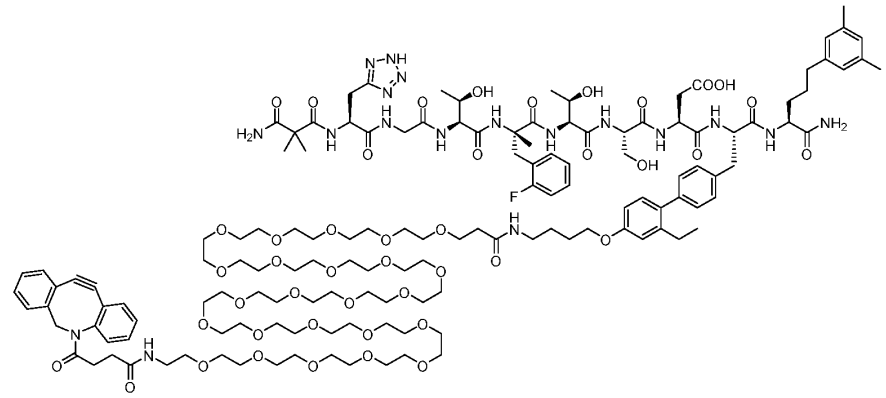
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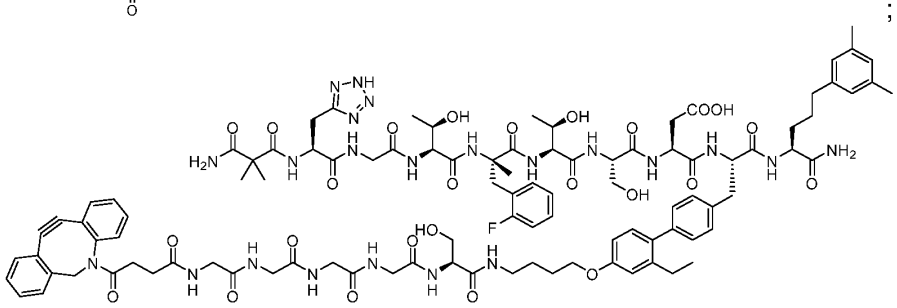
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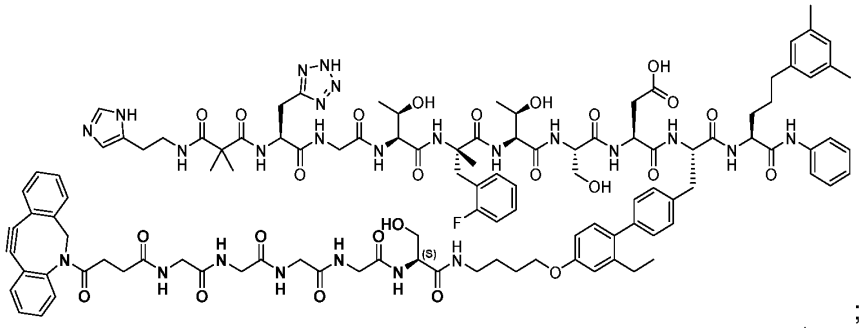
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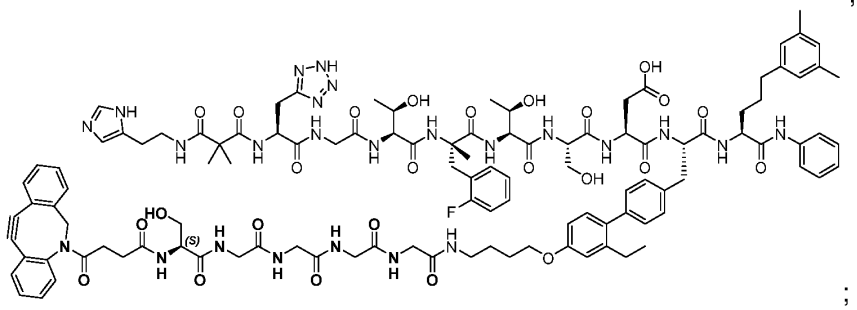
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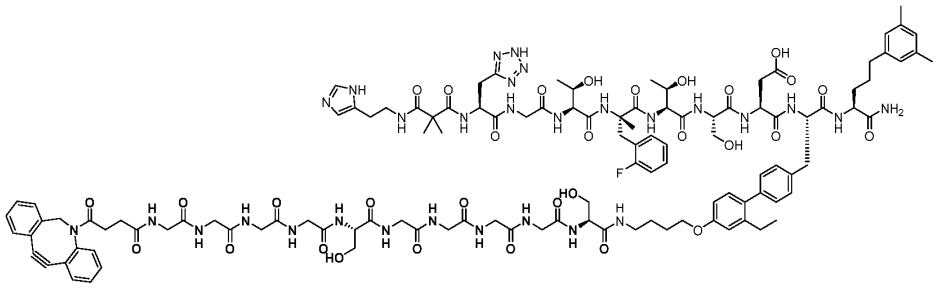
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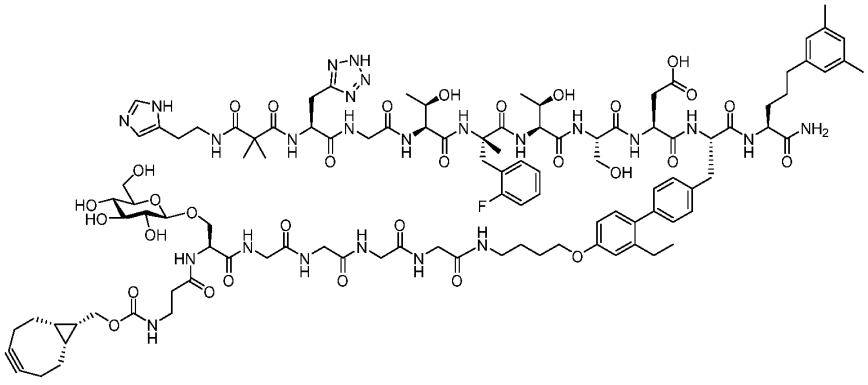
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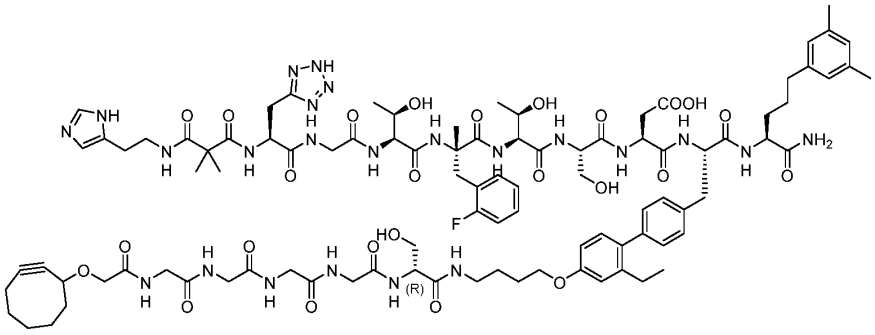
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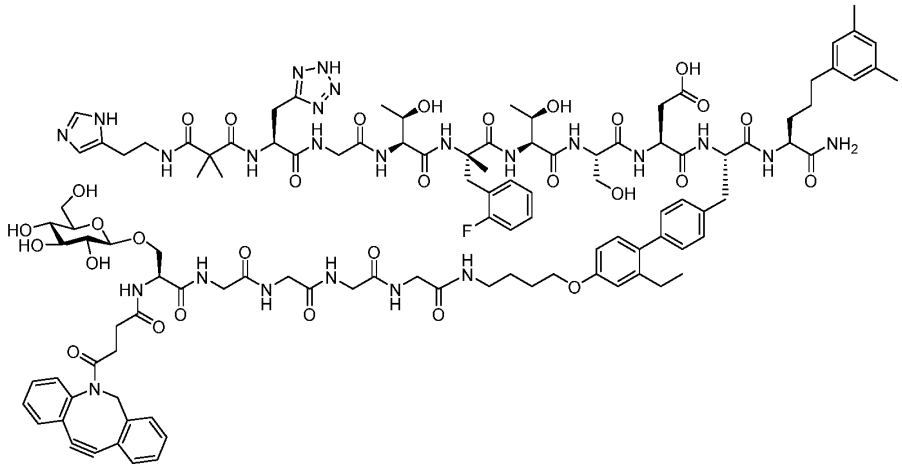
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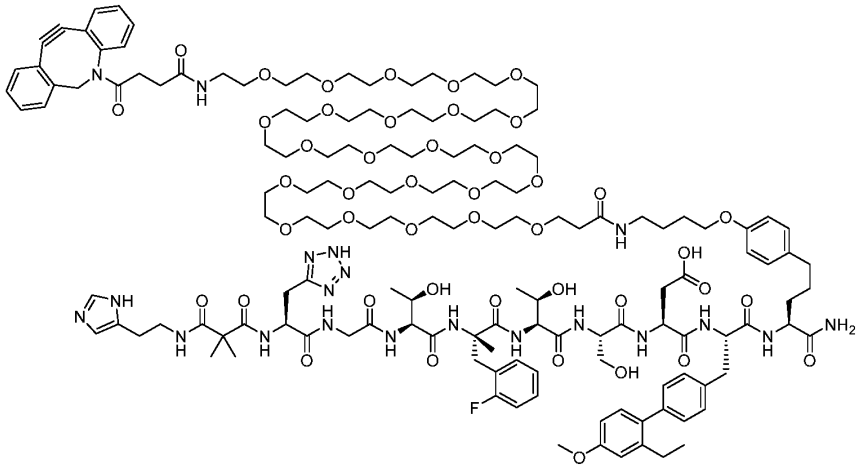
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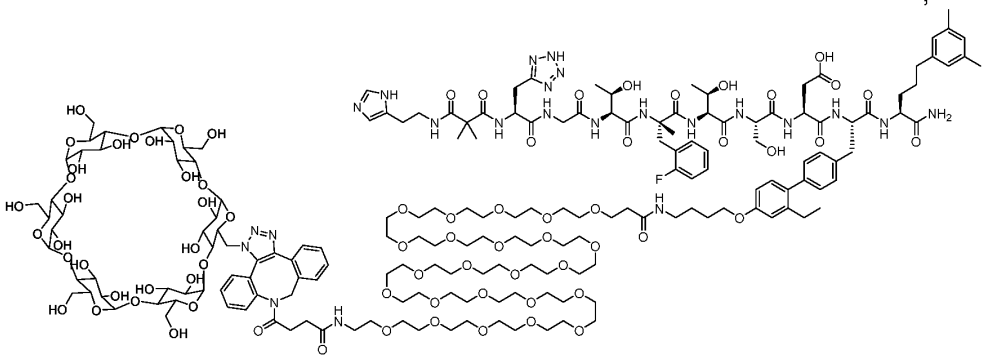
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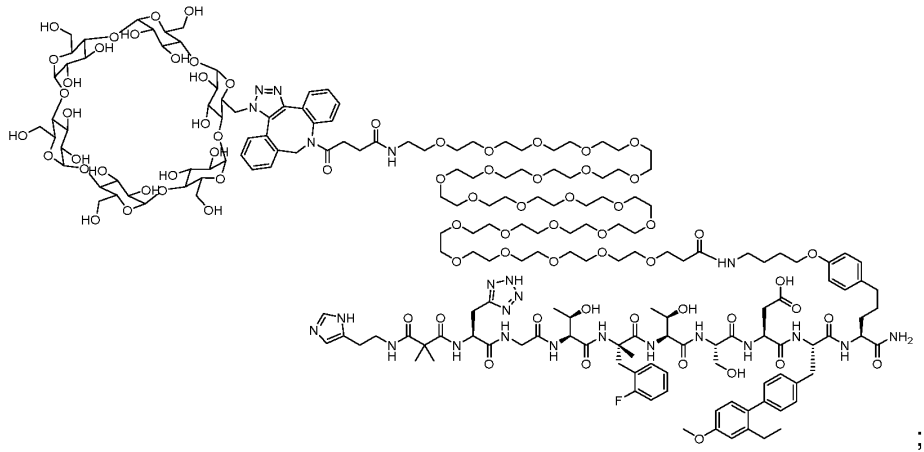
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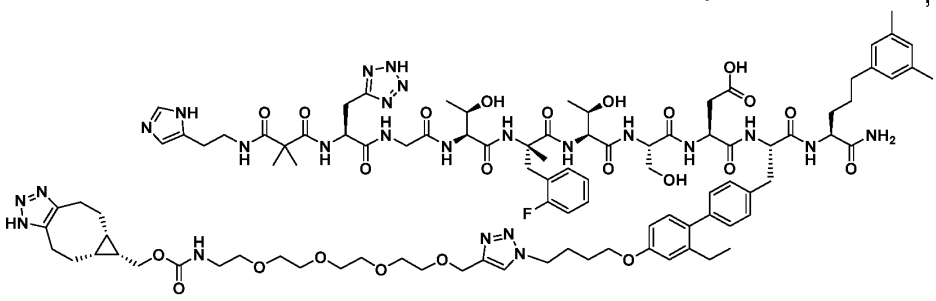
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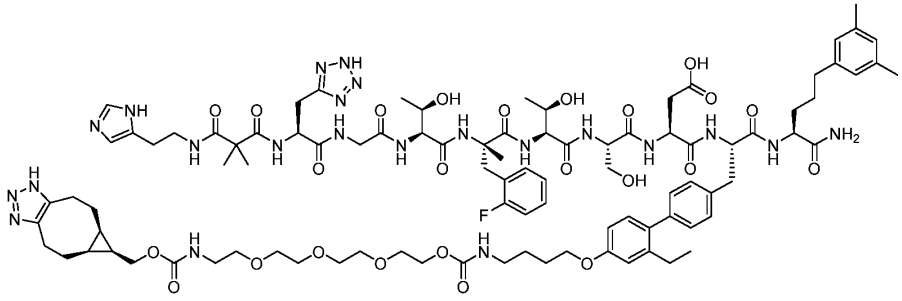
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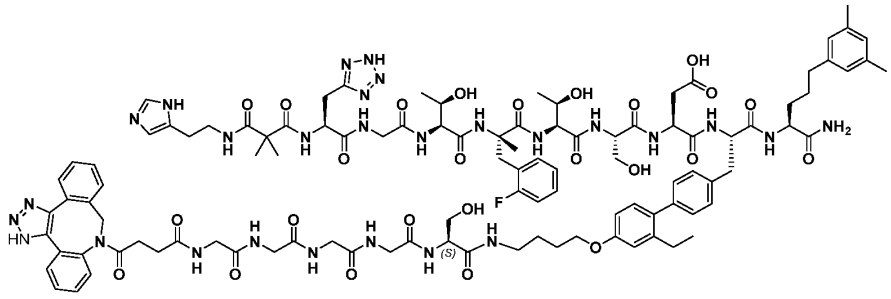
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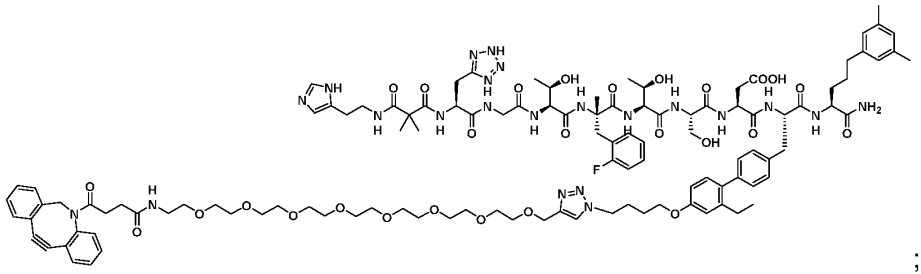
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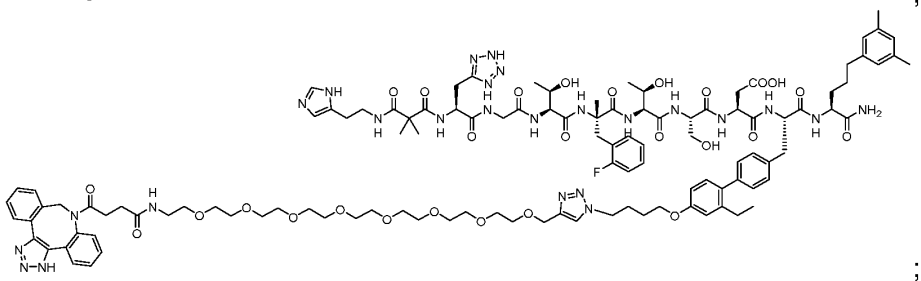


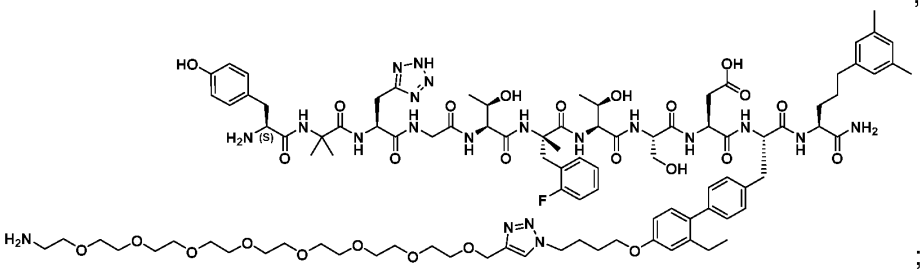
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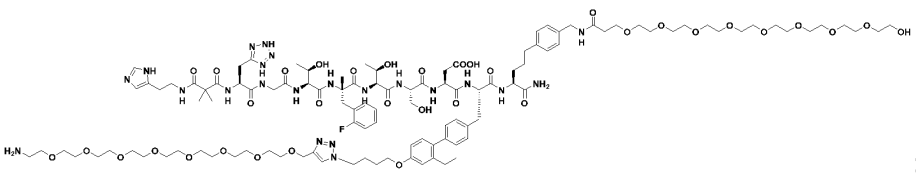


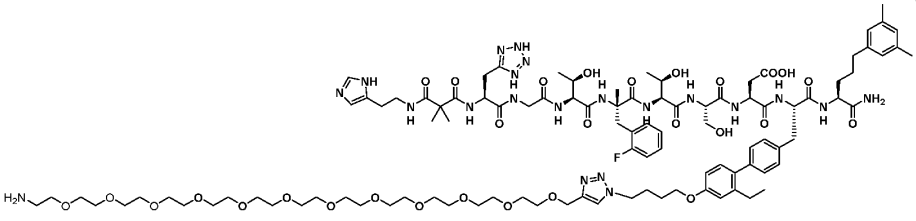
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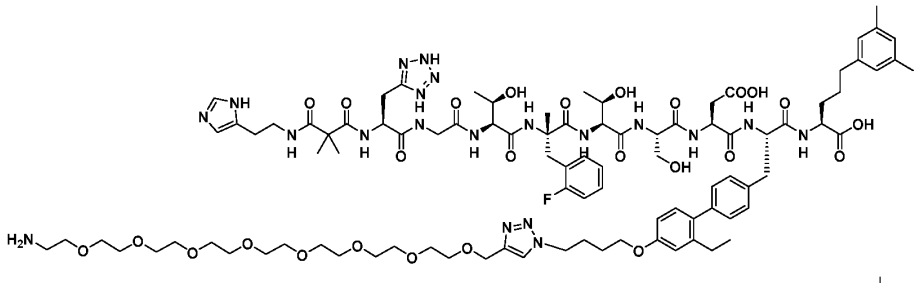

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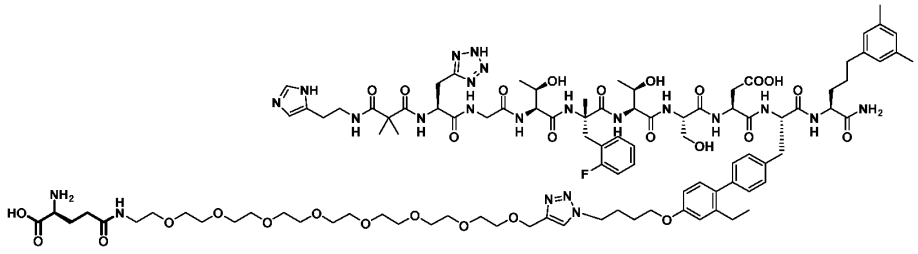

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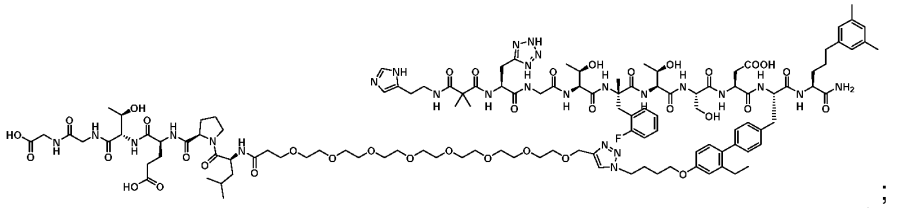

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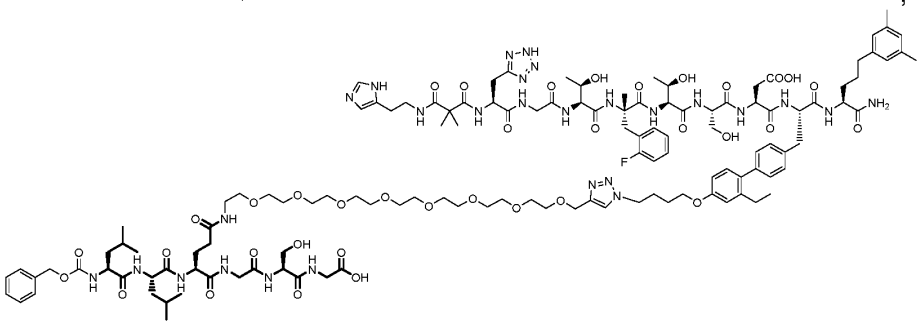

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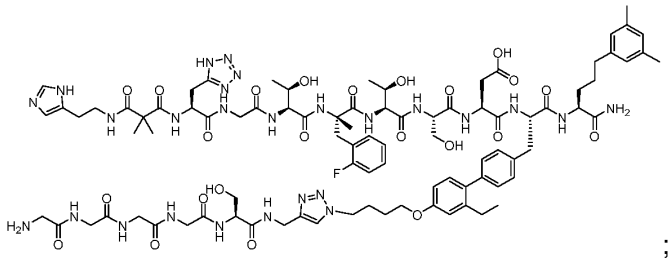

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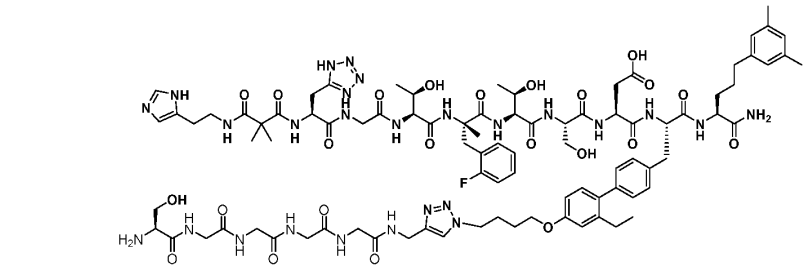

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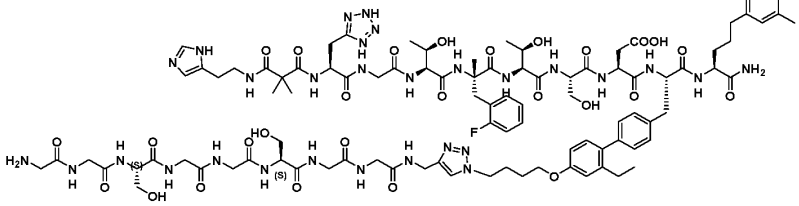

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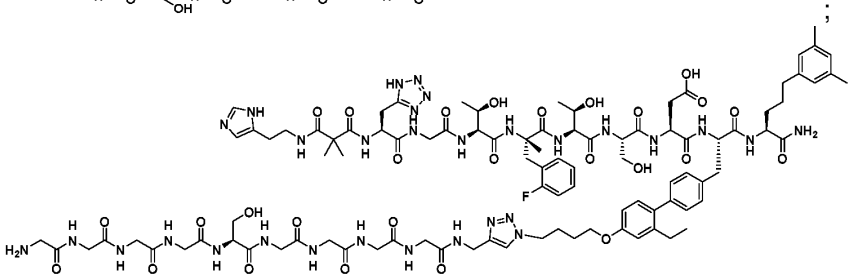

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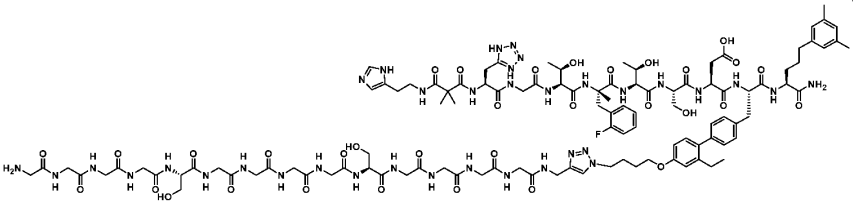
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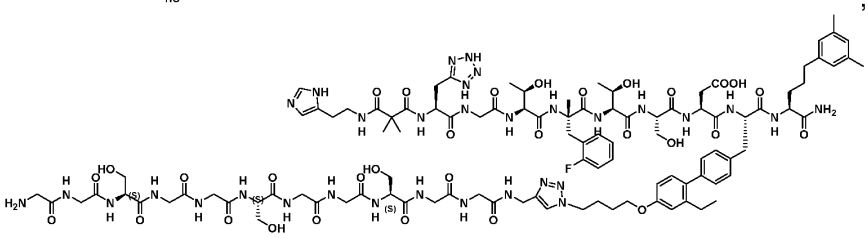
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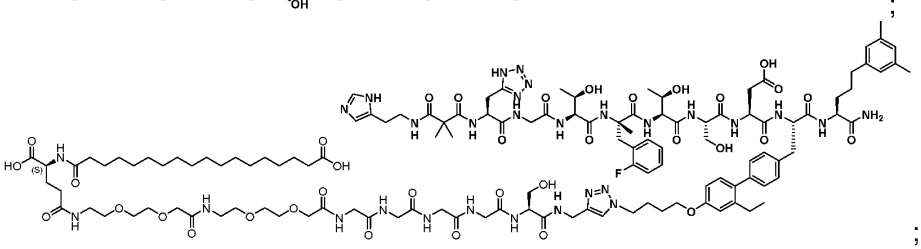
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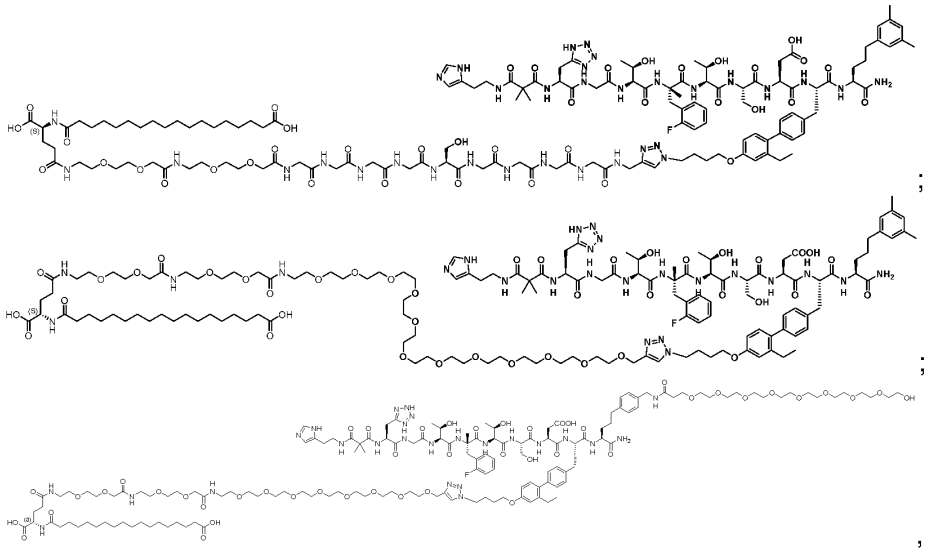
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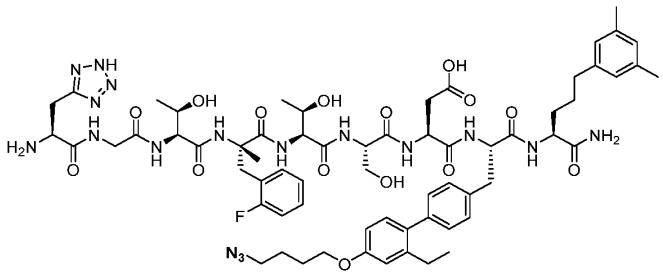


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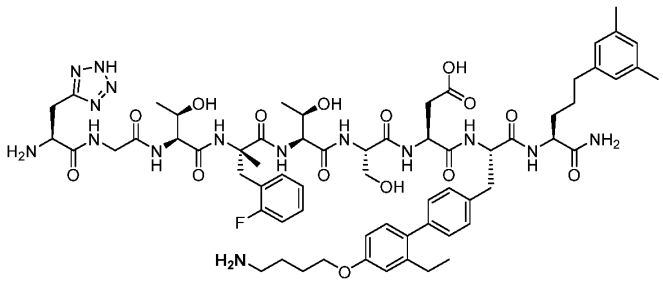
or a pharmaceutically acceptable salt thereof.

[00070] In another aspect, provided herein is an antibody-drug conjugate comprising a Glucagon-like peptide-1 receptor (GLP1R)-targeting antibody or an antigen-binding fragment thereof conjugated, optionally through a linker, to a payload having the structure selected from the group consisting of:

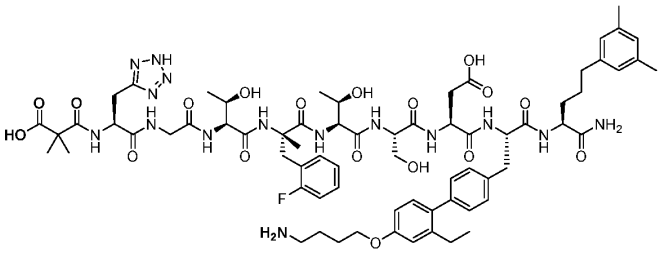
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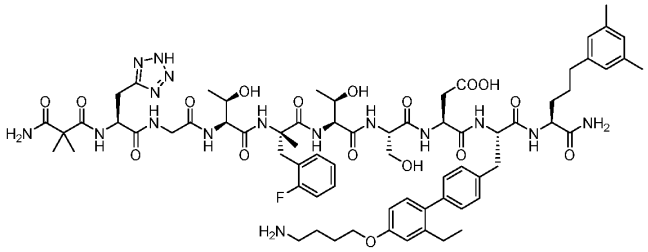


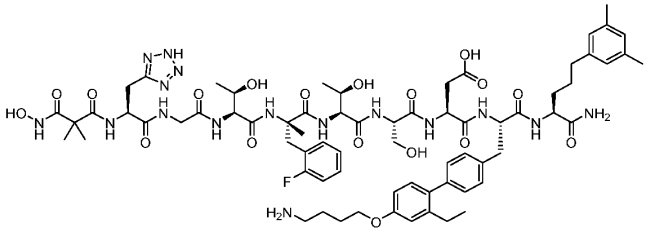
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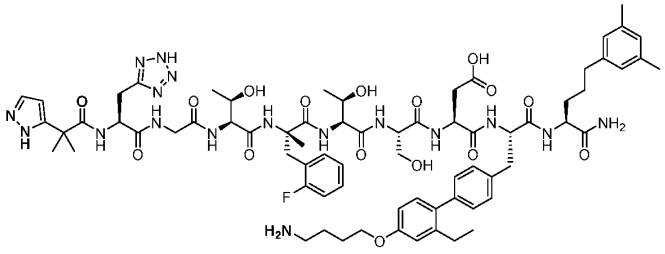


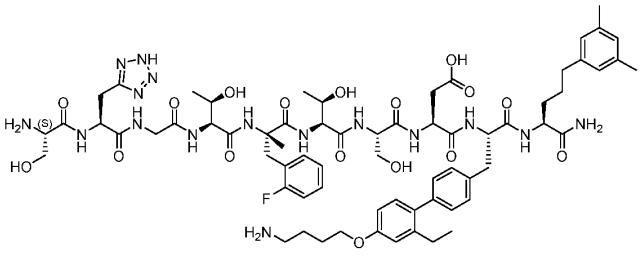
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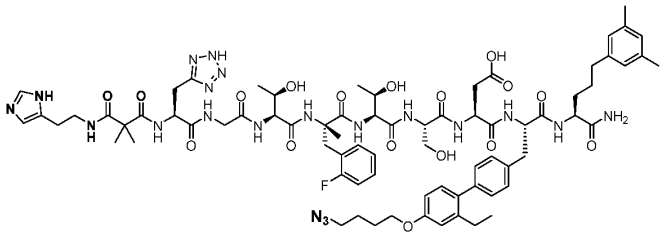

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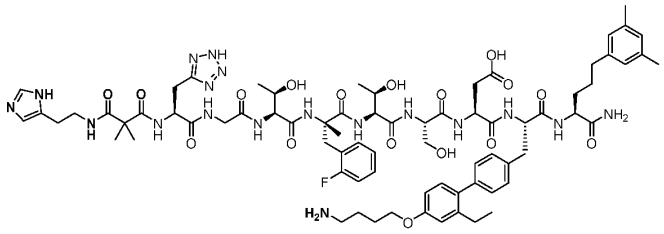

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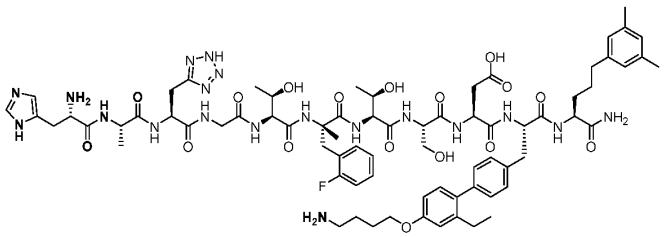

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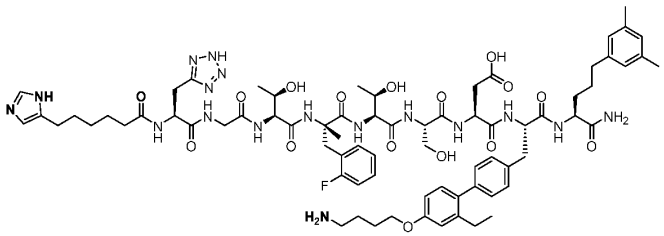

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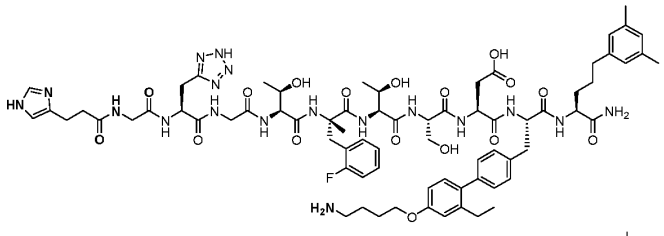

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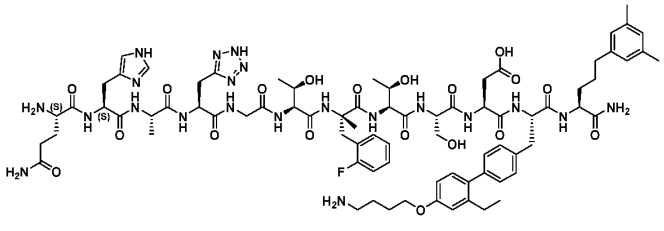

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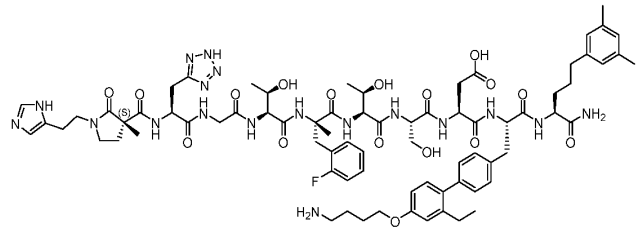

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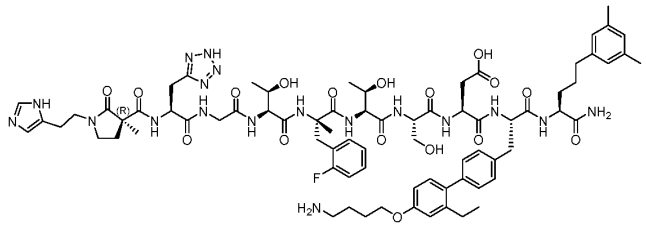

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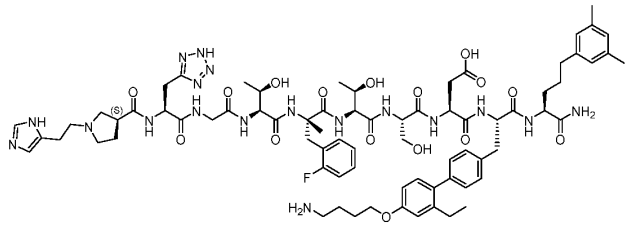

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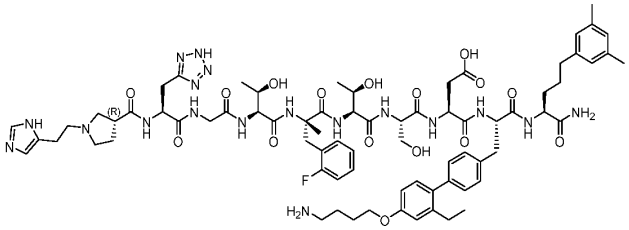

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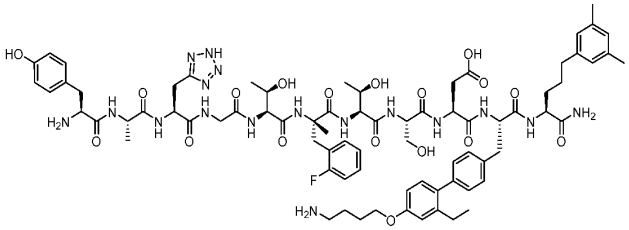

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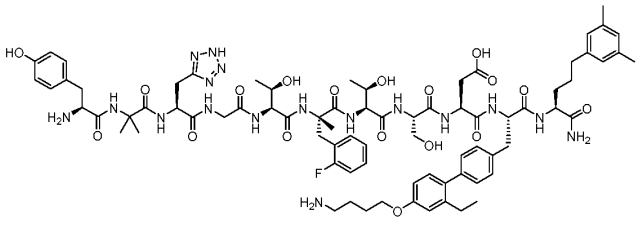

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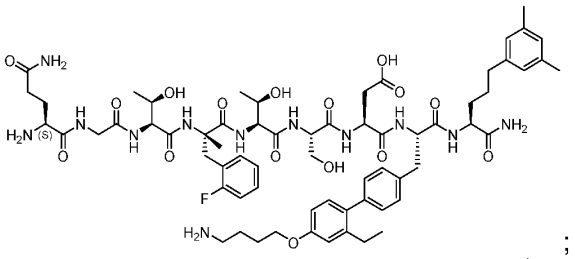

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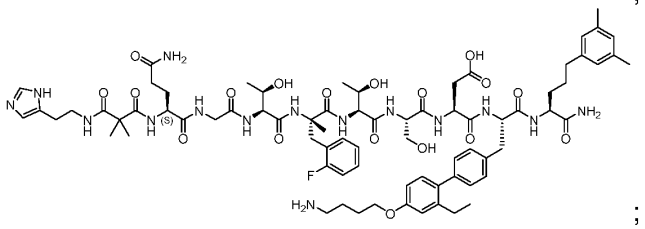

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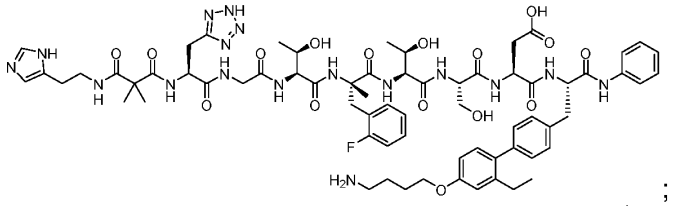

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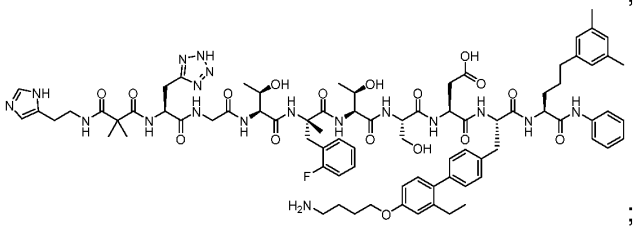

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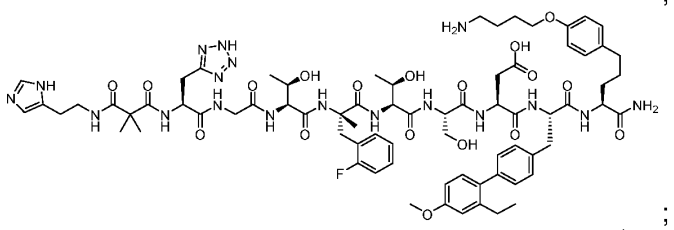

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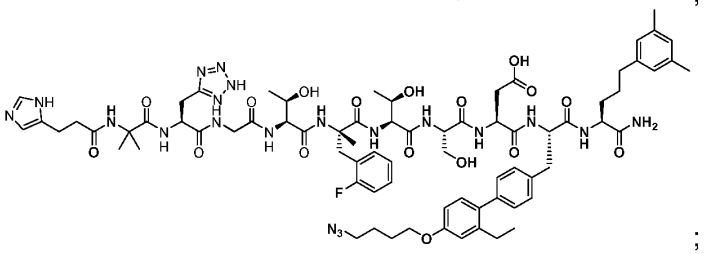

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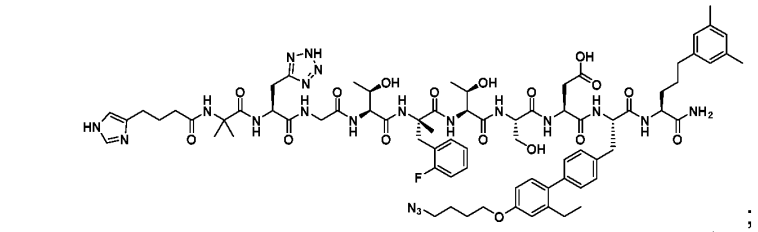

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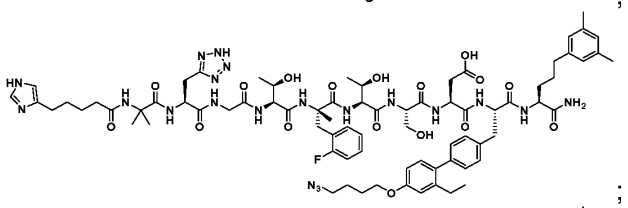

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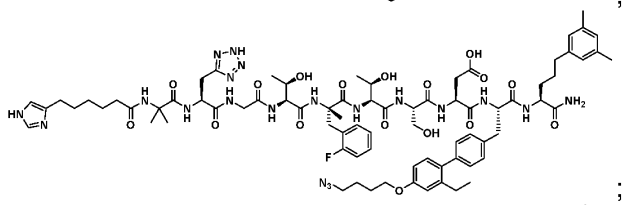

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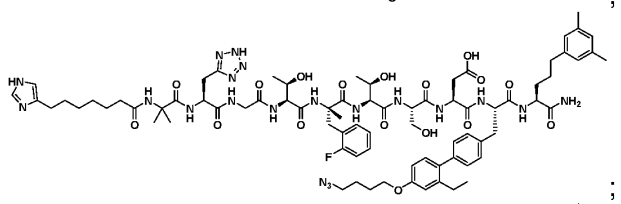

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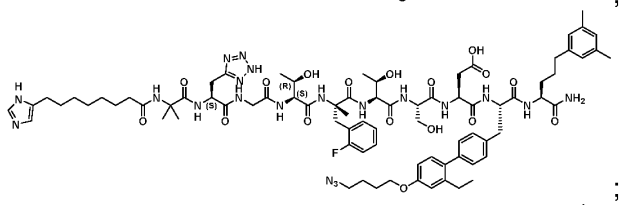

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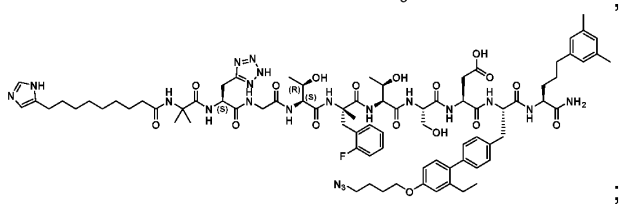

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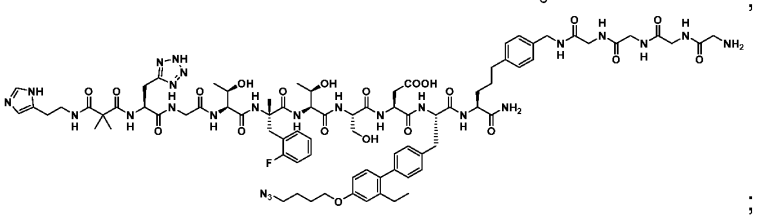

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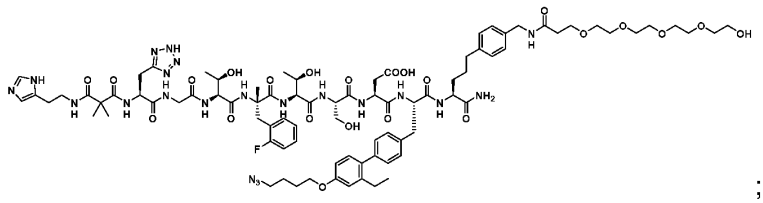
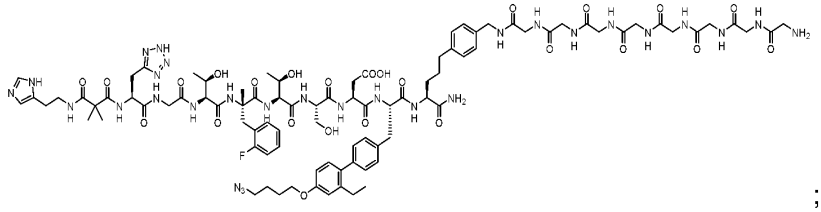
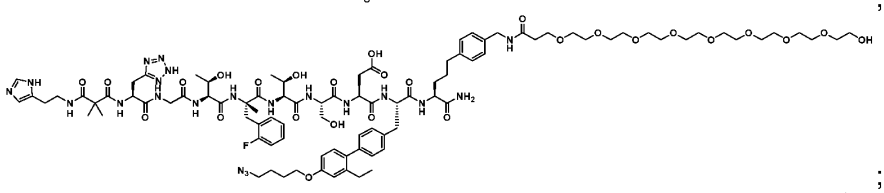
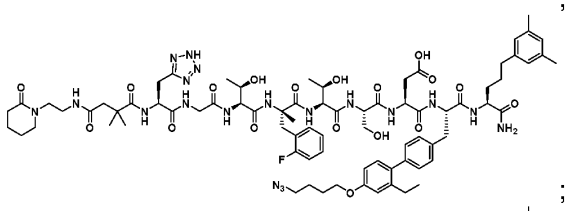
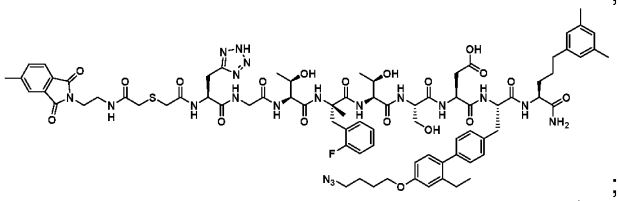
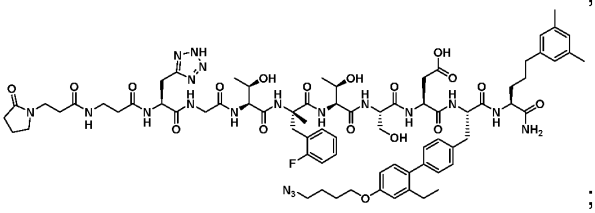
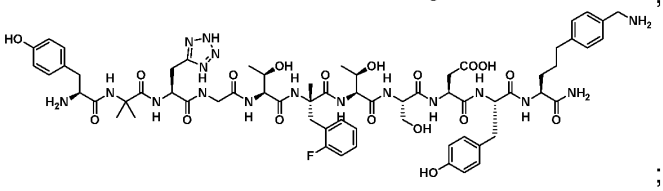

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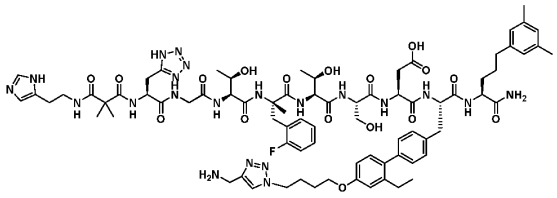

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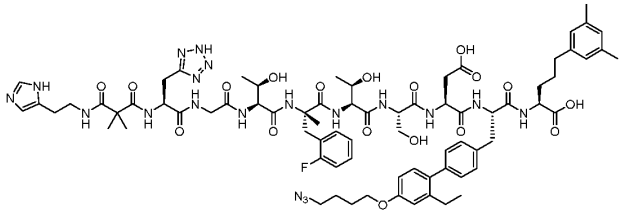

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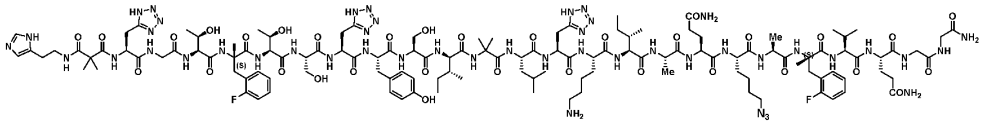

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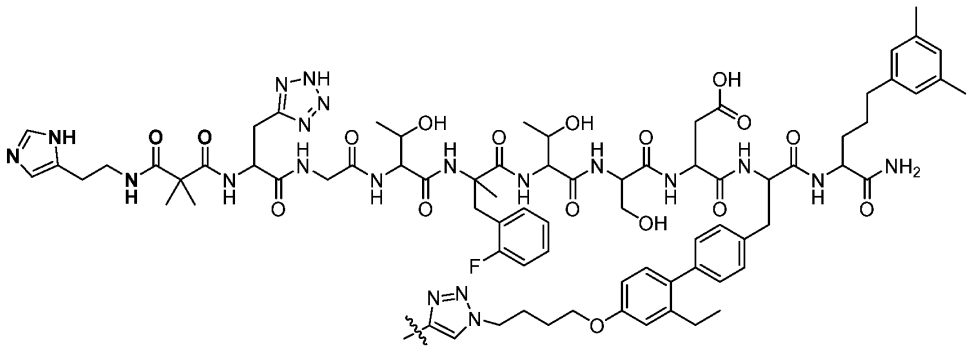
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or a pharmaceutically acceptable salt thereof.

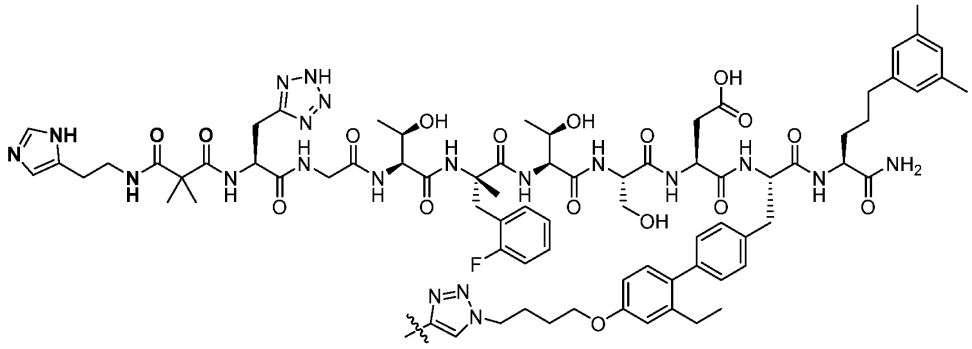
[00071] In yet another aspect, provided herein is an antibody-drug conjugate comprising a Glucagon-like peptide-1 receptor (GLP1R)-targeting antibody or an antigen-binding fragment thereof and a payload having the structure:



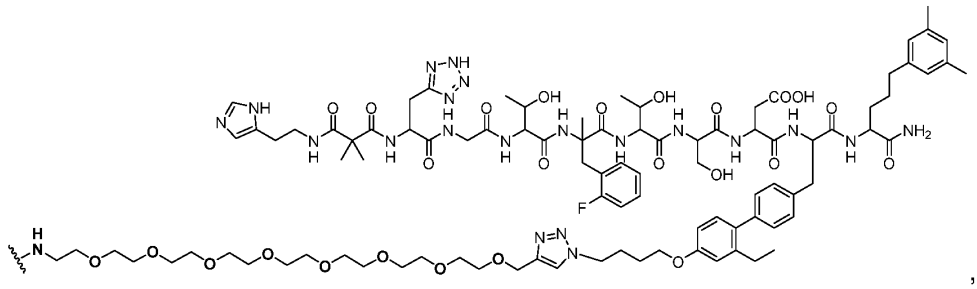
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wherein  is the point of attachment of the payload to the antibody or the antigen-binding fragment thereof directly or through a linker.

[00072] In one embodiment, the payload has the structure:

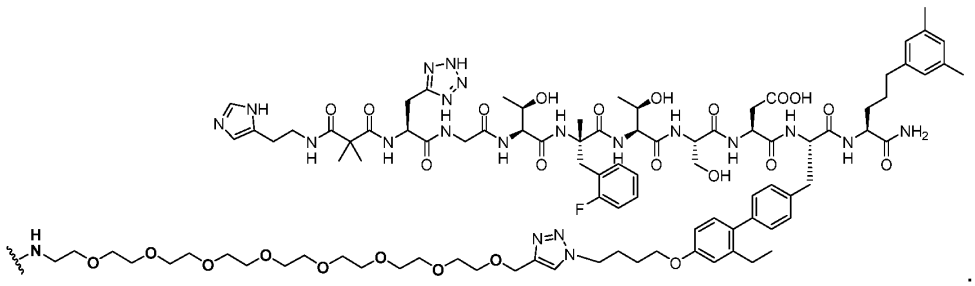


[00073] In yet another aspect, provided herein is an antibody-drug conjugate comprising a Glucagon-like peptide-1 receptor (GLP1R)-targeting antibody or an antigen-binding fragment thereof and a linker-payload having the structure:



wherein —§ is the point of attachment of the linker-payload to the antibody or the antigen-binding fragment thereof.

[00074] In one embodiments, the linker-payload has the structure:



[00075] These and other aspects of the present disclosure will become apparent to those skilled in the art after a reading of the following detailed description of the disclosure, including the appended claims.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[00076] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[00077] **Figure 1A** shows a schematic representation of an exemplary antibody-tethered drug conjugate (ATDC) design and its mechanism of action.

[00078] **Figure 1B** shows a schematic representation of a conventional antibody-drug conjugate (ADC) design and its mechanism of action.

[00079] **Figure 2** shows a model of an antibody-tethered drug conjugate having an antibody binding to extracellular domain (ECD) and a payload binding to the transmembrane domain (TMD).

[00080] **Figure 3A** shows a schematic representation of GLP1 (7-36) amide (SEQ ID NO: 4). The numbers above the sequence correspond to the amino acid positions in the proglucagon propeptide. The blue arrow indicates the dipeptidyl peptidase-4 (DPP-IV) cleavage site. The red arrows indicate the neutral endopeptidase (NEP) cleavage sites. The dashed arrows indicate cleavage sites by unknown endoprotease(s). The residues in orange are amino acids which, when substituted, reduce GLP1R binding and cAMP production. The residues in green are amino acids which, when substituted, reduce GLP1R binding.

[00081] **Figure 3B** shows a structure of GLP1R bound to GLP1 (Protein Data Bank ID: 3IOL). References of this structure may be found in Zhang et al. Nature 2017, Chepurny et al. JBC 2019, De Graaf et al. Pharmacological reviews 2016, and Manandhar and Ahn Journal of Medical Chemistry 2014, each of which is incorporated herein by reference in its entirety.

[00082] **Figure 4A** shows the sequence and structure of a GLP1 peptidomimetic, Peptide 5 (SEQ ID NO: 5). The numbers above the sequence correspond to the amino acid positions in the proglucagon propeptide.

[00083] **Figure 4B** shows superimposed structures of GLP1R bound to Peptide 5 (Protein Data Bank ID: 5NX2) and GLP1R bound to GLP1 (Protein Data Bank ID: 3IOL) using the GLP1R in 5NX2 as the template. Reference of the 5NX2 structure can be found in Jazayeri A, et al. Nature volume 546, pages 254–258 (2017), which is incorporated herein by reference in its entirety.

[00084] **Figure 5** shows a synthetic scheme for making GLP1 peptidomimetic payloads of the present disclosure. Solid Phase Peptide Synthesis on resin was established which efficiently generated the payloads of the present disclosure with good yields. Additional GLP1R peptidomimetic payloads were generated via systematic R1/R2/R3-modifications.

[00085] **Figures 6A-6D** demonstrate that the GLP1R peptidomimetic payloads of the present disclosure showed no activation in related GPCRs bioassays.

[00086] **Figures 7A-7B** show that shorter linker GLP1R ATDCs showed greater potency over the control ATDCs.

[00087] **Figure 8** shows that the lead linker-payload showed optimal *in vitro* ADME profile with no *in vitro* cardiotoxicity and mutagenic potential and its ATDC is highly stable in plasmas.

[00088] **Figure 9A** shows two methods for conjugating linker-payloads to an antibody of the present disclosure.

[00089] **Figure 9B** shows a representative hydrophobic interaction chromatography (HIC) graph of anti-GLP1R ATDC drug loading profile. HIC was used in the conjugatability screening to triage ATDCs that show low conjugation yields, low DAR, high aggregates and poor Biacore-binding.

[00090] **Figure 10** shows CRE-dependent luciferase reporter activity by anti-GLP1R ATDCs. Anti-GLP1R ATDCs showed better *in vitro* potency than isotype control ATDCs. Unconjugated mAbs did not activate hGLP1R cells (not shown). ATDCs did not activate Glucagon-like peptide-2 receptor (GLP2R), glucagon receptor (GCGR), or gastric inhibitory polypeptide receptor (GIPR) (not shown).

[00091] **Figure 11A** shows cyclic AMP response element (CRE)-dependent luciferase reporter activity by anti-GLP1R ATDCs in the presence of unconjugated anti-GLP1R antibodies. It shows that the unconjugated anti-GLP1R mAb concentrations <10 nM had no impact on anti-GLP1R ATDC activity. 100 nM unconjugated anti-GLP1R mAb reduced anti-GLP1R ATDC potency by 3.8-fold. The assay was performed by adding unconjugated anti-GLP1R mAb first, then immediately adding anti-GLP1R ATDC, and incubating for 4 hours.

[00092] **Figure 11B** shows the data corresponding to the graphs in **Figure 11A**.

[00093] **Figure 12** shows a schematic representation of an exemplary GLP1R Q-tag mAb - GLP1R agonist conjugate of the present disclosure.

[00094] **Figure 13** shows a general synthetic scheme for preparing GLP1 peptidomimetics according to the disclosure.

[00095] **Figure 14** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payloads P1 and P8 according to the disclosure.

[00096] **Figure 15** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payloads P2 and P9 according to the disclosure.

[00097] **Figure 16** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payloads P3, P4, P5, P6, P7, P11, P13, P14, P15, P16 and P17 according to the disclosure.

[00098] **Figures 17A and 17B** show a sequence for solid-supported synthesis of GLP1 peptidomimetic payloads P10, P12, P18, P19, P25, P26, P27, P28, P29, P30, P31, P36, P37, and P38 according to the disclosure.

[00099] **Figure 18** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payloads P20 and P21 according to the disclosure.

[000100] **Figure 19** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payloads P22 and P23 according to the disclosure.

[000101] **Figure 20** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payload P24 according to the disclosure.

[000102] **Figure 21** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payloads P32, P33, P34 and P35 according to the disclosure.

[000103] **Figure 22** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payload P39 according to the disclosure.

[000104] **Figure 23** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payload P40 according to the disclosure.

[000105] **Figure 24** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payload P41 according to the disclosure.

[000106] **Figure 25** shows a sequence for solid-supported synthesis of GLP1 peptidomimetic payload P42 according to the disclosure.

[000107] **Figure 26** shows a synthetic route for preparation of Linker-Payloads LP1, LP2, LP3, LP4 and LP5 according to the disclosure.

[000108] **Figure 27** shows a synthetic route for preparation of Linker-Payloads LP6 and LP7 according to the disclosure.

[000109] **Figure 28** shows a synthetic route for preparation of Linker-Payloads LP8, LP9, LP10 and LP11 according to the disclosure.

[000110] **Figure 29** shows a synthetic route for preparation of Linker-Payload LP12 according to the disclosure.

[000111] **Figure 30** shows a synthetic route for preparation of Linker-Payloads LP13 and LP14 according to the disclosure.

[000112] **Figure 31** shows a synthetic route for preparation of Linker-Payloads LP15 and LP18 according to the disclosure.

[000113] **Figure 32** shows a synthetic route for preparation of Linker-Payload LP17 according to the disclosure.

[000114] **Figure 33** shows a synthetic route for preparation of Linker-Payloads LP18 and LP20 according to the disclosure.

[000115] **Figure 34** shows a synthetic route for preparation of Linker-Payload LP19 according to the disclosure.

[000116] **Figure 35** shows a synthetic route for preparation of Linker-Payload LP21 according to the disclosure.

[000117] **Figure 36** shows a synthetic route for preparation of Linker-Payload LP22 according to the disclosure.

[000118] **Figure 37** shows a synthetic route for preparation of Linker-Payload LP23 according to the disclosure.

[000119] **Figure 38** shows a synthetic route for preparation of Linker-Payload LP24 according to the disclosure.

[000120] **Figure 39** shows a synthetic route for preparation of Linker-Payload LP25 according to the disclosure.

[000121] **Figure 40** shows a synthetic route for preparation of Linker-Payload LP26 according to the disclosure.

[000122] **Figure 41** shows a synthetic route for preparation of Linker-Payloads LP27 and LP28 according to the disclosure.

[000123] **Figure 42** shows a synthetic route for preparation of Linker-Payload LP29 according to the disclosure.

[000124] **Figure 43** shows a synthetic route for preparation of Linker-Payload LP30 according to the disclosure.

[000125] **Figure 44** shows a synthetic route for preparation of Linker-Payload LP31 according to the disclosure.

[000126] **Figure 45** shows a synthetic route for preparation of Linker-Payload LP32 according to the disclosure.

[000127] **Figure 46** shows a synthetic route for preparation of Linker-Payload LP33 according to the disclosure.

[000128] **Figure 47** shows a synthetic route for preparation of Linker-Payload LP34 according to the disclosure.

[000129] **Figure 48** shows a synthetic route for preparation of Linker-Payload LP35 according to the disclosure.

[000130] **Figure 49** shows a synthetic route for preparation of Linker-Payloads LP36, LP37, LP38, LP39, LP40, and LP41 according to the disclosure.

[000131] **Figure 50** shows a synthetic route for preparation of Linker-Payload LP42 according to the disclosure.

[000132] **Figure 51** shows a synthetic route for preparation of Linker-Payload LP43 according to the disclosure.

[000133] **Figure 52** shows a synthetic route for preparation of Linker-Payload LP44 according to the disclosure.

[000134] **Figure 53** shows a synthetic route for preparation of Linker-Payload LP45 according to the disclosure.

[000135] **Figure 54** shows a schematic of a general two-step conjugation procedure for the preparation of site-specific antibody-drug conjugates.

[000136] **Figure 55** shows a schematic of a general one-step conjugation procedure for the preparation of site-specific antibody-drug conjugates.

[000137] **Figure 56** shows the commander voltage protocol for electrophysiological study. From a holding potential of -80 mV, the voltage was first stepped to -50 mV for 80 ms for leak subtraction, and then stepped to +20 mV for 4800 ms to open hERG channels. After that, the voltage was stepped back down to -50 mV for 5000 ms, causing a "rebound" or tail current, which was measured and collected for data analysis. Finally, the voltage was stepped back to the holding potential (-80 mV, 1000 ms). Voltage command protocol was repeated every 20 sec and performed continuously during the test (vehicle control and test compound).

[000138] **Figure 57** shows *in vitro* stability of anti-GLP1R mAB2-LP11 over a 7-day, 37°C incubation in mouse, monkey and human plasma.

[000139] **Figure 58** shows the effects of GLP1R ATDCs on percent body weight changes in obese GLP1R humanized mice.

[000140] **Figure 59** shows the effects of GLP1R ATDCs on blood glucose levels in obese GLP1R humanized mice.

[000141] **Figure 60** is a schematic representation of a GLP1R ATDC according to an exemplary embodiment of the disclosure.

## DETAILED DESCRIPTION

[000142] The present disclosure provides, in some aspects, antibody-drug conjugates that specifically bind the glucagon-like peptide 1 receptor (GLP1R) protein. As described in the Background section above, GLP1R and its ligand GLP1 are highly validated targets for obesity and type 2 diabetes. However, no direct agonist antibodies have been identified for type 2 diabetes treatment. Single peptides with agonist activities on GLP1R are effective therapeutic agents for glucose control and body weight loss, but in-line peptide-antibody fusions are susceptible to proteolysis. In certain embodiments of the present disclosure, antibody-drug conjugates were generated that combine an antibody, or antigen-binding fragment thereof, specifically targeting the extracellular domain of GLP1R, with a GLP1 peptidomimetic functionally activating GLP1R. In certain embodiments, antibody-drug conjugates of the present disclosure have a longer drug duration with comparable or better weight and glucose reducing efficacy and minimized off-target side effects.

[000143] Detailed embodiments of the present disclosure are disclosed herein; however, it is to be understood that the disclosed embodiments are merely illustrative of the disclosure that may be embodied in various forms. In addition, each of the examples given in connection with the various embodiments of the disclosure is intended to be illustrative, and not restrictive. Therefore, specific structural and functional details disclosed herein are not to be interpreted as limiting, but merely as a representative basis for teaching one skilled in the art to variously employ the present disclosure.

### Definitions

[000144] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[000145] As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, a reference to “a method” includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure.

[000146] The terms “treat” or “treatment” of a state, disorder or condition include: (1) preventing, delaying, or reducing the incidence and/or likelihood of the appearance of at least one clinical or sub-clinical symptom of the state, disorder or condition developing in a subject that may be afflicted with or predisposed to the state, disorder or condition but does not yet experience or

display clinical or subclinical symptoms of the state, disorder or condition; or (2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof or at least one clinical or sub-clinical symptom thereof; or (3) relieving the disease, i.e., causing regression of the state, disorder or condition or at least one of its clinical or sub-clinical symptoms. The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician. In some embodiments, treatment comprises methods wherein cells are ablated in such manner where disease is indirectly impacted. In certain embodiments, treatment comprises depleting immune cells as a hematopoietic conditioning regimen prior to therapy.

[000147] A "subject" or "patient" or "individual" or "animal", as used herein, refers to humans, veterinary animals (e.g., cats, dogs, cows, horses, sheep, pigs, etc.) and experimental animal models of diseases (e.g., mice, rats). In a preferred embodiment, the subject is a human.

[000148] As used herein the term "effective" applied to dose or amount refers to that quantity of a compound or pharmaceutical composition that is sufficient to result in a desired activity upon administration to a subject in need thereof. Note that when a combination of active ingredients is administered, the effective amount of the combination may or may not include amounts of each ingredient that would have been effective if administered individually. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition being treated, the particular drug or drugs employed, the mode of administration, and the like.

[000149] The phrase "pharmaceutically acceptable salt", as used in connection with compositions of the disclosure, refers to any salt suitable for administration to a patient. Suitable salts include, but are not limited to, those disclosed in. Berge et al., "Pharmaceutical Salts", *J. Pharm. Sci.*, 1977, 66:1, incorporated herein by reference. Examples of salts include, but are not limited to, acid derived, base derived, organic, inorganic, amine, and alkali or alkaline earth metal salts, including but not limited to calcium salts, magnesium salts, potassium salts, sodium salts, salts of hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methane sulfonic acid, ethane sulfonic acid, p toluene sulfonic acid, salicylic acid, and the like. In some examples, a payload described herein comprises a tertiary amine, where the nitrogen atom in the tertiary amine is the atom through which the payload is bonded to a linker or a linker-spacer. In such instances, bonding to the tertiary amine of the payload yields a quaternary amine in the linker-

payload molecule. The positive charge on the quaternary amine can be balanced by a counter ion (e.g., chloro, bromo, iodo, or any other suitably charged moiety such as those described herein).

[000150] Ranges can be expressed herein as from "about" or "approximately" one particular value and/or to "about" or "approximately" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value.

[000151] By "comprising" or "containing" or "including" is meant that at least the named compound, element, particle, or method step is present in the composition or article or method, but does not exclude the presence of other compounds, materials, particles, or method steps, even if the other such compounds, material, particles, or method steps have the same function as what is named.

[000152] Compounds of the present disclosure include those described generally herein, and are further illustrated by the classes, subclasses, and species disclosed herein. As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001 , the entire contents of which are hereby incorporated by reference.

[000153] As used herein, the term "alkyl" is given its ordinary meaning in the art and may include saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In certain embodiments, a straight chain or branched chain alkyl has about 1–20 carbon atoms in its backbone (e.g., $C_1$–$C_{20}$ for straight chain, $C_2$–$C_{20}$ for branched chain), and alternatively, about 1–10 carbon atoms, or about 1 to 6 carbon atoms. In some embodiments, a cycloalkyl ring has from about 3–10 carbon atoms in their ring structure where such rings are monocyclic or bicyclic, and alternatively about 5, 6 or 7 carbons in the ring structure. In some embodiments, an alkyl group may be a lower alkyl group, wherein a lower alkyl group comprises 1–4 carbon atoms (e.g., $C_1$–$C_4$ for straight chain lower alkyls).

[000154] As used herein, the term "alkenyl" refers to an alkyl group, as defined herein, having one or more double bonds.

[000155] As used herein, the term "alkynyl" refers to an alkyl group, as defined herein, having one or more triple bonds.

[000156] The term "heteroatom" means one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon (including, any oxidized form of nitrogen, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen or; a substitutable nitrogen of a heterocyclic ring.

[000157] The term "halogen" means F, Cl, Br, or I; the term "halide" refers to a halogen radical or substituent, namely -F, -Cl, -Br, or -I.

[000158] The term "adduct", e.g., "a Diels-Alder adduct" of the present disclosure encompasses any moiety comprising the product of an addition reaction, e.g., a Diels-Alder reaction, independent of the synthetic steps taken to produce the moiety.

[000159] The term "covalent attachment" means formation of a covalent bond, i.e., a chemical bond that involves sharing of one or more electron pairs between two atoms. Covalent bonding may include different interactions, including but not limited to σ-bonding, π-bonding, metal-to-metal bonding, agostic interactions, bent bonds, and three-center two-electron bonds. When a first group is said to be "capable of covalently attaching" to a second group, this means that the first group is capable of forming a covalent bond with the second group, directly or indirectly, e.g., through the use of a catalyst or under specific reaction conditions. Non-limiting examples of groups capable of covalently attaching to each other may include, e.g., an amine and a carboxylic acid (forming an amide bond), a diene and a dienophile (via a Diels-Alder reaction), a maleimide and a thiol (forming a thio-maleimide), and an azide and an alkyne (forming a triazole via a 1,3-cycloaddition reaction).

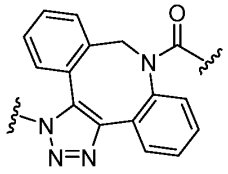
[000160] As described herein, compounds of the disclosure may contain "optionally substituted" moieties. In general, the term "substituted," whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this disclosure are preferably those that result in the formation of stable or chemically feasible compounds.

[000161] The term "stable," as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain

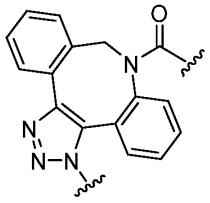
embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

[000162] Unless otherwise stated, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, (Z) and (E) double bond isomers, and (Z) and (E) conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the disclosure.

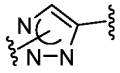
[000163] Unless otherwise stated, cyclic adducts, e.g., products of a cycloaddition reaction, e.g., an azide-acetylene cycloaddition reaction or a Diels-Alder reaction, depicted herein include all regioisomers, i.e., structural isomers that differ only in the position of a functional group or a substituent. By way of an example, the following structures represent triazole regioisomers, which differ only in the position of the substituent on the triazole ring:




and




. Triazole regioisomers may also be represented by the following structure:




.

[000164] Unless otherwise stated, all tautomeric forms of the compounds of the disclosure are within the scope of the disclosure.

[000165] Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a $^{11}C$- or $^{13}C$- or $^{14}C$ -enriched carbon are within the scope of this disclosure.

[000166] It is also to be understood that the mention of one or more method steps does not preclude the presence of additional method steps or intervening method steps between those steps expressly identified. Similarly, it is also to be understood that the mention of one or more components in a device or system does not preclude the presence of additional components or intervening components between those components expressly identified.

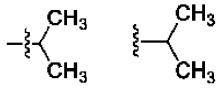
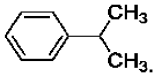
[000167] Unless otherwise stated, all crystalline forms of the compounds of the disclosure and salts thereof are also within the scope of the disclosure. The compounds of the disclosure may be isolated in various amorphous and crystalline forms, including without limitation forms which are anhydrous, hydrated, non-solvated, or solvated. Example hydrates include hemihydrates, monohydrates, dihydrates, and the like. In some embodiments, the compounds of the disclosure are anhydrous and non-solvated. By "anhydrous" is meant that the crystalline form of the compound contains essentially no bound water in the crystal lattice structure, i.e., the compound does not form a crystalline hydrate.

[000168] As used herein, "crystalline form" is meant to refer to a certain lattice configuration of a crystalline substance. Different crystalline forms of the same substance typically have different crystalline lattices (e.g., unit cells) which are attributed to different physical properties that are characteristic of each of the crystalline forms. In some instances, different lattice configurations have different water or solvent content. The different crystalline lattices can be identified by solid state characterization methods such as by X-ray powder diffraction (PXRD). Other characterization methods such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), dynamic vapor sorption (DVS), solid state NMR, and the like further help identify the crystalline form as well as help determine stability and solvent/water content.

[000169] Crystalline forms of a substance include both solvated (e.g., hydrated) and non-solvated (e.g., anhydrous) forms. A hydrated form is a crystalline form that includes water in the crystalline lattice. Hydrated forms can be stoichiometric hydrates, where the water is present in the lattice in a certain water/molecule ratio such as for hemihydrates, monohydrates, dihydrates, etc. Hydrated forms can also be non-stoichiometric, where the water content is variable and dependent on external conditions such as humidity.

[000170] In some embodiments, the compounds of the disclosure are substantially isolated. By "substantially isolated" is meant that a particular compound is at least partially isolated from impurities. For example, in some embodiments a compound of the disclosure comprises less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 2.5%, less than about 1%, or less than about 0.5% of impurities. Impurities generally include anything that is not the substantially isolated compound including, for example, other crystalline forms and other substances.

[000171] Certain groups, moieties, substituents, and atoms are depicted with a wavy line. The wavy line can intersect or cap a bond or bonds. The wavy line indicates the atom through

which the groups, moieties, substituents, or atoms are bonded. For example, a phenyl group that is substituted with a propyl group depicted as:  has the following structure:  .

[000172] The term “GLP1R” refers to the glucagon-like peptide 1 receptor and includes recombinant GLP1R protein or a fragment thereof. GLP1R has a sequence of 463 residues. Donnelly, Br J Pharmacol, 166(1):27–41 (2011). Glucagon-like peptide 1 (GLP1) is a 31-amino acid peptide hormone released from intestinal L cells following nutrient consumption. The binding of GLP1 to GLP1R potentiates glucose-induced secretion of insulin from pancreatic beta cells, increases insulin expression, inhibits beta-cell apoptosis, promotes beta-cell neogenesis, reduces glucagon secretion, delays gastric emptying, promotes satiety and increases peripheral glucose disposal.

[000173] The phrase "an antibody that binds GLP1R" or an "anti-GLP1R antibody" includes antibodies and antigen-binding fragments thereof that specifically recognize GLP1R.

[000174] An "agonist antibody," as used herein (or an "antibody that increases or enhances GLP1R activity"), is intended to refer to an antibody whose binding to GLP1R results in activation of at least one biological activity of GLP1R. For example, an agonist antibody of GLP1R may elicit stimulation of the adenylate cyclase pathway resulting in increased synthesis of cyclic AMP and release of insulin if the cell is a mammalian pancreatic beta cell. An agonist antibody of GLP1R may also reduce glucose levels upon administration to a subject in need thereof.

[000175] All amino acid abbreviations used in this disclosure are those accepted by the United States Patent and Trademark Office as set forth in 37 C.F.R. § 1 .822 (B)(J).

[000176] The term “protein” means any amino acid polymer having more than about 20 amino acids covalently linked via amide bonds. As used herein, “protein” includes biotherapeutic proteins, recombinant proteins used in research or therapy, trap proteins and other Fc-fusion proteins, chimeric proteins, antibodies, monoclonal antibodies, human antibodies, bispecific antibodies, antibody fragments, nanobodies, recombinant antibody chimeras, scFv fusion proteins, cytokines, chemokines, peptide hormones, and the like. Proteins can be produced using recombinant cell-based production systems, such as the insect bacculovirus system, yeast systems (e.g, Pichia sp.), mammalian systems (e.g., CHO cells and CHO derivatives like CHO-K1 cells).

[000177] All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species. Thus, the expression "GLP1R" means human GLP1R unless specified as being from a non-human species, e.g., "mouse GLP1R," "monkey GLP1R," etc.

[000178] The amino acid sequence of an antibody can be numbered using any known numbering schemes, including those described by Kabat et al., ("Kabat" numbering scheme); Al-Lazikani et al., 1997, *J. Mol. Biol.,* 273:927-948 ("Chothia" numbering scheme); MacCallum et al., 1996, *J. Mol. Biol.* 262:732-745 ("Contact" numbering scheme); Lefranc et al., Dev. Comp. *Immunol.*, 2003, 27:55-77 ("IMGT" numbering scheme); and Honegge and Pluckthun, *J. Mol. Biol.,* 2001 , 309:657-70 ("AHo" numbering scheme). Unless otherwise specified, the numbering scheme used herein is the Kabat numbering scheme. However, selection of a numbering scheme is not intended to imply differences in sequences where they do not exist, and one of skill in the art can readily confirm a sequence position by examining the amino acid sequence of one or more antibodies. Unless stated otherwise, the "EU numbering scheme" is generally used when referring to a residue in an antibody heavy chain constant region (e.g., as reported in Kabat et al., supra).

[000179] The term "glutaminyl-modified antibody" refers to an antibody with at least one covalent linkage from a glutamine side chain to a primary amine compound of the present disclosure. In particular embodiments, the primary amine compound is linked through an amide linkage on the glutamine side chain. In certain embodiments, the glutamine is an endogenous glutamine. In other embodiments, the glutamine is an endogenous glutamine made reactive by polypeptide engineering (e.g., via amino acid deletion, insertion, substitution, or mutation on the polypeptide). In additional embodiments, the glutamine is polypeptide engineered with an acyl donor glutamine-containing tag (e.g., glutamine-containing peptide tags, Q-tags or TGase recognition tag).

[000180] The term "TGase recognition tag" refers to a sequence of amino acids comprising an acceptor glutamine residue and that when incorporated into (e.g. appended to) a polypeptide sequence, under suitable conditions, is recognized by a TGase and leads to cross-linking by the TGase through a reaction between an amino acid side chain within the sequence of amino acids and a reaction partner. The recognition tag may be a peptide sequence that is not naturally present in the polypeptide comprising the TGase recognition tag. In some embodiments, the TGase recognition tag comprises at least one Gln. In some embodiments, the TGase recognition tag comprises an amino acid sequence XXQX, wherein X is any amino acid (e.g., conventional

amino acid Leu, Ala, Gly, Ser, Val, Phe, Tyr, His, Arg, Asn, Glu, Asp, Cys, Gin, He, Met, Pro, Thr, Lys, or Trp or nonconventional amino acid). In some embodiments, the acyl donor glutamine-containing tag comprises an amino acid sequence selected from the group consisting of LLQGG (SEQ ID NO: 6), LLQG (SEQ ID NO: 7), LSLSQG (SEQ ID NO: 8), GGGLLQGG (SEQ ID NO: 9), GLLQG (SEQ ID NO: 10), LLQ, GSPLAQSHGG (SEQ ID NO: 11), GLLQGGG (SEQ ID NO: 12), GLLQGG (SEQ ID NO: 13), GLLQ (SEQ ID NO: 14), LLQLLQGA (SEQ ID NO: 15), LLQGA (SEQ ID NO: 16), LLQYQGA (SEQ ID NO: 17), LLQGSG (SEQ ID NO: 18), LLQYQG (SEQ ID NO: 19), LLQLLQG (SEQ ID NO: 20), SLLQG (SEQ ID NO: 21), LLQLQ (SEQ ID NO: 22), LLQLLQ (SEQ ID NO: 23), and LLQGR (SEQ ID NO: 24). See for example, WO2012059882, the entire contents of which are incorporated herein.

[000181] The term “antibody,” as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen. The term “antibody” includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (e.g., IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2, and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region comprises one domain (CL1). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments, the FRs of the antibody (or antigen-binding portion thereof) can be identical to the human germline sequences, or can be naturally or artificially modified. An amino acid consensus sequence can be defined based on a side-by-side analysis of two or more CDRs.

[000182] The term “antibody,” as used herein, also includes antigen-binding fragments of full antibody molecules. The terms “antigen-binding portion” of an antibody, “antigen-binding fragment” of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody can be derived, e.g., from full antibody molecules using any suitable standard techniques such as

proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, e.g., commercial sources, DNA libraries (including, e.g., phage-antibody libraries), or can be synthesized. The DNA can be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[000183] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g., an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (e.g., monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[000184] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain can be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a VH domain associated with a VL domain, the VH and VL domains can be situated relative to one another in any suitable arrangement. For example, the variable region can be dimeric and contain VH-VH, VH-VL or VL-VL dimers.

[000185] Alternatively, the antigen-binding fragment of an antibody can contain a monomeric VH or VL domain.

[000186] In certain embodiments, an antigen-binding fragment of an antibody can contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that can be found within an antigen-binding fragment of an antibody of the present description include: (i) VH-CH1; (ii) VH-CH2; (iii) VH-CH3; (iv) VH-CH1-CH2; (V) VH-CH1-CH2- CH3; (vi) VH-CH2-CH3; (vii) VH-CL; (viii) VL-CH1; (ix) VL-CH2; (x) VL-CH3; (xi) VL-CH1-CH2; (xii) VL-CH1-CH2-CH3; (xiii) VL-CH2-CH3; and (xiv) VL-CL. In any configuration of variable and constant domains, including any of the exemplary configurations listed herein, the variable and constant domains can be either directly linked to one

another or can be linked by a full or partial hinge or linker region. A hinge region can consist of at least 2 (e.g., 5, 10, 15, 20, 40, 60, or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule.

[000187] Moreover, an antigen-binding fragment of an antibody of the present description can comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed herein in non-covalent association with one another and/or with one or more monomeric VH or VL domain (e.g., by disulfide bond(s)).

[000188] As with full antibody molecules, antigen-binding fragments can be monospecific or multispecific (e.g., bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, can be adapted for use in the context of an antigen-binding fragment of an antibody of the present description using routine techniques available in the art.

[000189] In certain embodiments, the antibodies of the description, e.g., anti-GLP1R antibodies, are human antibodies. The term “human antibody,” as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the description can include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term “human antibody,” as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[000190] The antibodies can, in some embodiments, be recombinant human antibodies. The term “recombinant human antibody,” as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (See, e.g., Taylor et al. (1992) *Nucl. Acids Res.* 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin

sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

[000191] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification. The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) *Molecular Immunology* 30: 105) to levels typically observed using a human IgG1 hinge. The instant description encompasses antibodies having one or more mutations in the hinge, CH2 or CH3 region which can be desirable, for example, in production, to improve the yield of the desired antibody form.

[000192] The antibodies of the description can be isolated or purified antibodies. An “isolated antibody” or “purified antibody,” as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an “isolated antibody” for purposes of the present description. For example, an antibody that has been purified from at least one component of a reaction or reaction sequence, is a “purified antibody” or results from purifying the antibody. An isolated antibody also includes an antibody in situ within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody or purified antibody can be substantially free of other cellular material and/or chemicals.

[000193] The antibodies disclosed herein can comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which

the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present description includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with given heavy and light chain variable region sequences, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the VH and/or VL domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, e.g., only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (i.e., a germline sequence that is different from the germline sequence from which the antibody was originally derived).

[000194] Furthermore, the antibodies of the present description can contain any combination of two or more germline mutations within the framework and/or CDR regions, e.g., wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, improved drug-to-antibody ratio (DAR) for antibody-drug conjugates, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present description.

[000195] The term "aglycosylated antibody" refers to an antibody that does not comprise a glycosylation sequence that might interfere with a transglutamination reaction, for instance an

antibody that does not have saccharide group at N297 on one or more heavy chains. In particular embodiments, an antibody heavy chain has an N297 mutation. In other words, the antibody is mutated to no longer have an asparagine residue at position 297 according to the EU numbering system as disclosed by Kabat et al. In particular embodiments, an antibody heavy chain has an N297Q or an N297D mutation. Such an antibody can be prepared by site-directed mutagenesis to remove or disable a glycosylation sequence or by site-directed mutagenesis to insert a glutamine residue at site apart from any interfering glycosylation site or any other interfering structure. Such an antibody also can be isolated from natural or artificial sources. Aglycosylated antibodies also include antibodies comprising a T299 or S298P or other mutations, or combinations of mutations that result in a lack of glycosylation.

[000196] The term "deglycosylated antibody" refers to an antibody in which a saccharide group at is removed to facilitate transglutaminase-mediated conjugation. Saccharides include, but are not limited to, N-linked oligosaccharides. In some embodiments, deglycosylation is performed at residue N297. In some embodiments, removal of saccharide groups is accomplished enzymatically, included but not limited to via PNGase.

[000197] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen can have more than one epitope. Thus, different antibodies can bind to different areas on an antigen and can have different biological effects. Epitopes can be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope can include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[000198] The terms "conjugated protein" or "conjugated antibody" as used herein refers to a protein or an antibody covalently linked to one or more chemical moieties. The chemical moiety can include an amine compound of the present disclosure. Linkers (**L**) and payloads (**P**) suitable for use with the present disclosure are described in detail herein. In particular embodiments, a conjugated antibody comprising a therapeutic moiety is an antibody-drug conjugate (ADC), also referred to as an antibody-payload conjugate, or an antibody-linker-payload conjugate.

[000199] The term "Drug-to-Antibody Ratio" or (DAR) is the average number of therapeutic moieties, e.g., drugs, conjugated to a binding agent of the present disclosure.

[000200] The term "Linker Antibody Ratio" or (LAR), also denoted as the lower case, in some embodiments, is the average number of reactive primary amine compounds conjugated to a

binding agent of the present disclosure. Such binding agents, e.g., antibodies, can be conjugated with primary amine compounds comprising, e.g., a suitable azide or alkyne. The resulting binding agent, which is functionalized with an azide or an alkyne can subsequently react with a therapeutic moiety comprising the corresponding azide or alkyne via the 1,3-cycloaddition reaction.

[000201] The phrase "pharmaceutically acceptable amount" refers to an amount effective or sufficient in treating, reducing, alleviating, or modulating the effects or symptoms of at least one health problem in a subject in need thereof. For example, a pharmaceutically acceptable amount of an antibody or antibody-drug conjugate is an amount effective for modulating a biological target using the antibody or antibody-drug-conjugates provided herein. Suitable pharmaceutically acceptable amounts include, but are not limited to, from about 0.001% up to about 10%, and any amount in between, such as about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% of an antibody or antibody-drug-conjugate provided herein.

[000202] The phrase "reaction pH" refers to the pH of a reaction after all reaction components or reactants have been added.

[000203] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule can, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[000204] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino

acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity can be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson (1994) Methods Mol. Biol. 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. In some embodiments, conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

[000205] Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) *Science* 256: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[000206] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another particular algorithm when comparing a sequence of the description to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, e.g., Altschul et al. (1990) *J. Mol. Biol.* 215:403-410 and Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-402.

**Protein-Drug Conjugate Compounds**

[000207] According to the foregoing objective and others, the present disclosure provides protein-drug conjugate compounds, e.g., antibody-drug conjugate compounds, and precursors and intermediates thereof, pharmaceutical compositions, and methods for treating certain diseases in a subject in need of such treatment. According to the disclosure, the protein-drug conjugate compounds provided herein comprise a binding agent conjugated with a therapeutic moiety, e.g., GLP1 peptidomimetics, as described herein.

[000208] In one aspect, the present disclosure provides compounds comprising a binding agent according to the present disclosure, (e.g., an antibody or a fragment thereof), conjugated to one or more GLP1 peptidomimetics via non-cleavable linker. Illustrative non-limiting examples include Formula (I) described herein. In specific embodiments of a protein-drug conjugate according to the disclosure, wherein the binding agent is an antibody, (e.g., a monoclonal antibody), the term "antibody drug conjugate" or ADC is optionally used.

[000209] In various embodiments, antibody drug conjugate or ADC of the disclosure is an antibody-tethered drug conjugate or ATDC. An ATDC is an antibody-drug conjugate wherein the drug is tethered to the antibody by a non-cleavable linker. In some embodiments, the non-cleavable linker in an ATDC of the present disclosure is stable after the ATDC is administered into the body, e.g., a human body. For example, the non-cleavable linker can be stable in plasma, e.g., in human plasma, stable upon binding cell surface, or stable upon antibody binding its target antigen and/or GLP1 peptidomimetic binding GLP1R. In some embodiments, the non-cleavable linker is more stable *in vivo* than either the payload or the antibody under the same physiological conditions. In some embodiments, an ATDC of the present disclosure may be degraded in the lysosome to release the payload, the linker-payload, and/or its ATDC metabolites/catabolites, which in certain embodiments are effective for GLP1R activation either locally or systematically.

[000210] In some embodiments, the ATDC is stable in plasma and degrades in the lysosome. In some embodiments, the ATDC is stable in plasma and does not degrade in the lysosome.

[000211] In one aspect, provided herein is a compound having a structure of Formula (A):

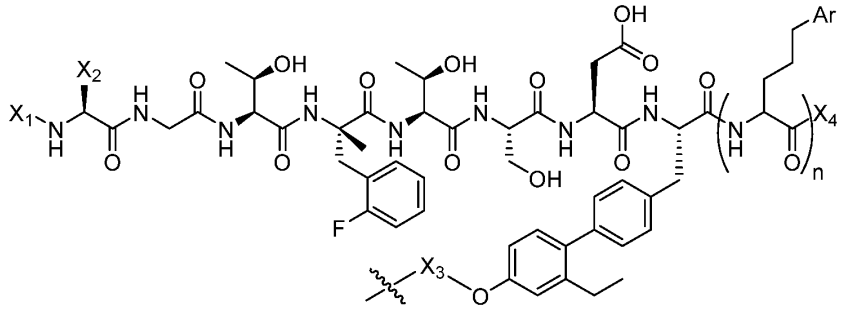
$$BA\text{-}(L\text{-}P)_m \quad (A),$$

wherein:

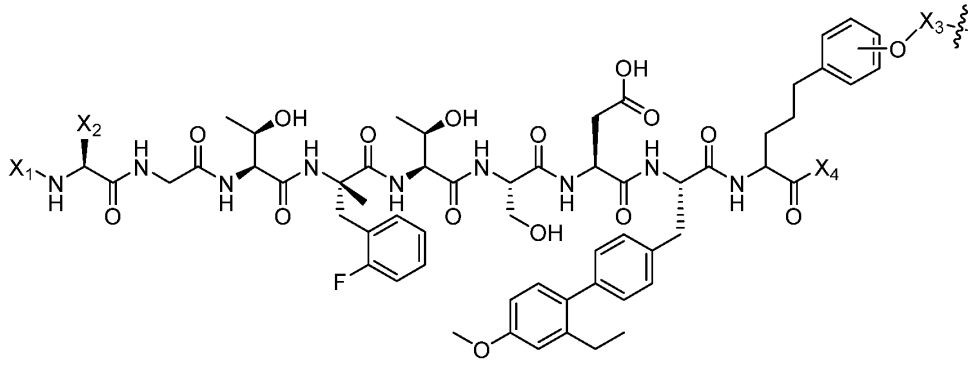
BA is an antibody or an antigen-binding fragment thereof;

L is a non-cleavable linker;

P is a payload having the structure selected from the group consisting of:

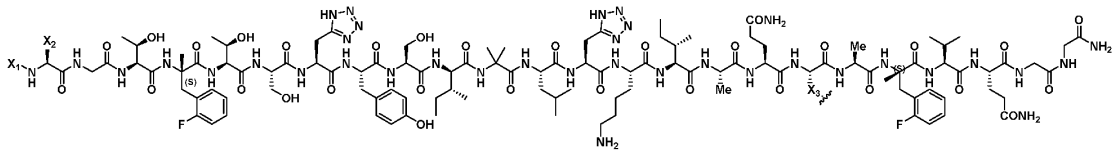


(P-IA),



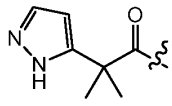
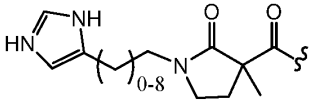
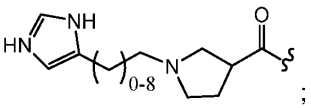
(P-IIA),

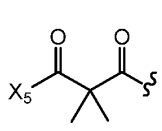
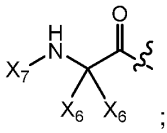
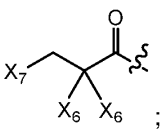
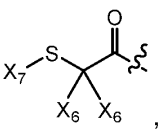
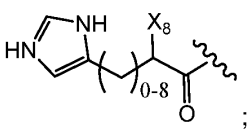
and

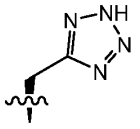
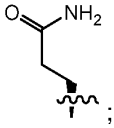


(P-IIIA),

wherein —≈— is the point of attachment of the payload to L;

$X_1$ is selected from H;  ;  ;  ;

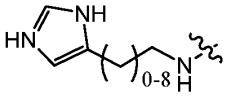
 ;  ;  ;  , and  ;

$X_2$ is selected from  and  ;

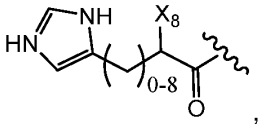
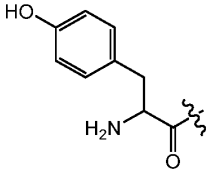
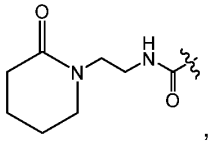
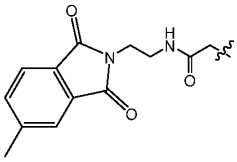
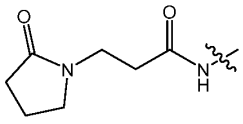
$X_3$ is selected from a bond, $–(CH_2)_{2-6}–NH–$, $–(CH_2)_{2-6}–Tr–$, and $–(CH_2)_{2-6}–Tr–(CH_2)_{1-6}–NH$, where Tr is a triazole moiety;

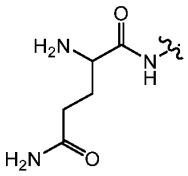
n is 0 or 1;

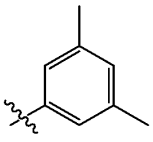
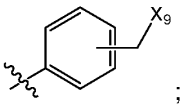
$X_4$ is selected from $-NH_2$, -OH and -N(H)(phenyl);

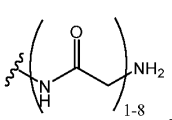
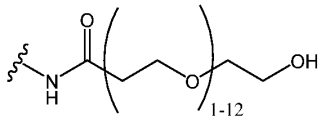
$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  ,  ,  ,  ,  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ;

Ar is selected from  and  ;

$X_9$ is selected from $-NH_2$,  , and  , and

m is an integer from 1 to 4

or a pharmaceutically acceptable salt thereof.

[000212] In one embodiment, m is 1. In one embodiment, m is an integer from 2 to 4. In one embodiment, m is 2.

[000213] In one aspect, the present disclosure provides a compound having a structure of Formula (I):

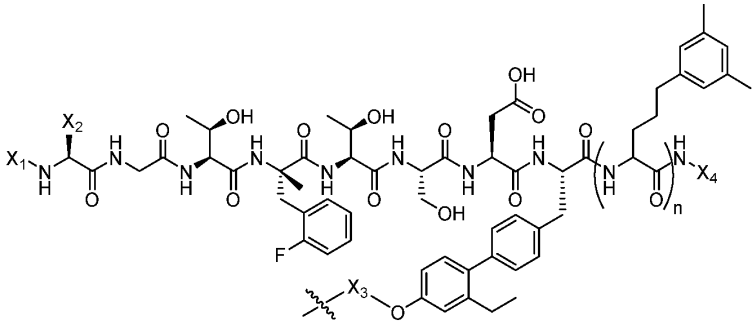
BA-L-P (I),

wherein:

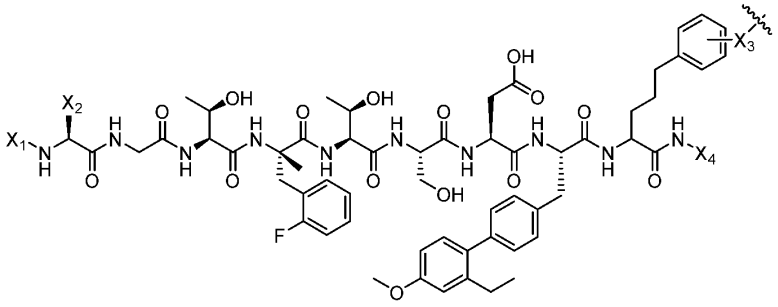
BA is an antibody or an antigen-binding fragment thereof;

L is a non-cleavable linker;

P is a payload having the structure selected from the group consisting of:

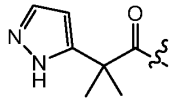
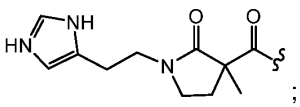
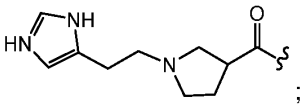


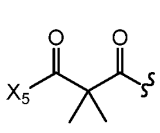
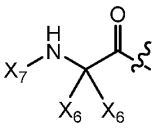
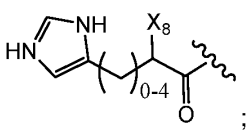
(P-I) and

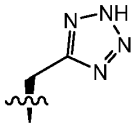
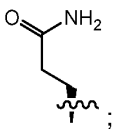


(P-II),

wherein —§— is the point of attachment of the payload to L;

$X_1$ is selected from H;  ;  ;  ;

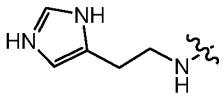
 ;  ; and  ;

$X_2$ is selected from  and  ;

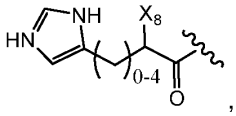
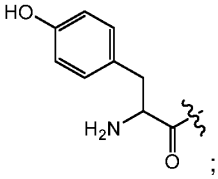
$X_3$ is selected from $–(CH_2)_{2\text{-}6}–NH–$ and $–(CH_2)_{2\text{-}6}–Tr–$, where Tr is a triazole moiety;

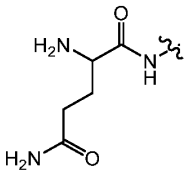
n is 0 or 1;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  , and  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ,

or a pharmaceutically acceptable salt thereof.

**Linker L**

[000214] In one embodiment, the linker L is a non-cleavable linker, i.e., a linker which is stable and provides a covalent connection between the antibody and the drug, e.g., between a GLP1R-targeting antibody and a GLP1 peptidomimetic payload P according to the present disclosure. In some embodiments, the non-cleavable linker L of the present disclosure is stable after the ATDC is administered into the body, e.g., a human body. For example, the linker L can be stable in plasma, e.g., in human plasma, stable upon binding cell surface, or stable upon antibody binding its target antigen and/or GLP1 peptidomimetic binding GLP1R. In some embodiments, the linker L is more stable *in vivo* than either the payload or the antibody under the same physiological conditions.

[000215] In one embodiment, the linker L has the structure of formula (L'):

-La-Y-Lp- (L'),

wherein La is a first linker covalently attached to the antibody or an antigen-binding fragment thereof;

Y is a group comprising a triazole, a Diels-Alder adduct, or a thiol-maleimide adduct, and

Lp is absent or a second linker covalently attached to the payload P according to the present disclosure, wherein when Lp is absent Y is also absent.

[000216] In one embodiment, Y is a group comprising a triazole.

[000217] In another embodiment, Y is a group comprising a Diels-Alder adduct.

[000218] In one embodiment, the linker L has the structure of formula (L'):

-La-Y-Lp- (L'),

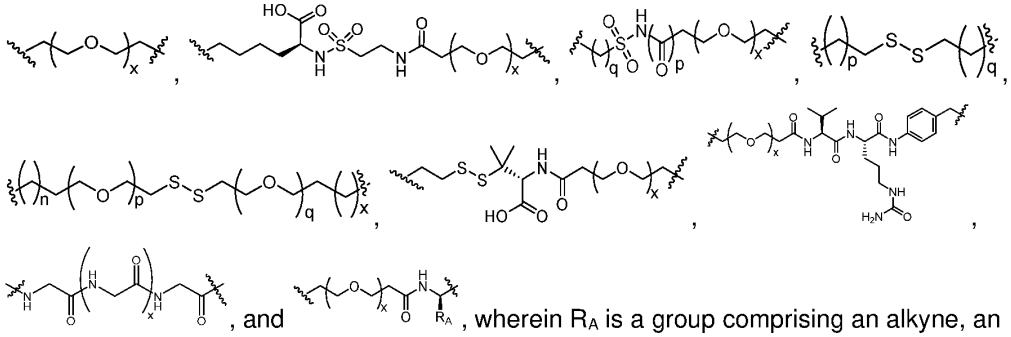
wherein La is a first linker covalently attached to the antibody or an antigen-binding fragment thereof;

Y is a group comprising a triazole, and

Lp is absent or a second linker covalently attached to the payload P according to the present disclosure.

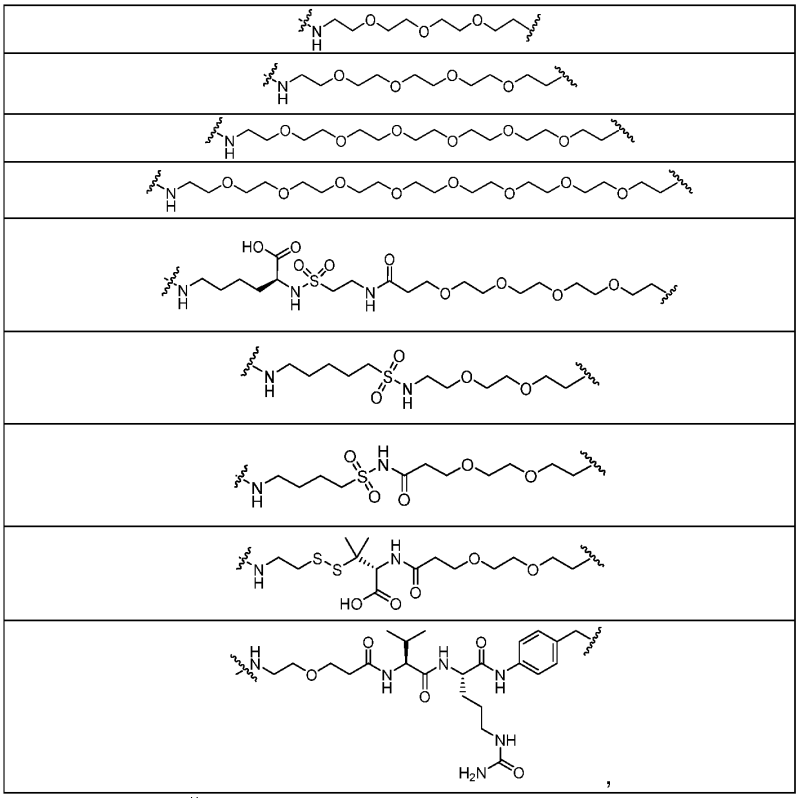
[000219] In one embodiment, La comprises $C_{1-6}$ alkyl, phenyl, -NH-, -C(O)-, $-(CH_2)_u$-NH-C(O)-, $-(CH_2)_u$-C(O)-NH-, $-(CH_2-CH_2-O)_v$-, $-(CH_2)_u-(O-CH_2-CH_2)_v$-C(O)-NH-, a peptide unit comprising from 2 to 4 amino acids, or combinations thereof, each of which may be optionally substituted with one or more of -S-, $-S(O_2)$-, -C(O)-, $-C(O_2)$-; and $CO_2H$, wherein subscripts u and v are independently an integer from 1 to 8.

[000220] In one embodiment, La is selected from the group consisting of:

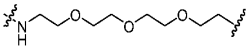


, and , wherein $R_A$ is a group comprising an alkyne, an azide, a tetrazine, a trans-cyclooctene, a maleimide, an amine, a ketone, an aldehyde, a carboxylic acid, an ester, a thiol, a sulfonic acid, a tosylate, a halide, a silane, a cyano group, a carbohydrate group, a biotin group, a lipid residue and wherein subscripts x, n, p and q are independently an integer from 0 to 12, and combinations thereof.

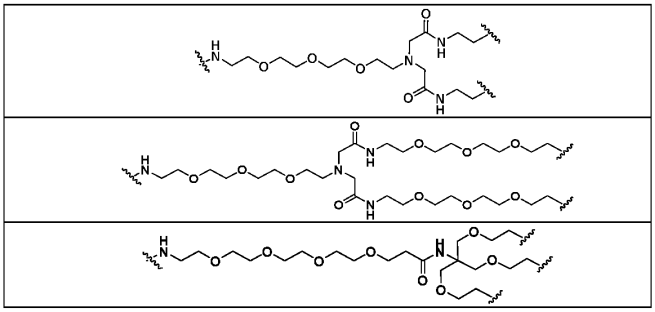
[000221] In one embodiment, -La- is selected from the group consisting of:

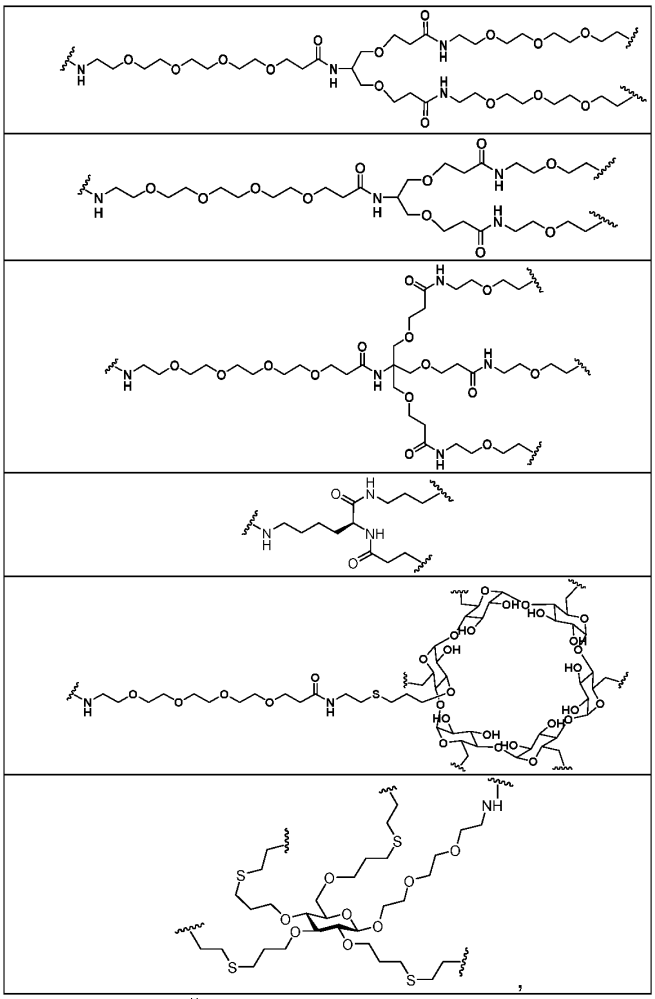


where the  is the amino point of attachment to a residue (e.g., a glutamine residue) of the antibody and/or the antigen-containing fragment thereof.

[000222] In one embodiment, -La- is  .

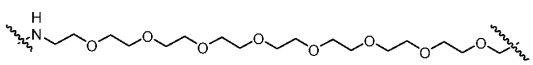
[000223] In another embodiment, -La- is selected from the group consisting of:

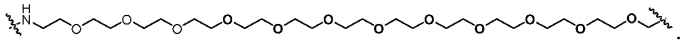




where the  is the amino point of attachment to a residue (e.g., a glutamine residue) of the antibody and/or the antigen-containing fragment thereof.

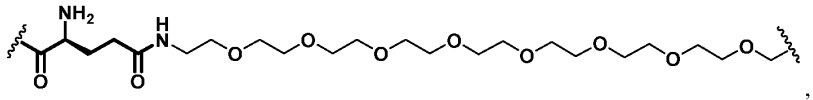
[000224] In some embodiments, La comprises a polyethylene glycol (PEG) segment having 1 to 36 -$CH_2CH_2O$- (EG) units. In some embodiments, the PEG segment comprises 4 EG units, or 8 EG units, or 12 EG units, or 24 EG units. In some embodiments, the PEG segment comprises 8 EG units. In some embodiments, La has a structure selected from the group consisting of

 and

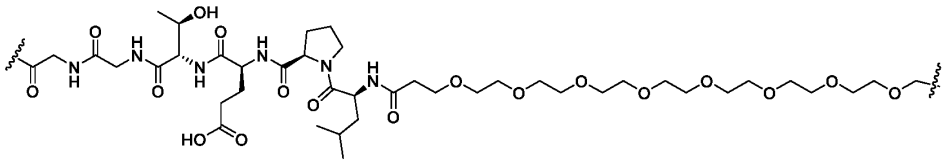
 .

[000225] In some embodiments, La comprises one or more amino acids selected from glycine, threonine, serine, glutamine, glutamic acid, alanine, valine, leucine, and proline and combinations thereof. In some embodiments, La comprises 1 to 10 glycines and 1 to 6 serines. In some embodiments, La comprises 4 glycines and 1 serine. In some embodiments, La is selected from the group consisting of Gly-Gly-Gly-Gly-Ser ($G_4S$) (SEQ ID NO: 1), Ser-Gly-Gly-Gly-Gly ($SG_4$) (SEQ ID NO: 2), Gly-Gly-Ser-Gly-Gly-Ser-Gly-Gly ($G_2S$-$G_2S$-$G_2$), and Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly ($G_4S$-$G_4$) (SEQ ID NO: 3).

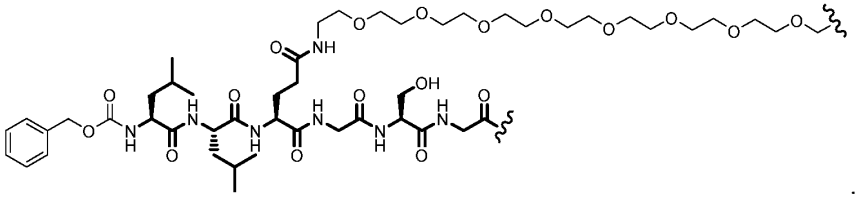
[000226] In some embodiments, La comprises a combination of a PEG segment having 1 to 36 EG units and one or more amino acids selected from glycine, threonine, serine, glutamine, glutamic acid, alanine, valine, leucine, and proline and combinations thereof. In some embodiments, La is selected from the group consisting of:



,

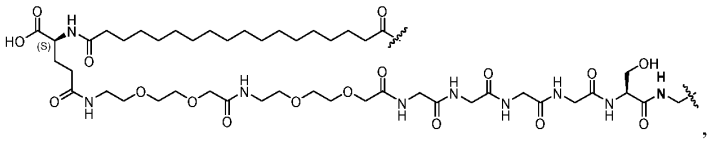


, and

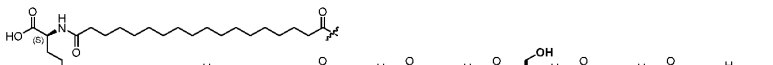


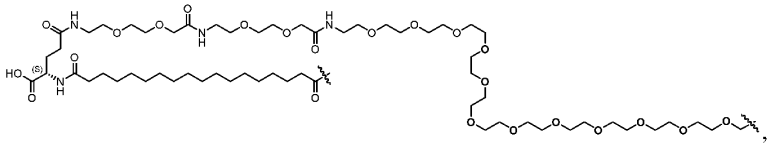
.

[000227] In some embodiments, La comprises a $-(CH_2)_{2\text{-}24}$- chain. In some embodiments, In some embodiments, La comprises a combination of a $-(CH_2)_{2\text{-}24}$- chain, a PEG segment having 1 to 36 EG units and one or more amino acids selected from glycine, threonine, serine, glutamine, glutamic acid, alanine, valine, leucine, and proline and combinations thereof. La is selected from the group consisting of:

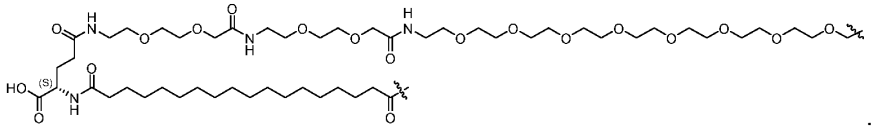


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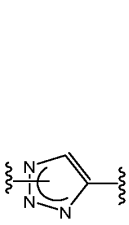


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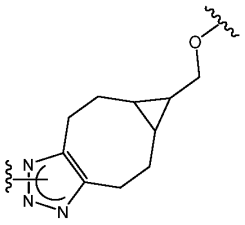


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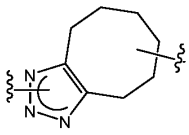
[000228] In one embodiment, Y has a structure selected from the group consisting of:



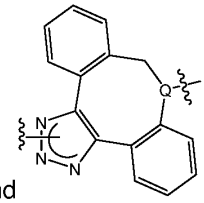
,



,



, and



, wherein Q is C or N.

[000229] In one embodiment, the linker L, or the first linker La, or the second linker Lp, comprises a polyethylene glycol (PEG) segment having 1 to 36 -$CH_2CH_2O$- (EG) units.

[000230] In one embodiment, the PEG segment comprises between 2 and 30 EG units, or between 4 and 24 EG units. In one embodiment, the PEG segment comprises 2 EG units, or 4 EG units, or 6 EG units, or 8 EG units, or 10 EG units, or 12 EG units, or 14 EG units, or 16 EG units, or 18 EG units, or 20 EG units, or 22 EG units, or 24 EG units.

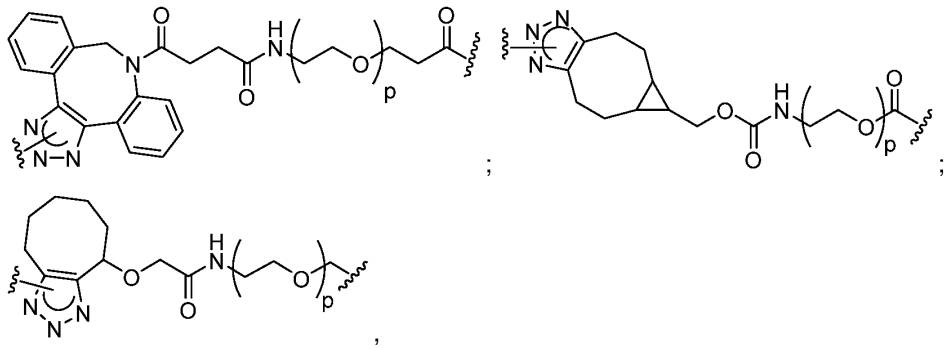
[000231] In one embodiment, the PEG segment comprises 4 EG units. In one embodiment, the PEG segment comprises 8 EG units. In one embodiment, the PEG segment comprises 12 EG units. In one embodiment, the PEG segment comprises 24 EG units.

[000232] In one embodiment, the PEG segment comprises 4 to 8 EG units. In one embodiment, the PEG segment comprises 4 EG units or 8 EG units.

[000233] In one embodiment, La comprises a PEG segment having 3 EG units.

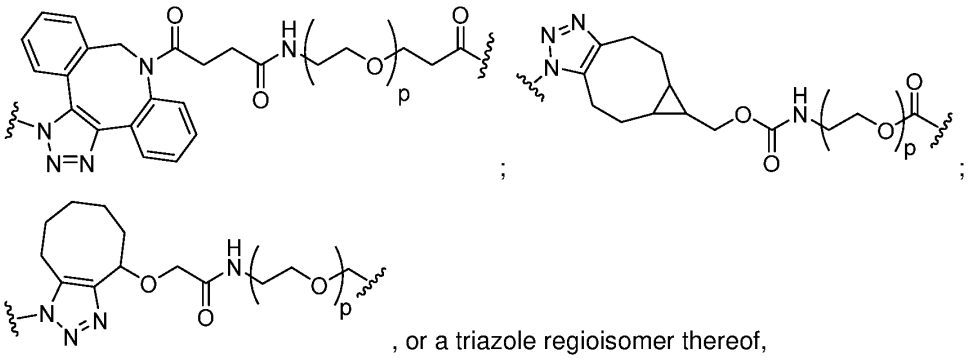
[000234] In one embodiment, Lp comprises a PEG segment having 4 EG units. In one embodiment, Lp comprises a PEG segment having 8 EG units.

[000235] In one embodiments, the Y-Lp has a structure selected from the group consisting of:



wherein p is an integer from 1 to 36.

[000236] In one embodiment, the Y-Lp has a structure selected from the group consisting of:



, or a triazole regioisomer thereof,

wherein p is an integer from 1 to 36.

[000237] In another embodiment, the linker L or the first linker La, or the second linker Lp, comprises one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline and combinations thereof.

[000238] In one embodiment, the linker L or the first linker La, or the second linker Lp, comprises from 1 to 10 glycines, or 1 glycine, or 2 glycines, or 3 glycines, or 4 glycines, or 5 glycines, or 6 glycines, or 7 glycines, or 8 glycines, or 9 glycines, or 10 glycines.

[000239] In one embodiment, the linker L or the first linker La, or the second linker Lp, comprises from 1 to 6 serines, or 1 serine, or 2 serines, or 3 serines, or 4 serines, or 5 serines, or 6 serines.

[000240] In one embodiment, the linker L or the first linker La, or the second linker Lp, comprises 1 to 10 glycines and 1 to 6 serines.

[000241] In one embodiment, the linker L or the first linker La, or the second linker Lp, comprises 4 glycines and 1 serine.

[000242] In one embodiment, the linker L or the first linker La, or the second linker Lp, is selected from the group consisting of Gly-Gly-Gly-Gly-Ser (G4S) (SEQ ID NO: 1), Ser-Gly-Gly-Gly-Gly (SG4) (SEQ ID NO: 2), and Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (G4S-G4S) (SEQ ID NO: 3).

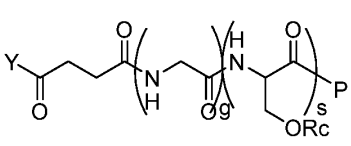
[000243] In some embodiments, one or more serine residues comprise a carbohydrate group, e.g., a glucose group.

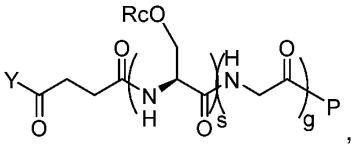
[000244] In one embodiment, the linker L or the first linker La, or the second linker Lp, comprises from 1 to 10 glutamic acids and from 1 to 10 glycines.

[000245] In some embodiments, the linker L or the first linker La, or the second linker Lp, comprises a combination of a polyethylene glycol (PEG) segment having 1 to 36 -$CH_2CH_2O$- (EG) units and one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline and combinations thereof.

[000246] In one embodiment, the linker L or the first linker La, or the second linker Lp, comprises a combination of a PEG segment having 1 to 36 EG units and 1 to 10 glycines. In one embodiment, the linker L or the first linker La, or the second linker Lp, comprises a combination of a PEG segment having 1 to 36 EG units and a group selected from Gly-Gly-Gly-Gly-Ser (G4S) (SEQ ID NO: 1), Ser-Gly-Gly-Gly-Gly (SG4) (SEQ ID NO: 2), and Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (G4S-G4S) (SEQ ID NO: 3).

[000247] In one embodiment, the linker L or the first linker La, or the second linker Lp, has a structure selected from the group consisting of:

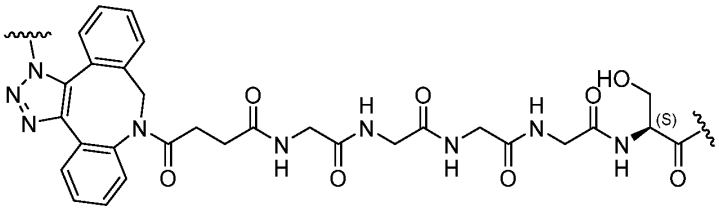


and


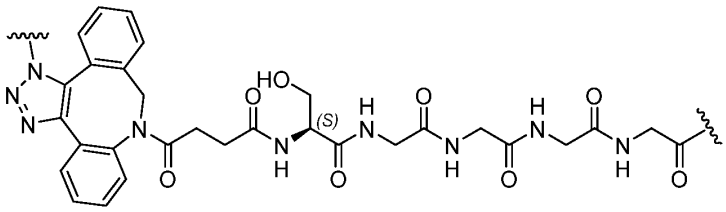
,

wherein Y is the group comprising a triazole, e.g., as shown above, and P is the payload, and wherein Rc is selected from hydrogen (H) and glucose, g is an integer from 1 to 10 and s is an integer from 0 to 4.

