FIG. 1A

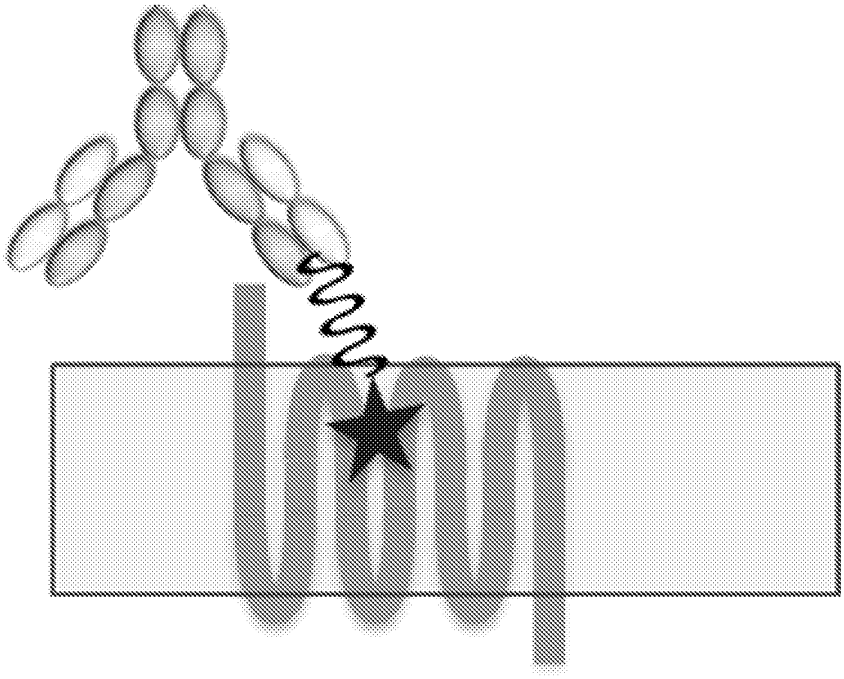


FIG. 1B

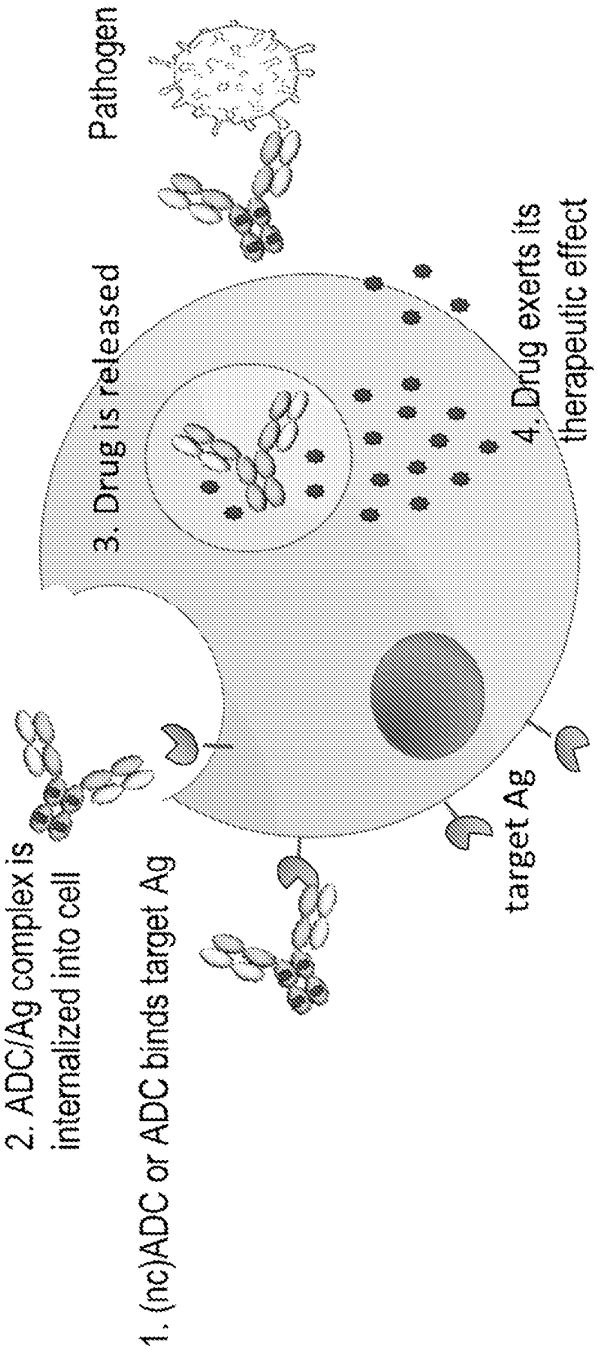

Pathogen
3. Drug is released
4. Drug exerts its therapeutic effect
2. ADC/Ag complex is internalized into cell
1. (nc)ADC or ADC binds target Ag
target Ag

FIG. 2

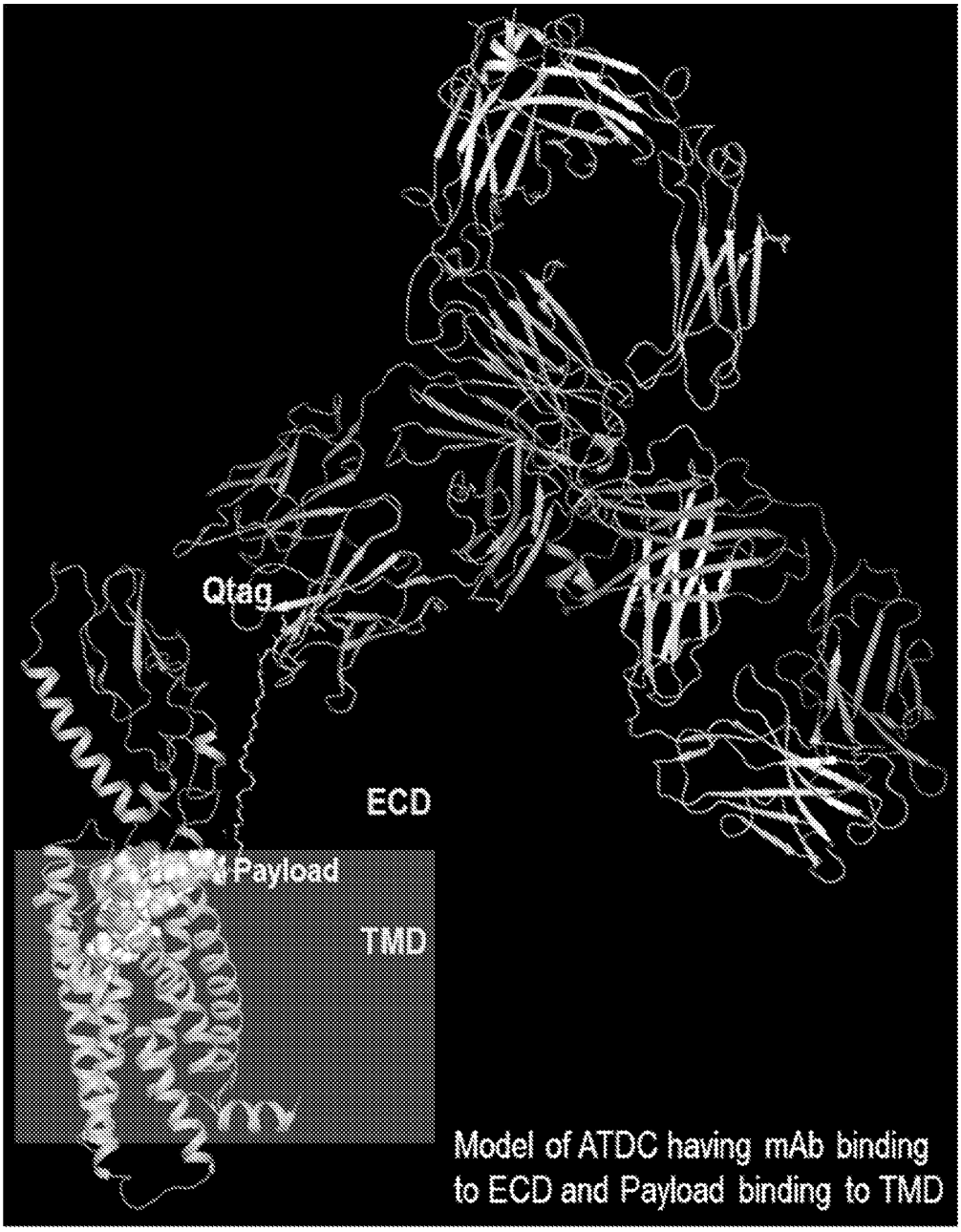

Qtag
ECD
Payload
TMD
Model of ATDC having mAb binding to ECD and Payload binding to TMD

FIG. 3A

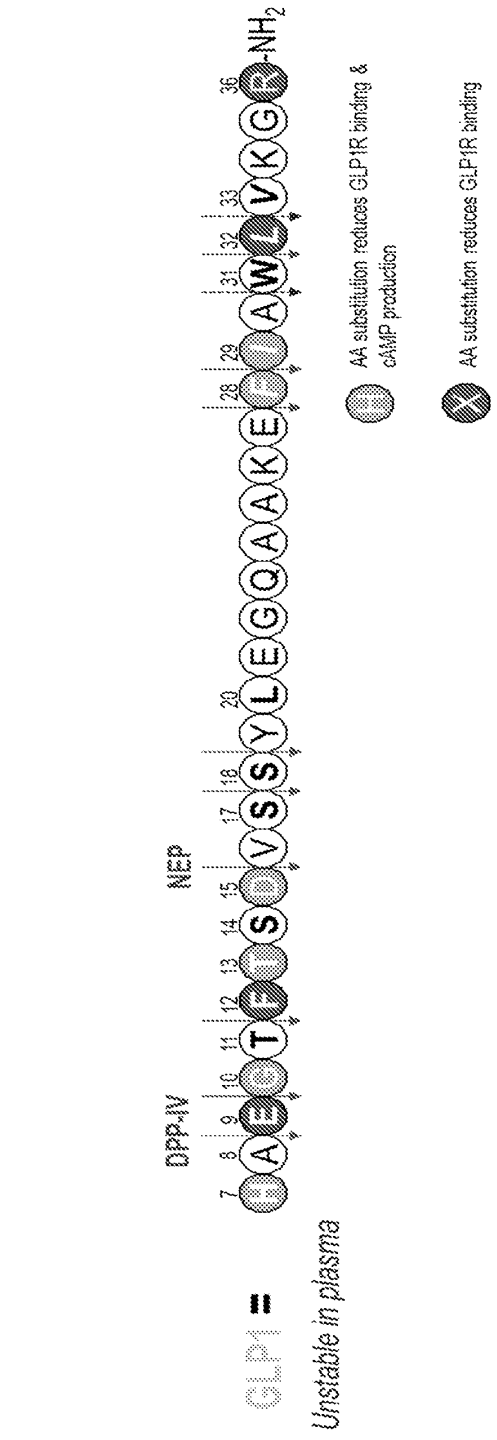
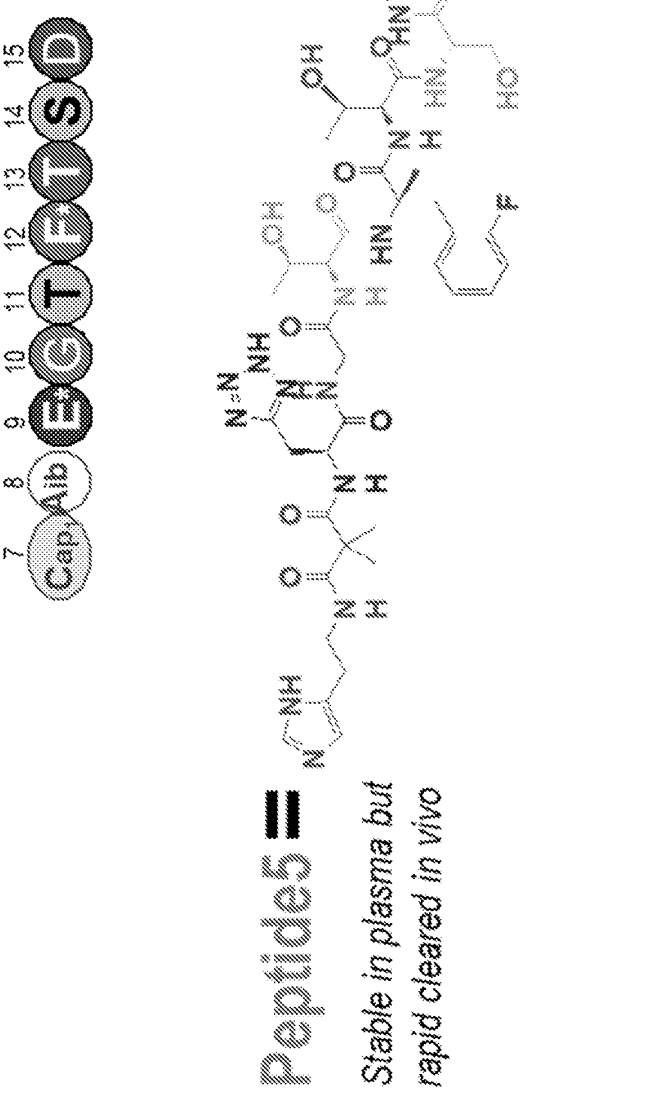

DPP-IV
NEP
GLP1 =
HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-NH2
7 8 9 10 11 12 13 14 15 17 18 20 28 29 31 32 33 36
Unstable in plasma
AA substitution reduces GLP1R binding & cAMP production
AA substitution reduces GLP1R binding

FIG. 3B



FIG. 4A


7 8 9 10 11 12 13 14 15
Cap. Aib E C T F T S D
Peptides
Stable in plasma but
rapid cleared in vivo

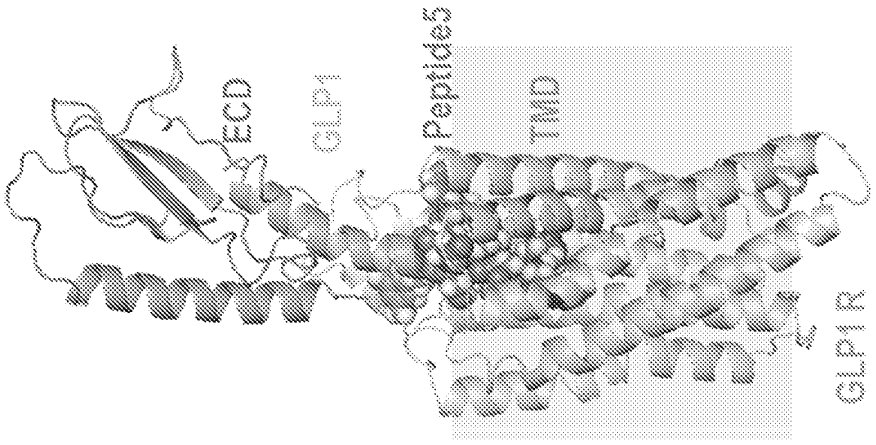


FIG. 4B

PDBID: 3IOL GLP1R/GLP1
PDBID: 5NX2 GLP1R/Peptide5
Nature volume 546, pages254–258(2017)

FIG. 5

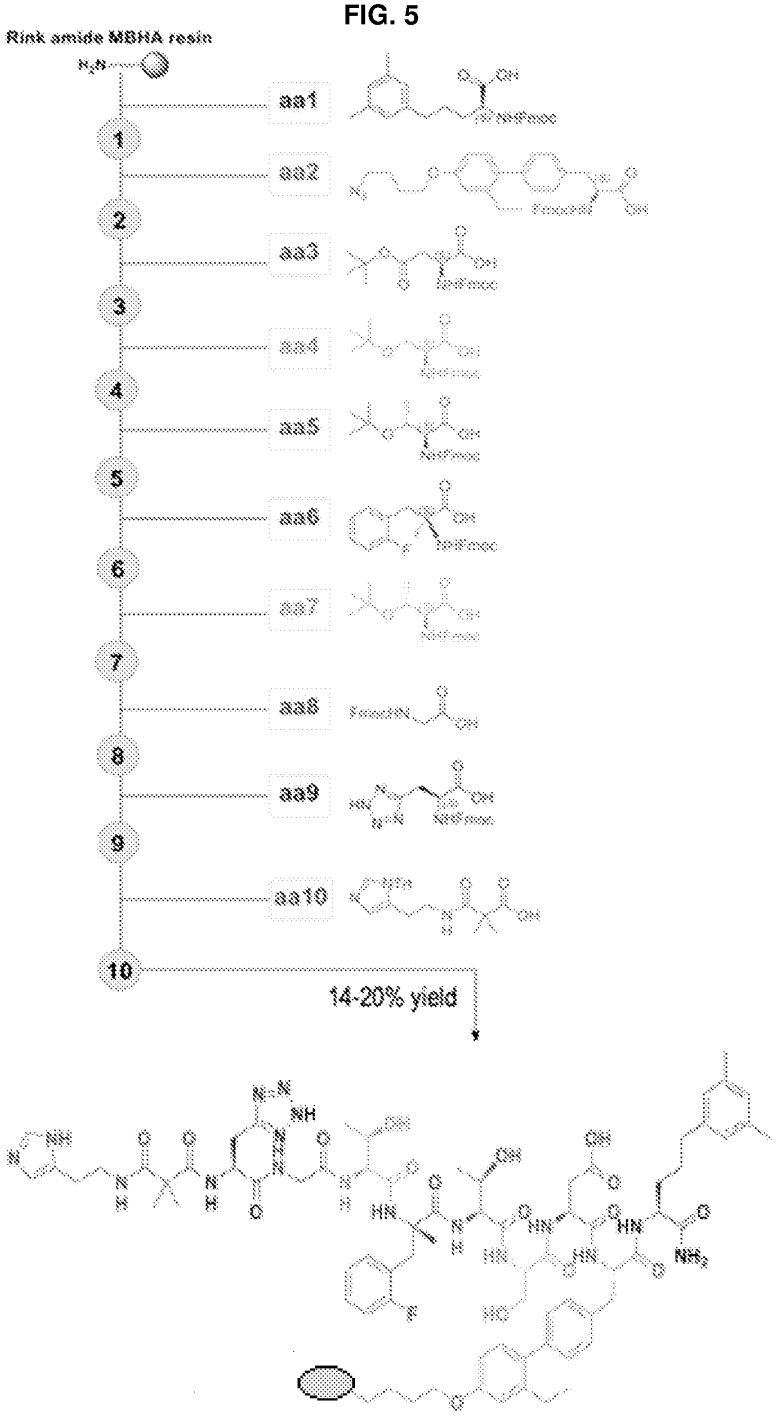

Rink amide MBHA resin
aa1
aa2
aa3
aa4
aa5
aa6
aa7
aa8
aa9
aa10
1
2
3
4
5
6
7
8
9
10
14-20% yield

FIG. 6A

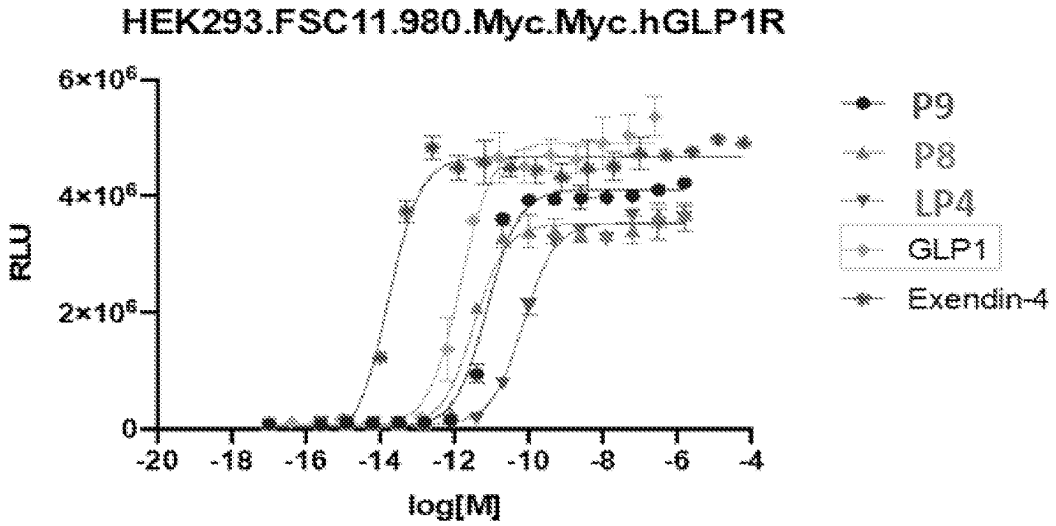

HEK293.FSC11.980.Myc.Myc.hGLP1R
RLU
6×10^6
4×10^6
2×10^6
0
-20
-18
-16
-14
-12
-10
-8
-6
-4
log[M]
P9
P8
LP4
GLP1
Exendin-4

FIG. 6B

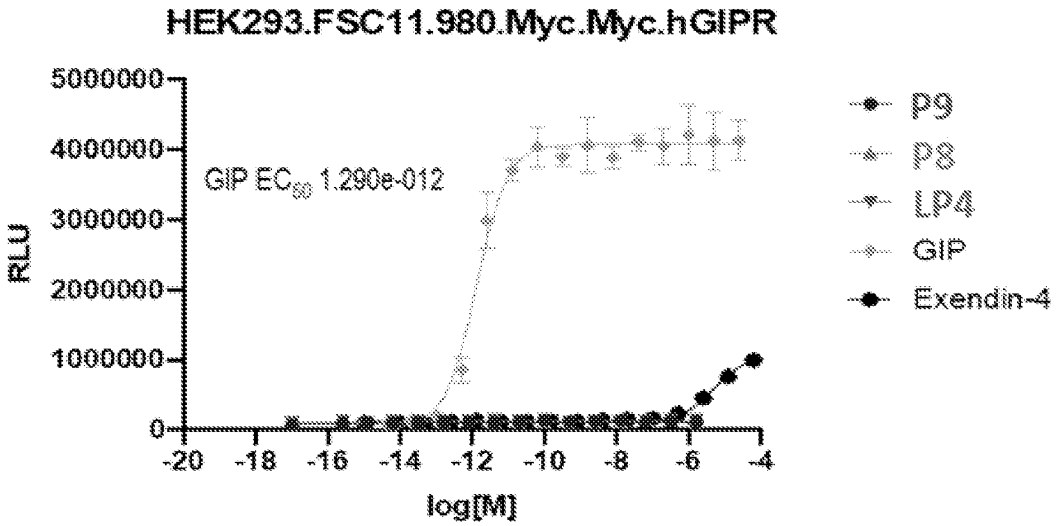

HEK293.FSC11.980.Myc.Myc.hGIPR
RLU
5000000
4000000
3000000
2000000
1000000
0
GIP EC50 1.290e-012
-20
-18
-16
-14
-12
-10
-8
-6
-4
log[M]
P9
P8
LP4
GIP
Exendin-4

FIG. 6C

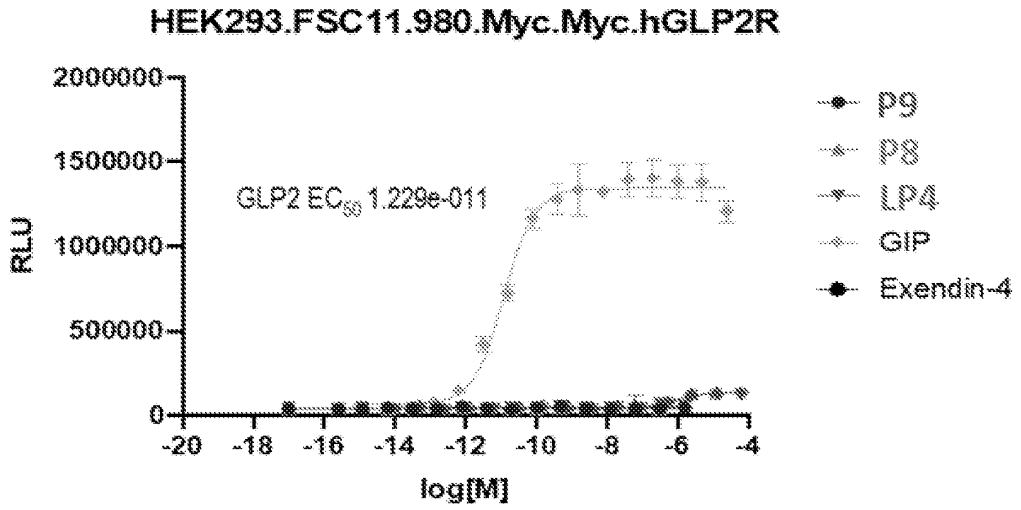

HEK293.FSC11.980.Myc.Myc.hGLP2R
2000000
1500000
1000000
500000
0
RLU
GLP2 EC50 1.229e-011
-20
-18
-16
-14
-12
-10
-8
-6
-4
log[M]
p9
P8
LP4
GIP
Exendin-4

