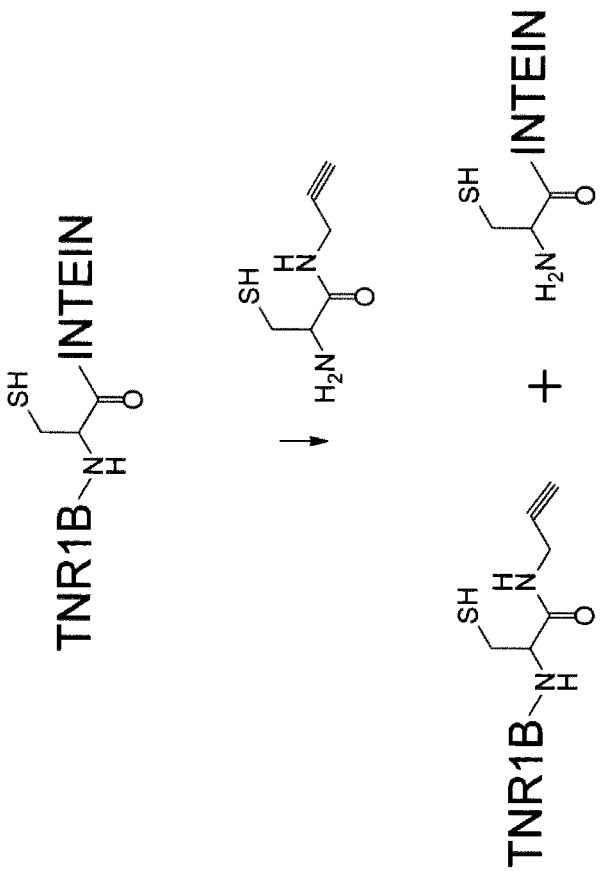


Figure 1

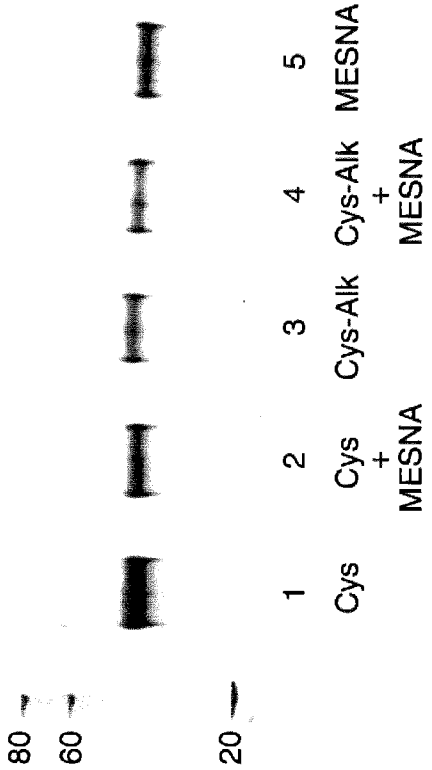


Figure 2

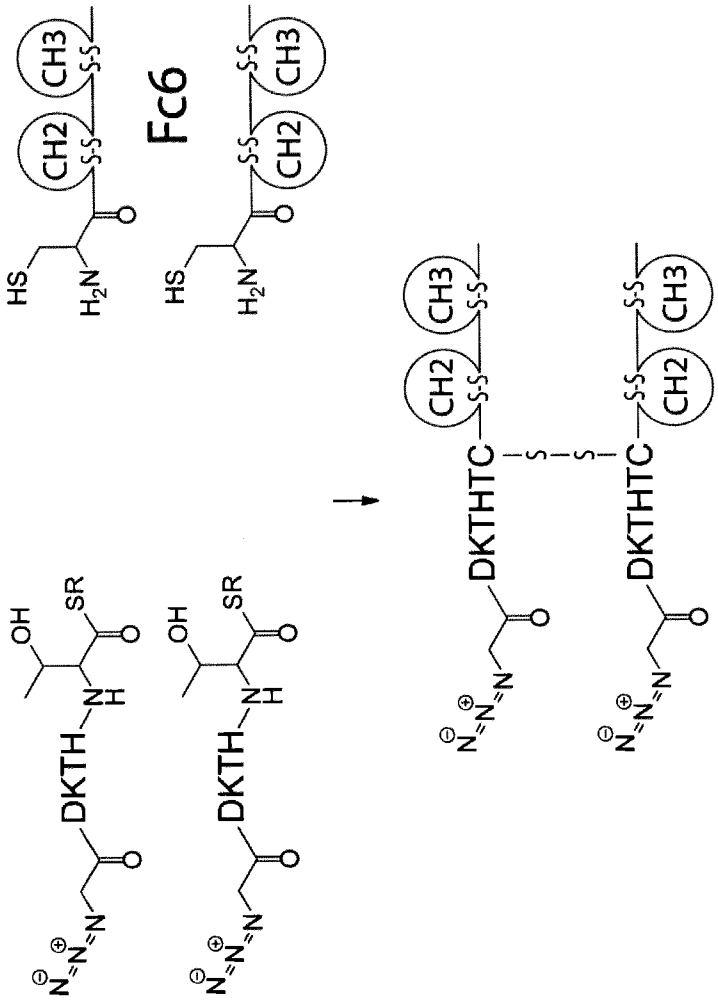


Figure 3

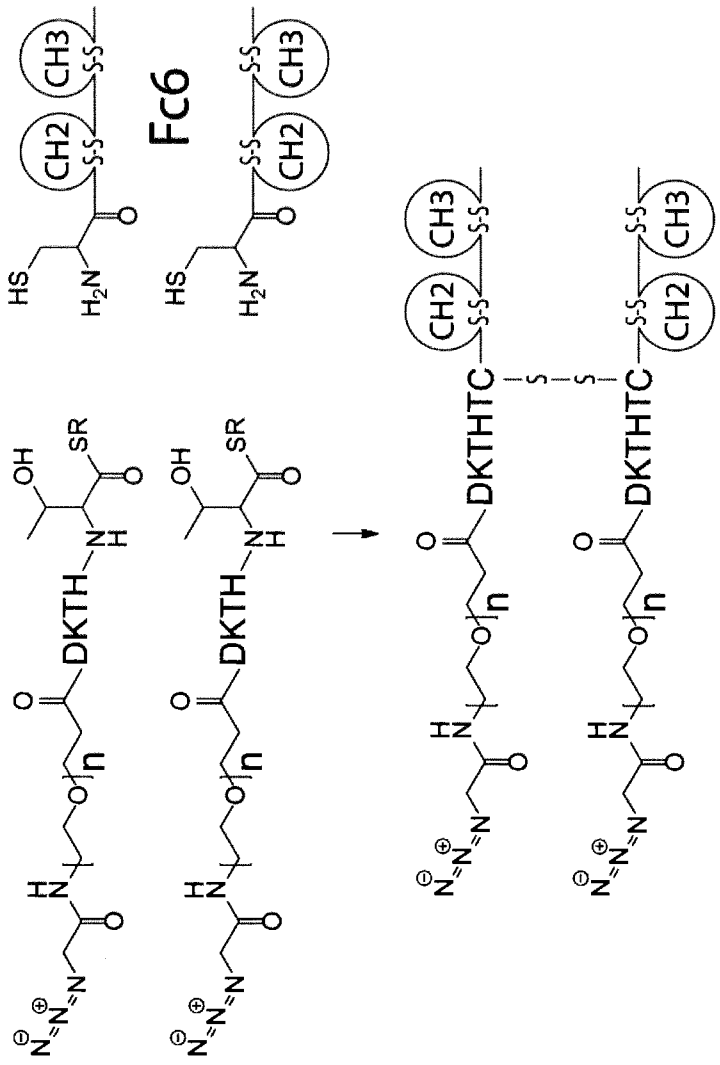


Figure 4

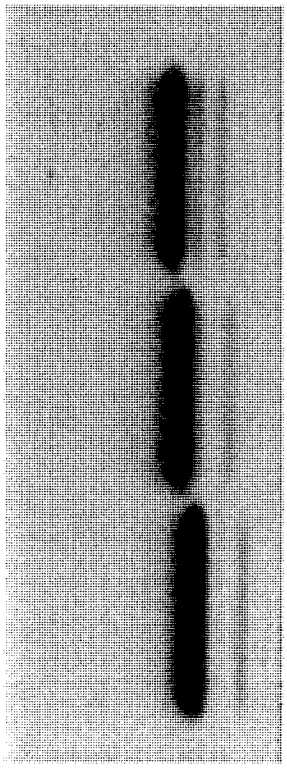


Figure 5

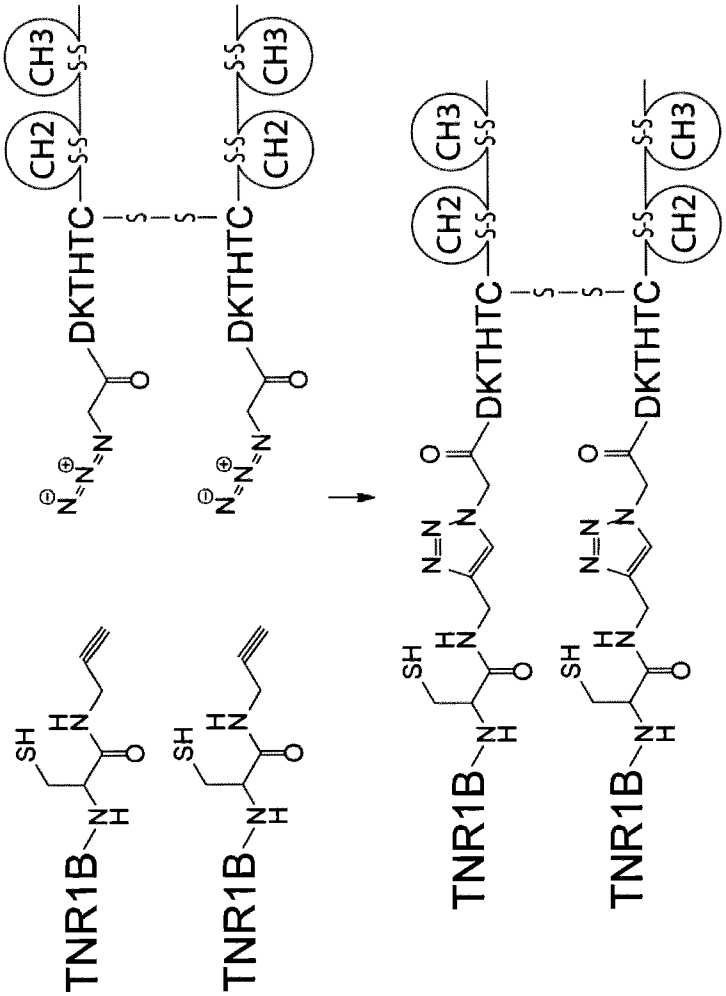


Figure 6

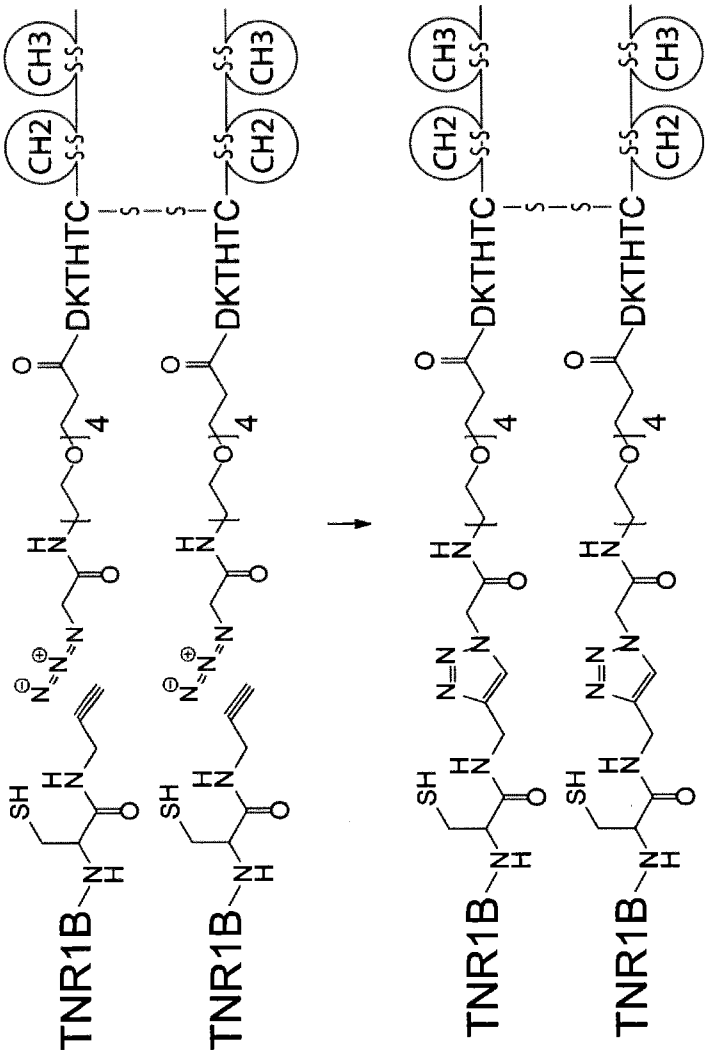


Figure 7

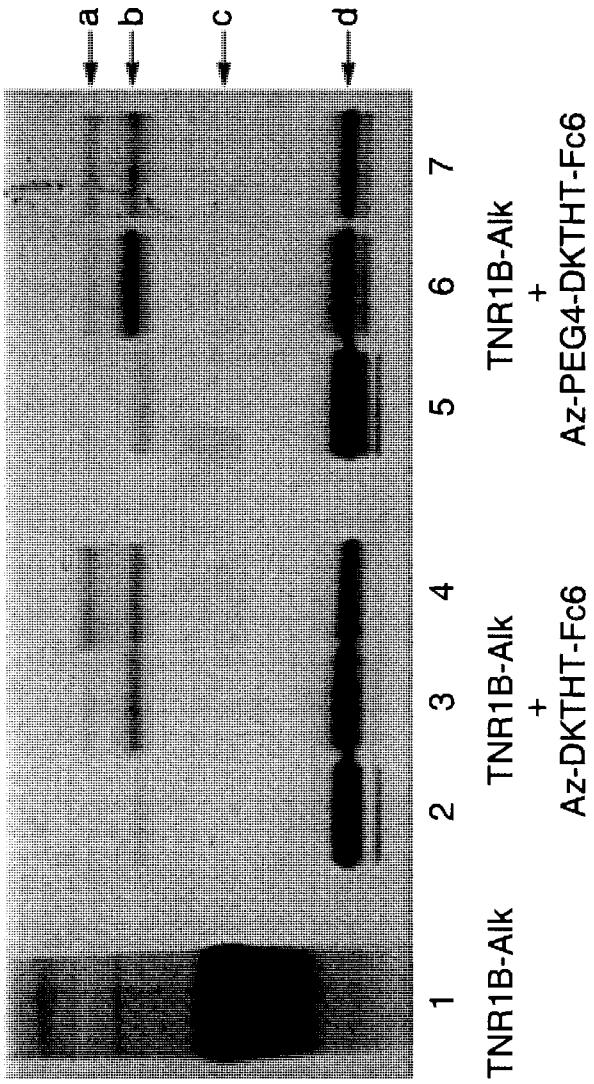

a
b
c
d
1
2
3
4
5
6
7
TNR1B-Alk
TNR1B-Alk
+
Az-DKTHT-Fc6
TNR1B-Alk
+
Az-PEG4-DKTHT-Fc6

Figure 8

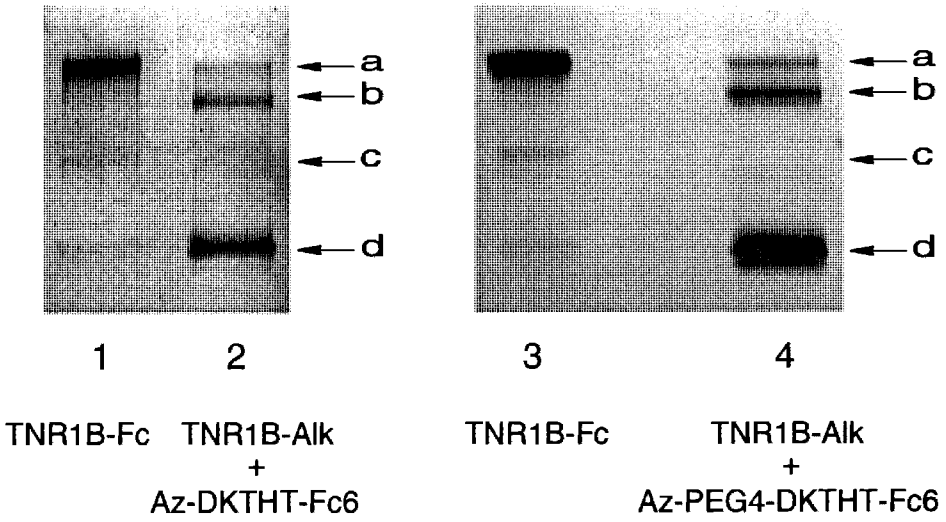

a
b
c
d
a
b
c
d
1
2
3
4
TNR1B-Fc
TNR1B-Alk
+
Az-DKTHT-Fc6
TNR1B-Fc
TNR1B-Alk
+
Az-PEG4-DKTHT-Fc6

