Fig. 1

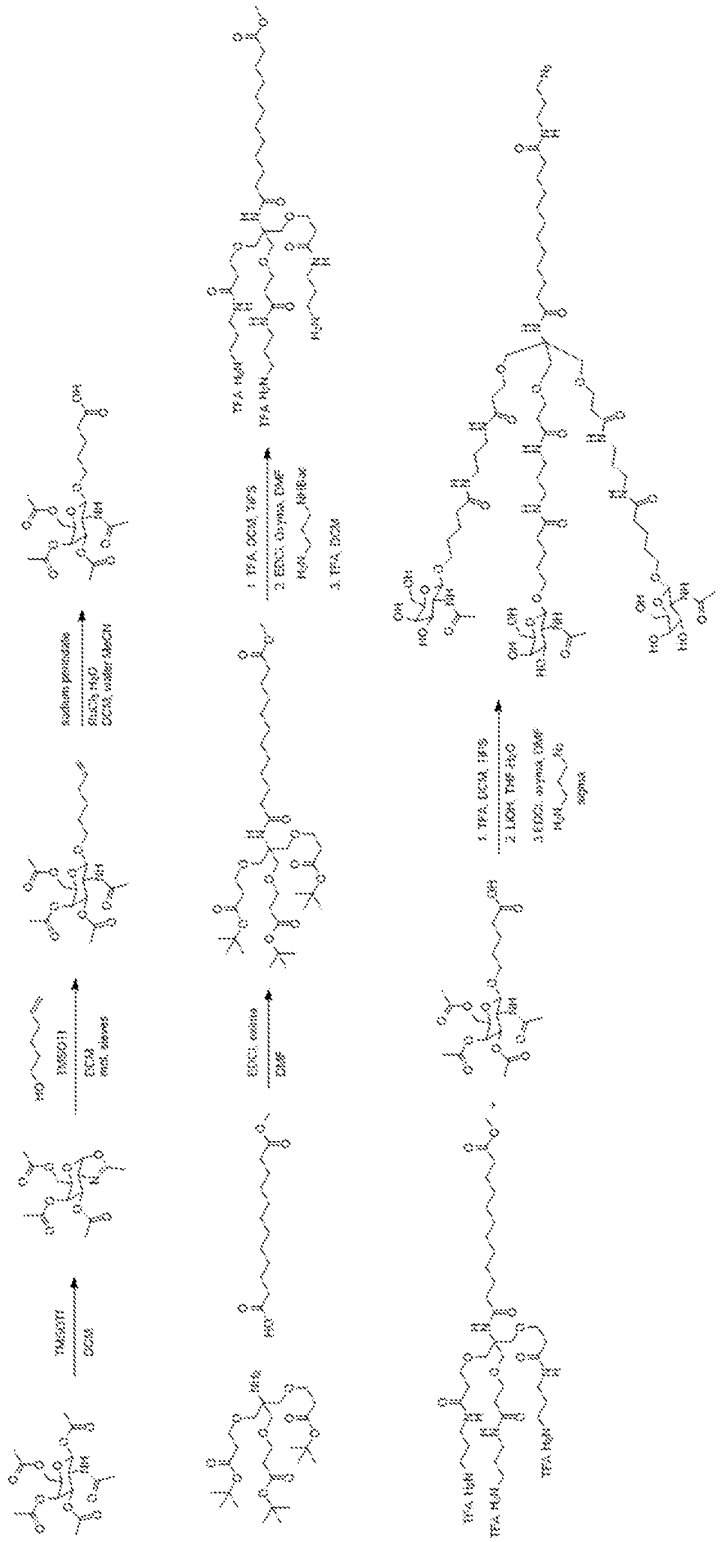


Fig. 2

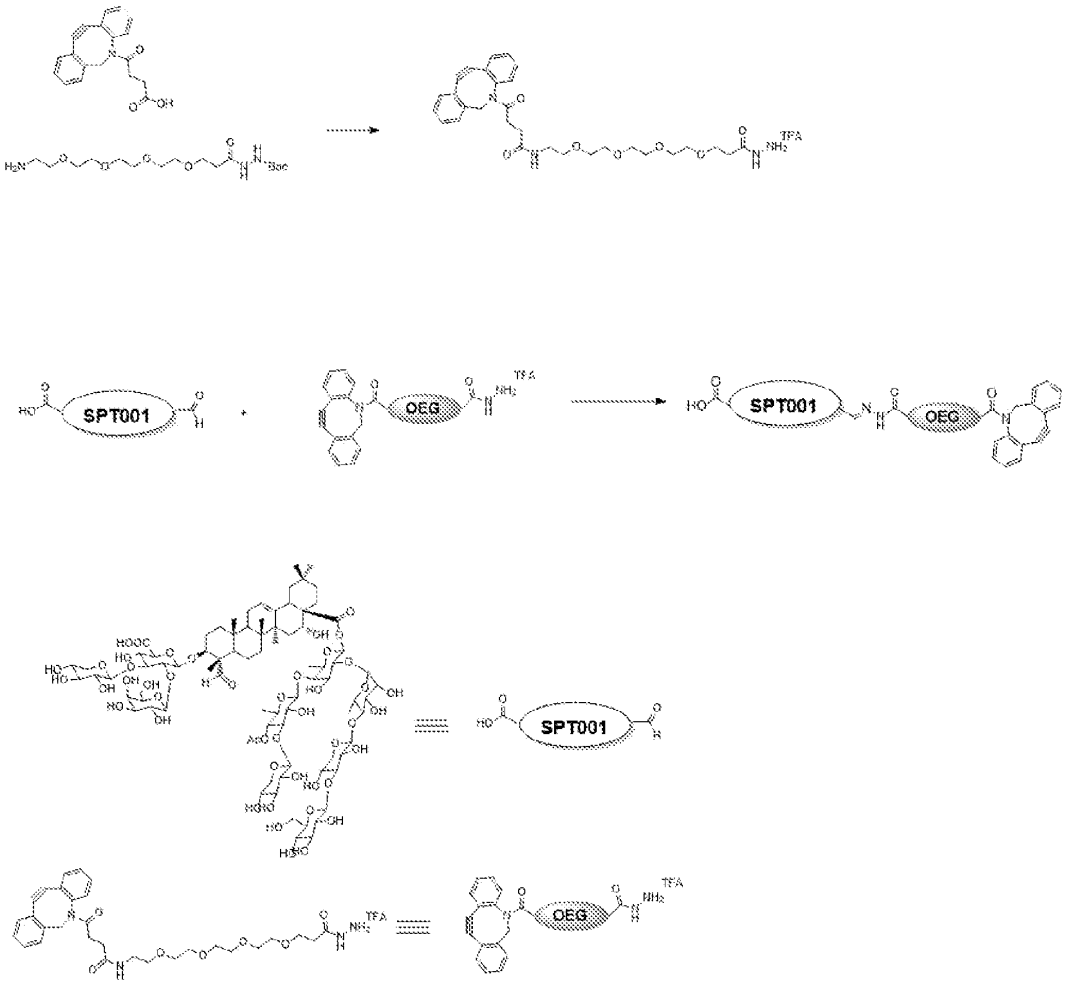


Fig. 3

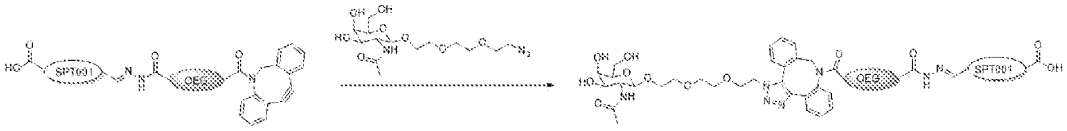


Fig. 4

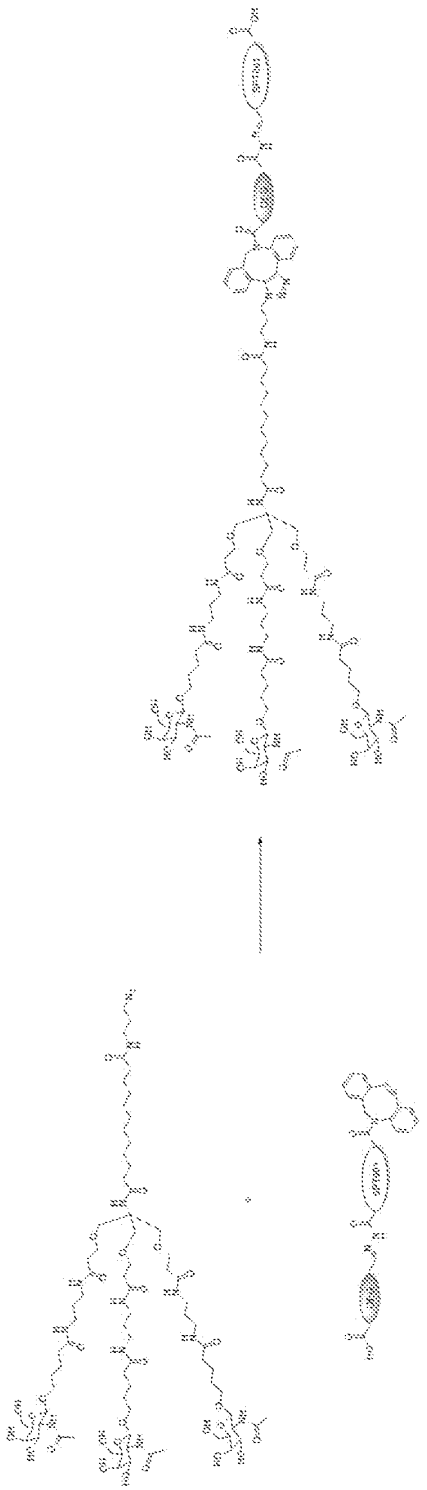


Fig. 5

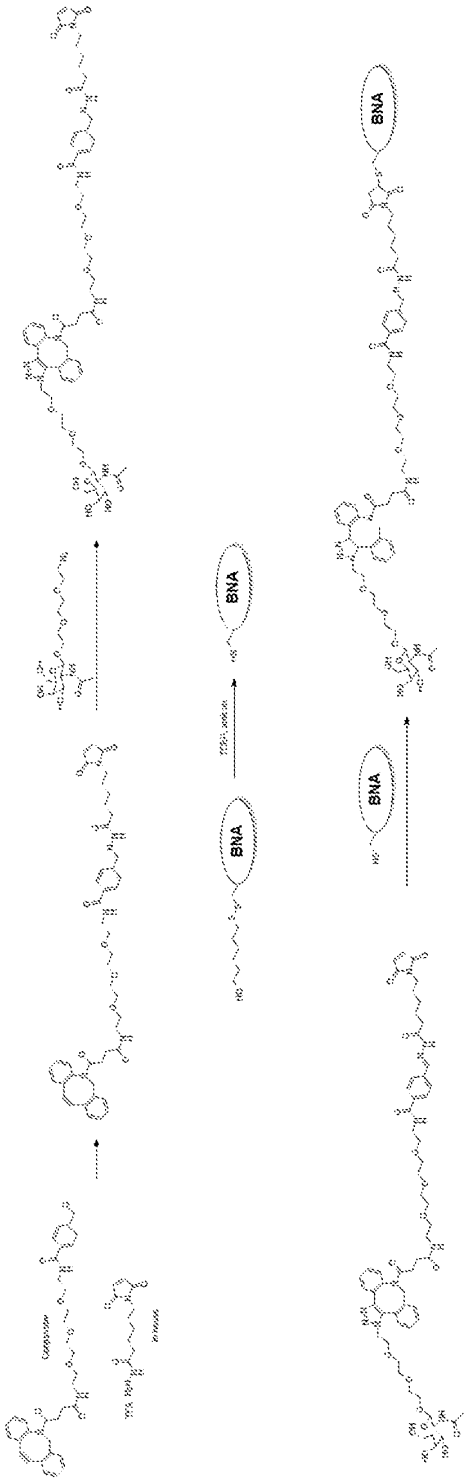


Fig. 6

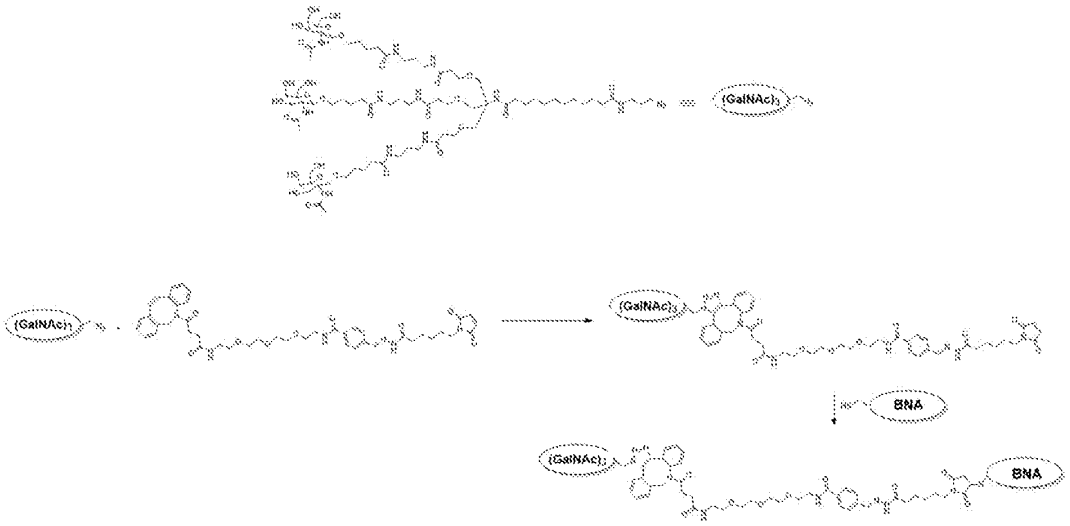


Fig. 7

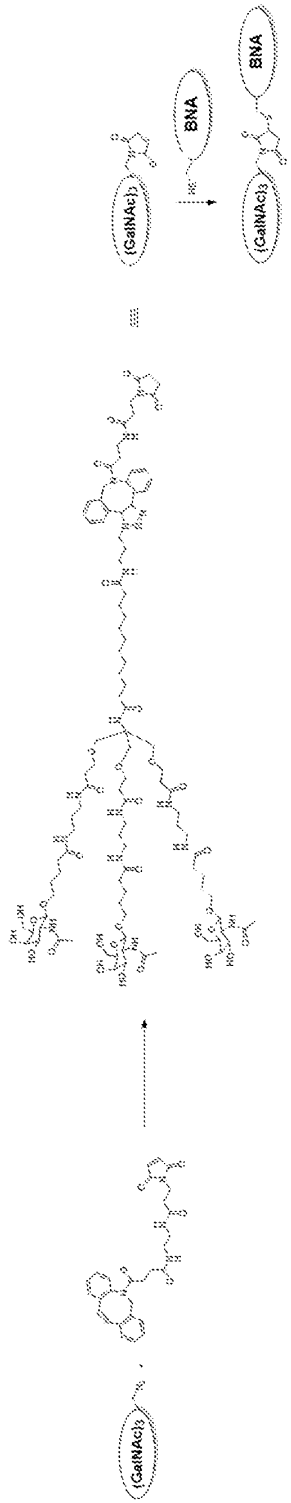


Fig. 8A

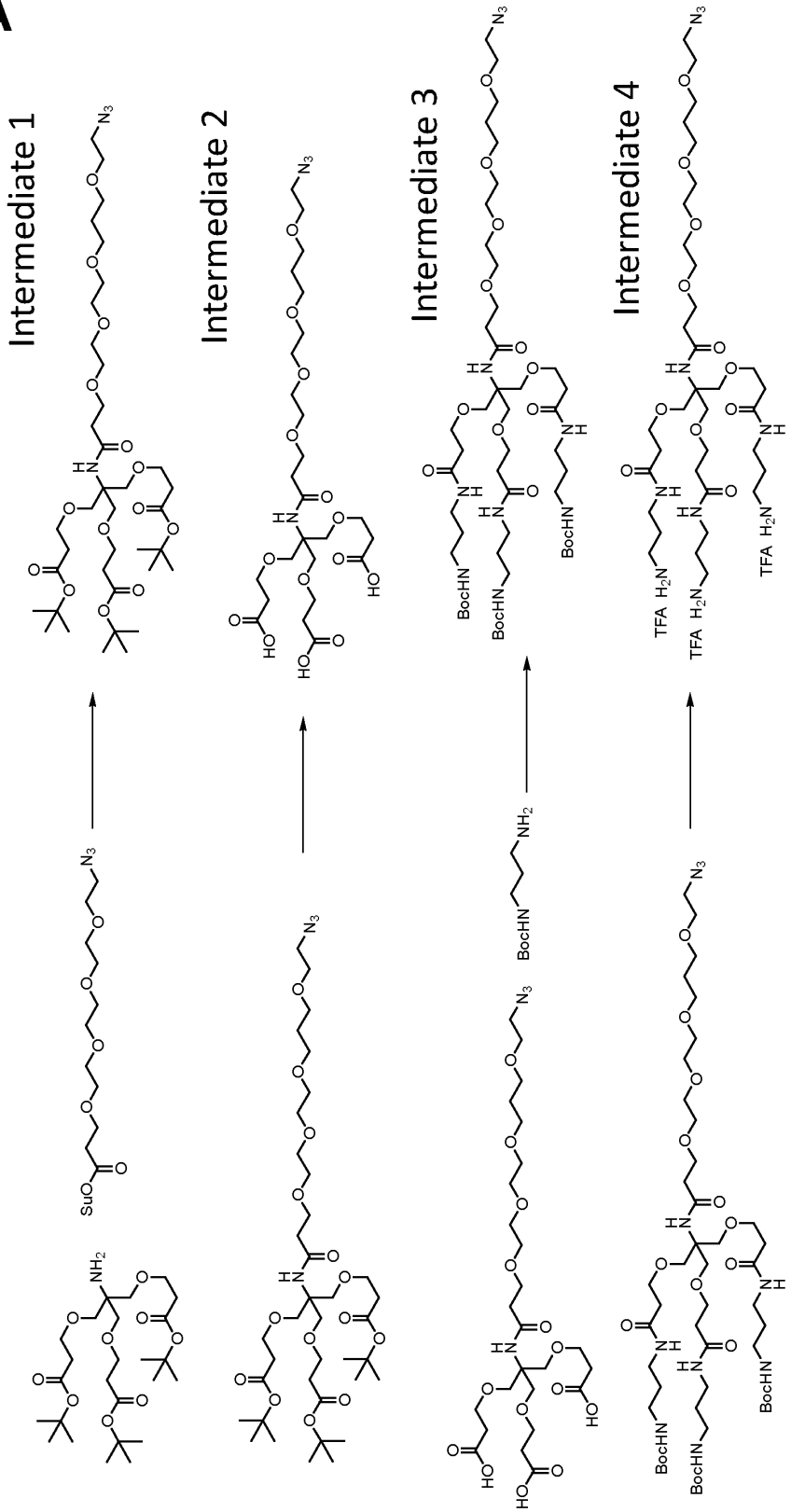

Intermediate 1
Intermediate 2
Intermediate 3
Intermediate 4

Fig. 8B

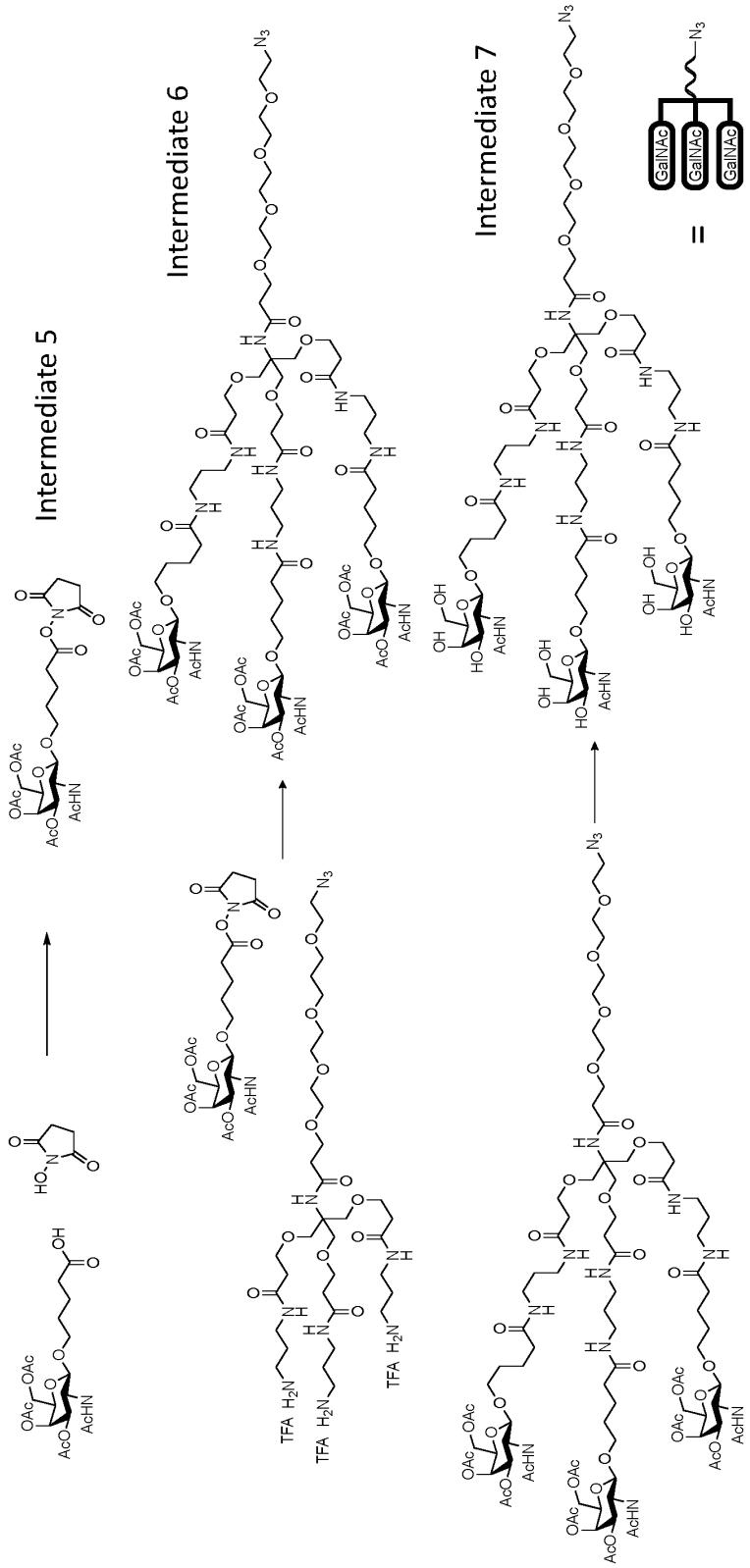