FIG. 6D

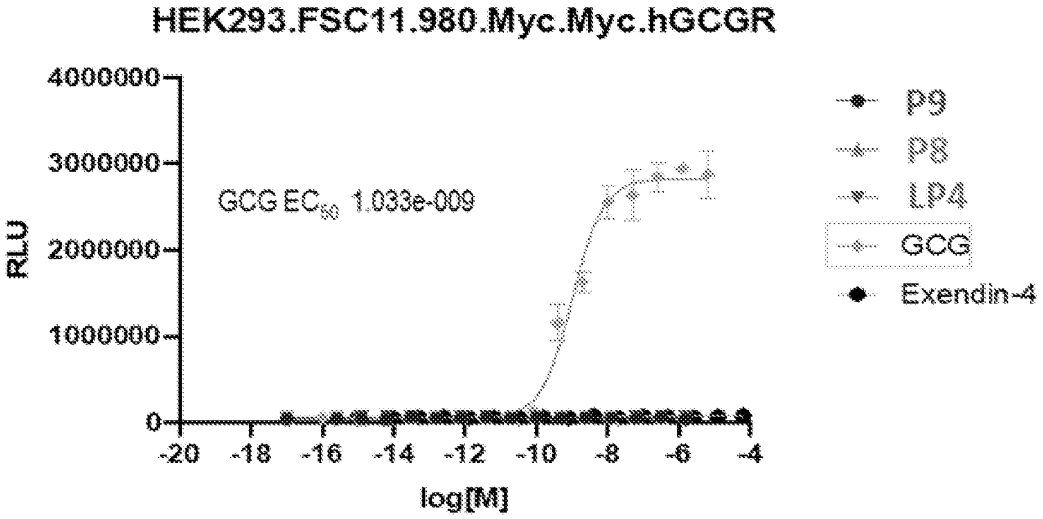

HEK293.FSC11.980.Myc.Myc.hGCGR
4000000
3000000
2000000
1000000
0
RLU
GCG EC50 1.033e-009
-20
-18
-16
-14
-12
-10
-8
-6
-4
log[M]
p9
P8
LP4
GCG
Exendin-4

FIG. 7A

ATDC	hGLP1R* EC_{50} (nM)	Ratio
Anti-GLP1R mAb1-4 PEG-GLP1R agonist	2.8E-11	90X
Isotype control mAb3-*4 PEG*-GLP1R agonist	2.5E-09	
Anti-GLP1R mAb1-8 PEG-GLP1R agonist	2.2E-11	136X
Isotype control mAb3-*8 PEG*-GLP1R agonist	2.9E-09	
Anti-GLP1R mAb1-12 PEG-GLP1R agonist	4.4E-11	53X
Isotype control mAb3-*12 PEG*-GLP1R agonist	2.3E-09	
Anti-GLP1R mAb1-24PEG-GLP1R agonist	4.9E-11	30X
Isotype control mAb3-*24PEG*-GLP1R agonist	1.9E-09	
GLP-1	4.7E-11	
Dulaglutide	9.5E-11	

FIG. 7B

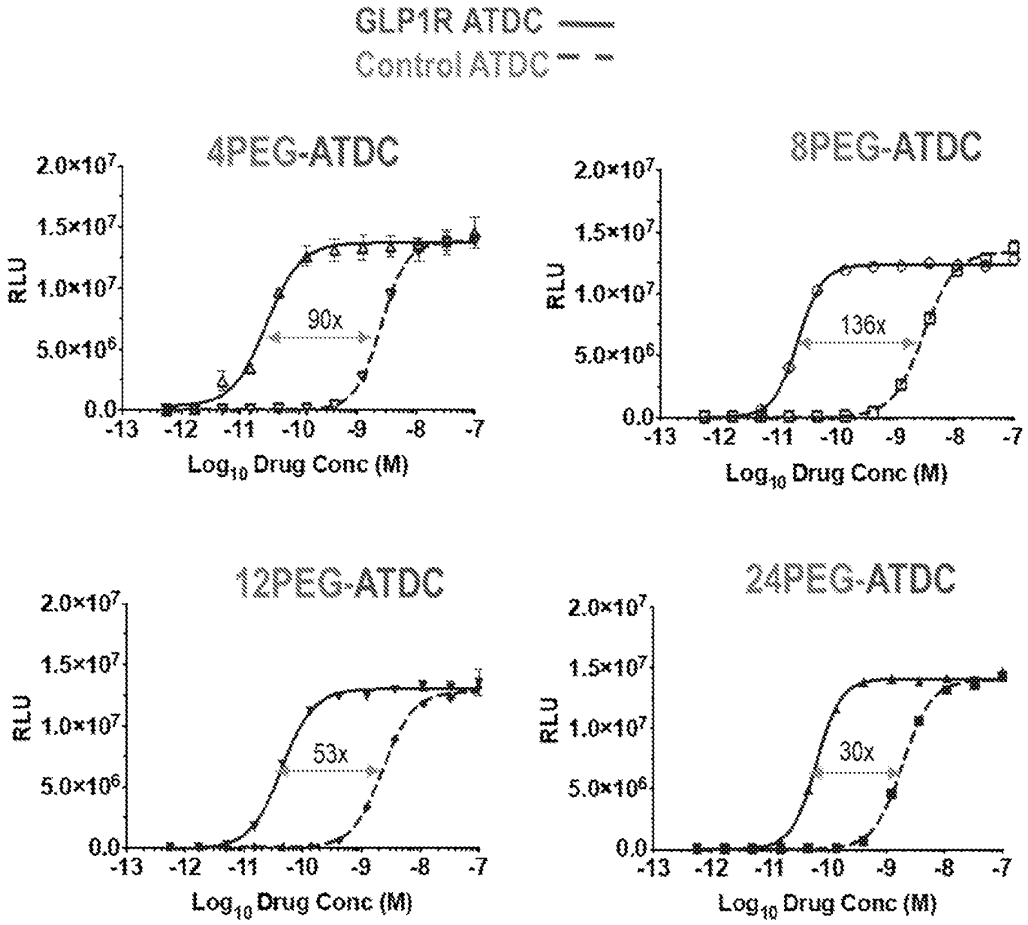

GLP1R ATDC
Control ATDC
4PEG-ATDC
8PEG-ATDC
12PEG-ATDC
24PEG-ATDC
RLU
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
-13 -12 -11 -10 -9 -8 -7
Log10 Drug Conc (M)
90x
136x
53x
30x

FIG. 8

Assay		Linker-Payload 11 (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
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) in the presence and absence of S9		Negative for mutagenicity

FIG. 9A

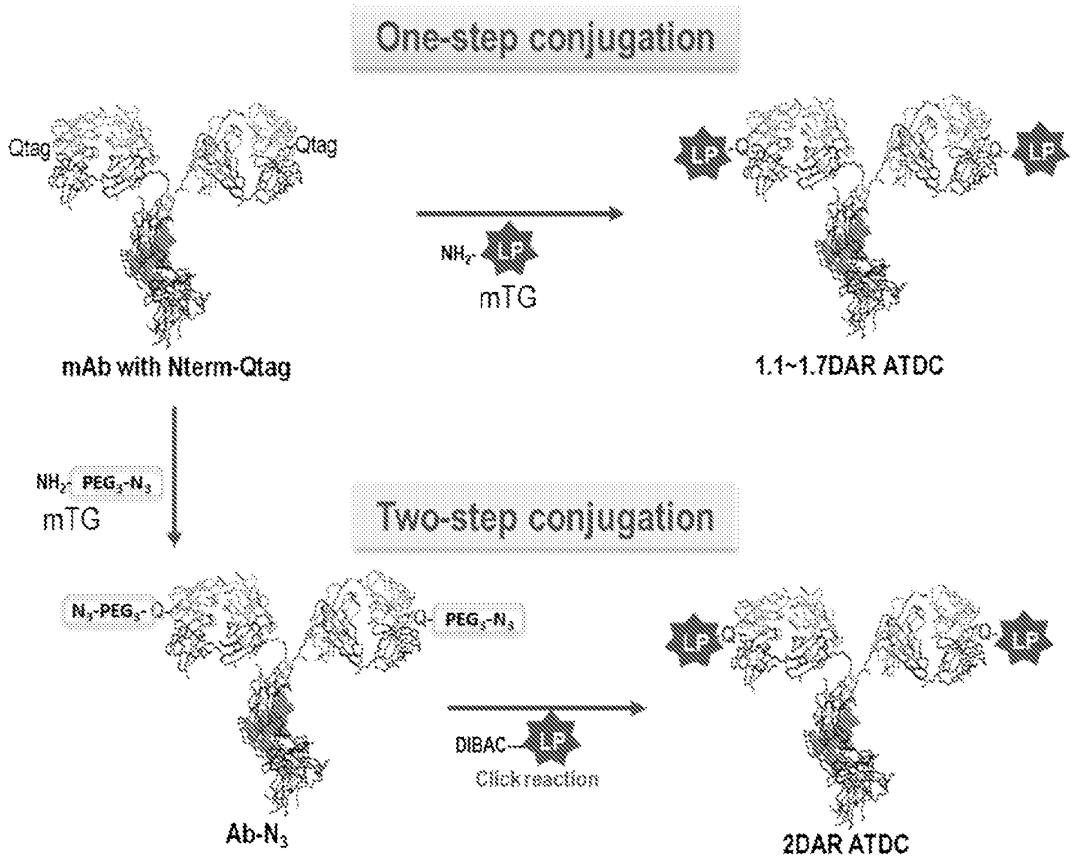

One-step conjugation
Qtag
Qtag
mAb with Nterm-Qtag
NH2-
LP
mTG
1.1~1.7DAR ATDC
NH2- PEG3-N3
mTG
Two-step conjugation
N3-PEG3-Q
PEG3-N3
Ab-N3
DIBAC-
LP
Click reaction
2DAR ATDC

FIG. 9B

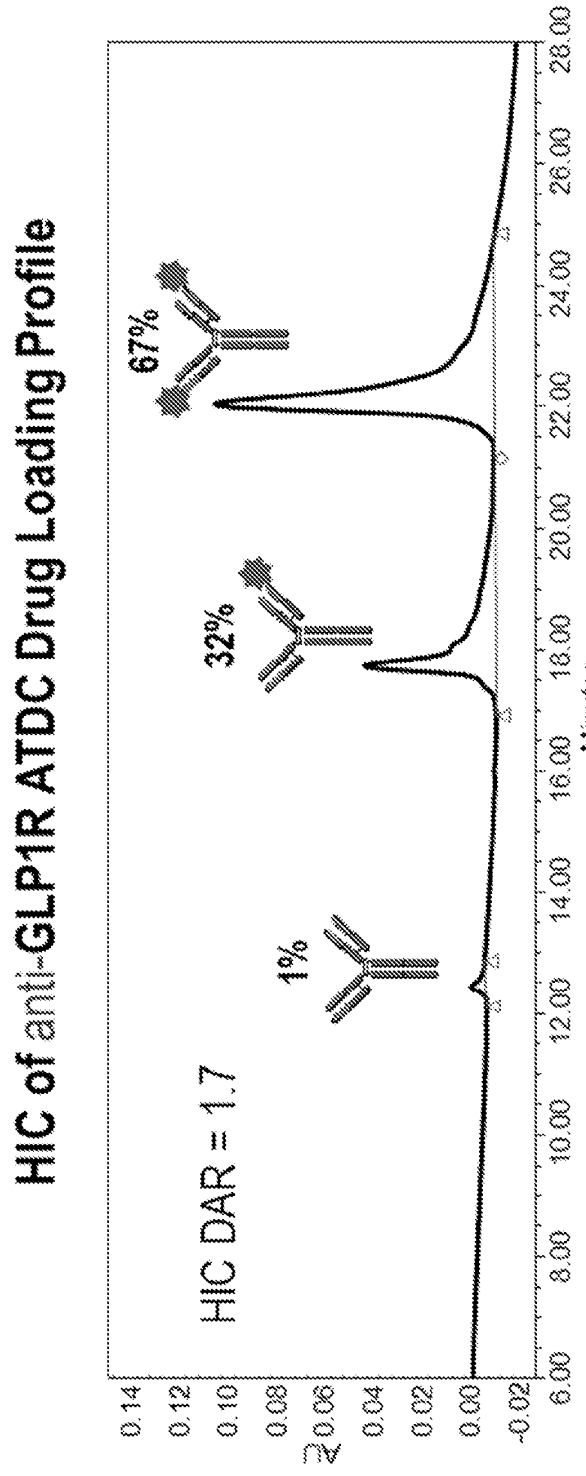
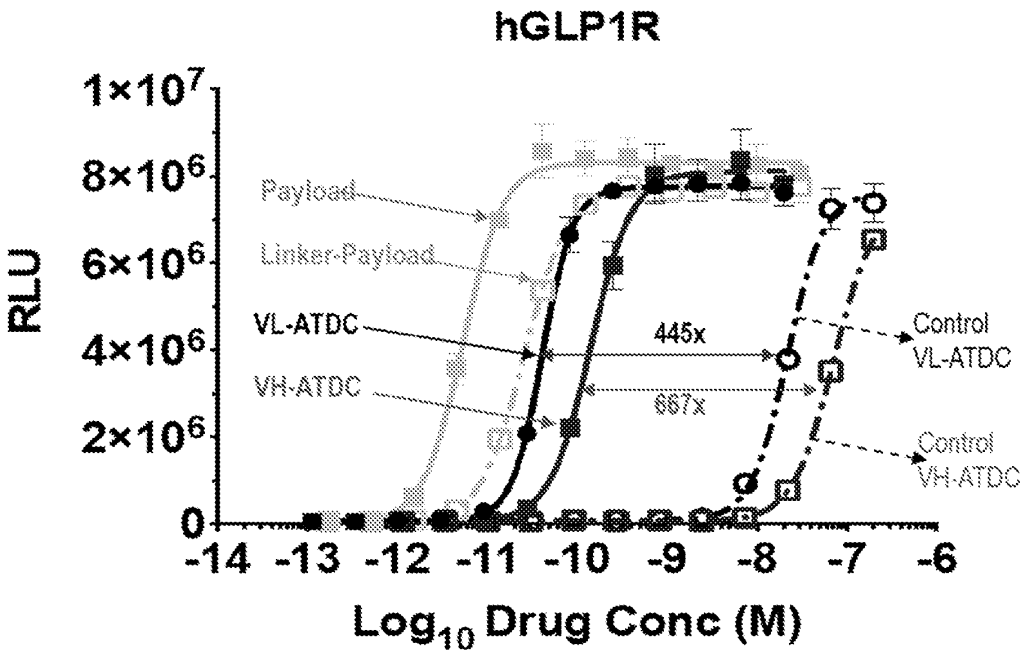

HIC of anti-GLP1R ATDC Drug Loading Profile
HIC DAR = 1.7
1%
32%
67%
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
Minutes
DAR: Drug to Antibody Ratio
LP: Linker payload
MTG: microbial Transglutaminase
HIC: Hydrophobic Interaction Chromatography

FIG. 10



Description	DAR	GLP1R LUC EC_{50} (pM)
Anti-GLP1R mAb2-VL-Qtag-LP11 (GLP1R-ATDC)	1.2	27
Isotype Control mAb2-VL-Qtag-LP11 (control ATDC)	1.6	12000
Anti-GLP1R mAb6-VH-Qtag-LP11 (GLP1R-ATDC)	1.8	110
Isotype Control mAb1-VH-Qtag-LP11 (Control ATDC)	1.6	75000
Linker-Payload		37
Payload		18
Control		28

FIG. 11A

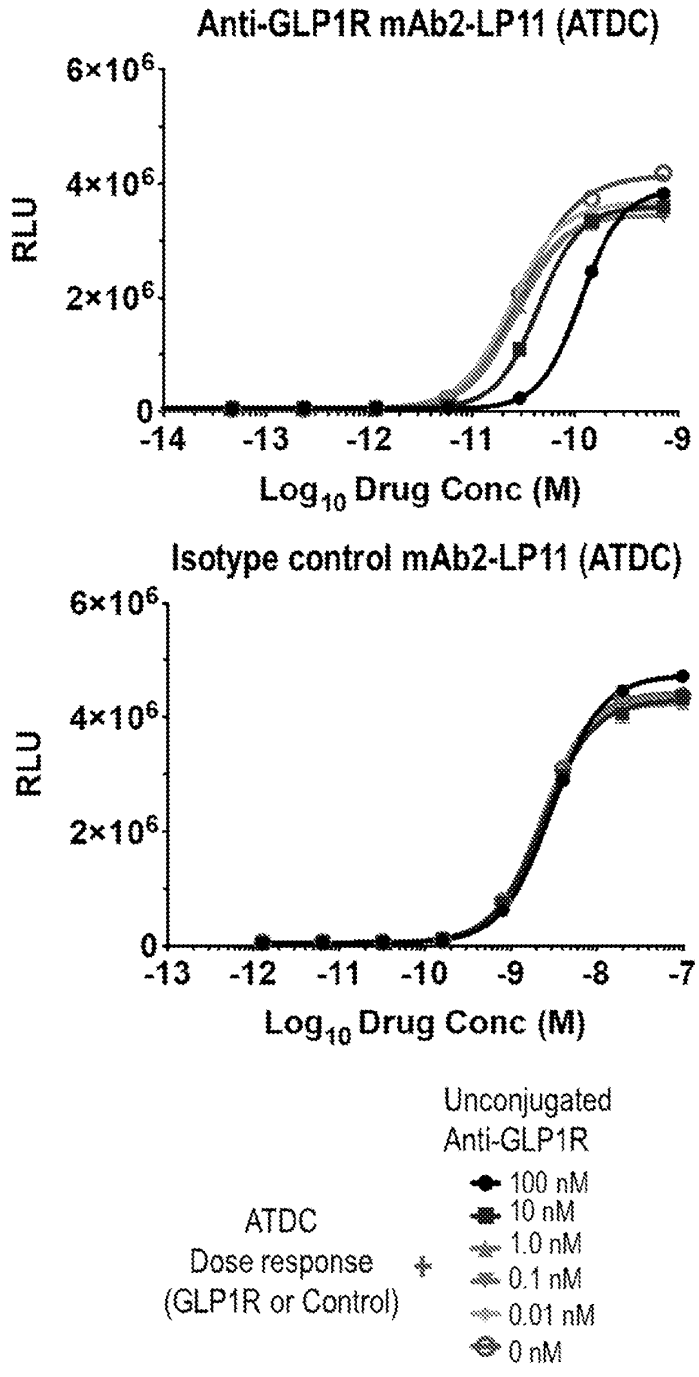

Anti-GLP1R mAb2-LP11 (ATDC)
RLU
6×10^6
4×10^6
2×10^6
0
-14
-13
-12
-11
-10
-9
Log10 Drug Conc (M)
Isotype control mAb2-LP11 (ATDC)
RLU
6×10^6
4×10^6
2×10^6
0
-13
-12
-11
-10
-9
-8
-7
Log10 Drug Conc (M)
ATDC
Dose response
(GLP1R or Control)
+
Unconjugated
Anti-GLP1R
100 nM
10 nM
1.0 nM
0.1 nM
0.01 nM
0 nM

FIG. 11B

Reagent		ATDC + mAb EC_{50} (M)	EC_{50} fold change compared to 0 nM anti-GLP1R parental mAb
Anti-GLP1R mAb2-LP11 (VL-Qtag-ATDC)	+ 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
		3.0E-11	1.0

FIG. 12

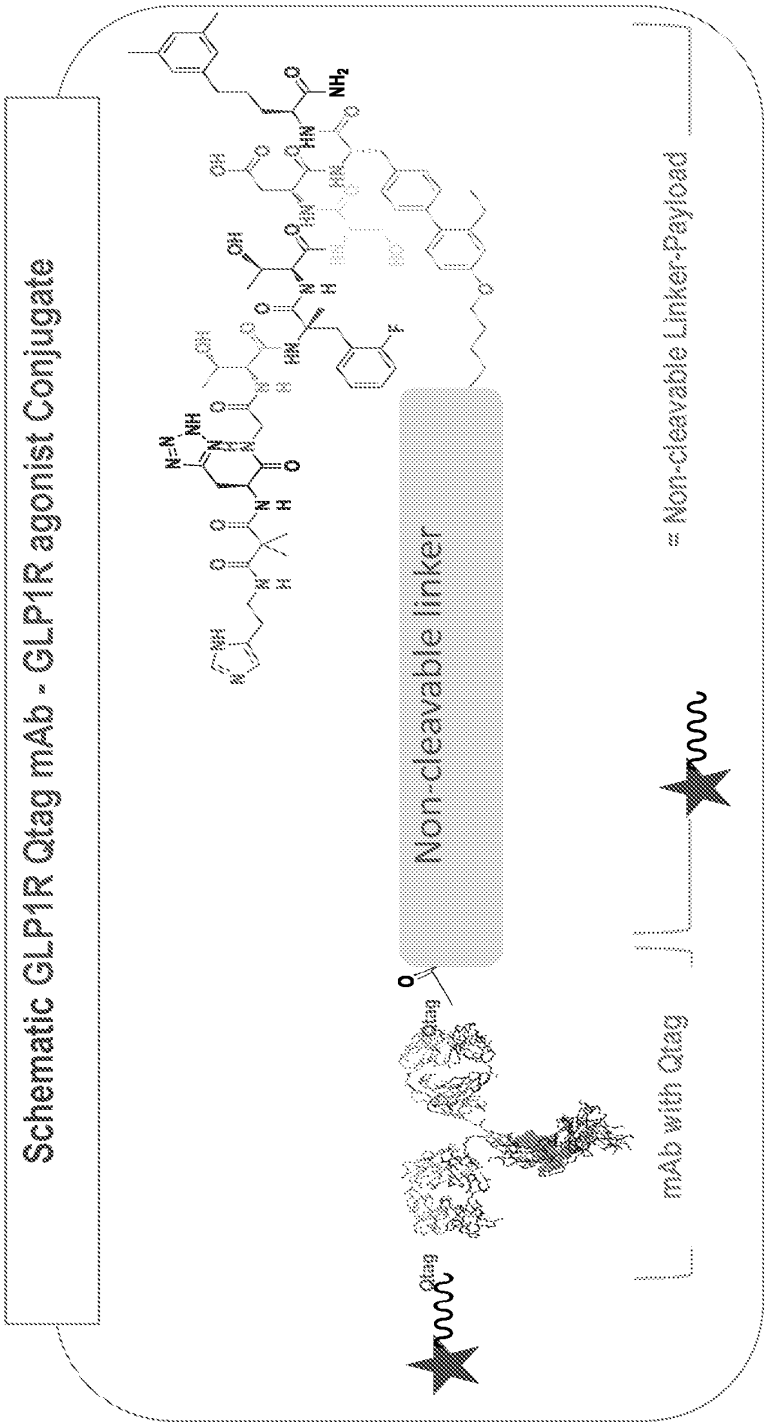

Schematic GLP1R Qtag mAb - GLP1R agonist Conjugate
= Non-cleavable Linker-Payload
Non-cleavable linker
mAb with Qtag
Qtag
Qtag

FIG. 13

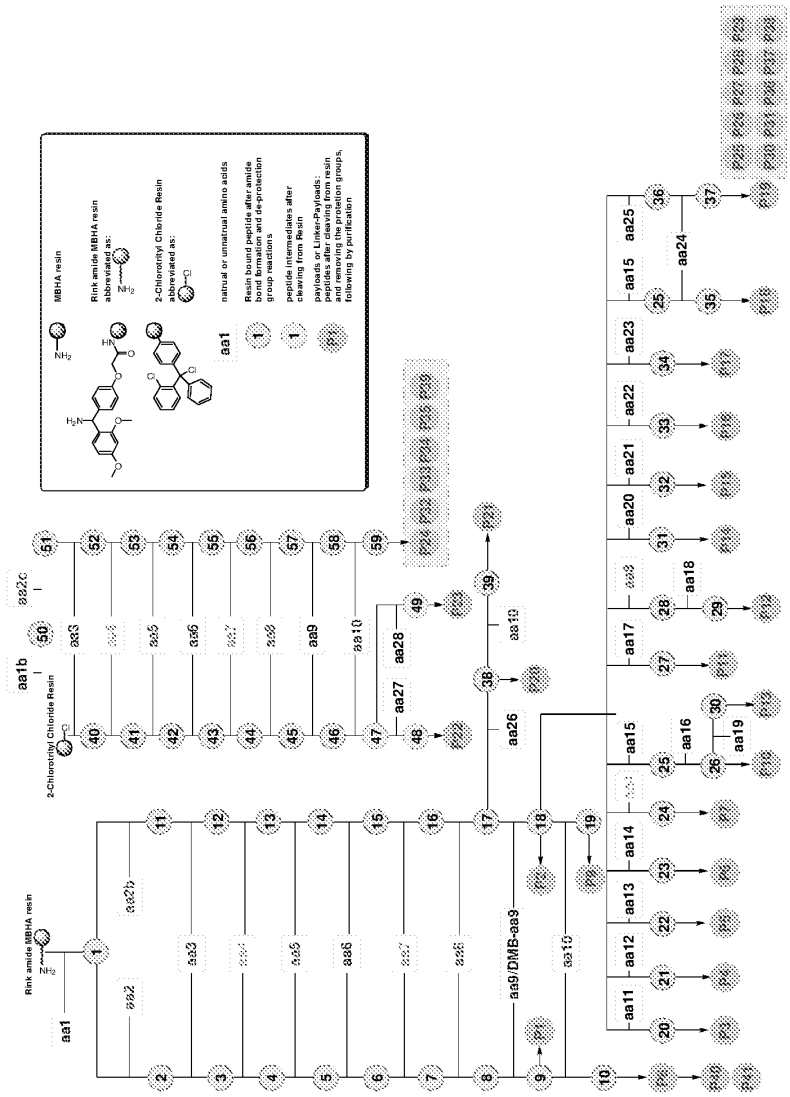

MBHA resin
Rink amide MBHA resin abbreviated as:
2-Chlorotrityl Chloride Resin abbreviated as:
natural or unnatural amino acids
Resin bound peptide after amide bond forming and de-protection group reactions
peptide intermediates after cleaving from Resin
payloads or Linker-Payloads peptide after cleaving from resin and removing the protetion groups, following by purification
Rink amide MBHA resin
2-Chlorotrityl Chloride Resin
aa9/DMB-aa9
aa10
aa26
aa27
aa28
aa15
aa16
aa18
aa19
aa24

