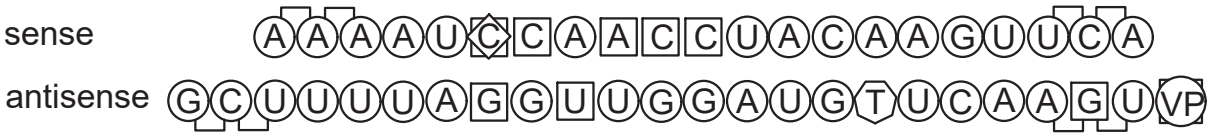
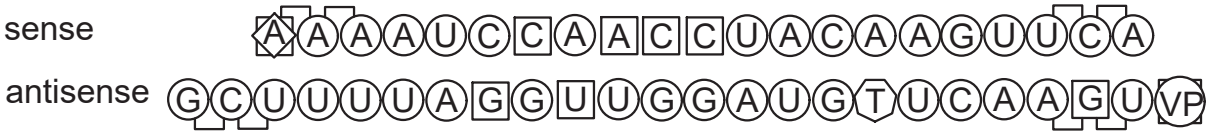
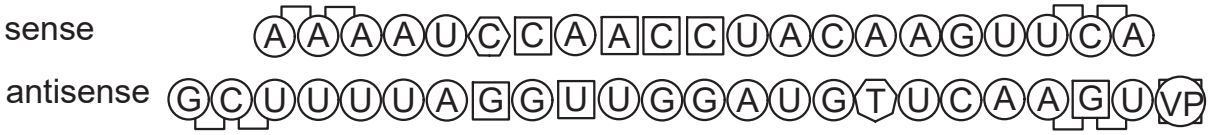
AD-454844



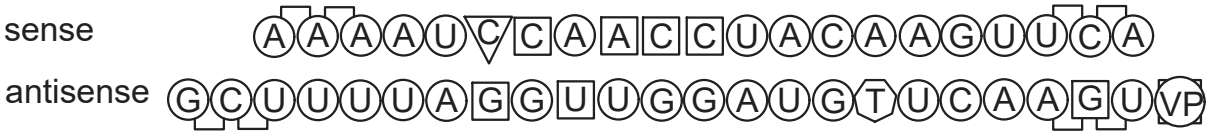
AD-1302922



AD-1302923



AD-1999409



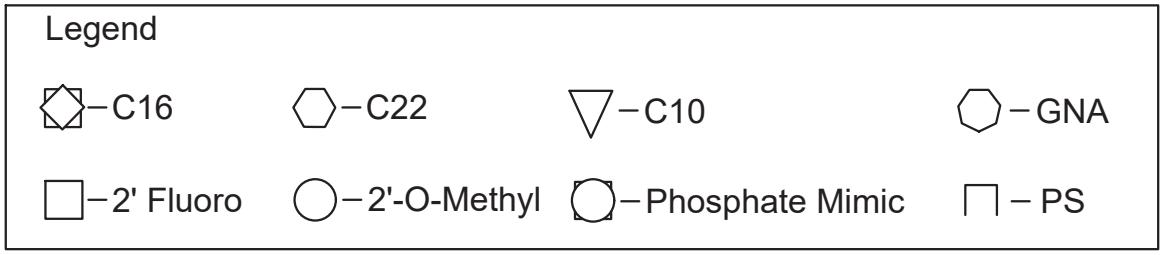


FIG. 1A

Type	Diluent						Drug Product					
Formulation	Original		F1		F2		Original DP		F1 DP		F2 DP	
APP DS	0						60 mg/mL					
Unit	Conc (g/L)	Conc. (mM)	Conc (g/L)	Conc. (mM)	Conc (g/L)	Conc. (mM)	Conc (g/L)	Conc. (mM)	Conc (g/L)	Conc. (mM)	Conc (g/L)	Conc. (mM)
di-sodium hydrogen phosphate	0.10	0.7	0	0	0	0	0.065	0.5	0	0	0	0
sodium dihydrogen phosphate	0.05	0.3	0	0	0	0	0.033	0.2	0	0	0	0
Sodium chloride	8.77	150.1	8.77	150.1	8.77	150.1	5.701	97.6	5.701	97.6	5.701	97.6
Potassium chloride	0.22	3.0	0.22	3.0	0	0	0.143	1.9	0.143	1.9	0	0
Magnesium chloride	0.16	0.8	0.16	0.8	0	0	0.104	0.5	0.104	0.5	0	0
Calcium chloride	0.21	1.4	0.21	1.4	0.21	1.4	1.911	13.0	1.911	13.0	1.911	13.0

FIG. 1B

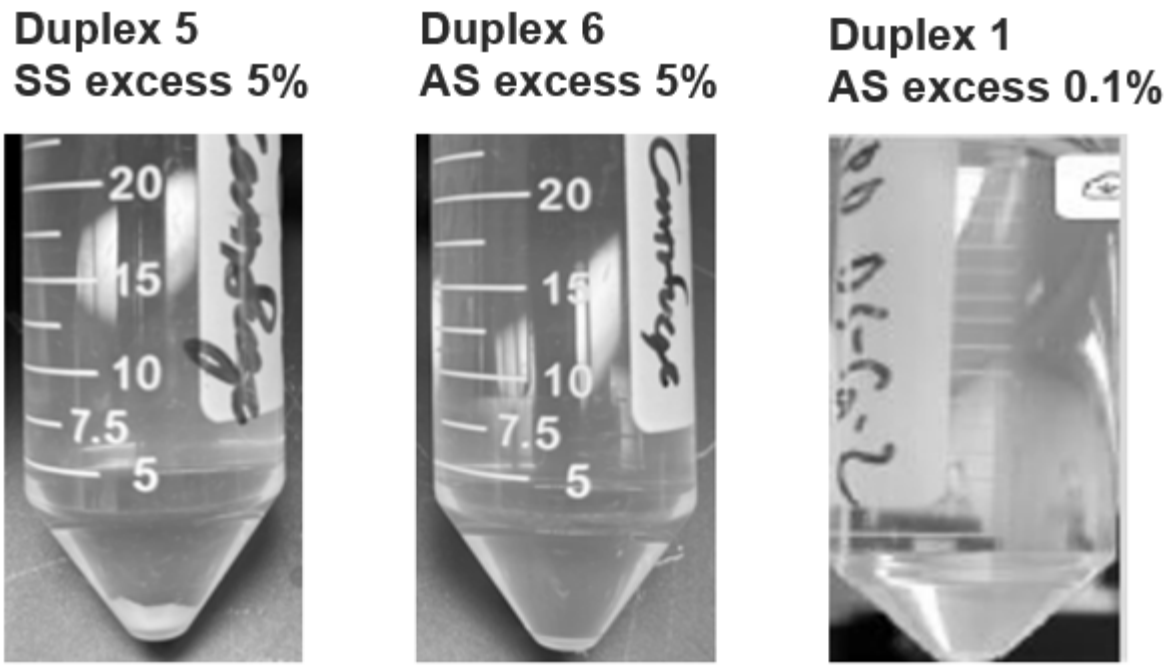

Duplex 5
SS excess 5%
20
15
10
7.5
5
Duplex 6
AS excess 5%
20
15
10
7.5
5
Duplex 1
AS excess 0.1%

FIG. 2

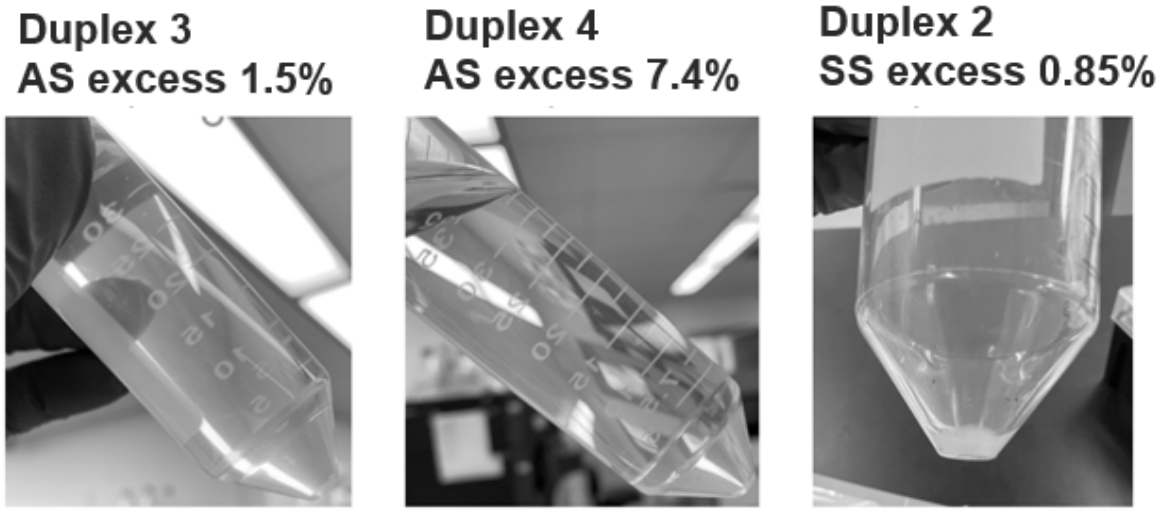

Duplex 3
AS excess 1.5%
Duplex 4
AS excess 7.4%
Duplex 2
SS excess 0.85%

FIG. 3

	Internal C16 - Mg	Internal C16 - Ca	Terminal C16	Internal C22	Internal C10	RODI
	✓	✓	✓	✓	×	-
Before						
After						

