Figure 1

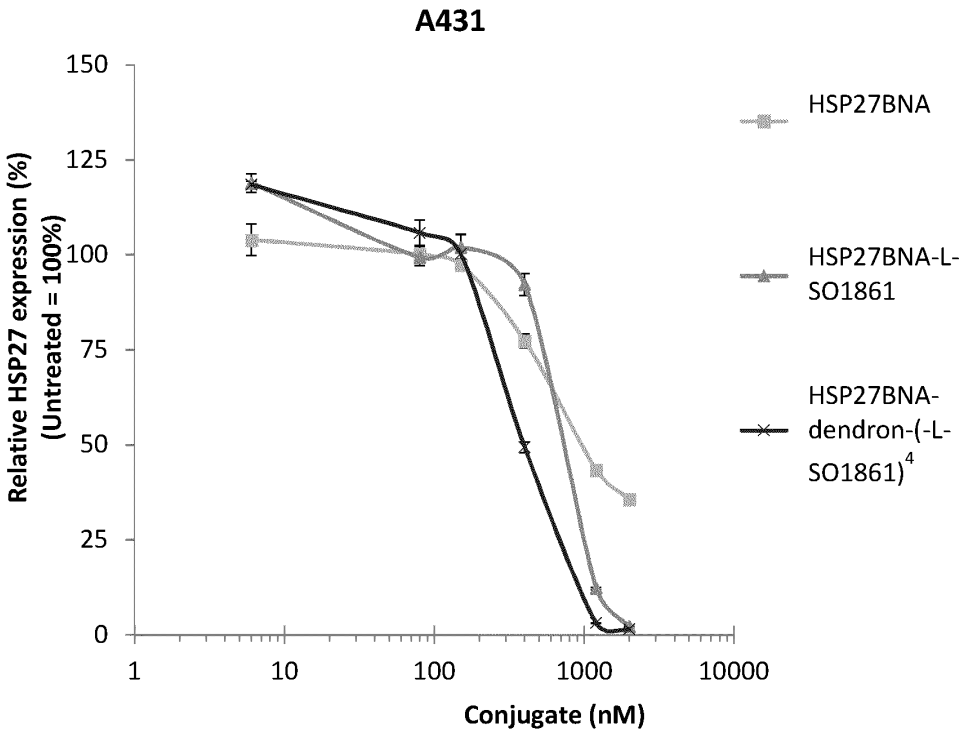

A431
Relative HSP27 expression (%)
(Untreated = 100%)
150
125
100
75
50
25
0
1
10
100
1000
10000
Conjugate (nM)
HSP27BNA
HSP27BNA-L-SO1861
HSP27BNA-dendron-(-L-SO1861)4

Figure 2

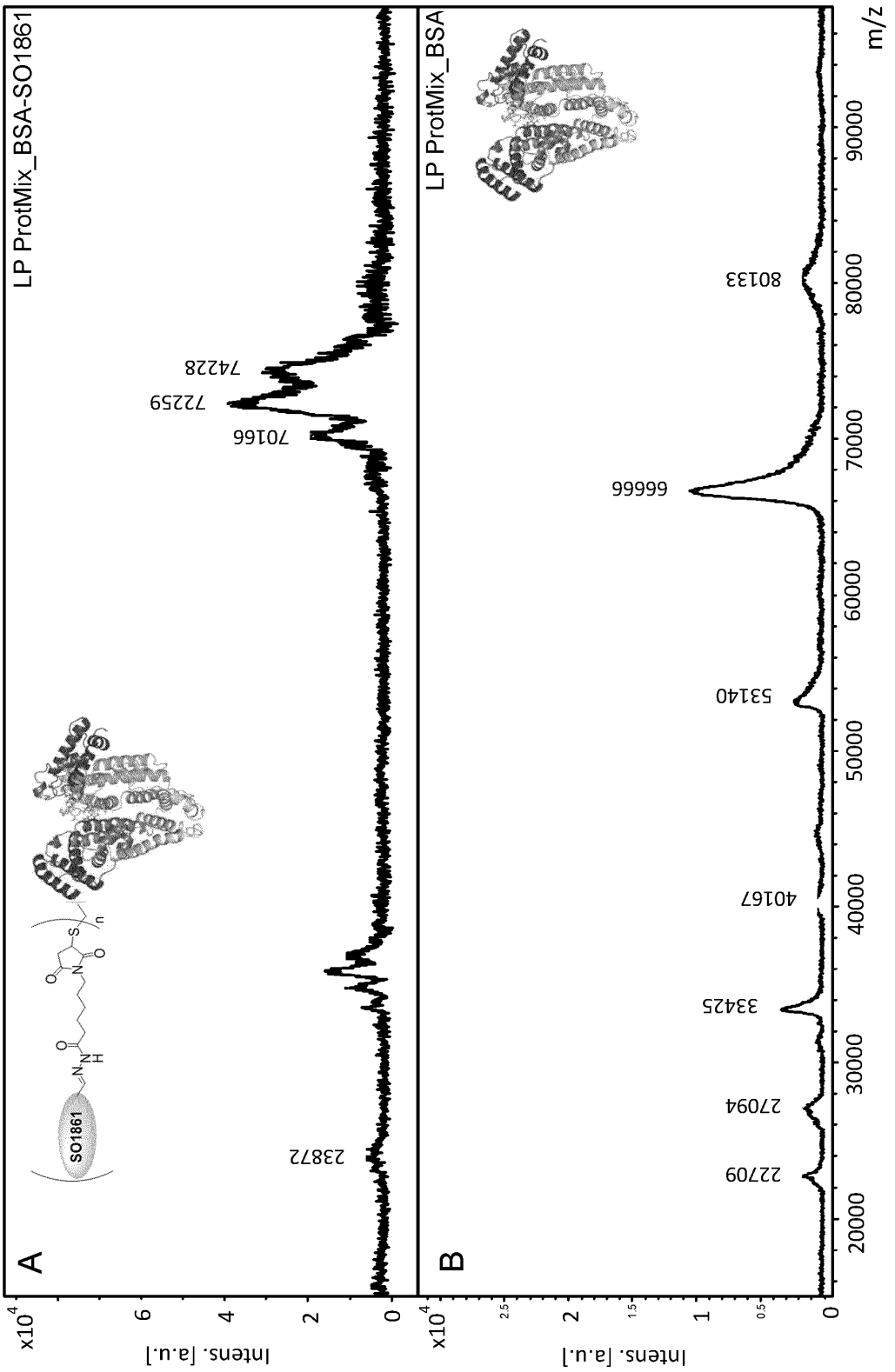

A
LP ProtMix_BSA-SO1861
SO1861
23872
70166
72259
74228
Intens. [a.u.]
x10^4
B
LP ProtMix_BSA
22709
27094
33425
40167
53140
66666
80133
m/z

Figure 3

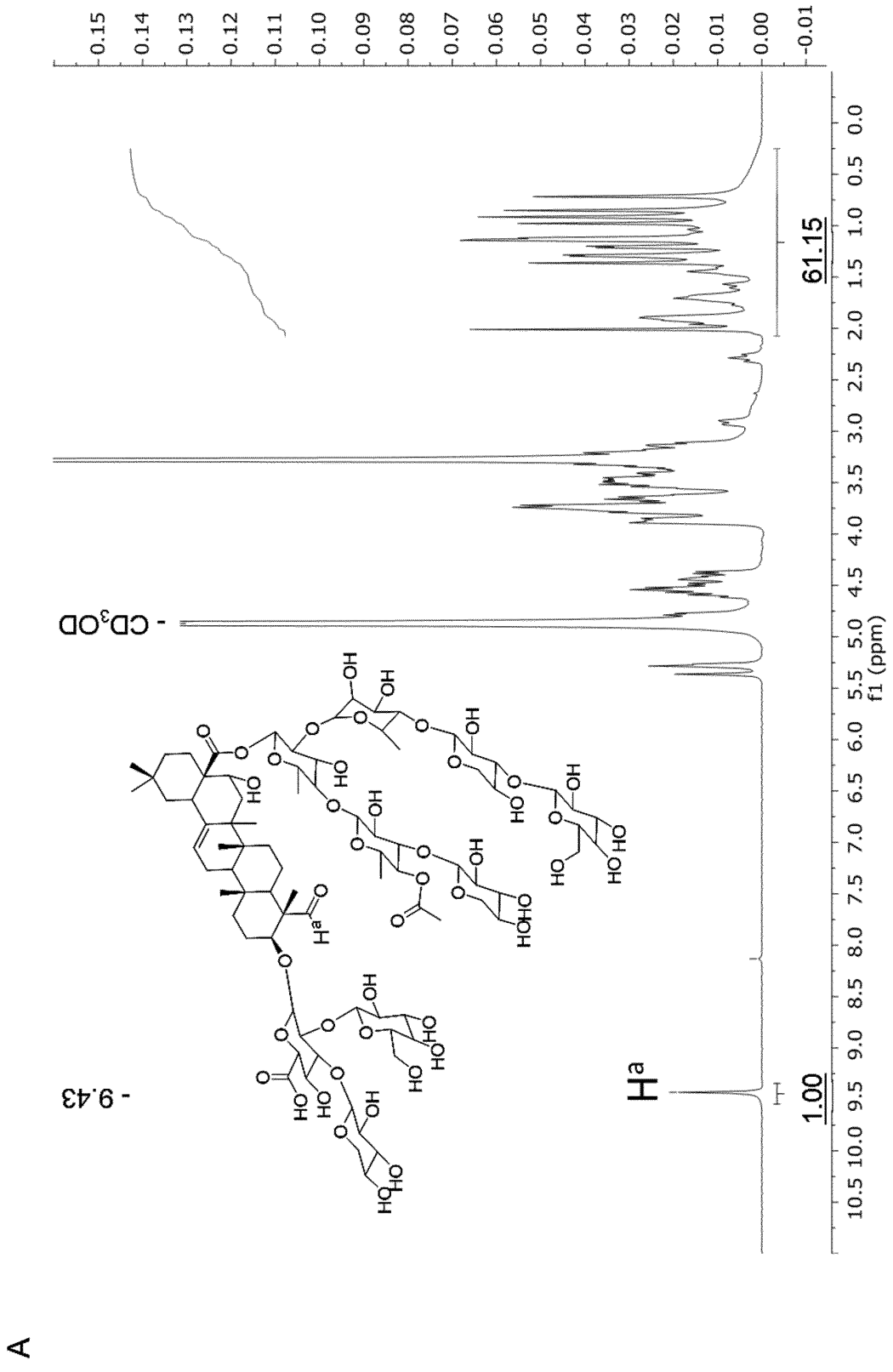


Figure 3

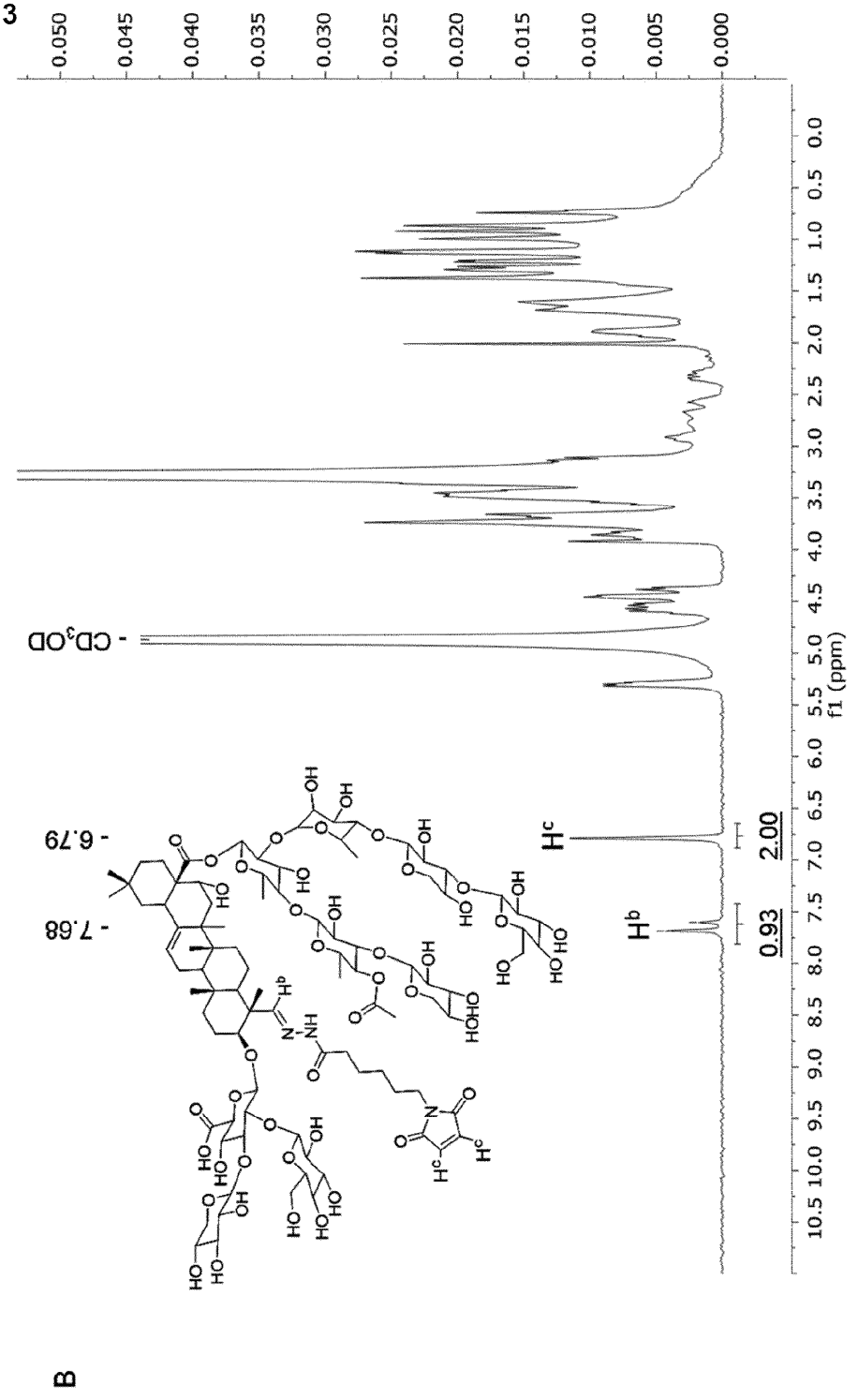


Figure 4

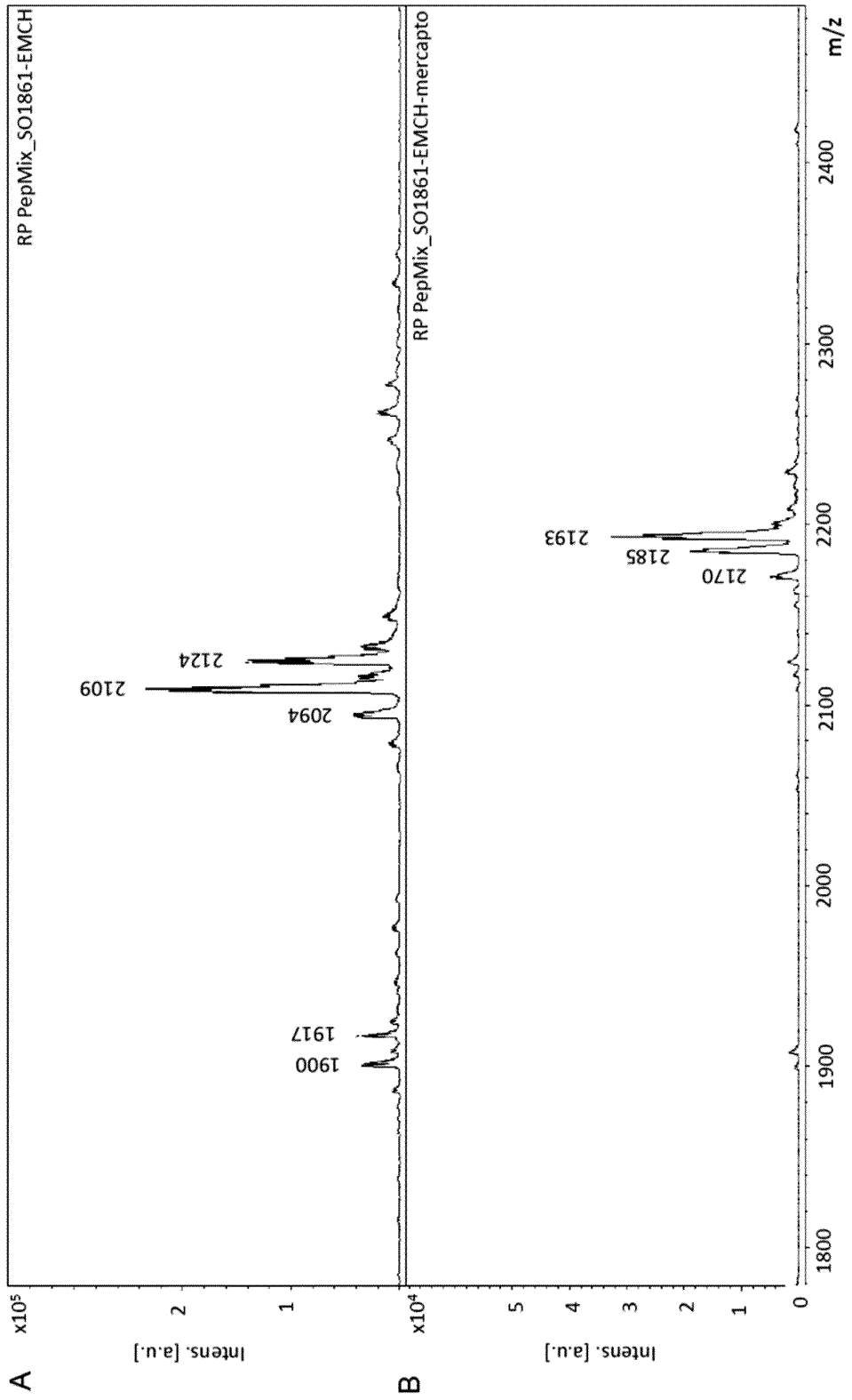

RP PepMix_SO1861-EMCH
RP PepMix_SO1861-EMCH-mercapto
A
B
x10⁵
x10⁴
Intens. [a.u.]
m/z
1800
1900
2000
2100
2200
2300
2400
1900
1917
2094
2109
2124
2170
2185
2193

Figure 5

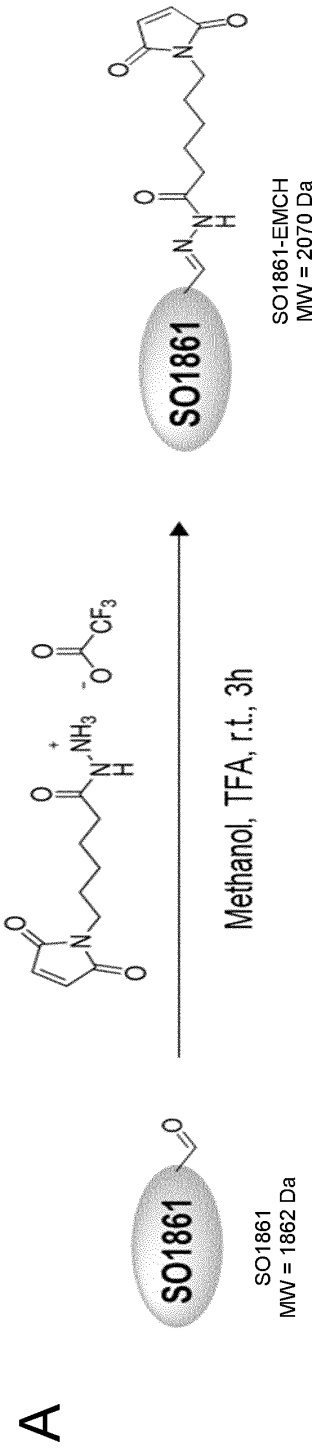

A
SO1861
SO1861
MW = 1862 Da
Methanol, TFA, r.t., 3h
SO1861
SO1861-EMCH
MW = 2070 Da

