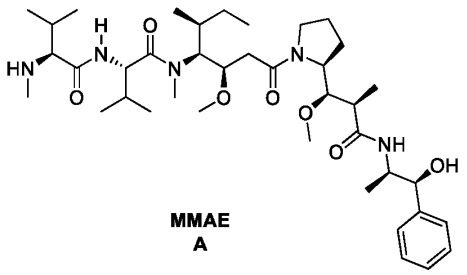
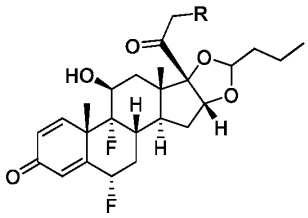
FIG. 1

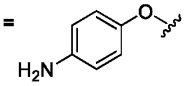


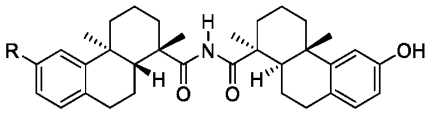
MMAE
A



B: R = NH_2

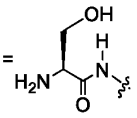
C: R =





D: R = NH_2

E: R =



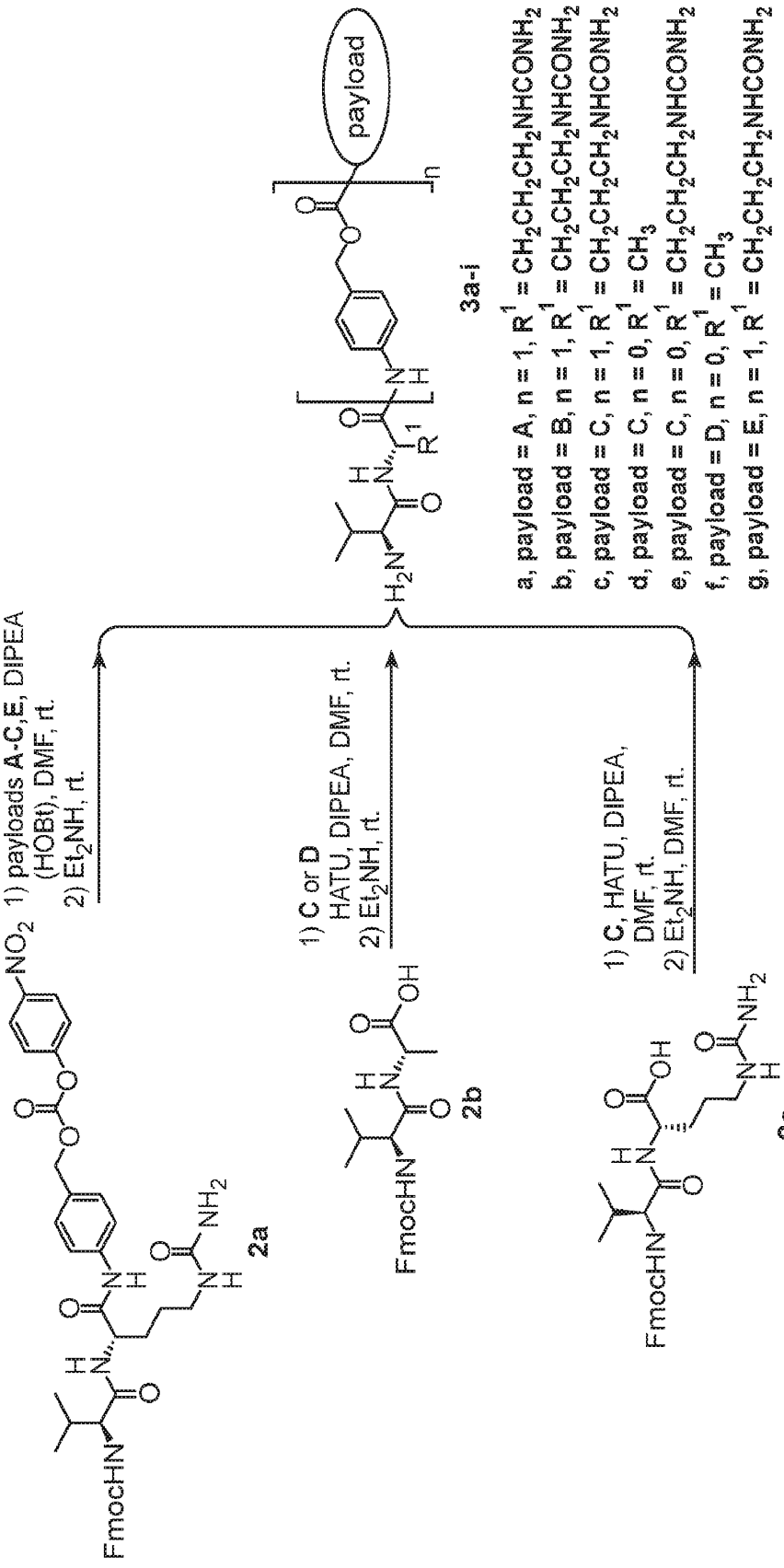

1) payloads A-C,E, DIPEA
(HOBt), DMF, rt.
2) Et2NH, rt.
1) C or D
HATU, DIPEA, DMF, rt.
2) Et2NH, rt.
1) C, HATU, DIPEA,
DMF, rt.
2) Et2NH, DMF, rt.
payload
3a-j
a, payload = A, n = 1, R1 = CH2CH2CH2NHCONH2
b, payload = B, n = 1, R1 = CH2CH2CH2NHCONH2
c, payload = C, n = 1, R1 = CH2CH2CH2NHCONH2
d, payload = C, n = 0, R1 = CH3
e, payload = C, n = 0, R1 = CH2CH2CH2NHCONH2
f, payload = D, n = 0, R1 = CH3
g, payload = E, n = 1, R1 = CH2CH2CH2NHCONH2
2a
2b
2c

FIG. 2

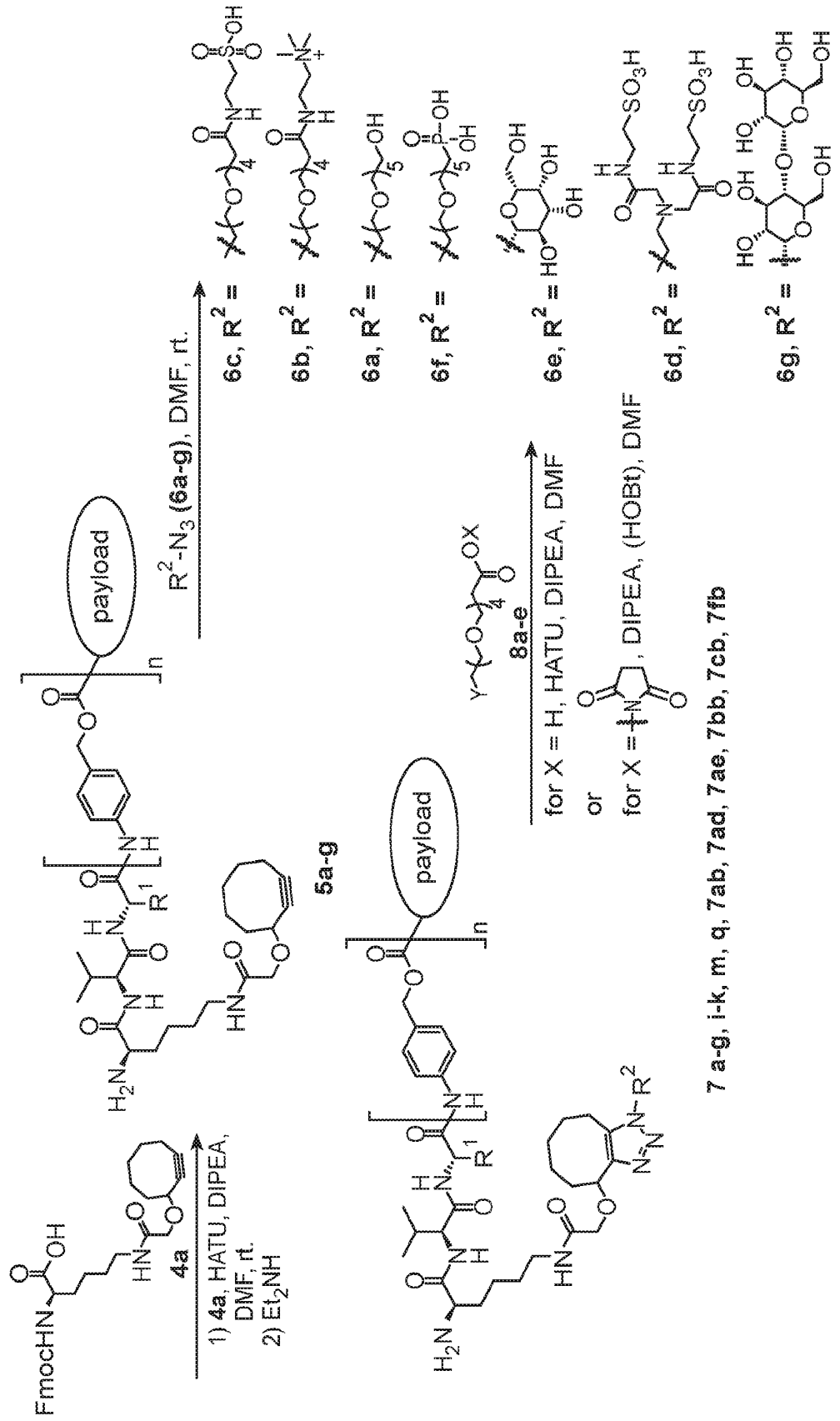
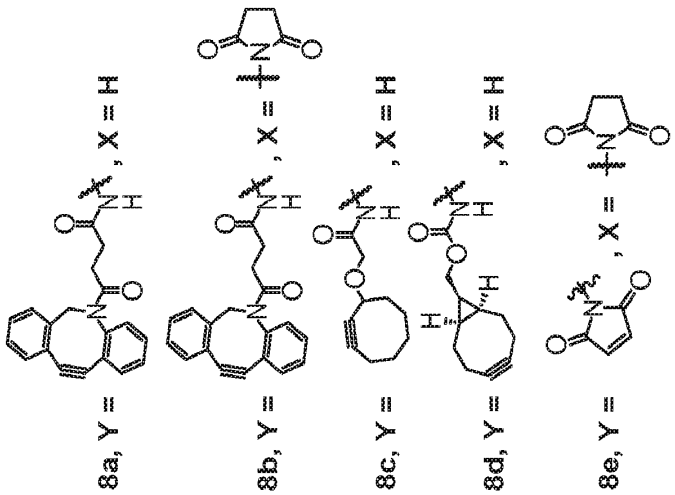

FmocHN
OH
HN
4a
1) 4a, HATU, DIPEA, DMF, rt.
2) Et2NH
payload
n
5a-g
R2-N3 (6a-g), DMF, rt.
6c, R2 =
6b, R2 =
6a, R2 =
6f, R2 =
6e, R2 =
6d, R2 =
6g, R2 =
8a-e
for X = H, HATU, DIPEA, DMF
or
for X =
, DIPEA, (HOBt), DMF
7 a-g, i-k, m, q, 7ab, 7ad, 7ae, 7bb, 7cb, 7fb

FIG. 2 (Cont.)


