Figure 1

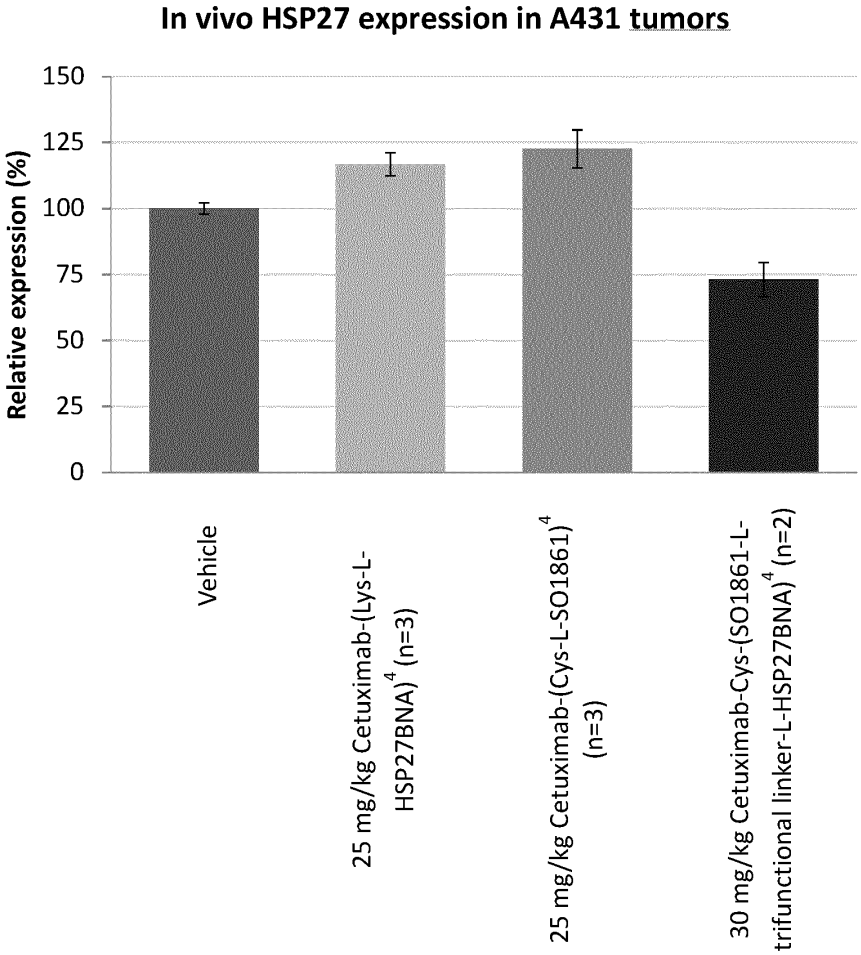

In vivo HSP27 expression in A431 tumors
Relative expression (%)
150
125
100
75
50
25
0
Vehicle
25 mg/kg Cetuximab-(Lys-L-HSP27BNA)4 (n=3)
25 mg/kg Cetuximab-(Cys-L-SO1861)4 (n=3)
30 mg/kg Cetuximab-Cys-(SO1861-L-trifunctional linker-L-HSP27BNA)4 (n=2)

Figure 2

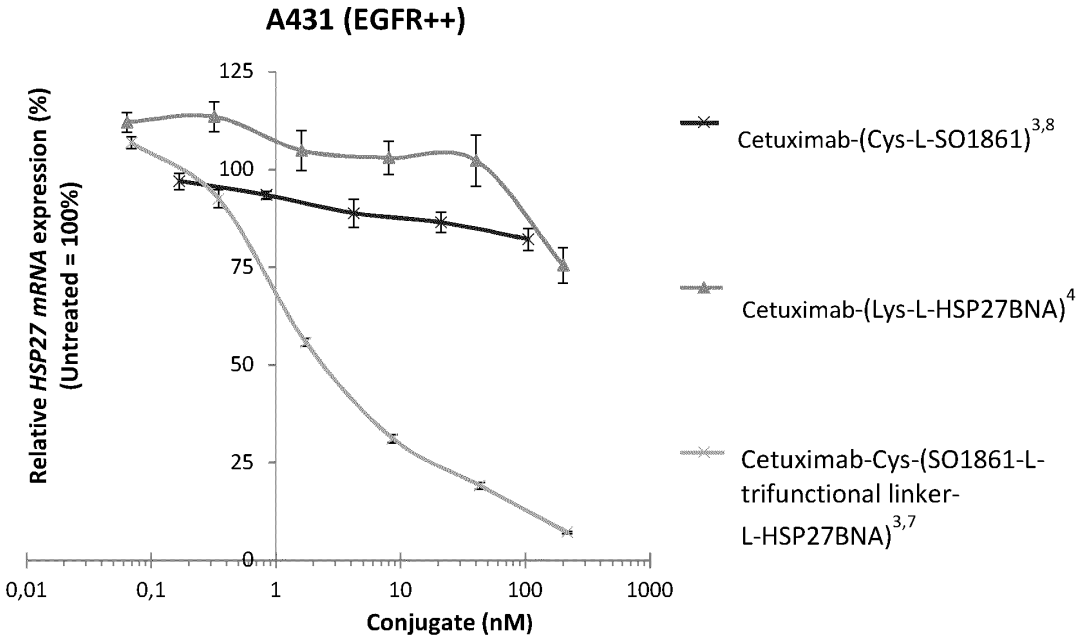


Figure 3

A

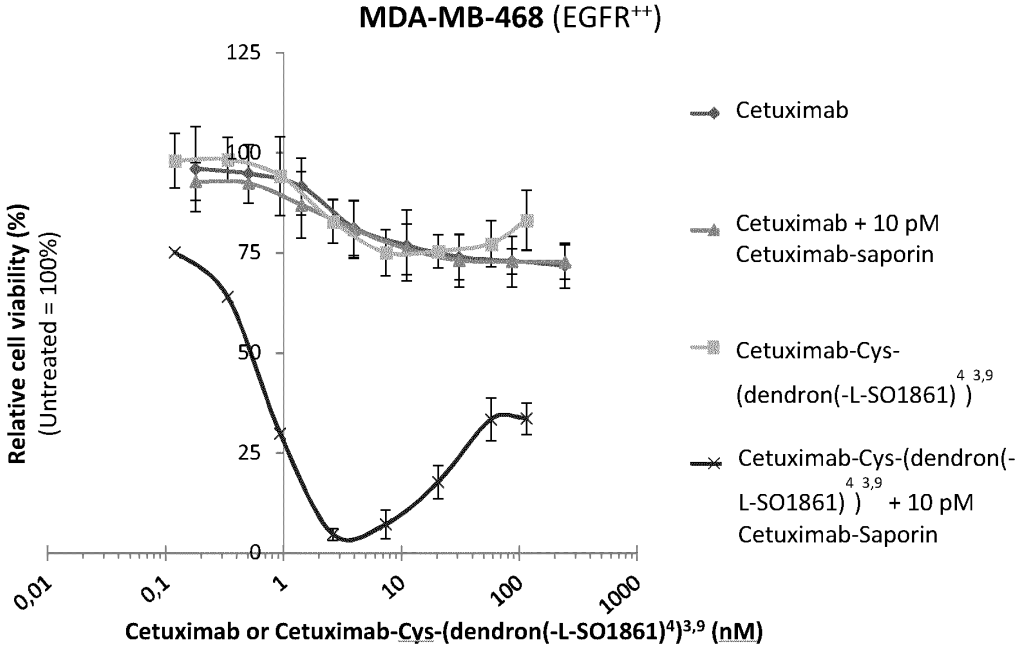
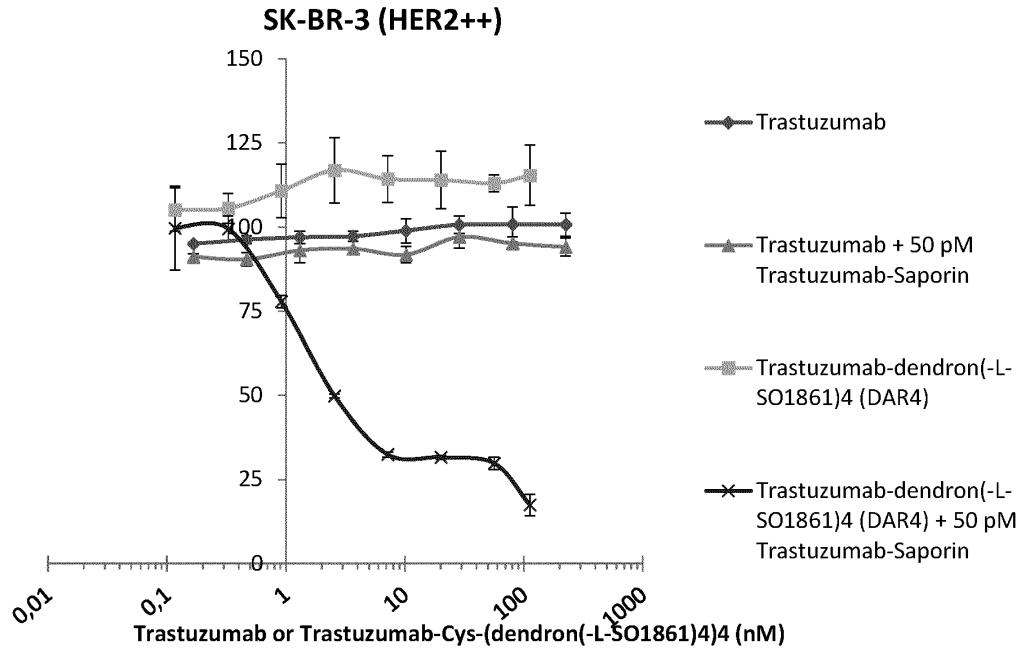


Figure 3

B


SK-BR-3 (HER2++)
150
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Trastuzumab or Trastuzumab-Cys-(dendron(-L-SO1861)4)4 (nM)
Trastuzumab
Trastuzumab + 50 pM Trastuzumab-Saporin
Trastuzumab-dendron(-L-SO1861)4 (DAR4)
Trastuzumab-dendron(-L-SO1861)4 (DAR4) + 50 pM Trastuzumab-Saporin

C

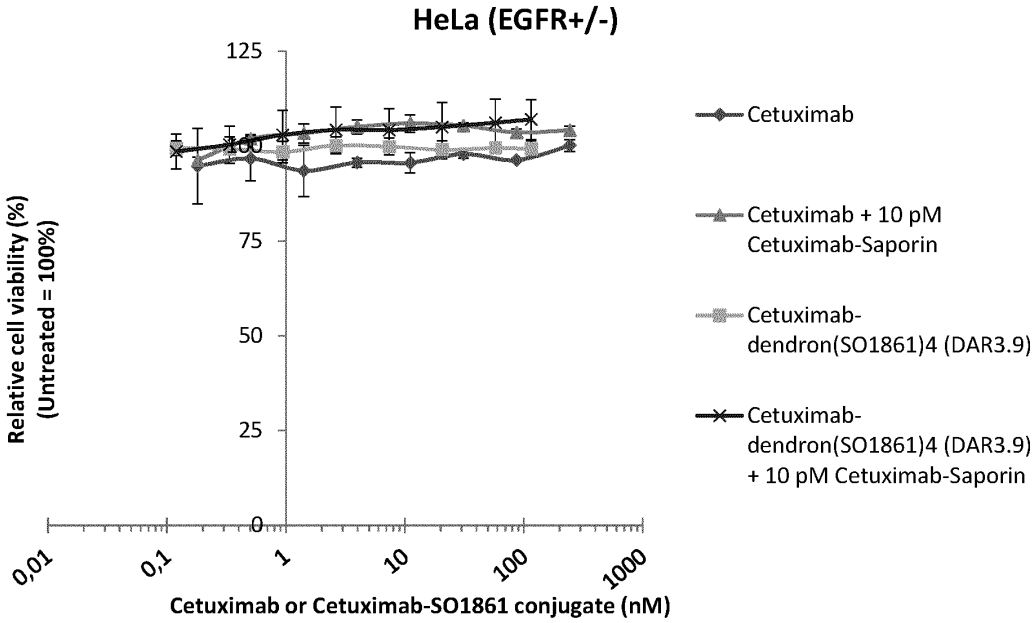

HeLa (EGFR+/-)
Relative cell viability (%) (Untreated = 100%)
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Cetuximab or Cetuximab-SO1861 conjugate (nM)
Cetuximab
Cetuximab + 10 pM Cetuximab-Saporin
Cetuximab-dendron(SO1861)4 (DAR3.9)
Cetuximab-dendron(SO1861)4 (DAR3.9) + 10 pM Cetuximab-Saporin

Figure 3
D

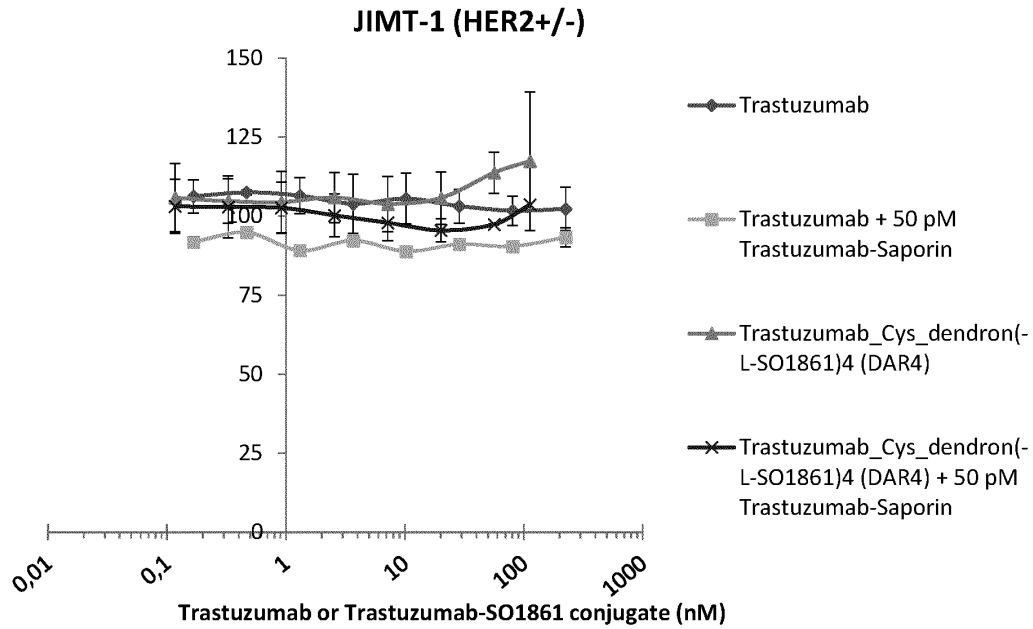


Figure 4

A

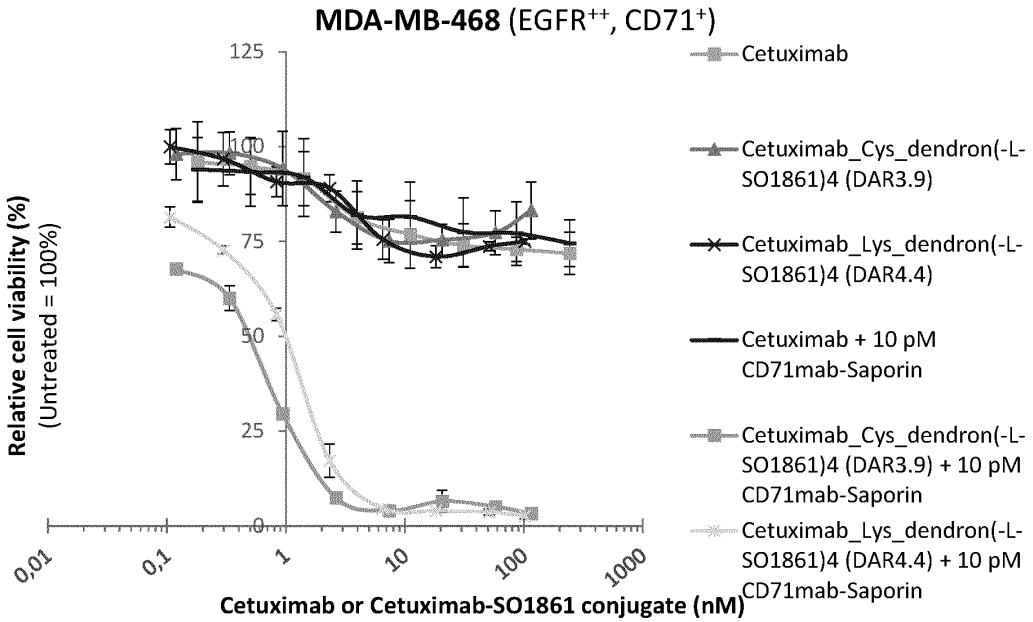
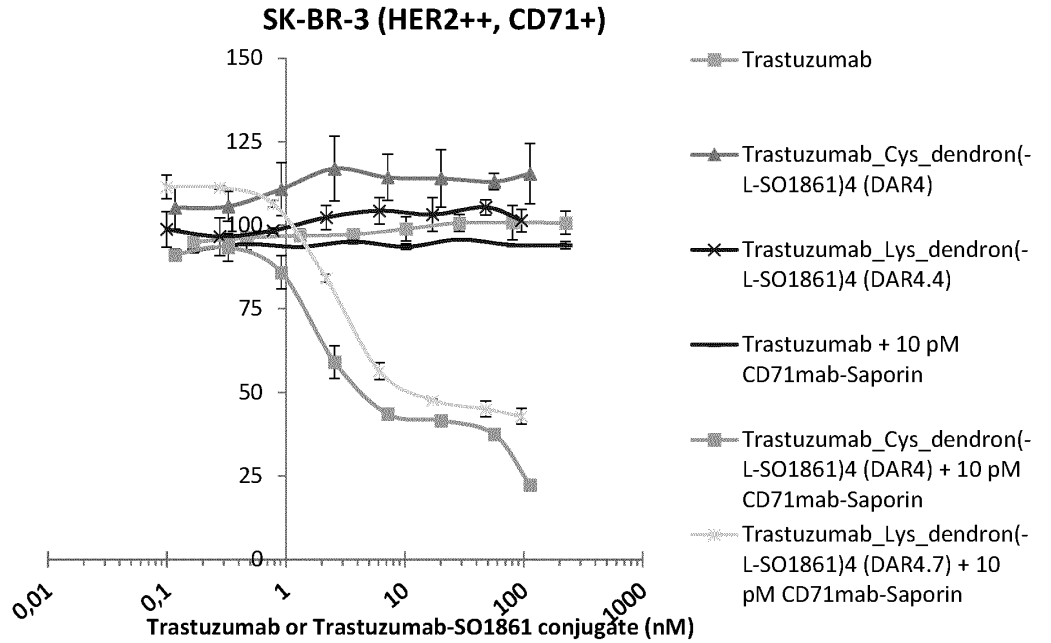


Figure 4

B


SK-BR-3 (HER2++, CD71+)
150
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Trastuzumab or Trastuzumab-SO1861 conjugate (nM)
Trastuzumab
Trastuzumab_Cys_dendron(-L-SO1861)4 (DAR4)
Trastuzumab_Lys_dendron(-L-SO1861)4 (DAR4.4)
Trastuzumab + 10 pM CD71mab-Saporin
Trastuzumab_Cys_dendron(-L-SO1861)4 (DAR4) + 10 pM CD71mab-Saporin
Trastuzumab_Lys_dendron(-L-SO1861)4 (DAR4.7) + 10 pM CD71mab-Saporin

C

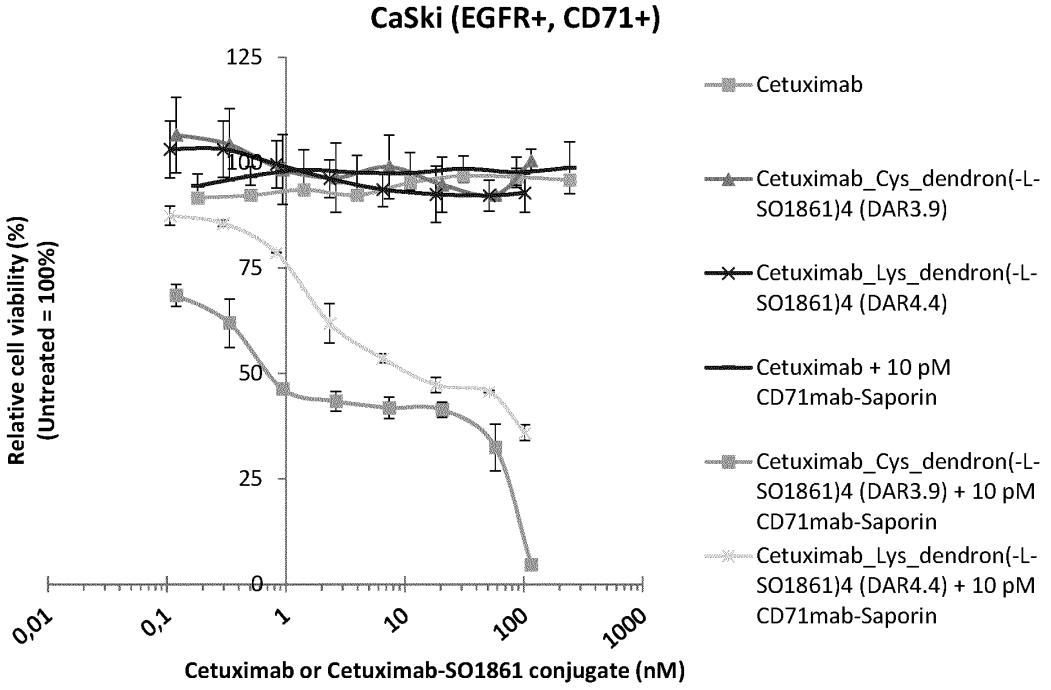

CaSki (EGFR+, CD71+)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Cetuximab or Cetuximab-SO1861 conjugate (nM)
Cetuximab
Cetuximab_Cys_dendron(-L-SO1861)4 (DAR3.9)
Cetuximab_Lys_dendron(-L-SO1861)4 (DAR4.4)
Cetuximab + 10 pM CD71mab-Saporin
Cetuximab_Cys_dendron(-L-SO1861)4 (DAR3.9) + 10 pM CD71mab-Saporin
Cetuximab_Lys_dendron(-L-SO1861)4 (DAR4.4) + 10 pM CD71mab-Saporin

Figure 4
D

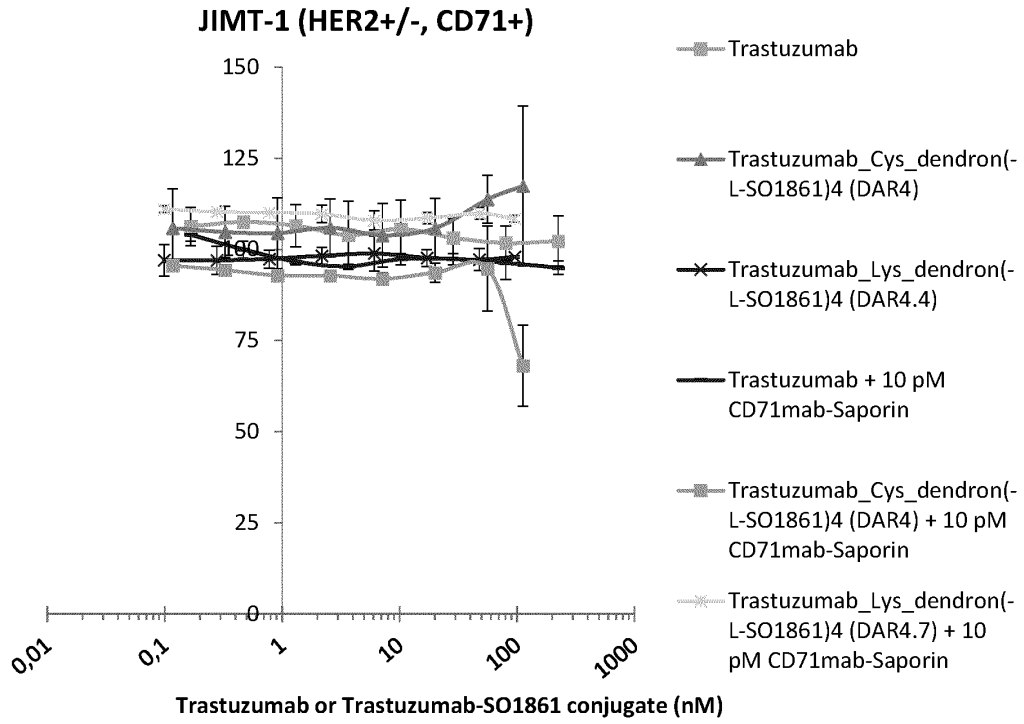


Figure 5

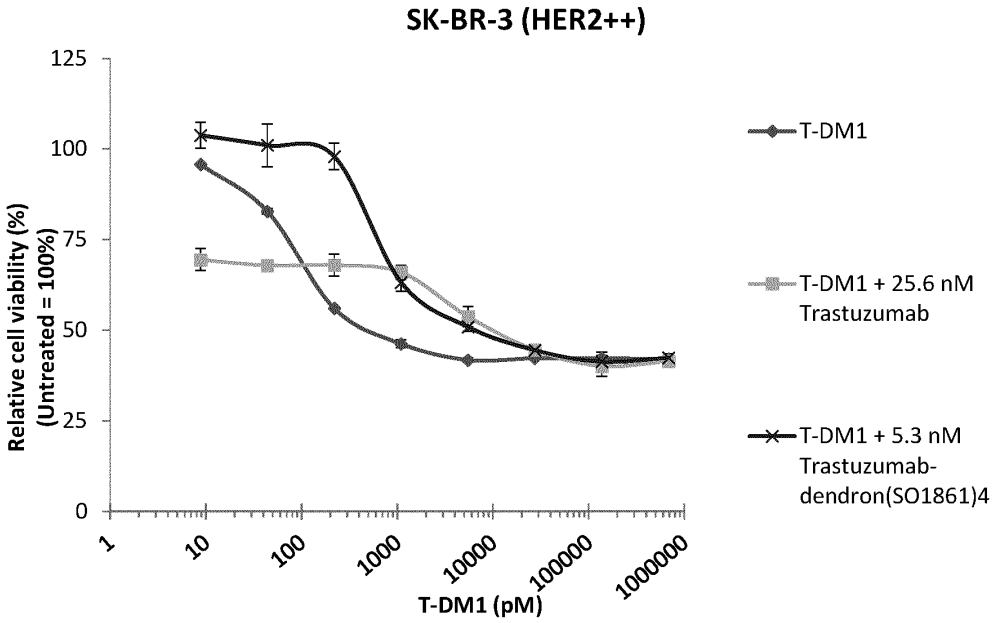


Figure 6

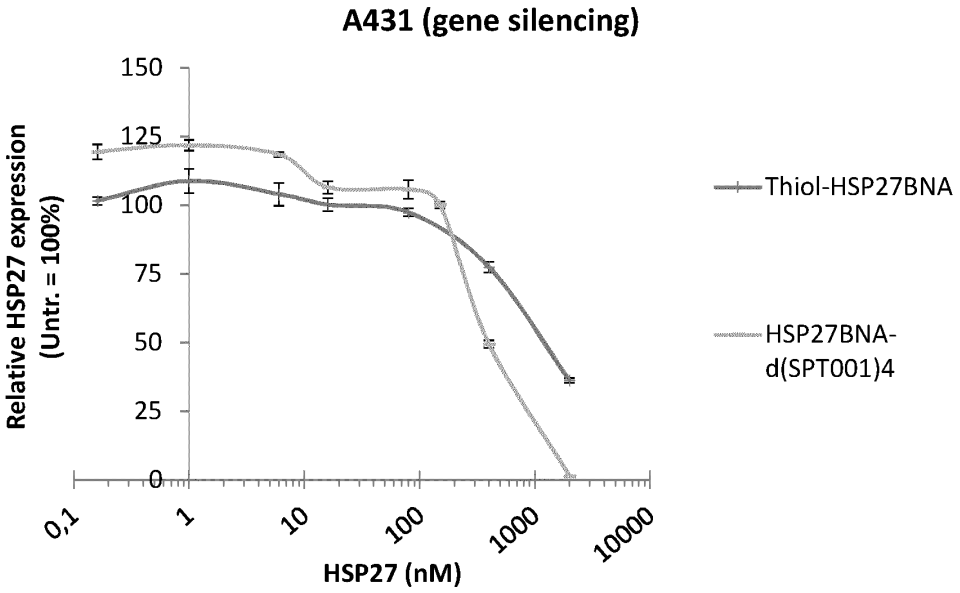

A431 (gene silencing)
Relative HSP27 expression (Untr. = 100%)
150
125
100
75
50
25
0
0,1
1
10
100
1000
10000
HSP27 (nM)
Thiol-HSP27BNA
HSP27BNA-d(SPT001)4

Figure 7

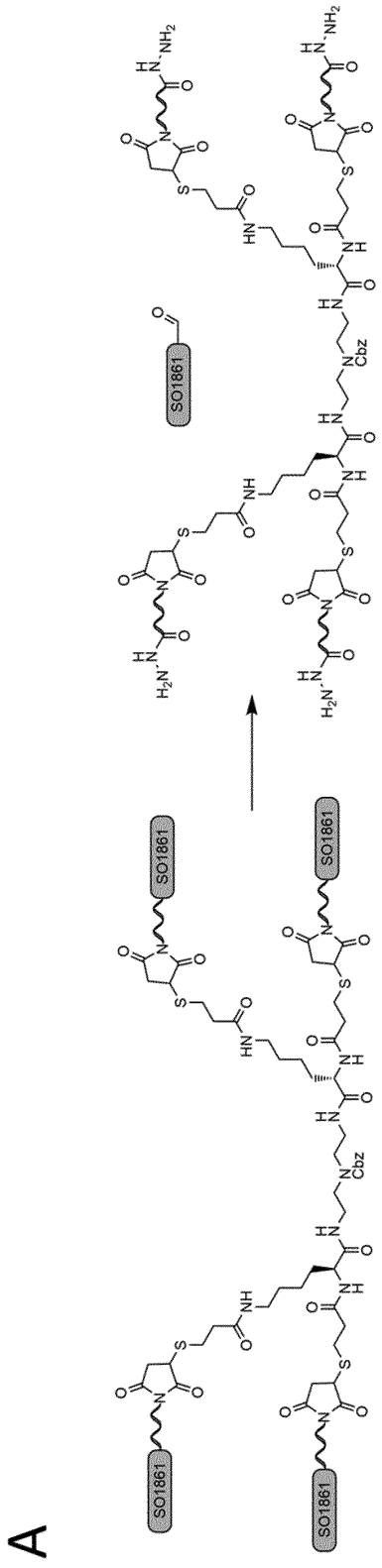


Figure 7

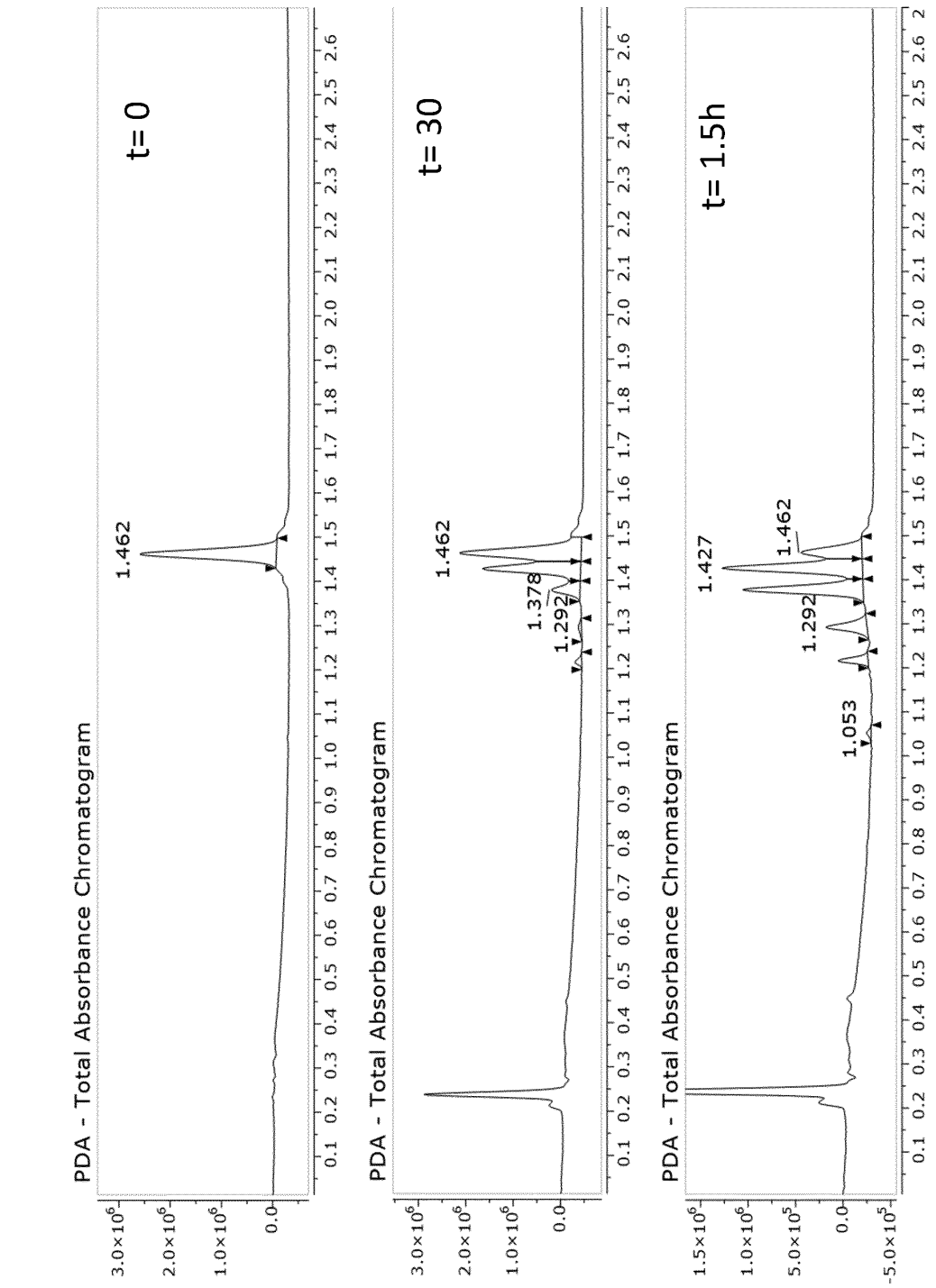

B
PDA - Total Absorbance Chromatogram
t= 0
1.462
PDA - Total Absorbance Chromatogram
t= 30
1.462
1.378
1.292
PDA - Total Absorbance Chromatogram
t= 1.5h
1.427
1.462
1.292
1.053