Figure 5

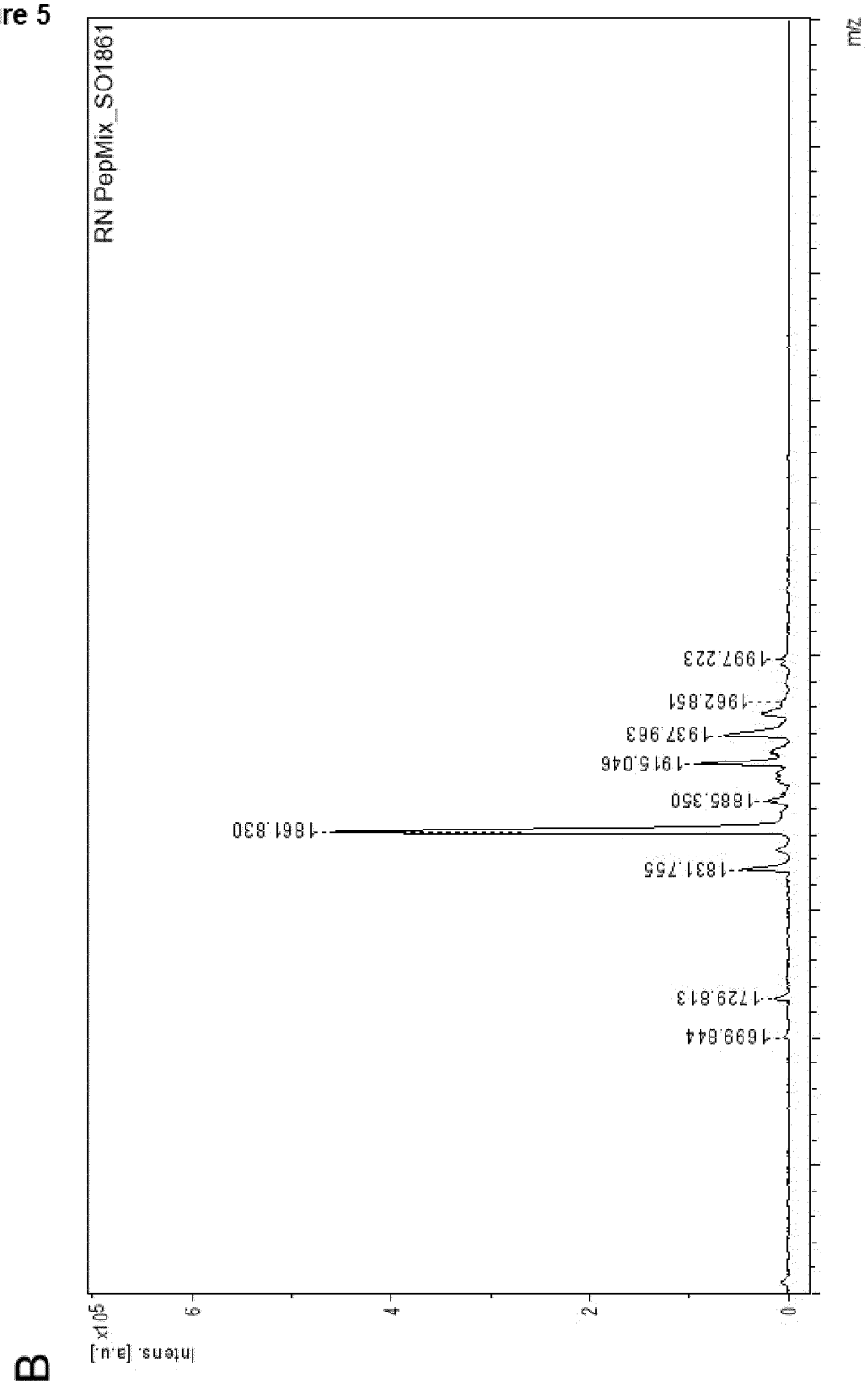
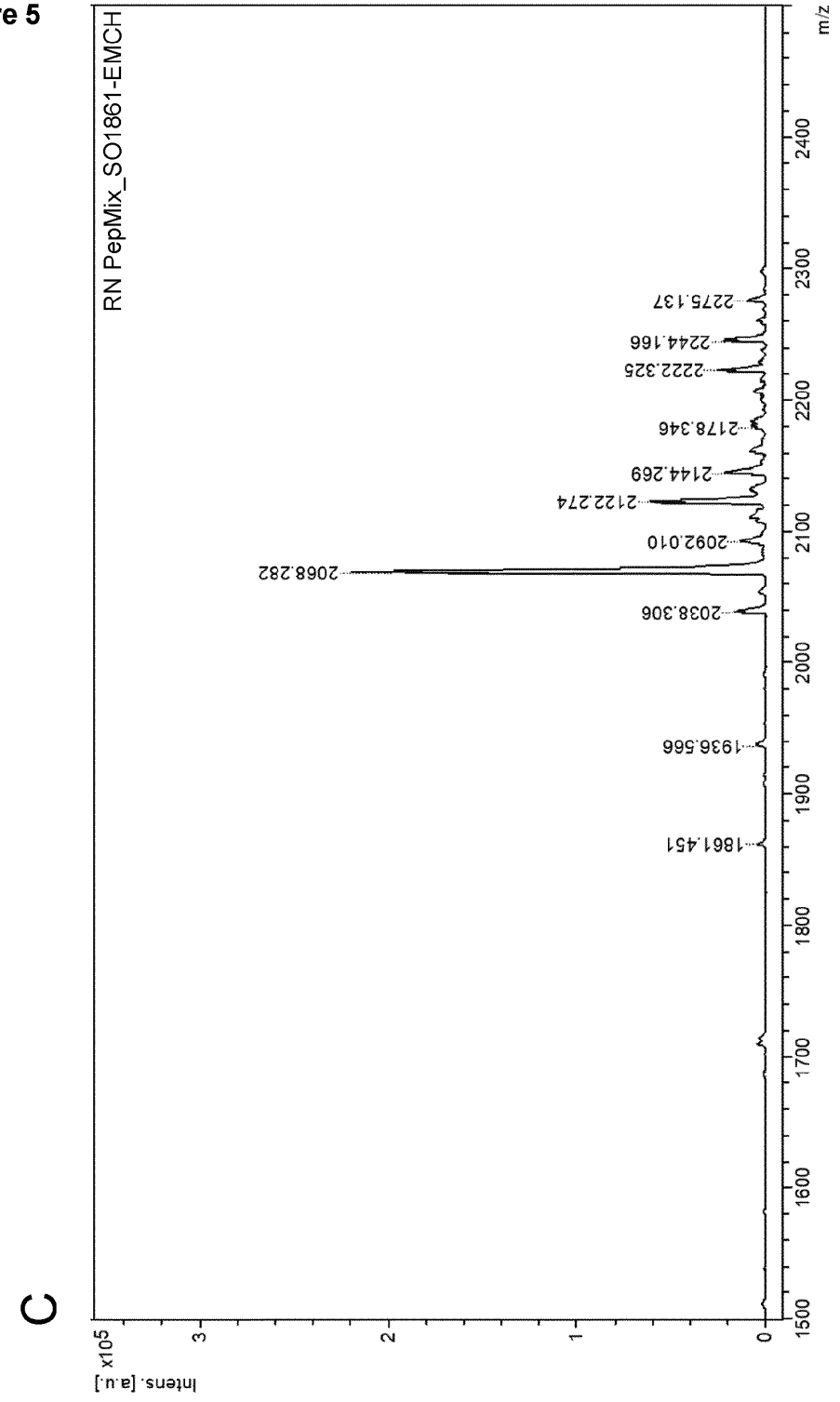

B
RN PepMix_SO1861
Intens. [a.u.]
x10^5
6
4
2
0
m/z
1997.223
1962.851
1937.963
1915.046
1885.350
1861.830
1831.755
1729.813
1699.844

Figure 5


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

C

Figure 6

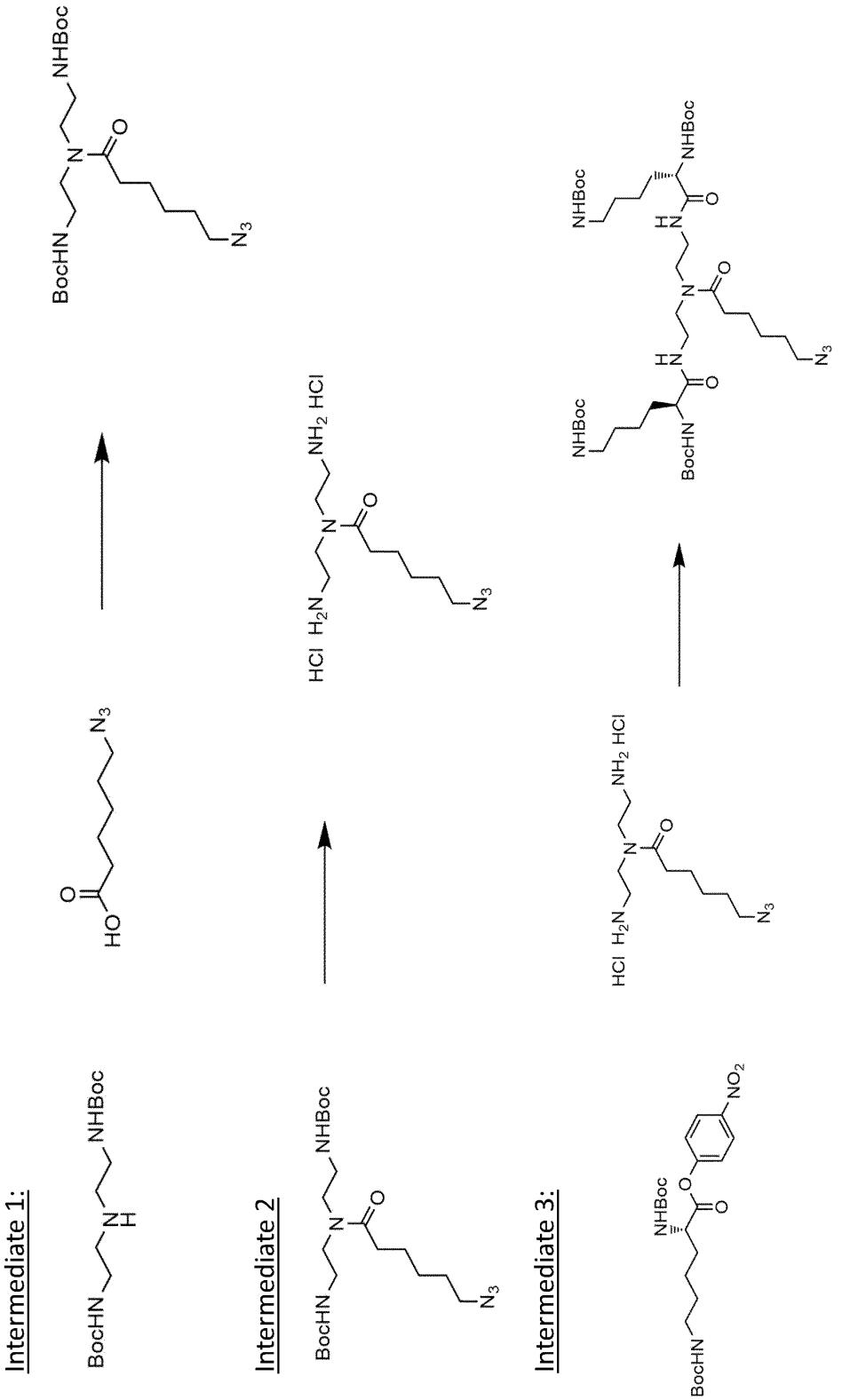

Intermediate 1:
Intermediate 2
Intermediate 3:
BocHN
NHBoc
HO
HCl H2N
NH2 HCl
N3
NO2
O

Figure 6

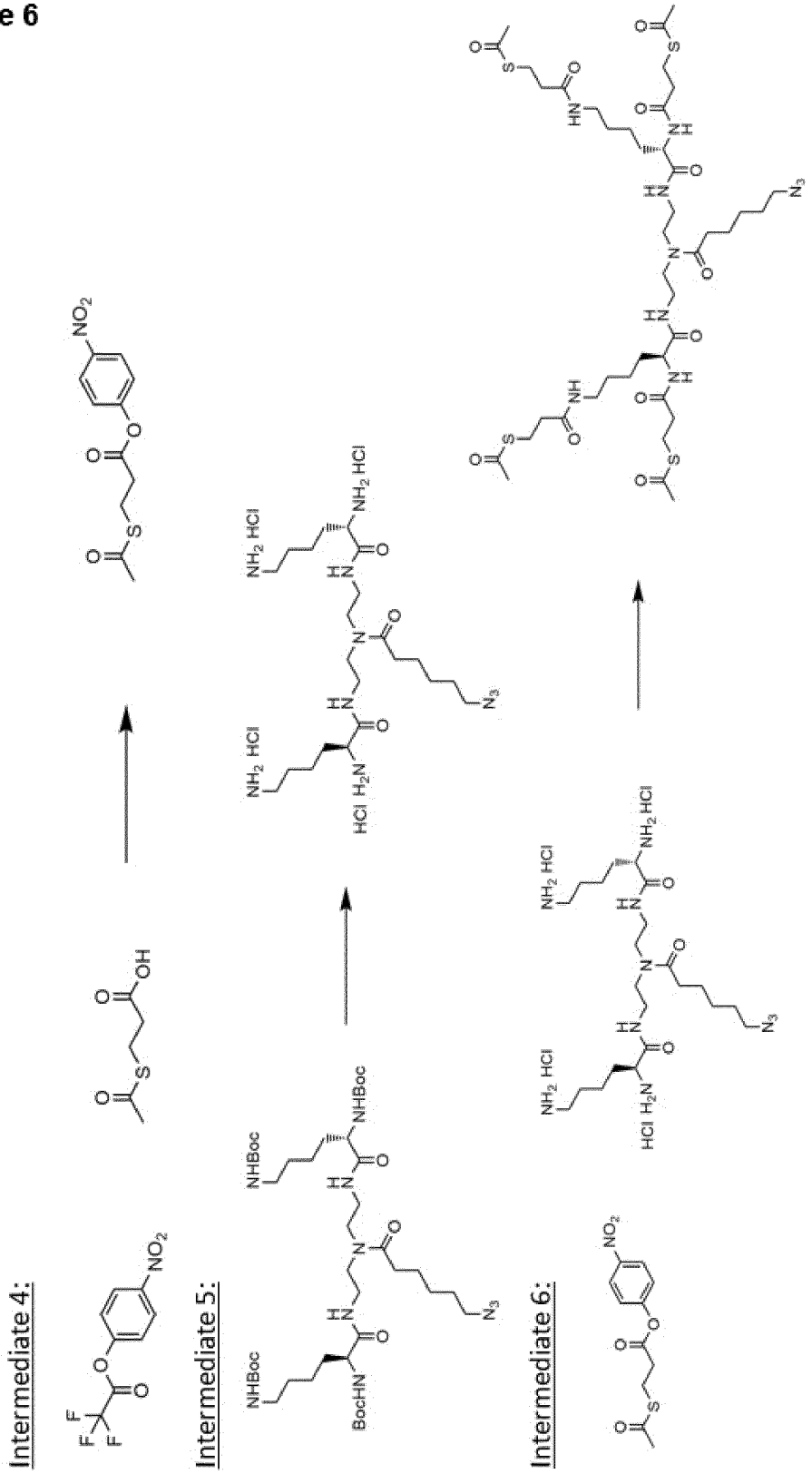


Figure 6

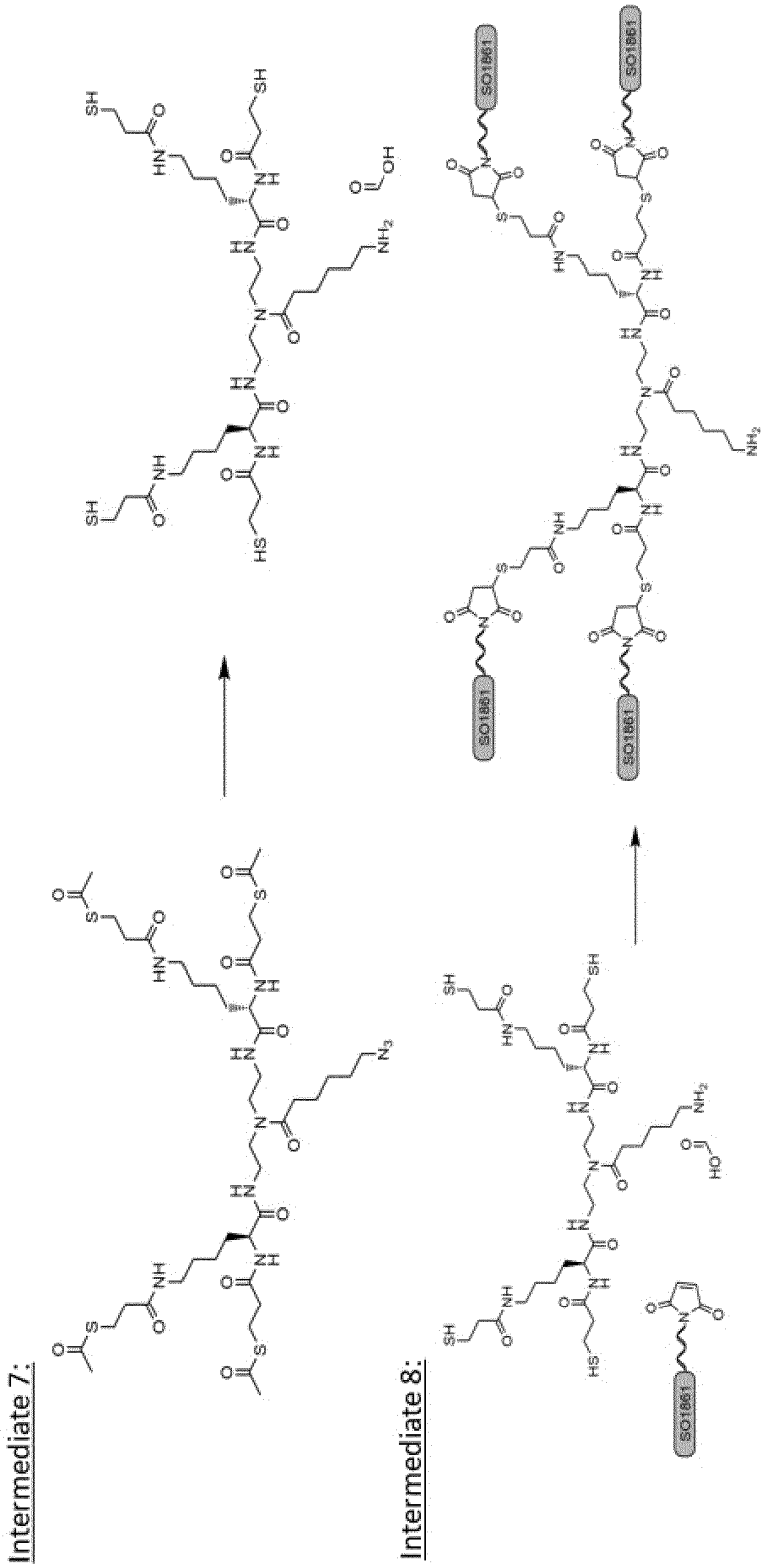


Figure 6

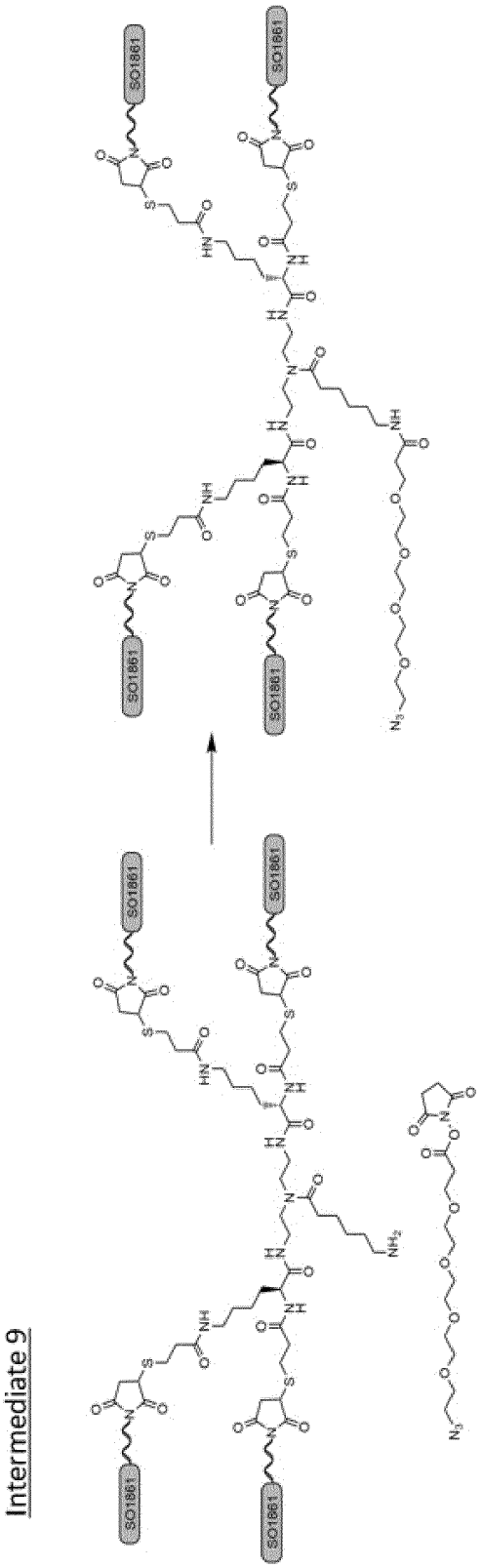
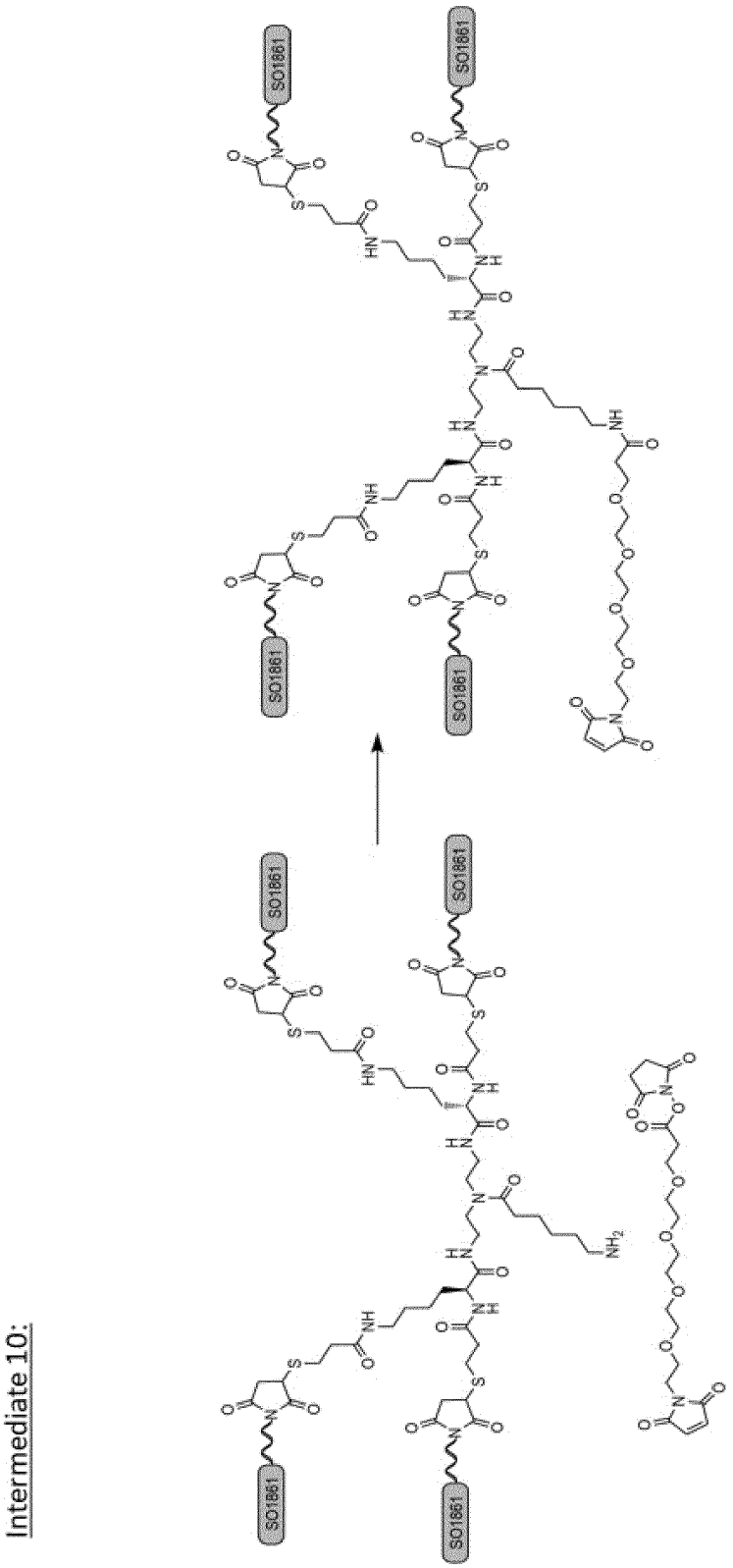