FIG. 4A

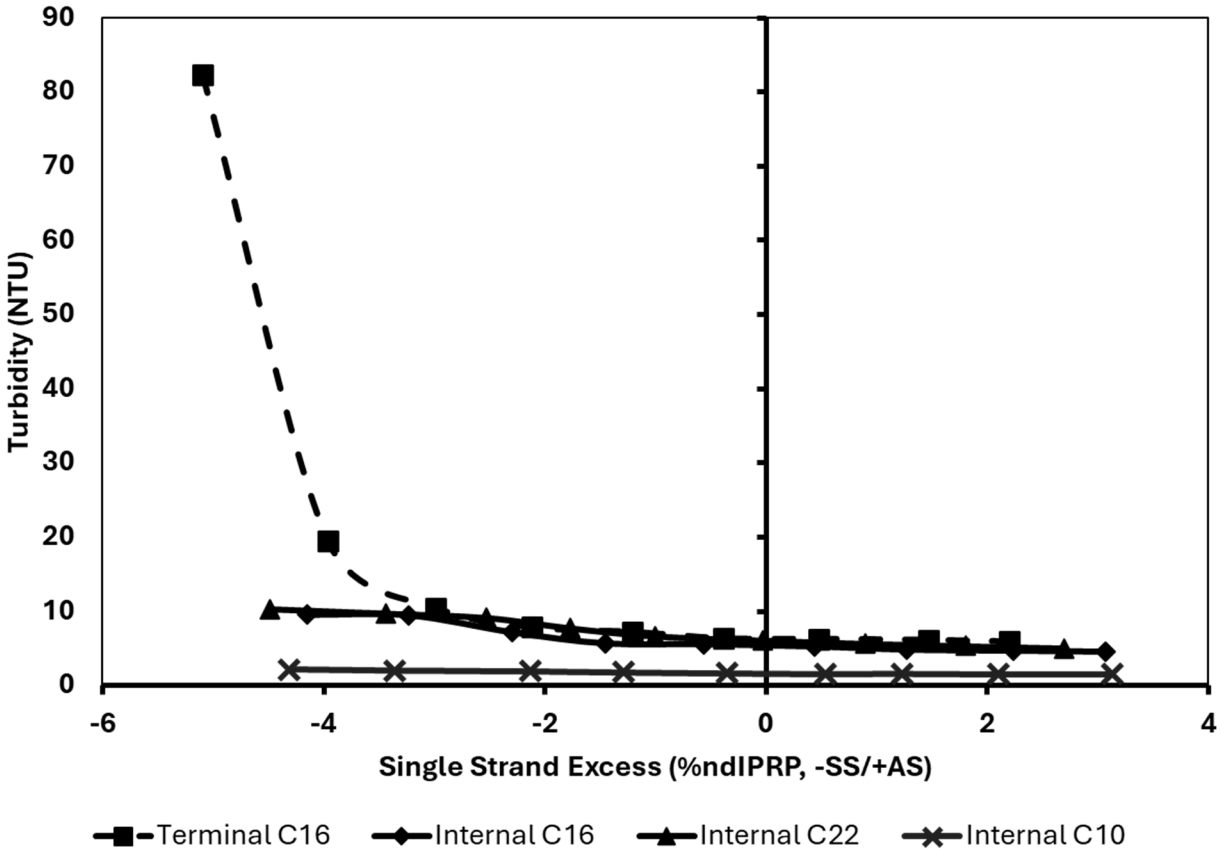

90
80
70
60
50
40
30
20
10
0
Turbidity (NTU)
-6
-4
-2
0
2
4
Single Strand Excess (%ndIPRP, -SS/+AS)
Terminal C16
Internal C16
Internal C22
Internal C10

FIG. 4B

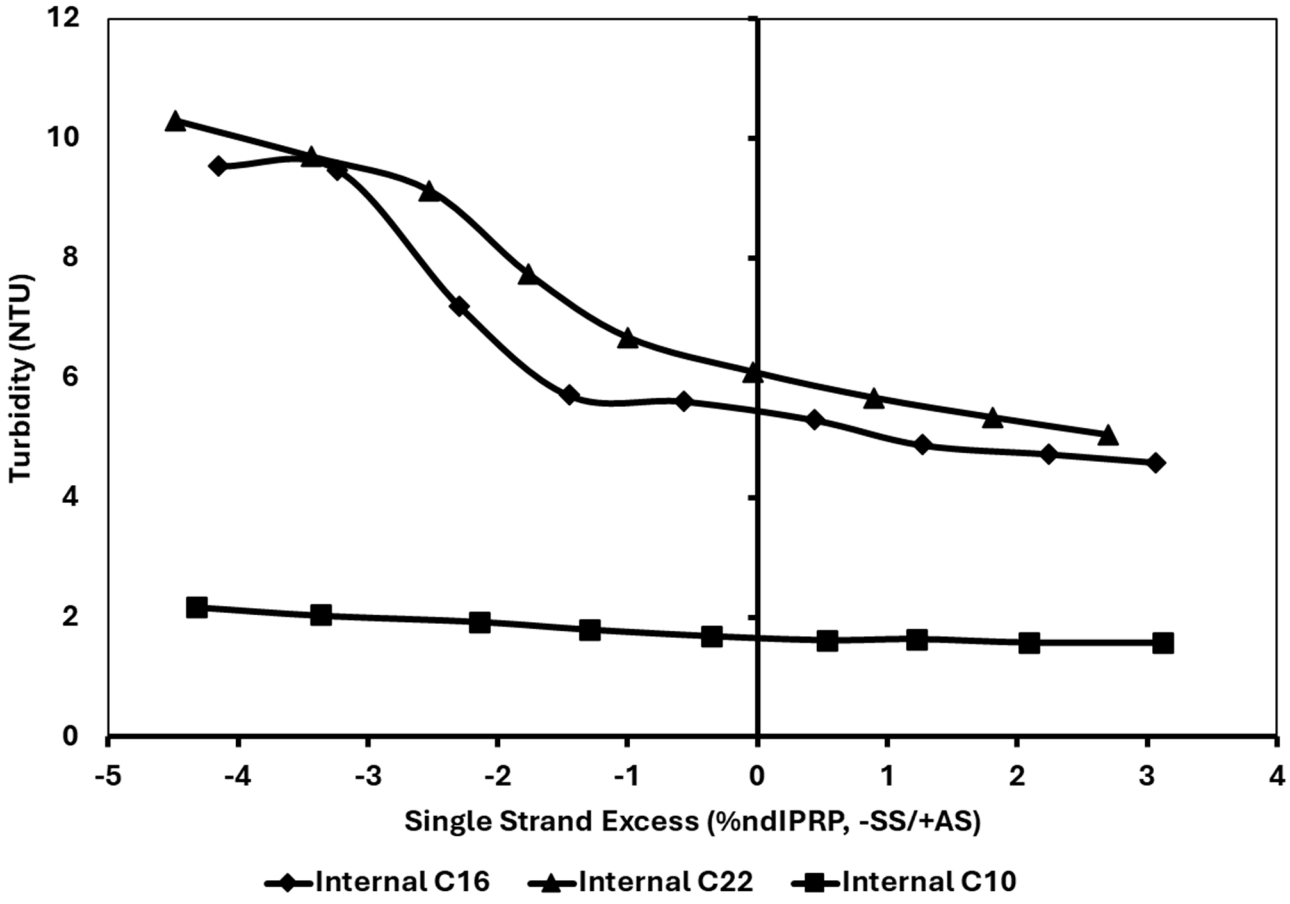

12
10
8
6
4
2
0
Turbidity (NTU)
-5
-4
-3
-2
-1
0
1
2
3
4
Single Strand Excess (%ndIPRP, -SS/+AS)
Internal C16
Internal C22
Internal C10