Intermediate 5
Intermediate 6
Intermediate 7
GalNAc
GalNAc
GalNAc
=

Fig. 9A

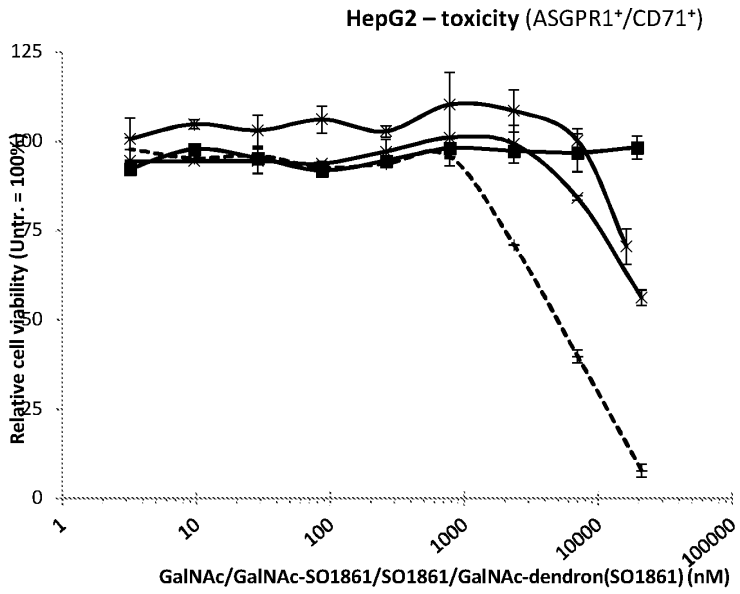

HepG2 – toxicity (ASGPR1+/CD71+)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
1
10
100
1000
10000
100000
GalNAc/GalNAc-SO1861/SO1861/GalNAc-dendron(SO1861) (nM)

Fig. 9B

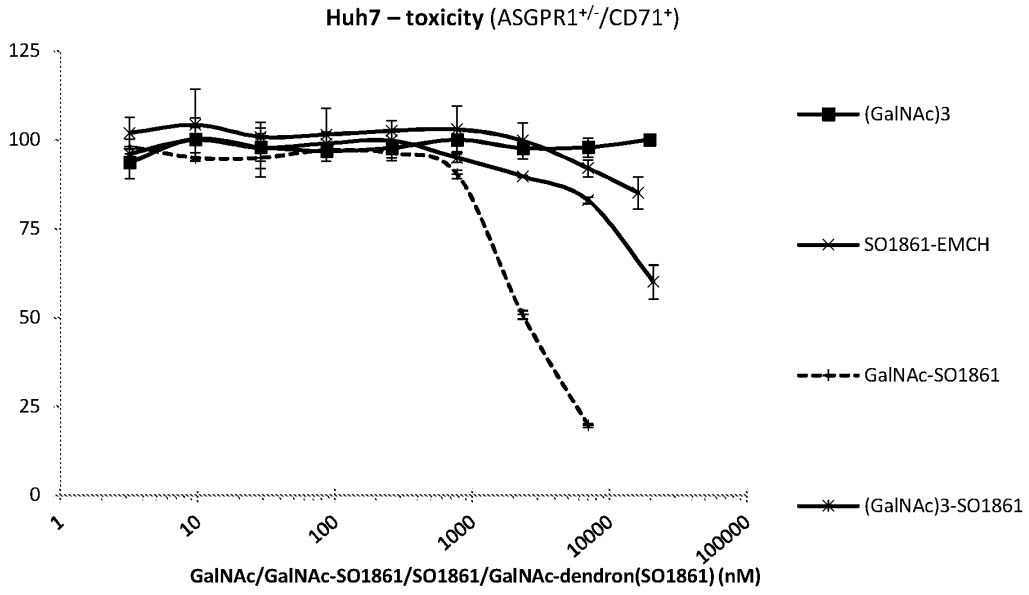

Huh7 – toxicity (ASGPR1+/-/CD71+)
125
100
75
50
25
0
1
10
100
1000
10000
100000
GalNAc/GalNAc-SO1861/SO1861/GalNAc-dendron(SO1861) (nM)
(GalNAc)3
SO1861-EMCH
GalNAc-SO1861
(GalNAc)3-SO1861

