
payload (small molecule
to macro-biomolecule)
pH-sensitive
Polymer
encapsulation
pH < pHt
payload release

FIG. 1

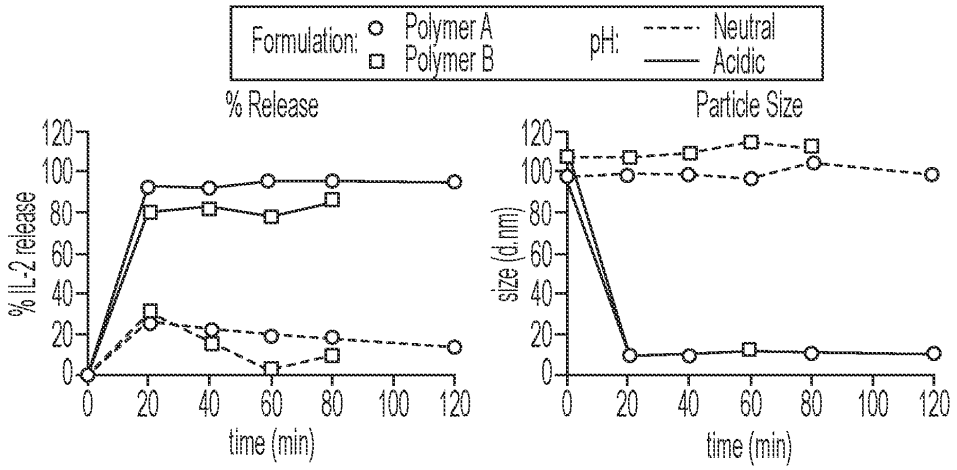

Formulation:
Polymer A
Polymer B
pH:
Neutral
Acidic
% Release
% IL-2 release
time (min)
Particle Size
size (d.nm)
time (min)

FIG. 2

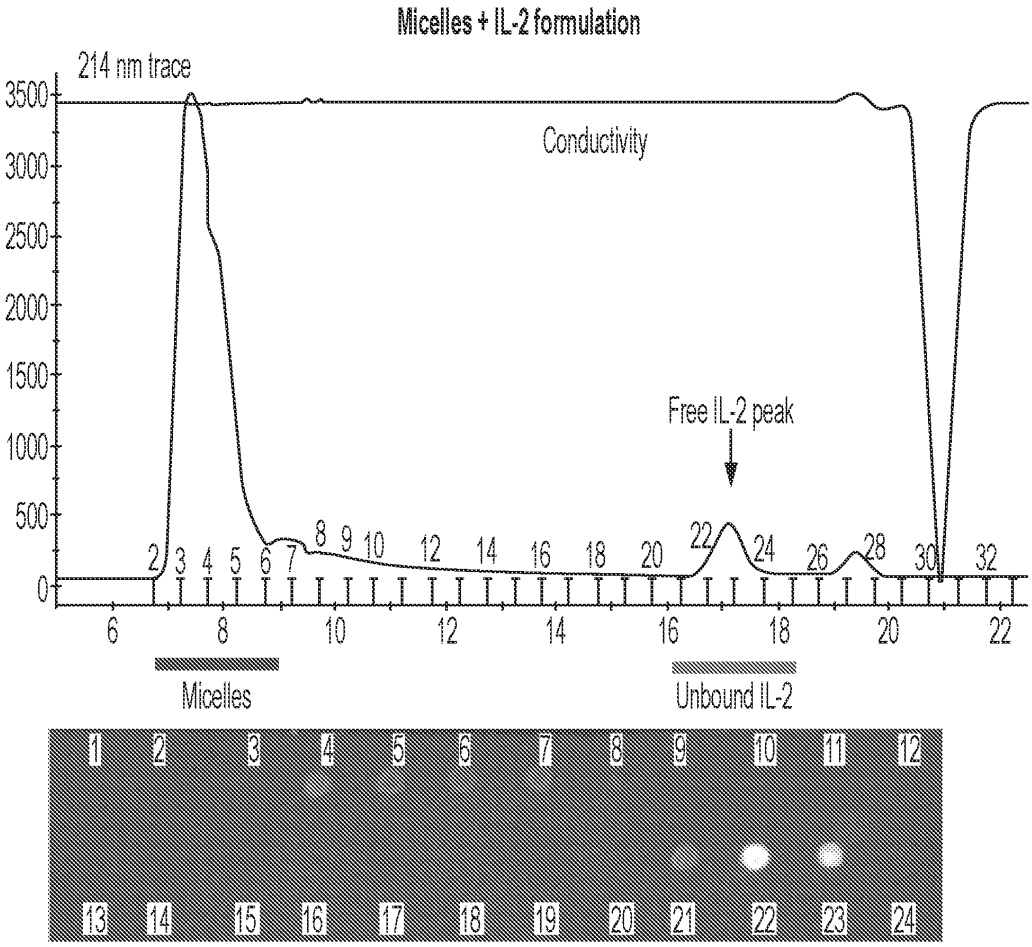

Micelles + IL-2 formulation
214 nm trace
Conductivity
Free IL-2 peak
Micelles
Unbound IL-2

FIG. 3

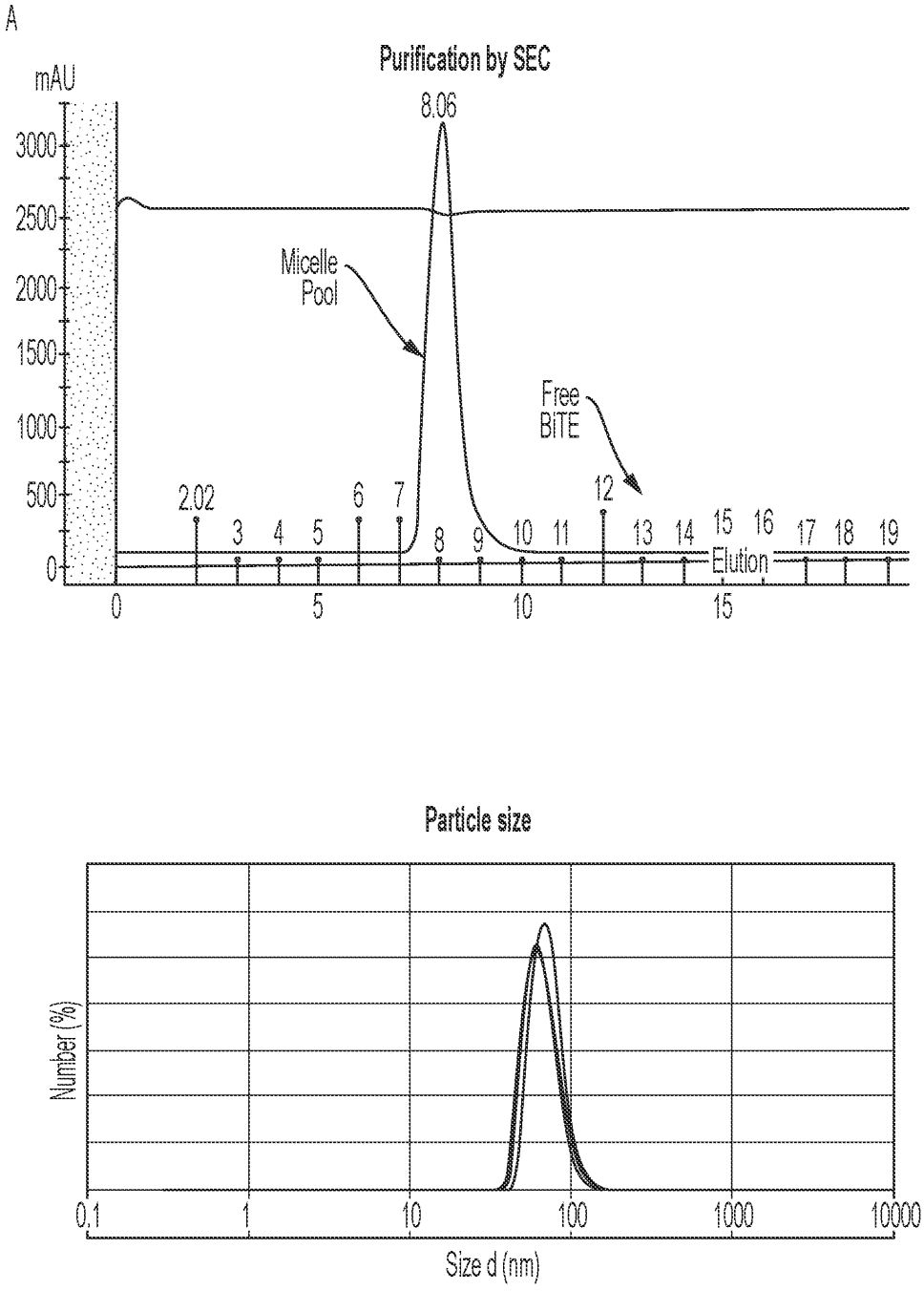
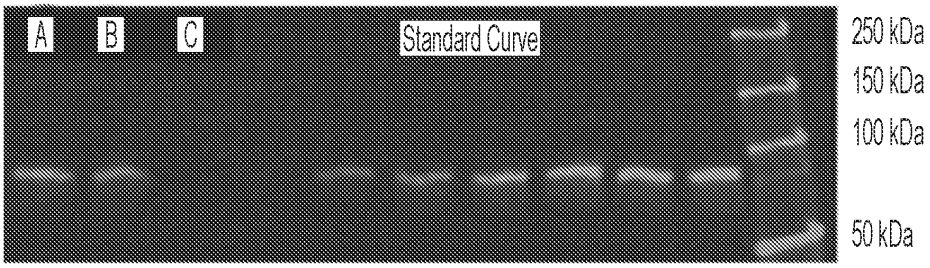

