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Supporting Information

Chemically Modified Poly(A) Analogs Targeting PABP: Structure Activity Relationship and Translation Inhibitory Properties

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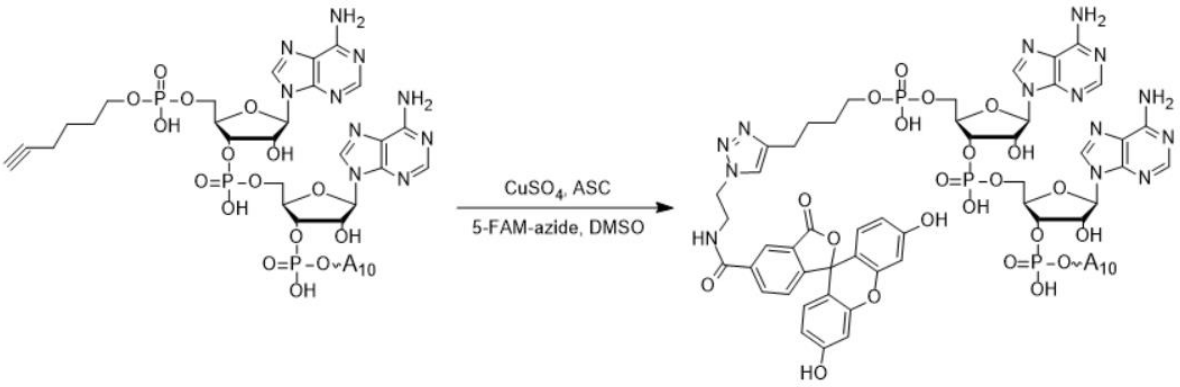


Figure S1. 5'-FAM-A_{12} synthesis via CuAAC click chemistry.

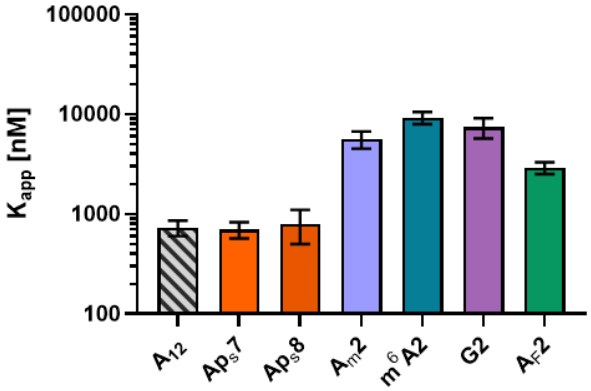


Figure S2. Apparent binding values ($K_{D,app}$) for complexes between PABP and poly(A) analogs containing different modifications in the middle part of the oligoadenylate chain.

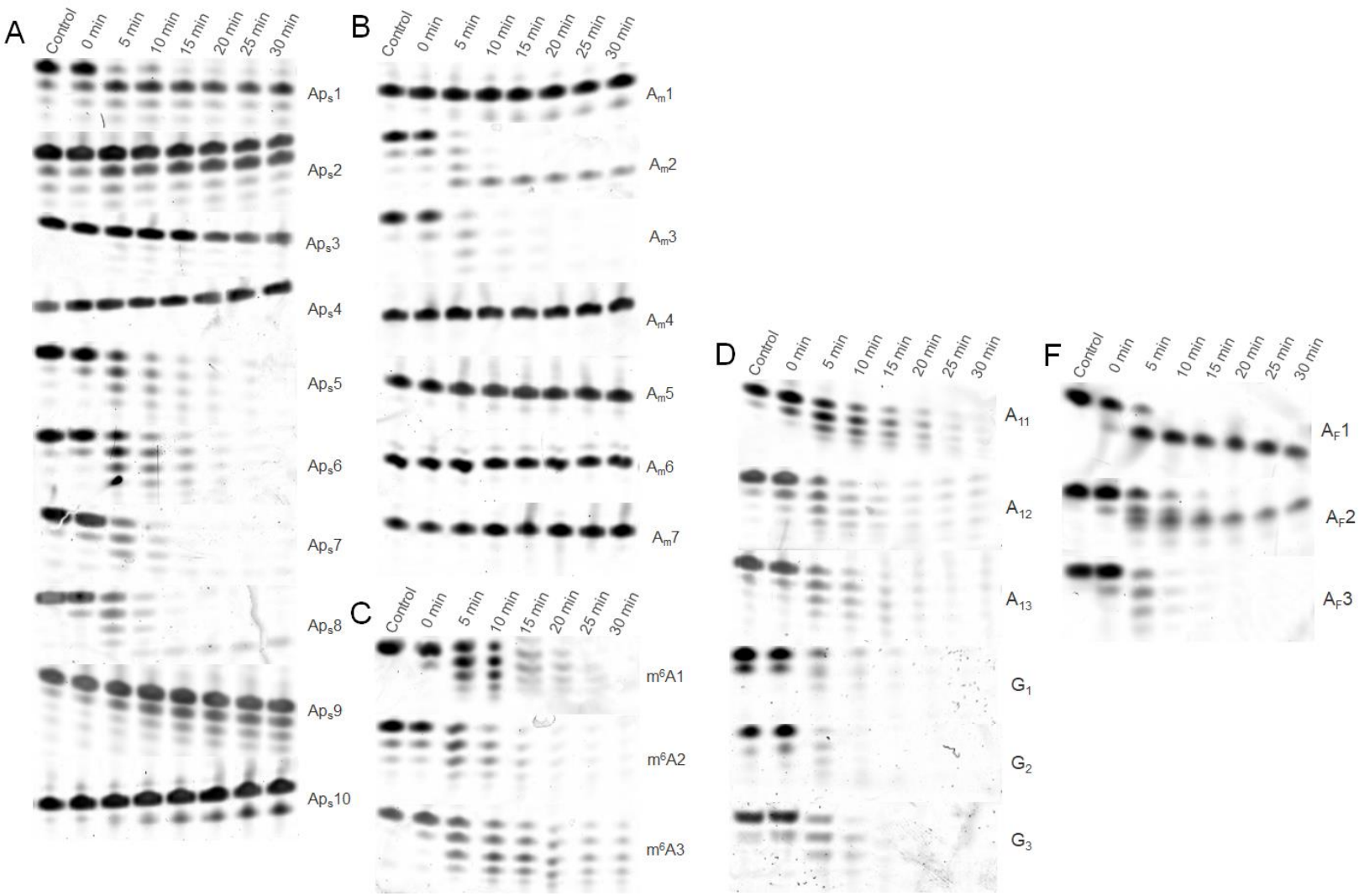
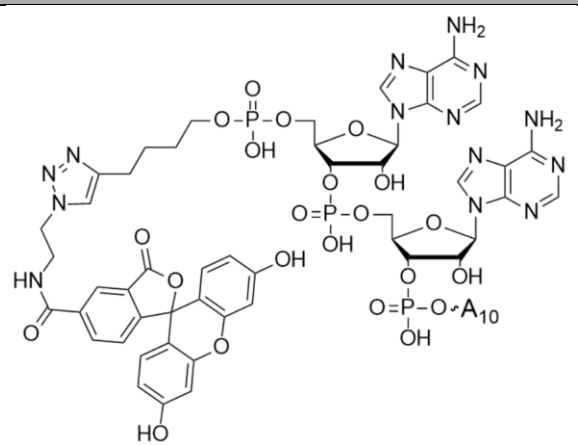
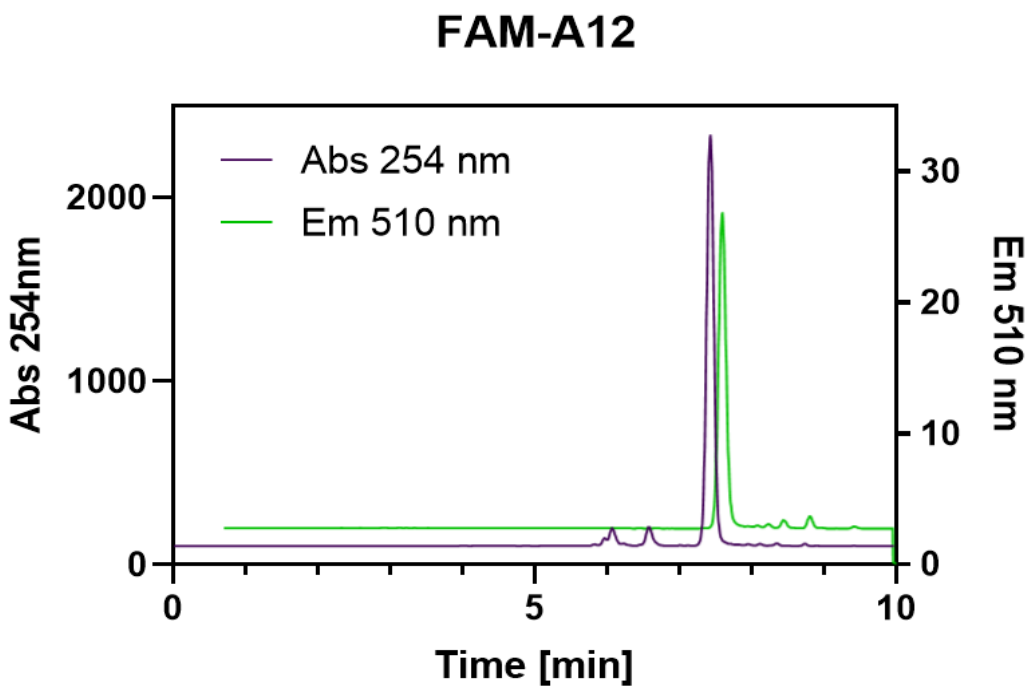
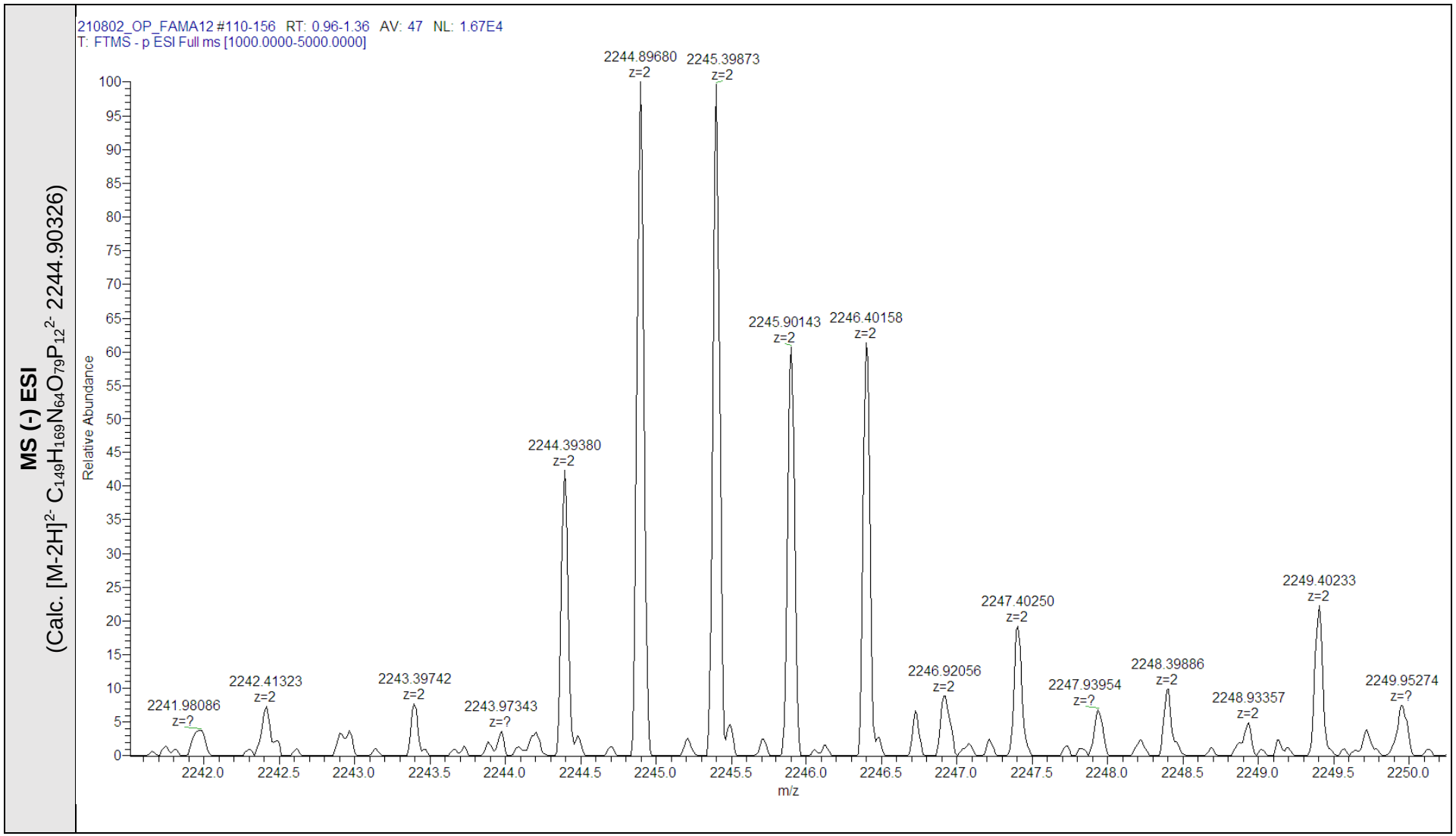