Figure 9

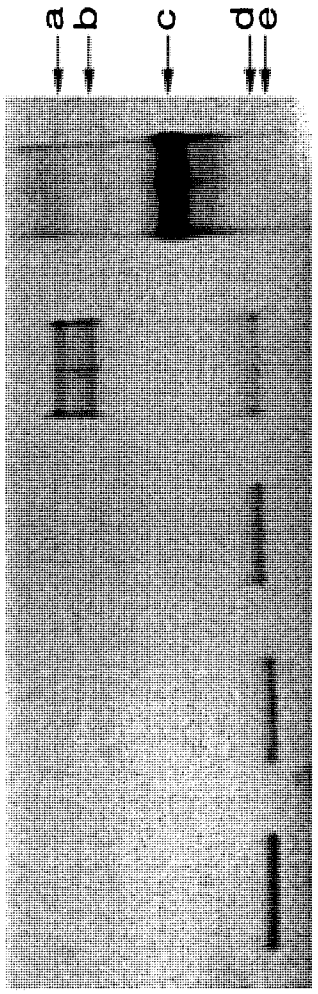

a
b
c
d
e
1
2
3
4
5
Fc6
Fc6 + TNR1B-Alk
Az-DKTHT-Fc6
Az-DKTHT-Fc6 + TNR1B-Alk
TNR1B-Alk

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

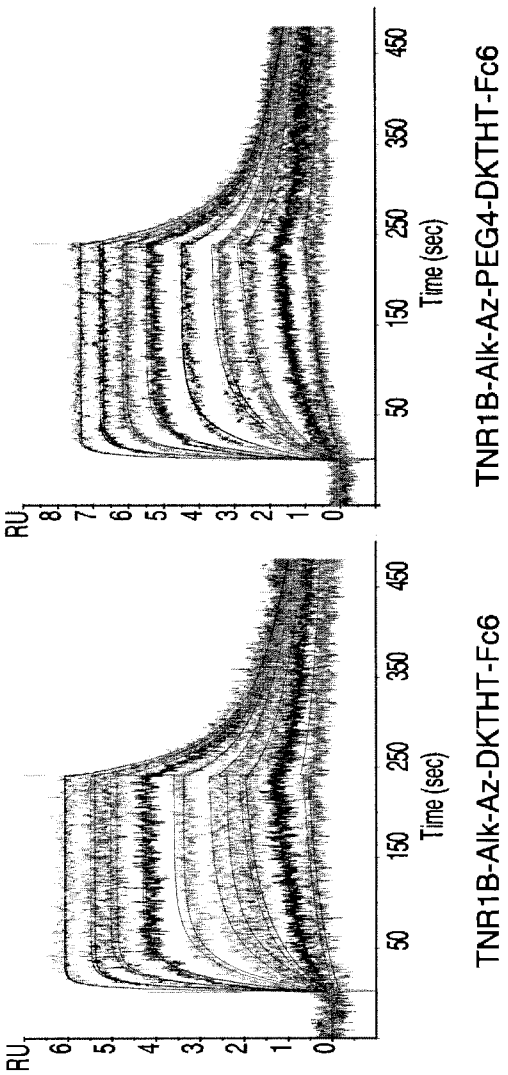


Figure 12

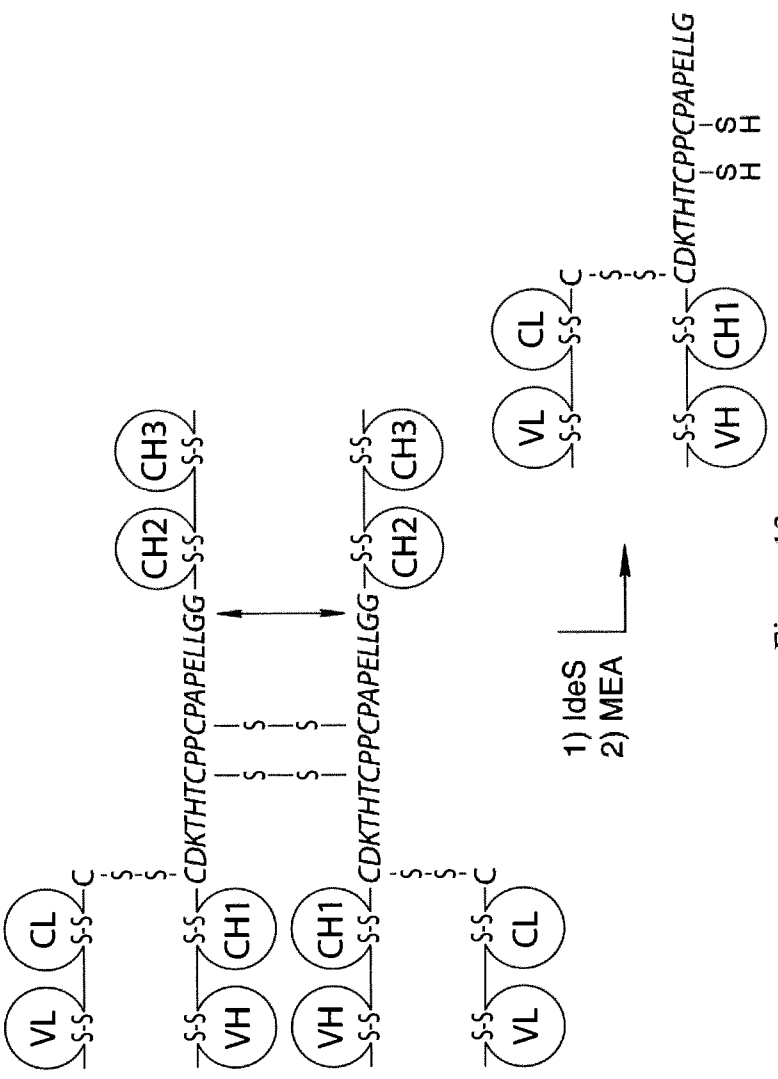


Figure 13

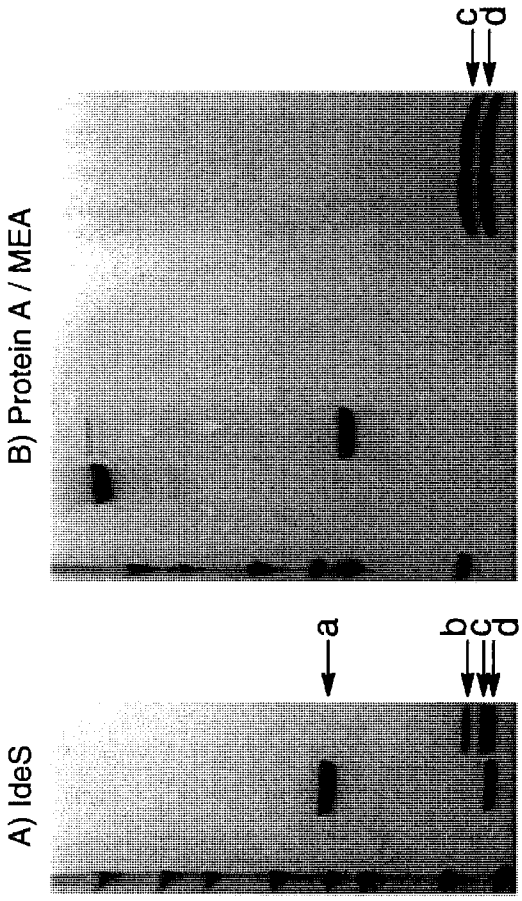

A) IdeS
B) Protein A / MEA
a
b
c
d
c
d
1 2
Mab Fab'2 + Fc'
3 4
Fab'2 Fab' (-DTT)
5 6
Fab'2 Fab' (+DTT)
Adalimumab-Fab'

Figure 14

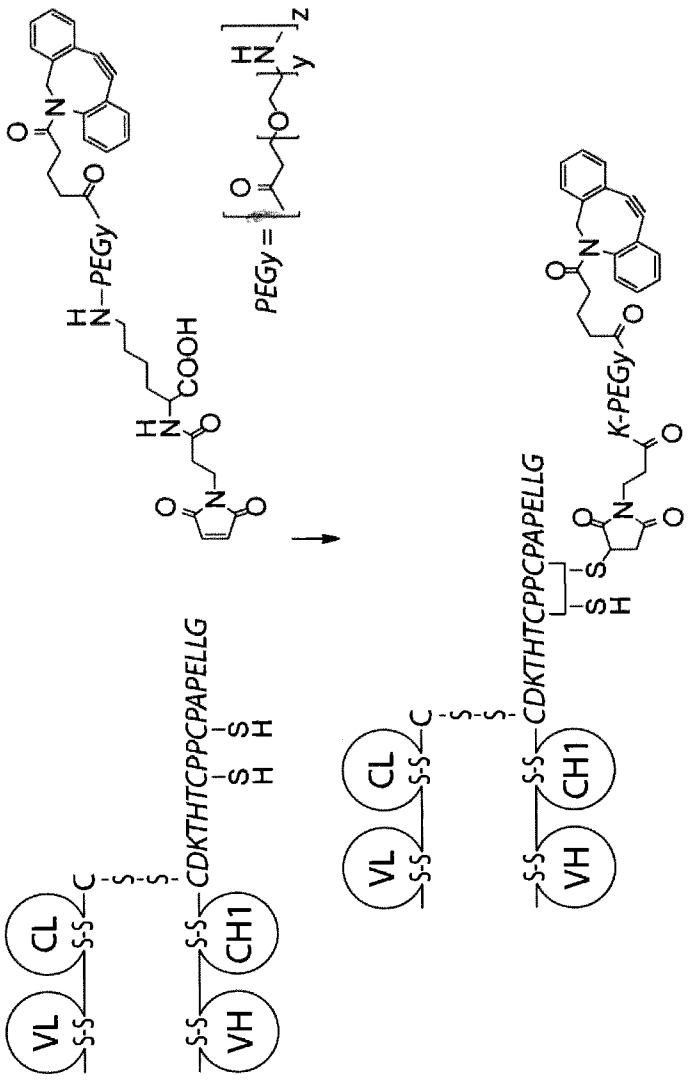


Figure 15

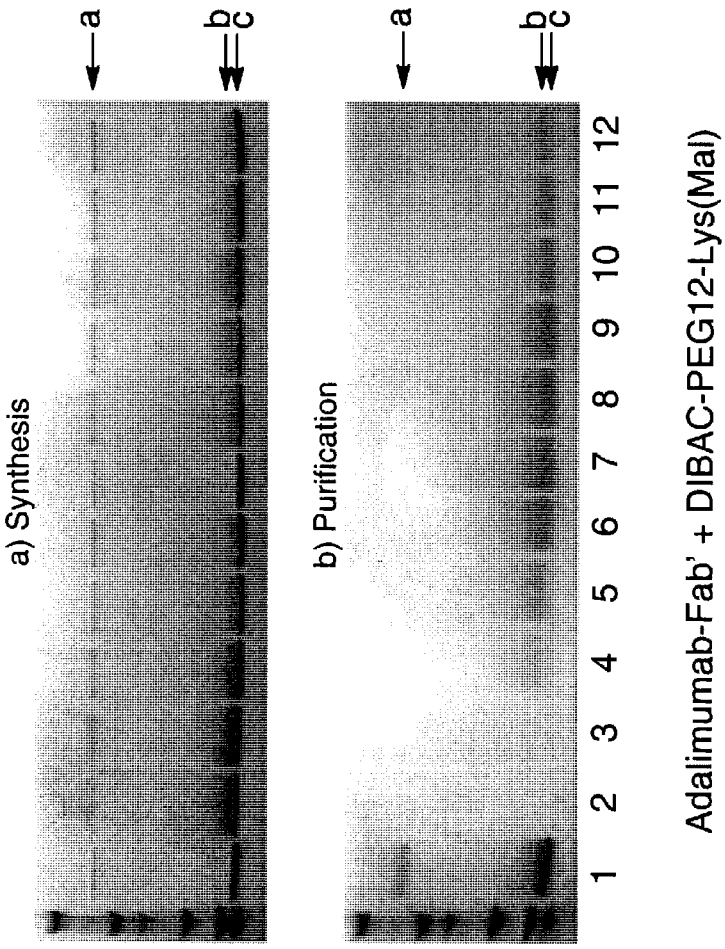
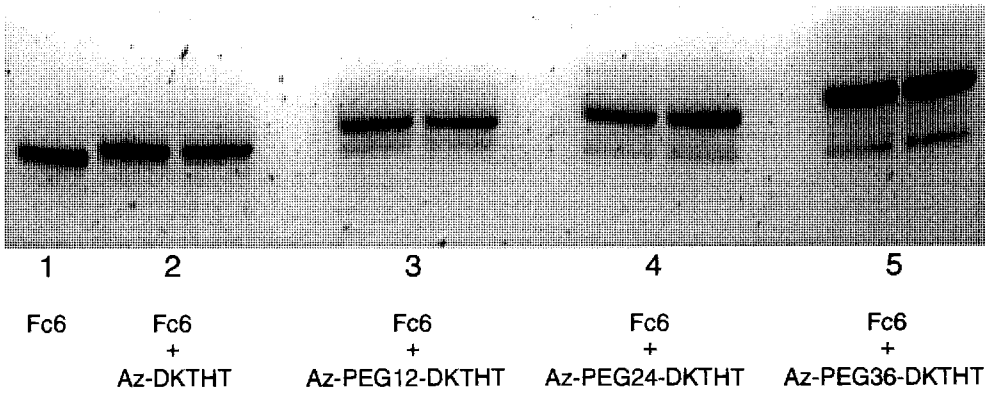

a) Synthesis
b) Purification
a
b
c
1 2 3 4 5 6 7 8 9 10 11 12
Adalimumab-Fab' + DIBAC-PEG12-Lys(Mal)

Figure 16



Az-PEGx-DKTHT-Fc6

Figure 17

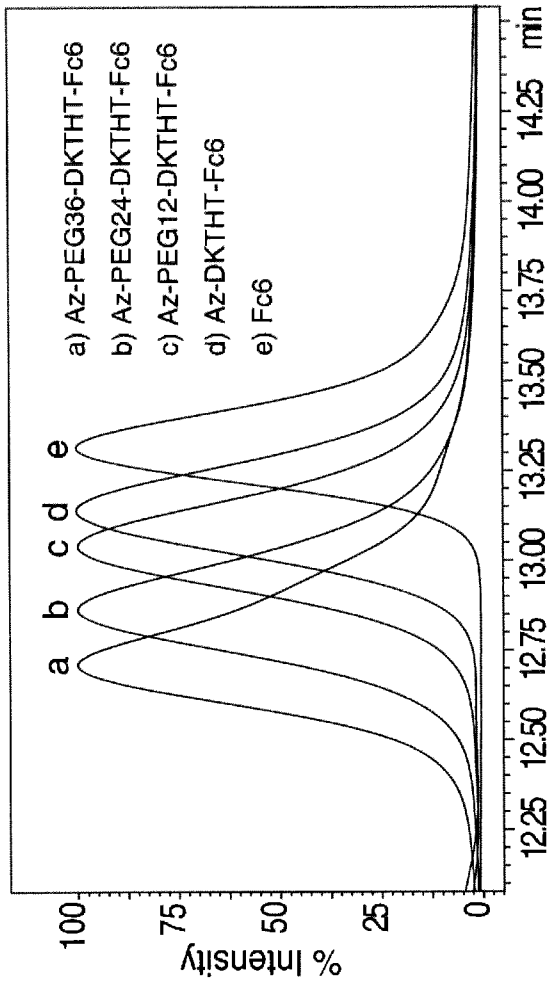

a) Az-PEG36-DKTHT-Fc6
b) Az-PEG24-DKTHT-Fc6
c) Az-PEG12-DKTHT-Fc6
d) Az-DKTHT-Fc6
e) Fc6
a b c d e
% Intensity
100
75
50
25
0
12.25 12.50 12.75 13.00 13.25 13.50 13.75 14.00 14.25 min
retention time