8a, Y =
, X = H
8b, Y =
, X =
8c, Y =
, X = H
8d, Y =
, X = H
8e, Y =
, X =

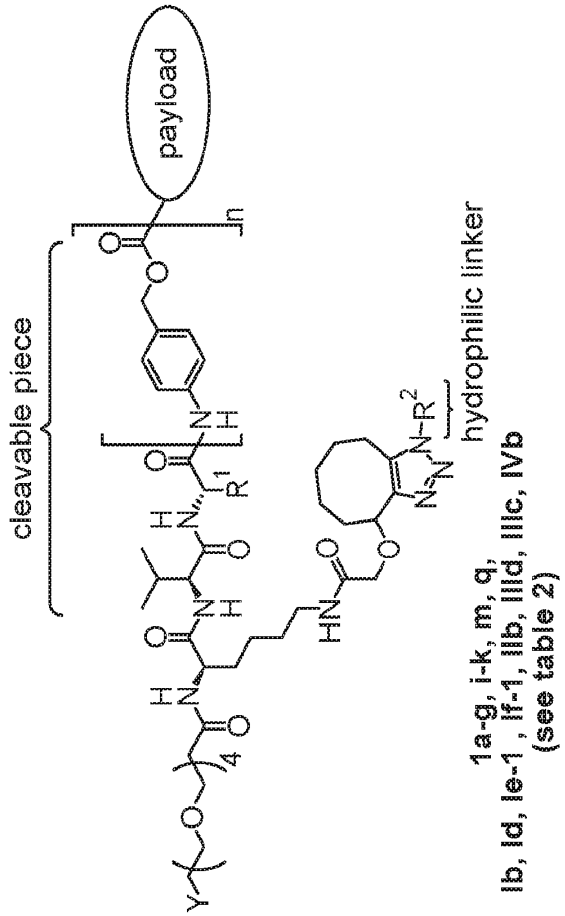

payload
n
cleavable piece
hydrophilic linker
1a-g, i-k, m, q,
Ib, Id, Ie-1, If-1, IIb, IIId, IIIc, IVb
(see table 2)

FIG. 2 (Cont. 1)

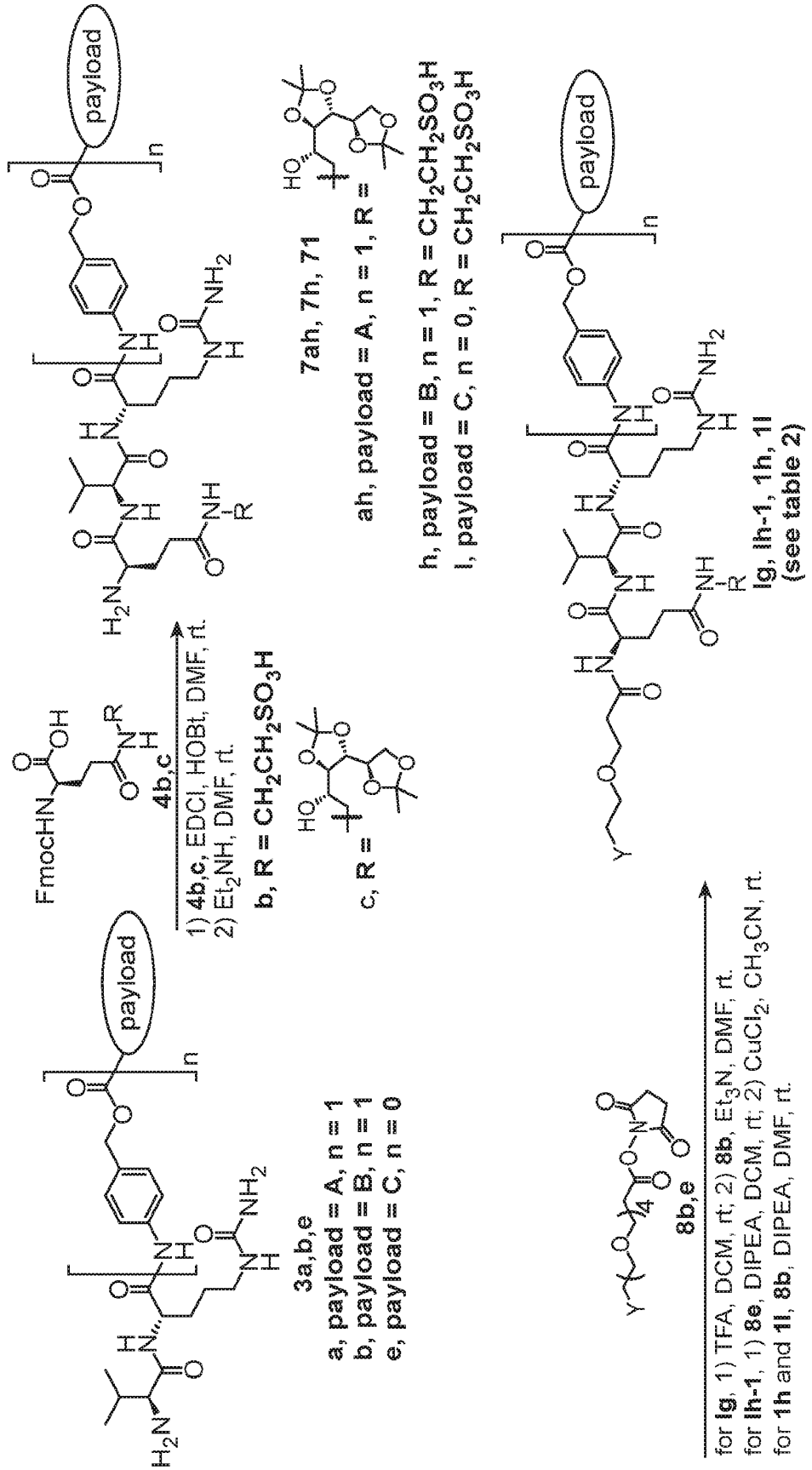

payload
n
FmocHN
4b,c
1) 4b,c, EDCI, HOBt, DMF, rt.
2) Et2NH, DMF, rt.
b, R = CH2CH2SO3H
c, R =
3a,b,e
a, payload = A, n = 1
b, payload = B, n = 1
e, payload = C, n = 0
7ah, 7h, 71
ah, payload = A, n = 1, R =
h, payload = B, n = 1, R = CH2CH2SO3H
l, payload = C, n = 0, R = CH2CH2SO3H
Ig, Ih-1, 1h, 1l
(see table 2)
8b,e
for Ig, 1) TFA, DCM, rt; 2) 8b, Et3N, DMF, rt.
for Ih-1, 1) 8e, DIPEA, DCM, rt; 2) CuCl2, CH3CN, rt.
for 1h and 1l, 8b, DIPEA, DMF, rt.

FIG. 3

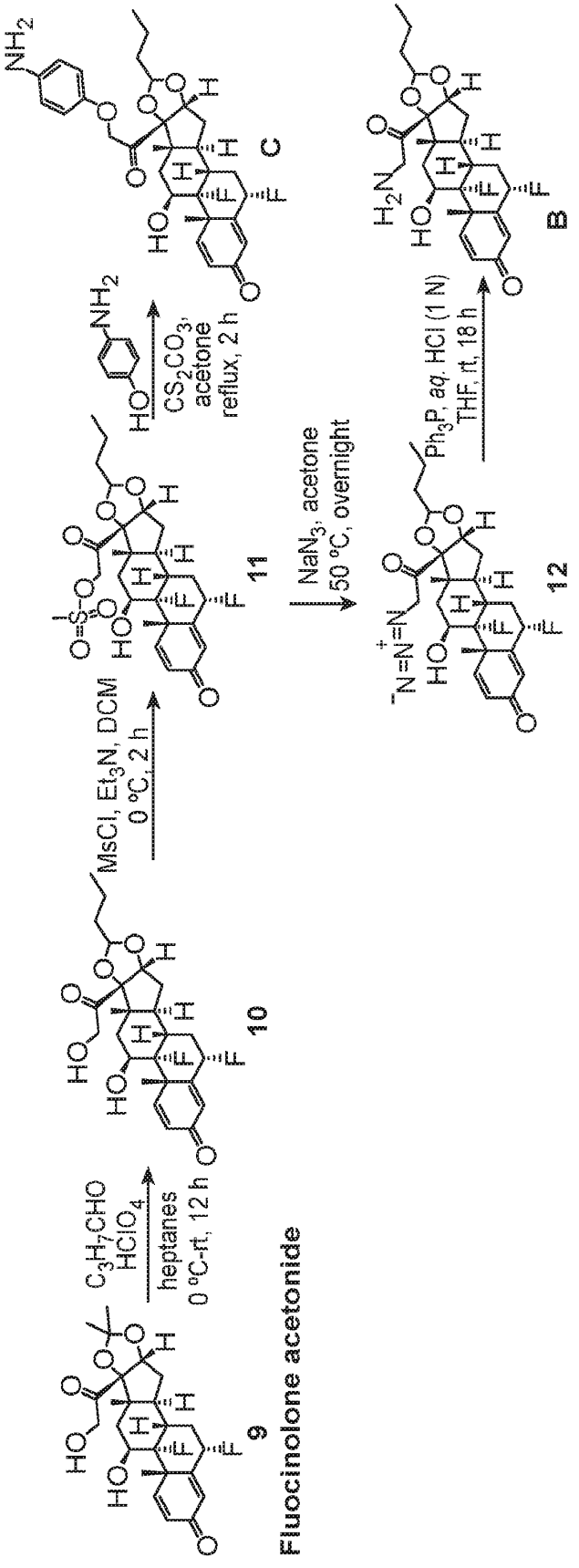

Fluocinolone acetonide
9
C_3H_7CHO
$HClO_4$
heptanes
0 °C-rt, 12 h
10
MsCl, Et_3N, DCM
0 °C, 2 h
11
CS_2CO_3,
acetone
reflux, 2 h
C
NaN_3, acetone
50 °C, overnight
12
Ph_3P, aq. HCl (1 N)
THF, rt, 18 h
B

