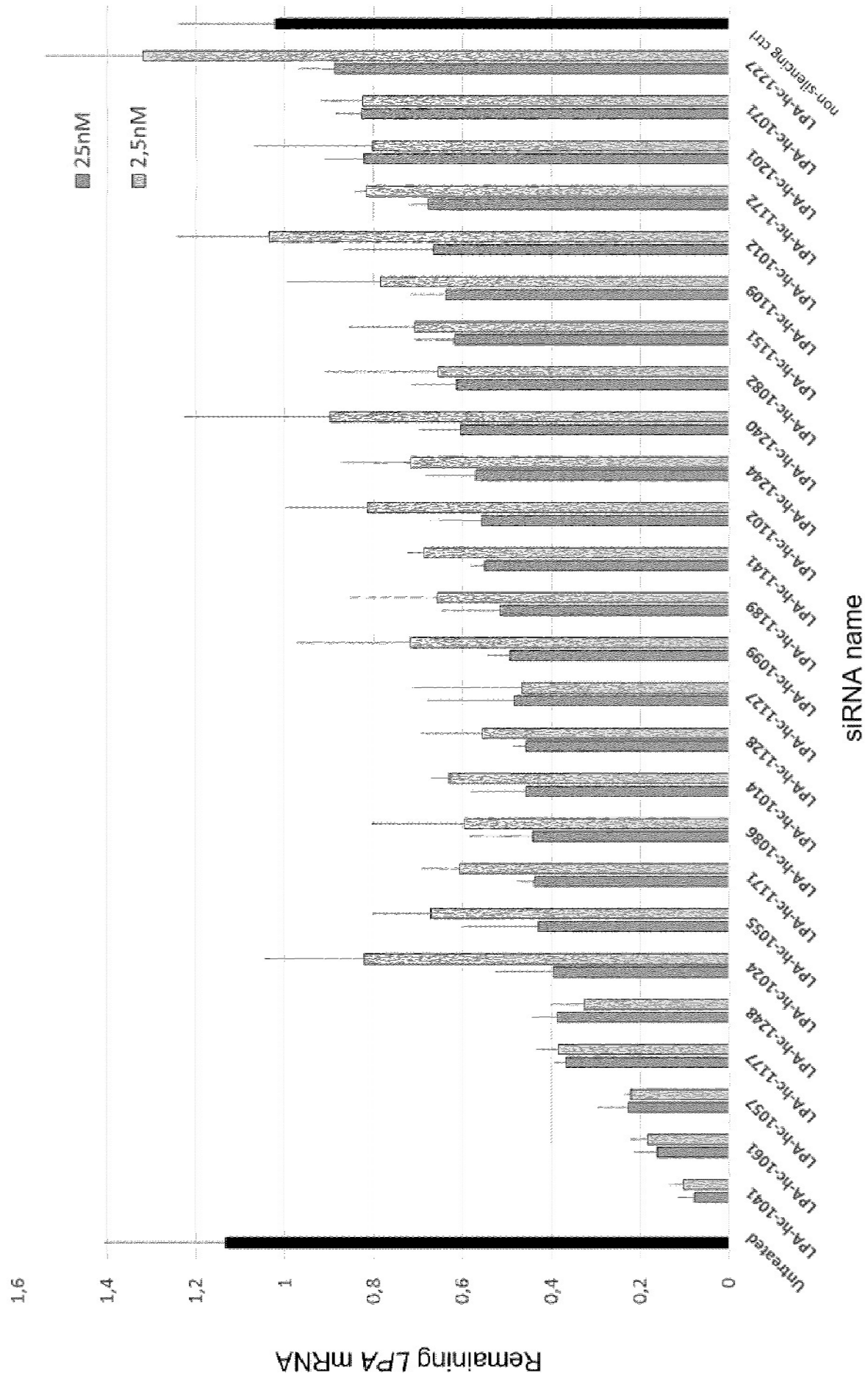

25nM
2,5nM
Remaining LPA mRNA
0
0,2
0,4
0,6
0,8
1
1,2
1,4
1,6
siRNA name
Untreated
LPA-hc-1041
LPA-hc-1061
LPA-hc-1057
LPA-hc-1177
LPA-hc-1248
LPA-hc-1024
LPA-hc-1055
LPA-hc-1171
LPA-hc-1086
LPA-hc-1014
LPA-hc-1128
LPA-hc-1127
LPA-hc-1099
LPA-hc-1189
LPA-hc-1141
LPA-hc-1102
LPA-hc-1244
LPA-hc-1240
LPA-hc-1082
LPA-hc-1151
LPA-hc-1109
LPA-hc-1012
LPA-hc-1172
LPA-hc-1201
LPA-hc-1071
LPA-hc-1227
non-silencing ctrl

Figure 1

Figure 2A

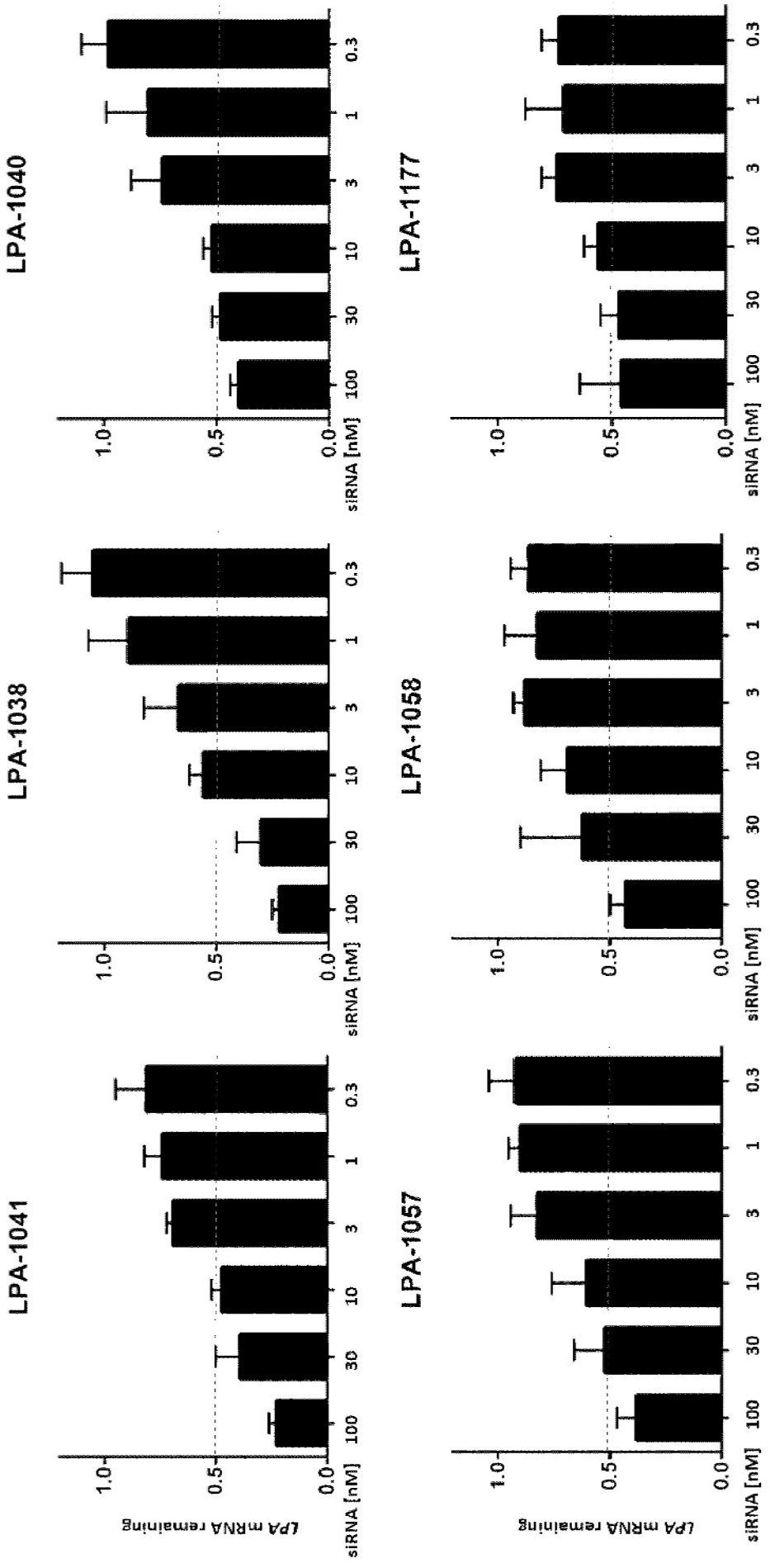
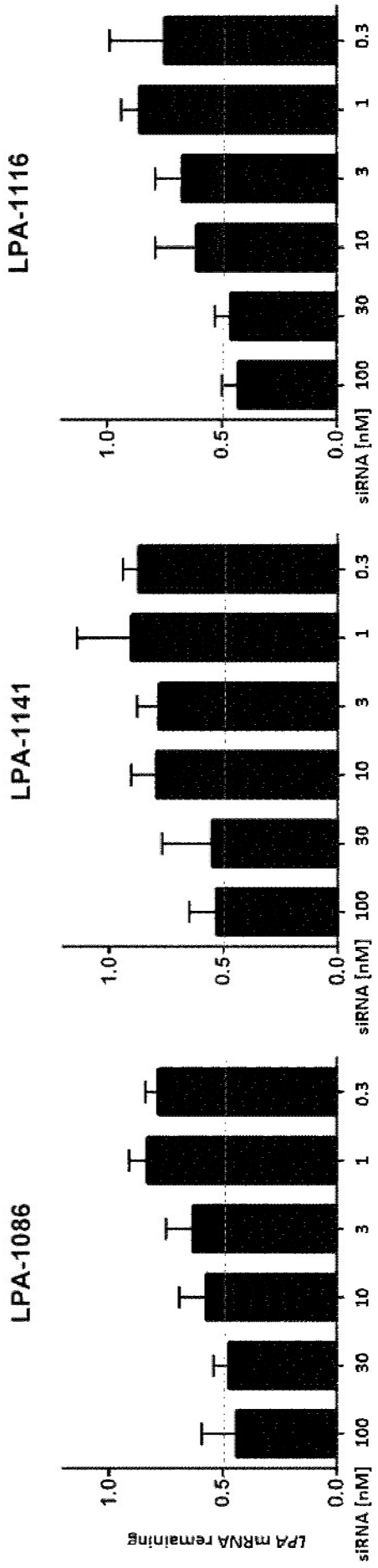
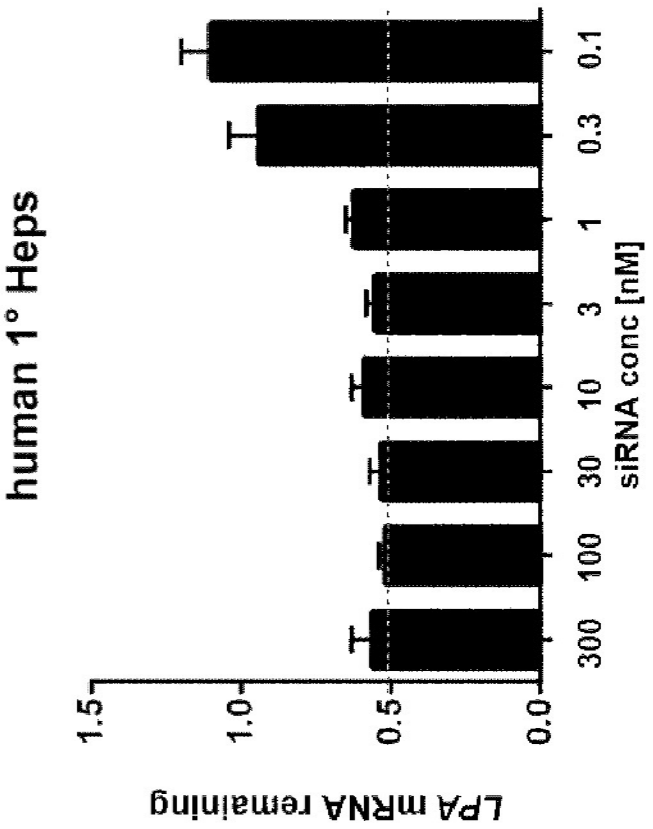

LPA-1040
LPA-1038
LPA-1041
LPA-1177
LPA-1058
LPA-1057
LPA mRNA remaining
siRNA [nM]
100
30
10
3
1
0.3
0.0
0.5
1.0

Figure 2B


LPA-1086
LPA-1141
LPA-1116
LPA mRNA remaining
1.0
0.5
0.0
siRNA [nM]
100
30
10
3
1
0.3


human 1° Heps
LPA mRNA remaining
1.5
1.0
0.5
0.0
300
100
30
10
3
1
0.3
0.1
siRNA conc [nM]

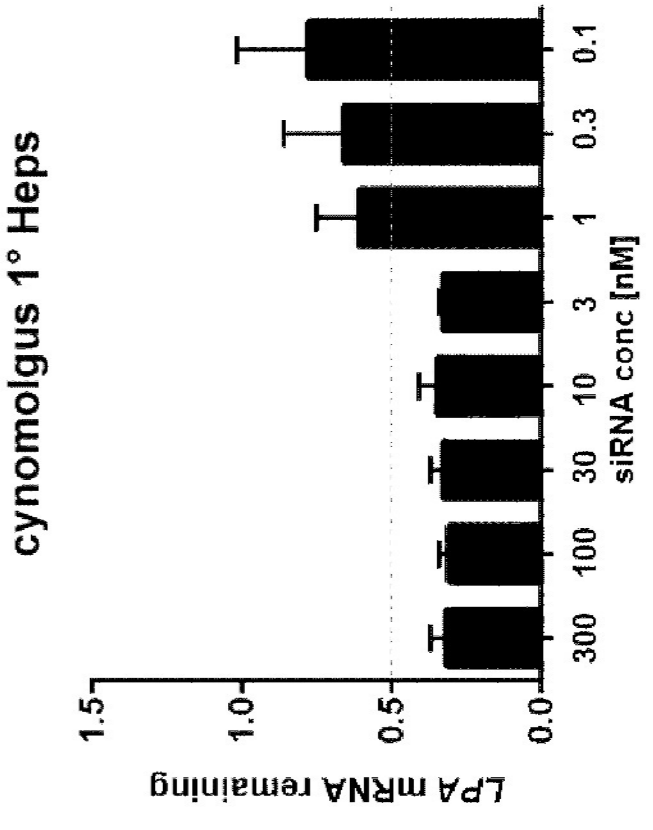
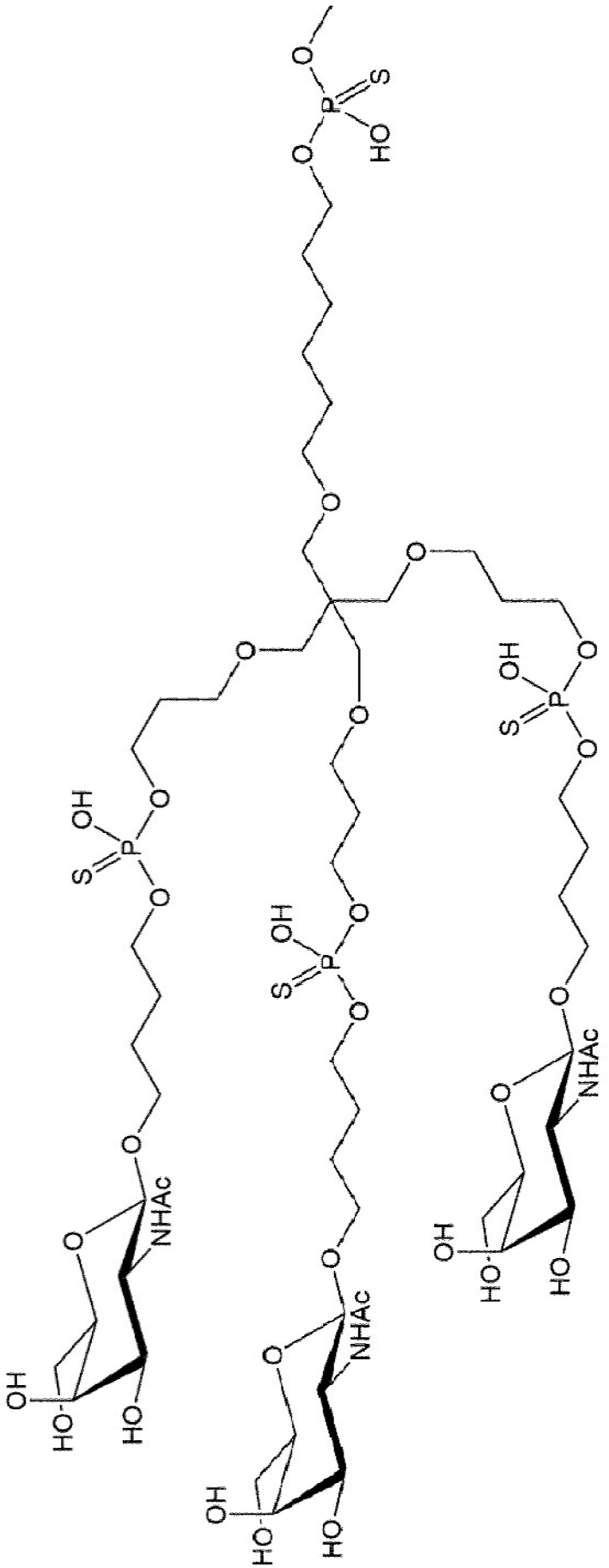

cynomolgus 1° Heps
LPA mRNA remaining
1.5
1.0
0.5
0.0
300
100
30
10
3
1
0.3
0.1
siRNA conc [nM]