FIG. 4C

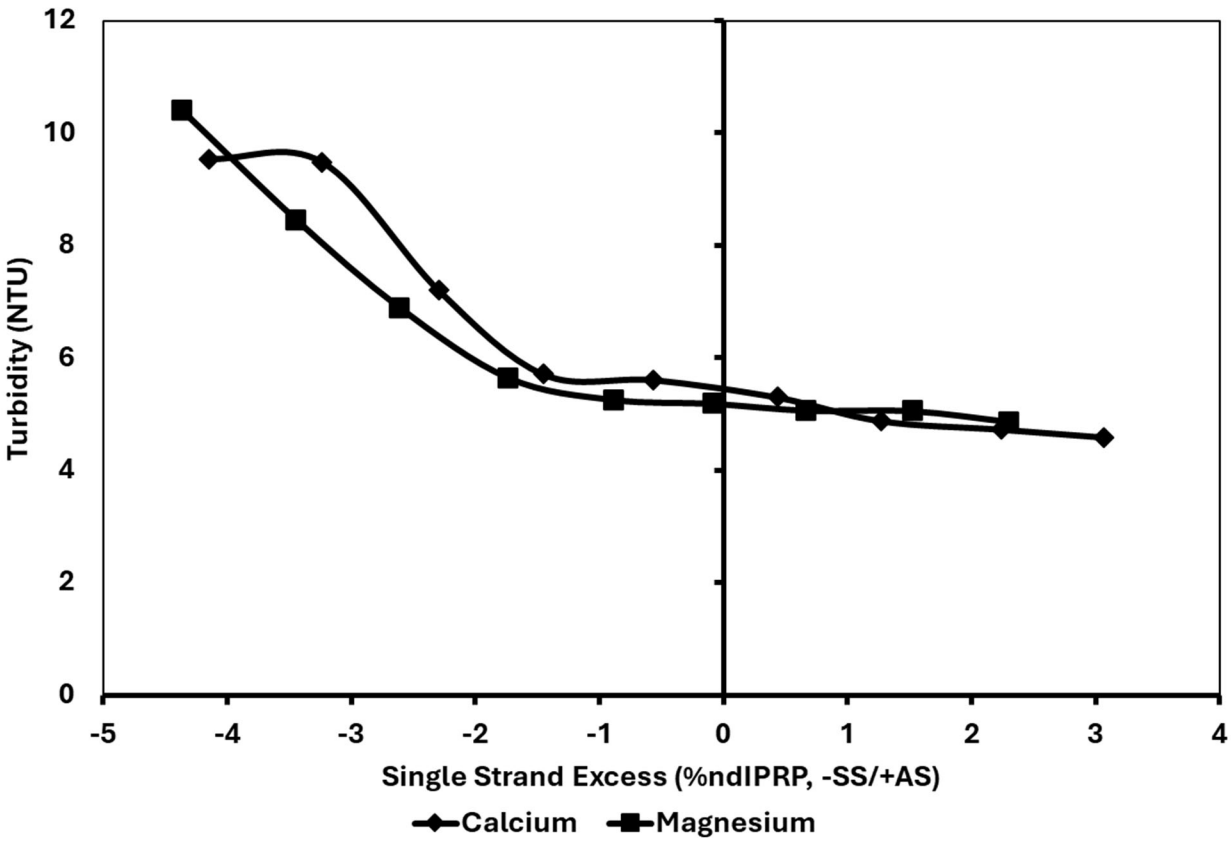

12
10
8
6
4
2
0
Turbidity (NTU)
-5
-4
-3
-2
-1
0
1
2
3
4
Single Strand Excess (%ndIPRP, -SS/+AS)
Calcium
Magnesium

FIG. 4D

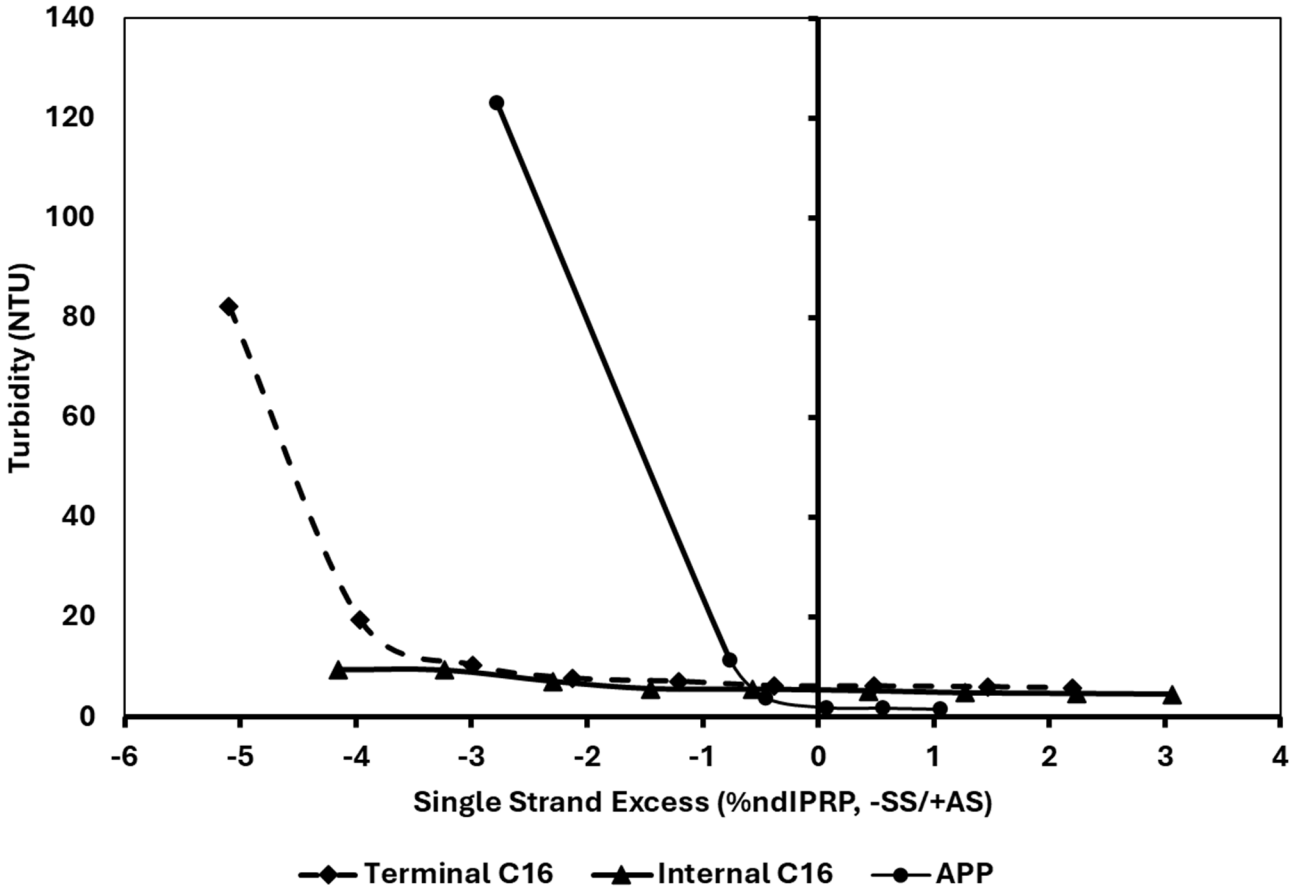
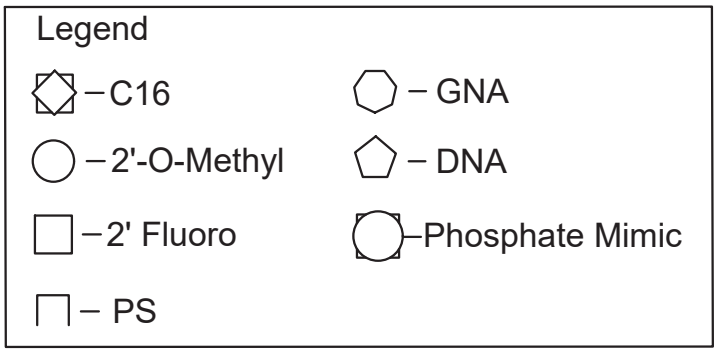
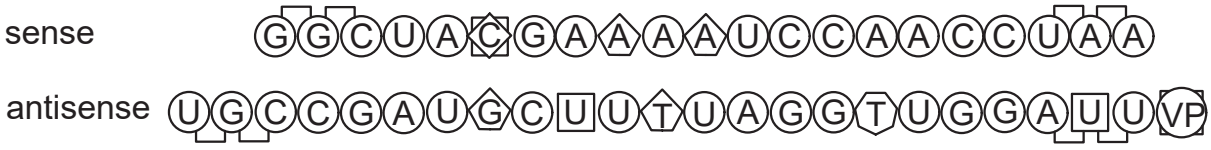

140
120
100
80
60
40
20
0
Turbidity (NTU)
-6
-5
-4
-3
-2
-1
0
1
2
3
4
Single Strand Excess (%ndIPRP, -SS/+AS)
Terminal C16
Internal C16
APP