A
Purification by SEC
mAU
3000
2500
2000
1500
1000
500
0
8.06
Micelle Pool
Free BiTE
2.02
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Elution
0
5
10
15
Particle size
Number (%)
0,1
1
10
100
1000
10000
Size d (nm)

FIG. 4

B

BiTE loading quantification by Western blot



A: Feed
B: Encapsulated
C: Unencapsulated

Characterization			
EE (%)	47	Size (nm)	69.4
DL (%)	8	PDI	0.06

FIG. 4

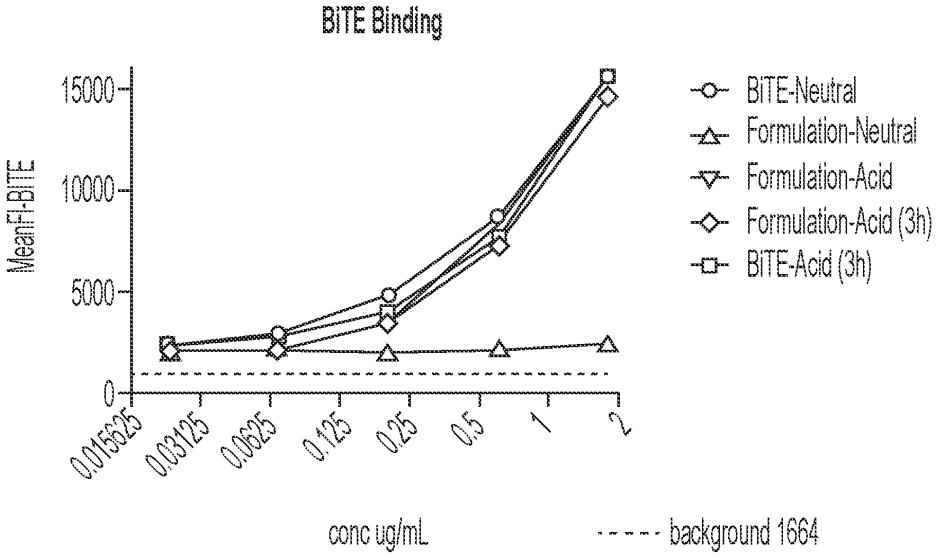

BiTE Binding
MeanFl-BiTE
15000
10000
5000
0
0.015625
0.03125
0.0625
0.125
0.25
0.5
1
2
conc ug/mL
BiTE-Neutral
Formulation-Neutral
Formulation-Acid
Formulation-Acid (3h)
BiTE-Acid (3h)
background 1664

FIG. 5

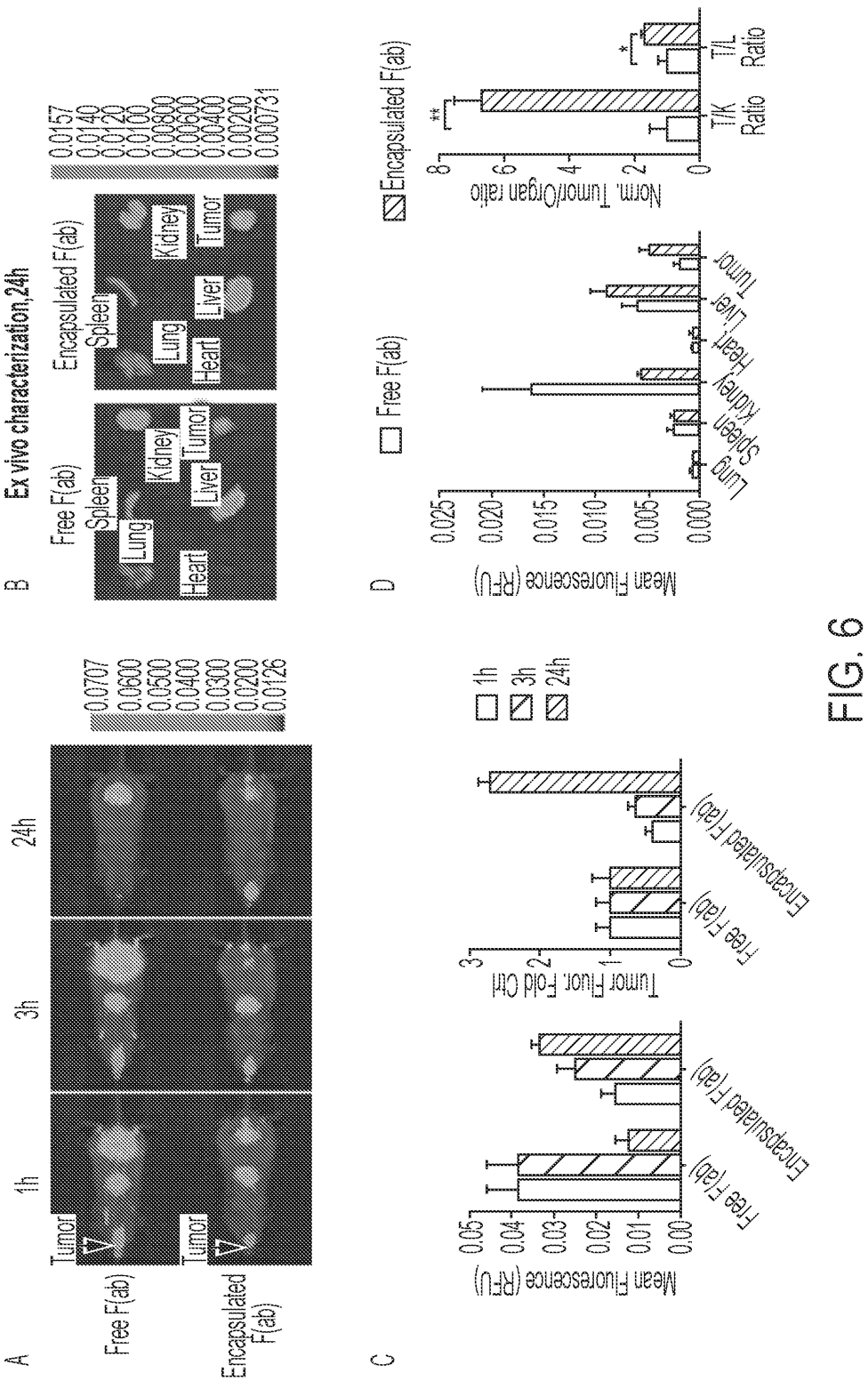

A
1h
3h
24h
Tumor
Free F(ab)
Encapsulated F(ab)
0.0707
0.0600
0.0500
0.0400
0.0300
0.0200
0.0126
B
Ex vivo characterization,24h
Free F(ab)
Encapsulated F(ab)
Spleen
Lung
Kidney
Tumor
Liver
Heart
0.0157
0.0140
0.0120
0.0100
0.00800
0.00600
0.00400
0.00200
0.000731
C
Mean Fluorescence (RFU)
Free F(ab)
Encapsulated F(ab)
Tumor Fluor. Fold Ctrl
1h
3h
24h
D
Free F(ab)
Encapsulated F(ab)
Mean Fluorescence (RFU)
Lung
Spleen
Kidney
Heart
Liver
Tumor
Norm. Tumor/Organ ratio
T/K Ratio
T/L Ratio

