Figure 1

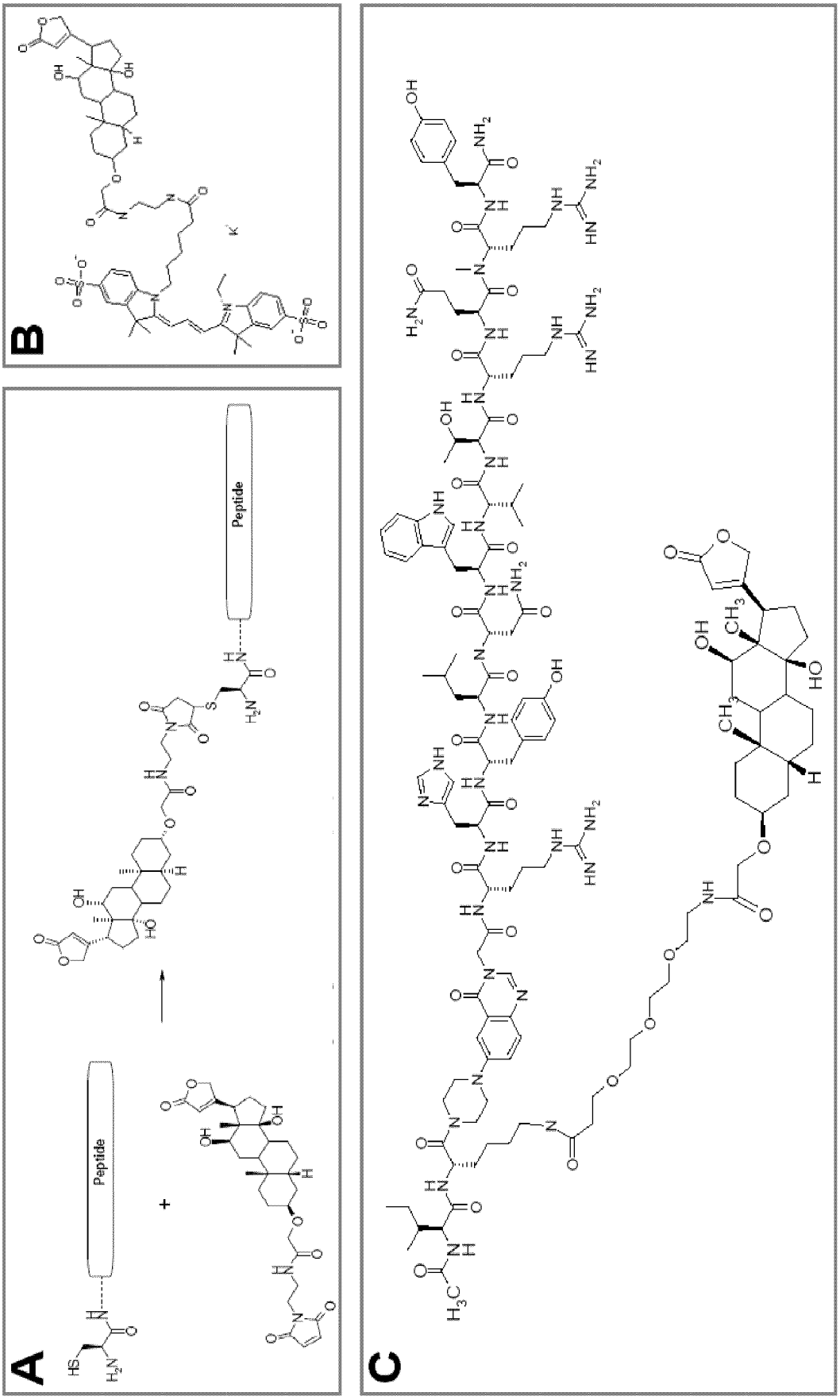


Figure 2

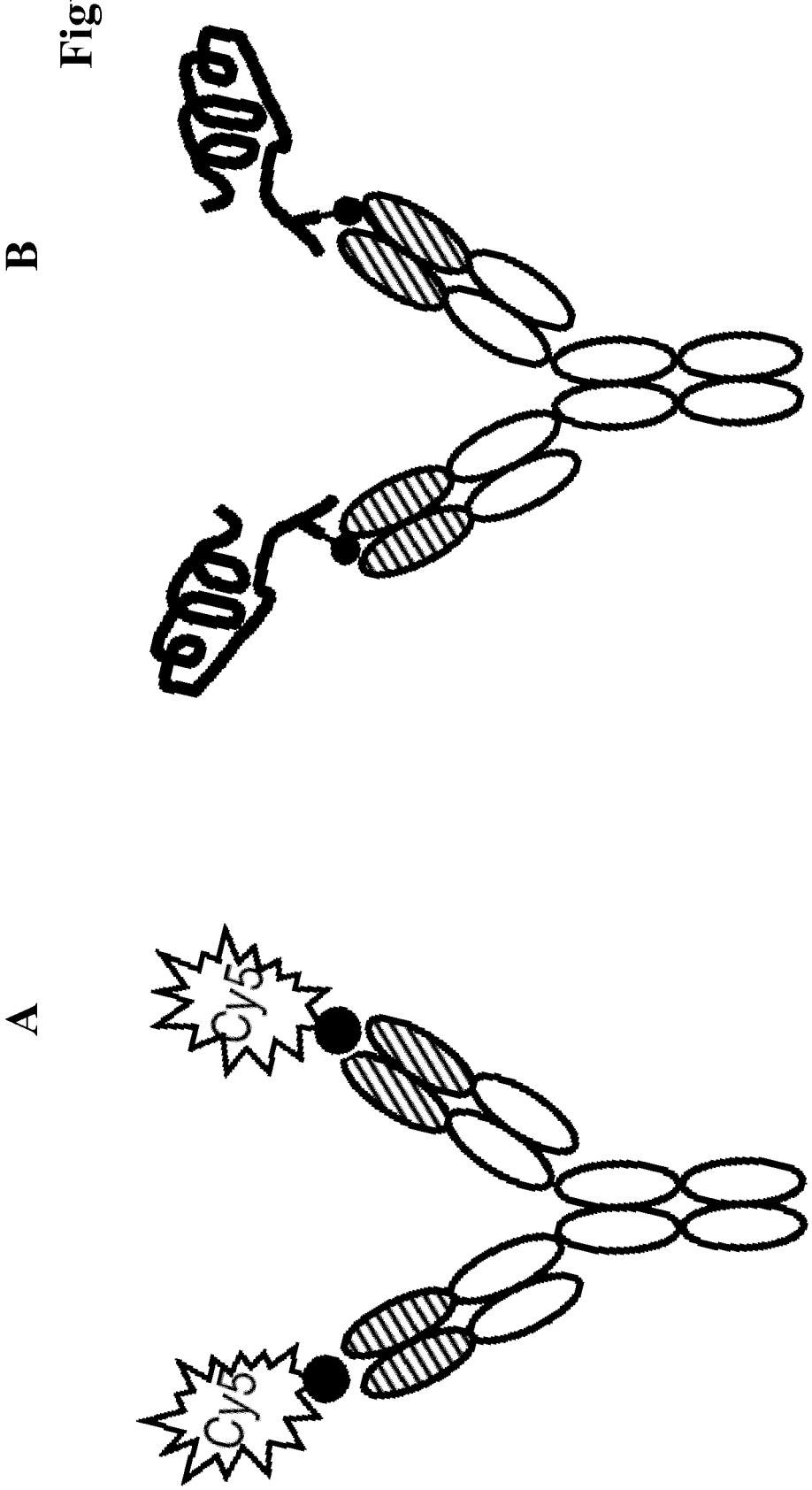


Figure 2

C

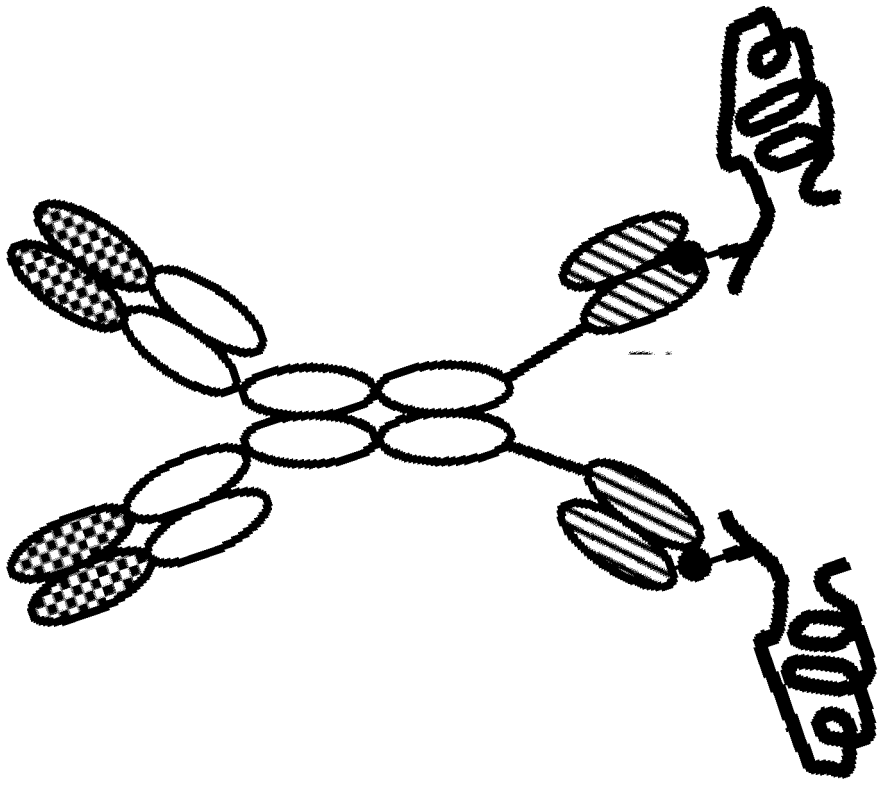


Figure 3

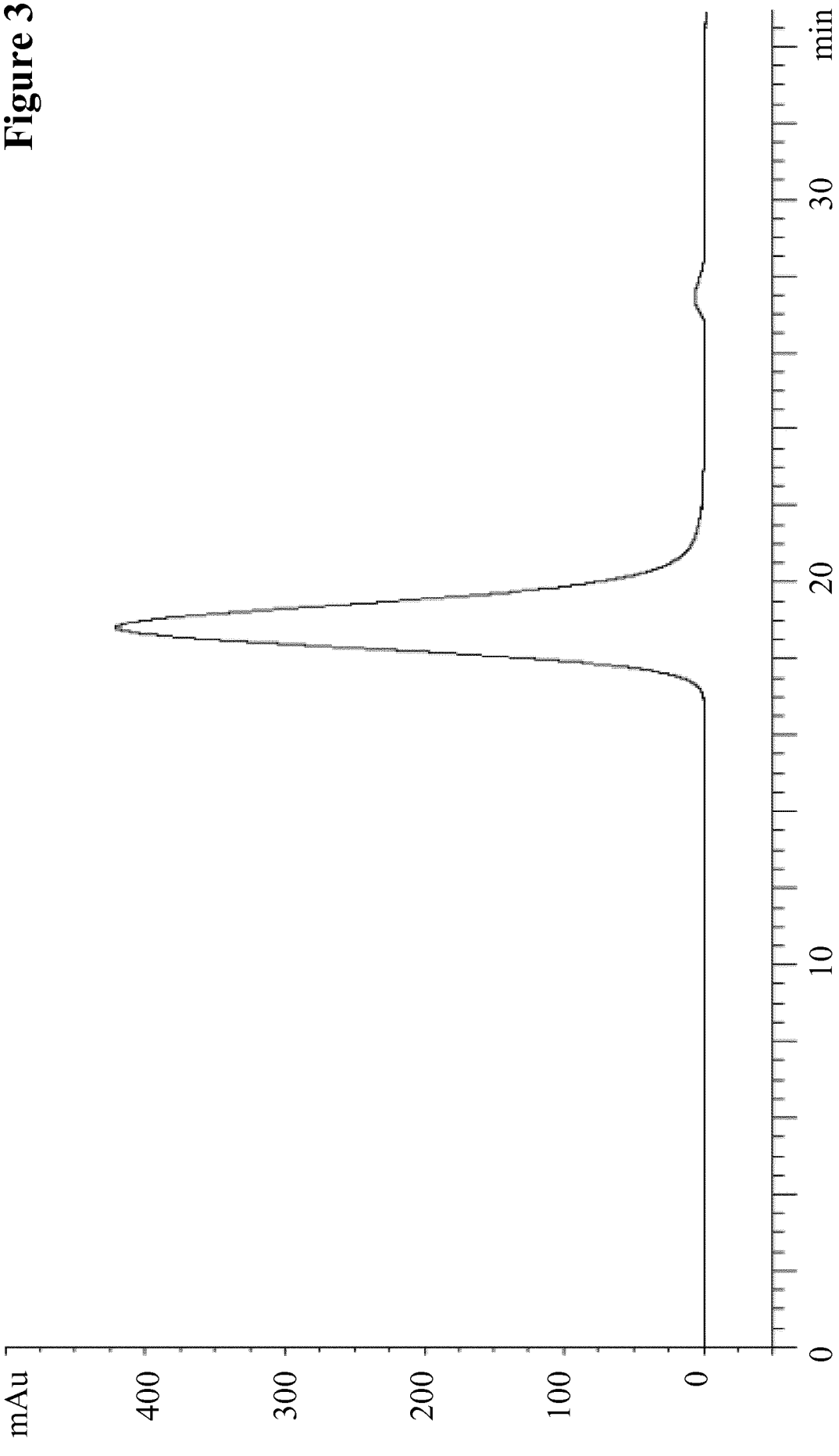
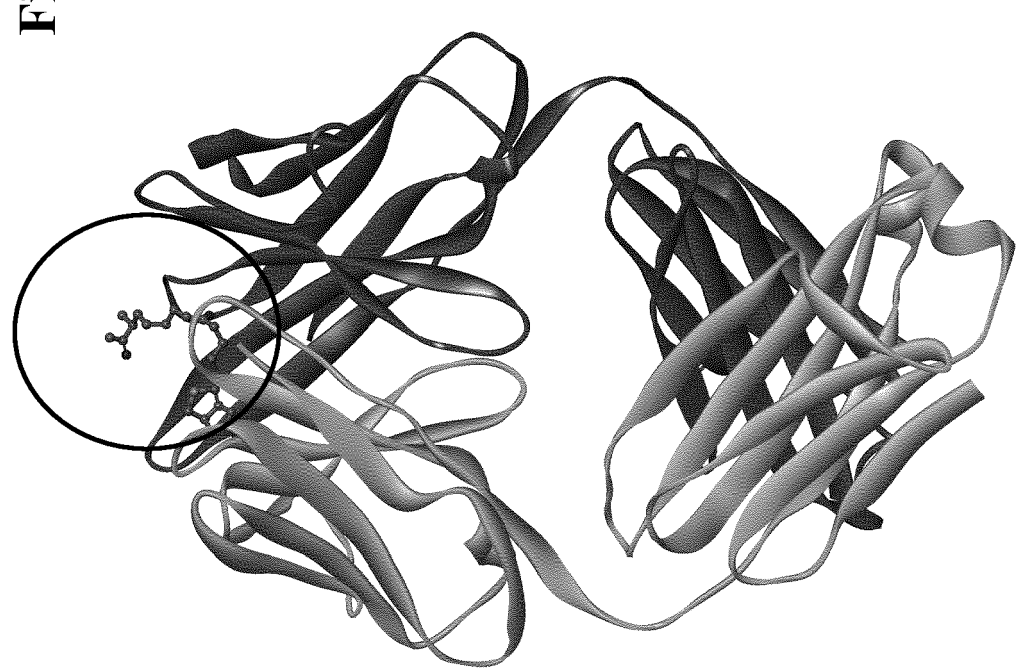


Figure 4



B



A

Figure 5

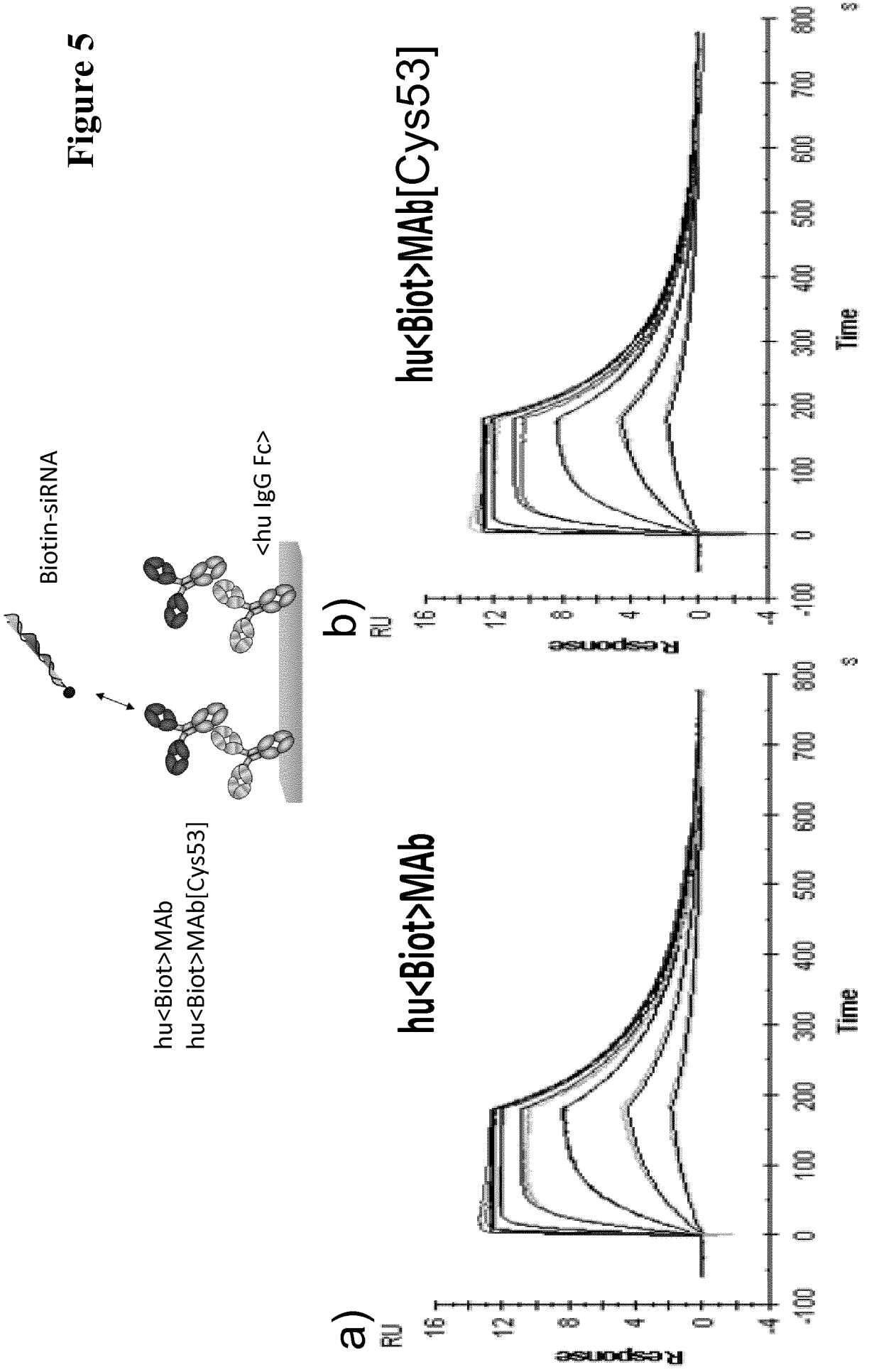

Biotin-siRNA
hu<Biot>MAb
hu<Biot>MAb[Cys53]
<hu IgG Fc>
a)
RU
hu<Biot>MAb
Response
Time
s
b)
RU
hu<Biot>MAb[Cys53]
Response
Time
s