Fig. 10A

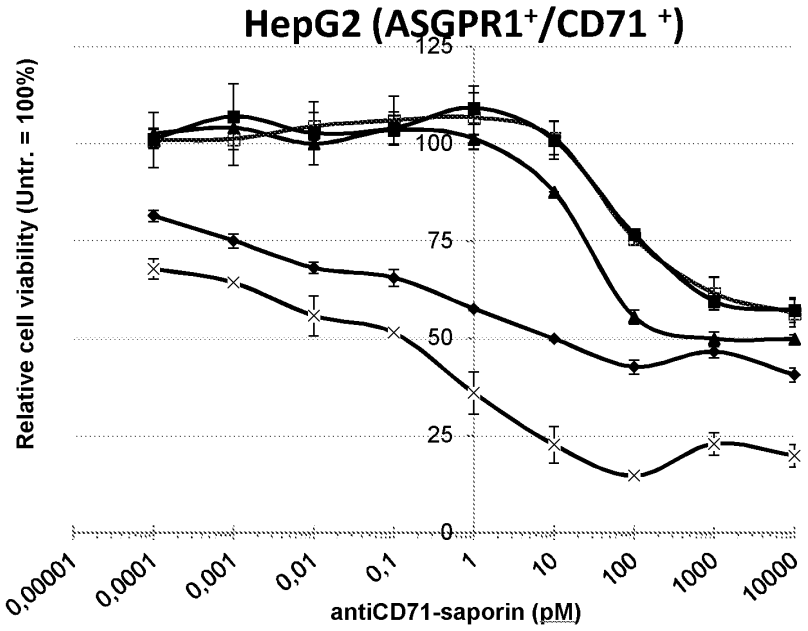


Fig. 10B

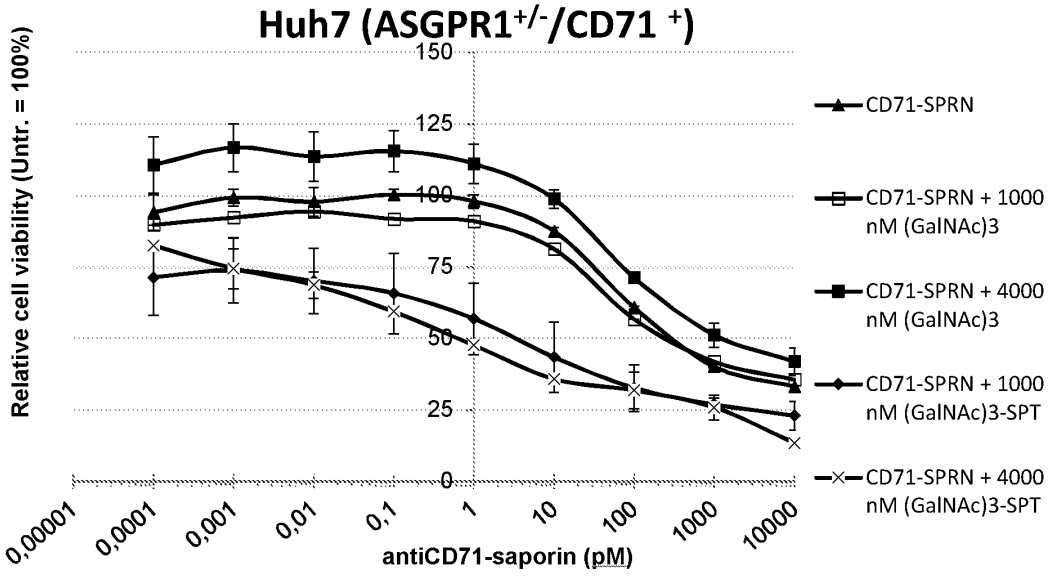


Fig. 11A

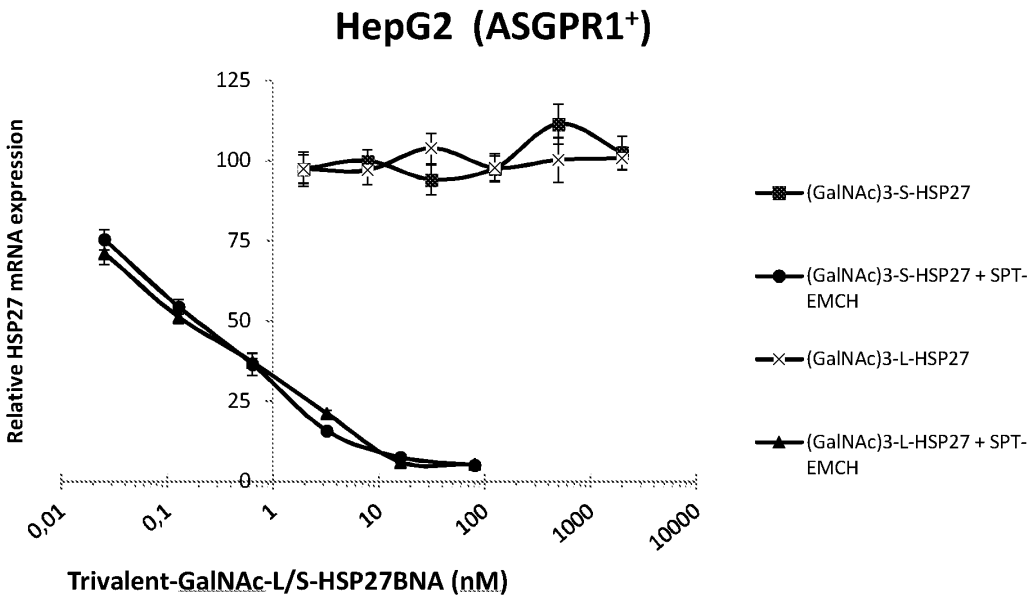


Fig. 11B

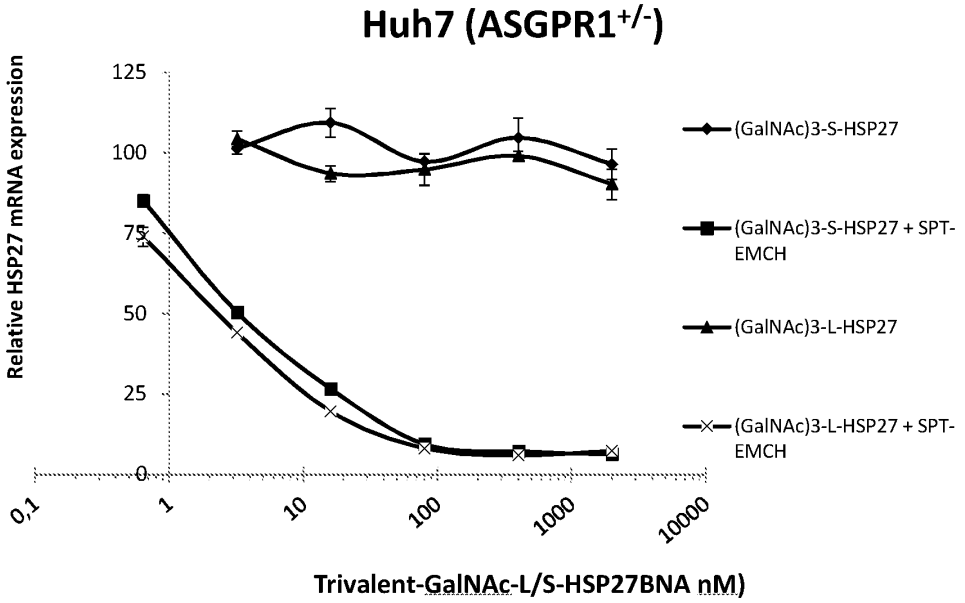


Fig. 12A

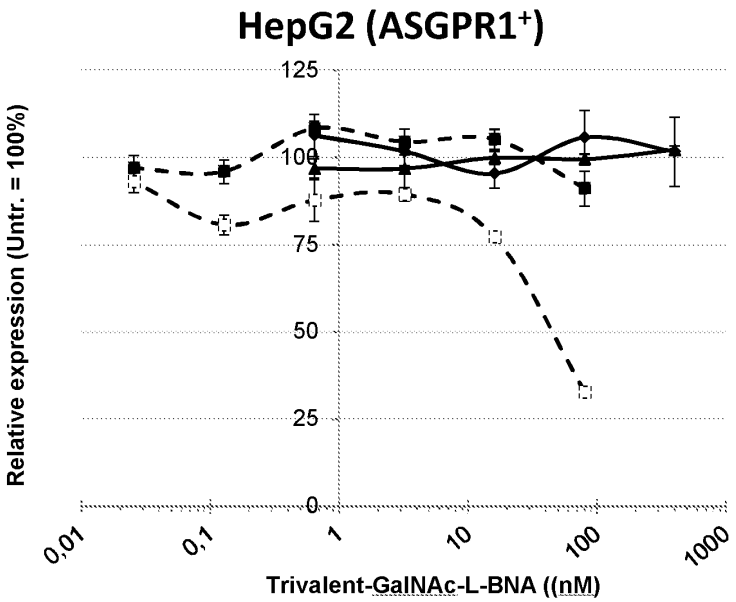


Fig. 12B

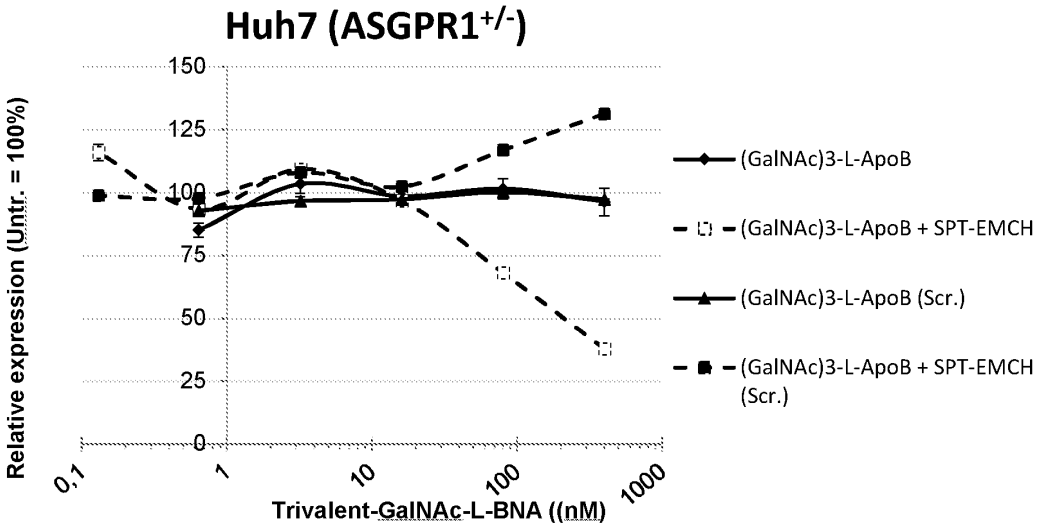


Fig. 13A

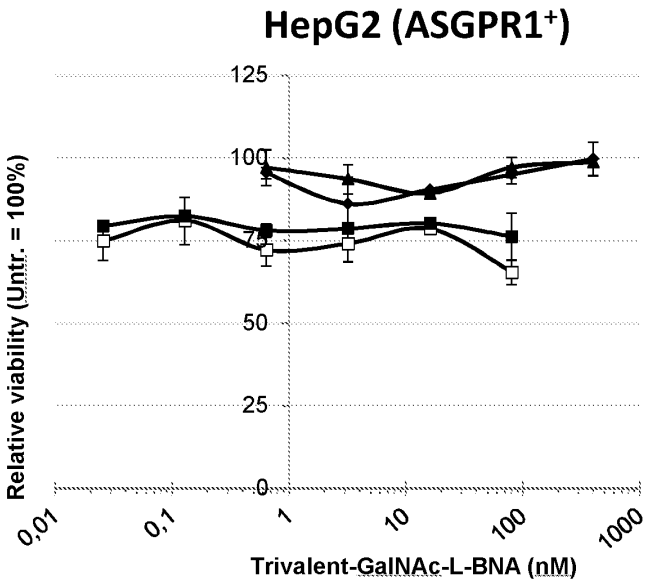


Fig. 13B

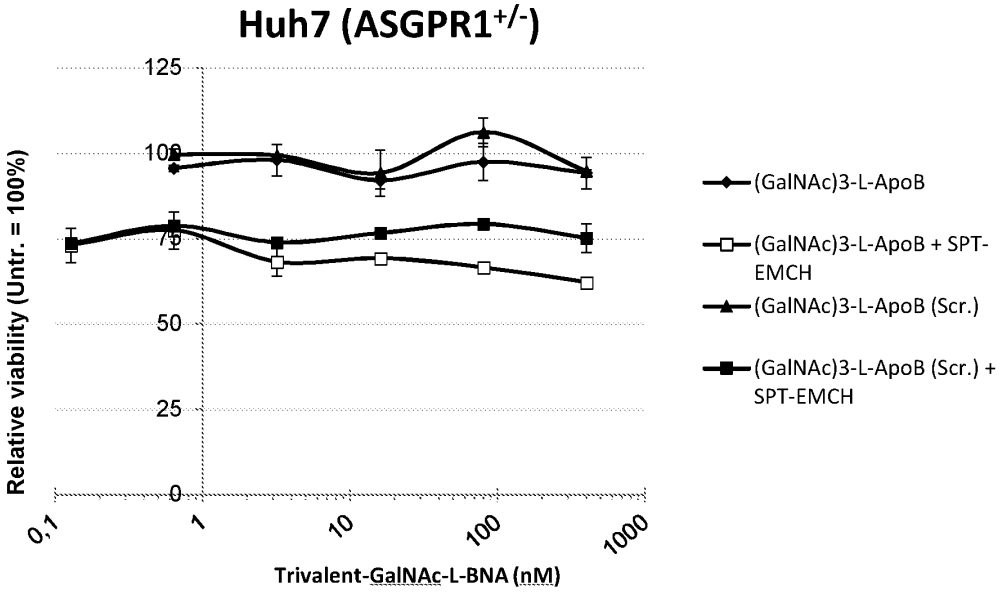


Fig. 14A

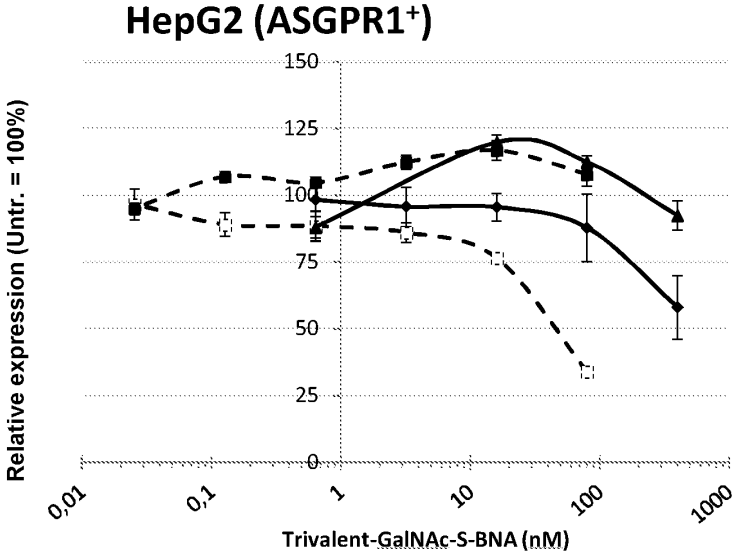


Fig. 14B

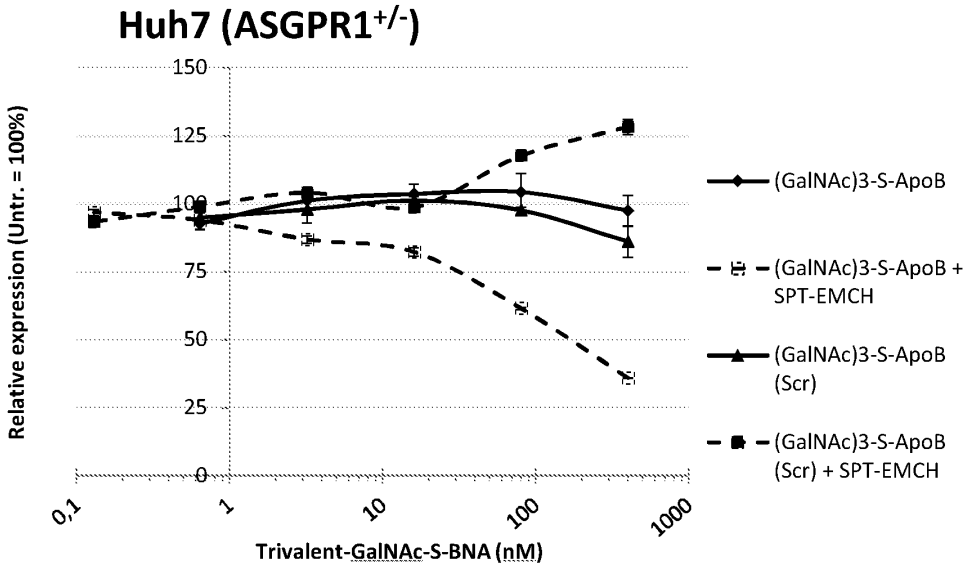


Fig. 15A

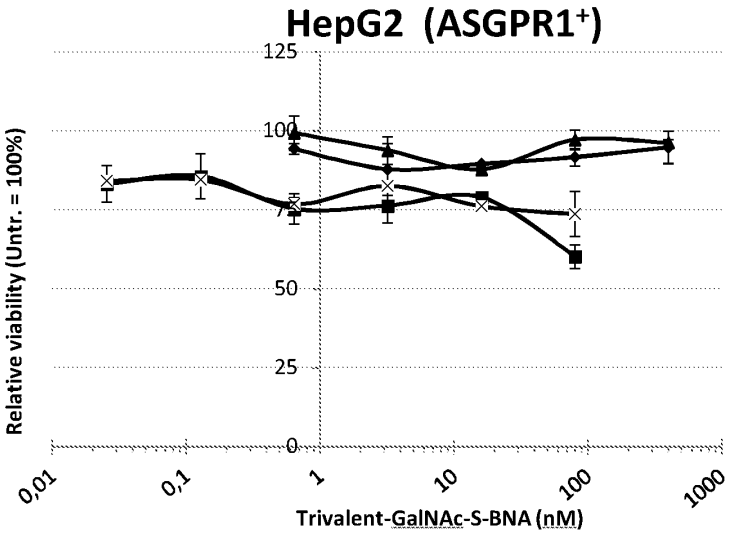


