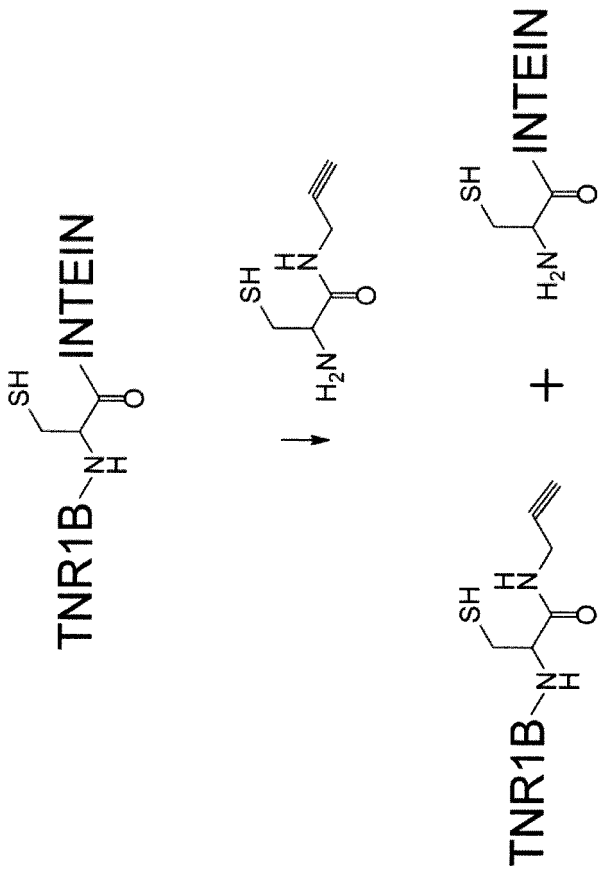


Figure 1

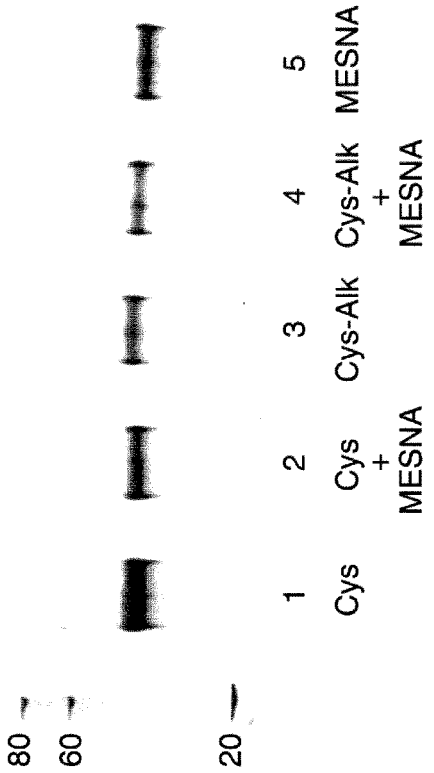


Figure 2

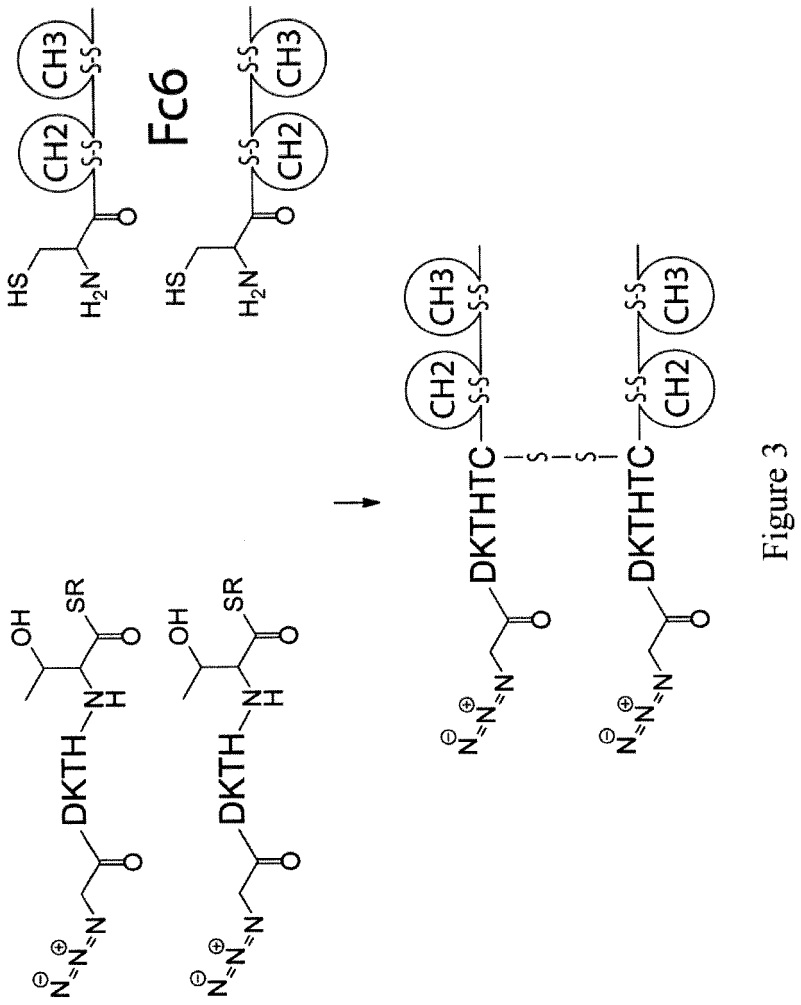


Figure 3

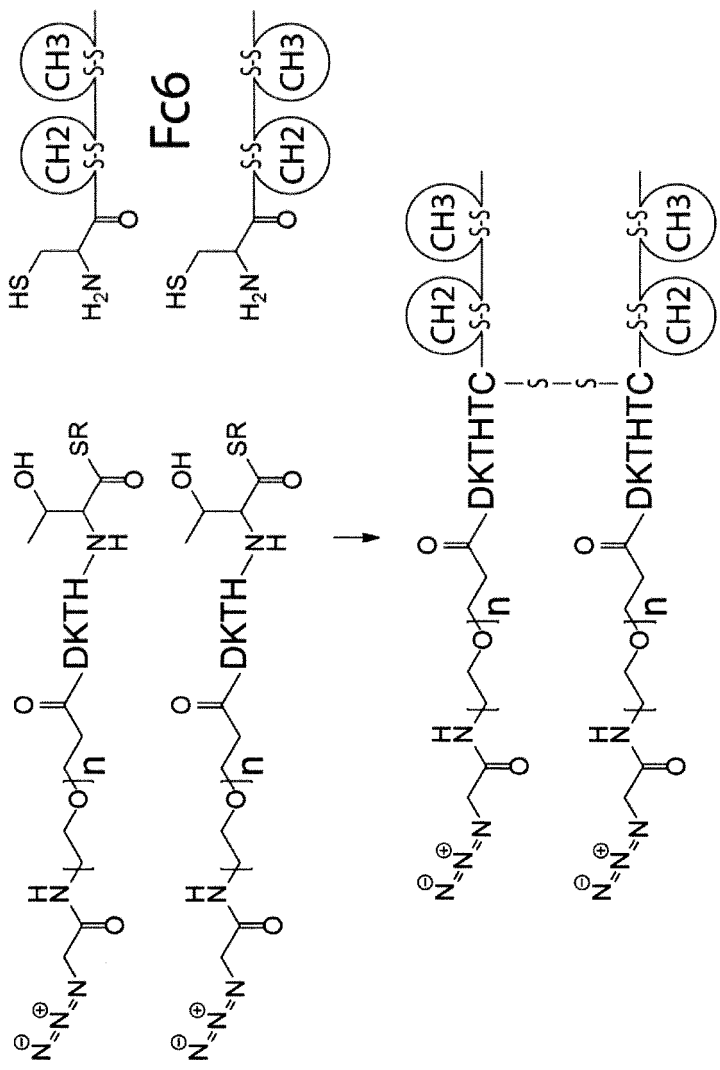


Figure 4

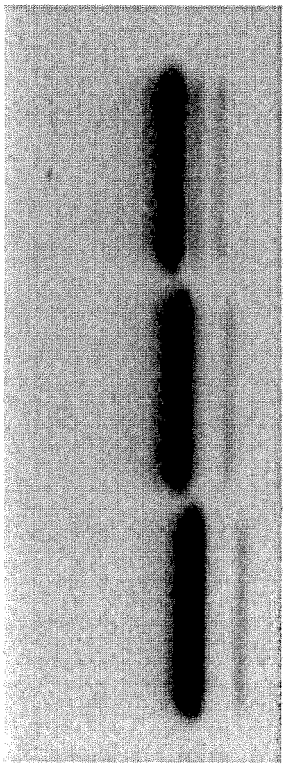


Figure 5

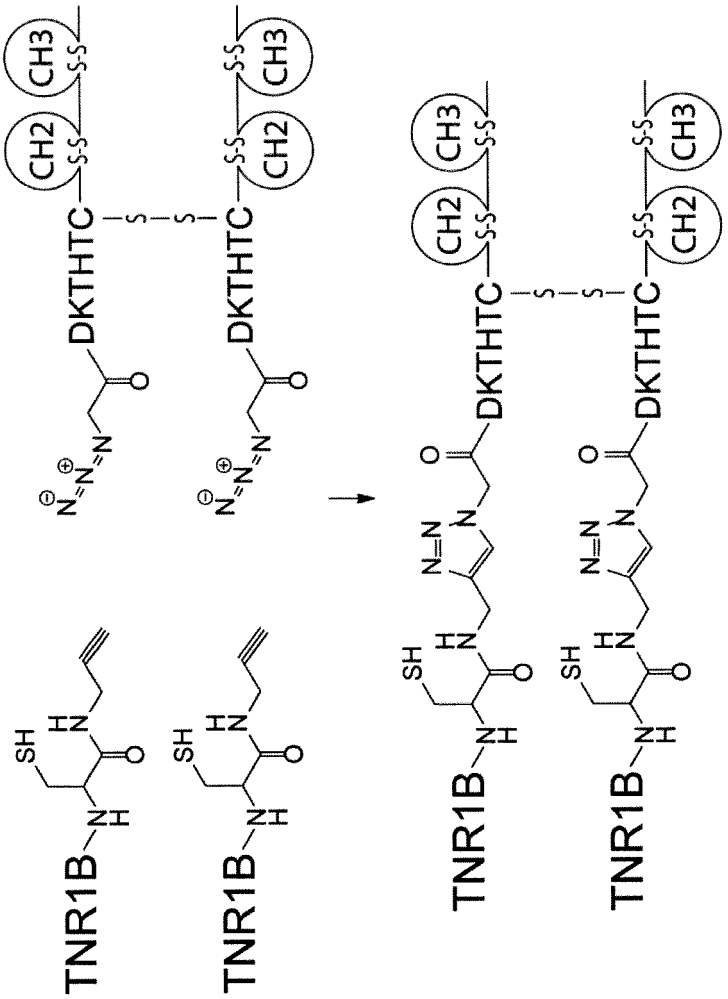

CH3
CH2
S-S
DKTHTC
S
TNR1B
SH
N
H
O
N=N
CH3
CH2
S-S
DKTHTC
S
TNR1B
SH
N
H
O
N=N

Figure 6

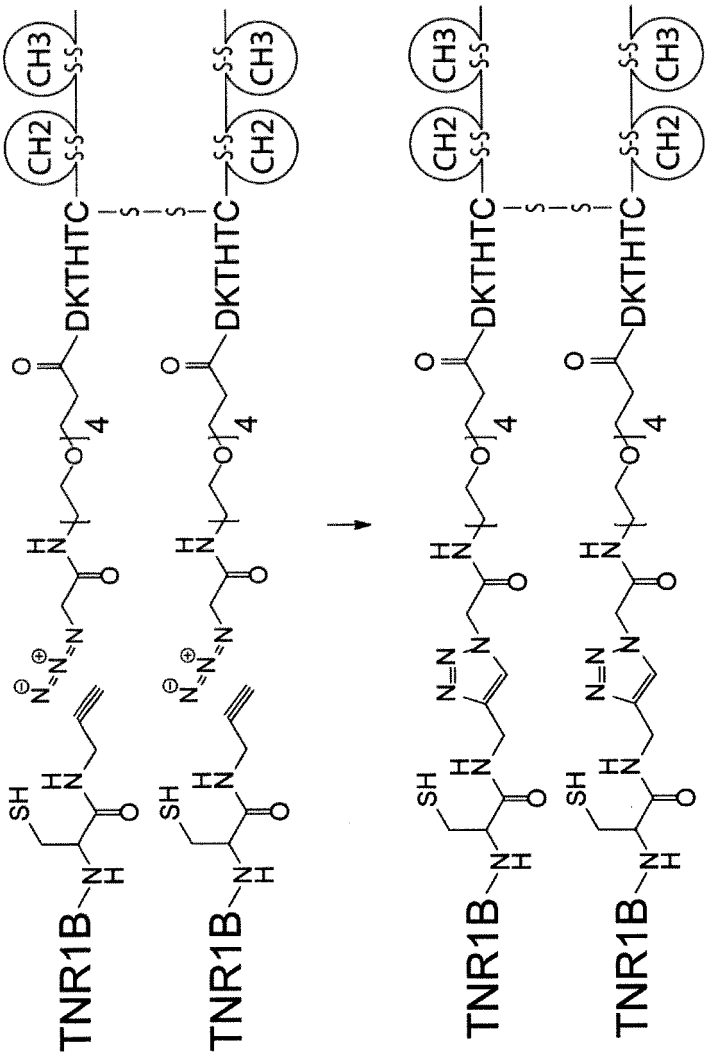


Figure 7

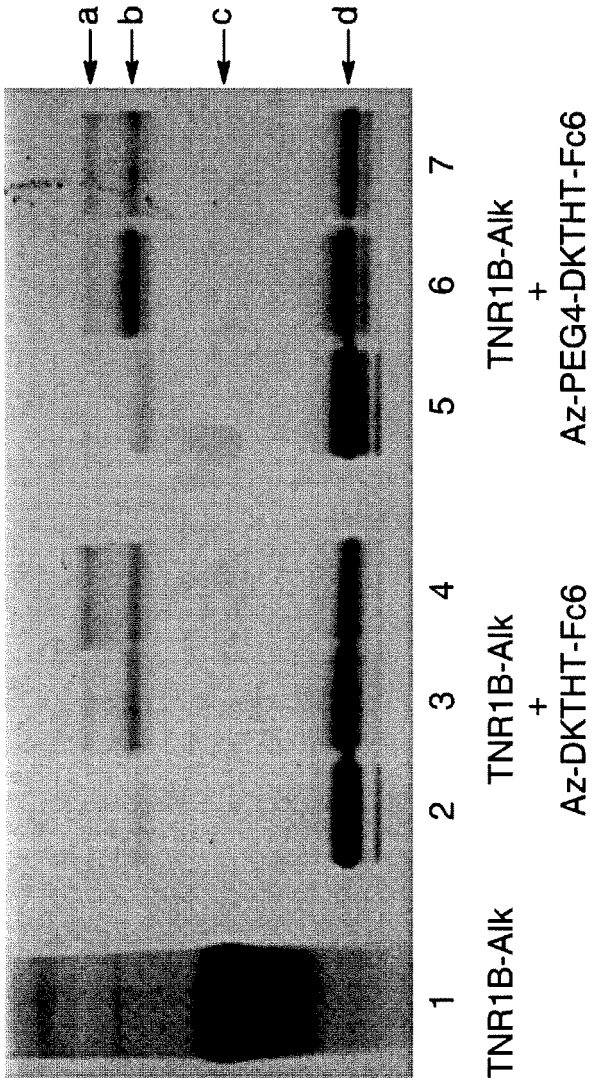

a
b
c
d
1
2
3
4
5
6
7
TNR1B-Alk
TNR1B-Alk + Az-DKTHT-Fc6
TNR1B-Alk + Az-PEG4-DKTHT-Fc6

Figure 8

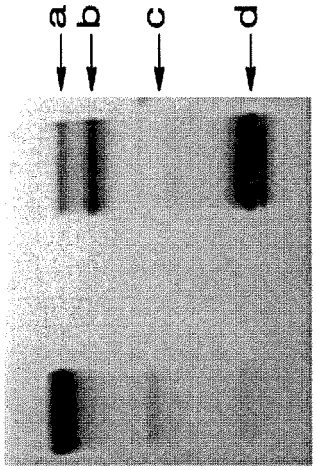

a
b
c
d
1
2
3
4
TNR1B-Fc
TNR1B-Alk
+
Az-DKTHT-Fc6
TNR1B-Fc
TNR1B-Alk
+
Az-PEG4-DKTHT-Fc6

Figure 9

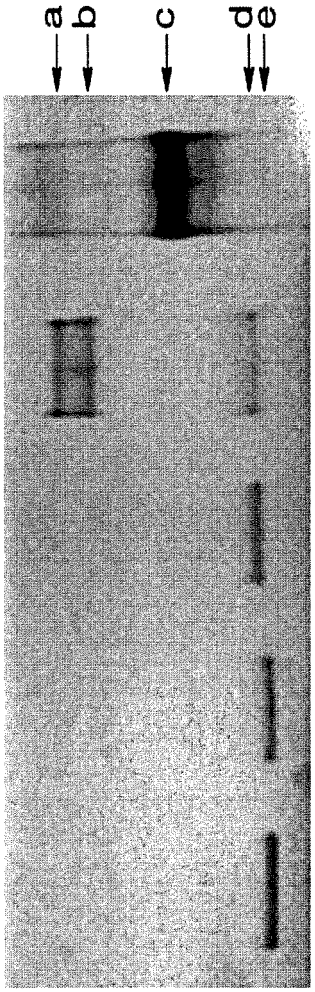

a
b
c
d
e
1
2
3
4
5
Fc6
Fc6 + TNR1B-Alk
Az-DKTHT-Fc6
Az-DKTHT-Fc6 + TNR1B-Alk
TNR1B-Alk