Figure 18

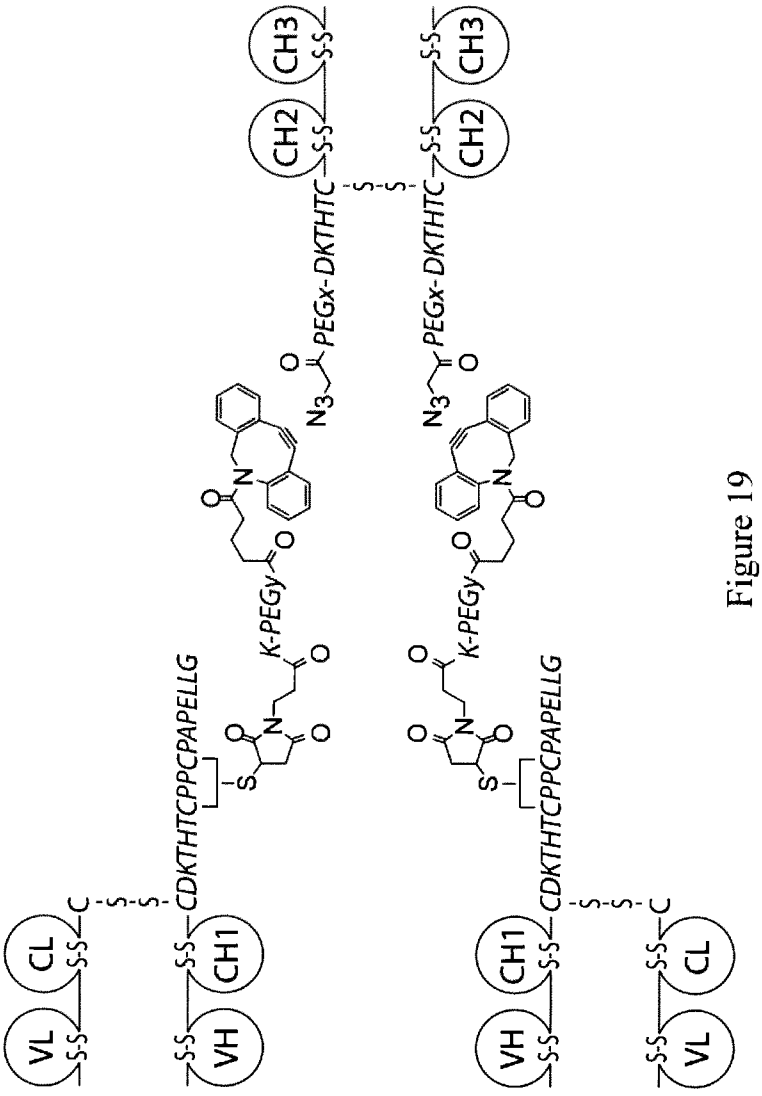

VL
CL
C
S-S
CDKTHTCPPCPAPELLG
VH
CH1
K-PEGy
PEGx-DKTHTC
CH2
CH3
N3

Figure 19

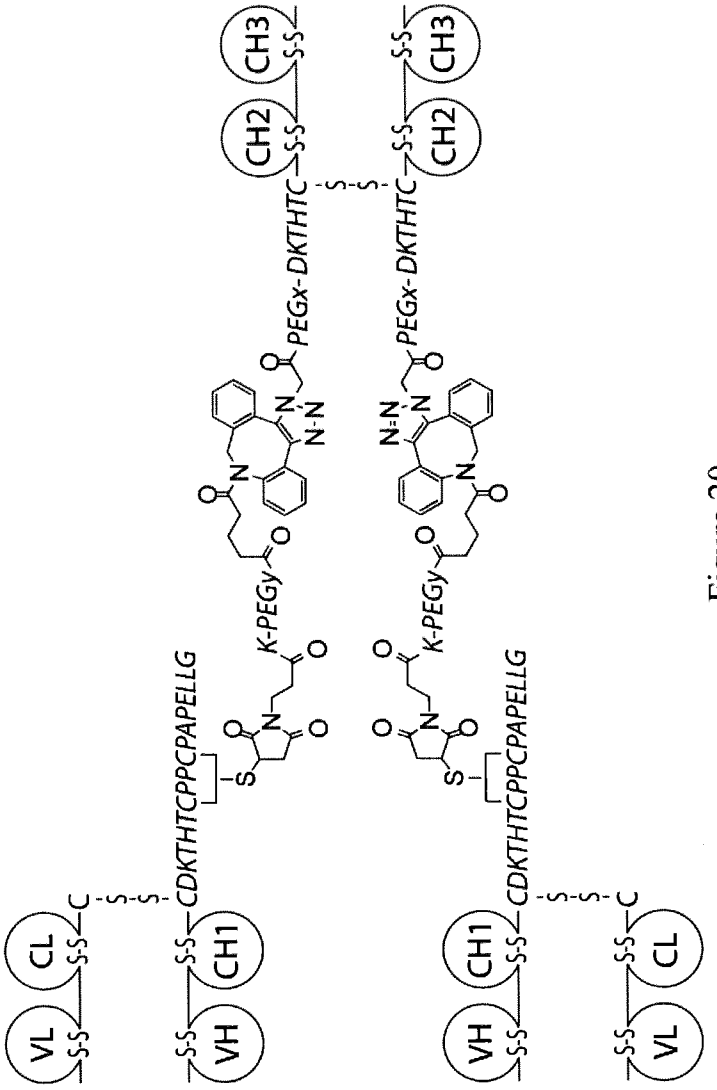
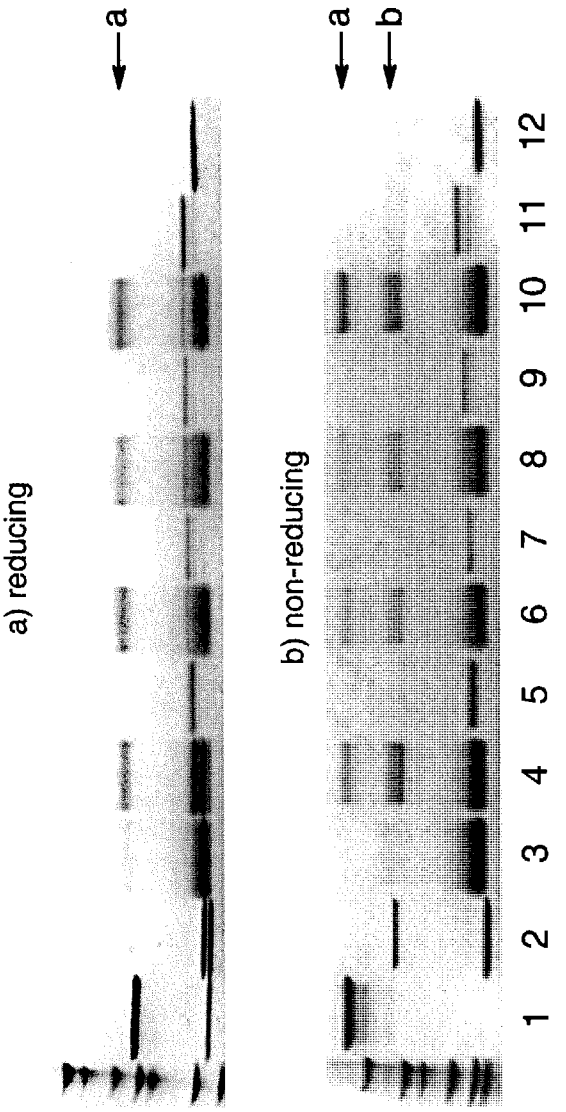
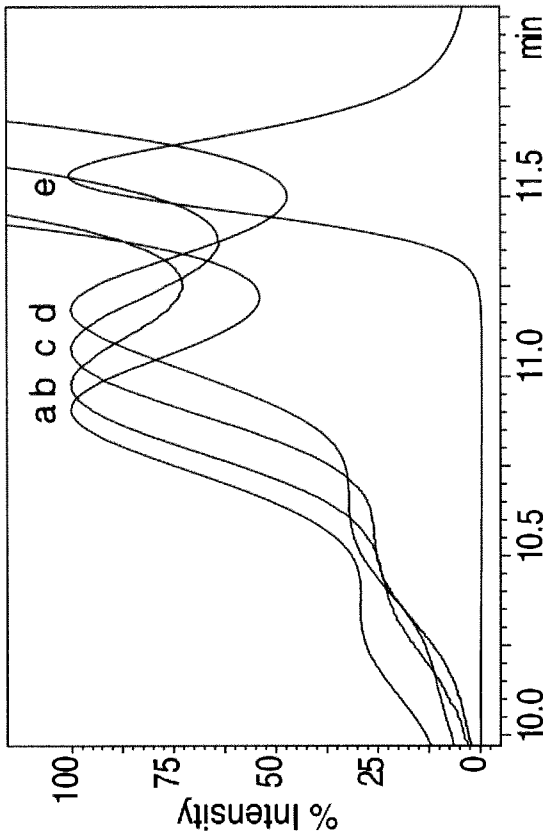

VL
CL
C
S-S
CDKTHTCPPCPAPELLG
VH
CH1
K-PEGy
PEGx-DKTHTC
CH2
CH3
N=N

Figure 20



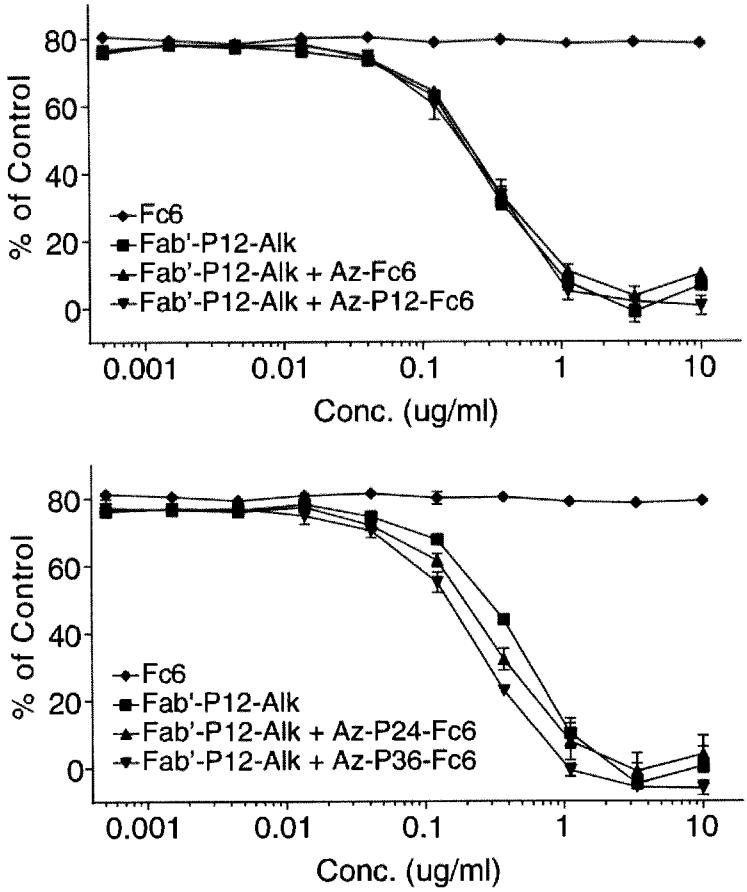
Adalimumab-Fab'-PEGy-Alk + Az-PEGx-DKTHT-Fc6

Figure 21



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

Figure 22



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

Figure 23

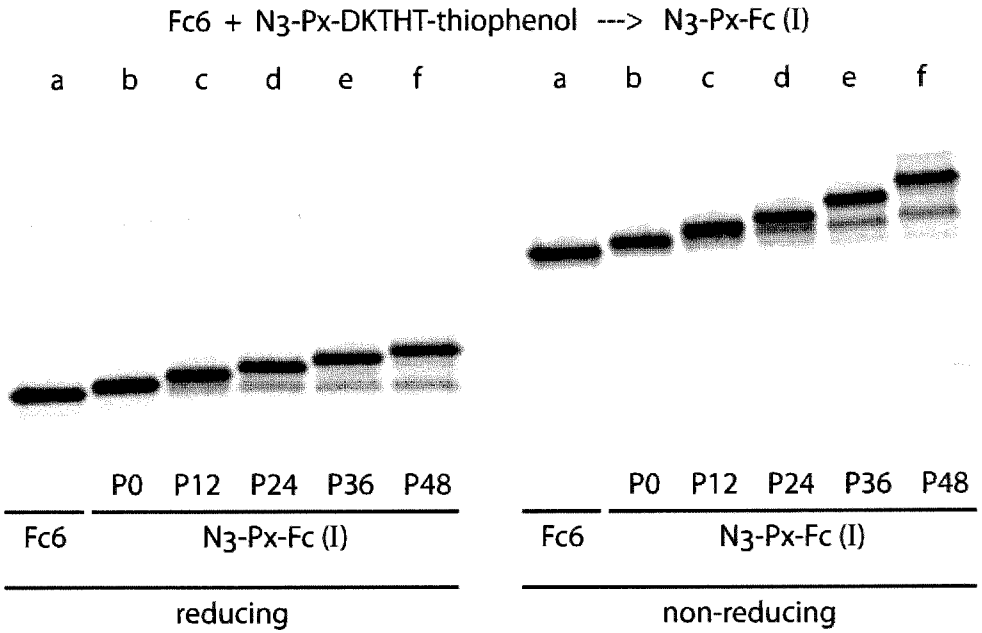

Fc6 + N3-Px-DKTHT-thiophenol ---> N3-Px-Fc (I)
a b c d e f
a b c d e f
P0 P12 P24 P36 P48
P0 P12 P24 P36 P48
Fc6
N3-Px-Fc (I)
Fc6
N3-Px-Fc (I)
reducing
non-reducing

Figure 24

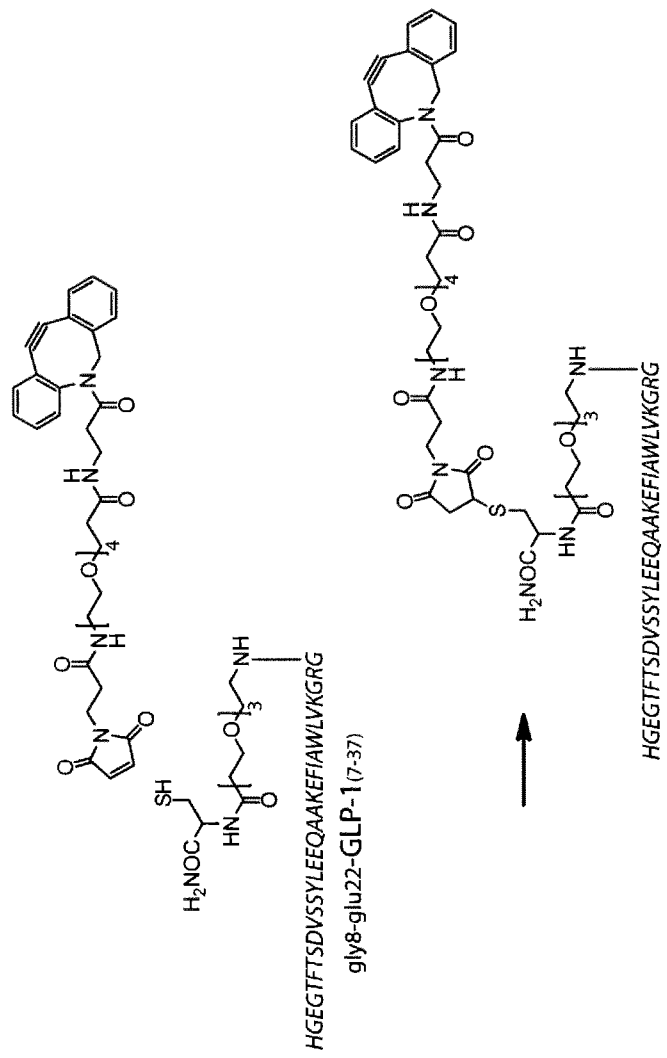

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)

Figure 25

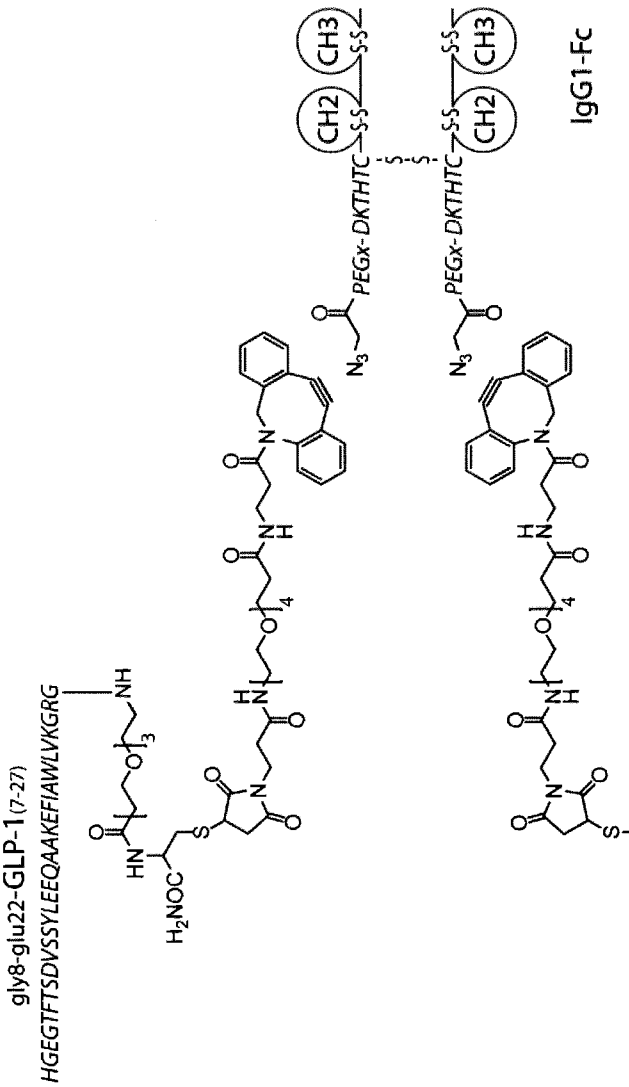

gly8-glu22-GLP-1(7-27)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
H2NOC
PEGx-DKTHTC
N3
CH2
CH3
S-S
IgG1-Fc
gly8-glu22-GLP-1(7-37)