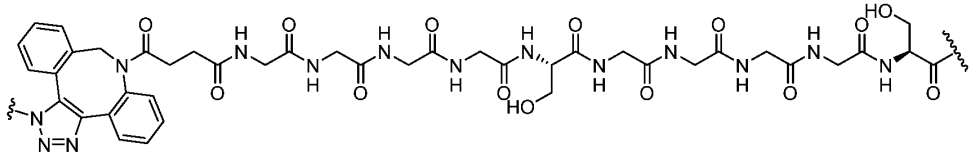
[000248] In one embodiment, the Y-Lp has a structure selected from the group consisting of:



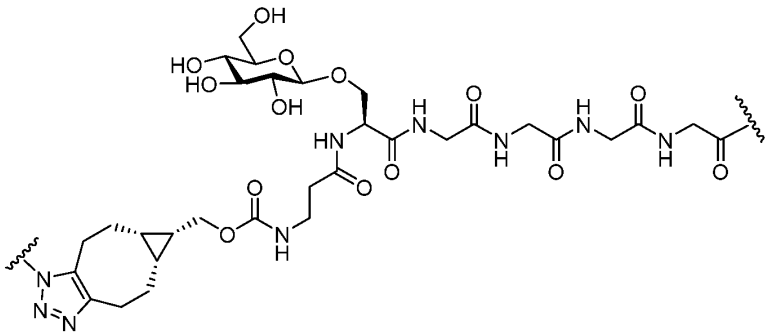
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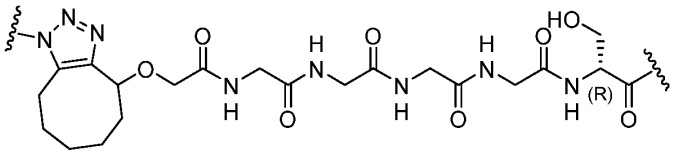
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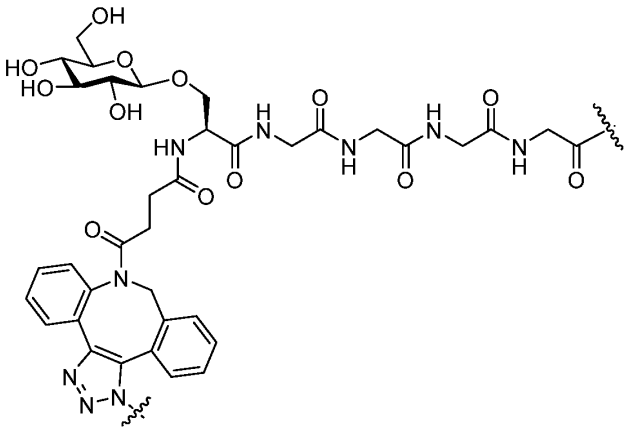
;



;



; and

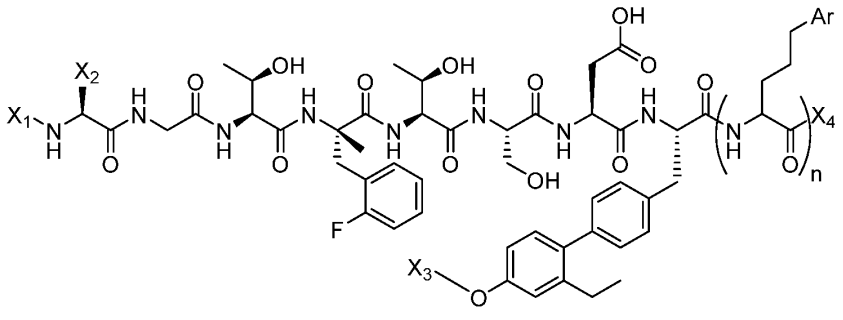


[000249] In one embodiment, the linker L comprises a cyclodextrin moiety.

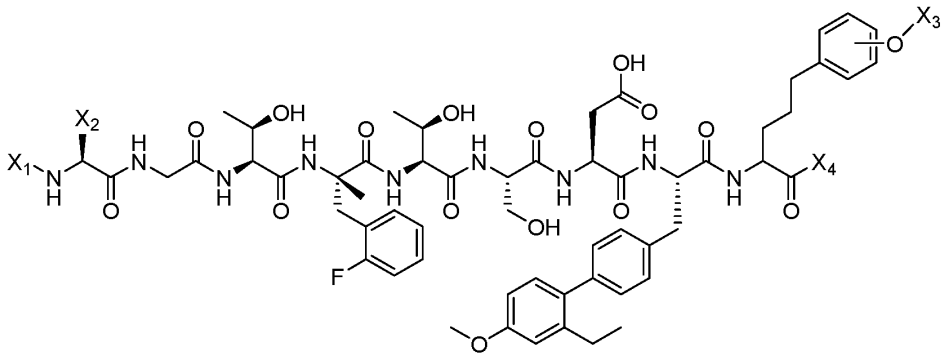
[000250] In some embodiments, the linker L is attached to the antibody or an antigen-binding fragment thereof via a glutamine residue. In some embodiments, the linker L is attached to the antibody or an antigen-binding fragment thereof via a lysine residue. In some embodiments, the linker L is attached to the antibody or an antigen-binding fragment thereof via a cysteine residue.

**Payloads P**

[000251] In one aspect, the payloads P according to the present disclosure have a structure of Formula selected from the group consisting of Formula (P-IB), Formula (P-IIB), and Formula (P-IIIB):

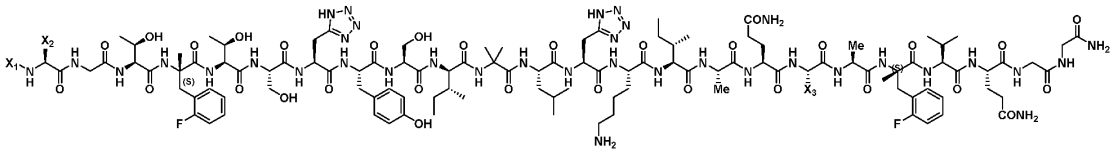


(P-IB),

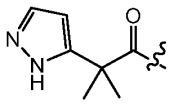
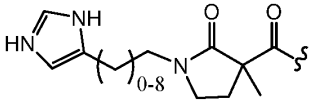
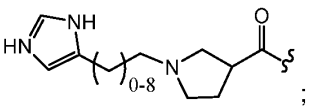


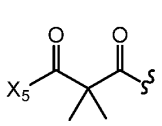
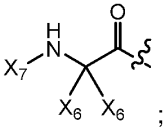
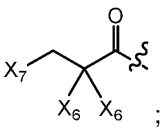
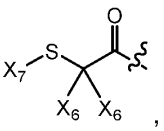
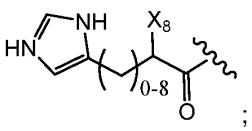
(P-IIB),

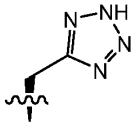
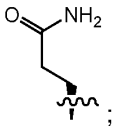
and



(P-IIIB), wherein:

$X_1$ is selected from H;  ;  ;  ;

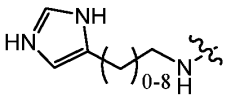
 ;  ;  ;  , and  ;

$X_2$ is selected from  and  ;

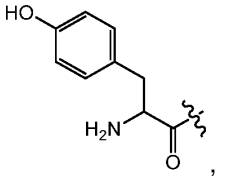
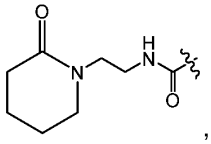
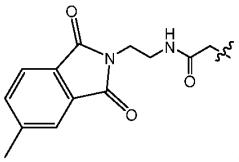
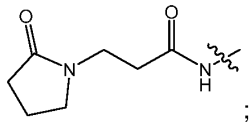
$X_3$ is selected from $-CH_3$, $-(CH_2)_{2-6}-NH_2$, $-(CH_2)_{2-6}-N_3$, and $-(CH_2)_{2-6}-Tr-(CH_2)_{1-6}-NH_2$, where Tr is a triazole moiety;

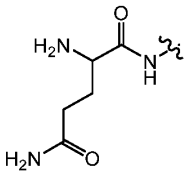
n is 0 or 1;

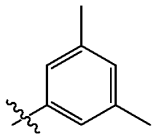
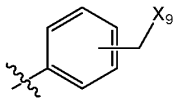
$X_4$ is selected from $-NH_2$, -OH and -N(H)(phenyl);

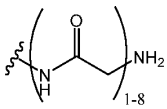
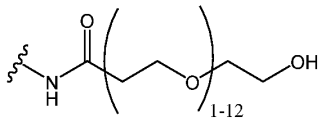
$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  ,  ,  ,  ,  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ;

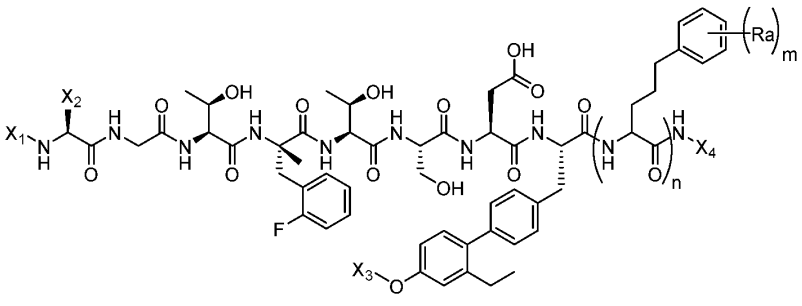
Ar is selected from  and  ;

$X_9$ is selected from $-NH_2$,  , and  , and

m is an integer from 1 to 4,

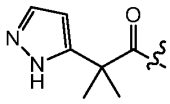
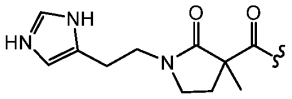
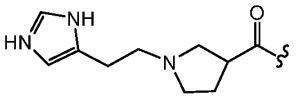
or a pharmaceutically acceptable salt thereof.

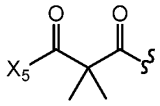
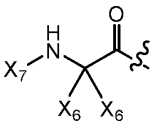
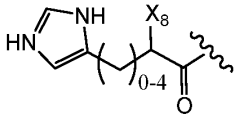
[000252] In one embodiment, the payloads P according to the present disclosure have a structure of Formula (II):

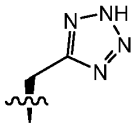
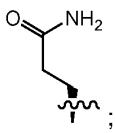


(II),

wherein:

$X_1$ is selected from H;  ;  ;  ;

 ;  ; and  ;

$X_2$ is selected from  and  ;

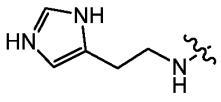
$X_3$ is selected from $–(CH_2)_{2-6}–NH_2$, $–(CH_2)_{2-6}–N_3$, and $-CH_3$, with the proviso that when $X_3$ is $-CH_3$, n is 1 and Ra in at least one occurrence is selected from $–(CH_2)_{2-6}–NH_2$ and $–(CH_2)_{2-6}–N_3$;

n is 0 or 1;

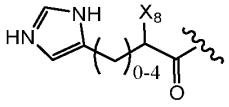
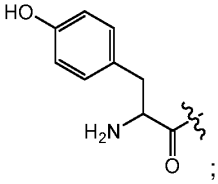
m is an integer from 0 to 3;

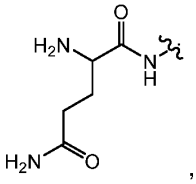
Ra is independently at each occurrence selected from $-CH_3$, $–(CH_2)_{2-6}–NH_2$, and $–(CH_2)_{2-6}–N_3$;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

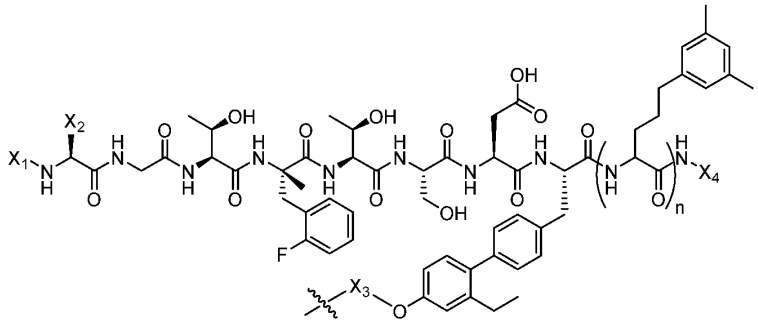
$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  , and  ;

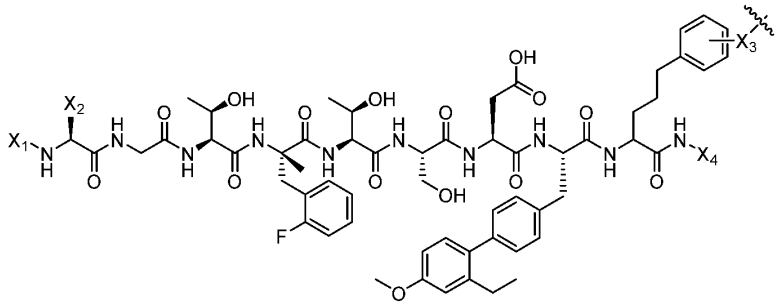
$X_8$ is selected from H, -OH, $-NH_2$, and  ,

and pharmaceutically acceptable salts thereof.

[000253] In one embodiment, the payload P has a structure selected from:

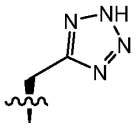
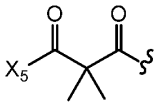
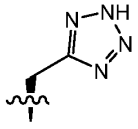
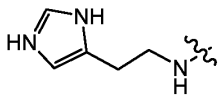
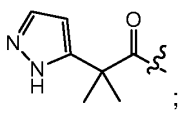
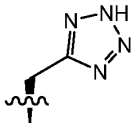


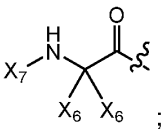
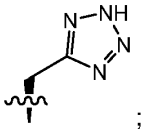
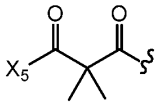
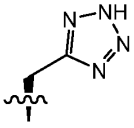
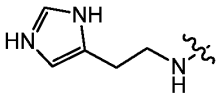
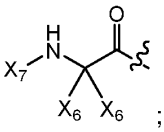
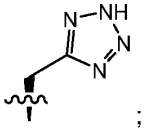
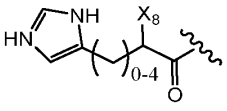
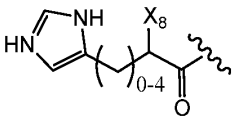
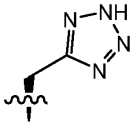
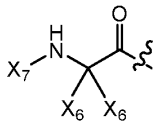
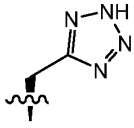
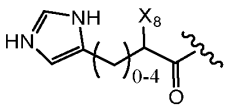
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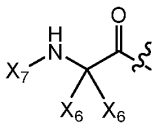
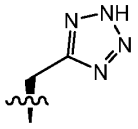
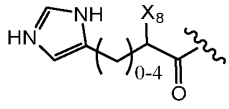
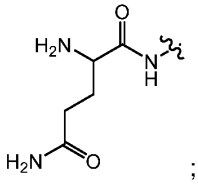
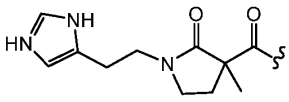
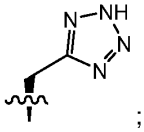
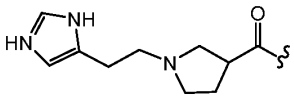
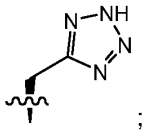
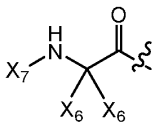
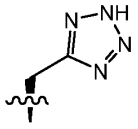
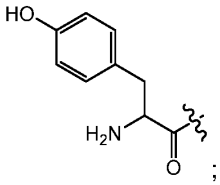
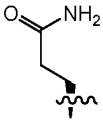


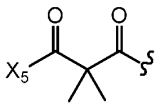
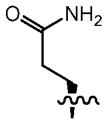
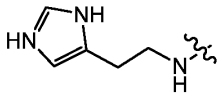
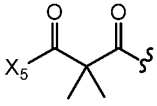
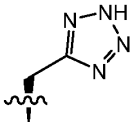
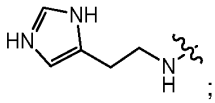
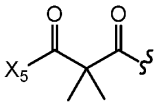
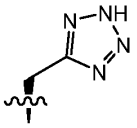
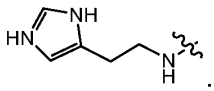
(P-II), where  indicates the point of attachment to a linker.

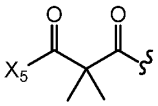
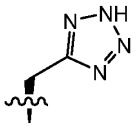
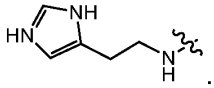
[000254] In one embodiment, the payload has the structure of formula (P-I), shown above, wherein

- $X_1$ is H; $X_2$ is  ; $X_3$ is selected from $–(CH_2)_{2-6}–NH–$ and $–(CH_2)_{2-6}–Tr–$, where Tr is a triazole moiety; n is 1, and $X_4$ is H;
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2-6}–NH–$; n is 1; $X_4$ is H, and $X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2-6}–NH–$; n is 1, and $X_4$ is H;

- $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and $-CH_2OH$, and $X_7$ is H;
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–Tr–$, where Tr is a triazole moiety; n is 1; $X_4$ is H, and $X_5$ is  ;
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and $-CH_3$; $X_7$ is  , and $X_8$ is $-NH_2$;
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H, and $X_8$ is H;
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H; $X_6$ is H at each occurrence; $X_7$ is  , and $X_8$ is H;

- $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and -$CH_3$; $X_7$ is  , and $X_8$ is  ;
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1, and $X_4$ is H;
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1, and $X_4$ is H;
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and -$CH_3$, and $X_7$ is  ;
- $X_1$ is H; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1, and $X_4$ is H;

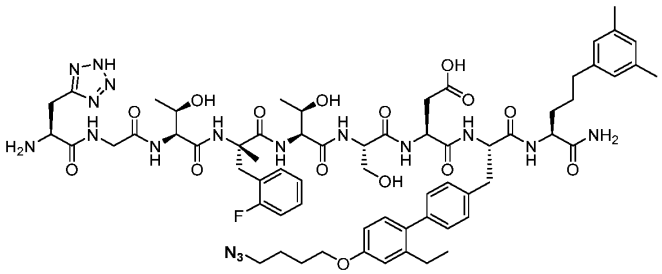
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1; $X_4$ is H, and $X_5$ is  ;
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 0; $X_4$ is phenyl, and $X_5$ is  ; and
- $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1; $X_4$ is phenyl, and $X_5$ is  .

[000255] In one embodiment, the payload has the structure of formula (P-II), shown above, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$;$X_4$ is H, and $X_5$ is  .

[000256] In one embodiment, the payloads P according to the present disclosure have a structure selected from:

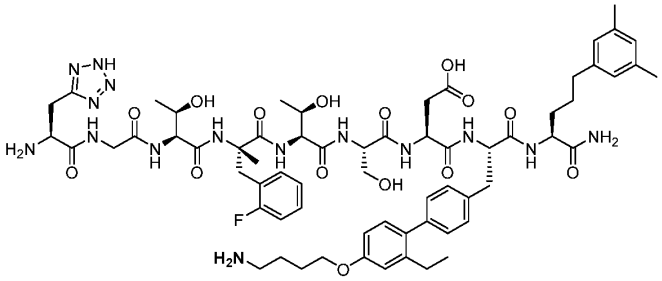
| P# | Structure |
|---|---|

P1



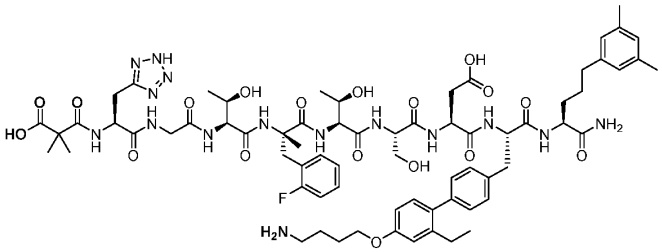
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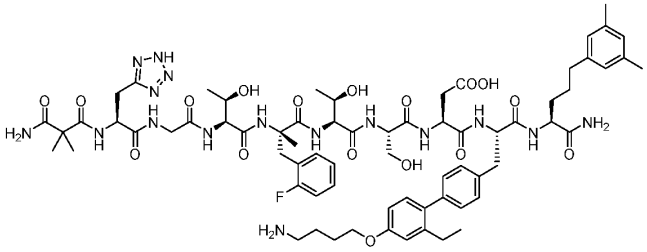
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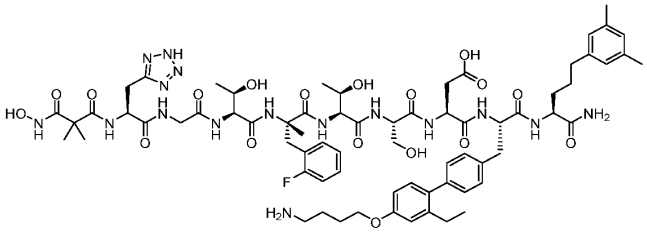
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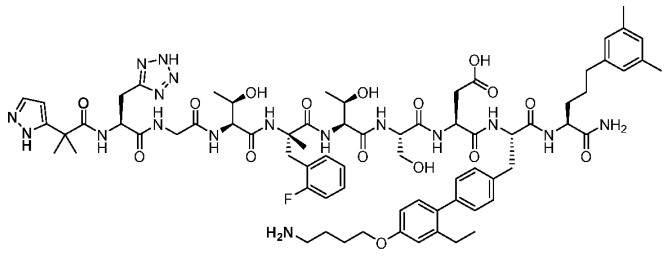


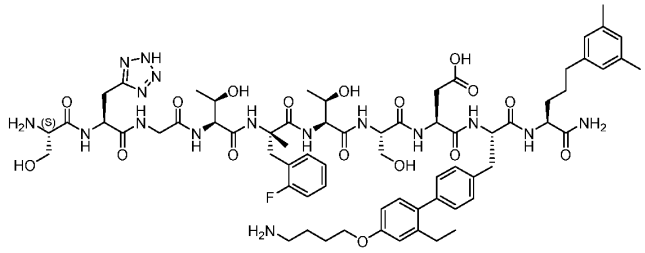
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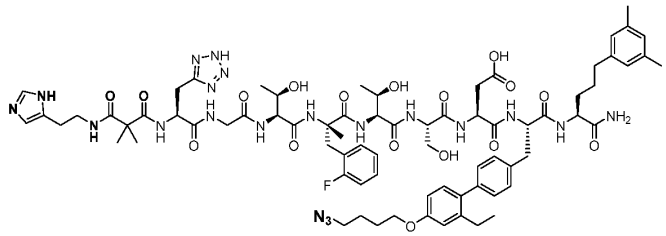
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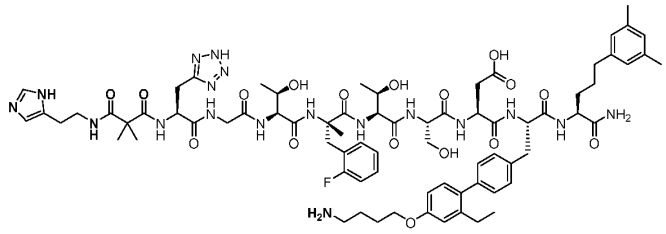


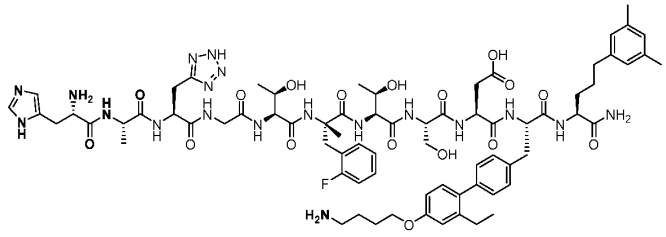
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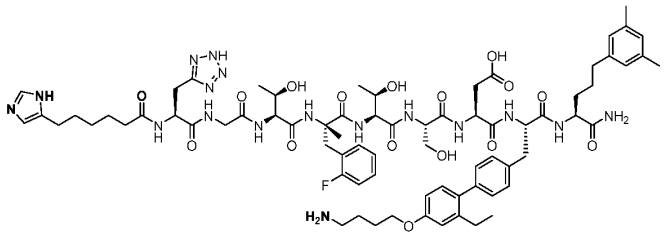
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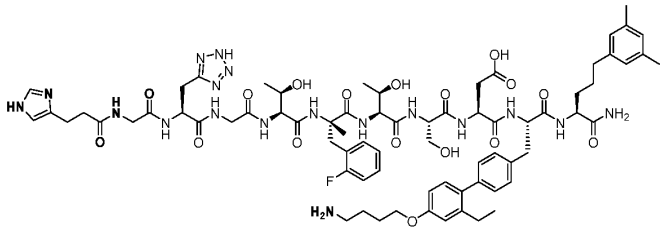
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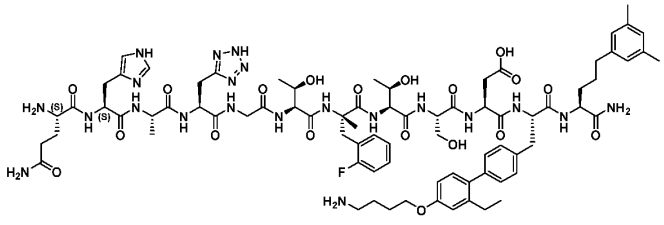
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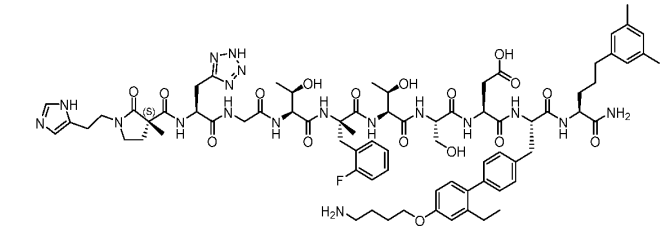
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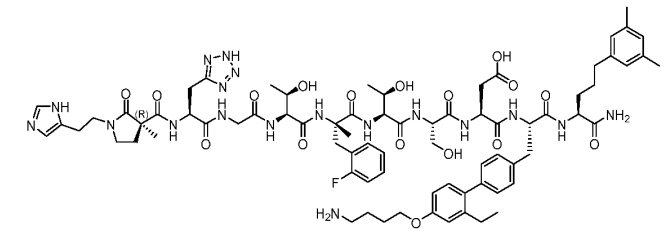
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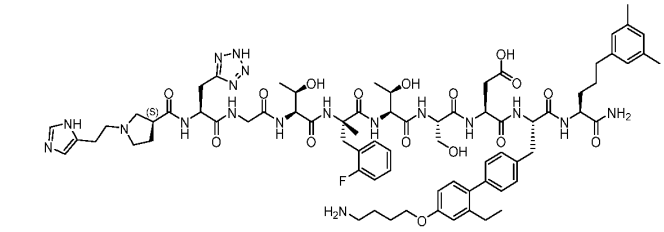
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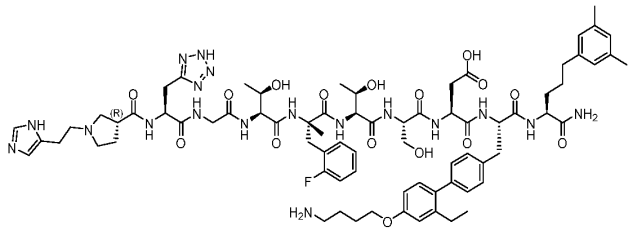
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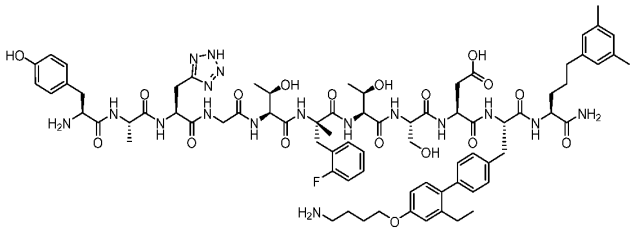
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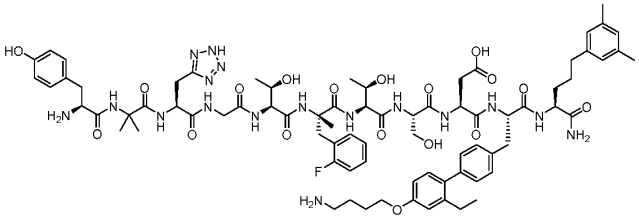
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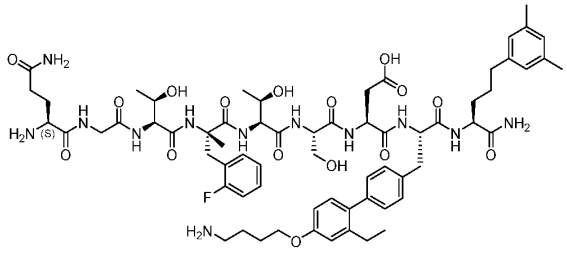
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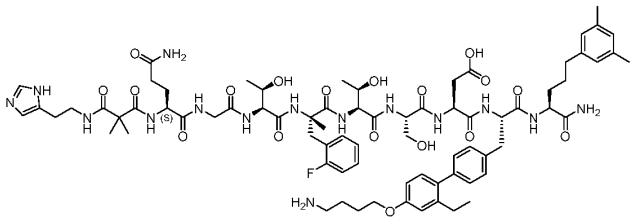
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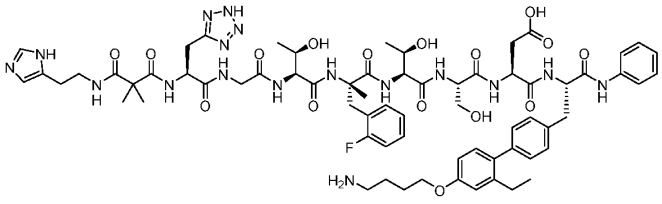
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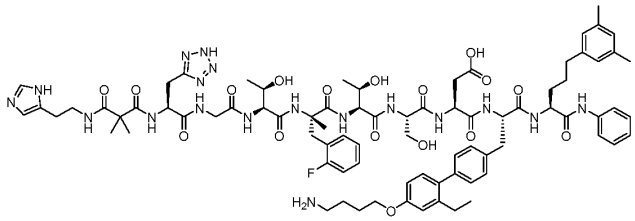
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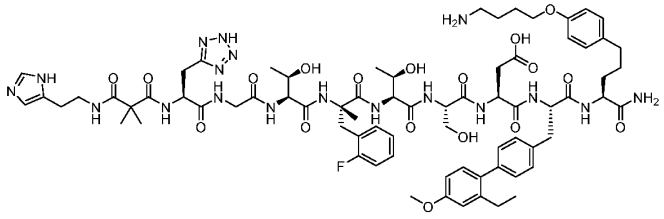
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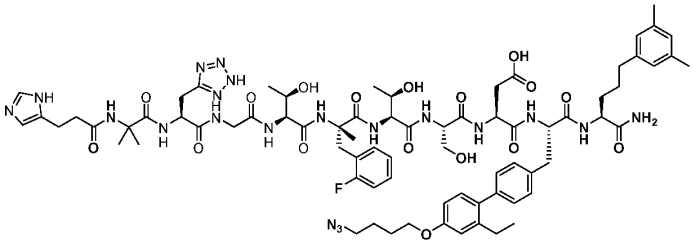
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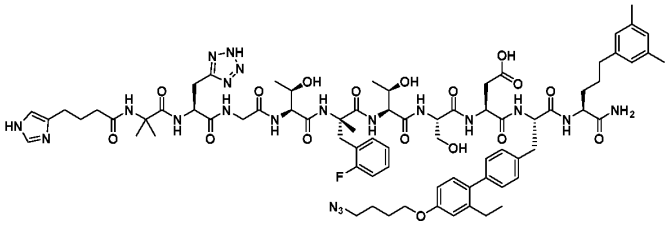
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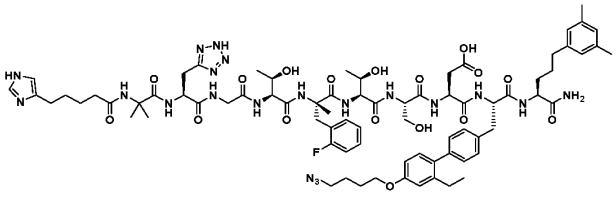
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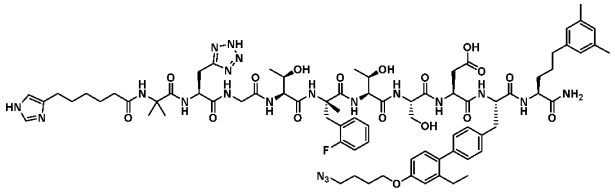
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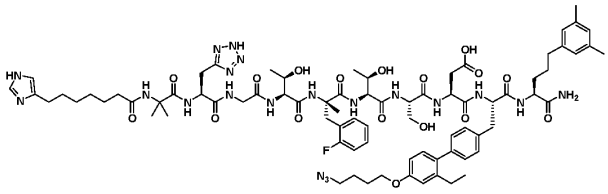
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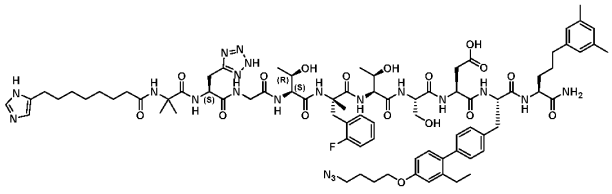
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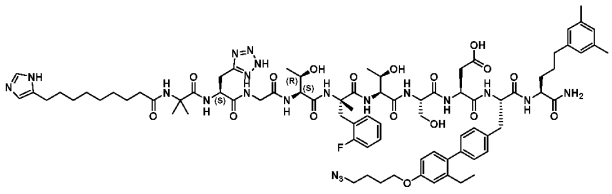
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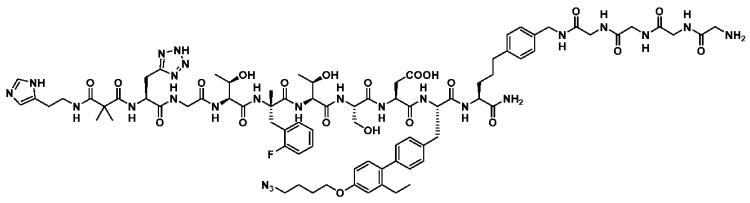
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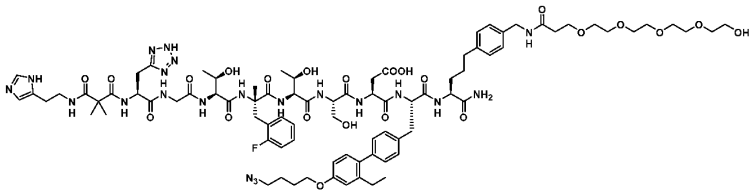
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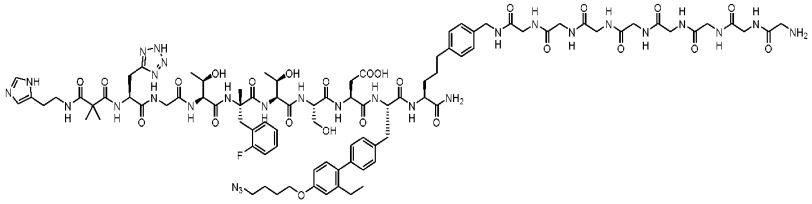
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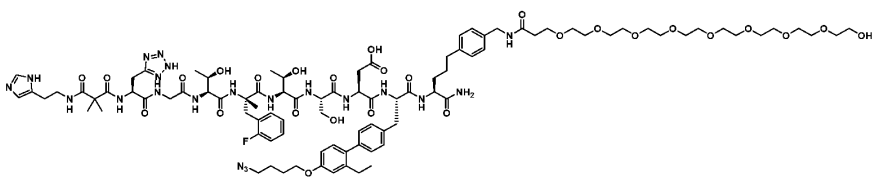
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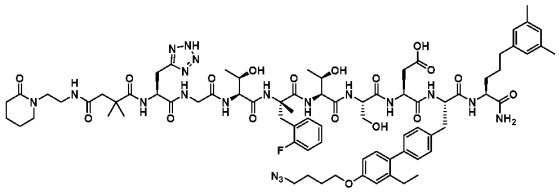
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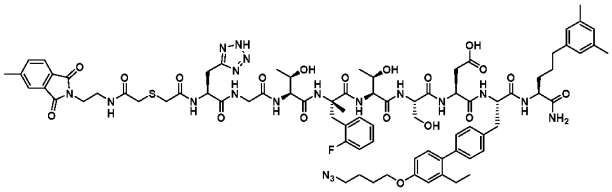
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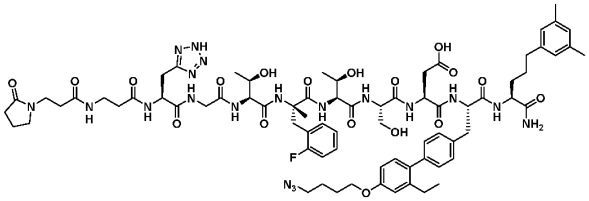
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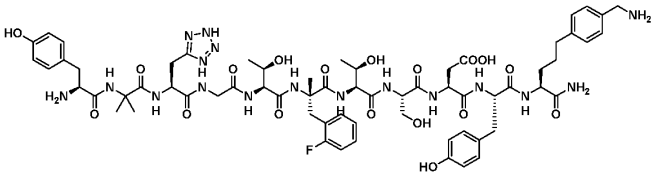
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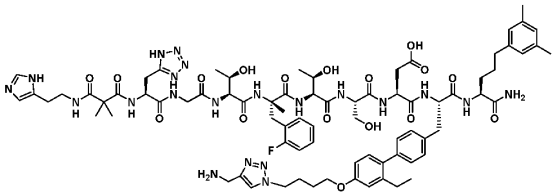
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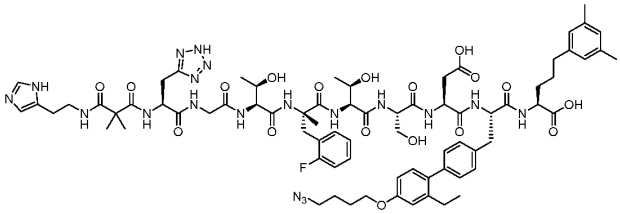
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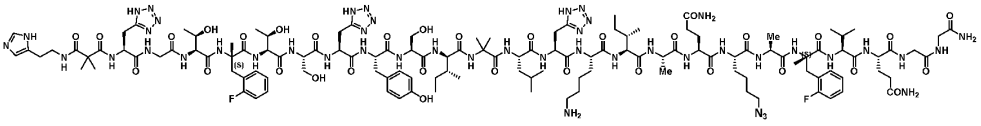
P37  ;

P38  ;

P39  ;

P40  ;

P41  , and

P42  .

[000257] In one embodiment, the payloads as described above have the following properties:

| P# | Molecular Formula | MW | M/Z 100% (M+H) | RT on HPLC (15 min) | CLogP | Plasma stability t½ (hr) |
|---|---|---|---|---|---|---|
| **P1** | $C_{65}H_{86}FN_{17}O_{15}$ | 1364.48 | 860.2 $[M+2H]^{2+}$ 1365.6 $[M+H]^{+}$ | 10.15 | 4.94 ± 0.99 | |
| **P2** | $C_{65}H_{88}FN_{15}O_{15}\cdot 2CF_3COOH$ | 1566.53 | 670.0 $[M+2H]^{2+}$ | 7.77 | 4.08 ± 0.98 | 10.7 |
| **P3** | $C_{70}H_{94}FN_{15}O_{18}\cdot CF_3COOH$ | 1566.61 | 1454.1 $[M+H]^{+}$ 727.3 $[M+2H]^{2+}$ | 8.10 | 4.54 ± 1.00 | >57.8 |
| **P4** | $C_{70}H_{95}FN_{16}O_{17}\cdot CF_3COOH$ | 1565.62 | 1452.4 $[M+H]^{+}$ 726.7 $[M+2H]^{2+}$ | 7.90 | 3.59 ± 1.00 | >57.8 |
| **P5** | $C_{70}H_{95}FN_{16}O_{18}$ | 1467.6 | 1468.0 $[M+H]^{+}$ 735.2 $[M+2H]^{2+}$ | 8.00 | 3.23 ± 1.00 | |
| **P6** | $C_{72}H_{96}FN_{17}O_{16}\cdot 2CF_3COOH$ | 1702.68 | 738.2 $[M+2H]^{2+}$ | 8.16 | 4.38 ± 1.00 | >57.8 |
| **P7** | $C_{68}H_{93}FN_{16}O_{17}\cdot 2CF_3COOH$ | 1653.61 | 713.8 $[M+2H]^{2+}$ | 7.58 | 3.16 ± 1.00 | |
| **P8** | $C_{75}H_{99}FN_{20}O_{17}$ | 1571.71 | 786.38 $[M+2H]^{2+}$ 1571.76 $[M+H]^{+}$ | 10.14 | 4.43 ± 1.02 | |
| **P9** | $C_{75}H_{101}FN_{18}O_{17}\cdot 2CF_3COOH$ | 1773.76 | 773.9 $[M+2H]^{2+}$ | 7.44 | 3.57 ± 1.01 | >57.8 |
| **P10** | $C_{74}H_{100}FN_{19}O_{17}\cdot 3CF_3COOH$ | 1888.77 | 516.4 $[M+3H]^{3+}$ 774.2 $[M+2H]^{2+}$ | 7.46 | 2.95 ± 1.02 | 1.8 |
| **P11** | $C_{74}H_{100}FN_{17}O_{16}\cdot 2CF_3COOH$ | 1730.74 | 752.3 $[M+2H]^{2+}$ | 7.72 | 5.04 ± 0.99 | >57.8 |
| **P12** | $C_{73}H_{97}FN_{18}O_{17}\cdot 2CF_3COOH$ | 1745.71 | 759.8 $[M+2H]^{2+}$ | 7.65 | 3.03 ± 1.01 | >57.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **P13** | $C_{79}H_{108}FN_{21}O_{19}\cdot 3CF_3COOH$ | 2016.9 | 559.1 $[M+3H]^{3+}$ 838.3 $[M+2H]^{2+}$ | 7.37 | 1.81 ± 1.04 | 16.9 |
| **P14** | $C_{76}H_{101}FN_{18}O_{17}$ | 1557.72 | 779.9 $[M+2H]^{2+}$ | 7.60 | 3.68 ± 1.01 | |
| **P15** | $C_{76}H_{101}FN_{18}O_{17}\cdot 2(CF_3COOH)$ | 1785.77 | 779.8 $[M+2H]^{2+}$ | 7.62 | 3.68 ± 1.01 | |
| **P16** | $C_{75}H_{101}FN_{18}O_{16}\cdot 2(CF_3COOH)$ | 1757.76 | 765.7$[M+2H]^{2+}$ | 7.18 | 3.68 ± 1.01 | |
| **P17** | $C_{75}H_{101}FN_{18}O_{16}\cdot 2(CF_3COOH)$ | 1757.76 | 766.3 $[M+2H]^{2+}$ | 7.36 | 3.68 ± 1.01 | |
| **P18** | $C_{77}H_{102}FN_{17}O_{18}$ | 1572.74 | 787.3 $[M+2H]^{2+}$ | 7.68 | 4.60 ± 1.01 | |
| **P19** | $C_{78}H_{104}FN_{17}O_{18}$ | 1586.76 | 794.4 $[M+2H]^{2+}$ | 7.81 | 4.95 ± 1.01 | >57.8 |
| **P20** | $C_{66}H_{91}FN_{12}O_{16}$ | 1327.5 | 1327.7 $[M+H]^{+}$ | 7.40 | 3.41 ± 1.00 | |
| **P21** | $C_{76}H_{104}FN_{15}O_{18}\cdot 2CF_3COOH$ | 1762.77 | 768.3 $[M+2H]^{2+}$ | 7.44 | 3.92 ± 0.97 | >57.8 |
| **P22** | $C_{68}H_{88}FN_{17}O_{16}$ | 1418.53 | 710.2 $[M+2H]^{2+}$ | 7.06 | 3.03 ± 0.99 | |
| **P23** | $C_{81}H_{105}FN_{18}O_{17}$ | 1621.81 | 811.9 $[M+2H]^{2+}$ | 8.30 | 6.00 ± 1.01 | |
| **P24** | $C_{74}H_{99}FN_{18}O_{18}\cdot 2CF_3COOH$ | 1775.73 | 774.8 $[M+2H]^{2+}$ | 11.00 (20 min) | 2.56 ± 1.01 | |
| **P25** | $C_{75}H_{99}FN_{20}O_{17}$ | 1570.8 | 786.7 $[M+2H]^{2+}$ | 9.54 | 4.58 ± 1.01 | |
| **P26** | $C_{76}H_{101}FN_{20}O_{17}$ | 1584.8 | 793.7 $[M+2H]^{2+}$ | 9.55 | 5.09 ± 1.01 | |
| **P27** | $C_{77}H_{103}FN_{20}O_{17}$ | 1598.8 | 800.39 $[M+2H]^{2+}$ | 9.58 | 5.54 ± 1.01 | |
| **P28** | $C_{78}H_{105}FN_{20}O_{17}$ | 1612.8 | 807.40 $[M+2H]^{2+}$ | 9.60 | 5.98 ± 1.01 | |
| **P29** | $C_{79}H_{107}FN_{20}O_{17}$ | 1626.8 | 814.40 $[M+2H]^{2+}$ | 9.61 | 6.51 ± 1.01 | |
| **P30** | $C_{81}H_{111}FN_{20}O_{17}$ | 1768.8 | 821.4 $[M+2H]^{2+}$ | 9.63 | 7.57 ± 1.01 | |
| **P31** | $C_{81}H_{111}FN_{20}O_{17}$ | 1654.8 | 828.8 $[M+2H]^{2+}$ | 9.80 | 7.57 ± 1.01 | |

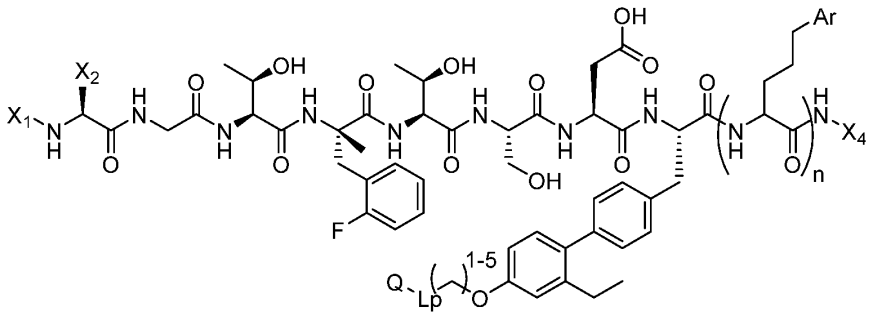
| | | | | | |
|---|---|---|---|---|---|
| **P32** | $C_{82}H_{110}FN_{25}O_{21}$ | 1799.8 | 900.91 $[M+2H]^{2+}$ | 7.23 | -0.30 ± 1.06 |
| **P33** | $C_{85}H_{118}FN_{21}O_{23}$ | 1819.9 | 911.40 $[M+2H]^{2+}$ | 7.96 | 0.25 ± 1.05 |
| **P34** | $C_{90}H_{122}FN_{29}O_{25}$ | 2027.9 | 1015.2 $[M+2H]^{2+}$ | 7.15 | -2.76 ± 1.10 |
| **P35** | $C_{93}H_{134}FN_{21}O_{27}$ | 1996.0 | 999.4 $[M+2H]^{2+}$ | 7.88 | -1.18 ± 1.07 |
| **P36** | $C_{78}H_{106}FN_{19}O_{18}$ | 1615.8 | 809.3 $[M+2H]^{2+}$ | 5.25 | 4.76 ± 1.02 |
| **P37** | $C_{80}H_{100}FN_{19}O_{19}S$ | 1681.7 | 842.3 $[M+2H]^{2+}$ | 4.84 | 7.01 ± 1.03 |
| **P38** | $C_{75}H_{100}FN_{19}O_{18}$ | 1573.8 | 788.20 $[M+2H]^{2+}$ | 9.93 | 3.49 ± 1.02 |
| **P39** | $C_{65}H_{86}FN_{17}O_{18}$ | 1411.63 | 1412.7 $[M+H]^{+}$, 707.3 $[M+2H]^{2+}$ | 6.89 | -0.24+/- 1.01 |
| **P40** | $C_{78}H_{104}FN_{21}O_{17}$ | 1625.79 | 814.2 $[M+2H]^{2+}$ | 6.94 | 2.98+/- 1.40 |
| **P41** | $C_{75}H_{98}FN_{19}O_{18}$ | 1571.73 | 787.3 $[M+2H]^{2+}$ | 9.66 | 5.40+/- 1.02 |
| **P42** | $C_{126}H_{190}F_2N_{46}O_{33}$ | 2913.46 | 1458.2 $[M+2H]^{2+}$ | 7.17 | -3.08+/- 1.57 |

[000258] In some embodiments, the payloads of the present disclosure are amenable to conjugation with a binding agent (e.g., antibody).

**Linker-Payloads (L-P)**

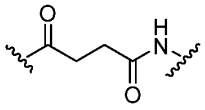
[000259] In one embodiment, the present disclosure provides reactive linker-payloads comprising payloads P as described above and linkers capable of covalently attaching to an antibody or an antigen-binding fragment thereof.

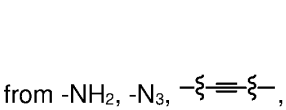
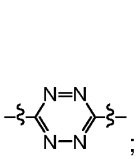
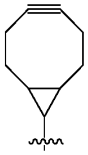
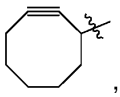
[000260] In one embodiment, the linker-payload according to the present disclosure has a structure of Formula (C):

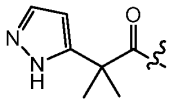
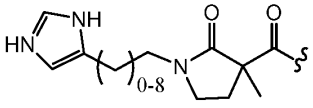
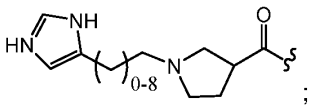
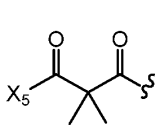
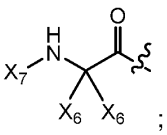
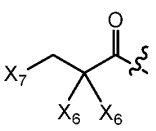
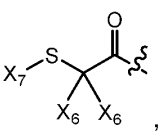
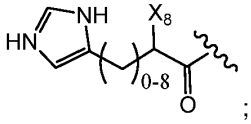


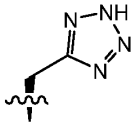
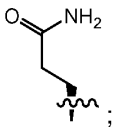
(C),

wherein:

$L_p$ is absent or a linker comprising one or more of  ; a carbamate group; a cyclodextrin; a polyethylene glycol (PEG) segment having 1 to 36 $-CH_2CH_2O-$ (EG) units; a $-(CH_2)_{2-24}-$ chain; a triazole; one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline, and combinations thereof;

Q is a moiety selected from $-NH_2$, $-N_3$,  ;  ;  ; and  , where A is C or N;

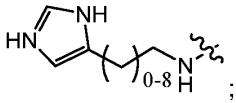
$X_1$ is selected from H;  ;  ;  ;  ;  ;  ;  , and  ;

$X_2$ is selected from  and  ;

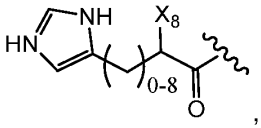
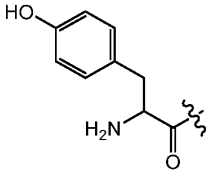
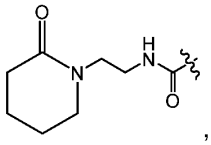
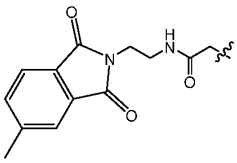
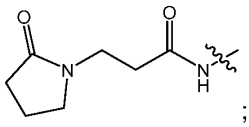
$X_3$ is selected from $-CH_3$, $-(CH_2)_{2-6}-NH_2$, $-(CH_2)_{2-6}-N_3$, and $-(CH_2)_{2-6}-Tr-(CH_2)_{1-6}-NH_2$, where Tr is a triazole moiety;

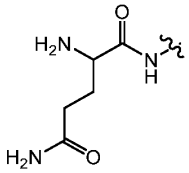
n is 0 or 1;

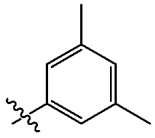
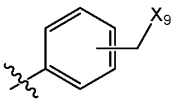
$X_4$ is selected from -$NH_2$, -OH and -N(H)(phenyl);

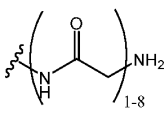
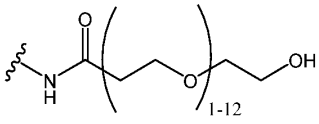
$X_5$ is selected from -OH, -$NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, -$CH_3$, and -$CH_2OH$;

$X_7$ is selected from H,  ,  ,  ,  ,  ;

$X_8$ is selected from H, -OH, -$NH_2$, and  ;

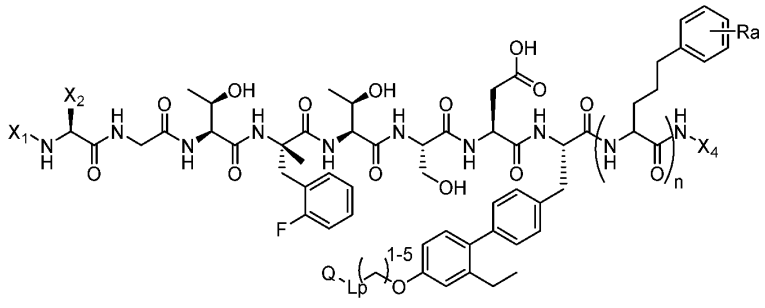
Ar is selected from  and  ;

$X_9$ is selected from -$NH_2$,  , and  , and

m is an integer from 1 to 4

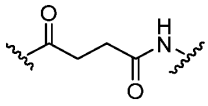
or a pharmaceutically acceptable salt thereof.

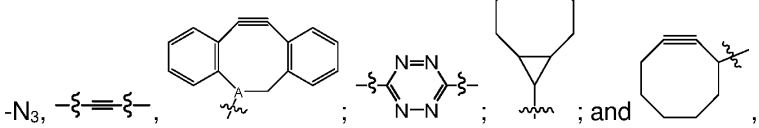
[000261] In one embodiment, the linker-payload according to the present disclosure has a structure of Formula (III):

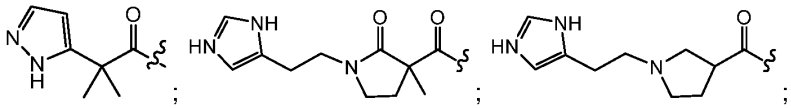


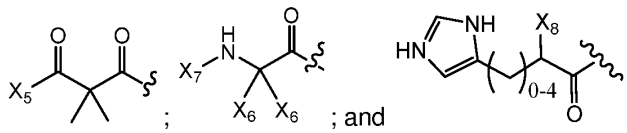
(III),

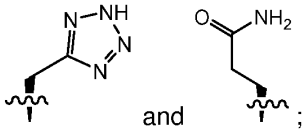
wherein:

$L_p$ is absent or a linker comprising one or more of  ; a carbamate group; a cyclodextrin; a polyethylene glycol (PEG) segment having 1 to 36 $-CH_2CH_2O-$ (EG) units; one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline, and combinations thereof;

Q is a moiety selected from $-N_3$,  ; and , where A is C or N;

$X_1$ is selected from H;  ;

 ; and  ;

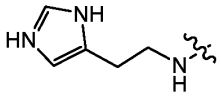
$X_2$ is selected from  and ;

$X_3$ is selected from $-(CH_2)_{2-6}-NH_2$, $-(CH_2)_{2-6}-N_3$, and $-CH_3$, with the proviso that when $X_3$ is $-CH_3$, n is 1 and Ra in at least one occurrence is selected from $-(CH_2)_{2-6}-NH_2$ and $-(CH_2)_{2-6}-N_3$;

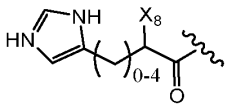
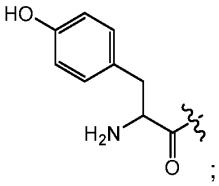
n is 0 or 1;

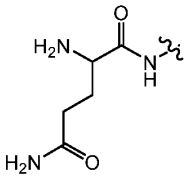
Ra is independently at each occurrence selected from H, $-CH_3$, $-(CH_2)_{2-6}-NH_2$, and $-(CH_2)_{2-6}-N_3$;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, -$NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, -$CH_3$, and -$CH_2OH$;

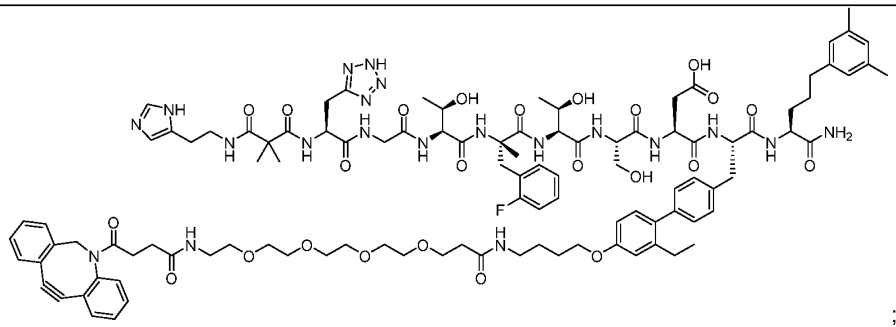
$X_7$ is selected from H,  , and  ;

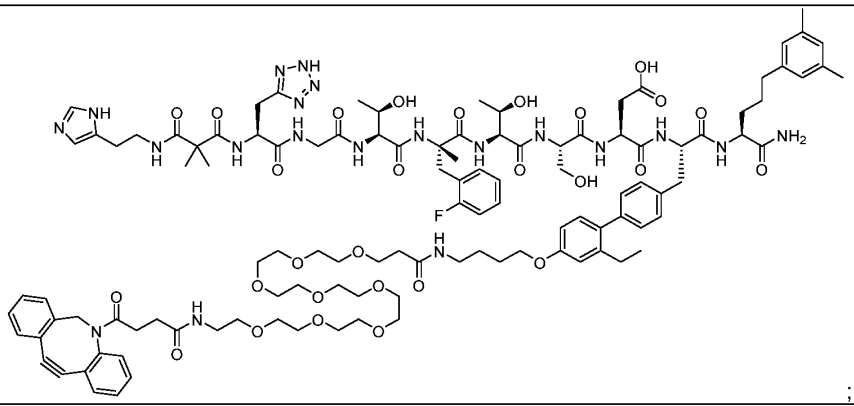
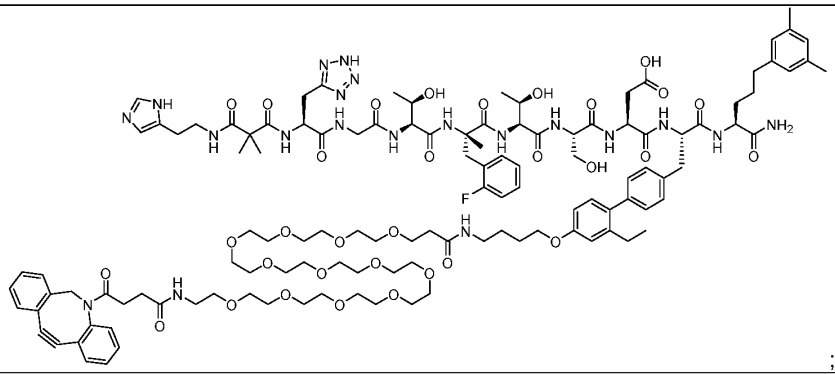
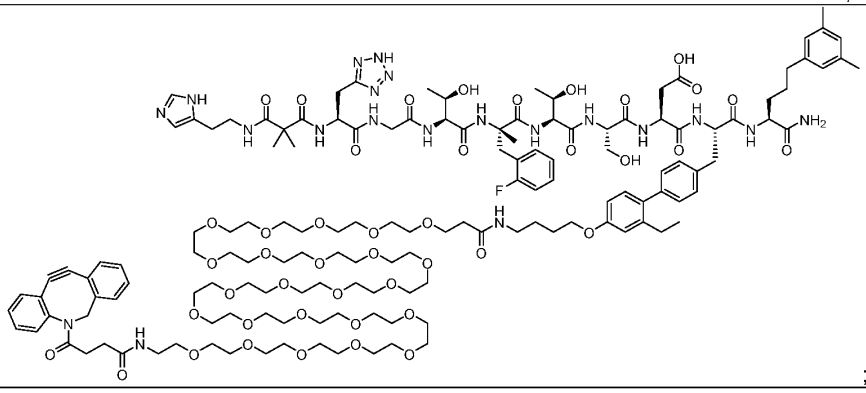
$X_8$ is selected from H, -OH, -$NH_2$, and  ,

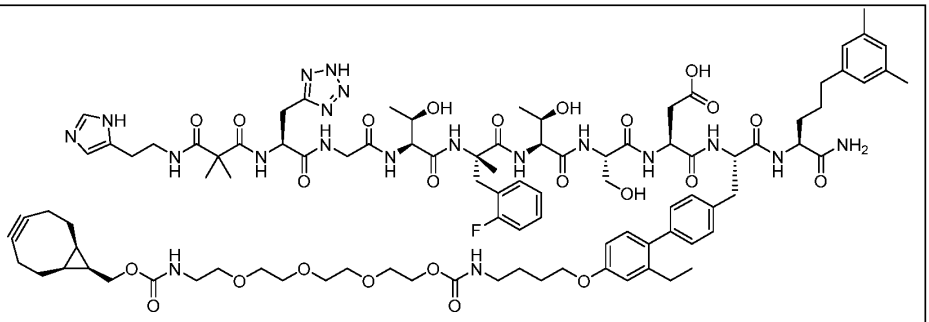
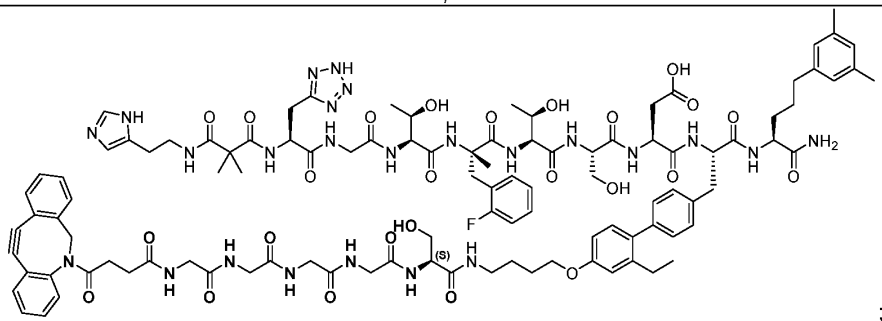
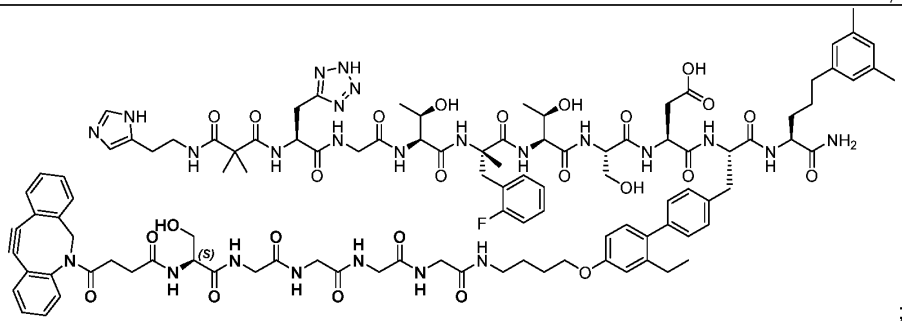
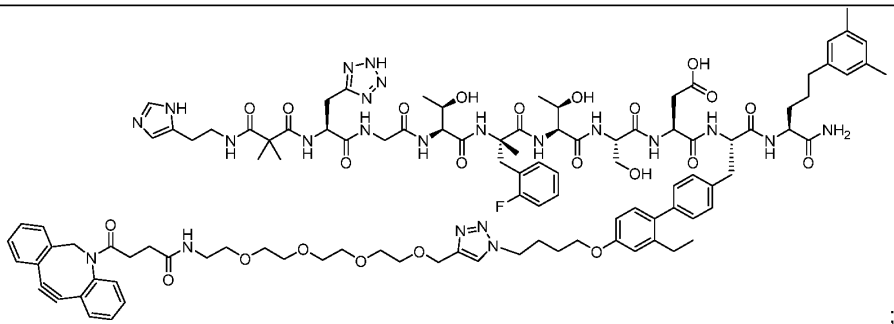
and pharmaceutically acceptable salts thereof.

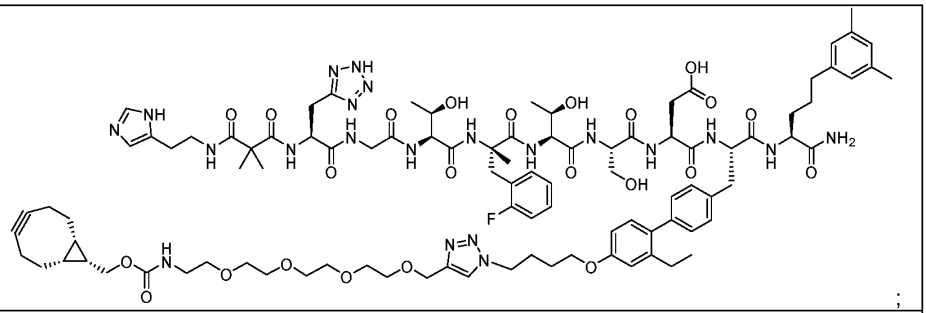
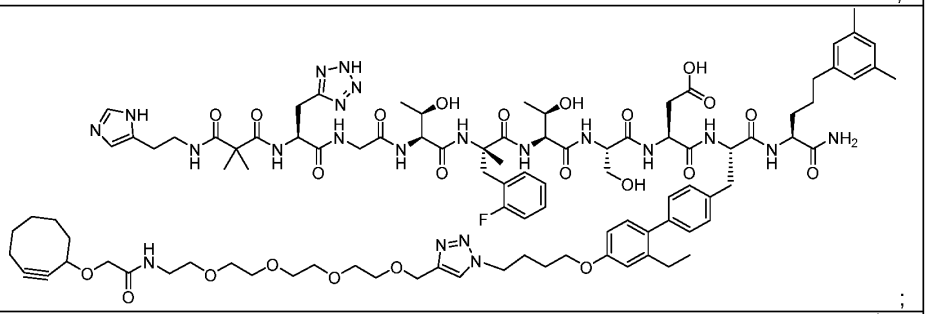
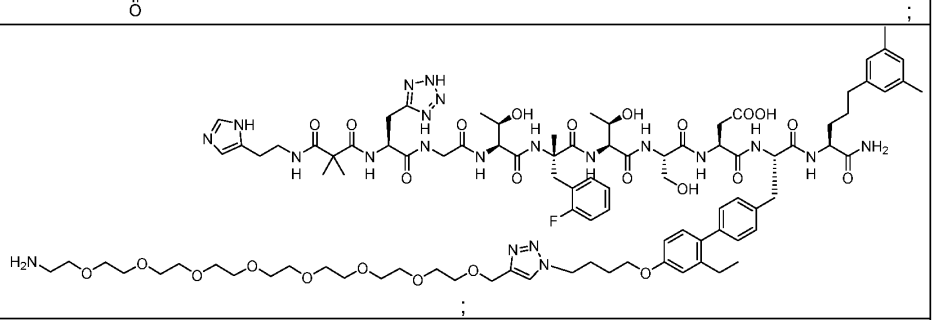
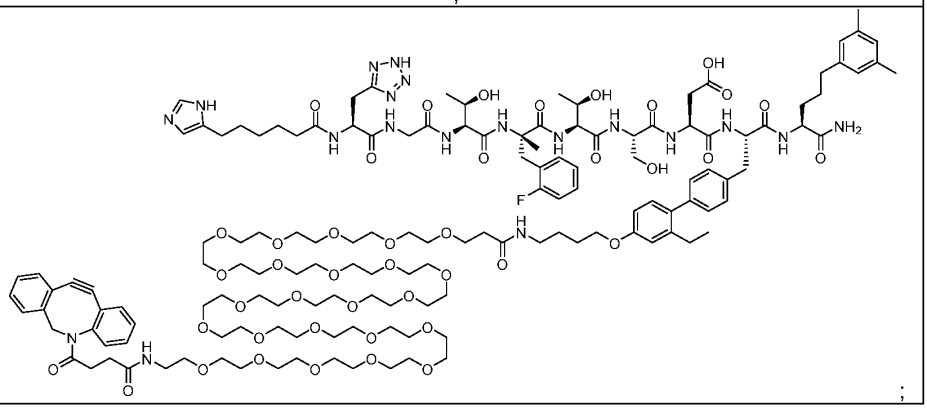
[000262] In one embodiment, the linker-payload LP comprises a cyclodextrin moiety. In some embodiments, the linker-payload LP comprising a cyclodextrin moiety exhibits GLP1R agonism activity.

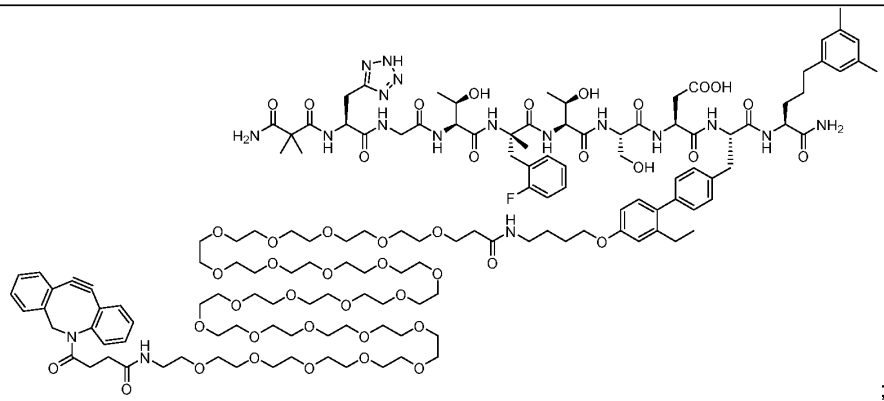
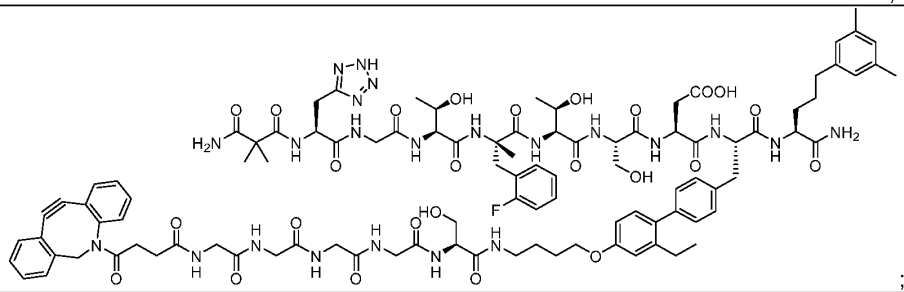
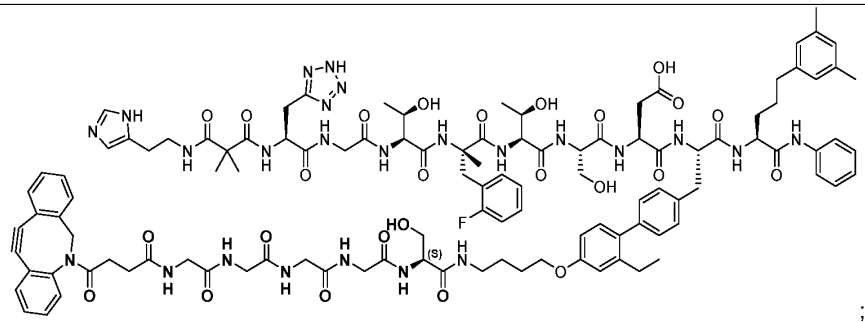
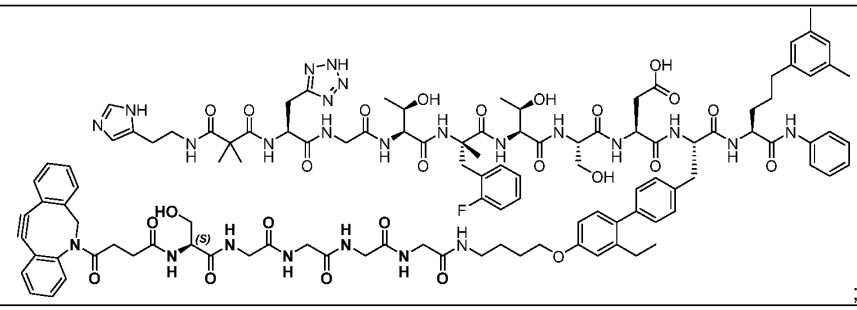
[000263] In one embodiment, the linker-payloads LP according to the present disclosure have the structure selected from the group consisting of:

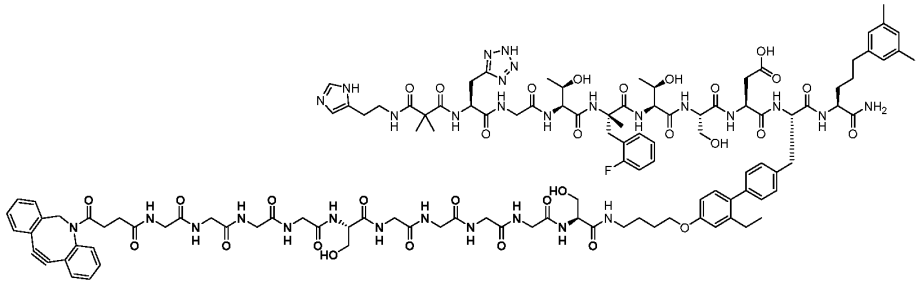
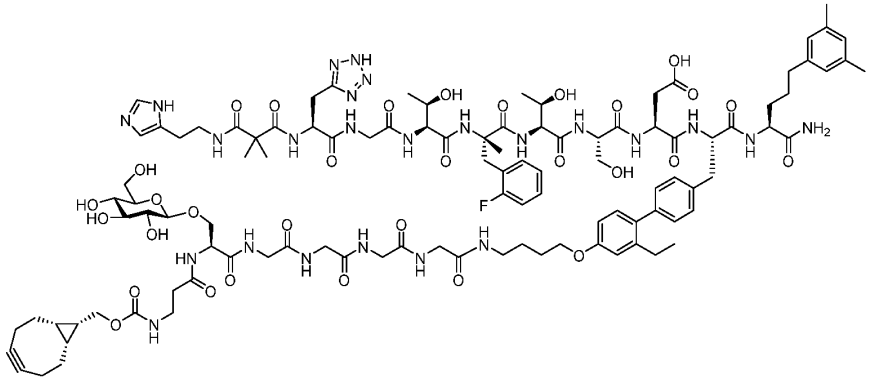
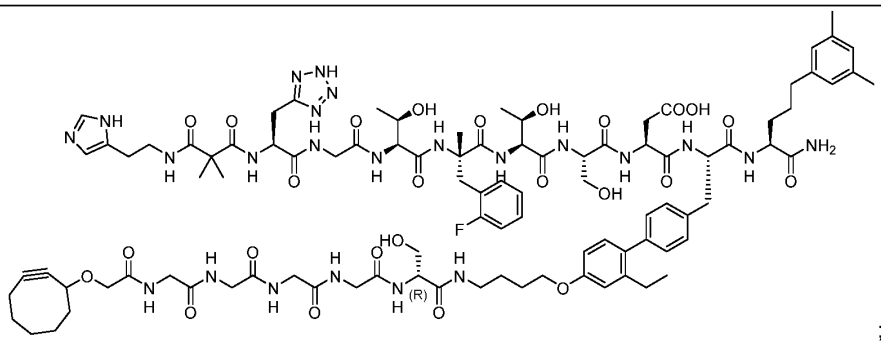
| LP# | Name | Structure |
|---|---|---|
| **LP1** | DIBAC-suc-PEG4- P9 |  ; |

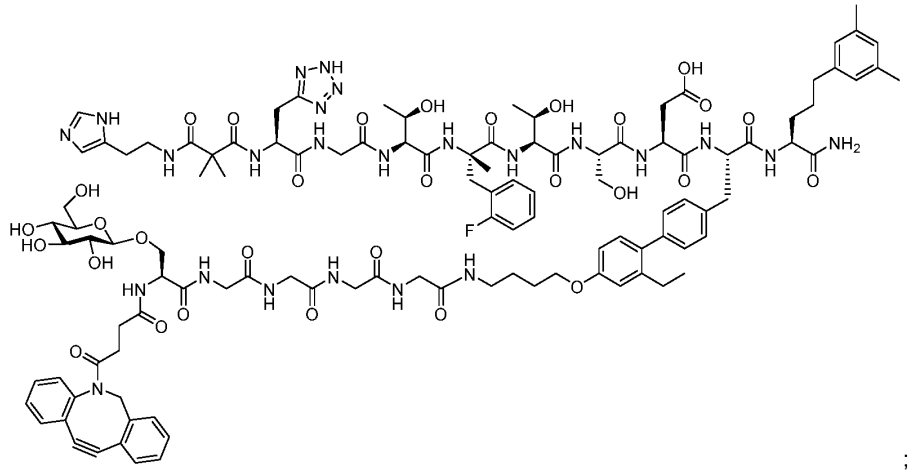
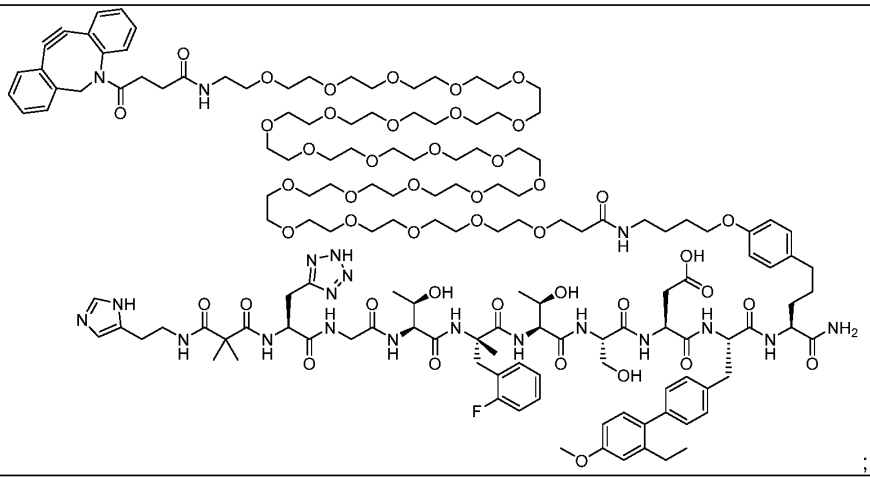
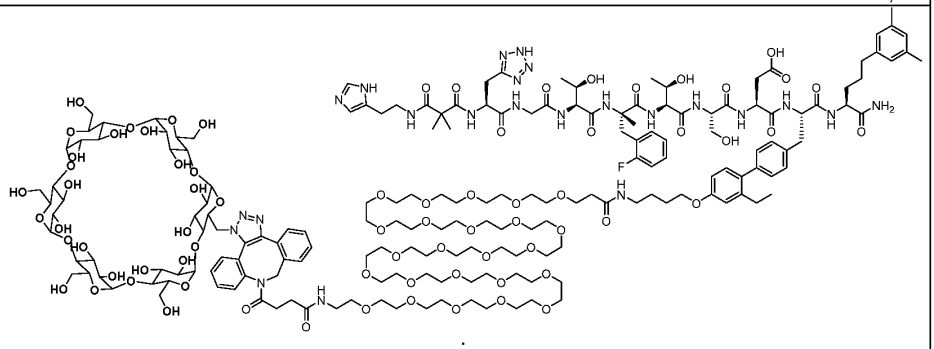
| | | |
|---|---|---|
| **LP2** | DIBAC-suc-PEG8-P9 |  ; |
| **LP3** | DIBAC-suc-PEG12-P9 |  ; |
| **LP4** | DIBAC-suc-PEG24-P9 |  ; |

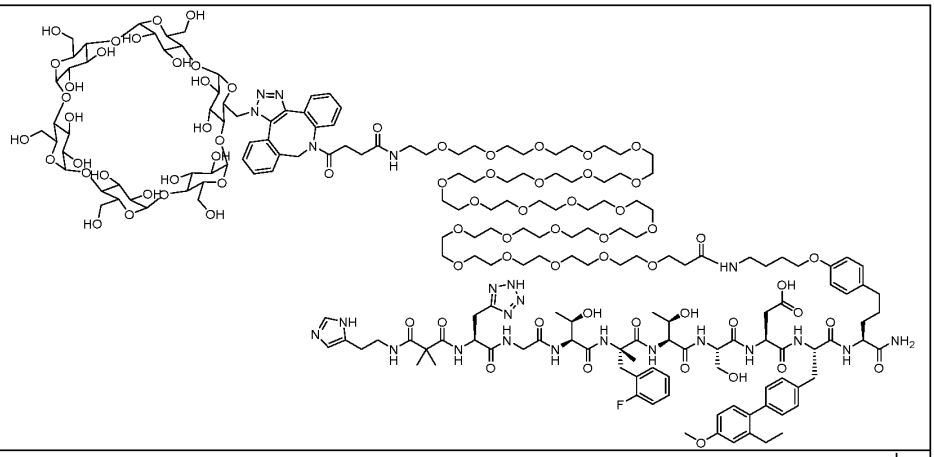
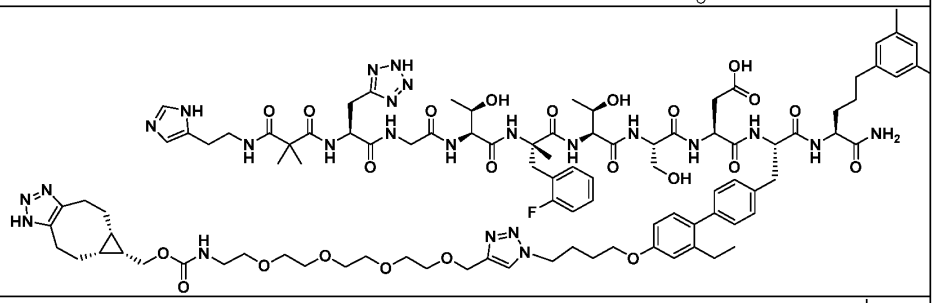
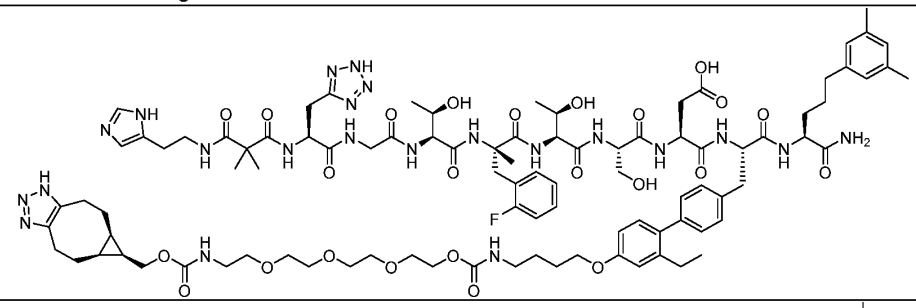
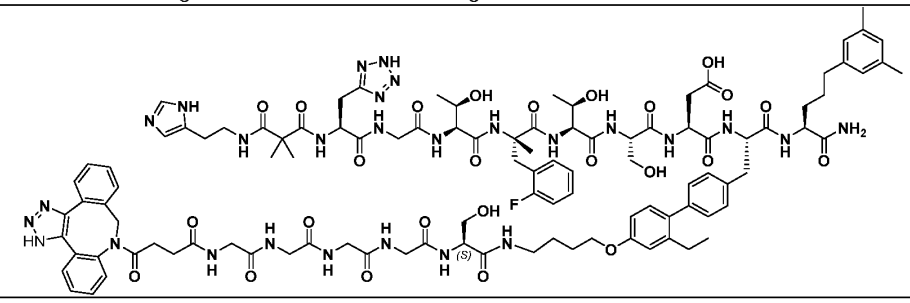
| | | |
|---|---|---|
| **LP5** | BCN-PEG4-carbamate-P9 |  ; |
| **LP6** | DIBAC-suc-G4S-P9 |  ; |
| **LP7** | DIBAC-suc-SG4-P9 |  ; |
| **LP8** | DIBAC-suc-PEG4-triazole-P8 |  ; |

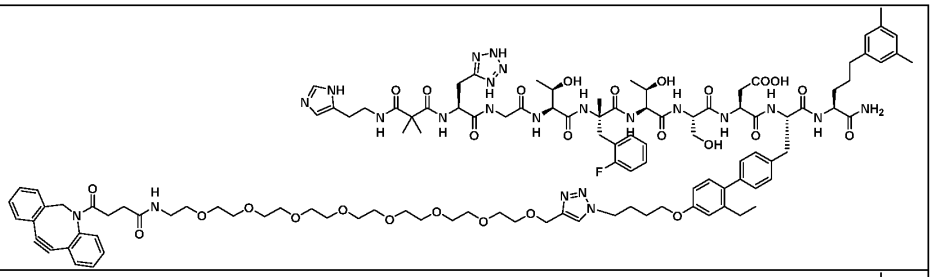
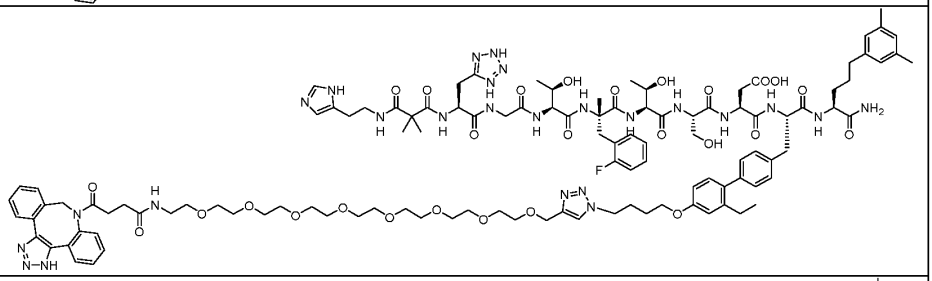
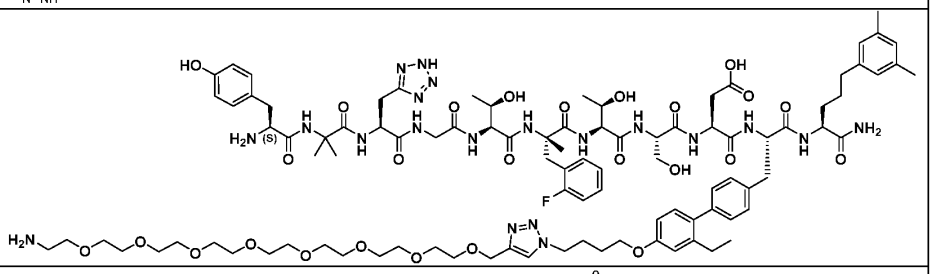
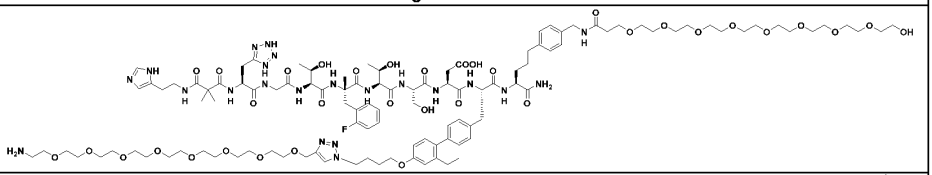
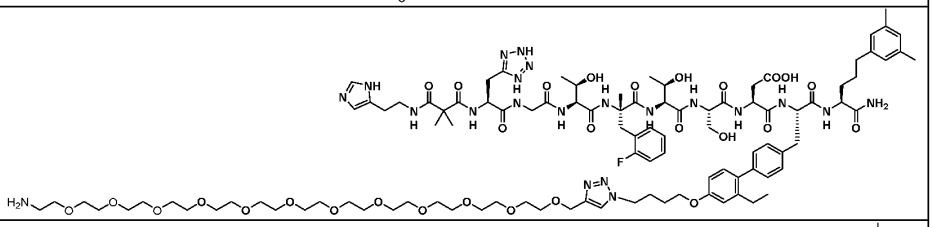
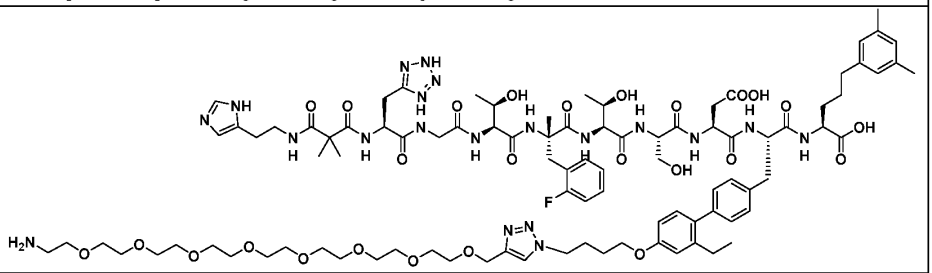
| | | |
|---|---|---|
| **LP9** | BCN-PEG4-triazole-P8 |  ; |
| **LP10** | COT-PEG4-triazole-P8 |  ; |
| **LP11** | NH2-PEG8-triazole-P8 |  ; |
| **LP12** | DIBAC-suc-PEG24-P11 |  ; |

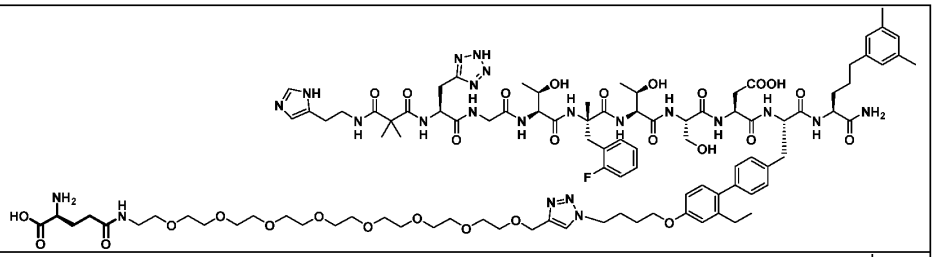
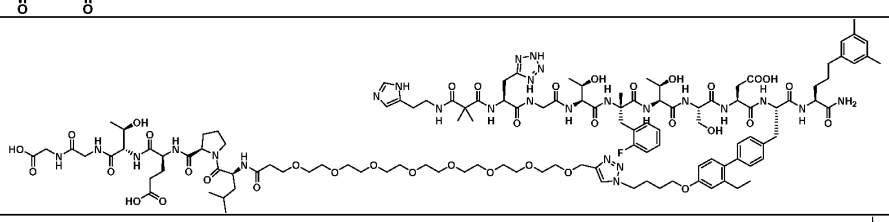
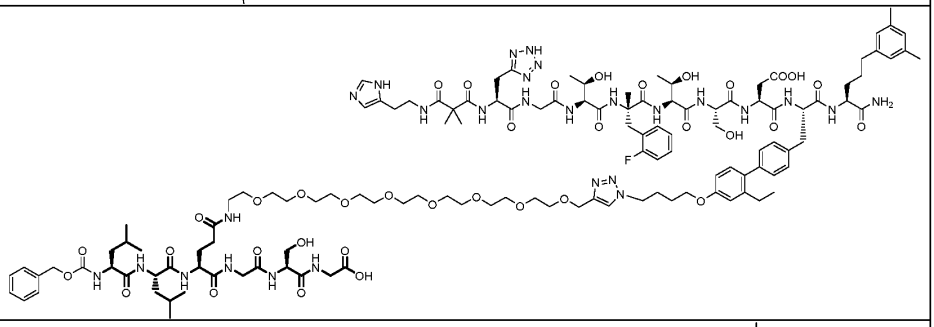
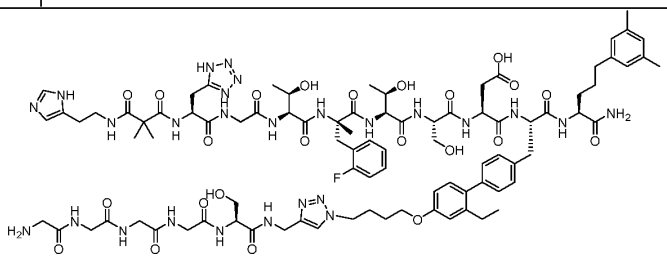
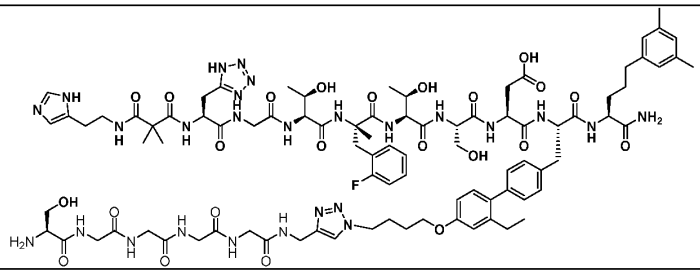
| | | |
|---|---|---|
| **LP13** | DIBAC-suc-PEG24-P4 |  ; |
| **LP14** | DIBAC-suc-G4S-P4 |  ; |
| **LP15** | DIBAC-suc-G4S-P23 |  ; |
| **LP16** | DIBAC-suc-SG4-P23 |  ; |

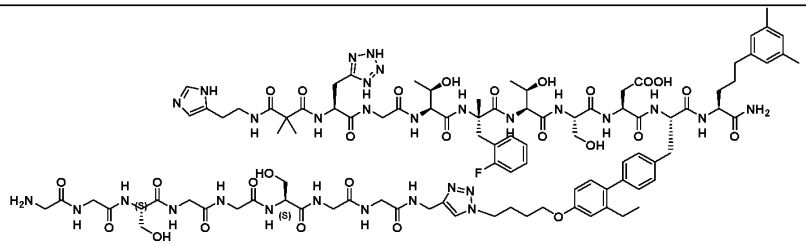
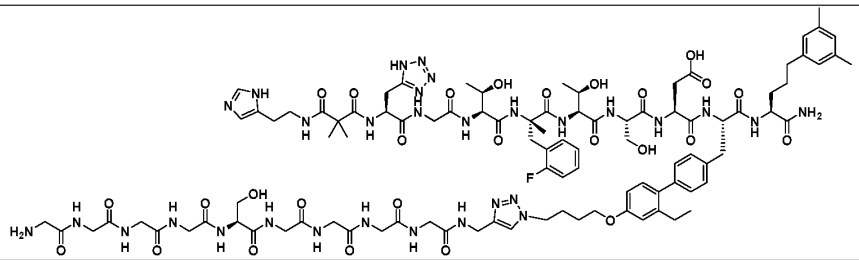
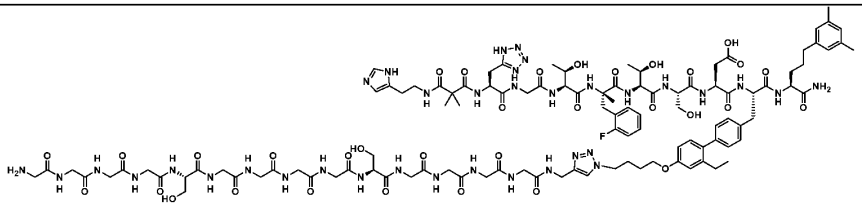
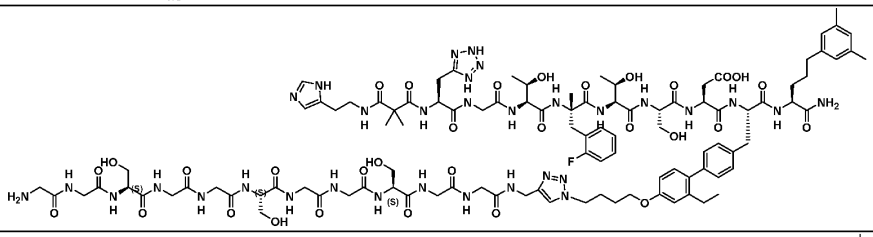
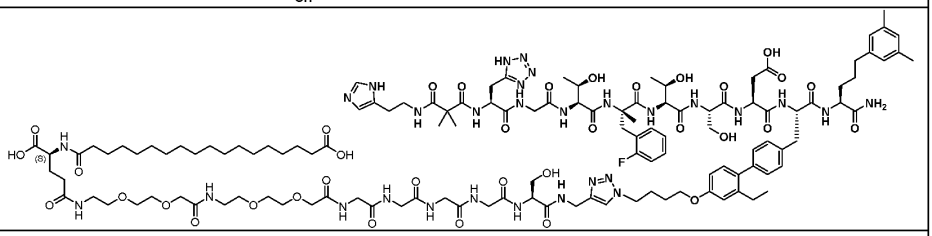
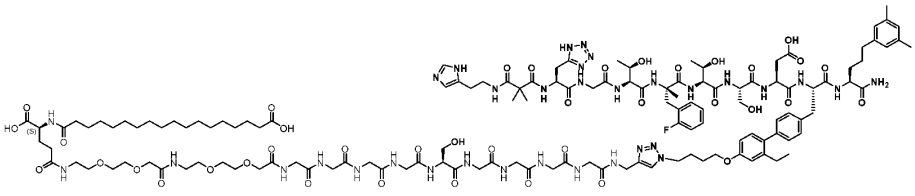
| | | |
|---|---|---|
| **LP17** | DIBAC-suc-$G_4$S-$G_4$S-P9 |  ; |
| **LP18** | BCN-$NHC_2H_4CO$-(glucose)$SG_4$-P9 |  ; |
| **LP19** | COT-$G_4$-(*R*)Ser-P9 |  ; |

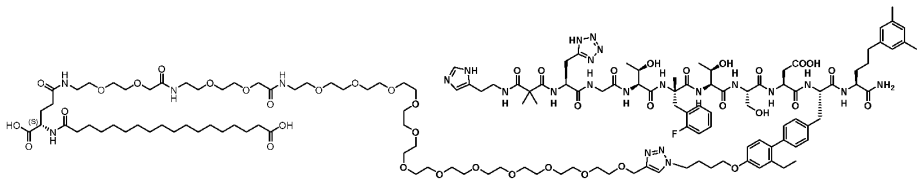
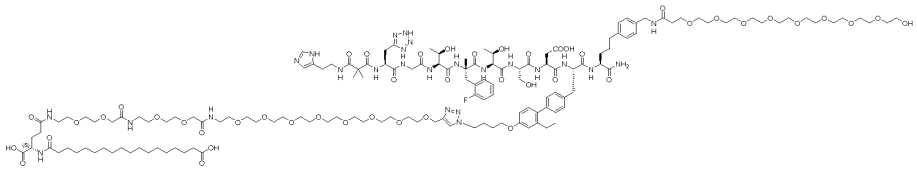
| | | |
|---|---|---|
| **LP20** | DIBAC-suc-(glucose)$SG_4$-P9 |  ; |
| **LP21** | DIBAC-suc-PEG24-P24 |  ; |
| **LP22** | cyclodextrin-triazole-DIBAC-suc-PEG24-P9 |  ; |

| | | |
|---|---|---|
| **LP23** | cyclodextrin-triazole-DIBAC-suc-PEG24-P24 |  |
| **LP24** | triazole-BCN-PEG4-triazole-P8 |  |
| **LP25** | triazole-BCN-PEG4-carbamate-P9 |  |
| **LP26** | triazole-DIBAC-G4S-P9 |  |

| | | |
|---|---|---|
| **LP27** | DIBAC-suc-PEG8-triazole-P8 |  |
| **LP28** | triazole-DIBAC-suc-PEG8-triazole-P8 |  |
| **LP29** | NH2-PEG8-triazole-P19 |  |
| **LP30** | NH2-PEG8-triazole-P35 |  |
| **LP31** | NH2-PEG8-triazole-P8 |  |
| **LP32** | NH2-PEG8-triazole-P8 acid |  |

| | | |
|---|---|---|
| **LP33** | E-PEG8-triazole-P8 |  |
| **LP34** | GGTEPL-PEG8-triazole-P8 |  |
| **LP35** | Cbz-LLQGSG-PEG8-triazole-P8 |  |
| **LP36** | G4S triazole-P40 |  |
| **LP37** | SG4-triazole-P40 |  |

| | | |
|---|---|---|
| **LP38** | G2SG2SG2 triazole-P40 |  |
| **LP39** | G4SG4 triazole-P40 |  |
| **LP40** | G4SG4SG4 triazole-P40 |  |
| **LP41** | G2SG2SG2SG2 triazole-P40 |  |
| **LP42** | C18-diacid-Glu-(AEEA)2-G4SG4-triazole-P40 |  |
| **LP43** | C18-diacid-Glu-(AEEA)2-G4SG4-triazole-P40 |  |

| | | |
|---|---|---|
| **LP44** | C18-diacid-Glu-(AEEA)2-NH2-PEG12-P40 |  |
| **LP45** | C18-diacid-Glu-(AEEA)2-NH2-PEG8-**P35** |  |

[000264] In one embodiment, the linker-payloads as described above have the following properties:

| LP# | Molecular Formula | MW | M/Z 100% (M+H) | RT on HPLC (min) | CLogP | Corresponding Payload |
|---|---|---|---|---|---|---|
| LP1 | $C_{105}H_{135}FN_{20}O_{24}$ | 2080.31 | 1041 $[M+2H]^{2+}$ | 15.16 (A) | 5.55 ± 1.08 | P9 |
| LP2 | $C_{113}H_{151}FN_{20}O_{28}$ | 2256.52 | 1129.2 $[M+2H]^{2+}$<br>753.0 $[M+3H]^{3+}$ | 15.27 (A) | 4.12 ± 1.10 | P9 |
| LP3 | $C_{121}H_{167}FN_{20}O_{32}$ | 2432.73 | 811.8 $[M+3H]^{3+}$<br>1217.2 $[M+2H]^{2+}$ | 15.12 (A) | 2.68 ± 1.12 | P9 |
| LP4 | $C_{145}H_{215}FN_{20}O_{44}$ | 2961.36 | 741.3 $[M+4H]^{4+}$<br>988.3 $[M+3H]^{3+}$ | 14.76 (A) | -1.61 ± 1.17 | P9 |
| LP5 | $C_{95}H_{130}FN_{19}O_{24}$ | 1941.16 | 971.5 $[M+2H]^{2+}$ | 10.12 (D) | 5.48 ± 1.05 | P9 |
| LP6 | $C_{105}H_{131}FN_{24}O_{25}$ | 2148.31 | 716.9 $[M+3H]^{3+}$ | 3.58 (E) | 4.24 ± 1.10 | P9 |
| LP7 | $C_{105}H_{131}FN_{24}O_{25}$ | 2148.31 | 1074.5 $[M+2H]^{2+}$ | 3.55 (E) | 4.24 ± 1.10 | P9 |
| LP8 | $C_{105}H_{133}FN_{22}O_{23}$ | 2090.31 | 1045.5 $[M+2H]^{2+}$ | 2.86 (E) | 5.95 ± 1.47 | P8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| LP9 | $C_{97}H_{132}FN_{21}O_{23}$ | 1979.21 | 990.5 $[M+2H]^{2+}$<br>660.7 $[M+3H]^{3+}$ | 9.89 (D) | 4.59 ± 1.44 | P8 |
| LP10 | $C_{96}H_{132}FN_{21}O_{23}$ | 1967.2 | 984.7 $[M+2H]^{2+}$ | 9.74 (D) | 7.75 ± 1.55 | P8 |
| LP11 | $C_{94}H_{136}FN_{21}O_{25}\cdot 2(CF_3COOH)$ | 2207.26 | 990.6 $[M+2H]^{2+}$<br>660.8 $[M+3H]^{3+}$<br>495.8 $[M+4H]^{4+}$ | 9.40 (B) | 0.52 ± 1.46 | P8 |
| LP12 | $C_{144}H_{214}FN_{19}O_{43}$ | 2918.34 | 974 $[M+3H]^{3+}$<br>1459.6 $[M+2H]^{2+}$ | 2.53 (E) | -0.14 ± 1.16 | P11 |
| LP13 | $C_{140}H_{209}FN_{18}O_{44}$ | 2867.25 | 717.8 $[M+4H]^{4+}$<br>956.9 $[M+3H]^{3+}$<br>1434.7 $[M+2H]^{2+}$ | 3.97 (E) | -1.59 ± 1.16 | P4 |
| LP14 | $C_{100}H_{125}FN_{22}O_{25}$ | 2054.19 | 1028.5 $[M+2H]^{2+}$ | 3.57 (E) | 4.26 ± 1.09 | P4 |
| LP15 | $C_{111}H_{135}FN_{24}O_{25}$ | 2224.4 | 1113.1 $[M+2H]^{2+}$ | 2.76 (E) | 6.67 ± 1.09 | P23 |
| LP16 | $C_{111}H_{135}FN_{24}O_{25}$ | 2224.4 | 1112.9 $[M+2H]^{2+}$ | 3.73 (E) | 6.67 ± 1.09 | P23 |
| LP17 | $C_{116}H_{148}FN_{29}O_{31}$ | 2463.59 | 1232.4 $[M+2H]^{2+}$ | 2.17 (E) | 0.93 ± 1.14 | P9 |
| LP18 | $C_{106}H_{145}FN_{24}O_{31}$ | 2270.43 | 757.7 $[M+3H]^{3+}$<br>1136.0 $[M+2H]^{2+}$ | 8.17 (D) | 0.36 ± 1.09 | P9 |
| LP19 | $C_{96}H_{130}FN_{23}O_{25}$ | 2025.2 | 1013.7 $[M+2H]^{2+}$ | 8.70 (B) | 2.38 ± 1.09 | P9 |
| LP20 | C111H141FN24O30 | 2310.45 | 1156.0 $[M+2H]^{2+}$ | 2.28 (E) | 2.54 ± 1.10 | P9 |
| LP21 | $C_{144}H_{213}FN_{20}O_{45}$ | 2963.34 | 741.6 $[M+4H]^{4+}$<br>988.5 $[M+3H]^{3+}$ | 14.49 (A) | -2.62 ± 1.17 | P24 |
| LP22 | $C_{181}H_{274}FN_{23}O_{73}$ | 3959.22 | 1321.0 $[M+3H]^{3+}$<br>991.3 $[M+4H]^{4+}$ | 11.97 (A) | N/A | P9 |
| LP23 | $C_{180}H_{272}FN_{23}O_{74}$ | 3961.2 | 991.4 $[M+4H]^{4+}$<br>1321.5 $[M+3H]^{3+}$ | 8.14 (D) | N/A | **P24** |

| | | | | | | |
|---|---|---|---|---|---|---|
| LP24 | $C_{97}H_{133}FN_{24}O_{23}$ | 2022.2 | 675.1 $[M+3H]^{3+}$<br>1012.0 $[M+2H]^{2+}$ | 3.56 (F) | 2.82 ± 1.45 | **P8** |
| LP25 | $C_{95}H_{131}FN_{22}O_{24}$ | 1984.2 | 662.4 $[M+3H]^{3+}$<br>1012.0 $[M+2H]^{2+}$ | 3.57 (F) | 3.71 ± 1.43 | **P9** |
| LP26 | $C_{105}H_{132}FN_{27}O_{25}$ | 2191.3 | 1096.7 $[M+2H]^{2+}$ | 3.26 (F) | 2.54 ± 1.53 | **P9** |
| LP27 | C113H149FN22O27 | 2266.5 | 756.03 $[M+3H]^{3+}$ | 9.17 (E) | 4.52 ± 1.49 | **P8** |
| LP28 | C113H150FN25O27. | 2423.57 | 2309.11 $[M+H]^{+}$<br>1155.56<br>$[M+2H]^{2+}$ | 4.20 (F) | 3.81+/- 1.50 | **P8** |
| LP29 | $C_{97}H_{139}FN_{20}O_{26}$. | 2248.3 | 1011.0 $[M+2H]^{2+}$ | 3.73 (F) | 1.90 ± 1.46 | **P19** |
| LP30 | $C_{112}H_{171}FN_{22}O_{35}$. | 2632.7 | 802.20 $[M+3H]^{3+}$ | 6.32 (D) | -5.09 ± 1.51 | **P35** |
| LP31 | $C_{102}H_{152}FN_{21}O_{29}$. | 2383.5 | 1078.6 $[M+2H]^{2+}$ | 7.48 (D) | -0.92 ± 1.48 | **P8** |
| LP32 | $C_{94}H_{135}FN_{20}O_{26}$. | 2208.24 | 991.0 $[M+2H]^{2+}$ | 7.56 (D) | 1.49+/- 1.46 | **P41** |
| LP33 | $C_{99}H_{143}FN_{22}O_{28}$ | 2108.32 | 1054.9 $[M+2H]^{2+}$ | 3.27 (E) | -0.23+/- 1.49 | **P8** |
| LP34 | $C_{119}H_{173}FN_{26}O_{36}$. | 2659.81 | 1282.6 $[M+2H]^{2+}$ | 4.34 (E) | -0.48+/- 1.56 | **P8** |
| LP35 | $C_{126}H_{182}FN_{27}O_{36}$. | 2783.97 | 1335.7 $[M+2H]^{2+}$<br>890.9 $[M+3H]^{3+}$<br>668.4 $[M+4H]^{4+}$ | 7.15 (D) | 2.72+/- 1.54 | **P8** |
| LP36 | C89H121FN26O23. | 2170.12 | 971.9 $[M+2H]^{2+}$ | 6.62 (D) | -0.54+/- 1.47 | **P40** |
| LP37 | $C_{89}H_{121}FN_{26}O_{23}$. | 2170.12 | 971.0 $[M+2H]^{2+}$ | 6.61 (D) | -0.61+/- 1.47 | **P40** |
| LP38 | $C_{96}H_{132}FN_{29}O_{27}$ | 2143.25 | 1072.30$[M+2H]^{2+}$<br>715.30 $[M+3H]^{3+}$ | 8.96 (D) | -2.88+/- 1.49 | **P40** |

| | | | | | | |
|---|---|---|---|---|---|---|
| LP39 | $C_{97}H_{133}FN_{30}O_{27}$ | 2170.28 | 1085.60 $[M+2H]^{2+}$ 725.30 $[M+3H]^{3+}$ | 8.97 (D) | -3.00+/- 1.50 | **P40** |
| LP40 | $C_{108}H_{150}FN_{35}O_{33}$ | 2485.56 | 1243.05 $[M+2H]^{2+}$ 829.03 $[M+3H]^{3+}$ | 6.50 (D) | -6.31+/- 1.55 | **P40** |
| LP41 | $C_{103}H_{143}FN_{32}O_{31}$ | 2344.43 | 1173.21 $[M+2H]^{2+}$ 782.47 $[M+3H]^{3+}$ | 6.54 (D) | -4.96+/- 1.52 | **P40** |
| LP42 | $C_{124}H_{182}FN_{29}O_{35}$ | 2657.94 | 1329.17 $[M+2H]^{2+}$ | 8.53 (D) | 3.04+/- 1.54 | **P40** |
| LP43 | $C_{132}H_{194}FN_{33}O_{39}$ | 2886.15 | 1443.21 [M+2H]2+ | 8.30 (D) | 0.58+/- 1.57 | **P40** |
| LP44 | $C_{137}H_{213}FN_{24}O_{41}$ | 2985.32 | 1436.4 $[M+2H]^{2+}$ | 9.30 (D) | 2.56+/- 1.56 | **P40** |
| LP45 | $C_{147}H_{232}FN_{25}O_{47}$ | 3120.55 | 781.1 $[M+4H]^{4+}$ | 8.14 (D) | -1.88+/- 1.58 | **P35** |

**Binding Agents**

[000265] In one embodiment, the effectiveness of the protein-drug conjugate embodiments described herein depend on the selectivity of the binding agent to bind its binding partner. In one embodiment of the present disclosure, the binding agent is any molecule capable of binding with some specificity to a given binding partner. In one embodiment, the binding agent is within a mammal where the interaction can result in a therapeutic use. In an alternative embodiment, the binding agent is *in vitro* where the interaction can result in a diagnostic use. In some aspects, the binding agent is capable of binding to a cell or cell population.

[000266] Suitable binding agents of the present disclosure include proteins that bind to a binding partner. Suitable binding agents include, but are not limited to, antibodies, lymphokines, hormones, growth factors, viral receptors, interleukins, or any other cell binding or peptide binding molecules or substances.

[000267] In one embodiment the binding agent is an antibody. In certain embodiments, the antibody is selected from monoclonal antibodies, polyclonal antibodies, antibody fragments (Fab, Fab', and F(ab)2, minibodies, diabodies, tribodies, and the like). Antibodies herein can be

humanized using methods described in US Patent No. 6,596,541 and US Publication No. 2012/0096572, each incorporated by reference in their entirety. In certain embodiments of the protein-drug conjugate compounds of the present disclosure, BA is a humanized monoclonal antibody. For example, BA can be a monoclonal antibody that binds GLP1R.

[000268] In the present disclosure, the antibody can be any antibody deemed suitable to the practitioner of skill. In some embodiments, a linker or linker-payload is attached to one or both heavy chains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to one or both heavy chain variable domains of the antibody or antigen-binding fragment thereof.

[000269] In some embodiments, a linker or linker-payload is attached to the N-terminus of one or both heavy chain variable domains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to the N-terminus of both heavy chain variable domains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to one or both light chains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to one or both light chain variable domains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to the N-terminus of one or both light chain variable domains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to the N-terminus of both light chain variable domains of the antibody or antigen-binding fragment thereof.

[000270] In some embodiments, a linker or linker-payload is attached to the C-terminus of one or both heavy chain variable domains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to the C-terminus of both heavy chain variable domains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to one or both light chains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to one or both light chain variable domains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to the C-terminus of one or both light chain variable domains of the antibody or antigen-binding fragment thereof. In some embodiments, a linker or linker-payload is attached to the C-terminus of both light chain variable domains of the antibody or antigen-binding fragment thereof.

[000271] In some embodiments, the antibody comprises at least one glutamine residue in at least one polypeptide chain sequence. In certain embodiments, the antibody comprises one or

more Gln295 residues. In certain embodiments, the antibody comprises two heavy chain polypeptides, each with one Gln295 residue. In further embodiments, the antibody comprises one or more glutamine residues at a site other than a heavy chain 295. Such antibodies can be isolated from natural sources or engineered to comprise one or more glutamine residues. Techniques for engineering glutamine residues into an antibody polypeptide chain are within the skill of the practitioners in the art. In certain embodiments, a glutamine residue is introduced to the N-terminus of an antibody polypeptide chain. In one embodiment, a glutamine residue is introduced to the N-terminus of one or both heavy chains of the antibody. In one embodiment, a glutamine residue is introduced to the N-terminus of both heavy chains of the antibody. In another embodiment, the glutamine residue is introduced to the N-terminus of one or both light chains of the antibody. In one embodiment, a glutamine residue is introduced to the N-terminus of both light chains of the antibody. In another embodiment, a glutamine residue is introduced to the N-terminus of one or both heavy chains and one or both light chains of the antibody.

[000272] In certain embodiments, a glutamine residue is introduced to the C-terminus of an antibody polypeptide chain. In one embodiment, a glutamine residue is introduced to the C-terminus of one or both heavy chains of the antibody. In one embodiment, a glutamine residue is introduced to the C-terminus of both heavy chains of the antibody. In another embodiment, the glutamine residue is introduced to the C-terminus of one or both light chains of the antibody. In one embodiment, a glutamine residue is introduced to the C-terminus of both light chains of the antibody. In another embodiment, a glutamine residue is introduced to the C-terminus of one or both heavy chains and one or both light chains of the antibody.

[000273] In certain embodiments, the antibody or antigen-binding fragment thereof has been modified to comprise a TGase recognition tag. Suitable TGase recognition tags include those described herein.

[000274] In certain embodiments, the antibody or antigen-binding fragment thereof has been modified to comprise a Q-tag at the N-terminus of one or both antibody light chains. In certain embodiments, the antibody or antigen-binding fragment thereof has been modified to comprise a Q-tag at the N-terminus of both antibody light chains.

[000275] In certain embodiments, the antibody or antigen-binding fragment thereof has been modified to comprise a Q-tag at the N-terminus of one or both antibody heavy chains. In certain embodiments, the antibody or antigen-binding fragment thereof has been modified to comprise a Q-tag at the N-terminus of both antibody heavy chains.

[000276] In certain embodiments, the antibody or antigen-binding fragment thereof has been modified to comprise a Q-tag at the C-terminus of one or both antibody light chains. In certain embodiments, the antibody or antigen-binding fragment thereof has been modified to comprise a Q-tag at the C-terminus of both antibody light chains.

[000277] In certain embodiments, the antibody or antigen-binding fragment thereof has been modified to comprise a Q-tag at the C-terminus of one or both antibody heavy chains. In certain embodiments, the antibody or antigen-binding fragment thereof has been modified to comprise a Q-tag at the C-terminus of both antibody heavy chains.

[000278] In certain embodiments, the antibody or antigen-binding fragment thereof is aglycosylated. In certain embodiments, the antibody antigen-binding fragment thereof is deglycosylated. In certain embodiments, the antibody antigen-binding fragment is a Fab fragment.

[000279] The antibody can be in any form known to those of skill in the art. In certain embodiments, the antibody comprises a light chain. In certain embodiments, the light chain is a kappa light chain. In certain embodiments, the light chain is a lambda light chain.

[000280] In certain embodiments, the antibody comprises a heavy chain. In some aspects, the heavy chain is an IgA. In some aspects, the heavy chain is an IgD. In some aspects, the heavy chain is an IgE. In some aspects, the heavy chain is an IgG. In some aspects, the heavy chain is an IgM. In some aspects, the heavy chain is an IgG1. In some aspects, the heavy chain is an IgG2. In some aspects, the heavy chain is an IgG3. In some aspects, the heavy chain is an IgG4. In some aspects, the heavy chain is an IgA1. In some aspects, the heavy chain is an IgA2.

[000281] In some embodiments, the antibody is an antibody fragment. In some aspects, the antibody fragment is an Fv fragment. In some aspects, the antibody fragment is a Fab fragment. In some aspects, the antibody fragment is a F(ab')2 fragment. In some aspects, the antibody fragment is a Fab' fragment. In some aspects, the antibody fragment is an scFv (sFv) fragment. In some aspects, the antibody fragment is an scFv-Fc fragment.

[000282] In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a polyclonal antibody.

[000283] In some embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a human antibody.

[000284] The antibody can have binding specificity for any antigen deemed suitable to those of skill in the art. In certain embodiments, the antigen is a transmembrane molecule (e.g., receptor) or a growth factor. Exemplary antigens include, but are not limited to, molecules such as GLP1R.

[000285] Some embodiments herein are target specific for therapeutic or diagnostic use.

[000286] In some embodiments, the binding agent is an anti-glucagon-like peptide 1 receptor (GLP1R), i.e., an anti-GLP1R antibody, or an antigen-binding fragment thereof. In some embodiments, the binding agent is an anti-GLP1R agonist antibody, or an antigen-binding fragment thereof.

[000287] In some embodiments, suitable anti-GLP1R antibodies are those disclosed in US Publication No. US20060275288A1, which is incorporated herein by reference in its entirety. Exemplary anti-GLP1R antibodies according to the present disclosure include 5A10 and 9A10.

[000288] In some embodiments, suitable anti-GLP1R antibodies include, but not limited to, AB9433-I, h38C2, PA5-111834, NLS1205, MAB2814, EPR21819, and glutazumab.

[000289] The present disclosure also provides nucleic acid molecules encoding anti-GLP1R antibodies or portions thereof. Also included within the scope of the present disclosure are recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-GLP1R antibody. For example, the present disclosure includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above. Further included within the scope of the present disclosure are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

[000290] In some embodiments, the present disclosure includes anti-GLP1R antibodies having a modified glycosylation pattern. In some embodiments, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[000291] In another aspect, the disclosure provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds GLP1R and a pharmaceutically acceptable carrier. In a related aspect, the disclosure includes a composition which is a combination of an anti-GLP1R antibody and a second therapeutic agent. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with an anti-GLP1R antibody. Additional combination therapies and co-formulations involving the anti-GLP1R antibodies of the present disclosure are disclosed elsewhere herein.

[000292] In another aspect, the disclosure provides therapeutic methods for targeting cells expressing GLP1R using an anti-GLP1R antibody of the disclosure, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-GLP1R antibody of the disclosure to a subject in need thereof. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is a pancreatic cell, a brain cell, a heart cell, a vascular tissue cell, a kidney cell, an adipose tissue cell, a liver cell, or a muscle cell. In one embodiment, the cell is a pancreatic cell or a brain cell.

[000293] In some cases, the anti-GLP1R antibodies (or antigen-binding fragments thereof) can be used to enhance GLP1R activity in the cells.

[000294] The present disclosure also includes the use of an anti-GLP1R antibody of the disclosure in the manufacture of a medicament for the treatment of a disease or disorder (e.g., type II diabetes and/or obesity) associated with GLP1R-expressing cells. In one aspect, the disclosure relates to a compound comprising an anti-GLP1R antibody or antigen-binding fragment, as disclosed herein, for use in medicine. In one aspect, the disclosure relates to a compound comprising an antibody-drug conjugate (ADC) as disclosed herein, for use in medicine.

[000295] In yet another aspect, the disclosure provides monospecific anti-GLP1R antibodies for diagnostic applications, such as, e.g., imaging reagents.

[000296] The disclosure further includes an antibody or antigen-binding fragment that competes for binding to human GLP1R with an antibody described herein.

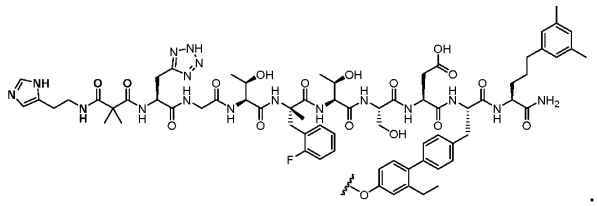
[000297] The disclosure furthermore includes an antibody or antigen-binding fragment, wherein the antibody or antigen-binding fragment thereof binds to the same epitope on human GLP1R as an antibody described herein.

[000298] In one aspect, the present disclosure provides antibody-drug conjugates comprising an anti-GLP1R antibody or antigen-binding fragment thereof as described above and a therapeutic agent (e.g., a GLP1 peptidomimetic). In some embodiments, the antibody or antigen-binding fragment and the payload are covalently attached via a linker, as discussed above. In various embodiments, the anti-GLP1R antibody or antigen-binding fragment can be any of the anti-GLP1R antibodies or fragments described herein.In some embodiments, the antibody-drug conjugates of the present disclosure are stable in plasma. Plasma stability may be determined using an *in vitro* or *in vivo* plasma stablity assay, such as those set forth in Example 8.2 or Example 10 below. In some embodiments, the antibody-drug conjugates of the present disclosure have a half life of longer than 4 days, longer than 5 days, longer than 6 days, longer

than 7 days, longer than 8 days, longer than 9 days, longer than 10 days, longer than 11 days, longer than 12 days, longer than 13 days, longer than 2 weeks, longer than 3 weeks, longer than 4 weeks, about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 month, about 3 month, about 4 month, about 5 month, about 6 month, between 5-10 days, between 8-12 days, between 10-15 days, between 12-18 days, between 15-20 days, between 20-30 days, between 1-2 weeks, between 2-3 weeks, between 3-4 weeks, between 4-6 weeks, between 5-8 weeks, between 6-10 weeks, between 1-2 months, between 1.5-3 months, between 2-4 months, between 2.5-5 months, between 3-6 months, or between 4-6 months in plasma.