Figure 7

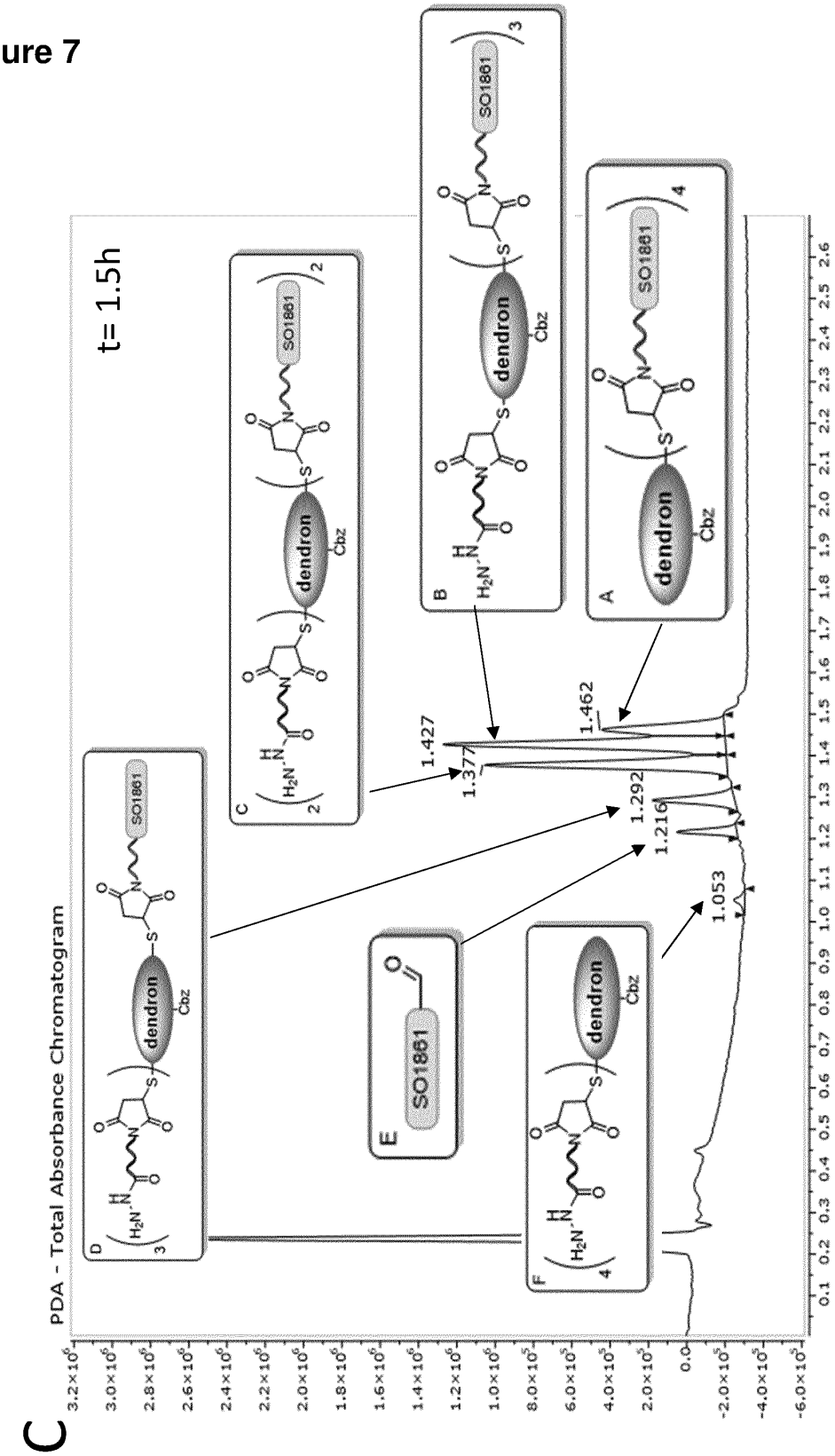
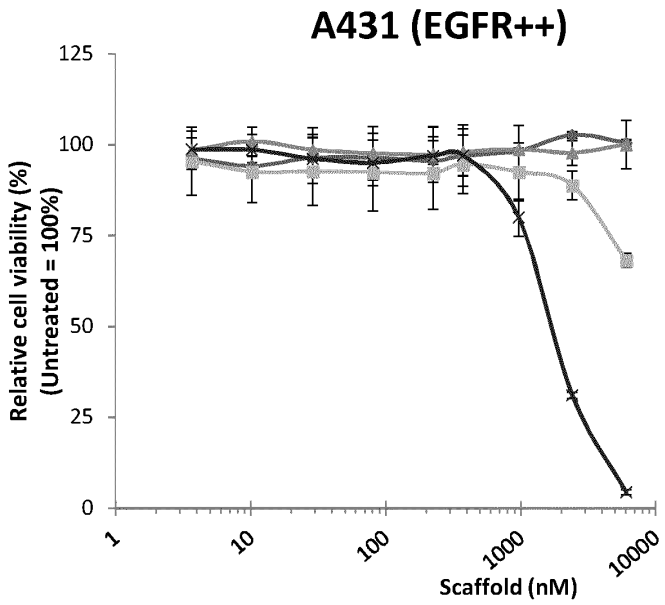

C
PDA - Total Absorbance Chromatogram
t= 1.5h
1.427
1.377
1.462
1.292
1.216
1.053
SO1861
dendron
Cbz

Figure 8

A


A431 (EGFR++)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
1
10
100
1000
10000
Scaffold (nM)

B

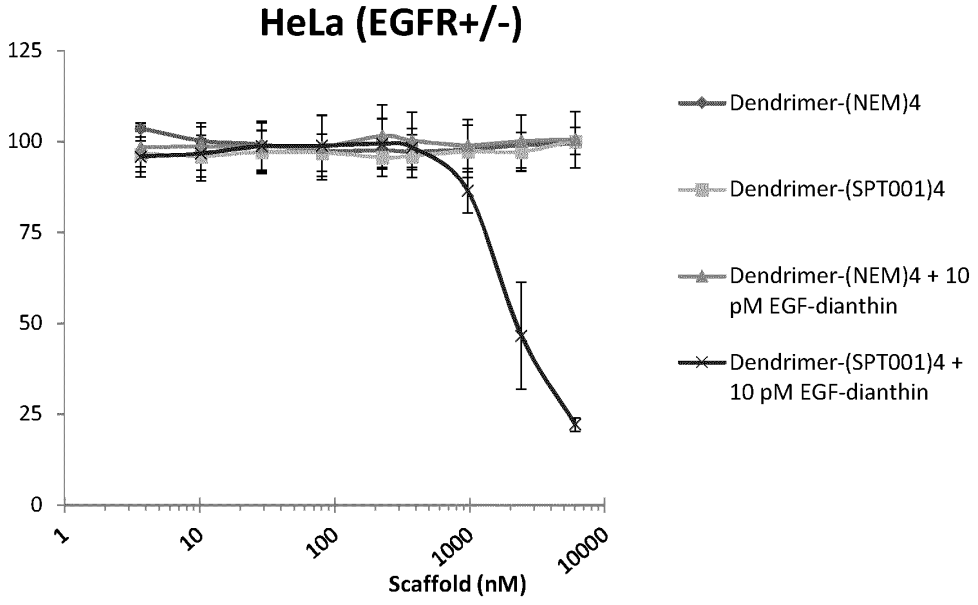
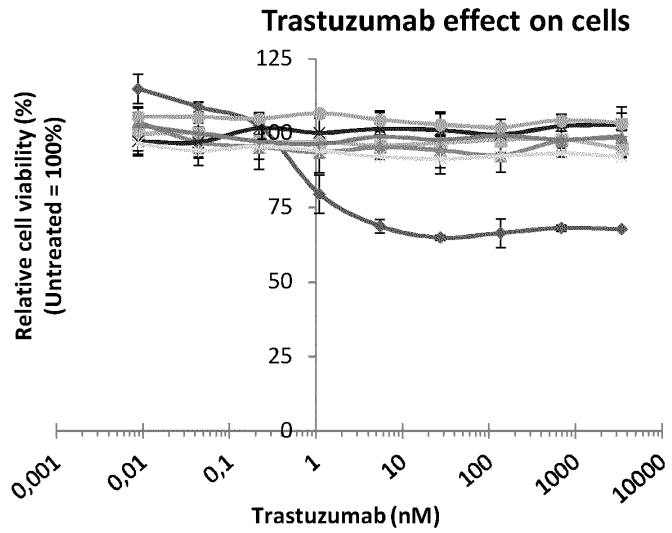

HeLa (EGFR+/-)
125
100
75
50
25
0
1
10
100
1000
10000
Scaffold (nM)
Dendrimer-(NEM)4
Dendrimer-(SPT001)4
Dendrimer-(NEM)4 + 10 pM EGF-dianthin
Dendrimer-(SPT001)4 + 10 pM EGF-dianthin

Figure 9

A


Trastuzumab 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
Trastuzumab (nM)

B

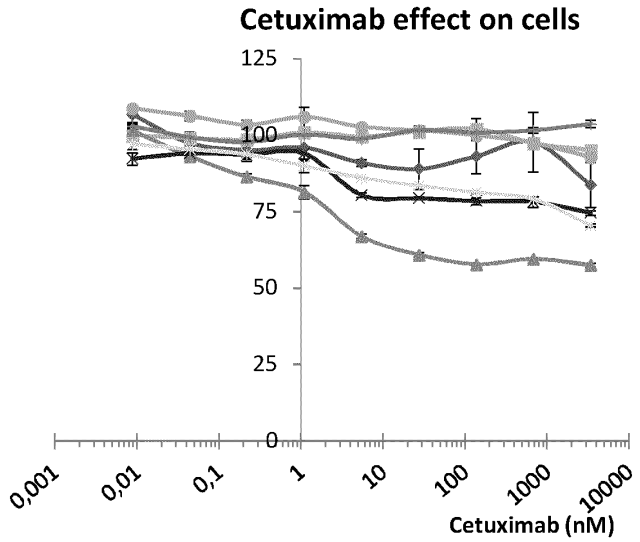

Cetuximab effect on cells
125
100
75
50
25
0
0,001
0,01
0,1
1
10
100
1000
10000
Cetuximab (nM)

Figure 9

C

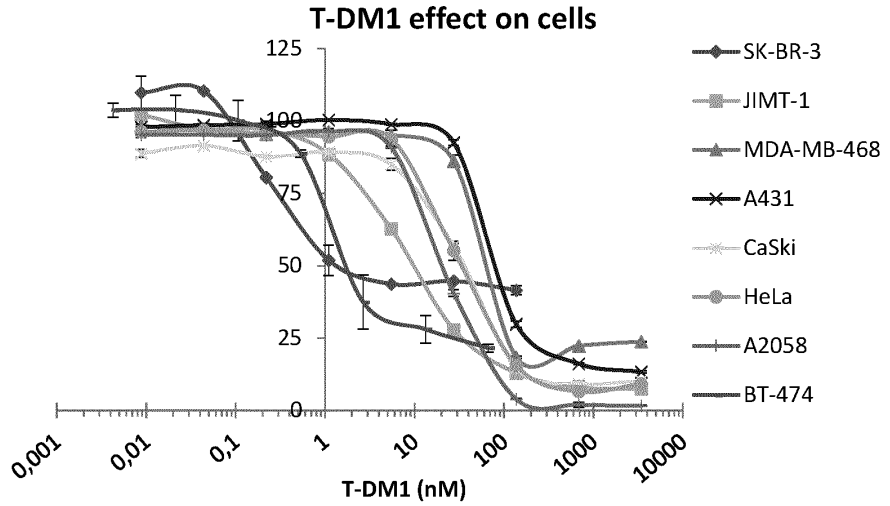


Figure 10

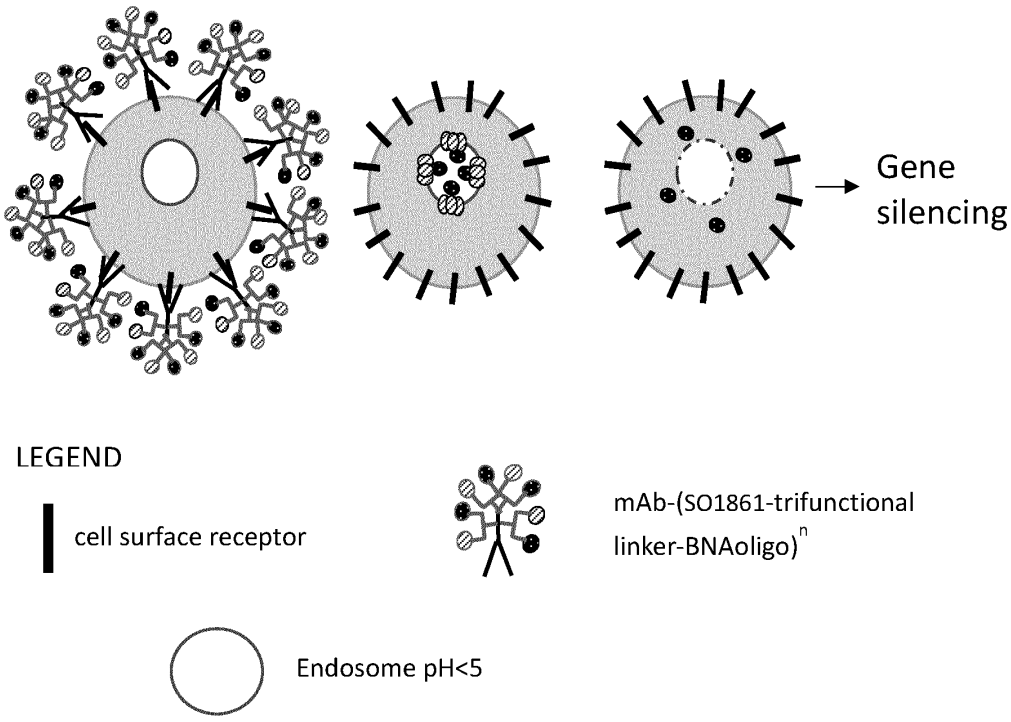


Figure 11

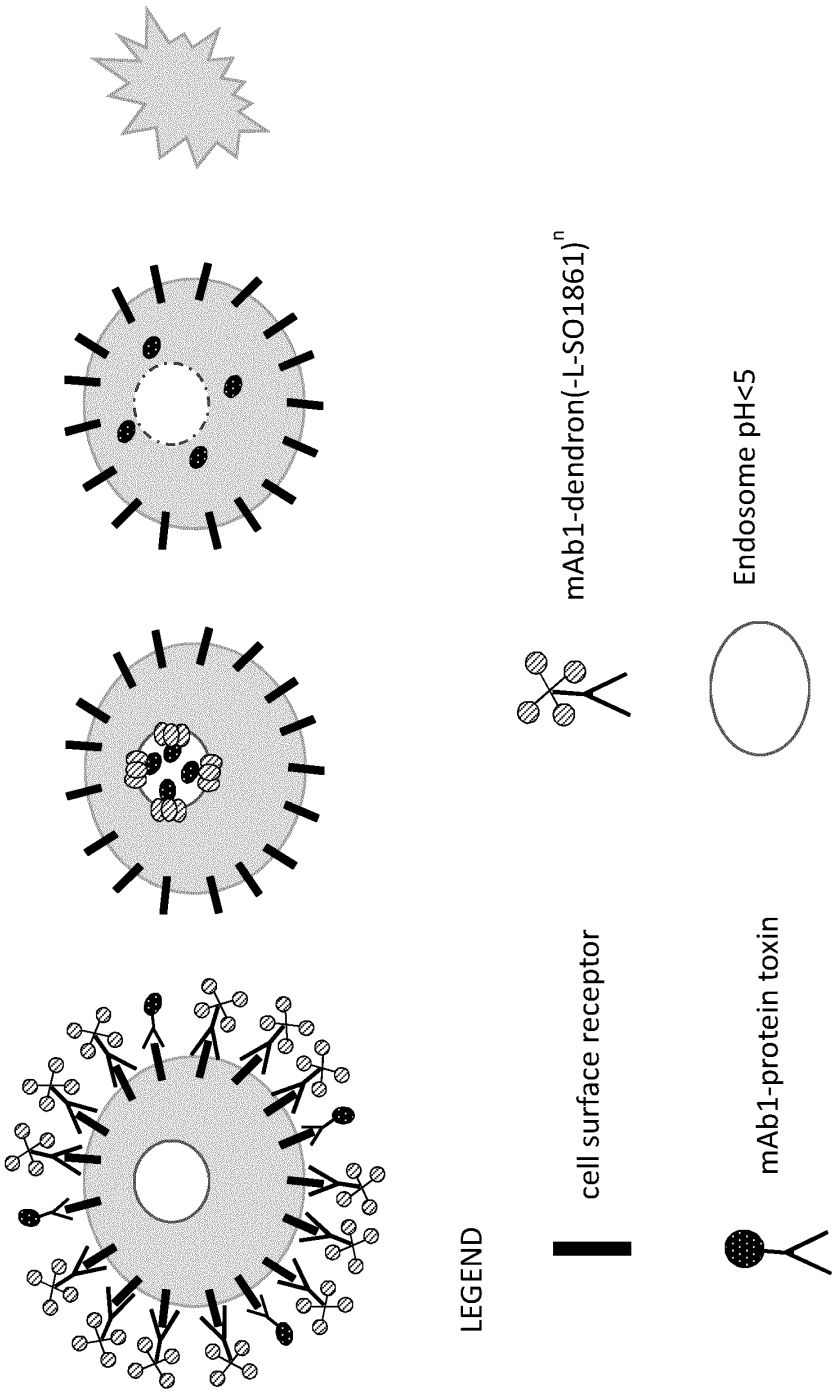

mAb1-dendron(-L-SO1861)n
Endosome pH<5
LEGEND
cell surface receptor
mAb1-protein toxin

Figure 12

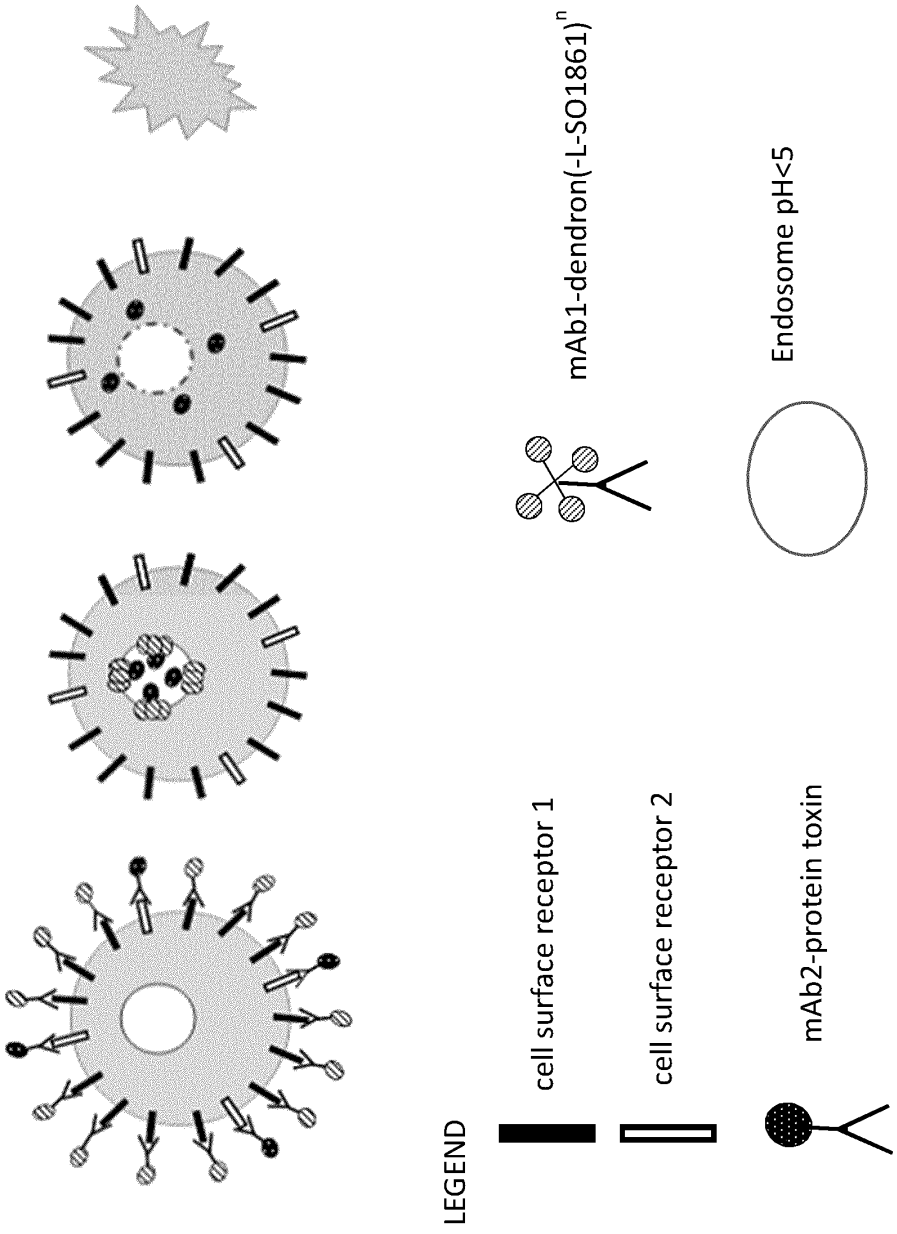

LEGEND
cell surface receptor 1
cell surface receptor 2
mAb2-protein toxin
mAb1-dendron(-L-SO1861)n
Endosome pH<5

Figure 13

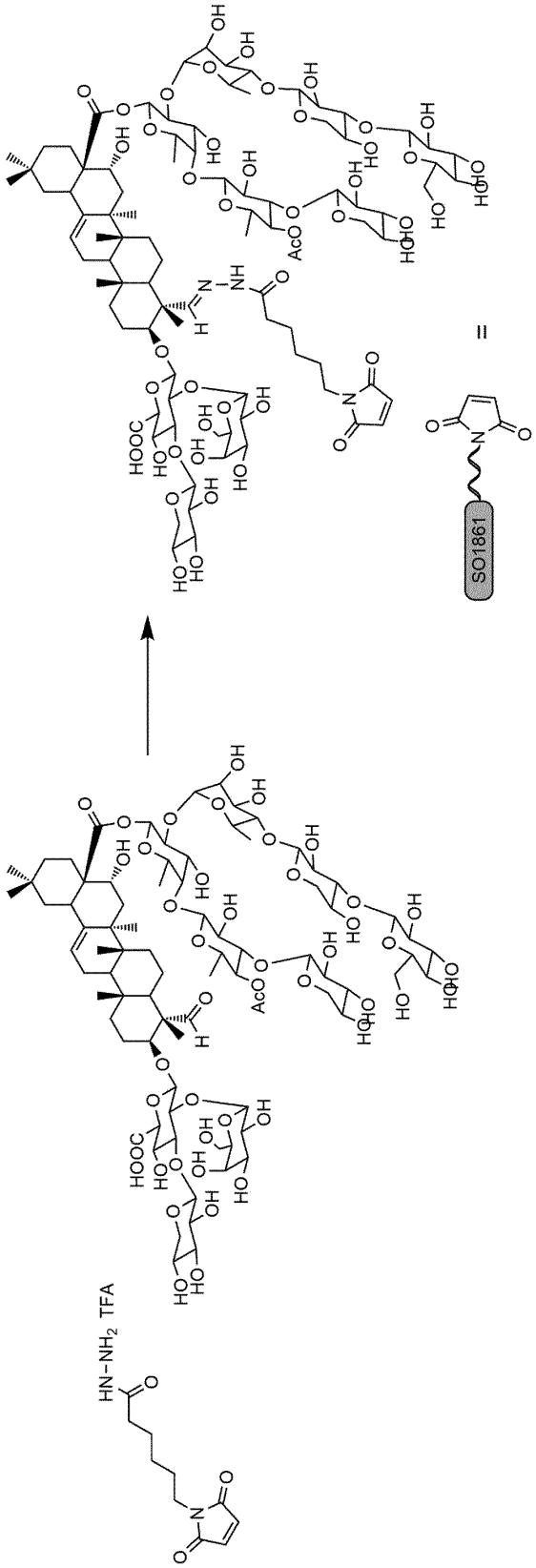


Figure 14

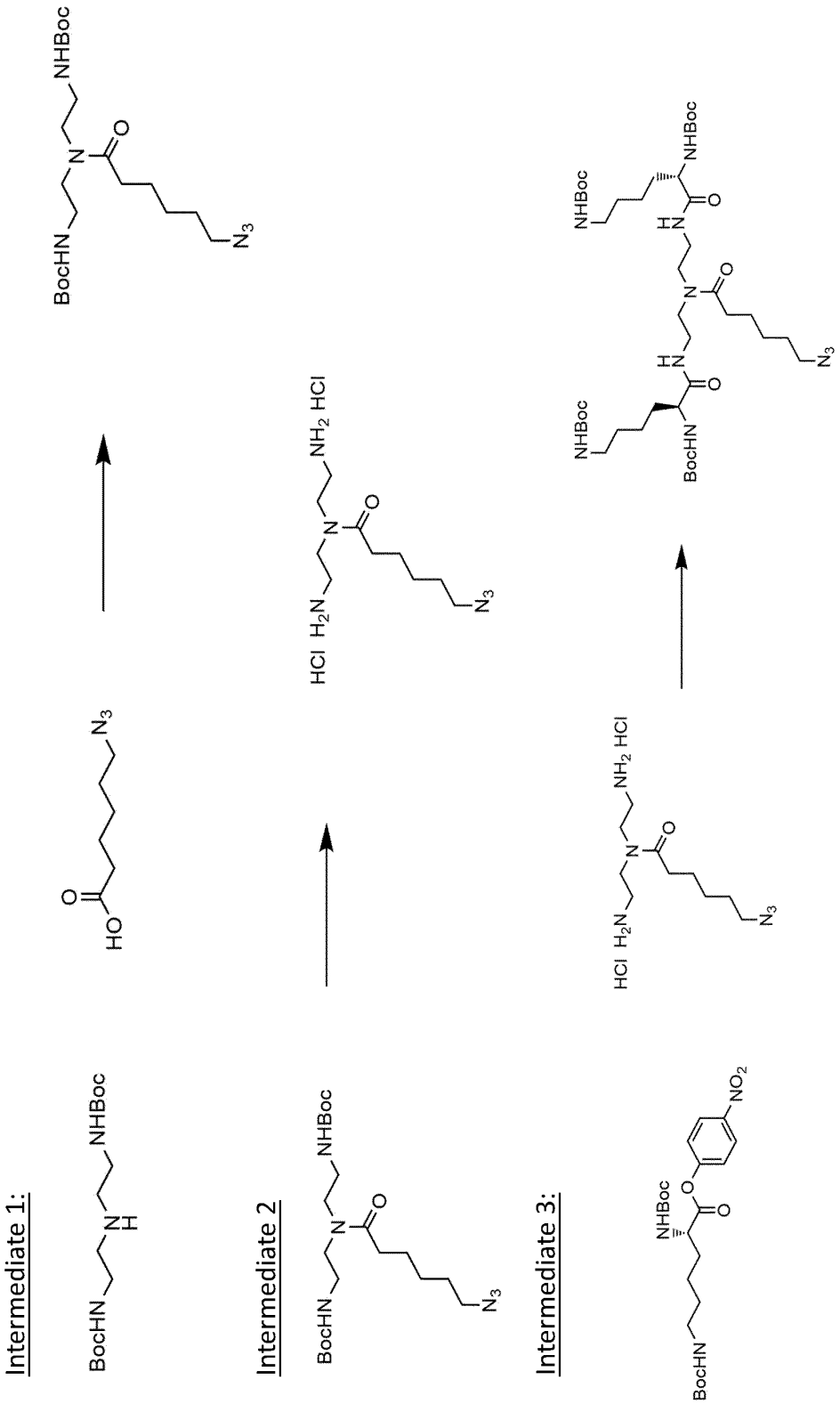

Intermediate 1:
BocHN
NHBoc
Intermediate 2
BocHN
NHBoc
Intermediate 3:
NHBoc
NO2
BocHN