Figure 26

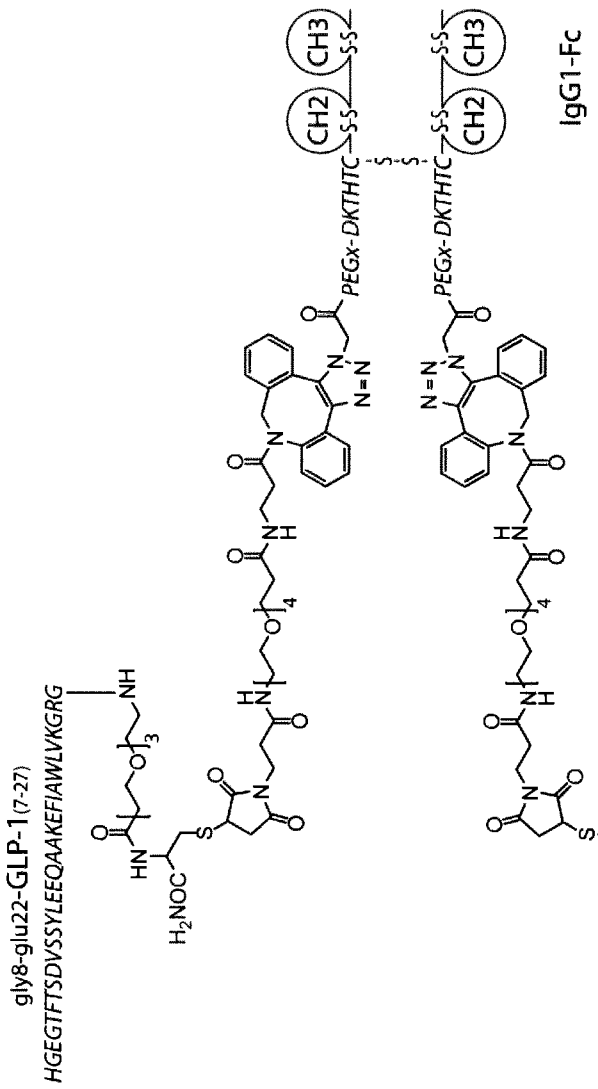

gly8-glu22-GLP-1(7-27)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
IgG1-Fc
gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG

Figure 27

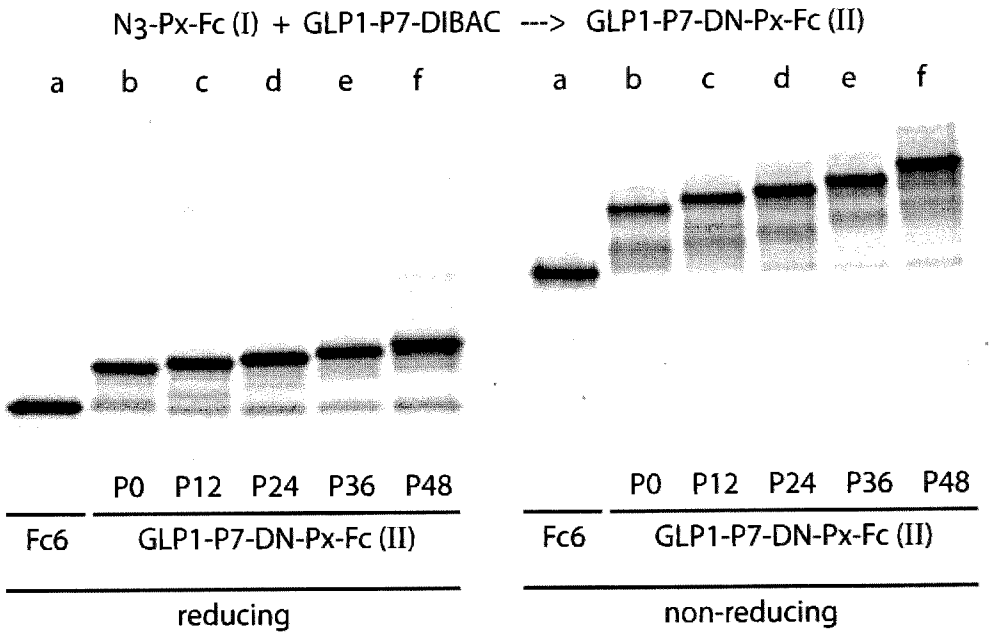

N3-Px-Fc (I) + GLP1-P7-DIBAC ---> GLP1-P7-DN-Px-Fc (II)
a b c d e f
a b c d e f
P0 P12 P24 P36 P48
Fc6
GLP1-P7-DN-Px-Fc (II)
reducing
P0 P12 P24 P36 P48
Fc6
GLP1-P7-DN-Px-Fc (II)
non-reducing

Figure 28

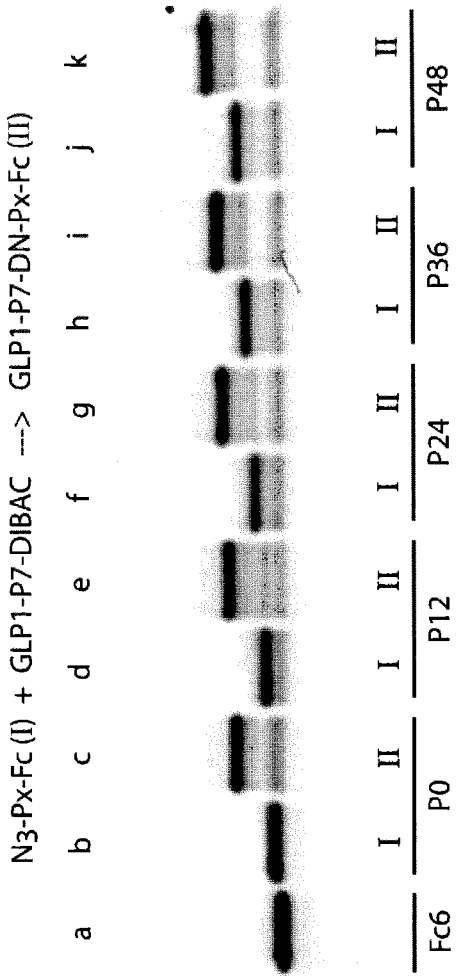

N3-Px-Fc (I) + GLP1-P7-DIBAC ---> GLP1-P7-DN-Px-Fc (II)
a b c d e f g h i j k
I II I II I II I II I II
Fc6 P0 P12 P24 P36 P48

Figure 29

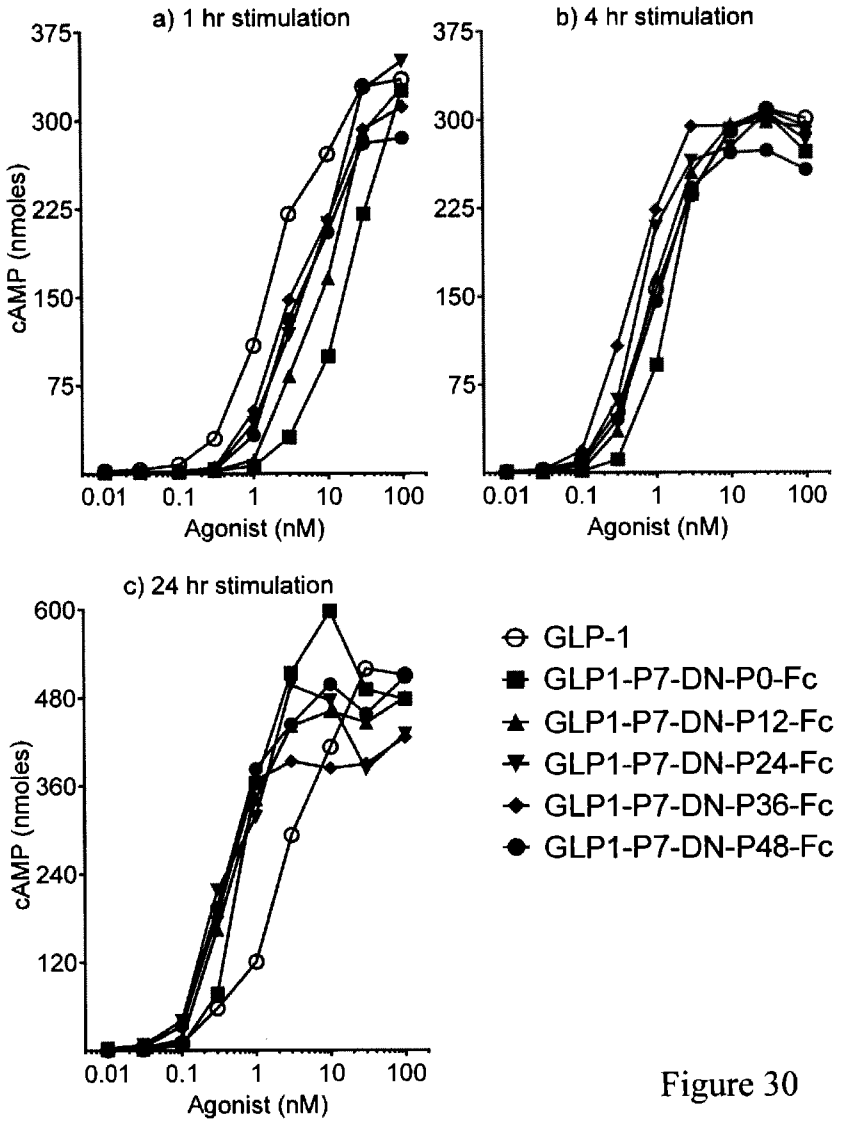

a) 1 hr stimulation
b) 4 hr stimulation
c) 24 hr stimulation
cAMP (nmoles)
Agonist (nM)
0.01
0.1
1
10
100
375
300
225
150
75
600
480
360
240
120
GLP-1
GLP1-P7-DN-P0-Fc
GLP1-P7-DN-P12-Fc
GLP1-P7-DN-P24-Fc
GLP1-P7-DN-P36-Fc
GLP1-P7-DN-P48-Fc

Figure 30

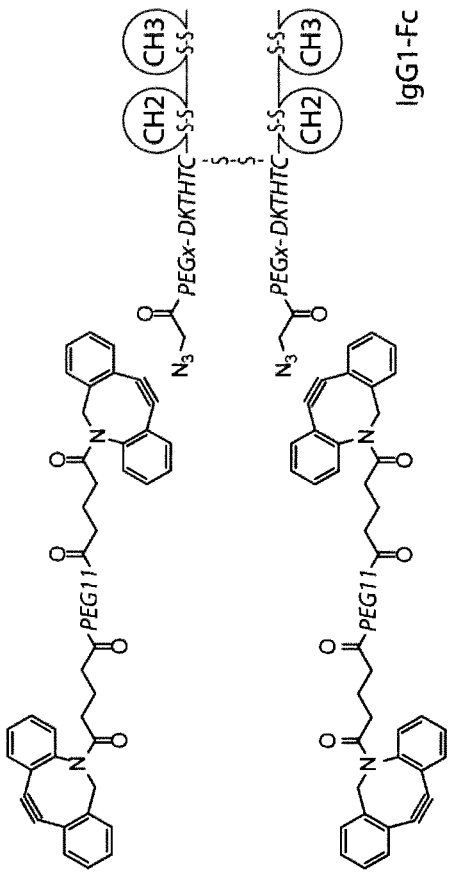

CH3
CH2
S-S
S-S
S-S
S-S
CH2
CH3
PEGx-DKTHTC
PEGx-DKTHTC
N3
N3
PEG11
PEG11
N
N
IgG1-Fc

Figure 31

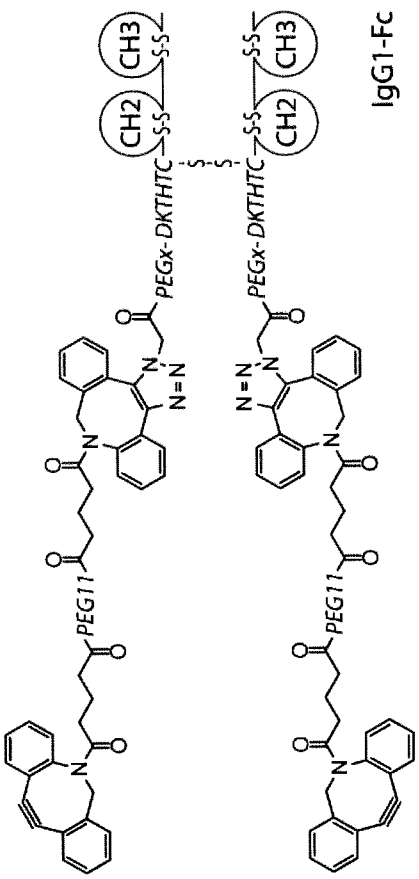


Figure 32

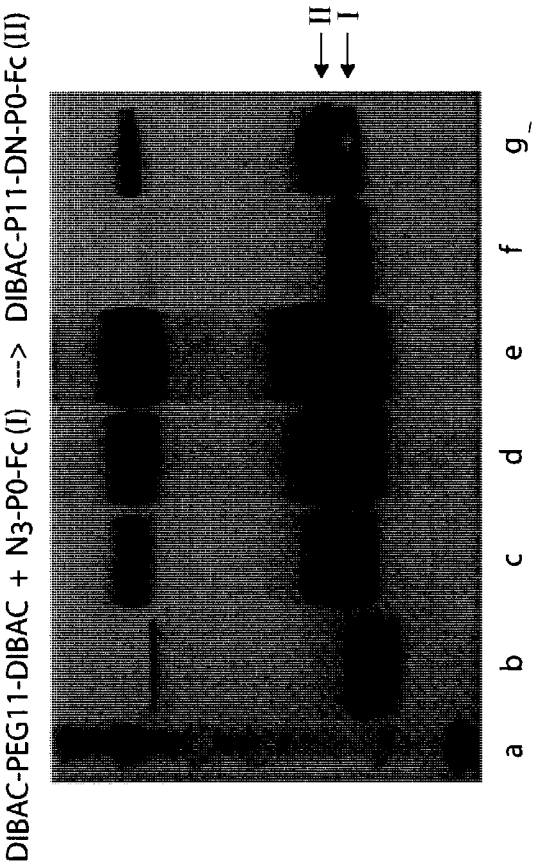


Figure 33

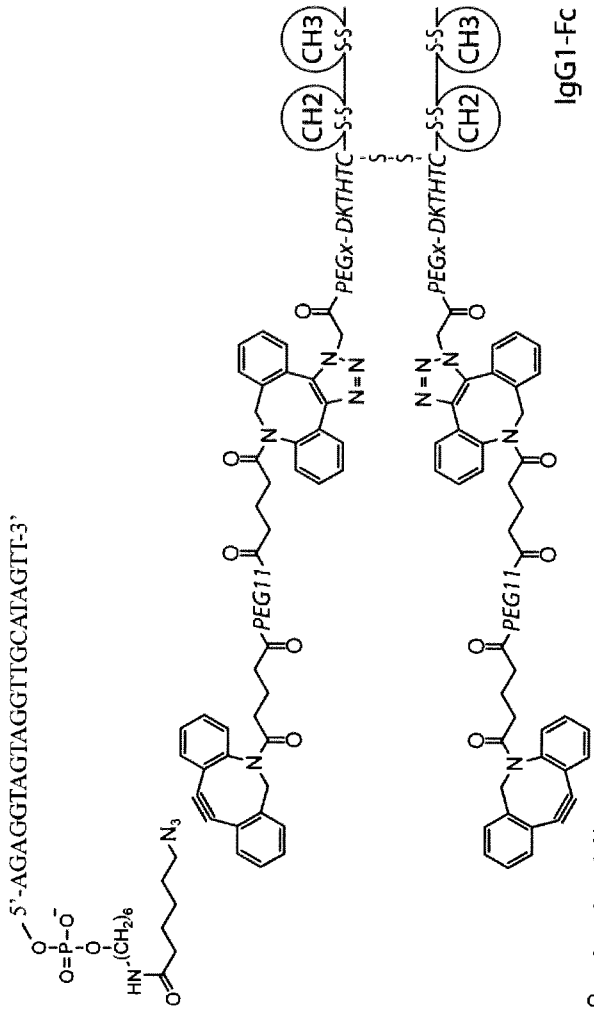

5'-AGAGGTAGTAGGTTGCATAGTT-3'
PEG11
PEGx-DKTHTC
CH2
CH3
IgG1-Fc
5'-AGAGGTAGTAGGTTGCATAGTT-3'