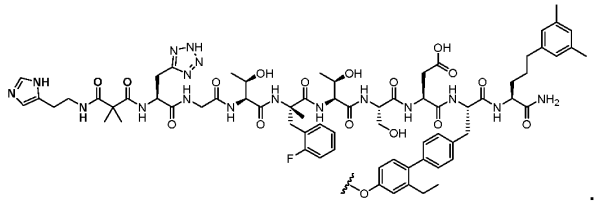
[000299] In some embodiments, the antibody-drug conjugates of the present disclosure bind to GLP1R with at least a 10-fold greater affinity than other G protein-coupled receptors (GPCRs). In some embodiments, the antibody-drug conjugates of the present disclosure bind to GLP1R with at least a 20-fold, 50-fold,100-fold, 500-fold, 1000-fold, 10,000-fold greater affinity than other G protein-coupled receptors (GPCRs). In some embodiments, such antibody-drug conjugates exhibit essentially undetectable binding against GPCRs other than GLP1R. Binding of the antibody-drug conjugates to a target molecule can be measured using a standard binding assay available in the relevant art, such as luciferase reporter assay, surface plasmon resonance assay, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, or Western blot assay. Examples of GPCRs other than GLP1R include, but are not limited to, GIPR, GLP2R and GCGR.

[000300] In certain embodiments, an antibody drug conjugate of the present disclosure comprises an anti-GLP1R antibody, or antigen-binding fragment thereof, conjugated with a linker payload, wherein the linker payload is attached to the antibody, or antigen-binding fragment thereof, at the N-terminus of a light chain. In one embodiment, an antibody drug conjugate of the present disclosure comprises an anti-GLP1R antibody, or antigen-binding fragment thereof, conjugated at the N-terminus of a light chain with a linker payload, wherein the payload has the following structure:



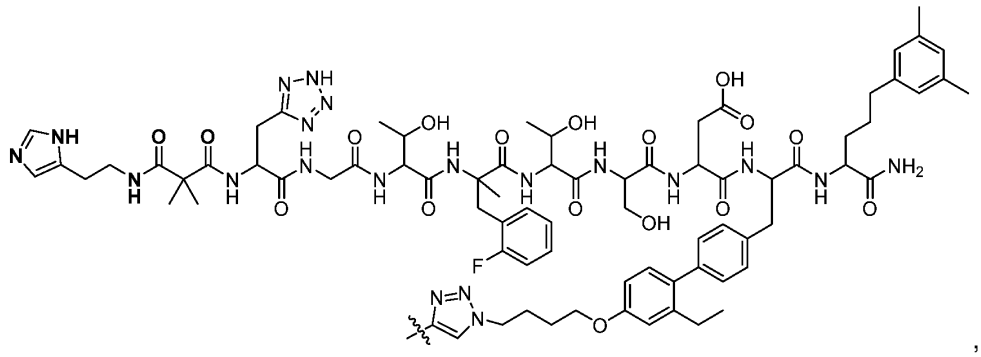
[000301] In certain embodiments, an antibody drug conjugate of the present disclosure comprises an anti-GLP1R antibody, or antigen-binding fragment thereof, conjugated with two

linker payloads, wherein each linker payload is attached to the antibody, or antigen-binding fragment thereof, at the N-terminus of a light chain. In one embodiment, an antibody drug conjugate of the present disclosure comprises an anti-GLP1R antibody, or antigen-binding fragment thereof, conjugated at the N-terminus of each light chain with a linker payload for a total of two linker payloads per each antibody, wherein the payload has the following structure:



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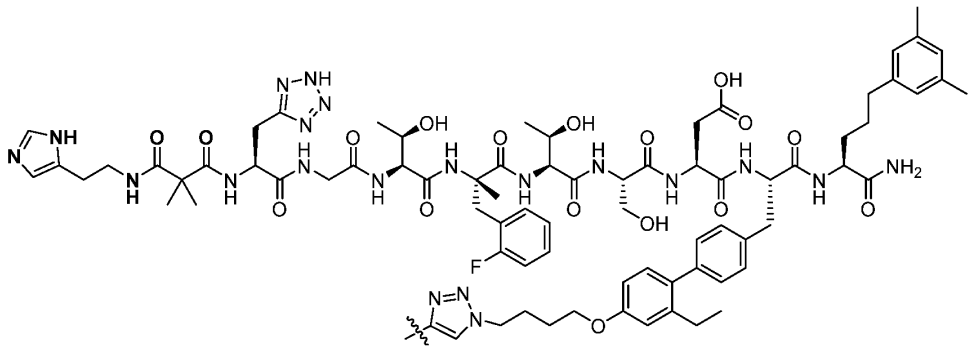
[000302] In yet another aspect, provided herein is an antibody-drug conjugate comprising a Glucagon-like peptide-1 receptor (GLP1R)-targeting antibody or an antigen-binding fragment thereof and a payload having the structure:



,

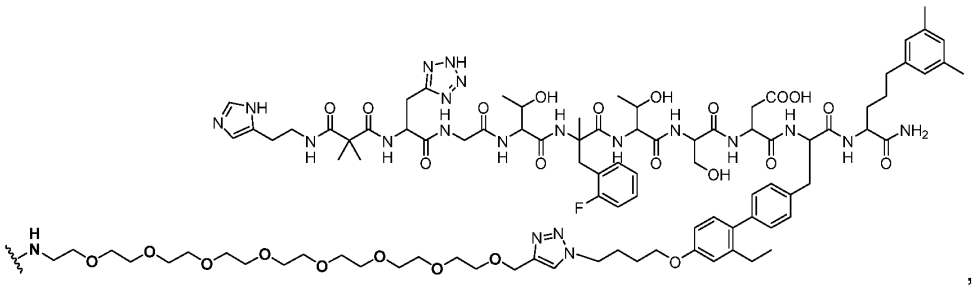
wherein  is the point of attachment of the payload to the antibody or the antigen-binding fragment thereof directly or through a linker.

[000303] In one embodiment, the payload has the structure:



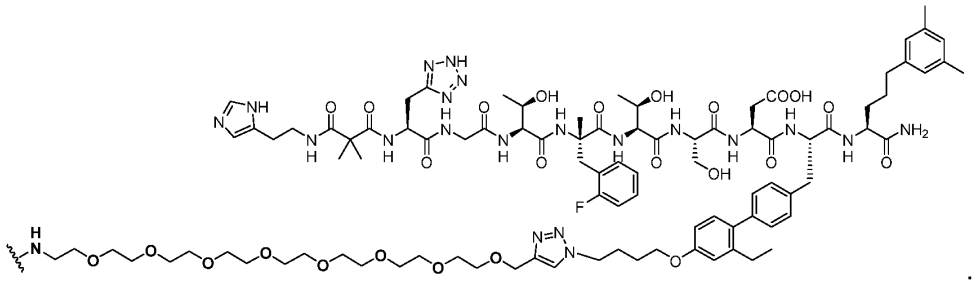
.

[000304] In yet another aspect, provided herein is an antibody-drug conjugate comprising a Glucagon-like peptide-1 receptor (GLP1R)-targeting antibody or an antigen-binding fragment thereof and a linker-payload having the structure:



wherein —§— is the point of attachment of the linker-payload to the antibody or the antigen-binding fragment thereof.

[000305] In one embodiments, the linker-payload has the structure:



**Antibody conjugation**

[000306] Techniques and linkers for conjugating to residues of an antibody or antigen binding fragment are known in the art. Exemplary amino acid attachments that can be used in the context of this aspect, *e.g.*, lysine (*see*, *e.g.*, US 5,208,020; US 2010/0129314; Hollander *et al.*, *Bioconjugate Chem.*, 2008, 19:358-361; WO 2005/089808; US 5,714,586; US 2013/0101546; and US 2012/0585592), cysteine (*see*, *e.g.*, US 2007/0258987; WO 2013/055993; WO 2013/055990; WO 2013/053873; WO 2013/053872; WO 2011/130598; US 2013/0101546; and US 7,750,116), selenocysteine (*see*, *e.g.*, WO 2008/122039; and Hofer *et al.*, *Proc. Natl. Acad. Sci., USA, 2008, 105*:12451-12456), formyl glycine (*see*, *e.g.*, Carrico *et al.*, *Nat. Chem. Biol.*, 2007, 3:321-322; Agarwal *et al.*, *Proc. Natl. Acad. Sci., USA, 2013, 110*:46-51, and Rabuka *et al.*, *Nat. Protocols*, 2012, *10*:1052-1067), non-natural amino acids (*see*, *e.g.*, WO 2013/068874, and WO 2012/166559), and acidic amino acids (*see*, *e.g.*, WO 2012/05982). Lysine conjugation can also proceed through NHS (N-hydroxy succinimide). Linkers can also be conjugated to cysteine

residues, including cysteine residues of a cleaved interchain disulfide bond, by forming a carbon bridge between thiols (see, e.g., US 9,951,141, and US 9,950,076). Linkers can also be conjugated to an antigen-binding protein via attachment to carbohydrates (*see, e.g.*, US 2008/0305497, WO 2014/065661, and Ryan *et al.*, *Food & Agriculture Immunol.*, 2001, *13*:127-130) and disulfide linkers (*see, e.g.*, WO 2013/085925, WO 2010/010324, WO 2011/018611, and Shaunak *et al.*, *Nat. Chem. Biol.*, 2006, *2*:312-313). Site specific conjugation techniques can also be employed to direct conjugation to particular residues of the antibody or antigen binding protein (*see, e.g.*, Schumacher *et al. J Clin Immunol* (2016) 36 (Suppl 1): 100). In specific embodiments discussed in more detail below, Site specific conjugation techniques, include glutamine conjugation via transglutaminase (*see e.g.*, Schibli, Angew Chemie Inter Ed. 2010, 49 ,9995).

[000307] Payloads according to the disclosure linked through lysine and/or cysteine, e.g., via a maleimide or amide conjugation, are included within the scope of the present disclosure.

[000308] In some embodiments, the protein-drug conjugates of the present disclosure are produced according to a two-step process, where Step 1 is lysine-based linker conjugation, e.g., with an NHS-ester linker, and Step 2 is a payload conjugation reaction (e.g., a 1,3-cycloaddition reaction).

[000309] In some embodiments, the protein-drug conjugates of the present disclosure are produced according to a two-step process, where Step 1 is cysteine-based linker conjugation, e.g., with a maleimide linker, and Step 2 is a payload conjugation reaction (e.g., a 1,3-cycloaddition reaction).

[000310] In some embodiments, the protein-drug conjugates of the present disclosure are produced according to a two-step process, where Step 1 is transglutaminase-mediated site specific conjugation and Step 2 is a payload conjugation reaction (e.g., a 1,3-cycloaddition reaction).

**Step 1: Transglutaminase Mediated Site Specific Conjugation**

[000311] In some embodiments, proteins (e.g., antibodies) may be modified in accordance with known methods to provide glutaminyl modified proteins. Techniques for conjugating antibodies and primary amine compounds are known in the art. Site specific conjugation techniques are employed herein to direct conjugation to glutamine using glutamine conjugation via transglutaminase (*see e.g.*, Schibli, *Angew Chemie* Inter Ed. 2010, 49, 9995).

[000312] Primary amine-comprising compounds (e.g., linkers La) of the present disclosure can be conjugated to one or more glutamine residues of a binding agent (e.g., a protein, e.g., an antibody, e.g., an anti-GLP1R antibody) via transglutaminase-based chemo-enzymatic

conjugation (*see, e.g.,* Dennler et al., *Protein Conjugate Chem.* 2014, 25, 569-578, and WO 2017/147542). For example, in the presence of transglutaminase, one or more glutamine residues of an antibody can be coupled to a primary amine linker compound. Briefly, in some embodiments, a binding agent having a glutamine residue (*e.g.*, a Gln295, i.e. Q295 residue) is treated with a primary amine-containing linker La in the presence of the enzyme transglutaminase. In certain embodiments, the binding agent is aglycosylated. In certain embodiments, the binding agent is deglycosylated.

[000313] In certain embodiments, the binding agent (e.g., a protein, e.g., an antibody) comprises at least one glutamine residue in at least one polypeptide chain sequence. In certain embodiments, the binding agent comprises two heavy chain polypeptides, each with one Gln295 residue. In further embodiments, the binding agent comprises one or more glutamine residues at a site other than a heavy chain 295.

[000314] In some embodiments, a binding agent, such as an antibody, can be prepared by site-directed mutagenesis to insert a glutamine residue at a site without resulting in disabled antibody function or binding. For example, included herein are antibodies bearing Asn297Gln (N297Q) mutation(s) as described herein. In some embodiments, an antibody having a Gln295 residue and/or an N297Q mutation contains one or more additional naturally occurring glutamine residues in their variable regions, which can be accessible to transglutaminase and therefore capable of conjugation to a linker or a linker-payload. An exemplary naturally occurring glutamine residue can be found, *e.g.*, at Q55 of the light chain. In such instances, the binding agent, e.g., antibody, conjugated via transglutaminase can have a higher than expected LAR value (*e.g.*, a LAR higher than 4). Any such antibodies can be isolated from natural or artificial sources.

**Step 2: Payload Conjugation Reaction**

[000315] In certain embodiments, linkers La according to the present disclosure comprise at least one first reactive group capable of further reaction after transglutamination. In these embodiments, the glutaminyl-modified protein (e.g., antibody) is capable of further reaction with a reactive payload compound P or a reactive linker-payload compound (e.g., Lp-P as disclosed herein), to form a protein-payload conjugate. More specifically, the reactive linker-payload compound Lp-P may comprise a second reactive group that is capable of reacting with the first reactive group of the linker La. In certain embodiments, a first or second reactive group according to the present disclosure comprises a moiety that is capable of undergoing a 1,3-cycloaddition reaction. In certain embodiments, the reactive group is an azide. In certain embodiments, the reactive group comprises an alkyne (e.g., a terminal alkyne, or an internal strained alkyne). In

certain embodiments of the present disclosure the reactive group is compatible with the binding agent and transglutamination reaction conditions.

[000316] In certain embodiments of the disclosure, linker La molecules comprise a first reactive group. In certain embodiments of the disclosure, linker La molecules comprise more than one reactive group.

[000317] In certain embodiments, the reactive linker-payload Lp-P comprises one payload molecule (n = 1). In certain other embodiments, the reactive linker-payload Lp-P comprises two or more payload molecules (n ≥ 2).

[000318] In certain embodiments of the disclosure, the drug-antibody ratio or DAR is from about 1 to about 30, or from about 1 to about 24, or from about 1 to about 20, or from about 1 to about 16, or from about 1 to about 12, or from about 1 to about 10, or from about 1 to about 8, or about 1, 2, 3, 4, 5, 6, 7, or 8 payload molecules per antibody. In some embodiments, the DAR is from 1 to 30. In some embodiments, the DAR is from 1 to 16. In some embodiments, the DAR is from 1 to 8. In some embodiments, the DAR is from 1 to 6. In certain embodiments, the DAR is from 2 to 4. In some cases, the DAR is from 2 to 3. In certain cases, the DAR is from 0.5 to 3.5. In some embodiments, the DAR is about 1, or about 1.5, or about 2, or about 2.5, or about 3, or about 3.5. In some embodiments, the DAR is 2. In some embodiments, the DAR is 4. In some embodiments, the DAR is 8.

[000319] In one aspect, the present disclosure provides a method of producing the compound having a structure of Formula (A):

$$BA\text{-}(L\text{-}P)_m \quad (A),$$

the method comprising the steps of:

a) contacting, in the presence of a transglutaminase, the BA comprising at least m glutamine residues Gln with at least m equivalents of compound L-P, and

b) isolating the produced compound of Formula (A).

[000320] In one aspect, the present disclosure provides a method of producing the compound having a structure of Formula (A):

$$BA\text{-}(L\text{-}P)_m \quad (A),$$

wherein the linker L has has the structure of formula (L'):

$$\text{-La-Y-Lp-} \quad (L'),$$

wherein La is a first linker covalently attached to the BA;

Y is a group comprising a triazole, and

Lp is a second linker covalently attached to the P,

the method comprising the steps of:

a) contacting, in the presence of a transglutaminase, the BA comprising at least m glutamine residues Gln with the first linker La comprising an azide or an alkyne moiety;

b) contacting the product of step a) with at least m equivalents of compound Lp-P, wherein the second linker Lp comprises an azide or an alkyne moiety, wherein La and Lp are capable of reacting to produce a triazole, and

c) isolating the produced compound of Formula (A).

[000321] In one aspect, the present disclosure provides a method of producing a compound having a structure according to Formula (I):

BA-L-P (I),

the method comprising the steps of:

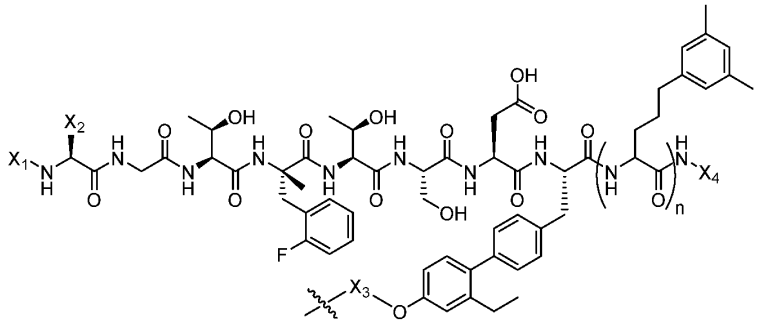
a) contacting, in the presence of a transglutaminase, the BA comprising at least one glutamine residue Gln with a compound L-P, and

b) isolating the produced compound of Formula (I),

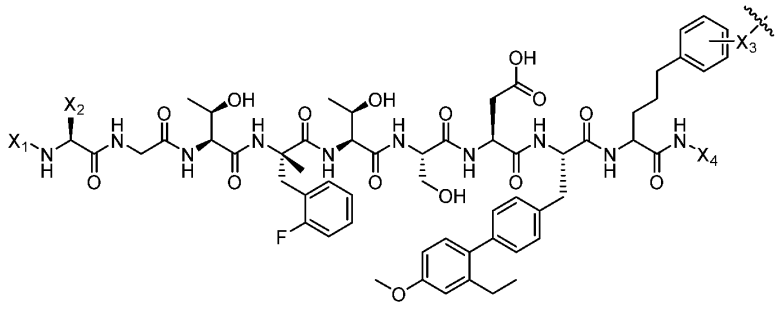
wherein BA is an antibody or an antigen-binding fragment thereof;

L is a non-cleavable linker;

P is a payload having the structure selected from the group consisting of:

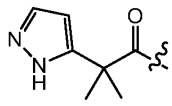
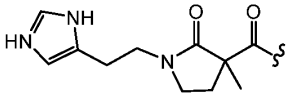
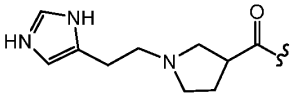


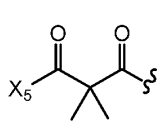
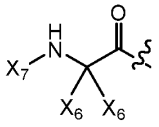
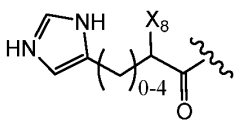
(P1) and

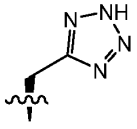
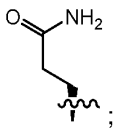


(P2),

wherein —≈— is the point of attachment of the payload to L;

$X_1$ is selected from H;  ;  ;  ;

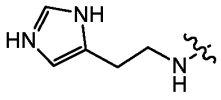
 ;  ; and  ;

$X_2$ is selected from  and  ;

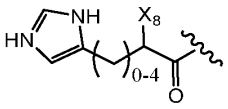
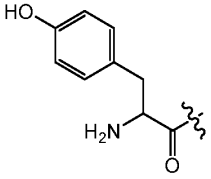
$X_3$ is selected from $–(CH_2)_{2\text{-}6}–NH–$ and $–(CH_2)_{2\text{-}6}–Tr–$, where Tr is a triazole moiety;

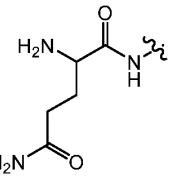
n is 0 or 1;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  , and  ;



$X_8$ is selected from H, -OH, $-NH_2$, and , or a pharmaceutically acceptable salt thereof.

[000322] In another aspect, the present disclosure provides a method of producing a compound having a structure according to Formula (I):

BA-L-P (I),

wherein the linker L has has the structure of formula (L'):

-La-Y-Lp- (L'),

wherein La is a first linker covalently attached to the BA;

Y is a group comprising a triazole, a Diels-Alder adduct, or a thio-maleimide adduct, and

Lp is a second linker covalently attached to the P,

the method comprising the steps of:

a) contacting, in the presence of a transglutaminase, the BA comprising at least one glutamine residue Gln with the first linker La comprising an azide or an alkyne moiety;

b) contacting the product of step a) with a compound Lp-P, wherein the second linker Lp comprises an azide or an alkyne moiety, wherein La and Lp are capable of reacting to produce a triazole, and

c) isolating the produced compound of Formula (I),

wherein BA, L', and P are as defined above.

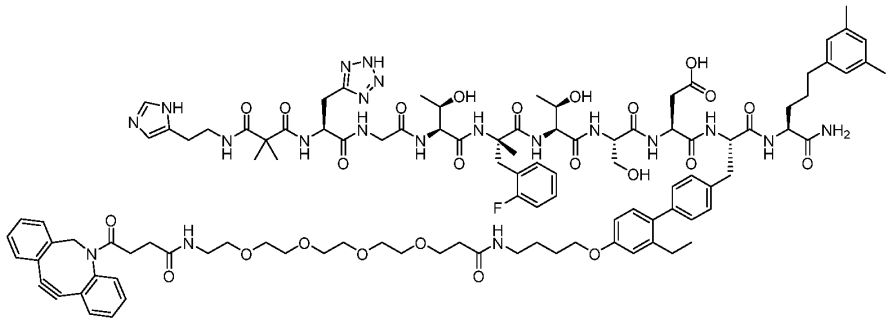
[000323] In one embodiment, Y is a group comprising a triazole.

[000324] In one embodiment, the glutamine residue Gln is naturally present in a CH2 or CH3 domain of the BA. In another embodiment, the glutamine residue Gln is introduced to the BA by modifying one or more amino acids. In one embodiment, the Gln is Q295 or N297Q.

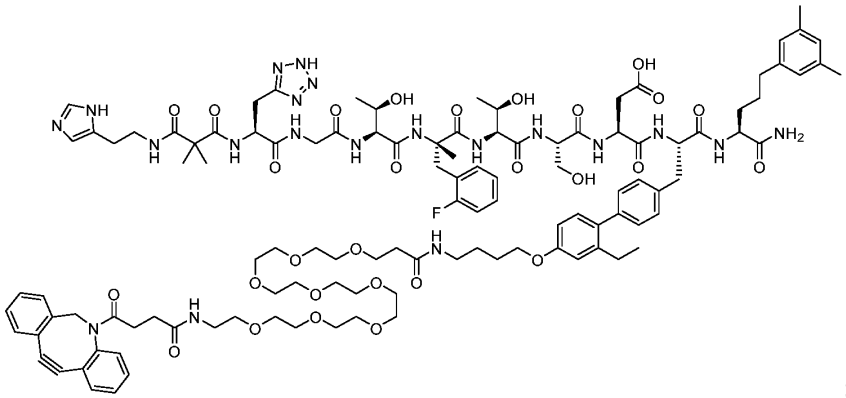
[000325] In one embodiment, the transglutaminase is microbial transglutaminase (MTG). In one embodiment, the transglutaminase is bacterial transglutaminase (BTG).

[000326] In one embodiment, the compound L-P for use in any of the above methods of producing the compounds of Formula (I) has a structure selected from the group consisting of:

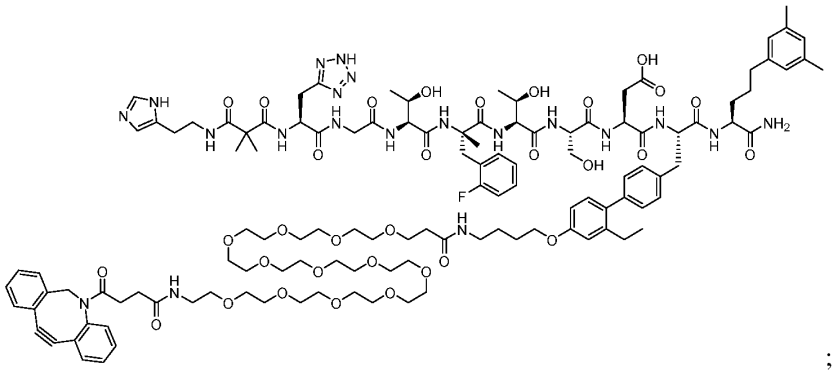
**Structure**



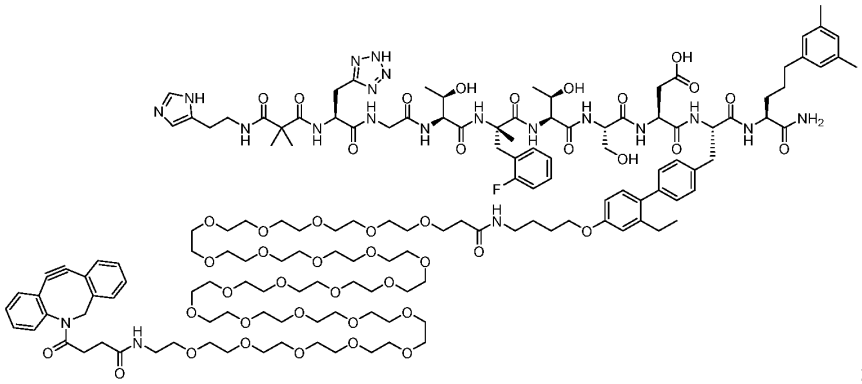
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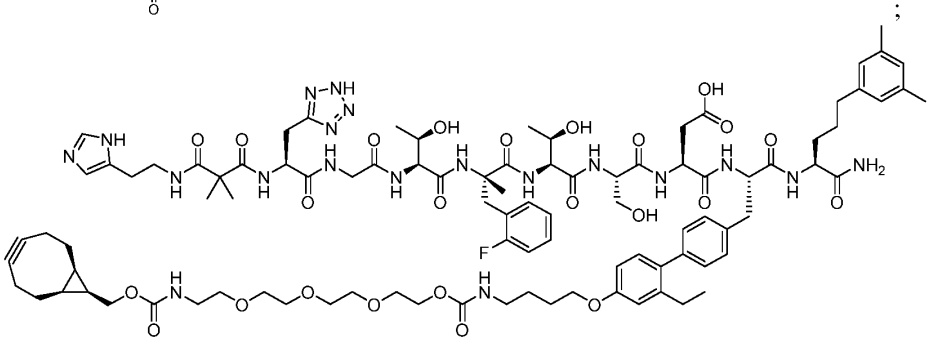
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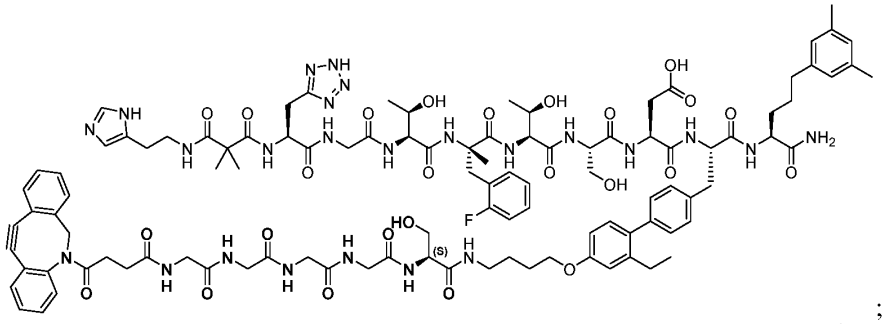
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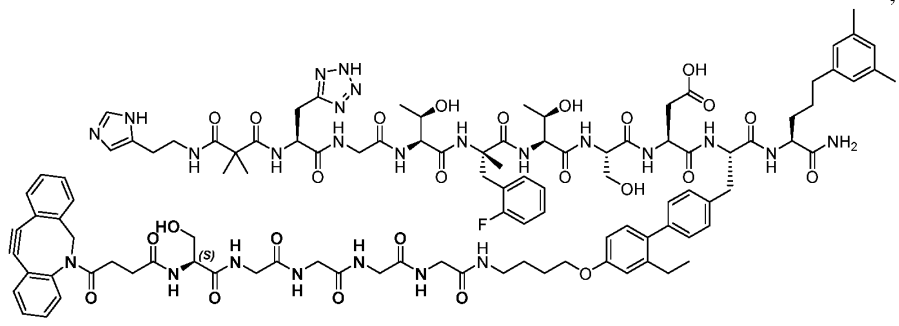
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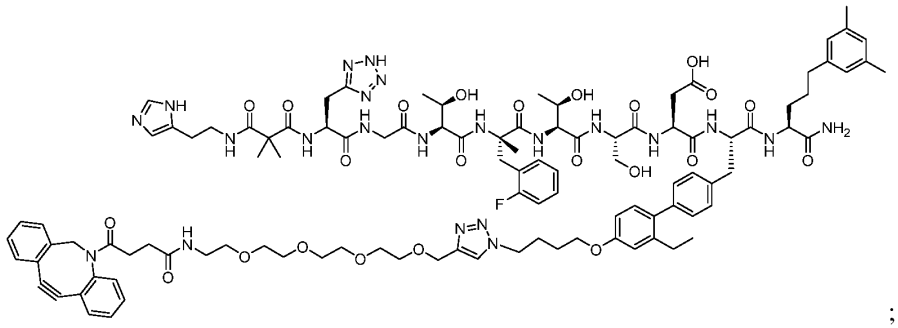
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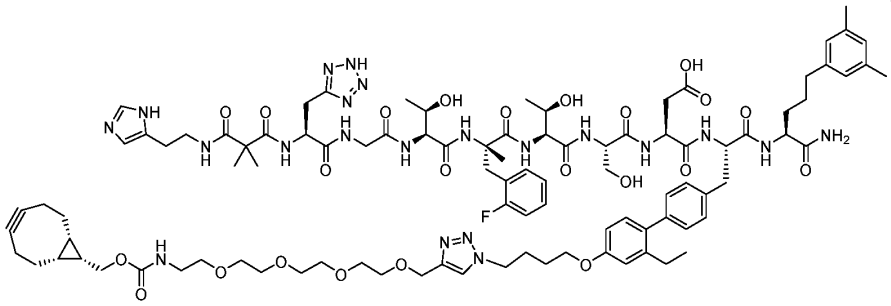
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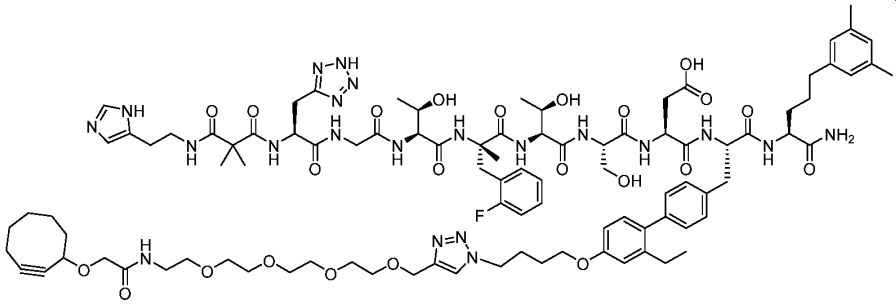
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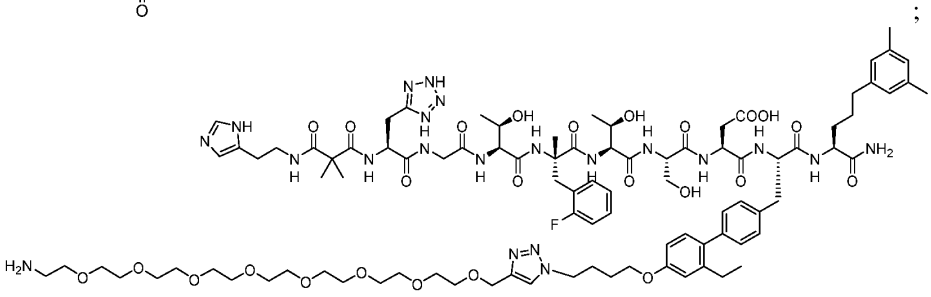
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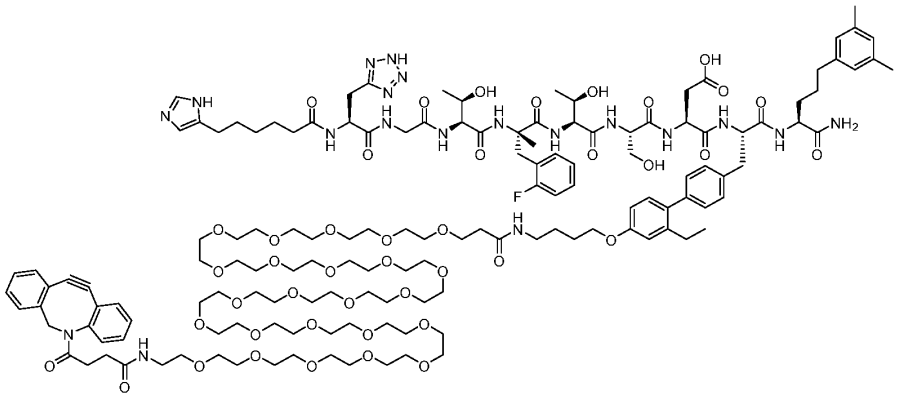
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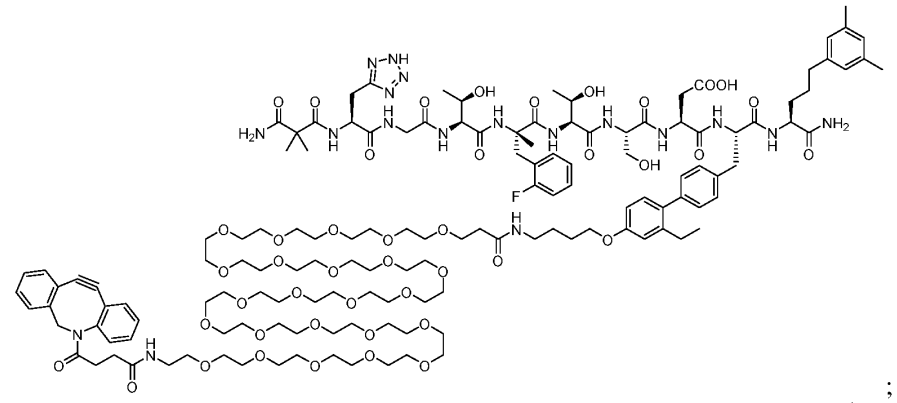
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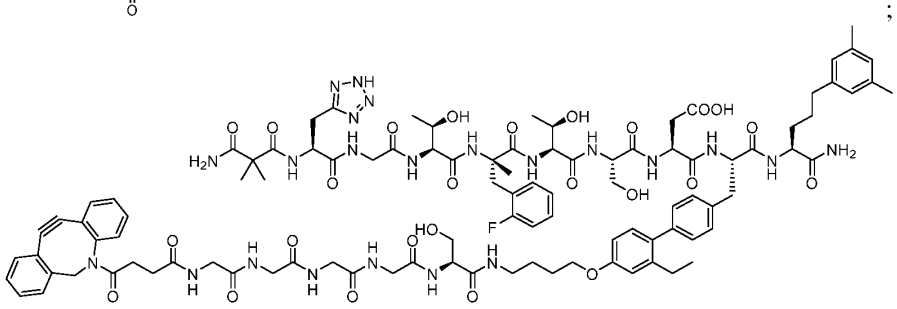
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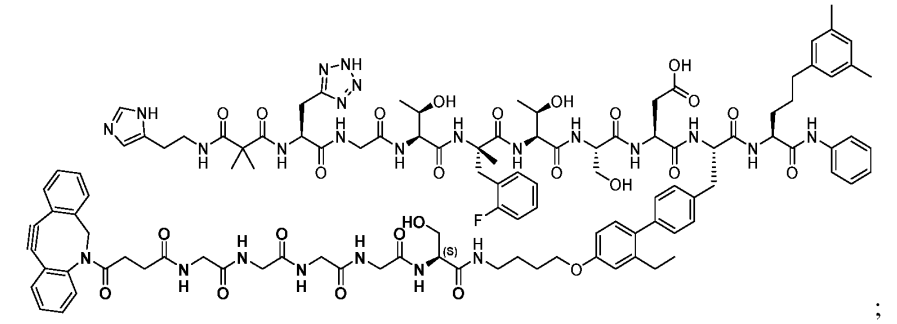
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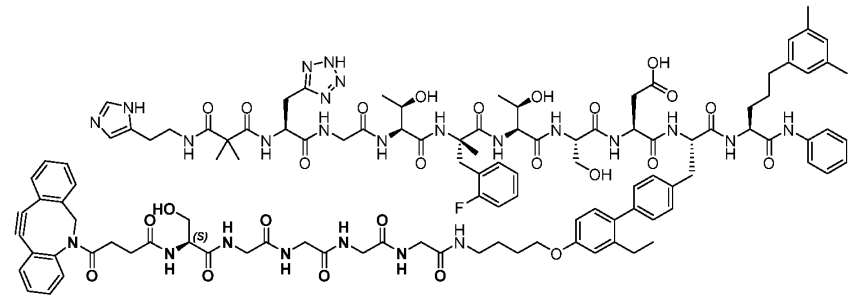
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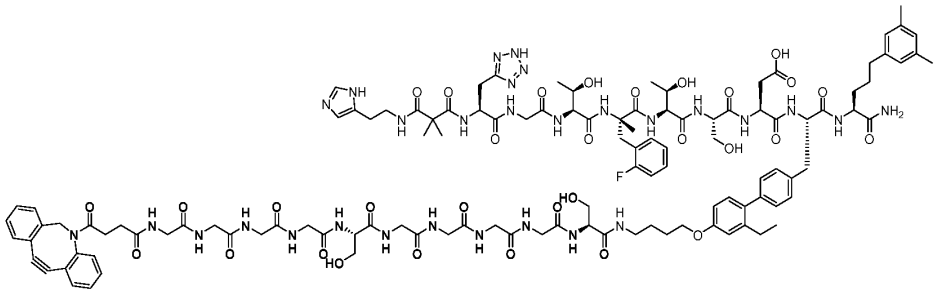
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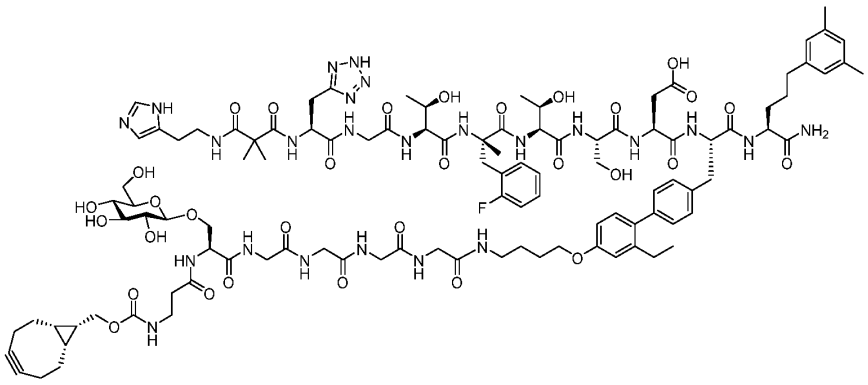
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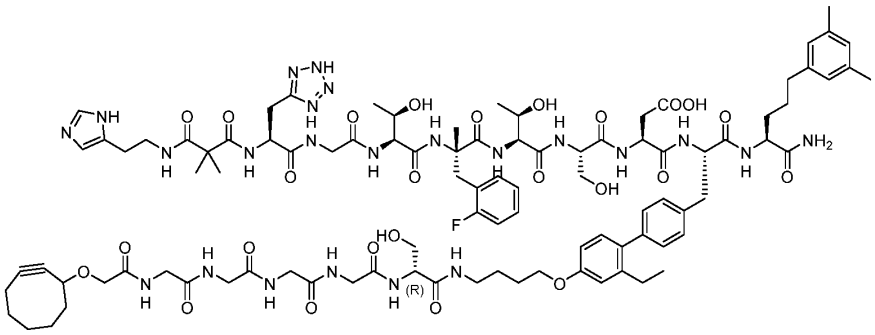
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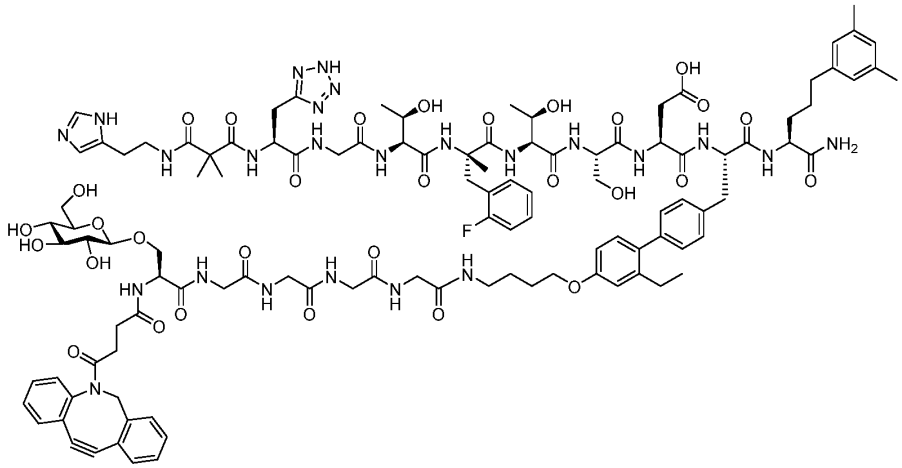
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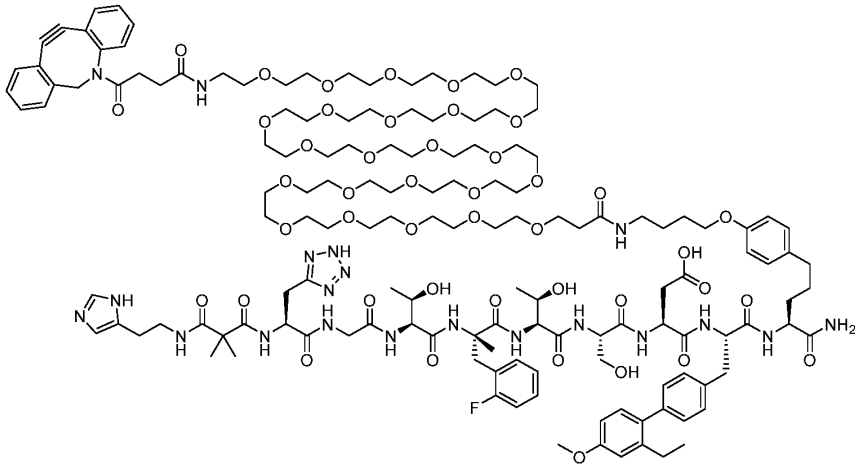
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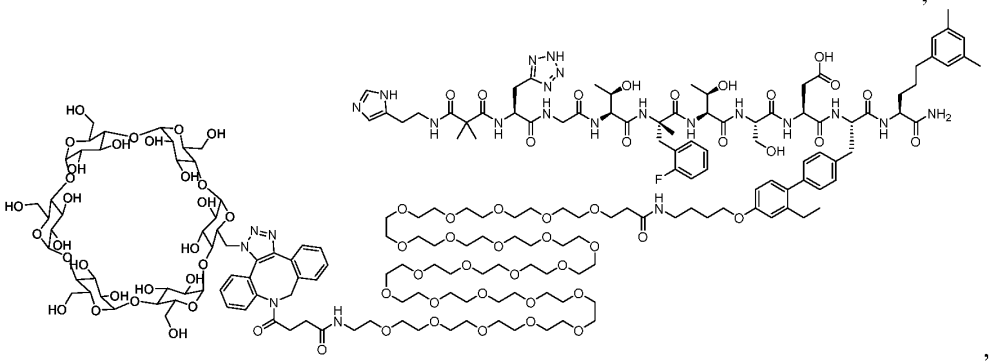
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,

or a pharmaceutically acceptable salt thereof.

**Therapeutic Formulation and Administration**

[000327] The present disclosure provides pharmaceutical compositions comprising the protein-drug conjugates of the present disclosure.

[000328] In one aspect, the present disclosure provides compositions comprising a population of protein-drug conjugates according to the present disclosure having a drug-antibody ratio (DAR) of about 0.5 to about 30.0.

[000329] In one embodiment, the composition has a DAR of about 1.0 to about 2.5.

[000330] In one embodiment, the composition has a DAR of about 2.

[000331] In one embodiment, the composition has a DAR of about 3.0 to about 4.5.

[000332] In one embodiment, the composition has a DAR of about 4.

[000333] In one embodiment, the composition has a DAR of about 6.5 to about 8.5.

[000334] In one embodiment, the composition has a DAR of about 8.

[000335] The compositions of the disclosure are formulated with suitable carriers, excipients, and other agents that provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™, Life Technologies, Carlsbad, CA), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[000336] The dose of a protein-drug conjugate administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The suitable dose is typically calculated according to body weight or body surface area. When a protein-drug conjugate of the present disclosure is used for therapeutic purposes in an adult patient, it may be advantageous to intravenously administer the protein-drug conjugate of the present disclosure normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering a protein-drug conjugate may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly.

Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (e.g., Mordenti et al., 1991, Pharmaceut. Res. 8:1351).

[000337] Various delivery systems are known and can be used to administer the pharmaceutical composition of the disclosure, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu et al., 1987, J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[000338] A pharmaceutical composition of the present disclosure can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present disclosure. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[000339] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present disclosure. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications

in subcutaneous delivery of a pharmaceutical composition of the present disclosure include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICKTM Autoinjector (Amgen, Thousand Oaks, CA), the PENLETTM (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRATM Pen (Abbott Labs, Abbott Park IL), to name only a few.

[000340] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[000341] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, e.g., by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[000342] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

**Therapeutic uses of the protein-drug conjugates, linker-payloads and payloads**

[000343] In another aspect, the compounds (e.g., an antibody-drug conjugate, a linker-payload and/or a payload), disclosed herein are useful, inter alia, for the treatment, prevention and/or amelioration of a disease, disorder or condition in need of such treatment.

[000344] In one aspect, the present disclosure provides a method of treating a condition in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a compound (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) according to the disclosure, or the composition comprising any compound according to the present disclosure.

[000345] In some embodiments, the compounds (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) disclosed herein are useful for treating any disease or disorder in which stimulation, activation and/or targeting of GLP1R would be beneficial. In particular, the anti-GLP1R antibody-drug conjugates of the present disclosure can be used for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by GLP1R expression or activity.

[000346] In some embodiments, the compounds (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) disclosed herein are useful for treating a GLP1R-associated condition. In some embodiments, the GLP1R-associated condition is Type 1 or Type 2 diabetes mellitus. The administered compound (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) may cause at least one of the following results: induction of insulin secretion, suppression of glucagon release, reduction of blood sugar, improvement of glycemic control, promotion of islet neogenesis, and delay of gastric emptying or potentiation of glucose resistant islets.

[000347] In some embodiments, the GLP1R-associated condition is a neurodegenerative disorder, a cognitive disorder, memory disorder or learning disorder. The neurodegenerative disorder may be, for example, dementia, senile dementia, mild cognitive impairment, Alzheimer-related dementia, Huntington's chores, tardive dyskinesia, hyperkinesias, mania, Morbus Parkinson, steel-Richard syndrome, Down's syndrome, myasthenia gravis, nerve trauma, brain trauma, vascular amyloidosis, cerebral hemorrhage I with amyloidosis, brain inflammation, Friedrich's ataxia, acute confusion disorder, amyotrophic lateral sclerosis, glaucoma and Alzheimer's disease.

[000348] In some embodiments, the GLP1R-associated condition is a liver disease. The liver disease may be, for example, non-alcoholic fatty liver disease (NAFLD), fatty liver, non-alcoholic steatohepatitis (NASH), and cirrhosis.

[000349] In some embodiments, the GLP1R-associated condition is a coronary artery disease. The coronary artery disease may be, for example, cardiomyopathy and myocardial infarction.

[000350] In some embodiments, the GLP1R-associated condition is a kidney disease. The kidney disease may be, for example, hypertension, or chronic kidney failure.

[000351] In some embodiments, the GLP1R-associated condition is an eating disorder. The eating disorder may be, for example, binge eating.

[000352] Without wishing to be bound by theory, the compounds (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) disclosed herein may be employed to attenuate the effects of apoptosis-mediated degenerative diseases of the central nervous system such as Alzheimer's Disease, Creutzfeld-Jakob Disease and bovine spongiform encephalopathy, chronic wasting syndrome and other prion mediated apoptotic neural diseases (*see*, e.g., Perry and Grieg (2004) Current Drug Targets 6:565-571). Administration of a compound (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) disclosed herein may also lead to down-modulation of βAPP and thereby ameliorate Aβ mono- or oligomer-mediated pathologies associated with Alzheimer's Disease (*see*, e.g., Perry et al. (2003) Journal of Neuroscience Research 72:603-612).

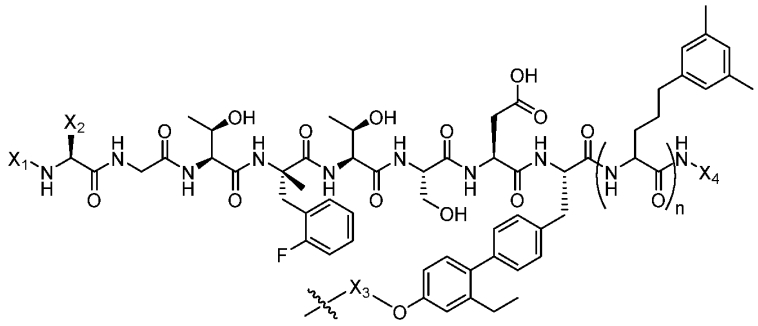
[000353] It is also contemplated that the compounds (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) disclosed herein may be used to improve learning and memory, for example, by enhancing neuronal plasticity and facilitation of cellular differentiation (*see*, During et al. (2003) Nature Medicine 9:1173-1179). Further, the compounds (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) disclosed herein may also be used to preserve dopamine neurons and motor function in Morbus Parkinson (*see*, e.g., Greig et al. (2005) Abstract 897.6, Society for Neuroscience, Washington, D.C.).

[000354] In some embodiments, the compounds (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) disclosed herein may also be used to treat a metabolic disorder. The metabolic disorder may be, for example, obesity, dyslipidemia, metabolic syndrome X, and pathologies emanating from islet insufficiency.

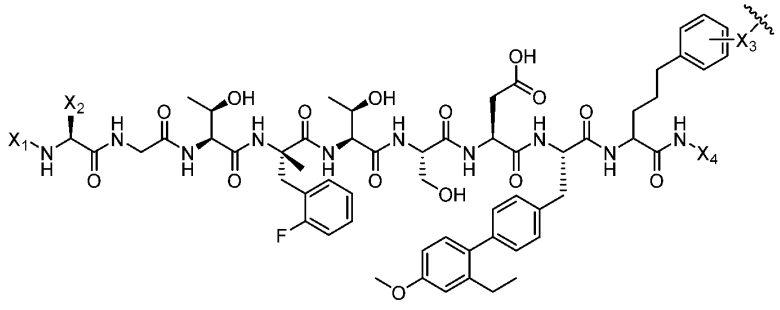
[000355] Additional diseases that may be treated by a compound (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) of the present disclosure include autoimmune diseases, in particular, those associated with inflammation, including, but not limited to, autoimmune diabetes, adult onset diabetes, morbid obesity, Metabolic Syndrome X and dyslipidemia. For example, the anti-GLP1R antibody-drug conjugate can be employed as a

growth factor for the promotion of islet growth in persons with autoimmune diabetes. The compounds (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) described herein may also be useful in the treatment of congestive heart failure.

[000356] In one aspect, the present disclosure provides a method of selectively targeting an antigen (e.g., GLP1R) on a surface of a cell with a compound. In one embodiment, the method of selectively targeting an antigen (e.g., GLP1R) on a surface of a cell with a compound comprises linking the compound to a targeted antibody. In one embodiment, the compound is a payload as described above. In one embodiment, the cell is a mammalian cell. In one embodiment, the cell is a human cell. In one embodiment, the cell is a pancreatic cell or a brain cell. In certain embodiments, the present disclosure provides a method of selectively targeting an antigen on a surface of a cell with a compound having the structure selected from the group consisting of:

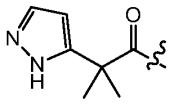
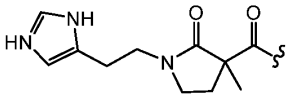
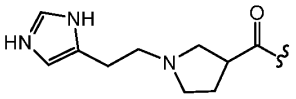


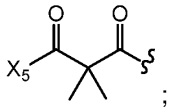
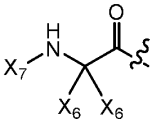
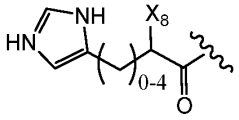
(P-I) and

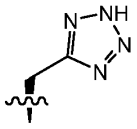
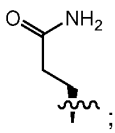


(P-II),

wherein —§— is the point of attachment of the compound to a linker L;

$X_1$ is selected from H;  ;  ;  ;

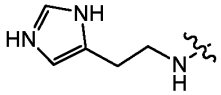
 ;  ; and  ;

$X_2$ is selected from  and  ;

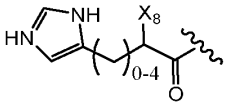
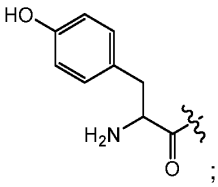
$X_3$ is selected from $–(CH_2)_{2\text{-}6}–NH–$ and $–(CH_2)_{2\text{-}6}–Tr–$, where Tr is a triazole moiety;

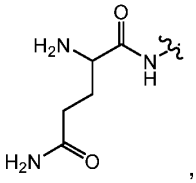
n is 0 or 1;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  , and  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ,

or a pharmaceutically acceptable salt thereof.

[000357] In certain embodiments, the present disclosure also includes the use of a compound (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) of the present disclosure in the manufacture of a medicament for the treatment of a disease or disorder (e.g., cancer) related to or caused by GLP1R-expressing cells. In one aspect, the present disclosure relates to a protein-drug conjugate comprising an anti-GLP1R antibody or antigen-binding fragment, as disclosed herein, for use in medicine. In one aspect, the present disclosure relates

to a compound comprising an antibody-drug conjugate (ADC) as disclosed herein, for use in medicine.

**Combination Therapies and Formulations**

[000358] The present disclosure provides methods which comprise administering a pharmaceutical composition comprising any of the exemplary protein-drug conjugates (e.g., antibody-drug conjugates), linker-payloads and payloads described herein in combination with one or more additional therapeutic agents.

[000359] Exemplary additional therapeutic agents that may be combined with or administered in combination with protein-drug conjugates (e.g., antibody-drug conjugates), linker-payloads and payloads of the present disclosure include, other GLP1R agonists (e.g., an anti-GLP1R antibody or a small molecule agonist of GLP1R or an anti-GLP1R antibody-drug conjugate). Non-limiting examples of GLP1R agonists include exenatide (Byetta, Bydureon), liraglutide (Victoza, Saxenda), lixisenatide (Lyxumia in Europe, Adlyxin in the United States), albiglutide (Tanzeum), dulaglutide (Trulicity), semaglutide (Ozempic), and taspoglutide.

[000360] Exemplary additional therapeutic agents may include dual or triple-agonists, including GLP1R/GIPR dual agonists, such as GLP1R/GCGR dual agonists, GLP1R/GIPR/GCGR triple-agonists.

[000361] Other agents that may be beneficially administered in combination with the protein-drug conjugates (e.g., antibody-drug conjugates), linker-payloads and payloads of the disclosure include those that are useful in the treatment of diabetes (e.g., type II diabetes), obesity, and/or other related metabolic diseases.

[000362] In some embodiments, the additional therapeutic agent is an antidiabetic agent. Any suitable antidiabetic agents can be used. Non-limiting examples of antidiabetic agents include insulin, insulin analogs (including insulin lispro, insulin aspart, insulin glulisine, isophane insulin, insulin zinc, insulin glargine, and insulin detemir), biguanides (including metformin, phenformin, and buformin), thiazolidinediones or TZDs (including rosiglitazone, pioglitazone, and troglitazone), sulfonylureas (including tolbutamide, acetohexamide, tolazamide, chlorpropamide, glipizide, glibenclamide, glimepiride, gliclazide, glyclopyramide, and gliquidone), meglitinides (including repaglinide and nateglinide), alpha-glucosidase inhibitors (including miglitol, acarbose, and voglibose), glucagon-like peptide analogs and agonists (including exenatide, liraglutide, semaglutide, taspoglutide, lixisenatide, albuglutide, and dulaglutide), gastric inhibitory peptide analogs, dipeptidyl peptidase-4 (DPP-4) inhibitors (including vildagliptin, sitagliptin, saxagliptin, linagliptin, alogliptin, septagliptin, teneligliptin, and gemigliptin), amylin agonist analogs,

sodium/glucose cotransporter 2 (SGLT2) inhibitors, glucokinase activators, squalene synthase inhibitors, other lipid lowering agents and aspirin. In some such embodiments, the antidiabetic agent is an oral antidiabetic agents (OAA) such as metformin, acarbose, or TZDs. In some such embodiments, the antidiabetic agent is metformin.

[000363] In some embodiments, the GLP1R agonist and one or more antidiabetic agents may be formulated into the same dosage form, such as a solution or suspension for parenteral administration.

[000364] The additional therapeutically active component(s) may be administered just prior to, concurrent with, or shortly after the administration of a compound of the present disclosure; (for purposes of the present disclosure, such administration regimens are considered the administration of an antigen-binding molecule "in combination with" an additional therapeutically active component).

[000365] The present disclosure includes pharmaceutical compositions in which protein-drug conjugates (e.g., antibody-drug conjugates), linker-payloads and/or payloads of the present disclosure are co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

**Administration Regimens**

[000366] According to certain embodiments of the present disclosure, multiple doses of a protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or a payload may be administered to a subject over a defined time course. The methods according to this aspect of the disclosure comprise sequentially administering to a subject multiple doses of a protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or a payload of the disclosure. As used herein, "sequentially administering" means that each dose of a protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or a payload is administered to the subject at a different point in time, e.g., on different days separated by a predetermined interval (e.g., hours, days, weeks or months). The present disclosure includes methods which comprise sequentially administering to the patient a single initial dose of a protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or a payload, followed by one or more secondary doses of the protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or payload, and optionally followed by one or more tertiary doses of the a protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or payload.

[000367] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or payload of the disclosure. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of the protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or payload, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of the protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or payload contained in the initial, secondary and/or tertiary doses varies from one another (e.g., adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (e.g., 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (e.g., "maintenance doses").

[000368] In one exemplary embodiment of the present disclosure, each secondary and/or tertiary dose is administered 1 to 26 (e.g., 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of a protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or payload which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[000369] The methods according to this aspect of the disclosure may comprise administering to a patient any number of secondary and/or tertiary doses of a protein-drug conjugate (e.g., an anti-GLP1R antibody-drug conjugate), linker-payload and/or payload. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[000370] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary

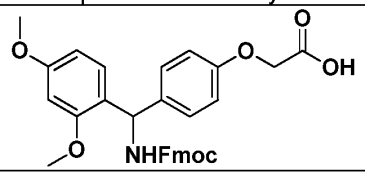
dose may be administered to the patient 1 to 2 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 4 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

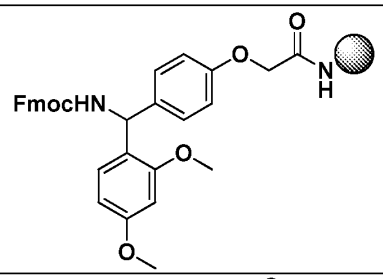
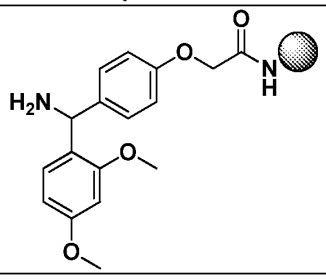
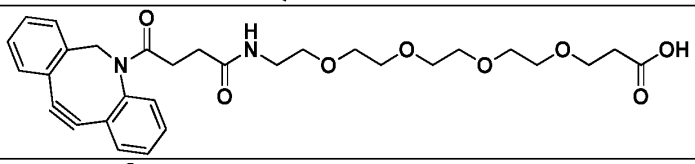
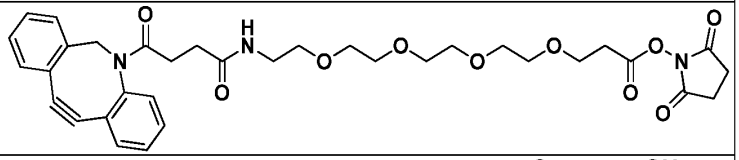
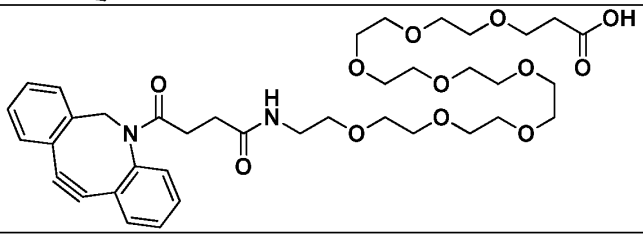
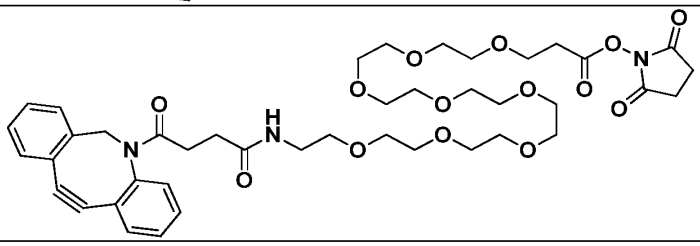
**EXAMPLES**

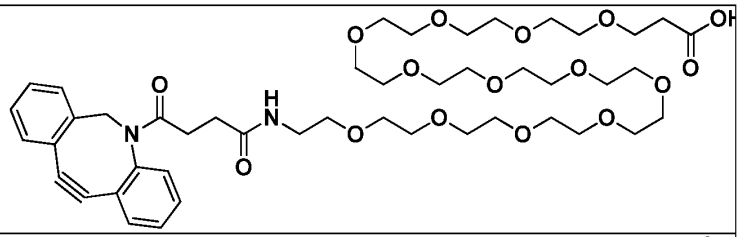
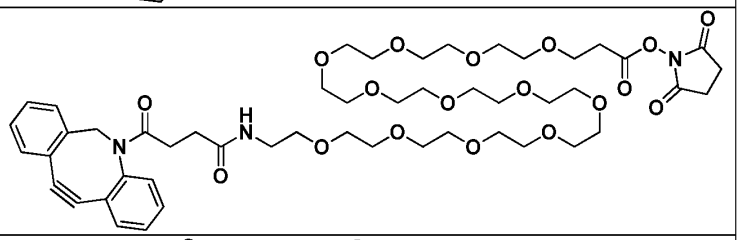
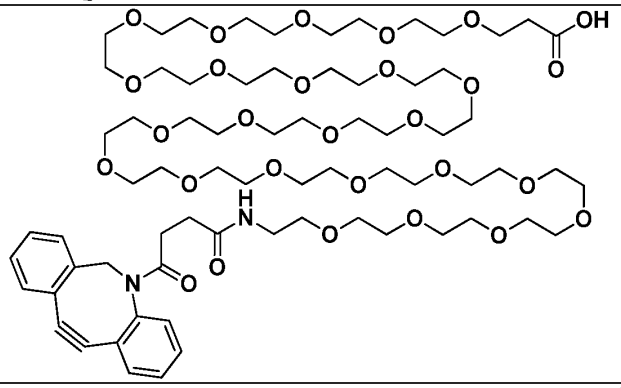
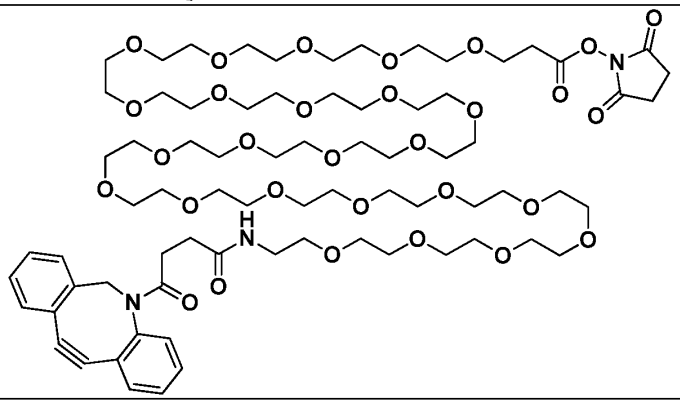
[000371] The following examples illustrate specific aspects of the instant description. The examples should not be construed as limiting, as the examples merely provide specific understanding and practice of the embodiments and their various aspects.

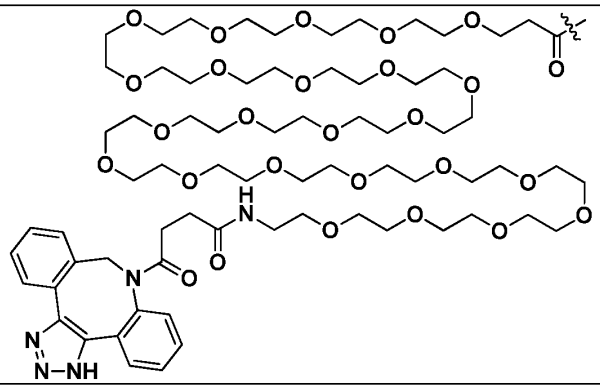
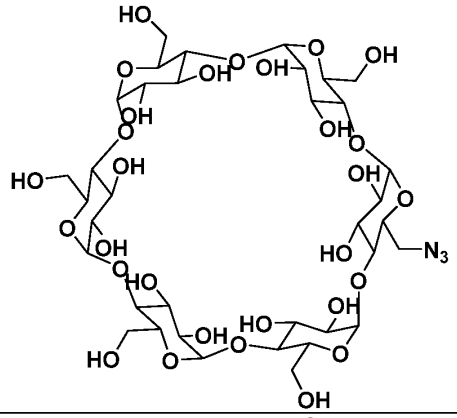
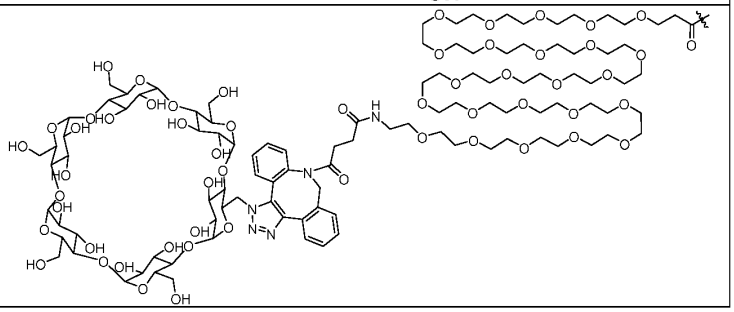
[000372] The abbreviations used in the Examples and throughout the specification are as follows:

| Abbreviation | Term |
|---|---|
| **aa# (e.g., aa1)** | amino acid number (e.g., amino acid 1) |
| **Ac** | acetyl |
| **ADC** | antibody-drug conjugation |
| **aq.** | aqueous |
| **Boc** | *t*-butoxycarbonyl |
| **CD** | cyclodextrin |
| **DCC** | dicyclohexylcarbodiimide |
| **DCM** | dichloromethane |
| **DIPEA** | diisopropylethylamine |
| **DMAP** | 4-Dimethylaminopyridine |
| **DMF** | N,N-Dimethylformamide |
| **DMSO** | dimethylsulfoxide |
| **EDCI** | 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride |
| **Et** | ethyl |
| **EtOAc** | Ethyl acetate |
| **EtOH** | ethanol |
| **$Et_3N$** | Triethylamine |
| **Fmoc** | 9-fluorenylmethoxycarbonyl |
| **FmocCl** | 9-Fluorenylmethyl chloroformate |
| **FmocOSu** | N-(9-Fluorenylmethoxycarbonyloxy)succinimide |
| **HATU** | *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate |
| **HOBt (HOBT)** | 1-hydroxybenzotriazole |

| | |
|---|---|
| **HOSu** | *N*-hydroxysuccinimide |
| **HPLC** | high-pressure liquid chromatography |
| **HRMS** | High-resolution mass spectrometry |
| **LCMS** | Liquid chromatography–mass spectrometry |
| **Me** | methyl |
| **MPM (PMB)** | p-methoxybenzyl |
| **Ms** | mesyl (methanesulfonyl) |
| **MS** | mass spectrometry |
| **4A MS** | 4A molecular sieves |
| **MW** | Molecule weight |
| **MeOH** | methanol |
| **NMR** | nuclear magnetic resonance |
| **PEG** | polyethylene glycol |
| **Ph** | phenyl |
| **Pr** | propyl |
| **psi** | pounds per square inch |
| **Py (pyr)** | pyridine |
| **PE** | Petroleum ether |
| **Resin** | MBHA resin (0.3~0.8mmol/g, 100~200 mesh, 1% DVB) |
| **$R_f$** | retention factor in chromatography |
| ***t*-Bu (*t*Bu)** | tert-butyl |
| ***t*-BuOMe (MTBE, TBME)** | Methyl tert-butyl ether |
| **TEA** | triethylamine |
| **TES** | triethylsilyl |
| **TFA** | trifluoroacetic acid |
| **Tfa** | trifluoroacetamide |
| **THF** | tetrahydrofuran |
| **Tr (Trt)** | trityl (triphenylmethyl) |
| **TRTCl** | triphenylmethyl chloride |
| **Ts (Tos)** | p-toluenesulfonyl |
| **Rink Amide Linker** |  |

| | |
|---|---|
| **Fmoc - Rink Amide MBHA Resin** |  |
| **Rink Amide MBHA Resin** |  |
| **DIBAC-PEG$_4$-acid** |  |
| **DIBAC-PEG$_4$-NHS** |  |
| **DIBAC-PEG$_8$-acid** |  |
| **DIBAC-PEG$_8$-NHS** |  |

| | |
|---|---|
| **DIBAC-PEG$_{12}$-acid** |  |
| **DIBAC-PEG$_{12}$-NHS** |  |
| **DIBAC-PEG$_{24}$-acid** |  |
| **DIBAC-PEG$_{24}$-NHS** |  |

| | |
|---|---|
| **Azido-DIBAC-PEG$_{24}$-linker** |  |
| **CD-N$_3$** |  |
| **CD-N$_3$-DIBAC-PEG$_{24}$-linker** |  |

**Example 1. Synthesis of small molecular payloads and linker-payloads**

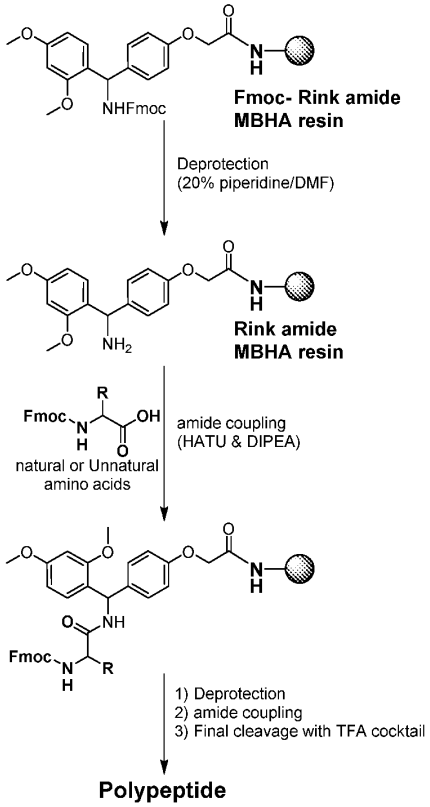
1.1 Solid phase peptide synthesis of peptidomimetic payloads

*General procedure of preparation of peptidomimetics (payloads) using SPPS approach*

[000373] **Scheme 1** depicts an assembly of peptidomimetic payloads according to the disclosure on resin. The peptides were assembled manually by a roller-mixer onto Fmoc SPPS (Solid phase peptide synthesis) using polypropylene columns equipped with a filter disc. A

sufficient quantity of Rink amide MBHA resin (loading: 0.5-0.6 mmol/g) was swollen in DMF or $CH_2Cl_2$ for 15 min.

**Scheme 1: General Scheme for assembling polypeptide payloads using SPPS strategy**




*Step 1: General procedure for removal of Fmoc from Fmoc-Rink amide MBHA resin*

[000374] The Fmoc-group on the resin was removed by incubation of resin with 20% piperidine in DMF (10-30 ml /100 mg of resin) for 5 to 15 min. The deprotected resin was filtered and washed with excess of DMF and DCM. After washing three times, the resin was incubated in a freshly distilled DMF (1 mL/100 mg of resin), under nitrogen atmosphere for 5 min.

*Step 2: General procedure for amide coupling on Rink amide MBHA resin*

[000375] For the amide coupling reaction with the SPPS-reactant, a DMF solution containing HATU (1.5-4 eq.), Fmoc-protected amino acid (1.5-5 eq. at 0.5M concentration), and DIPEA (5-10 eq.) were added to the resin. For the amide coupling reaction with a natural amino acid as a reactant, the Fmoc-amino acid (5 eq.), HATU (4.5 eq.) and DIPEA (10 eq.) were mixed with the resin; for the amide coupling reaction with an unnatural amino acid as a reactant, the Fmoc-amino

acid (1.5-2 eq.), HATU (1.5 eq.) and DIPEA (5.0 eq.) were mixed with the resin. The mixed resin mixture was then shaken for 1-3 hours under nitrogen atmosphere, and the coupling reactions were monitored using a ninhydrin test qualitative analysis. After attachment of the Fmoc-protected amino acid, the resin was then washed with DMF and DCM to generate the corresponding peptide bound resin.

*Step 3: General procedure for cleavage from resin followed by global deprotection*

[000376] The resin-bound peptidomimetic payloads were subjected to cleavage and deprotection with TFA cocktail as follows. A solution of TFA/water/triisopropylsilane (95:2.5:2.5) (10 mL per 100 mg of peptidyl-resin) was added to peptidyl-resins and the mixture was kept at room temperature. After 2-3 hours, the resin was filtered and rinsed by a cleavage solution. The combined filtrate was treated with cold t-BuOMe to precipitate the peptide. The suspension was centrifuged for 10 min (5000 R). The crude white powder was combined and purified by preparative HPLC.

[000377] The peptide chain elongation was performed by a number of iterations consisting of deprotection, washing, coupling, and washing procedures, (i.e. the resin was subjected to the reaction conditions for 1 hour each time, and the solution was drained and the resin was re-subjected to fresh reagents each time), as depicted in Scheme 1. Finally, the resulting Fmoc-protected peptidyl-resin was deprotected by 20% piperidine as described above and washed with DMF and DCM four times each. The resin bound peptide was dried under nitrogen flow for 10-15 minutes and subjected to cleavage/deprotection. Using the above protocol and suitable variations thereof, the peptidomimetics designed in the present disclosure were prepared, using Fmoc-SPPS approach. Furthermore, the resin bound peptidomimetics were cleaved and deprotected, purified and characterized using the following protocol.

<u>1.2 Preparative HPLC purification of the crude peptidomimetics:</u>

[000378] The preparative HPLC was carried out on a Shimadzu LC-8a Liquid chromatograph. A solution of crude peptide dissolved in DMF or water was injected into a column and eluted with a linear gradient of ACN in water. Different methods were used. (See General Information). The desired product eluted were in fractions and the pure peptidomimetics were obtained as amorphous with powders by lyophilization of respective HPLC fractions. In general, after the prep-HPLC purification, the overall recovery was found to be in the range of 40~50% yield.

[000379] **Preparative HPLC method A:** using FA condition (column: Xtimate C18 150*25mm*5μm; mobile phase: [water (0.225%FA)-ACN]; B%: 40%-70%, 7min) to afford a pure product.

[000380] **Preparative HPLC method B:** using TFA condition (column: YMC-Exphere C18 10μm 300*50mm 12nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to afford a pure product.

[000381] **Preparative HPLC method C:** using neutral condition (column: Phenomenex Gemini-NX 150*30 mm*5μm; mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 21%-51%, 11min) to afford a pure product.

[000382] **Preparative HPLC method D:** using neutral condition (column: Waters Xbridge 150*255u; mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 20%-50%, 7min) to afford a pure product.

[000383] **Preparative HPLC method E:** using FA condition (column: Phenomenex Luna C18 250*50 mm*10 μm; mobile phase: [water (0.225%FA)-ACN]; B%: 55%-86%, 21 min) to afford a pure product.

1.3 HPLC analysis of the purified peptidomimetics:

[000384] After purification by preparation HPLC as described above, each peptide was analyzed by analytical HPLC with using methods A, B, C, D, E, or F. The acquisition of chromatogram was carried out at 220 nm, using a PDA detector, in general, the purity of pure peptidomimetics obtained after Prep-HPLC purification was found to be >95%.

[000385] **HPLC method A (20 min):** Mobile Phase: 4.0 mL TFA in 4 L water (solvent A) and 3.2 mL TFA in 4 L acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 20 minutes and holding at 80% for 3.5 minutes at a flow rate of 1.0 mL/minutes; Column: Gemini-NX 5 μm 150*4.6 mm, C18, 110A Wavelength: UV 220 nm,254 nm; Column temperature: 30°C

[000386] **HPLC method B (15 min):** Mobile Phase: 2.75 mL/4L TFA in water (solvent A) and 2.5 mL/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 mL/min; Column: WELCH Ultimate LP-C18 150*4.6 mm 5μm; Wavelength: UV 220 nm, 215nm, 254nm; Column temperature: 40°C.

[000387] **HPLC method C (8 min):** Mobile Phase: 2.75 mL/4L TFA in water (solvent A) and 2.5 mL/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 7 minutes and holding at 80% for 0.48 minutes at a flow rate of 1.5 mL/min; Column: Ultimate XB-C18,3 μm, 3.0*50 mm; Wavelength: 220nm, 215nm, 254nm; Column temperature: 40°C.

[000388] **HPLC method D (15 min):** Mobile Phase: water containing 0.04% TFA (solvent A). and acetonitrile containing 0.02% TFA (solvent B), using the elution gradient 10% to 80% (solvent B) over 15 minutes and holding at 80% for 3.5 minutes at a flow rate of 1.5 mL/minutes; Column: YMC-Pack ODS-A 150*4.6 mm Wavelength: UV 220 nm, 254 nm; Column temperature: 30°C.

[000389] **HPLC method E (8 min):** Mobile Phase: 0.2 mL/1L NH3*$H_2O$ in water (solvent A) and acetonitrile (solvent B), using the elution gradient 0%-60% (solvent B) over 5 minutes and holding at 60% for 2 minutes at a flow rate of 1.2 ml/min; Column: Xbridge Shield RP-18, 5 µm, 2.1*50 mm. Wavelength: UV 220 nm, 254 nm; Column temperature: 30°C.

[000390] **HPLC method F (7 min):** Mobile Phase: 1.5 mL/4L TFA in water (solvent A) and 0.75 mL/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B). Column: Xtimate C18 2.1*30 mm; Wavelength: UV 220nm, 254nm; Column temperature: 50°C.

1.4 Characterization by Mass Spectrometry:

[000391] Each peptide was characterized by electrospray ionization mass spectrometry (ESI-MS), either in flow injection or LC/MS mode. In all cases, the experimentally measured molecular weight was within 0.5 Daltons of the calculated monoisotopic molecular weight. Using the above described protocol, all the crude/pure peptidomimetics were characterized by mass spectroscopy and in general, observed mass of peptidomimetic agreed with the calculated/theoretical mass, which confirms successful synthesis of desired peptidomimetics.

[000392] **LC-MS method A:** a MERCK (RP-18e 25-2mm) column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000393] **LC-MS method B:** a Xtimate (C18 2.1*30 mm, 3µm) column, with a flow rate of 0.8 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000394] **LC-MS method C:** a Chromolith (Flash RP-18e 25-3 mm) column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.04% TFA (solvent B) and water containing 0.06% TFA (solvent A).

[000395] **LC-MS method D:** Agilent, a Pursuit (5 C18 20*2.0 mm) column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000396] **LC-MS method E:** Waters Xbridge C18 30*2.0mm, 3.5 µm column, with a flow rate of 1.0 mL/min, eluting with a gradient of 5% to 95%. Mobile phase: A) 0.05% $NH_3H_2O$ in

Water; B) ACN. Gradient: 0% B increase to 95%B within 5.8 min; hold at 95%B for 1.1 min; then back to 0%B at 6.91 min and hold for 0.09 min.

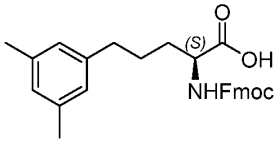
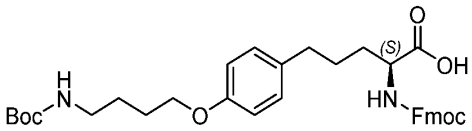
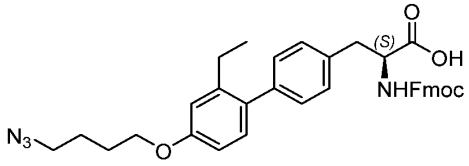
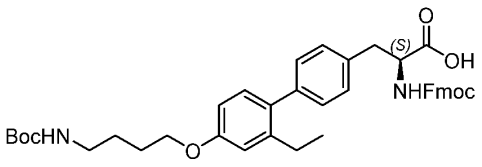
[000397] **LC-MS method F:** XBridge C18 3.5 µm 2.1*30 mm Column, with a flow rate of 1.0 mL/min, Mobile phase: 0.8mL/4L $NH_3 \cdot H_2O$ in water (solvent A) and acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 2 minutes and holding at 80% for 0.48 minutes.

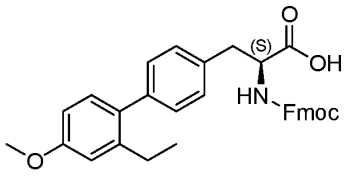
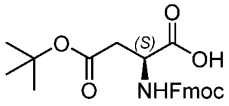
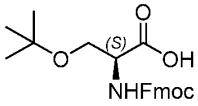
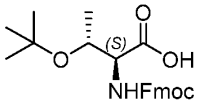
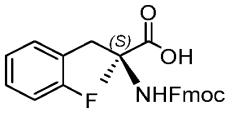
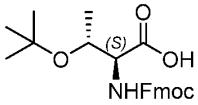
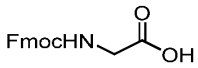
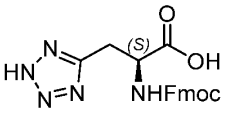
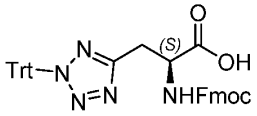
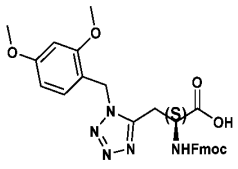
[000398] 1.5 HRMS analysis was performed on an Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System.

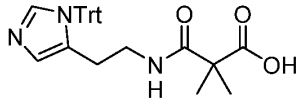
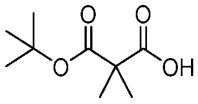
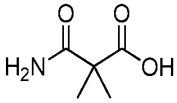
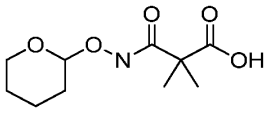
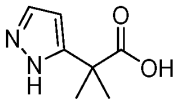
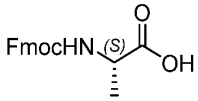
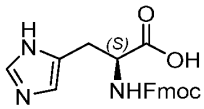
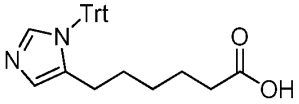
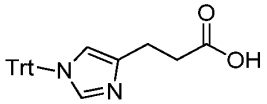
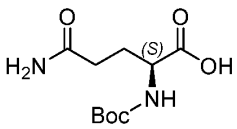
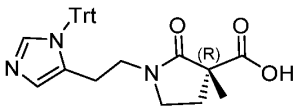
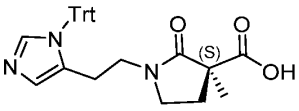
[000399] The mobile phase: 0.1% FA in water (solvent A) and ACN (solvent B); Elution Gradient: 5%-95% (solvent B) over 3 minutes and holding at 95% for 1 minute at a flow rate of 1 ml/minute; Column: Xbridge Shield RP 18 5 µm, 2.1*50mm Ion Source: AJS ESI source; Ion Mode: Positive; Nebulization Gas: Nitrogen; Drying Gas (N2) Flow: 8L/min; Nebulizer Pressure: 35psig; Gas Temperature: 325°C; Sheath gas Temperature: 350°C; Sheath gas flow: 11L/min; Capillary Voltage: 3.5KV; Fragmentor Voltage: 175 V.

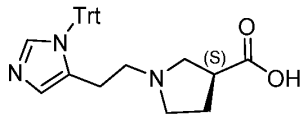
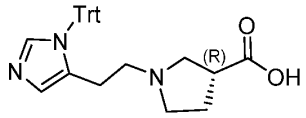
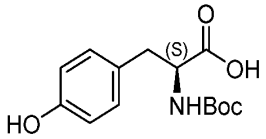
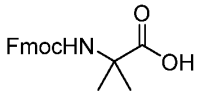
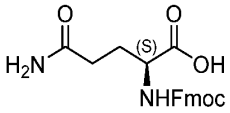
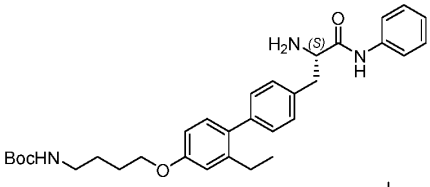
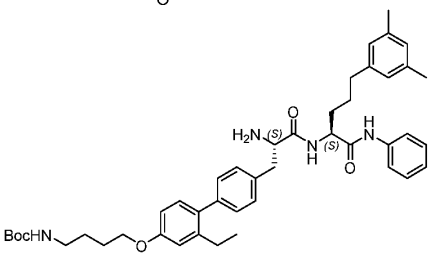
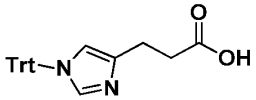
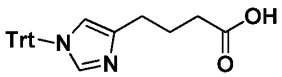
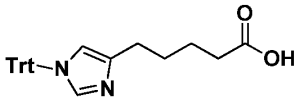
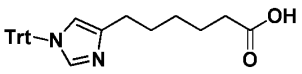
**Example 2. Synthesis of unnatural amino acids**

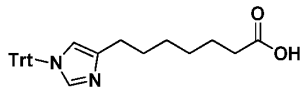
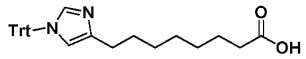
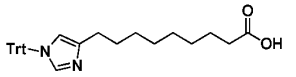
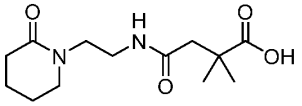
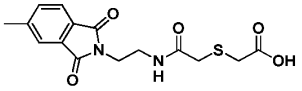
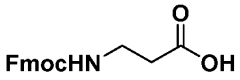
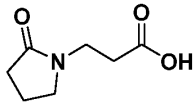
**Table 1. Structures of natural and unnatural amino acids aa1 – aa30**

| No. | Structure | Source* | MF | ESI-MS Calcd. M/Z | ESI-MS Found |
|---|---|---|---|---|---|
| aa1 |  | GB2551945a Jazayeri, A. et al. *Nature.* **2017**, *546, 254-258* | $C_{28}H_{29}NO_4$ | 443.2 | 466.1 $[M+Na]^+$ |
| aa1b |  | Prepared | $C_{35}H_{42}N_2O_7$ | 602.2 | 625.1 $[M+Na]^+$ |
| aa2 |  | Prepared | $C_{36}H_{36}N_4O_5$ | 604.2 | 627.3 $[M+Na]^+$ |
| aa2b |  | Prepared | $C_{41}H_{46}N_2O_7$ | 678.3 | 701.3 $[M+Na]^+$ |

| | | | | | |
|---|---|---|---|---|---|
| **aa2c** |  | Prepared | $C_{40}H_{43}N_2O_7$ | 662.77 | 663.5 $[M+H]^+$ |
| **aa3** |  | Commercial | | | |
| **aa4** |  | Commercial | | | |
| **aa5** |  | Commercial | | | |
| **aa6** |  | Commercial | | | |
| **aa7** |  | Commercial | | | |
| **aa8** |  | Commercial | | | |
| **aa9** |  | Ceretti, S. et al *Eur. J. Org. Chem.* **2004,** 4188-4196 | $C_{19}H_{17}N_5O_4$ | 379.1 | 402.0 $[M+Na]^+$ |
| **aa9a** |  | Prepared | $C_{38}H_{31}N_5O_4$ | 621.2 | |
| **aa9d** |  | Prepared | $C_{28}H_{27}N_5O_6$ | 529.20 | 530.4 $[M+H]^+$ |

| | | | | | |
|---|---|---|---|---|---|
| **aa10** |  | GB2551945a | $C_{29}H_{30}N_3O_3$ | 467.2 | 468.1 $[M+H]^+$ |
| **aa11** |  | Commercial | | | |
| **aa12** |  | WO2010/052253 | | | |
| **aa13** |  | Prepared | | | |
| **aa14** |  | Prepared | $C_7H_{11}N_2O_2$ | 154.1 | 155.0 $[M+H]^+$ |
| **aa15** |  | Commercial | | | |
| **aa16** |  | Commercial | | | |
| **aa17** |  | US2003/114668 | $C_{28}H_{27}N_2O_2$ | 422.22 | 423.2 $[M-H]^-$ |
| **aa18** |  | Crich, D. et al, *Org. Lett.* **2007,** 9, 4423-4426 | $C_{25}H_{23}N_2O_2$ | 382.18 | 383.2 $[M+H]^+$ |
| **aa19** |  | Commercial | | | |
| **aa20** |  | Prepared | $C_{30}H_{30}N_3O_3$ | 479.2 | 480.3 $[M+H]^+$ SFC-HPLC: RT = 2.346 min |
| **aa21** |  | Prepared | $C_{30}H_{30}N_3O_3$ | 479.2 | 480.3 $[M+H]^+$ SFC-HPLC: RT = 3.607 min |

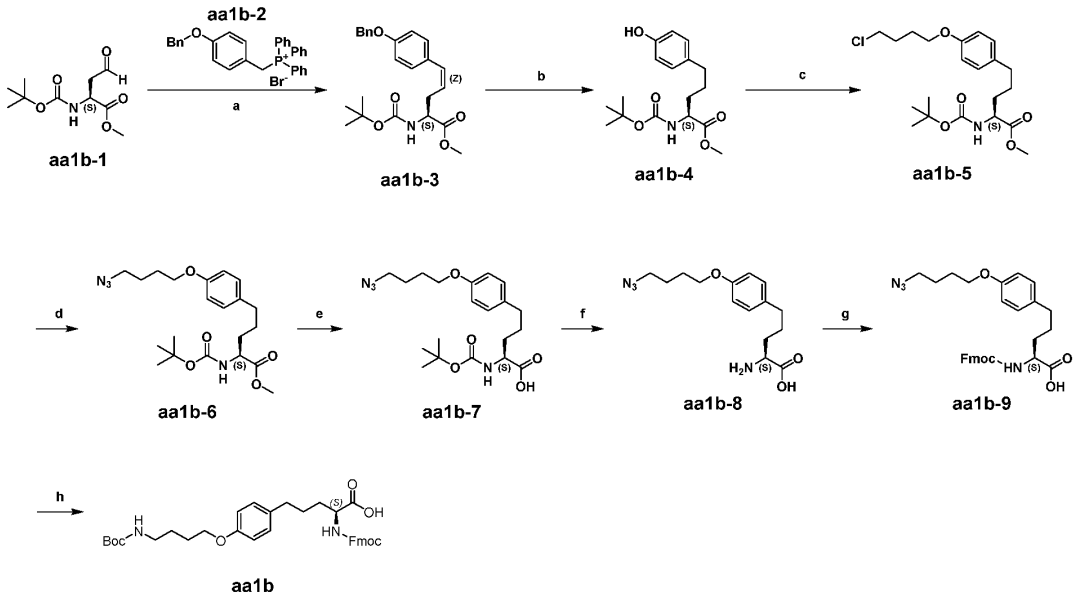
| | | | | | |
|---|---|---|---|---|---|
| **aa22** |  | Prepared | $C_{29}H_{30}N_3O_2$ | 451.2 | 452.3 $[M+H]^+$ |
| **aa23** |  | Prepared | $C_{29}H_{30}N_3O_2$ | 451.2 | 452.2 $[M+H]^+$ |
| **aa24** |  | Commercial | | | |
| **aa25** |  | Commercial | | | |
| **aa26** |  | Commercial | | | |
| **aa27** |  | Prepared | $C_{32}H_{41}N_3O_4$ | 531.3 | 554.1 $[M+Na]^+$ |
| **aa28** |  | Prepared | $C_{45}H_{58}N_4O_5$ | 734.4 | 757.5 $[M+Na]^+$. |
| **aa29** |  | Prepared | $C_{25}H_{22}N_2O_2$ | 382.17 | 381.17 $[M-H]^-$ |
| **aa30** |  | Prepared | $C_{26}H_{24}N_2O_2$ | 396.18 | 397.20 $[M-H]^-$ |
| **aa31** |  | Prepared | $C_{27}H_{26}N_2O_2$ | 410.20 | 411.3 $[M-H]^-$ |
| **aa32** |  | Prepared | $C_{28}H_{28}N_2O_2$ | 424.22 | 425.22 $[M-H]^-$ |

| | | | | | |
|---|---|---|---|---|---|
| **aa33** |  | Prepared | $C_{29}H_{30}N_2O_2$ | 438.23 | 439.23 $[M-H]^-$ |
| **aa34** |  | Prepared | $C_{30}H_{32}N_2O_2$ | 452.25 | 453.23 $[M-H]^-$ |
| **aa35** |  | Prepared | $C_{31}H_{34}N_2O_2$ | 466.26 | 465.25 $[M-H]^-$ |
| **aa36** |  | Prepared | $C_{13}H_{22}N_2O_4$ | 270.16 | 271.2 $[M+H]^+$ |
| **aa37** |  | Prepared | $C_{15}H_{16}N_2O_5S$ | 336.08 | 358.9 $[M+Na]^+$ |
| **aa38** |  | Commercial | $C_{18}H_{17}NO_4$ | 311.12 | / |
| **aa39** |  | Commercial | $C_7H_{11}NO_3$ | 157.07 | / |

2.1 Synthesis of unnatural amino acid (aa1b)

Scheme 2 outlines the synthesis of unnatural amino acid (aa1b):

**Scheme 2**




[000400] Reagents and conditions: a) 1-(benzyloxy)-4-(bromomethyl)benzene (aa1b-4) (1.0 eq.), t-BuOK (1.0 eq.), THF, 0-10 °C, 1 h, 67.93%; b) Pd/C (10%), $H_2$ (50 Psi), MeOH, 40 °C, 44

h; 94.24%; c) 1-chloro-4-iodobutane (1.5 eq.), $K_2CO_3$ (2.0 eq.), DMF, 50 °C, 16 h, 83.44%; d) $NaN_3$ (2.17 eq.), $K_2CO_3$ (2.0 eq.), DMF, 65 °C, 16 h, 98.44%; e) $LiOH·H_2O$ (2.0 eq.), THF/$H_2O$, 22 °C, 1 h, 100%; f) HCl/EtOAc, 22 °C, 1 h, 85.04%; g) aq. $NaHCO_3$ (0.43 M), Fmoc-osu, THF, 0-22 °C, 16 h, 92.11%; h) $(Boc)_2O$ (3.0 eq.), DIPEA (3.0 eq.), Pd/C (10%), $H_2$ (15 Psi), 4 h, 22.50%.

[000401] Aa1b-2 were prepared according to the detailed synthetic procedure found in Wu, X.Y.; Stockdill, J.L.; Park, P. K.; Samuel J. Danishefsky, S.J. Expanding the Limits of Isonitrile-Mediated Amidations: On the Remarkable Stereosubtleties of Macrolactam Formation from Synthetic Seco-Cyclosporins. *J. Am. Chem. Soc.* **2012**, 134, 2378–2384. Preparation of aa1b-1 was referred to at Du, J. J.; Gao, X. F.; Xin, L.M.; Lei, Z.; Liu, Z.; and Guo, J. Convergent Synthesis of N-Linked Glycopeptides via Aminolysis of ω-Asp p-Nitrophenyl Thioesters in Solution. Org. Lett. **2016**, *18*, 4828–4831.

*Step 1: Synthesis of (S,Z)-methyl 5-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)pent-4-enoate (aa1b-3)*

[000402] To a suspension of aa1b-2 (17.50 g, 32.43 mmol, 1.0 eq.) in THF (100 mL) was added t-BuOK (3.64 g, 32.43 mmol, 1.0 eq.) under nitrogen at 0 °C. The mixture was stirred at 0 °C for 30 min. A solution of aa1b-1 (7.5 g, 32.43 mmol, 1.0 eq.) in THF (50 mL) was added dropwise to the mixture. The reaction was warmed to 10 °C and stirred for 1 hr. Light yellow suspension was observed. The reaction was added to ice-water (300 mL) and extracted with EtOAc (200 mL × 2). The organic layers were combined and washed with brine (200 mL), dried over Na2SO4, filtered and concentrated to give crude as yellow oil. The residue was purified by flash silica gel chromatography (ISCO®; 80 g SepaFlash® Silica Flash Column, Eluent of 0~18% Ethylacetate/Petroleum ether gradient @ 50 mL/min) for 1.5 h with total volume 2.5 L to give aa1b-3 (9.7 g, 23.57 mmol, 67.93% yield) as a light yellow oil.

[000403] $^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.45 - 7.26 (m, 6H), 7.18 (br d, J=8.6 Hz, 1H), 6.92 (dd, J=8.7, 11.4 Hz, 2H), 6.56 - 6.34 (m, 1H), 5.98 - 5.83 (m,1H), 5.55 - 5.42 (m, 1H), 5.07 (d, J=2.2 Hz, 3H), 4.48 - 4.36 (m, 1H), 3.77 - 3.68 (m, 3H), 2.94 - 2.56 (m, 2H), 1.43 (s, 9H)

*Step 2: Synthesis of (S)-methyl 2-((tert-butoxycarbonyl)amino)-5-(4-hydroxyphenyl) pentanoate (aa1b-4)*

[000404] A solution of methyl aa1b-3 (9.7 g, 23.57 mmol, 1.0 eq.) and Pd/C (10% palladium on activated carbon, 1.0 g, 9.40 mmol) in MeOH (100 mL) was stirred at 40 °C under 50 Psi of hydrogen for 44 hr. Black suspension was observed. The reaction was filtered through a pad of

Celite, the cake was washed with MeOH (50 mL × 3). The filtrate was concentrated in vacuum to give **aa1b-4** (7.5 g, 22.22 mmol, 94.24% yield, 95.788% purity) as a gray solid.

[000405] $^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.05 - 6.92 (m, J=8.3 Hz, 2H), 6.79 - 6.70 (m, J=8.3 Hz, 2H), 5.59 (br s, 1H), 5.03 (br d, J=7.8 Hz, 1H), 4.37 - 4.26 (m, 1H), 3.71 (s, 3H), 2.61 - 2.47 (m, 2H), 1.81 (br s, 1H), 1.68 - 1.54 (m, 3H), 1.44 (s, 9H). LCMS (ESI): RT = 0.927 min, mass calcd. for $C_{17}H_{25}NO_5$ 323.17, m/z found 345.9 $[M+Na]^+$. Reverse phase LC-MS was carried out using method C.

*Step 3: Synthesis of (S)-methyl 2-((tert-butoxycarbonyl)amino)-5-(4-(4-chlorobutoxy)phenyl) pentanoate (aa1b-5)*

[000406] A solution of **aa1b-10** (7 g, 21.65 mmol, 1.0 eq.), **1-chloro-4-iodobutane** (7.09 g, 32.47 mmol, 1.5 eq.) and $K_2CO_3$ (5.98 g, 43.29 mmol, 2.0 eq.) in DMF (70 mL) was stirred at 50 °C for 16 hr. The combined reaction was added to ice-water (200 mL) and extracted with EtOAc (150 mL × 3). The organic layers were combined and washed with brine (150 mL × 2), dried over $Na_2SO_4$, filtered and concentrated to give crude as yellow oil. The residue was purified by flash silica gel chromatography (ISCO®; 80 g SepaFlash® Silica Flash Column, Eluent of 0~20% Ethyl acetate/Petroleum ether gradient @ 50 mL/min) for 1.5 h with total volume 2.5 L to give **aa1b-5** (8 g, 19.33 mmol, 83.44% yield) as a colorless oil.

[000407] $^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.04 (d, J=8.6 Hz, 2H), 6.79 (d, J=8.6 Hz, 2H), 4.95 (br d, J=7.3 Hz, 1H), 4.34 - 4.22 (m, 1H), 3.98 - 3.92 (m, 2H), 3.70 (s, 3H), 3.60 (t, J=6.2 Hz, 2H), 2.62 - 2.48 (m, 2H), 2.04 - 1.84 (m, 4H), 1.83 - 1.59 (m, 4H), 1.42 (s, 9H)

*Step 4: Synthesis of (S)-methyl 5-(4-(4-azidobutoxy)phenyl)-2-((tert-butoxycarbonyl)amino) pentanoate (aa1b-6)*

[000408] A mixture of **aa1b-5** (7.5 g, 18.12 mmol, 1.0 eq.), $NaN_3$ (2.56 g, 39.32 mmol, 2.17 eq.), $K_2CO_3$ (5.01 g, 36.24 mmol, 2.0 eq.) and KI (300.78 mg, 1.81 mmol, 0.1 eq.) in DMF (75 mL) was stirred at 65 °C for 16 hr. The reaction was added to ice-water (200 mL) and extracted with EtOAc (100 mL × 3). The organic layers were combined and washed with brine (100 mL × 3), dried over $Na_2SO_4$, filtered and concentrated to give **aa1b-6** (7.5 g, 17.84 mmol, 98.44% yield) as a yellow oil.

*Step 5: Synthesis of (S)-5-(4-(4-azidobutoxy)phenyl)-2-((tert-butoxycarbonyl)amino)pentanoic acid (aa1b-7)*

[000409] A solution of **aa1b-6** (7.5 g, 17.84 mmol, 1.0 eq.) and $LiOH·H_2O$ (1 M, 35.67 mL, 2.0 eq.) in THF (70 mL) was stirred at 22 °C for 1 hr. No change was observed. The reaction was concentrated in vacuum to remove THF. The reaction was adjusted to pH = 5 with 1N HCl and

extracted with EtOAc (10 mL × 2). The organic layers were combined and washed with brine (10 mL), dried over $Na_2SO_4$, filtered and concentrated to give **aa1b-7** (7.25 g, 17.84 mmol, 100.00% yield) as a colorless oil.

*Step 6: Synthesis of (S)-2-amino-5-(4-(4-azidobutoxy)phenyl)pentanoic acid hydrochloride (aa1b-8)*

[000410] A solution of **aa1b-7** (7.25 g, 17.84 mmol, 1.0 eq.) in 4M HCl/EtOAc (75 mL) was stirred at 22 °C for 1 hr. The reaction was concentrated in vacuum to give **aa1b-8** (5.2 g, 15.17 mmol, 85.04% yield, HCl) as a white solid.

*Step 7: Synthesis of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(4-(4-azidobutoxy) phenyl)pentanoic acid (aa1b-9)*

[000411] To a solution of aa1b-8 (5 g, 14.58 mmol, 1.0 eq., HCl) in THF (116 mL) was added $NaHCO_3$ (2.45 g, 29.17 mmol, 2.0 eq.) in $H_2O$ (58 mL), and then Fmoc-OSu (5.41 g, 16.04 mmol, 1.1 eq.) was added at 0 °C. The reaction mixture was stirred at 22 °C for 16 hr. No change was observed. The reaction was adjusted to pH=6 with 1N HCl and extracted with EtOAc (50 mL × 3). The organic layers were combined and washed with brine (50 mL), dried over $Na_2SO_4$, filtered and concentrated to give crude as yellow oil. The residue was purified by flash silica gel chromatography (ISCO®; 80 g SepaFlash® Silica Flash Column, Eluent of 0~5% MeOH/DCM gradient @ 50 mL/min) for 2.5 h with total volume 3 L to give **aa1b-9** (7 g, 12.20 mmol, 83.64% yield, 92.113% purity) as a yellow oil.

[000412] $^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.75 (br d, J=7.3 Hz, 2H), 7.60 - 7.49 (m, 2H), 7.41 - 7.34 (m, 2H), 7.29 (br t, J=7.5 Hz, 2H), 7.05 (br d, J=8.3 Hz, 2H), 6.78 (br d, J=8.3 Hz, 2H), 5.18 (br d, J=8.3 Hz, 1H), 4.40 (br d, J=6.8 Hz, 3H), 4.21 (br t, J=7.0 Hz, 1H), 3.98 - 3.88 (m, 2H), 3.34 (t, J=6.5 Hz, 2H), 2.57 (br d, J=6.1 Hz, 2H), 1.98 - 1.60 (m, 8H)

*Step 8: Synthesis of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(4-(4-((tert-butoxycarbonyl) amino) butoxy)phenyl)pentanoic acid (aa1b)*

[000413] A mixture of **aa1b-15** (7 g, 13.24 mmol, 1.0 eq.), DIPEA (5.13 g, 39.73 mmol, 6.92 mL, 3.0 eq.) and $Boc_2O$ (8.67 g, 39.73 mmol, 9.13 mL, 3.0 eq.) and Pd/C (10% palladium on activated carbon, 3.5 g, 32.9 mmol) in EtOAc (100 mL) was stirred at 25 °C under 15 PSi of $H_2$ for 4 hr. Black suspension was observed. The reaction was filtered through a pad of Celite, the cake was washed with EtOAc (50 mL × 3). The filtrate was washed with aq.$NH_4Cl$ (100 mL × 3), brine (100 mL), dried over $Na_2SO_4$, filtered and concentrated in vacuum to give crude as colorless oil. The crude was purified by prep-HPLC (column: Phenomenex Luna(2) C18 250*50 10 µm;mobile phase: [water(0.225%FA)-ACN];B%: 45%-78%,21.5min) to give **aa1b** (1.8 g, 2.99

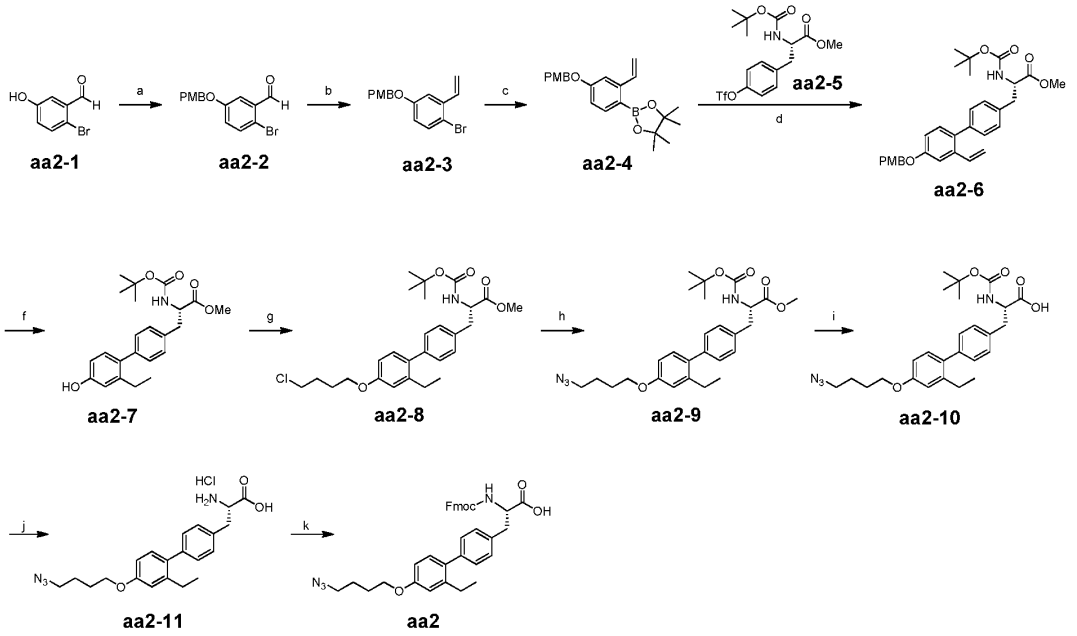
mmol, 22.50% yield) as a yellow oil. LCMS (ESI): RT = 0.954 min, mass calcd. for $C_{35}H_{42}N_2O_7Na$ 625.29, m/z found 625.1 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

[000414] $^1H$ NMR (400MHz, CHLOROFORM-d) δ = 7.75 (br d, J=7.6 Hz, 2H), 7.60 - 7.55 (m, 2H), 7.38 (br t, J=6.7 Hz, 2H), 7.29 (t, J=7.1 Hz, 2H), 7.04 (br d, J=8.1 Hz, 2H), 6.78 (d, J=7.4 Hz, 2H), 5.24 (br s, 1H), 4.40 (br d, J=6.6 Hz, 2H), 4.23 - 4.18 (m, 1H), 3.92 (br s, 2H), 3.21 - 3.07 (m, 2H), 2.61 - 2.51 (m, 2H), 1.97 - 1.84 (m, 2H), 1.82 - 1.54 (m, 8H), 1.43 (s, 9H)

2.2 The synthesis of unnatural amino acid (aa2)

Scheme 3 outlines the synthesis of unnatural amino acid (aa2):

**Scheme 3**




[000415] Reagents and Conditions: (a) $K_2CO_3$, PMB-Cl, DMF, 25 °C, 12h; (b) $Ph_3PCH_2Br$, t-BuOK, THF, 0-25 °C, 4 h, 71.91%; (c) $Pd(dppf)Cl_2$, KOAc, 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane, 1,4-dioxane, 80 °C, 20 h, 42.75%; (d) aa 2-6, $K_2CO_3$, $Pd(PPh_3)_4$, 1,4-dioxane and $H_2O$, 80 °C, 2.5 h, 76.20%; (e) Pd/C, $H_2$ (15 psi), MeOH, 25 °C, 48 h, 85.01%; (f) $K_2CO_3$, 1-chloro-4-iodo-butane, DMF, 50 °C, 12 h; (g) $K_2CO_3$, KI, $NaN_3$, DMF, 50 °C, 7 h;

[000416] The compound aa2-5 was prepared according to the following literature reference: I. Berezowska, N.N. Chung, C. Lemieux, B.C. Wilkes, and P.W. Schiller, Agonist vs Antagonist

Behavior of δ Opioid Peptides Containing Novel Phenylalanine Analogues in Place of Tyr. J. Med. Chem., Vol. 52, No. 21, 2009, 6941-6945.

*Step 1: Synthesis of 2-bromo-5-((4-methoxybenzyl)oxy)benzaldehyde (aa2-2)*

[000417] To a solution of **aa2-1** (10 g, 49.75 mmol, 1.0 eq.) and $K_2CO_3$ (10.31 g, 74.62 mmol, 1.5 eq.) in DMF (100 mL) was added PMB-Cl (11.69 g, 74.62 mmol, 10.16 mL, 1.5 eq.). The mixture was stirred at 25 °C for 12 hr. The reaction mixture was concentrated under reduced pressure. The residue was diluted with $H_2O$ (100 mL) and extracted with EtOAc (100 mL × 2). The combined organic layers was dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. Compound **aa2-2** (21 g, crude) was obtained as a white solid which was used directly in the next step without any further purification.

[000418] $^1$H NMR (400 MHz, DMSO-d6) δ = 10.16 (s, 1H), 7.69 (d, *J*=8.8 Hz, 1H), 7.43 - 7.34 (m, 3H), 7.28 (dd, *J*=3.3, 8.8 Hz, 1H), 6.99 - 6.90 (m, 2H), 5.10 (s, 2H), 3.75 (s, 3H).

*Step 2: Synthesis of 1-bromo-4-((4-methoxybenzyl)oxy)-2-vinylbenzene (aa2-3)*

[000419] To a solution of $Ph_3PCH_3Br$ (4.56 g, 12.77 mmol, 1.0 eq.) in THF (25 mL) was added *t*-BuOK (7.16 g, 63.83 mmol, 5.0 eq.) under nitrogen. The mixture was stirred for 1 hr at 0 °C. Then **aa2-2** (4.1 g, 12.77 mmol, 1.0 eq.) in THF (25 mL) was added dropwise. The mixture was stirred for 3 hr at 25 °C. The reaction mixture was diluted with $H_2O$ (100 mL) and extracted with EtOAc (100 mL × 2). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 5% Ethyl acetate/Petroleum ether gradient @ 30 mL/min) for 24 min with total volume 0.9 L. Compound **aa2-3** (2.93 g, 9.18 mmol, 71.91% yield) was obtained as a white solid.

*[000420]* $^1$H NMR (400 MHz, CHLOROFORM-d) δ = 7.46 - 7.29 (m, 3H), 7.18 - 7.09 (m, 1H), 7.06 - 6.96 (m, 1H), 6.95 - 6.86 (m, 2H), 6.81 - 6.70 (m, 1H), 5.72 - 5.59 (m, 1H), 5.40 - 5.29 (m, 1H), 5.03 - *4.90 (m, 2H), 3.86 - 3.74 (m, 3H).*

*Step 3: Synthesis of 2-(4-((4-methoxybenzyl)oxy)-2-vinylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (aa2-4)*

[000421] A solution of **aa2-3** (200 mg, 626.58 µmol, 1 eq.) in 1,4-dioxane (3 mL) was treated with $Pd(PPh_3)_4$ (72.41 mg, 62.66 µmol, 0.1 eq.) and KOAc (122.99 mg, 1.25 mmol, 2 eq.), and then 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (477.34 mg, 1.88 mmol, 3 eq.) was added. The mixture was stirred at 80 °C for 12 hr under nitrogen. The reaction mixture was diluted with brine (30 mL) and extracted with EtOAc (30 mL × 2). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated

under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 4 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 10% Ethyl acetate/Petroleum ether gradient @ 18 mL/min). Compound **aa2-4** (190 mg, 518.76 µmol, 82.79% yield) was obtained as a white solid.

[000422] $^1$H NMR (400 MHz, CHLOROFORM-d) δ = 7.75 (d, *J*=8.4 Hz, 1H), 7.55 (dd, *J*=10.9, 17.5 Hz, 1H), 7.36 (d, *J*=8.6 Hz, 2H), 7.22 (d, *J*=2.4 Hz, 1H), 6.97 - 6.89 (m, 2H), 6.87 (dd, *J*=2.4, 8.4 Hz, 1H), 5.67 (d, *J*=17.4 Hz, 1H), 5.26 (d, *J*=11.0 Hz, 1H), 5.03 (s, 2H), 3.82 (s, 3H), 1.34 (s, 12H).

*Step 4: Synthesis of (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(4'-((4-methoxybenzyl)oxy)-2'-vinyl-[1,1'-biphenyl]-4-yl)propanoate (aa2-6)*

[000423] A solution of aa2-4 (100 mg, 273.03 µmol, 1 eq.) in 1,4-dioxane (3 mL) and H2O (1 mL) was treated with $K_2CO_3$ (56.60 mg, 409.55 µmol, 1.5 eq.) and $Pd(PPh_3)_4$ (31.55 mg, 27.30 µmol, 0.1 eq.), and then **aa2-5** (116.69 mg, 273.03 µmol, 1 eq.) was added. The mixture was stirred at 80 °C for 2.5 hr under nitrogen. The reaction mixture was diluted with brine (30 mL) and extracted with EtOAc (30 mL × 2). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 4 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 10% Ethyl acetate/Petroleum ether gradient @ 18 mL/min) for 14 min with total volume 0.3 L. Compound **aa2-6** (100 mg, 193.20 µmol, 70.76% yield) was obtained as a yellow oil.

[000424] LCMS (ESI): RT = 0.950 min, mass calcd. for $C_{31}H_{35}NO_6Na$ 540.24, $[M+Na]^+$, m/z found 540.1 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

[000425] $^1$H NMR (400 MHz, CHLOROFORM-d) δ = 7.39 (d, *J* = 8.6 Hz, 2H), 7.27 - 7.25 (m, 2H), 7.23 (s, 2H), 7.17 (dd, *J* = 8.3, 16.2 Hz, 3H), 6.97 - 6.91 (m, 3H), 6.67 (dd, *J* = 11.0, 17.4 Hz, 1H), 5.67 (dd, *J* = 1.1, 17.4 Hz, 1H), 5.22 - 5.15 (m, 1H), 5.05 (s, 2H), 3.83 (s, 3H), 3.74 (s, 3H), 1.47 - 1.35 (m, 1H), 1.43 (s, 8H).

*Step 5: Synthesis of (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(2'-ethyl-4'-hydroxy-[1,1'-biphenyl]-4-yl)propanoate (aa2-7)*

[000426] To a solution of **aa2-6** (2.8 g, 5.41 mmol, 1.0 eq.) in MeOH (25 mL) was added Pd/C (300 mg, 10% palladium on activated carbon) and stirred for 48 hr at 25 °C under hydrogen (15 psi). The reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 30% Ethyl acetate/Petroleum ether, gradient @ 35 mL/min) for 22 min with total

volume 0.9 L. Compound **aa2-7** (1.85 g, 4.60 mmol, 85.01% yield, 99.3% purity) was obtained as a colorless oil.

[000427] LCMS (ESI): RT = 0.935 min, mass calcd. for $C_{23}H_{29}NO_5Na$ 422.20 $[M+Na]^+$, m/z found 422.1 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

[000428] $^1H$ NMR (400 MHz, CHLOROFORM-d) δ = 7.22 - 7.11 (m, 4H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.78 (d, *J* = 2.6 Hz, 1H), 6.69 (dd, *J* = 2.6, 8.2 Hz, 1H), 5.24 (s, 1H), 5.05 (br d, *J* = 8.4 Hz, 1H), 4.70 - 4.58 (m, 1H), 3.73 (s, 3H), 3.20 - 3.11 (m, 1H), 3.11 - 3.02 (m, 1H), 2.53 (q, *J* = 7.6 Hz, 2H), 1.42 (s, 9H), 1.08 (t, *J* = 7.5 Hz, 3H).

*Step 6: Synthesis of (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(4'-(4-chlorobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoate (aa2-8)*

[000429] To a solution of **aa2-7** (350 mg, 876.14 µmol, 1 eq.) and $K_2CO_3$ (242.18 mg, 1.75 mmol, 2.0 eq.) in DMF (5 mL) was added 1-chloro-4-iodo-butane (287.11 mg, 1.31 mmol, 1.5 eq.) at 25 °C. The mixture was stirred at 50 °C for 12 hr. The residue was diluted with brine (50 mL) and extracted with EtOAc (50 mL × 2). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 12 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 30% Ethyl acetate/Petroleum ether gradient @ 35 mL/min) for 14 min with total volume 0.4 L. Compound **aa2-8** (340 mg, crude) was obtained as a yellow oil.

[000430] LCMS (ESI): RT = 1.145 min, mass calcd. for $C_{27}H_{36}ClNO_5Na$ 512.22 $[M+Na]^+$, m/z found 512.2 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

*Step 7: Synthesis of (S)-methyl 3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-2-((tert-butoxycarbonyl)amino)propanoate (aa2-9)*

[000431] To a solution of **aa2-8** (1.6 g, 3.27 mmol, 1.0 eq.) in DMF (15 mL) was added $K_2CO_3$ (902.51 mg, 6.53 mmol, 2.0 eq.), KI (54.20 mg, 326.51 µmol, 0.1 eq.) and $NaN_3$ (460 mg, 7.08 mmol, 2.1 eq.). The mixture was stirred at 50 °C for 7 hr. The reaction mixture was diluted with brine 50 mL and extracted with EtOAc (50 mL × 2). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The water layers were quenched by addition of aqueous NaClO (1.0 M, 100 mL). Compound **aa2-9** (1.7 g, crude) was obtained as a yellow oil.

[000432] LCMS (ESI): RT = 1.140 min, mass calcd. for $C_{27}H_{36}N_4O_5Na$ 519.27, m/z found 519.3 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

*Step 8: Synthesis of (S)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-2-((tert-butoxycarbonyl)amino)propanoic acid (aa2-10)*

[000433] To a solution of **aa2-9** (1.7 g, 3.42 mmol, 1.0 eq.) in THF (12 mL) was added $LiOH·H_2O$ (287.31 mg, 6.85 mmol, 2.0 eq.) in $H_2O$ (6 mL) at 0 °C, and then the mixture was allowed to gradually warm to 25 °C and was stirred for 2 hr. The mixture was treated with EtOAc (30 mL) and extracted with water (25 mL × 2). The combined aqueous layers were acidified (1 M aqueous HCl) and extracted with EtOAc (50 mL × 3). The combined organic layer was dried by anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. Compound **aa2-10** (2.01 g, crude) was obtained as a yellow oil which was used directly in the next step without any further purification.

*Step 9: Synthesis of (S)-2-amino-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoic acid hydrochloride (aa2-11)*

[000434] Compound **aa2-10** (2.01 g, 4.17 mmol, 1.0 eq.) was dissolved in 4.0 M HCl/EtOAc (20 mL). The mixture was stirred at 25 °C for 1 hr. The reaction mixture was filtered. The filter cake was washed with EtOAc (30 ml) and dried under vacuum. Compound **aa2-11** (1 g, crude) was obtained as a white solid which was used directly in the next step without any further purification.

[000435] LCMS (ESI): RT = 1.121 min, mass calcd. for $C_{21}H_{27}N_4O_3$ 383.21 $[M+H]^+$, m/z found 383.2 $[M+H]^+$. Reverse phase LC-MS was carried out using method A.

Step 10: Synthesis of *(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoic acid* (aa2)

[000436] To a solution of **aa2-11** (1 g, 2.39 mmol, 1.0 eq.) in THF (15 mL) was added $NaHCO_3$ (401.07 mg, 4.77 mmol, 2.0 eq.) in $H_2O$ (8 mL), and then (2,5-dioxopyrrolidin-1-yl) 9H-fluoren-9-ylmethyl carbonate (805.23 mg, 2.39 mmol, 1.0 eq.) was added at 0 °C. The mixture was stirred for 12 hr at 25 °C. The reaction mixture was diluted with brine (100 mL) and acidified (1 M aqueous HCl) to pH = 2~3. The reaction mixture was extracted with EtOAc (30 mL × 2). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 10% Methanol/Dichloromethane @ 30 mL/min) for 16 min with total volume 0.6 L. Product **aa2** (1.3 g, 2.14 mmol, 89.52% yield, 99.4% purity) was obtained as a white foam.

