Figure 1

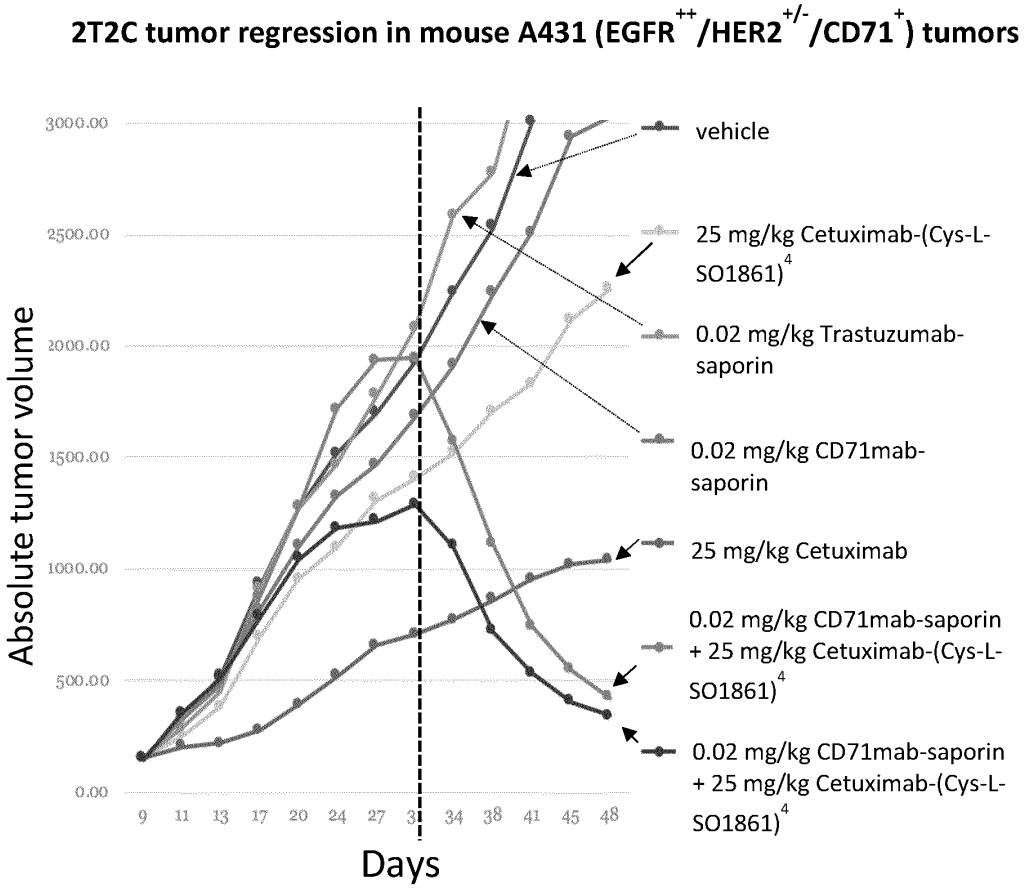

2T2C tumor regression in mouse A431 (EGFR++/HER2+/-/CD71+) tumors
Absolute tumor volume
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
9 11 13 17 20 24 27 31 34 38 41 45 48
Days
vehicle
25 mg/kg Cetuximab-(Cys-L-SO1861)4
0.02 mg/kg Trastuzumab-saporin
0.02 mg/kg CD71mab-saporin
25 mg/kg Cetuximab
0.02 mg/kg CD71mab-saporin + 25 mg/kg Cetuximab-(Cys-L-SO1861)4
0.02 mg/kg CD71mab-saporin + 25 mg/kg Cetuximab-(Cys-L-SO1861)4

Figure 2

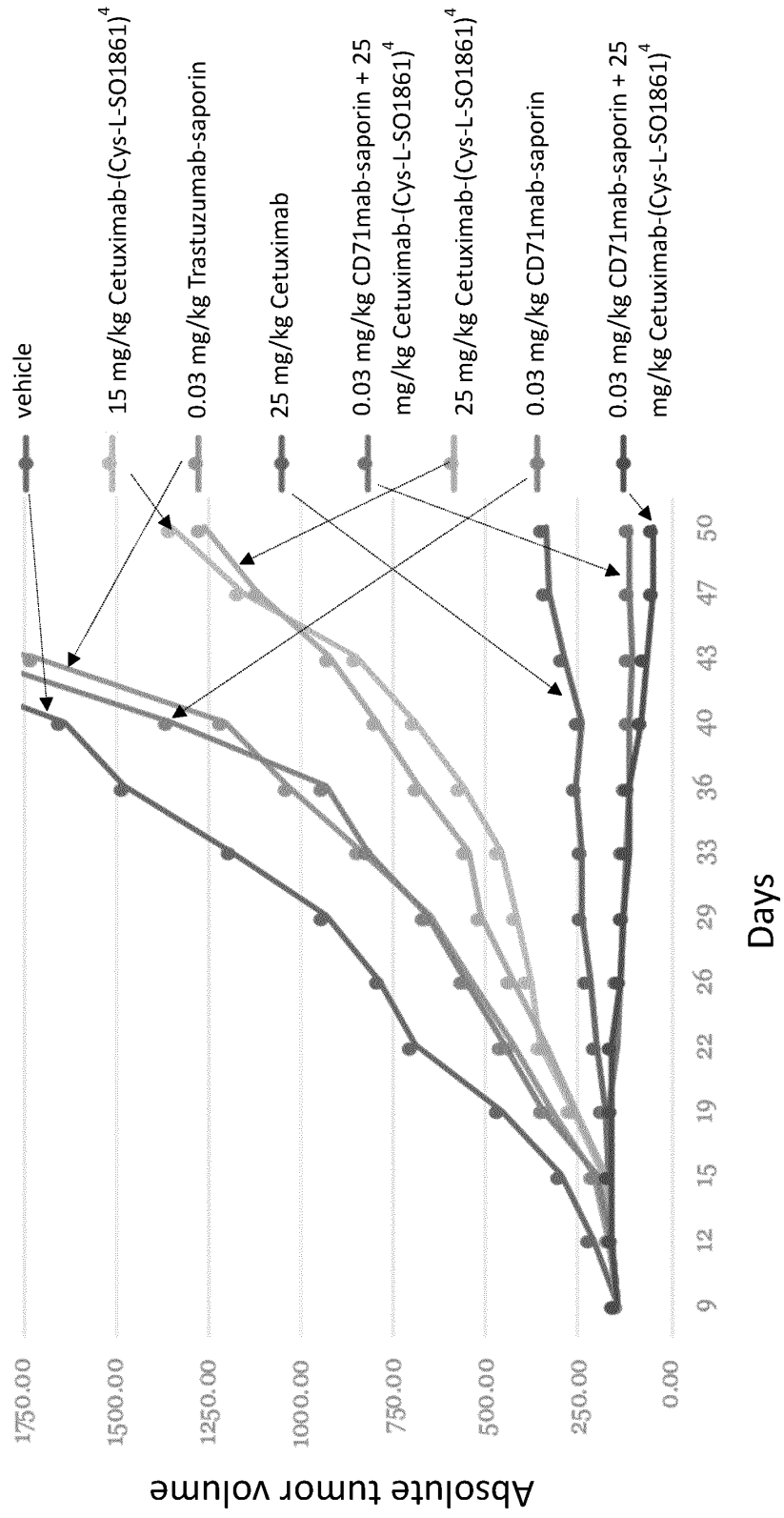
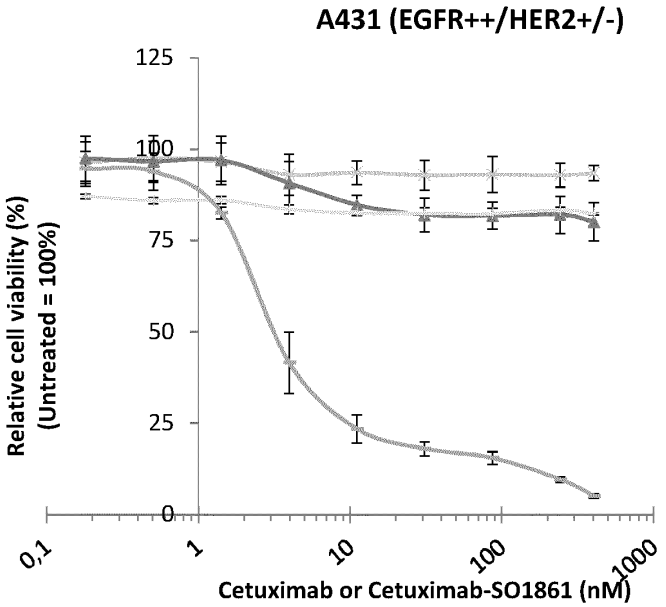

2T2C tumor regression and eradication in mouse A431 (EGFR++/HER2+/-/CD71+) tumors
vehicle
15 mg/kg Cetuximab-(Cys-L-SO1861)4
0.03 mg/kg Trastuzumab-saporin
25 mg/kg Cetuximab
0.03 mg/kg CD71mab-saporin + 25 mg/kg Cetuximab-(Cys-L-SO1861)4
25 mg/kg Cetuximab-(Cys-L-SO1861)4
0.03 mg/kg CD71mab-saporin
0.03 mg/kg CD71mab-saporin + 25 mg/kg Cetuximab-(Cys-L-SO1861)4
Absolute tumor volume
1750.00
1500.00
1250.00
1000.00
750.00
500.00
250.00
0.00
9 12 15 19 22 26 29 33 36 40 43 47 50
Days

Figure 3

A


A431 (EGFR++/HER2+/-)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
Cetuximab or Cetuximab-SO1861 (nM)

B

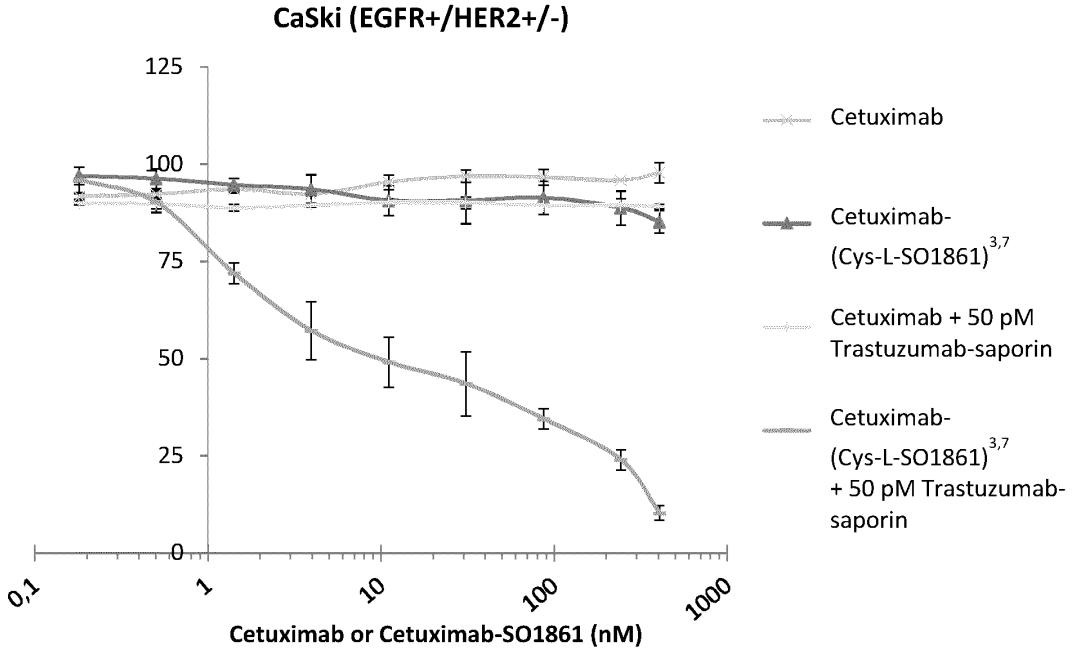
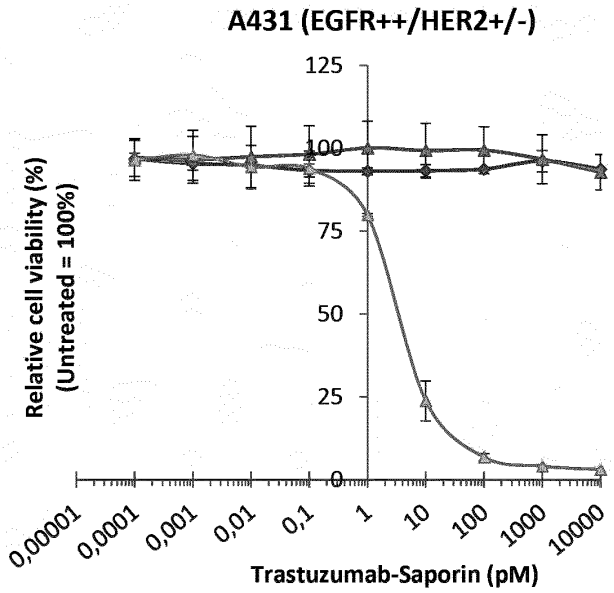

CaSki (EGFR+/HER2+/-)
125
100
75
50
25
0
0,1
1
10
100
1000
Cetuximab or Cetuximab-SO1861 (nM)
Cetuximab
Cetuximab-(Cys-L-SO1861)3,7
Cetuximab + 50 pM Trastuzumab-saporin
Cetuximab-(Cys-L-SO1861)3,7 + 50 pM Trastuzumab-saporin

Figure 3

C


A431 (EGFR++/HER2+/-)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
Trastuzumab-Saporin (pM)

D

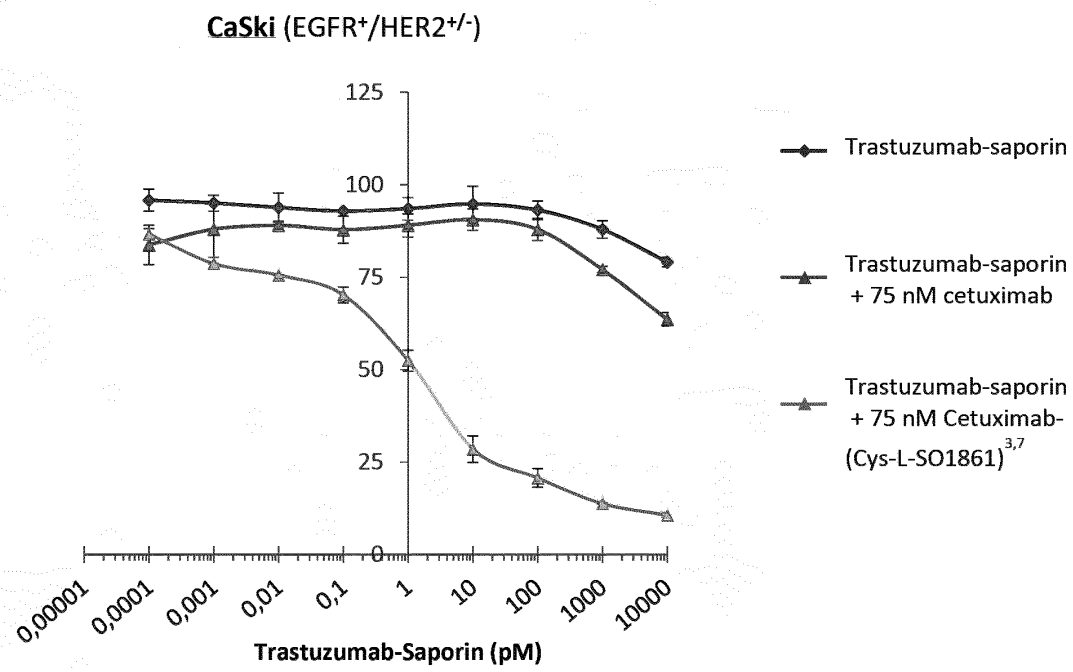
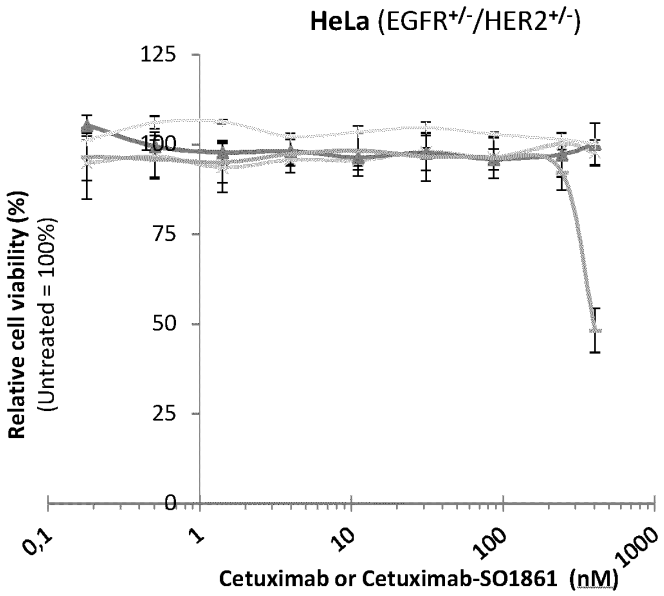

CaSki (EGFR+/HER2+/-)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
Trastuzumab-Saporin (pM)
Trastuzumab-saporin
Trastuzumab-saporin + 75 nM cetuximab
Trastuzumab-saporin + 75 nM Cetuximab-(Cys-L-SO1861)3,7

Figure 4

A


HeLa (EGFR+/-/HER2+/-)
125
100
75
50
25
0
Relative cell viability (%)
(Untreated = 100%)
0,1
1
10
100
1000
Cetuximab or Cetuximab-SO1861 (nM)

B

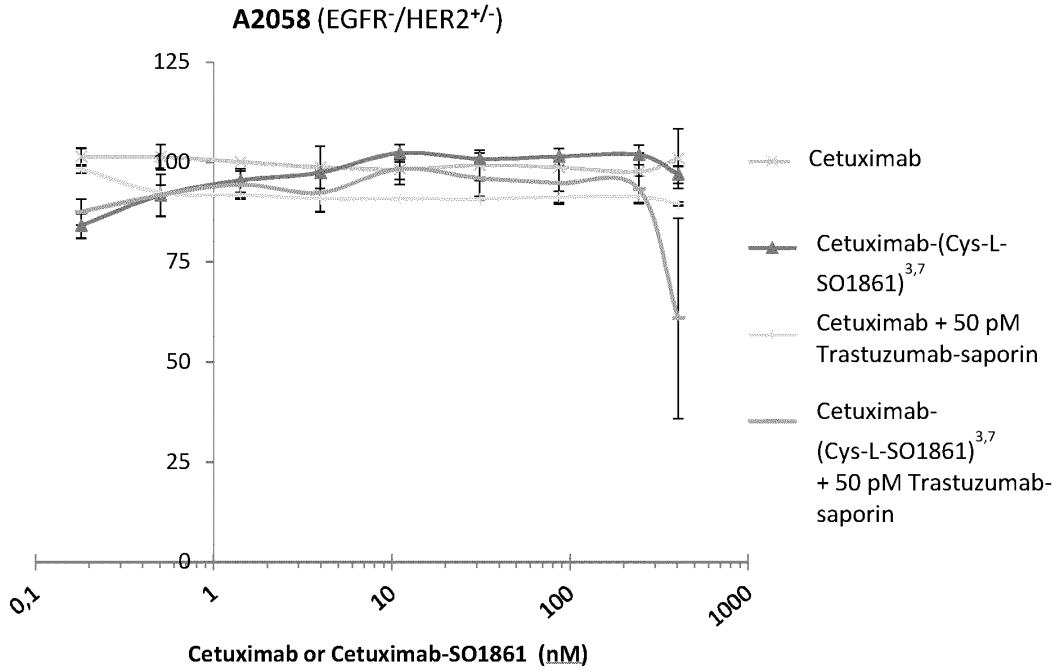
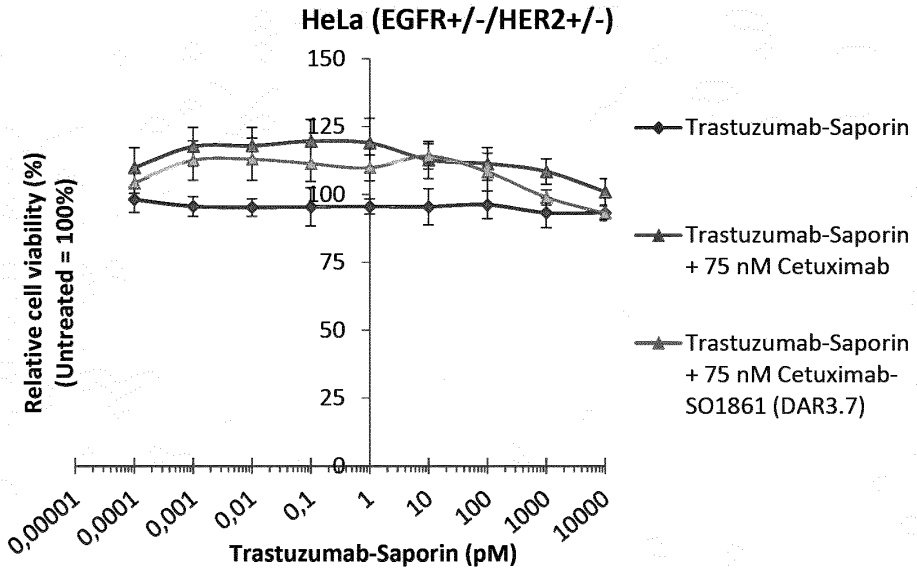

A2058 (EGFR-/HER2+/-)
125
100
75
50
25
0
0,1
1
10
100
1000
Cetuximab or Cetuximab-SO1861 (nM)
Cetuximab
Cetuximab-(Cys-L-SO1861)3,7
Cetuximab + 50 pM Trastuzumab-saporin
Cetuximab-(Cys-L-SO1861)3,7 + 50 pM Trastuzumab-saporin

Figure 4

C


HeLa (EGFR+/-/HER2+/-)
Relative cell viability (%)
(Untreated = 100%)
150
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
Trastuzumab-Saporin (pM)
Trastuzumab-Saporin
Trastuzumab-Saporin + 75 nM Cetuximab
Trastuzumab-Saporin + 75 nM Cetuximab-SO1861 (DAR3.7)

D

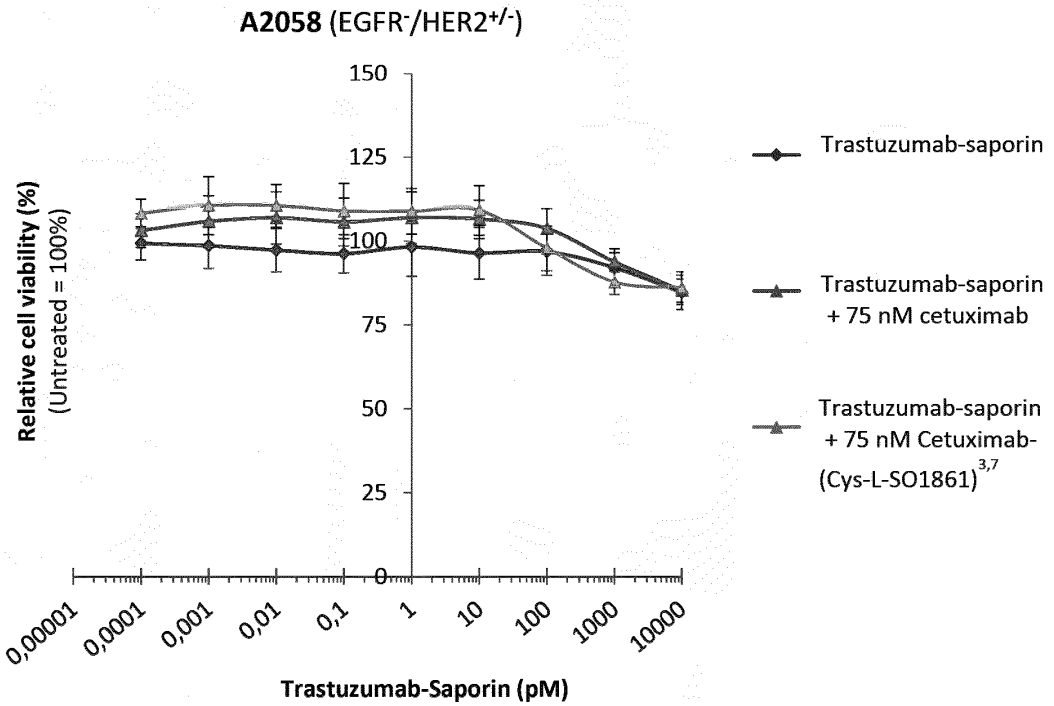
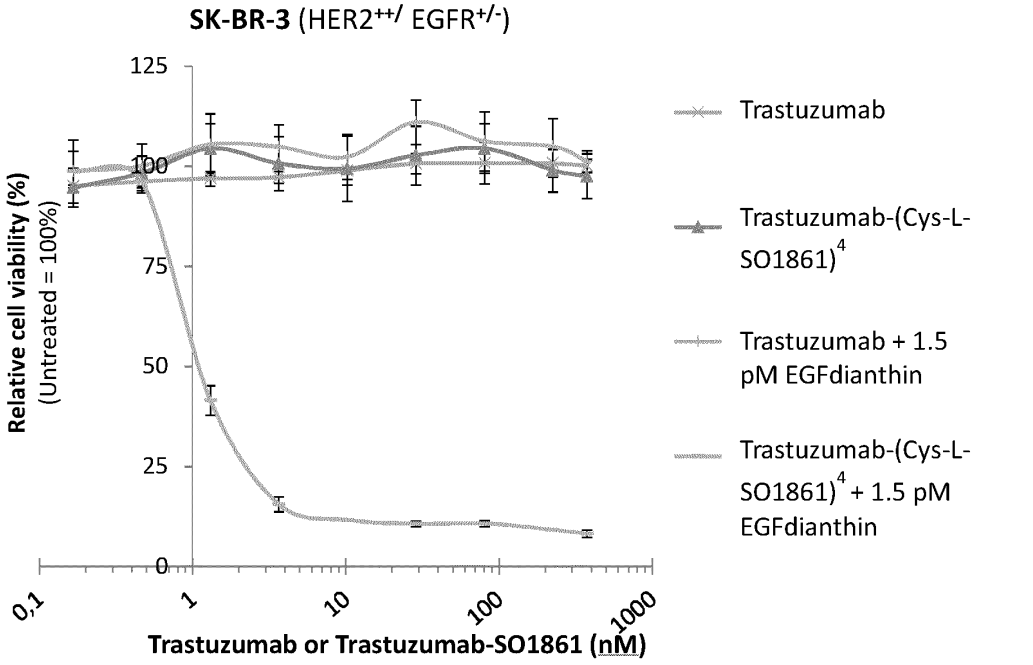

A2058 (EGFR-/HER2+/-)
Relative cell viability (%)
(Untreated = 100%)
150
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
Trastuzumab-Saporin (pM)
Trastuzumab-saporin
Trastuzumab-saporin + 75 nM cetuximab
Trastuzumab-saporin + 75 nM Cetuximab-(Cys-L-SO1861)3,7

Figure 5

A


SK-BR-3 (HER2++/ EGFR+/-)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
Trastuzumab or Trastuzumab-SO1861 (nM)
Trastuzumab
Trastuzumab-(Cys-L-SO1861)4
Trastuzumab + 1.5 pM EGFdianthin
Trastuzumab-(Cys-L-SO1861)4 + 1.5 pM EGFdianthin

B

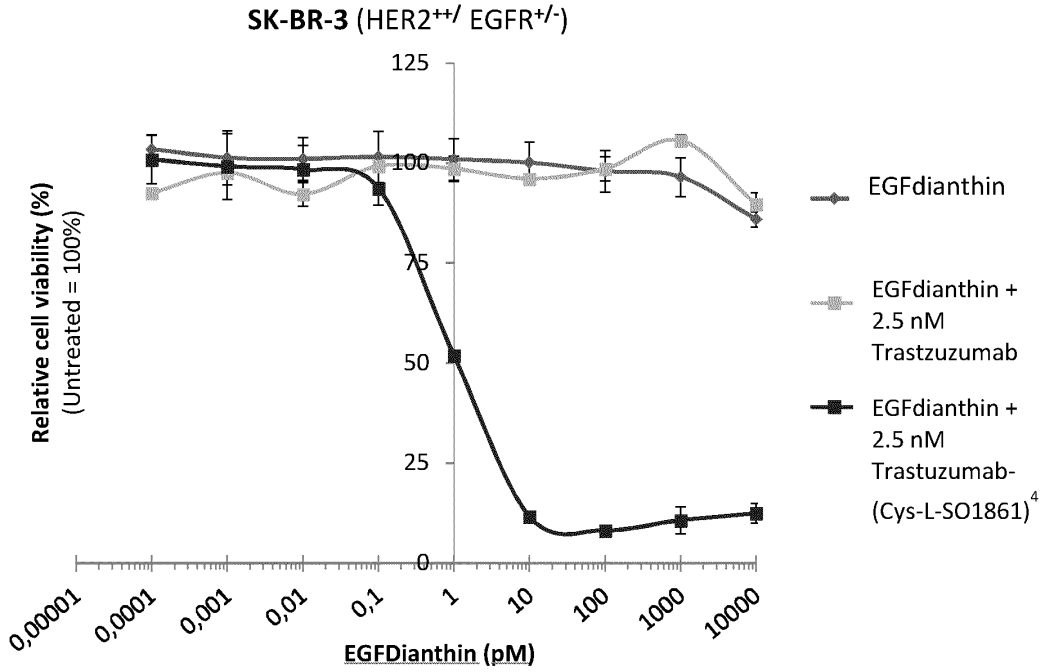
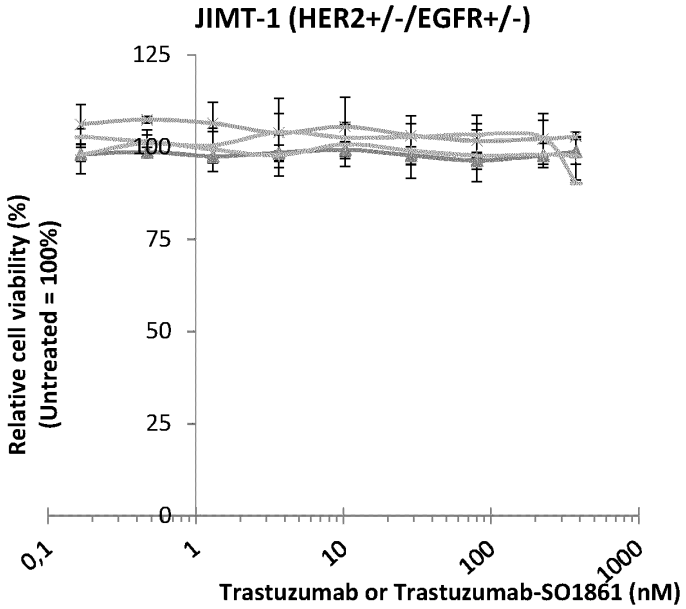

SK-BR-3 (HER2++/ EGFR+/-)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
EGFDianthin (pM)
EGFdianthin
EGFdianthin + 2.5 nM Trastzuzumab
EGFdianthin + 2.5 nM Trastuzumab-(Cys-L-SO1861)4

Figure 6

A


JIMT-1 (HER2+/-/EGFR+/-)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
Trastuzumab or Trastuzumab-SO1861 (nM)

B

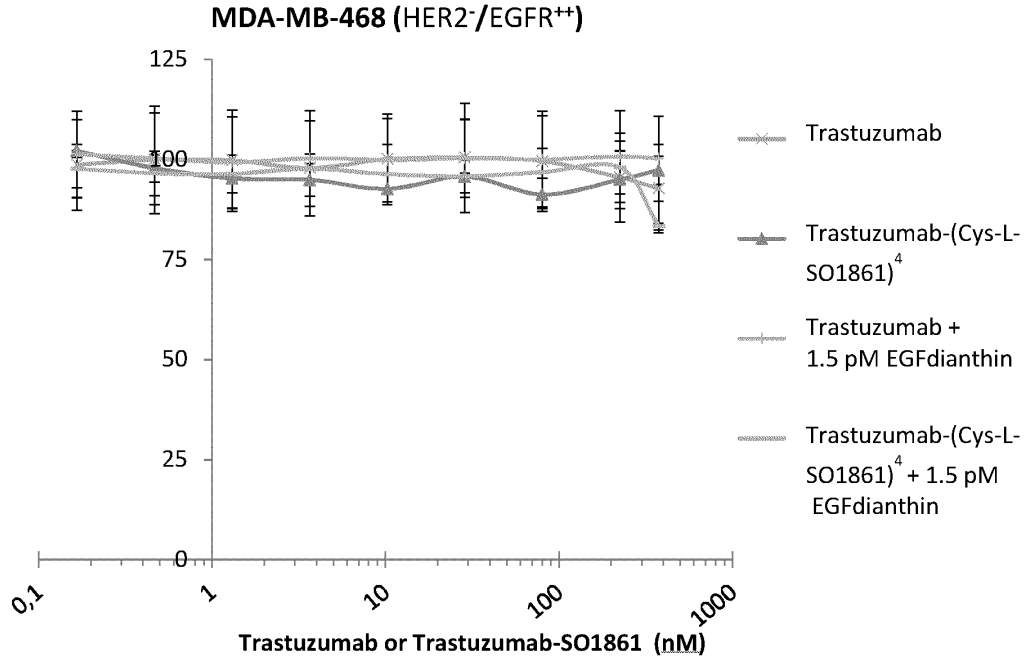
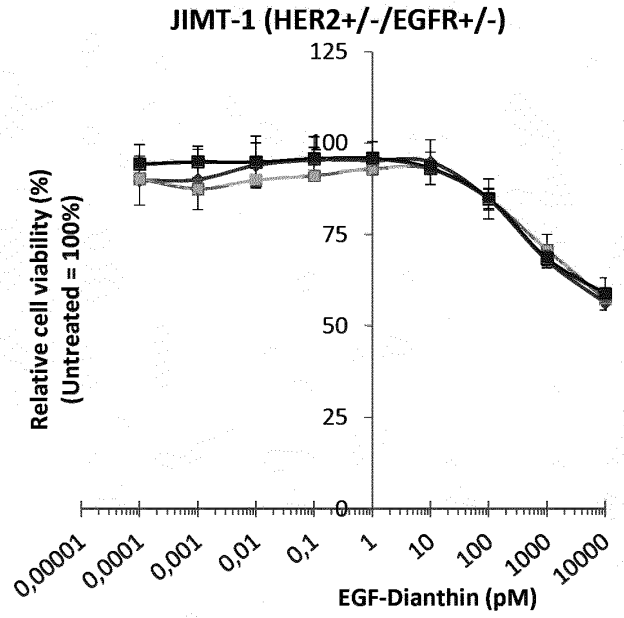

MDA-MB-468 (HER2-/EGFR++)
125
100
75
50
25
0
0,1
1
10
100
1000
Trastuzumab or Trastuzumab-SO1861 (nM)
Trastuzumab
Trastuzumab-(Cys-L-SO1861)4
Trastuzumab + 1.5 pM EGFdianthin
Trastuzumab-(Cys-L-SO1861)4 + 1.5 pM EGFdianthin

Figure 6

C


JIMT-1 (HER2+/-/EGFR+/-)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
EGF-Dianthin (pM)

D

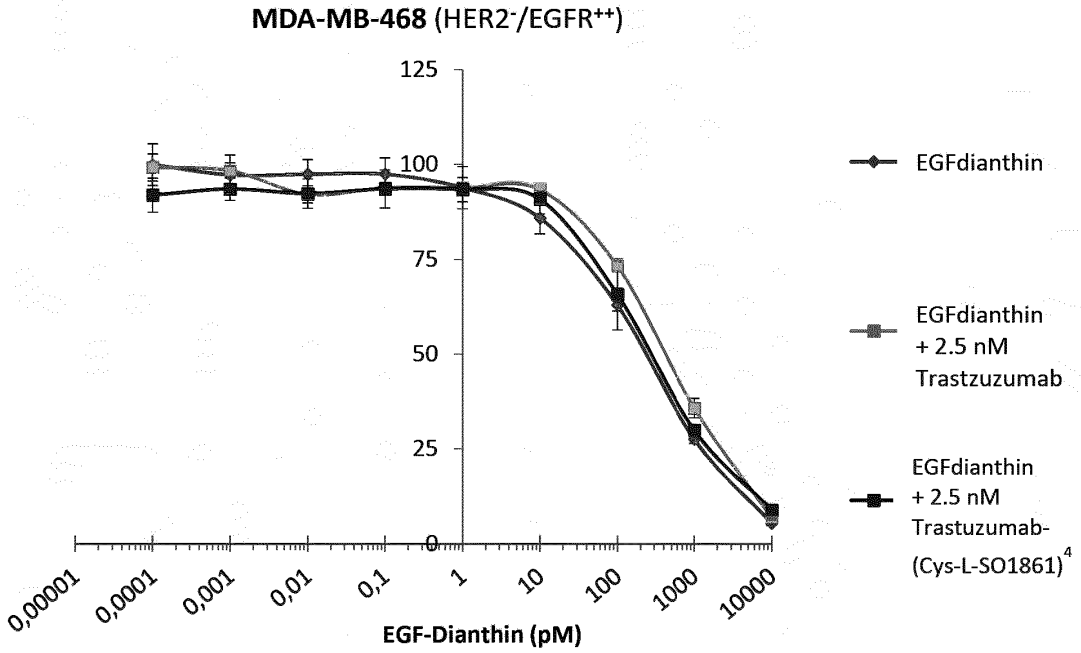
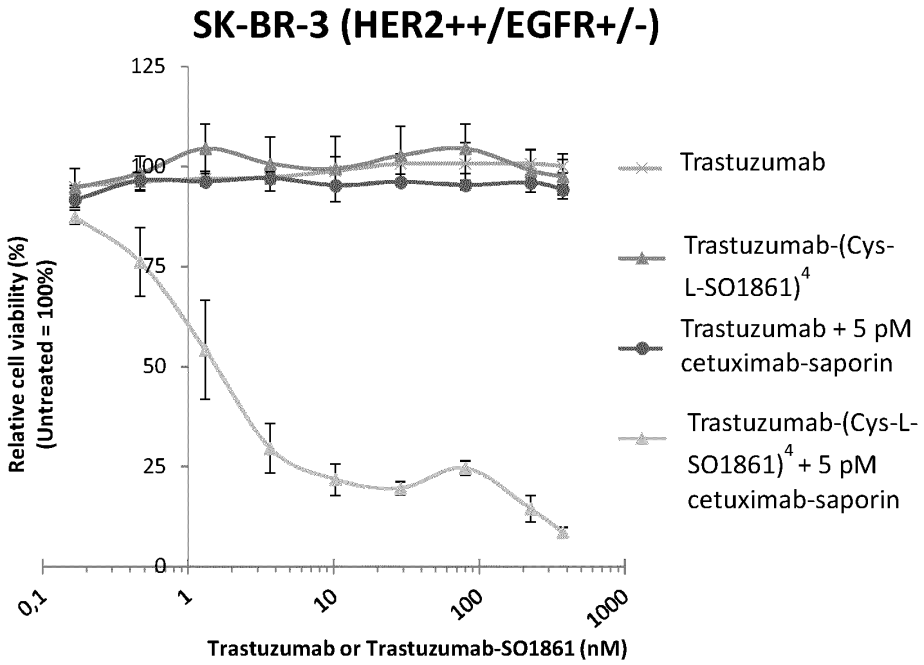

MDA-MB-468 (HER2-/EGFR++)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
EGF-Dianthin (pM)
EGFdianthin
EGFdianthin + 2.5 nM Trastzuzumab
EGFdianthin + 2.5 nM Trastuzumab-(Cys-L-SO1861)4

Figure 7

A


SK-BR-3 (HER2++/EGFR+/-)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
Trastuzumab or Trastuzumab-SO1861 (nM)
Trastuzumab
Trastuzumab-(Cys-L-SO1861)4
Trastuzumab + 5 pM cetuximab-saporin
Trastuzumab-(Cys-L-SO1861)4 + 5 pM cetuximab-saporin