Figure 3

Figure 4A



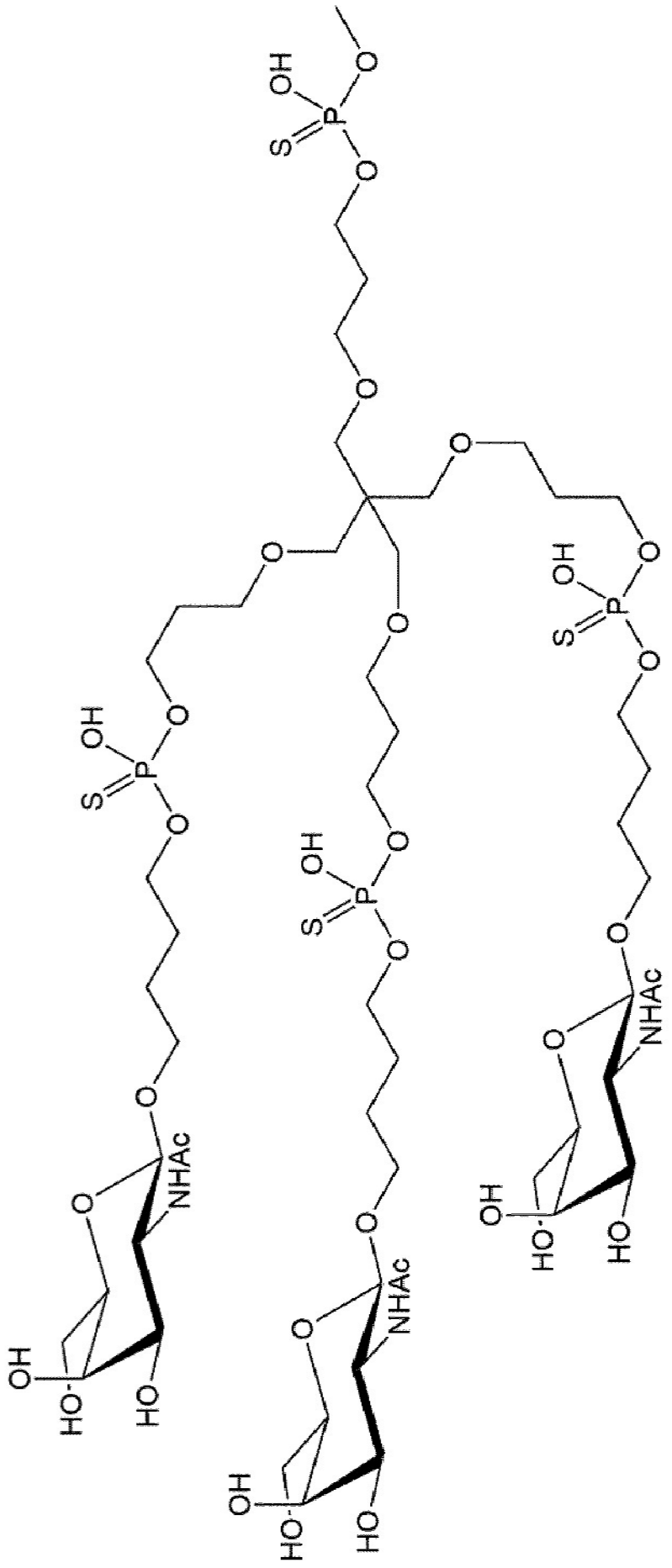


Figure 4B

Figure 5

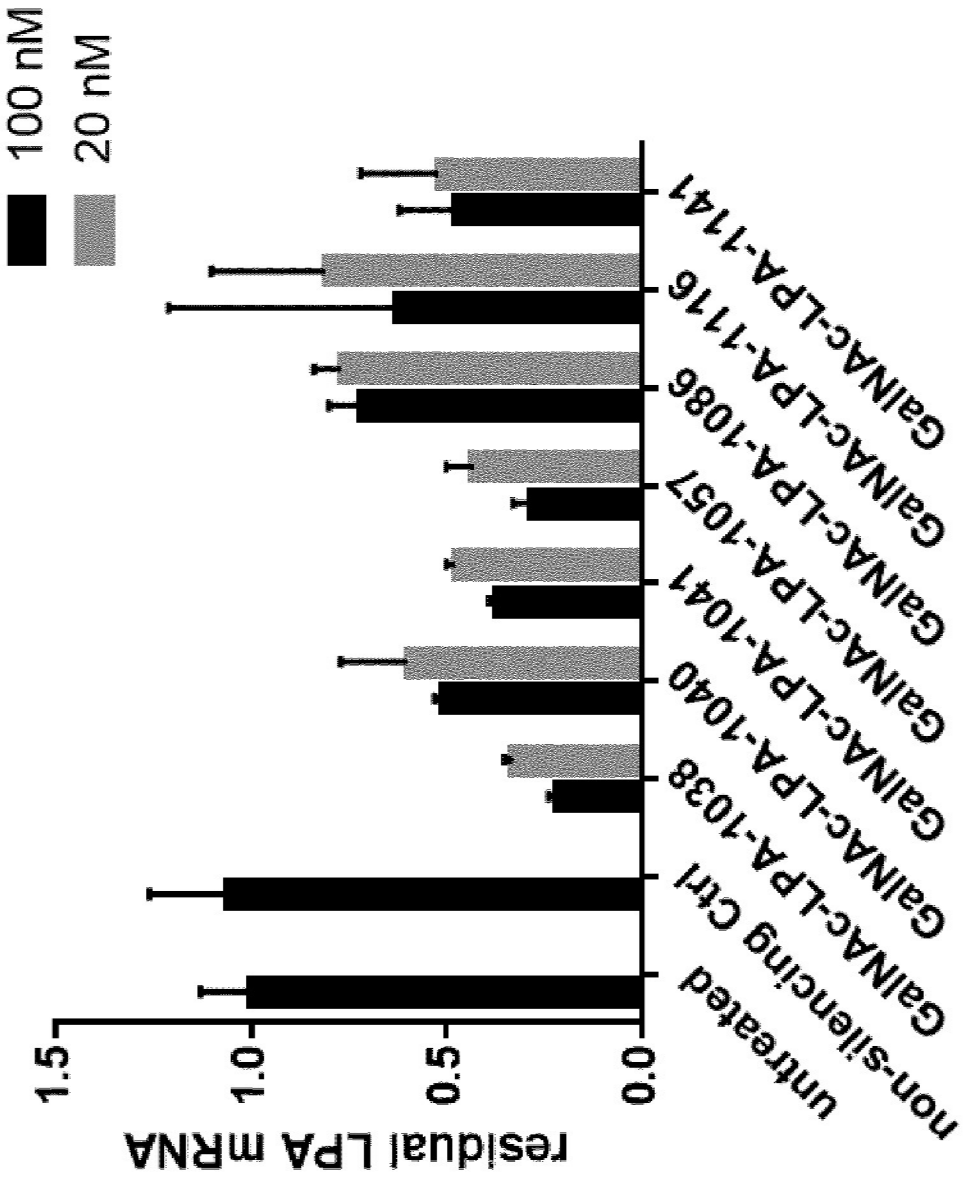

100 nM
20 nM
residual LPA mRNA
1.5
1.0
0.5
0.0
untreated
non-silencing Ctrl
GalNAc-LPA-1038
GalNAc-LPA-1040
GalNAc-LPA-1041
GalNAc-LPA-1057
GalNAc-LPA-1086
GalNAc-LPA-1116
GalNAc-LPA-1141

Figure 6

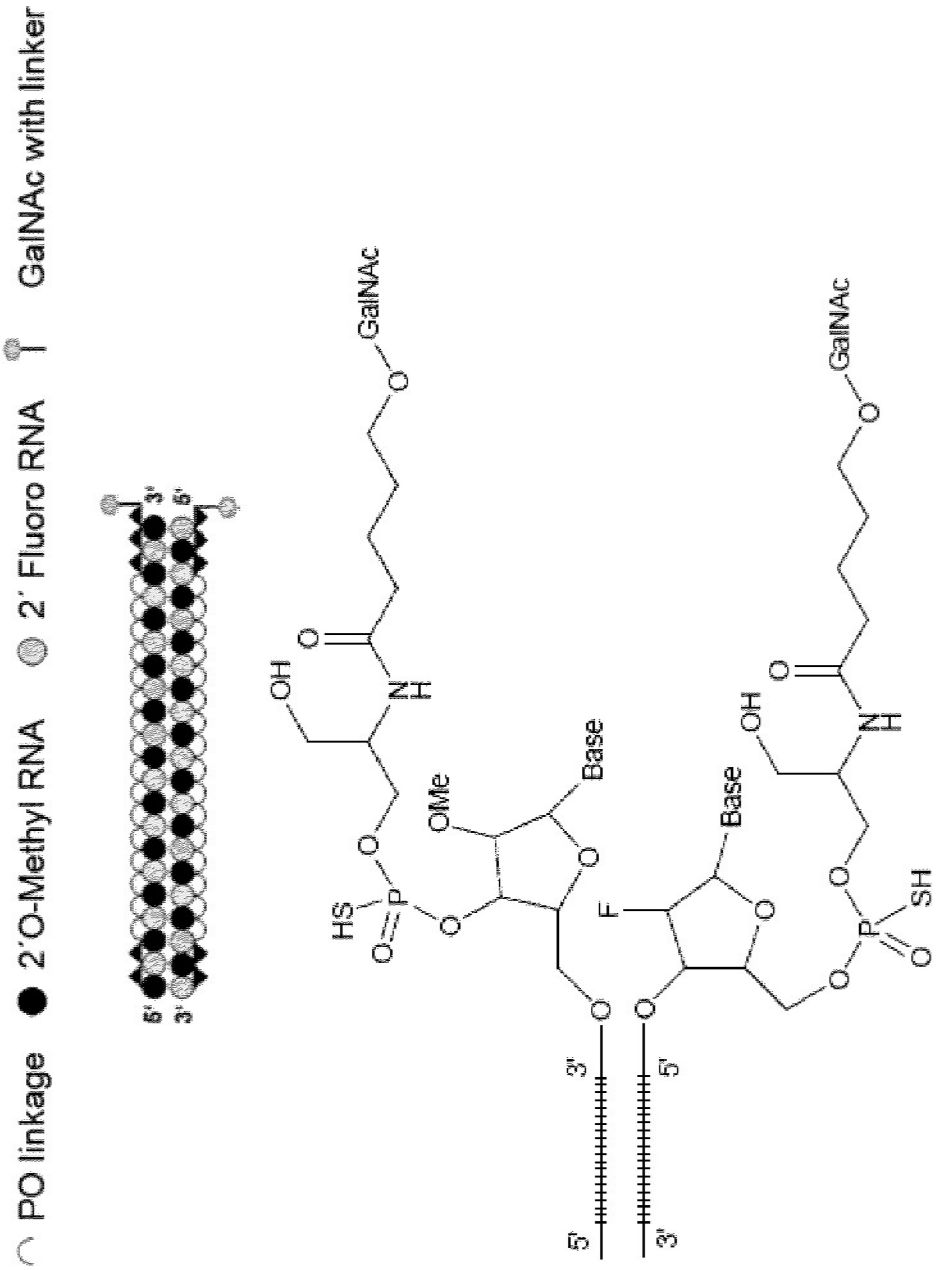

PS linkage
PO linkage
2´O-Methyl RNA
2´ Fluoro RNA
GalNAc with linker
5'
3'
3'
5'
GalNAc
OH
HS
OMe
Base
F
SH

Figure 7

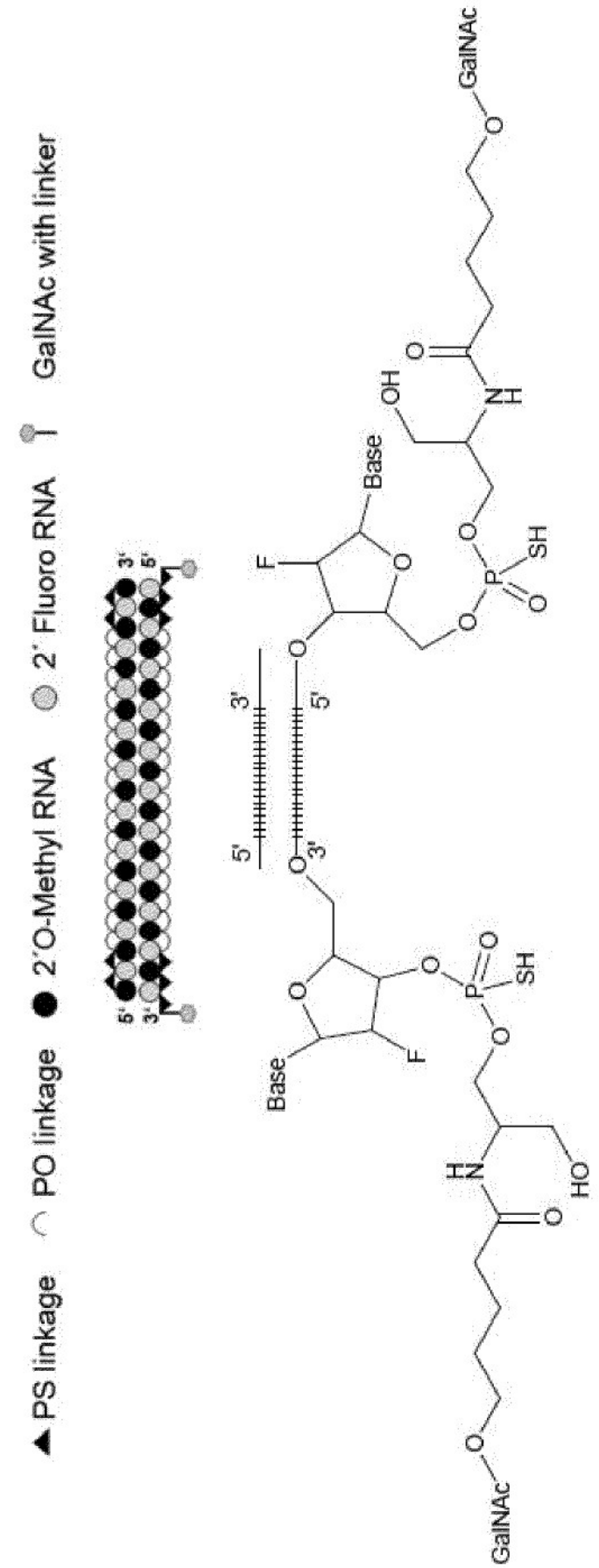

▲ PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
3'
5'
5'
3'
3'
5'
Base
F
O
P
SH
OH
HO
N
H
GalNAc

Figure 8

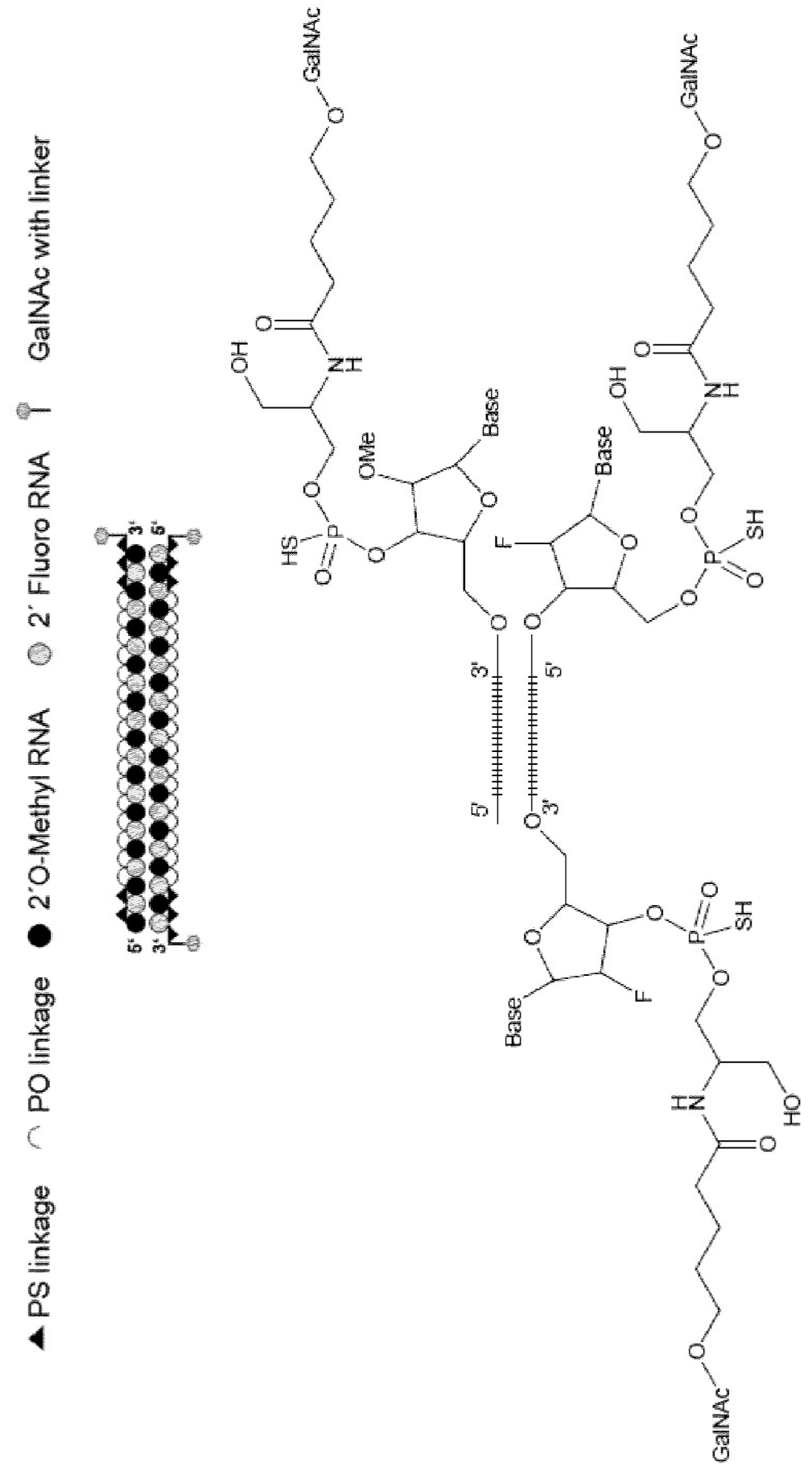

PS linkage
PO linkage
2´O-Methyl RNA
2´ Fluoro RNA
GalNAc with linker
5'
3'
3'
5'
GalNAc
OH
HS
OMe
Base
F
SH
HO

Figure 9

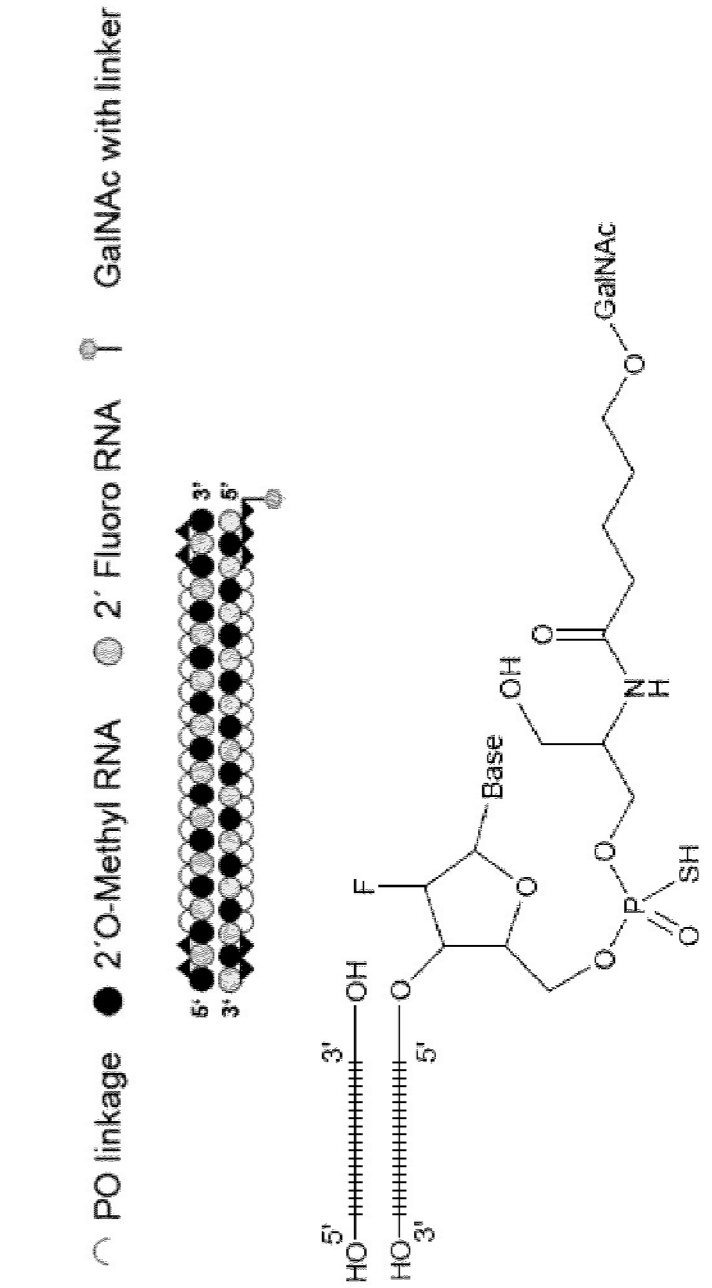

PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
3'
5'
5'
3'
HO
OH
3'
5'
HO
F
Base
O
O
O
O
P
SH
O
OH
O
N
H
O
GalNAc