Fig. 15B

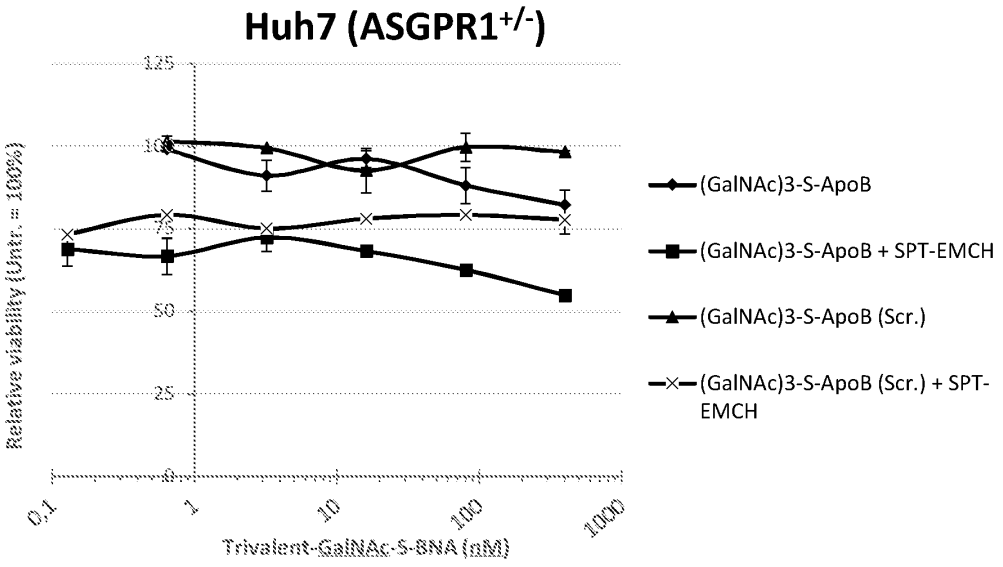


Fig. 16A

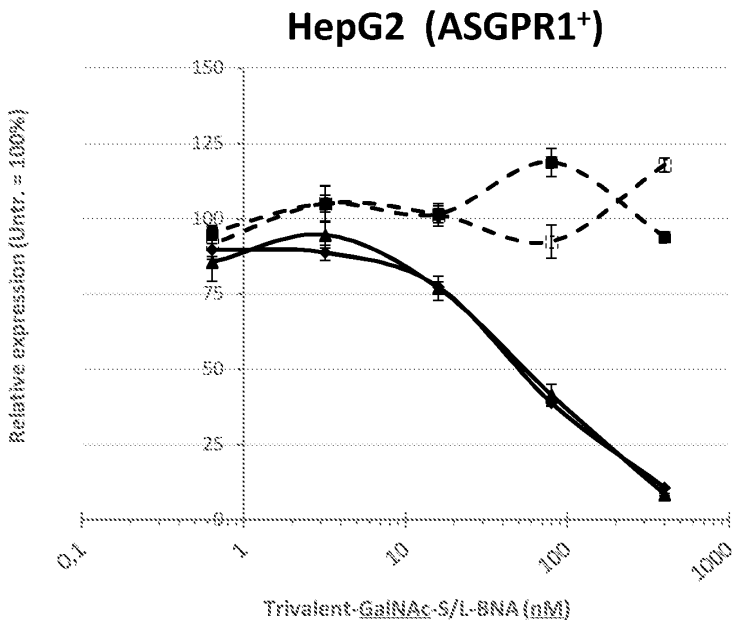


Fig. 16B

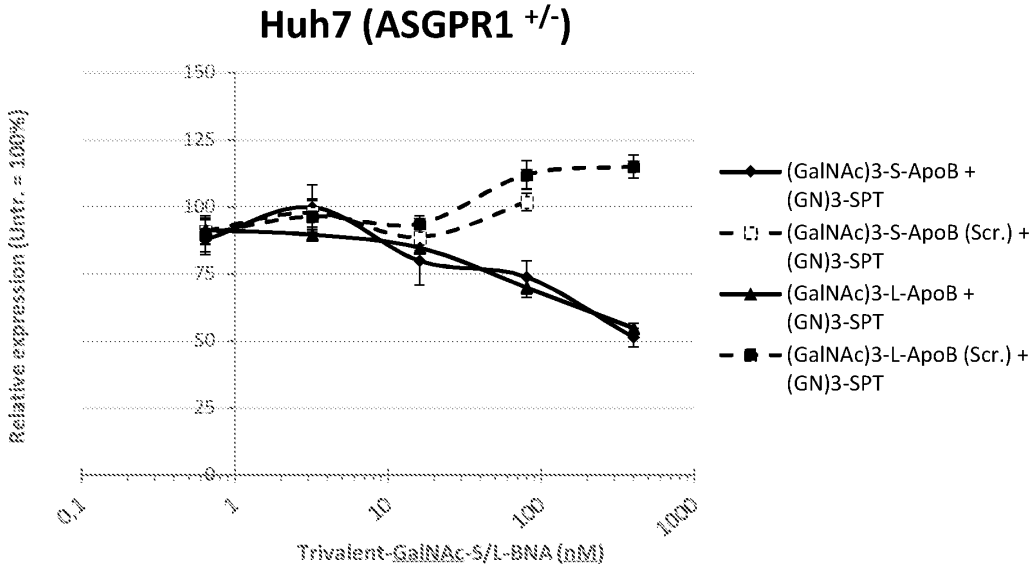


Fig. 17A

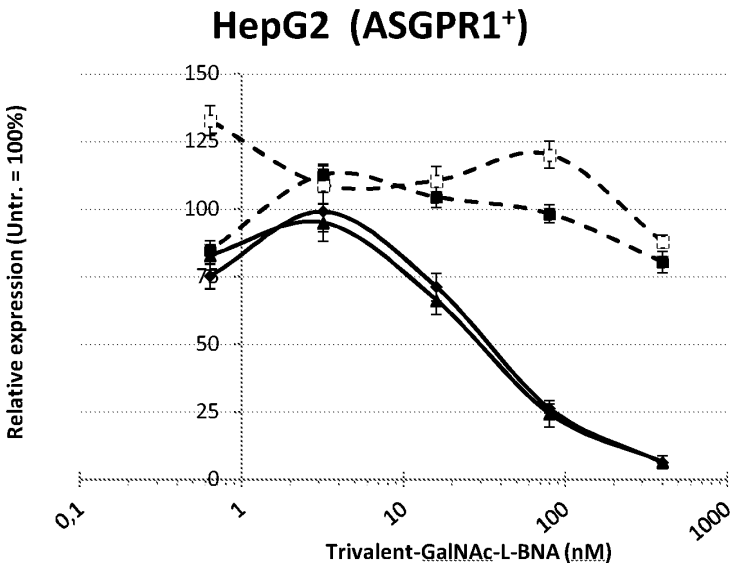


Fig. 17B

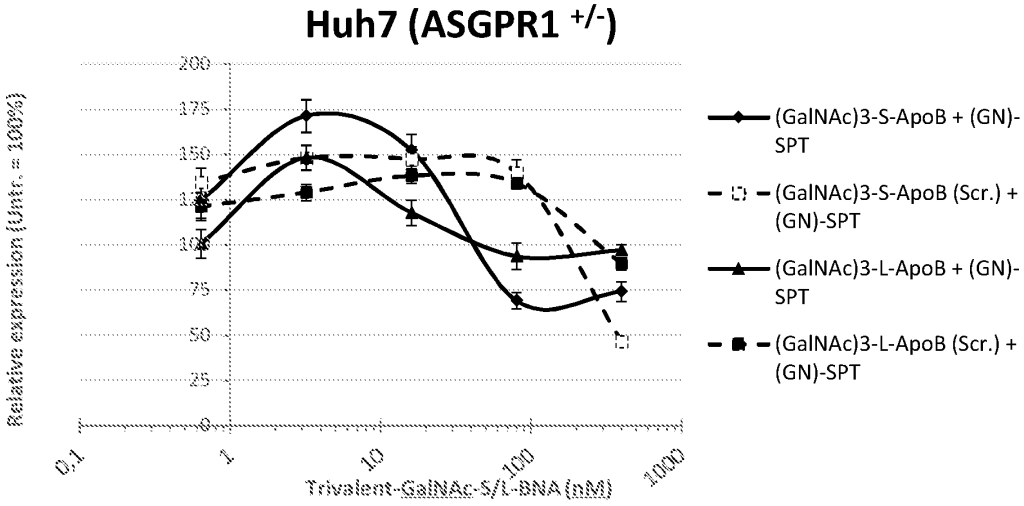


Fig. 18

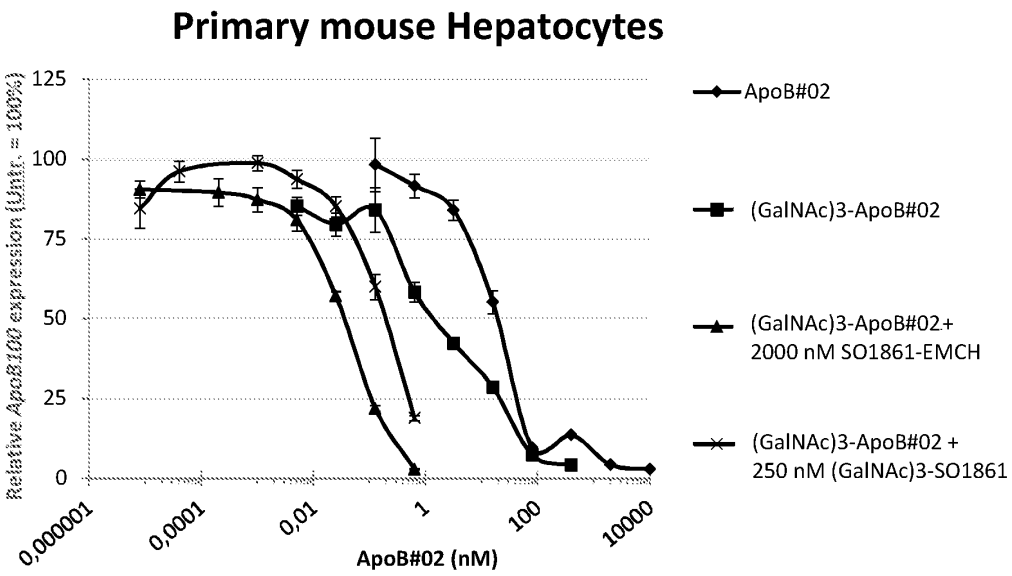

Primary mouse Hepatocytes
Relative ApoB100 expression (Unt. = 100%)
125
100
75
50
25
0
0,000001
0,0001
0,01
1
100
10000
ApoB#02 (nM)
ApoB#02
(GalNAc)3-ApoB#02
(GalNAc)3-ApoB#02+
2000 nM SO1861-EMCH
(GalNAc)3-ApoB#02 +
250 nM (GalNAc)3-SO1861