FIG. 4

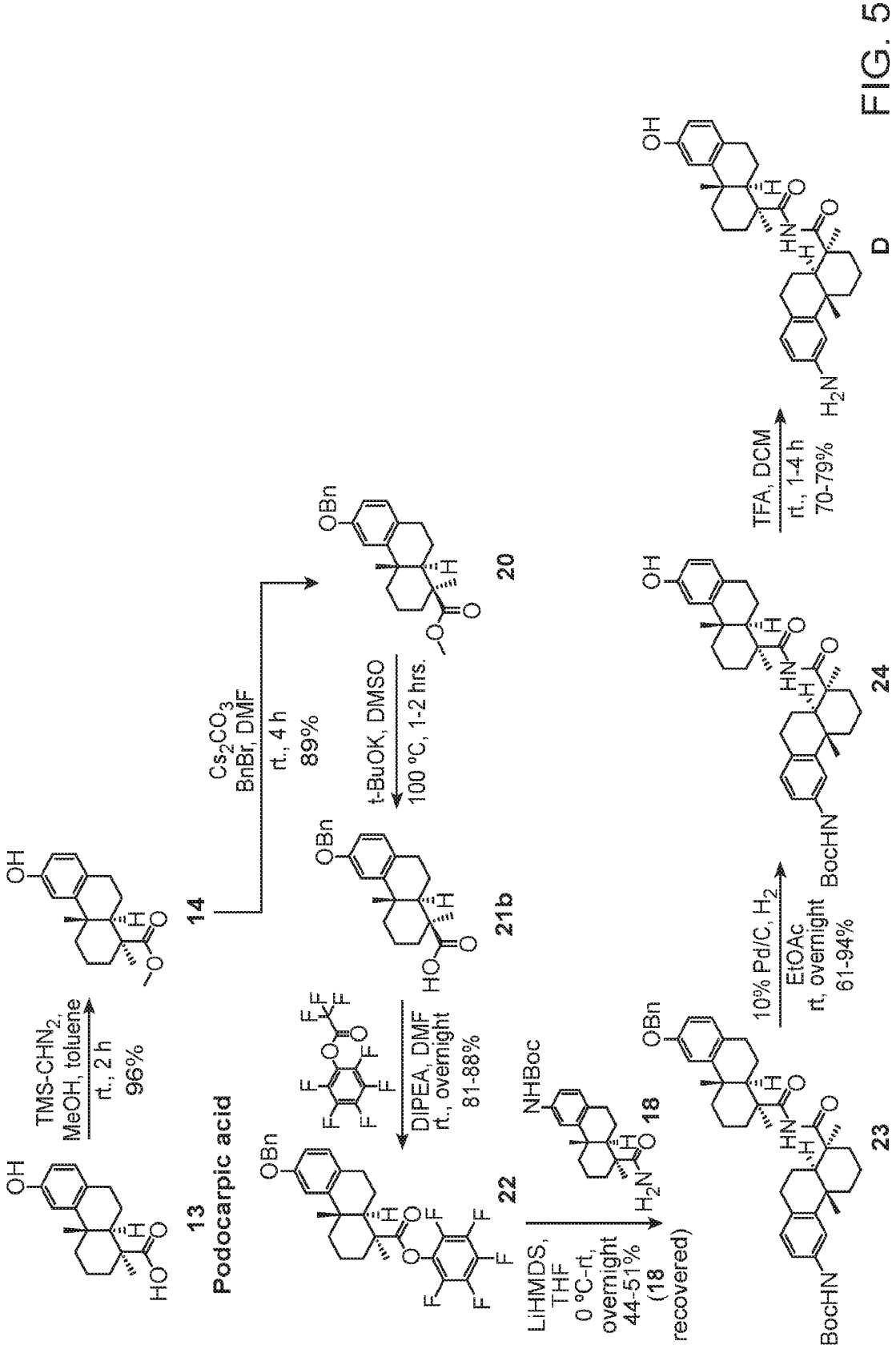
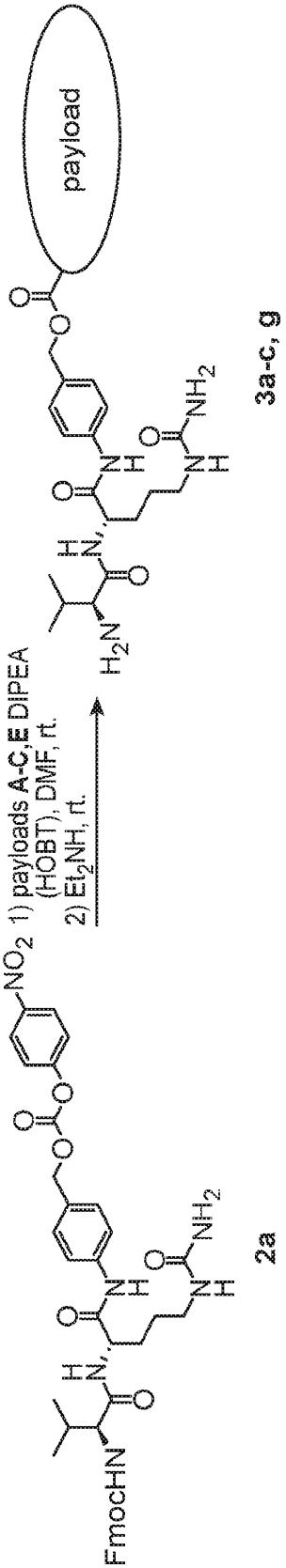

TMS-CHN2, MeOH, toluene
rt., 2 h
96%
13
Podocarpic acid
14
Cs2CO3
BnBr, DMF
rt., 4 h
89%
20
t-BuOK, DMSO
100 °C, 1-2 hrs.
21b
DIPEA, DMF
rt., overnight
81-88%
22
LiHMDS, THF
0 °C-rt, overnight
44-51% (18 recovered)
18
10% Pd/C, H2
EtOAc
rt, overnight
61-94%
23
TFA, DCM
rt., 1-4 h
70-79%
D
24
OH
OBn
NHBoc
BocHN
H2N

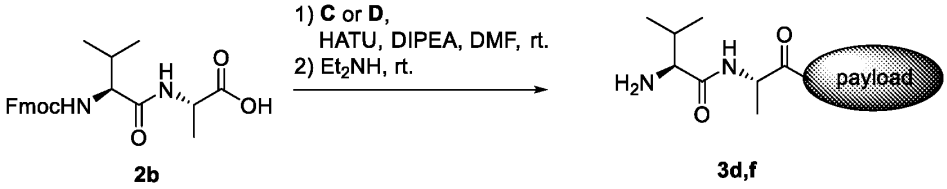
FIG. 5



Product	Payload
3a	A
3b	B
3c	C
3g	E

FIG. 6

FIG. 7



Product	Payload
3d	C
3f	D

FIG. 8

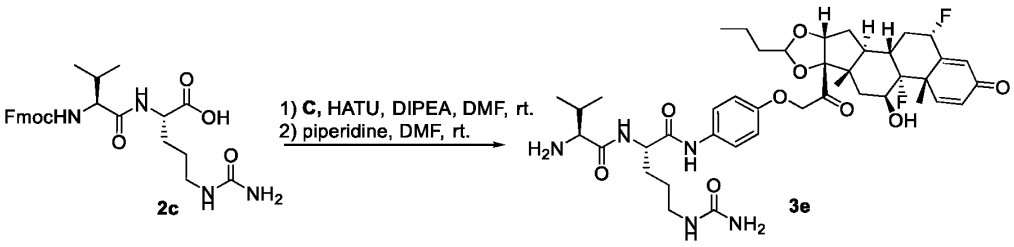


FIG. 9

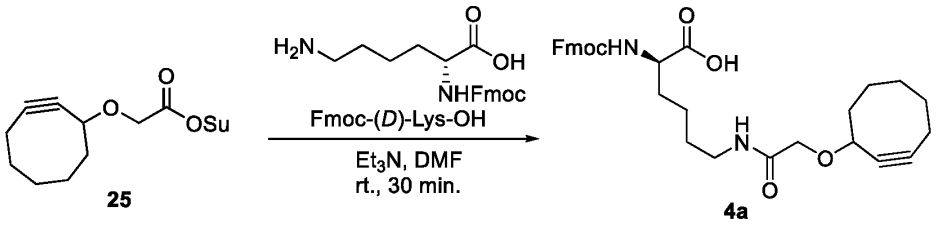

O
O
OSu
25
H2N
O
OH
NHFmoc
Fmoc-(D)-Lys-OH
Et3N, DMF
rt., 30 min.
FmocHN
O
OH
H
N
O
O
4a

FIG. 10

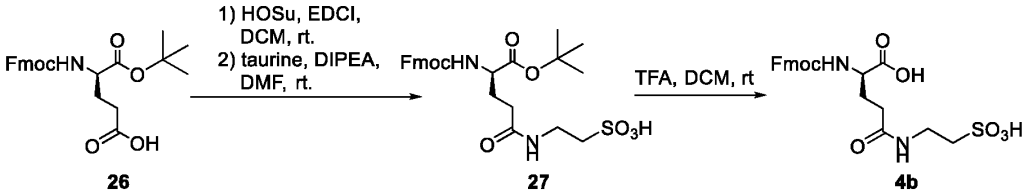
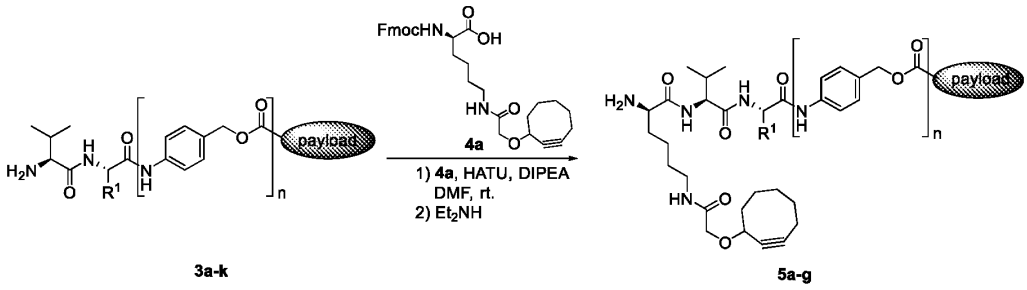
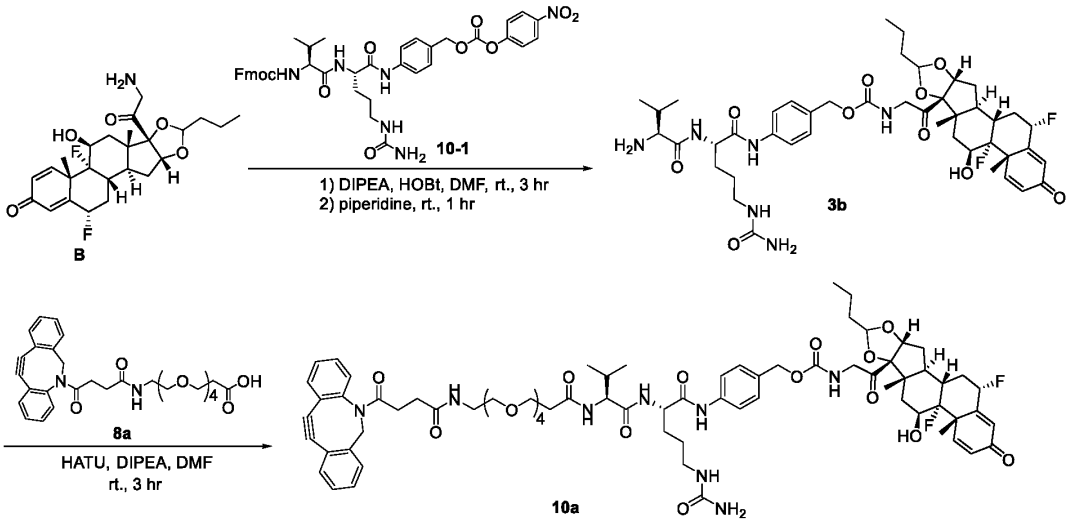

1) HOSu, EDCI,
DCM, rt.
2) taurine, DIPEA,
DMF, rt.
TFA, DCM, rt
FmocHN
OH
SO3H
26
27
4b

FIG. 11



Amine 3	Product 5	Payload	n	R^1
3a	**5a**	**A**	1	$(CH_2)_3NHCONH_2$
3b	**5b**	**B**	1	$(CH_2)_3NHCONH_2$
3c	**5c**	**C**	1	$(CH_2)_3NHCONH_2$
3d	**5d**	**C**	0	CH_3
3e	**5e**	**C**	0	$(CH_2)_3NHCONH_2$
3f	**5f**	**D**	0	CH_3
3g	**5g**	**E**	0	$(CH_2)_3NHCONH_2$

FIG. 11A


B
10-1
1) DIPEA, HOBt, DMF, rt., 3 hr
2) piperidine, rt., 1 hr
3b
8a
HATU, DIPEA, DMF
rt., 3 hr
10a