FIG. 6

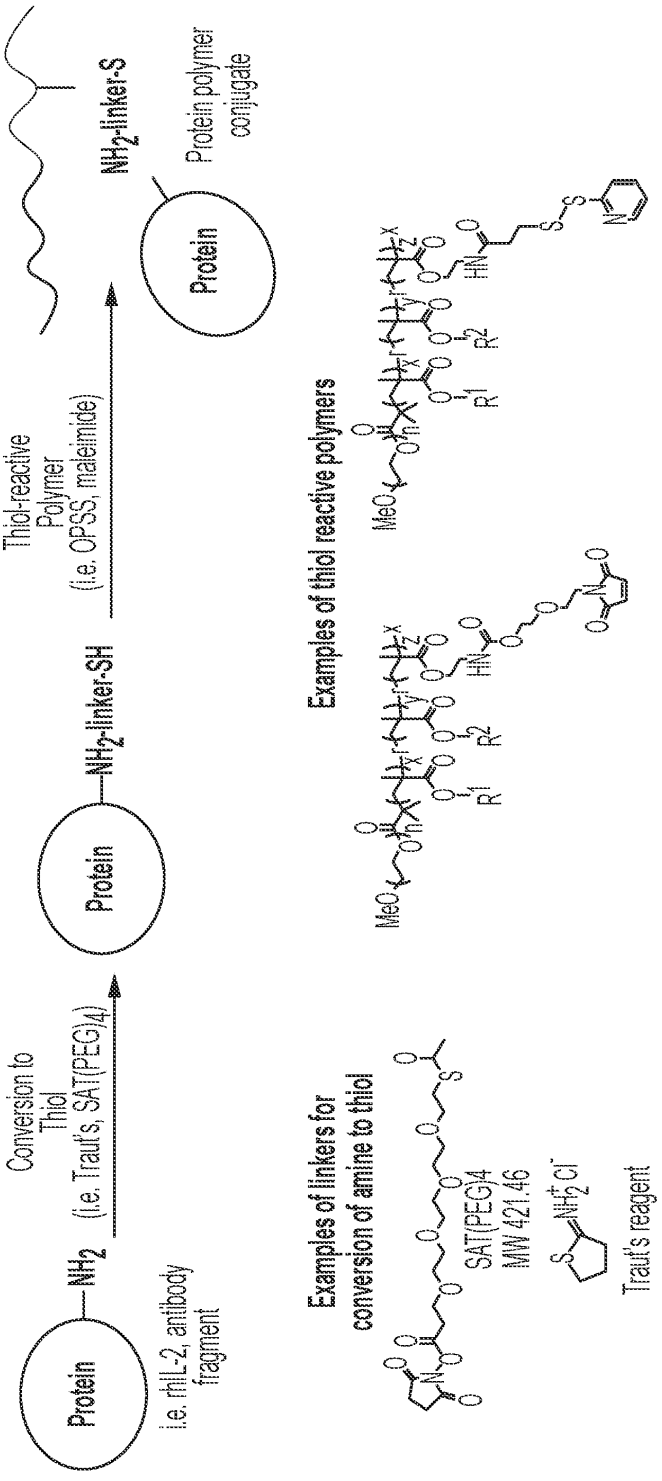

Protein
NH2
i.e. rhIL-2, antibody fragment
Conversion to Thiol
(i.e. Traut's, SAT(PEG)4)
Protein
NH2-linker-SH
Thiol-reactive Polymer
(i.e. OPSS, maleimide)
NH2-linker-S
Protein
Protein polymer conjugate
Examples of linkers for conversion of amine to thiol
SAT(PEG)4
MW 421.46
Traut's reagent
Examples of thiol reactive polymers

FIG. 7

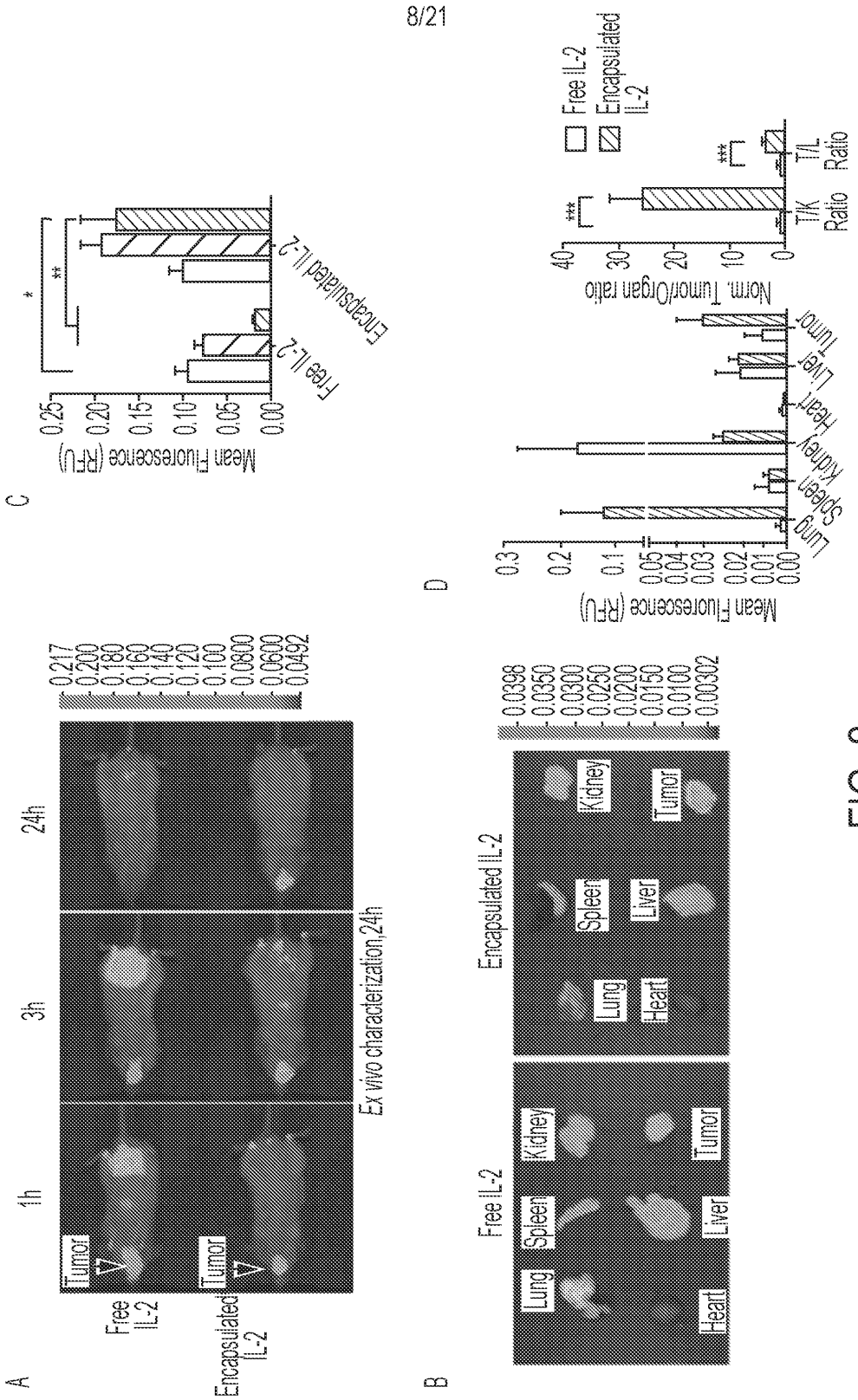

A
1h
3h
24h
Free IL-2
Encapsulated IL-2
Tumor
Ex vivo characterization,24h
0.217
0.200
0.180
0.160
0.140
0.120
0.100
0.0800
0.0600
0.0492
B
Free IL-2
Encapsulated IL-2
Lung
Spleen
Kidney
Heart
Liver
Tumor
0.0398
0.0350
0.0300
0.0250
0.0200
0.0150
0.0100
0.00302
C
Mean Fluorescence (RFU)
0.25
0.20
0.15
0.10
0.05
0.00
Free IL-2
Encapsulated IL-2
*
**
D
Mean Fluorescence (RFU)
0.3
0.2
0.1
0.05
0.04
0.03
0.02
0.01
0.00
Lung
Spleen
Kidney
Heart
Liver
Tumor
Norm. Tumor/Organ ratio
40
30
20
10
0
T/K Ratio
T/L Ratio
***
***
Free IL-2
Encapsulated IL-2