Figure 10

TNR1B
LPAQVAFTPY APEPGSTCRL REYYDQTAQM CCSKCSPGQH
AKVFCTKTSD TVCDSCEDST YTQLWNWVPE CLSCGSRCSS
DQVETQACTR EQNRICTCRP GWYCALSKQE GCRLCAPLRK
CRPGFGVARP GTETSDVVCK PCAPGTFSNT TSSTDICRPH
QICNVVAIPG NASMDAVCTS TSPTRSMAPG AVHLPQPVST
RSQHTQPTPE PSTAPSTSFL LPMGPSPPAE GSTGDGC

Fc6
CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV
LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG
QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC
SVMHEALHNH YTQKSLSLSP GK

TNR1B-Alk-Az-DKTHT-Fc6

Figure 11

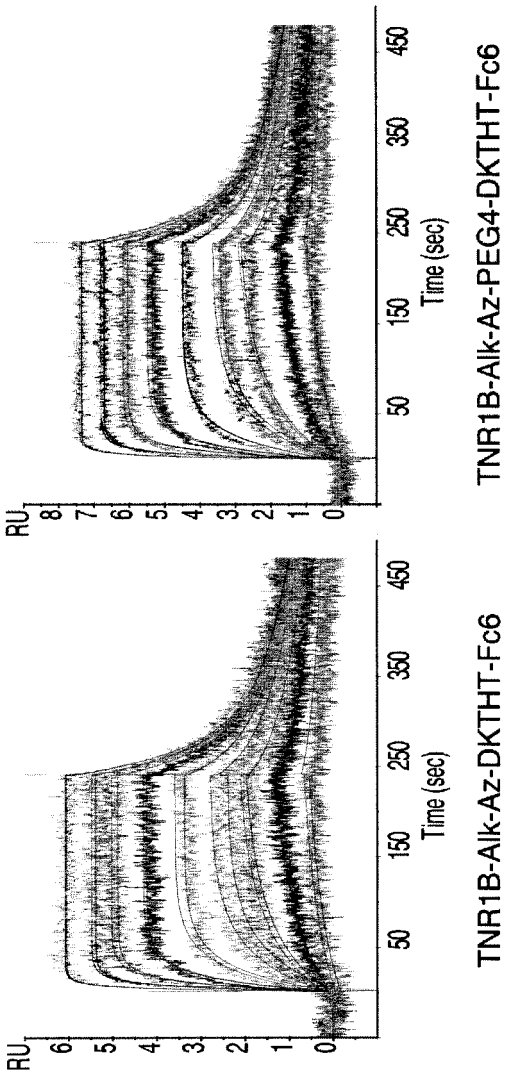


Figure 12

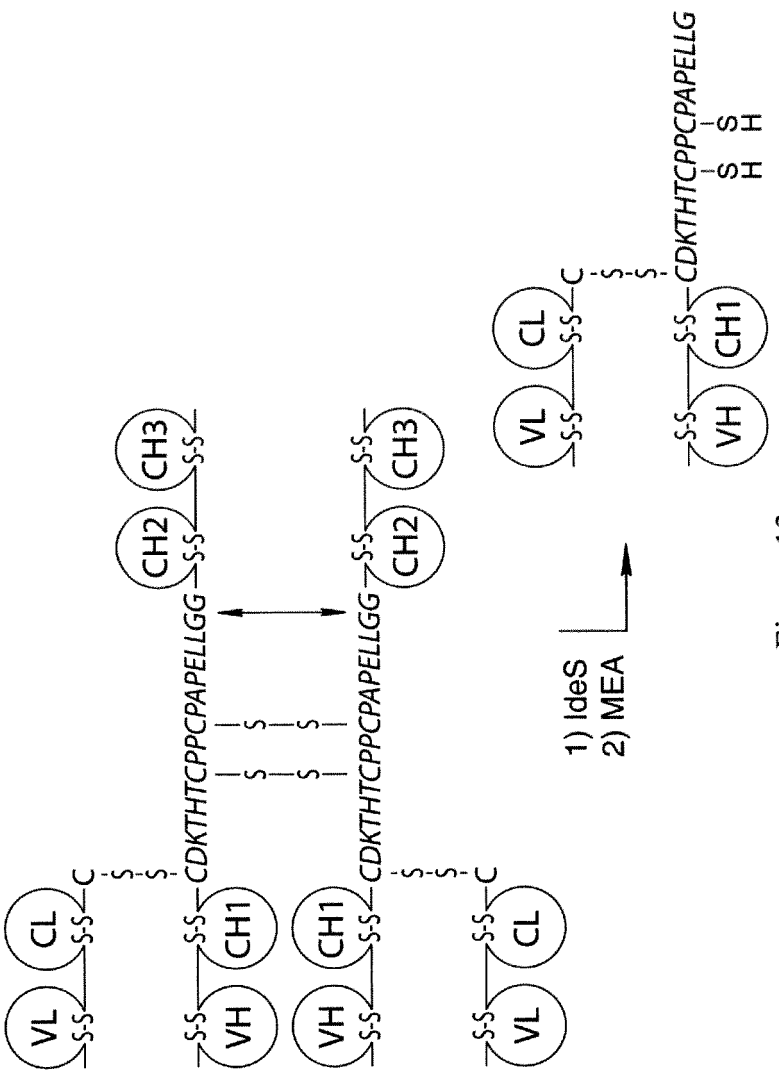

VL
CL
C
CDKTHTCPPCPAPELLGG
CH1
VH
CH2
CH3
1) IdeS
2) MEA
CDKTHTCPPCPAPELLG
H
S
S
H

Figure 13

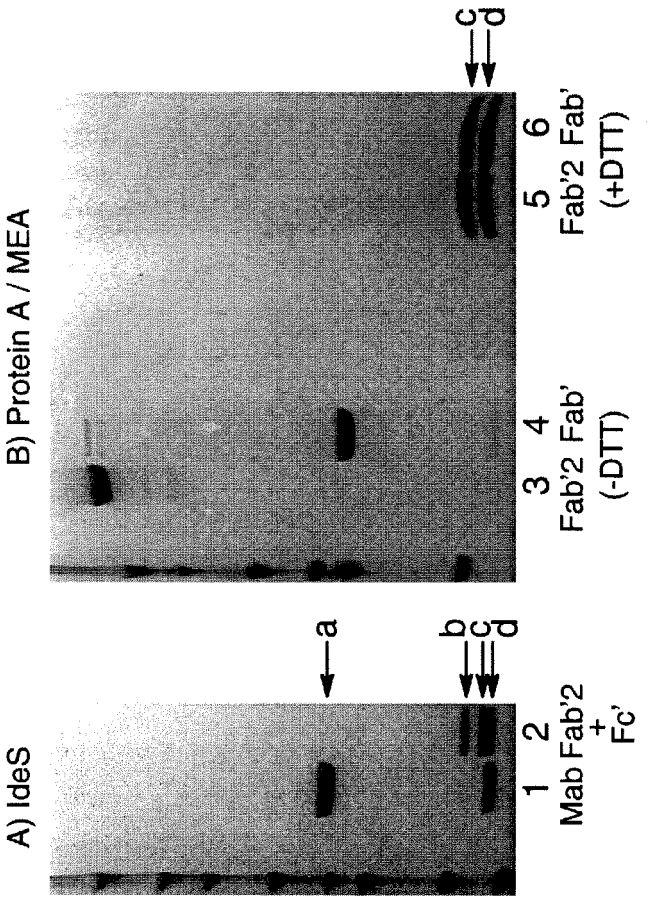

A) IdeS
B) Protein A / MEA
a
b
c
d
1 2
Mab Fab'2 + Fc'
3 4
Fab'2 Fab' (-DTT)
5 6
Fab'2 Fab' (+DTT)
Adalimumab-Fab'

Figure 14

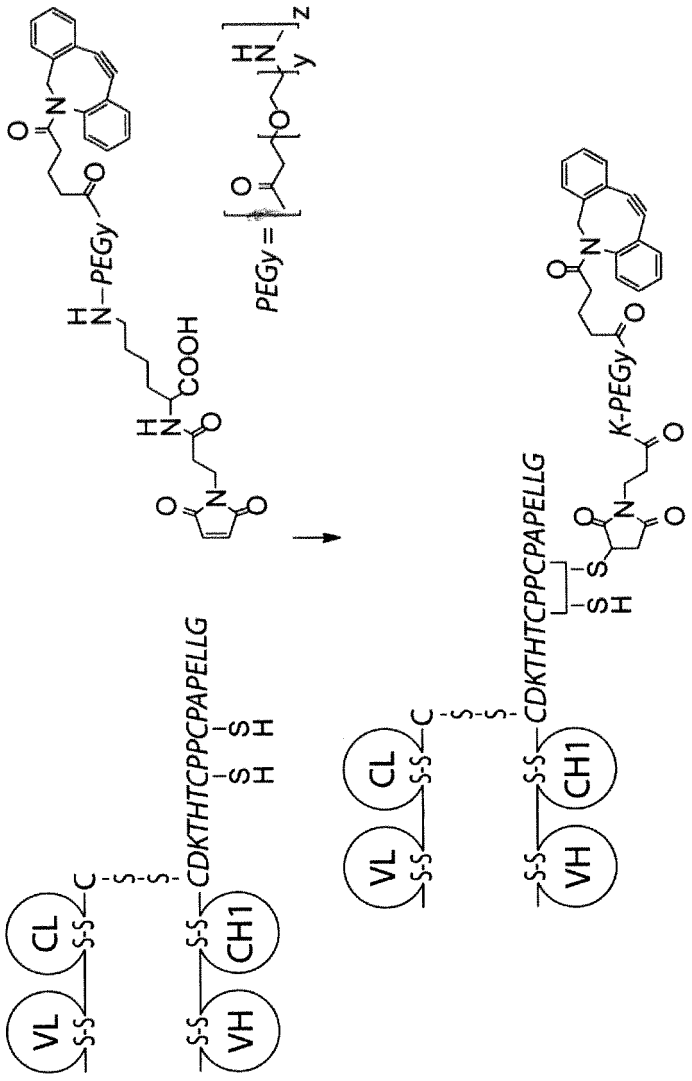


Figure 15

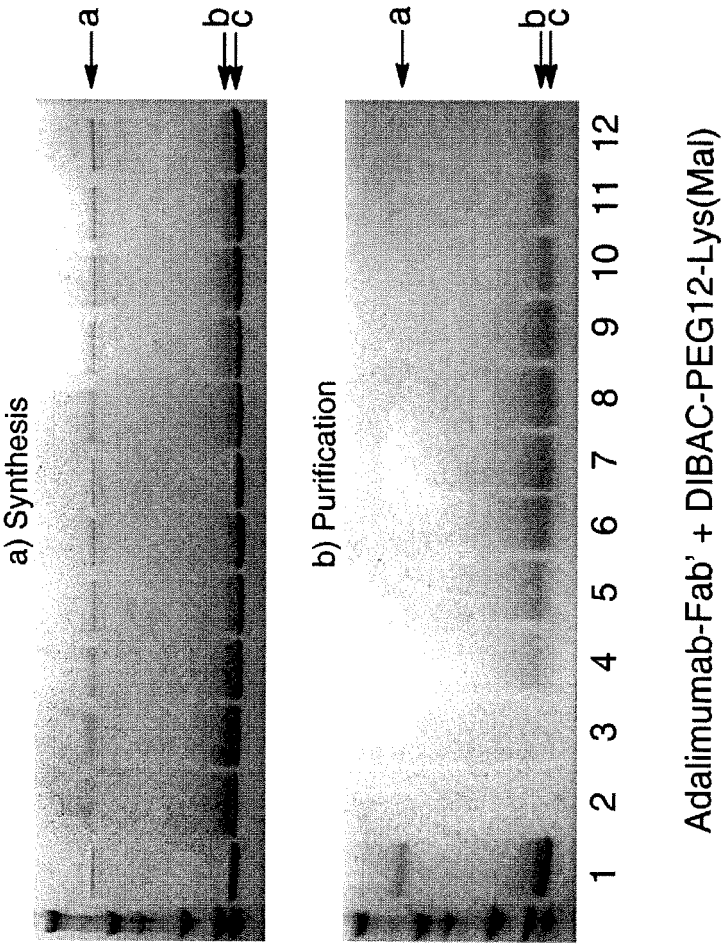
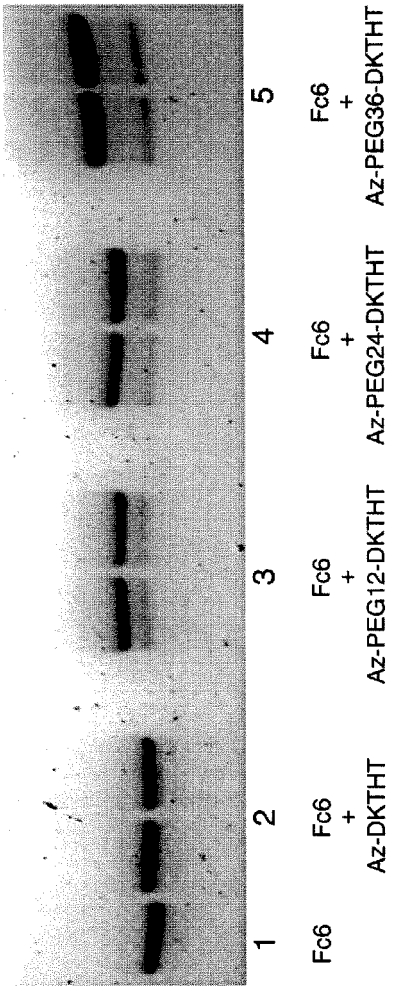

a) Synthesis
b) Purification
a
b
c
1 2 3 4 5 6 7 8 9 10 11 12
Adalimumab-Fab' + DIBAC-PEG12-Lys(Mal)

Figure 16



Az-PEGx-DKTHT-Fc6

Figure 17

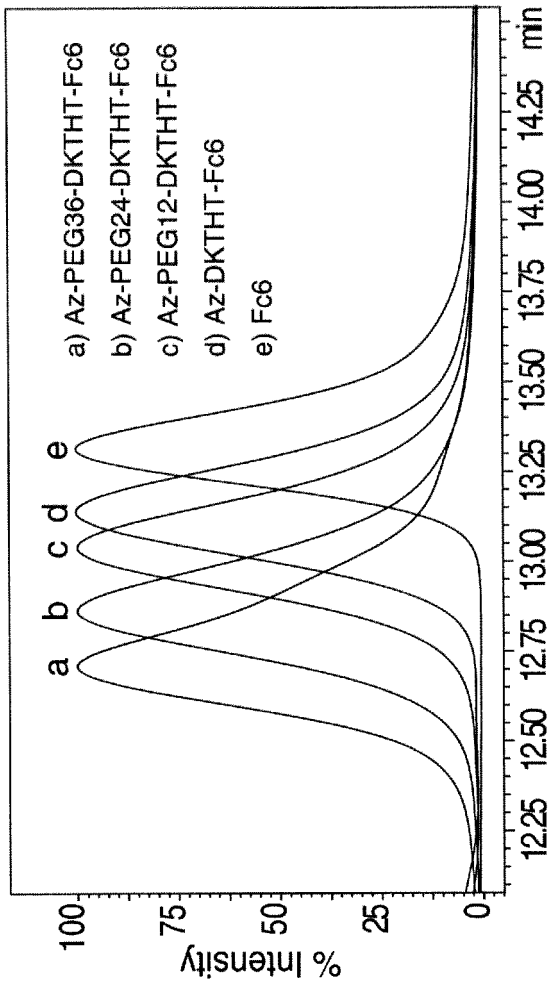

a) Az-PEG36-DKTHT-Fc6
b) Az-PEG24-DKTHT-Fc6
c) Az-PEG12-DKTHT-Fc6
d) Az-DKTHT-Fc6
e) Fc6
a b c d e
% Intensity
100
75
50
25
0
12.25 12.50 12.75 13.00 13.25 13.50 13.75 14.00 14.25 min
retention time