Figure 10

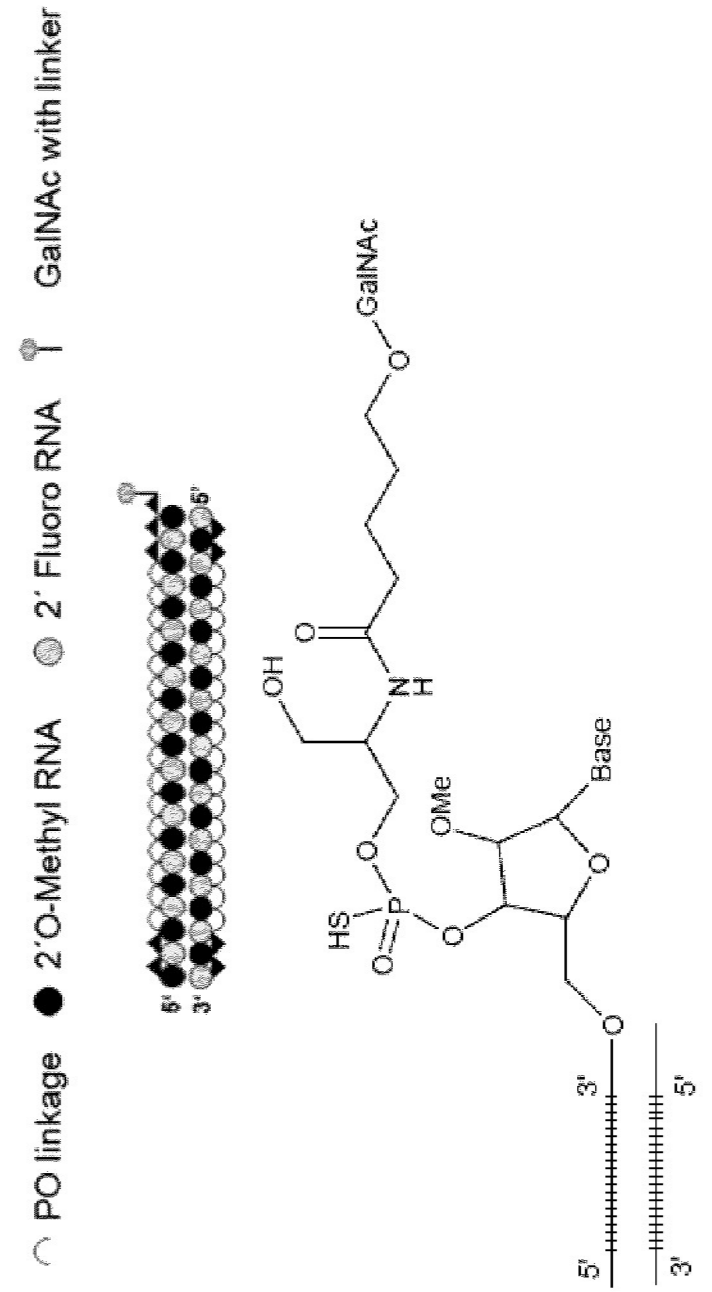

▲ PS linkage
PO linkage
2´O-Methyl RNA
2´ Fluoro RNA
GalNAc with linker
5'
3'
GalNAc
OH
HS
OMe
Base
5'
3'
3'
5'

Figure 11

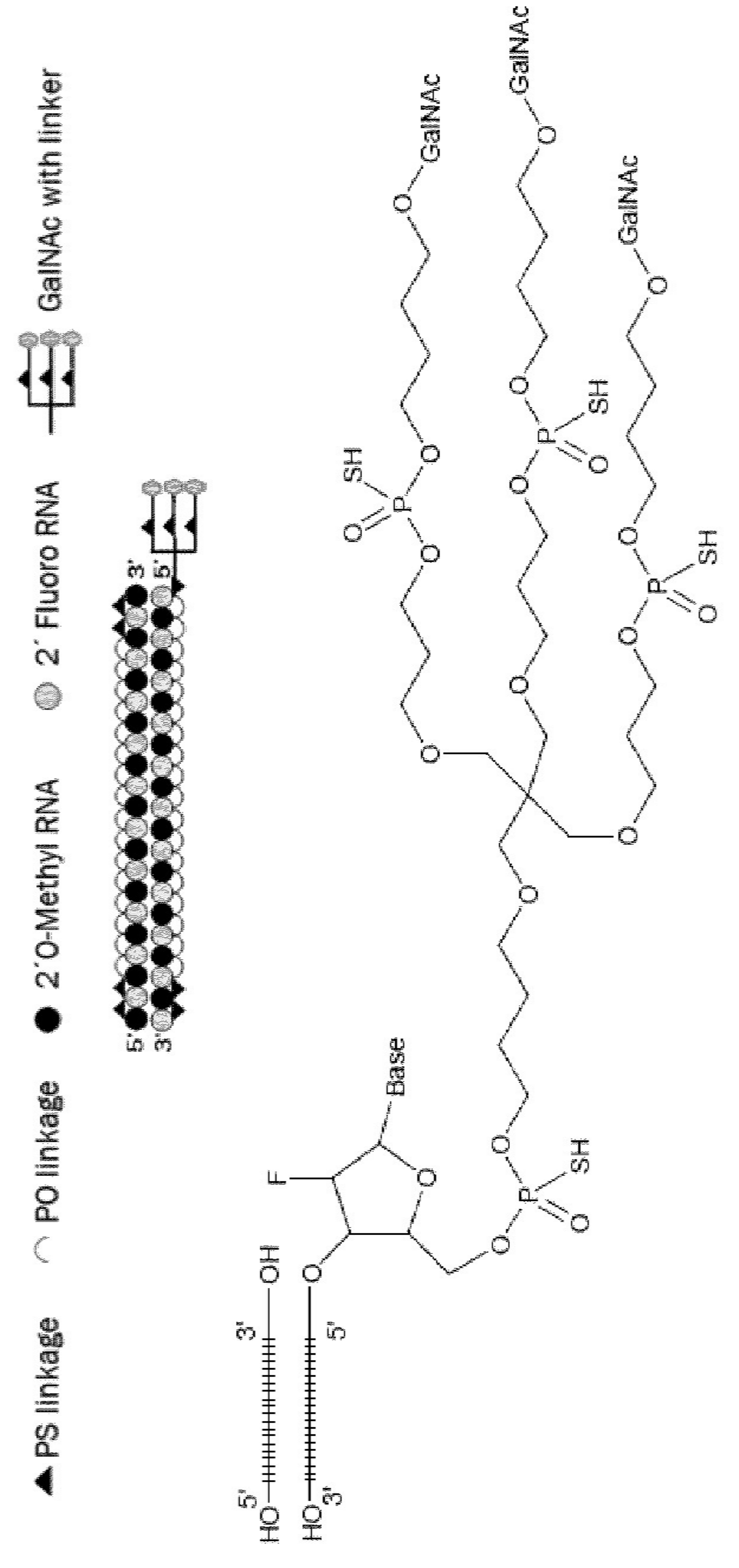

PS linkage
PO linkage
2´O-Methyl RNA
2´ Fluoro RNA
GalNAc with linker
5'
3'
HO
OH
F
Base
O
P
SH
GalNAc

Figure 12

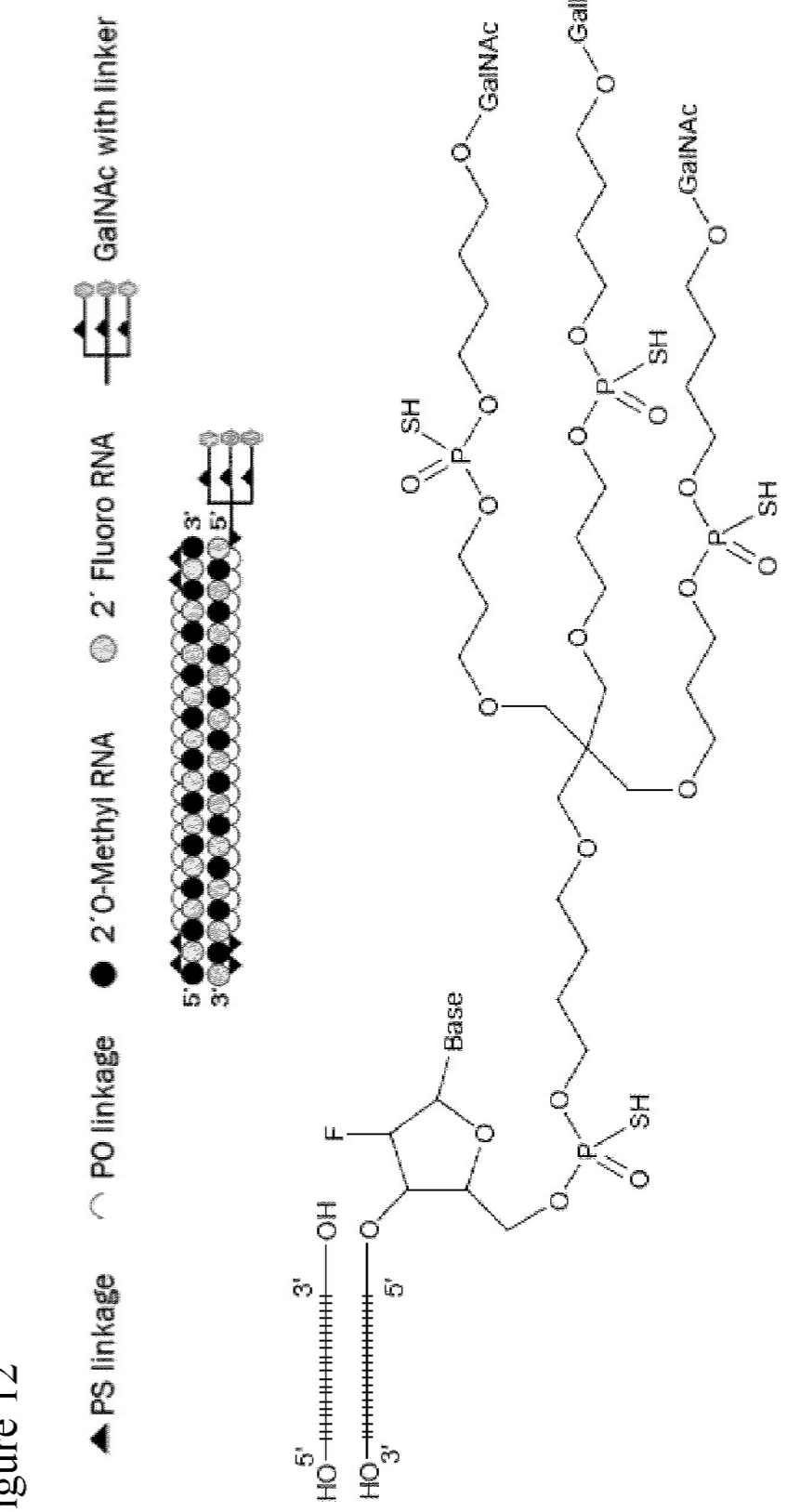

PS linkage
PO linkage
2´O-Methyl RNA
2´ Fluoro RNA
GalNAc with linker
5'
3'
3'
5'
HO
OH
Base
F
SH
GalNAc

Figure 13

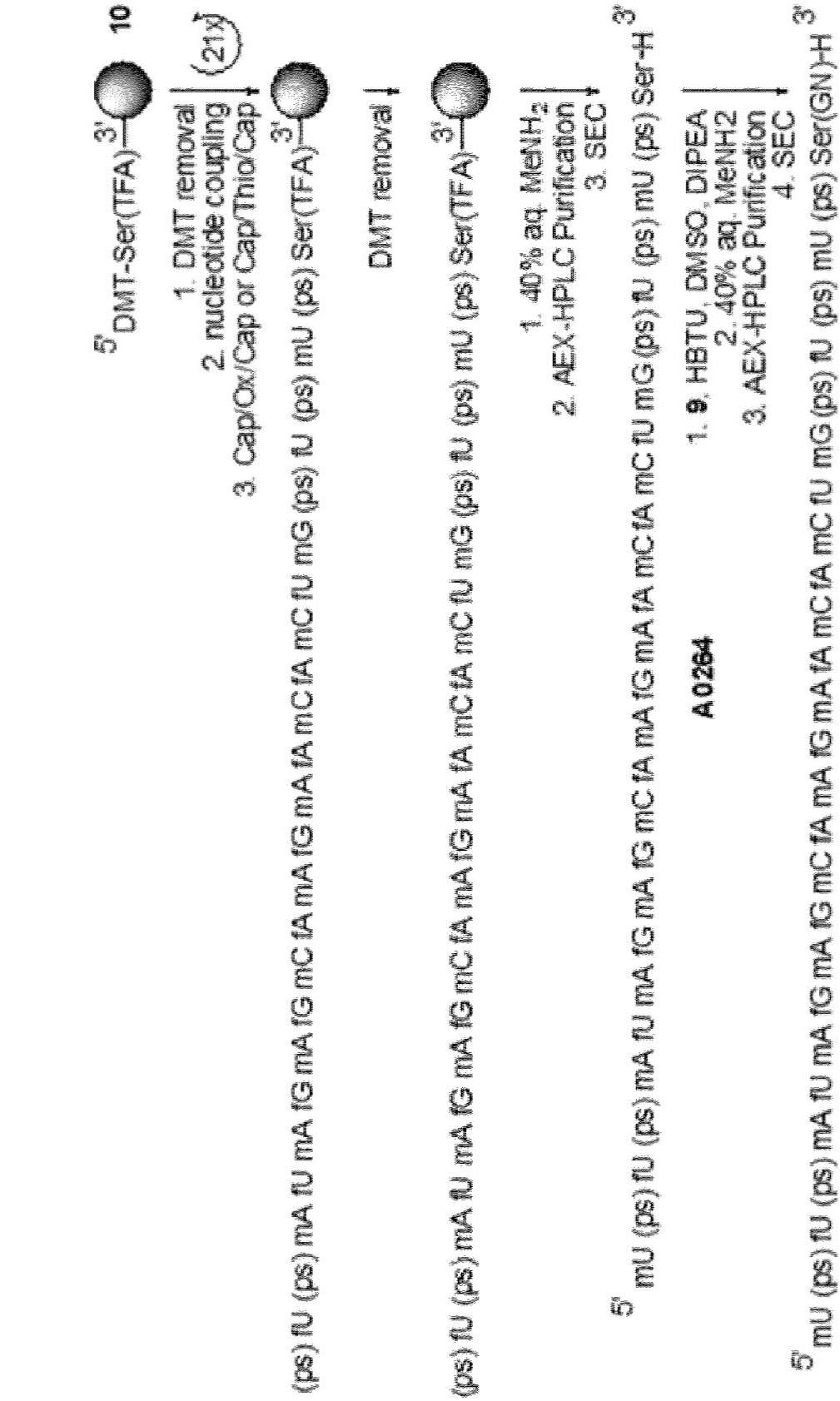

5' DMT-Ser(TFA) 3' 10
1. DMT removal
2. nucleotide coupling
3. Cap/Ox/Cap or Cap/Thio/Cap
(21x)
5' DMT-mU (ps) fU (ps) mA fU mA fG mA fG mC fA mA fG mA fA mC fA mC fU mG (ps) fU (ps) mU (ps) Ser(TFA) 3'
DMT removal
5' mU (ps) fU (ps) mA fU mA fG mA fG mC fA mA fG mA fA mC fA mC fU mG (ps) fU (ps) mU (ps) Ser(TFA) 3'
1. 40% aq. MeNH2
2. AEX-HPLC Purification
3. SEC
5' mU (ps) fU (ps) mA fU mA fG mA fG mC fA mA fG mA fA mC fA mC fU mG (ps) fU (ps) mU (ps) Ser-H 3'
A0264
1. 9, HBTU, DMSO, DIPEA
2. 40% aq. MeNH2
3. AEX-HPLC Purification
4. SEC
5' mU (ps) fU (ps) mA fU mA fG mA fG mC fA mA fG mA fA mC fA mC fU mG (ps) fU (ps) mU (ps) Ser(GN)-H 3'
A0268

Figure 14

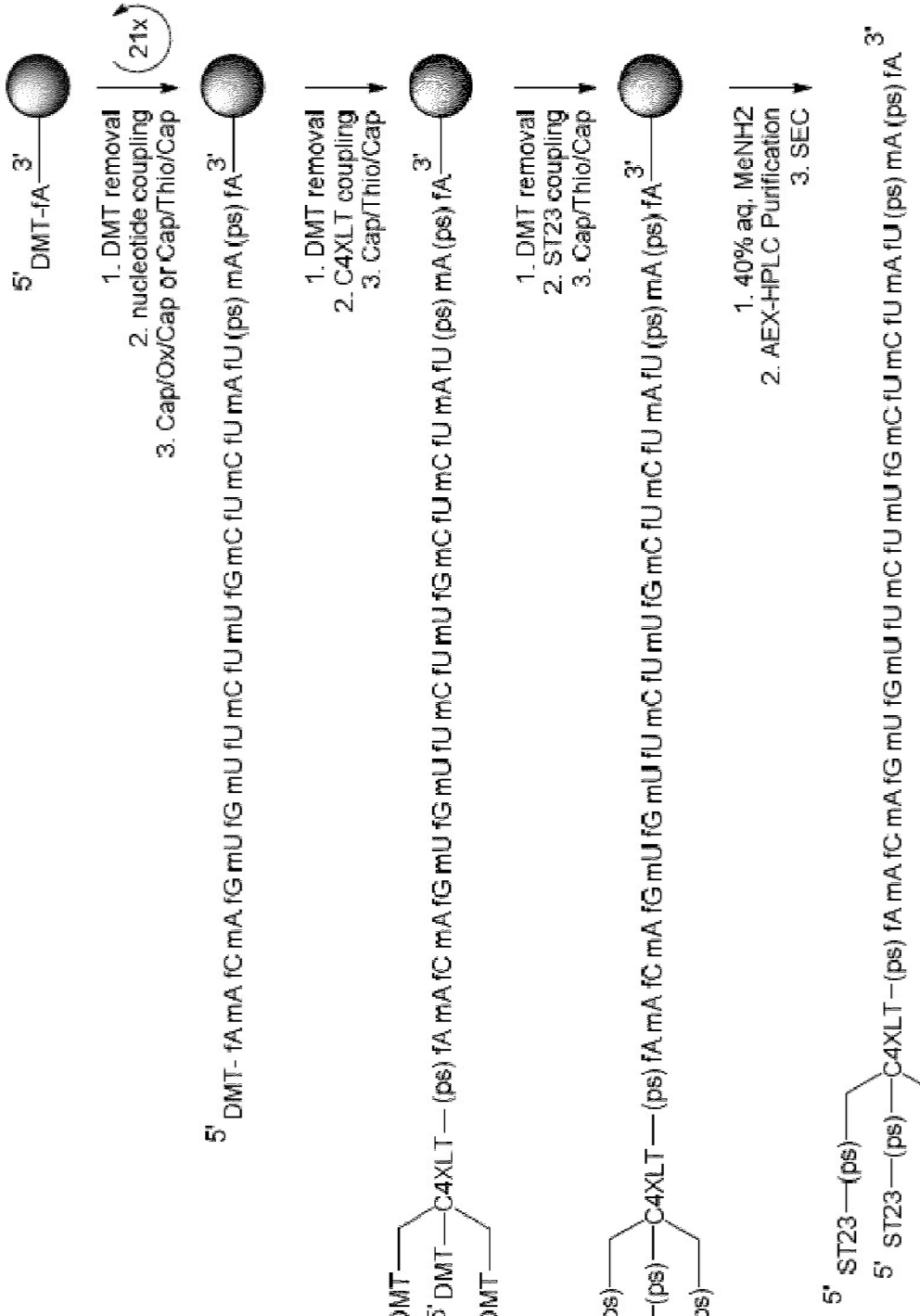

5' DMT-fA 3'
1. DMT removal
2. nucleotide coupling
3. Cap/Ox/Cap or Cap/Thio/Cap
21x
5' DMT- fA mA fC mA fG mU fG mU fU mC fU mU fG mC fU mC fU mA fU (ps) mA (ps) fA 3'
1. DMT removal
2. C4XLT coupling
3. Cap/Thio/Cap
5' DMT
5' DMT
5' DMT
C4XLT — (ps) fA mA fC mA fG mU fG mU fU mC fU mU fG mC fU mC fU mA fU (ps) mA (ps) fA 3'
1. DMT removal
2. ST23 coupling
3. Cap/Thio/Cap
5' ST23(Ac)3—(ps)
5' ST23(Ac)3—(ps)
5' ST23(Ac)3—(ps)
C4XLT — (ps) fA mA fC mA fG mU fG mU fU mC fU mU fG mC fU mC fU mA fU (ps) mA (ps) fA 3'
1. 40% aq. MeNH2
2. AEX-HPLC Purification
3. SEC
5' ST23 —(ps)
5' ST23 —(ps)
5' ST23 —(ps)
C4XLT –(ps) fA mA fC mA fG mU fG mU fU mU fG mC fU mC fU mA fU (ps) mA (ps) fA 3'
A0006