B

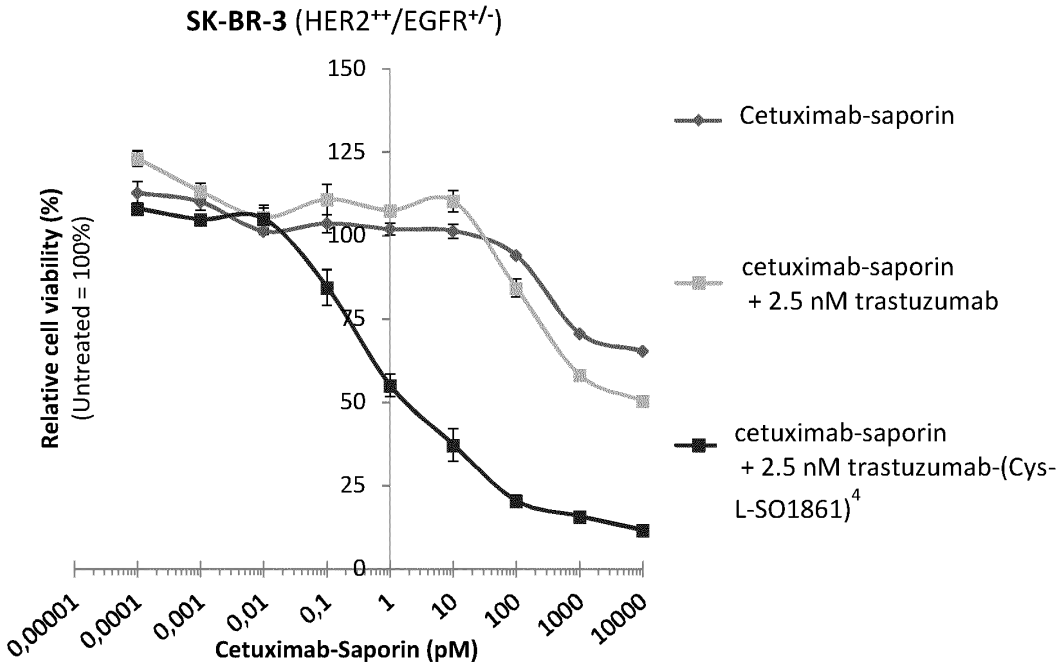
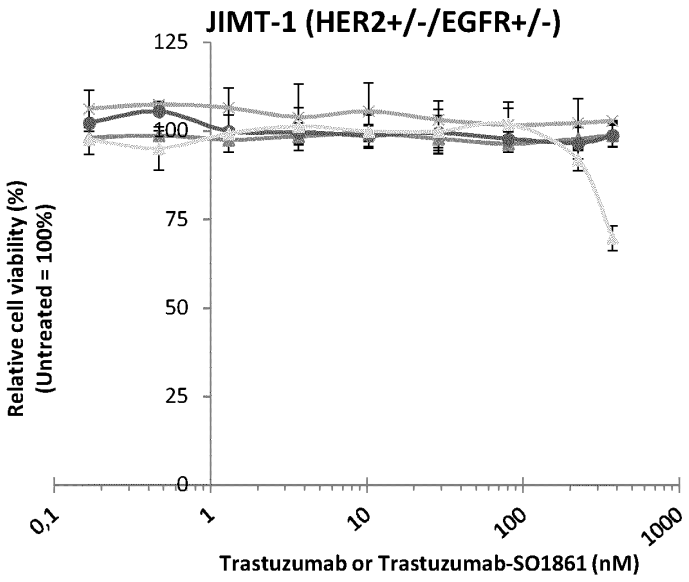

SK-BR-3 (HER2++/EGFR+/-)
Relative cell viability (%)
(Untreated = 100%)
150
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
Cetuximab-Saporin (pM)
Cetuximab-saporin
cetuximab-saporin + 2.5 nM trastuzumab
cetuximab-saporin + 2.5 nM trastuzumab-(Cys-L-SO1861)4

Figure 8

A


JIMT-1 (HER2+/-/EGFR+/-)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
Trastuzumab or Trastuzumab-SO1861 (nM)

B

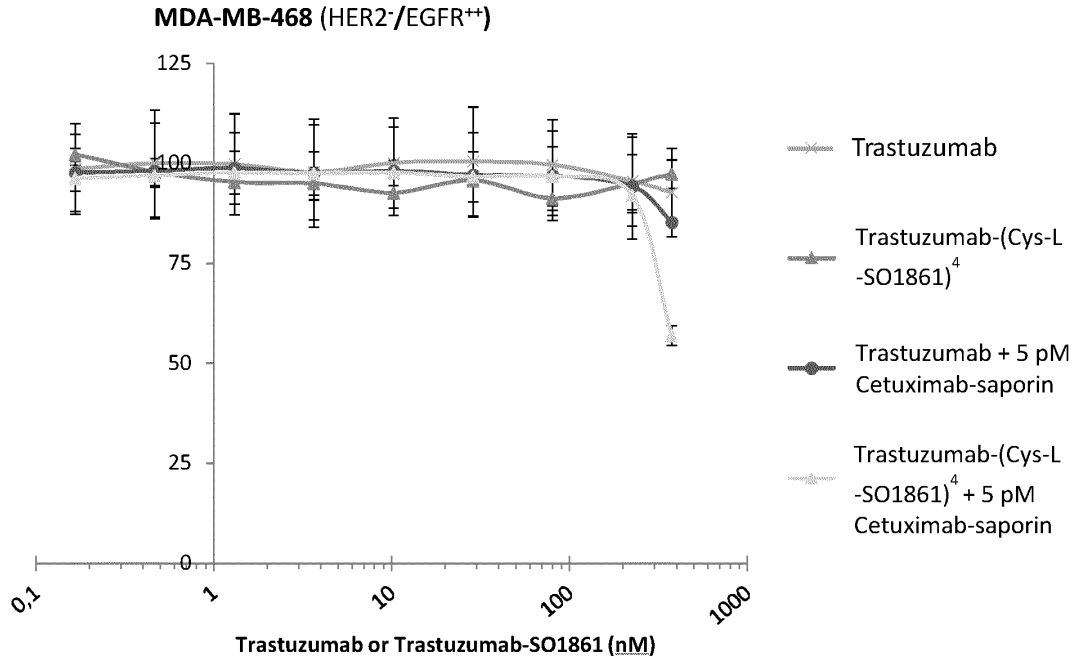
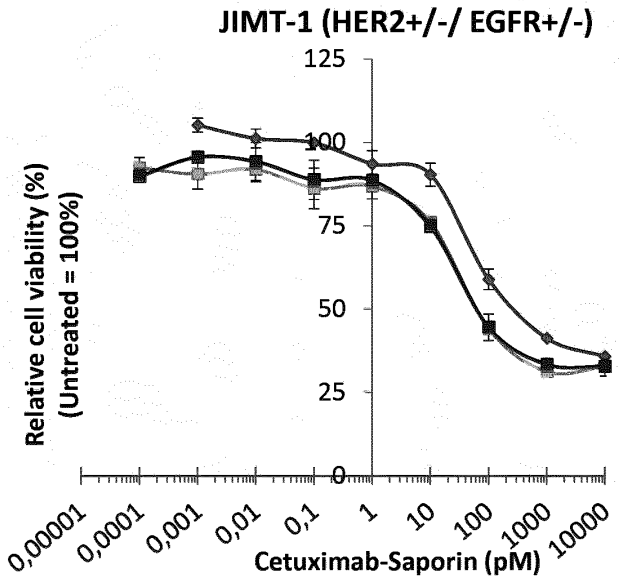

MDA-MB-468 (HER2-/EGFR++)
125
100
75
50
25
0
0,1
1
10
100
1000
Trastuzumab or Trastuzumab-SO1861 (nM)
Trastuzumab
Trastuzumab-(Cys-L-SO1861)4
Trastuzumab + 5 pM Cetuximab-saporin
Trastuzumab-(Cys-L-SO1861)4 + 5 pM Cetuximab-saporin

Figure 8

C


JIMT-1 (HER2+/-/ EGFR+/-)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
Cetuximab-Saporin (pM)

D

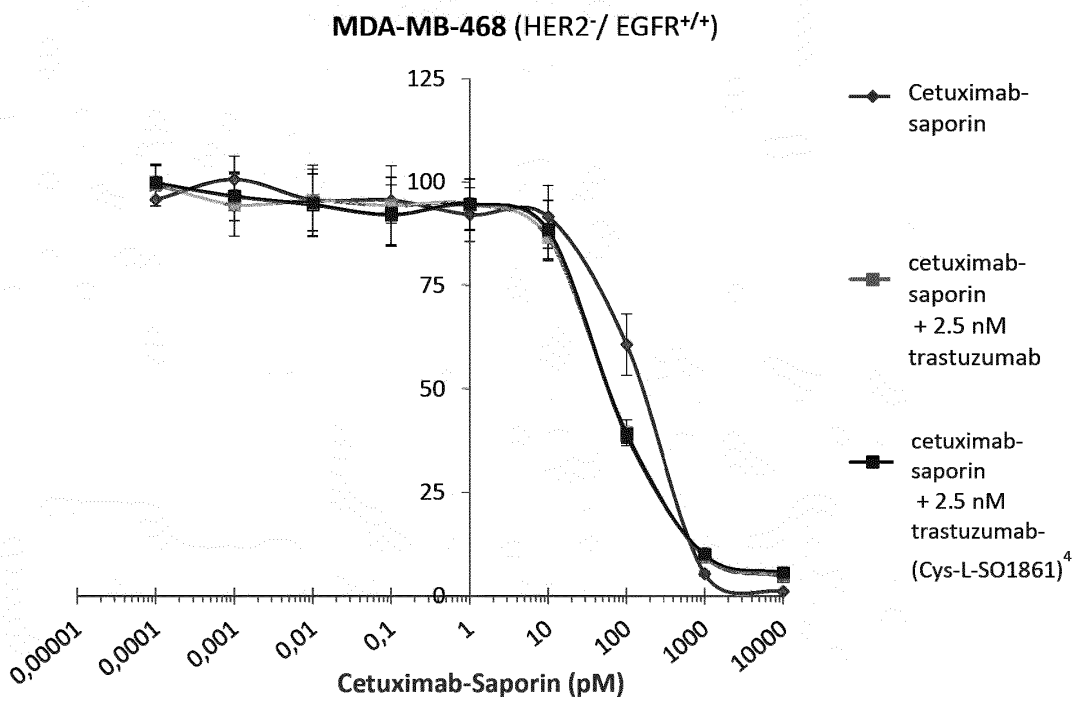
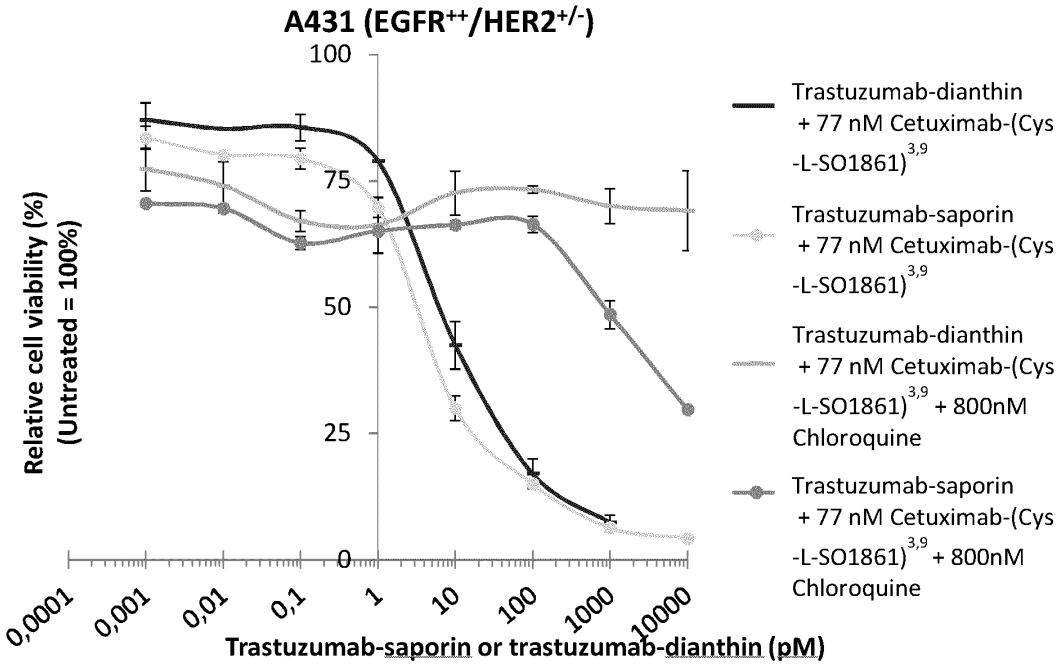

MDA-MB-468 (HER2- / EGFR+/+)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
Cetuximab-Saporin (pM)
Cetuximab-saporin
cetuximab-saporin + 2.5 nM trastuzumab
cetuximab-saporin + 2.5 nM trastuzumab-(Cys-L-SO1861)4

Figure 9

A


A431 (EGFR++/HER2+/-)
Relative cell viability (%) (Untreated = 100%)
100
75
50
25
0
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
Trastuzumab-saporin or trastuzumab-dianthin (pM)
Trastuzumab-dianthin + 77 nM Cetuximab-(Cys-L-SO1861)3,9
Trastuzumab-saporin + 77 nM Cetuximab-(Cys-L-SO1861)3,9
Trastuzumab-dianthin + 77 nM Cetuximab-(Cys-L-SO1861)3,9 + 800nM Chloroquine
Trastuzumab-saporin + 77 nM Cetuximab-(Cys-L-SO1861)3,9 + 800nM Chloroquine

B

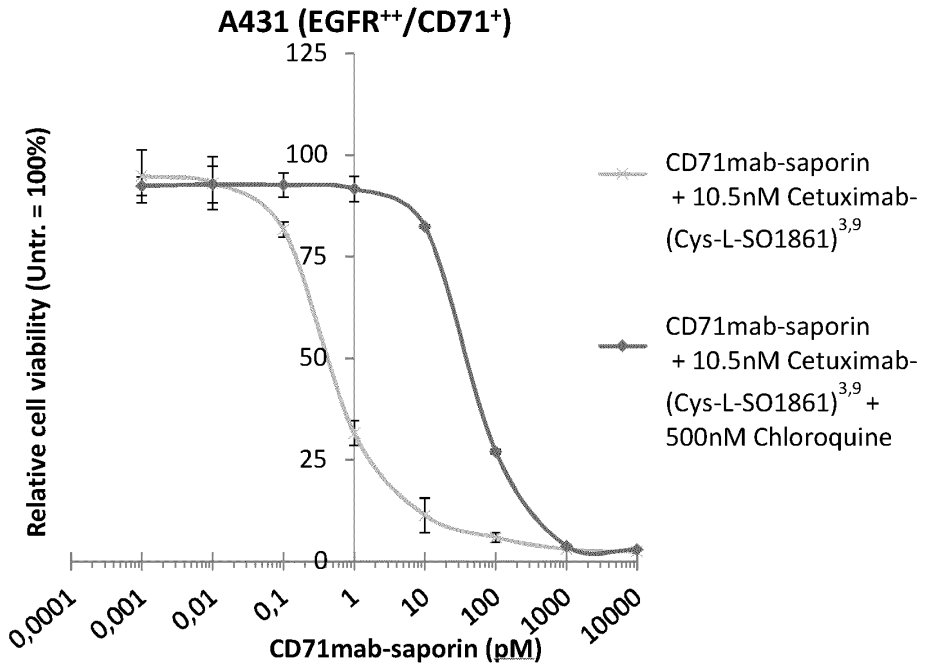
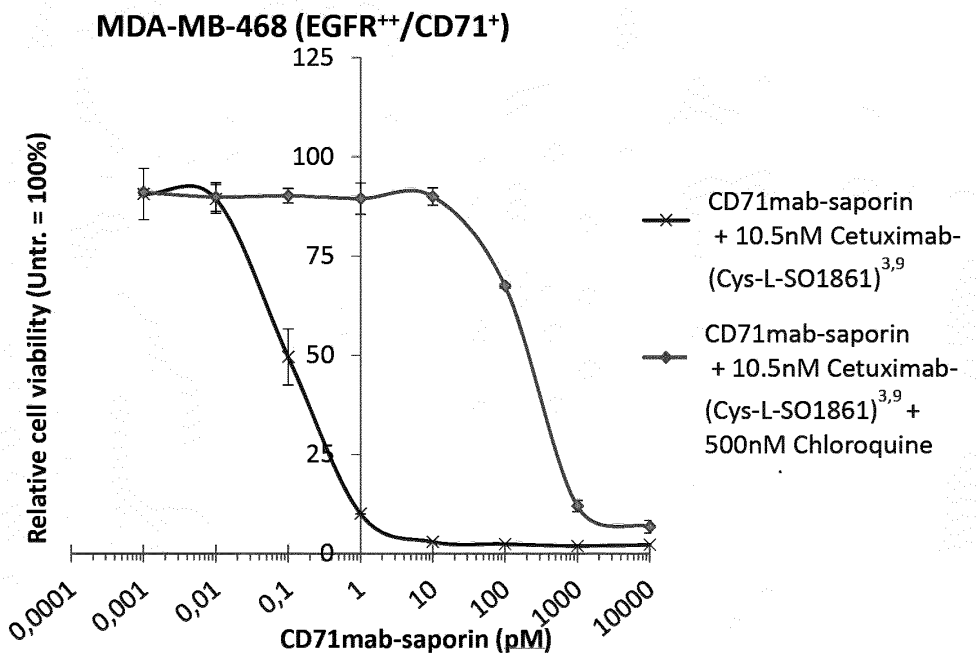

A431 (EGFR++/CD71+)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
CD71mab-saporin (pM)
CD71mab-saporin + 10.5nM Cetuximab-(Cys-L-SO1861)3,9
CD71mab-saporin + 10.5nM Cetuximab-(Cys-L-SO1861)3,9 + 500nM Chloroquine

Figure 9

C


MDA-MB-468 (EGFR++/CD71+)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
CD71mab-saporin (pM)
CD71mab-saporin + 10.5nM Cetuximab-(Cys-L-SO1861)3,9
CD71mab-saporin + 10.5nM Cetuximab-(Cys-L-SO1861)3,9 + 500nM Chloroquine

D

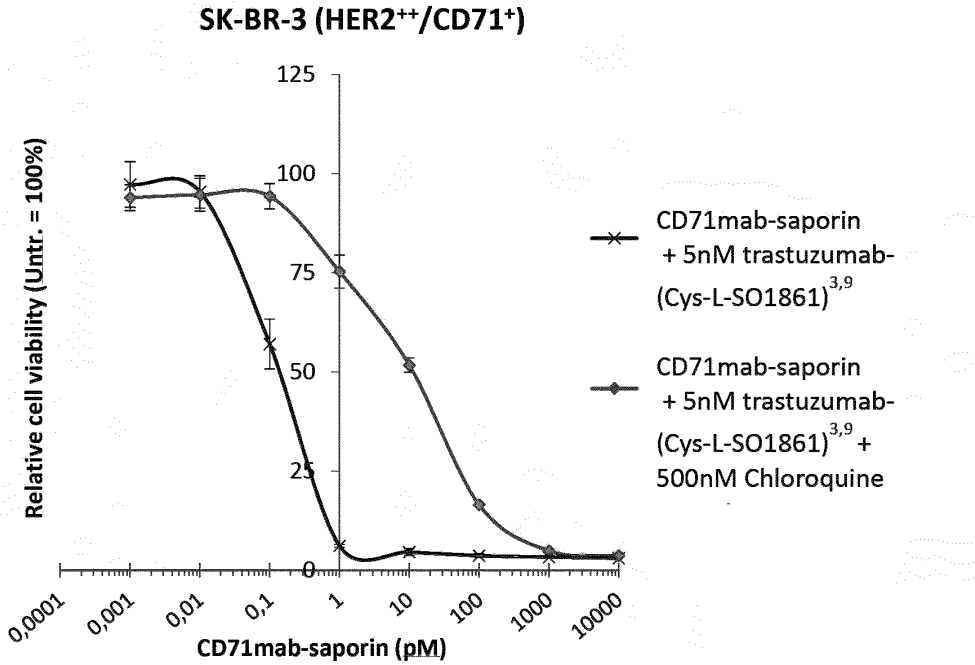
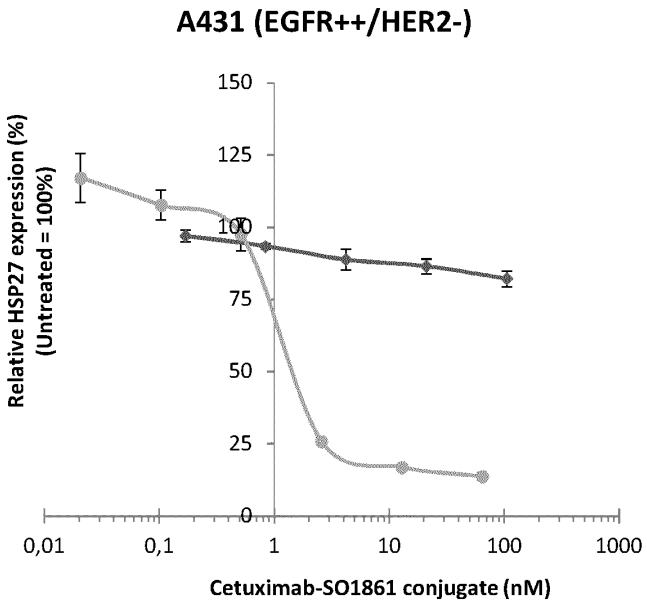

SK-BR-3 (HER2++/CD71+)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
CD71mab-saporin (pM)
CD71mab-saporin + 5nM trastuzumab-(Cys-L-SO1861)3,9
CD71mab-saporin + 5nM trastuzumab-(Cys-L-SO1861)3,9 + 500nM Chloroquine

Figure 10

A


A431 (EGFR++/HER2-)
Relative HSP27 expression (%)
(Untreated = 100%)
150
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Cetuximab-SO1861 conjugate (nM)

B

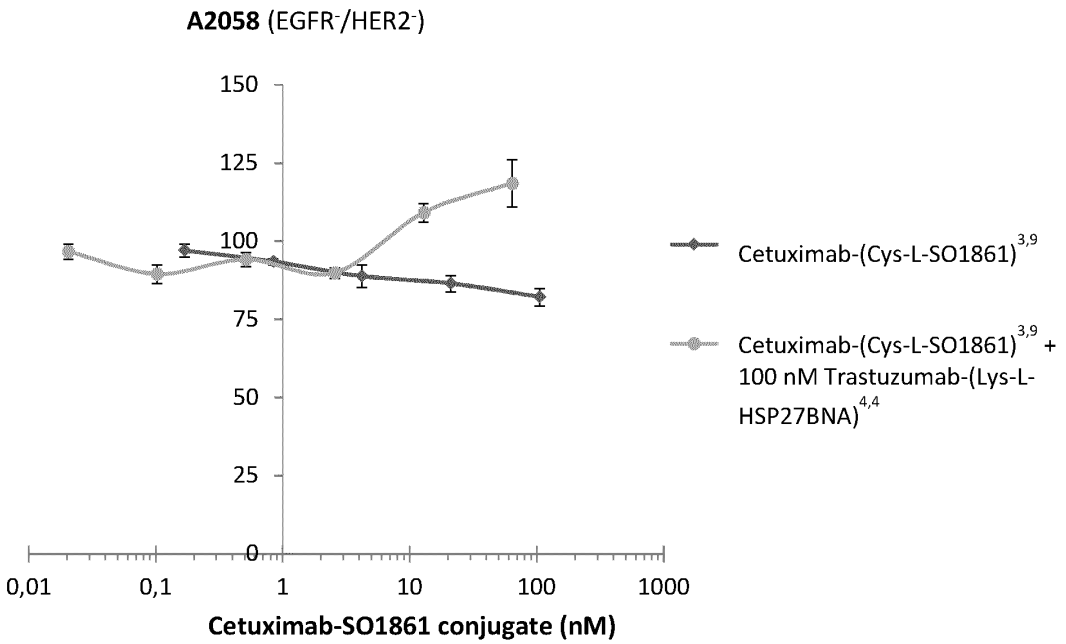
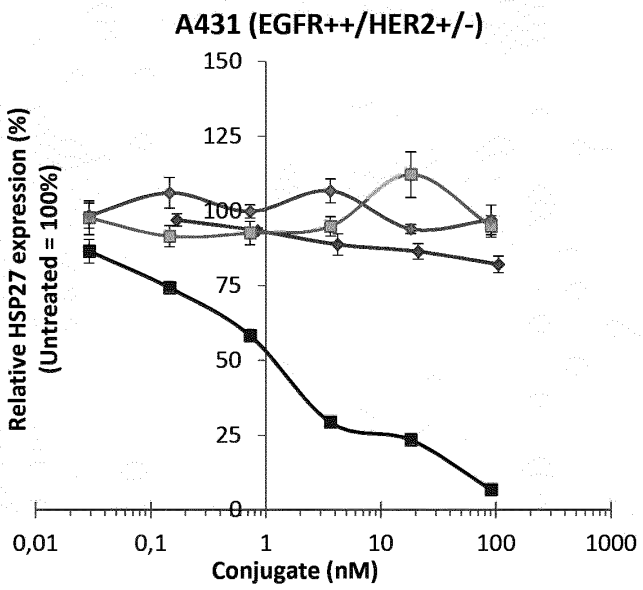

A2058 (EGFR-/HER2-)
150
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Cetuximab-SO1861 conjugate (nM)
Cetuximab-(Cys-L-SO1861)3,9
Cetuximab-(Cys-L-SO1861)3,9 + 100 nM Trastuzumab-(Lys-L-HSP27BNA)4,4

Figure 10

C


A431 (EGFR++/HER2+/-)
Relative HSP27 expression (%)
(Untreated = 100%)
150
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Conjugate (nM)

D

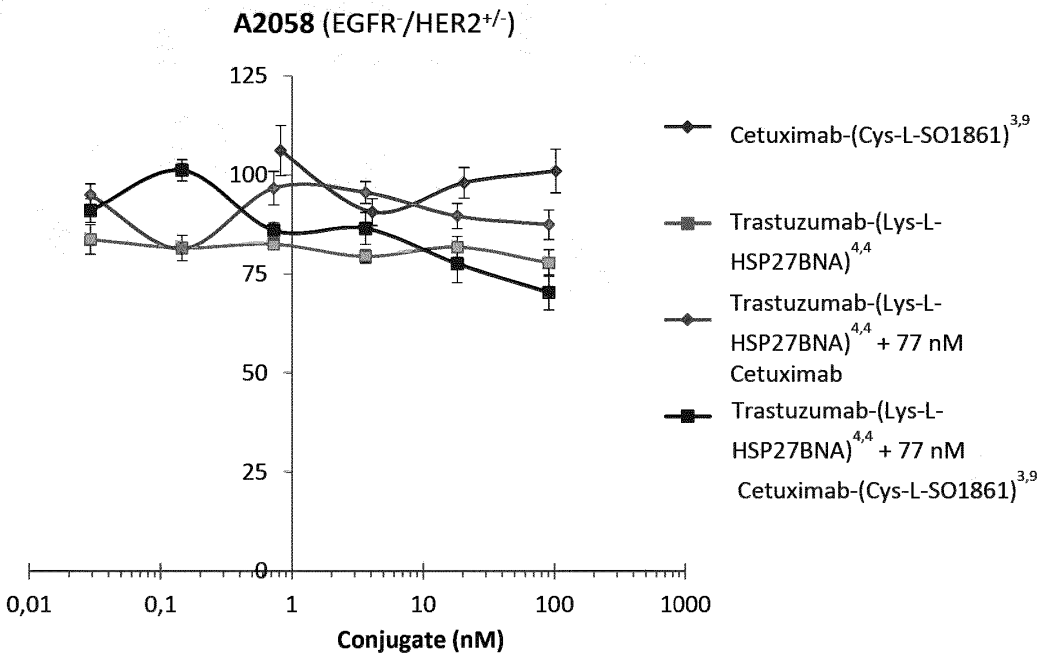
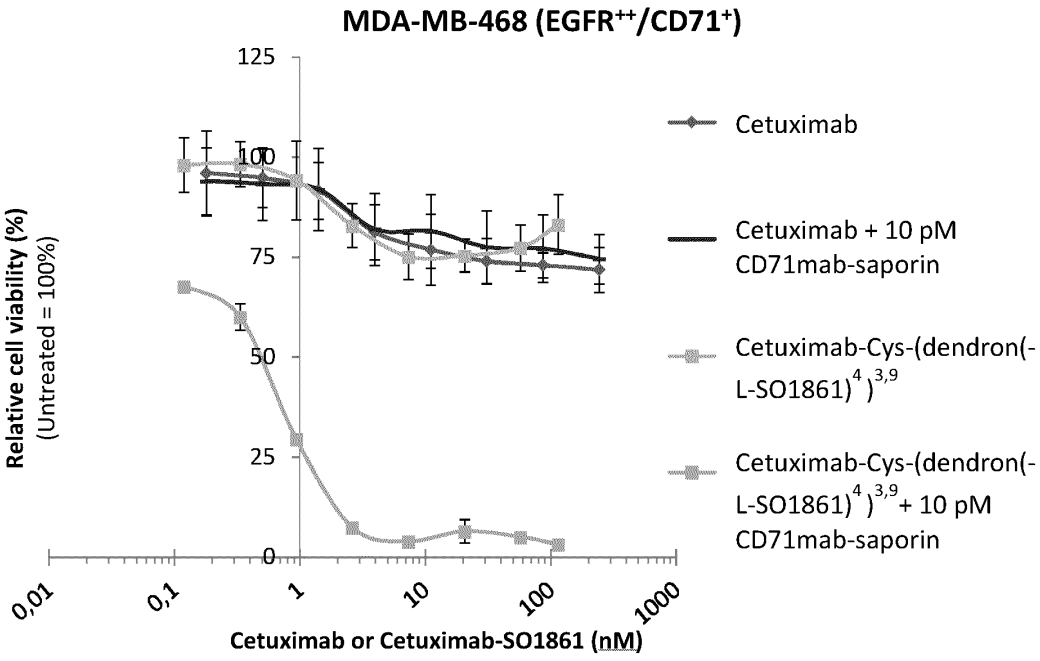

A2058 (EGFR-/HER2+/-)
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Conjugate (nM)
Cetuximab-(Cys-L-SO1861)3,9
Trastuzumab-(Lys-L-HSP27BNA)4,4
Trastuzumab-(Lys-L-HSP27BNA)4,4 + 77 nM Cetuximab
Trastuzumab-(Lys-L-HSP27BNA)4,4 + 77 nM Cetuximab-(Cys-L-SO1861)3,9

Figure 11

A


MDA-MB-468 (EGFR++/CD71+)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Cetuximab or Cetuximab-SO1861 (nM)
Cetuximab
Cetuximab + 10 pM CD71mab-saporin
Cetuximab-Cys-(dendron(-L-SO1861)4)3,9
Cetuximab-Cys-(dendron(-L-SO1861)4)3,9+ 10 pM CD71mab-saporin

B

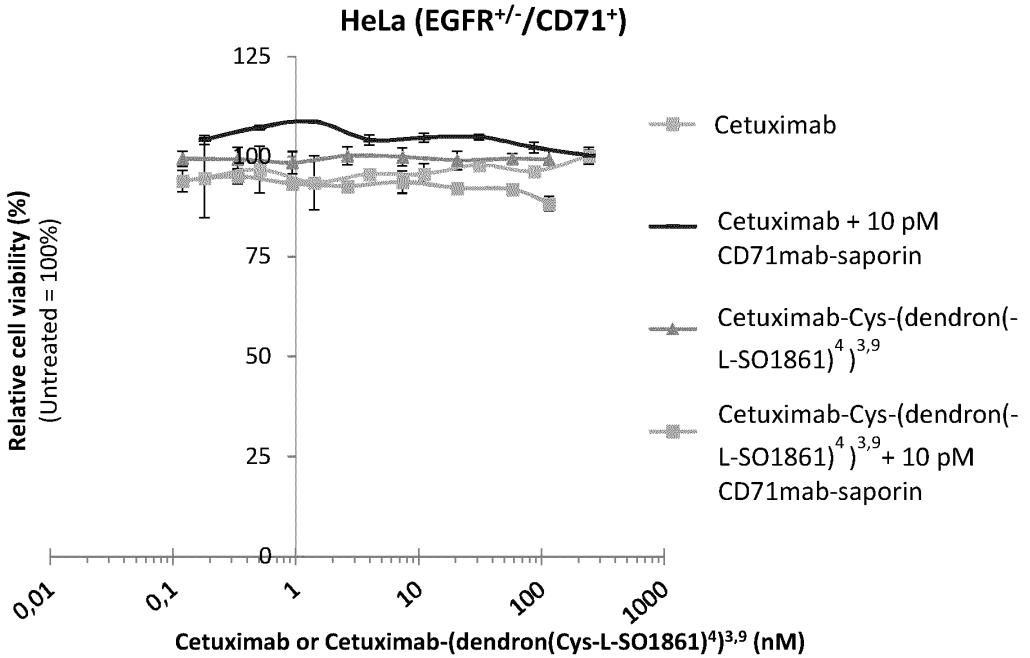
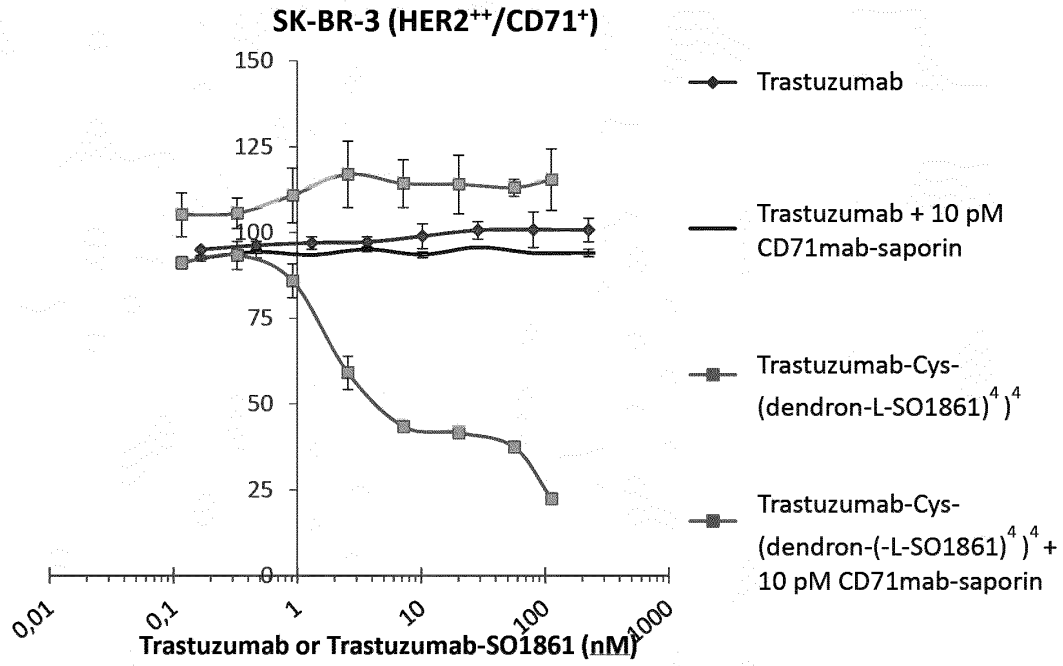

HeLa (EGFR+/-/CD71+)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Cetuximab or Cetuximab-(dendron(Cys-L-SO1861)4)3,9 (nM)
Cetuximab
Cetuximab + 10 pM CD71mab-saporin
Cetuximab-Cys-(dendron(-L-SO1861)4)3,9
Cetuximab-Cys-(dendron(-L-SO1861)4)3,9+ 10 pM CD71mab-saporin

Figure 11

C


SK-BR-3 (HER2++/CD71+)
150
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Trastuzumab or Trastuzumab-SO1861 (nM)
Trastuzumab
Trastuzumab + 10 pM CD71mab-saporin
Trastuzumab-Cys-(dendron-L-SO1861)4)4
Trastuzumab-Cys-(dendron-(-L-SO1861)4)4 + 10 pM CD71mab-saporin

D

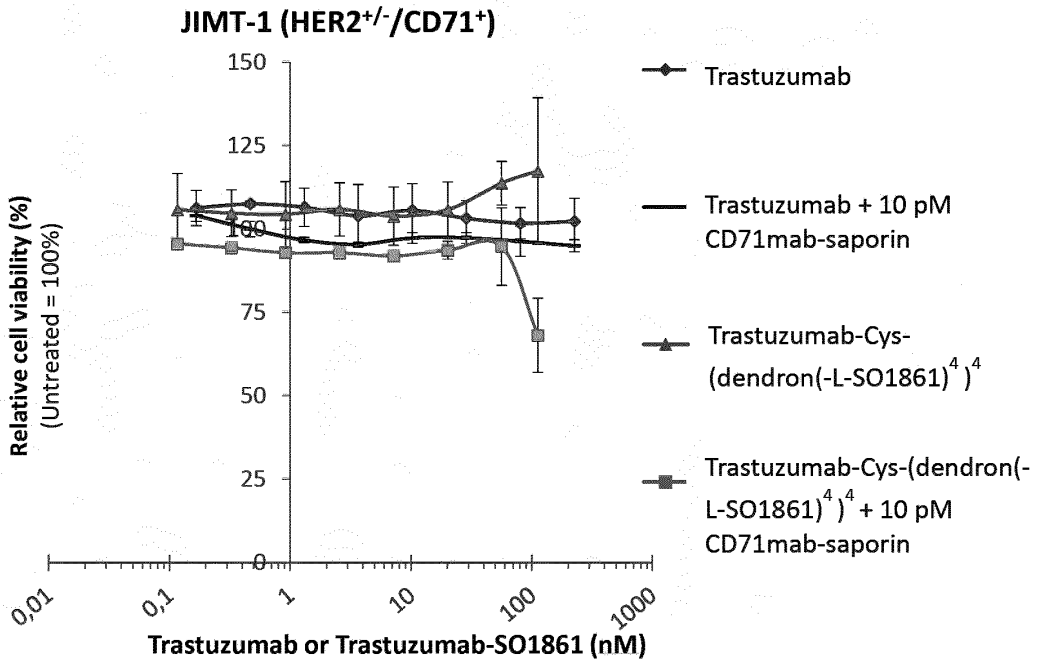

JIMT-1 (HER2+/-/CD71+)
Relative cell viability (%)
(Untreated = 100%)
150
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Trastuzumab or Trastuzumab-SO1861 (nM)
Trastuzumab
Trastuzumab + 10 pM CD71mab-saporin
Trastuzumab-Cys-(dendron(-L-SO1861)4)4
Trastuzumab-Cys-(dendron(-L-SO1861)4)4 + 10 pM CD71mab-saporin

