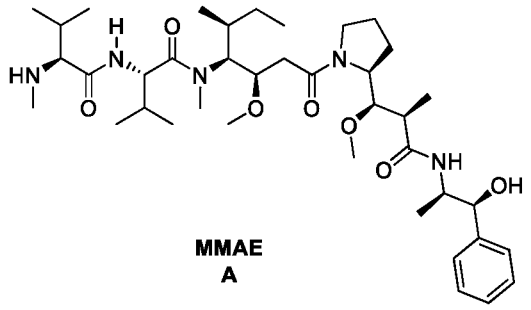
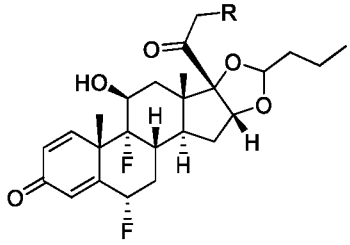
FIG. 1

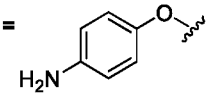


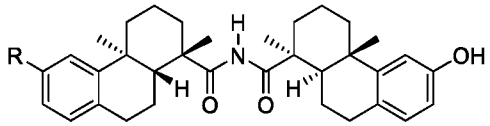
MMAE
A



B: R = $NH_2$

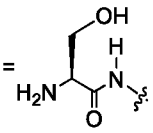
C: R =





D: R = $NH_2$

E: R =



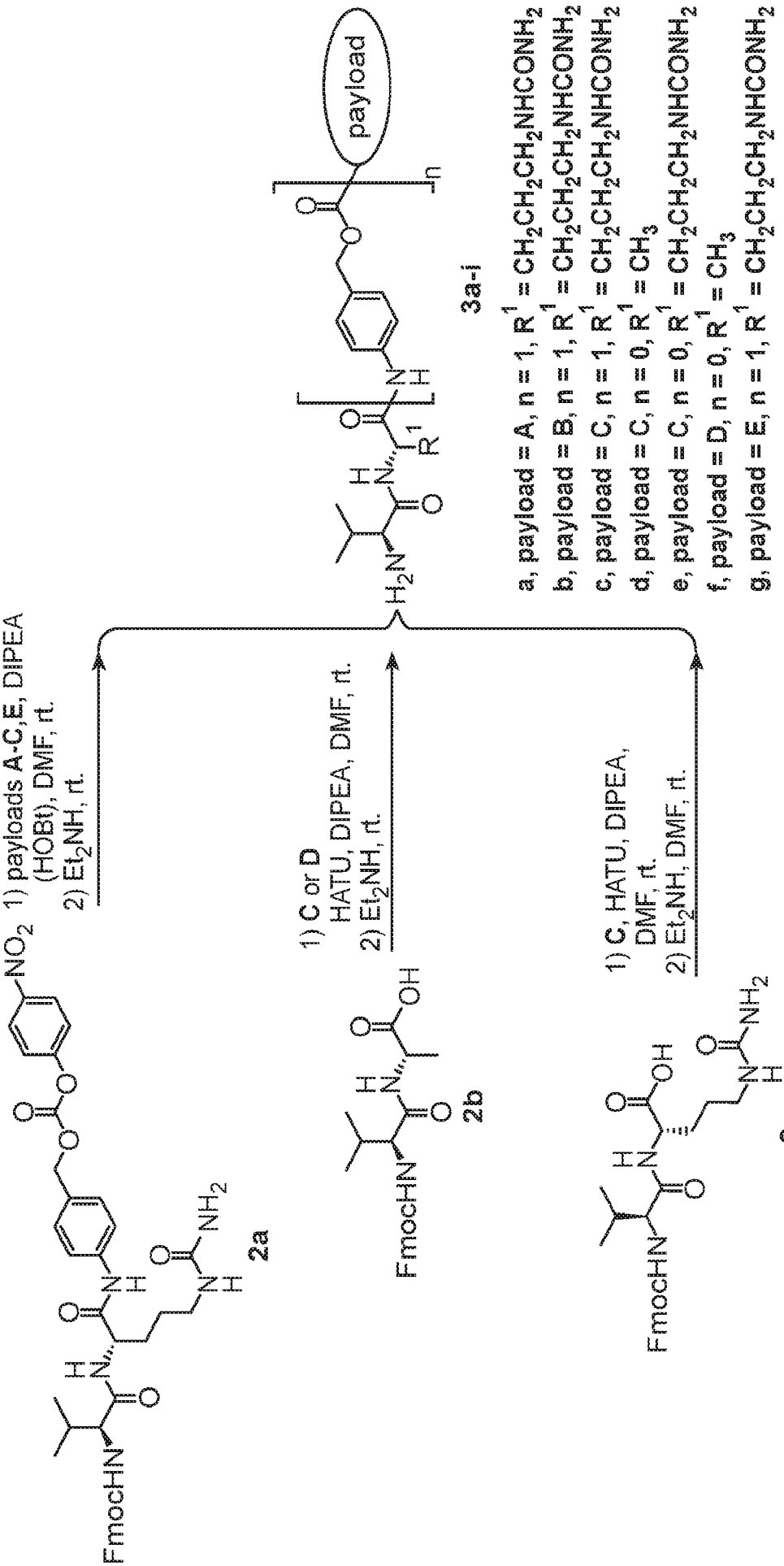

1) payloads A-C,E, DIPEA (HOBt), DMF, rt.
2) Et2NH, rt.
1) C or D HATU, DIPEA, DMF, rt.
2) Et2NH, rt.
1) C, HATU, DIPEA, DMF, rt.
2) Et2NH, DMF, rt.
payload
3a-i
a, payload = A, n = 1, R1 = CH2CH2CH2NHCONH2
b, payload = B, n = 1, R1 = CH2CH2CH2NHCONH2
c, payload = C, n = 1, R1 = CH2CH2CH2NHCONH2
d, payload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

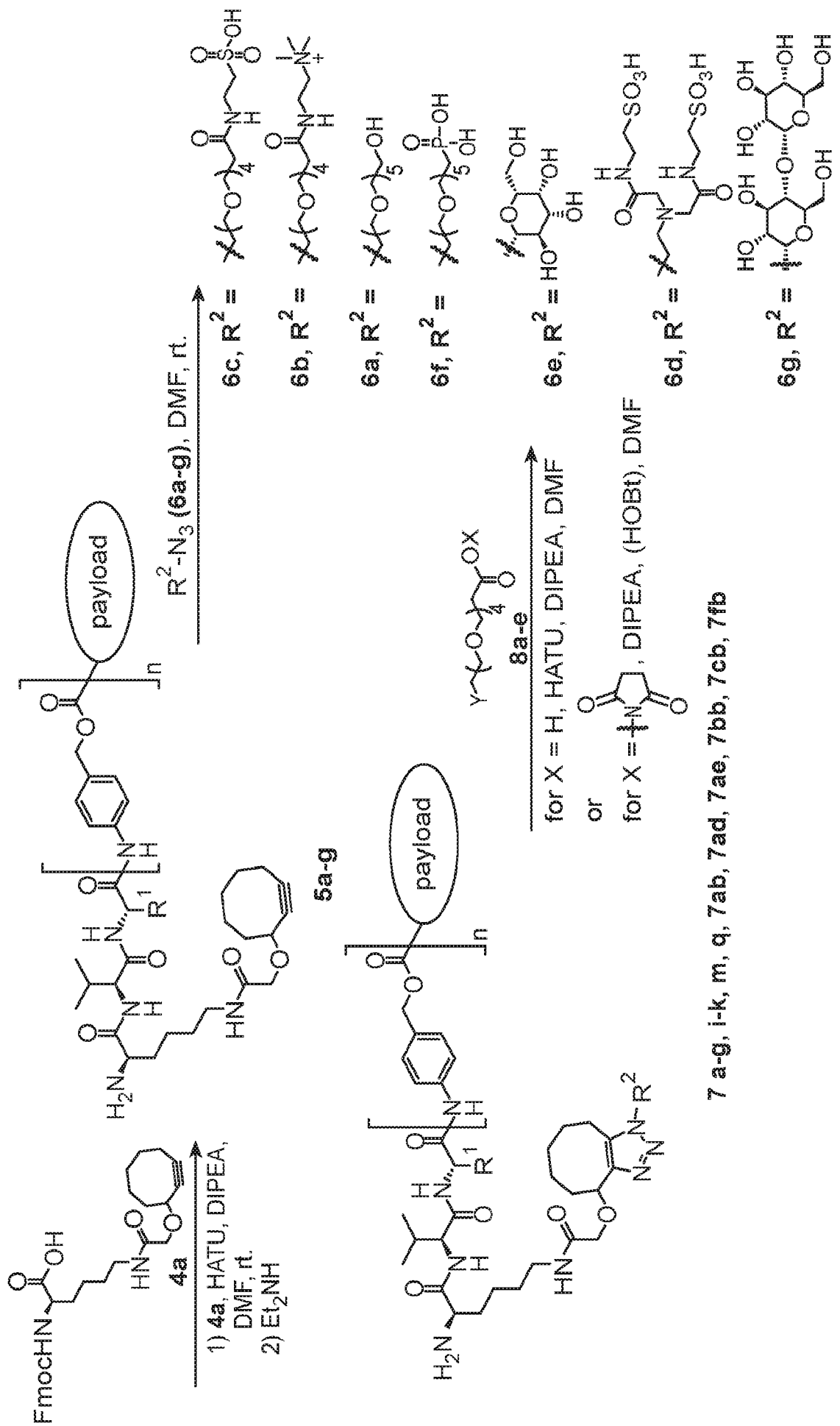
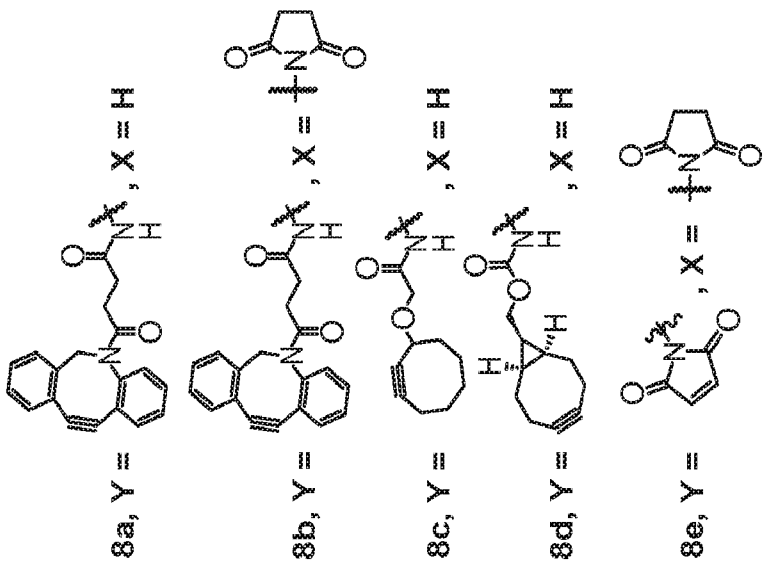

FmocHN
4a
1) 4a, HATU, DIPEA, DMF, rt.
2) Et2NH
H2N
payload
5a-g
R2-N3 (6a-g), DMF, rt.
6c, R2 =
6b, R2 =
6a, R2 =
6f, R2 =
6e, R2 =
6d, R2 =
6g, R2 =
8a-e
for X = H, HATU, DIPEA, DMF
or
for X = , DIPEA, (HOBt), DMF
7 a-g, i-k, m, q, 7ab, 7ad, 7ae, 7bb, 7cb, 7fb

FIG. 2 (Cont.)