Figure 15b

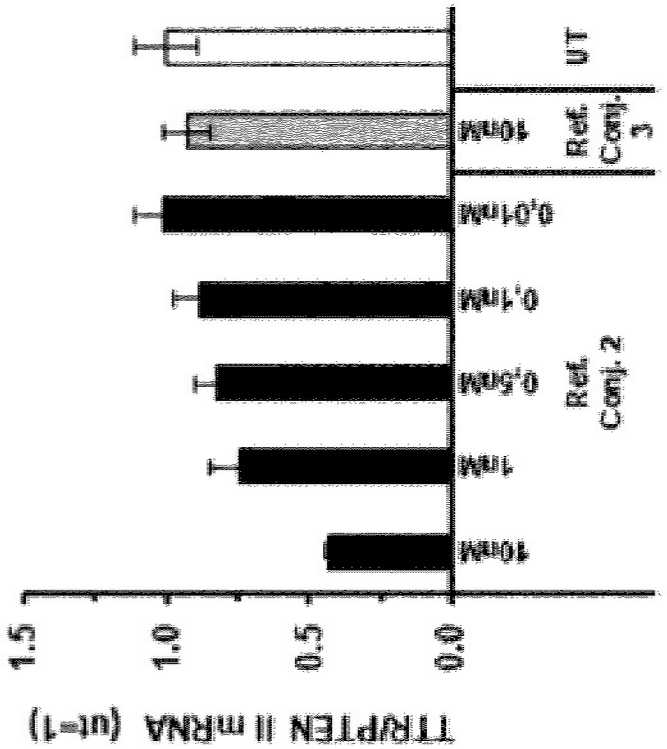
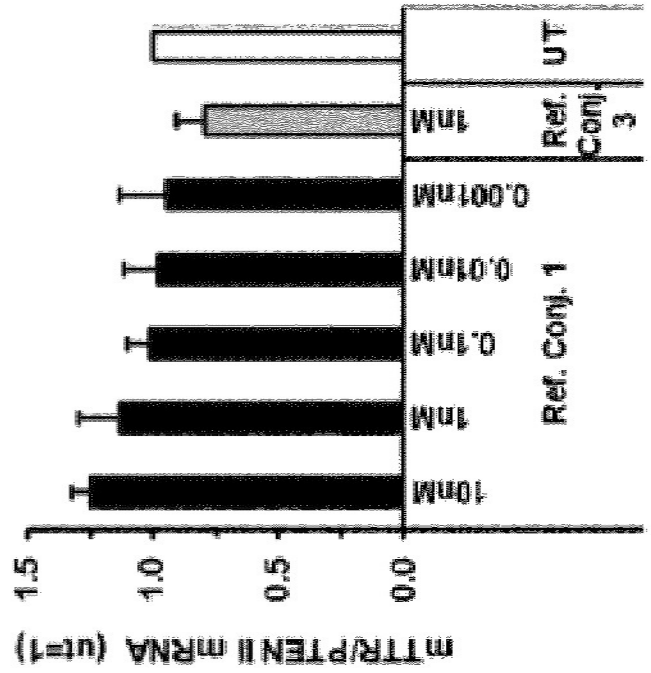


Figure 15a



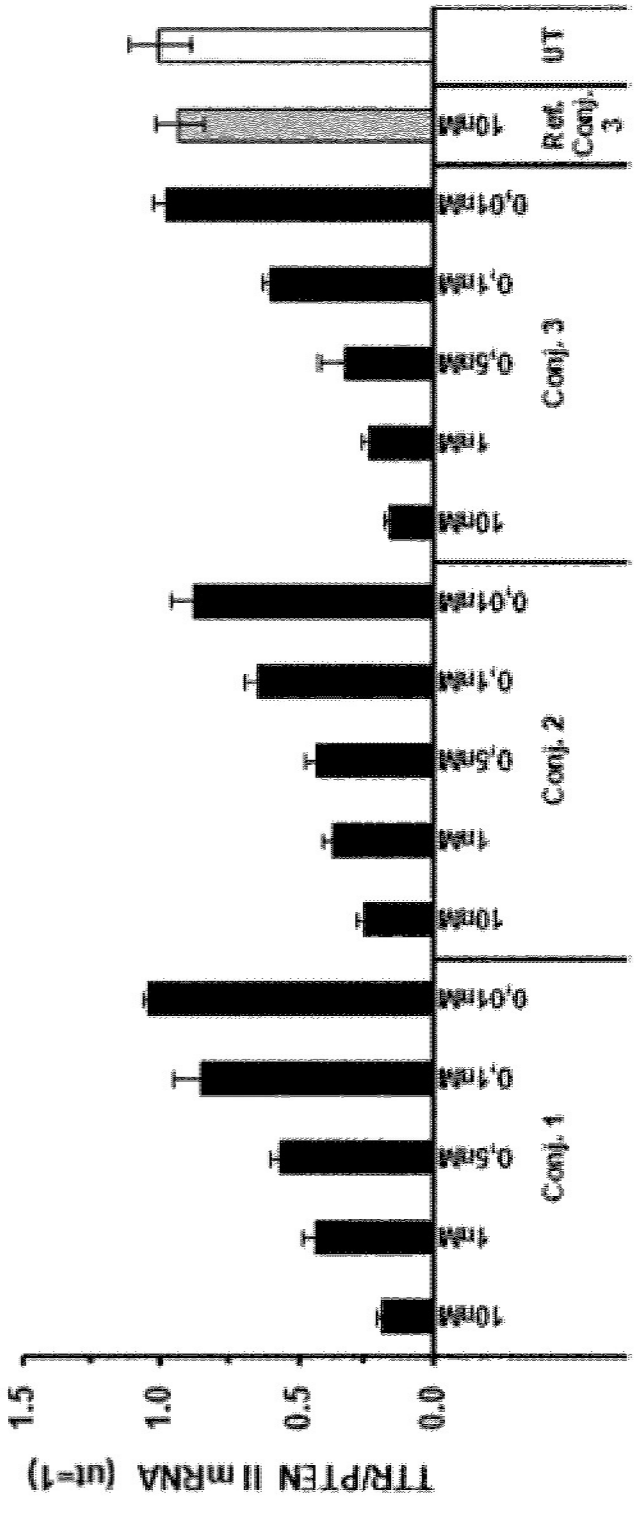

TTR/PTEN II mRNA (ut=1)
1.5
1.0
0.5
0.0
UT
Ref. Conj. 3
10nM
Conj. 3
0,01nM
0,1nM
0,5nM
1nM
10nM
Conj. 2
0,01nM
0,1nM
0,5nM
1nM
10nM
Conj. 1
0,01nM
0,1nM
0,5nM
1nM
10nM

Figure 15c

Figure 16

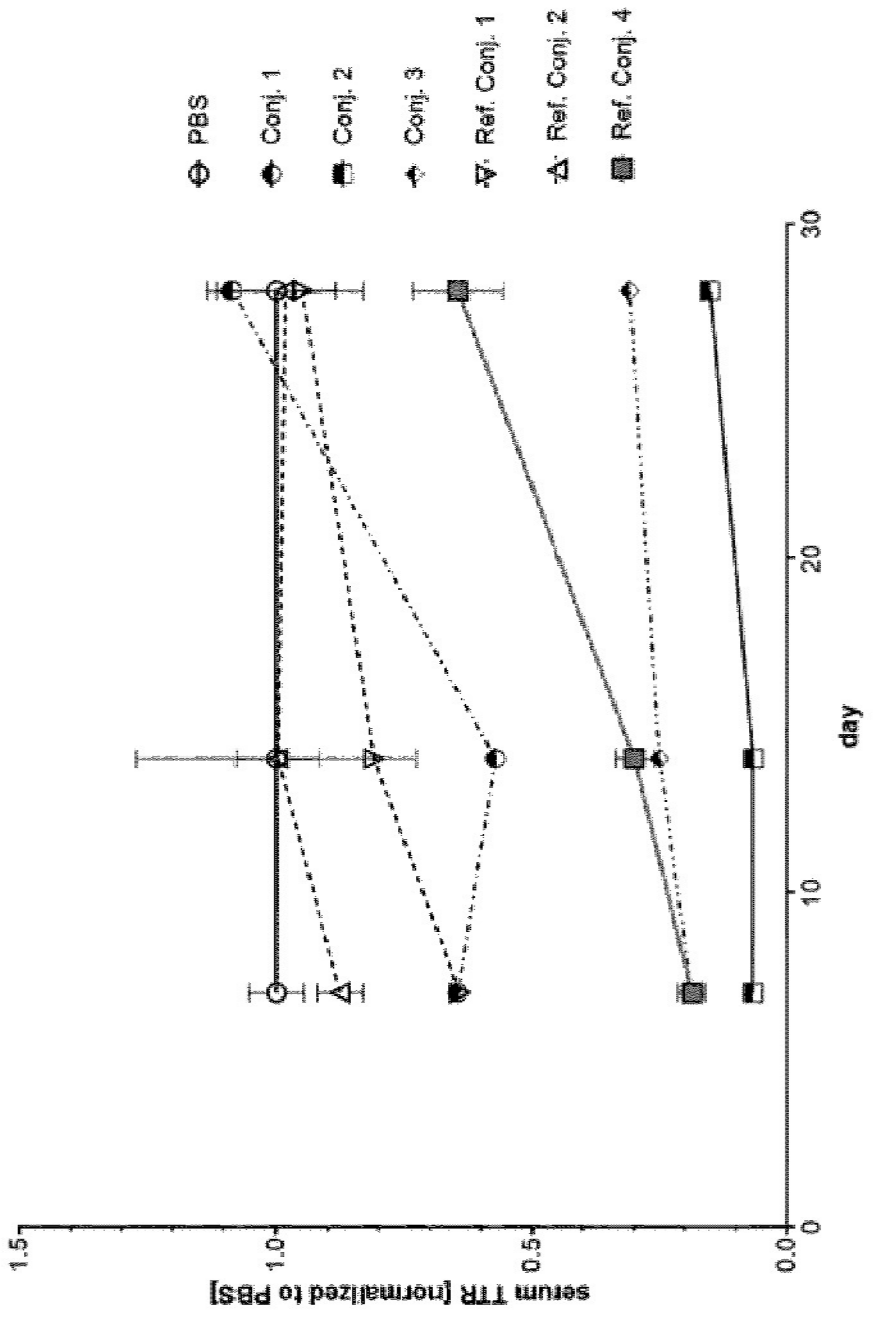

time course of serum TTR in C57BL/6 following
s.c. application of 1mg/kg siRNA-conjugate (mean ± SD, n=4)
PBS
Conj. 1
Conj. 2
Conj. 3
Ref. Conj. 1
Ref. Conj. 2
Ref. Conj. 4
serum TTR [normalized to PBS]
1.5
1.0
0.5
0.0
0
10
20
30
day

Figure 17

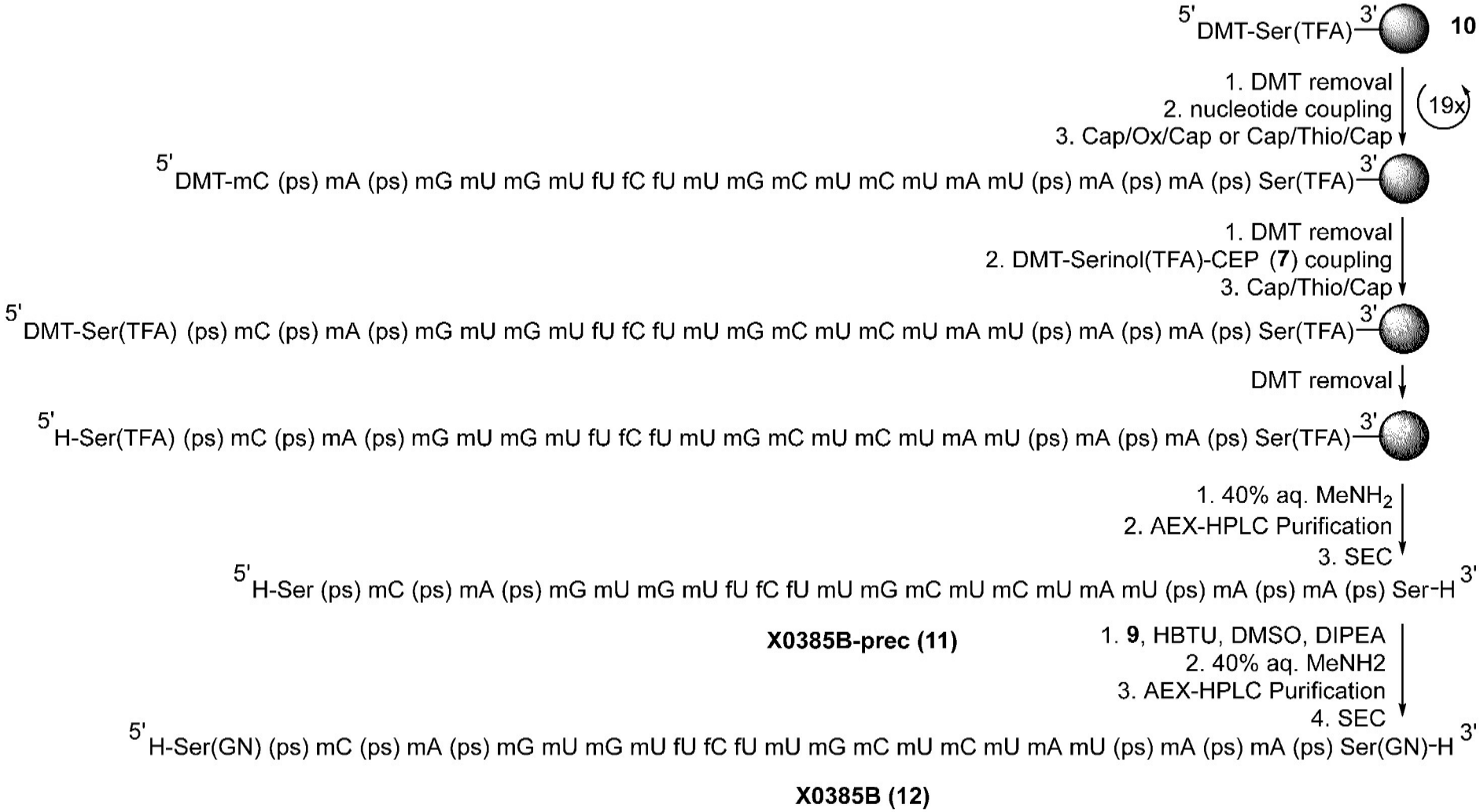

5' DMT-Ser(TFA) 3'
10
1. DMT removal
2. nucleotide coupling
3. Cap/Ox/Cap or Cap/Thio/Cap
19x
5' DMT-mC (ps) mA (ps) mG mU mG mU fU fC fU mU mG mC mU mC mU mA mU (ps) mA (ps) mA (ps) Ser(TFA) 3'
1. DMT removal
2. DMT-Serinol(TFA)-CEP (7) coupling
3. Cap/Thio/Cap
5' DMT-Ser(TFA) (ps) mC (ps) mA (ps) mG mU mG mU fU fC fU mU mG mC mU mC mU mA mU (ps) mA (ps) mA (ps) Ser(TFA) 3'
DMT removal
5' H-Ser(TFA) (ps) mC (ps) mA (ps) mG mU mG mU fU fC fU mU mG mC mU mC mU mA mU (ps) mA (ps) mA (ps) Ser(TFA) 3'
1. 40% aq. MeNH2
2. AEX-HPLC Purification
3. SEC
5' H-Ser (ps) mC (ps) mA (ps) mG mU mG mU fU fC fU mU mG mC mU mC mU mA mU (ps) mA (ps) mA (ps) Ser-H 3'
X0385B-prec (11)
1. 9, HBTU, DMSO, DIPEA
2. 40% aq. MeNH2
3. AEX-HPLC Purification
4. SEC
5' H-Ser(GN) (ps) mC (ps) mA (ps) mG mU mG mU fU fC fU mU mG mC mU mC mU mA mU (ps) mA (ps) mA (ps) Ser(GN)-H 3'
X0385B (12)