Figure 12

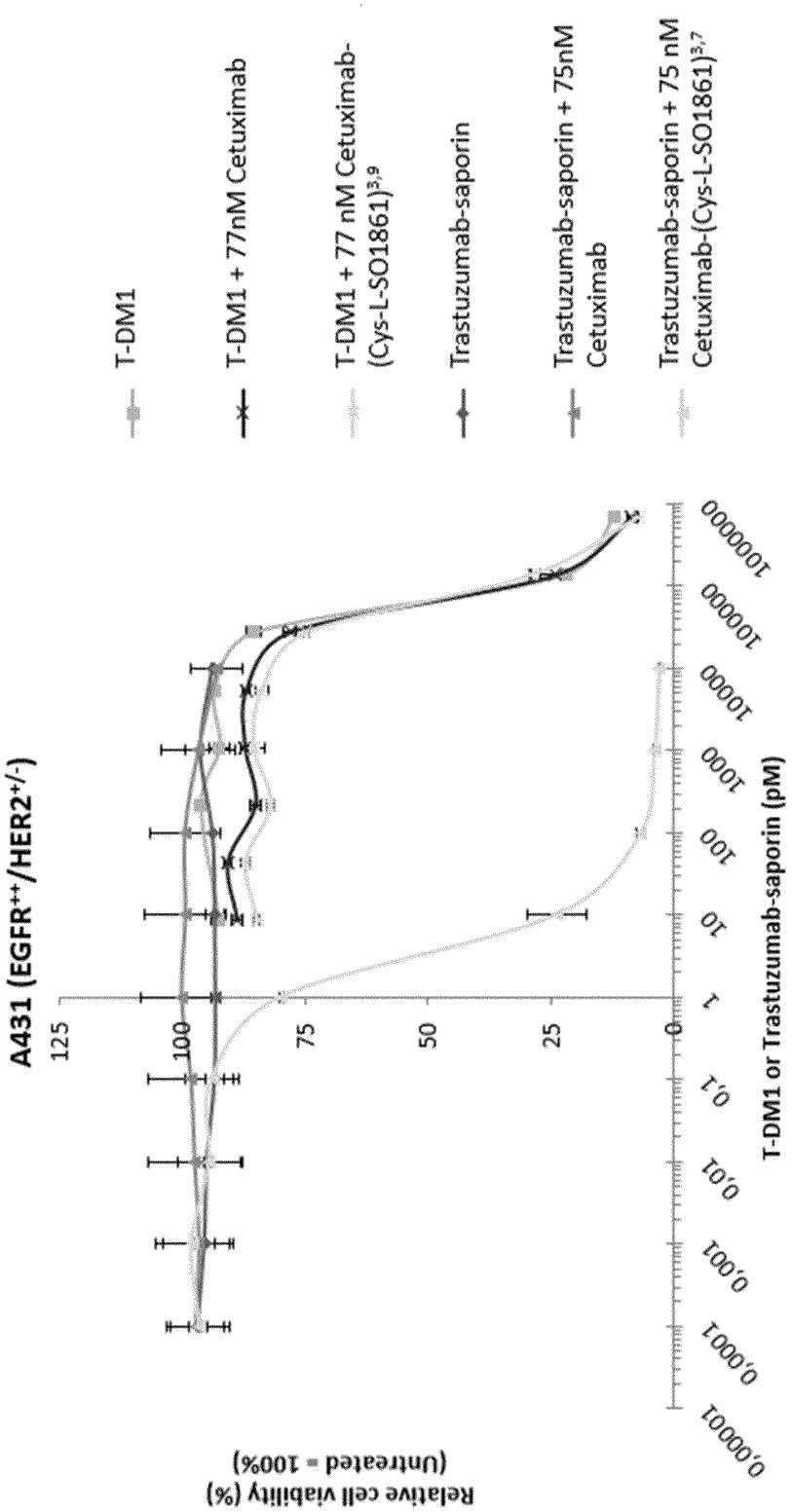
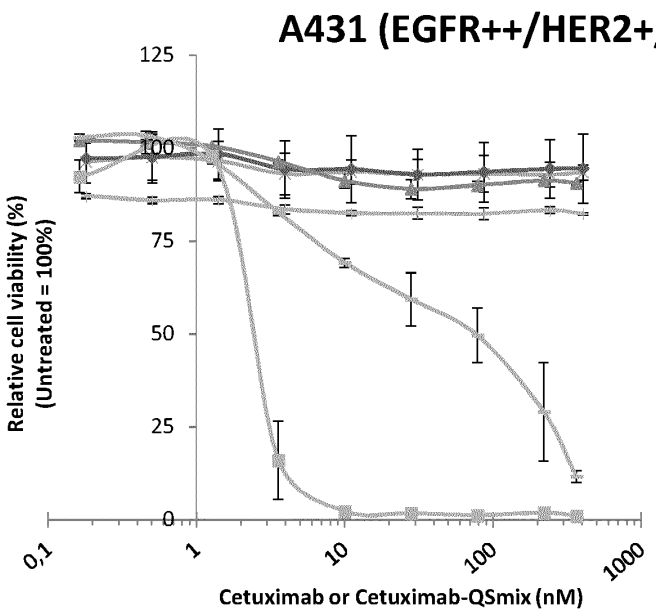

T-DM1
T-DM1 + 77nM Cetuximab
T-DM1 + 77 nM Cetuximab-(Cys-L-SO1861)3,9
Trastuzumab-saporin
Trastuzumab-saporin + 75nM Cetuximab
Trastuzumab-saporin + 75 nM Cetuximab-(Cys-L-SO1861)3,7
A431 (EGFR++/HER2+/-)
Relative cell viability (%) (Untreated = 100%)
125
100
75
50
25
0
T-DM1 or Trastuzumab-saporin (pM)
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
100000
1000000

Figure 13

A


A431 (EGFR++/HER2+/-/CD71+)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
Cetuximab or Cetuximab-QSmix (nM)

B

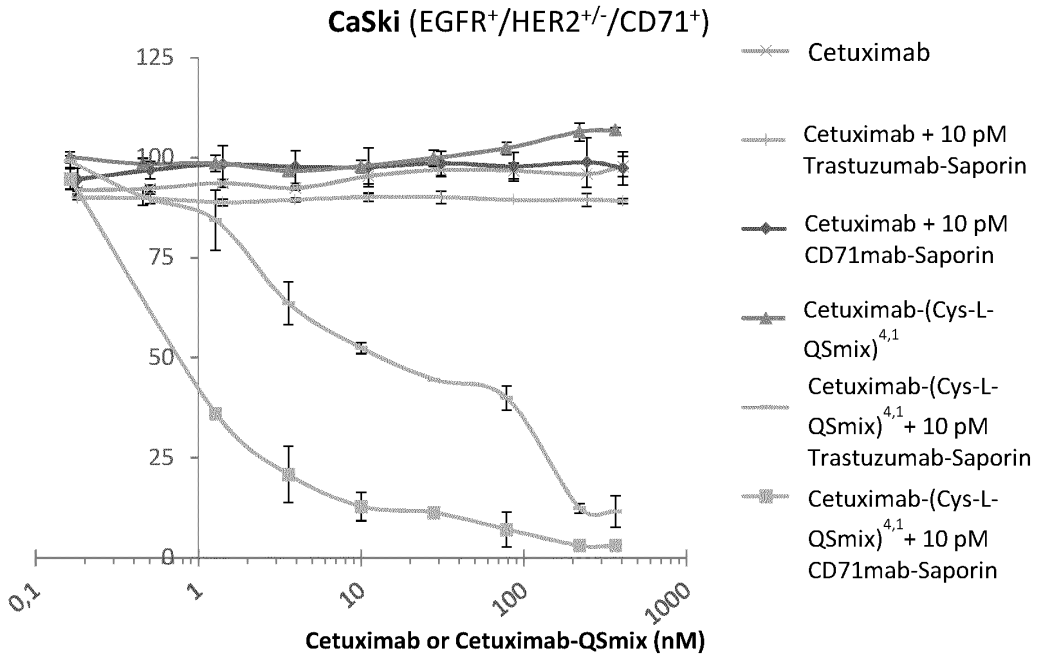

CaSki (EGFR+/HER2+/-/CD71+)
125
100
75
50
25
0
0,1
1
10
100
1000
Cetuximab or Cetuximab-QSmix (nM)
Cetuximab
Cetuximab + 10 pM Trastuzumab-Saporin
Cetuximab + 10 pM CD71mab-Saporin
Cetuximab-(Cys-L-QSmix)4,1
Cetuximab-(Cys-L-QSmix)4,1 + 10 pM Trastuzumab-Saporin
Cetuximab-(Cys-L-QSmix)4,1 + 10 pM CD71mab-Saporin

Figure 13

C

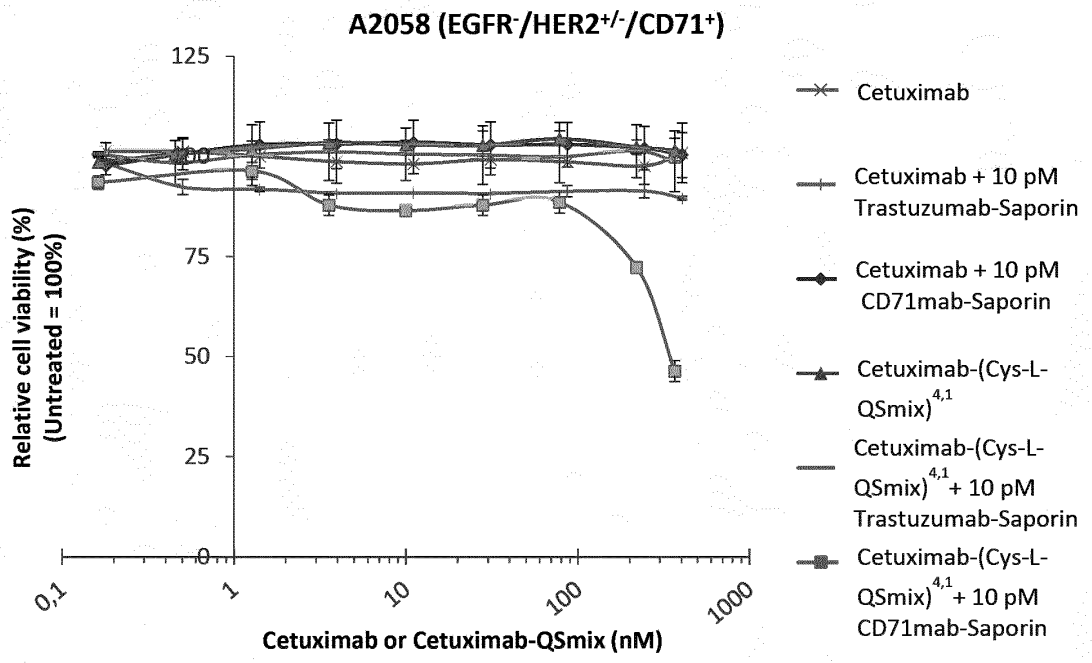


Figure 14

A

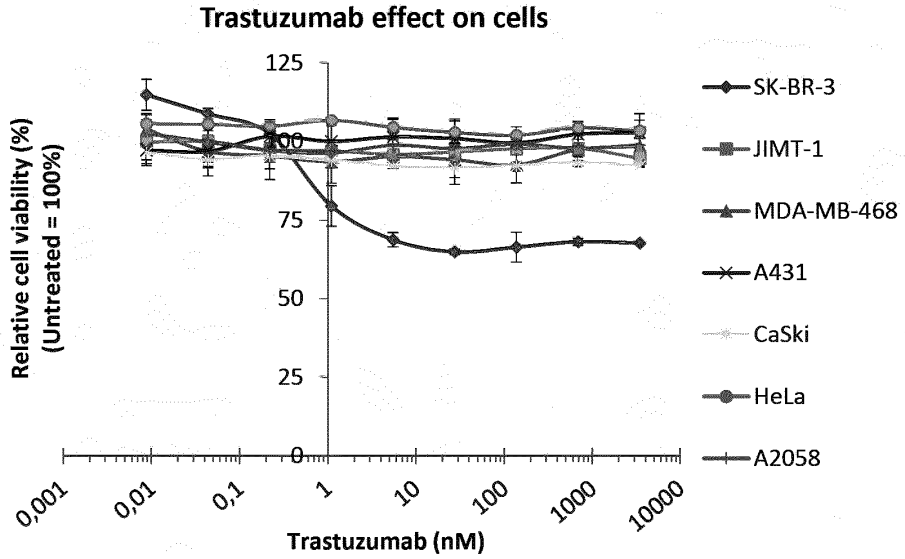
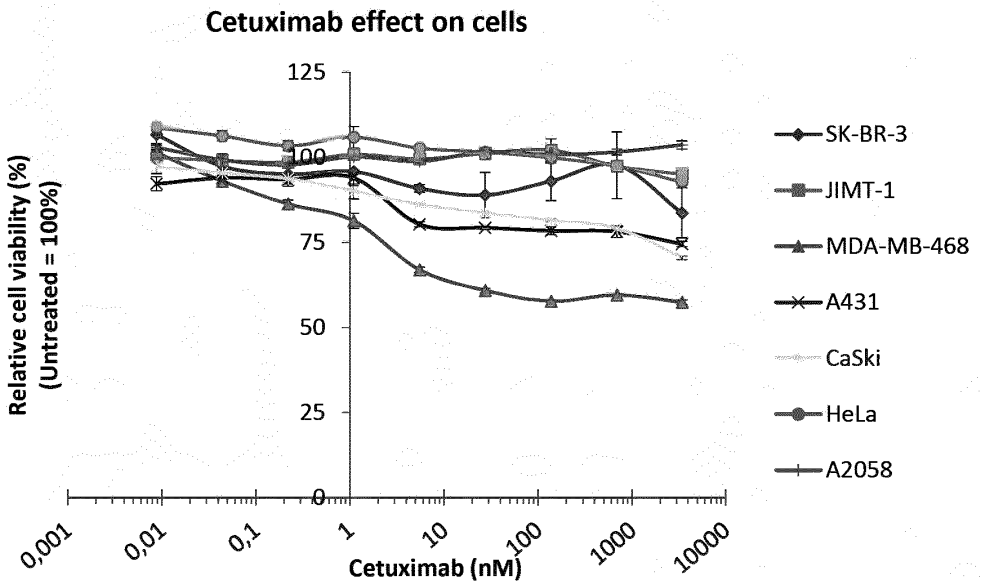


Figure 14

B


Cetuximab effect on cells
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,001
0,01
0,1
1
10
100
1000
10000
Cetuximab (nM)
SK-BR-3
JIMT-1
MDA-MB-468
A431
CaSki
HeLa
A2058

C

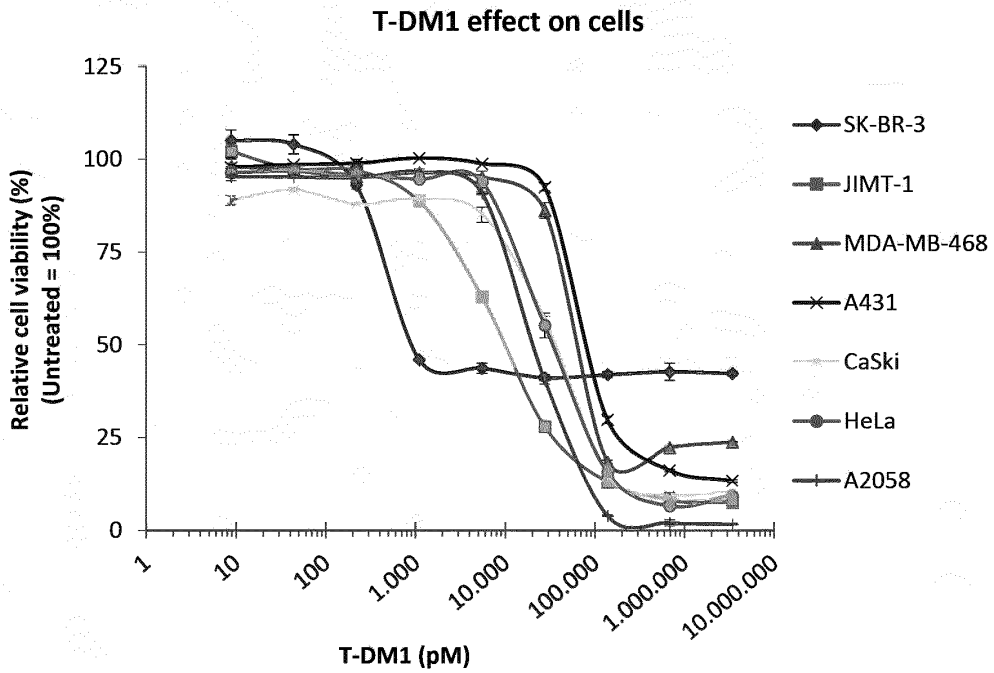

T-DM1 effect on cells
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
1
10
100
1.000
10.000
100.000
1.000.000
10.000.000
T-DM1 (pM)
SK-BR-3
JIMT-1
MDA-MB-468
A431
CaSki
HeLa
A2058

Figure 14

D

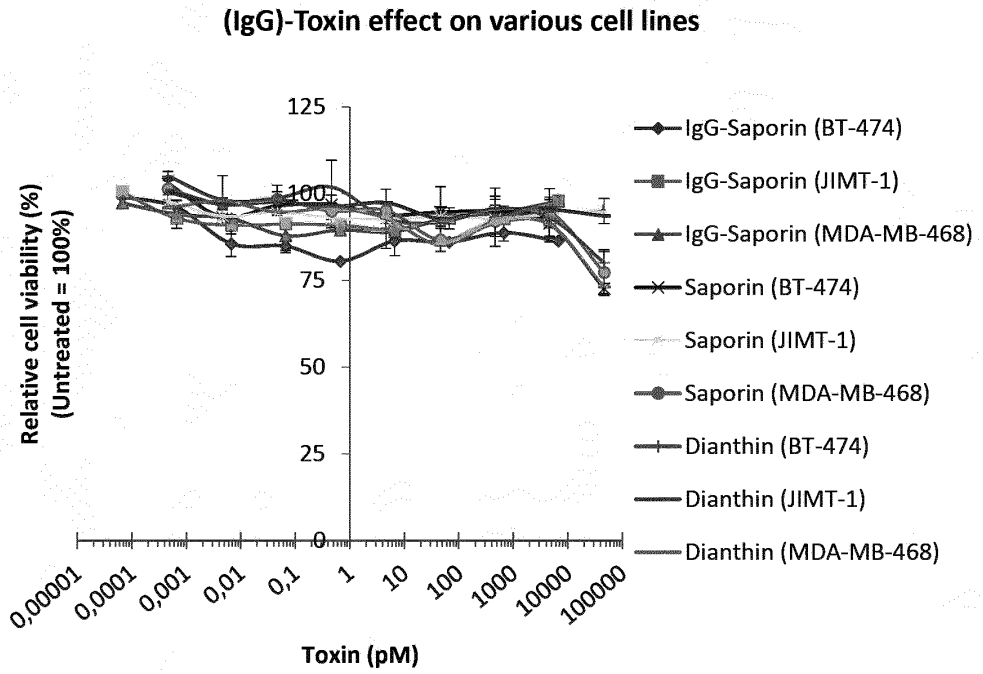

(IgG)-Toxin effect on various cell lines
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
100000
Toxin (pM)
IgG-Saporin (BT-474)
IgG-Saporin (JIMT-1)
IgG-Saporin (MDA-MB-468)
Saporin (BT-474)
Saporin (JIMT-1)
Saporin (MDA-MB-468)
Dianthin (BT-474)
Dianthin (JIMT-1)
Dianthin (MDA-MB-468)

Figure 15

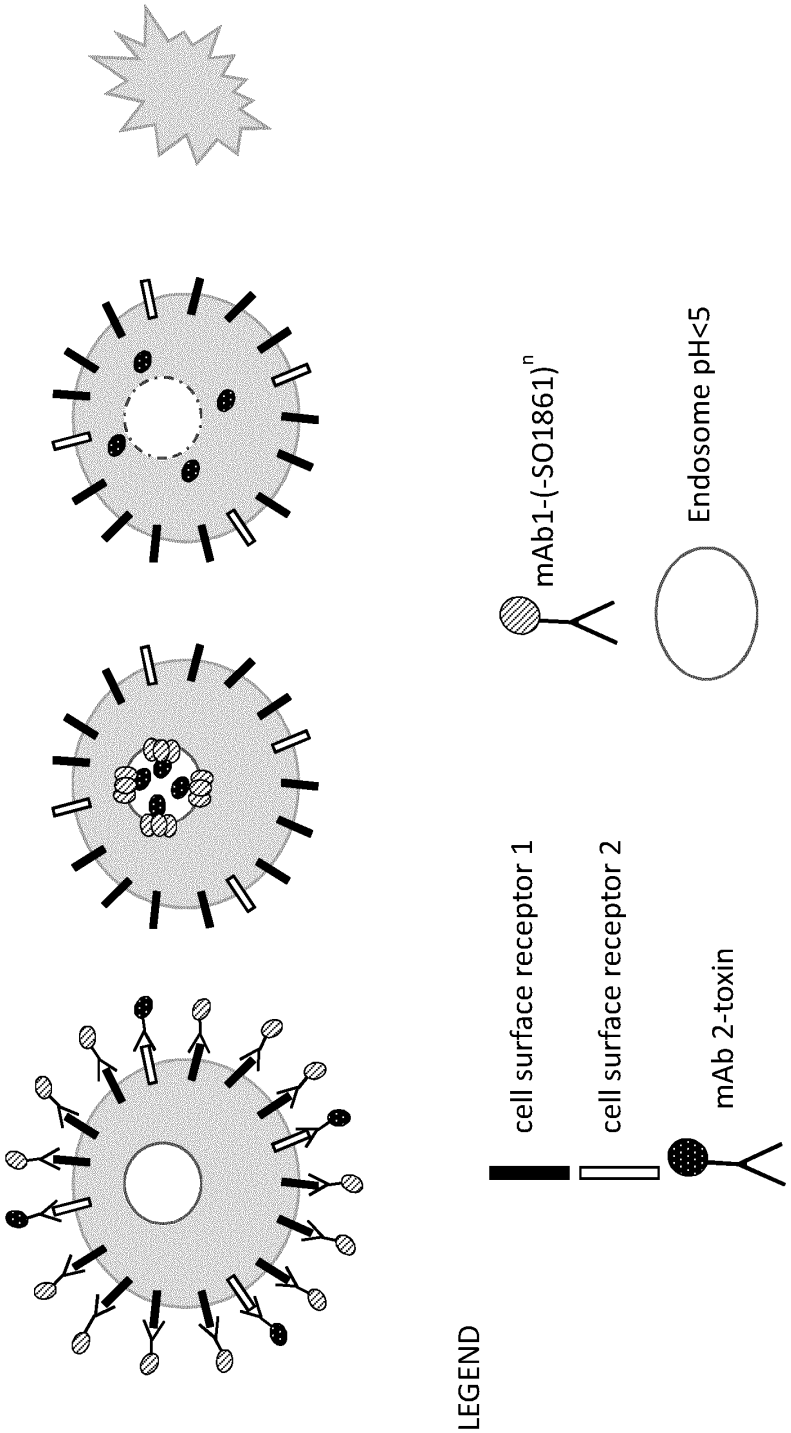

mAb1-(-SO1861)n
Endosome pH<5
cell surface receptor 1
cell surface receptor 2
mAb 2-toxin
LEGEND

Figure 16

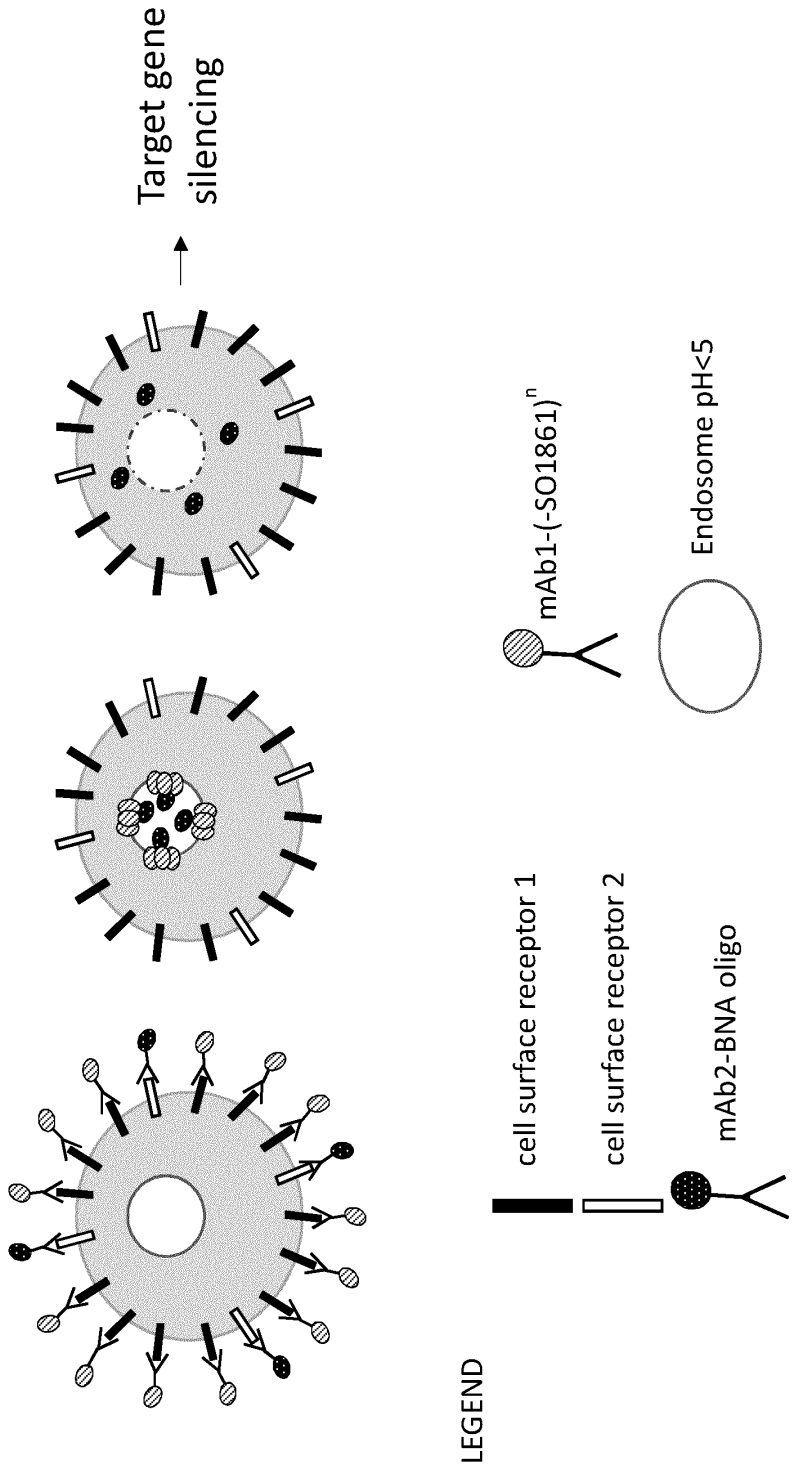

Target gene silencing
mAb1-(-SO1861)n
Endosome pH<5
cell surface receptor 1
cell surface receptor 2
mAb2-BNA oligo
LEGEND

Figure 17

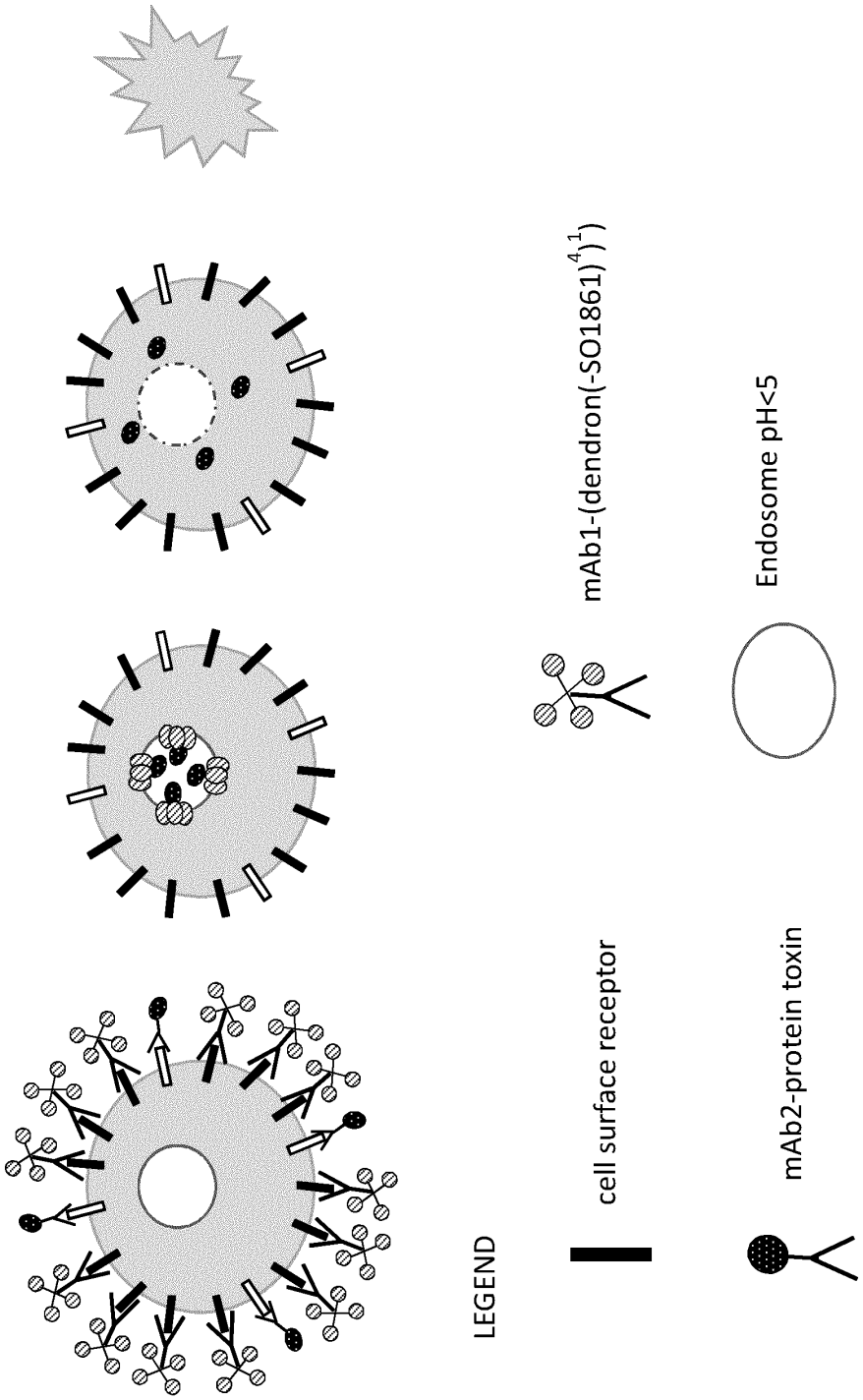

mAb1-(dendron(-SO1861)^{4})^{1})
Endosome pH<5
LEGEND
cell surface receptor
mAb2-protein toxin

Figure 18

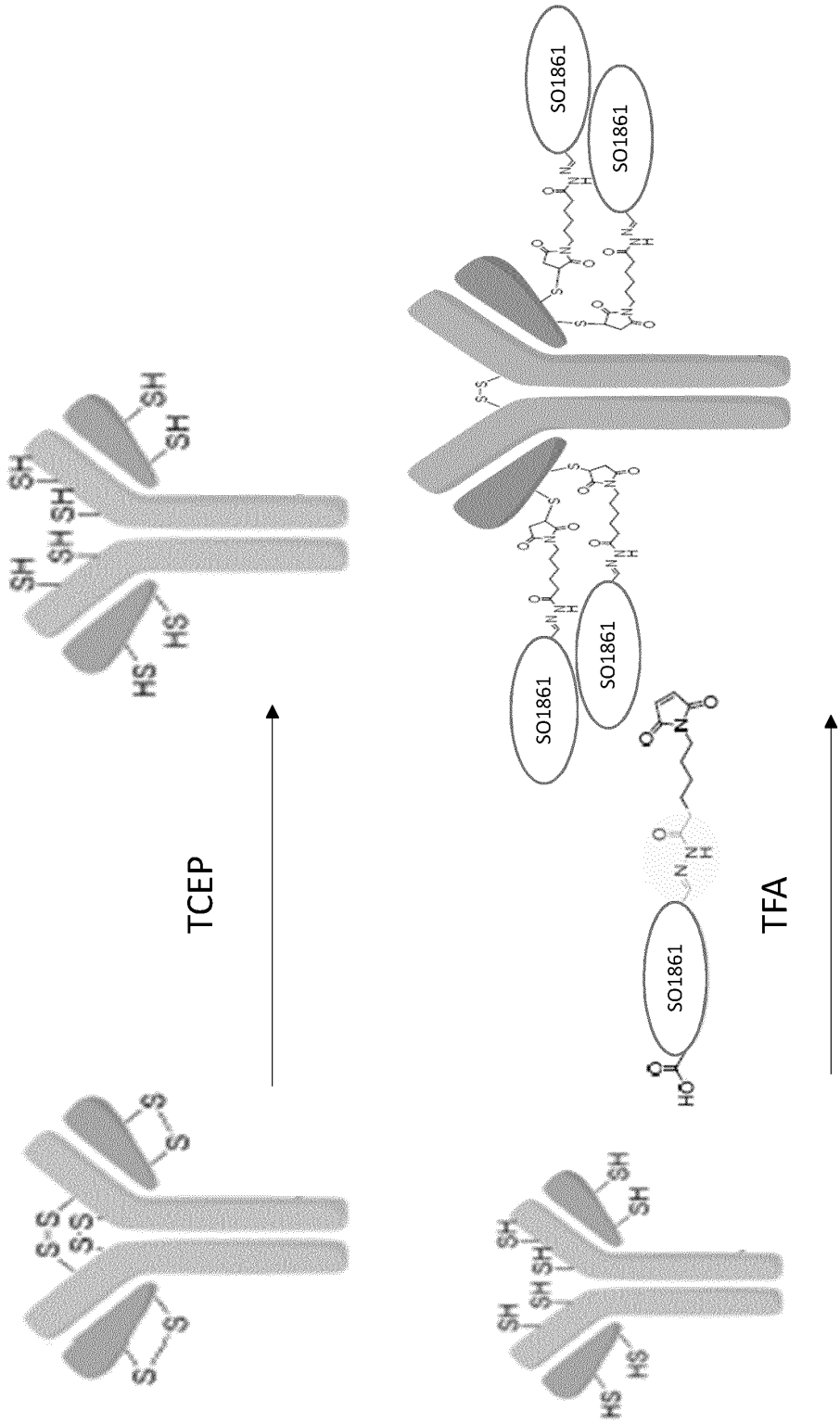


Figure 19

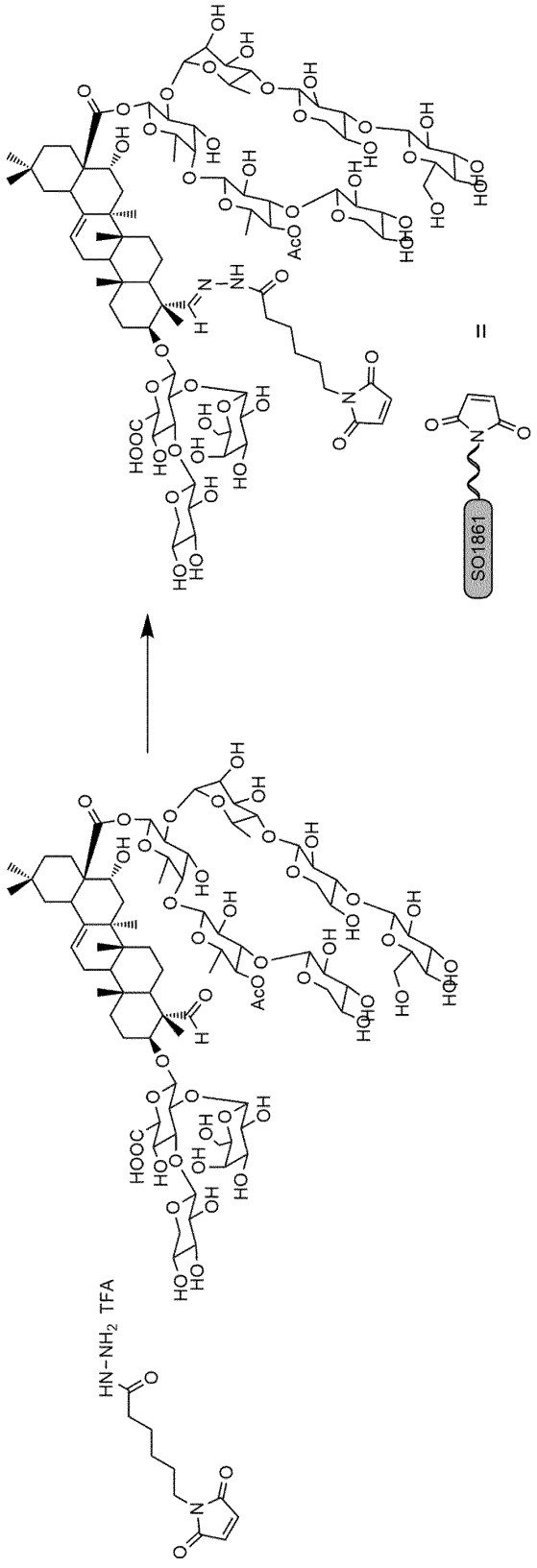


Figure 20

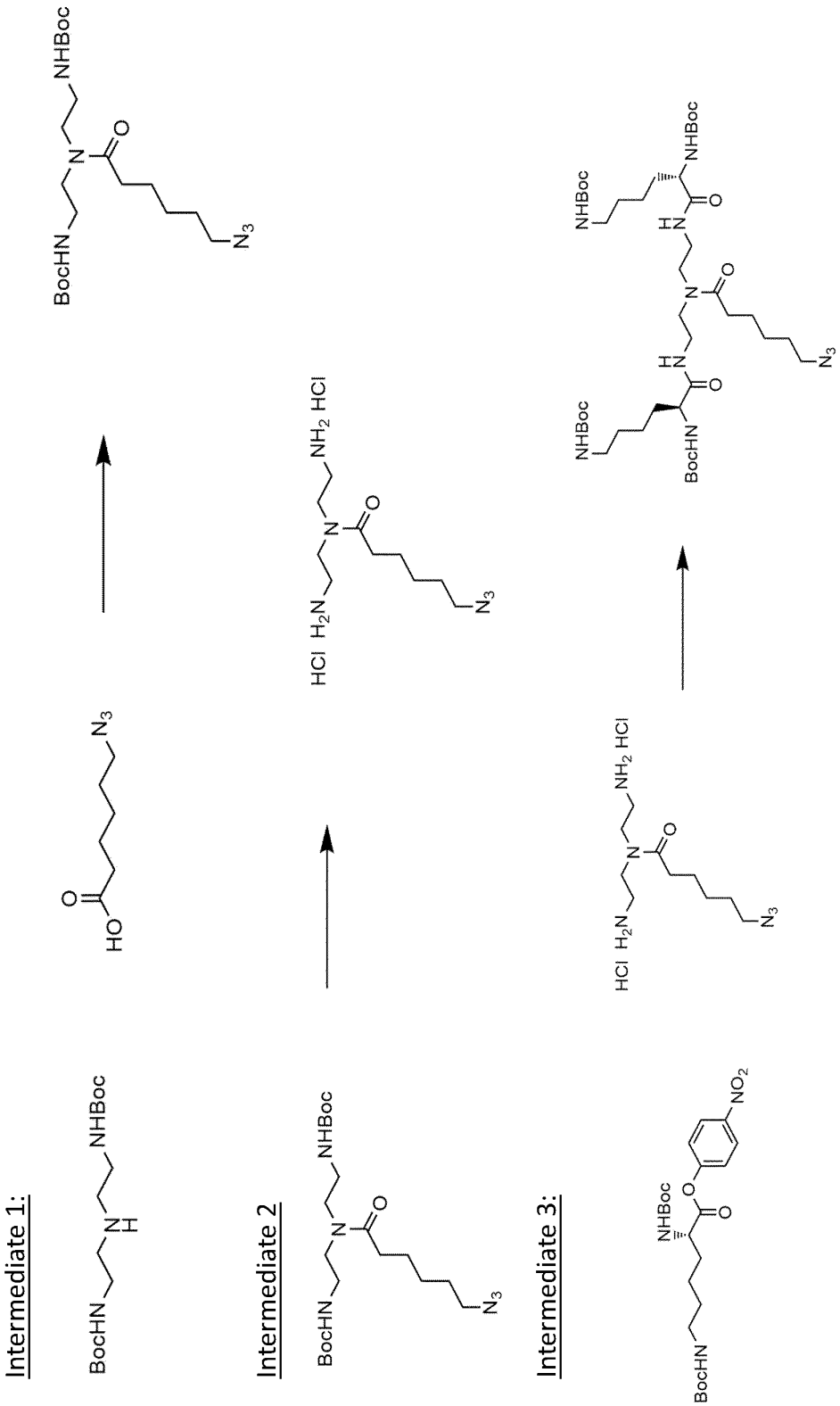

Intermediate 1:
BocHN
NHBoc
Intermediate 2
BocHN
NHBoc
Intermediate 3:
BocHN
NHBoc
NO2