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

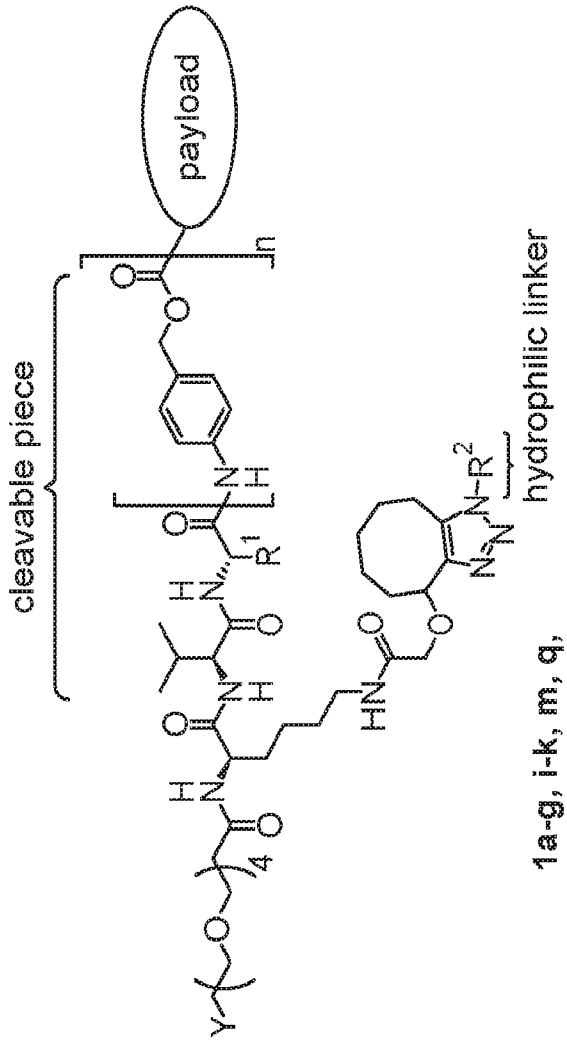

cleavable piece
payload
n
hydrophilic linker
1a-g, i-k, m, q,
Ib, Id, Ie-1, If-1, IIb, IIId, IIIc, IVb
(see table 2)

FIG. 2 (Cont. 1)

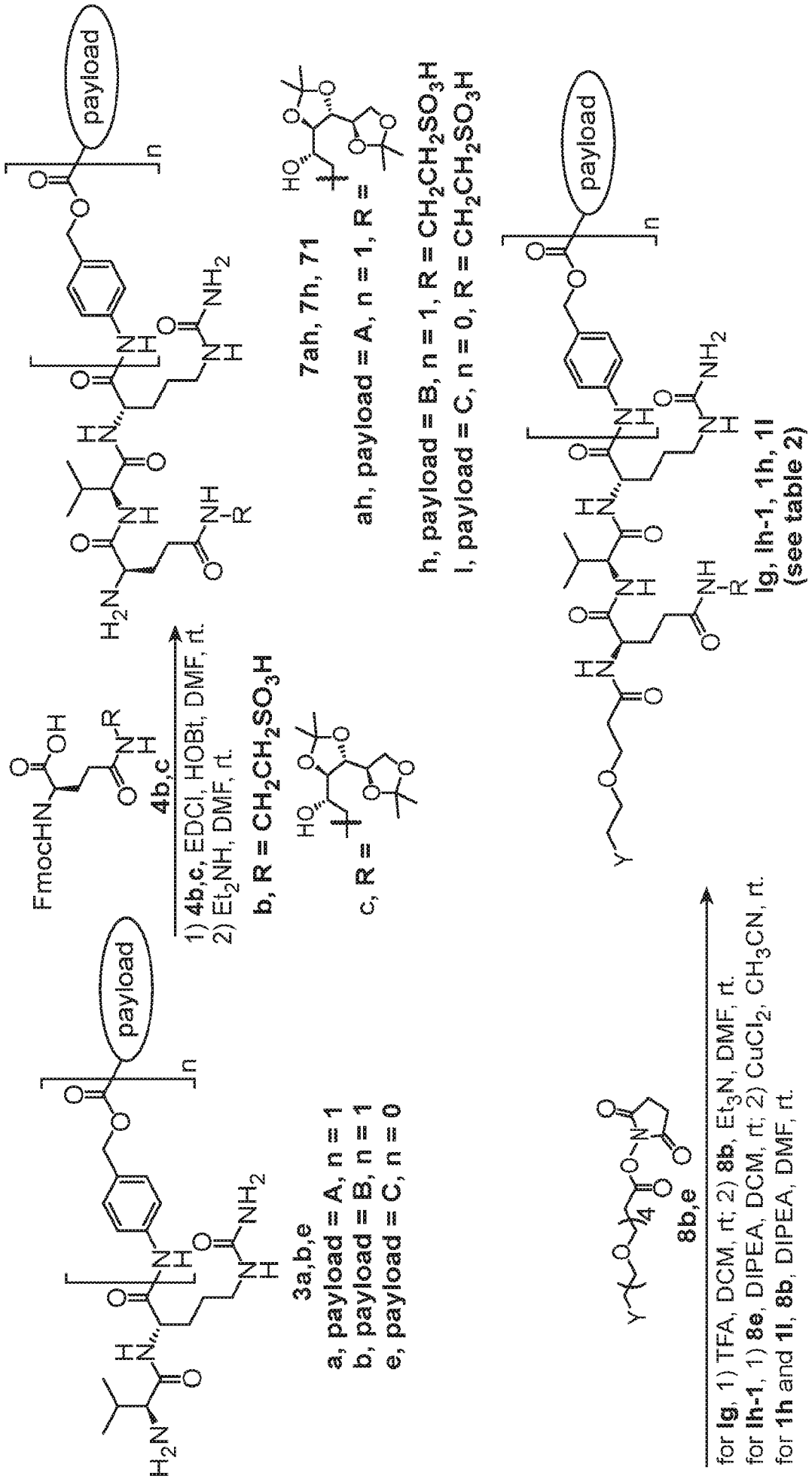

payload
n
FmocHN
OH
R
H
4b,c
1) 4b,c, EDCI, HOBt, DMF, rt.
2) Et2NH, DMF, rt.
b, R = CH2CH2SO3H
c, R =
HO
7ah, 7h, 71
ah, payload = A, n = 1, R =
h, payload = B, n = 1, R = CH2CH2SO3H
l, payload = C, n = 0, R = CH2CH2SO3H
3a,b,e
a, payload = A, n = 1
b, payload = B, n = 1
e, payload = C, n = 0
8b,e
Y
4
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.
Ig, Ih-1, 1h, 1l
(see table 2)

FIG. 3

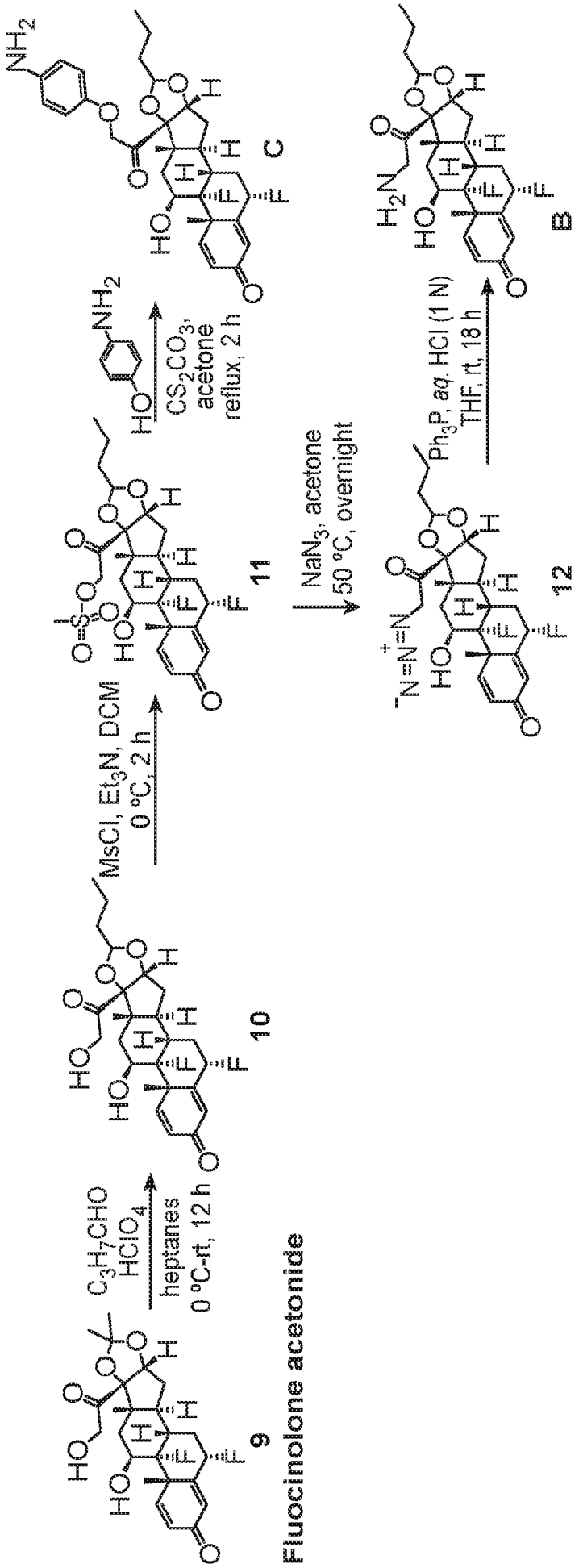

Fluocinolone acetonide
9
$C_3H_7CHO$
$HClO_4$
heptanes
0 °C-rt, 12 h
10
MsCl, $Et_3N$, DCM
0 °C, 2 h
11
$CS_2CO_3$,
acetone
reflux, 2 h
C
$NaN_3$, acetone
50 °C, overnight
12
$Ph_3P$, aq. HCl (1 N)
THF, rt, 18 h
B

FIG. 4

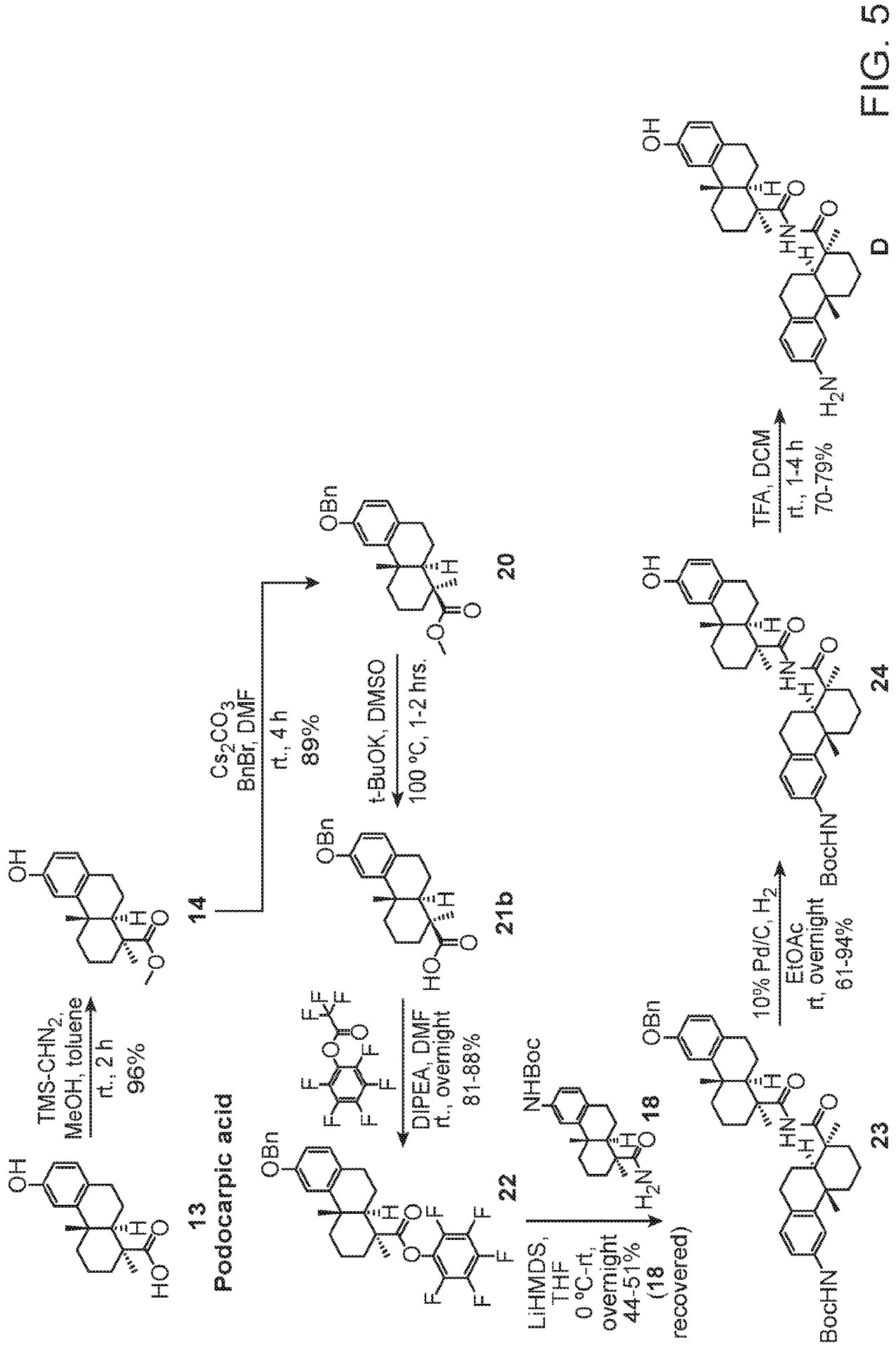
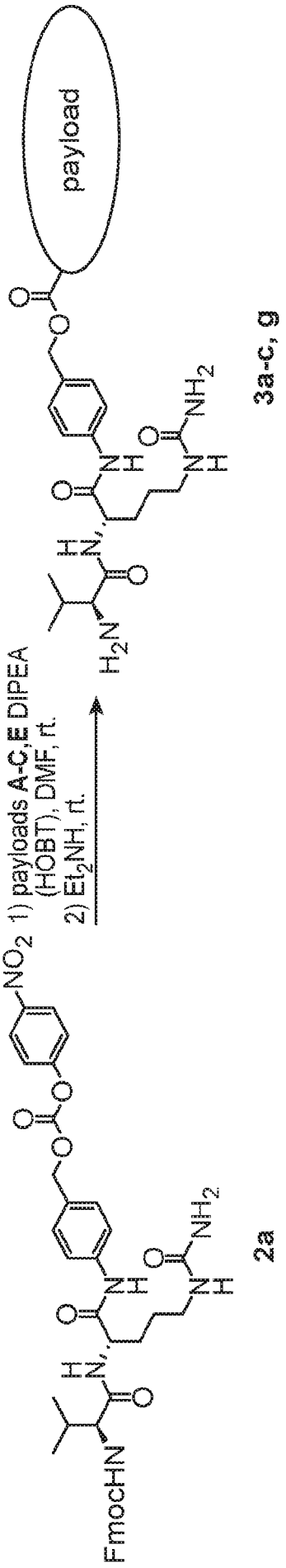