[000437] LCMS (ESI): RT = 1.113 min, mass calcd. for $C_{36}H_{36}N_4O_5Na$ 627.26 $[M+Na]^+$, m/z found 627.3 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

[000438] $^1H$ NMR (400MHz, DMSO-$d_6$) δ = 7.88 (d, *J* = 7.5 Hz, 2H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.66 (t, *J* = 6.9 Hz, 2H), 7.40 (dt, *J* = 2.3, 7.3 Hz, 2H), 7.34 - 7.25 (m, 4H), 7.14 (d, *J* = 8.2 Hz,

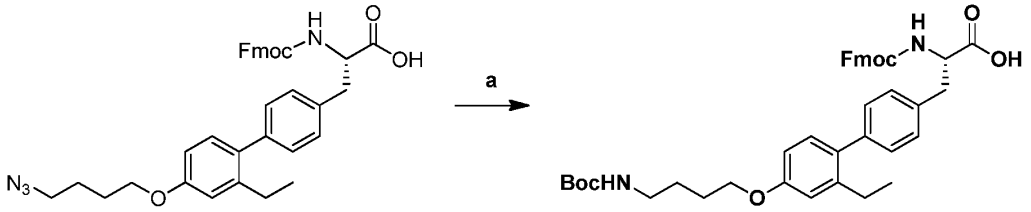
2H), 6.97 (d, *J* = 8.4 Hz, 1H), 6.83 (d, *J* = 2.4 Hz, 1H), 6.76 (dd, *J* = 2.5, 8.5 Hz, 1H), 4.27 - 4.17 (m, 3H), 4.17 - 4.13 (m, 1H), 4.03 - 3.98 (m, 2H), 3.42 (t, *J* = 6.7 Hz, 2H), 3.13 (br dd, *J* = 3.9, 13.8 Hz, 1H), 2.96 - 2.87 (m, 1H), 2.52 (d, *J* = 1.8 Hz, 2H), 2.43 (q, *J* = 7.4 Hz, 2H), 1.82 - 1.74 (m, 2H), 1.73 - 1.66 (m, 2H), 0.98 - 0.90 (m, 3H).

[000439] SFC: ee% = 97.95% - 2.05% = 95.9%; Method Comments: Column: Chiralcel OJ-3 100×4.6 mm I.D., 3μm; Mobile phase: A: CO2 B: ethanol (0.05% DEA); Gradient: from 5% to 40% of B in 4 min and hold 40% for 2.5 min, then 5% of B for 1.5min; Flow rate: 2.8 mL/min; Column temp.: 35℃; ABPR: 1500psi.

2.3 The synthesis of unnatural amino acid (aa2b)

[000440] Scheme 4 outlines the synthesis of unnatural amino acid (aa1b):

**Scheme 4**




[000441] Reagents and Conditions: (a) $H_2$, Pd/C, $(Boc)_2O$, DIPEA, MeOH, 20 °C, 12 h, 47%.

*Step 1: Synthesis of (R)-2-((((9H-fluoren-9-yl) methoxy) carbonyl) amino) -3- (4'-(4-((tert-butoxycarbonyl)amino)butoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl) propanoic acid (aa2b)*

[000442] To a solution of **aa2** (3.2 g, 5.29 mmol, 1.0 eq.) in MeOH (30 mL) was added Pd/C (700 mg, 10% palladium on activated carbon), DIPEA (1.37 g, 10.60 mmol, 1.84 mL, 2.0 *eq*) and $Boc_2O$ (2.31 g, 10.58 mmol, 2.43 mL, 2.0 *eq*). The mixture was stirred at 20 °C for 12 hr under hydrogen (15 psi). The reaction mixture was filtered through a small pad of Celite and the cake was rinsed with 40*5 mL of EtOAc (40 mL*5). Then brine (400 mL) was added and extracted with EtOAc (300 mL*2). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 250*50 mm*10 um; mobile phase: [water (0.225%FA)-ACN]; B%: 55%-86%, 21 min), the product was suspended in water (150 mL), the mixture frozen in a dry-ice/ethanol bath to afford the product **aa2b** (3.4 g, 5.01 mmol, 47.32% yield, 100% purity) as a white solid.

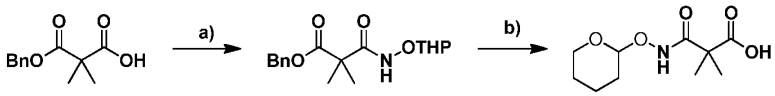
[000443] LCMS (ESI): RT = 0.922 min, mass calcd. for $C_{41}H_{46}N_2O_7Na$ 701.33 $[M+Na]^+$, m/z found 701.3 $[M+Na]^+$. Reverse phase LC-MS was carried out using method C.

[000444] $^1H$ NMR (400MHz, DMSO-d6) δ = 7.87 (d, J = 7.6 Hz, 2H), 7.69 - 7.60 (m, 2H), 7.43 - 7.36 (m, 2H), 7.33 - 7.20 (m, 4H), 7.15 - 7.05 (m, 3H), 6.95 (br d, J = 8.3 Hz, 1H), 6.89 - 6.70 (m, 4H), 4.28 (br dd, J = 5.9, 9.0 Hz, 1H), 4.17 - 4.09 (m, 2H), 4.00 - 3.91 (m, 3H), 3.19 - 3.09 (m, 2H), 3.02 - 2.88 (m, 2H), 2.46 - 2.38 (m, 2H), 1.73 - 1.62 (m, 2H), 1.56 - 1.48 (m, 2H), 1.37 (s, 9H), 0.93 (br t, J = 7.6 Hz, 3H).

2.4 The synthesis of unnatural amino acid (aa13)

[000445] Scheme 5 outlines the synthesis of unnatural amino acid (aa13):

**Scheme 5**




**aa13-1** **aa13-2** **aa13**

Reagents and conditions: (a) HATU (1.5 eq), DHP (1.0 eq), DIPEA (3.0 eq), DCM, 25°C, 2 h; (b) 10% Pd/C, MeOH, 25 °C, 5h.

[000446] aa13-1 was prepared according to WO2010/052253, the content of which are incorporated by reference herein in their entirety.

*Step 1: Synthesis of benzyl 2,2-dimethyl-3-oxo-3-(((tetrahydro-2H-pyran-2-yl)oxy)amino) propanoate (aa13-2)*

[000447] To a solution of **aa13-1** (200 mg, 899.94 µmol, 1 eq), HATU (513.28 mg, 1.35 mmol, 1.5 eq) and DIPEA (348.93 mg, 2.70 mmol, 470.26 µL, 3 eq) in DCM (10 mL) was stirred at 25 °C for 10 min. Otetrahydropyran-2-ylhydroxylamine (105.42 mg, 899.94 µmol, 1 eq) was added to the mixture and stirred at 25 °C for 2hr. The reaction was added DCM (5 mL) and washed with aq. $NH_4Cl$ (5 mL). The aqueous layer was separated and extracted with DCM (5 mL). The organic layers were combined and washed with brine (5 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuum to give crude as yellow oil, which was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~35% Ethylacetate/Petroleum ethergradient @ 30 mL/min) to give **aa13-2** (230 mg, 715.69 µmol, 79.53% yield) as a colorless oil.

[000448] $^1H$ NMR (400MHz, CHLOROFORM-d) δ = 9.28 - 9.14 (m, 1H), 7.38 - 7.30 (m, 5H), 5.16 (s, 2H), 4.87 (br s, 1H), 3.93 - 3.80 (m, 1H), 3.59 (br d, J=1.5 Hz, 1H), 1.76 (br s, 3H), 1.65 - 1.58 (m, 1H), 1.56 - 1.52 (m, 2H), 1.48 (s, 6H)

*Step 2: Synthesis of 2,2-dimethyl-3-oxo-3-(((tetrahydro-2H-pyran-2-yl)oxy)amino)propanoic acid (aa13)*

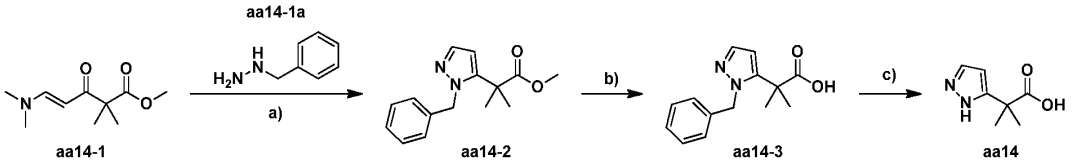
[000449] A black suspension of **aa13-2** (230 mg, 715.69 µmol, 1 eq) and 10% Pd/C (23 mg) in MeOH (5 mL) was stirred at 25 °C under 15 Psi of $H_2$ for 5 hr. The reaction was filtered through a pad of Celite, the cake was washed with MeOH (5 mL *3). The filtrate was concentrated in vacuum to give **aa13** (120 mg, 518.93 µmol, 72.51% yield) as colorless oil.

[000450] $^1$H NMR (400MHz, METHANOL-d4) δ = 4.90 - 4.88 (m, 1H), 4.05 (br s, 1H), 3.59 - 3.53 (m, 1H), 1.85 - 1.68 (m, 3H), 1.68 - 1.51 (m, 3H), 1.40 (s, 6H).

2.5 The synthesis of unnatural amino acid (aa14)

[000451] Scheme 6 outlines the synthesis of unnatural amino acid (aa14):

**Scheme 6**




[000452] Reagents and conditions: (a) aa-14-1a (1.0 eq), dioxane, 50°C, 15 h; (b) LiOH·$H_2O$ (2.0 eq), THF/H2O = 1/1, 50°C, 16 h; (c) 10% Pd/C, cat. TFA, EtOH

[000453] The compound **aa14-1** was prepared according to US2015/380666.

*Step 1: Synthesis of methyl 2-(1-benzyl-1H-pyrazol-5-yl)-2-methylpropanoate (aa14-3)*

[000454] A mixture of **aa14-1** (2.76 g, 13.85 mmol, 1 eq) and **aa14-1a** (2.20 g, 13.85 mmol, 1 eq) in dioxane (30 mL) was stirred at 50 °C for 15 hr. The reaction was filtered and the filtrate was concentrated in vacuum to give crude as brown oil. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~20% Ethylacetate/Petroleum ether gradient @ 25 mL/min) to give **aa14-2** (0.9 g, 3.48 mmol, 25.15% yield) as a light yellow oil.

[000455] $^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.53 (s, 1H), 7.29 - 7.26 (m, 1H), 7.23 - 7.16 (m, 1H), 6.98 (d, J=7.4 Hz, 2H), 6.22 (s, 1H), 5.23 (s, 2H), 3.26 (s, 3H), 1.56 (s, 6H)

*Step 2: Synthesis of methyl 2-(1-benzyl-1H-pyrazol-5-yl)-2-methylpropanoate (aa14-3)*

[000456] A mixture of **aa14-2** (700 mg, 2.71 mmol, 1 eq) and LiOH·$H_2O$ (1 M, 5.42 mL, 2 eq) in THF (5.4 mL) was stirred at 50 °C for 16 hr. The reaction was concentrated in vacuum to remove the THF. The reaction was diluted with water (5 mL) and MTBE (5 mL). The aqueous layer was separated and adjusted to pH=6 with 1N HCl. The organic layer was discarded. The

aqueous layer was extracted with EtOAc (5 mL*3). The organic layers were combined and washed with brine (5 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuum to give **aa14-3** (600 mg, 2.46 mmol, 90.64% yield) as a white solid.

[000457] $^1$H NMR (400MHz, CHLOROFORM-d) δ= 7.53 (s, 1H), 7.26 - 7.17 (m, 3H), 6.97 (d, J=7.4 Hz, 2H), 6.24 (s, 1H), 5.23 (s, 2H), 1.54 (s, 6H).

*Step 3: Synthesis of 2-methyl-2-(1H-pyrazol-5-yl)propanoic acid (aa14)*

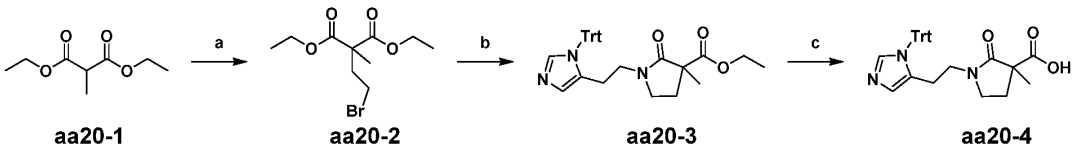
[000458] A mixture of **aa14-3** (500 mg, 2.05 mmol, 1 eq) , TFA (23.34 mg, 204.68 µmol, 15.15 µL, 0.1 eq) and 10% Pd/C (100 mg) in EtOH (5 mL) was stirred at 80 °C under 100 Psi of $H_2$ for 16 hr. The reaction was filtered through a pad of Celite, the cake was washed with EtOH (5 mL*3). The filtrate was concentrated in vacuum to give **aa14** (300 mg, 1.52 mmol, 74.27% yield, 78.11% purity) as a light yellow oil. LCMS (ESI): RT = 1.036 min, m/z calcd. for $C_7H_{11}N_2O_2$ $[M+H]^+$ 155.07, found 155.0. Reverse phase LC-MS was carried out using method A.

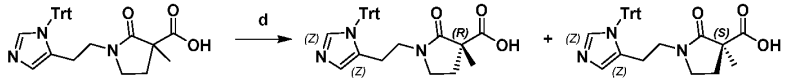
[000459] $^1$H NMR (400MHz, METHANOL-d4) δ= 7.57 - 7.50 (m, 1H), 6.26 (d, J=1.8 Hz, 1H), 1.56 (s, 6H).

2.6 The synthesis of unnatural amino acid [aa20: (R)-isomer and aa21: (S)-isomer]

[000460] Scheme 7 outlines the synthesis of unnatural amino acids (aa20) and (aa21):

**Scheme 7**




[000461] Reagents and conditions: (a) NaH (1.2 eq), dibromoethane (2 eq.), THF (25 ml), 0~100°C, 16h; (b) 2-(1-trityl-1H-imidazol-5-yl) ethanamine (0.9 eq), DMF (5 ml), 60°C, 16h; (c) LiOH (4 eq.), $H_2O$ (10 ml), THF (10 ml), 20°C, 16h; (d) SFC separation.

*Step 1: Synthesis of diethyl 2-(2-bromoethyl)-2-methylmalonate (aa20-2)*

[000462] A volume of THF (10 mL) was added to NaH (252.57 mg, 6.31 mmol, 60% purity, 1.1 *eq.*) under a $N_2$ atmosphere. The THF solution was cooled to 0°C. Compound **aa20-1** (1 g, 5.74 mmol, 980.39 µL, 1 *eq.*) in THF (5 mL) was added over 30 min with stirring. The reaction mixture was allowed to stir for 60 min at 20°C. The generated enolate was dripped into a 1, 2-

dibromoethane (2.16 g, 11.48 mmol, 866.23 µL, 2 *eq.*) in THF (10 mL) over 60 min with stirring under nitrogen atmosphere. The reaction mixture was then heated to 100°C in solvent for 14 hr. TLC indicated that the reactant was consumed, and one major new spot with lower polarity was detected. The residue was poured into 1N HCl to adjust pH 2~4. Then aqueous phase was extracted with ethyl acetate (30 mL*3). The combined organic phase was washed with brine (50 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuum. The residue was purified by flash silica gel chromatography (ISCO®; 24 g SepaFlash® Silica Flash Column, Eluent of 0~20% Ethyl acetate/Petroleum ether gradient @ 20 mL/min) for 40 min with 0.8 L solvent. The compound **aa20-2** (1.2 g, 4.27 mmol, 74.35% yield) was obtained as a pale yellow liquid.

[000463] $^1$H NMR (400 MHz, CHLOROFORM-d) δ 4.18 (q, J=7.20 Hz, 4H), 3.33-3.41 (m, 2H), 2.39-2.47 (m, 2H), 1.43 (s, 3H), 1.22 (s, 6H)

*Step 2: Synthesis of ethyl 3-methyl-2-oxo-1-(2-(1-trityl-1H-imidazol-5-yl)ethyl)pyrrolidine-3-carboxylate (aa20-3)*

[000464] To a solution of 2-(1-trityl-1H-imidazol-5-yl) ethanamine (1 g, 2.83 mmol, 1 *eq.*) in DMF (10 mL) was added **aa20-2** (874.95 mg, 3.11 mmol, 1.1 *eq.*). The mixture was stirred at 60°C for 16 hr. LCMS showed the material was consumed completely and the desired product was observed as the major. The residue was poured into water (100 mL). The aqueous phase was extracted with ethyl acetate (50 mL*3). The combined organic phase was washed with brine (100 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuum. The crude product was purified by C-18 reverse phase chromatography (ISCO®; 80 g, C-18 Column, Eluent of 0~100% acetonitrile/$H_2O$ gradient @ 40 mL/min, 60 min with total volume 2400 mL) to provide a yellow solid. The compound **aa20-3** (650 mg, 903.47 µmol, 31.93% yield, 70.55% purity) was obtained as a yellow solid.

[000465] LCMS (ESI): RT = 0.861 min, m/z calcd. for $C_{32}H_{34}N_3O_3$ 508.25 $[M+H]^+$, found 508.3. Reverse phase LC-MS was carried out using method A.

*Step 3: Synthesis of 3-methyl-2-oxo-1-(2-(1-trityl-1H-imidazol-5-yl)ethyl)pyrrolidine-3-carboxylic acid (aa20-4)*

[000466] To a mixture of **aa20-3** (650 mg, 1.28 mmol, 1 *eq.*) was added $LiOH.H_2O$ (268.67 mg, 6.40 mmol, 5 *eq.*) in $H_2O$ (10 mL) and THF (10 mL). The mixture was stirred at 20°C for 16 hr. LCMS showed the material was consumed completely and the desired product was observed as the major. The residue was poured into $H_2O$ (50 mL), added 5% $KHSO_4$ to adjust pH 2~3. The aqueous phase was extracted with ethyl acetate (50 mL*3). The combined organic phase was washed with brine (50 ml), dried with anhydrous $Na_2SO_4$, filtered and concentrated in vacuum.

The crude was purified by prep-HPLC (column: Phenomenex Synergi Max-RP 250*50mm*10 um; mobile phase: water (0.225% FA)-ACN; B%: 30%-60%, 60min) to give **aa20-4** (180 mg, 375.34 µmol, 29.31% yield) as a white solid.

[000467] LCMS (ESI): RT = 2.391 min, m/z calcd. for $C_{30}H_{30}N_3O_3$ 480.6, found 480.3 $[M+H]^+$. Reverse phase LC-MS was carried out using a Merck RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A)

[000468] $^1$H NMR (ES8586-496-P1B1) 1H NMR (400 MHz, METHANOL-d4) δ 7.92 (s, 1H), 7.35-7.42 (m, 9H), 7.12-7.19 (m, 6H), 6.97 (s, 1H), 3.39-3.64 (m, 3H), 3.32 (br d, J=3.75 Hz, 1H), 2.84(br t, J=5.95 Hz, 2H), 2.34-2.42 (m, 1H), 1.86 (td, J=7.99, 12.90 Hz, 1H), 1.25 (s, 3H)

*Step 4: Synthesis of (R)-3-methyl-2-oxo-1-(2-(1-trityl-1H-imidazol-5-yl)ethyl)pyrrolidine-3-carboxylic acid (aa20) and (S)-3-methyl-2-oxo-1-(2-(1-trityl-1H-imidazol-5-yl)ethyl)pyrrolidine-3-carboxylic acid (aa21)*

[000469] The absolute configurations of aa20 (*R*) and aa21 (*S*) were not confirmed.

[000470] **Compound aa20-4** (180 mg, 375.34 µmol) was purified by prep-SFC (column: DAICEL CHIRALPAK IC (250mm*30 mm, 10 µm; mobile phase: 0.1%$NH_3H_2O$ MEOH; B%: 50%-50%, 80min). Isomer **(*R*) aa20** (86 mg, 177.18 µmol, 94.41% yield, 98.8% purity) was obtained as a white solid, and isomer **(*S*) aa21** (85 mg, 175.29 µmol, 93.41% yield, 98.9% purity) was also obtained as a white solid.

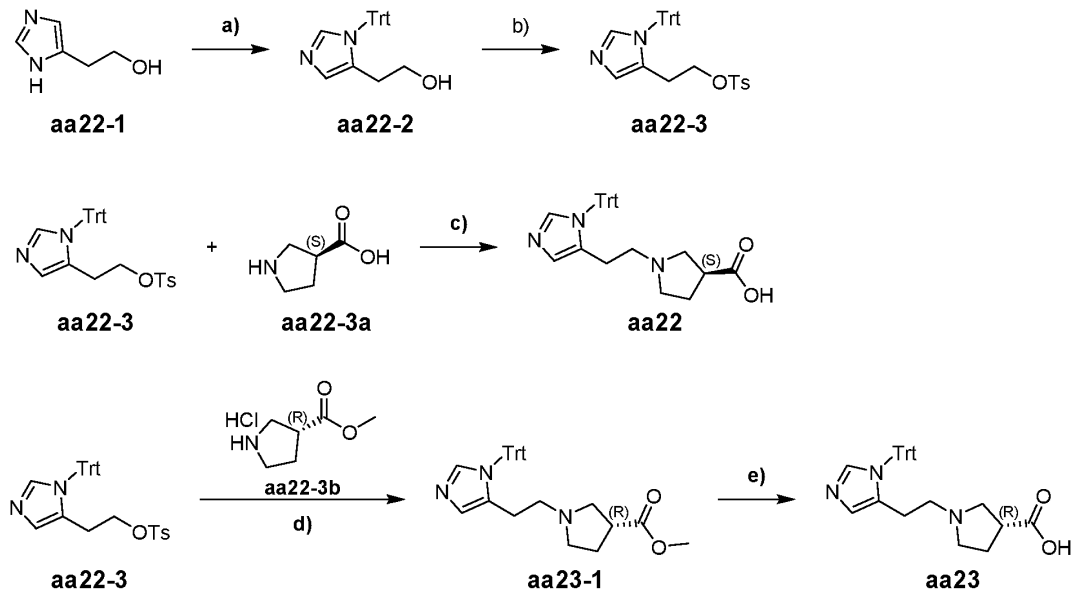
[000471] Isomer **aa20** on SFC-HPLC: RT = 2.346 min. HPLC conditions: Chiralpak IC-3 100×4.6 mm I.D., 3 µm, flow rate 2.8mL/min. eluting with a gradient of 40% methanol (0.05% DEA) (solvent B) and $CO_2$ (solvent A).

[000472] Isomer **aa21** on SFC-HPLC: RT = 3.607 min. HPLC conditions: Chiralpak IC-3 100×4.6 mm I.D., 3 µm, flow rate 2.8mL/min. eluting with a gradient of 40% methanol (0.05% DEA) (solvent B) and $CO_2$ (solvent A).

2.7 The synthesis of unnatural amino acid [aa22 (S)-isomer and aa23 (R)-isomer]

[000473] Scheme 8 outlines the synthesis of unnatural amino acids (aa22) and (aa23):

**Scheme 8**




[000474] Reagents and conditions: (a) TrtCl (1.2 eq.), $Et_3N$ (2 eq.), DMF, 20°C, 2 h, 31%; (b) TsCl (1.5 eq.), $Et_3N$ (3 eq.), DMAP (0.5 eq.), DCM, 20°C, 2 h, 66%; (c) aa22-3 (1.0eq.), aa22-3a (1.2 eq.), LiI (1.5 eq.), DIPEA (4 eq.), 60°C, 2 h, 17% yield; (d) aa22-3 (1.0eq.), aa22-3b (1.2 eq.), LiI (1.5 eq.), $K_2CO_3$ (4 eq.), 20°C, 3 h, 58% yield; (e) NaOH (2 eq.), MeOH/water, 20°C, 2h, 87% yield.

*Step 1: Synthesis of 2-(1-trityl-1H-imidazol-4-yl) ethanol (aa22-2)*

[000475] To a solution of **aa22-1** (2 g, 17.84 mmol, 1 eq) in DMF (40 mL) were added TEA (3.61 g, 35.67 mmol, 4.97 mL, 2 eq), [chloro(diphenyl)methyl]benzene (5.97 g, 21.40 mmol, 1.2 eq) at 20 °C. The reaction mixture was stirred for 2 hr at 20 °C. The reaction progress was monitored by TLC (DCM:MeOH = 10:1), which indicated that the starting material was consumed and one new spot was observed. The reaction mixture was quenched by $NaHCO_3$ (sat. aq., 20 mL) and extracted with EtOAc (50 mL*2). The combined organic layers were washed with brine (20 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethyl acetate/Petroleum ether then 0~10% MeOH (0.5% TEA additive) /DCM gradient @ 30 mL/min) for 25 min with total volume 1.6 L. Compound **aa22-2** (2.2 g, 5.59 mmol, 31.32% yield, 90% purity) was obtained as a white solid.

[000476] LCMS: (ESI): RT = 1.397 min, m/z calcd. for $C_{24}H_{23}N_2O$ 355.2, found 355.2 $[M+H]^+$; Reverse phase LC-MS was carried out using method B.

[000477] $^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.39 - 7.31 (m, 10H), 7.17 - 7.11 (m, 6H), 6.63 - 6.57 (m, 1H), 3.89 (t, J=5.6 Hz, 2H), 3.64 (br s, 1H), 2.76 (t, J=5.5 Hz, 2H).

*Step 2: Synthesis of 2-(1-trityl-1H-imidazol-4-yl)ethyl 4-methylbenzenesulfonate (aa22-3)*

[000478] To a solution of **aa22-2** (2.2 g, 5.59 mmol, 1 eq) in DCM (20 mL) were added TEA (1.70 g, 16.76 mmol, 2.33 mL, 3 eq), 4-methylbenzenesulfonyl chloride (1.60 g, 8.38 mmol, 1.5 *eq*) and DMAP (341.23 mg, 2.79 mmol, 0.5 *eq*) at 20 °C. The reaction mixture was stirred for 2 hr at 20 °C. The reaction progress was monitored by LC-MS which indicated no starting material remained and formation of desired product. The reaction mixture was quenched by $NaHCO_3$ (sat. aq., 50 mL) and extracted with EtOAc (50 mL*2). The combined organic layers were washed with brine (20 mLx2), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 24g SepaFlash® Silica Flash Column, Eluent of 0~5% MeOH (4% TEA additive)/DCM gradient @ 20 mL/min) for 35 min with total volume 1.2 L. Compound **aa22-3** (2.1 g, 3.72 mmol, 66.52% yield, 90% purity) was obtained as a brown solid. LCMS: (ESI): RT = 0.775 min, m/z calcd. for $C_{31}H_{28}N_2O_3SNa$ 531.2 $[M+H]^+$, found 531.1. Reverse phase LC-MS was carried out using method D.

[000479] $^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.73 (d, J=8.3 Hz, 2H), 7.38 - 7.28 (m, 12H), 7.15 - 7.08 (m, 6H), 6.61 (s, 1H), 4.27 (t, J=7.0 Hz, 2H), 2.91 - 2.86 (m, 2H), 2.42 (s, 3H).

*Step 3: Synthesis of (R)-1-(2-(1-trityl-1H-imidazol-4-yl)ethyl)pyrrolidine-3-carboxylic acid (aa22)*

[000480] To a solution of **aa22-3** (500 mg, 983.03 μmol, 1 *eq.*) in DMF (2 mL) were added **aa22-3a** (135.81 mg, 1.18 mmol, 1.2 *eq.*), LiI (197.36 mg, 1.47 mmol, 56.55 μL, 1.5 *eq.*), and DIPEA (508.20 mg, 3.93 mmol, 684.91 μL, 4.0 *eq.*). Then the mixture was heated to 60 °C and stirred at 60 °C for 2 hr. LCMS showed that reactant was consumed completely and the desired MS was detected. The reaction mixture was quenched by addition of water (10 mL), and then diluted with EtOAc (20 mL), then extracted with EtOAc (20 mL * 2). The aqueous layers were acidfied with 2M HCl to pH 6. Then the aqueous phase was extracted with DCM (20 mL * 5). The combined organic layers were concentrated to give the residue. The crude product was purified by reversed-phase HPLC (neutral condition). Product **aa22** (80 mg, 168.31 μmol, 17.12% yield, 95% purity) was obtained as an off-white solid. LCMS (ESI): RT = 1.986 min mass calcd. for $C_{29}H_{30}N_3O_2$ 452.23, m/z found 452.3 $[M+H]^+$. Reverse phase LC-MS was carried out using method A.

*Step 4: Synthesis of methyl (3S)-1-[2-(1-tritylimidazol-4-yl)ethyl]pyrrolidine-3-carboxylate (aa23-1)*

[000481] To a solution of **aa22-3** (750 mg, 1.47 mmol, 1 eq.) in DMF (5 mL) was added $K_2CO_3$ (815.17 mg, 5.90 mmol, 4 eq.), **aa22-3b** (293.05 mg, 1.77 mmol, 1.2 eq., HCl) and LiI (296.04 mg, 2.21 mmol, 84.83 µL, 1.5 eq.) at 20 °C. The reaction mixture was stirred for 3 hr at 20 °C. The reaction progress was monitored by LC-MS, which indicated no starting material remained and formation of desired product. The mixture was cooled to 25 °C and poured into water (30 mL) and stirred for 5 min. The aqueous phase was extracted with ethyl acetate (50 mL*2). The combined organic phase was washed with brine (15 mL*2), dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuum. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethyl acetate/Petroleum ether then 0~10% MeOH (0.5% TEA additive) /DCM gradient @ 30 mL/min) for 25 min with total volume 1.6 L. Product **aa23-1** (420 mg, 856.99 µmol, 58.12% yield, 95% purity) was obtained as a brown oil.

[000482] LCMS: (ESI): RT = 0.717 min, m/z calcd. for $C_{30}H_{31}N_3O_2$ 466.2 $[M+H]^+$, found 465.9 ; LC-MS Conditions: Reverse phase LC-MS was carried out using method D.

[000483] $^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.40 - 7.30 (m, 10H), 7.20 - 7.09 (m, 6H), 6.58 (s, 1H), 3.75 - 3.64 (m, 3H), 3.10 - 2.95 (m, 2H), 2.83 - 2.74 (m, 5H), 2.72 - 2.64(m, 1H), 2.55 (q, J=8.0 Hz, 1H), 2.14 - 2.07 (m, 2H).

*Step 5: Synthesis of methyl (3S)-1-[2-(1-tritylimidazol-4-yl)ethyl]pyrrolidine-3-carboxylate (aa23)*

[000484] To a solution of **aa23-1** (390.00 mg, 837.66 µmol, 1 eq.) in MeOH (3 mL) and Water (3 mL) was added NaOH (67.01 mg, 1.68 mmol, 2 eq.) at 20 °C. The reaction mixture was stirred for 2 hr at 20 °C. The reaction progress was monitored by LCMS which indicated no starting material remained and formation of desired product. After completion, the reaction mixture was cooled to room temperature. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was adjusted to pH=6 with 1M HCl and dried by lyophilization. The residue was triturated with DCM/MeOH=10/1 (15 mL*3). The combined organic phase was dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuum. Product **aa23** (350 mg, 736.34 µmol, 87.90% yield, 95% purity) was obtained as a brown solid.

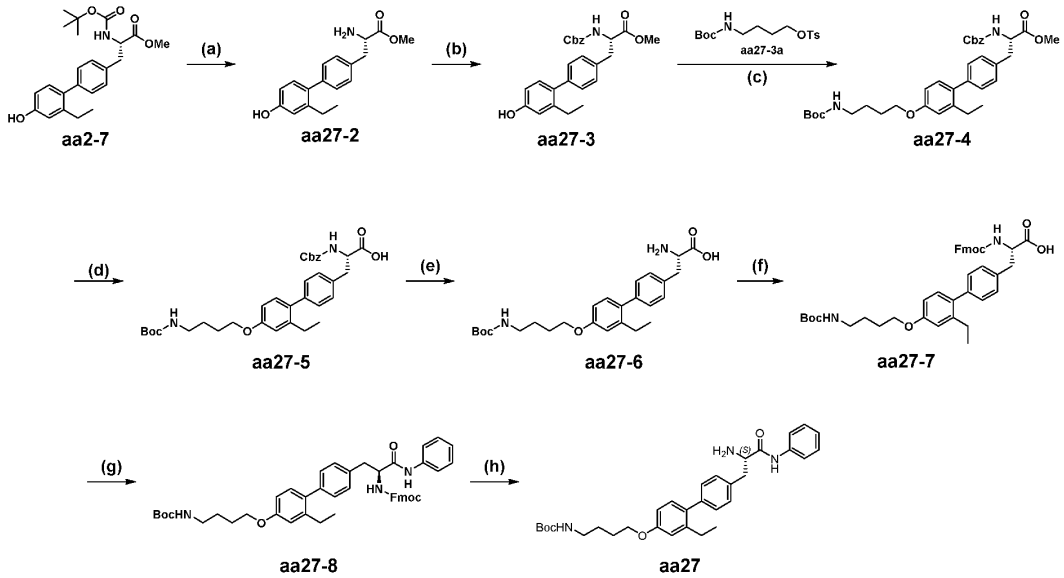
[000485] LCMS: (ESI): RT = 0.708 min, m/z calcd. for $C_{29}H_{30}N_3O_2$ 452.2 $[M+H]^+$, found 451.9; Reverse phase LC-MS was carried out using method D.

[000486] $^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.38 (s, 1H), 7.36 - 7.31 (m, 9H), 7.12 (dd, J=3.3, 6.3 Hz, 6H), 6.64 (s, 1H), 3.83 (br s, 1H), 3.56 (br s, 1H), 3.20 (br d, J=18.5 Hz,2H), 3.05-2.96 (m, 4H), 2.65 - 2.61 (m, 1H), 2.42-2.30 (m, 1H), 2.18-2.09 (m, 1H).

2.8 The synthesis of unnatural amino acid (aa27)

[000487] Scheme 9 outlines the synthesis of unnatural amino acid (aa27):

**Scheme 9**




[000488] Reagents and conditions: (a) HCl/dioxane, 0-25 °C, 2 h, 100%; (b) CbzCl (1.2 eq.), HOSu (1.3 eq.), DIPEA (2 eq.), DCM, 0-20 °C, 12 h, 96%; (c) 3a (2 eq.), $K_2CO_3$ (2 eq.), 60 °C, 12 h, 56%; (d) LiOH (2.0 eq.), THF, $H_2O$, 20 °C, 12h, 94%; (e) $H_2$, $Pd(OH)_2$, AcOH, MeOH, 20 °C, 12h; (f) FmocOSu (1.2 eq.), $NaHCO_3$ (2.0 eq.), THF, $H_2O$, 0-20 °C, 12h, 44%; (g) $PhNH_2$ (1.1 eq.), DIC (1.0 eq.), HOBt (1.0 eq.), DCM, 0-20 °C, 1h, 76%; (h) piperidine (5.0 eq.), DMF, 20 °C, 1h, 65%.

*Step 1: Synthesis of methyl (2S)-2-amino-3-[4-(2-ethyl-4-hydroxy-phenyl)phenyl]propanoate (aa27-2)*

[000489] **Compound aa27-1** (3.5 g, 8.76 mmol, 1 *eq.*) was dissolved in HCl/dioxane (4 M, 100 mL, 45.65 *eq.*) at 0°C. The mixture was stirred at 25°C for 2 hr. The reaction progress was monitored by LCMS. The reaction mixture was concentrated under reduced pressure to give the crude product. **Compound aa27-2** (2.6 g, crude) was obtained as a colorless oil.

[000490] LCMS (ESI): RT = 0.772 min, mass calcd. for $C_{18}H_{22}NO_3$, 300.16 $[M+H]^+$, m/z found 300.00 $[M+H]^+$. Reverse phase LC-MS was carried out using method A.

*Step 2: Synthesis of methyl (2S)-2-(benzyloxycarbonylamino)-3-[4-(2-ethyl-4-hydroxy-phenyl) phenyl]propanoate (aa27-3)*

[000491] To a solution of HOSu (1.25 g, 10.86 mmol, 1.3 *eq.*) in DCM (50 mL) was added DIPEA (3.24 g, 25.05 mmol, 4.36 mL, 3 *eq.*) and CbzCl (1.57 g, 9.19 mmol, 1.31 mL, 1.1 *eq.*) at 0°C. After stirred at 25°C for 2 hr, then **Compound aa27-2** (2.5 g, 8.35 mmol, 1 *eq.*) was added and the mixture was stirred at 20 °C for 10 hr. The reaction progress was monitored by LC-MS and TLC. The mixture was diluted with DCM (30 mL) and washed with $H_2O$ (30 mL*2). The organic layer was dried over $Na_2SO_4$, filtered and concentrated to give a residue, then purified by column chromatography ($SiO_2$, Petroleum ether/Ethyl acetate=2/1). **Compound aa27-3** (3.5 g, 8.07 mmol, 96.68% yield) was obtained as a yellow oil.

[000492] LCMS (ESI): RT = 0.992 min, mass calcd. for C26H27NNaO5+, 456.18 [M+Na]+, m/z found 456.2 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

[000493] $^1H$ NMR (400 MHz, DMSO-*d6*) δ ppm 9.35 (s, 1 H) 7.88 (br d, *J*=8.07 Hz, 1 H) 7.21 - 7.38 (m, 7 H) 7.15 (br d, *J*=8.07 Hz, 2 H) 6.93 (d, *J*=8.31 Hz, 1 H) 6.70 (d, *J*=2.20 Hz, 1 H) 6.63 (dd, *J*=8.07, 2.45 Hz, 1 H) 4.99 (br s, 2 H) 4.24 - 4.36 (m, 1 H) 3.60 - 3.65 (m, 3 H) 3.07 (br dd, *J*=13.69, 4.89 Hz, 1 H) 2.91 (br dd, *J*=13.57, 10.39 Hz, 1 H) 2.40 - 2.48 (m, 2 H) 0.99 (t, *J*=7.58 Hz, 3 H).

*Step 3: Synthesis of (S)-methyl 2-(((benzyloxy)carbonyl)amino)-3-(4'-(4-((tert-butoxycarbonyl) amino)butoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoate (aa27-4)*

[000494] To a solution of **aa27-3** (3.3 g, 7.61 mmol, 1 *eq.*) and **aa27-3a** (5.23 g, 15.23 mmol, 2 *eq.*) in DMF (30 mL) was added $K_2CO_3$ (2.10 g, 15.23 mmol, 2 *eq.*). The mixture was stirred at 60°C for 12 hr. The reaction progress was monitored by LC-MS and TLC. The reaction mixture was diluted with EtOAc (100 mL) and washed with $H_2O$ (80 mL*3). The organic layer was dried over $Na_2SO_4$, filtered and concentrated to give a residue, then purified by column chromatography ($SiO_2$, Petroleum ether/Ethyl acetate=10/1 to 2/1). **Compound aa27-4** (2.9 g, 4.32 mmol, 56.70% yield, 90% purity) was obtained as a yellow oil.

[000495] LCMS (ESI): RT = 1.140 min, mass calcd. for $C_{35}H_{44}N_2NaO_7$, 627.30 $[M+Na]^+$, m/z found 627.2 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

*Step 4: Synthesis of (S)-2-(((benzyloxy)carbonyl)amino)-3-(4'-(4-((tert-butoxycarbonyl) amino) butoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoic acid (aa27-5)*

[000496] To a solution of **aa27-4** (2.7 g, 4.46 mmol, 1 *eq.*) in THF (30 mL) were added a solution of $LiOH·H_2O$ (374.72 mg, 8.93 mmol, 2 *eq.*) in $H_2O$ (15 mL). The mixture was stirred at 20 °C for 2 hr. The reaction progress was monitored by LC-MS. The mixture was adjusted to pH 3~4 with 1M HCl and extracted with EtOAc (20 mL*3). The organic layer was dried over $Na_2SO_4$,

filtered and concentrated to give the product. **Compound aa27-5** (2.5 g, 4.23 mmol, 94.79% yield) was obtained as a yellow oil.

[000497] LCMS (ESI): RT = 1.082 min, mass calcd. for $C_{34}H_{42}N_2NaO_7$, 613.29, m/z found 613.2 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

[000498] $^1H$ NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.29 - 7.36 (m, 5 H) 7.14 - 7.21 (m, 4 H) 7.07 (br d, *J*=8.56 Hz, 1 H) 6.81 (s, 1 H) 6.73 (br d, *J*=7.83 Hz, 1 H) 5.27 (br d, *J*=7.58 Hz, 1 H) 5.05 - 5.16 (m, 2 H) 4.68 - 4.75 (m, 1 H) 4.50 - 4.68 (m, 2 H) 3.99 (br s, 2 H) 3.20 - 3.28 (m, 2 H) 2.53 (q, *J*=7.42 Hz, 2 H) 1.62 - 1.68 (m, 2 H) 1.51 - 1.57 (m, 2 H) 1.44 (br s, 9 H) 1.07 (t, *J*=7.46 Hz, 3 H).

*Step 5: Synthesis of (2S)-2-amino-3-[4-[4-[4-(tert-butoxycarbonylamino)butoxy]-2-ethyl-phenyl] phenyl]propanoic acid (aa27-6)*

[000499] To a solution of **aa27-5** (1.5 g, 2.54 mmol, 1 *eq.*) in MeOH (30 mL) were added $Pd(OH)_2/C$ (300 mg, 213.62 µmol, 10% purity) and AcOH (105.00 mg, 1.75 mmol, 0.1 mL). The mixture was stirred under $H_2$ at 20 °C for 12 hr. The reaction progress was monitored by LC-MS. The mixture was filtered and concentrated to give the crude product. **Compound aa27-6** (1.16 g, crude) was obtained as a yellow oil.

[000500] LCMS (ESI): RT = 0.890 min, mass calcd. for $C_{26}H_{36}N_2NaO_5$, 479.25, m/z found 479.1 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

*Step 6: Synthesis of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4'-(4-((tert-butoxycarbonyl)amino)butoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoic acid (aa27-7)*

[000501] To a solution of **aa27-6** (1.16 g, 2.54 mmol, 1 *eq.*) in THF (15 mL) and $H_2O$ (8 mL) were added $NaHCO_3$ (426.64 mg, 5.08 mmol, 197.52 µL, 2 *eq.*) and Fmoc-OSu (1.03 g, 3.05 mmol, 1.2 *eq.*) at 0 °C. The mixture was stirred 20 °C for 12 hr. The reaction progress was monitored by LC-MS. The mixture was adjusted to pH 3~4 and extracted with EtOAc (20 mL*2). The organic layer was dried over $Na_2SO_4$, filtered and concentrated to give a residue, then purified by prep-HPLC (AcOH condition; MeCN/$H_2O$, 0~100%). **Compound aa27-7** (950 mg, 1.12 mmol, 44.09% yield, 80% purity) was obtained as a colorless oil.

[000502] LCMS (ESI): RT = 1.142 min, mass calcd. for $C_{41}H_{46}N_2NaO_7$, 701.32, m/z found 701.0 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

[000503] HPLC: RT = 4.33 min, Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 2 minutes at a flow rate of 1.2 ml/min; Column: Ultimate C18 3.0*50mm,3 µm.

[000504] SFC: RT = 0.598 min, Column: Chiralpak AD-3 50×4.6 mm I.D., 3 µm; Mobile phase: A: CO2 B: ethanol (0.05% DEA) Isocratic: 40% B; Flow rate: 4mL/min.

[000505] $^1$H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.29 - 7.36 (m, 5 H) 7.14 - 7.21 (m, 4 H) 7.07 (br d, *J*=8.56 Hz, 1 H) 6.81 (s, 1 H) 6.73 (br d, *J*=7.83 Hz, 1 H) 5.27 (br d, *J*=7.58 Hz, 1 H) 5.05 - 5.16 (m, 2 H) 4.68 - 4.75 (m, 1 H) 4.50 - 4.68 (m, 2 H) 3.99 (br s, 2 H) 3.20 - 3.28 (m, 2 H) 2.53 (q, *J*=7.42 Hz, 2 H) 1.62 - 1.68 (m, 2 H) 1.51 - 1.57 (m, 2 H) 1.44 (br s, 9 H) 1.07 (t, *J*=7.46 Hz, 3 H).

*Step 7: Synthesis of 9H-fluoren-9-ylmethyl N-[(1S)-2-anilino-1-[[4-[4-[4-(tert-butoxycarbonylamino)butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-oxo-ethyl]carbamate (aa27-8)*

[000506] To a solution of **aa27-7** (250mg, 368.29 µmol, 1 *eq.*) and HOBt (49.76 mg, 368.29 µmol, 1 *eq.*) in DCM (1.5 mL) were added aniline (36.01 mg, 386.71 µmol, 35.31 µL, 1.05 *eq.*) and a solution of DIC (46.48 mg, 368.29 µmol, 57.03 µL, 1 *eq.*) in DCM (0.5 mL) at 0°C. The mixture was stirred at 20°C for 1 hr. The reaction progress was monitored by LC-MS. The mixture was diluted with DCM (20 mL) and washed with $H_2O$ (10 mL*2). The organic layer was dried over $Na_2SO_4$, filtered and concentrated to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 12 g SepaFlash® Silica Flash Column, Eluent of 0~50% Ethyl acetate/Petroleum ether gradient @ 20 mL/min). **Compound aa27-8** (220 mg, 283.05 µmol, 76.86% yield, 97% purity) was obtained as a white solid.

[000507] LCMS (ESI): RT = 1.198 min, mass calcd. for $C_{47}H_{51}N_3NaO_6$ 776.37, m/z found 777.3 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A.

[000508] HPLC: RT = 8.07 min, Reverse phase HPLC analysis was carried out using method D.SFC: RT = 2.186 min; Column: Chiralcel OD-3 50×4.6 mm I.D., 3 µm; Mobile phase: A: CO2 B: ethanol (0.05% DEA); Isocratic: 40% B; Flow rate: 4mL/min.

[000509] $^1$H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.74 (br d, *J*=7.58 Hz, 2 H) 7.51 - 7.58 (m, 3 H) 7.30 - 7.40 (m, 4 H) 7.28 (br d, *J*=7.58 Hz, 4 H) 7.17 - 7.24 (m, 4 H) 7.07 - 7.11 (m, 1 H) 7.02 (d, *J*=8.31 Hz, 1 H) 6.81 (d, *J*=2.45 Hz, 1 H) 6.72 (dd, *J*=8.31, 2.45 Hz, 1 H) 5.57 (br s, 1 H) 4.32 - 4.69 (m, 4 H) 4.20 (t, *J*=6.72 Hz, 1 H) 3.99 (t, *J*=6.24 Hz, 2 H) 3.06 - 3.29 (m, 4 H) 2.49 (q, *J*=7.42 Hz, 2 H) 1.77 - 1.87 (m, 2 H) 1.68 (dt, *J*=14.55, 7.15 Hz, 2 H) 1.44 (s, 9 H) 1.03 (t, *J*=7.58 Hz, 3 H).

*Step 8: Synthesis of tert-butyl N-[4-[4-[4-[(2S)-2-amino-3-anilino-3-oxo-propyl]phenyl]-3-ethyl-phenoxy]butyl]carbamate (aa27)*

[000510] To a solution of **aa27-8** (220 mg, 291.81 µmol, 1 *eq.*) in DMF (2 mL) was added a solution of piperidine (124.24 mg, 1.46 mmol, 5 *eq.*). The mixture was stirred at 20 °C for 1 hr.

The reaction progress was monitored by LC-MS. The mixture was diluted with EtOAc (30 mL) and washed with $H_2O$ (10 mL*3). The organic layer was dried over $Na_2SO_4$, filtered and concentrated to give a residue, then purified by column chromatography ($SiO_2$, EtOAc:MeOH = 1:0 to 5:1). **Compound aa27** (105 mg, 191.56 μmol, 65.65% yield, 97% purity) was obtained as a yellow foam.

[000511] LCMS (ESI): RT = 0.953 min, mass calcd. for $C_{32}H_{41}N_3NaO_4$ 554.30, m/z found 554.1 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A. HPLC: RT = 9.13 min, Reverse phase HPLC analysis was carried out using method C.

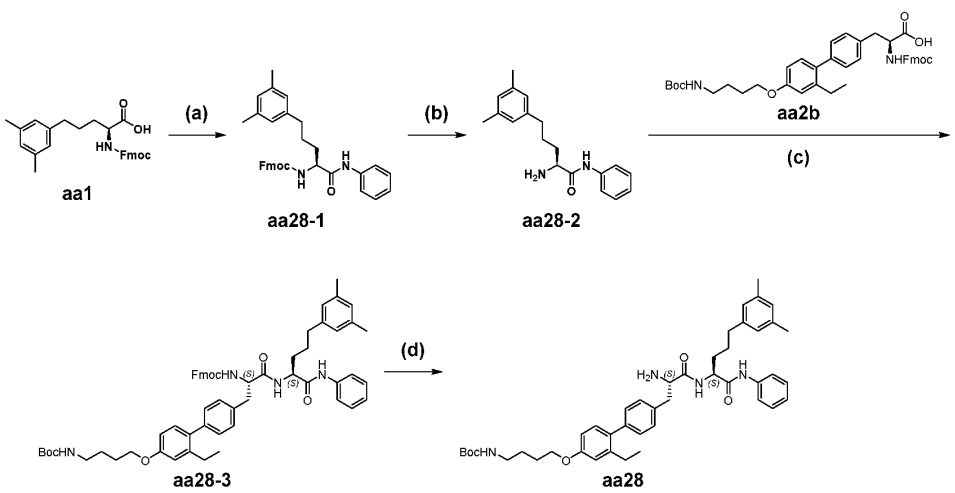
[000512] SFC: RT = 3.185 min, Column: Chiralpak AS-3 100×4.6 mm I.D., 3μm; Mobile phase: A: $CO_2$ B:ethanol (0.1%ethanolamine) Gradient: from 5% to 40% of B in 4.5min and hold 40% for 2.5 min, then 5% of B for 1 min; Flow rate: 2.8mL/min.

[000513] $^1$H NMR (400 MHz, CHLOROFORM-d) δ ppm 9.45 (s, 1 H) 7.60 (br d, J=7.83 Hz, 2 H) 7.33 (t, J=7.83 Hz, 2 H) 7.20 - 7.29 (m, 4 H) 7.06 - 7.13 (m, 2 H) 6.84 (d, J=2.20 Hz, 1 H) 6.75 (dd, J=8.31, 2.45 Hz, 1 H) 4.68 (br s, 1 H) 4.01 (t, J=6.11 Hz, 2 H) 3.82 (br d, J=5.38 Hz, 1 H) 3.41 (br dd, J=13.82, 3.30 Hz, 1 H) 3.13 - 3.27 (m, 2 H) 2.84 (br dd, J=13.82, 9.90 Hz, 1 H) 2.56 (q, J=7.50 Hz, 2 H) 1.79 - 1.86 (m, 2 H) 1.69 (quin, J=7.15 Hz, 2 H) 1.45 (s, 9 H) 1.09 (t, J=7.58 Hz, 3 H)

2.9 The synthesis of unnatural amino acid (aa28)

[000514] Scheme 10 outlines the synthesis of unnatural amino acid (aa28):

**Scheme 10**



[000515] Reagents and conditions: (a) $PhNH_2$ (1.1 eq.), DIC (1.0 eq.), HOBT(1.0 eq.), DCM, 0-20 °C, 1h; (b) piperidine (3.0 eq.), DMF, 20 °C, 12h, 52%; (c) 3a (1 eq.), DIC (1.0 eq.), HOBT (1.0 eq.), DCM, 0-20 °C, 1 h, 61%; (d) piperidine (15.0 eq.), DMF, 20 °C, 0.5 h, 41%.

*Step 1: Synthesis of 9H-fluoren-9-ylmethyl N-[(1S)-4-(3,5-dimethylphenyl)-1-(phenylcarbamoyl) butyl]carbamate (aa28-1)*

[000516] To a solution of **aa1** (300 mg, 676.39 µmol, 1 *eq.*) and HOBt (91.40 mg, 676.39 µmol, 1 *eq.*) in DCM (1.5 mL) were added aniline (66.14 mg, 710.21 µmol, 64.84 µL, 1.05 *eq.*) at 0 °C. A solution of DIC (85.36 mg, 676.39 µmol, 1 *eq.*) in DCM (0.5 mL) was added to the mixture. The reaction was stirred at 20 °C for 1 hr. The reaction progress was monitored by LC-MS. The mixture was diluted with DCM (20 mL) and washed with $H_2O$ (10 mL*2). The organic layer was dried over $Na_2SO_4$, filtered and concentrated to give the crude product. **Compound aa28-1** (400 mg, crude) was obtained as a yellow solid.

[000517] LCMS (ESI): RT = 1.178 min, mass calcd. for $C_{34}H_{35}N_2O_3^+$ 519.26 $[M+H]^+$, m/z found 519.1 $[M+H]^+$. Reverse phase LC-MS was carried out using method A.

*Step 2: Synthesis of (2S)-2-amino-5-(3,5-dimethylphenyl)-N-phenyl-pentanamide (aa28-2)*

[000518] To a solution of **aa28-1** (400 mg, 771.24 µmol, 1 *eq.*) in DMF (2 mL) were added piperidine (197.01 mg, 2.31 mmol, 3 *eq*) at 20°C. The reaction was stirred at 20 °C for 12 hr. The reaction progress was monitored by LC-MS and TLC. The mixture was diluted with EtOAc (30 mL) and washed with $H_2O$ (10 mL*3). The organic layer was dried over $Na_2SO_4$, filtered and concentrated to give a residue, then purified by flash silica gel chromatography (ISCO®; 12 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethyl acetate/Petroleum ethergradient @ 20 mL/min). **Compound aa28-2** (150 mg, 399.79 µmol, 51.84% yield, 79% purity) was obtained as a yellow foam. LCMS (ESI): RT = 0.861 min, mass calcd. for $C_{19}H_{25}N_2O$ 297.2 $[M+H]^+$, m/z found 297.1 $[M+H]^+$. Reverse phase LC-MS was carried out using method A.

[000519] $^1H$ NMR (400 MHz, CD3Cl) δ ppm 9.46 (br s, 1 H), 7.60 (d, J=7.83 Hz, 2 H), 7.33 (t, J=7.95 Hz, 2 H), 7.07 - 7.14 (m, 1 H), 6.78 - 6.86 (m, 4 H), 3.51 (dd, J=7.95, 4.28 Hz, 1 H), 2.57 - 2.64 (m, 2 H), 2.29 (s, 6 H), 1.67 - 1.81 (m, 4 H).

*Step 3: Synthesis of 9H-fluoren-9-ylmethyl N-[(1S)-1-[[4-[4-[4-(tert-butoxycarbonylamino) butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-4-(3,5-dimethylphenyl)-1-(phenylcarbamoyl) butyl]amino]-2-oxo-ethyl]carbamate (aa28-3)*

[000520] To a solution of **aa2b** (343.52 mg, 506.06 µmol, 1 *eq.*) and HOBt (68.38 mg, 506.06 µmol, 1 *eq*) in DCM (2.5 mL) were added **aa28-2** (150 mg, 506.06 µmol, 1 *eq.*) at 0 °C. A solution of DIC (63.86 mg, 506.06 µmol, 1 *eq.*) in DCM (0.5 mL) was added to the mixture. The

reaction was stirred at 20 °C for 1 hr. The reaction progress was monitored by TLC. The mixture was diluted with DCM (20 mL) and washed with $H_2O$ (10 mL*2). The organic layer was dried over $Na_2SO_4$, filtered and concentrated to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 12 g SepaFlash® Silica Flash Column, Eluent of 0~45% Ethylacetate/Petroleum ether gradient @ 20 mL/min). **Compound aa28-3** (400 mg, 309.23 µmol, 61.11% yield, 74% purity) was obtained as a yellow solid. LCMS (ESI): RT = 6.617 min, mass calcd. for $C_{60}H_{68}N_4NaO_7$ 979.50, m/z found 979.8 $[M+Na]^+$. Reverse phase LC-MS was carried out using method B.

[000521] $^1$H NMR (400 MHz, $CD_3Cl$) δ ppm 7.72 - 7.80 (m, 2 H), 7.49 - 7.57 (m, 3 H), 7.35 - 7.41 (m, 2 H), 7.20 - 7.33 (m, 5 H), 7.08 - 7.20 (m, 4 H), 6.96 - 7.08 (m, 2 H), 6.76 - 6.84 (m, 2 H), 6.68 - 6.75 (m, 3 H), 5.32 - 5.47 (m, 1 H), 4.64 (br s, 1 H), 4.52 - 4.60 (m, 1 H), 4.42 - 4.51 (m, 2 H), 4.37 (br s, 1 H), 4.13 - 4.23 (m, 1 H), 4.00 (t, J=6.24 Hz, 2 H), 3.05 - 3.27 (m, 4 H), 2.44 - 2.61 (m, 4 H), 2.20 - 2.25 (m, 6 H), 1.82 - 1.89 (m, 2 H), 1.62 - 1.75 (m, 6 H), 1.46 (s, 9 H), 1.01 - 1.09 (m, 3 H).

*Step 4: Synthesis of tert-butyl N-[4-[4-[4-[(2S)-2-amino-3-[[(1S)-4- (3,5-dimethylphenyl)-1-(phenylcarbamoyl)butyl]amino]-3-oxo-propyl]phenyl]-3-ethyl-phenoxy]butyl]carbamate (aa28)*

[000522] To a solution of **aa28-3** (380 mg, 396.99 µmol, 1 *eq.*) in DMF (2.5 mL) were added piperidine (507.06 mg, 5.95 mmol, 15 *eq.*) at 20°C. The reaction was stirred at 20 °C for 0.5 hr. The reaction progress was monitored by LC-MS. The mixture was diluted with ACN (2 mL) and purified by prep-HPLC (HCl condition; column: Agela ASB 150*25mm*5 µm; mobile phase: [water (0.05%HCl) - ACN]; B%: 55%-85%, 8min). **Compound aa28** (125 mg, 164.97 µmol, 41.56% yield, 97% purity) was obtained as a little yellow foam.

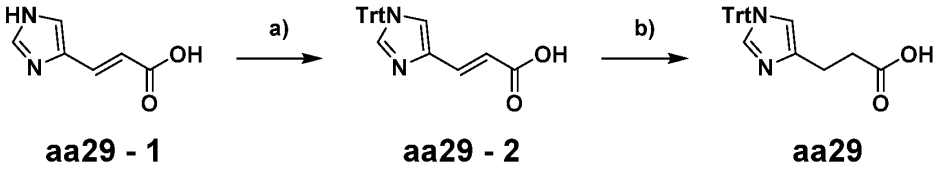
[000523] LCMS (ESI): RT = 1.061 min, mass calcd. for $C_{45}H_{58}N_4NaO_5$ 757.43, m/z found 757.5 $[M+Na]^+$. Reverse phase LC-MS was carried out using method A. HPLC: RT = 10.66 min, Reverse phase HPLC analysis was carried out using method C. SFC: RT = 2.161 min, Column: Chiralpak AD-3 50*4.6 mm I.D., 3µm; Mobile phase: A: $CO_2$ B:iso-propanol (0.05% DEA); Gradient: from 5% to 40% of B in 2 min and hold 40% for 1.2 min, then 5% of B for 0.8 min; Flow rate: 4mL/min.

[000524] $^1$H NMR (400 MHz,CD3OD) δ ppm 7.55 (br d, J=8.07 Hz, 2 H), 7.24 - 7.31 (m, 4 H), 7.14 (d, J=8.07 Hz, 2 H), 7.05 - 7.11 (m, 1 H), 6.95 (d, J=8.31 Hz, 1 H), 6.81 (d, J=2.45 Hz, 1 H), 6.78 (s, 3 H), 6.68 - 6.72 (m, 1 H), 4.54 (t, J=6.72 Hz, 1 H), 4.20 (t, J=6.85 Hz, 1 H), 4.00 (t, J=6.11 Hz, 2 H), 3.25 - 3.30 (m, 1 H), 3.12 (br t, J=6.85 Hz, 3 H), 2.54 - 2.63 (m, 2 H), 2.51 (q, J=7.50 Hz, 2 H), 2.22 (s, 6 H), 1.62 - 1.95 (m, 8 H), 1.44 (s, 9 H), 1.03 (t, J=7.58 Hz, 3 H).

2.10 The synthesis of unnatural amino acid (aa29)

[000525] Scheme 11 outlines the synthesis of unnatural amino acid (aa29):

**Scheme 11**




**aa29 - 1** **aa29 - 2** **aa29**

**Reagents and conditions:** (a) TrtCl (1.0 eq.), $Et_3N$ (3.0 eq.), DMF, 20 oC, 12 h, 29.05%; (b)Pd/C (10 wt %), $H_2$, EtOH, 20 oC, 2 h.

*Step 1: Synthesis of (E)-3-(1-trityl-1H-imidazol-5-yl) acrylic acid (aa29-2)*

[000526] Compounds **aa29 - 1** (2 g, 14.48 mmol, 1.0 eq.), TEA (4.40 g, 43.44 mmol, 6.05 mL, 3.0 eq.) and TrtCl (4.04 g, 14.48 mmol, 1.0 eq.) in DMF (34 mL) were stirred at 20 °C for 12 hr. TLC (DCM/MeOH=10/1) showed the reaction was complete. The mixture was diluted with $CH_2Cl_2$, washed with $H_2O$ (3 *50 mL) and citric acid solution (3* 50mL). The organic phase was evaporated and purified by column chromatography (eluent: $CH_2Cl_2$/MeOH, 9:1). Compound **aa29 - 2** (1.6 g, 4.21 mmol, 29.05% yield) was obtained as a white solid.

[000527] $^1$H NMR (400 MHz, $CD_3OD$) δ 7.57 (s, 1 H), 7.51 (d, J=15.77 Hz, 1 H), 7.38 - 7.45 (m, 9 H), 7.32 (s, 1 H), 7.15 -7.22 (m, 6 H), 6.45 (d, J=15.65 Hz, 1 H) ppm.

*Step 2: Synthesis of 3-(1-trityl-1H-imidazol-5-yl) propanoic acid (aa29)*

[000528] To a solution of compound **aa29 - 2** (1.6 g, 4.21 mmol, 1 eq.) in EtOH (20 mL) was added Pd/C (1 g, 10% purity) under $N_2$ atmosphere. The suspension was degassed and purged with $H_2$ for 3 times. The mixture was stirred under $H_2$ (15 Psi.) at 20°C for 2 h. After completion, the mixture was filtered and the filtrate was concentrated to give the product. Compound **aa29** (1.2 g, 3.14 mmol, 74.60% yield) was obtained as a white solid.

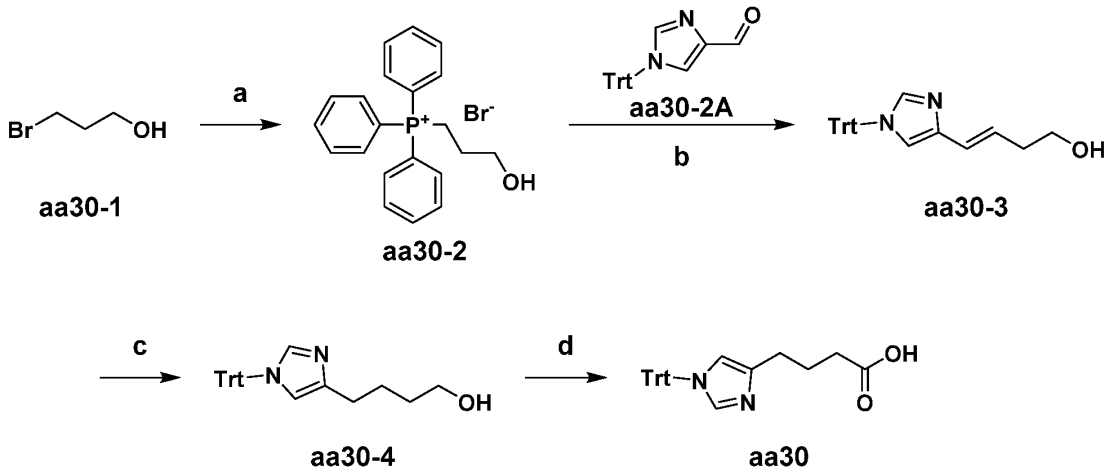
[000529] LCMS (ESI): RT =0.802 min, mass calcd. for $C_{25}H_{21}N_2O_2$ 381.17[M-H]$^-$, found 381.17 [M-H]$^-$, Reverse phase LCMS was carried out using Waters Xbridge C18 30*2.0mm, 3.5 µm, with a flow rate of 1.2 ml/min, eluting with a gradient of 5% to 95% acetonitrile containing ACN (solvent B) and water containing 0.05% $NH_3H_2O$ in Water (solvent A).

[000530] $^1$H NMR (400 MHz, $CD_3OD$) δ 7.34 - 7.39 (m, 4 H), 7.22 - 7.39 (m, 4 H), 7.11 - 7.14 (m, 3 H), 7.06 - 7.20 (m, 2 H), 7.08 (d, *J*=7.25 Hz, 4 H), 2.90 (t, *J*=7.32 Hz, 1 H), 2.80 (t, *J*=7.38 Hz, 1 H), 2.51 - 2.62 (m, 2 H) ppm.

2.11 The synthesis of unnatural amino acid (aa30)

[000531] Scheme 12 outlines the synthesis of unnatural amino acid (aa30):

**Scheme 12**




**Reagents and conditions:** (a) $PPh_3$ (1.2 eq.), toluene, 110 °C, 12 h; b) **aa30-2A** (1.5 eq.), LiHMDS (4.0 eq.), THF, -20-25 °C, 12 h; c) $H_2$, Pd/C, MeOH, 25 °C, 2 h; d) TEMPO (1.5 eq.), PIDA (2.5 eq.), MeCN/$H_2O$ (3:2), 25 °C, 2 h;

*Step 1: Synthesis of (3-hydroxypropyl)triphenylphosphonium bromide (aa30-2)*

[000532] To a solution of 3-bromopropan-1-ol (10 g, 71.95 mmol, 6.49 mL, 1 eq.) in toluene (100 mL) was added $PPh_3$ (22.65 g, 86.34 mmol, 1.2 eq.). The mixture was stirred at 100 °C under $N_2$ for 12 h. The reaction progress was monitored by LCMS. After completion, the reaction was cooled to 0 °C and filtered, the filter cake was washed with toluene (10 mL *3), then dried under reduced pressure. Compound **aa30 - 2** (20.7 g, crude) was obtained as a white solid.

[000533] LCMS (ESI): RT = 0.758 min, mass calcd. for $C_{21}H_{22}OP^+$, 321.14 $[M]^+$, found 321.0 $[M]^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20 * 2.0 mm Wavelength: UV 220 nm & 254 nm; Column temperature: 50 °C; MS ionization: ESI.

[000534] $^1H$ NMR (400 MHz, $CD_3Cl$) δ 7.83 - 7.65 (m, 15H), 4.94 (br s, 1H), 3.87 - 3.73 (m, 4H), 1.83 (m, 2H) ppm.

*Step 2: Synthesis of (E)-4-(1-trityl-1H-imidazol-4-yl)but-3-en-1-ol (aa30-3)*

[000535] To a solution of **aa30 - 2** (10.67 g, 26.60 mmol, 1.5 eq.) in THF (50 mL) was added LiHMDS (1 M, 70.92 mL, 4 eq.). The mixture was stirred at 0 °C for 0.5 h, then a solution of **aa30-2A** (1-tritylimidazole-4-carbaldehyde, 6 g, 17.73 mmol, 1.0 eq.) in THF (60 mL) was added to the above mixture and the resulting mixture was stirred at 25 °C for 12 h. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and concentrated under reduced pressure to give a residue, then the residue was re-dissolved in dioxane (50 mL) and the final solution was stirred at 100 °C for 12 h, after that, the solution was concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 80 g SepaFlash® Silica Flash Column, Eluent of 0~80% Ethylacetate/Petroleum ethergradient @ 60 mL/min). Compound **aa30 - 3** (650 mg, crude) was obtained as a yellow solid.

[000536] LCMS (ESI): RT = 0.846 min, mass calcd. for $C_{26}H_{24}N_2ONa$, 403.19 $[M+Na]^+$, found 403.1 $[M+Na]^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20 * 2.0 mm Wavelength: UV 220 nm & 254 nm; Column temperature: 50 °C; MS ionization: ESI.

[000537] $^1H$ NMR (400 MHz, $CD_3OD$) δ 7.68 - 7.57 (m, 1H), 7.40 - 7.37 (m, 9H), 7.18 - 7.13 (m, 6H), 6.83 (d, $J$ = 1.0 Hz, 1H), 6.36 - 6.14 (m, 2H), 3.62 (t, $J$ = 6.7 Hz, 2H), 2.36 (q, $J$ = 6.6 Hz, 2H) ppm.

*Step 3: Synthesis of 4-(1-trityl-1H-imidazol-4-yl)butan-1-ol (aa30-4)*

[000538] To a solution of **aa30 - 3** (650 mg, 1.71 mmol, 1 eq.) in MeOH (5 mL) was added Pd/C (50 mg, 489.61 mmol, 10% purity). The mixture was stirred at 25 °C for 2 h under $H_2$. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 25 g SepaFlash® Silica Flash Column, Eluent of 0~50% Ethyl acetate/Petroleum ether gradient @ 40 mL/min) for 8 min with total volume. Compound **aa30 - 4** (450 mg, 1.18 mmol, 68.87% yield, 100% purity) was obtained as a white solid.

[000539] LCMS (ESI): RT = 0.850 min, mass calcd. for $C_{26}H_{26}N_2ONa$, 405.20 $[M+Na]^+$, found 405.2 $[M+Na]^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20 * 2.0 mm Wavelength: UV 220 nm & 254 nm; Column temperature: 50 °C; MS ionization: ESI.

*Step 4: Synthesis of 4-(1-trityl-1H-imidazol-4-yl)butanoic acid (aa30)*

[000540] To a solution of **aa30 - 4** (450 mg, 1.18 mmol, 1 eq.) in MeCN (5 mL) and $H_2O$ (7 mL) was added TEMPO (277.51 mg, 1.76 mmol, 1.5 eq.) and PIDA (Iodobenzene diacetate (3240-34-4), 947.35 mg, 2.94 mmol, 2.5 eq.). The mixture was stirred at 25 °C for 12 h. The reaction progress was monitored by LCMS. After completion, to the mixture was added citric acid (aq. 15 mL) to adjust pH = 4, then extracted with EtOAc (15 mL*2), the organic layers were collected, dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure to give a residue. Then the residue was triturated by MTBE. Compound **aa30** (250 mg, crude) was obtained as a white solid.

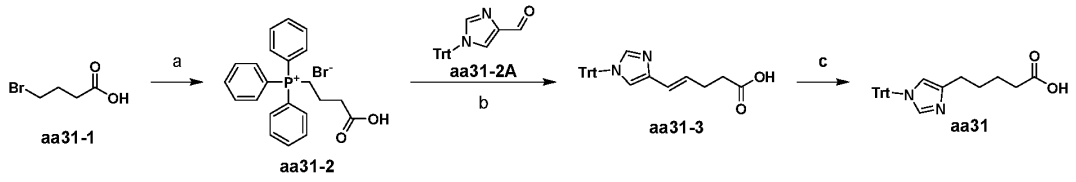
[000541] LCMS (ESI): RT = 2.484 min, mass calcd. for $C_{26}H_{25}N_2O_2$, 397.18 $[M+H]^+$, found 397.2 $[M+H]^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode; Wavelength 220 nm & 254 nm, Oven Temperature 50 °C.

[000542] $^1H$ NMR (400 MHz, $CD_3OD$) δ 8.69 (d, $J$ = 1.6 Hz, 1H), 7.53 - 7.38 (m, 9H), 7.28 - 7.14 (m, 7H), 2.75 (t, $J$ = 7.5 Hz, 2H), 2.34 (t, $J$ = 7.1 Hz, 2H), 2.00 - 1.87 (m, 2H) ppm.

2.12 The synthesis of unnatural amino acid (aa31)

[000543] Scheme 13 outlines the synthesis of unnatural amino acid (aa31):

**Scheme 13**




**Reagents and conditions:** (a) $PPh_3$ (1.2 eq.), toluene, 110 °C, 12 h; b) **aa31-2A** (1.5 eq.), tBuOK (3.0 eq.), THF, 0-25 °C, 12 h; c) $H_2$, Pd/C, MeOH, 25 °C, 2 h;

*Step 1: Synthesis of (3-carboxypropyl)triphenylphosphonium bromide (aa31-2)*

[000544] To a solution of 4-bromobutanoic acid (5 g, 29.94 mmol, 6.76 mL, 1 eq.) in toluene (70 mL) was added PPh3 (8.64 g, 32.93 mmol, 1.1 eq.). The mixture was stirred at 110 °C under N2 for 12 h. The reaction progress was monitored by TLC and LCMS. Upon completion, the reaction mixture was cooled to 0 °C, then filtered and washed with toluene (10 mL *3), the filter cake was collected and dried under reduced pressure. Compound **aa31-2** (5.6 g, crude) was obtained as a white solid.

[000545] LCMS (ESI): RT = 1.076 min, mass calcd. for $C_{22}H_{22}O_2P^+$, 349.14[M]$^+$, found 349.2 [M]$^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 1.35 minutes and holding at 80% for 0.9 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1 * 30 mm, 3 mm; Wavelength: UV 220 nm & 254 nm Column temperature: 50 °C; MS ionization: ESI.

[000546] $^1$H NMR (400 MHz, $CD_3Cl$) δ 7.91 - 7.66 (m, 15H), 3.31 - 3.22 (m, 2H), 2.55 (t, *J* = 6.3 Hz, 2H), 1.84 (m, 2H) ppm.

*Step 2: Synthesis of (E)-5-(1-trityl-1H-imidazol-4-yl)pent-4-enoic acid (aa31-3)*

[000547] To a solution of **aa31-2** (3.81 g, 8.87 mmol, 1.5 eq.) in THF (20 mL) was added tBuOK (1.99 g, 17.73 mmol, 3 eq.) at 0 °C under $N_2$, the mixture was stirred at 0 °C for 30 min, then a solution of **aa31-2A** (2 g, 5.91 mmol, 1 eq.) in THF (20 mL) was added to the above mixture at 0 °C and the final mixture was stirred at 25 °C for 12 h. The reaction progress was monitored by LCMS. Upon completion, to the mixture was added citric acid to adjust pH=4, then extracted with EtOAc (50 mL*2), the organic layers were collected, dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 80 g SepaFlash® Silica Flash Column, Eluent of 0~10% MeOH/DCM@ 40 mL/min). Compound **aa31-3** (4 g, crude) was obtained as a light yellow foam.

[000548] LCMS (ESI): RT = 1.487 min, mass calcd. for $C_{27}H_{25}N_2O_2$, 409.18 [M+H]$^+$, found 409.3 [M+H]$^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 1.35 minutes and holding at 80% for 0.9 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1 * 30 mm, 3 mm; Wavelength: UV 220nm & 254nm; Column temperature: 50 °C; MS ionization: ESI.

*Step 3: Synthesis of 5-(1-trityl-1H-imidazol-4-yl)pentanoic acid (aa31)*

[000549] To a solution of **aa31-3** (3 g, 7.34 mmol, crude purity, 1 eq.) in MeOH (40 mL) was added Pd/C (300 mg, 489.61 mmol, 10% purity). The mixture was stirred at 25 °C for 2 h under H2. The reaction progress was monitored by LCMS. Upon completion, the mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0~10% MeOH/DCM@ 40 mL/min) for 7 min with total volume. After that, the product was triturated by TBME. Compound **aa31** (290 mg, 692.32 mmol, 71.05% yield, 98% purity) was obtained as a white solid.

[000550] LCMS (ESI): RT = 1.805 min, mass calcd. for $C_{27}H_{27}N_2O_2$, 411.20 [M+H]$^+$, found 411.3 [M+H]$^+$. LCMS conditions: 0.8mL/4L $NH_3{\cdot}H_2O$ in water (solvent A) and acetonitrile (solvent

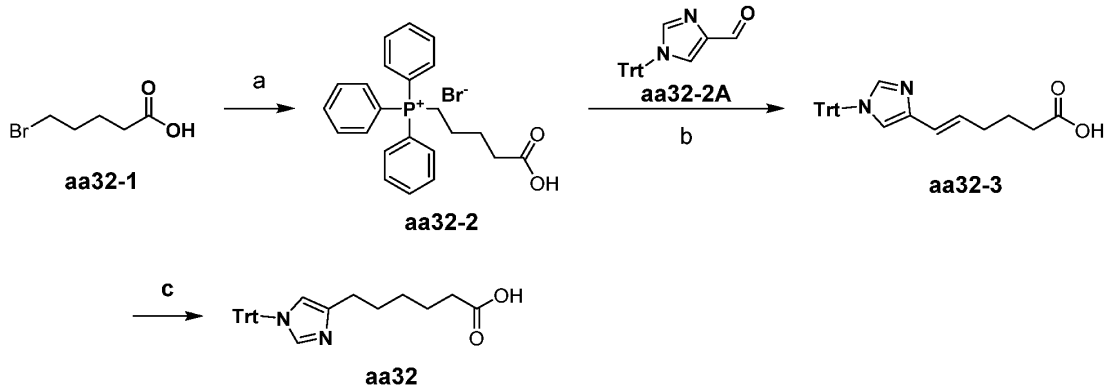
B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: XBridge C18 3.5 mm 2.1 * 30 mm; Wavelength: UV 220 nm & 254 nm; Column temperature: 50 °C; MS ionization: ESI.

[000551] $^1$H NMR (400 MHz, $CD_3Cl$) δ 7.65 (s, 1H), 7.44 - 7.34 (m, 9H), 7.16 (dd, *J* = 2.9, 6.8 Hz, 6H), 6.77 (s, 1H), 2.58 (br t, *J* = 6.8 Hz, 2H), 2.28 (t, *J* = 7.1 Hz, 2H), 1.70 - 1.55 (m, 4H) ppm.

2.13 The synthesis of unnatural amino acid (aa32)

[000552] Scheme 14 outlines the synthesis of unnatural amino acid (aa32):

**Scheme 14**




**Reagents and conditions:** (a) $PPh_3$ (1.2 eq.), toluene, 110 °C, 12 h; b) **aa32-2A** (1.5 eq.), tBuOK (3.0 eq.), THF, 0-25 °C, 12 h; c) $H_2$, Pd/C, MeOH, 25 °C, 2 h;

*Step 1: Synthesis of (4-carboxybutyl)triphenylphosphonium bromide (aa32-2)*

[000553] To a solution of 5-bromopentanoic acid (10 g, 55.24 mmol, 6.76 mL, 1 eq.) in toluene (100 mL) was added $PPh_3$ (15.94 g, 60.76 mmol, 1.1 eq.). The mixture was stirred at 110 °C for 12 h under $N_2$. The reaction progress was monitored by LCMS. Upon completion, the reaction mixture was cooled to 0 °C, then filtered and washed with toluene (10 mL *3), the filter cake was collected and dried under reduced pressure. Compound **aa32-2** (16.4 g, 36.99 mmol, 66.97% yield) was obtained as a white solid.

[000554] LCMS (ESI): RT = 1.076 min, mass calcd. for $C_{22}H_{22}O_2P^+$, 349.14 $[M+H]^+$, found 349.2 $[M+H]^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 1.35 minutes and

holding at 80% for 0.9 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1 * 30 mm, 3 mm; Wavelength: UV 220 nm & 254 nm Column temperature: 50 °C;

[000555] $^1$H NMR (400 MHz, $CD_3Cl$) δ 7.91 - 7.66 (m, 15H), 3.31 - 3.22 (m, 2H), 2.55 (t, *J* = 6.3 Hz, 2H), 1.86-1.82 (m, 4H) ppm.

*Step 2: Synthesis of (E)-6-(1-trityl-1H-imidazol-4-yl)hex-5-enoic acid (aa32-3)*

[000556] To a solution of **aa32-2** (7.86 g, 17.73 mmol, 1.5 eq.) in THF (60 mL) was added t-BuOK (3.98 g, 35.46 mmol, 3 eq.) at 0 °C under $N_2$. The mixture was stirred at 0 °C for 30 min. A solution of **aa32-2A** (4 g, 11.82 mmol, 1 eq.) in THF (40 mL) was added to the above mixture at 0 °C and the resulting mixture was stirred at 25 °C for 12 h. The reaction progress was monitored by LCMS. Upon completion, to the mixture was added citric acid to adjust pH=4, then extracted with EtOAc (50 mL*2), the organic layers were collected, dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 80 g SepaFlash® Silica Flash Column, Eluent of 0~10% MeOH/DCM@ 40 mL/min). Compound **aa32-3** (4 g, crude) was obtained as a light yellow foam.

[000557] LCMS (ESI): RT = 2.739 min, mass calcd. for $C_{28}H_{27}N_2O_2$, 423.20[M+H]$^+$, found 423.2 [M+H]$^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 1.35 minutes and holding at 80% for 0.9 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1 * 30 mm, 3 mm; Wavelength: UV 220 nm & 254 nm; Column temperature: 50 °C; MS ionization: ESI.

*Step 3: Synthesis of 6-(1-trityl-1H-imidazol-4-yl)hexanoic acid (aa32)*

[000558] To a solution of **aa32-3** (4 g, 9.47 mmol, 1 eq.) in MeOH (40 mL) was added Pd/C (300 mg, 489.61 mmol, 10% purity). The mixture was stirred at 25 °C for 2 h under $H_2$. The reaction progress was monitored by LCMS. Upon completion, the mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0~10% MeOH/DCM@ 40 mL/min). After that, the product was triturated by TBME. Compound aa32 (660 mg, 1.51 mmol, 15.93% yield, 97% purity) was obtained as a white solid.

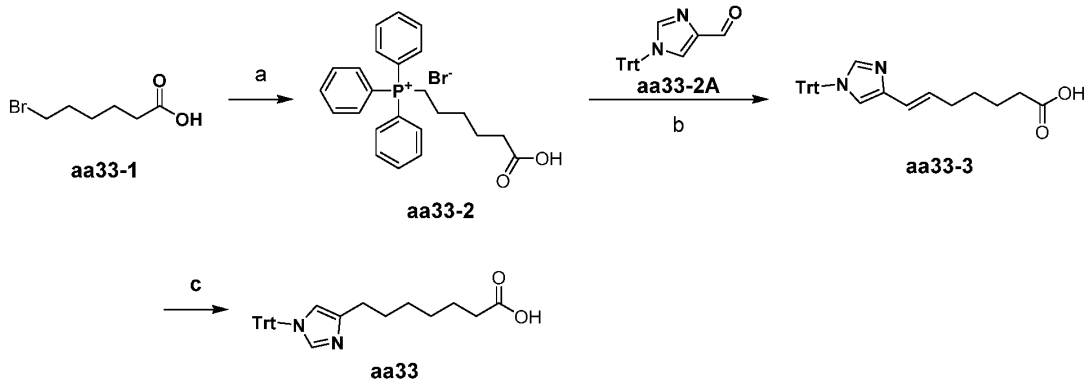
[000559] LCMS (ESI): RT = 2.807 min, mass calcd. for $C_{28}H_{29}N_2O_2$, 425.22 [M+H]$^+$, found 425.2 [M+H]$^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6.0 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1 * 30 mm, 3 mm; Wavelength: UV 220 nm & 254 nm Column temperature: 50 °C; MS ionization: ESI.

[000560] $^1$H NMR (400 MHz, $CD_3OD$) δ 7.43 (d, *J* = 1.3 Hz, 1H), 7.41 - 7.34 (m, 9H), 7.19 - 7.11 (m, 6H), 6.65 (s, 1H), 2.52 (t, *J* = 7.4 Hz, 2H), 2.24 (t, *J* = 7.4 Hz, 2H), 1.60 (m, 4H), 1.38 - 1.27 (m, 2H) ppm.

2.14 The synthesis of unnatural amino acid (aa33)

[000561] Scheme 15 outlines the synthesis of unnatural amino acid (aa33):

**Scheme 15**




**Reagents and conditions:** (a) $PPh_3$ (1.2 eq.), toluene, 110 °C, 12 h; b) **aa33-2A** (1.5 eq.), *t*BuOK (3.0 eq.), THF, 0-25 °C, 12 h; c) $H_2$, Pd/C, MeOH, 25 °C, 2 h;

*Step 1: Synthesis of (5-carboxypentyl)triphenylphosphonium bromide (aa33-2)*

[000562] To a solution of 6-bromohexanoic acid (5 g, 25.63 mmol, 1 eq.) in toluene (50 mL) was added $PPh_3$ (7.06 g, 26.92 mmol, 1.05 eq.). The resulting solution was stirred at 120 °C over 12 h. After completion, the reaction mixture was cooled to 0 °C, then filtered and washed with toluene (10 mL *3), the filter cake was collected and dried under reduced pressure. Compound **aa33-2** (6.85 g, 14.47 mmol, 56.44% yield, 96.6% purity) was obtained as a white solid.

[000563] LCMS (ESI): RT = 0.916 min, m/z calcd. for $C_{24}H_{26}PO_2^+$ 377.17, found 377.1 $[M-Br]^+$. Reverse phase LCMS was carried out using a Xtimate C18, 2.1 * 30 mm 3 mm, SN: 3U411301530 column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

*Step 2: Synthesis of (E)-7-(1-trityl-1H-imidazol-4-yl)hept-6-enoic acid (aa33-3)*

[000564] To a solution of **aa33-2** (4.05 g, 8.87 mmol, 2 eq.) in THF (20 mL) was added tBuOK (1 M, 22.16 mL, 5 eq.) at 0 °C under $N_2$, the mixture was stirred at 0 °C for 30 min, then a solution of **aa33-2A** (1.5 g, 4.43 mmol, 1 eq.) in THF (20 mL) was added to the above mixture

at 0 °C and the final mixture was stirred at 25 °C for 12 h. The reaction progress was monitored by LCMS. After completion, the mixture was added citric acid to adjust pH=4, then extracted with EtOAc (50 mL*2), the organic layers were collected, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give a residue. The crude product was used into the next step without any further purification. Compound **aa33-3** (3.8 g, crude) was obtained as a yellow syrup.

[000565] LCMS (ESI): RT = 2.904 min and 3.068 min, mass calcd. for $C_{29}H_{29}N_2O_2$ 437.22, found 437.3 $[M+H]^+$; Reverse phase LCMS was carried out using a Chromolith Flash RP-C18 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

*Step 3: Synthesis of 7-(1-trityl-1H-imidazol-4-yl)heptanoic acid (aa33)*

[000566] To a solution of **aa33-3** (3 g, 6.87 mmol, 1 eq.) in MeOH (30 mL) was added Pd/C (300 mg, 489.61 mmol, 10% purity). The mixture was stirred at 25 °C for 2 h under $H_2$. The reaction progress was monitored by LCMS. After completion, the residue was purified by prep-HPLC (column: Boston Prime C18 150 * 25 mm * 5 mm; mobile phase: [water (0.05% ammonia hydroxide v/v)-ACN]; B%: 22%-45%, 7 min). Compound aa33 (200 mg, 456.04 mmol, 6.64% yield, 100% purity) was obtained as a white solid.

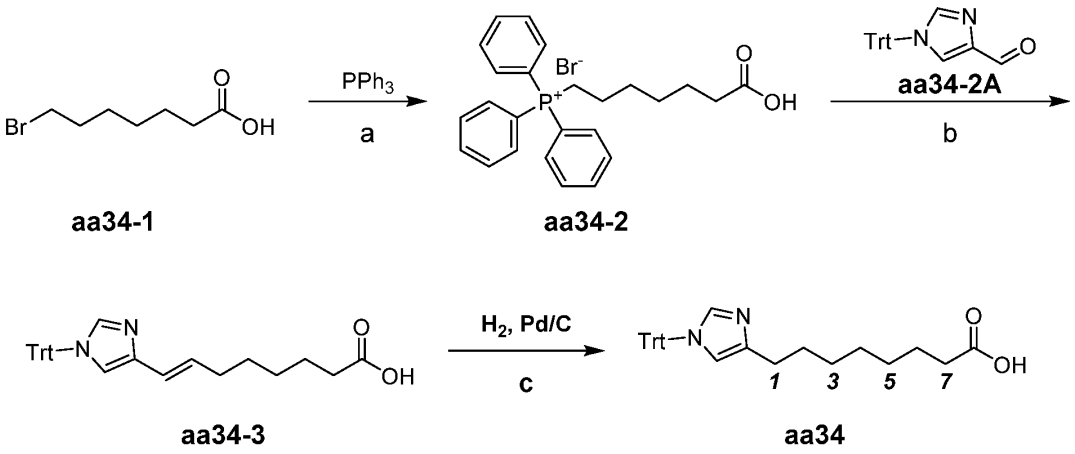
[000567] LCMS (ESI): RT = 2.231min, mass calcd. for $C_{29}H_{31}N_2O_2$, 439.23 $[M+H]^+$, found 439.3 $[M+H]^+$. LCMS conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode; Wavelength 220 nm & 254 nm, Oven Temperature 50 °C.

[000568] $^1H$ NMR (400 MHz, $CD_3OD$) δ 7.45 - 7.31 (m, 10H), 7.19 - 7.11 (m, 6H), 6.64 (s, 1H), 2.52 (t, $J$ = 7.3 Hz, 2H), 2.24 (t, $J$ = 7.4 Hz, 2H), 1.65 - 1.52 (m, 4H), 1.38- 1.26 (m, 4H) ppm.

2.15 The synthesis of unnatural amino acid (aa34)

[000569] Scheme 16 outlines the synthesis of unnatural amino acid (aa34):

**Scheme 16**




**Reagents and conditions:** (a) $PPh_3$ (1.2 eq.), toluene, 110 °C, 12 h; b) **aa34-2A** (1.5 eq.), *t*BuOK (3.0 eq.), THF, 0-25 °C, 12 h; c) $H_2$, Pd/C, MeOH, 25 °C, 2 h;

*Step 1: Synthesis of 7-[BLAH(triphenyl)-λ5-phosphanyl]heptanoic acid (aa34-2)*

[000570] To a solution of **aa34-1** (10 g, 47.83 mmol, 1 eq) in toluene (100 mL) was added $PPh_3$ (13.80 g, 52.61 mmol, 1.1 eq). The mixture was stirred at 110 °C for 12 hr. After completion, the reaction was filtered under reduced pressure to give a residue. The crude product was used to the next step without further purification. Compound **aa34-2** (22.8 g, 43.73 mmol, 91.43% yield, 90.410% purity) was obtained as a yellow oil.

[000571] LCMS (ESI): RT = 0.792 min, m/z calcd. for $C_{25}H_{28}O_2P^+$ 391.18 $[M]^+$, found 391.1 $[M]^+$, LC-MS Conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

*Step 2: Synthesis of (E)-8-(1-tritylimidazol-4-yl)oct-7-enoic acid (aa34-3)*

[000572] To a solution of **aa34-2** (10.45 g, 22.16 mmol, 1.5 eq.) in THF (150 mL) was added t-BuOK (4.97 g, 44.33 mmol, 3 eq) at 0 °C under $N_2$, the mixture was stirred at 0 °C then a solution of **aa34-2A** (5 g, 14.78 mmol, 1 eq) in THF (20 mL) added to the above mixture at 0 °C and the final mixture was stirred at 25 °C for 12 hr. After completion, the mixture was added citric acid to adjust pH=4, then extracted with EtOAc (200 mL*2), the organic layers were collected, dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give the target **aa34-3** (14.5 g, crude) as a white solid.

[000573] LCMS (ESI): RT = 0.870 min, m/z calcd. for $C_{30}H_{31}N_2O_2$ 451.23 $[M+H]^+$, $C_{30}H_{30}N_2O_2Na$ 473.23 $[M+Na]^+$, found 473.1 $[M+Na]^+$, LC-MS Conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

*Step 3: Synthesis of 8-(1-tritylimidazol-4-yl)octanoic acid (aa34)*

[000574] To a solution of **aa34-3** (14 g, 15.54 mmol, 50% purity, 1 eq) in MeOH (200 mL) was added Pd/C (4 g, 10% purity) and $H_2$. The mixture was stirred at 25 °C for 3 hr. After completion, the mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by C-18 reverse phase chromatography (ISCO®; 120 g SepaFlash® C-18 Column, Eluent of 60~70% acetonitrile/$H_2O$ gradient @ 80 mL/min, 40 min with total volume 2 L) to give a residue (neutral). Compound **aa34** (2.1 g, 4.53 mmol, 29.15% yield, 97.6% purity) was obtained as a white solid.

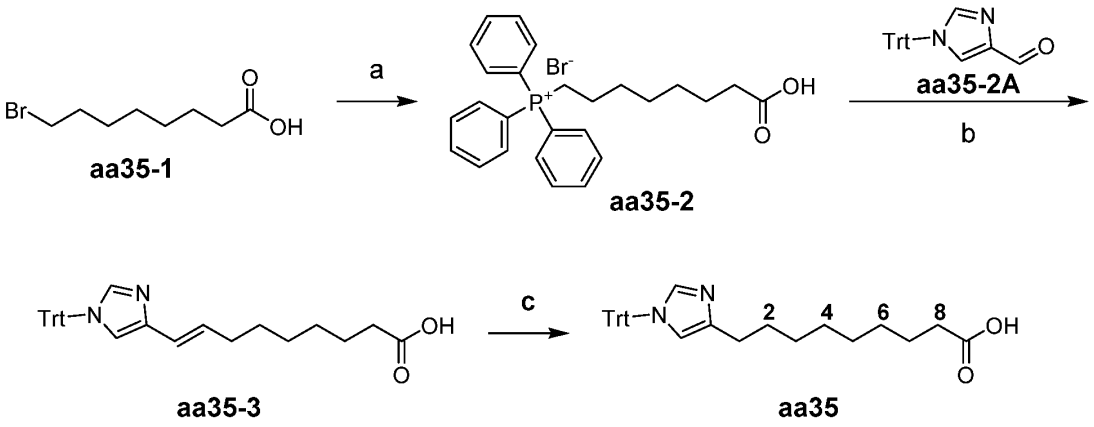
[000575] LCMS (ESI): RT = 2.956 min, mass calcd. for $C_{30}H_{33}N_2O_2$ 453.25 $[M+H]^+$, found 453.3 $[M+H]^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6.0 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1*30 mm,3 µm; Wavelength: UV 220 nm & 254 nm Column temperature: 50°C; MS ionization: ESI.

[000576] $^1H$ NMR (400MHz, $CD_3OD$) δ 7.47 - 7.33 (m, 10H), 7.22 - 7.10 (m, 6H), 6.64 (s, 1H), 2.52 (t, *J*=7.4 Hz, 2H), 2.26 (t, *J*=7.4 Hz, 2H), 1.69 - 1.48 (m, 4H), 1.37 - 1.26 (m, 6H) ppm.

2.16 The synthesis of unnatural amino acid (aa35)

[000577] Scheme 17 outlines the synthesis of unnatural amino acid (aa35):

**Scheme 17**




**Reagents and conditions:** (a) $PPh_3$ (1.2 eq.), toluene, 110 °C, 20 h; b) **aa35-2A** (1.5 eq.), *t*BuOK (3.0 eq.), THF, 0-25 °C, 20 h; c) $H_2$, Pd/C, MeOH, 25 °C, 10 h;

*Step 1: Synthesis of (7-carboxyheptyl)triphenylphosphonium bromide (aa35-2)*

[000578] To a solution of **aa35-1** (15 g, 71.74 mmol, 1 eq.) in toluene (150 mL) was added $PPh_3$ (20.70 g, 78.92 mmol, 1.1 eq.). The mixture was stirred at 110 °C under $N_2$ for 20 h. After completion, the reaction mixture was cooled to 0 °C, then filtered and washed with toluene (10 mL *3), the filter cake was collected and dried under reduced pressure. The residue was triturated with THF (200 mL). Then the mixture was filtered, and the filter cake was dried to give the product **aa35-2** (20 g, 40.31 mmol, 56.18% yield, 95% purity) as a white solid.

[000579] LCMS: (ESI): Rt = 2.175 min, mass calcd. for $C_{26}H_{30}O_2P^+$ $[M+H]^+$ 405.20, found 405.70; Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

*Step 2: Synthesis of (E)-9-(1-trityl-1H-imidazol-4-yl)non-8-enoic acid (aa35-3)*

[000580] To a solution of **aa35-2** (10.45 g, 22.17 mmol, 1.5 eq.) in THF (150 mL) was added t-BuOK (4.97 g, 44.34 mmol, 3.0 eq.) at 0°C. Then the mixture was stirred at 0°C for 30 min. A solution of aa35-2A (5 g, 14.78 mmol, 1 eq.) in THF (50 mL) was added. Then the mixture was stirred at 20°C for 12 h. After completion, the reaction mixture was quenched by addition H2O (100 mL) at 0°C, and then extracted with EtOAc (150 mL * 2). The combined organic layers were washed with Sat. NaCl 100 mL (50 mL*2), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by flash silica

gel chromatography (ISCO®; 80 g Special Flash® Silica Flash Column, Eluent of 0~20% Ethyl acetate/Petroleum ether gradient @ 60 mL/min) to give the product **aa35-3** (1.4 g, crude) as a light-yellow oil.

[000581] LCMS: (ESI): Rt = 3.310 min, mass calcd. for $C_{31}H_{33}N_2O_2$ $[M+H]^+$ 465.25, found 465.20 $[M+H]^+$ ; Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3 mm, with a flow rate of 0.8 ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

*Step 3: Synthesis of 9-(1-tritylimidazol-4-yl)nonanoic acid (aa35)*

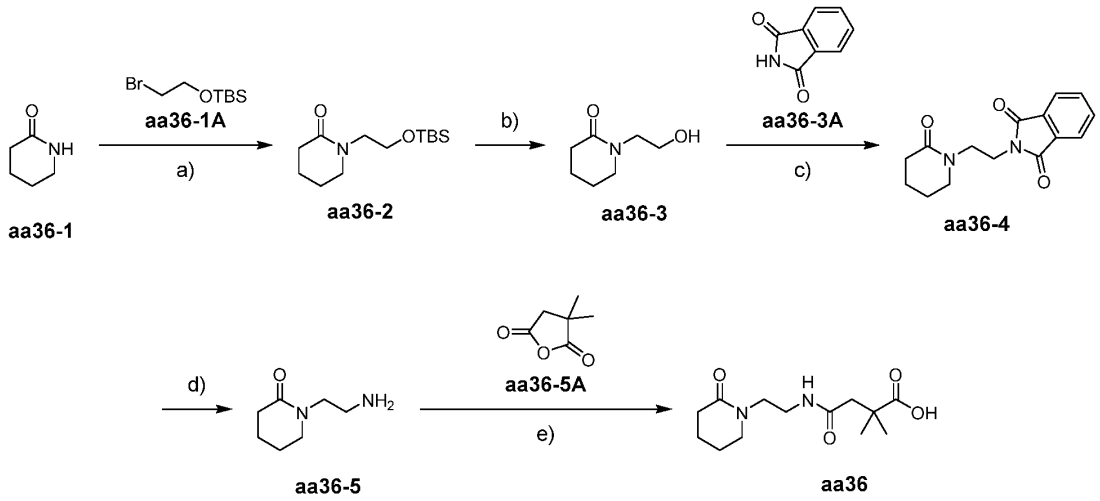
[000582] To a solution of **aa35-3** (1.4 g, 3.01 mmol, 1 eq.) in MeOH (40 mL) was added Pd/C (0.2 g, 10% purity). The mixture was stirred at 25 °C under $H_2$ for 3 hr. After completion, the mixture was filtered and concentrated under reduced pressure to give a residue. The crude product was purified by prep-HPLC (column: C18 spherical 20-35 um, 100A, 12 g; mobile phase: [Water-ACN]; B%: 0%-90%, 15 min) to give the product **aa35** (0.12 g, 244.31 µmol, 8.11% yield, 95% purity) was obtained as a white solid.

[000583] LCMS: (ESI): Rt = 3.257min, mass calcd. for $C_{31}H_{35}N_2O_2$ $[M+H]^+$ 467.26, found 467.20 $[M+H]^+$ ; Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3 mm, with a flow rate of 0.8 ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

2.17 The synthesis of unnatural amino acid (aa36)

[000584] Scheme 18 outlines the synthesis of unnatural amino acid (aa36):

**Scheme 18**




**Reagents and conditions:** (a) aa36-1A (1 eq.), NaH (1.1 eq.), THF, 0-70 °C, 12.5 h; b) HCl/MeOH (2 mL, 30%, v/v), MeOH (3 mL), 0 °C, 0.5 h; c) aa36-3A (1.5 eq,), $PPh_3$ (1.5 eq.), DIAD (1.5 eq.), THF (10 mL), 0-20 °C, 2 h; d) $NH_2$-$NH_2$.$H_2O$ (10 eq.), MeOH, 45 °C, 2 h; e) **aa36-5A** (1.1 eq.), DMF (2 mL), 20 °C, 12 h

*Step 1: Synthesis of 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperidin-2-one (aa36-2)*

[000585] To a solution of **aa36-1** (6 g, 60.53 mmol, 1 eq) in THF (300 mL) was added NaH (2.66 g, 66.58 mmol, 60% purity, 1.1 eq) in portions at 0 °C. The mixture was stirred at 20 °C for 0.5 h and **aa36-1A** (14.48 g, 60.53 mmol, 1 eq) was added. The reaction mixture was heated to 70 °C for 12 h. LCMS showed the desired product was observed. The mixture was cooled to r.t., quenched by water (150 mL) and extracted with EtOAc (150 mL x 2). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography ($SiO_2$, Petroleum ether/Ethyl acetate = 1/1). Compound **aa36-2** (2.7 g, 10.49 mmol, 17.33% yield) was obtained as a colorless oil.

[000586] LCMS (ESI): RT = 0.974 min, mass calcd. for $C_{13}H_{27}NO_2SiH$ 258.18, found 258.2 $[M+H]^+$; Reverse phase LCMS was carried out using a Chromolith Flash Agilent Pursult 5 C18 20*2.0 mm, with a flow rate of 1.5 mL/min, eluting with a gradient of 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes.

[000587] $^1$H NMR (400MHz, DMSO-*d6*) 3.64 (t, *J*=6.0 Hz, 2H), 3.32 - 3.29 (m, 4H), 2.16 (t, *J*=6.0 Hz, 2H), 1.74 - 1.57 (m, 4H), 0.83 (s, 9H), 0.00 (s, 6H) ppm.

*Step 2: Synthesis of 1-(2-hydroxyethyl)piperidin-2-one (aa36-3)*

[000588] To a solution of *aa36-2* (2.7 g, 10.49 mmol, 1 eq) in MeOH (12 mL) was added a solution of HCl/MeOH (v/v=30%, 8 mL) at 0 °C. The solution was stirred at 0 °C for 0.5 h. After

completion, the solution was concentrated in vacuo. The residue was purified by column chromatography ($SiO_2$, DCM/MeOH=10/1). The crude product **aa36-3** (1.4 g, 9.73 mmol, 92.76% yield, 99.5% purity) was obtained as a yellow solid which was used into the next step without further purification.

[000589] $^1$H NMR (400MHz, DMSO-*d6*) 4.13 - 4.11 (m, 1H), 3.52 - 3.44 (m, 2H), 3.34-3.29 (m, 4H), 2.20 (t, *J*=6.0 Hz, 2H), 1.75 - 1.61 (m, 4H) ppm.

[000590] LCMS (ESI): RT = 0.448-0.574 min, mass calcd. for $C_7H_{13}NO_2H$ 144.09, found 144.2 $[M+H]^+$; Reverse phase LCMS was carried out using a Chromolith Flash Agilent Pursult 5 C18 20*2.0mm, with a flow rate of 1.5 mL/min, eluting with a gradient of 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes.

*Step 3: Synthesis of 2-(2-(2-oxopiperidin-1-yl)ethyl)isoindoline-1,3-dione (aa36-4)*

[000591] To a solution of **aa36-3** (1.5 g, 10.48 mmol, 1 eq) and **aa36-3A** (1.85 g, 12.57 mmol, 1.2 eq) in THF (40 mL) was added $PPh_3$ (4.12 g, 15.71 mmol, 1.5 eq) and DIAD (3.18 g, 15.71 mmol, 3.06 mL, 1.5 eq) at 0 °C. The resulting mixture was stirred at 20 °C for 2 h. LCMS showed the desired product was observed. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography ($SiO_2$, Petroleum ether/Ethyl acetate=1/1). Compound **aa36-4** (0.15 g, 550.87 µmol, 5.26% yield) was obtained as a white solid.

[000592] LCMS (ESI): RT = 0.764 min, mass calcd. for $C_{15}H_{16}N_2O_3H$ 273.12, found 273.1 [M+H]+; Reverse phase LCMS was carried out using a Chromolith Flash Agilent Pursult 5 C18 20*2.0 mm, with a flow rate of 1.5 mL/min, eluting with a gradient of 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes.

[000593] $^1$H NMR (400MHz, DMSO-*d6*) 7.83 - 7.65 (m, 4H), 3.68 - 3.58 (m, 2H), 3.45 - 3.39 (m, 2H), 3.21 (t, *J*=6.0 Hz, 2H), 1.89 (t, *J*=6.4 Hz, 2H), 1.64 - 1.56 (m, 2H), 1.55 - 1.47 (m, 2H) ppm.

*Step 4: Synthesis of 1-(2-aminoethyl)piperidin-2-one (aa36-5)*

[000594] To a solution of **aa36-4** (0.15 g, 550.87 µmol, 1 eq) in MeOH (10 mL) was added $NH_2NH_2{\cdot}H_2O$ (275.76 mg, 5.51 mmol, 267.73 µL, 10 eq). The solution was stirred at 45 °C for 2 h. After completion, the solution was cooled to r.t. and concentrated in vacuo. The crude product **aa36-5** (0.07 g, crude) was obtained as a colorless oil, which was used into the next step without further purification.

[000595] $^1$H NMR (400MHz, DMSO-*d6*) 3.27-3.22 (m, 6H), 2.62 (t, *J*=6.8 Hz, 2H), 2.17 (t, *J*=6.0 Hz, 2H), 1.76 - 1.58 (m, 4H) ppm.

*Step 5: Synthesis of 2,2-dimethyl-4-oxo-4-((2-(2-oxopiperidin-1-yl)ethyl)amino)butanoic acid (aa36)*

[000596] To a solution of **aa36-5** (0.07 g, 492.27 µmol, 1 eq) in DMF (2 mL) was added **aa36-5A** (69.38 mg, 541.50 µmol, 60.86 µL, 1.1 eq). The solution was stirred at 20 °C for 12 h. LCMS showed the desired product was observed. The solution was concentrated in vacuo. The residue was purified by prep-HPLC (FA condition; column: Phenomenex Synergi C18 150*30 mm*4um; mobile phase: [water (0.225%FA)-ACN]; B%: 15%-25%, 11min). Compound **aa36** (20 mg, 73.99 µmol, 15.03% yield) was obtained as a white solid.

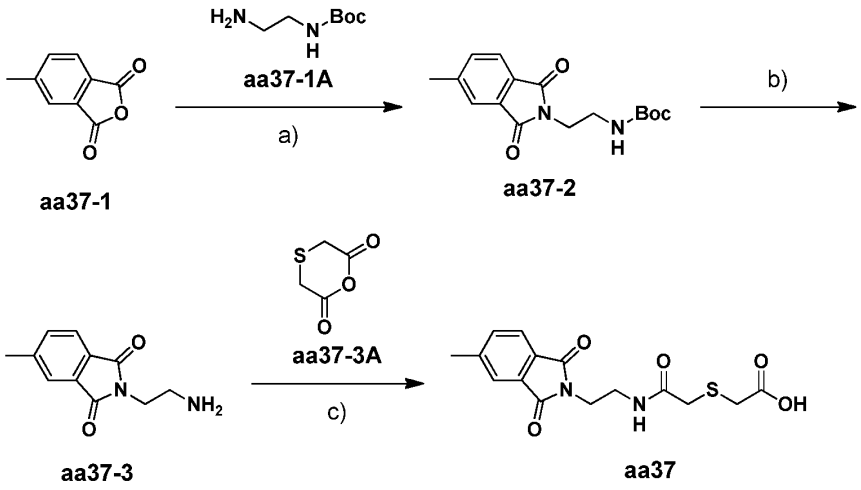
[000597] LCMS (ESI): RT = 0.19 min, mass calcd. for $C_{13}H_{22}N_2O_4H$ 271.16, found 271.1 $[M+H]^+$; Reverse phase LCMS was carried out using a Chromolith Flash Agilent Pursult 5 C18 20*2.0mm, with a flow rate of 1.5 mL/min, eluting with a gradient of 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes.

[000598] $^1$H NMR (400MHz, METHANOL-*d4*) 3.51 - 3.44 (m, 2H), 3.43 - 3.35 (m, 4H), 2.47 (s, 2H), 2.35 (t, *J*=6.0 Hz, 2H), 1.91 - 1.76 (m, 4H), 1.25 (s, 6H) ppm.

2.18 The synthesis of unnatural amino acid (aa37)

[000599] Scheme 19 outlines the synthesis of unnatural amino acid (aa37):

**Scheme 19**




**Reagents and conditions:** (a) **aa37-1A** (1.1 eq.), toluene, 120 °C, 12 h, 46% yield; b) HCl/MeOH (4M), 25 °C, 12 h; c) **aa37-3A** (1.5 eq,), DIPEA (2.0 eq.), DMF (10 mL), 25 °C, 12 h, 43% yield.

*Step 1: Synthesis of tert-butyl N-[2-(5-methyl-1,3-dioxo-isoindolin-2-yl)ethyl]carbamate (aa37-2)*

[000600] To a solution of **aa37-1** (500 mg, 3.08 mmol, 1 eq) in toluene (10 mL) was added **aa37-1A** (543.46 mg, 3.39 mmol, 532.80 µL, 1.1 eq). The mixture was stirred at 120 °C for 12 hr. After completion, the reaction mixture was concentrated under vacuum to give the crude product, which was triturated with MTBE/PE (1:1) at 25 ºC for 1h. Compound **aa37-2** (460 mg, 1.44 mmol, 46.56% yield, 95% purity) was obtained as a yellow solid.

[000601] LCMS (ESI): RT = 0.898 min, m/z calcd. for $C_{16}H_{21}N_2O_4$ 305.14 $[M+H]^+$, $C_{16}H_{21}N_2O_4Na$ 327.14 $[M+Na]^+$, found 327.2 $[M+Na]^+$. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

*Step 2: Synthesis of 2-(2-aminoethyl)-5-methyl-isoindoline-1,3-dione (aa37-3)*

[000602] To a solution of **aa37-2** (460 mg, 1.51 mmol, 1 eq) was added HCl/MeOH (4 M, 377.87 µL, 1 eq). The mixture was stirred at 25 °C for 12 hr. After completion, the reaction was concentrated in vacuum to give the crude product **aa37-3** (300 mg, crude, HCl salt) was obtained as a white solid.

[000603] LCMS (ESI): RT = 0.638 min, m/z calcd. for $C_{11}H_{13}N_2O_2$ 205.09 $[M+H]^+$, found 205.0 $[M+H]^+$. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000604] $^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.99 (br s, 2H), 7.26 - 7.21 (m, 1H), 3.56 (br s, 2H), 2.77 (br s, 4H), 2.08 (s, 3H) ppm.

*Step 3: Synthesis of2-[2-[2-(5-methyl-1,3-dioxo-isoindolin-2-yl)ethylamino]-2-oxo-ethyl] sulfanylacetic acid (aa37)*

[000605] To a solution of **aa37-3** (250 mg, 1.22 mmol, 1 eq) and **aa37-3A** (194.11 mg, 1.47 mmol, 1.2 eq) in DMF (4 mL) was added DIPEA (316.42 mg, 2.45 mmol, 426.45 µL, 2 eq). The mixture was stirred at 25 °C for 12 hr. The reaction mixture was partitioned between EtOAc (50 mL) and water (60 mL). The water layer was acidified to pH=4 by 1 N HCl solution. The organic phase was separated, washed with brine (30 mL x 3), and dried over anhydrous $Na_2SO_4$. The resulting solution was concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 12 g SepaFlash® Silica Flash Column, Eluent of 0~30% Ethyl acetate/Petroleum ether gradient @ 35 mL/min). Compound **aa37** (200 mg, 535.14 µmol, 43.72% yield, 90% purity) was obtained as a white solid.

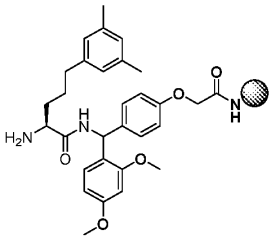
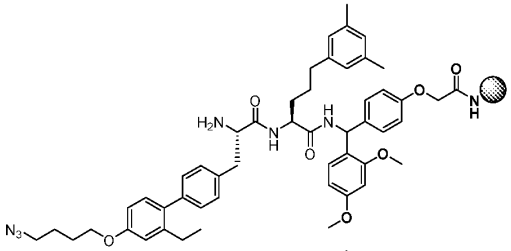
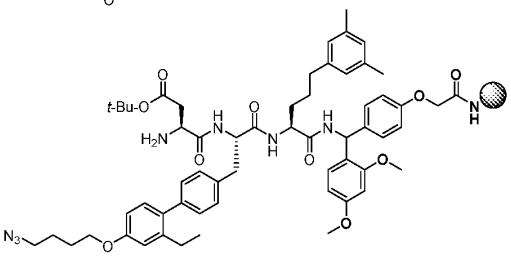
[000606] LCMS (ESI): RT = 0.757 min, m/z calcd. for $C_{15}H_{16}N_2O_5SNa$ 359.08 $[M+Na]^+$, found 358.9 $[M+Na]^+$. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

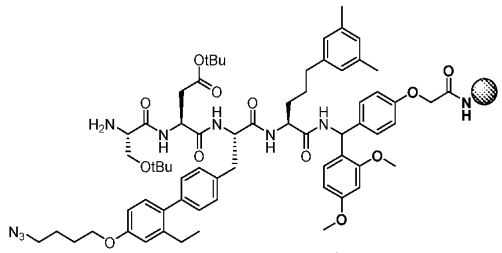
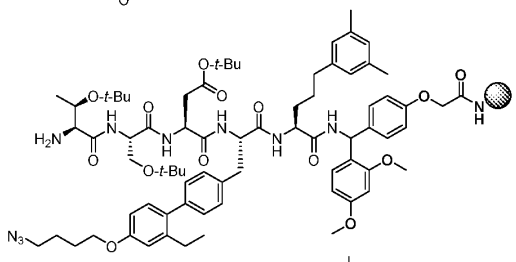
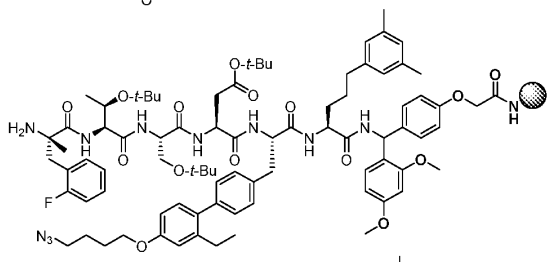
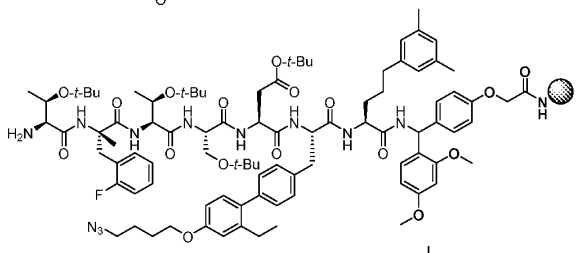
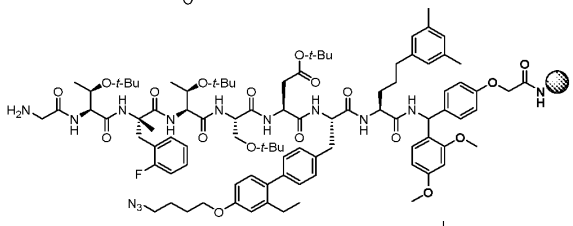
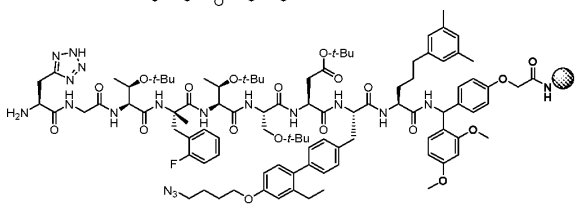
[000607] $^1H$ NMR (400MHz, CHLOROFORM-*d*) δ = 7.69 - 7.60 (m, 1H), 7.55 (s, 1H), 7.43 (br d, *J*=7.3 Hz, 1H), 3.82 - 3.70 (m, 2H), 3.52 - 3.41 (m, 2H), 2.86 (s, 2H), 2.76 (s, 2H), 2.38 (s, 3H) ppm.

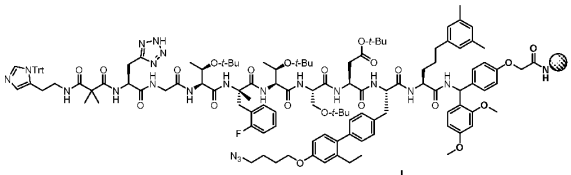
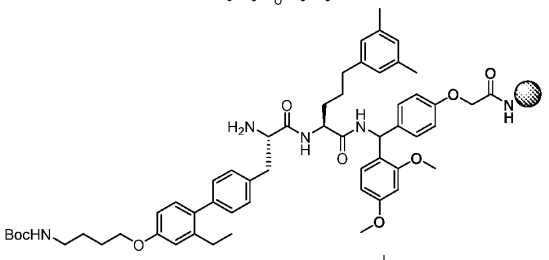
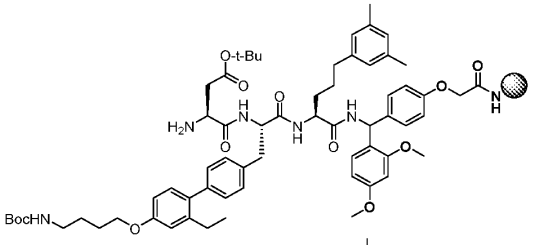
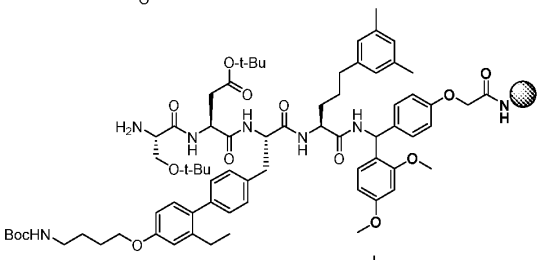
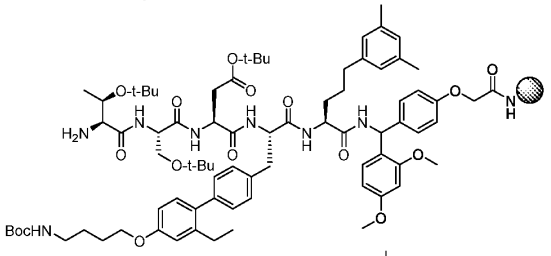
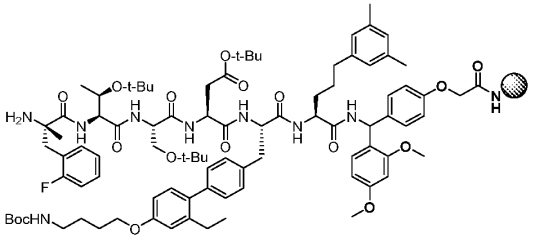
**Example 3. Synthesis of GLP1 Peptidomimetics**

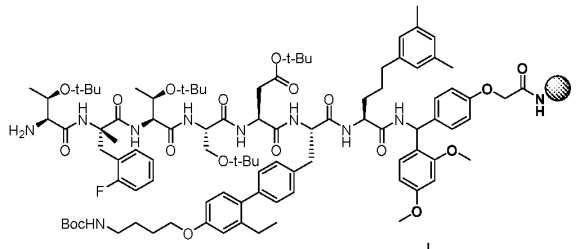
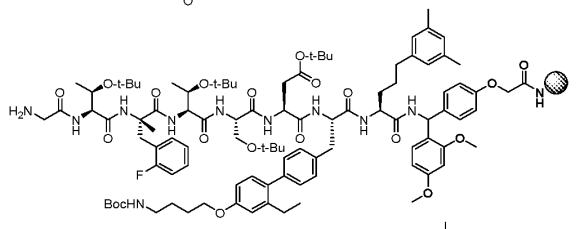
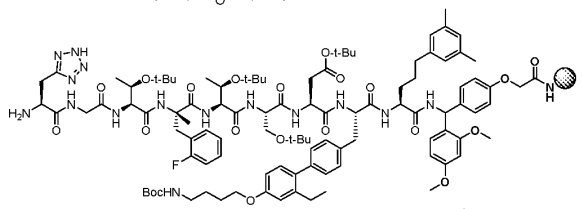
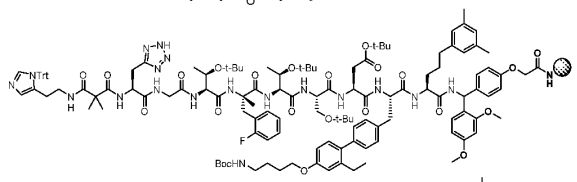
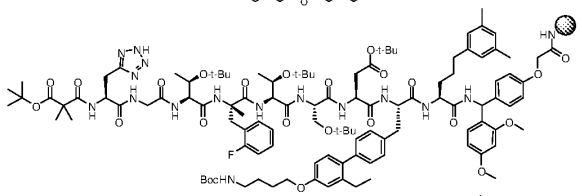
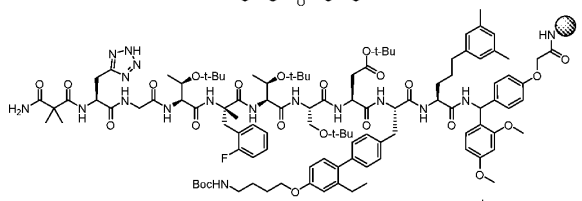
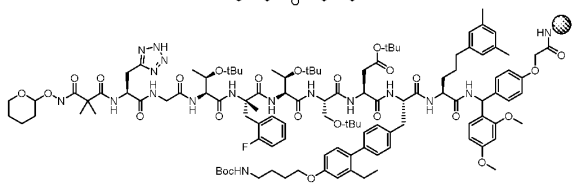
[000608] The general synthetic scheme for making GLP1 peptidomimetic payloads according to the present disclosure is shown as Figure 13. Table 2, shown below, depicts the structures of Rink amide MBHA resin bound peptides and intermediates 1-59.