FIG. 4E

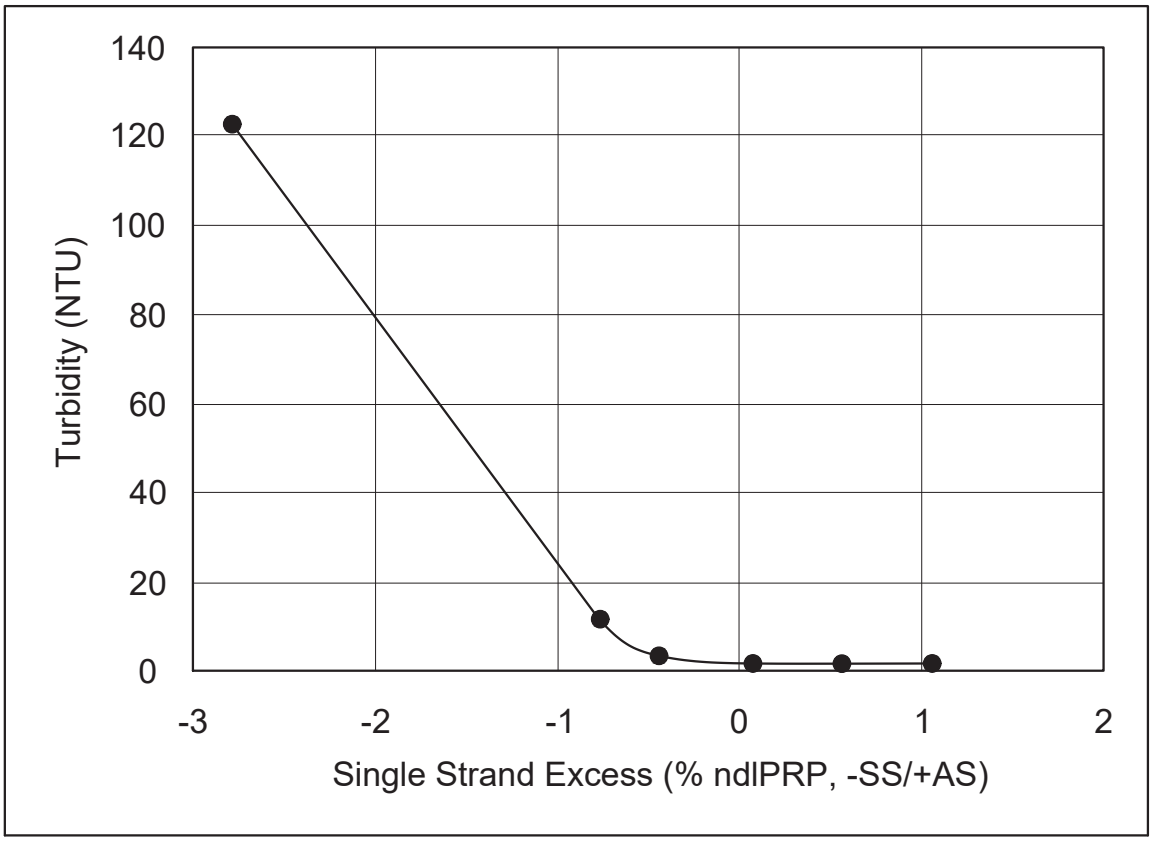


AD-961583



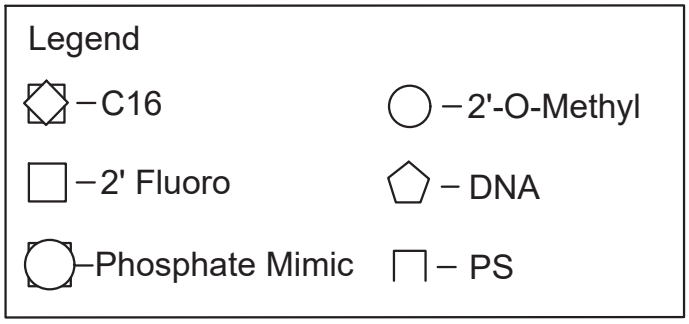
Antisense Addition	0uL	200uL	250uL	300uL	350uL	400uL	RODI
Single Strand excess	2.8% SS	0.8% SS	0.5% SS	0.1% AS	0.6% AS	1.1% AS	-
Before Antisense Addition							
After Antisense Addition							

FIG. 5A

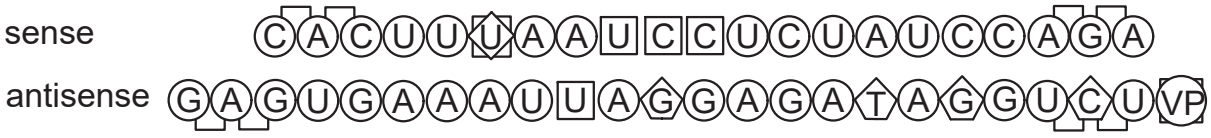


Antisense Excess	-2.8%	-0.8%	-0.5%	0.1%	0.6%	1.1%
Turbidity (NTU)	123	11.4	3.8	1.9	1.7	1.5

FIG. 5B



AD-1395762



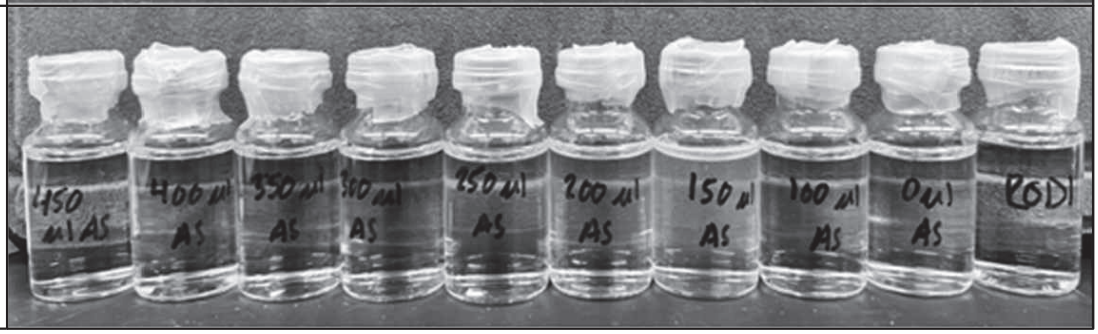
Antisense Addition	450uL	400uL	350uL	300uL	250uL	200uL	150uL	100uL	0uL	RODI
Single Strand excess	5.9% AS	4.3% AS	2.8% AS	1.4% AS	1.9% AS	0.5% SS	1.7% SS	3.2% SS	5.8% SS	-
Before Antisense Addition										
After Antisense Addition										

FIG. 6A

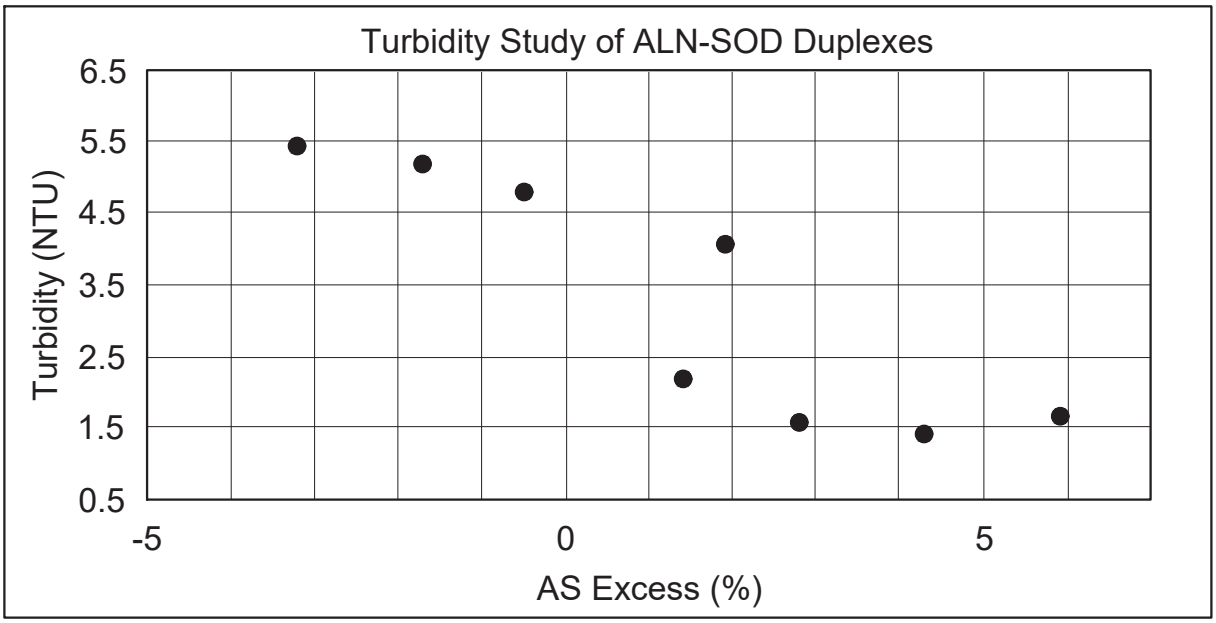
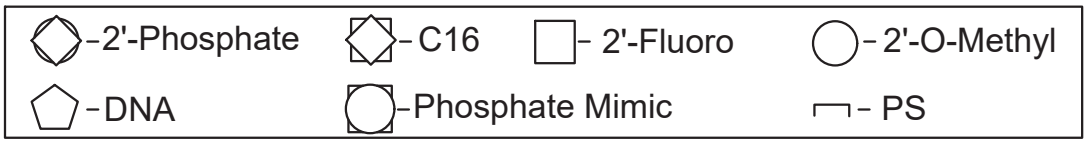

Turbidity Study of ALN-SOD Duplexes
Turbidity (NTU)
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-5
0
5
AS Excess (%)

FIG. 6B



AD-1498524

sense GCGACCCUGGAAAAGCUGAUA

antisense GCCGCUGGGACCUUUUCGACUAUVP

AS addition (uL)	0	50	100	150	200	250	300	350	400	450
Single strand excess	4.43% SS	2.0% SS	0.1% SS	1.9% AS	4.4% AS	6.8% AS	8.9% AS	11.9% AS	13.6% AS	16.1% AS
Before AS addition										
After AS addition										