Figure 34

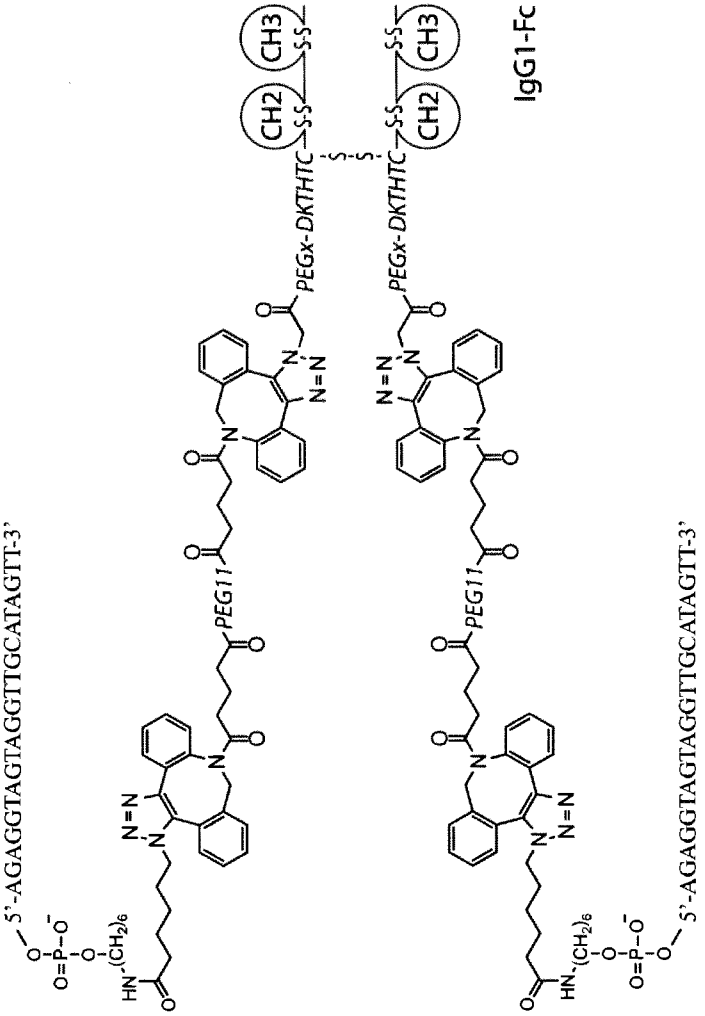

5'-AGAGGTAGTAGGTTGCATAGTT-3'
PEG11
PEGx-DKTHTC
CH2
CH3
IgG1-Fc

Figure 35

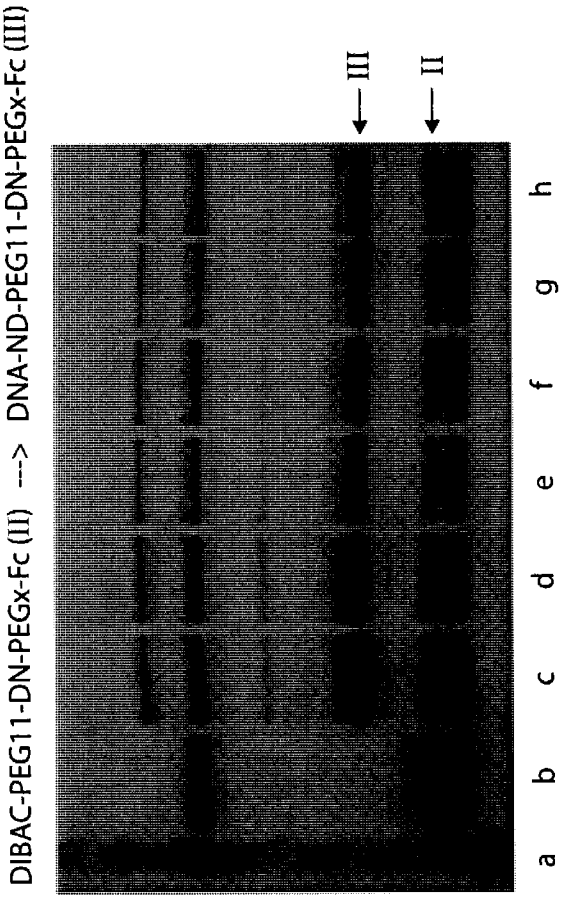

N3-DNA + DIBAC-PEG11-DN-PEGx-Fc (II) --> DNA-ND-PEG11-DN-PEGx-Fc (III)
III
II
a
b
c
d
e
f
g
h