TMS-CHN2, MeOH, toluene
rt., 2 h
96%
13
Podocarpic acid
14
Cs2CO3 BnBr, DMF
rt., 4 h
89%
20
t-BuOK, DMSO
100 °C, 1-2 hrs.
21b
DIPEA, DMF
rt., overnight
81-88%
22
LiHMDS, THF
0 °C-rt, overnight
44-51% (18 recovered)
18
10% Pd/C, H2
EtOAc
rt, overnight
61-94%
23
TFA, DCM
rt., 1-4 h
70-79%
D
24
OBn
OH
NHBoc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

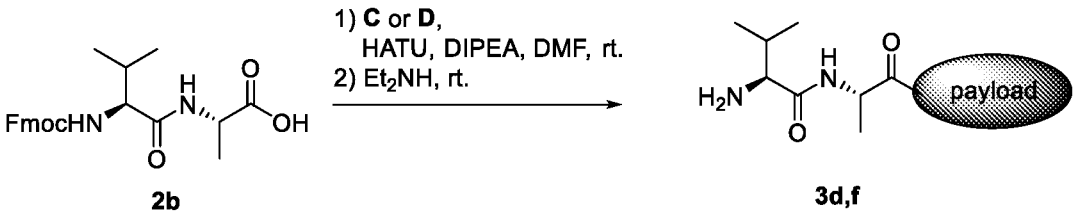
FIG. 5




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

FIG. 6

FIG. 7




| Product | Payload |
|---|---|
| 3d | C |
| 3f | D |

FIG. 8

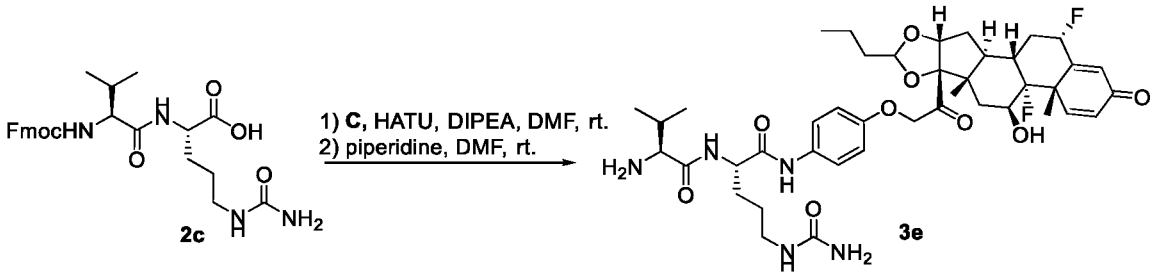


FIG. 9

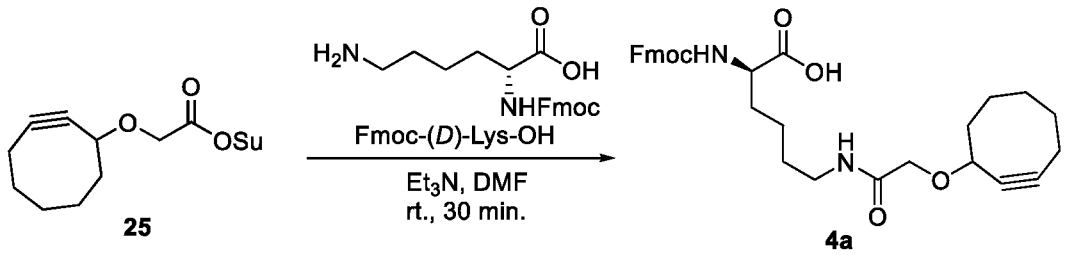

O
O
OSu
25
H2N
O
OH
NHFmoc
Fmoc-(D)-Lys-OH
Et3N, DMF
rt., 30 min.
FmocHN
O
OH
H
N
O
O
4a

FIG. 10

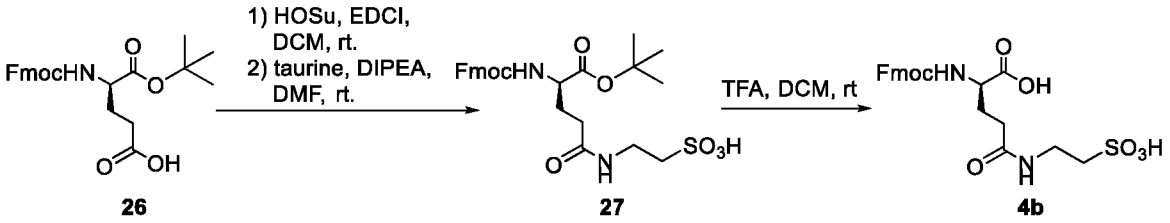
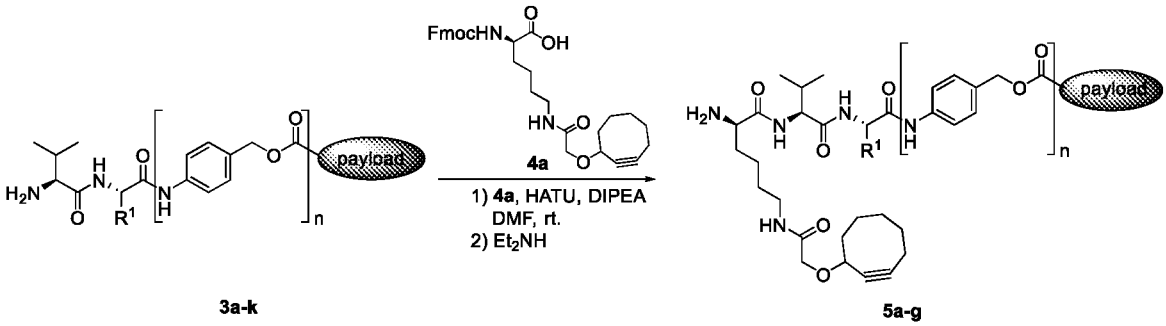
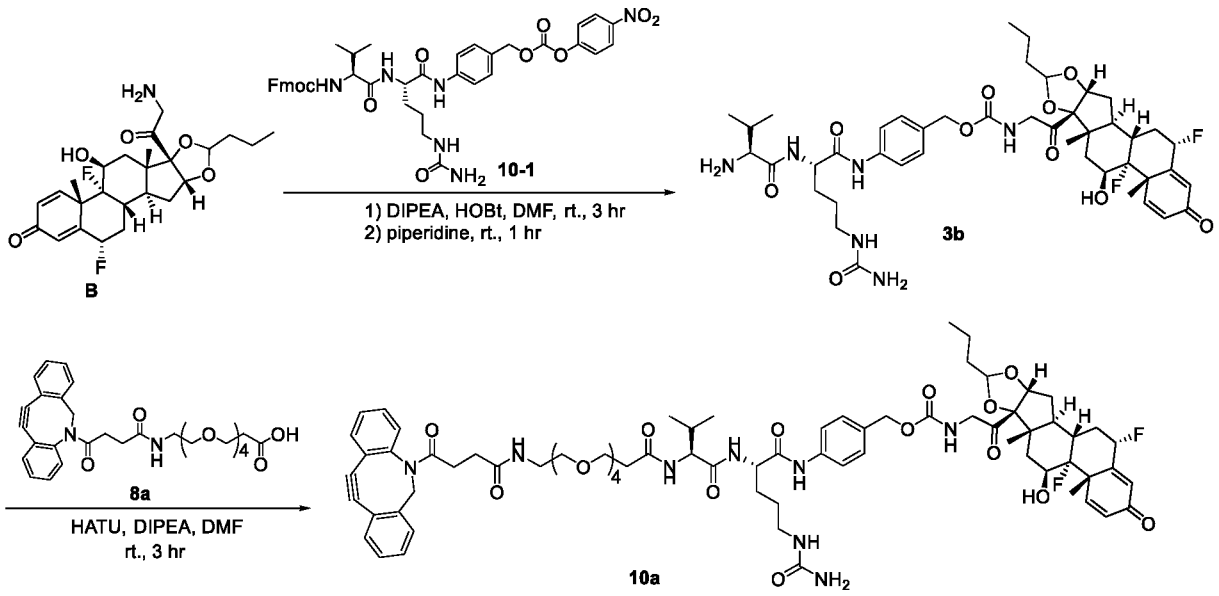

1) HOSu, EDCI,
DCM, rt.
2) taurine, DIPEA,
DMF, rt.
TFA, DCM, rt
FmocHN
OH
SO3H
26
27
4b

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

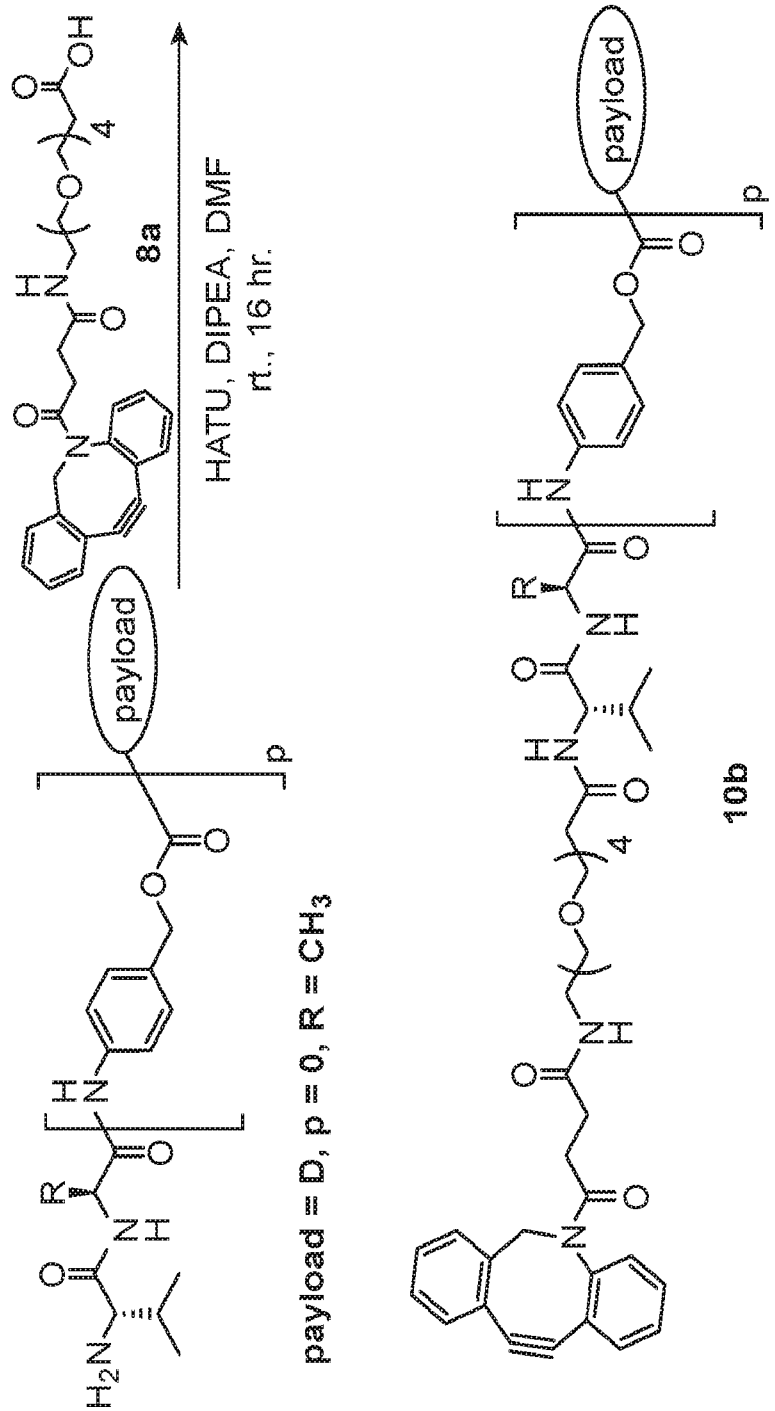
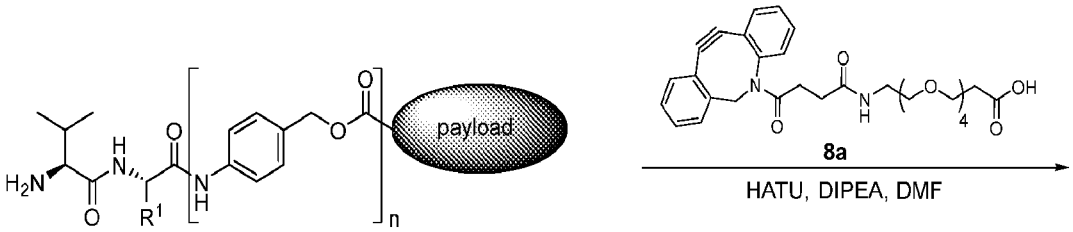
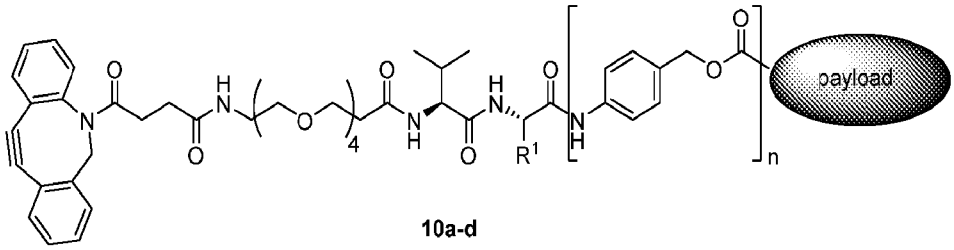