Figure S3. Urea polyacrylamide gel electrophoresis separation of products of degradation by CNOT7 deadenylase. Poly(A) analogs were incubated with CNOT7 for 30 minutes in 37°C. Reaction was stopped every 5 minutes by diluting a portion of the reaction mixture with loading dye and EDTA. Control sample was incubated for 30 minutes in 37°C without enzyme. A) Thiophosphate modification. B) 2'O-Me ribose modification. C) m^6A nucleobase modification. D) Guanosine for adenosine substitution and different length of unmodified analogs. E) 2'F ribose modification.

FAM-A_{12}	
Chemical structure	
RP HPLC	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T: FTMS - p ESI Full ms [1000.0000-5000.0000]
Relative Abundance
m/z
2241.98086 z=?
2242.41323 z=2
2243.39742 z=2
2243.97343 z=?
2244.39380 z=2
2244.89680 z=2
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2248.93357 z=2
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2249.95274 z=?

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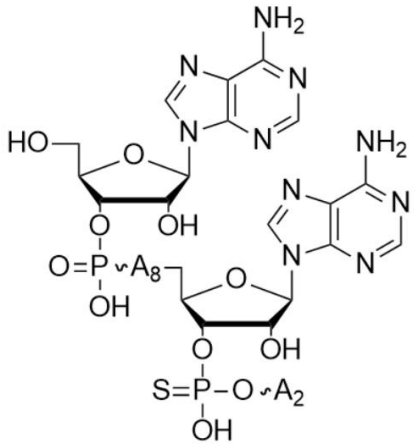
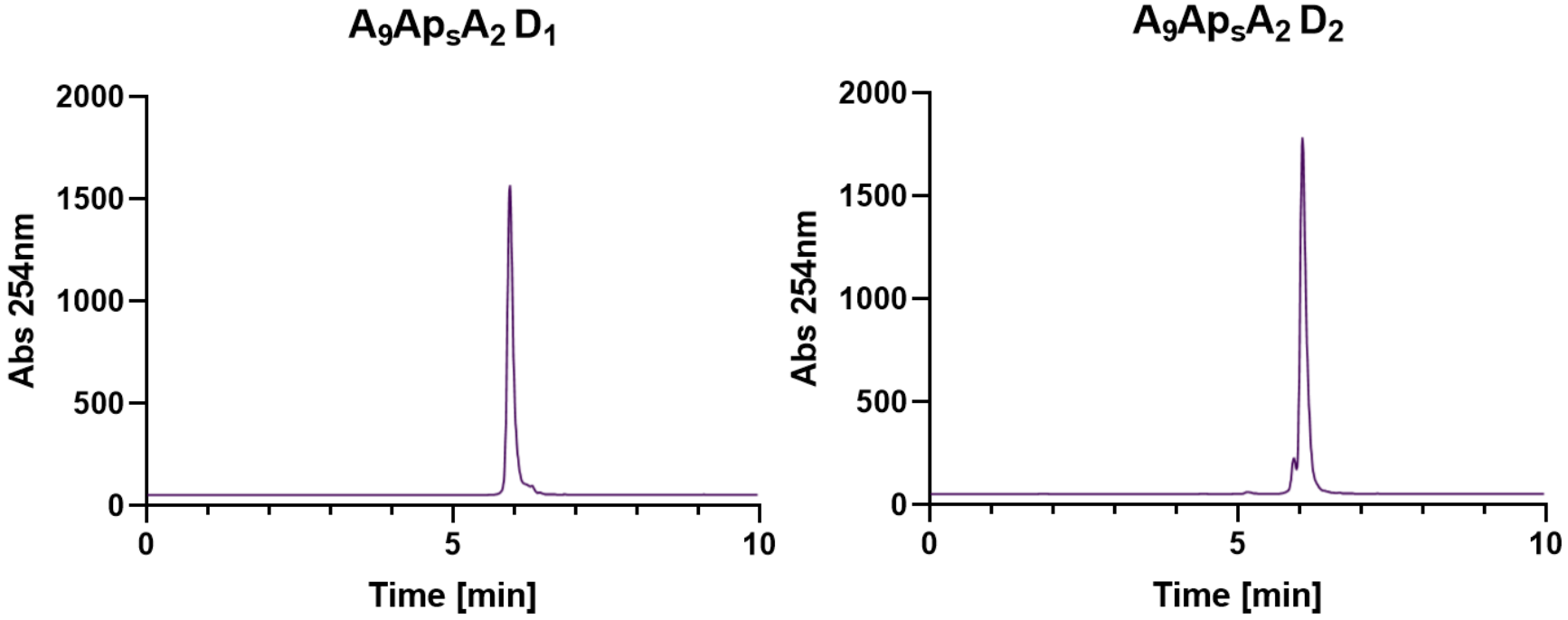
Chemical structure	
RP HPLC	

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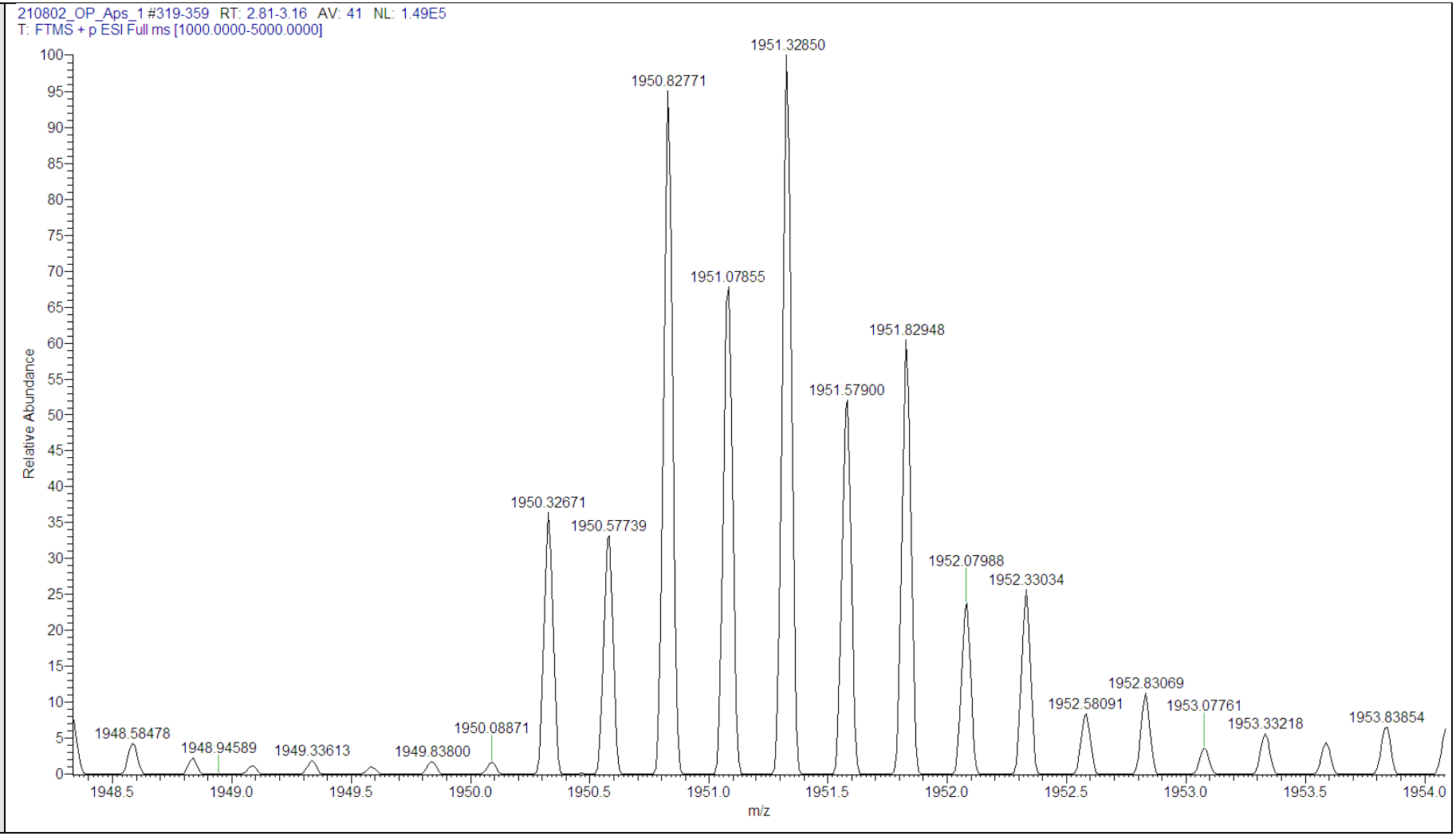