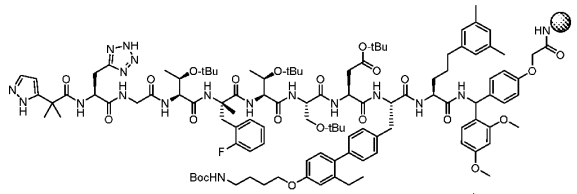
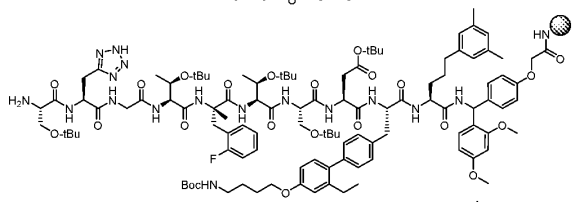
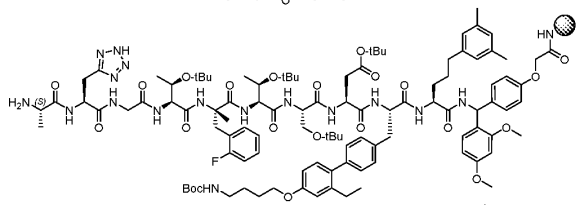
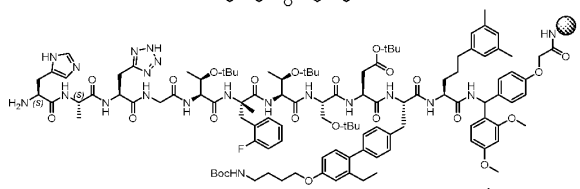
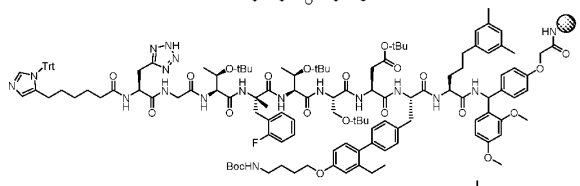
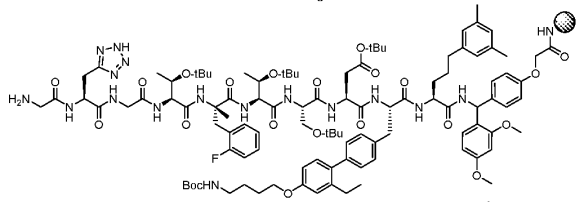
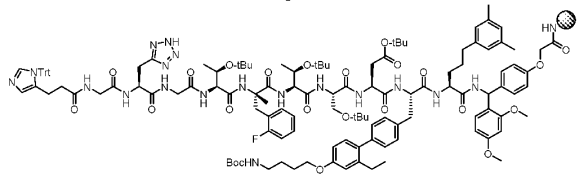
**Table 2**

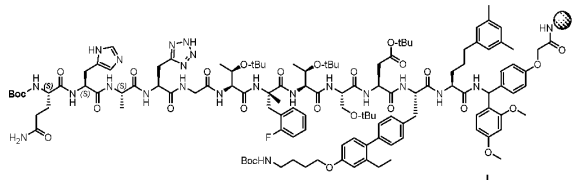
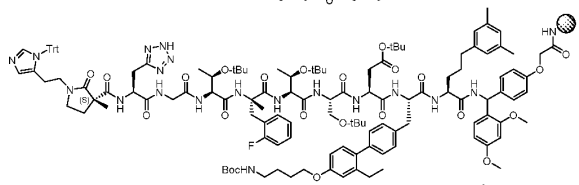
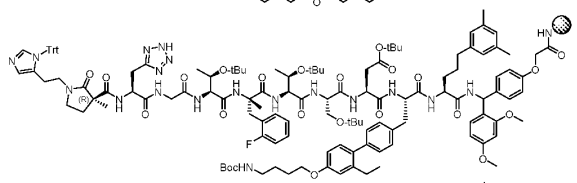
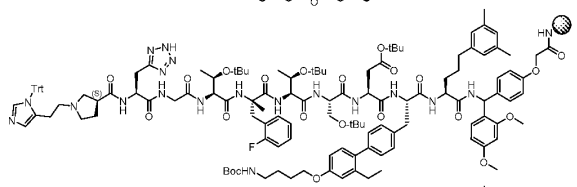
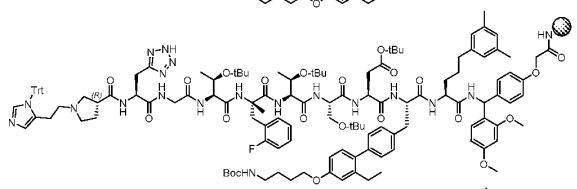
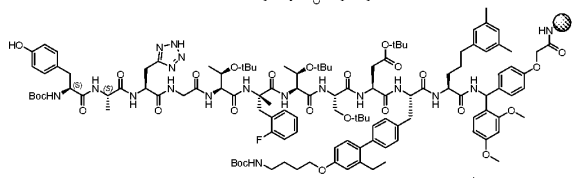
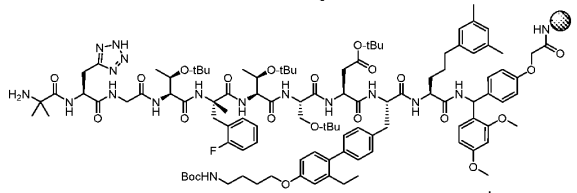
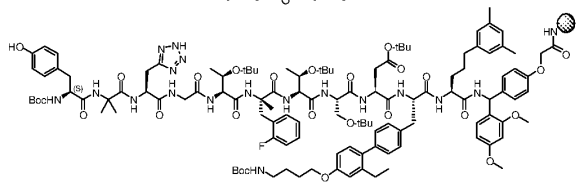
| Compound No. | Structure | MF [M-resin-$C_{17}H_{17}NO_4$+H] | MW (Cal.) |
|---|---|---|---|
| 1 |  | $C_{13}H_{20}N_2O$ | 220.31 |
| 2 |  | $C_{34}H_{44}N_6O_3$ | 584.75 |
| 3 |  | $C_{42}H_{57}N_7O_6$ | 755.95 |

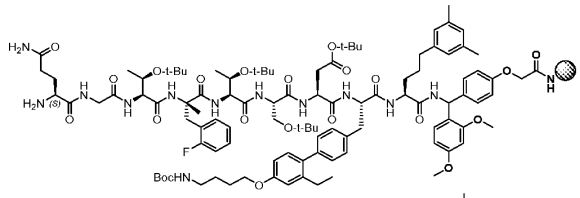
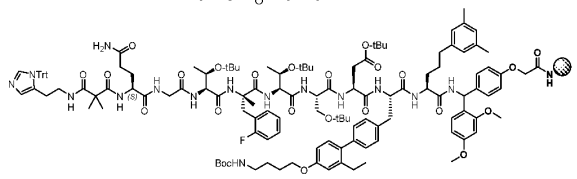
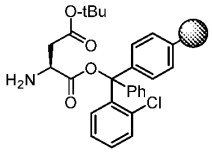
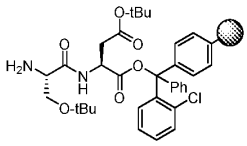
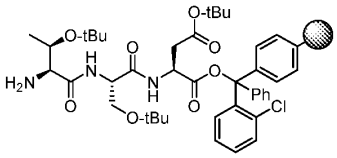
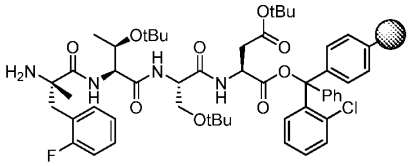
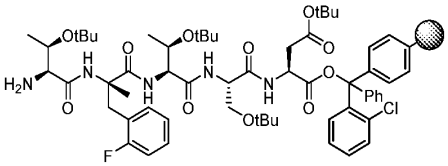
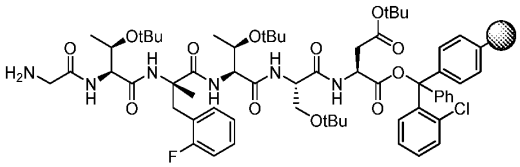
| | | | |
|---|---|---|---|
| 4 |  | $C_{49}H_{70}N_8O_8$ | 899.13 |
| 5 |  | $C_{57}H_{85}N_9O_{10}$ | 1056.34 |
| 6 |  | $C_{67}H_{95}FN_{10}O_{11}$ | 1235.53 |
| 7 |  | $C_{75}H_{110}FN_{11}O_{13}$ | 1392.74 |
| 8 |  | $C_{77}H_{113}FN_{12}O_{14}$ | 1449.79 |
| 9 |  | $C_{81}H_{118}FN_{17}O_{15}$ | 1588.91 |

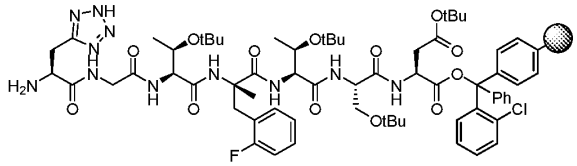
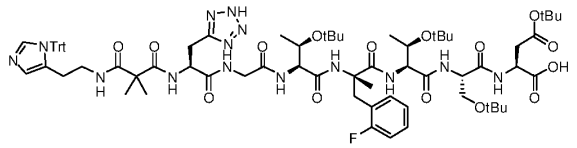
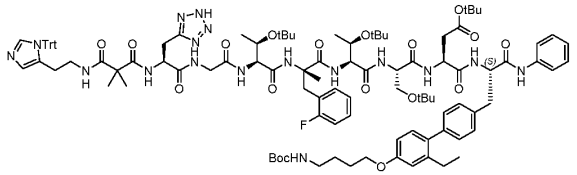
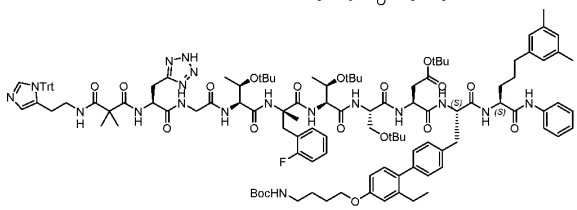
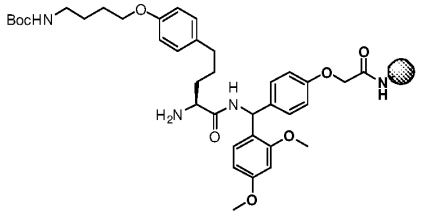
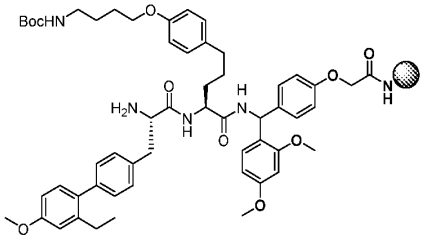
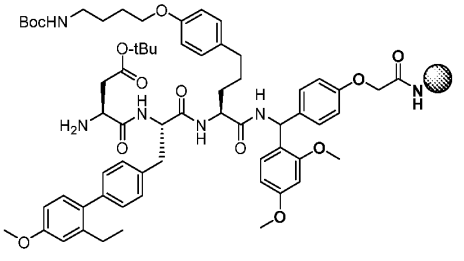
| | | | |
|---|---|---|---|
| 10 |  | $C_{110}H_{145}FN_{20}O_{17}$ | 2038.45 |
| 11 |  | $C_{39}H_{54}N_4O_5$ | 658.87 |
| 12 |  | $C_{47}H_{67}N_5O_8$ | 830.06 |
| 13 |  | $C_{54}H_{80}N_6O_{10}$ | 973.24 |
| 14 |  | $C_{62}H_{95}N_7O_{12}$ | 1130.45 |
| 15 |  | $C_{72}H_{105}FN_8O_{13}$ | 1309.64 |

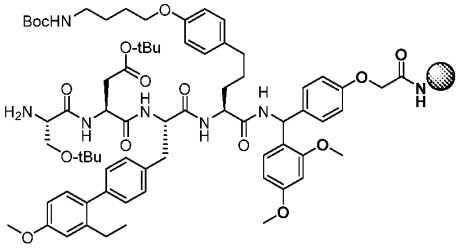
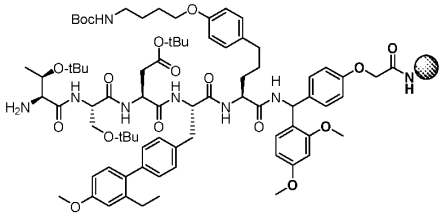
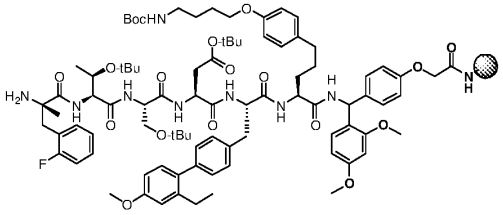
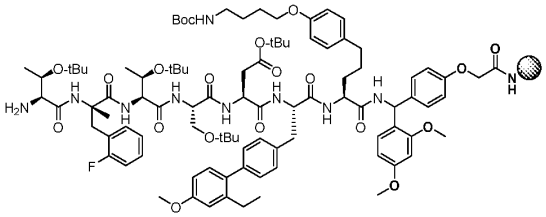
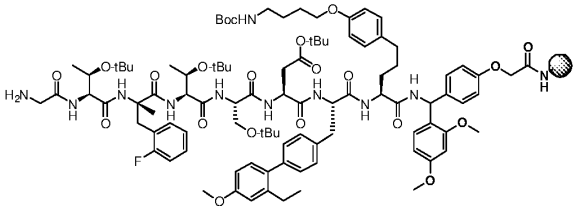
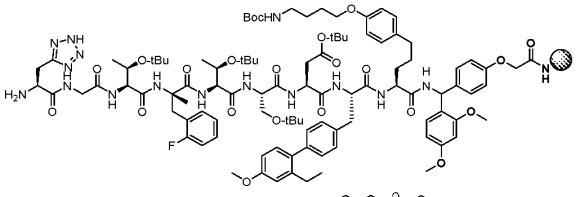
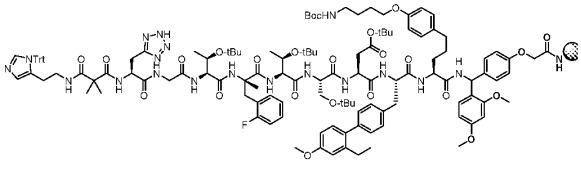
| | | | |
|---|---|---|---|
| 16 |  | $C_{80}H_{120}FN_9O_{15}$ | 1466.85 |
| 17 |  | $C_{82}H_{123}FN_{10}O_{16}$ | 1523.91 |
| 18 |  | $C_{86}H_{128}FN_{15}O_{17}$ | 1663.02 |
| 19 |  | $C_{115}H_{155}FN_{18}O_{19}$ | 2112.56 |
| 20 |  | $C_{95}H_{142}FN_{15}O_{20}$ | 1833.23 |
| 21 |  | $C_{91}H_{135}FN_{16}O_{19}$ | 1776.14 |
| 22 |  | $C_{96}H_{142}FN_{16}O_{21}$ | 1875.25 |

| | | | |
|---|---|---|---|
| 23 |  | $C_{93}H_{136}FN_{17}O_{18}$ | 1799.18 |
| 24 |  | $C_{93}H_{141}FN_{16}O_{19}$ | 1806.21 |
| 25 |  | $C_{89}H_{133}FN_{16}O_{18}$ | 1734.10 |
| 26 |  | $C_{95}H_{140}FN_{19}O_{19}$ | 1871.24 |
| 27 |  | $C_{114}H_{154}FN_{17}O_{18}$ | 2069.54 |
| 28 |  | $C_{88}H_{131}FN_{16}O_{18}$ | 1720.08 |
| 29 |  | $C_{113}H_{151}FN_{18}O_{19}$ | 2084.52 |

| | | | |
|---|---|---|---|
| 30 |  | $C_{105}H_{156}FN_{21}O_{23}$ | 2099.49 |
| 31 |  | $C_{116}H_{155}FN_{18}O_{19}$ | 2124.58 |
| 32 |  | $C_{116}H_{155}FN_{18}O_{19}$ | 2124.58 |
| 33 |  | $C_{115}H_{155}FN_{18}O_{18}$ | 2096.57 |
| 34 |  | $C_{115}H_{155}FN_{18}O_{18}$ | 2096.57 |
| 35 |  | $C_{103}H_{150}FN_{17}O_{22}$ | 1997.39 |
| 36 |  | $C_{90}H_{135}FN_{16}O_{18}$ | 1748.13 |
| 37 |  | $C_{104}H_{152}FN_{17}O_{22}$ | 2011.42 |

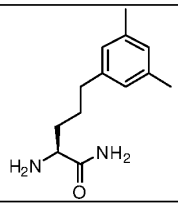
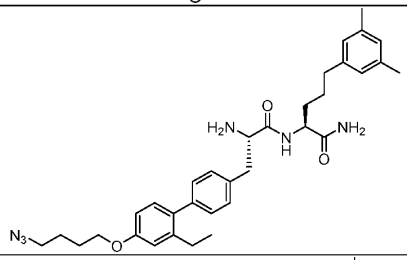
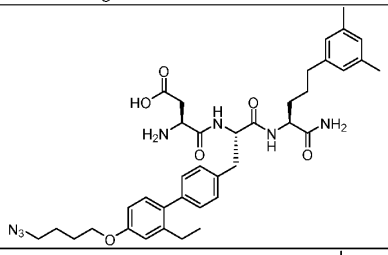
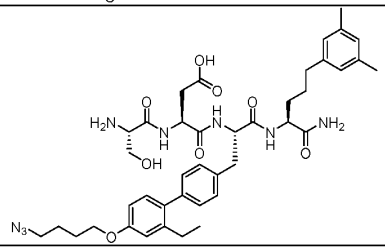
| | | | |
|---|---|---|---|
| **38** |  | $C_{87}H_{131}FN_{12}O_{18}$ | 1652.04 |
| **39** |  | $C_{116}H_{158}FN_{15}O_{20}$ | 2101.58 |
| **40** |  | **[M-resin-ClTrt+H]** $C_8H_{15}NO_4$ | 189.21 |
| **41** |  | **[M-resin-ClTrt+H]** $C_{15}H_{28}N_2O_6$ | 332.39 |
| **42** |  | **[M-resin-ClTrt+H]** $C_{23}H_{43}N_3O_8$ | 489.60 |
| **43** |  | **[M-resin-ClTrt+H]** $C_{33}H_{53}FN_4O_9$ | 668.79 |
| **44** |  | **[M-resin-ClTrt+H]** $C_{41}H_{68}FN_5O_{11}$ | 826.00 |
| **45** |  | **[M-resin-ClTrt+H]** $C_{43}H_{71}FN_6O_{12}$ | 883.06 |

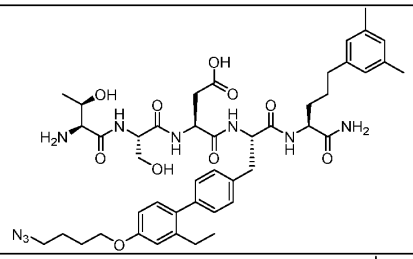
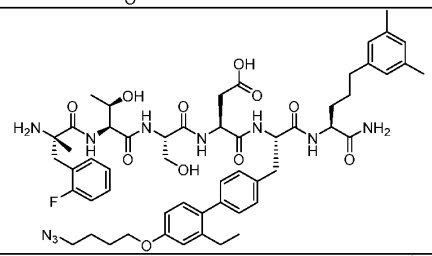
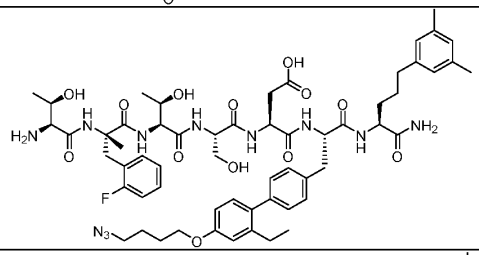
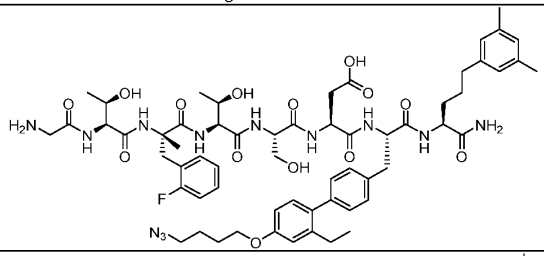
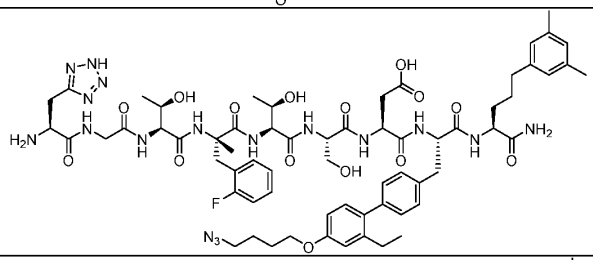
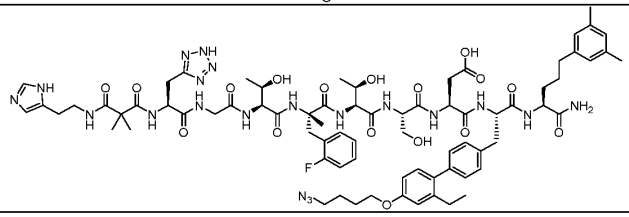
| | | | |
|---|---|---|---|
| 46 |  | [M-resin-ClTrt+H] $C_{47}H_{76}FN_{11}O_{13}$ | 1022.17 |
| 47 |  | $C_{76}H_{103}FN_{14}O_{15}$ | 1471.71 |
| 48 |  | $C_{108}H_{142}FN_{17}O_{18}$ | 1985.38 |
| 49 |  | $C_{121}H_{159}FN_{18}O_{19}$ | 2188.66 |
| 50 |  | $C_{20}H_{33}N_3O_4$ | 379.49 |
| 51 |  | $C_{38}H_{52}N_4O_6$ | 660.3887 |
| 52 |  | $C_{46}H_{65}N_5O_9$ | 831.4782 |

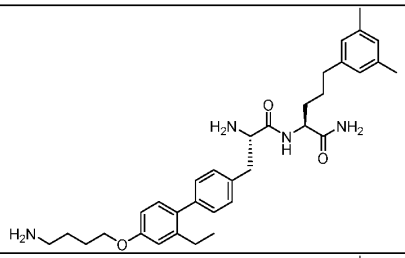
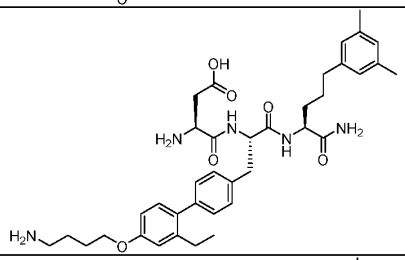
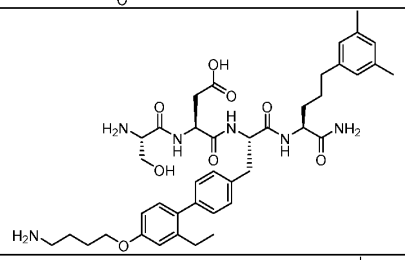
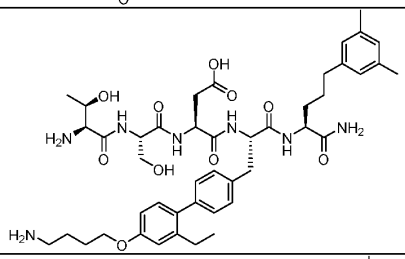
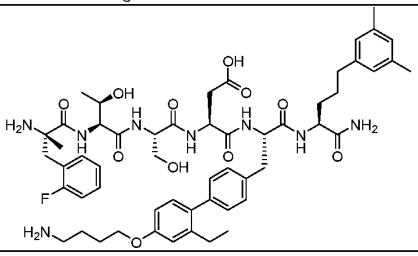
| | | | |
|---|---|---|---|
| 53 |  | $C_{53}H_{78}N_6O_{11}$ | 974.5729 |
| 54 |  | $C_{61}H_{93}N_7O_{13}$ | 1131.68 |
| 55 |  | $C_{71}H_{103}FN_8O_{14}$ | 1310.76 |
| 56 |  | $C_{79}H_{118}FN_9O_{16}$ | 1467.868 |
| 57 |  | $C_{81}H_{121}FN_{10}O_{17}$ | 1524.8895 |
| 58 |  | $C_{85}H_{126}FN_{15}O_{18}$ | 1663.9389 |
| 59 |  | $C_{114}H_{153}FN_{18}O_{20}$ | 2113.149 |

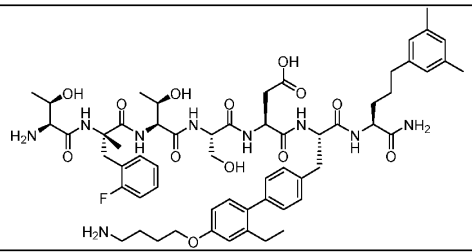
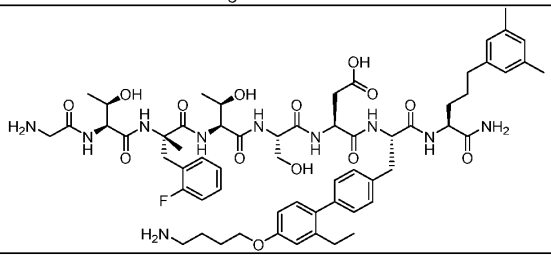
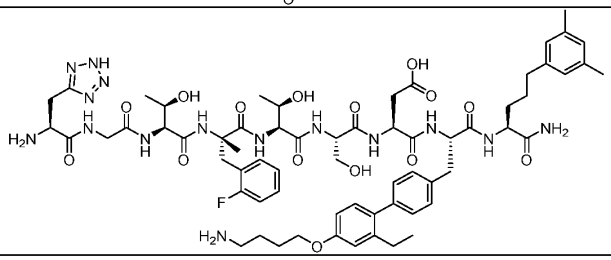
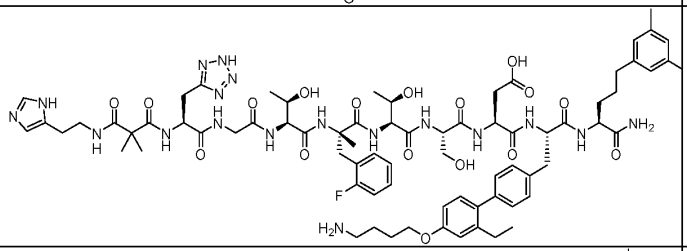
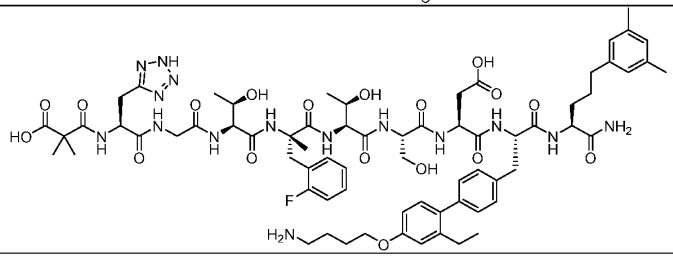
[000609] The Rink amide MBHA resin bound peptides and intermediates (1 ~ 59) were analyzed by LC-MS after cleavage from the Resin. Table 2B, below, summarizes these intermediates.

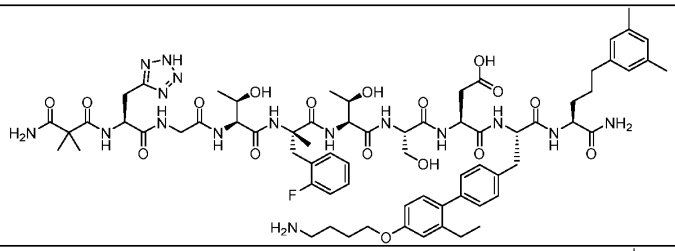
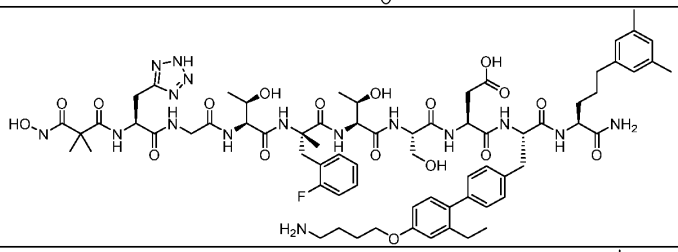
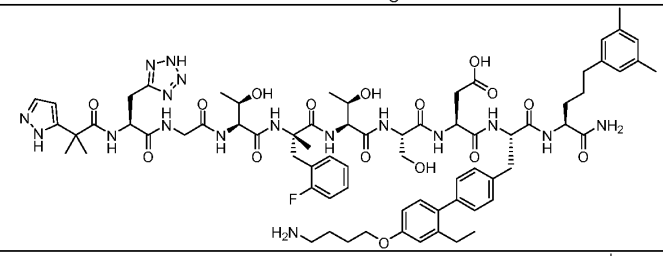
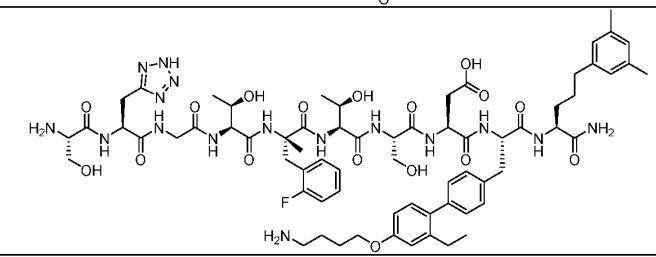
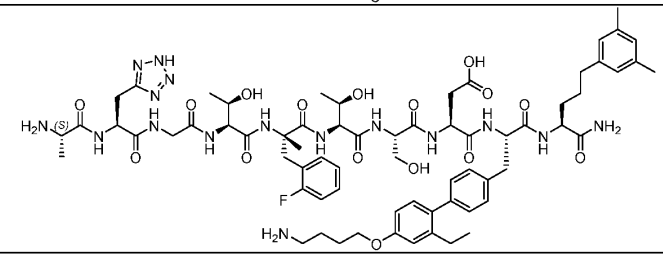
**Table 2B. Intermediates Cleaved from MBHA Resin**

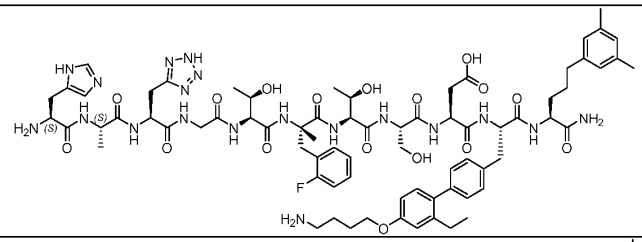
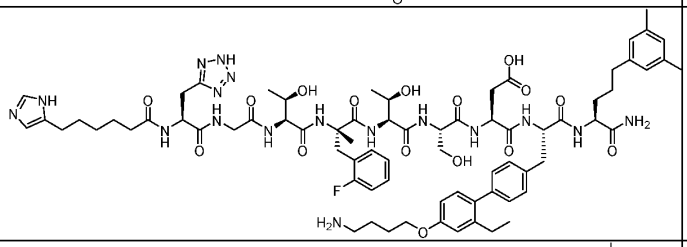
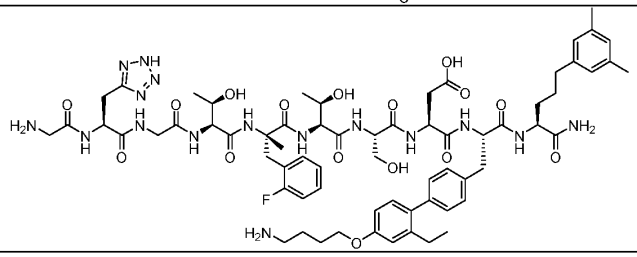
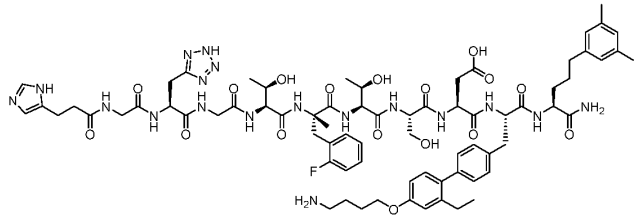
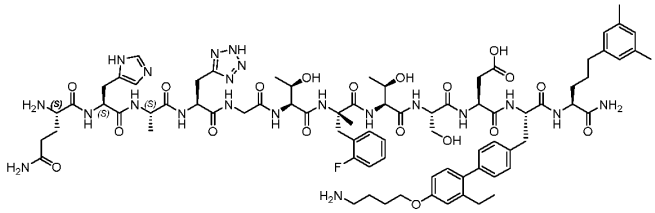
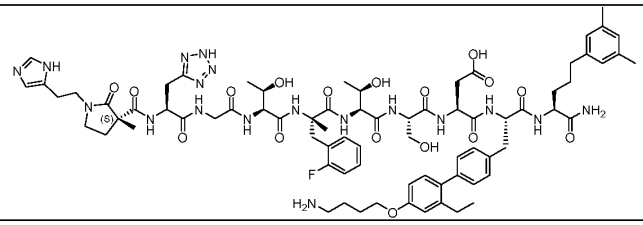
| No. | Structure | MF | MW (Cal.) | MS (m/z) |
|---|---|---|---|---|
| 1 |  | $C_{13}H_{20}N_2O$ | 220.3 | 221.4 $[M+H]^+$ |
| 2 |  | $C_{34}H_{44}N_6O_3$ | 584.8 | 585.3 $[M+H]^+$ 607.30 $[M+Na]^+$ |
| 3 |  | $C_{38}H_{49}N_7O_6$ | 699.4 | 700.4 $[M+H]^+$ |
| 4 |  | $C_{41}H_{54}N_8O_8$ | 786.4 | 787.4 $[M+H]^+$ |

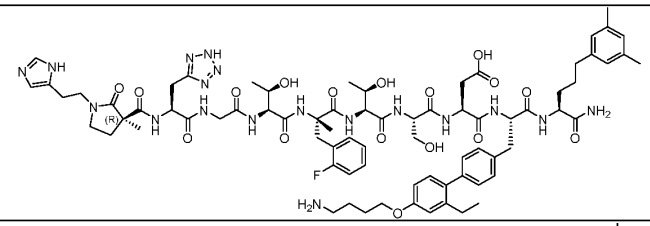
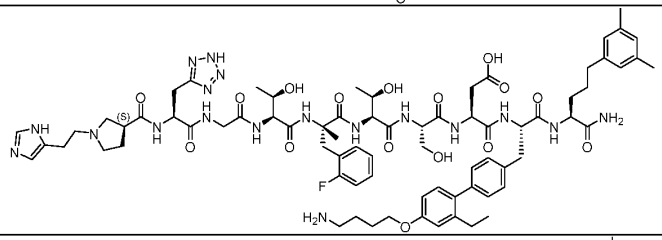
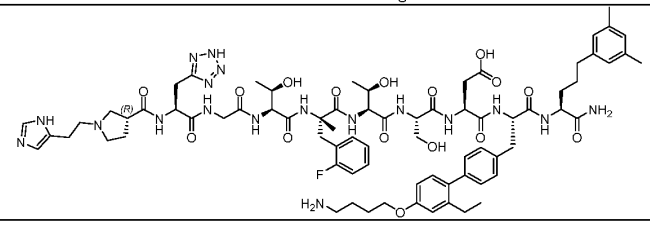
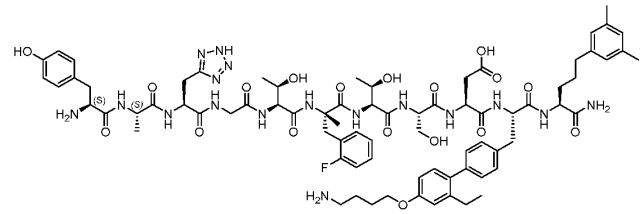
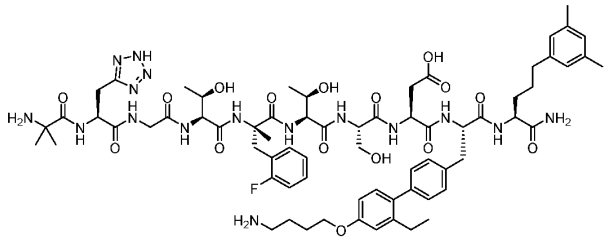
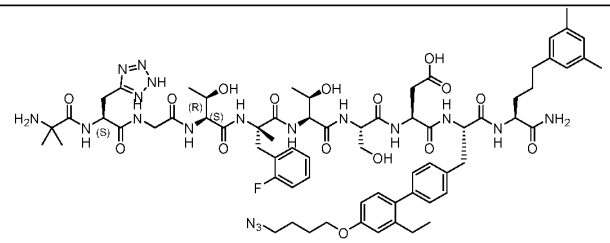
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|---|---|---|---|---|
| 5 |  | $C_{45}H_{61}N_9O_{10}$ | 887.5 | 888.5 $[M+H]^+$ |
| 6 |  | $C_{55}H_{71}FN_{10}O_{11}$ | 1066.7 | 1067.7 $[M+H]^+$ |
| 7 |  | $C_{59}H_{78}FN_{11}O_{13}$ | 1167.6 | 1168.2 $[M+H]^+$ |
| 8 |  | $C_{61}H_{81}FN_{12}O_{14}$ | 1224.6 | 1225.9 $[M+H]^+$ |
| 9 |  | $C_{65}H_{86}FN_{17}O_{15}$ | 1363.6 | 1364.9 $[M+H]^+$ |
| 10 |  | $C_{75}H_{99}FN_{20}O_{17}$ | 1570.7 | 787.1 $[M+2H]^{2+}$ |

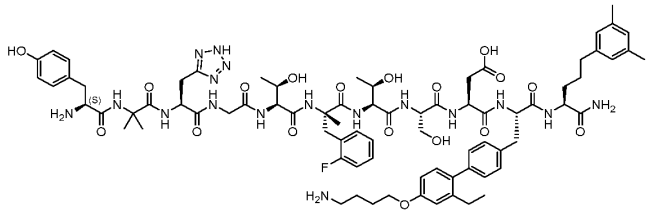
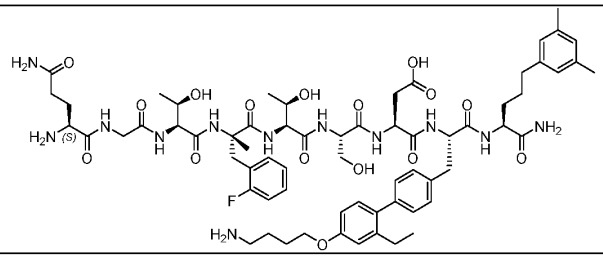
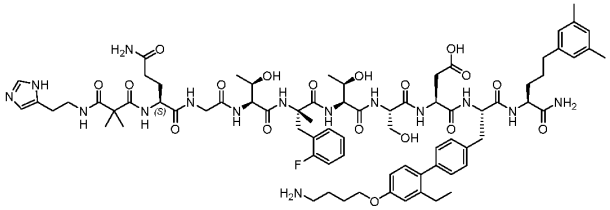
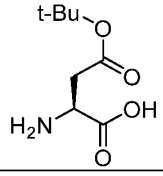
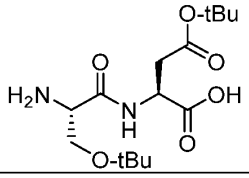
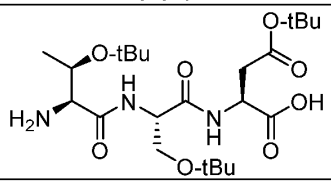
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| **11** |  | $C_{34}H_{46}N_4O_3$ | 558.4 | 559.4<br>$[M+H]^+$ |
| **12** |  | $C_{38}H_{51}N_5O_6$ | 673.4 | 674.2<br>$[M+H]^+$ |
| **13** |  | $C_{41}H_{56}N_6O_8$ | 760.4 | 761.4<br>$[M+H]^+$<br>381.3<br>$[M+2H]^{2+}$ |
| **14** |  | $C_{45}H_{63}N_7O_{10}$ | 861.5 | 862.9<br>$[M+H]^+$<br>431.9<br>$[M+2H]^{2+}$ |
| **15** |  | $C_{55}H_{73}FN_8O_{11}$ | 1040.5 | 521.6<br>$[M+2H]^{2+}$ |

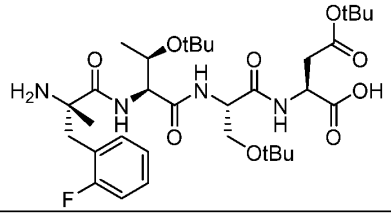
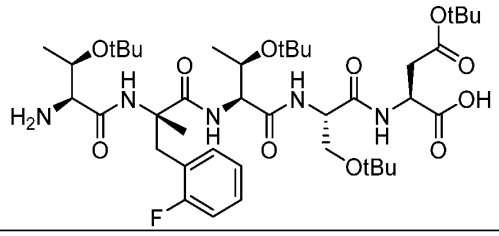
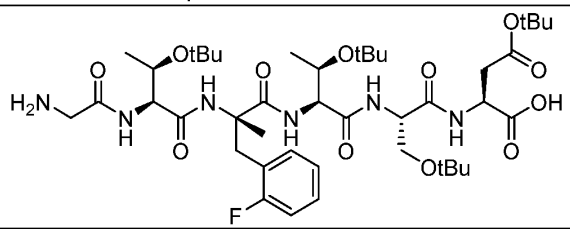
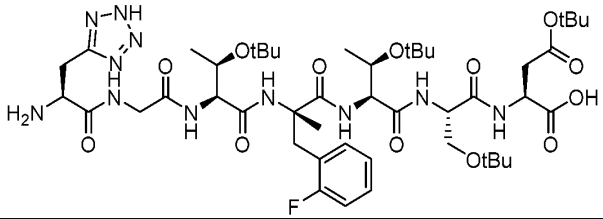
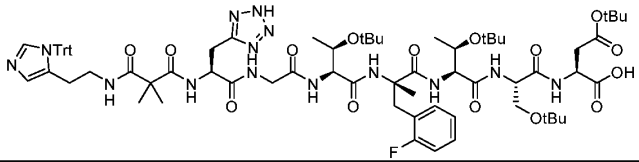
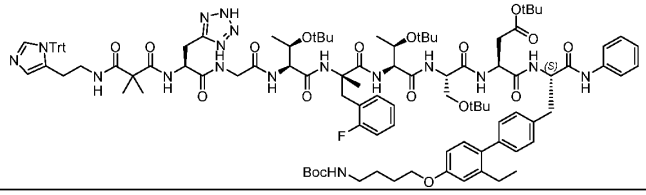
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| **16** |  | $C_{59}H_{80}FN_9O_{13}$ | 1141.6 | 572.0 $[M+2H]^{2+}$ |
| **17** |  | $C_{61}H_{83}FN_{10}O_{14}$ | 1198.6 | 600.6 $[M+2H]^{2+}$ |
| **18** |  | $C_{65}H_{88}FN_{15}O_{15}$ | 1337.7 | 670.1 $[M+2H]^{2+}$ |
| **19** |  | $C_{75}H_{101}FN_{18}O_{17}$ | 1544.8 | 773.7 $[M+2H]^{2+}$ |
| **20** |  | $C_{70}H_{94}FN_{15}O_{18}$ | 1451.7 | 726.9 $[M+2H]^{2+}$ |

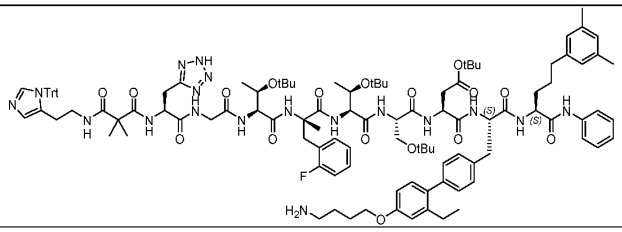
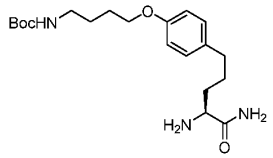
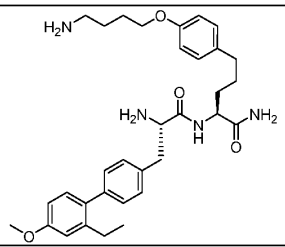
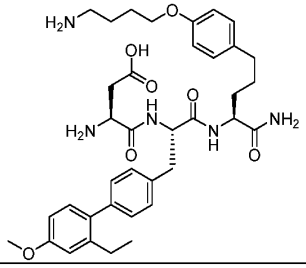
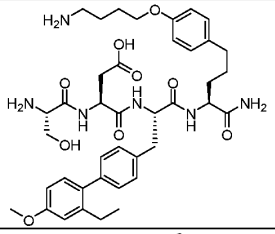
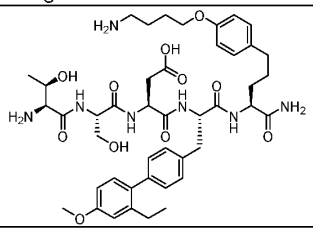
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| **21** |  | $C_{70}H_{95}FN_{16}O_{17}$ | 1450.7 | 1452.1 $[M+H]^+$ |
| **22** |  | $C_{70}H_{94}FN_{16}O_{18}$ | 1465.7 | 734.4 $[M+2H]^{2+}$ |
| **23** |  | $C_{72}H_{96}FN_{17}O_{16}$ | 1473.7 | 738.2 $[M+2H]^{2+}$ |
| **24** |  | $C_{68}H_{93}FN_{16}O_{17}$ | 1424.7 | 713.8 $[M+2H]^{2+}$ |
| **25** |  | $C_{68}H_{93}FN_{16}O_{16}$ | 1408.7 | 705.8 $[M+2H]^{2+}$ |

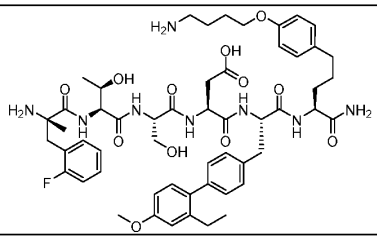
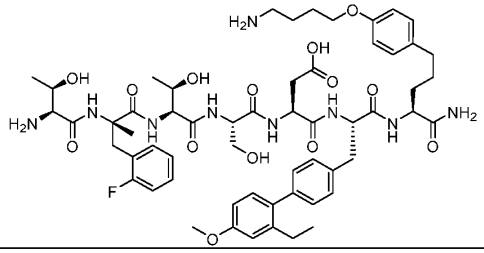
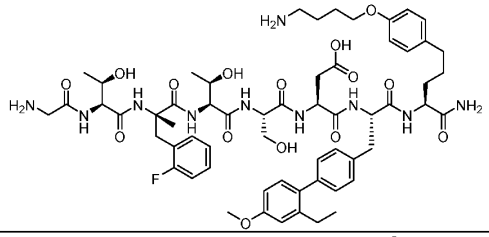
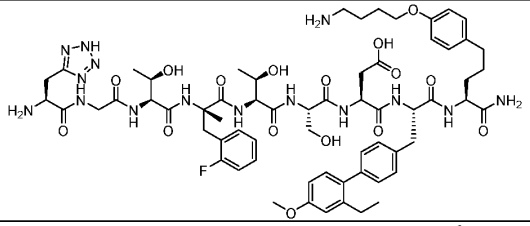
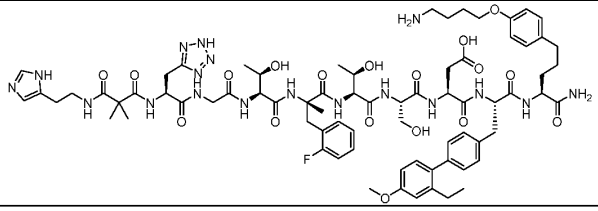
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| **26** |  | $C_{74}H_{100}FN_{19}O_{17}$ | 1545.8 | 773.9 $[M+2H]^{2+}$ |
| **27** |  | $C_{74}H_{100}FN_{17}O_{16}$ | 1501.8 | 752.0 $[M+2H]^{2+}$ |
| **28** |  | $C_{67}H_{91}FN_{16}O_{16}$ | 1394.7 | 698.6 $[M+2H]^{2+}$ |
| **29** |  | $C_{73}H_{97}FN_{18}O_{17}$ | 1516.7 | 759.7 $[M+2H]^{2+}$ |
| **30** |  | $C_{79}H_{108}FN_{21}O_{19}$ | 1673.8 | 838.2 $[M+2H]^{2+}$ |
| **31** |  | $C_{76}H_{101}FN_{18}O_{17}$ | 1556.8 | 779.7 $[M+2H]^{2+}$ |

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| **32** |  | $C_{76}H_{101}FN_{18}O_{17}$ | 1556.8 | 779.8 $[M+2H]^{2+}$ |
| **33** |  | $C_{75}H_{101}FN_{18}O_{16}$ | 1528.8 | 765.8 $[M+2H]^{2+}$ |
| **34** |  | $C_{75}H_{101}FN_{18}O_{16}$ | 1528.8 | 765.8 $[M+2H]^{2+}$ |
| **35** |  | $C_{77}H_{102}FN_{17}O_{18}$ | 1571.8 | 787.4 $[M+2H]^{2+}$ |
| **36** |  | $C_{69}H_{95}FN_{16}O_{16}$ | 1422.7 | 712.7 $[M+2H]^{2+}$ |
| **36A** |  | $C_{69}H_{93}FN_{18}O_{16}$ | 1448.7 | 1450.4 $[M+H]^{+}$ |

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| **37** |  | $C_{78}H_{104}FN_{17}O_{18}$ | 1585.8 | 794.3 $[M+2H]^{2+}$ |
| **38** |  | $C_{66}H_{91}FN_{12}O_{16}$ | 1326.7 | 664.6 $[M+2H]^{2+}$ |
| **39** |  | $C_{76}H_{104}FN_{15}O$ | 1533.8 | 768.3 $[M+2H]^{2+}$ |
| **40** |  | $C_8H_{15}NO_4$ | 189.2 | / |
| **41** |  | $C_{15}H_{28}N_2O_6$ | 332.2 | 333.0 $[M+H]^+$ |
| **42** |  | $C_{23}H_{43}N_3O_8$ | 489.3 | 490.2 $[M+H]^+$ |

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| **43** |  | $C_{33}H_{53}FN_4O_9$ | 668.4 | 669.3 $[M+H]^+$ |
| **44** |  | $C_{41}H_{68}FN_5O_{11}$ | 825.5 | 826.5 $[M+H]^+$ |
| **45** |  | $C_{43}H_{71}FN_6O_{12}$ | 882.5 | 883.4 $[M+H]^+$ |
| **46** |  | $C_{47}H_{76}FN_{11}O_{13}$ | 1021.6 | 1022.5 $[M+H]^+$ |
| **47** |  | $C_{76}H_{103}FN_{14}O_{15}$ | 1470.8 | 1471.8 $[M+H]^+$ |
| **48** |  | $C_{108}H_{142}FN_{17}O_{18}$ | 1984.1 | 993.8 $[M+2H]^{2+}$ |

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|---|---|---|---|---|
| **49** |  | $C_{116}H_{151}FN_{18}O_{17}$ | 2087.1 | 1044.8 $[M+2H]^{2+}$ |
| **50** |  | $C_{20}H_{33}N_3O_4$ | 379.5 | / |
| **51** |  | $C_{33}H_{44}N_4O_4$ | 560.3 | 583.3 $[M+Na]^+$ |
| **52** |  | $C_{37}H_{49}N_5O_7$ | 675.4 | 676.1 $[M+H]^+$ |
| **53** |  | $C_{40}H_{54}N_6O_9$ | 762.4 | 763.4 $[M+H]^+$ |
| **54** |  | $C_{44}H_{61}N_7O_{11}$ | 863.4 | 864.4 $[M+H]^+$ |

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|---|---|---|---|---|
| **55** |  | $C_{54}H_{71}FN_8O_{12}$ | 1042.5 | 522.5 $[M+2H]^{2+}$ |
| **56** |  | $C_{58}H_{78}FN_9O_{14}$ | 1143.6 | 573.0 $[M+2H]^{2+}$ |
| **57** |  | $C_{60}H_{81}FN_{10}O_{15}$ | 1200.6 | 601.5 $[M+2H]^{2+}$ |
| **58** |  | $C_{64}H_{86}FN_{15}O_{16}$ | 1339.6 | 671.0 $[M+2H]^{2+}$ |
| **59** |  | $C_{74}H_{99}FN_{18}O_{18}$ | 1546.7 | 774.6 $[M+2H]^{2+}$ |

[000610] Figure 14 depicts the sequence of steps for solid support synthesis of GLP1 peptidomimetic payloads P1 and P8 according to the disclosure.

3.1 Preparation of (3S,6S,9R,12S,15S,21S)-21-amino-3-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl) -1-oxopentan-2-yl)amino)-3-(4'-(4-aminobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-12-(2-fluorobenzyl)-9,15-bis((R)-1-hydroxyethyl)-6-(hydroxymethyl)-

12-methyl-5,8,11,14,17,20-hexaoxo-22-(2H-tetrazol-5-yl)-4,7,10,13,16,19-hexaazadocosan-1-oic acid (P1)

[000611] The corresponding **Fmoc-protected aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1** peptidyl **Rink Amide MBHA Resin** (**9**, 16.59 µmol) was prepared as described in the general procedure of SPPS. The resin-bound peptide **9** was cleaved following the general procedure to give the crude product as a white solid. This crude product was dissolved in DMF (1 mL), and piperidine (14.13 mg, 165.94 µmol, 16.39 µL, 10.0 *eq.*) was added in one portion at 20 °C under nitrogen. The mixture was stirred at 20 °C for 2 hours. The mixture was concentrated in vacuum to give the residue. The residue was purified by prep-HPLC (column: mobile phase: [water (0.1%TFA)-ACN]; B%: 30%-60%, 60min) to afford pure product. The product was suspended in water (10 mL), the mixture frozen in a dry-ice/ethanol bath, and then lyophilized to dryness to afford the desired product **P1** (1.02 mg, 7.48e-1 µmol, 4.50% yield, 100% purity) as a white solid. HRMS (ESI): mass calcd. for $C_{65}H_{87}FN_{17}O_{15}$ 1364.6552, m/z found 1364.4887 $[M+H]^+$.

[000612] HPLC: RT = 10.15 min, Reverse phase HPLC was carried out using a MERCK, RP-18e 25-2mm column, with a flow rate of 1.2 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

3.2 Preparation of (8S,14S,17S,20S,23S,26S)-8-((2H-tetrazol-5-yl)methyl)-26-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-aminobutoxy)-2'-ethyl-[1,1'-biphenyl] -4-yl)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl)-23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21,24-octaoxo-3,7,10,13,16,19,22,25-octaazaoctacosan-28-oic acid (P8)

[000613] The corresponding **aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1** peptidyl **Rink Amide MBHA Resin** (**10**, 100.99 µmol) was assembled as described in the general procedure of SPPS. The resin-bound peptide **10** was cleaved following the general procedure to give the crude product as a white solid. The crude was purified by preparative HPLC using column: Luna 200*25 mm, C18, 10 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 30%-60%, 60min to afford pure product. The product was suspended in water (10 mL), the mixture frozen in a dry-ice/acetone bath, and then lyophilized to dryness to afford the desired product **P8** (25.00 mg, 17.49 µmol, 17.32% yield, 100% purity) as a white solid.

[000614] HRMS (ESI): mass calcd for $C_{75}H_{100}FN_{20}O_{17}$ 1571.7559, m/z found 1571.7589 $[M+H]^+$.

[000615] HPLC: RT = 10.14 min. Reverse phase HPLC was carried out using a YMC-Pack ODS-A 150*4.6 mm,5µm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to

80% acetonitrile containing 0.062% TFA (solvent B) and water containing 0.068% TFA (solvent A).

[000616] Figure 15 depicts the sequence of steps for solid support synthesis of GLP1 peptidomimetic payloads P2 and P9 according to the disclosure.

3.3 Preparation of (8S,14S,17S,20R,23S,26S)-8-((2H-tetrazol-5-yl)methyl)-26-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-aminobutoxy)-2'-ethyl-[1,1'-biphenyl] -4-yl)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl)-23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21,24-octaoxo-3,7,10,13, 16,19,22,25-octaazaoctacosan-28-oic acid (P2)

[000617] The corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (18, 74.75 µmol) was prepared as described in the general procedure of SPPS. The resin-bound peptide 18 was cleaved following the general procedure to give the crude product as a white solid. The crude product was sent to prep-HPLC (column: mobile phase: [water (0.1%TFA)-ACN];B%: 20%-50%,60min) to afford pure product. The product was suspended in water (100 mL), the mixture frozen in a dry-ice/acetone bath, and then lyophilized to dryness to afford the desired product P2 (11.56 mg, 8.45 µmol, 11.27% yield, 97.84% purity) was obtained as a white solid.

[000618] LCMS (ESI): RT = 0.830 min, mass calcd. for C65H88FN15O15 1337.66 [M-4tBu-Boc+6H]+ 669.83 [M-4tBu-Boc+7H]2+, found 670.0 [M-4tBu-Boc+7H]2+. Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.04% TFA (solvent B) and water containing 0.06% TFA (solvent A).

[000619] HPLC: RT = 7.77 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40℃.

3.4 Preparation of (8S,14S,17S,20S,23S,26S)-8-((2H-tetrazol-5-yl)methyl)-26-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-aminobutoxy)-2'-ethyl-[1,1'-biphenyl] -4-yl)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl)-23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21,24-octaoxo-3,7,10,13, 16,19,22,25-octaazaoctacosan-28-oic acid (P9)

[000620] The corresponding aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (19) was prepared as described in the general procedure of SPPS. The resin-

bound peptide 19 was cleaved following the general procedure to give the crude product as a white solid. The crude product was sent to prep-HPLC (TFA: mobile phase: [water (0.075%TFA)-ACN]; B%: 15%-45%, 55 min) to afford pure product. The product was suspended in water (20 mL), the mixture frozen in a dry-ice/acetone bath, and then lyophilized to dryness to afford the desired product P9 (125 mg, 79.82 µmol, 7.70% yield, 98.7% purity) was obtained as a white solid.

[000621] LCMS (ESI): RT = 0.843 min, mass calcd. for C75H102FN18O17 1545.77 [M-4tBu-Boc+6H]+ 773.385 [M-4tBu-Boc+7H]2+, found 773.9 [M-4tBu-Boc+7H]2+. Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.04% TFA (solvent B) and water containing 0.06% TFA (solvent A).

[000622] Figure 16 depicts the sequence of steps for solid support synthesis of GLP1 peptidomimetic payloads P3, P4, P5, P6, P7, P11, P13, P14, P15, P16 and P17 according to the disclosure.

3.5 Preparation of 3-[[(1S)-2-[[2-[[(1S,2R)-1-[[(1S)-2-[[(1S,2R)-1-[[(1S)-2-[[(1S)-2-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl) butyl]amino]-2-oxo-ethyl]amino]-1-(carboxymethyl)-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl]carbamoyl]-2-hydroxy-propyl]amino]-1-[(2-fluorophenyl)methyl]-1-methyl-2-oxo-ethyl]carbamoyl]-2-hydroxy-propyl]amino]-2-oxo-ethyl]amino]-2-oxo-1-(2H-tetrazol-5-ylmethyl)ethyl]amino]-2,2-dimethyl-3-oxo-propanoic acid (P3)

[000623] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (18, 152.96 µmol) and aa11 (57.58 mg, 305.91 µmol, 2.0 eq.), the corresponding aa11-aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (20, 119 µmol) was prepared as described in the general procedure of SPPS.

[000624] The corresponding resin-bound peptide 20 (119 µmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water(0.1%TFA)-ACN];B%: 15%-45%, 55 min) to provide P3 (8 mg, 5.47 µmol, 3.58% yield, 99.37% purity) as a light yellow solid.

[000625] HPLC: RT =8.10 min. HPLC conditions: YMC-Pack ODS-A 150*4.6 mm, 5 µm column, flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.12% TFA (solvent B) and water containing 0.1% TFA (solvent A).

[000626] LCMS: (ESI): RT = 0.841 min, m/z calcd. for C18H21NO2 1452.69 [M+H]+, found 727.3 [M+2H]2+; LCMS conditions: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

3.6 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-2-[(3-amino-2,2-dimethyl-3-oxo-propanoyl)amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P4)

[000627] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin compound 18 (192.73 µmol) and aa12 (75.82 mg, 578.18 µmol, 3 eq.), the corresponding aa12-aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (21, 192.73 µmol) was obtained as described in the general procedure of SPPS.

[000628] The corresponding resin-bound peptide 21 (192.73 µmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water(0.1%TFA)-ACN];B%: 15%-45%,55 min) to provide P4 (35 mg, 24.03 µmol, 8.73% yield, 99.66% purity) as a light yellow solid.

[000629] LCMS: (ESI): RT = 0.861 min, mass calcd. for C76H103FN18O17 726.36 m/z [M+2H]2+; found 726.7 m/z [M+2H]2+; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000630] LCMS: (ESI): RT = 0.854 min, mass calcd. for C76H103FN18O17 726.36 m/z [M+2H]2+; found 726.7 m/z [M+2H]2+; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000631] HPLC: RT = 7.90 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40°C.

3.7 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-

[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-(hydroxyamino)-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino] -2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (P5)

[000632] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin compound 18 (152.96 µmol) and aa13 (60 mg, 259.47 µmol, 1.7 eq.), the corresponding aa13-aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (22, 152.67 µmol) was obtained as described in the general procedure of SPPS.

[000633] The corresponding resin-bound peptide 22 was cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water(0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P5 (4 mg, 2.66 µmol, 42.12% yield, 97.7% purity) as a light yellow solid.

[000634] LCMS: (ESI): RT = 0.705 min, mass calcd. for $C_{70}H_{96}FN_{16}O_{18}$ 733.85 m/z $[M+2H]^{2+}$; found 718.2 m/z $[M-2OH+4H]^{2+}$; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000635] LCMS: (ESI): RT = 0.847 min, mass calcd. for $C_{70}H_{96}FN_{16}O_{18}$ 733.85 m/z $[M+2H]^{2+}$; found 735.2 m/z $[M+2H]^{2+}$; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000636] HPLC: RT = 8.00 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40°C.

3.8 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[2-methyl-2-(1H-pyrazol-5-yl) propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (P6)

[000637] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin compound 18 (82.60 µmol) and aa14 (25.47 mg, 165.19 µmol, 2 eq.),

the corresponding aa14-aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (23, 82.60 µmol) was obtained as described in the general procedure of SPPS.

[000638] The corresponding resin-bound peptide 23 (82.60 µmol) was cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P6 (9 mg, 6.10 µmol, 7.40% yield, 97.34% purity) as a light yellow solid.

[000639] LCMS: (ESI): RT = 0.856 min, mass calcd. for C72H98FN17O16 737.87 m/z [M-4tBu-Boc-C17H17NO4+8H]2+, rink amide (C17H17NO4, exact mass = 299.12); found 738.2 m/z [M-4tBu-Boc-C17H17NO4+8H]2+, rink amide (C17H17NO4, exact mass = 299.12); LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000640] LCMS: (ESI): RT = 0.856 min, mass calcd. for C72H98FN17O16 737.87 m/z [M-4tBu-Boc-C17H17NO4+8H]2+, rink amide (C17H17NO4, exact mass = 299.12); found 738.2 m/z [M-4tBu-Boc-C17H17NO4+8H]2+, rink amide (C17H17NO4, exact mass = 299.12); LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000641] HPLC: RT = 8.16 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40°C.

3.9 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-2-[[(2S)-2-amino-3-hydroxy-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P7)

[000642] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (18, 76.48 µmol) and aa4 (87.98 mg, 229.44 µmol, 3 eq.). The corresponding aa4-aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (24, 76.48 µmol) was obtained as described in the general procedure of SPPS.

[000643] The corresponding resin-bound peptide 24 (76.48 µmol) was cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-

HPLC (column: Waters Xbridge Prep OBD C18 150*40 mm*10 μm; mobile phase: [water(0.1%TFA)-ACN];B%: 28%-58%,30min) to provide P7 (17 mg, 11.16 μmol,14.59% yield, 93.57% purity) as a white solid.

[000644] LCMS: (ESI): RT = 2.675 min, m/z calcd. for C68H95FN16O17, 713.35 [M+2H]2+, m/z found 713.8 [M+2H]2+; Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B),using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min;

[000645] LCMS: (ESI): RT = 0.757 min, m/z calcd. for C68H95FN16O17, 713.35 [M+2H]2+, m/z found 713.8 [M+2H]2+; Reverse phase LCMS was carried out using a Merck RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solventA);

[000646] HPLC (Rt = 7.58 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

3.10 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[6-(1H-imidazol-5-yl) hexanoylamino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P11)

[000647] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (18, 74.75 μmol) and aa17 (70.13 mg, 165.19 μmol, 2 eq.), the corresponding aa17-aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (27, 74.75 μmol) was obtained as described in the general procedure of SPPS.

[000648] The corresponding resin-bound peptide compound 27 (74.75 μmol) was cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 μm 300*50 mm, 12 nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P7 (9 mg, 5.80 μmol, 7.05% yield, 96.92% purity) as a white solid.

[000649] HPLC: RT =7.72 min. HPLC conditions: YMC-Pack ODS-A 150*4.6 mm, 5 μm column, flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.12% TFA (solvent B) and water containing 0.1% TFA (solvent A).

[000650] LCMS: (ESI): RT = 0.828 min, m/z calcd. for C18H21NO2 1502.75 [M+H]+, found 752.3 [M+2H]2+; LCMS conditions: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

3.11 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-2-[[(2S)-2-[[(2S)-2-[[(2S)-2,5-diamino-5-oxo-pentanoyl]amino]-3-(1H-imidazol-4-yl)propanoyl]amino]propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxybutanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P13)

[000651] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin compound 18 (82.60 µmol), aa15 (77.14 mg, 247.79 µmol, 3 eq.), aa16 (93.40 mg, 247.49 µmol, 3 eq.), and aa19 (40.63 mg, 165.00 µmol, 2 eq.), the corresponding aa19-aa16-aa15-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (30, 82.50 µmol) was prepared as described in the general procedure of SPPS .

[000652] The corresponding resin-bound peptide 30 (82.50 µmol) was cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P13 (10 mg, 5.69 µmol, 6.90% yield, 95.28% purity) as a white solid.

[000653] LCMS: (ESI): RT = 0.807 min, mass calcd. for C79H110FN21O19 837.9 m/z [M-4tBu-2Boc- C17H17NO4+9H]2+, rink amide (C17H17NO4, exact mass = 299.12); found 838.4 m/z [M-4tBu-Boc- C17H17NO4+9H]2+, rink amide (C17H17NO4, exact mass = 299.12); LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000654] LCMS: (ESI): RT = 0.820 min, mass calcd. for C79H110FN21O19 837.9 m/z [M-4tBu-2Boc- C17H17NO4+9H]2+, rink amide (C17H17NO4, exact mass = 299.12); found 838.3 m/z [M-4tBu-Boc- C17H17NO4+9H]2+, rink amide (C17H17NO4, exact mass = 299.12); LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000655] HPLC: RT = 7.37 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10

minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40°C.

3.12 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[(3R)-1-[2-(1H-imidazol-5-yl) ethyl]-3-methyl-2-oxo-pyrrolidine-3-carbonyl]amino]-3-(2H-tetrazol-5-yl) propanoyl] amino] acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (P14)

[000656] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (18, 82.60 µmol) and aa20 (79.22 mg, 165.19 µmol, 2 eq.), the corresponding aa20-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (31, 82.60 µmol) was prepared as described in the general procedure of SPPS.

[000657] The corresponding resin-bound peptide 31 (82.60 µmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P14 (8 mg, 3.85 µmol, 6.2% yield, 93.37% purity) as a white solid.

[000658] LCMS: (ESI): RT = 0.840 min, mass calcd. for C76H103FN18O17 779.39 m/z [M+2H]2+; found 779.9 m/z [M+2H]2+; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000659] LCMS: (ESI): RT = 0.864 min, mass calcd. for C76H103FN18O17 779.39 m/z [M+2H]2+; found 779.9 m/z [M+2H]2+; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000660] HPLC: RT = 7.60 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40°C.

3.13 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[(3R)-1-[2-(1H-imidazol-5-yl) ethyl]-3-methyl-2-oxo-pyrrolidine-3-carbonyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]

acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (P15)

[000661] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (18, 82.60 µmol) and aa21 (79.22 mg, 165.19 µmol, 2 eq.), the corresponding aa21-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (32, 82.60 µmol) was prepared as described in the general procedure of SPPS.

[000662] The corresponding resin-bound peptide 32 (82.60 µmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P15 (10.5 mg, 6.54 µmol, 7.91% yield, 96.96% purity) as a white solid.

[000663] LCMS: (ESI): RT = 0.828 min, mass calcd. for C76H103FN18O17 779.39 m/z [M+2H]2+; found 779.8 m/z [M+2H]2+; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000664] LCMS: (ESI): RT = 0.830 min, mass calcd. for C76H103FN18O17 779.39 m/z [M+2H]2+; found 779.8 m/z [M+2H]2+; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000665] HPLC: RT = 7.62 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40°C.

3.14 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[(3S)-1-[2-(1H-imidazol-5-yl) ethyl]pyrrolidine-3-carbonyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino] butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P16)

[000666] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (18 (141.13 µmol) and aa22 (127.46 mg, 282.27 µmol, 2.0 eq.), the corresponding aa22-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (33, 141.13 µmol) was prepared as described in the general procedure of SPPS.

[000667] The corresponding resin-bound peptide 33 (141.13 µmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water(0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P16 (15 mg, 9.71 µmol, 6.88% yield, and 99% purity) as a white solid.

[000668] LCMS (ESI): RT = 2.361 min, m/z calcd. for C75H103FN18O16, 1530.76 M-Boc-4tBu+2H]2+, m/z found 765.8, Mobile Phase:1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80%(solvent B)over 2.5 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min.ESI source, Positive ion mode; Wavelength 220nm&254nm,OvenTemperature 50°C

[000669] LCMS (ESI): RT = 0.755 min, m/z calcd. for C75H103FN18O16, 1530.76 M-Boc-4tBu+2H]2+, m/z found 765.8, Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B),using the elution gradient 5%-95%(solvent B) over0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm Wavelength: UV 220nm;Column temperature: 50℃;MS ionization: ESI

[000670] HPLC: RT = 7.18 min Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm Wavelength: UV 220nm, 215nm& 254nm Column temperature: 40°C.

3.15 Preparation of (3S,9S,12S,15S,18S,21S)-tert-butyl1-((R)-1-(2-(1H-imidazol-5-yl)ethyl) pyrrolidin-3-yl)-3-((2H-tetrazol-5-yl)methyl)-21-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan -2-yl)amino)-3-(4'-(4-aminobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl) -12-(2-fluorobenzyl)-9,15-bis((R)-1-hydroxyethyl)-18-(hydroxymethyl)-12-methyl-1,4,7,10,13, 16,19-heptaoxo-2,5,8,11,14,17,20-heptaazatricosan-23-oate (P17)

[000671] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin compound 18 (101.97 µmol) and aa23 (80 mg, 177.16 µmol, 1.74 eq.), the corresponding aa23-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (34, 101.88 µmol) was prepared as described in the general procedure of SPPS.

[000672] The corresponding resin-bound peptide 34 (101.88 µmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P17 (18 mg, 11.65 µmol, 11.43% yield, 99% purity) as a white solid.

[000673] LCMS (ESI): RT = 0.828 min, m/z calcd. for C75H103FN18O16, 1530.76 M-Boc-4tBu+2H]2+, m/z found 765.8, Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95%(solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm Wavelength: UV 220nm; Column temperature: 50℃; MS ionization: ESI

[000674] HPLC: RT = 7.36 min Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm Wavelength: UV 220nm, 215nm& 254nm Column temperature: 40°C.

[000675] Figures 17A and 17B depict the sequence of steps for solid support synthesis of GLP1 peptidomimetic payloads P10, P12, P18, P19, P25, P26, P27, P28, P29, P30, P31, P36, P37, and P38 according to the disclosure.

3.16 Preparation of [[(2S)-2-[[(2S)-2-amino-3-(1H-imidazol-5-yl)propanoyl]amino]propanoyl] amino] -3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl] amino]-4-oxo-butanoic acid (P10)

[000676] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (18, 137.66 µmol), the corresponding aa16-aa15-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (26, 137.28 µmol) was prepared as described in the general procedure of SPPS by elongating the peptide with aa15 (128.58 mg, 412.99 µmol, 3 eq.) and then aa16 (155.42 mg, 411.83 µmol, 3 eq.).

[000677] The corresponding resin-bound peptide 26 (137.28 µmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P10 (16 mg, 10.33 µmol, 7.55% yield, 99.85% purity) as a white solid.

[000678] LCMS (ESI): RT = 0.808 min, mass calcd. for C74H100FN19O17 1545.75 [M-4tBu-Boc+6H]+ 773.88 [M-4tBu-Boc+7H]2+, found 774.2 [M-4tBu-Boc+7H]2+. Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.04% TFA (solvent B) and water containing 0.06% TFA (solvent A).

[000679] HPLC: RT = 7.46 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10

minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40 °C.

3.17 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3-hydroxy-5-methyl-phenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[2-[3-(1H-imidazol-4-yl)propanoylamino]acetyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (P12)

[000680] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (18, 82.60 µmol), the corresponding aa18-aa8-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (29, 82.60 µmol) was prepared as described in the general procedure of SPPS by elongating the peptide with aa8 (73.67 mg, 247.79 µmol, 3 eq.) and then aa18 (63.18 mg, 165.19 µmol, 2 eq.).

[000681] The corresponding resin-bound peptide 29 (82.60 µmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P12 (11 mg, 7.09 µmol, 8.59% yield, 97.99% purity) as a white solid.

[000682] LCMS: (ESI): RT = 0.828 min, mass calcd. for C72H96FN18O18 759.86 m/z [M+2H]2+; found 759.7 m/z [M+2H]2+; [M+2H]2+; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000683] LCMS: (ESI): RT = 0.828 min, mass calcd. for C72H96FN18O18 759.86 m/z [M+2H]2+; found 759.8 m/z [M+2H]2+; [M+2H]2+; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000684] HPLC: RT = 7.65 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40°C.

3.18 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-2-[[(2S)-2-amino-3-hydroxy-propanoyl]amino]-3-(2H-tetrazol-5-

yl)propanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P18)

[000685] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin compound 18 (76.48 µmol), the corresponding aa24-aa15-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin compound 35 (76.48 µmol) was prepared as described in the general procedure of SPPS by elongating the peptide with aa15 (71.43 mg, 229.44 µmol, 3 eq.) and then aa24 (64.54 mg, 229.44 µmol, 3 eq.).

[000686] The corresponding resin-bound peptide 35 (76.48 µmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: YMC-Exphere C18 10 µm 300*50 mm, 12 nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to provide P18 (16 mg, 10.15 µmol, 11.76 % yield, 99.77% purity) as a white solid.

[000687] LCMS: (ESI): RT = 0.762 min, m/z calcd. for C77H104FN17O18, 786.89 [M+2H]2+, m/z found 787.3 [M+2H]2+; Reverse phase LCMS was carried out using a Merck RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000688] HPLC (Rt = 7.68 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 mL/min.

3.19 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-2-[[2-[[(2S)-2-amino-3-(4-hydroxyphenyl) propanoyl] amino]-2-methyl-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (P19)

[000689] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin compound 18 (81.07 µmol), the corresponding aa24-aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin compound 37 (81.07 µmol) was prepared as described in the general procedure of SPPS by elongating the peptide with aa25 (79.13 mg, 243.20 µmol, 3 eq.) and then aa24 (68.41 mg, 243.20 µmol, 3 eq.).

[000690] The corresponding resin-bound peptide 37 was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-

HPLC (column: Phenomenex Gemini-NX 150*30 mm*5 µm; mobile phase: [water (0.04%$NH_3H_2O$+10mM $NH_4HCO_3$)-ACN]; B%: 10%-50%, 15min) to provide P19 (9 mg, 5.62 µmol, 7.03% yield, 99% purity) as a white solid.

[000691] LCMS: (ESI): RT = 0.841 min, m/z calcd. for $C_{78}H_{106}FN_{17}O_{18}$, 793.90 $[M+2H]^{2+}$, m/z found 794.2 $[M+2H]^{2+}$; Reverse phase LCMS was carried out using a Merck RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000692] Crude HPLC: (Rt = 7.81 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

[000693] LCMS: (ESI): RT = 0.813 min, m/z calcd. for $C_{78}H_{106}FN_{17}O_{18}$, 793.90 $[M+2H]^{2+}$, m/z found 794.4 $[M+2H]^{2+}$; Reverse phase LCMS was carried out using a Merck RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000694] HPLC: Rt = 7.81 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

3.20 Preparation of (3S,6S,9S,12S,15S,21S)-21-((2H-tetrazol-5-yl)methyl)-3-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-12-(2-fluorobenzyl)-9,15-bis((R)-1-hydroxyethyl)-6-(hydroxymethyl)-28-(1H-imidazol-5-yl)-12,24,24-trimethyl-5,8,11,14,17,20,23,26-octaoxo-4,7,10,13,16,19,22,25-octaazaoctacosan-1-oic acid (P25)

[000695] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (1.03 mmol), aa25 (669 mg, 206 mmol, 2.0 eq.), the corresponding aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin compound 36A (1.03 mmol) was prepared as described in the general procedure of SPPS.

[000696] To a mixture of Compound 36A (0.1 g, 50.70 µmol, 1.0 eq.) in DMF (20 mL) was added a solution of compound aa29 (58.17 mg, 152.11 µmol, 3.0 eq.), PyBOP (73.88 mg, 141.96 µmol, 2.8 eq.) and DIPEA (39.32 mg, 304.21 µmol, 52.99 µl, 6.0 eq) in DMF (20 mL) in one portion at 20°C. The mixture was bubbled with N2 at 20°C for 2 hours. The mixture was filtered, and the collected resin was washed with DMF (50 mL*3), DCM (50 mL*3) to give the crude product on solid phase, which was subjected to acidic cleavage by using TFA cocktail (6 mL of TFA, 0.4 mL of water, 0.3 mL of triisopropylsilane, 120 mg of phenol). The mixture was filtered and the filtrate

was diluted with t-BuOMe (1000 mL) to give a precipitate, which was centrifuged (5000 R) for 10 min. The residue was purified by prep-HPLC (column: Phenomenex Gemini-NX C18 80*3 0mm*5 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 10%-60%, 20min) to give the product P25 (2.3 mg, 1.33 µmol, 2.63% yield, 91% purity) as a white solid

[000697] LCMS (ESI): RT = 4.006 min, m/z calcd. for $C_{75}H_{100}FN_{20}O_{17}$ 1571.76 $[M+H]^+$, found 786.20 $[M+2H]^{2+}$, Mobile Phase:1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 2.5 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode; Wavelength 220nm&254nm, Oven Temperature 50°C.

[000698] HPLC: RT=9.54 min, 98.48% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

3.21 Preparation of (3S,6S,9S,12S,15S,21S)-21-((2H-tetrazol-5-yl)methyl)-3-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-12-(2-fluorobenzyl)-9,15-bis((R)-1-hydroxyethyl)-6-(hydroxymethyl)-29-(1H-imidazol-5-yl)-12,24,24-trimethyl-5,8,11,14,17,20,23,26-octaoxo-4,7,10,13,16,19,22,25-octaazanonacosan-1-oic acid (P26)

[000699] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin (1.03 mmol), aa25 (669 mg, 206 mmol, 2.0 eq.), the corresponding aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin compound 36A (1.03 mmol) was prepared as described in the general procedure of SPPS.

[000700] The Resin bound compound 36A (50 mg, 25.35 µmol, 1 eq.) was subjected to acidic cleavage by using TFA cocktail (5 mL, TFA/TIPS/H2O = 95:2.5:2.5). The mixture was filtered, and the filtrate was diluted with t-BuOMe (50 mL) and then centrifuged (5000 R) for 10 min to give a crude product aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 (50 mg, crude) as a white solid.

[000701] LCMS (ESI): RT = 3.968 min, m/z calcd. for C69H95FN18O16 1449.70, found 1449.7 [M+H]+, Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 2.5 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode; Wavelength 220 nm, 254 nm, Oven Temperature 50 °C.

[000702] To a solution of aa30 (34.19 mg, 86.23 µmol, 2.5 eq.) in DMF (4 mL) was added PyBOP (39.49 mg, 75.88 µmol, 2.2 eq.) and DIPEA (26.75 mg, 206.96 µmol, 36.05 µL, 6 eq.), the mixture was stirred at 25 °C for 10 min, then aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 (50 mg, 34.49 µmol, 1 eq.) was added, and the final mixture was stirred for 2 h at 25 °C. The reaction progress was monitored by LCMS. After completion, the mixture was triturated by TBME (25 mL) to give a crude product, which was added into a solution of H2O (0.1 mL), triisopropylsilane (77.10 mg, 486.88 µmol, 0.1 mL, 14.83 eq.) and TFA (2.77 g, 24.31 mmol, 1.8 mL, 740.69 eq.). The mixture was stirred at 25 °C for 1 h. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and then triturated by TBME (50 mL) to give a crude product, which was purified by prep-HPLC (column: Gemini NX C18 5 µm * 10 * 150 mm; mobile phase: [water (0.1% TFA)-ACN]; B%: 10%-60%, 30 min) to give **P26** (7.72 mg, 4.77 µmol, 14.54% yield, 98% purity) as a white solid.

[000703] LCMS (ESI): RT = 3.991 min, mass calcd. for $C_{76}H_{103}FN_{20}O_{17}$, 793.38 $[M+H]^+$, found 793.7 $[M+H]^+$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6.0 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: X timate C18 2.1 * 30 mm, 3 µm; Wavelength: UV 220 nm & 254 nm Column temperature: 50 °C; MS ionization: ESI.

[000704] HPLC: RT = 9.55 min, HPLC conditions: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: WELCH Ultimate LP-C18 150 * 4.6 mm 5 µm; Wavelength: UV 220 nm, 215 nm, 254 nm; Column temperature: 40 °C.

3.22 Preparation of (3S,6S,9S,12S,15S,21S)-21-((2H-tetrazol-5-yl)methyl)-3-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-12-(2-fluorobenzyl)-9,15-bis((R)-1-hydroxyethyl)-6-(hydroxymethyl)-30-(1H-imidazol-5-yl)-12,24,24-trimethyl-5,8,11,14,17,20,23,26-octaoxo-4,7,10,13,16,19,22,25-octaazatriacontan-1-oic acid (P27)

[000705] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin (1.03 mmol), aa25 (669 mg, 206 mmol, 2.0 eq.), the corresponding aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin compound 36A (1.03 mmol) was prepared as described in the general procedure of SPPS.

[000706] To a mixture of Compound 36A (120 mg, 60.84 µmol, 1 eq.) in DMF (4 mL) was added a solution of aa31 (62.44 mg, 152.11 µmol, 2.5 eq.), PyBOP (69.66 mg, 133.85 µmol, 2.2

eq.) and DIPEA (47.18 mg, 365.05 µmol, 63.59 µL, 6 eq.) in DMF (10 mL) in one portion at 20°C, and the final mixture was bubbled with N2 at 20 °C for 2 h and repeat this progress for twice. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and washed with DMF (10 mL * 4) and DCM (10 mL * 4) to give the crude product on solid phase, which was subjected to acidic cleavage by using TFA cocktail (5 mL, TFA/TIPS/H2O = 95:2.5:2.5). The mixture was filtered, and the filtrate was diluted with t-BuOMe (50 mL) to give a precipitate, which was centrifuged (5000 R) for 10 min. The residue was purified by prep-HPLC (column: Phenomenex Gemini NX C18 150 * 40 mm * 5 µm; mobile phase: [water (0.1% TFA)-ACN]; B%: 0%-45%, 30 min) to give the product P27 (2.4 mg, 1.47 µmol, 2.48% yield, 98% purity) as a white solid

[000707] LCMS (ESI): RT = 4.071 min, m/z calcd. for $C_{77}H_{105}FN_{20}O_{17}$ 800.39 $[M+2H]^{2+}$, found 800.8, Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 2.5 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode; Wavelength 220 nm & 254 nm, Oven Temperature 50 °C.

[000708] HPLC: RT = 9.58 min, HPLC conditions: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: WELCH Ultimate LP-C18 150 * 4.6 mm 5 µm; Wavelength: UV 220 nm, 215 nm, 254 nm; Column temperature: 40 °C.

3.23 Preparation of (3S,6S,9S,12S,15S,21S)-21-((2H-tetrazol-5-yl)methyl)-3-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-12-(2-fluorobenzyl)-9,15-bis((R)-1-hydroxyethyl)-6-(hydroxymethyl)-31-(1H-imidazol-4-yl)-12,24,24-trimethyl-5,8,11,14,17,20,23,26-octaoxo-4,7,10,13,16,19,22,25-octaazahentriacontan-1-oic acid (P28)

[000709] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin (1.03 mmol), aa25 (669 mg, 206 mmol, 2.0 eq.), the corresponding aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin compound 36A (1.03 mmol) was prepared as described in the general procedure of SPPS.

[000710] To a mixture of Compound 36A (120 mg, 60.84 µmol, 1 eq.) in DMF (4 mL) was added a solution of aa32 (64.57 mg, 152.10 µmol, 2.5 eq.), PyBOP (69.65 mg, 133.85 µmol, 2.2 eq.) and DIPEA (39.32 mg, 304.20 µmol, 52.99 µL, 5 eq.) in DMF (10 mL) in one portion at 20°C, and the final mixture was bubbled with N2 at 20 °C for 2 h and repeat this progress for twice. The

reaction progress was monitored by LCMS. After completion, the mixture was filtered and washed with DMF (10 mL * 4) and DCM (10 mL * 4) to give the crude product on solid phase, which was subjected to acidic cleavage by using TFA cocktail (5 mL, TFA/TIPS/H2O = 95:2.5:2.5). The mixture was filtered, and the filtrate was diluted with t-BuOMe (50 mL) to give a precipitate, which was centrifuged (5000 R) for 10 min. The residue was purified by prep-HPLC (column: Phenomenex Gemini-NX 150 * 30 mm * 5 µm; mobile phase: [water (0.05% ammonia hydroxide v/v)-ACN]; B%: 0%-45%, 30 min) to give the product P28 (8.5 mg, 5.21 µmol, 10.34% yield, 99% purity) as a white solid

[000711] LCMS (ESI): RT = 4.035 min, m/z calcd. for $C_{78}H_{107}FN_{20}O_{17}$ 807.40 $[M+2H]^{2+}$, found 807.8, Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 2.5 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode; Wavelength 220 nm & 254 nm, Oven Temperature 50 °C.

[000712] HPLC: RT = 9.60 min, HPLC conditions: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: WELCH Ultimate LP-C18 150 * 4.6 mm 5 µm; Wavelength: UV 220 nm, 215 nm, 254 nm; Column temperature: 40 °C.

3.24 Preparation of (3S,6S,9S,12S,15S,21S)-21-((2H-tetrazol-5-yl)methyl)-3-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-12-(2-fluorobenzyl)-9,15-bis((R)-1-hydroxyethyl)-6-(hydroxymethyl)-32-(1H-imidazol-4-yl)-12,24,24-trimethyl-5,8,11,14,17,20,23,26-octaoxo-4,7,10,13,16,19,22,25-octaazadotriacontan-1-oic acid (P29)

[000713] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin (1.03 mmol), aa25 (669 mg, 206 mmol, 2.0 eq.), the corresponding aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin compound 36A (1.03 mmol) was prepared as described in the general procedure of SPPS.

[000714] To a mixture of Compound 36A (120 mg, 60.84 µmol, 1 eq.) in DMF (4 mL) was added a solution of aa33 (64.04 mg, 146.02 µmol, 2.4 eq.), PyBOP (63.32 mg, 121.68 µmol, 2 eq.) and DIPEA (39.32 mg, 304.21 µmol, 52.99 µL, 5 eq.) in DMF (10 mL) in one portion at 20°C, and the final mixture was bubbled with N2 at 20 °C for 2 h and repeat this progress for twice. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and washed with DMF (10 mL * 4) and DCM (10 mL * 4) to give the crude product on solid phase, which was

subjected to acidic cleavage by using TFA cocktail (5 mL, TFA/TIPS/H2O = 95:2.5:2.5). The mixture was filtered, and the filtrate was diluted with t-BuOMe (50 mL) to give a precipitate, which was centrifuged (5000 R) for 10 min. The residue was purified by prep-HPLC (column: Phenomenex Gemini-NX 150 * 30 mm * 5 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-55%, 20 min) to give the product P29 (5 mg, 3.05 µmol, 5.22% yield, 99.4% purity) as a white solid

[000715] LCMS (ESI): RT = 3.997 min, m/z calcd. for $C_{77}H_{103}FN_{20}O_{17}$, 814.40 $[M+2H]^{2+}$, found 814.9, Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 2.5 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode; Wavelength 220 nm & 254 nm, Oven Temperature 50 °C.

[000716] HPLC: RT = 9.61 min, HPLC conditions: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: WELCH Ultimate LP-C18 150 * 4.6 mm 5 µm; Wavelength: UV 220 nm, 215 nm, 254 nm; Column temperature: 40 °C.

3.25 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[2-[8-(1H-imidazol-5-yl) octanoylamino]-2-methyl-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl] amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P30)

[000717] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin (1.03 mmol), aa25 (669 mg, 206 mmol, 2.0 eq.), the corresponding aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin compound 36A (1.03 mmol) was prepared as described in the general procedure of SPPS.

[000718] To a mixture of Compound 36A (500 mg, 126.75 µmol, 50% purity, 1 eq) in DMF (4 mL) was added a solution of aa34 (286.84 mg, 633.77 µmol, 5 eq), HATU (86.75 mg, 228.16 µmol, 1.8 eq) and DIPEA (65.53 mg, 507.02 µmol, 88.31 µL, 4 eq) in DMF (20 mL) in one portion at 20°C, and the final mixture was bubbled with N2 at 20 °C for 2 h. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and washed with DMF (10 mL * 4) and DCM (10 mL * 4) to give the crude product on solid phase, which was subjected to acidic cleavage by using TFA cocktail (10 mL, TFA/TIPS/H2O = 95:2.5:2.5). The mixture was filtered,

and the filtrate was diluted with t-BuOMe (100 mL) to give a precipitate, which was centrifuged (5000 R) for 10 min. The residue was purified by prep-HPLC (column: Boston Green ODS 150*30 mm*5 µm; mobile phase: [water (0.1%TFA)-ACN];B%: 22%-62%,9min) to give the product P30 (7.68 mg, 4.62 µmol, 4.45% yield, 98.82% purity) as a white solid

[000719] LCMS (ESI): RT = 3.998 min, m/z calcd. for $C_{80}H_{110}FN_{20}O_{17}$ 1641.83 $[M+H]^+$, $C_{80}H_{111}FN_{20}O_{17}$ 821.4 $[M+2H]^{2+}$, found 821.8 [M+2H]2+. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000720] HPLC: RT =9.63 min. HPLC conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min; Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm.

3.26 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[2-[9-(1H-imidazol-5-yl) nonanoylamino]-2-methyl-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl] amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P31)

[000721] Starting from the corresponding aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin (1.03 mmol), aa25 (669 mg, 206 mmol, 2.0 eq.), the corresponding aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin compound 36A (1.03 mmol) was prepared as described in the general procedure of SPPS.

[000722] To a mixture of Compound 36A (125 mg, 63.38 µmol, 1 eq.) in DMF (4 mL) was added a solution of aa35 (57.37 mg, 126.75 µmol, 2.0 eq.), HATU (43.38 mg, 114.08 µmol, 1.8 eq.) and DIPEA (32.76 mg, 253.51 µmol, 44.16 µl, 4.0 eq.) in DMF (10 mL) in one portion at 20°C, and the final mixture was bubbled with N2 at 20 °C for 2 h. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and washed with DMF (10 mL * 4) and DCM (10 mL * 4) to give the crude product on solid phase, which was subjected to acidic cleavage by using TFA cocktail (5 mL, TFA/TIPS/H2O = 95:2.5:2.5). The mixture was filtered, and the filtrate was diluted with t-BuOMe (50 mL) to give a precipitate, which was centrifuged (5000 R) for 10 min. The residue was purified by prep-HPLC (column: Boston Green ODS 150*30 mm*5 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 25%-65%, 9min) to give the product P31 (7.0 mg, 4.23 µmol, 6.69% yield, 100% purity) as a white solid.

[000723] LCMS (ESI): RT = 4.023 min, m/z calcd. for $C_{75}H_{100}FN_{20}O_{17}$ 1571.76 $[M+H]^+$, 786.38 $[M+2H]^{2+}$, found 786.20 $[M+2H]^{2+}$, Mobile Phase:1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 2.5 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode; Wavelength 220nm, 254nm, OvenTemperature 50°C.

[000724] HPLC: RT=9.80 min, 100% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

3.27 Preparation of (9S,15S,18S,21S,24S,27S)-9-((2H-tetrazol-5-yl)methyl)-27-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-18-(2-fluorobenzyl)-15,21-bis((R)-1-hydroxyethyl)-24-(hydroxymethyl)-6,6,18-trimethyl-4,7,10,13,16,19,22,25-octaoxo-1-(2-oxopiperidin-1-yl)-3,8,11,14,17,20,23,26-octaazanonacosan-29-oic acid (P36)

[000725] To a mixture of aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin (75 mg, 19.87 µmol, 50% purity, 1 eq) in DMF (2 mL) was added a solution of aa36 (20 mg, 73.99 µmol, 3.72 eq), HATU (15.11 mg, 39.74 µmol, 2 eq) and DIPEA (25.68 mg, 198.71 µmol, 34.61 µL, 10 eq) in DMF (10 mL) in one portion at 20°C, and the final mixture was bubbled with N2 at 20 °C for 2 h. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and washed with DMF (10 mL × 4) and DCM (10 mL × 4) to give the crude product on solid phase, which was subjected to acidic cleavage by using TFA cocktail (5 mL, TFA/TIPS/H2O = 95:2.5:2.5). The mixture was filtered, and the filtrate was diluted with t-BuOMe (50 mL) to give a precipitate, which was centrifuged (5000 R) for 10 min. The residue was purified by prep-HPLC (column: Welch Xtimate C18 100*40mm*3 µm; mobile phase: [water(0.075%TFA)-ACN];B%: 50%-80%,10min) to give the product P36 (2.4 mg, 1.48 µmol, 7.47% yield, 100% purity) as a white solid.

[000726] LCMS (ESI): RT = 4.416 min, mass calcd. for $C_{95}H_{122}N_{20}O_{22}FH$ 1917.11 $[M+H]^+$, $C_{95}H_{122}N_{20}O_{22}F$ 809.1 $[M+2H]^{2+}$, found 809.3 [M+2H]2+; Reverse phase LCMS was carried out using a Chromolith Flash column 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B)over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate 3 µm, C18, 2.1*30 mm.

[000727] HPLC: RT = 5.24 min. HPLC conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80%

(solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min; Column: Ultimate XB- C18, 3 µm,3.0*50 mm.

3.28 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[2-[2-[2-(5-methyl-1,3-dioxo-isoindolin-2-yl)ethylamino]-2-oxo-ethyl]sulfanylacetyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino] -3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P37)

[000728] To a mixture of aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin (100 mg, 26.49 µmol, 1 eq) in DMF (2 mL) was added a solution of aa36 (44.56 mg, 132.47 µmol, 5 eq), HATU (18.13 mg, 47.69 µmol, 1.8 eq) and DIPEA (13.70 mg, 105.98 µmol, 18.46 µL, 4 eq) in DMF (10 mL) in one portion at 20°C, and the final mixture was bubbled with N2 at 20 °C for 2 h. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and washed with DMF (10 mL × 4) and DCM (10 mL × 4) to give the crude product on solid phase, which was subjected to acidic cleavage by using TFA cocktail (5 mL, TFA/TIPS/H2O = 95:2.5:2.5). The mixture was filtered, and the filtrate was diluted with t-BuOMe (50 mL) to give a precipitate, which was centrifuged (5000 R) for 10 min. The residue was purified by prep-HPLC (column: Waters Xbridge BEH C18 100*25mm*5 µm; mobile phase: [water(0.1%TFA)-ACN];B%: 20%-80%,15min) to give the product P37 (4.81 mg, 2.86 µmol, 10.86% yield, 100% purity) as a white solid.

[000729] LCMS (ESI): RT = 4.602 min, m/z calcd. for $C_{80}H_{101}FN_{19}O_{19}S$ 1682.71 $[M+H]^+$, $C_{80}H_{100}FN_{19}O_{19}S$ 841.85 $[M+2H]^{2+}$, found 842.3 $[M+2H]^{2+}$. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000730] HPLC: RT =4.844 min. HPLC conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm.

3.29 Preparation of (3S,6S,9S,12S,15S,21S)-21-((2H-tetrazol-5-yl)methyl)-3-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-12-(2-fluorobenzyl)-9,15-bis((R)-1-hydroxyethyl)-6-(hydroxymethyl)-12-methyl-5,8,11,14,17,20,23,27-octaoxo-29-(2-oxopyrrolidin-1-yl)-4,7,10,13,16,19,22,26-octaazanonacosan-1-oic acid (P38)

[000731] To a mixture of aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin (0.2 g, 52.99 µmol, 50% purity, 1.0 eq.) in DMF (2 mL) was added a solution of aa38 (82.48 mg, 264.94 µmol, 5.0 eq.), HATU (90.66 mg, 238.45 µmol, 4.5 eq.) and DIPEA (68.48 mg, 529.88 µmol, 92.29 µL, 10.0 eq.) in DMF (10 mL) in one portion at 20°C, and the final mixture was bubbled with N2 at 20 °C for 2 h. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and washed with DMF (10 mL × 4) and DCM (10 mL × 4) to give the crude product on solid phase, which was swelled again with 20% piperidine/DMF (20 mL) and bubbled with N2 at 20°C for 2 hr. After completion, the mixture was filtered, and the collected resin was washed with DMF (100 mL×3), DCM (100 mL×3) to give the crude product aa38-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 on solid phase (52.99 µmol).

[000732] To a mixture of aa38-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1 peptidyl Rink Amide MBHA Resin (52.60 µmol, 1 eq.) in DMF (2 mL) was added a solution of aa39 (41.33 mg, 262.98 µmol, 5.0 eq.), HATU (90.00 mg, 236.69 µmol, 4.5 eq.) and DIPEA (67.98 mg, 525.97 µmol, 91.61 µL, 10.0 eq.) in DMF (10 mL) in one portion at 20°C, and the final mixture was bubbled with N2 at 20 °C for 2 h. The reaction progress was monitored by LCMS. After completion, the mixture was filtered and washed with DMF (10 mL × 4) and DCM (10 mL × 4) to give the crude product on solid phase, which was subjected to acidic cleavage by using TFA cocktail (10 mL, TFA/TIPS/H2O = 95:2.5:2.5). The mixture was filtered, and the filtrate was diluted with t-BuOMe (100 mL) to give a precipitate, which was centrifuged (5000 R) for 10 min. The residue was purified by prep-HPLC (column: Boston Green ODS 150*30 mm*5 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 36%-76%, 9min) and prep-HPLC (column: Waters X-bridge BEH C18 100*25mm*5 µm; mobile phase: [water (0.05%$NH_3H_2O$)-ACN]; B%: 5%-49%, 11min) to give the product P38 (3.0 mg, 1.71 µmol, 3.26% yield, 90% purity) as a white solid. LCMS (ESI): RT = 4.313 min, m/z calcd. for $C_{75}H_{102}FN_{19}O_{18}$ 1575.76 $[M+H]^+$, 788.38 $[M+2H]^{2+}$, found 788.20 $[M+2H]^{2+}$. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate 3 µm, C18,2.1*30 mm; HPLC: RT=9.93 min, 90% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

[000733] Figure 18 depicts the sequence of steps for solid support synthesis of GLP1 peptidomimetic payloads P20 and P21 according to the disclosure.

3.30 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-2,5-diamino-5-oxopentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxybutanoyl] amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P20)

[000734] Starting from the corresponding aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (17, 175.17 µmol) and aa26 (193.59 mg, 525.51 µmol, 3 eq.), the corresponding aa26-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (38, 175.17 µmol) was prepared as described in the general procedure of SPPS.

[000735] The corresponding resin-bound peptide 38 (175.17 µmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150*40mm*10 µm; mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 0%-90%,25min) to provide P20 (4.65 mg, 3.35 µmol, 95.77% purity) as a white solid.

[000736] LCMS: (ESI): RT = 0.789 min, mass calcd. for $C_{66}H_{93}FN_{12}O_{16}$ 664.34 m/z $[M+2H]^{2+}$; found 665.0 m/z $[M+2H]^{2+}$; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000737] LCMS: (ESI): RT = 1.362 min, mass calcd. for $C_{66}H_{92}FN_{12}O_{16}$ 1327.67 m/z $[M+H]^+$; found 1327.7 m/z $[M+H]^+$; LC-MS: Mobile Phase:1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B),using the gradient10%-80%(solvent B)over 2 minutes and holding at 80% for 0.48 minutes at a flow rate of 0.8ml/min.

[000738] HPLC: RT = 7.40 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40 °C.

3.31 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-5-amino-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-5-oxo-pentanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P21)

[000739] Starting from the corresponding aa26-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin compound 38 (175.17 μmol) and aa10 (163.80 mg, 350.34 μmol, 2 eq.), the corresponding aa10-aa26-aa8-aa7-aa6-aa5-aa4-aa3-aa2b-aa1 peptidyl Rink Amide MBHA Resin (39, 175.17 μmol) was prepared as described in the general procedure of SPPS.

[000740] The corresponding resin-bound peptide 39 (175.17 μmol) was further cleaved following the general procedure to give the crude product as a white solid. The crude was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150*40mm*10 μm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 20min) to provide P21 (20 mg, 12.94 μmol, 7.39% yield, 99.31% purity) as a white solid.

[000741] LCMS: (ESI): RT = 0.833 min, mass calcd. for $C_{76}H_{106}FN_{15}O_{18}$ 767.89 m/z $[M+2H]^{2+}$; found 768.3 m/z $[M+2H]^{2+}$; LC-MS: MERCK, RP-18e 25-2mm column, flow rate 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000742] HPLC: RT = 7.44 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 μm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40°C.

[000743] Figure 19 depicts the sequence of steps for solid support synthesis of GLP1 peptidomimetic payloads P22 and P23 according to the disclosure.

3.32 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-anilino-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P22)

[000744] The corresponding **aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3** peptidyl **2-Chlorotrityl Chloride Resin bound 47** was prepared as described in the general procedure of SPPS.

[000745] To a mixture of the corresponding resin-bound peptide (**47**, 553.98 μmol) was added TFE (2.61 g, 26.09 mmol, 1.88 mL, 47.09 eq.) and AcOH (1.97 g, 32.83 mmol, 1.88 mL, 59.26 eq.) in DCM (8 mL) in one portion at 25 °C under $N_2$. The mixture was shaked at 25 °C for 2 hours. LCMS trace showed that the reaction was complete. The mixture was filtered, and the cake was washed with DCM (5 mL × 3). The filtrate was concentrated in vacuum to give a yellow oil, which was diluted with water (5 mL). The mixture was adjusted to pH=8 with aq. sat. $NaHCO_3$,

yellow solids were precipitated. The mixture was filtered, and the cake was washed with water (5 mL × 2), dried in vacuum to give crude product (550 mg, crude) as a yellow solid. The crude was purified by prep-HPLC (column: Xtimate C18 150*25mm*5 µm; mobile phase: [water (0.225%FA)-ACN]; B%: 48%- 58%, 11min) to give compound **47** (220 mg, 122.65 µmol, 36.10% yield, 82.05% purity) as an off-white solid.

[000746] LCMS (ESI): RT = 1.073 min, mass calcd. for $C_{76}H_{104}FN_{14}O_{15}$ 1471.78 m/z [M-$C_{19}H_{13}Cl$+2H]$^+$, m/z found 1471.65 ; [M-$C_{19}H_{13}Cl$+2H]$^+$, rink 2-[chloro(diphenyl)methyl]benzene ($C_{19}H_{13}Cl$, exact mass = 276.1); Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000747] LCMS (ESI): RT = 1.079 min, mass calcd. for $C_{76}H_{104}FN_{14}O_{15}$ 1471.78 m/z [M-$C_{19}H_{13}Cl$+2H]$^+$, m/z found 1471.8 ; [M-$C_{19}H_{13}Cl$+2H]$^+$, rink 2-[chloro(diphenyl)methyl]benzene ($C_{19}H_{13}Cl$, exact mass = 276.1); Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000748] HPLC: RT=10.96 min. HPLC: Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%- 80%(solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min

[000749] To a solution of compound **47** (37.37 µmol) and HOBt (5.05 mg, 37.37 µmol, 1 eq.) in $CHCl_3$ (0.225 mL) and DMF (0.025 mL) was added **aa27** (19.87 mg, 37.37 µmol, 1 eq.) at 20 °C. A solution of DIC (4.72 mg, 37.37 µmol, 5.79 µL, 1 eq.) in $CHCl_3$ (0.225 mL) and DMF (0.025 mL) were added to the mixture. The reaction was stirred at 20 °C for 16 hours. LCMS trace showed that the reaction was complete. The reaction was concentrated in vacuum to give crude product **48** (74.2 mg, 37.37 µmol) as a brown oil, which was used to the next step without further purification.

[000750] LCMS (ESI): RT = 1.215 min, mass calcd. for $C_{108}H_{144}FN_{17}O_{18}$ 993.05 m/z [M+2H]$^{2+}$, m/z found 993.8 m/z [M+2H]$^{2+}$; Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000751] To a mixture of compound **48** (74.2 mg, 37.37 µmol, 1 eq.) was added triisopropylsilane (137.30 mg, 867.01 µmol, 178.08 µL, 23.20 eq.) in TFA (2 mL) and $H_2O$ (0.06

mL) in one portion at 25 °C under $N_2$. The mixture was standing at 15 °C for 2.5 hours. LCMS trace showed that the reaction was complete. The mixture was concentrated in vacuum to give crude as a yellow oil. The crude was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150*30 mm, 10 µm; mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 28%-58%, 11min) to give compound **P22** (7.5 mg, 5.18 µmol, 13.86% yield, 97.96% purity) as a white solid.

[000752] LCMS (ESI): RT = 0.811 min, mass calcd. for $C_{68}H_{90}FN_{17}O_{16}$ 709.84 m/z $[M+2H]^{2+}$, m/z found 710.2 m/z $[M+2H]^{2+}$; Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000753] HPLC: RT=7.06 min. HPLC: Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%- 80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min.

3.33 Preparation of (3S)-4-[[(1S)-1-[[4-[4-(4-aminobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-4-(3,5-dimethylphenyl)-1-(phenylcarbamoyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P23)

[000754] To a solution of **47** (27.18 µmol) and HOBt (3.67 mg, 27.18 µmol, 1 eq.) in $CHCl_3$ (0.225 mL) and DMF (0.025 mL) was added **aa28** (19.98 mg, 27.18 µmol, 1 eq.) at 20 °C. A solution of DIC (3.43 mg, 27.18 µmol, 4.21 µL, 1 eq.) in $CHCl_3$ (0.225 mL) and DMF (0.025 mL) were added to the mixture. The reaction was stirred at 20 °C for 16 hours. LCMS trace showed that the reaction was complete. The reaction was concentrated in vacuum to give crude product **49** (59.49 mg, 27.18 µmol) as a brown oil, which was used to the next step without further purification.

[000755] LCMS (ESI): RT = 1.223 min, mass calcd. for $C_{116}H_{154}FN_{18}O_{17}$ 1045.09 m/z $[M-Boc+3H]^{2+}$, m/z found 1044.8 m/z $[M-Boc+2H]^{2+}$; Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000756] To a mixture of compound **49** (59.49 mg, 27.18 µmol, 1 eq.) was added triisopropylsilane (131.07 mg, 827.67 µmol, 0.17 mL, 30.45 eq.) in TFA (6 mL) and $H_2O$ (0.17 mL)

in one portion at 15 °C under $N_2$. The mixture was standing at 15 °C for 2.5 hours. LCMS trace showed that the reaction was complete. The mixture was concentrated in vacuum to give crude as yellow oil. The crude was purified by prep-HPLC (column: Xtimate C18 10 µm, 250 mm*50 mm; mobile phase: [water (0.04%$NH_3H_2O$+10mM NH4HCO3)-ACN]; B%: 25%-55%, 8min) to give compound **P23** (7.5 mg, 4.60 µmol, 16.91% yield, 99.38% purity) as a white solid.

[000757] LCMS (ESI): RT = 0.875 min, mass calcd. for $C_{81}H_{107}FN_{18}O_{17}$ 811.4 m/z $[M+2H]^{2+}$, m/z found 811.9 m/z $[M+2H]^{2+}$ ; Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000758] LCMS (ESI): RT = 0.882 min, mass calcd. for $C_{81}H_{107}FN_{18}O_{17}$ 811.4 m/z $[M+2H]^{2+}$, m/z found 811.8 m/z $[M+2H]^{2+}$ ; Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000759] HPLC: RT=8.30 min. HPLC: Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%- 80%(solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

[000760] Figure 20 depicts the sequence of steps for solid support synthesis of GLP1 peptidomimetic payload P24 according to the disclosure.

3.34 Preparation of (3S)-4-[[(1S)-2-[[(1S)-4-[4-(4-aminobutoxy)phenyl]-1-carbamoyl-butyl]amino]-1-[[4-(2-ethyl-4-methoxy-phenyl)phenyl]methyl]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2R,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl) ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino] acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (P24)

[000761] The corresponding **aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2c-aa1b** peptidyl **Rink Amide MBHA Resin** (**59)** was prepared as described in the general procedure of SPPS.

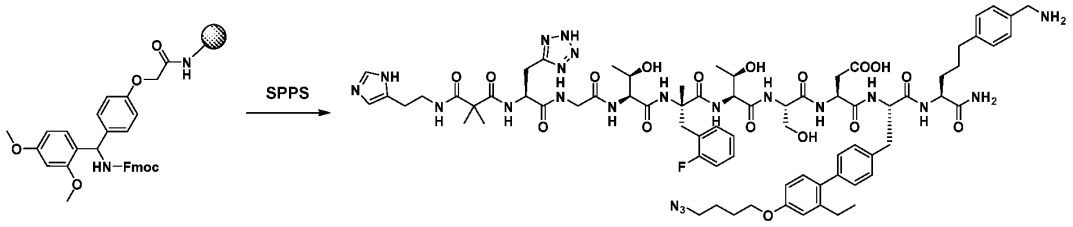
[000762] The corresponding resin-bound peptide **59** was further cleaved following the general procedure to give the crude product as a white solid. The crude product was purified by prep-HPLC (column: mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 15%-45%, 55 min) to afford pure product. The product was suspended in water (20 mL), the mixture frozen in a dry-

ice/acetone bath, and then lyophilized to dryness to afford the desired product. Compound **P24** (50 mg, 29.69 µmol, 6.76% yield, 98.686% purity, TFA salt) was obtained as a white solid.

[000763] LCMS (ESI): RT = 0.821 min, mass calcd. for $C_{74}H_{99}FN_{18}O_{18}$ 1546.74 $[M+H]^+$, 773.4 $[M+H]^{2+}$, m/z found 774.8 $[M+H]^{2+}$. Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000764] Figure 21 depicts the sequence of steps for solid support synthesis of GLP1 peptidomimetic payloads P32, P33, P34, and P35 according to the disclosure.

3.35 Preparation of *(8S,14S,17S,20S,23S,26S)-8-((2H-tetrazol-5-yl)methyl)-26-(((S)-1-(((S)-1-amino-5-(4-(aminomethyl)phenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl)-23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21,24-octaoxo-3,7,10,13,16,19,22,25-octaazaoctacosan-28-oic acid* (P24A)




**Rink Amide MBHA Resin** **P24A**

[000765] The peptide elongation was performed on a 0.5 mmol scale using Liberty Lite Automated Microwave Peptide Synthesizer. To a polypropylene solid-phase reaction vessel was added **Rink Amide MBHA Resin** (0.5 mmol, 1 *eq.*). The resin was washed (swelled) two times as follows: to the reaction vessel was added DMF (10 mL) through the top of the vessel upon which the mixture was agitated for 5 minutes before the solvent was drained through the frit.

[000766] The general coupling reaction of each amino acid was carried out after general removal of Fmoc group procedure. **A)** General removal of Fmoc group procedure: To the reaction vessel containing the resin from the previous step was added piperidine: DMF (1:4 v/v, 5 mL). The mixture was agitated under microwave at 90 °C for 2 min and then the solution was drained through the frit. The resin was washed five times as follows: for each wash, DMF (5 mL) was

added through the top of the vessel and the resulting mixture was periodically agitated for 0.5 minutes before the solution was drained through the frit. **B)** General coupling reaction procedure:

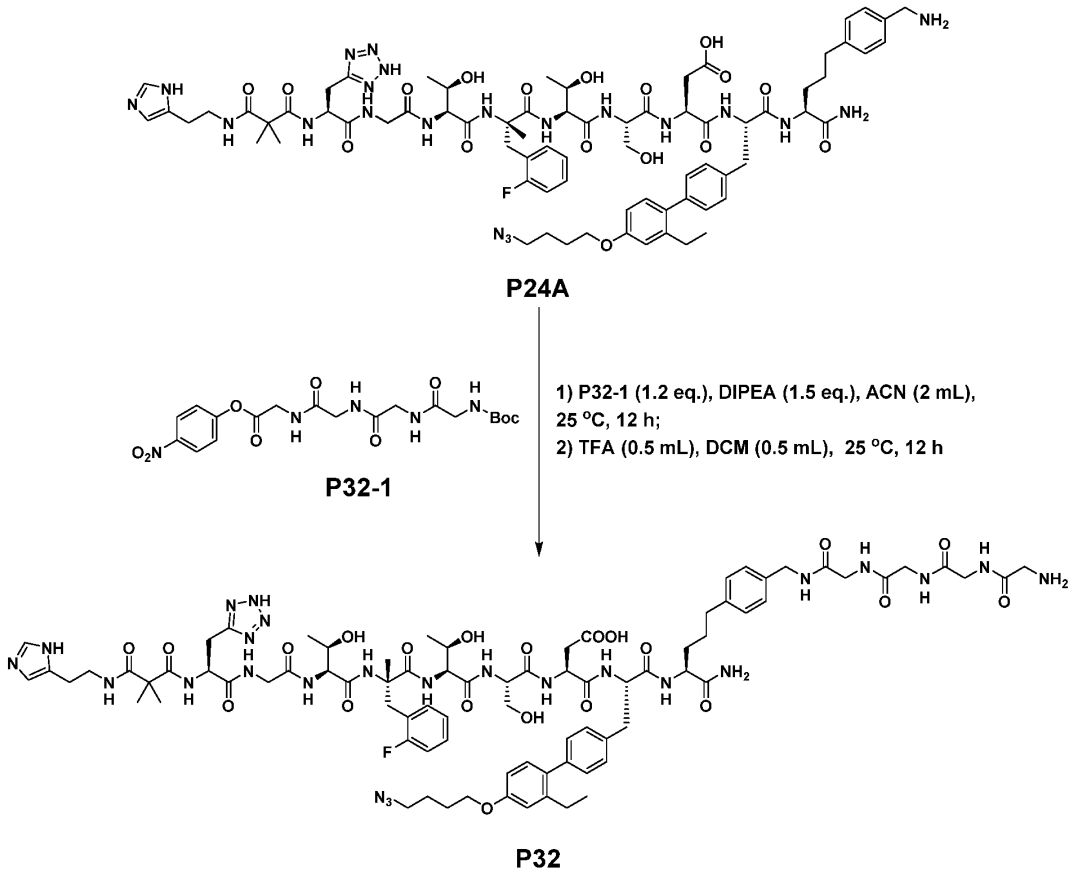
[000767] To the reaction vessel was added the amino acid (0.2 M in DMF, 12.5 mL, 5 eq.), then DIC (0.5 M in DMF, 4 mL, 4 eq.) and oxyma (0.5 M in DMF, 2 mL, 2 eq.). The mixture was agitated under microwave at 90°C for 10 min, then the reaction solution was drained through the frit. The resin was washed four times as follows: for each wash, DMF (8 mL) was added through the top of the vessel and the resulting mixture was periodically agitated for 0.5 minutes before the solution was drained through the frit. After completion of synthe sis, resin was thoroughly rinsed with DMF (6 x 6 mL) then $CH_2Cl_2$ (6 x 6 mL). The resulting resin was subjected to acidic cleavage by using TFA cocktail (TFA/TIPS/$H_2O$ = 95:2.5:2.5) for 2 hours, then filtered and the filtrate was diluted with *t*-BuOMe to give a precipitate, which was centrifuged (5000 R) for 10 min and decanted to give a crude product.

[000768] The corresponding **aa10-aa9-aa8-aa7-aa6-aa5-aa4-aa3-aa2-aa1b** peptidyl **Rink Amide MBHA Resin** was prepared as described in the general procedure of SPPS. The crude product was purified by prep-HPLC (column: Phenomenex Gemini-NX C18 80*30 mm*5 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 5%-55%, 8 min) to afford pure product **P24A** (50 mg, 31.79 µmol, 41.80% yield) as a white solid.

[000769] LCMS (ESI): RT = 2.913 min, m/z calcd. $C_{75}H_{101}FN_{20}O_{17}$ 786.37, found 786.9 $[M+2H]^{2+}$. LCMS conditions: 1.5ML/4LTFA in water (solvent A) and 0.75ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate 3 µm, C18, 2.1*30 mm.

[000770] HPLC: RT=7.44 min, 99.18% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6mm, 5 µm; 2.75ML/4L TFA in water (solvent A) and 2.5ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

3.36 Preparation of *(8S,14S,17S,20S,23S,26S)-8-((2H-tetrazol-5-yl)methyl)-26-(((S)-1-(((S)-1-amino-5-(4-(13-amino-3,6,9,12-tetraoxo-2,5,8,11-tetraazatridecyl)phenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl)-23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21,24-octaoxo-3,7,10,13,16,19,22,25-octaazaoctacosan-28-oic acid* (P32)



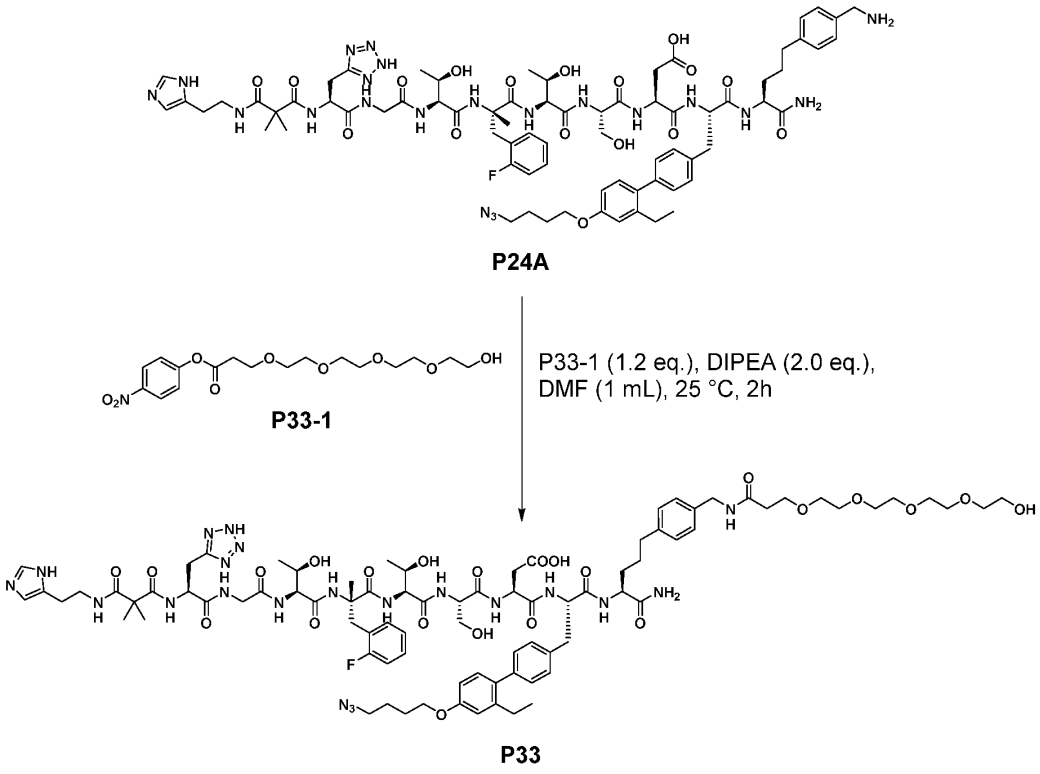

[000771] To a solution of **P24A** (20 mg, 12.72 µmol, 1 *eq.*) in DMF (2 mL) were added **P32-1** (7.13 mg, 15.26 µmol, 1.2 *eq.*) and DIPEA (3.29 mg, 25.43 µmol, 4.43 µL, 2.0 *eq.*). Then the solution was stirred at 20°C for 2hr. After completion, water (6 mL) was added and the mixture was lyophilized to give a white solid (25 mg, crude), which was added in DCM (2.5 mL), followed by the addition of TFA (3.85 g, 33.77 mmol, 2.5 mL, 2567.52 *eq.*). Then the solution was stirred at 20°C for 2hr. After completion, the solvent of the solution was removed under reduced pressure to give the crude. It was purified by prep-HPLC (column: Phenomenex Gemini-NX 80*40 mm*3 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 5%-45%, 30 min). **P32** (7.2 mg, 3.60 µmol, 27.36% yield, 90% purity) was obtained as a white solid.

[000772] LCMS: (ESI): Rt = 2.880 min, mass calcd. for $C_{82}H_{112}FN_{25}O_{21}$ 901.20, found 900.91 $[M+2H]^{2+}$; Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3mm, with a

flow rate of 0.8 ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000773] HPLC: RT=7.23 min, 100% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm; 2.75ML/4L TFA in water (solvent A) and 2.5ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

3.37 Preparation of *(8S,14S,17S,20S,23S,26S)-8-((2H-tetrazol-5-yl)methyl)-26-(((S)-1-(((S)-1-amino-5-(4-(17-hydroxy-3-oxo-6,9,12,15-tetraoxa-2-azaheptadecyl)phenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl)-23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21,24-octaoxo-3,7,10,13,16,19,22,25-octaazaoctacosan-28-oic acid* (P33)



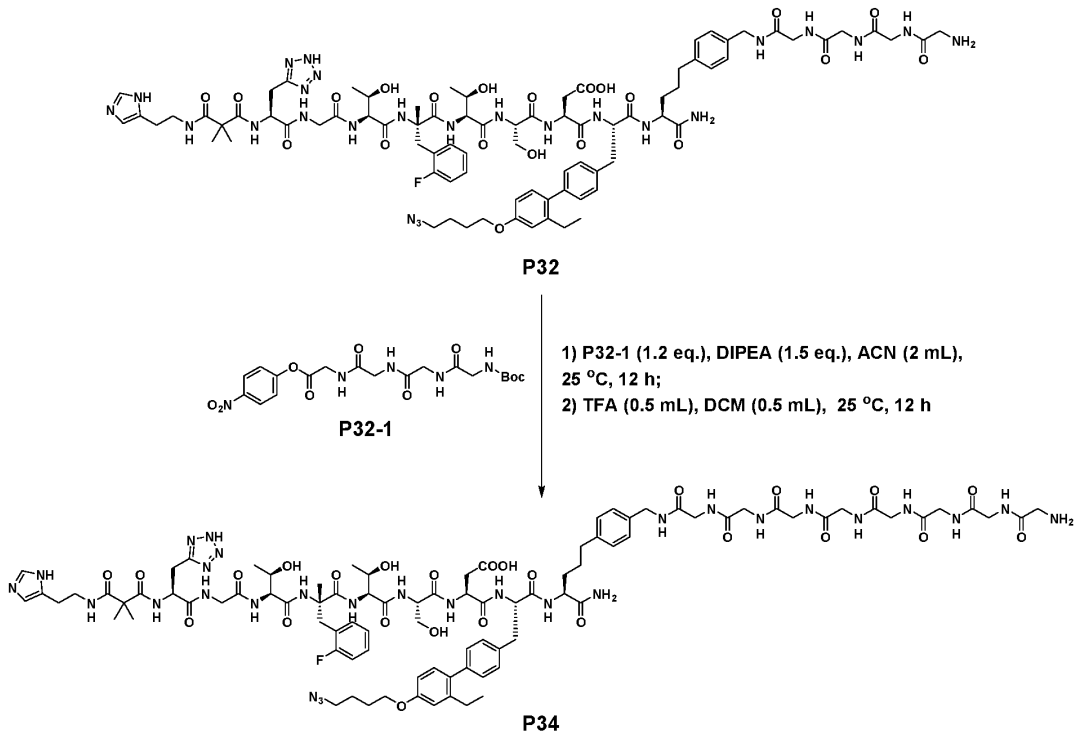

[000774] To a solution of **P24A** (10 mg, 6.36 µmol, 1 *eq.*) in DMF (0.3 mL) were added **P33-1** (2.96 mg, 7.63 µmol, 1.2 *eq.*) and DIPEA (1.64 mg, 12.72 µmol, 2.22 µL, 2.0 *eq*). Then the

solution was stirred at 20°C for 2hr. After completion, the reaction mixture was purified by prep-HPLC (TFA condition, column: Boston Green ODS 150*30 mm*5 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 14%-54%,9 min) to afford **P33** (2.0 mg, 1.13 µmol, 17.69% yield, 95% purity) as a white solid.