Figure 20

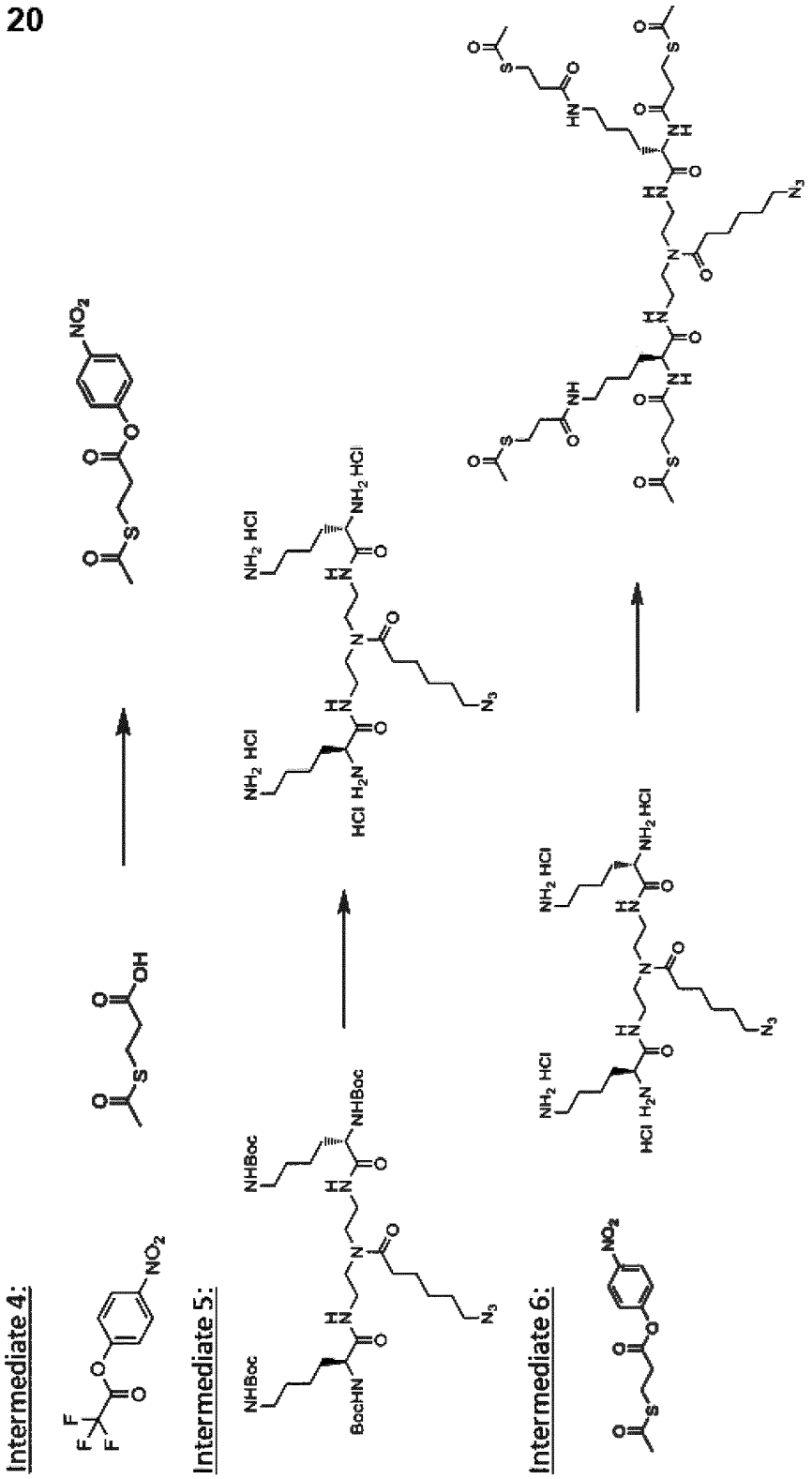


Figure 20

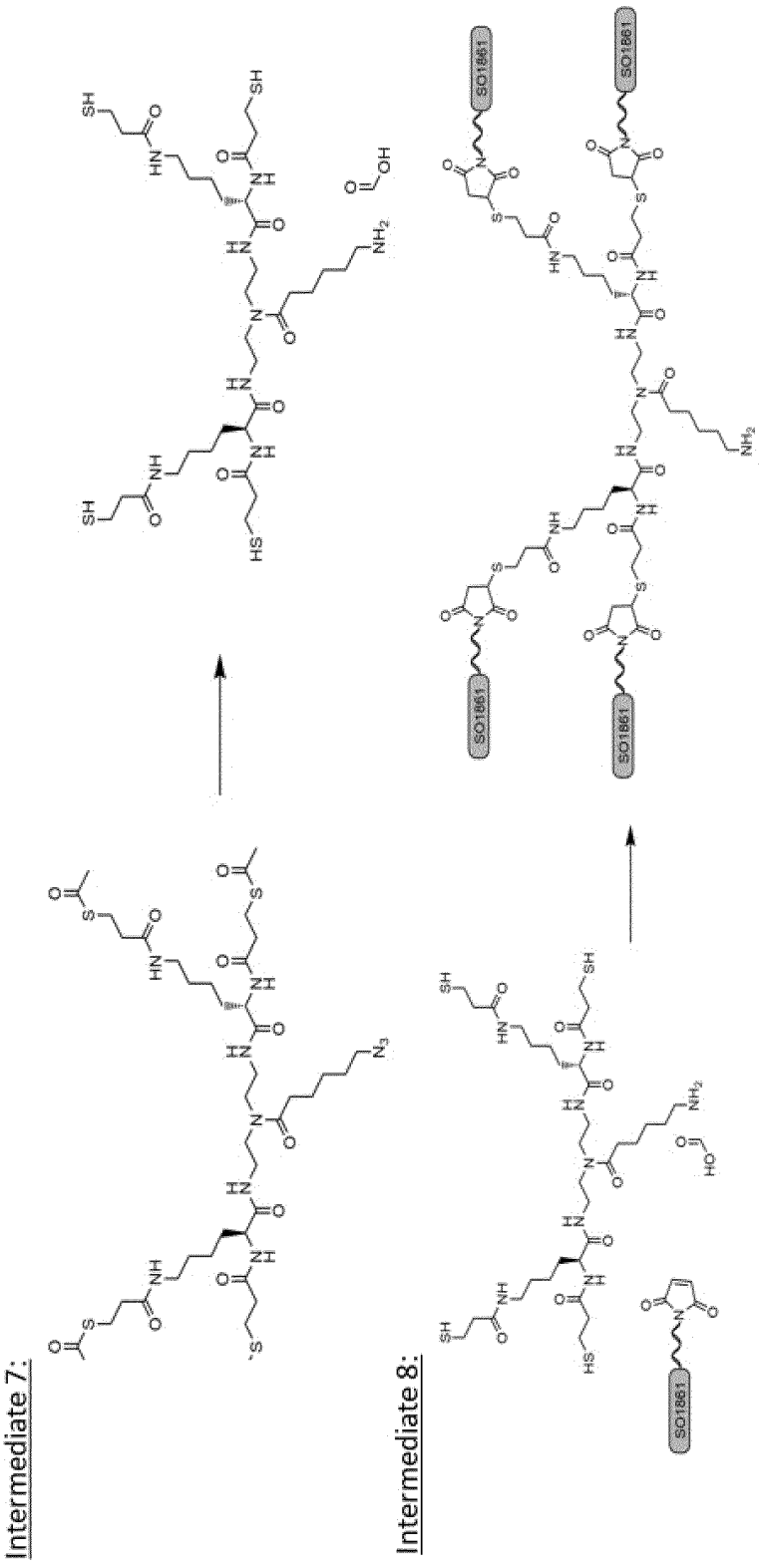


Figure 20

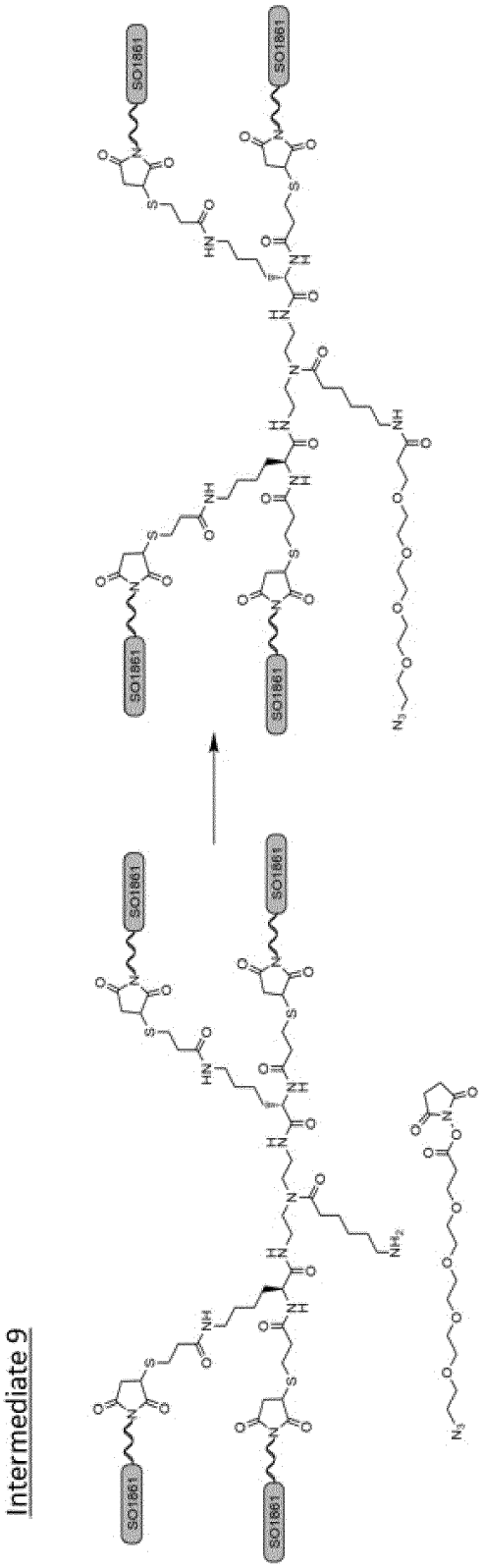
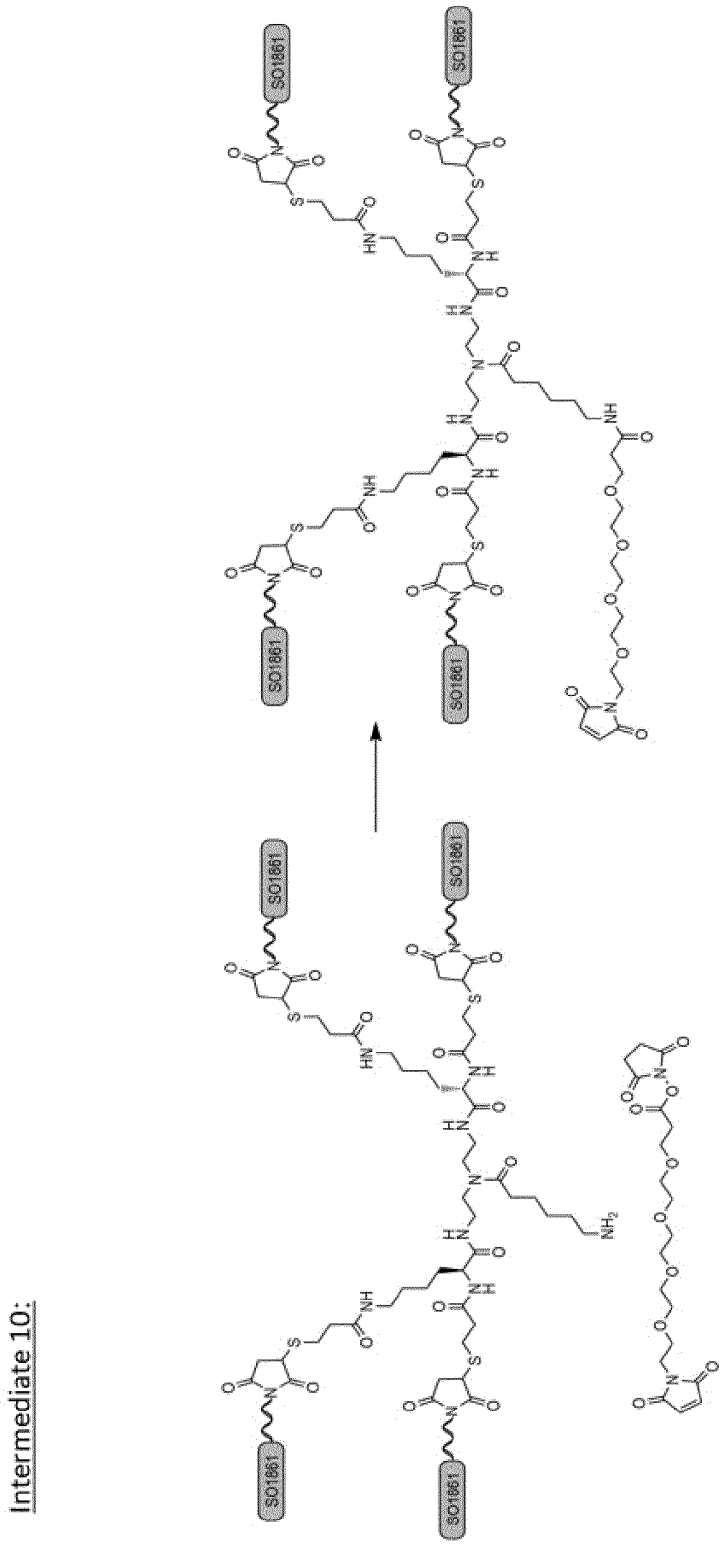


Figure 20



Intermediate 10:

Figure 20

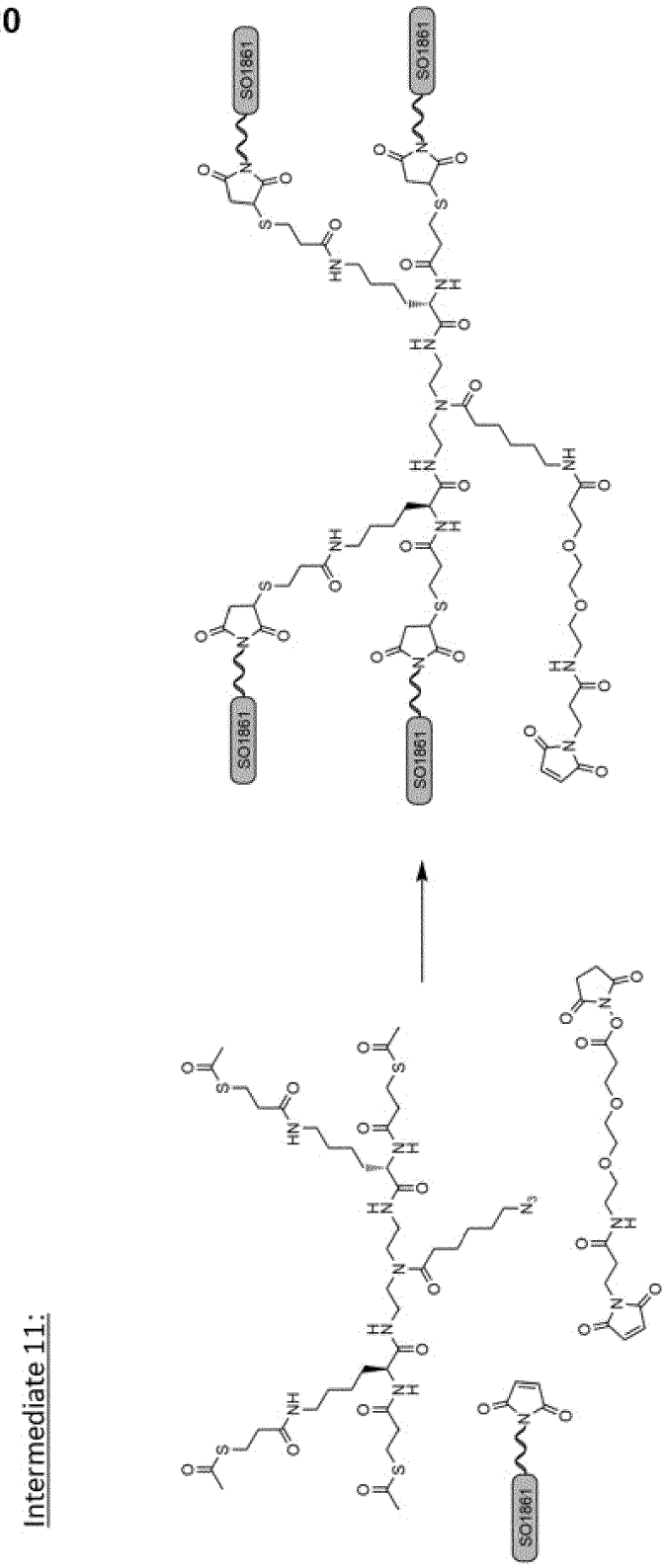
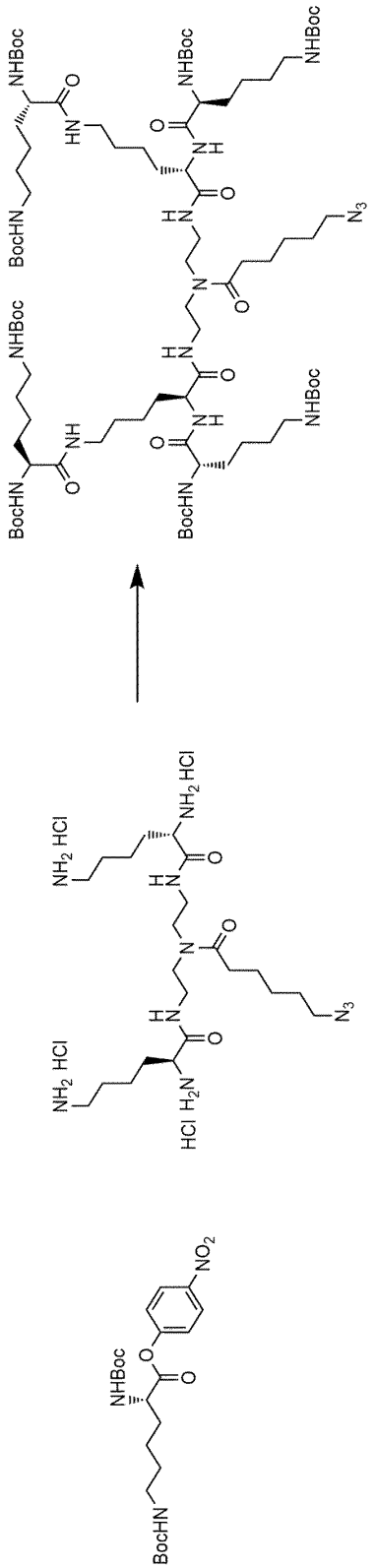


Figure 21



Intermediate 1:

Figure 21

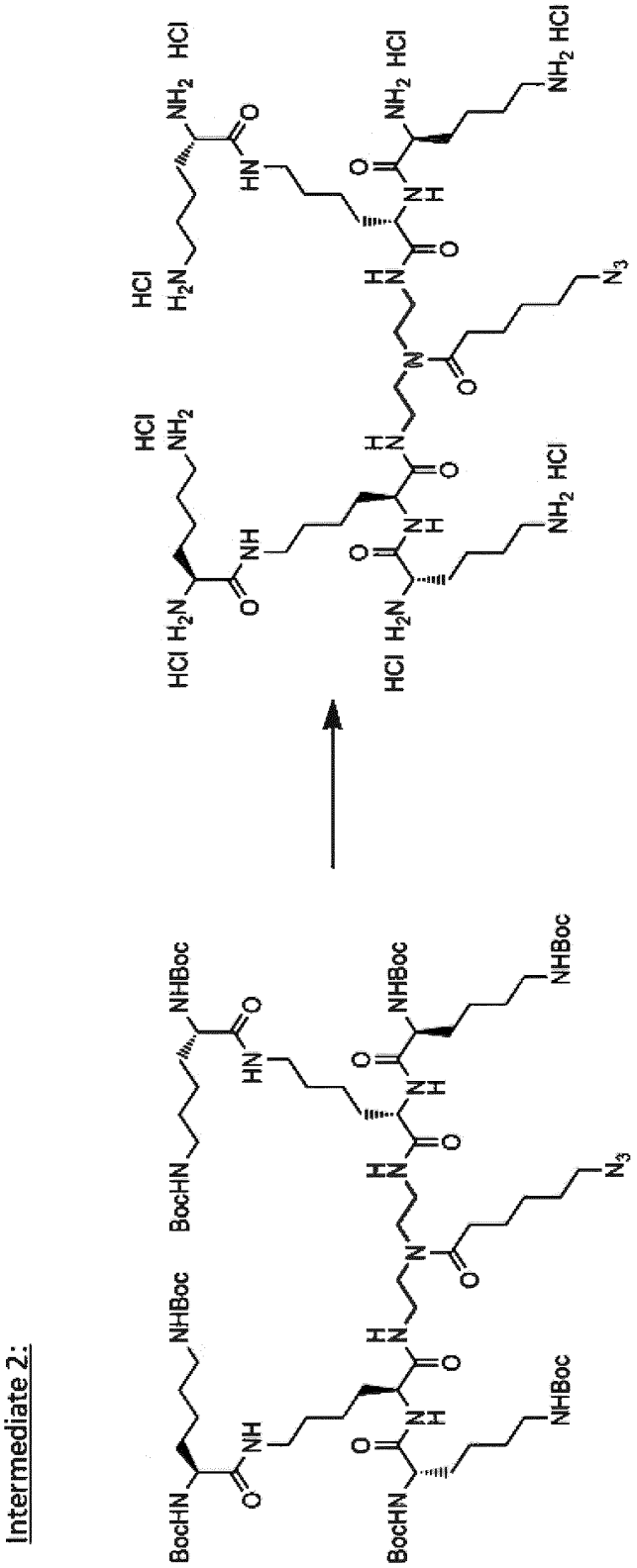


Figure 21

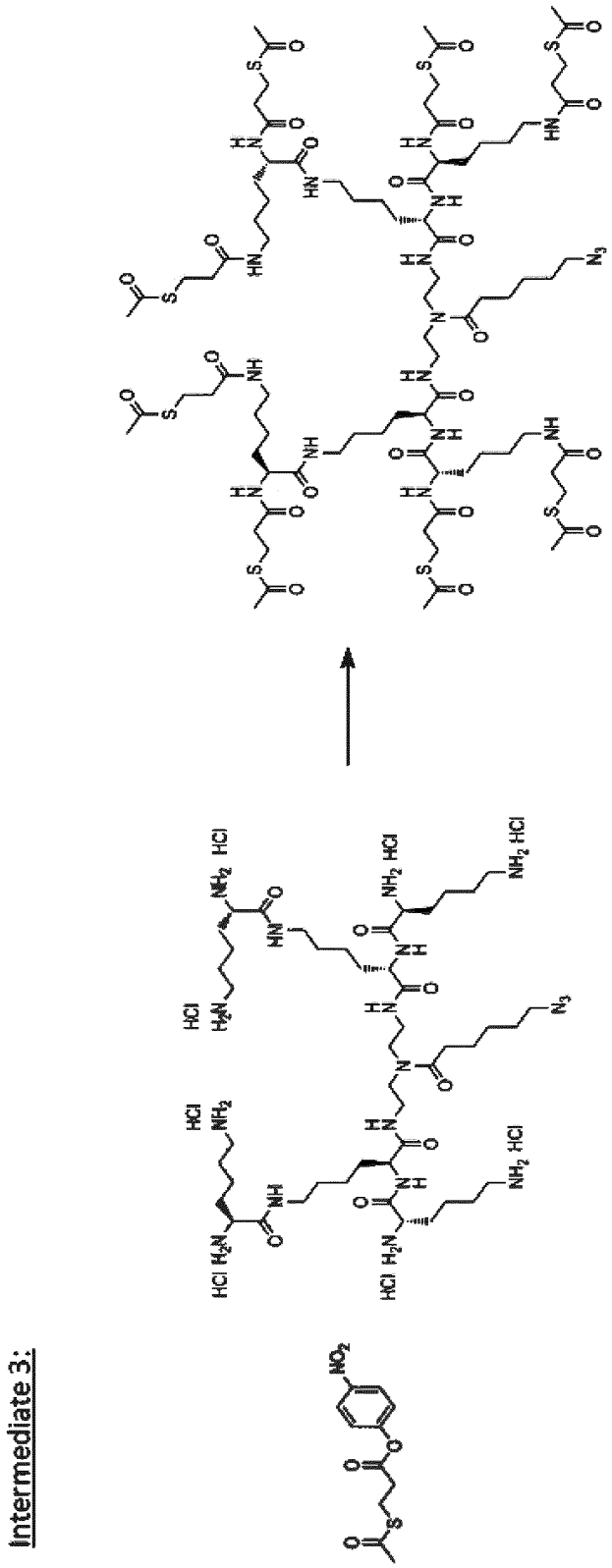
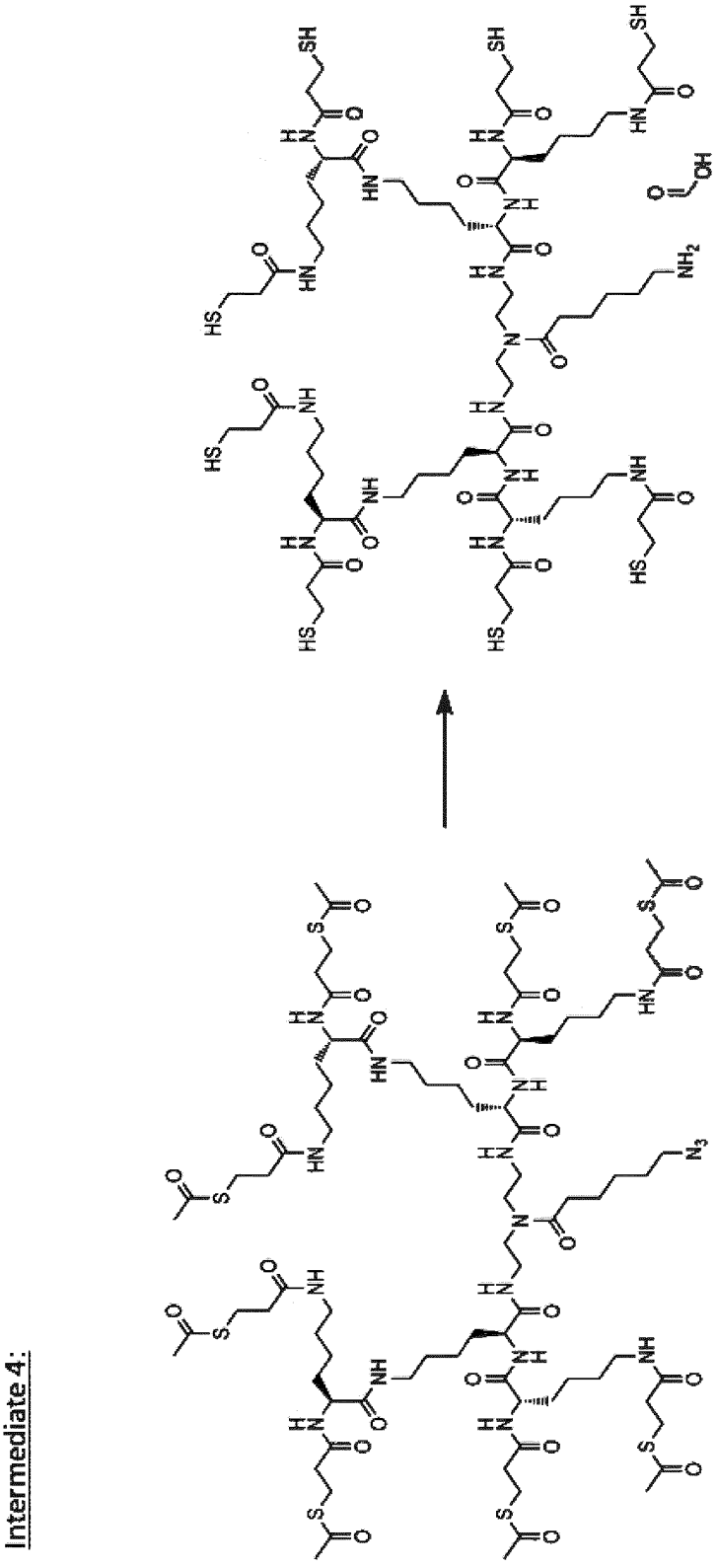


Figure 21



Intermediate 4:

Figure 21

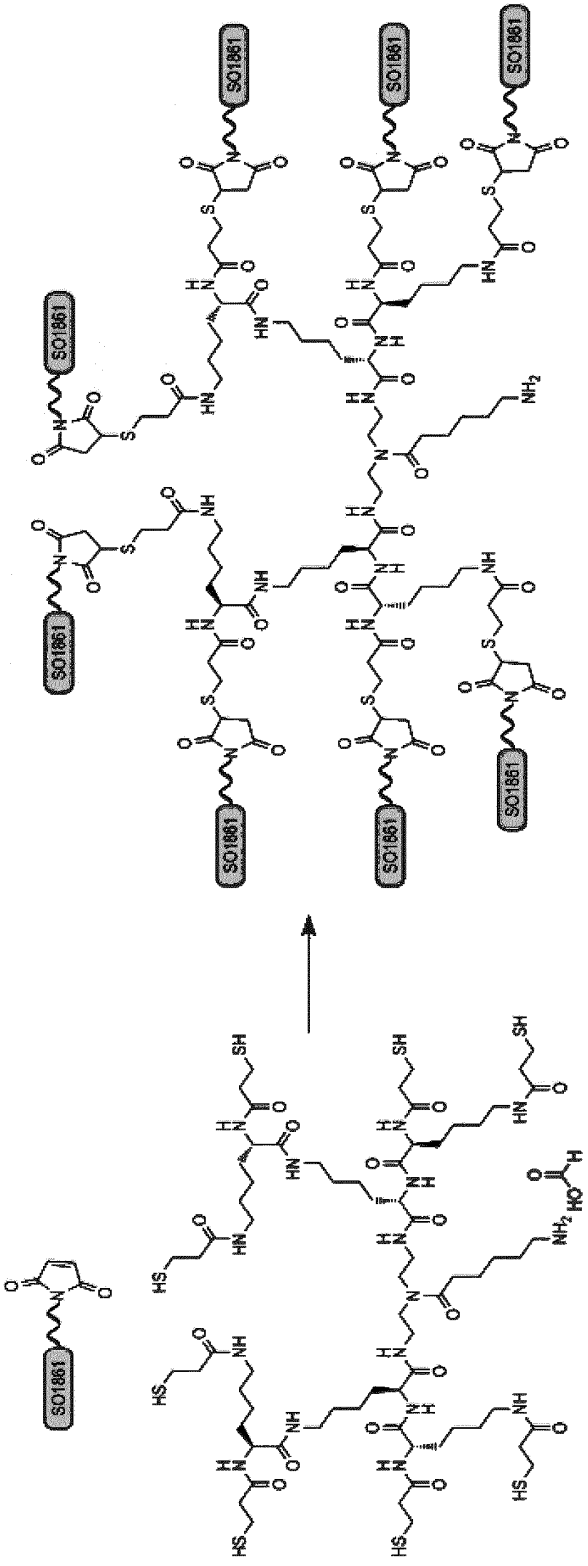


Figure 22

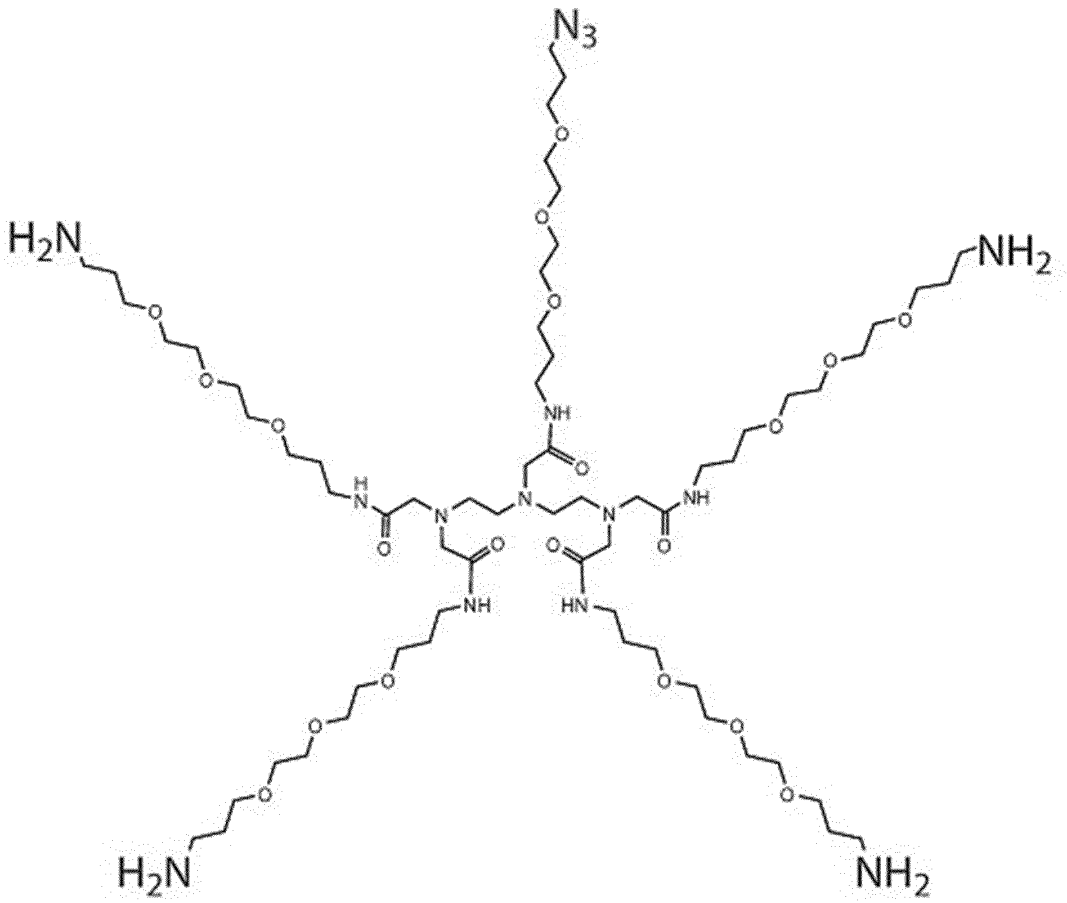


Figure 23

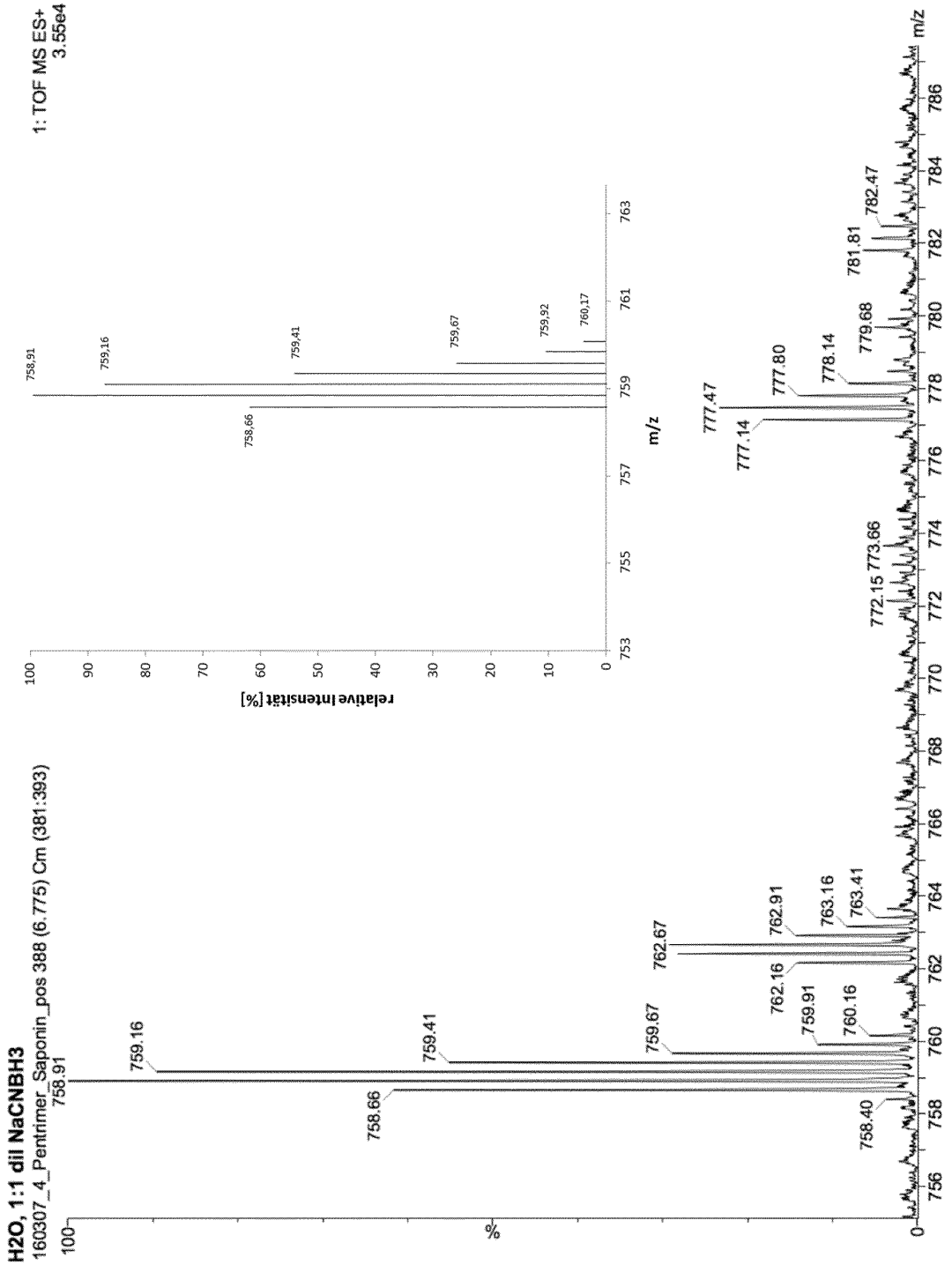

H2O, 1:1 dil NaCNBH3
160307_4_Pentrimer_Saponin_pos 388 (6.775) Cm (381:393)
1: TOF MS ES+
3.55e4
relative Intensität [%]
m/z
%
758.91
759.16
759.41
758.66
759.67
762.67
762.91
763.16
763.41
762.16
759.91
760.16
758.40
772.15
773.66
777.14
777.47
777.80
778.14
779.68
781.81
782.47

Figure 24

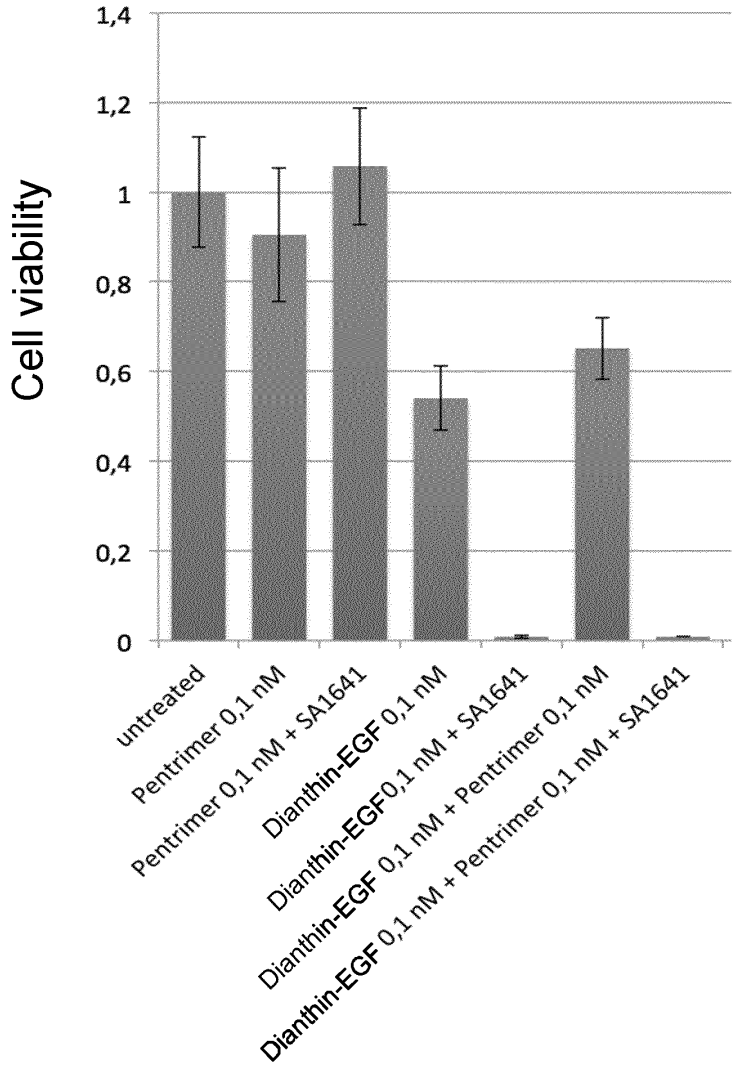

Cell viability
1,4
1,2
1
0,8
0,6
0,4
0,2
0
untreated
Pentrimer 0,1 nM
Pentrimer 0,1 nM + SA1641
Dianthin-EGF 0,1 nM
Dianthin-EGF 0,1 nM + SA1641
Dianthin-EGF 0,1 nM + Pentrimer 0,1 nM
Dianthin-EGF 0,1 nM + Pentrimer 0,1 nM + SA1641