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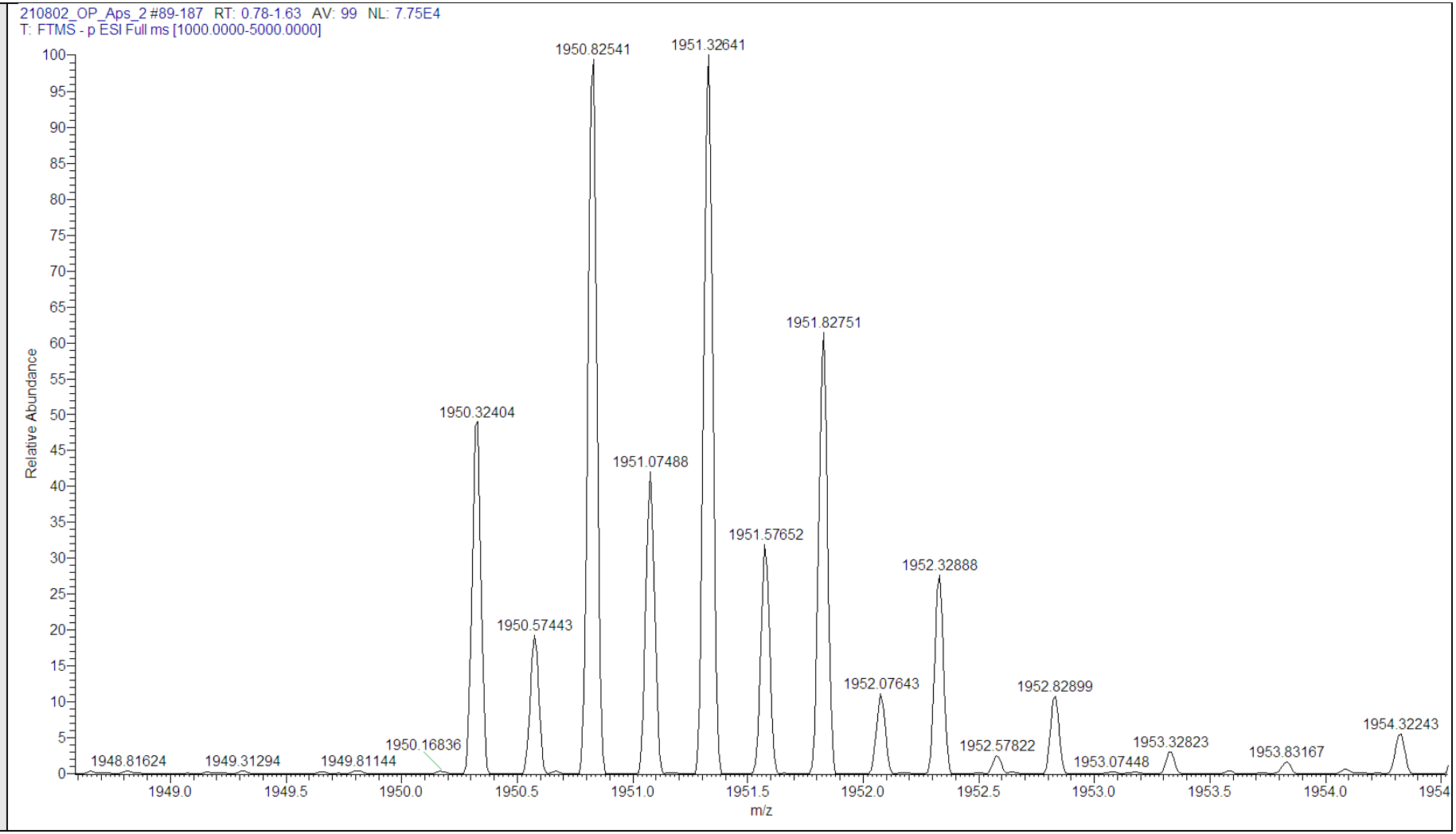

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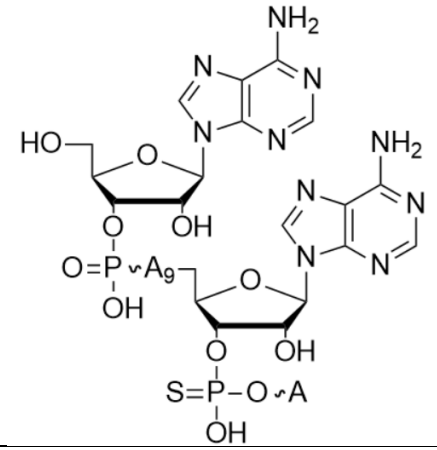
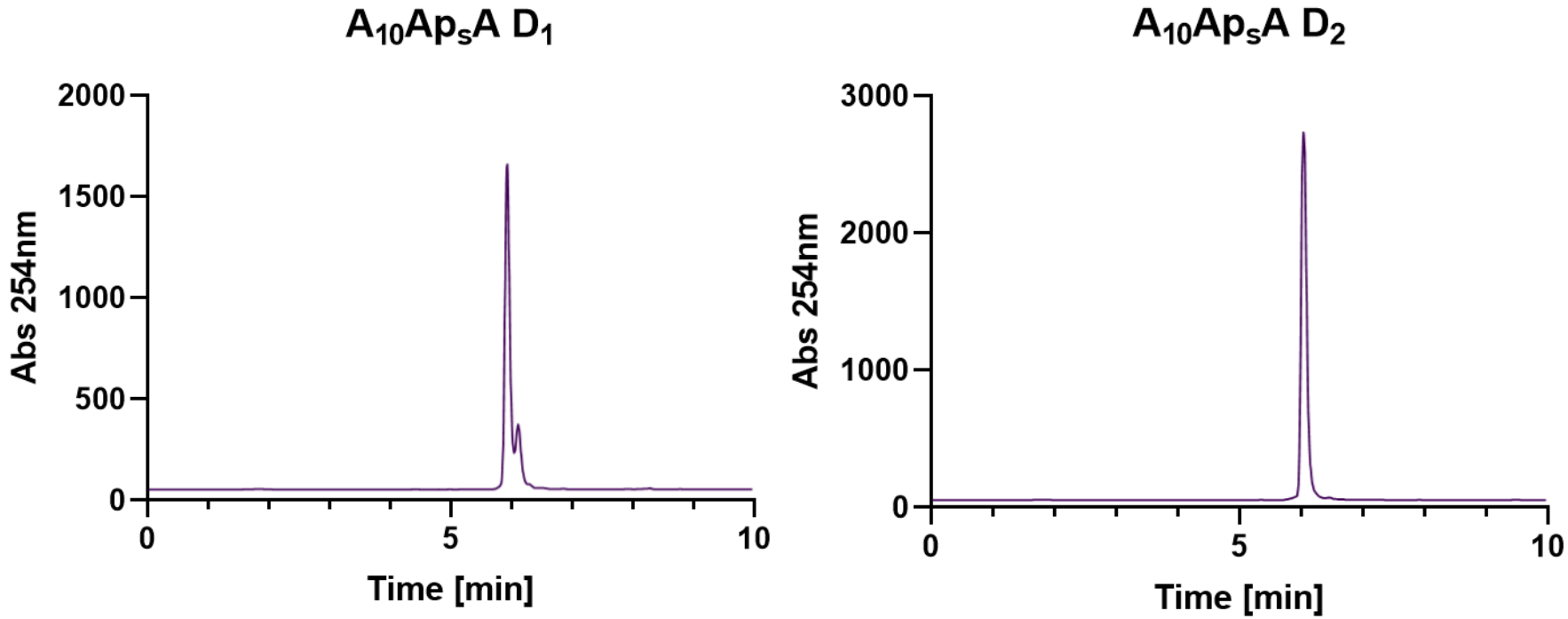
Chemical structure	
RP HPLC	

MS (-) ESI

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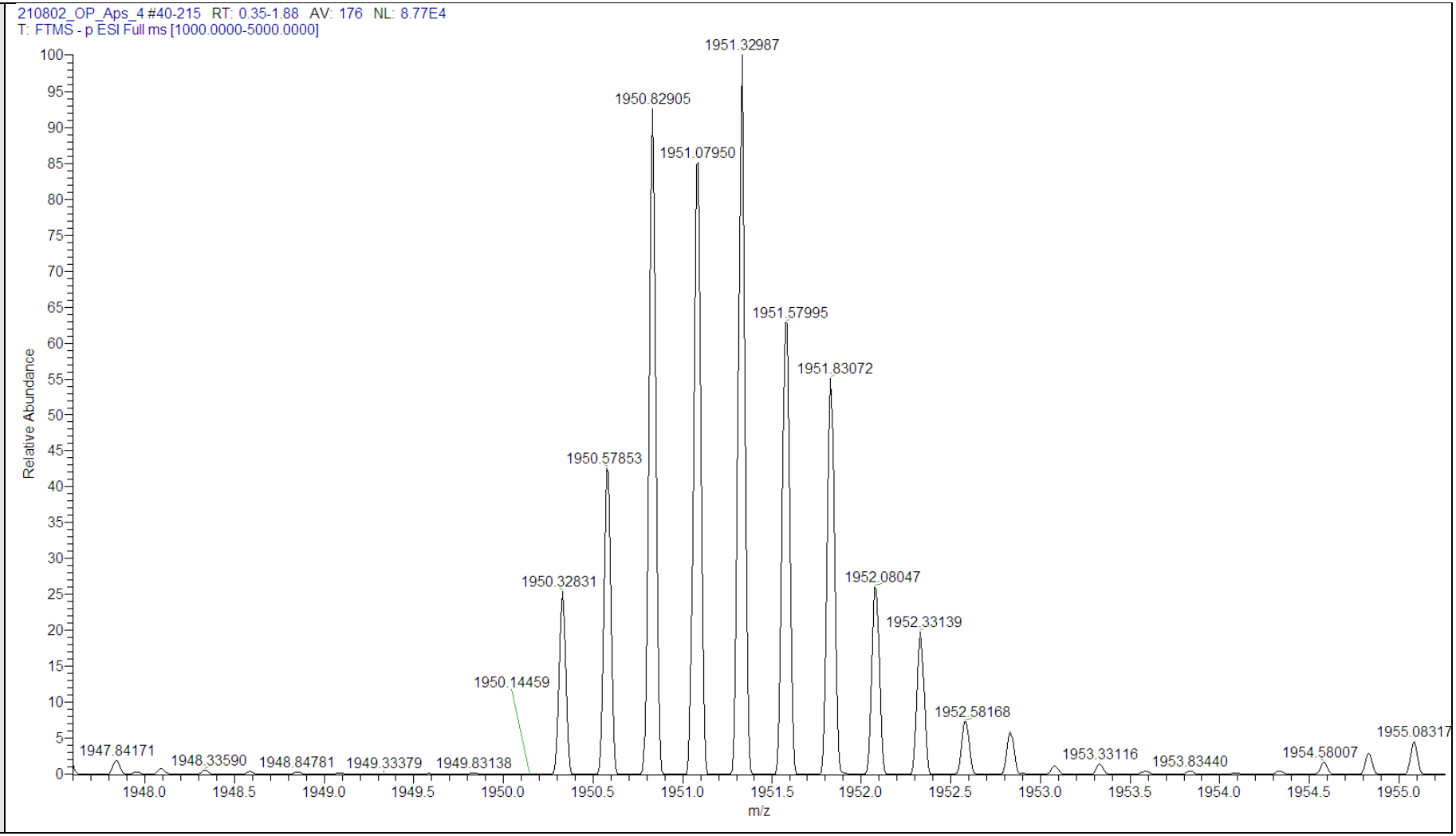