Fig. 19

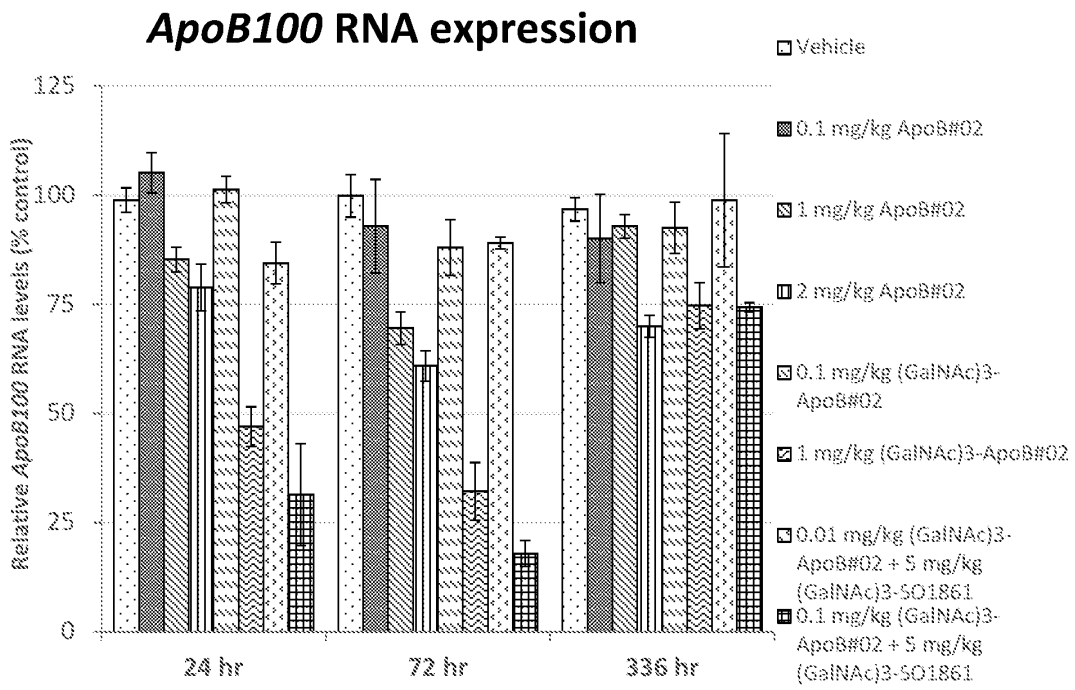

ApoB100 RNA expression
Relative ApoB100 RNA levels (% control)
125
100
75
50
25
0
24 hr
72 hr
336 hr
Vehicle
0.1 mg/kg ApoB#02
1 mg/kg ApoB#02
2 mg/kg ApoB#02
0.1 mg/kg (GalNAc)3-ApoB#02
1 mg/kg (GalNAc)3-ApoB#02
0.01 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
0.1 mg/kg (GalNAc)3-ApoB#02 + 5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

Fig. 20

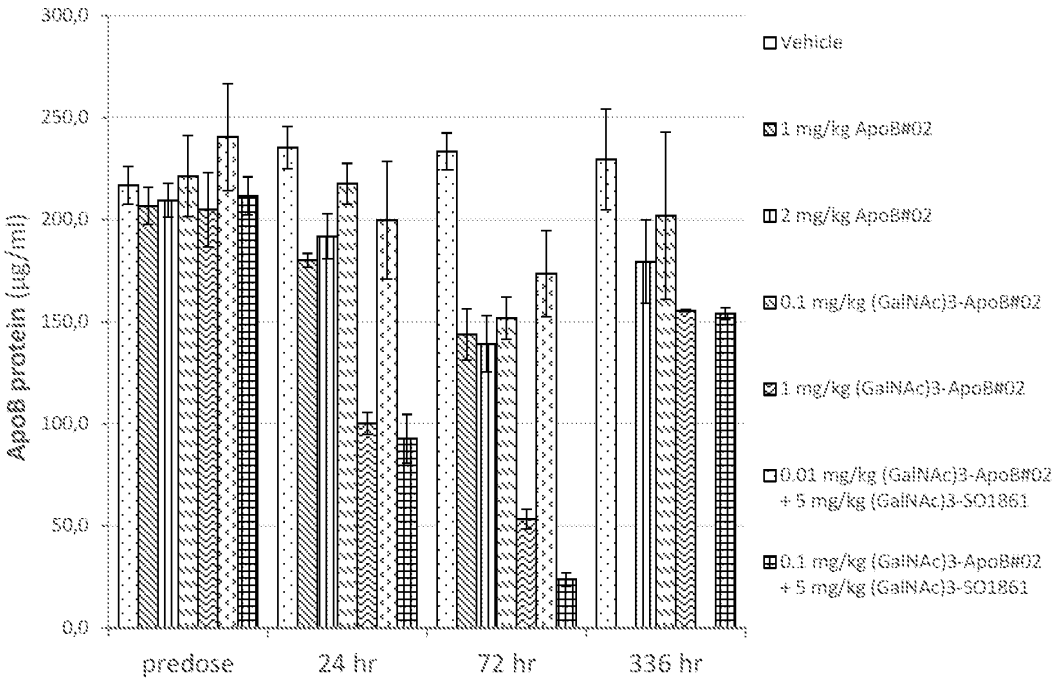

300,0
250,0
200,0
150,0
100,0
50,0
0,0
ApoB protein (µg/ml)
predose
24 hr
72 hr
336 hr
Vehicle
1 mg/kg ApoB#02
2 mg/kg ApoB#02
0.1 mg/kg (GalNAc)3-ApoB#02
1 mg/kg (GalNAc)3-ApoB#02
0.01 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
0.1 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861

Fig. 21

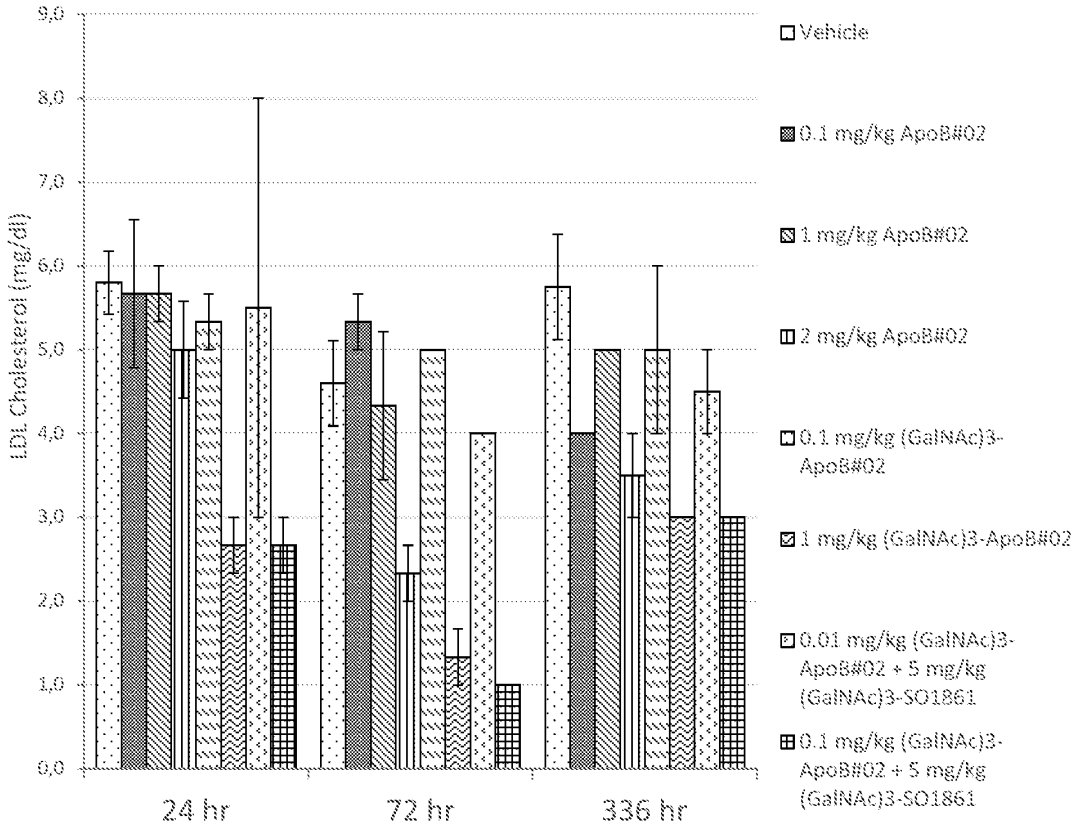

9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
LDL Cholesterol (mg/dl)
24 hr
72 hr
336 hr
Vehicle
0.1 mg/kg ApoB#02
1 mg/kg ApoB#02
2 mg/kg ApoB#02
0.1 mg/kg (GalNAc)3-ApoB#02
1 mg/kg (GalNAc)3-ApoB#02
0.01 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
0.1 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861

Fig. 22

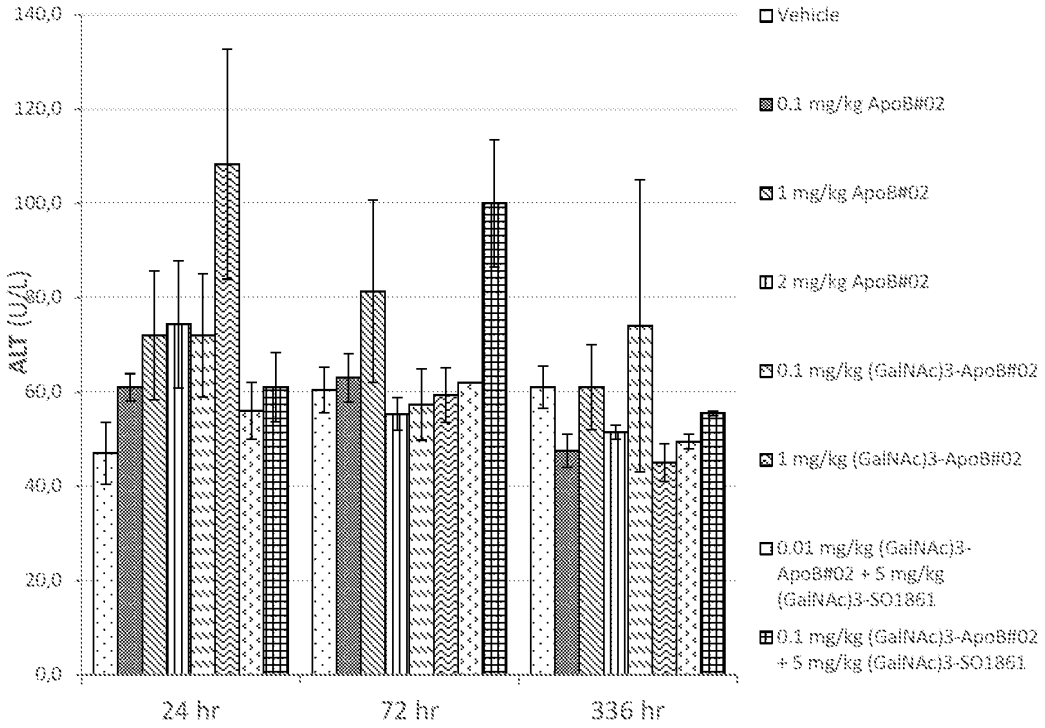

140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
ALT (U/L)
24 hr
72 hr
336 hr
Vehicle
0.1 mg/kg ApoB#02
1 mg/kg ApoB#02
2 mg/kg ApoB#02
0.1 mg/kg (GalNAc)3-ApoB#02
1 mg/kg (GalNAc)3-ApoB#02
0.01 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861
0.1 mg/kg (GalNAc)3-ApoB#02 + 5 mg/kg (GalNAc)3-SO1861

Fig. 23

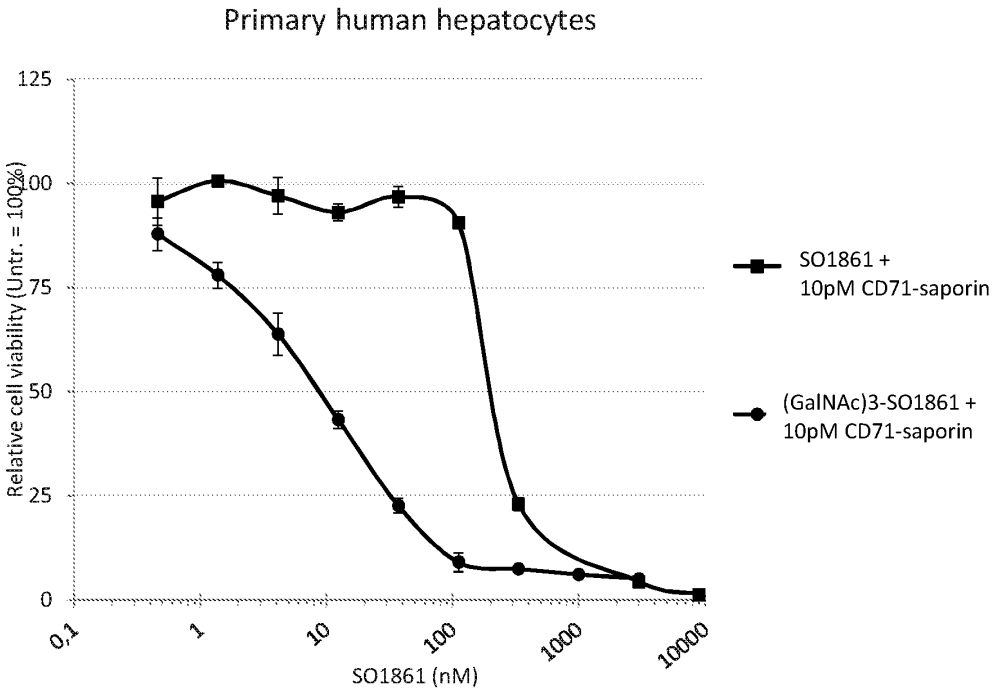

Primary human hepatocytes
125
100
75
50
25
0
Relative cell viability (Untr. = 100%)
0,1
1
10
100
1000
10000
SO1861 (nM)
SO1861 +
10pM CD71-saporin
(GalNAc)3-SO1861 +
10pM CD71-saporin

Fig. 24

A

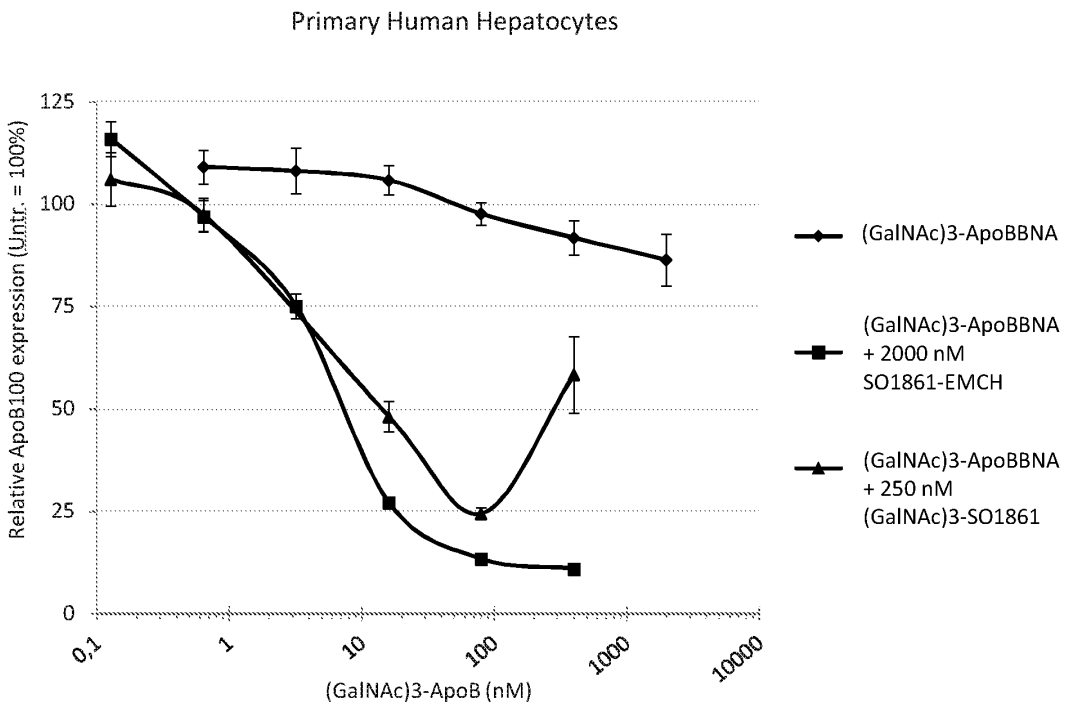

Primary Human Hepatocytes
Relative ApoB100 expression (Untr. = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
10000
(GalNAc)3-ApoB (nM)
(GalNAc)3-ApoBBNA
(GalNAc)3-ApoBBNA + 2000 nM SO1861-EMCH
(GalNAc)3-ApoBBNA + 250 nM (GalNAc)3-SO1861