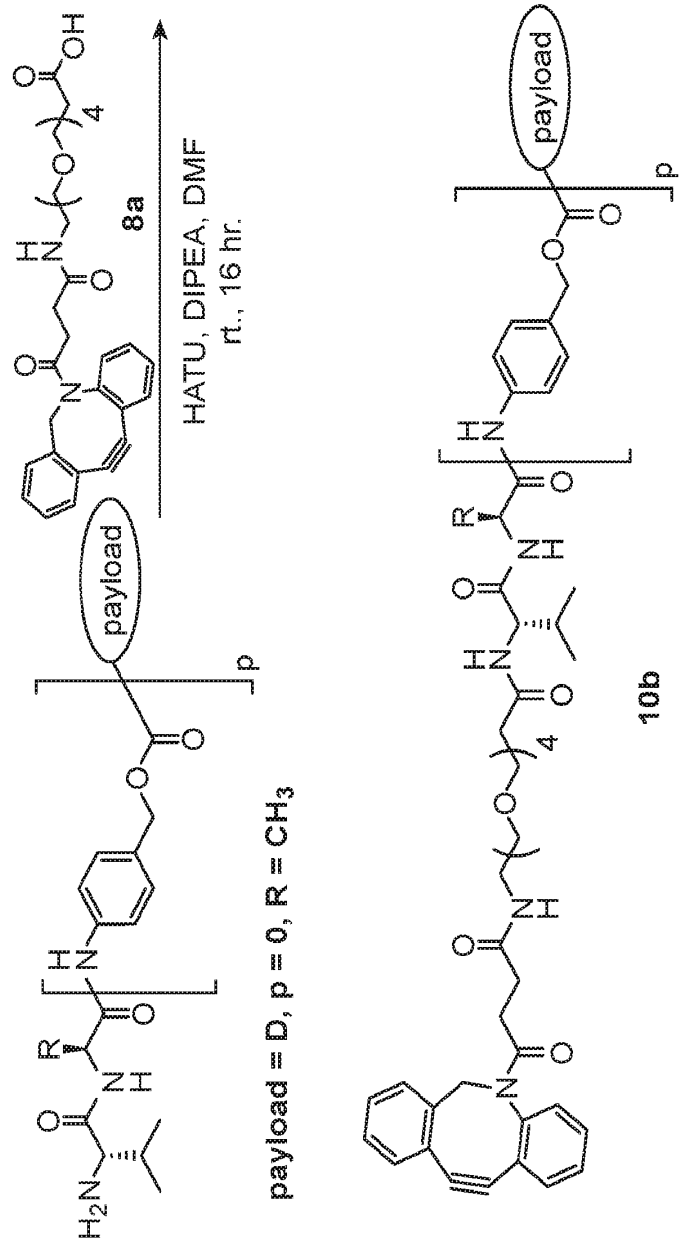
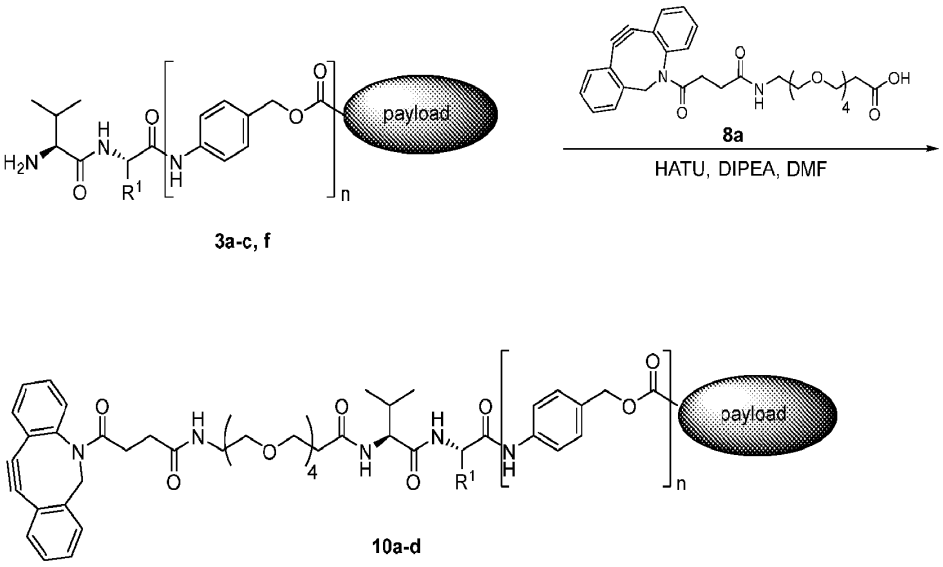


FIG. 11B

FIG. 11C



Amine 3	Product 10	Payload	n	R^1
3a	**10d**	**A**	1	$(CH_2)_3NHCONH_2$
3b	**10a**	**B**	1	$(CH_2)_3NHCONH_2$
3c	**10c**	**C**	1	$(CH_2)_3NHCONH_2$
3f	**10b**	**D**	0	CH_3

FIG. 12



30
CAS: 944251-24-5

6c

FIG. 13

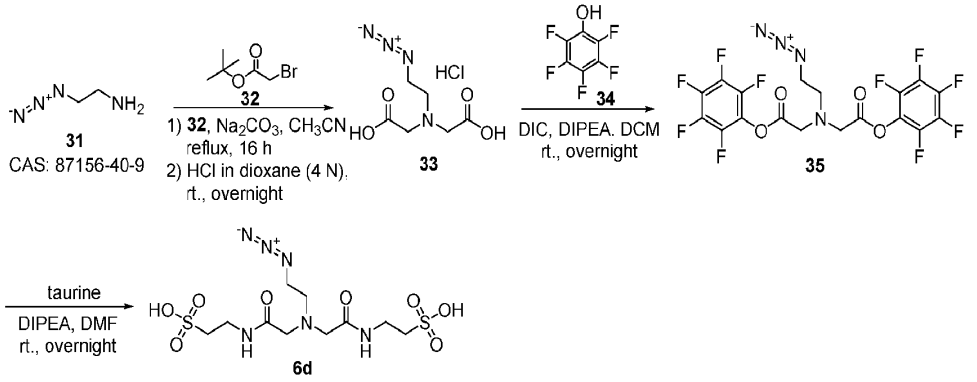


FIG. 13A

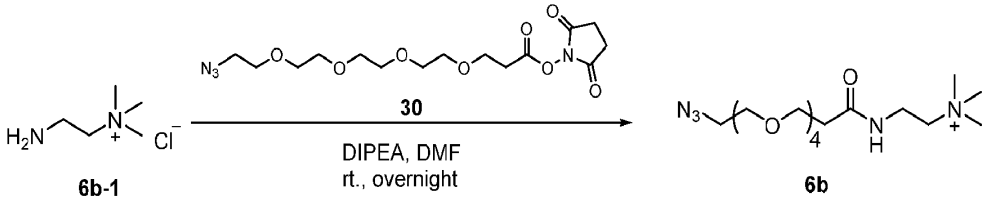
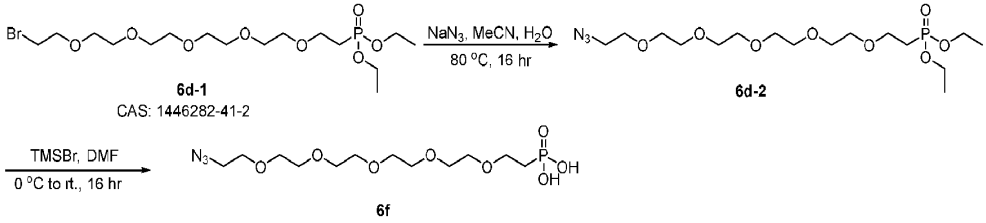
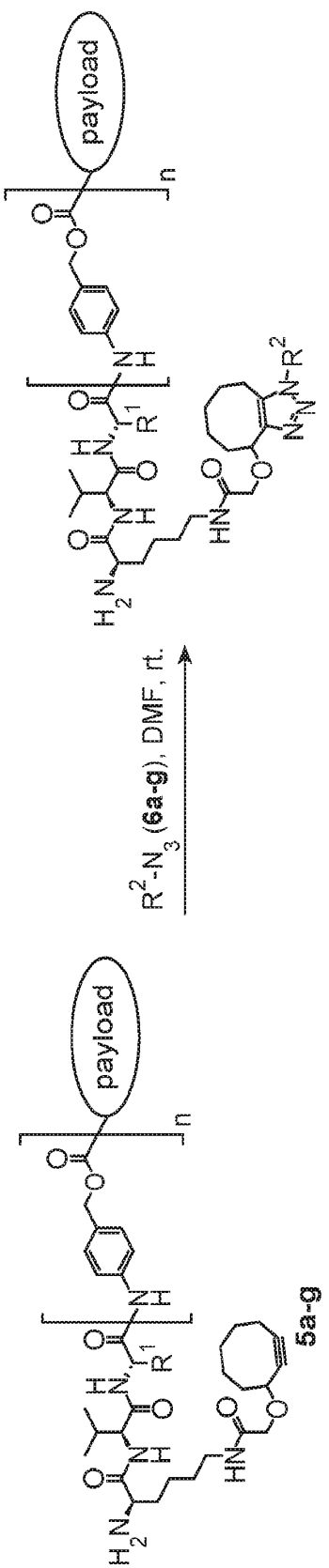


FIG. 13B





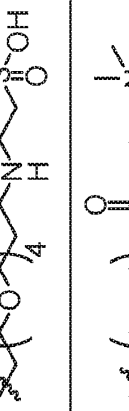
COT 5	Azide 6	Product 7	Payload	n	R^1	R^2
5a	6a	7a	A	1	$(CH_2)_3NHCONH_2$	
5a	6c	7c	A	1	$(CH_2)_3NHCONH_2$	
5a	6b	7ab	A	1	$(CH_2)_3NHCONH_2$	
5a	6f	7ad	A	1	$(CH_2)_3NHCONH_2$	

FIG. 14

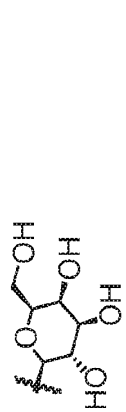
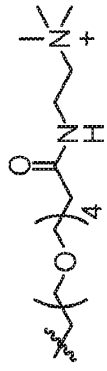
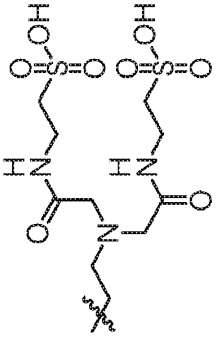
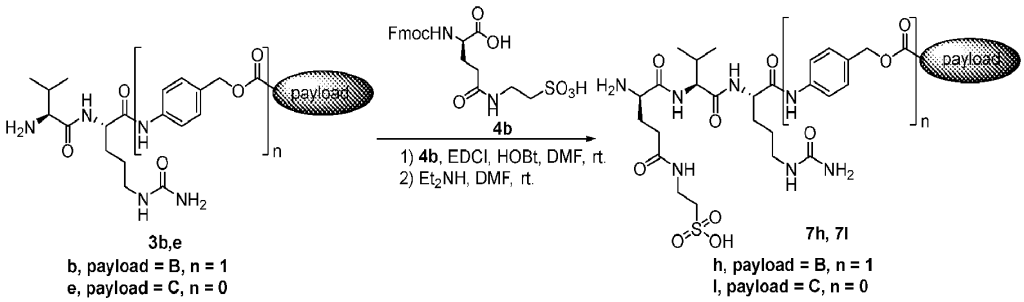
5a	6e	7ae	A	1	
5b	6c	7ba	B	1	$(CH_2)_3NHCONH_2$
5b	6b	7bb	B	1	
5b	6d	7f	B	1	
5c	6c	7i	C	1	$(CH_2)_3NHCONH_2$
5d	6c	7j	C	0	CH_3
5e	6c	7k	C	0	$(CH_2)_3NHCONH_2$

FIG. 14 (Cont.)