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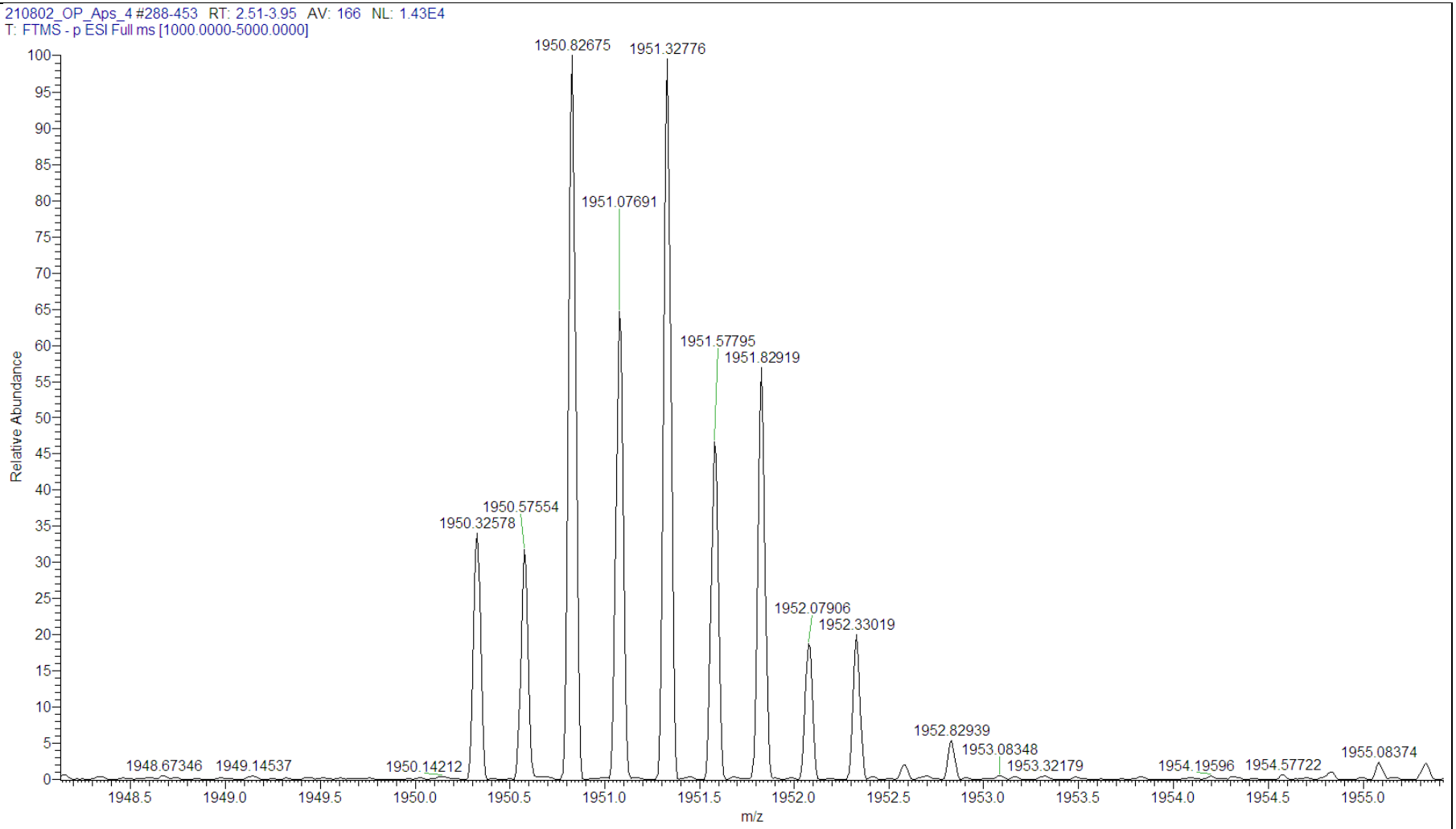

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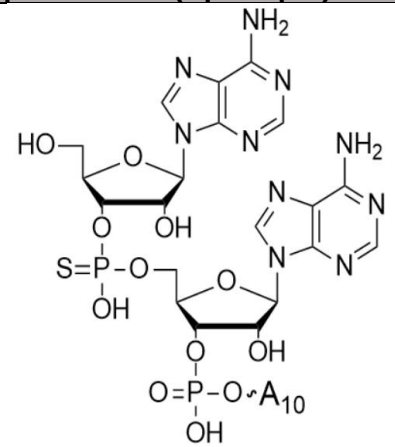
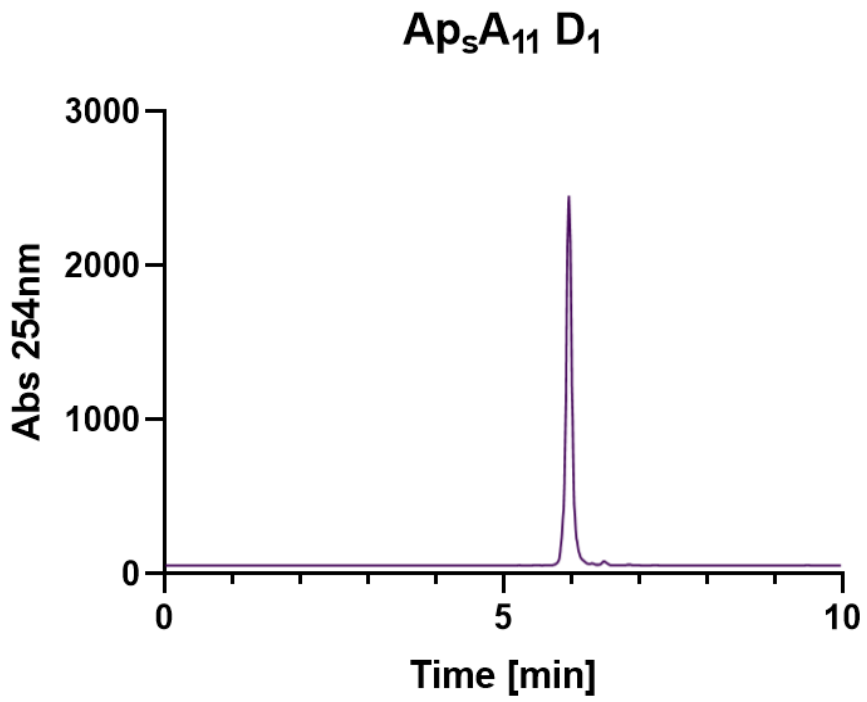
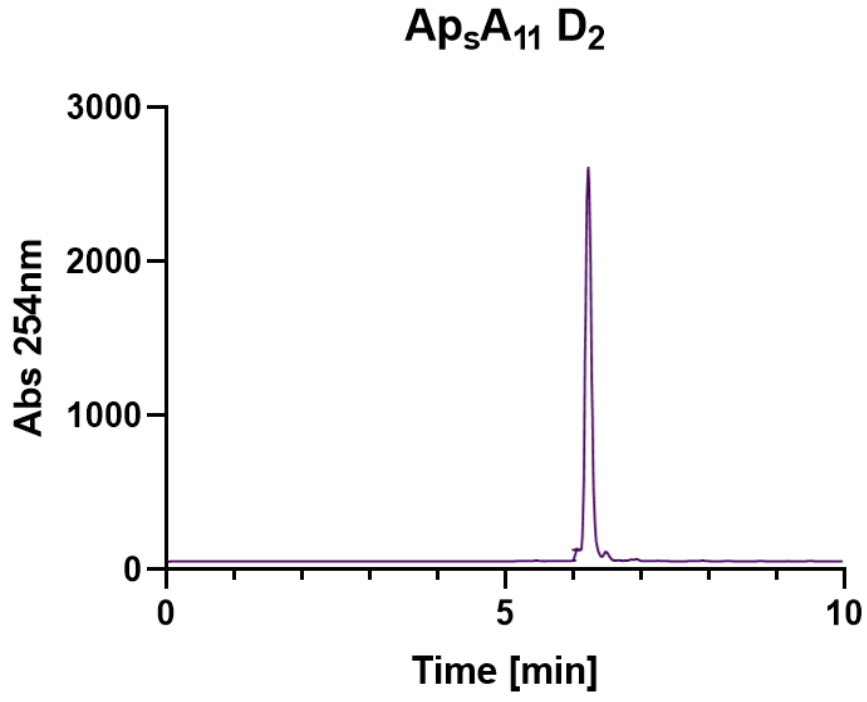
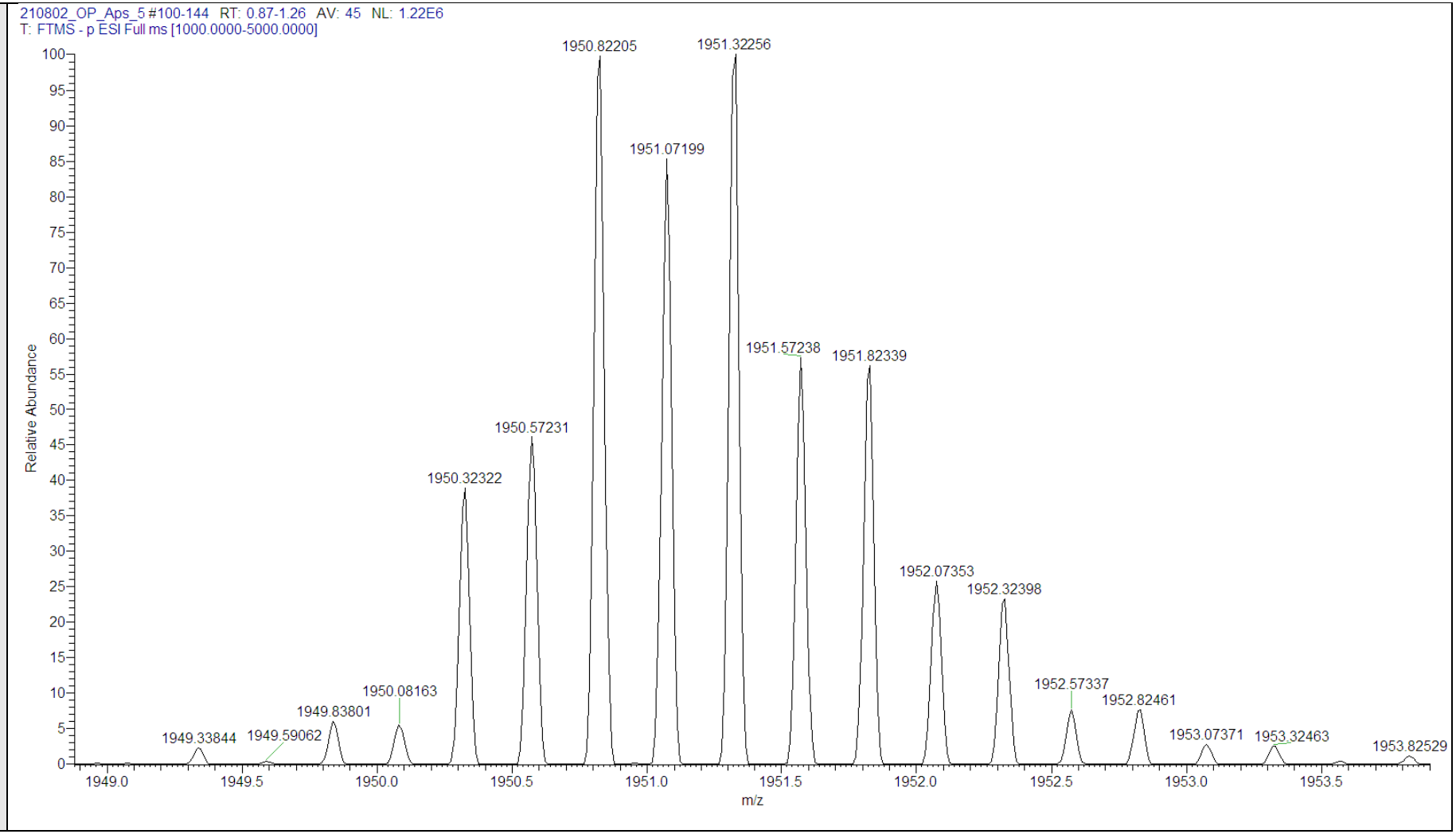
Chemical structure	
RP HPLC	 

MS (-) ESI

D1 (Calc. $[M-2H]^{2-}$ $C_{120}H_{144}N_{60}O_{69}P_{11}S^{2-}$ 1950.82385)