FIG. 7A

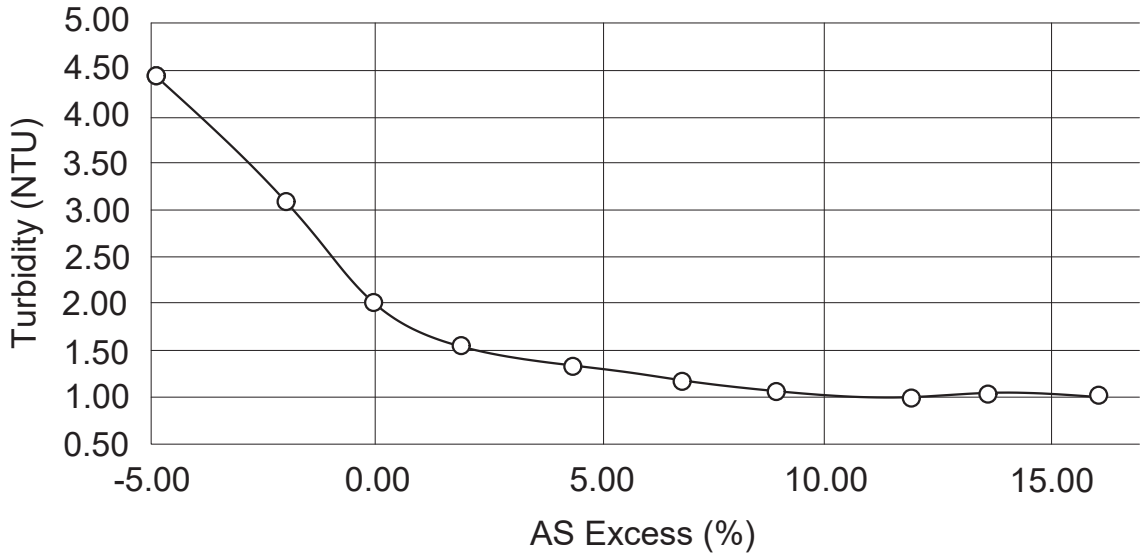


FIG. 7B

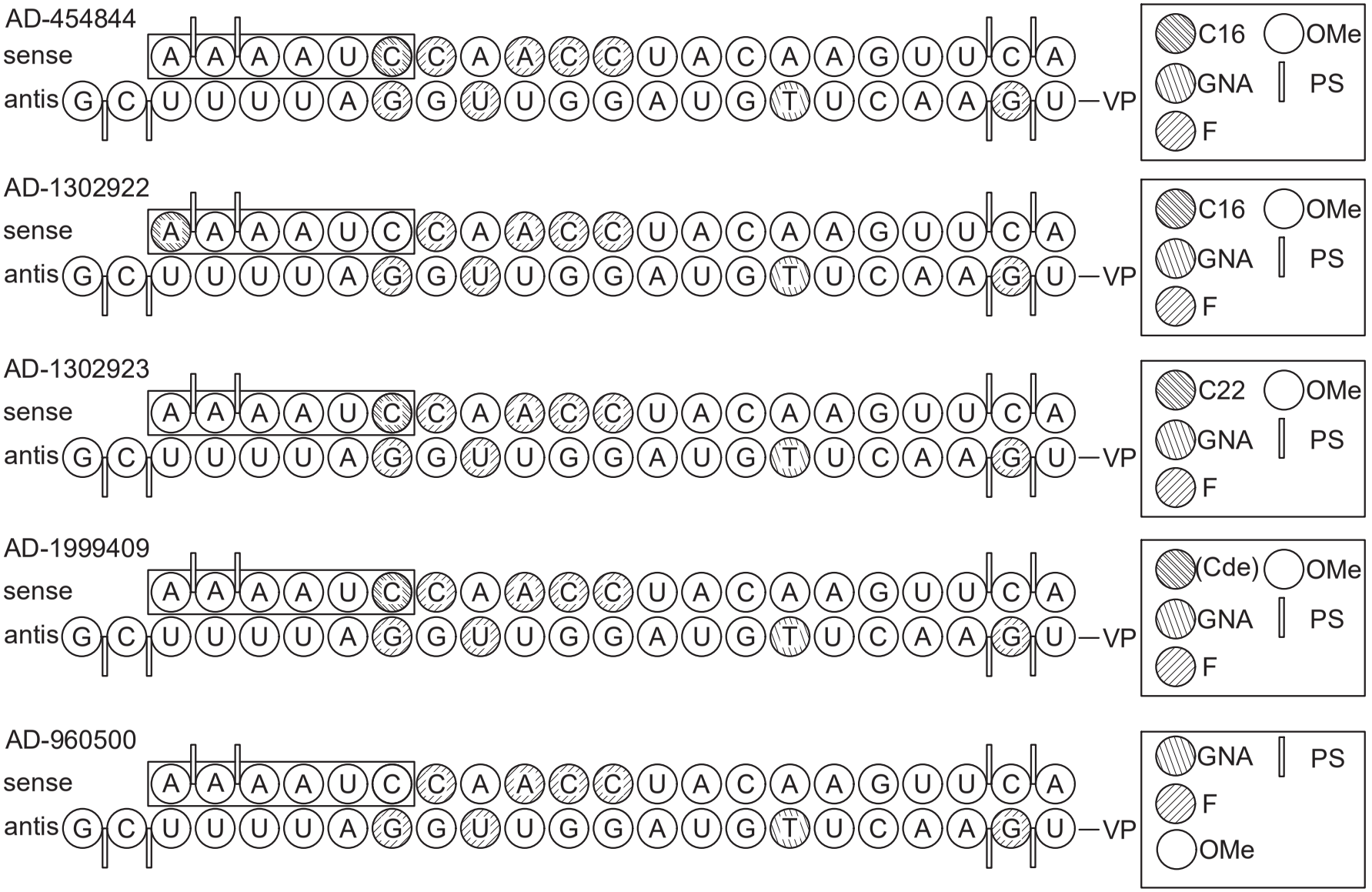

AD-454844
sense
antis
AAAAUCCAACCUACAAGUUCA
GCUUUUAGGUUGGAUGTUCAAGU
VP
C16
OMe
GNA
PS
F
AD-1302922
sense
antis
AAAAUCCAACCUACAAGUUCA
GCUUUUAGGUUGGAUGTUCAAGU
VP
C16
OMe
GNA
PS
F
AD-1302923
sense
antis
AAAAUCCAACCUACAAGUUCA
GCUUUUAGGUUGGAUGTUCAAGU
VP
C22
OMe
GNA
PS
F
AD-1999409
sense
antis
AAAAUCCAACCUACAAGUUCA
GCUUUUAGGUUGGAUGTUCAAGU
VP
(Cde)
OMe
GNA
PS
F
AD-960500
sense
antis
AAAAUCCAACCUACAAGUUCA
GCUUUUAGGUUGGAUGTUCAAGU
VP
GNA
PS
F
OMe

FIG. 8

Duplex	AD-960500	AD-454844	AD-454844	AD-1302922	AD-1302922	AD-1302923	AD-1999409	RODI
Sense Strand Lipid Chemistry	Non-Lipid	Internal C16	Internal C16	Terminal C16	Terminal C16	Internal C22	Internal C10	-
Cation Exchange	Ca	Ca	Mg	Ca	Mg	Ca	Ca	-
Precipitate Observation	-	+	+	++++	++	+	-	-
Duplex after Cation Exchange								

FIG. 9A

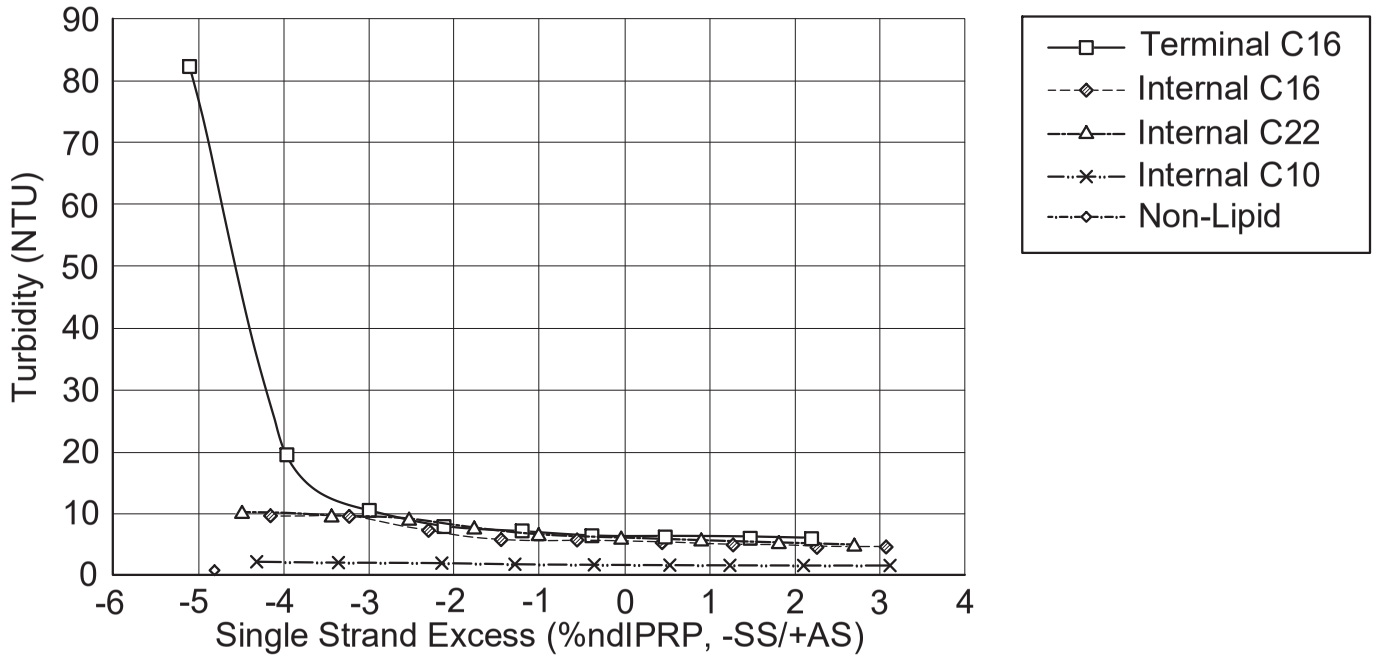


FIG. 9B

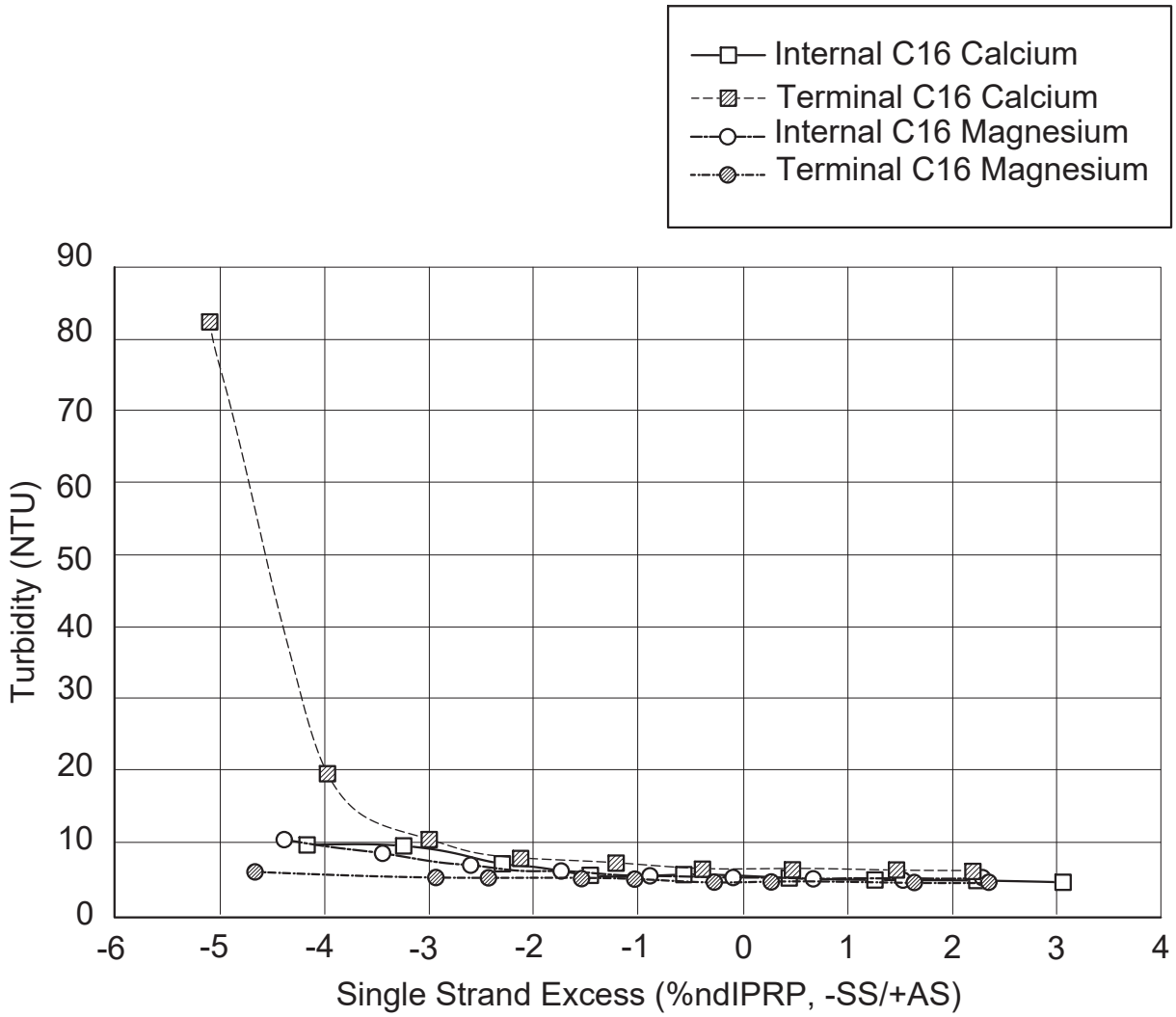

Internal C16 Calcium
Terminal C16 Calcium
Internal C16 Magnesium
Terminal C16 Magnesium
Turbidity (NTU)
90
80
70
60
50
40
30
20
10
0
-6
-5
-4
-3
-2
-1
0
1
2
3
4
Single Strand Excess (%ndIPRP, -SS/+AS)

FIG. 9C

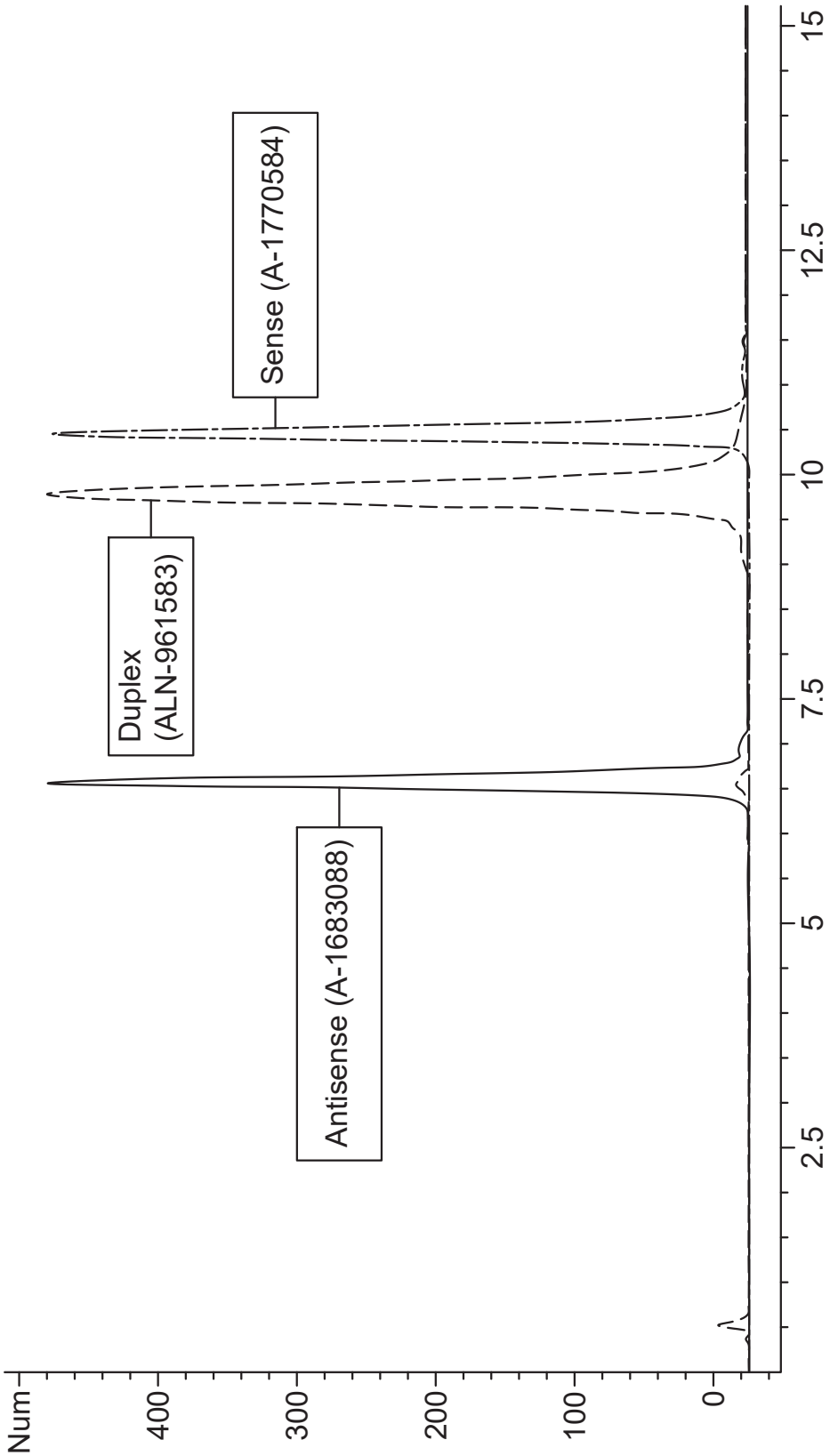

Num
400
300
200
100
0
2.5
5
7.5
10
12.5
15
Antisense (A-1683088)
Duplex (ALN-961583)
Sense (A-1770584)