[000775] LCMS: (ESI): Rt = 3.383 min, mass calcd. for $C_{85}H_{120}FN_{21}O_{23}$ 911.40, found 911.40 $[M+2H]^{2+}$; Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000776] HPLC: RT=7.96 min, 95.47% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm; 2.75ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

3.38 Preparation of *(8S,14S,17S,20S,23S,26S)-8-((2H-tetrazol-5-yl)methyl)-26-(((S)-1-(((S)-1-amino-5-(4-(29,29-dimethyl-3,6,9,12,15,18,21,24,27-nonaoxo-28-oxa-2,5,8,11,14,17,20,23,26-nonaazatriacontyl)phenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl) -23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21,24-octaoxo-3,7,10,13,16,19,22,25-octaazaoctacosan-28-oic acid* (P34)



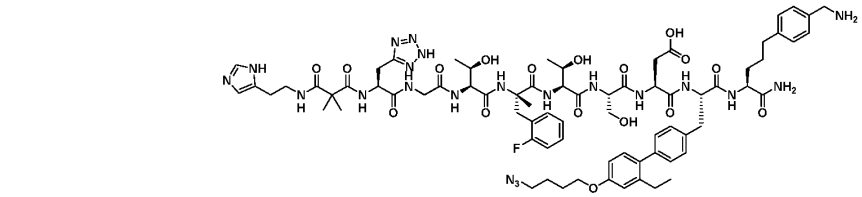

[000777] To a solution of **P32** (5 mg, 2.78 µmol, 1 *eq*) in DMF (1 mL) were added **P32-1** (1.56 mg, 3.33 µmol, 1.2 *eq*) and DIPEA (717.64 ug, 5.55 µmol, 9.67e-1 µL, 2.0 *eq.*). Then the solution was stirred at 20°C for 2hr. After completion, water (6 mL) was added and the mixture was lyophilized to give a white solid (25 mg, crude), which was added in DCM (0.2 mL), followed by the addition of TFA (256.67 mg, 2.25 mmol, 166.67 µL, 958.60 *eq.*). Then the solution was stirred at 20°C for 2hr. After completion, the solvent of the solution was removed under reduced pressure to give the crude. It was purified by prep-HPLC (column: Waters Xbridge BEH C18 100*25 mm*5 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 10%). **P34** (1.72 mg, 7.63e-1 µmol, 32.49% yield, 90% purity) was obtained as a white solid.

[000778] LCMS: (ESI): Rt = 2.760 min, mass calcd. for $C_{90}H_{124}FN_{29}O_{25}$ 1014.98, found 1015.20 $[M+2H]^{2+}$; Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

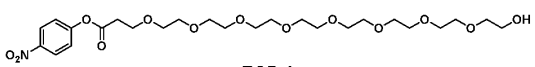
[000779] HPLC: RT=7.15 min, 100% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6mm, 5 µm; 2.75ML/4 LTFA in water (solvent A) and 2.5ML/4L TFA in acetonitrile (solvent

B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

3.39 Preparation of *(8S,14S,17S,20S,23S,26S)-8-((2H-tetrazol-5-yl)methyl)-26-(((S)-1-(((S)-1-amino-5-(4-(17-hydroxy-3-oxo-6,9,12,15-tetraoxa-2-azaheptadecyl)phenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl)-23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21,24-octaoxo-3,7,10,13,16,19,22,25-octaazaoctacosan-28-oic acid* (P35)

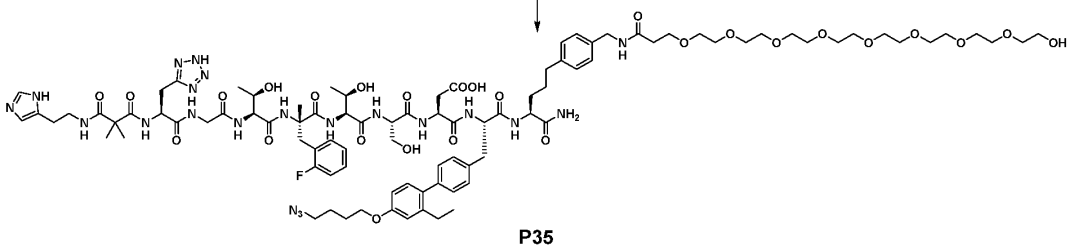


P24A



P35-1

P35-1 (1.2 eq.), DIPEA (2.0 eq.), DMF (1 mL), 25 °C, 2h



P35

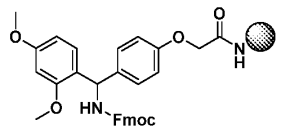
[000780] To a solution of **P24A** (20 mg, 12.72 µmol, 1 *eq.*) in DMF (0.3 mL) were added **P35-1** (10.75 mg, 19.08 µmol, 1.5 *eq.*) and DIPEA (3.29 mg, 25.43 µmol, 4.43 µL, 2.0 *eq.*). Then the solution was stirred at 20°C for 2hr. After completion, the reaction mixture was purified by prep-HPLC (column: Boston Green ODS 150*30 mm*5 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 14%-54%,9min) to afford **P33** (20 mg, 10.01 µmol, 78.75% yield, 100% purity) as a white solid.

[000781] LCMS: (ESI): Rt = 3.325 min, mass calcd. for $C_{93}H_{136}FN_{21}O_{27}$ 998.98, found 999.40 $[M+2H]^{2+}$; Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000782] HPLC: RT=7.88min, 100% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm; 2.75ML/4L TFA in water (solvent A) and 2.5ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

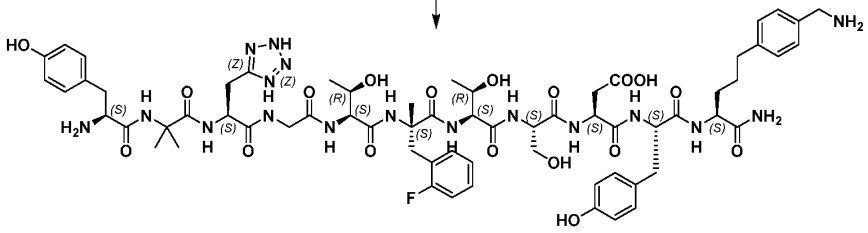
[000783] Figure 22 depicts the sequence for synthesis of **P39**.

Preparation of *(3S,6S,9S,12S,15S,21S,27S)-21-((2H-tetrazol-5-yl)methyl)-27-amino-3-(((S)-1-(((S)-1-amino-5-(4-(aminomethyl)phenyl)-1-oxopentan-2-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamoyl)-12-(2-fluorobenzyl)-9,15-bis((R)-1-hydroxyethyl)-6-(hydroxymethyl)-28-(4-hydroxyphenyl)-12,24,24-trimethyl-5,8,11,14,17,20,23,26-octaoxo-4,7,10,13,16,19,22,25-octaazaoctacosan-1-oic acid* (P39)




**Rink Amide MBHA Resin**

SPPS on Automated Microwave Peptide Synthesizer



**P39**

[000784] The peptide elongation was performed on a 0.5 mmol scale using Liberty Lite Automated Microwave Peptide Synthesizer. Following the standard operation on peptide

synthesizer: a) De-protection: a solution of 20% piperidine/DMF (5 mL) was added to the resin vessel, agitated with $N_2$ for 2 min at 90 °C. Then drained the vessel and washed with DMF (3 mL×3) at 20°C. b) Coupling (each amino acid reacted for triple with 5.0 eq.): a solution of amino acid (2.5 mmol, 5 *eq.*) in DMF (5 mL), DIC (2 mL) and oxyma (1 mL) were added to the vessel and agitated with $N_2$ for 10 min at 90°C. Repeat a) and b) for all amino acids. The resin was subjected to acidic cleavage by using TFA cocktail (TFA/TIPS/$H_2O$ = 95:2.5:2.5), then filtered and the filtrate was diluted with t-BuOMe to give a precipitate, which was centrifuged (5000 R) for 10 min to give the crude product.

[000785] The corresponding **aa24-aa25-aa9-aa8-aa7-aa6-aa5-aa4-aa3-Y-aa1b** peptidyl **Rink Amide MBHA Resin** was prepared as described in the general procedure of SPPS. The crude product was purified by *prep*-HPLC (column: Boston Prime C18 150*30 mm*5 µm; mobile phase: [water (0.05% ammonia hydroxide v/v)-ACN]; B%: 0%-35%, 9 min) to afford pure product **P39** (15 mg, 9.35 µmol, 9.35% yield, 88% purity) as a white solid. The product was further purified by *prep*-HPLC (column: Welch Xtimate C18 100*40 mm*3 µm; mobile phase: [water (0.075%TFA)-ACN]; B%: 5%-45%, 12min) to give the product (10 mg, 6.96 µmol, 65.51% yield, 98.26% purity) was obtained as a white solid.

[000786] LCMS (ESI): RT = 1.573 min, m/z calcd. for C65H87FN17O18 [M+H]+ 1412.63, C65H88FN17O18 $[M+2H]^{2+}$ 707.81, found $C_{65}H_{87}FN_{17}O_{18}$ $[M+H]^+$ 1412.70, $C_{65}H_{88}FN_{17}O_{18}$ $[M+2H]^{2+}$ 707.30 found. LCMS conditions: Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

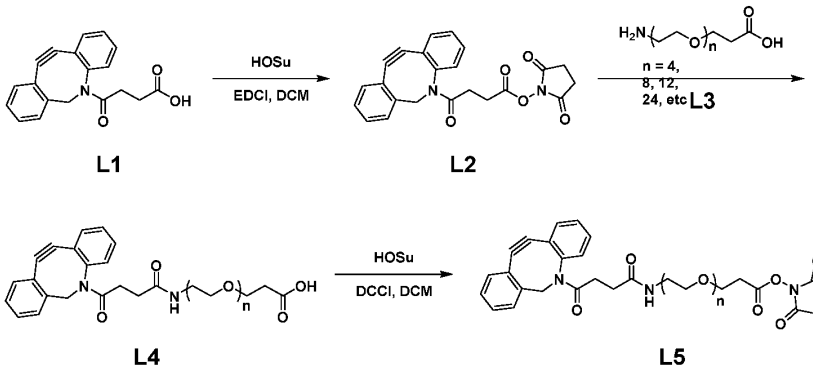
[000787] HPLC: RT=4.72 min, 98.26% purity LC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm; 2.75ML/4L TFA in water (solvent A) and 2.5ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

**Example 4. Synthesis of Linkers**

4.1 Preparation of PEG linkers

[000788] Scheme 20, below, depicts synthesis of PEGn linkers (n=4, 8, 12 and 24):

**Scheme 20**




[000789] Synthesis of PEGn linker with **n = 8** is provided as an example; linkers of other lengths were prepared in the same fashion. Synthesis of **L1 ((11,12-Didehydrodibenzo[b,f]azocin-5(6H)-yl)-4-oxobutanoic acid)** was performed according to L.S. Campbell-Verduyn, L. Mirfeizi, A.K. Schoonen, R.A. Dierckx, P.H. Elsinga, and B.L. Feringa, *Strain-Promoted Copper-Free "Click" Chemistry for $^{18}F$ Radiolabeling of Bombesin.* Angew. Chem. Int. Ed., Vol. 50, No. 47, 2011, 11117-11120.

*Step 1: Synthesis of 2,5-dioxopyrrolidin-1-yl 4-(didehydrodibenzo[b,f]azocin-5(6H)-yl)-4-oxobutanoate (L2)*

[000790] To a solution of **L1** (0.35 g, 1.15 mmol, 1 eq.) in DCM (4 mL) were added HOSu (158.31 mg, 1.38 mmol, 1.2 eq.) and EDCI (263.70 mg, 1.38 mmol, 1.2 eq.). The mixture was stirred at 20 °C for 1 hr. TLC (PE:EtOAc = 1:1) indicated that the complete consumption of reactant. The mixture was filtered and concentrated to give a residue. The residue was purified by column chromatography ($SiO_2$, Petroleum ether/Ethyl acetate = 3/1 to 1/1). The desired compound **L2** (450 mg, 1.12 mmol, 97.56% yield) was obtained as a white solid.

*Step 2: Synthesis of 3-[2-[2-[2-[2-[2-[2-[2-[2-[[4-(39-azatricyclohexadeca-(4),1(5),2(6),3(7),31,33-hexaen-9-yn-39-yl)-4-oxo-butanoyl] amino] ethoxy] ethoxy] ethoxy] ethoxy] ethoxy] ethoxy] ethoxy] ethoxy] propanoic acid (L4)*

[000791] To a solution of **L2** (54.68 mg, 135.90 µmol, 1.5 *eq.*) in DMF (0.5 mL) was added **L3** (n = 8, 40 mg, 90.60 µmol, 1 *eq.*) and DIPEA (58.54 mg, 452.99 µmol, 78.90 µL, 5 *eq.*). The mixture was stirred at 25 °C for 1 hr. LCMS trace indicated that the complete consumption of reactant and the formation of the desired mass. The mixture was filtered and purified by prep-HPLC (AcOH 0.3%, MeCN/$H_2O$, 0~43%, 25 mL/min, 15 min). The desired **L4** (60 mg, 74.09 µmol, 81.78% yield, 90% purity) was obtained as a pale-yellow oil.

[000792] LCMS (ESI): RT = 0.900 min, mass calcd. for $C_{38}H_{53}N_2O_{12}$ 729.36, m/z found 729.3 $[M+H]^+$. Reverse phase LC-MS was carried out using a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

*Step 3: Synthesis of (2,5-dioxopyrrolidin-1-yl) 3-[2-[2-[2-[2-[2-[2-[2-[2-[[4-(43-azatricyclohexadeca-(4),1(5),2(6),3(7),33,35-hexaen-11-yn-43-yl)-4-oxo-butanoyl] amino] ethoxy] ethoxy] ethoxy] ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]propanoate (L5)*

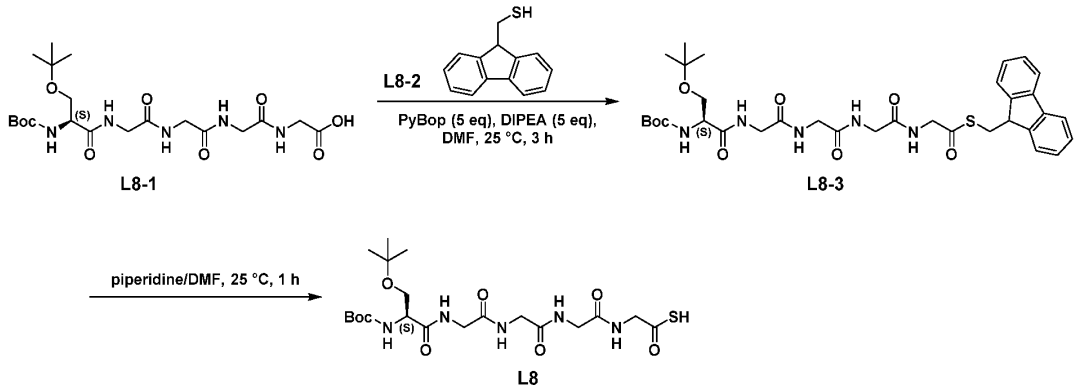
[000793] To a solution of **L4** (20 mg, 27.44 µmol, 1 *eq.*) in DCM (0.5 mL) was added HOSu (6.32 mg, 54.88 µmol, 2 *eq.*) and DCC (8.49 mg, 41.16 µmol, 8.33 µL, 1.5 *eq.*). The mixture was stirred at 25 °C for 1 hr. LCMS trace indicated that the complete consumption of reactant and the formation of the desired mass. The mixture was filtered and purified by prep-HPLC (AcOH 0.3%, MeCN/$H_2O$, 0~65%, 18 mL/min, 15 min). The desired compound **L5** (18 mg, 17.87 µmol, 65.13% yield, 82% purity) was obtained as a pale-yellow oil.

[000794] LCMS (ESI): RT = 0.920 min, mass calcd. for $C_{42}H_{55}N_3O_{14}$ 825.37, m/z found 848.2 $[M+Na]^+$. Reverse phase LC-MS was carried out using a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

4.2 Preparation of peptide linker L8

[000795] Scheme 21, below, depicts synthesis of SG4-SH peptide linker L8:

**Scheme 21**




[000796] See the following references D. Crich, K. Sana, and S. Guo, *Amino Acid and Peptide Synthesis and Functionalization by the Reaction of Thioacids with 2,4-Dinitrobenzenesulfonamides.* Org. Lett., Vol. 9, No. 22, 2007, 4423-4426 and X.Y. Wu, J.L. Stockdill, P.K. Park, J. Samuel, and S.J. Danishefsky, *Expanding the Limits of Isonitrile-Mediated*

*Amidations: On the Remarkable Stereosubtleties of Macrolactam Formation from Synthetic Seco-Cyclosporins.* J. Am. Chem. Soc., Vol. 134, No. 4, 2012, 2378-2384.

*Step 1: (S)-S-((9H-fluoren-9-yl)methyl) 6-(tert-butoxymethyl)-2,2-dimethyl-4,7,10,13,16-pentaoxo-3-oxa-5,8,11,14,17-pentaazanonadecane-19-thioate (L8-3)*

[000797] To a solution of **L8-1** (700 mg, 1.43 mmol, 1 eq.) in DMF (7 mL) were added 4A MOLECULAR SIEVE (1 g) and **L8-2** (455.40 mg, 2.14 mmol, 1.5 eq.) and the mixture was stirred at -20 °C. After 15 min, PyBOP (1.12 g, 2.14 mmol, 1.5 eq.) and DIPEA (369.63 mg, 2.86 mmol, 498.15 µL, 2 eq.) were added and the reaction mixture was stirred at -20°C for 1.5h. LCMS trace showed that the reaction converted completely. The reaction was diluted with EtOAc (30 mL) and filtered, the cake was washed with EtOAc (10 mL*2). The filtrate was washed with aq.$NH_4Cl$ (20 mL), water (20 mL), brine (20 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuum to give crude as yellow oil. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0~20% MeOH/DCM gradient @ 30 mL/min) to give **L8-3** (700 mg, 814.78 µmol, 56.98% yield, 79.594% purity) as a light-yellow oil.

[000798] LCMS: (ESI): RT = 0.882 min, m/z calcd. for $C_{29}H_{38}N_5O_6S$, 584.25 $[M\text{-}Boc+2H]^{2+}$, m/z found 584.3 $[M\text{-}Boc+2H]^{2+}$; Reverse phase LCMS was carried out using a Merck RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000799] $^1H$ NMR (400MHz, METHANOL-d4) ð = 7.78 (d, J=7.3 Hz, 2H), 7.66 (d, J=7.2 Hz, 2H), 7.42 - 7.29 (m, 4H), 4.15 (d, J=5.0 Hz, 2H), 4.04 (s, 2H), 3.92 (d, J=4.3 Hz, 6H), 3.68 - 3.57 (m, 4H), 3.38 - 3.35 (m, 3H), 1.46 (s, 9H), 1.19 (s, 10H).

*Step 2: (S)-6-(tert-butoxymethyl)-2,2-dimethyl-4,7,10,13,16-pentaoxo-3-oxa-5,8,11,14,17-pentaazanonadecane-19-thioic S-acid (L8)*

[000800] To a solution of **L8-3** (560 mg, 818.94 µmol, 1 eq.) in THF (7 mL) was added piperidine (139.46 mg, 1.64 mmol, 161.75 µL, 2 eq.) at 20 °C. The reaction was stirred at 20 °C for 2 h. LCMS trace showed that the reaction converted completely. The reaction was added MTBE (50 mL), white solids were precipitated. The mixture was filtered to give a crude as an off-white solid. The crude was purified by prep-HPLC (reversed-phase column, 40 g, 0%~35% 0.4% AcOH in water/ACN, 15 min) to give **L8** (180 mg, 252.88 µmol, 30.88% yield, 71.028% purity) as an off-white solid.

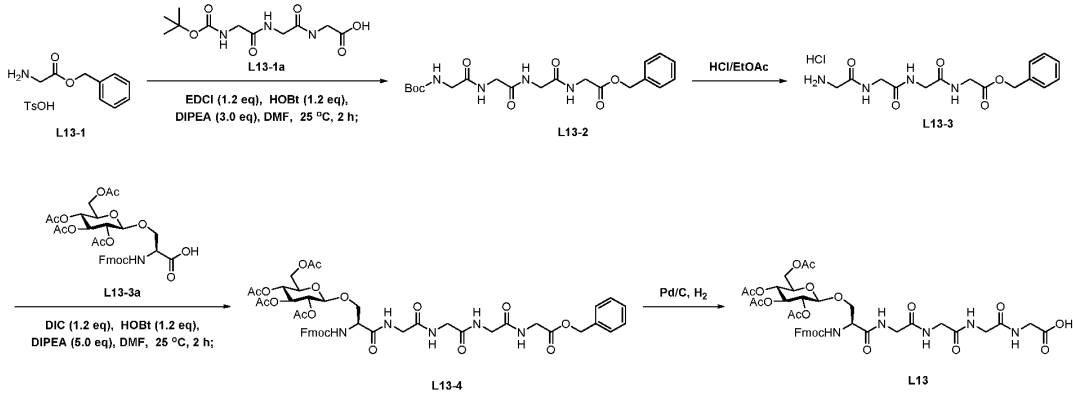
[000801] LCMS: (ESI): RT = 0.709 min, m/z calcd. for $C_{15}H_{28}N_5O_6S$, 406.18 $[M\text{-}Boc+2H]^+$, m/z found 406.2 $[M\text{-}Boc+2H]^+$; Reverse phase LCMS was carried out using a Merck RP-18e 25-

2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

4.3 Preparation of peptide linker L9

[000802] Scheme 22, below, depicts synthesis of SG4-SH peptide linker L8:

**Scheme 22**




[000803] Glycopeptide **L13-3a**, was synthesized according to J.J. Du, X.F. Gao, L.M. Xin, Z. Lei, Z. Liu, and J. Guo, *Convergent Synthesis of N-Linked Glycopeptides via Aminolysis of ω-Asp p-Nitrophenyl Thioesters in Solution.* Org. Lett., Vol. 18, No. 19, 2016, 4828-4831. Synthesis of L13-1awas performed according to Y. A. Naumovich, I.S. Golovanov, A.Y. Sukhorukov, and S.L. Loffe, *Addition of HO-Acids to N ,N -Bis(oxy)enamines: Mechanism, Scope and Application to the Synthesis of Pharmaceuticals.* Eur. J. Org. Chem., Vol. 2017, No. 4, 2017, 6209-6227.

*Step 1: Synthesis of benzyl 2,2-dimethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate (L13-2)*

[000804] To a solution of **L13-1a** (10 g, 34.57 mmol, 1 eq.) in DMF (60 mL) was added HOBt (5.61 g, 41.48 mmol, 1.2 eq.), DIPEA (22.34 g, 172.84 mmol, 30.10 mL, 5 eq.) and EDCI (7.95 g, 41.48 mmol, 1.2 eq.) at 20 °C. The mixture was stirred at 20 °C for 15 min, **L13-1** (11.08 g, 32.84 mmol, 0.95 eq.) was added and the reaction mixture was stirred at 20 °C for 2 h. The reaction progress was monitored by LC-MS, which indicated no starting material was remained and formation of desired product. The mixture was quenched with a saturated solution of $NaHCO_3$ (20 mL) and brine (20 mL x 3), the solid precipitation was collected and washed with PE (20 mL x 2), concentrated under reduced pressure to give **L13-2** (13.5 g, 29.38 mmol, 85.00% yield, 95% purity) as a white solid.

[000805] LCMS: (ESI): RT = 0.714 min, m/z calcd. for $C_{20}H_{28}N_4O_7Na$ 459.2 $[M+Na]^+$, found 459.1; LC-MS Conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

[000806] $^1$H NMR (400MHz, DMSO-d6) δ = 8.31 (br t, J=5.7 Hz, 1H), 8.19 (br t, J=5.8 Hz, 1H), 8.05 (br t, J=5.3 Hz, 1H), 7.43 - 7.29 (m, 5H), 7.04 - 6.95 (m, 1H), 5.13 (s, 2H), 3.90 (d, J=5.9 Hz, 2H), 3.75 (d, J=5.6 Hz, 4H), 3.58 (br d, J=6.0 Hz, 2H), 1.38 (s, 9H).

*Step 2: Synthesis of benzyl 2-(2-(2-(2-aminoacetamido)acetamido)acetamido)acetate hydrochloride (L13-3)*

[000807] A solution of HCl/EtOAc (4 M, 14.80 mL, 4 eq.) was added to **L13-2** (7 g, 14.80 mmol, 1 eq, HCl) dropwise at 20 °C. The mixture was stirred at 20 °C for 10 min. The reaction progress was monitored by LC-MS, which indicated no starting material was remained and formation of desired product. The mixture was concentrated in vacuum and lyophilization to provide the **L13-3** (5.5 g, 10.33 mmol, 69.77% yield, 70% purity, HCl) as a white solid.

[000808] LCMS: (ESI): RT = 0.479 min, m/z calcd. for $C_{15}H_{21}N_4O_5$ 337.1 $[M+H]^+$, found 337.1 ; LC-MS Conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

*Step 3: Synthesis of (2R,3R,4S,5R,6R)-2-(((S)-16-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3,6,9,12,15-pentaoxo-1-phenyl-2-oxa-5,8,11,14-tetraazaheptadecan-17-yl)oxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (L13-4)*

[000809] To a solution of **L13-3a** (2.9 g, 4.41 mmol, 1 eq.) in DMF (20 mL) was added HOBt (715.03 mg, 5.29 mmol, 1.2 eq.), DIPEA (3.42 g, 26.46 mmol, 4.61 mL, 6 eq.) and DIC (667.82 mg, 5.29 mmol, 819.42 µL, 1.2 eq.) at 20 °C. The mixture was stirred at 20 °C for 15 min, **L13-3** (3.29 g, 6.61 mmol, 1.5 eq, HCl) was added and the reaction mixture was stirred at 20 °C for 12 h. The reaction progress was monitored by LC-MS, which indicated no starting material was remained and formation of desired product. The mixture was quenched with water (40 mL), and extracted with ethyl acetate (40 mL x 2). The organic layer was washed with water and brine (30 mL x 3), dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuum. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent

of 0~5% MeOH/DCM) for 25 min with total volume 1.6 L to provide **L13-4** (2.1 g, 1.72 mmol, 39.04% yield, 80% purity) as a white solid.

[000810] LCMS: (ESI): RT = 1.937 min, m/z calcd. for $C_{47}H_{54}N_5O_{18}$ 976.3 $[M+H]^+$, found 976.3 ; LC-MS Conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B),using the elution gradient 10%-80% (solvent B) over 2.0 minutes and holding at 80% for 0.48 minutes at a flow rate of 0.8 ml/min; Column: Xtimate 3 μm,C18,2.1*30 mm.

$^1$H NMR (400MHz, CHLOROFORM-d) δ = 7.83 - 7.73 (m, 2H), 7.59 (br d, J=7.5 Hz, 2H), 7.47 - 7.28 (m, 10H), 7.21 - 7.03 (m, 2H), 5.87 (br d, J=5.5 Hz, 1H), 5.27 - 4.89 (m,5H), 4.61 - 4.38 (m, 4H), 4.29 - 3.81 (m, 13H), 2.10 - 1.98 (m, 12H).

*Step 4: Synthesis of (S)-1-(9H-fluoren-9-yl)-3,6,9,12,15-pentaoxo-5-((((2R,3R,4S,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)-2-oxa-4,7,10,13,16-pentaazaoctadecan-18-oic acid (L13)*

[000811] To a solution of **L13-4** (2.1 g, 2.15 mmol, 1 eq.) in EtOAc (16 mL) and MeOH (2 mL) was added Pd/C (300 mg, 430.35 μmol, 10.35 μL, 10% purity, 0.20 eq.) at 20 °C and the mixture was stirred at 20 °C for 4 hr under $H_2$ (4.35 mg, 2.15 mmol, 1 eq.) (15 psi). The reaction progress was monitored by LC-MS, which indicated no starting material was remained and formation of desired product. The mixture was filtered and the filtered cake was washed with MeOH (10 mL x 3), concentrated in vacuum to provide **L13** (1.2 g, 1.29 mmol, 59.81% yield, 95% purity) as a white solid.

[000812] LCMS: (ESI): RT = 0.788 min, m/z calcd. for $C_{40}H_{48}N_5O_{18}$ 886.3 $[M+H]^+$, found 886.3 ; LC-MS Conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

[000813] $^1$H NMR (400MHz, METHANOL-d4) δ = 7.81 (br d, J=7.4 Hz, 2H), 7.73 - 7.63 (m, 2H), 7.44 - 7.37 (m, 2H), 7.36 - 7.29 (m, 2H), 5.31 - 5.18 (m, 1H), 5.03 (t, J=9.8 Hz,1H),4.94 - 4.89 (m, 2H), 4.51 - 4.20 (m, 5H), 4.16 - 4.08 (m, 1H), 4.06 - 3.75 (m, 11H), 2.05 - 1.98 (m, 12H).

**Example 5. Synthesis of GLP1 peptidomimetic linker-payloads**

[000814] Figure 26 depicts synthesis of linker-payloads LP1, LP2, LP3, LP4, and LP5 according to the disclosure.

5.1 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[3-[2-[2-[2-[2-[[4-(124-azatricyclohexadeca-8(14), 9(15), 10(16),12(18),68,70(73)-hexaen-33-yn-124-yl)-4-oxo-butanoyl]amino] ethoxy] ethoxy]

ethoxy] ethoxy]propanoylamino]butoxy]-2-ethyl-72-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (LP1)

[000815] To a solution of **P9** (9.46 mg, 14.56 µmol, 1.5 *eq.*) in DMF (0.5 mL) was added **L5** (n = 4, 15 mg, 9.70 µmol, 1 *eq.*) and DIPEA (6.27 mg, 48.52 µmol, 8.45 µL, *5 eq.*). The mixture was stirred at 25 °C for 1 hr. LCMS trace indicated that the complete consumption of reactant and the formation of the desired mass. The mixture was filtered and purified by prep-HPLC (TFA condition; column: Phenomenex Gemini-NX 150*30 mm*5 µm; mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 30%-60%, 11min) to give **LP1** (4.84 mg, 2.23 µmol, 23.02% yield, 96% purity) as a white solid.

[000816] LCMS (ESI): RT = 0.944 min, mass calcd. for $C_{105}H_{135}FN_{20}O_{24}$ 2078.99, m/z found 1041.0 $[M+2H]^{2+}$. Reverse phase LC-MS was carried out using a Merck RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.04% TFA (solvent B) and water containing 0.06% TFA (solvent A).

5.2 Preparation of 3S)-4-[[(1S)-1-[[4-[4-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[4-(132-azatricyclohexadeca-8(14),9(15),10(16),12(18),76,78(81)-hexaen-33-yn-132-yl)-4-oxo-butanoyl]amino]ethoxy] ethoxy] ethoxy] ethoxy] ethoxy] ethoxy] ethoxy] propanoylamino]butoxy]-2-ethyl-80-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl] amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl) propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP2)

[000817] Starting from **P9** (18 mg, 21.79 µmol, 2.25 *eq.*) and using the same procedure as described in Example 1, the desired **LP2** (3.41 mg, 1.41 µmol, 14.63% yield, 94% purity) was obtained as a white solid. Water solubility of **LP2** was assessed and determined to be equal to or greater than 60 nM.

[000818] LCMS (ESI): RT = 0.949 min, mass calcd. for $C_{113}H_{151}FN_{20}O_{28}$ 2255.10, m/z found 1128.9 $[M+2H]^{2+}$. Reverse phase LC-MS was carried out using a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000819] HPLC: RT = 15.273 min. Reverse phase HPLC was carried out using a Gemini-NX 5 µm 150*4.6 mm, C18, 110A column, with a flow rate of 1.0mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.12% TFA (solvent B) and water containing 0.12% TFA (solvent A).

5.3 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[4-(140-azatricyclohexadeca-8(14),9(15),10(16),12(18),84,86(89)-hexaen-33-yn-140-yl)-4-oxo-butanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]butoxy]-2-ethyl-88-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl -4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino] butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP3)

[000820] Starting from **P9** (14.59 mg, 14.56 µmol, 1.5 *eq.*) and using the same procedure as described in Example 1, the desired product **LP3** (4.68 mg, 1.68 µmol, 17.27% yield, 87.1% purity) was obtained as a white solid.

[000821] HPLC condition: RT = 15.120 min, Reverse phase HPLC was carried out using a Gemini-NX 5u C18 110A 150*4.6 mm column, with a flow rate of 1.0 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.1% TFA (solvent B) and water containing 0.1% TFA (solvent A).

5.4 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[4-(164-azatricyclohexadeca-8,10(16),12(18),14(108),15(109),110(113)-hexaen-33-yn-164-yl)-4-oxo-butanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]butoxy]-2-ethyl-112-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl) propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP4)

[000822] Starting from **P9** (20 mg, 12.94 µmol, 1.0 eq.) and using the same procedure as described in Example 1, the desired product **LP4** (5.58 mg, 1.76 µmol, 13.59% yield, 93.29% purity) was obtained as a white solid.

[000823] LCMS (ESI): RT = 0.912 min, mass calcd. for $C_{145}H_{215}FN_{20}O_{44}$ 2961.36 $[M+H]^+$, 987.514 $[M+3H]^{3+}$, m/z found 988.3 $[M+3H]^{3+}$. Reverse phase LC-MS was carried out using a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000824] HRMS (ESI): mass calcd for $C_{145}H_{216}FN_{20}O_{44}$ 2960.5263 $[M+H]^+$, 1480.7632 $[M+2H]^{2+}$, 987.5088 $[M+3H]^{3+}$, m/z found 2960.53 $[M+H]^+$, 1481.26 $[M+2H]^{2+}$, 987.8517 $[M+3H]^{3+}$.

5.5 Preparation of 8S,14S,17S,20S,23S,26S)-8-((2H-tetrazol-5-yl)methyl)-26-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-((1-((1R,8S,9s)-bicyclo[6.1.0] non-4-yn-9-yl)-3,17-dioxo-2,7,10,13,16-pentaoxa-4,18-diazadocosan-22-yl)oxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl)-23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21,24-octaoxo-3,7,10,13, 16,19,22,25-octaazaoctacosan-28-oic acid (LP5)

[000825] To a solution of **P9** (12 mg, 7.76 µmol, 1 *eq.*) in DMF (0.5 mL) were added **L6** (8.30 mg, 15.53 µmol, 2.0 *eq.*) and TEA (1.57 mg, 15.53 µmol, 2.16 µL, 2.0 *eq.*). Then the mixture was stirred at 20°C for 12 h. LCMS trace showed that most of reactant was consumed completely and the desired MS was detected. It was purified by prep-HPLC (column: Phenomenex Gemini-NX 150*30 mm*5 µm; mobile phase: [water (0.225%FA)-ACN]; B%: 0%-55%, 45min). Compound **LP5** (4 mg, 2.00 µmol, 25.75% yield, 97% purity) was obtained as a white solid.

[000826] LCMS: (ESI): Rt = 4.087 min, mass calcd. for $C_{95}H_{132}FN_{19}O_{24}$ $[M+2H]^{2+}$ 971.5, m/z found 971.5 $[M+2H]^{2+}$ ; Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000827] HPLC: (ESI): Rt = 10.12 min, Reverse phase LCMS was carried out using Column: YMC-Pack ODS-A 150*4.6 mm, with a flow rate of 1.5 ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000828] Figure 27 depicts synthesis of linker-payloads LP6 and LP7 according to the disclosure.

5.6 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[[(2S)-2-[[2-[[2-[[2-[[2-[[4-(128-azatricyclohexadeca-8(14),9(15),10(16),12(18),63,65(68)-hexaen-33-yn-128-yl)-4-oxo-butanoyl]amino]acetyl] amino]acetyl]amino]acetyl]amino]acetyl]amino]-3-hydroxy-propanoyl]amino]butoxy]-2-ethyl -67-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl) ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-

yl)propanoyl] amino]acetyl] amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP6)

[000829] To an oven-dried vial were charged **L7** (163.54 mg, 323.48 µmol, 2.5 *eq.*) and **P9** (200 mg, 129.39 µmol, 1 *eq.*). A stock solution of HOBt (69.93 mg, 517.56 µmol, 4 *eq.*), DIPEA (50.17 mg, 388.17 µmol, 3 *eq.*) in DMF (1 mL) and $I_2$ (39.41 mg, 155.27 µmol, 1.2 *eq.*) in DMF (1 mL) was added to the vial. The reaction mixture was stirred at 15 °C for 12 h. The reaction progress was monitored by LCMS trace. The mixture was filtered, then precipitated by added EtOAc (20 mL). After filtration, the crude product **protected G4S-P9** (261 mg, 116.45 µmol, 90.00% yield, 90% purity) was obtained as a pale-yellow foam.

[000830] LCMS (ESI): RT = 3.283 min, mass calcd. for $C_{95}H_{136}FN_{23}O_{25}^{2+}$ 1009.005 $[M+2H\text{-}Boc]^{2+}$, m/z found 1009.5 $[M+2H]^{2+}$. LCMS conditions: Flash RP-18e 25-2mm, with a flow rate of 0.8 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000831] To an oven-dried vial was charged **protected G4S-P9** (261 mg, 129.39 µmol, 1 *eq.*) and DCM (5 mL). TFA (6.70 g, 58.75 mmol, 4.35 mL, 454.08 *eq.*) was added to the vial. The reaction mixture was stirred at 20 °C for 2 h. The reaction progress was monitored by LCMS. The reaction mixture was concentrated and purified by prep-HPLC (TFA condition; column: Waters Xbridge Prep OBD C18 150*40mm*10µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 0%-70%, 30min). **G4S-P9** (206 mg, 98.61 µmol, 76.21% yield, 100% purity, 2 TFA) was obtained as a white foam.

[000832] LCMS (ESI): RT = 2.497 min, mass calcd. for $C_{95}H_{136}FN_{23}O_{25}^{2+}$ 930.945 $[M+2H]^{2+}$, m/z found 931.0 $[M+2H]^{2+}$. LCMS conditions: Chromolith Flash RP-C18 25-3 mm, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80%acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000833] To an oven-dried vial were charged **DIBAC-suc-OSu** (39.68 mg, 98.61 µmol, 1 *eq.*) and **G4S-P9** (206 mg, 98.61 µmol, 1 *eq.*, 2 TFA). DMF (2 mL) and DIPEA (38.23 mg, 295.83 µmol, 3 *eq.*) were added to the vial. The reaction mixture was stirred at 20 °C for 1 h. The reaction progress was monitored by LC-MS. The reaction was fitered and purified by prep-HPLC (neutral condition; column: Waters Xbridge Prep OBD C18 150*40mm*10 µm; mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 0%-50%, 30min.). **LP6** (112 mg, 52.13 µmol, 52.87% yield, 100% purity) was obtained as a white foam.

[000834] LCMS (ESI): RT = 2.419 min, mass calcd. for $C_{105}H_{133}FN_{24}O_{25}^{2+}$ 1074.49 $[M+2H]^{2+}$, m/z found 1074.8 $[M+2H]^{2+}$. LCMS conditions: Waters Xbridge C18 30*2.0mm, 3.5 µm Mobile

phase: A) 0.05%NH3H2O in Water; B) ACN. Gradient: 0%B increase to 95%B within 5.8 min; hold at 95%B for 1.1 min; then back to 0%B at 6.91 min and hold for 0.09 min. Flow rate 1.0 mL/min.

[000835] HPLC: RT =3.58 min. HPLC conditions: Mobile Phase: 0.2ML/1L $NH_3$·$H_2O$ in water (solvent A) and acetonitrile (solvent B),using the elution gradient 0%-60% (solvent B) over 5 minutes and holding at 60% for 2 minutes at a flow rate of 1.2 ml/min; Column: Xbridge Shield RP-18, 5μm,2.1*50mm.

[000836] $^1$H NMR (400 MHz, ACETONITRILE-$d_3$) δ ppm 8.40 (s, 1 H) 7.52 (br t, *J*=6.82 Hz, 2 H) 7.35 - 7.46 (m, 3 H) 7.27 - 7.34 (m, 2 H) 7.19 - 7.25 (m, 1 H) 7.05 - 7.15 (m, 4 H) 6.95 (br d, *J*=5.75 Hz, 4 H) 6.86 - 6.91 (m, 1 H) 6.74 - 6.82 (m, 5 H) 6.70 (br d, *J*=8.13 Hz, 1 H) 4.98 (br d, *J*=14.51 Hz, 1 H) 4.61 (br s, 1 H) 4.38 - 4.46 (m, 2 H) 4.29 (br s, 2 H) 4.16 - 4.21 (m, 1 H) 4.09 - 4.12 (m, 2 H) 3.89 - 4.01 (m, 6 H) 3.79 - 3.88 (m, 10 H) 3.57 - 3.77 (m, 7 H) 3.28 - 3.36 (m, 3 H) 3.16 (br s, 4 H) 3.09 (br s, 1 H) 2.82 - 2.89 (m, 1 H) 2.76 (br d, *J*=3.75 Hz, 2 H) 2.61 - 2.70 (m, 1 H) 2.37 - 2.48 (m, 6 H) 2.16 (s, 6 H) 1.53 - 1.81 (m, 8 H) 1.28 (br d, *J*=3.38 Hz, 3 H) 1.17 - 1.22 (m, 6 H) 1.11 (br d, *J*=6.00 Hz, 6 H) 0.92 (br t, *J*=7.44 Hz, 3 H).

5.7 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[[2-[[2-[[2-[[(2S)-2-[[4-(128-azatricyclohexadeca-8(14),9(15),10(16),12(18),63,65(68)-hexaen-33-yn-128-yl)-4-oxo-butanoyl]amino]-3-hydroxypropanoyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]butoxy]-2-ethyl-67-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino] butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (LP7)

[000837] Starting from **L8** (50 mg, 32.35 μmol, 1 eq.) and **P9** (32.71 mg, 64.70 μmol, 2 eq.), **LP7** (15 mg, 6.74 μmol, 53.21% yield, 96.47% purity) was obtained as a white solid using the same procedure as described in Example 6.

[000838] LCMS: (ESI): RT = 0.845 min, m/z calcd. for $C_{105}H_{133}FN_{24}O_{25}$, 1075.5 $[M+2H]^{2+}$, m/z found 1074.6 $[M+2H]^{2+}$; Reverse phase LCMS was carried out using a Merck RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000839] HPLC: RT = 3.55 min. Mobile Phase: Mobile Phase: 0.5 ML/1L NH3H2O in water (solvent A) and acetonitrile (solvent B), using the elution gradient 0%-60% (solvent B) over 5 minutes and holding at 60% for 2 minutes at a flow rate of 1.2 ml/min.

[000840] Figure 28 depicts synthesis of linker-payloads LP8, LP9, LP10 and LP11 according to the disclosure.

5.8 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[[4-(2-azatricyclo[10.4.0.04,9]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl)-4-oxobutanoyl]amino]ethoxy]ethoxy]ethoxy] ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (LP8)

[000841] To a solution of **P8** (20 mg, 12.73 µmol, 1 *eq.*) and **L9** (8.83 mg, 38.18 µmol, 3.0 *eq.*) in $H_2O$ (0.25 mL) and *t*-BuOH (0.5 mL) were added $CuSO_4 \cdot 5H_2O$ (635.45 µg, 2.55 µmol, 0.2 *eq.*) and sodium;(2R)-2-[(1S)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2H-furan-3-olate (1.01 mg, 5.09 µmol, 0.4 *eq*). The mixture was stirred at 20°C for 1.5 h. LCMS trace showed the material was disappeared and the desired product was observed as the major. The green solution was filtered to give the crude product. The crude product was purified by reversed phase HPLC (ISCO®; 20 g C18® Silica Flash Column, Eluent of 0~60.1%$CH_3CN/H_2O$ (0.4%AcOH) gradient @ 18 mL/min). The desired fluent was lyophilized in freeze dryer to give compound **61** (15 mg, 8.24 µmol, 64.78% yield, 99.090% purity) as a white solid.

[000842] LCMS (ESI): RT = 0.764 min, mass calcd. for $C_{23}H_{43}N_{11}O_6$ 901.95, m/z found 902.3 $[M+H]^+$. LC-MS method A: a Xtimate C18 2.1*30 mm, 3µm column, with a flow rate of 1.2 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.75 ML/4L TFA (solvent B) and 1.5 ML/4L TFA in water (solvent A).

[000843] To a solution of **61** (17 mg, 9.43 µmol, 1 *eq.*) and **DIBAC-suc-OSu** (5.05 mg, 12.55 µmol, 1.33 *eq.*) in DMF (2 mL) was added DIPEA (2.44 mg, 18.86 µmol, 3.28 µL, 2.0 *eq.*). The solution was stirred at 15 °C for 1 h. LCMS showed the reaction was converted completely and the desired product was observed. The solution was filtered and purified by prep-HPLC (neutral condition: Column: Durashell C18(L) 100*10mm*5µm; Mobile phase: [water (10mM NH4HCO3)-ACN]; B%: 12%-52%, 12min). The desired fluent was lyophilized in freeze dryer to give **LP8** (7.0 mg, 3.21 µmol, 30.42% yield, 95.745% purity) as a white solid.

[000844] LCMS (ESI): RT = 1.337 min, m/z calcd. for $C_{105}H_{135}FN_{22}O_{23}$ $[M+2H]^{2+}$ 1045.50, found 1046.1. LCMS conditions: 0.8mL/4L $NH_3 \cdot H_2O$ in water (solvent A) and acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 2 minutes and holding at 80% for 0.48 minutes

at a flow rate of 1 ml/min; Column: XBridge C18 3.5μm 2.1*30 mm; Wavelength: UV 220nm&254nm; Column temperature: 50℃;

[000845] HPLC: RT = 2.868 min, Mobile Phase: 0.2mL/1L $NH_3 \cdot H_2O$ in water (solvent A) and acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 2 minutes at a flow rate of 0.8 ml/min; Column: Xbridge Shield C18, 5μm, 2.1*50mm;

5.9 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[[(1S,8R)-9-bicyclo[6.1.0]non-4-ynyl]methoxycarbonylamino]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP9)

[000846] To a solution of compound **61** (14.55 mg, 8.07 μmol, 1 *eq.)* in DMF (2.0 mL) were added (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate (**BCN-CO-PNP)** (5.81 mg, 18.41 μmol, 2.28 *eq.)* and DIPEA (2.09 mg, 16.14 μmol, 2.81 μL, 2.0 *eq.)*. The solution was purified by prep-HPLC (FA condition) (Column: Phenomenex Gemini-NX 150*30 mm*5 μm; mobile phase: [water (0.225%FA)-ACN]; B%: 0%-55%, 45min) to give **LP9** (3.6 mg, 1.79 μmol, 22.21% yield, 98.54% purity) was obtained as a white solid.

[000847] LCMS (ESI): RT = 4.040 min, mass calcd. for $C_{97}H_{134}FN_{21}O_{23}$ $[M+2H]^{2+}$ 989.89, found 990.6. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B)over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate 3μm,C18,2.1*30 mm;

[000848] HPLC: RT= 9.89 min, Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 μm.

5.10 Preparation of (3S)-4-[[(1S)-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-1-[[4-[4-[4-[4-[2-[2-[2-[2-[(2-cyclooct-2-yn-1-yloxyacetyl)amino]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]

butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP10)

[000849] To a solution of **61** (14 mg, 7.76 µmol, 1 *eq*) in DMF (0.5 mL) were added **2,5-dioxopyrrolidin-1-yl 2-(cyclooct-2-yn-1-yloxy)acetate** (5.18 mg, 18.53 µmol, 2.39 *eq*) and DIPEA (2.01 mg, 15.53 µmol, 2.70 µL, 2.0 *eq*). The solution was stirred at 20 °C for 1 hr. LCMS showed the reaction was converted completely and the desired product was observed. The mixture was diluted with CH3CN, and the crude product was purified by prep-HPLC (FA condition: Column: Phenomenex Gemini-NX 150*30 mm*5 µm; Mobile phase: [water (0.225%FA)-ACN]; B%: 5%-55%, 35 min). The desired fluent was lyophilized in freeze dryer to give **LP10** (2.6 mg, 1.26 µmol, 13.08% yield, 95.26% purity) as a white solid.

[000850] LCMS (ESI): RT = 3.805 min, m/z calcd. for $C_{96}H_{134}FN_{21}O_{23}$ $[M+2H]^{2+}$ 983.99, found 984.7. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B)over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate 3 µm,C18,2.1*30 mm.

[000851] HPLC: RT= 9.74 min, Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm,5 µm; HPLC: RT= 9.89 min, Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm.

5.11 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl] -2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl) ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino] acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl] amino]-4-oxo-butanoic acid (LP11)

[000852] A mixture of **P8** (10 mg, 6.36 µmol, 1 eq.), sodium ascorbate (504.18 µg, 2.54 µmol, 0.4 eq.) and $CuSO_4 \cdot 5H_2O$ (317.72 µg, 1.27 µmol, 0.2 eq.) in t-BuOH (0.8 mL) and $H_2O$ (0.4 mL) were stirred at 20 °C for 2h. LCMS showed **P8** was consumed completely. The reaction was filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Genimi

NX C18 150*40mm*5 μm; mobile phase: [water (0.1%TFA)-ACN]; B%: 0%-60%, 25min) to give **LP11** (4.32 mg, 2.18 μmol, 34.31% yield) was obtained as a white solid.

[000853] LCMS: (ESI): RT = 3.121 min, m/z calcd. for $C_{94}H_{138}FN_{21}O_{25}$, 990.01 $[M+2H]^{2+}$, m/z found 990.6 $[M+2H]^{2+}$; Mobile Phase: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B),using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min.

[000854] HPLC (ES8584-1120-P1C1) was attached. RT= 3.75 min, 99.72% purity. HPLC method: Column: Ultimate XB-C18,3 μm,3.0*50mm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80%(solvent B) over 7 minutes and holding at 80% for 0.5 minutes at a flow rate of 1.5 ml/min.

[000855] Figure 29 depicts synthesis of linker-payload LP12 according to the disclosure.

5.12 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[4-(162-azatricyclohexadeca-6(12),7(13),8(14),10(16),109,111(114)-hexaen-31-yn-162-yl)-4-oxo-butanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]butoxy]-2-ethyl-113-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S, 3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[6-(1H-imidazol-5-yl) hexanoylamino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP12)

[000856] To a solution of **P11** (30 mg, 14.65 μmol, 1 eq., TFA) and **L11** (22.43 mg, 14.65 μmol, 1 eq.) in DMF (1.5 mL) was added DIPEA (9.47 mg, 73.25 μmol, 12.76 μL, 5 eq.) at 15 °C. The mixture was stirred at 15 °C for 1h. LCMS trace showed that the reaction was complete. The mixture was filtered to give crude as yellow oil, and the residue was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150*40mm*10μm; mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 0%-80%, 30min) to give **LP12** (25 mg, 8.18 μmol, 47.72% yield, 95.49% purity) as a white solid.

[000857] LCMS: (ESI): RT = 0.970 min, m/z calcd. for $C_{144}H_{217}FN_{19}O_{43}$, 973.18 $[M+3H]^{3+}$, m/z found 974.0 $[M+3H]^{3+}$; Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000858] HPLC: Rt = 2.53; Mobile Phase: 0.2ML/1L $NH_3H_2O$ in water (solvent A) and acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 5 minutes and

holding at 80% for 2 minutes at a flow rate of 1.2 ml/min Column: Xbridge Shield RP-18,5 µm, 2.1*50mm.

[000859] Figure 30 depicts synthesis of linker-payloads LP13 and LP14 according to the disclosure.

5.13 Preparation of (3S)-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-2-[(3-amino-2,2-dimethyl-3-oxo-propanoyl)amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl] amino]-3-hydroxy-propanoyl]amino]-4-[[(1S)-1-[[4-[4-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[4-(157-azatricyclohexadeca-8(14),9(15),10(16),12(18),104, 106(109)-hexaen-31-yn-157-yl)-4-oxobutanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]butoxy]-2-ethyl-108-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoic acid (LP13)

[000860] To a solution of **P4** (10 mg, 6.89 µmol, 1 eq.) and **L12** (10.55 mg, 6.89 µmol, 1 eq.) in DMF (0.5 mL) was added DIPEA (4.45 mg, 34.44 µmol, 6.00 µL, 5 eq.) at 15 °C. The mixture was stirred at 15 °C for 1h. LCMS showed that the reaction was converted completely. The mixture was filtered to give crude as a yellow oil, which was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150*30 mm, 10 µm; mobile phase: [water(10mM $NH_4HCO_3$)-ACN]; B%: 10%-50%, 55 min) to give **LP13** (11 mg, 3.84 µmol, 36.67% yield) as a white solid.

[000861] LCMS: (ESI): RT = 0.965 min, m/z calcd. for $C_{140}H_{213}FN_{18}O_{44}$, 717.38 $[M+4H]^{2+}$, m/z found 717.8 $[M+4H]^{4+}$; Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000862] HPLC: RT = 3.97; Mobile Phase: 0.2ML/1L $NH_3H_2O$ in water (solvent A) and acetonitrile (solvent B), using the elution gradient 0%-60% (solvent B) over 5 minutes and holding at 60% for 2 minutes at a flow rate of 1.2 ml/minColumn: Xbridge Shield RP-18,5 µm,2.1*50mm Wavelength: 220nm&215nm&254nm.

5.14 Preparation of (3S)-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-2-[(3-amino-2,2-dimethyl-3-oxo-propanoyl)amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl] amino]-3-hydroxy-propanoyl]amino]-4-[[(1S)-1-[[4-[4-[4-[[(2S)-2-[[2-[[2-[[2-[[4-(121-azatricyclohexadeca-8(14),9(15),10(16),12(18),59,61(64)-hexaen-31-yn-121-yl)-4-oxo-butanoyl]

amino]acetyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]-3-hydroxy-propanoyl]amino] butoxy]-2-ethyl-63-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl] amino]-2-oxo-ethyl]amino]-4-oxo-butanoic acid (LP14)

[000863] To a solution of **P4** (40 mg, 27.56 µmol, 1 eq.) in DMF (0.5 mL) were added HOBt (14.89 mg, 110.22 µmol, 4 eq.), DIPEA (10.68 mg, 82.67 µmol, 14.40 µL, 3 eq.) and **L7** (34.83 mg, 68.89 µmol, 2.5 eq.); then $I_2$ (8.39 mg, 33.07 µmol, 6.66 µL, 1.2 eq.) in DMF (0.5 mL) was added. The reaction mixture was stirred at 20 °C for 16 hr. To the reaction was added EtOAc (15 mL) and white solids were precipitated. The mixture was centrifuged for 3 min (5000 R) to get the solid. Then the solid was dissolved in $H_2O$ (10 mL) and CAN (2 mL). The solution was lyophilized to give the crude **protected compound 62** (59 mg, 23.01 µmol, 83.50% yield, 75% purity) as a white solid.

[000864] LCMS (ESI): RT = 3.882 min, mass calcd. for $C_{90}H_{130}FN_{21}O_{25}$ 1923.94 961.9$[M+2H]^{2+}$, m/z found 962.5 $[M+2H]^{2+}$; Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B),using the elution gradient 10%-80% (solvent B) over 3 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1*30 mm, 3 µm; Wavelength: UV 220 nm; Column temperature: 50℃; MS ionization: ESI

[000865] To a solution of **protected compound 62** (59 mg, 30.68 µmol, 1 *eq.*) in DCM (0.5 mL) was added TFA (0.5 mL) at 0 °C. Then the mixture was warmed to 20°C and stirred at 20°C for 2hr. LCMS trace showed the reactant was consumed completely and the desire MS was detected. The solvent was removed under reduced pressure at 30°C to give the crude. The residue was purified by prep-HPLC (column: Phenomenex Gemini-NX C18 75*30 mm*3 µm; mobile phase: [water (0.075%TFA)-ACN]; B%: 0%-60%, 35 min.) to give **compound 62** (11 mg, 5.91 µmol, 19.28% yield, 95% purity) as a white solid.

[000866] LCMS (ESI): RT = 2.936 min, mass calcd. for $C_{81}H_{114}FN_{21}O_{23}$ 1767.82 884.2 $[M+2H]^{2+}$, m/z found 884.2 $[M+2H]^{2+}$; Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min. ESI source, Positive ion mode; Wavelength 220 nm&254 nm, OvenTemperature 50°C

[000867] HPLC: RT = 6.05 min Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80 %( solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm; Wavelength: UV 220nm&215nm&254nm; Column temperature

[000868] To a solution of **compound 62** (11 mg, 6.23 μmol, 1 *eq.*) and **DIBAC-suc-OSu** (2.76 mg, 6.85 μmol, 1.1 *eq.*) in DMF (0.5 mL) was added DIPEA (4.02 mg, 31.13 μmol, 5.42 μL, 5.0 *eq.*) at 20 °C. Then the mixture was stirred at 20 °C for 2 hr. LCMS trace showed the reactant was consumed completely and the desired MS was detected. It was purified by prep-HPLC (column: Phenomenex Gemini-NX C18 75*30 mm*3 μm; mobile phase: [water (10 mM $NH_4HCO_3$)-ACN]; B%: 0%-60%, 35 min). **LP14** (10 mg, 4.62 μmol, 74.28% yield, 95% purity) was obtained as a white solid.

[000869] LCMS (ESI): RT = 0.939 min, mass calcd. for $C_{100}H_{127}FN_{22}O_{25}$ 2054.92, m/z found 1028.0 $[M+2H]^{2+}$ Mobile Phase: 1.5 mL/4L TFA in water (solvent A) and 0.75 mL/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95%(solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm Wavelength: UV 220 nm; Column temperature: 50℃.

[000870] HPLC: RT = 3.75 min Mobile Phase: water (solvent A) and acetonitrile (solvent B), using the elution gradient 0%-60% (solvent B) over 5 minutes and holding at 60% for 2 minutes at a flow rate of 1.2 ml/min; Column: Xbridge Shield RP-18, 5μm, 2.1*50 mm; Wavelength: UV 220 nm, 215 nm&254 nm; Column temperature: 40°C.

[000871] Figure 31 depicts synthesis of linker-payloads LP15 and LP16 according to the disclosure.

5.15 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[[(2S)-2-[[2-[[2-[[2-[[2-[[4-(134-azatricyclohexadeca-11(19),12(20),13(21),15(23),69,71(74)-hexaen-38-yn-134-yl)-4-oxobutanoyl]amino]acetyl] amino]acetyl]amino]acetyl]amino]acetyl]amino]-3-hydroxy-propanoyl]amino]butoxy]-2-ethyl-73-phenyl]phenyl]methyl]-2-[[(1S)-4-(3,5-dimethylphenyl)-1-(phenylcarbamoyl)butyl] amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl] amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methylpropanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP15)

[000872] To an oven-dried vial were charged L7 (47.33 mg, 93.62 μmol, 2.5 eq.) and P23 (65 mg, 37.45 μmol, 1 eq., TFA). A stock solution of HOBt (20.24 mg, 149.78 μmol, 4 eq.), DIPEA (14.52 mg, 112.34 μmol, 19.57 μL, 3 eq.) in DMF (0.5 mL) and I2 (11.40 mg, 44.94 μmol, 9.05 μL, 1.2 eq.) in DMF (2 mL) was added to the vial. The reaction mixture was stirred at 20 °C for 16 h. LCMS trace showed that the reaction was complete. The reaction was added EtOAc (15 mL) and white solids were precipitated. The mixture was centrifuged for 3 min (5000 R) to give the crude product 63 (60 mg, 27.82 μmol, 74.29% yield, and 97.06% purity) as a white solid.

[000873] LCMS (ESI): RT = 0.940 min, mass calcd. for $C_{96}H_{132}FN_{23}O_{23}$ 997 m/z [M-Boc+3H]2+, m/z found 997.5 m/z [M-Boc+3H]2+ ; Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000874] A solution of 63 (60 mg, 28.66 µmol, 1 eq.) in TFA (1 mL) and DCM (1 mL) were stirred at 20 °C for 2h. LCMS trace showed that the reaction was complete. The reaction was concentrated in vacuum to give crude as yellow oil. The crude was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150*40mm*10µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 0%-60%, 30min.) to give 64 (45 mg, 22.72 µmol, 79.27% yield, 97.81% purity) as a white solid.

[000875] LCMS (ESI): RT = 0.890 min, mass calcd. for $C_{92}H_{124}FN_{23}O_{23}$ 968.96 m/z [M+2H]2+, m/z found 969.5 m/z [M+2H]2+; Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000876] HPLC (ES8584-719-P1C1) was attached. RT=4.18 min., 97.81% purity. HPLC method A: Mobile phase: 1.0% ACN in water (0.1% TFA) to 5% ACN in water (0.1% TFA) in 1 min; then from 5% ACN in water (0.1% TFA) to 100% ACN (0.1% TFA) in 5 minutes; hold at 100% ACN (0.1% TFA) for 2 minutes; back to 1.0% CAN in water (0.1% TFA) at 8.01min, and hold two minutes.Flow rate: 1.2ml/min.

[000877] To a solution of 64 (40 mg, 19.50 µmol, 1 eq., TFA) and DIBAC-suc-OSu (7.85 mg, 19.50 µmol, 1 eq.) in DMF (1.5 mL) was added DIPEA (12.60 mg, 97.51 µmol, 16.98 µL, 5 eq.) at 18 °C. The reaction was stirred at 18 °C for 2h. LCMS trace showed that the reaction was complete. The crude was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150*40 mm*10 µm; mobile phase: [water (10mM NH4HCO3)-ACN]; B%: 0%-50%, 35min.) to give LP15 (27 mg, 12.07 µmol, 55.75% yield, 99.42% purity) as a white solid.

[000878] LCMS (ESI): RT = 0.948 min, mass calcd. for $C_{111}H_{137}FN_{24}O_{25}$ 1112.51 m/z $[M+2H]^{2+}$, m/z found 1113.1 m/z $[M+2H]^{2+}$ ; Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000879] HPLC: Reverse phase 0.2ML/1L $NH_3 \cdot H_2O$ in water (solvent A) and acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 5 minutes and holding at 80% for 2 minutes at a flow rate of 1.2 ml/min Column: Xbridge Shield RP-18, 5 μm, 2.1*50 mm Wavelength: 220 nm&215 nm&254 nm, Column temperature: 40 °C

5.16 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[[2-[[2-[[2-[[2-[[(2S)-2-[[4-(134-azatricyclohexadeca-11(19),12(20),13(21),15(23),69,71(74)-hexaen-38-yn-134-yl)-4-oxo-butanoyl]amino]-3-hydroxypropanoyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]butoxy]-2-ethyl-73-phenyl]phenyl]methyl]-2-[[(1S)-4-(3,5-dimethylphenyl)-1-(phenylcarbamoyl)butyl] amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl] amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (LP16)

[000880] Starting from P23 (70 mg, 43.16 μmol, 1 eq), L8 (43.64 mg, 86.32 μmol, 2 eq) and DIBAC-suc-OSu (3.92 mg, 9.75 μmol, 1 eq), LP16 (10 mg, 4.25 μmol, 34.87% yield, 94.53% purity) as a white solid was prepared using the same procedure as described in Example 15.

[000881] LCMS: (ESI): RT = 0.845 min, mass calcd. for $C_{111}H_{137}FN_{24}O_{25}$ 1112.51 $[M+2H]^{2+}$, m/z found 1112.9$[M+2H]^{2+}$; Mobile Phase: 0.8mL/4L $NH_3.H_2O$ in water (solvent A) and acetonitrile (solvent B),using the elution gradient 10%-80%(solvent B) over 2 minutes and holding at 80% for 0.48minutes at a flow rate of 1ml/min;

[000882] HPLC: RT=3.73 min, 94.53% purity. Mobile Phase: 2.0ML/4L $NH_3H_2O$ in water (solvent A) and Aetonitrile (solvent B), using the elution gradient 0%-60% (solvent B) over 4minutes and holding at 60% for 2 minutes at a flow rate of 1.2ml/min.

[000883] Figure 32 depicts synthesis of linker-payload LP17 according to the disclosure.

5.17 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[[(2S)-2-[[2-[[2-[[2-[[2-[[(2S)-2-[[2-[[2- [[2-[[2-[[4-(144-azatricyclohexadeca-8(14),9(15),10(16),12(18),68,70(73)-hexaen-33-yn-144-yl)-4-oxo-butanoyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]-3-hydroxy-propanoyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]-3-hydroxy-propanoyl]amino]butoxy]-2-ethyl-72-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino] butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP17)

[000884] To an oven-dried vial were charged L7 (26.17 mg, 51.76 μmol, 2 eq.), P9 (40.00 mg, 25.88 μmol, 1 eq.) and 4A MS (5 mg). A stock solution of HOBt (6.99 mg, 51.76 μmol, 2 eq.), DIPEA (5.02 mg, 38.82 μmol, 6.76 μL, 1.5 eq.) in DMF (0.1 mL) and I2 (3.94 mg, 15.53 μmol, 3.13 μL, 0.6 eq.) in DMF (0.1 mL) was added to the vial. The reaction mixture was stirred at 15 °C for 48 h. The reaction progress was monitored by LC-MS. The mixture was filtered, then precipitated by added EtOAc (3 mL). After filtration, protected G4S-P9 (50 mg, 22.31 μmol, 86.20% yield, 90% purity) was obtained as a white foam.

[000885] LCMS (ESI): RT = 0.907 min, mass calcd. for $C_{95}H_{136}FN_{23}O_{25}^{2+}$ 1009.005 $[M+2H]^{2+}$, m/z found 1010.4 $[M+2H]^{2+}$. LCMS conditions: a Merck, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000886] HPLC: RT =8.87 min. HPLC conditions: YMC-Pack ODS-A 150*4.6 mm, 5 mm column, flow rate 1.5 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.12% TFA (solvent B) and water containing 0.1% TFA (solvent A).

[000887] To an oven-dried vial were charged protected G4S-P9 (35 mg, 17.35 μmol, 1 eq.) and DCM (1 mL). TFA (1.54 g, 13.51 mmol, 1 mL, 778.42 eq.) was added to the vial. The reaction mixture was stirred at 20 °C for 3 h. The reaction progress was monitored by LC-MS. LCMS (ESI): RT = 0.832 min, mass calcd. for $C_{95}H_{136}FN_{23}O_{25}^{2+}$ 930.945 $[M+2H]^{2+}$, m/z found 931.3 $[M+2H]^{2+}$. LCMS conditions: a Merck, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A). The reaction mixture was concentrated to give the crude product 67 (35 mg, crude) as a yellow solid.

[000888] To an oven-dried vial were charged L7 (19.02 mg, 37.61 μmol, 2 eq.) and compound 67 (35 mg, 18.81 μmol, 1 eq.). A stock solution of HOBt (10.16 mg, 75.23 μmol, 4 eq.) and DIPEA (7.29 mg, 56.42 μmol, 9.83 μL, 3 eq.) in DMF (0.5 mL) and I2 (5.73 mg, 22.57 μmol, 4.55 μL, 1.2 eq.) in DMF (0.5 mL) was added to the vial. The reaction mixture was stirred at 15 °C for 24 h. The reaction progress was monitored by LC-MS. The mixture was filtered, then precipitated by added EtOAc (6 mL). After filtration, the crude product protected G4S-G4S-P9 (40 mg, 13.38 μmol, 71.12% yield, 78% purity) was obtained as a pale yellow foam.

[000889] LCMS (ESI): RT = 3.400 min, mass calcd. for $C_{106}H_{153}FN_{28}O_{31}^{2+}$ 1166.56, m/z found 1166.9 $[M+2H]^{2+}$. LCMS conditions: a Merck, RP-18e 25-2mm column, with a flow rate of 0.8 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000890] HPLC: RT =8.10 min. HPLC conditions: YMC-Pack ODS-A 150*4.6 mm, 5 mm column, flow rate 1.5 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.12% TFA (solvent B) and water containing 0.1% TFA (solvent A).

[000891] To an oven-dried vial were charged protected G4S-G4S-P9 (40 mg, 17.15 μmol, 1 eq.) and DCM (2 mL). TFA (3.08 g, 27.01 mmol, 2 mL) was added to the vial. The reaction mixture was stirred at 20 °C for 2 h. The reaction progress was monitored by LC-MS. The reaction was concentrated to give a residue, then purified by prep-HPLC (TFA condition; column: Waters Xbridge Prep OBD C18 150*30 mm, 10 μm; mobile phase: [water(0.1%TFA)-ACN];B%: 0%-60%,16min). Compound 68 (15 mg, 6.62 μmol, 38.58% yield, 96% purity) was obtained as a white foam.

[000892] LCMS (ESI): RT = 0.744 min, mass calcd. for $C_{97}H_{137}FN_{28}O_{29}^{2+}$ 1088.505 $[M+2H]^{2+}$, m/z found 1089.2 $[M+2H]^{2+}$. LCMS conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

[000893] HPLC: RT =3.89 min. HPLC conditions: Mobile phase:1.0% ACN in water (0.1%TFA) to 5% ACN in water (0.1%TFA) in 1 min;then from 5% ACN in water (0.1%TFA) to 100% ACN (0.1%TFA) in 5 minutes; hold at 100% ACN (0.1%TFA) for 2 minutes;back to 1.0% ACN in water (0.1%TFA) at 8.01min,and hold two minutes.Flow rate:1.2ml/min.

[000894] To a solution of DIBAC-suc-OSu (3.37 mg, 8.36 μmol, 1.3 eq.) in DMF (0.3 mL) was added compound 68 (14 mg, 6.43 μmol, 1 eq.) and DIPEA (4.16 mg, 32.17 μmol, 5.60 μL, 5 eq.). The mixture was stirred at 20 °C for 2 hr. The reaction progress was monitored by LC-MS. The reaction mixture was diluted with DMSO (2 mL) and purified by prep-HPLC (neutral condition, column: Waters Xbridge Prep OBD C18 150*40mm*10 μm; mobile phase: [water(10mM NH4HCO3)-ACN];B%: 0%-60%,55 min). LP17 (1.73 mg, 0.65 μmol, 10.15% yield, 93% purity) was obtained as a white foam.

[000895] LCMS (ESI): RT = 2.531 min, mass calcd. for $C_{116}H_{150}FN_{29}O_{31}^{2+}$ 1232.05 $[M+2H]^{2+}$, m/z found 1232.4 $[M+2H]^{2+}$. LCMS conditions: Mobile phase: A) 0.05%$NH_3H_2O$ in Water; B) ACN. Gradient : 0%B increase to95%B within 5.8 min; hold at 95%B for 1.1 min; then back to 0%B at 6.91 min and hold for 0.09 min. Flow rate 1.0 mL/min; Column: Waters Xbridge C18 30*2.0mm,3.5 μm.

[000896] HPLC: RT =2.17 min. HPLC conditions: Mobile Phase: 0.2ML/1L $NH_3H_2O$ in water (solvent A) and acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 5

minutes and holding at 80% for 2 minutes at a flow rate of 1.2 ml/min; Column: Xbridge Shield RP-18,5 µm,2.1*50mm.

[000897] Figure 33 depicts synthesis of linker-payloads LP18 and LP20 according to the disclosure.

5.18 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[[2-[[2-[[2-[[(2S)-2-[3-[[(1S,8R)-9-bicyclo[6.1.0]non-4-ynyl]methoxycarbonylamino]propanoylamino]-3-[(2R,4S,5S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]oxypropanoyl]amino]acetyl]amino]acetyl]amino]acetyl] amino]acetyl]amino]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino] butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]asmino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (LP18)

[000898] To a solution of L13 (22.92 mg,25.88 µmol, 2 eq.), PyBOP (13.47 mg, 25.88 µmol, 2 eq.) and DIPEA (6.69 mg, 51.76 µmol, 9.01 µL, 4 eq.) in DMF (0.1 mL) was stirred at 25°C for 5 min. A solution of P9 (20 mg, 12.94 µmol, 1 eq.) in DMF (0.1 mL) was added to the mixture, the reaction was stirred at 25 °C for 5 min. The mixture was diluted with EtOAc (15 mL) to give precipitate, which was centrifuged for 3 min (5000 R) to give the crude product as a white solid. The residue was diluted with water (2 mL) and ACN (2 mL). The solution was dried by lyophilization to give compound 69 (25 mg, 8.53 µmol, 65.95% yield, 82.384% purity) as a white solid.

[000899] LCMS: (ESI): RT = 2.376 min, m/z calcd. for $C_{115}H_{148}FN_{23}O_{34}$, 1207.03 $[M+2H]^{2+}$, m/z found 1207.4 $[M+2H]^{2+}$; Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B),using the elution gradient 10%-80% (solvent B) over 3 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min.

[000900] To a solution of compound 69 (20 mg, 8.29 µmol, 1 eq.) in DMF (2 mL) was added $NH_2NH_2.H_2O$ (488.04 µg, 8.29 µmol, 0.2 mL, 85% purity, 1 eq.) at 25 °C. The reaction was stirred at 25 °C for 1h. LCMS showed that the reaction converted completely. The mixture was filtered to give a residue, which was purified by prep.-HPLC (column: Phenomenex Gemini-NX 150*30 mm*5 µm; mobile phase:[water(0.075%TFA)-ACN];B%: 0%-60%,30min) to give compound 70 (15 mg, 7.38 µmol, 71.28% yield, 99.58% purity) as a white solid.

[000901] LCMS: (ESI): RT = 0.791 min, m/z calcd. for $C_{92}H_{130}FN_{23}O_{28}$, 1011.97 $[M+2H]^{2+}$, m/z found 1012.2 $[M+2H]^{2+}$; Reverse phase LCMS was carried out using a Merck RP-18e 25-

2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000902] HPLC: Rt = 3.47 min. Mobile phase: 1.0% ACN in water (0.1%TFA) to 5% ACN in water (0.1%TFA) in 1 min;then from 5% ACN in water(0.1%TFA) to 100% ACN (0.1%TFA) in 5 minutes; hold at 100% ACN (0.1%TFA) for 2 minutes; back to 1.0% ACNin water (0.1%TFA) at 8.01min, and hold two minutes. Flow rate: 1.2ml/min.

[000903] A solution of compound 70 (18 mg, 8.90 µmol, 1 eq.) and L14 (6.88 mg, 17.79 µmol, 2 eq.) in PBS buffer (0.45 mL, pH=8.2) and DMF (0.9 mL) was stirred at 25 °C for 6h. LCMS showed the reaction was complete. The reaction was filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Gemini-NX 150*30 mm*5 µm; mobile phase: [water (0.225%FA)-ACN]; B%: 0%-60%, 35min) to give LP18 (7.5 mg, 3.24 µmol, 36.45% yield, 98.18% purity) as a white solid.

[000904] LCMS: (ESI): RT = 1.595 min, m/z calcd. for $C_{106}H_{147}FN_{24}O_{31}$, 1135.53 $[M+2H]^{2+}$, m/z found 1136.0 $[M+2H]^{2+}$; Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B),using the elution gradient 10%-80% (solvent B) over 2 minutes and holding at 80% for 0.48 minutes at a flow rate of 0.8 ml/min; Chromolith Flash RP-C18 2.1-30 mm

[000905] HPLC: RT=8.17 min, 98.18% purity. HPLC method A: Column: YMC-Pack ODS-A150*4.6 mm,5 µm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80%(solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min.

5.19 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[[2-[[2-[[2-[[2-[[(2S)-2-[[4-(153-azatricyclohexadeca-8(18),10(20),12(22),15(25),77(79),81(85)-hexaen-42(44)-yn-153-yl)-4-oxo-butanoyl]amino]-3-[6-[[4-(153-azatricyclohexadeca-8(18),10(20),12(22),15(25),77(79),81(85)-hexaen-42(44)-yn-153-yl)-4-oxo-butanoyl]oxymethyl]-3,4,5-trihydroxytetrahydropyran-2-yl]oxy-propanoyl] amino]acetyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]butoxy]-2-ethyl-84-phenyl] phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxopropanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl] amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxybutanoyl]amino] -3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP20)

[000906] To a solution of 70 (7.90 mg, 18.54 µmol, 2.5 eq) was added DIPEA (4.79 mg, 37.07 µmol, 6.46 µL, 5 eq) in DMF (0.5 mL) at 25 °C. The reaction was stirred at 25 °C for 16h. Then H2O (2.5 mL) was added and it was stirred at 25 °C for 2h. LCMS showed the reaction was

complete. The reaction was filtered to give a residue as a yellow solution. The residue was purified by prep-HPLC (column: Phenomenex Gemini-NX 150*30 mm*5 μm; mobile phase: [water (0.04%$NH_3H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 17%-47%, 8min) to give LP20 (3.7 mg, 1.52 μmol, 20.82% yield, 95.093% purity) as a white solid.

[000907] LCMS: (ESI): RT = 1.328 min, m/z calcd. for $C_{111}H_{143}FN_{24}O_{30}$, 1155.52 $[M+2H]^{2+}$, m/z found 1156.0 $[M+2H]^{2+}$; Mobile Phase: 0.8mL/4L $NH_3.H_2O$ in water (solvent A) and acetonitrile (solvent B),using the elution gradient 10%-80% (solvent B) over 2 minutes and holding at 80% for 0.48minutes at a flow rate of 1ml/min; Column: XBridge C18 3.5μm 2.1*30 mm; Wavelength:UV 220nm & 254nm; Column temperature: 50℃.

[000908] HPLC: RT=1.328 min, 92.31% purity. HPLC method A: Mobile Phase: 0.8mL/4L NH3.H2O in water (solvent A) and acetonitrile (solvent B), using the elution gradient 0%-60% (solvent B) over 5 minutes and holding at 60% for 0.48minutes at a flow rate of 1ml/min; Column: Xbridge Shield RP18 5μm 2.1*50mm; Wavelength: UV 220nm & 254nm.

[000909] Figure 34 depicts synthesis of linker-payload LP19 according to the disclosure.

5.20 Preparation of (3S)-4-[[(1S)-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-1-[[4-[4-[4-[[(2R)-2-[[2-[[2-[[2-[[2-[(2-cyclooct-2-yn-1-yloxyacetyl)amino]acetyl]amino]acetyl]amino] acetyl]amino]acetyl]amino]-3-hydroxypropanoyl]amino]butoxy]-2-ethyl-phenyl]phenyl] methyl]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxypropanoyl]amino]-4-oxo-butanoic acid (LP19)

[000910] To a colorless solution of P9 (25 mg, 16.17 μmol, 1 eq), L15 (16.35 mg, 32.35 μmol, 2.0 eq) and HOBt (4.37 mg, 32.35 μmol, 2.0 eq), DIPEA (3.14 mg, 24.26 μmol, 4.23 μL, 1.5 eq) in DMF (0.3 mL) was added a solution of I2 (2.87 mg, 11.32 μmol, 2.28 μL, 0.7 eq) in DMF (0.25 mL). The solution was stirred at 20 °C for 1 hr. LCMS trace showed the reaction was converted completely and the desired product was observed. The solution was treated with EtOAc (25 mL), the formed precipitate was collected by centrifuged for 5 min (5000 R). The collected white solid was lyophilized in freeze dryer to give compound 71 (45 mg, 21.11 μmol, 93.25% yield, 94.65% purity) as a white solid.

[000911] LCMS (ESI): RT = 0.880 min, m/z calcd. for $C_{95}H_{126}FN_{23}O_{23}$ $[M-Boc+2H]^{2+}$ 958.97, found 959.0. LC-MS method A: a Xtimate C18 2.1*30 mm, 3μm column, with a flow rate of 1.2 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.75 ML/4L TFA (solvent B) and 1.5 ML/4L TFA in water (solvent A).

[000912] HPLC: RT = 4.43 min. Mobile phase: 1.0% ACN in water (0.1%TFA) to 5% ACN in water (0.1%TFA) in 1 min; then from 5% ACN in water (0.1%TFA) to 100% ACN (0.1%TFA) in 5 minutes; hold at 100% ACN (0.1%TFA) for 2 minutes; back to 1.0% ACN in water (0.1%TFA) at 8.01min, and hold two minutes. Flow rate: 1.2ml/min

[000913] To a solution of 71 (35 mg, 17.35 µmol, 1 eq) in DCM (1 mL) was added TFA (1.54 g, 13.51 mmol, 1 mL, 778.42 eq). The solution was stirred at 20 °C for 1 hr. LCMS trace showed the reaction was converted completely and the desired product was observed. The solution was concentrated in vacuum to give a residue. The residue was purified by reversed phase HPLC (0.4%AcOH) (20 g column, Eluent of 0~ 44% $CH_3CN/H_2O$, gradient @ 25 mL/min). The desired fluent was lyophilized in freeze dryer to give 72 (18 mg, 8.91 µmol, 51.35% yield, 92.108% purity) as a white solid.

[000914] LCMS (ESI): RT = 0.815 min, m/z calcd. for $C_{86}H_{120}FN_{23}O_{23}$ [M+2H]2+ 930.94, found 931.2. LC-MS method A: a Xtimate C18 2.1*30 mm, 3µm column, with a flow rate of 1.2 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.75 ML/4L TFA (solvent B) and 1.5 ML/4L TFA in water (solvent A).

[000915] To a solution of 72 (17 mg, 9.13 µmol, 1 eq) and L16 (5.10 mg, 18.27 µmol, 2.0 eq) in DMF (0.5 mL) was added DIPEA (2.36 mg, 18.27 µmol, 3.18 µL, 2.0 eq). The solution was stirred at 20 °C for 1 hr. LCMS trace showed the reaction was converted completely and the desired product was observed. The solution was purified by prep-HPLC (FA condition: Column: Phenomenex Gemini-NX 150*30 mm*5 µm; mobile phase: [water (0.1%TFA)-ACN]; B%: 0%-50%, 35min). The desired fluent was lyophilized in freeze dryer to give LP19 (8.36 mg, 4.12 µmol, 36.34% yield, 99.72% purity) as a white solid.

[000916] LCMS (ESI): RT = 3.379 min, m/z calcd. for $C_{96}H_{132}FN_{23}O_{25}$ $[M+2H]^{2+}$ 1012.98, found 1013.7. LCMS conditions: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B)over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate 3 µm,C18,2.1*30 mm;

[000917] HPLC: RT= 8.70 min, Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm.

[000918] Figure 35 depicts synthesis of linker-payload LP21 according to the disclosure.

5.21 Preparation of (3S)-4-[[(1S)-2-[[(1S)-4-[4-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[4-(163-azatricyclohexadeca-7(13),8(14),9(15),11(17),108,110(113)-hexaen-

33-yn-163-yl)-4-oxo-butanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]butoxy]phenyl]-1-carbamoyl-butyl]amino]-1-[[4-(2-ethyl-4-methoxy-phenyl)phenyl]methyl]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2R,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino] acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP21)

[000919] To a mixture of P24 (15 mg, 9.69 µmol, 1 eq.) and L17 (22.25 mg, 14.54 µmol, 1.5 eq.) in DMF (1 mL) was added DIPEA (6.26 mg, 48.46 µmol, 8.44 µL, 5 eq.) in one portion at 25 °C under nitrogen. The mixture was stirred at 25 °C for 2 h. LCMS trace showed that the reaction was converted completely. The reaction mixture was concentrated in vacuum to give residue. The residue was purified by prep-HPLC (column: mobile phase: [water(10mM NH4HCO3)-ACN]; B%: 20%-50%, 55 min) to give pure product. The product was suspended in water (10 mL), the mixture frozen in a dry-ice/ethanol bath, and then lyophilized to dryness to afford the desired product LP21 (8.2 mg, 2.70 µmol, 27.86% yield, 97.5798% purity) as a white solid.

[000920] LCMS (ESI): RT = 0.924 min, mass calcd. for $C_{144}H_{213}FN_{20}O_{45}$ 2961.50 $[M+H]^+$, 987.167 $[M+3H]^{3+}$, m/z found 988.8 $[M+3H]^{3+}$. Reverse phase LCMS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.04% TFA (solvent B) and water containing 0.06% TFA (solvent A).

[000921] HPLC condition: RT = 14.496 min, Reverse phase HPLC was carried out using a Gemini-NX 5u C18 110A 150*4.6 mm column, with a flow rate of 1.0 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.1% TFA (solvent B) and water containing 0.1% TFA (solvent A).

[000922] Figure 36 depicts synthesis of linker-payload LP22 according to the disclosure.

5.22 Preparation of (3S)-4-[[(1S)-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-1-[[4-[4-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[4-[202-[[131,132,133,134,135,136,137,138,139,140,141,142-dodecahydroxy-126,127,128,129,130-pentakis(hydroxymethyl)-240,241,242,243,244,245,246,247,248,249,250,251-dodecaoxaheptacyclodotetracontan-125-yl]methyl]-187,189,202,203-tetrazatetracyclononadeca-8(14),10(16),11(17),12(18),112(114),115(117),123,187(189)-octaen -203-yl]-4-oxo-

butanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]butoxy]-2-ethyl-116-phenyl]phenyl]methyl]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2R,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP22)

[000923] To a mixture of compound LP4 (5 mg, 1.69 µmol, 1 eq.) in DMF (0.3 mL) was added and compound L18 (cyclodextrin-$N_3$, 4.38 mg, 4.39 µmol, 2.6 eq.) in one portion at 25°C under N2. The mixture was stirred at 25 °C for 12 hours. LCMS and HPLC trace showed the reaction were complete. The residue was purified by prep-HPLC (neutral: column: Waters Xbridge 150*25 5u; mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 20%-50%, 7min) to give LP22 (1.66 mg, 4.03e-1 µmol, 23.88% yield, 96.149% purity) as a white solid.

[000924] HPLC: RT = 11.976 min, Reverse phase HPLC was carried out using a Gemini-NX 5u C18 110A 150*4.6 mm column, with a flow rate of 1.0 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.1% TFA (solvent B) and water containing 0.1% TFA (solvent A).

[000925] LCMS (ESI): RT = 0.877 min, mass calcd. for $C_{74}H_{120}N_3O_{30}$ 3956.84 [M+H]+, 1318.95 [M+3H]3+, found 1320.2 [M+3H]3+. Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.04% TFA (solvent B) and water containing 0.06% TFA (solvent A).

[000926] Figure 37 depicts synthesis of linker-payload LP23 according to the disclosure.

5.23 Preparation of (3S)-4-[[(1S)-2-[[(1S)-1-carbamoyl-4-[4-[4-[3-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[4-[201-[[130,131,132,133,134,135,136,137,138,139,140, 141-dodecahydroxy-125,126,127,128,129-pentakis(hydroxymethyl)-239,240,241,242,243, 244, 245,246,247,248,249,250-dodecaoxaheptacyclodotetracontan-124-yl]methyl]-186,188,201, 202-tetrazatetracyclononadeca-7(13),9(15),10(16),11(17), 112(114),115(117),122, 186 (188)-octaen-202-yl]-4-oxo-butanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]butoxy]phenyl]butyl]amino]-1-[[4-(2-ethyl-4-methoxy-phenyl)phenyl]methyl]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-

dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino] butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP23)

[000927] Starting from LP21 (9.3 mg, 3.10 µmol, 1 eq.) and L18 (cyclodextrin-$N_3$, 3.7 mg, 3.7 µmol, 1.2 eq.) LP23 (3.5 mg, 8.84e-1 µmol, 50.00% yield, 100% purity) was obtained as a white solid following the same procedure in Example 22.

[000928] HPLC condition: RT = 8.14 min, Reverse phase HPLC was carried out using a YMC-Pack ODS-A 150*4.6 mm, 5 µm, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.1% TFA (solvent B) and water containing 0.1% TFA (solvent A).

[000929] LCMS (ESI): RT = 0.868 min, mass calcd. for $C_{75}H_{102}FN_{18}O_{17}$ 3958.82 [M+H]+ 1319.61 [M+H]3+, found 1321.5 [M+H]3+. Reverse phase LC-MS was carried out using a Chromolith Flash RP-18e 25-3 mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.04% TFA (solvent B) and water containing 0.06% TFA (solvent A).

[000930] Figure 38 depicts synthesis of linker-payload LP24 according to the disclosure.

5. 24 Preparation of (3S)-4-[[(1S)-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-1-[[4-[2-ethyl-4-[4-[4-[2-[2-[2-[2-[[(68S,69R,71S)-106,107,108-triazatricyclododeca-72(107),106(108)-dien-71-yl]methoxycarbonylamino]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-120-yl]butoxy] -61-phenyl]phenyl]methyl]-2-oxoethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP24)

[000931] A mixture of **LP9** (1.13 mg, 0.571 µmol, 1 eq.) and $NaN_3$ (44.54 µg, 0.685 µmol, 1.2 eq.) in DMSO (0.1 mL) was stirred at 37 °C for 16 h. LCMS showed the reaction was complete. The crude product **LP24** (1.15 mg, 0.569 µmol, 100.00% yield) was sent for the bio-assay directly without any further purification.

[000932] LCMS: (ESI): RT = 3.561 min, m/z calcd. for $C_{97}H_{135}FN_{24}O_{23}$, 1011.51 $[M+2H]^{2+}$, found 1012.0 $[M+2H]^{2+}$; Reverse phase LC-MS was carried out using method B.

[000933] Figure 39 depicts synthetic route of GLP-1R agonist Linker-payloads (LP25)

5.25 Preparation of (3S)-4-[[(1S)-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-1-[[4-[2-ethyl-4-[4-[2-[2-[2-[2-[[(65R,66S,68R)-102,103,104-triazatricyclododeca-69(103),102(104)-dien-68-yl]methoxycarbonylamino]ethoxy]ethoxy]ethoxy]ethoxycarbonylamino]butoxy]-59-phenyl]phenyl]methyl]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methylpropanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP25)

[000934] A mixture of **LP5** (1.12 mg, 0.577 µmol, 1 eq.) and $NaN_3$ (45.01 µg, 0.692 µmol, 1.2 eq.) in DMSO (0.1 mL) was stirred at 37°C for 16h. LCMS showed the reaction was complete. The crude product **LP25** (1.14 mg, 4.42e-1 µmol, 76.68% yield, 77% purity) was sent for the bio-assay directly without any further purification.

[000935] LCMS: (ESI): RT = 3.571 min, m/z calcd. for $C_{105}H_{134}FN_{27}O_{25}$, 992.49 $[M+2H]^{2+}$, found 993.0 $[M+2H]^{2+}$; Reverse phase LC-MS was carried out using method B.

[000936] Figure 40 depicts synthetic route of GLP-1R agonist Linker-payloads (LP26)

5.26 Preparation of (3S)-4-[[(1S)-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-1-[[4-[2-ethyl-4-[4-[[(2S)-3-hydroxy-2-[[2-[[2-[[2-[[2-[[4-oxo-4-(112,113,114,131-tetrazatetracyclononadeca-8(14),10(16),11(17),12(18),61(64),65,73(113),112(114)-octaen-131-yl)butanoyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]propanoyl]amino]butoxy]-63-phenyl]phenyl]methyl]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP26)

[000937] A mixture of **LP18** (1 mg, 4.65e-1 µmol, 1 eq.) and $NaN_3$ (36.31 µg, 0.559 µmol, 1.2 eq.) in DMSO (0.1 mL) was stirred at 37°C for 16h. LCMS showed the reaction was complete. The crude product **LP26** (1.02 mg, 0.465 µmol, 100.00% yield) was sent for the bio-assay directly without any further purification.

[000938] LCMS: (ESI): RT = 3.259 min, m/z calcd. for $C_{105}H_{134}FN_{27}O_{25}$, 1096.0, found 1096.7 $[M+2H]^{2+}$; Reverse phase LC-MS was carried out using method F.

[000939] Figure 41 depicts synthetic route of GLP-1R agonist Linker-payloads (LP27 and LP28)

5.27 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[4-(2-azatricyclo[10.4.0.04,9] hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl)-4-oxo-butanoyl]amino]ethoxy]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl] phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino] -3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl) propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP27)

[000940] To a solution of **LP11** (70 mg, 35.37 µmol, 1 eq) and **DIBAC-suc-OSu** (19.92 mg, 49.51 µmol, 1.4 eq) in DMF (0.5 mL) was added DIPEA (9.14 mg, 70.74 µmol, 12.32 µL, 2 eq). The mixture was stirred at 25 °C for 1 hr. LCMS showed the reaction was converted completely and the desired product was observed. The solution was filtered and purified by prep-HPLC (neutral condition. column: Phenomenex Gemini-NX 80*30 mm*3 µm; mobile phase: [water (10mM NH4HCO3)-ACN];B%: 25%-55%,9min). The desired fluent was lyophilized in freeze dryer to give **LP27** (24.87 mg, 10.56 µmol, 29.86% yield, 96.235% purity) as a white solid.

[000941] LCMS (ESI): RT = 2.316 min, m/z calcd. for $C_{113}H_{150}FN_{22}O_{27}$ 2266.09 $[M+H]^+$, 756.03 $[M+3H]^{3+}$, found 756.3 $[M+3H]^{3+}$, LC-MS Conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

[000942] HPLC: RT =9.17 min. HPLC conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min; Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm.

5.28 Preparation of (3S)-4-[[(1S)-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-1-[[4-[2-ethyl-4-[4-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[4-oxo-4-(3,4,5,13-tetrazatetracyclo[13.4.0.02,6.07,12]nonadeca-1(15),2(6),3,7(12),8,10,16,18-octaen-13-yl) butanoyl]amino]ethoxy] ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]phenyl]phenyl]methyl]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl] amino]acetyl] amino]butanoyl]amino]-2-methyl-

propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP28)

[000943] To a solution of **LP27** (3 mg, 1.32 µmol, 1 eq) in DMSO (0.5 mL) was added $NaN_3$ (258.14 ug, 3.97 µmol, 3 eq). The mixture was stirred at 25 °C for 1 hr. LC-MS showed **LP27** was consumed completely and one main peak with desired mass was detected. The reaction was added $NH_4Cl$ solution (5 mL). The aqueous layer was separated and extracted with EtOAc (5 mL*2). The organic layers were combined and washed with water/brine=1/1 (400 mL*2), dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuum to give product as brown oil. The residue was purified by prep-HPLC (TFA condition; column: Waters Xbridge BEH C18 100*25mm*5 µm; mobile phase: [water(0.075%TFA)-ACN];B%: 15%-55%,16min) to give **LP28** (1.02 mg, 4.23e-1 µmol, 31.99% yield, 95.869% purity) was obtained as a white solid.

[000944] LCMS (ESI): RT = 3.512 min, m/z calcd. for $C_{113}H_{151}FN_{25}O_{27}$ 2309.11 [M+H]+, $C_{113}H_{152}FN_{25}O_{27}$ 1155.56 $[M+2H]^{2+}$, found 1155.5 $[M+2H]^{2+}$. LC-MS Conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

[000945] HPLC: RT = 4.024 min. HPLC conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min; Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm.

[000946] Figure 42 depicts synthetic route of GLP-1R agonist Linker-payloads (LP29)

Step 1: Preparation of tert-butyl (3S)-4-[[(1S)-2-[[(1S)-1-[[[4-(2-amino-2-oxo-ethoxy)phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-oxo-ethyl]amino]-3-[[(2S)-3-tert-butoxy-2-[[(2S,3R)-3-tert-butoxy-2-[[(2S)-2-[[(2S,3R)-3-tert-butoxy-2-[[2-[[(2S)-2-[[2-(9H-fluoren-9-ylmethoxycarbonylamino)-2-methyl-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (LP29-2)

[000947] To a solution of **LP29-1A** (86.20 mg, 264.94 µmol, 1 eq) in DMF (10 mL) was added HATU (181.33 mg, 476.89 µmol, 1.8 eq) and DIPEA (136.96 mg, 1.06 mmol, 184.59 µL, 4 eq) at 25°C over 10 min. Then the solution was added into **LP29-1** (1 g, 264.94 µmol, 50% purity, 1 eq), and the mixture was bubbled with $N_2$ at 20 °C for 2 h. After completion, the mixture was filtered, and the collected resin was washed with DMF (30 mL*3), DCM (30 mL*3) to give the

crude product on solid phase, which was swelled in 20% piperidine/DMF (10 mL), and the mixture was bubbled with $N_2$ at 25°C for 2 hr. After completion, the mixture was filtered, and the collected resin was washed with DMF (100 mL*3), DCM (100 mL*3) to give the crude product bound on resin **LP29-2** (264.94 µmol, crude) as a white solid.

[000948] LCMS (ESI): RT = 3.923 min, m/z calcd. for $C_{102}H_{141}FN_{19}O_{20}$ 1448.70, found 1450.40 $[M-4*tBu-C_{17}H_{17}NO_4+7H]^+$. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step 2: Synthesis of tert-butyl (3S)-4-[[(1S)-2-[[(1S)-1-[[[4-(2-amino-2-oxo-ethoxy)phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-oxo-ethyl]amino]-3-[[(2S)-3-tert-butoxy-2-[[(2S,3R)-3-tert-butoxy-2-[[(2S)-2-[[(2S,3R)-3-tert-butoxy-2-[[2-[[(2S)-2-[[2-[[(2S)-2-(tert-butoxycarbonylamino)-3-(4-tert-butoxyphenyl)propanoyl]amino]-2-methyl-propanoyl] amino] -3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (LP29-3)

[000949] To a solution of **LP29-2A** (222.39 mg, 659.12 µmol, 5 eq) in DMF (5 mL) was added HATU (90.22 mg, 237.28 µmol, 1.8 eq) and DIPEA (68.15 mg, 527.30 µmol, 91.85 µL, 4 eq) at 25° over 10 min. Then the solution was added into **LP29-2** (131.82 µmol, 1 eq), and the mixture was bubbled with $N_2$ at 25 °C for 1 h. After completion, the mixture was filtered, and the collected resin was washed with DMF (50 mL*3), DCM (50 mL*3) to give the crude product bound on resin **LP29-3** (131.82 µmol, crude) as a yellow solid.

[000950] LCMS (ESI): RT = 3.990 min, m/z calcd. for $C_{78}H_{102}FN_{19}O_{18}$ 806.88, found 807.3 $[M-5tBu-Boc-C_{17}H_{17}NO_4]^{2+}$. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step 3: Synthesis of tert-butyl (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy] -2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-[[[4-(2-amino-2-oxo-ethoxy)phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino] -3-[[(2S)-3-tert-butoxy-2-[[(2S,3R)-3-tert-butoxy-2-[[(2S)-2-[[(2S,3R)-3-tert-butoxy-2-[[2-[[(2S)-2-[[2-[[(2S)-2-(tert-butoxycarbonylamino)-3-(4-tert-butoxyphenyl)propanoyl]amino]-2-methyl-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino] -3-(2-

fluorophenyl)-2-methyl-propanoyl]amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (LP29-4)

[000951] To a solution of **LP29-3** (22.23 µmol, 1 eq) and **LP29-3A** (17.78 mg, 43.64 µmol, 2 eq) in DMF (5 mL) was added SODIUM ASCORBATE (21.61 mg, 109.09 µmol, 5 eq), TBTA (11.58 mg, 21.82 µmol, 1 eq) and CuI (20.78 mg, 109.09 µmol, 5 eq). The mixture was stirred at 25 °C for 2 hr. After completion, the mixture was filtered, and the collected resin was washed with DMF (50 mL*3), DCM (50 mL*3) to give the crude product bound on resin **LP29-4** (22.23 µmol, crude) as a green solid.

[000952] LCMS (ESI): RT = 3.085 min, m/z calcd. for $C_{97}H_{139}FN_{20}O_{26}$ 1010.51, found 1011.0 $[M+2H]^{2+}$, LC-MS Conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

Step 4: Synthesis of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl] phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino] -3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-2-[[(2S,3R)-2-[[2-[[(2S)-2-[[2-[[(2S)-2-amino-3-(4-hydroxyphenyl) propanoyl]amino]-2-methyl-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl] amino]-3-hydroxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-hydroxy -butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP29)

[000953] The resin bound compound **LP29-4** (22.23 µmol, 1 eq) was subjected to acidic cleavage by using a TFA cocktail (TFA/TIPS/$H_2O$ = 95:2.5:2.5, 10 mL), the mixture was shaken at 25 °C for 2 hours. The mixture was filtered and the filtrate was diluted with t-BuOMe (100 mL) at 0 oC to give a precipitate, which was centrifuged (5000 R) for 10 min. The residue was purified by prep-HPLC (column: Boston Green ODS 150*30 mm*5 µm;mobile phase: [water(0.1%TFA)-ACN];B%: 17%-57%,9min) to give the product **LP29** (7.55 mg, 3.69 µmol, 16.58% yield, 98.63% purity) as a white solid.

[000954] LCMS (ESI): RT = 3.133 min, m/z calcd. for $C_{97}H_{139}FN_{20}O_{26}$ 1010.51, found 1011.0 $[M+2H]^{2+}$. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvents A).

[000955] HPLC: RT = 3.77 min. HPLC conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80%

(solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min; Column: YMC-Pack ODS-A 150*4.6 mm, 5 μm.

[000956] Figure 43 depicts synthetic route of GLP-1R agonist Linker-payloads (LP30)

5.30 Preparation of (8S,14S,17S,20S,23S,26S)-8-((2H-tetrazol-5-yl)methyl)-26-(((S)-3-(4'-(4-(4-(25-amino-2,5,8,11,14,17,20,23-octaoxapentacosyl)-1H-1,2,3-triazol-1-yl)butoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((S)-1-amino-5-(4-(23-hydroxy-3-oxo-6,9,12,15,18,21-hexaoxa-2-azatricosyl) phenyl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl)-23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21,24-octaoxo-3,7,10,13,16,19,22,25-octaazaoctacosan-28-oic acid (LP30)

[000957] To a solution of **P35** (15 mg, 7.51 μmol, 1 *eq.*) in $H_2O$ (0.09 mL) was added a solution of $CuSO_4.5H_2O$ (1.88 mg, 7.51 μmol, 1.0 *eq.*), sodium;(2R)-2-[(1S)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2H-furan-3-olate (1.49 mg, 7.51 μmol, 1.0 *eq.*) in DMSO (0.03 mL), and followed by TBTA (1.99 mg, 3.76 μmol, 0.5 *eq.*) in $H_2O$ (0.03 mL) and a solution of **LP30-1** (6.12 mg, 15.02 μmol, 2.0 *eq.*) in DMSO (0.03 mL). The mixture was stirred at 30 °C for 2.5 hr. LCMS showed the desired MS was detected. The reaction mixture was purified by prep-HPLC (column: Waters X bridge BEH C18 100*25mm*5 μm; mobile phase: [water (0.1%TFA)-ACN]; B%: 5%-42.5%, 12min) to afford **LP30** (2 mg, 8.15e-1 μmol, 10.85% yield, 98% purity) as a white solid.

[000958] LCMS (ESI): RT = 2.628 min, mass calcd. for $C_{112}H_{174}FN_{22}O_{35}$ 2406.23 $[M+H]^+$, 802.74 $[M+3H]^{3+}$, found 802.20 $[M+3H]^{3+}$. LC-MS Conditions: Mobile Phase:1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6.0 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate3 μm, C18,2.1*30 mm. Wave length: UV 220nm&254nm; Column temperature: 50°C.

[000959] HPLC: RT=6.32 min, 98% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 μm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

[000960] Figure 44 depicts synthetic route of GLP-1R agonist Linker-payloads (LP31)

5.31 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-[2-[2-(2-aminoeth-oxy) ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl) butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]

amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP31)

[000961] To a solution of **P8** (40 mg, 25.45 µmol, 1 eq) and **LP31-1** (29.71 mg, 50.90 µmol, 2 eq) in DMSO (0.1 mL) and $H_2O$ (0.4 mL) was added TBTA (6.75 mg, 12.72 µmol, 0.5 eq), $CuSO_4.5H_2O$ (6.35 mg, 25.45 µmol, 1 eq) and SODIUM ASCORBATE (5.04 mg, 25.45 µmol, 1 eq). The mixture was stirred at 37 °C for 4 hr. LC-MS showed the desired mass was detected. The green solution was filtered to give the crude product. The residue was purified by prep-HPLC (TFA condition. column: Welch Xtimate C18 100*40mm*3 µm; mobile phase: [water (0.075%TFA)-ACN]; B%: 23%-53%, 10min) to afford **LP31** (batch 1: 11.59 mg, 5.21 µmol, 20.48% yield, 96.94% purity) and (batch 2: 11.13 mg, 4.95 µmol, 19.45% yield, 95.86% purity) as yellow gum.

[000962] Batch 1: LCMS (ESI): RT = 3.160 min, m/z calcd. for $C_{102}H_{153}FN_{21}O_{29}$ 2155.10 $[M+H]^+$, $C_{102}H_{154}FN_{21}O_{29}$ 1078.05 $[M+2H]^{2+}$, found 1078.6 $[M+2H]^{2+}$.LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000963] HPLC: RT =7.48 min. HPLC Conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min; Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm.

[000964] Batch 2: LCMS (ESI): RT = 3.147 min, m/z calcd. for $C_{102}H_{153}FN_{21}O_{29}$ 2155.10 $[M+H]^+$, $C_{102}H_{154}FN_{21}O_{29}$ 1078.05 $[M+2H]^{2+}$, found 1078.6 $[M+2H]^{2+}$. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000965] HPLC: RT =7.48 min. HPLC Conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min; Column: YMC-Pack ODS-A 150*4.6 mm, 5 µm.

[000966] Figure 45 depicts synthetic route of GLP-1R agonist Linker-payloads (LP32)

5.32 Preparation of (2S)-2-[[(2S)-3-[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]-2-[[(2S)-3-carboxy-2-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-

yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]propanoyl]amino]propanoyl]amino]-5-(3,5-dimethylphenyl)pentanoic acid (LP32)

[000967] To a solution of LP31-1 (35 mg, 22.25 µmol, 1 eq) in $H_2O$ (1 mL) was added a solution of $CuSO_4.5H_2O$ (5.56 mg, 22.25 µmol, 1 eq) and NaVc (4.41 mg, 22.25 µmol, 1 eq), a solution of TBTA (5.90 mg, 11.13 µmol, 0.5 eq) and a solution of **P41** (18.14 mg, 44.51 µmol, 2 eq) in DMSO (0.25 mL). The mixture was stirred at 40 °C for 12 hr. The reaction mixture was diluted with $H_2O$ (3 mL) and ACN (2 mL), then the mixture was filtered. The filtrate was purified by prep-HPLC (column: Welch Xtimate C18 100*40mm*3 µm; mobile phase: [water (TFA)-ACN]; B%: 20%-60%,10 min) to give the LP31 (1 mg, 0.46 µmol, 10.11% yield, 91% purity).

[000968] LCMS (ESI): RT =2.910 min, m/z calcd. for C94H136FN20O26 991.05[M+H]+, found 990.9 [M+H]+.LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000969] HPLC: RT =7.56 min; Mobile Phase: 2.75 ML /4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 100% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A150*4.6 mm, 5 µm.

[000970] Figure 46 depicts synthetic route of GLP-1R agonist Linker-payloads (LP33)

Step 1: Synthesis of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[(4S)-5-tert-butoxy-4-(9H-fluoren-9-ylmethoxycarbonylamino)-5-oxo-pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP33-2)

[000971] To a solution of LP11 (10 mg, 5.05 µmol, 1 *eq.*) and **LP33-1** (2.9 mg, 5.5 µmol, 1 *eq.*) in DMF (2 mL) was added DIPEA (1.96 mg, 15.16 µmol, 2.64 µl, 3 *eq.*). The mixture was stirred at 20 °C for 12 hr. LC-MS showed **LP11** was consumed completely and one main peak with desired mass was detected. LCMS (ESI): RT = 4.030 min, m/z calcd. for $C_{118}H_{163}O_{30}N_{22}F$ 796.6$[M+3H]^{3+}$, found 796.5. Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L

TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1*30 mm, 3 µm; Wavelength: UV 220nm, 254nm; Column temperature: 50℃; MS ionization: ESI. The reaction was purified by prep-HPLC (column: O-phenomenex clarity RP 150*10mm*5 µm; mobile phase: [water (0.075%TFA)-ACN]; B%: 20%-70%, 20min) to give the product **LP33-2** (8 mg, 3.12 µmol, 61.70% yield, 93% purity).

[000972] LCMS (ESI): RT = 4.003 min, m/z calcd. for C118H163O30N22F 1194.17[M+2H]+/2, found 1194.3.Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1*30 mm, 3 µm; Wavelength: UV 220nm, 254nm; Column temperature: 50℃; MS ionization: ESI.

Step 2: (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[(4S)-4-amino-5-tert-butoxy-5-oxopentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP33-3)

[000973] To a solution of LP32-2 (8 mg, 3.35 µmol, 1 *eq.*) in THF (0.5 mL) was added N-ethylethanamine (2.45 mg, 33.52 µmol, 3.45 µl, 10 *eq.*). The mixture was stirred at 20 °C for 3 hr. LC-MS showed LP32-3 was consumed completely and one main peak with desired mass was detected. LCMS (ESI): RT = 3.070 min, m/z calcd. for $C_{118}H_{153}O_{28}N_{22}F$ 1082.5$[M+2H]^{2+}$, found 1082.9. Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1*30 mm, 3 µm; Wavelength: UV 220nm, 254nm; Column temperature: 50℃; MS ionization: ESI. The reaction mixture was concentrated under reduced pressure to give **LP33-3** (7 mg, crude) as a colourless oil.

Step 3: (2S)-2-amino-5-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[4-[4-[4-[(2S)-3-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-[[(2S)-3-carboxy-2-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-

propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]propanoyl]amino]-3-oxo-propyl]phenyl]-3-ethyl-phenoxy]butyl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-5-oxo-pentanoic acid (LP33)

[000974] To a solution of **LP33-3** (7 mg, 3.23 µmol, 1 *eq.*) in DCM (0.5 mL) was added TFA (770.00 mg, 6.75 mmol, 500.00 µl, 2088.06 *eq.*). The mixture was stirred at 20 °C for 1 hr. LC-MS showed LP32-4 was consumed completely and one main peak with desired mass was detected. LCMS (ESI): RT = 2.977 min, m/z calcd. for $C_{99}H_{145}O_{28}N_{22}F$ 1054.52$[M+2H]^{2+}$, found 1054.9. Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1*30 mm, 3 µm; Wavelength: UV 220nm, 254nm; Column temperature: 50℃; MS ionization: ESI. The reaction mixture was filtered to give a residue. The residue was purified by prep-HPLC (column: O-phenomenex clarity RP 150*10mm*5 µm; mobile phase: [water (0.075%TFA)-ACN]; B%: 15%-65%, 25min) give the product **LP33** (2.1 mg, 9.66e-1 µmol, 29.87% yield, 97% purity) as a white solid.

[000975] LCMS (ESI): RT = 2.950 min, m/z calcd. for $C_{99}H_{145}O_{28}N_{22}F$ 1054.52[M+2H]+/2, found 1054.9.Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1*30 mm, 3 µm; Wavelength: UV 220nm, 254nm; Column temperature: 50℃; MS ionization: ESI.

[000976] UPLC RT = 3.277 min Mobile Phase: 0.05% TFA in water (solvent A) and 0.05% TFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over 5minutes, later holding at 95% for 3 minutes at a flow rate of 0.6 ml/minutes; Column: Waters ACQUITY UPLC HSS T3 1.8um, 2.1x100mm.

[000977] Figure 47 depicts synthetic route of GLP-1R agonist Linker-payloads (LP34)

Step 1: Preparation of [(2-chlorophenyl)-diphenyl-methyl] 2-[[2-(9H-fluoren-9-ylmethoxycarbonylamino)acetyl]amino]acetate (LP34-2)

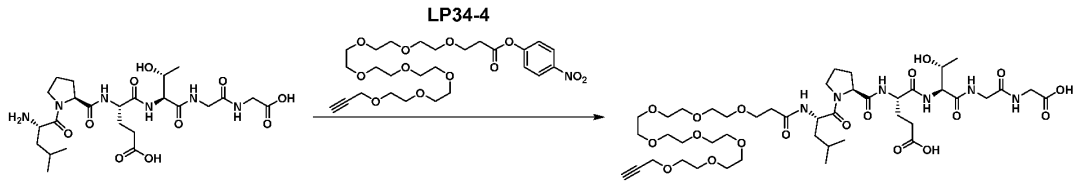
[000978] To a mixture **LP34-1** (20 g, 21.01 mmol, 32.8% purity, 1 eq.) in DMF (200 mL) was shaked for 30 min and a solution of 2-[[2-(9H-fluoren-9-ylmethoxycarbonylamino)acetyl]amino]acetic acid (37.23 g, 105.06 mmol, 5 eq.) and DIPEA (27.16 g, 210.11 mmol, 36.60 mL, 10 eq.) in DMF (200 mL) was added. The resulting mixture was shaked for 12 h at 20 °C. The resulting mixture was shaked for 12 h at 20 °C. The mixture

was added MeOH (100 mL) and shaked for another 2 h. The crude product WUXI-262-2 (26.68 g, crude) was used into the next step without further purification as a yellow solid.

Step 2: Preparation of (4S)-4-[[(2S)-1-[(2S)-2-amino-4-methyl-pentanoyl]pyrrolidine-2-carbonyl]amino]-5-[[(1S,2R)-1-[[2-(carboxymethylamino)-2-oxo-ethyl]carbamoyl]-2-hydroxy-propyl]amino]-5-oxo-pentanoic acid (LP34-3)

[000979] The solid-phase peptide synthesis was carried on Liberty Lite - Automated Microwave Peptide Synthesizer. The **LP34-2 Resin** (0.43 mmol, 1 *eq.*) was swollen with DMF (10 mL) for 300S on standard. Following the standard operation on peptide synthesizer: a) De-protection: a solution of 20% piperidine/DMF (5 mL) was added to the resin vessel, agitated with $N_2$ for 2 min at 90 °C. Then drained the vessel and washed with DMF (3 mL×3) at 20°C. b) Coupling (each amino acid reacted for triple with 5.0 eq.): a solution of amino acid (2.5 mmol, 5 *eq.*) in DMF (5 mL), DIC (2 mL) and oxyma (1 mL) were added to the vessel and agitated with $N_2$ for 10 min at 90°C. Repeat a) and b) for all amino acids. The resin was subjected to acidic cleavage by using TFA cocktail (TFA/TIPS/$H_2O$ = 95:2.5:2.5), then filtered and the filtrate was diluted with t-BuOMe to give a precipitate, which was centrifuged (5000 R) for 10 min to afford product **P34-3** (0.6 g, crude, TFA) as a white solid. $^1$H NMR (400MHz, DMSO-d6) 13.13 - 11.71 (m, 2H), 8.52 - 7.97 (m, 7H), 7.85 - 7.59 (m, 1H), 5.06 (br s, 1H), 4.48 - 4.28 (m, 2H), 4.26 - 4.16 (m, 1H), 4.12 (br s, 1H), 4.04 - 3.94 (m, 1H), 3.90 - 3.68 (m, 5H), 2.35 - 1.72 (m, 9H), 1.64 - 1.44 (m, 2H), 1.03 (d, J=6.3 Hz, 3H), 0.96 - 0.87 (m, 6H).

Step 3: Preparation of (4S)-5-[[(1S,2R)-1-[[2-(carboxymethylamino)-2-oxo-ethyl]carbamoyl]-2-hydroxy-propyl]amino]-4-[[(2S)-1-[(2S)-4-methyl-2-[3-[2-[2-[2-[2-[2-[2-(2-prop-2-ynoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]pentanoyl]pyrrolidine-2-carbonyl]amino]-5-oxo-pentanoic acid (LP34-5)




[000980] To a solution of **LP34-3** (45 mg, 65.54 µmol, 1 eq., TFA) and **LP34-4** (36.54 mg, 65.54 µmol, 1 eq.) in DMF (2 mL) was added DIPEA (16.94 mg, 131.07 µmol, 22.83 µl, 2 eq.). The solution was stirred at 20 °C for 1 h. LCMS showed the desired product was observed. (ESI): RT = 2.455 min, mass calcd. for $C_{47}H_{77}N_{19}O_{19}N_6$ 992.08 $[M+H]^+$, found 991.6 $[M+H]^+$. Reverse phase LCMS was carried out using a Chromolith Flash 1.5 ML/4L TFA in water (solvent A) and

0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate3 µm,C18,2.1*30 mm; The mixture was diluted with CH3CN (2 mL). The residue was purified by prep-HPLC (TFA condition; column: Welch Xtimate C18 100*40mm*3 µm; mobile phase: [water (0.075%TFA)-ACN]; B%: 15%-45%, 10 min). LP34-5 (50 mg, 49.54 µmol, 75.59% yield, 98.2% purity) was obtained as colorless oil confirmed by LCMS: ES17478-97-P1D2, HPLC: ES17478-97-p1C1 and HNMR: ES17478-97-P1B1.

[000981] (ESI): RT = 3.158 min, mass calcd. for $C_{47}H_{77}N_{19}O_{19}N_6$ 992.08 $[M+H]^+$, found 991.6 [M+H]+Reverse phase LCMS was carried out using a Chromolith Flash 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 0%-60% (solvent B)over 6 minutes and holding at 60% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate3 µm,C18,2.1*30 mm;

[000982] HPLC: RT = 3.69 min. HPLC conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 1%-100% (solvent B) over 10 minutes and holding at 100% for 5 minutes at a flow rate of 1.5 ML/min; Column: Ultimate XB- C18,3 µm,3.0*50mm.

[000983] $^1$H NMR (400MHz, DMSO-d6) 8.20 - 8.06 (m, 4H), 7.61 (d, J=8.0 Hz, 1H), 4.62 - 4.50 (m, 1H), 4.34 (br dd, J=4.4, 8.0 Hz, 1H), 4.31 - 4.24 (m, 1H), 4.19 (dd, J=4.0, 8.0 Hz, 1H), 4.14 (d, J=2.4 Hz, 2H), 4.04 - 3.96 (m, 1H), 3.82 - 3.73 (m, 4H), 3.60 - 3.47 (m, 34H), 2.41 - 2.25 (m, 4H), 1.99 - 1.82 (m, 4H), 1.80 - 1.69 (m, 1H), 1.67 - 1.56 (m, 1H), 1.50 - 1.35 (m, 2H), 1.32 - 1.22 (m, 1H), 1.03 (d, J=6.4 Hz, 3H), 0.88 (t, J=7.2 Hz, 6H).

Step 4: Preparation of (4S)-4-[[(2R)-1-[(2S)-2-[3-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[5-[4-[4-[(2S)-3-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-[[(2S)-3-carboxy-2-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]propanoyl]amino]-3-oxo-propyl]phenyl]-3-ethyl-phenoxy]pentyl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]-4-methyl-pentanoyl]pyrrolidine-2-carbonyl]amino]-5-[[(1S,2R)-1-[[2-(carboxymethylamino)-2-oxo-ethyl]carbamoyl]-2-hydroxy-propyl]amino]-5-oxo-pentanoic acid (LP34)

[000984] To a solution of **P8** (15 mg, 9.54 µmol, 1 eq.), **LP34-5** (19.00 mg, 19.17 µmol, 2.01 eq.) in DMSO (0.8 mL) and H2O (0.8 mL) were added $CuSO_4.5H_2O$ (2.38 mg, 9.54 µmol, 1 eq.), SODIUM ASCORBATE (1.89 mg, 9.54 µmol, 1 eq.) and 1-(1-benzyltriazol-4-yl)-N,N-bis[(1-

benzyltriazol-4-yl)methyl]methanamine (2.5 mg, 4.77 μmol, 0.5 eq.). The resulting mixture was stirred at 25 °C for 3 h. LCMS showed the desired product was observed. (ESI): RT = 3.677 min, mass calcd. for $C_{120}H_{175}N_{26}O_{36}FH$ 2562.25[M+H]$^+$, 1282.6 [M+2H]$^{2+}$, found 1282.2 [M+2H]$^{2+}$. Reverse phase LCMS was carried out using a Chromolith Flash 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B)over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate3 μm,C18,2.1*30 mm; The mixture was diluted with MeOH (2 mL), filtered and the filtrate was sent to Prep-HPLC. The residue was purified by prep-HPLC (TFA condition). Column: Welch Xtimate C18 100*40mm*3 μm; mobile phase: [water (0.075%TFA)-ACN]; B%: 28%-58%, 8min. LP34 (8.9 mg, 3.33 μmol, 34.85% yield, 96.3% purity) was obtained as a white solid.

[000985] LCMS RT = 3.682 min, mass calcd. for $C_{120}H_{175}N_{26}O_{36}FH$ 2562.25[M+H]$^+$, 1282.6 [M+2H]$^{2+}$, found 1282.2 [M+2H]$^{2+}$. Reverse phase LCMS was carried out using a Chromolith Flash 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B)over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate3 μm,C18,2.1*30 mm.

[000986] HPLC: RT = 4.34 min. HPLC conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 1%-100% (solvent B) over 10 minutes and holding at 100% for 5 minutes at a flow rate of 1.5 ML/min; Column: Ultimate XB- C18,3 μm,3.0*50mm.

[000987] Figure 48 depicts synthetic route of GLP-1R agonist Linker-payloads (LP35)

Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-[[(4S)-4-[[(2S)-2-[[(2S)-2-(benzyloxycarbonylamino)-4-methyl-pentanoyl]amino]-4-methyl-pentanoyl]amino]-5-[[2-[[(1S)-2-(carboxymethylamino)-1-(hydroxymethyl)-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl] triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl) butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP35)

[000988] A solution of **LP11** (6.0 mg, 2.72 μmol, 1.0 eq., 2TFA) and PBS buffer (K-free, 100 mM, pH=7.20) (7.2 mL) was measured pH as 7.2. **LP35-1** (9.62 mg, 13.59 μmol, 5.0 eq.) and MTGase (Ajinomoto-TI, 51.6 mg) were added. The resulting mixture was stirred at 37 °C for 16 h. The reaction progress was monitored by LCMS. Upon completion, the reaction mixture was

quenched with aqueous AcOH solution (1% v/v, 7.2 mL). The obtained solid was rinsed with DMF/$H_2O$ (3 mL, 1/1), and then the filtrate was purified by prep-HPLC (column: O-Xbridge C18 150*10mm*5 μm; mobile phase: [water (0.075%TFA)-ACN]; B%: 10%-65%, 20min) to afford LP35 (2.62 mg, 0.926 μmol, 34.1% yield, 98.3% purity, TFA salt) as a white solid.