Figure 18

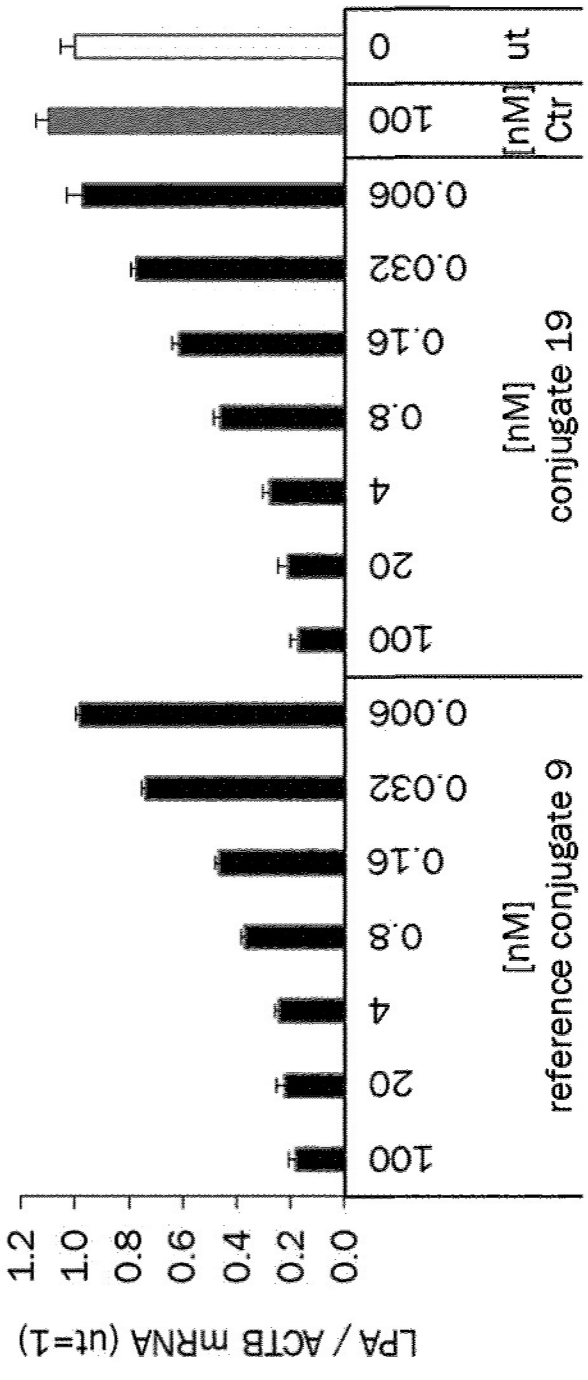

LPA / ACTB mRNA (ut=1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
100
20
4
0.8
0.16
0.032
0.006
[nM]
reference conjugate 9
100
20
4
0.8
0.16
0.032
0.006
[nM]
conjugate 19
100
[nM]
Ctr
0
ut

Figure 19A

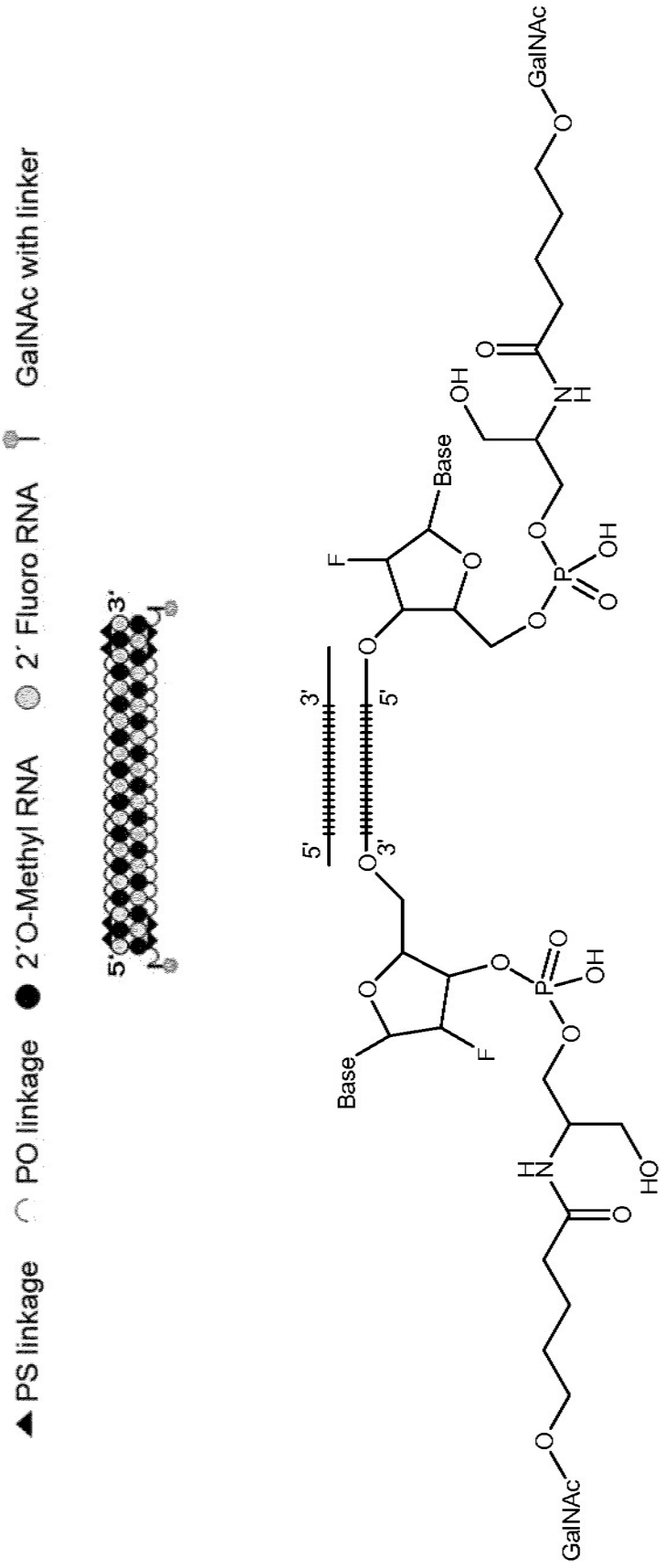

PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
Base
F
OH
HO
GalNAc
NH
O
P

Figure 19B

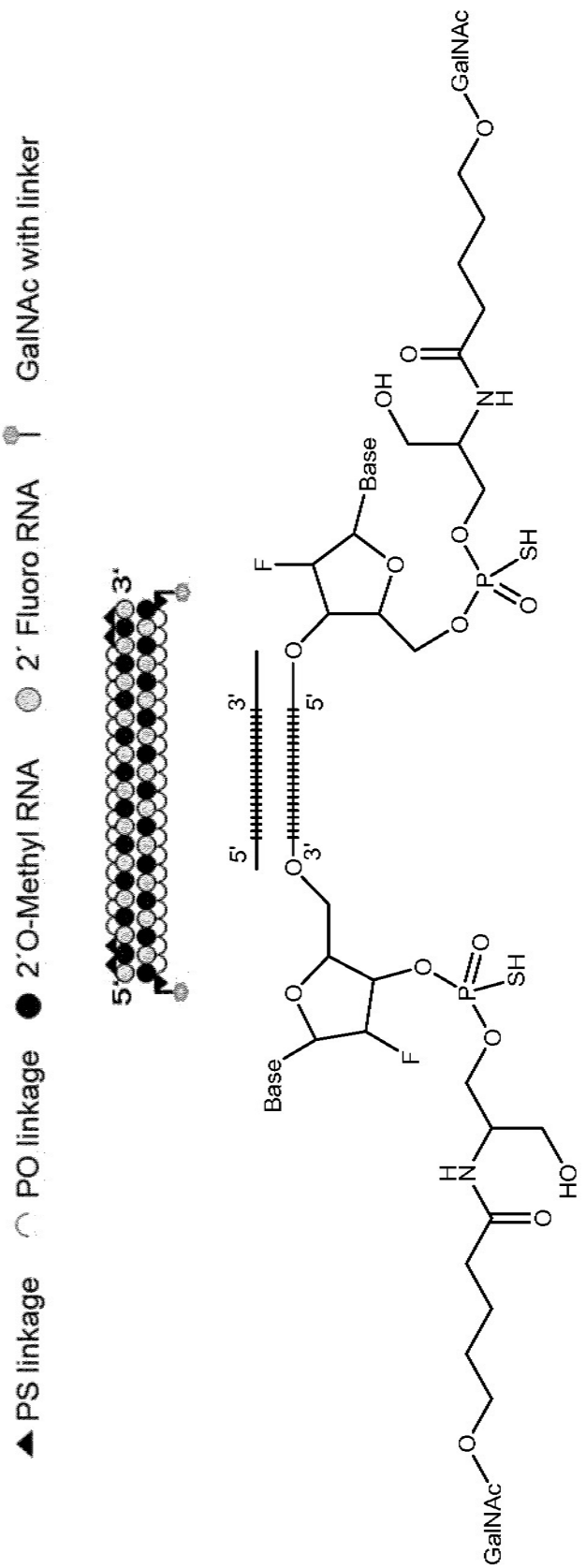

▲ PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
Base
F
OH
SH
HO
GalNAc

Figure 20

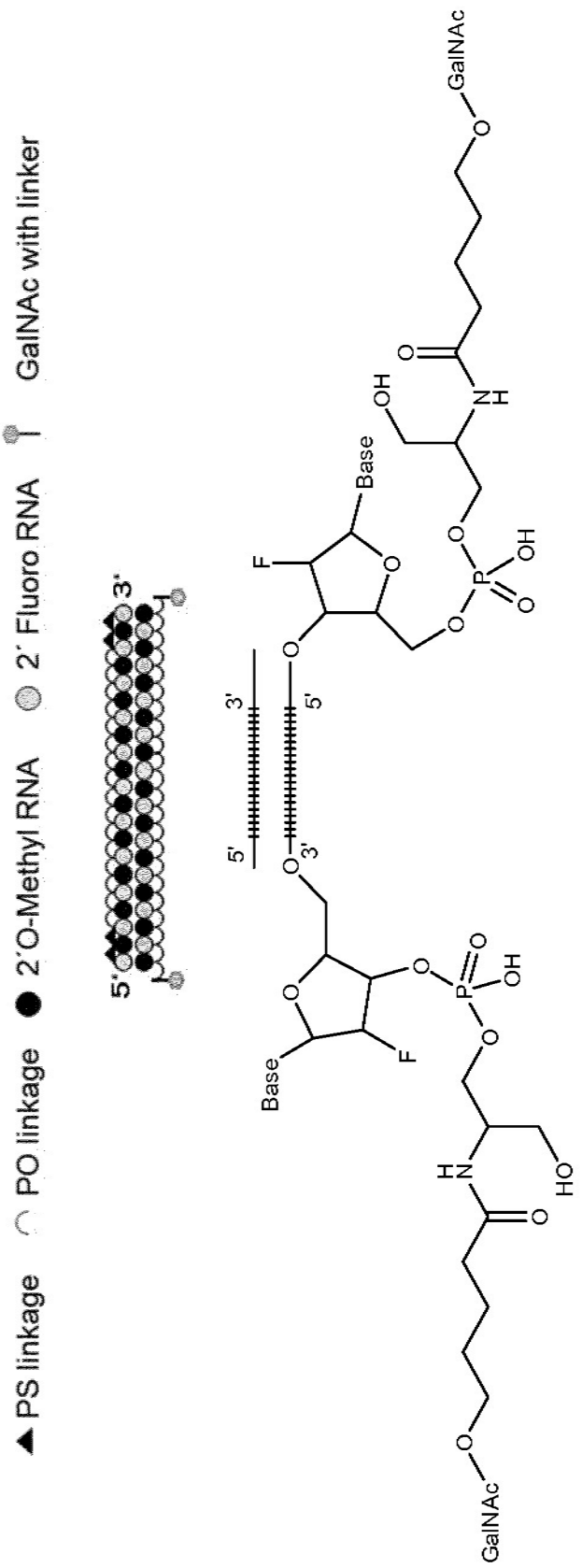

▲ PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
Base
F
OH
HO
GalNAc
H
N
O
P

Figure 21

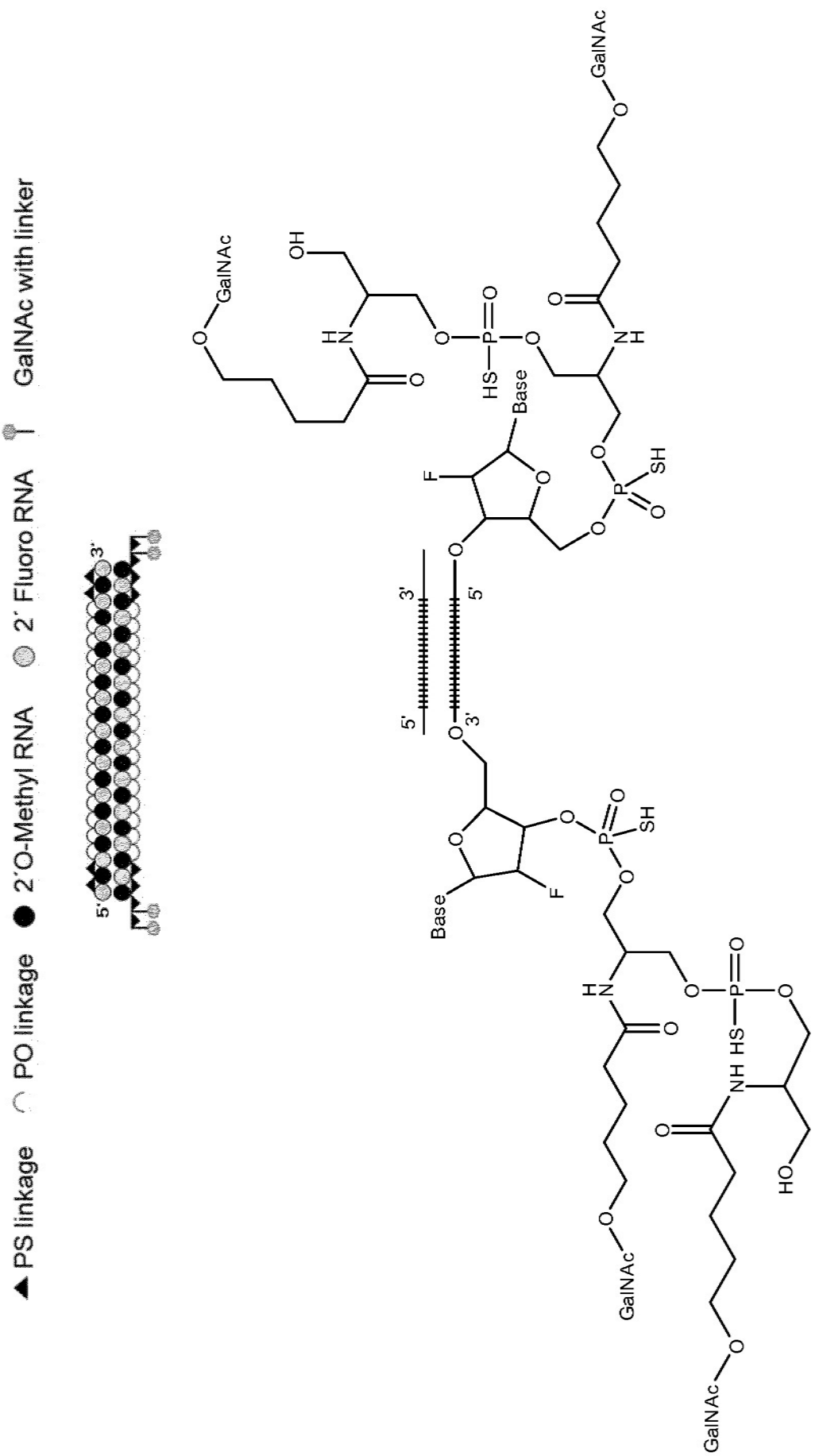

PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
GalNAc
OH
HS
Base
F
SH
HO
NH

Figure 22

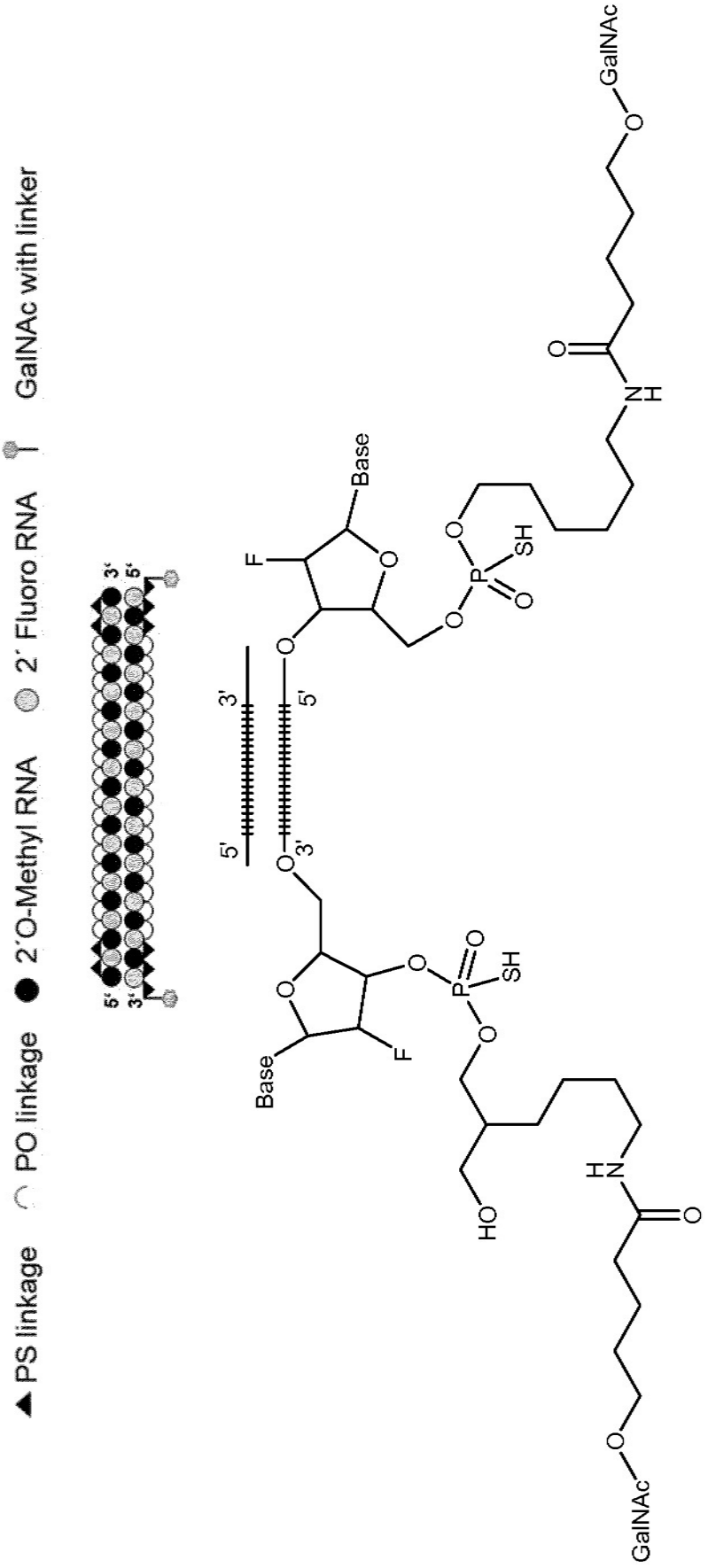

PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
Base
F
SH
HO
H
N
O
GalNAc

Figure 23

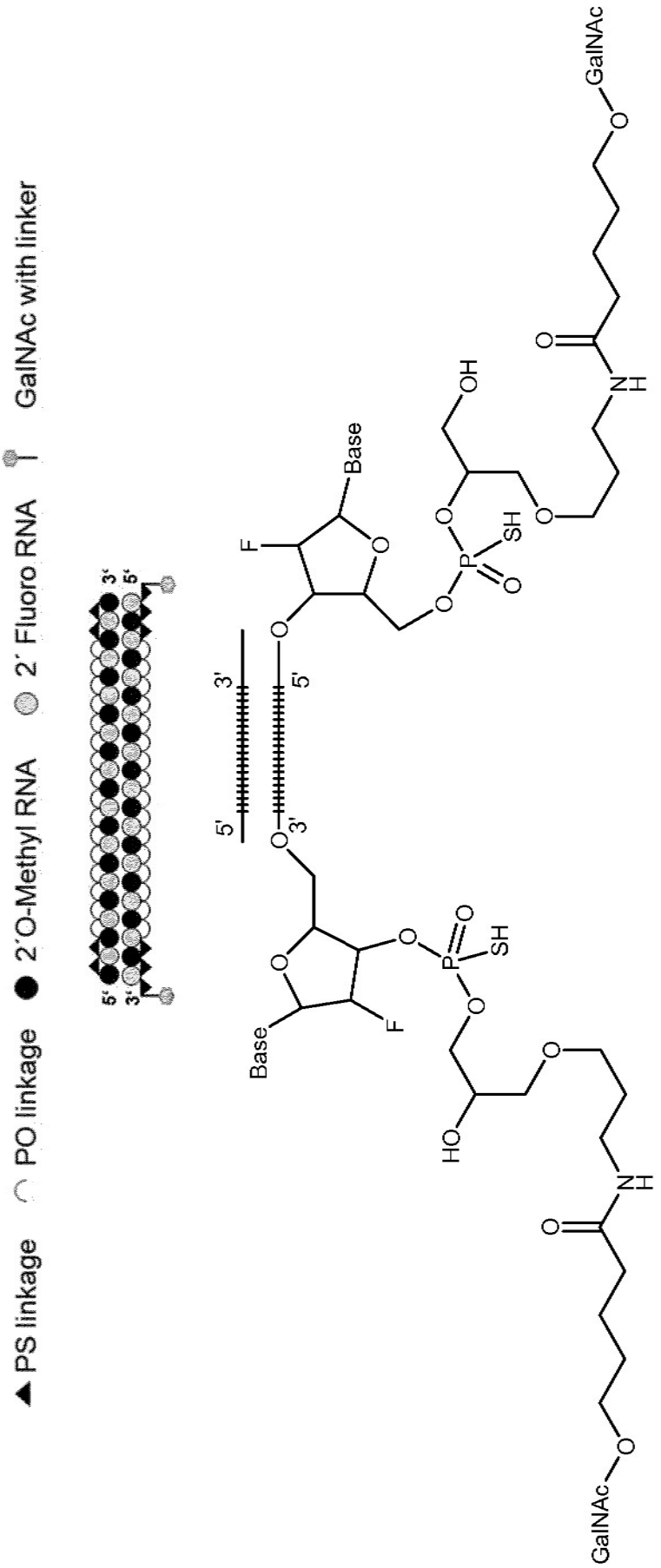

▲ PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
3'
5'
Base
F
O
P
SH
OH
HO
N
H
GalNAc