Figure 14

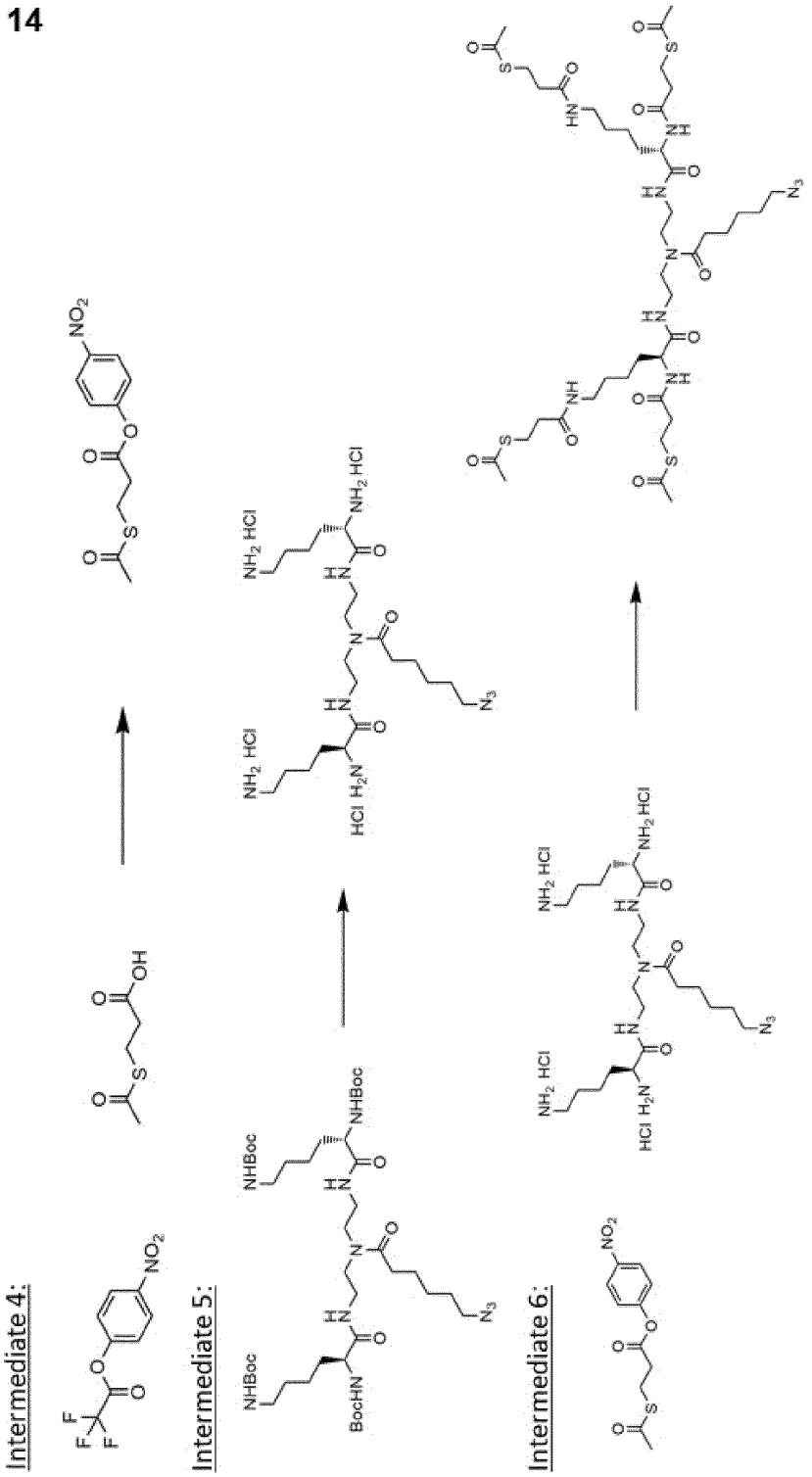


Figure 14

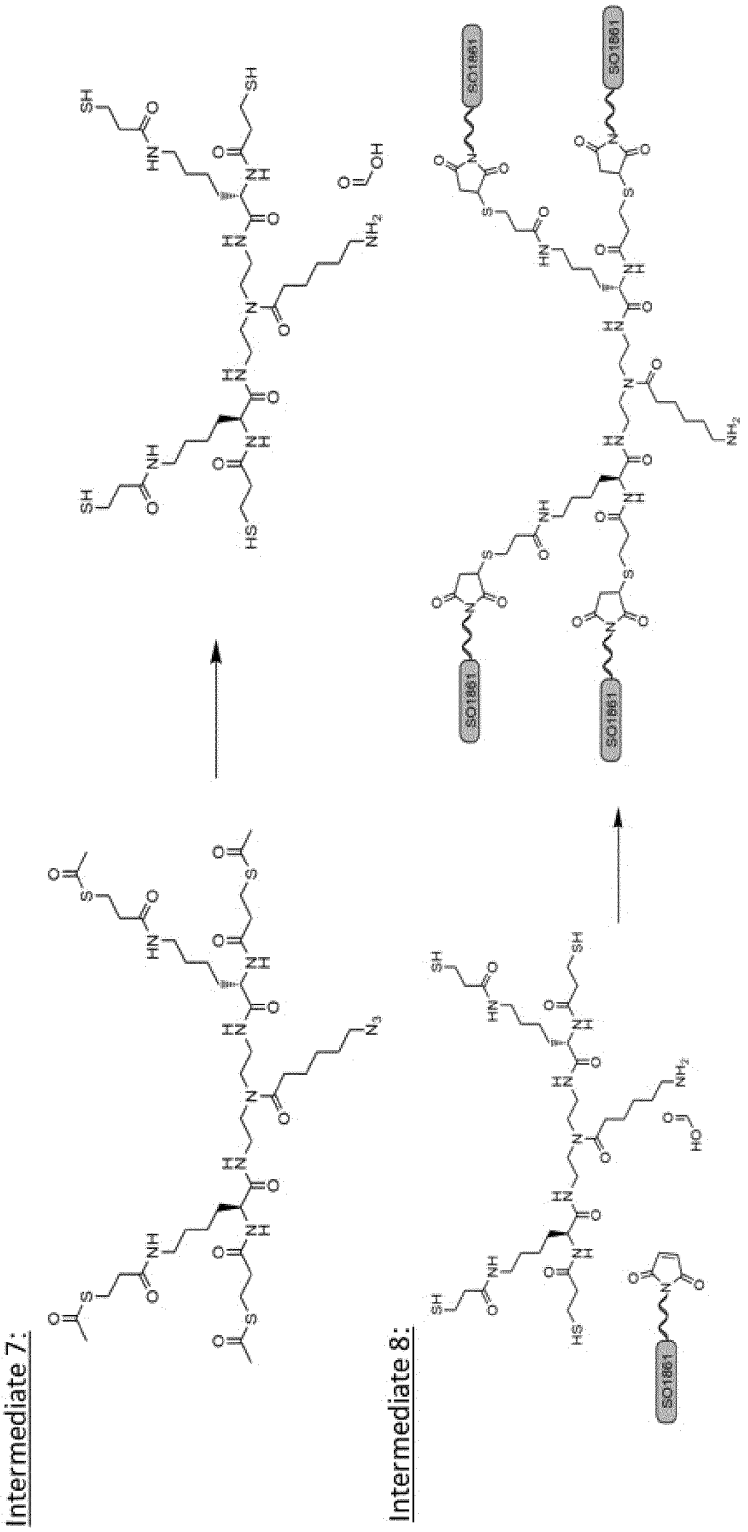


Figure 14

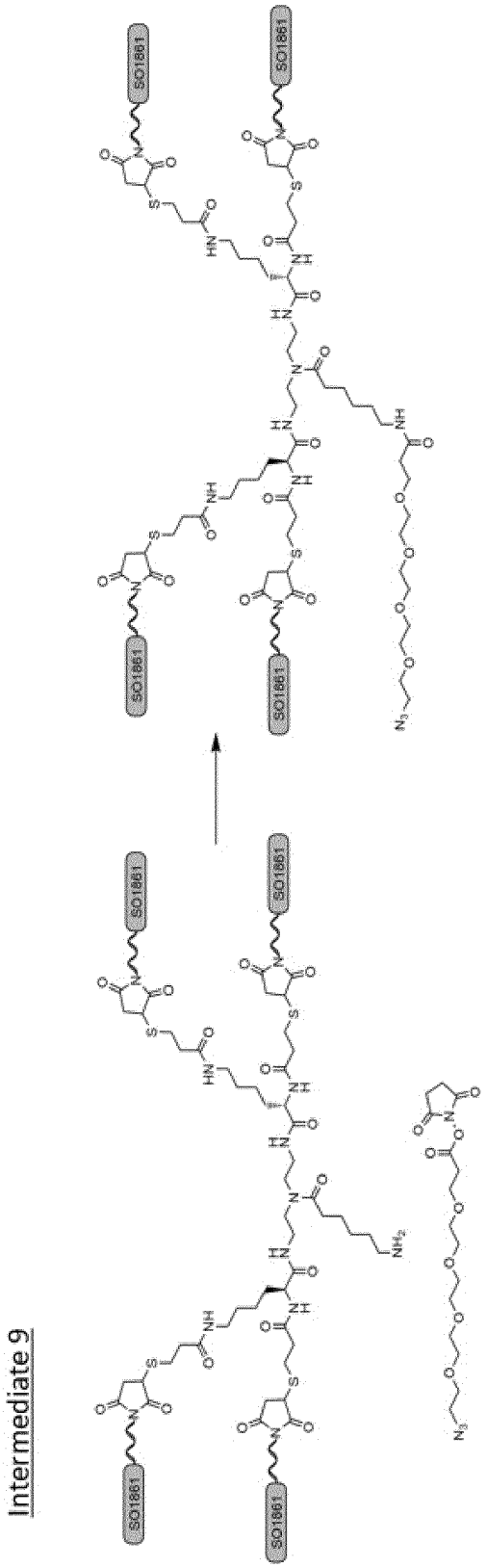
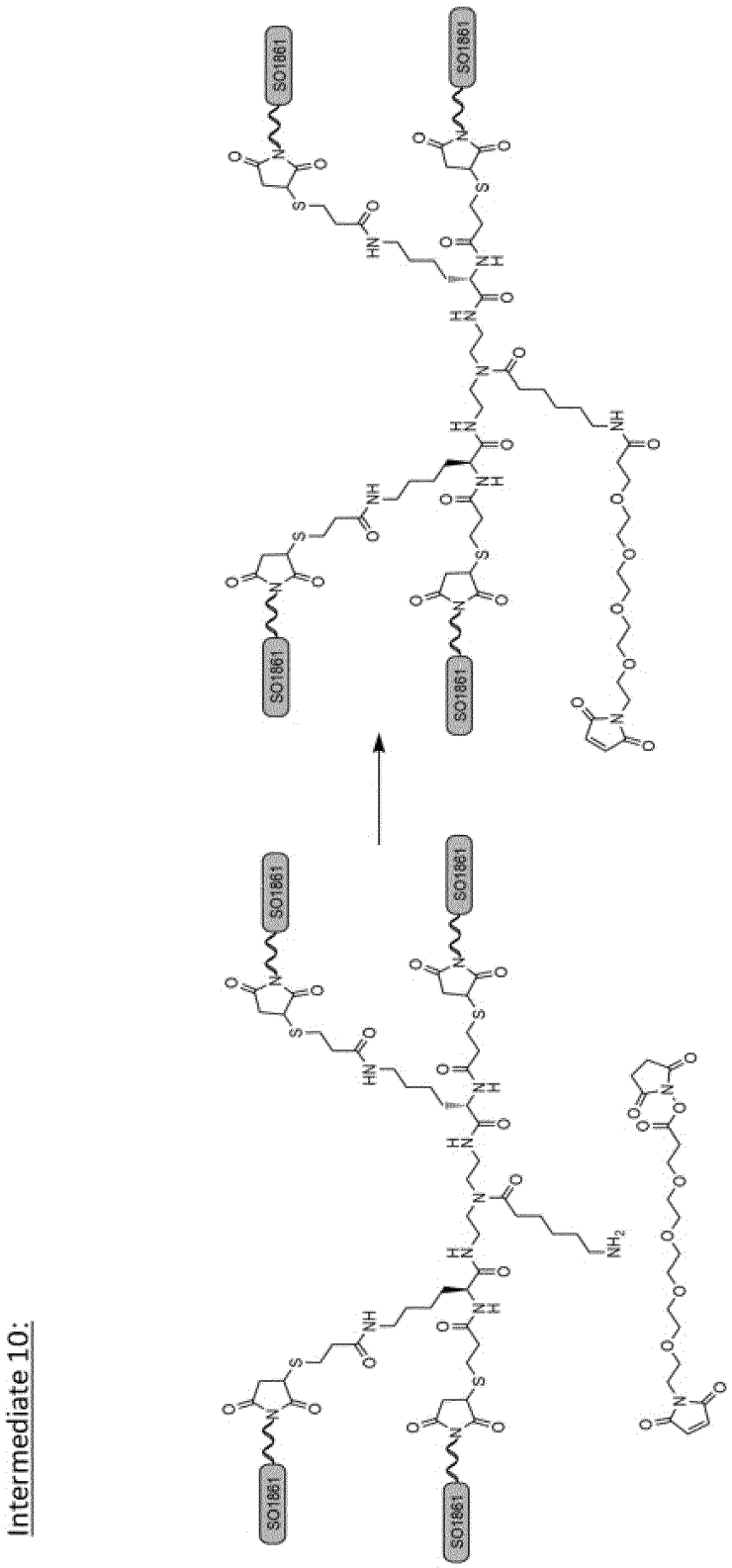
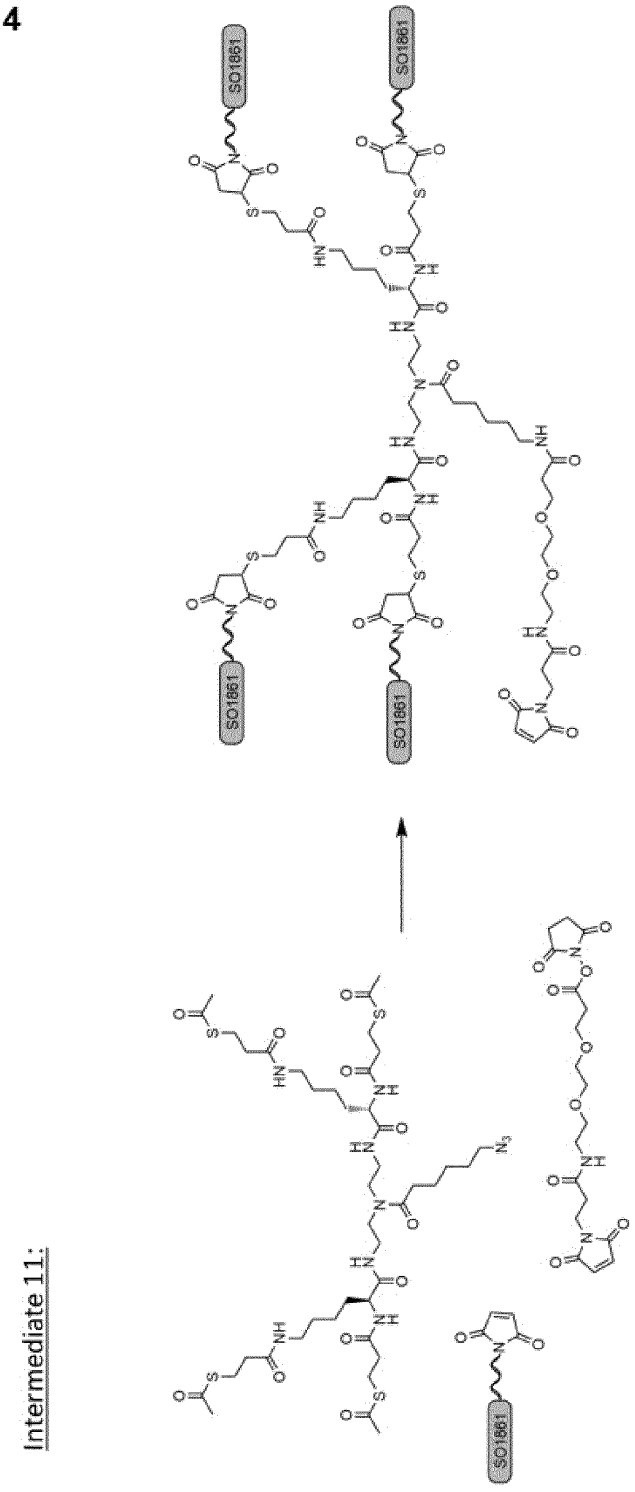


Figure 14



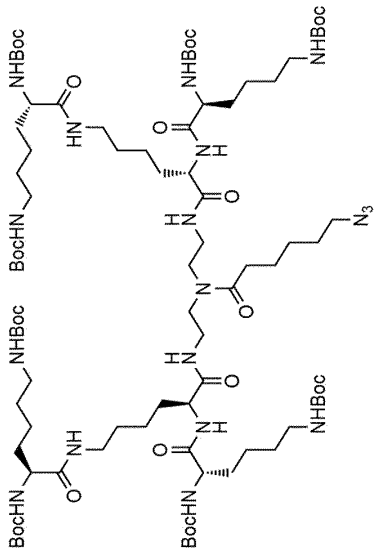
Intermediate 10:

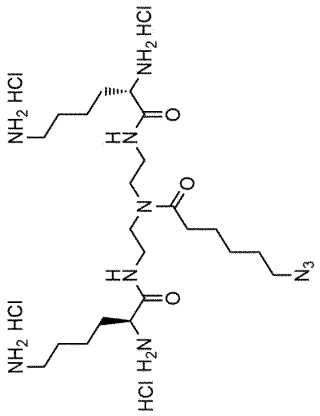
Figure 14



Intermediate 11:

Figure 15





Intermediate 1:

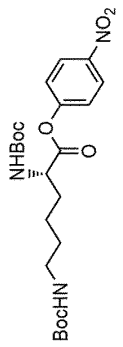


Figure 15

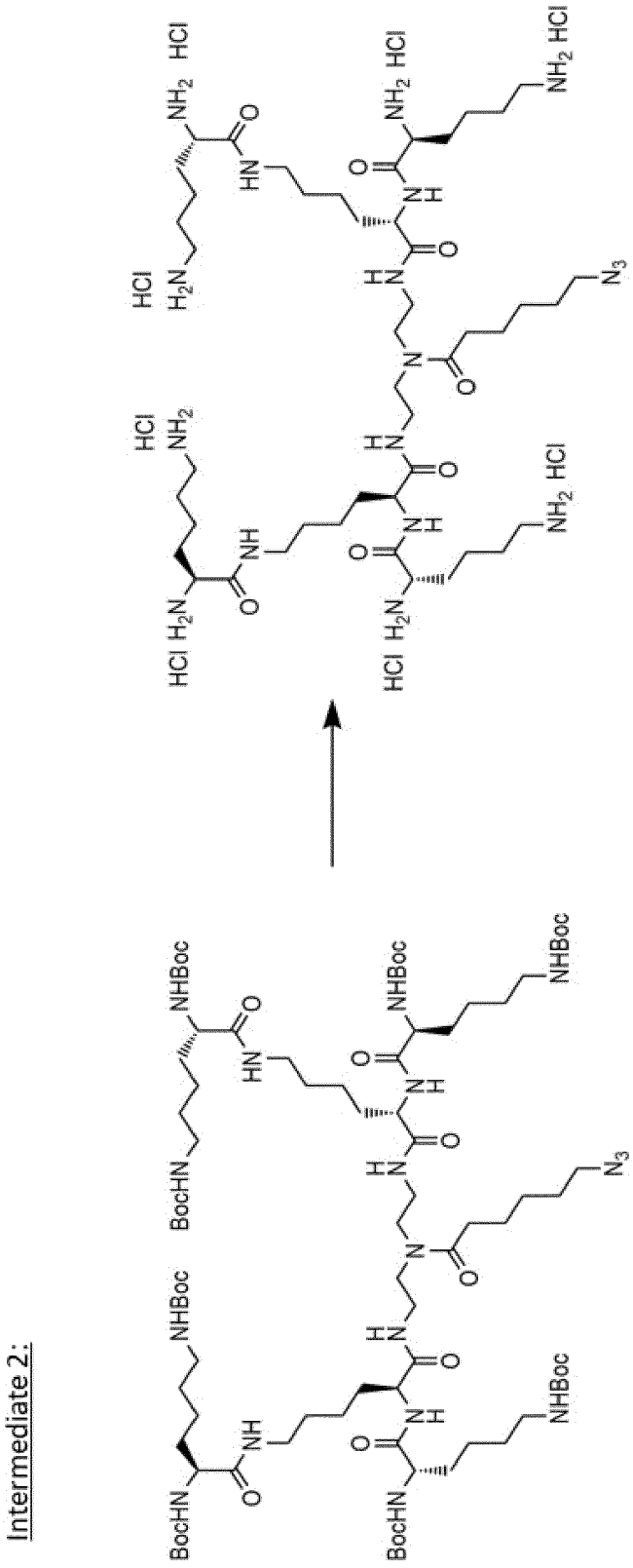


Figure 15

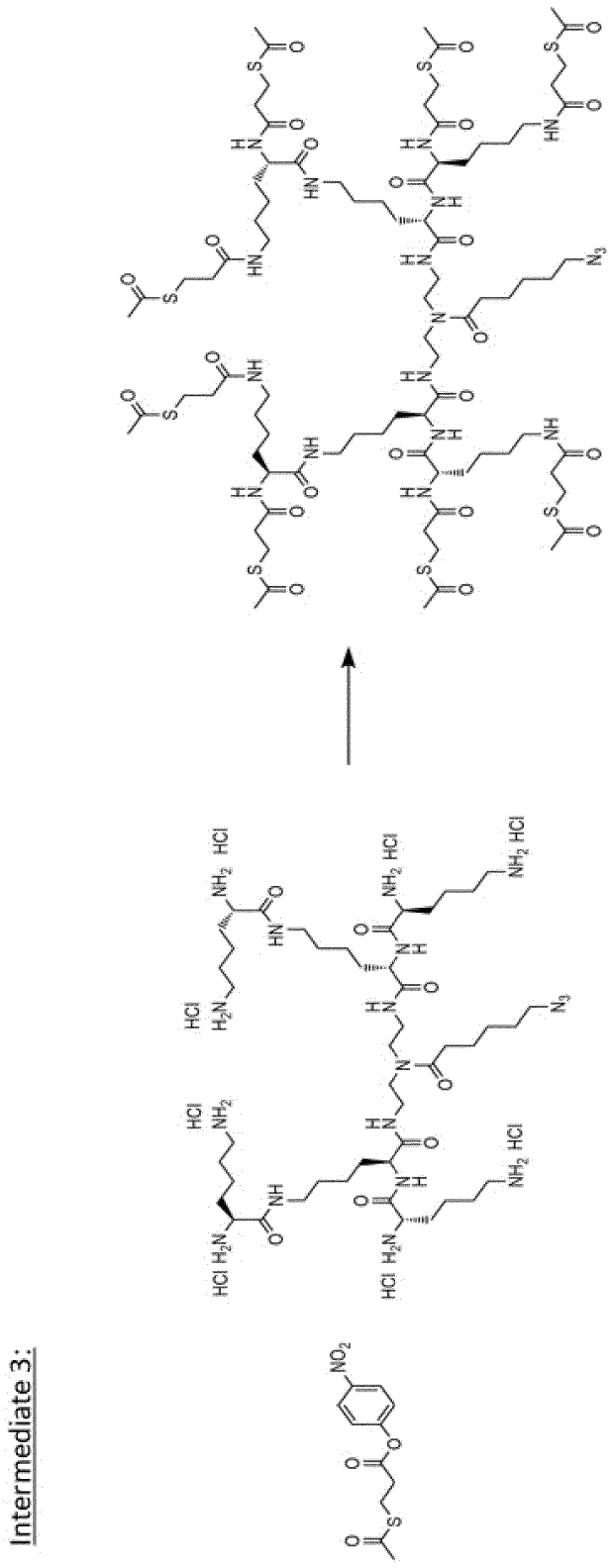


Figure 15

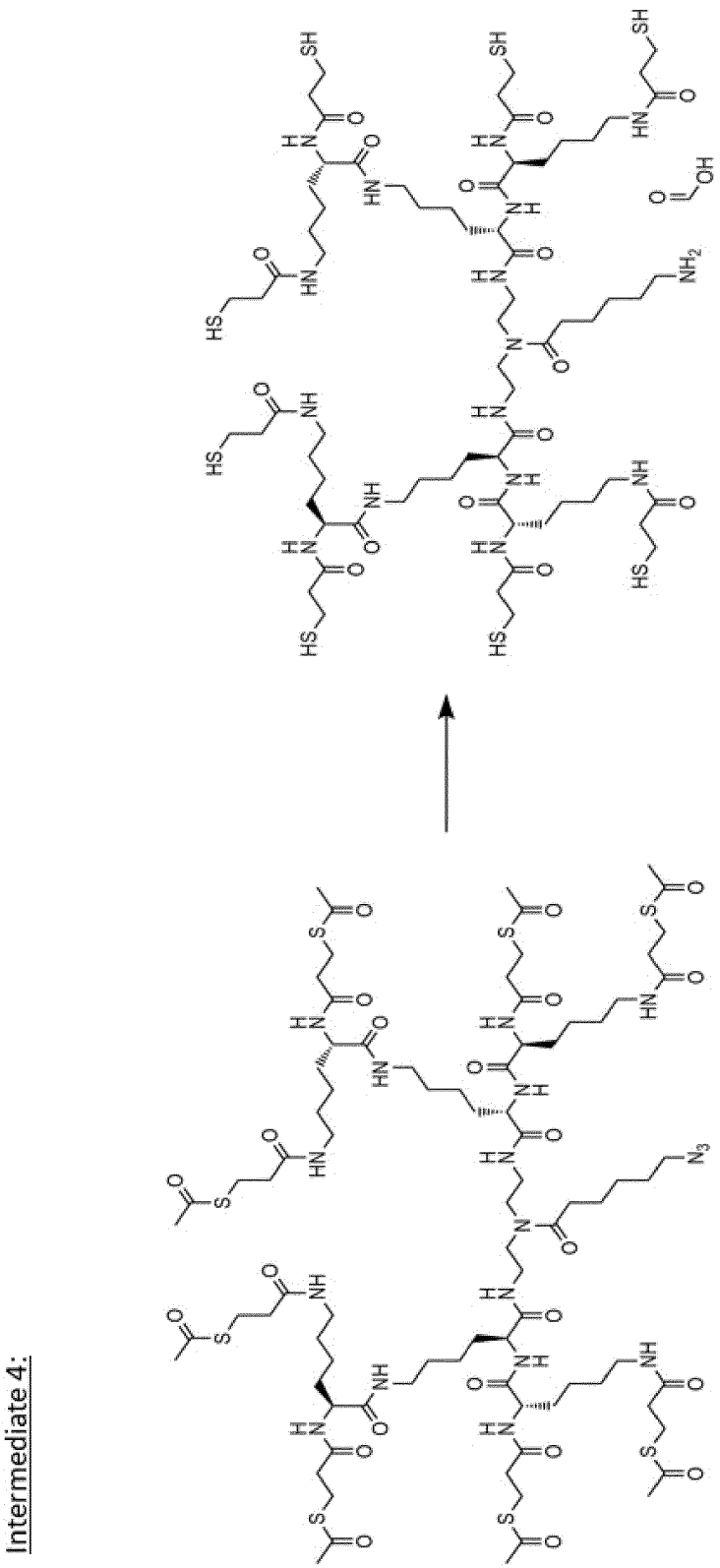


Figure 15

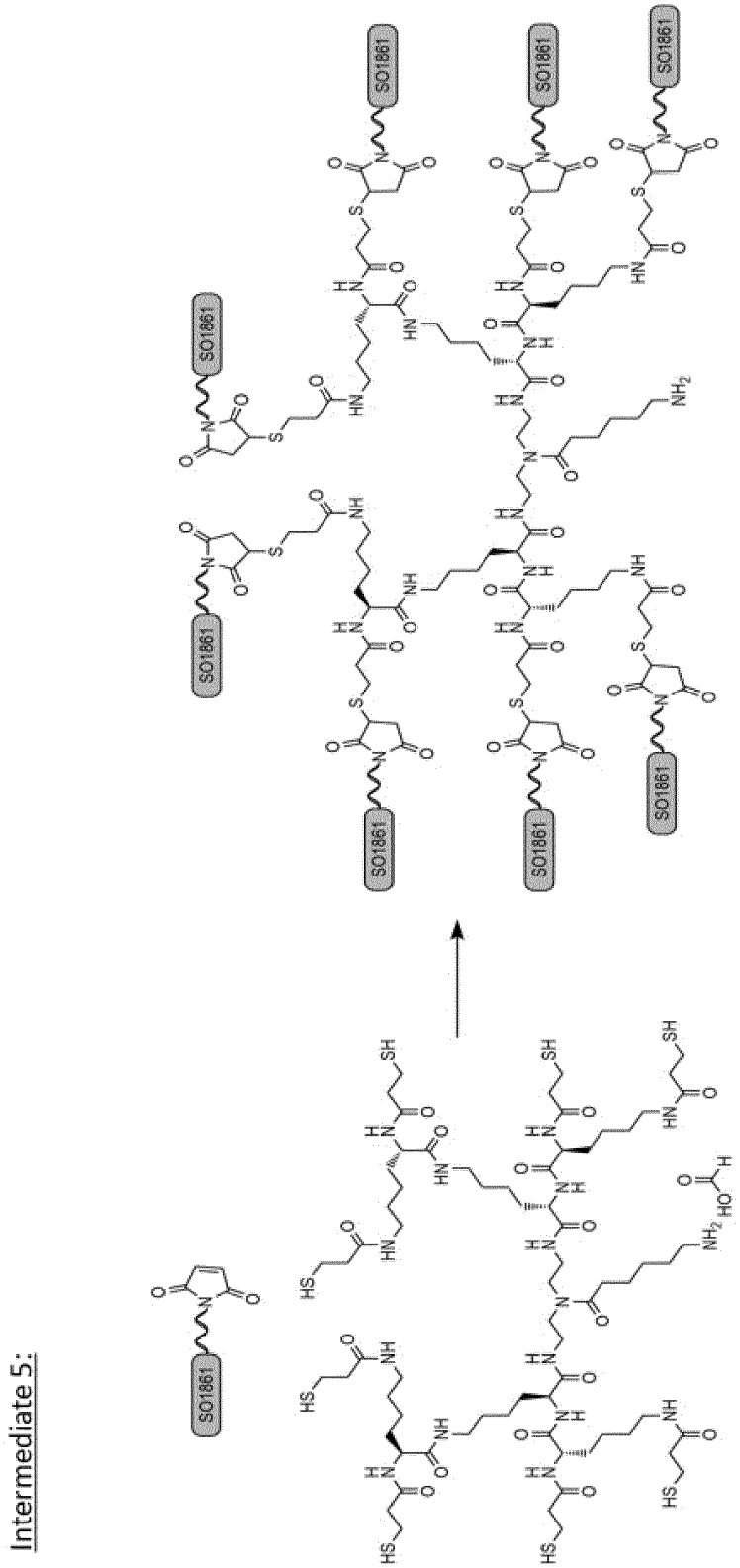


Figure 16

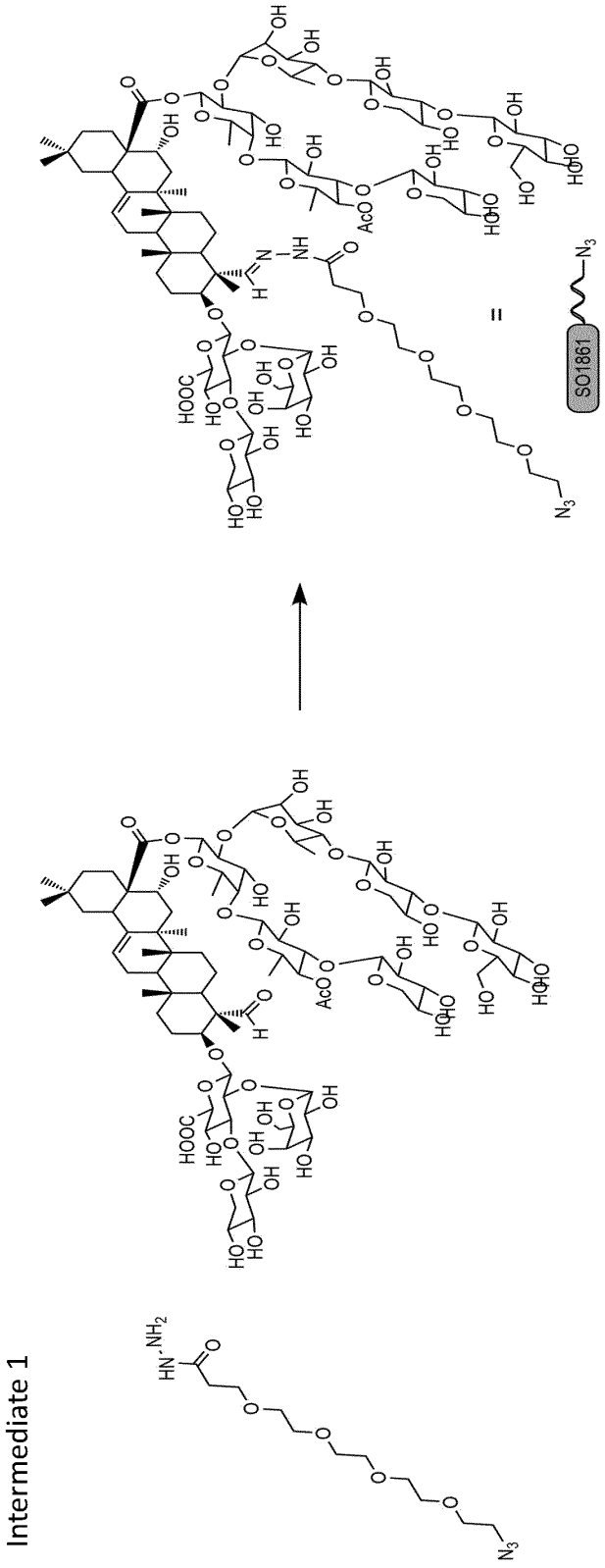


Figure 16

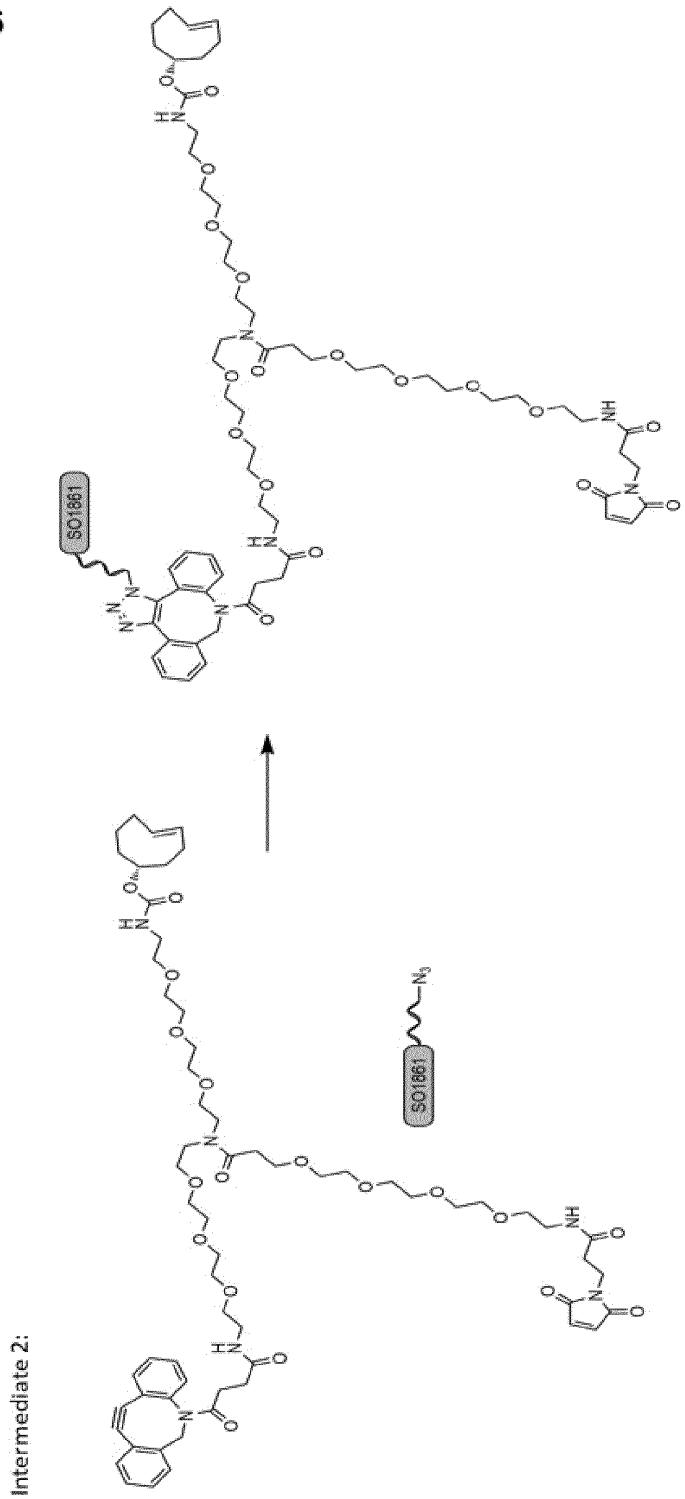


Figure 16

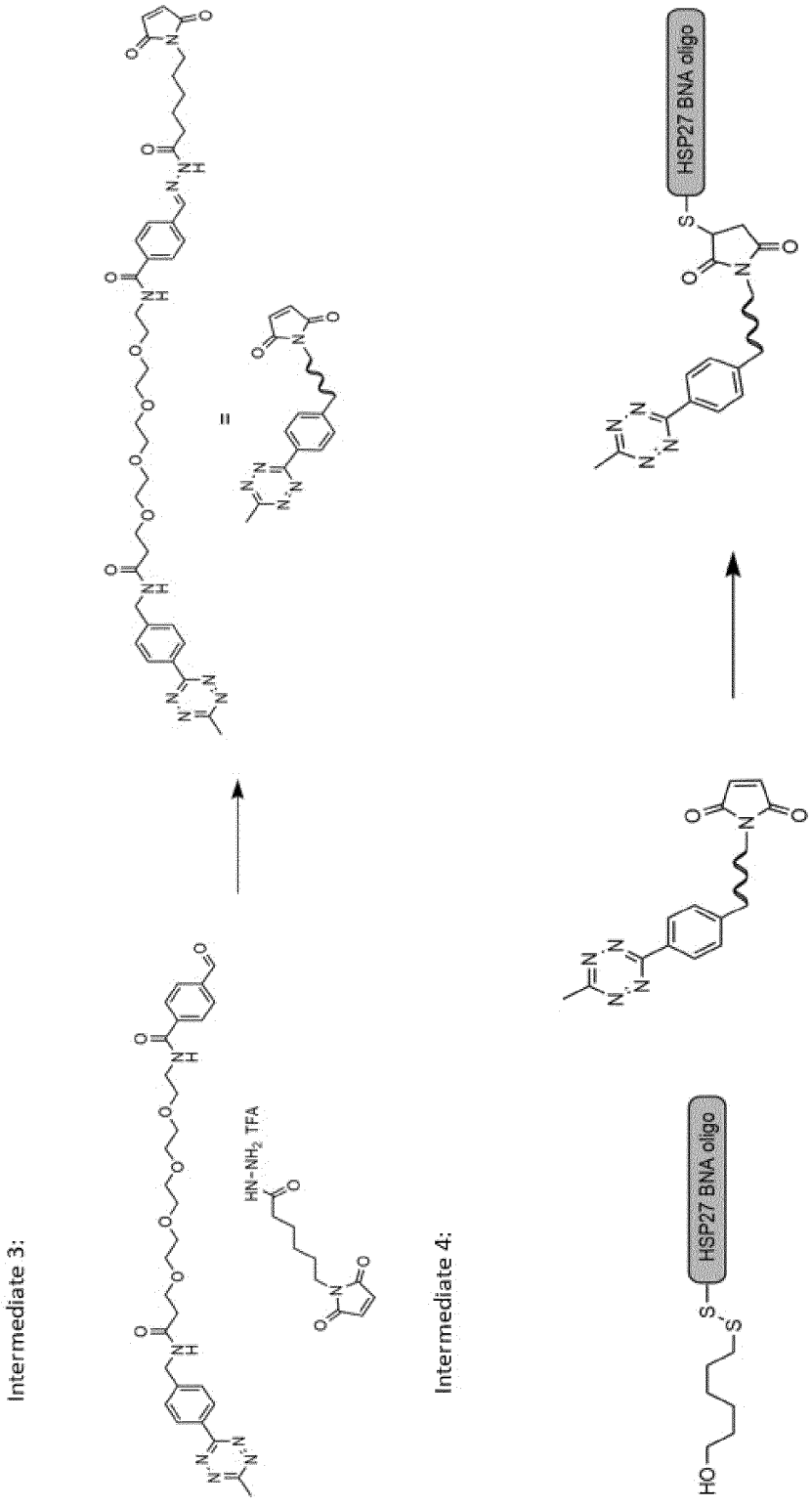
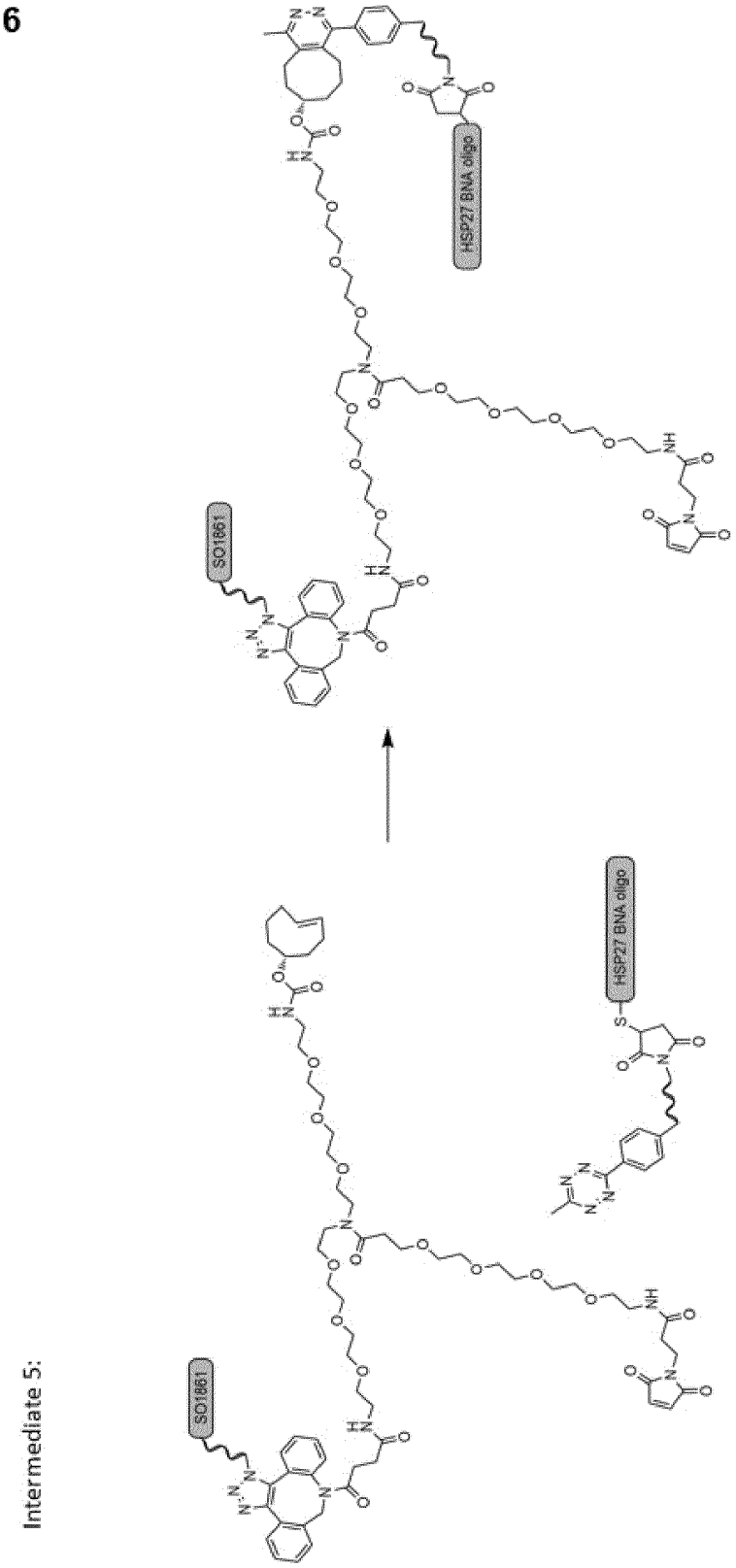

Intermediate 3:
HN-NH2 TFA
Intermediate 4:
HSP27 BNA oligo
HSP27 BNA oligo
HO

Figure 16



Intermediate 5:

Figure 17

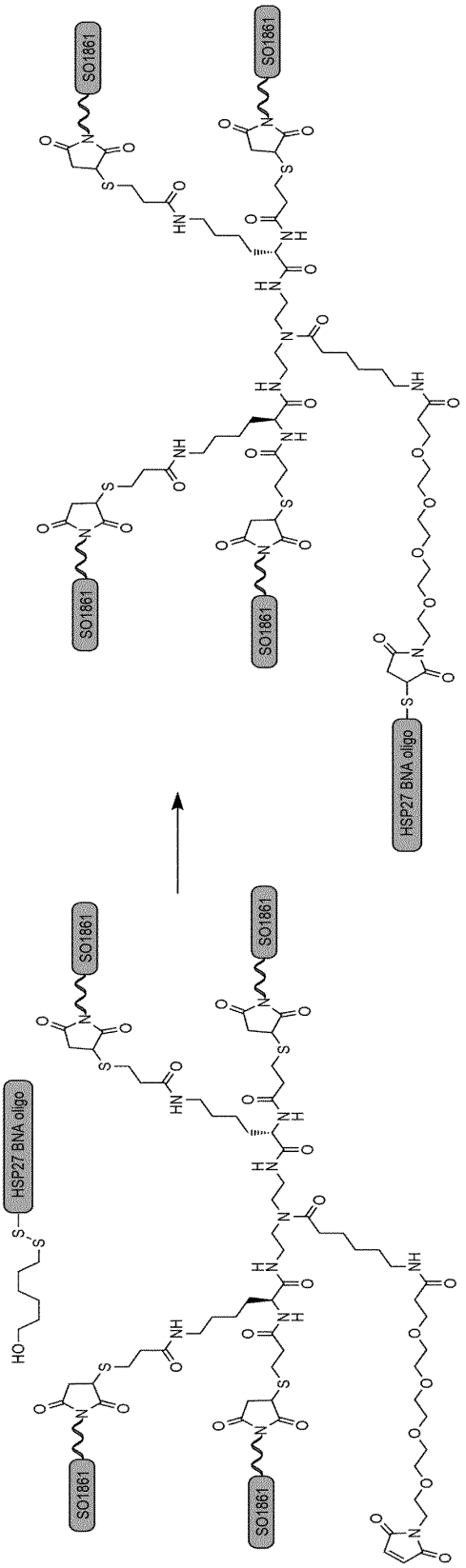


Figure 18

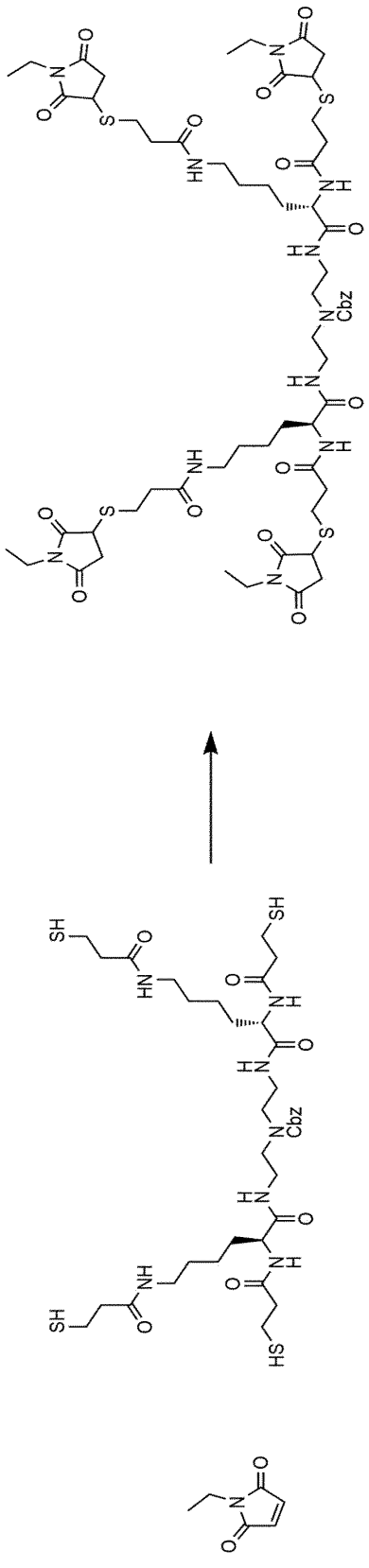


Figure 19

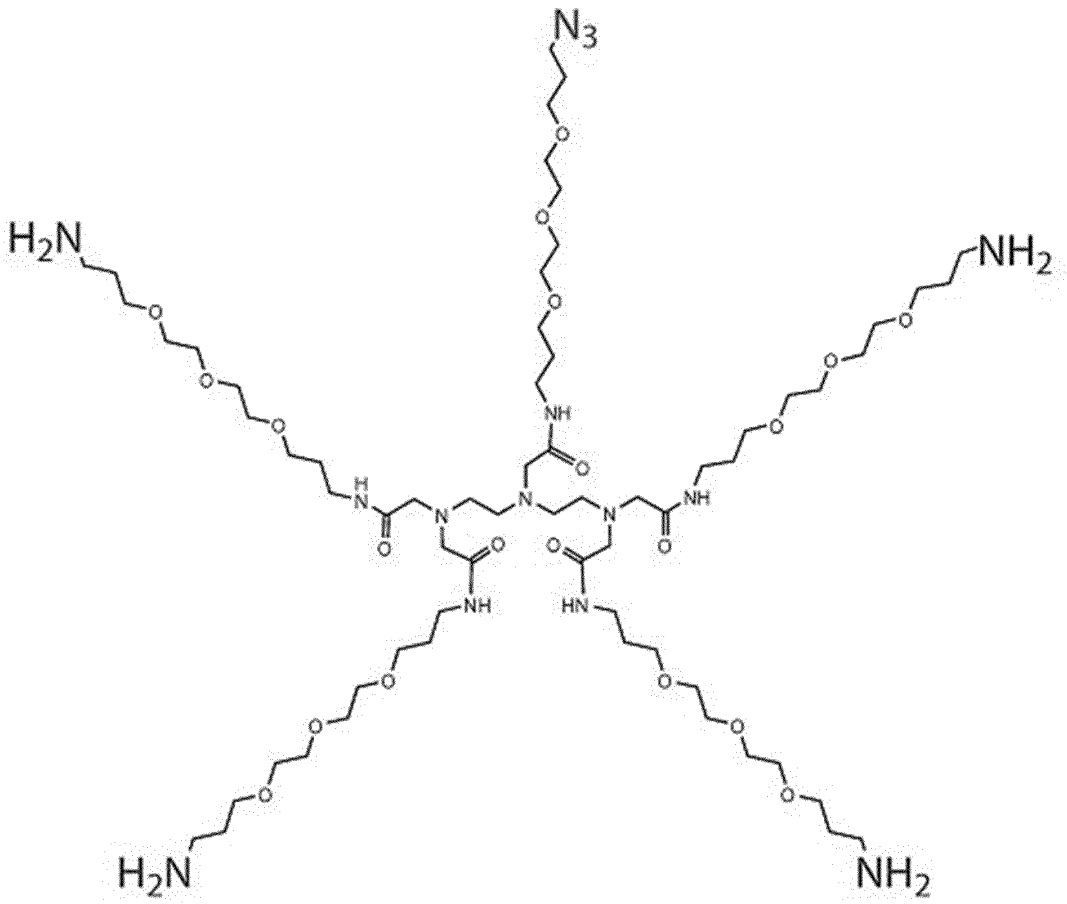


Figure 20

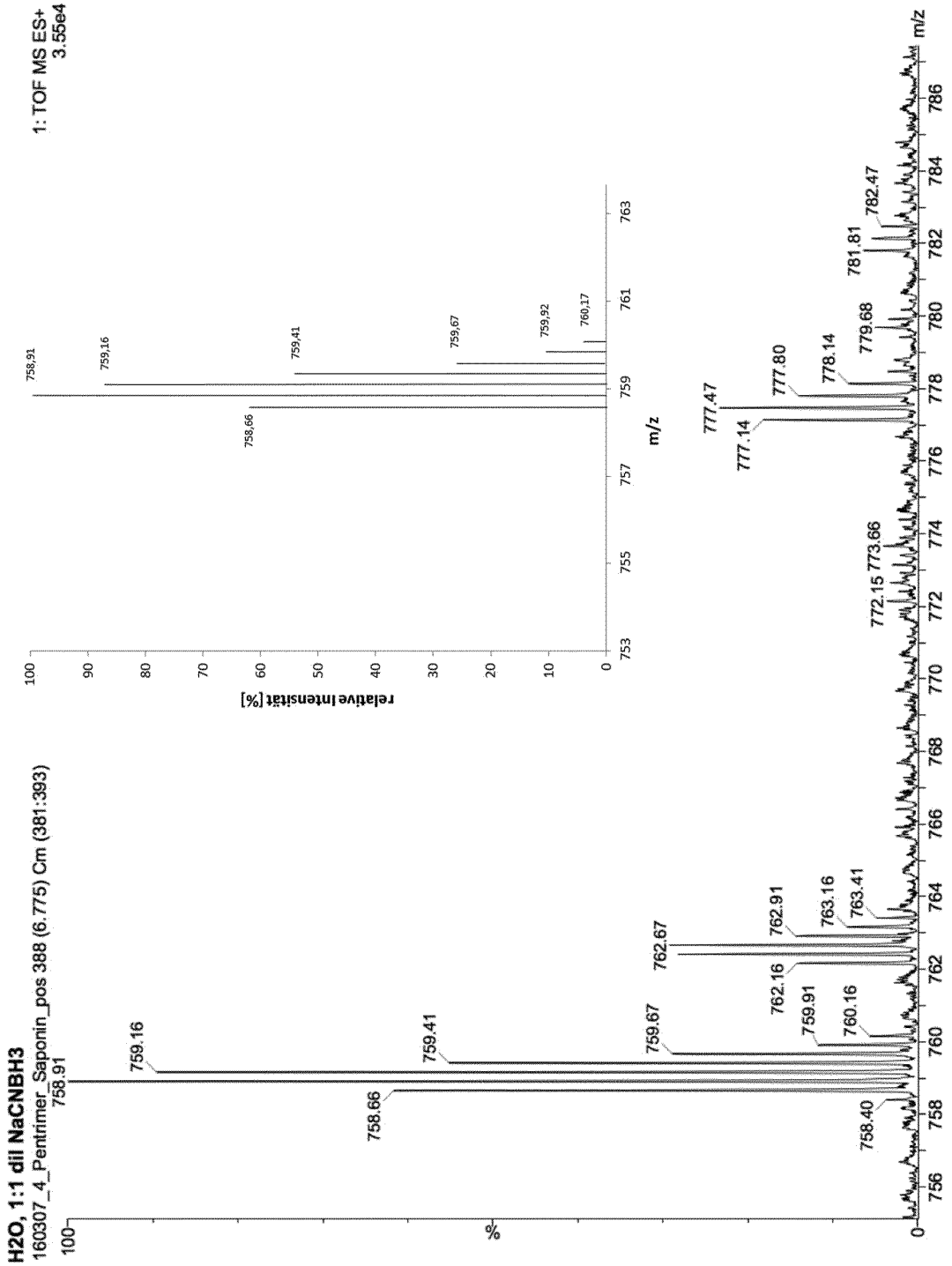

H2O, 1:1 dil NaCNBH3
160307_4_Pentrimer_Saponin_pos 388 (6.775) Cm (381:393)
1: TOF MS ES+
3.55e4
relative Intensität [%]
m/z
%
758.91
759.16
759.41
758.66
759.67
762.67
762.91
763.16
763.41
762.16
759.91
760.16
758.40
772.15
773.66
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777.14
777.80
778.14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

Figure 21

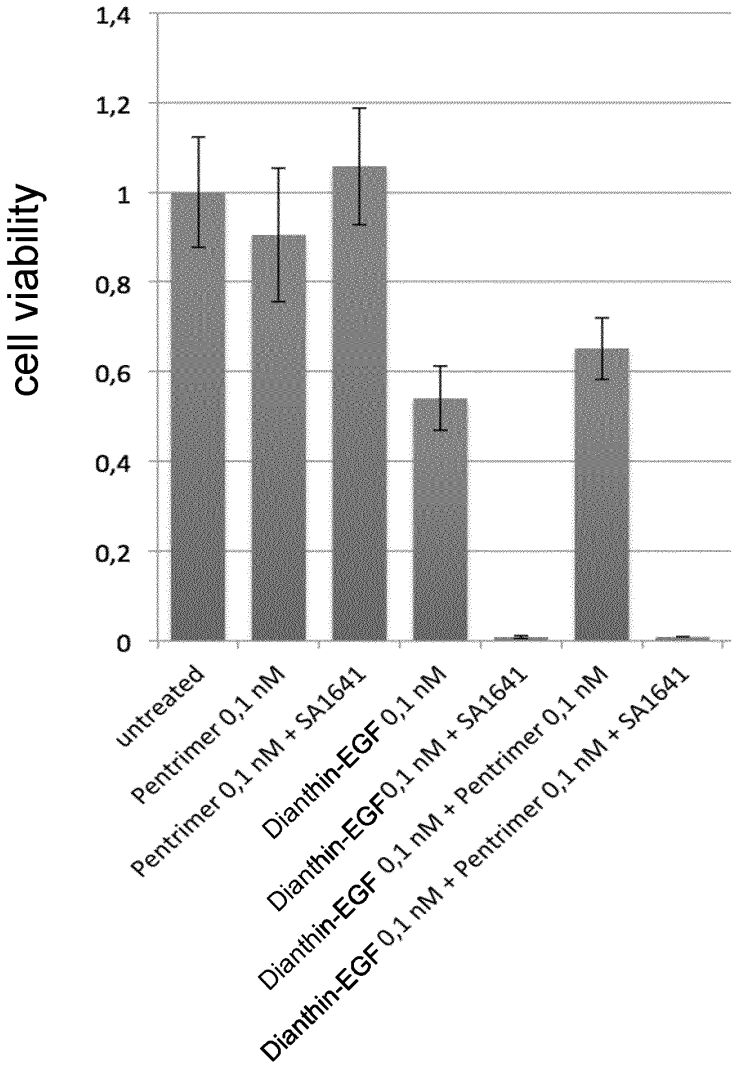

cell viability
1,4
1,2
1
0,8
0,6
0,4
0,2
0
untreated
Pentrimer 0,1 nM
Pentrimer 0,1 nM + SA1641
Dianthin-EGF 0,1 nM
Dianthin-EGF 0,1 nM + SA1641
Dianthin-EGF 0,1 nM + Pentrimer 0,1 nM
Dianthin-EGF 0,1 nM + Pentrimer 0,1 nM + SA1641

Figure 22

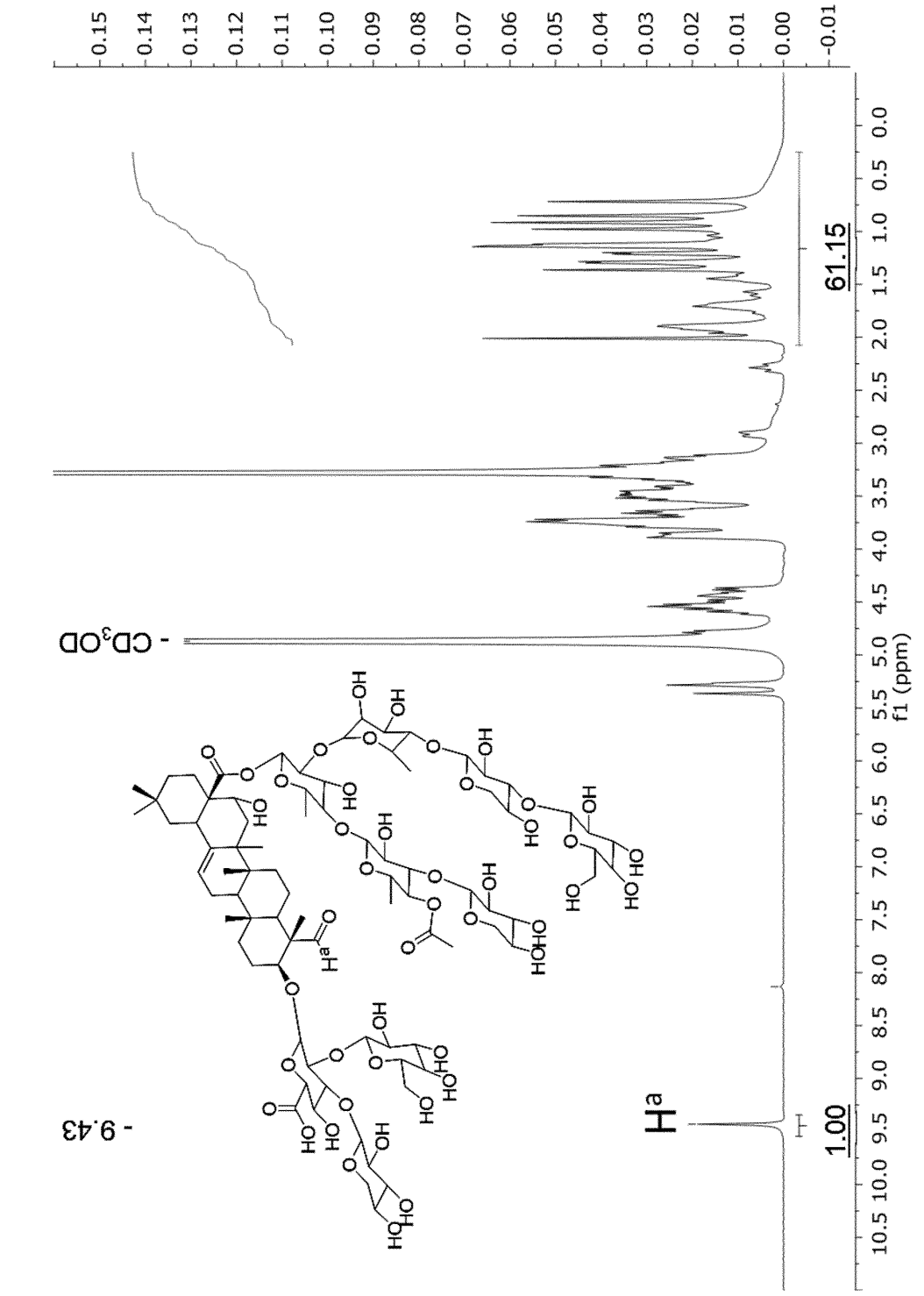
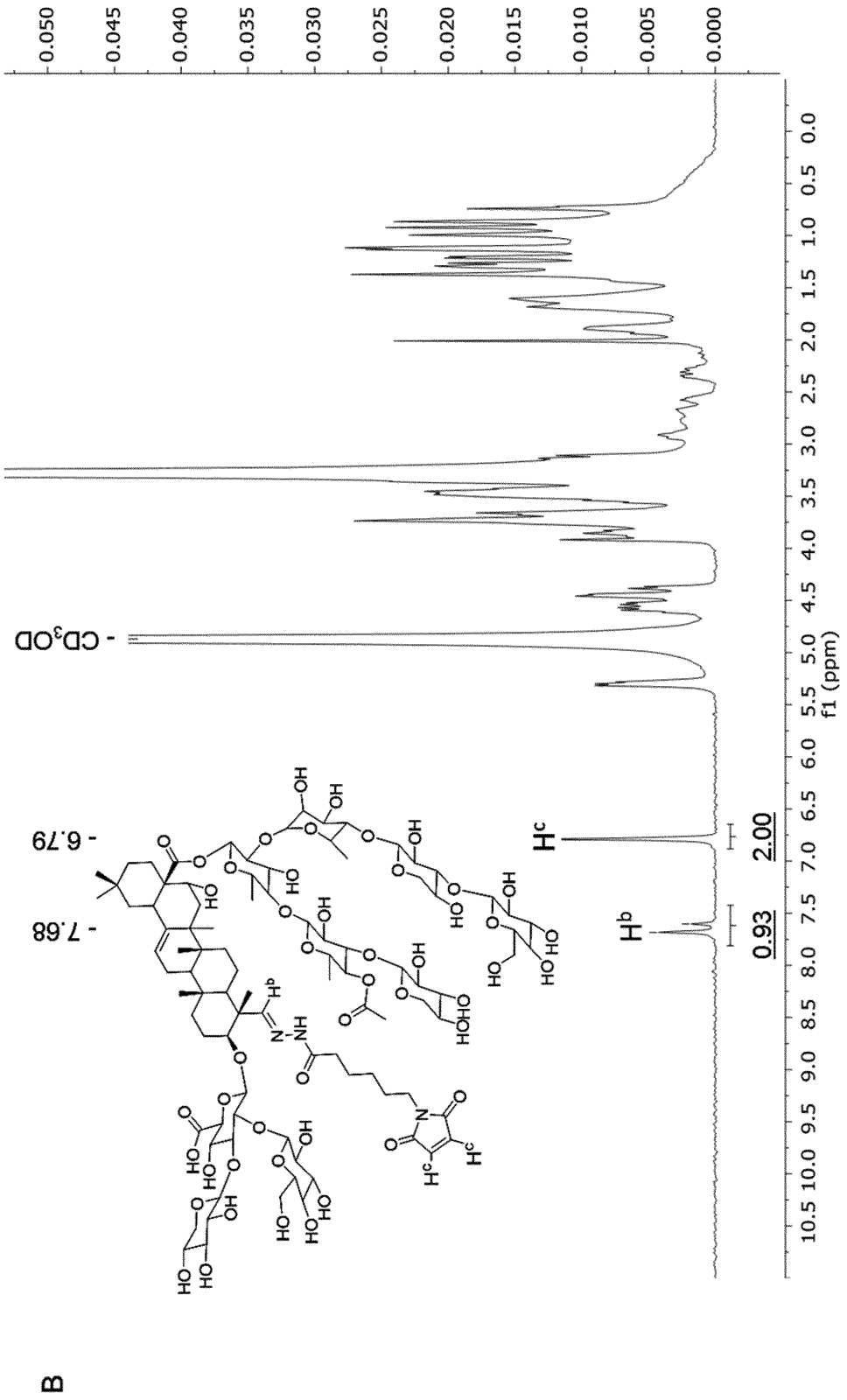


Figure 22


0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
10.5
f1 (ppm)
CD3OD
7.68
6.79
Hc
Hb
0.93
2.00
Hb
Hc
Hc

B

Figure 23

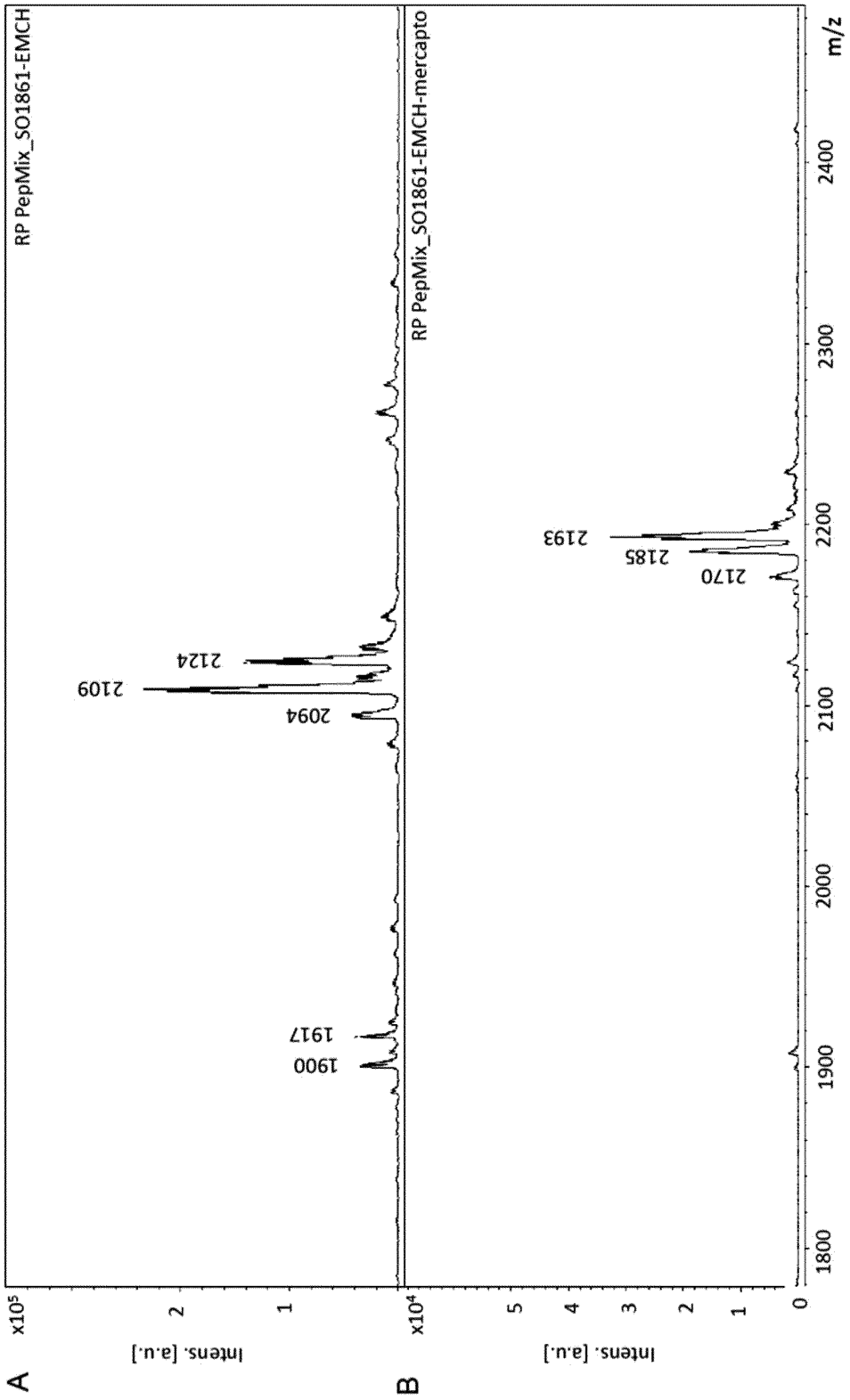

A
B
RP PepMix_SO1861-EMCH
RP PepMix_SO1861-EMCH-mercapto
Intens. [a.u.]
x10⁵
x10⁴
m/z
1800
1900
2000
2100
2200
2300
2400
1900
1917
2094
2109
2124
2170
2185
2193

Figure 24

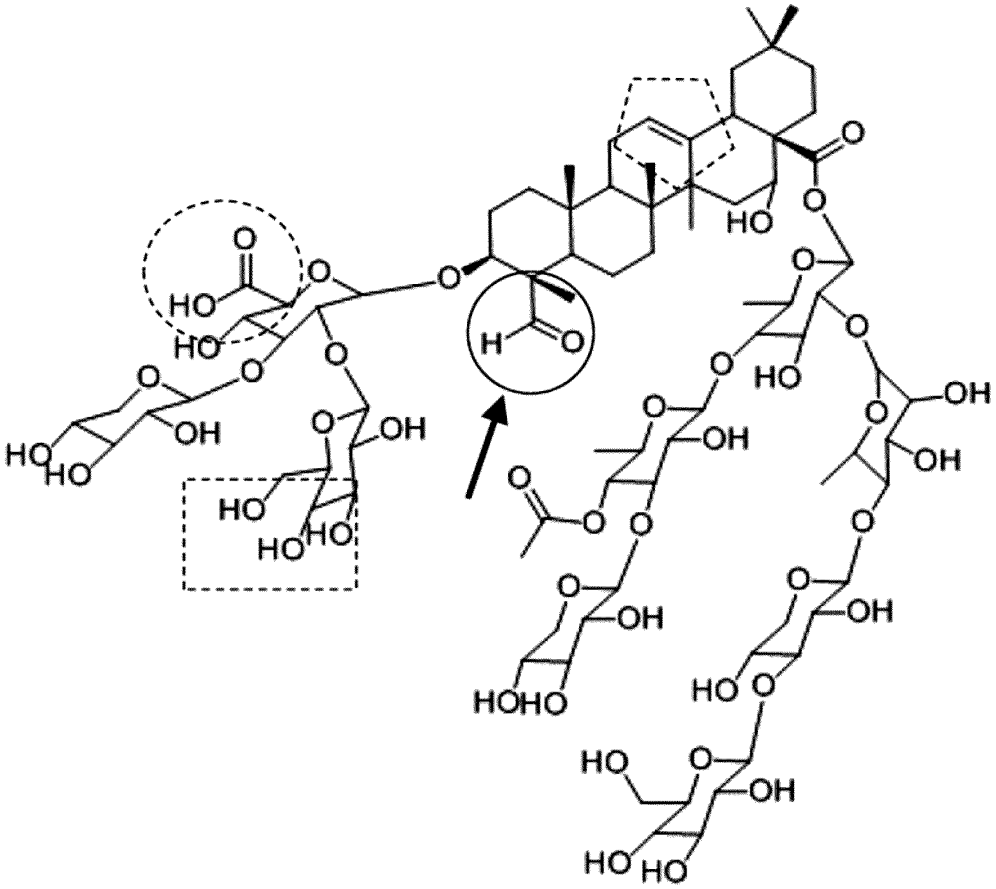


Figure 25

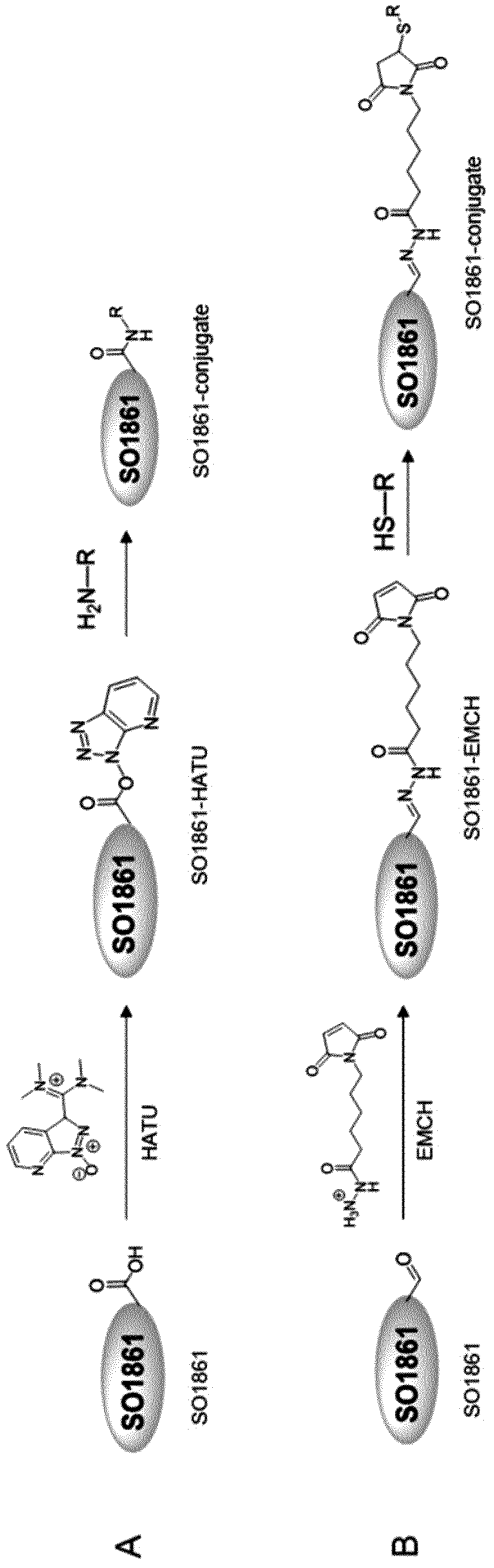

A
SO1861
HATU
SO1861-HATU
H_2N—R
SO1861-conjugate
B
SO1861
EMCH
SO1861-EMCH
HS—R
SO1861-conjugate

Figure 26

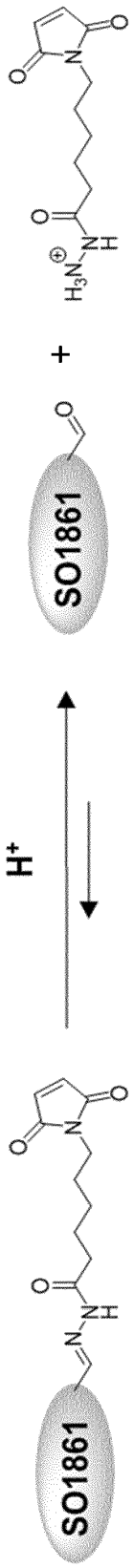
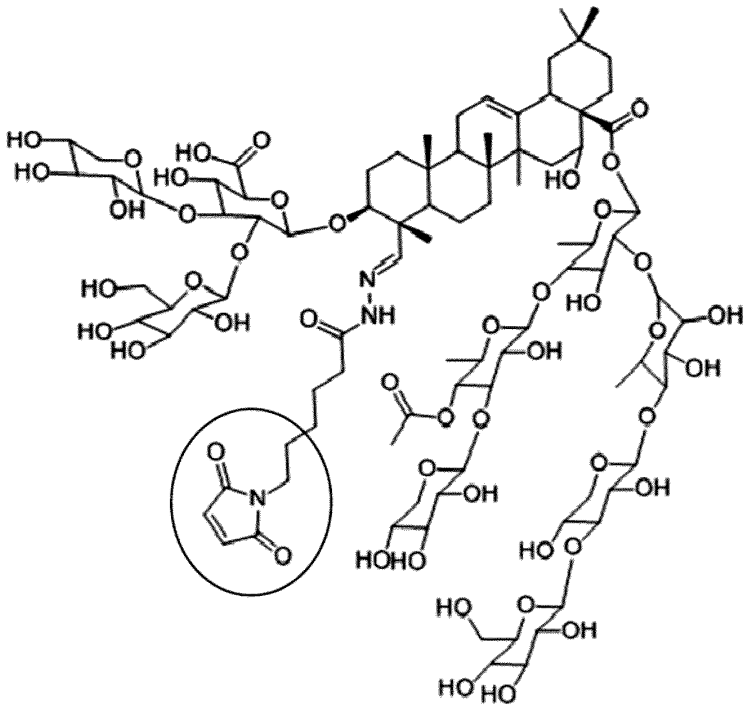


Figure 27

A



B

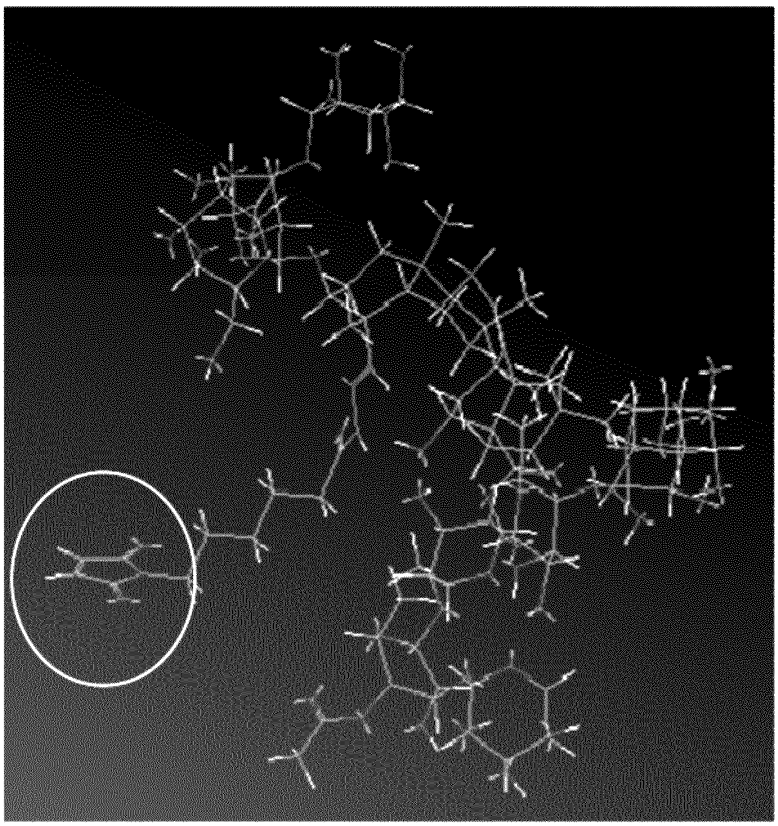


Figure 28

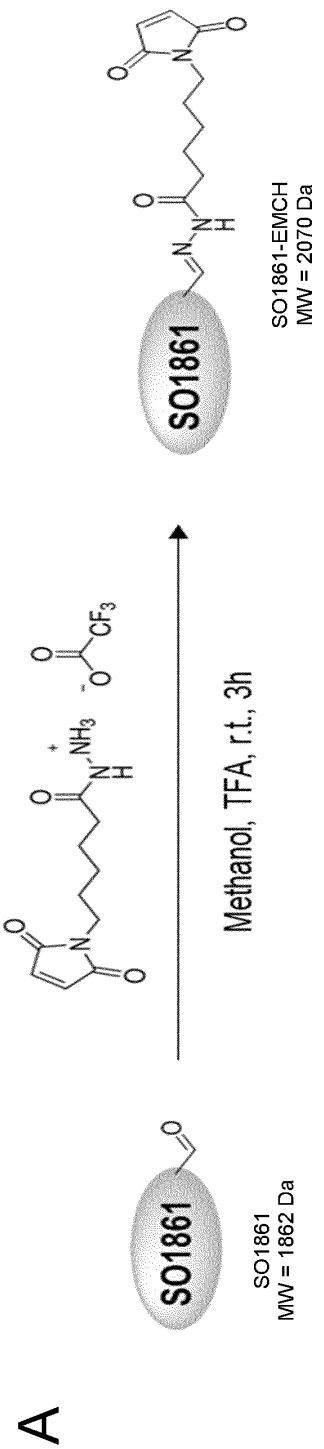

A
SO1861
SO1861
MW = 1862 Da
Methanol, TFA, r.t., 3h
SO1861
SO1861-EMCH
MW = 2070 Da