Figure 6



Intermediate 10:

Figure 6

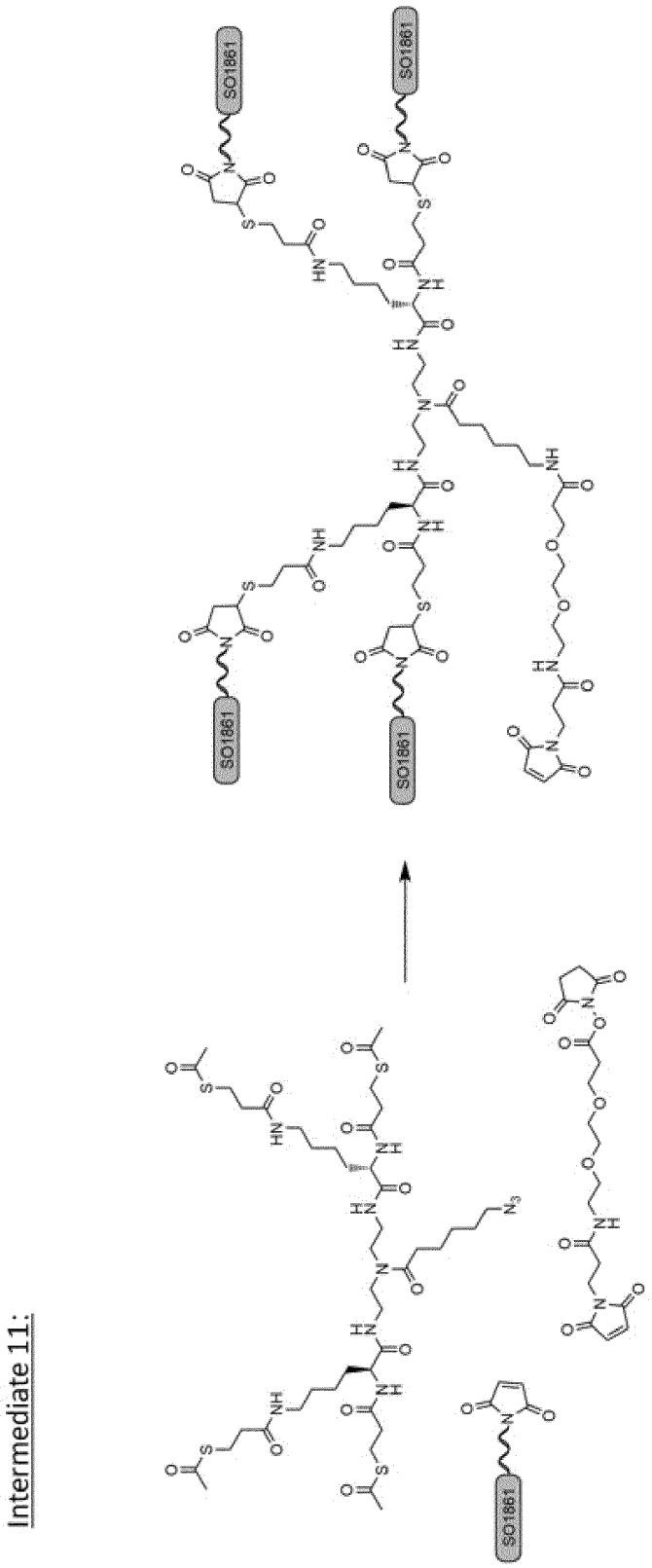
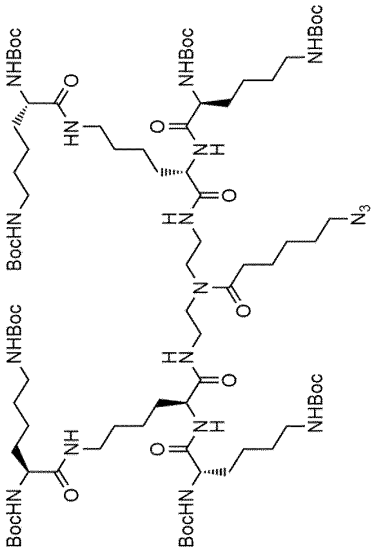
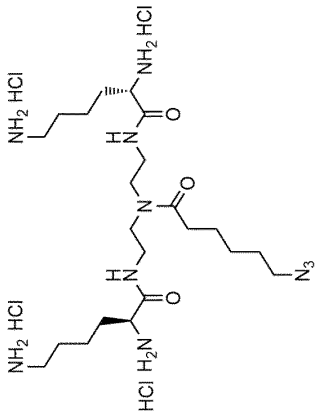


Figure 7





Intermediate 1:

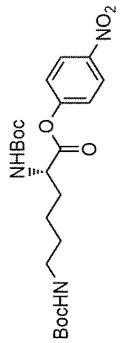


Figure 7

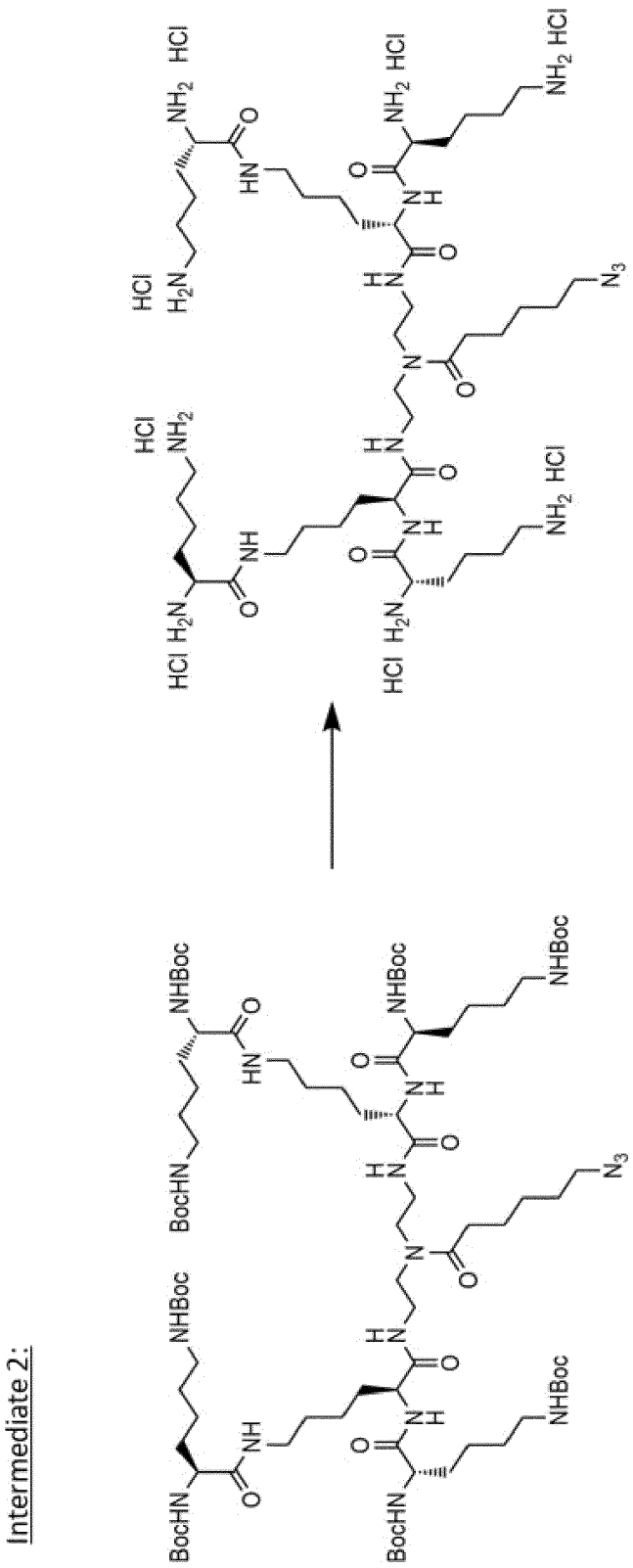


Figure 7

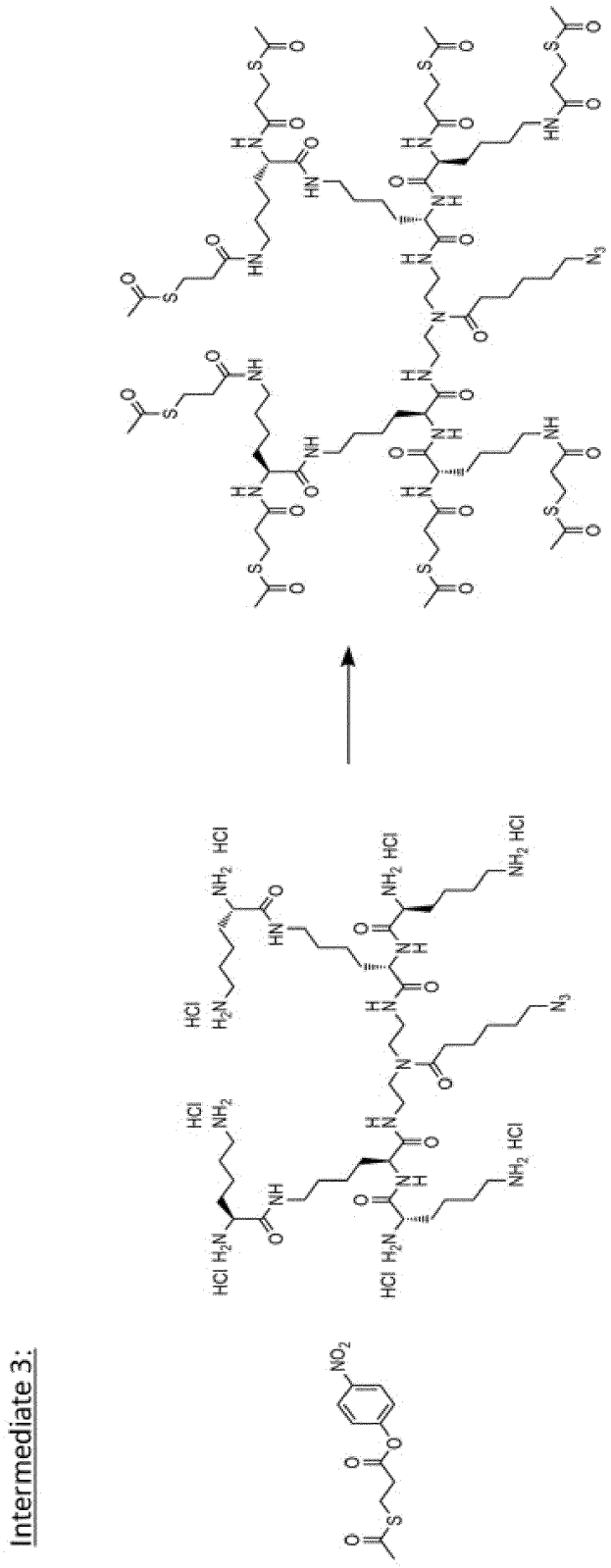
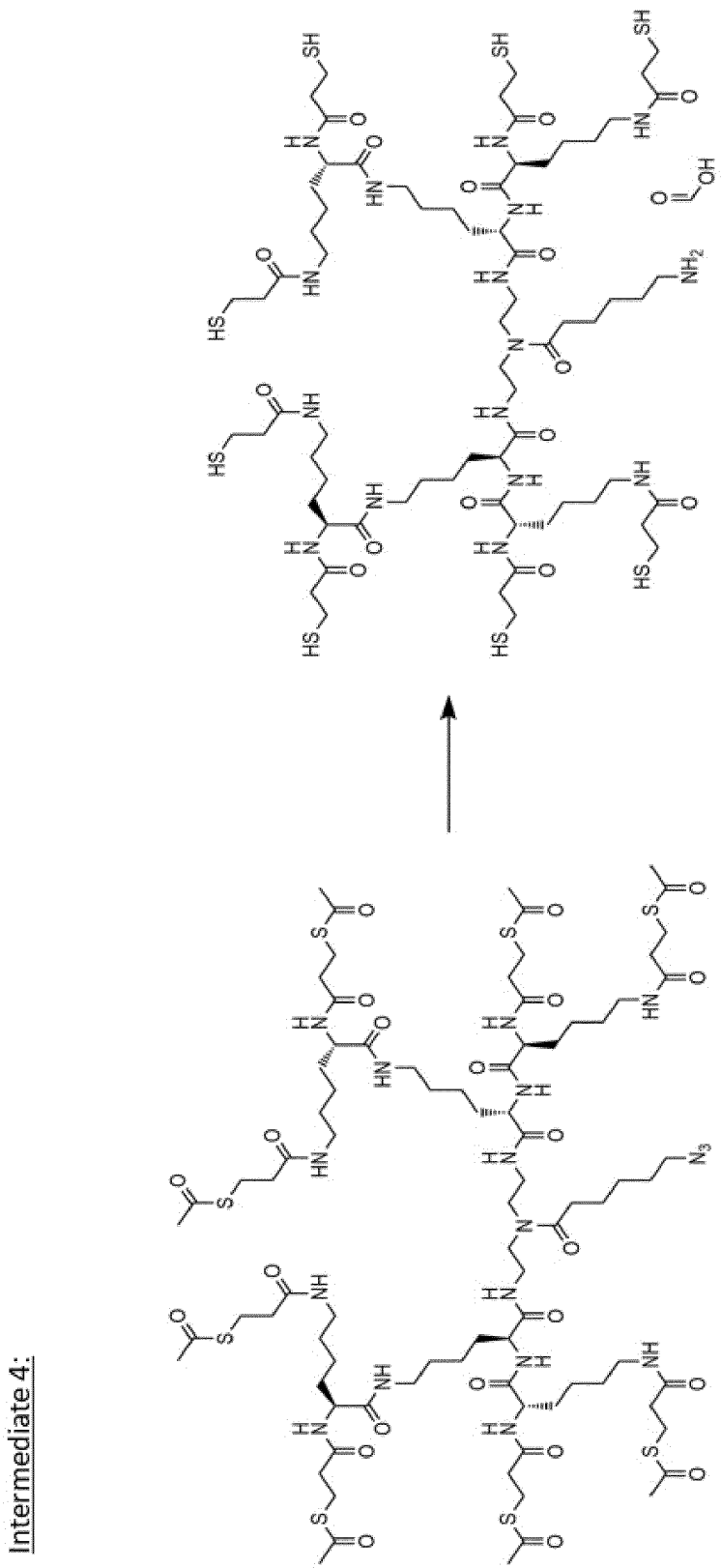


Figure 7



Intermediate 4:

Figure 7

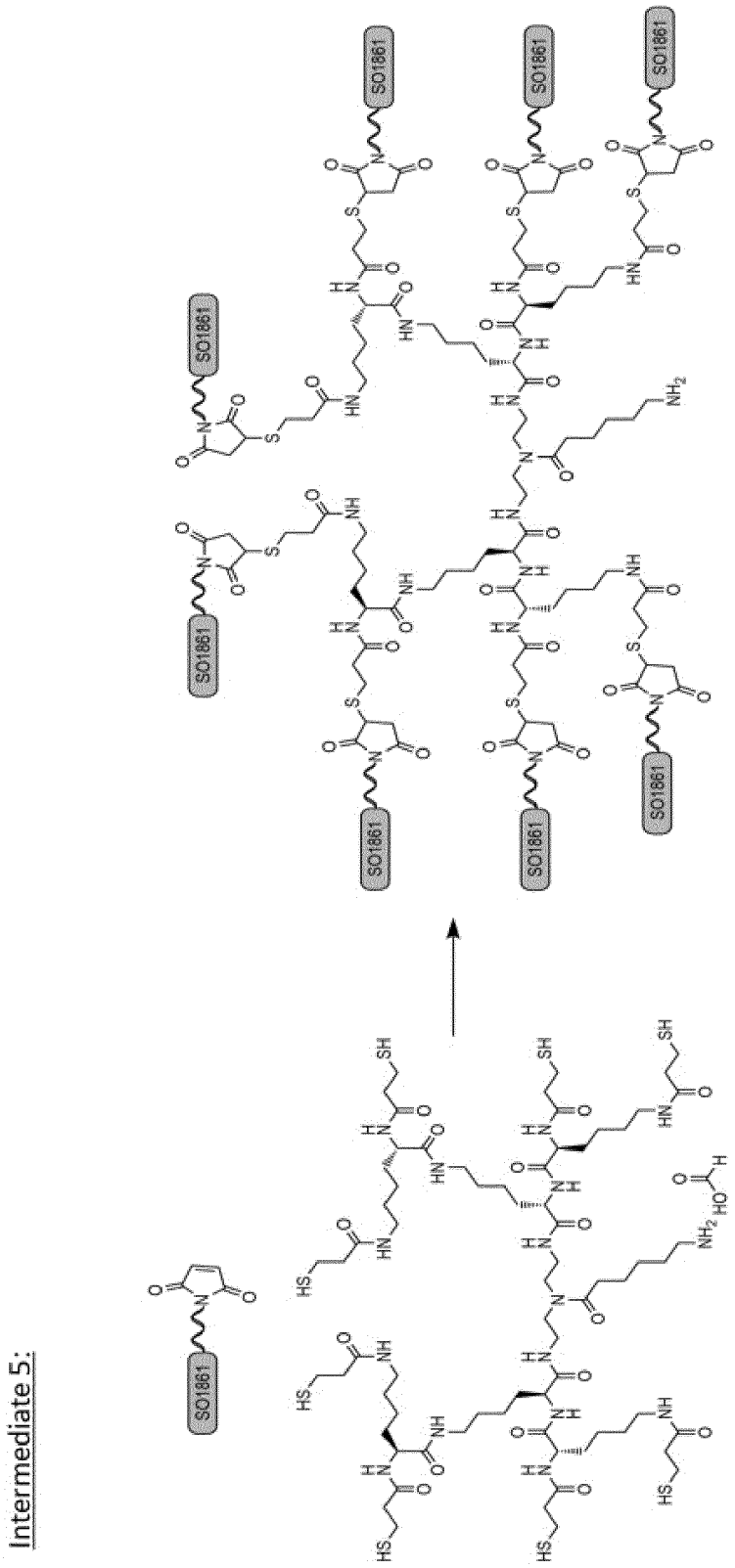


Figure 8

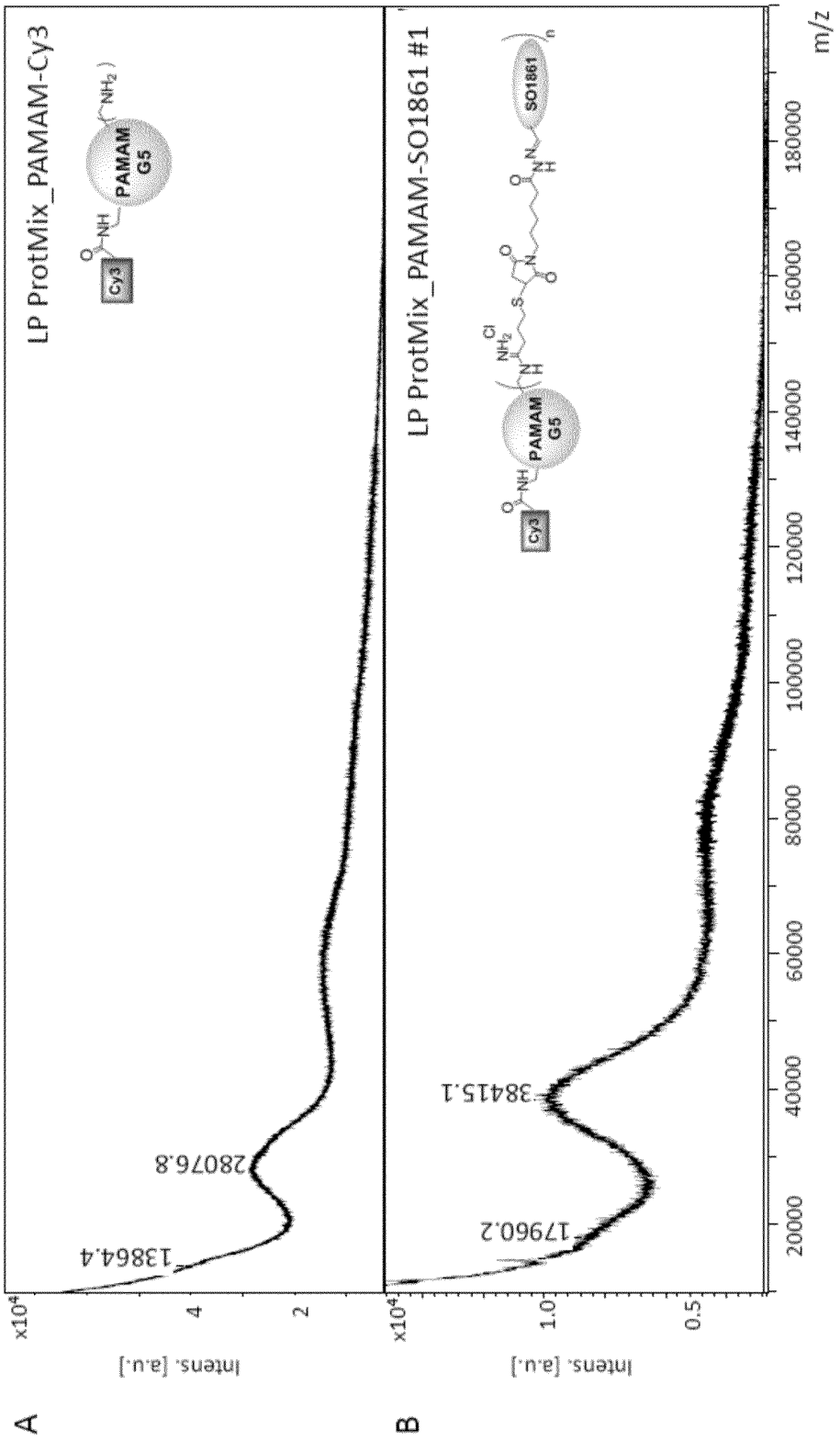

A
LP ProtMix_PAMAM-Cy3
PAMAM G5
Cy3
13864.4
28076.8
Intens. [a.u.]
x10^4
4
2
B
LP ProtMix_PAMAM-SO1861 #1
PAMAM G5
Cy3
SO1861
17960.2
38415.1
Intens. [a.u.]
x10^4
1.0
0.5
20000
40000
60000
80000
100000
120000
140000
160000
180000
m/z