Figure 25

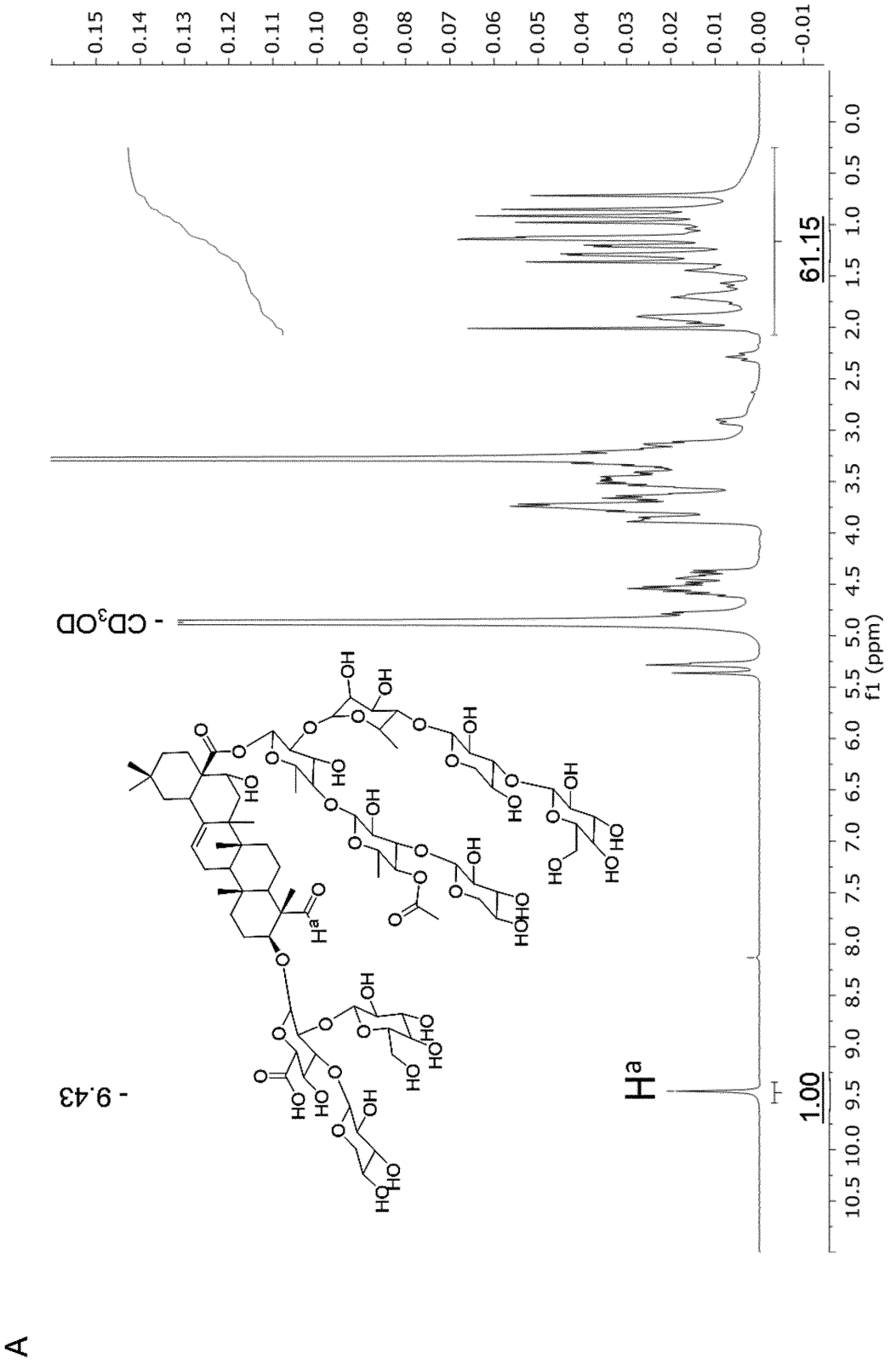


Figure 25

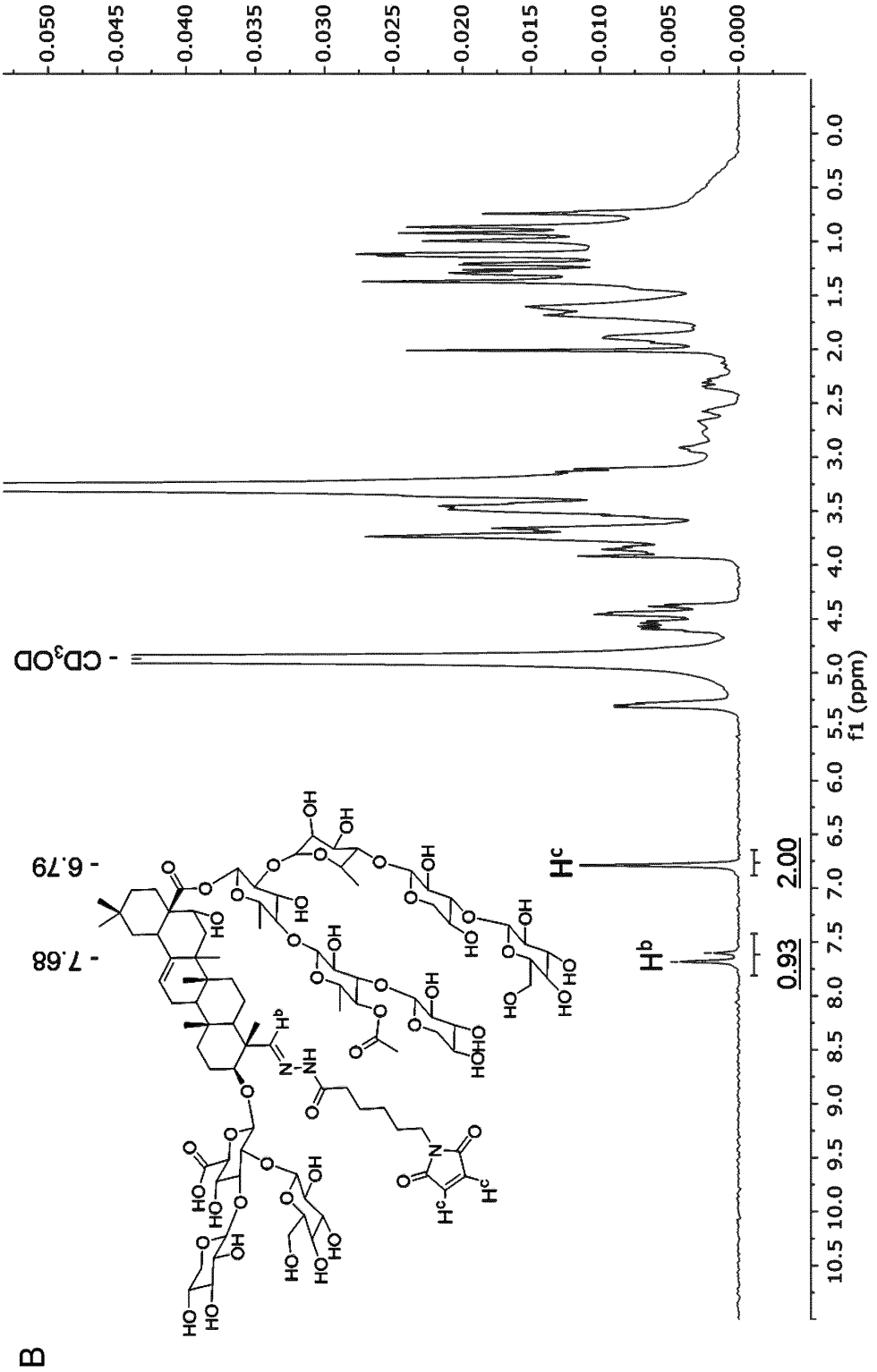


Figure 26

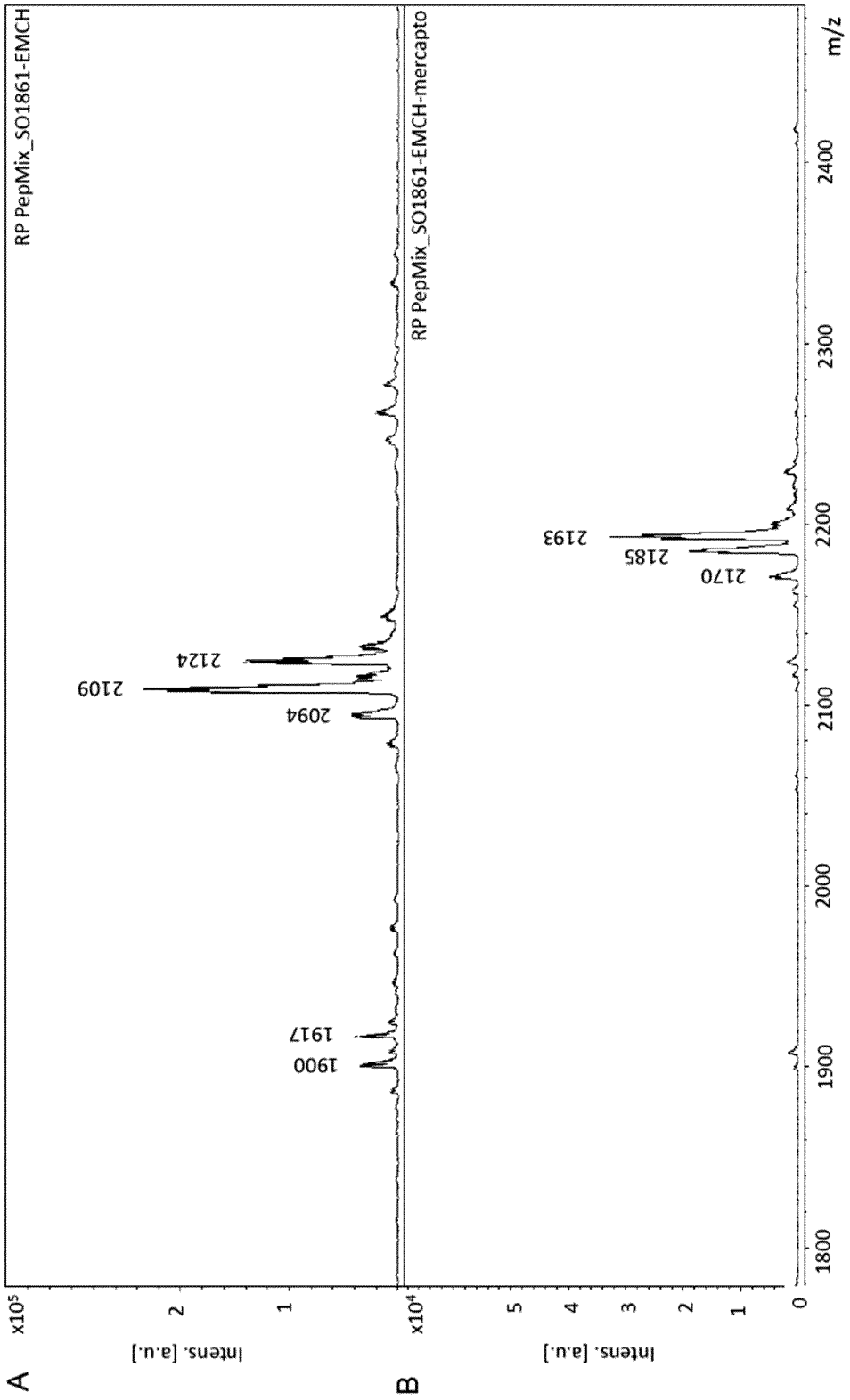

A
B
RP PepMix_SO1861-EMCH
RP PepMix_SO1861-EMCH-mercapto
Intens. [a.u.]
x10⁵
x10⁴
m/z
1800
1900
2000
2100
2200
2300
2400
1917
1900
2094
2109
2124
2170
2185
2193

Figure 27

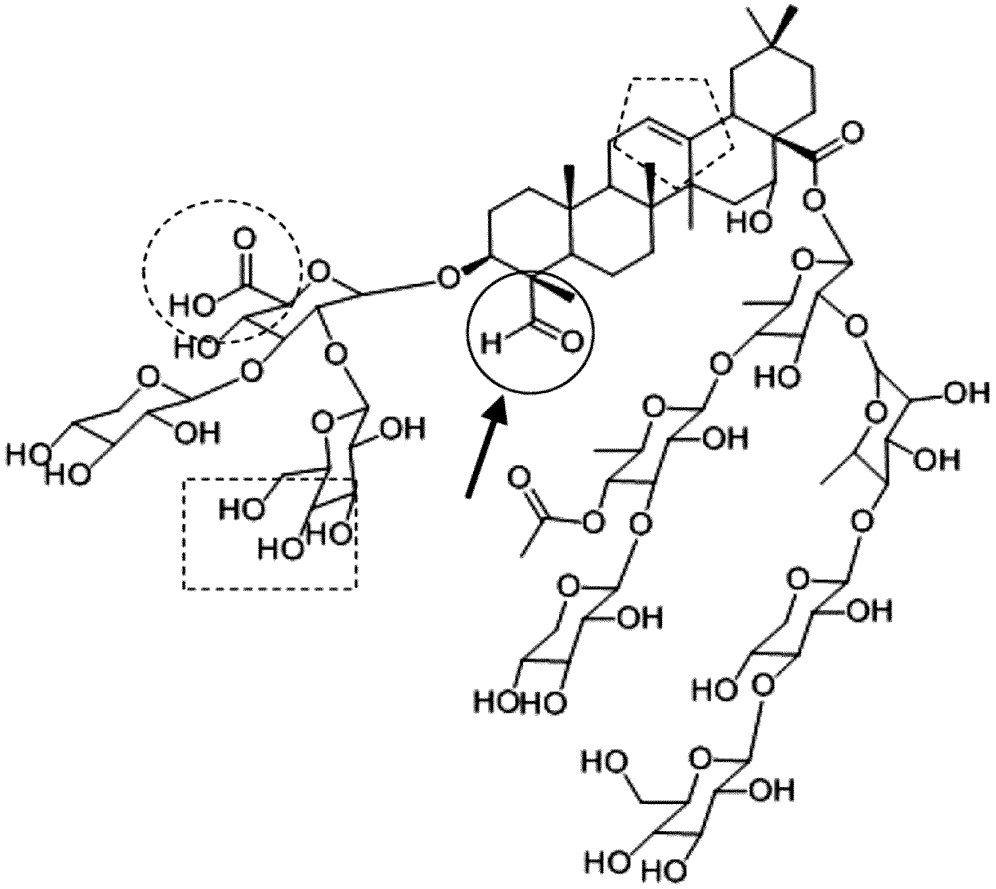


Figure 28

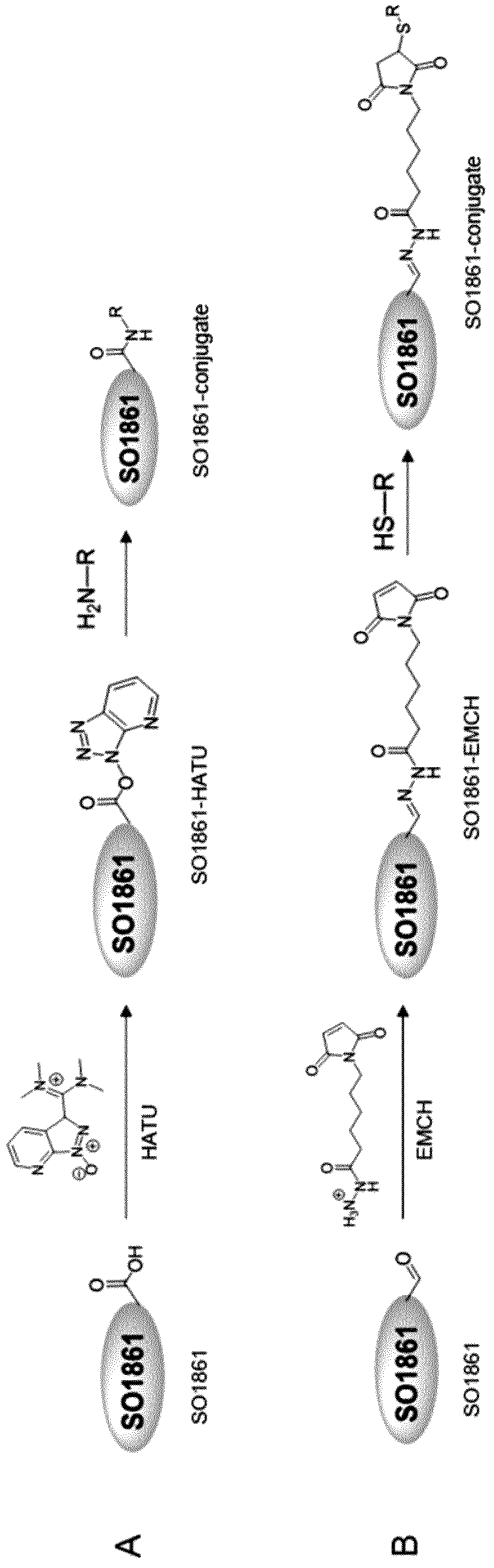

A
SO1861
HATU
SO1861-HATU
H_2N—R
SO1861-conjugate
B
SO1861
EMCH
SO1861-EMCH
HS—R
SO1861-conjugate

Figure 29

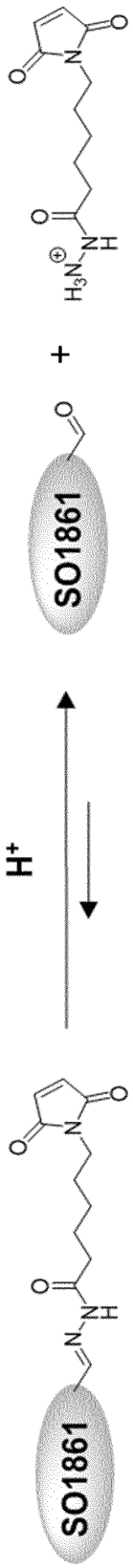
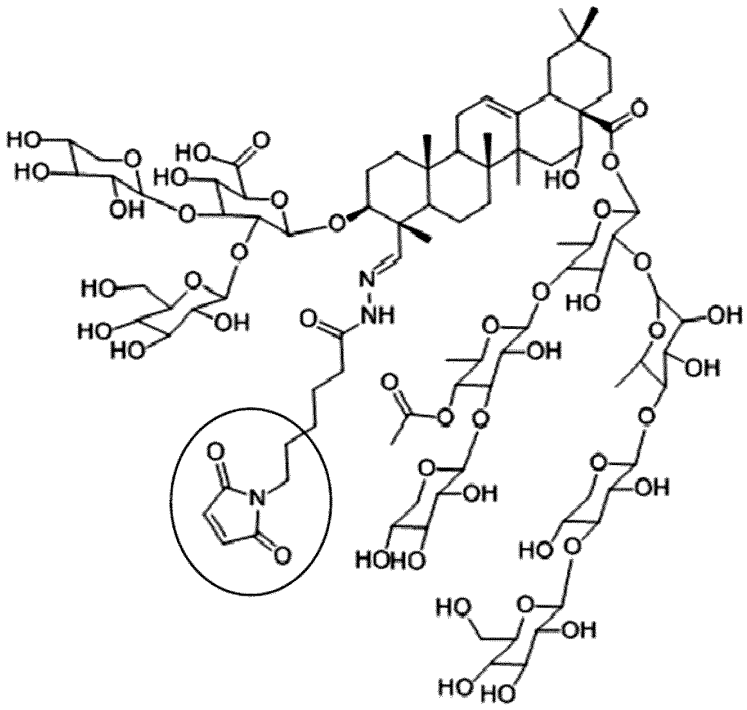


Figure 30

A



B

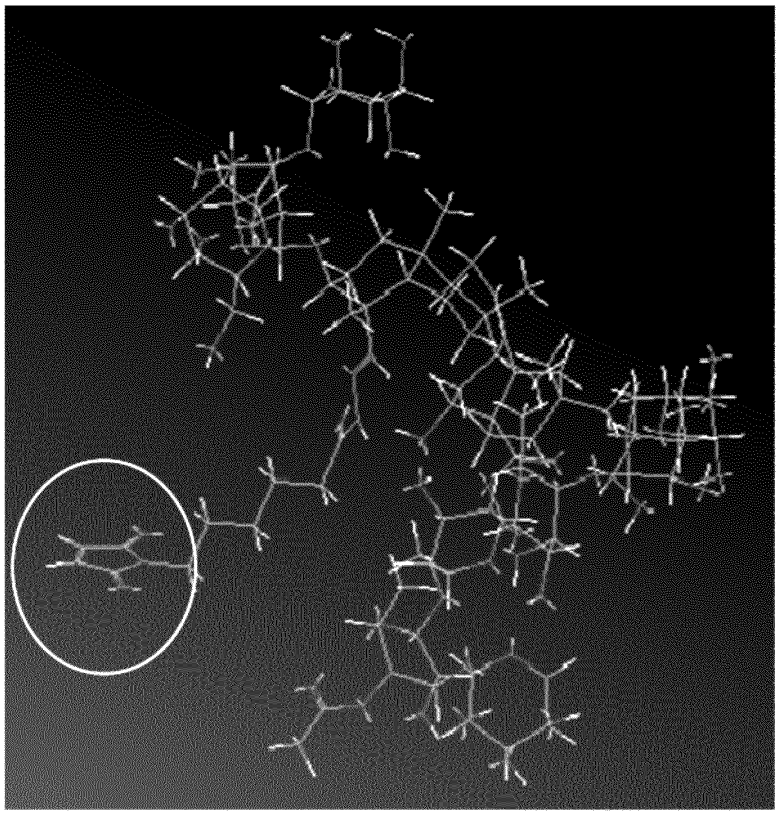


Figure 31

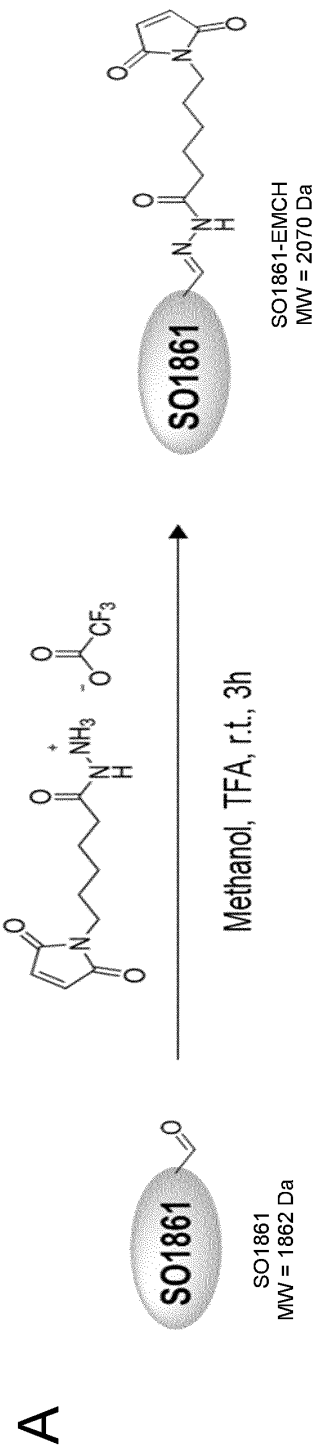

A
SO1861
SO1861
MW = 1862 Da
Methanol, TFA, r.t., 3h
SO1861
SO1861-EMCH
MW = 2070 Da

Figure 31

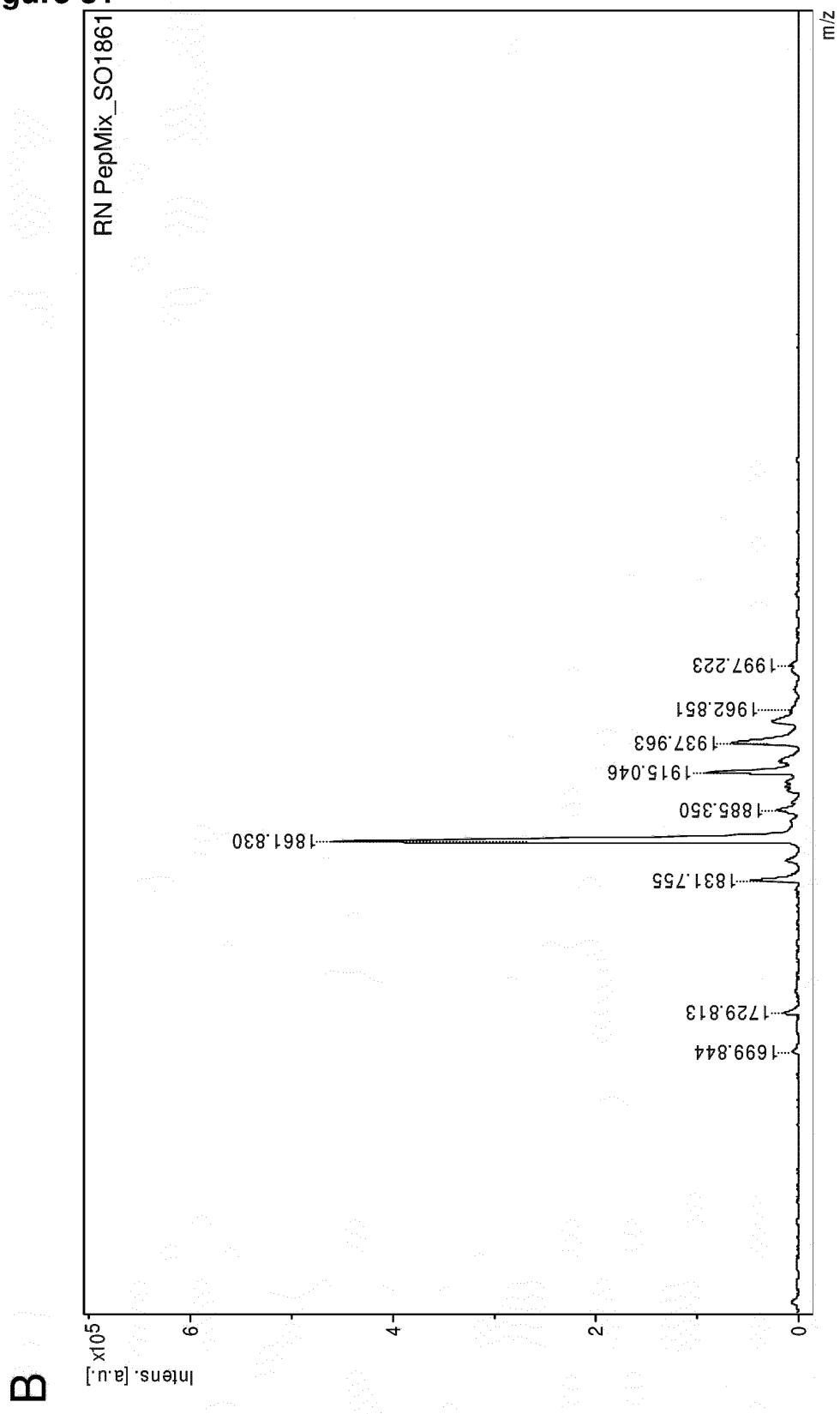

B
RN PepMix_SO1861
Intens. [a.u.]
x10^5
6
4
2
0
m/z
1997.223
1962.851
1937.963
1915.046
1885.350
1861.830
1831.755
1729.813
1699.844

Figure 31

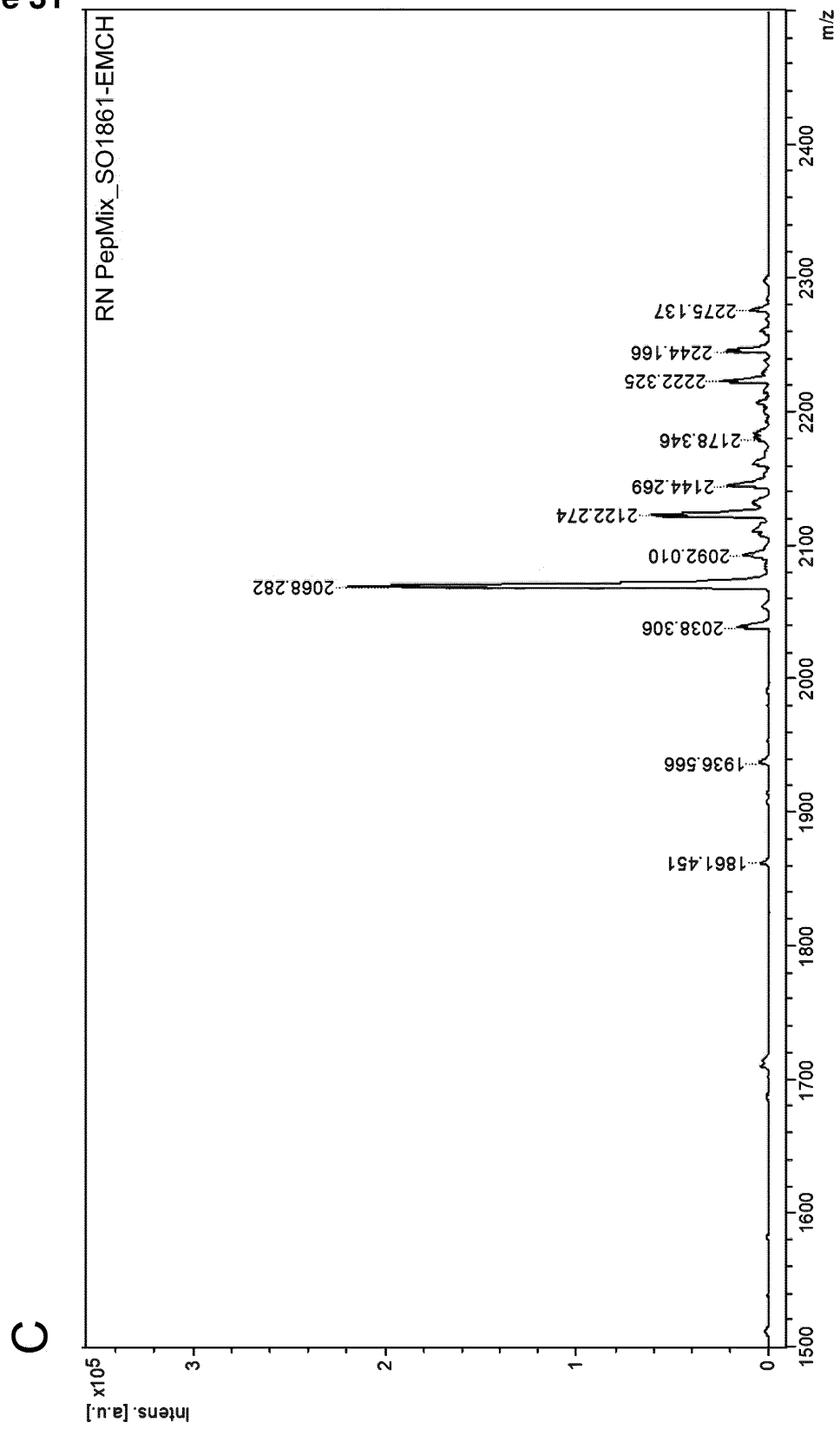

C
RN PepMix_SO1861-EMCH
Intens. [a.u.]
x10^5
3
2
1
0
m/z
1500
1600
1700
1800
1900
2000
2100
2200
2300
2400
1861.451
1936.566
2038.306
2068.282
2092.010
2122.274
2144.269
2178.346
2222.325
2244.166
2275.137

Figure 32

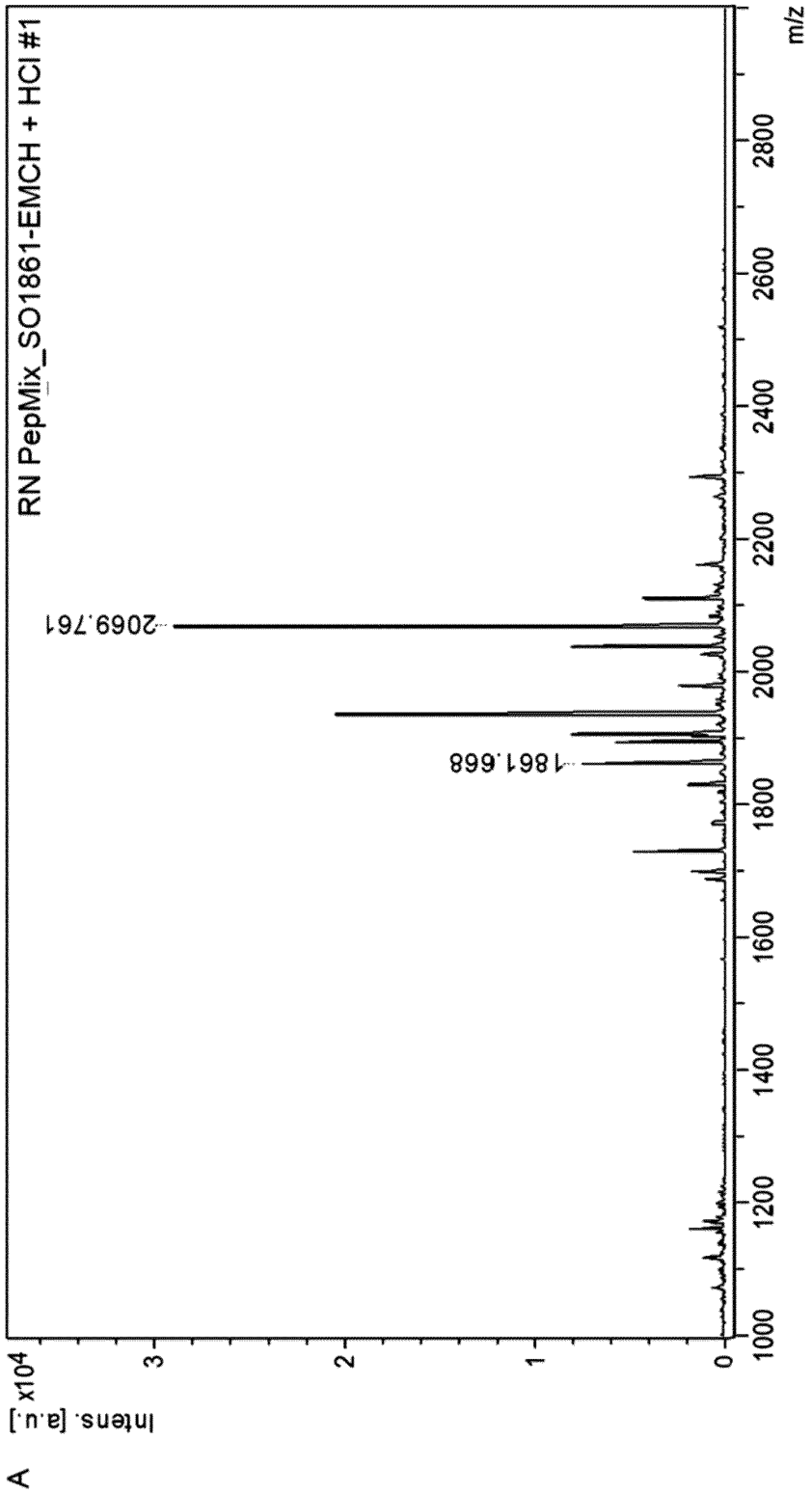

A
Intens. [a.u.]
x10^4
3
2
1
0
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
m/z
RN PepMix_SO1861-EMCH + HCl #1
2069.761
1861.668

Figure 32

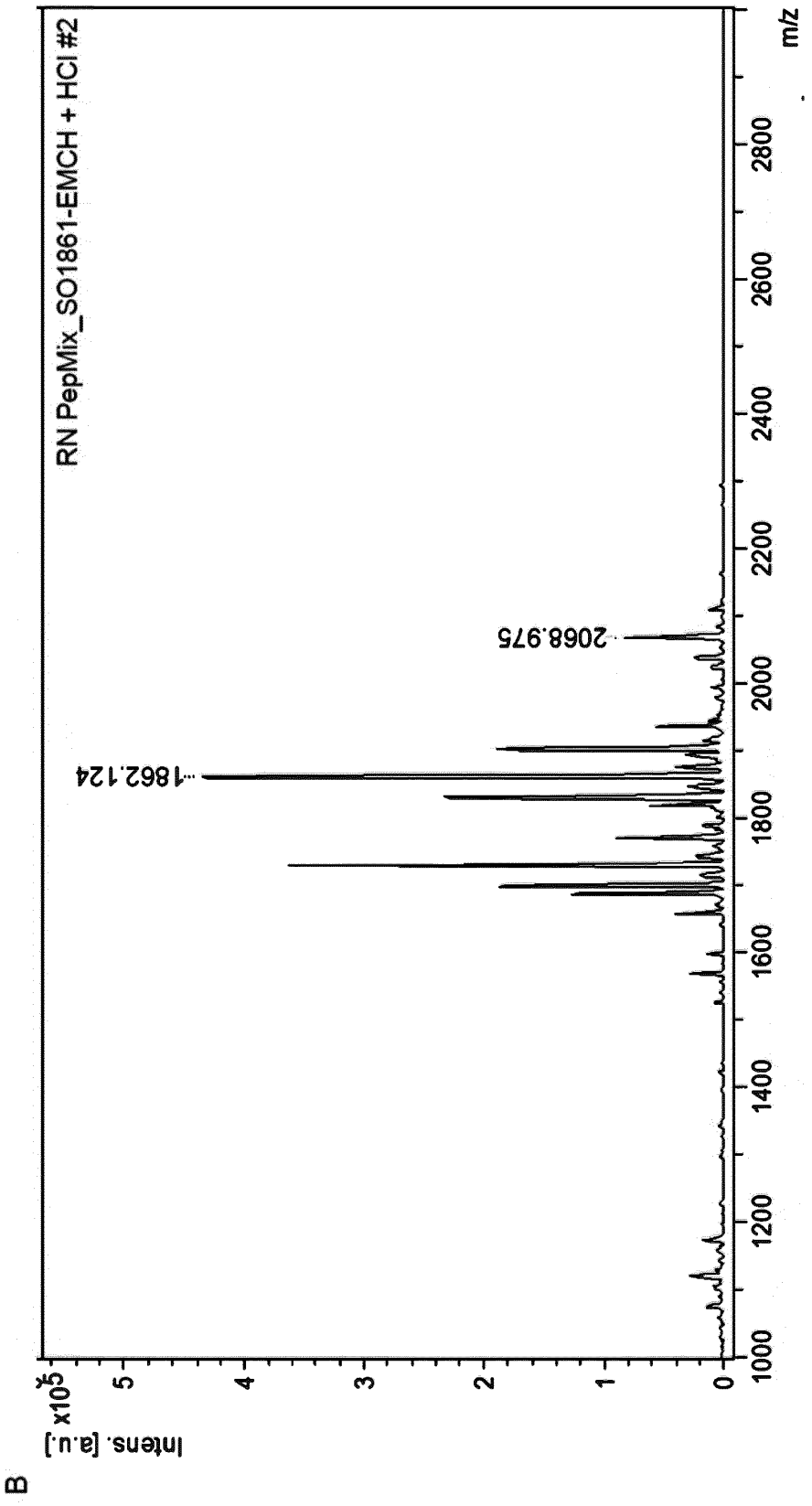

B
RN PepMix_SO1861-EMCH + HCl #2
Intens. [a.u.]
x10^5
0
1
2
3
4
5
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
m/z
1862.124
2068.975