Fig. 24

B

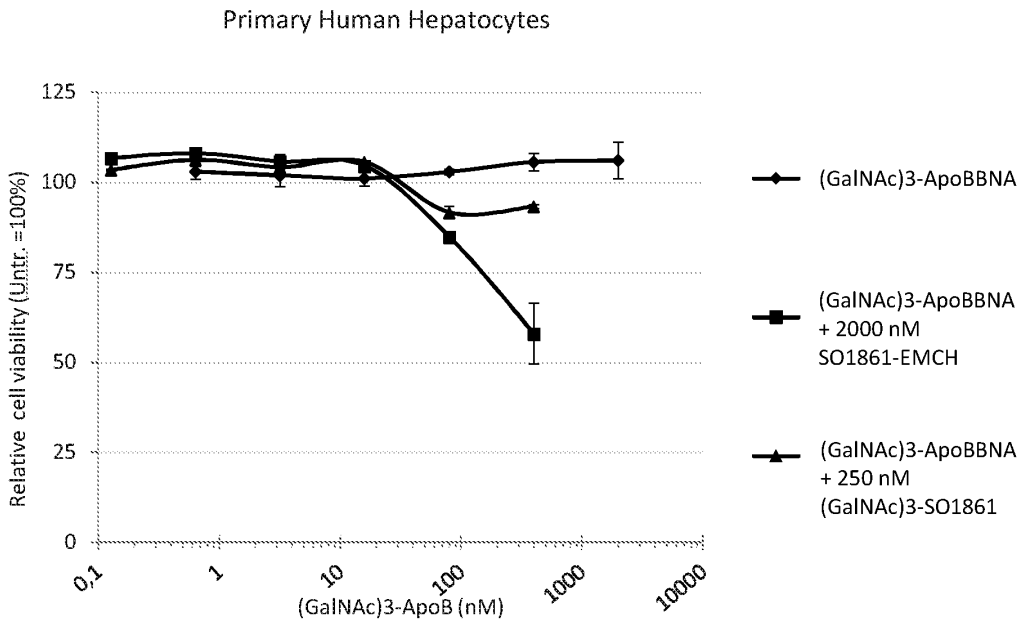

Primary Human Hepatocytes
Relative cell viability (Untr. =100%)
125
100
75
50
25
0
0,1
1
10
100
1000
10000
(GalNAc)3-ApoB (nM)
(GalNAc)3-ApoBBNA
(GalNAc)3-ApoBBNA + 2000 nM SO1861-EMCH
(GalNAc)3-ApoBBNA + 250 nM (GalNAc)3-SO1861