Figure 8

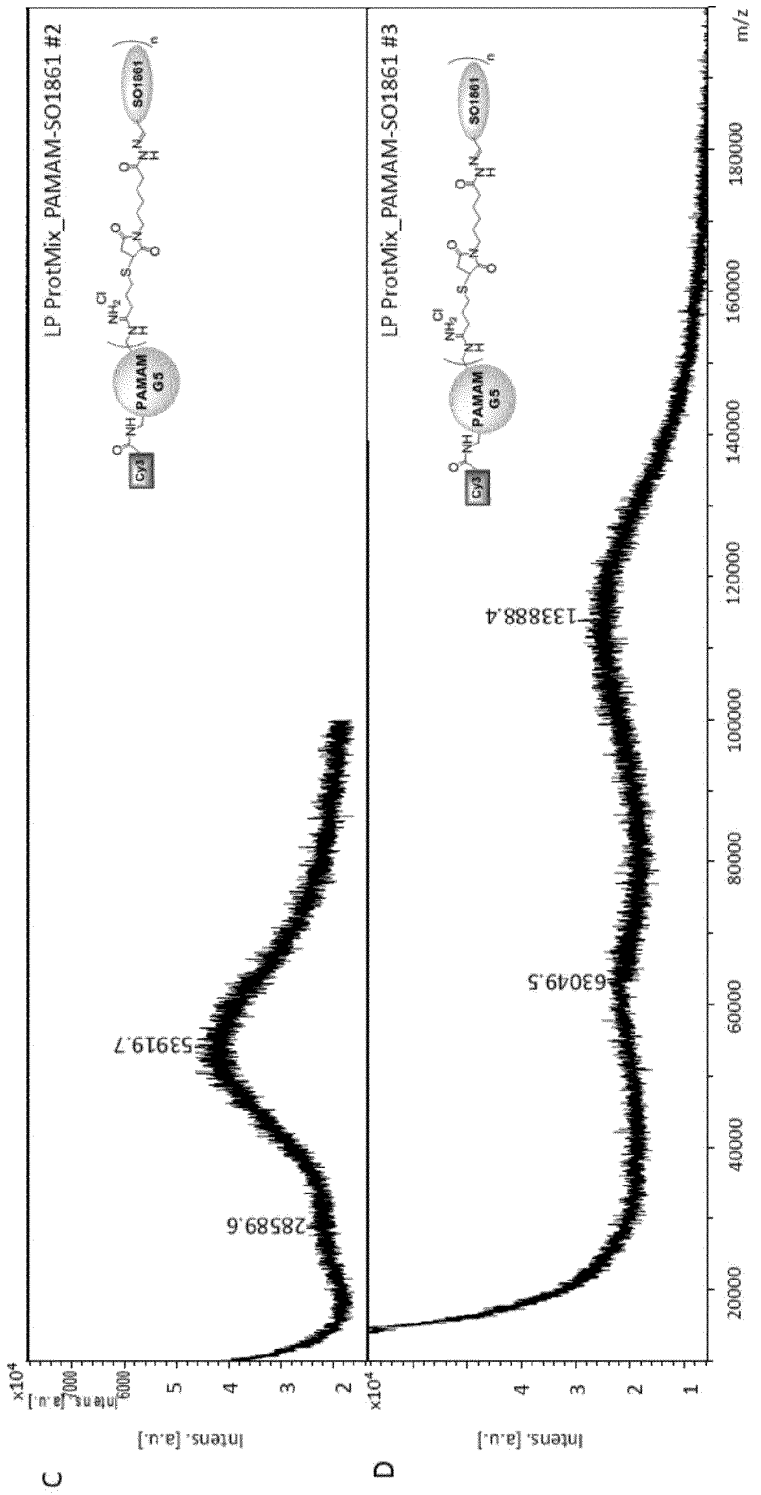

LP ProtMix_PAMAM-SO1861 #2
LP ProtMix_PAMAM-SO1861 #3
PAMAM G5
SO1861
Cy3
285896
539197
630495
1338884
Intens. [a.u.]
m/z
C
D

Figure 9

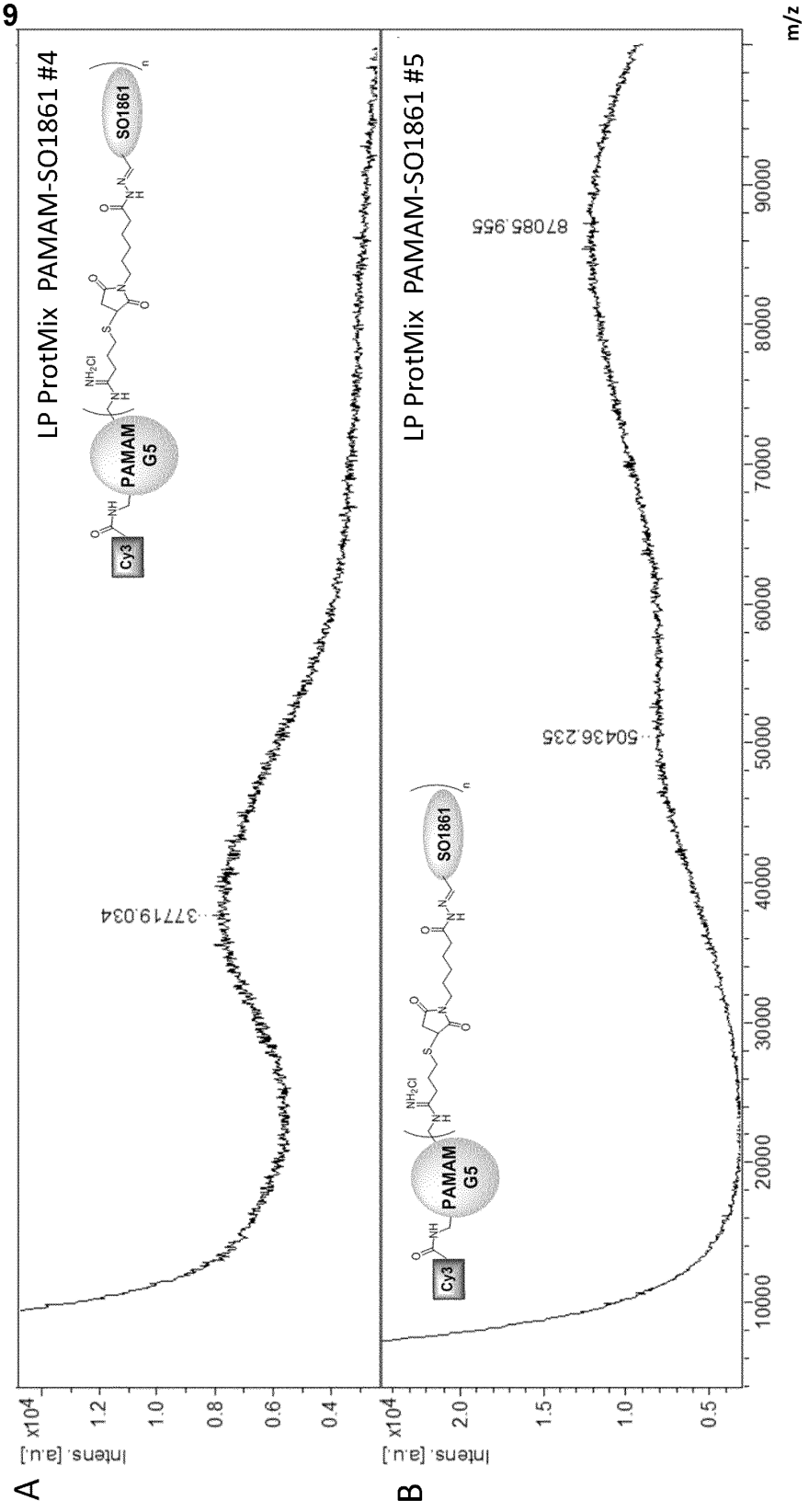

A
LP ProtMix PAMAM-SO1861 #4
Intens. [a.u.]
x10^4
1.2
1.0
0.8
0.6
0.4
37719.034
Cy3
PAMAM G5
SO1861
B
LP ProtMix PAMAM-SO1861 #5
Intens. [a.u.]
x10^4
2.0
1.5
1.0
0.5
87085.955
50436.235
10000
20000
30000
40000
50000
60000
70000
80000
90000
m/z

Figure 10

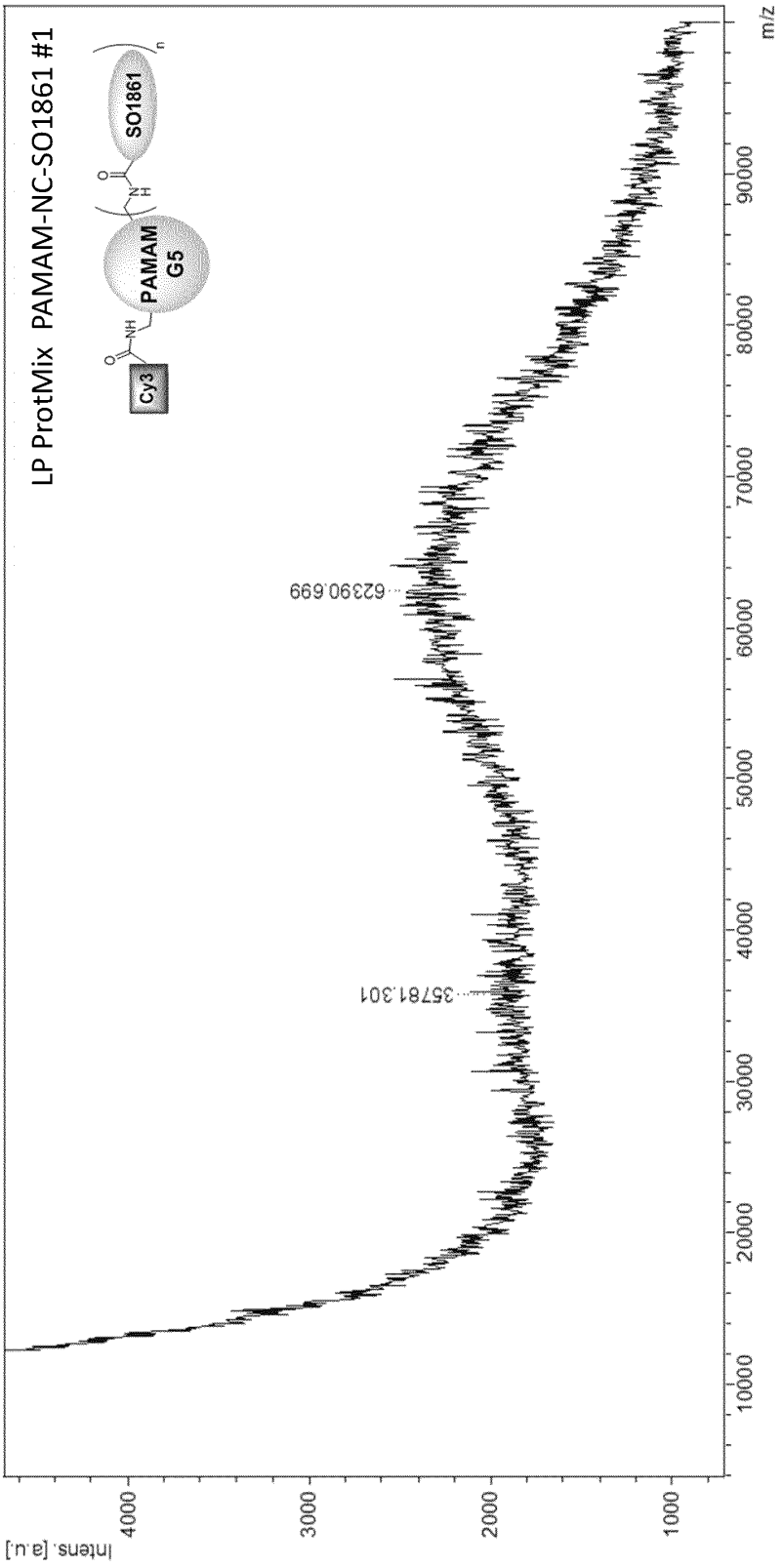

LP ProtMix PAMAM-NC-SO1861 #1
SO1861
PAMAM
G5
Cy3
62390.699
35781.301
Intens. [a.u.]
4000
3000
2000
1000
m/z
10000
20000
30000
40000
50000
60000
70000
80000
90000

Figure 11

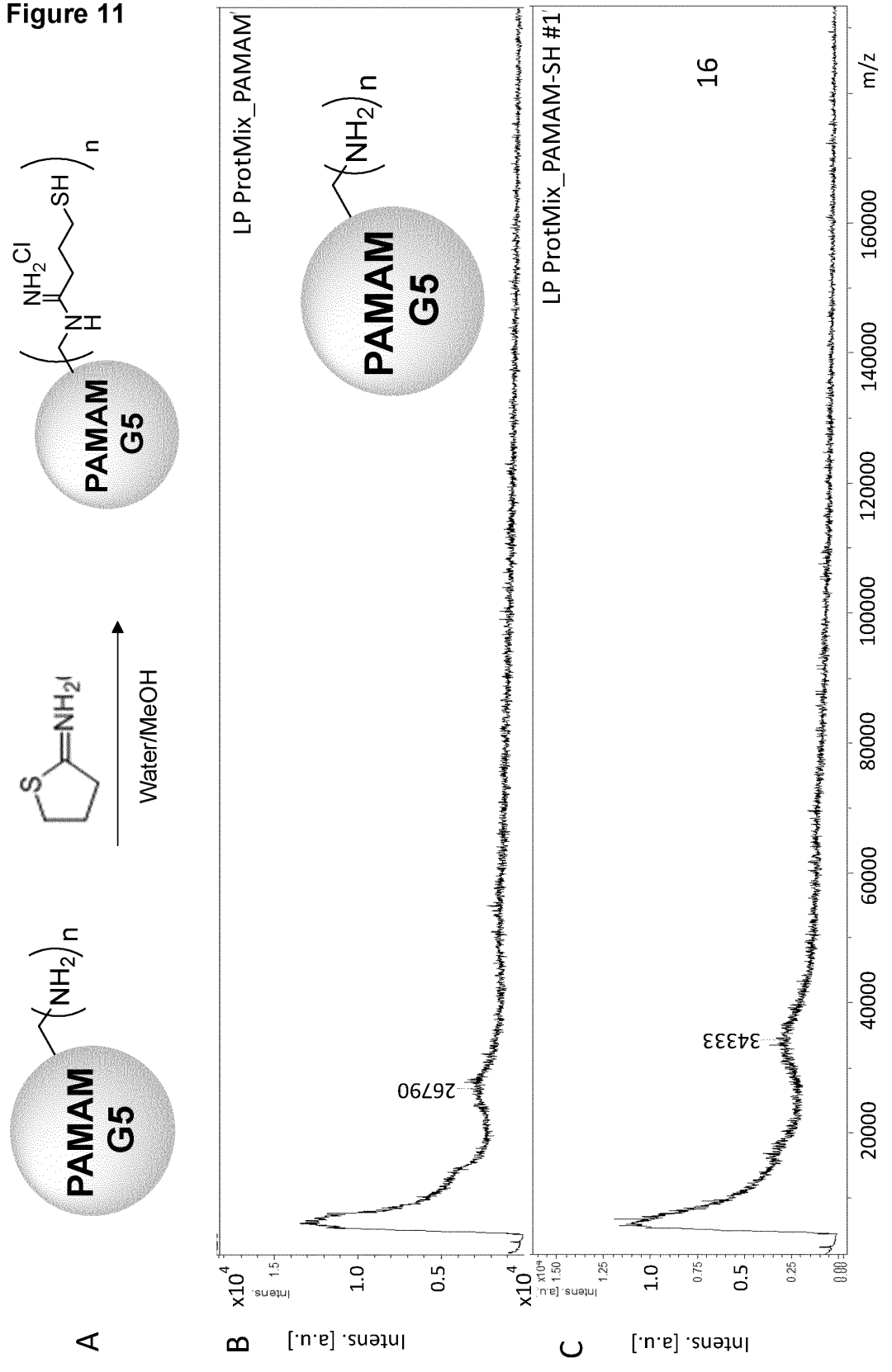

A
PAMAM G5
(NH2)n
Water/MeOH
PAMAM G5
NH2Cl
NH
SH
n
B
LP ProtMix_PAMAM'
PAMAM G5
(NH2)n
26790
Intens. [a.u.]
x10^4
1.5
1.0
0.5
C
LP ProtMix_PAMAM-SH #1'
16
34333
Intens. [a.u.]
x10^4
1.50
1.25
1.0
0.75
0.5
0.25
0.00
20000
40000
60000
80000
100000
120000
140000
160000
m/z

Figure 11

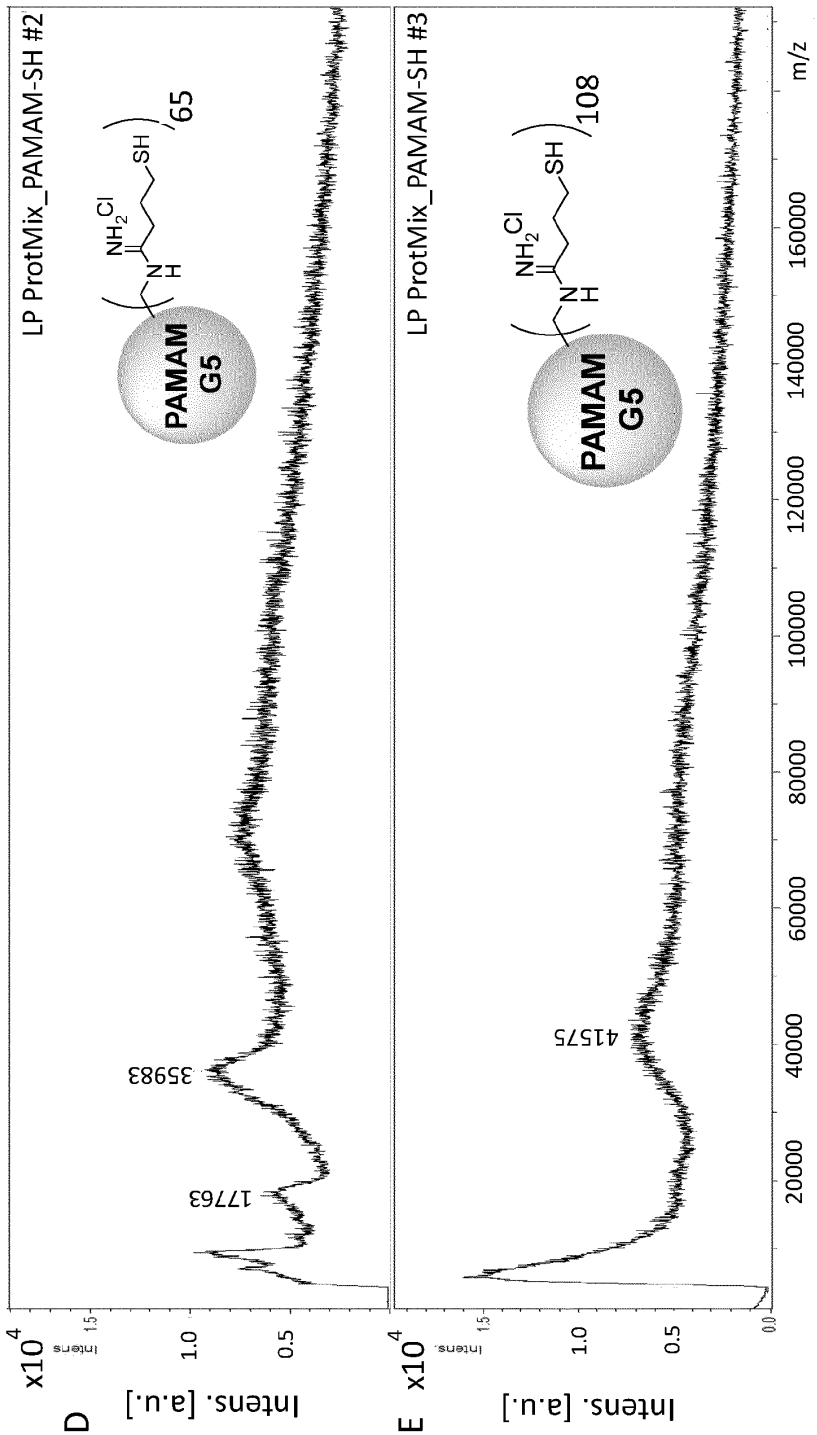

D
LP ProtMix_PAMAM-SH #2
Intens. [a.u.]
x10^4
17763
35983
PAMAM G5
65
E
LP ProtMix_PAMAM-SH #3
Intens. [a.u.]
41575
PAMAM G5
108
m/z
20000
40000
60000
80000
100000
120000
140000
160000