Figure 36

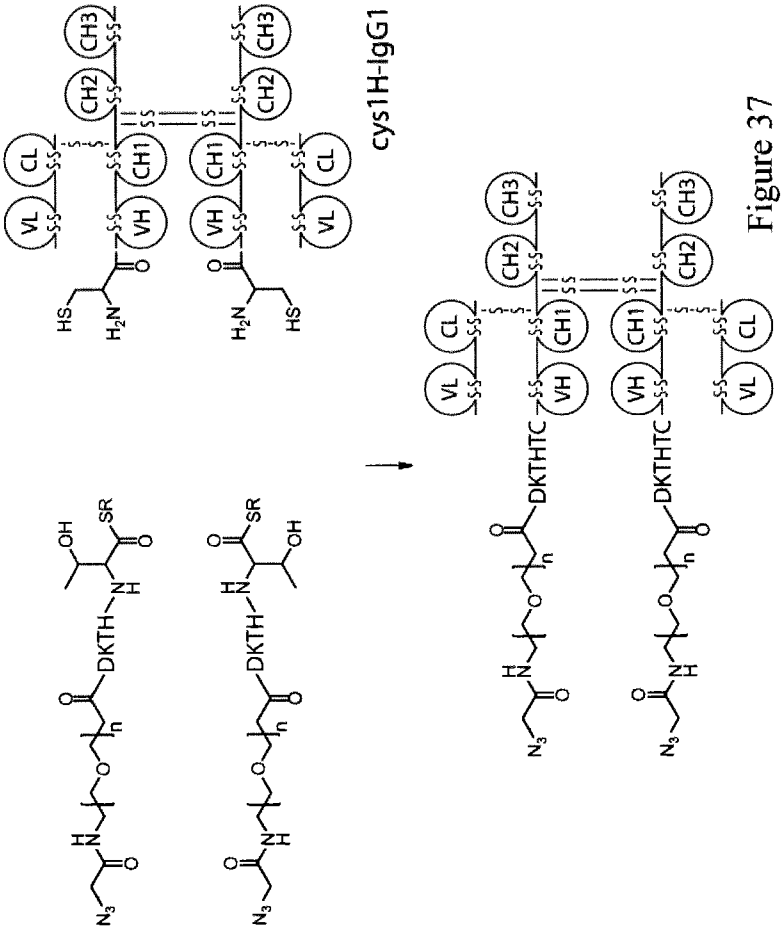


Figure 37

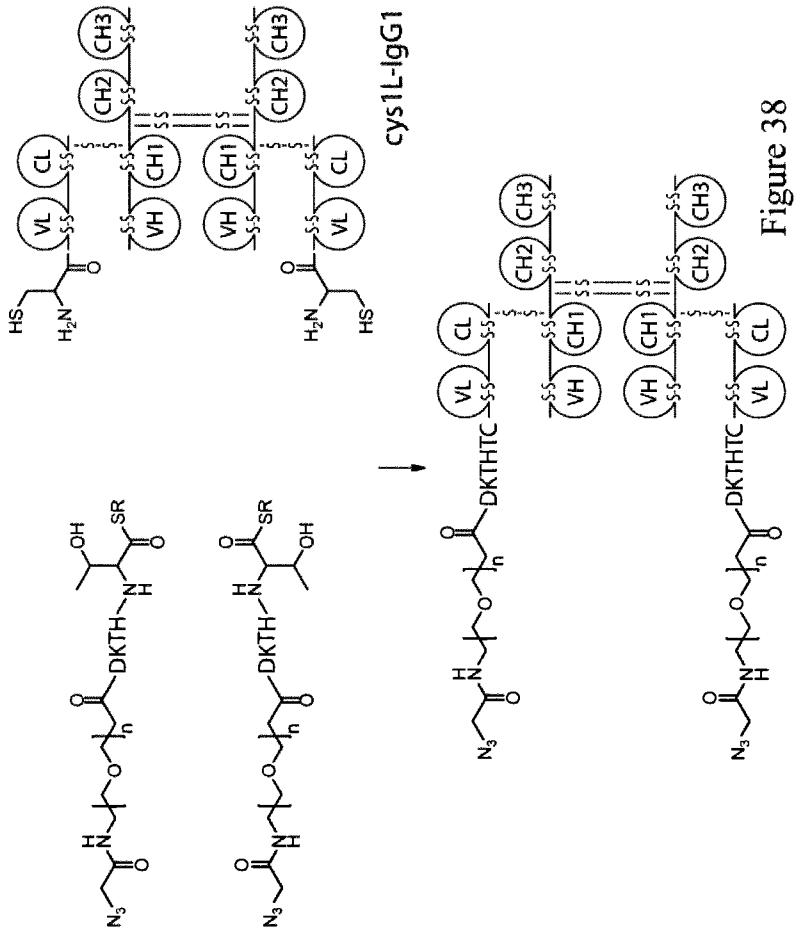


Figure 38

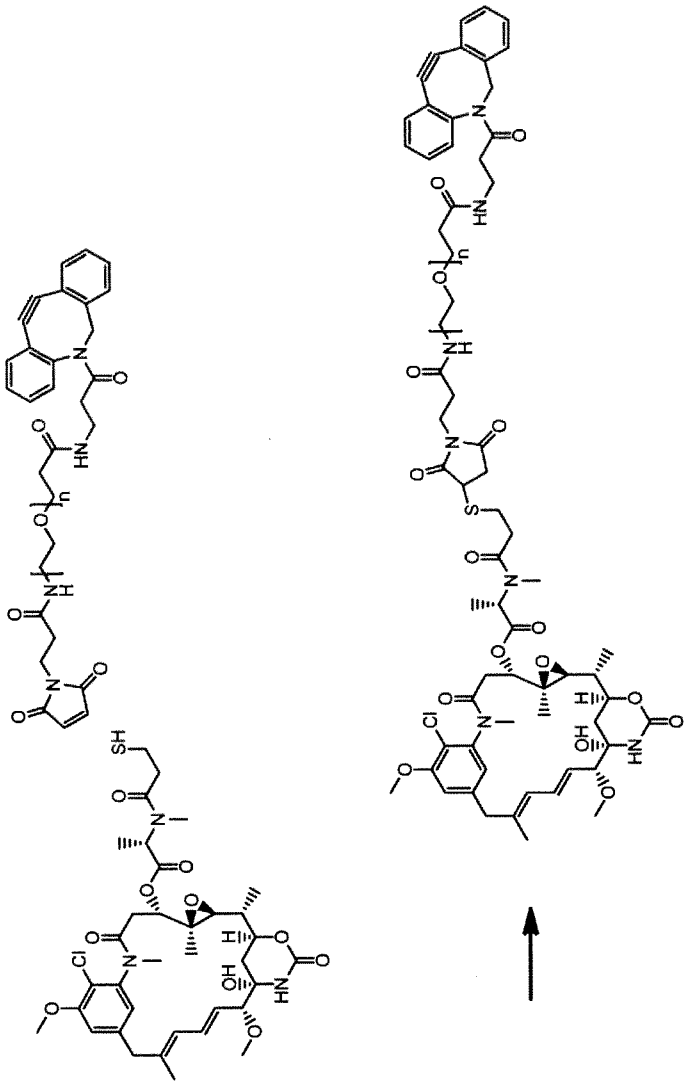


Figure 39

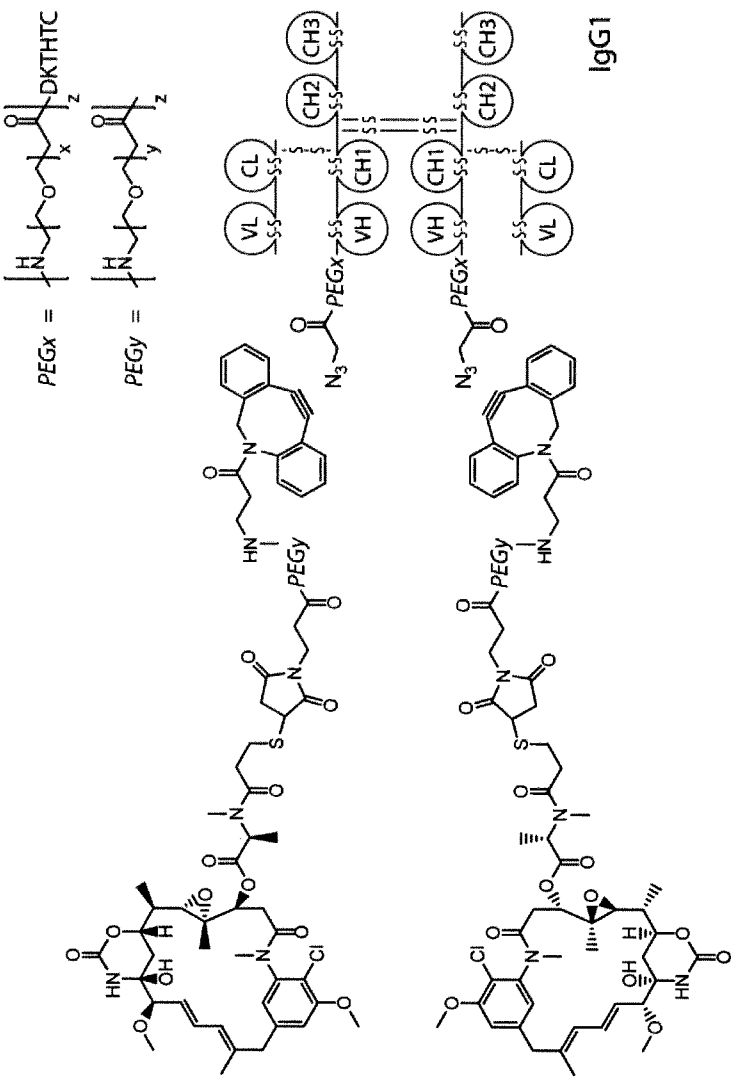

PEGx =
PEGy =
DKTHTC
IgG1
VL
CL
VH
CH1
CH2
CH3
PEGx
PEGy
N3
HN
S
O
N
Cl
OH
HO

Figure 40

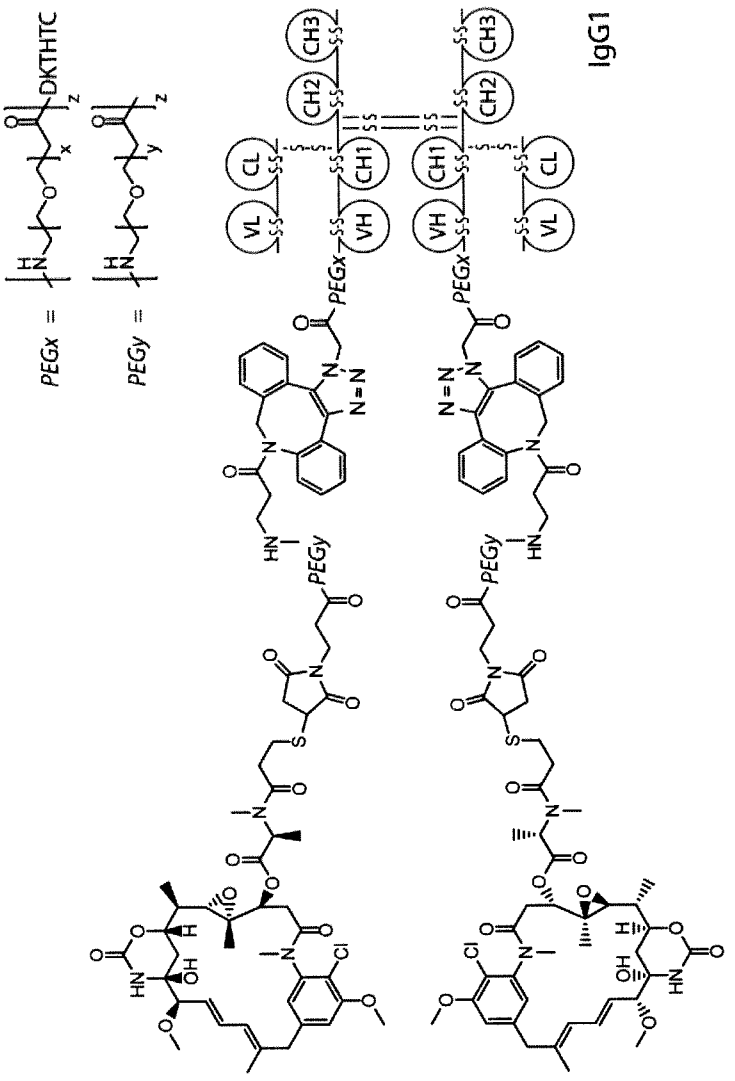


Figure 41

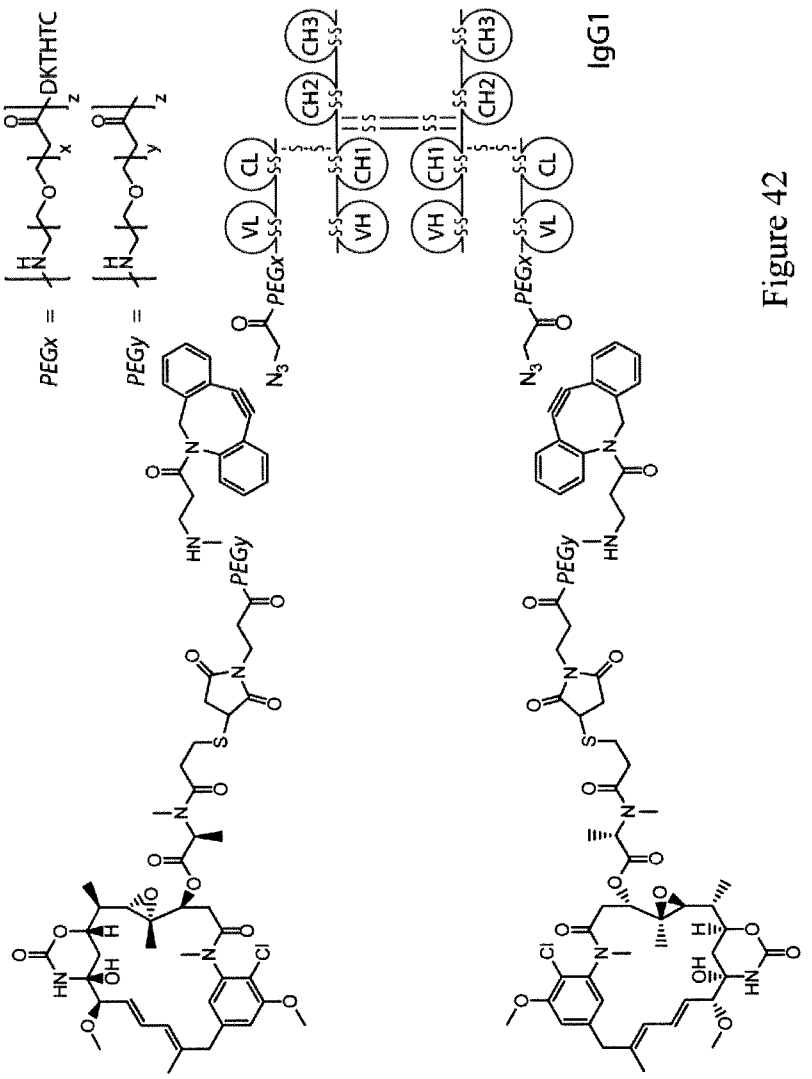


Figure 42

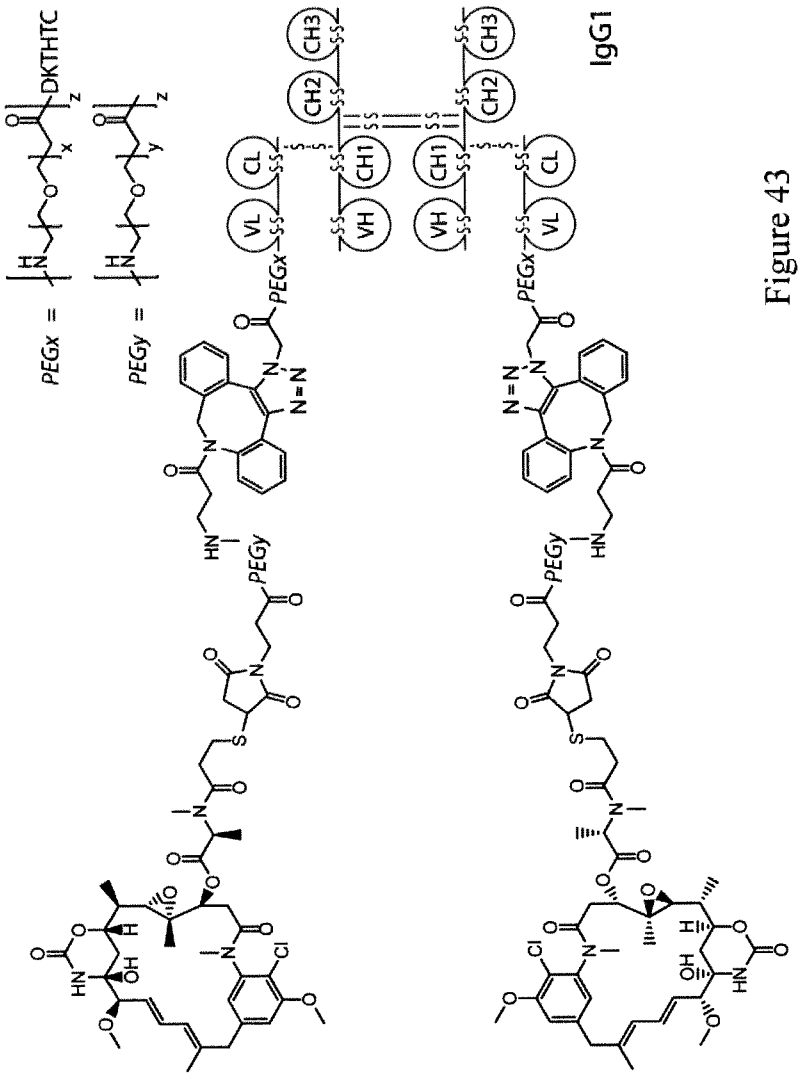


Figure 43

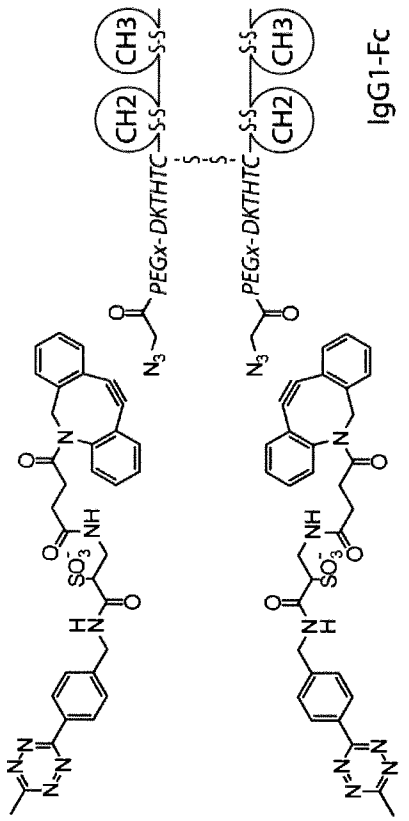


Figure 44

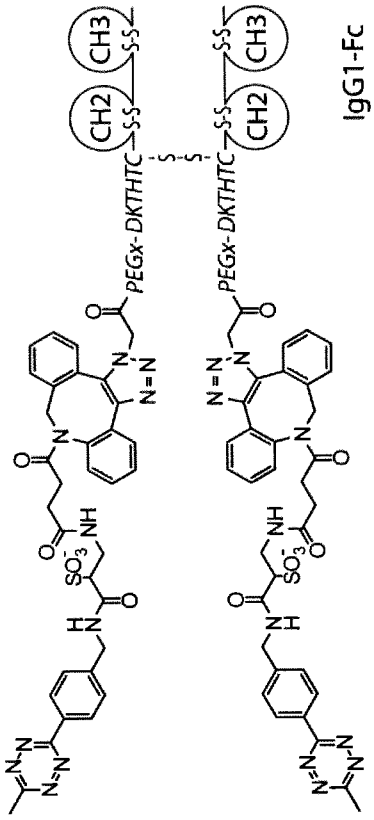


Figure 45

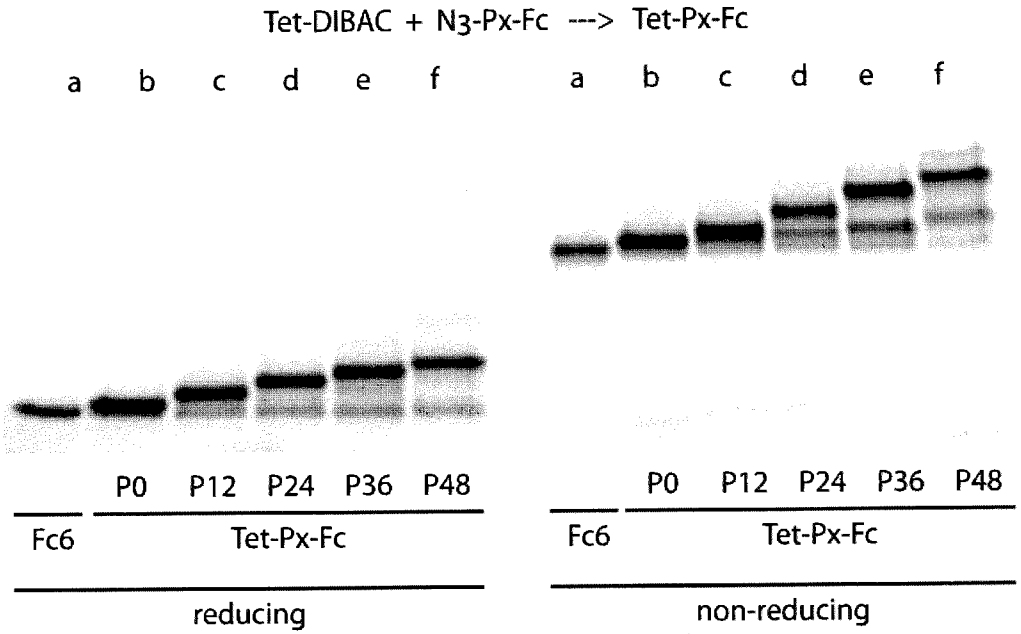

Tet-DIBAC + N3-Px-Fc ---> Tet-Px-Fc
a b c d e f
a b c d e f
P0 P12 P24 P36 P48
Fc6
Tet-Px-Fc
reducing
P0 P12 P24 P36 P48
Fc6
Tet-Px-Fc
non-reducing

Figure 46

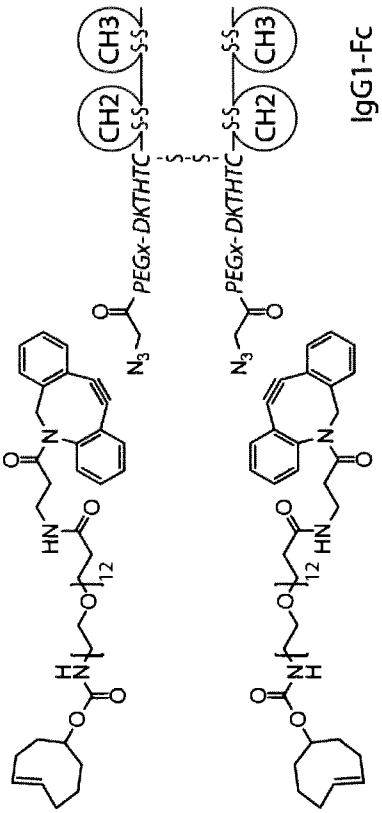


Figure 47

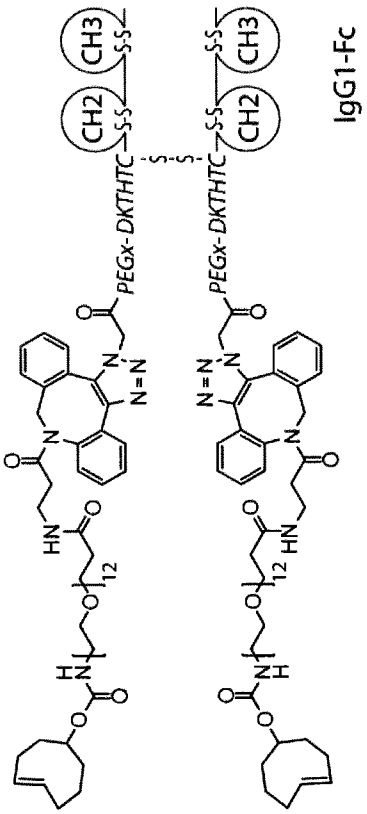


Figure 48

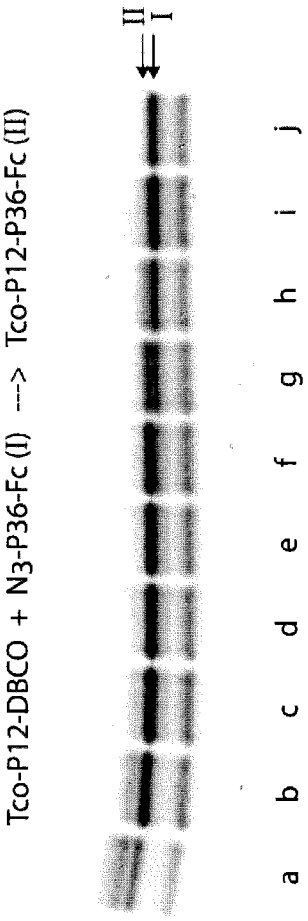


Figure 49

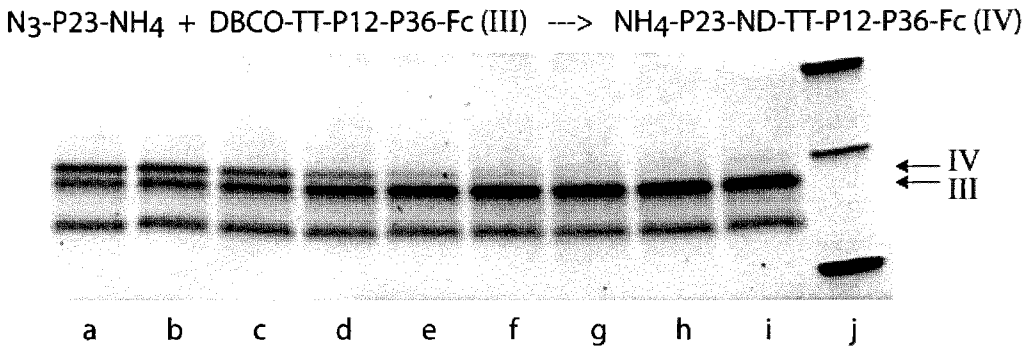

N3-P23-NH4 + DBCO-TT-P12-P36-Fc (III) ---> NH4-P23-ND-TT-P12-P36-Fc (IV)
IV
III
a
b
c
d
e
f
g
h
i
j

Figure 50

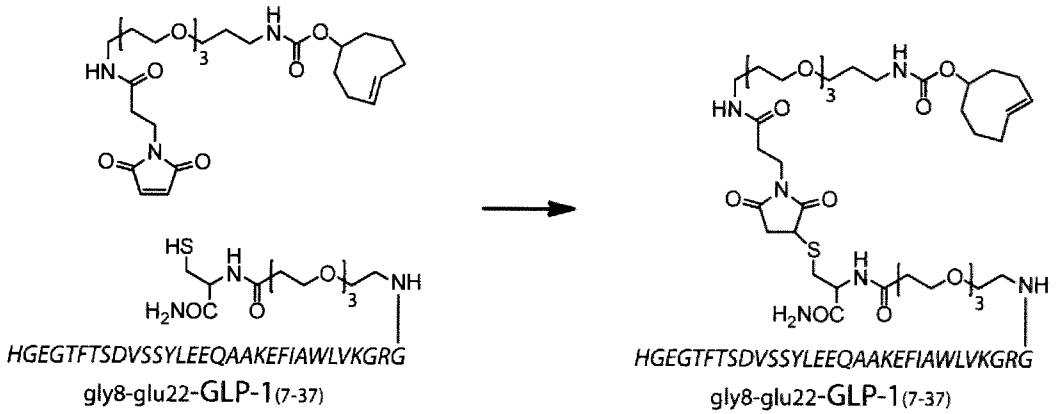

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)

Figure 51

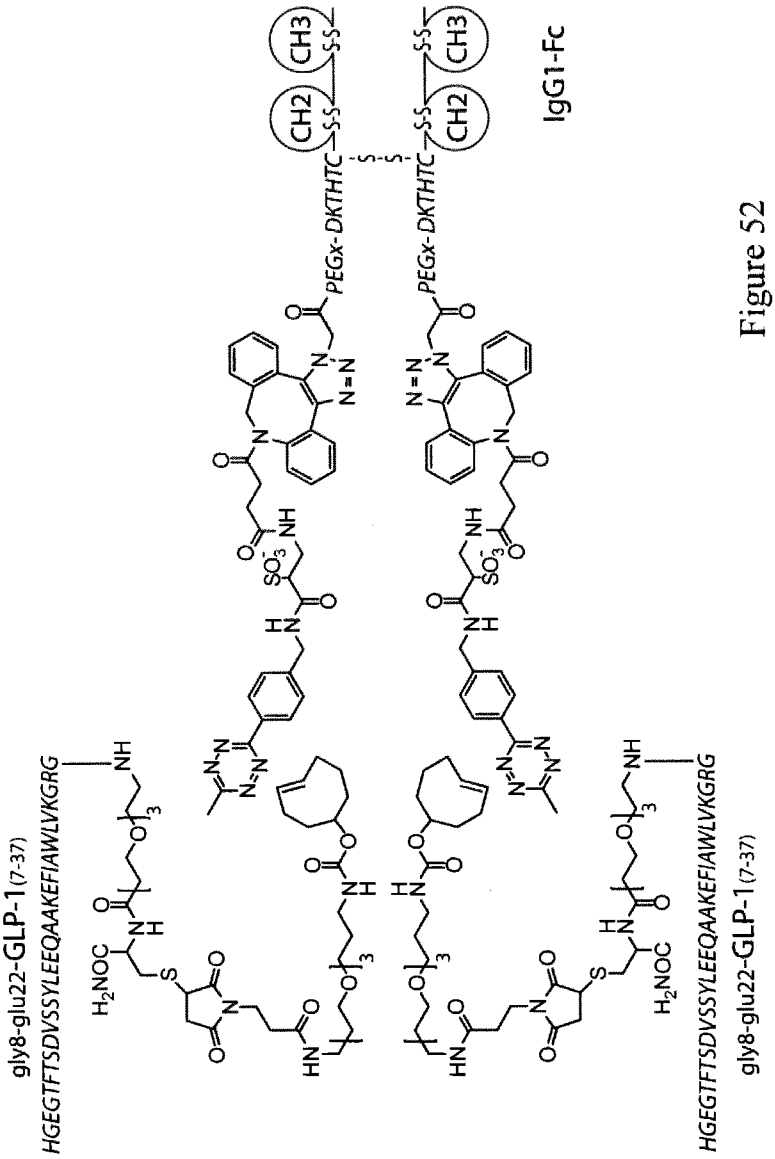

gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
IgG1-Fc

Figure 52

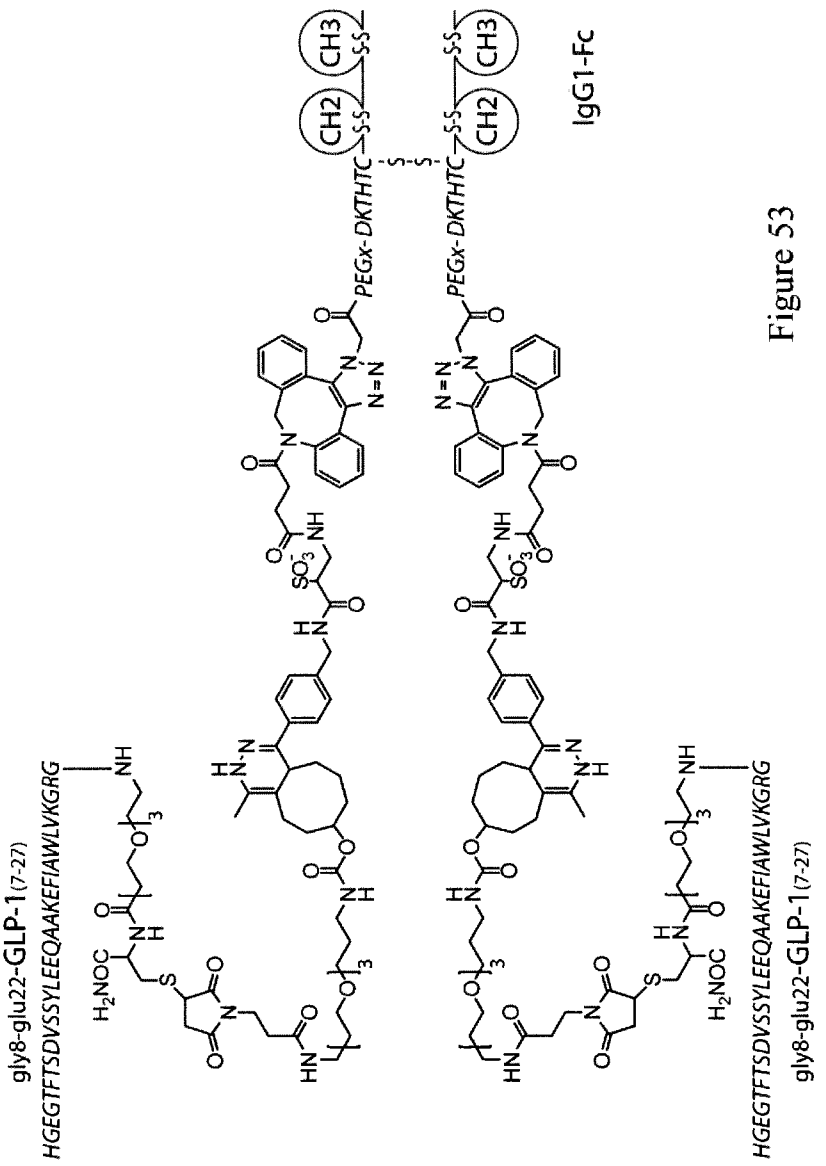

gly8-glu22-GLP-1(7-27)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
IgG1-Fc