5c	6b	7cb	C	1	$(CH_2)_3NHCONH_2$
5f	6c	7m	D	0	CH_3
5f	6b	7fb	D	0	CH_3
5g	6c	7q	E	1	$(CH_2)_3NHCONH_2$

FIG. 14 (Cont. 1)

FIG. 15

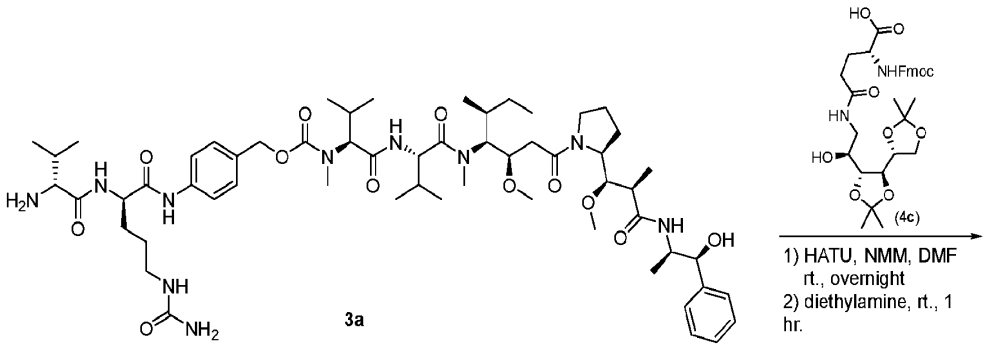


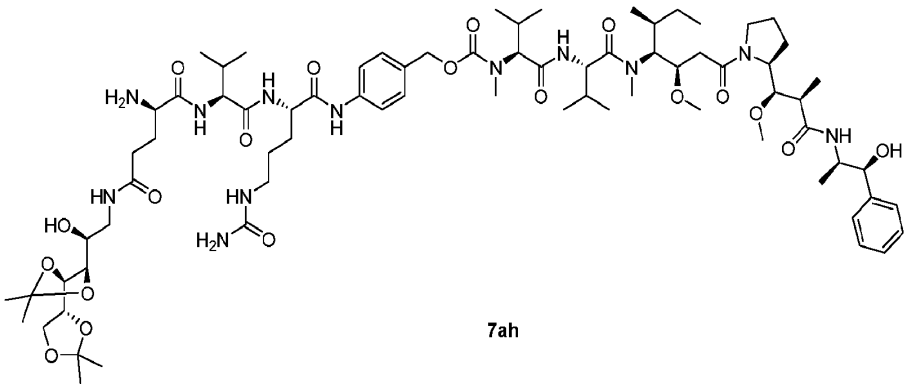
3b,e
b, payload = B, n = 1
e, payload = C, n = 0

h, payload = B, n = 1
l, payload = C, n = 0

3	7	Payload	n
3b	7h	B	1
3e	7l	C	0

FIG. 15A





7ah

FIG. 15B

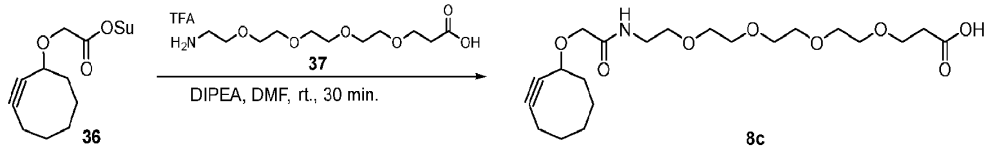
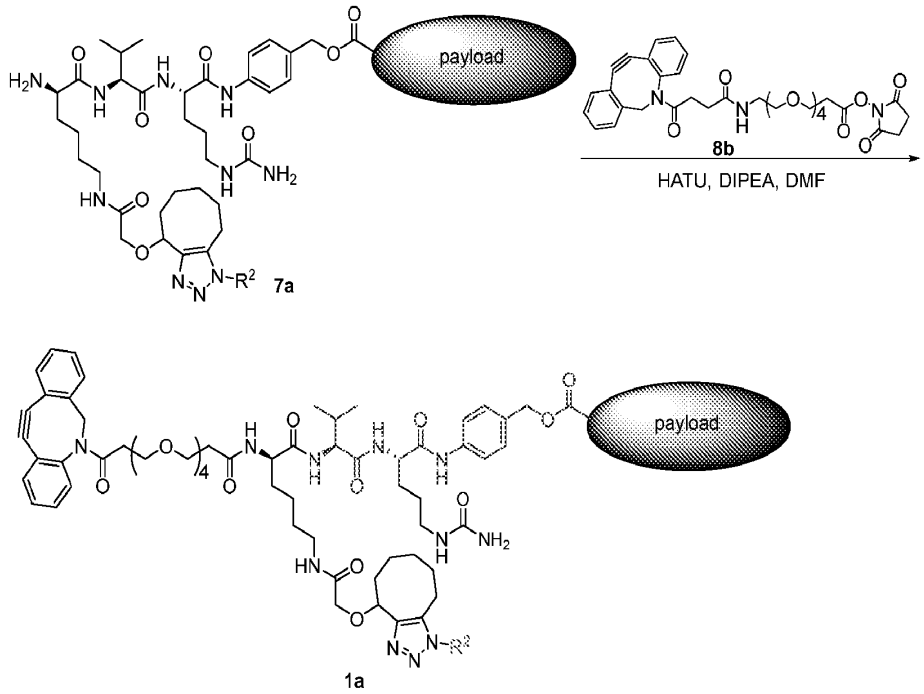
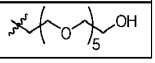
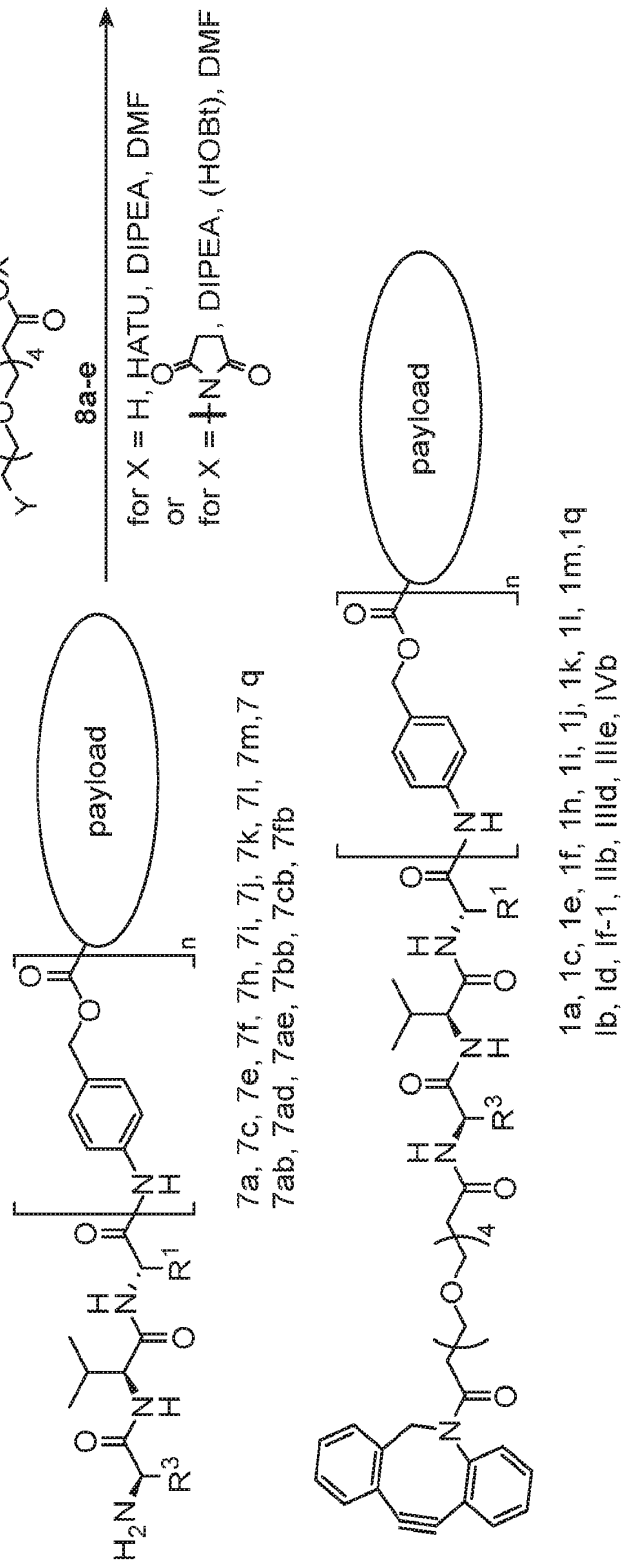


FIG. 16



Amine 7	Product 1	Payload	R^2
7a	1a	A	



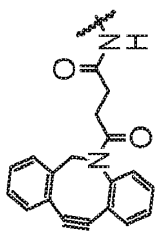
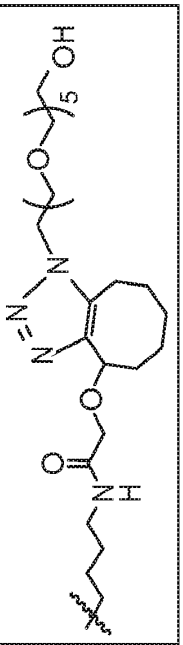
Amine 7	Product 1	Payload	Y	n	R^1	R^3
7a	1a	A		1	$(CH_2)_3NHCONH_2$	

FIG. 17

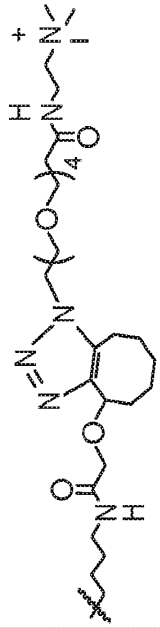
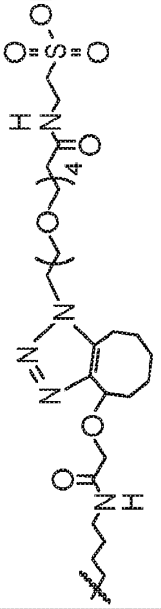
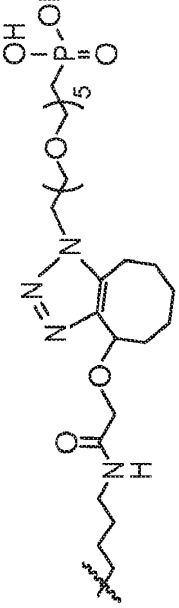
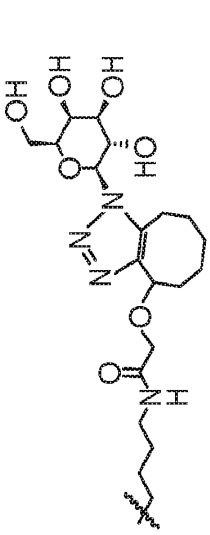
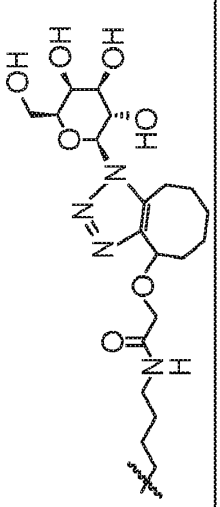
7ab	Ib	A		1	$(CH_2)_3NHCONH_2$
7c	1c	A		1	$(CH_2)_3NHCONH_2$
7ad	Id	A		1	$(CH_2)_3NHCONH_2$
7ae	Ie-1	A		1	$(CH_2)_3NHCONH_2$
7ae	If-1	A		1	$(CH_2)_3NHCONH_2$

FIG. 17 (Cont.1)