[000989] LCMS: (ESI): RT = 3.62 min, m/z calcd. for $C_{126}H_{184}FN_{27}O_{36}$ 1335.17 $[M+2H]^{2+}$, found 1335.6; 100% purity at 220 nm. LCMS conditions: Xtimate C18 2.1*30 mm, 3 μm; 1.5 mL/4 L TFA in water (solvent A) and 0.75 mL/4 L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 mL/min.

[000990] UPLC: RT = 7.15 min, 98.38% purity at 220 nm. UPLC method: Waters ACQUITY UPLC BEH C18 1.7um,2.1*100mm; 0.05% TFA in 1L water (solvent A) and 0.05% TFA in 1L acetonitrile (solvent B), using the elution gradient 3%-100% (solvent B) over 11 minutes and holding at 100% for 4 minutes at a flow rate of 0.4 mL/minute.

[000991] Figure 49 depicts synthetic route of GLP-1R agonist Linker-payloads (LP36, LP37, LP38, LP39, LP40, LP41)

5.36 Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[[[(2S)-2-[[2-[[2-[[2-[(2-aminoacetyl)amino] acetyl]amino]acetyl]amino]acetyl]amino]-3-hydroxy-propanoyl]amino]methyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(1H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP36)

[000992] The solid-phase peptide synthesis was carried out on the Liberty Lite - Automated Microwave Peptide Synthesizer. The **LP36-1** (0.43 mmol, 1 *eq.*) was swollen with DMF (10 mL) for 300S on standard. Following the standard operation on peptide synthesizer: a) De-protection: a solution of 20% piperidine/DMF (5 mL) was added to the resin vessel, agitated with $N_2$ for 2 min at 90 °C. Then drained the vessel and washed with DMF (3 mL×3) at 20°C. b) Coupling (each amino acid reacted for triple with 5.0 eq.): a solution of amino acid (2.5 mmol, 5 *eq.*) in DMF (5 mL), DIC (2 mL) and oxyma (1 mL) were added to the vessel and agitated with $N_2$ for 10 min at 90°C. Repeat a) and b) for all amino acids. The resin was subjected to acidic cleavage by using TFA cocktail (TFA/TIPS/$H_2O$ = 95:2.5:2.5), then filtered and the filtrate was diluted with t-BuOMe to give a precipitate, which was centrifuged (5000 R) for 10 min to give the crude product.

[000993] **LP36** was prepared as described in the general procedure of SPPS. The crude product was purified by prep-HPLC (column: Welch Xtimate C18 100*40mm*3 μm; mobile phase: [water (0.075%TFA)-ACN];B%: 0%-40%,15min) to afford pure product **LP36** (13.9 mg, 6.25 μmol, 1.44% yield, 97.58% purity, 2TFA) as a white solid.

[000994] LCMS: (ESI): Rt = 2.715 min, m/z calcd. For $C_{89}H_{123}FN_{26}O_{23}$, 971.46, $[M+2H]^{2+}$ ; found 971.9 $[M+2H]^{2+}$ ; Reverse phase LCMS was carried out using Chromolith Flash RPC1825-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solventB)and water containing 0.04% TFA (solvent A).

[000995] HPLC: RT = 6.62 min. HPLC Conditions: Mobile phase:1.0% ACN in water (0.1%TFA) to 5% ACN in water (0.1%TFA) in 1 minutes; then from 5% ACN in water(0.1%TFA) to 100% ACN (0.1%TFA) in 5 minutes; hold at 100% ACN (0.1%TFA) for 2 minutes;back to 1.0% ACN in water (0.1%TFA) at 8.01minutes,and hold two minutes.Flow rate:1.2ml/minColumn: Ultimate XB-C18,3 μm,3.0*50mm

[000996] HRMS (ESI): m/z calcd for $C_{89}H_{121}FN_{26}O_{23}$ 1941.91 $[M+H]^{+}$, 971.46 $[M+2H]^{2+}$, found 1942.9299 $[M+H]^{+}$, 971.9736 $[M+2H]^{2+}$.

[000997] UPLC: RT = 5.824 min. conditions: Mobile Phase: 0.05% TFA in 1L water (solvent A) and 0.05% TFA in 1L acetonitrile (solvent B), using the elution gradient 30%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 0.4 mL/minute; Column: Waters ACQUITY UPLC HSS T3 1.8um,2.1*100mm;

[000998] 5.37 LP37 was obtained as the same with LP36. The crude product was purified by prep-HPLC (column: Welch Xtimate C18 100*40mm*3 μm; mobile phase: [water (0.075%TFA)-ACN];B%: 0%-40%,15min) to afford pure product **LP37** (5 mg, 2.50 μmol, 1.25% yield, 97% purity) as a white solid.

[000999] LCMS: (ESI): Rt = 2.800 min, mass calcd. for $C_{89}H_{121}FN_{26}O_{23}$ ; found 971.45 $[M/2+H]^{+}$ found 971.0; Reverse phase LCMS was carried out using Chromolith Flash RPC1825-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B)and water containing 0.04% TFA (solvent A).

[0001000] HPLC: RT= 6.61 min. Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min; Column: WELCH Ultimate LP-C18 150*4.6 mm 5 μm; Wavelength: UV 220nm&215nm&254nm; Column temperature: 40℃

[0001001] HRMS-TOF: $C_{89}H_{122}N_{26}O_{23}F$ [M+H] = 1942.9573. The mobile phase: 0.1% FA in water (solvent A) and 0.05% FA in ACN (solvent B);Elution Gradient: 5%-95% (solvent B) over

1.3 minutes and holding at 95% for 0.7 minutes at a flow rate of 1.2 ml/minute; Column: Agilent Poroshell HPH-C182.7um, 2.1*50mm; Ion Source: AJS ESI source; Ion Mode: Positive; Nebulization Gas: Nitrogen; Drying Gas (N2) Flow: 8L/min; Nebulizer Pressure: 35psi;Gas Temperature: 325oC;Sheath gas Temperature: 350oC;Sheath gas flow: 11L/min; Capillary Voltage: 3.5KV; Fragmentor Voltage: 300 V.

[0001002] UPLC: RT=4.595 min, mass calcd. Mobile Phase: 0.05% TFA in 1L water (solvent A) and 0.05% TFA in 1L acetonitrile (solvent B), using the elution gradient 0%-95% (solvent B) over 6 minutes and holding at 95% for 4 minutes at a flow rate of 0.6 mL/minute; Column: Waters ACQUITY UPLC HSS T3 2.1*50mm,1.8μm; Column temp:40℃

[0001003] 5.38 LP38 was obtained as the same with LP36. The crude product was purified by prep-HPLC (column: Welch X timate C18 100*40mm*3 μm; mobile phase: [water (0.075%TFA)-ACN]; B%: 15%-45%, 15min) to afford pure product LP38 (52.17 mg, 8.87 μmol, 2.53% yield, 95% purity) as a white solid.

[0001004] LCMS: (ESI): Rt = 2.723 min, mass calcd. for $C_{96}H_{134}FN_{29}O_{27}$ $[M+2H]^{2+}$ 1071.99, $C_{96}H_{135}FN_{29}O_{27}$ $[M+3H]^{3+}$ 714.99; found 1072.40 $[M+2H]^{2+}$ found 715.30 $[M+3H]^{3+}$; Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[0001005] HPLC RT=9.00 min, 95% purity. HPLC method A**:** Column: YMC-Pack ODS-A 150*4.6 mm, 5 μm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min;

[0001006] 5.39 LP39 was obtained as the same with LP36. The crude product was purified by prep-HPLC (column: Welch Xtimate C18 100*40mm*3 μm; mobile phase: [column: Waters X bridge BEH C18 100*25mm*5 μm; mobile phase: [water (0.05%NH3H2O)-ACN]; B%: 5%-32%, 11min) o afford pure product **LP39** (20 mg, 8.75 μmol, 2.50% yield, 95% purity) as a white solid.

[0001007] LCMS: (ESI): Rt = 2.711 min, mass calcd. for $C_{97}H_{135}FN_{30}O_{27}$ $[M+2H]^{2+}$ 1085.49, $C_{97}H_{136}FN_{30}O_{27}$ $[M+3H]^{3+}$ 724.30; found 1085.90 $[M+2H]^{2+}$ found725.30[M+3H]3+; Reverse phase LCMS was carried out using Chromolith Flash RP-C1825-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[0001008] HPLC RT=8.99 min, 95% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 μm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent

B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min;

[0001009] 5.40 LP40 was obtained as the same with LP36. The crude product was purified by prep-HPLC (column: Welch X timate C18 100*40mm*3 μm; mobile phase: [water (0.075%TFA)-ACN]; B%: 6%-46%, 20min) to afford pure product **LP40** (3.5 mg, 1.38 μmol, 3.94e-1% yield, 97.99% purity) as a white solid.

[0001010] LCMS: (ESI): Rt = 2.740 min, mass calcd. for $C_{108}H_{152}FN_{35}O_{33}$ $[M+2H]^{2+}$ 1243.05, $C_{108}H_{153}FN_{35}O_{33}$ $[M+3H]^{3+}$ 829.03; found 829.4$[M+2H]^{2+}$ found 1243.30$[M+3H]^{3+}$; Reverse phase LCMS was carried out using X Bridge C18 3.5 μm 2.1*30 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile (solvent B) and NH3·H2O in water (solvent A).

[0001011] HPLC RT=6.50 min, 97.99% purity; HPLC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 μm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min;

[0001012] 5.41 LP41 was obtained as the same with LP36. The crude product was purified by prep-HPLC (column: Welch Xtimate C18 100*40mm*3 μm; mobile phase: [water (0.075%TFA)-ACN]; B%: 6%-46%, 20 min) to afford pure product **LP41** (4.5 mg, 1.89 μmol, 0.54% yield, 98.52% purity) as a white solid.

[0001013] LCMS: (ESI): Rt = 2.590 min, mass calcd. for $C_{103}H_{145}FN_{32}O_{31}$ $[M+2H]^{2+}$ 1173.21, $C_{103}H_{146}FN_{32}O_{31}$ $[M+3H]^{3+}$ 782.47; found 1172.90 $[M+2H]^{2+}$ found 782.30 $[M+3H]^{3+}$; Reverse phase LCMS was carried out using X Bridge C18 3.5 μm 2.1*30 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile (solvent B) and NH3·H2O in water (solvent A).

[0001014] HPLC RT=6.54 min, 98.52% purity; HPLC method A: Column: YMC-Pack ODS-A 150*4.6 mm, 5 μm; 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

[0001015] Figure 50 depicts synthetic route of GLP-1R agonist Linker-payloads (LP42)

Step 1: Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[[[(2S)-2-[[2-[[2-[[2-[[2-[2-[2-[[2-[2-[2-[[(4S)-5-tert-butoxy-4-[(18-tert-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]acetyl]amino]-3-hydroxy-propanoyl]amino]methyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-

3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(1H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP42-2)

[0001016] To a solution of **LP42-1** (45.75 mg, 54.07 µmol, 1.5 eq.) and HATU (16.45 mg, 43.25 µmol, 1.2 eq.) in DMF (2 mL) was added DIPEA (13.98 mg, 108.13 µmol, 18.83 µL, 3 eq). The mixture was stirred at 20°C for 0.1 hr. **LP36** (70 mg, 36.04 µmol, 1 eq.) was added and the mixture was stirred at 20°C for 1 hr. LCMS showed the reaction converted completely. The reaction mixture was added into MTBE (40 mL) dropwise. The precipitated yellow solid was collected by centrifuged. **LP42-2** (110 mg, 25.49 µmol, 70.71% yield, 64.18% purity) was obtained as a light-yellow solid.

[0001017] LCMS (ESI): RT = 4.670 min, m/z calcd. For C132H199FN29O35 1385.23 [M+2H]2+; m/z found 1385.8; LCMS Conditions: Mobile Phase:1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. Column: Xtimate C18 2.1*30 mm, 3 µm.

Step 2: Preparation of 18-[[(1S)-4-[2-[2-[2-[2-[2-[2-[[2-[[2-[[2-[[(1S)-2-[[1-[4-[4-[4-[(2S)-3-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-[[(2S)-3-carboxy-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(1H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]propanoyl]amino]-3-oxo-propyl]phenyl]-3-ethyl-phenoxy]butyl]triazol-4-yl]methylamino]-1-(hydroxymethyl)-2-oxo-ethyl] amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethoxy] ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylamino]-1-carboxy-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (LP42)

[0001018] To a solution of **LP42-2** (110 mg, 25.49 µmol, 64.18% purity, 1 eq.) in DCM (1 mL) was added TFA (1.54 g, 13.51 mmol, 1 mL, 529.95 eq.). The mixture was stirred at 20°C for 1 hr. LCMS showed the reaction converted completely. The reaction mixture was purified by prep-HPLC (column: Welch Xtimate C18 100*40mm*3µm; mobile phase: [water (0.075%TFA)-ACN]; B%: 10%-50%, 40min) to give the dedired compound **LP42** (24 mg, 8.68 µmol, 17.03% yield, 96.11% purity) as a white solid.

[0001019] LCMS: (ESI): Rt = 3.943 min, m/z calcd. For $C_{124}H_{184}FN_{29}O_{35}$ 1329.17 $[M+2H]^{2+}$; m/z found 1329.6; Reverse phase LCMS was carried out using Chromolith Flash RPC1825-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[0001020] HPLC: RT = 8.52 min. HPLC conditions: Mobile phase: 1.0% ACN in water (0.1%TFA) to 5% ACN in water (0.1%TFA) in 1 minutes; then from 5% ACN in water (0.1%TFA) to 100% ACN (0.1%TFA) in 5 minutes; hold at 100% ACN (0.1%TFA) for 2 minutes; back to 1.0% ACN in water (0.1%TFA) at 8.01minutes, and hold two minutes. Flow rate: 1.2ml/min. Column: Ultimate XB-C18, 3 µm, 3.0*50mm

[0001021] HRMS (ESI): m/z calcd for $C_{124}H_{183}FN_{29}O_{35}$ 2657.33 $[M+H]^{+}$, 1329.665 $[M+2H]^{2+}$, found 2657.33 $[M+H]^{+}$, 1329.6703 $[M+2H]^{2+}$.

[0001022] UPLC: RT = 5.411 min. conditions: Mobile Phase: 0.05% TFA in 1L water (solvent A) and 0.05% TFA in 1L acetonitrile (solvent B), using the elution gradient 0%-95% (solvent B) over 6 minutes and holding at 95% for 4 minutes at a flow rate of 0.4 mL/minute; Column: Waters ACQUITY UPLC HSS T3 1.8 um, 2.1*100mm.

[0001023] Figure 51 depicts synthetic route of GLP-1R agonist Linker-payloads (LP43)

Step 1: Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[[[2-[[2-[[2-[[(2S)-2-[[2-[[2-[[2-[[2-[2-[2-[2-[[2-[2-[[(4S)-5-tert-butoxy-4-[(18-tert-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino]ethoxy] ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]amino]acetyl]amino]acetyl] amino]acetyl]amino]acetyl]amino]-3-hydroxy-propanoyl]amino]acetyl]amino]acetyl]amino]acetyl] amino]acetyl]amino]methyl] triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl] amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(1H-tetrazol-5-yl) propanoyl]amino]acetyl]amino]butanoyl] amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl] amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP43-1)

[0001024] To a solution of **LP42-1** (41 mg, 18.89 µmol, 1 eq.) and HATU (8.62 mg, 22.67 µmol, 1.2 eq.) in DMF (0.3 mL) was added DIPEA (7.32 mg, 56.67 µmol, 9.87 µL, 3 eq). The mixture was stirred at 20°C for 0.1 hr. **LP39** (23.98 mg, 28.34 µmol, 1.5 eq.) was added and the mixture was stirred at 20°C for 1 hr. LCMS showed the reaction converted completely. The reaction mixture was added into MTBE (40 mL) dropwise. The precipitated yellow solid was collected by centrifuged. **LP43-1** (80 mg, 12.56 µmol, 66.49% yield, 47.08% purity) as a light yellow solid.

[0001025] LCMS (ESI): RT = 4.603 min, m/z calcd. For $C_{140}H_{213}FN_{33}O_{39}$ 999.85 $[M+3H]^{3+}$; m/z found 1000.3; LCMS Conditions: Mobile Phase: 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. Column: Xtimate C18 2.1*30 mm, 3 µm.

Step 3: Preparation of 18-[[(1S)-4-[2-[2-[2-[2-[2-[2-[[2-[[2-[[2-[[2-[[(1S)-2-[[2-[[2-[[2-[[2-[[1-[4-[4-[4-[(2S)-3-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-[[(2S)-3-carboxy-2-[[(2S)-2-[[(2S,3R)-2-[[(2S)-3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(1H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl] amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino] propanoyl]amino]-3-oxo-propyl]phenyl]-3-ethyl-phenoxy]butyl]triazol-4-yl]methylamino]-2-oxo-ethyl] amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-1-(hydroxymethyl)-2-oxo-ethyl] amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethyl]amino]-2-oxo-ethoxy] ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylamino]-1-carboxy-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (LP43)

[0001026] To a solution of **LP43-1** (80 mg, 12.56 µmol, 47.08% purity, 1 eq.) in DCM (0.7 mL) was added TFA (2.31 g, 20.26 mmol, 1.5 mL, 1612.83 eq). The mixture was stirred at 20°C for 3 hr. LCMS showed the reaction converted completely. The residue was purified by prep-HPLC (column: O-phenomenex clarity RP 150*10mm*5µm; mobile phase: [water (0.075% TFA)-ACN]; B%: 30%-52%, 20min) to provide the desired compound **LP43** (1.8 mg, 0.571 µmol, 4.55% yield, 91.60% purity) as a white solid.

[0001027] LCMS: (ESI): RT = 3.883 min, m/z calcd. For $C_{132}H_{196}FN_{33}O_{39}$ 1443.21 $[M+2H]^{2+}$; m/z found 1443.8; Reverse phase LCMS was carried out using Chromolith Flash RPC1825-3 mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solventB) and water containing 0.04% TFA (solvent A).

[0001028] HPLC: RT = 8.30 min. HPLC conditions: Mobile phase: 1.0% ACN in water (0.1%TFA) to 5% ACN in water (0.1%TFA) in 1 minutes; then from 5% ACN in water (0.1%TFA) to 100% ACN (0.1%TFA) in 5 minutes; hold at 100% ACN (0.1%TFA) for 2 minutes; back to 1.0% ACN in water (0.1%TFA) at 8.01minutes, and hold two minutes. Flow rate: 1.2ml/min Column: Ultimate XB-C18, 3 µm, 3.0*50mm.

[0001029] HRMS (ESI): m/z calcd for $C_{132}H_{195}FN_{33}O_{39}$ 2885.42 $[M+H]^+$ , 1442.21 $[M+2H]^{2+}$, found 1442.21 $[M+H]^+$, 1443.7161 $[M+2H]^{2+}$.

[0001030] UPLC: RT = 5.316 min. conditions: Mobile Phase: 0.05% TFA in 1L water (solvent A) and 0.05% TFA in 1L acetonitrile (solvent B), using the elution gradient 0%-95% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 0.4 mL/minute; Column: Waters ACQUITY UPLC HSS T3 1.8um, 2.1*100mm.

[0001031] Figure 52 depicts synthetic route of GLP-1R agonist Linker-payloads (LP44)

Step 1: Preparation of (8S,14S,17S,20S,23S,26S)-8-((1H-tetrazol-5-yl)methyl)-26-(((S)-1-(((S)-1-amino-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-3-(4'-(4-(4-((S)-60-(tert-butoxycarbonyl)-81,81-dimethyl-39,48,57,62,79-pentaoxo-2,5,8,11,14,17,20,23,26,29,32,35,41,44,50,53,80-heptadecaoxa-38,47,56,61-tetraazadooctacontyl)-1H-1,2,3-triazol-1-yl)butoxy) -2'-ethyl-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-17-(2-fluorobenzyl)-14,20-bis((R)-1-hydroxyethyl)-23-(hydroxymethyl)-1-(1H-imidazol-5-yl)-5,5,17-trimethyl-4,6,9,12,15,18,21, 24-octaoxo-3,7,10,13,16,19,22,25-octaazaoctacosan-28-oic acid (LP44-2)

[0001032] To a solution of **LP30** (40 mg, 18.56 μmol, 1 *eq.*) in DMF (1 mL) were added DIPEA (7.2 mg, 55.68 μmol, 10 μL, 3 *eq.*) and **LP44-1** (22 mg, 22.27 μmol, 1.2 *eq.*). The resulting mixture was stirred at 20 °C for 0.5 h. LCMS showed the reaction was complete and the desired product was observed. The mixture was poured into ice-cooled MTBE, then filtered and the filter-cake was dried in vacuum. **LP44-2** (55 mg, crude) was obtained as a colorless oil.

[0001033] LCMS (ESI): RT = 6.396min, mass calcd. for $C_{145}H_{230}FN_{24}O_{41}$ 2982.66 $[M+H]^+$, 746.44 $[M+4H]^{4+}$, found 747.2 $[M+4H]^{4+}$. Reverse phase LCMS was carried out using a Chromolith Flash 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate3 μm, C18,2.1*30 mm.

Step 2: Preparation of 18-[[(1S)-4-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[4-[4-[4-[(2S)-3-[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-[[(2S)-3-carboxy-2-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl) ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(1H-tetrazol-5-yl)propanoyl]amino] acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]propanoyl]amino]-3-oxo-propyl]phenyl]-3-ethyl-phenoxy]butyl] triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy] ethylamino]-1-carboxy-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (LP44-2)

[0001034] A solution of **LP44-2** (55 mg, 18.26 μmol, 1 eq.) in TFA (0.5 mL) and DCM (0.5 mL) was stirred at 20 °C for 1 h. LCMS showed the reaction completed and the desired product

was observed. (ESI): RT = 4.219 min, mass calcd. for $C_{137}H_{214}FN_{24}O_{41}$ 2870.53$[M+H]^+$, 1436.27$[M+2H]^{2+}$, found 1436.2 $[M+2H]^{2+}$. Reverse phase LCMS was carried out using a Chromolith Flash 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate 3 µm, C18, 2.1*30 mm. The reaction solution was concentrated in vacuum. The residue was purified by prep-HPLC (TFA condition; column: O-phenomenex clarity RP 150*10mm*5µm; mobile phase: [water (0.075%TFA)-ACN]; B%: 40%). The desired compound **LP44** (12 mg, 4.05 µmol, 22.19% yield, 96.97% purity) was obtained as a white solid.

[0001035] LCMS (ESI): RT = 4.243 min, mass calcd. for $C_{137}H_{214}FN_{24}O_{41}$ 2870.53 $[M+H]^+$, 1436.27 $[M+2H]^{2+}$, found 1436.4 $[M+2H]^{2+}$. Reverse phase LCMS was carried out using a Chromolith Flash 1.5 ML/4L TFA in water (solvent A) and 0.75 ML/4L TFA in acetonitrile (solvent B), using the gradient 10-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min; Column: Xtimate3 µm, C18, 2.1*30 mm.

[0001036] HPLC RT = 9.13 min. HPLC conditions: Mobile Phase: 2.75 ML/4L TFA in water (solvent A) and 2.5 ML/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ML/min; Column: Ultimate XB- C18,3 µm,3.0*50mm.

[0001037] Figure 53 depicts synthetic route of GLP-1R agonist Linker-payloads (LP45)

Step 1: Preparation of (3S)-4-[[(1S)-1-[[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-[2-[[2-[2-[2-[[2-[2-[2-[[(4S)-5-tert-butoxy-4-[(18-tert-butoxy-18-oxo-octadecanoyl)amino]-5-oxo-pentanoyl]amino] ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-[[(1S)-1-carbamoyl-4-[4-[[3-[2-[2-[2-[2-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy] ethoxy]propanoylamino]methyl]phenyl]butyl]amino]-2-oxo-ethyl]amino]-3-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl) propanoyl]amino]acetyl]amino]butanoyl] amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (LP45-2)

[0001038] To a solution of **LP29** (210 mg, 87.33 µmol, 1 eq.) in DMF (0.5 mL) were added DIPEA (22.57 mg, 174.66 µmol, 30.42 µL, 2 eq) and **LP45-1** (168.93 mg, 174.66 µmol, 2 eq). The mixture was stirred at 25°C for 1 hr. LCMS showed **LP29** was consumed completely and one main peak with the desired mass was detected. The reaction mixture was partitioned in $H_2O$ (20

mL) and extracted with EtOAc (10 mL×3). The organic phase was separated, washed with brine (10 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give a residue. The desired compound **LP45-2** (200 mg, crude) was obtained as a yellow oil.

[0001039] LCMS (ESI): RT = 5.154 min, mass calcd. for $C_{155}H_{248}FN_{25}O_{47}$ 3231.78 $[M+H]^+$, $C_{155}H_{251}FN_{25}O_{47}$ 1077.9 $[M+3H]^{3+}$, found 1078.45 $[M+3H]^{3+}$. LCMS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 5% to 95%acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step 2: Preparation of 18-[[(1S)-4-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[2-[[1-[4-[4-[4-[(2S)-3-[[(1S)-1-carbamoyl-4-[4-[[3-[2-[2-[2-[2-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]propanoylamino]methyl]phenyl]butyl]amino]-2-[[(2S)-3-carboxy-2-[[(2S)-2-[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[(2S,3R)-3-hydroxy-2-[[2-[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(2H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]propanoyl]amino]-3-oxo-propyl]phenyl]-3-ethyl -phenoxy]butyl]triazol-4-yl]methoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylamino]-1-carboxy-4-oxo-butyl]amino]-18-oxo-octadecanoic acid (LP45)

[0001040] To a solution of **LP45-2** (200 mg, 61.87 μmol, 1 eq) in 1,1,1,3,3,3-HEXAFLUORO-2-PROPANOL (2 mL). The mixture was stirred at 90 °C for 2 hr under microwave. LCMS showed **LP45-2** was consumed completely and one main peak with desired mass was detected. LCMS (ESI): RT = 3.575 min, mass calcd. for $C_{147}H_{233}FN_{25}O_{47}$ 3119.65 $[M+H]^+$, $C_{147}H_{235}FN_{25}O_{47}$ 780.7 $[M+4H]^{4+}$, found 781.0 $[M+4H]^{4+}$. LCMS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80%acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A). The reaction mixture was concentrated under vacuum to give crude. The residue was purified by prep-HPLC (column: YMC-Actus Triart C18 150*30 mm*5μm; mobile phase: [water (0.1%TFA)-ACN]; B%: 36%-56%, 10.5 min) to obtain **LP45** (18.03 mg, 5.75 μmol, 9.29% yield, 99.51% purity) as a white solid.

[0001041] LCMS (ESI): RT = 3.550 min, m/z calcd. for $C_{147}H_{233}FN_{25}O_{47}$ 3119.65 $[M+H]^+$, $C_{147}H_{235}FN_{25}O_{47}$ 780.7 $[M+4H]^{4+}$, found 781.0 $[M+4H]^{4+}$. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80%acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

**Example 6. Preparative HPLC purification of the crude peptidomimtics**

[0001042] The preparative HPLC was carried out on a Shimadzu LC-8a Liguid chromatograph. A solution of crude peptide dissolved in DMF or water was injected into a column and eluted with a linear gradient of ACN in water. Different methods were used. (See General Information). The desired product eluted were in fractions and the pure peptidomimetics were obtained as amorphous with powders by lyophilization of respective HPLC fractions. In general, after the prep-HPLC purification, the overall recovery was found to be in the range of 40~50% yield.

[0001043] **Preparative HPLC method A:** using FA condition (column: Xtimate C18 150*25mm*5 µm; mobile phase: [water (0.225%FA)-ACN]; B%: 40%-70%, 7min) to afford a pure product.

[0001044] **Preparative HPLC method B:** using TFA condition (column: YMC-Exphere C18 10 µm 300*50mm 12nm; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-45%, 55 min) to afford a pure product.

[0001045] **Preparative HPLC method C:** using neutral condition (column: Phenomenex Gemini-NX 150*30 mm*5 µm; mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 21%-51%, 11min) to afford a pure product.

[0001046] **Preparative HPLC method D:** using neutral condition (column: Waters Xbridge 150*255u; mobile phase: [water (10mM $NH_4HCO_3$)-ACN]; B%: 20%-50%, 7min) to afford a pure product.

[0001047] **Preparative HPLC method E:** using FA condition (column: Phenomenex Luna C18 250*50 mm*10 um; mobile phase: [water (0.225%FA)-ACN]; B%: 55%-86%, 21 min) to afford a pure product.

**Example 7. Preparation of Antibody-Drug Conjugates**

7.1 General Site-Specific Conjugation

[0001048] This example demonstrates two methods for site–specific ATDC conjugation, generally, of a payload to an antibody comprising a Q-tag thereof. ATDCs 1-30 were prepared with LP1-LP11 and anti-GLP1R antibodies mAb1-mAb13 or control antibodies mAb1- mAb6 as summarized in Table 3 below. The ES-MS results and DAR values of the ADCs according to the disclosure are summarized in Table 3.

**Table 3. Site-specific GLP1 Antibody-Drug Conjugates**

| Target Ab | Ab # | ADC | | | | ATDC # |
|---|---|---|---|---|---|---|
| | | LP # | Yield | LCMS DAR | MS (DAR2) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **GLP1R** | COMP mAb 1 | LP1 | 20% | 1.4 | 103081 F(ab')2 | 1 |
| **non-target molecule (Cont 1)** | Isotype control mAb 3 | LP1 | 50% | 2.0 | 150044 | 2 |
| **GLP1R** | COMP mAb 1 | LP2 | 35% | 1.5 | 103435 F(ab')2 | 3 |
| **non-target molecule (Cont 1)** | Isotype control mAb 3 | LP2 | 70% | 2.0 | 150396 | 4 |
| **GLP1R** | COMP mAb 1 | LP3 | 40% | 1.4 | 151304 | 5 |
| **non-target molecule (Cont 1)** | Isotype control mAb 3 | LP3 | 30% | 2.0 | 150748 | 6 |
| **GLP1R** | COMP mAb 1 | LP4 | 93% | 2.0 | 155254 | 7 |
| **GLP1R** | mAb 7 | LP4 | 91% | 2.0 | 149325 | 8 |
| **GLP1R** | mAb 8 | LP4 | 50% | 4.2 | 156816 (DAR4) | 9 |
| **GLP1R** | mAb 9 | LP4 | 97% | 3.3 | 154870 | 10 |
| **GLP1R** | mAb 2 | LP4 | 99% | 2.4 | 155367 | 11 |
| **non-target molecule (Cont 2)** | control mAb 4 | LP4 | 40% | 2.0 | 151752 | 12 |
| **non-target molecule (Cont 3)** | control mAb 5 | LP4 | 95% | 2.0 | 155027 | 13 |
| **non-target molecule (Cont 4)** | control mAb 6 | LP4 | 95% | 2.2 | 155060 | 14 |
| **non-target molecule (Cont 1)** | Isotype control mAb 3 | LP4 | 55% | 2.0 | 151789 | 15 |
| **GLP1R** | COMP mAb 1 | LP6 | 30% | 1.1 | 150757 | 16 |
| **GLP1R** | mAb 10 | LP6 | 8% | 2.9 | 153054 | 17 |
| **GLP1R** | mAb 2 | LP6 | 77% | 2.5 | 153738 | 18 |
| **non-target molecule (Cont 3)** | control mAb 5 | LP6 | 28% | 2.0 | 153389 | 19 |
| **GLP1R** | mAb 2 | LP9 | 44% | 2.2 | 153413 | 20 |
| **GLP1R** | mAb 2 | LP11 | 72% | 1.2 | 152981 | 21 |
| **GLP1R** | mAb 6 | LP11 | 69% | 1.6 | 152994 | 22 |
| **GLP1R** | mAb 11 | LP11 | 64% | 1.5 | 151878 | 23 |
| **GLP1R** | mAb 3 | LP11 | 68% | 1.5 | 151876 | 24 |
| **GLP1R** | mAb 12 | LP11 | 55% | 1.6 | 153132 | 25 |
| **GLP1R** | mAb 5 | LP11 | 49% | 1.4 | 152661 | 26 |
| **GLP1R** | mAb 13 | LP11 | 46% | 1.7 | 152769 | 27 |
| **GLP1R** | mAb 4 | LP11 | 41% | 1.5 | 152735 | 28 |
| **GLP1R** | Isotype control mAb 3 | LP4 | 61% | 1.7 | 155264 | 31 |
| **GLP1R** | mAb 14 | LP11 | 25% | 1.4 | 153549 | 32 |
| **GLP1R** | mAb 15 | LP11 | 53% | 1.3 | 153514 | 33 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **GLP1R** | mAb 16 | LP11 | 66% | 1.1 | 153096 | 34 |
| **GLP1R** | mAb 17 | LP11 | 47% | 1.3 | 152660 | 35 |
| **GLP1R** | mAb 18 | LP11 | 25% | 1.1 | 154279 | 36 |
| **GLP1R** | mAb 19 | LP11 | 62% | 0.9 | 154246 | 37 |
| **GLP1R** | mAb 20 | LP11 | 27% | 1.3 | 154623 | 38 |
| **GLP1R** | mAb 21 | LP11 | 12% | 1.0 | 154614 | 39 |
| **GLP1R** | mAb 6 | LP11 | 67% | 1.6 | 152994 | 40 |
| **GLP1R** | mAb 2 | LP11 | 82% | 1.4 | 152989 | 41 |
| **GLP1R** | mAb 3 | LP11 | 60% | 1.6 | 151882 | 42 |
| **GLP1R** | mAb 4 | LP11 | 60% | 1.7 | 152731 | 43 |
| **GLP1R** | mAb 5 | LP11 | 69% | 1.7 | 152660 | 44 |
| **GLP1R** | mAb 3 | LP11 | 73% | 1.7 | 25811 (LC-DAR1) | 45 |
| **GLP1R** | mAb 15 | LP11 | 69% | 1.5 | 25759 (LC-DAR1) | 46 |
| **GLP1R** | mAb 16 | LP11 | 86% | 1.3 | 26660 (LC-DAR1) | 47 |
| **GLP1R** | mAb 17 | LP11 | 81% | 1.5 | 26007 (LC-DAR1) | 48 |
| **GLP1R** | mAb 19 | LP11 | 86% | 1.0 | 26146 (LC-DAR1) | 49 |
| **GLP1R** | mAb 3 | LP11 | 78% | 1.7 | 151865 | 50 |
| **GLP1R** | mAb 17 | LP11 | 63% | 1.7 | 152637 | 51 |
| **GLP1R** | mAb 3 | LP11 | n/a | 2.1 | 148977 | 52 |
| **GLP1R** | mAb 17 | LP11 | n/a | 2.1 | 149754 | 53 |
| **GLP1R** | mAb 3 | LP11 | 81% | 2.2 | 148971 | 54 |
| **GLP1R** | mAb 17 | LP11 | 60% | 2.1 | 149752 | 55 |
| **GLP1R** | mAb 3 | LP11 | 78% | 2.2 | 148978 | 56 |
| **GLP1R** | mAb 17 | LP11 | 80% | 2.1 | 149753 | 57 |
| **non-target molecule (Cont 5)** | Isotype control mAb 1 | LP11 | 65% | 1.7 | 151998 | 29 |
| **non-target molecule (Cont 6)** | Isotype control mAb 2 | LP11 | 62% | 1.5 | 151995 | 30 |
| **non-target molecule (Cont 6)** | Isotype control mAb 2 | LP11 | 80% | 1.7 | 151996 | 58 |
| **non-target molecule (Cont 6)** | Isotype control mAb 2 | LP11 | 91% | 1.6 | 26410 (LC-DAR1) | 59 |
| **C.Difficile (Cont 7)** | Isotype control mAb 7 | LP11 | 64% | 1.1 | n/a | 60 |
| **hANGPTL4 (Cont 8)** | Isotype control mAb 8 | LP11 | 52% | 1.3 | n/a | 61 |
| **EGFRvIII (Cont 9)** | Isotype control mAb 9 | LP11 | 51% | 1.6 | n/a | 62 |

7.2 General Two-Step Conjugation Protocol

[0001049] This method includes two step process shown in Figure 54. The first step is microbial transglutaminase (MTG) mediated attachment of a First Linker (La), e.g., a small molecular amine, e.g., an azide-PEG3-amine, to the antibody, wherein an excess of the amine reagent is used to avoid potential cross-linking of antibody chains. The second step attaches the alkyne-linked payload linker payload (LP) to the Azido-tagged conjugate via a strain-promoted azide–alkyne cycloaddition (SPAAC).

[0001050] The generic procedures are following.

*Step 1: making a site-specific azido-functionalized antibody drug conjugate containing two azido groups.*

[0001051] Anti-GLP1R human IgG antibody or isotype control antibody containing Q-tag was mixed with 100-200 molar equivalent of azido-PEG3-amine (L1, MW 218.26 g/mol). The resulting solution was mixed with transglutaminase (Zedira, Darmstadt, Germany, 1U MTG per mg of antibody) resulting in a final concentration of the antibody at 1-10 mg/mL. The reaction mixture was incubated at 25-37°C for 4-72 hours with gently shaking while reaction was monitored by ESI-MS. Upon the completion, the excess amine and MTG were removed by size exclusion chromatography (SEC) or protein A column chromatography. The conjugate was characterized by UV-Vis, SEC and ESI-MS. The azido linkers attached antibody (Ab-$N_3$) resulting in a 402 Da mass increase for the DAR2 conjugate. Conjugate's monomer purity was >95%.

*Step 2: making site-specific conjugates in Table 1 via [2+3] click reactions between azido-functionalized antibodies (Ab-$N_3$) and an alkyne containing linker-payload.*

[0001052] A site-specific antibody drug conjugate was prepared by incubating azido-functionalized antibody (Ab-$N_3$, 1-20 mg/mL) in PBS (pH7.4) with ≥3 molar equivalents of a linker-payload dissolved in an organic solvent, such as DMSO or DMA (10-20 mg/mL) to have the reaction mixture containing 5-15% organic solvent (v/v), at 25-37oC for 1-48 hours while gently shaking. The reaction was monitored by ESI-MS. Upon completion, the excess amount of linker-payload and protein aggregates were removed by size exclusion chromatography (SEC). The purified conjugate was concentrated, sterile filtered and characterized by UV-Vis, SEC, HIC and ESI-MS. Conjugates monomer purity was >95%.

[0001053] In a specific example, 36 mg anti-GLP1R mouse IgG antibody COMP mAb 1 containing a heavy chain N-term Q-tag was mixed with 150 molar equivalents of azido-PEG3-amine (L1, MW 218.26 g/mol). The resulting solution was mixed with microbial transglutaminase (1U mTG per mg of antibody, Zedira, Darmstadt, Germany) resulting in a final concentration of the antibody at 4.0 mg/mL. The reaction mixture was incubated at 37°C for 18 hours while gently

shaking while monitored by ESI-MS. Upon the completion, the excess amine and mTG were removed by size exclusion chromatography (SEC). The concentrated site-specific antibody azido conjugate (2.9 mg/mL) in PBS (pH7.4) was mixed with 5 molar equivalents of linker-payload (LP4) in 10 mg/mL of DMSO. Additional DMSO was added to 10% total DMSO (v/v), and the solution was set at 25°C for 22 hours with gently shaking. The reaction was monitored by ESI-MS. Upon completion, the excess amount of linker-payload and protein aggregates were removed by size exclusion chromatography (SEC). The purified conjugate was concentrated, sterile filtered and characterized by UV-Vis, SEC, HIC and ESI-MS. Conjugate monomer purity was 99.7%. The drug attached antibody resulting in a 5931 Da mass increase for the DAR2 conjugate.

S7.3 General One-Step Conjugation Protocol

[0001054] This method includes microbial transglutaminase (MTG) mediated attachment of Linker payload (LP) to the antibody, shown in Figure 55.

[0001055] The generic procedure is following.

[0001056] Anti-GLP1R human IgG antibody or isotype control antibody containing Q-tag was mixed with 10-20 molar equivalent of linker payload (LP11, MW 1979.24 g/mol); Tris-HCl was added to elevate pH to around pH7.4. The resulting solution was mixed with transglutaminase (Zedira, Darmstadt, Germany, 1U MTG per mg of antibody) resulting in a final concentration of the antibody at 1-10 mg/mL. The reaction mixture was incubated at 25-37°C for 4-72 hours with gently shaking while reaction was monitored by ESI-MS. Upon the completion, the excess amount of linker-payload, protein aggregates and MTG were removed by size exclusion chromatography (SEC). The purified conjugate was sterile filtered and characterized by UV-Vis, SEC, HIC and ESI-MS. Conjugates monomer purity was >95%.

[0001057] In a specific example, 1.5 mg anti-GLP1R human IgG antibody mAb 6 containing a heavy chain N-term Q-tag was mixed with 15 molar equivalents of linker-payload (LP11) in 10 mg/mL of DMSO. Additional DMSO was added to a 10% total DMSO (v/v), followed by 1M Tris-HCl pH8 to bring the Tris concentration to 25mM (pH ~8). The resulting solution was mixed with transglutaminase (Zedira, Darmstadt, Germany, 1U MTG per mg of antibody) resulting in a final concentration of the antibody at 3.0 mg/mL. The solution was set at 37°C for 23 hours with gently shaking. The reaction was monitored by ESI-MS. Upon completion, the excess amount of linker-payload, protein aggregates and MTG were removed by size exclusion chromatography (SEC). The purified conjugate was concentrated, sterile filtered and characterized by UV-Vis, SEC, HIC and ESI-MS. The drug attached antibody resulting in a 3924 Da mass increase for the DAR2 conjugate.

**Example 8. *In vitro* characterization of payloads and linker-payloads**

8.1 Human GLP1R cAMP Accumulation Assay (Cyclic AMP determination)

[0001058] The functional activity of the test compounds for agonizing GLP1R were evaluated using a cell-based assay. For the assay, HEK293 cells over-expressing full length human GLP-1R were utilized and the downstream cAMP accumulation was assessed as a measure of human GLP-1R stimulation. For the assay, compounds were 5-fold serially diluted in DMSO with a starting concentration of 1µM in PP-384 microplates using an automated Bravo liquid handling platform. Diluted compounds were transferred to OptiPlates (100 nL/well) using an Echo liquid handler. HEK293/hGLP1R cells were thawed in 37˚C water-bath, washed 2 times with HBSS and re-suspended in assay buffer (HBSS + 5mM HEPES + 500µM IBMX + 0.1% BSA). After the compounds were added, HEK293 cells were then seeded at 1 x $10^5$ cells/well (10µL) into the 384 OptiPlates containing diluted compounds. After centrifugation, the assay plate was incubated at 23˚C for 30min. The reaction was terminated by adding 10µL of lysis buffer containing D2-cAMP and a cAMP-antibody from the cyclic AMP immunoassay kit (Cisbio, Cat# 62AM4PEJ). Following a one-hour incubation, assay plates were read on an EnVision plate reader at 665/615nm. The levels of cAMP per well were calculated using a standard curve generated by GraphPad Prism. Percent activity was calculated according to the formula (% Activity=100%* (cAMP level-LC) / (HC-LC)). $EC_{50}$ values were fitted from a four-parameter logistic equation over a 10-point response curve (GraphPad Prism).

[0001059] As shown in Table 4, the test payloads and linker-payloads demonstrated cAMP activation with $EC_{50}$ values ranging from 39pM to > 11 nM, with most agonizing GLP1R with $EC_{50}$ values of < 1 nM. The reference compound, GLP1, demonstrated cAMP activation with an $EC_{50}$ value of 28 pM.

**Table 4. Activity of Payloads and Linker-Payloads in the cAMP Accumulation Assay**

| P# | cAMP $EC_{50}$ (nM) |
|---|---|
| **GLP1** | 0.028 |
| **P3** | 1.433 |
| **P4** | 0.156 |
| **P5** | 0.115 |
| **P6** | 0.853 |
| **P7** | 2.055 |
| **P8** | 0.497 |
| **P9** | 0.050 |
| **P10** | 0.047 |
| **P11** | 1.028 |

| | |
|---|---|
| **P12** | 0.039 |
| **P13** | 0.914 |
| **P14-S** | 0.727 |
| **P15-R** | 0.117 |
| **P16-S** | 0.544 |
| **P17-R** | 11.240 |
| **P18** | 0.370 |
| **P19** | 0.156 |
| **P20** | 0.370 |
| **P21** | 0.427 |
| **P22** | 0.603 |
| **P23** | 0.198 |
| **P24** | 0.136 |
| **LP5** | 0.044 |
| **LP6** | 0.436 |
| **LP7** | 0.378 |
| **LP8** | 0.412 |
| **LP9** | 0.078 |
| **LP10** | 0.982 |
| **LP15** | 0.649 |
| **LP16** | 1.038 |
| **LP11** | 0.318 |
| **LP18** | 0.458 |
| **LP19** | 0.558 |
| **LP20** | 0.314 |
| **LP22** | 2.706 |
| **LP23** | 0.534 |

8.2 Plasma stability

[0001060] The plasma stability of payloads and linker payloads of the disclosure were measured. For the assay, pooled frozen plasma (human, mouse or monkey) was thawed in cold water or a 37°C water bath prior to experiment. Plasma was centrifuged at 4000 rpm for 5 min if any clots were observed, they were subsequently removed. If required, the pH was adjusted to 7.4 ± 0.1. For the preparation of compounds and positive controls, a 1 mM intermediate solution was prepared by diluting 10 µL of their stock solution with 90 µL DMSO; a 1 mM intermediate solution of the positive control (Propantheline) was prepared by diluting 10 µL of its stock solution with 90 µL ultrapure water. Subsequently, 100 µM dosing solution for each compound was prepared by diluting 20 µL of the intermediate solution (1 mM) with 180 µL 45%MeOH/$H_2O$. 98 µL of plasma was spiked with 2 µL of dosing solution (100 µM) to achieve the final concentration of 2 µM in duplicate. Samples were then incubated at 37°C in a water bath. Four sets of time points were collected: (A) for the 2 hours test, the samples were collected at 0, 10, 30, 60 and

120 min; (B) for the 24 hours test the samples were collected at 0, 10, 60, 240 and 1440 min; (C) for the for 3 days test, the samples were collected at 0, 24, 48 and 72 hours (D) for the for 7 days test, the samples were collected at 0, 24, 48, 72, 96, 120, 144 and 168 hours. At each time point, 400 µL of stop solution (200 ng/mL tolbutamide plus 200 ng/mL labetalol in 50% ACN/MeOH) was added to precipitate protein and mixed thoroughly. Sample plates were then centrifuged at 4,000 rpm for 10 min. An aliquot of supernatant (50 µL) was transferred from each well and mixed with 100 µL ultra-pure water. The samples were then shaken at 800 rpm for approximately 10 min before submitting for LC-MS/MS analysis.

[0001061] As shown in Table 5A, most of the test payloads and linker-payloads were highly stable in human plasma, having $T_{1/2}$ values of > 48 hours in a one-day plasma assay and > 14 days in a 7-day plasma assay; the reference compound, GLP1, is unstable in human plasma and demonstrated a $T_{1/2}$ value of < 10 minutes. As shown in Table 5B, The one linker payload of the disclosure (LP11) tested in monkey plasma had a $T_{1/2}$ value of >49.4 hours. Two linker payloads of the disclosure (LP11 and LP23) that were tested in mouse plasma stability had $T_{1/2}$ values of either >6 days in the 3 day assay or >4 hours in the 2 hour assay.

**Table 5A. Human plasma stability of Payloads and Linker-Payloads**

| P# | Human plasma stability assay T½ (hr) | Tested time* |
|---|---|---|
| P2 | 10.74 | B |
| P3 | >57.81 | A |
| P4 | >57.81 | A |
| P6 | >57.81 | A |
| P8 | >57.81 | A |
| P9 | >57.81 | A |
| P10 | 1.83 | B |
| P11 | >57.81 | A |
| P12 | >57.81 | A |
| P13 | 16.9 | A |
| P19 | >57.81 | A |
| P21 | >173.4 | C |
| P23 | 10.74 | B |
| P41 | >57.81 | A |
| LP11 | >173.4 (human) | C |
| LP11 | >49.4 (monkey) | C |
| LP11 | >173.4 (mouse) | C |
| LP23 | >4.8 hr (mouse)<br>>4.8 hr (human) | B |
| LP24 | >404.7 hr | D |
| LP25 | 284.8 hr | D |

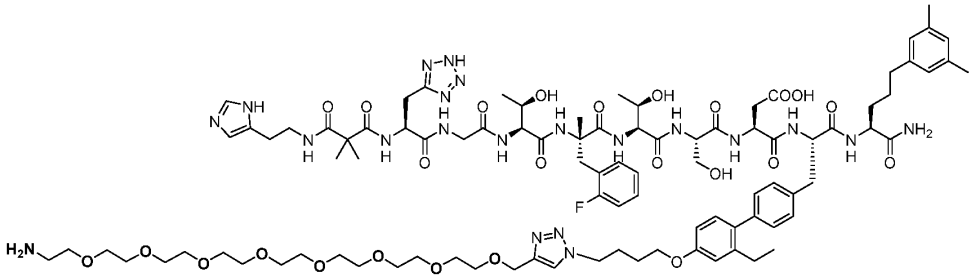
| | | |
|---|---|---|
| **LP26** | **>404.7 hr** | **D** |
| **LP28** | **>173.4** | **C** |
| **LP29** | **>173.4** | **C** |
| **LP34** | **>173.4** | **C** |

*Tested for 1 day in A; 2 hrs in B; 3 days in C; 7 days in D.

**Table 5B. Monkey and Mouse plasma stability of Linker-Payloads**

| Species | P# | plasma stability T½ | Assay time |
|---|---|---|---|
| **Monkey** | **LP11** | >49.4 hr | 3 days |
| **Mouse** | **LP11** | >6 days | 3 days |
| | **LP23** | >4 hr | 2 hr |

[0001062] Table 5C, below, summarizes tested parameters for Linker-Payload LP11 having the structure shown below.



(LP11)

Table 5C. LP11 Characterization

| **Assay** | | **Linker-Payload LP11** |
|---|---|---|
| GLP1R LUC $EC_{50}$ (nM) | | 0.016 nM |
| GLP2R, GIPR, or GCGR $EC_{50}$ (nM) | | Completely inactive |
| c-AMP $EC_{50}$ | | 0.318 nM |
| Plasma Stability | Mouse T½ | > 7 days |
| | Monkey $T_{1/2}$ | > 2 days |
| | Human T½ | > 7 days |
| Human Liver microsome | $T_{1/2}$ | >145 min |
| | $CL_{int(mic)}$ | < 9.6 µL/min/mg |

| | | |
|---|---|---|
| | $CL_{int(liver)}$ | < 8.6 mL/min/kg |
| Water solubility | | 60 mM |
| Protein binding | | 97.97% |
| hERG $IC_{50}$ | | > 100 uM |
| Ames (TA98 and TA100)<br>in the presence and absence of S9 | | Negative for mutagenicity |

8.3 hERG assay

[0001063] To determine if compounds of the disclosure had impacts on hERG potassium channels activity, cell based assay was performed. For the assay, CHO cells stably expressing hERG potassium channels were plated and cultured for at least 2 days in a humidified and air-controlled (5% $CO_2$) incubator at 37 °C prior to use in electrophysiological experiments. Cells were then harvested using TrypLE and resuspended in physiological solution (10mM HEPES, 80mM NaCl, 4mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 5mM Glucose, 60mM NMDG, pH 7.4). Test compounds were dissolved in water to obtain stock solutions for different test concentrations. Stock solutions were further diluted into external electrophysiological recording solution (10mM HEPES, 140mM NaCl, 4mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 5mM Glucose, pH 7.4) to achieve final concentrations for testing. Recordings were performed at room temperature using a Nanion SyncroPatch 384PE, a 384-well automated patch-clamp platform (Internal recording solution: 10mM HEPES, 10mM NaCl, 10mM KCl, 110mM KF, 10mM EGTA, pH 7.2). A schematic of the voltage command protocol used for electrophysiological recordings is shown in Figure 56. One 40 µL addition of the vehicle was applied, followed by a 300s baseline recording period. Then 40 µL doses of compounds were added at each concentration with an exposure time of no less than 300s. Recordings were required to pass quality control over the duration of the recording or the well was abandoned, and the compound was retested, all automatically set by PatchControl. Five concentrations (0.3 µM, 3 µM, 10.00 µM, 30.00 µM and 100.00 µM) were tested for each compound as well as a positive control (Amitriptyline). A minimum of 2 replicates per concentration were obtained. Data analysis was carried out using DataControl, Excel 2013 (Microsoft) and GraphPad Prism 5.0. Curve-fitting and $IC_{50}$ value calculations were performed by GraphPad Prism 5.0. If the inhibition obtained at the lowest concentration tested was over 50%, or at the highest concentration tested was less than 50%, the $IC_{50}$ value was reported as less

than lowest concentration, or higher than highest concentration, respectively. The $IC_{50}$ values of the test compounds on whole cell hERG currents were summarized in Table 6.

**Table 6. $IC_{50}$ of the test compounds on whole cell hERG currents**

| Compound ID | $IC_{50}$ (μM) | HillSlope | N |
|---|---|---|---|
| Amitriptyline | 2.90 | 1.25 | 3 |
| LP10 | >100.00 | - | 2 |
| LP11 | >100.00 | - | 2 |

8.4 Ames assay

[0001064] The objective of this Mini-Ames study was to evaluate the test article LP11, for its ability to induce reverse mutations in the genome of strains of *Salmonella typhimurium* TA98 and TA100 in the presence and absence of metabolic activation (S9 mixture).

[0001065] The Mini-Ames assay was conducted for the test article in the presence and absence of the S9 mixture, concurrently with the negative/solvent control (DMSO) using six wells and positive controls (2 μg/well TA98 alone, 0.2 μg/well TA100 alone or 0.4μg/well TA98 + TA100 in the presence of S9) using three wells. The tested dose levels for the test article, in the presence and absence of S9 mix, were 1000, 400, 160, 64, 25, 10, 4, and 1.5 μg per well, with each dose tested in triplicate. The study was conducted using fresh cultures of the bacterial strains and fresh test article formulations.

[0001066] For test article LP11, no obvious cytotoxicity was observed at any dose level with or without S9 mix in any tester strain. No obvious precipitates (by naked eye after the incubation period) were observed at any dose level with or without S9 mix in any tester strain.

[0001067] LP11 did not induce more than 2.0-fold increase in the two strains TA98 or TA100 in the mean number of revertant colonies at any dose level relative to the concurrent negative/solvent control, either in the presence or absence of S9 mix. No dose response was observed either.

[0001068] For both tester strains used in this study, the mean number of revertant colonies observed for the negative/solvent control was comparable to the laboratory historical negative control data. All positive controls induced the expected increase of greater than three-fold in the mean number of revertant colonies, in the presence and absence of S9 mix, when compared to the concurrent negative/solvent control, thereby confirming the responsiveness of the strains.

[0001069] The genotypes of all the tester strains used in this assay were confirmed. It was concluded that this Mini-Ames study was valid and LP11 was negative for mutagenicity under the conditions of this study.

8.5 *In Vitro* ADME

[0001070] To assess the *in vitro* ADME properties of the LP, microsomal stability assay was performed to determine intrinsic clearance, and plasma protein binding assay was performed to understand the distribution potential. The results are listed in Tables 7 and 8 below.

**Table 7. Liver Microsome Stability**

| Sample Name | $T_{1/2}$ (min) | $CL_{int(mic)}$ (µL/min/mg) | $CL_{int(liver)}$ (mL/min/kg) |
|---|---|---|---|
| LP11 | >145 | <9.6 | <8.6 |
| Testosterone | 16.2 | 85.7 | 77.2 |

[0001071] An assay was performed to determine the plasma protein binding of compounds of the disclosure. For the assay, on the day of experiment, the plasma was thawed in cold water and centrifuged at 3220 rpm for 5 minutes to remove any clots. The pH value of the resulting plasma was checked

[0001072] The 96-well equilibrium dialysis device (Cat # 1006) and HTD 96 a/b cellulose membrane strips with molecular mass cutoff of 12-14 kDa (Cat # 1101) were obtained from HTDialysis LLC, Gales Ferry, CT) and the dialysis device was assembled following the manufacturer's instructions. (<http://www.htdialysis.com/>).

[0001073] For the dialysis, the test compound and control compound were dissolved in DMSO to achieve 10 mM stock solutions. DMSO working solutions were prepared at 400 µM. To prepare the loading matrix, compound working solutions (5 µL) were added in a 1:200 ratio to blank matrix (995 µL) and mixed thoroughly. To prepare the time zero (T0) samples to be used for recovery determination, 50 µL aliquots of loading matrix were transferred in triplicate to the sample collection plate. The samples were immediately matched with opposite blank buffer to obtain a final volume of 100 µL of 1:1 matrix/dialysis buffer (v/v) in each well. 500 µL of stop solution were added to these T0 samples. This was then stored at 2-8°C pending further processes along with other post-dialysis samples. To load the dialysis device, an aliquot of 150 µL of the loading matrix was transferred to the donor side of each dialysis well in triplicate, and 150 µL of the dialysis buffer was loaded to the receiver side of the well. The dialysis plate was placed in a humidified incubator at 37 °C with 5% $CO_2$ on a shaking platform that rotated slowly

(about 100 rpm) for 4 hours. At the end of the dialysis, aliquots of 50 µL of samples were taken from both the buffer side and the matrix side of the dialysis device. These samples were transferred into new 96-well plates. Each sample was mixed with an equal volume of opposite blank matrix (buffer or matrix) to reach a final volume of 100 µL of 1:1 matrix/dialysis buffer (v/v) in each well. All samples were further processed by adding 500 µL of stop solution containing internal standards. The mixture was vortexed and centrifuged at 4000 rpm for about 20 minutes. An aliquot of 100 µL of supernatant of all the samples was then removed for LC-MS/MS analysis. The single blank samples were prepared by transferring 50 µL of blank matrix to a 96 well plate and adding 50 µL of blank PBS buffer to each well. The blank plasma must match the species of plasma used in the plasma side of the well. Then the matrix-matched samples were further processed by adding 500 µL of stop solution containing internal standards, following the same sample processing method as the dialysis samples.

[0001074] The percent unbound, percent bound, and percent recovery values were calculated using the following equations:

$$\%Unbound = 100 \times \frac{[F]}{[T]}$$

$$\%Bound = 100 \times \left(1 - \frac{[F]}{[T]}\right)$$

$$\%Recovery = 100 \times \left(\frac{[F] + [T]}{[T_0]}\right),$$

where [F] is the analyte concentration or peak area ratio of analyte/internal standard on the buffer (receiver) side of the membrane, [T] is the analyte concentration or peak area ratio of analyte/internal standard on the matrix (donor) side of the membrane, and [T0] is the analyte concentration or the peak area ratio of analyte/internal standard in the loading matrix sample at time zero. Table 8, below, summarizes the plasma protein binding assay results.

**Table 8. Plasma Protein Binding Assay Results**

| Compound ID | Species / Matrix | %Unbound | % Unbound SD | %Bound | %Recovery | % Recovery SD |
|---|---|---|---|---|---|---|
| Warfarin | Human Plasma | 1.25 | 0.2 | 98.75 | 98.3 | 3.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| LP11 | Human Plasma | 2.03 | 0.4 | 97.97 | 89.2 | 1.7 |

**EXAMPLE 9. Activities of GLP1R peptidomimetic payloads and linker-payloads against GPCR family members**

[0001075] To test the activity of GLP1R agonist payloads and GLP1R agonist linker-payloads (LPs) *in vitro*, a cell-based cAMP responsive luciferase reporter assay was developed. Human embryonic kidney cells (HEK293) expressing a cyclic AMP response element (CRE)-luciferase reporter were generated that express either Myc-tagged full length human GLP1R (amino acids 1 to 463 of accession number NP_002053); referred to as HEK293/Myc-hGLP1R/Cre-Luc cell line), full length human gastric inhibitory polypeptide receptor (GIPR) (amino acids 1 to 466 of accession number NP_000155.1; referred to as HEK293/Myc-hGIPR/Cre-Luc cell line), full length human glucagon-like peptide 2 receptor (GLP2R) (amino acids 1to 553 of accession number NP_004237; referred to as HEK293/Myc-hGLP2R/Cre-Luc cell line) or full length human glucagon receptor (GCGR) (amino acids 1 to 477 of accession number NP_000151.1; referred to as HEK293/Myc-hGCGR/Cre-Luc cell line) using standard methods for the generation of stable cell lines. Cell surface expression of the receptors was confirmed by flow cytometry, using an antibody recognizing the Myc tag.

[0001076] For the bioassay, cells were plated at a density of 20,000 cells/well in 80 µL of Opti-MEM supplemented with 0.1% FBS in a 96-well clear bottom white plates (Corning, #356693). Cells were incubated overnight at 37°C in 5% $CO_2$. Test reagents, including payload, a linker payload, and positive control ligands [human GLP1 (R&D Systems, #5374), human GIP (R&D Systems, #2084), human GCG (R&D Systems, #6927), or human GLP2 (R&D Systems, #2258)], were serially diluted in Opti-MEM with 0.1% FBS and were then added to the cells at 10µL/well for each concentration tested. Plates were incubated for 5.5h at 37°C in 5% $CO_2$. For end-point measurement, 100µL/well of One-Glo reagent (Promega, #E6051) was added and plates were kept at room temperature for 5-10 minutes. Luciferase activity was measured on Envision Multilabel Plate Reader (Perkin Elmer) in Luminescent mode. The relative light units (RLU) values were obtained and the results were analyzed using nonlinear regression with GraphPad Prism software (GraphPad).

[0001077] As shown in Table 9 and Figures 6A-6D, the payloads and linker-payload demonstrated activation in the HEK293/Myc-hGLP1R/Cre-Luc cell line with $EC_{50}$ values ranging from 3.32 pM to 71.5 pM. The payloads and linker payload did not demonstrate an measurable

activity in the other cell lines evaluated. All of the positive control reference ligands (human GLP1, GIP, GLP2, and GCG) activated individual cell lines as expected.

**Table 9. CRE-Dependent Reporter Activity by GLP1R payload and linker-payload Agonists in GLP1R, GIPR, GLP2R and GCGR cell lines**

| Compound | HEK293/Myc-hGLP1R/Cre-Luc $EC_{50}$ (M) | HEK293/Myc-hGIPR/Cre-Luc $EC_{50}$ (M) | HEK293/Myc-hGLP2R/Cre-Luc $EC_{50}$ (M) | HEK293/Myc-hGCGR/Cre-Luc $EC_{50}$ (M) |
|---|---|---|---|---|
| P9 | 3.32E-12 | N/A | N/A | N/A |
| P8 | 7.60E-12 | N/A | N/A | N/A |
| LP4 | 7.15E-11 | N/A | N/A | N/A |
| LP11 | 1.6E-11 | N/A | N/A | N/A |
| Reference* | 1.46E-12 | 1.29E-12 | 1.23E-11 | 1.03E-9 |

N/A = Not Active

Reference for GLP1R assay = GLP1

Reference for GIPR assay = GIP

Reference for GLP2R assay = GLP2

Reference for GCGR assay = GCG

**EXAMPLE 10. *In vitro* plasma stability**

[0001078] To determine the plasma stability of ATDC anti-GLP1R mAB2-LP11 bearing GLP1R agonist P8, the ATDCs was incubated *in vitro* with the plasma from different species and the drug to antibody ratio (DAR) was evaluated. Anti-GLP1R mAB2 is a biotinylated anti-Fc antibody.

[0001079] The ATDC solution was spiked into pooled C57BL/6 mouse, cynomolgus monkey (Cyno), or IgG depleted human plasma (BioIVT) to a final concentration of 50 µg/mL, and subsequently incubated at 37°C on ThermoMixer C (Eppendorf, Cat# 2231000574). A 100-µL aliquot was removed at times 0, 1, 2, 3 and 7 days and then immediately stored frozen at -80°C until analysis.

[0001080] For DAR analysis, the ATDC was purified from plasma samples by immunoaffinity capture using a DynaMag-2 magnetic rack (Life Technologies, Cat#12321D). First, biotinylated anti-human Fc antibody (Regeneron generated reagent) was immobilized on Dynabeads M280 streptavidin beads (Invitrogen, Cat#60210). Each plasma sample containing the ATDC was mixed at 950 rpm with 0.5 mg of the beads at room temperature for 2 hours with gentle shaking. The beads were then washed three times with 500 µL of HBS-EP pH 7.4 buffer (GE Healthcare,

Cat#BR100188), once with 500 µL water and once with 500 µL of 10% acetonitrile (VWR Chemicals, Cat#BDH83640.100E) in water. Following the washes, the ATDC was eluted by incubating the beads with 70 µL of 1% formic acid in 30:70 acetonitrile:water (v/v) for 15 minutes at room temperature. Fifty µL eluted samples were further reduced by adding 50 µL 10 mM TCEP (Sigma, Cat 646547-10X1ML) and incubated at 37°C for 20 min in ThermoMixer C.

[0001081] The reduced ATDC samples were then injected onto a 0.3 x 50 mm 1.7 µm BEH300 C4 column (Waters, Cat# 186009260) for separation and detected by Synapt G2-Si Mass Spectrometer (Waters). The flow rate used was 10 µL/min (mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile). The HPLC gradient eluted ATDC between 3.5-6.5 minutes corresponding to 25-40 % of mobile phase B. The acquired spectra were deconvoluted using MaxEnt1 software (Waters) with the following parameters: Mass range: 20-60 kDa; m/z range: 800-2500 Da; Resolution: 1.0 Da/channel; Width at half height: 0.8 Da; Minimum intensity ratios: 33%; Iteration max: 15. DAR was calculated based on peak intensity corresponding to individual DAR species in the deconvoluted spectra.

[0001082] No significant change in DAR was observed for anti-GLP1R mAb2-LP11 after a 7-day incubation in mouse, cynomolgus monkey or IgG depleted human plasma. The results are presented in Table 10 and Figure 57.

**Table 10. *In vitro* stability of anti-GLP1R mAB2-LP11 over a 7-day, 37°C incubation in mouse, monkey and human plasma**

| Time (Days) | DAR in Mouse Plasma | DAR in Monkey Plasma | DAR in Human Plasma |
|---|---|---|---|
| 0 | 1.1 | 1.0 | 0.97 |
| 1 | 1.1 | 0.99 | 0.93 |
| 2 | 1.1 | 1.0 | 0.90 |
| 3 | 1.1 | 0.99 | 0.95 |
| 7 | 1.2 | 1.0 | 0.99 |

**EXAMPLE 11. Luciferase reporter assay**

[0001083] To test the activity of GLP1R agonist payloads, GLP1R agonist linker-payloads (LPs), and anti-GLP1R antibody tethered drug conjugates (ATDCs) of the disclosure, a cell-based cAMP responsive luciferase reporter assay was developed. To generate the assay cell line, the firefly luciferase gene was placed under the control of a cAMP response element (CRE) located upstream of a minimal promoter and transfected into HEK293 cells and referred to herein as HEK293/CRE-Luc cells. HEK293/CRE-Luc cells were then engineered to express full-length

human GLP1R (amino acids 1 to 463 of accession number NP_002053) and are referred to herein as HEK293/CRE-Luc/hGLP1R cells.

[0001084] For the assays, cells were seeded into 96 well plates at 10,000 or 20,000 cells/well in assay media (Optimem, 0.1% BSA, 100 units/ml Penicillin, 100 ug/ml Streptomycin, 292 µg/ml L-glutamine) and incubated overnight. Three-fold serial dilutions of free payloads or LPs were prepared in 100% DMSO, transferred to fresh assay media, and added to the cells at a final constant DMSO concentration of 0.2%. The last well in the plate served as a blank control containing only the assay media and 0.2% DMSO (untreated well) and was plotted as a continuation of the 3-fold serial dilution. Four to six hours later, luciferase activity was determined after the addition of One-Glo™ reagent (Promega, Cat# E6130) to each well. Relative light units (RLUs) were measured on an Envision luminometer (PerkinElmer) and $EC_{50}$ values were determined using a four- parameter logistic equation over a 12-point dose response curve (GraphPad Prism). The signal to noise (S/N) was determined by taking the ratio of the highest RLU on the dose response curve to the RLU in the untreated wells. $EC_{50}$ and S/N values are summarized in Table 11. Data was generated across several experiments (A, B, and D) with P8 serving as a reference standard in each experiment to calculate the payload relative potency [(P8 $EC_{50}$/payload $EC_{50}$)*100] and relative S/N [(payload SN/ P8 SN)*100].

[0001085] As shown in Table 11, payload and linker-payload $EC_{50}$ values ranged from 3.97 pM to 1.95 nM and relative potency (% P8) ranged from 0.3% to 133.8% in HEK293/CRE-Luc/hGLP1R cells. Most tested payloads reached similar max activity with relative S/N values (% P8) ranging from 68.6% to 116.27%. The GLP1 ligand was also included for reference and increased CRE-dependent luciferase activity in HEK293/CRE-Luc/hGLP1R cells with an $EC_{50}$ of 14.3 pM, relative potency of 37.2%, and relative S/N of 96.8%. All tested agonists had minimal impact on luciferase activity in the absence of hGLP1R expression (HEK293/CRE-Luc cells), with S/N values ≤ 1.7 and $EC_{50}$ values > 200.0 nM.

**Table 11. CRE-Dependent Reporter Activity by GLP1R payload and linker-payload Agonists in HEK293/CRE-Luc/hGLP1R cells**

| Molecule | HEK293/CRE-LUC/hGLP1R | | | | HEK293/CRE-LUC | | |
|---|---|---|---|---|---|---|---|
| | $EC_{50}$ | Relative Potency (%P8 $EC_{50}$) | S/N | Relative Signal:Noise (%P8) | $EC_{50}$ | S/N | Experiment |
| P9 | 3.97E-12 | 133.8 | 75.2 | 114.3 | >2E-07 | 1.3 | A |
| P23 | 5.15E-12 | 103.0 | 57.1 | 86.9 | >2E-07 | 1.6 | A |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| P8 | 5.31E-12 | 100.0 | 65.8 | 100.0 | >2E-07 | 1.5 | A |
| P12 | 1.14E-11 | 46.5 | 76.4 | 116.2 | >2E-07 | 1.2 | A |
| P15-R | 1.32E-11 | 40.3 | 67.8 | 103.1 | >2E-07 | 1.1 | A |
| P10 | 1.40E-11 | 38.0 | 45.1 | 68.6 | >2E-07 | 1.2 | A |
| GLP1 | 1.43E-11 | 37.2 | 63.6 | 96.8 | >2E-07 | 1.3 | A |
| LP11 | 1.61E-11 | 33.1 | 74.5 | 113.3 | >2E-07 | 1.3 | A |
| P8 | 2.0E-11 | 100.0 | 327.9 | 100.0 | NT | NT | B |
| P8 | 2.73E-11 | 100.0 | 235.9 | 100.0 | NT | NT | D |
| P4 | 8.37E-11 | 6.3 | 66.9 | 101.8 | >2E-07 | 1.6 | A |
| LP4 | 1.27E-10 | 4.2 | 63.8 | 97.1 | >2E-07 | 1.7 | A |
| P13 | 3.57E-10 | 1.5 | 69.5 | 105.6 | >2E-07 | 1.2 | A |
| P6 | 6.02E-10 | 0.9 | 60.7 | 92.3 | >2E-07 | 1.0 | A |
| P11 | 6.85E-10 | 0.8 | 54.8 | 83.4 | >2E-07 | 1.1 | A |
| P21 | 7.38E-10 | 0.7 | 58.8 | 89.5 | >2E-07 | 1.3 | A |
| P3 | 1.95E-09 | 0.3 | 46.3 | 70.3 | >2E-07 | 1.2 | A |

NT = not tested
> = $EC_{50}$ values could not be determined with accuracy because the binding did not reach saturation within the tested antibody concentration range. $EC_{50}$ is reported as greater than the highest tested concentration

[0001086] GLP1R agonist linker payloads were conjugated to anti-hGLP1R antibodies via N-terminal heavy or light chain Q tags. Several resulting anti-GLP1R antibody tethered drug conjugates (ATDCs) were tested for activity in the HEK293/CRE-Luc/hGLP1R reporter assay as described above for the free GLP1R payload and linker-payload agonists. As shown in Table 12, anti-GLP1R ATDCs increased CRE-dependent luciferase reporter activity in HEK293/CRE-Luc/hGLP1R cells with $EC_{50}$ values ranging from 21.7 pM to 112 pM and relative potency values (% P8) ranging from 14.5% to 126%. Most tested ATDCs reached similar max activity with relative S/N values (% P8) ranging from 87.4% to 158.4%. The anti-GLP1R ATDCs were inactive in reporter cells that did not express hGLP1R (HEK293/CRE-Luc). Non-binding ATDCs tended to be less active than the anti-GLP1R ATDCs with $EC_{50}$ values ranging from 1.92 nM to 74.6 nM and relative potency values (% P8) ranging from <0.1% to 1.4%. A selected set of results are presented in Figure 10.

**Table 12. CRE-Dependent Reporter Activity by anti-GLP1R ATDCs in HEK293/CRE-Luc/hGLP1R cells**

| Test Article | | LP | HEK293/CRE-Luc/hGLP1R | HEK293/CRE-Luc |
|---|---|---|---|---|

| | mAb Q tag | | $EC_{50}$ | Relative Potency (%P8 $EC_{50}$) | S/N | Relative Signal:Noise (%P8) | Experiment | $EC_{50}$ | S/N |
|---|---|---|---|---|---|---|---|---|---|
| Anti-GLP1R mAB2 | VL N-term | LP11 | 2.73E-11 | 59.5 | 101.9 | 158.4 | A | >2.0E-08 | 1.2 |
| Anti-GLP1R mAB6 | VH N-term | LP11 | 1.12E-10 | 14.5 | 62.5 | 97.1 | A | >2.0E-08 | 0.9 |
| GLP1 | None | None | 2.75E-11 | 58.9 | 88.7 | 137.9 | A | >2.0E-08 | 1.4 |
| P8 | None | None | 1.62E-11 | 100.0 | 64.3 | 100.0 | A | >2.0E-08 | 1.3 |
| Isotype Control mAb1 | VH N-term | LP11 | 7.46E-08 | <0.1% | 69.9 | 108.7 | A | >2.0E-08 | 1.6 |
| Isotype Control mAb2 | VL N-term | LP11 | 1.24E-08 | 0.1 | 69.4 | 107.8 | A | >2.0E-08 | 1.2 |
| COMP Anti-GLP1R mAb1 | VH N-term | LP4 | 6.29E-11 | 55.3 | 284.1 | 87.4 | D | >2.0E-8 | 1.6 |
| COMP Anti-GLP1R mAb1 | VH N-term | LP3 | 4.40E-11 | 62.1 | 271.7 | 115.2 | D | NT | NT |
| COMP Anti-GLP1R mAb1 | VH N-term | LP1 | 2.82E-11 | 96.7 | 213.0 | 90.3 | D | NT | NT |
| COMP Anti-GLP1R mAb1 | VH N-term | LP2 | 2.17E-11 | 126.0 | 227.3 | 96.4 | D | NT | NT |
| P8 | None | None | 2.73E-11 | 100.0 | 235.9 | 100.0 | D | NT | NT |
| Isotype Control mAb3 | VH N-term | LP4 | 1.92E-09 | 1.4 | 252.0 | 106.8 | D | NT | NT |
| Isotype Control mAb3 | VH N-term | LP3 | 2.31E-09 | 1.2 | 218.8 | 92.8 | D | NT | NT |
| Isotype Control mAb3 | VH N-term | LP1 | 2.54E-09 | 1.1 | 235.0 | 99.6 | D | NT | NT |
| Isotype Control mAb3 | VH N-term | LP2 | 2.94E-09 | 0.9 | 285.0 | 120.8 | D | NT | NT |

NT = not tested
> = $EC_{50}$ values could not be determined with accuracy because the binding did not reach saturation within the tested antibody concentration range. $EC_{50}$ is reported as greater than the highest tested concentration

[0001087] In a separate experiment, the ability of unconjugated anti-GLP1R antibodies to compete for anti-GLP1R ATDC activity was assessed in the HEK293/CRE-Luc/hGLP1R reporter assay. In this experiment, reporter cells were incubated with a dose titration of the anti-GLP1R ATDC in the absence or presence of a constant amount (0.01, 0.1, 1.0, 10, or 100 nM) of the unconjugated anti-GLP1R antibody. The $EC_{50}$ values are reported in Table 13, and the fold-change in the $EC_{50}$ value relative to the ATDC alone condition ($EC_{50}$ fold-change) was calculated as follows: $EC_{50}$ of ATDC + unconjugated mAb/$EC_{50}$ of ATDC alone. As shown in Table 13, unconjugated mAb concentrations up to 10 nM had minimal impact on anti-GLP1R ATDC activity with the $EC_{50}$ fold-change values less than or equal to 1.5. The highest tested unconjugated antibody concentration of 100 nM reduced the $EC_{50}$ value by 4.0-fold. A non-binding control ATDC was not impacted by the presence of unconjugated anti-GLP1R antibody.

**Table 13. CRE-Dependent Reporter Activity by anti-GLP1R ATDCs in the presence of unconjugated anti-GLP1R antibodies**

| ATDC | Unconjugated mAb constant concentration | ATDC $EC_{50}$ (M) | $EC_{50}$ fold-change |
|---|---|---|---|
| Anti-GLP1R mAb2 | + 100 nM mAb | 1.2E-10 | 4.0 |
| | + 10 nM mAb | 4.4E-11 | 1.5 |
| | + 1 nM mAb | 2.9E-11 | 1.0 |
| | + 0.1 nM mAb | 2.7E-11 | 0.9 |
| | + 0.01 nM mAb | 2.4E-11 | 0.8 |
| | 0 nM mAb | 3.0E-11 | 1.0 |
| Isotype Control mAb2 | + 100 nM mAb | 3.0E-09 | 1.3 |
| | + 10 nM mAb | 2.4E-09 | 1.0 |
| | + 1 nM mAb | 2.2E-09 | 0.9 |
| | + 0.1 nM mAb | 2.50-09 | 1.1 |
| | + 0.01 nM mAb | 2.30E-09 | 1 |
| | 0 nM mAb | 2.30E-09 | 1 |

**EXAMPLE 12. Effects of anti-GLP1R mAb ATDCs on body weight and blood glucose in diet-induced obese GLP1R humanized mice**

[0001088] To determine body weight and blood glucose lowering effects of three anti-GLP1R antibody-tethered-drug conjugates (ATDCs) in obese animals, mice homozygous for the expression of human GLP1R in place of mouse GLP1R (referred to as GLP1R humanized mice) were placed on high-fat diet (60% kcal% fat) for 6 months. Forty-four, 9-month-old male, GLP1R humanized mice were stratified into six groups of five to eight mice, based on their day 0 body weights. After the stratification, each group was subcutaneously administered with 25 mg/kg of COMP anti-GLP1R mAb1-LP4 (n=8), anti-GLP1R mAb3-LP11 (n=8), anti-GLP1R mAb4-LP11 (n=5), control anti-GLP1R mAb (n=7), isotype control mAb ATDC (n=8) or reference compound (n=8) on day 0.

[0001089] The control anti-GLP1R mAb used in this study is a high-affinity anti-GLP1R antibody, which does not activate or inactivate GLP1R, without any drug conjugation. The control anti-GLP1R mAb is antibody 5A10 described in US Publication No. US20060275288A1, which is incorporated herein by reference in its entirety. The control anti-GLP1R mAb does not have a Q-tag. COMP anti-GLP1R mAb1- LP4 comprises the same control anti-GLP1R mAb with a N-terminal heavy chain Q-tag. The isotype control mAb ATDC is a GLP1

peptide mimetic described herein, conjugated to an antibody that does not bind to any protein in GLP1R humanized mice. The reference compound has identical amino acid sequences to dulaglutide in GLP1 analogue and linker segments, but was made with a hFc with three mutations (P16E; V17A; G19 insert).

[0001090] On days three and seven post administration and weekly from day fourteen to day fifty-six, body weights of the animals were recorded, and their blood glucose levels were measured with a handheld glucometer. Mean ± SEM of percent changes in body weight from day 0 at each time point was calculated for each group and are shown in Table 14. Mean ± SEM of blood glucose levels at each time point was calculated for each group and are shown in Table 15. Statistical analyses were performed by two-way ANOVA followed by Bonferroni post-hoc tests, comparing the control antibody group to the other five groups. The results are also presented in Figures 39 and 40.

[0001091] Body weights and blood glucose levels were stable in animals administered with the control anti-GLP1R mAb, with nominal handling (i.e., bleeding, cage changes) related fluctuations. Compared to animals in the control anti-GLP1R mAb group, animals administered with the isotype control mAb ATDC showed no significant differences in percent body weight change or blood glucose levels. In animals administered with the reference compound, weight reductions were observed on days 3 and 7, whereas glucose was reduced only on day 3. In animals administered anti-GLP1R mAb ATDCs, weight reductions lasted for 42, 56, and 28 days, depending on which GLP1R ATDC was dosed, respectively, while glucose was lowered for 7 days for all GLP1R ATDCs tested.

[0001092] In conclusion, single administration of the three anti-GLP1R mAb ATDCs tested leads to long-term weight loss in obese animals.

**Table 14. Effects of GLP1R ATDCs on percent body weight changes in obese GLP1R humanized mice**

| Time (day) | Control anti-GLP1R mAb | | Isotype control mAb ATDC | | Reference compound | | COMP anti-GLP1R mAb mAb1- LP4 | | Anti-GLP1R mAb mAb3-LP11 | | Anti-GLP1R mAb mAb4-LP11 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | -2.2 | 0.6 | -1.9 | 0.6 | -11.4** | 0.3 | -8.8 | 0.7 | -7.8 | 0.2 | -8.3 | 0.4 |
| 7 | -2.6 | 1.2 | -2.0 | 1.1 | -11.4** | 0.9 | -14.5**** | 0.6 | -11.3** | 0.9 | -11.3* | 0.8 |
| 14 | -3.3 | 1.5 | -2.2 | 1.8 | -6.6 | 1.4 | -19.6**** | 2.0 | -15.9**** | 1.7 | -13.2** | 1.7 |
| 20 | -2.2 | 1.1 | -1.8 | 2.0 | -4.8 | 1.4 | -18.0**** | 2.6 | -15.4**** | 2.0 | -10.9* | 3.0 |
| 28 | -0.7 | 1.3 | -1.5 | 2.1 | -2.3 | 1.1 | -13.7**** | 3.3 | -13.0**** | 2.3 | -8.9* | 4.1 |
| 35 | -0.3 | 1.5 | -1.4 | 2.3 | -0.9 | 1.4 | -11.2*** | 3.1 | -13.0**** | 3.0 | -7.2 | 4.0 |
| 42 | 0.1 | 1.4 | 0.7 | 2.3 | 0.7 | 1.5 | -8.3** | 2.0 | -12.6**** | 3.1 | -4.3 | 2.9 |
| 49 | -2.0 | 1.6 | 0.3 | 2.6 | 0.3 | 1.3 | -6.5 | 1.8 | -12.1*** | 2.8 | -3.1 | 2.0 |
| 56 | -1.7 | 1.1 | -0.7 | 2.1 | 0.1 | 1.6 | -5.0 | 1.5 | -13.2**** | 3.5 | -2.6 | 1.8 |

*$P$<0.05, **$P$<0.01, ***$P$<0.001, ****$P$<0.0001, compared to the control anti-GLP1R mAb group.

**Table 15. Effects of GLP1R ATDCs on blood glucose levels in obese GLP1R humanized mice**

| Time (day) | Control anti-GLP1R mAb | | Isotype control mAb ATDC | | Reference compound | | COMP anti-GLP1R mAb mAb1- LP4 | | Anti-GLP1R mAb mAb3-LP11 | | Anti-GLP1R mAb mAb4-LP11 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| 0 | 215 | 6 | 211 | 14 | 220 | 14 | 214 | 12 | 209 | 12 | 221 | 14 |
| 3 | 212 | 13 | 205 | 15 | 129**** | 10 | 144**** | 10 | 157** | 8 | 143*** | 8 |
| 7 | 202 | 12 | 198 | 13 | 163 | 6 | 158* | 10 | 154* | 9 | 155* | 16 |
| 14 | 186 | 5 | 196 | 9 | 206 | 12 | 167 | 8 | 162 | 7 | 178 | 9 |
| 20 | 200 | 10 | 191 | 10 | 198 | 8 | 176 | 7 | 184 | 7 | 205 | 4 |
| 28 | 207 | 11 | 213 | 16 | 204 | 13 | 197 | 9 | 190 | 8 | 188 | 8 |
| 35 | 219 | 12 | 220 | 12 | 207 | 11 | 211 | 10 | 200 | 10 | 222 | 17 |
| 42 | 208 | 11 | 217 | 17 | 229 | 12 | 208 | 7 | 196 | 12 | 224 | 10 |
| 49 | 226 | 15 | 207 | 12 | 228 | 13 | 215 | 10 | 214 | 12 | 226 | 13 |
| 56 | 217 | 14 | 198 | 12 | 205 | 12 | 196 | 10 | 204 | 4 | 229 | 16 |

*$P$<0.05, **$P$<0.01, ***$P$<0.001, ****$P$<0.0001, compared to the control anti-GLP1R mAb group.

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* * *

[0001093] As various changes can be made in the above-described subject matter without departing from the scope and spirit of the present disclosure, it is intended that all subject matter contained in the above description, or defined in the appended claims, be interpreted as descriptive and illustrative of the present disclosure. Many modifications and variations of the present disclosure are possible in light of the above teachings. Accordingly, the present description is intended to embrace all such alternatives, modifications, and variances which fall within the scope of the appended claims.

[0001094] All patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated by reference in their entirety as if physically present in this specification.

WHAT IS CLAIMED IS:

1. A compound having a structure of Formula (A):

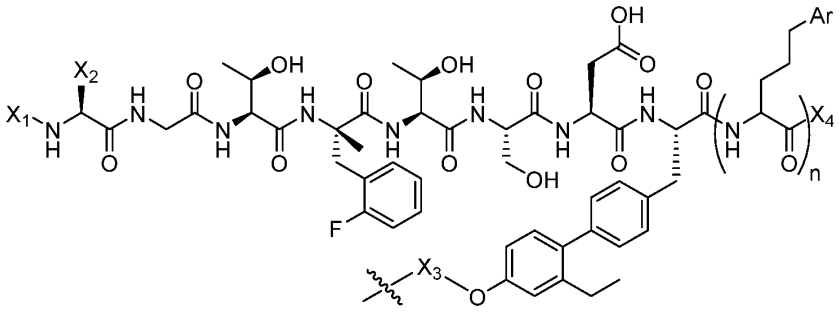
$$BA\text{-}(L\text{-}P)_m \quad (A),$$

wherein:

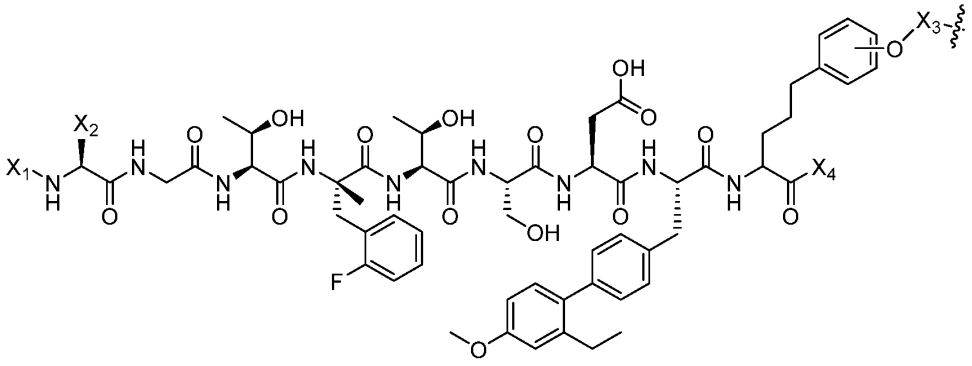
BA is an antibody or an antigen-binding fragment thereof;

L is a non-cleavable linker;

P is a payload having the structure selected from the group consisting of:

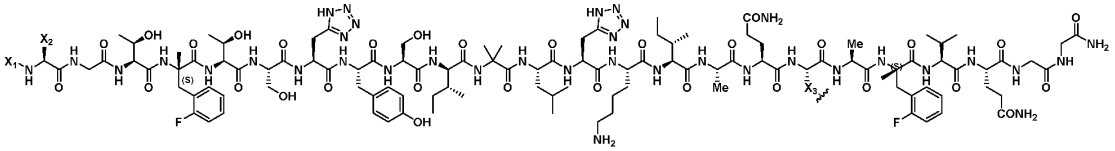


(P-IA),



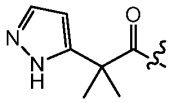
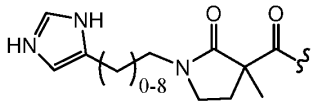
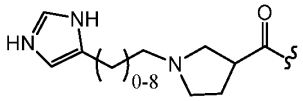
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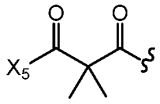
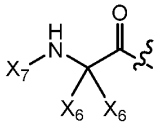
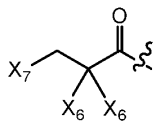
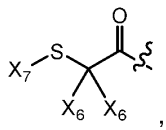
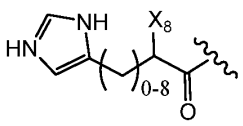
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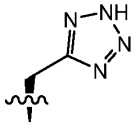
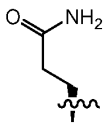


(P-IIIA),

wherein —§ is the point of attachment of the payload to L;

$X_1$ is selected from H;  ;  ;  ;

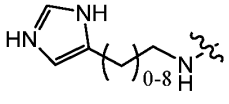
 ;  ;  ;  , and  ;

$X_2$ is selected from  and  ;

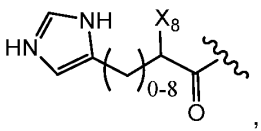
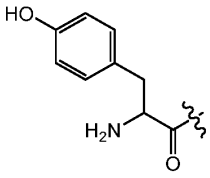
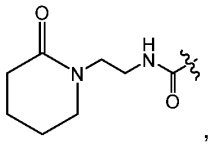
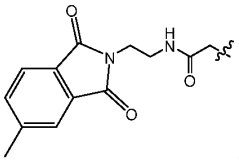
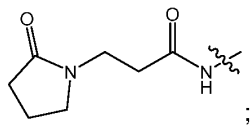
$X_3$ is selected from a bond, $–(CH_2)_{2\text{-}6}–NH–$, $–(CH_2)_{2\text{-}6}–Tr–$, and $–(CH_2)_{2\text{-}6}–Tr–(CH_2)_{1\text{-}6}–NH$, where Tr is a triazole moiety;

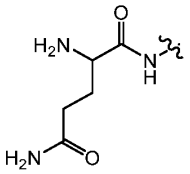
n is 0 or 1;

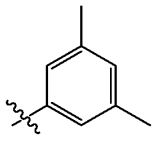
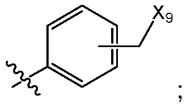
$X_4$ is selected from $-NH_2$, -OH and -N(H)(phenyl);

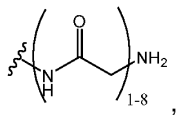
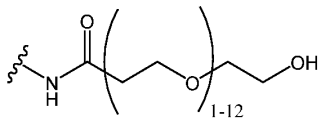
$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  ,  ,  ,  ,  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ;

Ar is selected from  and  ;

$X_9$ is selected from -$NH_2$,  , and  , and

m is an integer from 1 to 4

or a pharmaceutically acceptable salt thereof.

2. A compound having a structure of Formula (I):

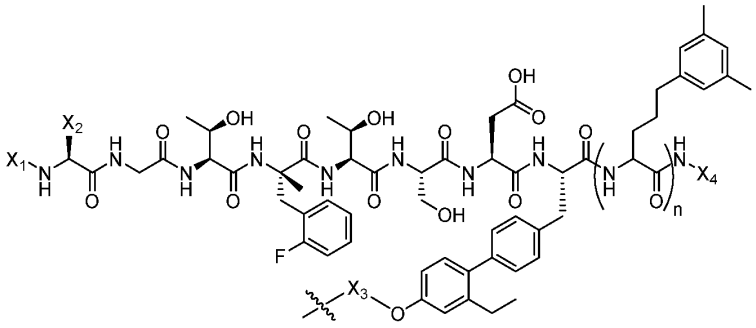
BA-L-P (I),

wherein:

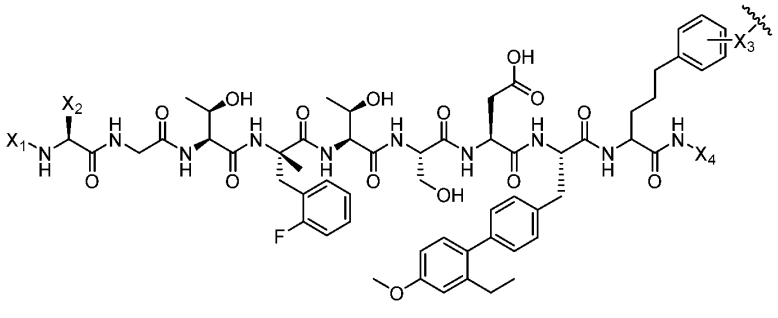
BA is an antibody or an antigen-binding fragment thereof;

L is a non-cleavable linker;

P is a payload having the structure selected from the group consisting of:

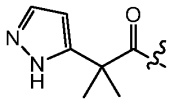
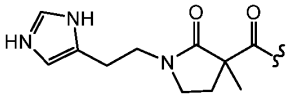
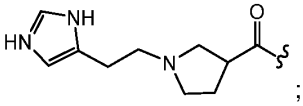


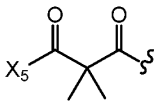
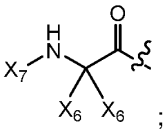
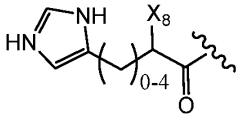
(P-I) and

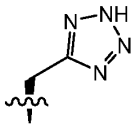
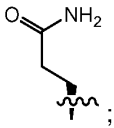


(P-II),

wherein —§– is the point of attachment of the payload to L;

$X_1$ is selected from H;  ;  ;  ;

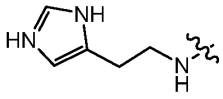
 ;  ; and  ;

$X_2$ is selected from  and  ;

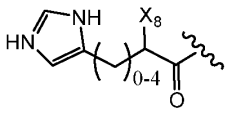
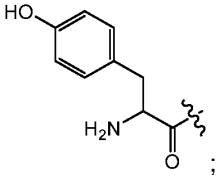
$X_3$ is selected from $–(CH_2)_{2\text{-}6}–NH–$ and $–(CH_2)_{2\text{-}6}–Tr–$, where Tr is a triazole moiety;

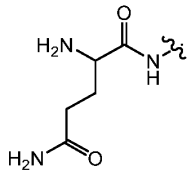
n is 0 or 1;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  , and  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ,

or a pharmaceutically acceptable salt thereof.

3. The compound of claim 1 or 2, wherein BA is a Glucagon-like peptide-1 receptor (GLP1R)-targeting antibody or an antigen-binding fragment thereof.

4. The compound of claim 3, wherein the GLP1R-targeting antibody is a GLP1R agonist antibody.

5. The compound of claim 4, wherein the GLP1R-targeting antibody is 5A10, 9A10, AB9433-I, h38C2, PA5-111834, NLS1205, MAB2814, EPR21819, or glutazumab.

6. The compound of any one of claims 1-5, wherein the linker L is attached to one or both heavy chains of the BA.

7. The compound of claim 6, wherein the linker L is attached to one or both heavy chain variable domains of the BA.

8. The compound of any one of claims 1-7, wherein the linker L is attached to one or both light chains of the BA.

9. The compound of claim 8, wherein the linker L is attached to one or both light chain variable domains of the BA.

10. The compound of any of claims 1-9, wherein the linker L is attached to BA via a glutamine residue.

11. The compound of claim 10, wherein the glutamine residue is introduced to the N-terminus of one or both heavy chains of the BA.

12. The compound of claim 10 or 11 , wherein the glutamine residue is introduced to the N-terminus of one or both light chains of the BA.

13. The compound of claim 10, wherein the glutamine residue is naturally present in a CH2 or CH3 domain of the BA.

14. The compound of claim 10, wherein the glutamine residue is introduced to the BA by modifying one or more amino acids.

15. The compound of claim 14, wherein the glutamine residue is Q295 or N297Q.

16. The compound of any of claims 1-10, wherein the linker L is attached to BA via a lysine residue.

17. The compound of any of claims 1-16, wherein the antibody or antigen-binding fragment thereof is aglycosylated.

18. The compound of any of claims 1-16, wherein the antibody or antigen-binding fragment thereof is deglycosylated.

19. The compound of any of claims 1-18, wherein the antigen-binding fragment is an Fab fragment.

20. The compoud of any of claims 1 and 3-19, wherein m is 1.

21. The compoud of any of claims 1 and 3-19, wherein m is an integer from 2 to 4.

22. The compoud of claim 20, wherein m is 2.

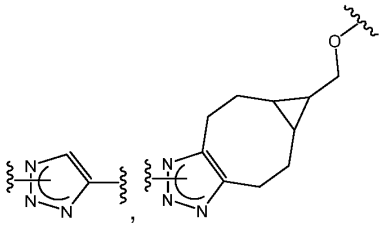
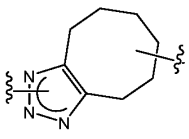
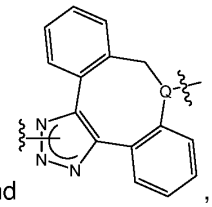
23. The compound of any of claims 1-22, wherein the linker L has the structure of formula (L'):

-La-Y-Lp- (L'),

wherein La is a first linker covalently attached to the BA;
Y is a group comprising a triazole, and
Lp is absent or a second linker covalently attached to the P, wherein when Lp is absent, Y is also absent.

24. The compound of claim 23, wherein Y-Lp is absent.

25. The compound of claim 23, wherein Y has a structure selected from the group consisting of:

 , ,  , and  , wherein Q is C or N.

26. The compound of claim 23 or 24, wherein Lp comprises a polyethylene glycol (PEG) segment having 1 to 36 -$CH_2CH_2O$- (EG) units.

27. The compound of claim 26, wherein the PEG segment comprises between 2 and 30 EG units.

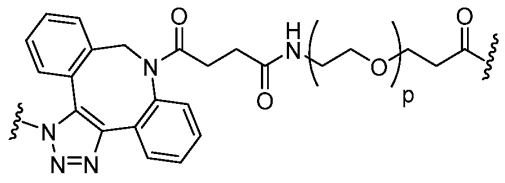
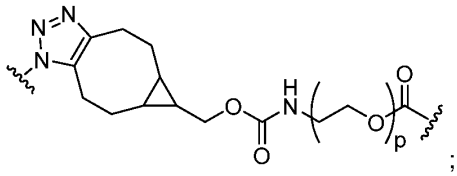
28. The compound of claim 26 or 27, wherein the PEG segment comprises between 4 and 24 EG units.

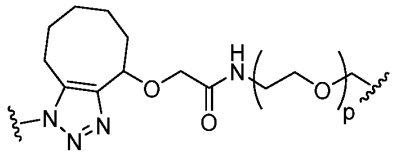
29. The compound of any of claims 26-28, wherein the PEG segment comprises 4 EG units, or 8 EG units, or 12 EG units, or 24 EG units.

30. The compound of any of claims 26-29, wherein the PEG segment comprises 4 EG units.

31. The compound of any of claims 26-29, wherein the PEG segment comprises 8 EG units.

32. The compound of claim 23, wherein Y-Lp has a structure selected from the group consisting of:

 ;  ;



, or a triazole regioisomer thereof,

wherein p is an integer from 1 to 36.

33. The compound of claim 23 or 24, wherein the Lp comprises one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline and combinations thereof.

34. The compound of claim 33, wherein the Lp comprises 1 to 10 glycines.

35. The compound of claim 33, wherein the Lp comprises 1 to 6 serines.

36. The compound of claim 33, wherein the Lp comprises 1 to 10 glycines and 1 to 6 serines.

37. The compound of any of claims 33-36, wherein the Lp comprises 4 glycines and 1 serine.

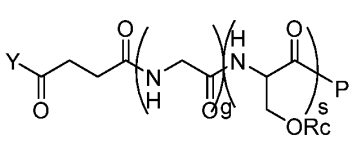
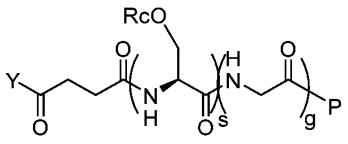
38. The compound of any of claims 33-37, wherein the Lp is selected from the group consisting of Gly-Gly-Gly-Gly-Ser ($G_4S$) (SEQ ID NO: 1), Ser-Gly-Gly-Gly-Gly ($SG_4$) (SEQ ID NO: 2), and Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser ($G_4S$-$G_4S$) (SEQ ID NO: 3).

39. The compound of any of claims 33-38, wherein the Lp comprises a combination of a PEG segment having 1 to 36 EG units and one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline and combinations thereof.

40. The compound of any of claims 35-38, wherein the serine residue comprises a carbohydrate group.

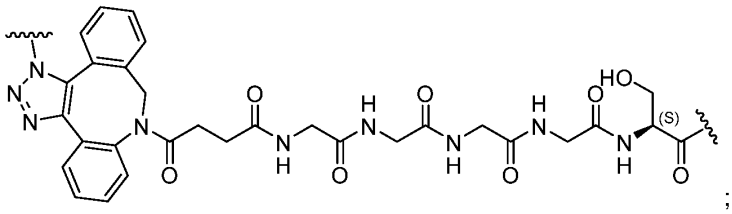
41. The compound of any of claims 35-40, wherein the serine residue comprises a glucose group.

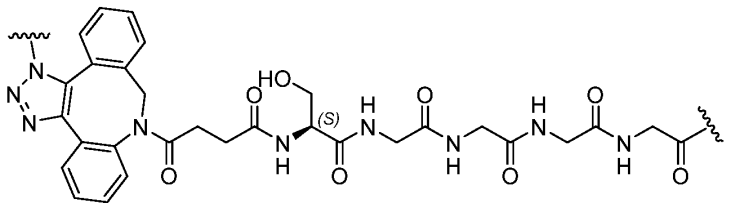
42. The compound of any of claims 33-41 wherein Lp has a structure selected from the group consisting of:

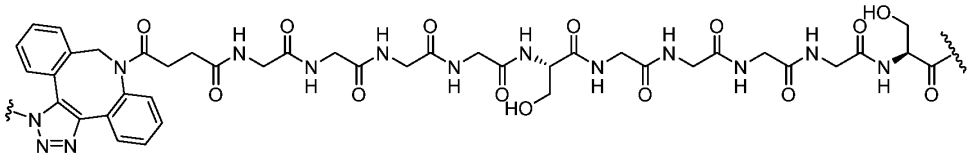

and

,

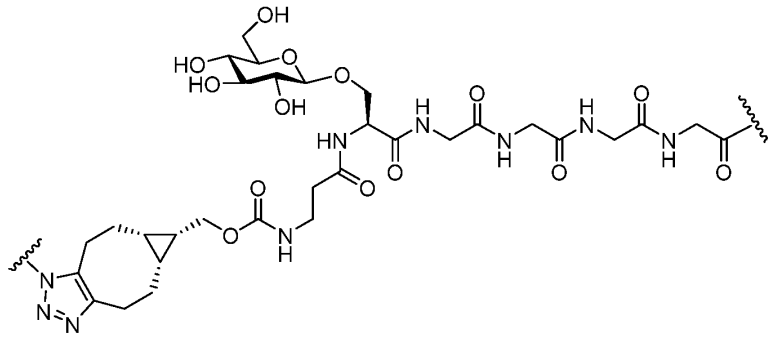
wherein Y is the group comprising a triazole and P is the payload, and wherein Rc is selected from H and glucose, g is an integer from 1 to 10 and s is an integer from 0 to 4.

43. The compound of any of claims 33-41 wherein Y-Lp has a structure selected from the group consisting of:

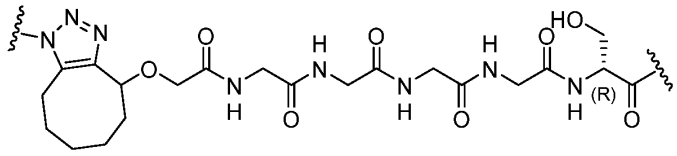

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;

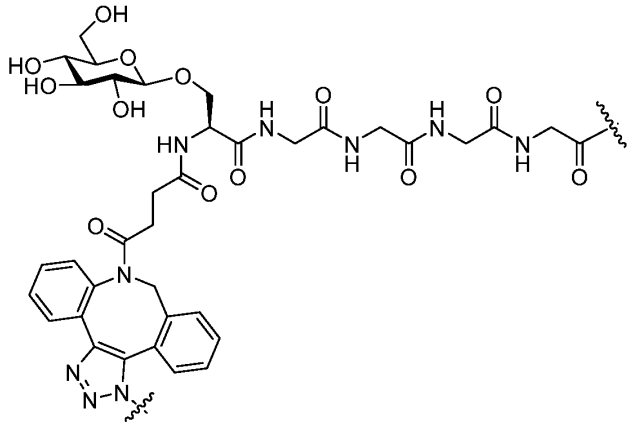

;



;



; and



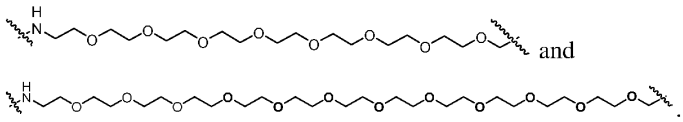
, or a triazole regioisomer thereof.

44. The compound of any one of claims 23-43, wherein La comprises a polyethylene glycol (PEG) segment having 1 to 36 -$CH_2CH_2O$- (EG) units.

45. The compound of claim 44, wherein the PEG segment comprises 4 EG units, or 8 EG units, or 12 EG units, or 24 EG units.

46. The compound of claim 45, wherein the PEG segment comprises 8 EG units.

47. The compound of any one of claims 23-46, wherein La has a structure selected from the group consisting of:

 and

.

48. The compound of any one of claims 23-45, wherein La comprises one or more amino acids selected from glycine, threonine, serine, glutamine, glutamic acid, alanine, valine, leucine, and proline and combinations thereof.

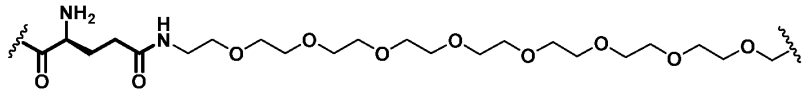
49. The compound of claim 46, wherein the La comprises 1 to 10 glycines and 1 to 6 serines.

50. The compound of any one of claims 46-49, wherein the La comprises 4 glycines and 1 serine.

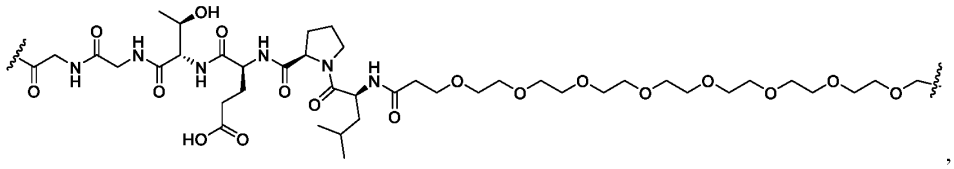
51. The compound of any of claims 46-50, wherein the La is selected from the group consisting of Gly-Gly-Gly-Gly-Ser ($G_4S$) (SEQ ID NO: 1), Ser-Gly-Gly-Gly-Gly ($SG_4$) (SEQ ID NO: 2), Gly-Gly-Ser-Gly-Gly-Ser-Gly-Gly ($G_2S$-$G_2S$-$G_2$), and Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly ($G_4S$-$G_4$) (SEQ ID NO: 3).

52. The compound of claim 46, wherein La comprises a combination of a PEG segment having 1 to 36 EG units and one or more amino acids selected from glycine, threonine, serine, glutamine, glutamic acid, alanine, valine, leucine, and proline and combinations thereof.

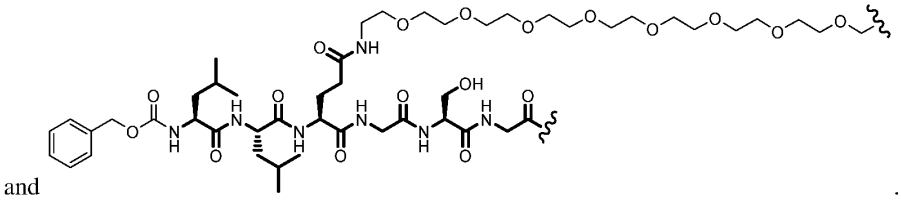
53. The compound of any of claims 46-52, wherein the La is selected from the group consisting of:



,



,

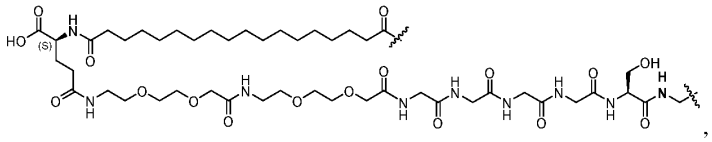


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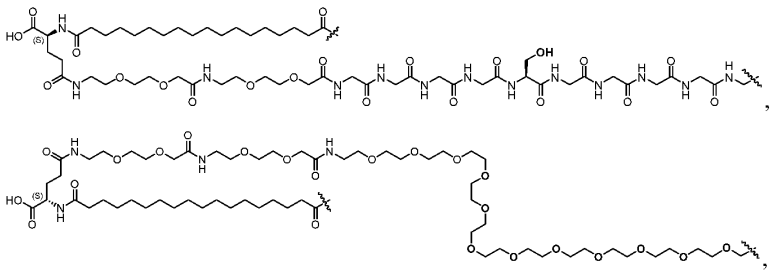
54. The compound of any one of claims 23-53, wherein La comprises a $-(CH_2)_{2\text{-}24}-$ chain.

55. The compound of claim 54, wherein La comprises a combination of a $-(CH_2)_{2\text{-}24}-$ chain, a PEG segment having 1 to 36 EG units and one or more amino acids selected from glycine, threonine, serine, glutamine, glutamic acid, alanine, valine, leucine, and proline and combinations thereof.

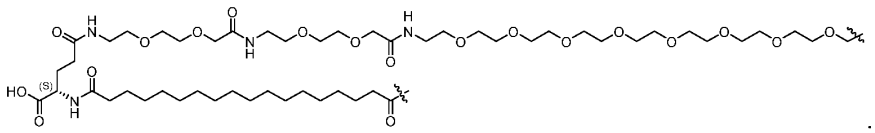
56. The compound of claim 55, wherein the La is selected from the group consisting of:



,

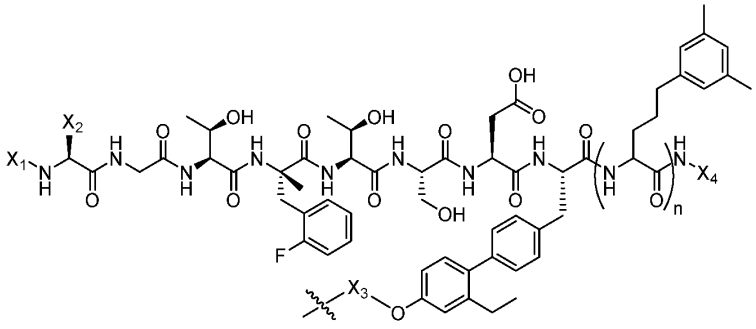


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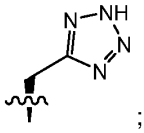


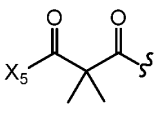
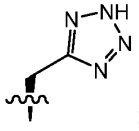
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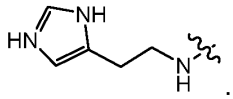
57. The compound of any of claims 1-43, wherein P has the structure



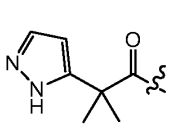
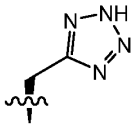
(P-I).

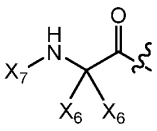
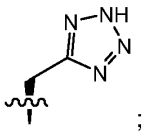
58. The compound of any of claims 1-44, wherein $X_1$ is H; $X_2$ is  ; $X_3$ is selected from $–(CH_2)_{2-6}–NH–$ and $–(CH_2)_{2-6}–Tr–$, where Tr is a triazole moiety; n is 1, and $X_4$ is H.

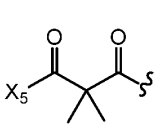
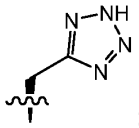
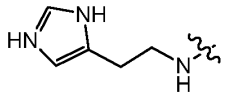
59. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2-6}–NH–$; n is 1; $X_4$ is H, and $X_5$ is selected from -OH, $-NH_2$, -NH-OH, and

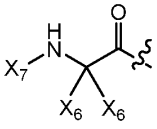
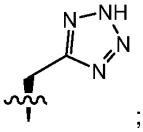
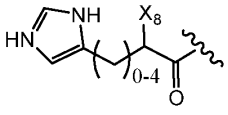


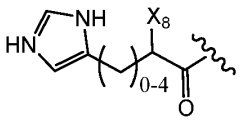
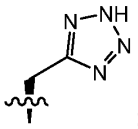
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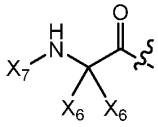
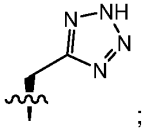
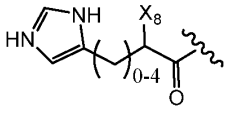
60. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1, and $X_4$ is H.

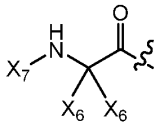
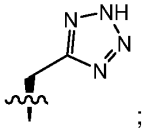
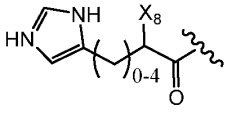
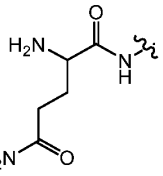
61. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and $-CH_2OH$, and $X_7$ is H.

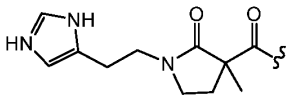
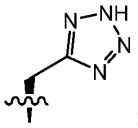
62. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–Tr–$, where Tr is a triazole moiety; n is 1; $X_4$ is H, and $X_5$ is  .

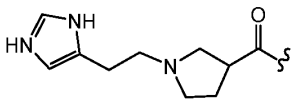
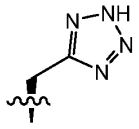
63. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and $-CH_3$; $X_7$ is  , and $X_8$ is $-NH_2$.

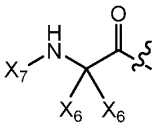
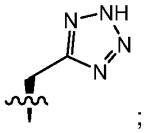
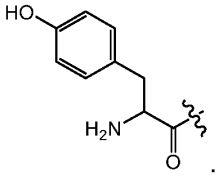
64. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H, and $X_8$ is H.

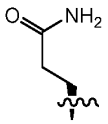
65. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H; $X_6$ is H at each occurrence; $X_7$ is  , and $X_8$ is H.

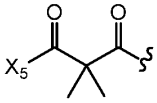
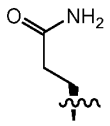
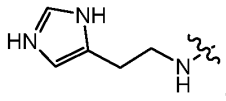
66. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and $-CH_3$; $X_7$ is  , and $X_8$ is  .

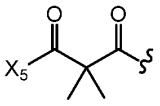
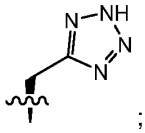
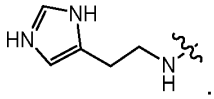
67. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1, and $X_4$ is H.

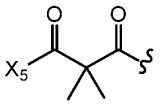
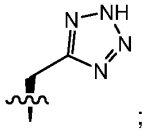
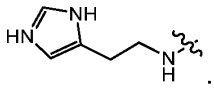
68. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1, and $X_4$ is H.

69. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H; $X_6$ is independently at each occurrence selected from H and $-CH_3$, and $X_7$ is  .

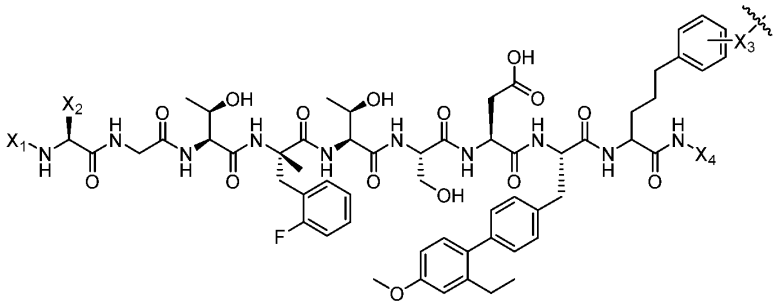
70. The compound of any of claims 1-44, wherein $X_1$ is H; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1, and $X_4$ is H.

71. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $–(CH_2)_{2\text{-}6}–NH–$; n is 1; $X_4$ is H, and $X_5$ is  .

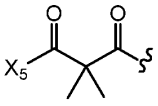
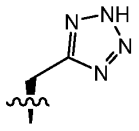
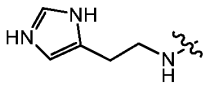
72. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 0; $X_4$ is phenyl, and $X_5$ is  .

73. The compound of any of claims 1-44, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$; n is 1; $X_4$ is phenyl, and $X_5$ is  .

74. The compound of any of claims 1-43, wherein P has the structure

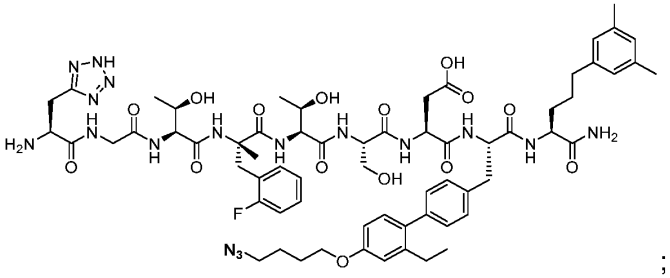


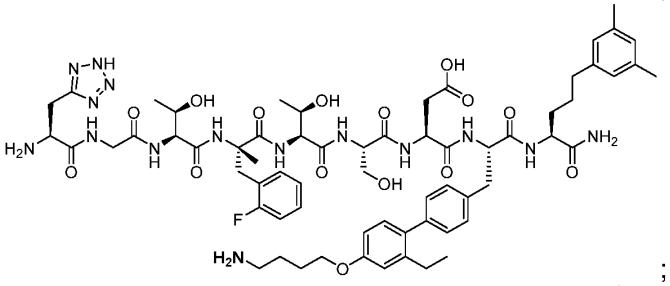
(P-II).

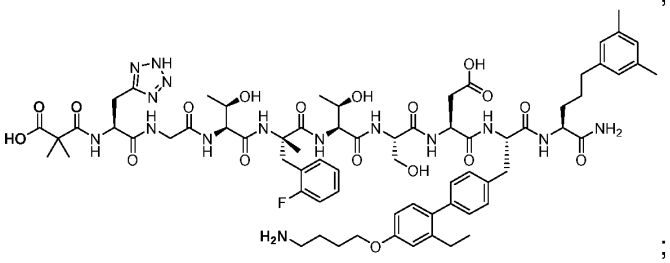
75. The compound of claim 74, wherein $X_1$ is  ; $X_2$ is  ; $X_3$ is $-(CH_2)_{2\text{-}6}-NH-$;$X_4$ is H, and $X_5$ is  .