Figure 33

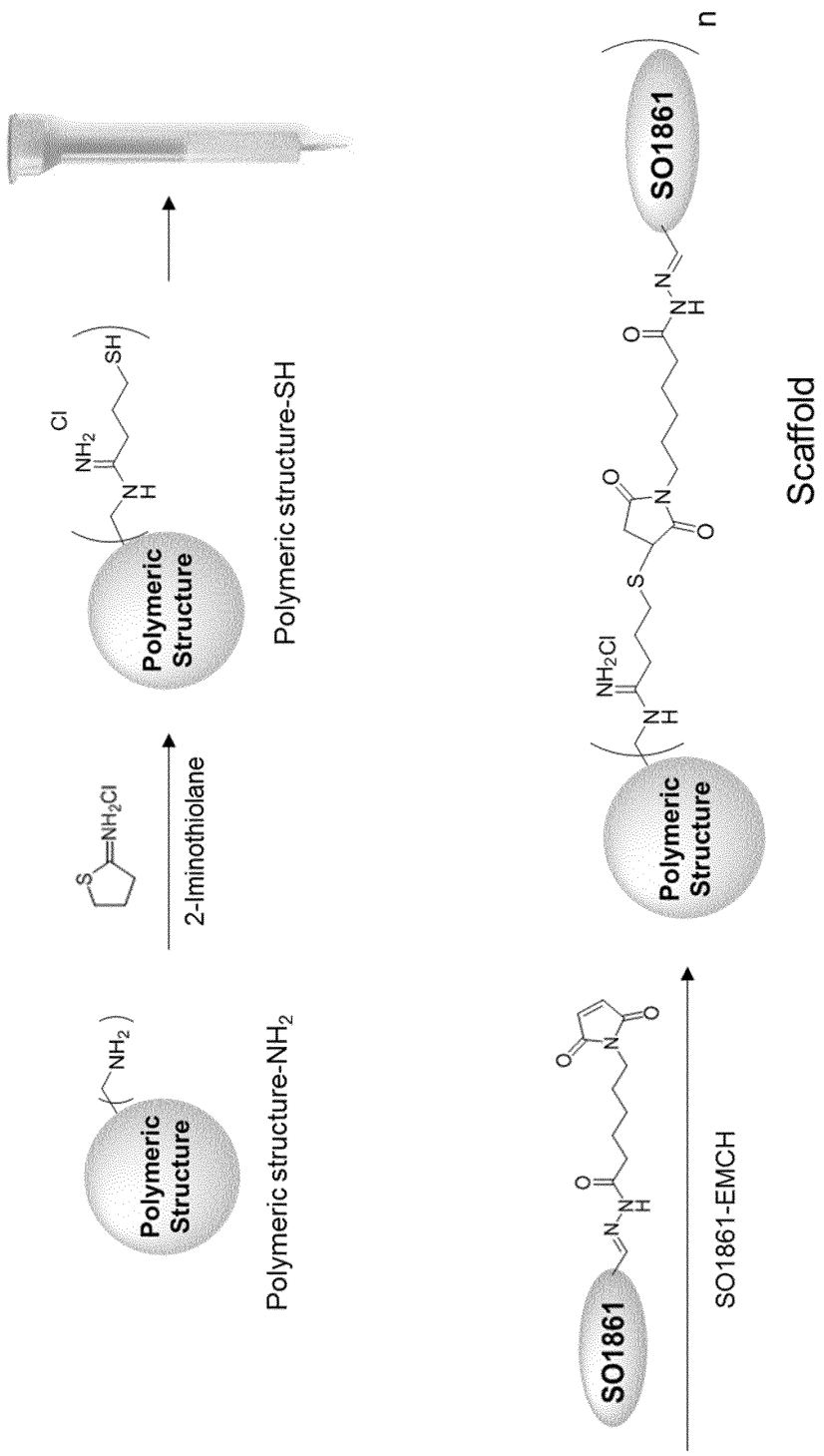

Polymeric structure-NH2
2-Iminothiolane
Polymeric structure-SH
SO1861-EMCH
Scaffold
Polymeric Structure
SO1861
n

Figure 34

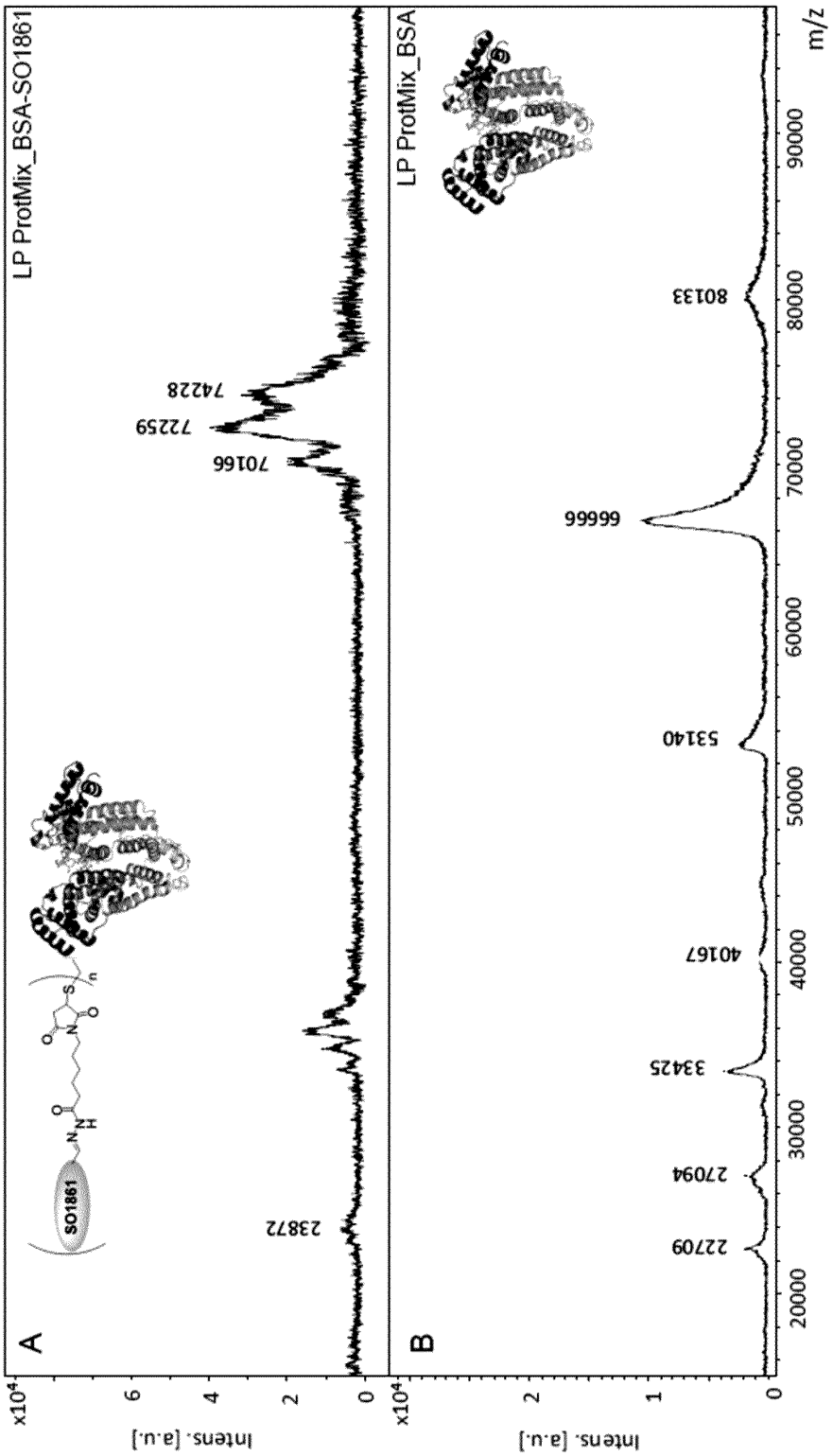

A
LP ProtMix_BSA-SO1861
SO1861
23872
70166
72259
74228
Intens. [a.u.]
x10⁴
B
LP ProtMix_BSA
22709
27094
33425
40167
53140
66666
80133
m/z
Intens. [a.u.]
x10⁴

Figure 35

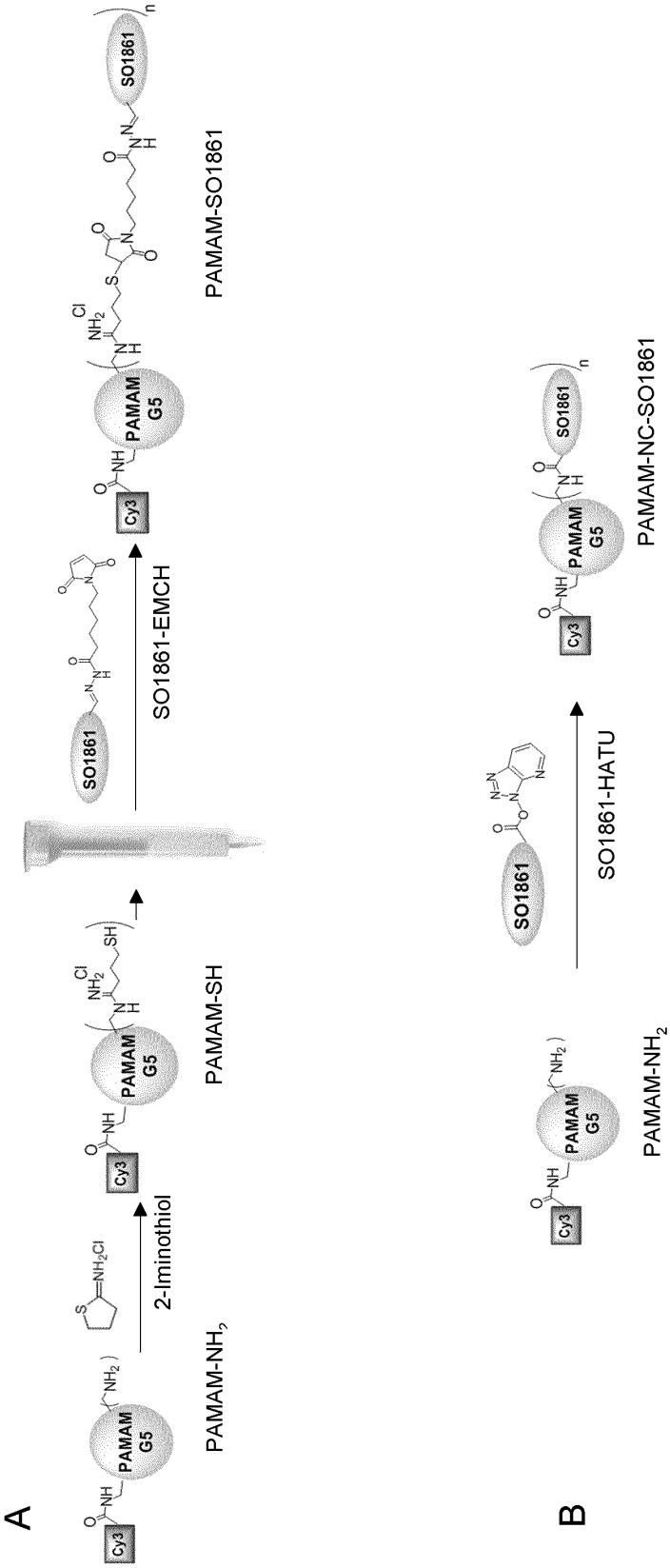

A
PAMAM-NH2
2-Iminothiol
PAMAM-SH
SO1861-EMCH
PAMAM-SO1861
B
PAMAM-NH2
SO1861-HATU
PAMAM-NC-SO1861

Figure 36

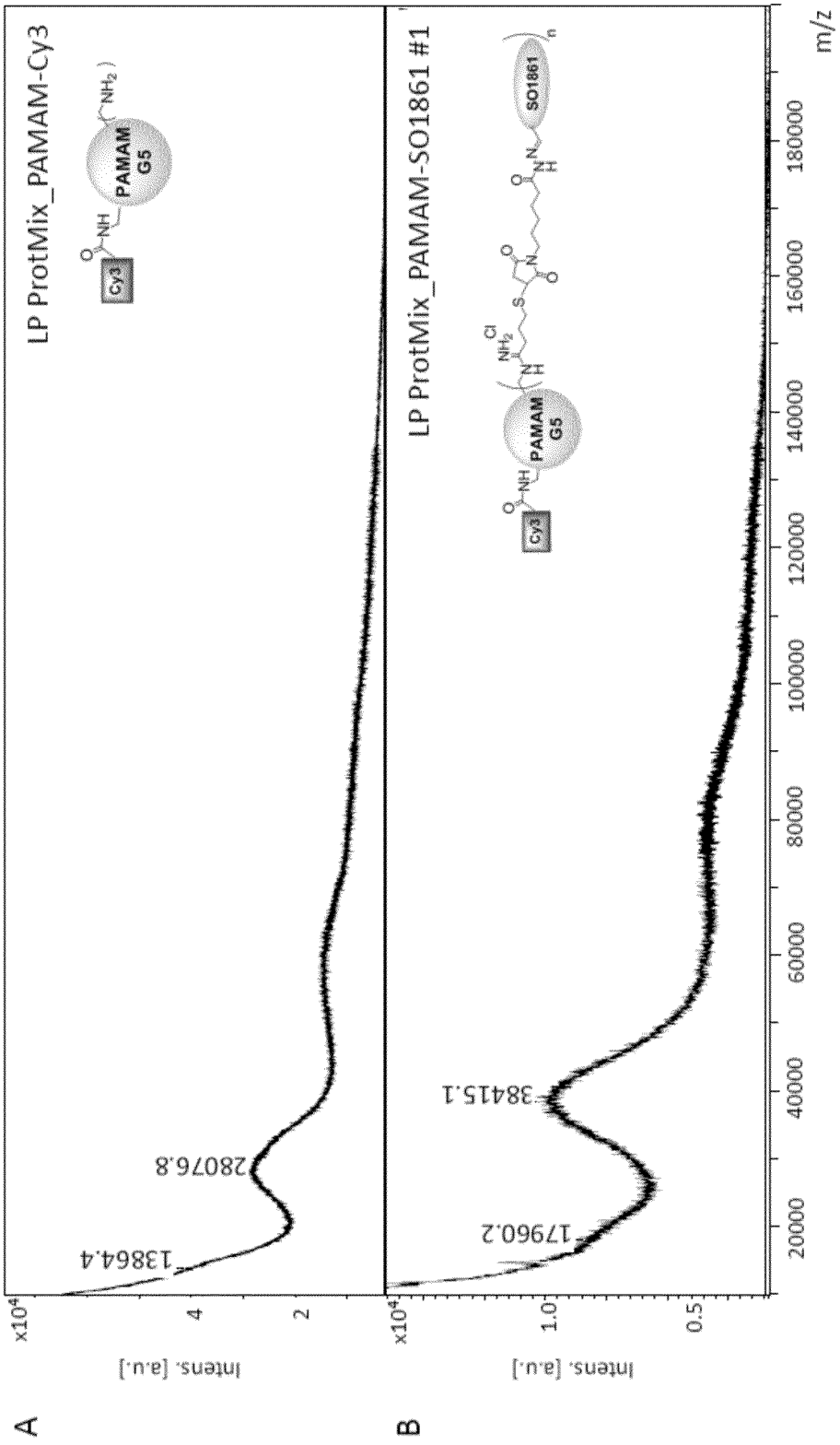

A
B
LP ProtMix_PAMAM-Cy3
LP ProtMix_PAMAM-SO1861 #1
PAMAM G5
Cy3
SO1861
13864.4
28076.8
17960.2
38415.1
Intens. [a.u.]
x10^4
m/z
20000
40000
60000
80000
100000
120000
140000
160000
180000

Figure 36

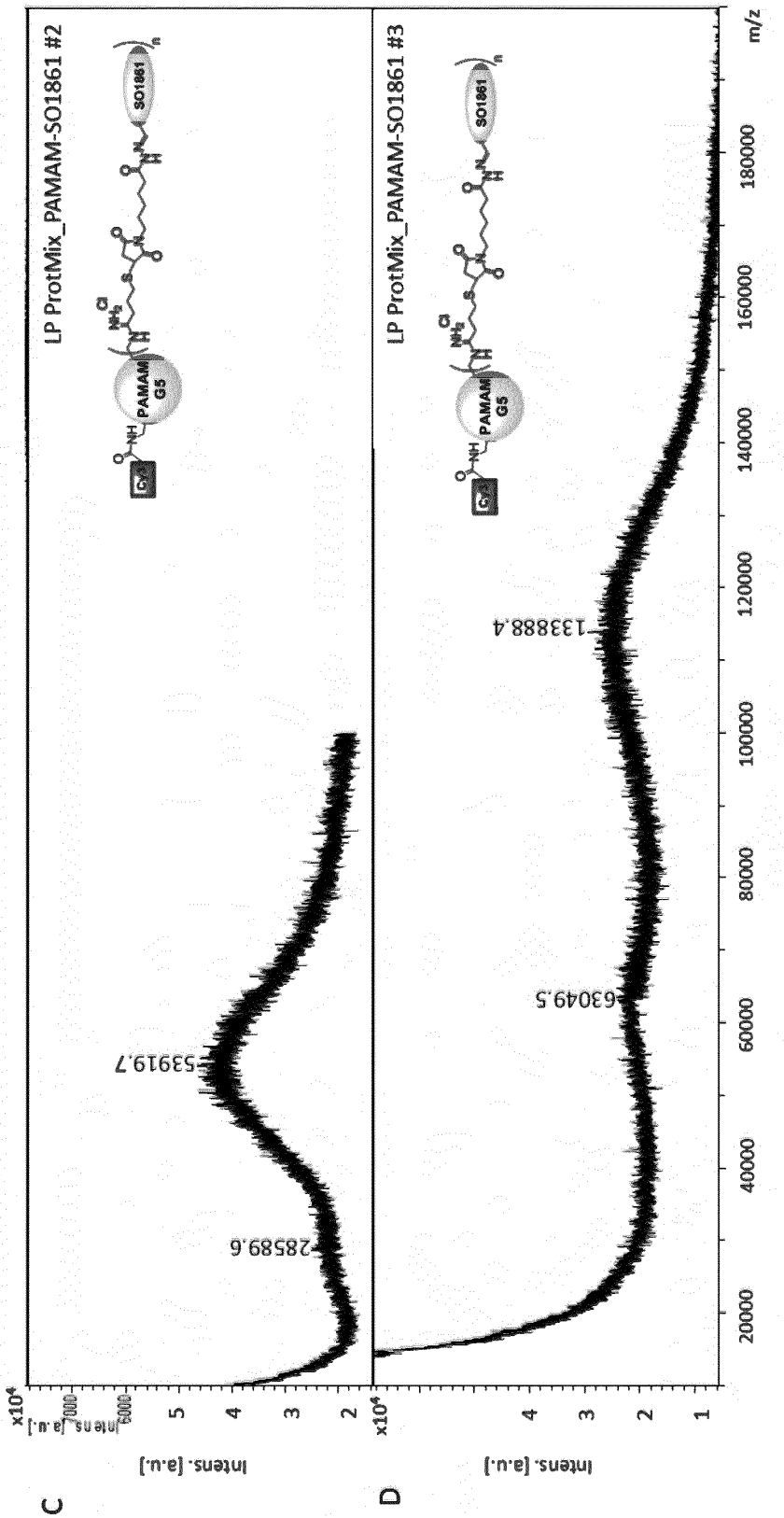

LP ProtMix_PAMAM-SO1861 #2
LP ProtMix_PAMAM-SO1861 #3
PAMAM G5
SO1861
285896
539197
630495
1338884
Intens. [a.u.]
m/z
C
D

Figure 37

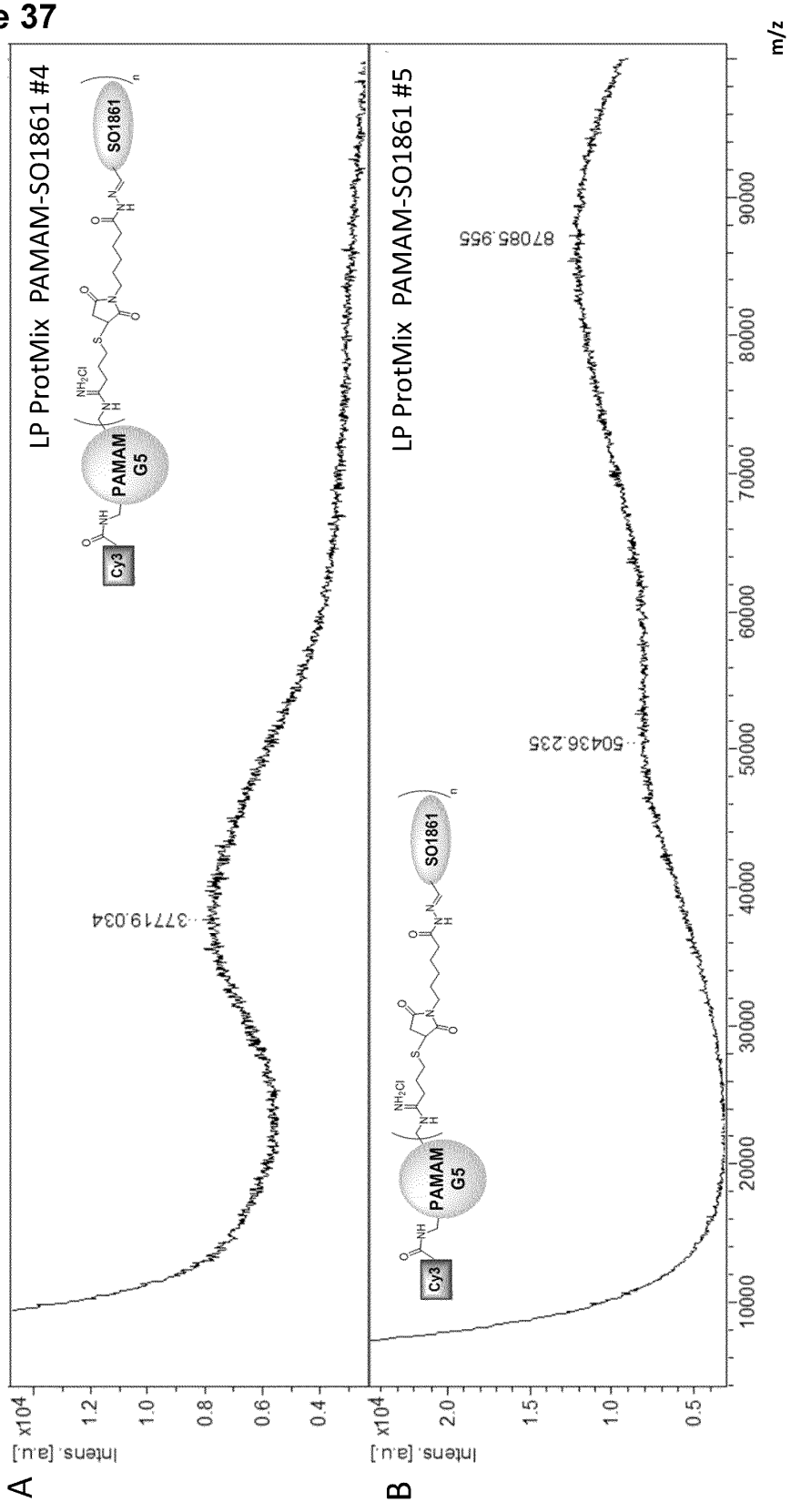

A
LP ProtMix PAMAM-SO1861 #4
Intens. [a.u.]
x10^4
1.2
1.0
0.8
0.6
0.4
37719.034
SO1861
PAMAM G5
Cy3
B
LP ProtMix PAMAM-SO1861 #5
Intens. [a.u.]
x10^4
2.0
1.5
1.0
0.5
87085.955
50436.235
SO1861
PAMAM G5
Cy3
10000
20000
30000
40000
50000
60000
70000
80000
90000
m/z

Figure 38

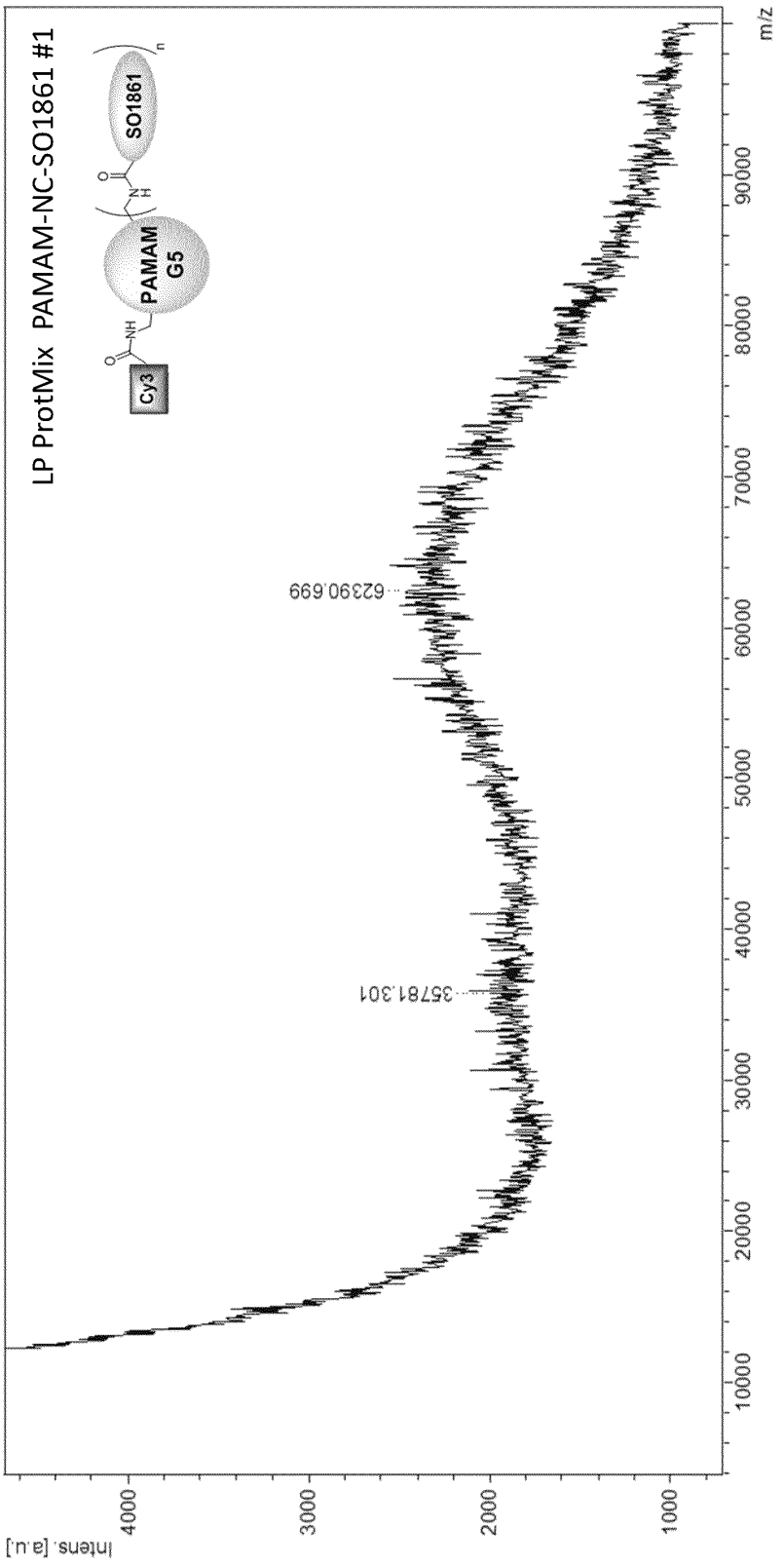

LP ProtMix PAMAM-NC-SO1861 #1
SO1861
PAMAM G5
Cy3
62390.699
35781.301
Intens. [a.u.]
m/z

Figure 39

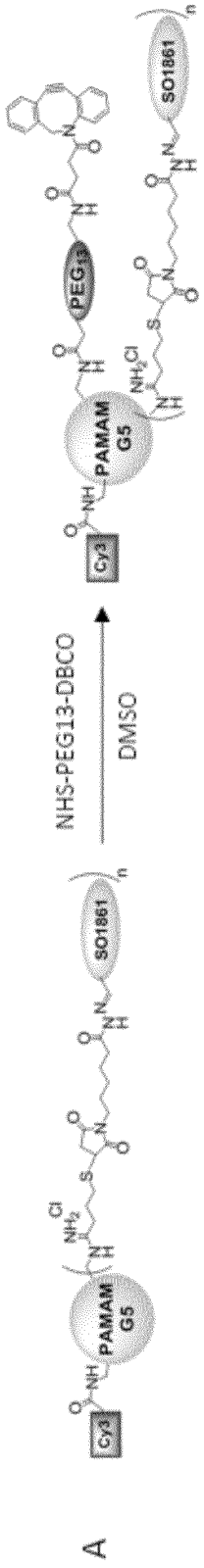


Figure 39

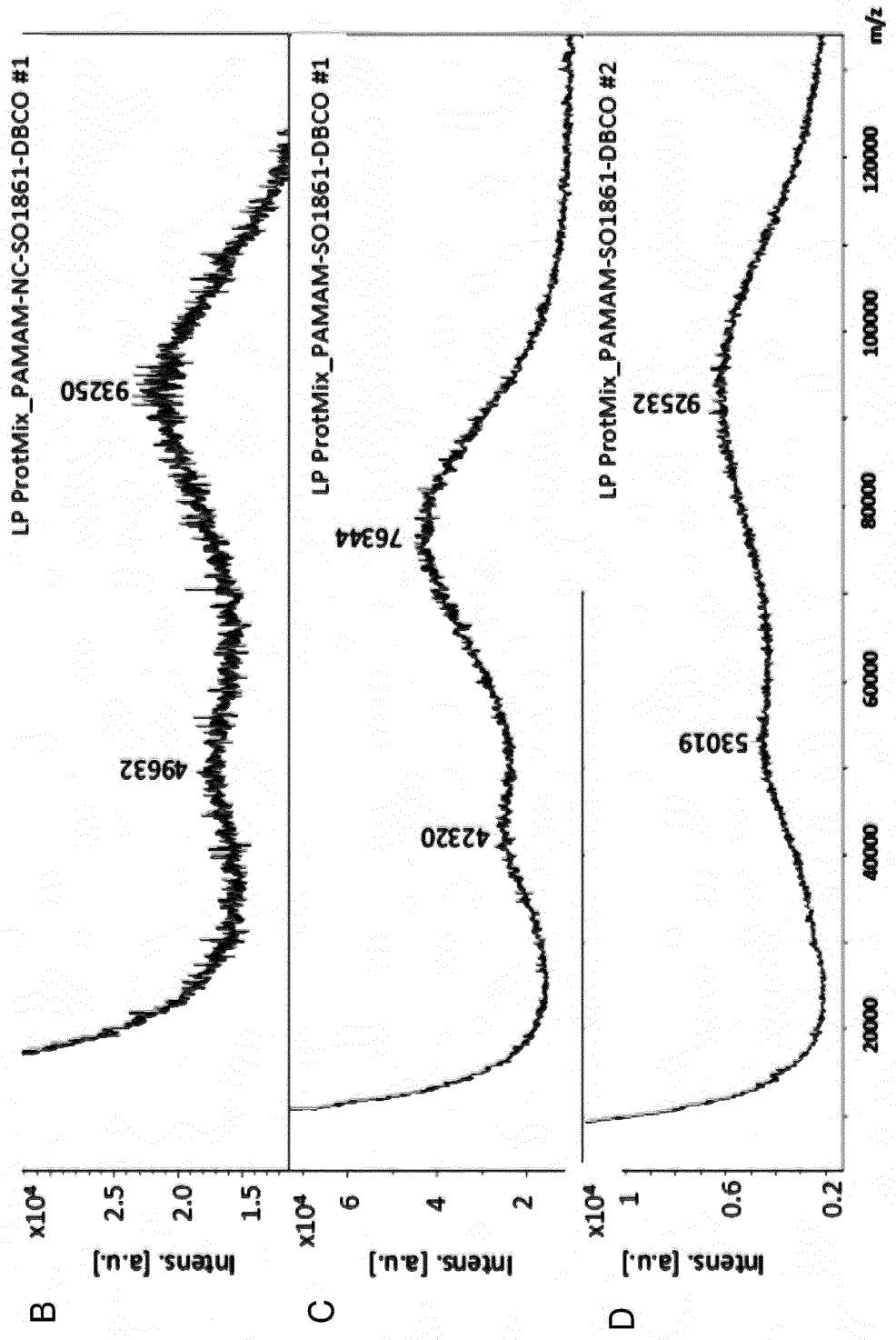

B
C
D
LP ProtMix_PAMAM-NC-SO1861-DBCO #1
LP ProtMix_PAMAM-SO1861-DBCO #1
LP ProtMix_PAMAM-SO1861-DBCO #2
93250
49632
76344
42320
92532
53019
Intens. [a.u.]
x10⁴
m/z
20000
40000
60000
80000
100000
120000

Figure 40

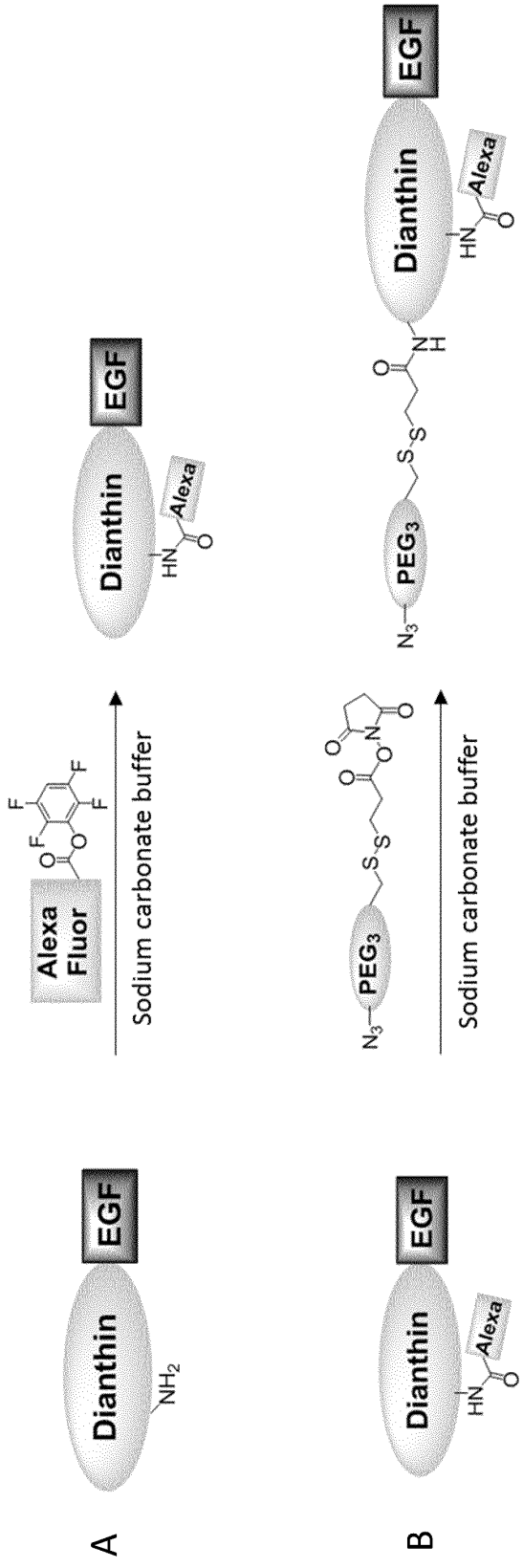

EGF
Dianthin
NH2
Alexa Fluor
Sodium carbonate buffer
Alexa
HN
O
PEG3
N3
S
A
B

Figure 40

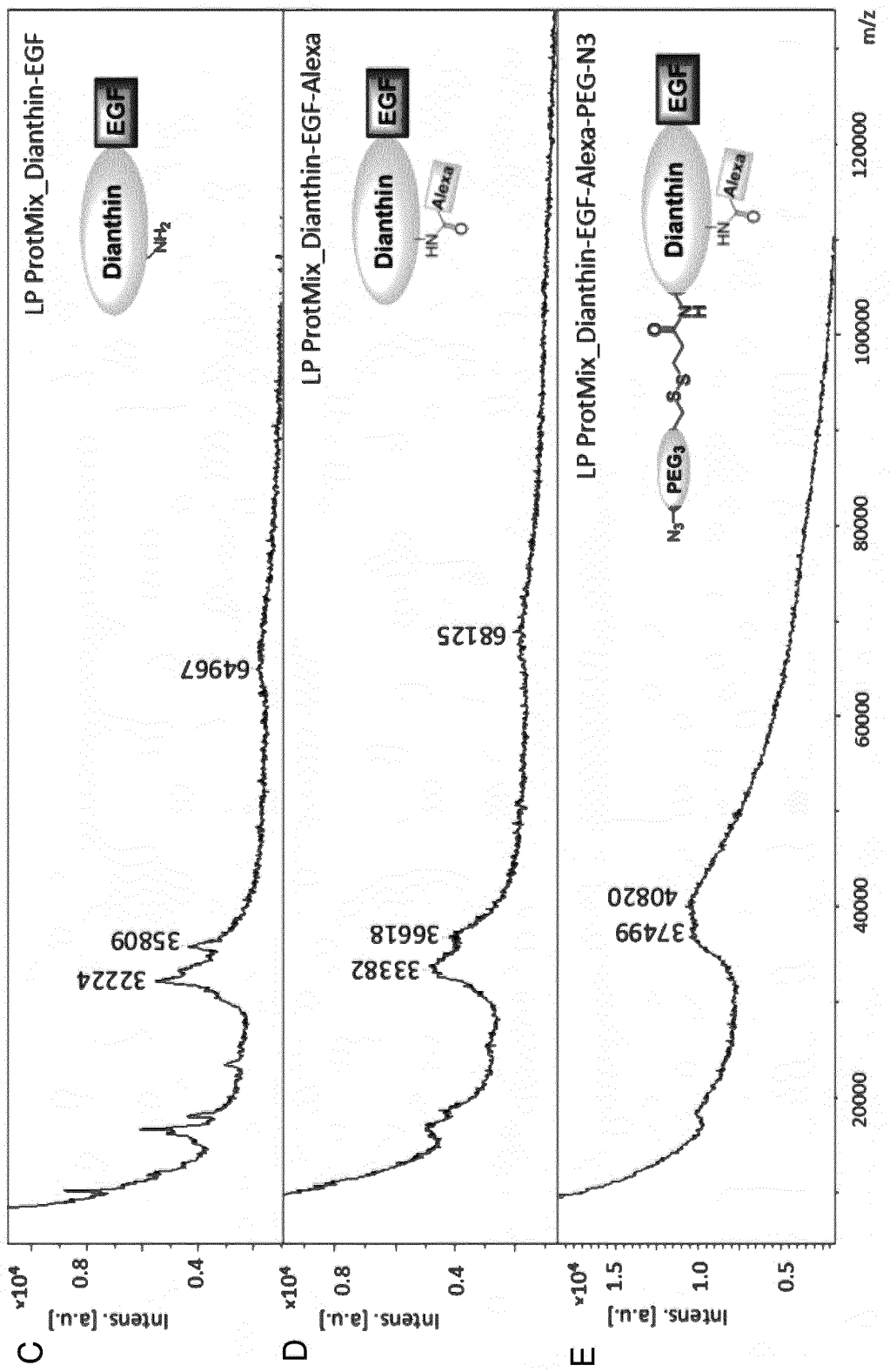

LP ProtMix_Dianthin-EGF
Dianthin
EGF
NH2
64967
35809
32224
C
Intens. [a.u.]
x10^4
0.8
0.4
LP ProtMix_Dianthin-EGF-Alexa
Dianthin
EGF
Alexa
HN
O
68125
36618
33382
D
Intens. [a.u.]
x10^4
0.8
0.4
LP ProtMix_Dianthin-EGF-Alexa-PEG-N3
Dianthin
EGF
Alexa
HN
O
H
N
O
S
S
PEG3
N3
37499
40820
E
Intens. [a.u.]
x10^4
1.5
1.0
0.5
20000
40000
60000
80000
100000
120000
m/z

Figure 41

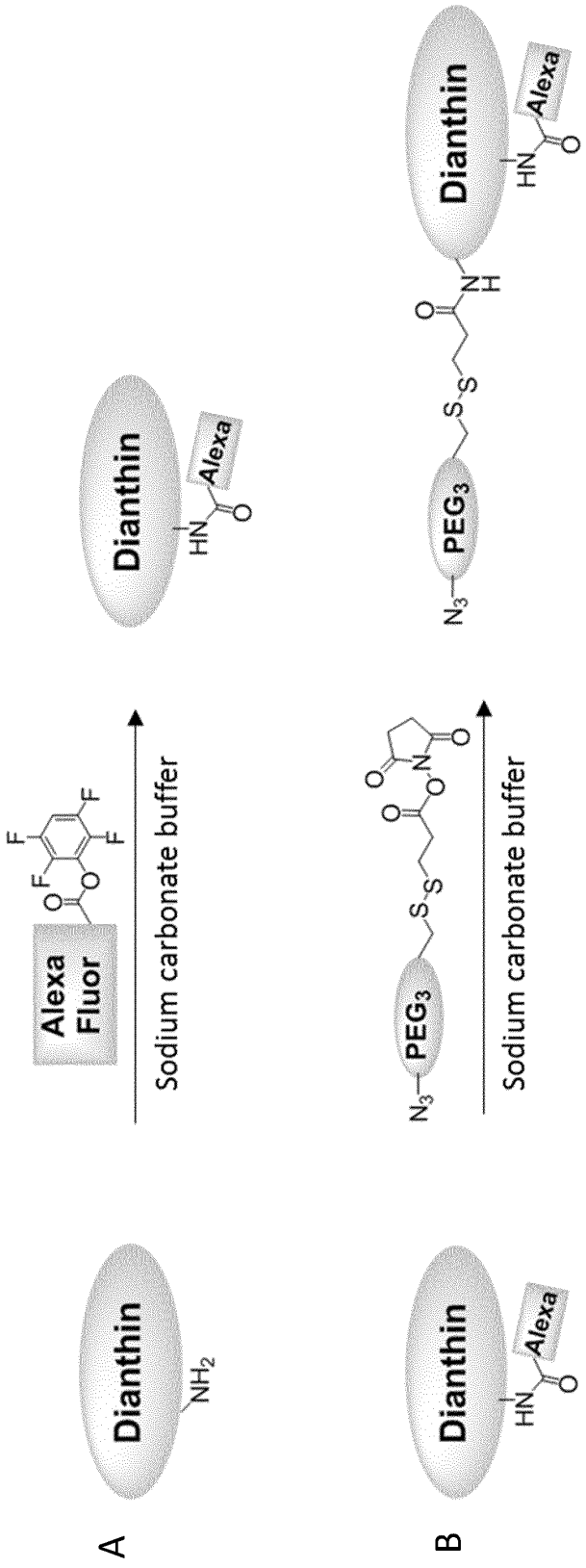

Dianthin
NH2
Alexa Fluor
Sodium carbonate buffer
Dianthin
HN
Alexa
A
Dianthin
NH2
PEG3
N3
Sodium carbonate buffer
Dianthin
HN
Alexa
B