Figure 12

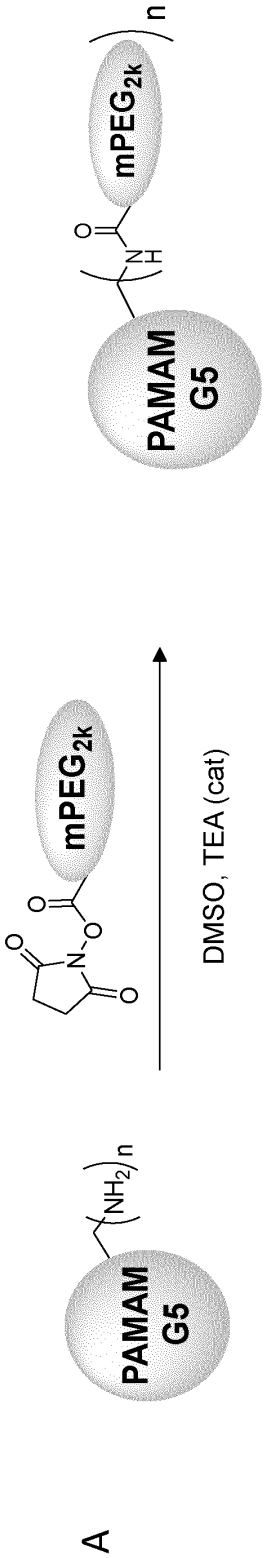


Figure 12

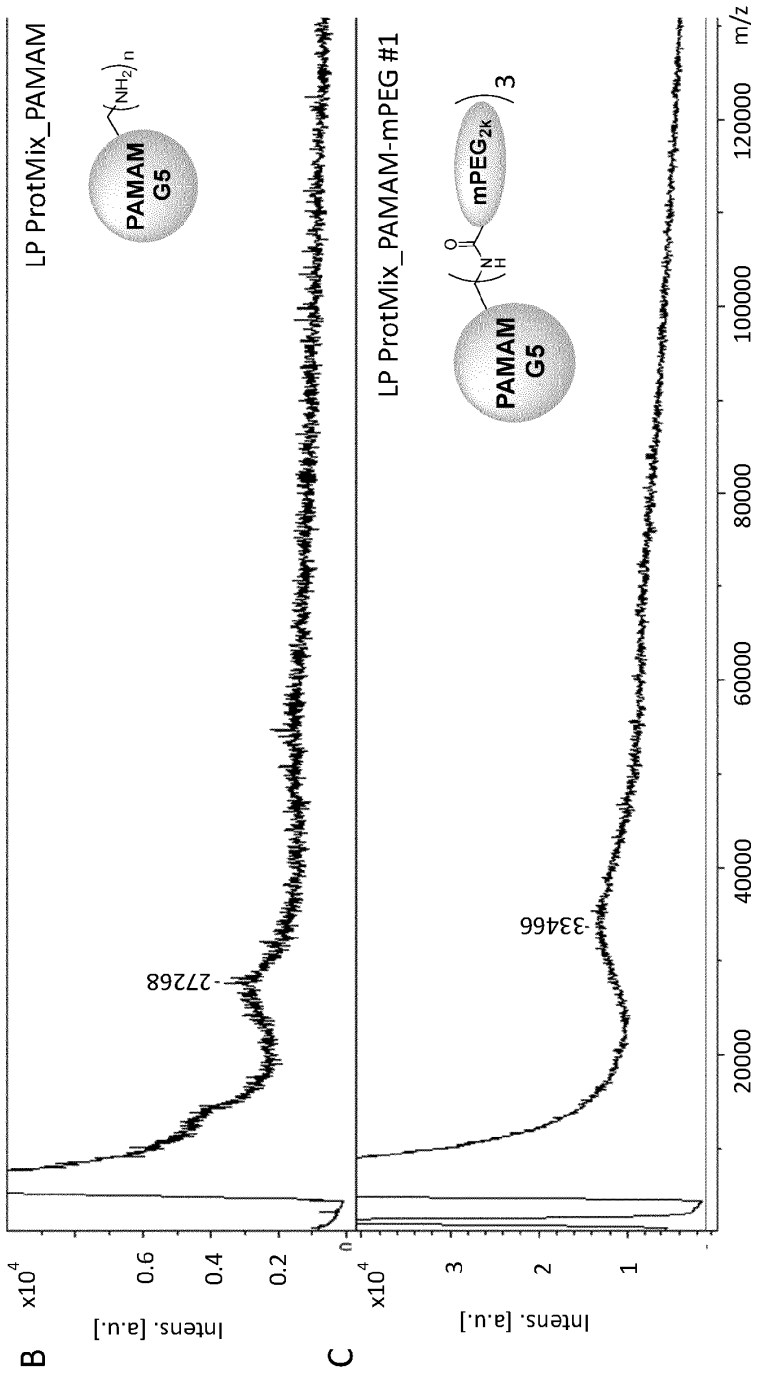

B
LP ProtMix_PAMAM
PAMAM G5
$(NH_2)_n$
27268
Intens. [a.u.]
x10^4
0.6
0.4
0.2
0
C
LP ProtMix_PAMAM-mPEG #1
PAMAM G5
mPEG2k
3
33466
Intens. [a.u.]
x10^4
3
2
1
20000
40000
60000
80000
100000
120000
m/z

Figure 12

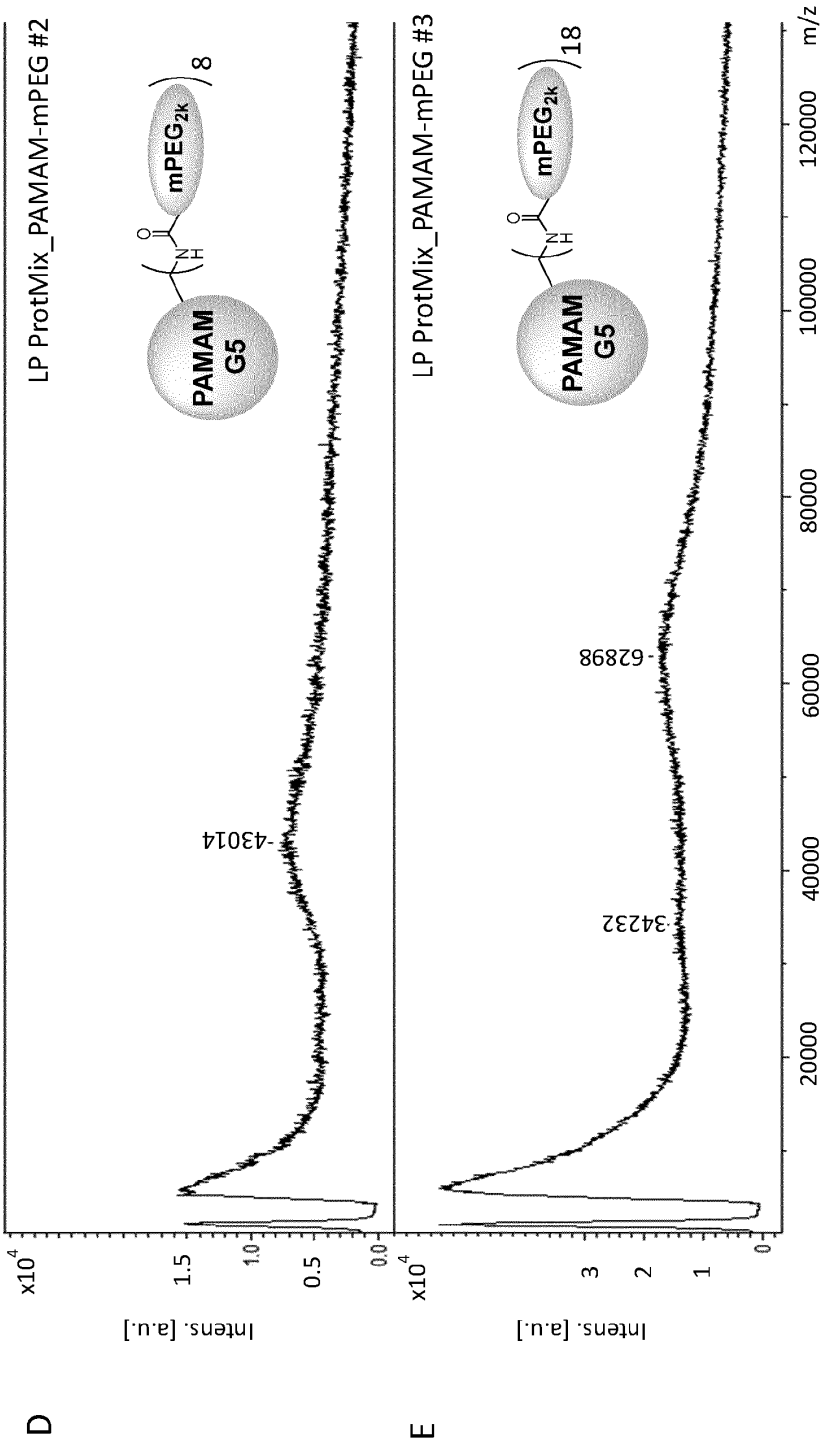
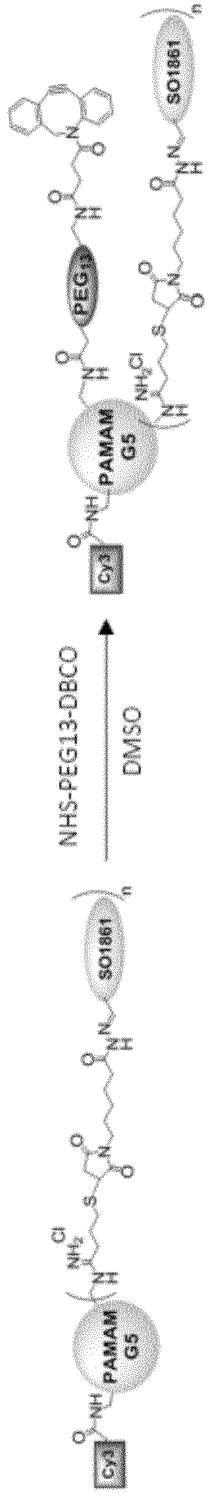

LP ProtMix_PAMAM-mPEG #2
PAMAM G5
mPEG2k
8
43014
Intens. [a.u.]
x10^4
1.5
1.0
0.5
0.0
D
LP ProtMix_PAMAM-mPEG #3
PAMAM G5
mPEG2k
18
62898
34232
Intens. [a.u.]
x10^4
3
2
1
0
E
20000
40000
60000
80000
100000
120000
m/z

Figure 13


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

A

Figure 13

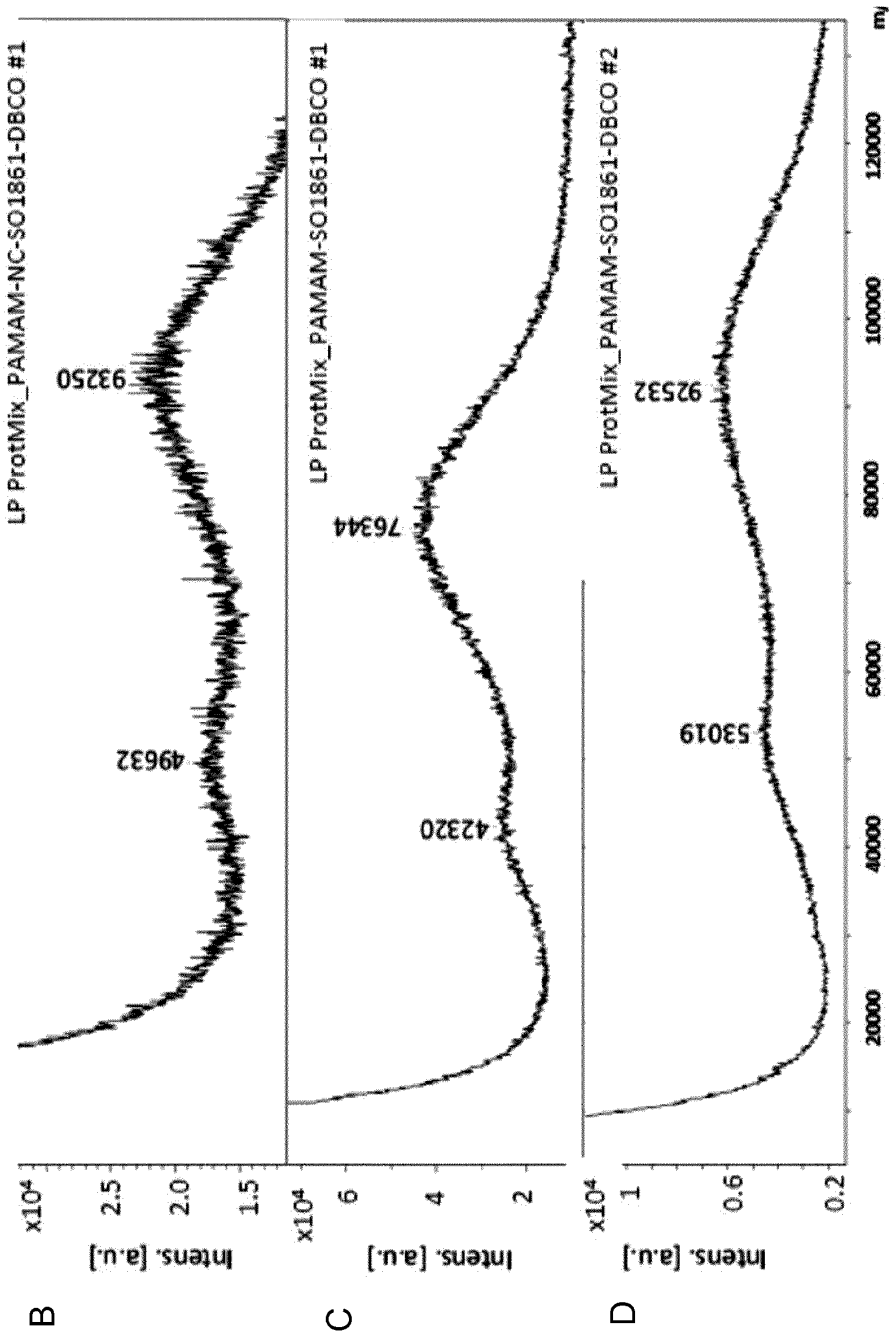

B
C
D
Intens. [a.u.]
x10⁴
2.5
2.0
1.5
6
4
2
1
0.6
0.2
LP ProtMix_PAMAM-NC-SO1861-DBCO #1
LP ProtMix_PAMAM-SO1861-DBCO #1
LP ProtMix_PAMAM-SO1861-DBCO #2
93250
49632
76344
42320
92532
53019
20000
40000
60000
80000
100000
120000
m/

Figure 14

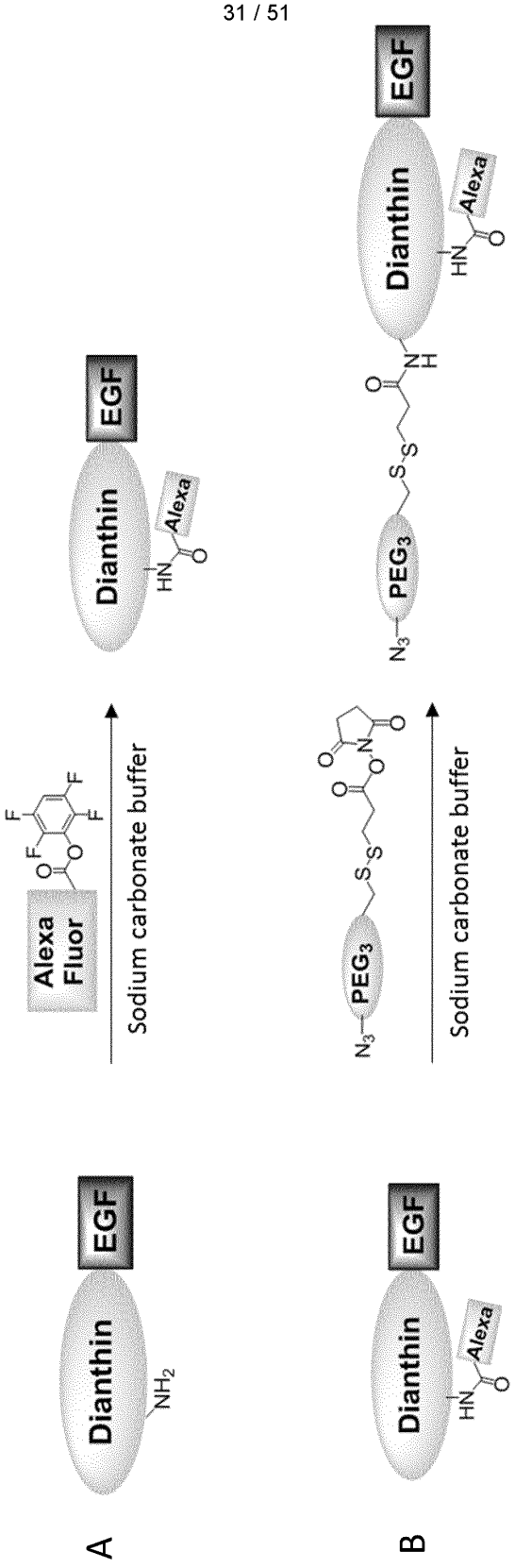

EGF
Dianthin
NH2
A
Alexa Fluor
Sodium carbonate buffer
EGF
Dianthin
Alexa
HN
O
F
F
F
F
O
O
EGF
Dianthin
Alexa
HN
O
N
H
O
S
S
PEG3
N3
B
Sodium carbonate buffer
O
O
N
O
O
S
S
PEG3
N3
EGF
Dianthin
Alexa
HN
O

Figure 14

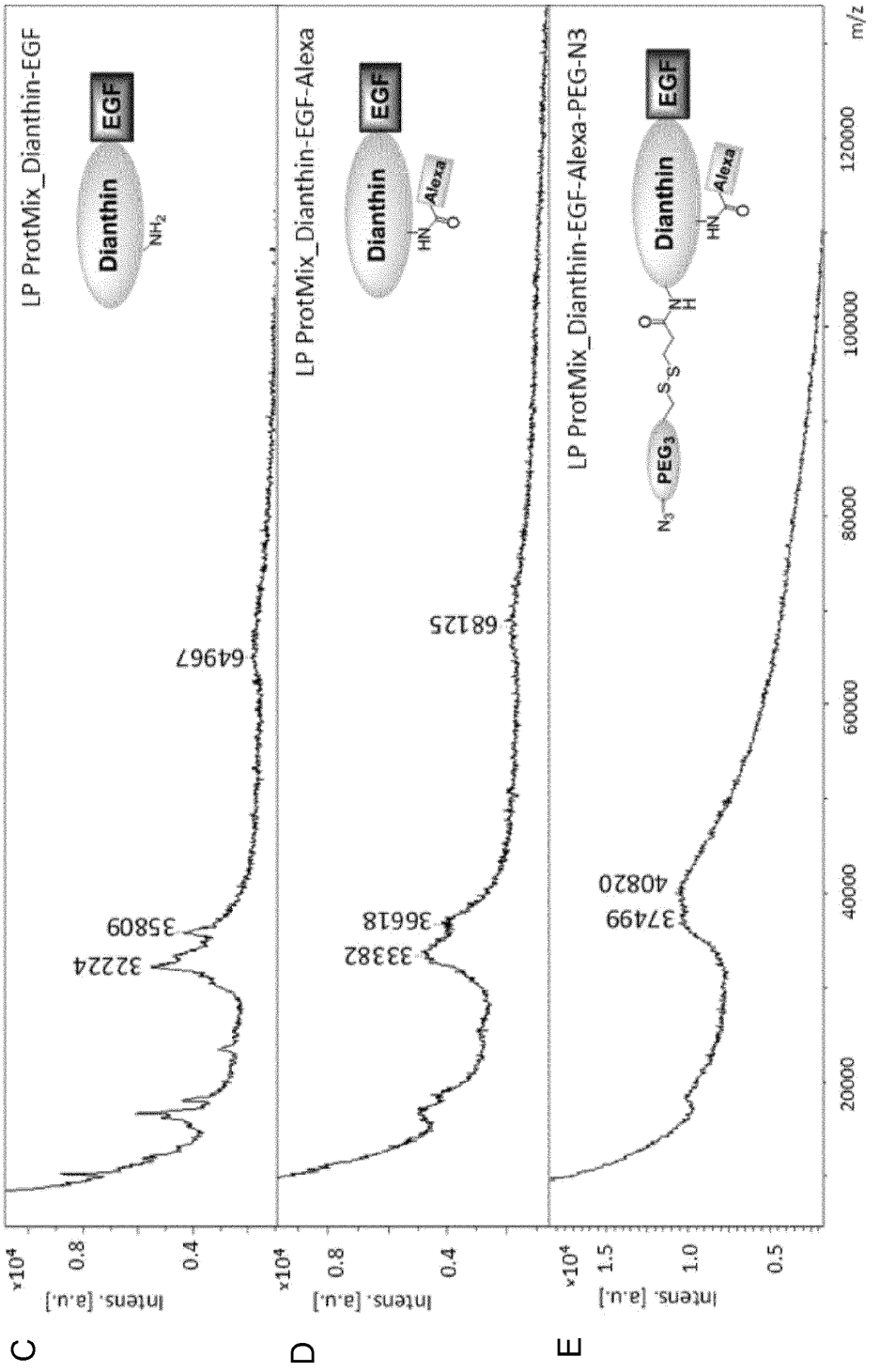

LP ProtMix_Dianthin-EGF
Dianthin
EGF
NH2
64967
35809
32224
Intens. [a.u.]
x10^4
0.8
0.4
C
LP ProtMix_Dianthin-EGF-Alexa
Dianthin
EGF
Alexa
HN
O
68125
36618
33382
Intens. [a.u.]
x10^4
0.8
0.4
D
LP ProtMix_Dianthin-EGF-Alexa-PEG-N3
Dianthin
EGF
Alexa
HN
O
N
H
O
S
S
PEG3
N3
40820
37499
Intens. [a.u.]
x10^4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

Figure 15

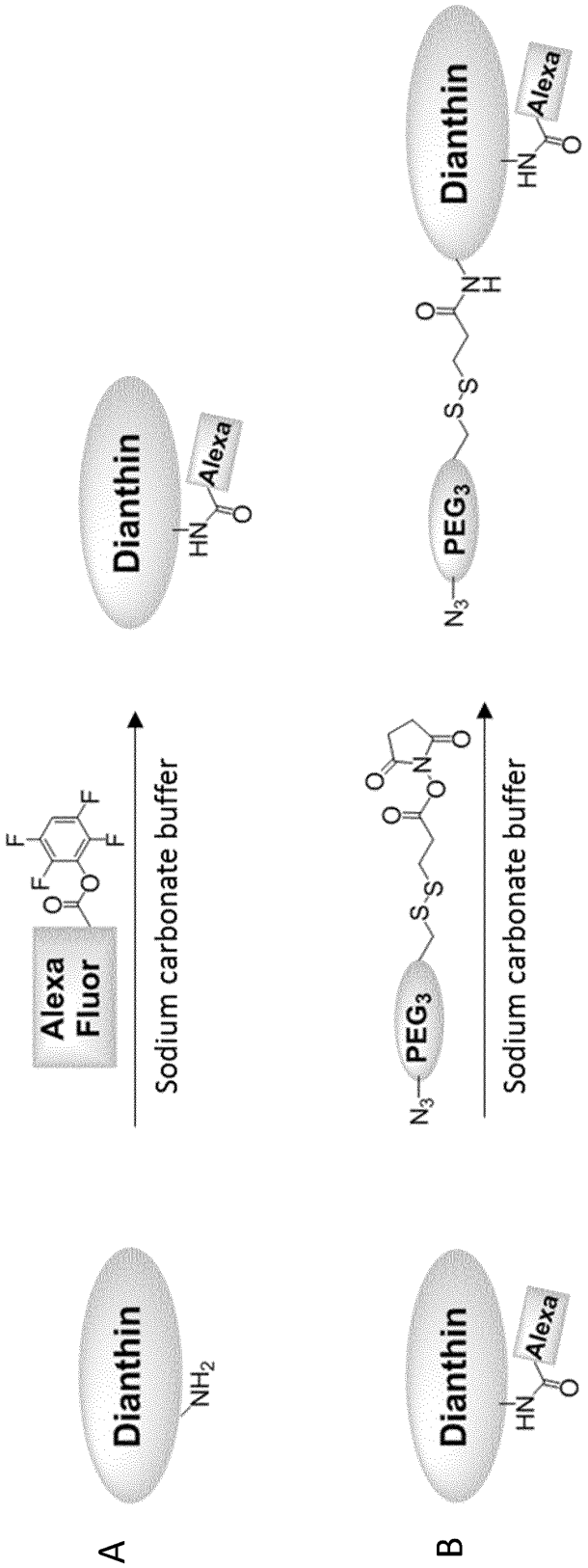

A
Dianthin
NH2
Alexa Fluor
Sodium carbonate buffer
Dianthin
Alexa
HN
B
Dianthin
PEG3
N3
Sodium carbonate buffer
Dianthin
Alexa
HN