Figure 6

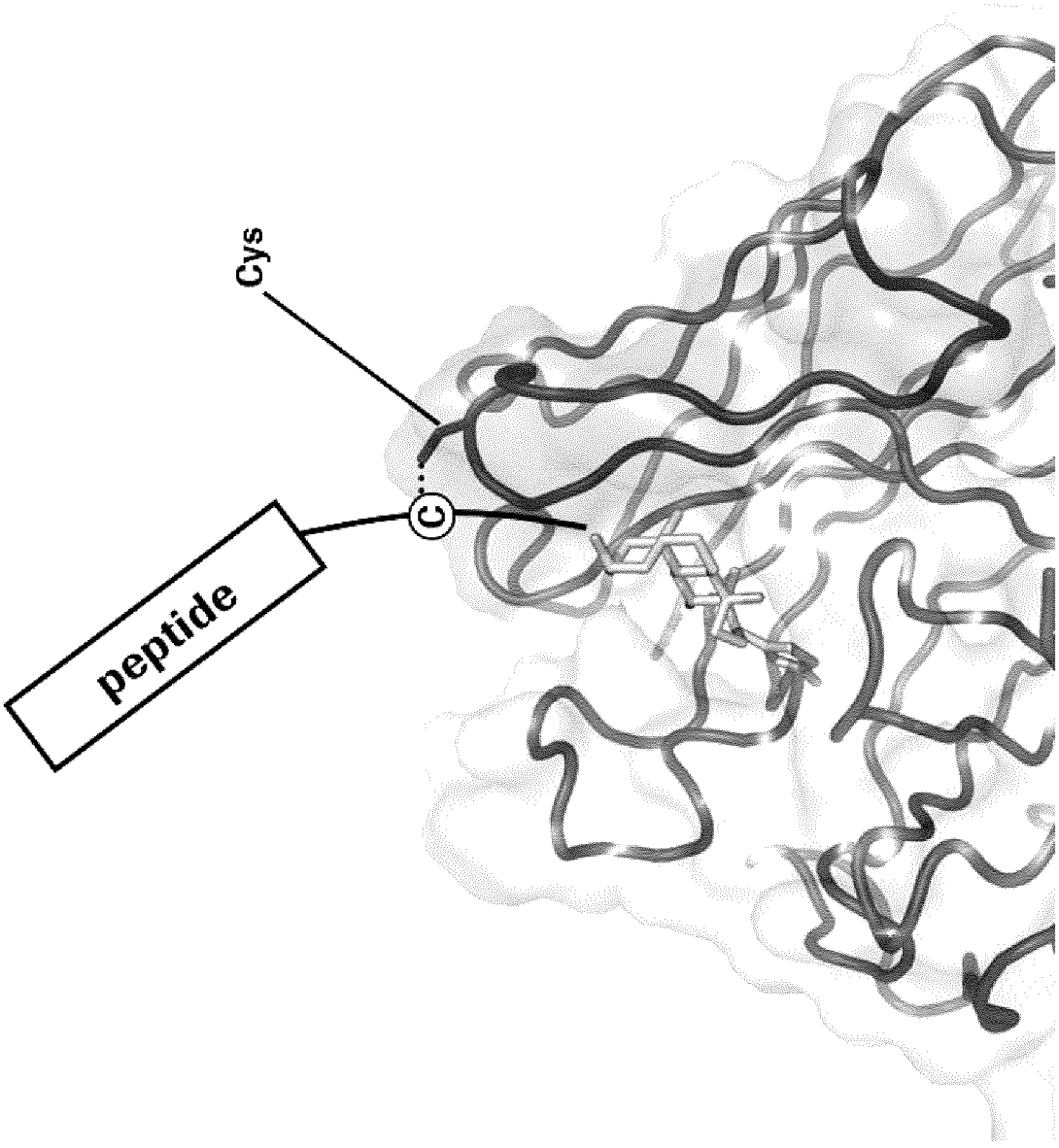


Figure 7

A
HS
10 kDa
reducing and non-reducing
SDS-PAGE conditions
B
S-S
S-S
150 kDa
10 kDa
non-reducing
reducing
SDS-PAGE conditions