Figure 53

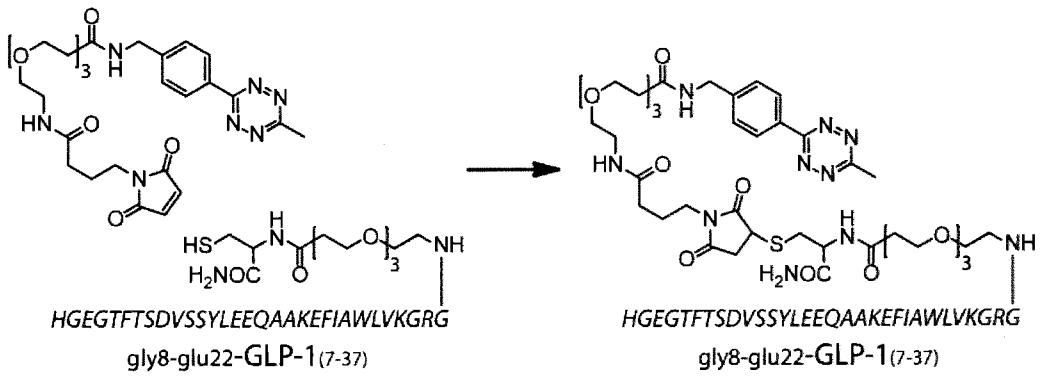

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
gly8-glu22-GLP-1(7-37)

Figure 54

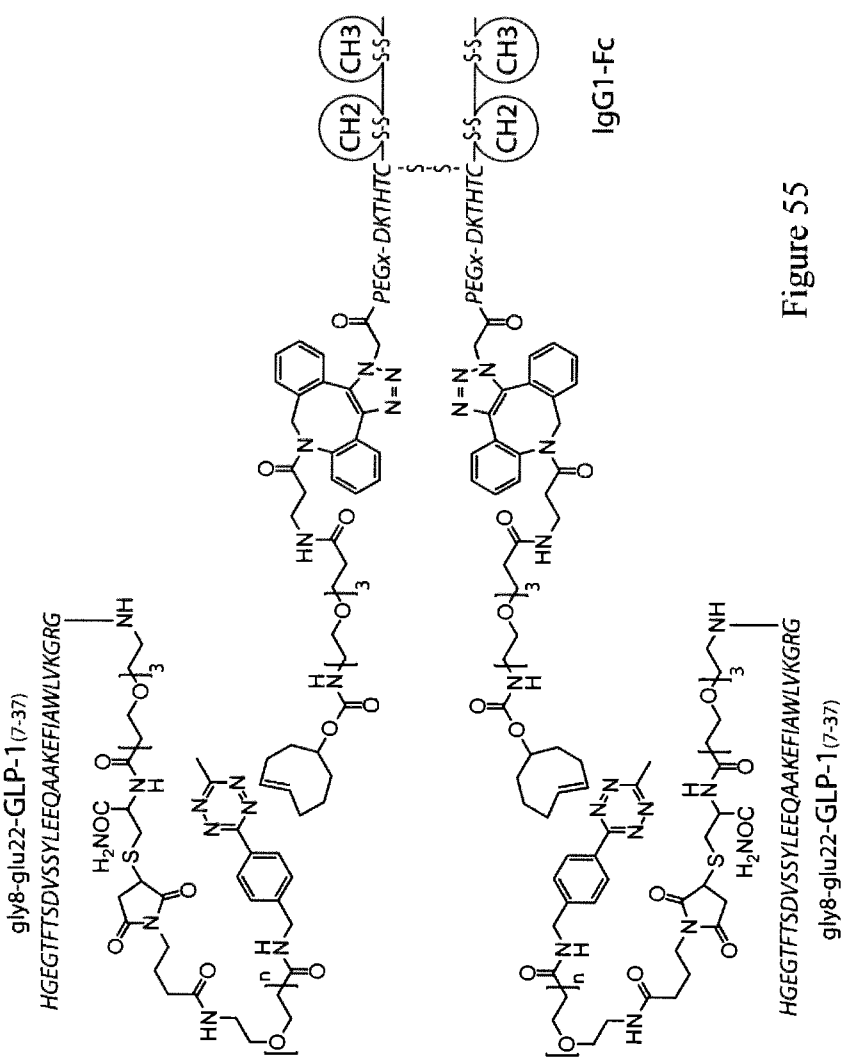

gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
IgG1-Fc

Figure 55

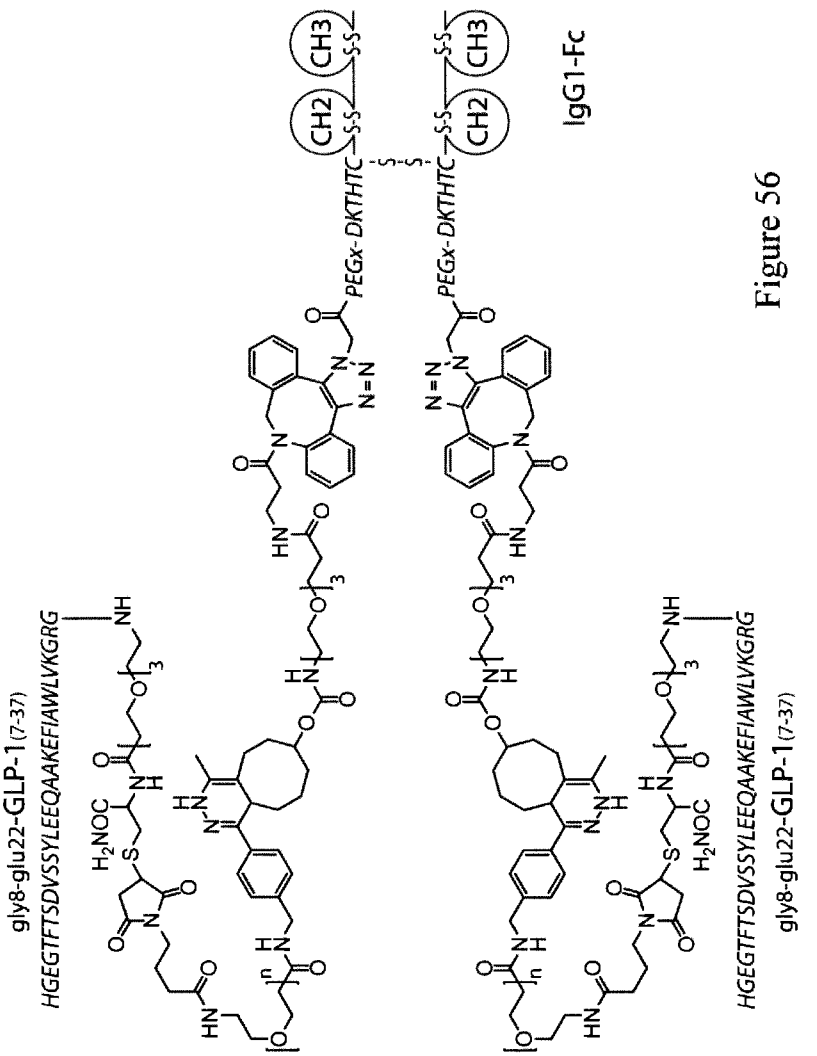

gly8-glu22-GLP-1(7-37)
HGEGTFTSDVSSYLEEQAAKEFIAWLVKGRG
PEGx-DKTHTC
CH2
CH3
IgG1-Fc

Figure 56

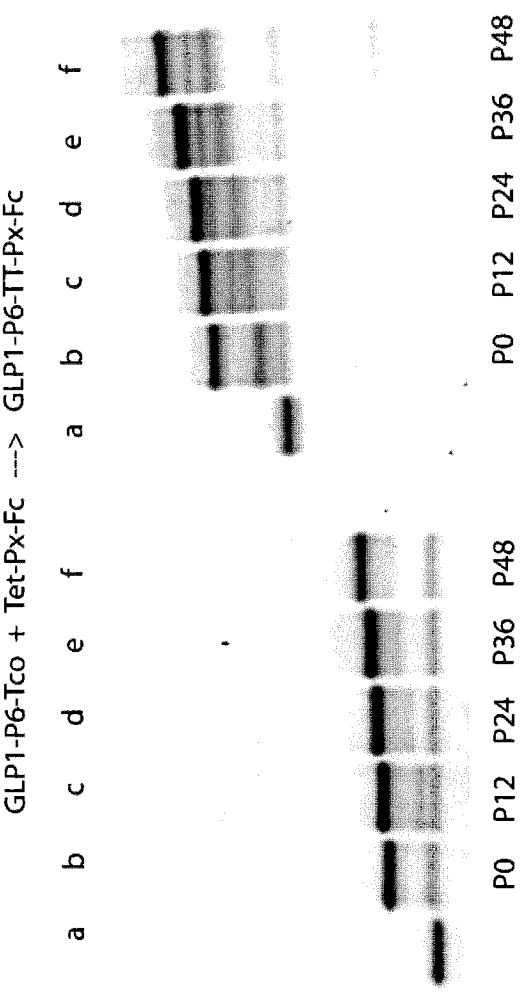

GLP1-P6-Tco + Tet-Px-Fc --> GLP1-P6-TT-Px-Fc
a b c d e f
a b c d e f
Fc6
P0 P12 P24 P36 P48
GLP1-P6-TT-Px-Fc
reducing
Fc6
P0 P12 P24 P36 P48
GLP1-P6-TT-Px-Fc
non-reducing

Figure 57

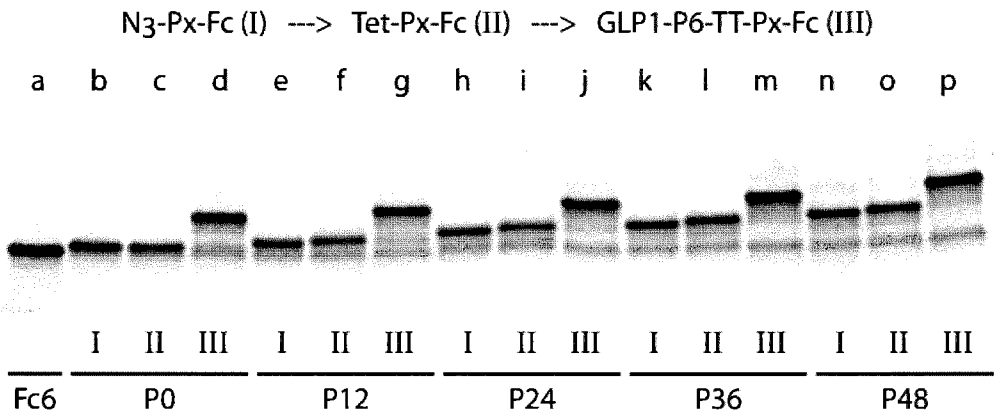

N3-Px-Fc (I) ---> Tet-Px-Fc (II) ---> GLP1-P6-TT-Px-Fc (III)
a b c d e f g h i j k l m n o p
I II III I II III I II III I II III I II III
Fc6
P0
P12
P24
P36
P48

Figure 58

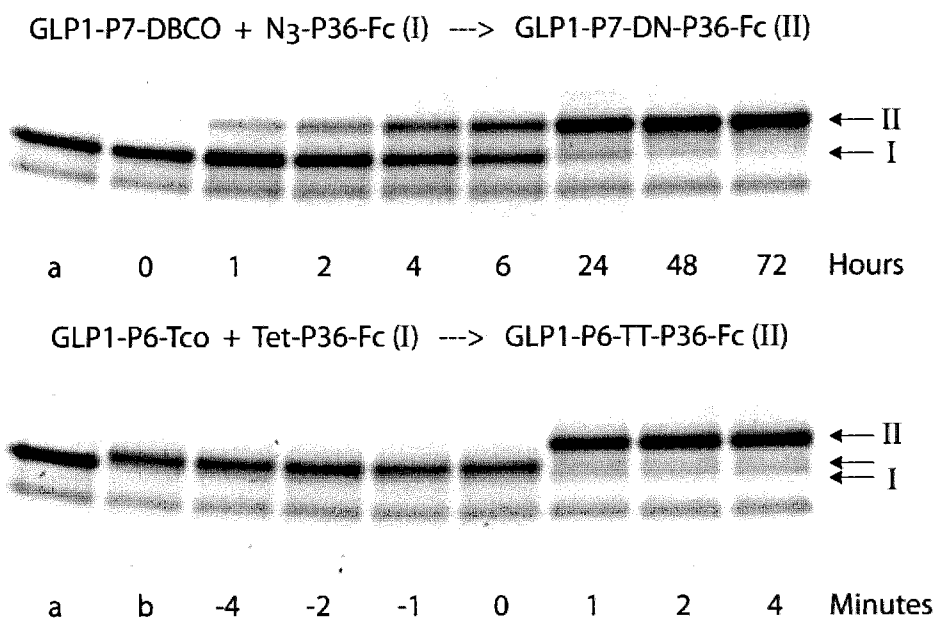

GLP1-P7-DBCO + N3-P36-Fc (I) ---> GLP1-P7-DN-P36-Fc (II)
II
I
a 0 1 2 4 6 24 48 72 Hours
GLP1-P6-Tco + Tet-P36-Fc (I) ---> GLP1-P6-TT-P36-Fc (II)
II
I
a b -4 -2 -1 0 1 2 4 Minutes

Figure 59

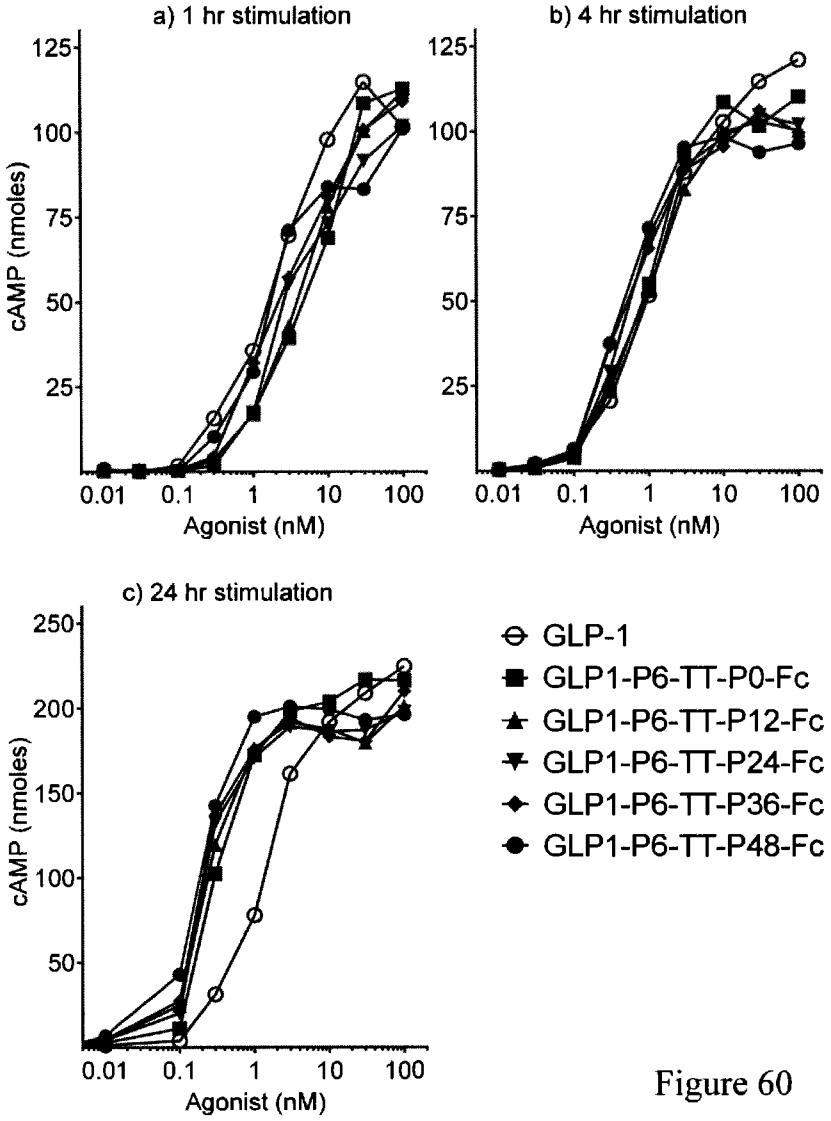

a) 1 hr stimulation
b) 4 hr stimulation
c) 24 hr stimulation
cAMP (nmoles)
Agonist (nM)
0.01
0.1
1
10
100
25
50
75
100
125
150
200
250
GLP-1
GLP1-P6-TT-P0-Fc
GLP1-P6-TT-P12-Fc
GLP1-P6-TT-P24-Fc
GLP1-P6-TT-P36-Fc
GLP1-P6-TT-P48-Fc