Figure 15

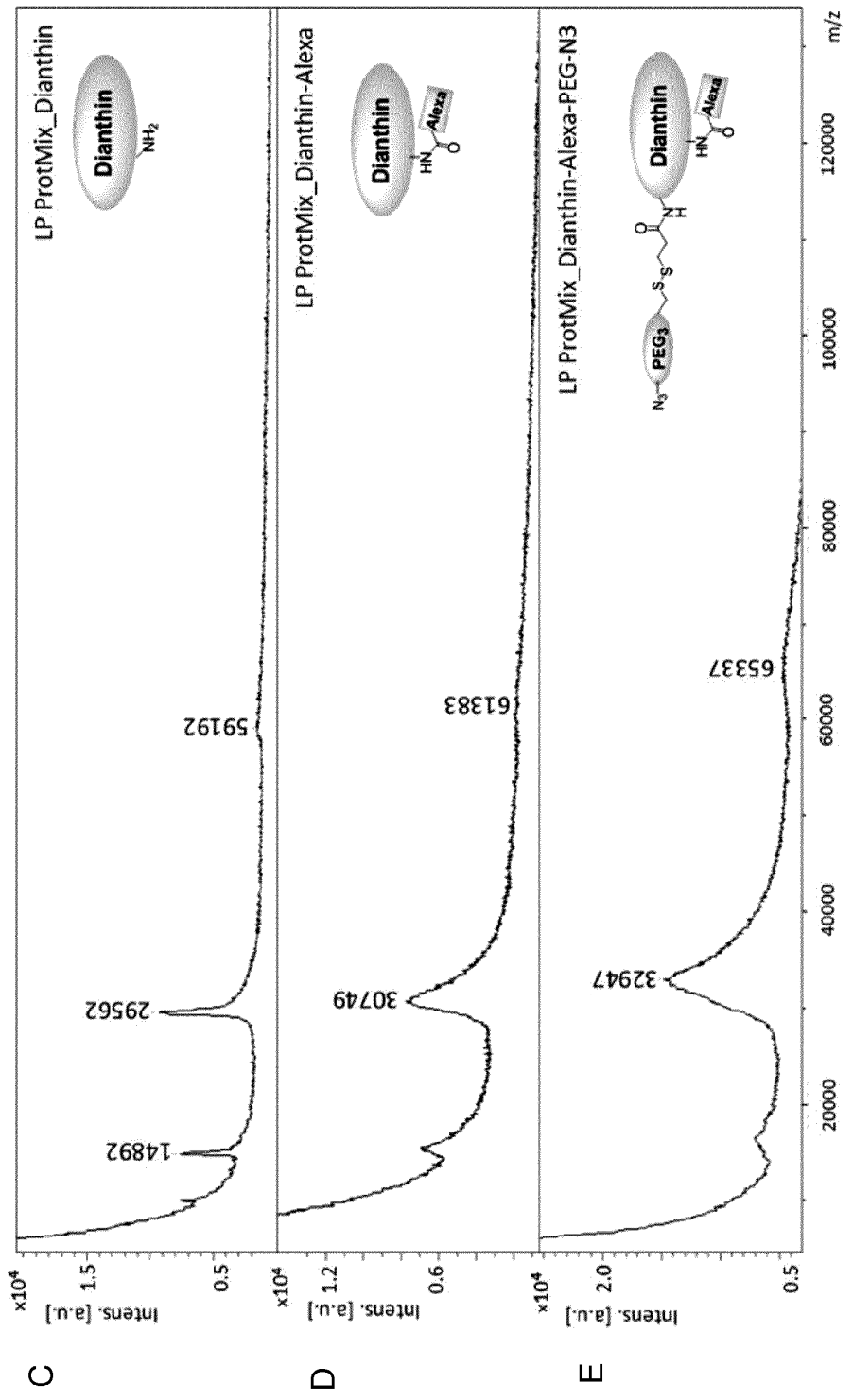

LP ProtMix_Dianthin
Dianthin
NH2
14892
29562
59192
LP ProtMix_Dianthin-Alexa
Dianthin
Alexa
HN
O
30749
61383
LP ProtMix_Dianthin-Alexa-PEG-N3
Dianthin
Alexa
HN
O
N
H
O
S
S
PEG3
N3
32947
65337
Intens. [a.u.]
x10^4
0.5
1.5
0.6
1.2
2.0
20000
40000
60000
80000
100000
120000
m/z
C
D
E

Figure 16

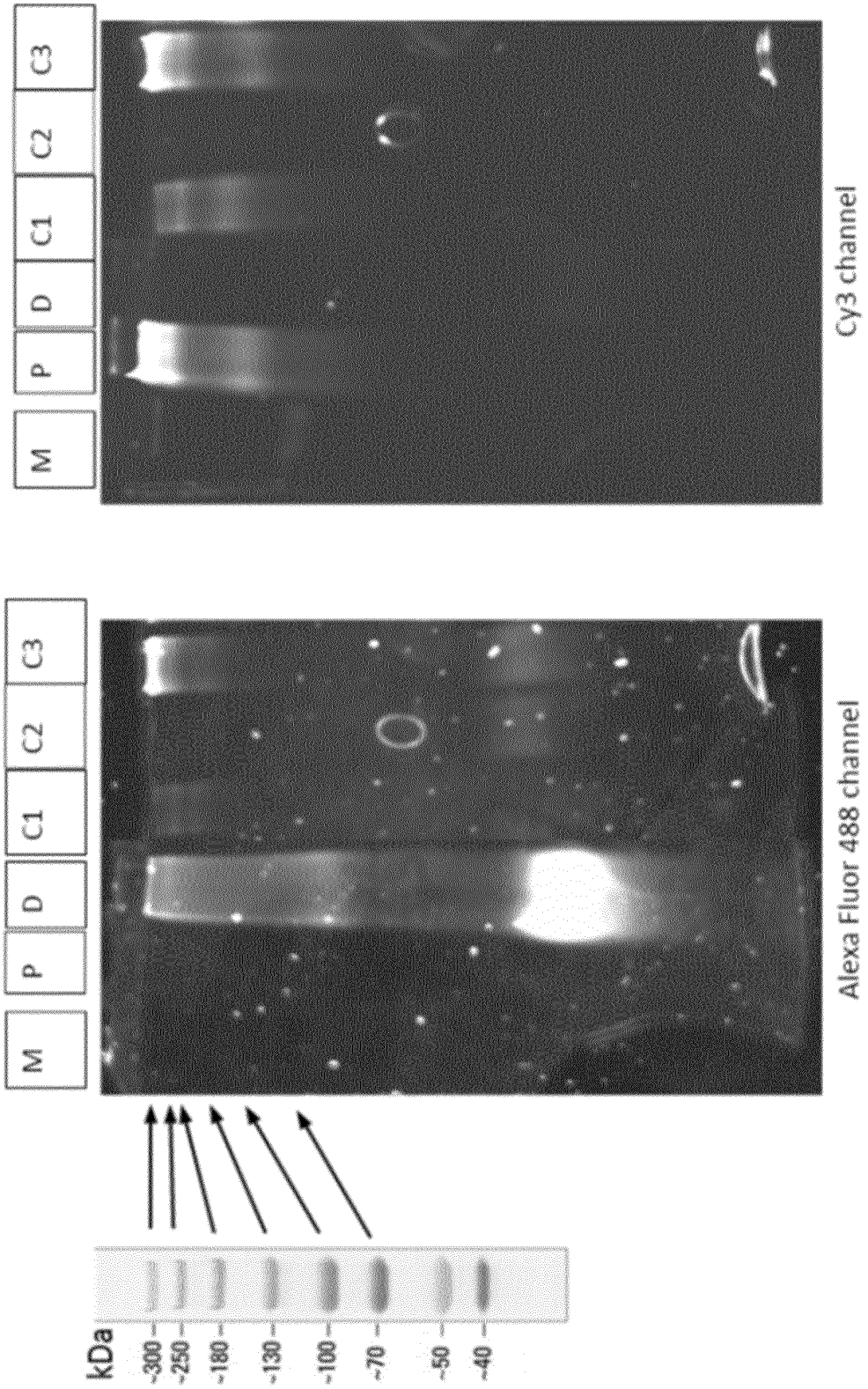

M
P
D
C1
C2
C3
Cy3 channel
Alexa Fluor 488 channel
kDa
~300
~250
~180
~130
~100
~70
~50
~40

Figure 17

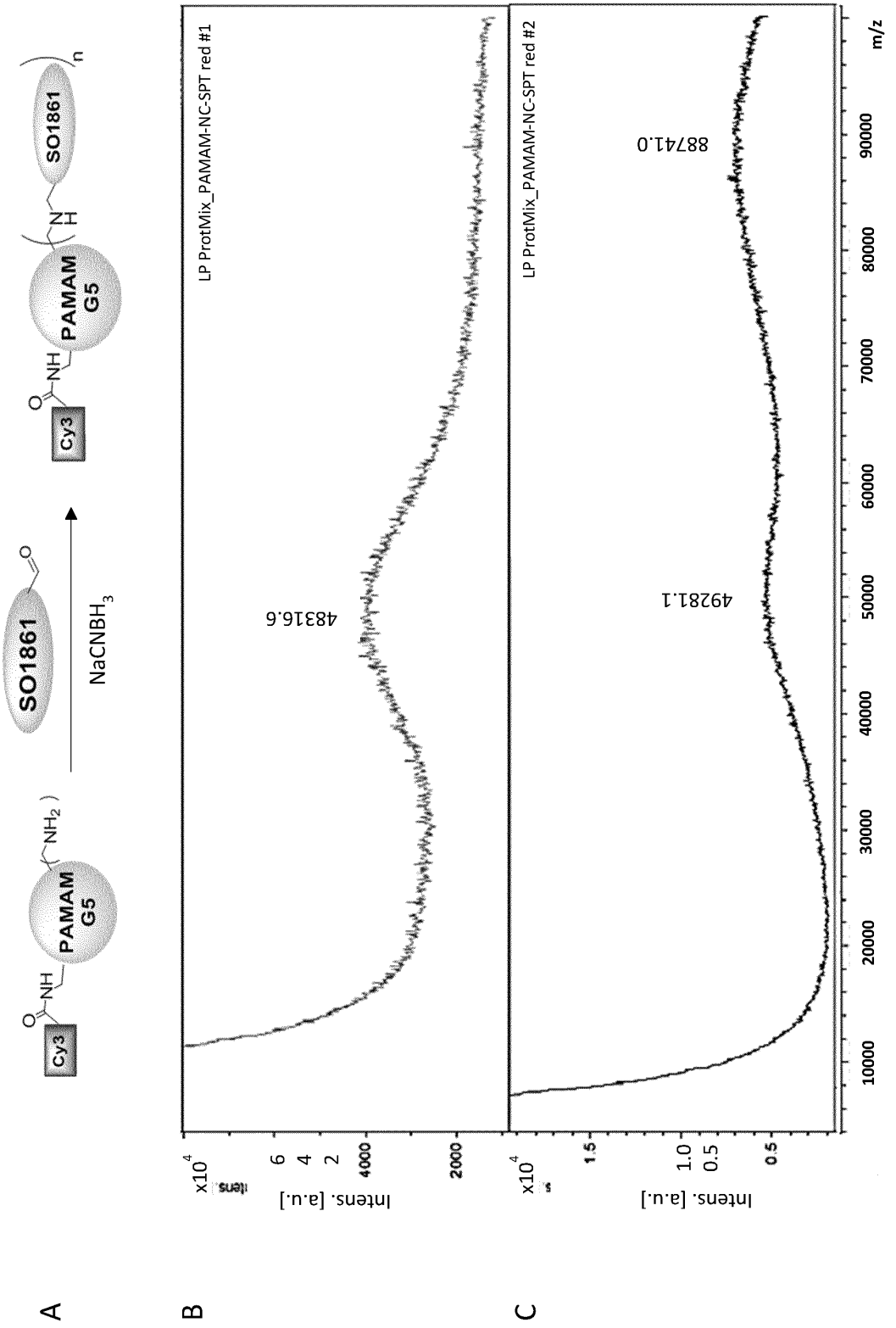

A
B
C
Cy3
PAMAM G5
NH
NH2
NaCNBH3
SO1861
n
LP ProtMix_PAMAM-NC-SPT red #1
48316.6
LP ProtMix_PAMAM-NC-SPT red #2
49281.1
88741.0
Intens. [a.u.]
x10^4
m/z
10000
20000
30000
40000
50000
60000
70000
80000
90000

Figure 18

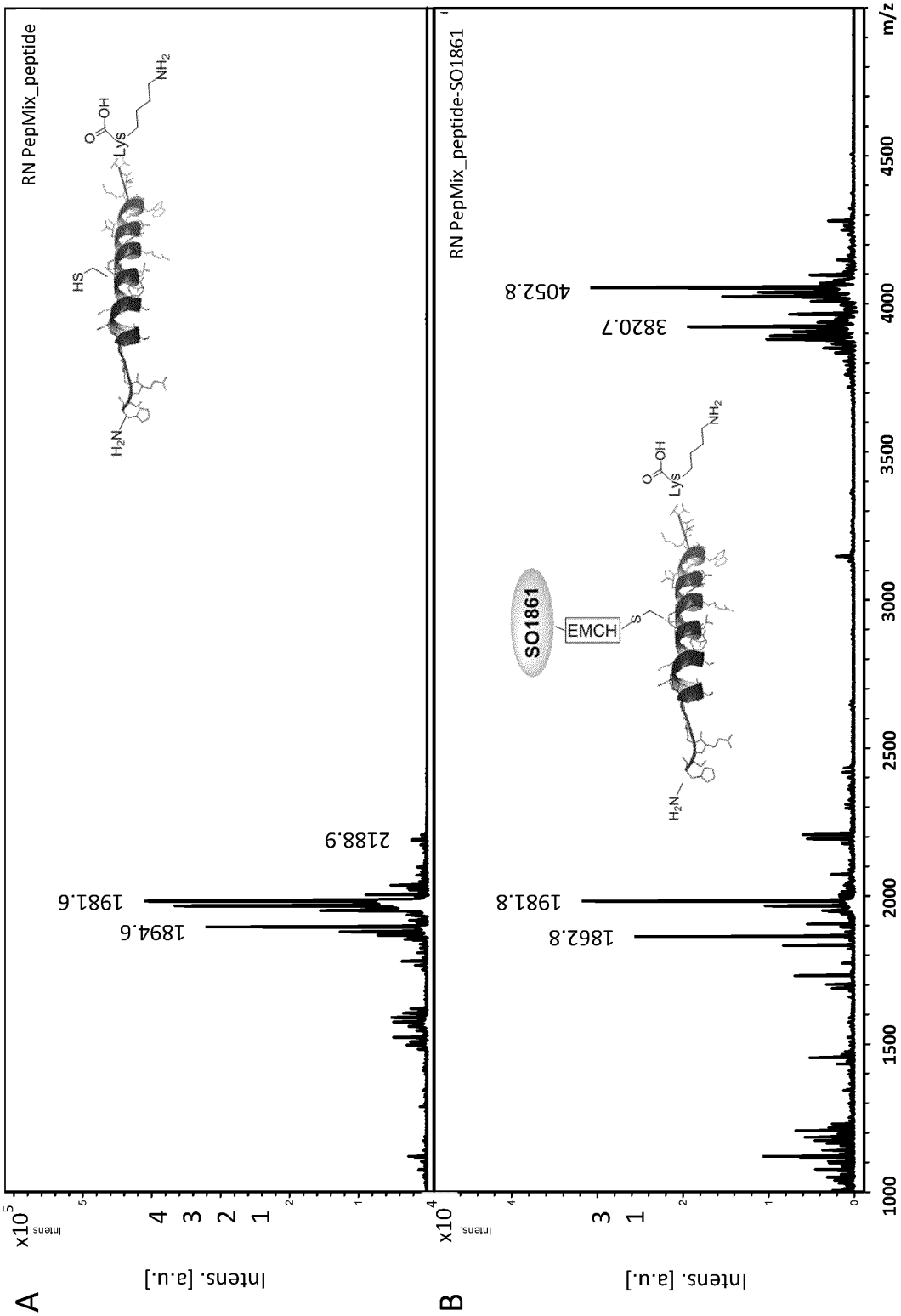

A
RN PepMix_peptide
Intens. [a.u.]
x10^5
Intens.
2188.9
1981.6
1894.6
B
RN PepMix_peptide-SO1861
Intens. [a.u.]
x10^4
Intens.
4052.8
3820.7
1981.8
1862.8
SO1861
EMCH
Lys
m/z
1000
1500
2000
2500
3000
3500
4000
4500

Figure 19

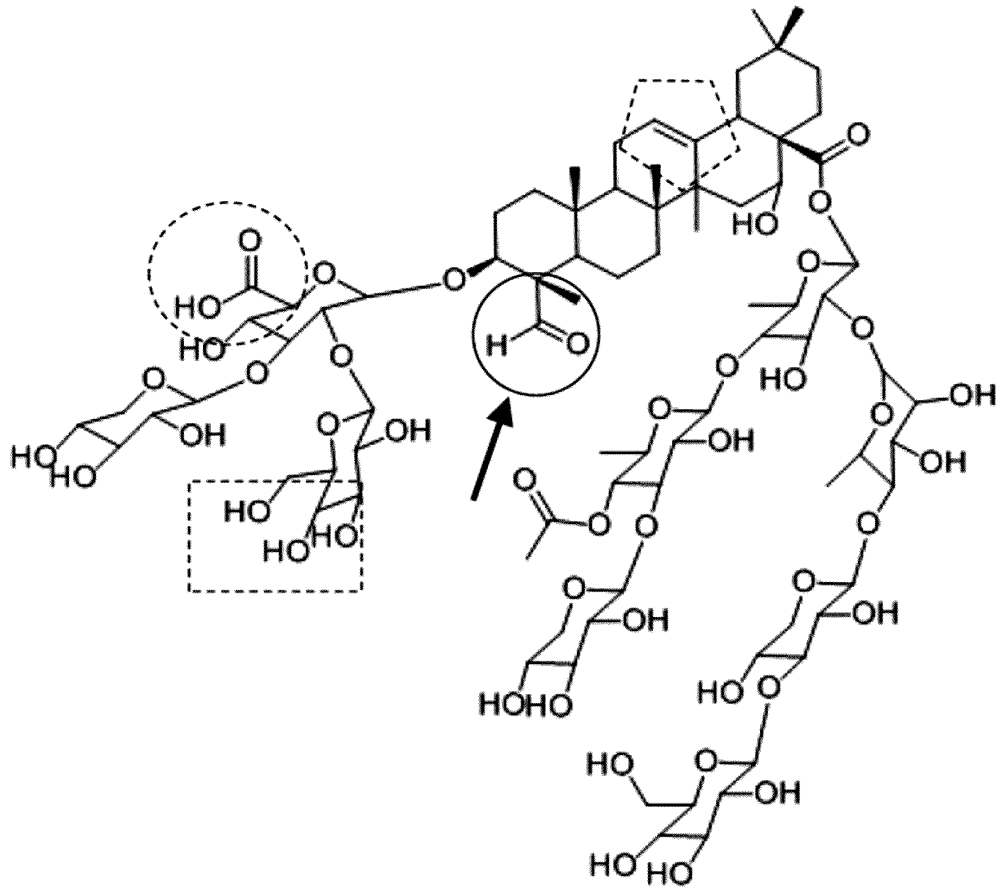


Figure 20

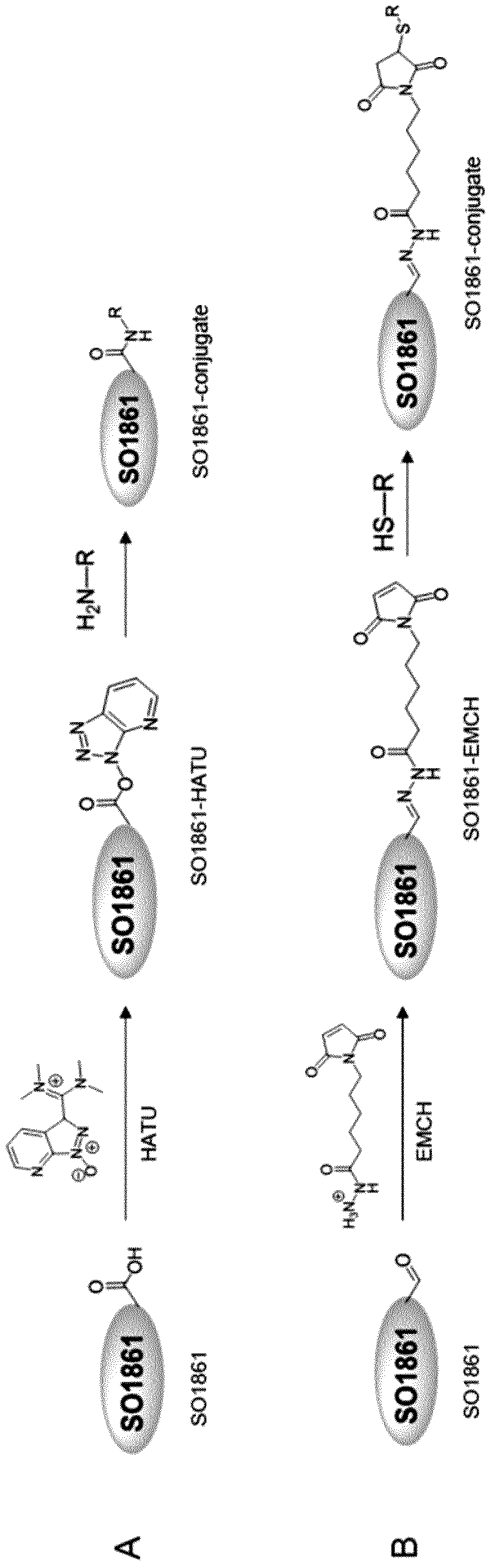

A
SO1861
HATU
SO1861-HATU
H_2N—R
SO1861-conjugate
B
SO1861
EMCH
SO1861-EMCH
HS—R
SO1861-conjugate