FIG. 11B

FIG. 11C



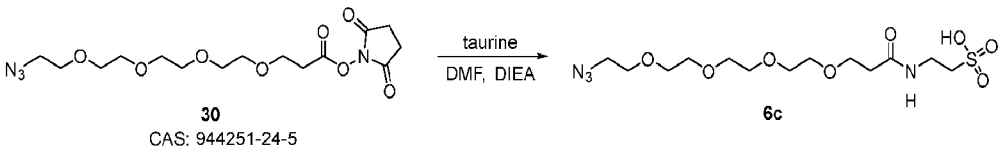

3a-c, f




10a-d

| 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

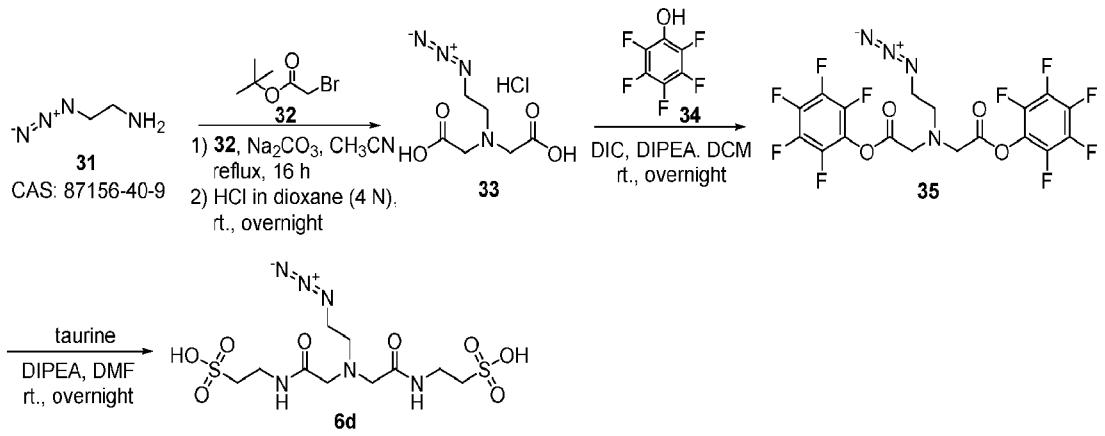



FIG. 13A

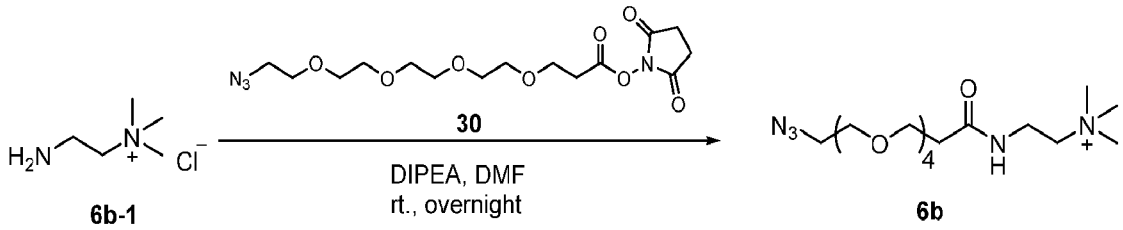
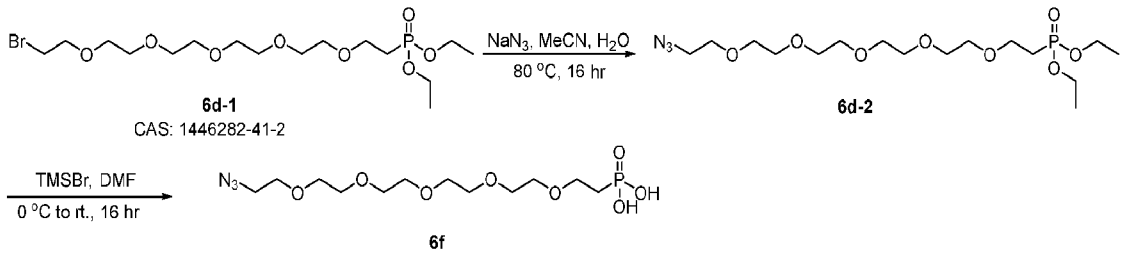
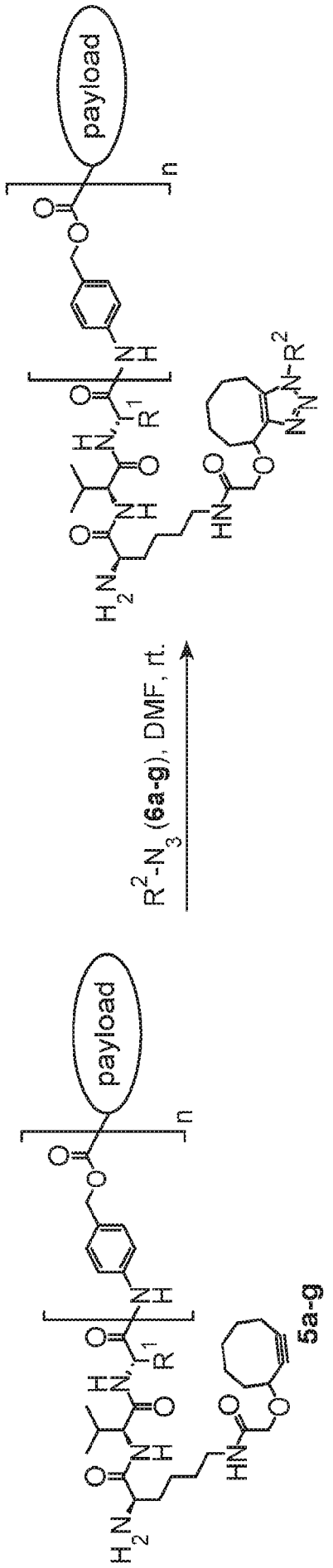



FIG. 13B






**7a, 7c, 7e, 7f, 7i, 7j, 7j, 7k, 7m, 7q, 7ab, 7ad, 7ae, 7bb, 7cb, 7fb**

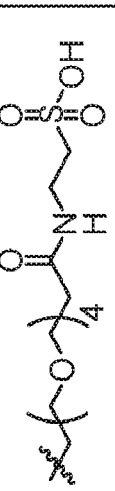
| COT 5 | Azide 6 | Product 7 | Payload | n | $R^1$ | $R^2$ |
|---|---|---|---|---|---|---|
| 5a | 6a | 7a | A | 1 | $(CH_2)_3NHCONH_2$ |  |
| 5a | 6c | 7c | A | 1 | $(CH_2)_3NHCONH_2$ |  |
| 5a | 6b | 7ab | A | 1 | $(CH_2)_3NHCONH_2$ |  |
| 5a | 6f | 7ad | A | 1 | $(CH_2)_3NHCONH_2$ |  |

FIG. 14

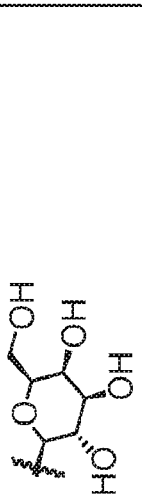
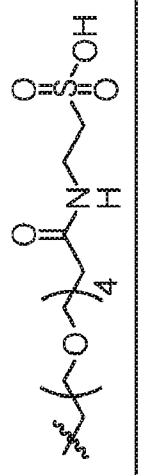
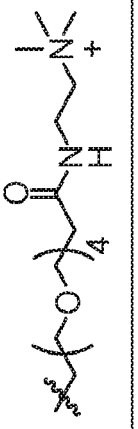
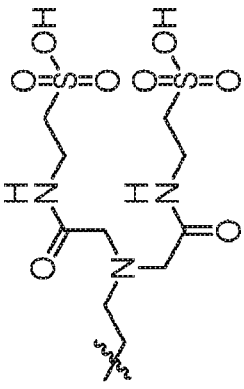
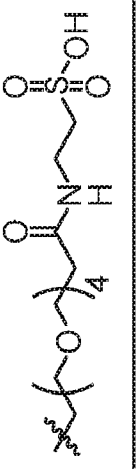
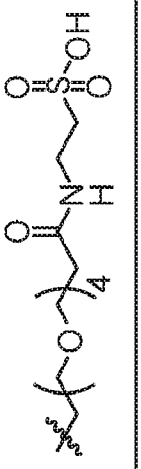
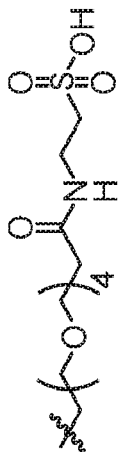
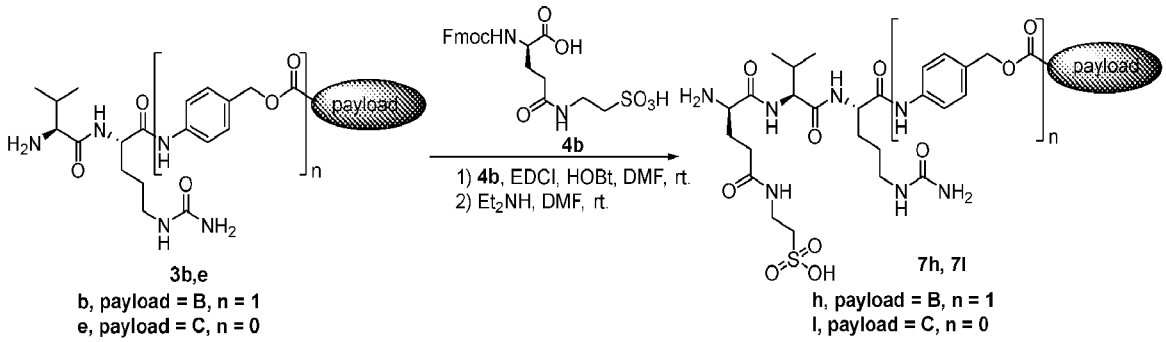
| 5a | 6e | 7ae | A | 1 | $(CH_2)_3NHCONH_2$ |  |
|---|---|---|---|---|---|---|
| 5b | 6c | 7ba | B | 1 | $(CH_2)_3NHCONH_2$ |  |
| 5b | 6b | 7bb | B | 1 | $(CH_2)_3NHCONH_2$ |  |
| 5b | 6d | 7f | B | 1 | $(CH_2)_3NHCONH_2$ |  |
| 5c | 6c | 7i | C | 1 | $(CH_2)_3NHCONH_2$ |  |
| 5d | 6c | 7j | C | 0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