FIG. 8

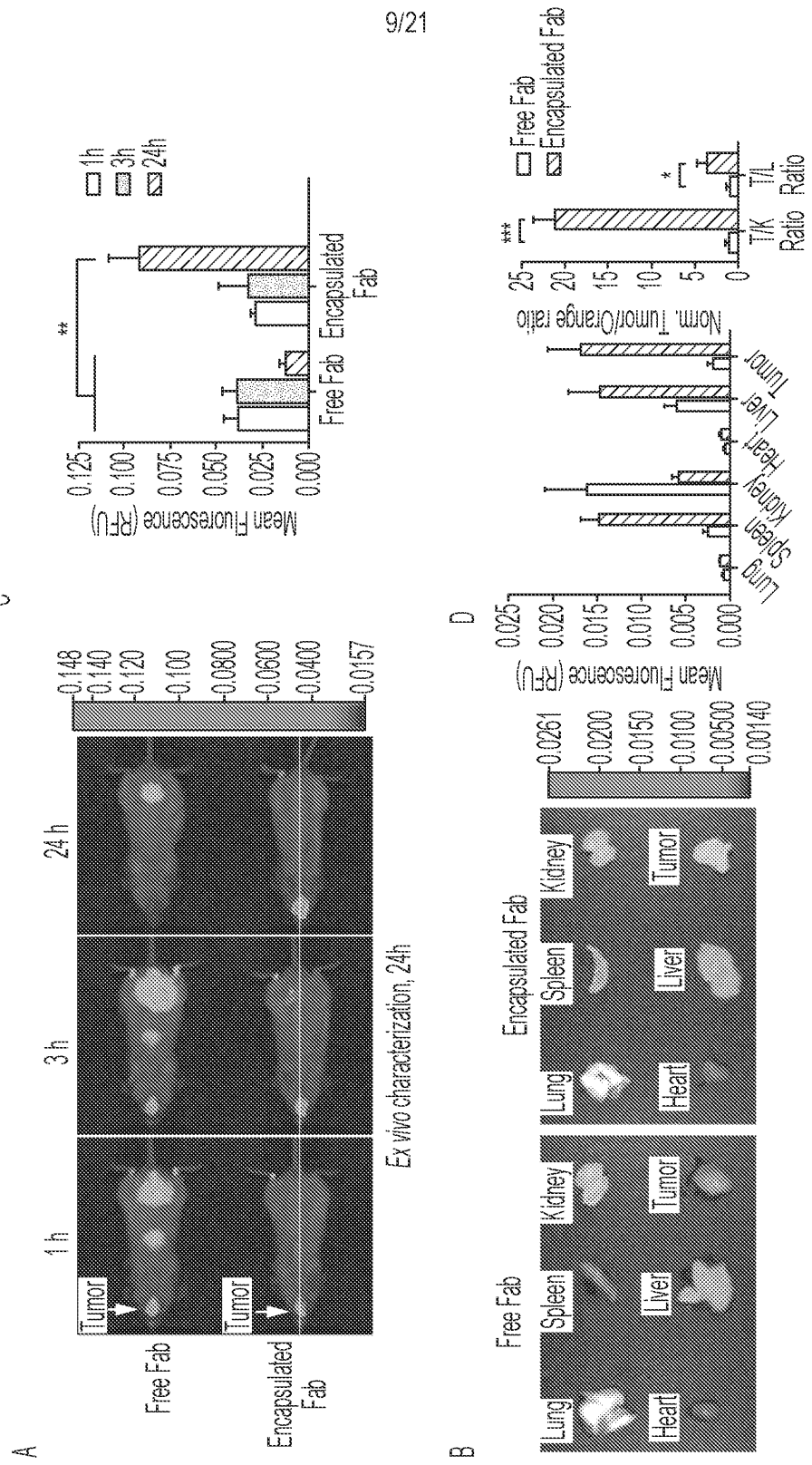

A
1 h
3 h
24 h
Tumor
Free Fab
Encapsulated Fab
0.148
0.140
0.120
0.100
0.0800
0.0600
0.0400
0.0157
Ex vivo characterization, 24h
B
Free Fab
Encapsulated Fab
Lung
Spleen
Kidney
Heart
Liver
Tumor
0.0261
0.0200
0.0150
0.0100
0.00500
0.00140
C
1h
3h
24h
**
Mean Fluorescence (RFU)
0.125
0.100
0.075
0.050
0.025
0.000
Free Fab
Encapsulated Fab
D
Free Fab
Encapsulated Fab
***
*
T/K Ratio
T/L Ratio
25
20
15
10
5
0
Norm. Tumor/Orange ratio
Lung
Spleen
Kidney
Heart
Liver
Tumor
Mean Fluorescence (RFU)
0.025
0.020
0.015
0.010
0.005
0.000

FIG. 9

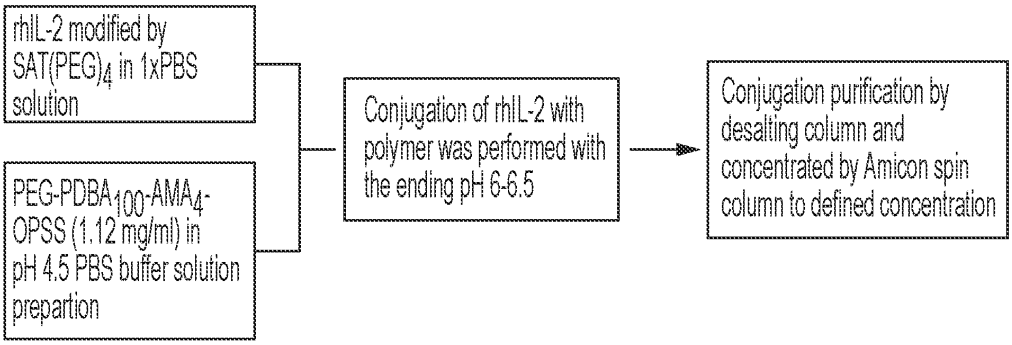

rhIL-2 modified by SAT(PEG)4 in 1xPBS solution
PEG-PDBA100-AMA4-OPSS (1.12 mg/ml) in pH 4.5 PBS buffer solution prepartion
Conjugation of rhIL-2 with polymer was performed with the ending pH 6-6.5
Conjugation purification by desalting column and concentrated by Amicon spin column to defined concentration

FIG. 10

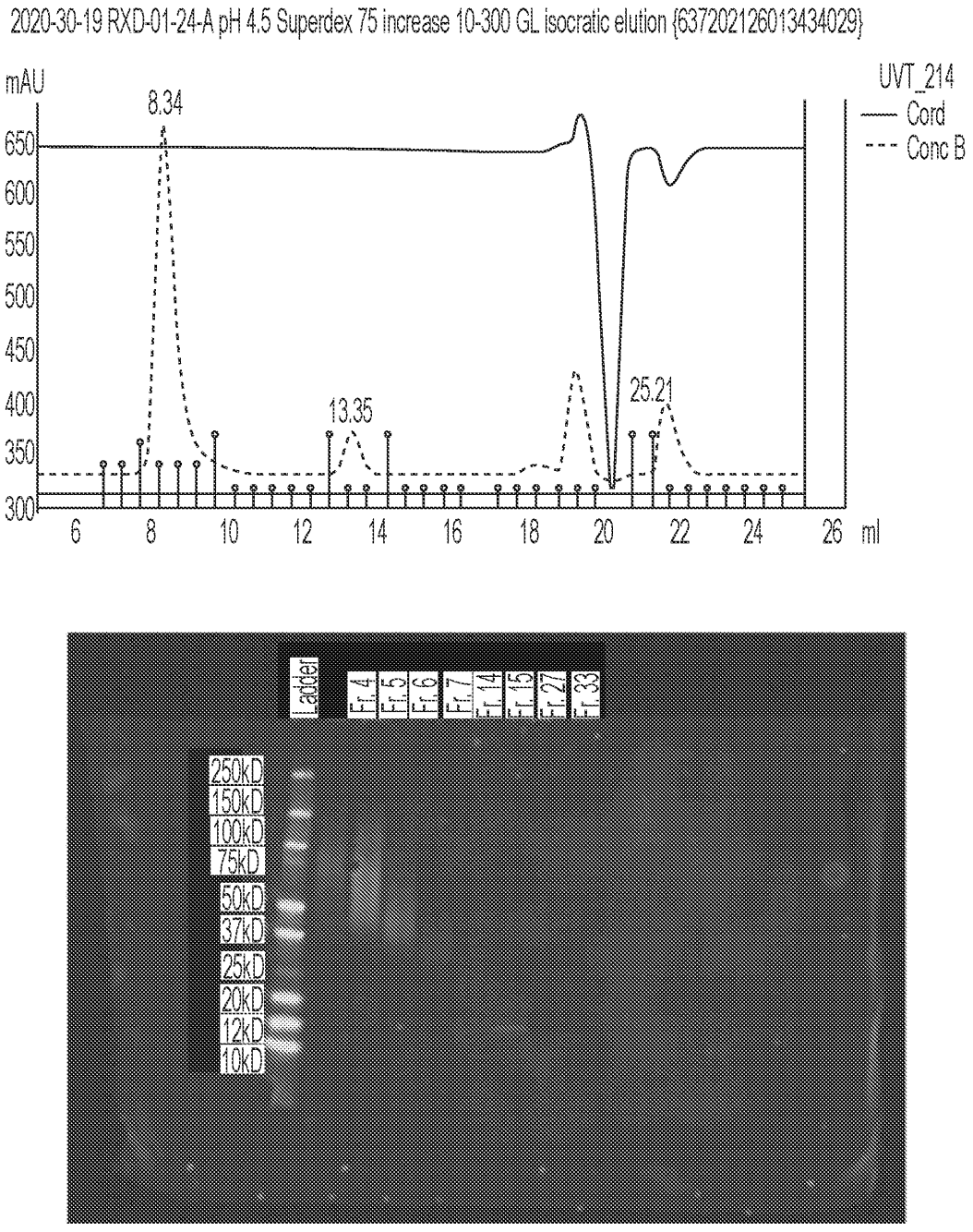

2020-30-19 RXD-01-24-A pH 4.5 Superdex 75 increase 10-300 GL isocratic elution (Default_2020-11-19_14-34-29_637202126013434029)
mAU
650
600
550
500
450
400
350
300
8.34
13.35
25.21
UVT_214
Cond
Conc B
6
8
10
12
14
16
18
20
22
24
26
ml
Ladder
Fr.4
Fr.5
Fr.6
Fr.7
Fr.14
Fr.15
Fr.27
Fr.33
250kD
150kD
100kD
75kD
50kD
37kD
25kD
20kD
12kD
10kD