FIG. 10A

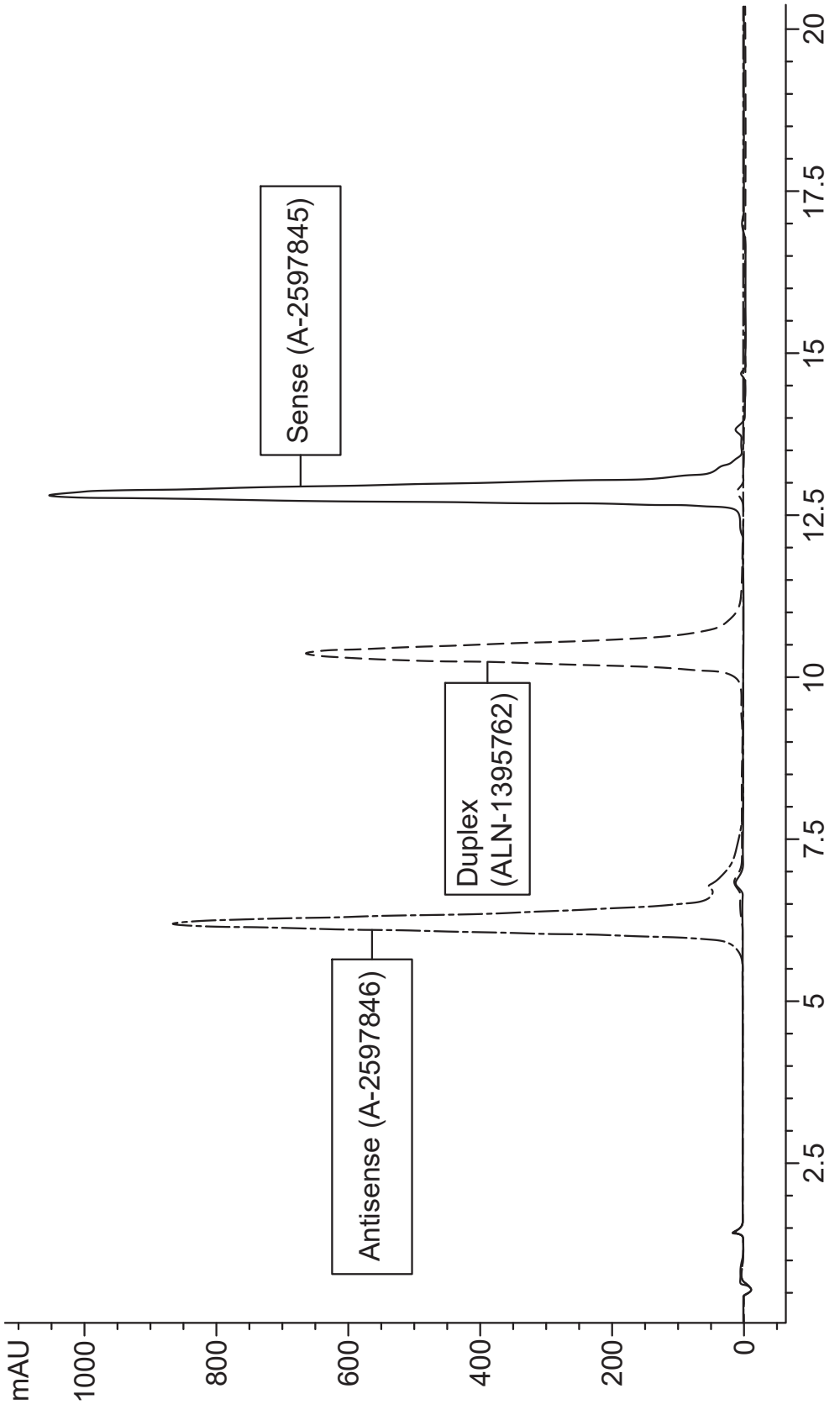

mAU
1000
800
600
400
200
0
2.5
5
7.5
10
12.5
15
17.5
20
Sense (A-2597845)
Duplex (ALN-1395762)
Antisense (A-2597846)

FIG. 10B

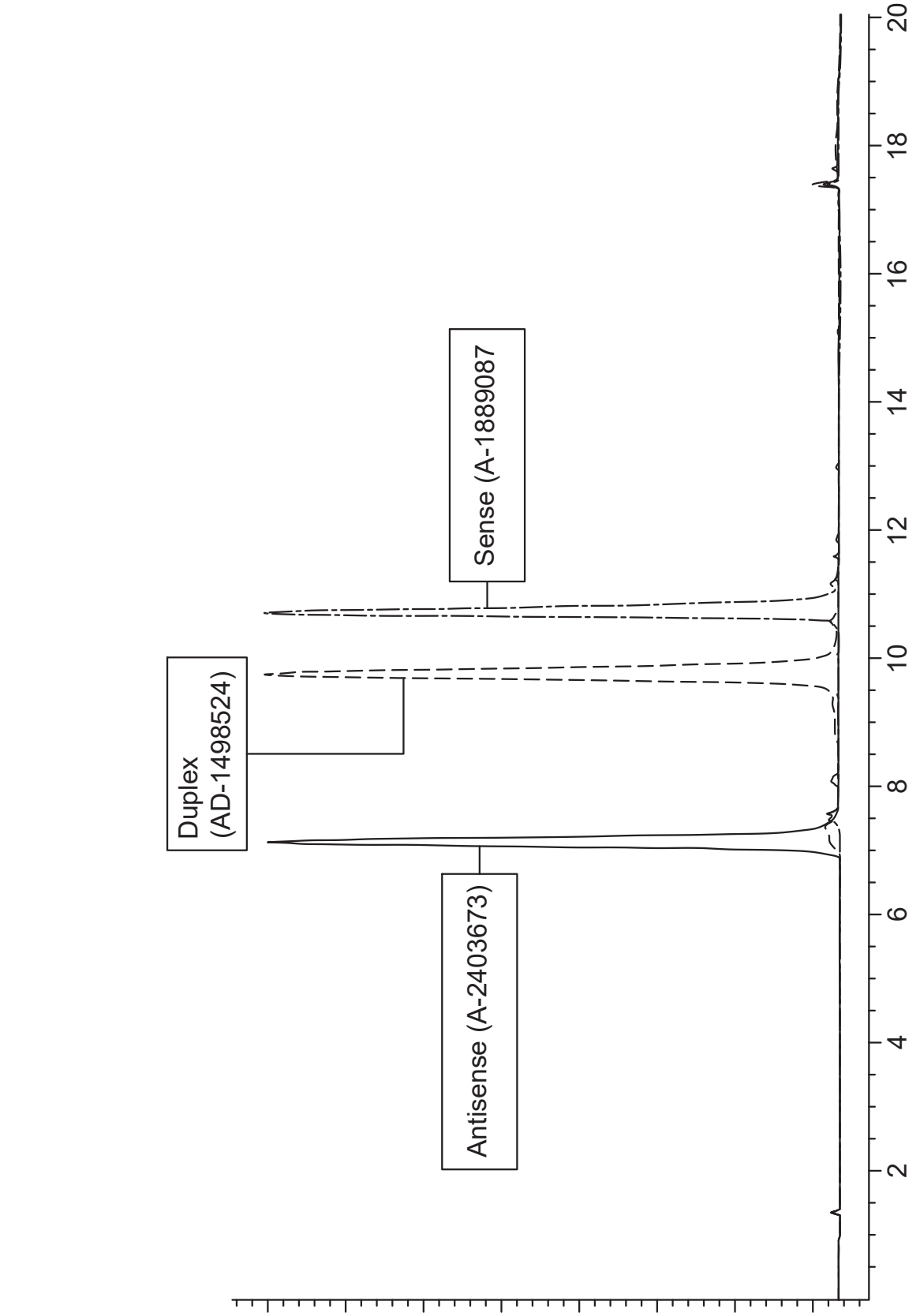
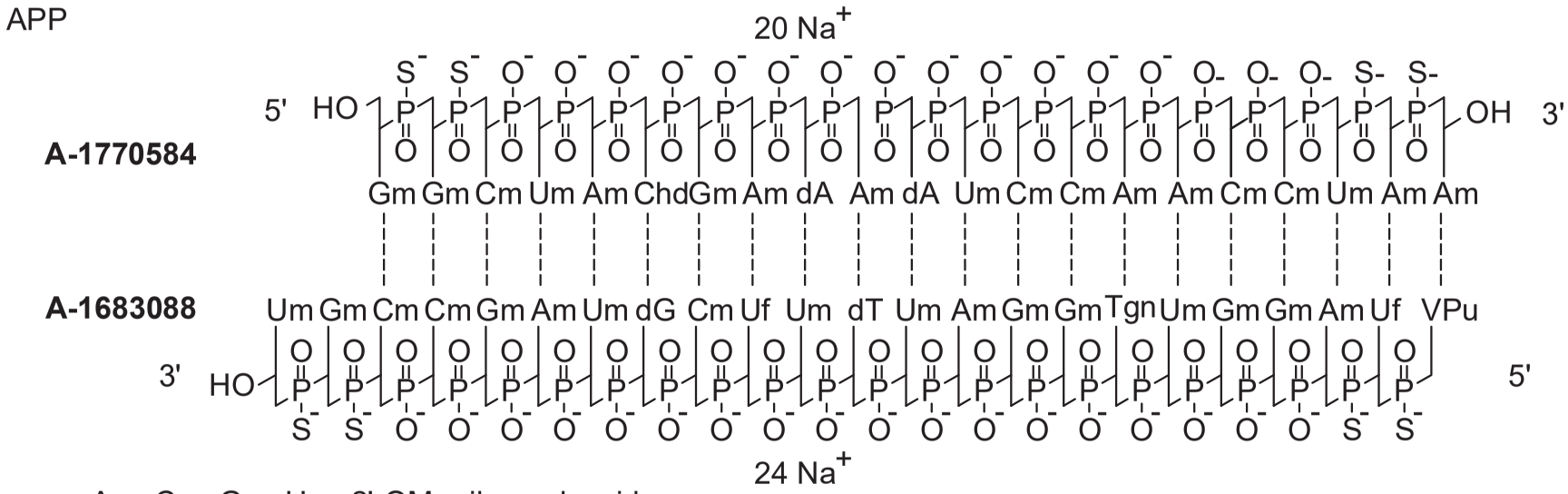
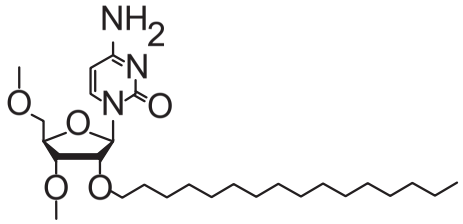