Figure 8

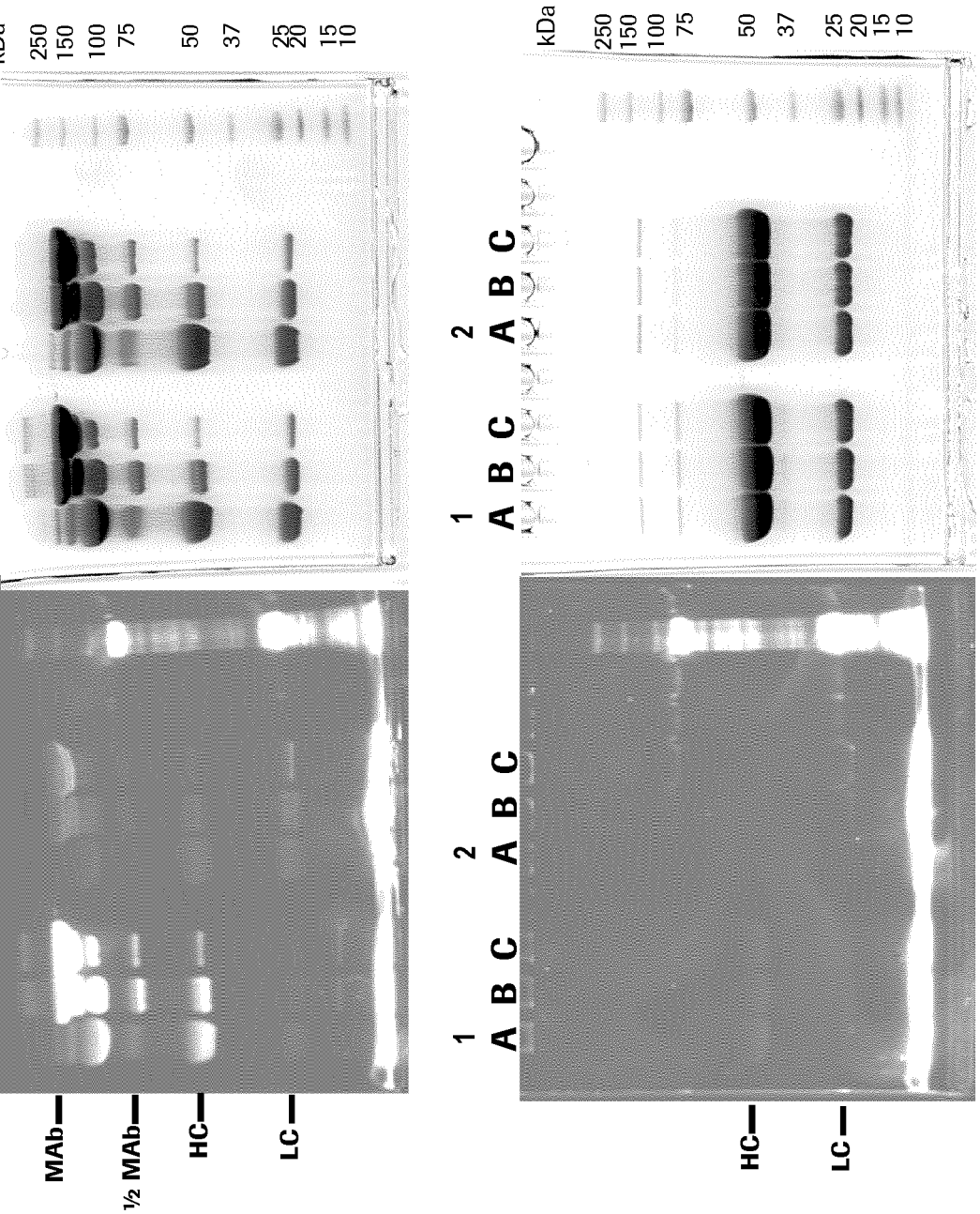


Figure 9

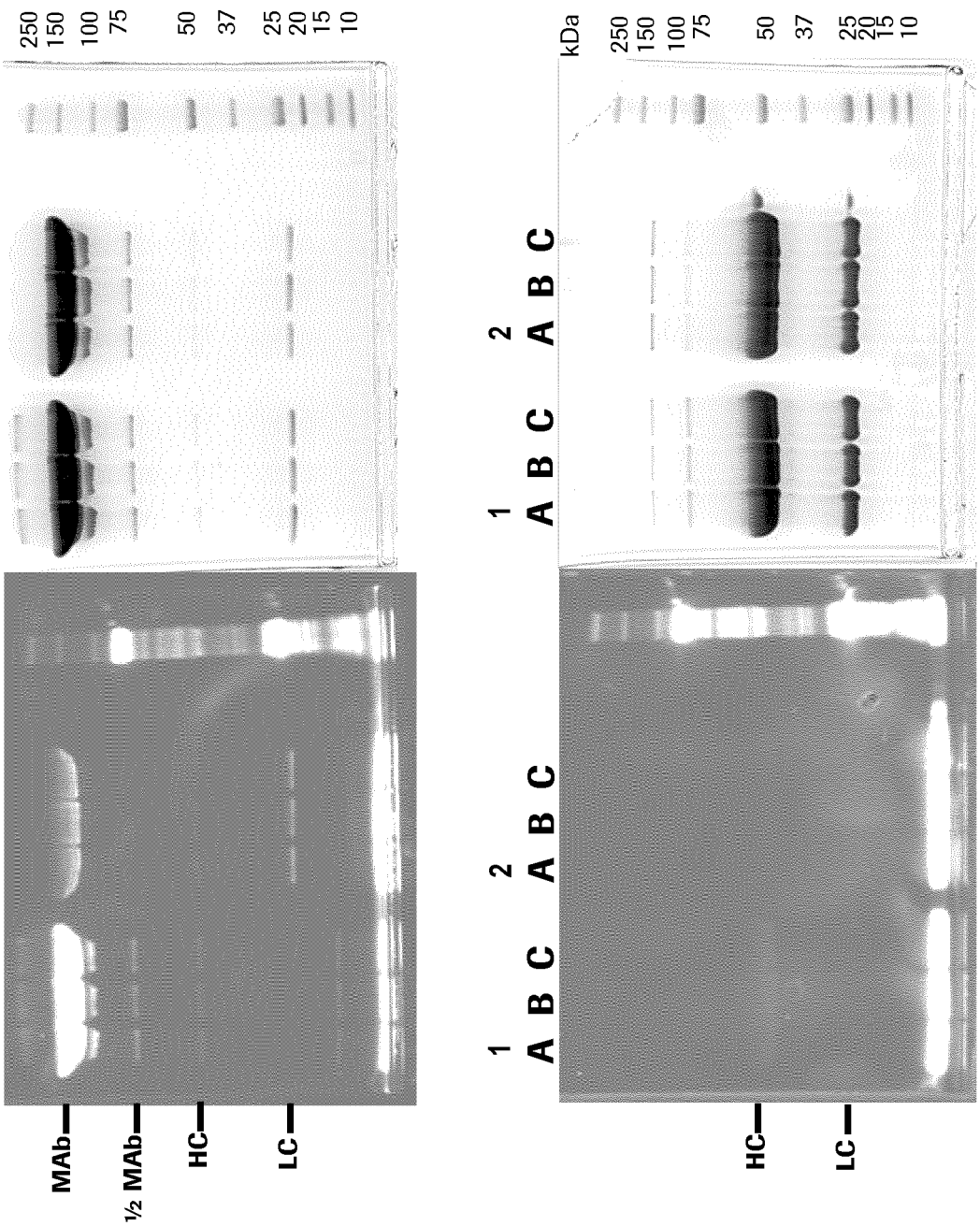


Figure 10

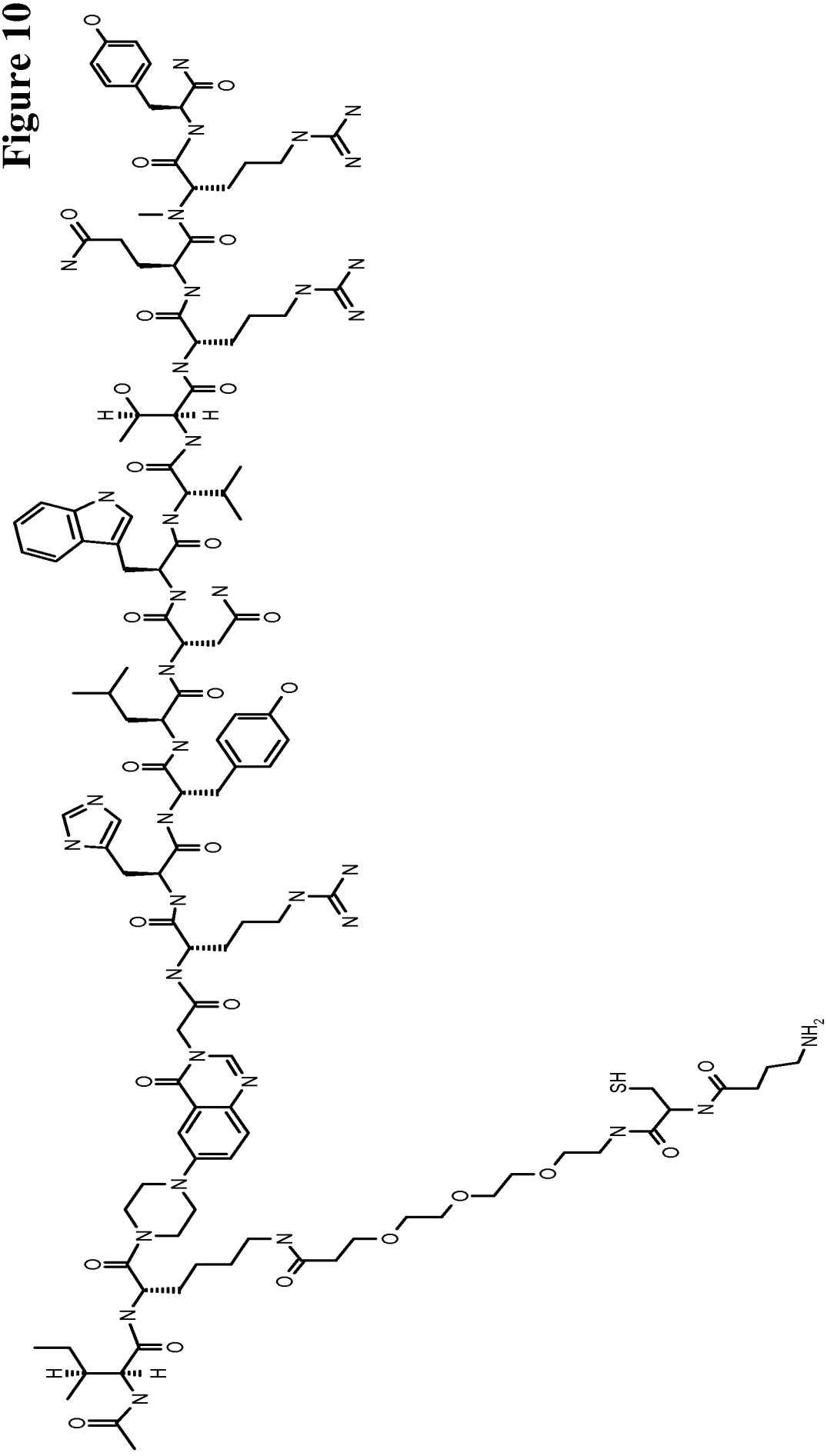


Figure 11

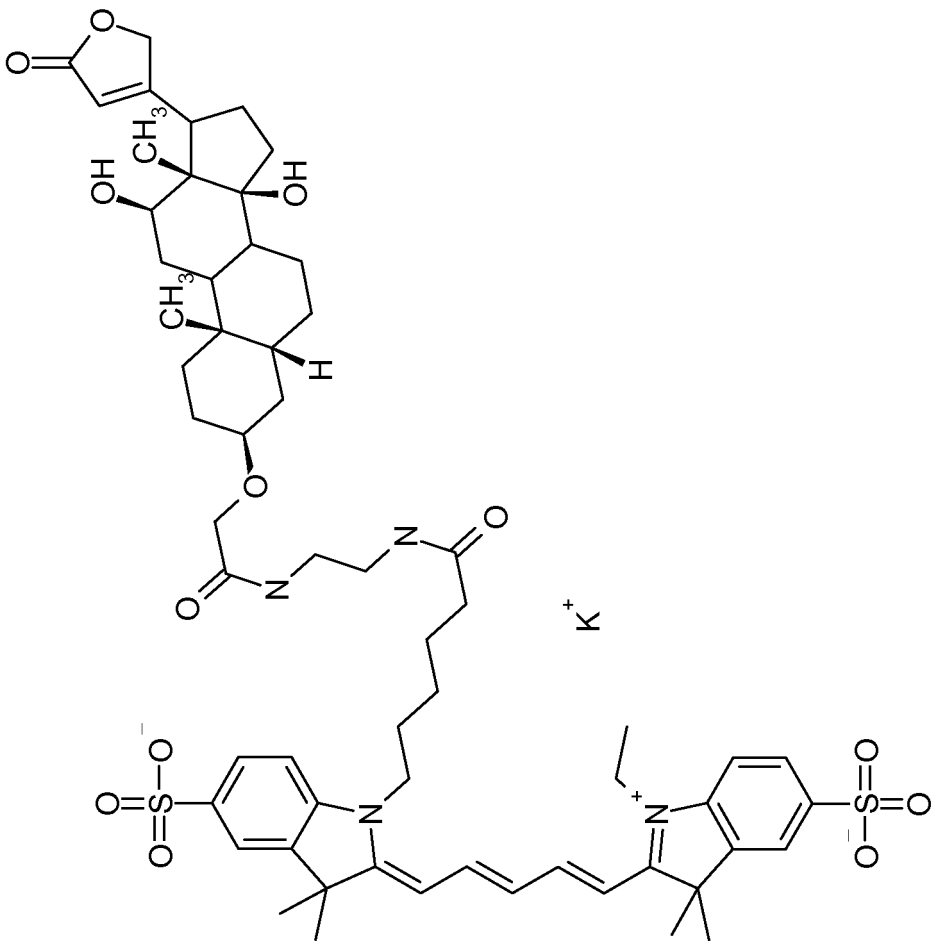


Figure 12

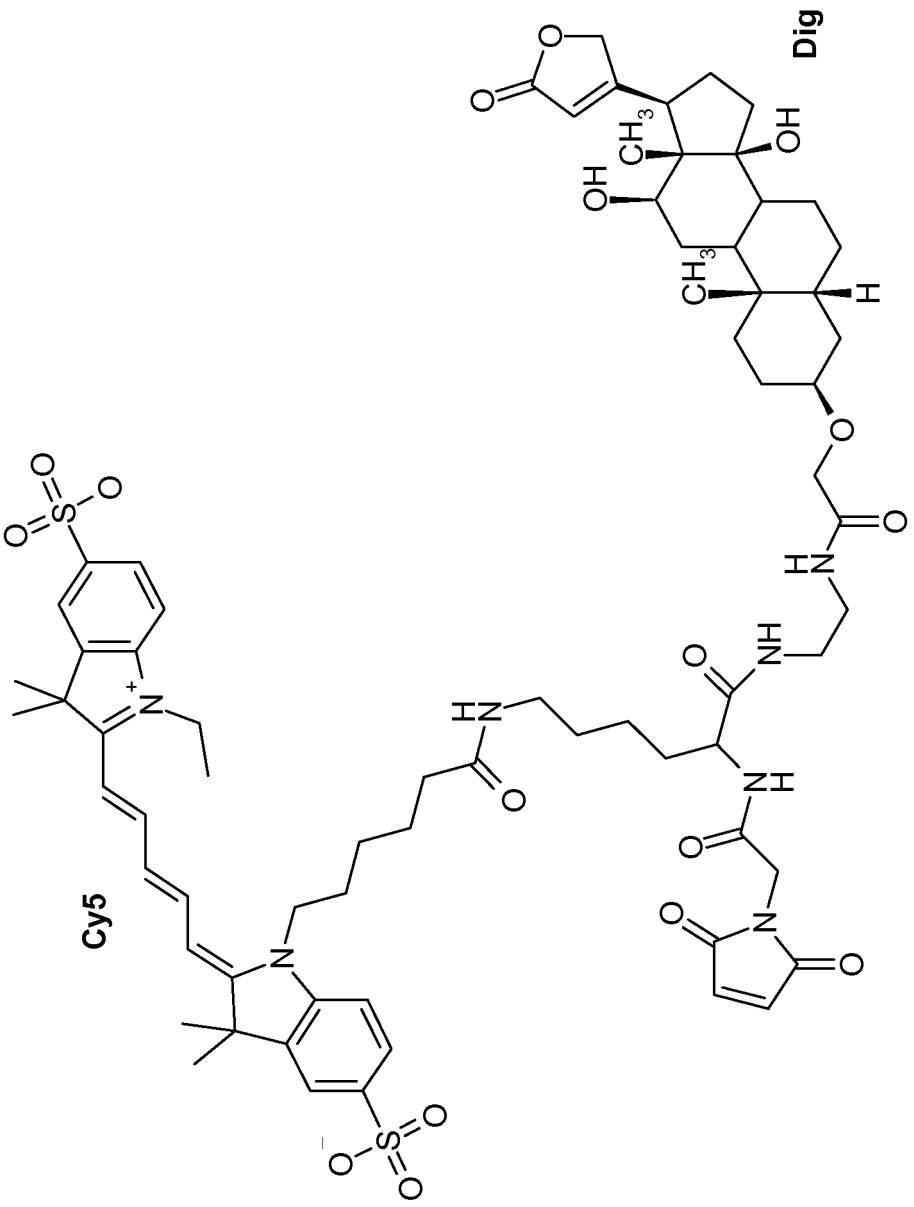


Figure 13

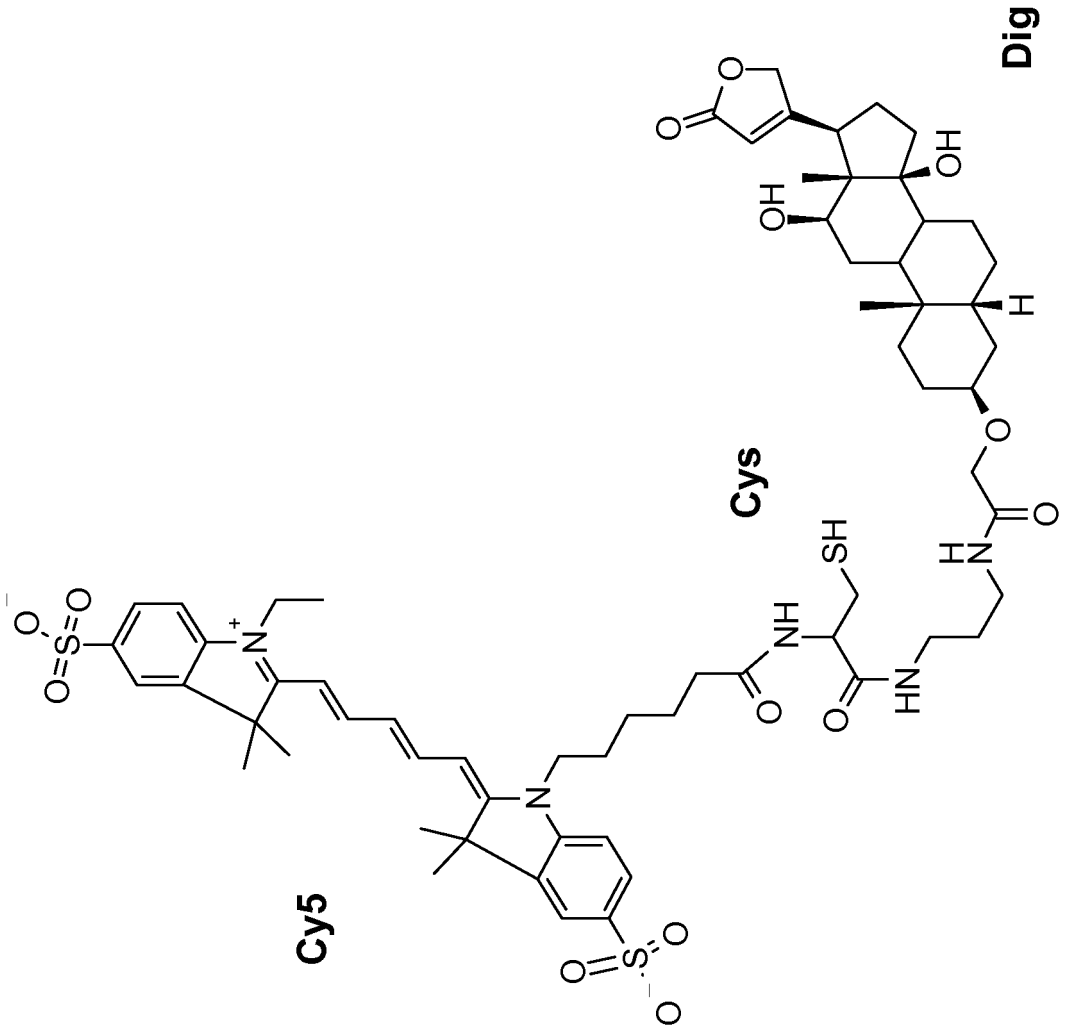


Figure 14

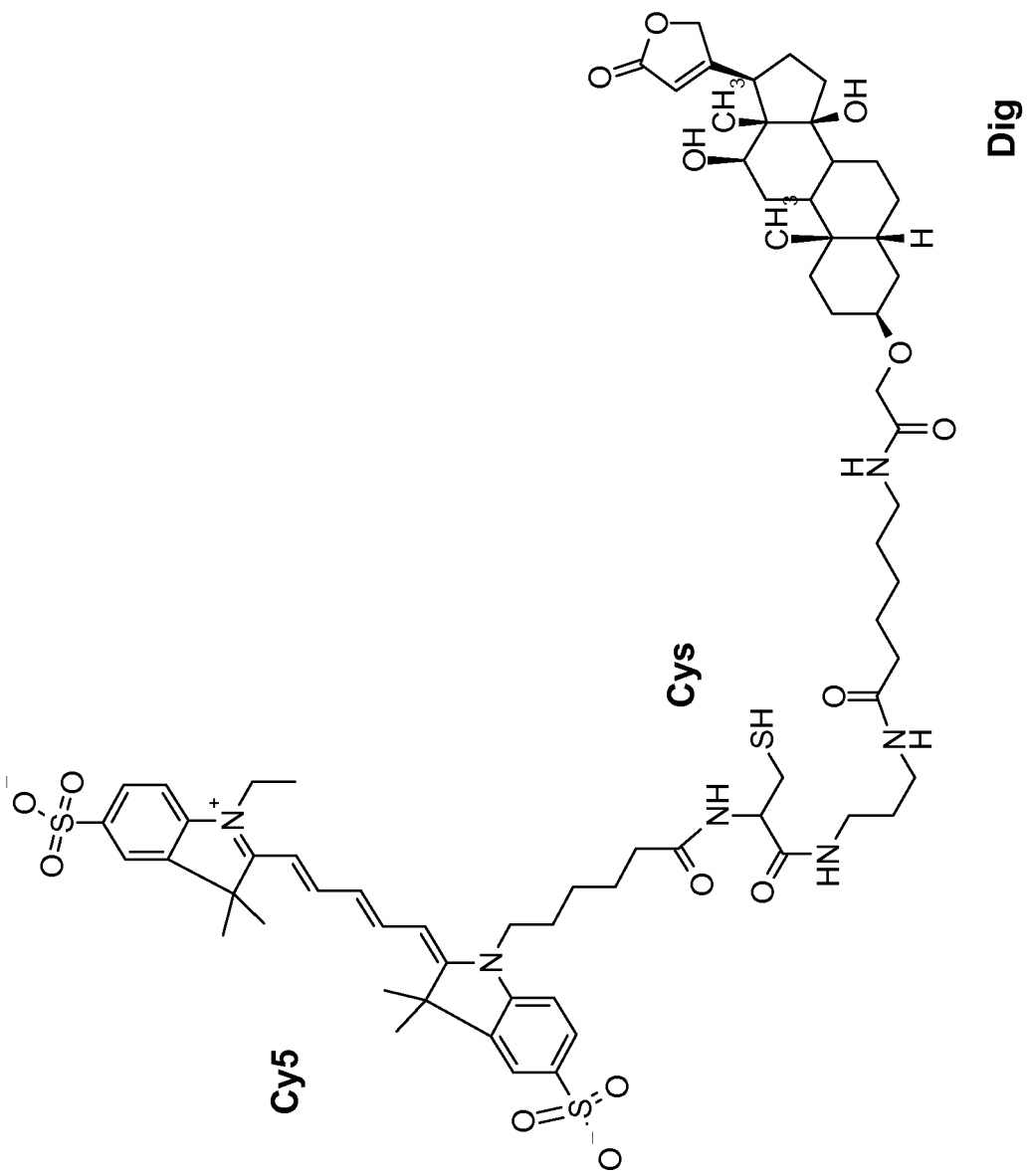


Figure 15

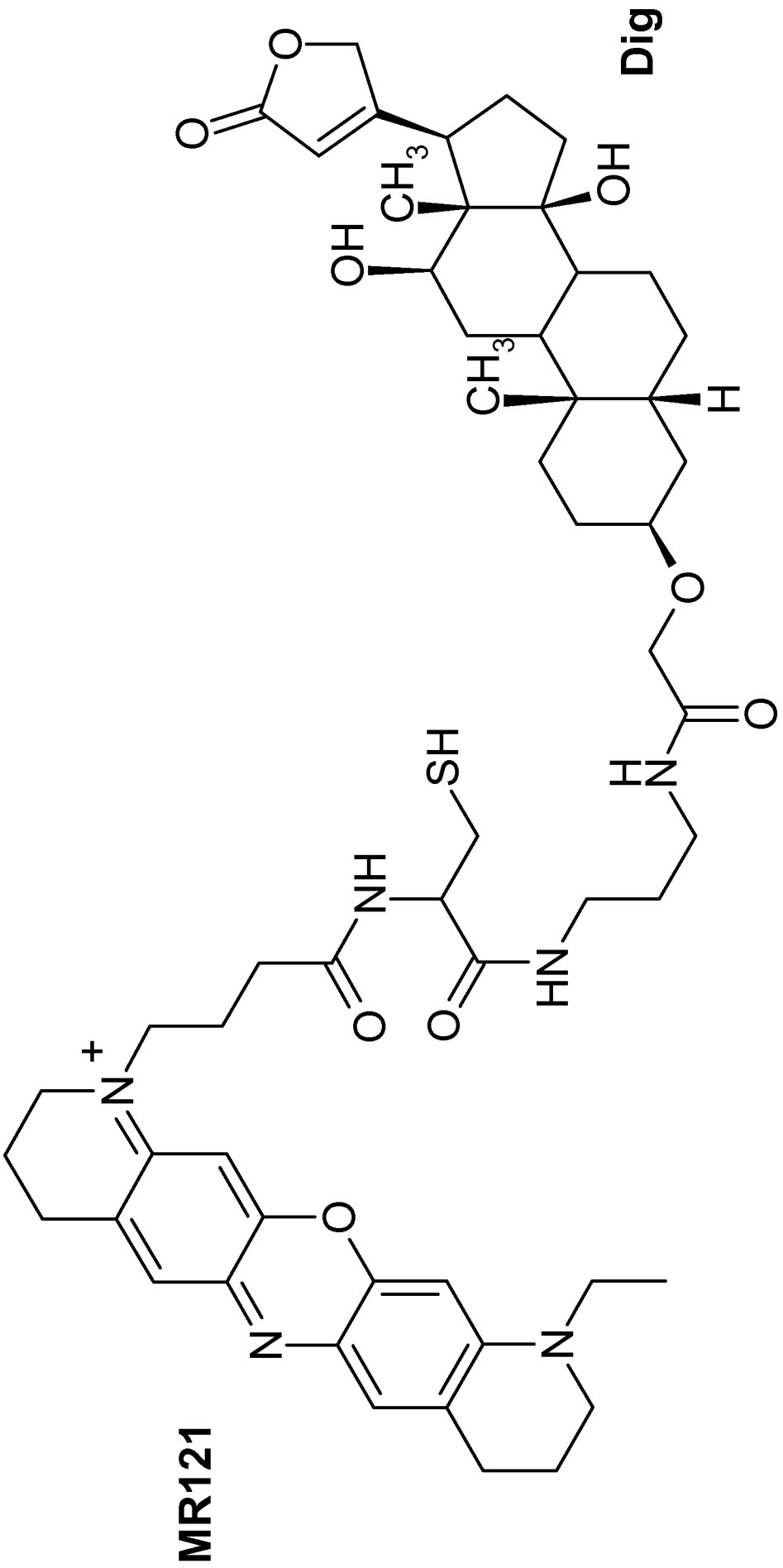


Figure 16

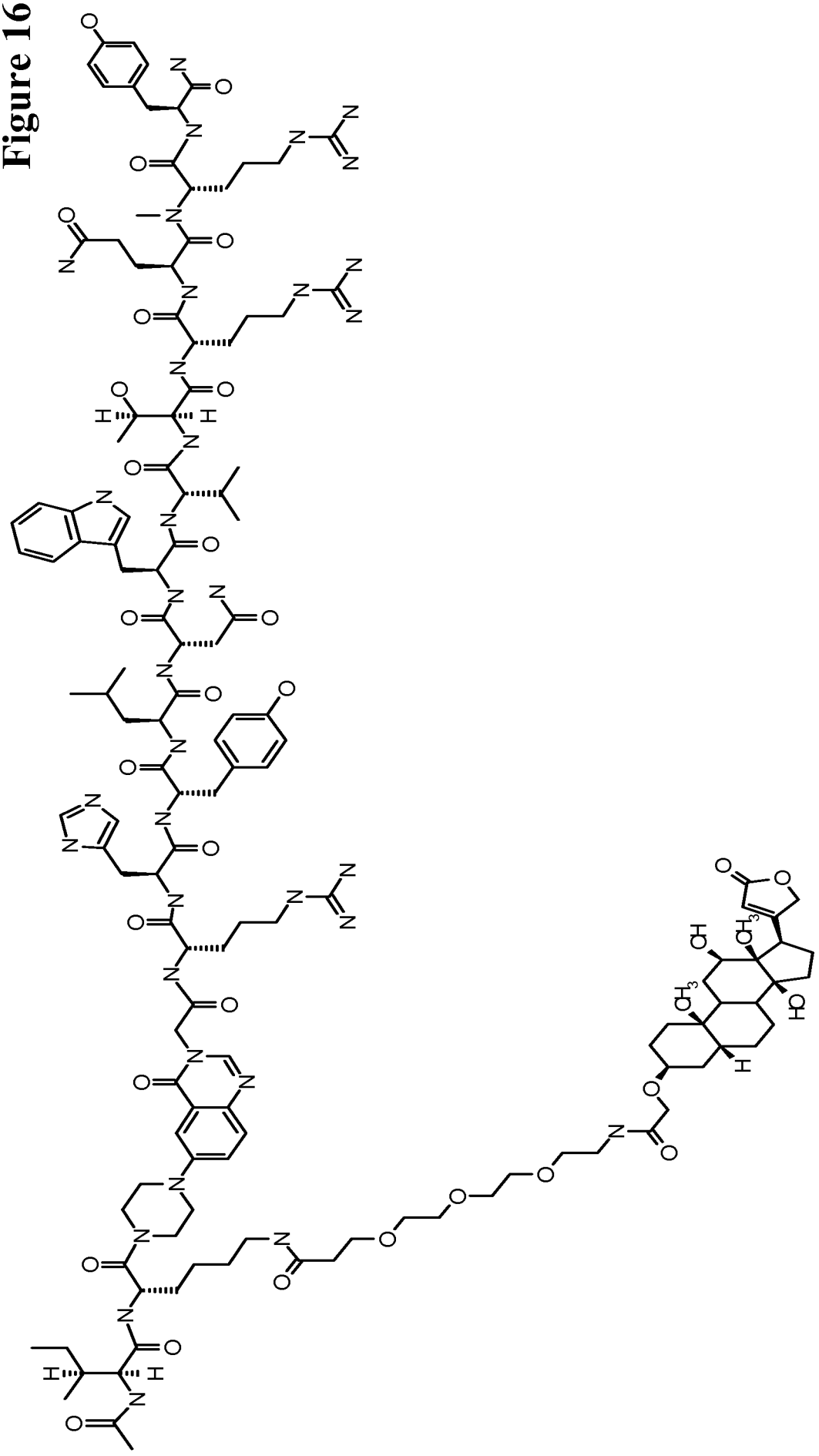


Figure 17

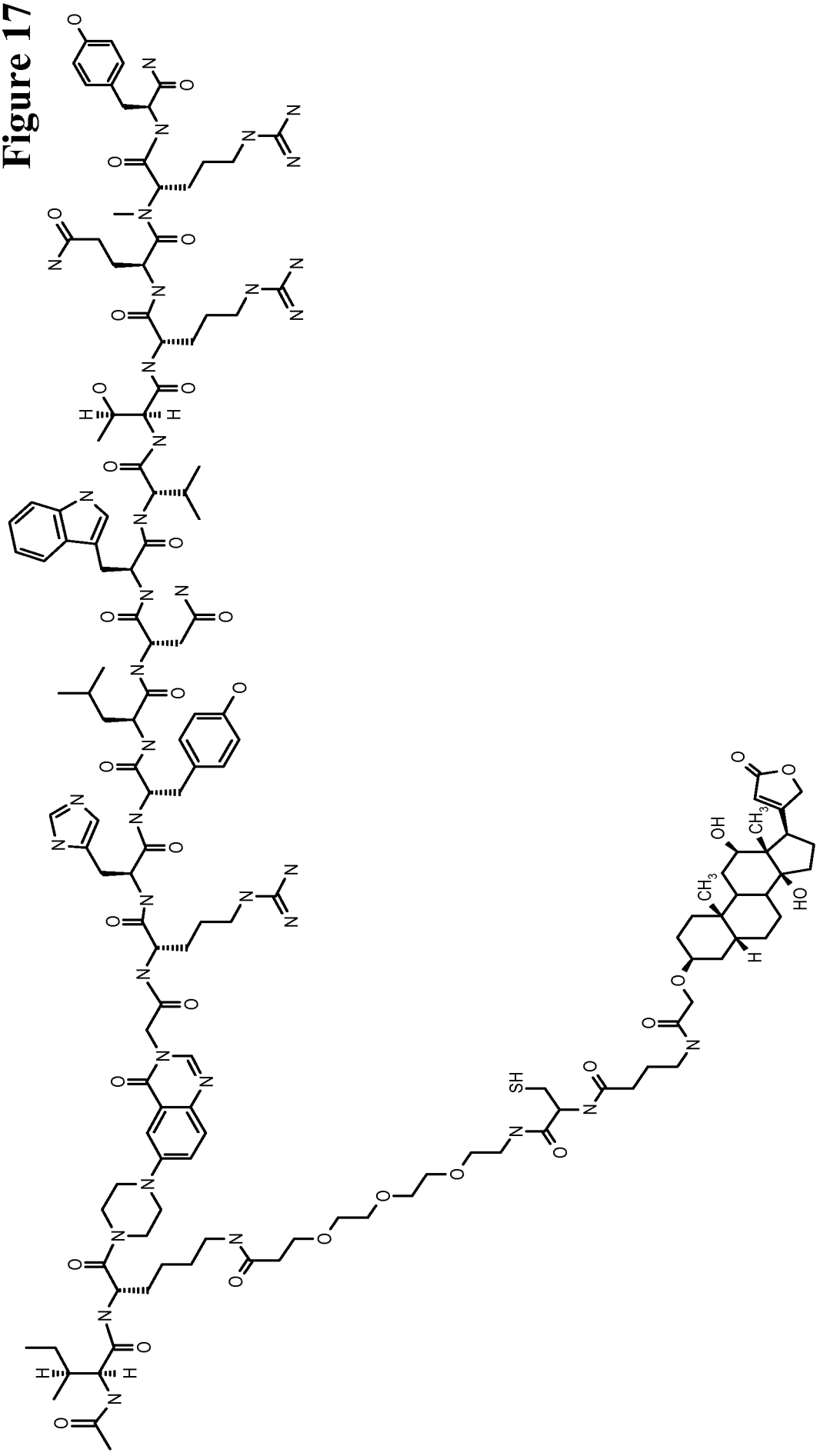


Figure 18

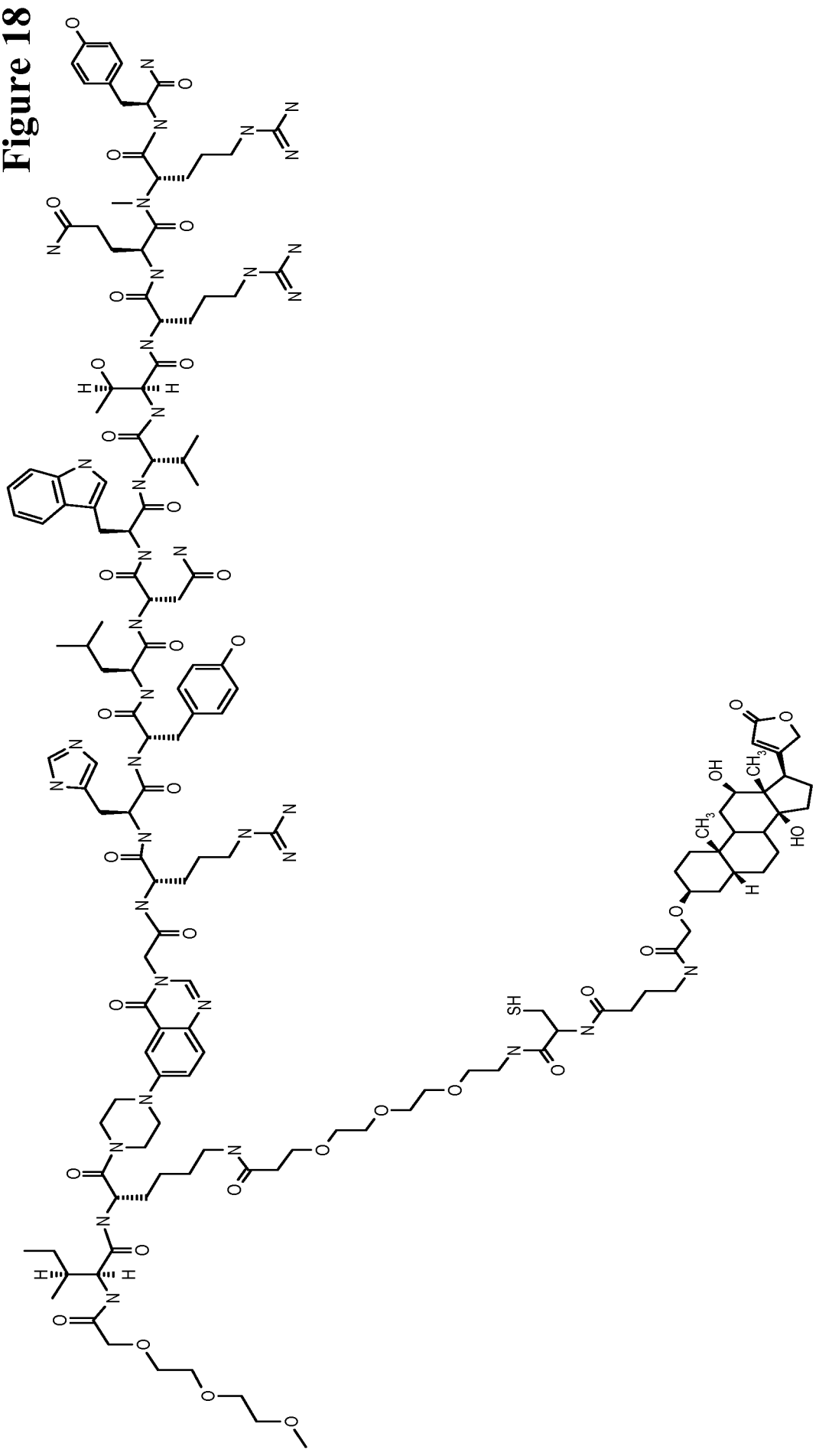


Figure 19

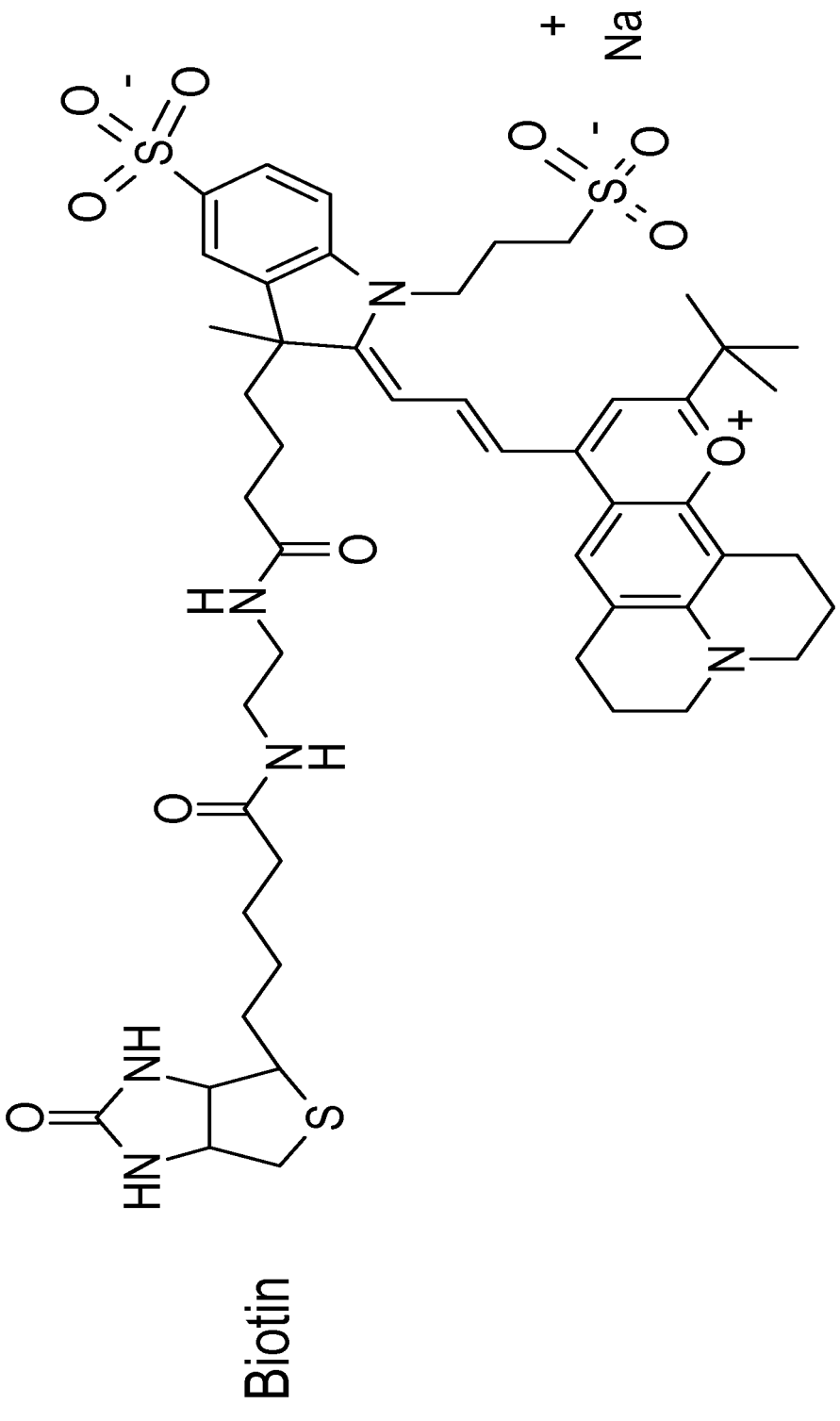


Figure 20

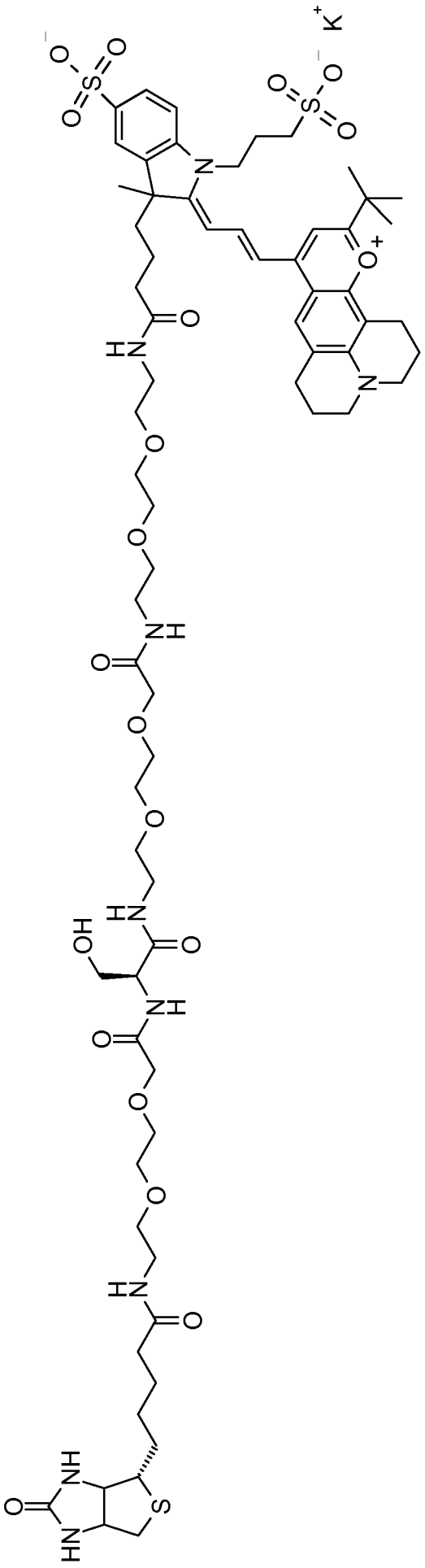


Figure 21

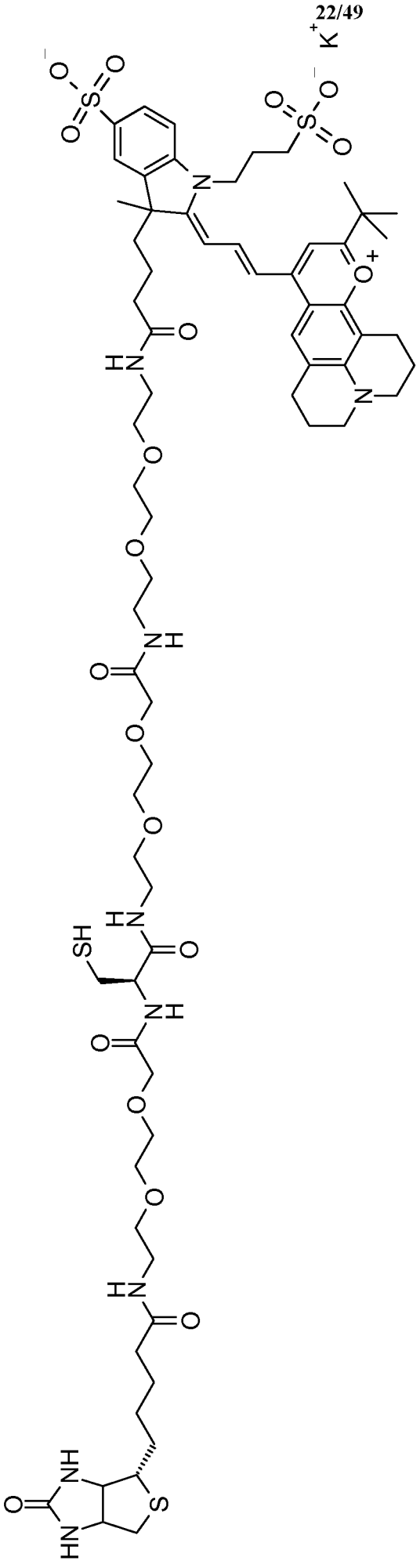


Figure 22

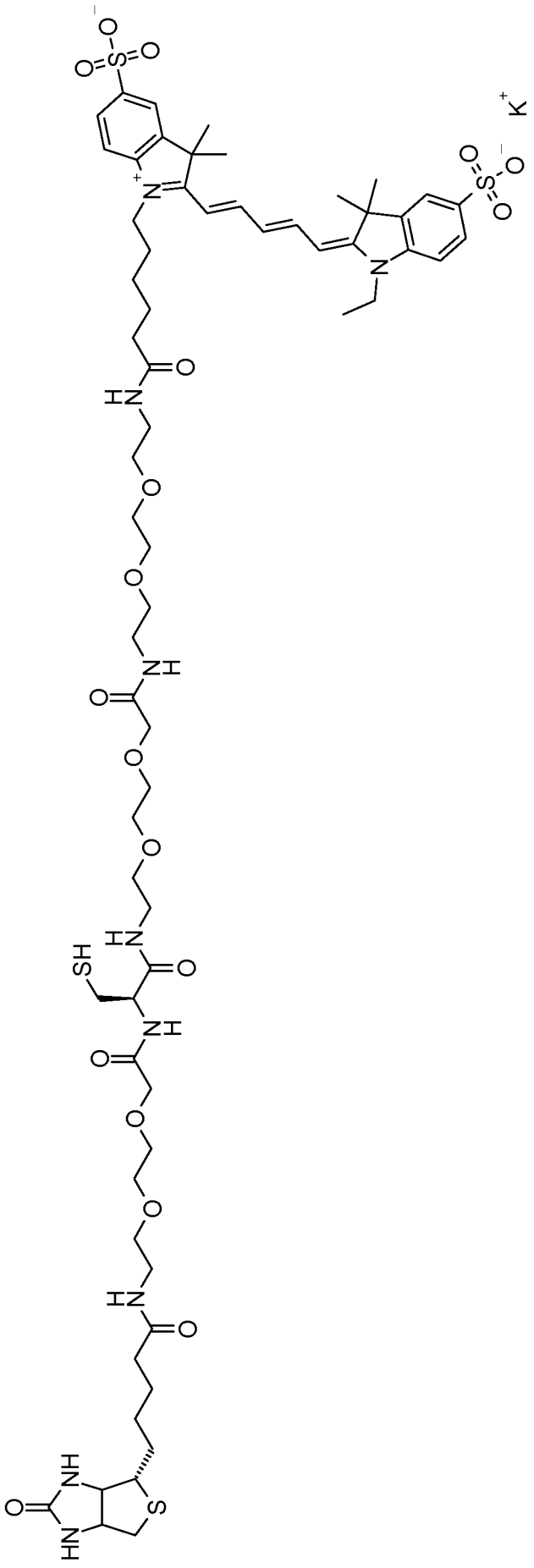


Figure 23

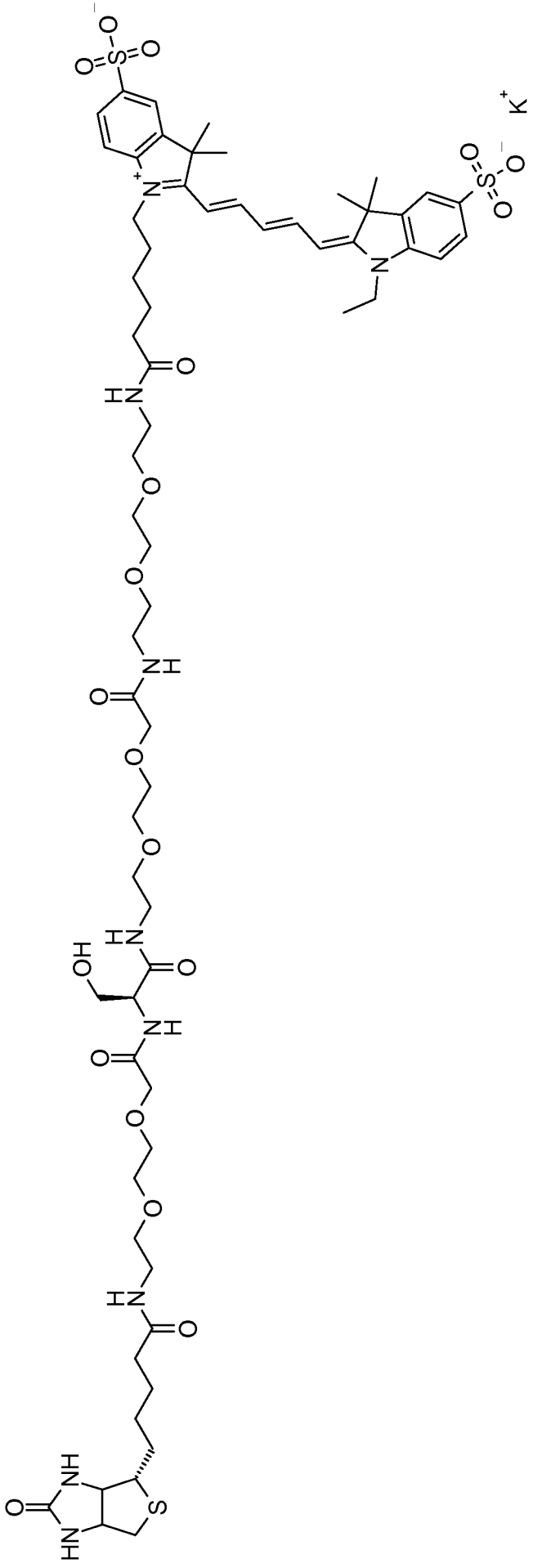


Figure 24