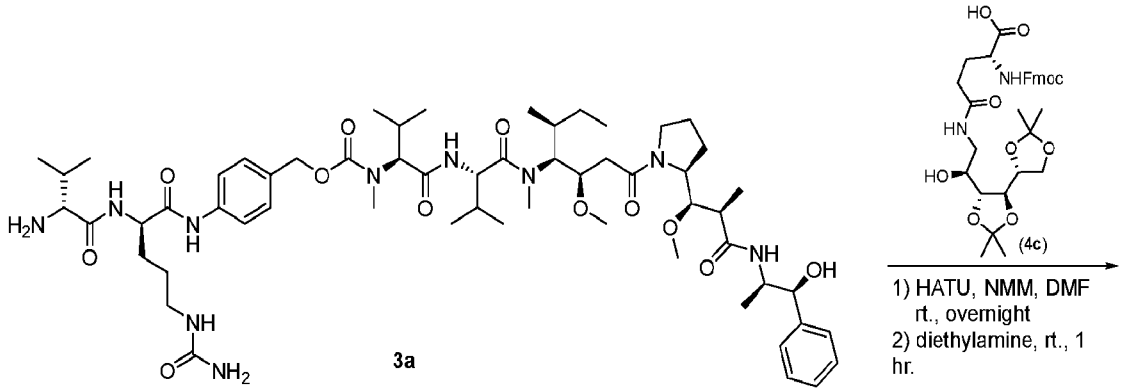
FIG. 14 (Cont. 1)

FIG. 15




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

FIG. 15A



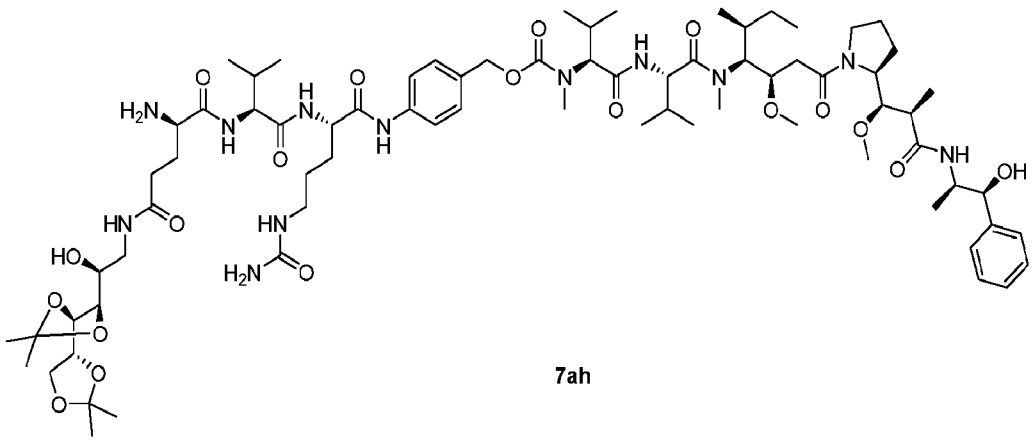



FIG. 15B

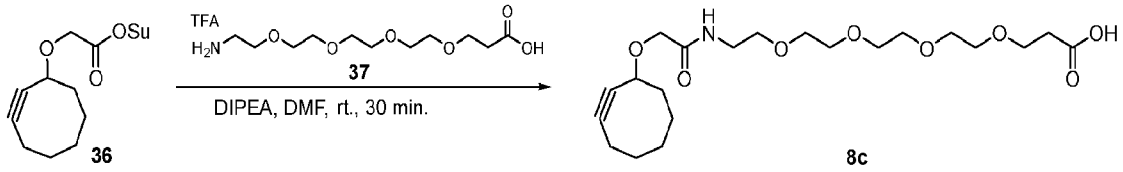
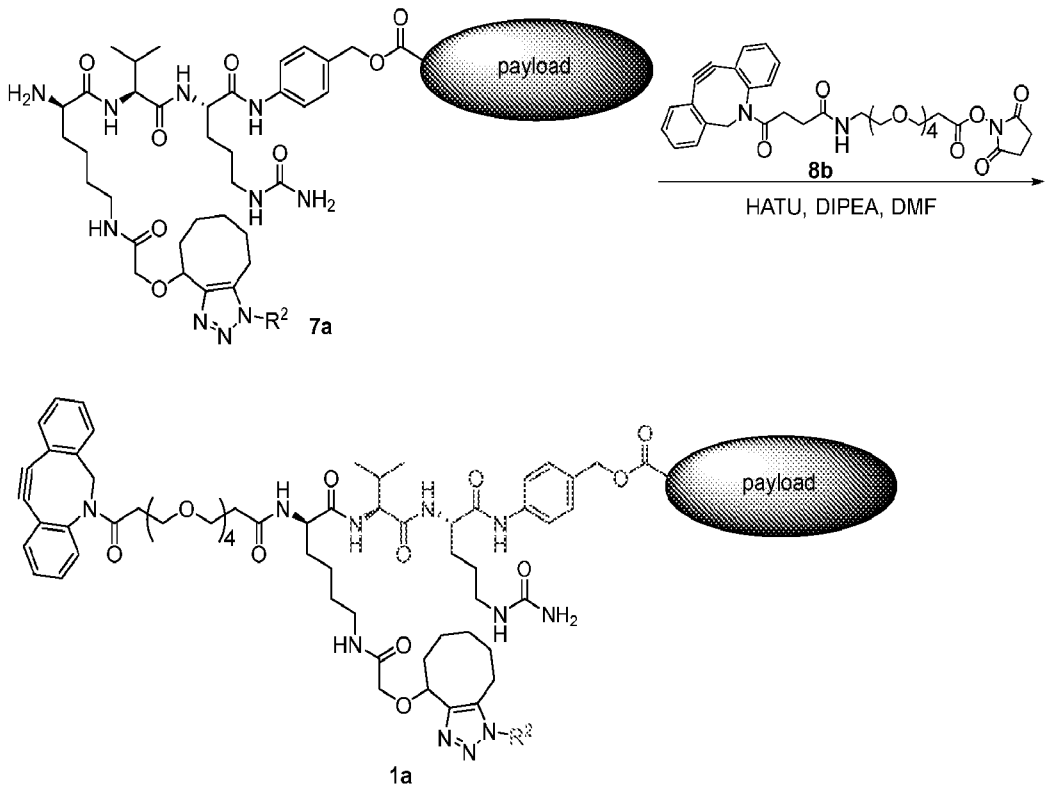
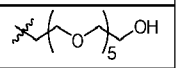
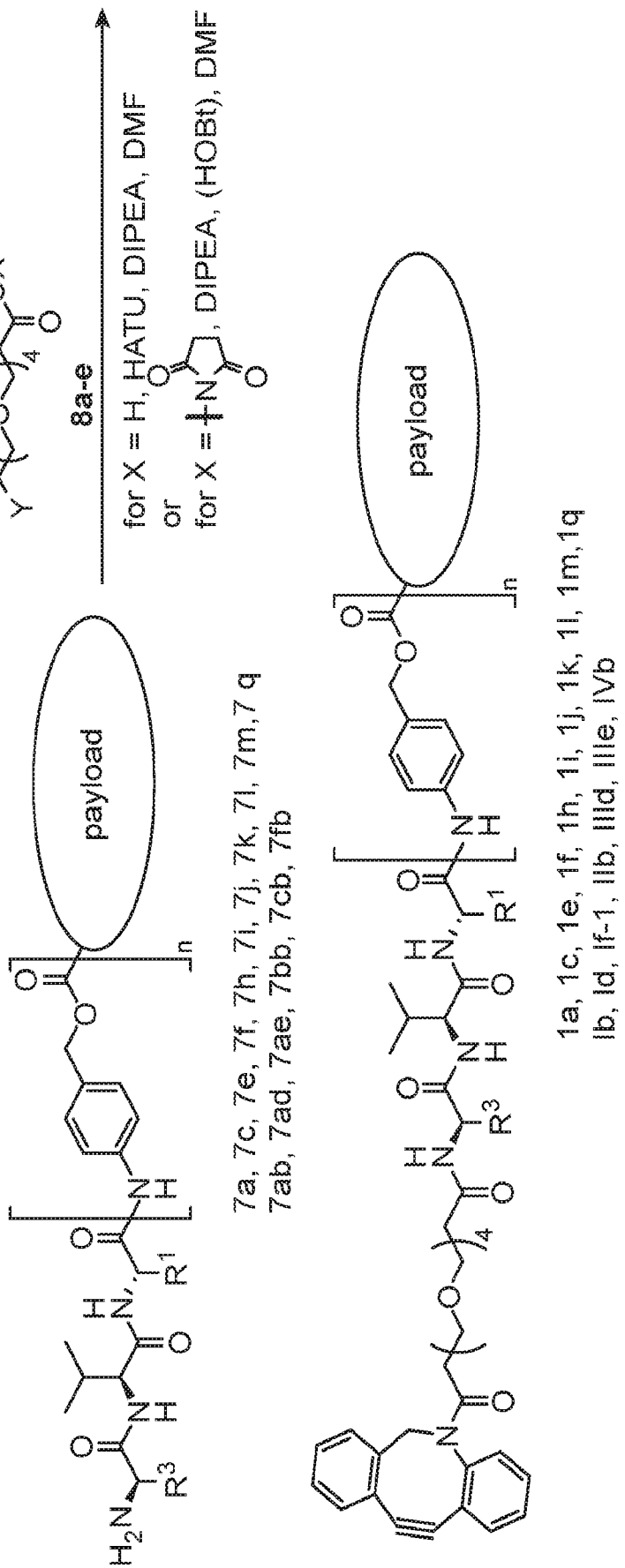



FIG. 16




| Amine 7 | Product 1 | Payload | $R^2$ |
|---|---|---|---|
| 7a | 1a | A |  |



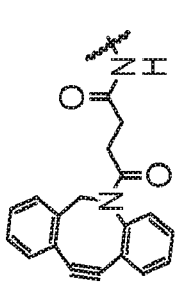
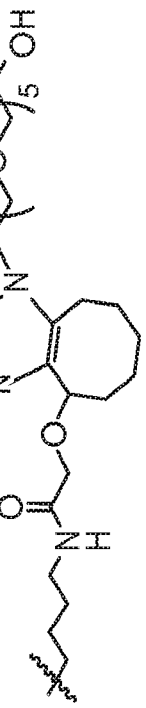

| Amine 7 | Product 1 | Payload | Y | n | $R^1$ | $R^3$ |
|---|---|---|---|---|---|---|
| 7a | 1a | A |  | 1 | $(CH_2)_3NHCONH_2$ |  |

FIG. 17

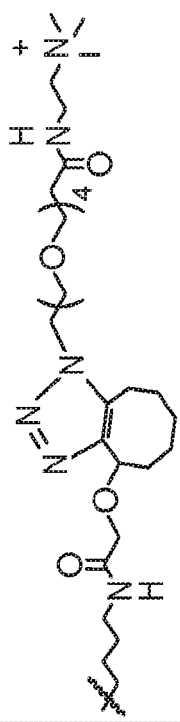
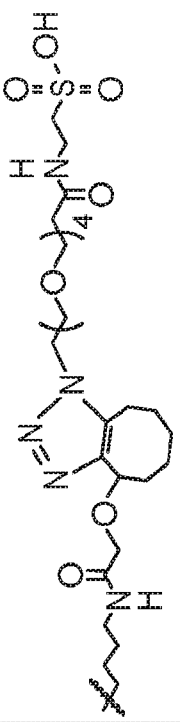
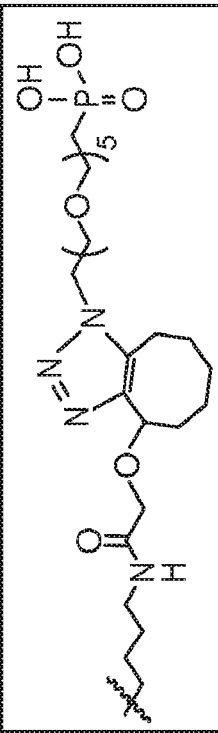
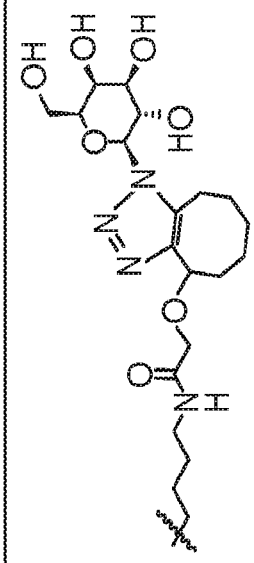
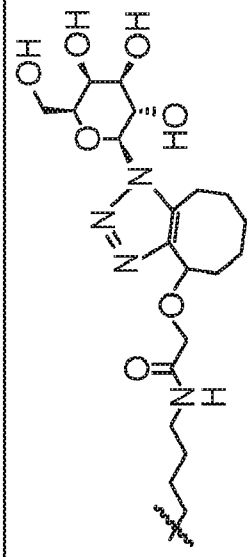
| | | | | | |
|---|---|---|---|---|---|
| 7ab | Ib | A | 1 | $(CH_2)_3NHCONH_2$ |  |
| 7c | 1c | A | 1 | $(CH_2)_3NHCONH_2$ |  |
| 7ad | Id | A | 1 | $(CH_2)_3NHCONH_2$ |  |
| 7ae | Ie-1 | A | 1 | $(CH_2)_3NHCONH_2$ |  |
| 7ae | If-1 | A | 1 | $(CH_2)_3NHCONH_2$ |  |