Figure 24

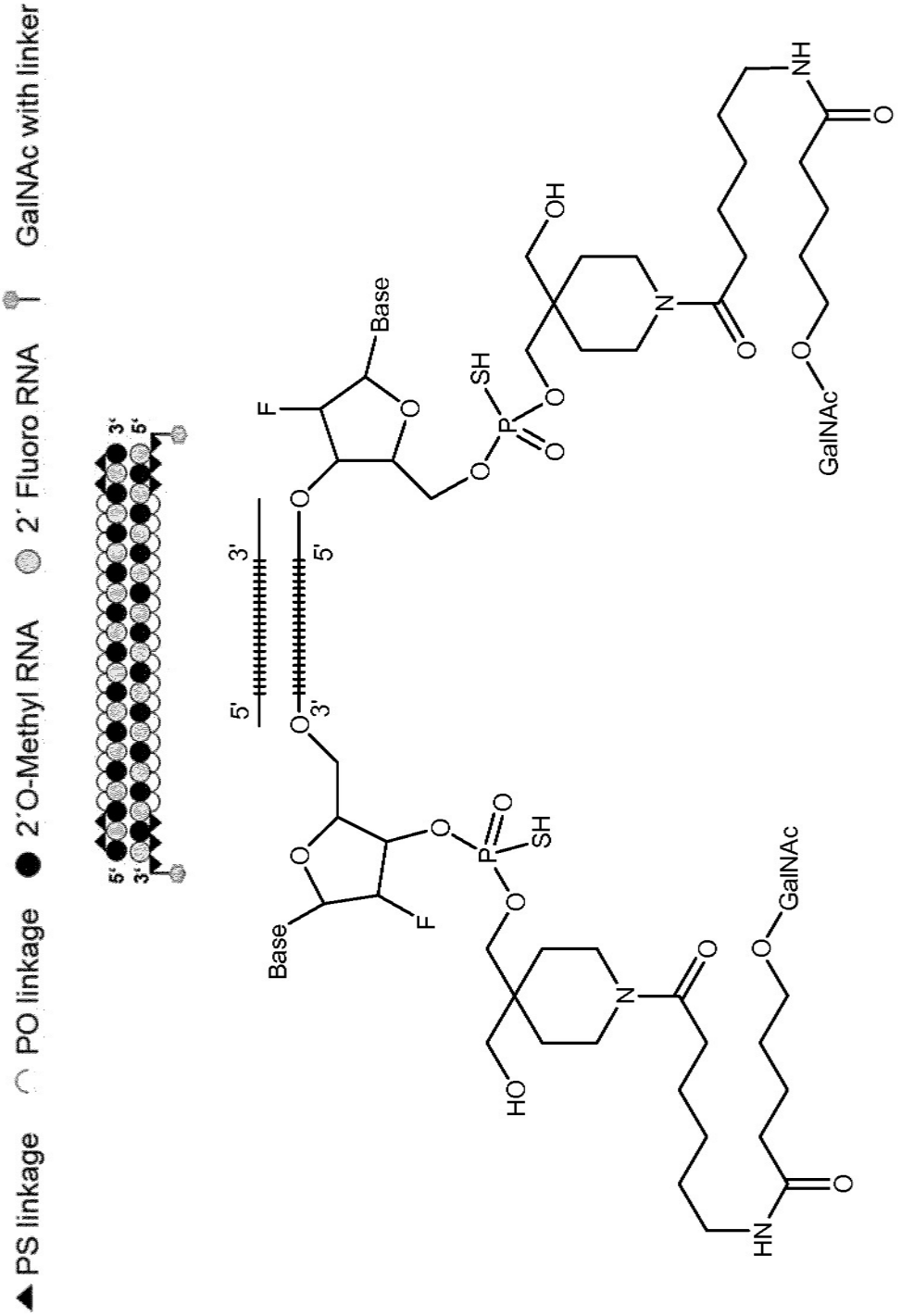

PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
Base
F
O
P
SH
OH
HO
N
NH
HN
GalNAc

Figure 25

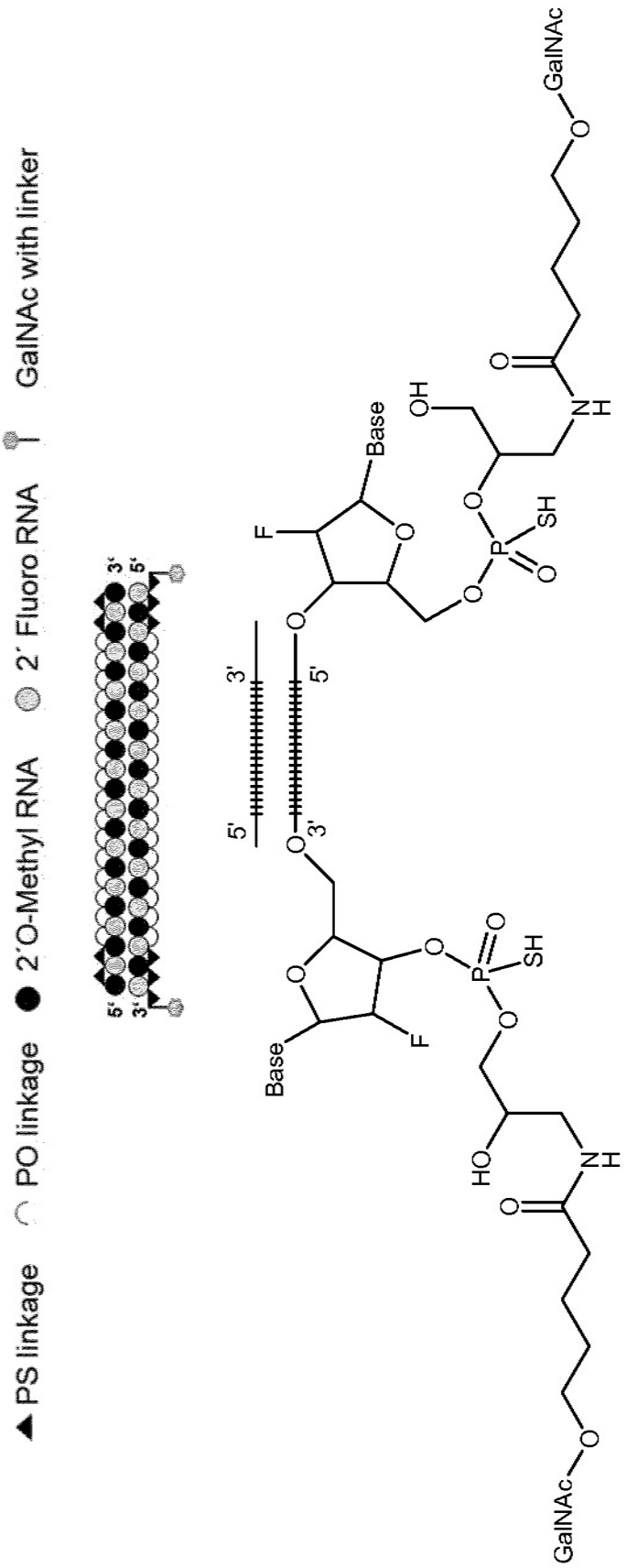

▲ PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
3'
5'
Base
F
O
P
SH
OH
HO
N
H
GalNAc

Figure 26

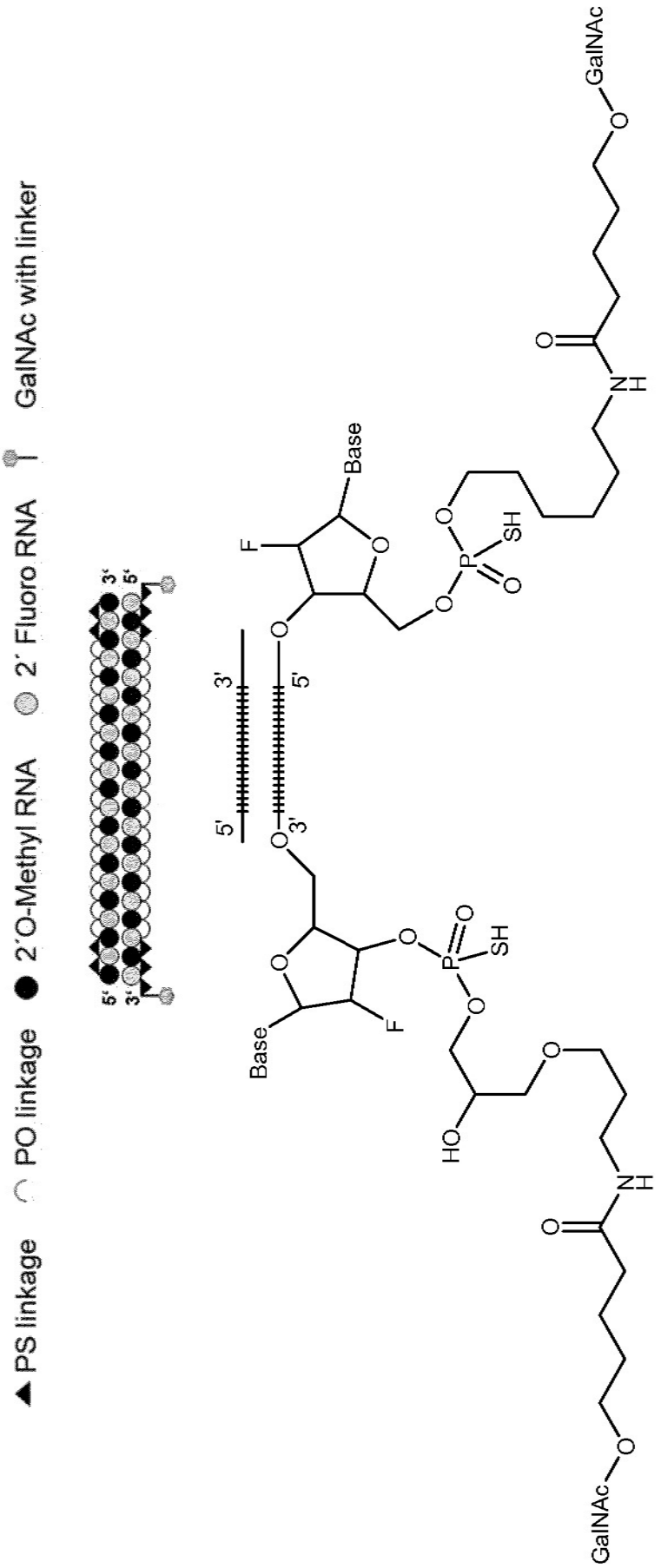

PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
Base
F
O
P
SH
HO
N
H
GalNAc

Figure 27

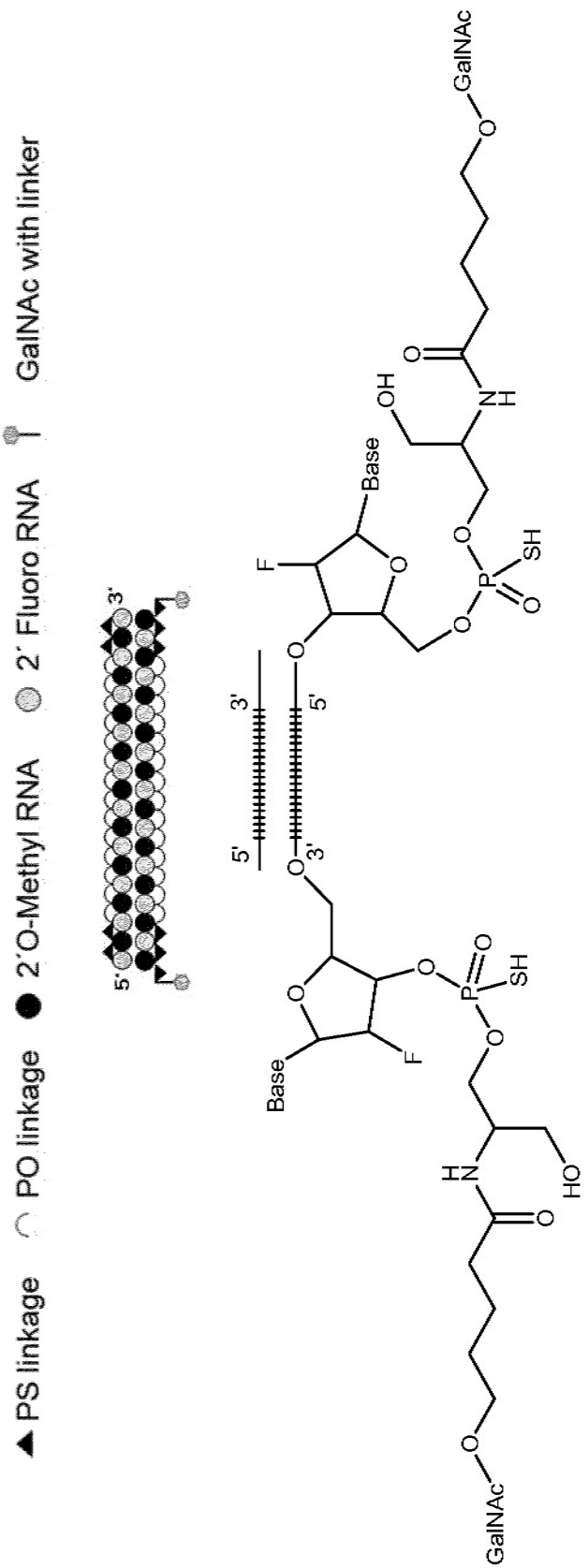

▲ PS linkage
PO linkage
2'O-Methyl RNA
2' Fluoro RNA
GalNAc with linker
5'
3'
Base
F
O
SH
HO
OH
N
H
GalNAc

Figure 28

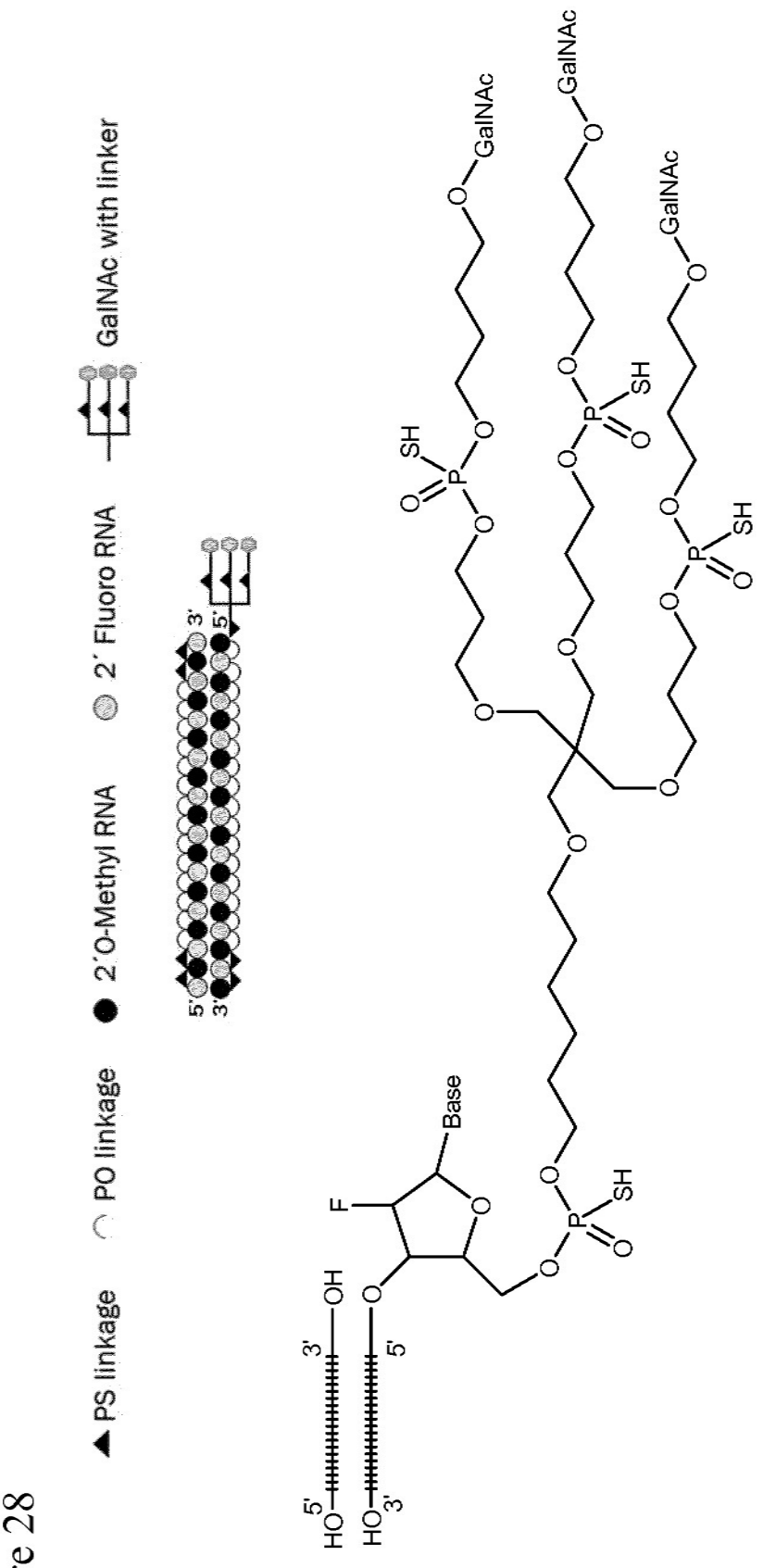

PS linkage
PO linkage
2´O-Methyl RNA
2´ Fluoro RNA
GalNAc with linker
5'
3'
HO
OH
F
Base
SH
GalNAc