FIG. 11

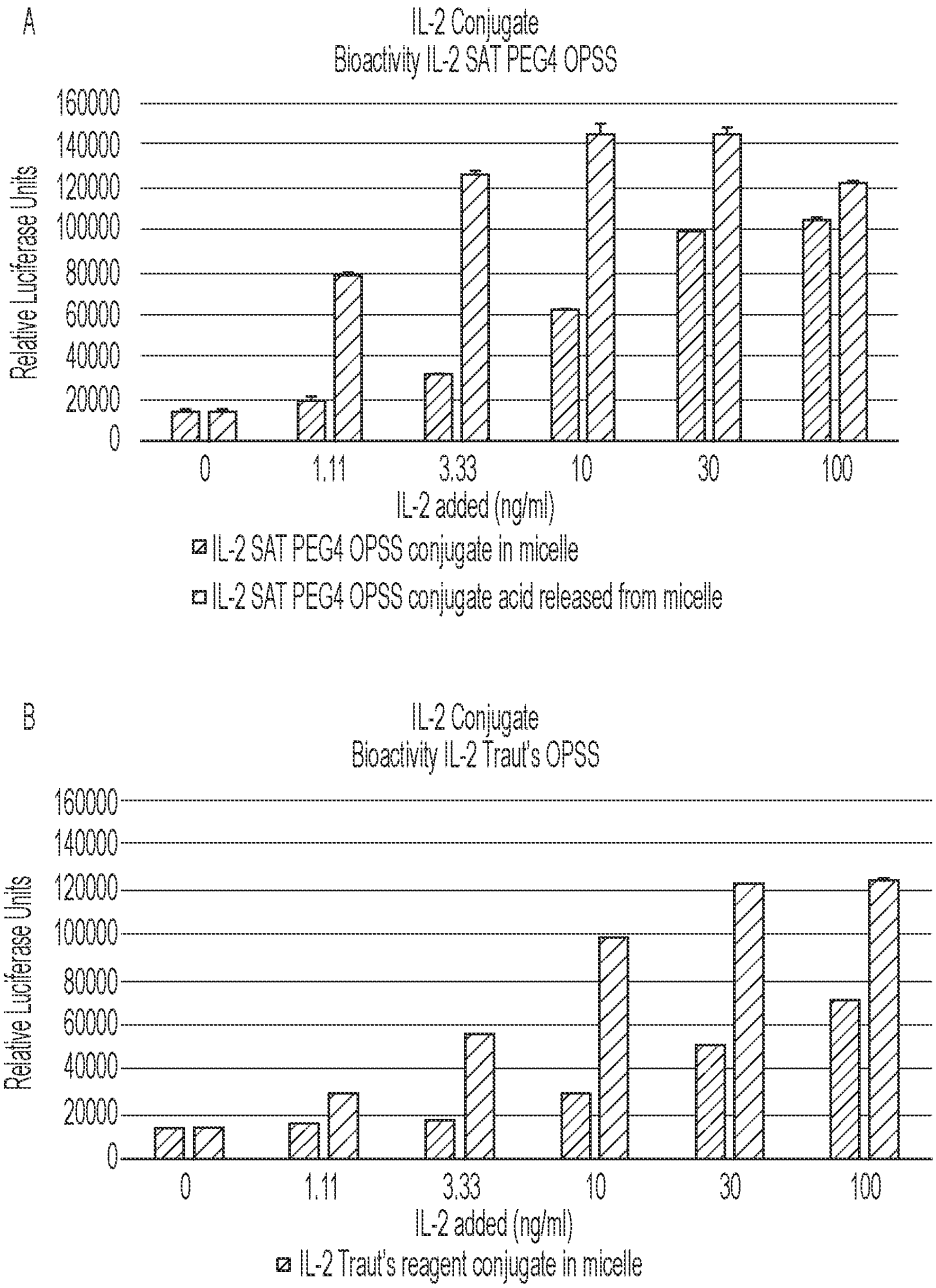
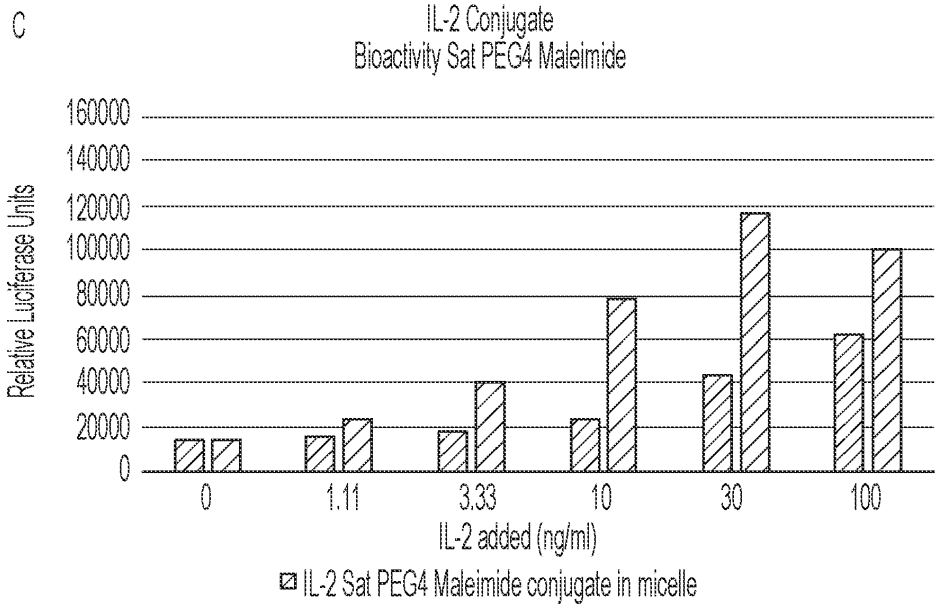

A
IL-2 Conjugate
Bioactivity IL-2 SAT PEG4 OPSS
Relative Luciferase Units
160000
140000
120000
100000
80000
60000
40000
20000
0
0
1.11
3.33
10
30
100
IL-2 added (ng/ml)
IL-2 SAT PEG4 OPSS conjugate in micelle
IL-2 SAT PEG4 OPSS conjugate acid released from micelle
B
IL-2 Conjugate
Bioactivity IL-2 Traut's OPSS
Relative Luciferase Units
160000
140000
120000
100000
80000
60000
40000
20000
0
0
1.11
3.33
10
30
100
IL-2 added (ng/ml)
IL-2 Traut's reagent conjugate in micelle

FIG. 12

C



D

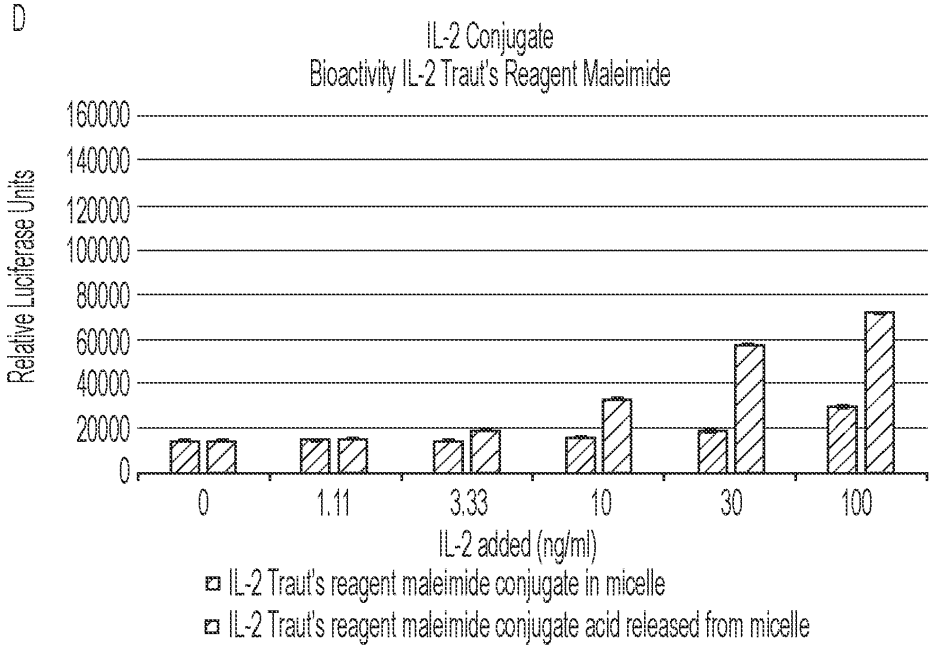


FIG. 12
Continued

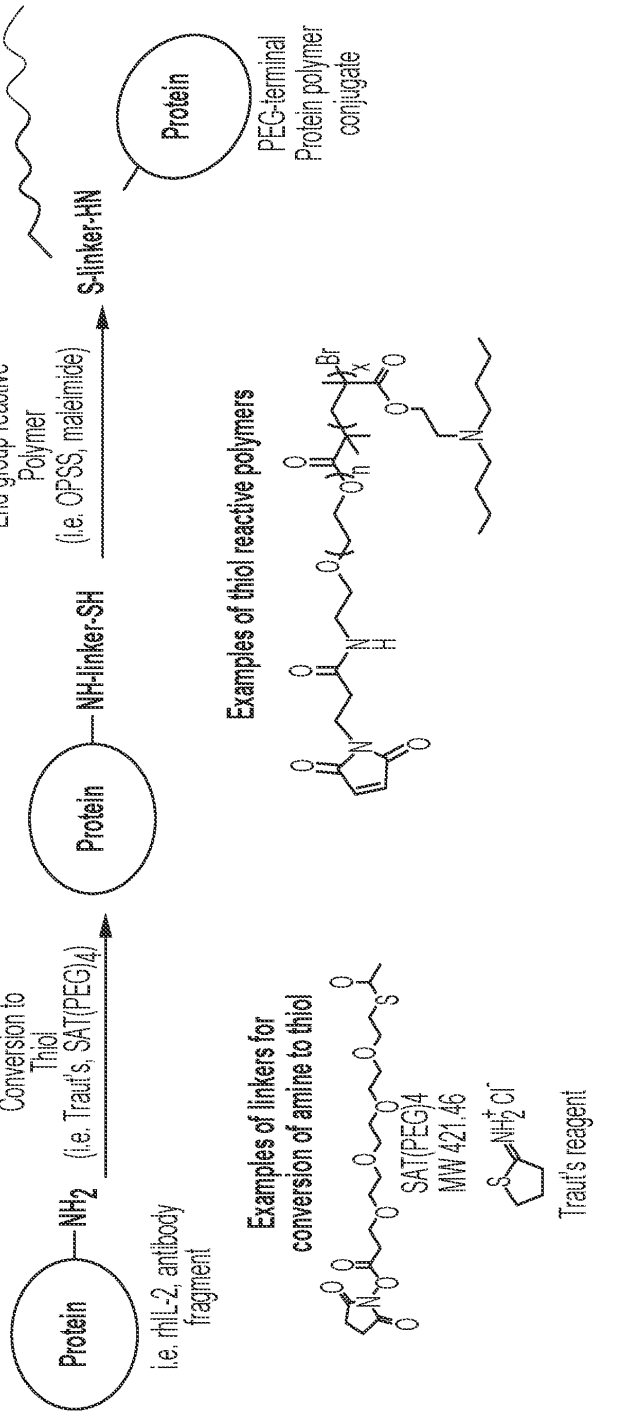

Protein
NH2
i.e. rhIL-2, antibody fragment
Conversion to Thiol
(i.e. Traut's, SAT(PEG)4)
Protein
NH-linker-SH
End-group-reactive Polymer
(i.e. OPSS, maleimide)
S-linker-HN
Protein
PEG-terminal Protein polymer conjugate
Examples of linkers for conversion of amine to thiol
SAT(PEG)4
MW 421.46
Traut's reagent
Examples of thiol reactive polymers