FIG. 14

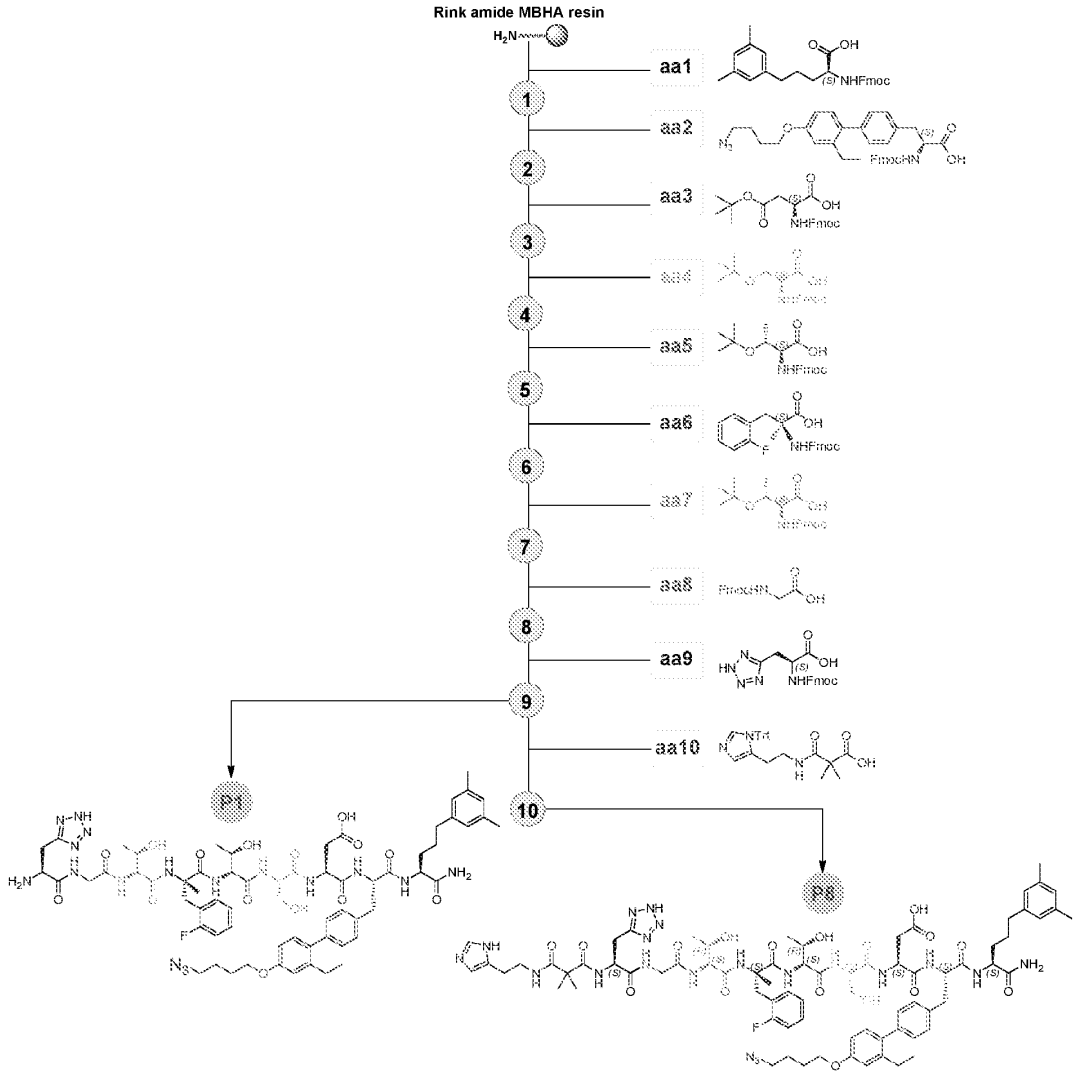

Rink amide MBHA resin
aa1
aa2
aa3
aa4
aa5
aa6
aa7
aa8
aa9
aa10
1
2
3
4
5
6
7
8
9
10
P1
P2

FIG. 15

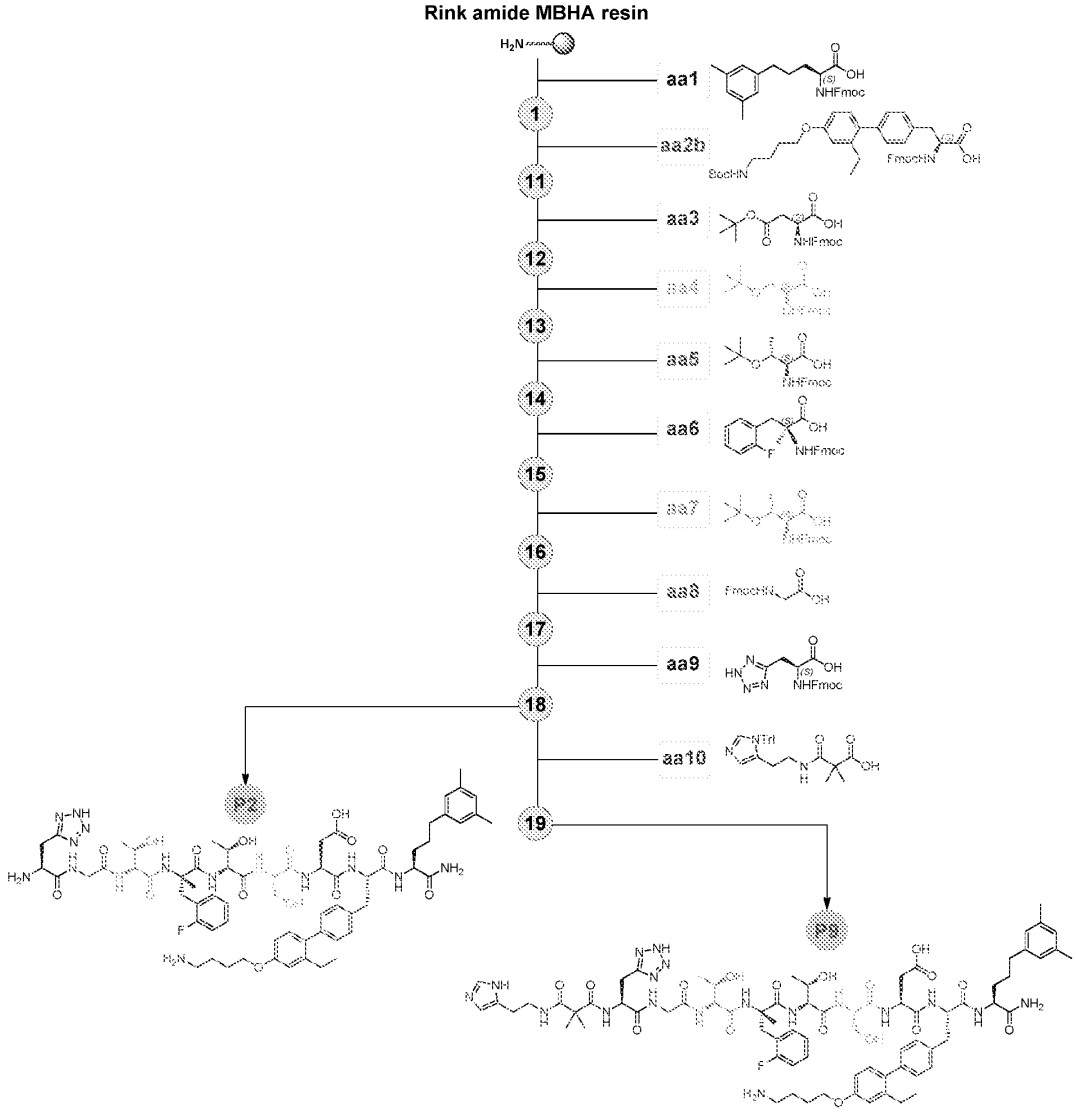
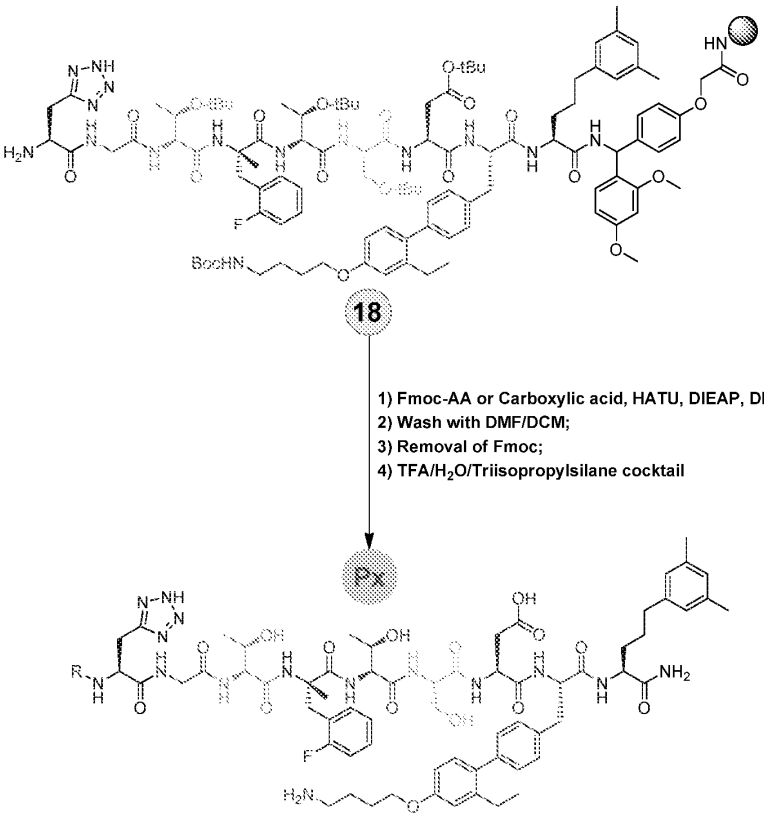

Rink amide MBHA resin
H2N
aa1
1
aa2b
11
aa3
12
aa4
13
aa5
14
aa6
15
aa7
16
aa8
17
aa9
18
aa10
19
P2
P3

FIG. 16



Px	P3	P4	P5	P6	P7	P11
R	aa11	aa12	aa13	aa14	aa4	aa17

Px	P13	P14, P15	P16, P17
R	Q-H-A	aa21 / aa20	aa22 / aa23

FIG. 17A

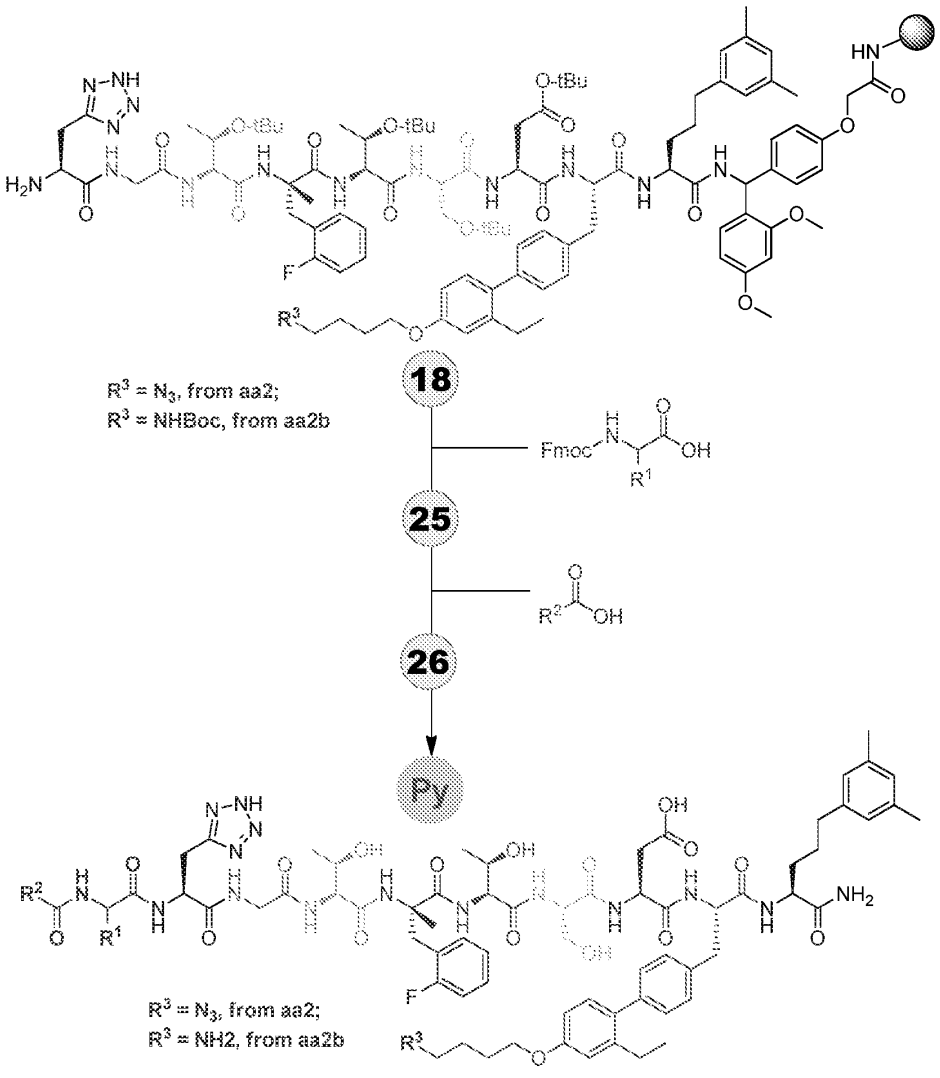

R3 = N3, from aa2;
R3 = NHBoc, from aa2b
18
25
26
Py
R3 = N3, from aa2;
R3 = NH2, from aa2b

FIGURE 17B

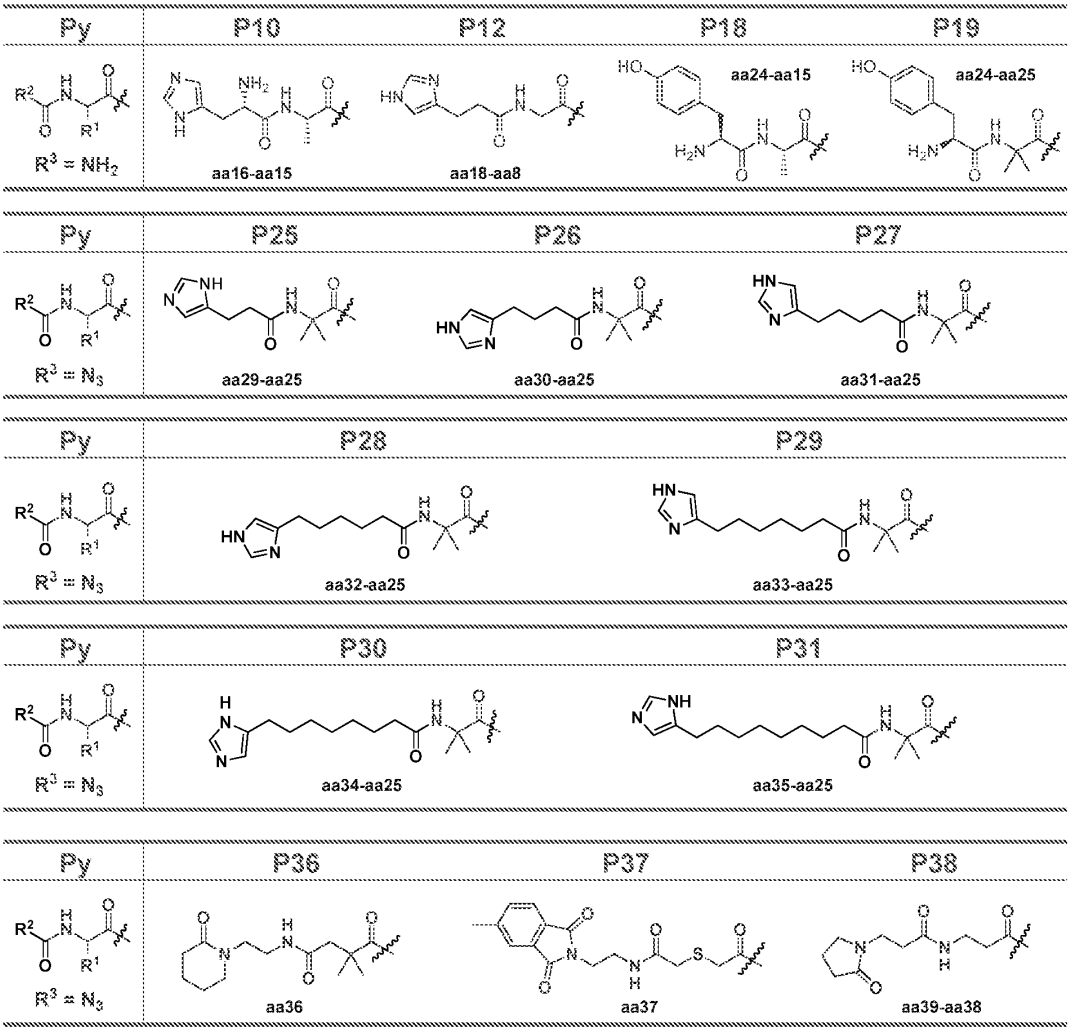

Py
P10
P12
P18
P19
R3 = NH2
aa16-aa15
aa18-aa8
aa24-aa15
aa24-aa25
Py
P25
P26
P27
R3 = N3
aa29-aa25
aa30-aa25
aa31-aa25
Py
P28
P29
R3 = N3
aa32-aa25
aa33-aa25
Py
P30
P31
R3 = N3
aa34-aa25
aa35-aa25
Py
P36
P37
P38
R3 = N3
aa36
aa37
aa39-aa38

FIG. 18

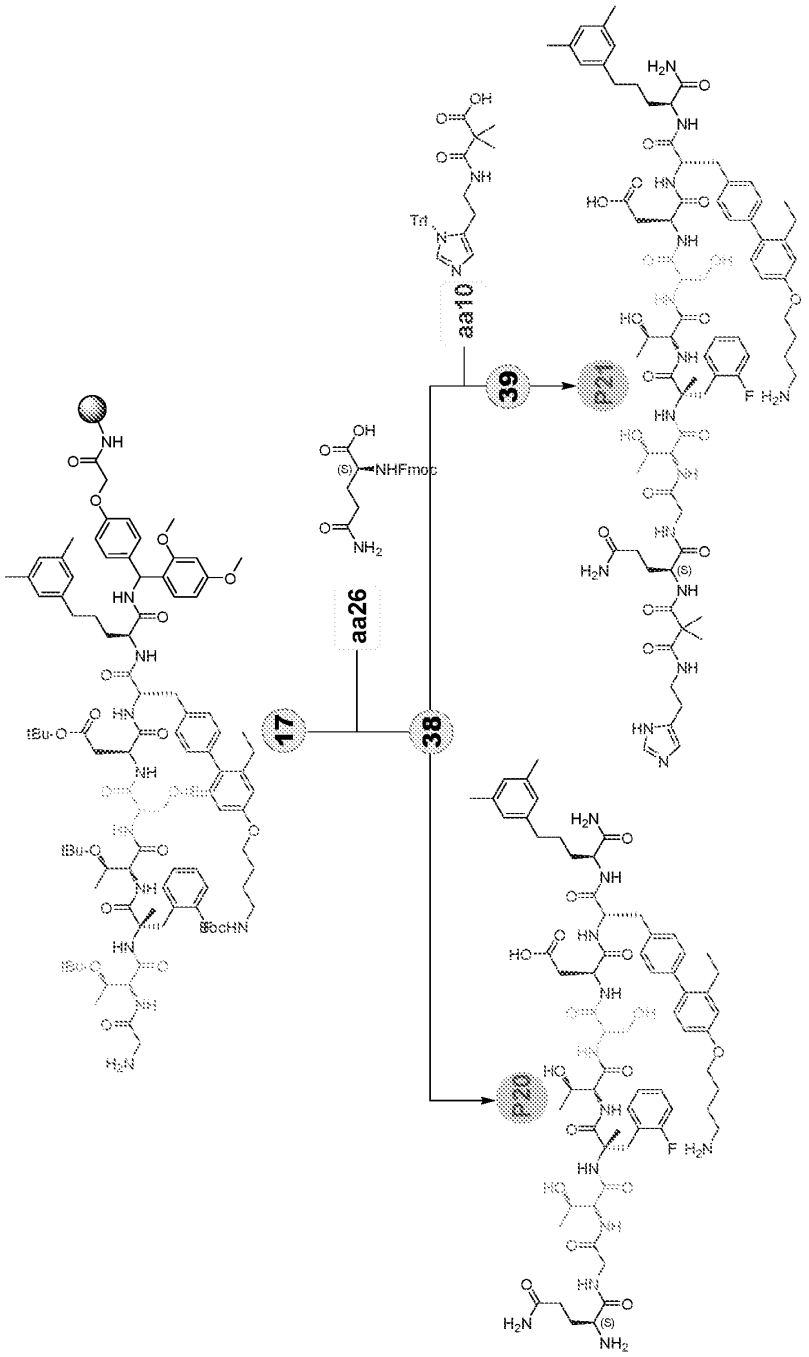


FIG. 19

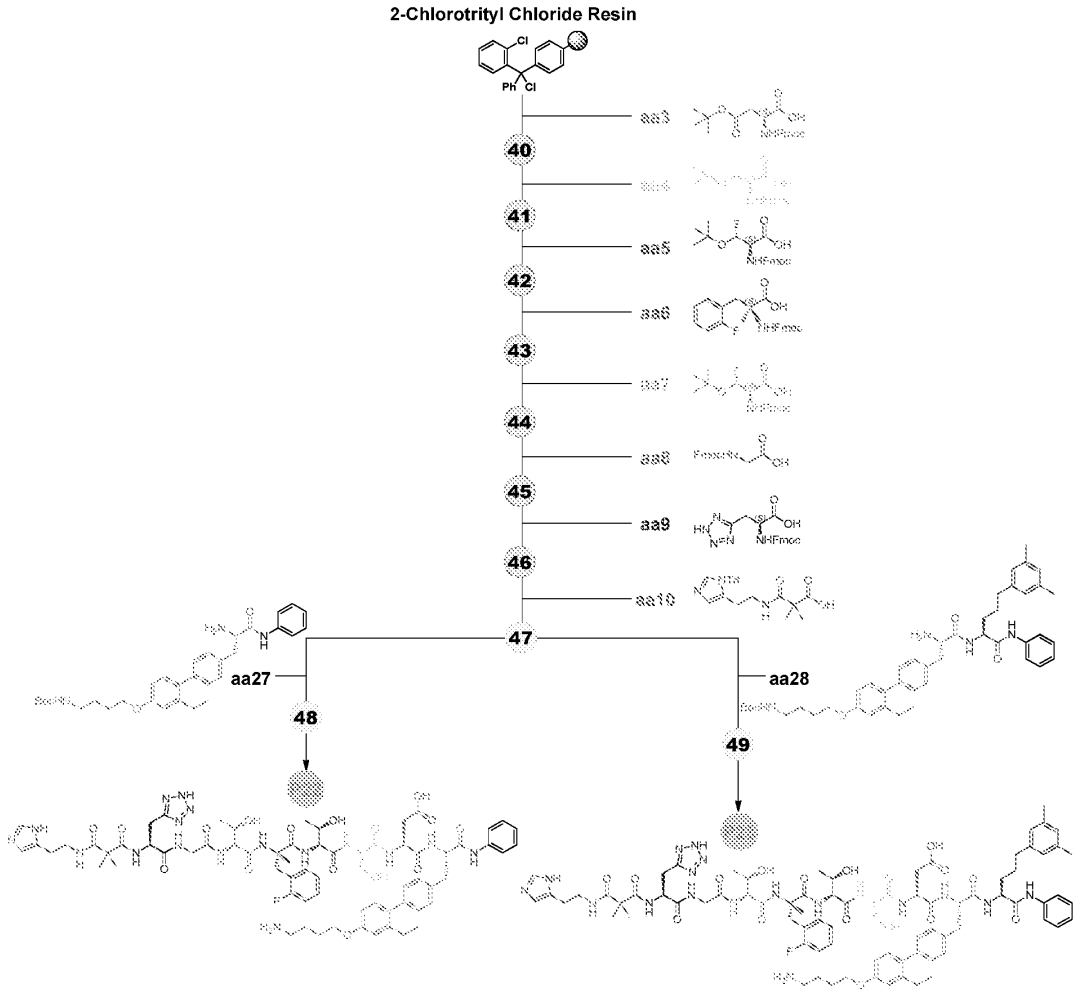

2-Chlorotrityl Chloride Resin
aa3
40
41
aa5
42
aa6
43
44
45
aa9
46
aa10
47
aa27
aa28
48
49