Figure 29

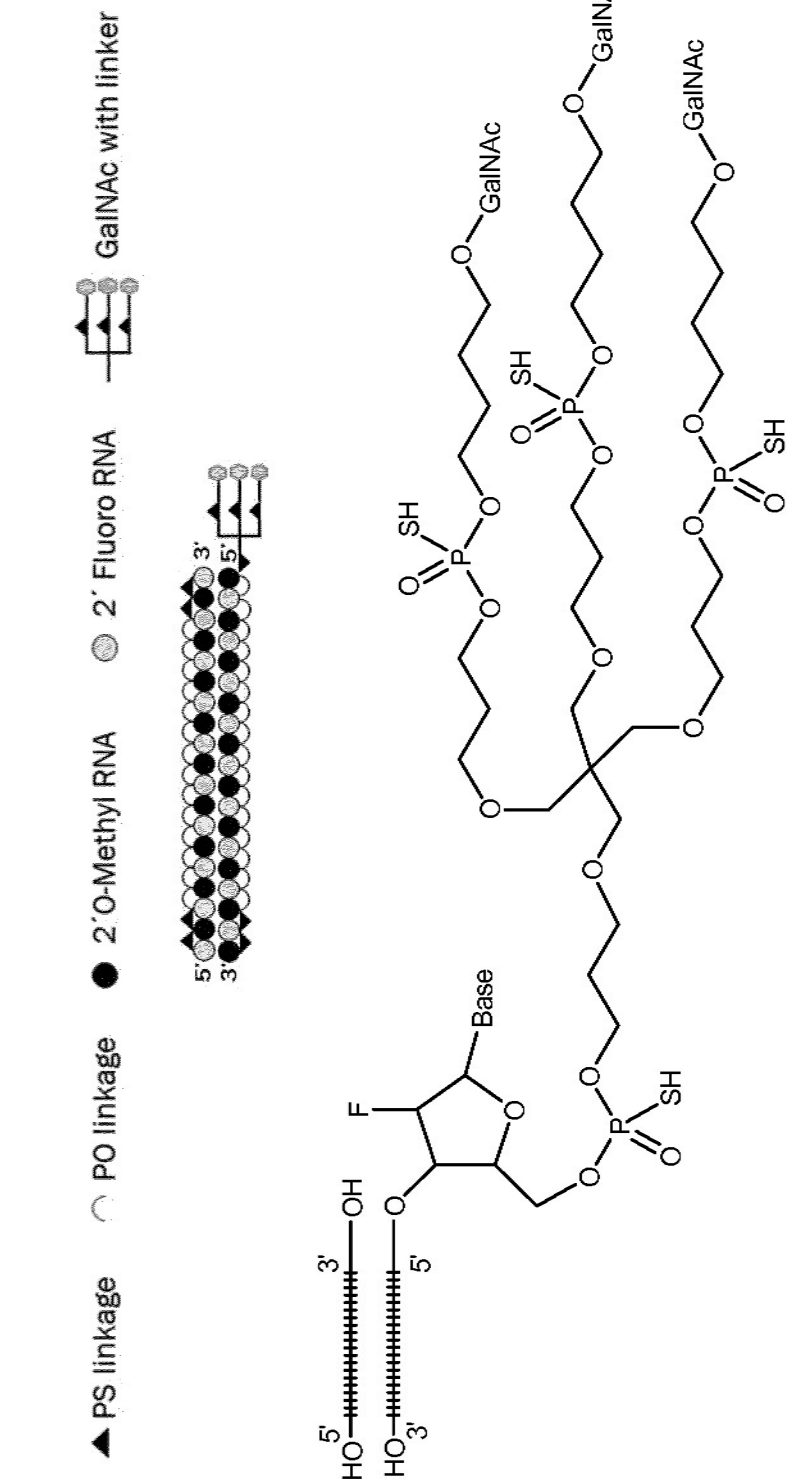
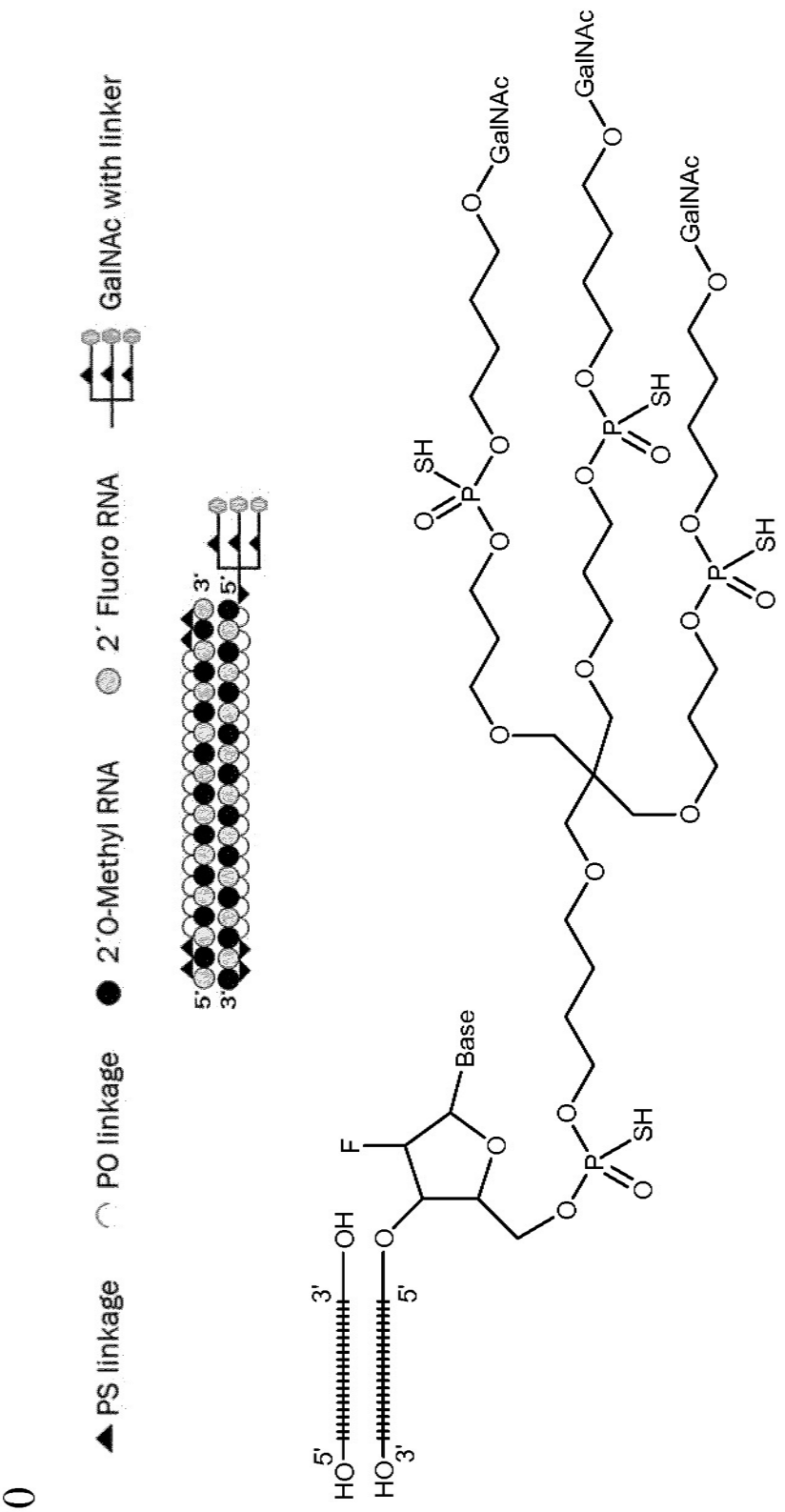

PS linkage
PO linkage
2´O-Methyl RNA
2´ Fluoro RNA
GalNAc with linker
5'
3'
HO
OH
F
Base
SH
GalNAc

Figure 30


PS linkage
PO linkage
2´O-Methyl RNA
2´ Fluoro RNA
GalNAc with linker
5'
3'
HO
OH
Base
F
SH
GalNAc

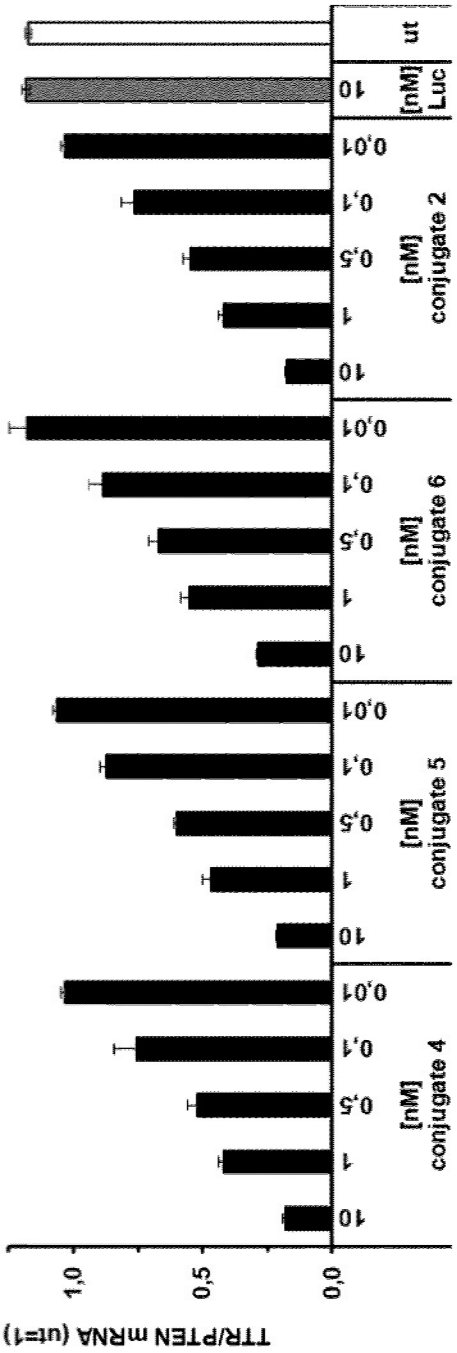


Figure 31A

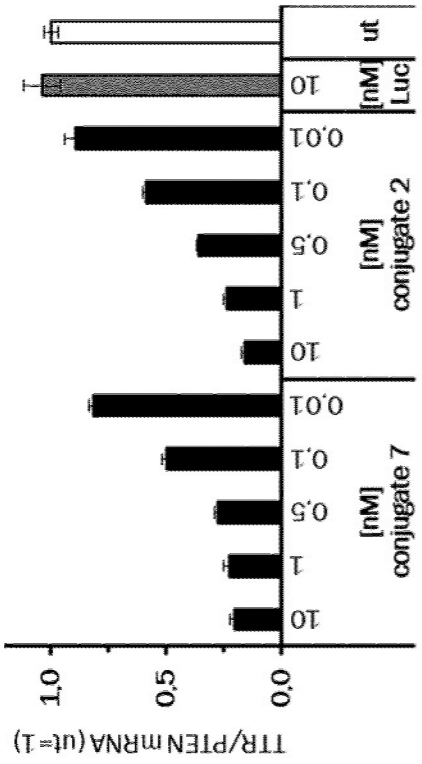


Figure 31B

Figure 32A

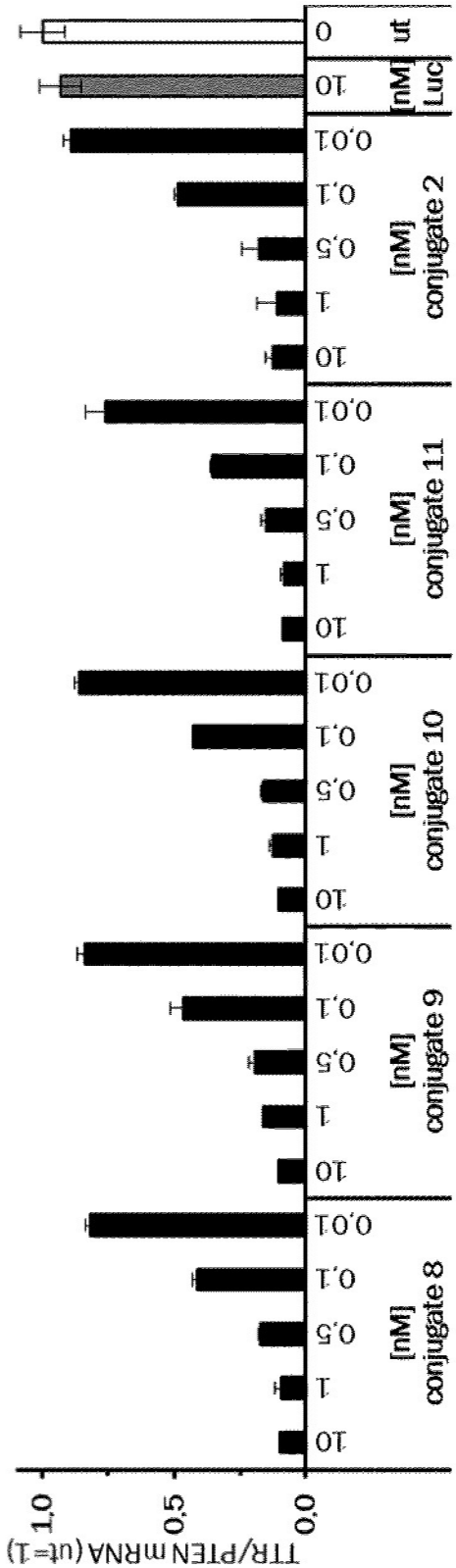
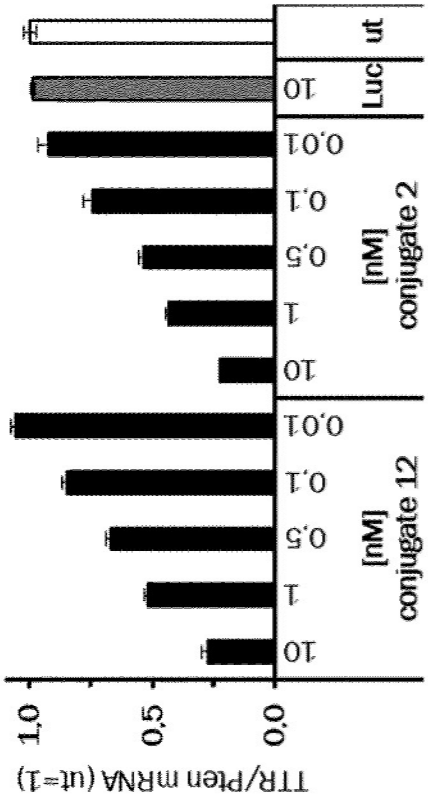


Figure 32B



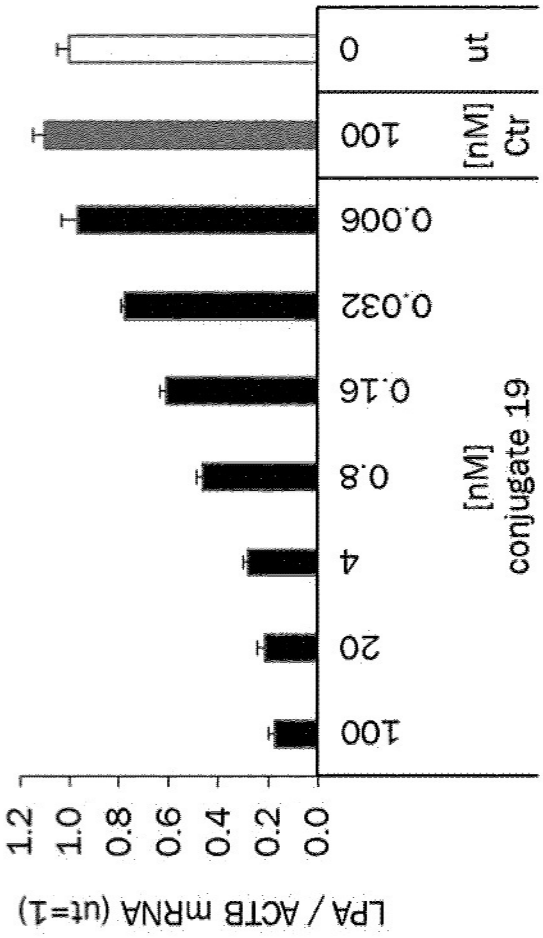

LPA / ACTB mRNA (ut=1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
100
20
4
0.8
0.16
0.032
0.006
[nM]
conjugate 19
100
[nM]
Ctr
0
ut

Figure 33

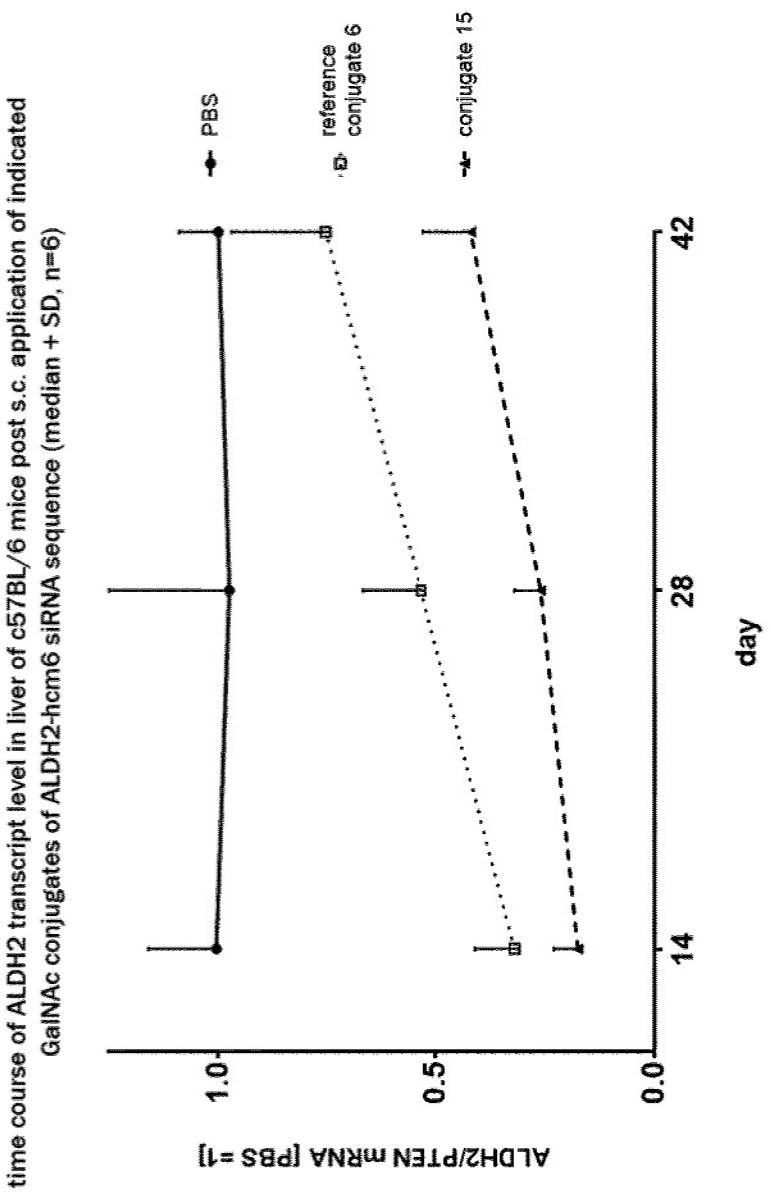

time course of ALDH2 transcript level in liver of c57BL/6 mice post s.c. application of indicated GalNAc conjugates of ALDH2-hcm6 siRNA sequence (median + SD, n=6)
PBS
reference conjugate 6
conjugate 15
ALDH2/PTEN mRNA [PBS =1]
1.0
0.5
0.0
14
28
42
day