Figure 28

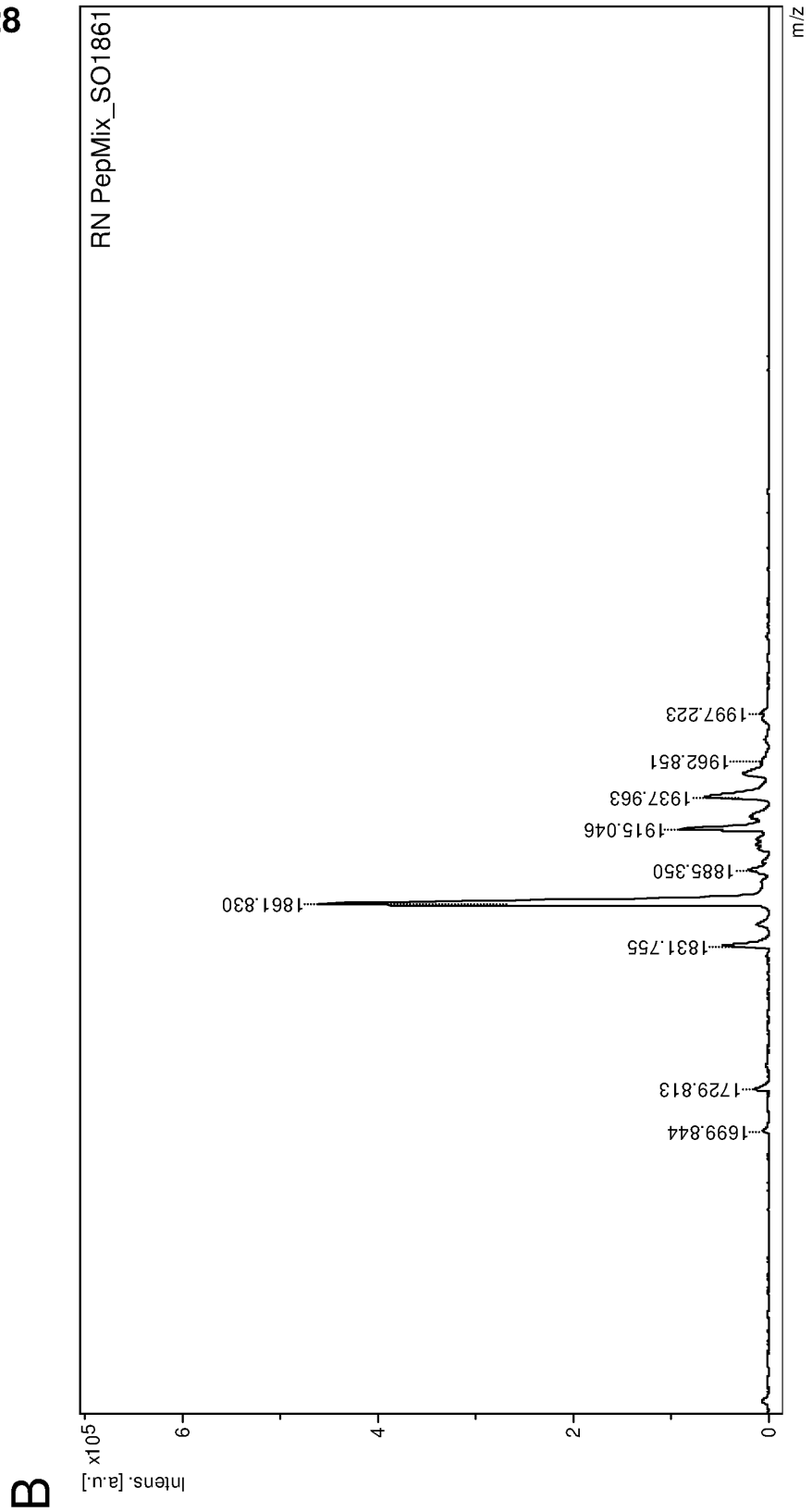

B
RN PepMix_SO1861
Intens. [a.u.]
x10^5
6
4
2
0
m/z
1997.223
1962.851
1937.963
1915.046
1885.350
1861.830
1831.755
1729.813
1699.844

Figure 28

C

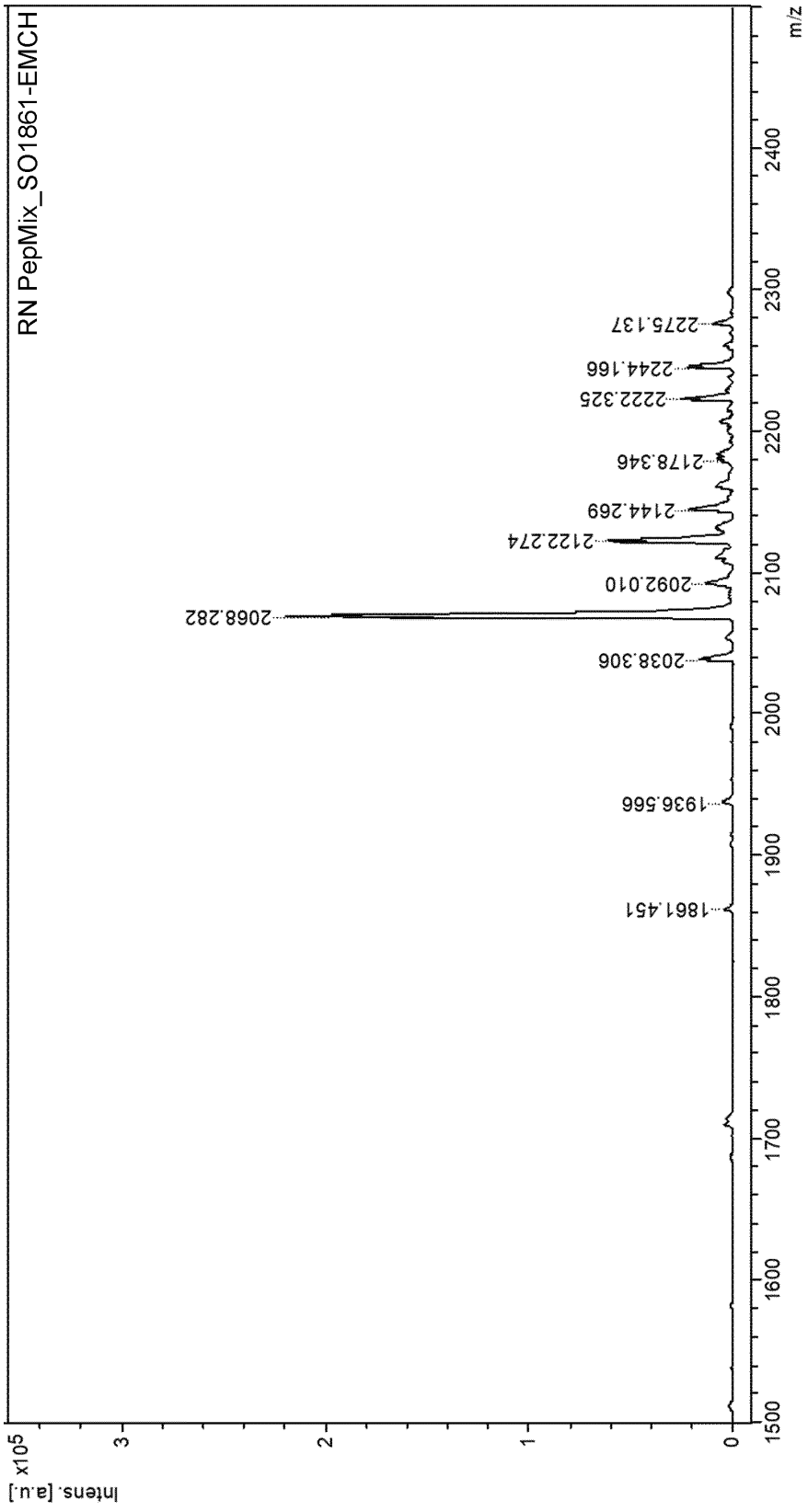

RN PepMix_SO1861-EMCH
Intens. [a.u.]
x10^5
3
2
1
0
1500
1600
1700
1800
1900
2000
2100
2200
2300
2400
m/z
1861.451
1936.566
2038.306
2068.282
2092.010
2122.274
2144.269
2178.346
2222.325
2244.166
2275.137

Figure 29

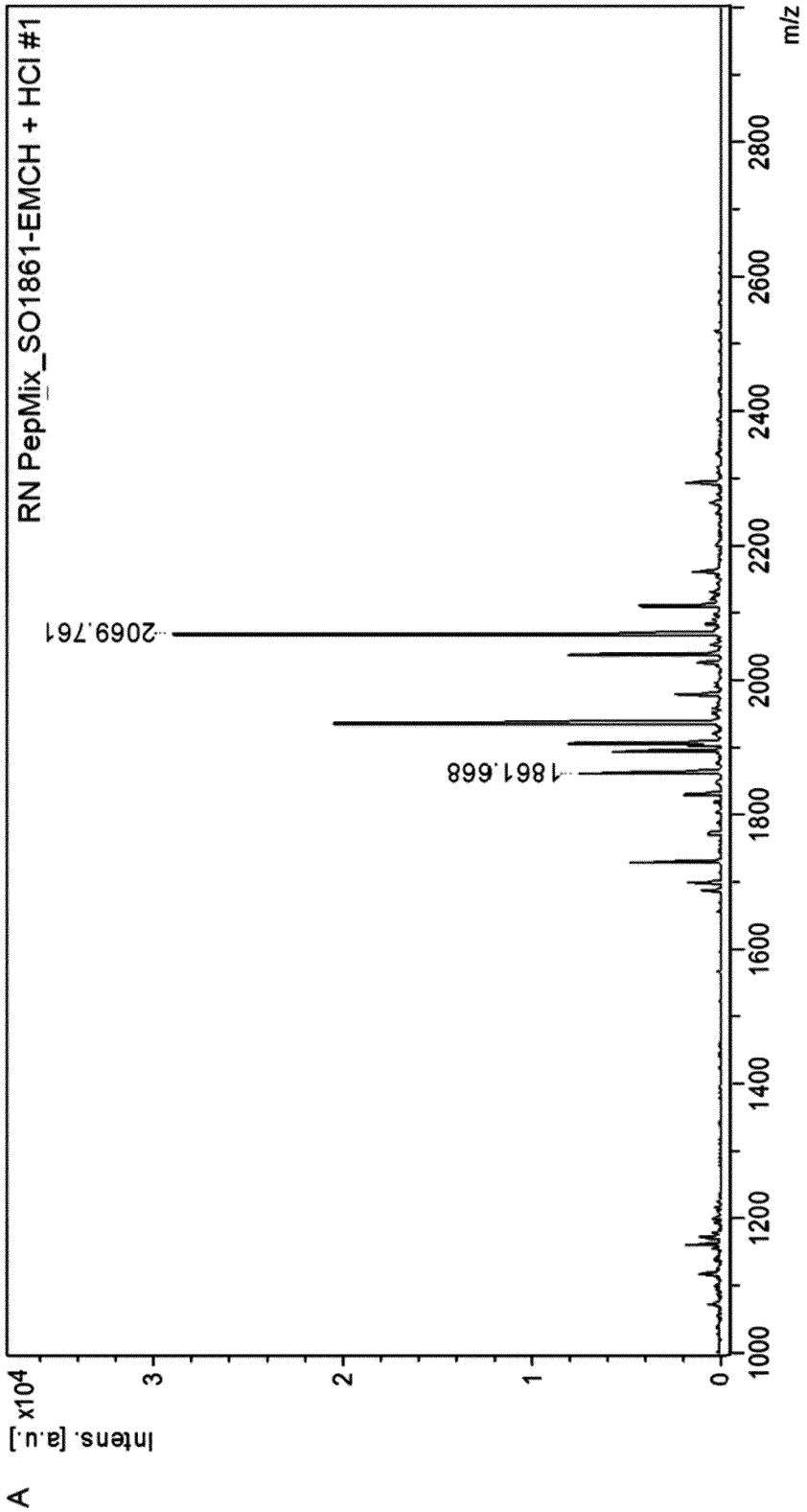

A
Intens. [a.u.]
x10^4
3
2
1
0
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
m/z
RN PepMix_SO1861-EMCH + HCl #1
2069.761
1861.668

Figure 29

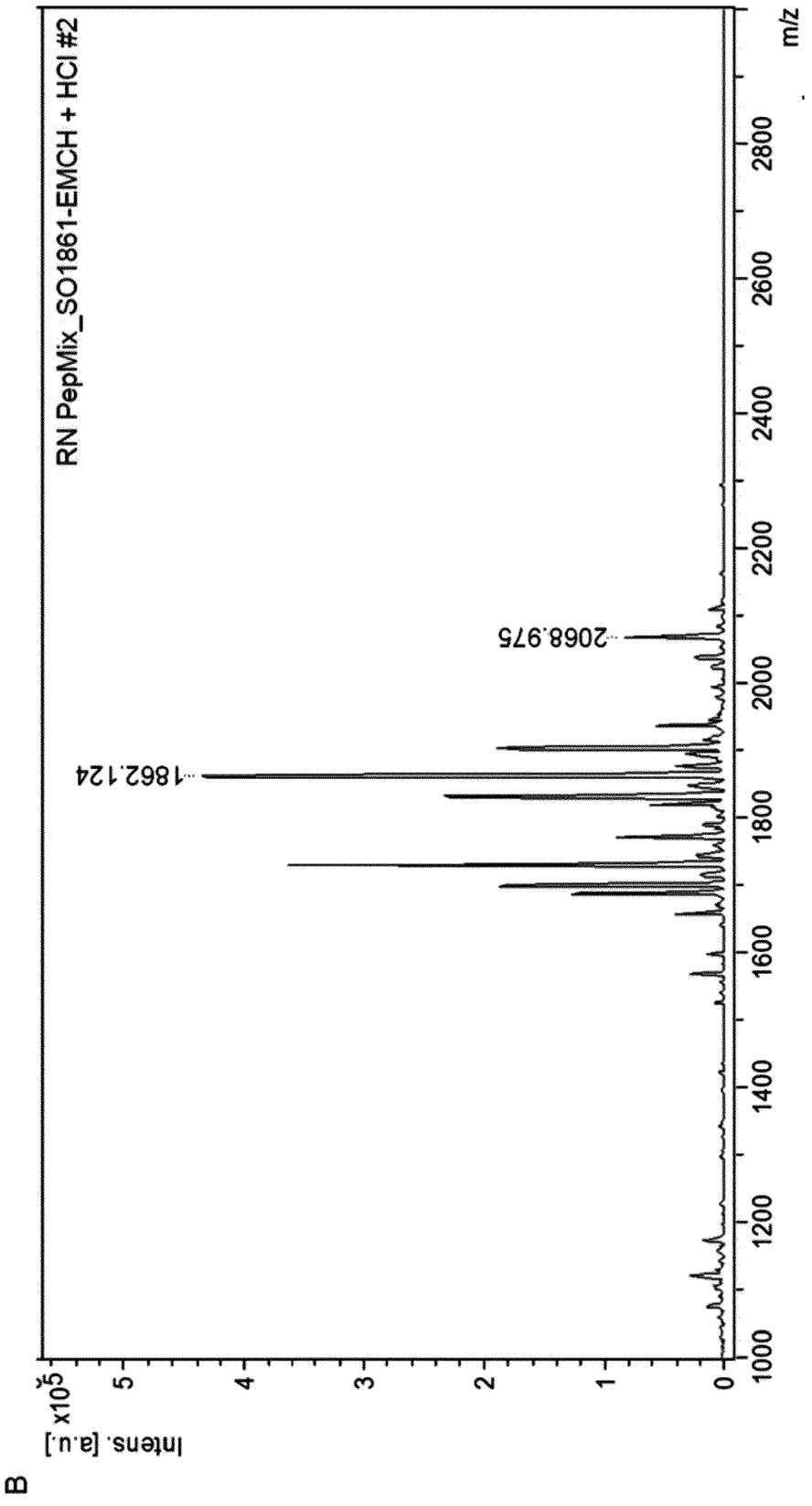

B
RN PepMix_SO1861-EMCH + HCl #2
Intens. [a.u.]
x10^5
m/z
1862.124
2068.975

Figure 30

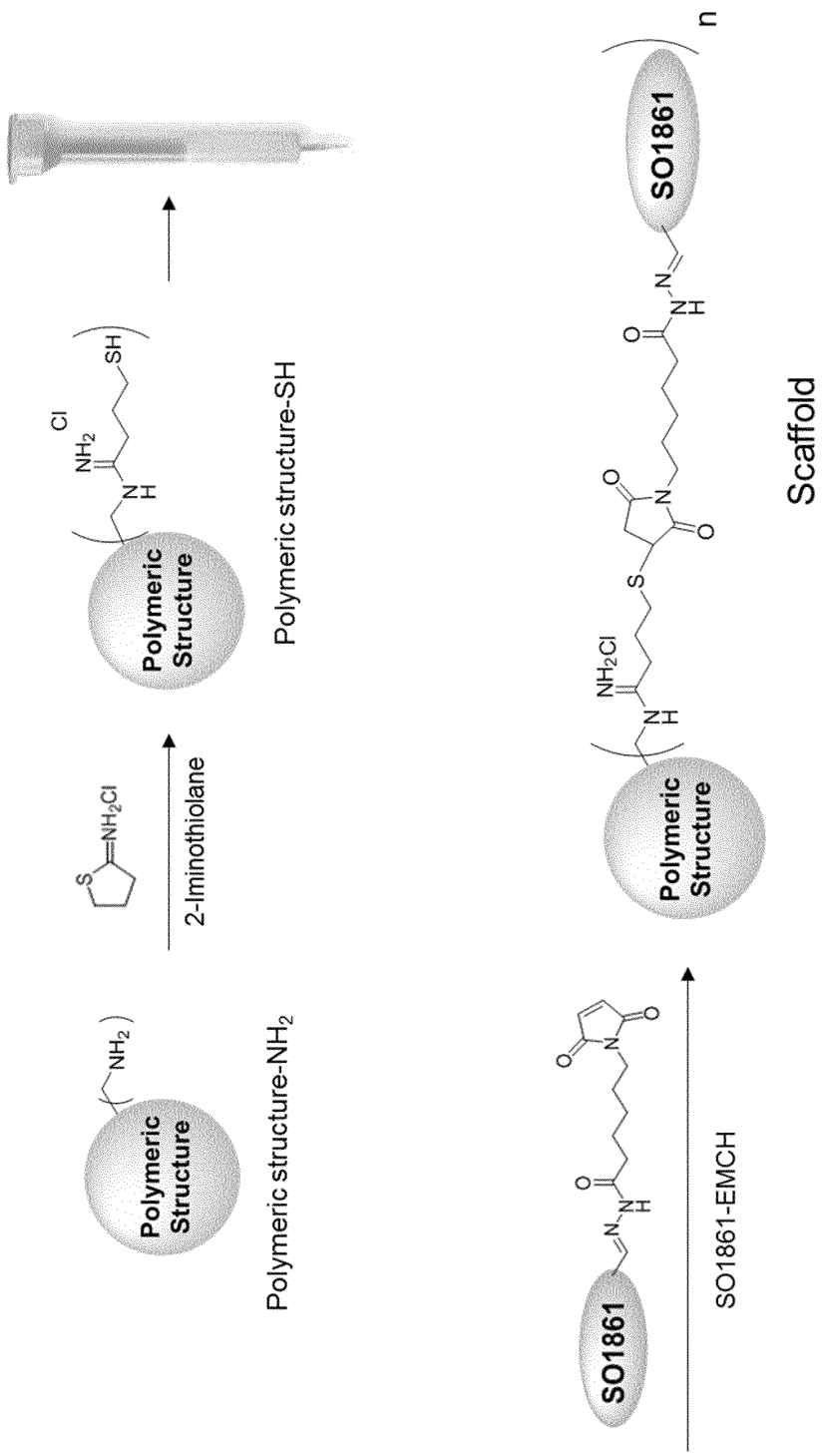

Polymeric structure-NH2
2-Iminothiolane
Polymeric structure-SH
SO1861-EMCH
Scaffold
Polymeric Structure
SO1861
n

Figure 31

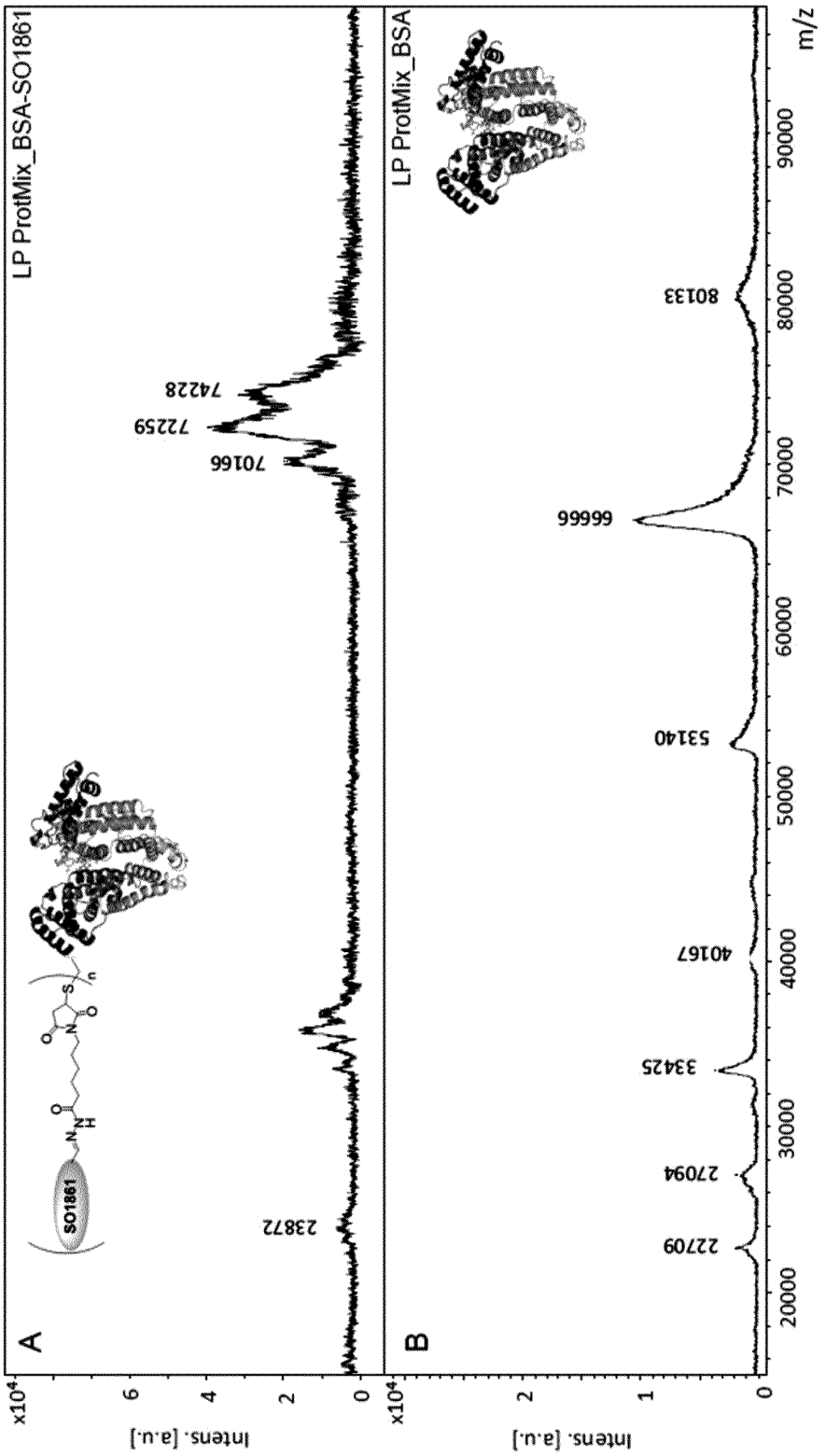

LP ProtMix_BSA-SO1861
A
SO1861
23872
70166
72259
74228
Intens. [a.u.]
x10⁴
LP ProtMix_BSA
B
22709
27094
33425
40167
53140
66666
80133
m/z
Intens. [a.u.]
x10⁴

Figure 32

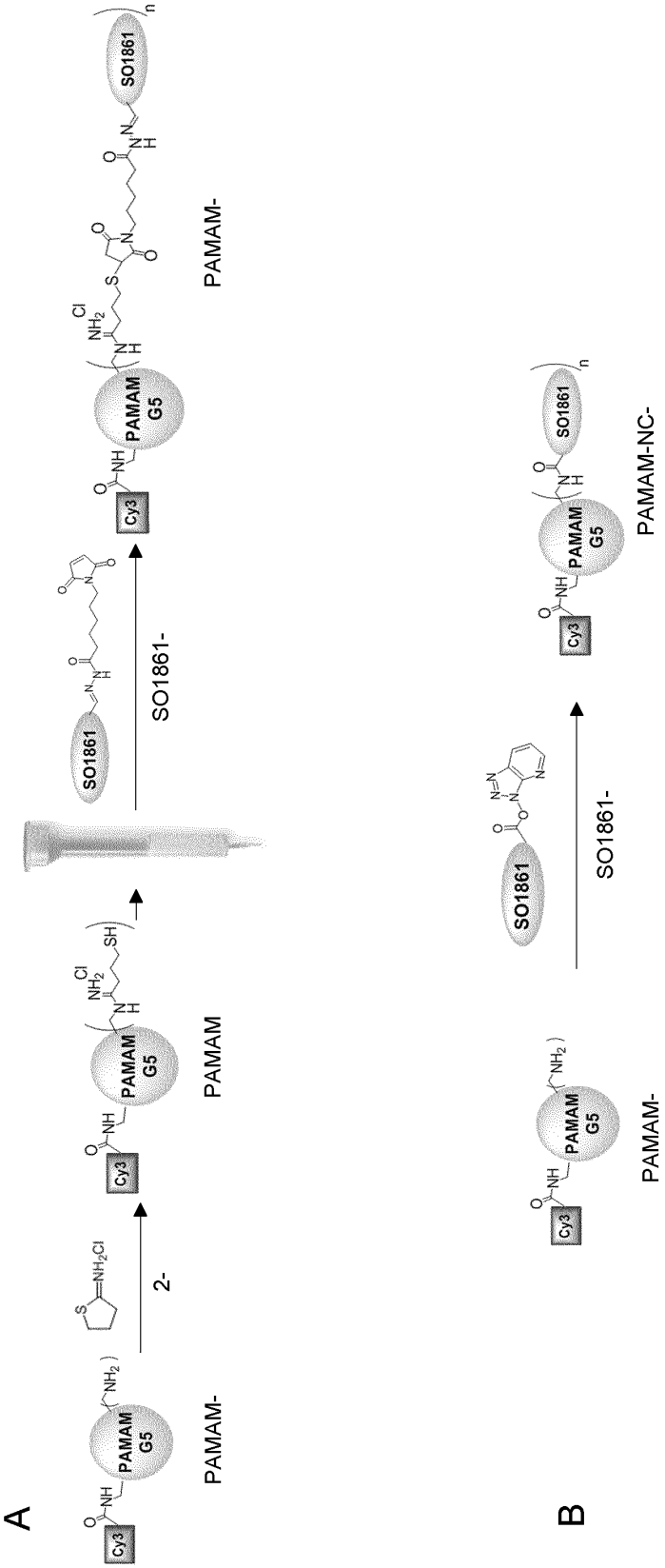

A
PAMAM-
PAMAM G5
Cy3
2-
PAMAM
SO1861-
SO1861
PAMAM-
B
SO1861
SO1861-
PAMAM-NC-

Figure 33

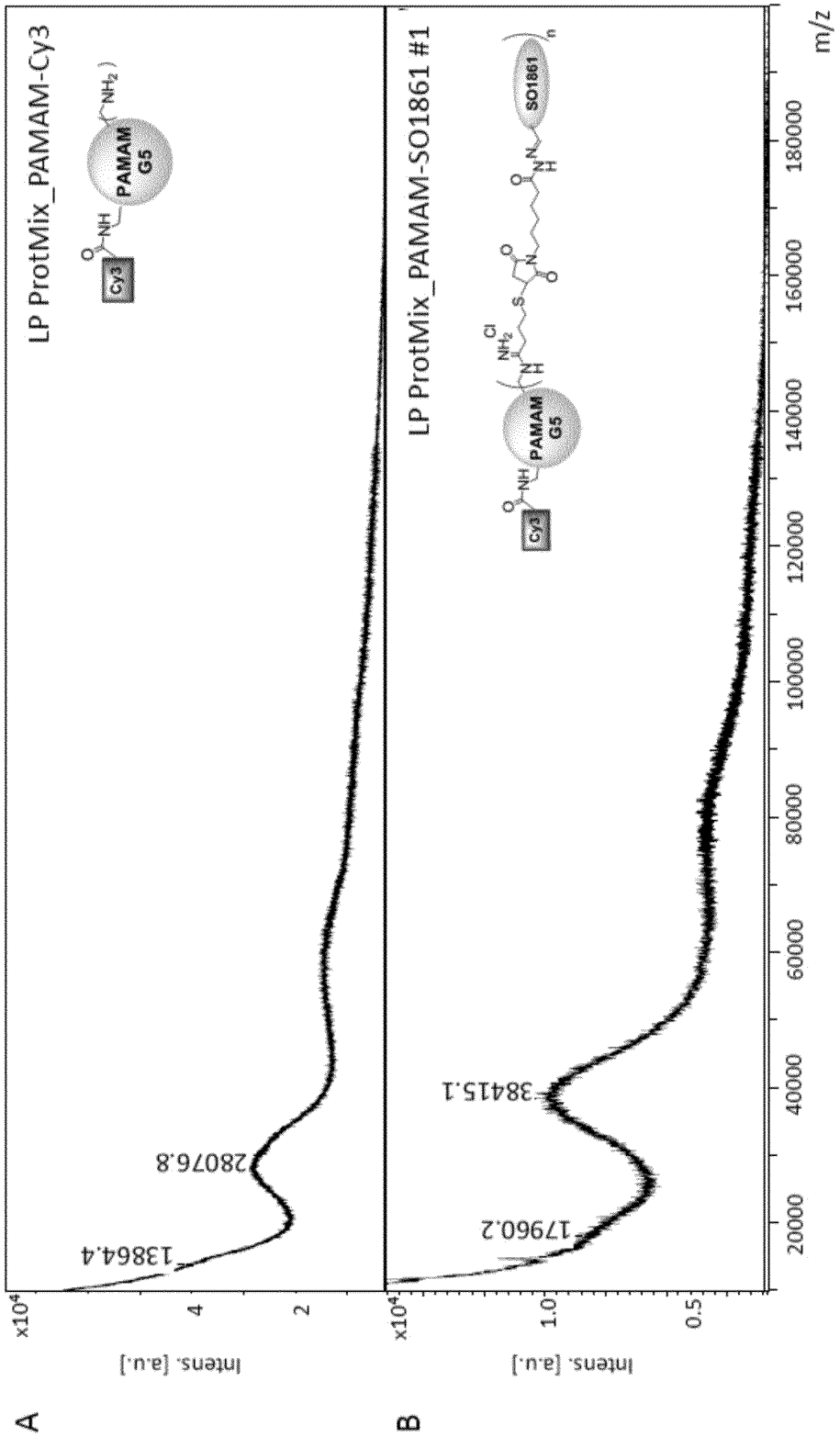

A
B
LP ProtMix_PAMAM-Cy3
LP ProtMix_PAMAM-SO1861 #1
Intens. [a.u.]
x10⁴
m/z
13864.4
28076.8
17960.2
38415.1
PAMAM G5
Cy3
SO1861

Figure 33

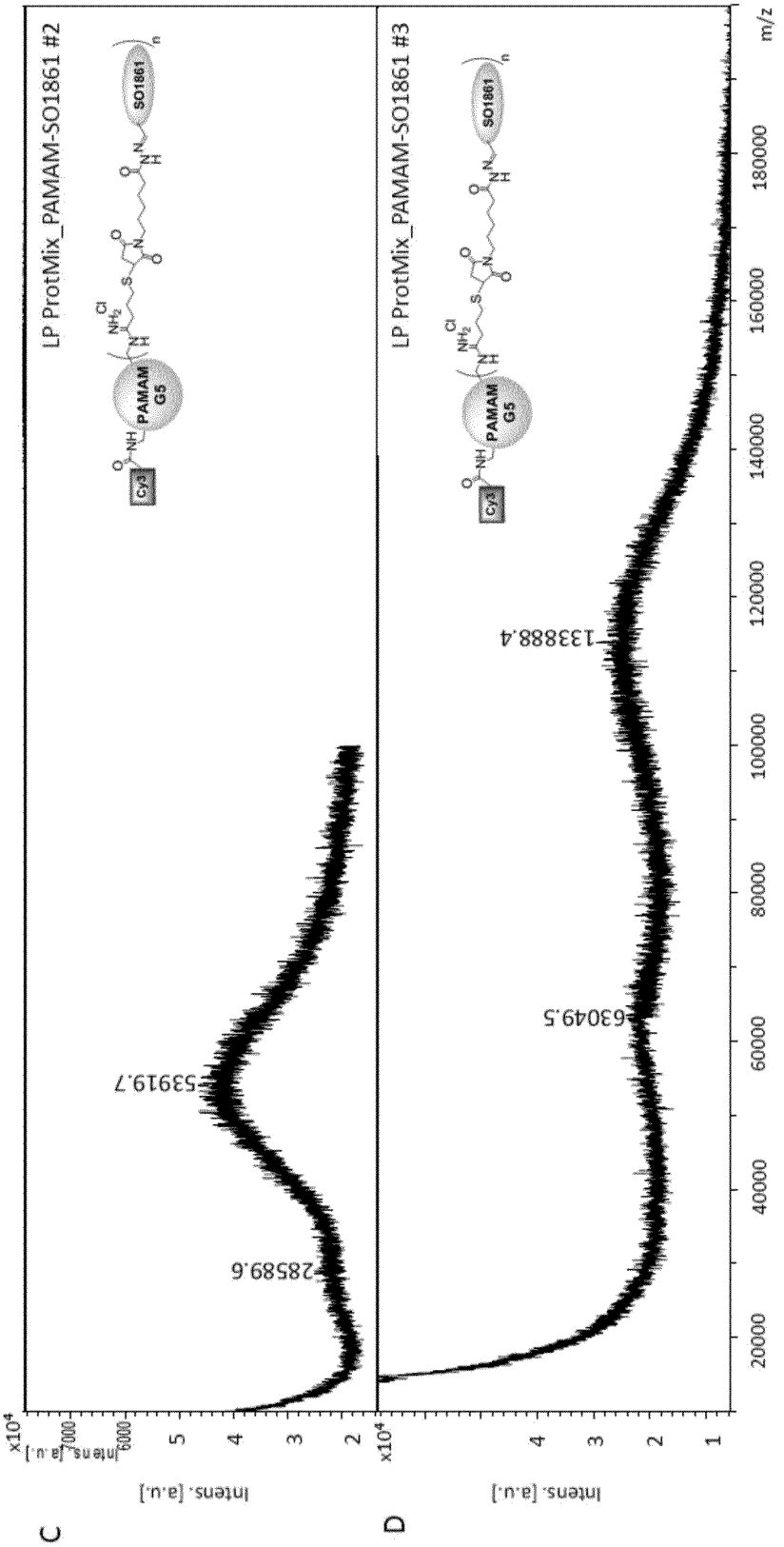

LP ProtMix_PAMAM-SO1861 #2
LP ProtMix_PAMAM-SO1861 #3
PAMAM G5
SO1861
Cy3
285589.6
539197
630495
1338884
Intens. [a.u.]
m/z
C
D