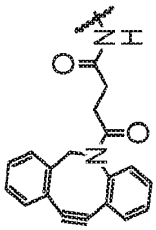
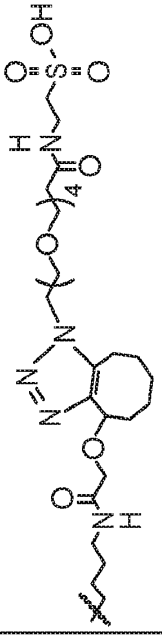
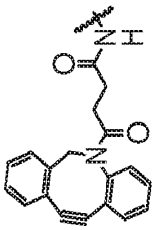
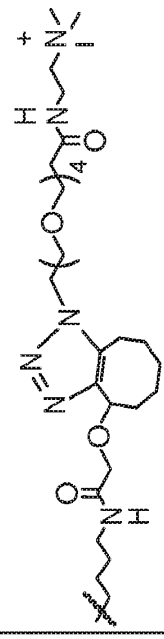
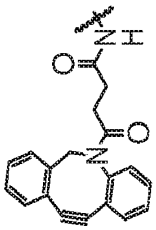
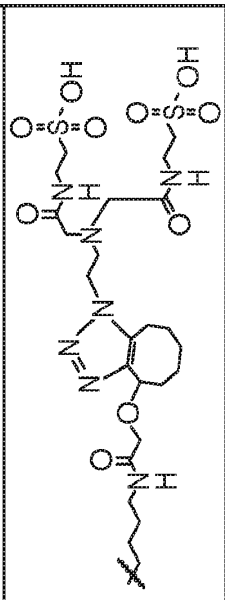
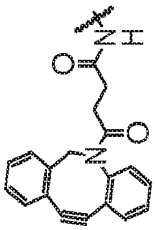
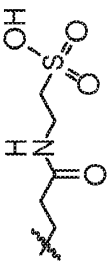
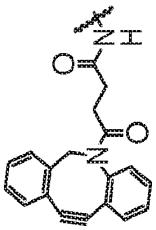
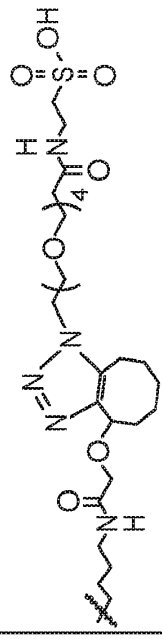
Amine 7	Product 1	Payload	Y	n	R^1	R^3
7e	1e	B		1	$(CH_2)_3NHCONH_2$	
7bb	IIb	B		1	$(CH_2)_3NHCONH_2$	
7f	1f	B		1	$(CH_2)_3NHCONH_2$	
7h	1h	B		1	$(CH_2)_3NHCONH_2$	
7i	1i	C		1	$(CH_2)_3NHCONH_2$	

FIG. 17 (Cont.2)

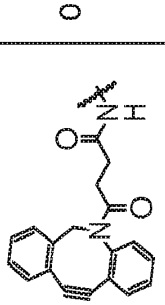
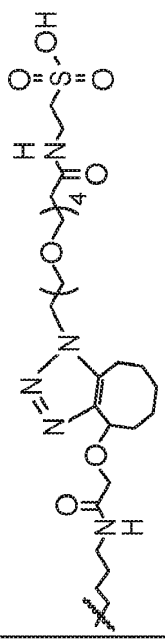
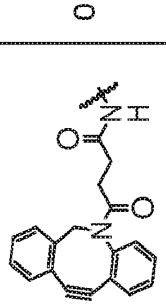
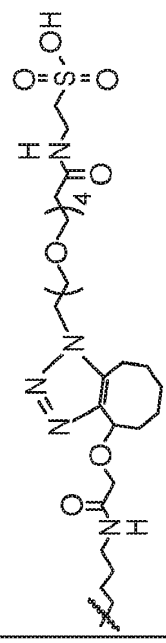
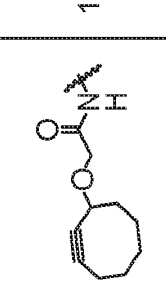
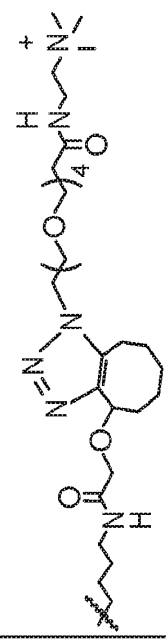
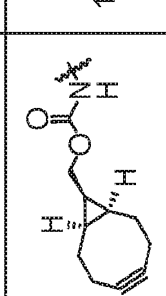
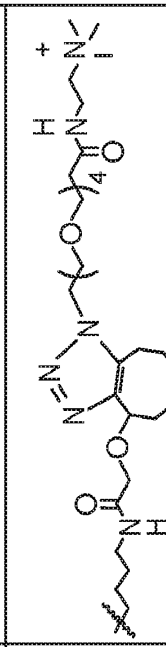
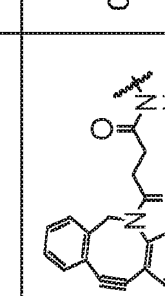
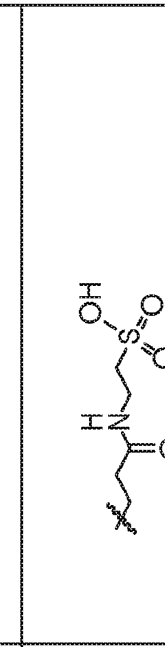
7j	1j	C		0	CH_3	
7k	1k	C		0	$(CH_2)_3NHCONH_2$	
7cb	IIId	C		1	$(CH_2)_3NHCONH_2$	
7cb	IIIe	C		1	$(CH_2)_3NHCONH_2$	
7l	1l	C		0	$(CH_2)_3NHCONH_2$	

FIG. 17 (Cont.3)

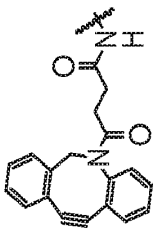
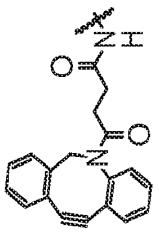
7m	1m	D		0	CH_3
7fb	IVb	D		0	CH_3
7q	1q	E		1	$(CH_2)_3NHCONH_2$

FIG. 17 (Cont.4)

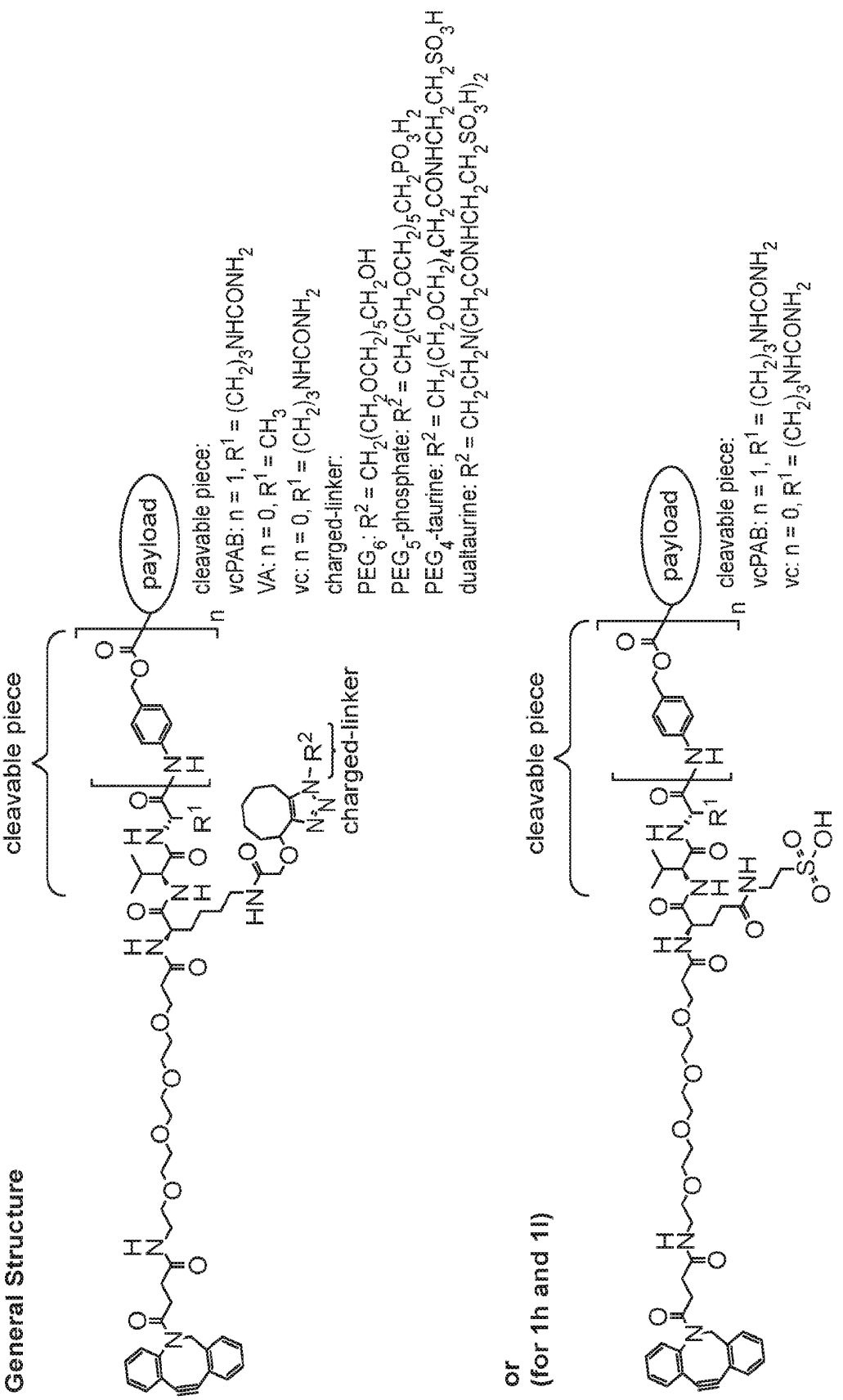
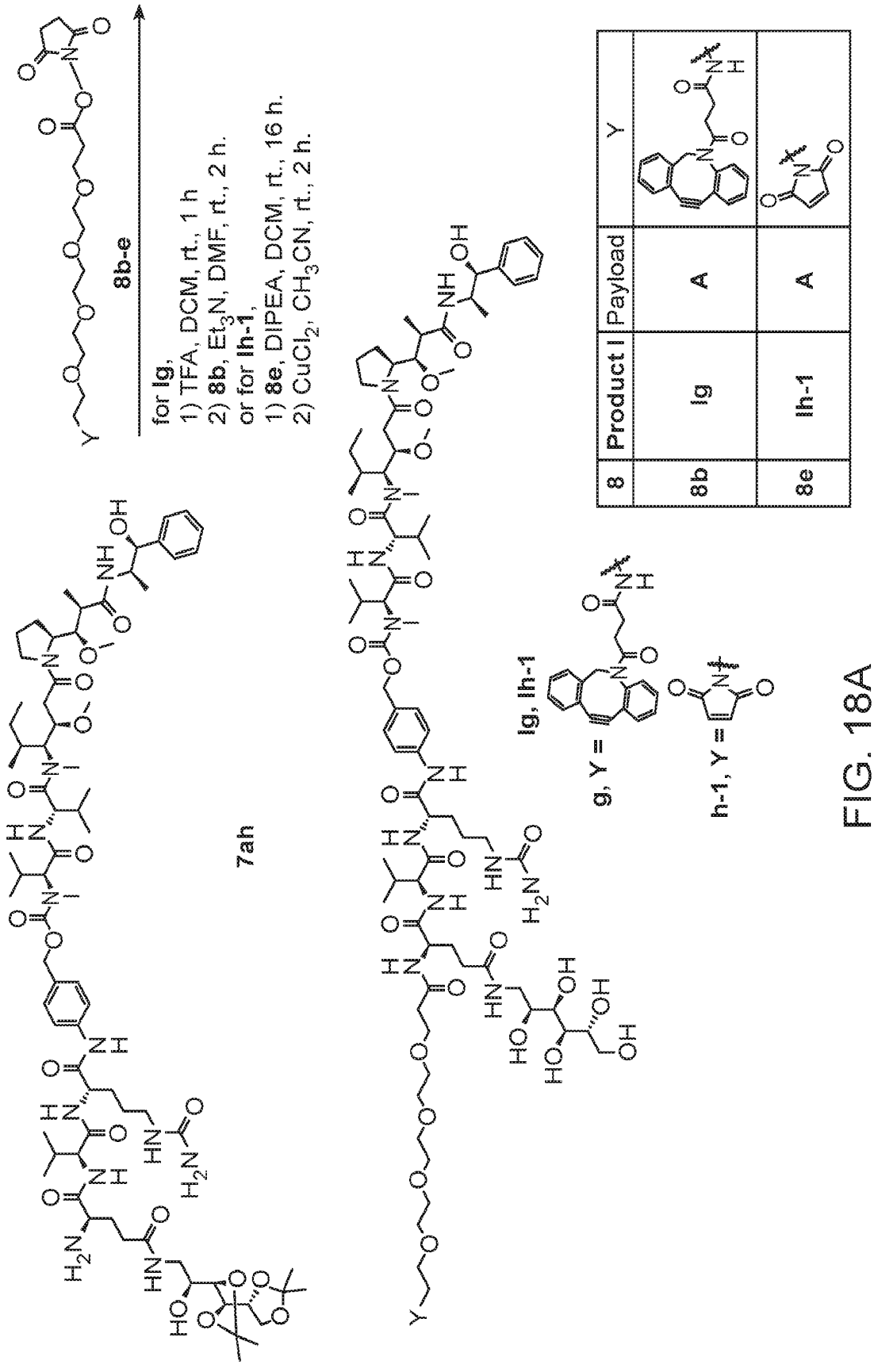