Figure 18

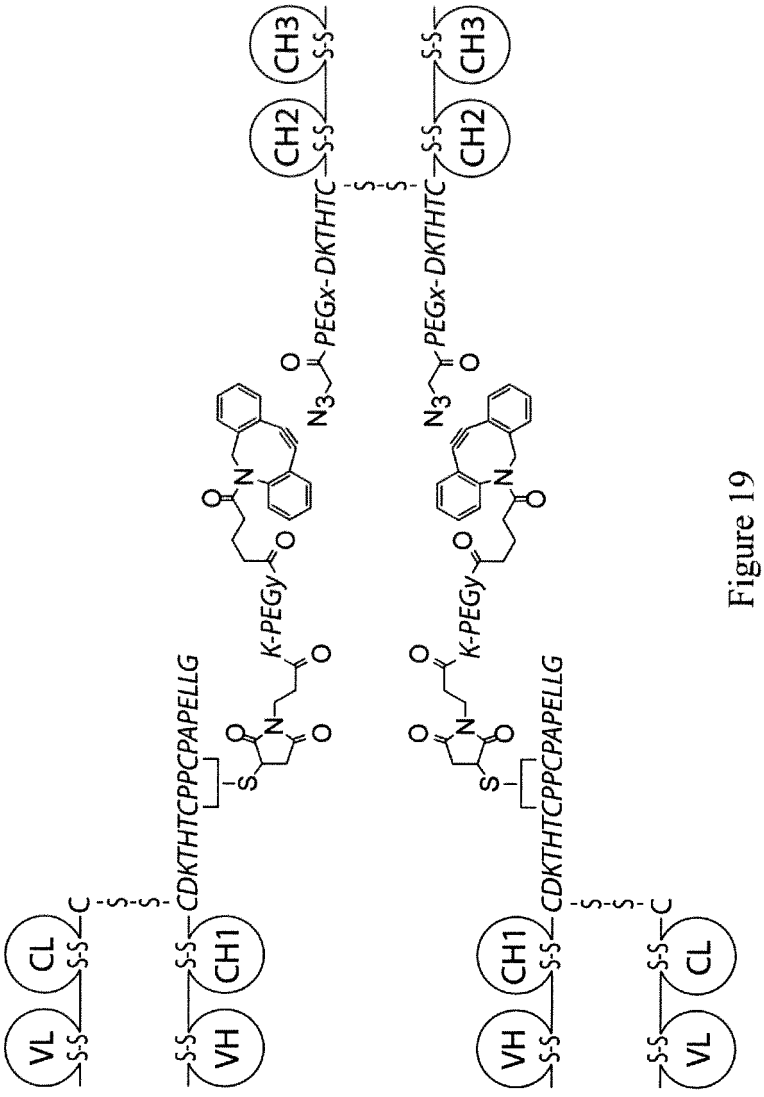

VL
CL
C
S-S
CDKTHTCPPCPAPELLG
VH
CH1
K-PEGy
PEGx-DKTHTC
CH2
CH3

Figure 19

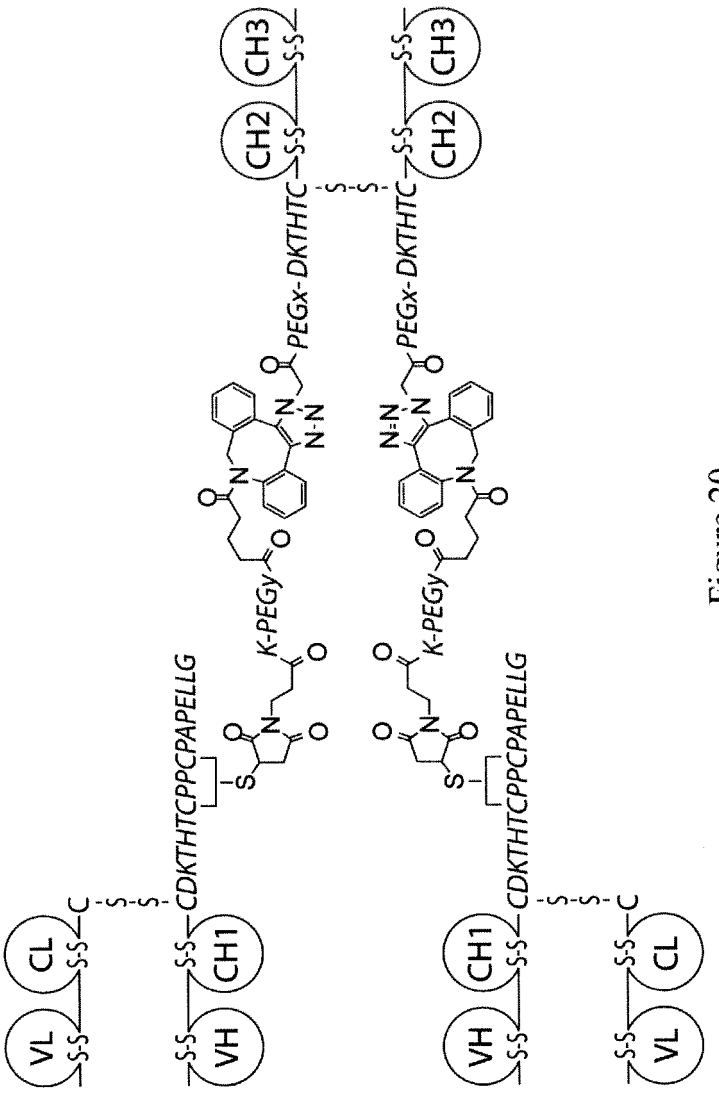
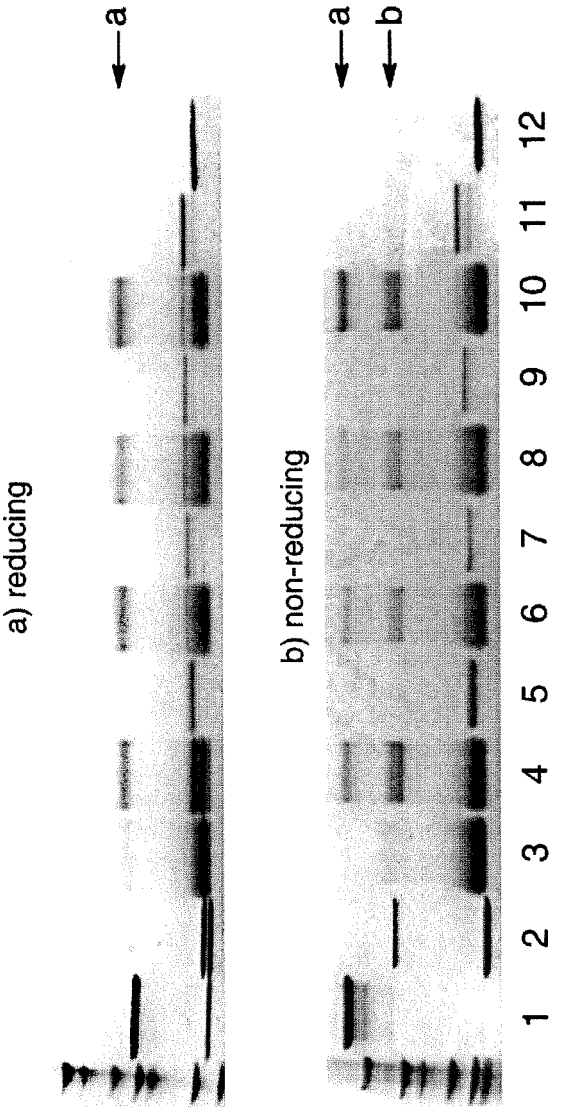
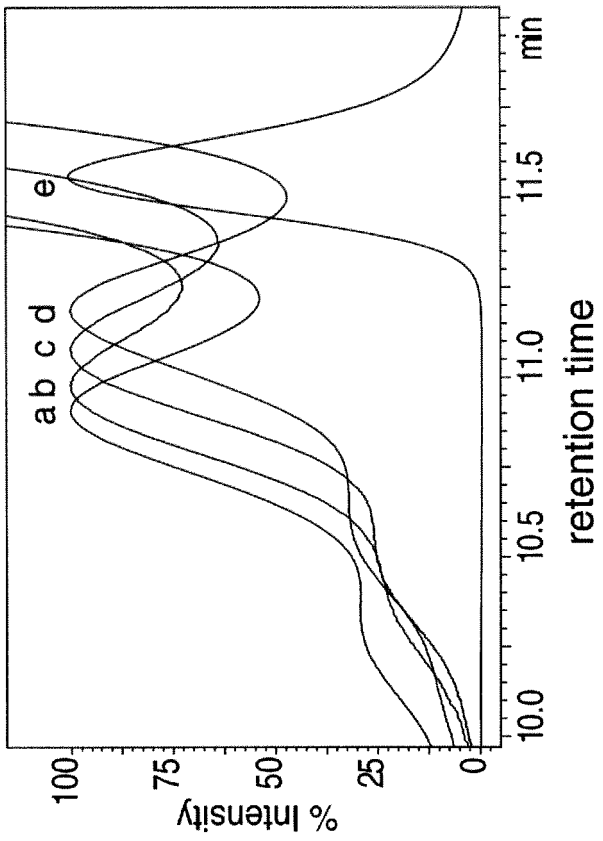

VL
CL
S-S
C
CDKTHTCPPCPAPELLG
VH
CH1
K-PEGy
PEGx-DKTHTC
CH2
CH3

Figure 20



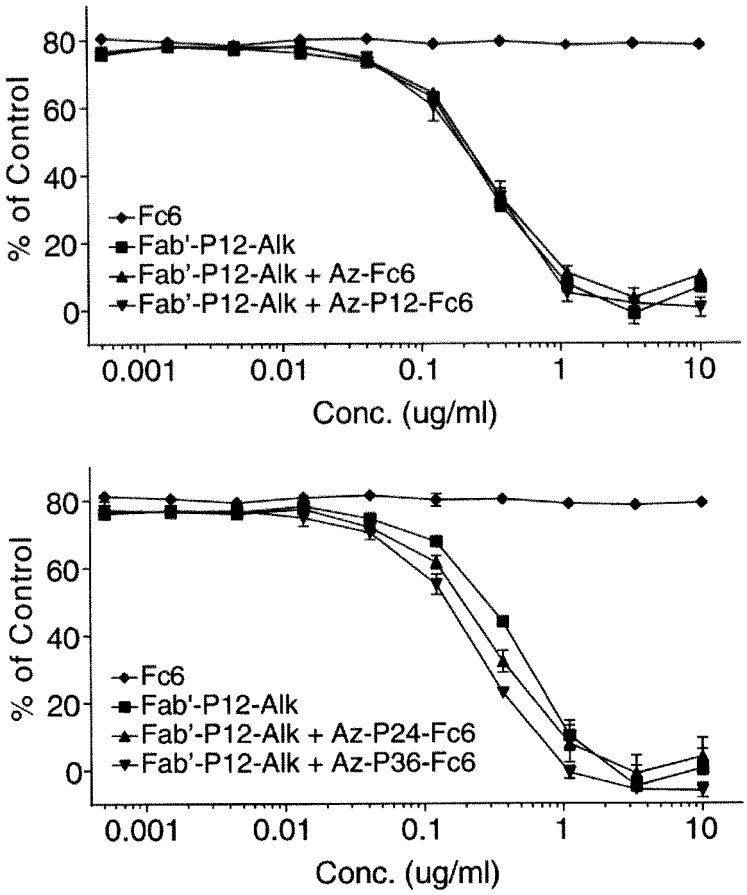
Adalimumab-Fab'-PEGy-Alk + Az-PEGx-DKTHT-Fc6

Figure 21



Fab'-PEGy-Alk-Az-PEGx-DKTHT-Fc6

Figure 22



Fab'-PEGy-Alk + Az-PEGx-DKTHT-Fc6

Figure 23

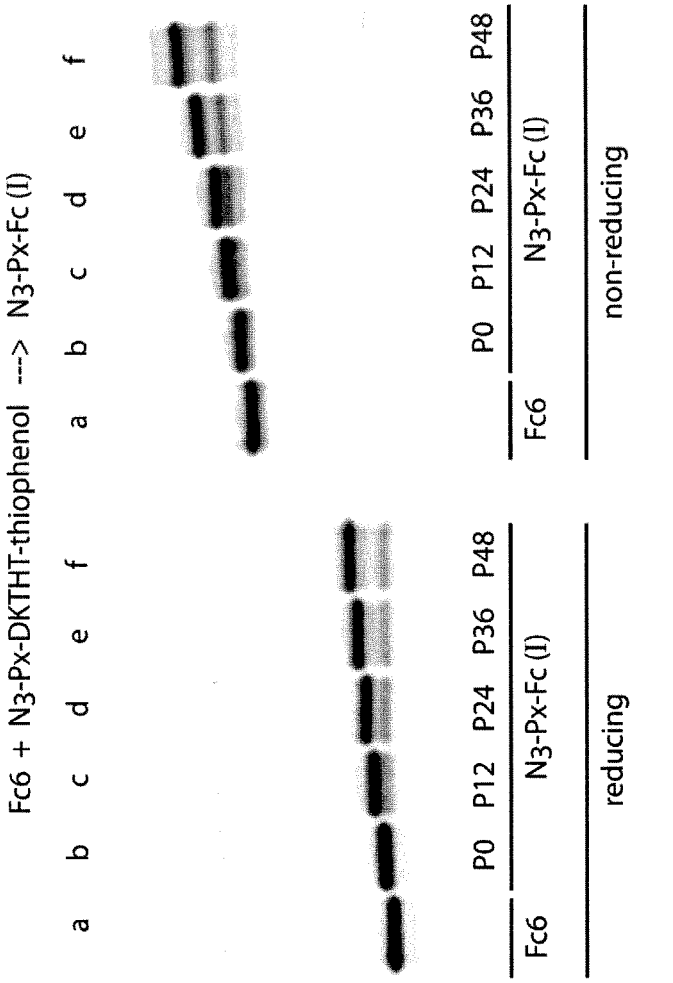

Fc6 + N3-Px-DKTHT-thiophenol --> N3-Px-Fc (I)
a b c d e f
a b c d e f
P0 P12 P24 P36 P48
Fc6
N3-Px-Fc (I)
reducing
P0 P12 P24 P36 P48
Fc6
N3-Px-Fc (I)
non-reducing

Figure 24

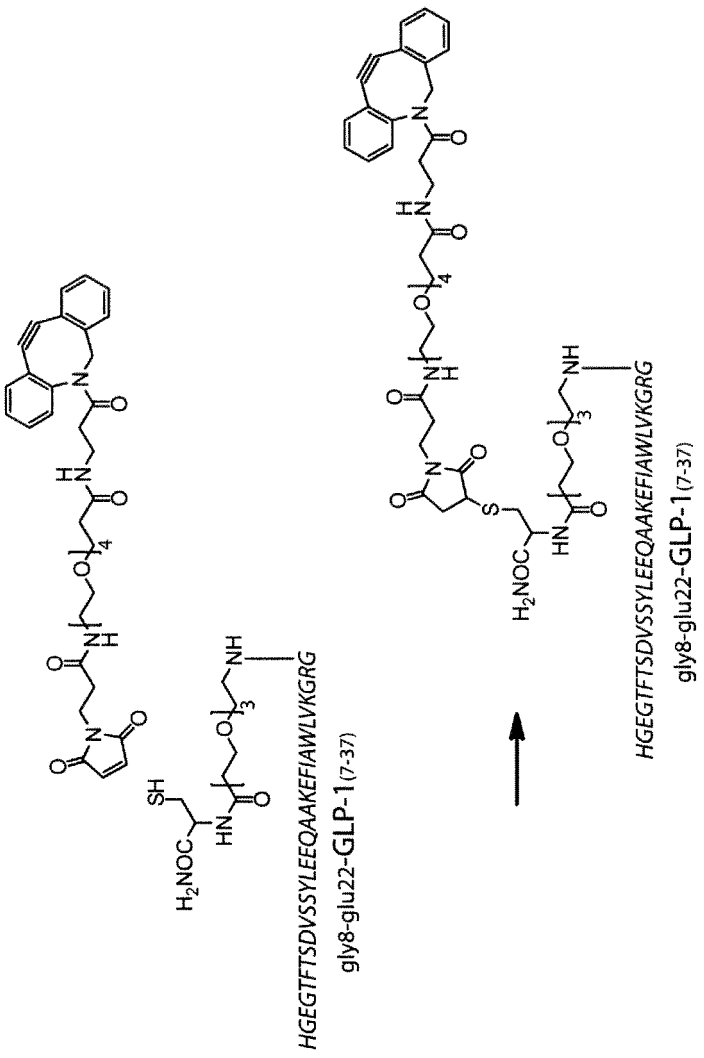

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)

Figure 25

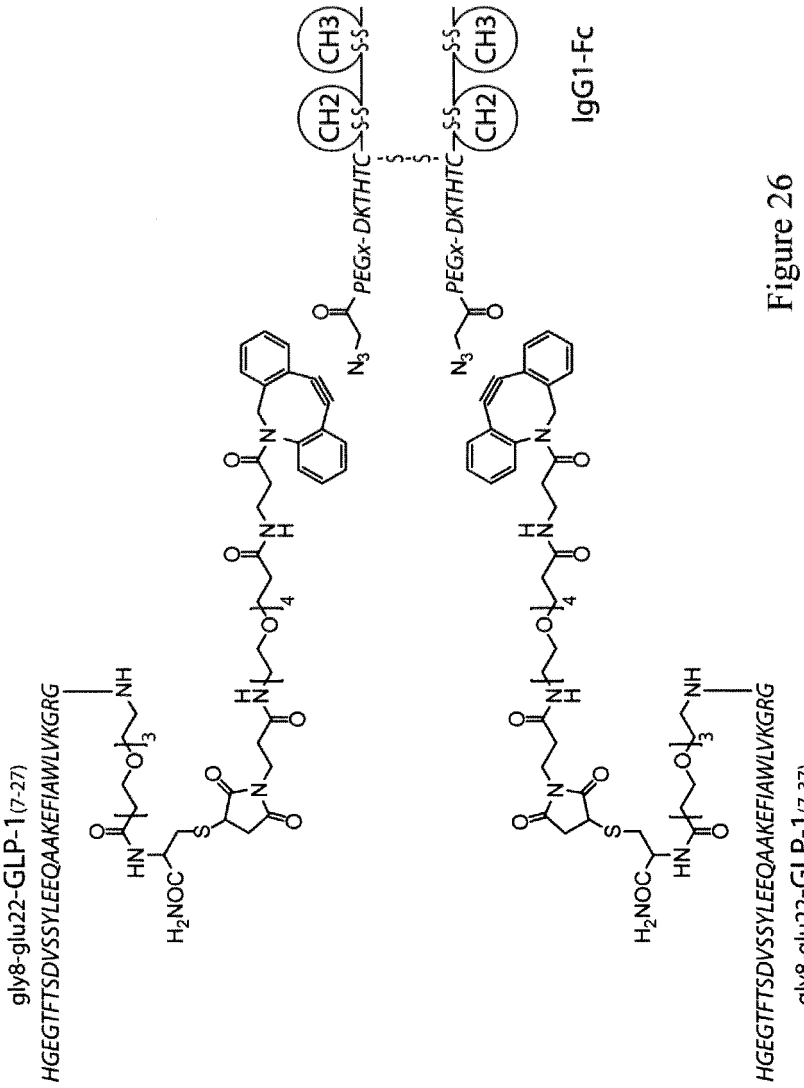

gly8-glu22-GLP-1(7-27)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
IgG1-Fc
gly8-glu22-GLP-1(7-37)