Figure 34

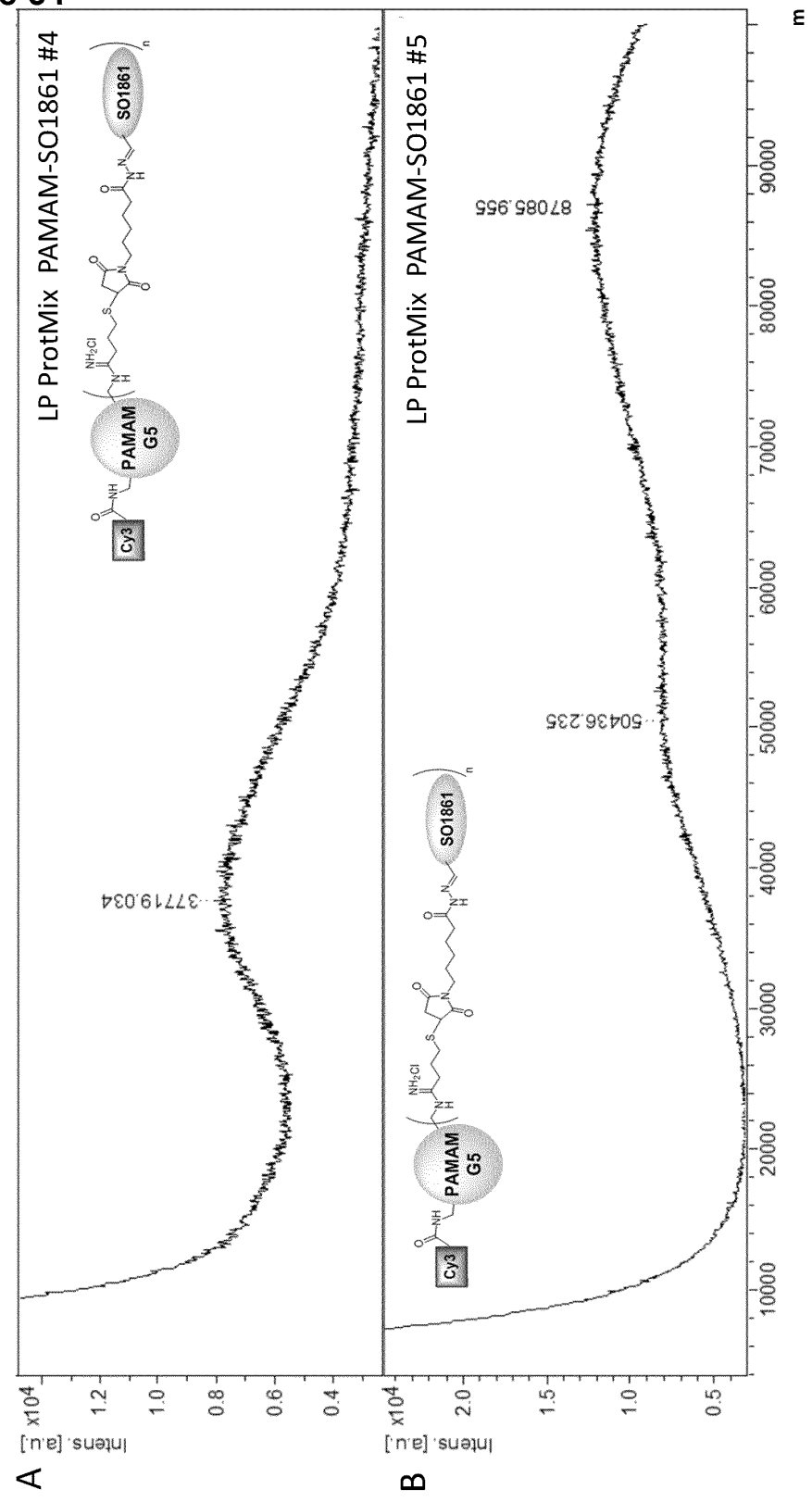

A
LP ProtMix PAMAM-SO1861 #4
Intens. [a.u.]
x10^4
1.2
1.0
0.8
0.6
0.4
37719.034
PAMAM G5
Cy3
SO1861
B
LP ProtMix PAMAM-SO1861 #5
Intens. [a.u.]
x10^4
2.0
1.5
1.0
0.5
87085.955
50436.235
10000
20000
30000
40000
50000
60000
70000
80000
90000
m

Figure 35

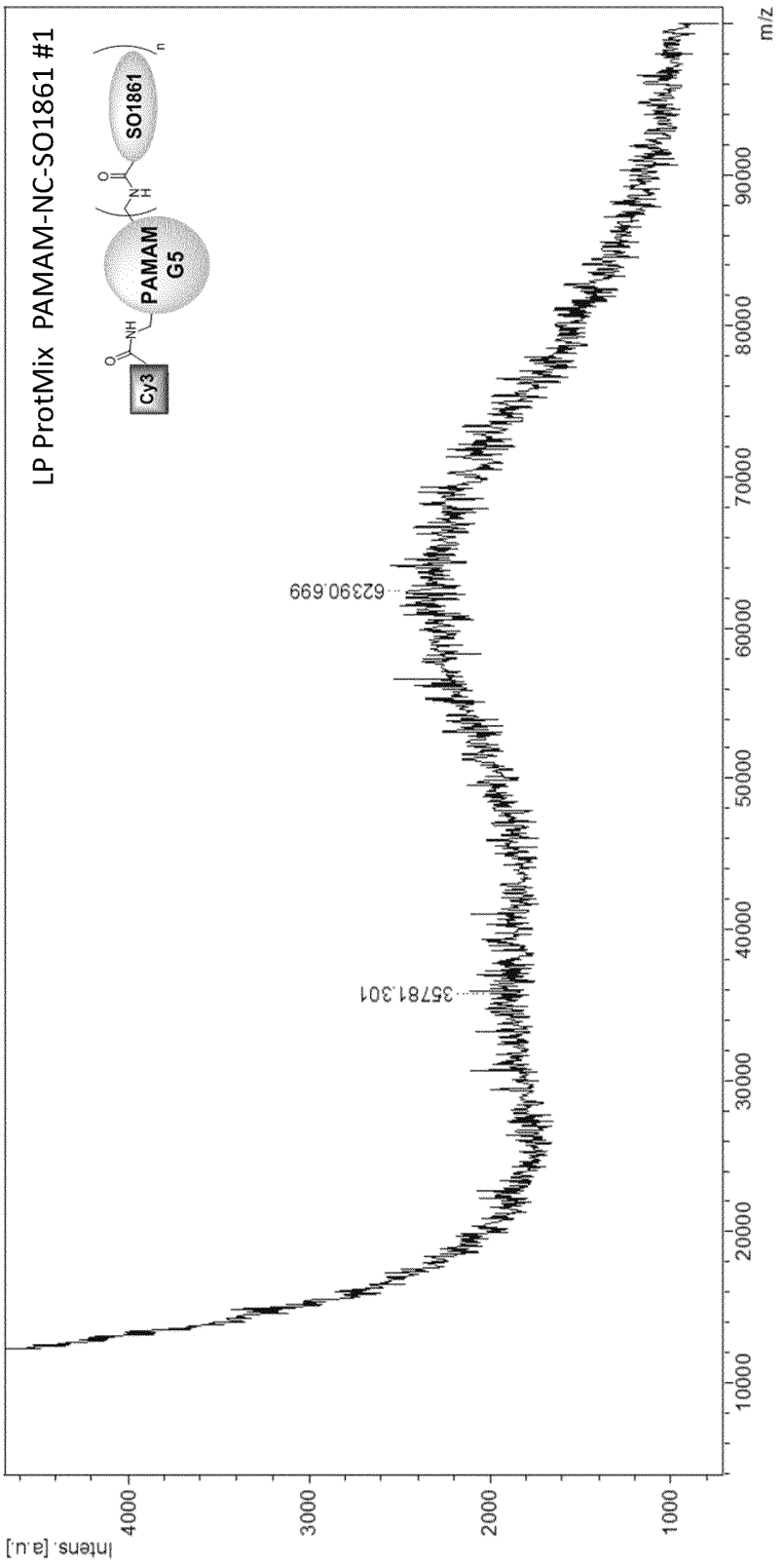
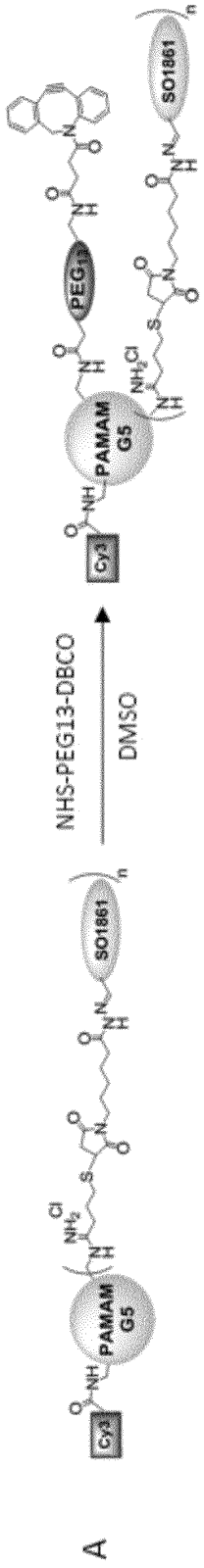

LP ProtMix PAMAM-NC-SO1861 #1
SO1861
PAMAM G5
Cy3
62390.699
35781.301
Intens. [a.u.]
4000
3000
2000
1000
m/z
10000
20000
30000
40000
50000
60000
70000
80000
90000

Figure 36


SO1861
PEG13
PAMAM G5
Cy3
NHS-PEG13-DBCO
DMSO
SO1861
PAMAM G5
Cy3

A

Figure 36

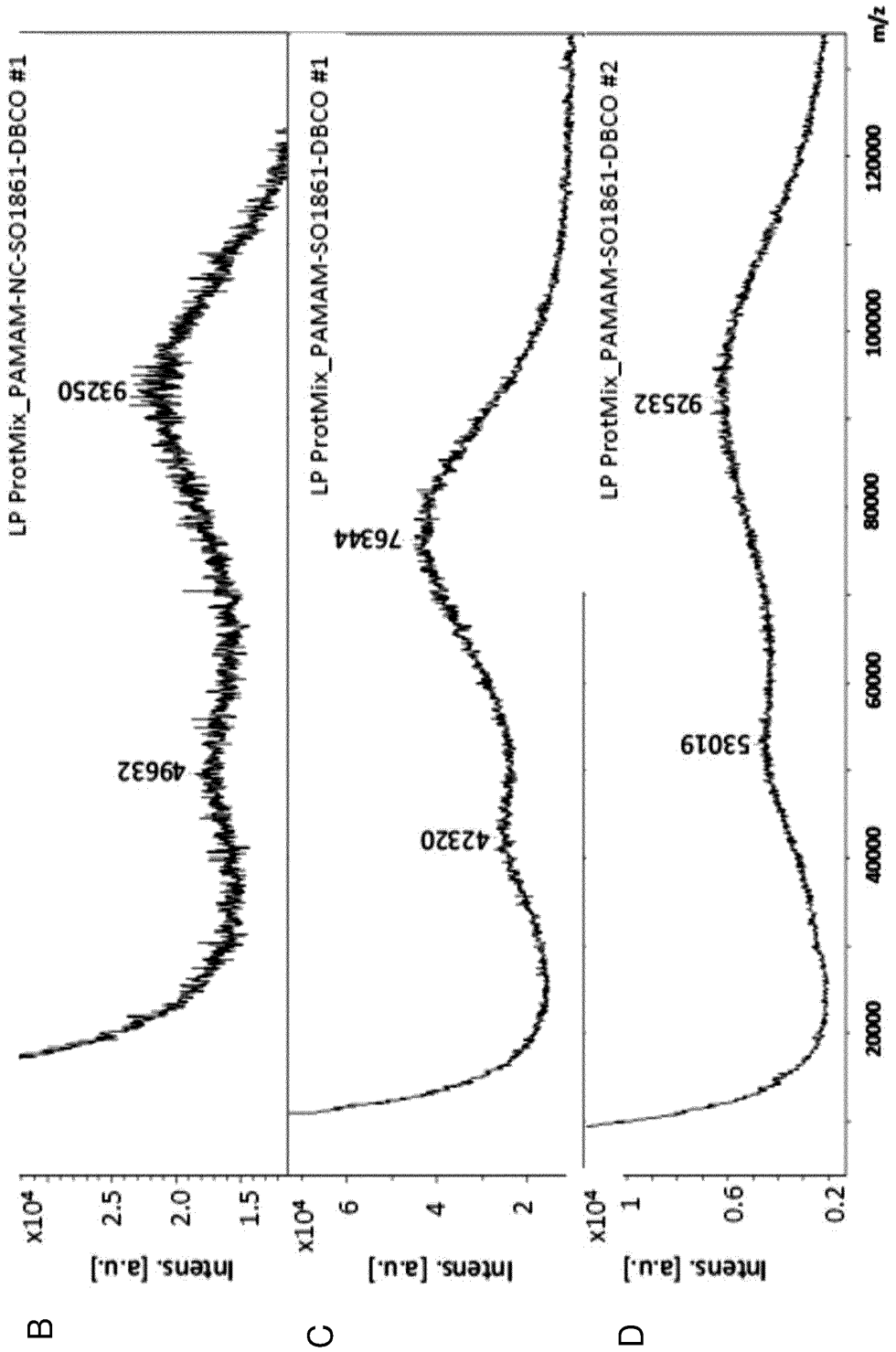

B
LP ProtMix_PAMAM-NC-SO1861-DBCO #1
9325O
4963 2
Intens. [a.u.]
x10⁴
2.5
2.0
1.5
C
LP ProtMix_PAMAM-SO1861-DBCO #1
7634 4
4232O
Intens. [a.u.]
x10⁴
6
4
2
D
LP ProtMix_PAMAM-SO1861-DBCO #2
9253 2
5301 9
Intens. [a.u.]
x10⁴
1
0.6
0.2
20000
40000
60000
80000
100000
120000
m/z

Figure 37

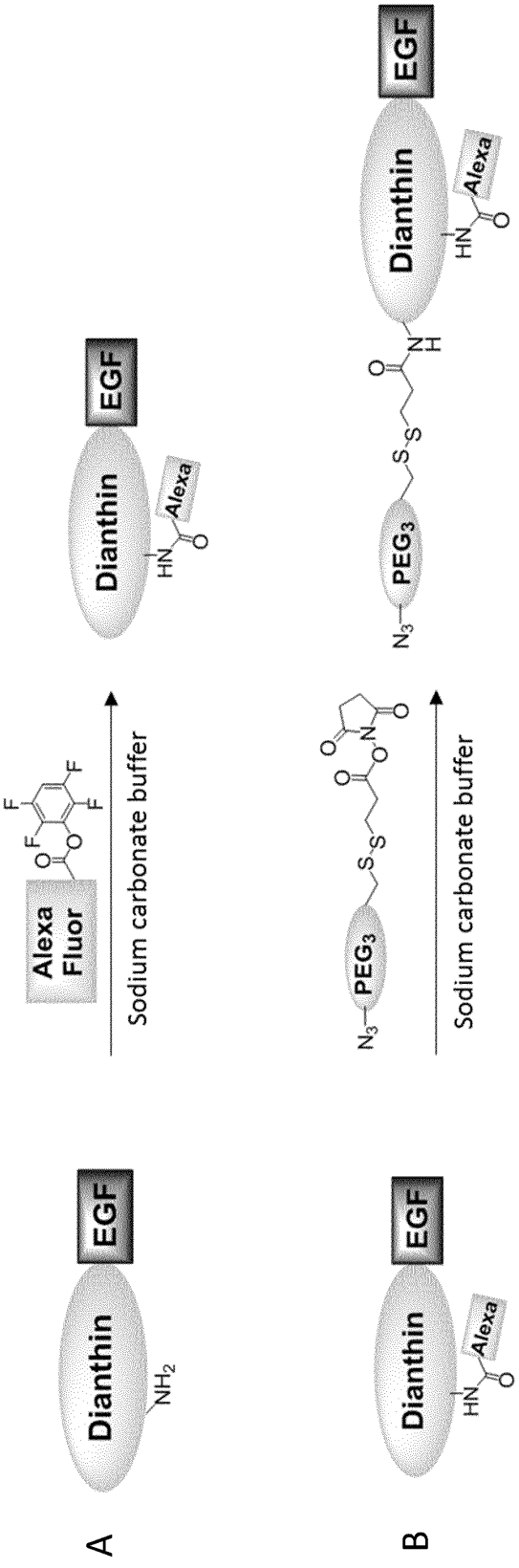

EGF
Dianthin
NH2
A
Alexa Fluor
Sodium carbonate buffer
EGF
Dianthin
HN
Alexa
B
PEG3
N3
Sodium carbonate buffer
EGF
Dianthin
HN
Alexa

Figure 37

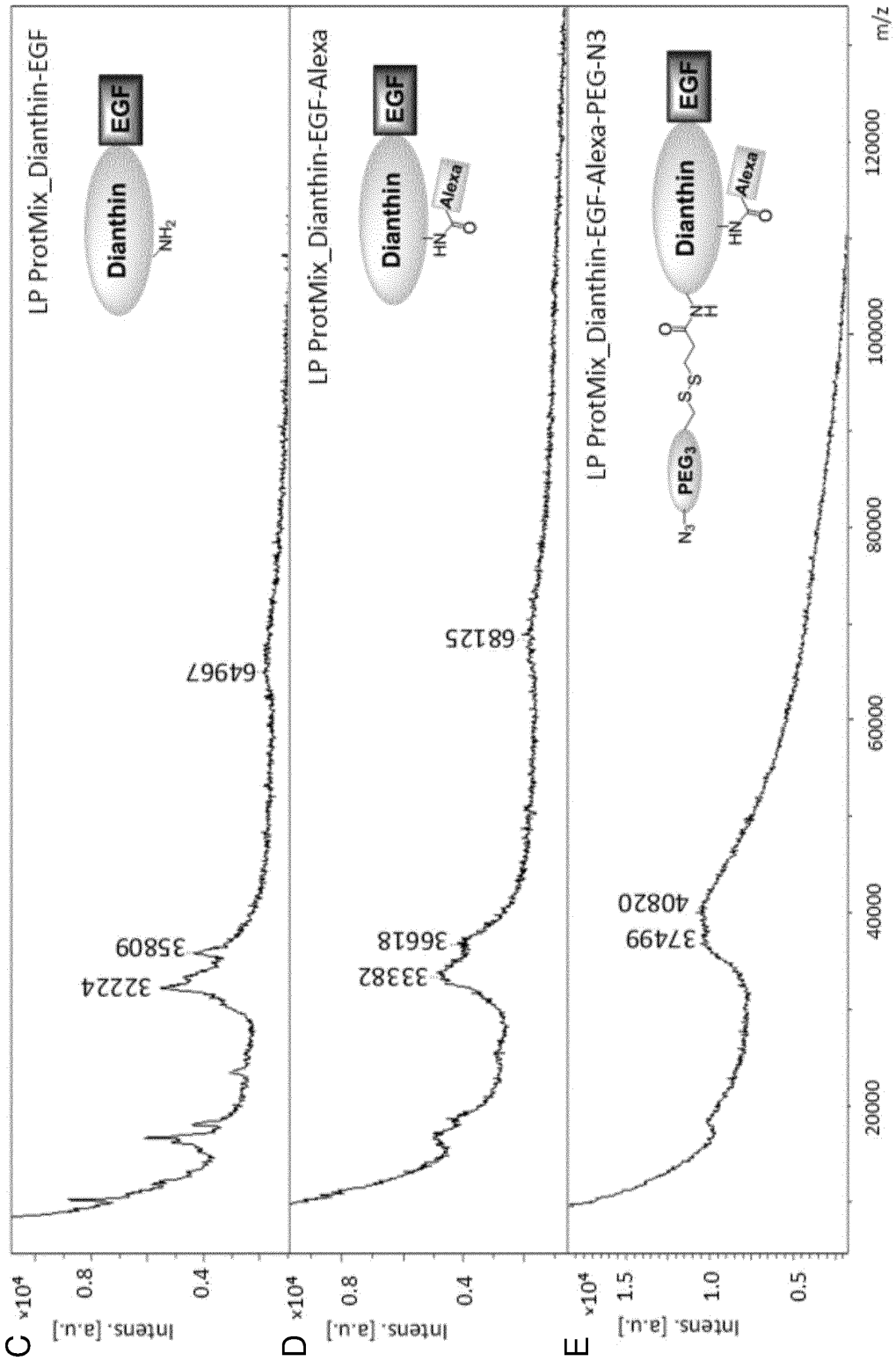

LP ProtMix_Dianthin-EGF
EGF
Dianthin
NH2
64967
35809
32224
LP ProtMix_Dianthin-EGF-Alexa
EGF
Dianthin
Alexa
HN
O
68125
36618
33382
LP ProtMix_Dianthin-EGF-Alexa-PEG-N3
EGF
Dianthin
Alexa
HN
O
N
H
O
S
S
PEG3
N3
40820
37499
C
D
E
×10⁴
Intens. [a.u.]
0.8
0.4
1.5
1.0
0.5
20000
40000
60000
80000
100000
120000
m/z

Figure 38

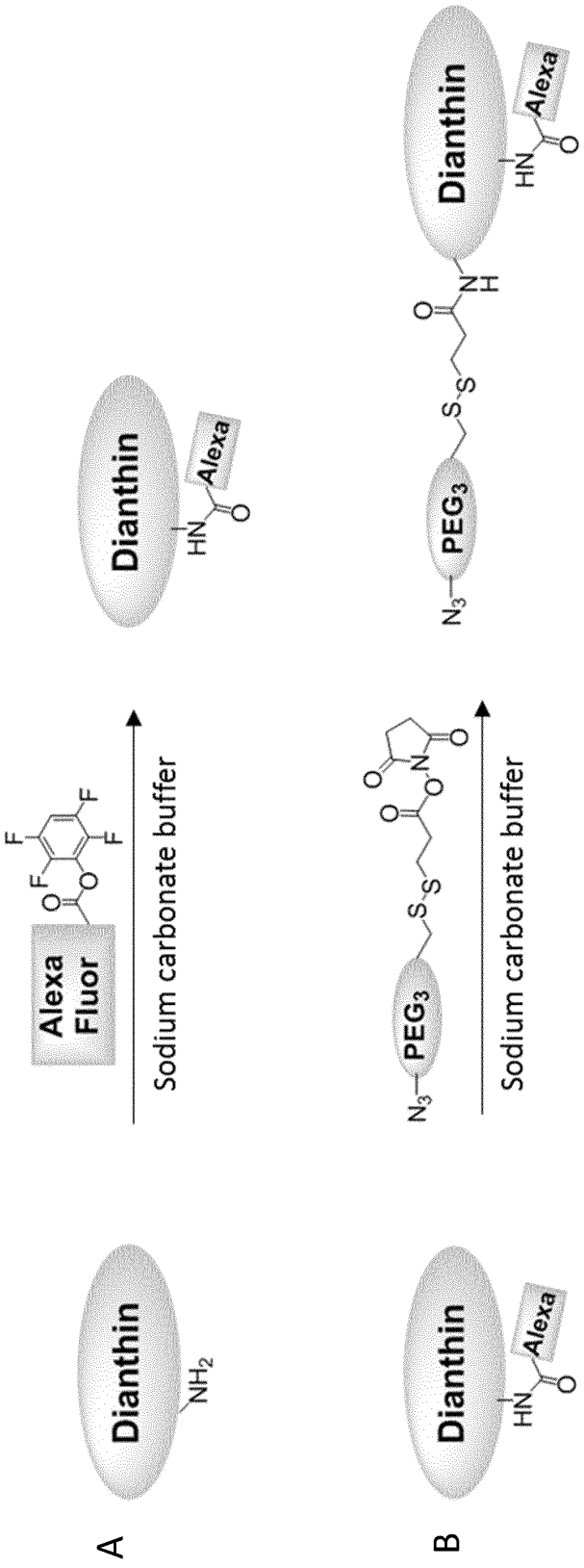

A
Dianthin
NH2
Alexa Fluor
Sodium carbonate buffer
Dianthin
Alexa
HN
O
B
Dianthin
HN
Alexa
O
NH
O
S
S
PEG3
N3
PEG3
N3
Sodium carbonate buffer
Dianthin
Alexa
HN
O

Figure 38

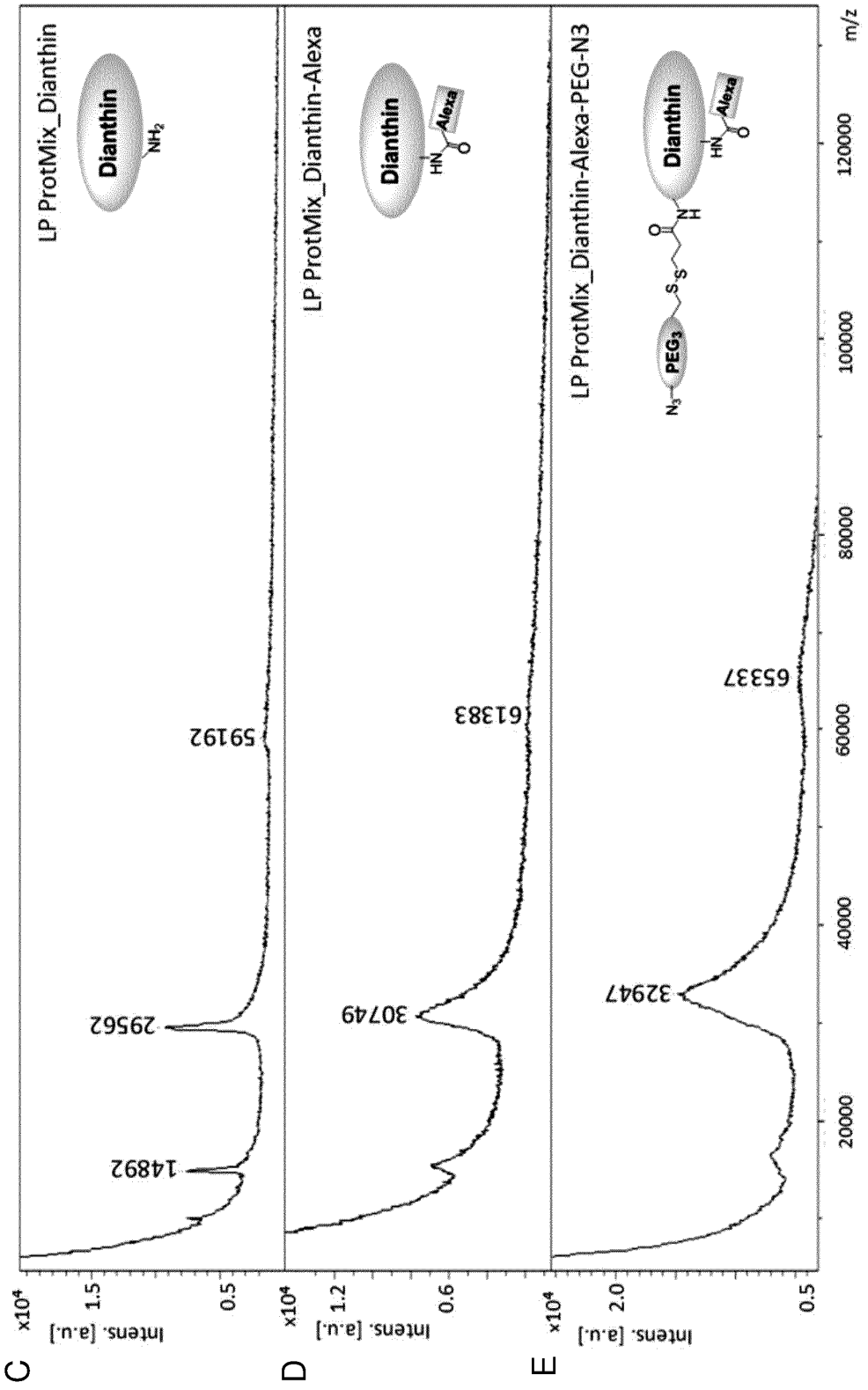

LP ProtMix_Dianthin
Dianthin
NH2
14892
29562
59192
LP ProtMix_Dianthin-Alexa
Dianthin
Alexa
HN
O
30749
61383
LP ProtMix_Dianthin-Alexa-PEG-N3
Dianthin
Alexa
HN
O
N
H
S
S
PEG3
N3
32947
65337
C
D
E
Intens. [a.u.]
x10^4
1.5
0.5
1.2
0.6
2.0
0.5
20000
40000
60000
80000
100000
120000
m/z

Figure 39

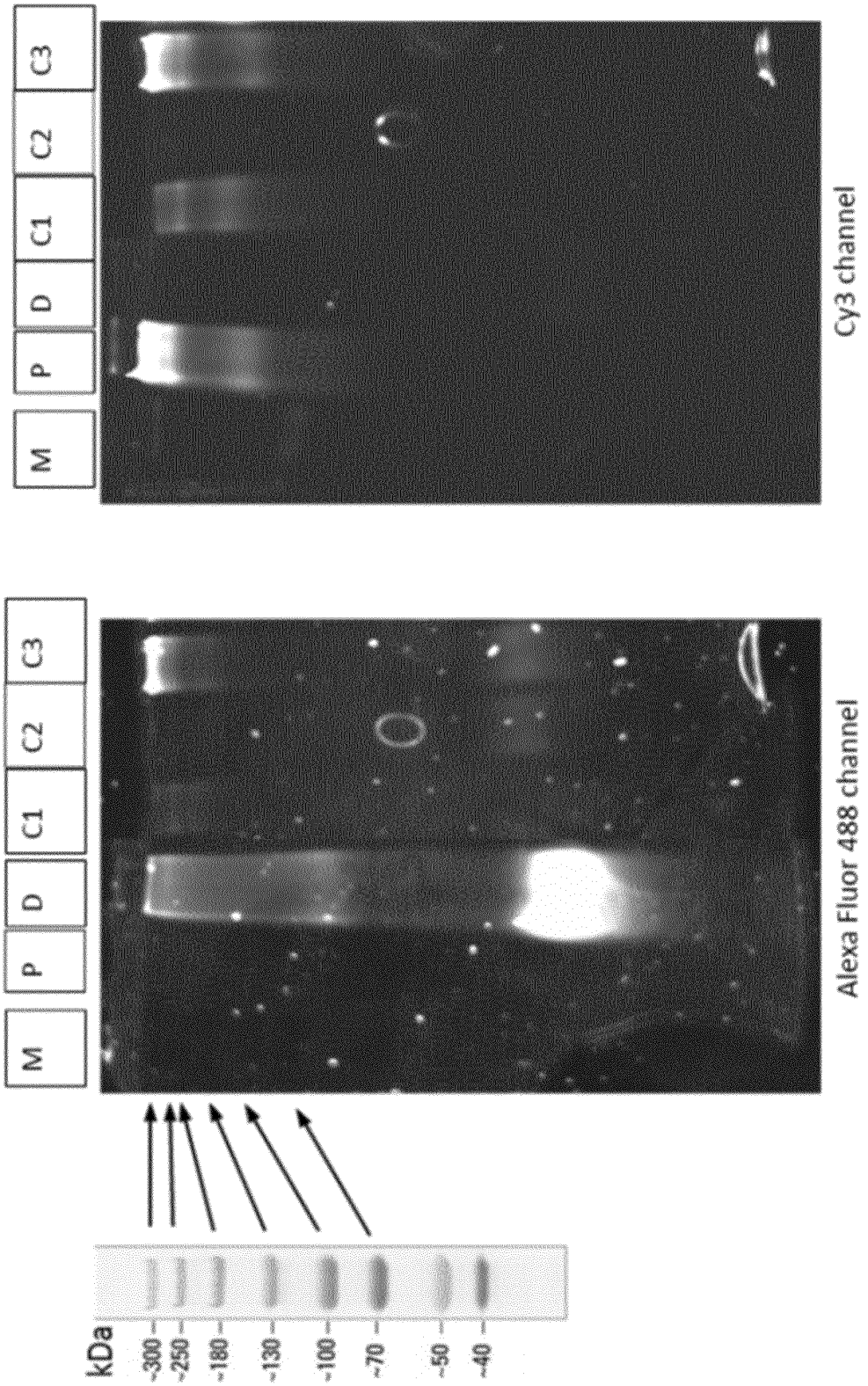

M
P
D
C1
C2
C3
Cy3 channel
Alexa Fluor 488 channel
kDa
~300
~250
~180
~130
~100
~70
~50
~40

Figure 40

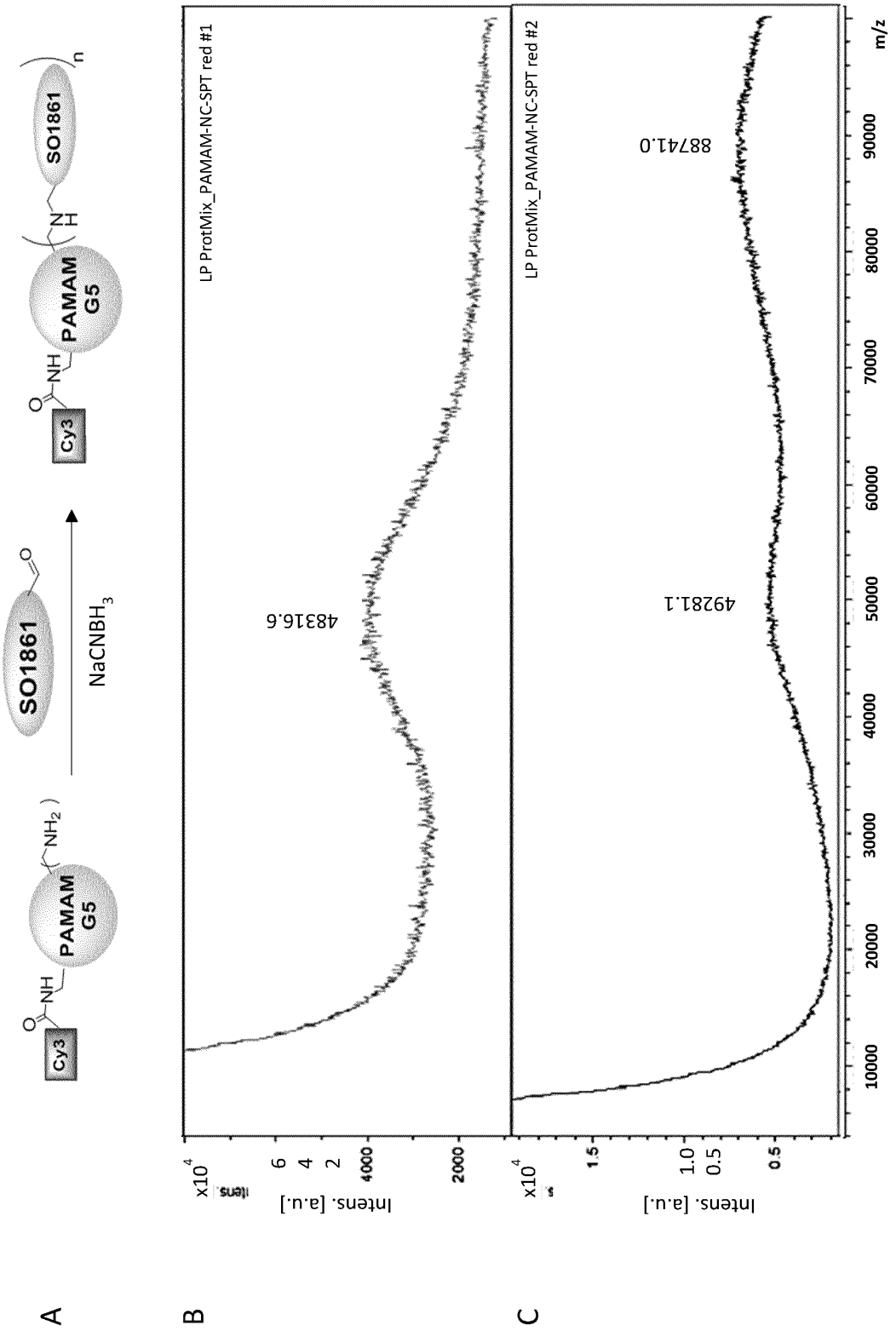

A
Cy3
PAMAM G5
NH
NH2
NaCNBH3
SO1861
Cy3
PAMAM G5
SO1861
n
B
LP ProtMix_PAMAM-NC-SPT red #1
48316.6
Intens. [a.u.]
x10^4
C
LP ProtMix_PAMAM-NC-SPT red #2
49281.1
88741.0
Intens. [a.u.]
m/z