FIG. 17 (Cont.1)

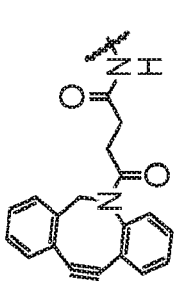
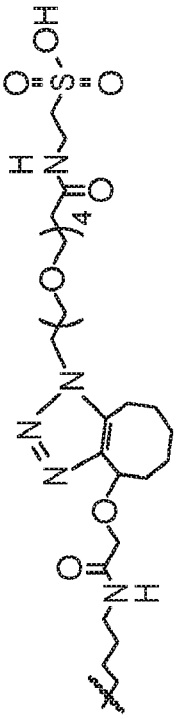
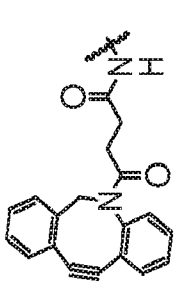
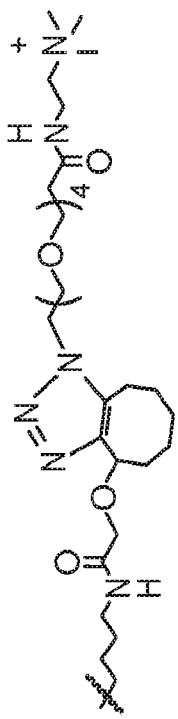
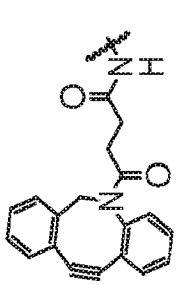
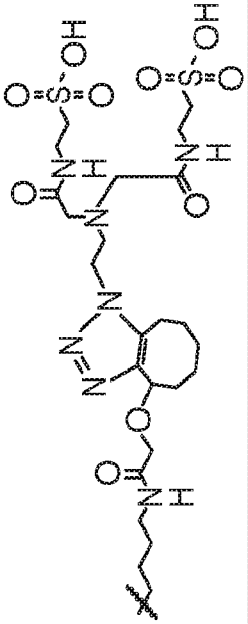
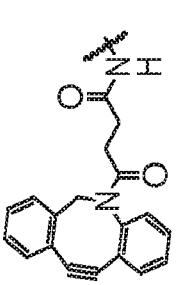
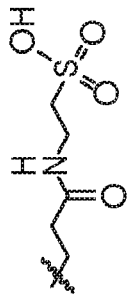
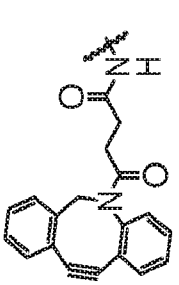
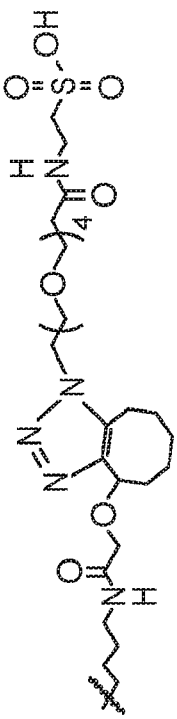
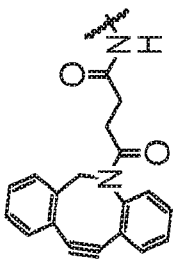
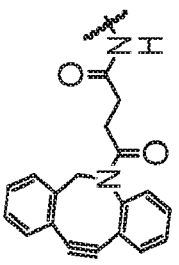
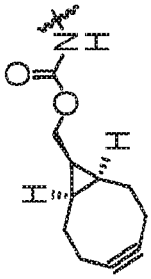
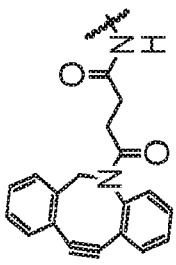
| Amine 7 | Product 1 | Payload | Y | n | $R^1$ | $R^3$ |
|---|---|---|---|---|---|---|
| 7e | 1e | B |  | 1 | $(CH_2)_3NHCONH_2$ |  |
| 7bb | IIb | B |  | 1 | $(CH_2)_3NHCONH_2$ |  |
| 7f | 1f | B |  | 1 | $(CH_2)_3NHCONH_2$ |  |
| 7h | 1h | B |  | 1 | $(CH_2)_3NHCONH_2$ |  |
| 7i | 1i | C |  | 1 |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

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 | $(CH_2)_3NHCONH_2$ |

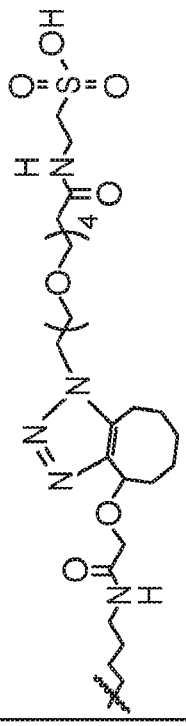
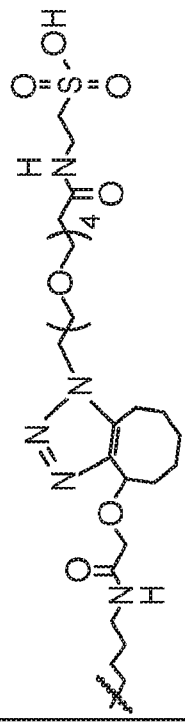
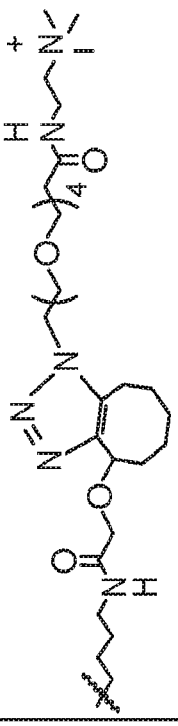
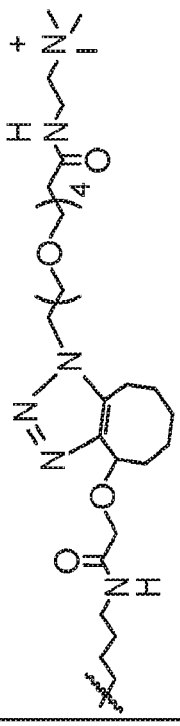
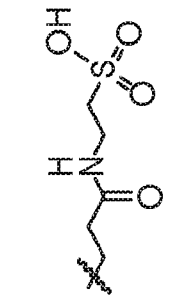






FIG. 17 (Cont.3)

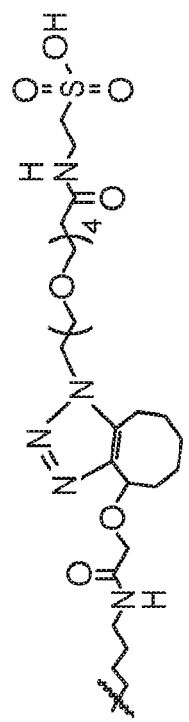
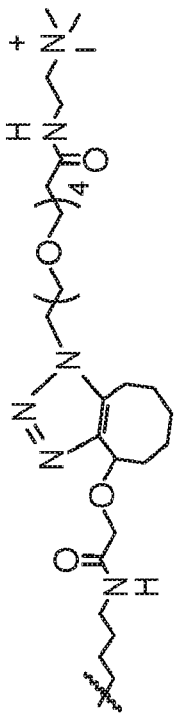
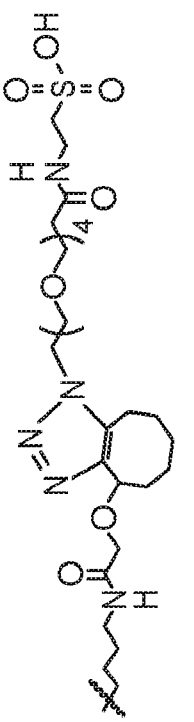
| | | | | | |
|---|---|---|---|---|---|
| 7m | 1m | D | 0 | $CH_3$ |  |
| 7fb | IVb | D | 0 | $CH_3$ |  |
| 7q | 1q | E | 1 | $(CH_2)_3NHCONH_2$ |  |

FIG. 17 (Cont.4)