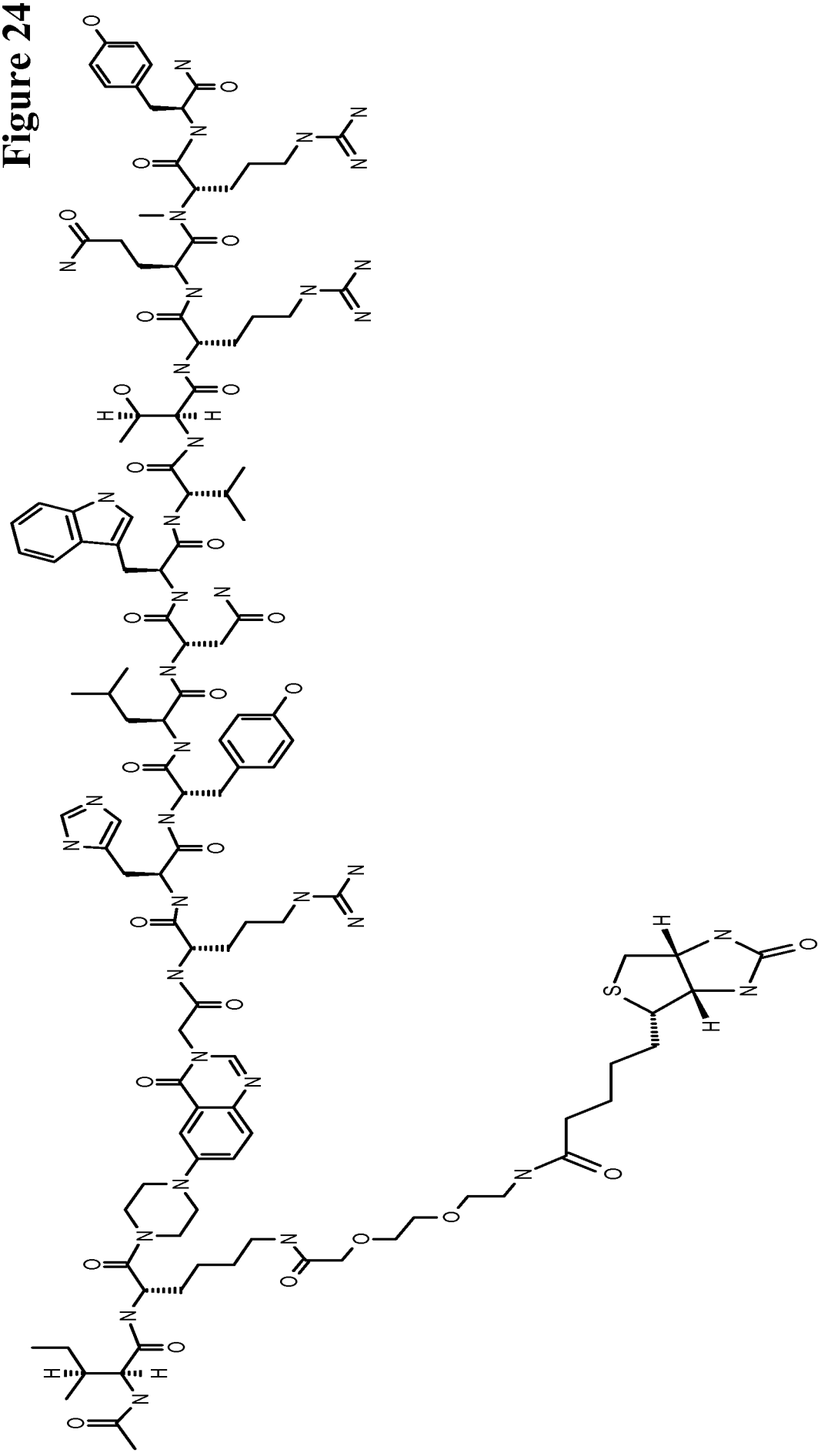


Figure 25

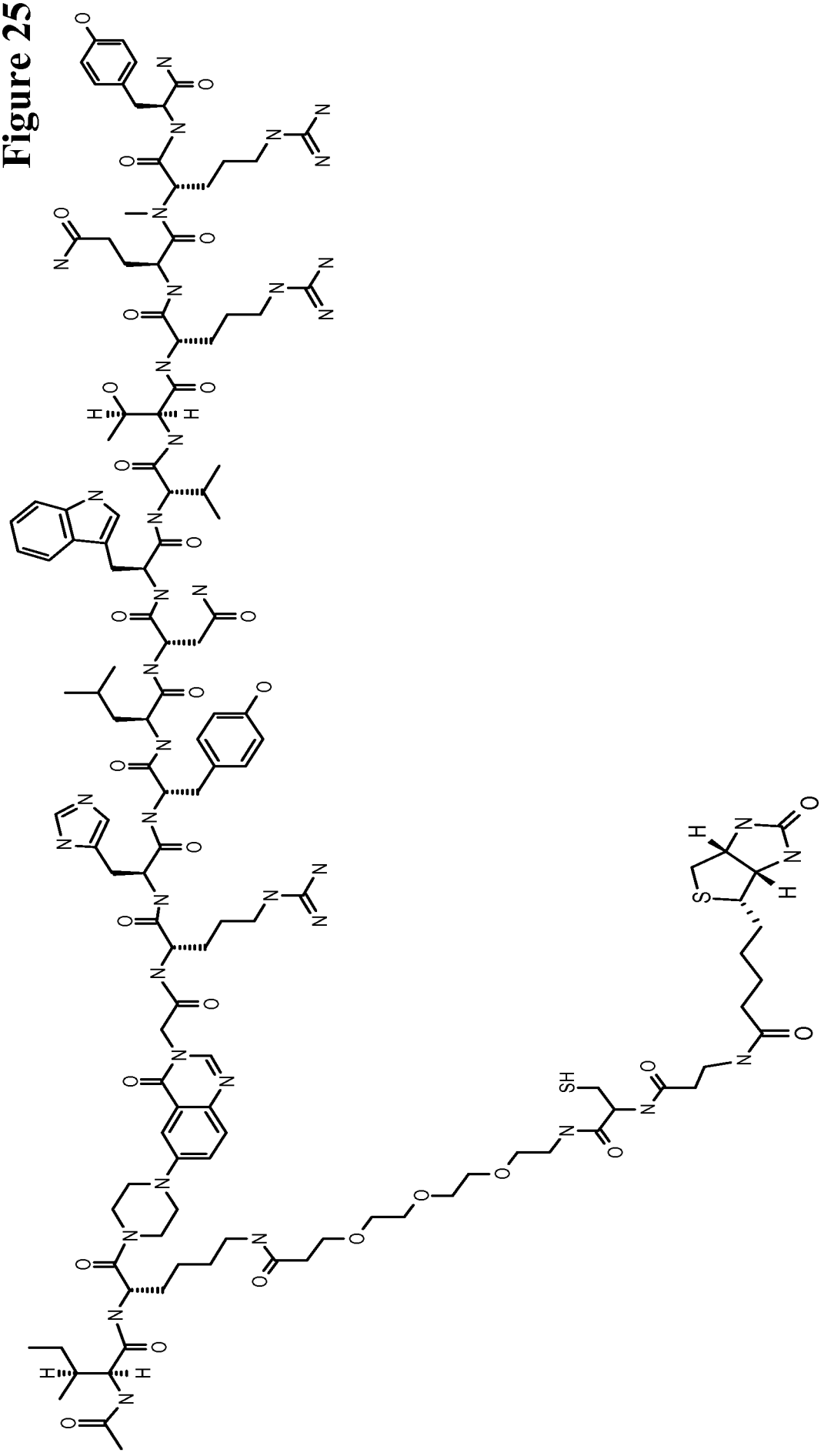


Figure 26

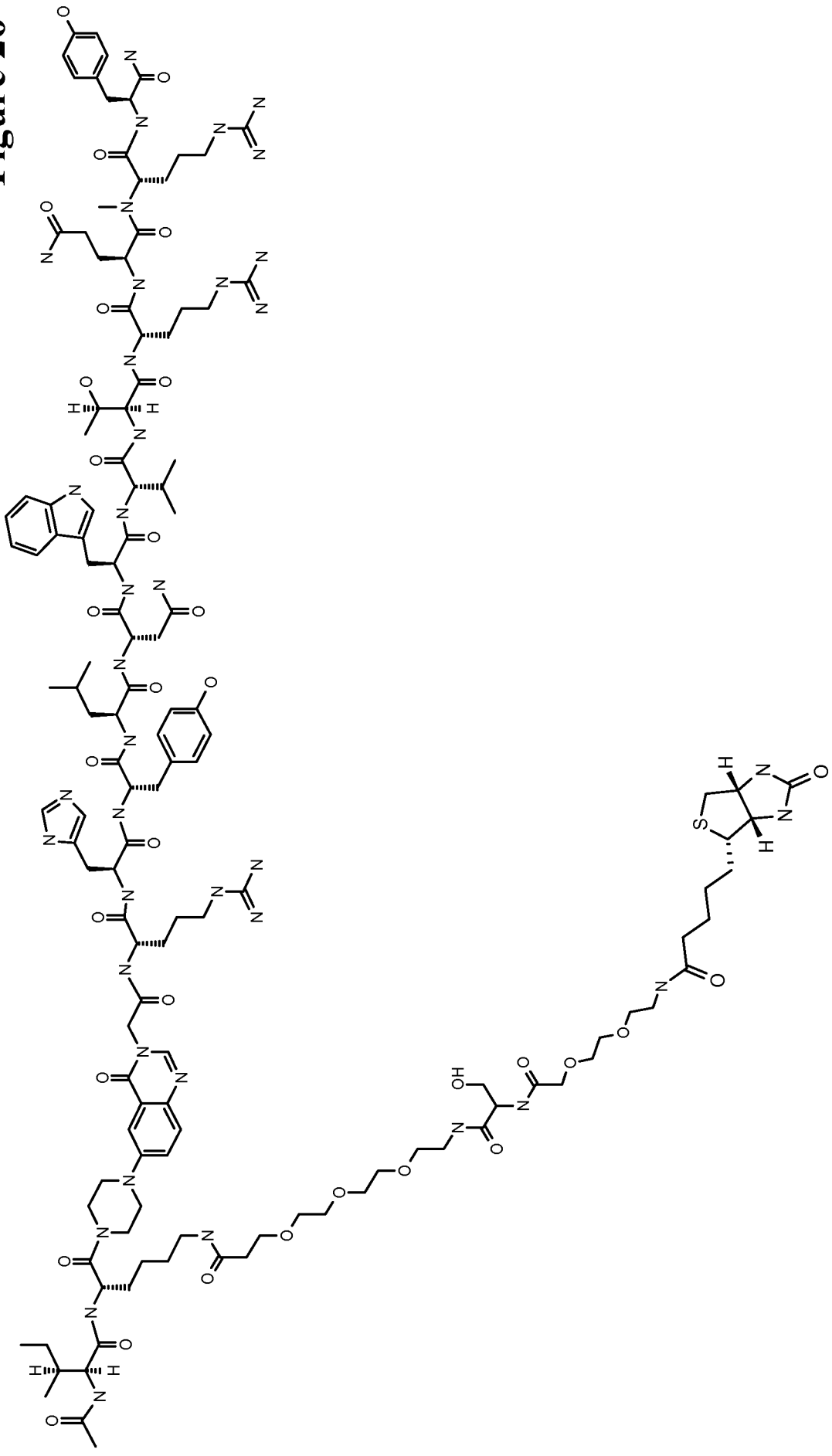


Figure 27

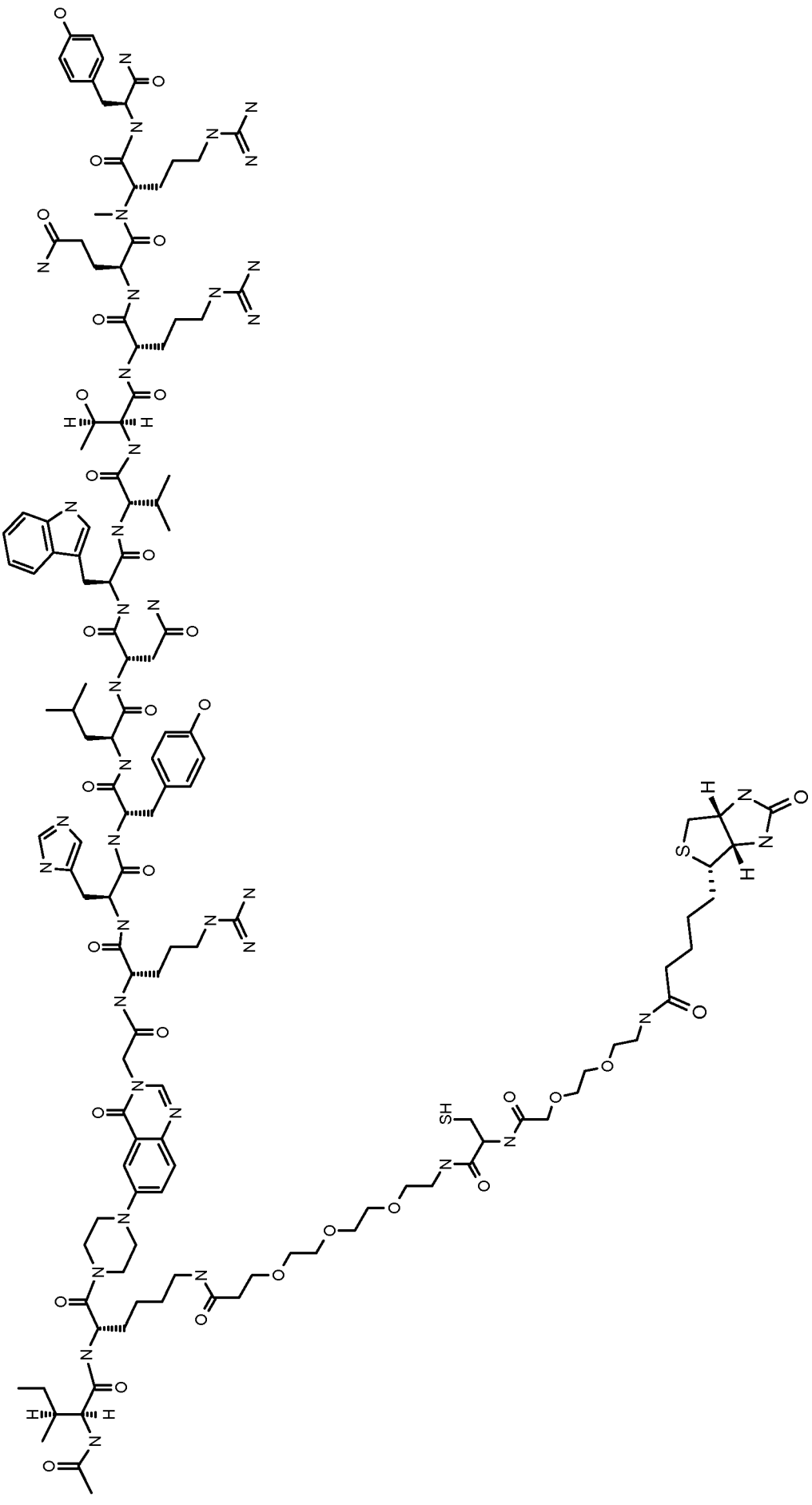


Figure 28

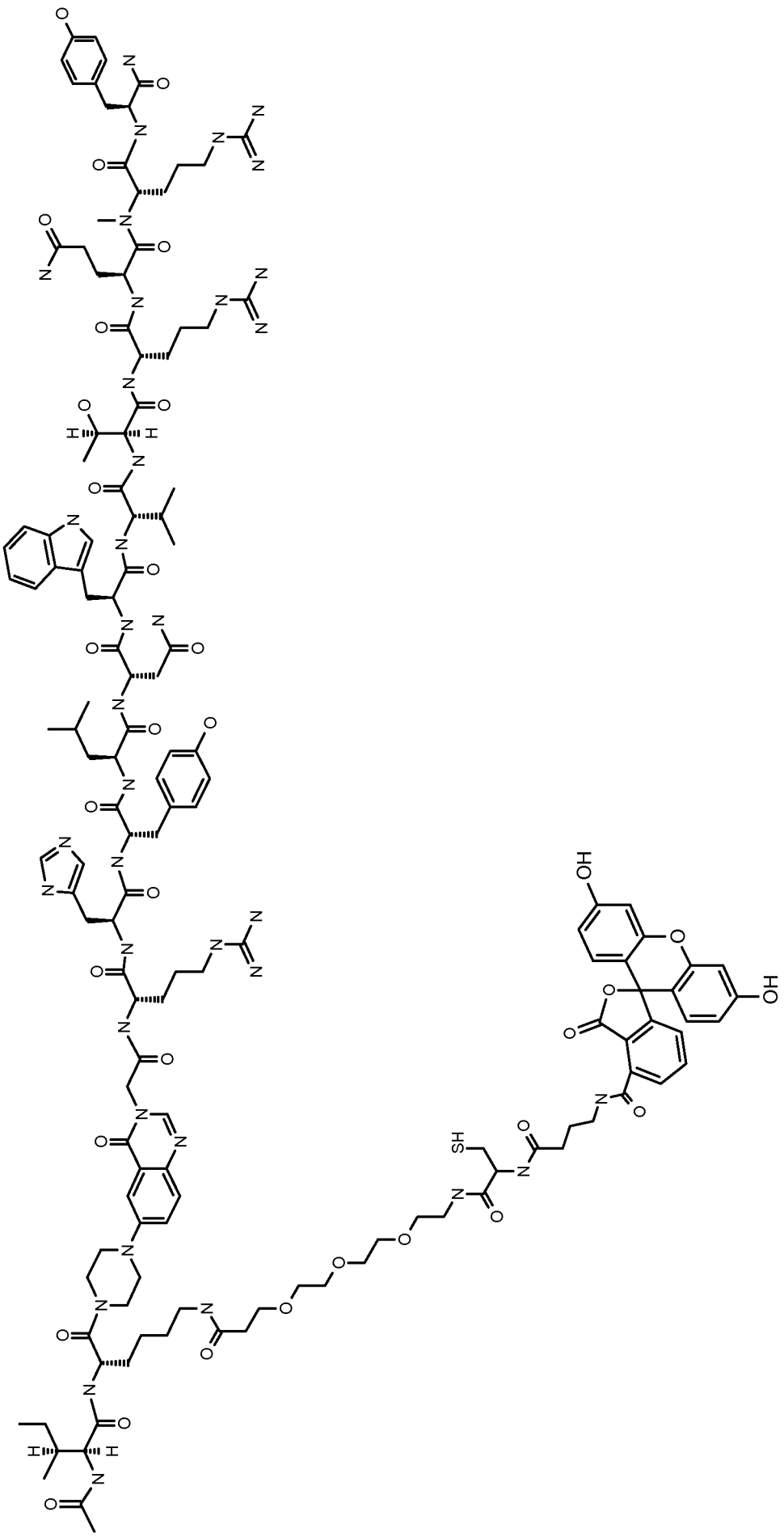


Figure 29

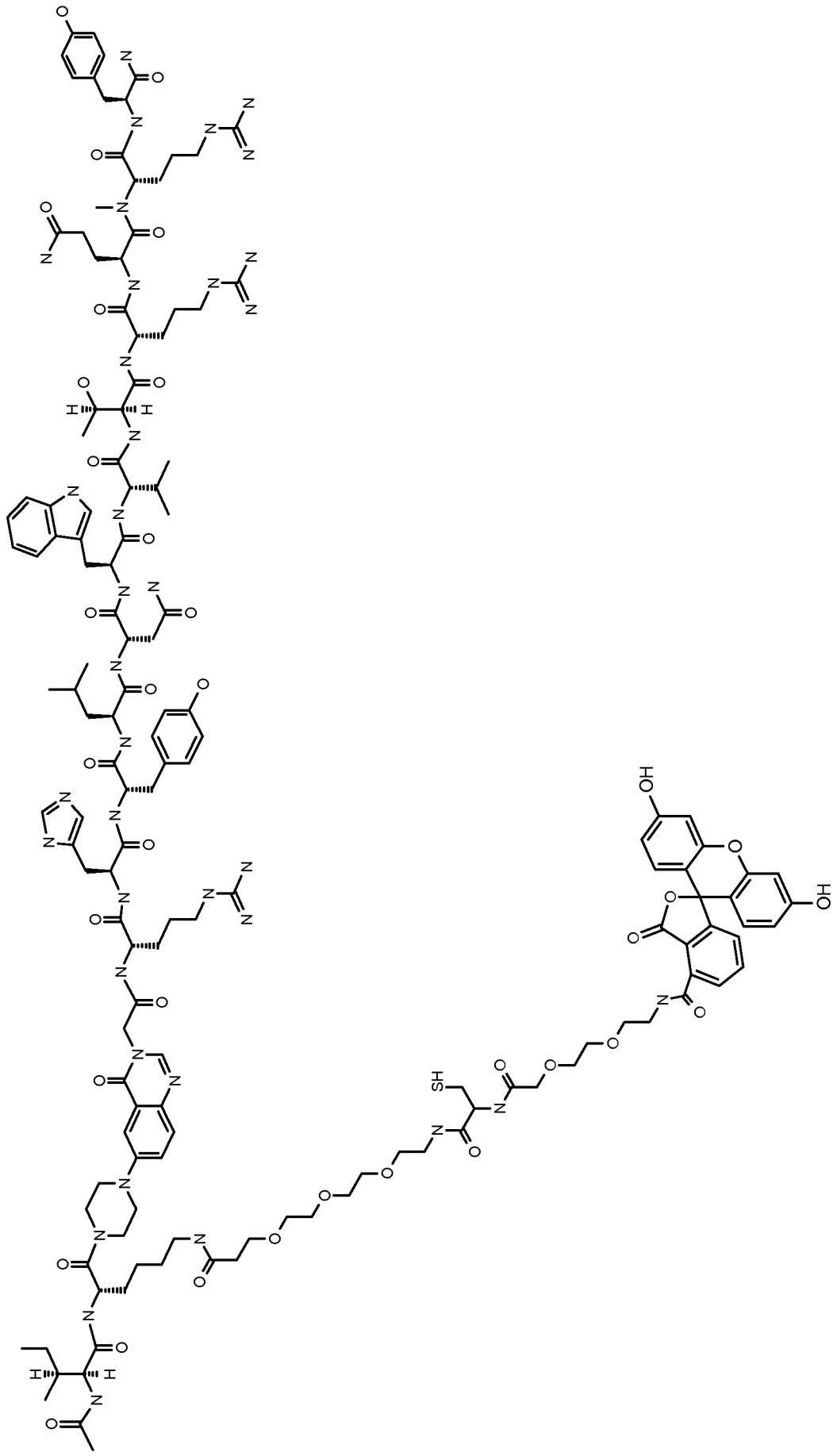


Figure 30

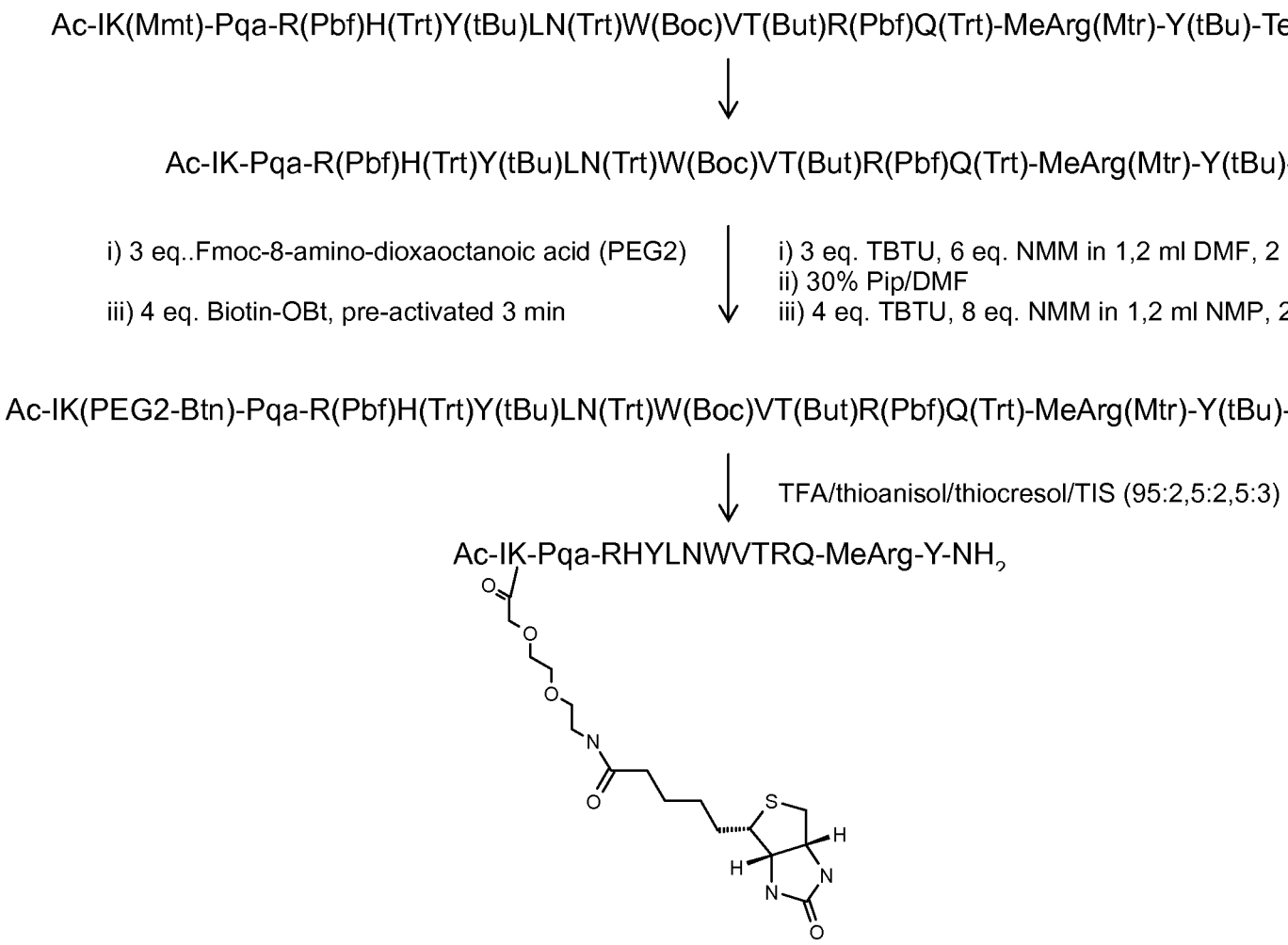

Ac-IK(Mmt)-Pqa-R(Pbf)H(Trt)Y(tBu)LN(Trt)W(Boc)VT(But)R(Pbf)Q(Trt)-MeArg(Mtr)-Y(tBu)-TentaGel S RAM resin
i) DCM/HFIP/TFE/TIS (6,5:2:1:0,5), 2 x 1h
ii) DMF wash
Ac-IK-Pqa-R(Pbf)H(Trt)Y(tBu)LN(Trt)W(Boc)VT(But)R(Pbf)Q(Trt)-MeArg(Mtr)-Y(tBu)-TentaGel S RAM resin
i) 3 eq..Fmoc-8-amino-dioxaoctanoic acid (PEG2)
iii) 4 eq. Biotin-OBt, pre-activated 3 min
i) 3 eq. TBTU, 6 eq. NMM in 1,2 ml DMF, 2 x 30 min
ii) 30% Pip/DMF
iii) 4 eq. TBTU, 8 eq. NMM in 1,2 ml NMP, 2 h
Ac-IK(PEG2-Btn)-Pqa-R(Pbf)H(Trt)Y(tBu)LN(Trt)W(Boc)VT(But)R(Pbf)Q(Trt)-MeArg(Mtr)-Y(tBu)-TentaGel S RAM resin