Figure 41

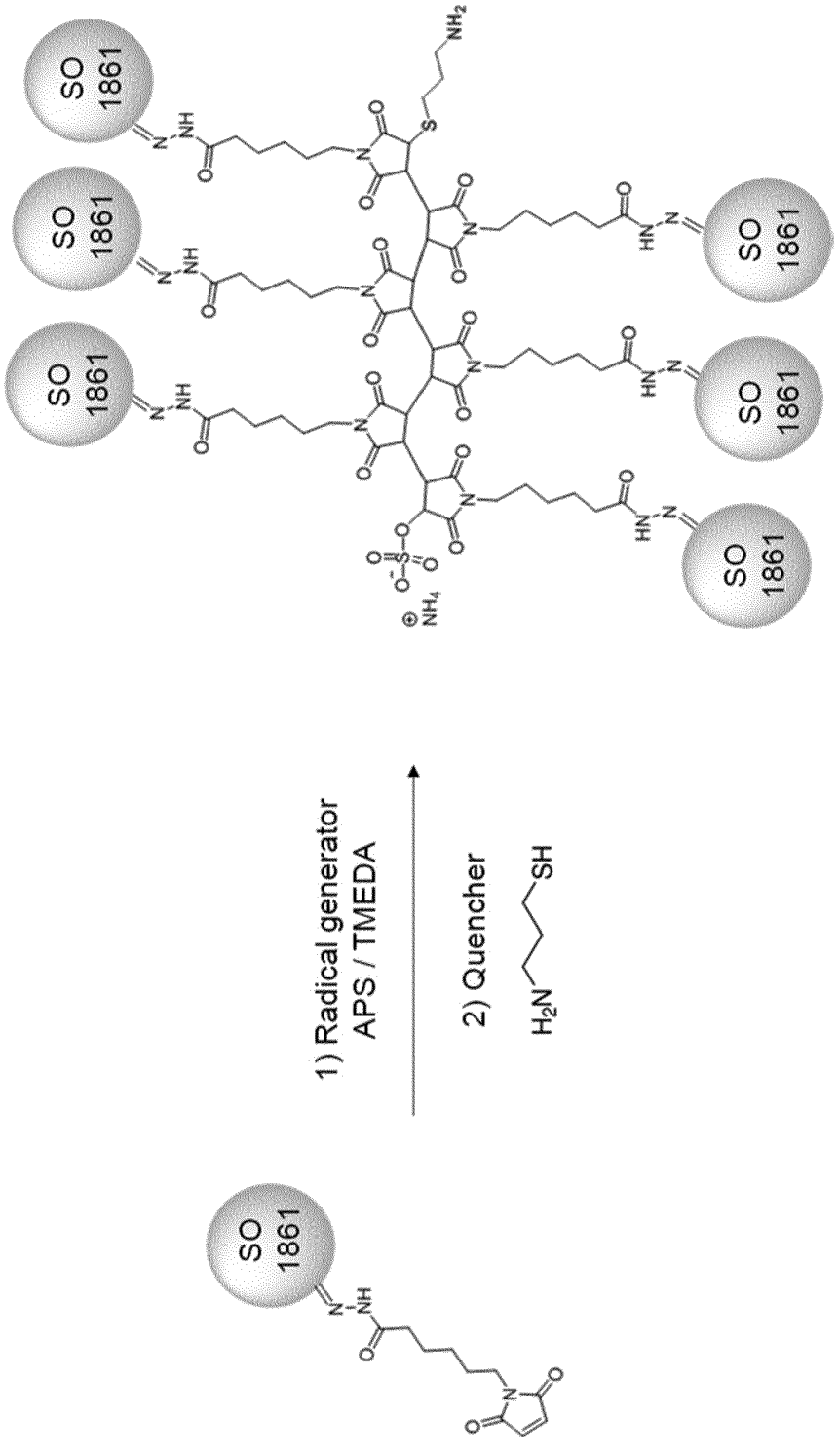


Figure 42

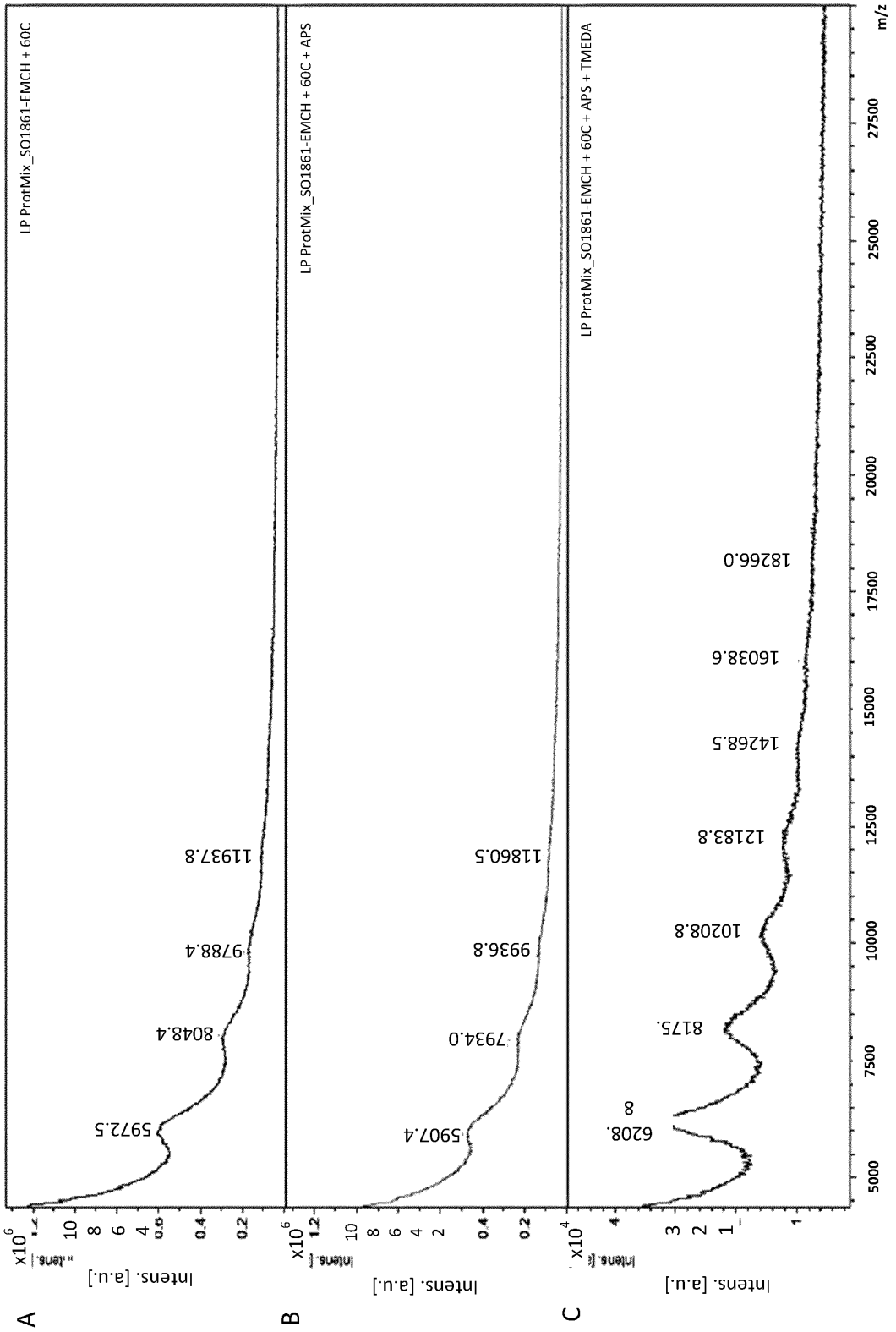

A
B
C
LP ProtMix_SO1861-EMCH + 60C
LP ProtMix_SO1861-EMCH + 60C + APS
LP ProtMix_SO1861-EMCH + 60C + APS + TMEDA
Intens. [a.u.]
m/z
5972.5
8048.4
9788.4
11937.8
5907.4
7934.0
9936.8
11860.5
6208.8
8175.
10208.8
12183.8
14268.5
16038.6
18266.0
5000
7500
10000
12500
15000
17500
20000
22500
25000
27500

Figure 43

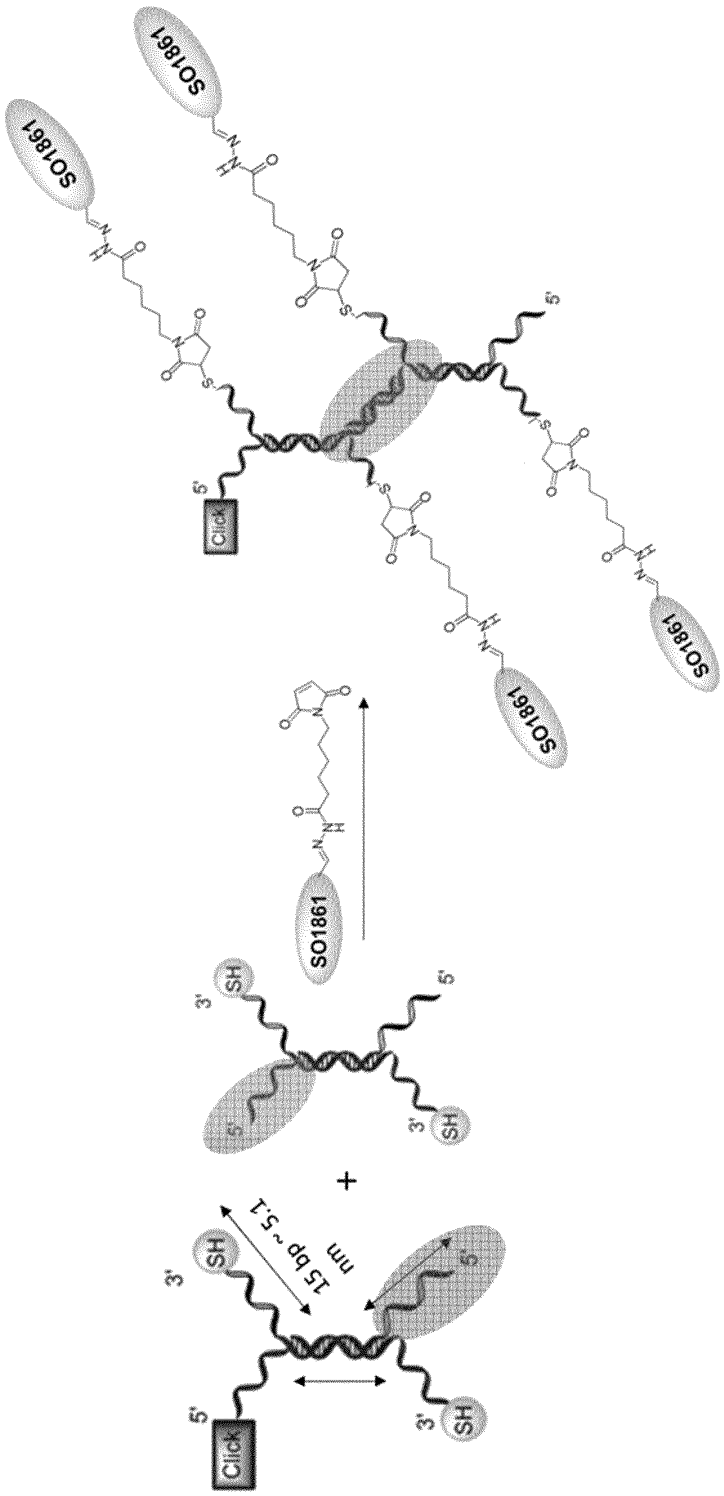

SO1861
SO1861
SO1861
SO1861
SO1861
5'
Click
SO1861
SH
3'
5'
5'
3'
SH
+
SH
3'
15 bp ~ 5,1
nm
5'
5'
Click
3'
SH

Figure 44

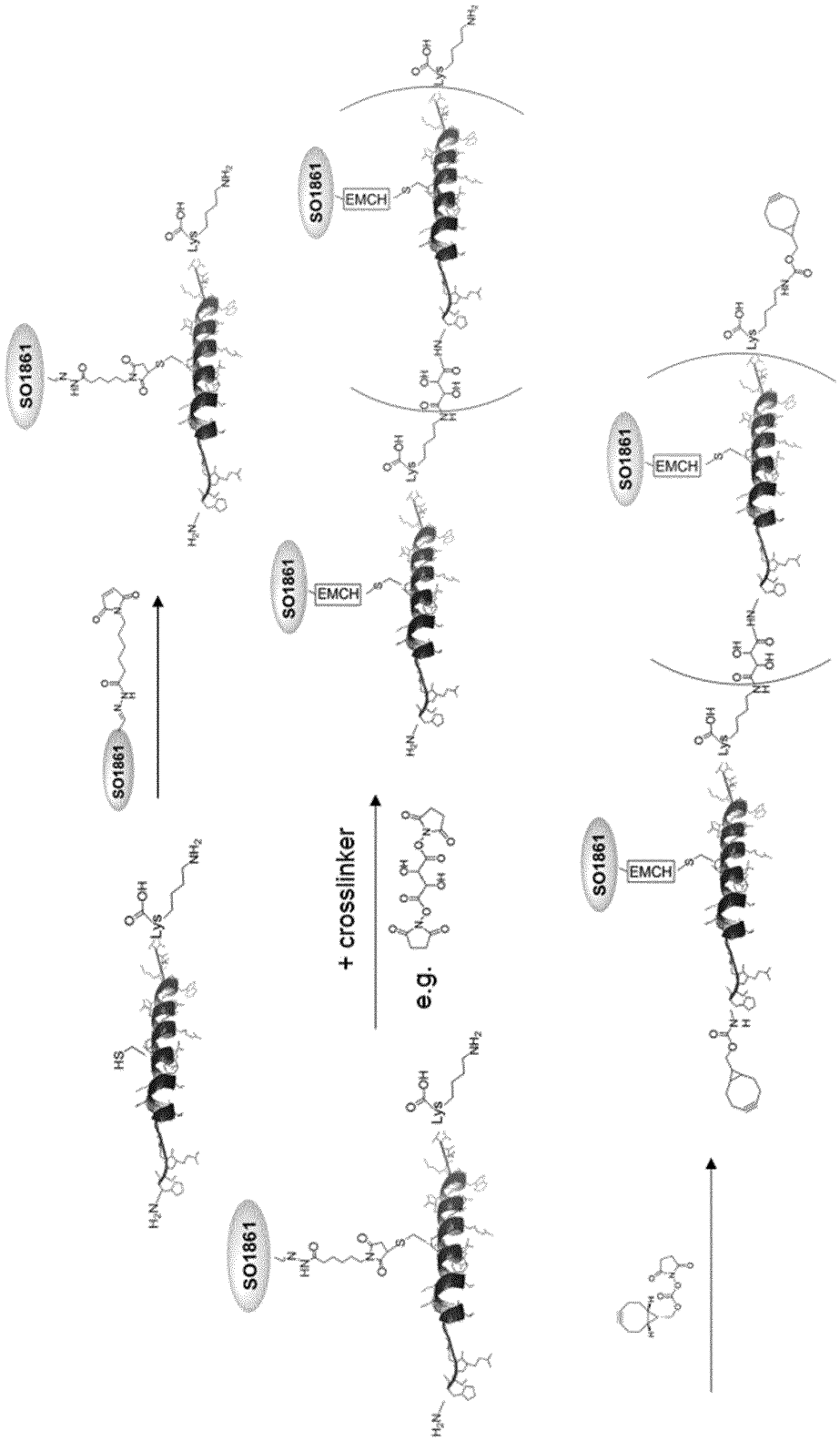


Figure 45

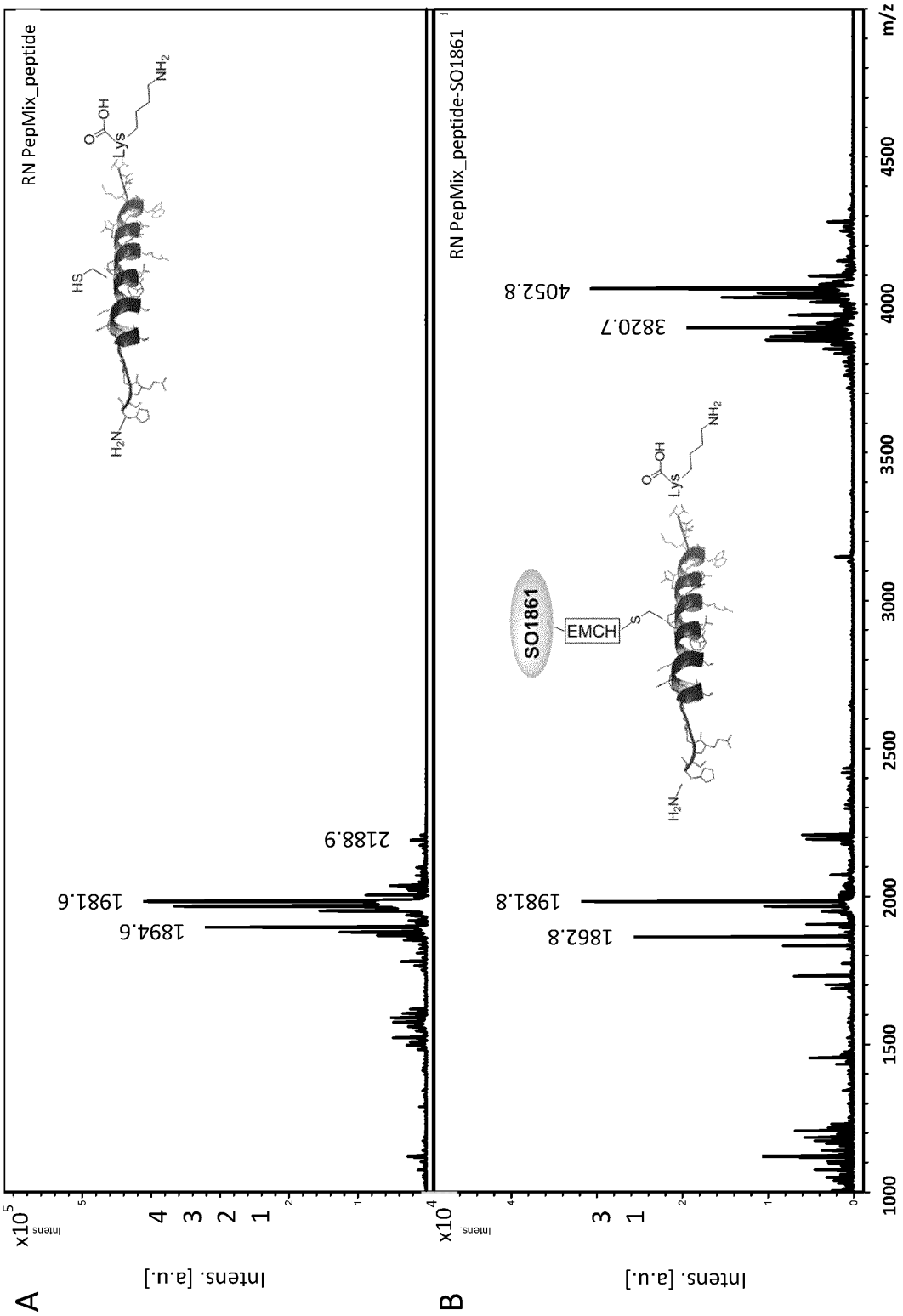

A
Intens. [a.u.]
x10^5
RN PepMix_peptide
2188.9
1981.6
1894.6
B
Intens. [a.u.]
x10^4
RN PepMix_peptide-SO1861
4052.8
3820.7
1981.8
1862.8
SO1861
EMCH
Lys
m/z
1000
1500
2000
2500
3000
3500
4000
4500

Figure 46

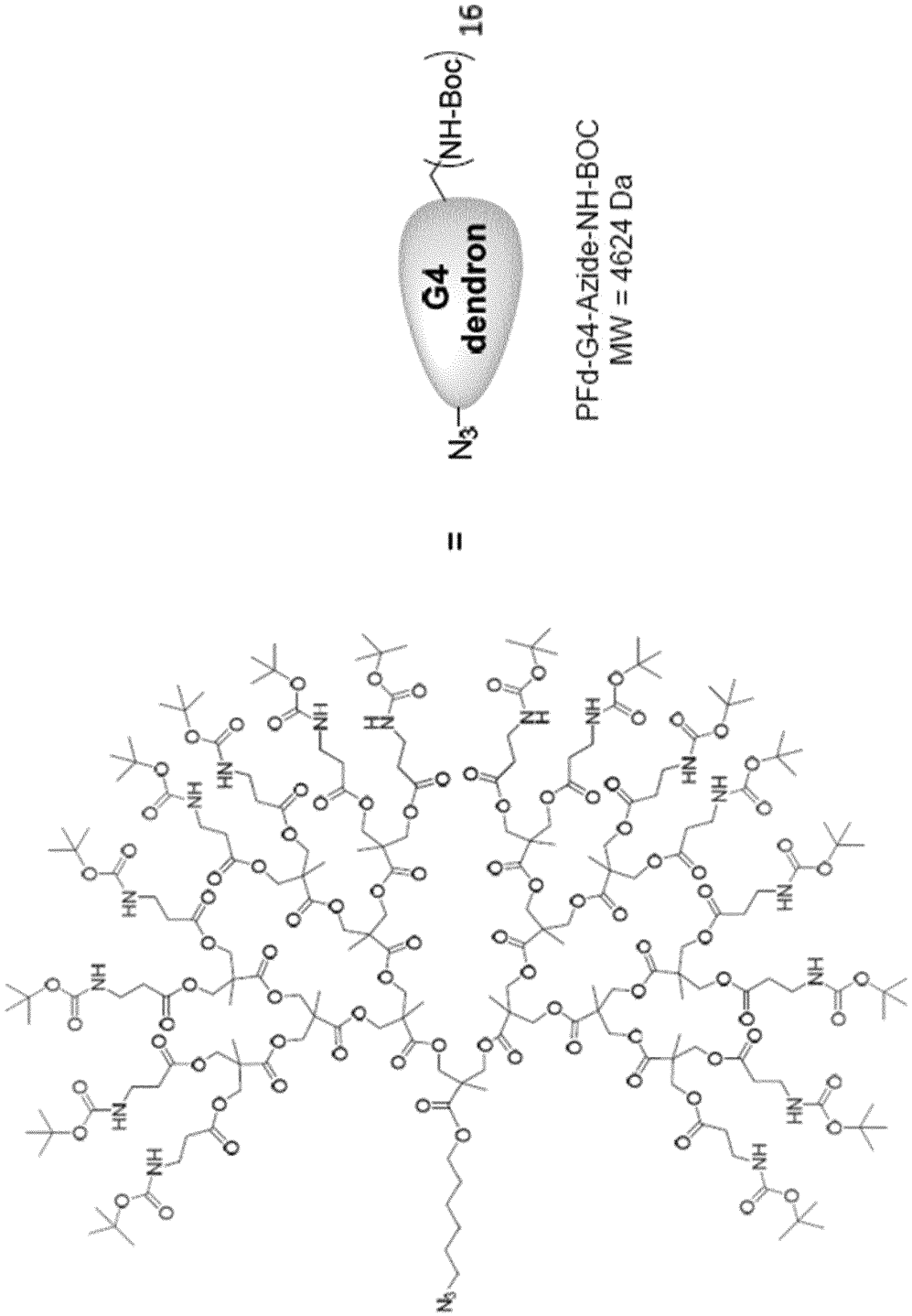


Figure 47

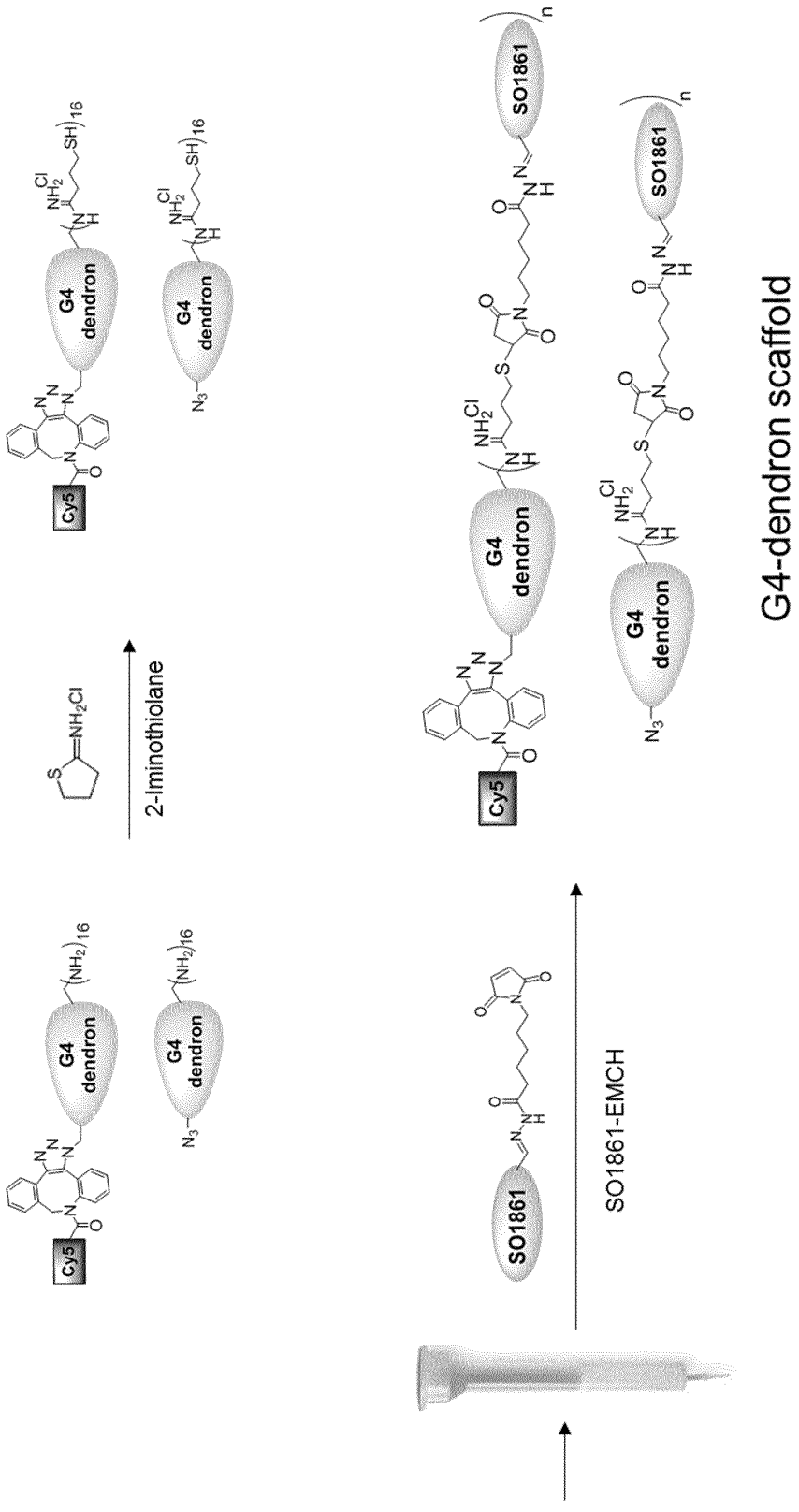

G4 dendron
Cy5
2-Iminothiolane
SO1861
SO1861-EMCH
G4-dendron scaffold

Figure 48

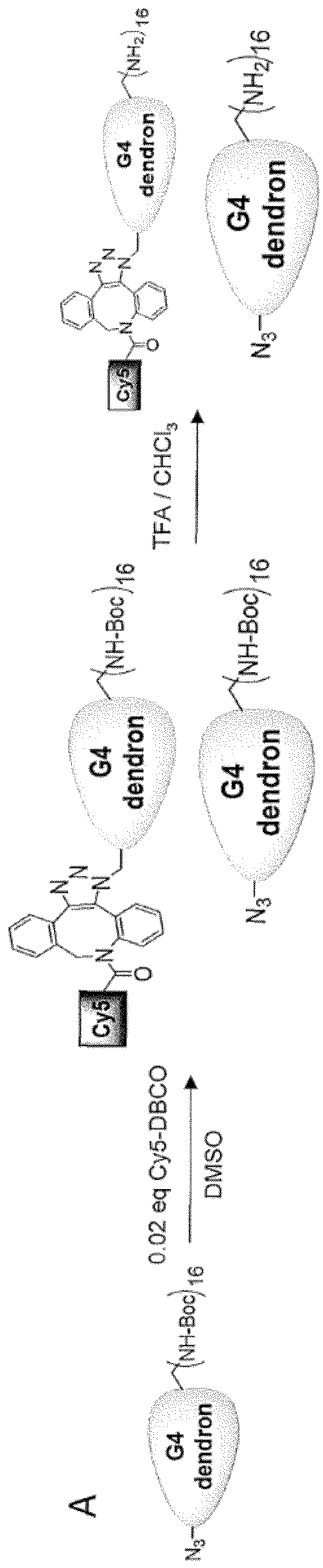

A
G4 dendron
N_3
$(NH\text{-}Boc)_{16}$
0.02 eq Cy5-DBCO
DMSO
Cy5
TFA / $CHCl_3$
$(NH_2)_{16}$

Figure 48

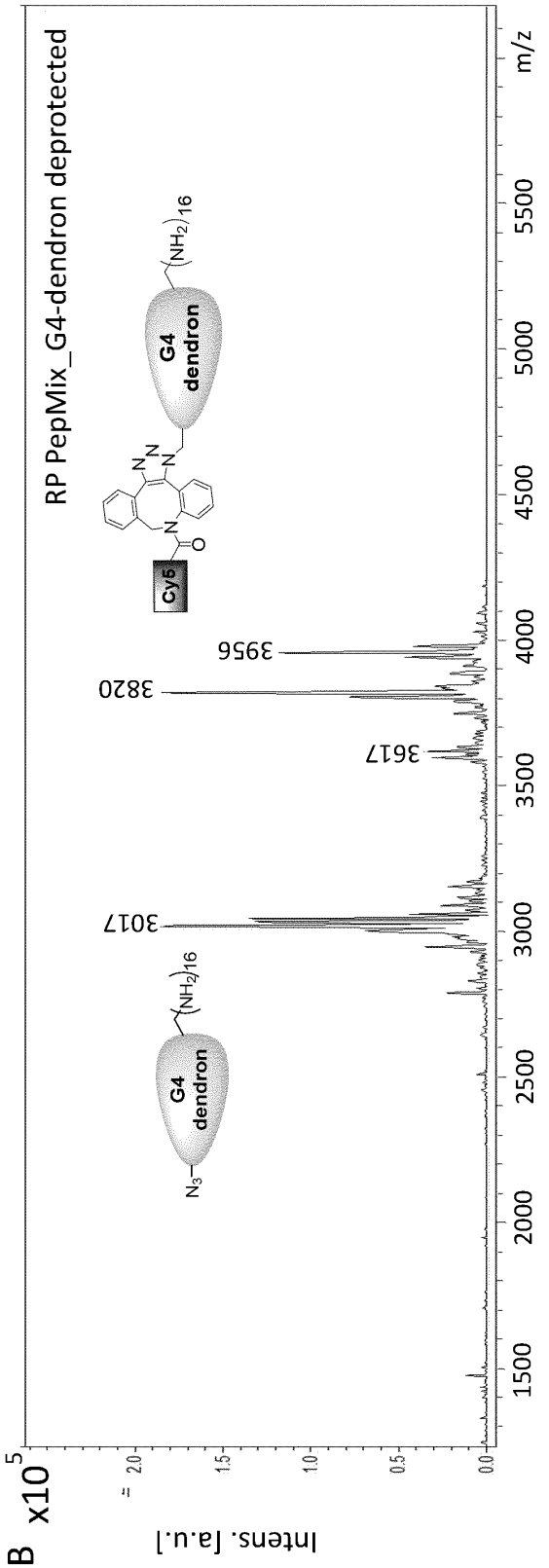

RP PepMix_G4-dendron deprotected
B x10^5
Intens. [a.u.]
m/z
3956
3820
3617
3017
G4 dendron
(NH2)16
N3
Cy5