Figure 21

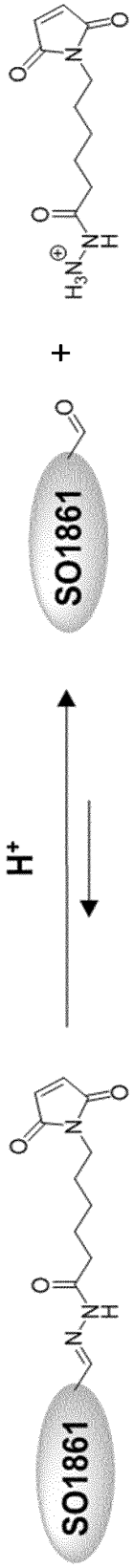
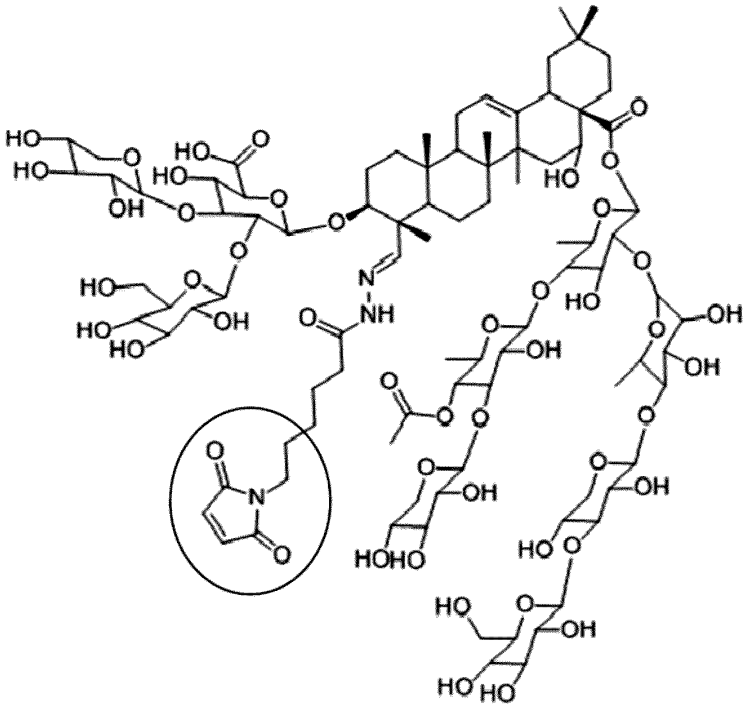


Figure 22

A



B

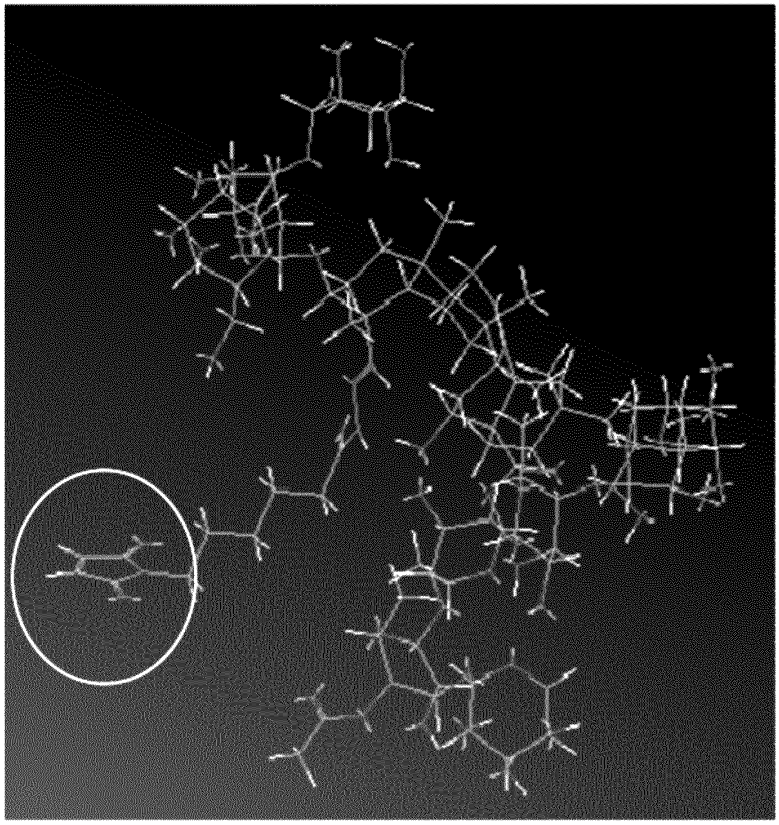


Figure 23

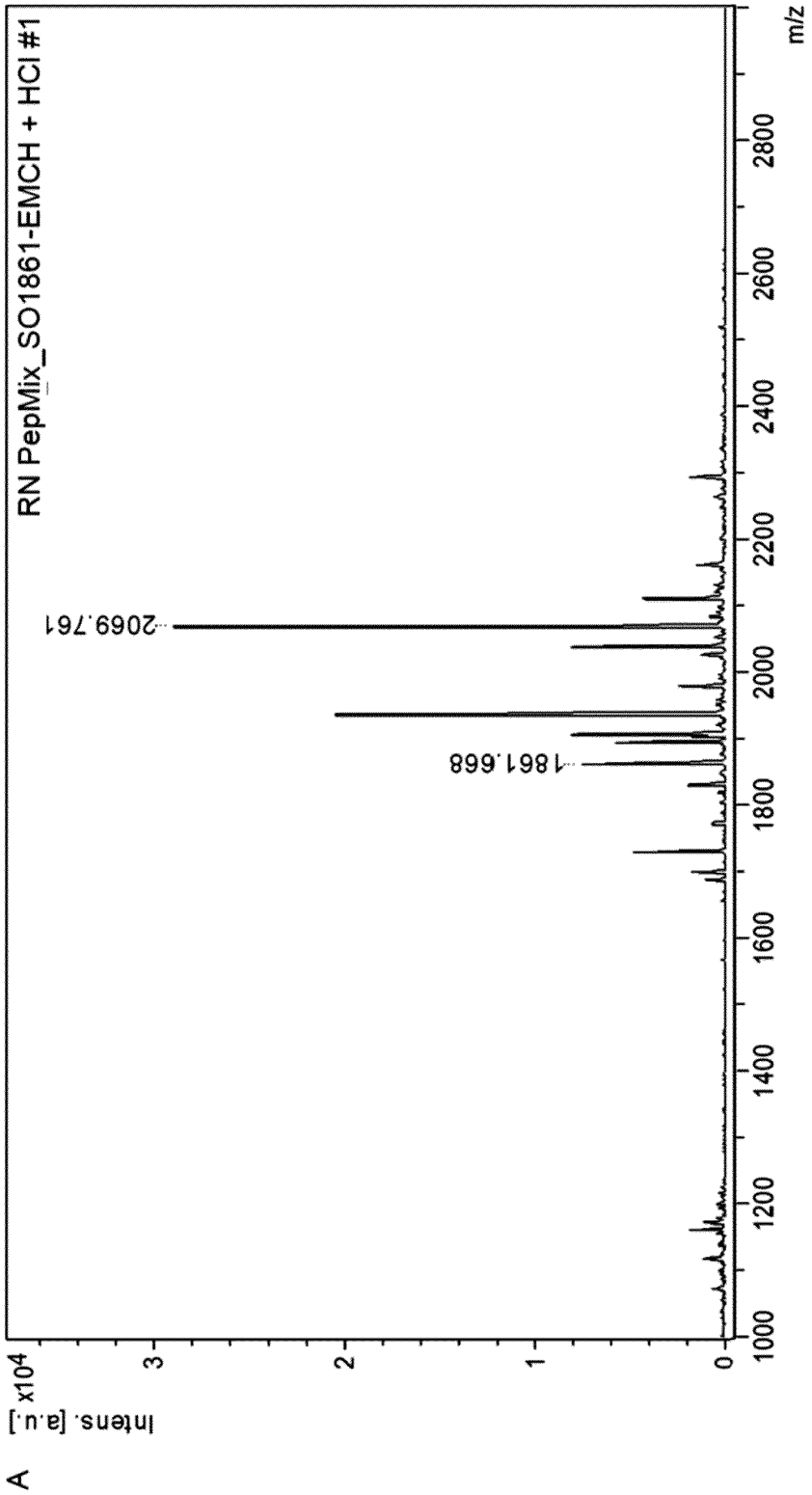

A
Intens. [a.u.]
x10^4
3
2
1
0
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
m/z
RN PepMix_SO1861-EMCH + HCl #1
2069.761
1861.668

Figure 23

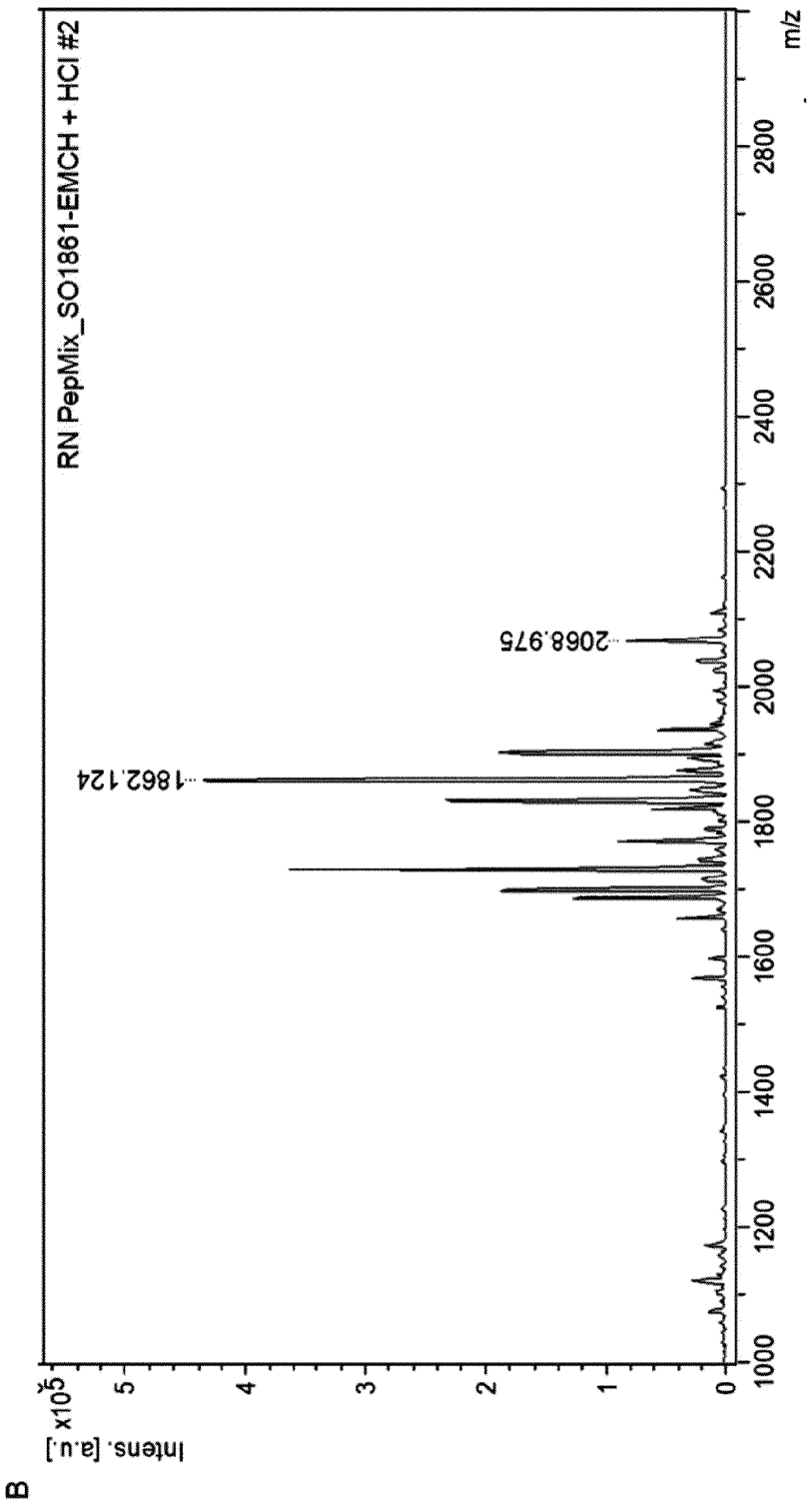

B
RN PepMix_SO1861-EMCH + HCl #2
Intens. [a.u.]
x10^5
0
1
2
3
4
5
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
m/z
1862.124
2068.975

Figure 24

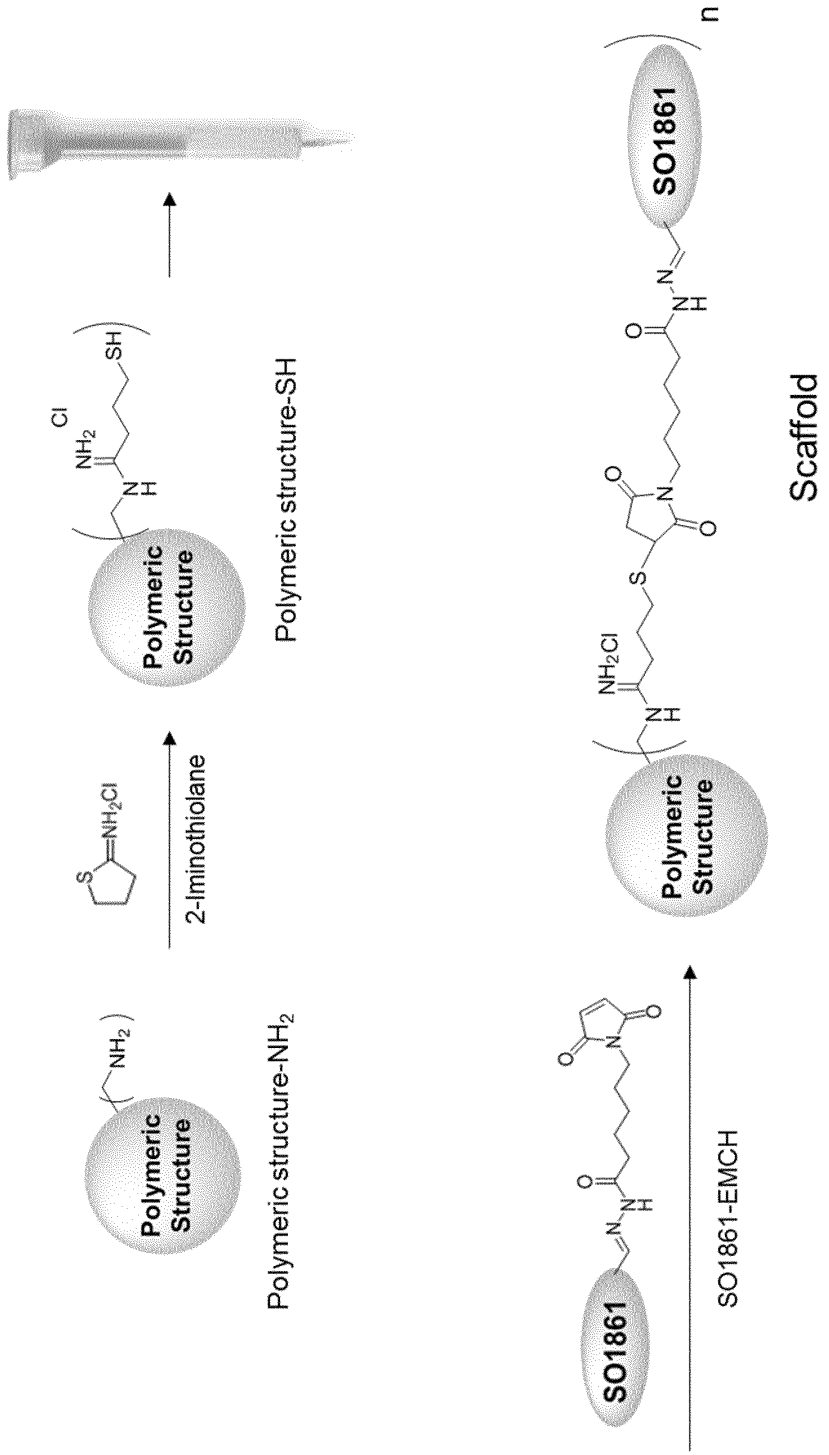

Polymeric structure-NH2
2-Iminothiolane
Polymeric structure-SH
SO1861-EMCH
Scaffold
Polymeric Structure
SO1861
n