FIG. 13

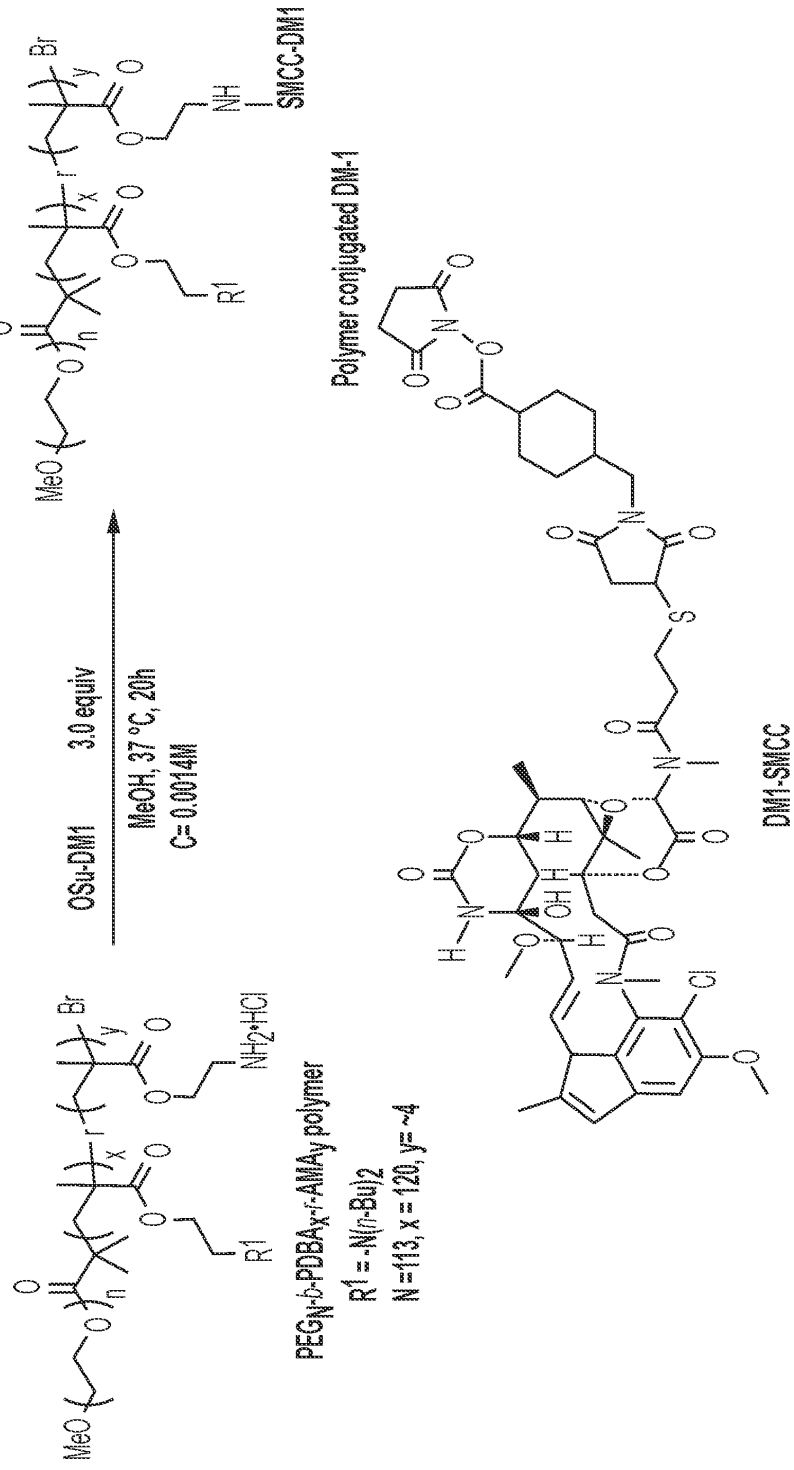

OSu-DM1
3.0 equiv
MeOH, 37 °C, 20h
C= 0.0014M
PEGN-b-PDBAx-r-AMAy polymer
R1 = -N(n-Bu)2
N=113, x = 120, y= ~4
Polymer conjugated DM-1
SMCC-DM1
DM1-SMCC