Figure 60

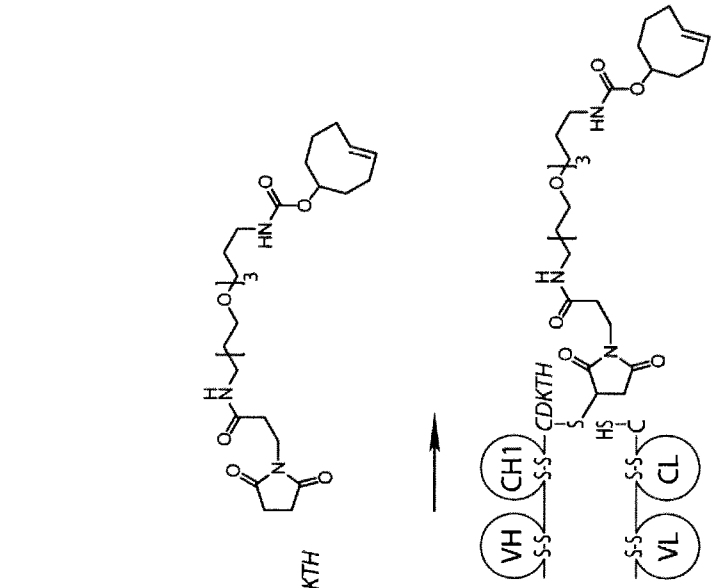

VL
CL
C
S-S
S-S
S-S
CDKTH
CH1
VH
TCEP
VH
CH1
CDKTH
HS
HS
C
VL
CL
HN
O
N
O
O
O
3
VH
CH1
CDKTH
HS
C
VL
CL

Figure 61

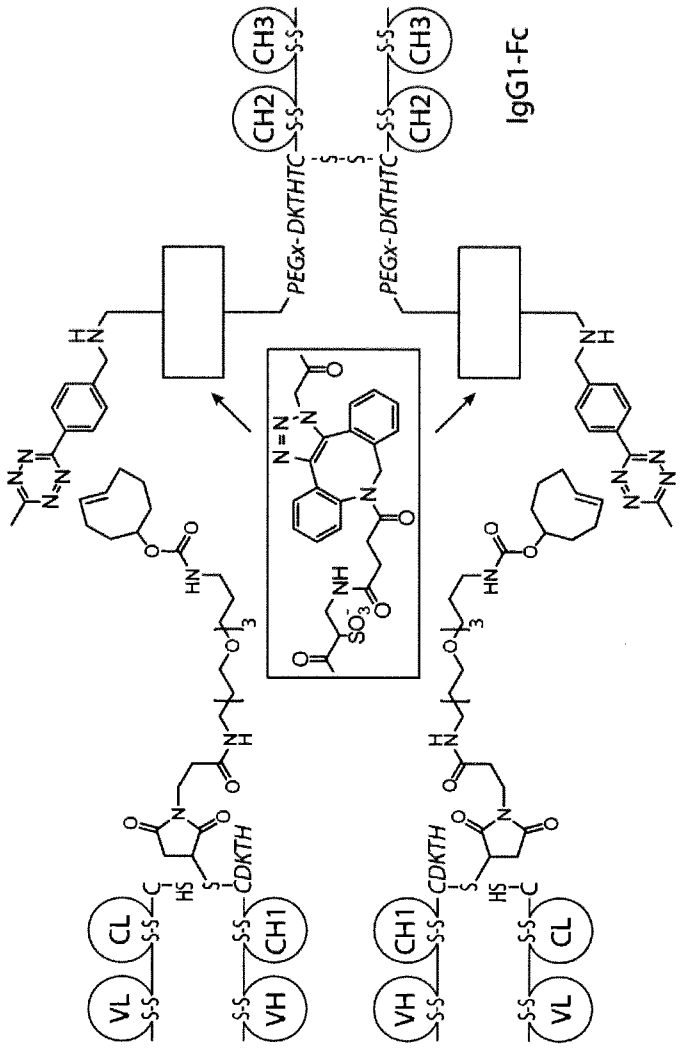


Figure 62

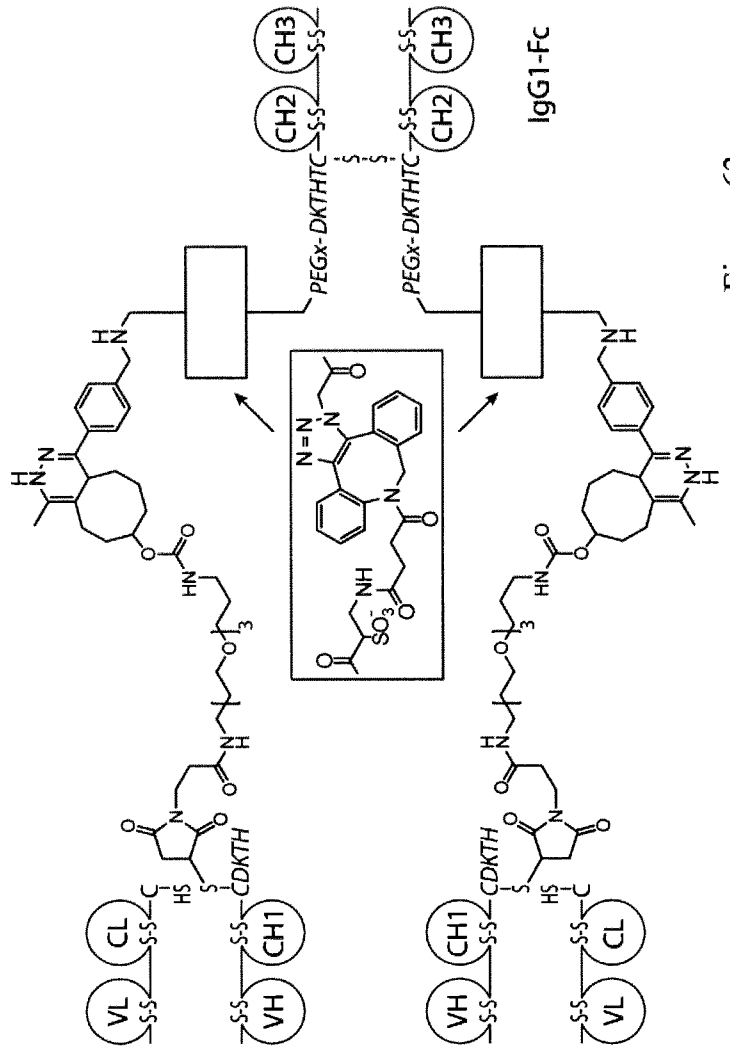

CH3
CH2
PEGx-DKTHTC
S-S
S-S
PEGx-DKTHTC
CH2
CH3
IgG1-Fc
VL
CL
VH
CH1
CDKTH
HS
VH
CH1
CDKTH
HS
VL
CL

Figure 63

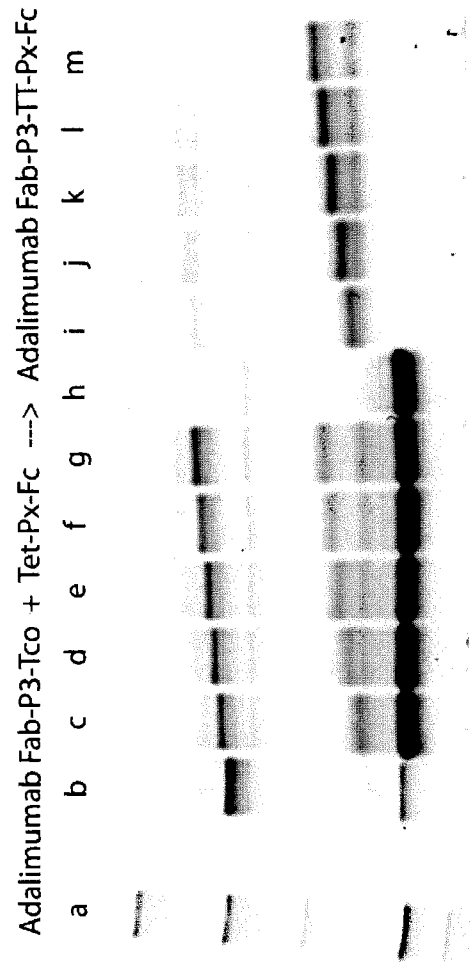

Adalimumab Fab-P3-Tco + Tet-Px-Fc ---> Adalimumab Fab-P3-TT-Px-Fc
a b c d e f g h i j k l m
M
Mab
P0 P12 P24 P36 P48
Fab-P3-TT-Px-Fc
Fab
P0 P12 P24 P36 P48
Tet-Px-Fc

Figure 64

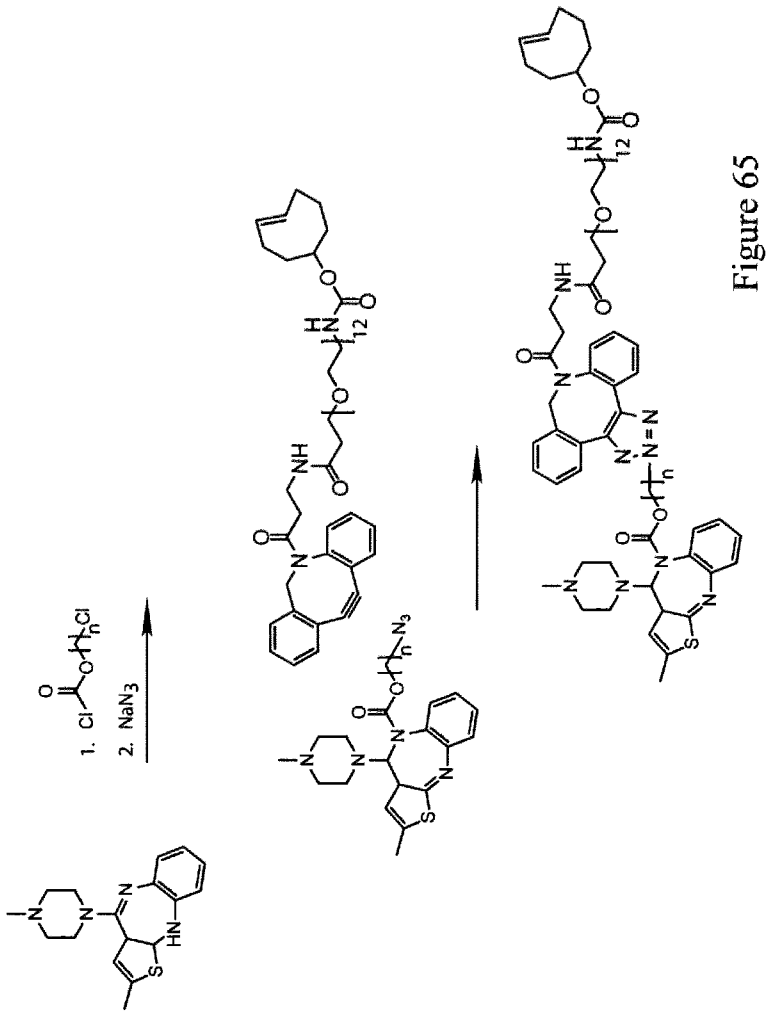


Figure 65

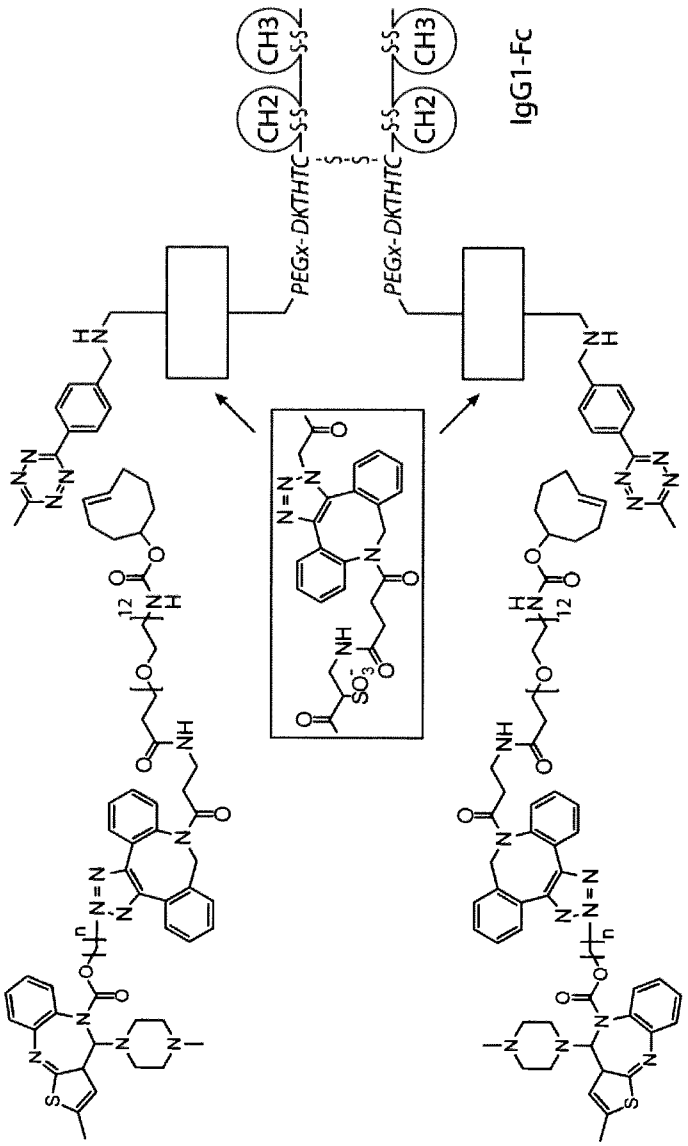


Figure 66

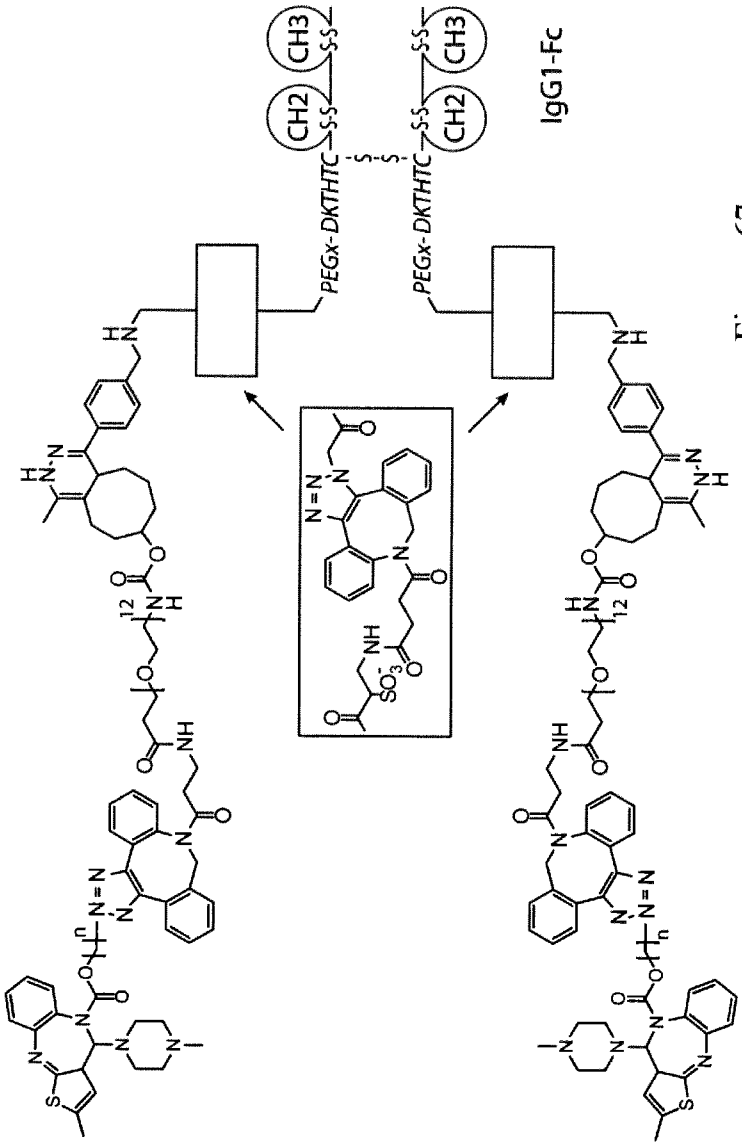


Figure 67