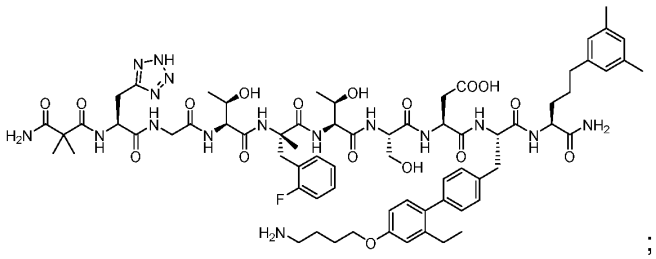
76. The compound of any of claims 1-75, wherein P has the structure selected from the group consisting of:

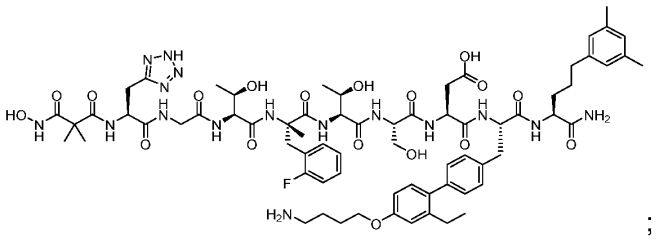
Structure

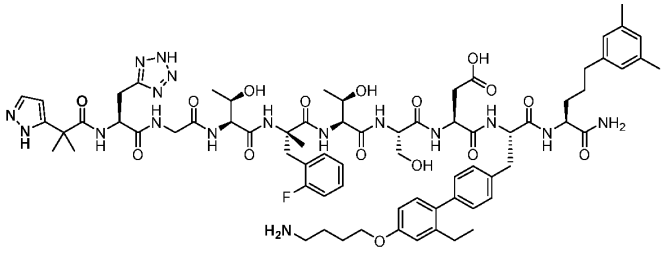

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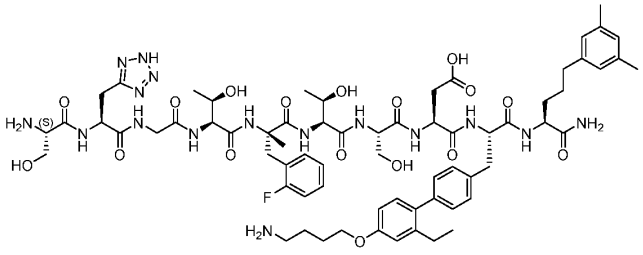

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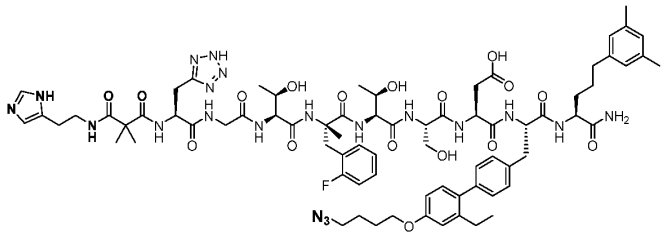

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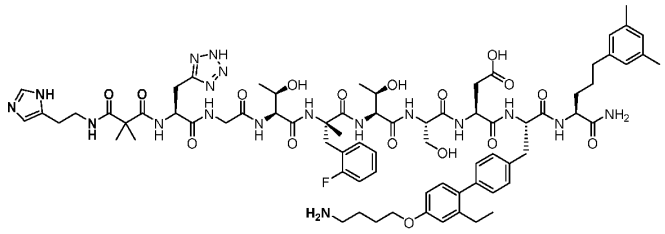

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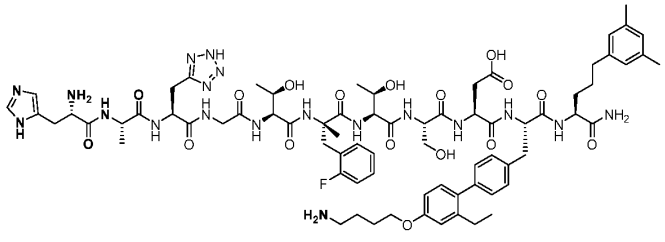

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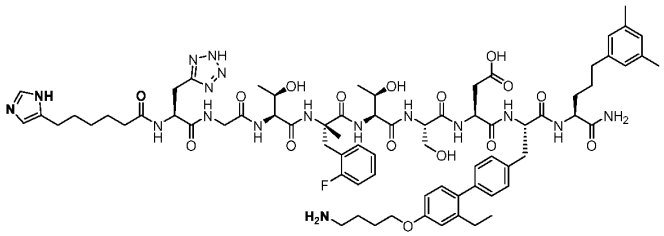

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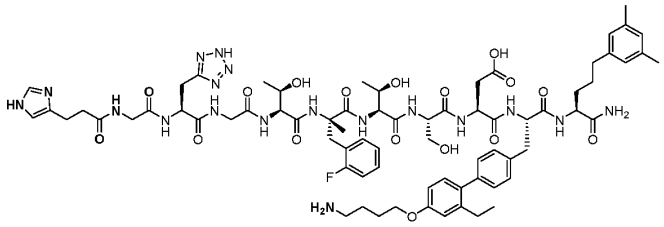

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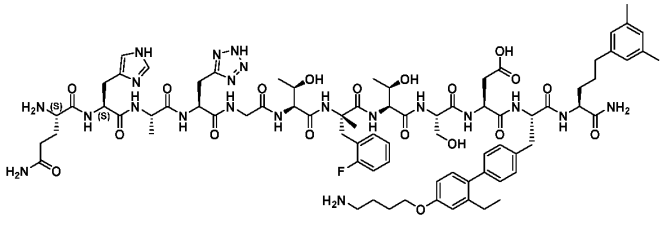

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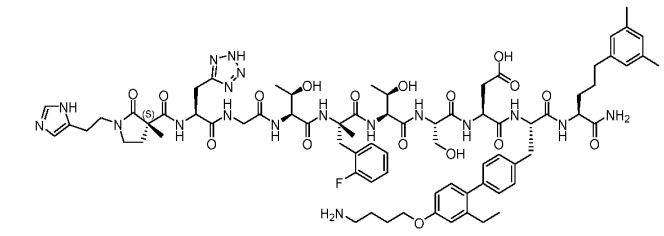

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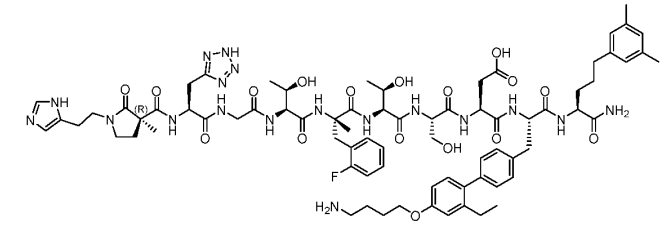

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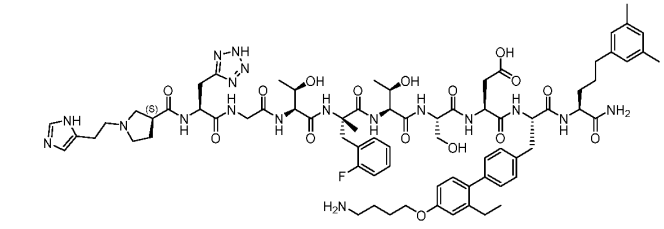

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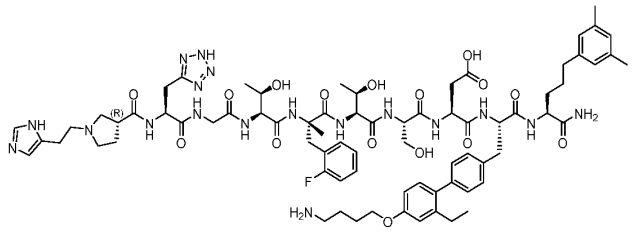

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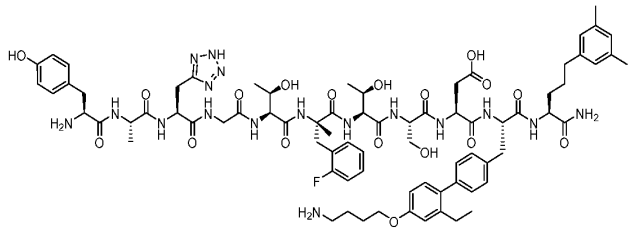

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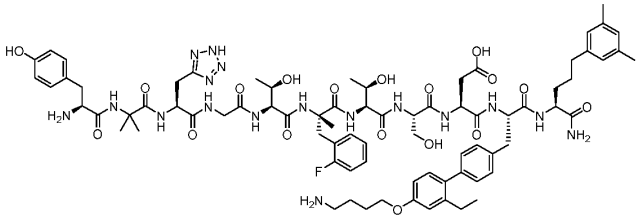

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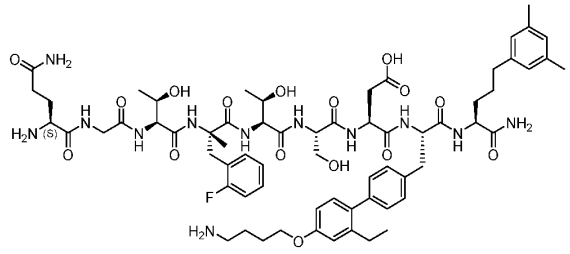

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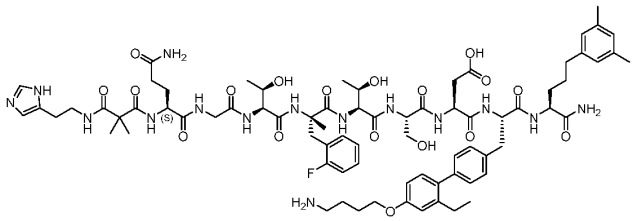

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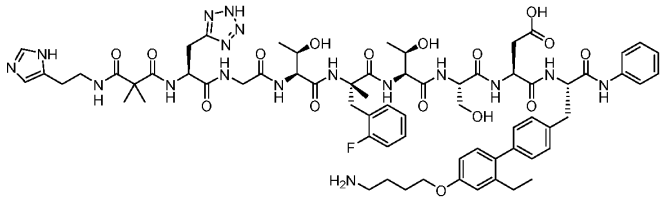

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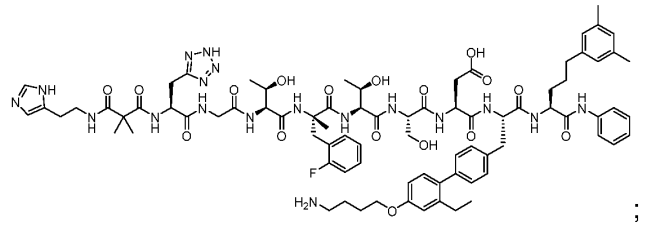

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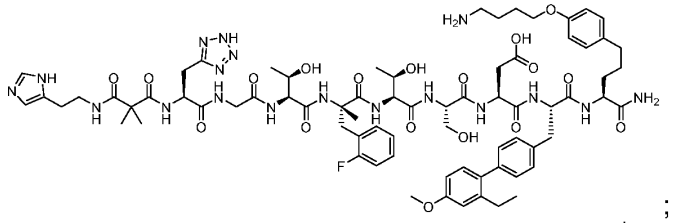

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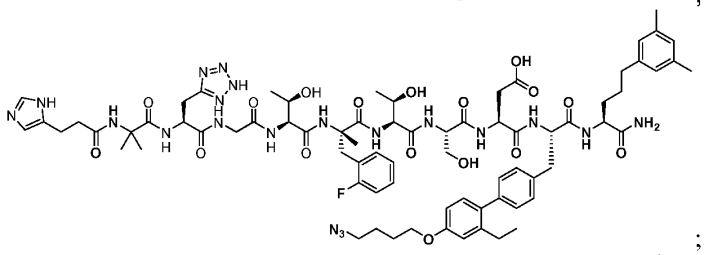

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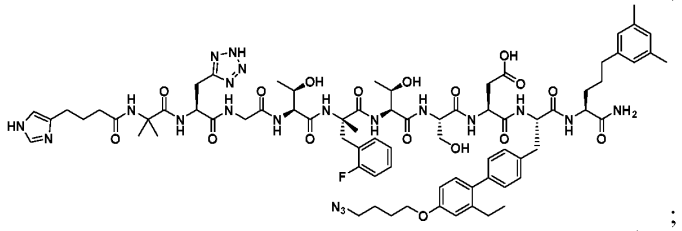

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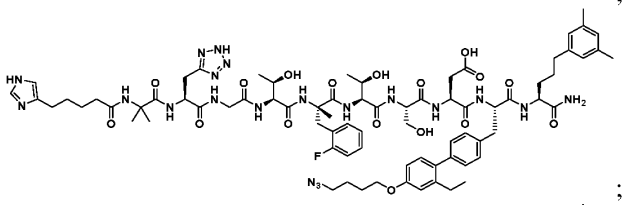

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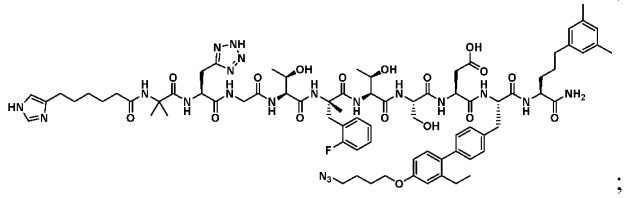

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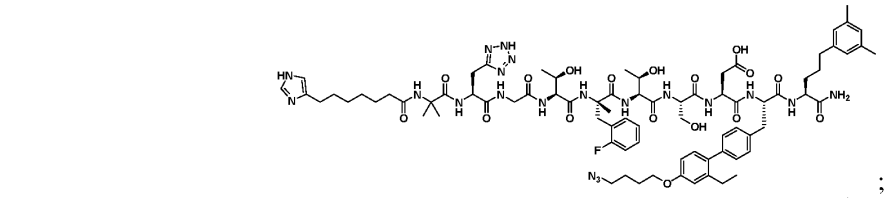

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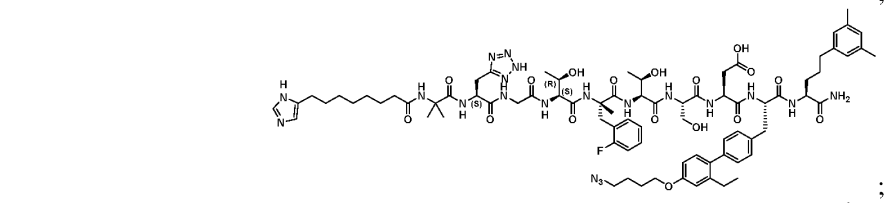

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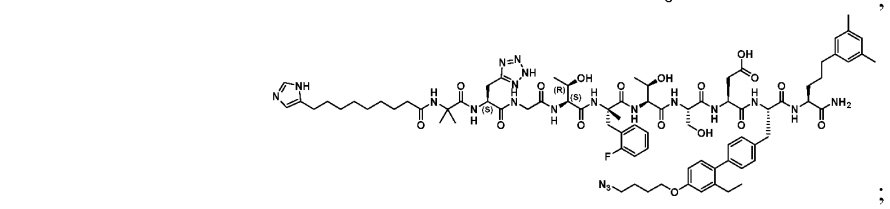

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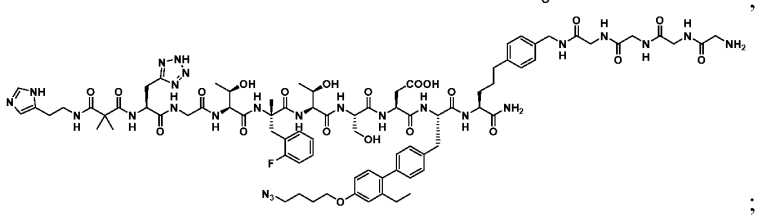

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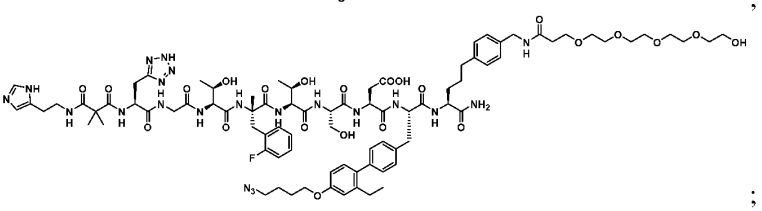

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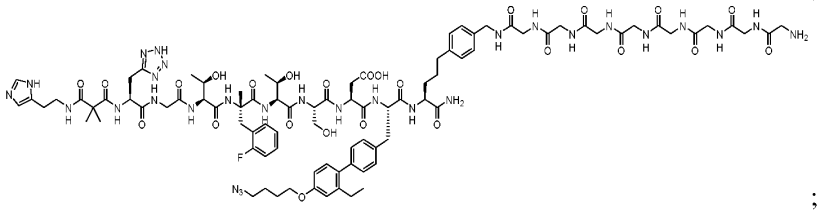

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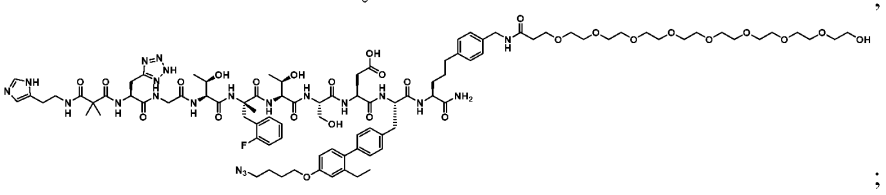

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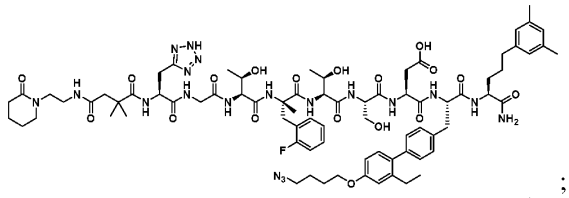

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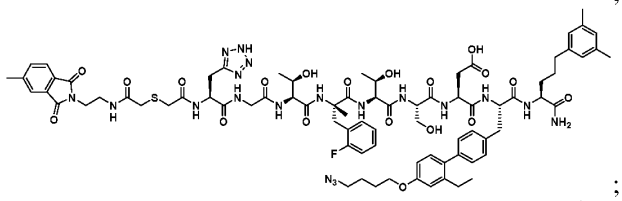

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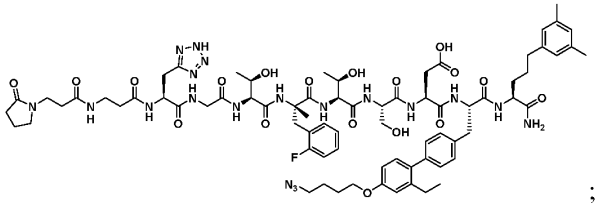

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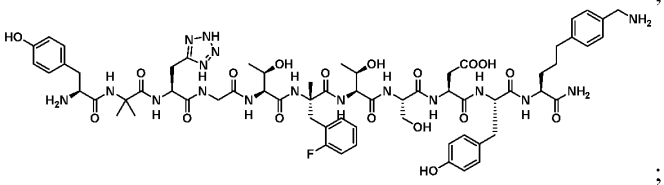

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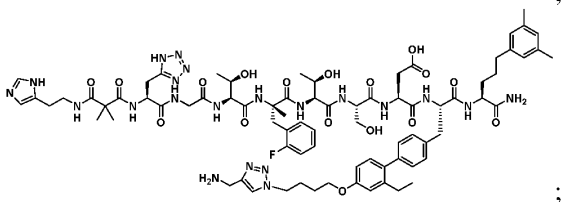

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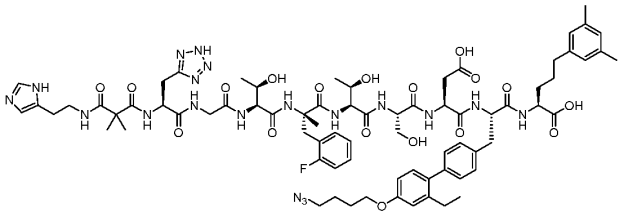

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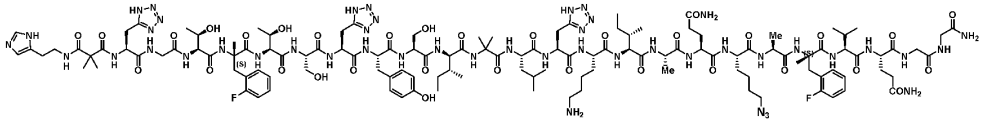

;


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;


;


, and


.

77. The compound of any of claims 1-76, wherein the compound has a half life of longer than 7 days in plasma.

78. The compound of any of claims 1-77, wherein the compound does not bind to G protein-coupled receptors (GPCRs) other than GLP1R.

79. A pharmaceutical composition comprising the compound of any of claims 1-78.

80. A pharmaceutical dosage form comprising the compound of any of claims 1-78.

81. A method of selectively targeting GLP1R on a surface of a cell with the compound according to any of claims 1-78.

82. The method of claim 81, wherein the cell is a mammalian cell.

83. The method of claim 81 or 82, wherein the cell is a human cell.

84. The method of any of claims 81-83, wherein the cell is a pancreatic cell, a brain cell, a heart cell, a vascular tissue cell, a kidney cell, an adipose tissue cell, a liver cell, or a muscle cell.

85. A method of enhancing GLP1R activity in an individual in need thereof comprising administering to the individual an effective amount of the compound of any of claims 1-78, the composition of claim 79, or the dosage form of claim 80.

86. A method of lowering blood glucose levels in an individual in need thereof comprising administering to the individual an effective amount of the compound of any of claims 1-78, the composition of claim 79, or the dosage form of claim 80.

87. A method of lowering body weight in an individual in need thereof comprising administering to the individual an effective amount of the compound of any of claims 1-78, the composition of claim 79, or the dosage form of claim 80.

88. A method of treating a GLP1R-associated condition in an individual in need thereof comprising administering to the individual an effective amount of the compound of any of claims 1-78, the composition of claim 79, or the dosage form of claim 80.

89. The method of claim 88, wherein the GLP1R-associated condition is type II diabetes, obesity, liver disease, coronary artery disease, or kidney disease.

90. The method of claim 88, wherein the GLP1R-associated condition is type II diabetes and/or obesity.

91. The method of any of claims 81-89, wherein the compound of any of claims 1-78, the composition of claim 79, or the dosage form of claim 80 is administered subcutaneously, intravenously, intradermally, intraperitoneally, or intramuscularly.

92. A method of producing the compound of claim 1 having a structure of Formula (A):

$$BA\text{-}(L\text{-}P)_m \quad (A),$$

the method comprising the steps of:
a) contacting, in the presence of a transglutaminase, the BA comprising at least m glutamine residues Gln with at least m equivalents of compound L-P, and
b) isolating the produced compound of Formula (A).

93. A method of producing the compound of claim 2 having a structure of Formula (I):

$$BA\text{-}L\text{-}P \quad (I),$$

the method comprising the steps of:
a) contacting, in the presence of a transglutaminase, the BA comprising at least one glutamine residue Gln with a compound L-P, and
b) isolating the produced compound of Formula (I).

94. A method of producing the compound of claim 1 having a structure of Formula (A):

$$BA\text{-}(L\text{-}P)_m \quad (A),$$

wherein the linker L has has the structure of formula (L'):

-La-Y-Lp- (L'),

wherein La is a first linker covalently attached to the BA;
Y is a group comprising a triazole, and
Lp is a second linker covalently attached to the P,
the method comprising the steps of:
a) contacting, in the presence of a transglutaminase, the BA comprising at least m glutamine residues Gln with the first linker La comprising an azide or an alkyne moiety;
b) contacting the product of step a) with at least m equivalents of compound Lp-P, wherein the second linker Lp comprises an azide or an alkyne moiety, wherein La and Lp are capable of reacting to produce a triazole, and
c) isolating the produced compound of Formula (A).

95. A method of producing the compound of claim 2 having a structure of Formula (I):

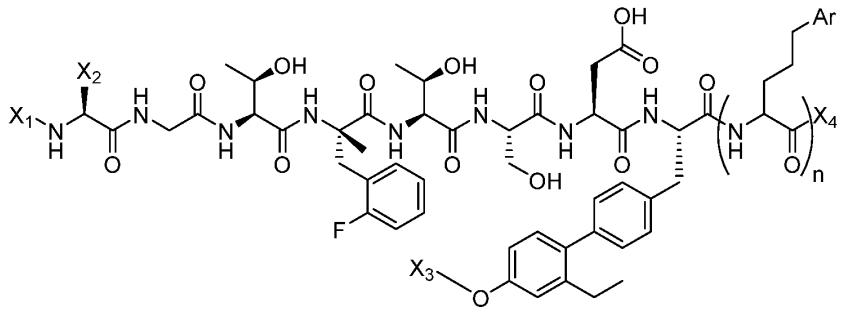
BA-L-P (I),

wherein the linker L has has the structure of formula (L'):

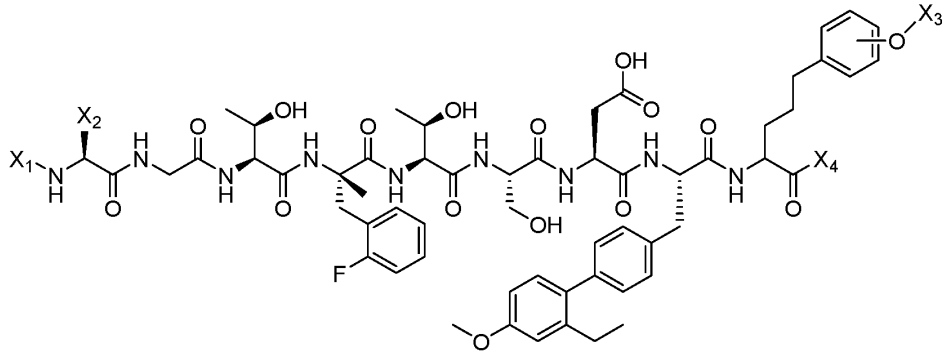
-La-Y-Lp- (L'),

wherein La is a first linker covalently attached to the BA;
Y is a group comprising a triazole, and
Lp is a second linker covalently attached to the P,
the method comprising the steps of:
a) contacting, in the presence of a transglutaminase, the BA comprising at least one glutamine residue Gln with the first linker La comprising an azide or an alkyne moiety;
b) contacting the product of step a) with a compound Lp-P, wherein the second linker Lp comprises an azide or an alkyne moiety, wherein La and Lp are capable of reacting to produce a triazole, and
c) isolating the produced compound of Formula (I).

96. A compound having a structure selected from the group consisting of Formula (P-IB), Formula (P-IIB), and Formula (P-IIIB):

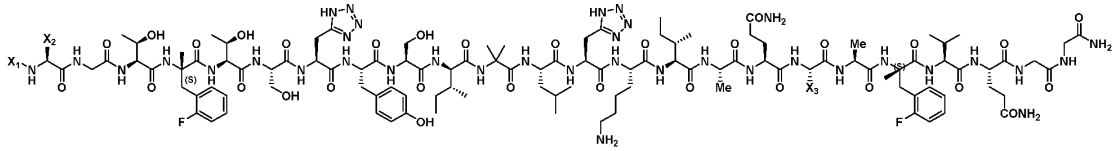


(P-IB),

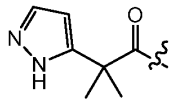
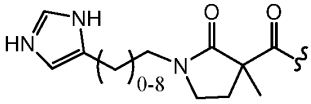
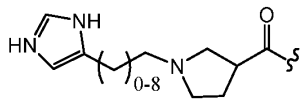


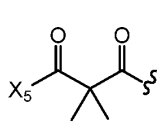
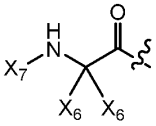
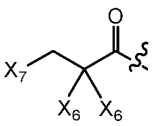
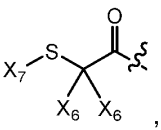
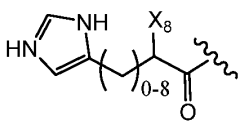
(P-IIB),

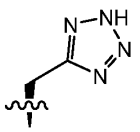
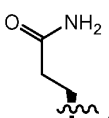
and



(P-IIIB), wherein:

$X_1$ is selected from H;  ;  ;  ;

 ;  ;  ;  , and  ;

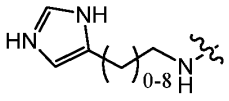
 and  ;

$X_2$ is selected from

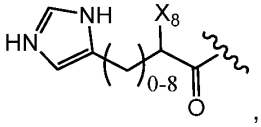
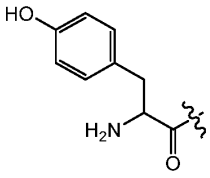
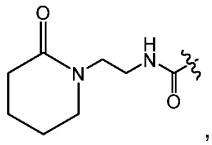
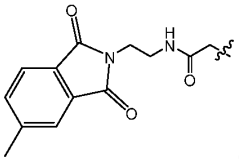
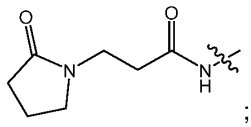
$X_3$ is selected from -$CH_3$, –$(CH_2)_{2-6}$–$NH_2$, –$(CH_2)_{2-6}$–$N_3$, and –$(CH_2)_{2-6}$–Tr–$(CH_2)_{1-6}$–$NH_2$, where Tr is a triazole moiety;

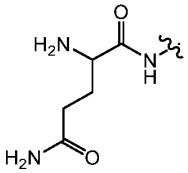
n is 0 or 1;

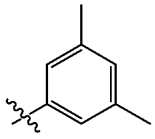
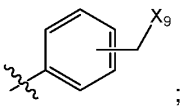
$X_4$ is selected from $-NH_2$, -OH and -N(H)(phenyl);

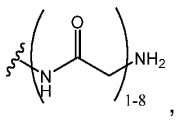
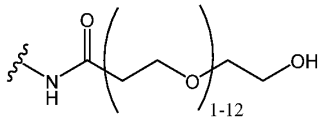
$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

$X_7$ is selected from H,  ,  ,  ,  ,  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ;

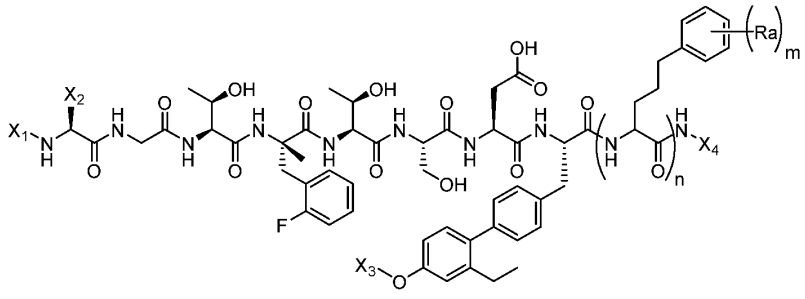
Ar is selected from  and  ;

$X_9$ is selected from $-NH_2$,  , and  , and

m is an integer from 1 to 4

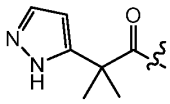
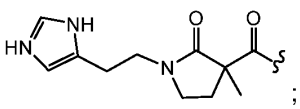
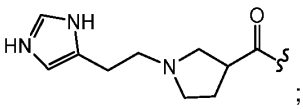
or a pharmaceutically acceptable salt thereof.

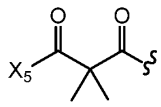
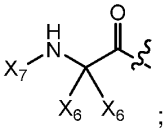
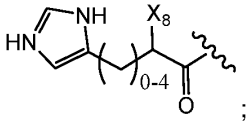
97. A compound having a structure of Formula (II):

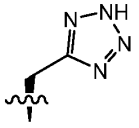
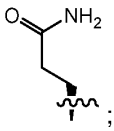


(II),

wherein:

$X_1$ is selected from H;  ;  ;  ;

 ;  ; and  ;

$X_2$ is selected from  and  ;

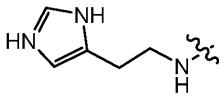
$X_3$ is selected from $–(CH_2)_{2\text{-}6}–NH_2$, $–(CH_2)_{2\text{-}6}–N_3$, and $-CH_3$, with the proviso that when $X_3$ is $-CH_3$, n is 1 and Ra in at least one occurrence is selected from $–(CH_2)_{2\text{-}6}–NH_2$ and $–(CH_2)_{2\text{-}6}–N_3$;

n is 0 or 1;

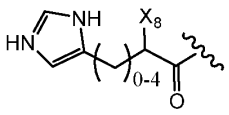
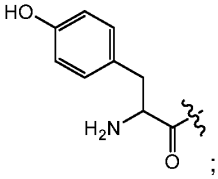
m is an integer from 0 to 3;

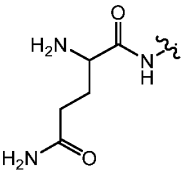
Ra is independently at each occurrence selected from $-CH_3$, $–(CH_2)_{2\text{-}6}–NH_2$, and $–(CH_2)_{2\text{-}6}–N_3$;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

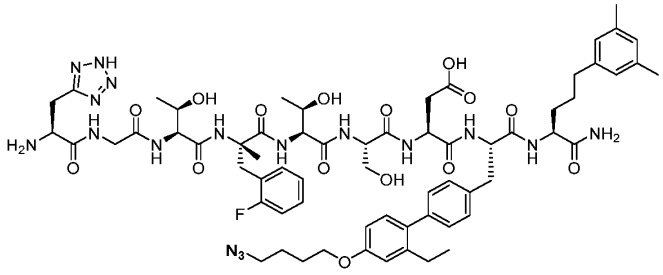
$X_7$ is selected from H,  , and  ;

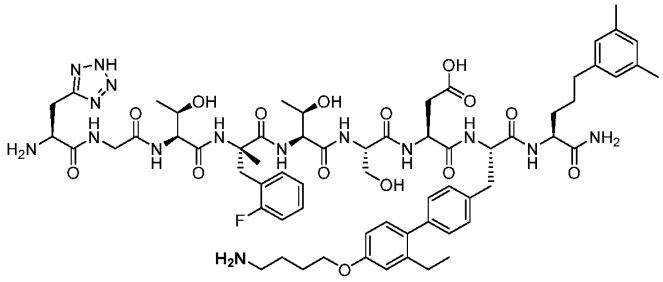


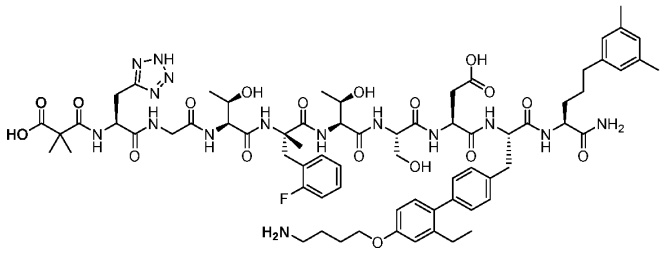
$X_8$ is selected from H, -OH, $-NH_2$, and , and pharmaceutically acceptable salts thereof.

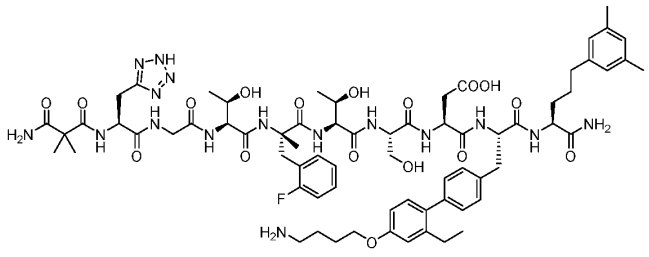
98. The compound of claim 96, wherein P has the structure selected from the group consisting of:

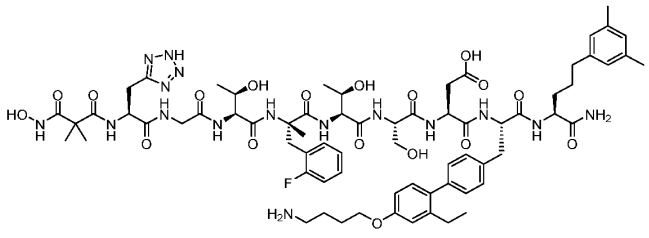
Structure

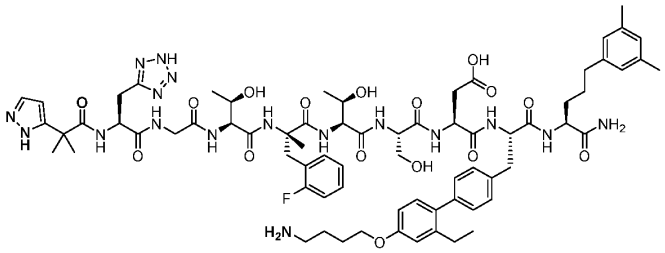

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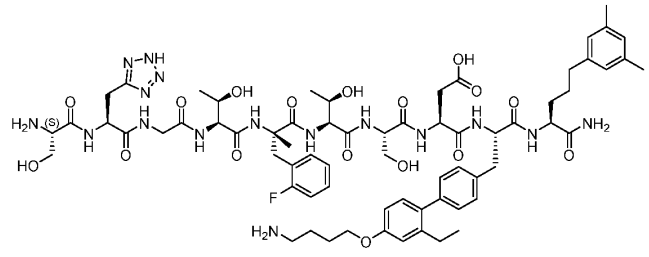

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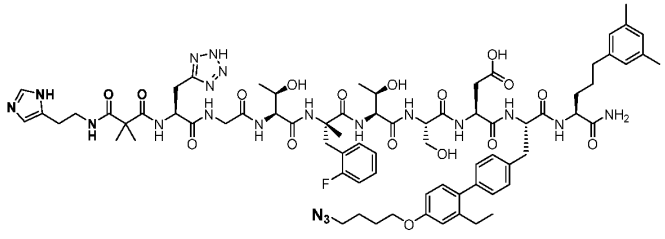

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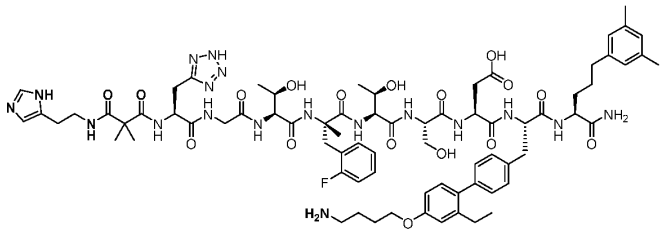

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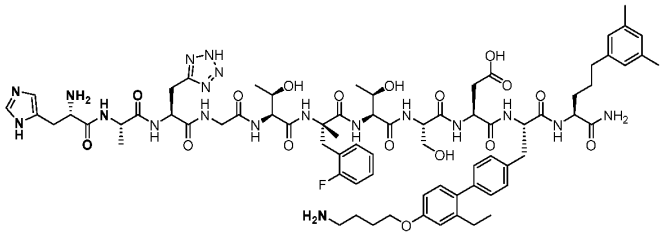

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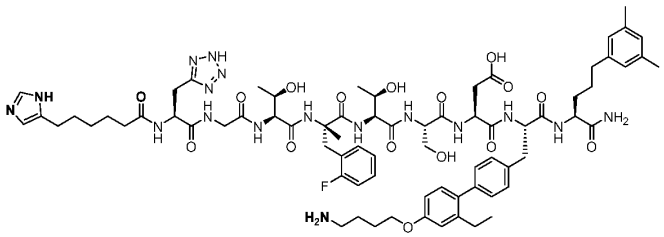

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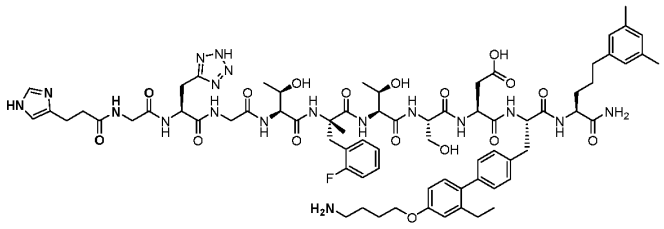

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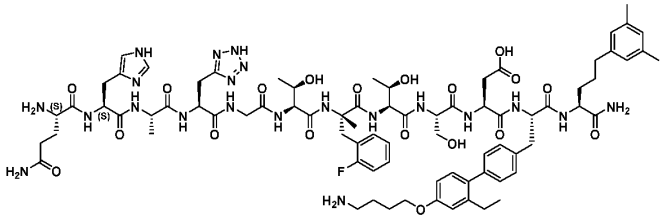

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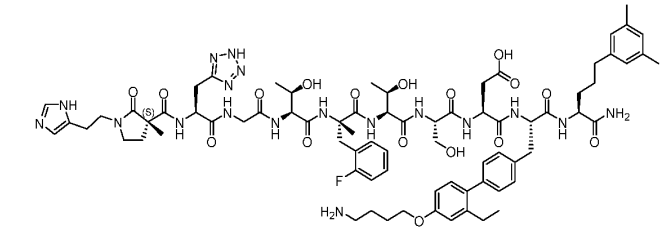

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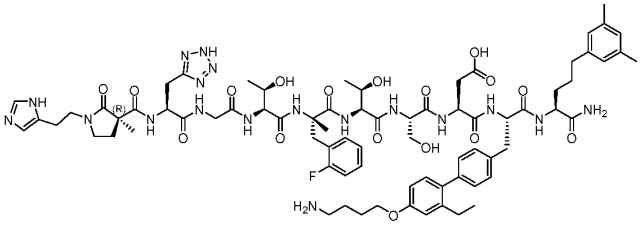

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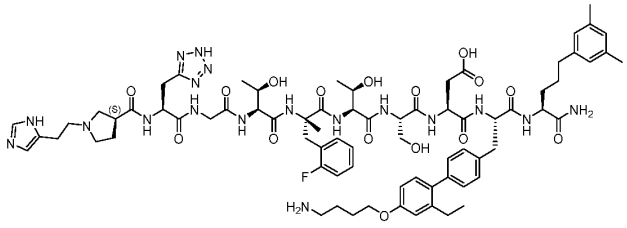

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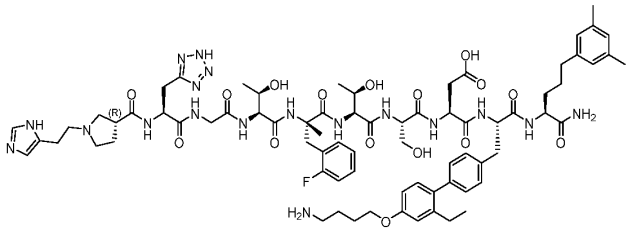

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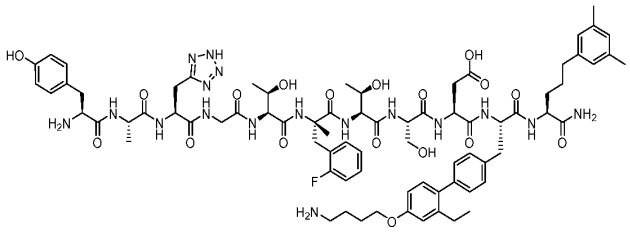

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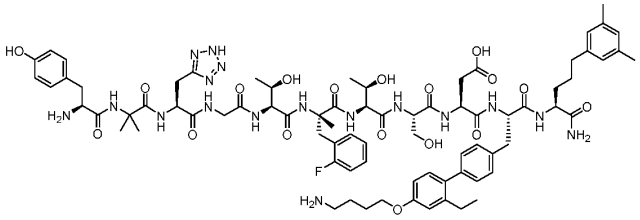

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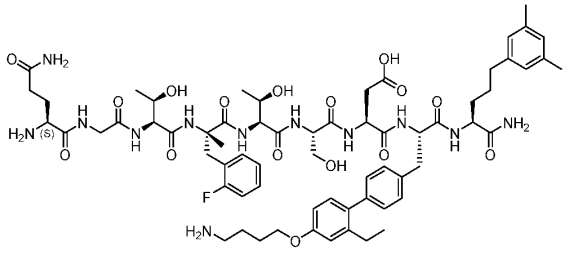

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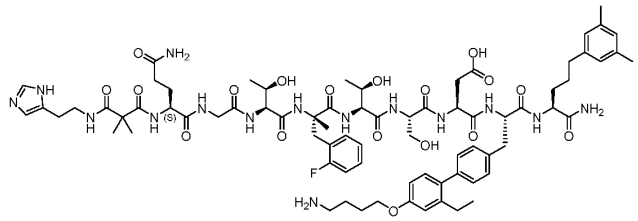

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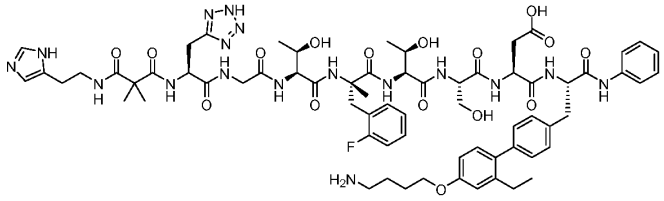

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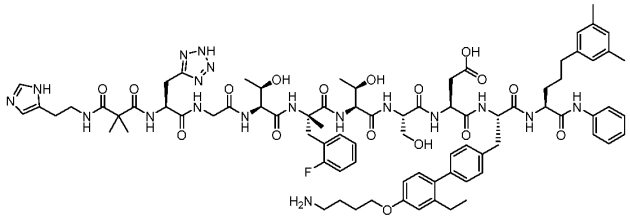

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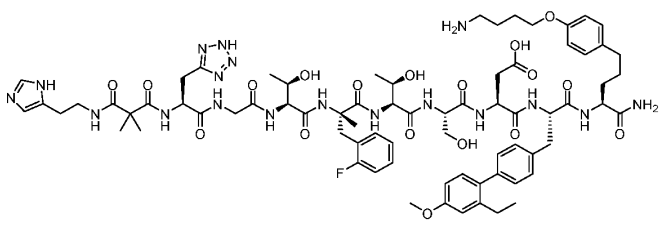

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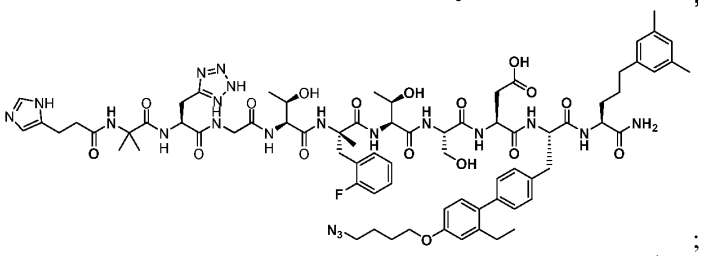

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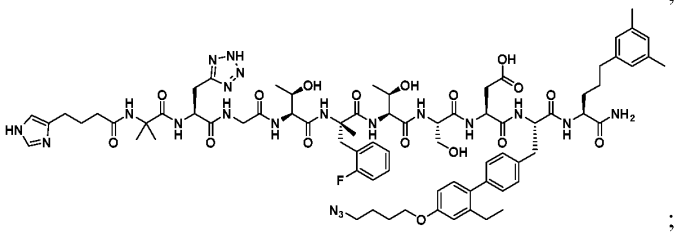

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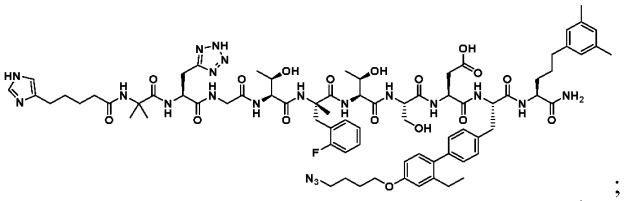

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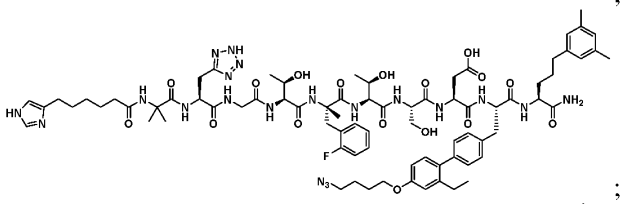

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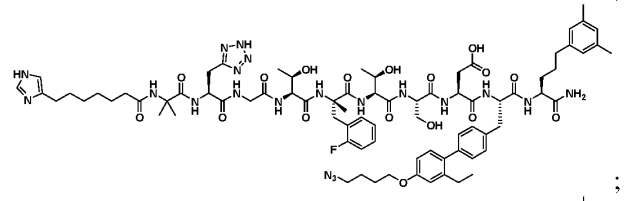

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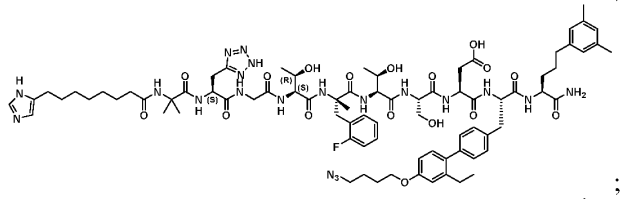

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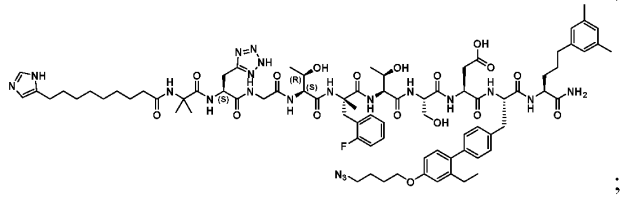

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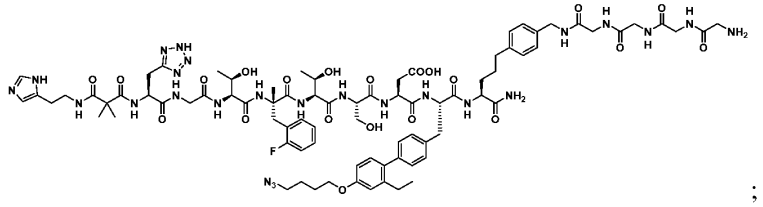

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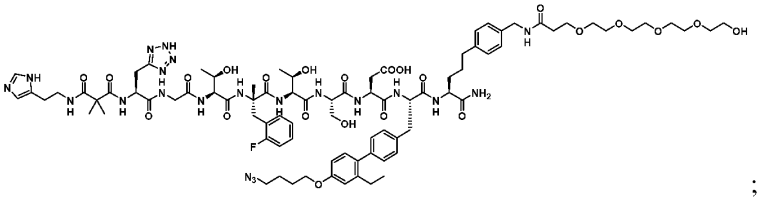

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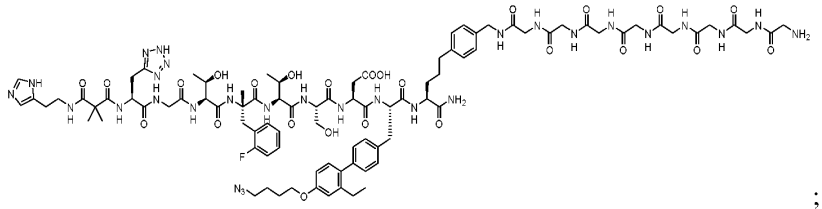

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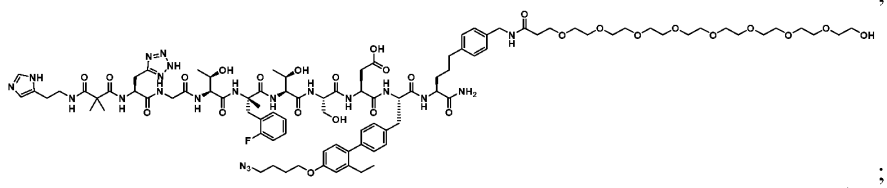

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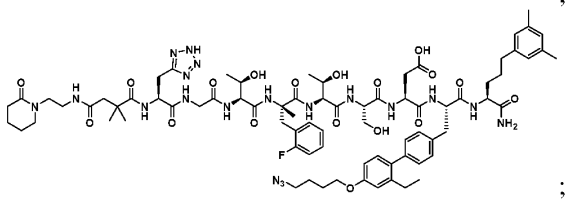

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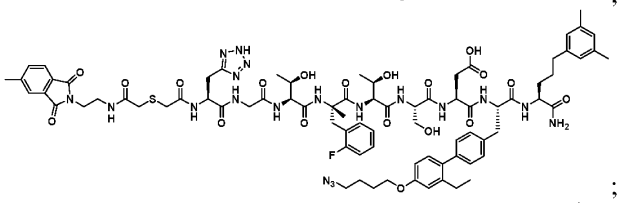

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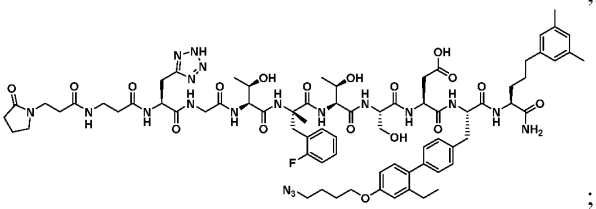

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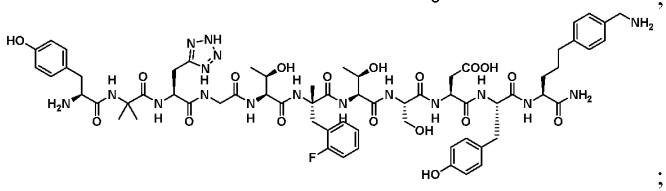

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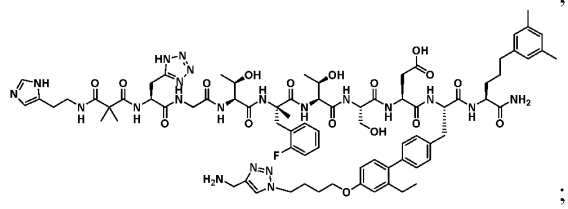

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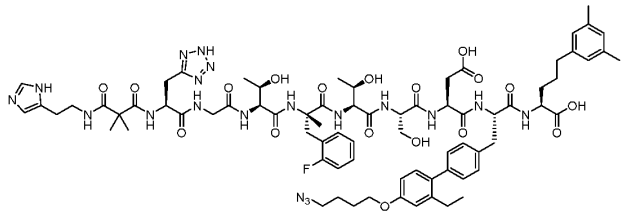

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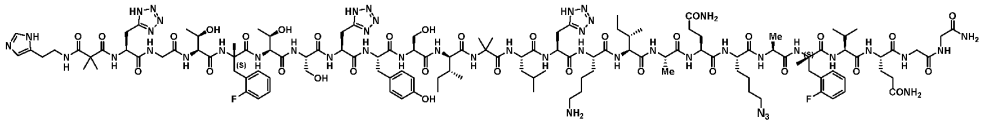

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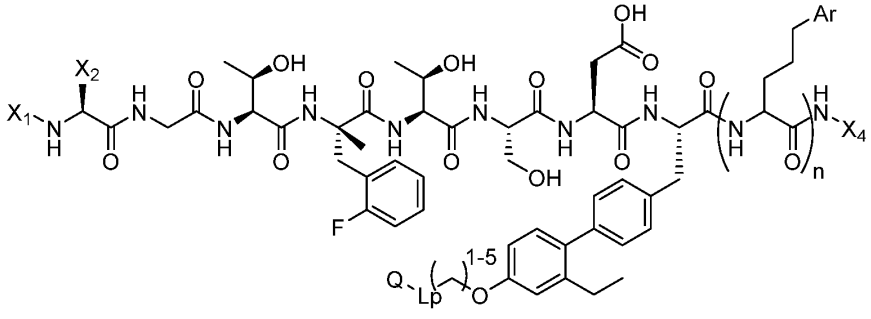


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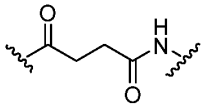
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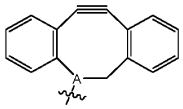
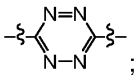
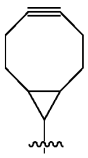
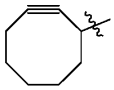
99. A compound having a structure of Formula (C):

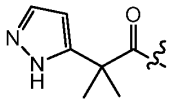
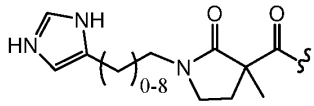
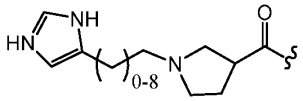


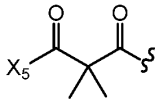
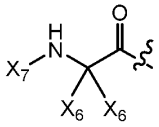
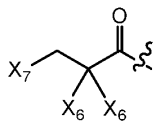
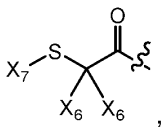
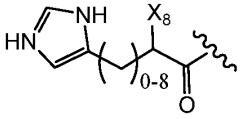
(C),

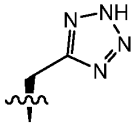
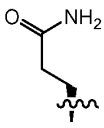
wherein:

$L_p$ is absent or a linker comprising one or more of  ; a carbamate group; a cyclodextrin; a polyethylene glycol (PEG) segment having 1 to 36 $-CH_2CH_2O-$ (EG) units; a $-(CH_2)_{2-24}-$ chain; a triazole; one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline, and combinations thereof;

Q is a moiety selected from $-NH_2$, $-N_3$, ,  ;  ;  ; and  , where A is C or N;

$X_1$ is selected from H;  ;  ;  ;

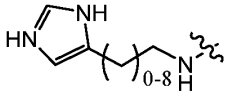
 ;  ;  ;  , and  ;

$X_2$ is selected from  and  ;

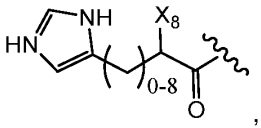
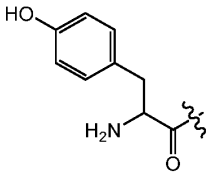
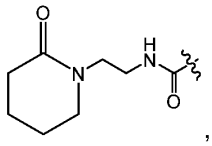
$X_3$ is selected from -$CH_3$, –$(CH_2)_{2\text{-}6}$–$NH_2$, –$(CH_2)_{2\text{-}6}$–$N_3$, and –$(CH_2)_{2\text{-}6}$–Tr–$(CH_2)_{1\text{-}6}$–$NH_2$, where Tr is a triazole moiety;

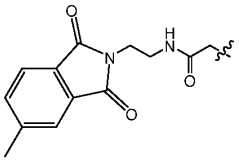
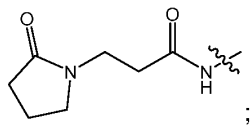
n is 0 or 1;

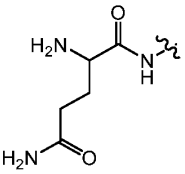
$X_4$ is selected from -$NH_2$, -OH and -N(H)(phenyl);

$X_5$ is selected from -OH, -$NH_2$, -NH-OH, and  ;

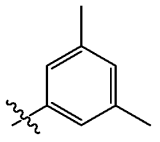
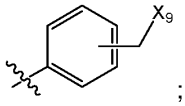
$X_6$ is independently at each occurrence selected from H, -OH, -$CH_3$, and -$CH_2OH$;

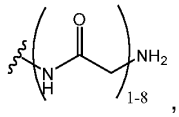
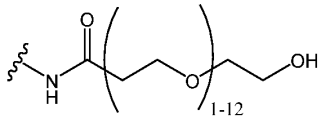
$X_7$ is selected from H,  ,  ,  ,

 ,  ;



$X_8$ is selected from H, -OH, -$NH_2$, and  ;

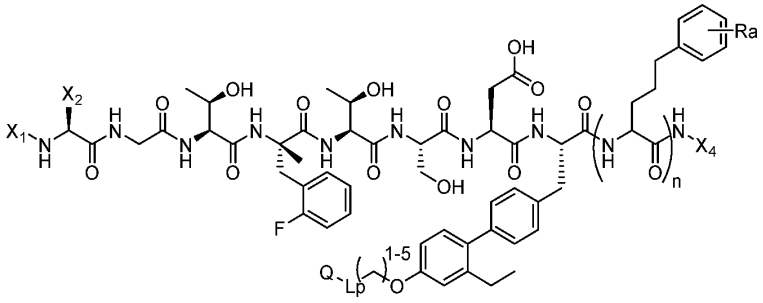
Ar is selected from  and  ;

$X_9$ is selected from -$NH_2$,  , and  , and

m is an integer from 1 to 4

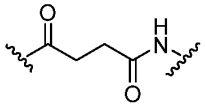
or a pharmaceutically acceptable salt thereof.

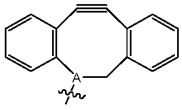
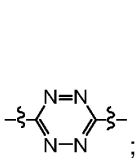
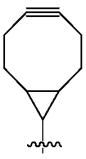
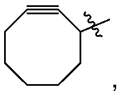
100. A compound having a structure of Formula (III):



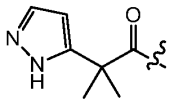
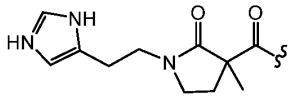
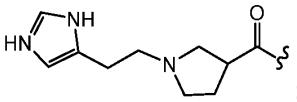
(III),

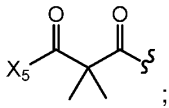
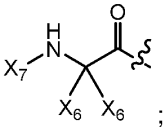
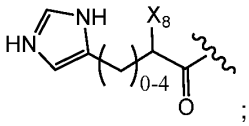
wherein:

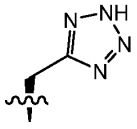
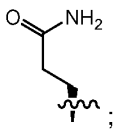
$L_p$ is absent or a linker comprising one or more of  ; a carbamate group; a cyclodextrin; a polyethylene glycol (PEG) segment having 1 to 36 -$CH_2CH_2O$- (EG) units; one or more amino acids selected from glycine, serine, glutamic acid, alanine, valine, and proline, and combinations thereof;

Q is a moiety selected from -$N_3$, ,  ;  ;  ; and  ,

where A is C or N;

$X_1$ is selected from H;  ;  ;  ;

 ;  ; and  ;

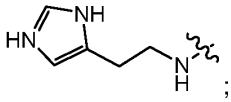
$X_2$ is selected from  and  ;

$X_3$ is selected from $–(CH_2)_{2\text{-}6}–NH_2$, $–(CH_2)_{2\text{-}6}–N_3$, and $-CH_3$, with the proviso that when $X_3$ is $-CH_3$, n is 1 and Ra in at least one occurrence is selected from $–(CH_2)_{2\text{-}6}–NH_2$ and $–(CH_2)_{2\text{-}6}–N_3$;

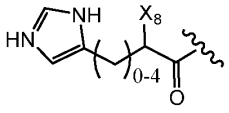
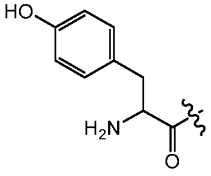
n is 0 or 1;

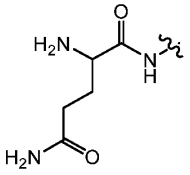
Ra is independently at each occurrence selected from H, $-CH_3$, $–(CH_2)_{2\text{-}6}–NH_2$, and $–(CH_2)_{2\text{-}6}–N_3$;

$X_4$ is selected from H and phenyl;

$X_5$ is selected from -OH, $-NH_2$, -NH-OH, and  ;

$X_6$ is independently at each occurrence selected from H, -OH, $-CH_3$, and $-CH_2OH$;

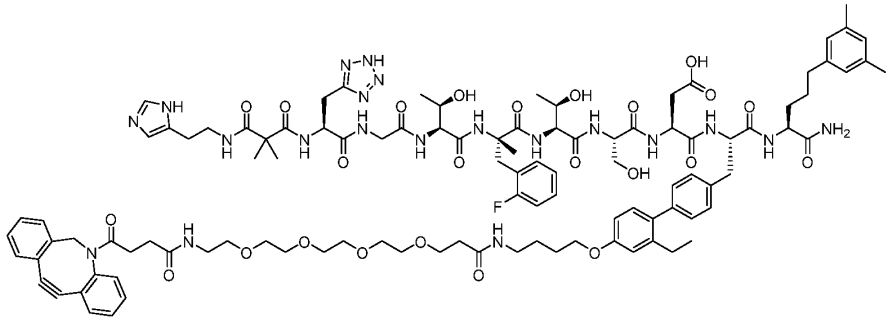
$X_7$ is selected from H,  , and  ;

$X_8$ is selected from H, -OH, $-NH_2$, and  ,

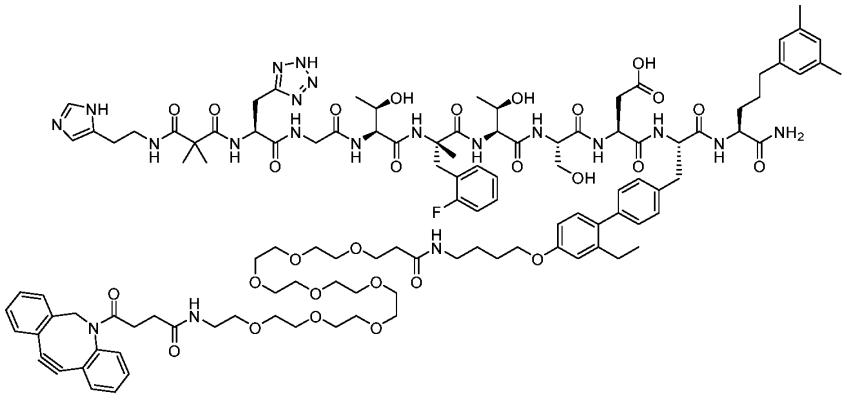
and pharmaceutically acceptable salts thereof.

101. The compound of claim 99 having the structure selected from the group consisting of:

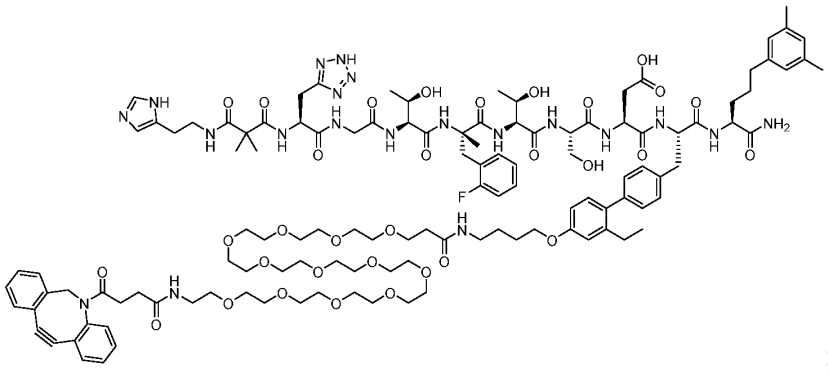
**Structure**



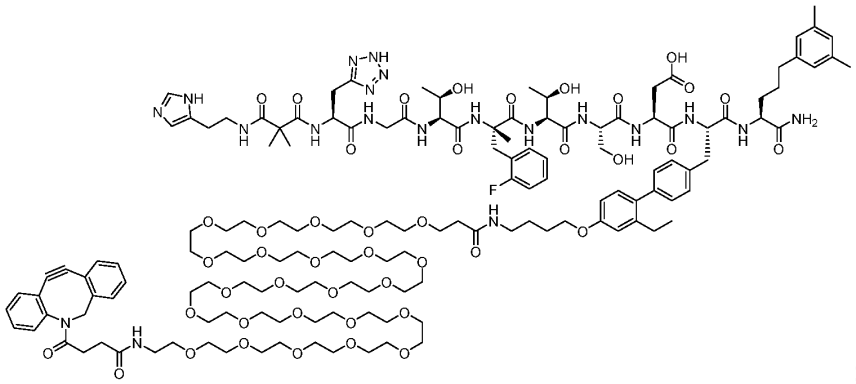
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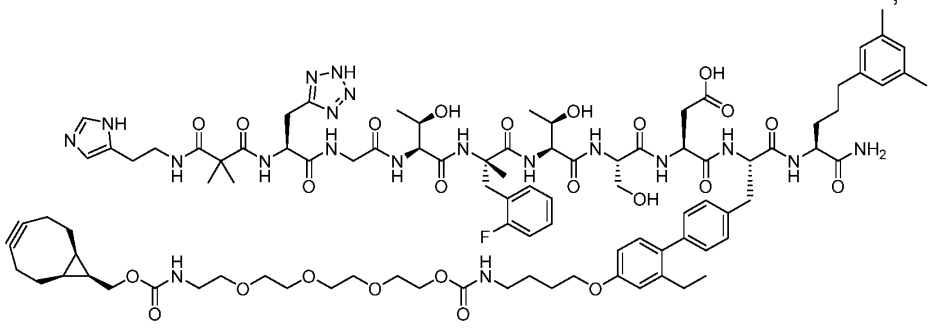
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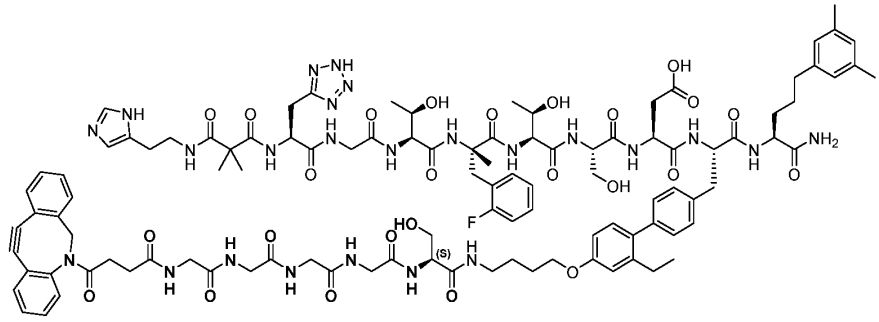
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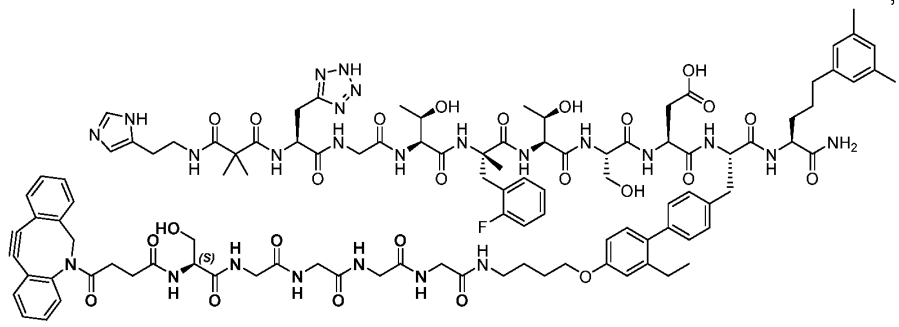
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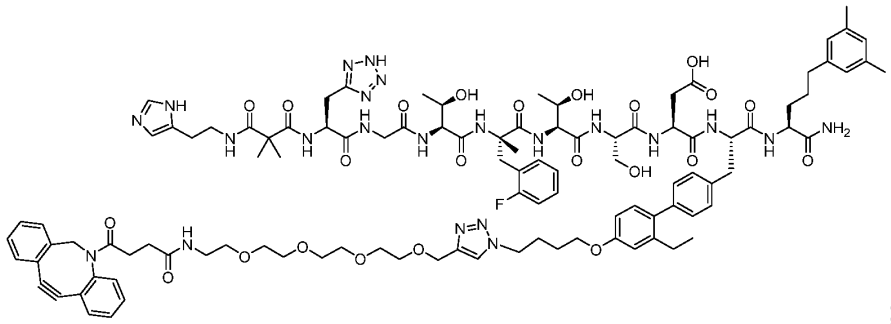
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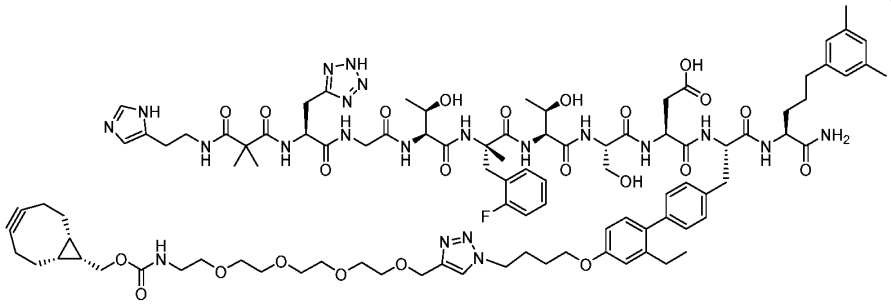
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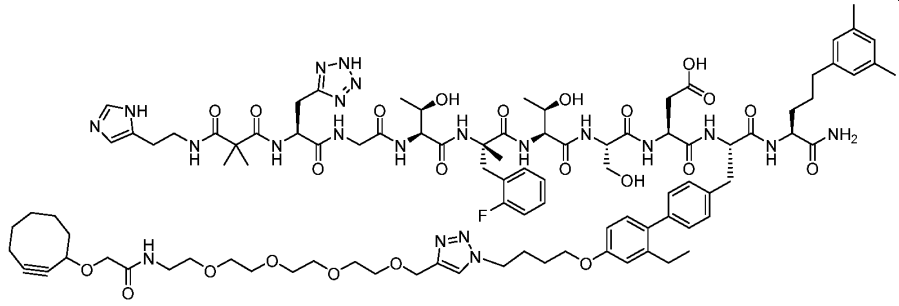
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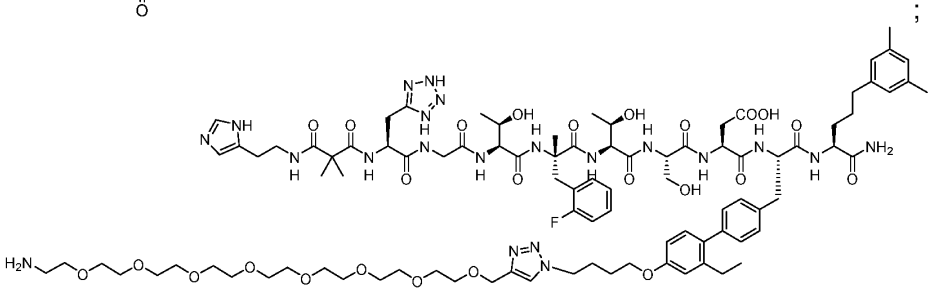
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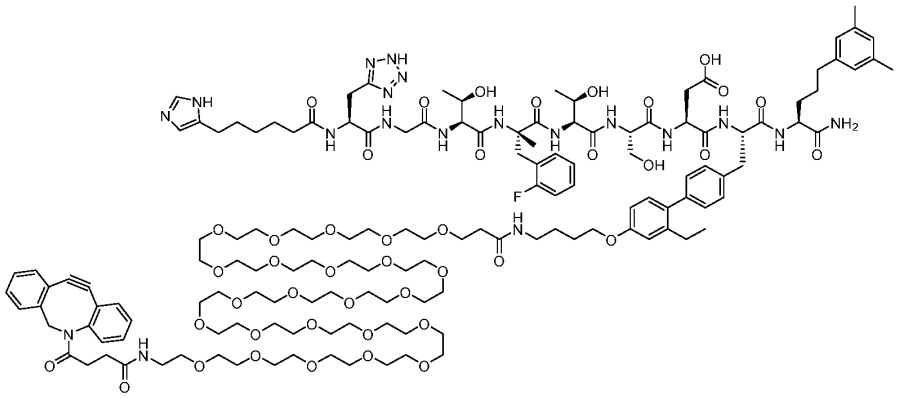
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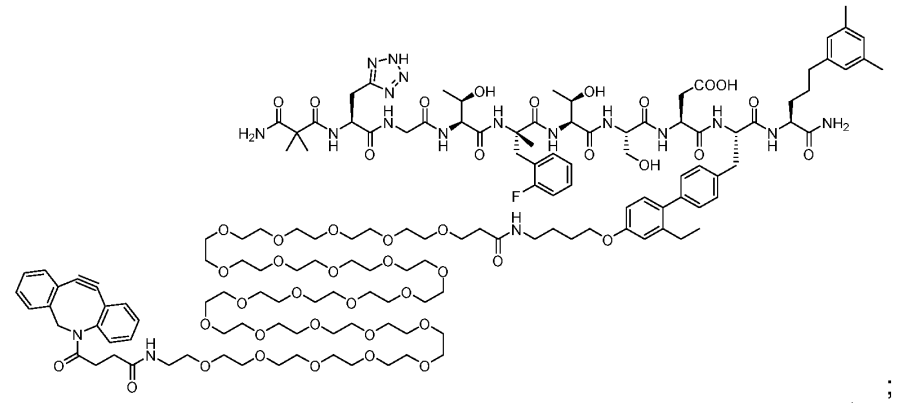
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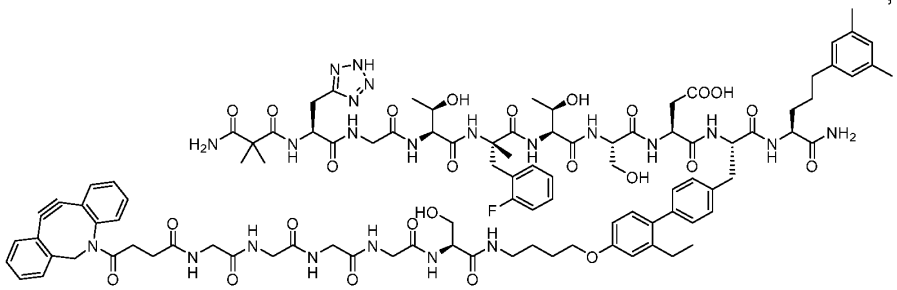
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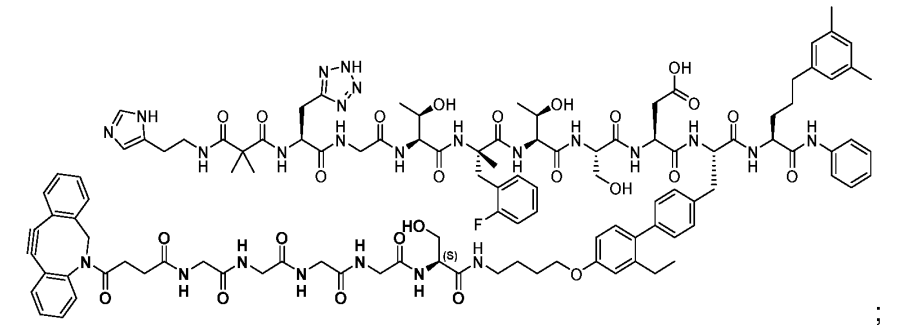
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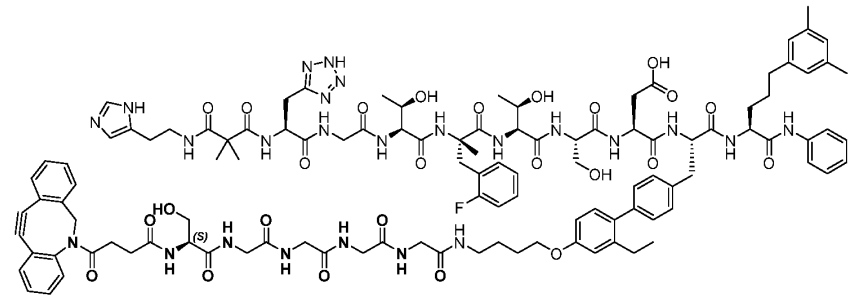
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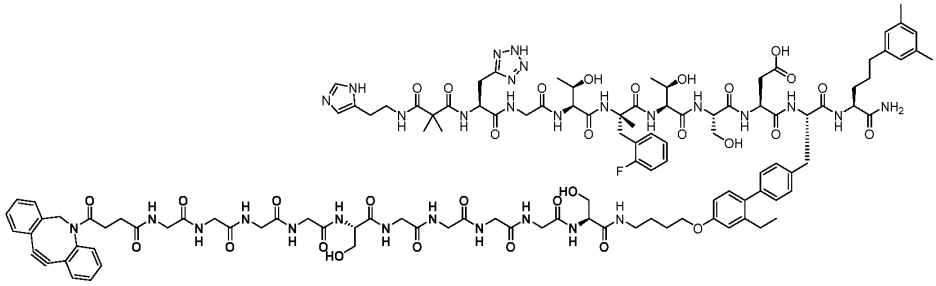
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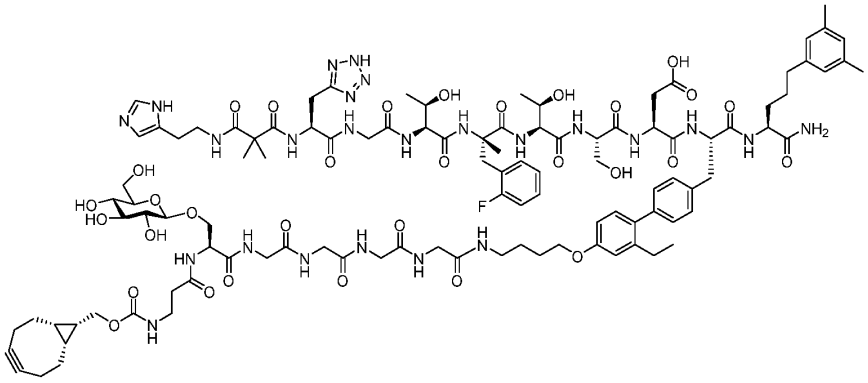
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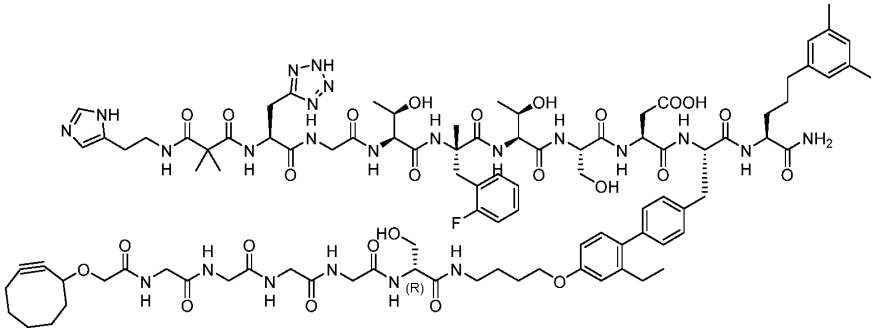
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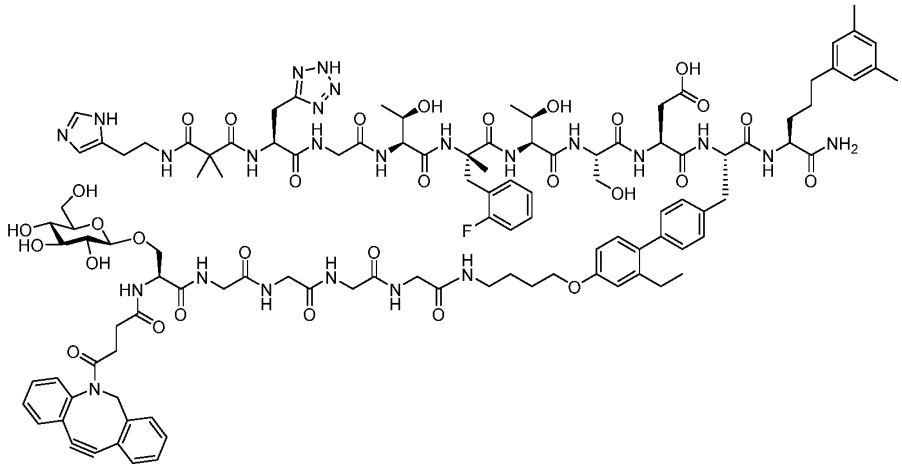
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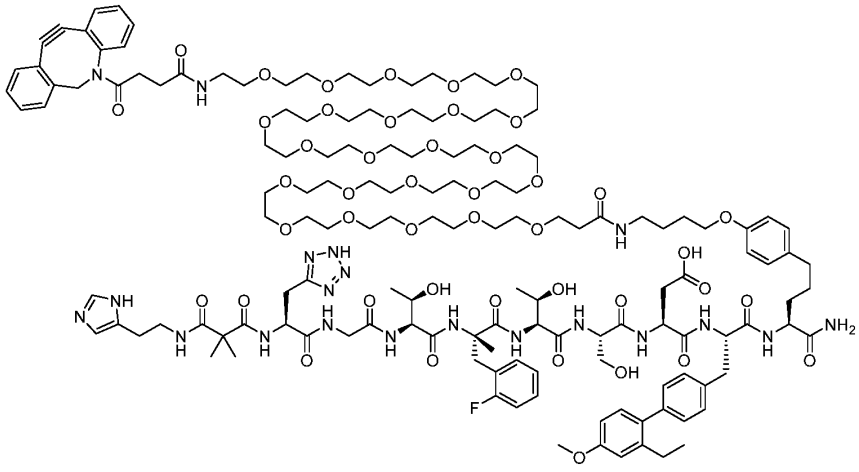
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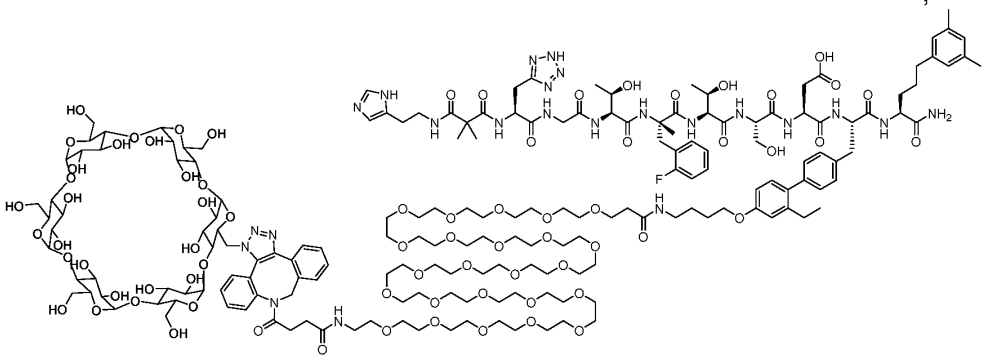
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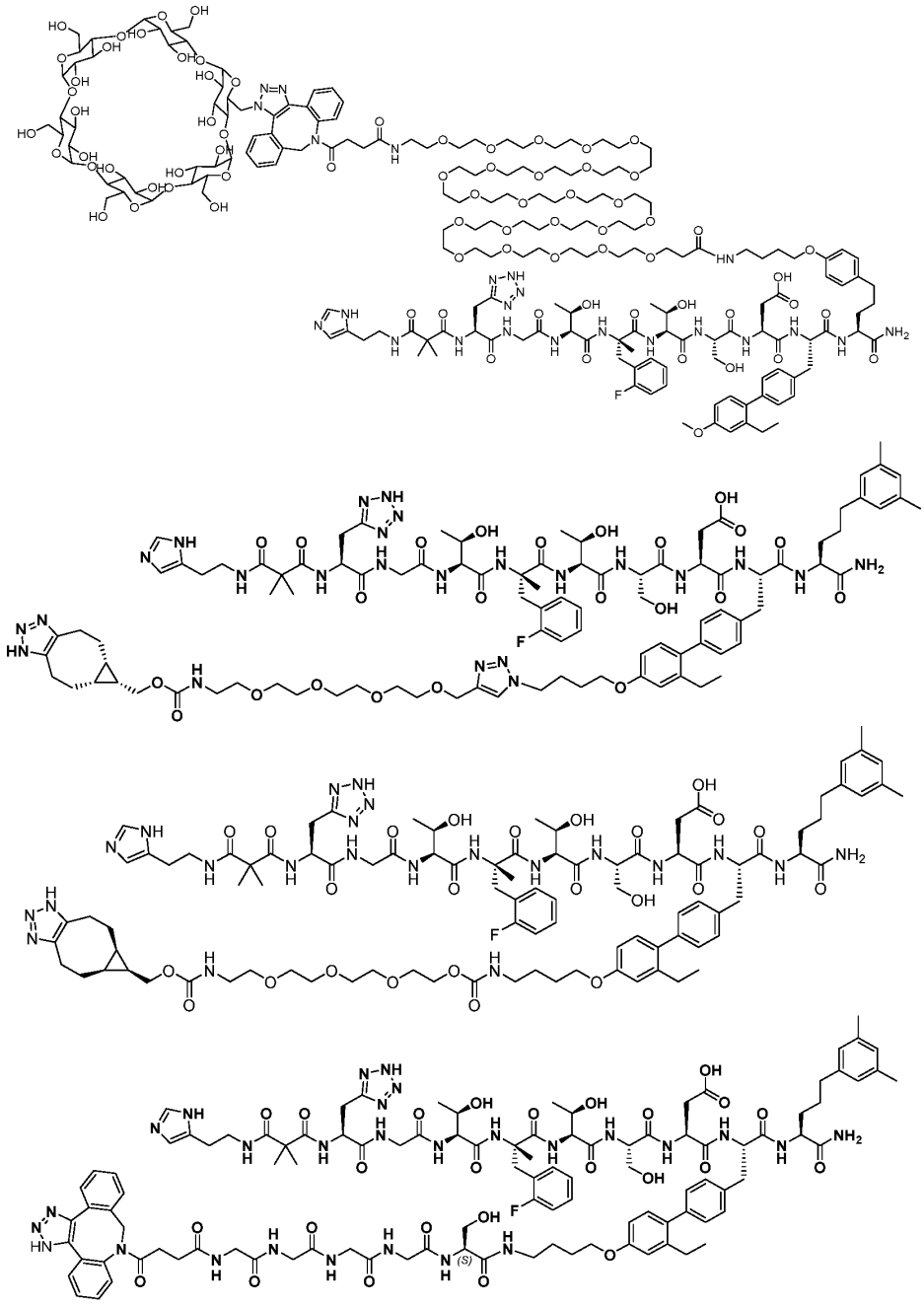
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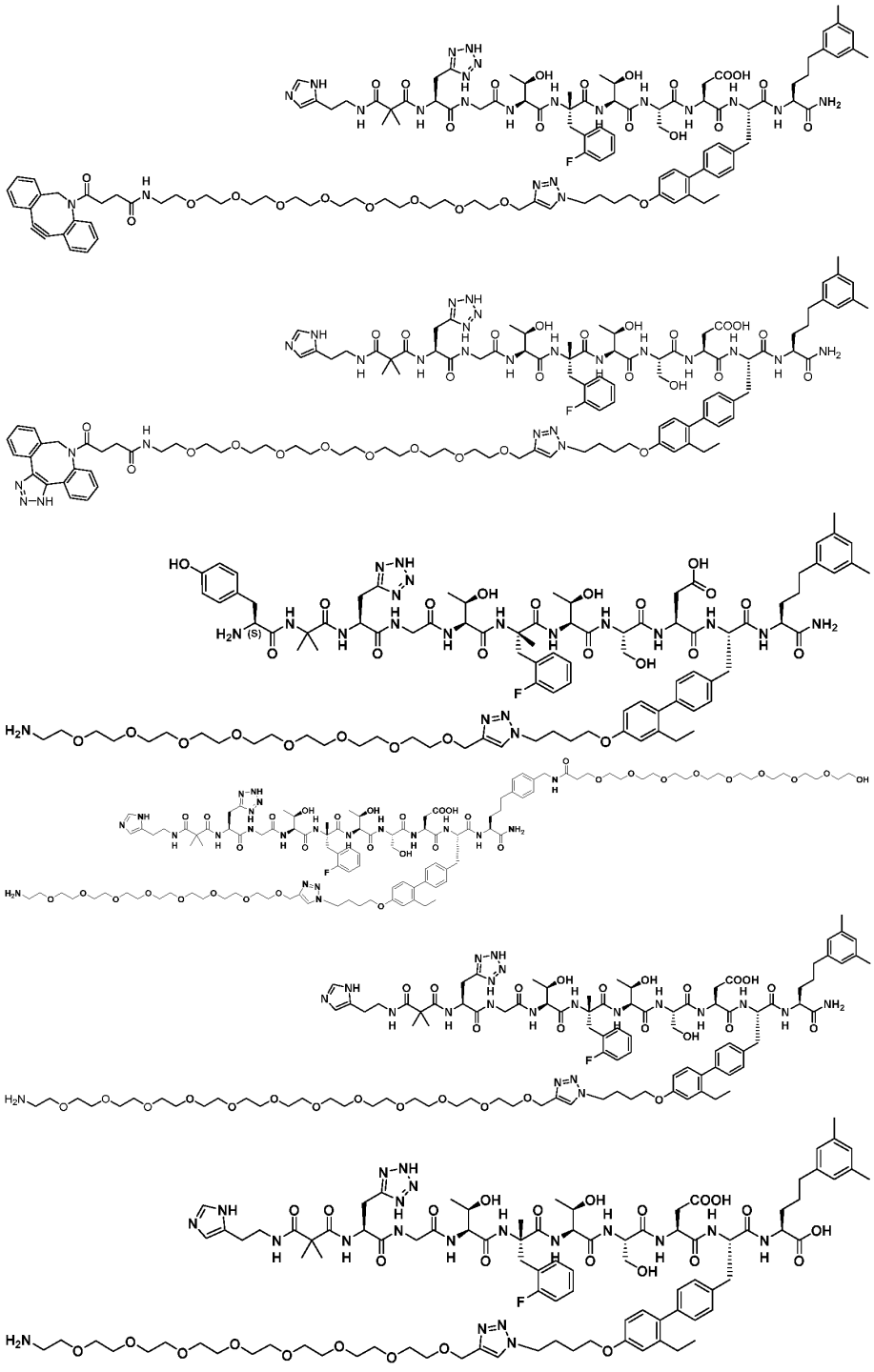


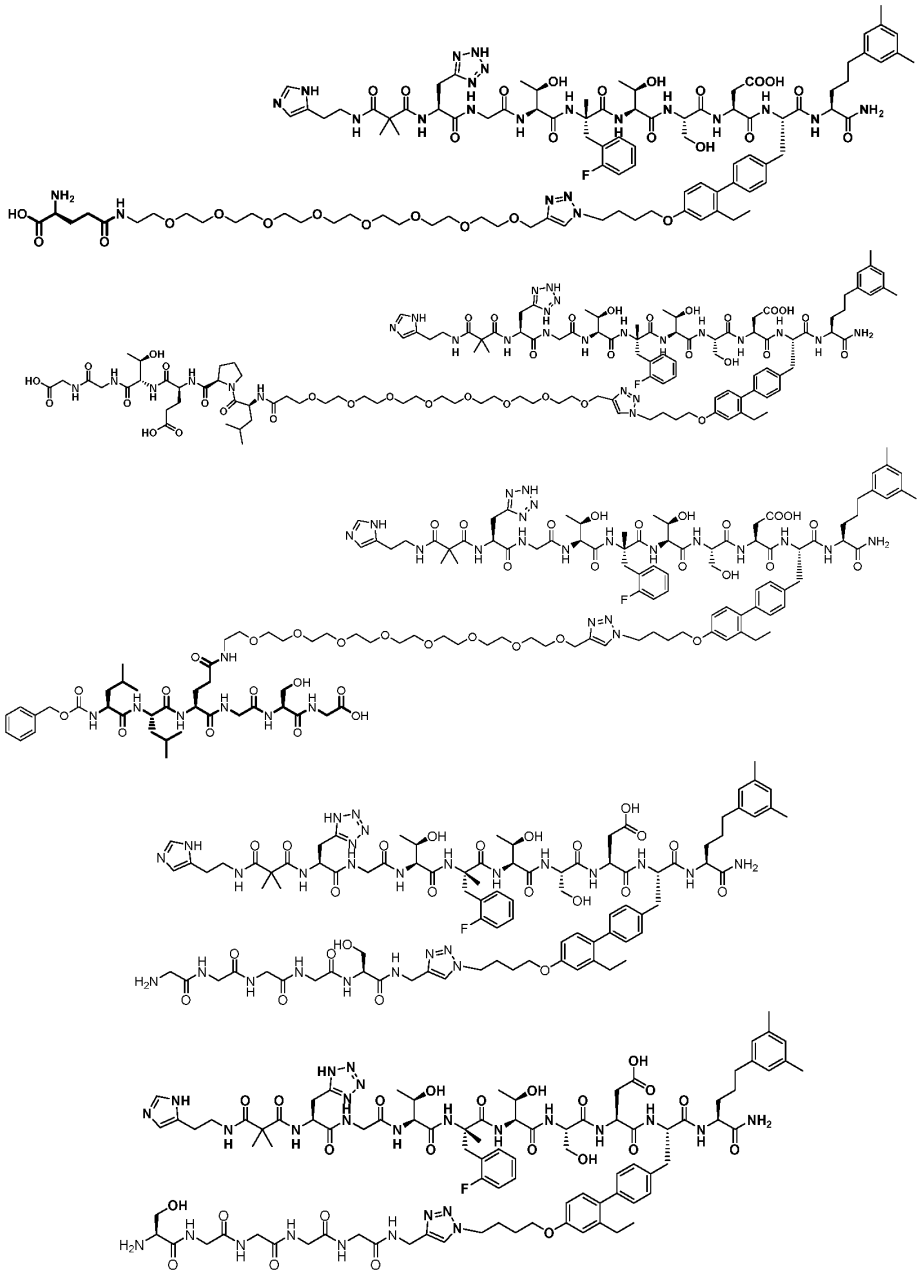
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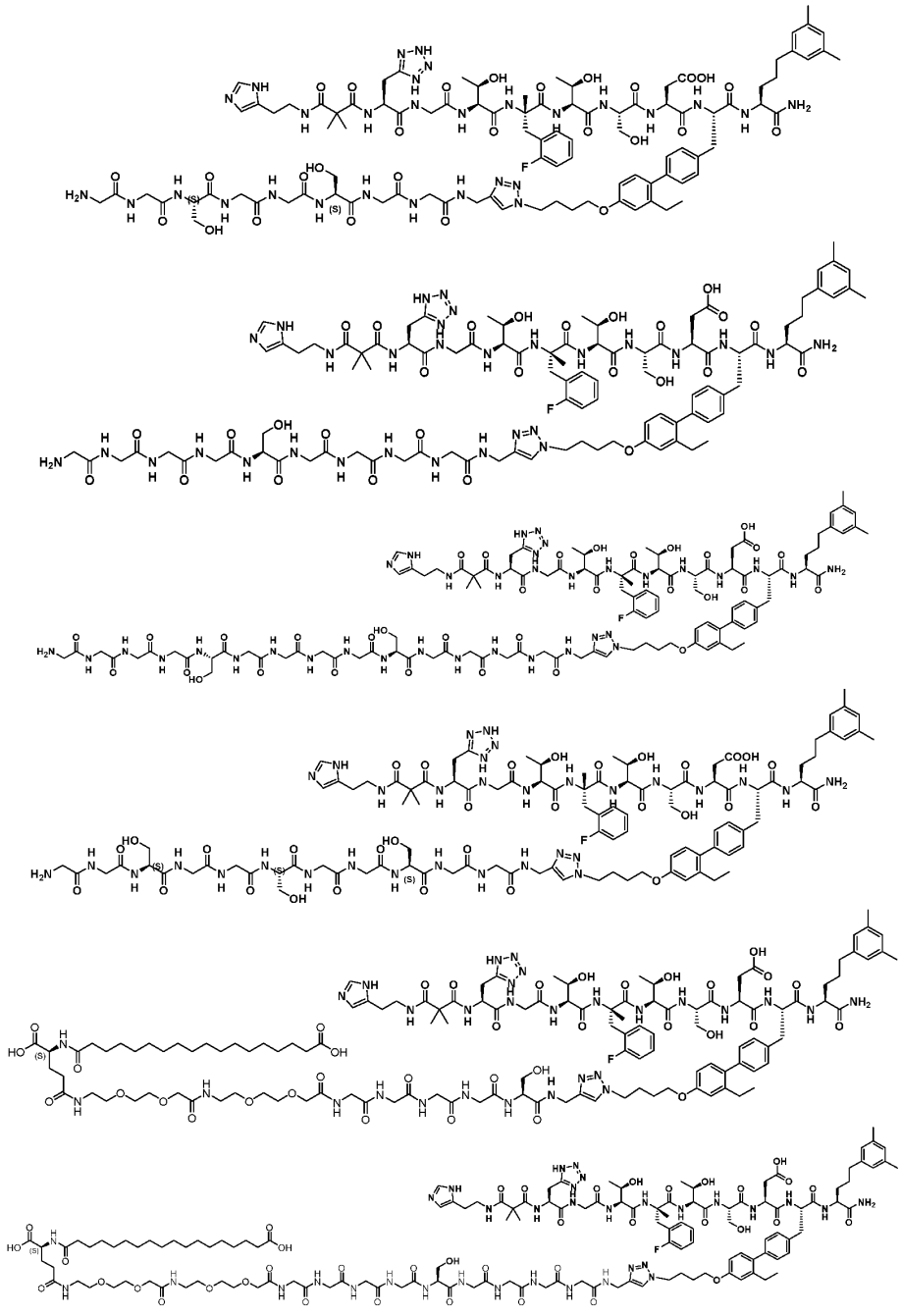


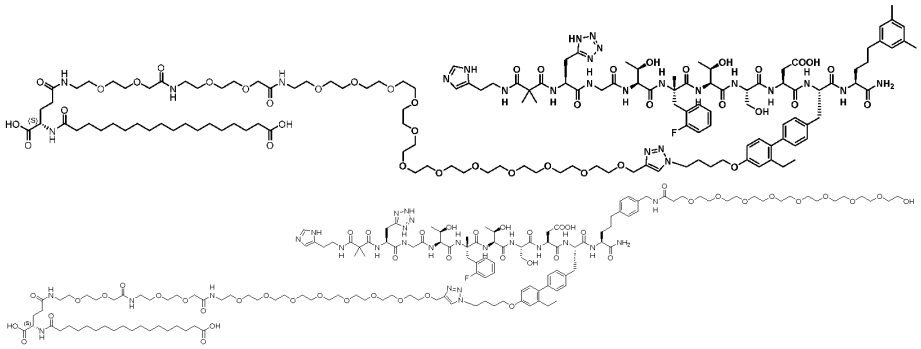
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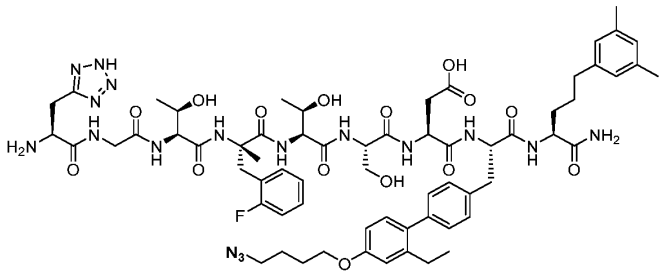




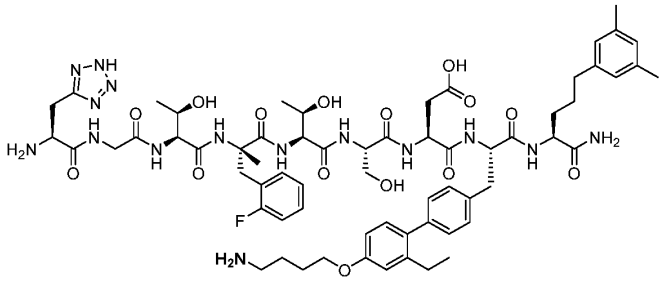
or a pharmaceutically acceptable salt thereof.

102. An antibody-drug conjugate comprising a Glucagon-like peptide-1 receptor (GLP1R)-targeting antibody or an antigen-binding fragment thereof conjugated, optionally through a linker, to a payload having the structure selected from the group consisting of:

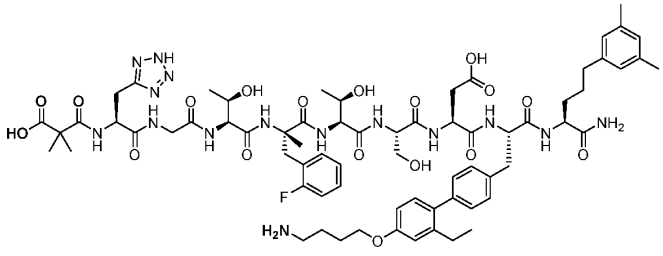
Structure



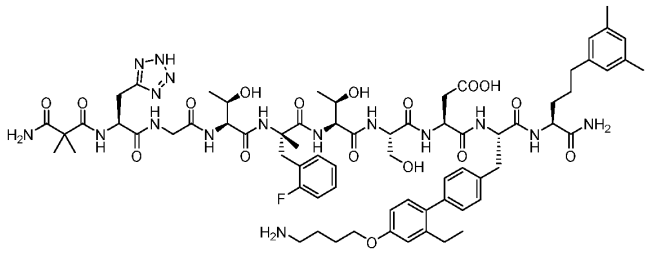
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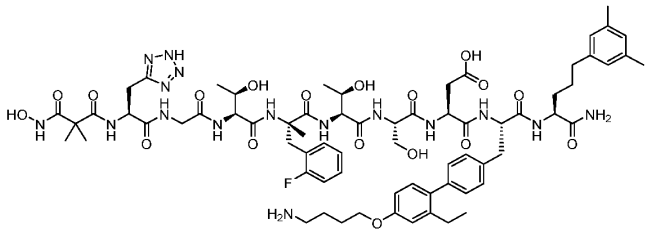


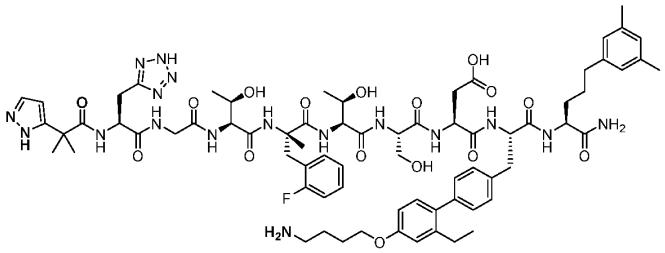
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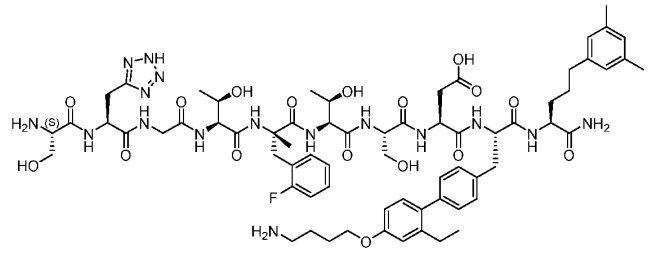


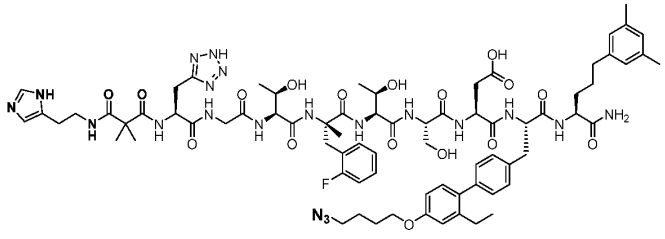
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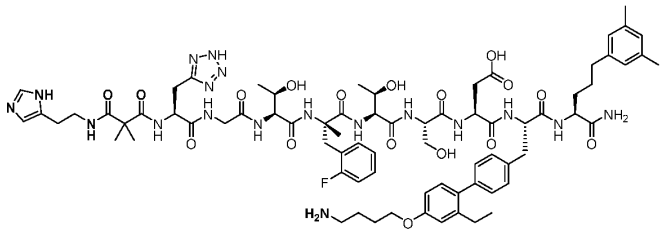

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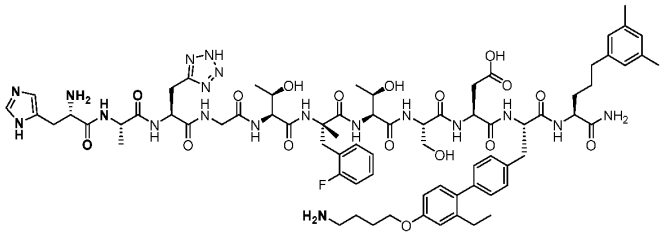

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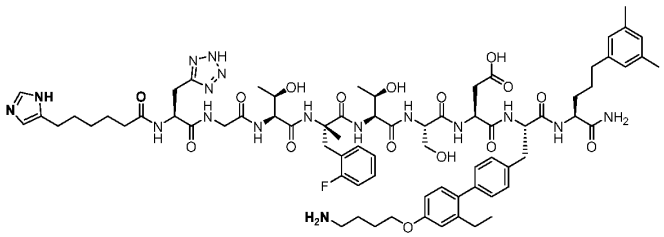

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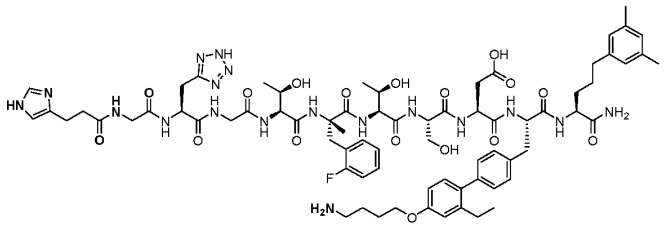

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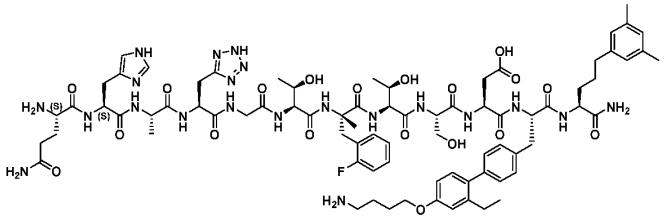

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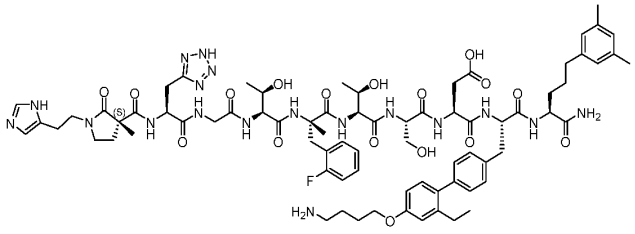

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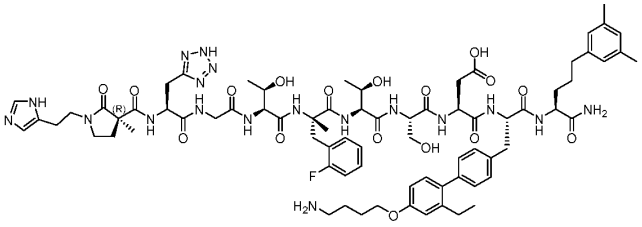

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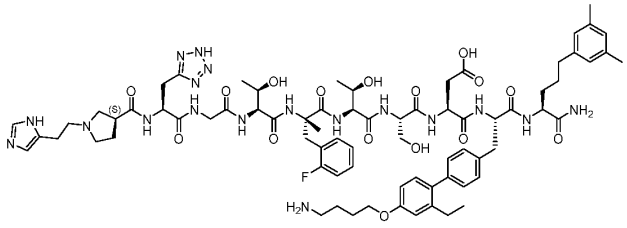

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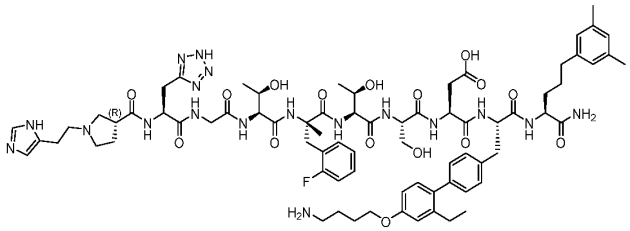

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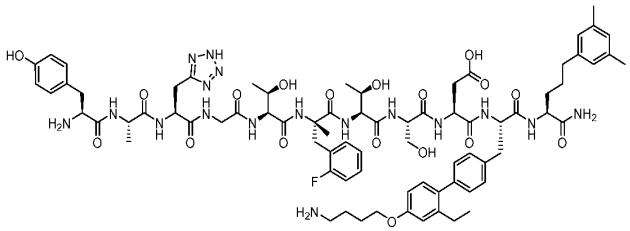

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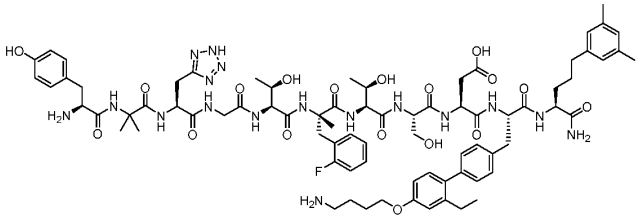

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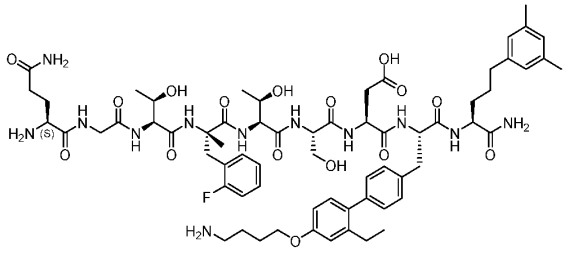

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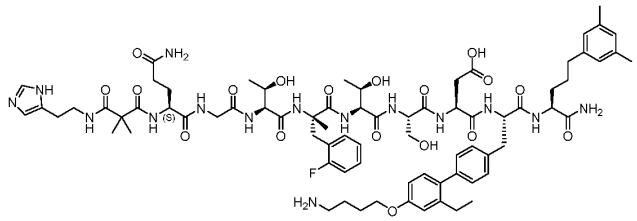

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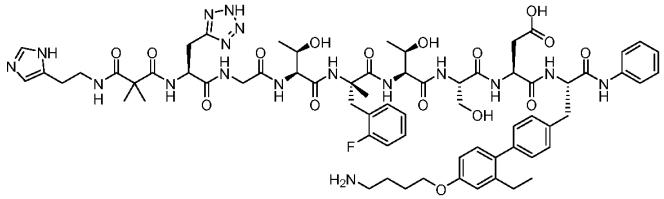

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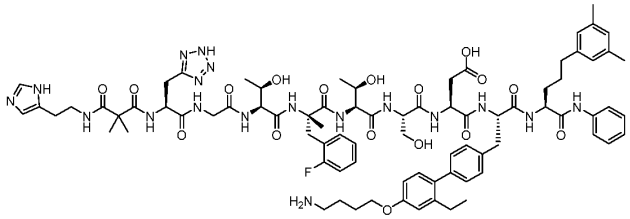

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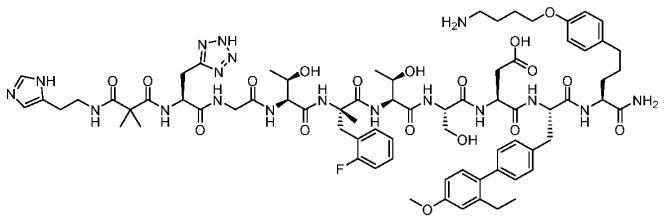

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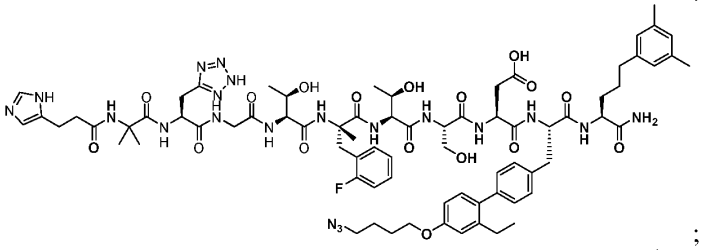

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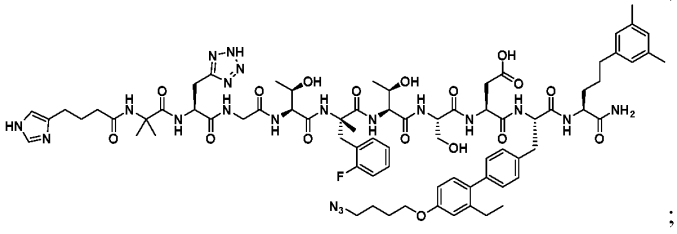

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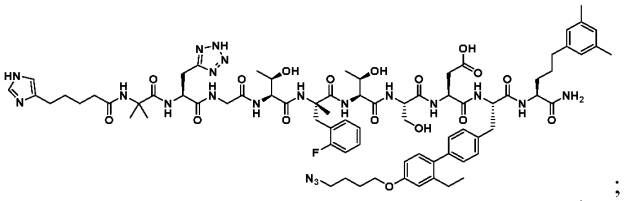

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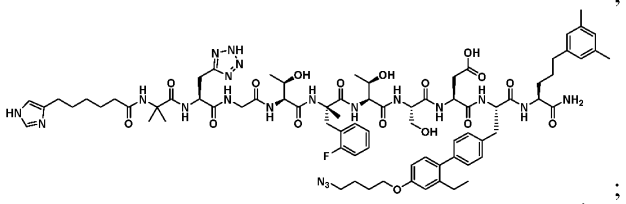

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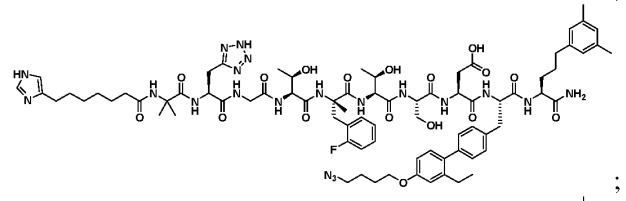

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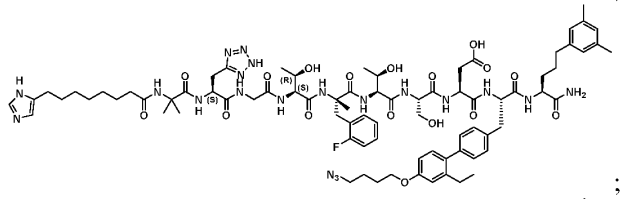

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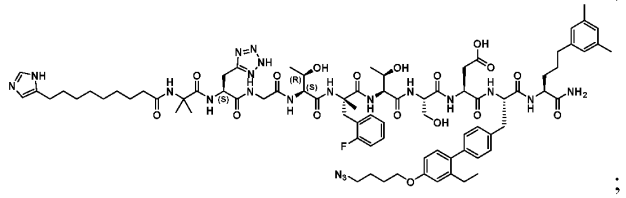

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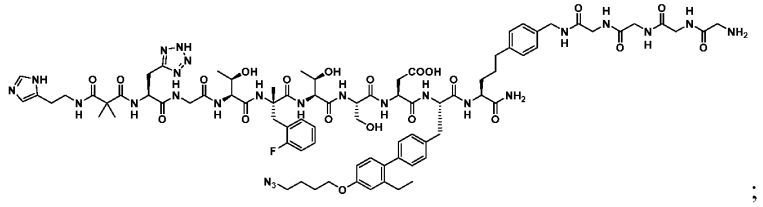

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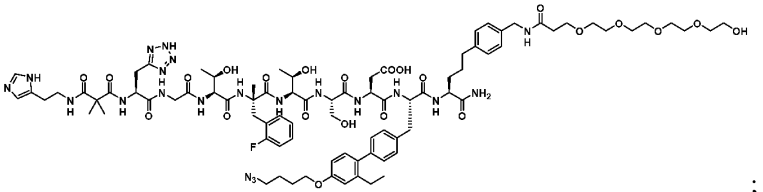

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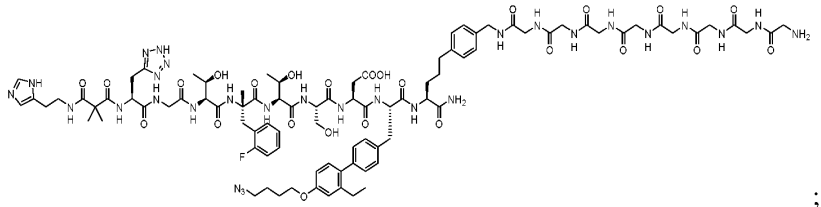

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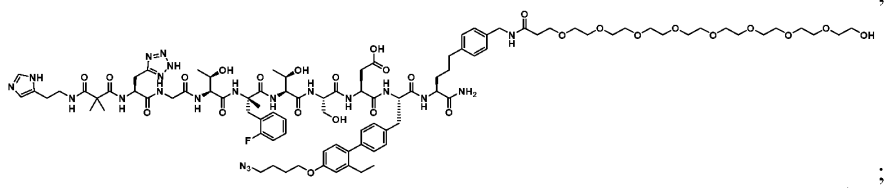

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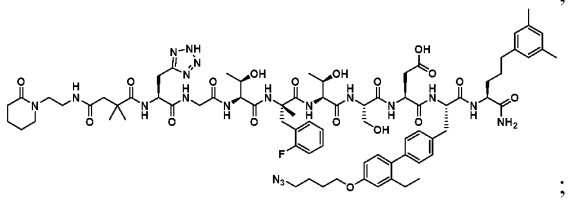

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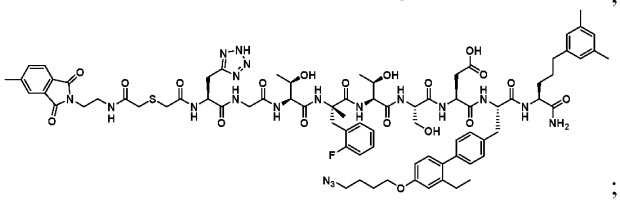

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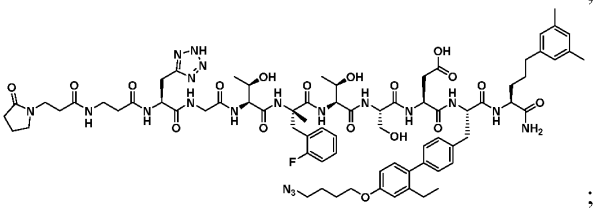

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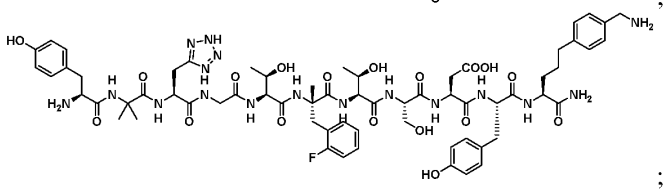

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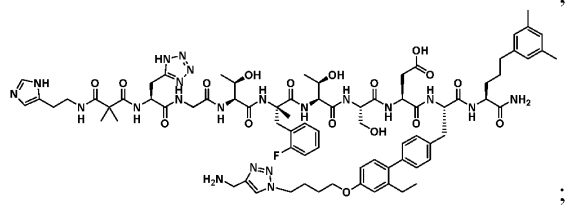

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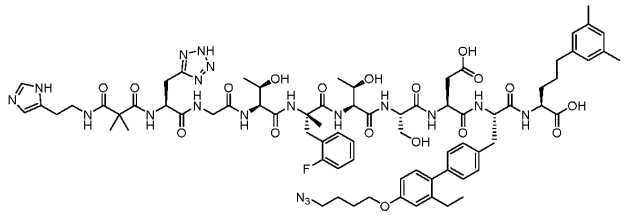

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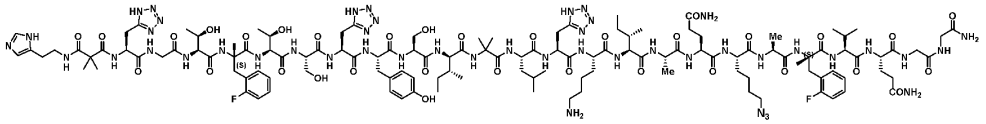

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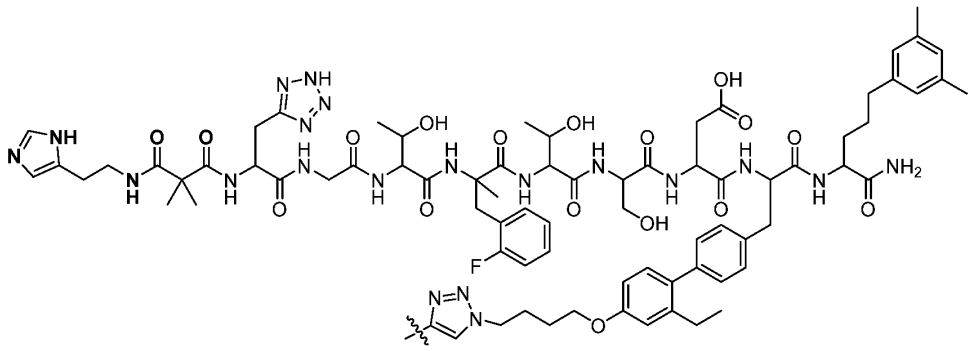
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or a pharmaceutically acceptable salt thereof.

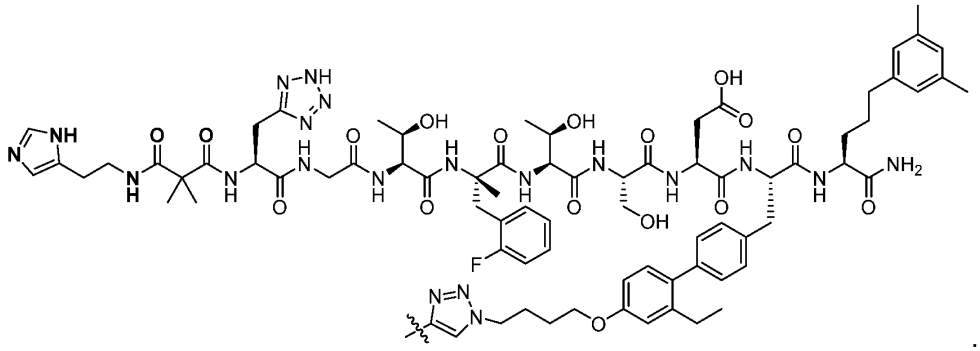
103. An antibody-drug conjugate comprising a Glucagon-like peptide-1 receptor (GLP1R)-targeting antibody or an antigen-binding fragment thereof and a payload having the structure:



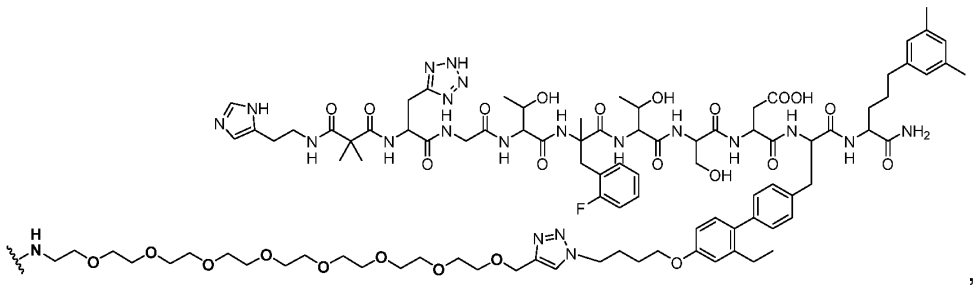
,

wherein  is the point of attachment of the payload to the antibody or the antigen-binding fragment thereof directly or through a linker.

104. The antibody-drug conjugate of claim 103,wherein the payload has the structure:



105. An antibody-drug conjugate comprising a Glucagon-like peptide-1 receptor (GLP1R)-targeting antibody or an antigen-binding fragment thereof and a linker-payload having the structure:



wherein —≈— is the point of attachment of the linker-payload to the antibody or the antigen-binding fragment thereof.

106. The antibody-drug conjugate of claim 105, wherein the linker-payload has the structure:

