Fig. 1
A

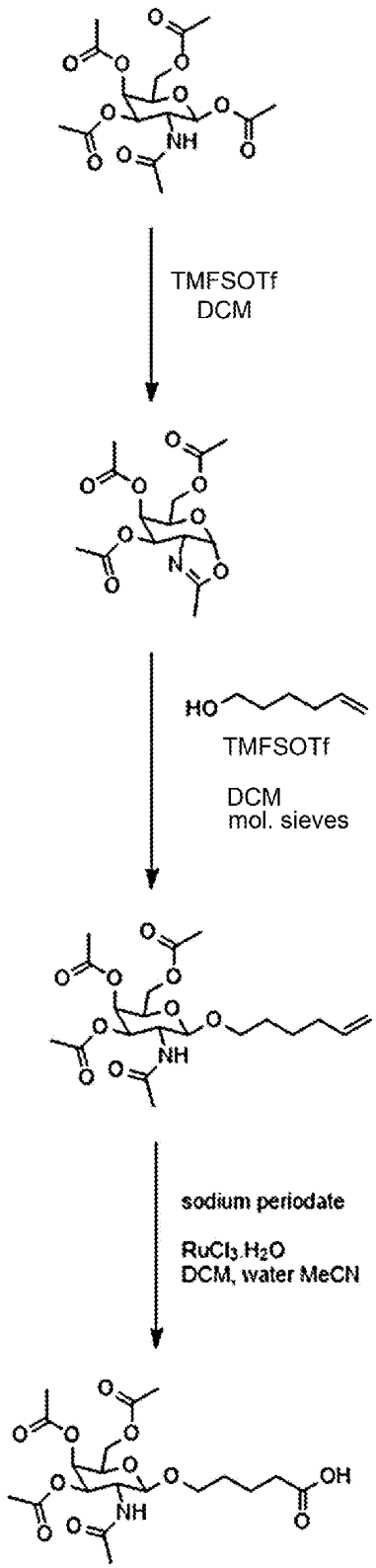

TMFSOTf
DCM
HO
TMFSOTf
DCM
mol. sieves
sodium periodate
RuCl3.H2O
DCM, water MeCN
OH

Fig. 1

B

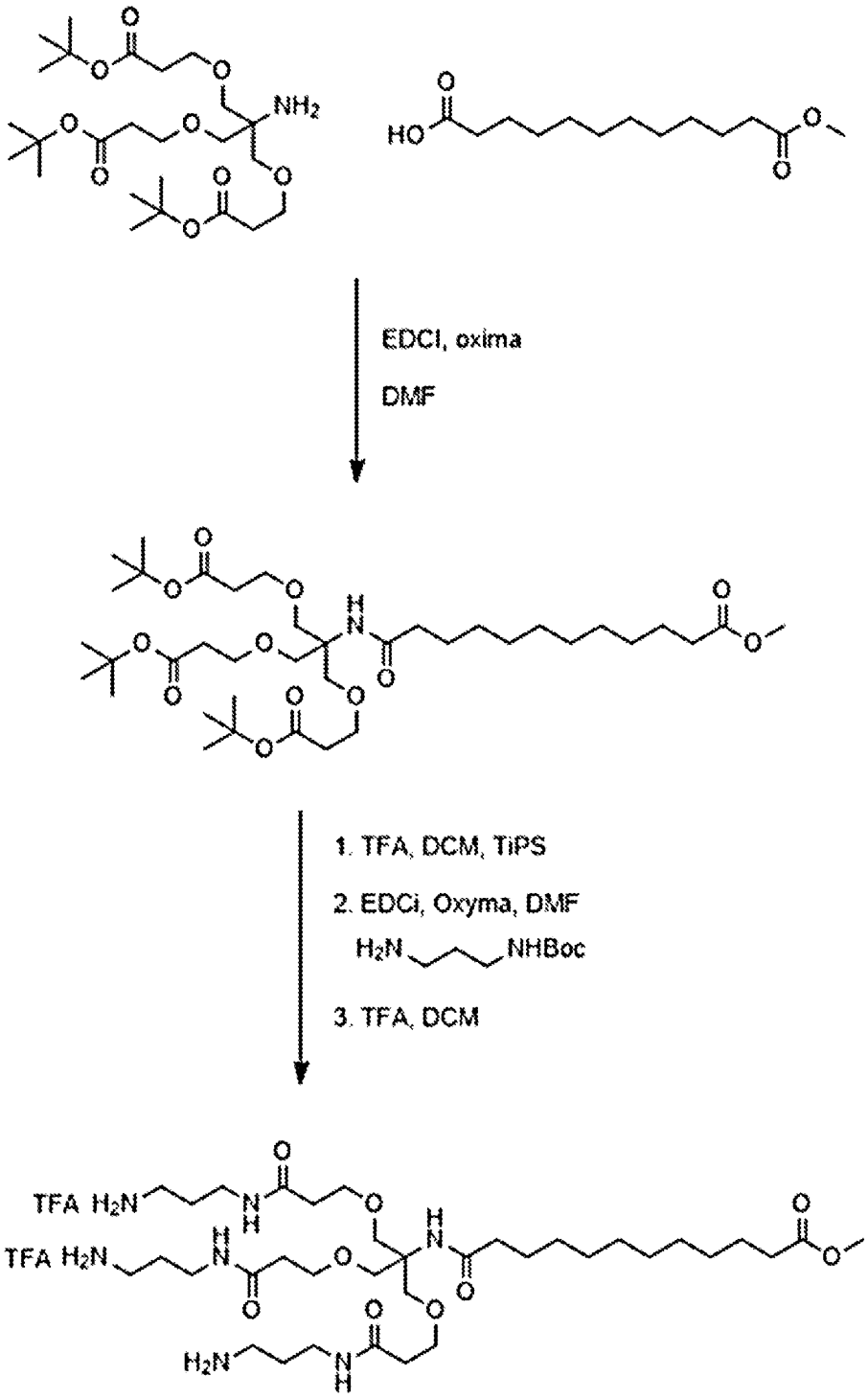

EDCI, oxima
DMF
1. TFA, DCM, TiPS
2. EDCi, Oxyma, DMF
H2N NHBoc
3. TFA, DCM
TFA H2N
TFA H2N
H2N

Fig. 1

C

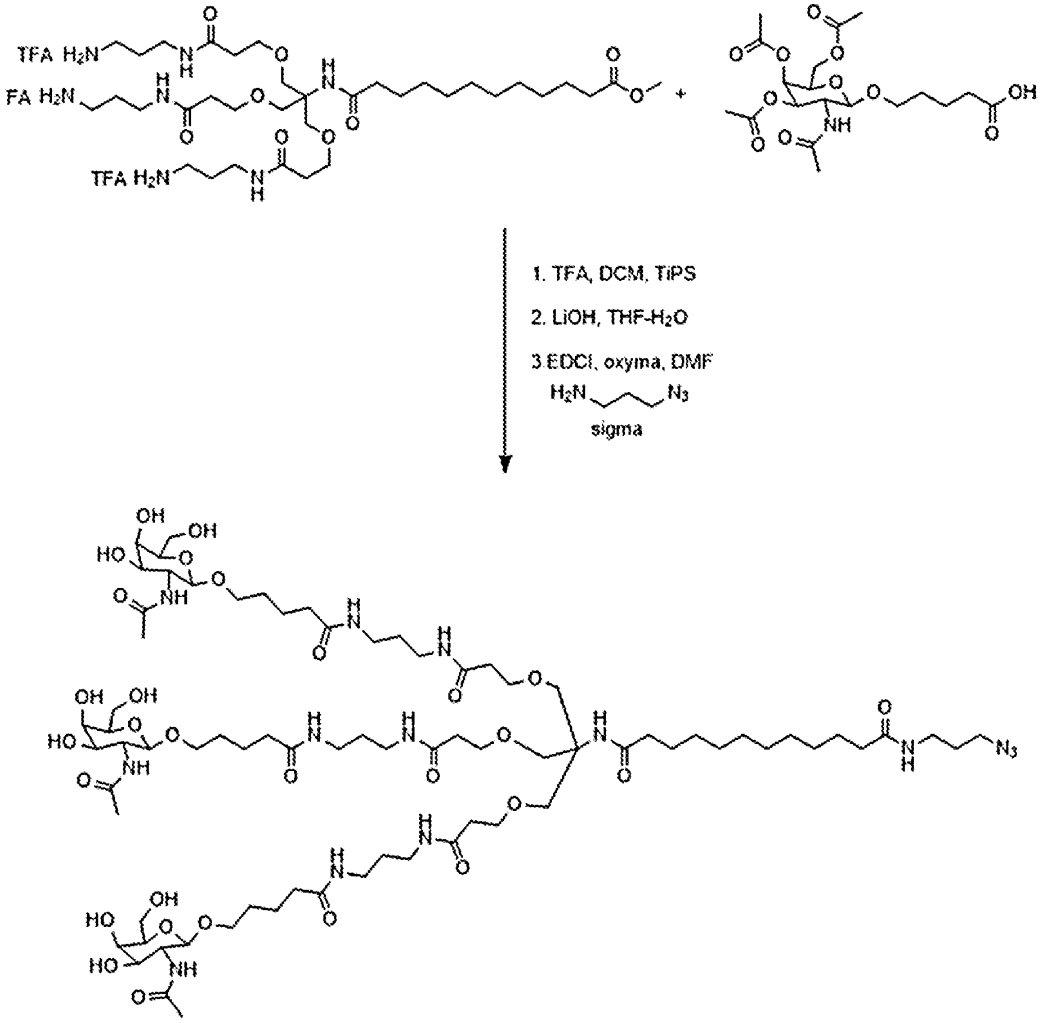

TFA H2N
FA H2N
TFA H2N
1. TFA, DCM, TiPS
2. LiOH, THF-H2O
3 EDCI, oxyma, DMF
H2N N3
sigma

Fig. 2
A

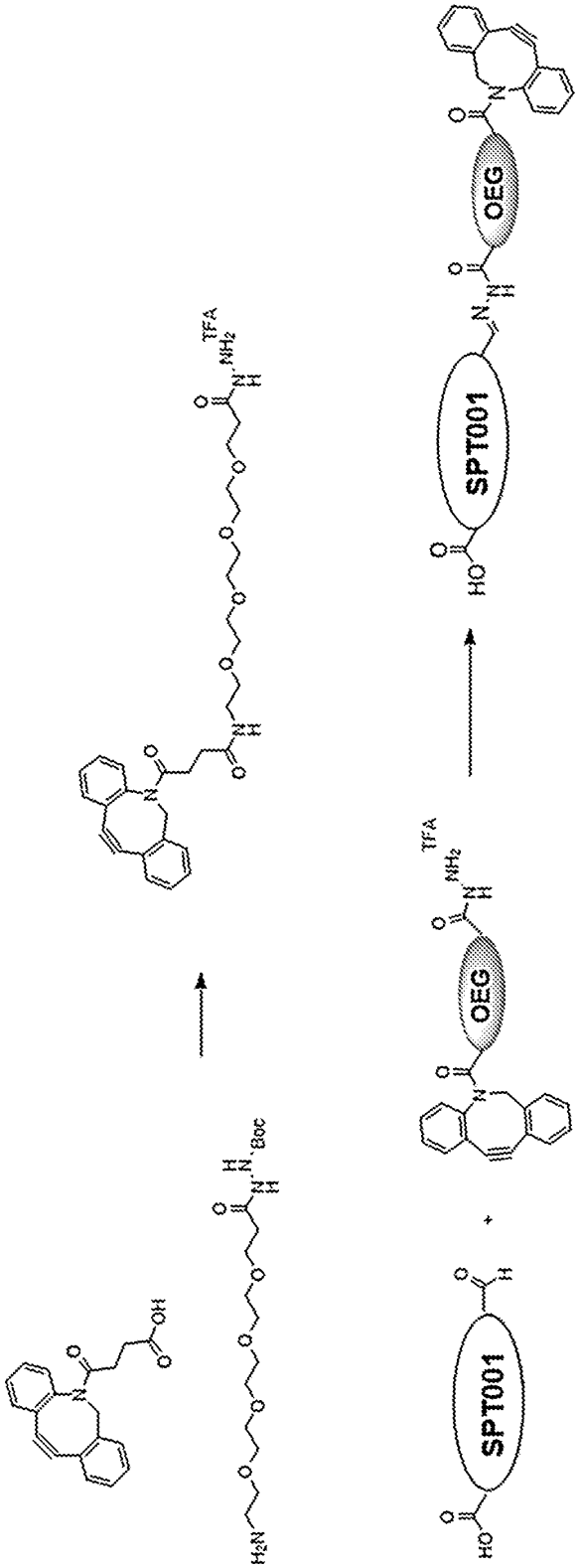


Fig. 2
B

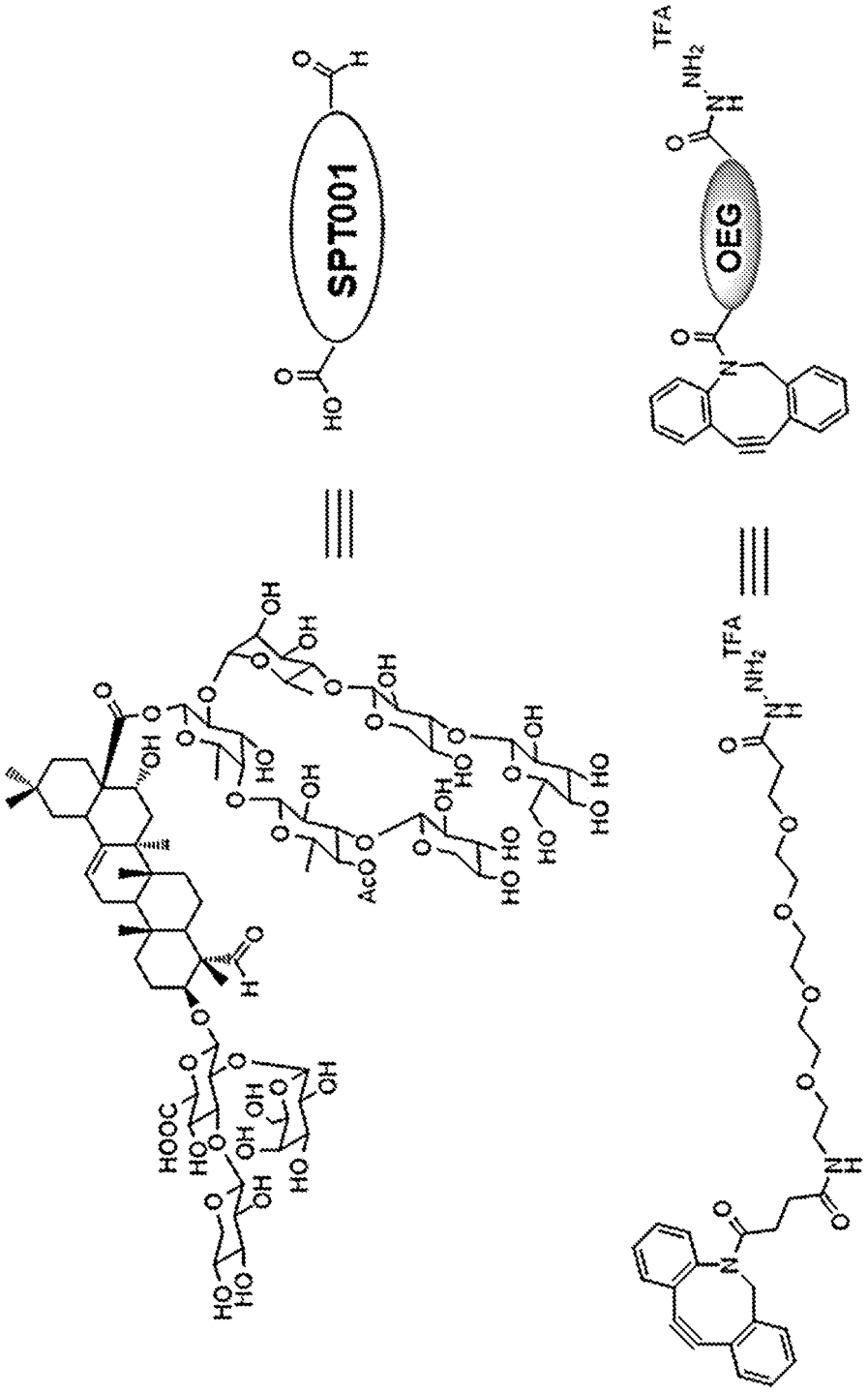


Fig. 3

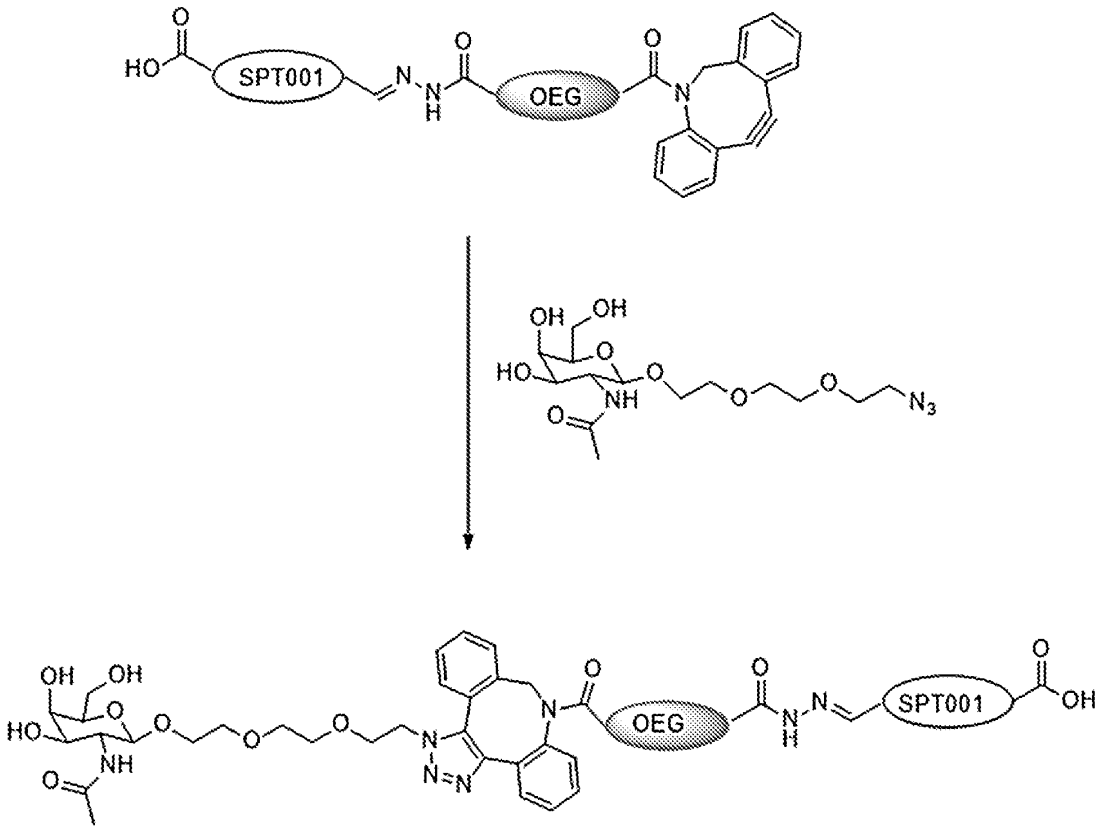

HO
SPT001
N
H
N
O
OEG
N
OH
OH
O
HO
NH
O
O
O
O
N3
OH
OH
O
HO
NH
O
O
O
O
N
N
N
O
OEG
O
N
H
N
SPT001
O
OH

Fig. 4

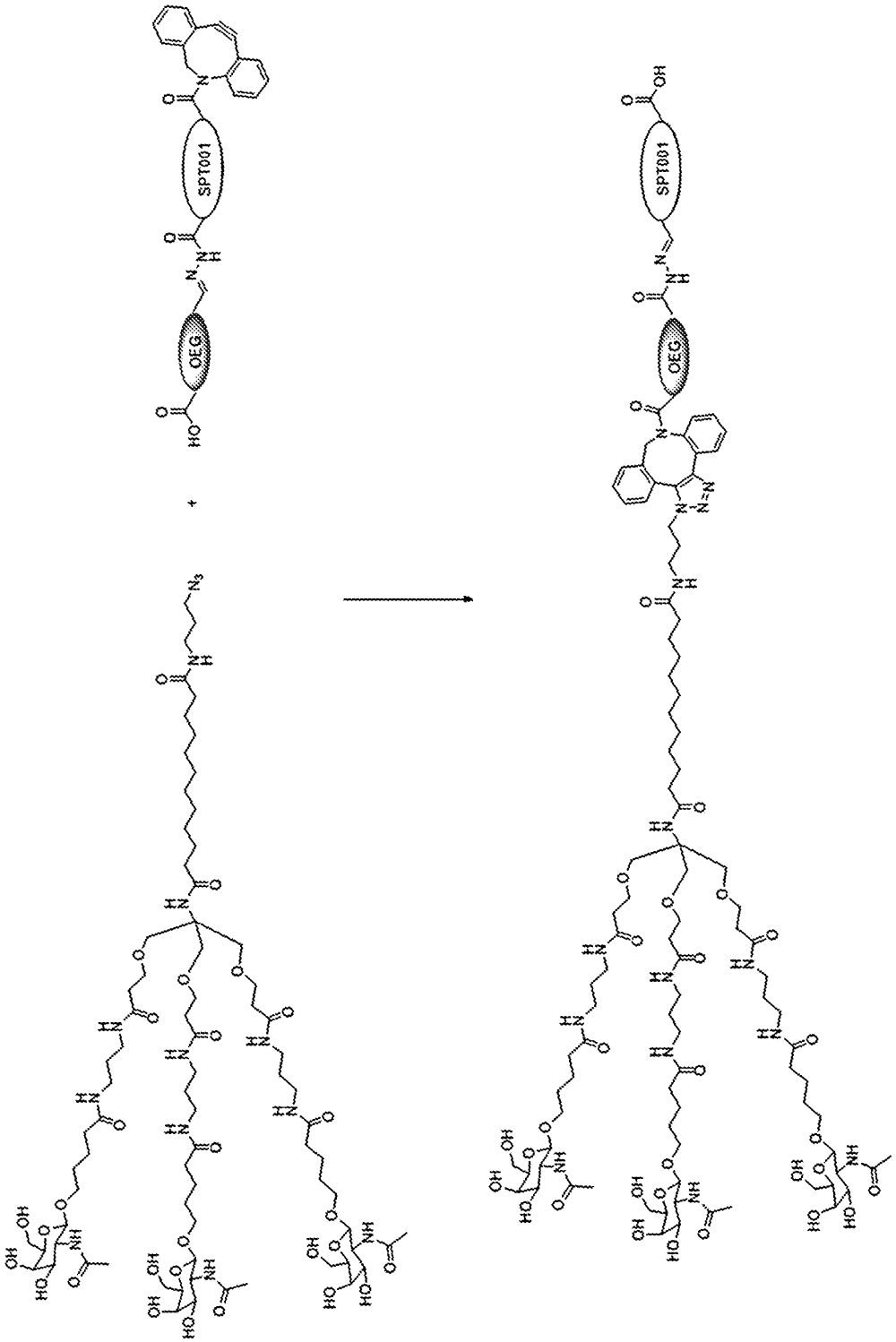


Fig. 5

A

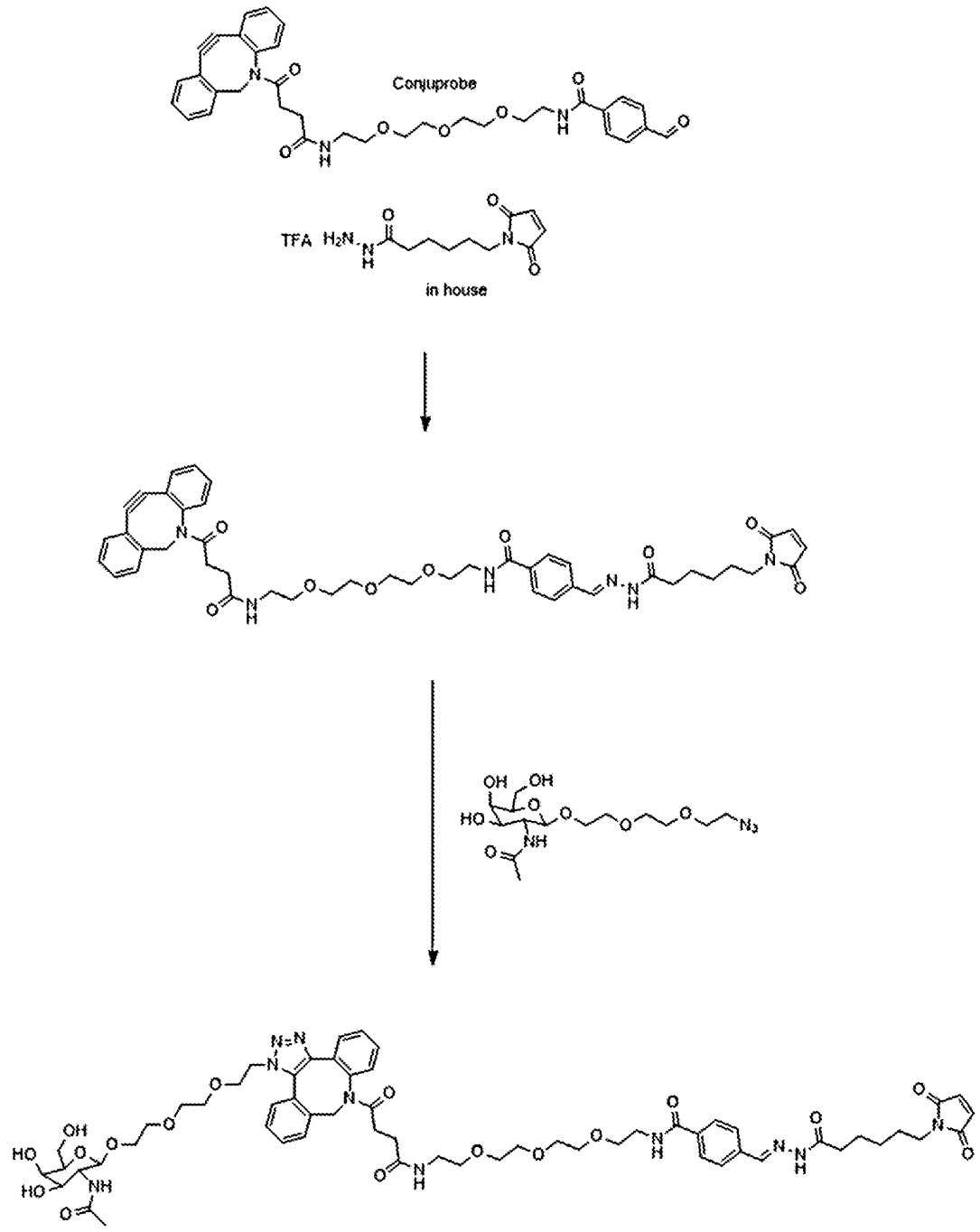


Fig. 5

B

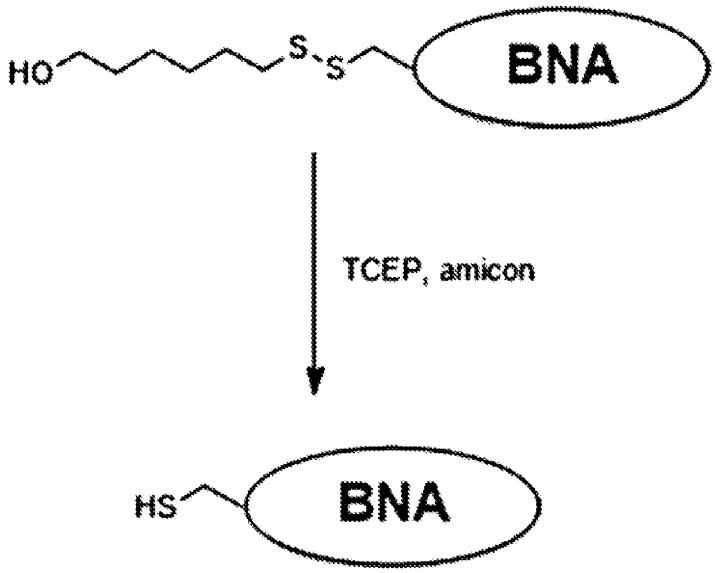


Fig. 5
C

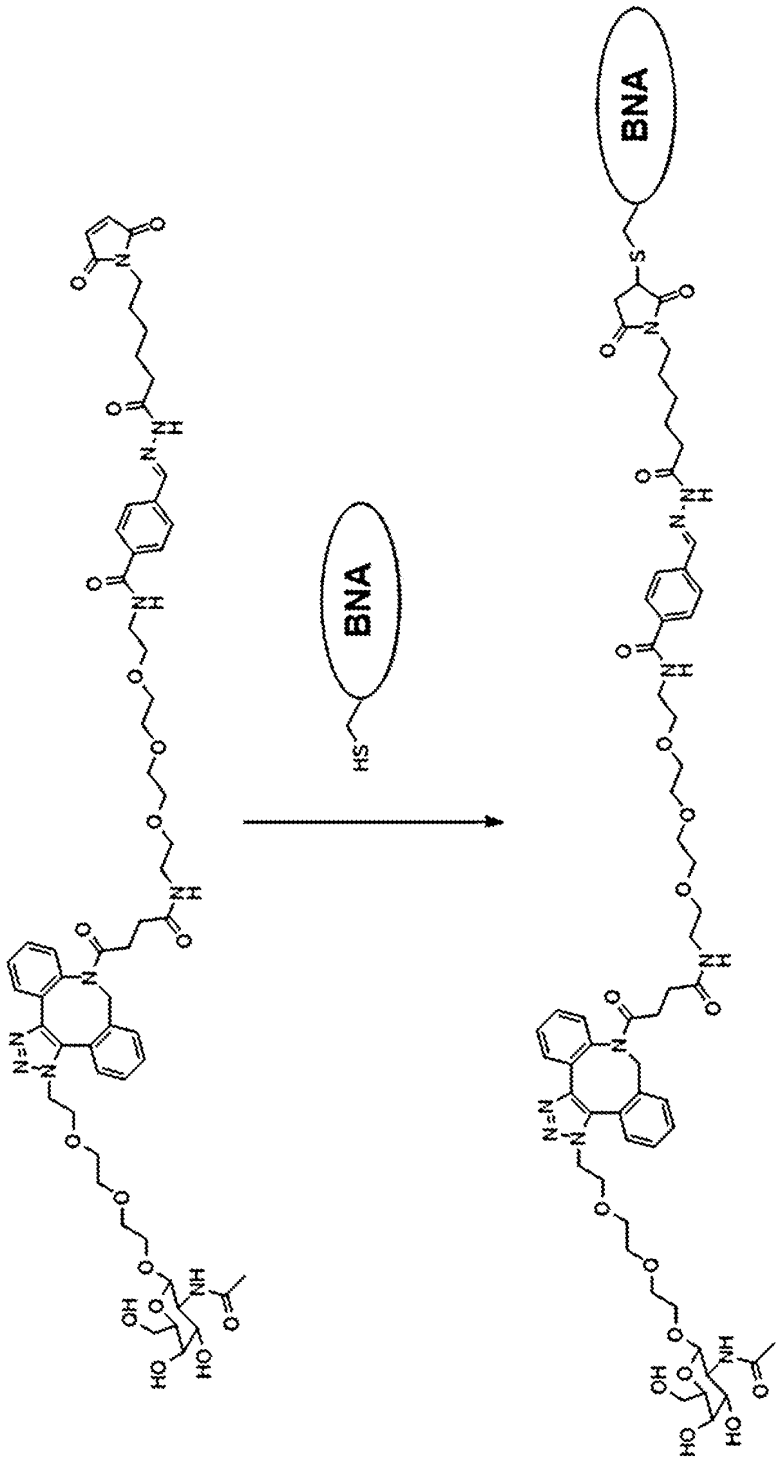


Fig. 6
A

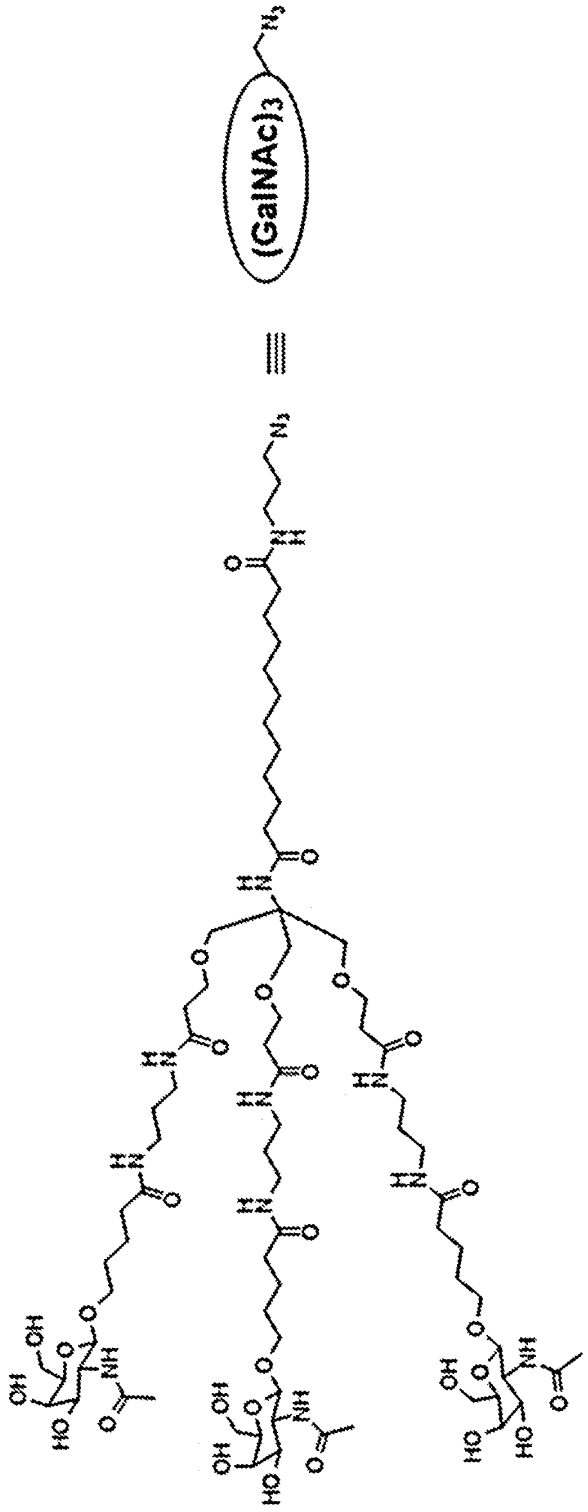


Fig. 6
B

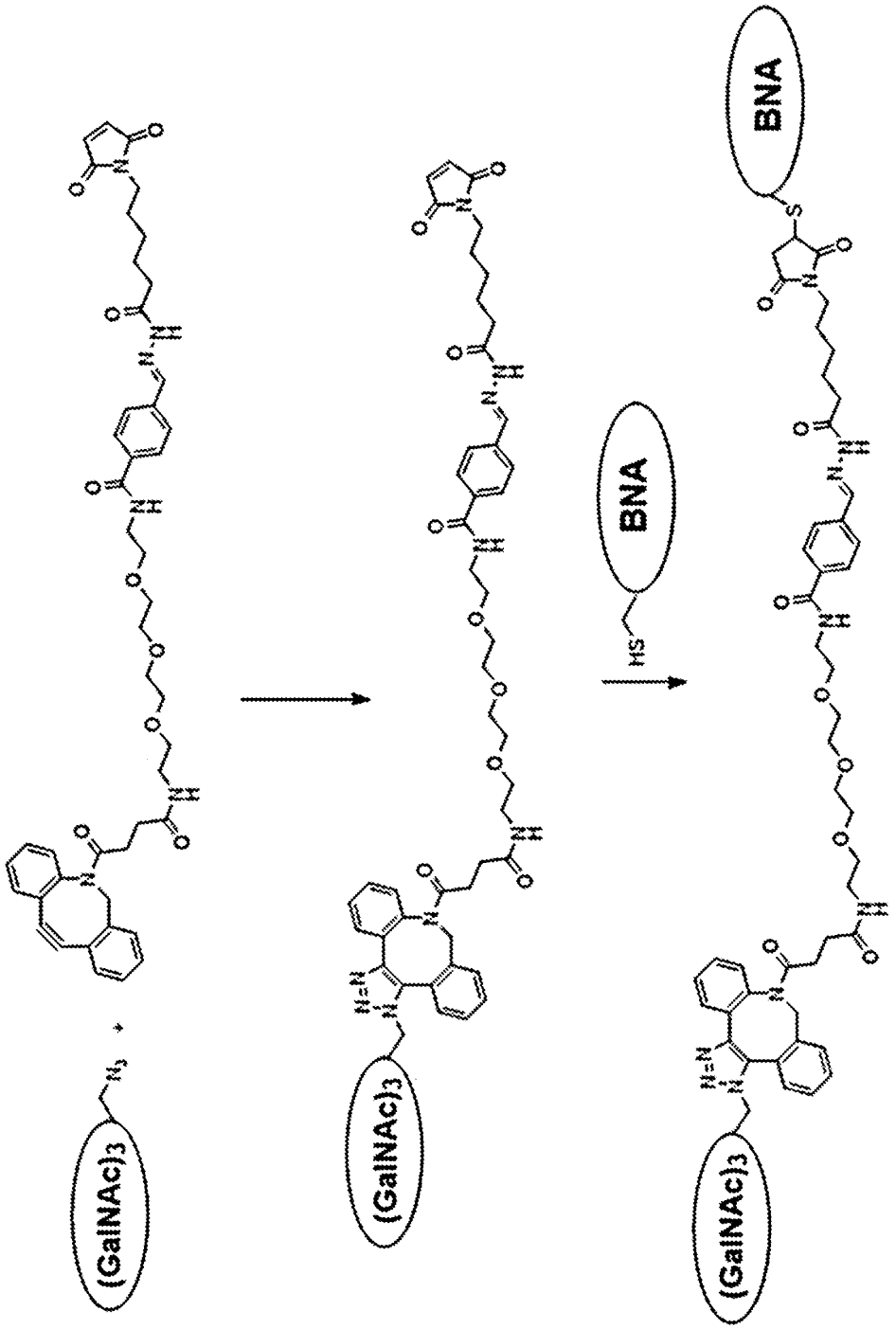


Fig. 7

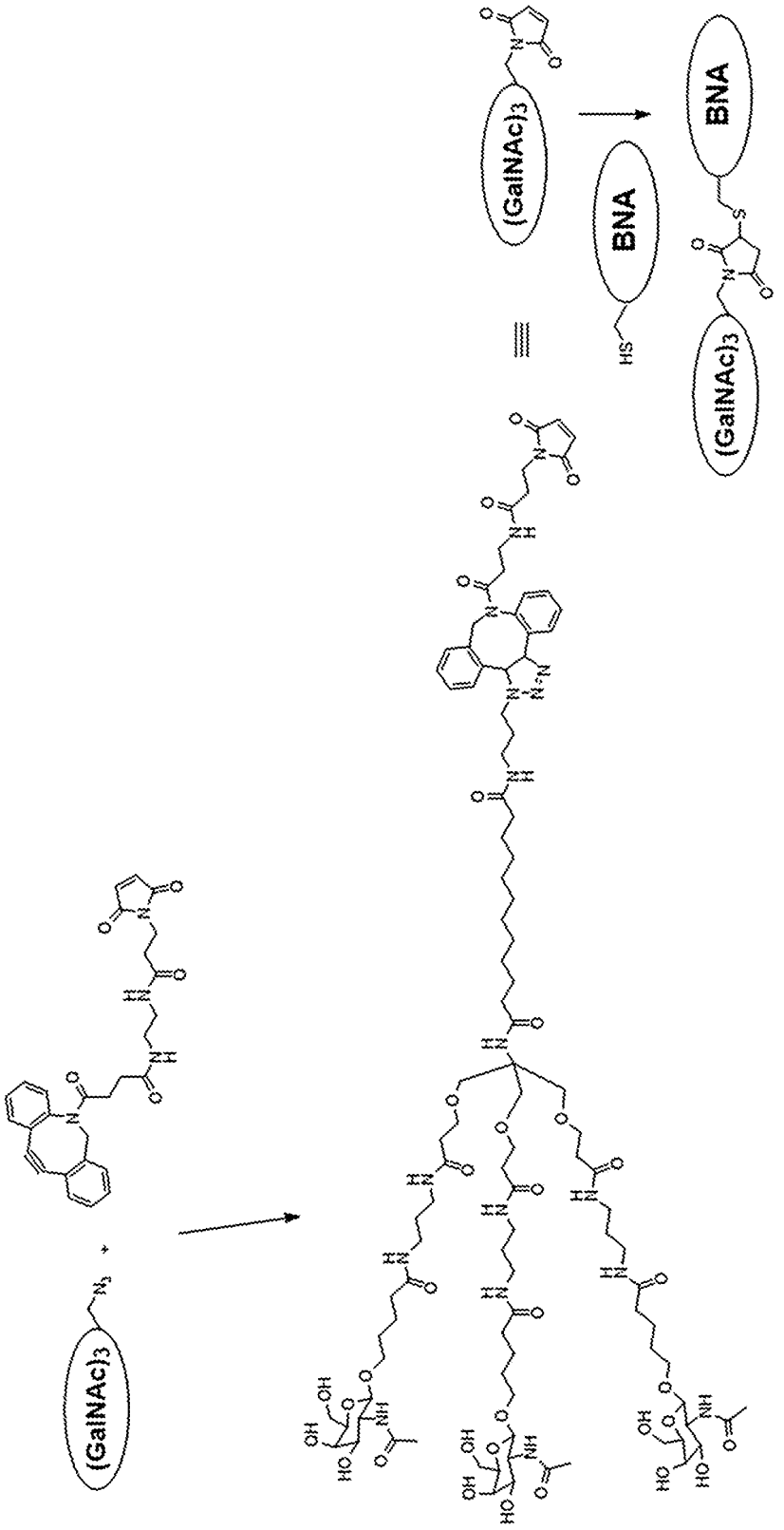


Fig. 8
A

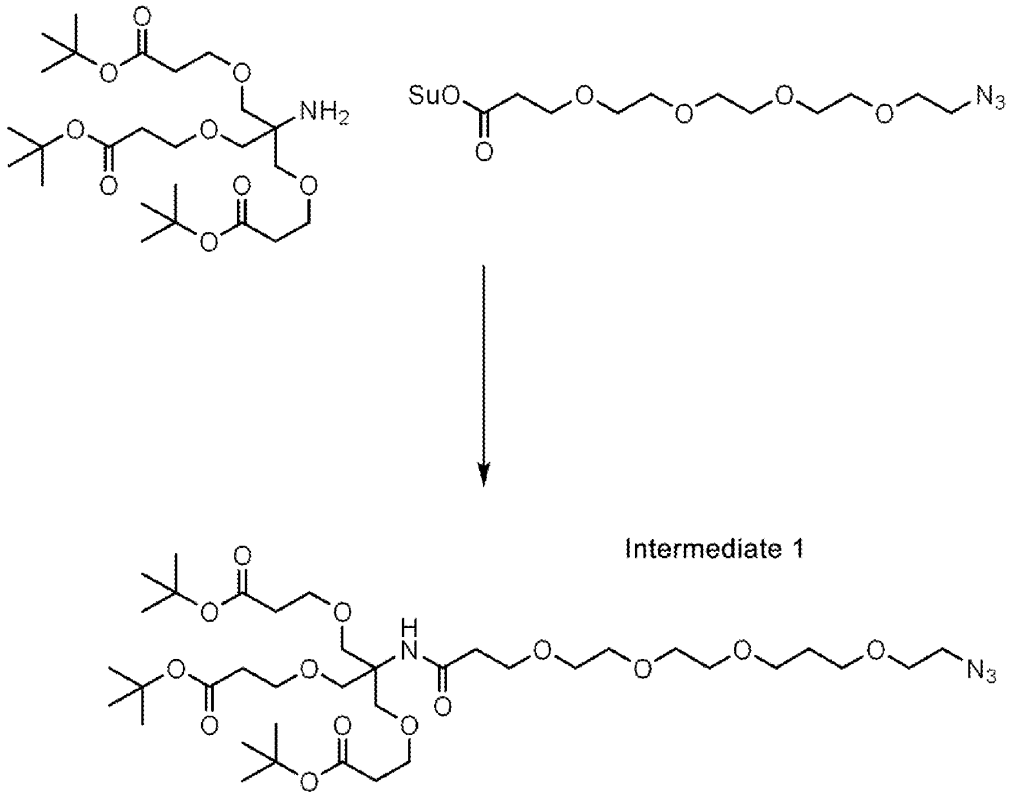


Fig. 8
B

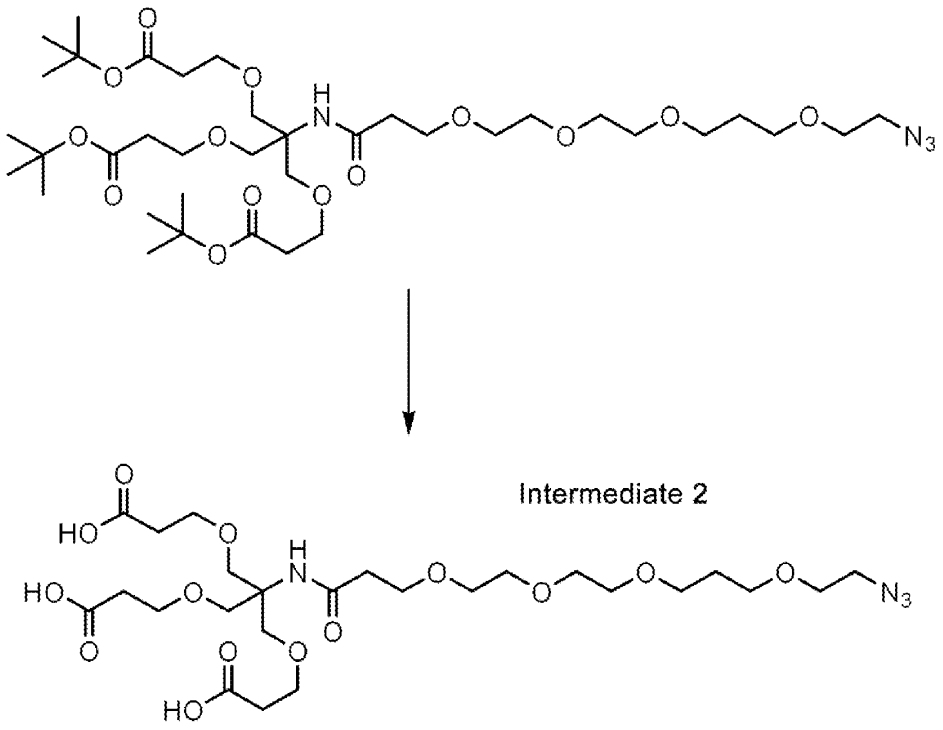


Fig. 8
C

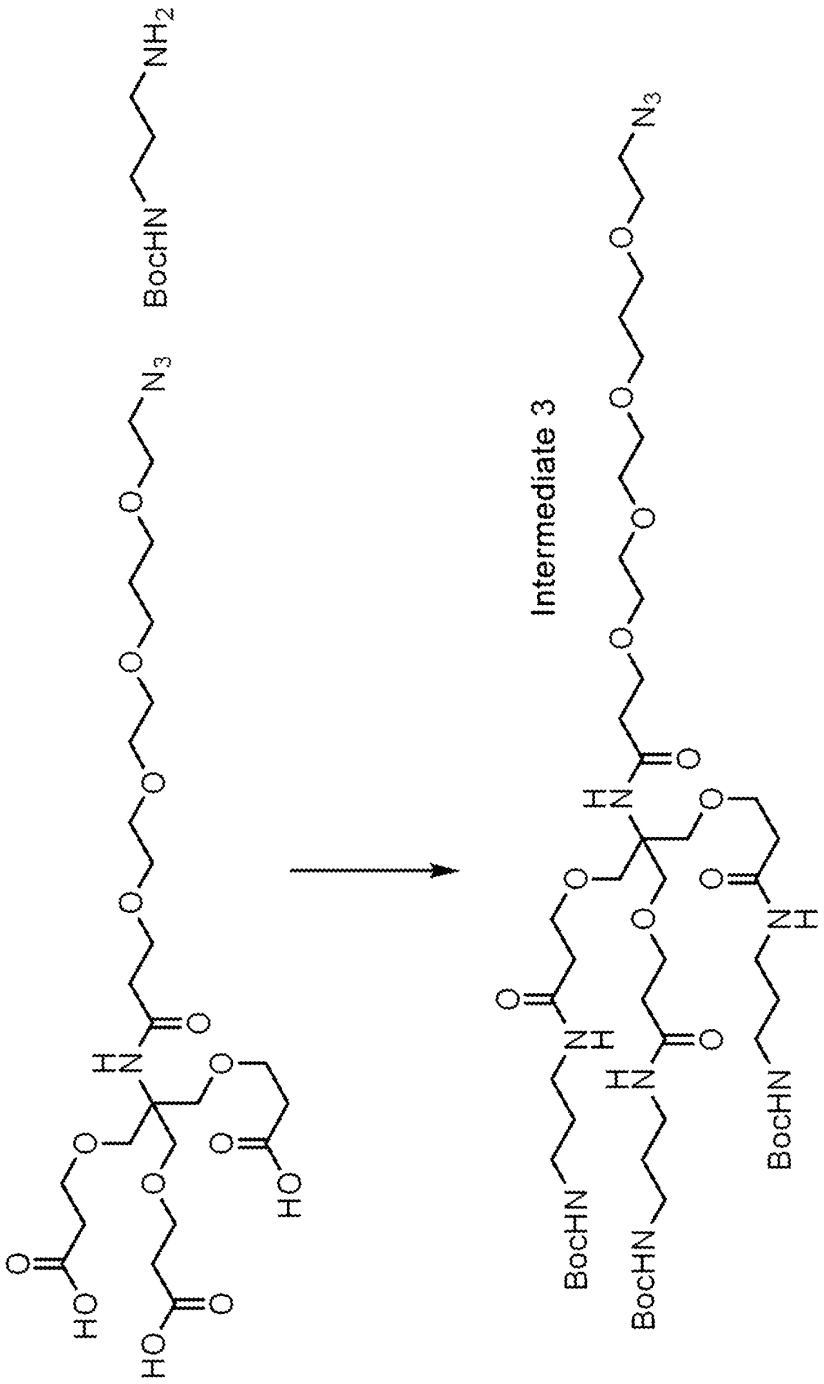


Fig. 8
D

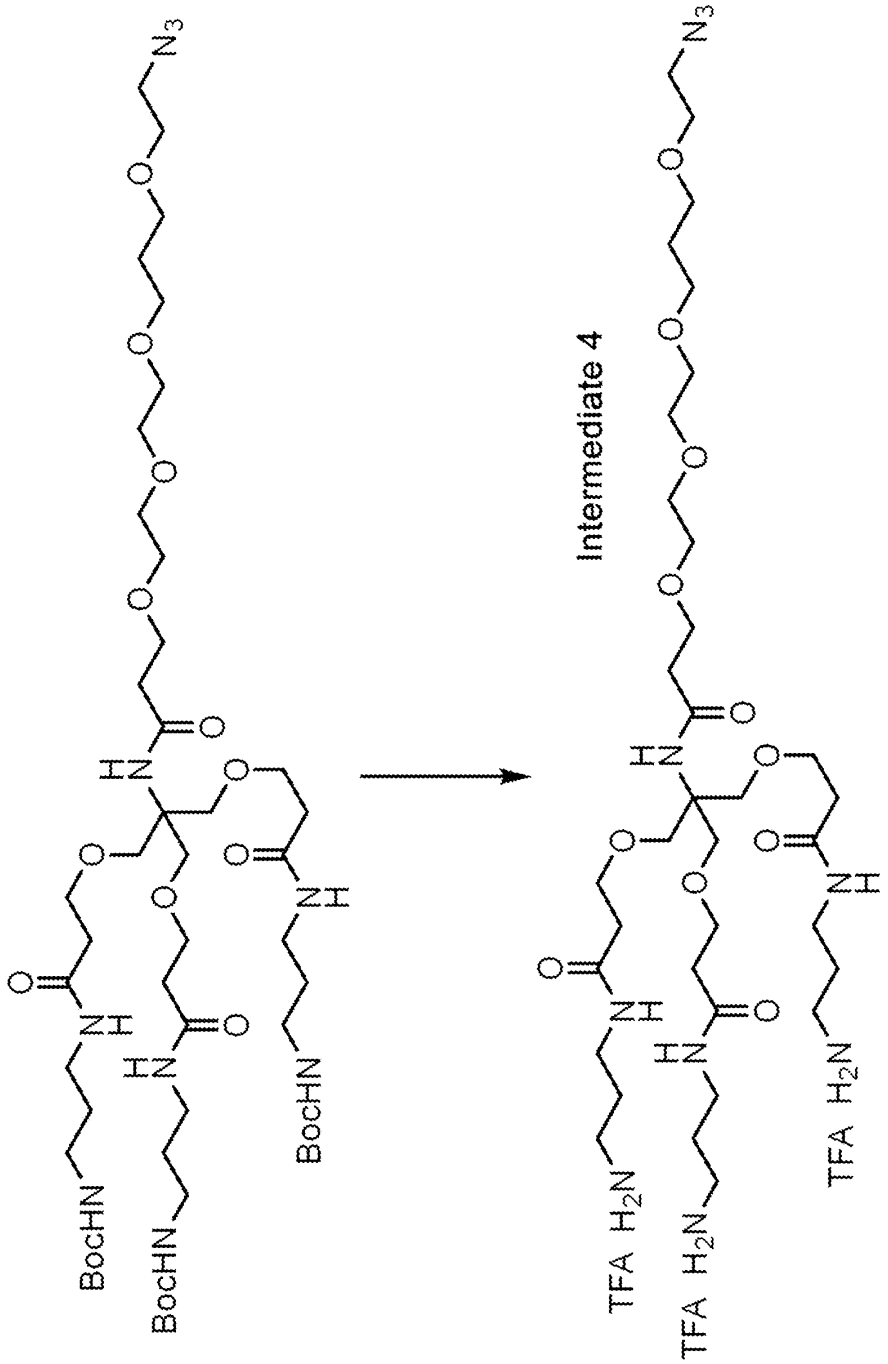
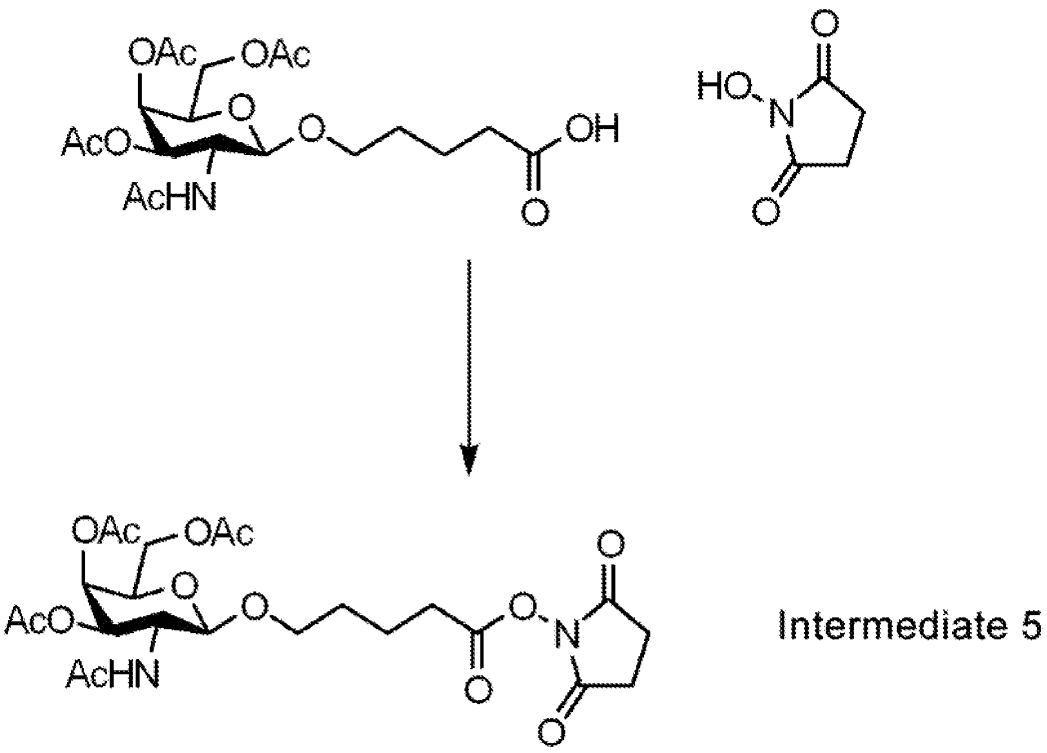


Fig. 8
E



Intermediate 5

Fig. 8
F

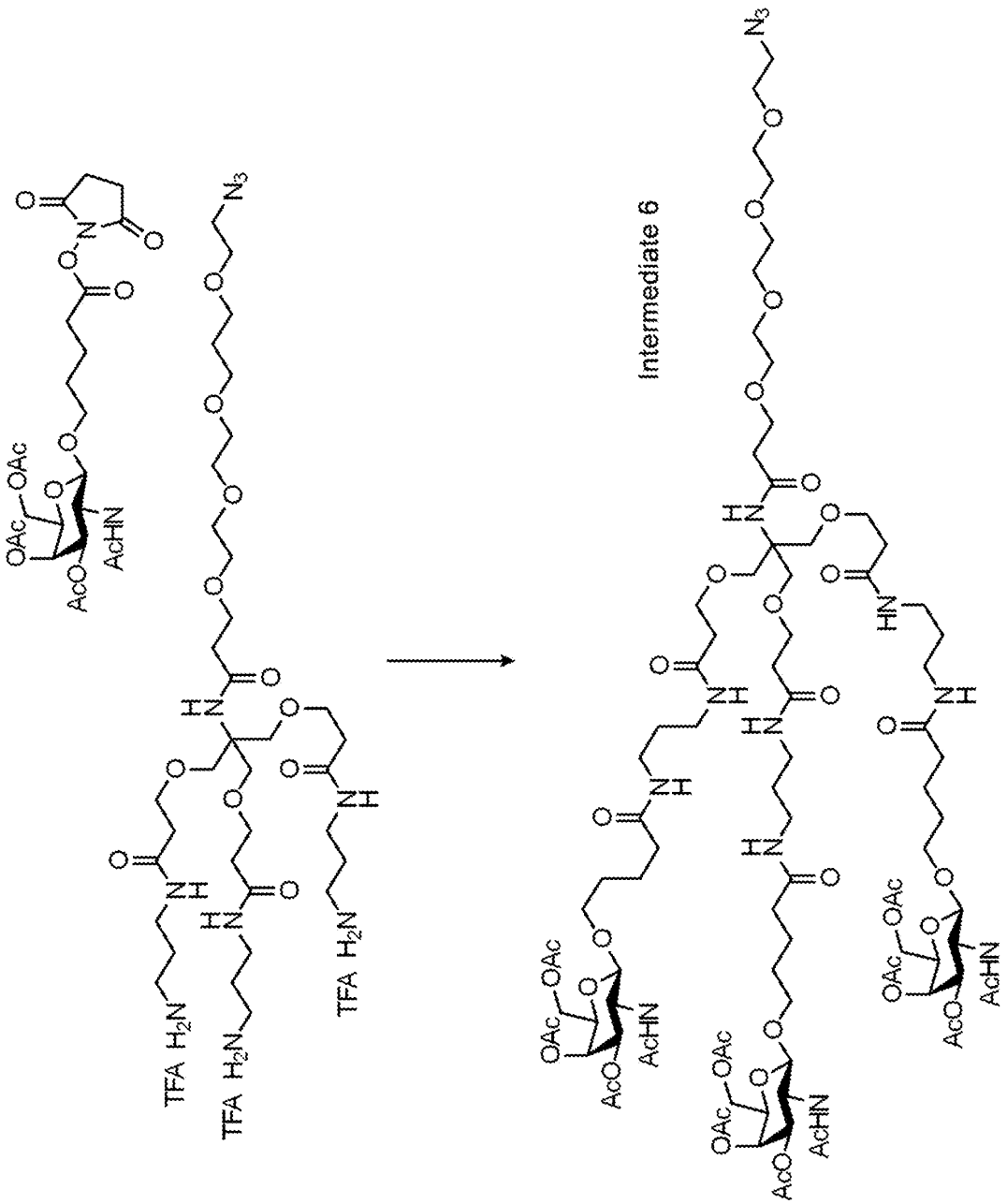


Fig. 8
G

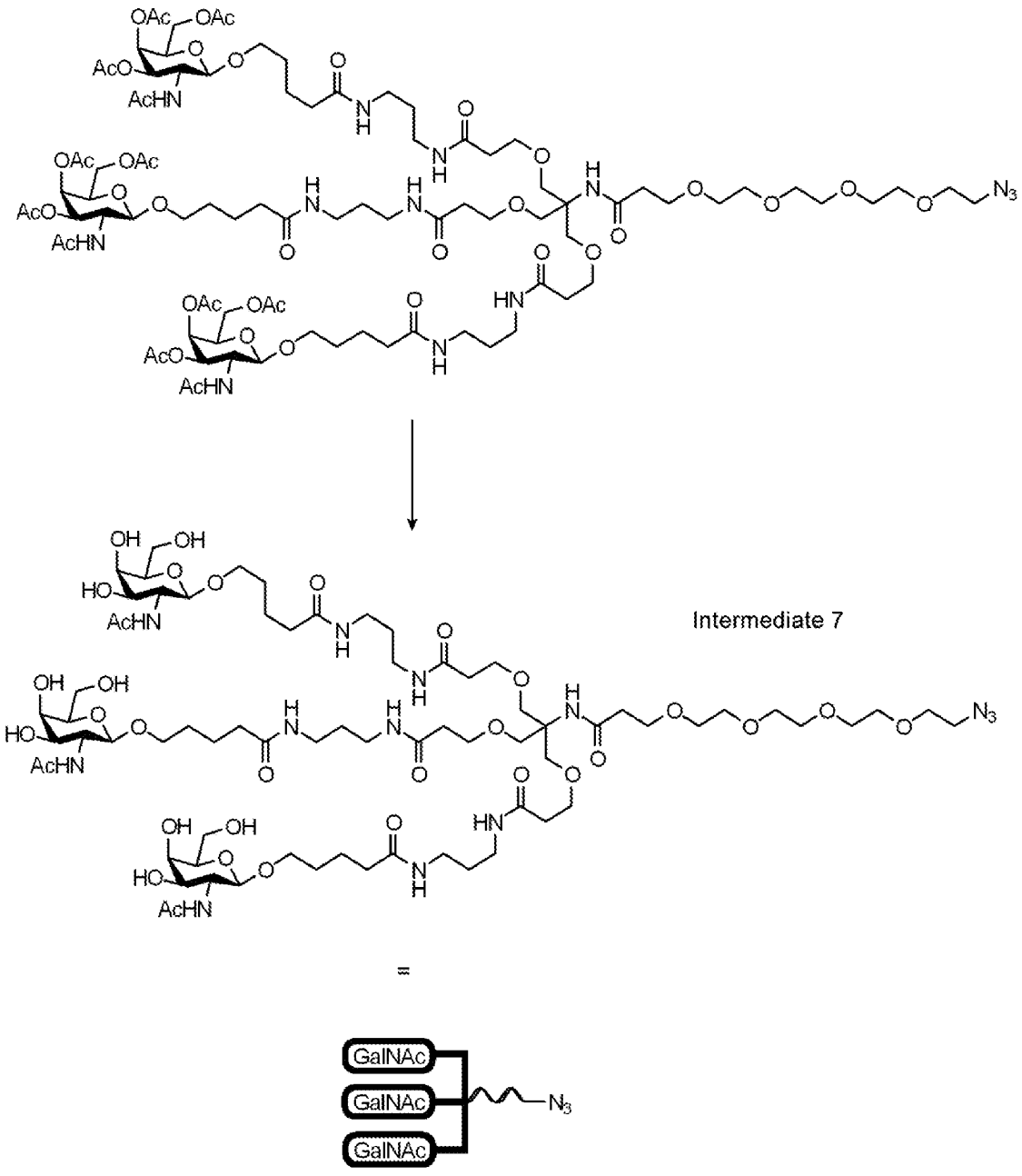
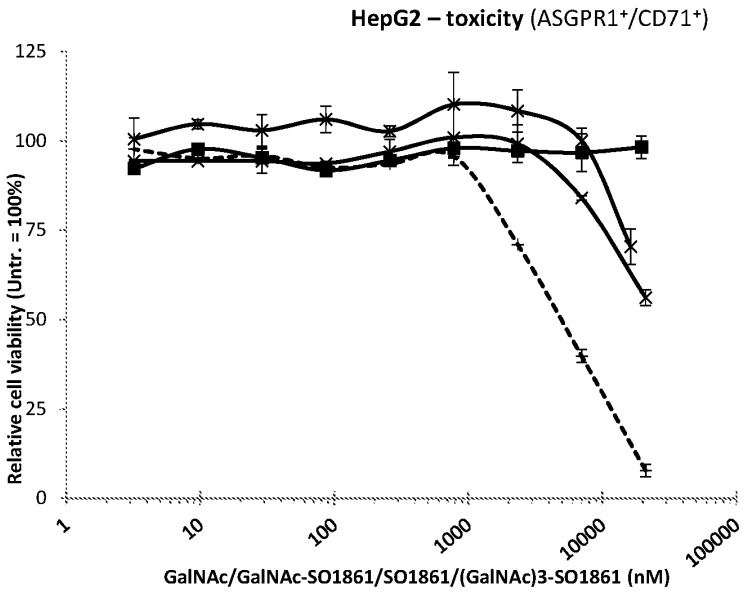


Fig. 9

A


HepG2 – toxicity (ASGPR1+/CD71+)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
1
10
100
1000
10000
100000
GalNAc/GalNAc-SO1861/SO1861/(GalNAc)3-SO1861 (nM)

B

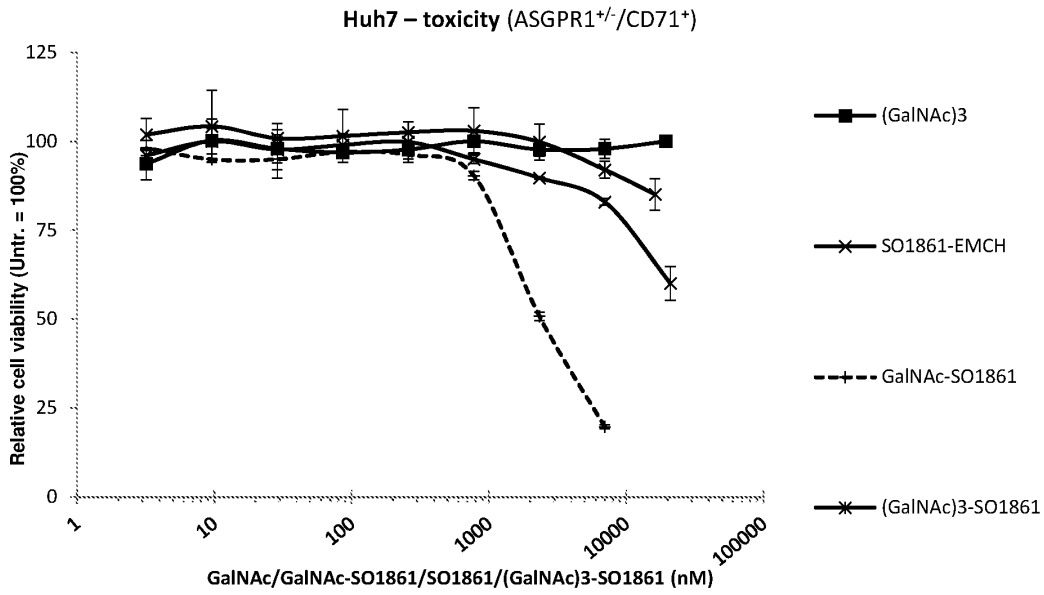
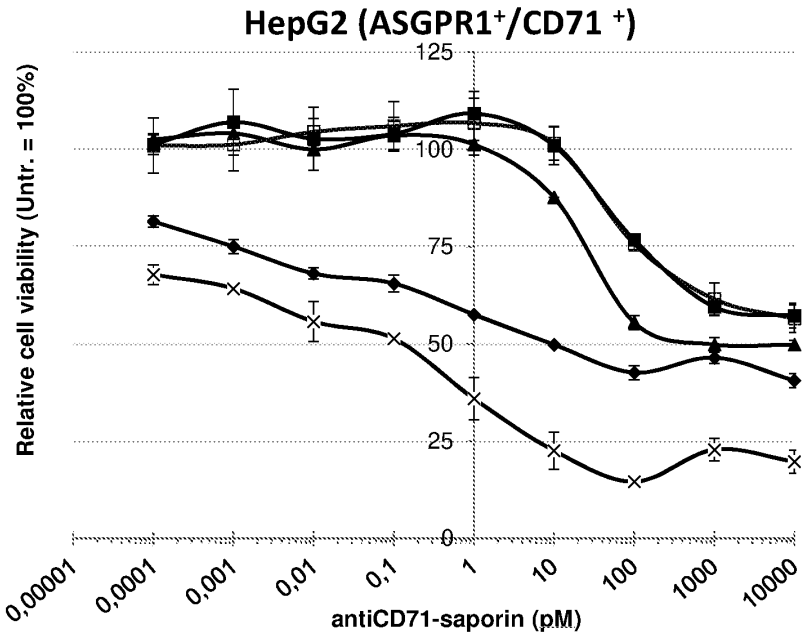

Huh7 – toxicity (ASGPR1+/-/CD71+)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
1
10
100
1000
10000
100000
GalNAc/GalNAc-SO1861/SO1861/(GalNAc)3-SO1861 (nM)
(GalNAc)3
SO1861-EMCH
GalNAc-SO1861
(GalNAc)3-SO1861

Fig. 10

A


HepG2 (ASGPR1+/CD71 +)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
antiCD71-saporin (pM)

B

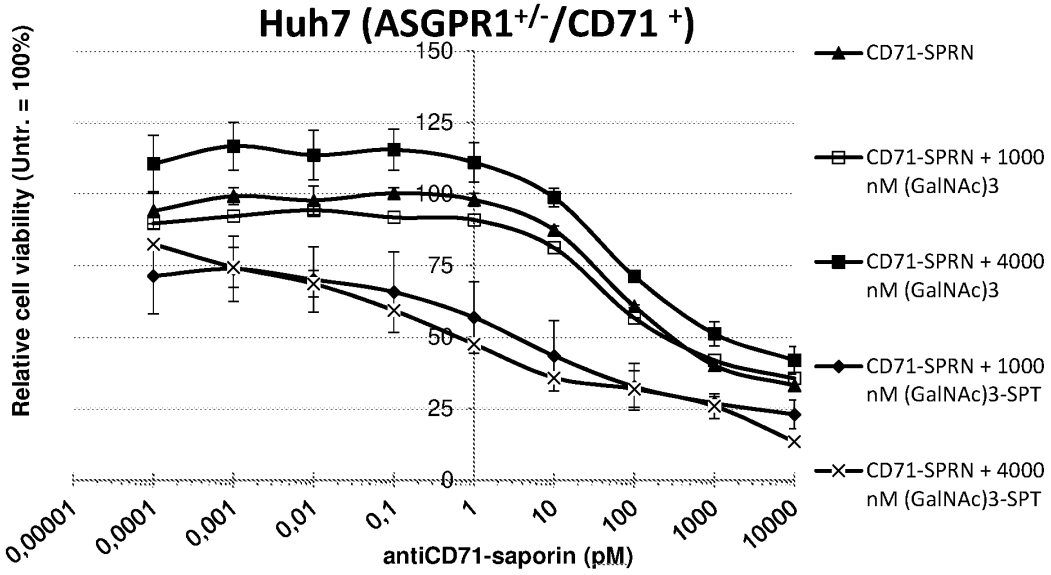
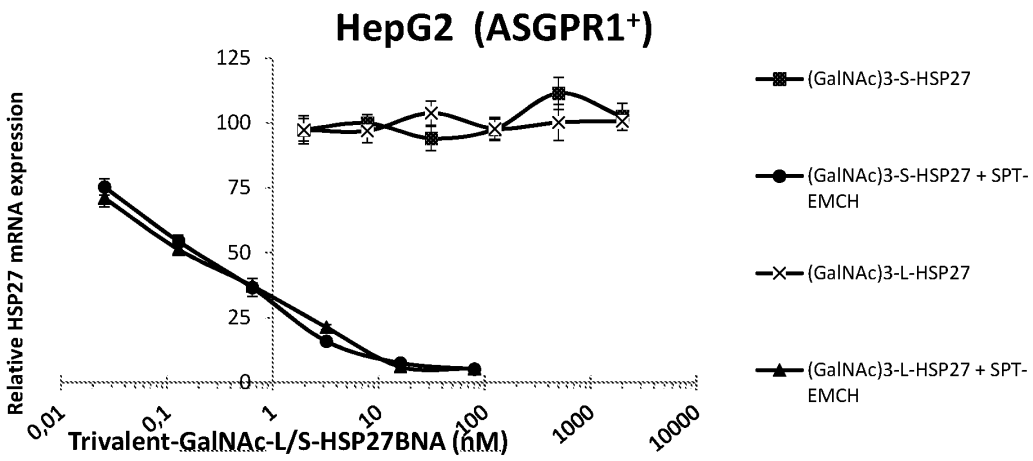

Huh7 (ASGPR1+/-/CD71 +)
Relative cell viability (Untr. = 100%)
150
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
antiCD71-saporin (pM)
CD71-SPRN
CD71-SPRN + 1000 nM (GalNAc)3
CD71-SPRN + 4000 nM (GalNAc)3
CD71-SPRN + 1000 nM (GalNAc)3-SPT
CD71-SPRN + 4000 nM (GalNAc)3-SPT

Fig. 11

A


HepG2 (ASGPR1+)
Relative HSP27 mRNA expression
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
10000
Trivalent-GalNAc-L/S-HSP27BNA (nM)
(GalNAc)3-S-HSP27
(GalNAc)3-S-HSP27 + SPT-EMCH
(GalNAc)3-L-HSP27
(GalNAc)3-L-HSP27 + SPT-EMCH

B

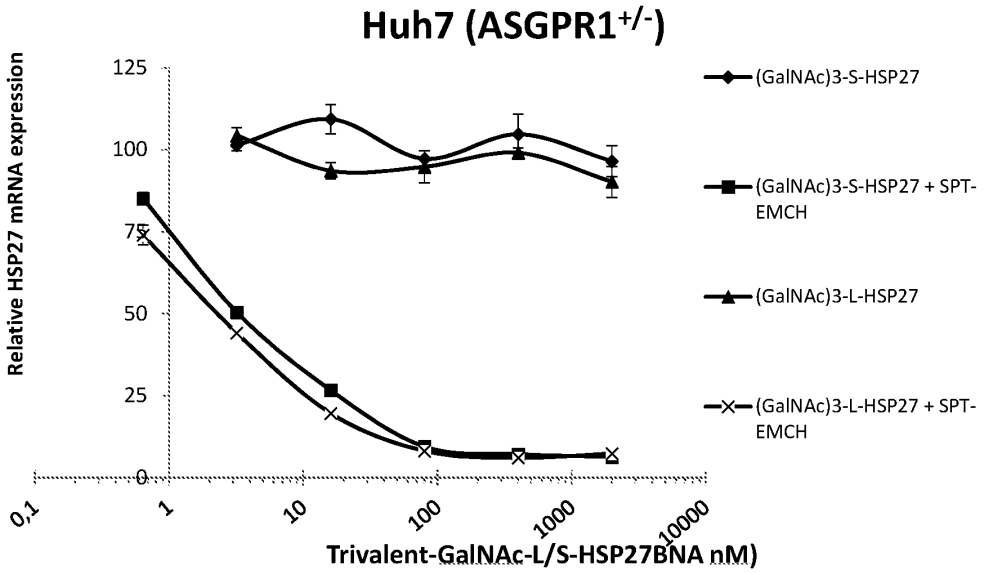
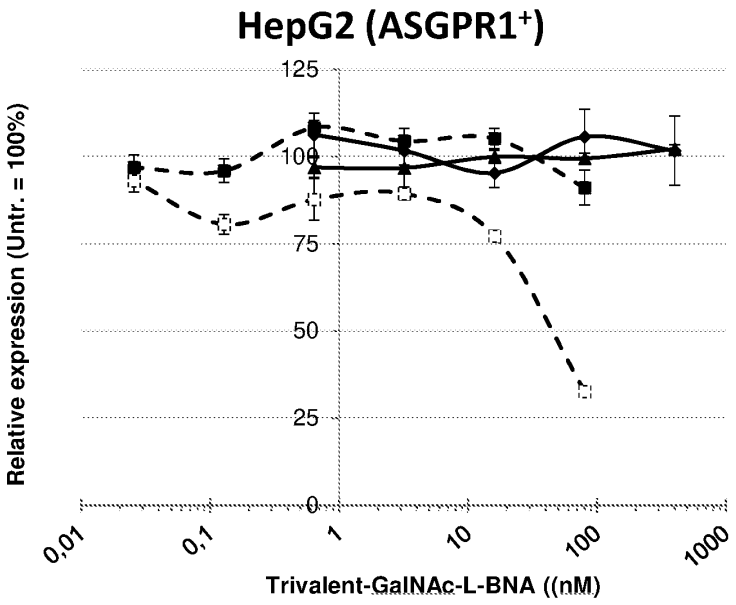

Huh7 (ASGPR1+/-)
Relative HSP27 mRNA expression
125
100
75
50
25
0
0,1
1
10
100
1000
10000
Trivalent-GalNAc-L/S-HSP27BNA nM)
(GalNAc)3-S-HSP27
(GalNAc)3-S-HSP27 + SPT-EMCH
(GalNAc)3-L-HSP27
(GalNAc)3-L-HSP27 + SPT-EMCH

Fig. 12

A


HepG2 (ASGPR1+)
Relative expression (Untr. = 100%)
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Trivalent-GalNAc-L-BNA ((nM)

B

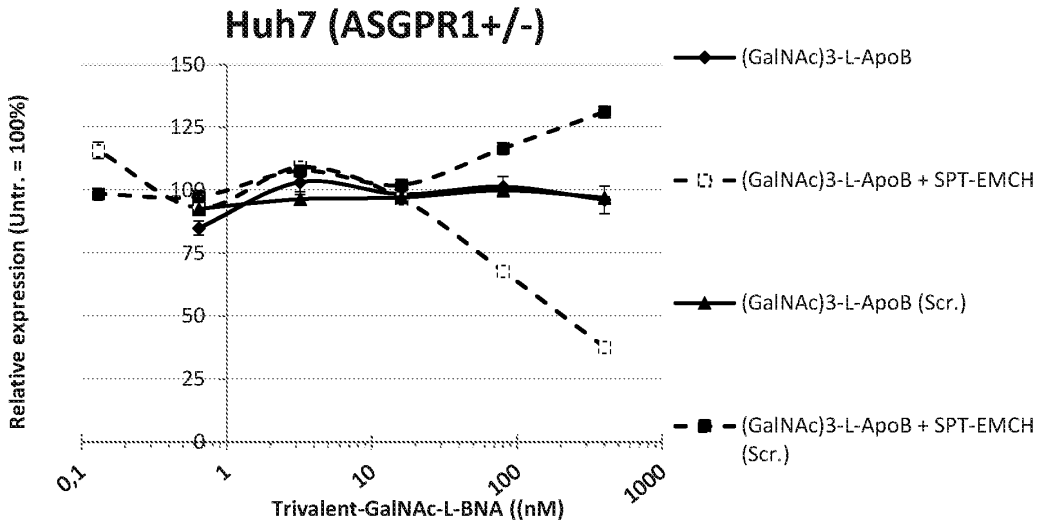
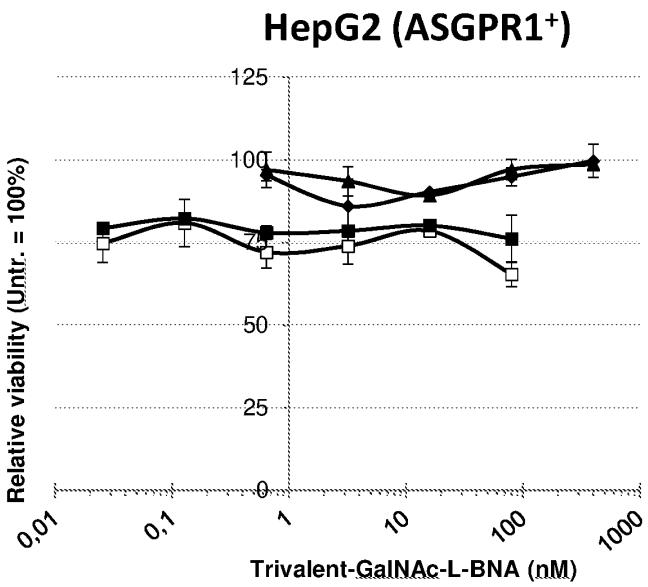

Huh7 (ASGPR1+/-)
Relative expression (Untr. = 100%)
150
125
100
75
50
25
0
0,1
1
10
100
1000
Trivalent-GalNAc-L-BNA ((nM)
(GalNAc)3-L-ApoB
(GalNAc)3-L-ApoB + SPT-EMCH
(GalNAc)3-L-ApoB (Scr.)
(GalNAc)3-L-ApoB + SPT-EMCH (Scr.)

Fig. 13

A


HepG2 (ASGPR1+)
Relative viability (Untr. = 100%)
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Trivalent-GalNAc-L-BNA (nM)

B

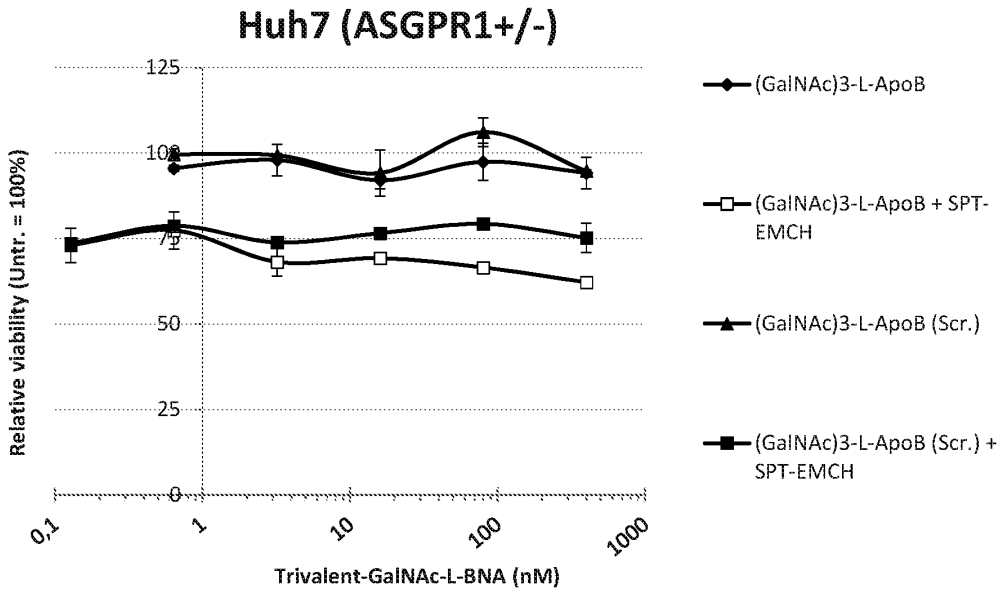
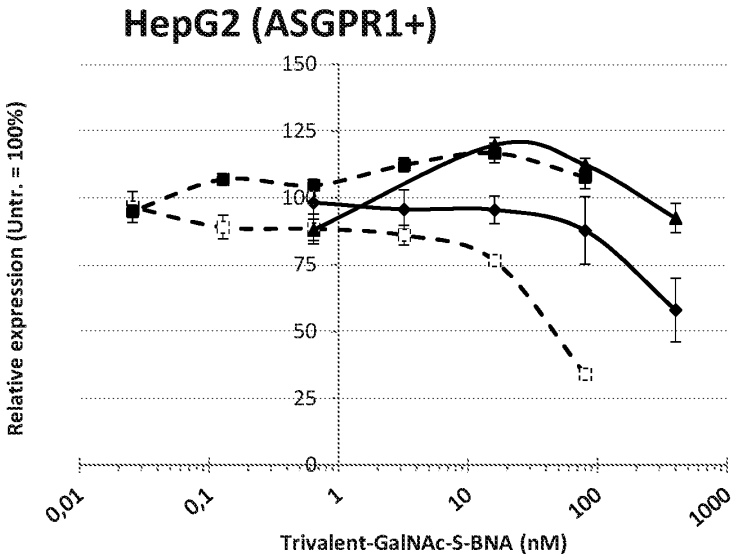

Huh7 (ASGPR1+/-)
Relative viability (Untr. = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
Trivalent-GalNAc-L-BNA (nM)
(GalNAc)3-L-ApoB
(GalNAc)3-L-ApoB + SPT-EMCH
(GalNAc)3-L-ApoB (Scr.)
(GalNAc)3-L-ApoB (Scr.) + SPT-EMCH

Fig. 14

A


HepG2 (ASGPR1+)
Relative expression (Untr. = 100%)
150
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Trivalent-GalNAc-S-BNA (nM)

B

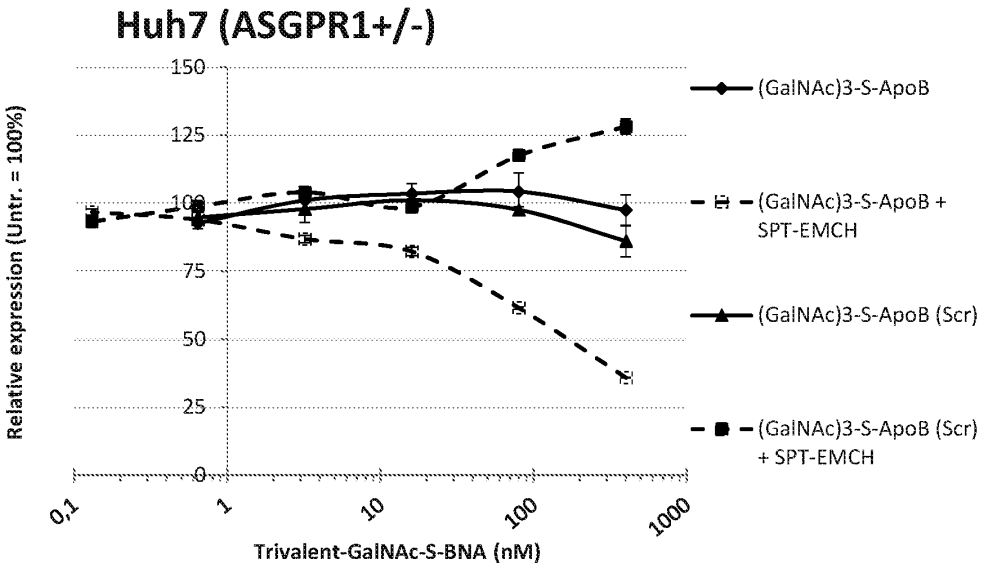
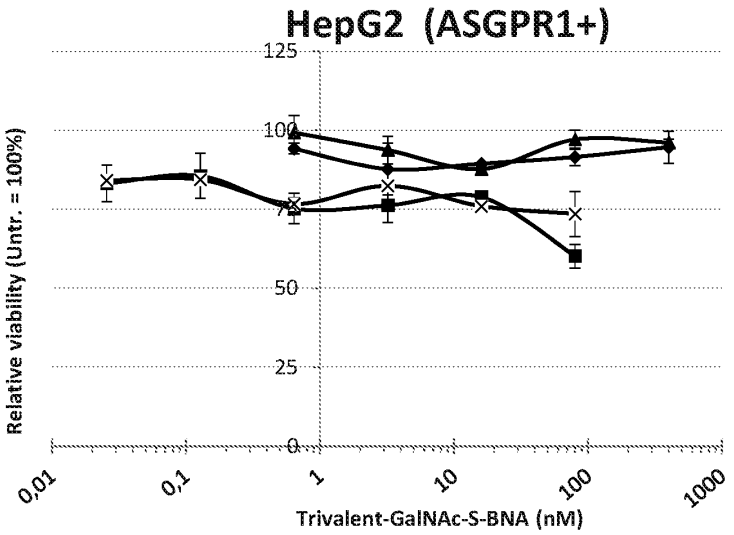

Huh7 (ASGPR1+/-)
Relative expression (Untr. = 100%)
150
125
100
75
50
25
0
0,1
1
10
100
1000
Trivalent-GalNAc-S-BNA (nM)
(GalNAc)3-S-ApoB
(GalNAc)3-S-ApoB + SPT-EMCH
(GalNAc)3-S-ApoB (Scr)
(GalNAc)3-S-ApoB (Scr) + SPT-EMCH

Fig. 15

A


HepG2 (ASGPR1+)
Relative viability (Untr. = 100%)
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Trivalent-GalNAc-S-BNA (nM)

B

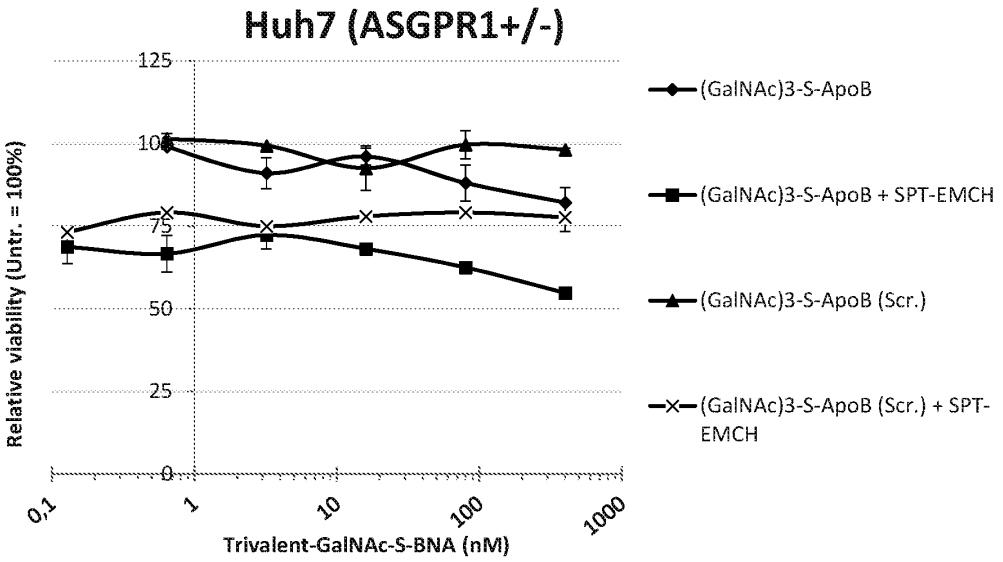
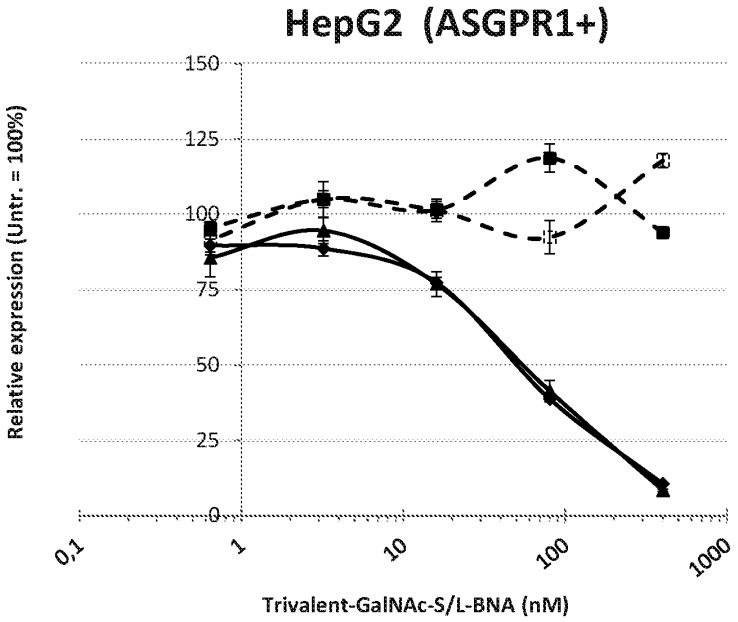

Huh7 (ASGPR1+/-)
Relative viability (Untr. = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
Trivalent-GalNAc-S-BNA (nM)
(GalNAc)3-S-ApoB
(GalNAc)3-S-ApoB + SPT-EMCH
(GalNAc)3-S-ApoB (Scr.)
(GalNAc)3-S-ApoB (Scr.) + SPT-EMCH

Fig. 16

A


HepG2 (ASGPR1+)
Relative expression (Untr. = 100%)
150
125
100
75
50
25
0
0,1
1
10
100
1000
Trivalent-GalNAc-S/L-BNA (nM)

B

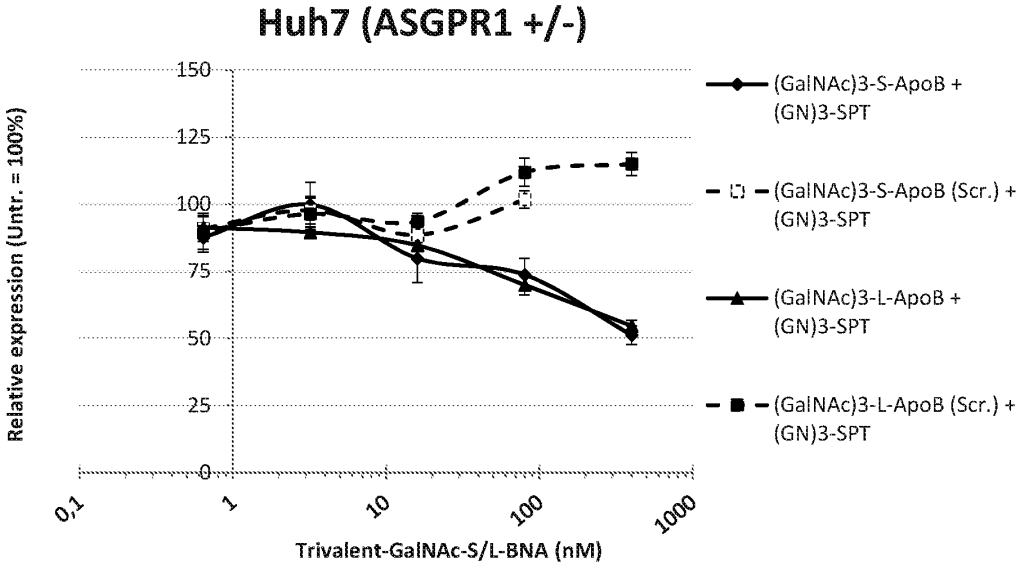

Huh7 (ASGPR1 +/-)
Relative expression (Untr. = 100%)
150
125
100
75
50
25
0
0,1
1
10
100
1000
Trivalent-GalNAc-S/L-BNA (nM)
(GalNAc)3-S-ApoB + (GN)3-SPT
(GalNAc)3-S-ApoB (Scr.) + (GN)3-SPT
(GalNAc)3-L-ApoB + (GN)3-SPT
(GalNAc)3-L-ApoB (Scr.) + (GN)3-SPT

Fig. 16
C

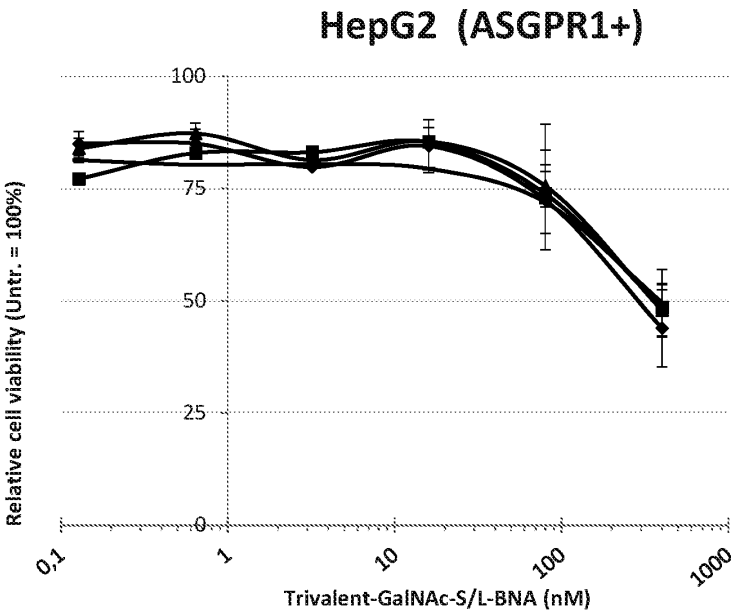


Fig. 16
D

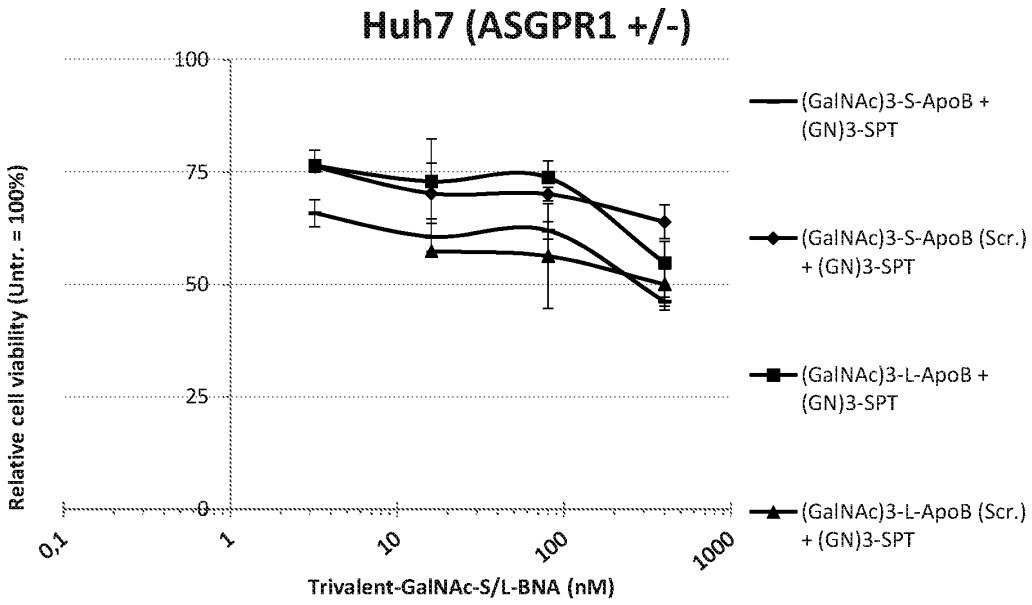
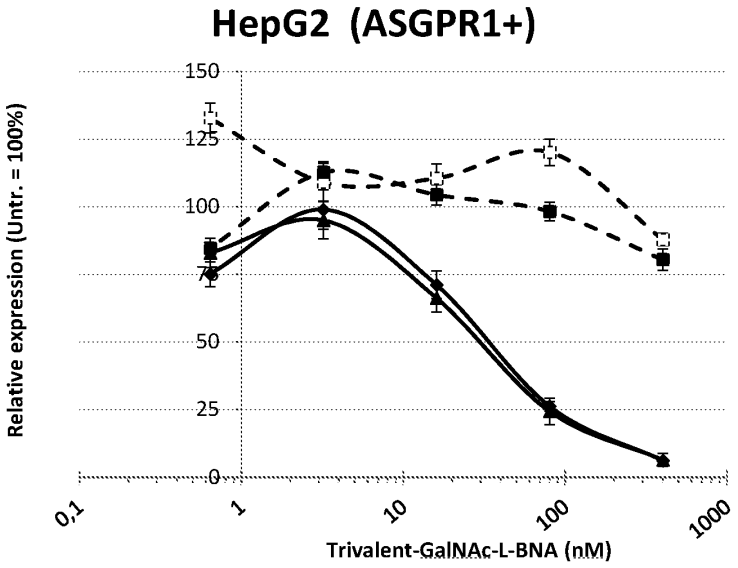


Fig. 17

A


HepG2 (ASGPR1+)
Relative expression (Untr. = 100%)
150
125
100
75
50
25
0
0,1
1
10
100
1000
Trivalent-GalNAc-L-BNA (nM)

B

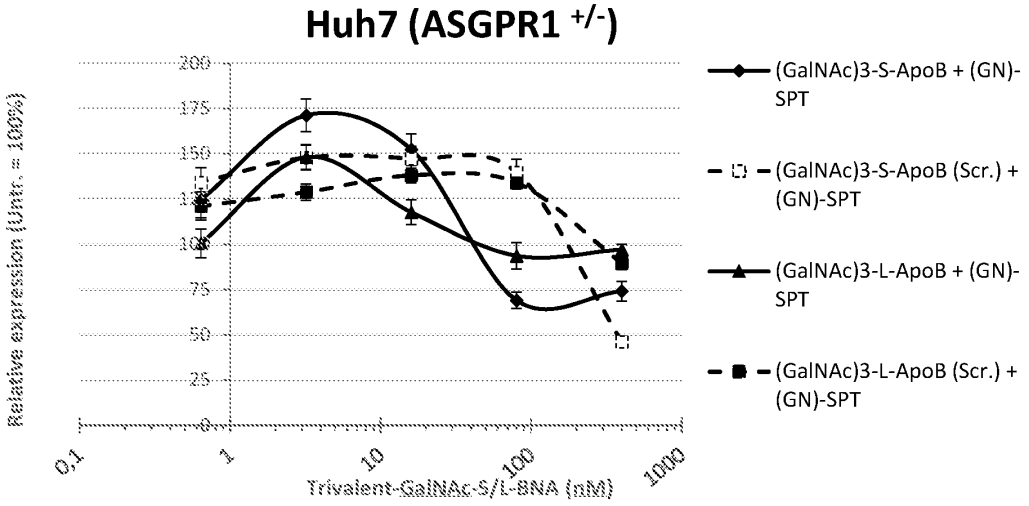
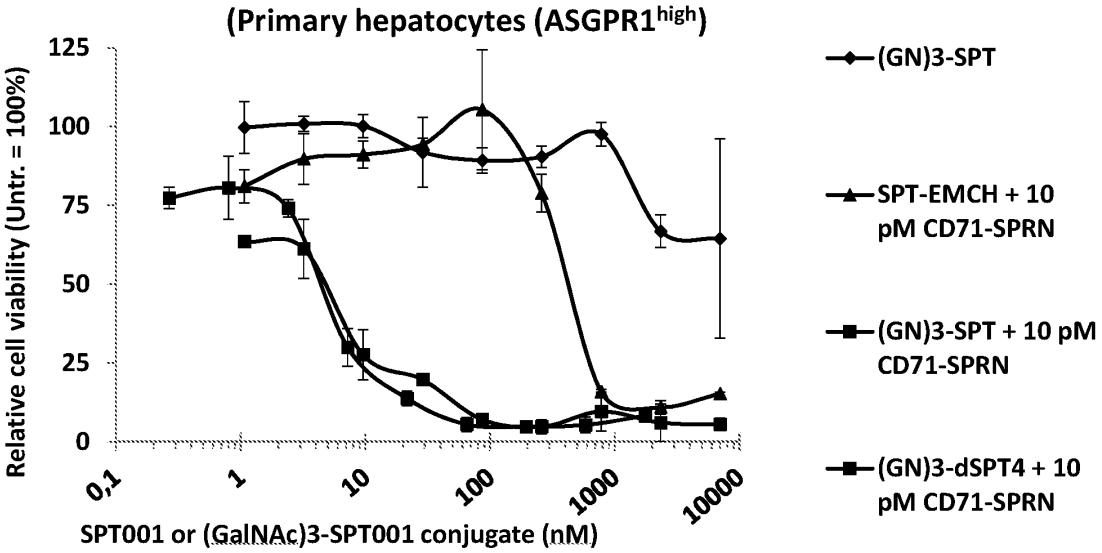

Huh7 (ASGPR1 +/-)
Relative expression (Untr. = 100%)
200
175
150
125
100
75
50
25
0
0,1
1
10
100
1000
Trivalent-GalNAc-S/L-BNA (nM)
(GalNAc)3-S-ApoB + (GN)-SPT
(GalNAc)3-S-ApoB (Scr.) + (GN)-SPT
(GalNAc)3-L-ApoB + (GN)-SPT
(GalNAc)3-L-ApoB (Scr.) + (GN)-SPT

FIG. 18

A


(Primary hepatocytes (ASGPR1high)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
10000
SPT001 or (GalNAc)3-SPT001 conjugate (nM)
(GN)3-SPT
SPT-EMCH + 10 pM CD71-SPRN
(GN)3-SPT + 10 pM CD71-SPRN
(GN)3-dSPT4 + 10 pM CD71-SPRN

B

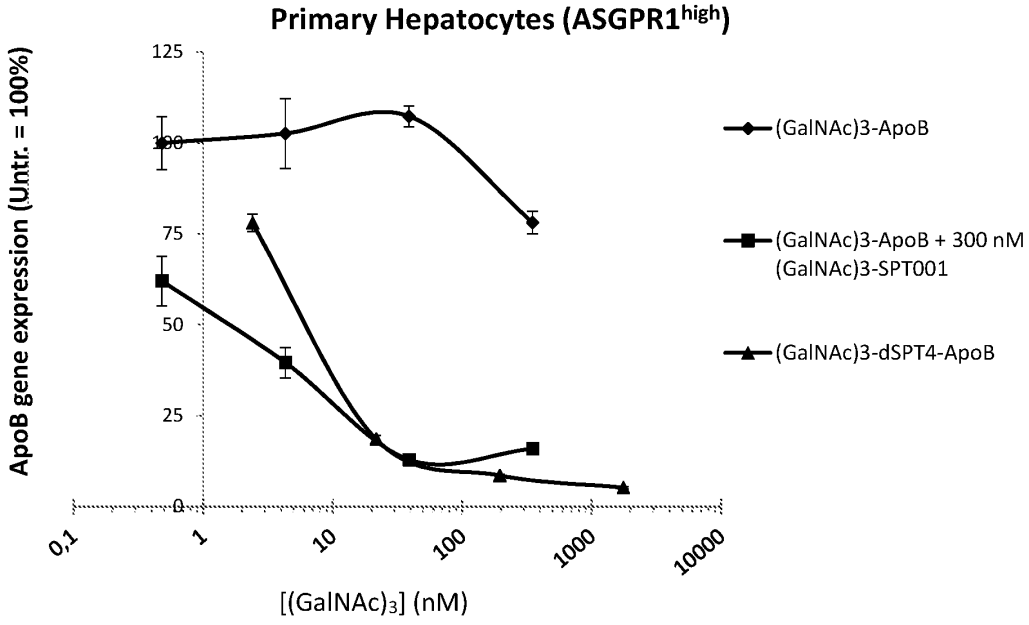
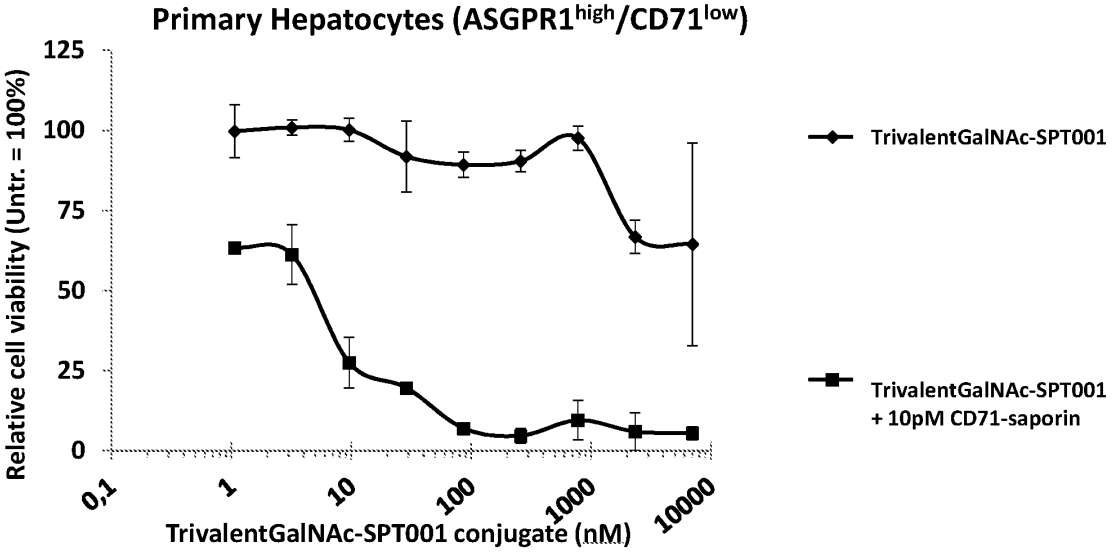

Primary Hepatocytes (ASGPR1high)
ApoB gene expression (Untr. = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
10000
[(GalNAc)3] (nM)
(GalNAc)3-ApoB
(GalNAc)3-ApoB + 300 nM (GalNAc)3-SPT001
(GalNAc)3-dSPT4-ApoB

FIG. 19

A


Primary Hepatocytes (ASGPR1high/CD71low)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
10000
TrivalentGalNAc-SPT001 conjugate (nM)
TrivalentGalNAc-SPT001
TrivalentGalNAc-SPT001
+ 10pM CD71-saporin

B

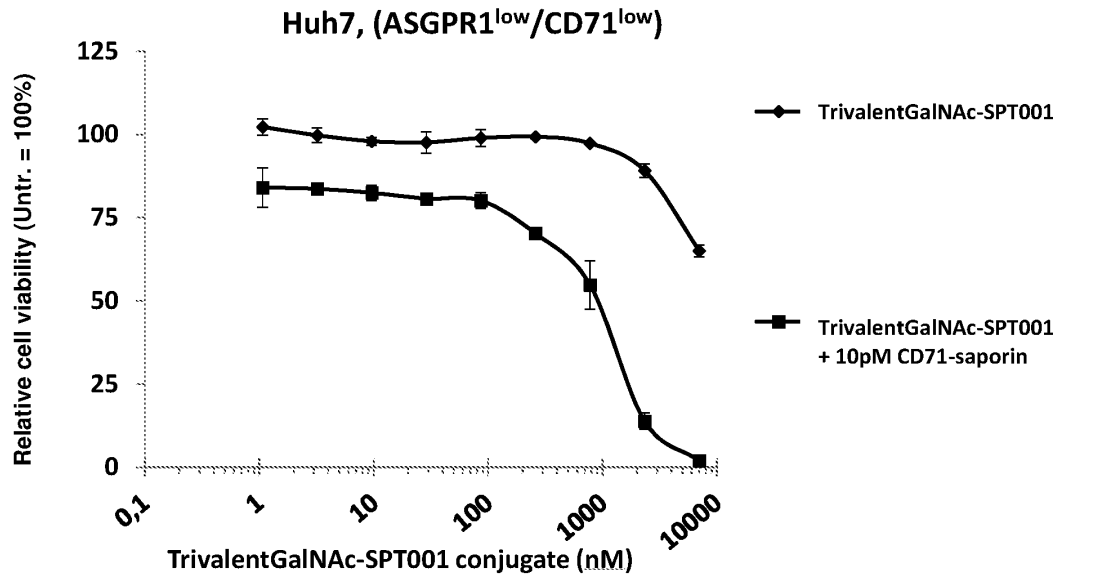
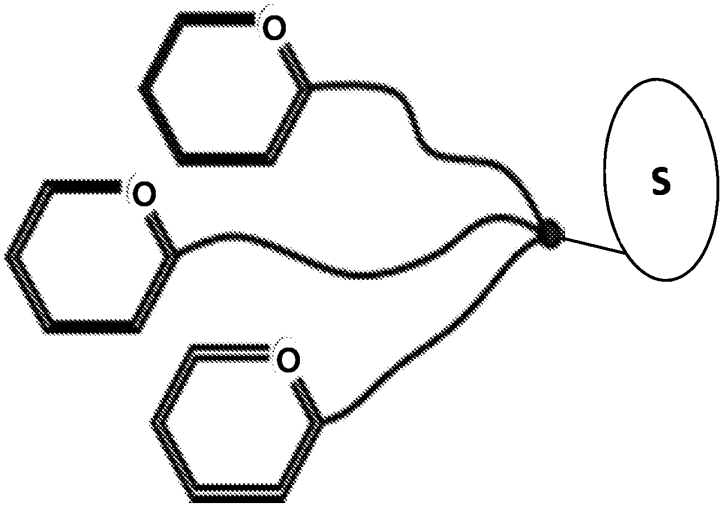

Huh7, (ASGPR1low/CD71low)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
10000
TrivalentGalNAc-SPT001 conjugate (nM)
TrivalentGalNAc-SPT001
TrivalentGalNAc-SPT001
+ 10pM CD71-saporin

FIG. 19

C

Trivalent GalNAc-SPT001 conjugate



D

Anti-CD71 antibody – saporin conjugate

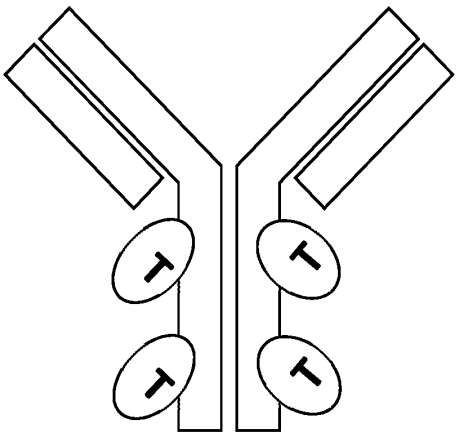


Fig. 19

E

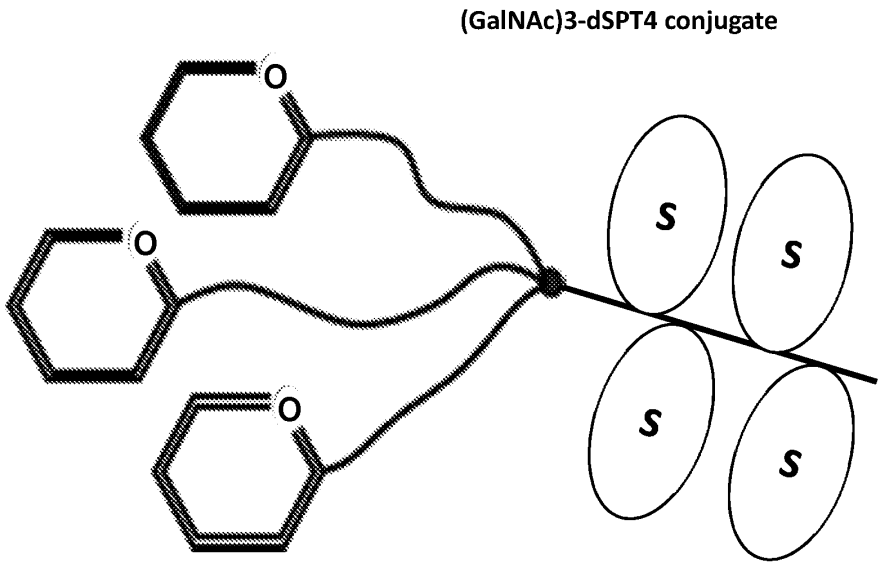
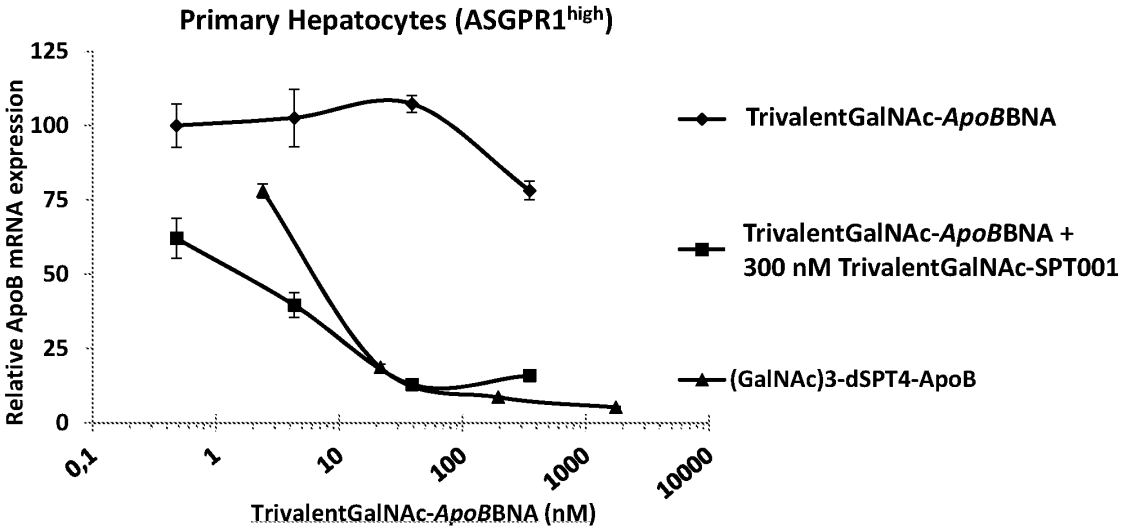


FIG. 20

A


Primary Hepatocytes (ASGPR1high)
Relative ApoB mRNA expression
125
100
75
50
25
0
0,1
1
10
100
1000
10000
TrivalentGalNAc-ApoBBNA (nM)
TrivalentGalNAc-ApoBBNA
TrivalentGalNAc-ApoBBNA + 300 nM TrivalentGalNAc-SPT001
(GalNAc)3-dSPT4-ApoB

B

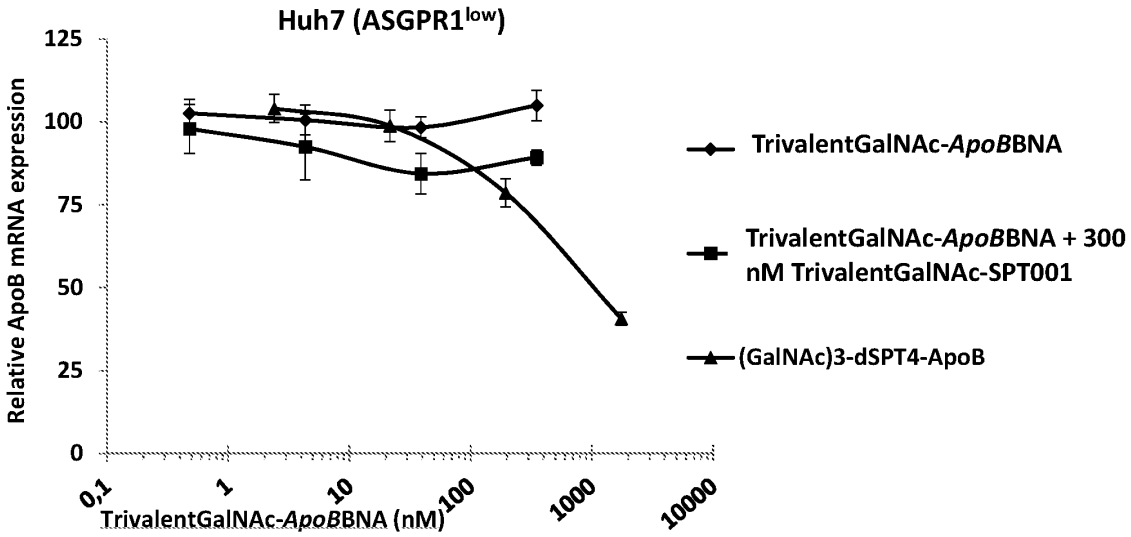
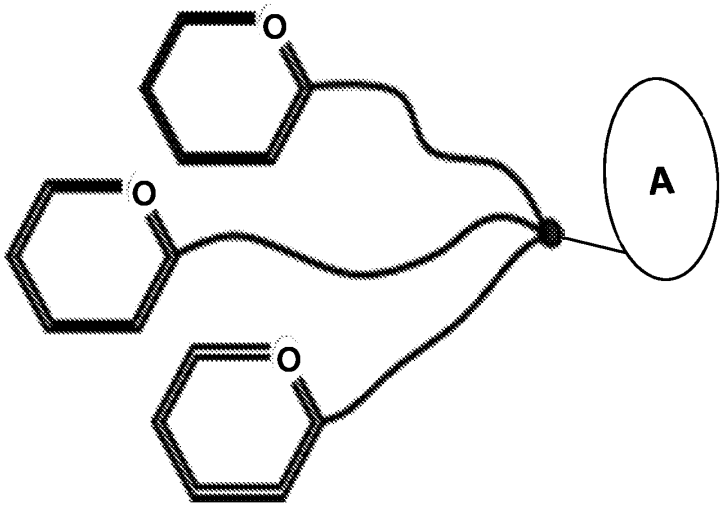

Huh7 (ASGPR1low)
Relative ApoB mRNA expression
125
100
75
50
25
0
0,1
1
10
100
1000
10000
TrivalentGalNAc-ApoBBNA (nM)
TrivalentGalNAc-ApoBBNA
TrivalentGalNAc-ApoBBNA + 300 nM TrivalentGalNAc-SPT001
(GalNAc)3-dSPT4-ApoB

FIG. 20

C

Trivalent GalNAc-*ApoB*BNA conjugate



D

Trivalent GalNAc-*ApoB*BNA-SPT001 conjugate

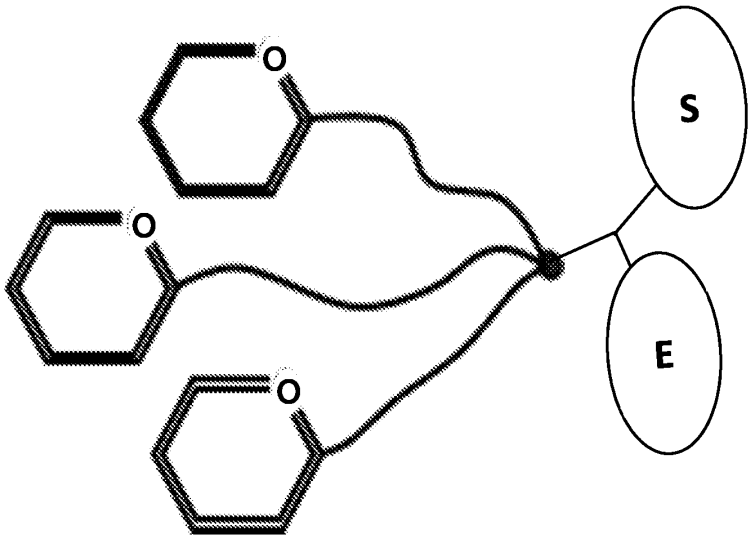


Fig. 20
E

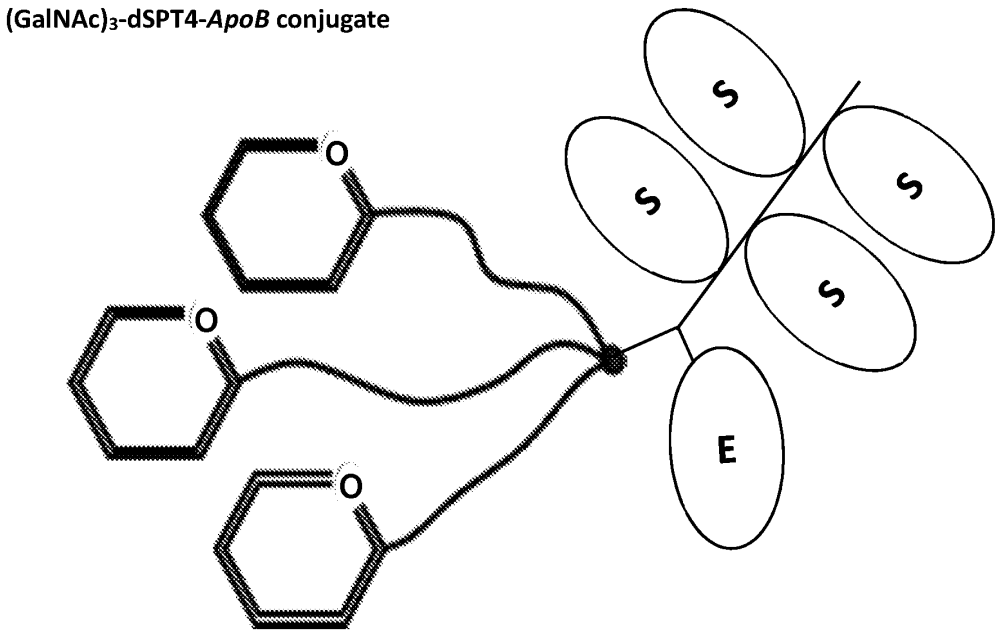
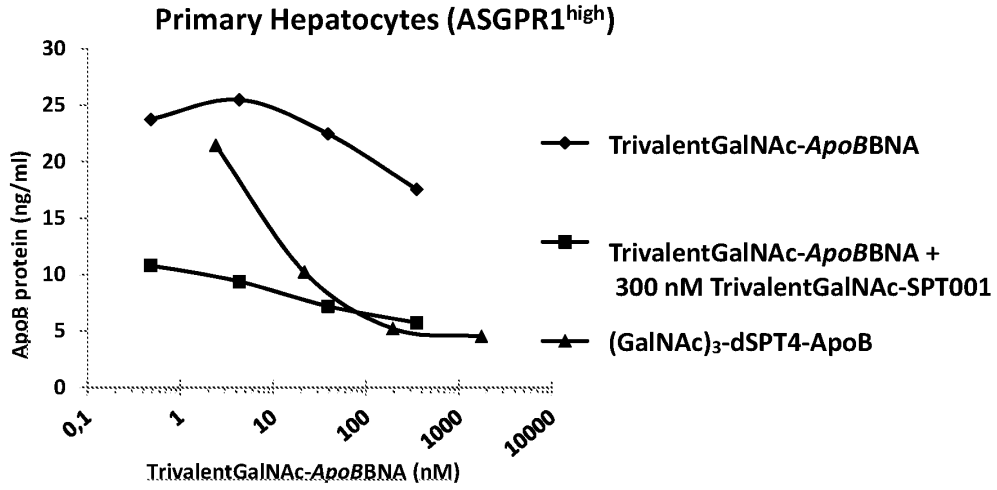


FIG. 21
A


Primary Hepatocytes (ASGPR1high)
ApoB protein (ng/ml)
30
25
20
15
10
5
0
0,1
1
10
100
1000
10000
TrivalentGalNAc-ApoBBNA (nM)
TrivalentGalNAc-ApoBBNA
TrivalentGalNAc-ApoBBNA + 300 nM TrivalentGalNAc-SPT001
(GalNAc)3-dSPT4-ApoB

B

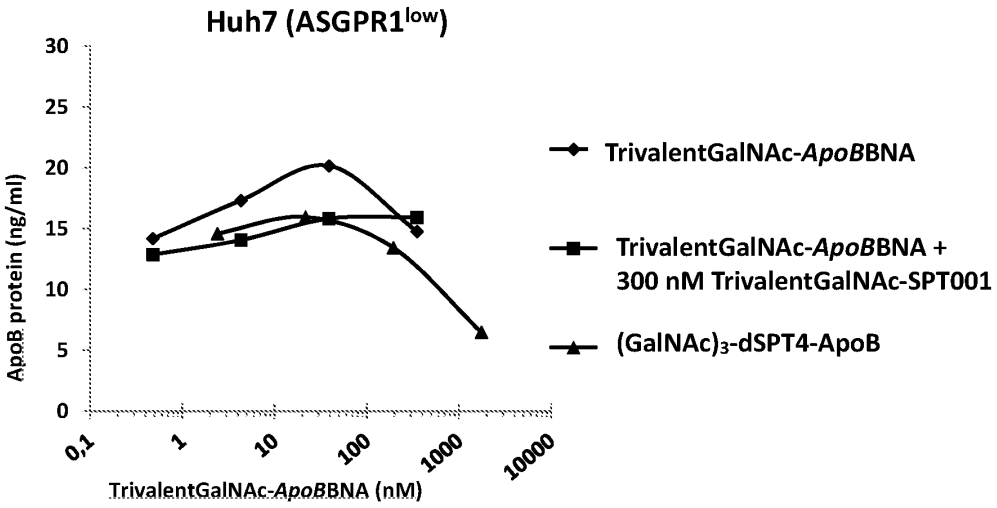

Huh7 (ASGPR1low)
ApoB protein (ng/ml)
30
25
20
15
10
5
0
0,1
1
10
100
1000
10000
TrivalentGalNAc-ApoBBNA (nM)
TrivalentGalNAc-ApoBBNA
TrivalentGalNAc-ApoBBNA + 300 nM TrivalentGalNAc-SPT001
(GalNAc)3-dSPT4-ApoB

Fig. 22
A

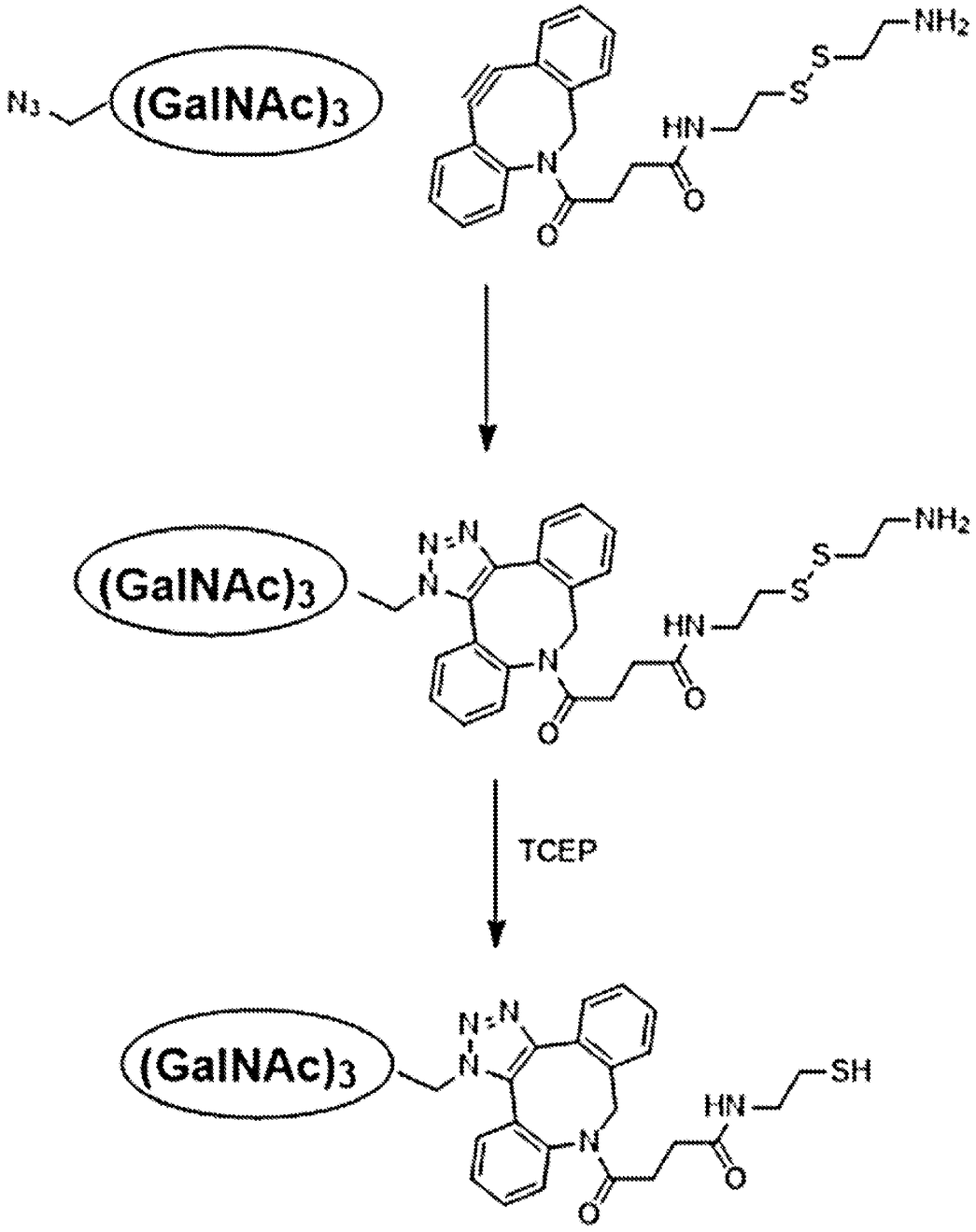

N3
(GalNAc)3
NH2
S
S
HN
N
O
O
(GalNAc)3
N=N
N
NH2
S
S
HN
N
O
O
TCEP
(GalNAc)3
N=N
N
SH
HN
N
O
O

Fig. 22
B

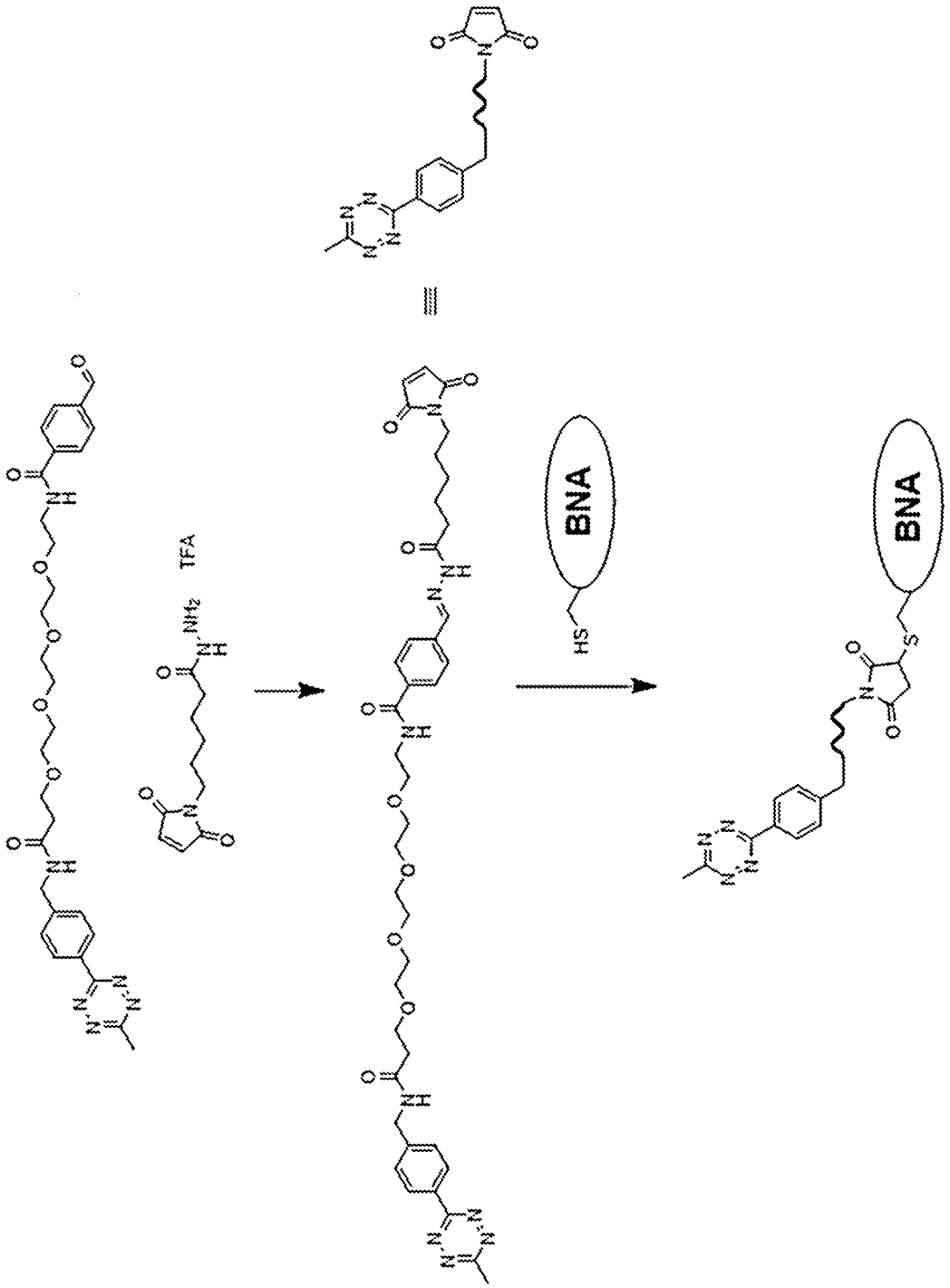


Fig. 22
C

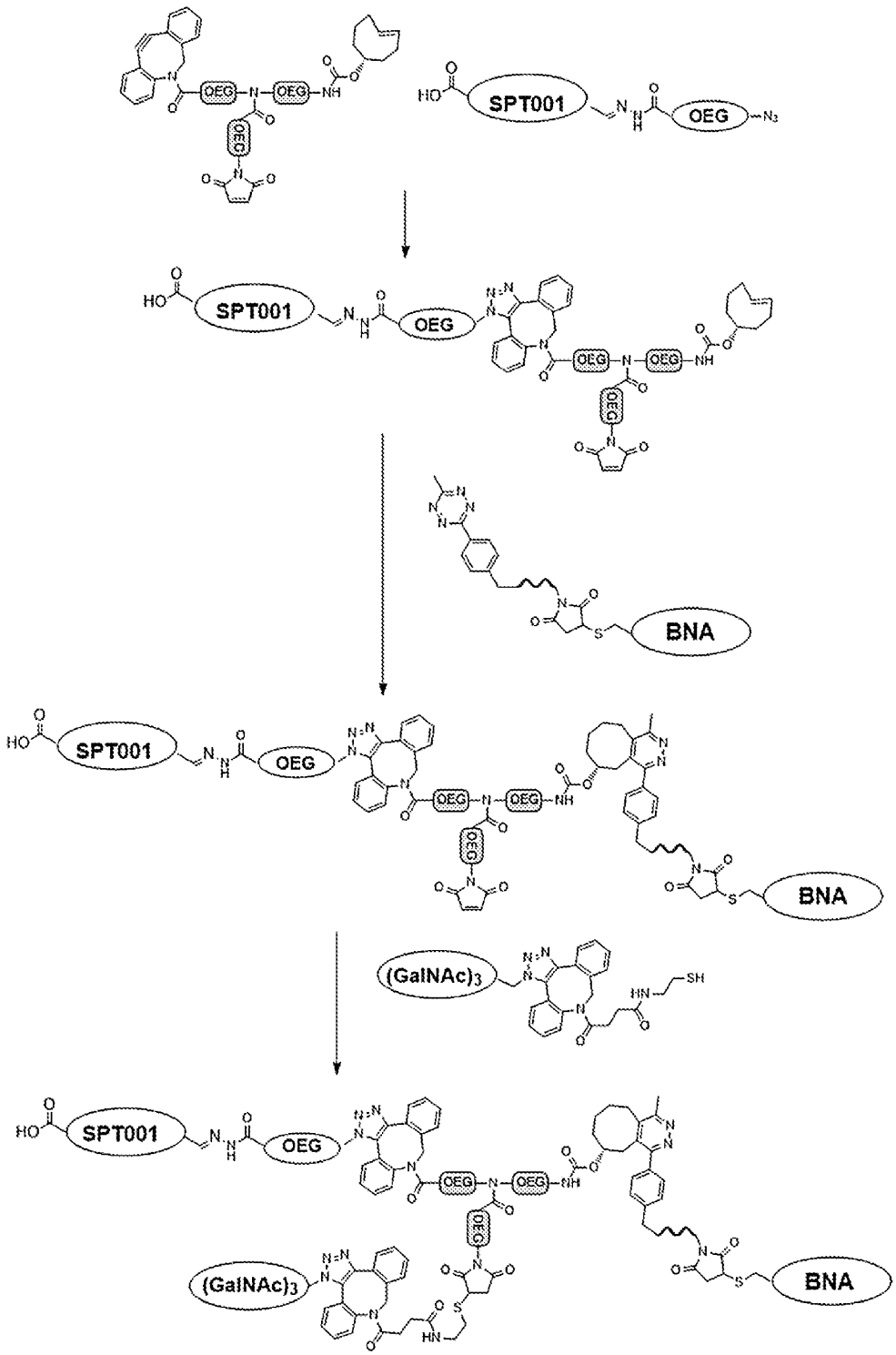


FIG. 23

A

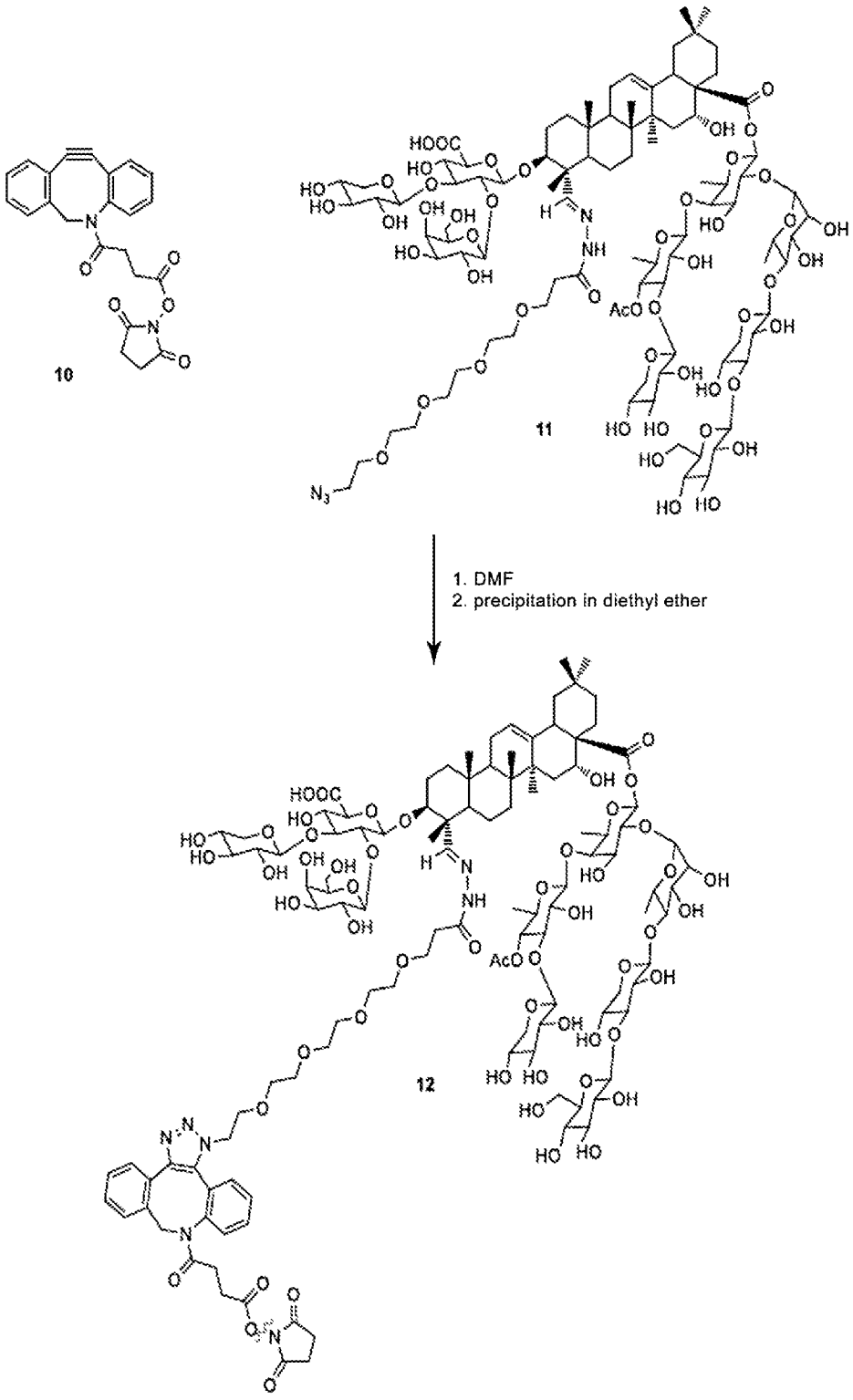


FIG. 23

B

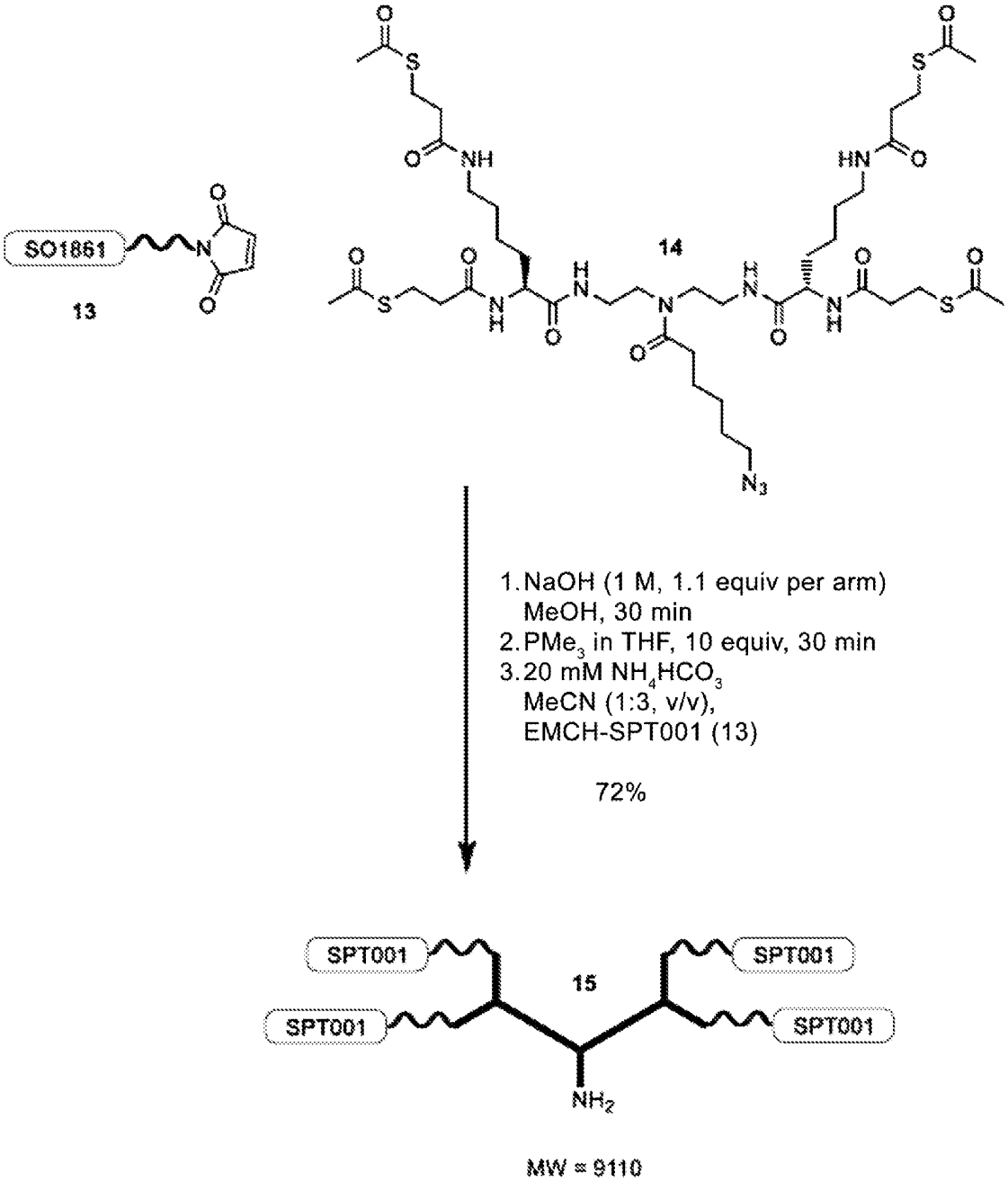

SO1861
13
14
1. NaOH (1 M, 1.1 equiv per arm)
MeOH, 30 min
2. PMe3 in THF, 10 equiv, 30 min
3. 20 mM NH4HCO3
MeCN (1:3, v/v),
EMCH-SPT001 (13)
72%
SPT001
SPT001
SPT001
SPT001
15
MW ≈ 9110

FIG. 23

C

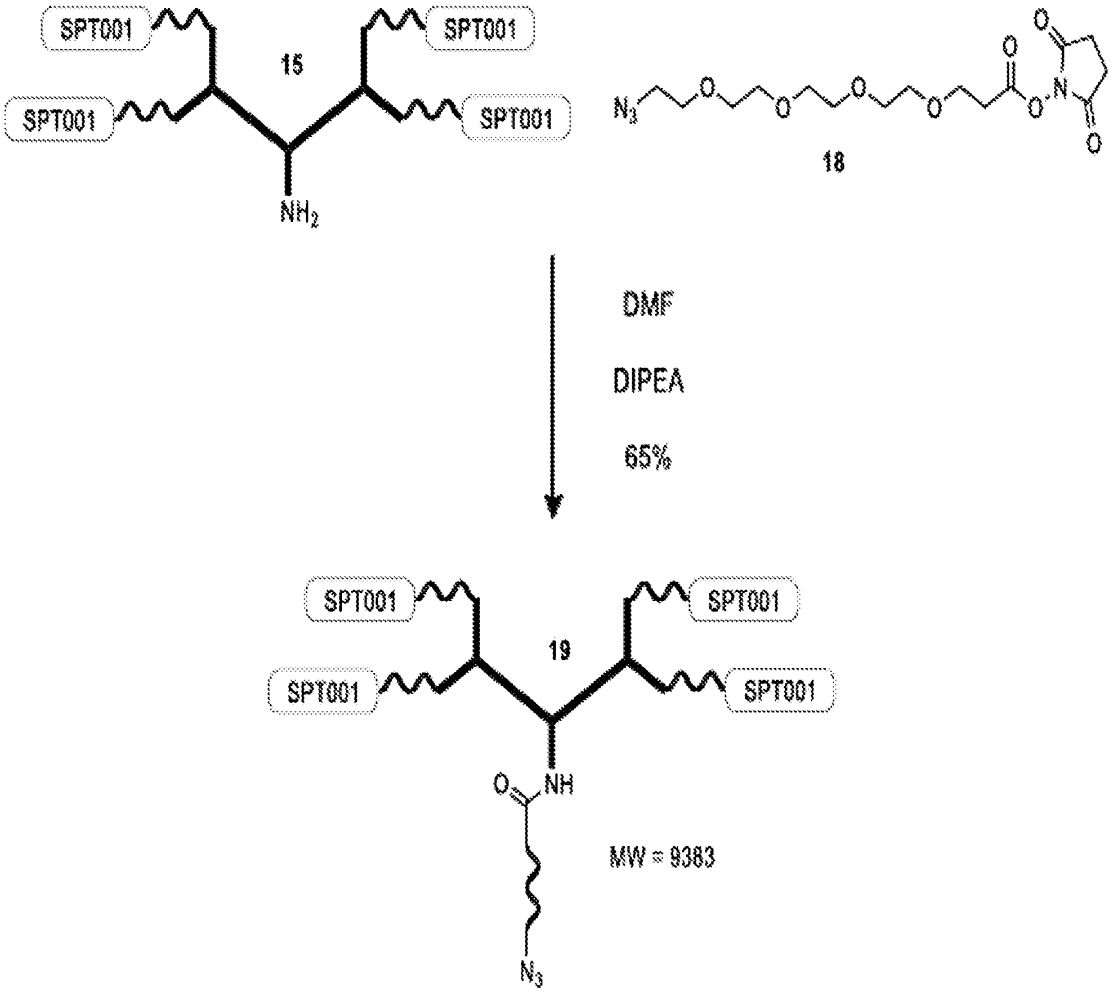

SPT001
SPT001
15
SPT001
SPT001
NH2
N3
18
DMF
DIPEA
65%
SPT001
SPT001
19
SPT001
SPT001
O
NH
MW = 9383
N3

FIG. 23

D

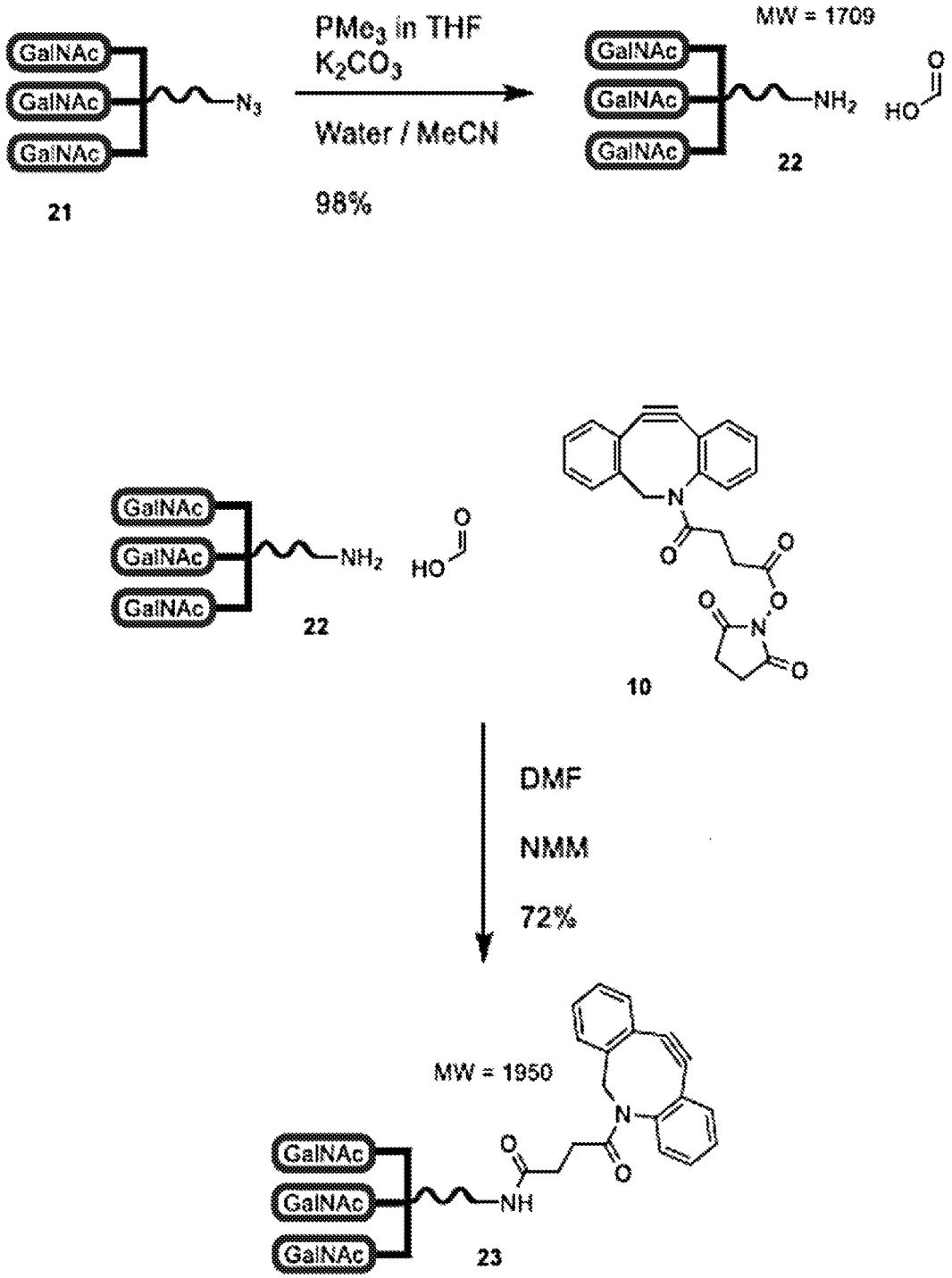

GalNAc
GalNAc
GalNAc
N3
21
PMe3 in THF
K2CO3
Water / MeCN
98%
MW = 1709
NH2
HO
O
22
10
DMF
NMM
72%
MW = 1950
NH
23

FIG. 23

E

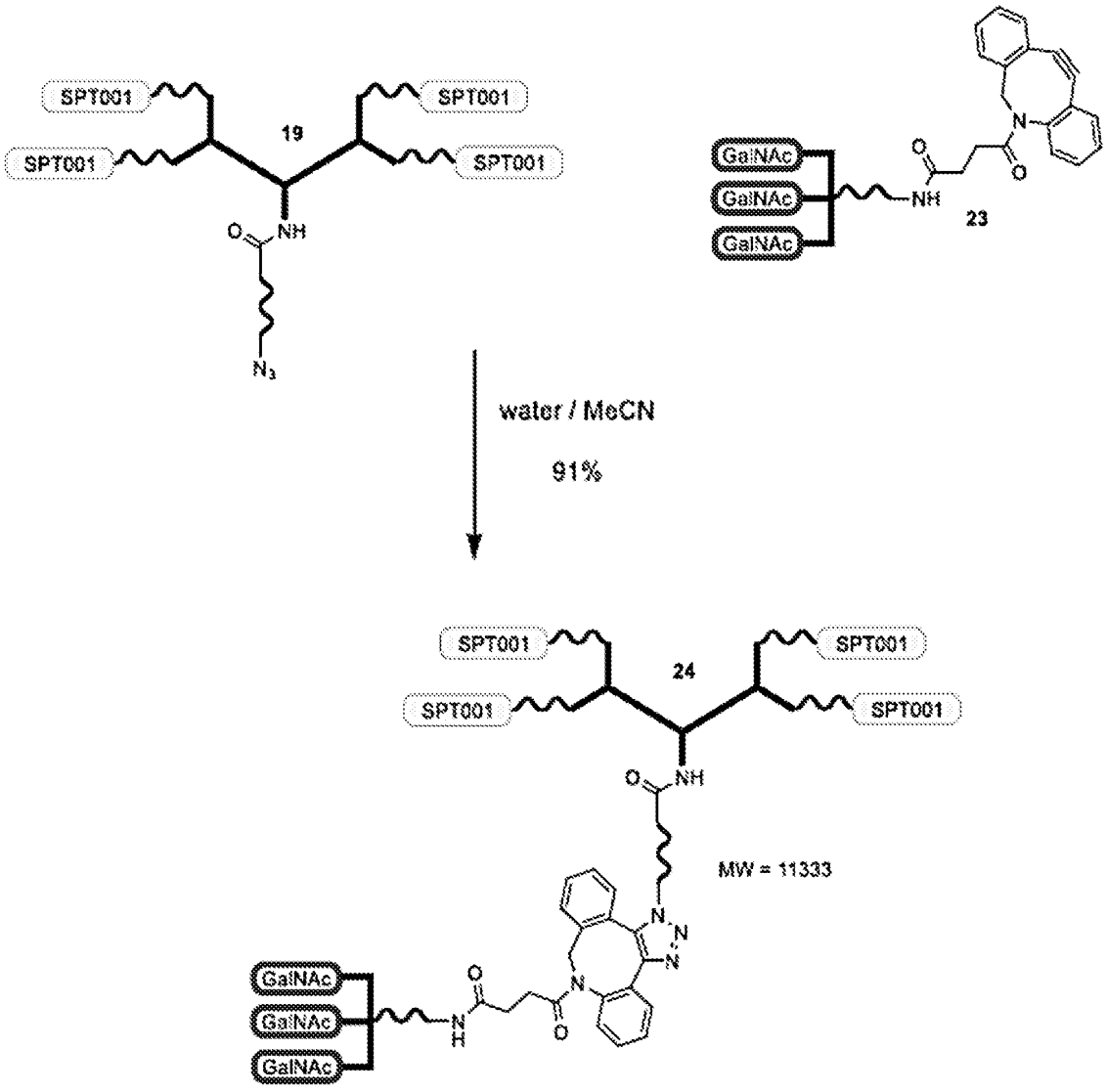

SPT001
SPT001
SPT001
SPT001
19
O
NH
N3
GalNAc
GalNAc
GalNAc
O
NH
O
N
23
water / MeCN
91%
SPT001
SPT001
SPT001
SPT001
24
O
NH
MW = 11333
N
N
N
GalNAc
GalNAc
GalNAc
O
N
H
O
N
O

FIG. 24

A

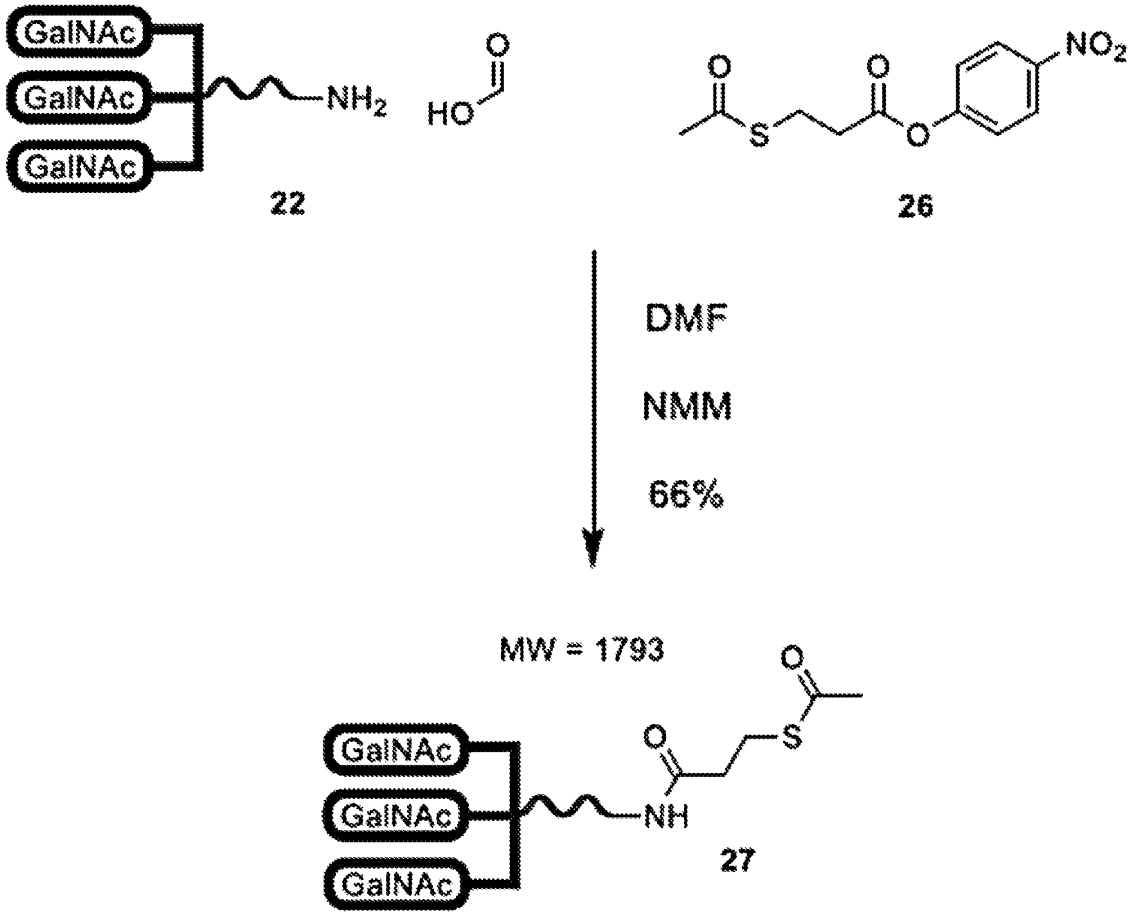

GalNAc
GalNAc
GalNAc
NH2
HO
O
22
O
S
O
O
NO2
26
DMF
NMM
66%
MW = 1793
GalNAc
GalNAc
GalNAc
O
NH
O
S
27

FIG. 24

B

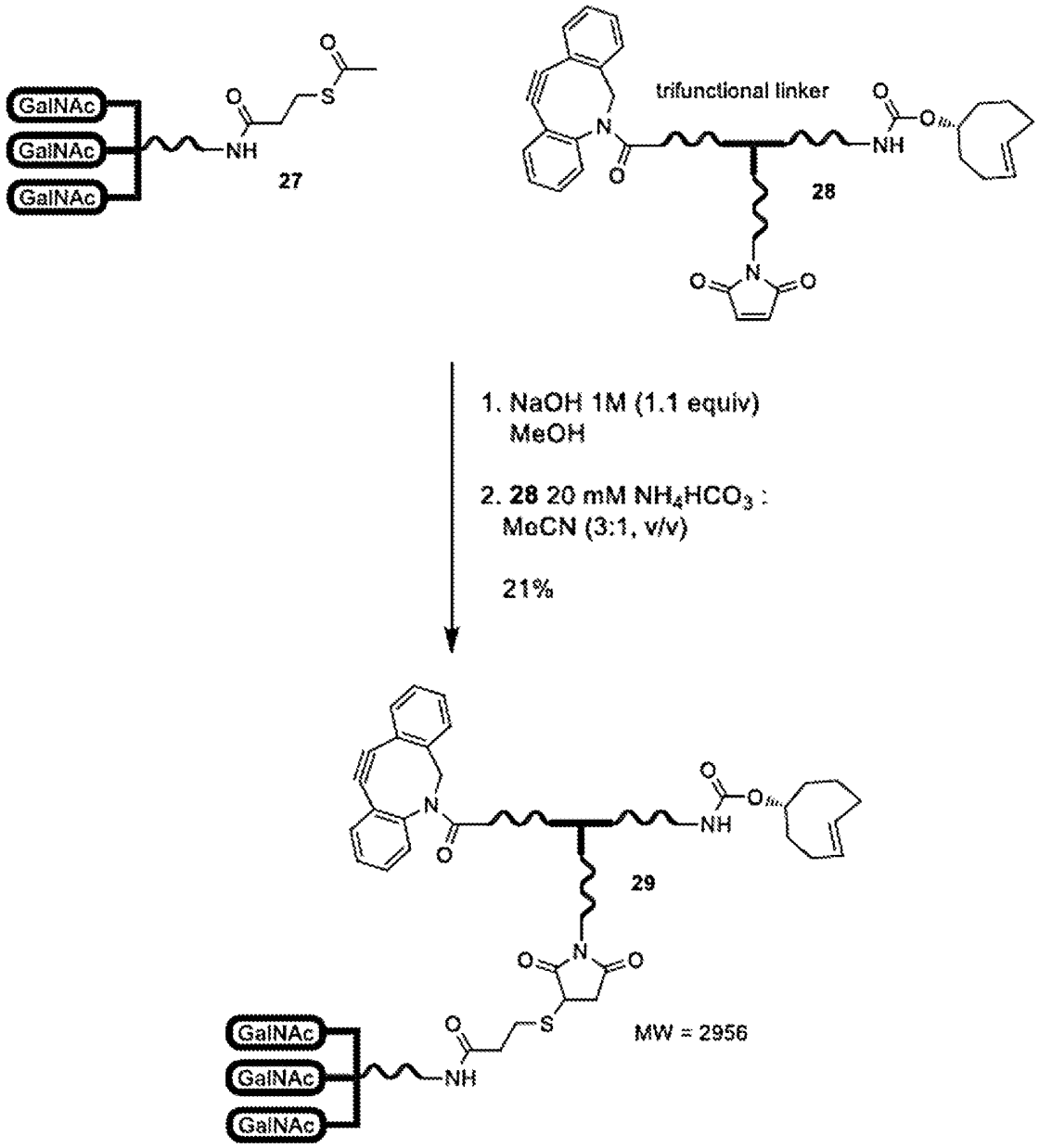

GalNAc
GalNAc
GalNAc
NH
27
trifunctional linker
NH
28
1. NaOH 1M (1.1 equiv)
MeOH
2. 28 20 mM NH4HCO3 :
MeCN (3:1, v/v)
21%
29
MW = 2956

FIG. 24
C

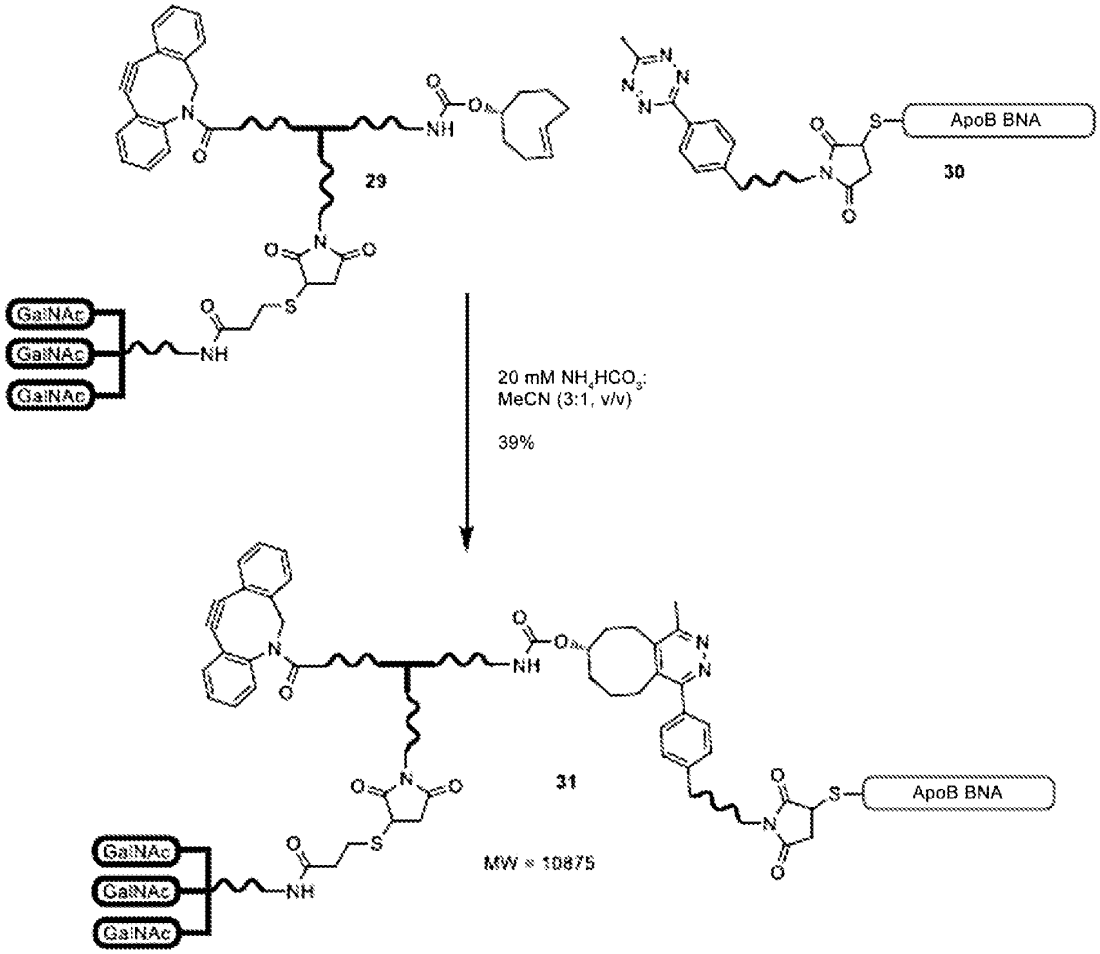

29
30
ApoB BNA
GalNAc
GalNAc
GalNAc
20 mM NH4HCO3:
MeCN (3:1, v/v)
39%
31
MW = 10875
ApoB BNA
GalNAc
GalNAc
GalNAc

FIG. 24
D

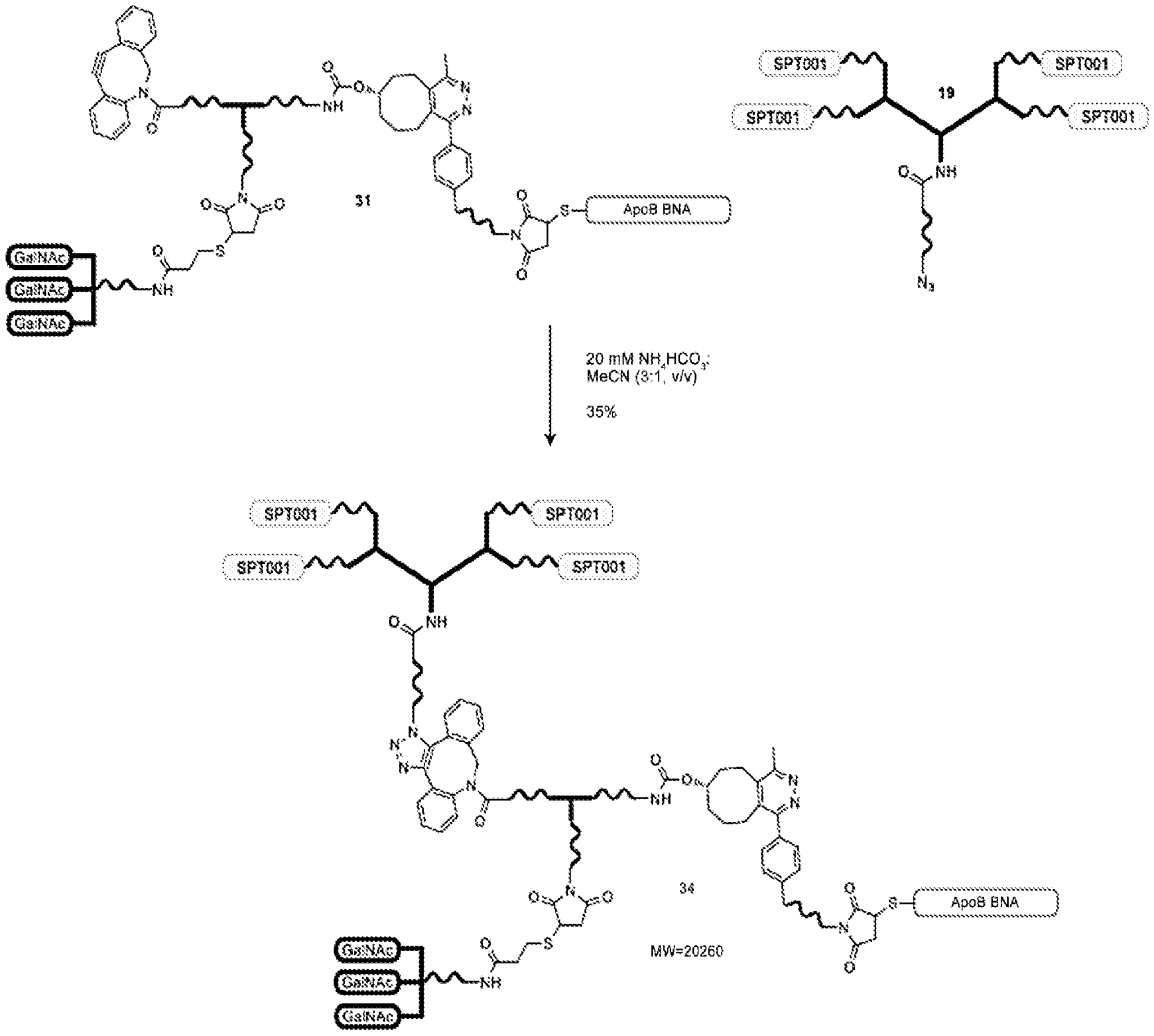

SPT001
SPT001
SPT001
SPT001
19
NH
N_3
GalNAc
GalNAc
GalNAc
NH
31
ApoB BNA
20 mM NH_4HCO_3:
MeCN (3:1, v/v)
35%
SPT001
SPT001
SPT001
SPT001
NH
34
MW=20260
ApoB BNA
GalNAc
GalNAc
GalNAc
NH

Fig. 25

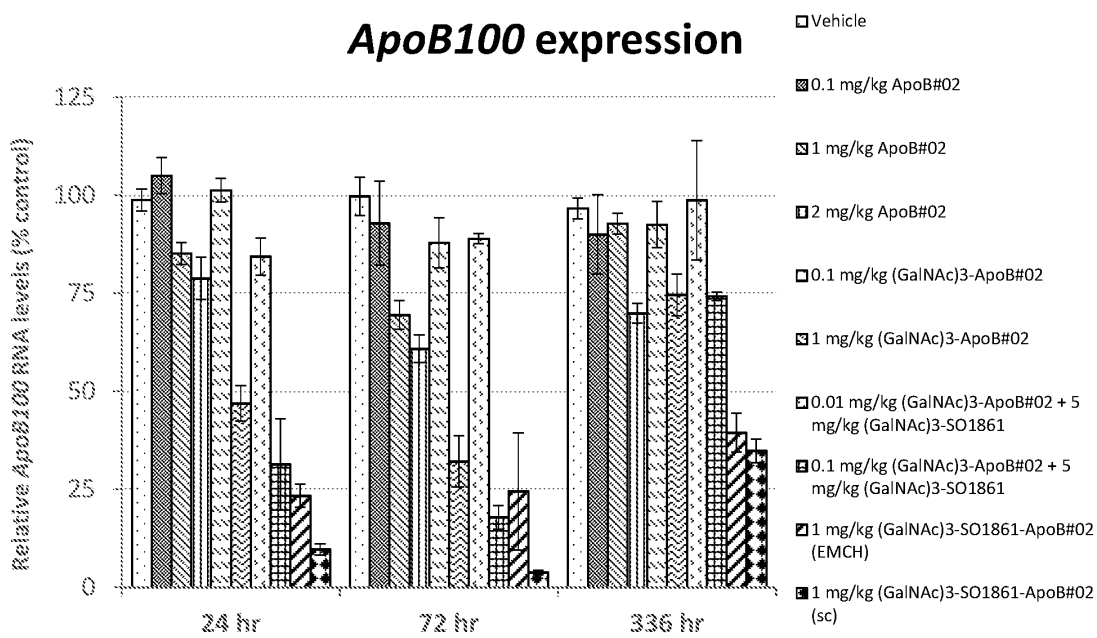

ApoB100 expression
Relative ApoB100 RNA levels (% control)
125
100
75
50
25
0
24 hr
72 hr
336 hr
Vehicle
0.1 mg/kg ApoB#02
1 mg/kg ApoB#02
2 mg/kg ApoB#02
0.1 mg/kg (GalNAc)3-ApoB#02
1 mg/kg (GalNAc)3-ApoB#02
0.01 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
0.1 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (EMCH)
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)

Fig. 26

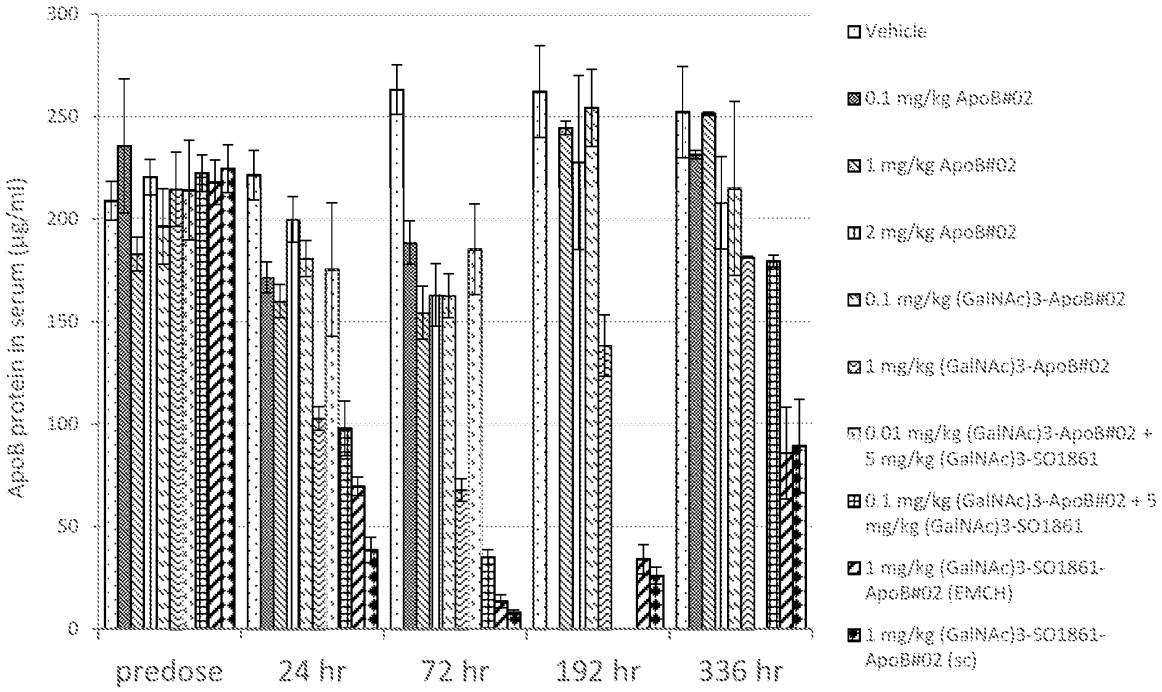

ApoB protein in serum (µg/ml)
300
250
200
150
100
50
0
predose
24 hr
72 hr
192 hr
336 hr
Vehicle
0.1 mg/kg ApoB#02
1 mg/kg ApoB#02
2 mg/kg ApoB#02
0.1 mg/kg (GalNAc)3-ApoB#02
1 mg/kg (GalNAc)3-ApoB#02
0.01 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
0.1 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (EMCH)
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)

Fig. 27

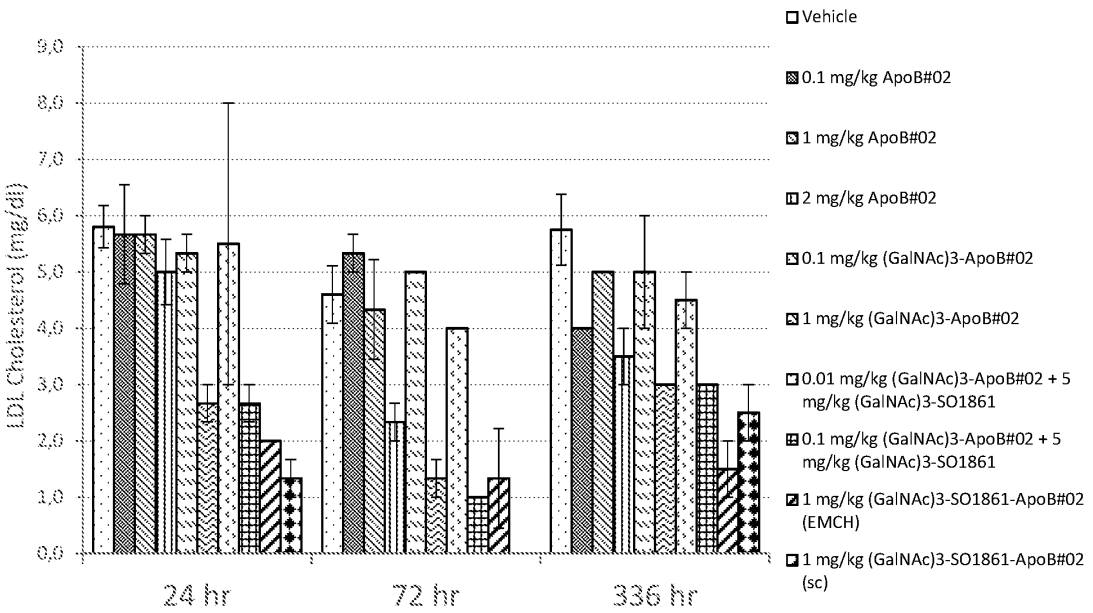

LDL Cholesterol (mg/dl)
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
24 hr
72 hr
336 hr
Vehicle
0.1 mg/kg ApoB#02
1 mg/kg ApoB#02
2 mg/kg ApoB#02
0.1 mg/kg (GalNAc)3-ApoB#02
1 mg/kg (GalNAc)3-ApoB#02
0.01 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
0.1 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (EMCH)
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)

Fig. 28

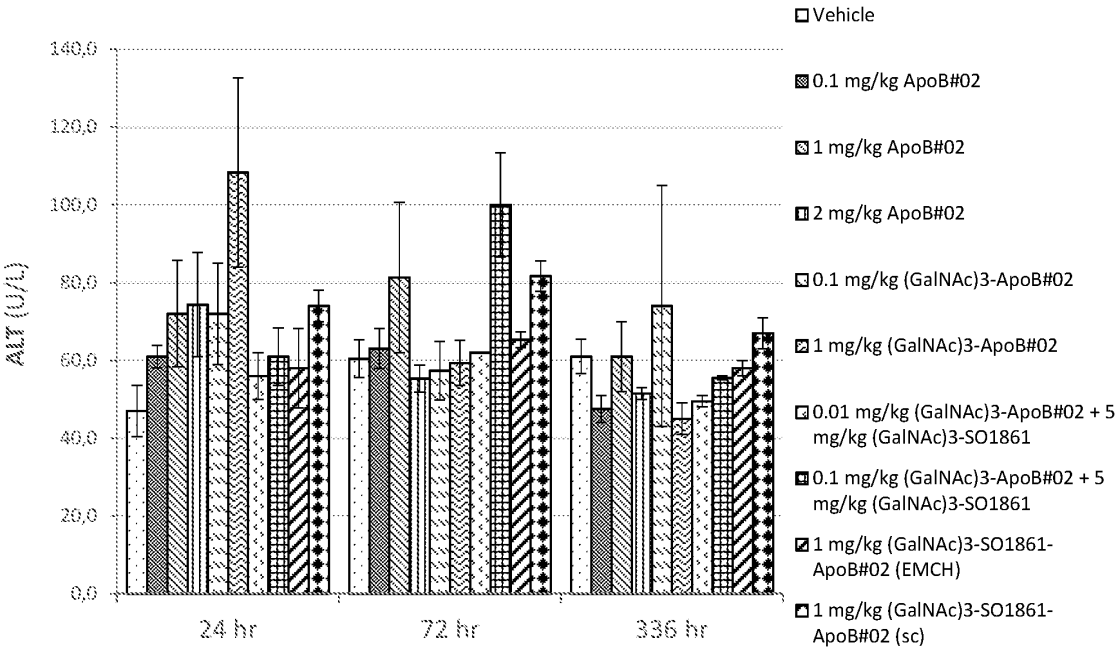

ALT (U/L)
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
24 hr
72 hr
336 hr
Vehicle
0.1 mg/kg ApoB#02
1 mg/kg ApoB#02
2 mg/kg ApoB#02
0.1 mg/kg (GalNAc)3-ApoB#02
1 mg/kg (GalNAc)3-ApoB#02
0.01 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
0.1 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (EMCH)
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)

Fig. 29

A

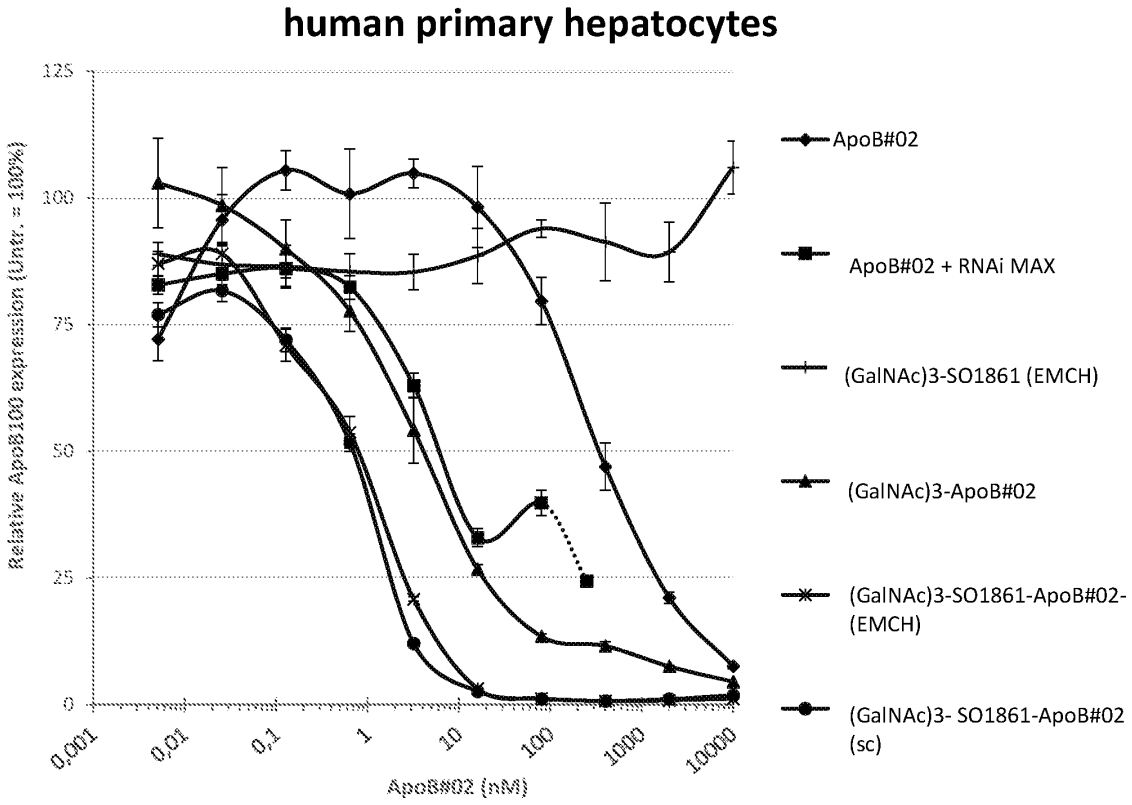

human primary hepatocytes
Relative ApoB100 expression (Untr. = 100%)
125
100
75
50
25
0
0,001
0,01
0,1
1
10
100
1000
10000
ApoB#02 (nM)
ApoB#02
ApoB#02 + RNAi MAX
(GalNAc)3-SO1861 (EMCH)
(GalNAc)3-ApoB#02
(GalNAc)3-SO1861-ApoB#02-(EMCH)
(GalNAc)3- SO1861-ApoB#02 (sc)

Fig. 29

B

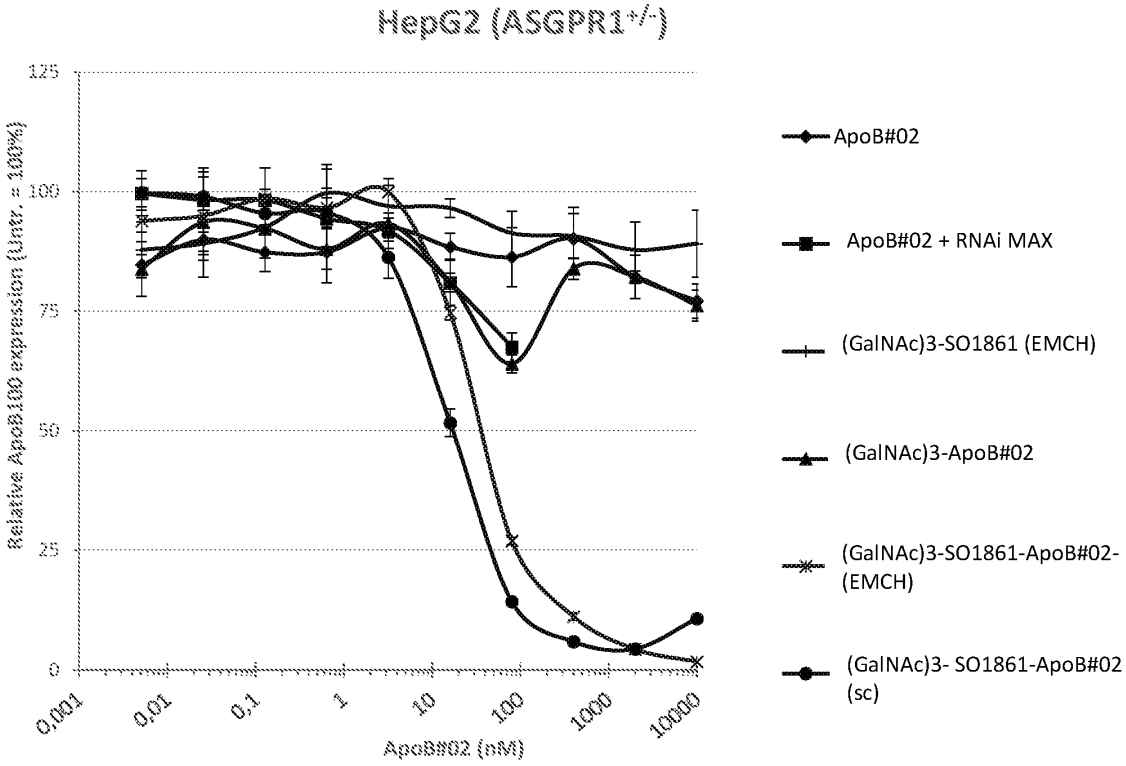

HepG2 (ASGPR1+/-)
Relative ApoB100 expression (Untr. = 100%)
125
100
75
50
25
0
0,001
0,01
0,1
1
10
100
1000
10000
ApoB#02 (nM)
ApoB#02
ApoB#02 + RNAi MAX
(GalNAc)3-SO1861 (EMCH)
(GalNAc)3-ApoB#02
(GalNAc)3-SO1861-ApoB#02-(EMCH)
(GalNAc)3- SO1861-ApoB#02 (sc)

Fig. 29

C

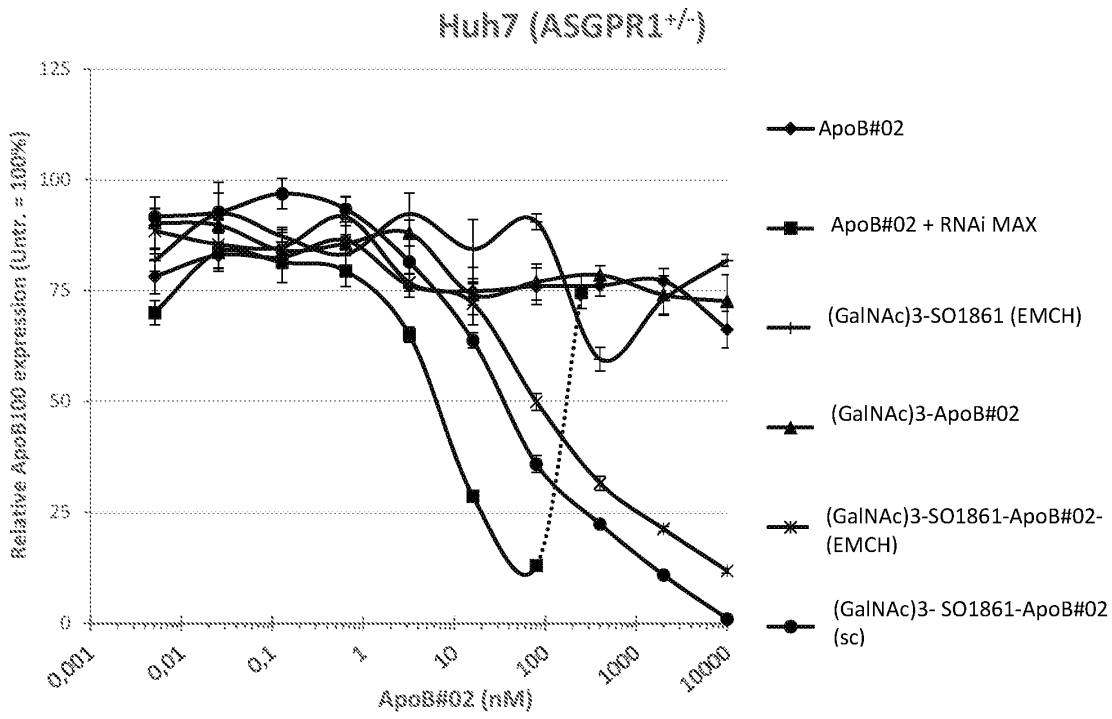

Huh7 (ASGPR1+/-)
Relative ApoB100 expression (Untr. = 100%)
125
100
75
50
25
0
0,001
0,01
0,1
1
10
100
1000
10000
ApoB#02 (nM)
ApoB#02
ApoB#02 + RNAi MAX
(GalNAc)3-SO1861 (EMCH)
(GalNAc)3-ApoB#02
(GalNAc)3-SO1861-ApoB#02-(EMCH)
(GalNAc)3- SO1861-ApoB#02 (sc)

Fig. 30

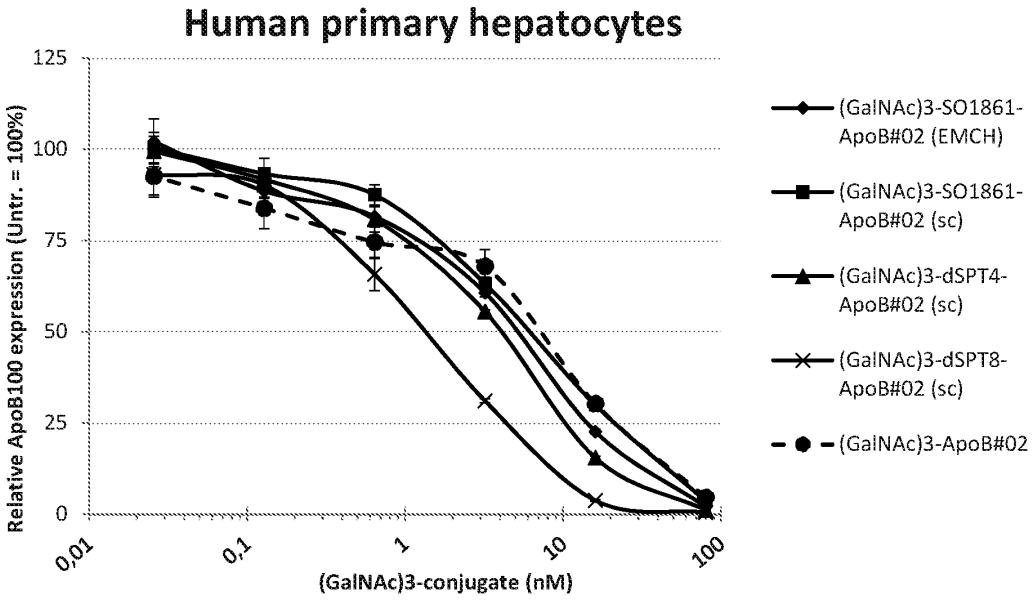

Human primary hepatocytes
Relative ApoB100 expression (Untr. = 100%)
125
100
75
50
25
0
0,01
0,1
1
10
100
(GalNAc)3-conjugate (nM)
(GalNAc)3-SO1861-ApoB#02 (EMCH)
(GalNAc)3-SO1861-ApoB#02 (sc)
(GalNAc)3-dSPT4-ApoB#02 (sc)
(GalNAc)3-dSPT8-ApoB#02 (sc)
(GalNAc)3-ApoB#02

Fig. 31

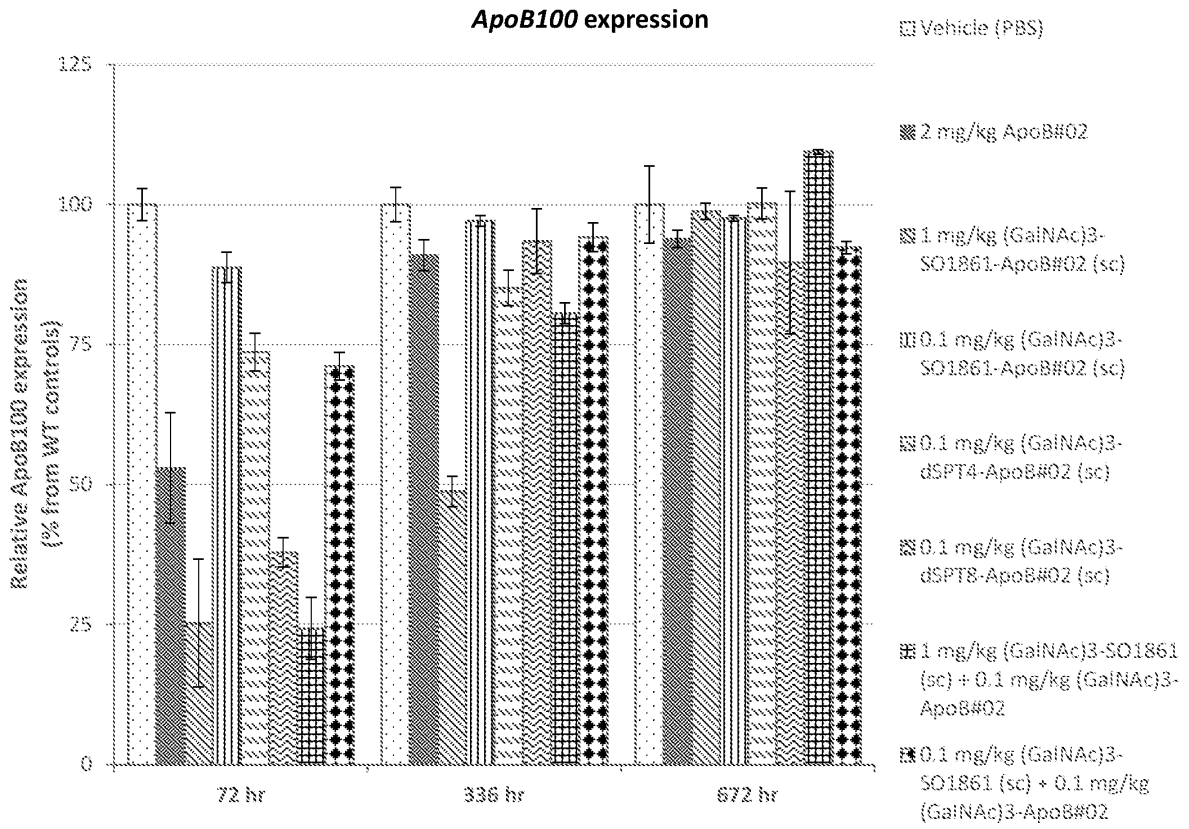

ApoB100 expression
Relative ApoB100 expression (% from WT controls)
125
100
75
50
25
0
72 hr
336 hr
672 hr
Vehicle (PBS)
2 mg/kg ApoB#02
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-dSPT4-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-dSPT8-ApoB#02 (sc)
1 mg/kg (GalNAc)3-SO1861 (sc) + 0.1 mg/kg (GalNAc)3-ApoB#02
0.1 mg/kg (GalNAc)3-SO1861 (sc) + 0.1 mg/kg (GalNAc)3-ApoB#02

Fig. 32

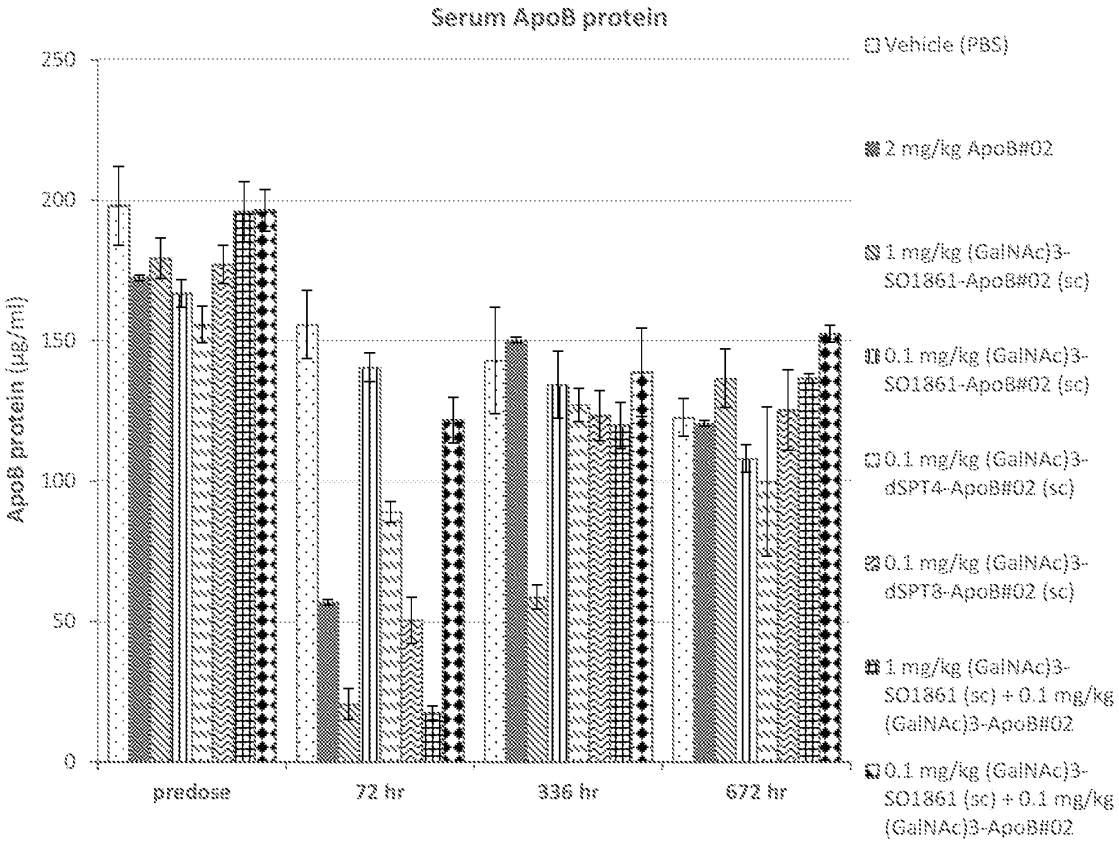

Serum ApoB protein
ApoB protein (µg/ml)
250
200
150
100
50
0
predose
72 hr
336 hr
672 hr
Vehicle (PBS)
2 mg/kg ApoB#02
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-dSPT4-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-dSPT8-ApoB#02 (sc)
1 mg/kg (GalNAc)3-SO1861 (sc) + 0.1 mg/kg (GalNAc)3-ApoB#02
0.1 mg/kg (GalNAc)3-SO1861 (sc) + 0.1 mg/kg (GalNAc)3-ApoB#02

Fig. 33

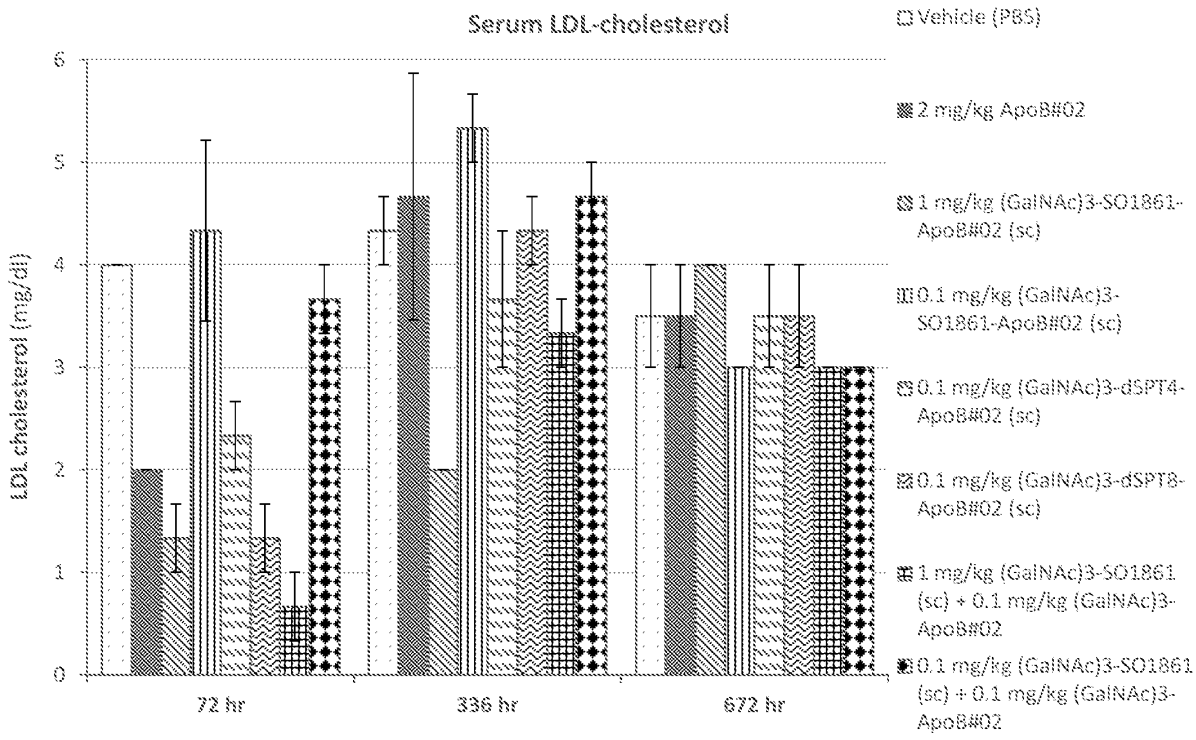

Serum LDL-cholesterol
LDL cholesterol (mg/dl)
0
1
2
3
4
5
6
72 hr
336 hr
672 hr
Vehicle (PBS)
2 mg/kg ApoB#02
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-dSPT4-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-dSPT8-ApoB#02 (sc)
1 mg/kg (GalNAc)3-SO1861 (sc) + 0.1 mg/kg (GalNAc)3-ApoB#02
0.1 mg/kg (GalNAc)3-SO1861 (sc) + 0.1 mg/kg (GalNAc)3-ApoB#02

Fig. 34

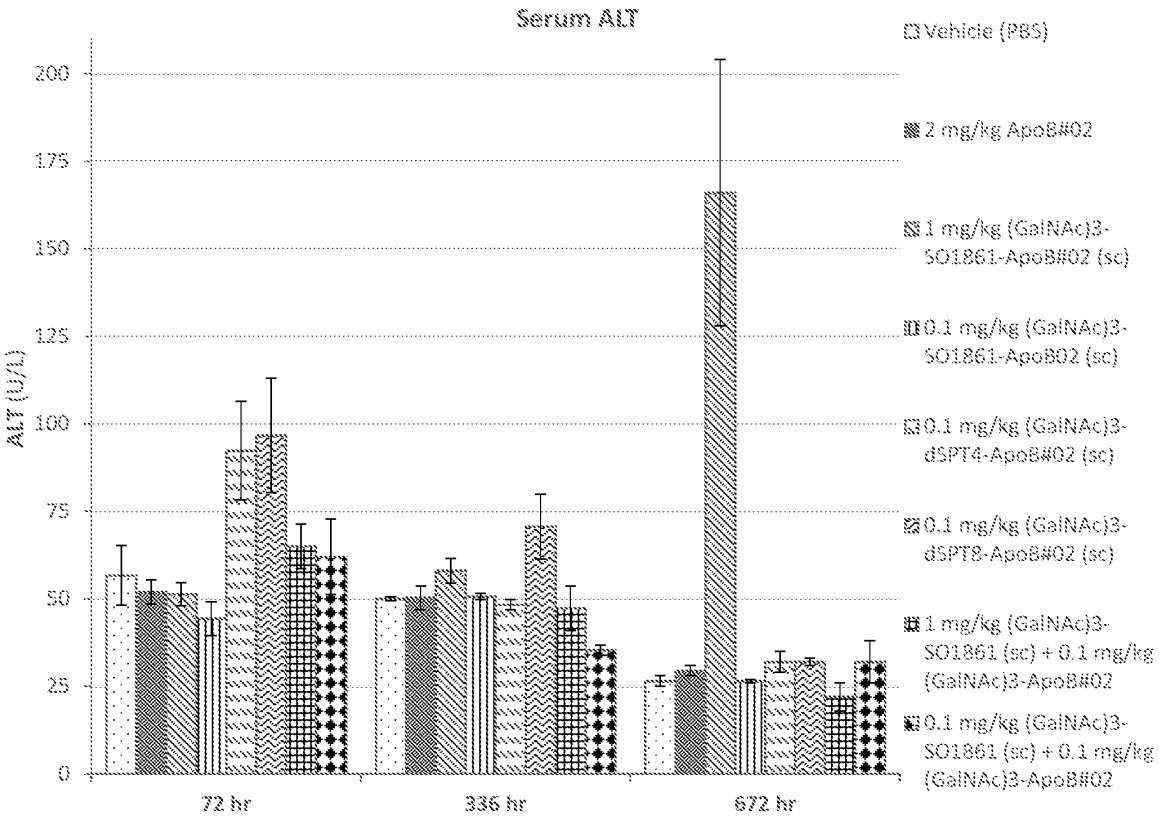

Serum ALT
ALT (U/L)
200
175
150
125
100
75
50
25
0
72 hr
336 hr
672 hr
Vehicle (PBS)
2 mg/kg ApoB#02
1 mg/kg (GalNAc)3-SO1861-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-SO1861-ApoB02 (sc)
0.1 mg/kg (GalNAc)3-dSPT4-ApoB#02 (sc)
0.1 mg/kg (GalNAc)3-dSPT8-ApoB#02 (sc)
1 mg/kg (GalNAc)3-SO1861 (sc) + 0.1 mg/kg (GalNAc)3-ApoB#02
0.1 mg/kg (GalNAc)3-SO1861 (sc) + 0.1 mg/kg (GalNAc)3-ApoB#02

Fig. 35
A

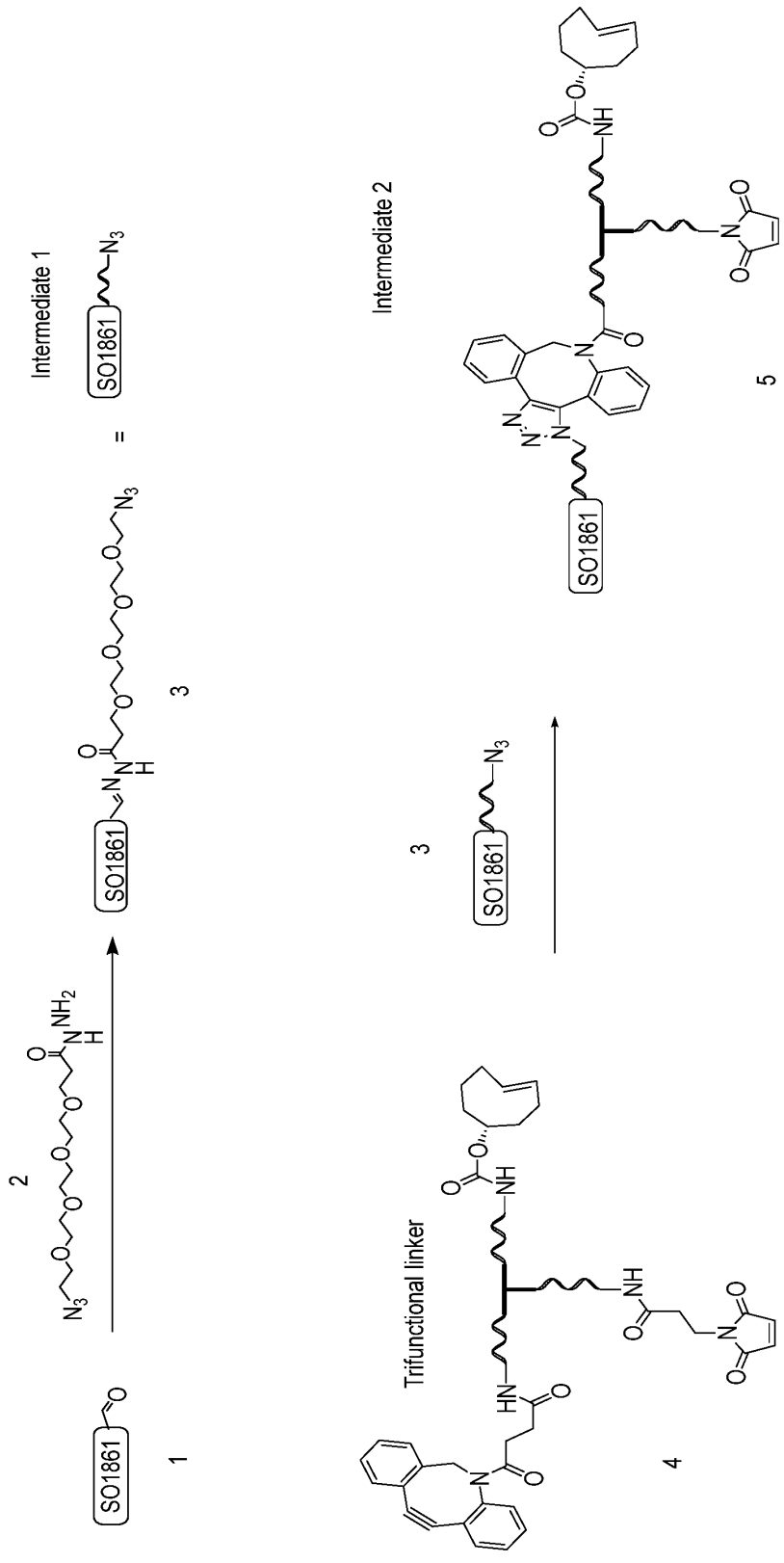

Intermediate 1
SO1861
N3
=
SO1861
N3
3
SO1861
O
1
2
N3
NH2
Intermediate 2
SO1861
5
Trifunctional linker
4
3
SO1861
N3

Fig. 35
B

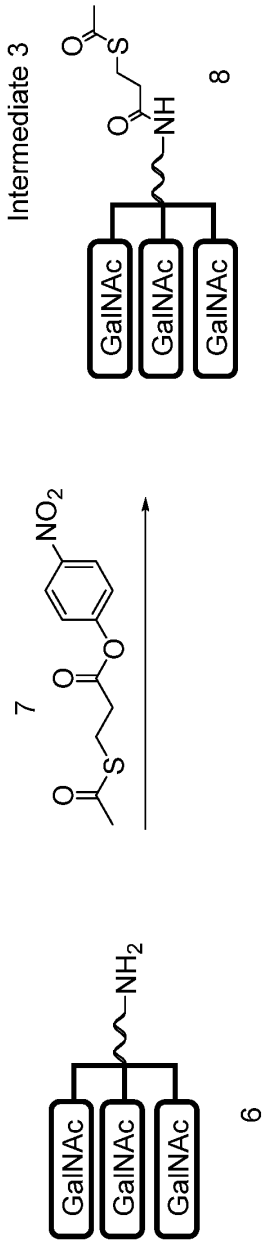


Fig. 35
C

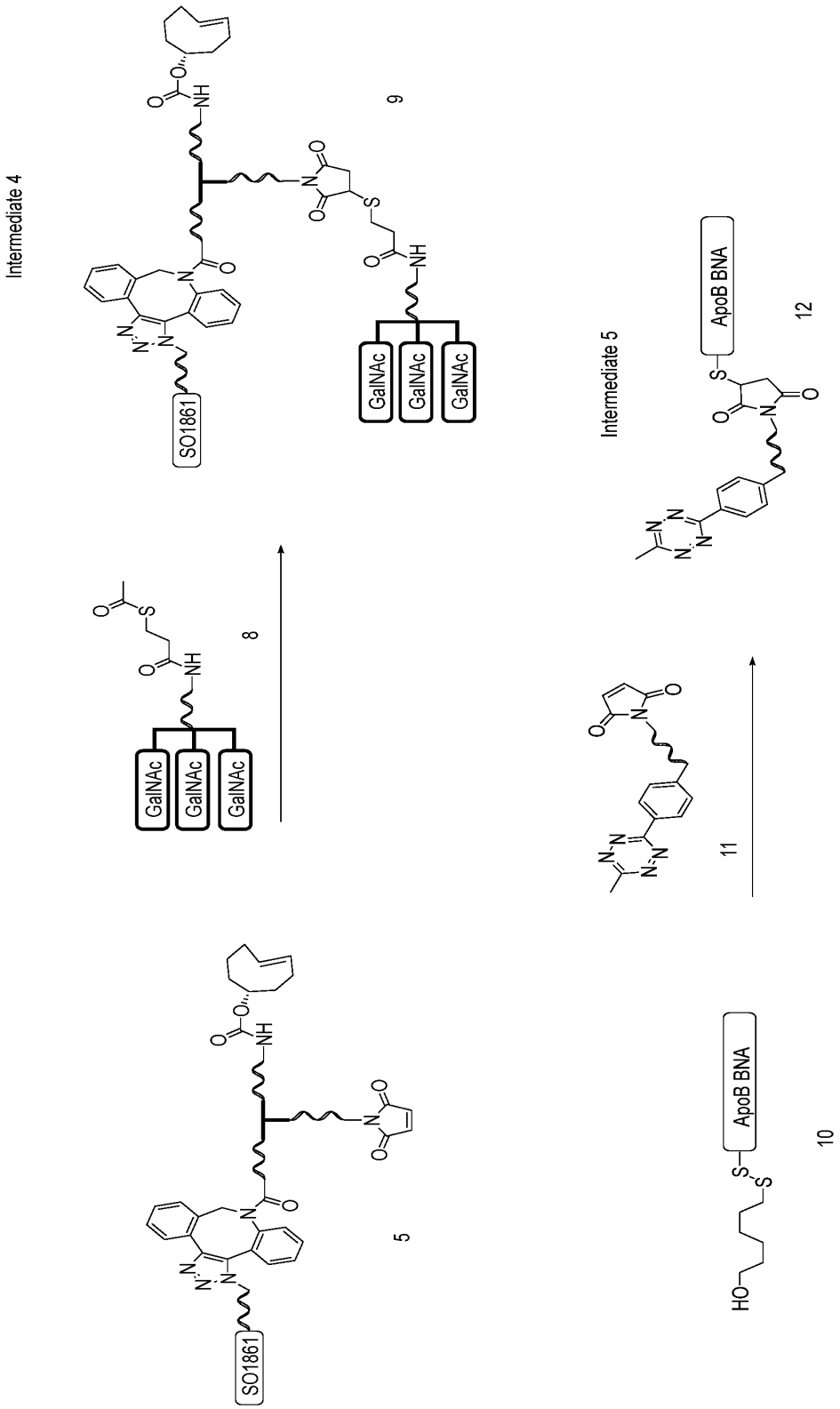

Intermediate 4
SO1861
GalNAc
GalNAc
GalNAc
9
8
5
Intermediate 5
ApoB BNA
12
11
ApoB BNA
HO
10

Fig. 35
D

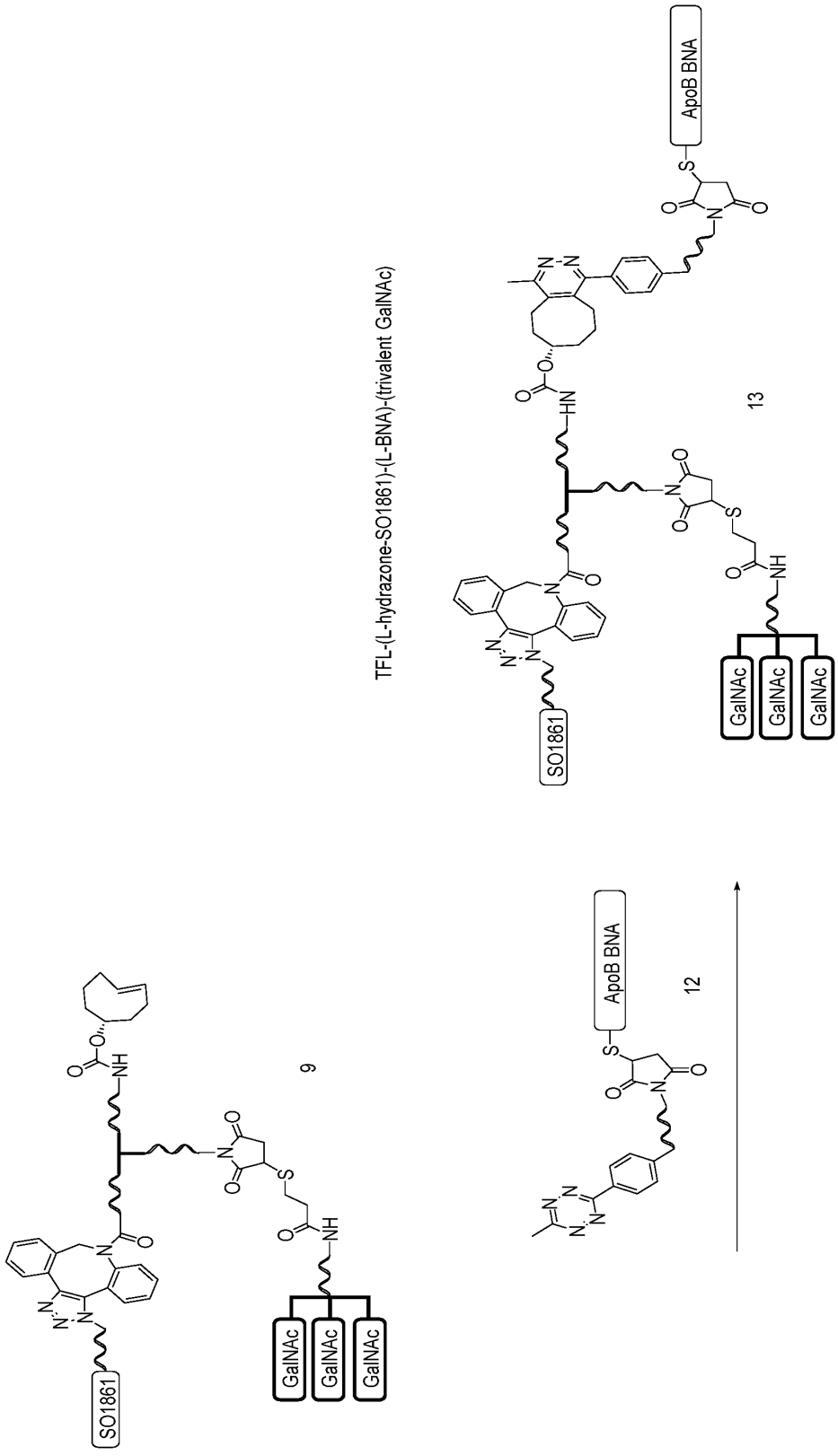

TFL-(L-hydrazone-SO1861)-(L-BNA)-(trivalent GalNAc)
ApoB BNA
SO1861
GalNAc
GalNAc
GalNAc
12
13
9

Fig. 36
A

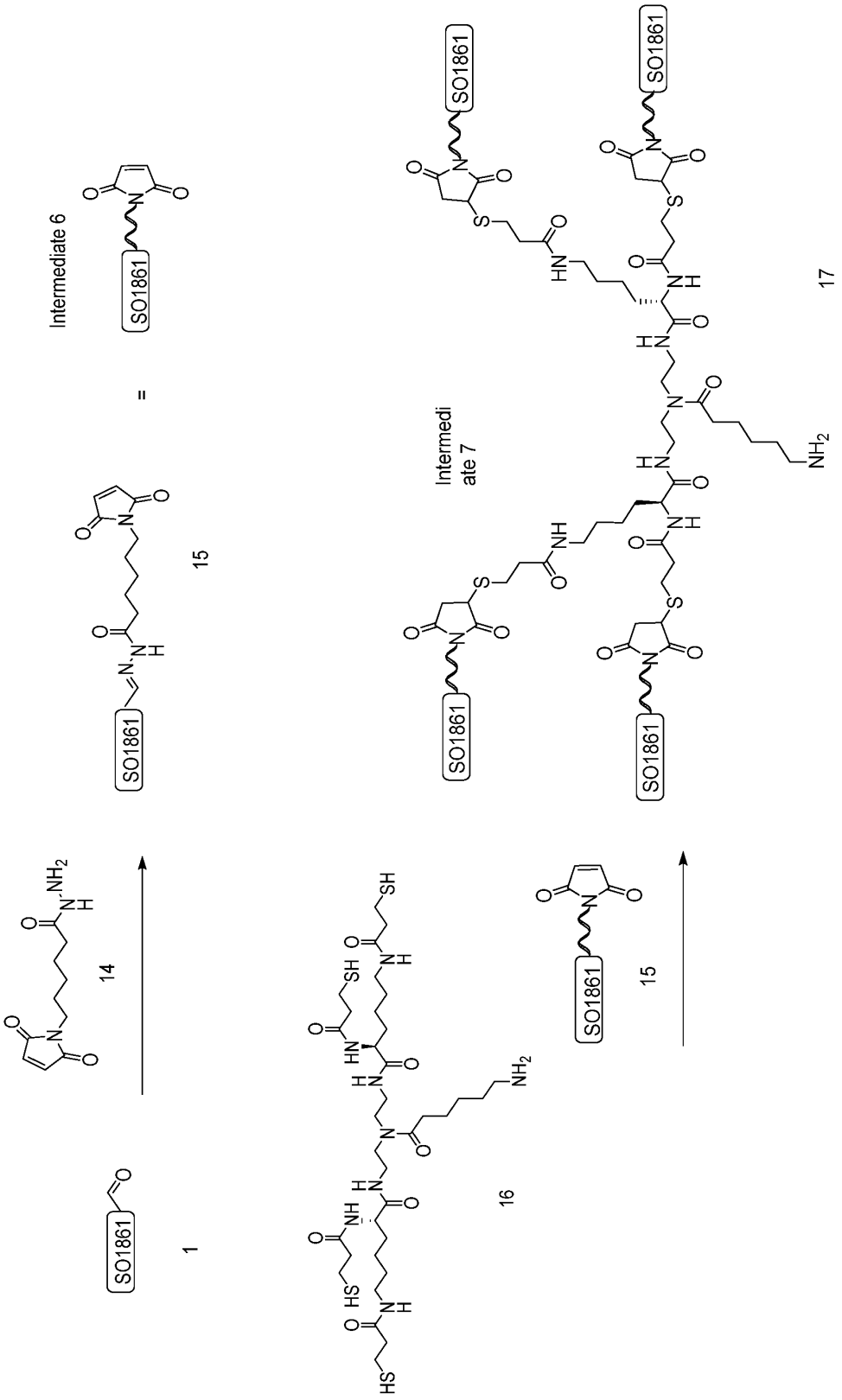


Fig. 36
B

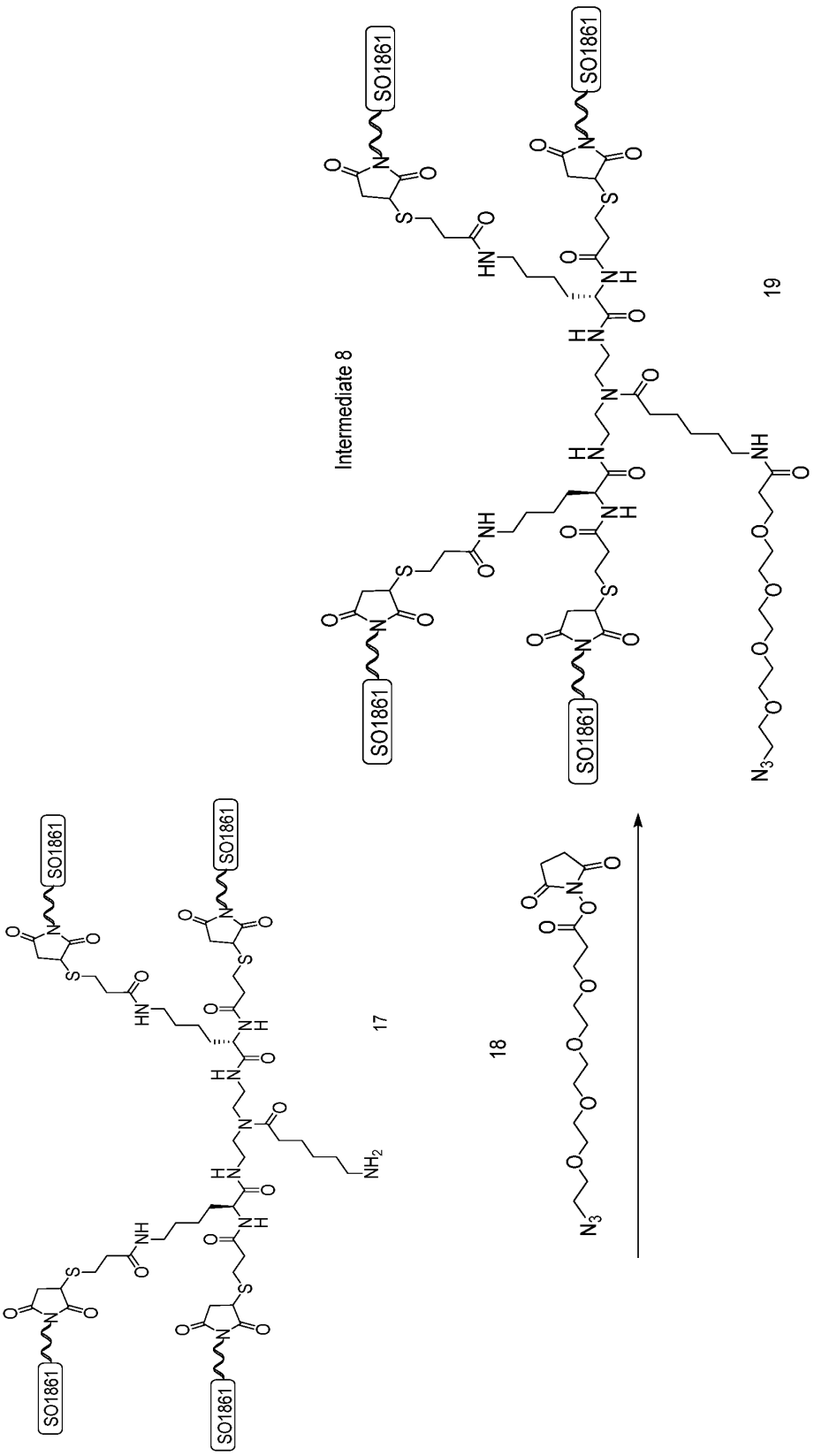


Fig. 36
C

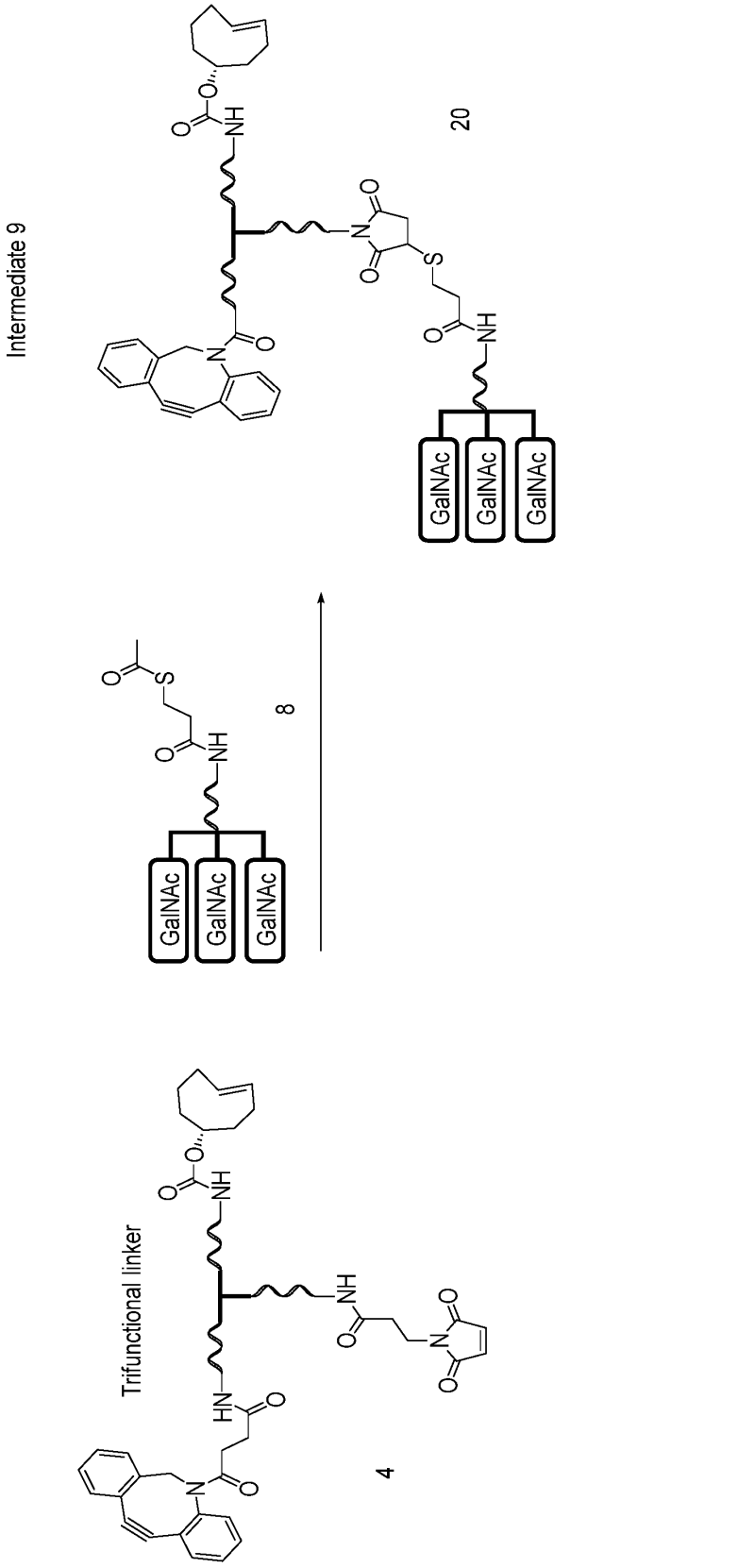

Intermediate 9
20
GalNAc
GalNAc
GalNAc
8
GalNAc
GalNAc
GalNAc
Trifunctional linker
4

Fig. 36
D

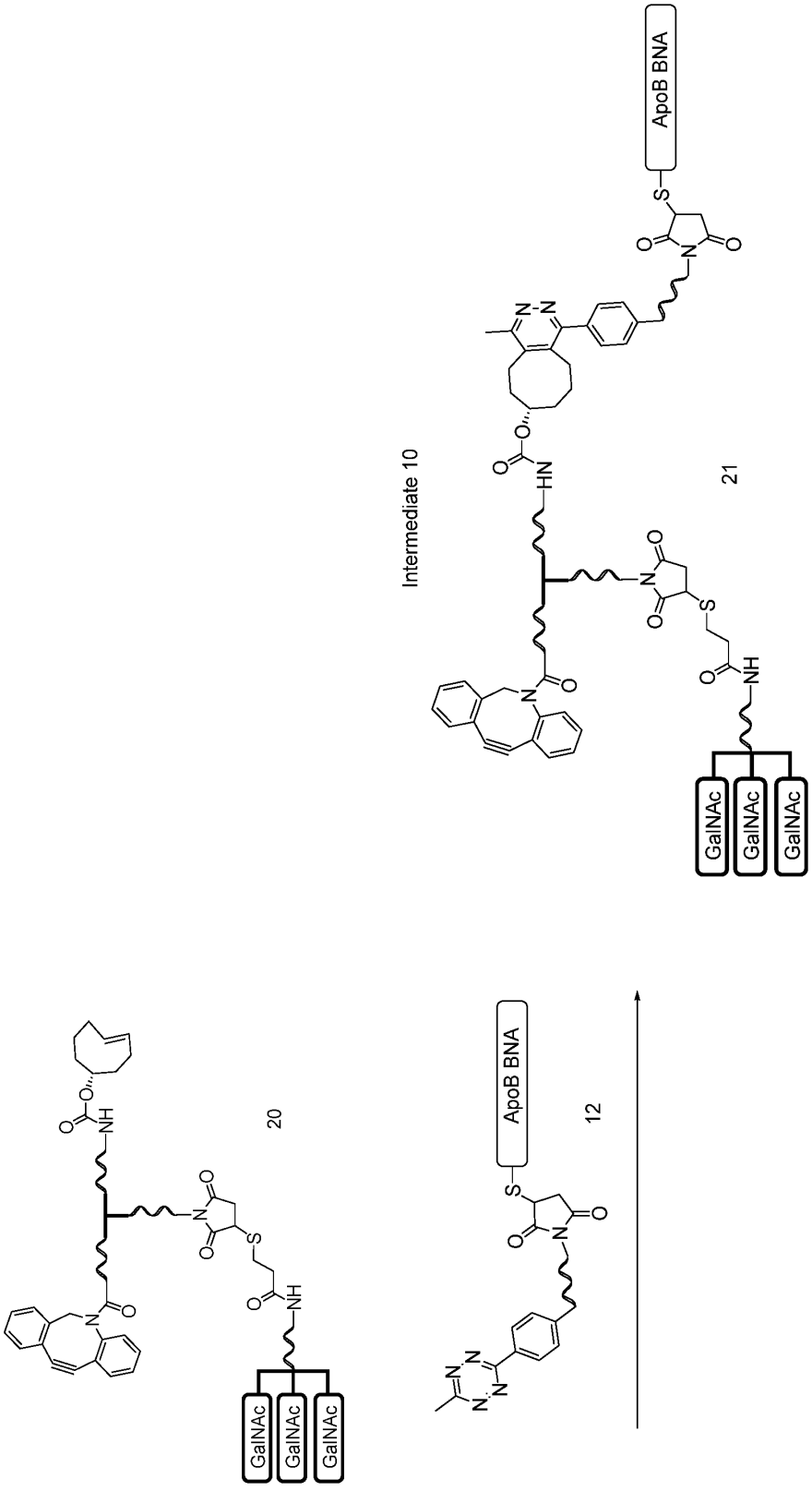


Fig. 36
E

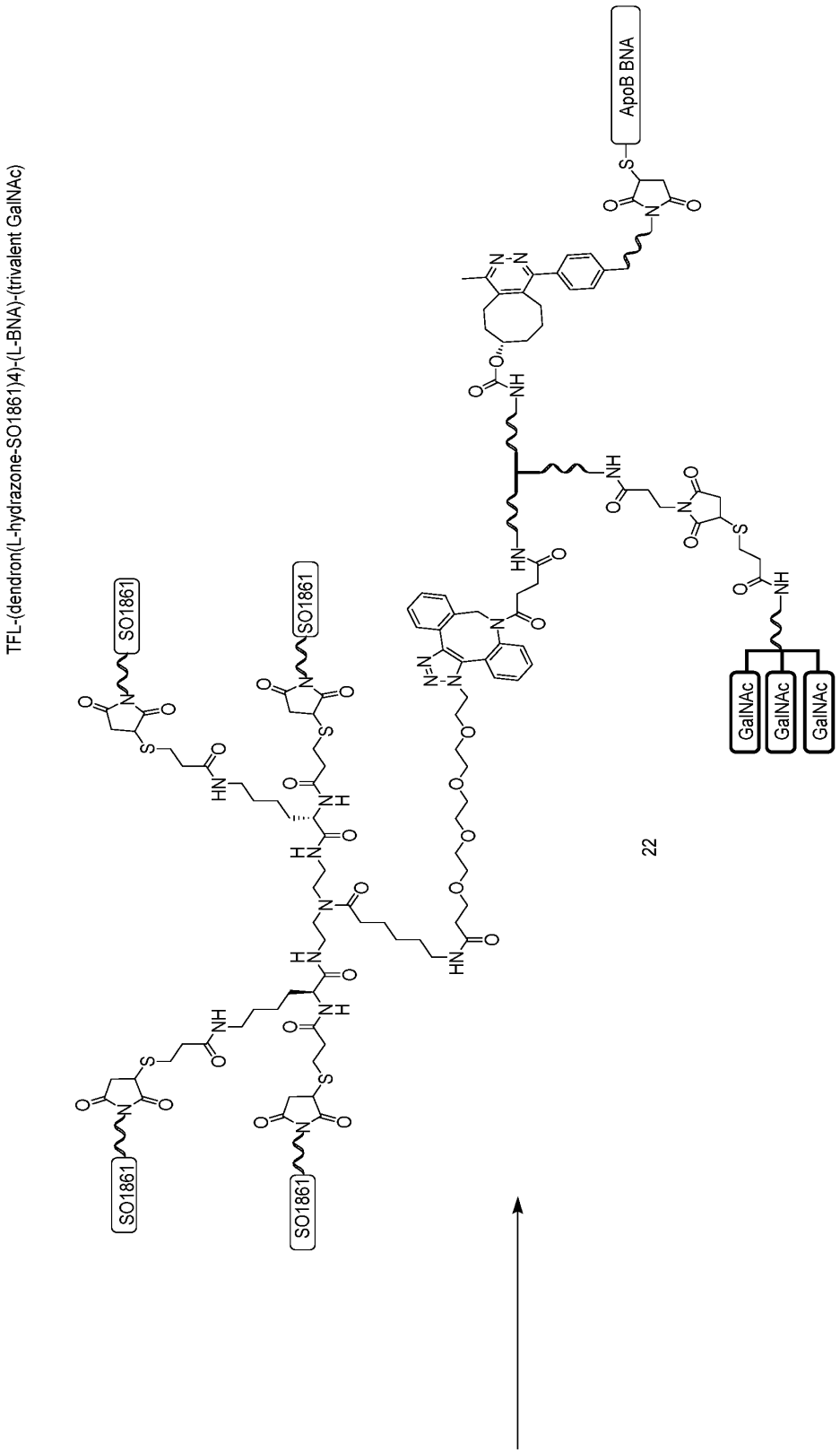

TFL-(dendron(L-hydrazone-SO1861)4)-(L-BNA)-(trivalent GalNAc)
ApoB BNA
SO1861
SO1861
SO1861
SO1861
GalNAc
GalNAc
GalNAc
22

Fig. 36
E (continued)

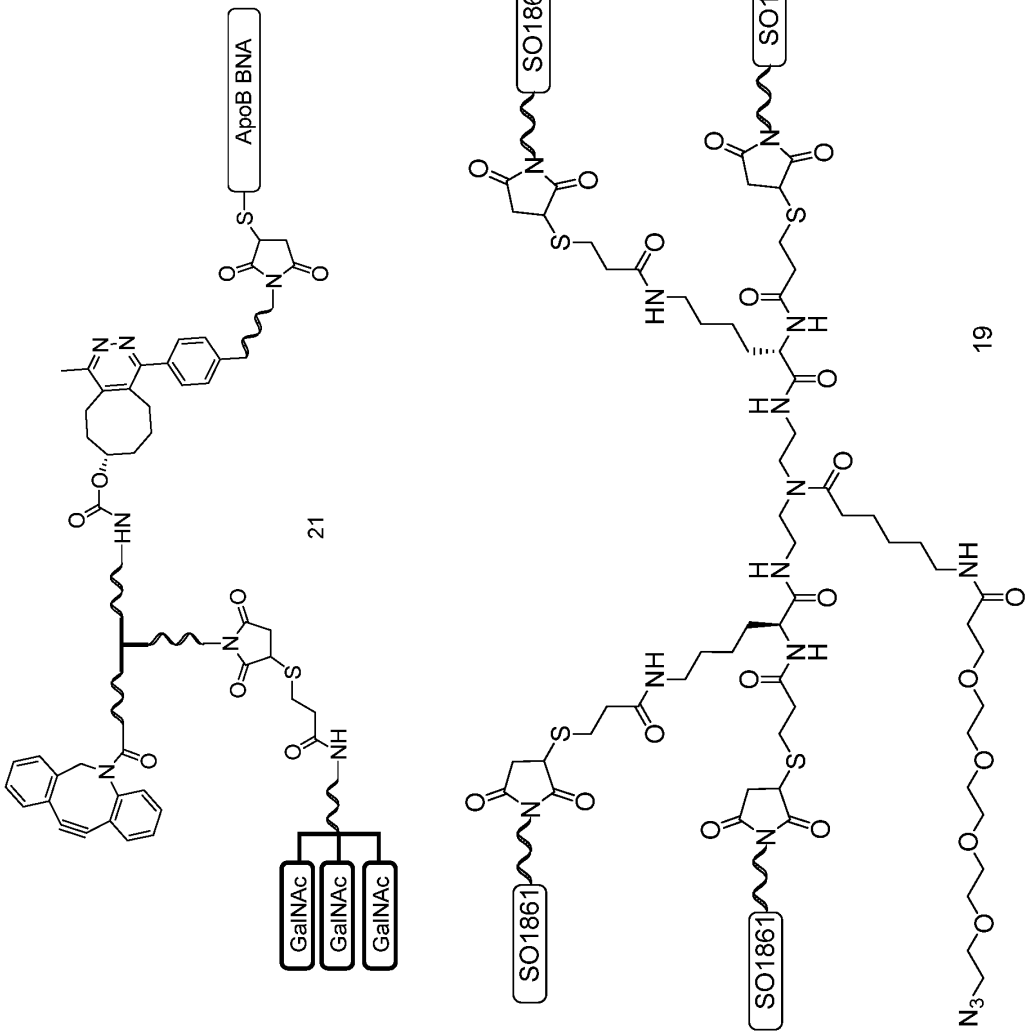


Fig. 37
A

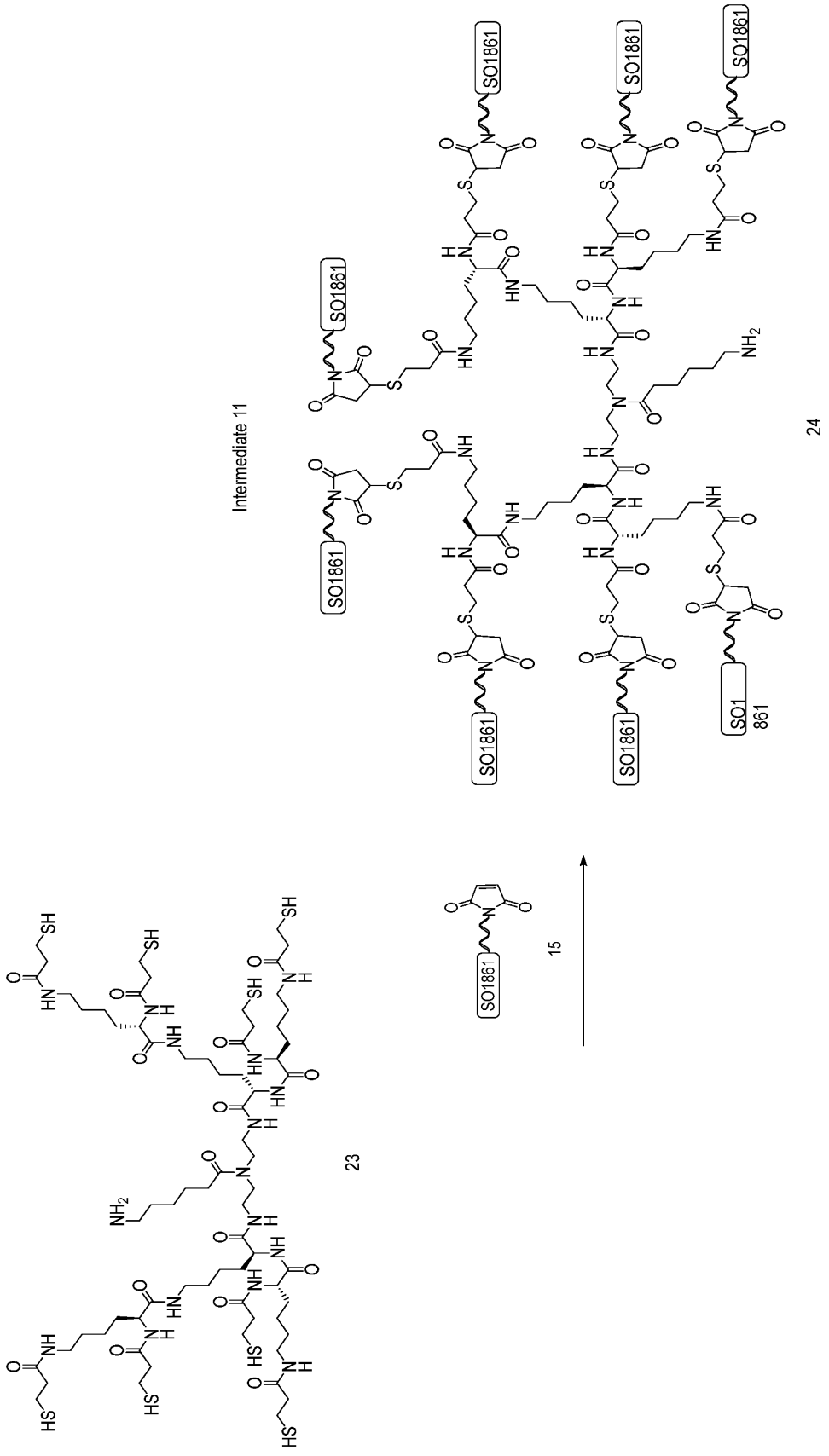


Fig. 37
B

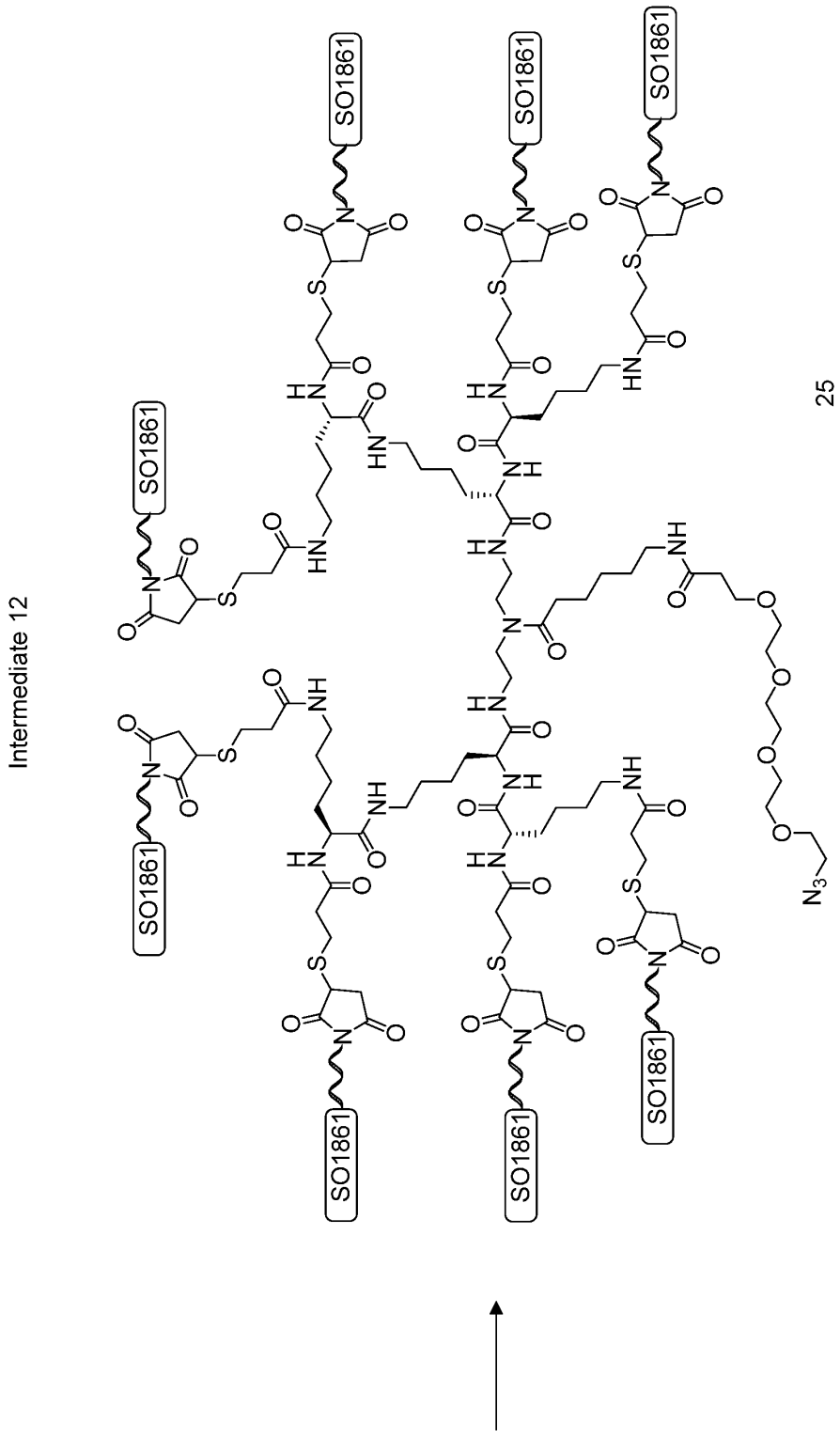

Intermediate 12
SO1861
SO1861
SO1861
SO1861
SO1861
SO1861
25
N_3

Fig. 37
B (continued)

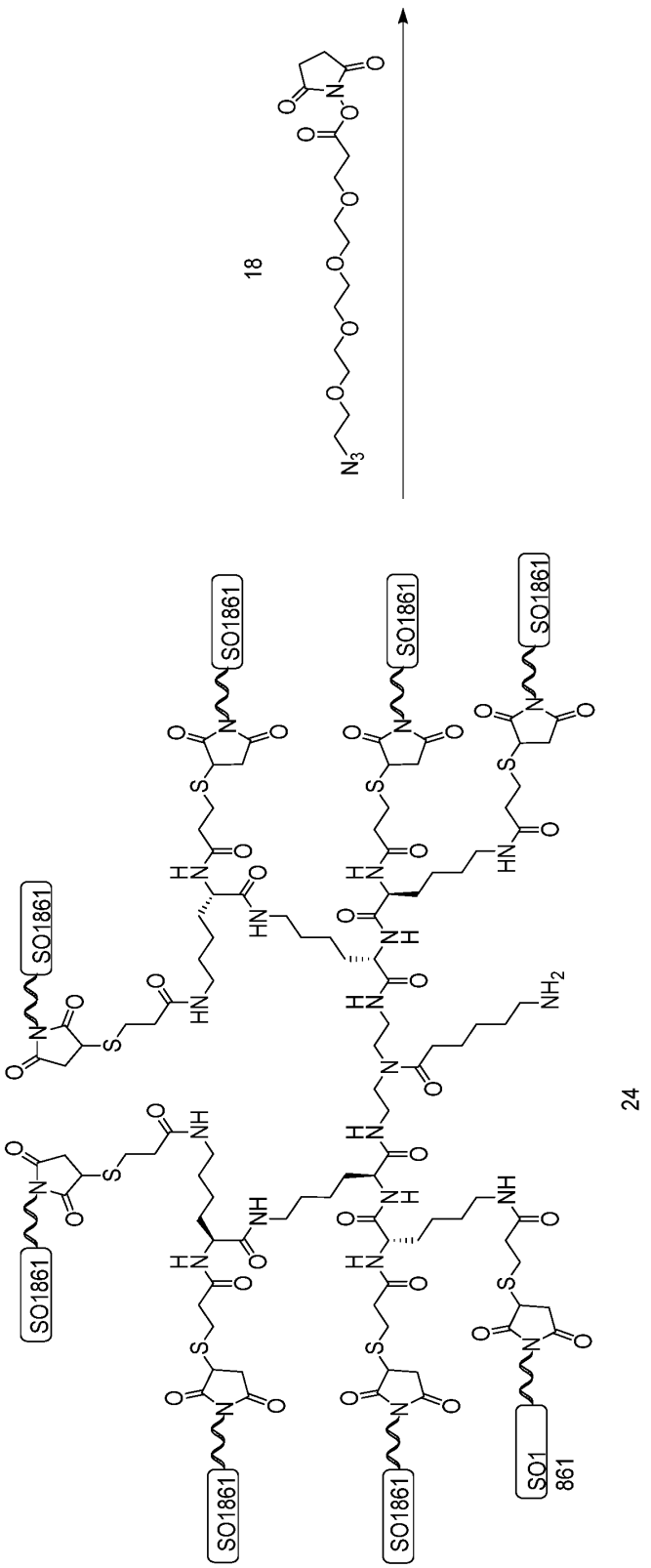


Fig. 37
C

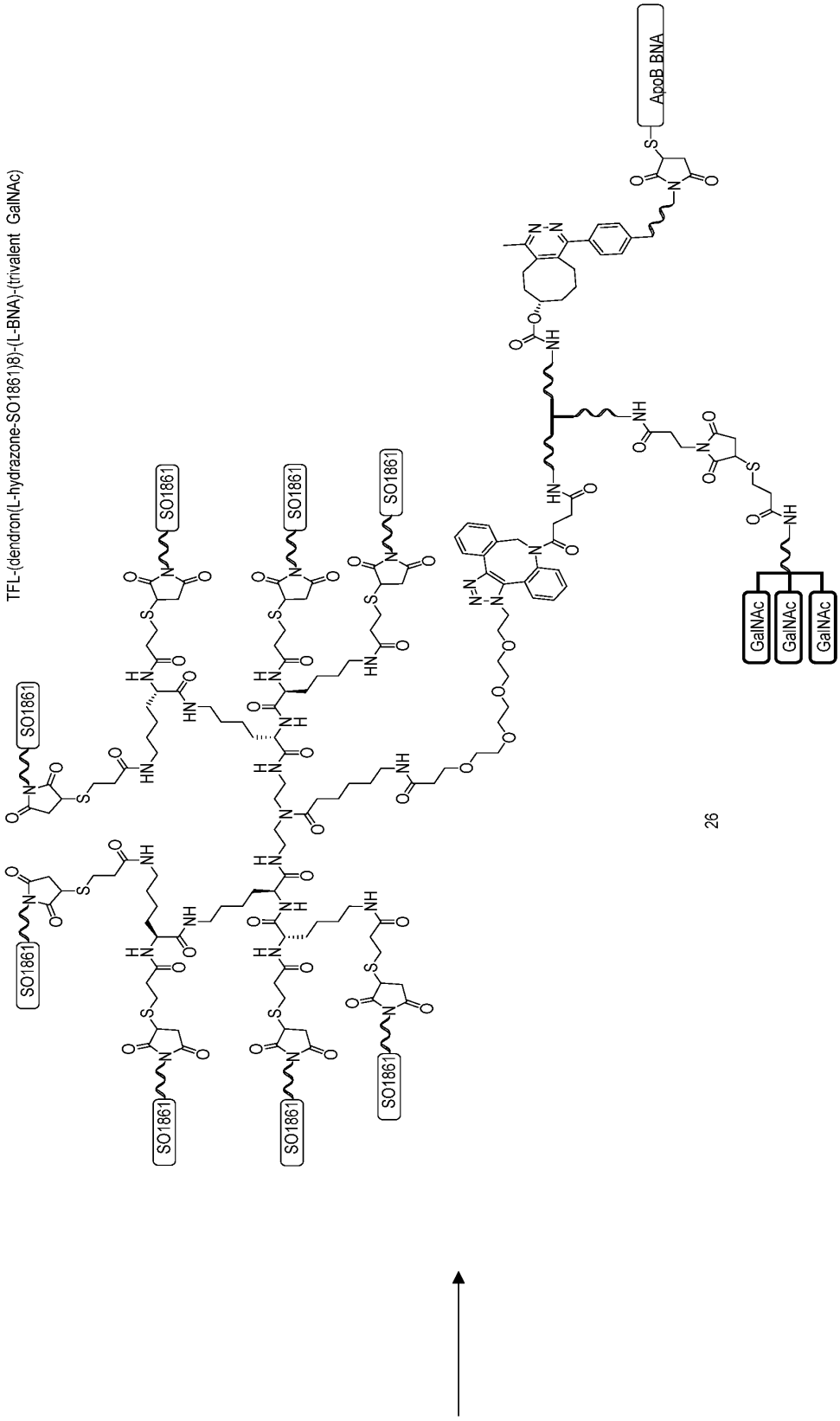

TFL-(dendron(L-hydrazone-SO1861)8)-(L-BNA)-(trivalent GalNAc)
ApoB BNA
SO1861
GalNAc
26

Fig. 37
C (continued)

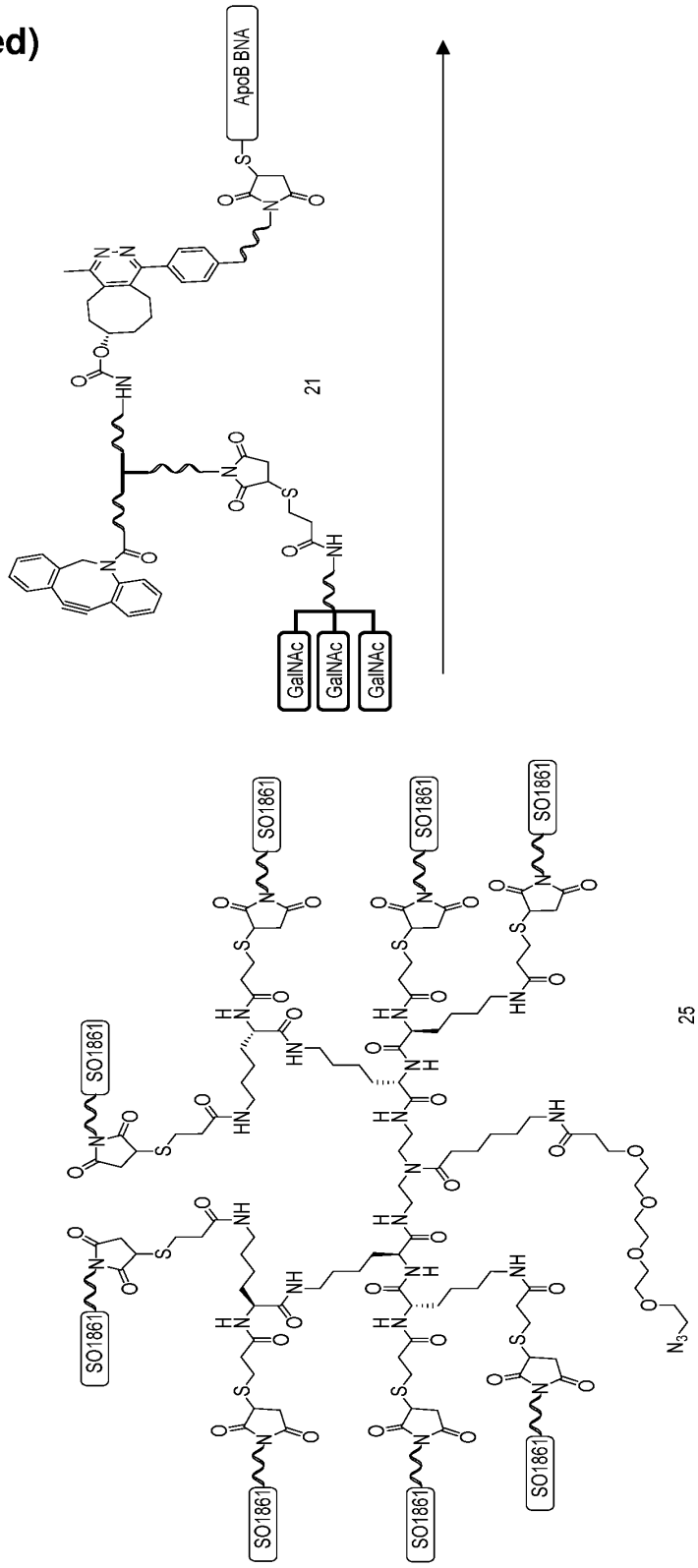


Fig. 38
A

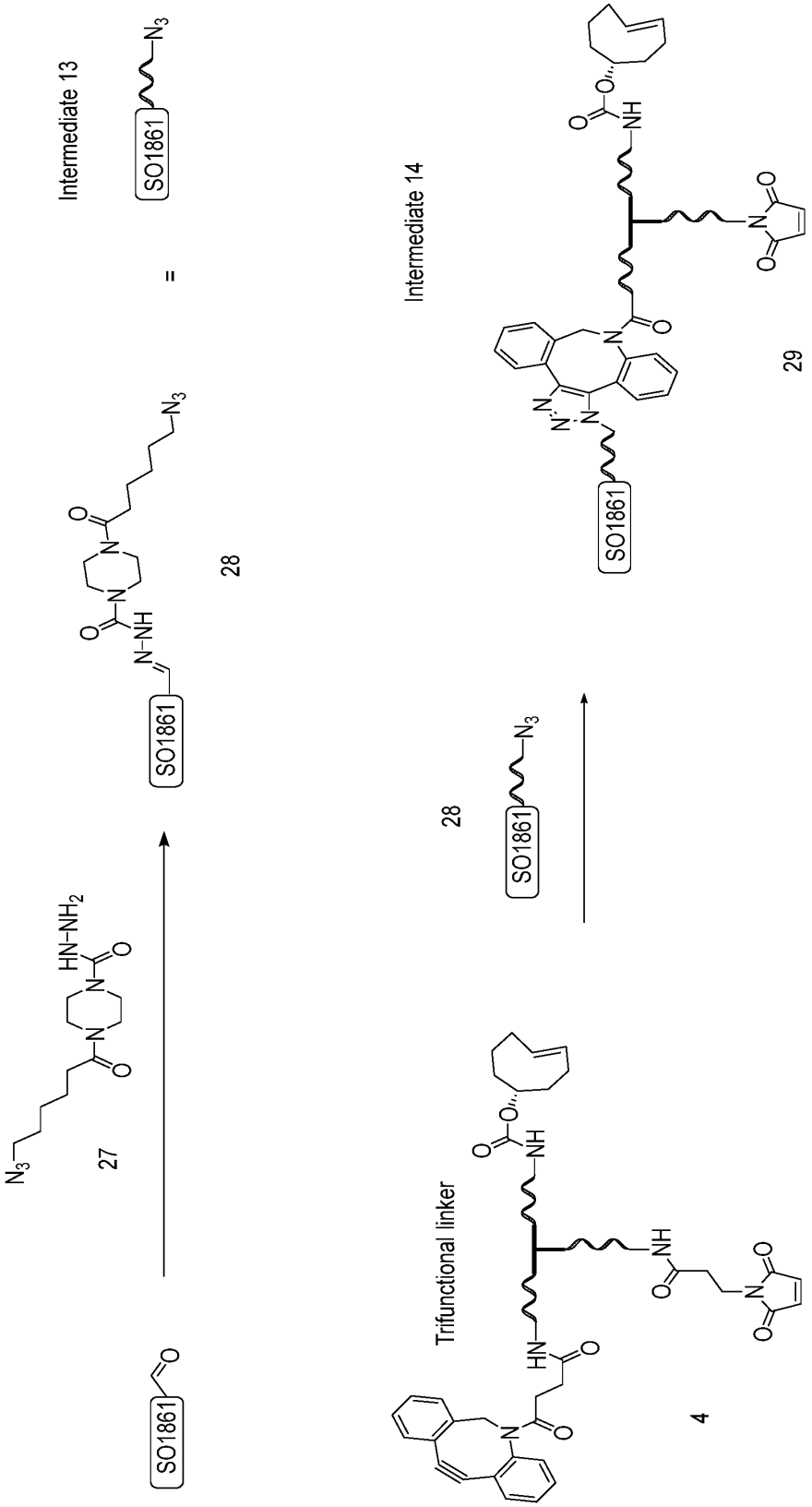

Intermediate 13
SO1861
N3
=
28
27
HN-NH2
Intermediate 14
29
Trifunctional linker
4

Fig. 38
B

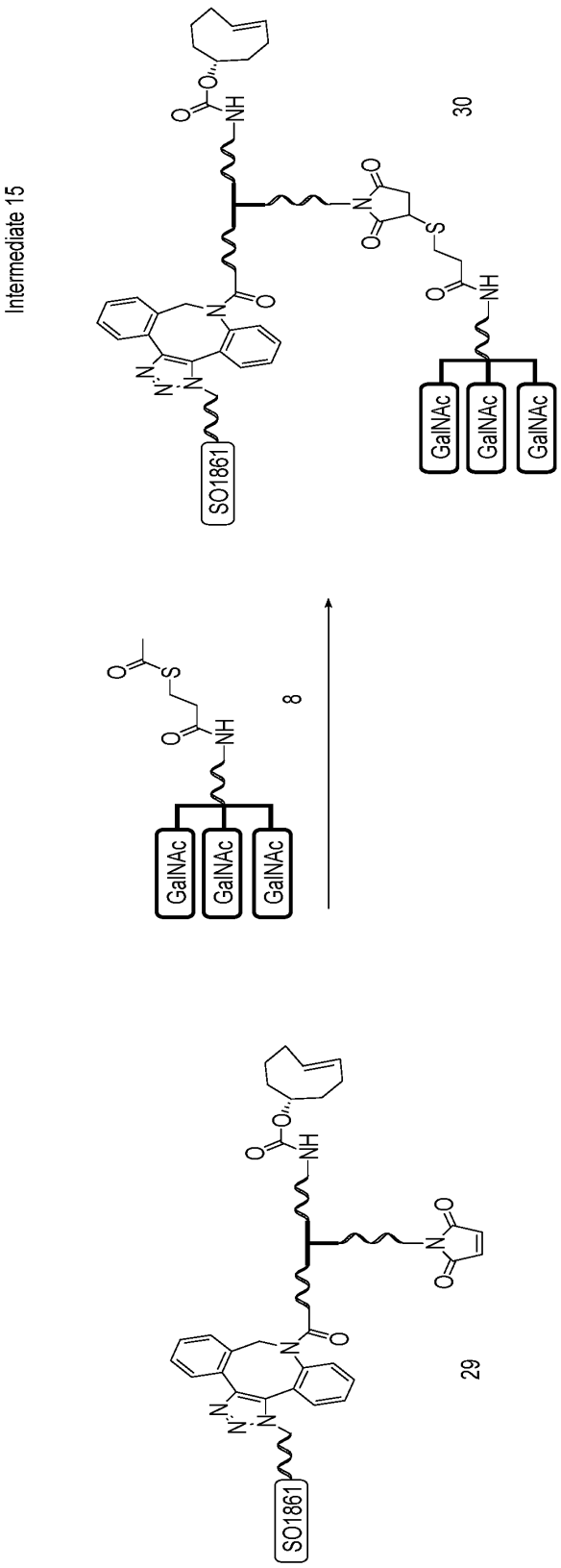

Intermediate 15
30
SO1861
GalNAc
GalNAc
GalNAc
8
GalNAc
GalNAc
GalNAc
29
SO1861

Fig. 38
C

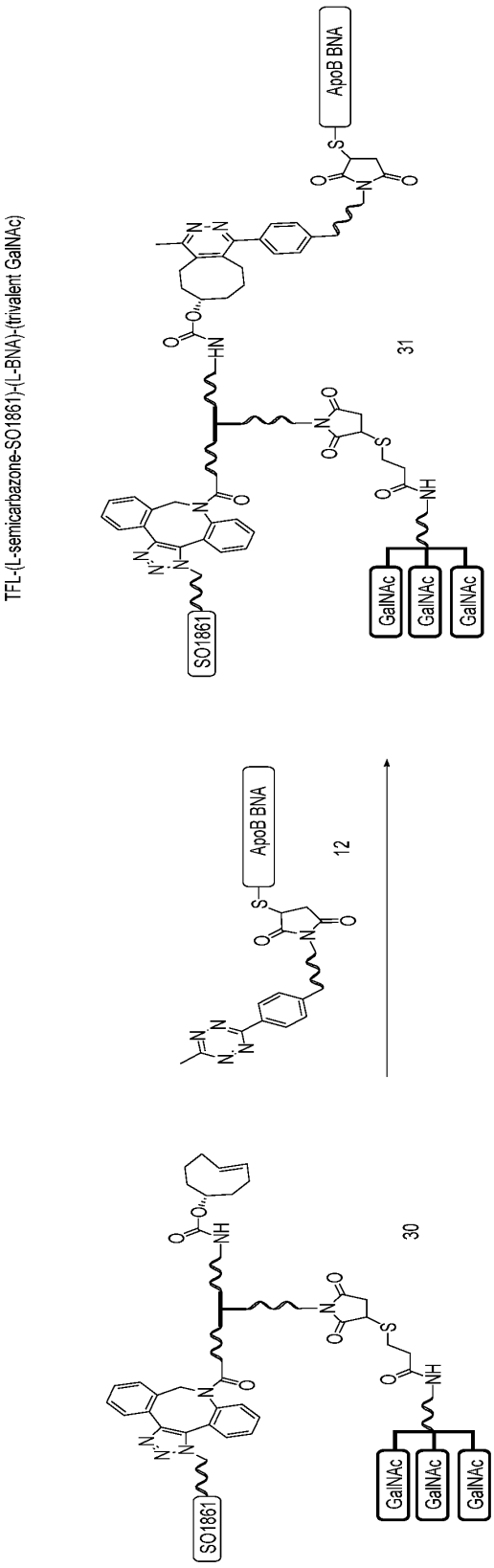

TFL-(L-semicarbazone-SO1861)-(L-BNA)-(trivalent GalNAc)
ApoB BNA
31
GalNAc
GalNAc
GalNAc
SO1861
ApoB BNA
12
30
GalNAc
GalNAc
GalNAc
SO1861

Fig. 39
A

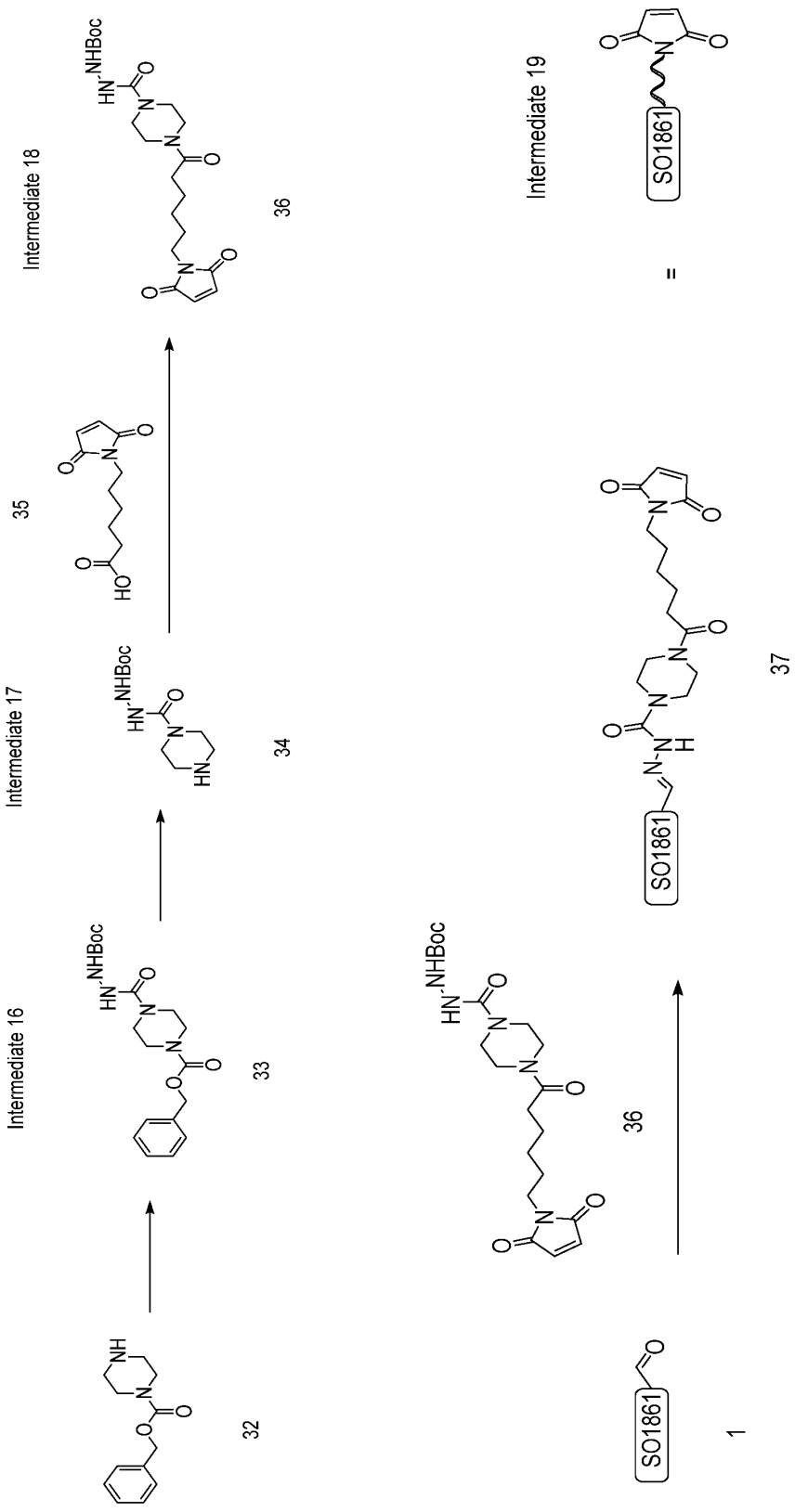

Intermediate 16
Intermediate 17
Intermediate 18
Intermediate 19
SO1861
NHBoc
HN
HO
32
33
34
35
36
37
1
=

Fig. 39
B

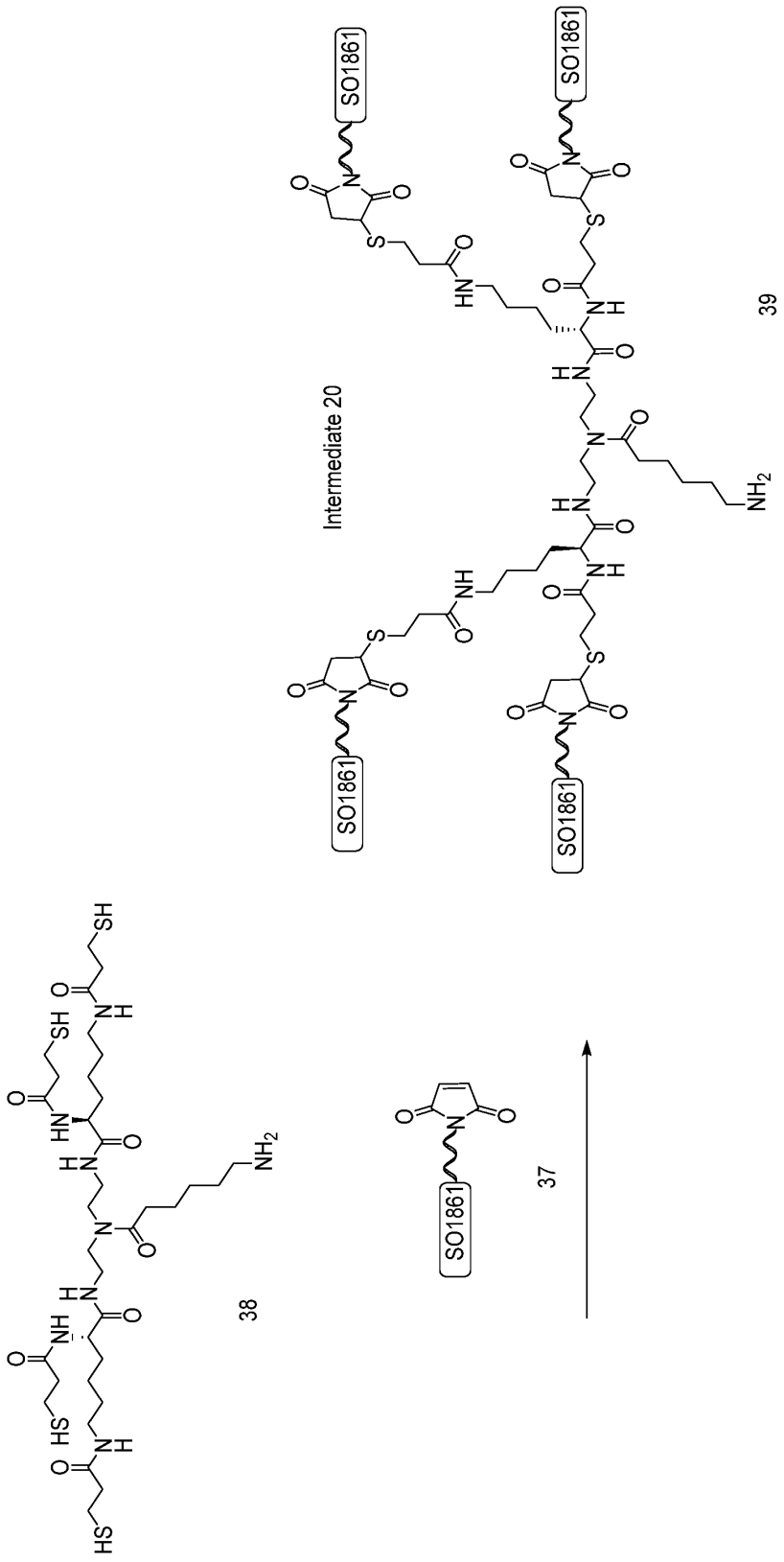


Fig. 39
C

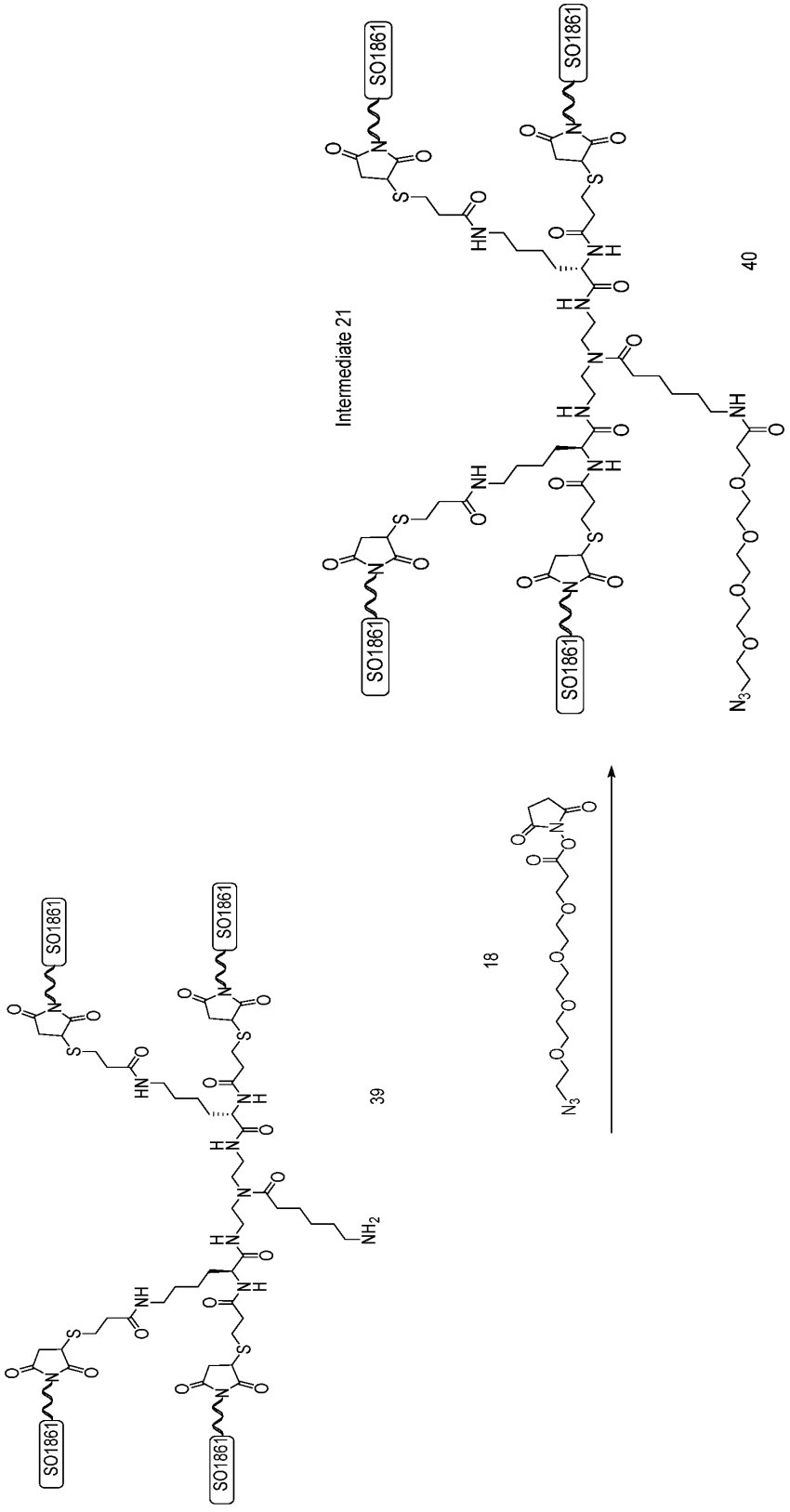


Fig. 39
D

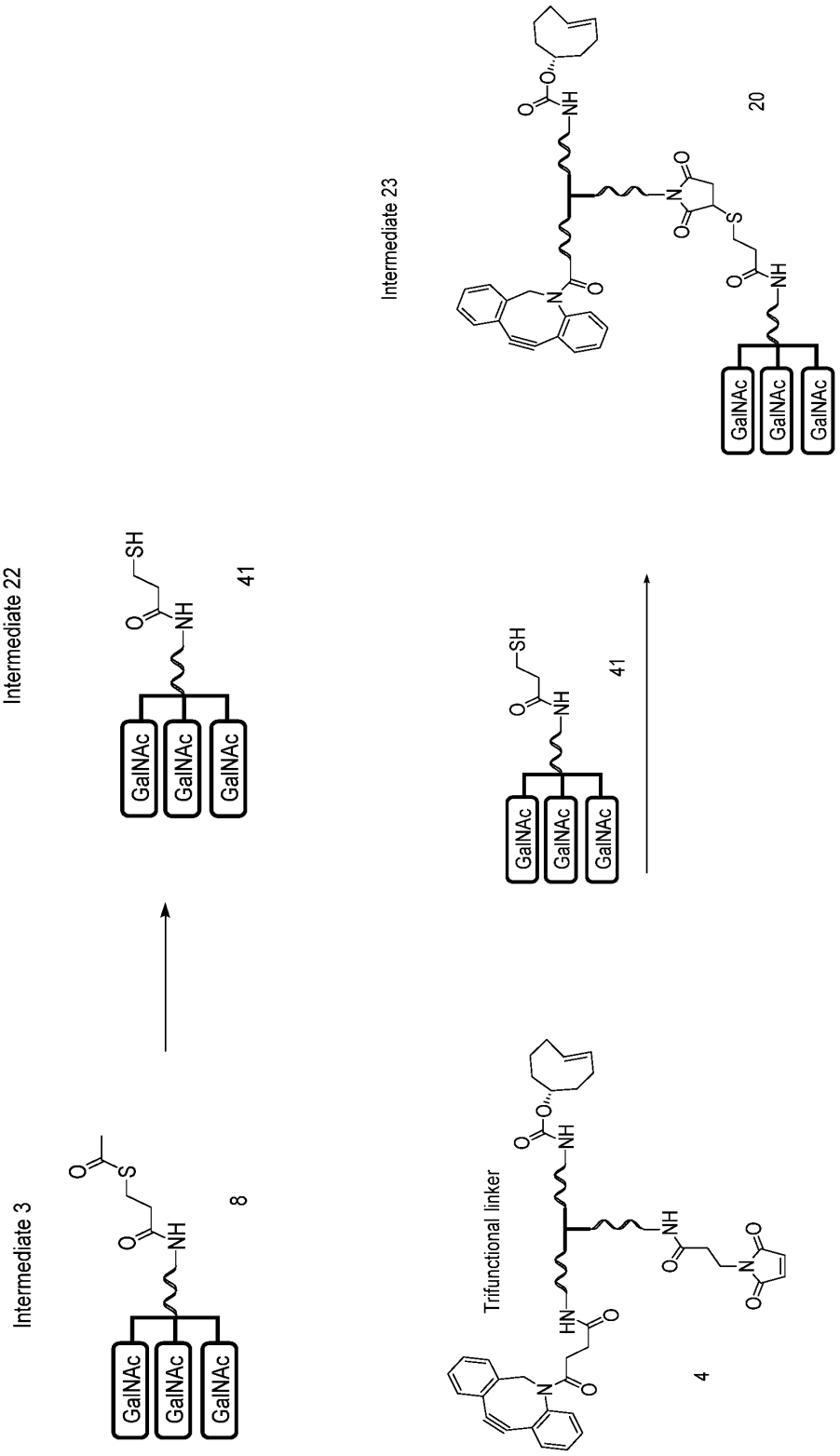

Intermediate 3
8
Intermediate 22
41
Trifunctional linker
4
41
Intermediate 23
20
GalNAc
GalNAc
GalNAc

Fig. 39
E

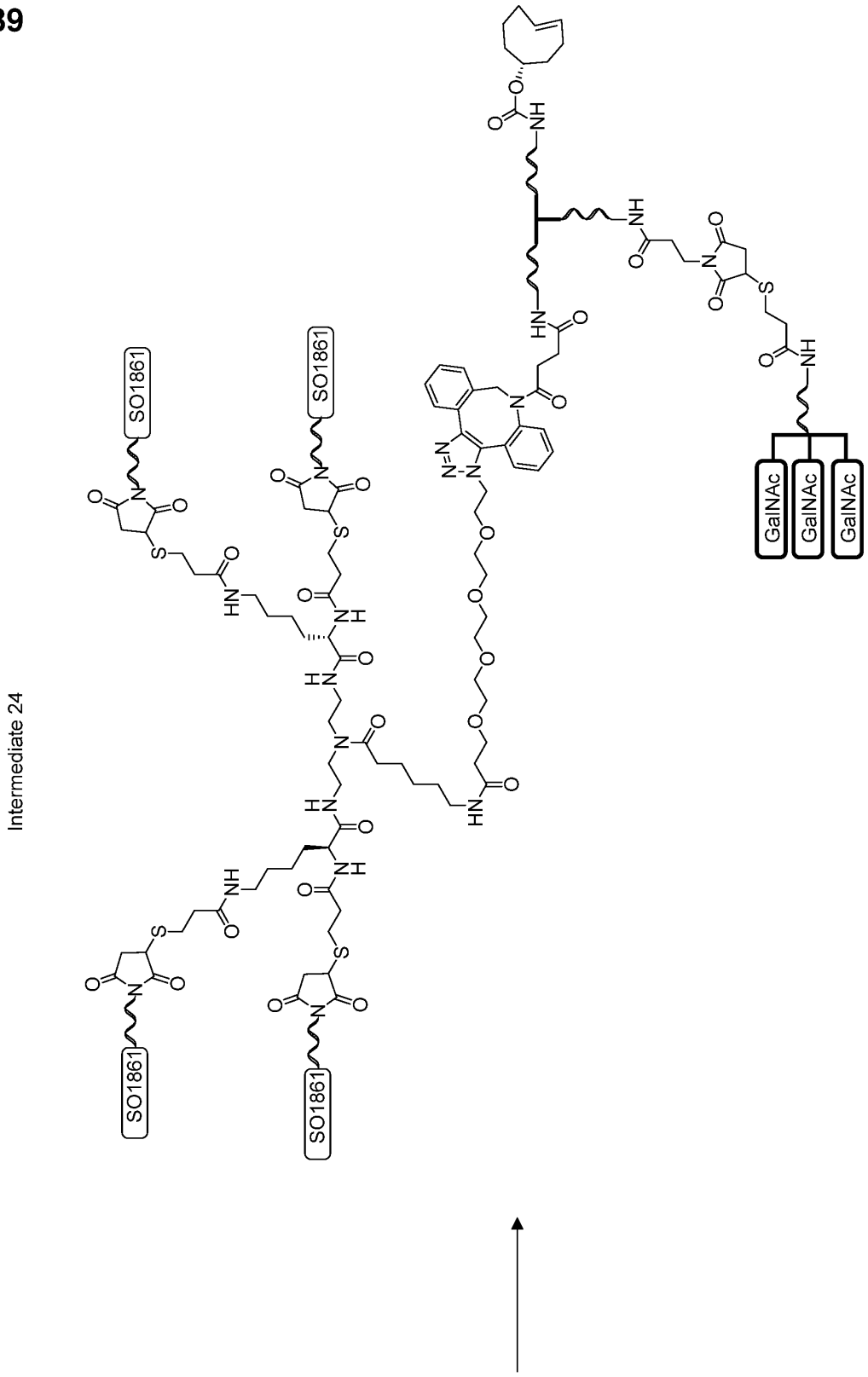
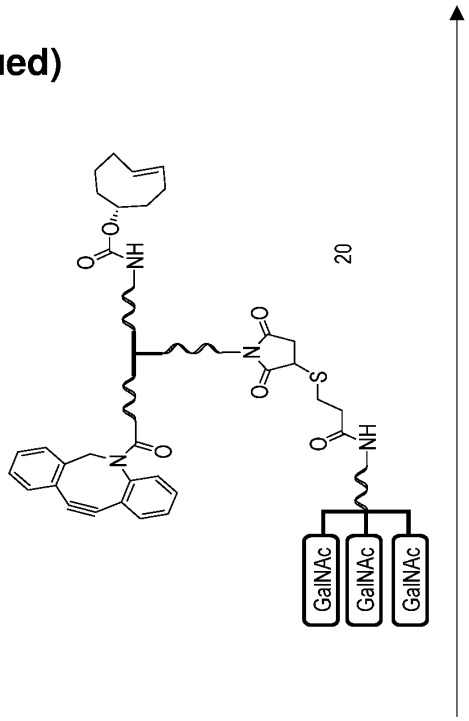

Intermediate 24
SO1861
SO1861
SO1861
SO1861
GalNAc
GalNAc
GalNAc

Fig. 39
E (continued)



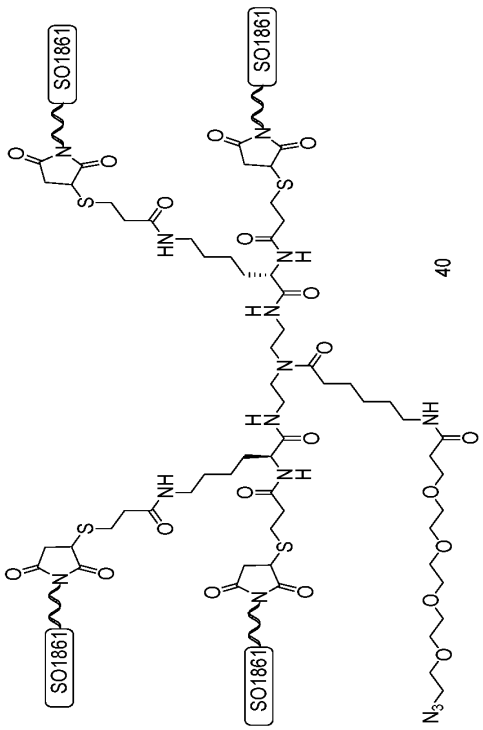


Fig. 39
F

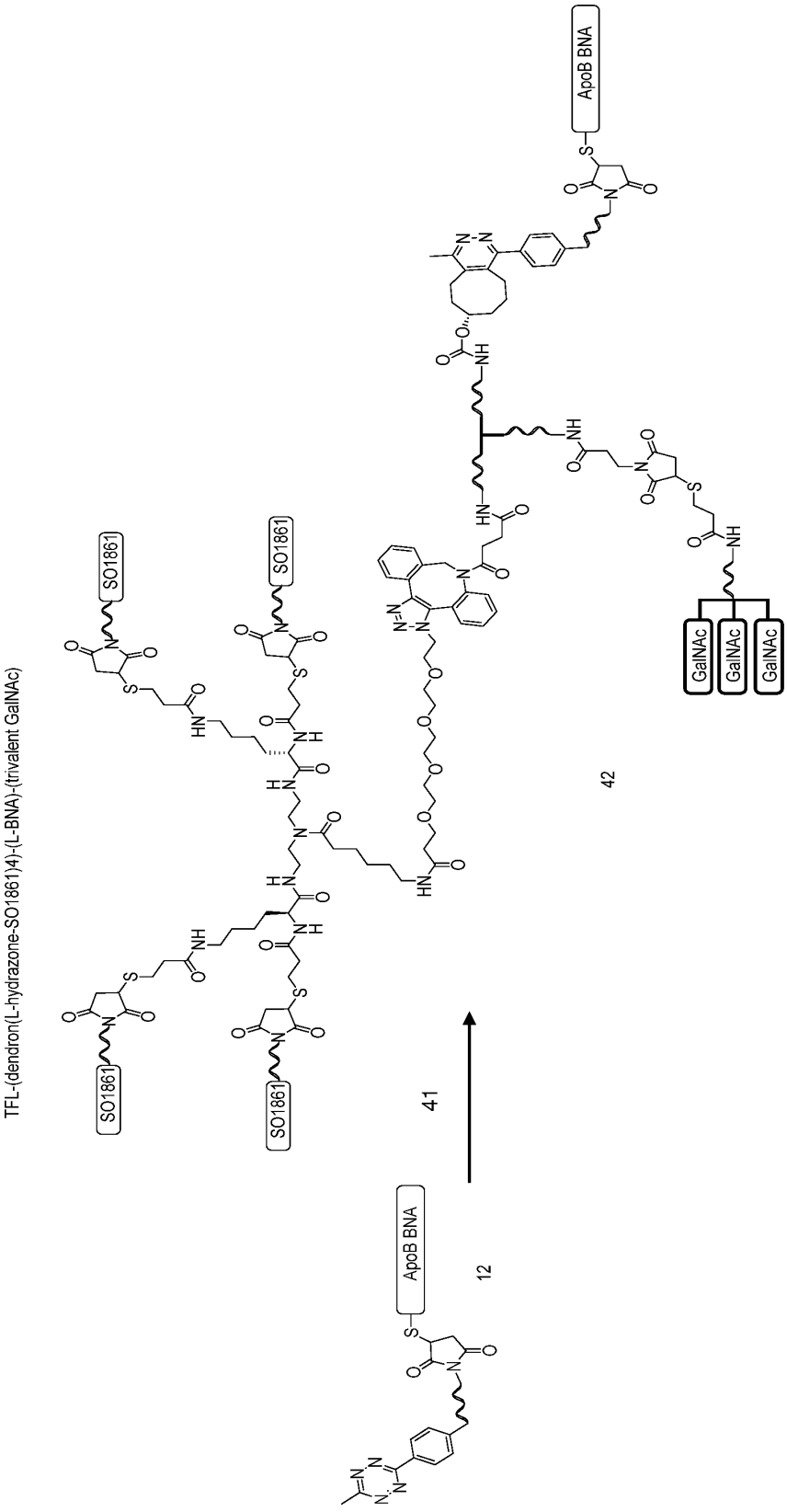

TFL-(dendron(L-hydrazone-SO1861)4)-(L-BNA)-(trivalent GalNAc)
SO1861
SO1861
SO1861
SO1861
ApoB BNA
12
41
ApoB BNA
GalNAc
GalNAc
GalNAc
42

Fig. 40
A

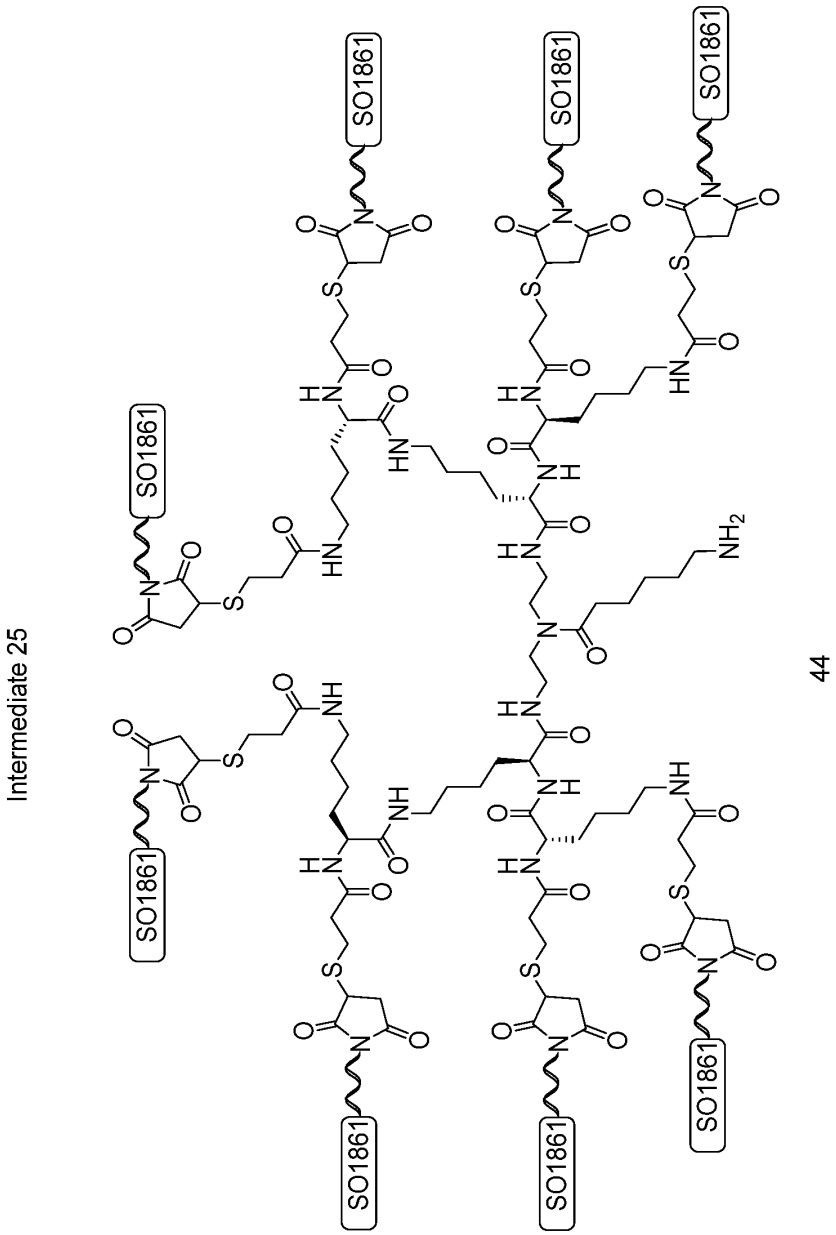
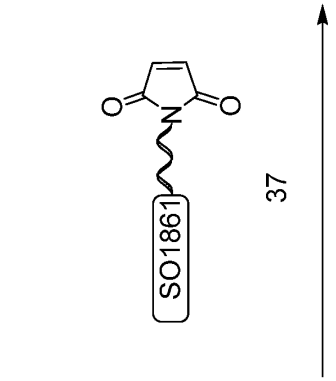


Fig. 40
A (continued)



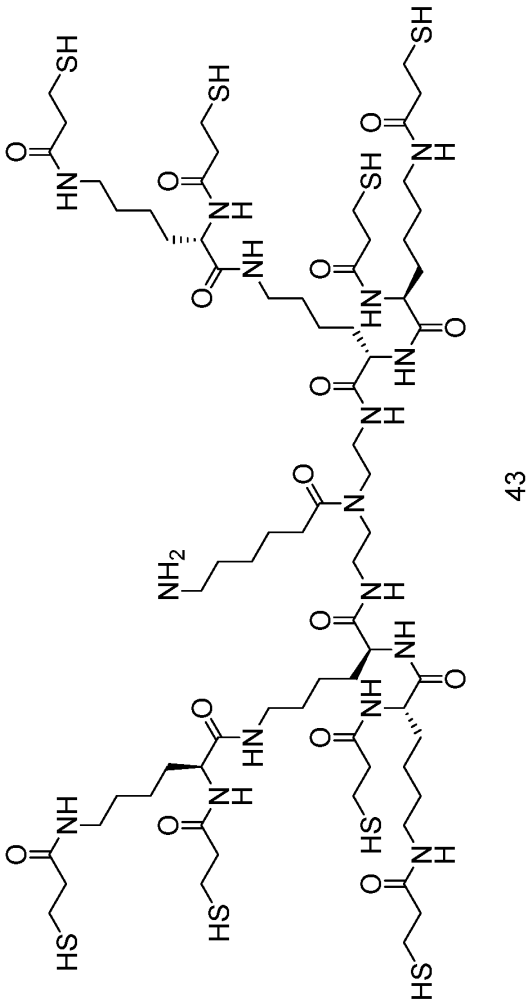
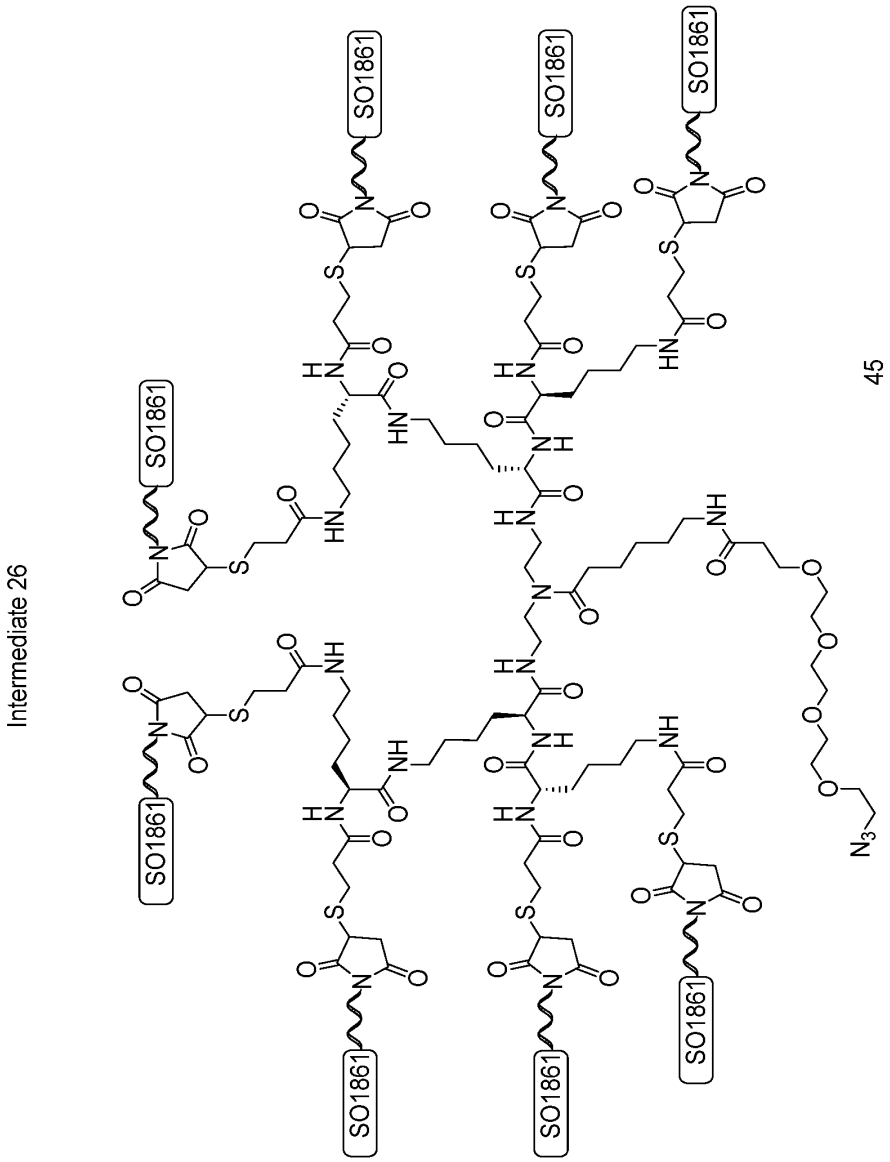
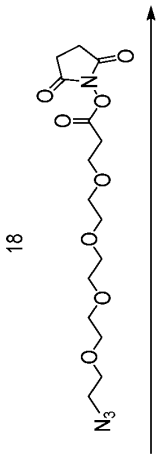


Fig. 40
B



Intermediate 26

Fig. 40
B (continued)



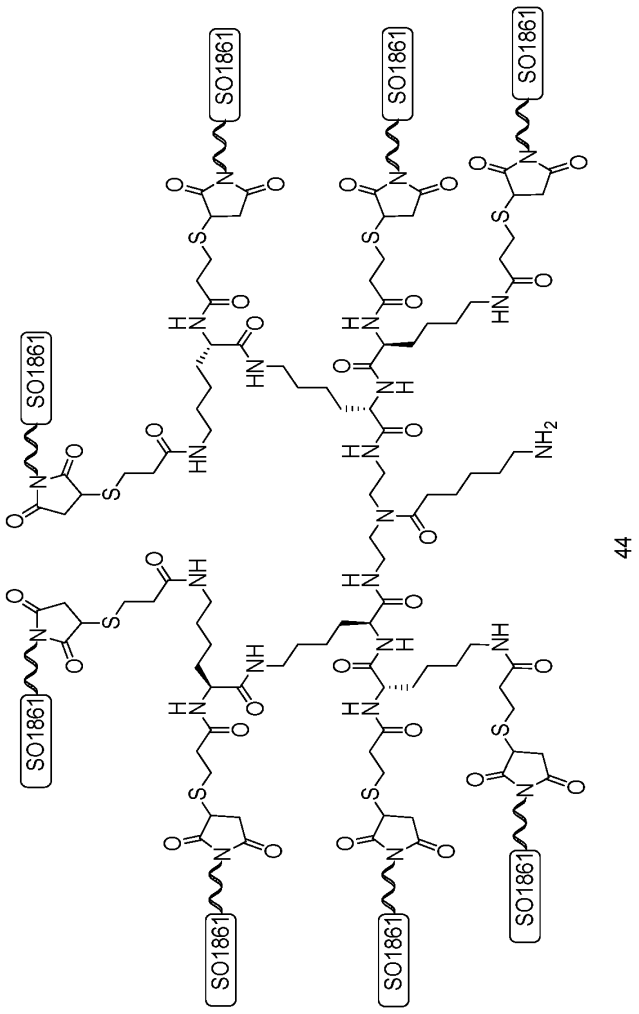


Fig. 40
C

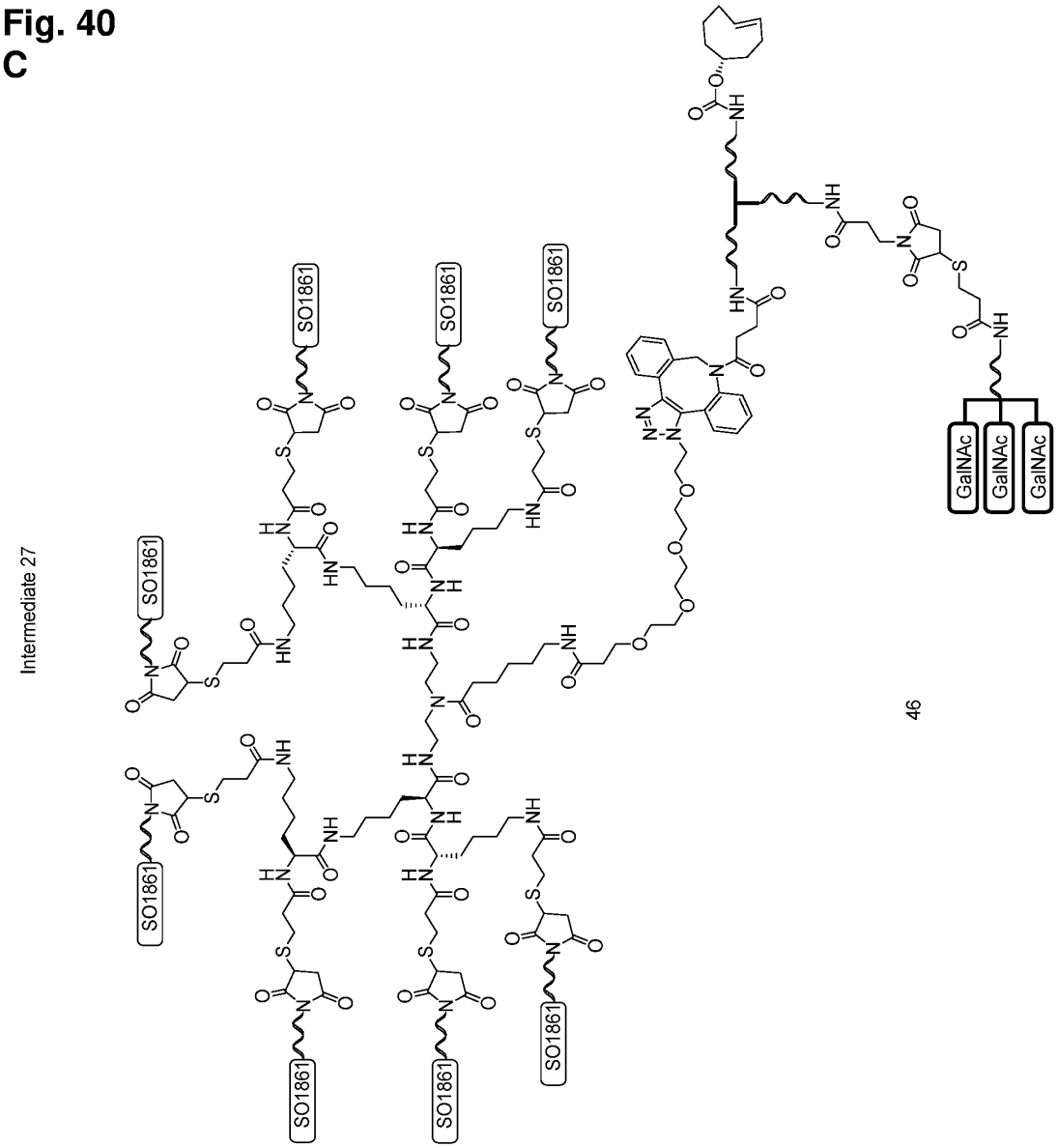


Fig. 40
C (continued)

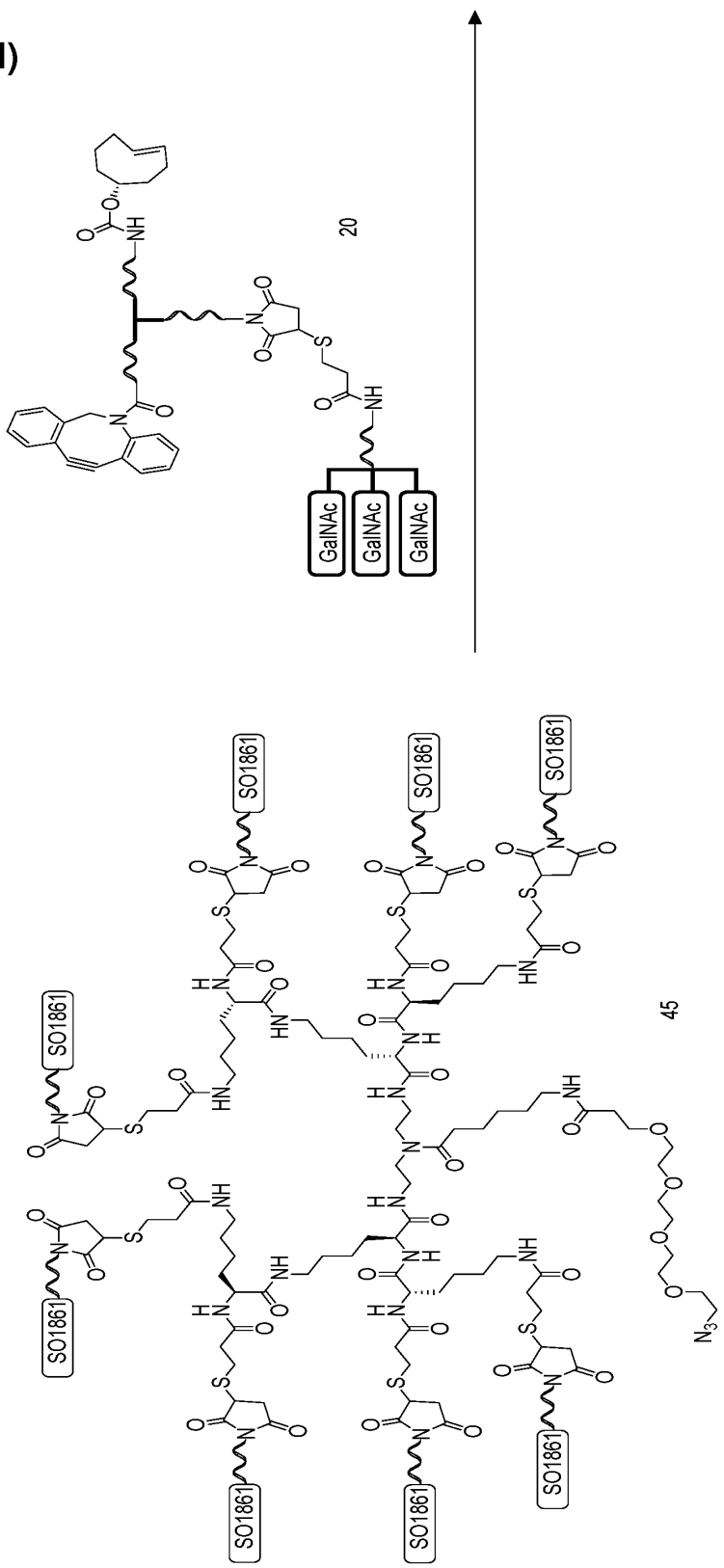


Fig. 40
D

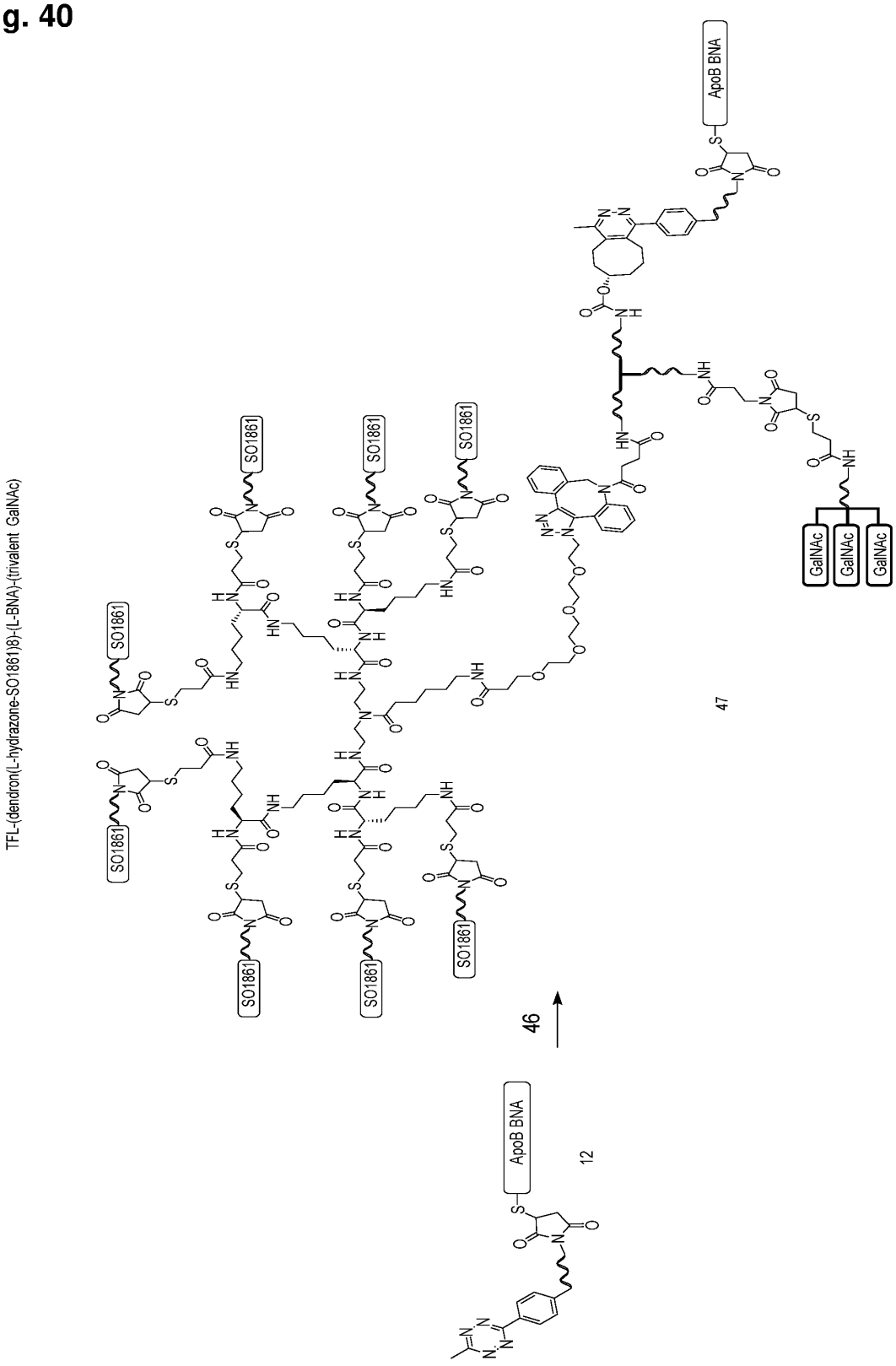

TFL-(dendron(L-hydrazone-SO1861)8)-(L-BNA)-(trivalent GalNAc)
SO1861
ApoB BNA
GalNAc
12
46
47

Fig. 41

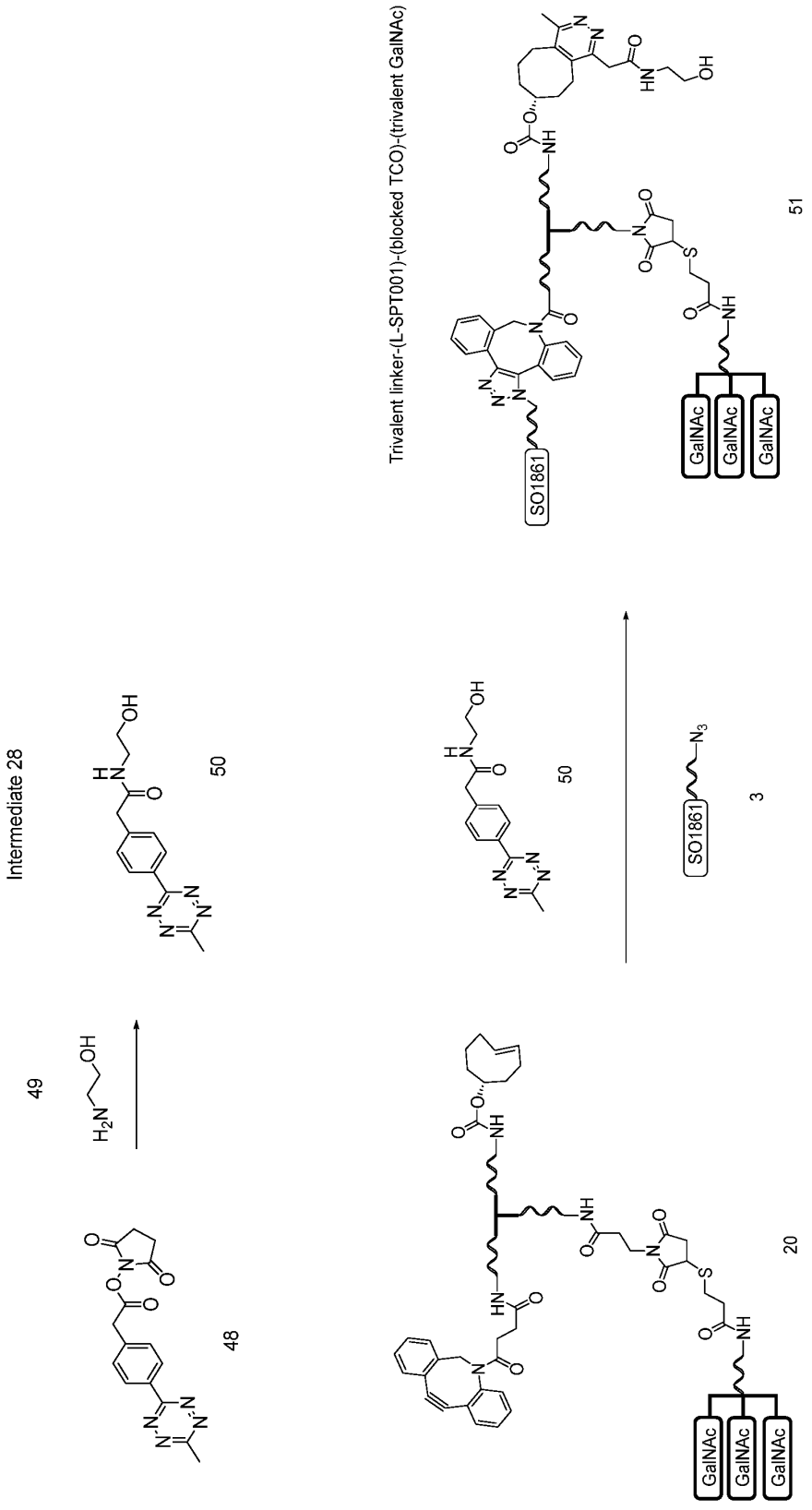

Intermediate 28
49
48
50
50
3
20
51
Trivalent linker-(L-SPT001)-(blocked TCO)-(trivalent GalNAc)
SO1861
GalNAc
GalNAc
GalNAc

Fig. 42A

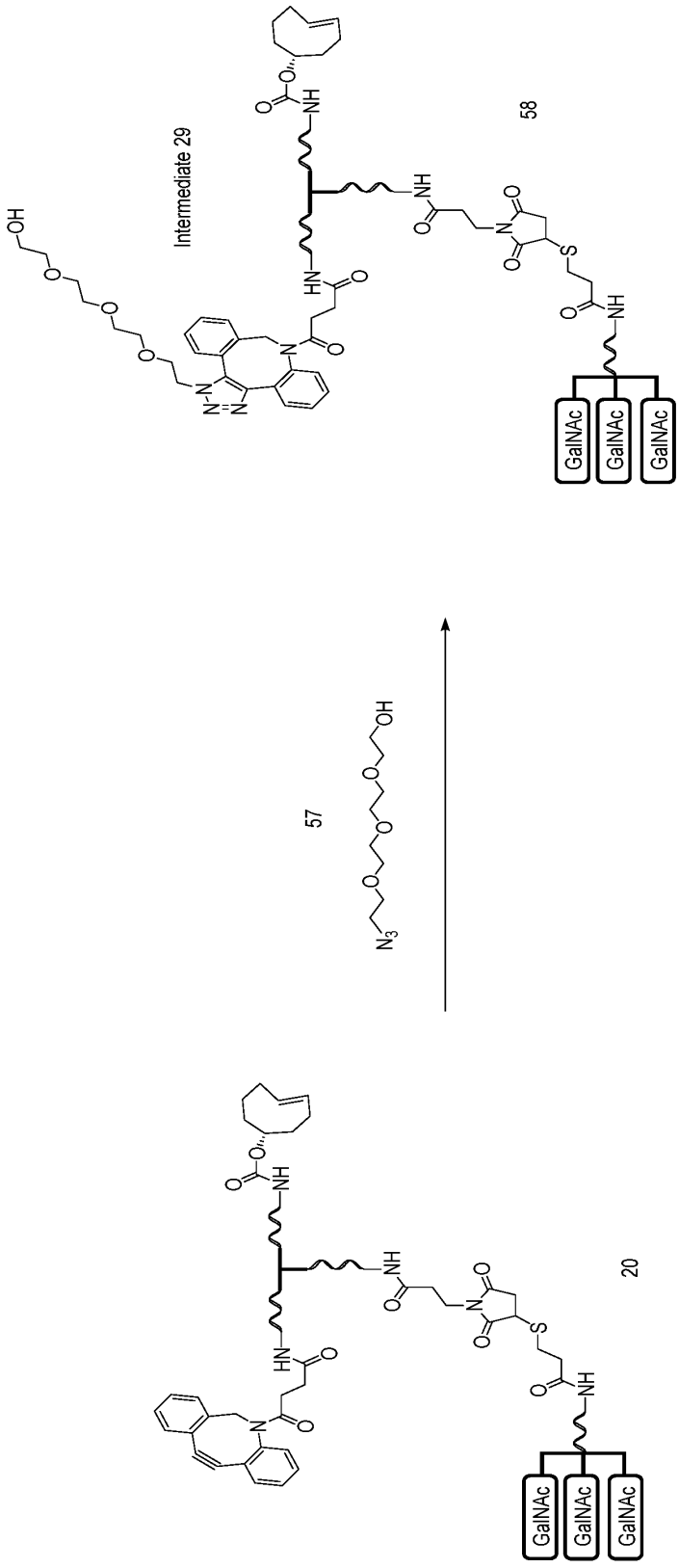


Fig. 42B

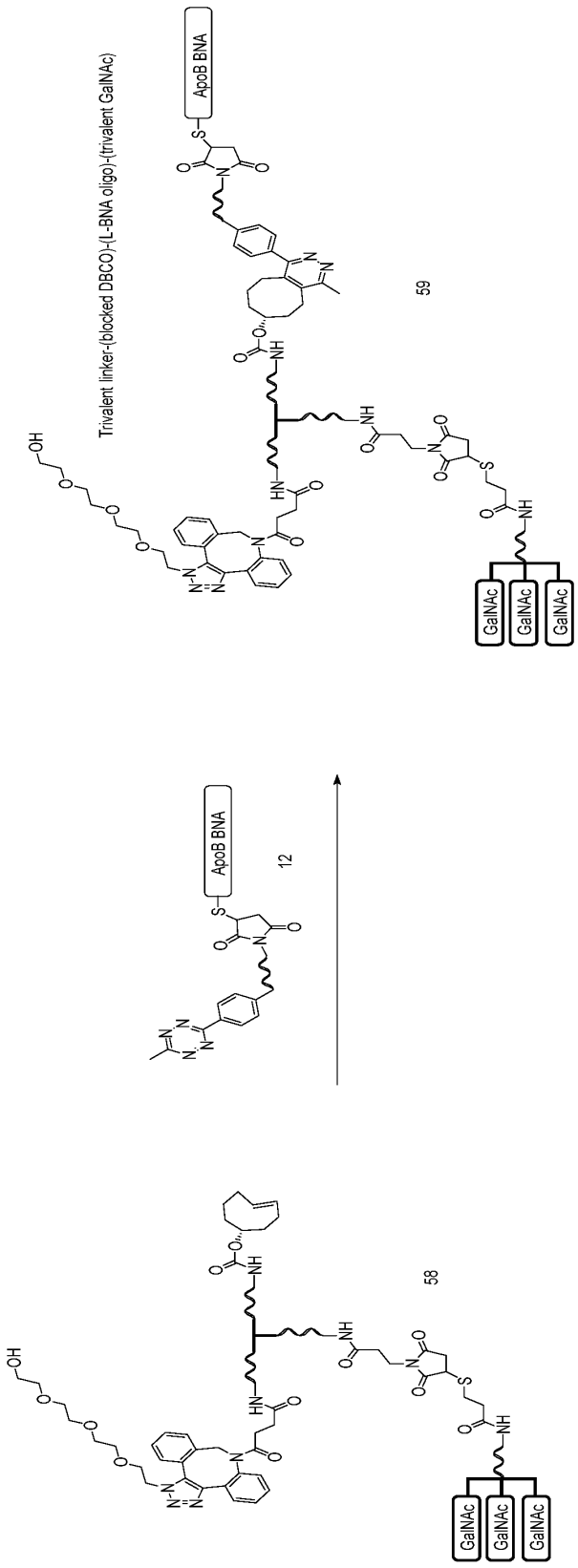

Trivalent linker-(blocked DBCO)-(L-BNA oligo)-(trivalent GalNAc)
ApoB BNA
59
GalNAc
GalNAc
GalNAc
ApoB BNA
12
58
GalNAc
GalNAc
GalNAc

Fig. 43
A

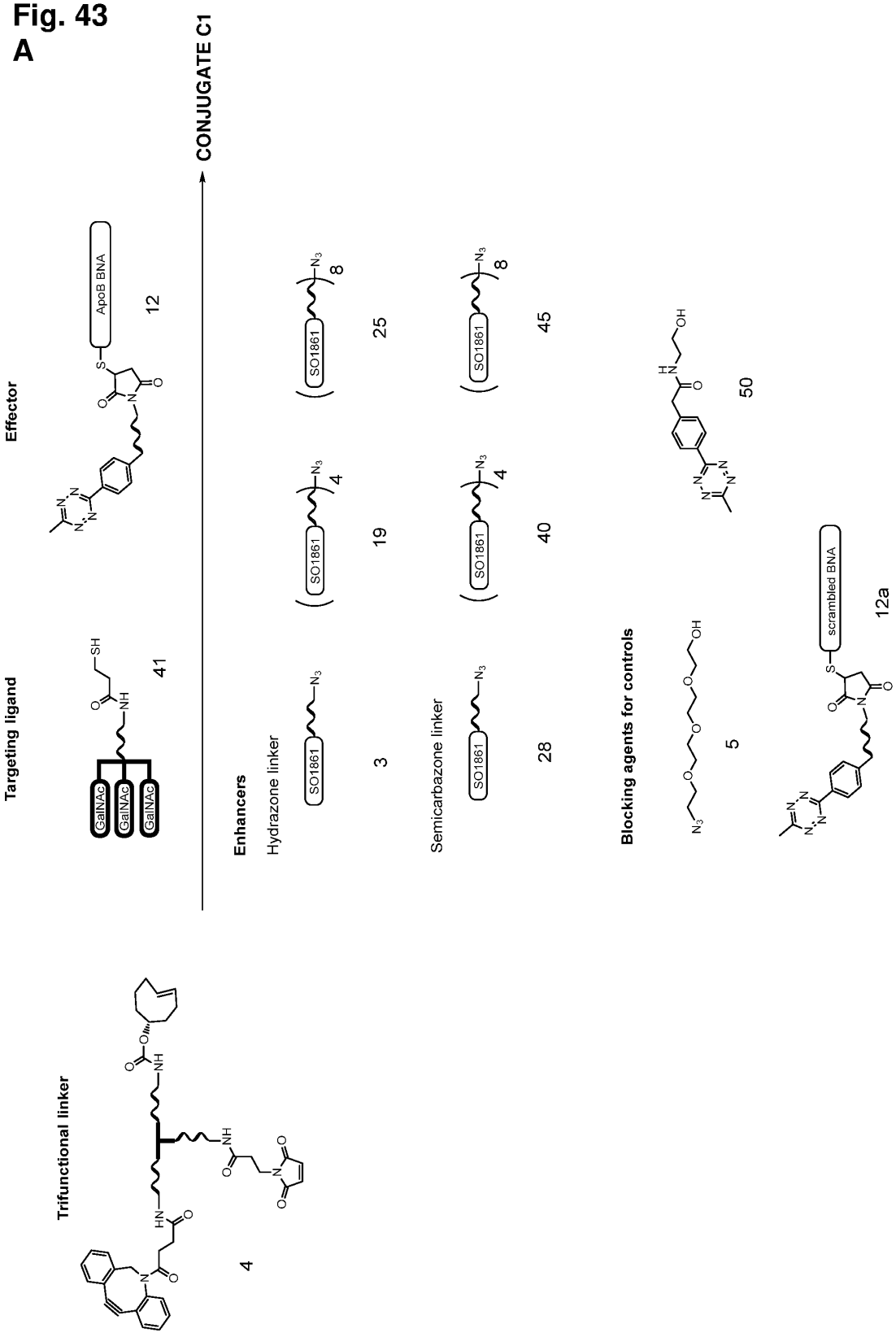
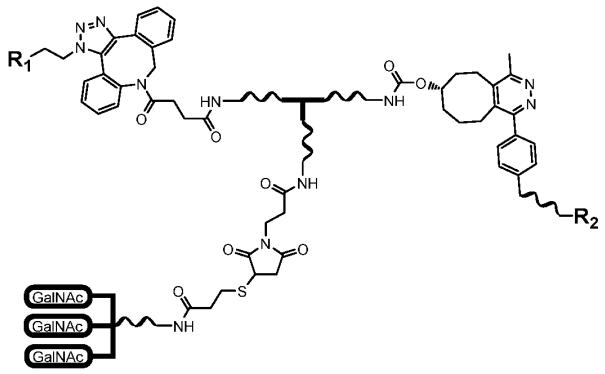

Effector
ApoB BNA
12
CONJUGATE C1
Targeting ligand
GalNAc
GalNAc
GalNAc
41
Enhancers
Hydrazone linker
SO1861
3
SO1861
19
SO1861
25
Semicarbazone linker
SO1861
28
SO1861
40
SO1861
45
Blocking agents for controls
5
50
Trifunctional linker
4
scrambled BNA
12a

Fig. 43
B

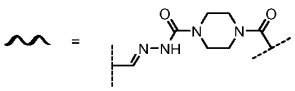
CONJUGATE C1 =



Hydrazone linker



Semicarbazone linker



R_1 =	SO1861	13, 13a, 51	R_1 =	SO1861	31, 31a, 54
R_1 =	SO1861 (4)	22, 22a, 52	R_1 =	SO1861 (4)	42, 42a, 55
R_1 =	SO1861 (8)	26, 26a, 53	R_1 =	SO1861 (8)	47, 47a, 56
R_1 =	HO-(O-)3	59, 59a			

R_2 =	ApoB BNA	13, 22. 26, 31, 42, 47, 59
R_2 =	scrambled BNA	13a, 22a,, 26a, 31a, 42a, 47a. 59a
R_2 =	OH	51, 52, 53, 54, 55, 56