TFA/thioanisol/thiocresol/TIS (95:2,5:2,5:3)
Ac-IK-Pqa-RHYLNWVTRQ-MeArg-Y-NH2
O
O
O
N
O
S
H
H
N
N
O

Figure 31

Ac-IK-Pqa-R(Pbf)H(Trt)Y(tBu)LN(Trt)W(Boc)VT(But)R(Pbf)Q(Trt)-MeArg(Mtr)-Y(tBu)-TentaGel S RAM resin

i) 3 eq.. Fmoc-12-amino-4,7,10-trioxadodecanoic acid (PEG3) ↓ i) 3 eq. HATU, 3 eq. NMM in 1,2 ml DMF, 2 x 30 min; ii) 30% Pip/DMF

Ac-IK(PEG3)-Pqa-R(Pbf)H(Trt)Y(tBu)LN(Trt)W(Boc)VT(But)R(Pbf)Q(Trt)-MeArg(Mtr)-Y(tBu)-TentaGel S RAM resin

i) 2 eq. Fmoc-Cys(Trt)-OH
iii) 2 eq. Fmoc-ß-Ala-OH)
iv) 4 eq. Biotin-OBt, pre-activated 3 min
↓
i) 2 eq. TBTU, 2 eq. NMM, 2 x 30 min
ii) 30% Pip/DMF
iii) 2 eq. TBTU, 2 eq. NMM, 2 x 30 min
iv) 4 eq. TBTU, 8 eq. NMM in 1,2 ml NMP, 2 h

Ac-IK(PEG3-C(Trt)-ß-A-Btn)-Pqa-R(Pbf)H(Trt)Y(tBu)LN(Trt)W(Boc)VT(But)R(Pbf)Q(Trt)-MeArg(Mtr)-Y(tBu)-TentaGel S RAM resin

↓ TFA/thioanisol/thiocresol/TIS (95:2,5:2,5:3)