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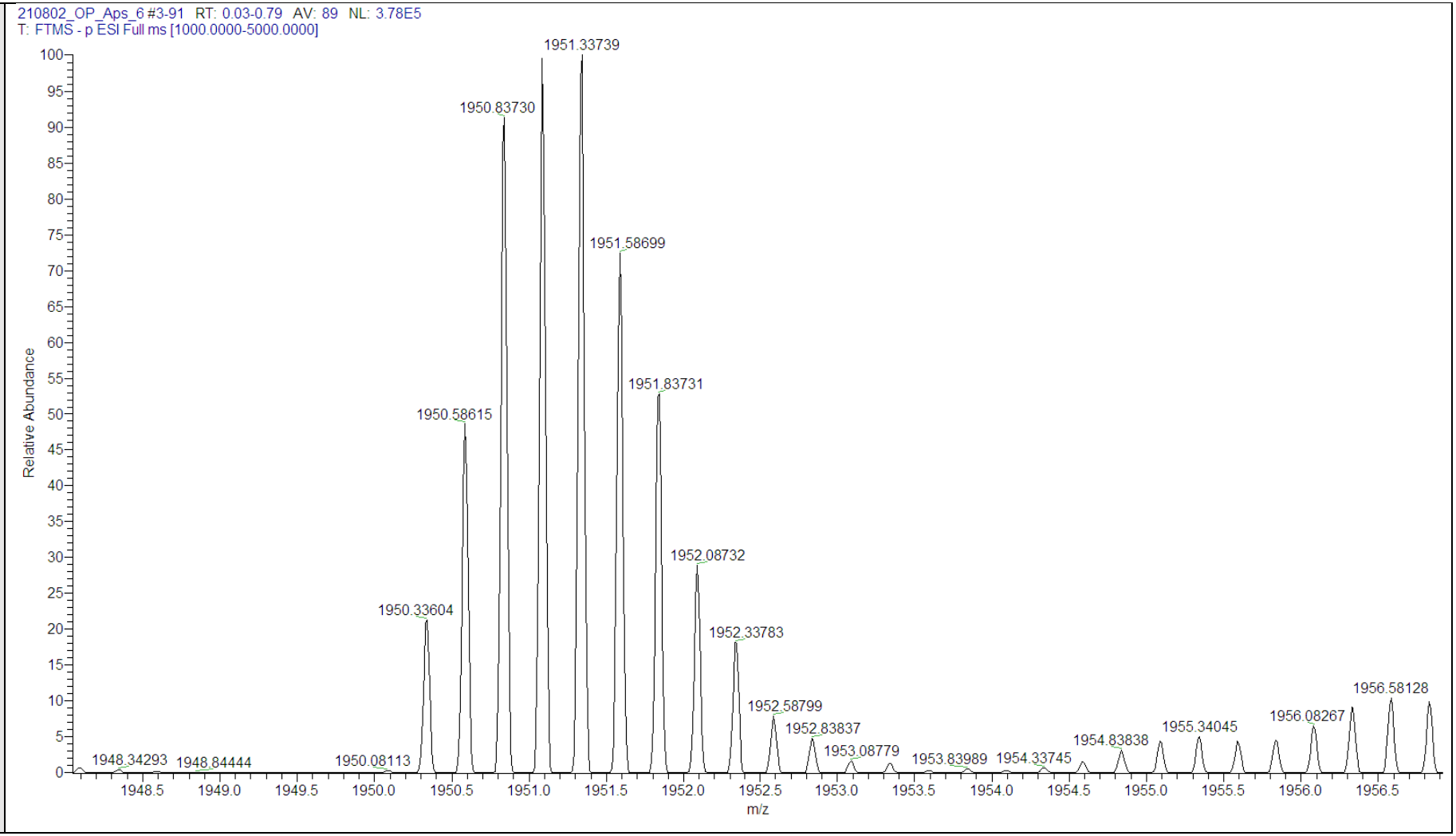

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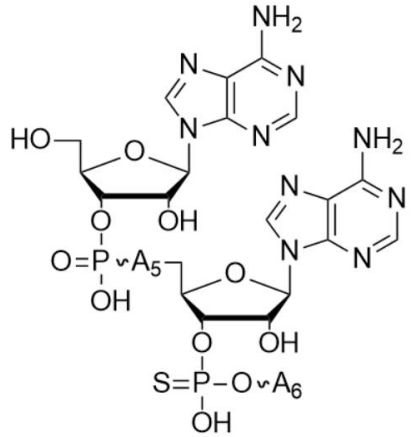
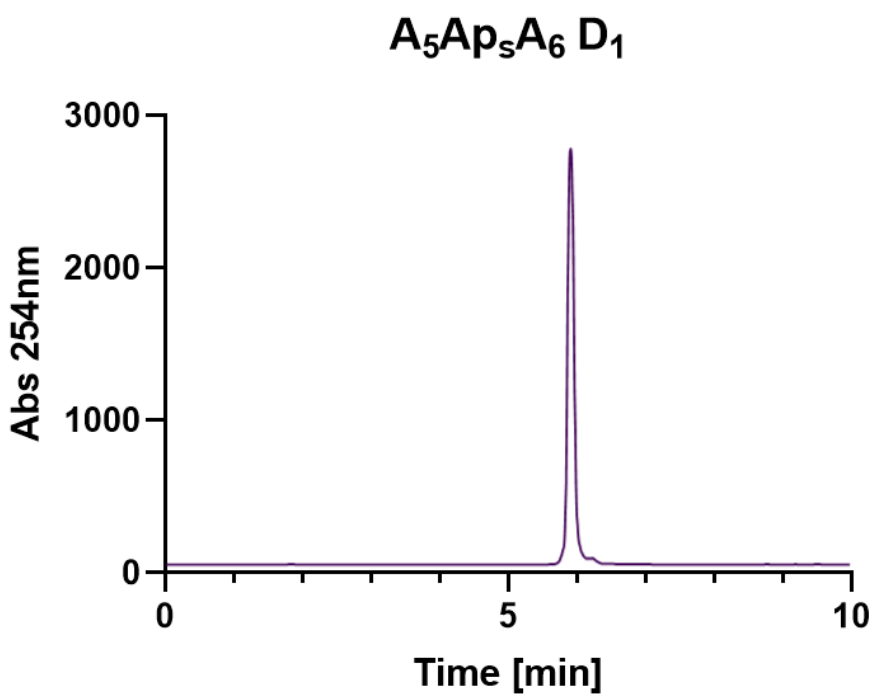
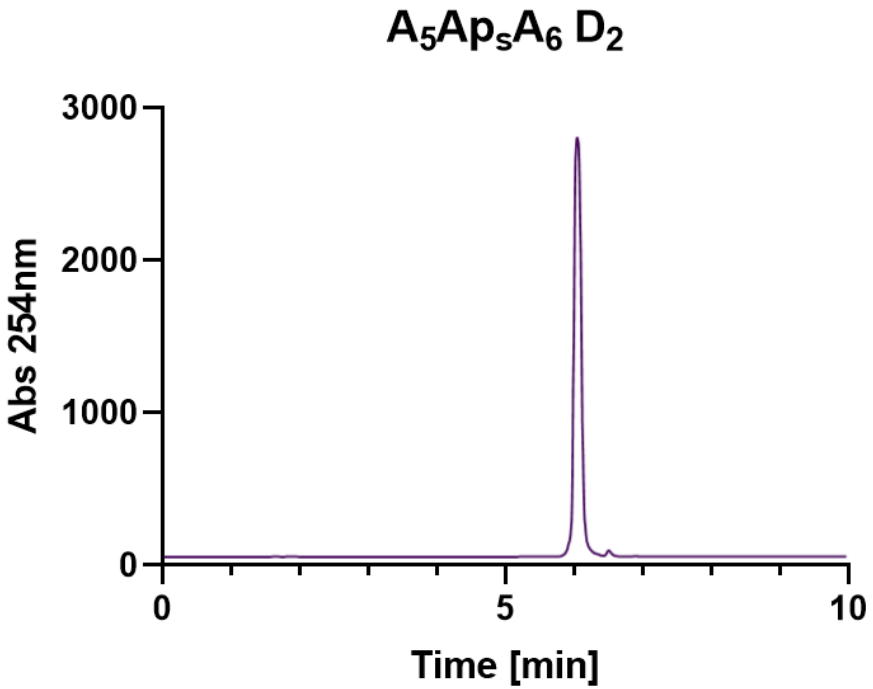
Chemical structure	
RP HPLC	 

MS (-) ESI

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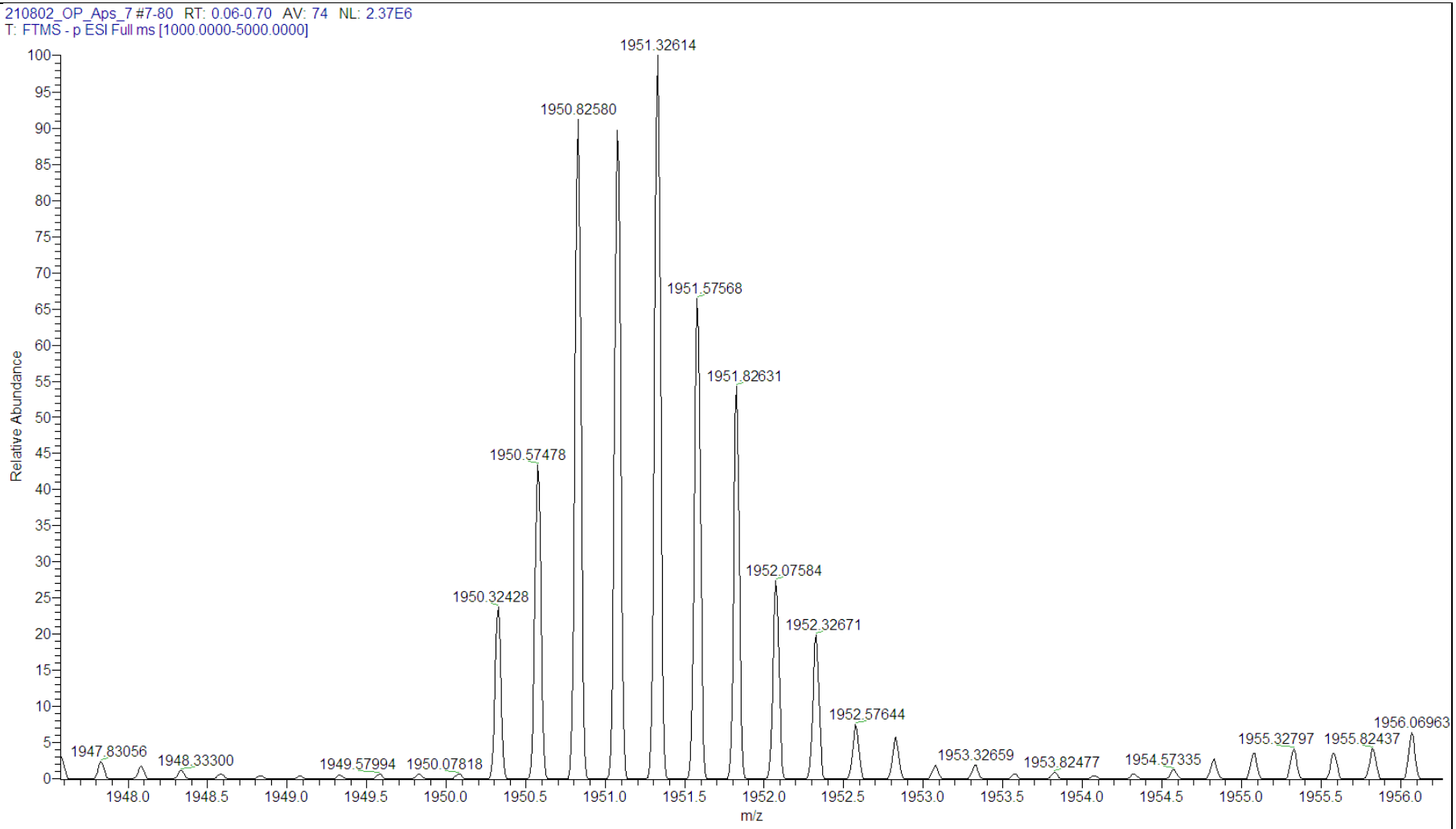