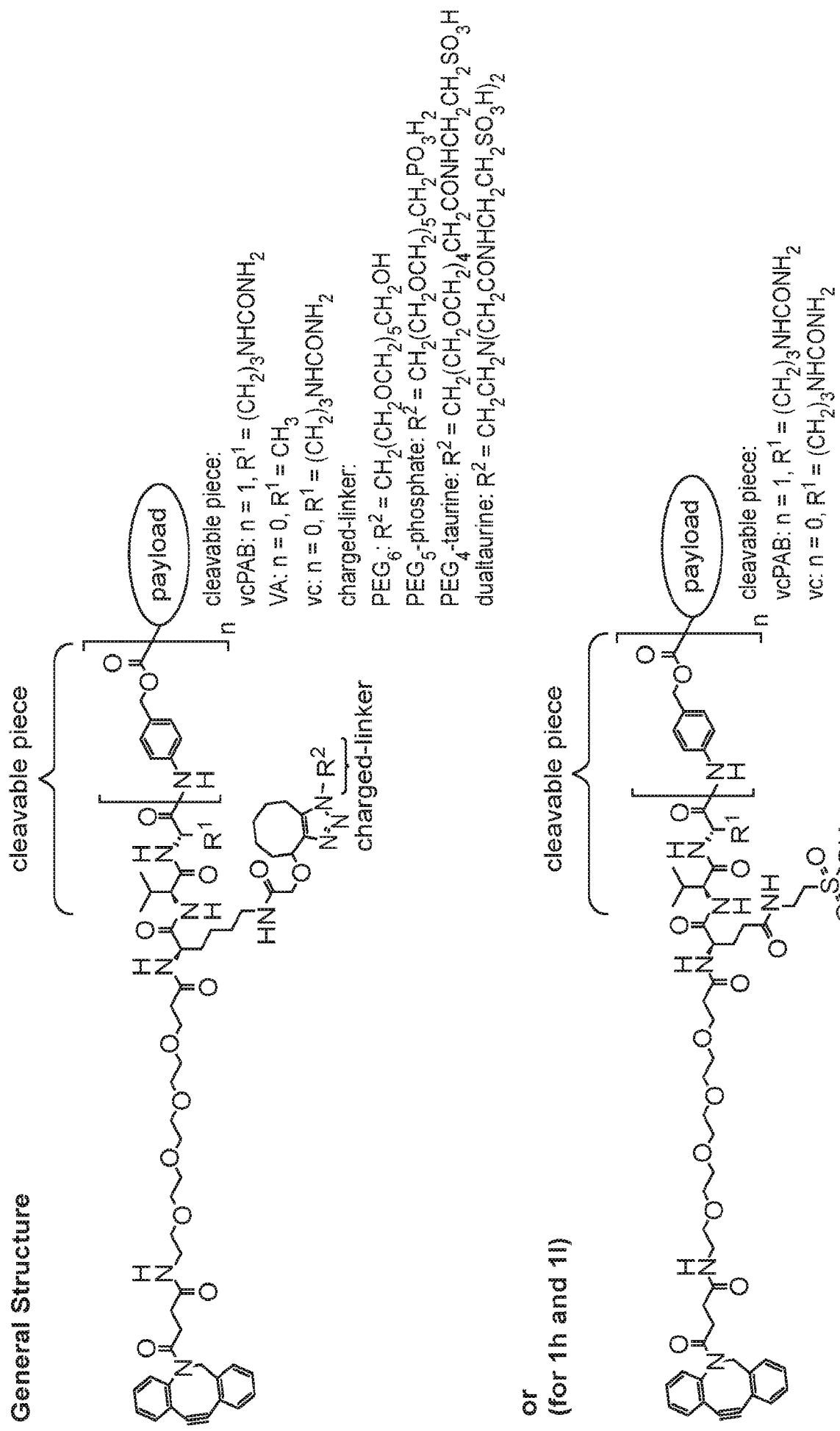
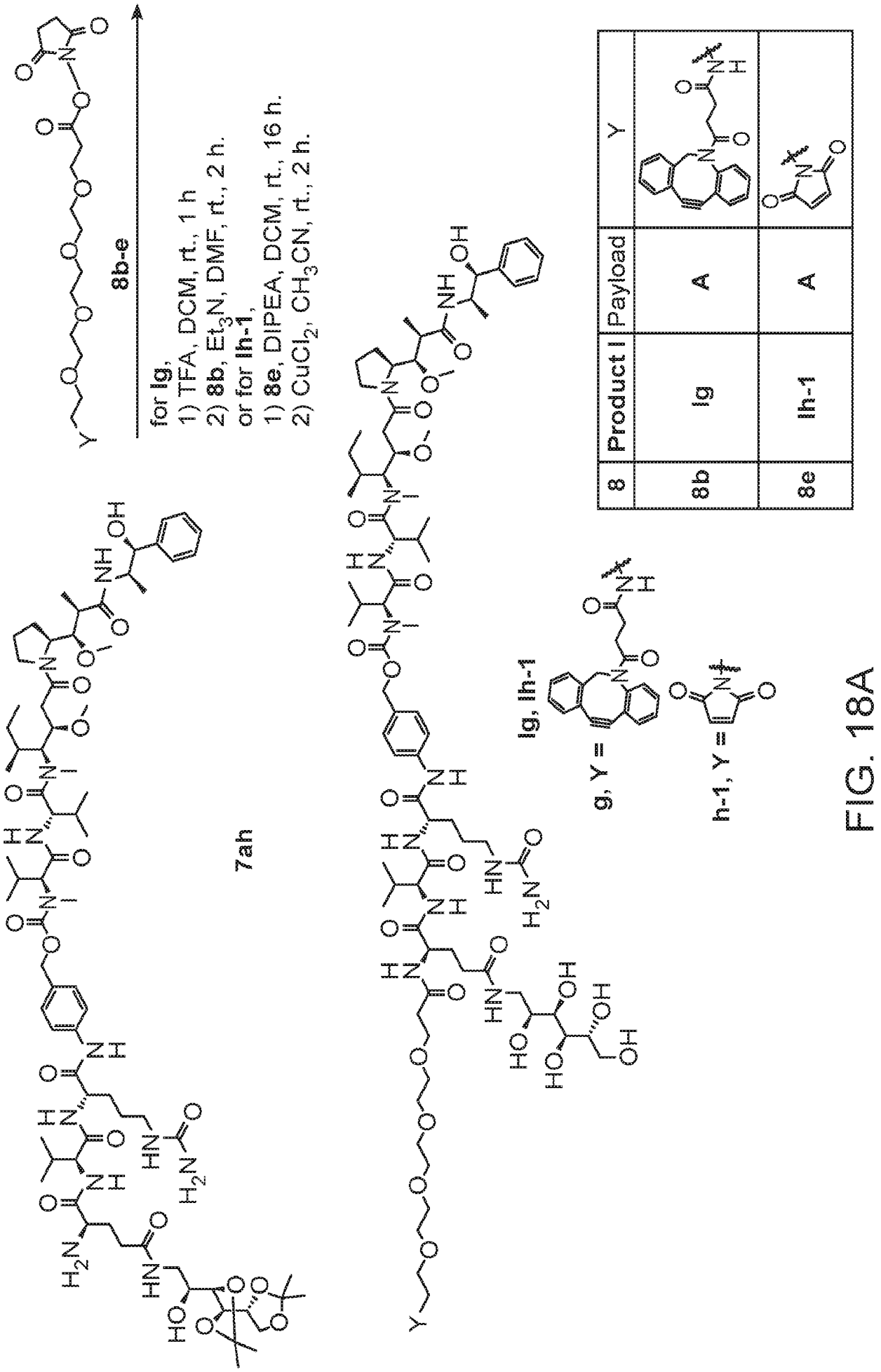

General Structure
cleavable piece
payload
n
cleavable piece:
vcPAB: n = 1, R1 = (CH2)3NHCONH2
VA: n = 0, R1 = CH3
vc: n = 0, R1 = (CH2)3NHCONH2
charged-linker:
PEG6: R2 = CH2(CH2OCH2)5CH2OH
PEG5-phosphate: R2 = CH2(CH2OCH2)5CH2PO3H2
PEG4-taurine: R2 = CH2(CH2OCH2)4CH2CONHCH2CH2SO3H
dualtaurine: R2 =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

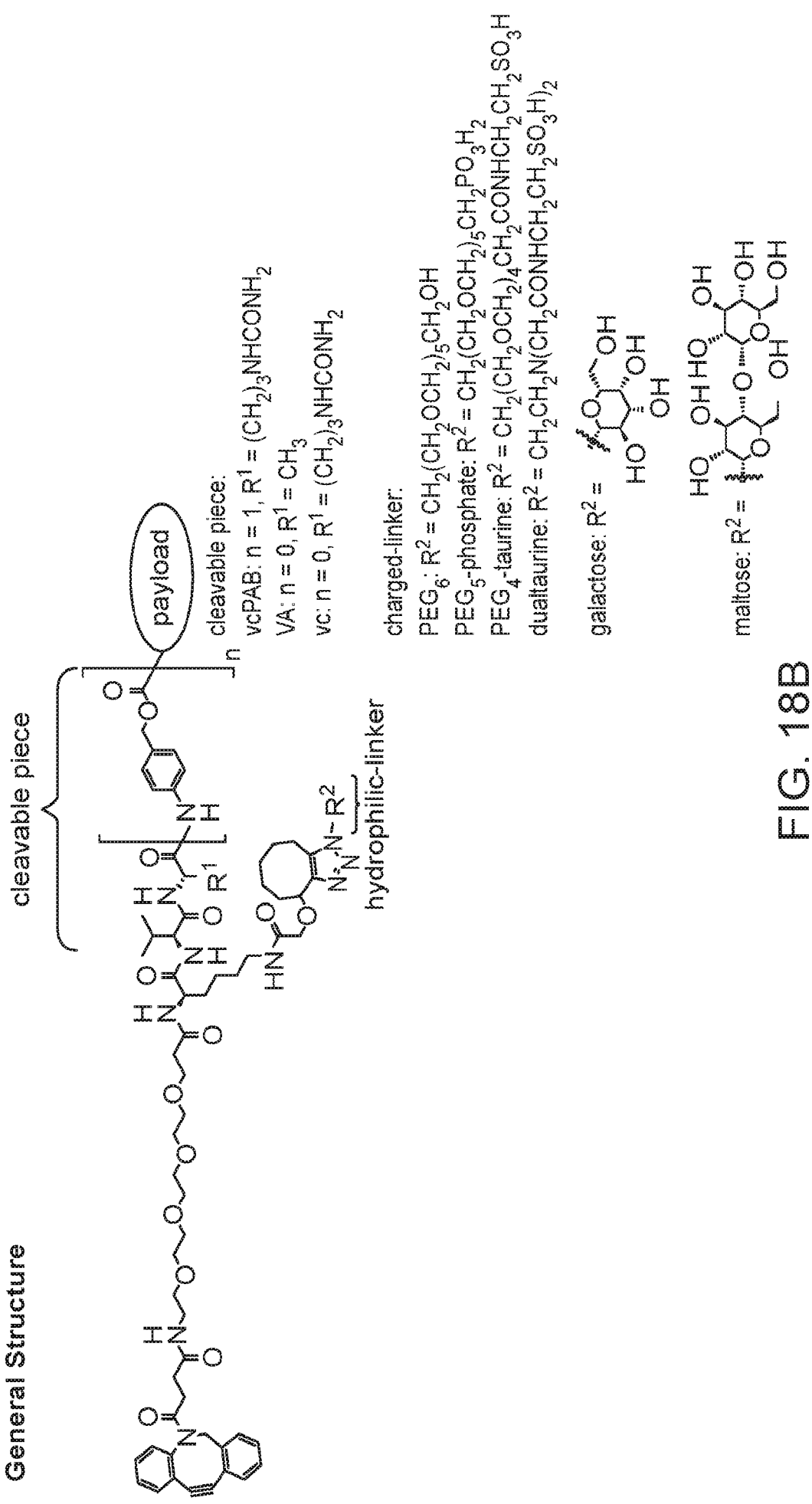

General Structure
cleavable piece
payload
cleavable piece:
vcPAB: n = 1, R1 = (CH2)3NHCONH2
VA: n = 0, R1 = CH3
vc: n = 0, R1 = (CH2)3NHCONH2
hydrophilic-linker
charged-linker:
PEG6: R2 = CH2(CH2OCH2)5CH2OH
PEG5-phosphate: R2 = CH2(CH2OCH2)5CH2PO3H2
PEG4-taurine: R2 = CH2(CH2OCH2)4CH2CONHCH2CH2SO3H
dualtaurine: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

FIG. 18B

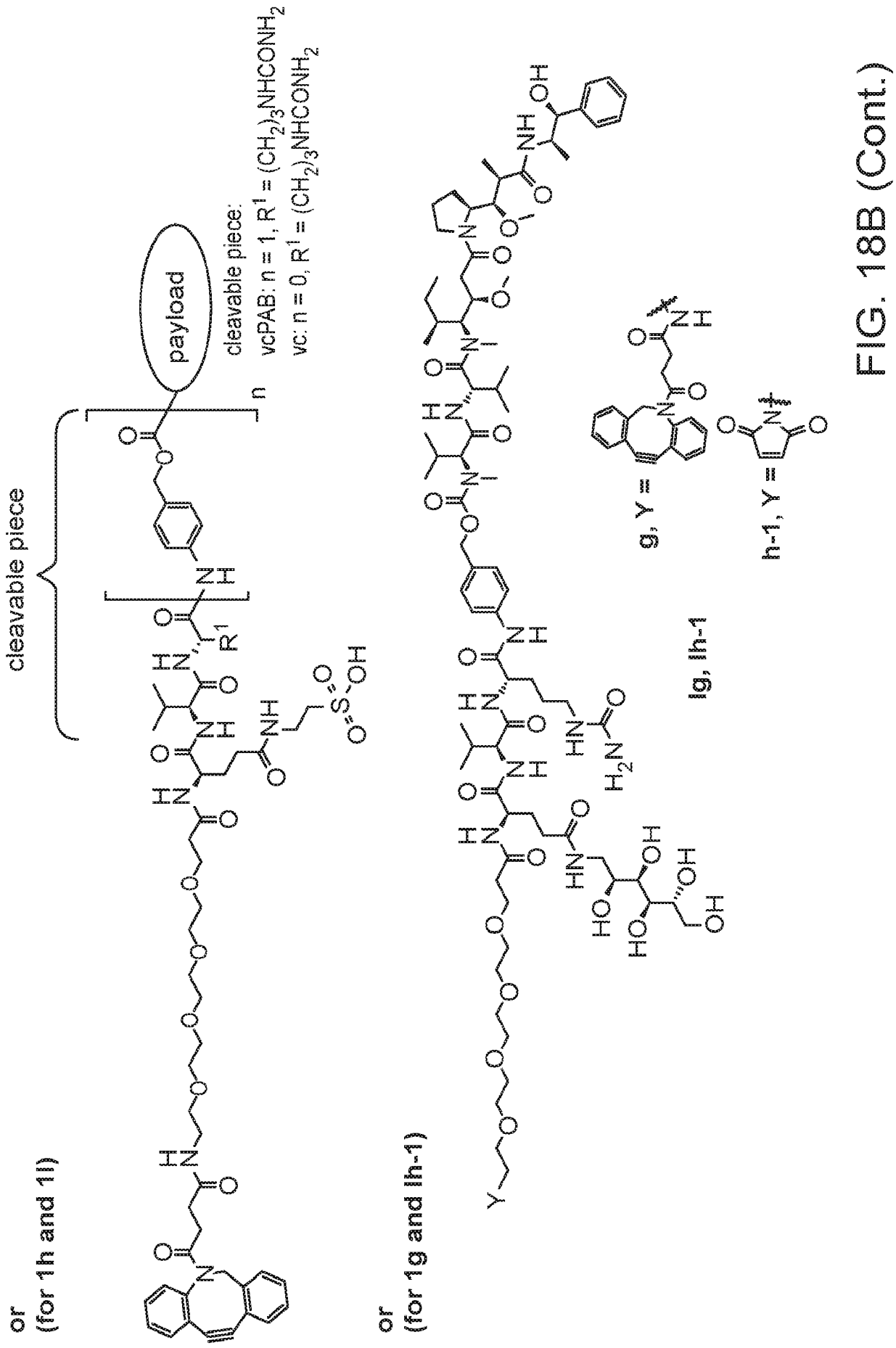

cleavable piece
payload
cleavable piece:
vcPAB: n = 1, R1 = (CH2)3NHCONH2
vc: n = 0, R1 = (CH2)3NHCONH2
or
(for 1h and 1i)
or
(for 1g and Ih-1)
g, Y =
Ih-1, Y =
Ig, Ih-1

FIG. 18B (Cont.)