Figure 26

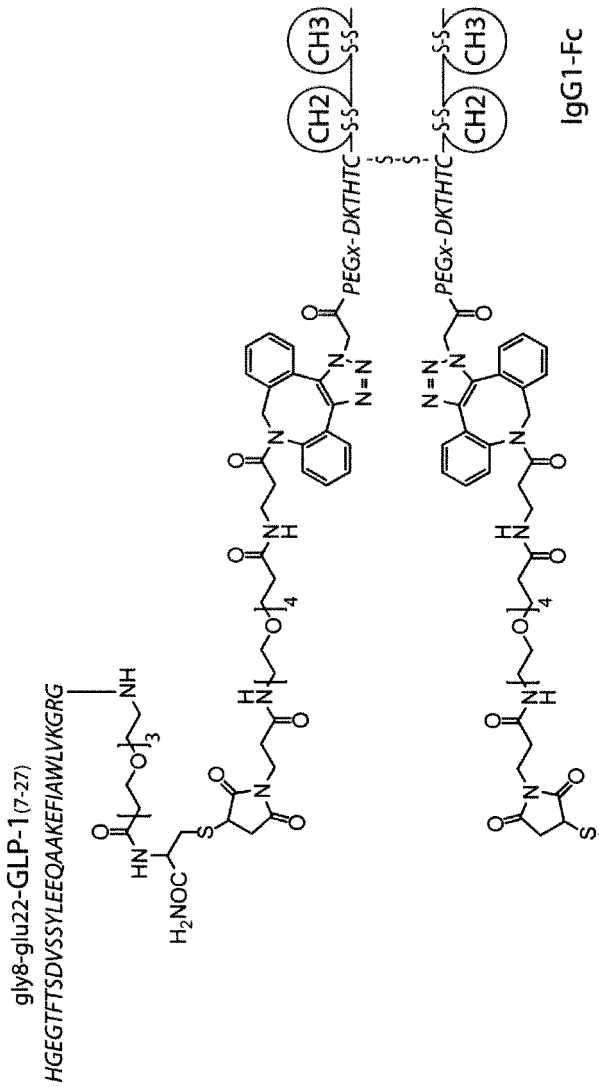

gly8-glu22-GLP-1(7-27)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
IgG1-Fc
gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG

Figure 27

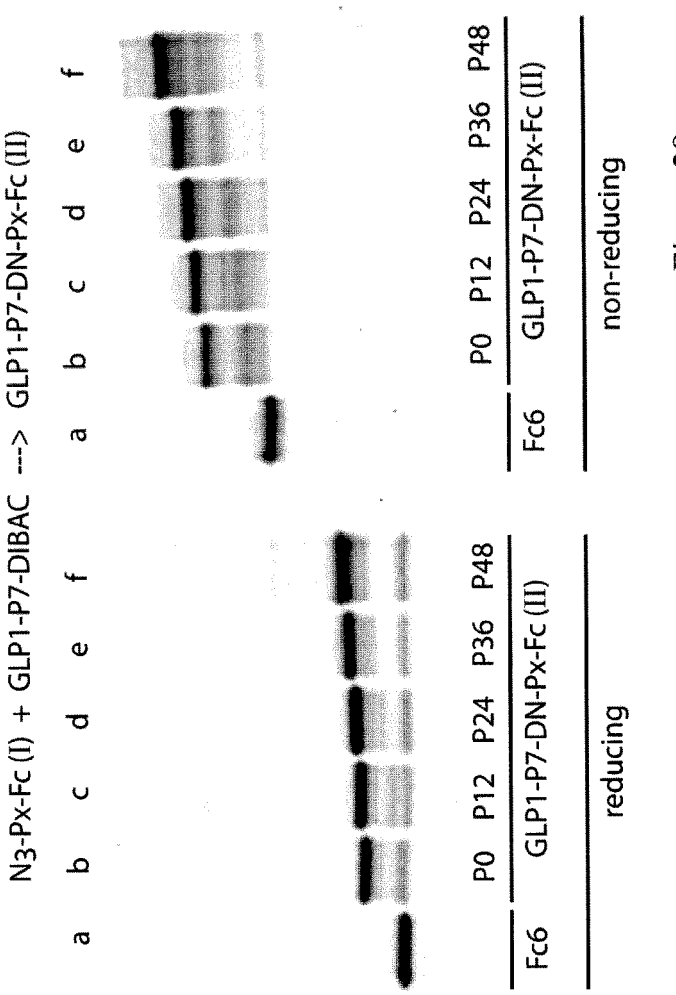

N3-Px-Fc (I) + GLP1-P7-DIBAC ---> GLP1-P7-DN-Px-Fc (II)
a b c d e f
a b c d e f
Fc6 P0 P12 P24 P36 P48
GLP1-P7-DN-Px-Fc (II)
reducing
Fc6 P0 P12 P24 P36 P48
GLP1-P7-DN-Px-Fc (II)
non-reducing

Figure 28

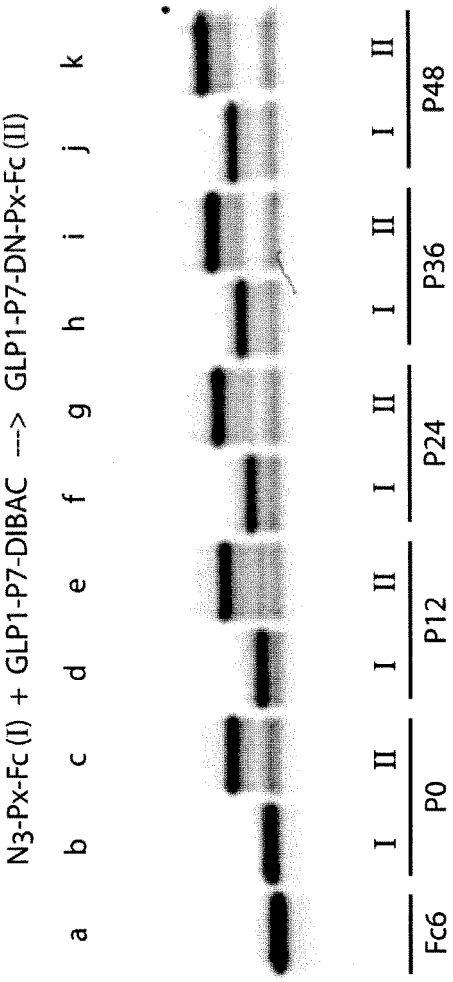

N3-Px-Fc (I) + GLP1-P7-DIBAC ---> GLP1-P7-DN-Px-Fc (II)
a b c d e f g h i j k
Fc6
I II P0
I II P12
I II P24
I II P36
I II P48

Figure 29

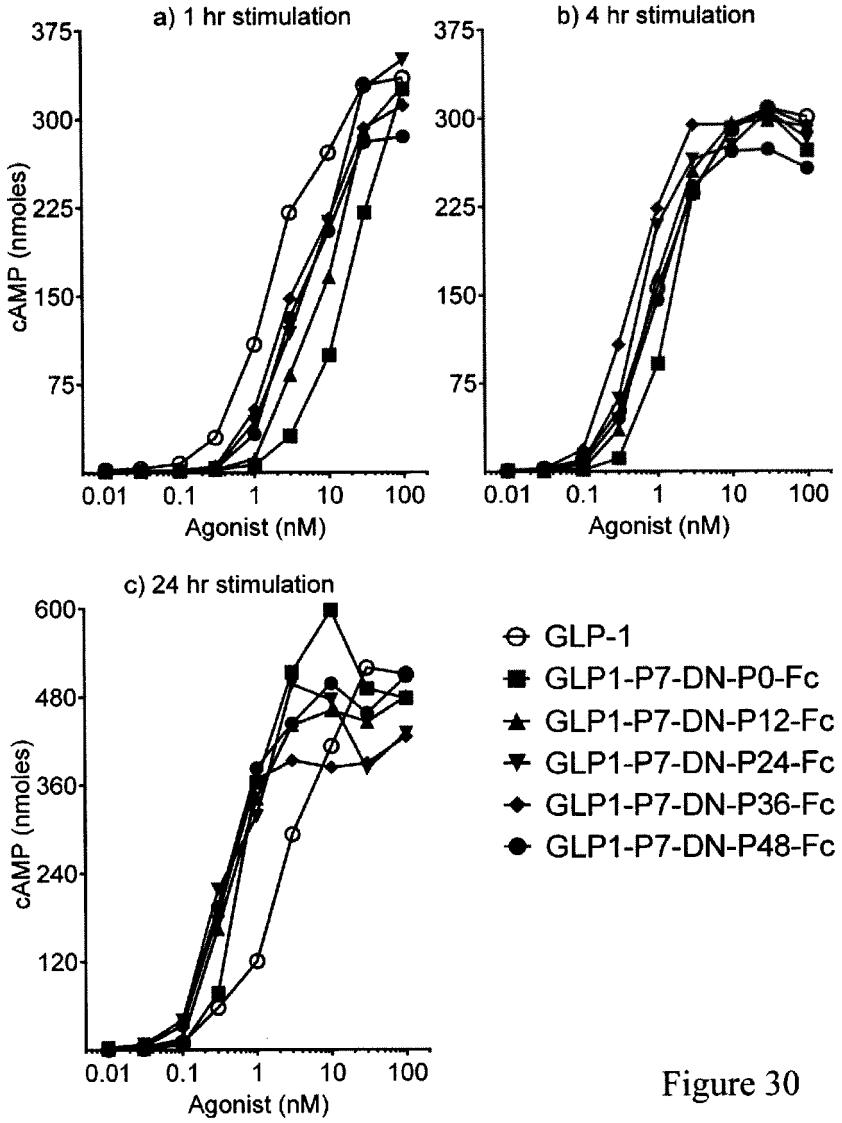

a) 1 hr stimulation
b) 4 hr stimulation
c) 24 hr stimulation
cAMP (nmoles)
Agonist (nM)
0.01
0.1
1
10
100
375
300
225
150
75
600
480
360
240
120
GLP-1
GLP1-P7-DN-P0-Fc
GLP1-P7-DN-P12-Fc
GLP1-P7-DN-P24-Fc
GLP1-P7-DN-P36-Fc
GLP1-P7-DN-P48-Fc