Figure 41

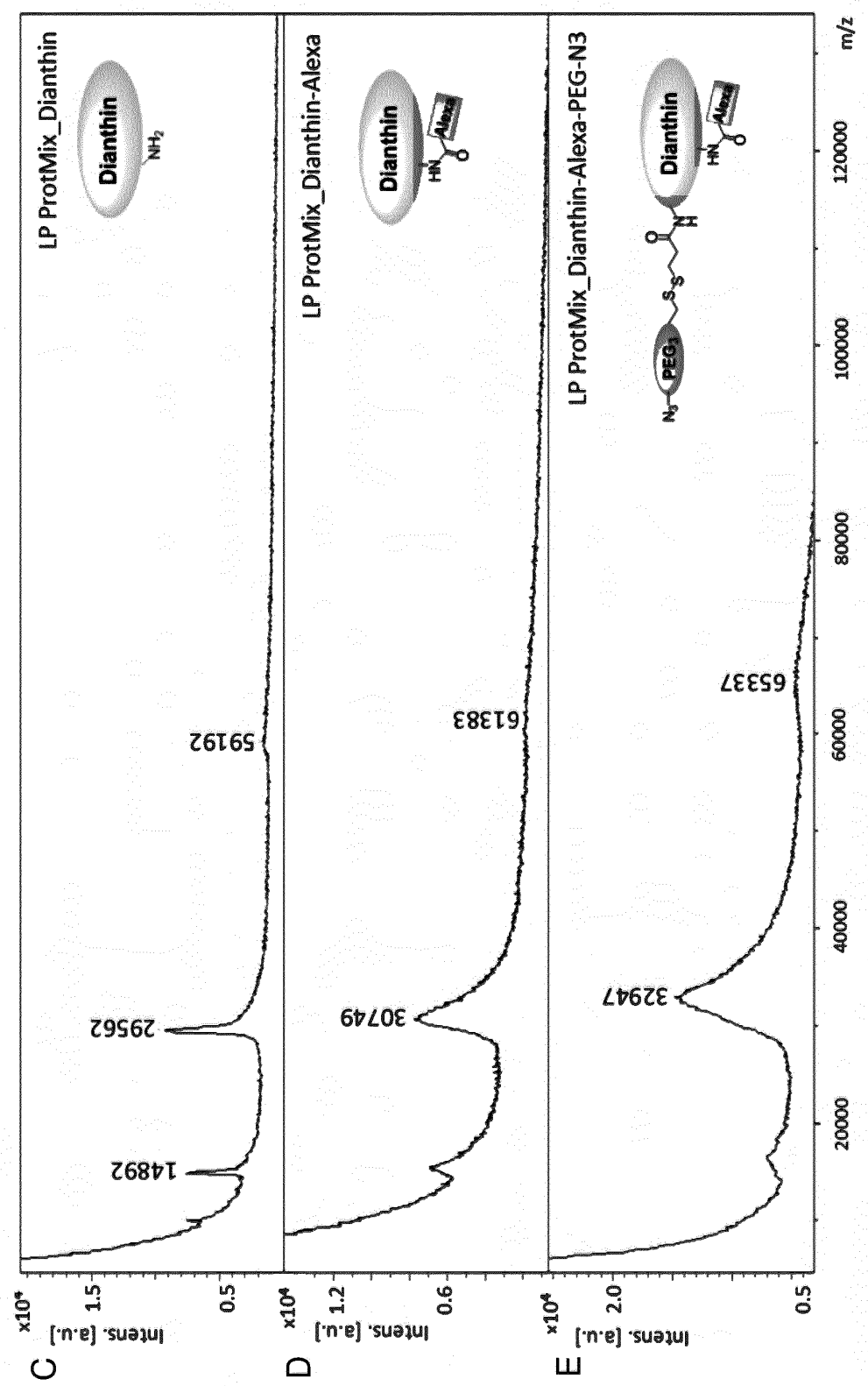

LP ProtMix_Dianthin
Dianthin
14892
29562
59192
LP ProtMix_Dianthin-Alexa
Dianthin
Alexa
30749
61383
LP ProtMix_Dianthin-Alexa-PEG-N3
Dianthin
Alexa
PEG3
32947
65337
Intens. [a.u.]
x10⁴
m/z
20000
40000
60000
80000
100000
120000
C
D
E

Figure 42

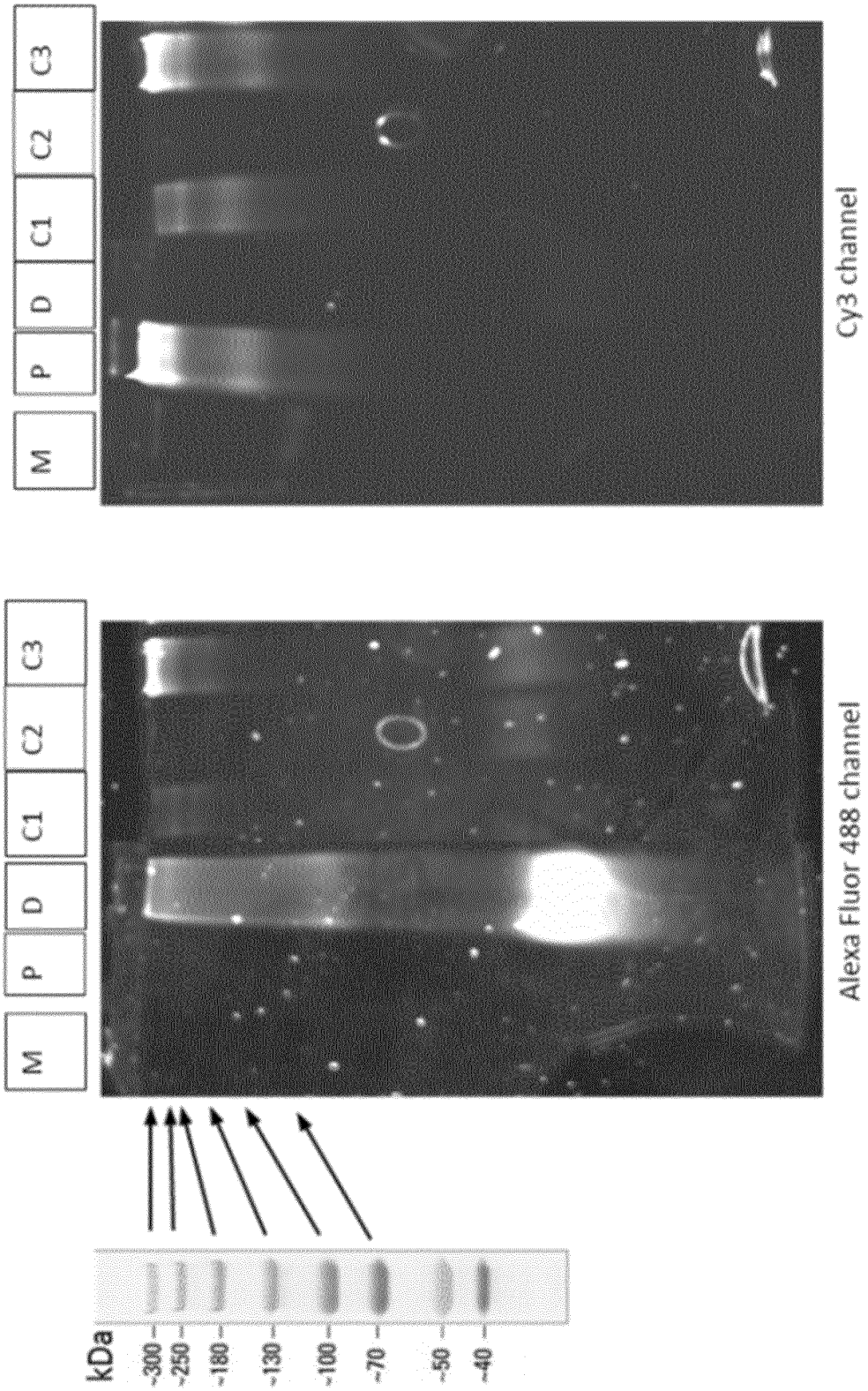

M
P
D
C1
C2
C3
Cy3 channel
Alexa Fluor 488 channel
kDa
~300
~250
~180
~130
~100
~70
~50
~40

Figure 43

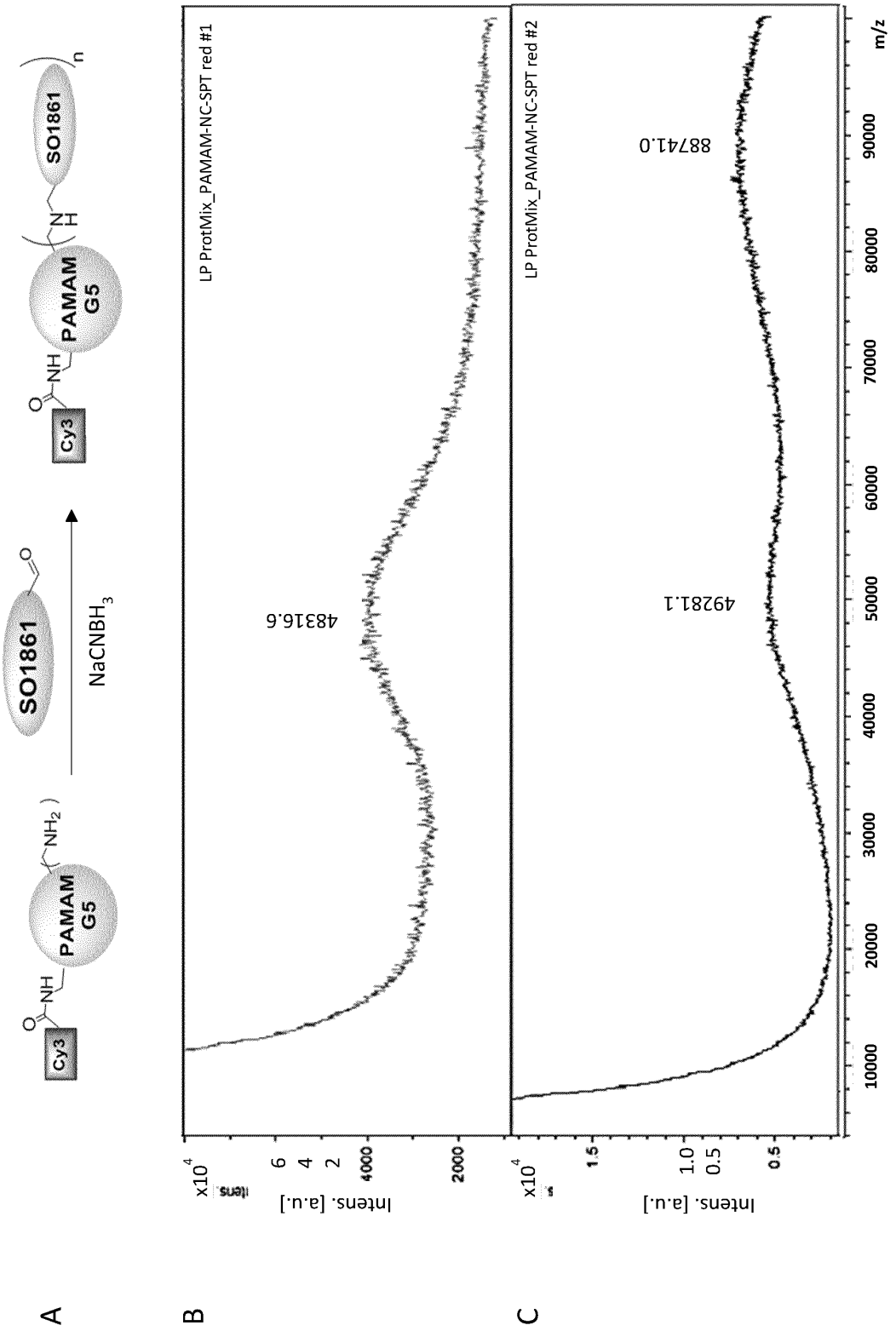

A
B
C
Cy3
PAMAM G5
NH
NH2
SO1861
NaCNBH3
n
LP ProtMix_PAMAM-NC-SPT red #1
48316.6
Intens. [a.u.]
x10^4
LP ProtMix_PAMAM-NC-SPT red #2
88741.0
49281.1
m/z

Figure 44

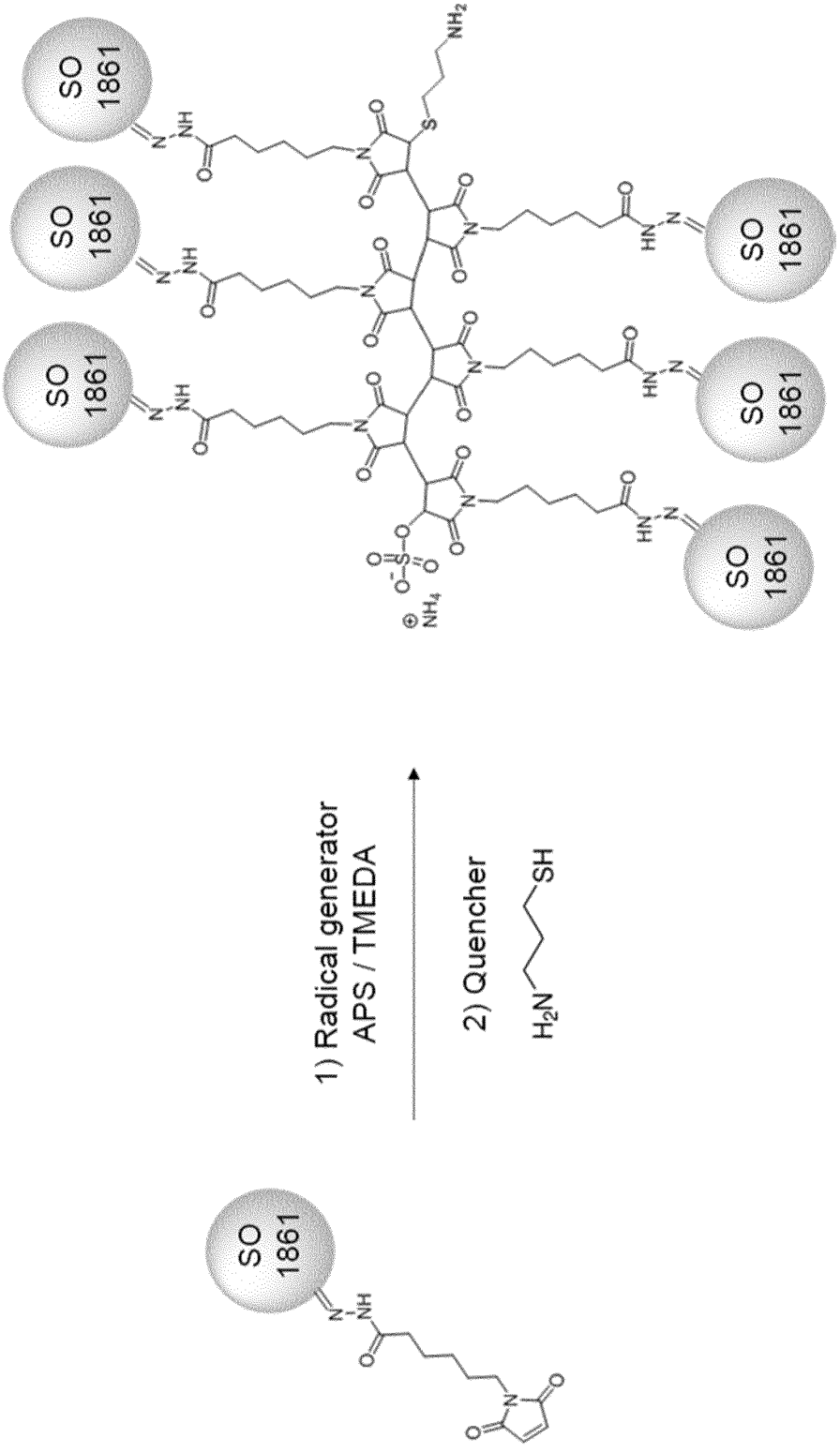


Figure 45

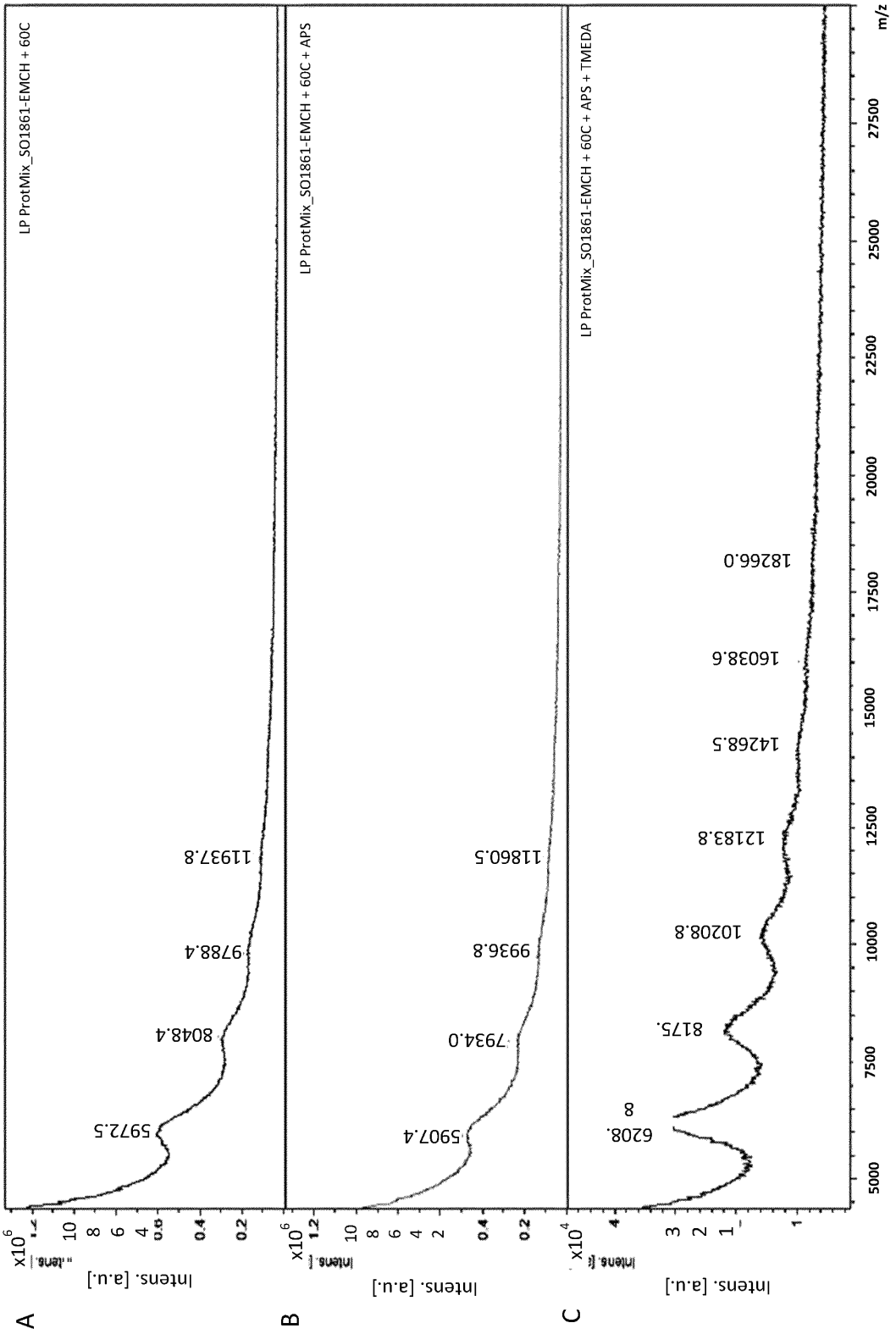

A
B
C
Intens. [a.u.]
LP ProtMix_SO1861-EMCH + 60C
LP ProtMix_SO1861-EMCH + 60C + APS
LP ProtMix_SO1861-EMCH + 60C + APS + TMEDA
5972.5
8048.4
9788.4
11937.8
5907.4
7934.0
9936.8
11860.5
6208.8
8175.
10208.8
12183.8
14268.5
16038.6
18266.0
m/z
5000
7500
10000
12500
15000
17500
20000
22500
25000
27500

Figure 46

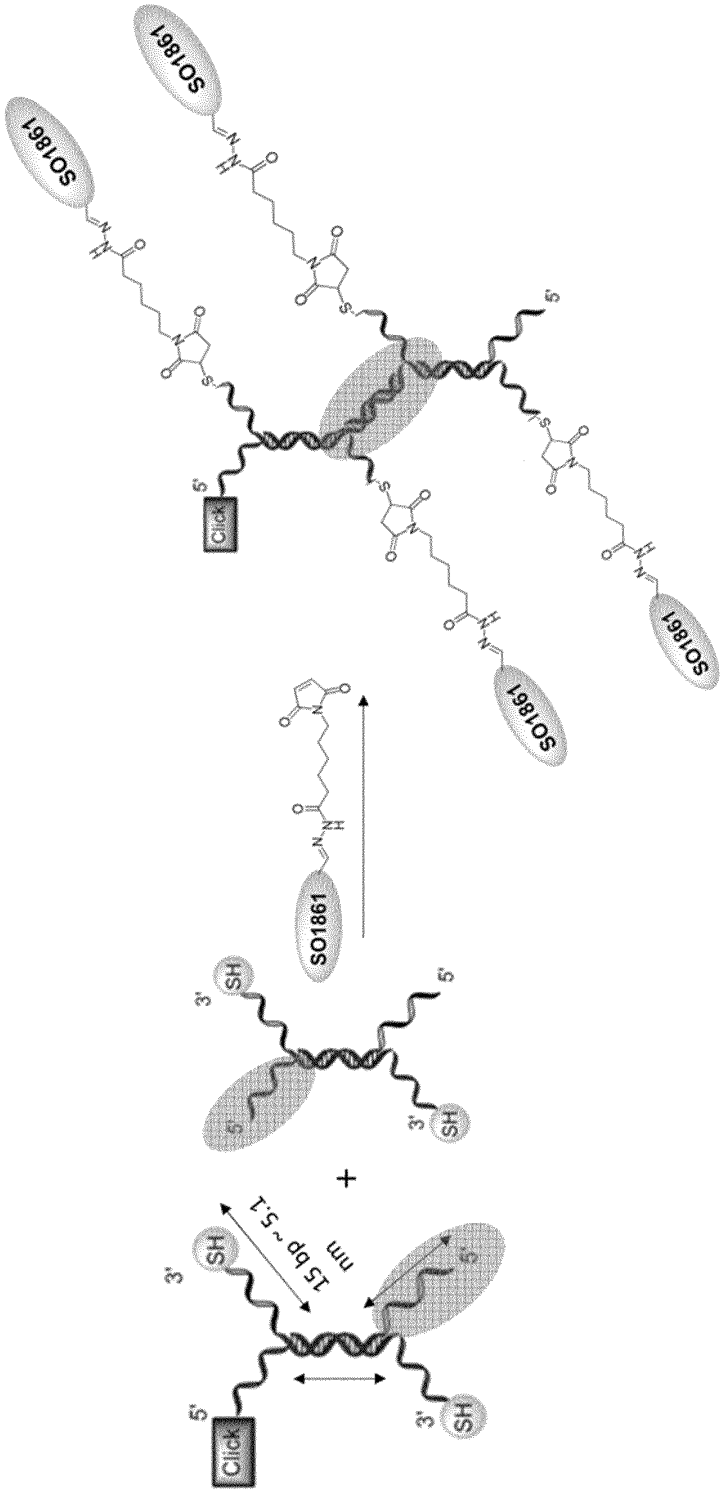

SO1861
SO1861
SO1861
SO1861
SO1861
5'
Click
SO1861
SH
3'
5'
5'
3'
SH
+
SH
3'
15 bp ~ 5.1
nm
5'
5'
Click
3'
SH

Figure 47

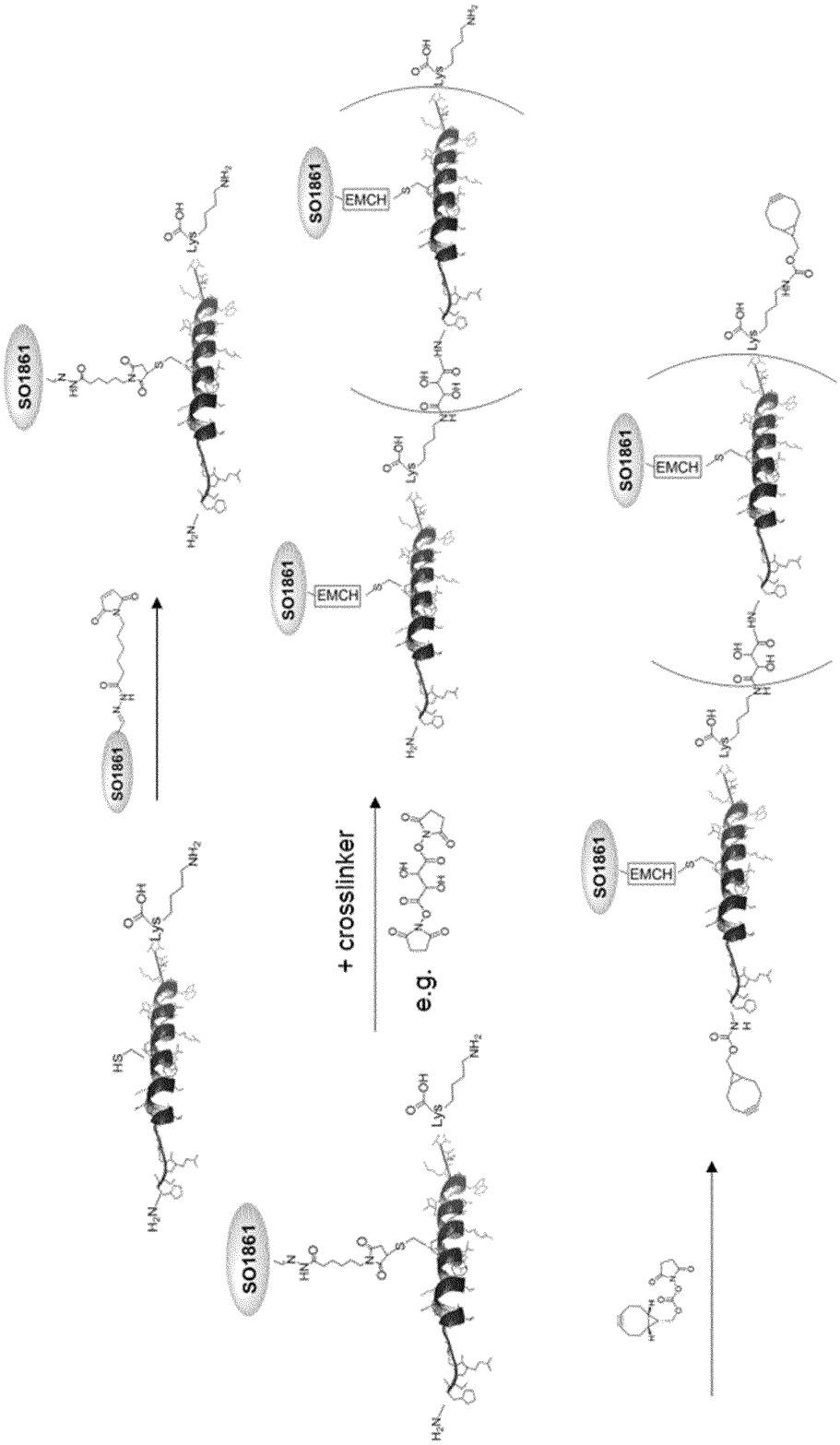


Figure 48

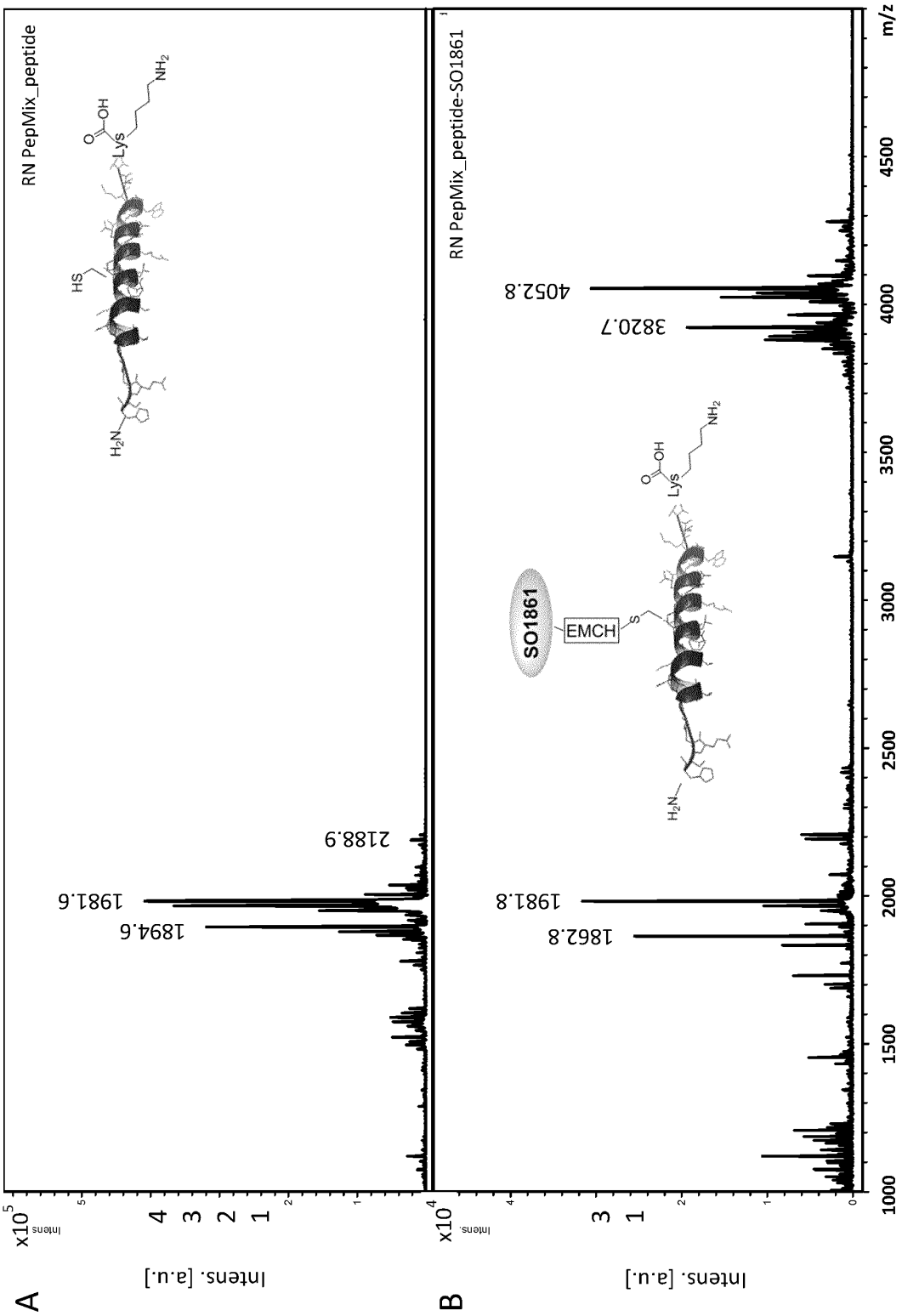

A
RN PepMix_peptide
Intens. [a.u.]
x10^5
2188.9
1981.6
1894.6
B
RN PepMix_peptide-SO1861
Intens. [a.u.]
x10^4
4052.8
3820.7
1981.8
1862.8
SO1861
EMCH
Lys
m/z
1000
1500
2000
2500
3000
3500
4000
4500

Figure 49

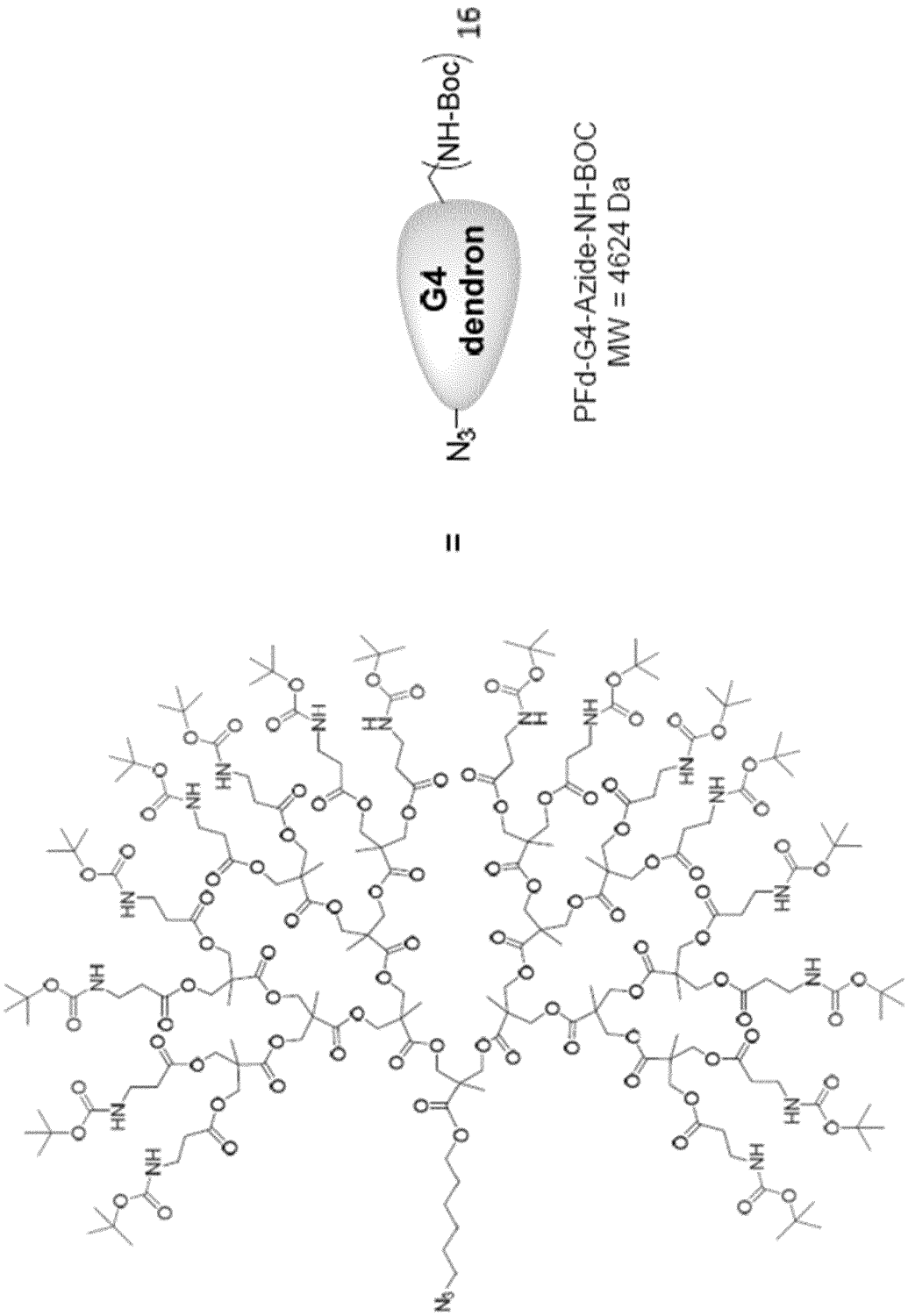


Figure 50

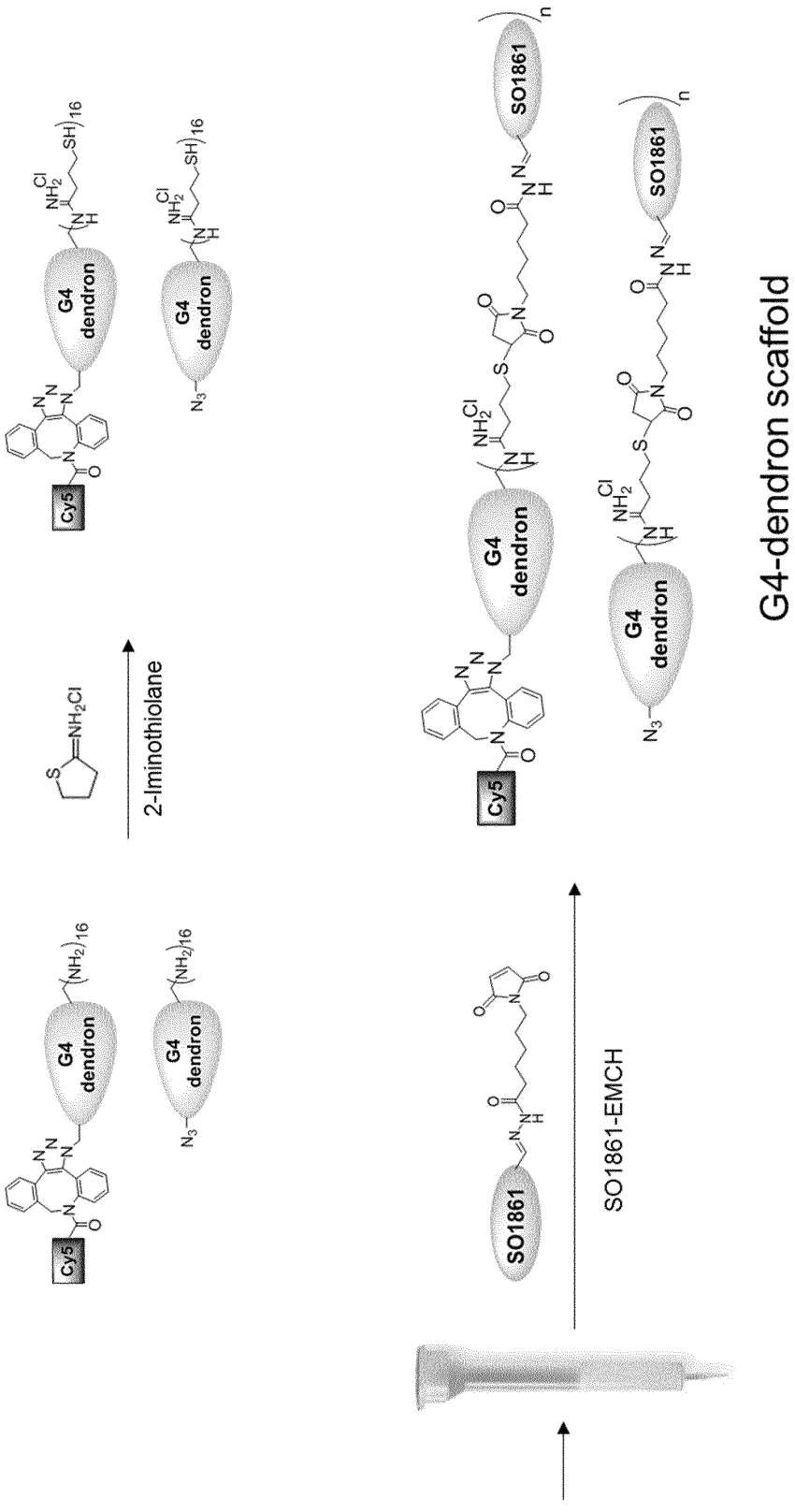

G4 dendron
Cy5
2-Iminothiolane
SO1861
SO1861-EMCH
G4-dendron scaffold

Figure 51

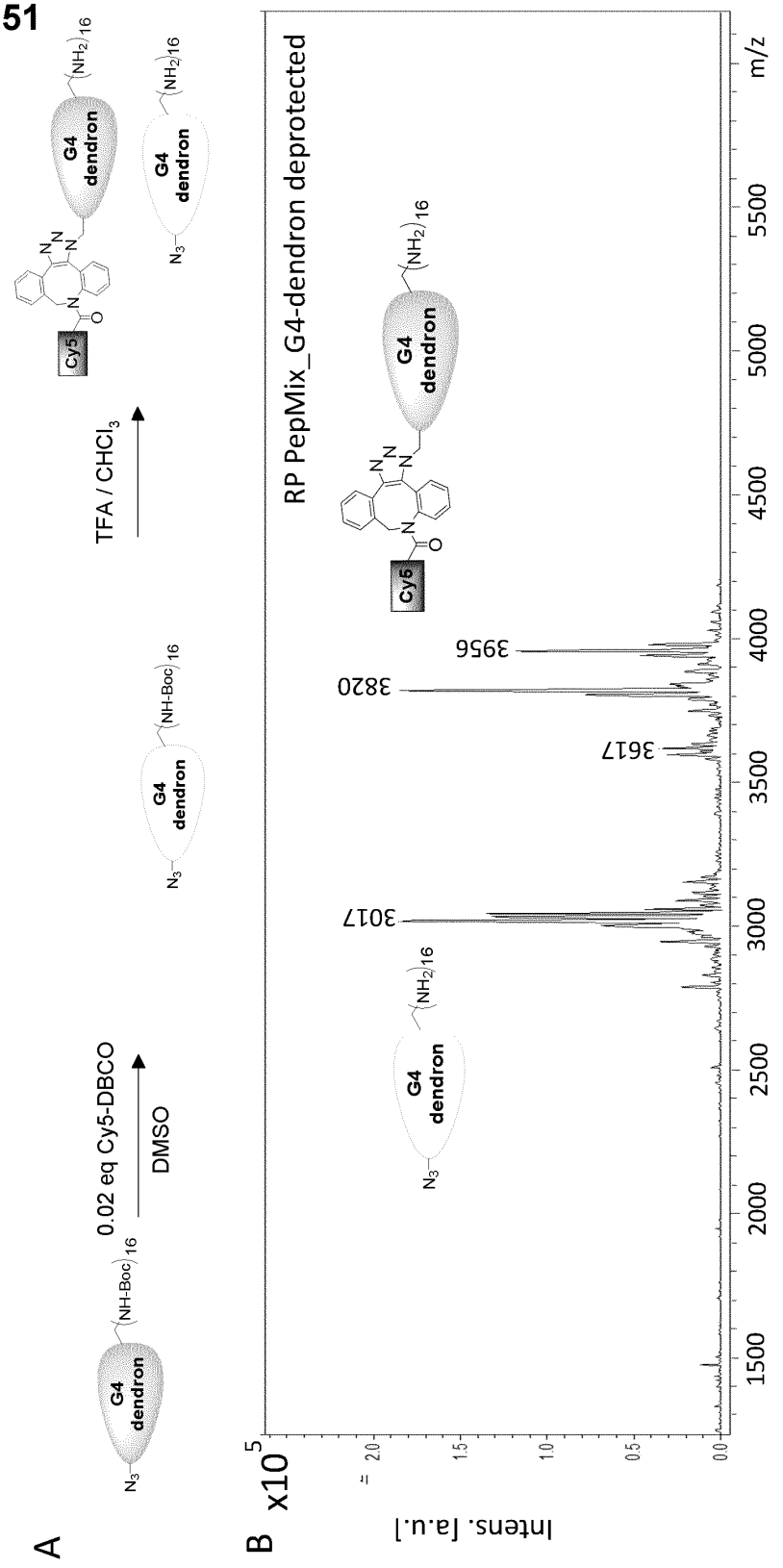

A
G4 dendron
(NH-Boc)16
N3
0.02 eq Cy5-DBCO
DMSO
TFA / CHCl3
(NH2)16
Cy5
B
RP PepMix_G4-dendron deprotected
x10^5
Intens. [a.u.]
m/z
3017
3617
3820
3956