FIG. 20

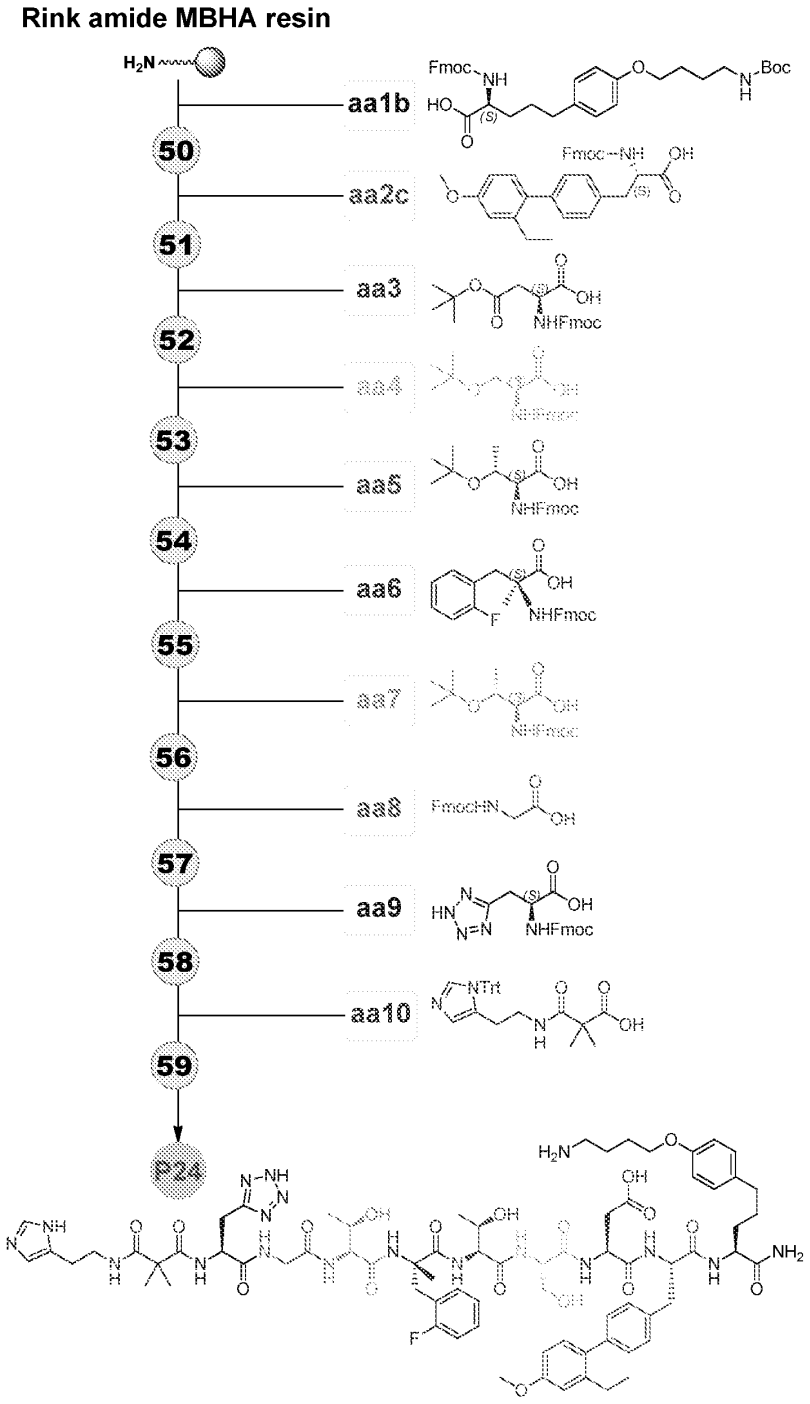

Rink amide MBHA resin
aa1b
50
aa2c
51
aa3
52
aa4
53
aa5
54
aa6
55
aa7
56
aa8
57
aa9
58
aa10
59
P-24

FIG. 21

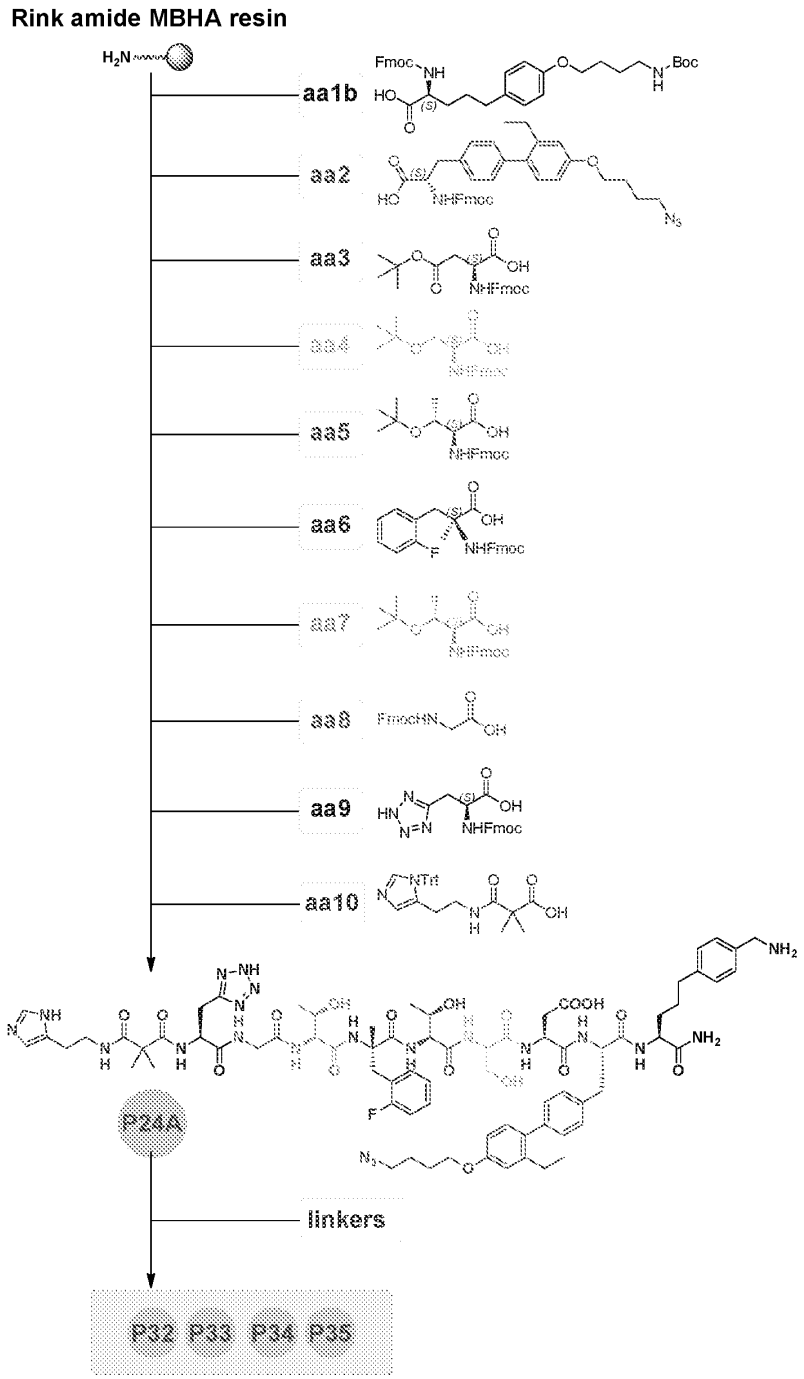

Rink amide MBHA resin
aa1b
aa2
aa3
aa4
aa5
aa6
aa7
aa8
aa9
aa10
P24A
linkers
P32 P33 P34 P35

FIG. 22

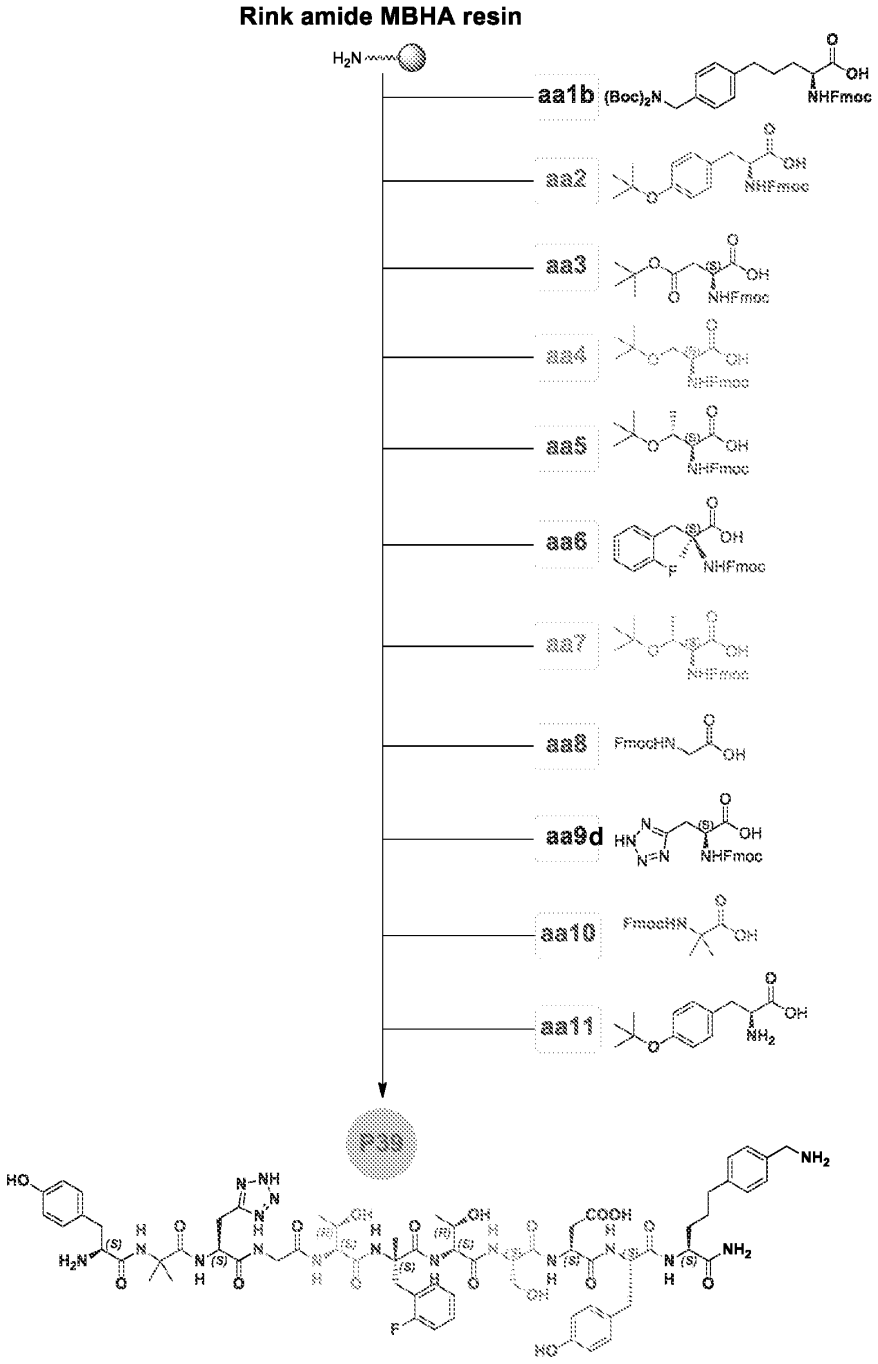

Rink amide MBHA resin
aa1b
aa2
aa3
aa4
aa5
aa6
aa7
aa8
aa9d
aa10
aa11
PEG

FIG. 23

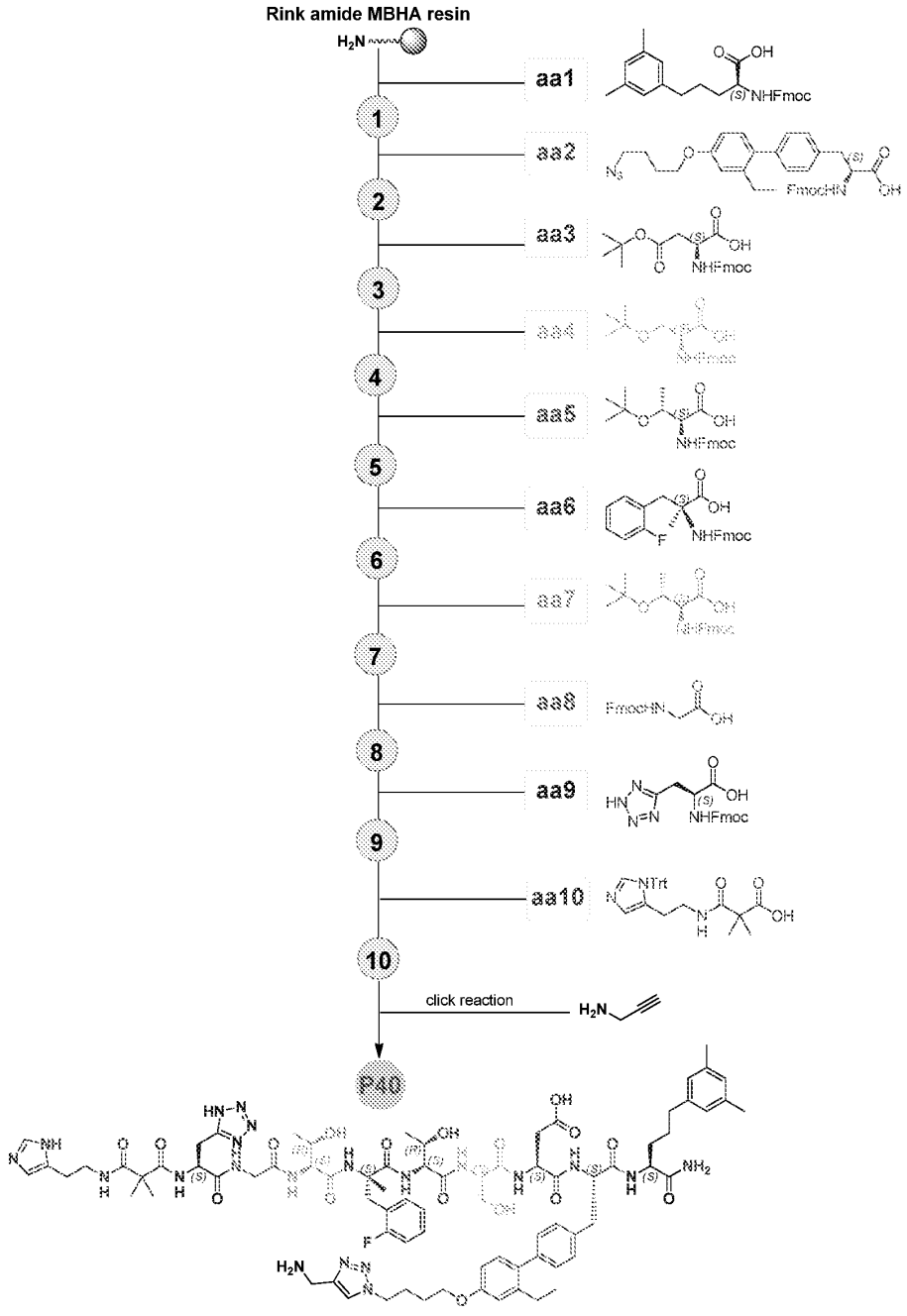

Rink amide MBHA resin
aa1
aa2
aa3
aa4
aa5
aa6
aa7
aa8
aa9
aa10
1
2
3
4
5
6
7
8
9
10
click reaction
P40

FIG. 24

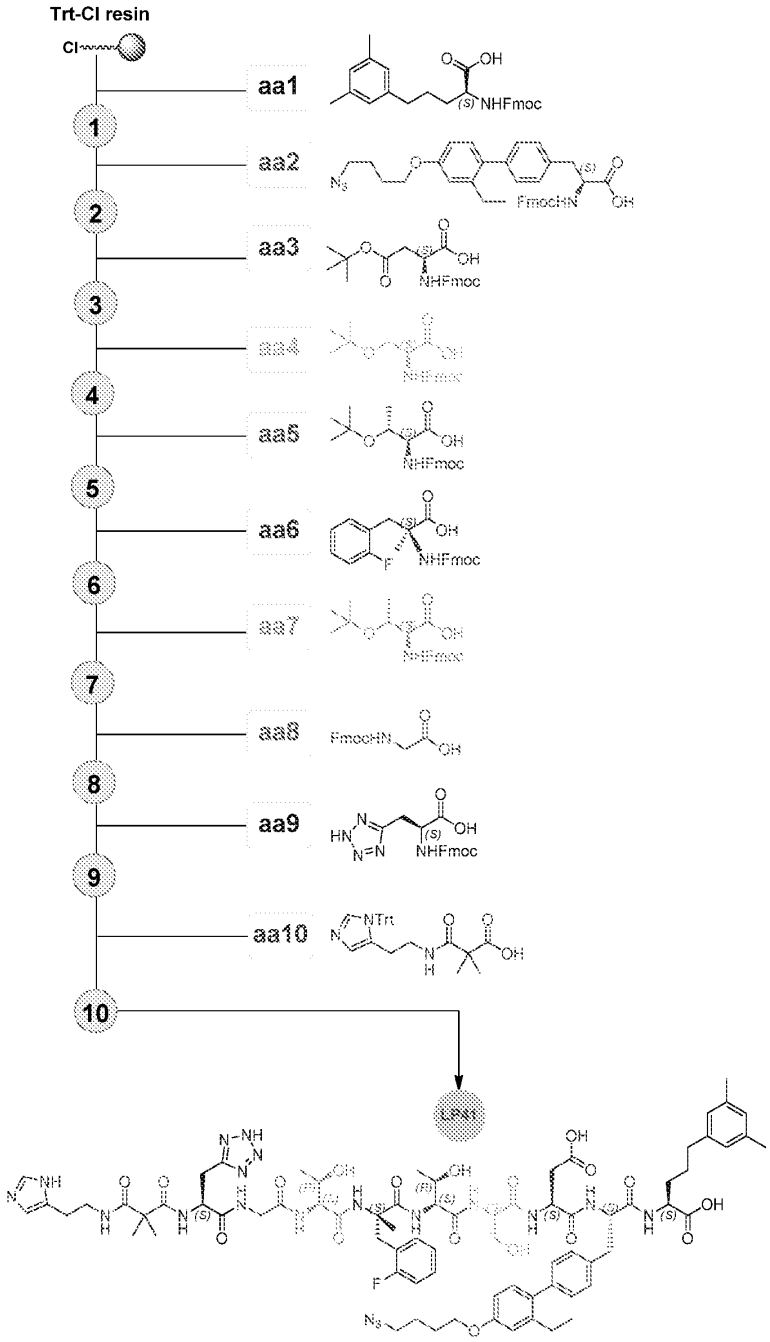

Trt-Cl resin
Cl
aa1
aa2
aa3
aa4
aa5
aa6
aa7
aa8
aa9
aa10
1
2
3
4
5
6
7
8
9
10
LPN1

FIG. 25

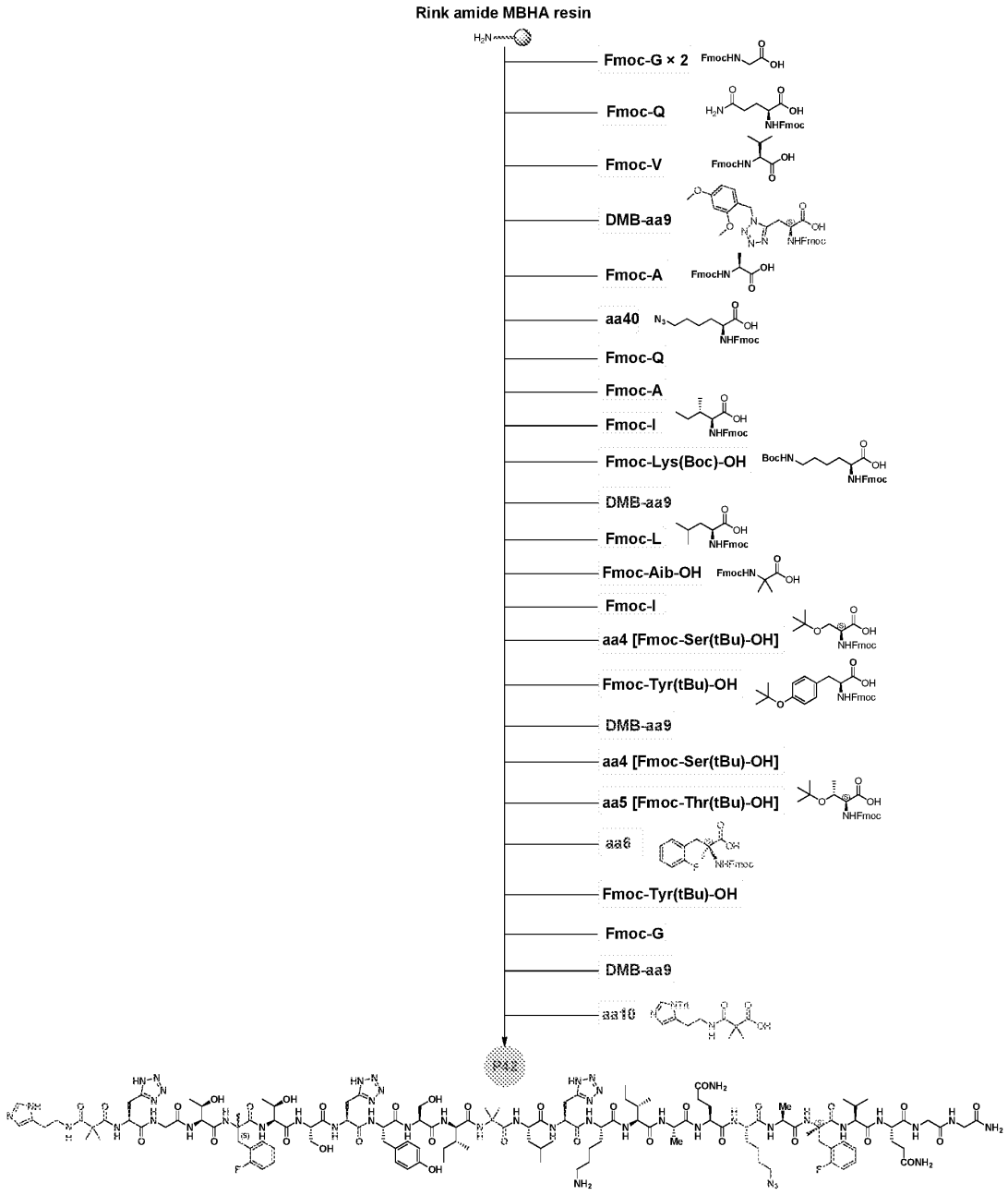

Rink amide MBHA resin
Fmoc-G × 2
Fmoc-Q
Fmoc-V
DMB-aa9
Fmoc-A
aa40
Fmoc-Q
Fmoc-A
Fmoc-I
Fmoc-Lys(Boc)-OH
DMB-aa9
Fmoc-L
Fmoc-Aib-OH
Fmoc-I
aa4 [Fmoc-Ser(tBu)-OH]
Fmoc-Tyr(tBu)-OH
DMB-aa9
aa4 [Fmoc-Ser(tBu)-OH]
aa5 [Fmoc-Thr(tBu)-OH]
aa6
Fmoc-Tyr(tBu)-OH
Fmoc-G
DMB-aa9
aa10

FIG. 26

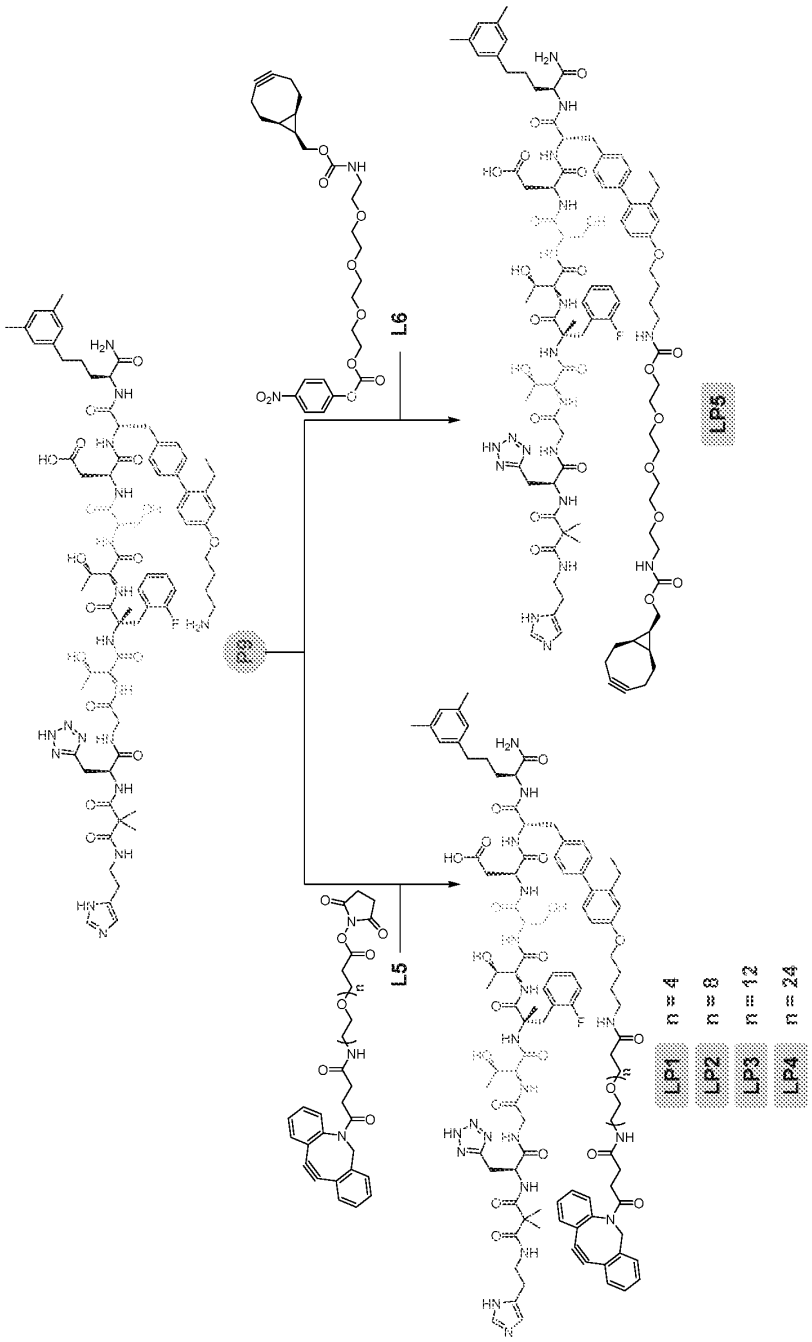


FIG. 27

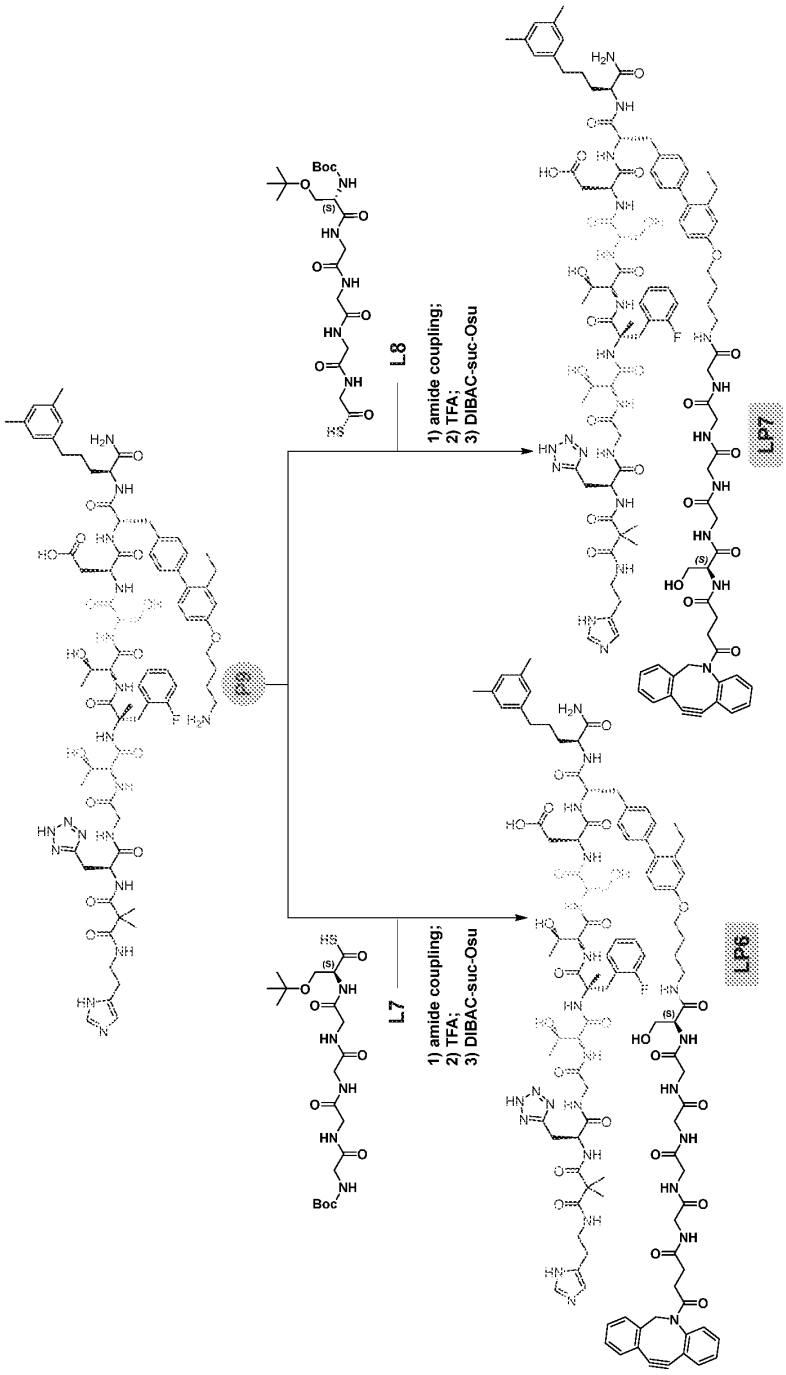

L8
1) amide coupling;
2) TFA;
3) DIBAC-suc-Osu
LP7
L7
1) amide coupling;
2) TFA;
3) DIBAC-suc-Osu
LP6