FIG. 14

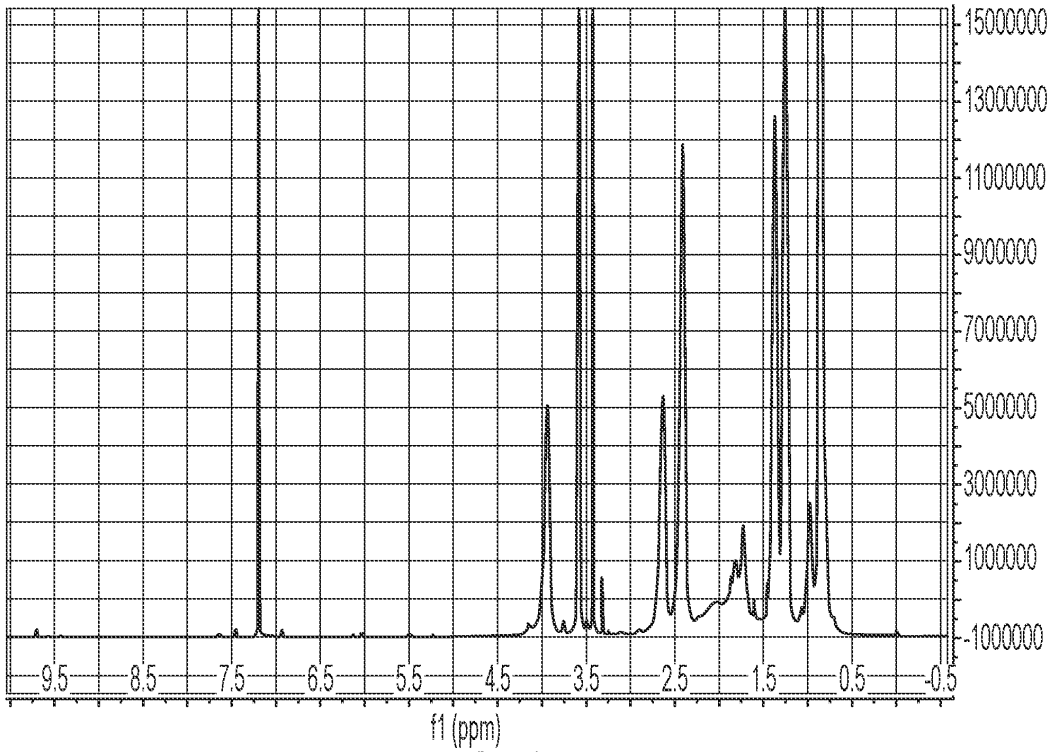


FIG. 15A

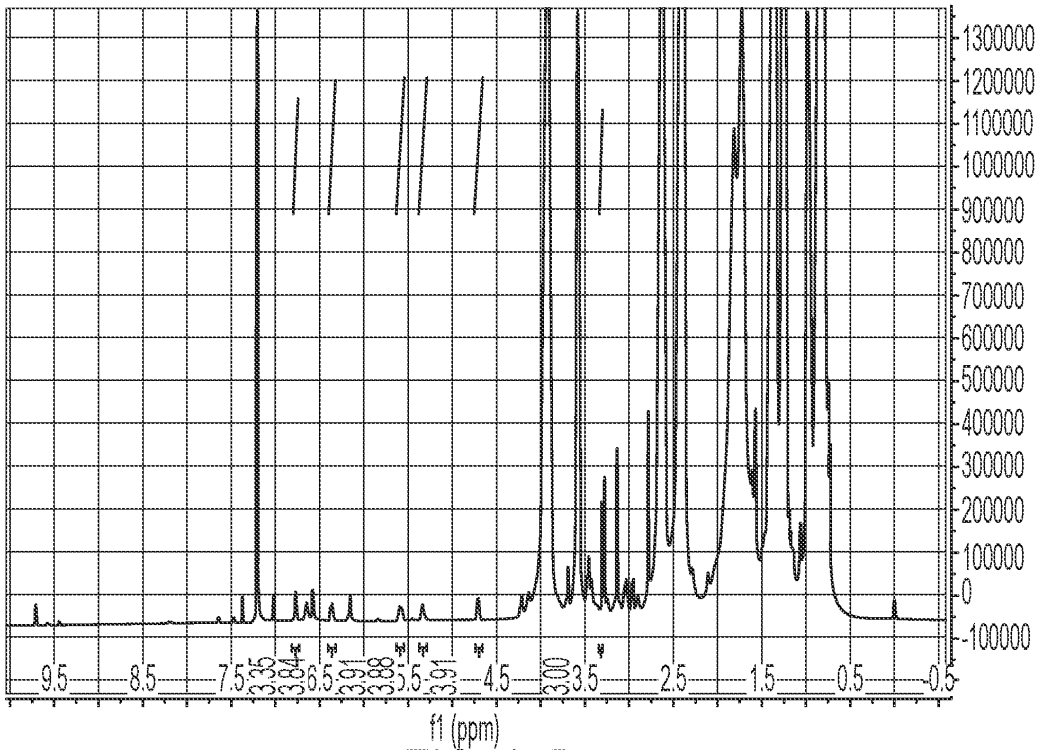


FIG. 15B

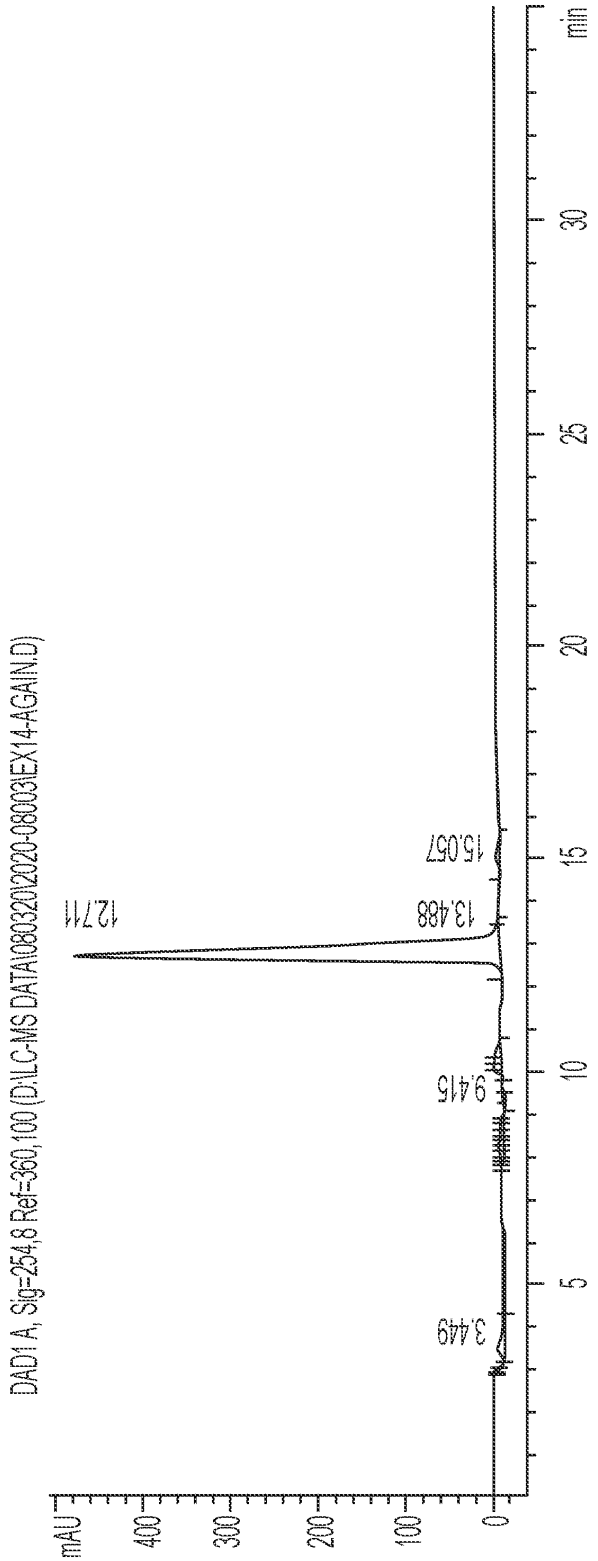

DAD1 A, Sig=254,8 Ref=360,100 (D:\LC-MS DATA\080320\2020-08003\EX14-AGAIN.D)
mAU
400
300
200
100
0
min
5
10
15
20
25
30
3.449
9.415
12.711
13.488
15.057

FIG. 15C

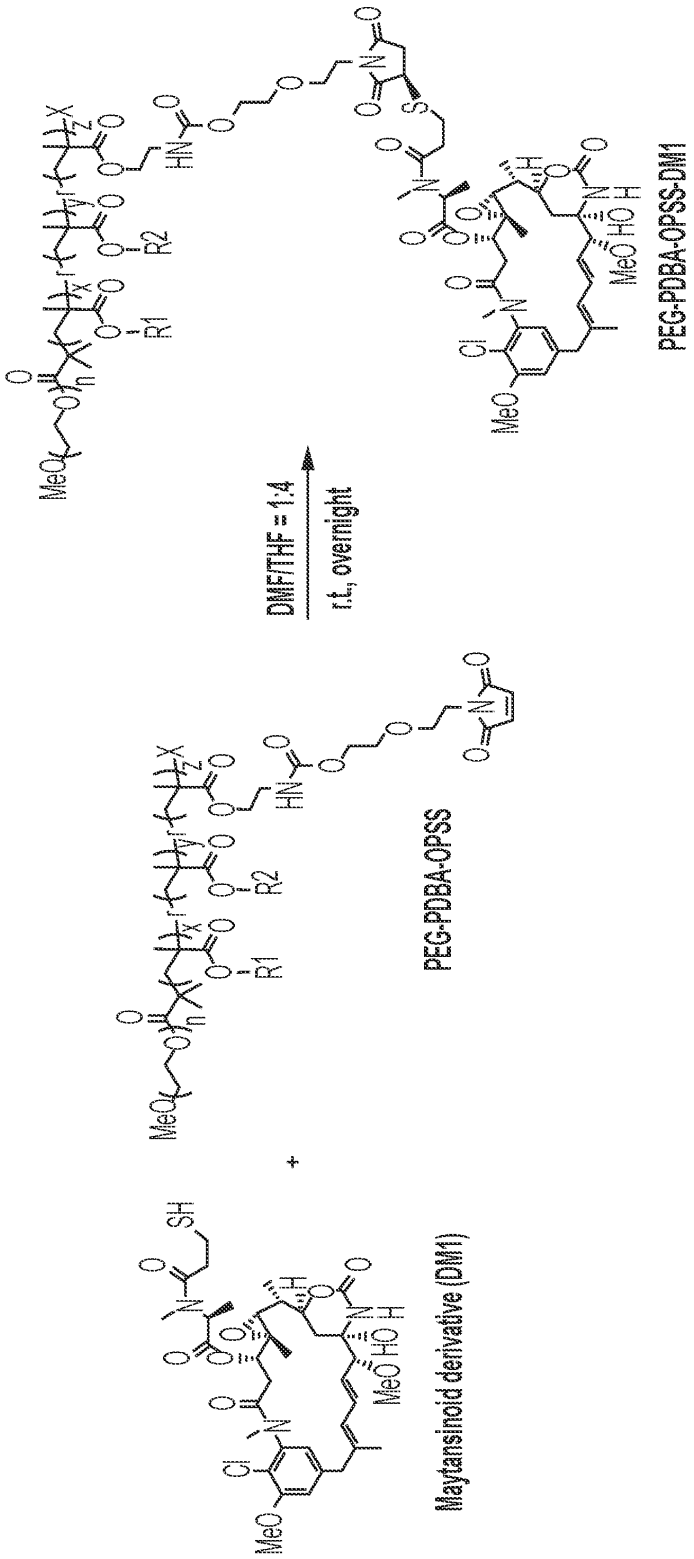

PEG-PDBA-OPSS
Maytansinoid derivative (DM1)
DMF/THF = 1:4
r.t., overnight
PEG-PDBA-OPSS-DM1