Fig. 25

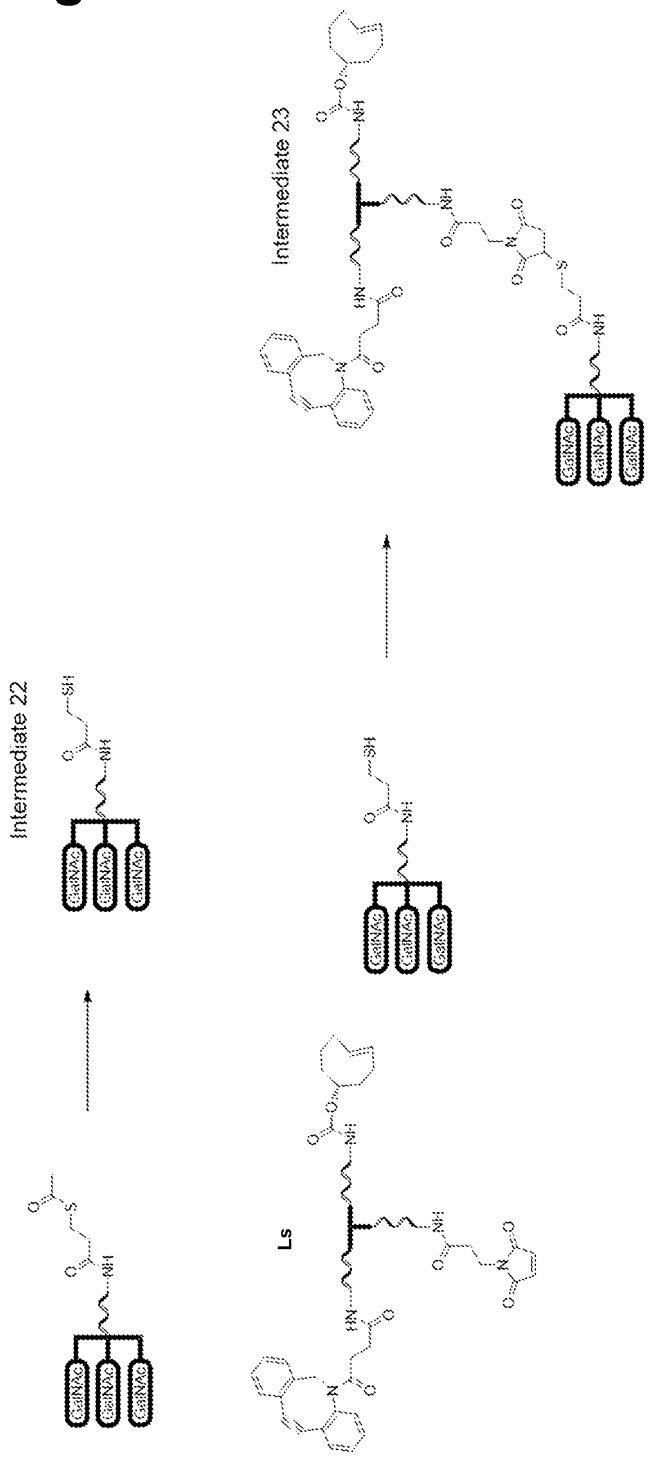


Fig. 26

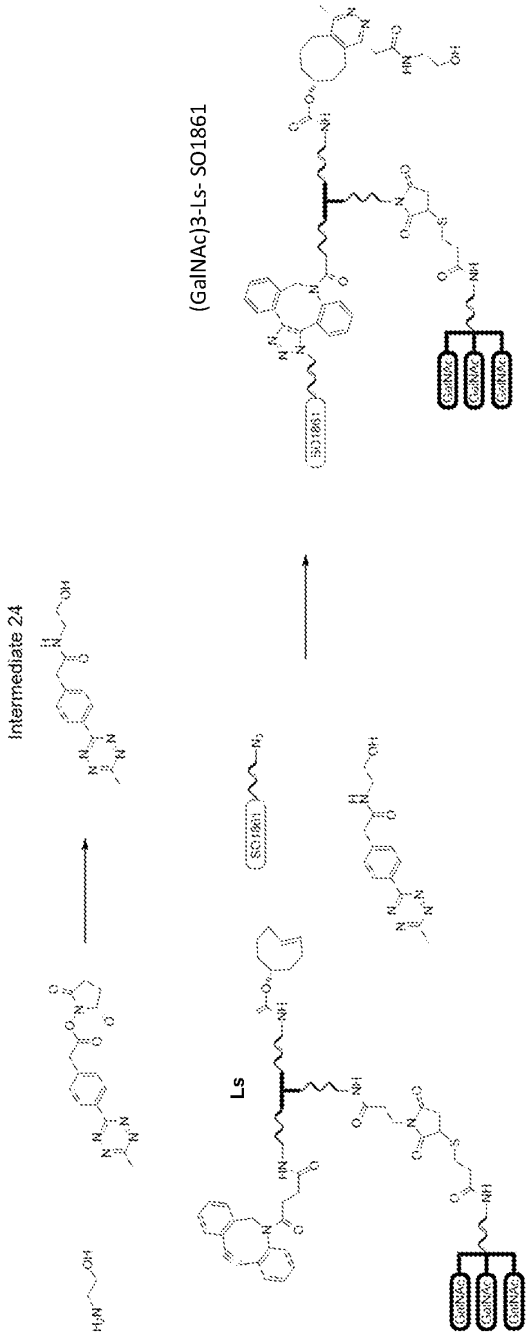


Fig. 27

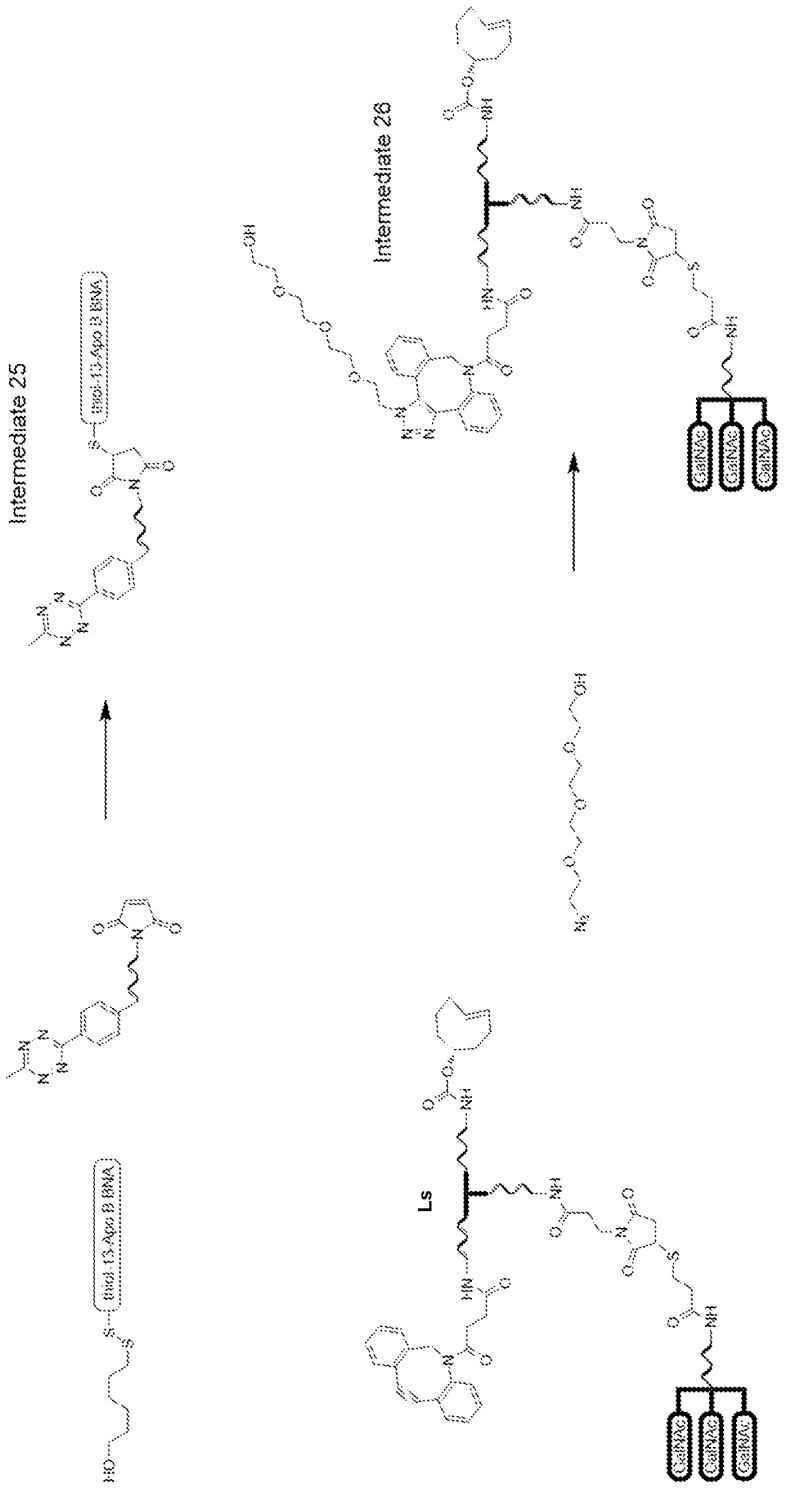


Fig. 28

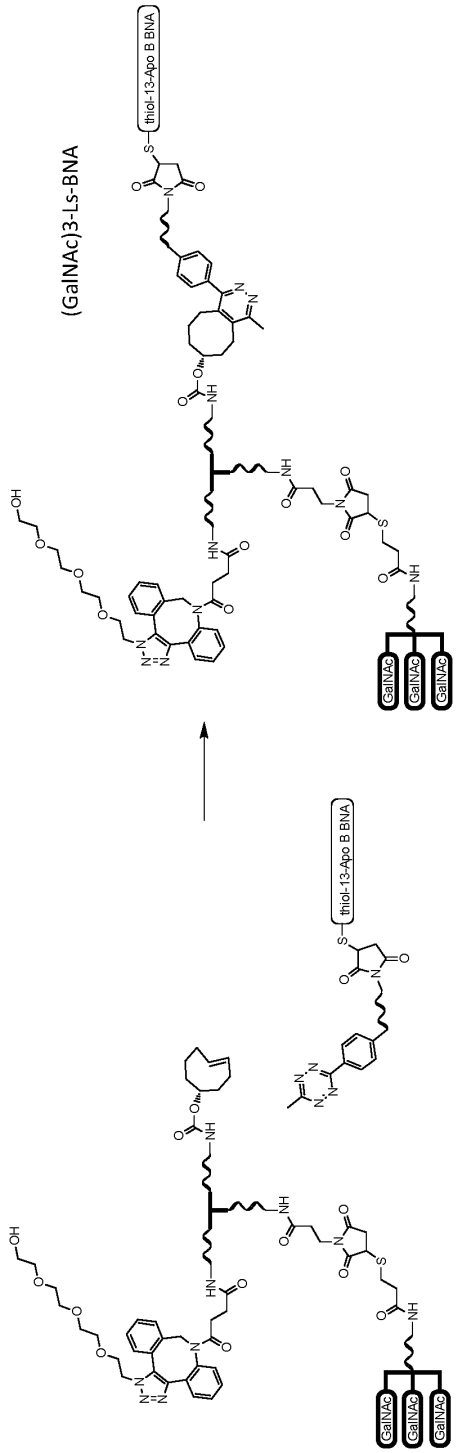
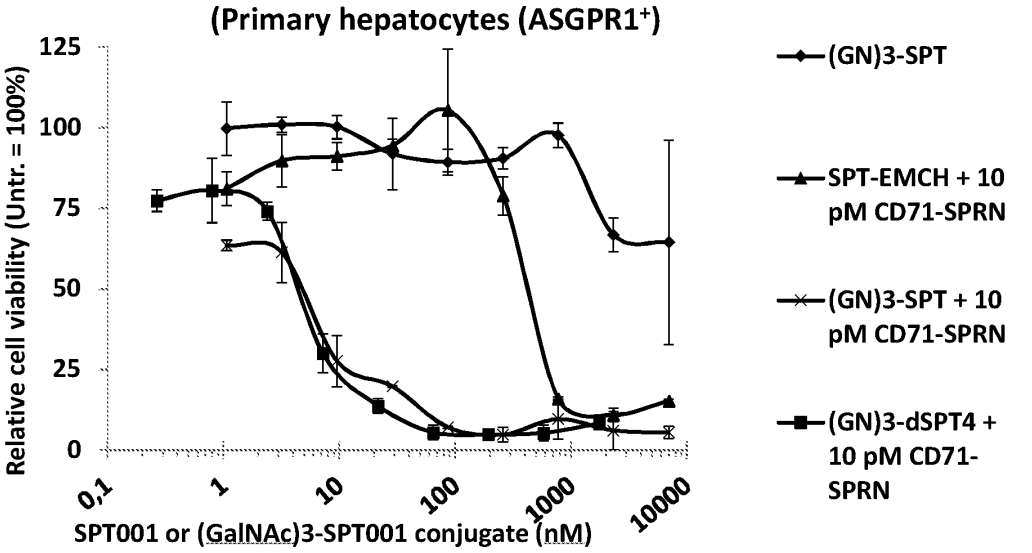

(GalNAc)3-Ls-BNA
thiol-13-Apo B BNA
GalNAc
GalNAc
GalNAc
thiol-13-Apo B BNA
GalNAc
GalNAc
GalNAc

FIG. 29

A


(Primary hepatocytes (ASGPR1+)
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

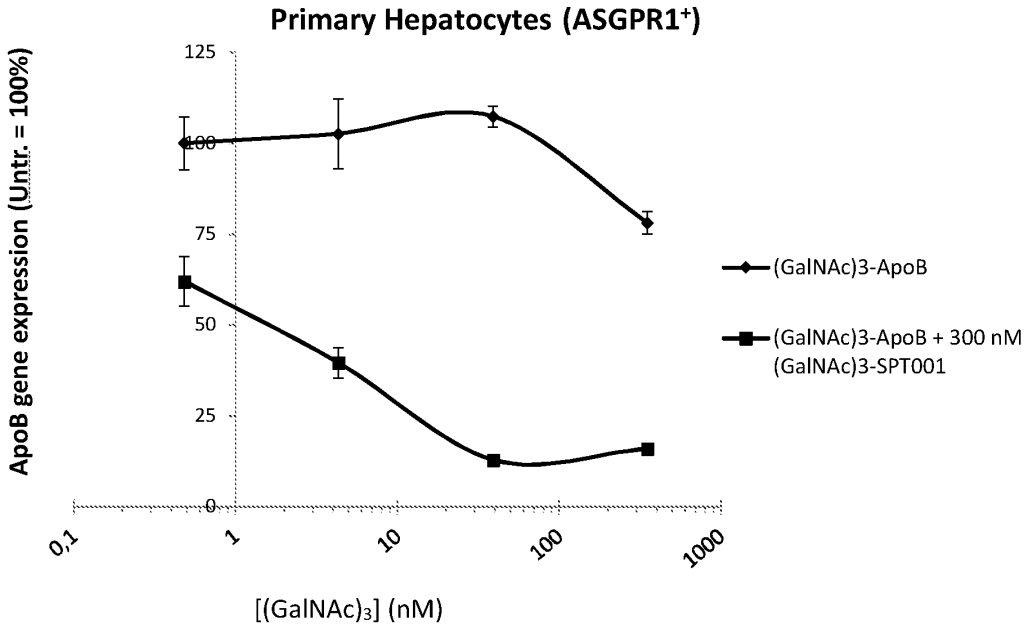
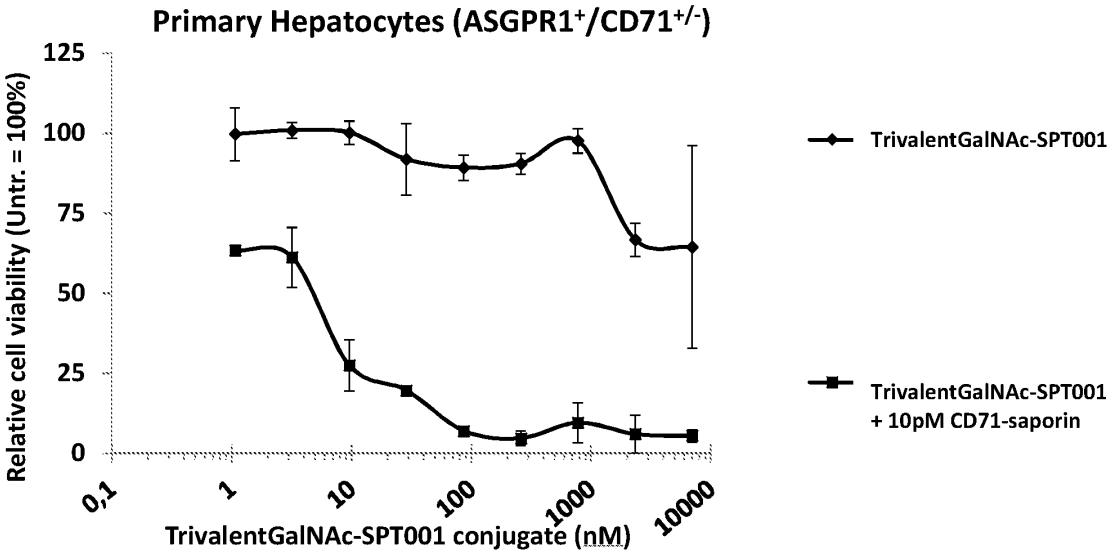

Primary Hepatocytes (ASGPR1+)
ApoB gene expression (Untr. = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
[(GalNAc)3] (nM)
(GalNAc)3-ApoB
(GalNAc)3-ApoB + 300 nM (GalNAc)3-SPT001

FIG. 30

A


Primary Hepatocytes (ASGPR1+/CD71+/-)
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

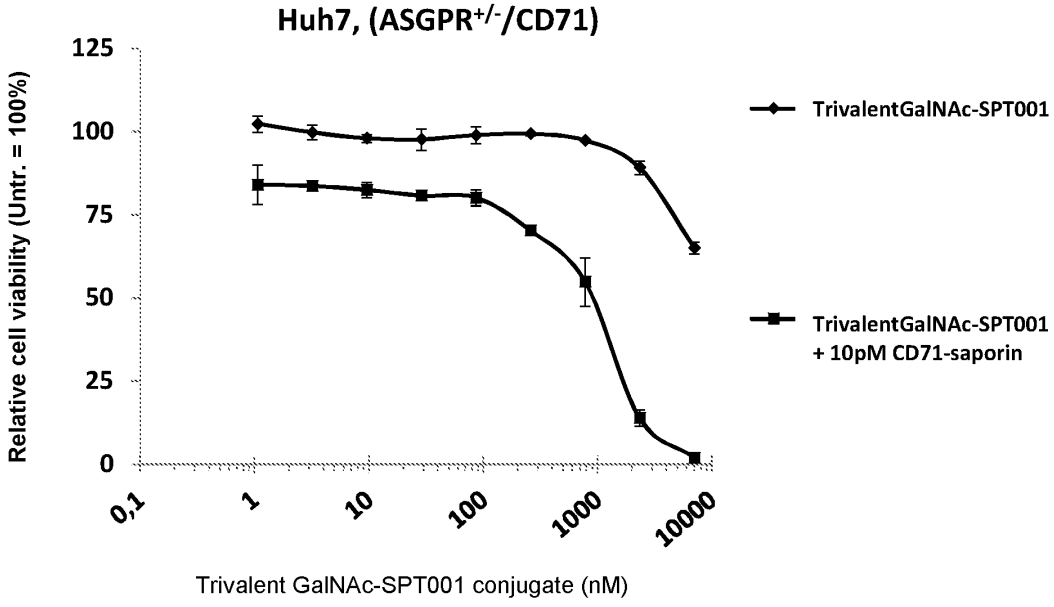
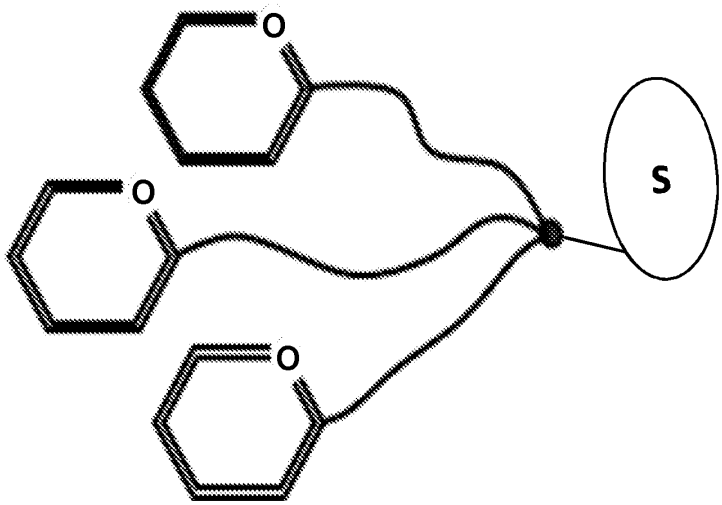

Huh7, (ASGPR+/-/CD71)
Relative cell viability (Untr. = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
10000
Trivalent GalNAc-SPT001 conjugate (nM)
TrivalentGalNAc-SPT001
TrivalentGalNAc-SPT001
+ 10pM CD71-saporin

FIG. 30

C

Trivalent GalNAc-SPT001 conjugate



D

Anti-CD71 antibody – saporin conjugate

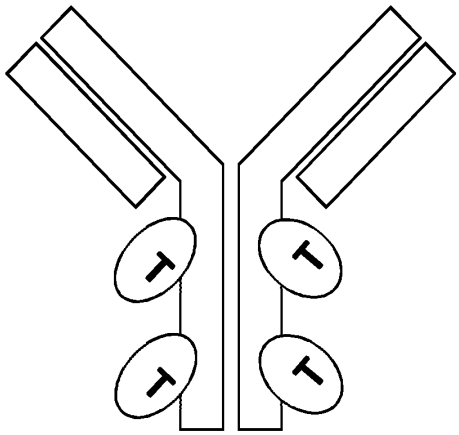
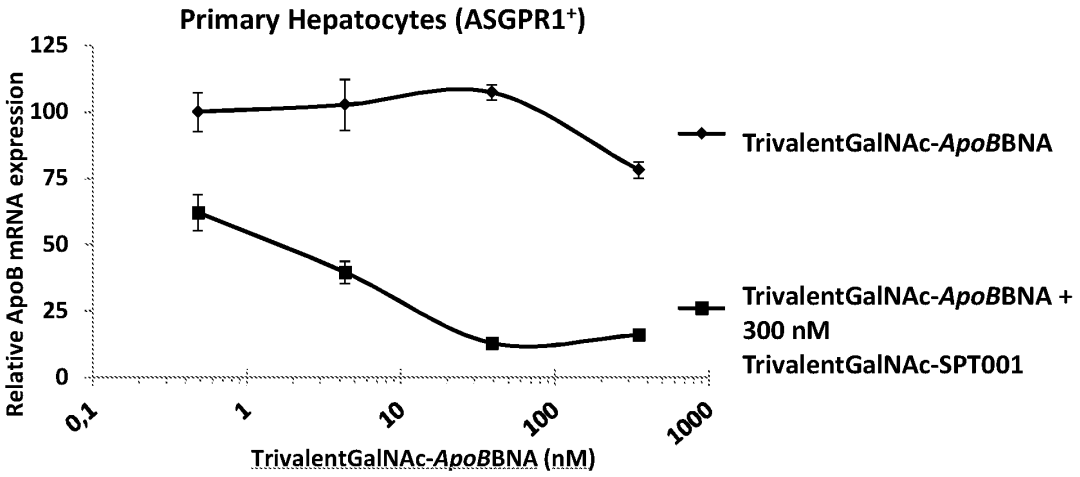


FIG. 31

A


Primary Hepatocytes (ASGPR1+)
Relative ApoB mRNA expression
125
100
75
50
25
0
0,1
1
10
100
1000
TrivalentGalNAc-ApoBBNA (nM)
TrivalentGalNAc-ApoBBNA
TrivalentGalNAc-ApoBBNA + 300 nM TrivalentGalNAc-SPT001