FIG. 28

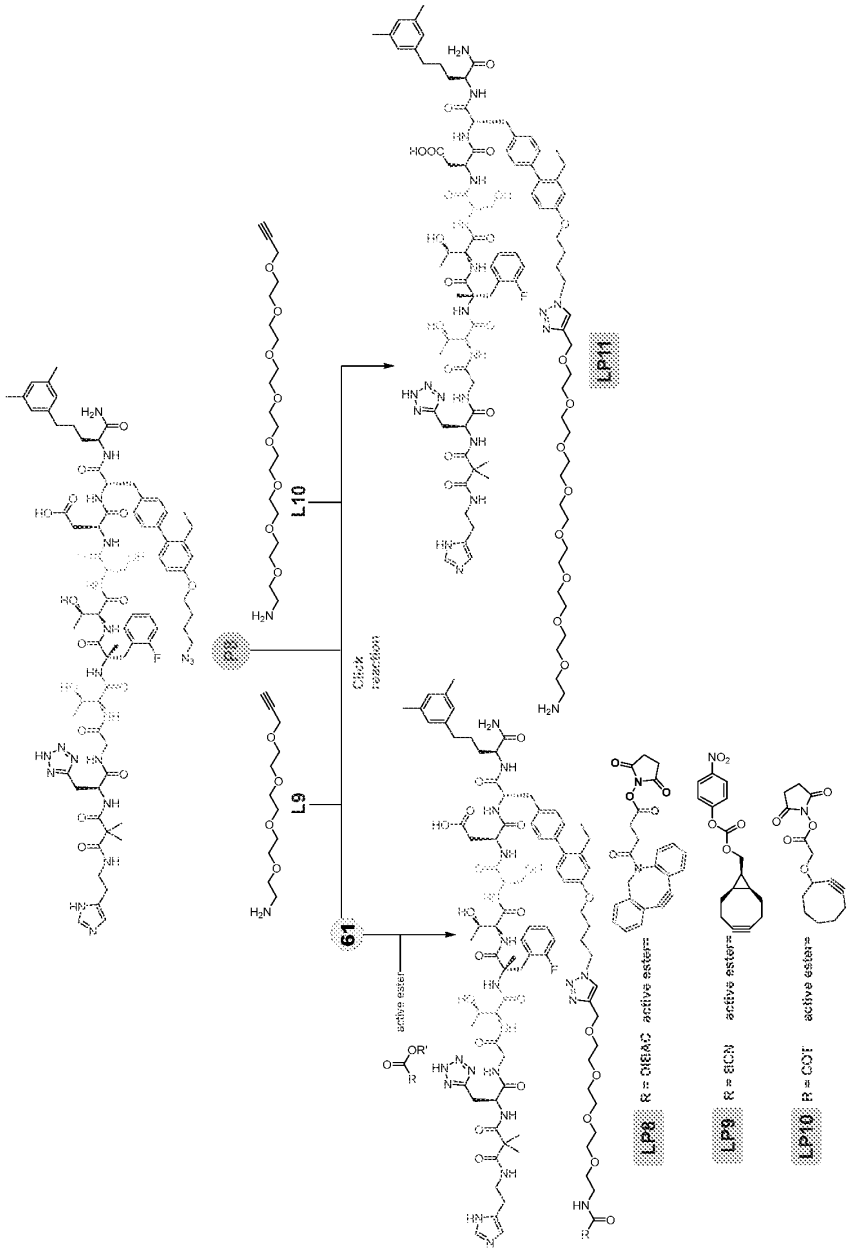

L10
L9
61
Click reaction
active ester
LP11
LP8
R = OMeAC active ester
LP9
R = BCN active ester
LP10
R = COT active ester