MS (-) ESI

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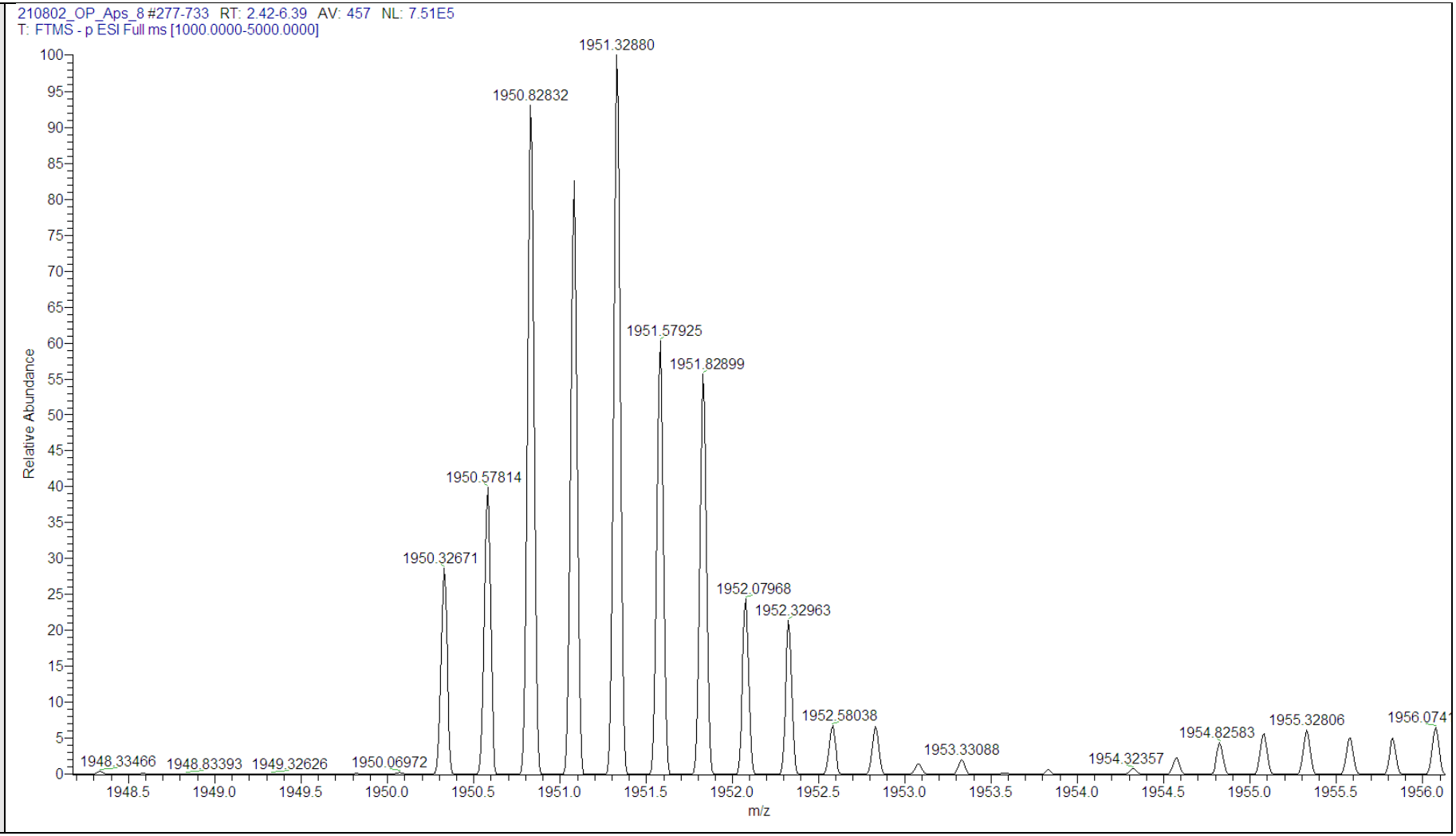

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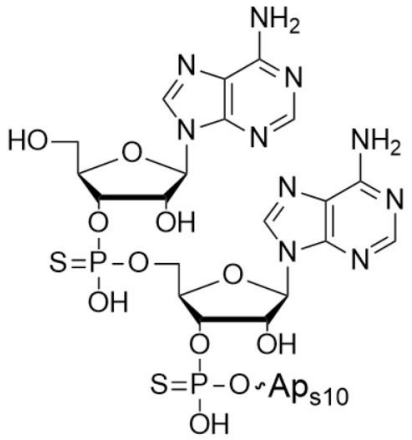
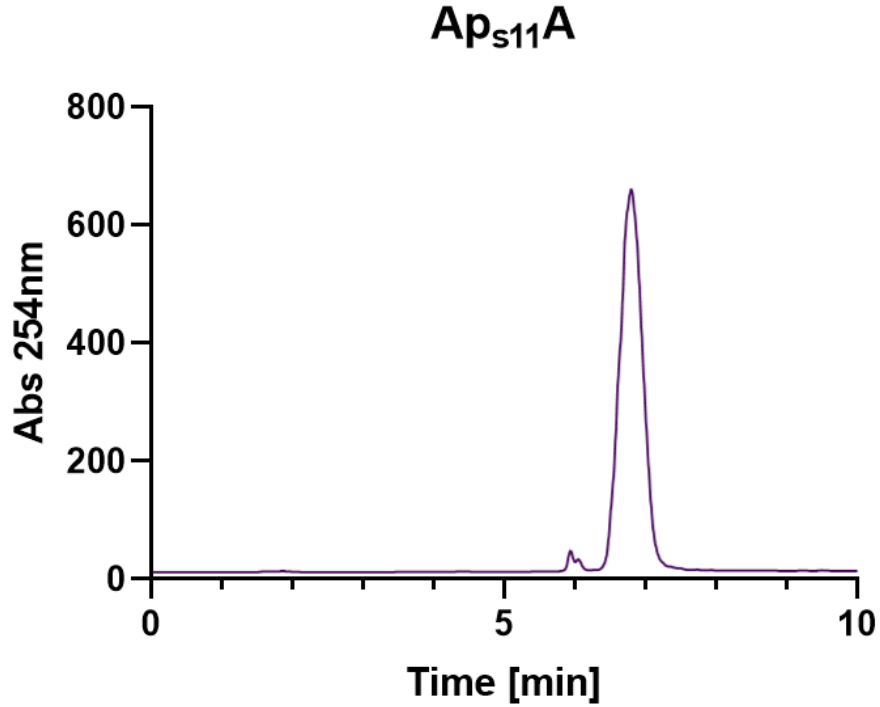
Chemical structure	
RP HPLC	

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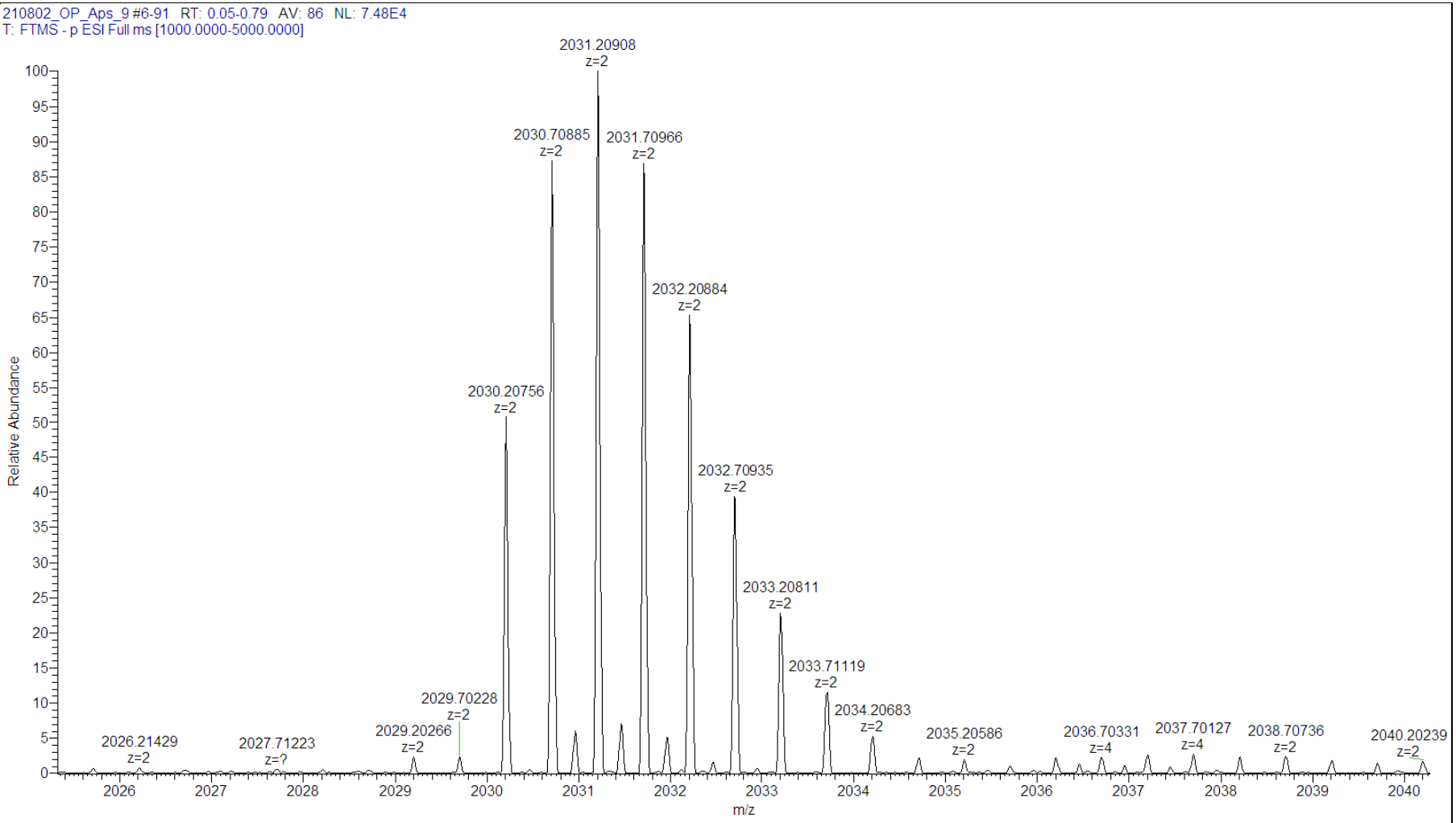
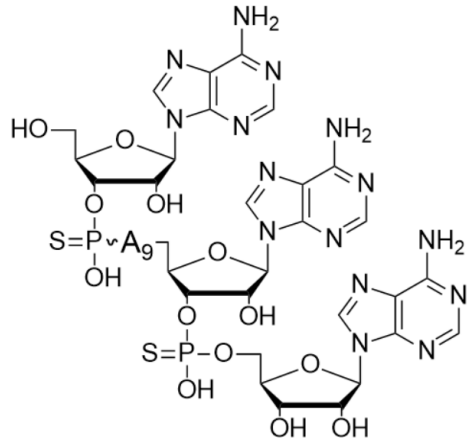
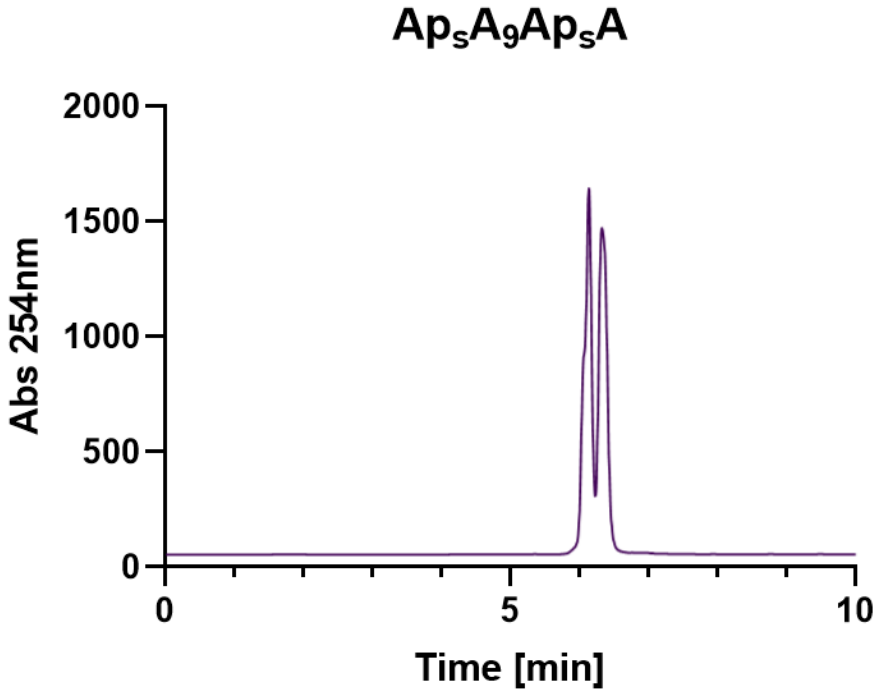