Figure 49

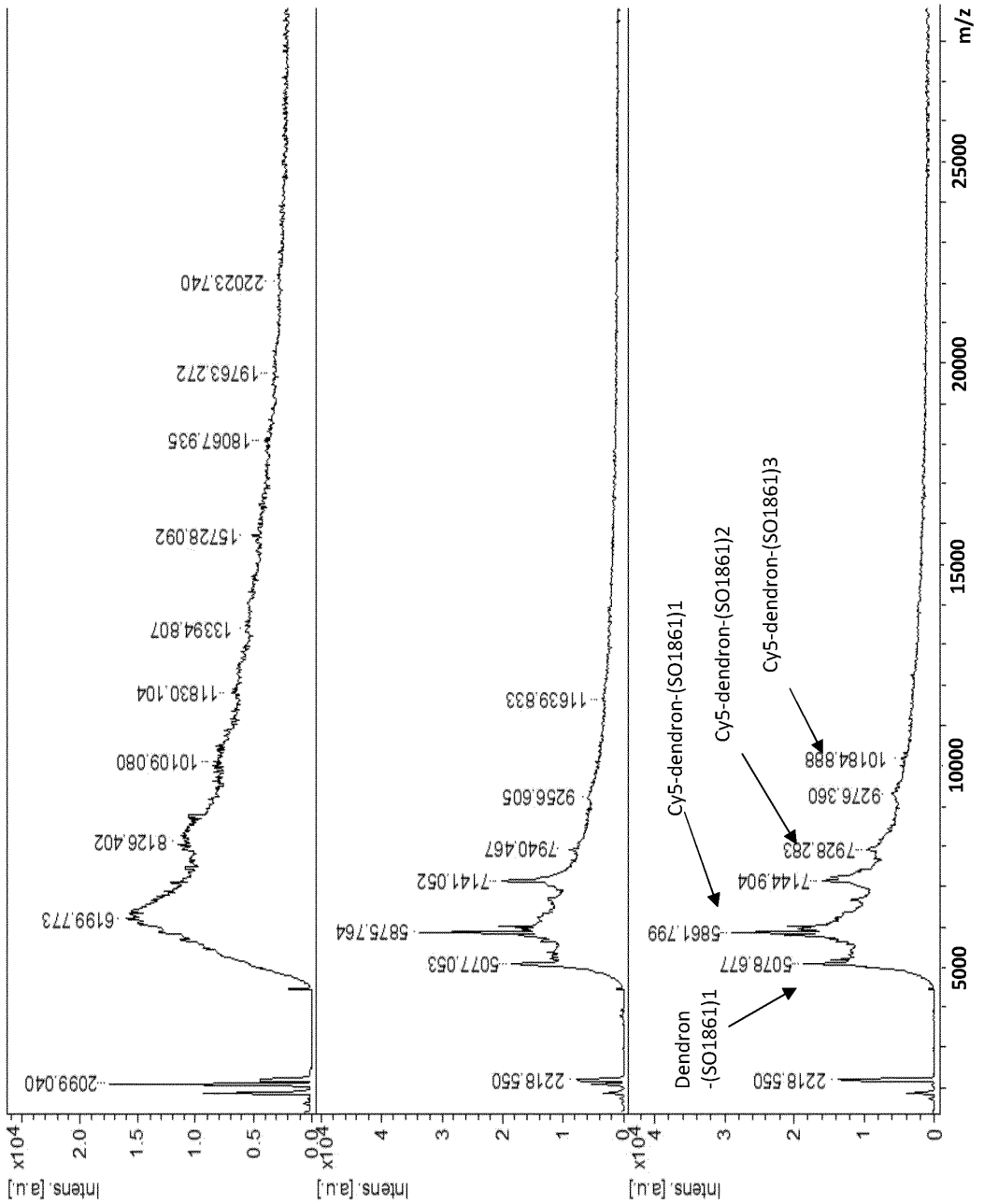
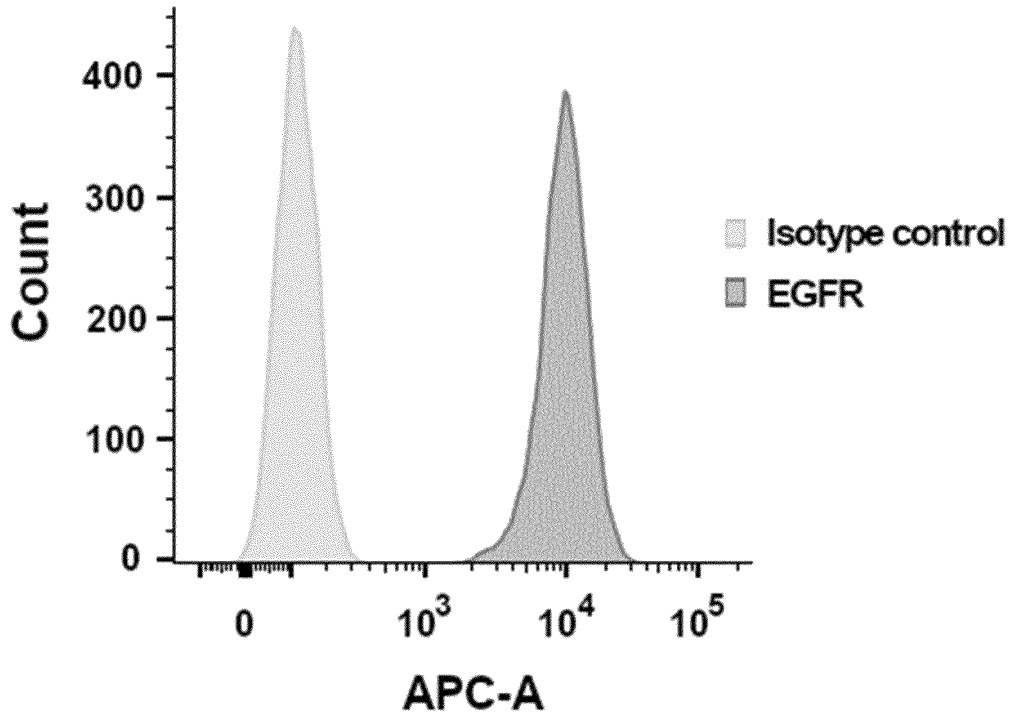

A
B
C
Intens. [a.u.]
x10^4
m/z
2099.040
6199.773
8126.402
10109.080
11830.104
13394.807
15728.092
18067.935
19763.272
22023.740
2218.550
5077.053
5875.764
7141.052
7940.467
9256.605
11639.833
Dendron -(SO1861)1
5078.677
5861.799
Cy5-dendron-(SO1861)1
7144.904
Cy5-dendron-(SO1861)2
7928.283
9276.360
Cy5-dendron-(SO1861)3
10184.888
5000
10000
15000
20000
25000

Figure 50

A


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

B

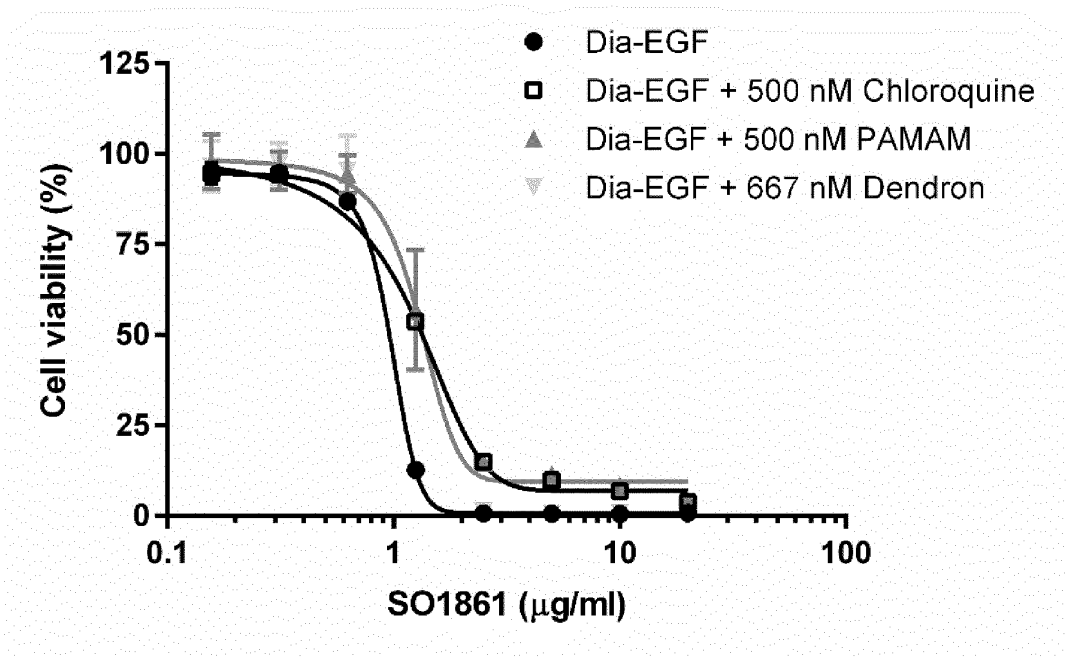
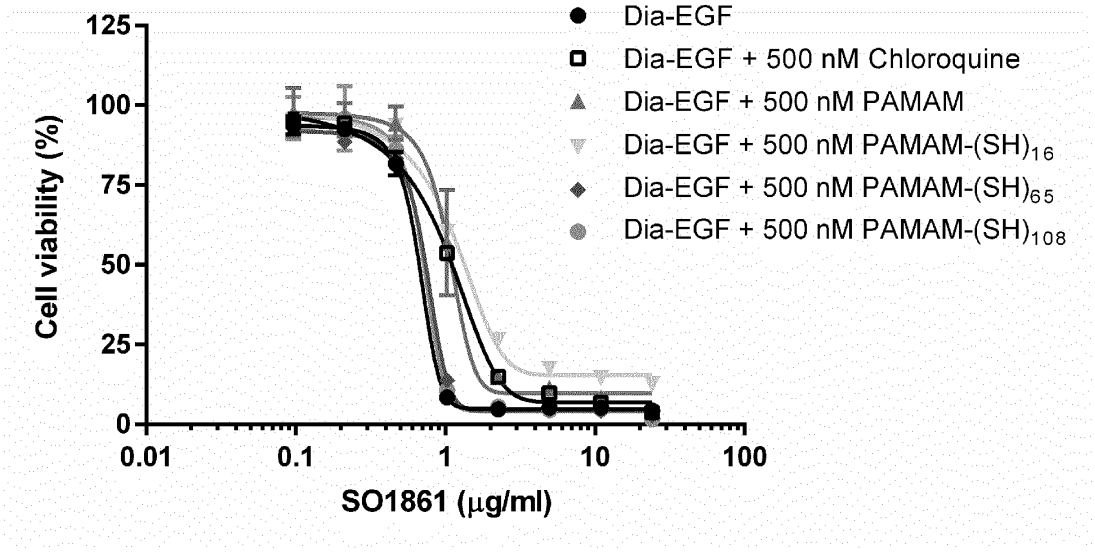

Cell viability (%)
125
100
75
50
25
0
0.1
1
10
100
SO1861 (μg/ml)
Dia-EGF
Dia-EGF + 500 nM Chloroquine
Dia-EGF + 500 nM PAMAM
Dia-EGF + 667 nM Dendron

Figure 50

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

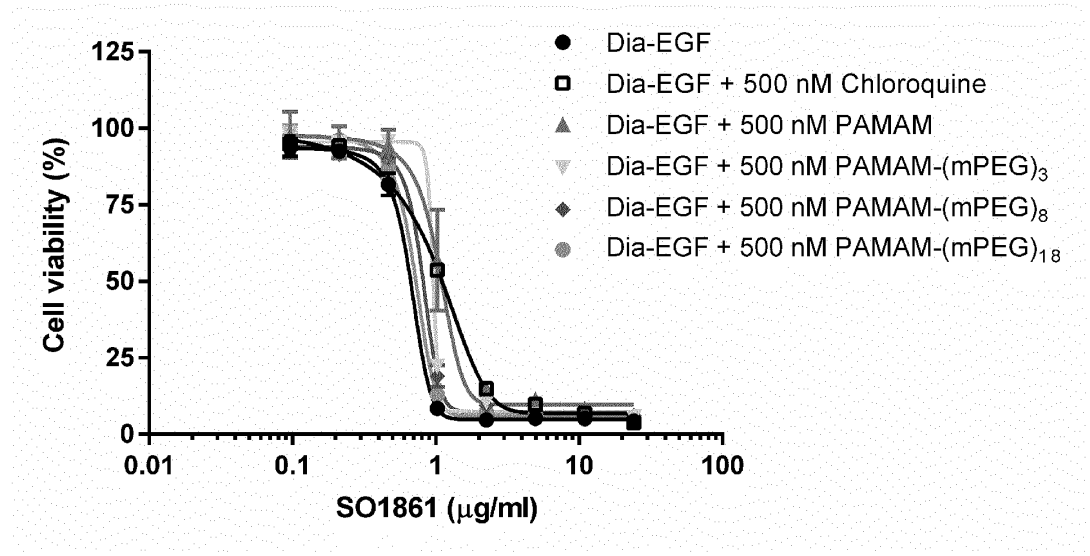

Cell viability (%)
125
100
75
50
25
0
0.01
0.1
1
10
100
SO1861 (µg/ml)
Dia-EGF
Dia-EGF + 500 nM Chloroquine
Dia-EGF + 500 nM PAMAM
Dia-EGF + 500 nM PAMAM-(mPEG)3
Dia-EGF + 500 nM PAMAM-(mPEG)8
Dia-EGF + 500 nM PAMAM-(mPEG)18

Figure 51

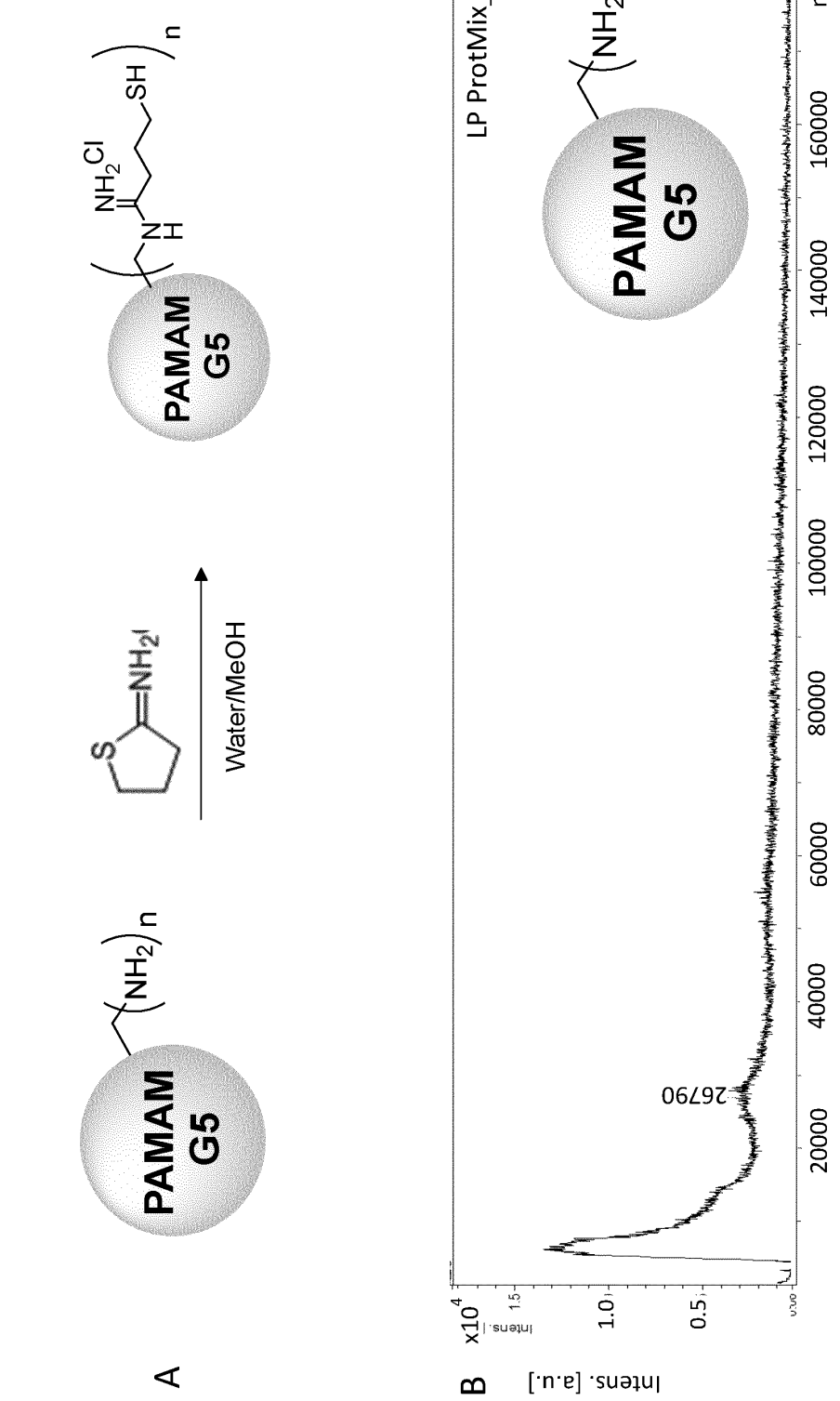

A
PAMAM G5
(NH2)n
Water/MeOH
PAMAM G5
B
LP ProtMix_PAMAM
PAMAM G5
26790
Intens. [a.u.]
x10^4
m/z

Figure 51

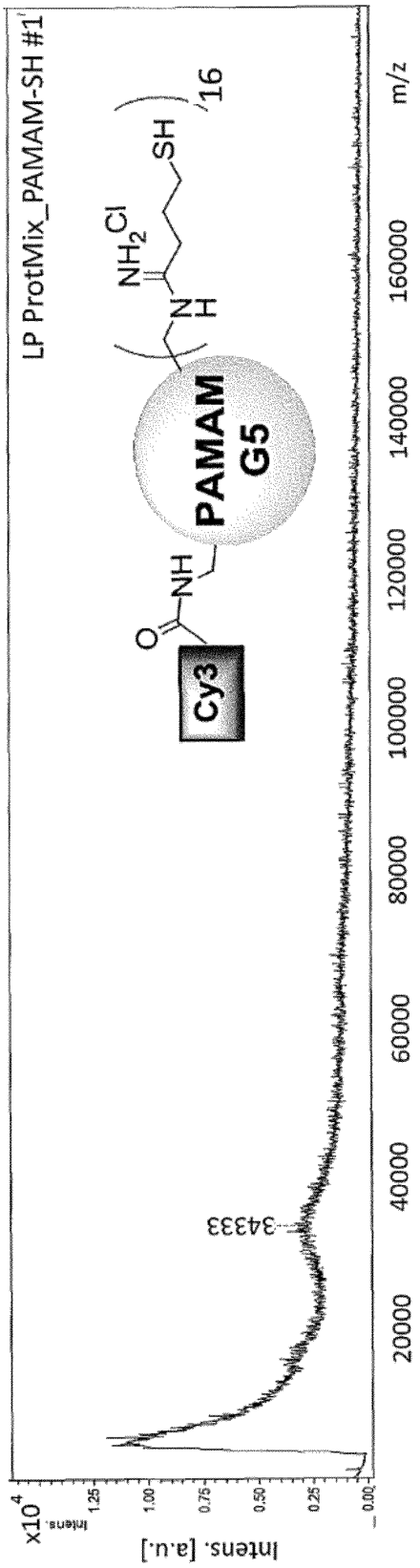

C
LP ProtMix_PAMAM-SH #1
Intens. [a.u.]
x10^4
Intens.
1.25
1.00
0.75
0.50
0.25
0.00
34333
20000
40000
60000
80000
100000
120000
140000
160000
m/z
Cy3
O
NH
PAMAM
G5
NH2
Cl
N
H
SH
16

Figure 51

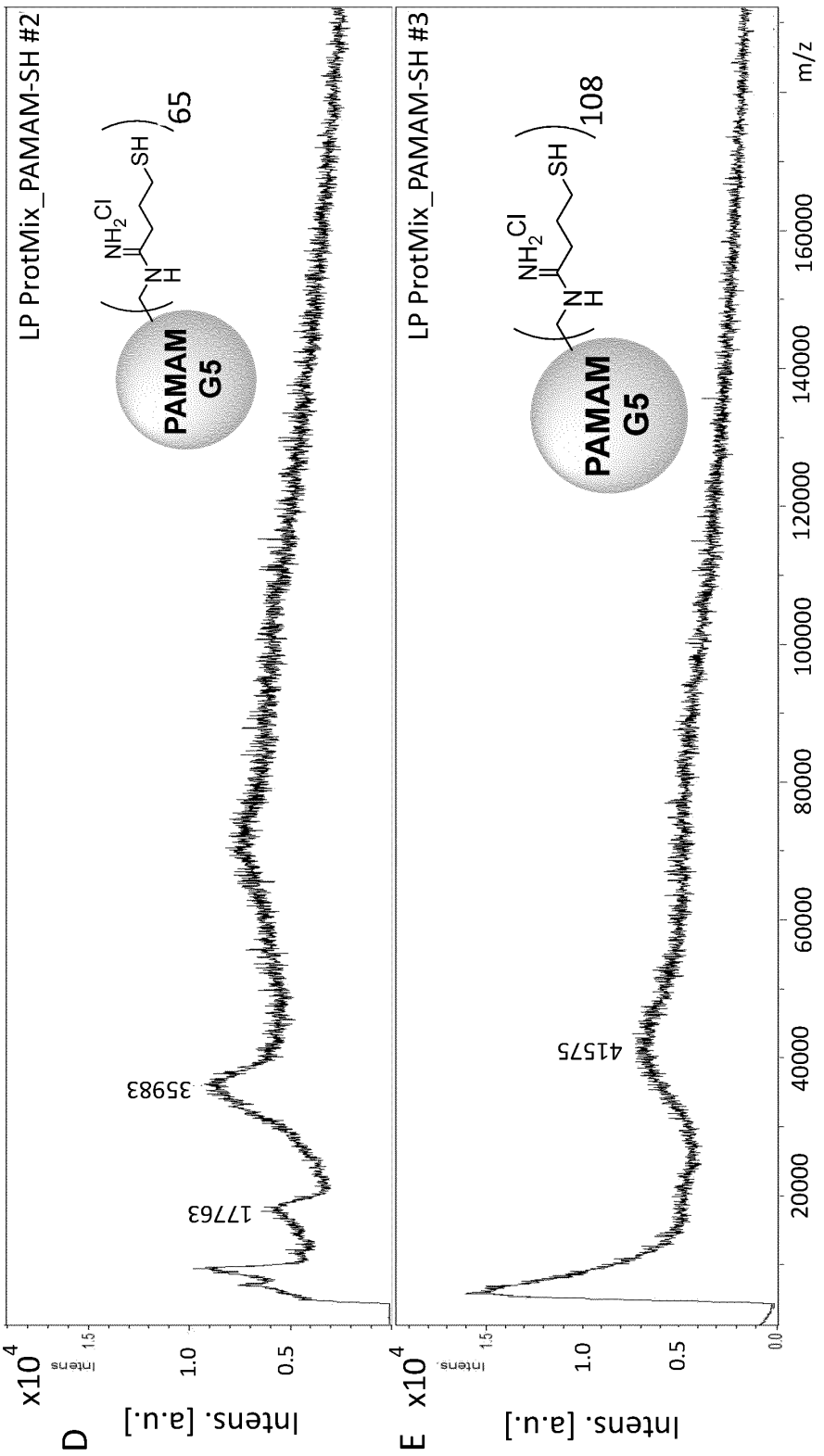

LP ProtMix_PAMAM-SH #2
PAMAM G5
65
17763
35983
D
x10^4
Intens. [a.u.]
LP ProtMix_PAMAM-SH #3
PAMAM G5
108
41575
E
x10^4
Intens. [a.u.]
m/z
20000
40000
60000
80000
100000
120000
140000
160000

Figure 52

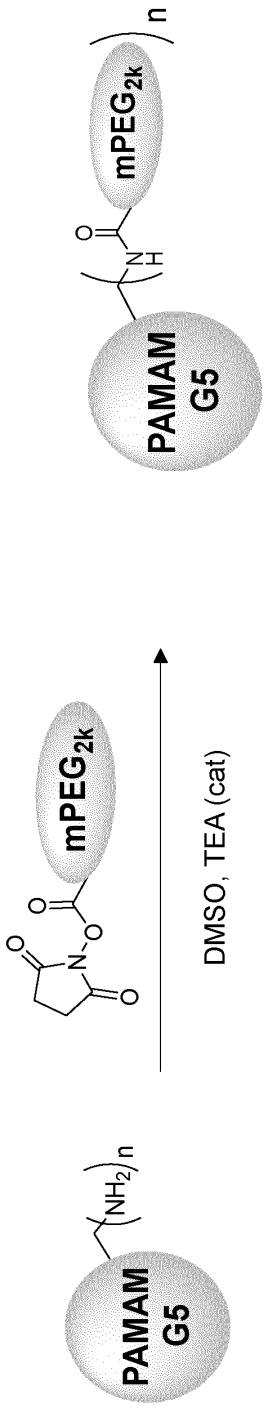

mPEG2k
n
O
N
H
PAMAM
G5
mPEG2k
O
O
N
O
O
O
DMSO, TEA (cat)
PAMAM
G5
(NH2)n
A

Figure 52

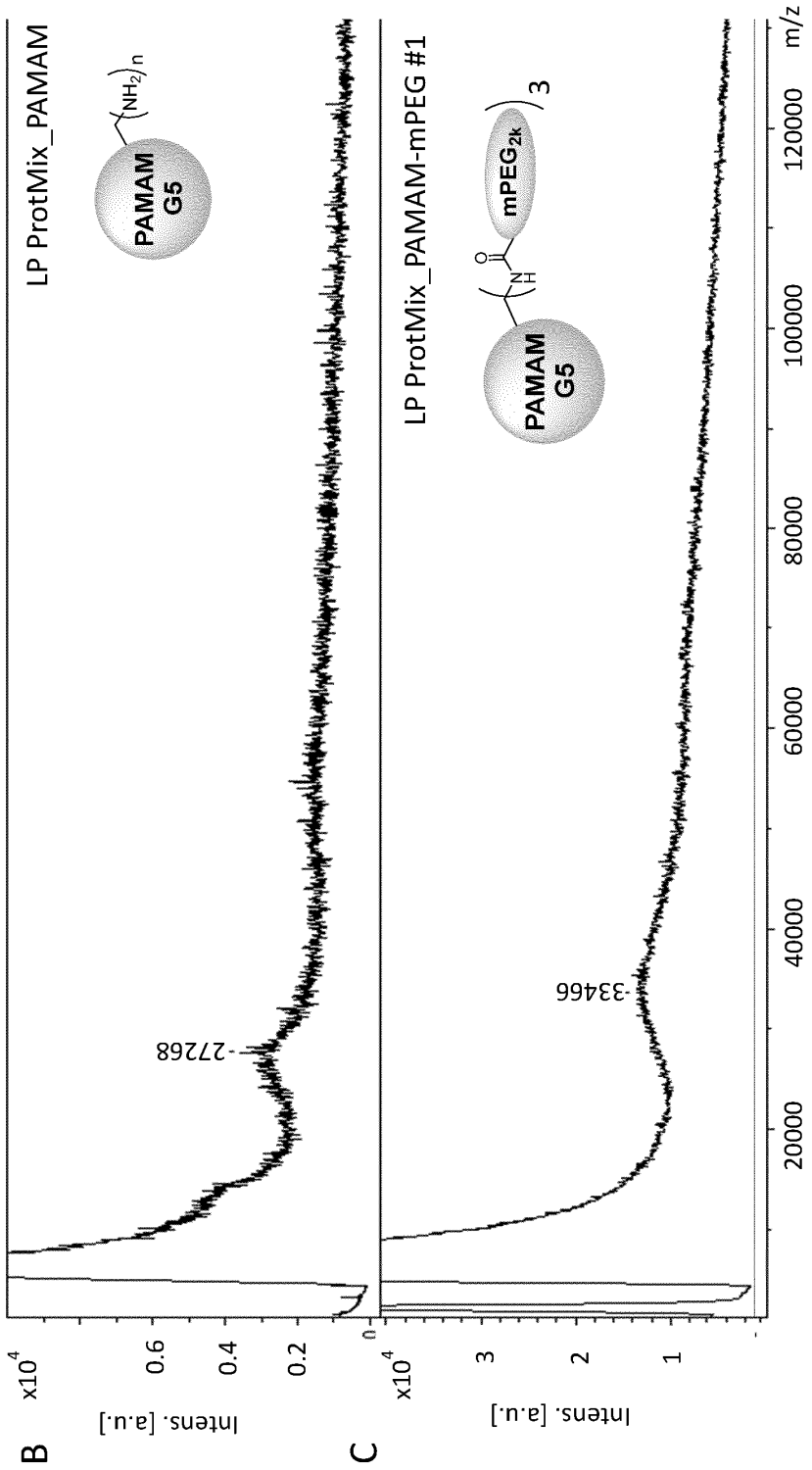

B
C
LP ProtMix_PAMAM
LP ProtMix_PAMAM-mPEG #1
PAMAM G5
mPEG2k
27268
33466
Intens. [a.u.]
x10^4
m/z
20000
40000
60000
80000
100000
120000

Figure 52

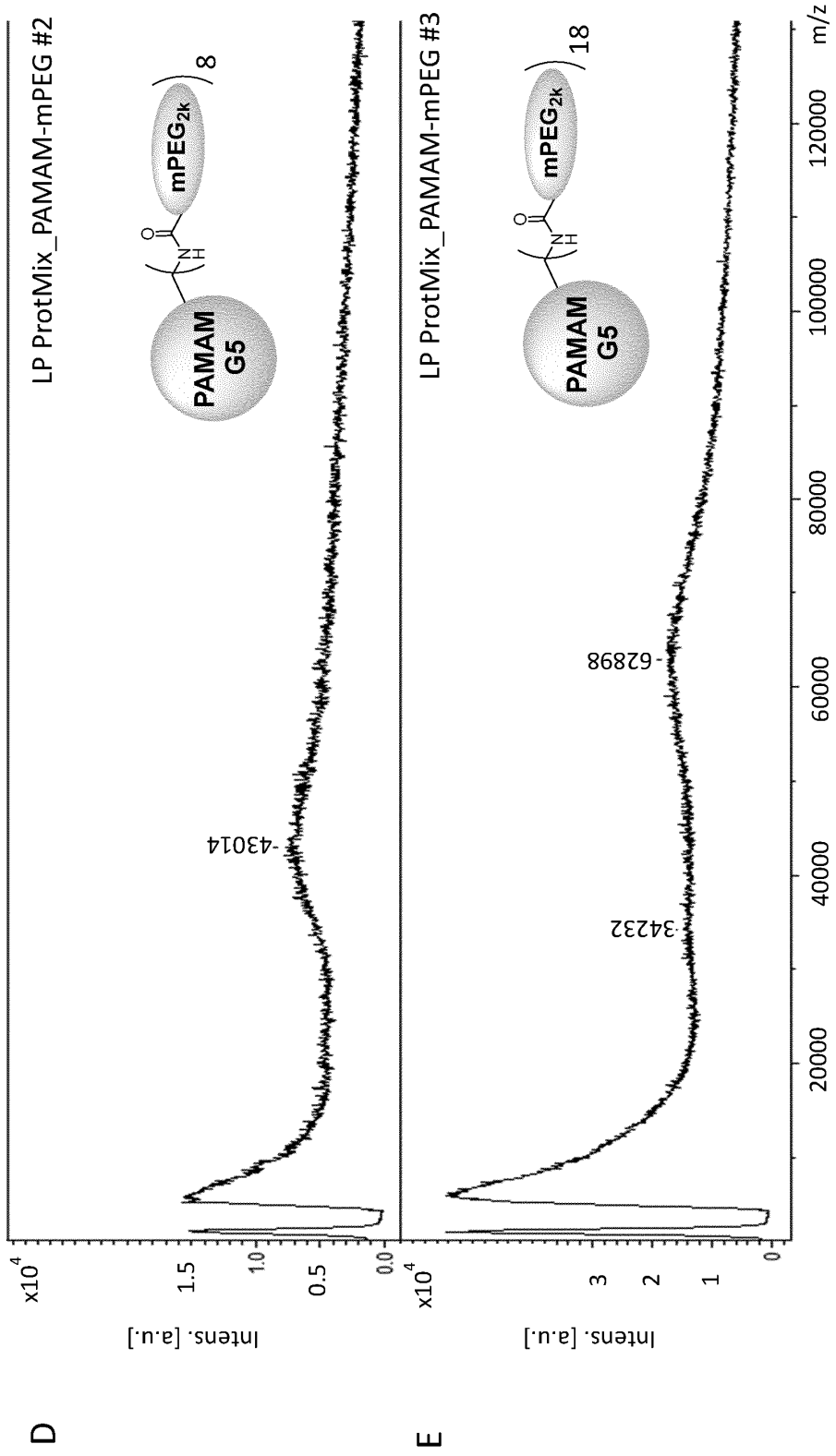

D
LP ProtMix_PAMAM-mPEG #2
PAMAM G5
mPEG2k
8
43014
Intens. [a.u.]
x10^4
1.5
1.0
0.5
0.0
E
LP ProtMix_PAMAM-mPEG #3
PAMAM G5
mPEG2k
18
34232
62898
Intens. [a.u.]
x10^4
3
2
1
0
20000
40000
60000
80000
100000
120000
m/z

Figure 53

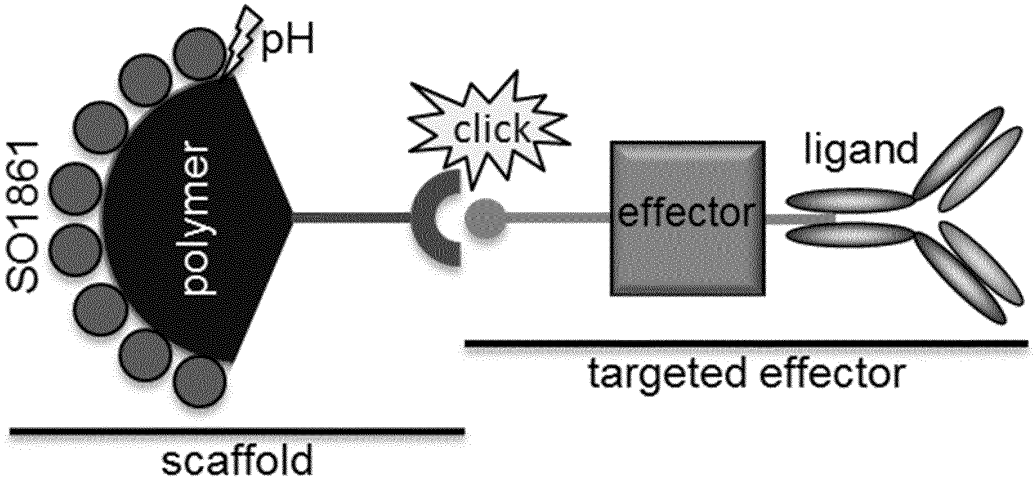
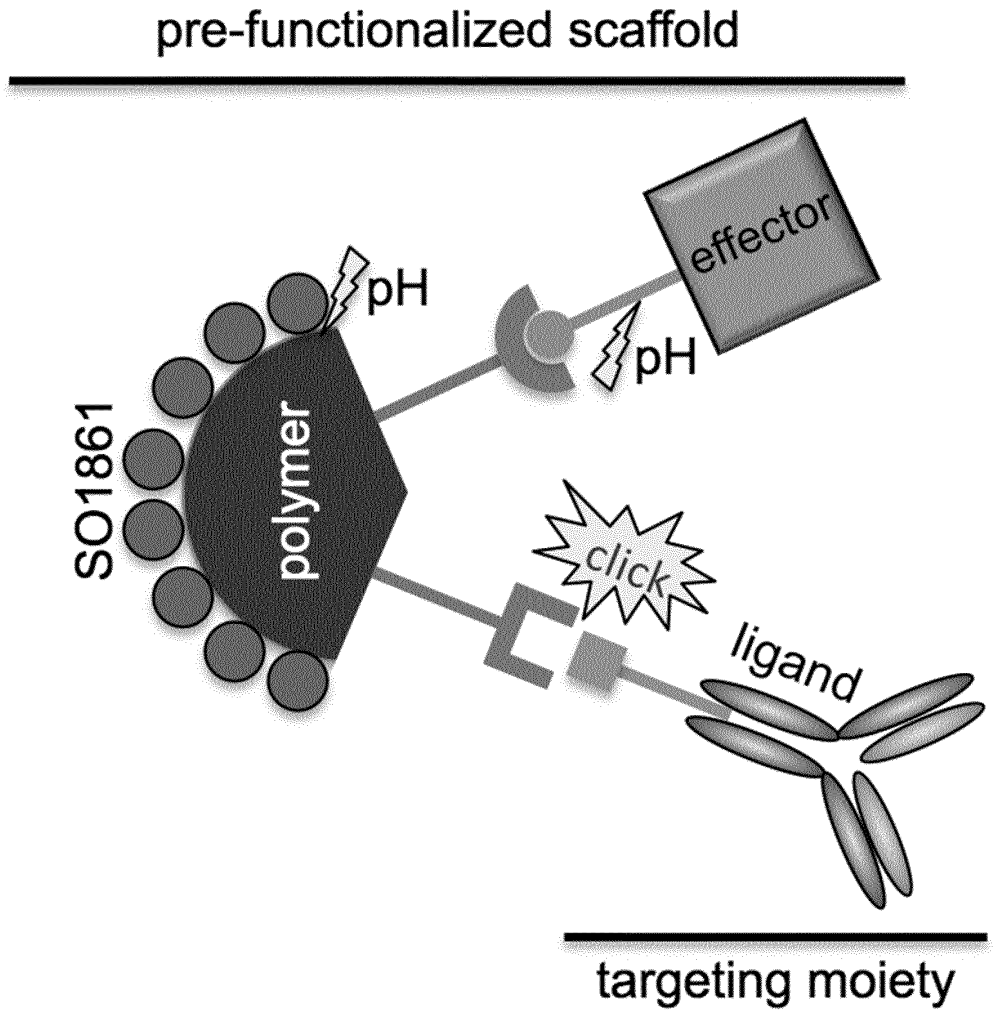

pH
click
ligand
SO1861
polymer
effector
targeted effector
scaffold

Figure 54


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