FIG. 29

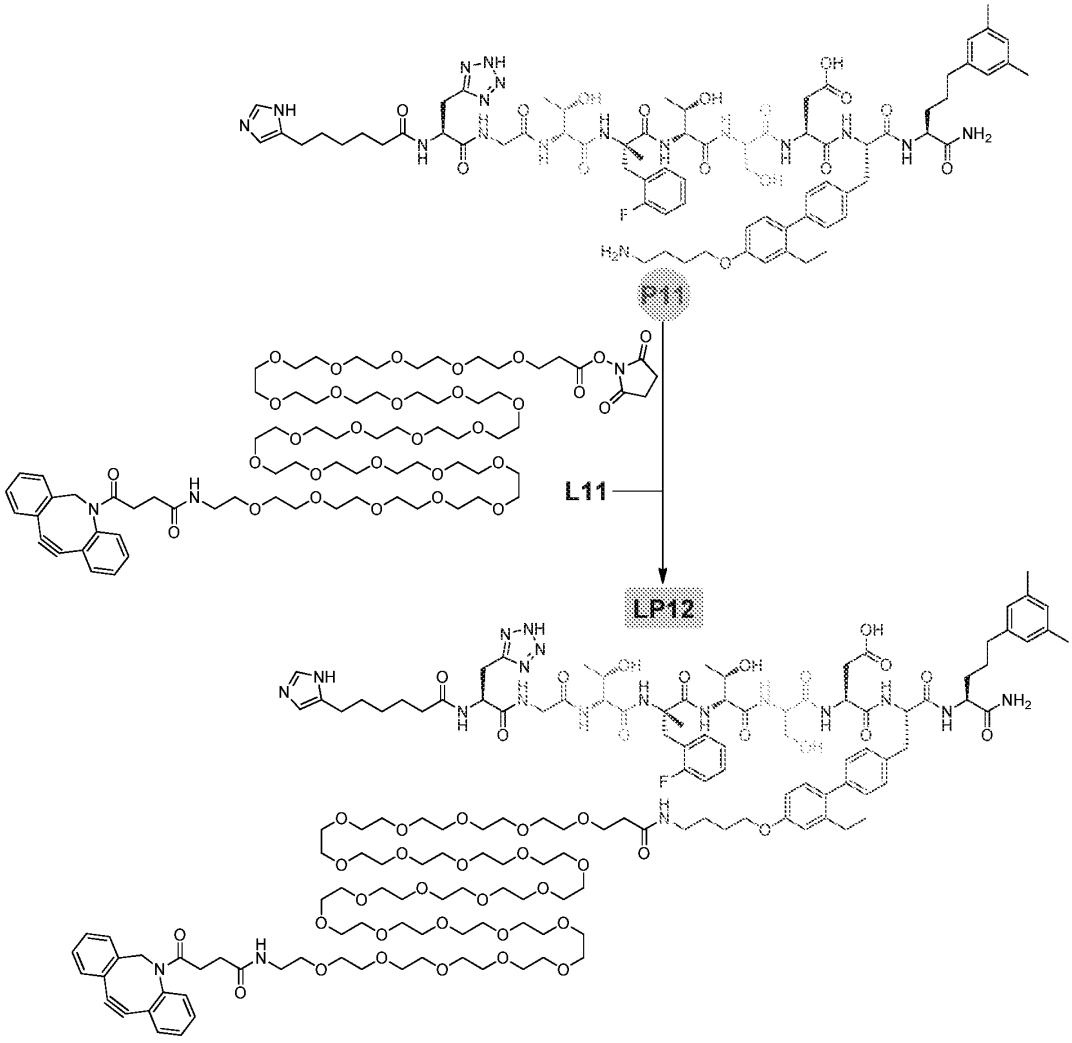


FIG. 30

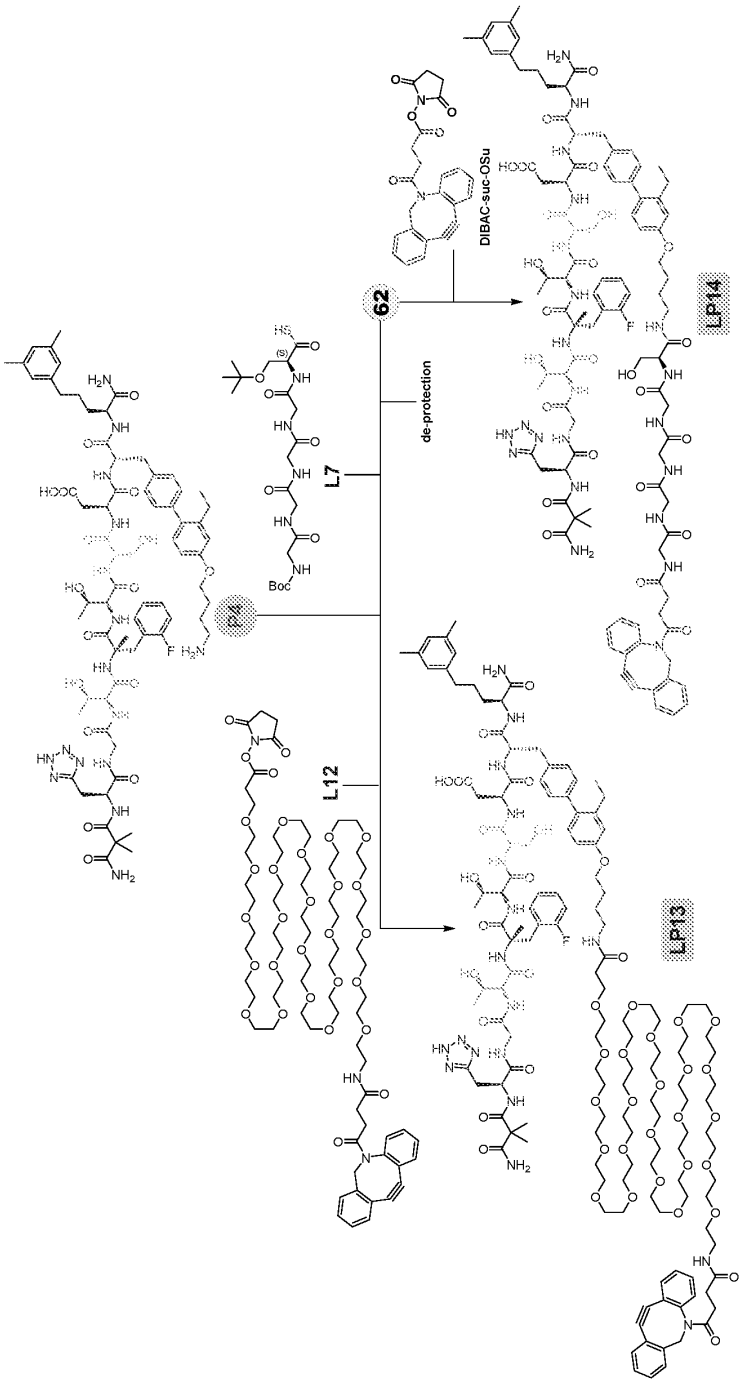


FIG. 31

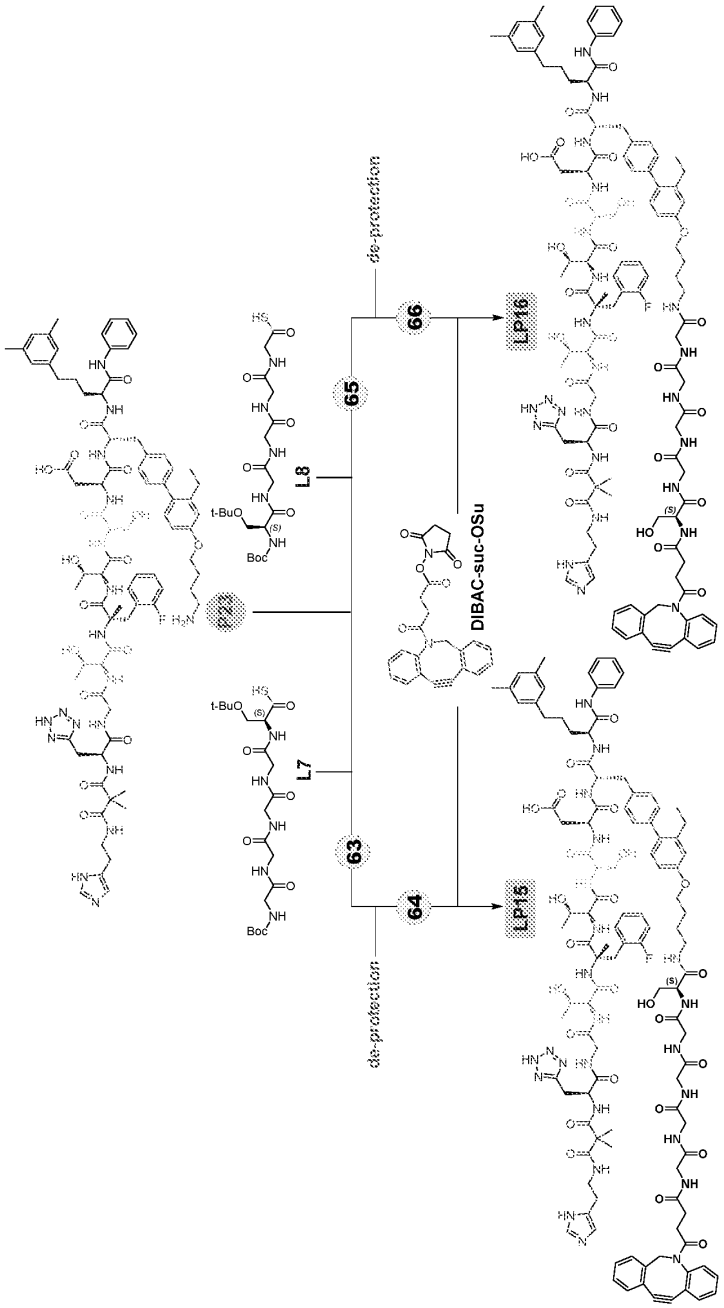

de-protection
66
LP16
65
L8
DIBAC-suc-OSu
63
L7
64
LP15
de-protection

FIG. 32

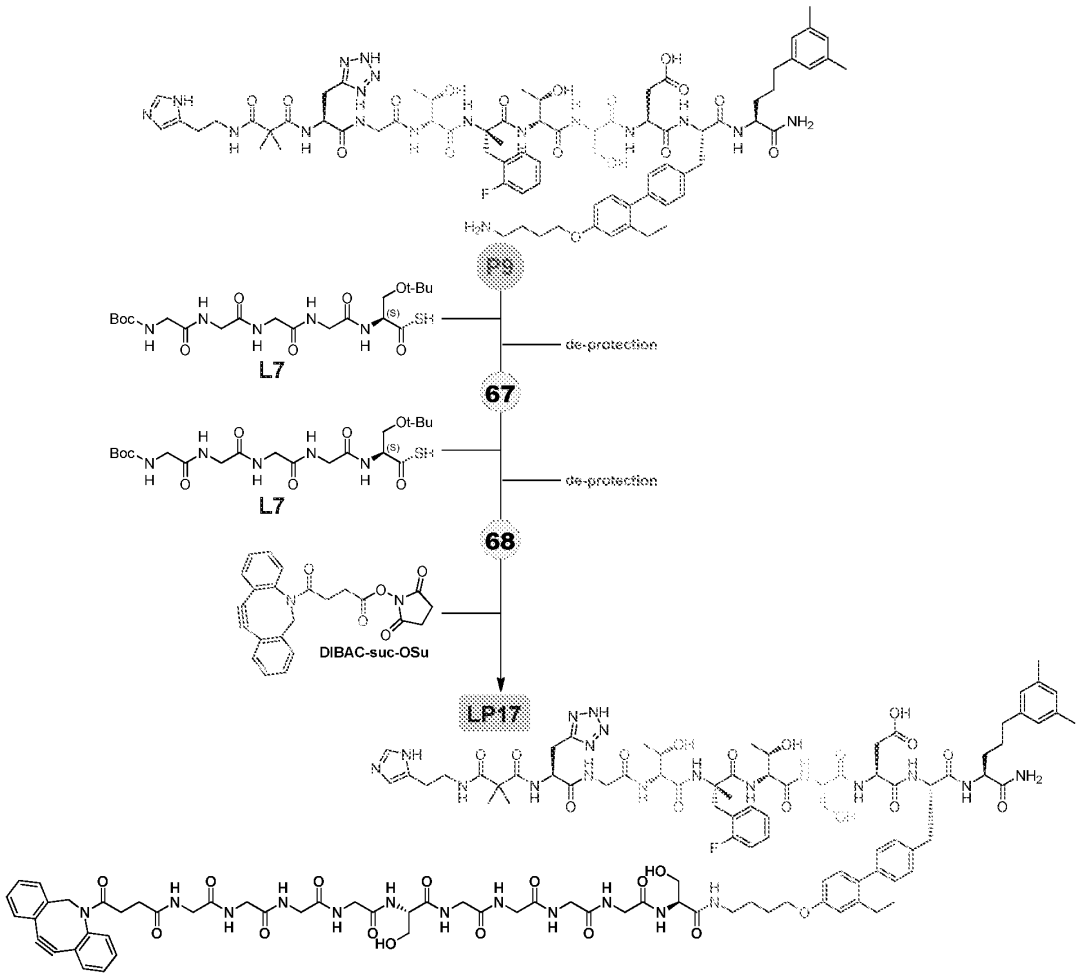


FIG. 33

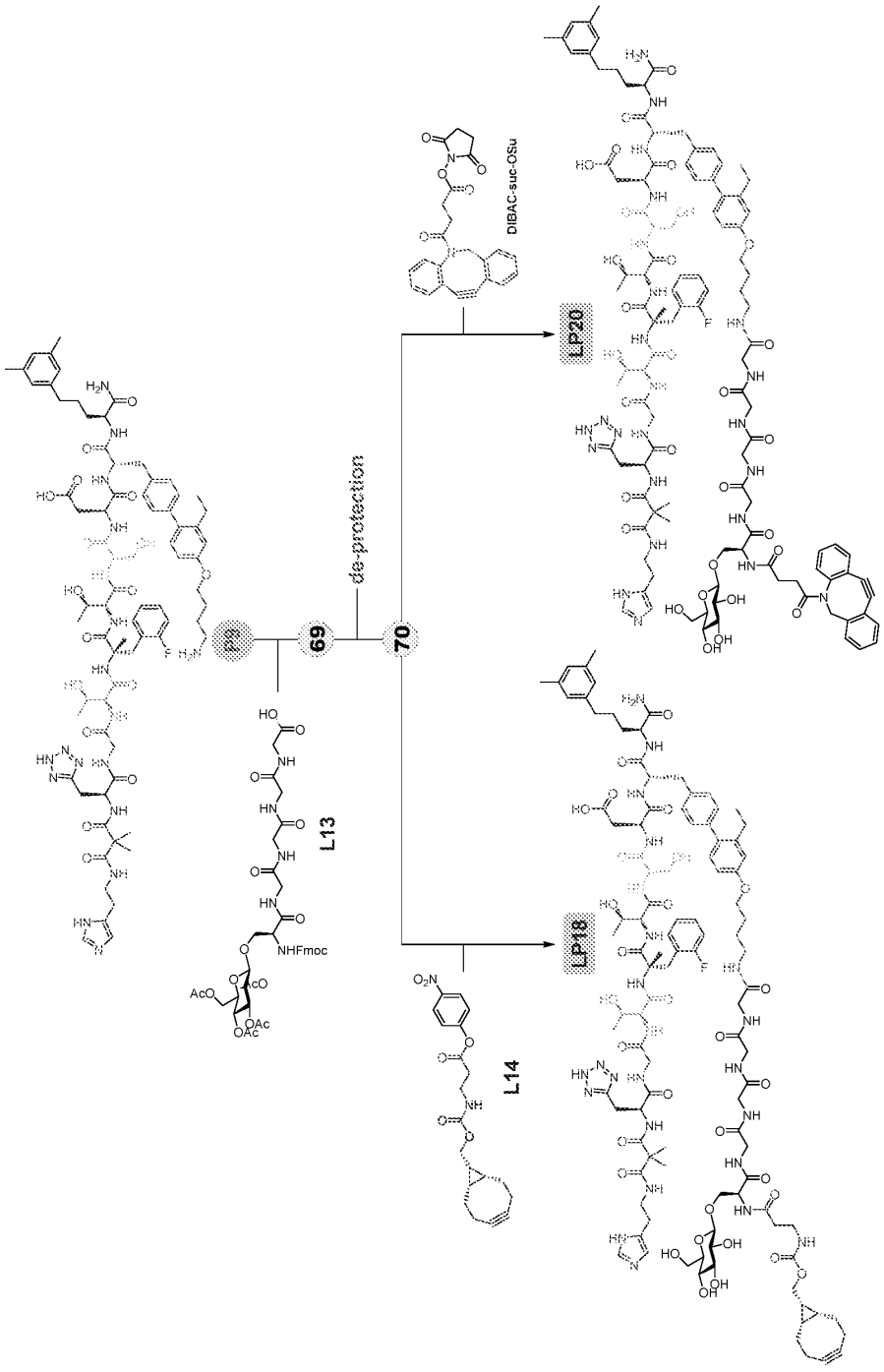


FIG. 34

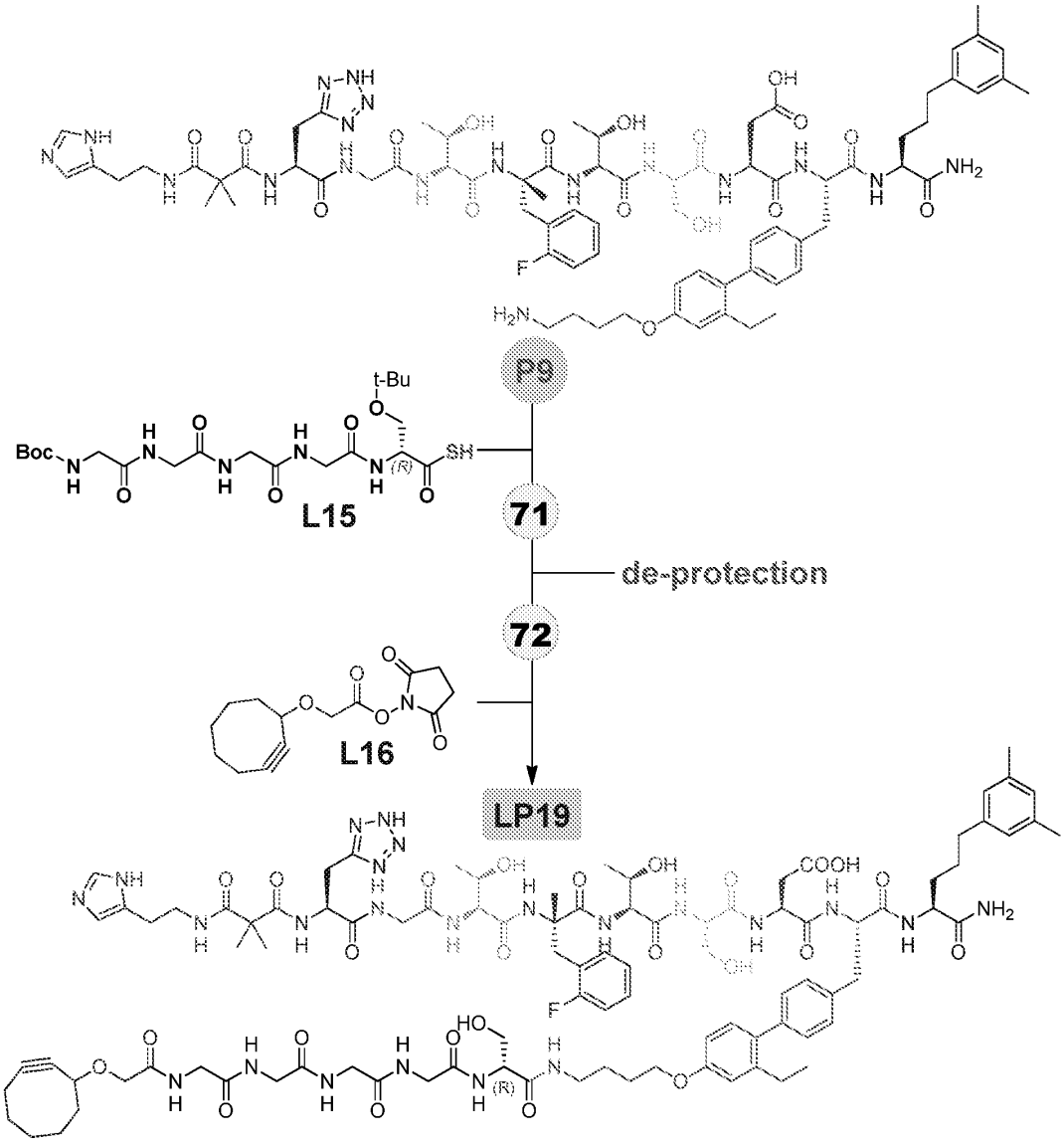


FIG. 35

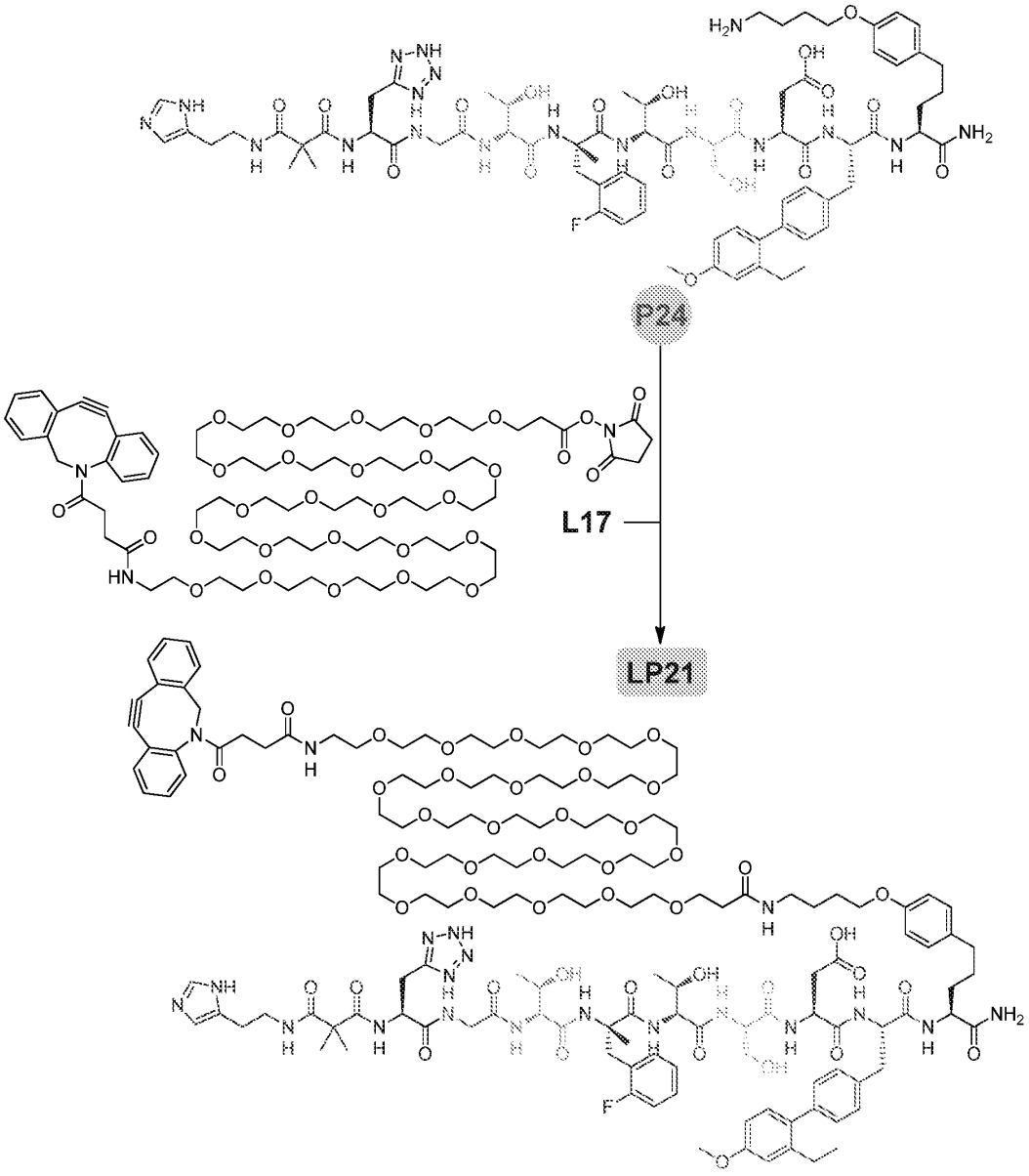


FIG. 36

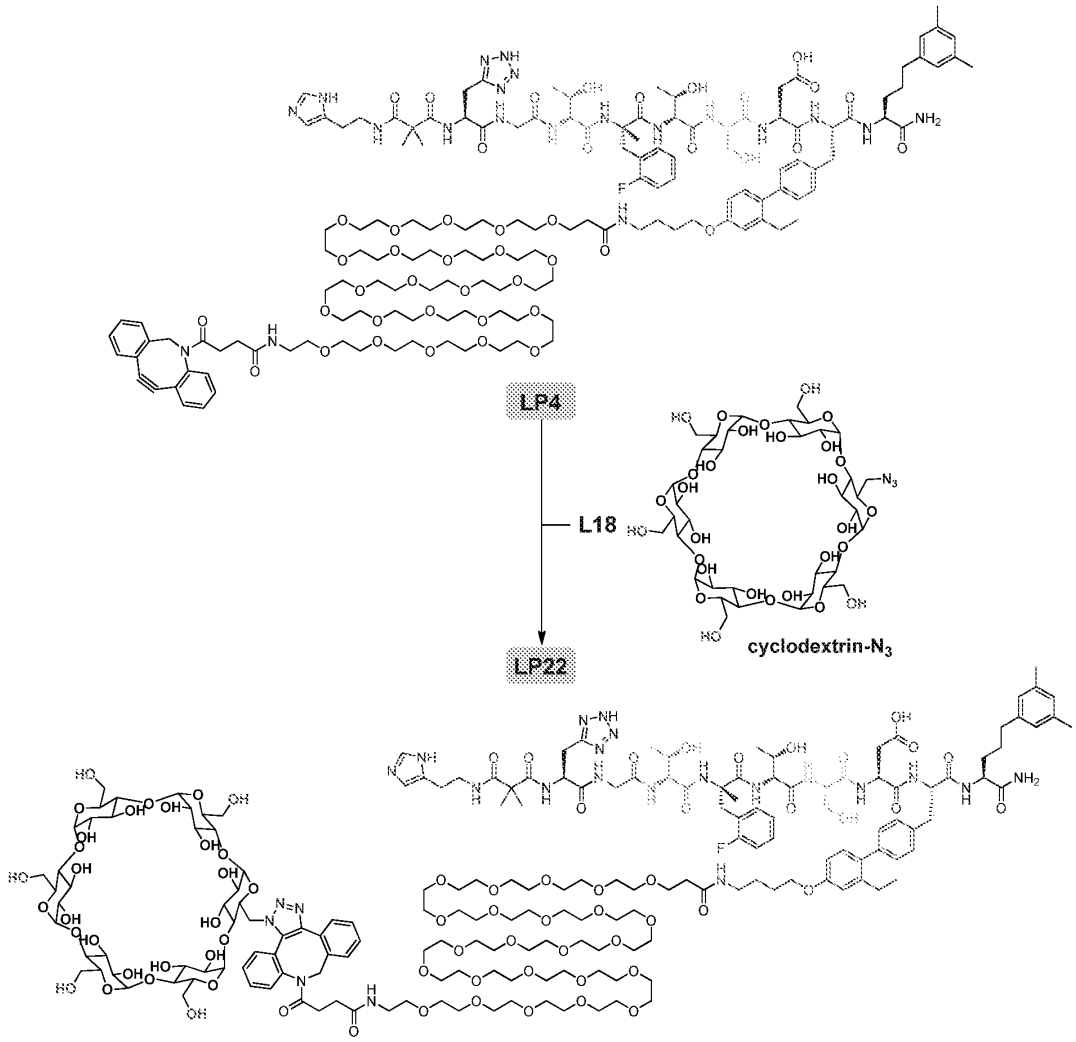


FIG. 37

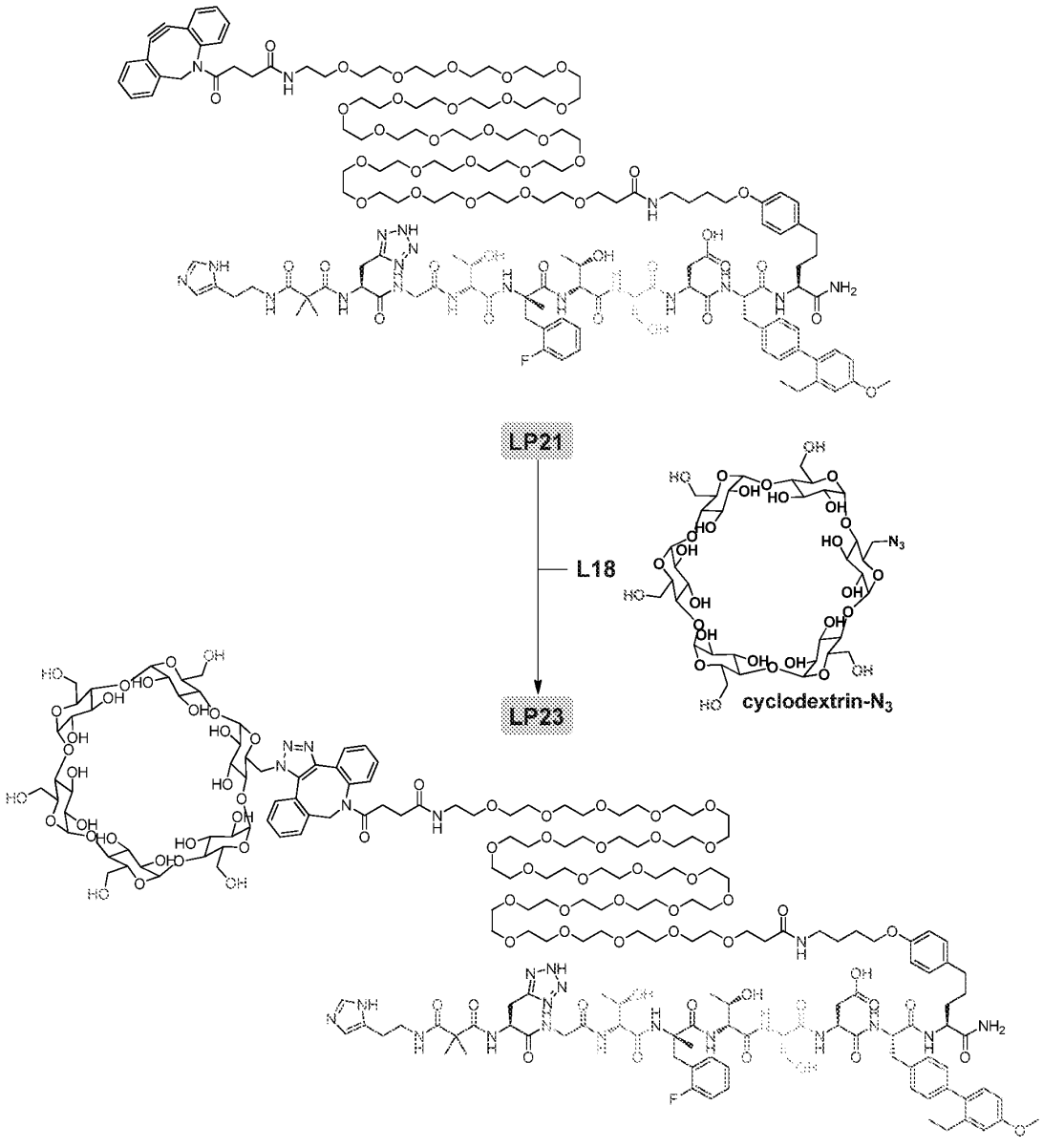


FIG. 38

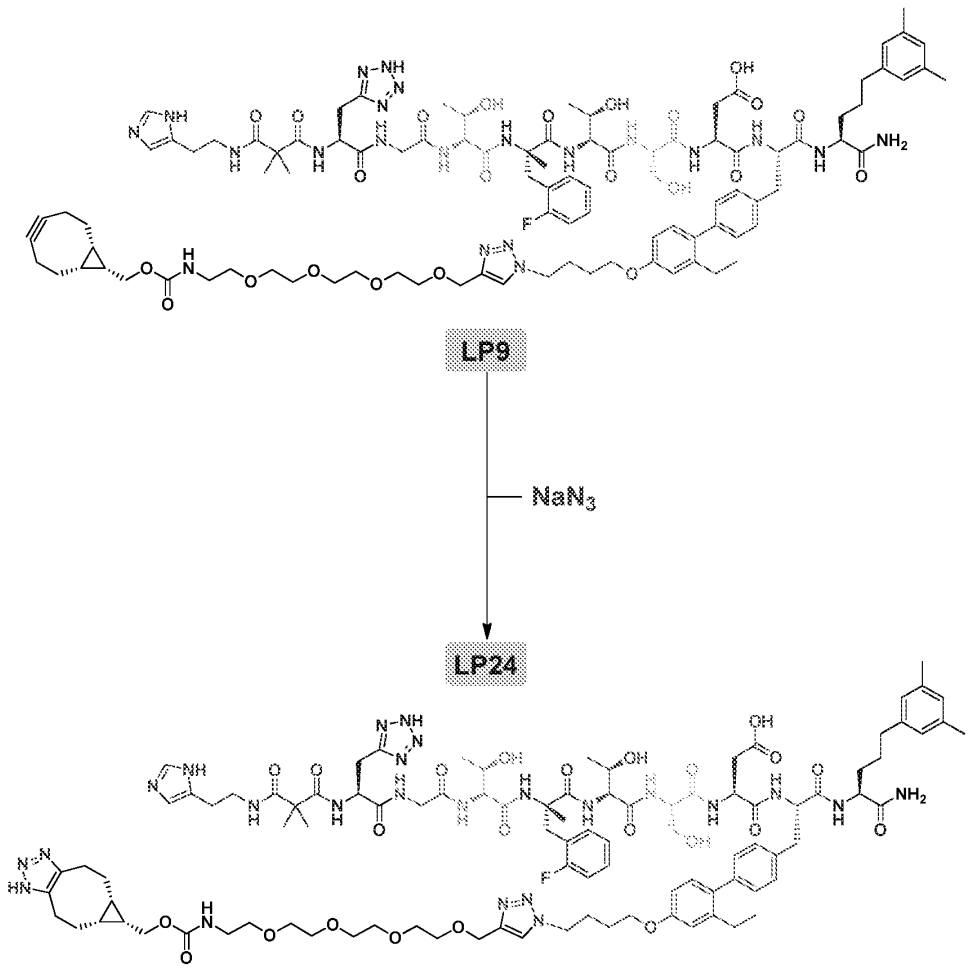


FIG. 39

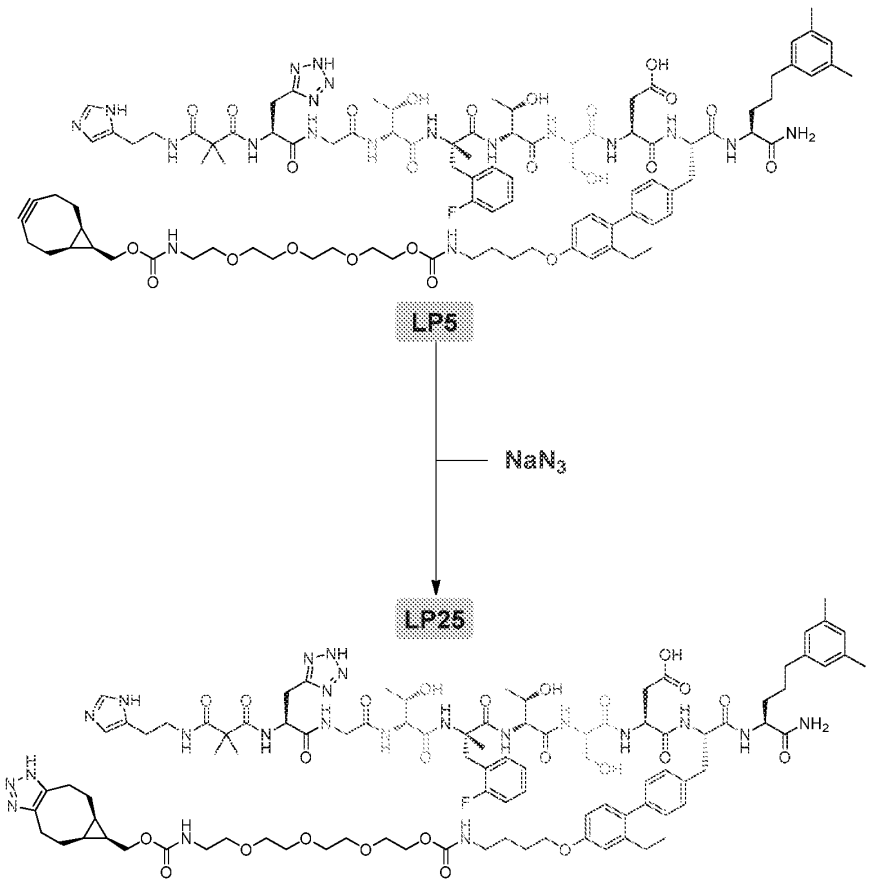


FIG. 40

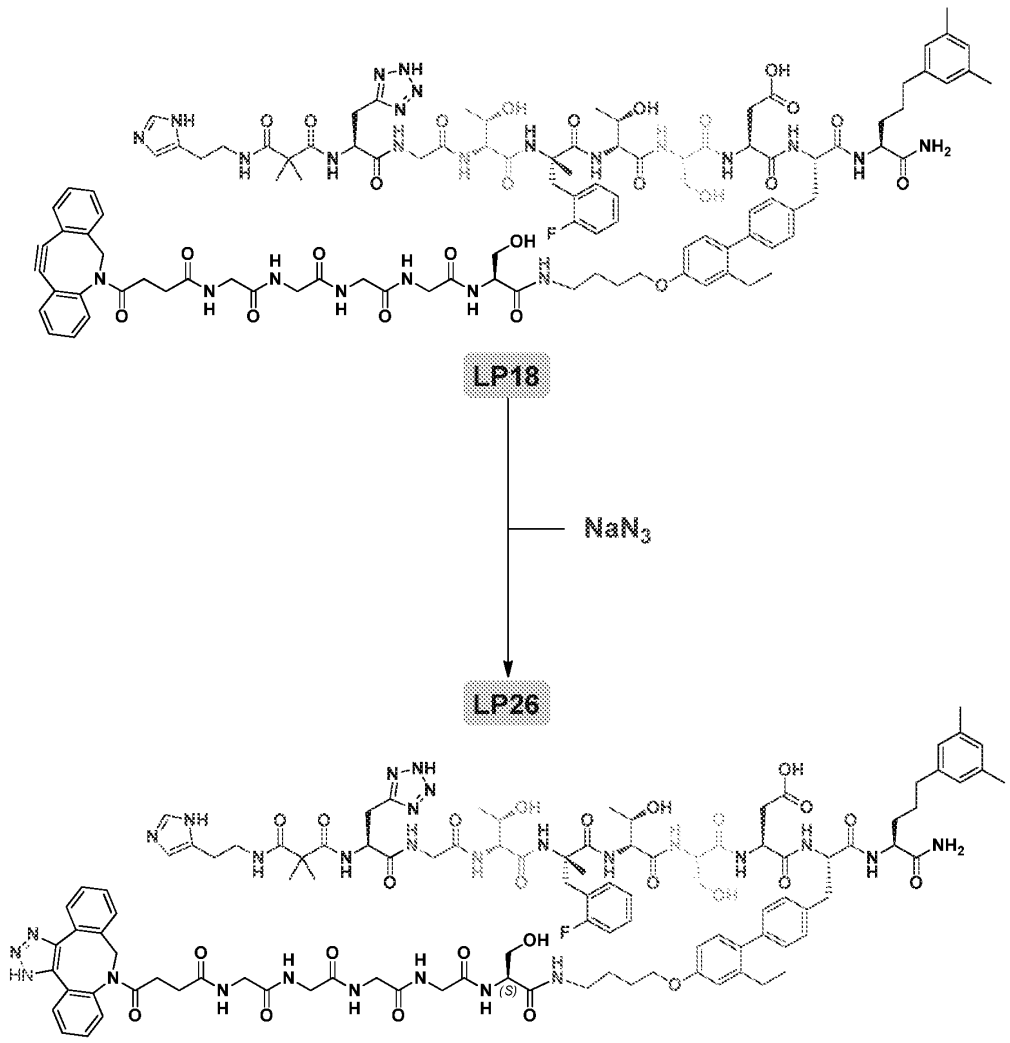


FIG. 41

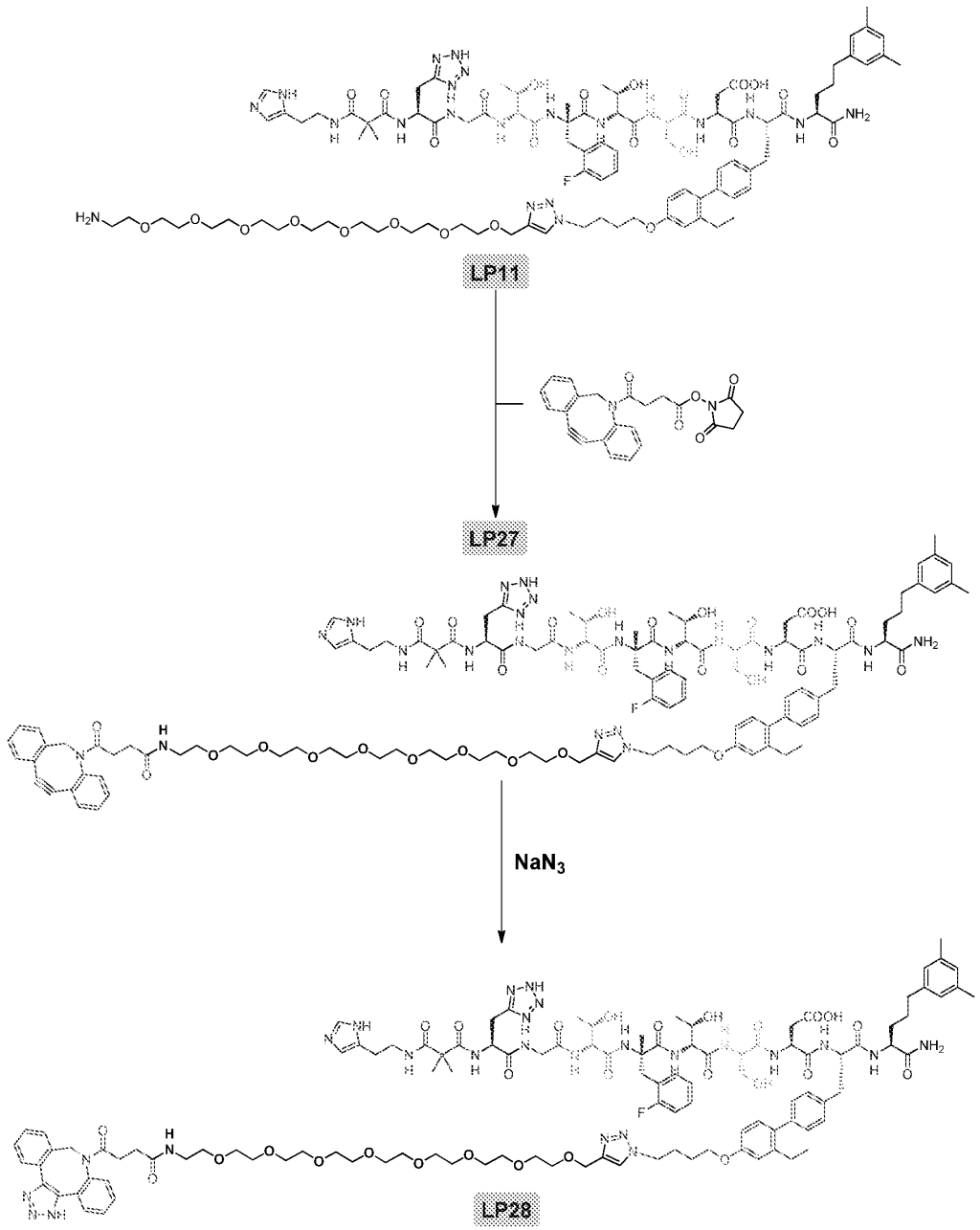


FIG. 42

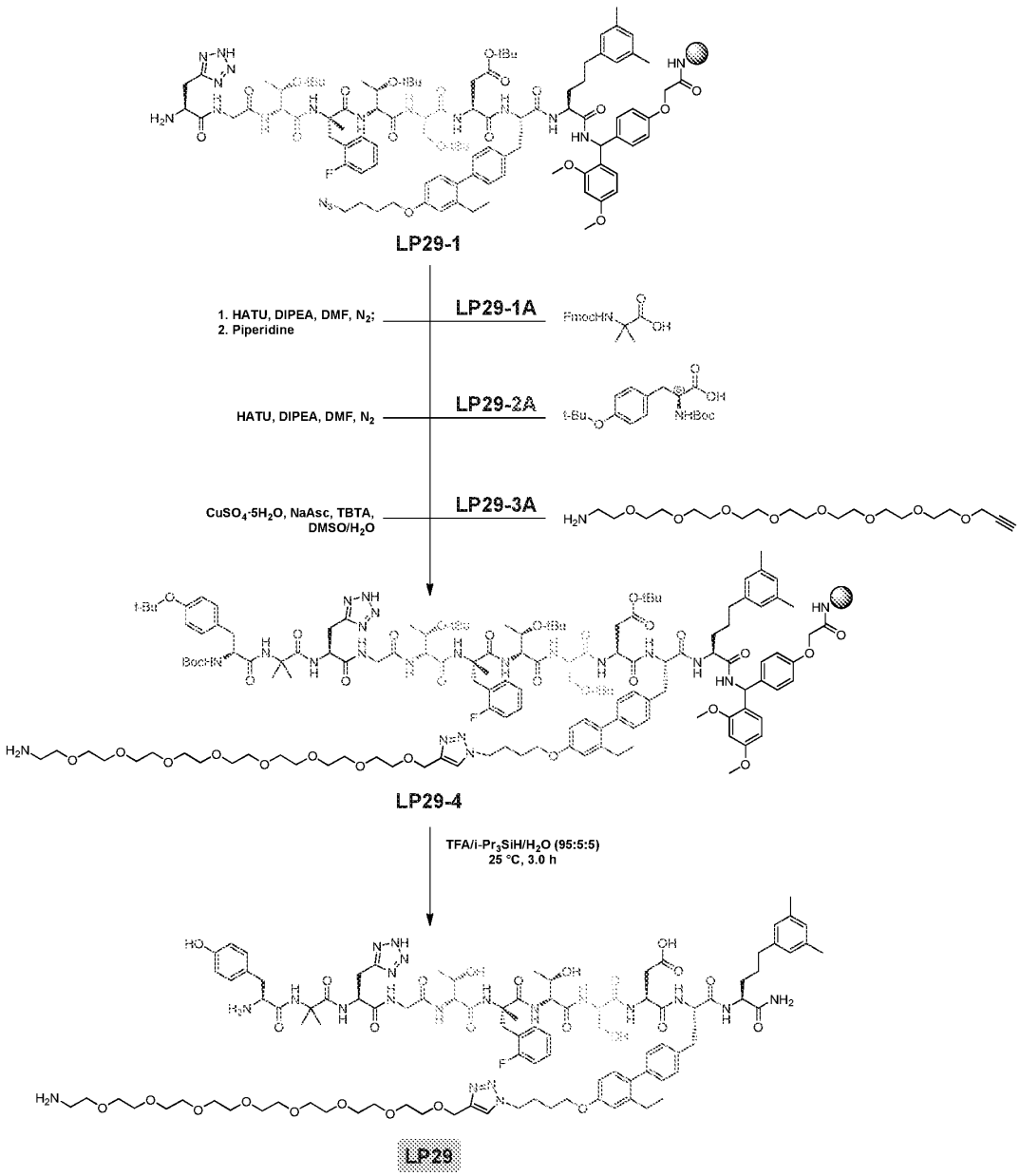

LP29-1
1. HATU, DIPEA, DMF, N2;
2. Piperidine
LP29-1A
HATU, DIPEA, DMF, N2
LP29-2A
CuSO4·5H2O, NaAsc, TBTA,
DMSO/H2O
LP29-3A
LP29-4
TFA/i-Pr3SiH/H2O (95:5:5)
25 °C, 3.0 h
LP29