$Ap_sA_9Ap_sA$ (Ap_s10)	
Chemical structure	
RP HPLC	

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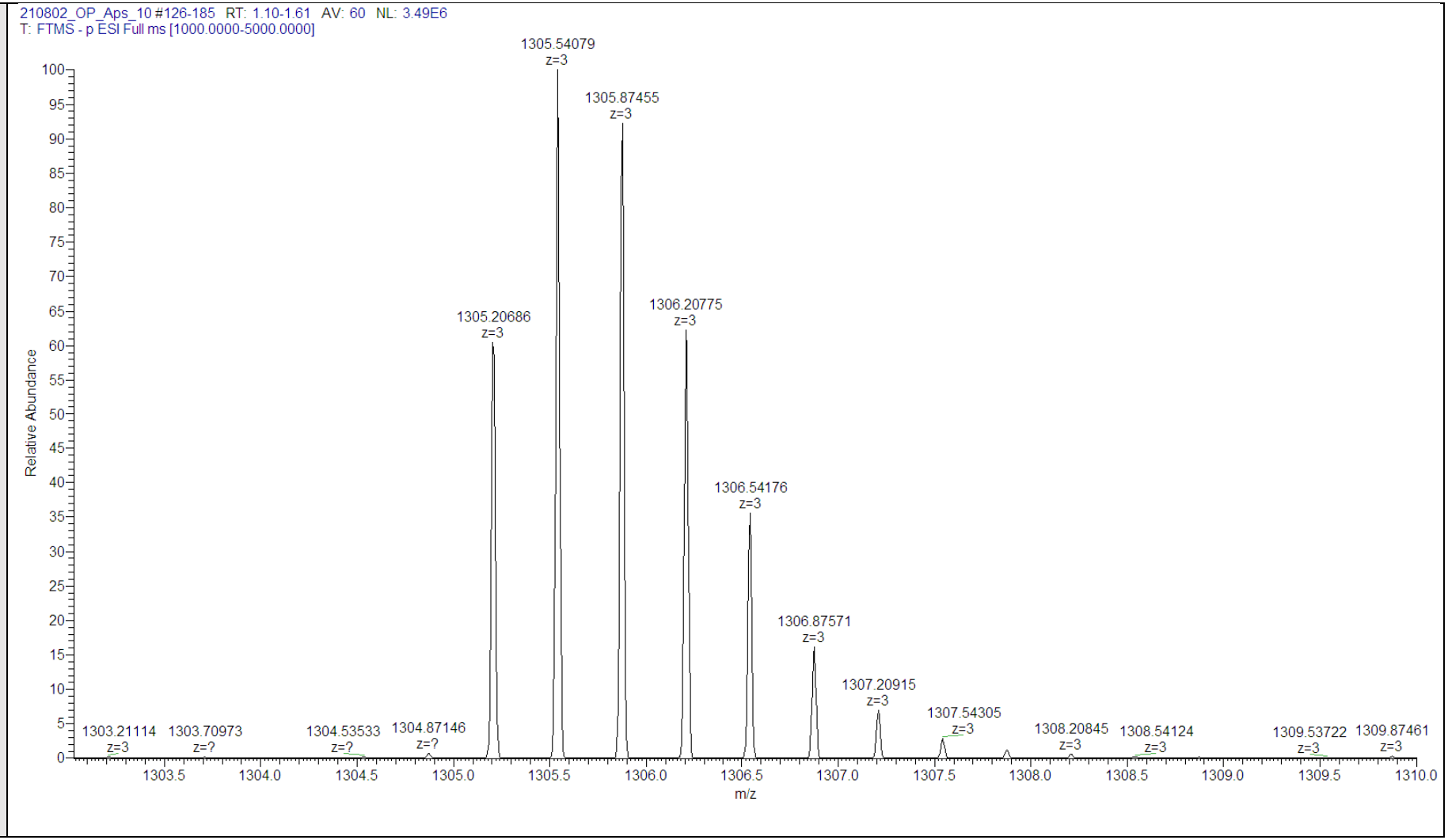

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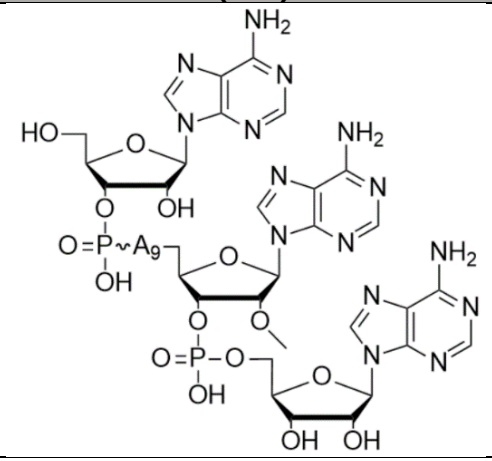
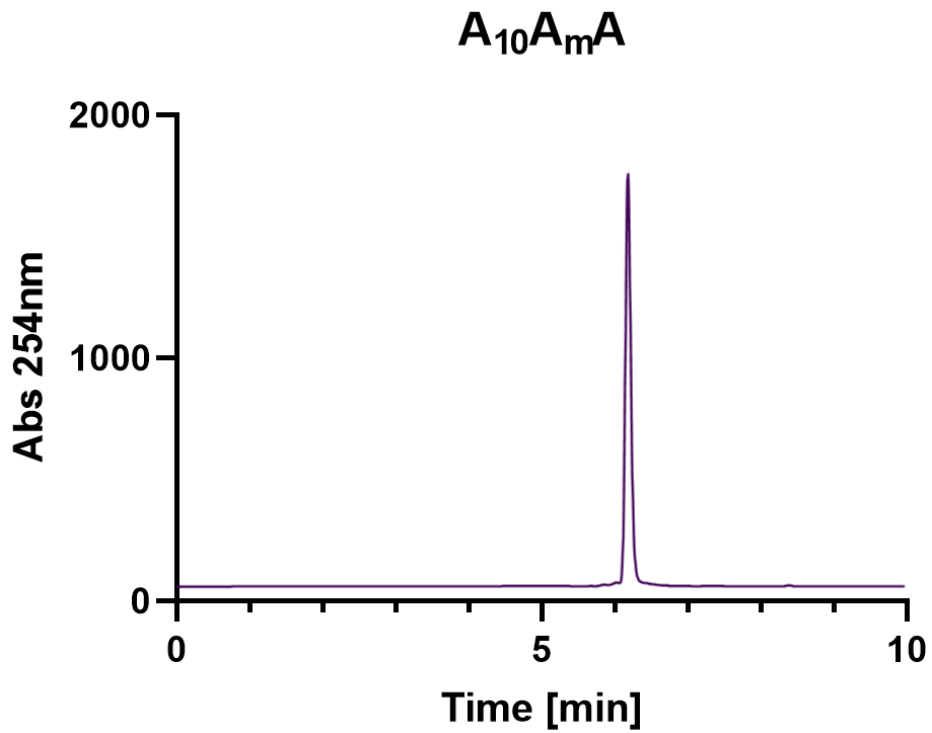
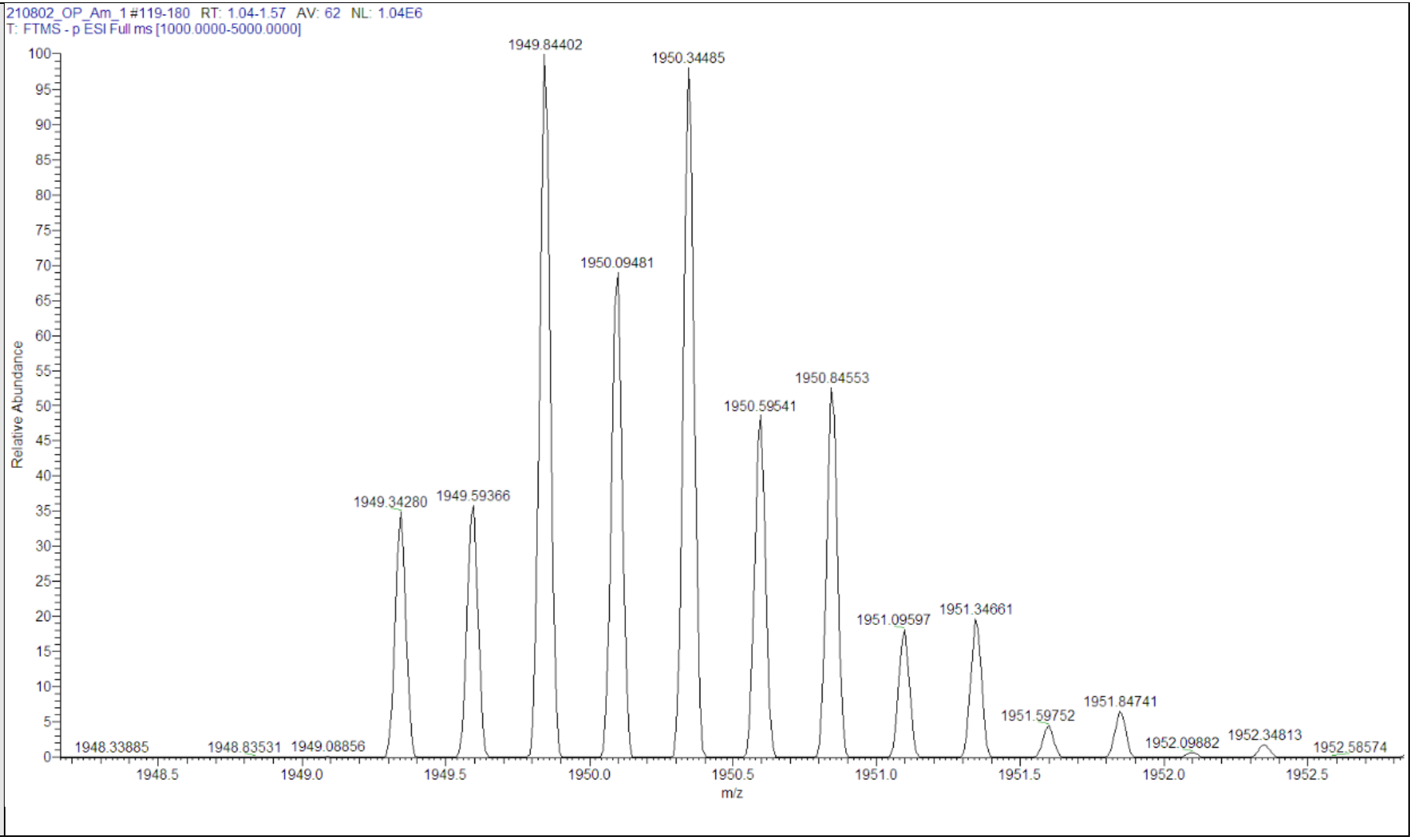
Chemical structure	
RP HPLC	