Ac-IK-Pqa-RHYLNWVTRQ-MeArg-Y-NH_2

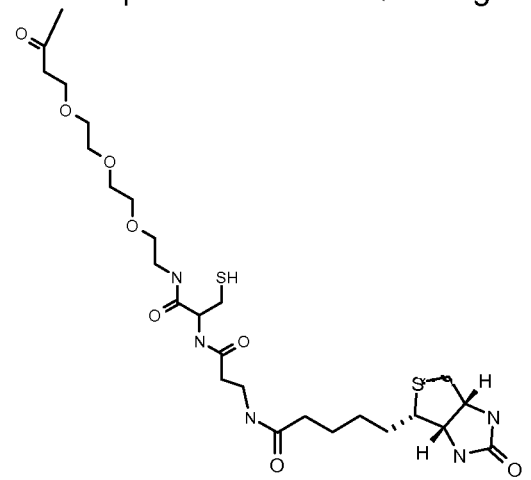


Figure 32

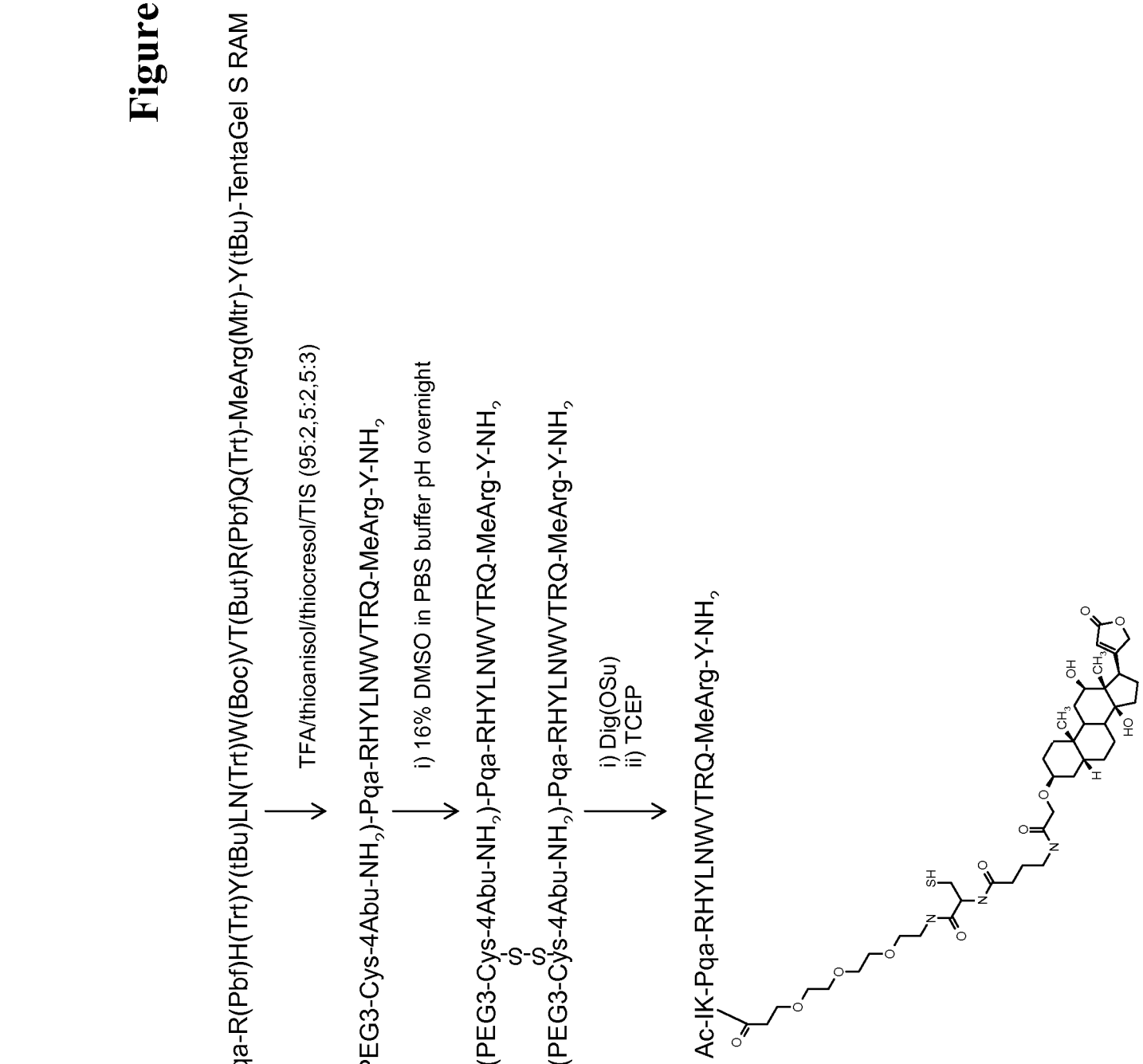

Ac-IK(PEG3-C(Trt)-4Abu-NH2)-Pqa-R(Pbf)H(Trt)Y(tBu)LN(Trt)W(Boc)VT(But)R(Pbf)Q(Trt)-MeArg(Mtr)-Y(tBu)-TentaGel S RAM resin
TFA/thioanisol/thiocresol/TIS (95:2,5:2,5:3)
Ac-IK(PEG3-Cys-4Abu-NH2)-Pqa-RHYLNWVTRQ-MeArg-Y-NH2
i) 16% DMSO in PBS buffer pH overnight
Ac-IK(PEG3-Cys-4Abu-NH2)-Pqa-RHYLNWVTRQ-MeArg-Y-NH2
S-S
Ac-IK(PEG3-Cys-4Abu-NH2)-Pqa-RHYLNWVTRQ-MeArg-Y-NH2
i) Dig(OSu)
ii) TCEP
Ac-IK-Pqa-RHYLNWVTRQ-MeArg-Y-NH2

Figure 33

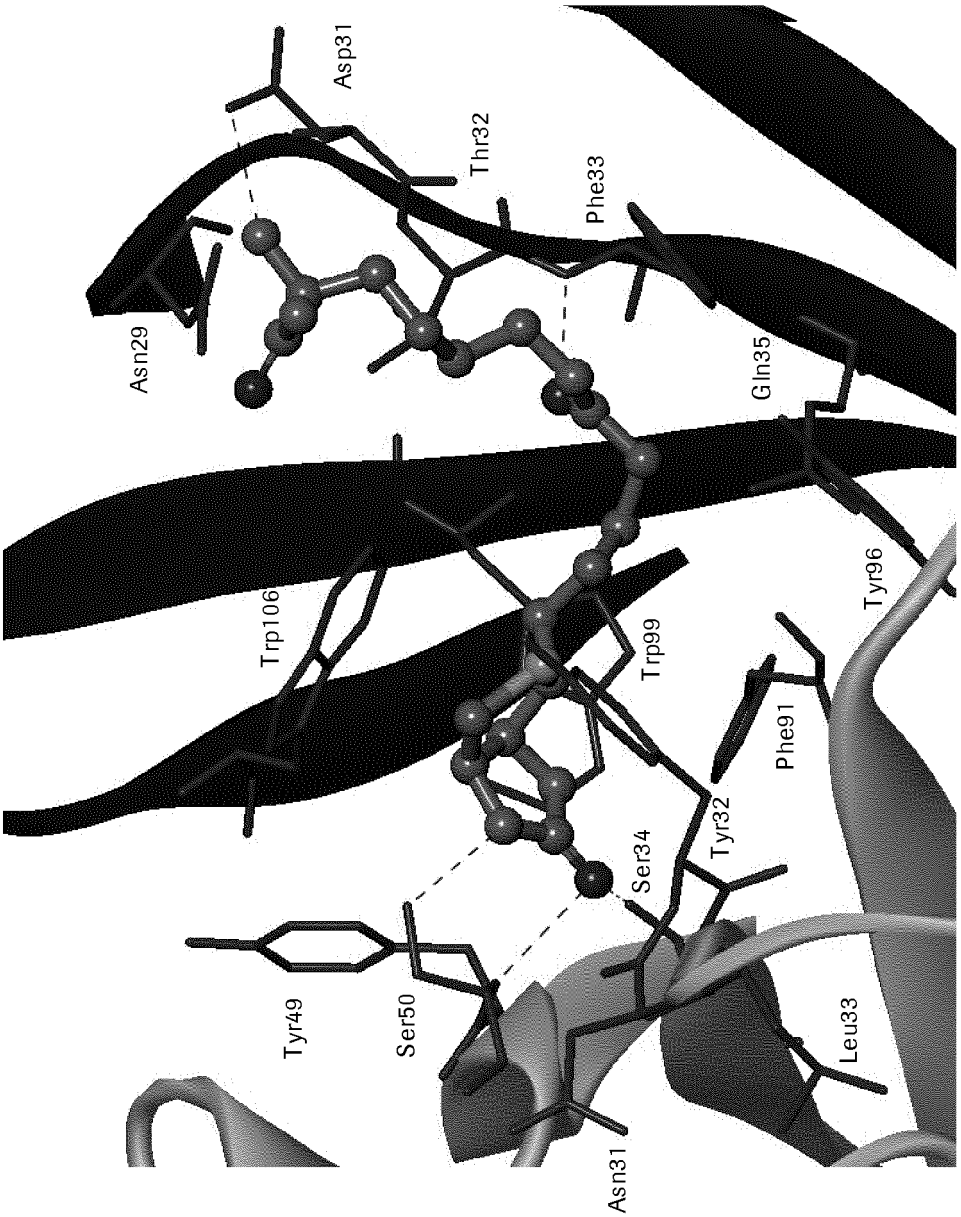

Asn29
Asp31
Thr32
Phe33
Gln35
Trp106
Trp99
Tyr96
Phe91
Tyr32
Ser34
Tyr49
Ser50
Leu33
Asn31

Figure 34A

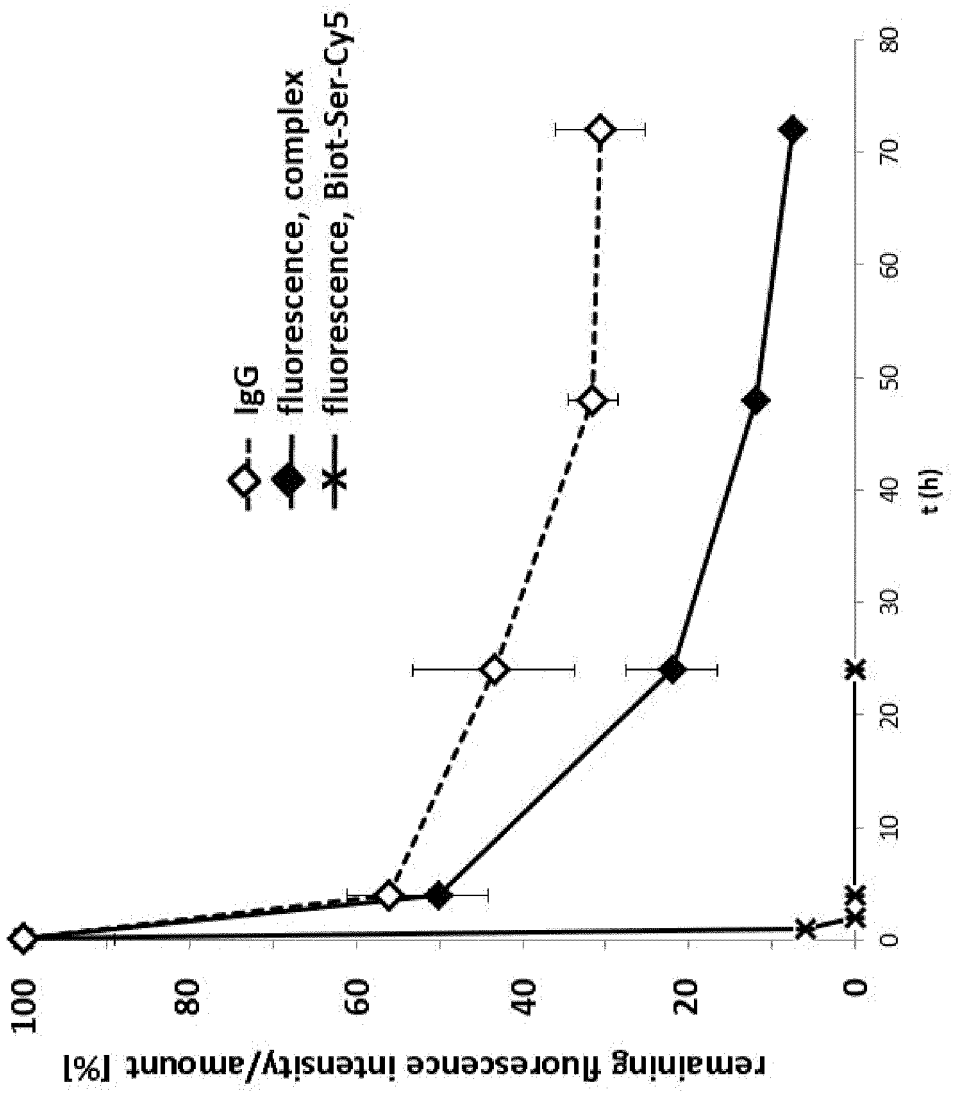

remaining fluorescence intensity/amount [%]
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
t (h)
IgG
fluorescence, complex
fluorescence, Biot-Ser-Cy5

Figure 34B

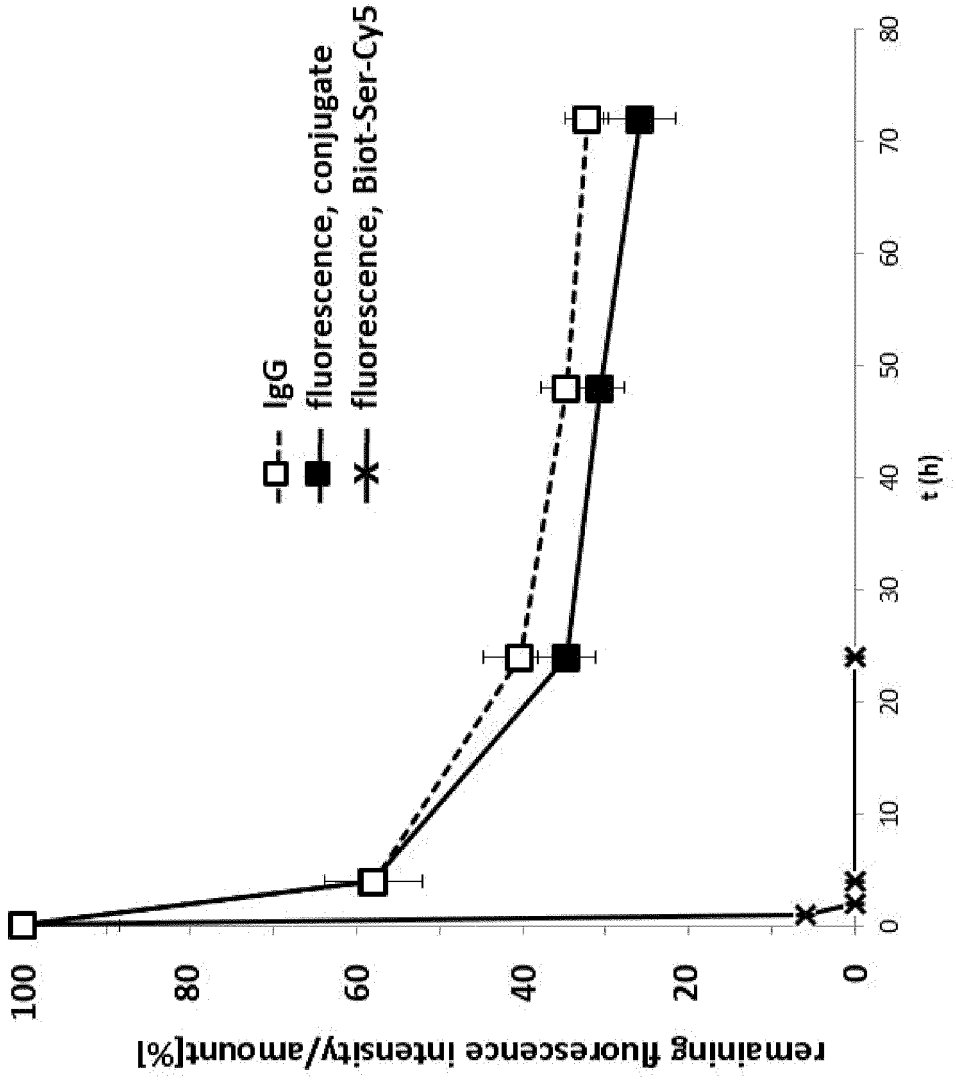
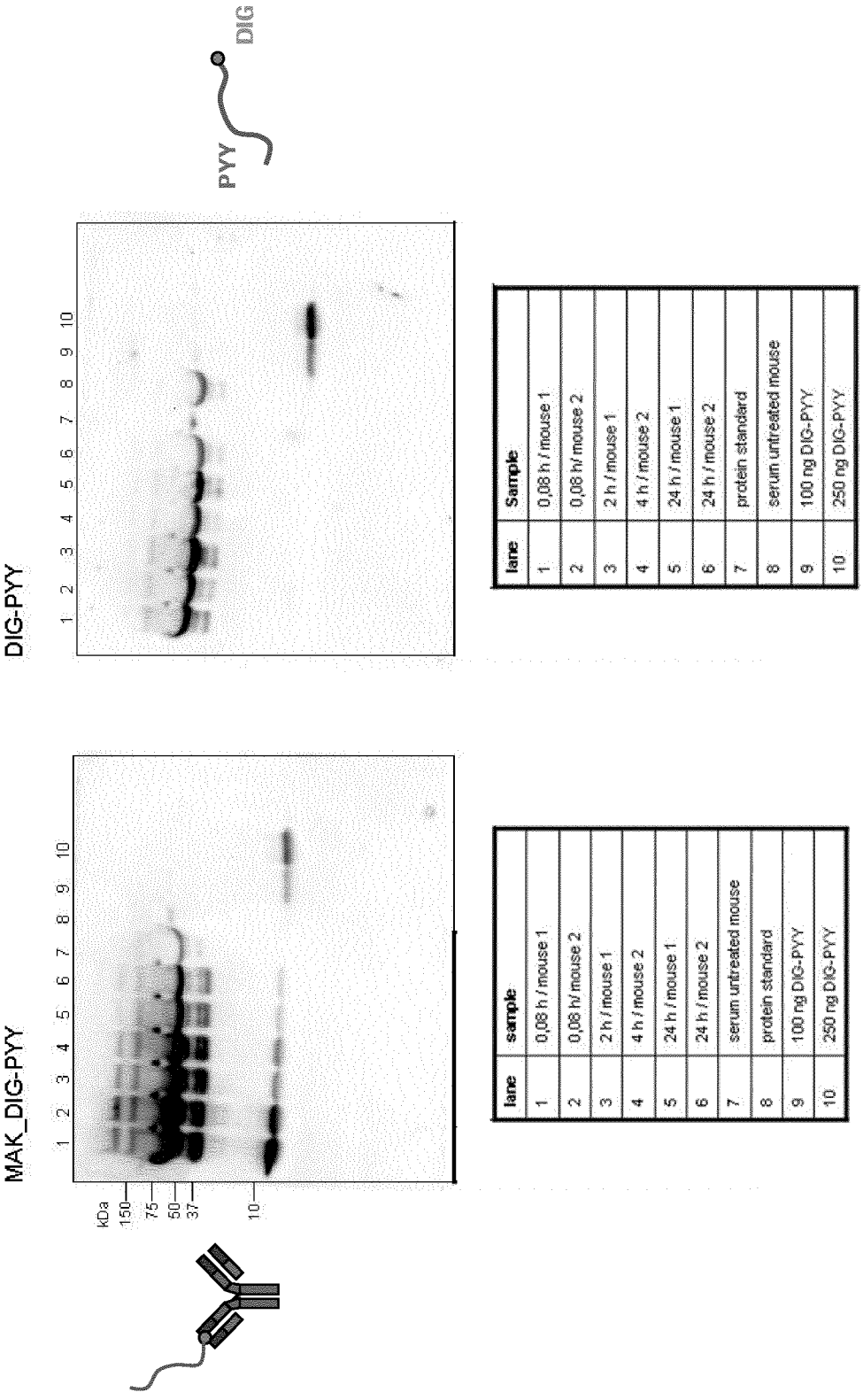

IgG
fluorescence, conjugate
fluorescence, Biot-Ser-Cy5
remaining fluorescence intensity/amount[%]
t (h)
0
20
40
60
80
100
0
10
20
30
40
50
60
70
80

Figure 35



lane	sample
1	0,08 h / mouse 1
2	0,08 h/ mouse 2
3	2 h / mouse 1
4	4 h / mouse 2
5	24 h / mouse 1
6	24 h / mouse 2
7	serum untreated mouse
8	protein standard
9	100 ng DIG-PYY
10	250 ng DIG-PYY

lane	Sample
1	0,08 h / mouse 1
2	0,08 h/ mouse 2
3	2 h / mouse 1
4	4 h / mouse 2
5	24 h / mouse 1
6	24 h / mouse 2
7	protein standard
8	serum untreated mouse
9	100 ng DIG-PYY
10	250 ng DIG-PYY