Figure 34

Figure 35

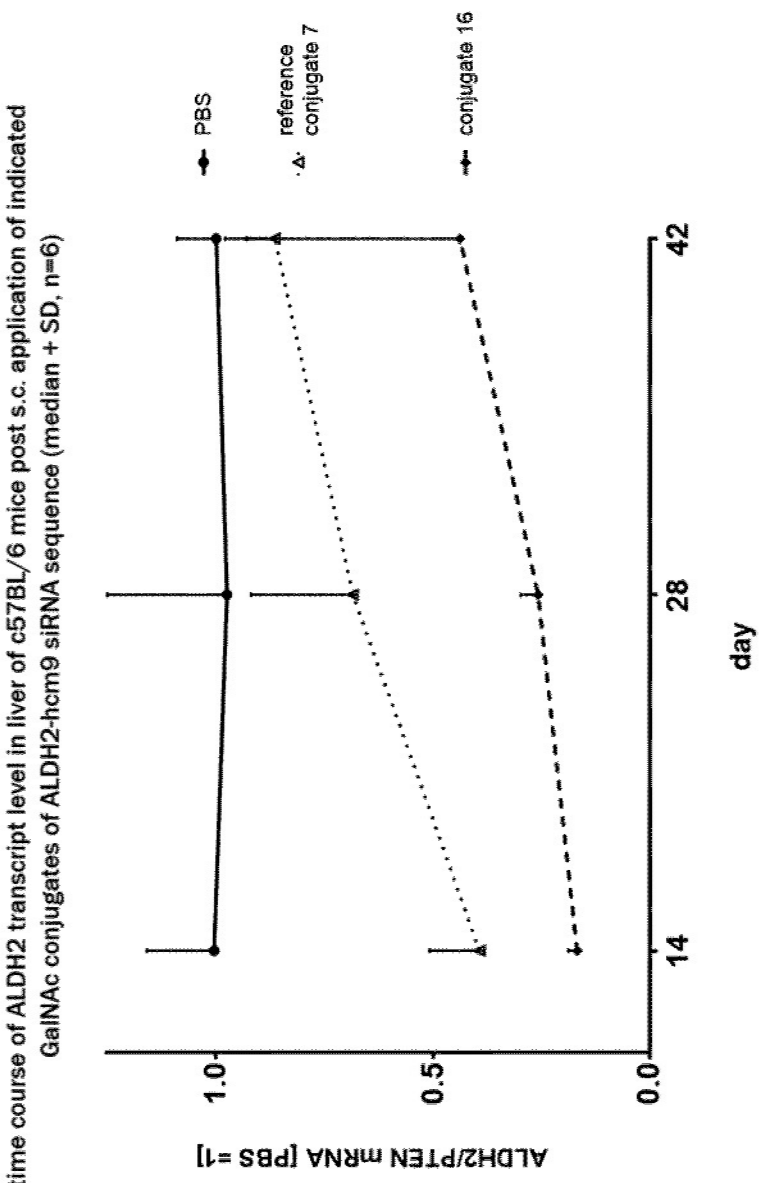

time course of ALDH2 transcript level in liver of c57BL/6 mice post s.c. application of indicated GalNAc conjugates of ALDH2-hcm9 siRNA sequence (median + SD, n=6)
PBS
reference conjugate 7
conjugate 16
ALDH2/PTEN mRNA [PBS =1]
1.0
0.5
0.0
14
28
42
day

Figure 36

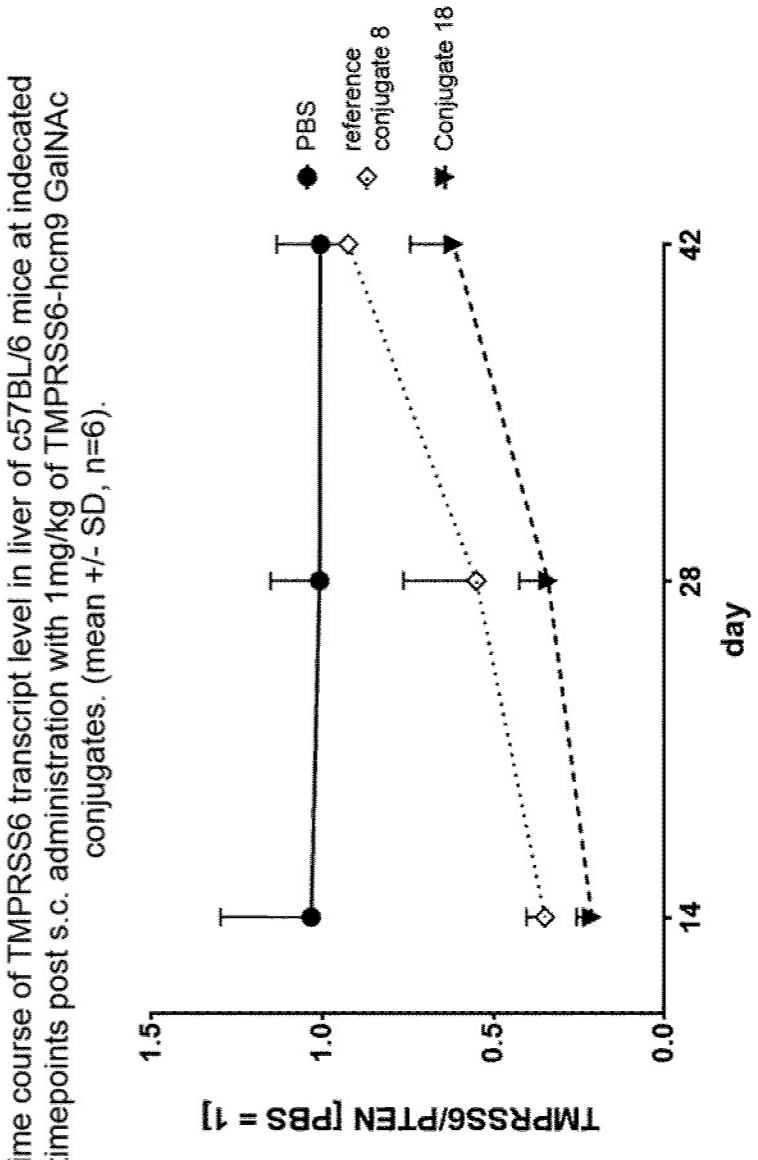

Time course of TMPRSS6 transcript level in liver of c57BL/6 mice at indecated timepoints post s.c. administration with 1mg/kg of TMPRSS6-hcm9 GalNAc conjugates. (mean +/- SD, n=6).
PBS
reference conjugate 8
Conjugate 18
TMPRSS6/PTEN [PBS = 1]
1.5
1.0
0.5
0.0
14
28
42
day

Figure 37

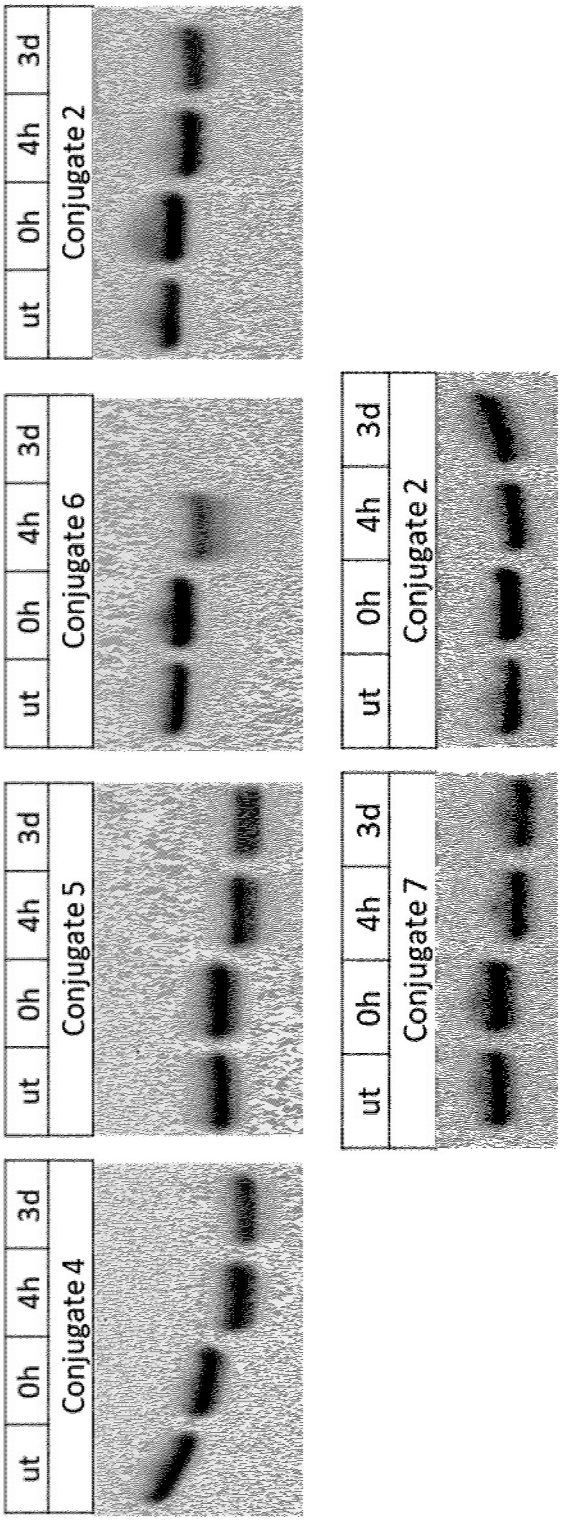

ut
0h
4h
3d
Conjugate 4
Conjugate 5
Conjugate 6
Conjugate 2
Conjugate 7
Conjugate 2

Figure 38

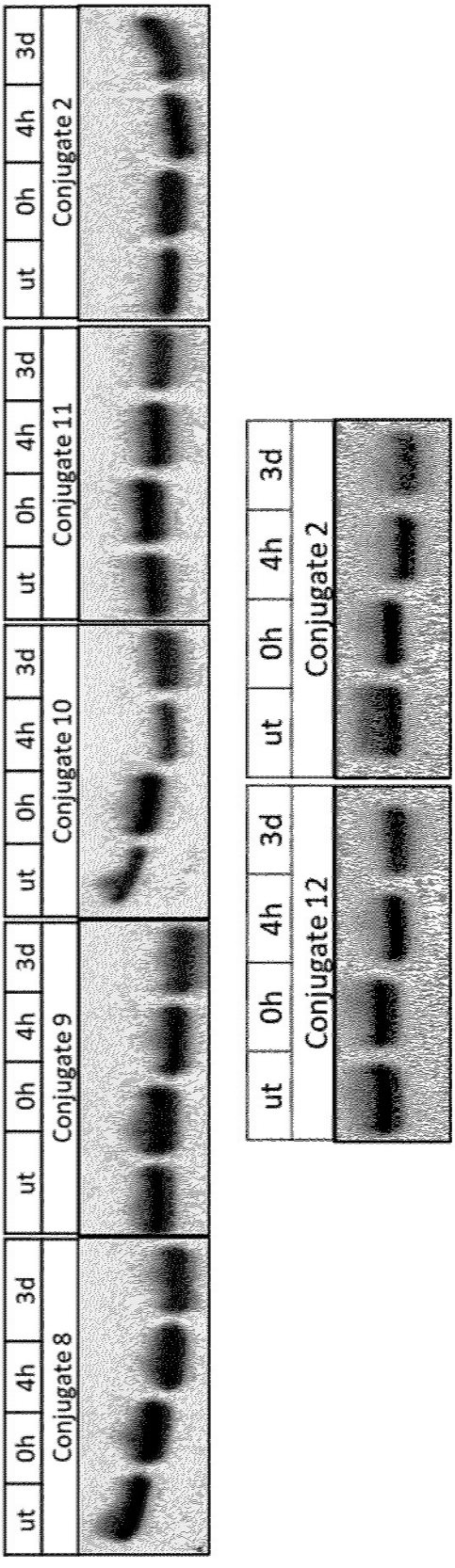

ut
0h
4h
3d
Conjugate 8
Conjugate 9
Conjugate 10
Conjugate 11
Conjugate 2
Conjugate 12

Figure 39

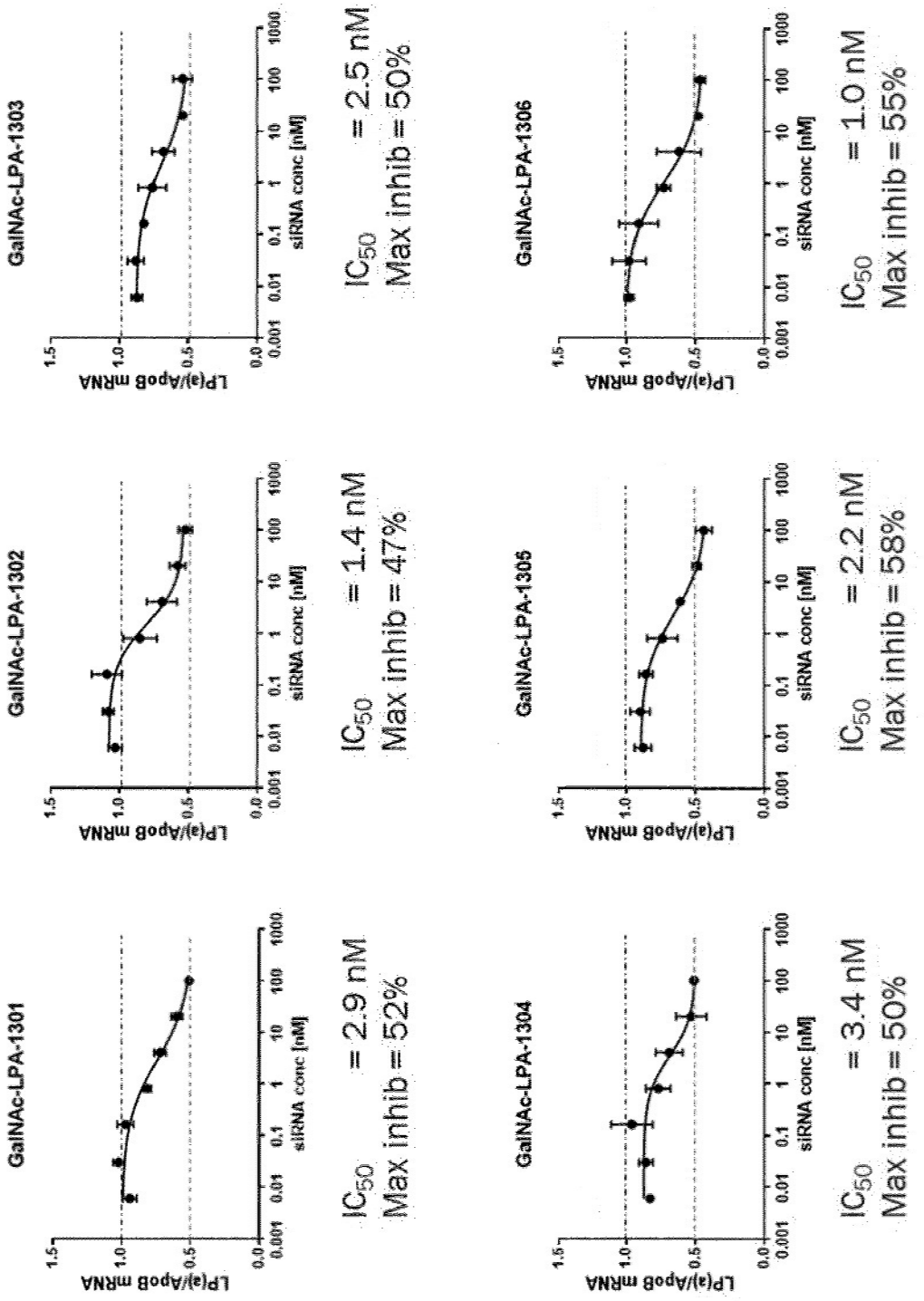

GalNAc-LPA-1301
GalNAc-LPA-1302
GalNAc-LPA-1303
GalNAc-LPA-1304
GalNAc-LPA-1305
GalNAc-LPA-1306
Lp(a)/Apob mRNA
siRNA conc [nM]
IC50 = 2.9 nM
Max inhib = 52%
IC50 = 1.4 nM
Max inhib = 47%
IC50 = 2.5 nM
Max inhib = 50%
IC50 = 3.4 nM
Max inhib = 50%
IC50 = 2.2 nM
Max inhib = 58%
IC50 = 1.0 nM
Max inhib = 55%

Figure 40A

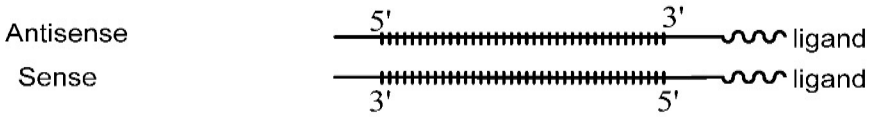


Figure 40B

Figure 40C

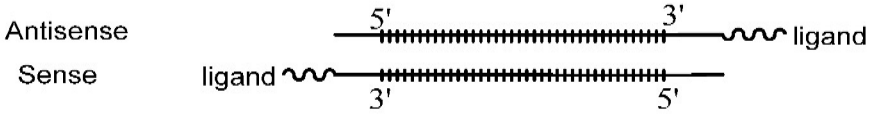


Figure 40D

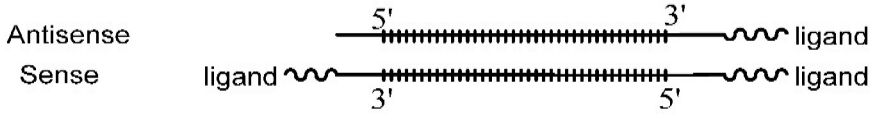


Figure 40E