Figure 30

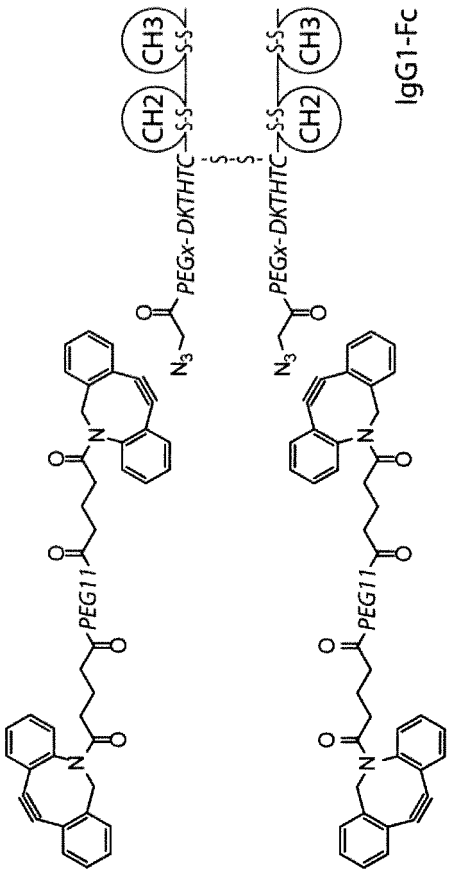


Figure 31

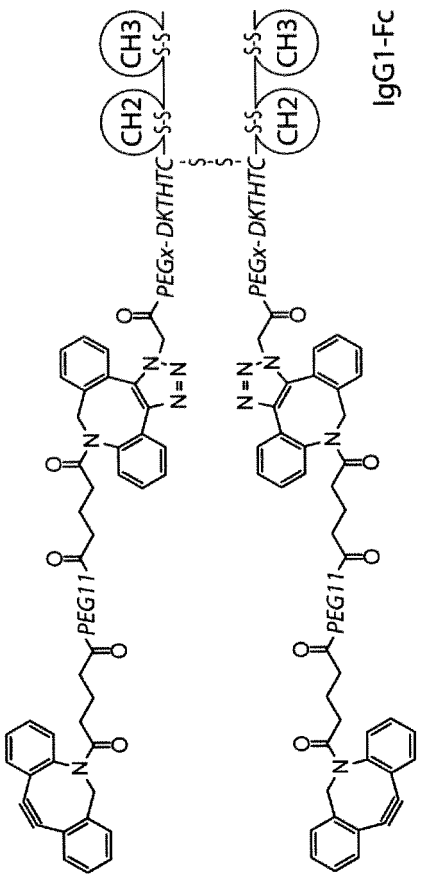

CH3
CH2
S-S
PEGx-DKTHTC
S-S
S-S
PEGx-DKTHTC
CH2
CH3
S-S
IgG1-Fc
N=N
N=N
PEG11
PEG11

Figure 32

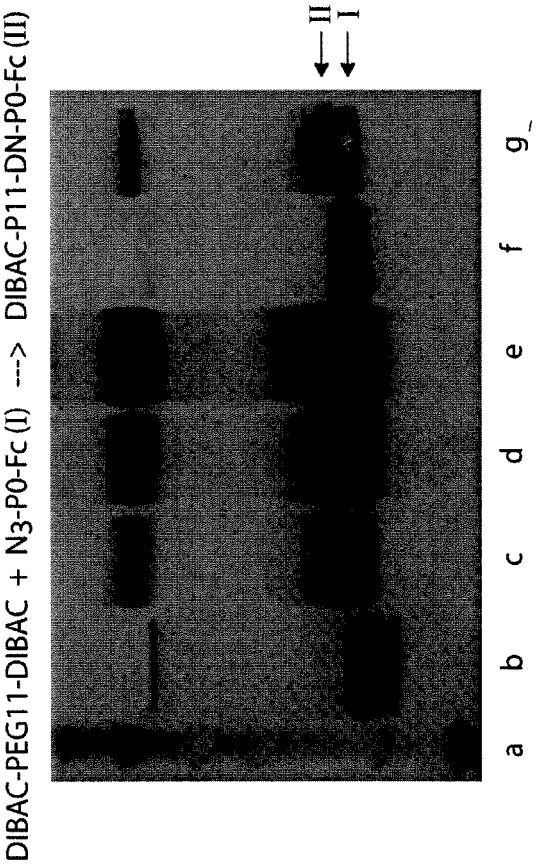


Figure 33

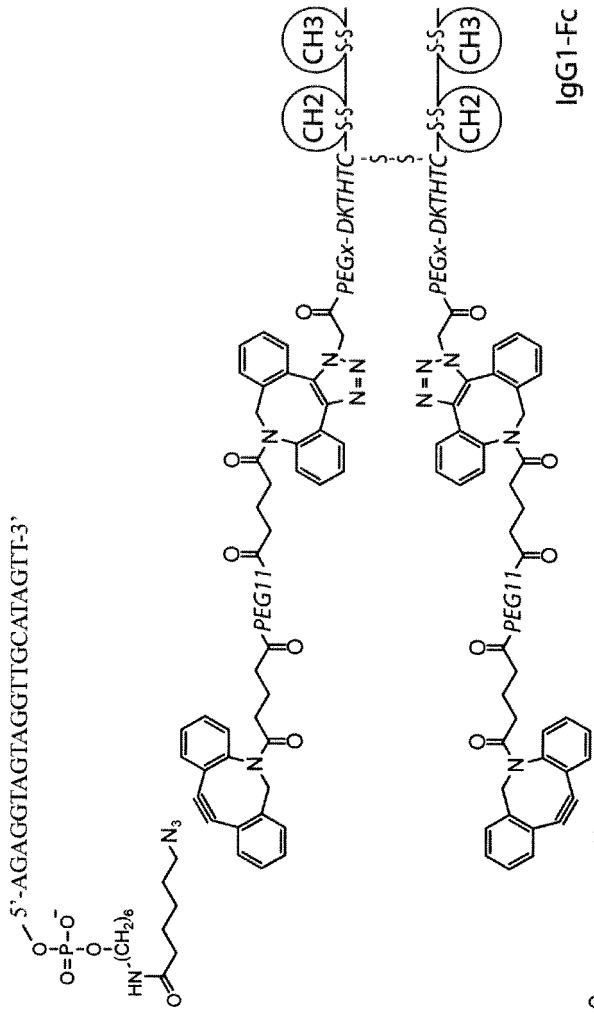

5'-AGAGGTAGTAGGTTGCATAGTT-3'
PEG11
PEGx-DKTHTC
CH2
CH3
IgG1-Fc

Figure 34

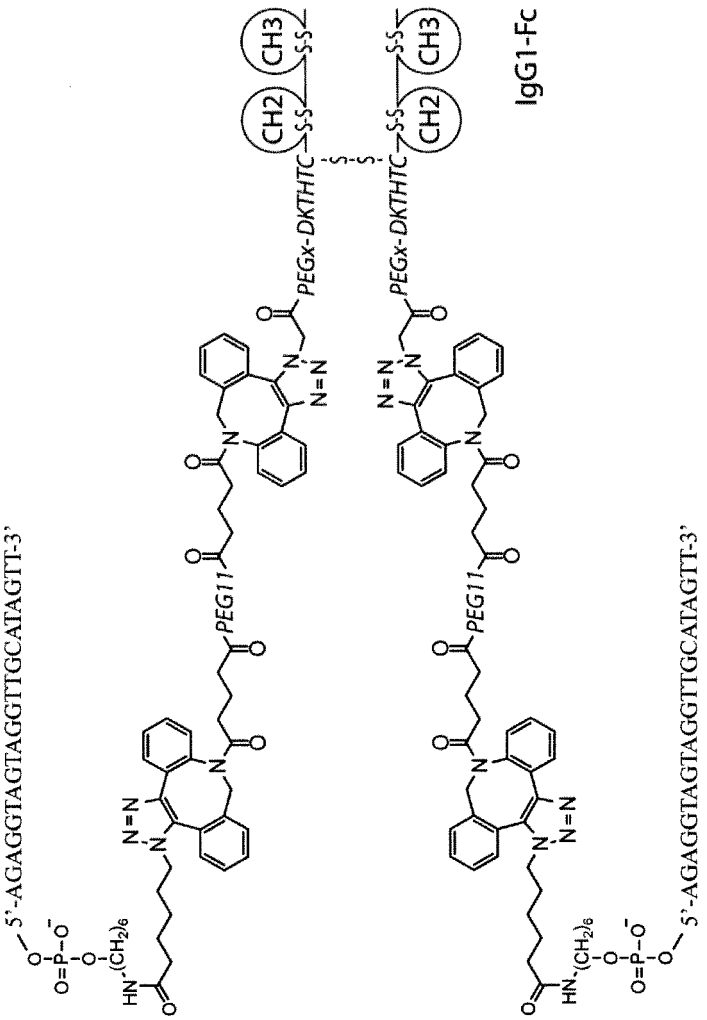

5'-AGAGGTAGTAGGTTGCATAGTT-3'
PEG11
PEGx-DKTHTC
CH2
CH3
IgG1-Fc

Figure 35

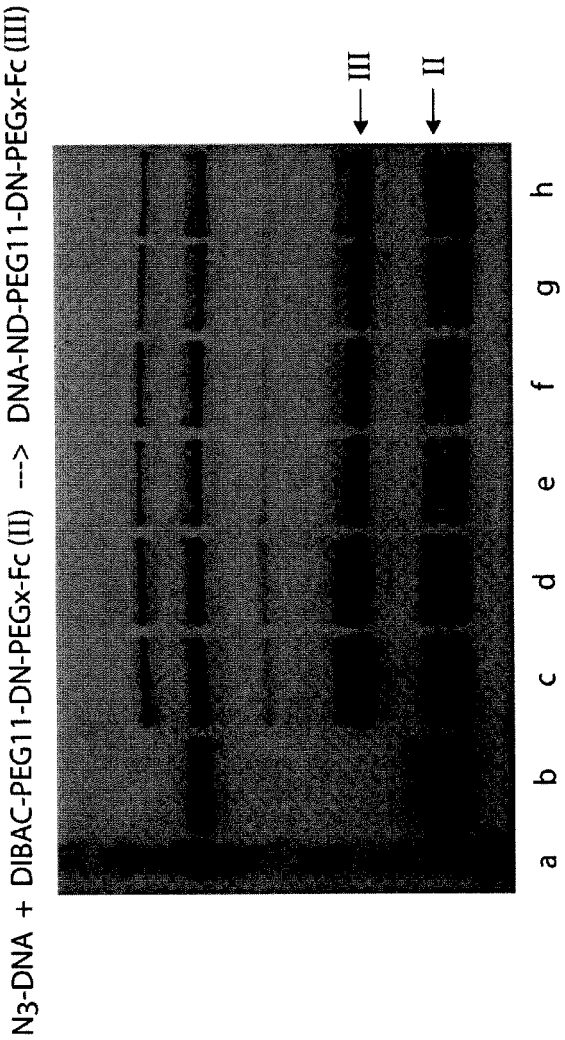

N3-DNA + DIBAC-PEG11-DN-PEGx-Fc (II) --> DNA-ND-PEG11-DN-PEGx-Fc (III)
III
II
a
b
c
d
e
f
g
h