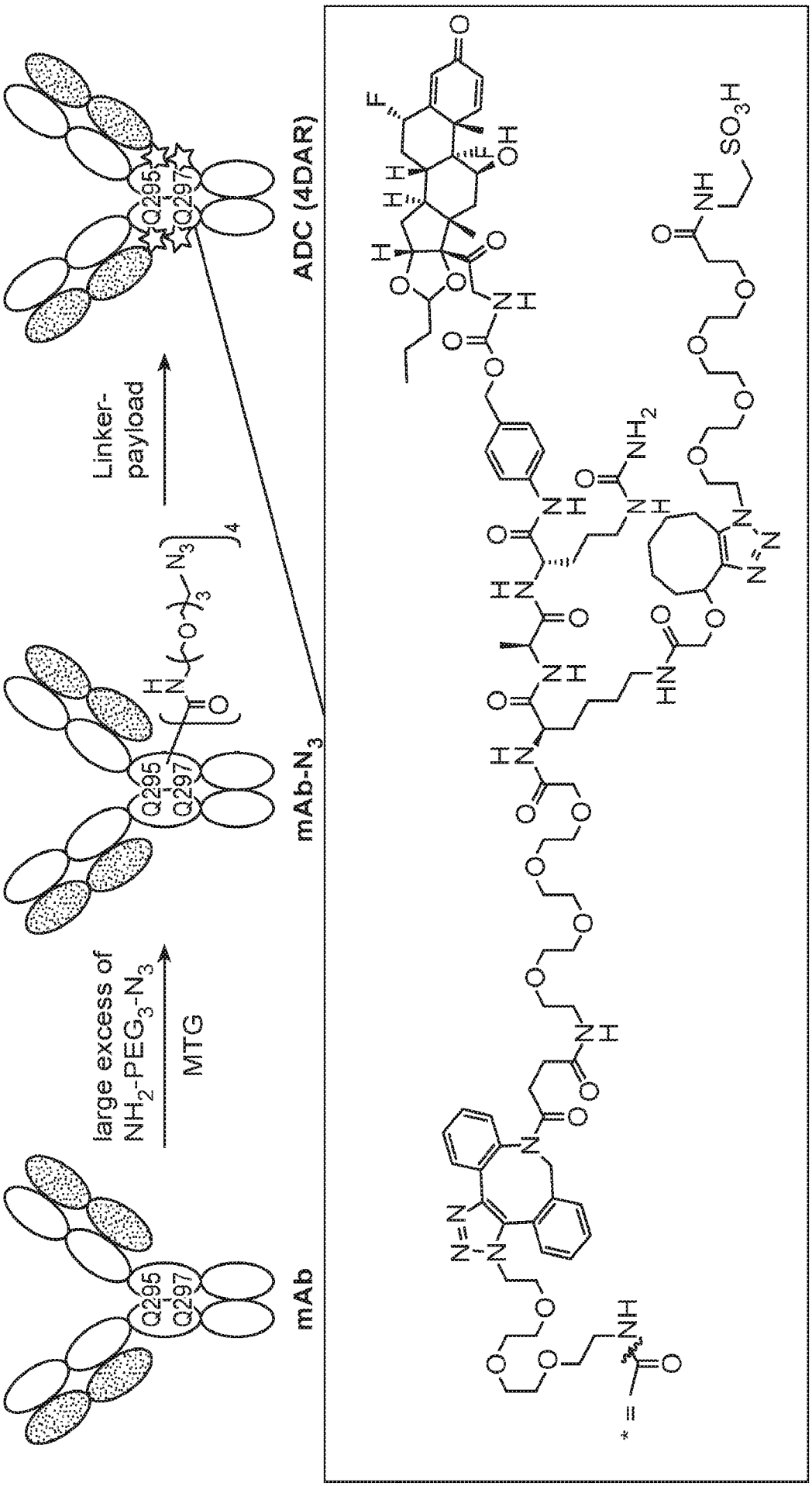

mAb
large excess of
NH2-PEG3-N3
MTG
mAb-N3
Linker-
payload
ADC (4DAR)
Q295
Q297

FIG. 19

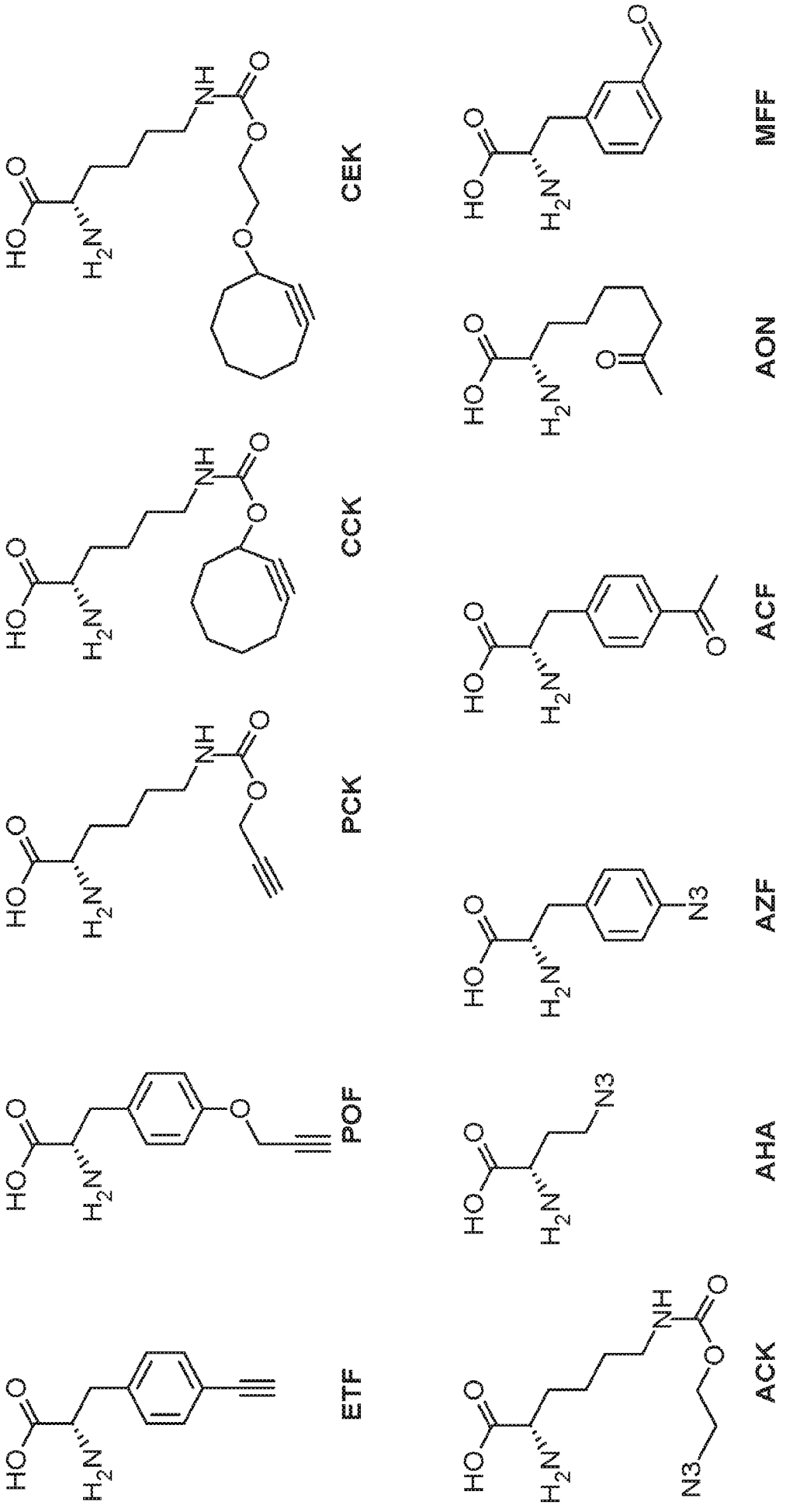



FIG. 20

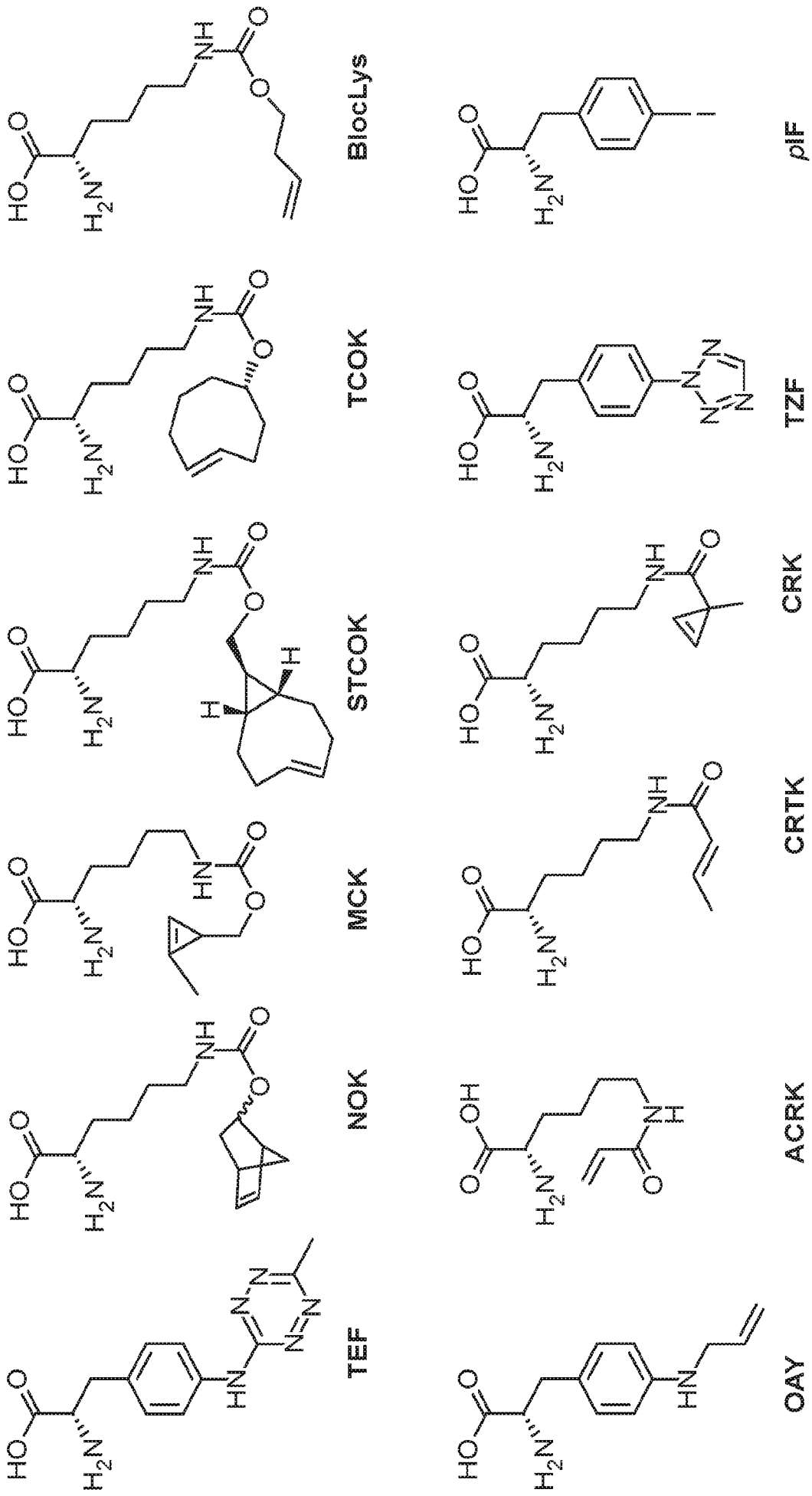



FIG. 20 (Cont.)