FIG. 16

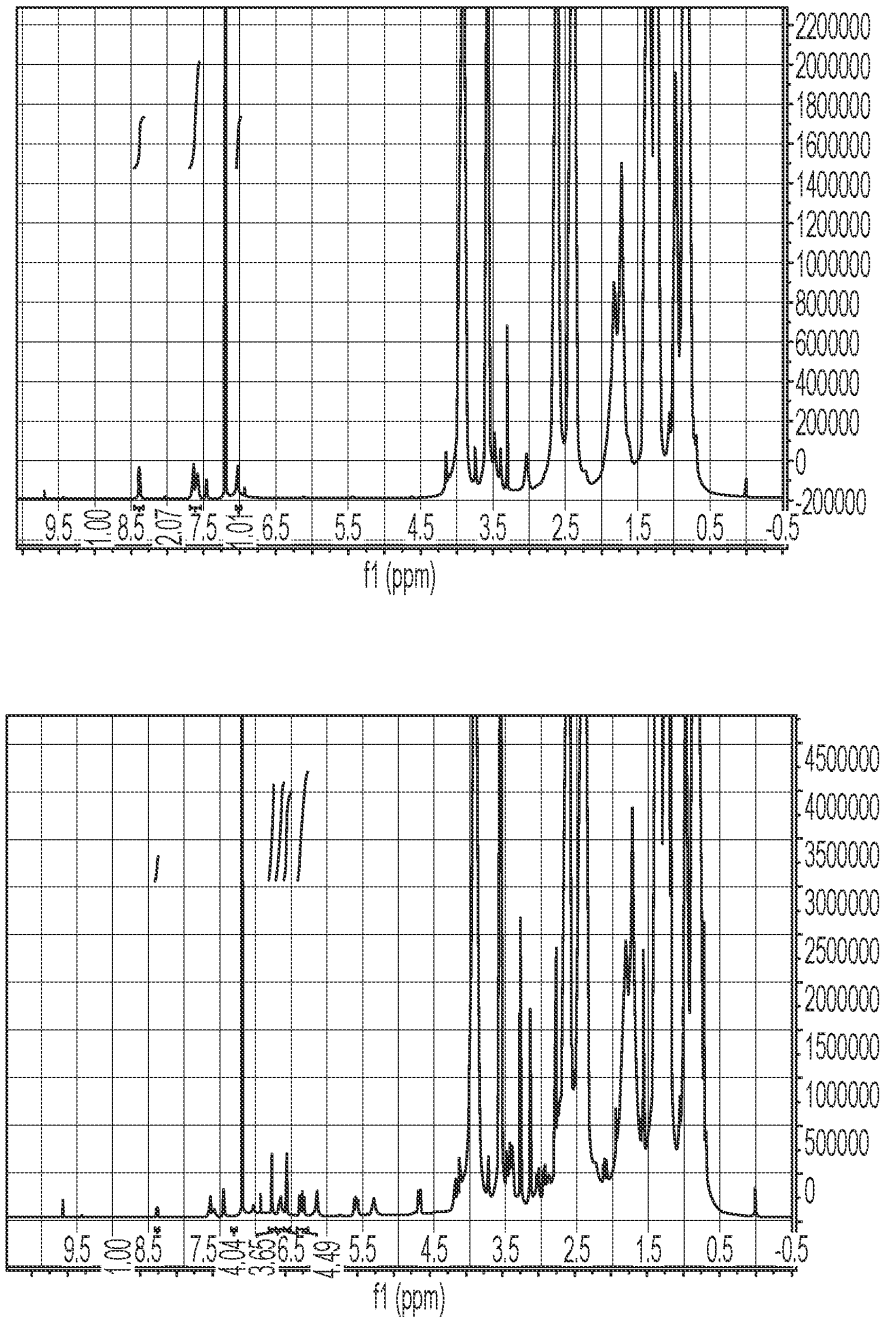

2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-200000
9.5
1.00
8.5
2.07
7.5
1.01
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
f1 (ppm)
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
9.5
1.00
8.5
7.5
4.04
3.69
6.5
4.49
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
f1 (ppm)

FIG. 17

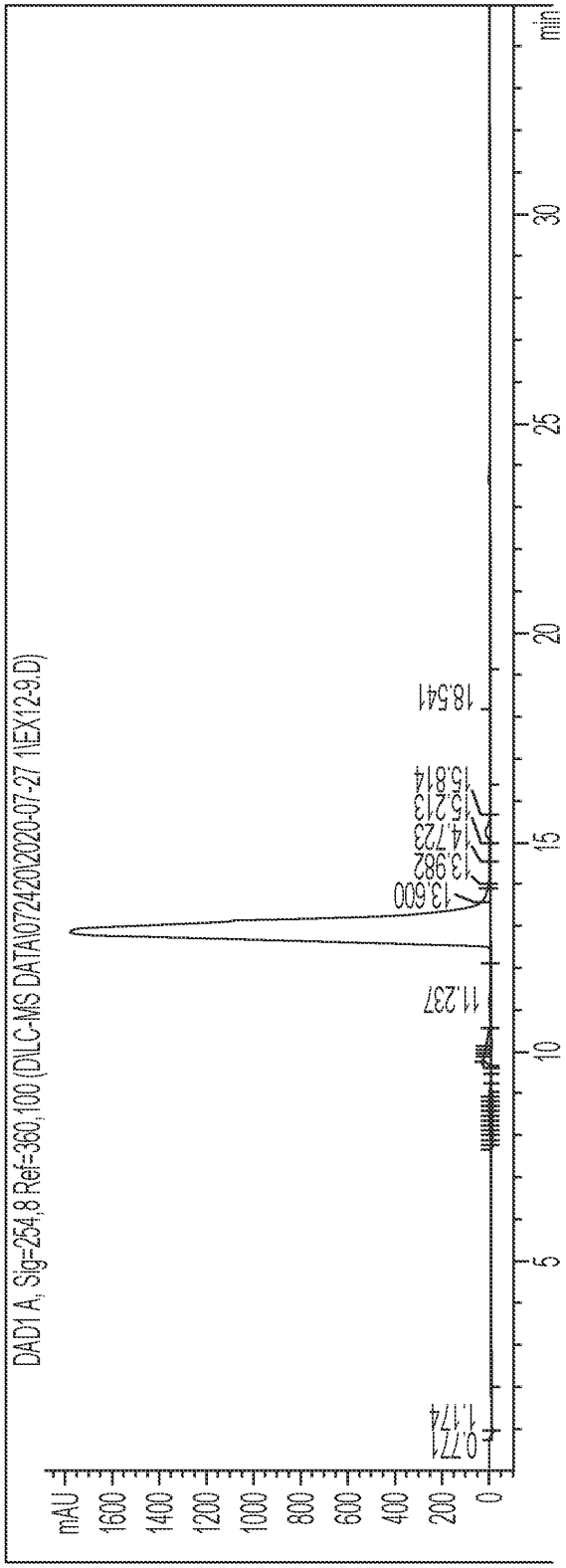

DAD1 A, Sig=254,8 Ref=360,100 (DILC-MS DATA\072420\2020-07-27 11EX12-9.D)
mAU
1600
1400
1200
1000
800
600
400
200
0
min
5
10
15
20
25
30
0.771
1.174
11.237
13.600
13.982
14.723
15.213
15.814
18.541

FIG. 17C

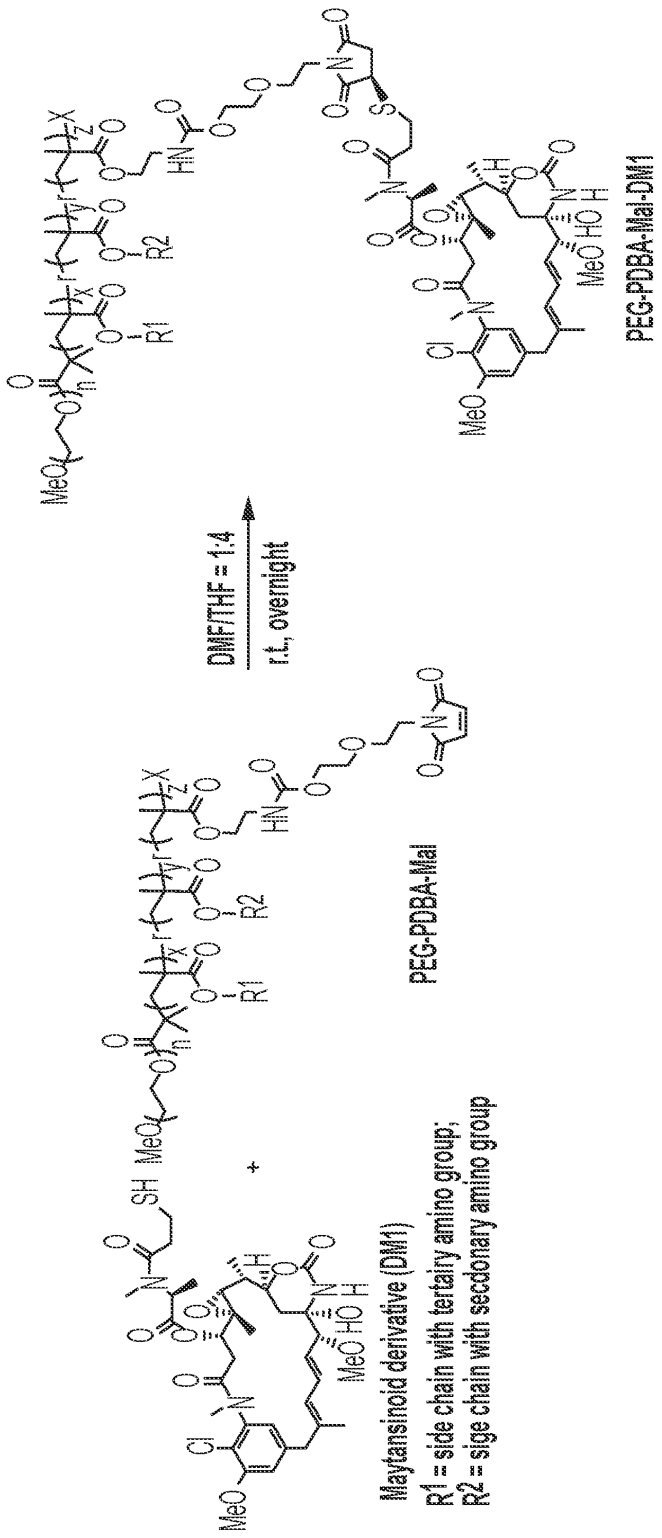

DMF/THF = 1:4
r.t., overnight
PEG-PDBA-Mal
PEG-PDBA-Mal-DM1
Maytansinoid derivative (DM1)
R1 = side chain with tertiary amino group;
R2 = sige chain with secdonary amino group

FIG. 18