Figure 36

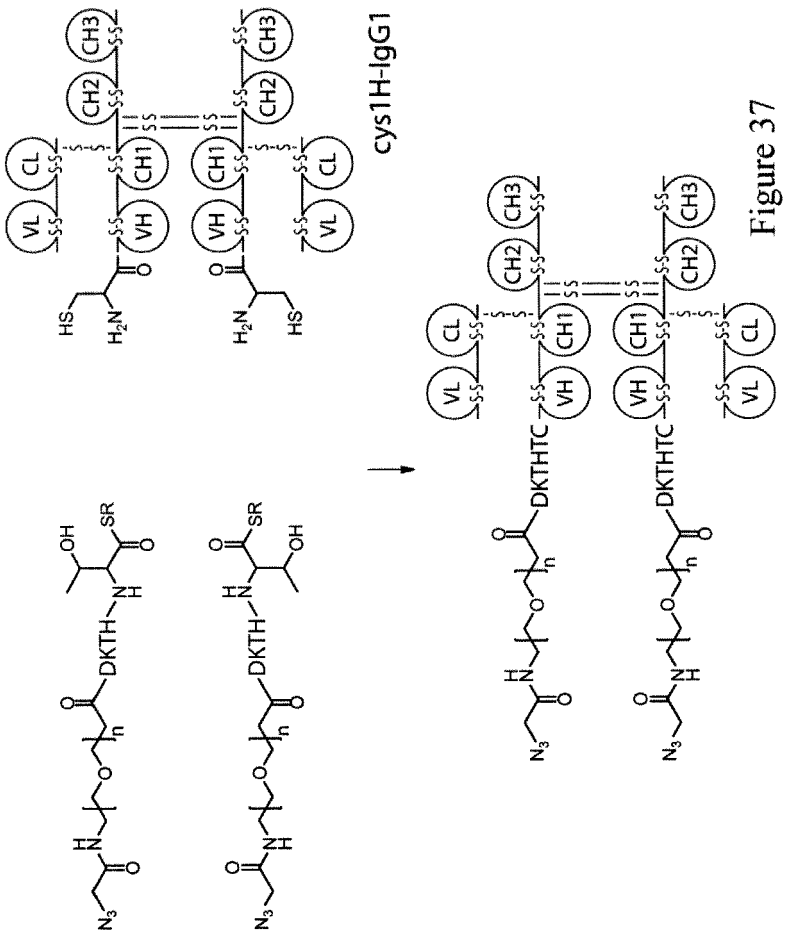


Figure 37

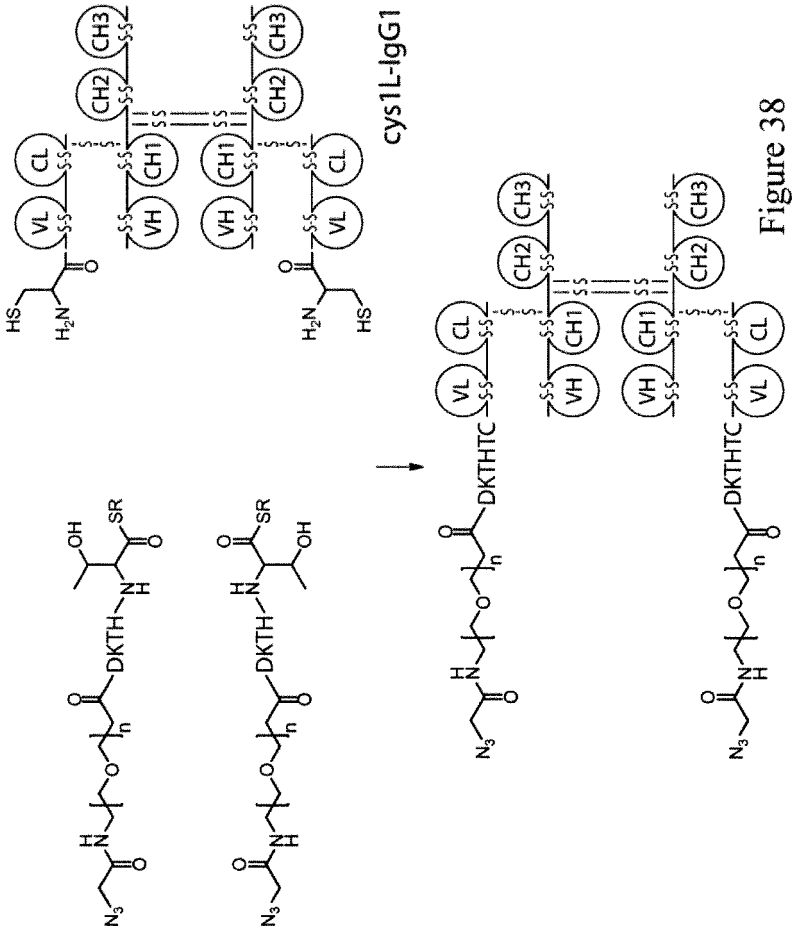


Figure 38

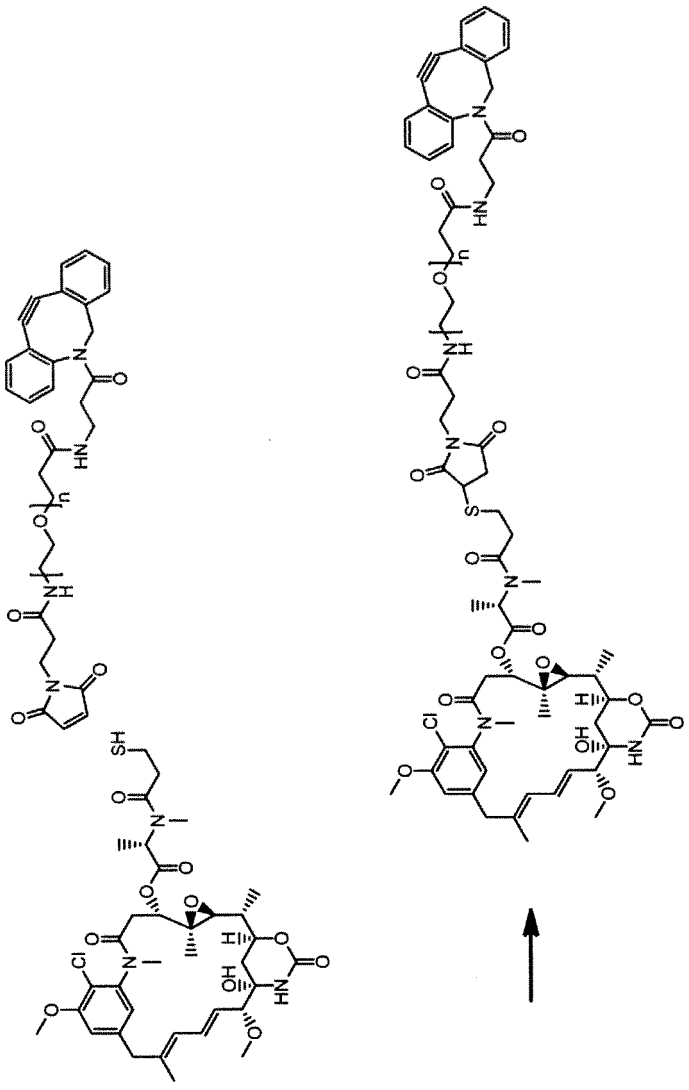


Figure 39

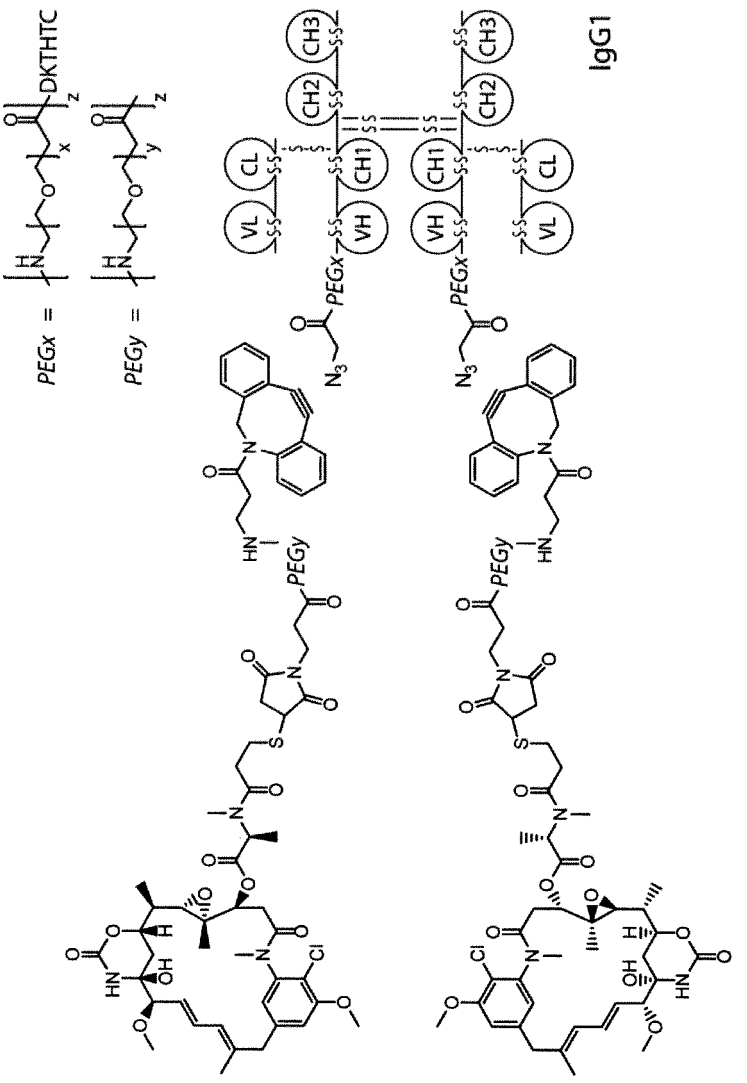


Figure 40

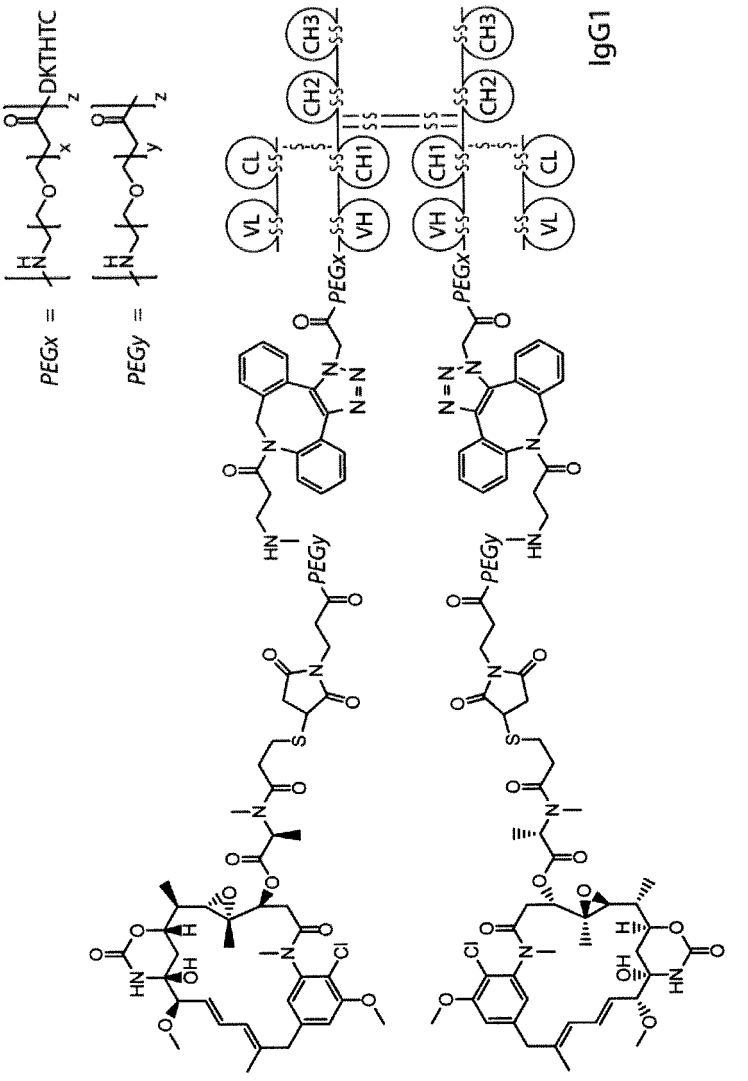


Figure 41

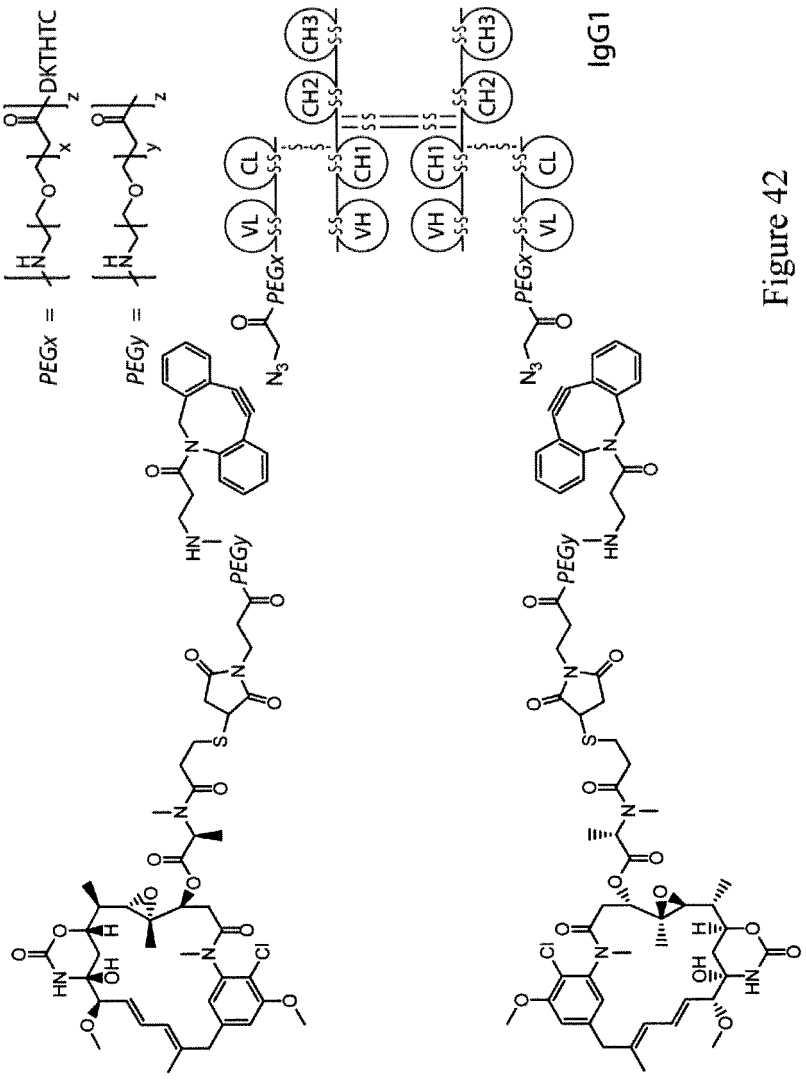


Figure 42

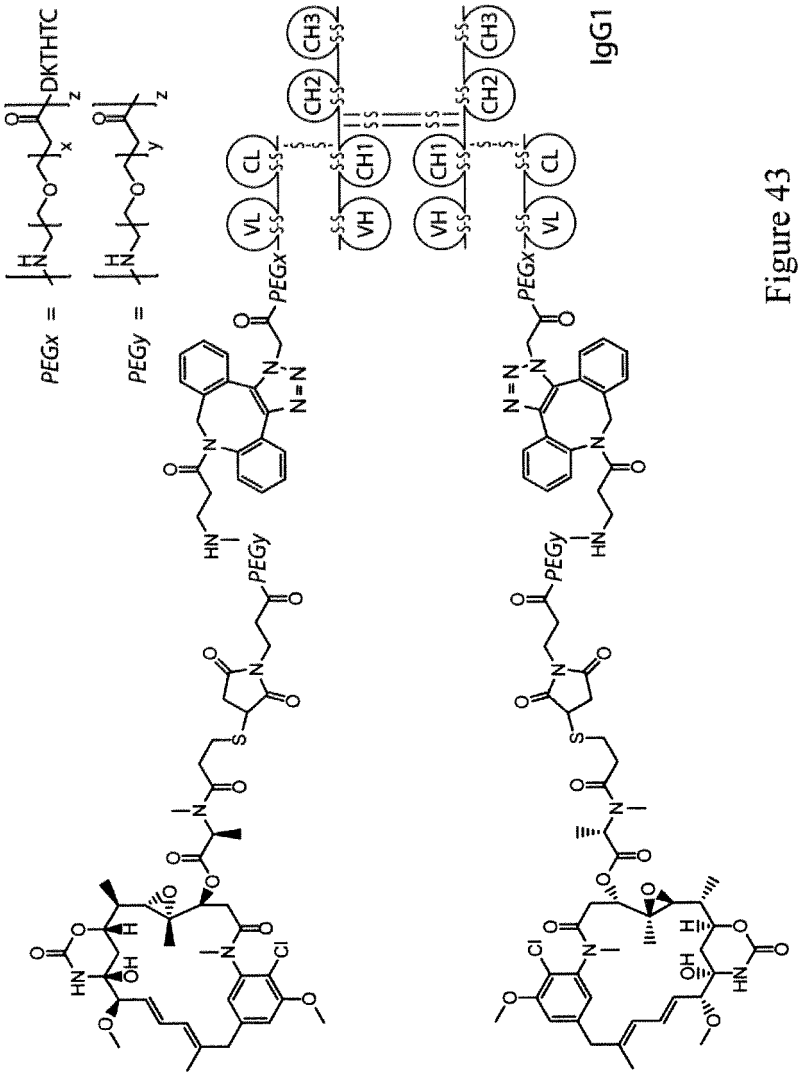


Figure 43

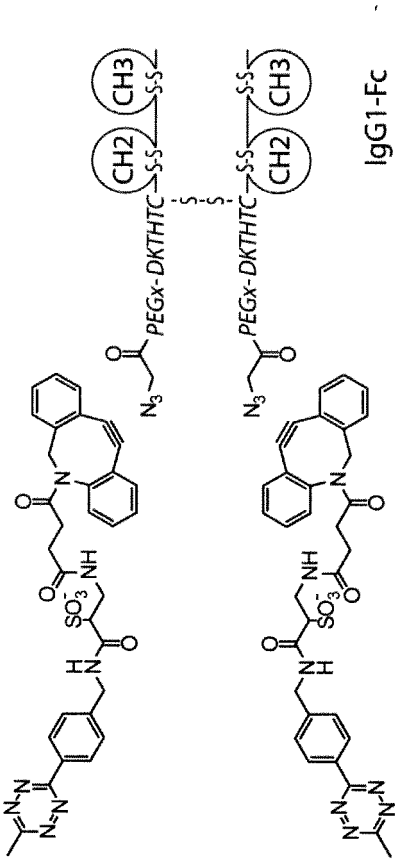


Figure 44

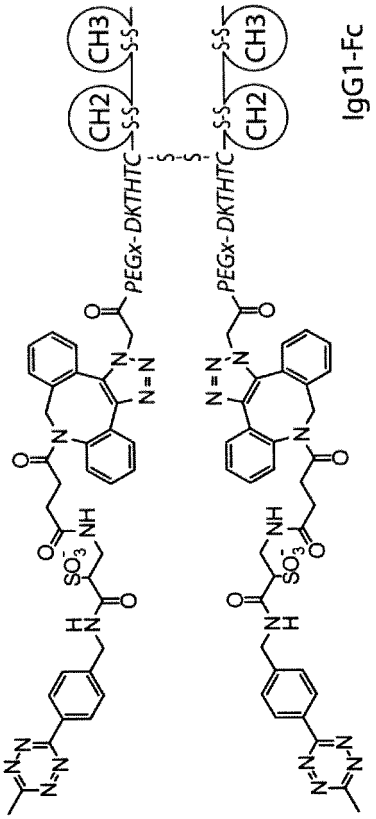


Figure 45

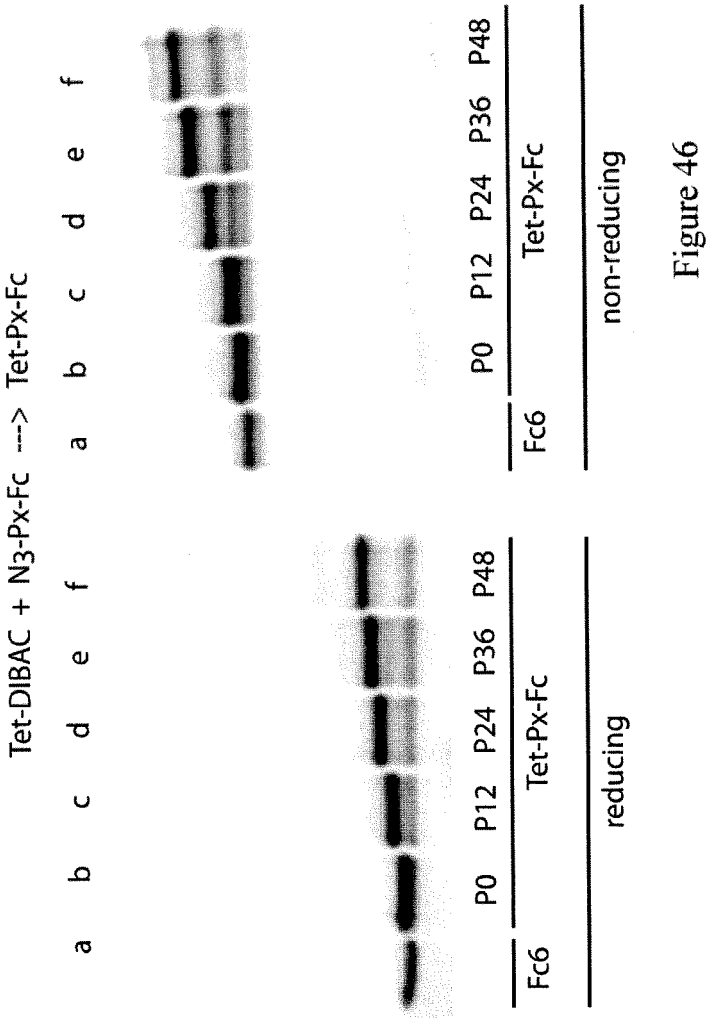

Tet-DIBAC + N3-Px-Fc --> Tet-Px-Fc
a b c d e f
a b c d e f
Fc6
P0 P12 P24 P36 P48
Tet-Px-Fc
reducing
Fc6
P0 P12 P24 P36 P48
Tet-Px-Fc
non-reducing

Figure 46

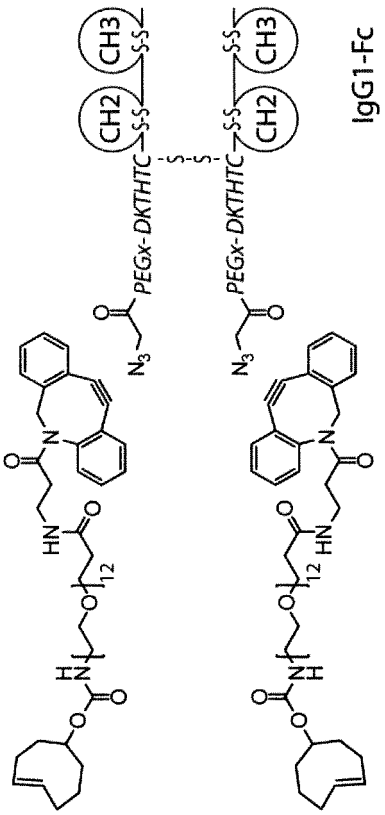


Figure 47

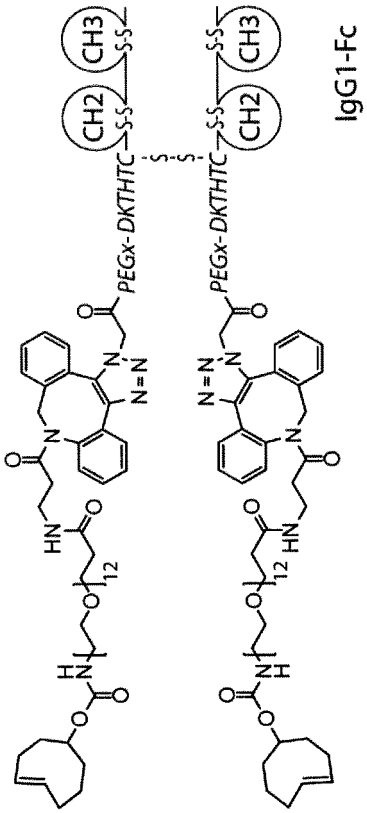


Figure 48

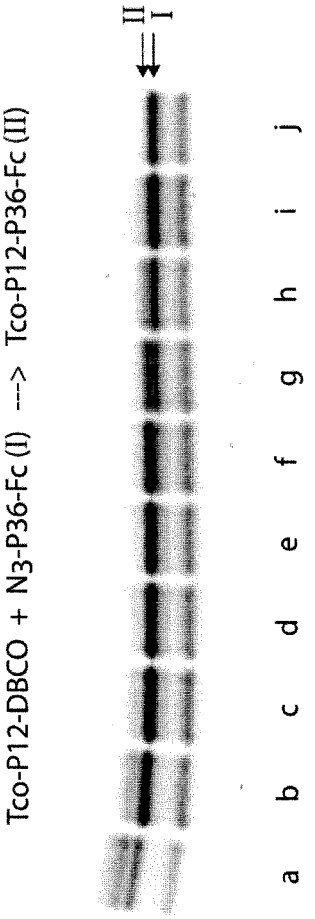


Figure 49

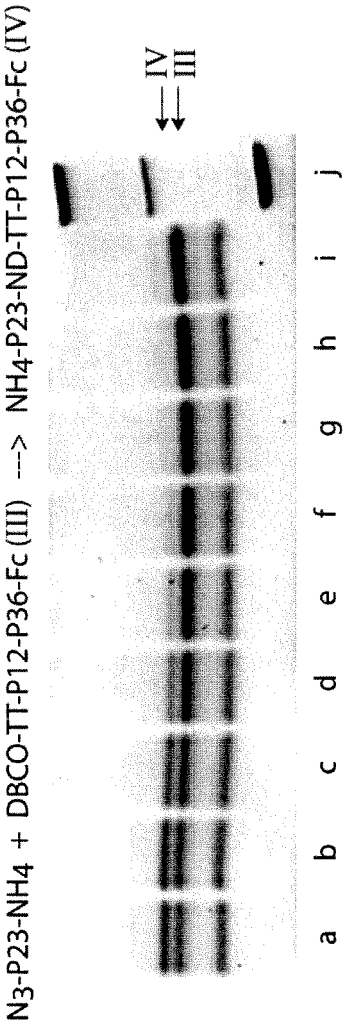

N3-P23-NH4 + DBCO-TT-P12-P36-Fc (III) ---> NH4-P23-ND-TT-P12-P36-Fc (IV)
IV
III
a b c d e f g h i j

Figure 50

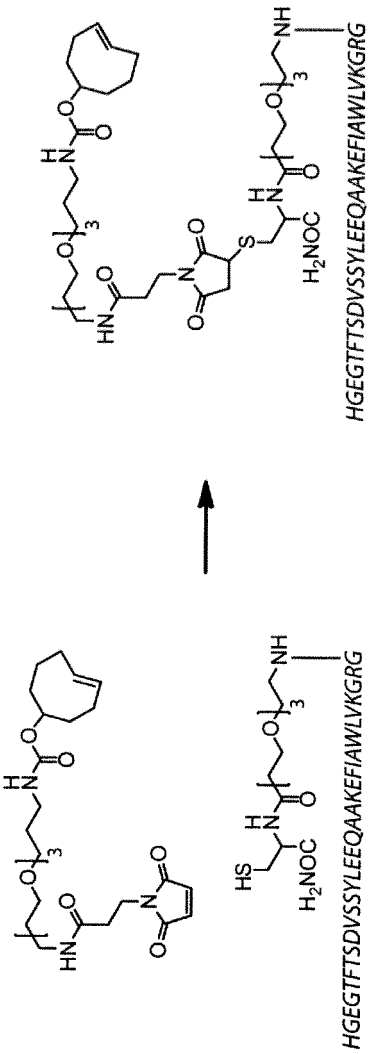

HS
H2NOC
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)
H2NOC
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)

Figure 51

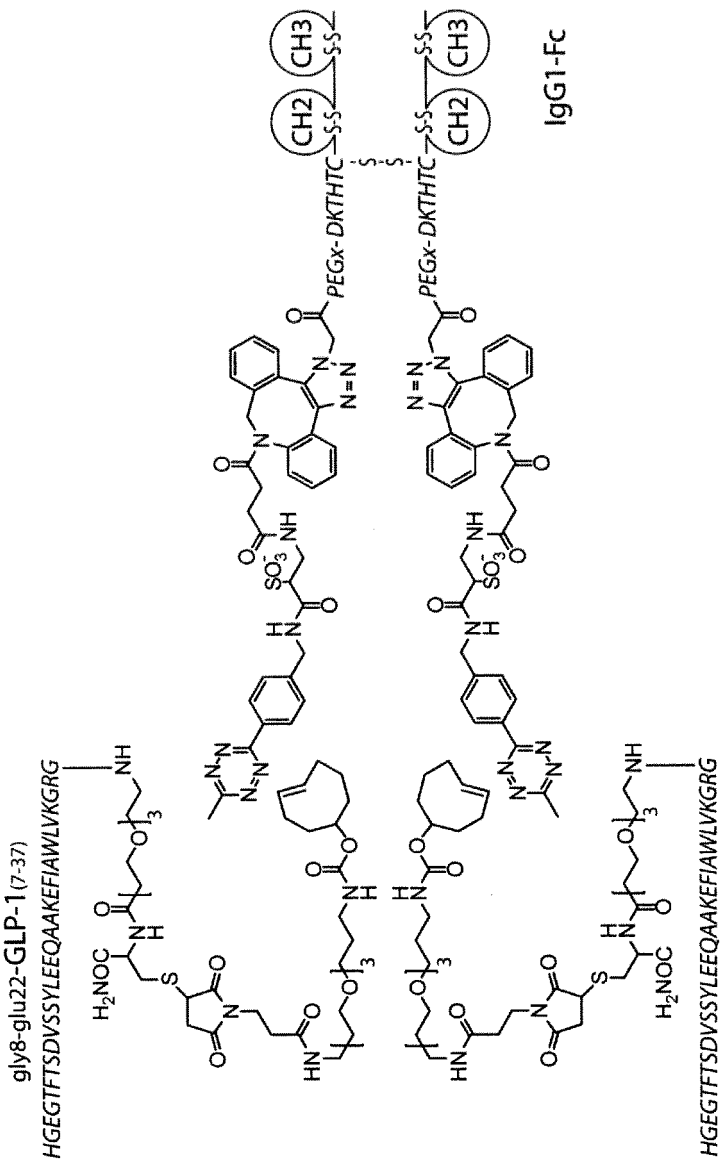

gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
IgG1-Fc

Figure 52

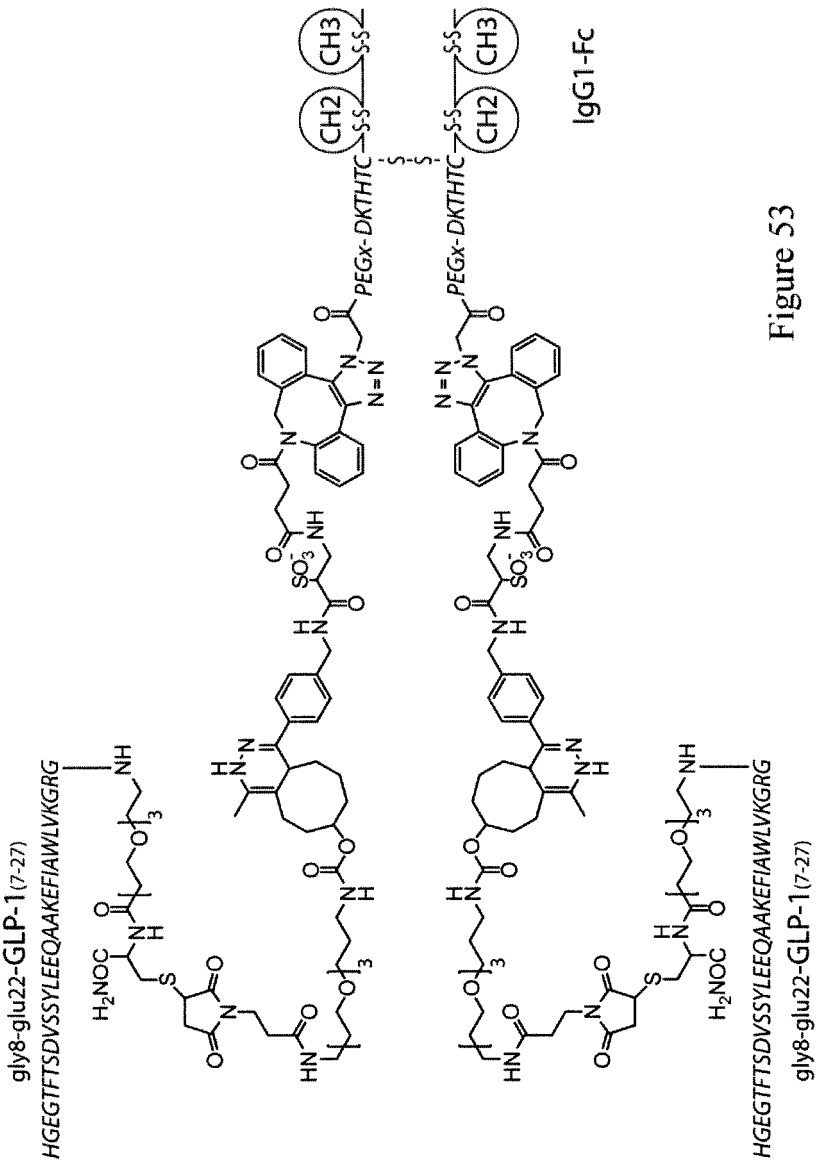

gly8-glu22-GLP-1(7-27)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
IgG1-Fc