General Structure
cleavable piece
payload
n
cleavable piece:
vcPAB: n = 1, R1 = (CH2)3NHCONH2
VA: n = 0, R1 = CH3
vc: n = 0, R1 = (CH2)3NHCONH2
charged-linker:
PEG6: R2 = CH2(CH2OCH2)5CH2OH
PEG5-phosphate: R2 = CH2(CH2OCH2)5CH2PO3H2
PEG4-taurine: R2 = CH2(CH2OCH2)4CH2CONHCH2CH2SO3H
dualtaurine: R2 = CH2CH2N(CH2CONHCH2CH2SO3H)2
charged-linker
or
(for 1h and 1l)
cleavable piece
payload
n
cleavable piece:
vcPAB: n = 1, R1 = (CH2)3NHCONH2
vc: n = 0, R1 = (CH2)3NHCONH2

FIG. 18



8	Product I	Payload	Y
8b	Ig	A	
8e	Ih-1	A	

FIG. 18A

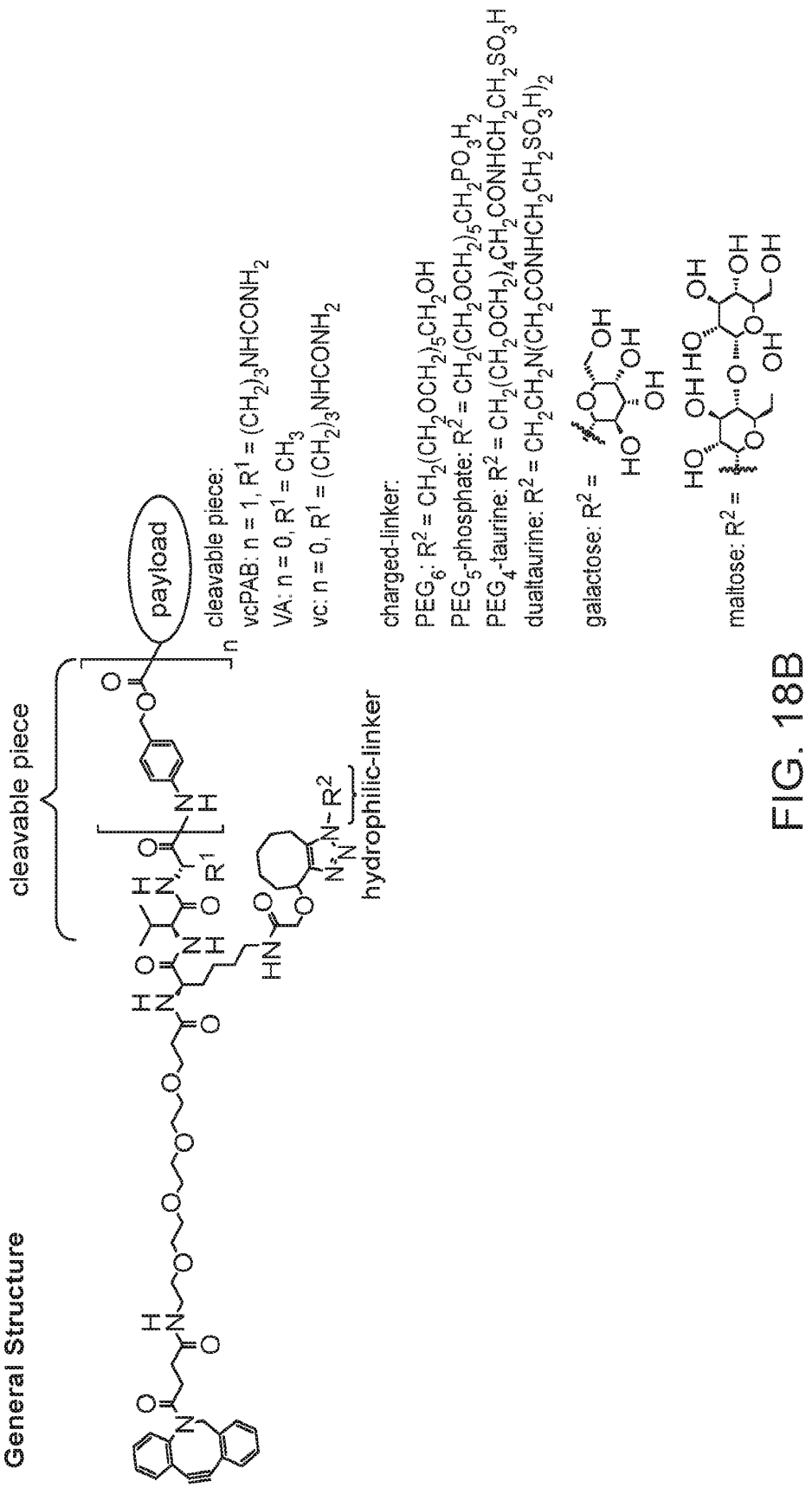

General Structure
cleavable piece
payload
n
hydrophilic-linker
cleavable piece:
vcPAB: n = 1, R1 = (CH2)3NHCONH2
VA: n = 0, R1 = CH3
vc: n = 0, R1 = (CH2)3NHCONH2
charged-linker:
PEG6: R2 = CH2(CH2OCH2)5CH2OH
PEG5-phosphate: R2 = CH2(CH2OCH2)5CH2PO3H2
PEG4-taurine: R2 = CH2(CH2OCH2)4CH2CONHCH2CH2SO3H
dualtaurine: R2 = CH2CH2N(CH2CONHCH2CH2SO3H)2
galactose: R2 =
maltose: R2 =

FIG. 18B

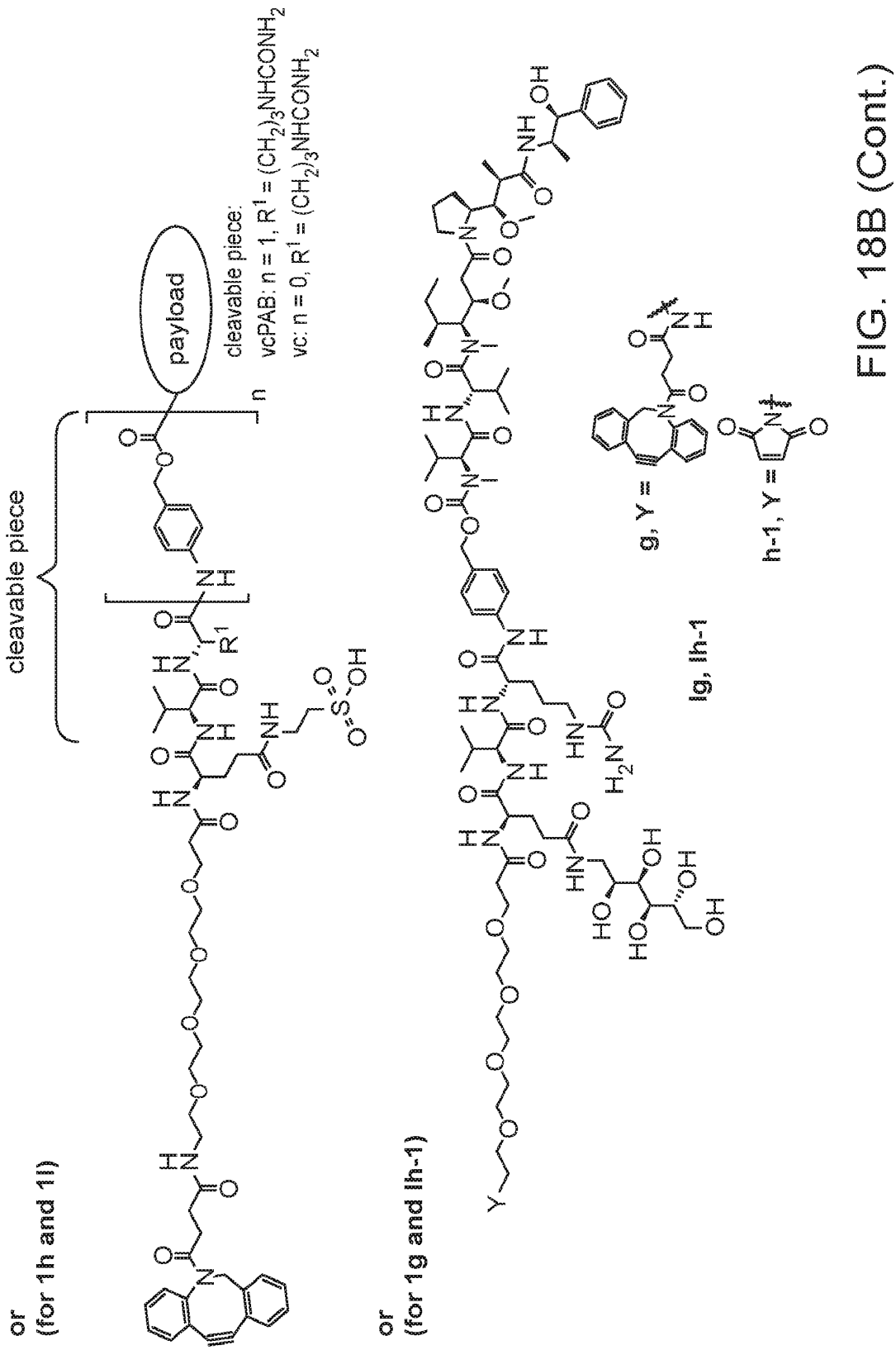

cleavable piece
payload
cleavable piece:
vcPAB: n = 1, R1 = (CH2)3NHCONH2
vc: n = 0, R1 = (CH2)3NHCONH2
n
or
(for 1h and 1l)
or
(for 1g and Ih-1)
g, Y =
h-1, Y =
Ig, Ih-1

FIG. 18B (Cont.)