B

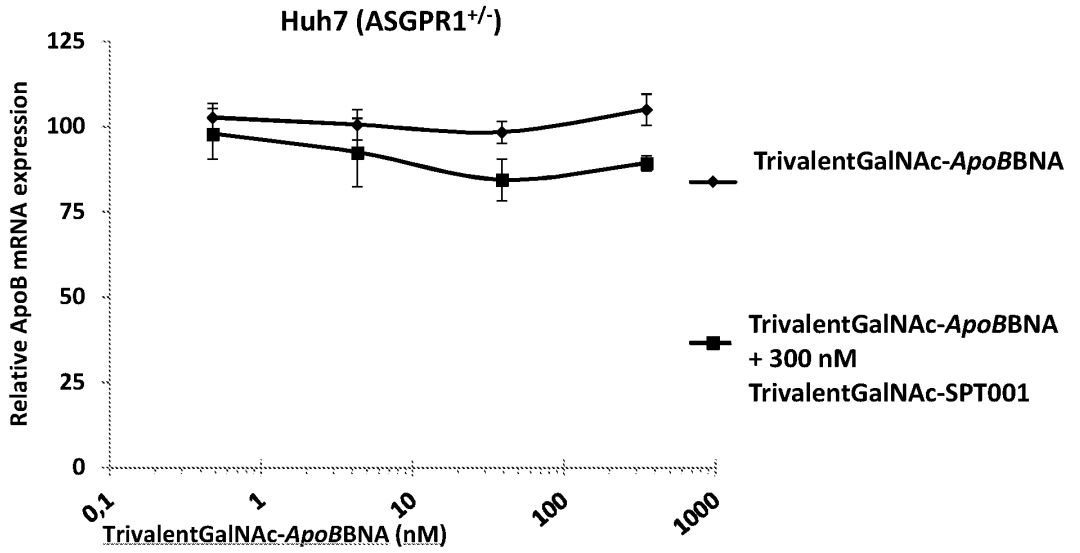

Huh7 (ASGPR1+/-)
Relative ApoB mRNA expression
125
100
75
50
25
0
0,1
1
10
100
1000
TrivalentGalNAc-ApoBBNA (nM)
TrivalentGalNAc-ApoBBNA
TrivalentGalNAc-ApoBBNA + 300 nM TrivalentGalNAc-SPT001

FIG. 31
C

Trivalent GalNAc-*ApoB* BNA conjugate

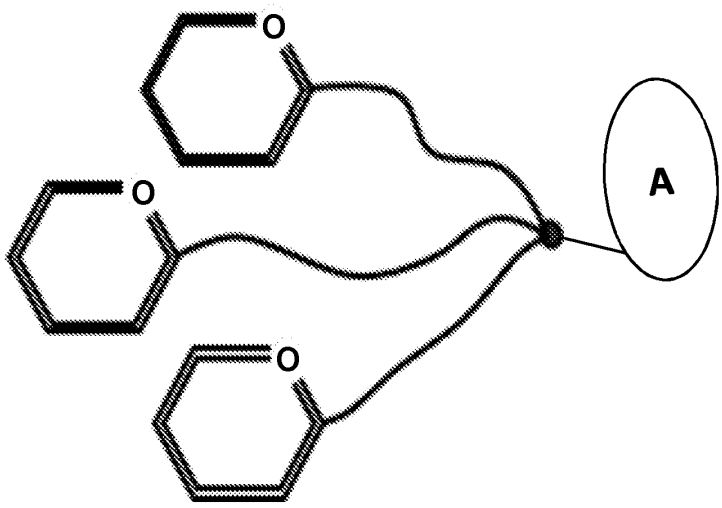
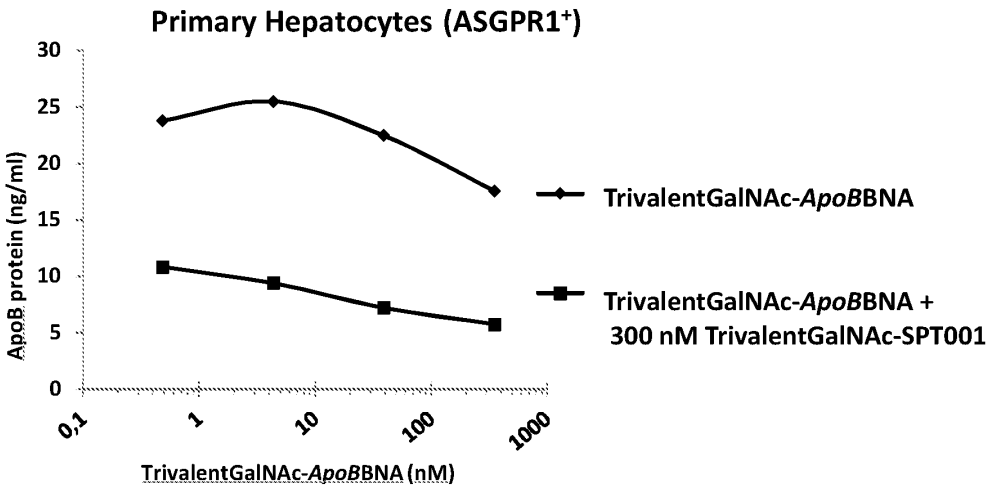


FIG. 32

A


Primary Hepatocytes (ASGPR1+)
ApoB protein (ng/ml)
30
25
20
15
10
5
0
0,1
1
10
100
1000
TrivalentGalNAc-ApoBBNA (nM)
TrivalentGalNAc-ApoBBNA
TrivalentGalNAc-ApoBBNA +
300 nM TrivalentGalNAc-SPT001

B

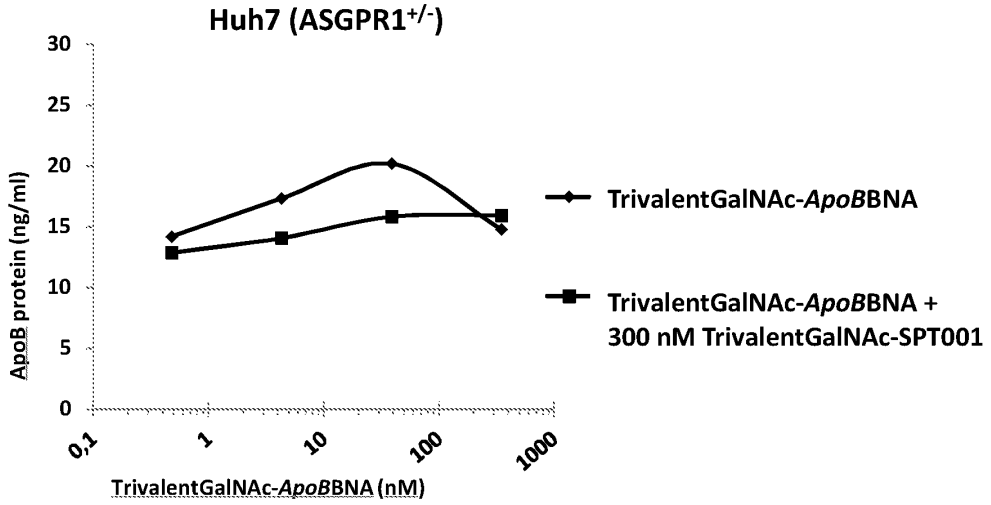

Huh7 (ASGPR1+/-)
ApoB protein (ng/ml)
30
25
20
15
10
5
0
0,1
1
10
100
1000
TrivalentGalNAc-ApoBBNA (nM)
TrivalentGalNAc-ApoBBNA
TrivalentGalNAc-ApoBBNA +
300 nM TrivalentGalNAc-SPT001

FIG. 33A

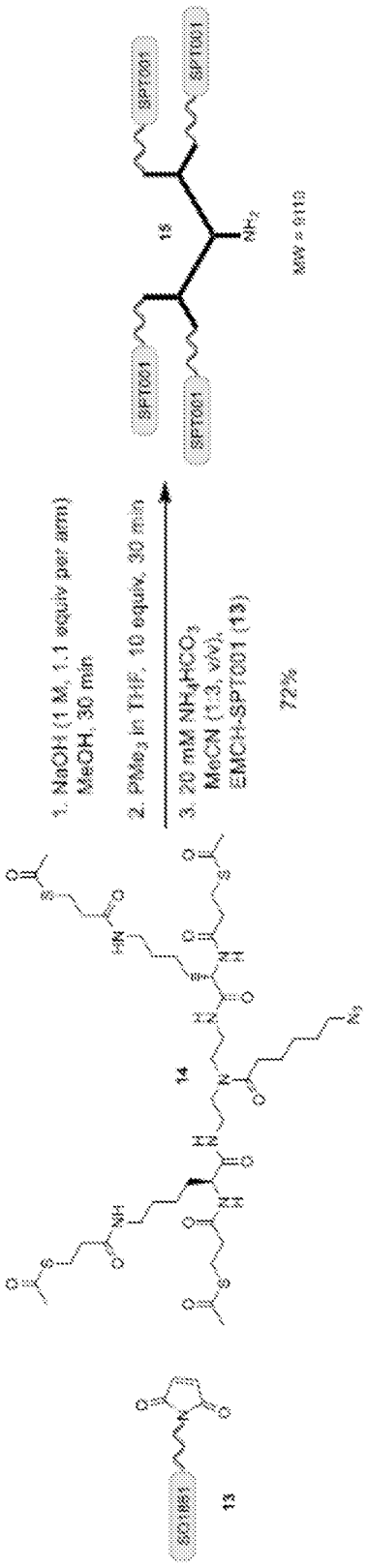

1. MeOH (1 M, 1.1 equiv per unit)
MeOH, 30 min
2. PMe3 in THF, 10 equiv, 30 min
3. 20 mM NH4HCO3
MeCN (1:3, v/v)
EMCH-SPT001 (13)
72%
SPT001
SPT001
SPT001
SPT001
15
MW = 9115
14
13

FIG. 33B

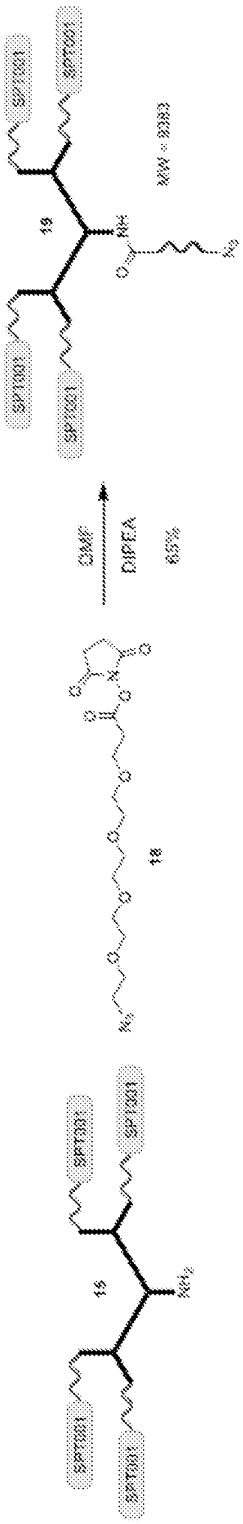
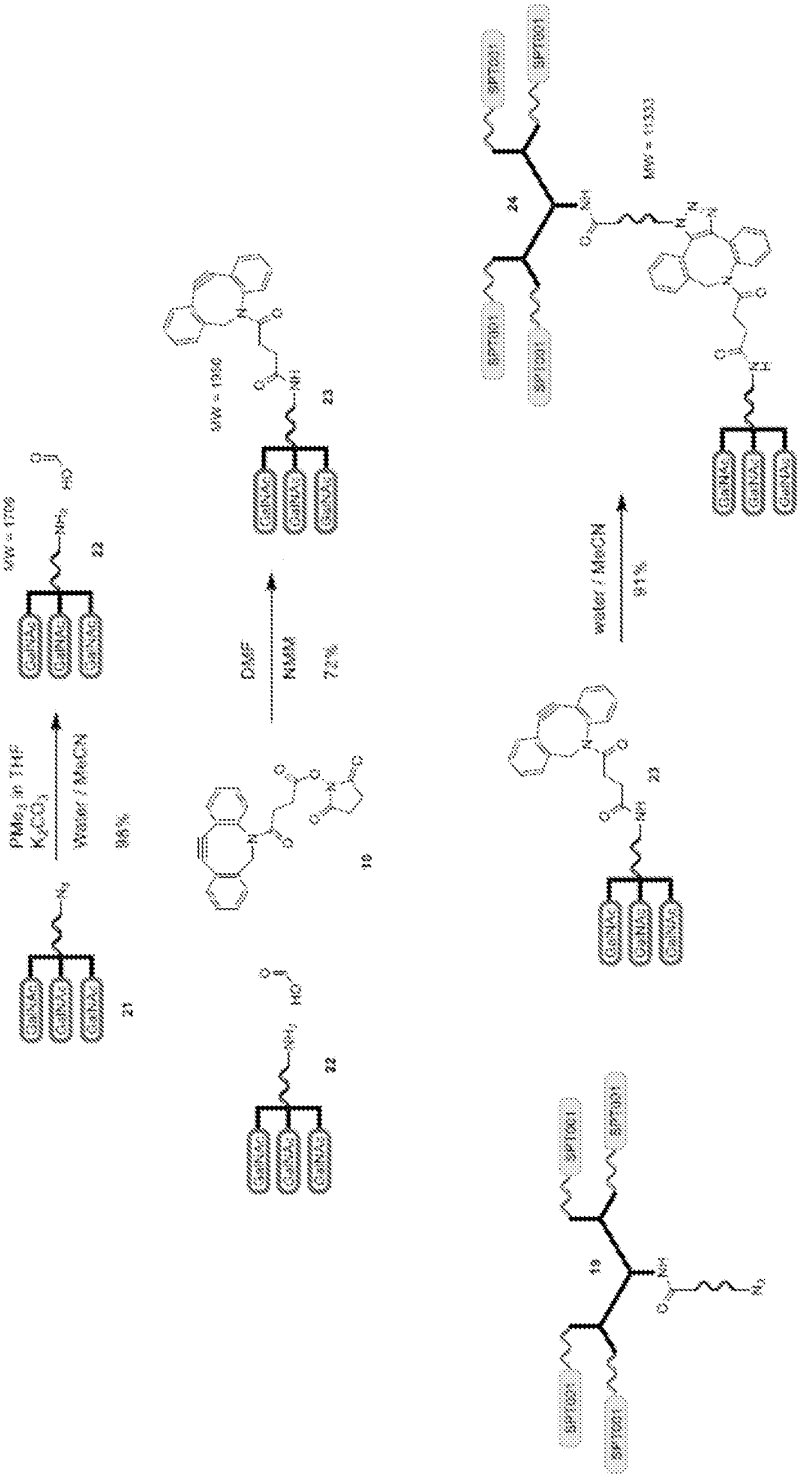

SPT001
SPT001
SPT001
SPT001
19
NH
MW ~ 2383
DMF
DIPEA
65%
18
SPT001
SPT001
SPT001
SPT001
15
NH2

FIG. 33C


MW = 1709
PMe3 in THF
K2CO3
Water / MeCN
86%
21
22
MW = 1909
DMF
NMM
73%
23
19
water / MeCN
81%
24
MW = 11533
SPT001
SPT002
SPT003
SPT004
SPT005
SPT006
SPT007
SPT008