Figure 36

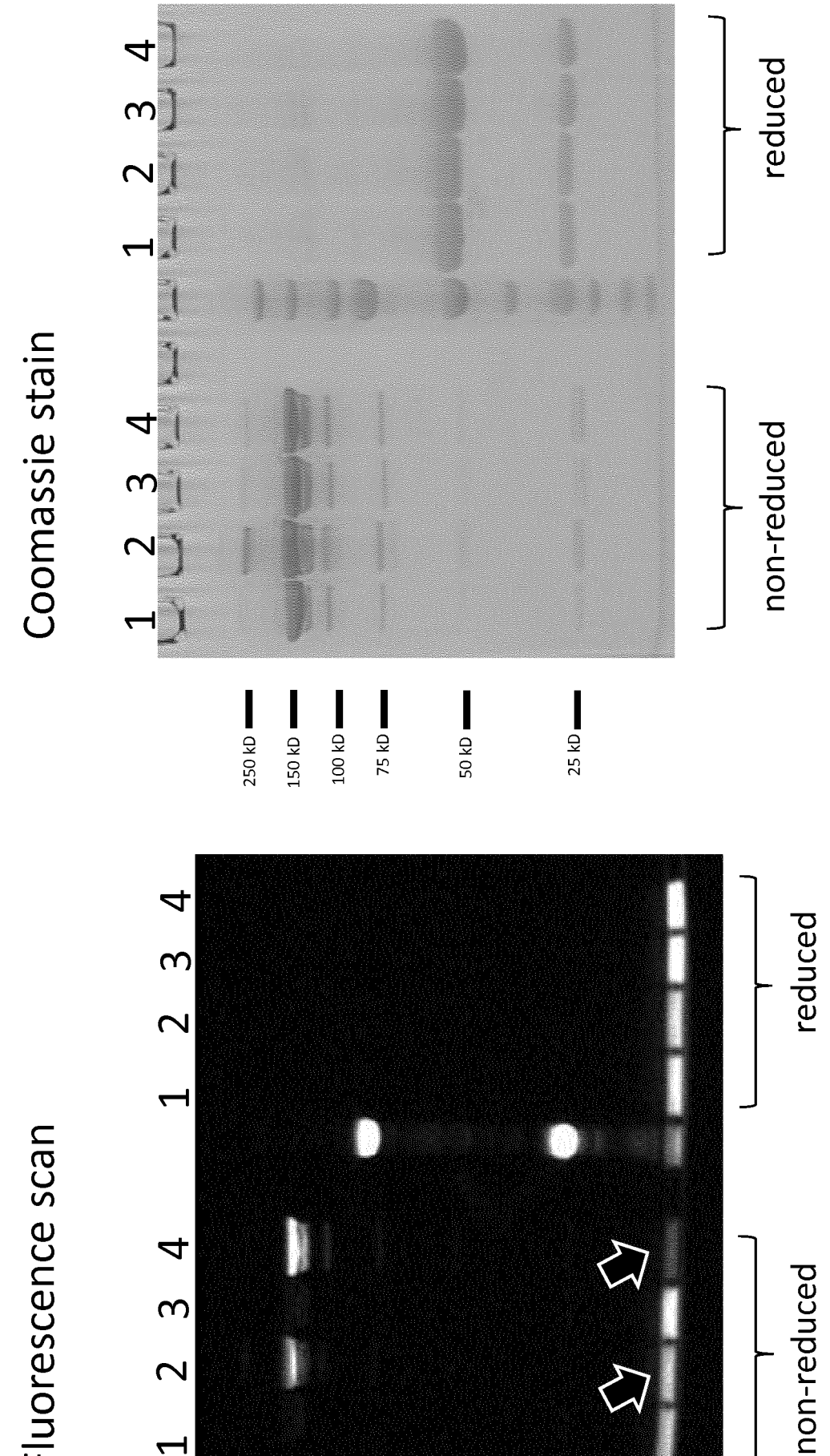

Coomassie stain
1 2 3 4
1 2 3 4
non-reduced
reduced
250 kD
150 kD
100 kD
75 kD
50 kD
25 kD
Fluorescence scan
1 2 3 4
1 2 3 4
non-reduced
reduced

Figure 37

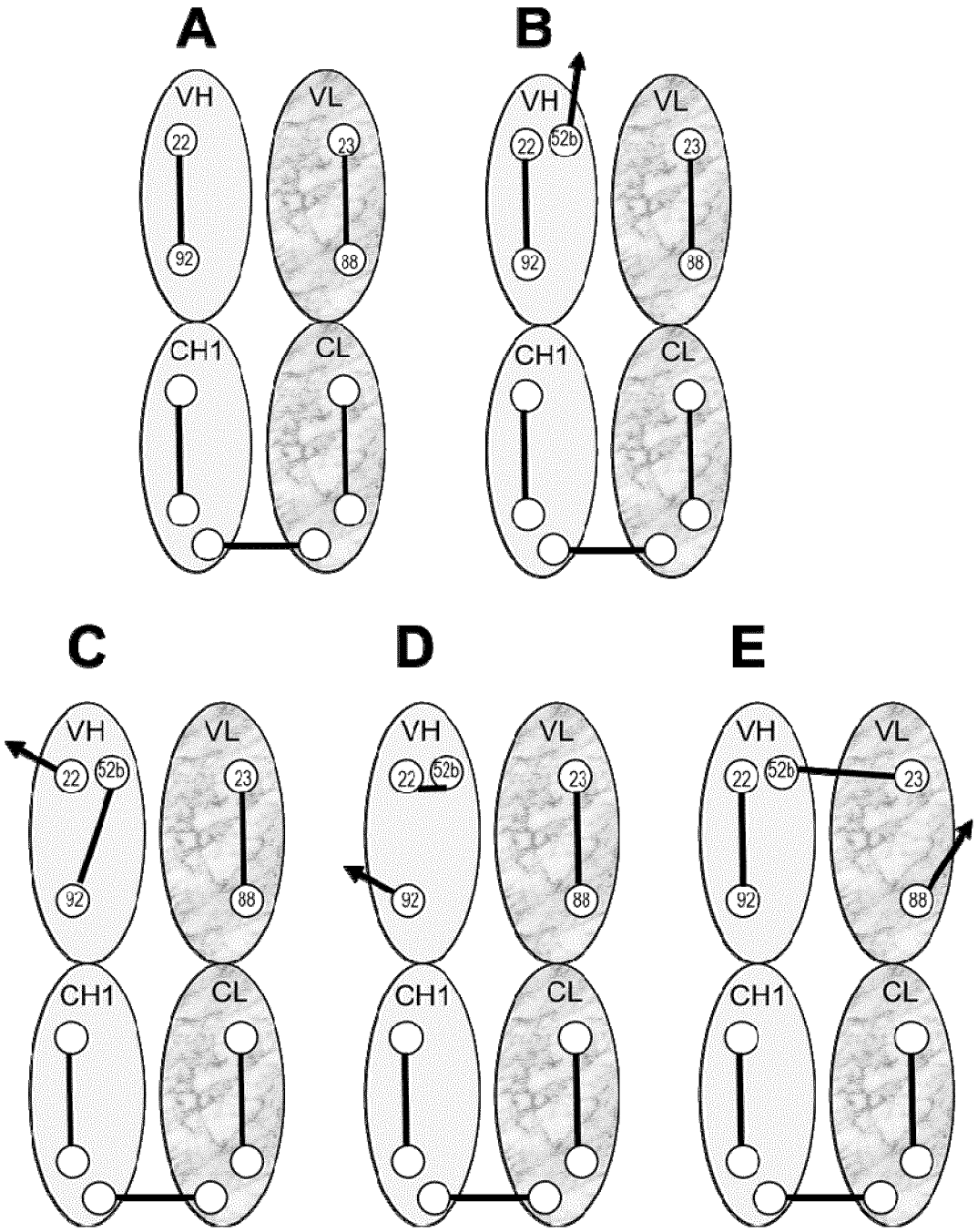

A
VH
VL
22
23
92
88
CH1
CL
B
VH
VL
22
52b
23
92
88
CH1
CL
C
VH
VL
22
52b
23
92
88
CH1
CL
D
VH
VL
22
52b
23
92
88
CH1
CL
E
VH
VL
22
52b
23
92
88
CH1
CL

Figure 38

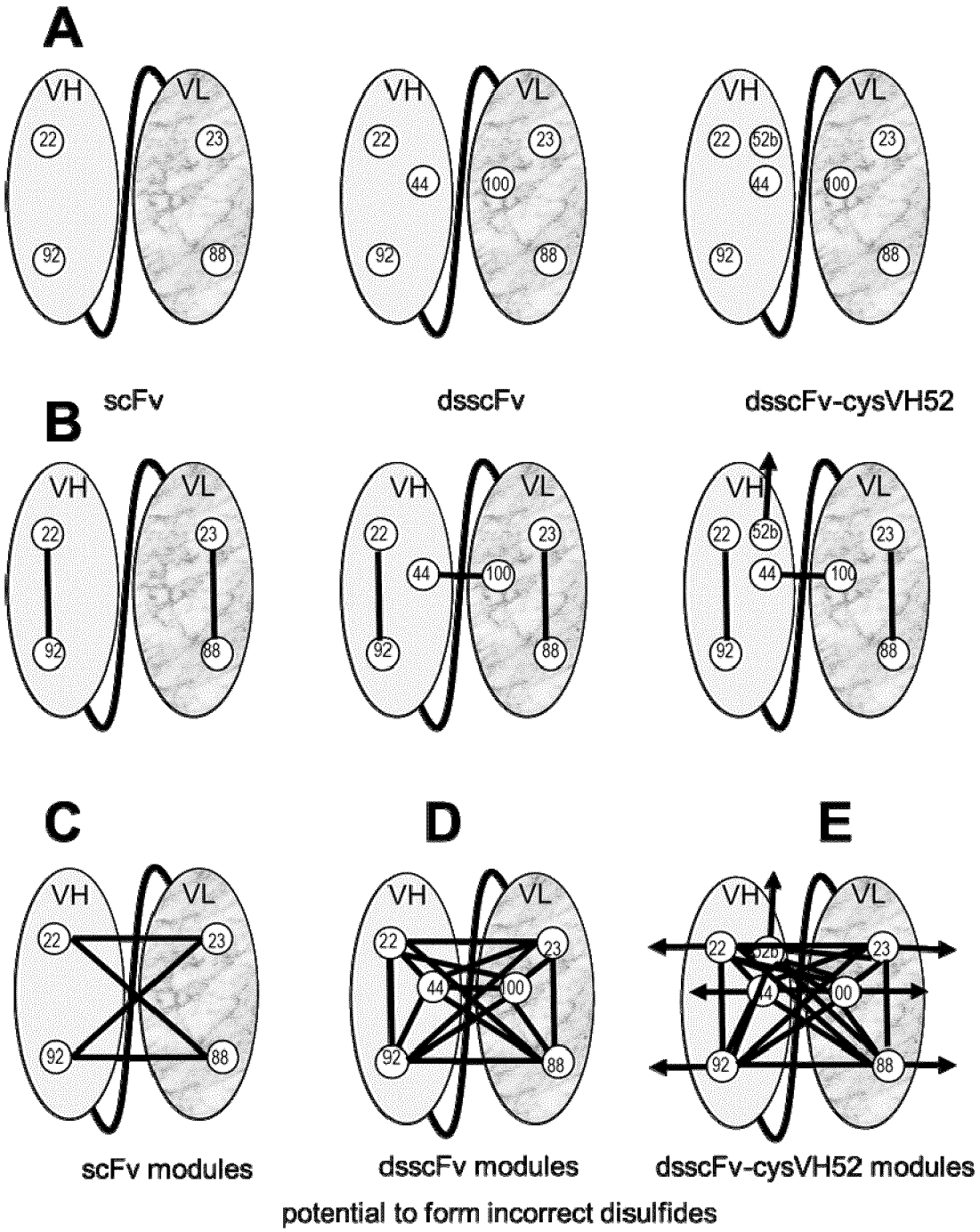

A
VH
VL
22
23
92
88
44
100
52b
scFv
dsscFv
dsscFv-cysVH52
B
C
D
E
scFv modules
dsscFv modules
dsscFv-cysVH52 modules
potential to form incorrect disulfides

Figure 39

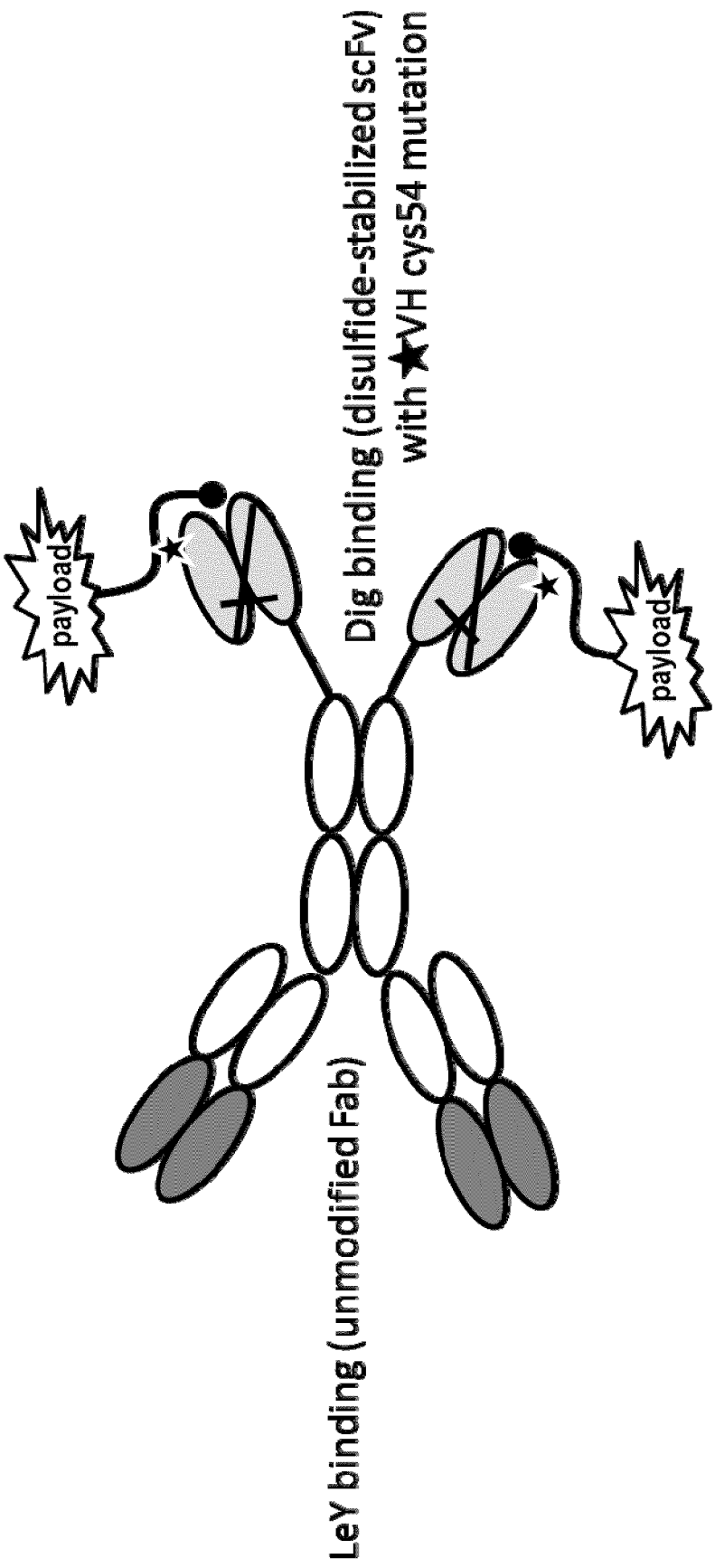
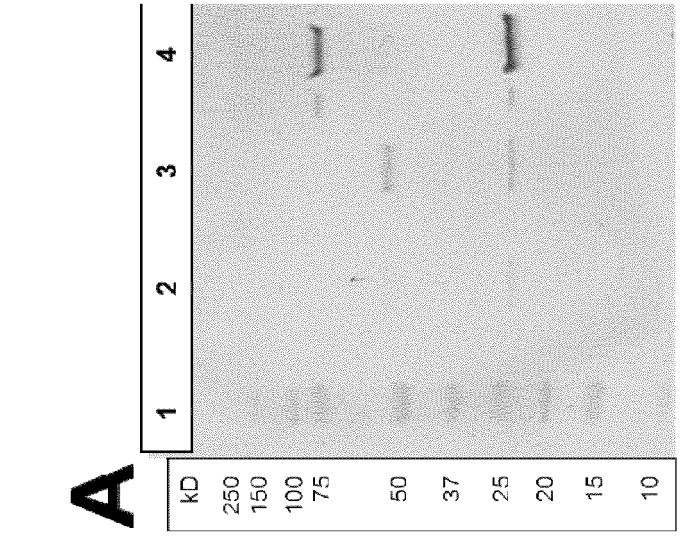
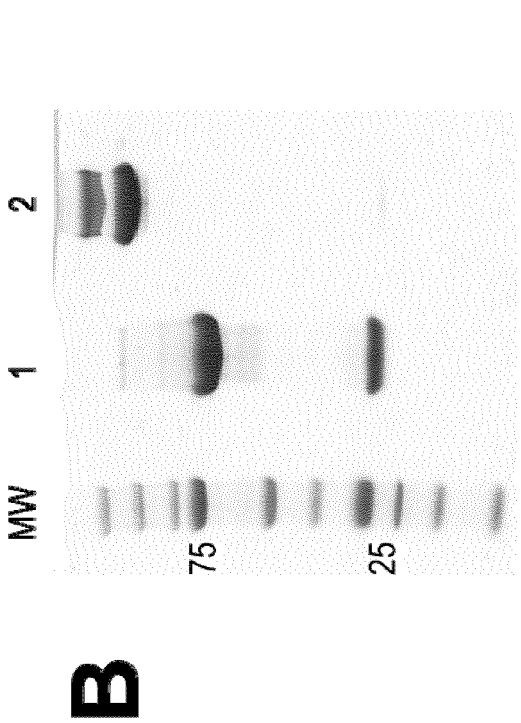

payload
Dig binding (disulfide-stabilized scFv)
with ★VH cys54 mutation
payload
LeY binding (unmodified Fab)

Figure 40


A
1
2
3
4
kD
250
150
100
75
50
37
25
20
15
10


B
MW
1
2
75
25

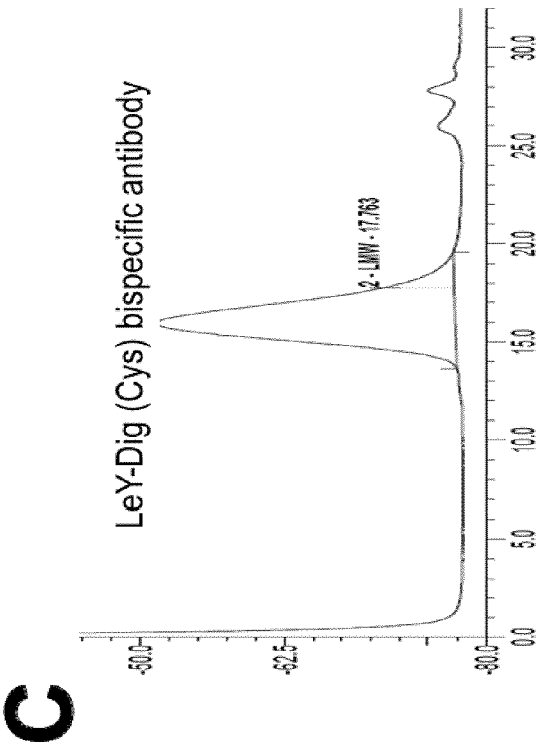
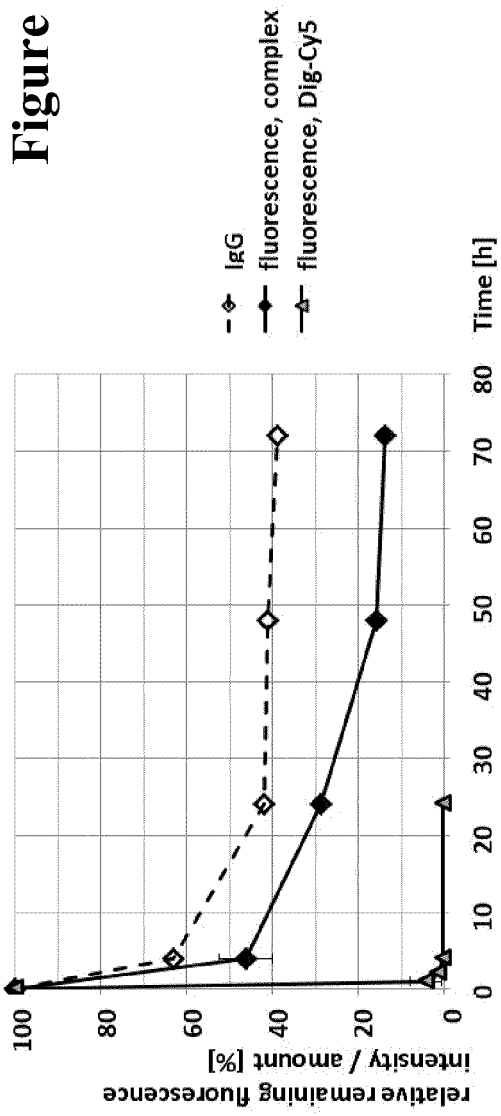

C
LeY-Dig (Cys) bispecific antibody
2 - LMW - 17.763
-50.0
-62.5
-80.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0

Figure 41


IgG
fluorescence, complex
fluorescence, Dig-Cy5
Time [h]
relative remaining fluorescence intensity / amount [%]
0
20
40
60
80
100
0
10
20
30
40
50
60
70
80