Figure 52

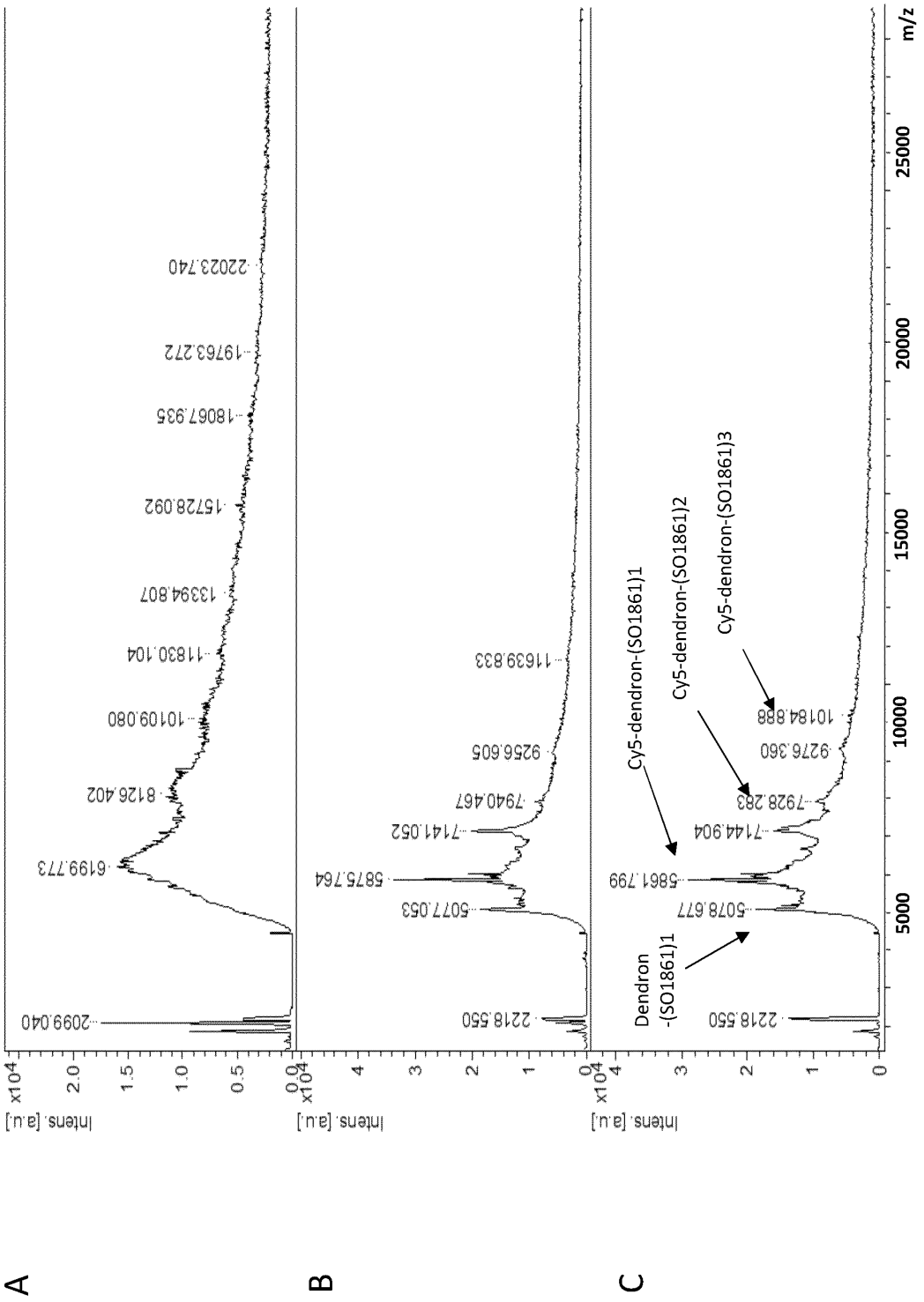
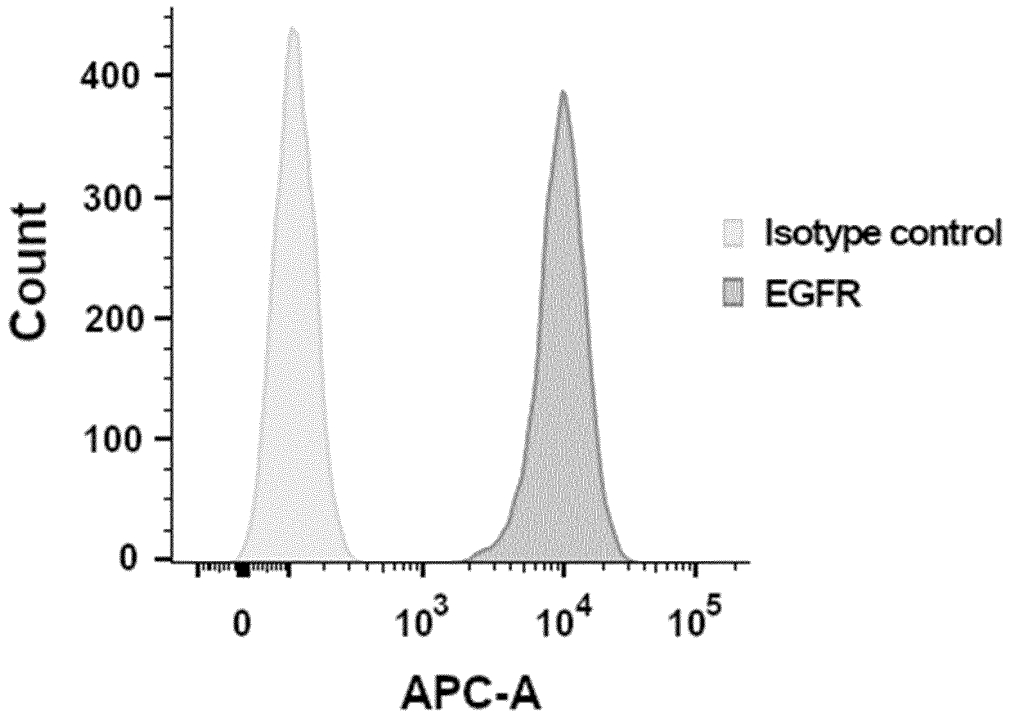

A
B
C
Intens. [a.u.]
m/z
2099.040
6199.773
8126.402
10109.080
11830.104
13394.807
15728.092
18067.935
19763.272
22023.740
2218.550
5077.053
5875.764
7141.052
7940.467
9256.605
11639.833
Dendron -(SO1861)1
Cy5-dendron-(SO1861)1
Cy5-dendron-(SO1861)2
Cy5-dendron-(SO1861)3
5078.677
5861.799
7144.904
7928.283
9276.360
10184.888

Figure 53

A


Count
400
300
200
100
0
0
10^3
10^4
10^5
APC-A
Isotype control
EGFR

B

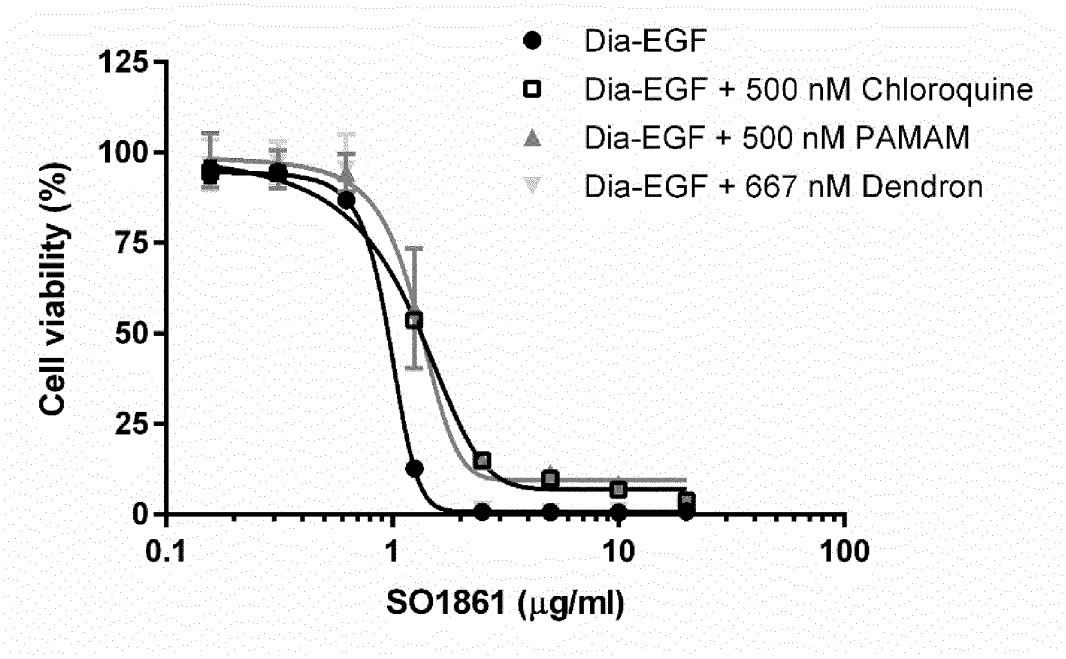
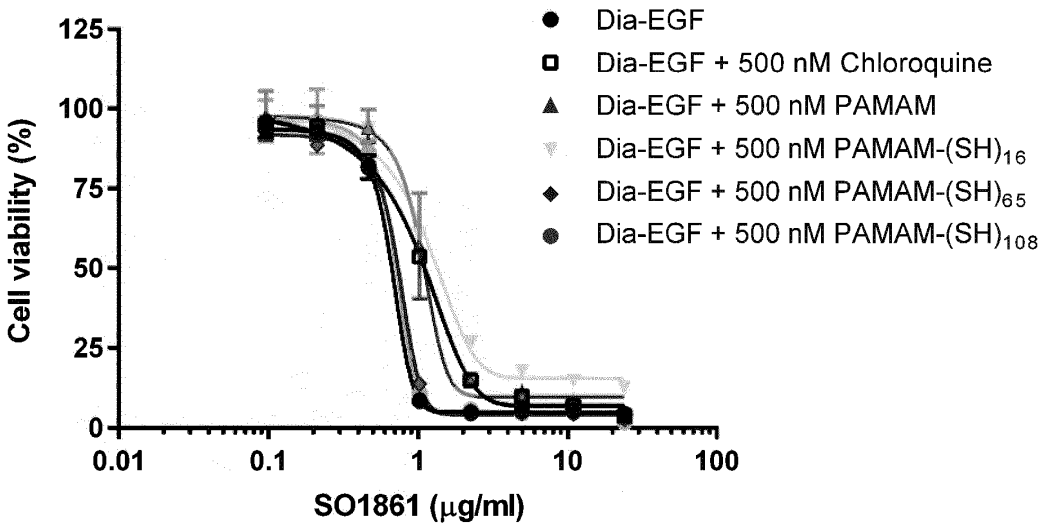

Cell viability (%)
125
100
75
50
25
0
0.1
1
10
100
SO1861 (μg/ml)
Dia-EGF
Dia-EGF + 500 nM Chloroquine
Dia-EGF + 500 nM PAMAM
Dia-EGF + 667 nM Dendron

Figure 53

C


Cell viability (%)
125
100
75
50
25
0
0.01
0.1
1
10
100
SO1861 (µg/ml)
Dia-EGF
Dia-EGF + 500 nM Chloroquine
Dia-EGF + 500 nM PAMAM
Dia-EGF + 500 nM PAMAM-(SH)16
Dia-EGF + 500 nM PAMAM-(SH)65
Dia-EGF + 500 nM PAMAM-(SH)108

D

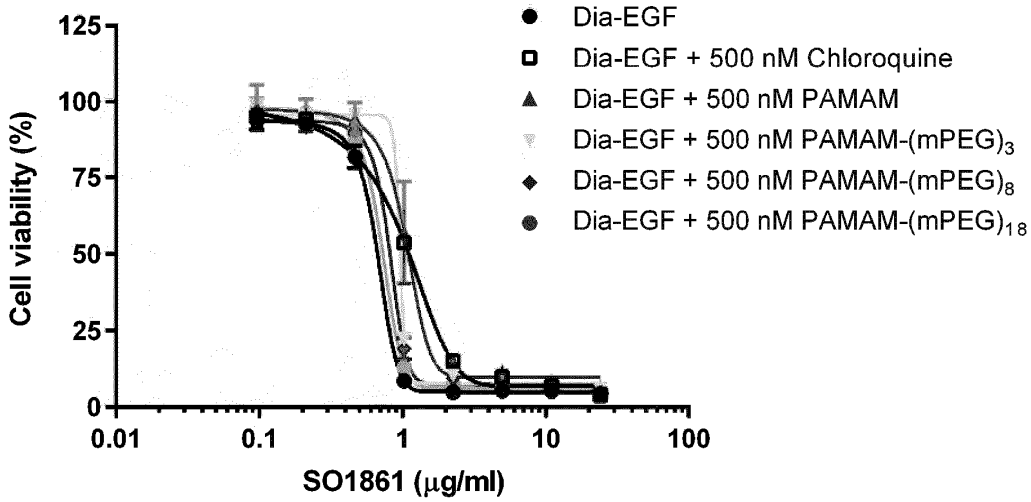

Cell viability (%)
125
100
75
50
25
0
0.01
0.1
1
10
100
SO1861 (µg/ml)
Dia-EGF
Dia-EGF + 500 nM Chloroquine
Dia-EGF + 500 nM PAMAM
Dia-EGF + 500 nM PAMAM-(mPEG)3
Dia-EGF + 500 nM PAMAM-(mPEG)8
Dia-EGF + 500 nM PAMAM-(mPEG)18

Figure 54

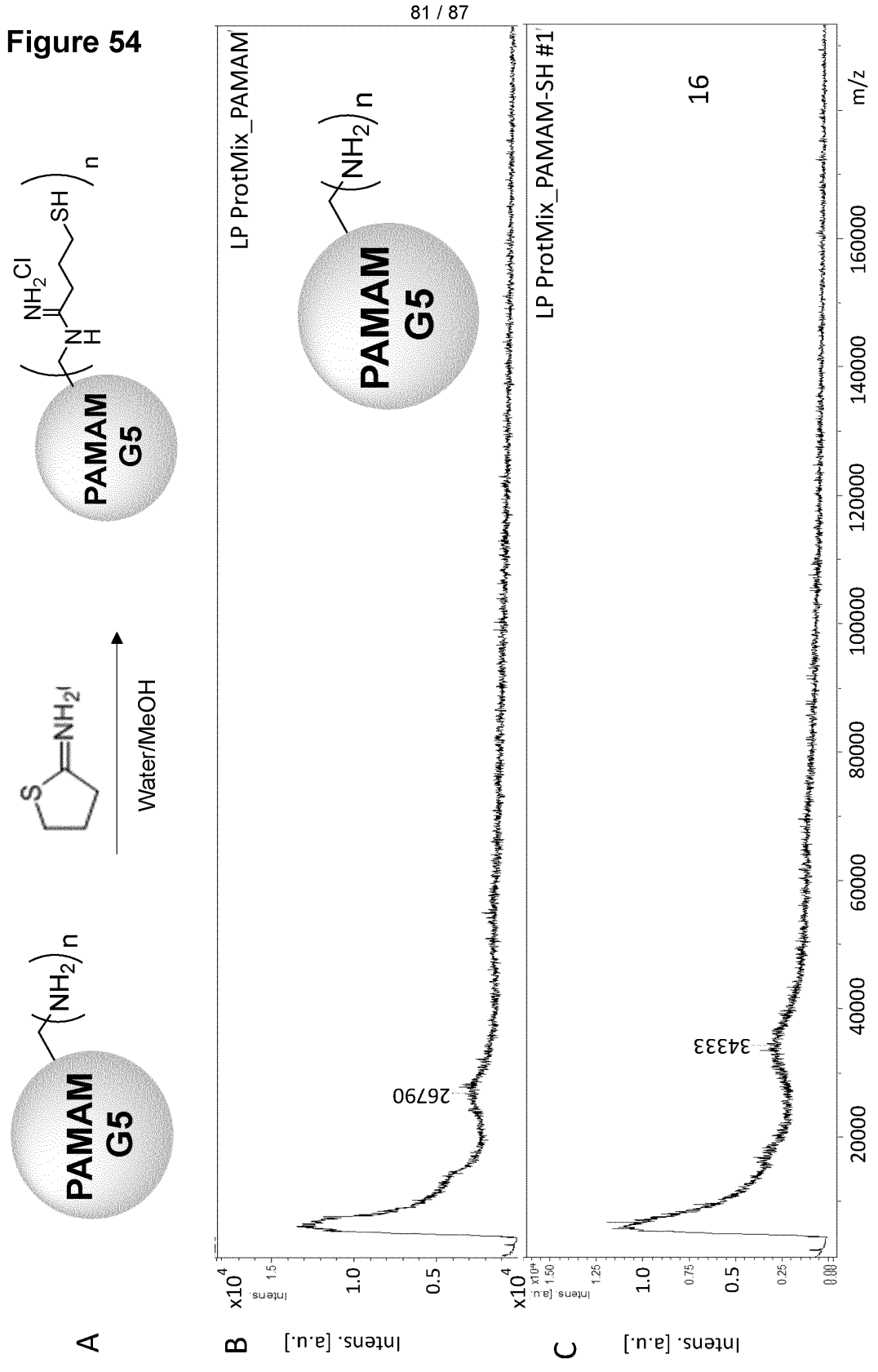

A
PAMAM G5
(NH2)n
Water/MeOH
PAMAM G5
(NH2)n
B
LP ProtMix_PAMAM
26790
Intens. [a.u.]
C
LP ProtMix_PAMAM-SH #1
16
34333
Intens. [a.u.]
m/z
20000
40000
60000
80000
100000
120000
140000
160000

Figure 54

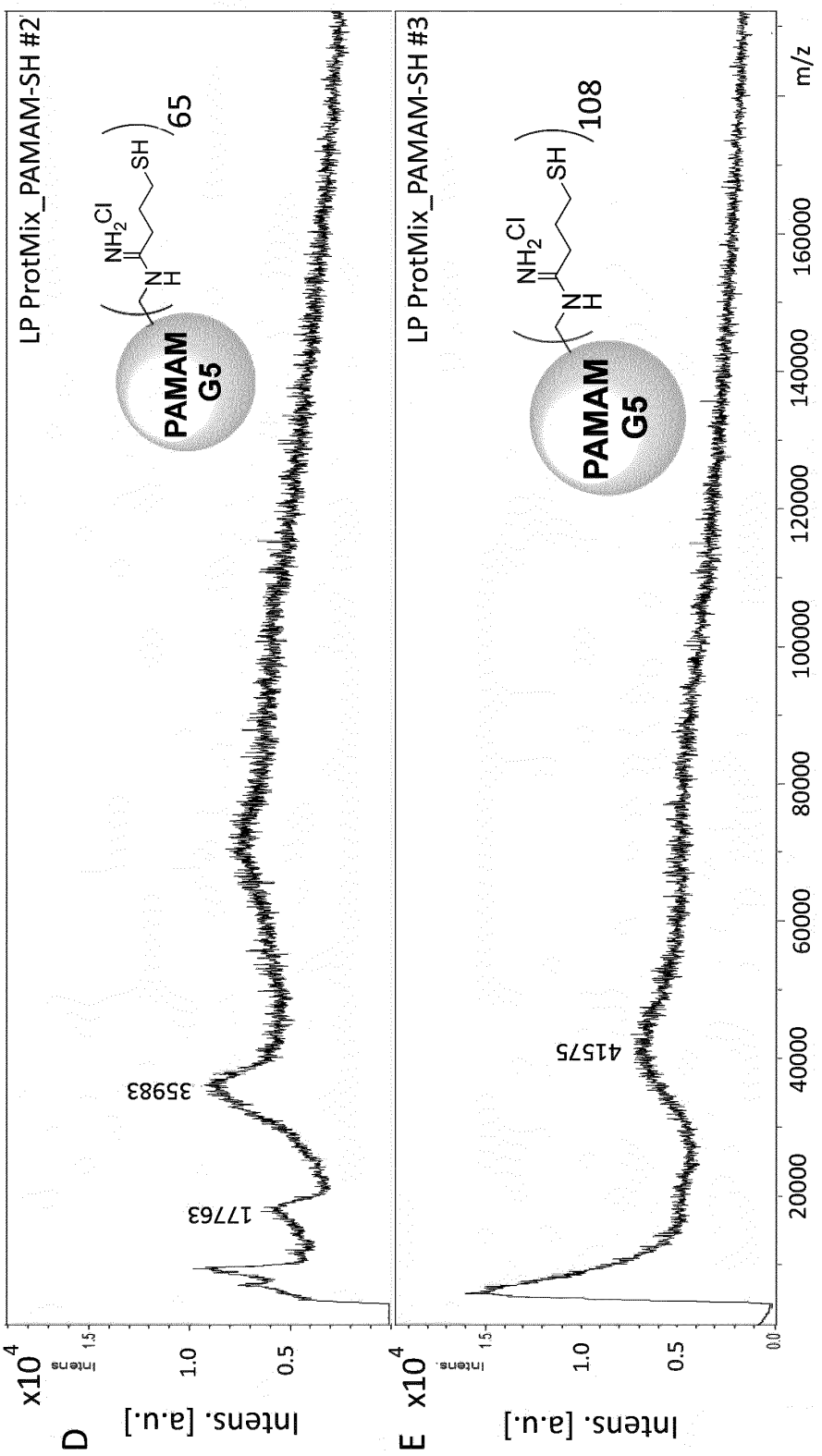

D
E
LP ProtMix_PAMAM-SH #2
LP ProtMix_PAMAM-SH #3
PAMAM G5
65
108
17763
35983
41575
Intens. [a.u.]
x10^4
m/z
20000
40000
60000
80000
100000
120000
140000
160000
0.5
1.0
1.5

Figure 55

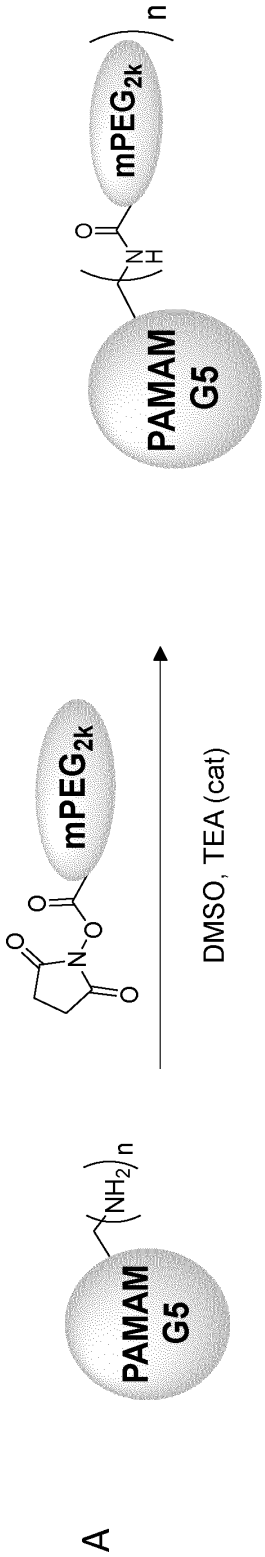


Figure 55

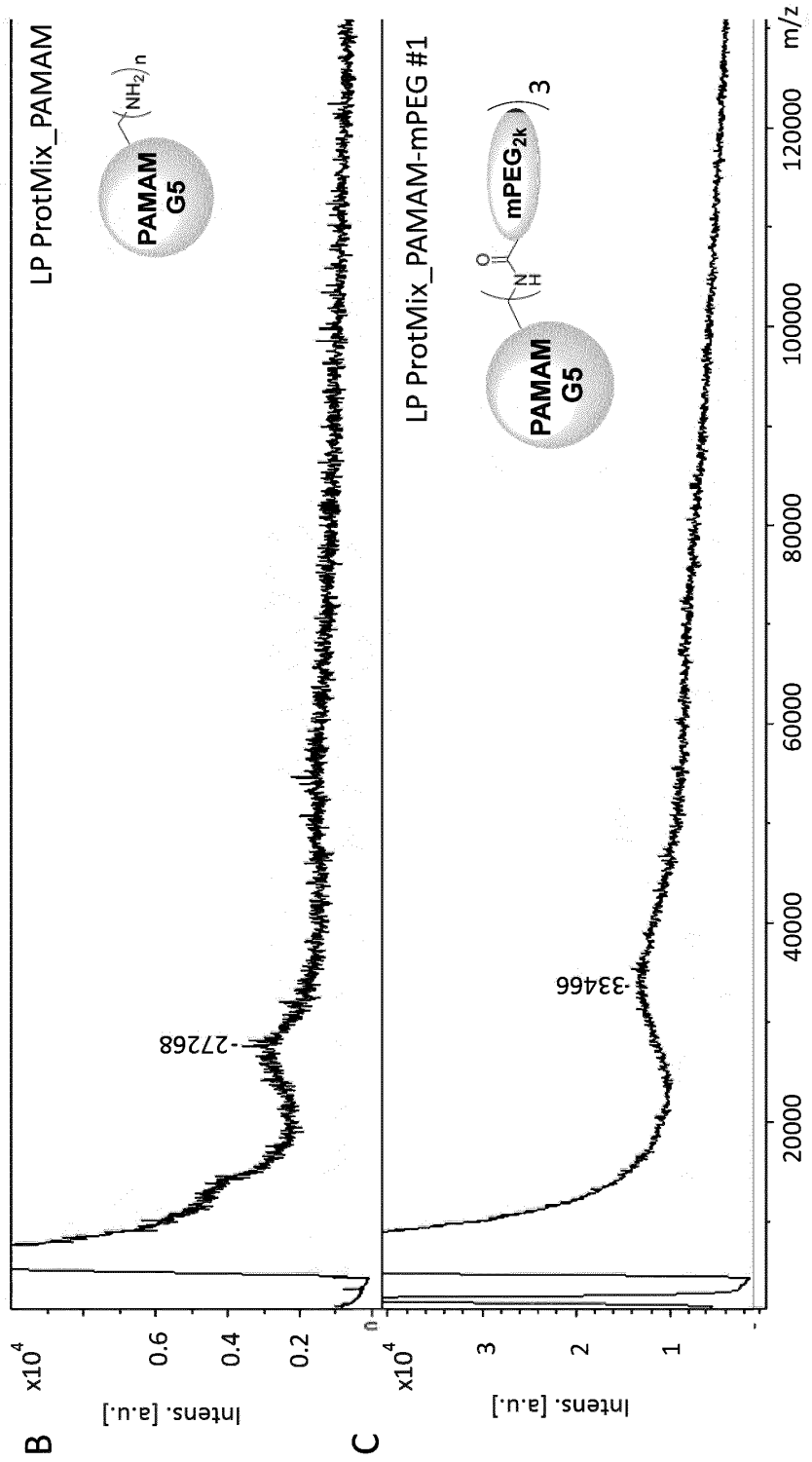

B
LP ProtMix_PAMAM
PAMAM G5
$(NH_2)_n$
27268
Intens. [a.u.]
x10^4
0.6
0.4
0.2
C
LP ProtMix_PAMAM-mPEG #1
PAMAM G5
mPEG2k
3
33466
Intens. [a.u.]
x10^4
3
2
1
20000
40000
60000
80000
100000
120000
m/z

Figure 55

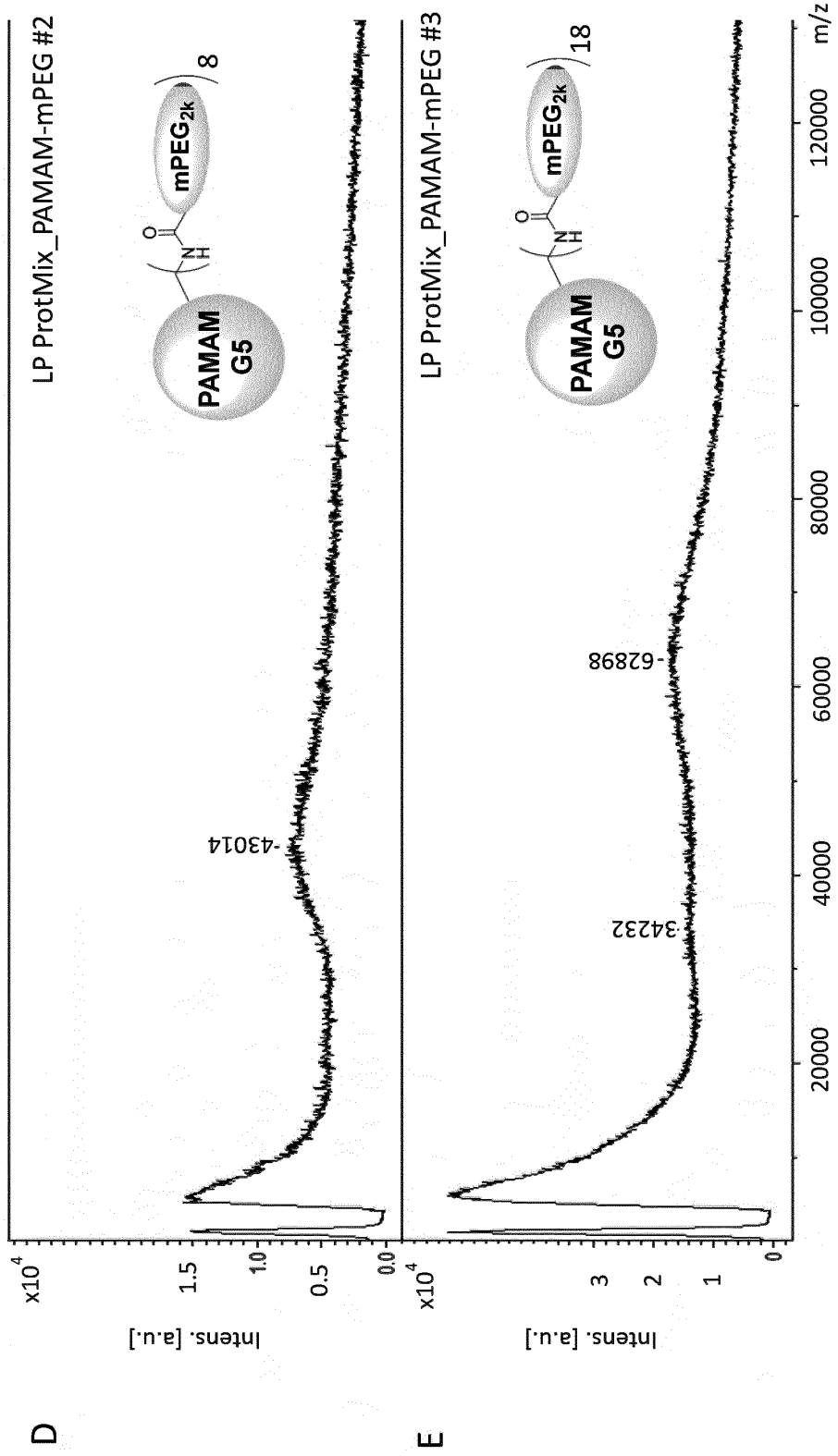

LP ProtMix_PAMAM-mPEG #2
PAMAM G5
mPEG2k
8
43014
x10^4
Intens. [a.u.]
1.5
1.0
0.5
0.0
D
LP ProtMix_PAMAM-mPEG #3
PAMAM G5
mPEG2k
18
62898
34232
x10^4
Intens. [a.u.]
3
2
1
0
E
20000
40000
60000
80000
100000
120000
m/z

Figure 56

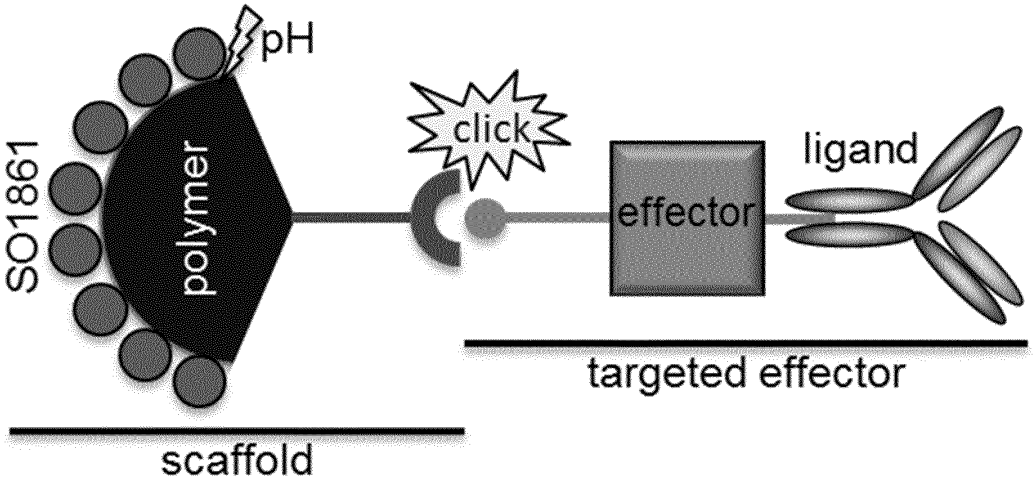
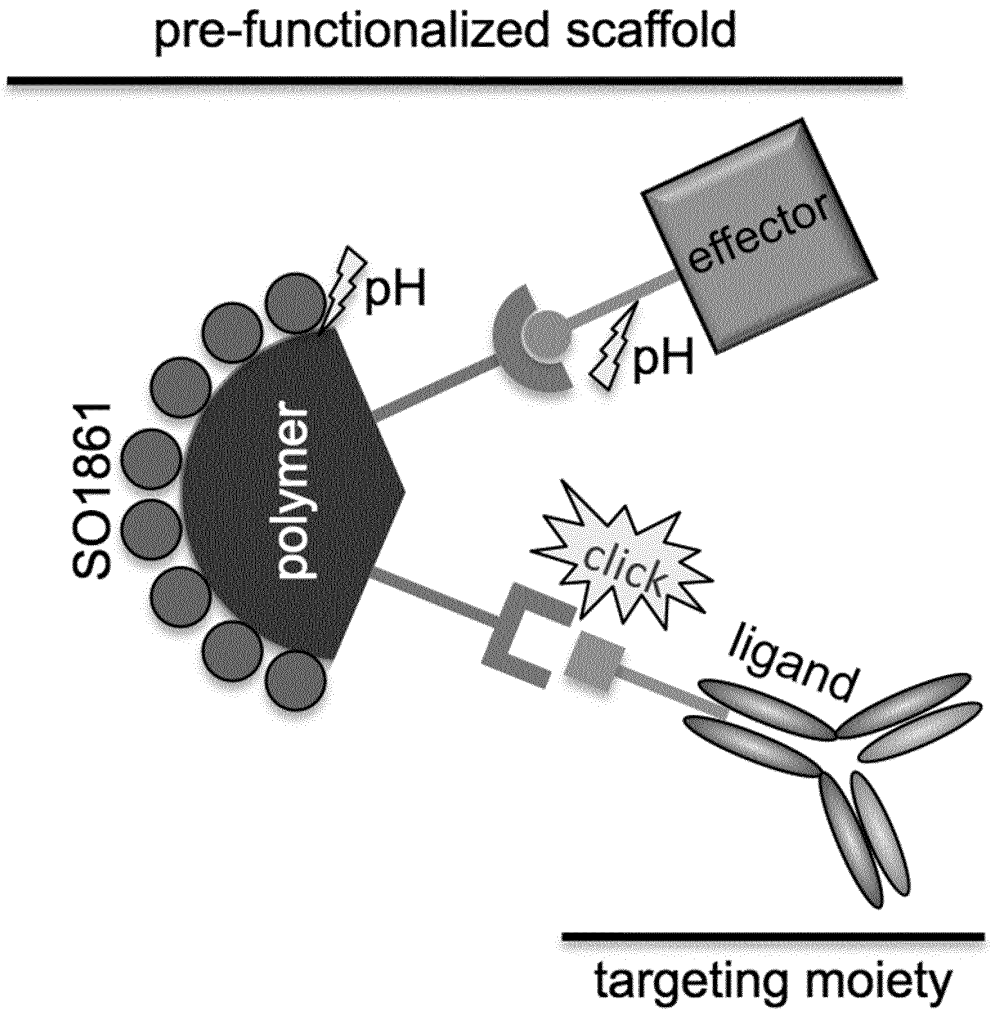

pH
click
ligand
SO1861
polymer
effector
targeted effector
scaffold

Figure 57


pre-functionalized scaffold
effector
pH
pH
SO1861
polymer
click
ligand
targeting moiety