Figure 53

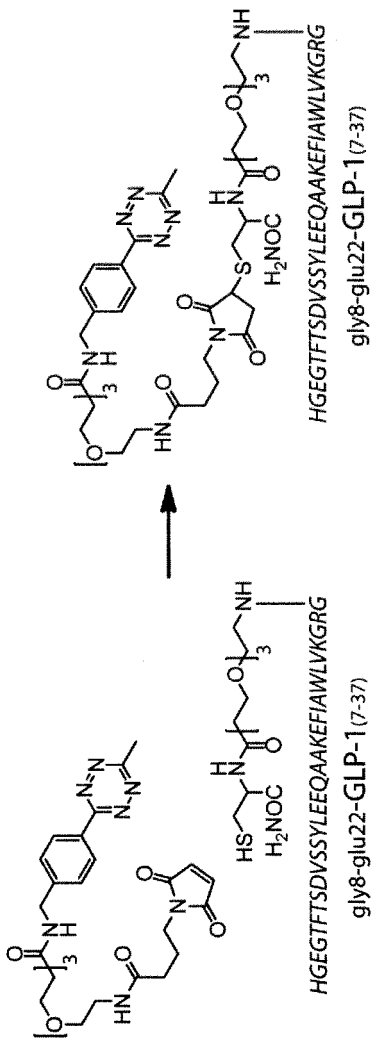

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)

Figure 54

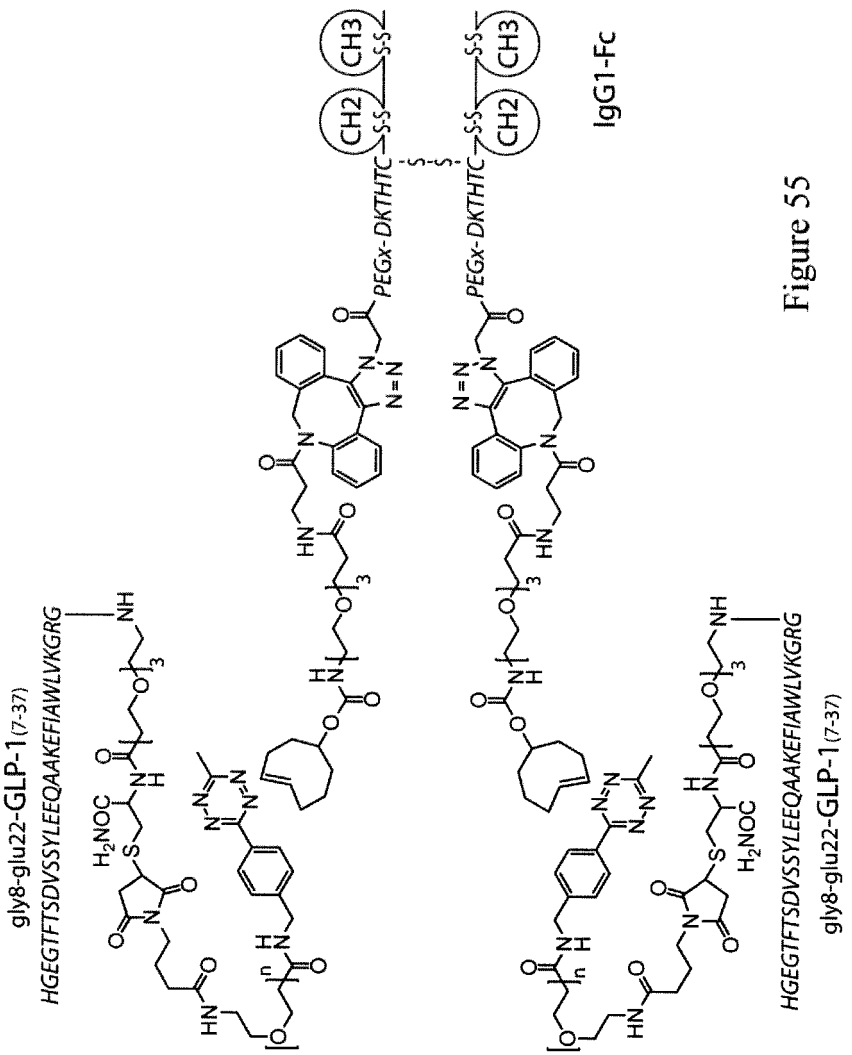

gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
S-S
IgG1-Fc

Figure 55

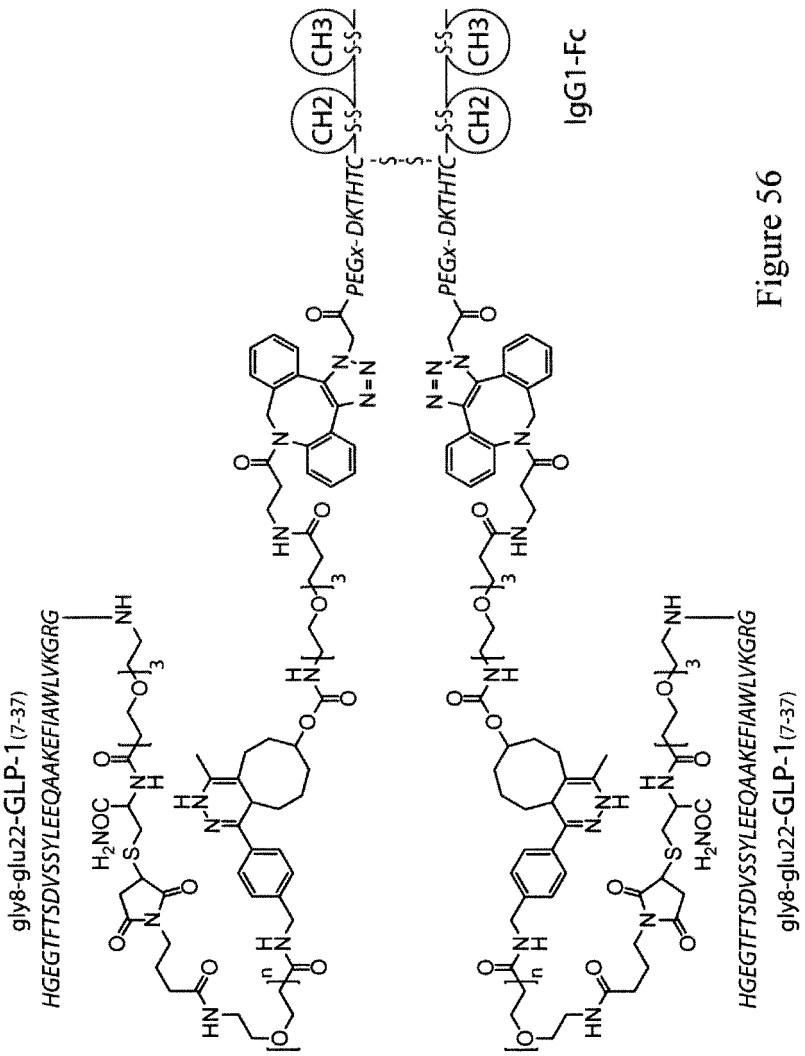

gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
IgG1-Fc

Figure 56

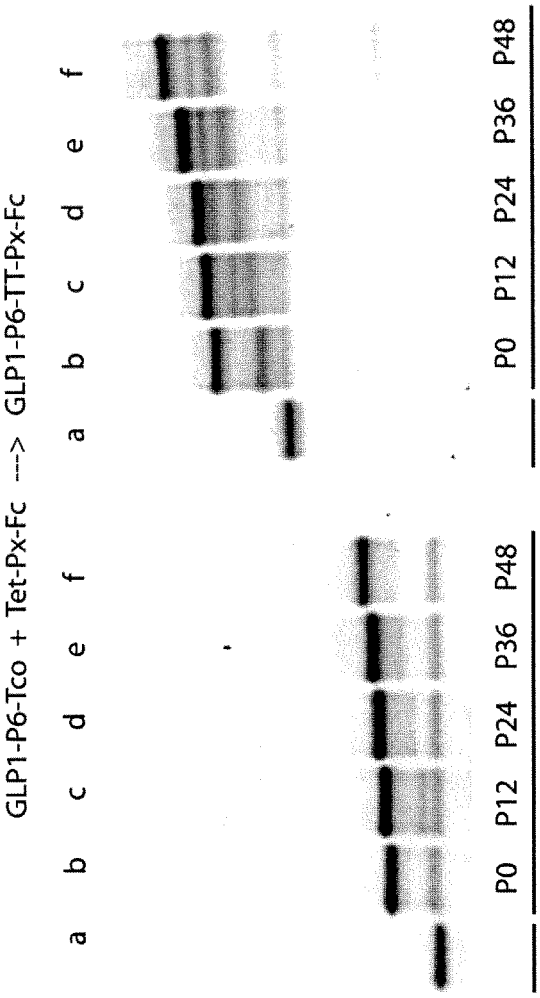

GLP1-P6-Tco + Tet-Px-Fc --> GLP1-P6-TT-Px-Fc
a b c d e f
a b c d e f
Fc6
P0 P12 P24 P36 P48
GLP1-P6-TT-Px-Fc
reducing
Fc6
P0 P12 P24 P36 P48
GLP1-P6-TT-Px-Fc
non-reducing

Figure 57

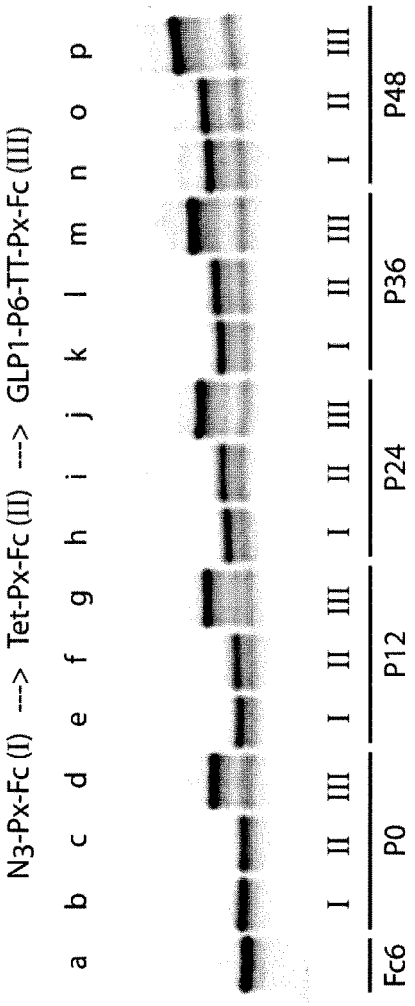

N3-Px-Fc (I) ---> Tet-Px-Fc (II) ---> GLP1-P6-TT-Px-Fc (III)
a b c d e f g h i j k l m n o p
Fc6
P0
P12
P24
P36
P48
I II III I II III I II III I II III I II III I II III

Figure 58

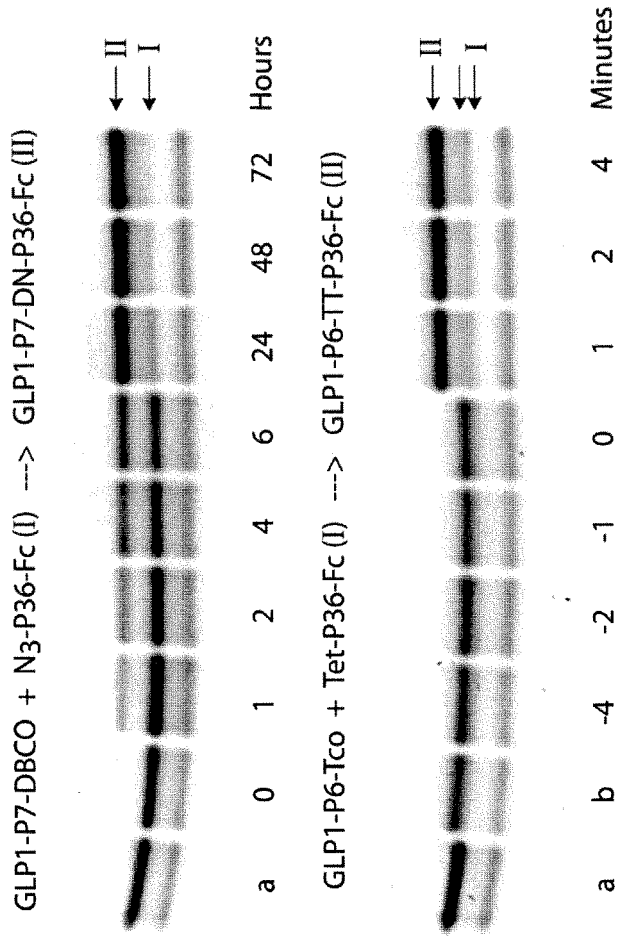

GLP1-P7-DBCO + N3-P36-Fc (I) --> GLP1-P7-DN-P36-Fc (II)
II
I
a 0 1 2 4 6 24 48 72 Hours
GLP1-P6-Tco + Tet-P36-Fc (I) --> GLP1-P6-TT-P36-Fc (II)
II
I
a b -4 -2 -1 0 1 2 4 Minutes

Figure 59

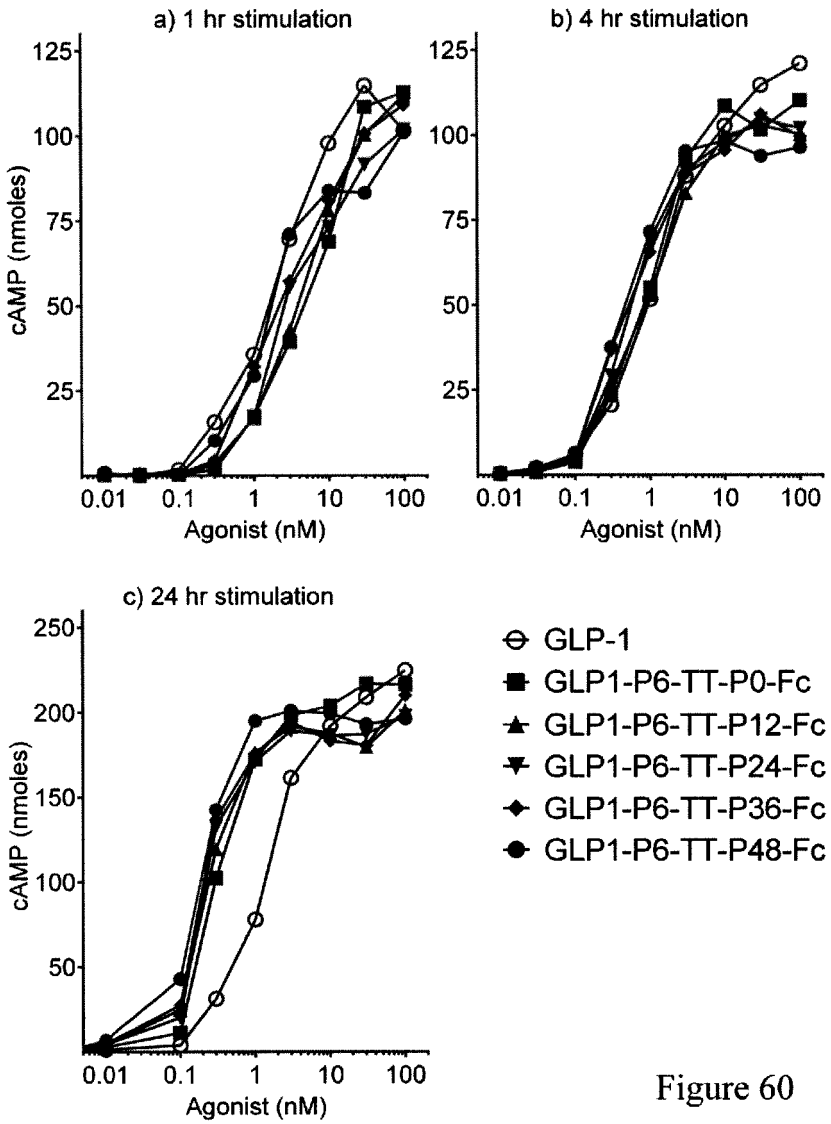

a) 1 hr stimulation
b) 4 hr stimulation
c) 24 hr stimulation
cAMP (nmoles)
Agonist (nM)
0.01
0.1
1
10
100
25
50
75
100
125
150
200
250
GLP-1
GLP1-P6-TT-P0-Fc
GLP1-P6-TT-P12-Fc
GLP1-P6-TT-P24-Fc
GLP1-P6-TT-P36-Fc
GLP1-P6-TT-P48-Fc