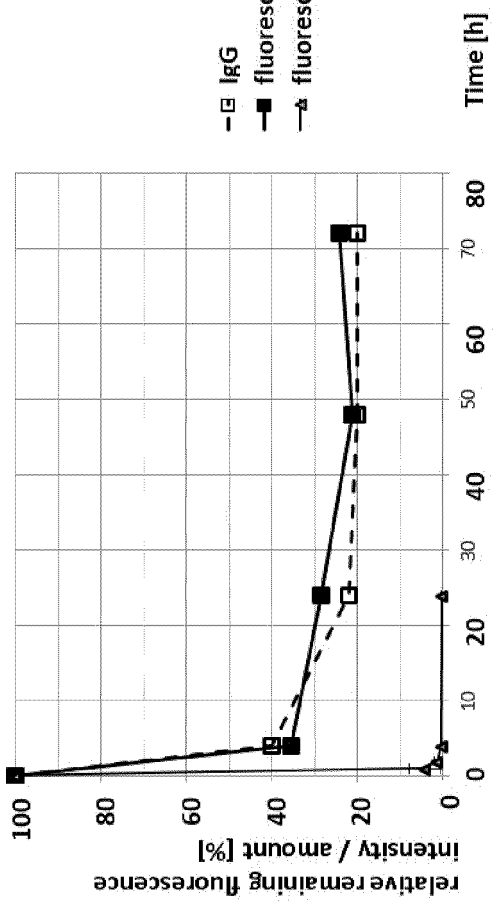

IgG
fluorescence, conjugate
fluorescence, Dig-Cy5
Time [h]
relative remaining fluorescence intensity / amount [%]
0
20
40
60
80
100
0
10
20
30
40
50
60
70
80

Figure 42

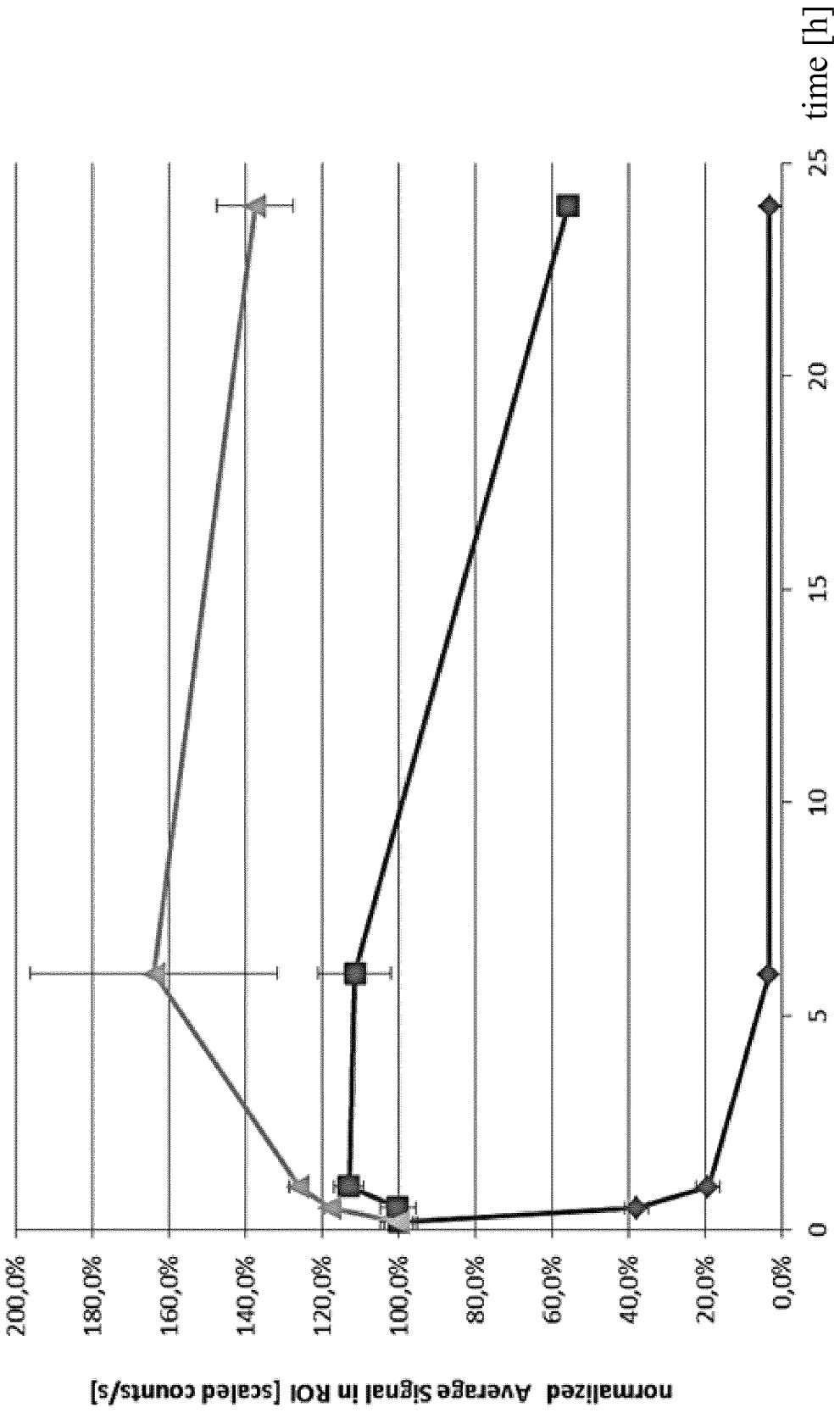
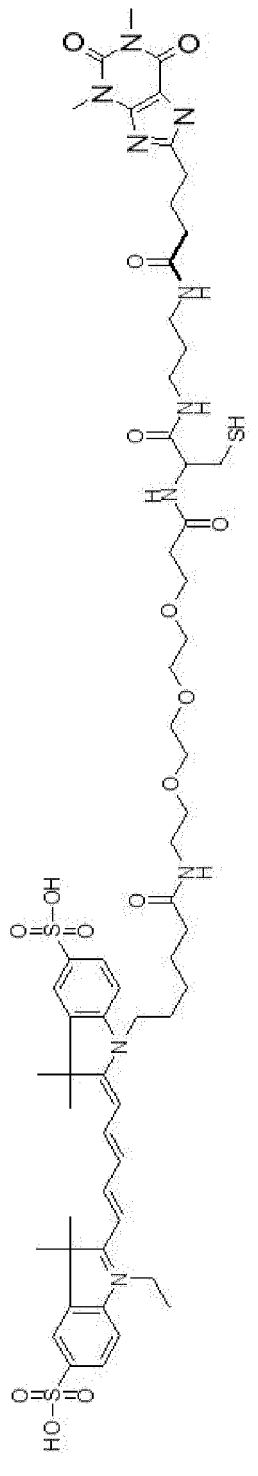

normalized Average Signal in ROI [scaled counts/s]
200,0%
180,0%
160,0%
140,0%
120,0%
100,0%
80,0%
60,0%
40,0%
20,0%
0,0%
0
5
10
15
20
25
time [h]

Figure 43

a)



Formula Weight : 1269,55(5)
Formula : $C_{59}H_{84}N_{10}O_{15}S_3$

b)

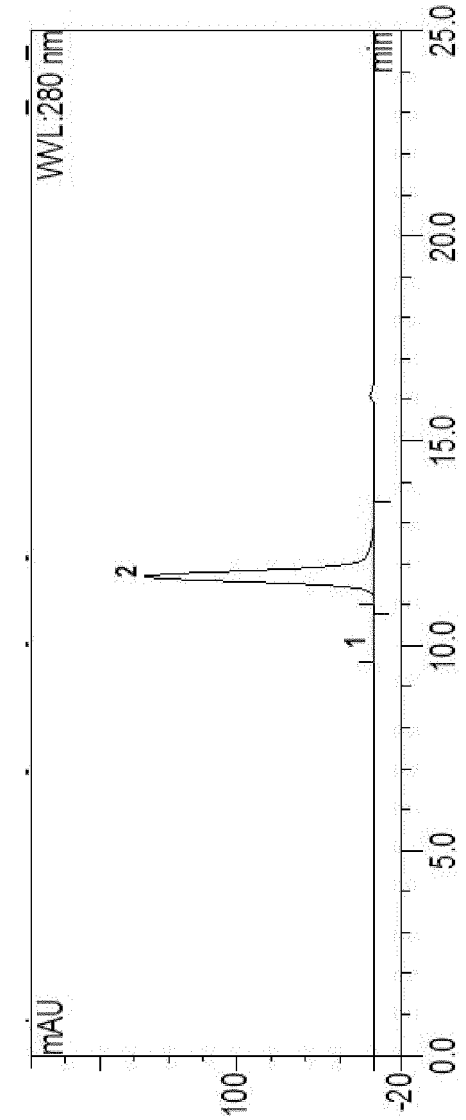


Figure 43 continued

c)

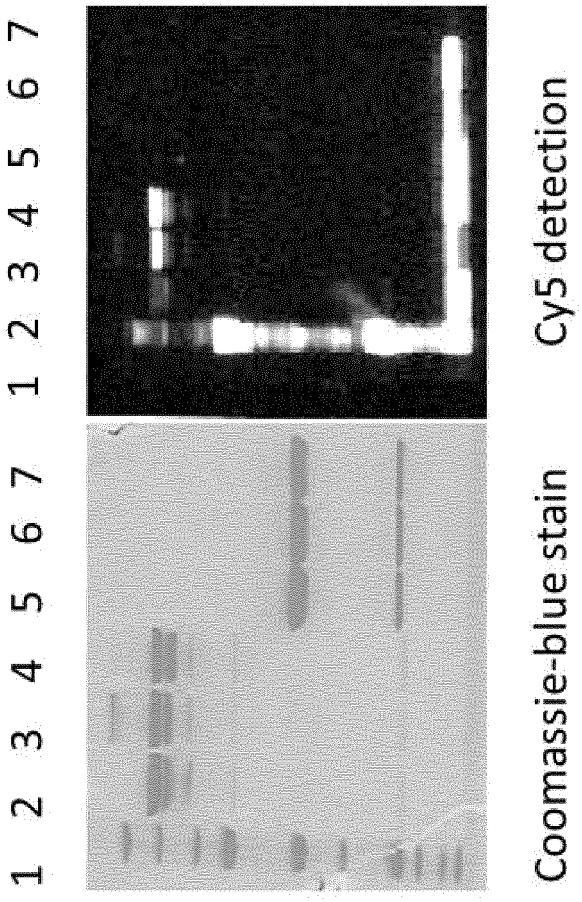


Figure 44

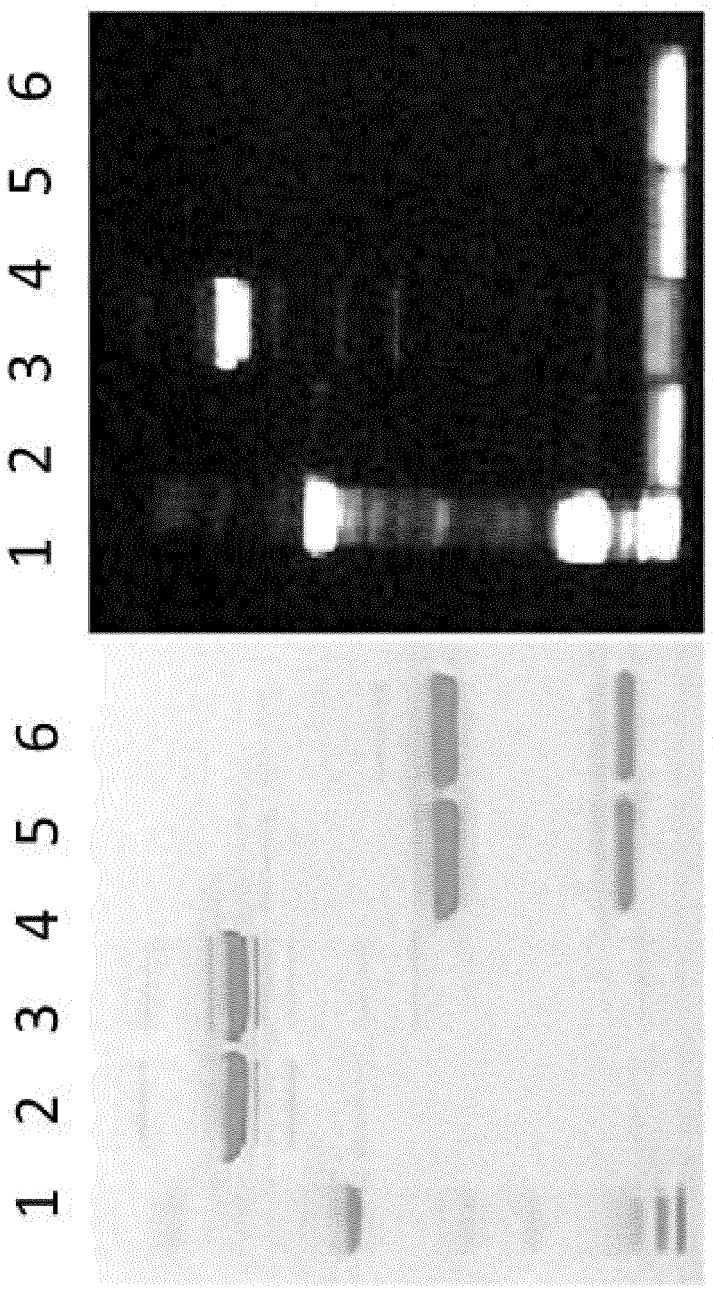


Figure 45

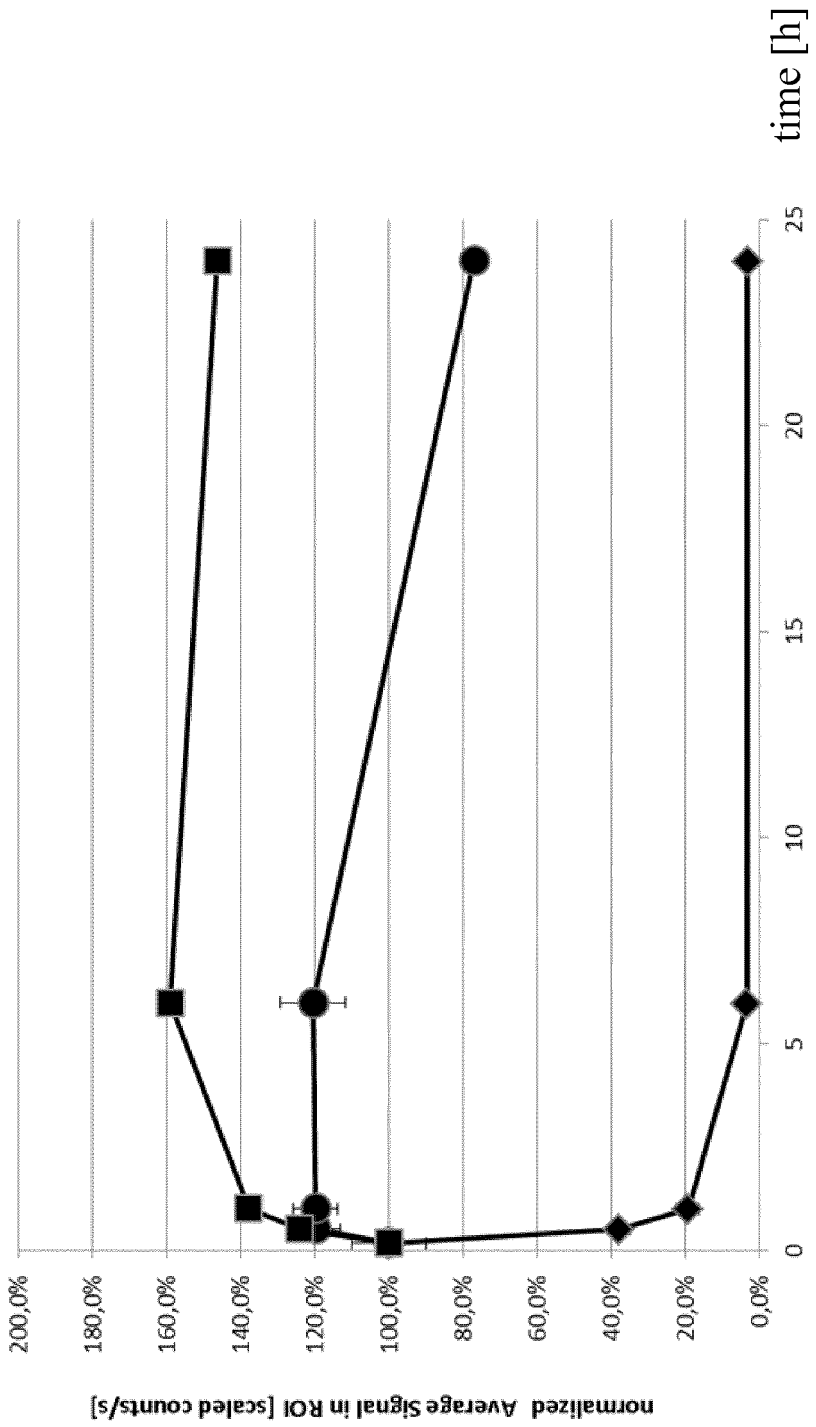
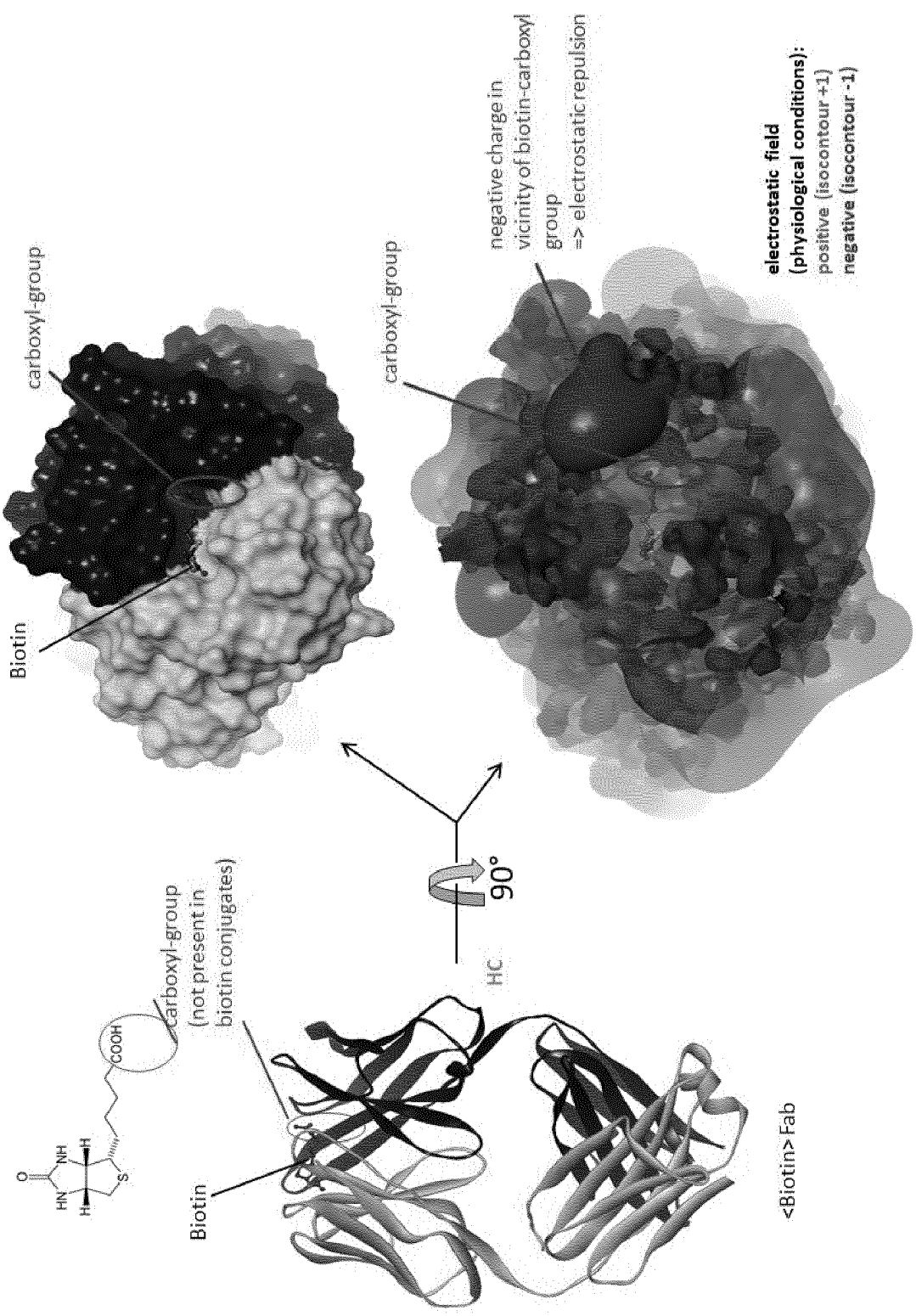

normalized Average Signal in ROI [scaled counts/s]
200,0%
180,0%
160,0%
140,0%
120,0%
100,0%
80,0%
60,0%
40,0%
20,0%
0,0%
0
5
10
15
20
25
time [h]

Figure 46


carboxyl-group
Biotin
carboxyl-group
negative charge in vicinity of biotin-carboxyl group
=> electrostatic repulsion
electrostatic field
(physiological conditions):
positive (isocontour +1)
negative (isocontour -1)
90°
HC
carboxyl-group (not present in biotin conjugates)
Biotin
<Biotin> Fab