Figure 25

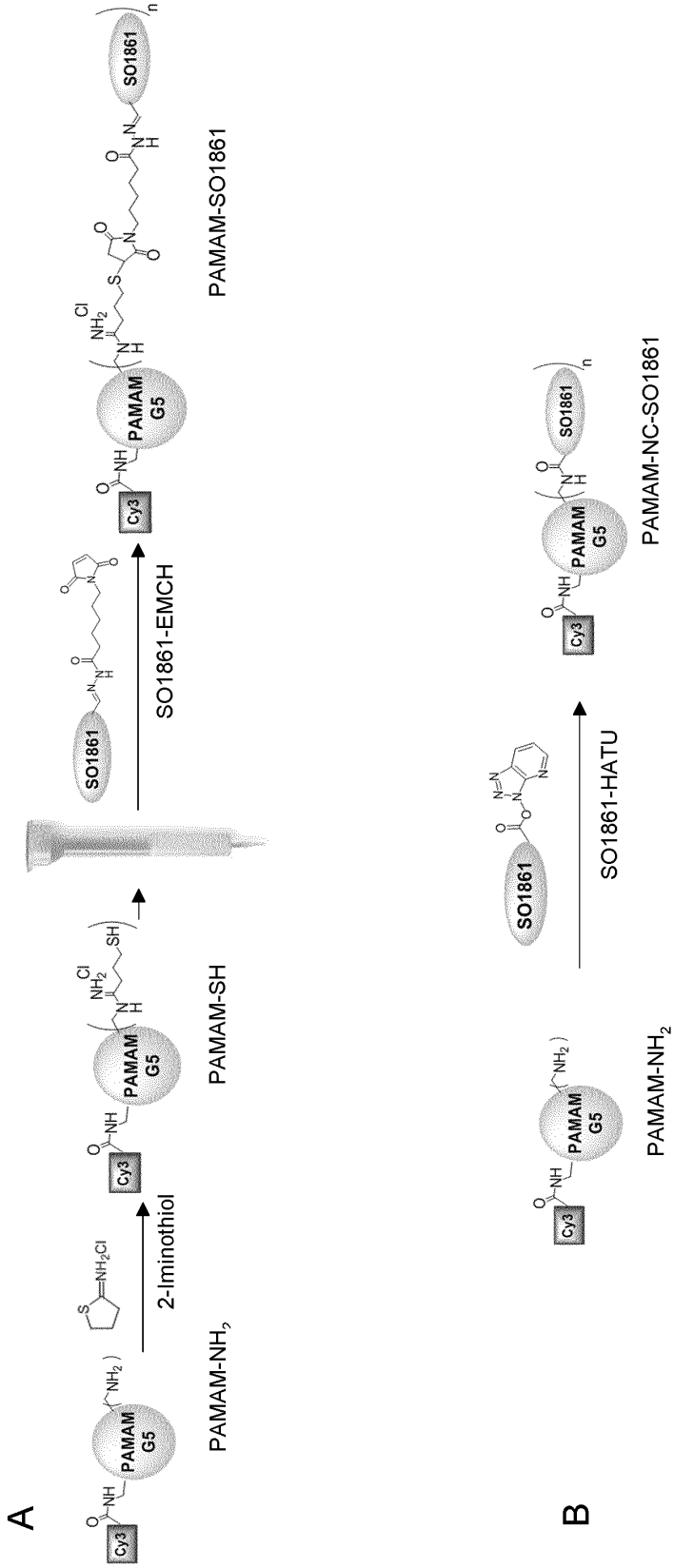

A
PAMAM-NH2
2-Iminothiol
PAMAM-SH
SO1861-EMCH
PAMAM-SO1861
B
PAMAM-NH2
SO1861-HATU
PAMAM-NC-SO1861
PAMAM G5
Cy3
SO1861

Figure 26

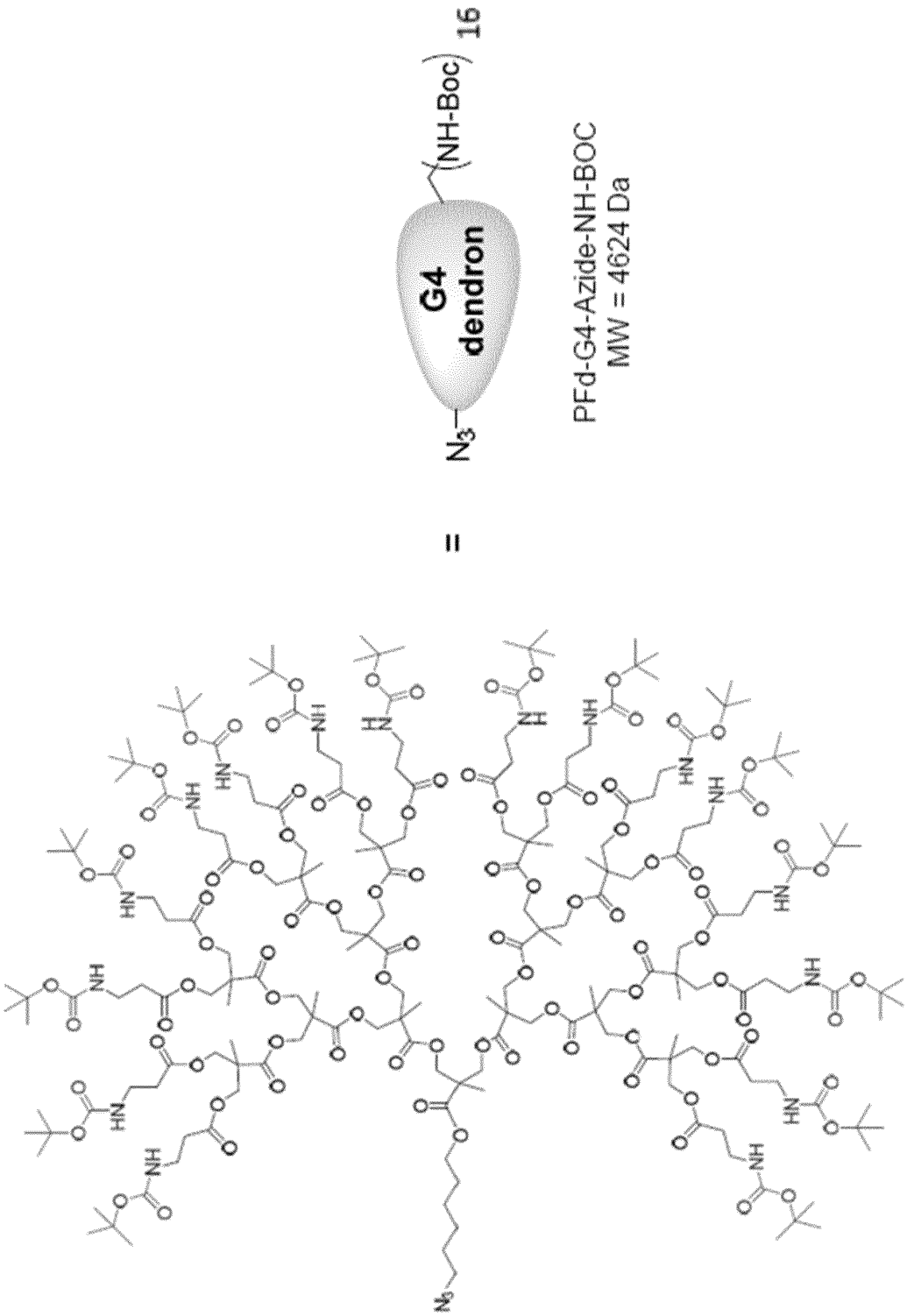


Figure 27

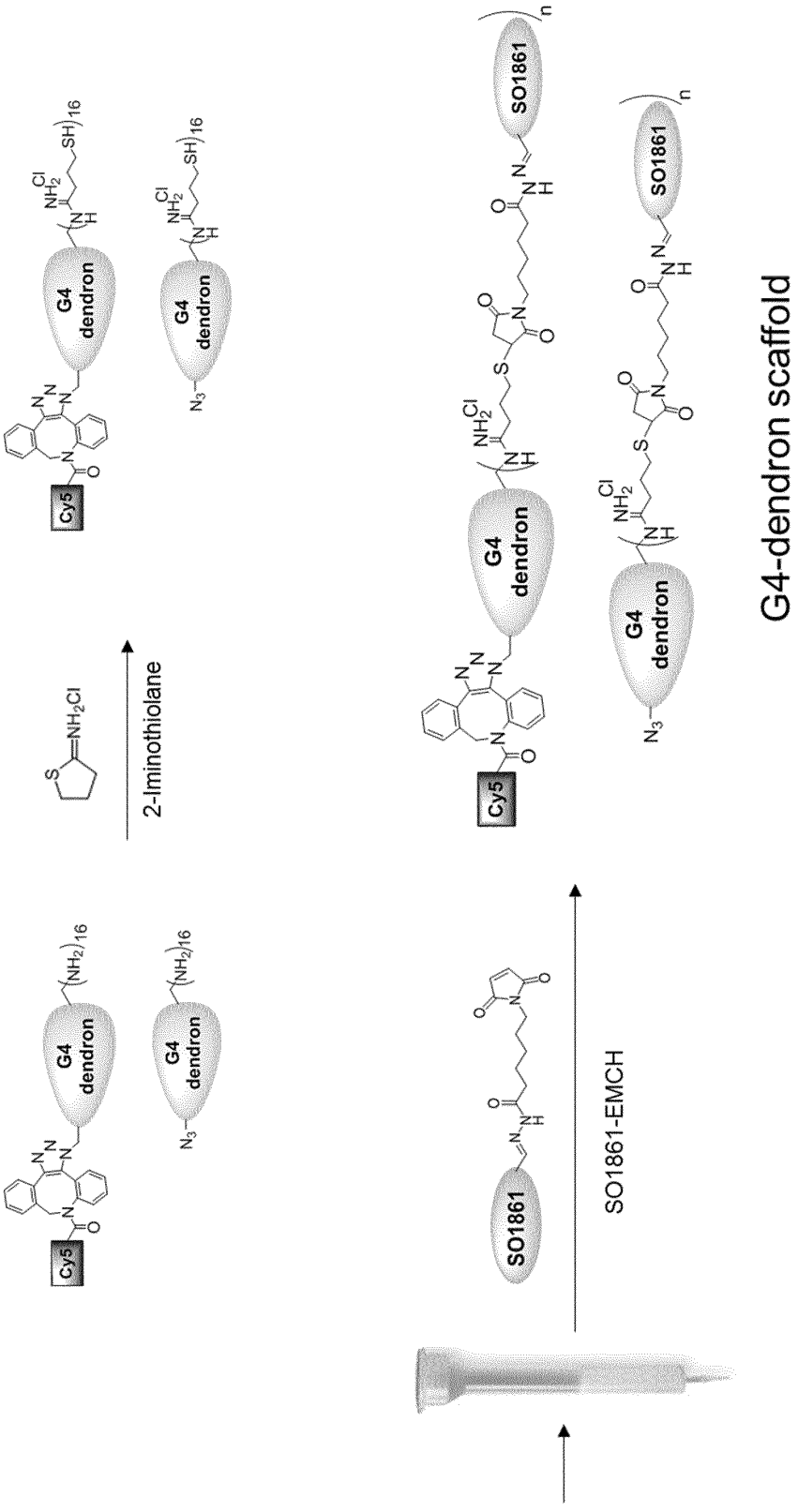

G4 dendron
Cy5
2-Iminothiolane
SO1861
SO1861-EMCH
G4-dendron scaffold

Figure 28

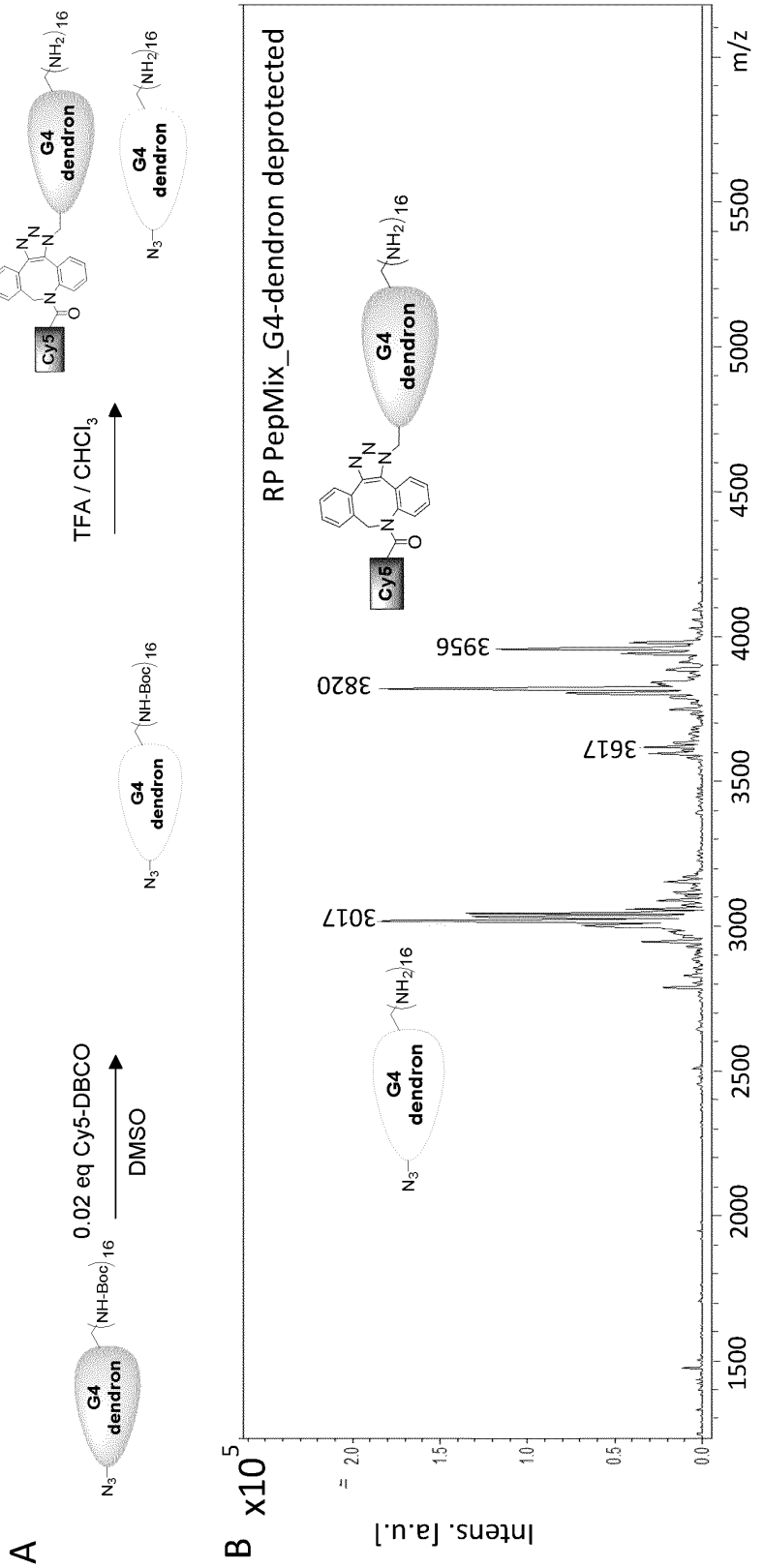

A
G4 dendron
(NH-Boc)16
N3
0.02 eq Cy5-DBCO
DMSO
Cy5
(NH2)16
TFA / CHCl3
B
RP PepMix_G4-dendron deprotected
x10^5
Intens. [a.u.]
m/z
3017
3617
3820
3956

Figure 29

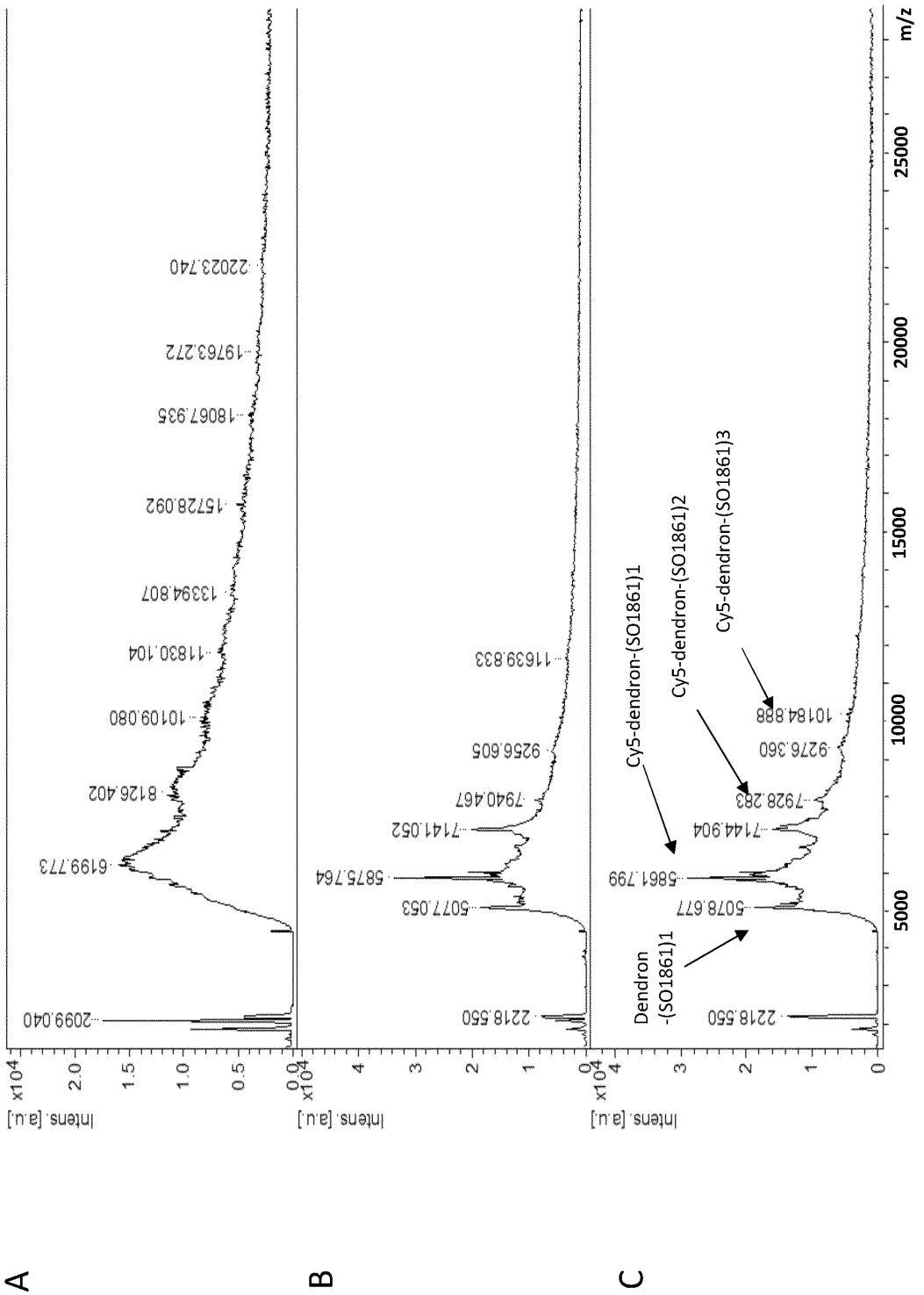
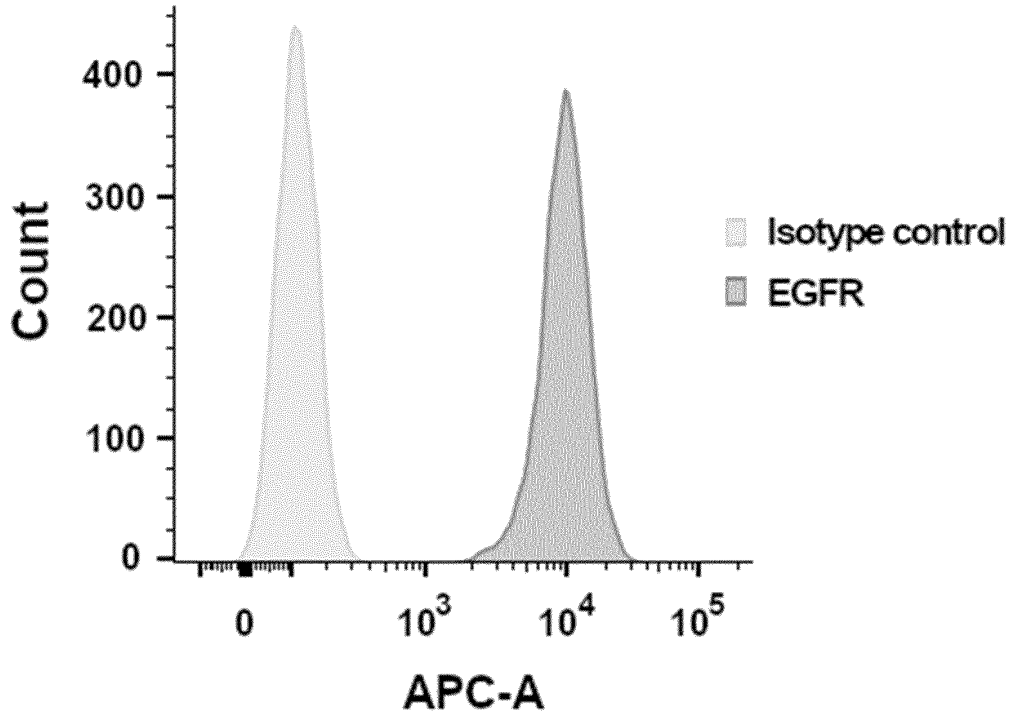

A
B
C
Intens. [a.u.]
x10^4
m/z
2099.040
6199.773
8126.402
10109.080
11830.104
13394.807
15728.092
18067.935
19763.272
22023.740
2218.550
5077.053
5875.764
7141.052
7940.467
9256.605
11639.833
Dendron -(SO1861)1
Cy5-dendron-(SO1861)1
Cy5-dendron-(SO1861)2
Cy5-dendron-(SO1861)3
5078.677
5861.799
7144.904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

Figure 30

A


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

B

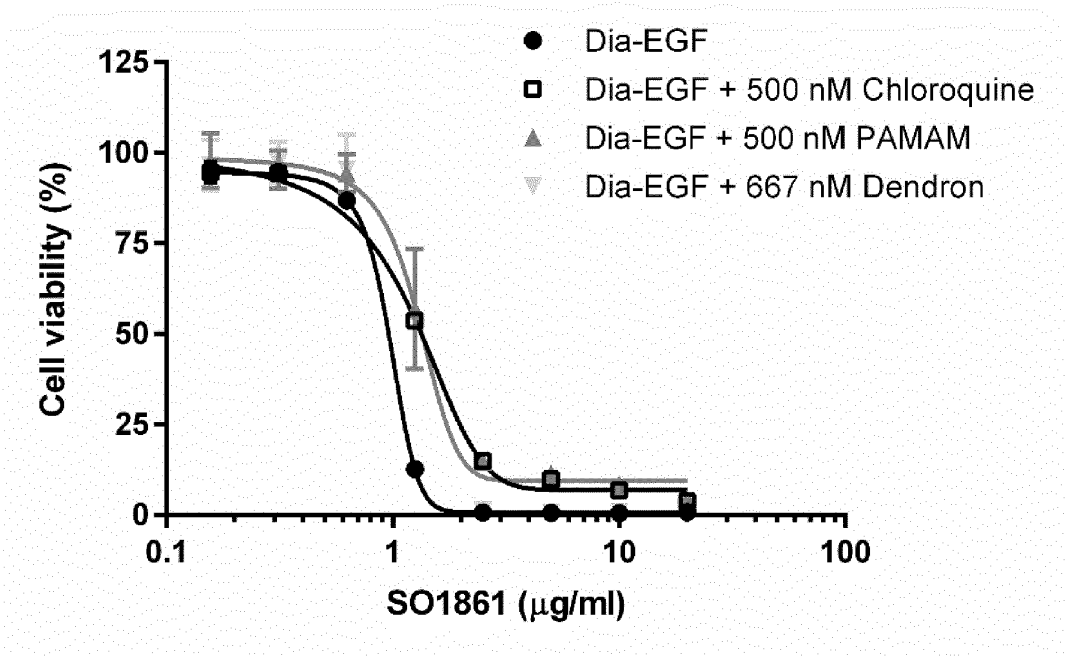
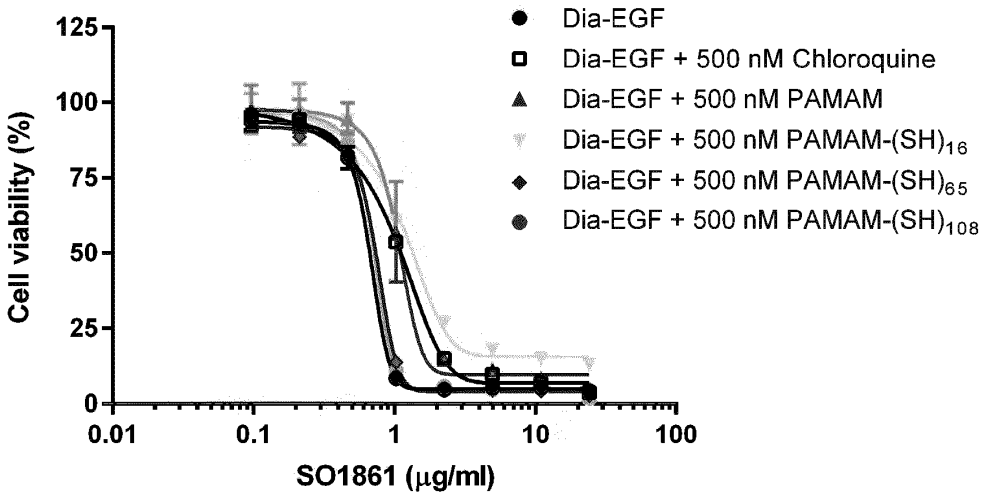
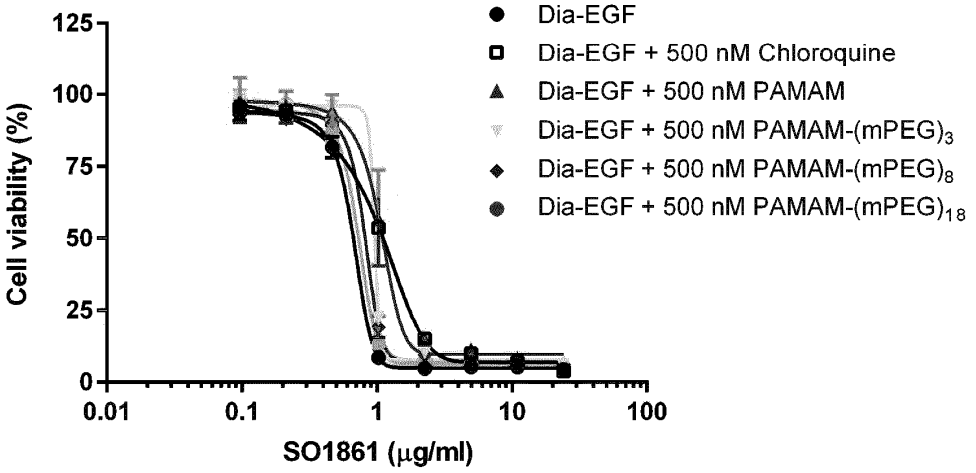

Cell viability (%)
125
100
75
50
25
0
0.1
1
10
100
SO1861 (μg/ml)
Dia-EGF
Dia-EGF + 500 nM Chloroquine
Dia-EGF + 500 nM PAMAM
Dia-EGF + 667 nM Dendron

Figure 30

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