FIG. 43

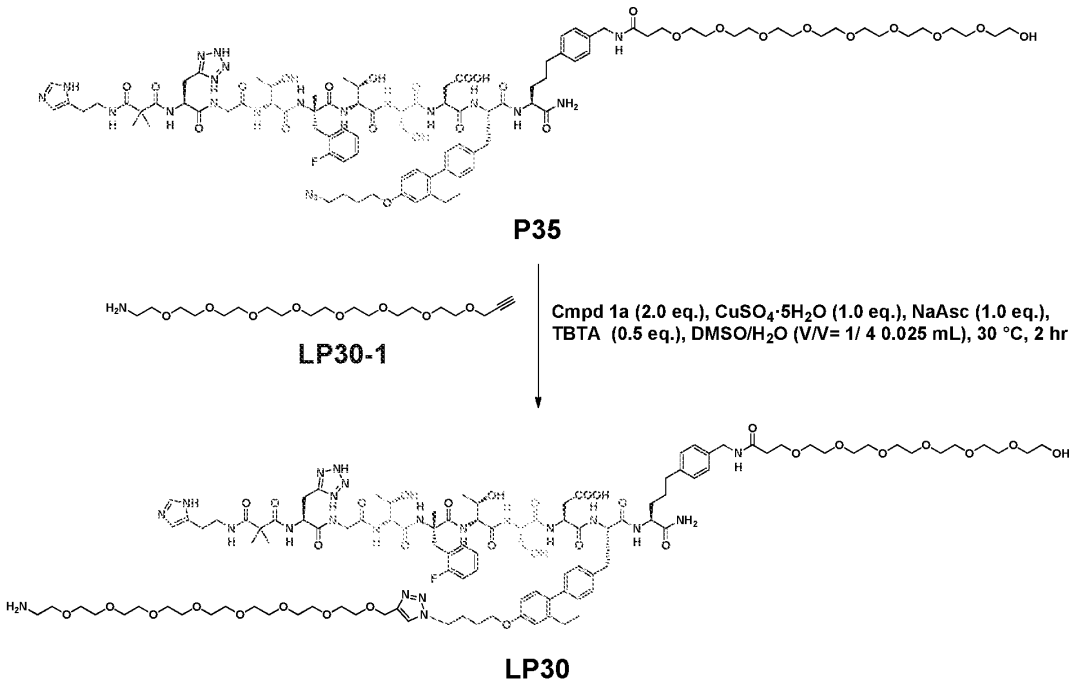

P35
LP30-1
Cmpd 1a (2.0 eq.), $CuSO_4 \cdot 5H_2O$ (1.0 eq.), NaAsc (1.0 eq.),
TBTA (0.5 eq.), DMSO/H_2O (V/V= 1/ 4 0.025 mL), 30 °C, 2 hr
LP30