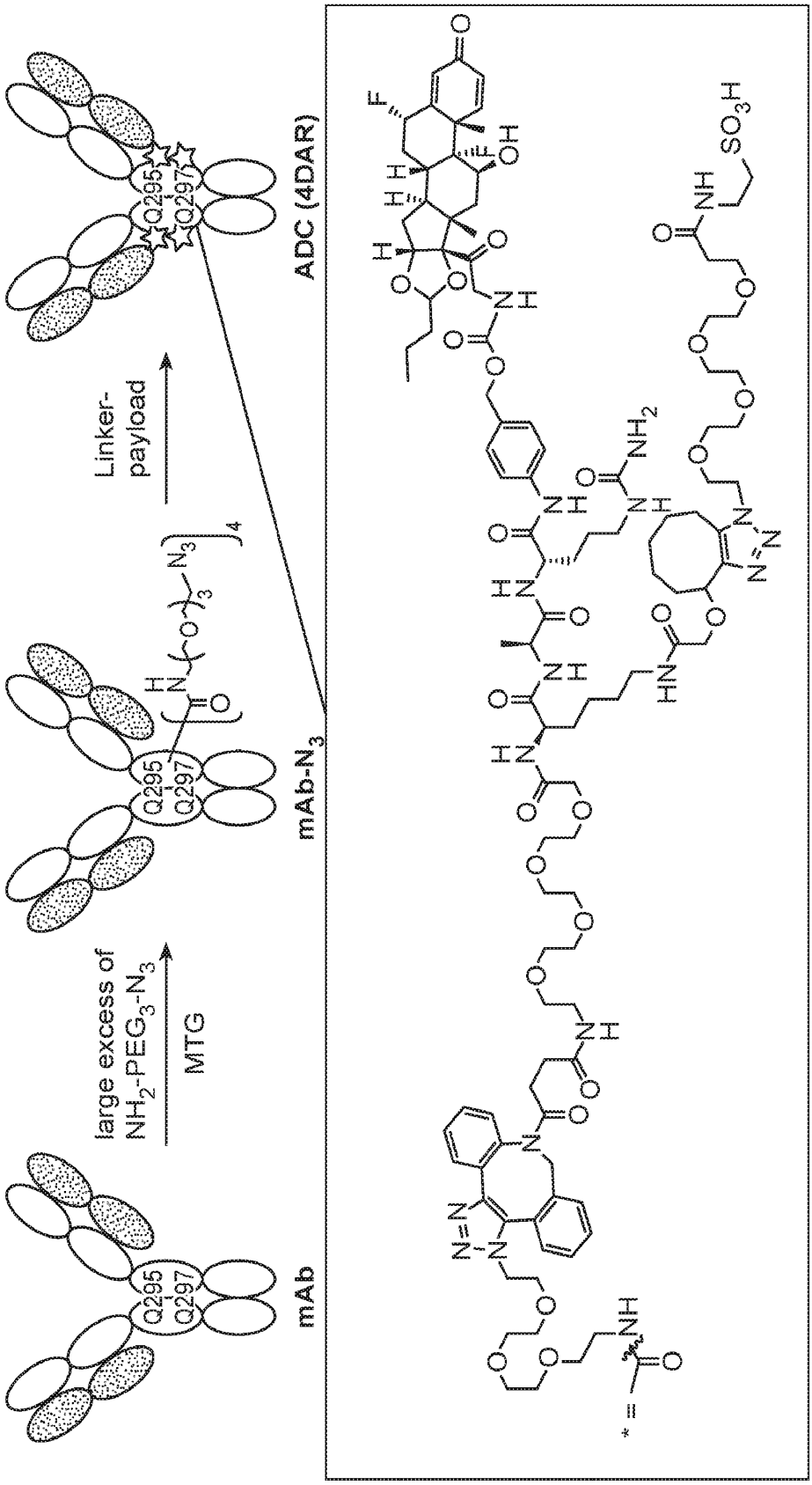

mAb
large excess of
NH2-PEG3-N3
MTG
mAb-N3
Linker-
payload
ADC (4DAR)
Q295
Q297

FIG. 19

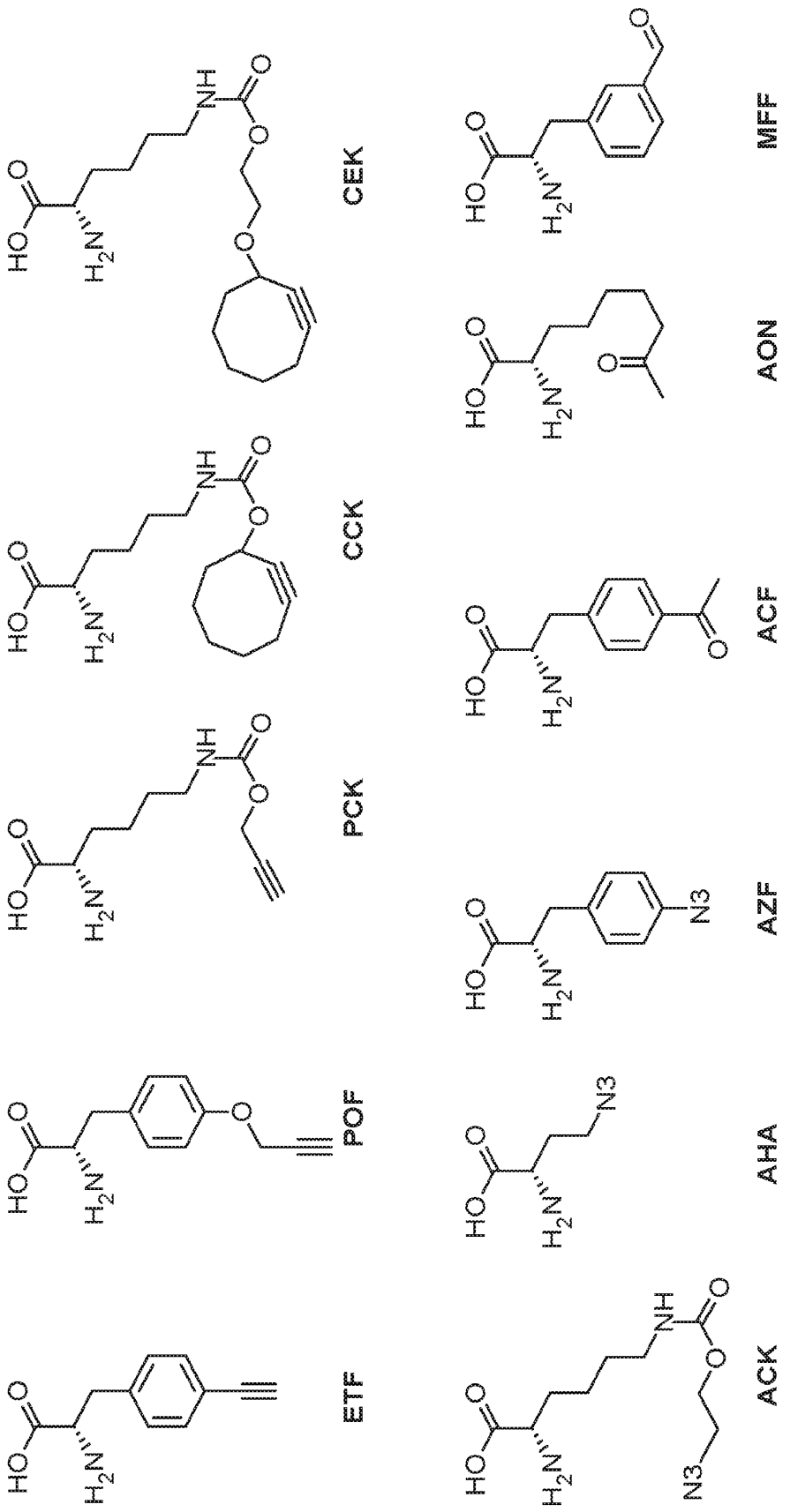


FIG. 20

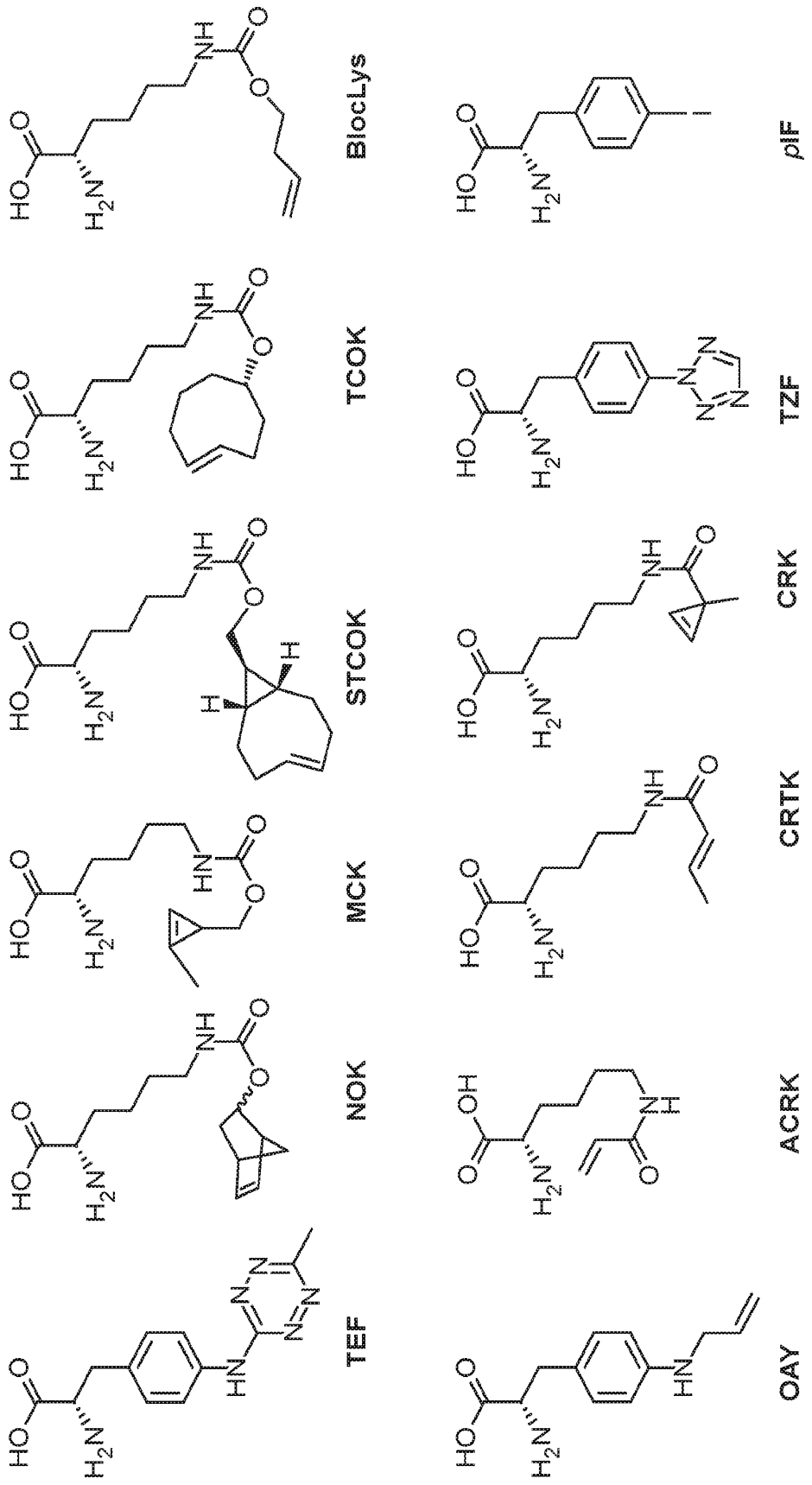


FIG. 20 (Cont.)