Num
3500
3000
2500
2000
1500
1000
500
0
2
4
6
8
10
12
14
16
18
20
Duplex
(AD-1498524)
Sense (A-1889087
Antisense (A-2403673)

FIG. 10C

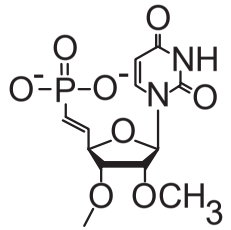
APP



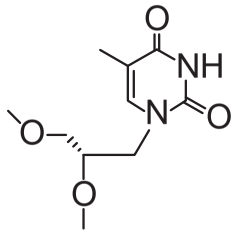
Am, Cm, Gm, Um=2'-OMe ribonucleosides
dA, dG, dT=2' deoxyribonucleosides
Uf=2'-F uridine



Chd=2'-*O*-hexadecyl cytidine



VPu=vinylphosphonate 2'-OMe uridine

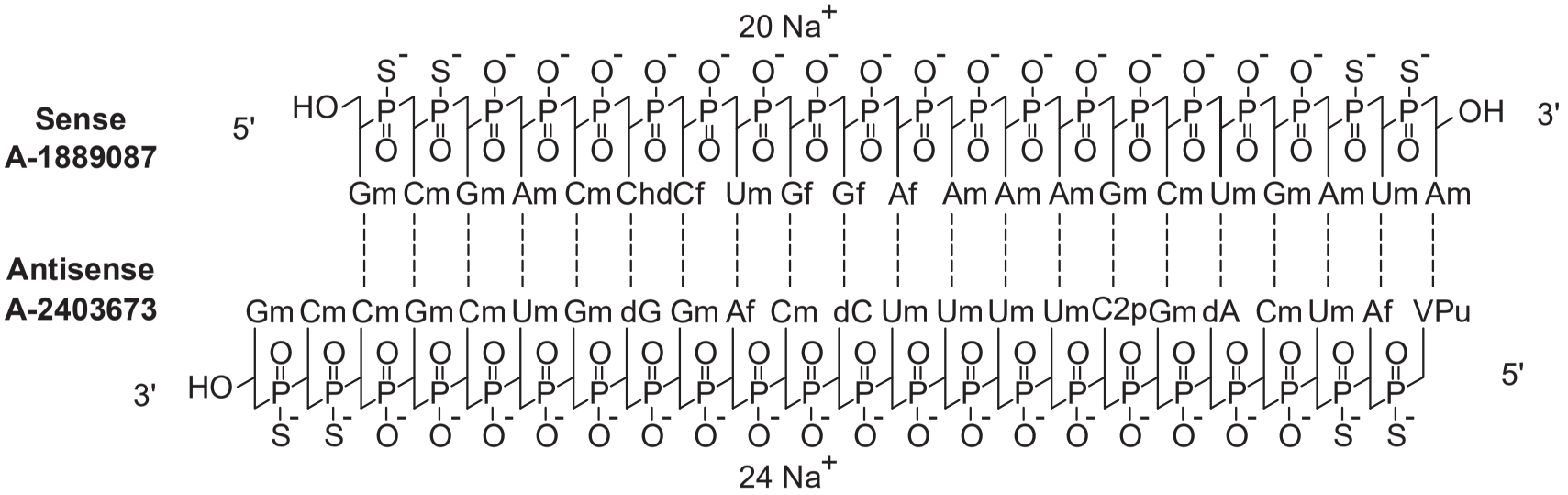


Tgn=1-((2S)-1-(2,3-dihydroxypropyl))thymine

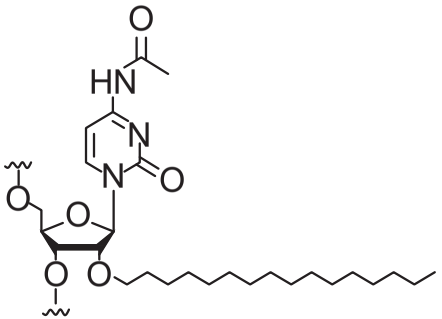
- Molecular Formula of the sodium salt: $C_{471}H_{578}N_{163}F_2O_{291}P_{43}S_8Na_{44}$
- Formula Weight of the sodium form: 15,816.50 Da
- Molecular Formula of the H-form: $C_{471}H_{622}N_{163}F_2O_{291}P_{43}S_8$
- Formula Weight of the H-form: 14,849.30Da

FIG. 11A

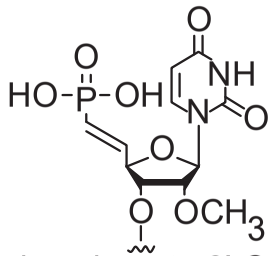
HTT



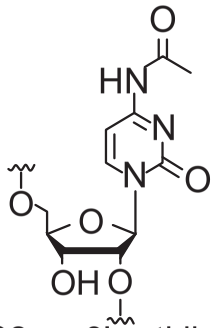
Am, Um, Cm, Gm = 2'-OMe ribonucleosides
dA, dC, dG = 2'-deoxyribonucleosides
Af, Cf, Gf = 2'-F ribonucleosides



Chd = 2'-*O*-hexadecylcytidine



VPu = vinylphosphonate 2'-O-methyluridine

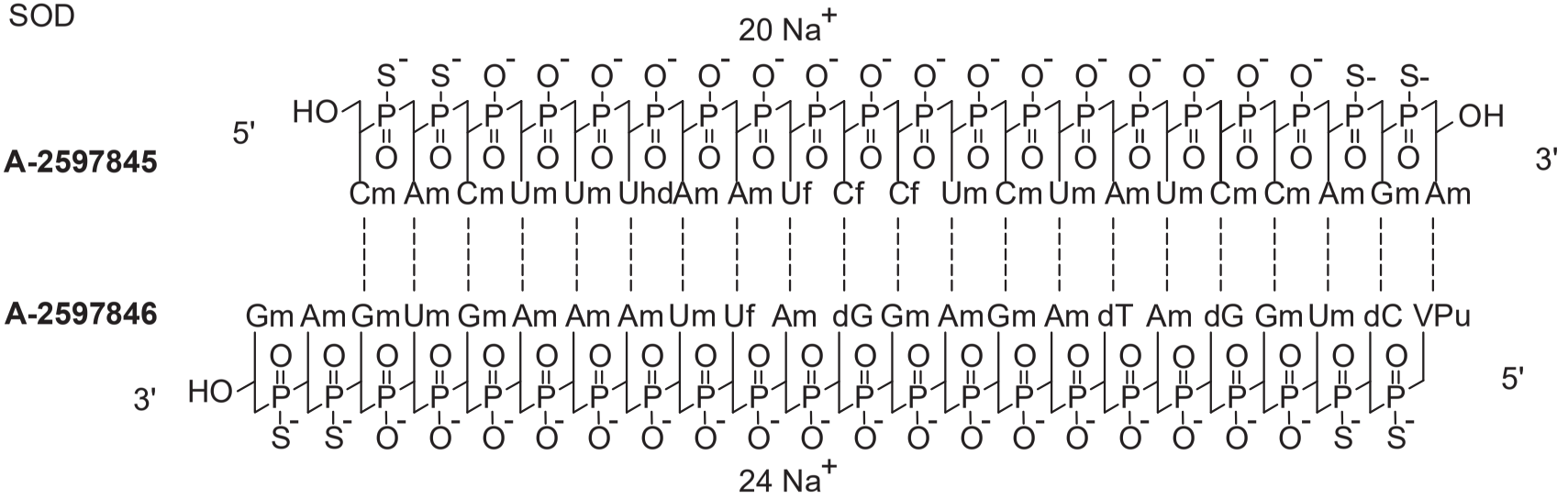


C2p = 3'-cytidine

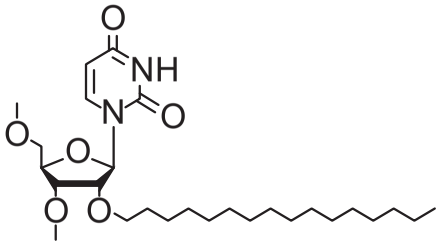
- Molecular Formula of the sodium salt form: $C_{468}H_{569}F_6N_{166}O_{289}P_{43}S_8Na_{44}$
- Formula Weight of the sodium form: 15,857.42 Da
- Molecular Formula of the H-form: $C_{468}H_{613}F_6N_{166}O_{289}P_{43}S_8$
- Formula Weight of the H-form: 14,890.21Da

FIG. 11B

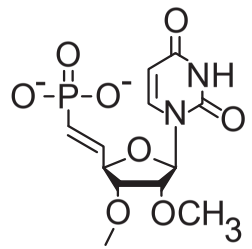
SOD



Am, Cm, Gm, Um=2'-OMe ribonucleosides
dC, dG, dT=2'-deoxyribonucleosides
Cf, Uf,=2'-F ribonucleosides



Uhd=2'-*O*-hexadecyl uridine



VPu=vinylphosphonate 2'-OMe uridine

- Molecular Formula of the sodium salt: $C_{472}H_{574}F_4N_{165}O_{289}P_{43}S_8Na_{44}$
- Formula Weight of the sodium form: 15,858.50 Da
- Molecular Formula of the H-form: $C_{472}H_{618}F_4N_{165}O_{289}P_{43}S_8$
- Formula Weight of the H-form: 14,891.29 Da

FIG. 11C