Figure 60

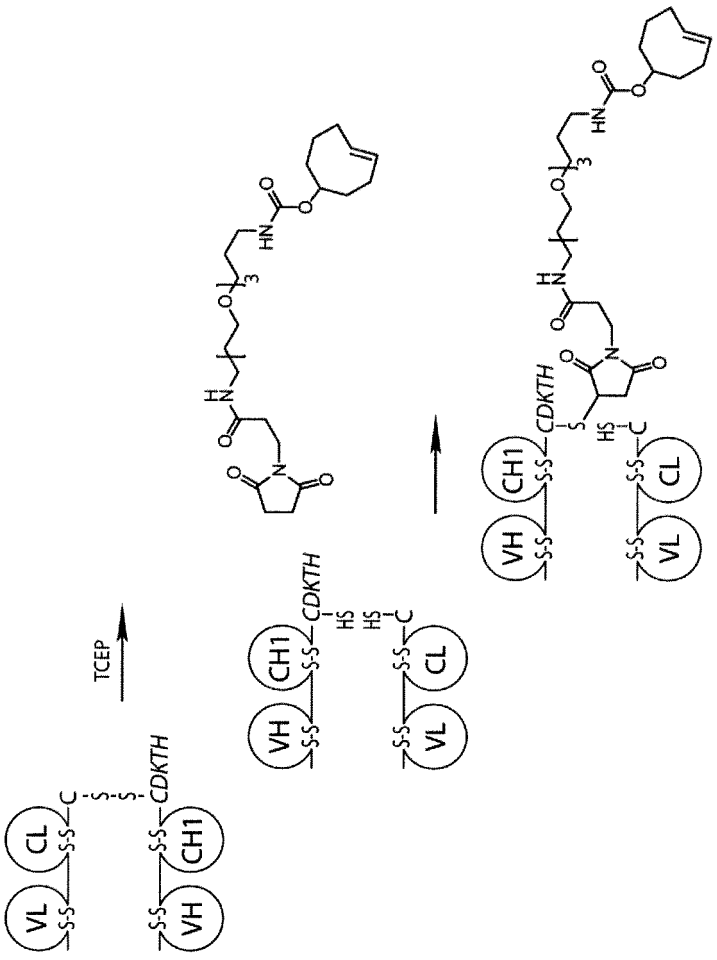


Figure 61

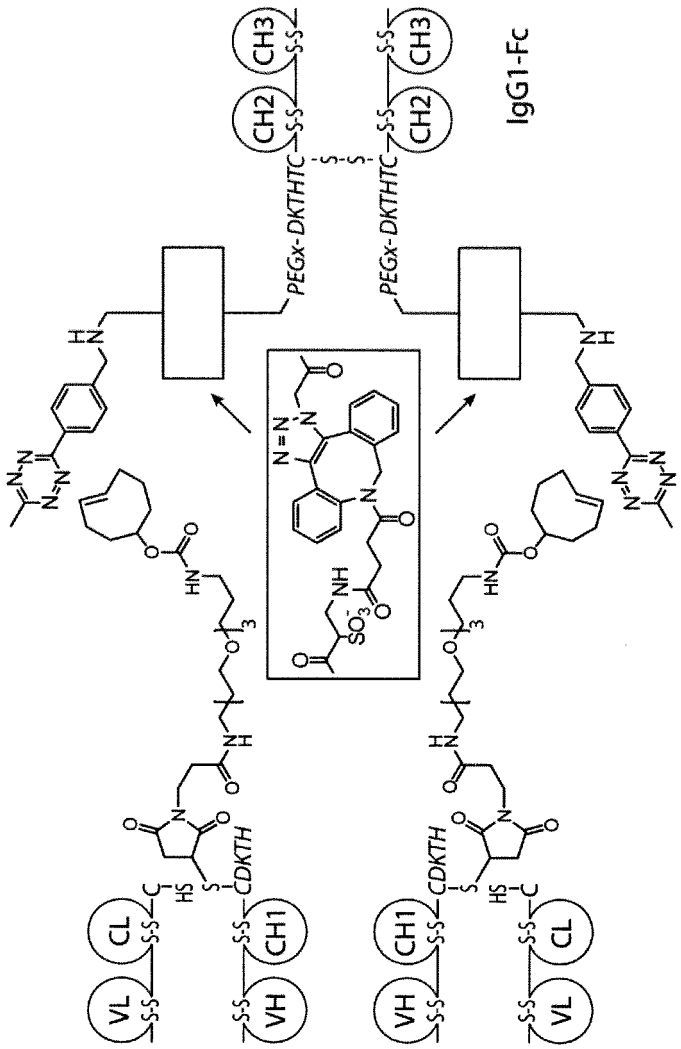

IgG1-Fc
PEGx-DKTHTC
PEGx-DKTHTC
CH3
CH2
CH3
CH2
CDKTH
CDKTH
CL
CH1
VH
VL
VH
CH1
VL
CL

Figure 62

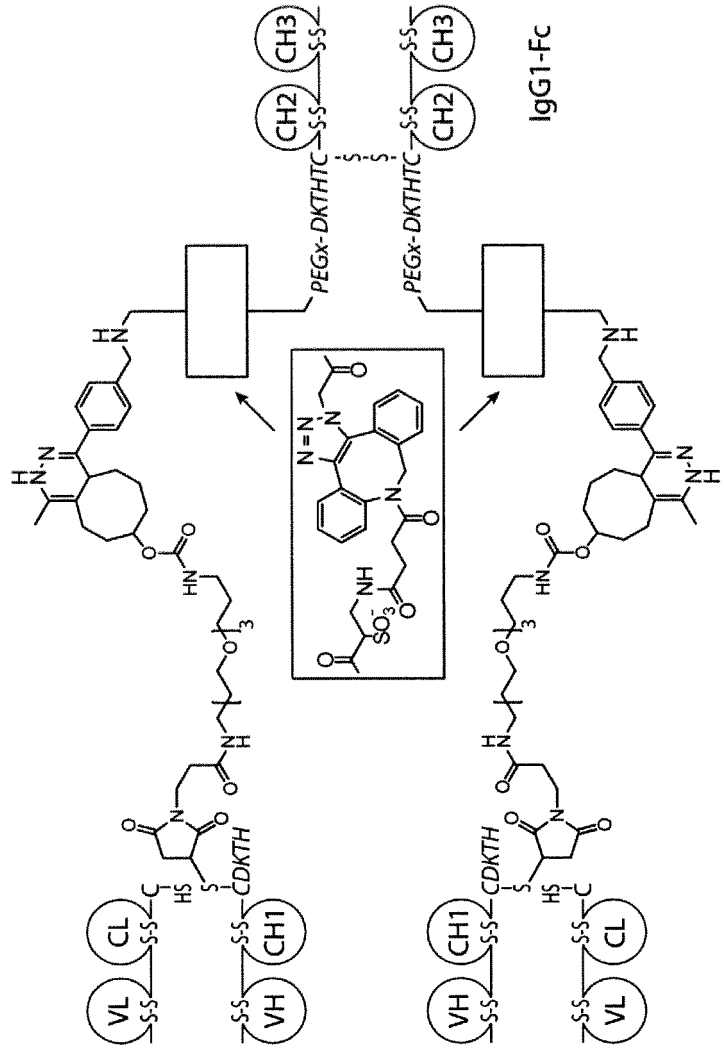


Figure 63

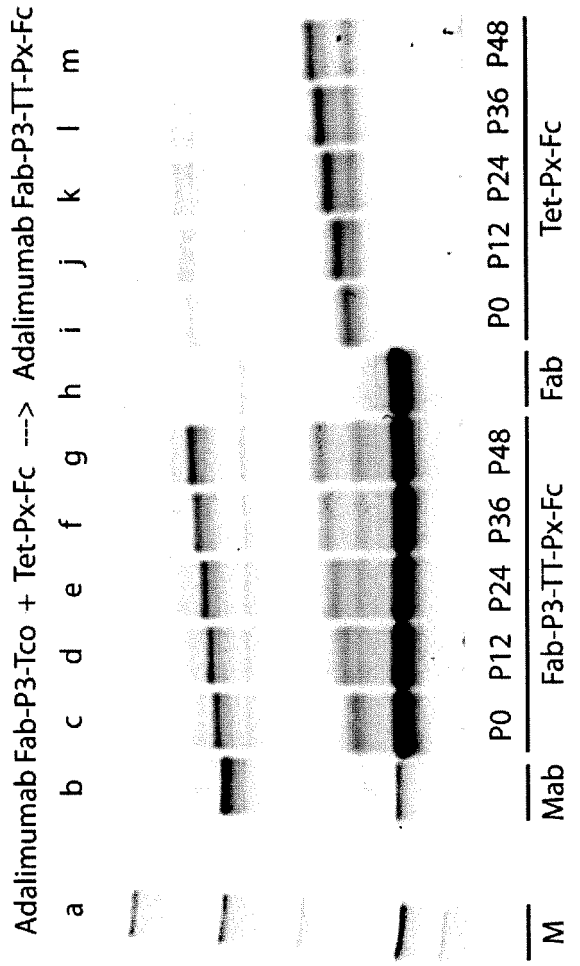

Adalimumab Fab-P3-Tco + Tet-Px-Fc ---> Adalimumab Fab-P3-TT-Px-Fc
a b c d e f g h i j k l m
M
Mab
P0 P12 P24 P36 P48
Fab-P3-TT-Px-Fc
Fab
P0 P12 P24 P36 P48
Tet-Px-Fc

Figure 64

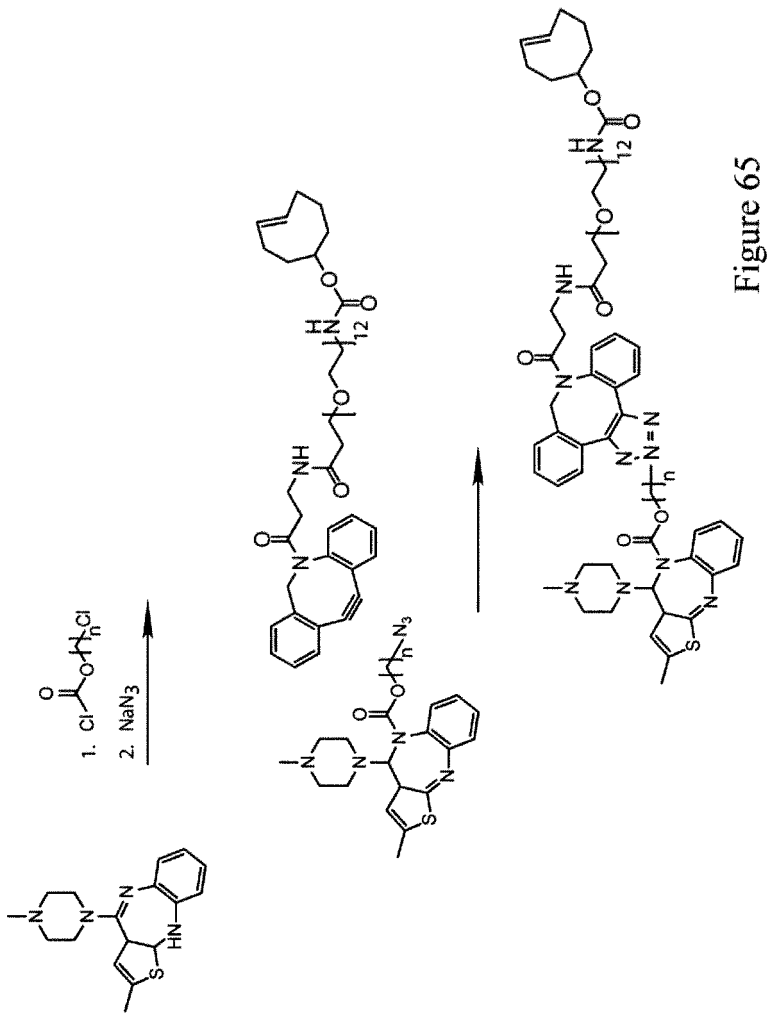


Figure 65

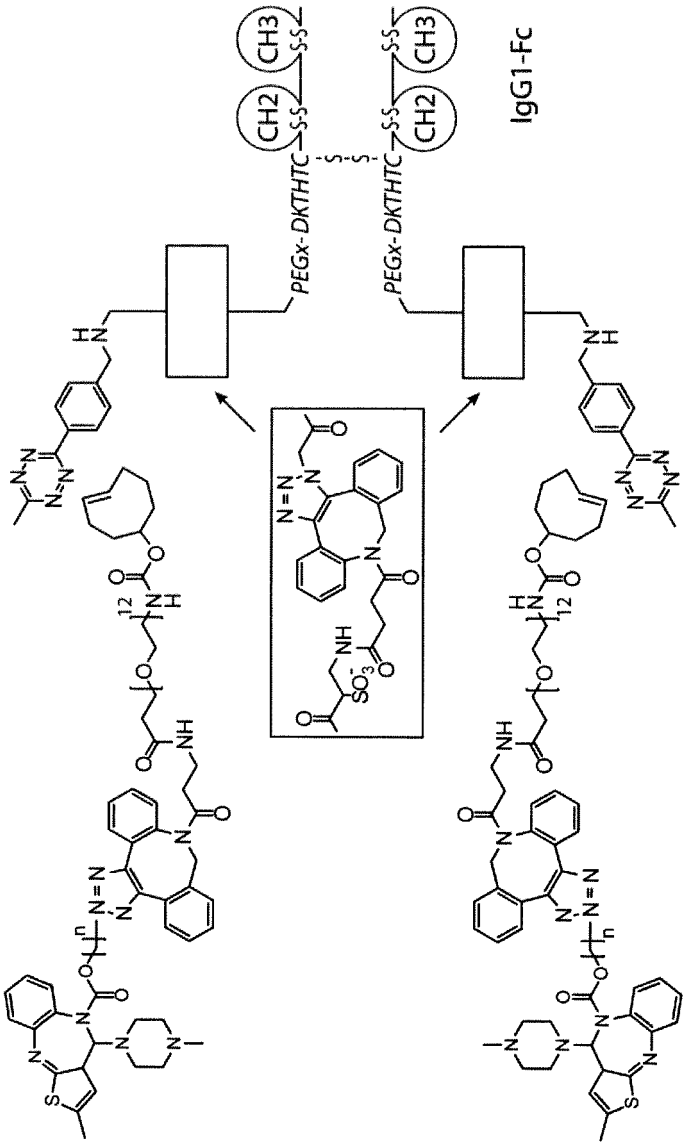


Figure 66

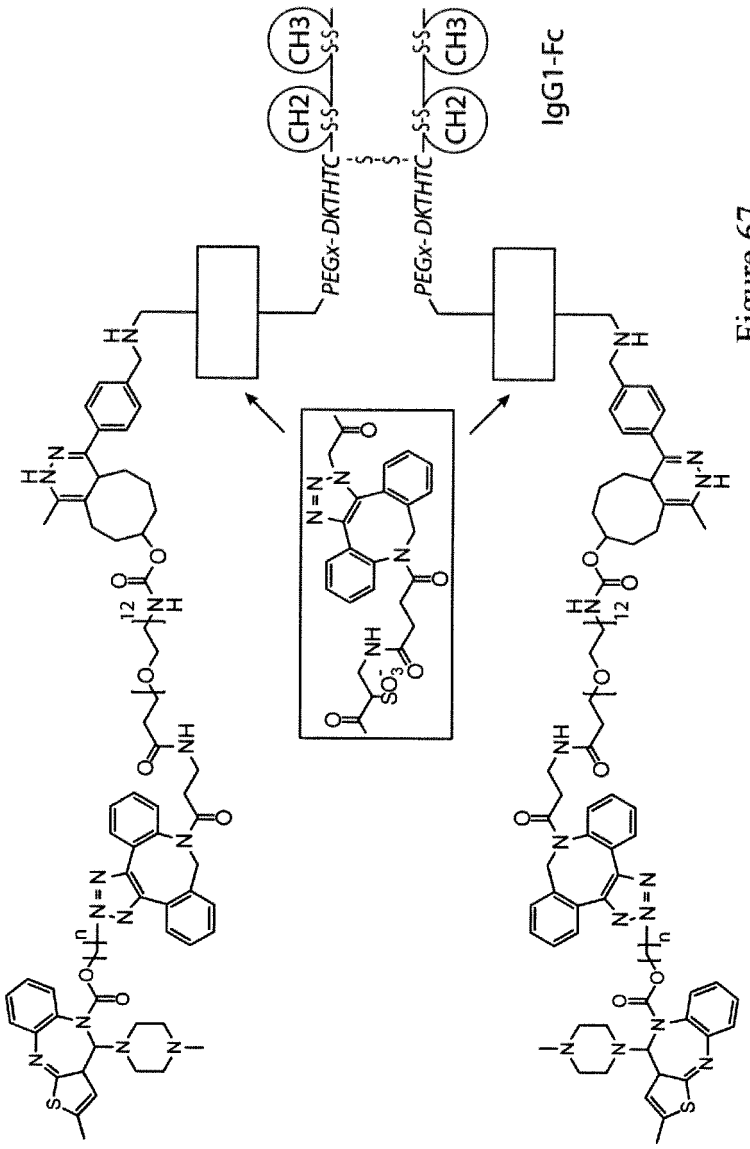


Figure 67