FIG. 44

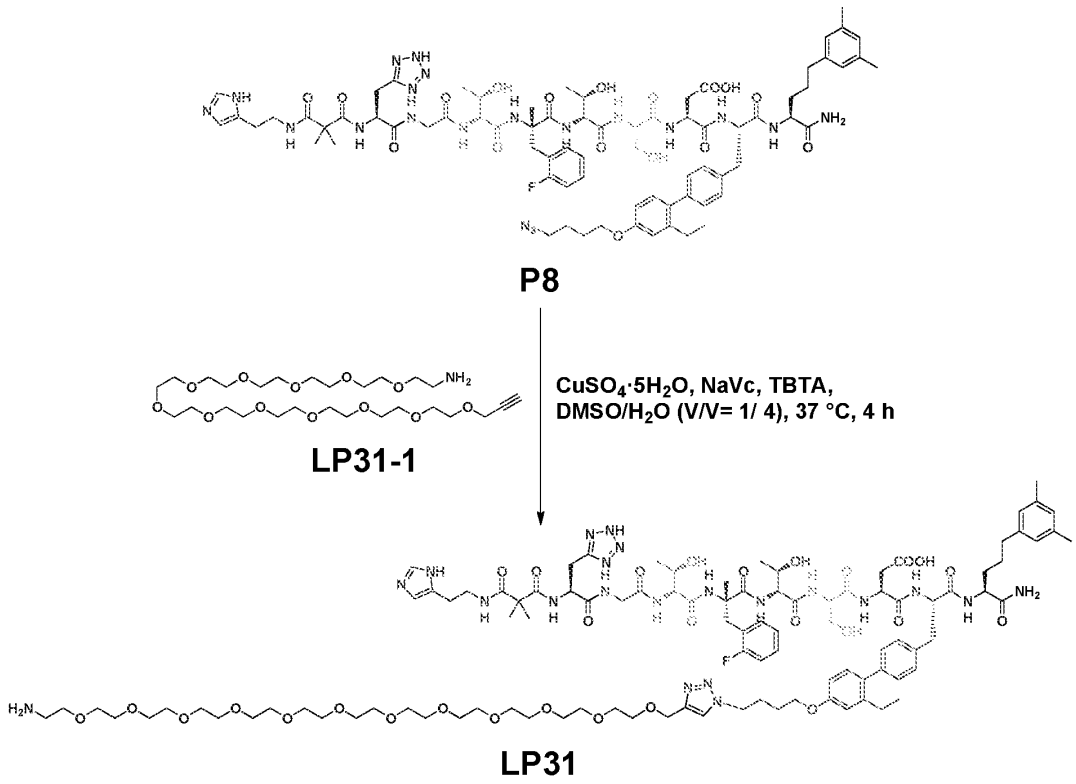


FIG. 45

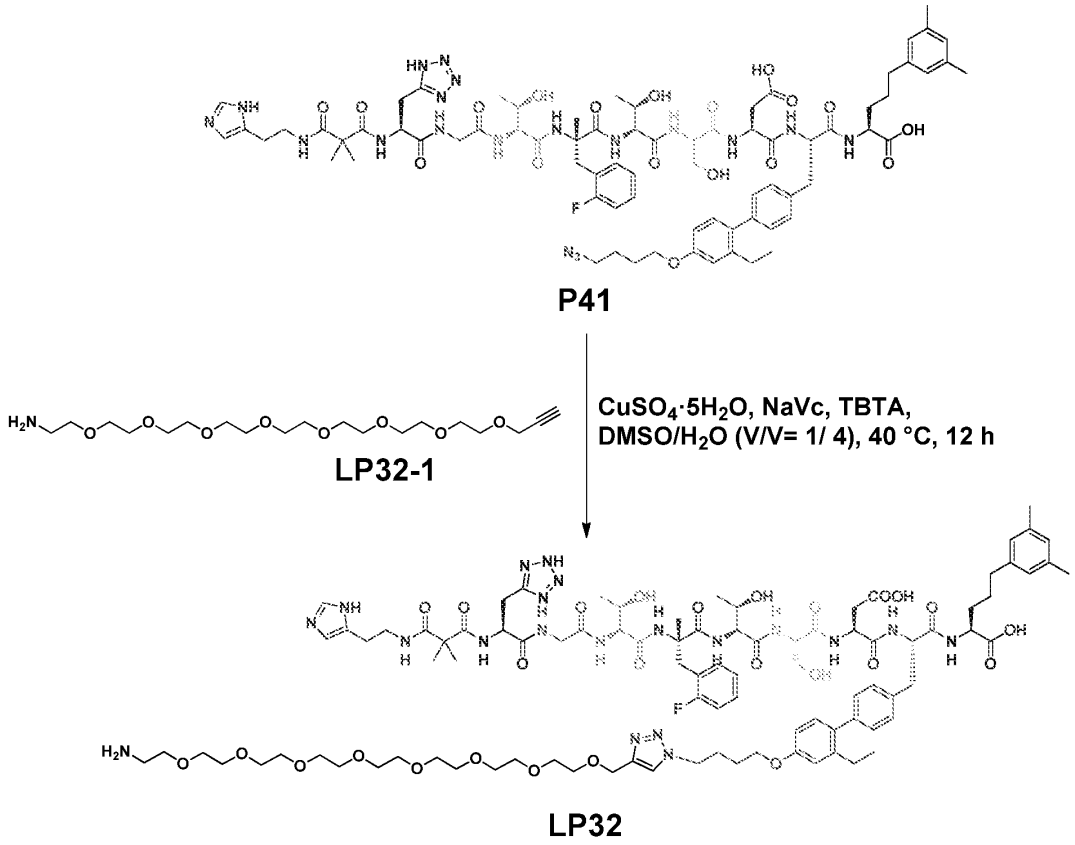


FIG. 46

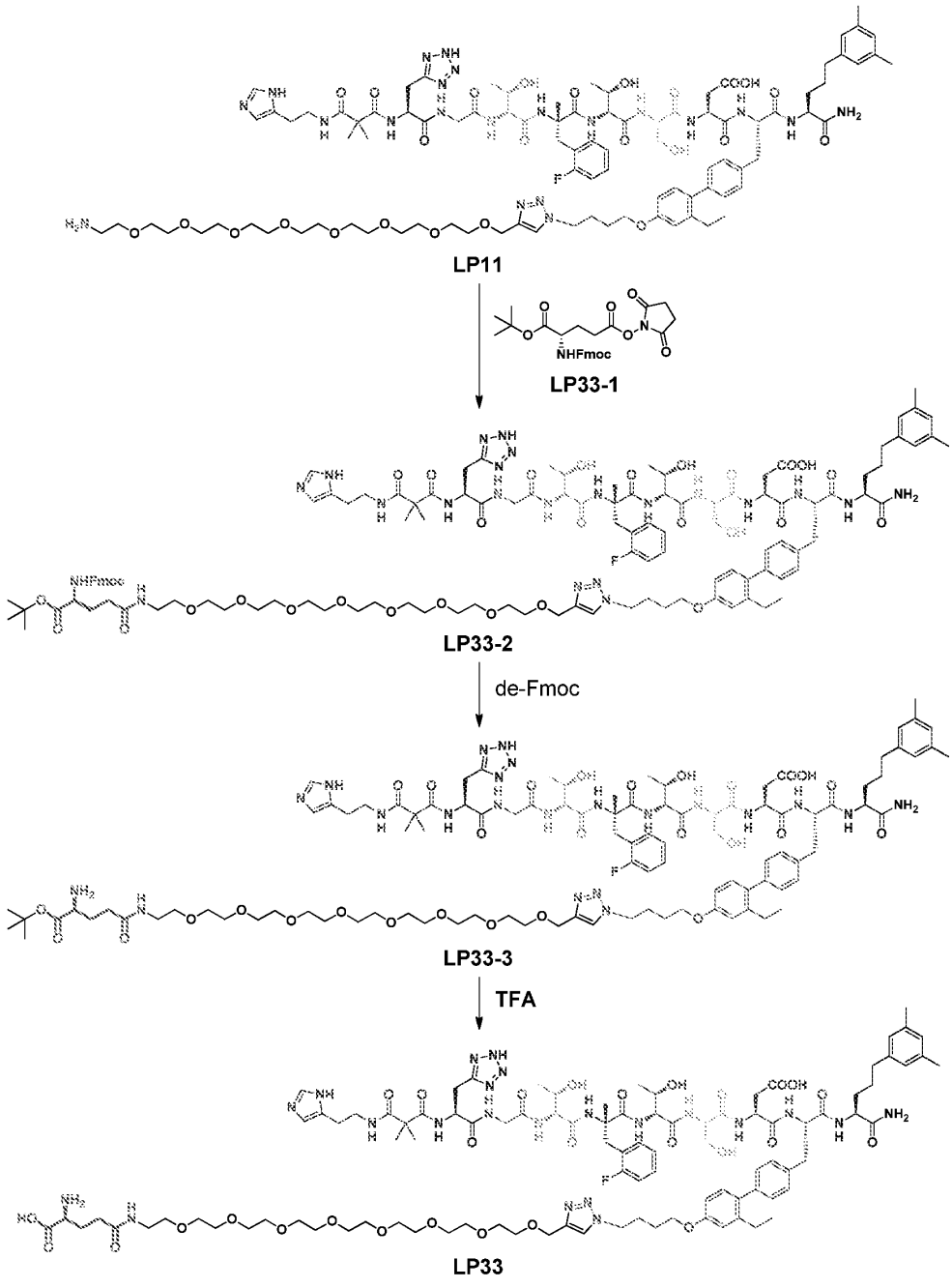


FIG. 47

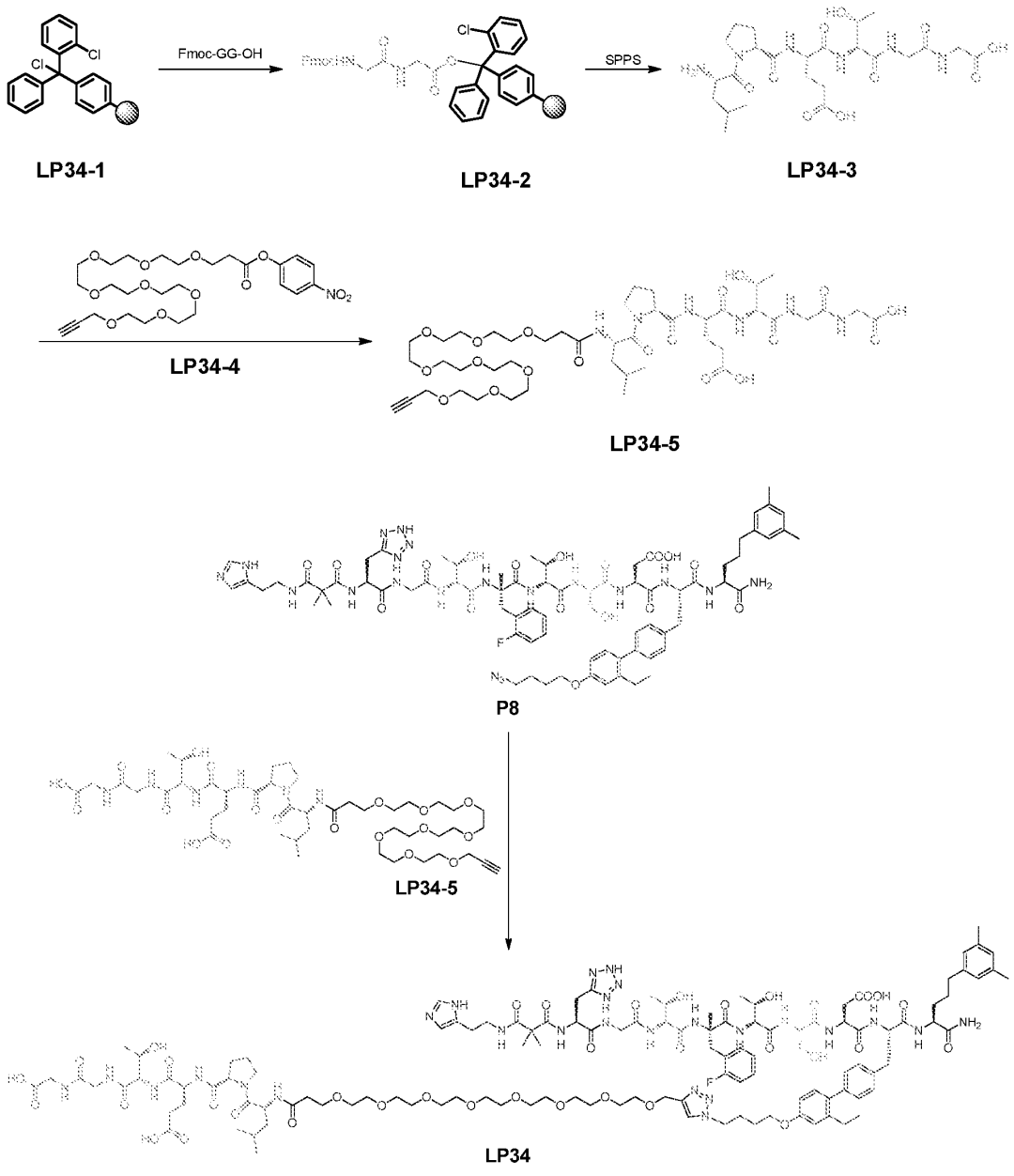


FIG. 48

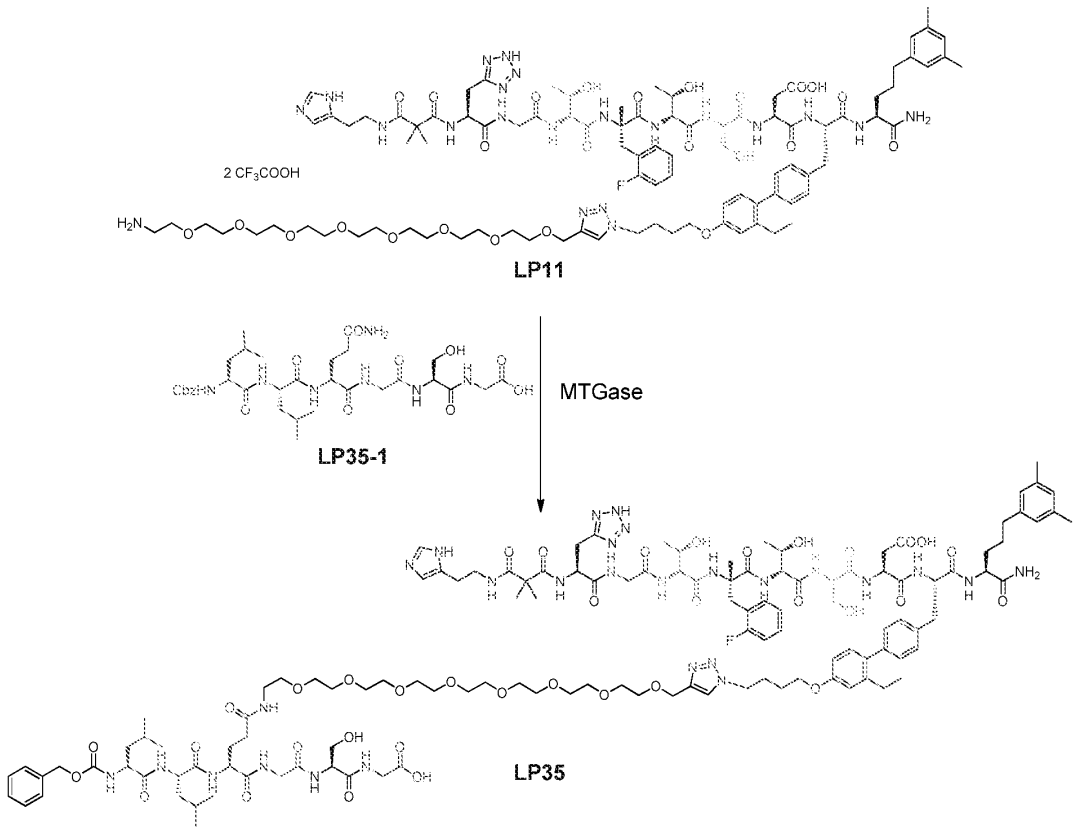


FIG. 49

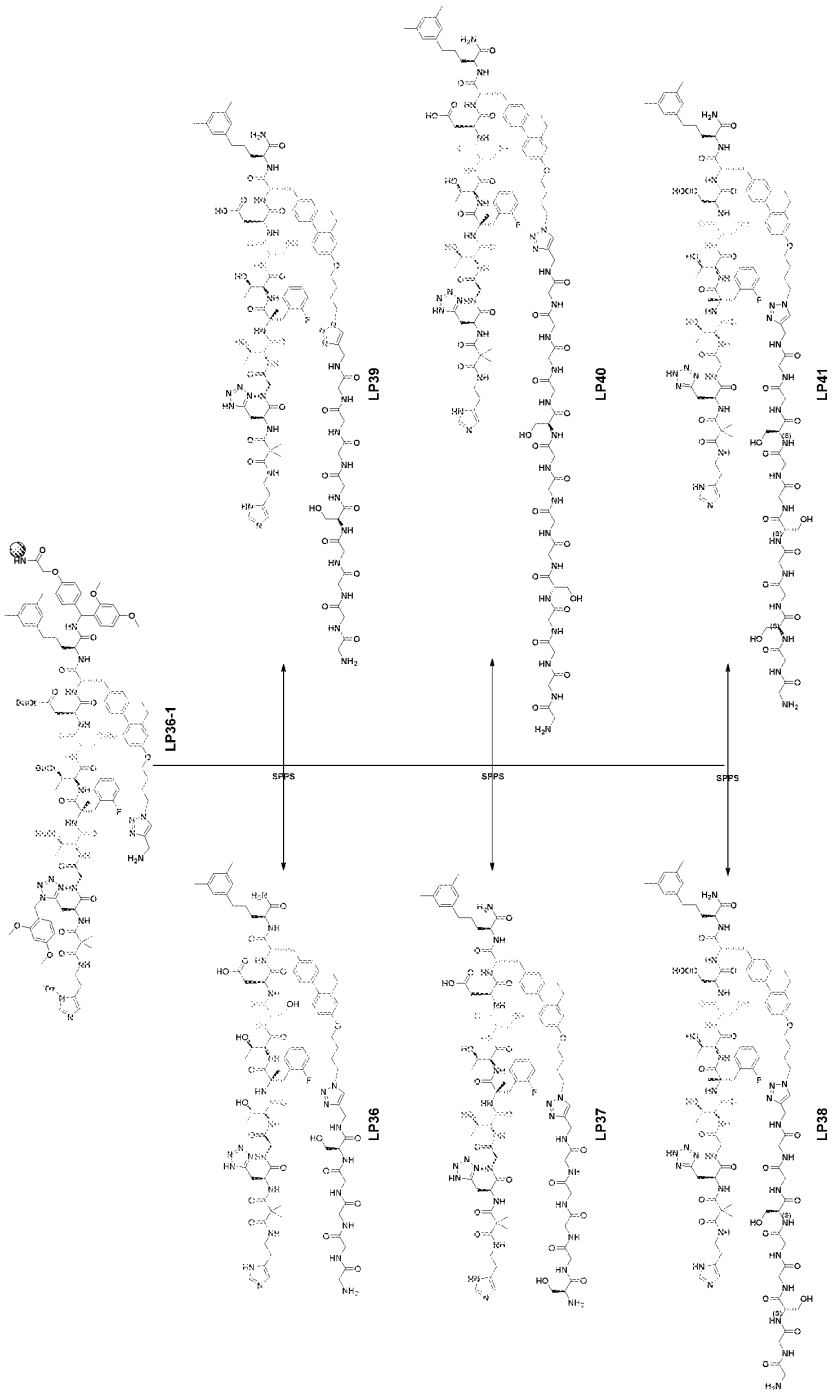


FIG. 50

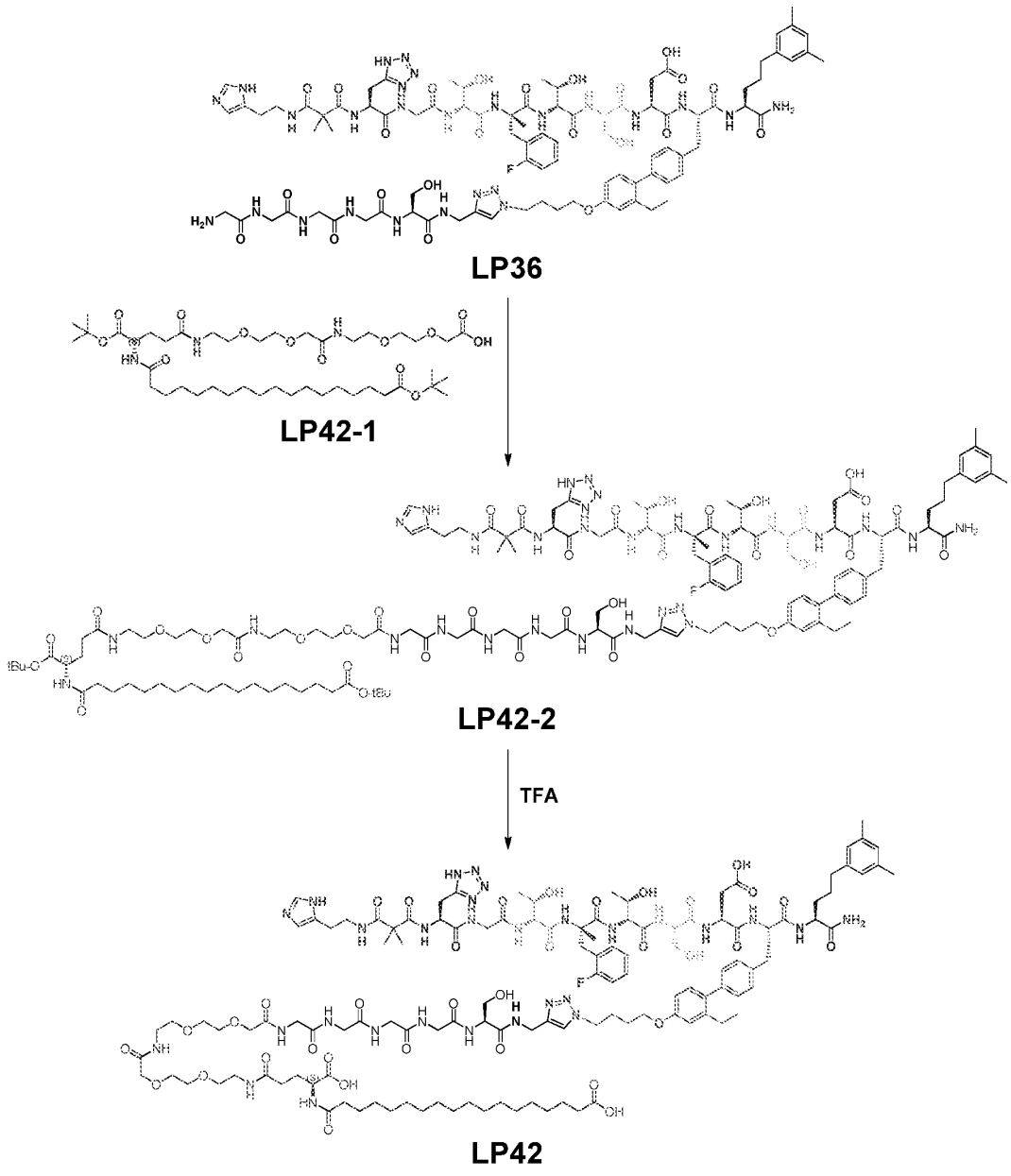


FIG. 51

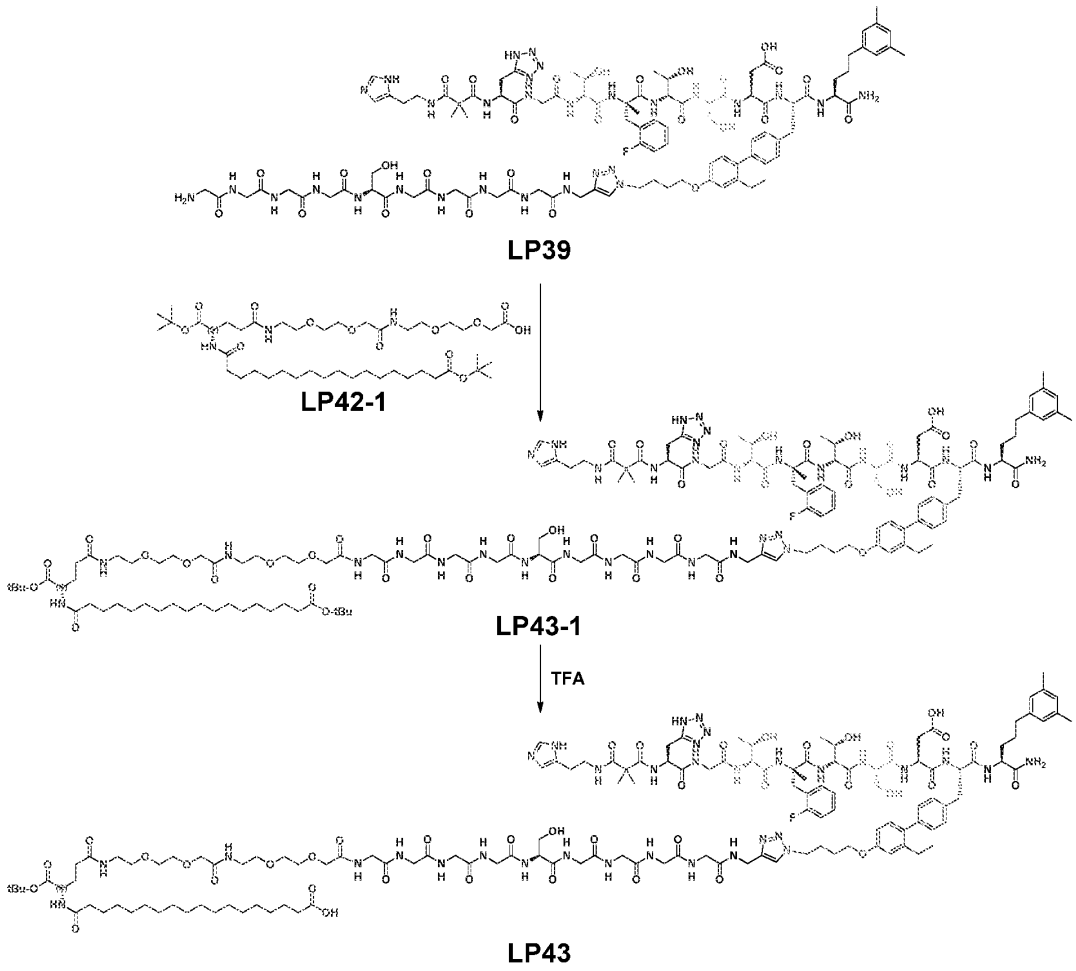


FIG. 52

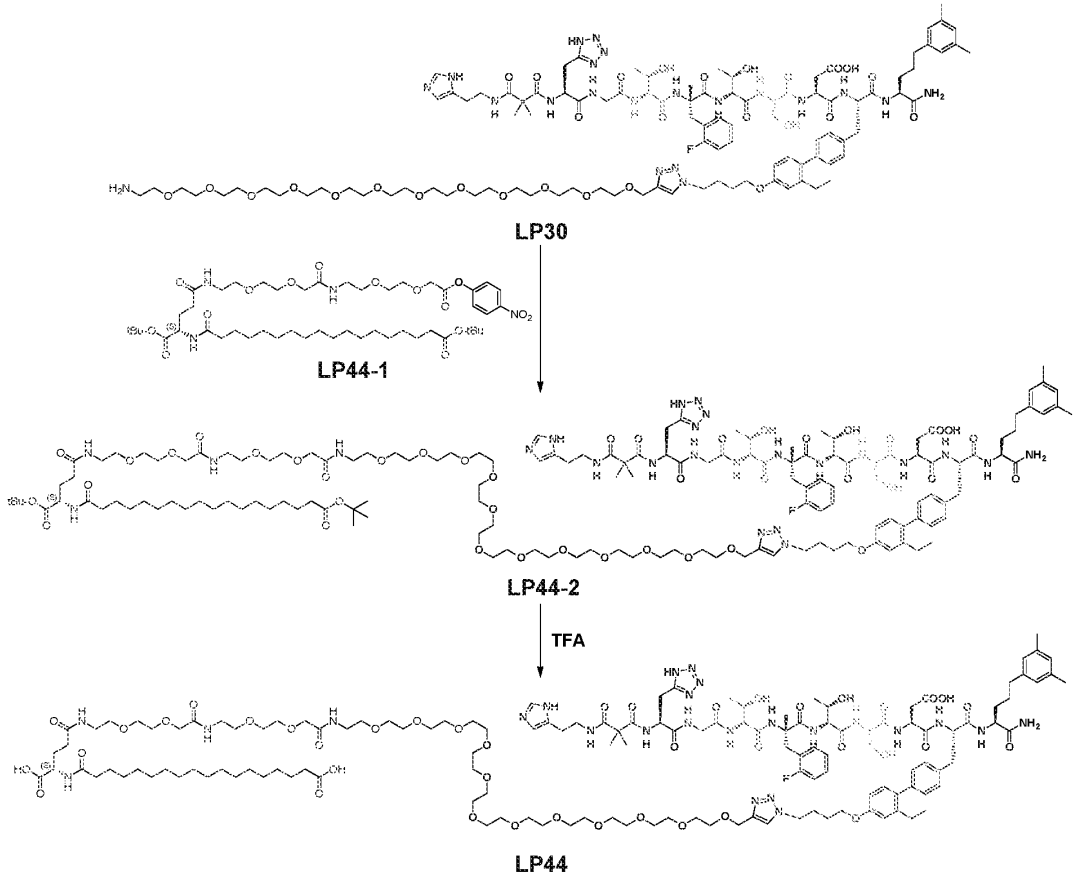


FIG. 53



FIG. 54

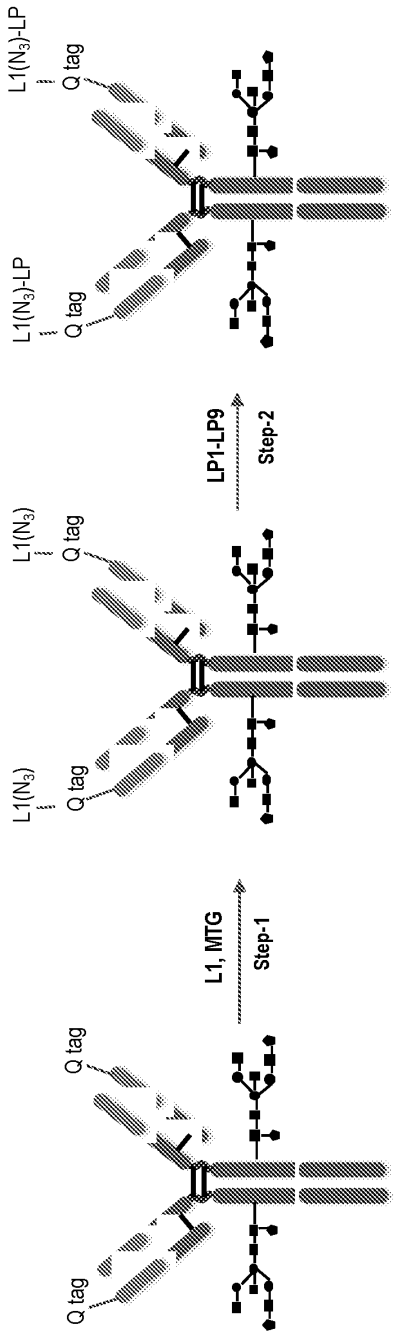

L1(N3)-LP
Q tag
L1(N3)-LP
Q tag
LP1-LP9
Step-2
L1(N3)
Q tag
L1(N3)
Q tag
L1, MTG
Step-1
Q tag
Q tag

FIG. 55

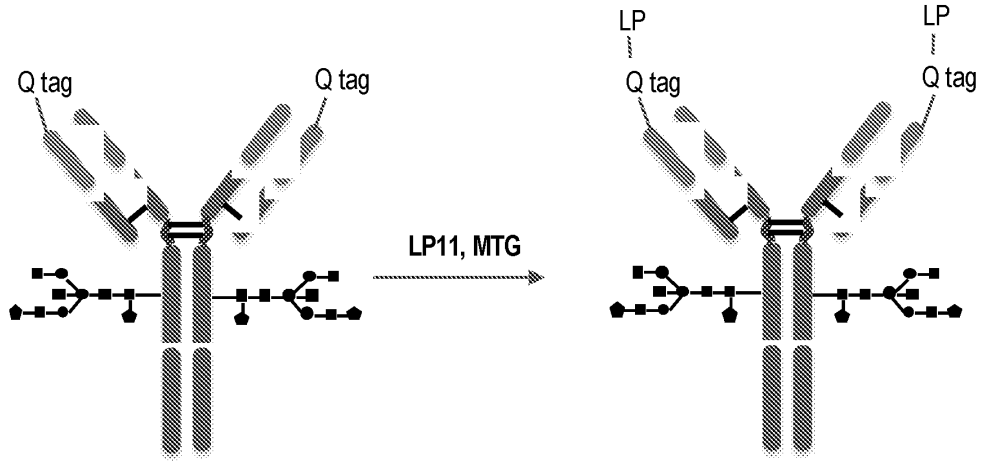


FIG. 56

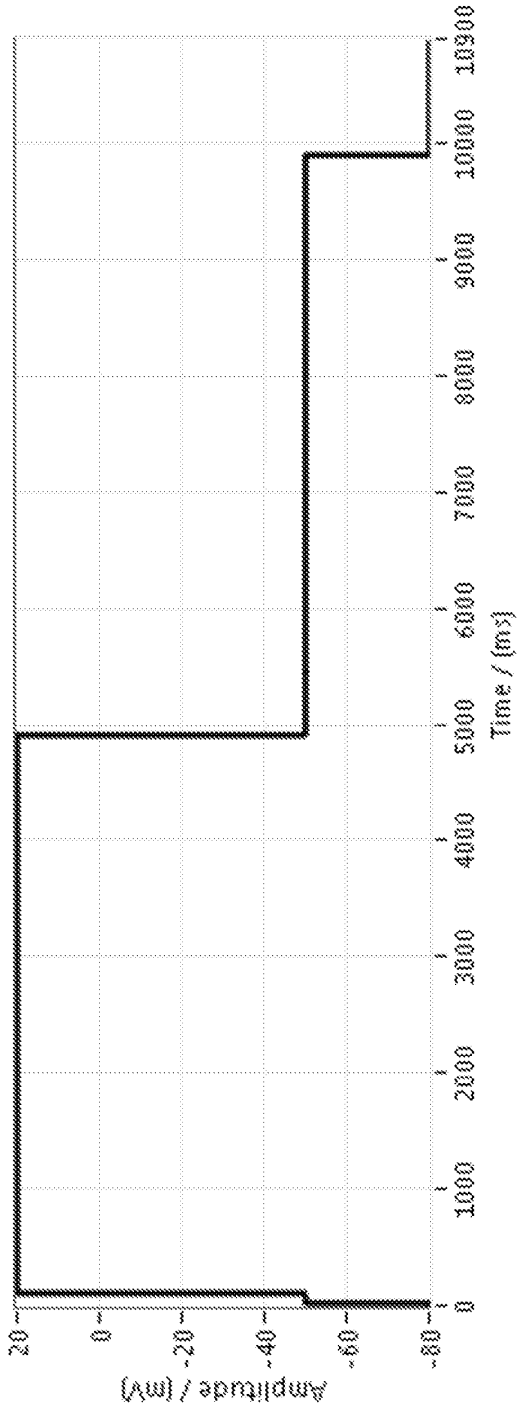

Amplitude / (mV)
20
0
-20
-40
-60
-80
Time / (ms)
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
10900

FIG. 57

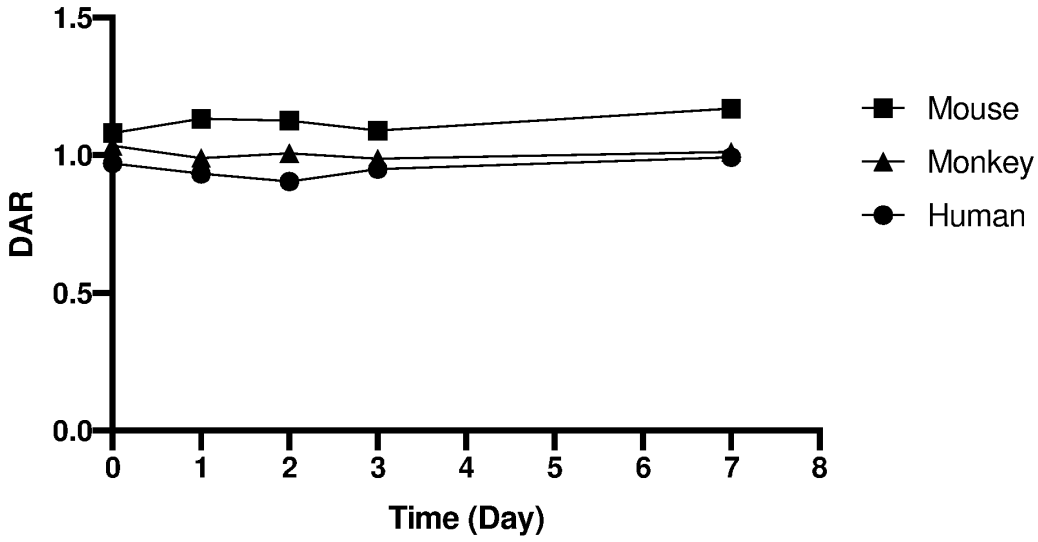


FIG. 58

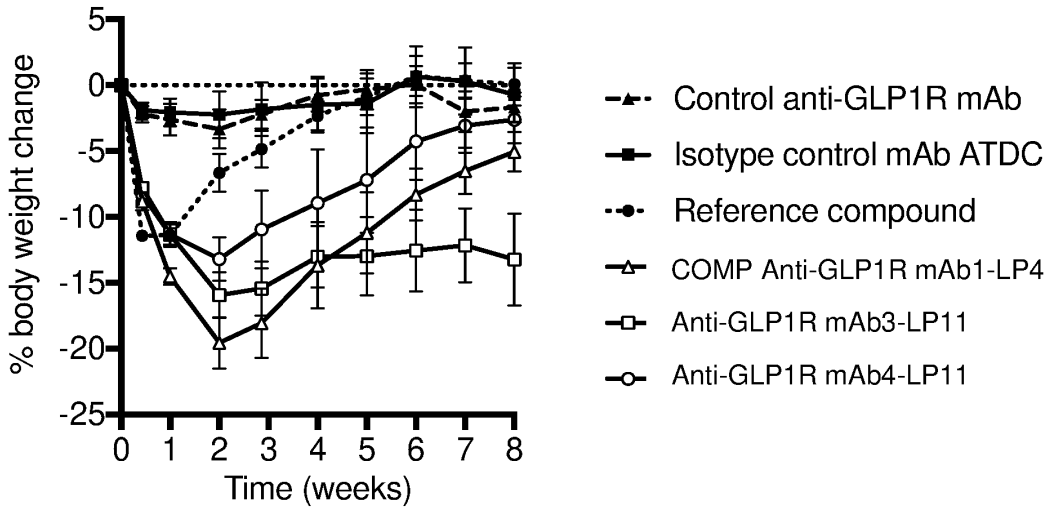

% body weight change
5
0
-5
-10
-15
-20
-25
0 1 2 3 4 5 6 7 8
Time (weeks)
Control anti-GLP1R mAb
Isotype control mAb ATDC
Reference compound
COMP Anti-GLP1R mAb1-LP4
Anti-GLP1R mAb3-LP11
Anti-GLP1R mAb4-LP11

FIG. 59

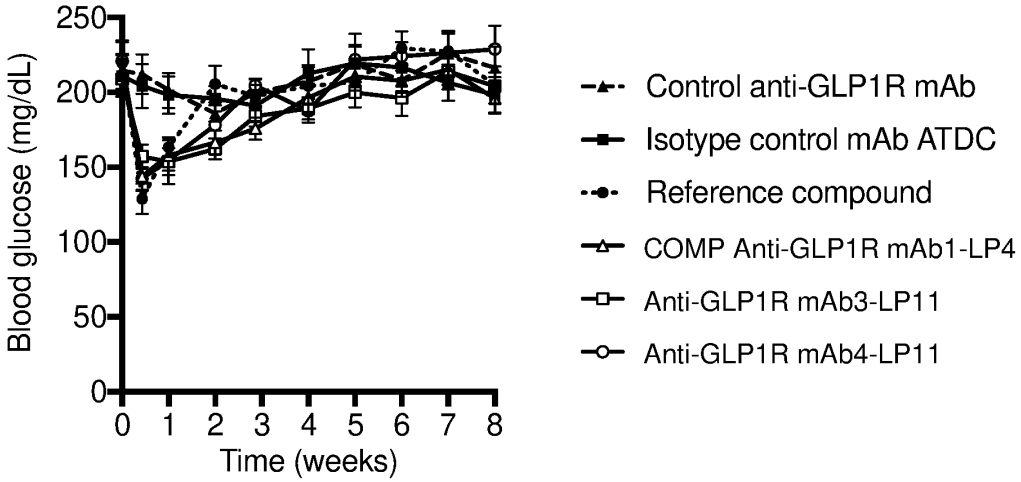
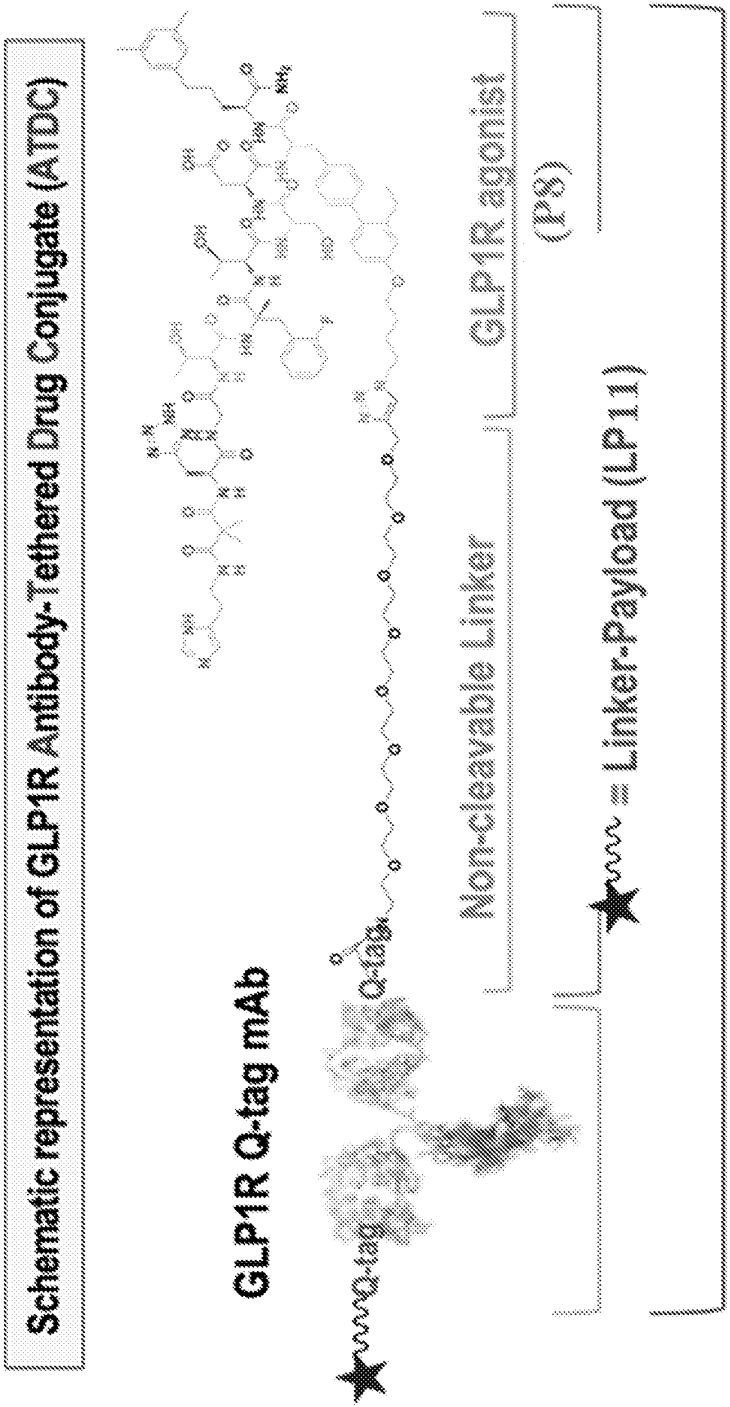

Blood glucose (mg/dL)
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8
Time (weeks)
Control anti-GLP1R mAb
Isotype control mAb ATDC
Reference compound
COMP Anti-GLP1R mAb1-LP4
Anti-GLP1R mAb3-LP11
Anti-GLP1R mAb4-LP11

FIG. 60


Schematic representation of GLP1R Antibody-Tethered Drug Conjugate (ATDC)
GLP1R Q-tag mAb
Q-tag
Q-tag
Non-cleavable Linker
GLP1R agonist
(P8)
= Linker-Payload (LP11)
GLP1R-ATDC