MS (-) ESI

(Calc. $[M-2H]^{2-}$ $C_{121}H_{146}N_{60}O_{70}P_{11}^{2-}$ 1949.8431)



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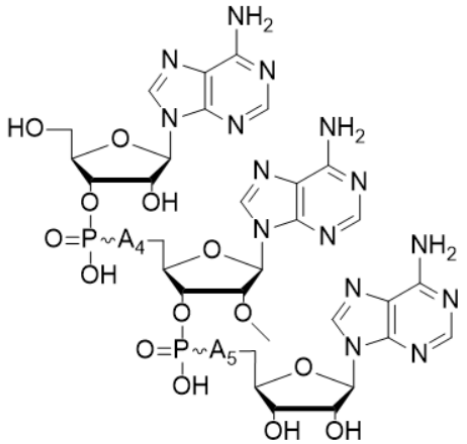
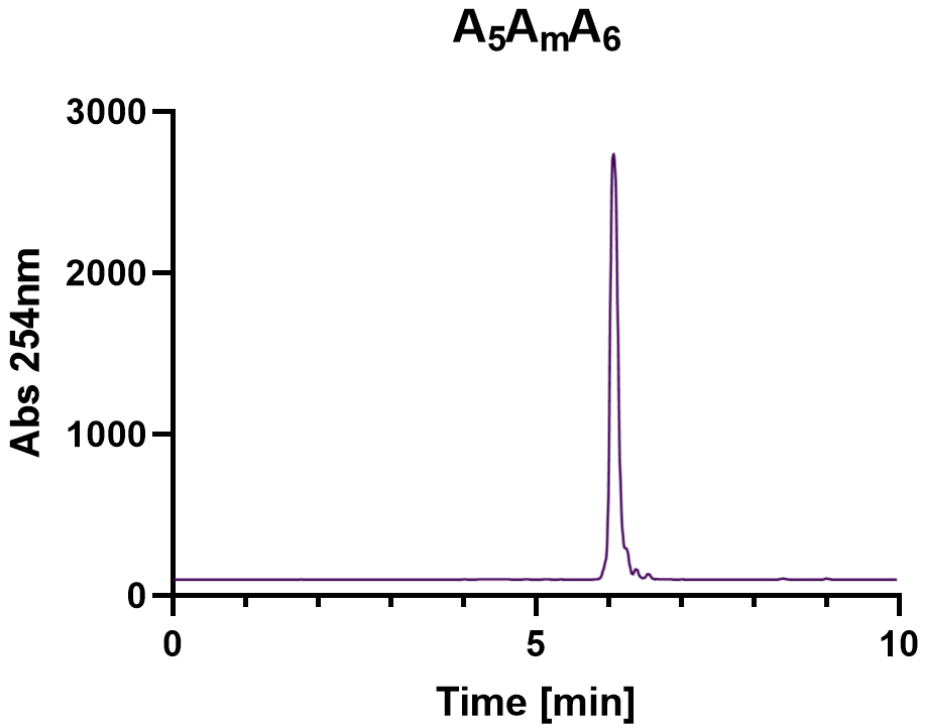
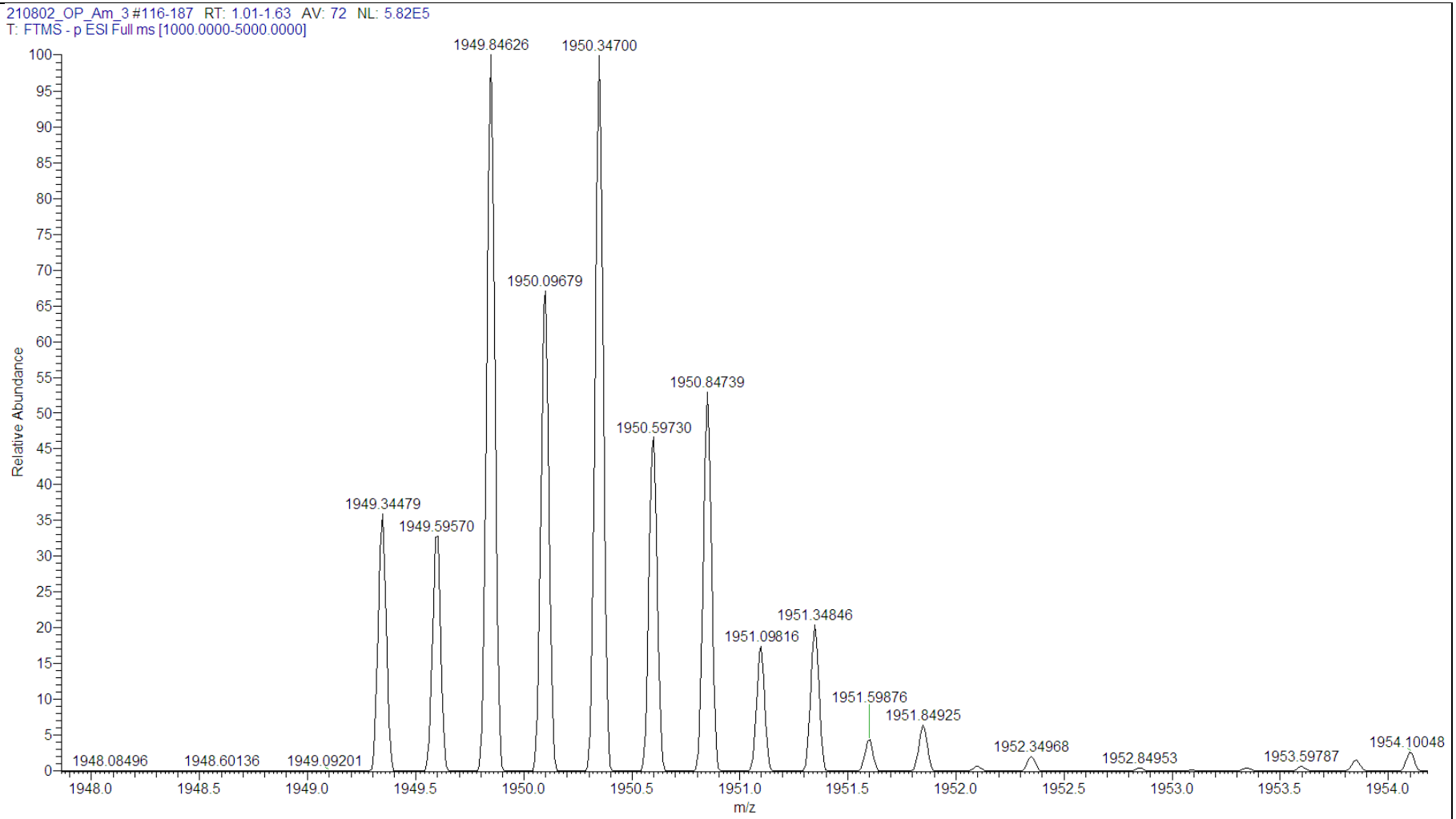
Chemical structure	
RP HPLC	

MS (-) ESI

(Calc. $[M-2H]^{2-}$ $C_{121}H_{146}N_{60}O_{70}P_{11}^{2-}$ 1949.8431)



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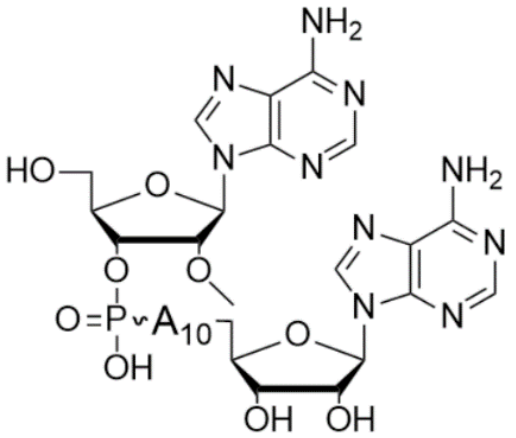
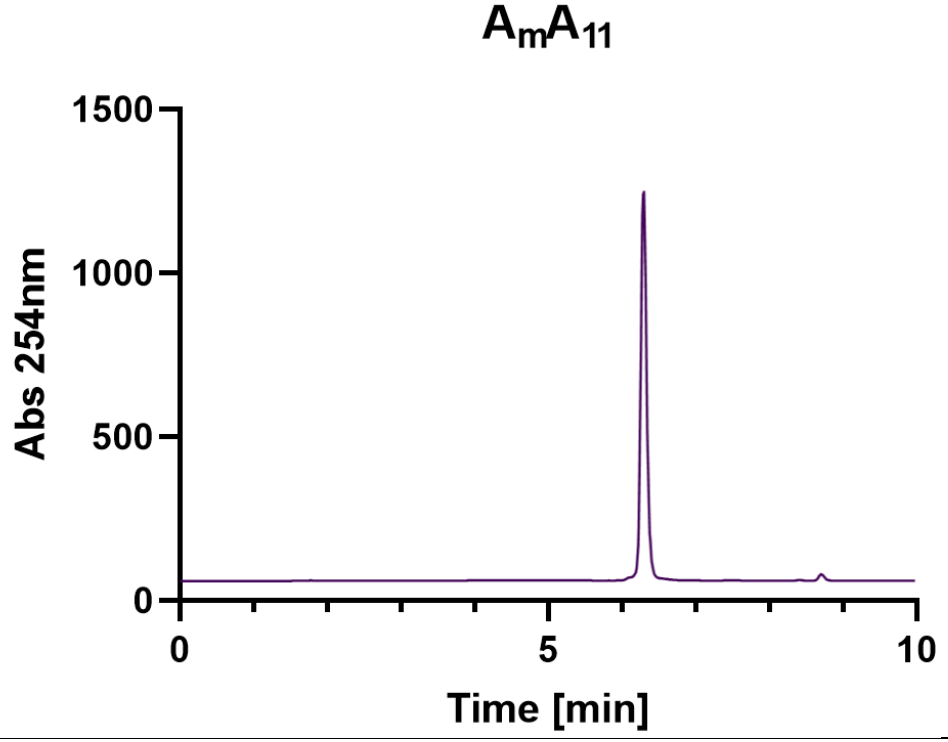
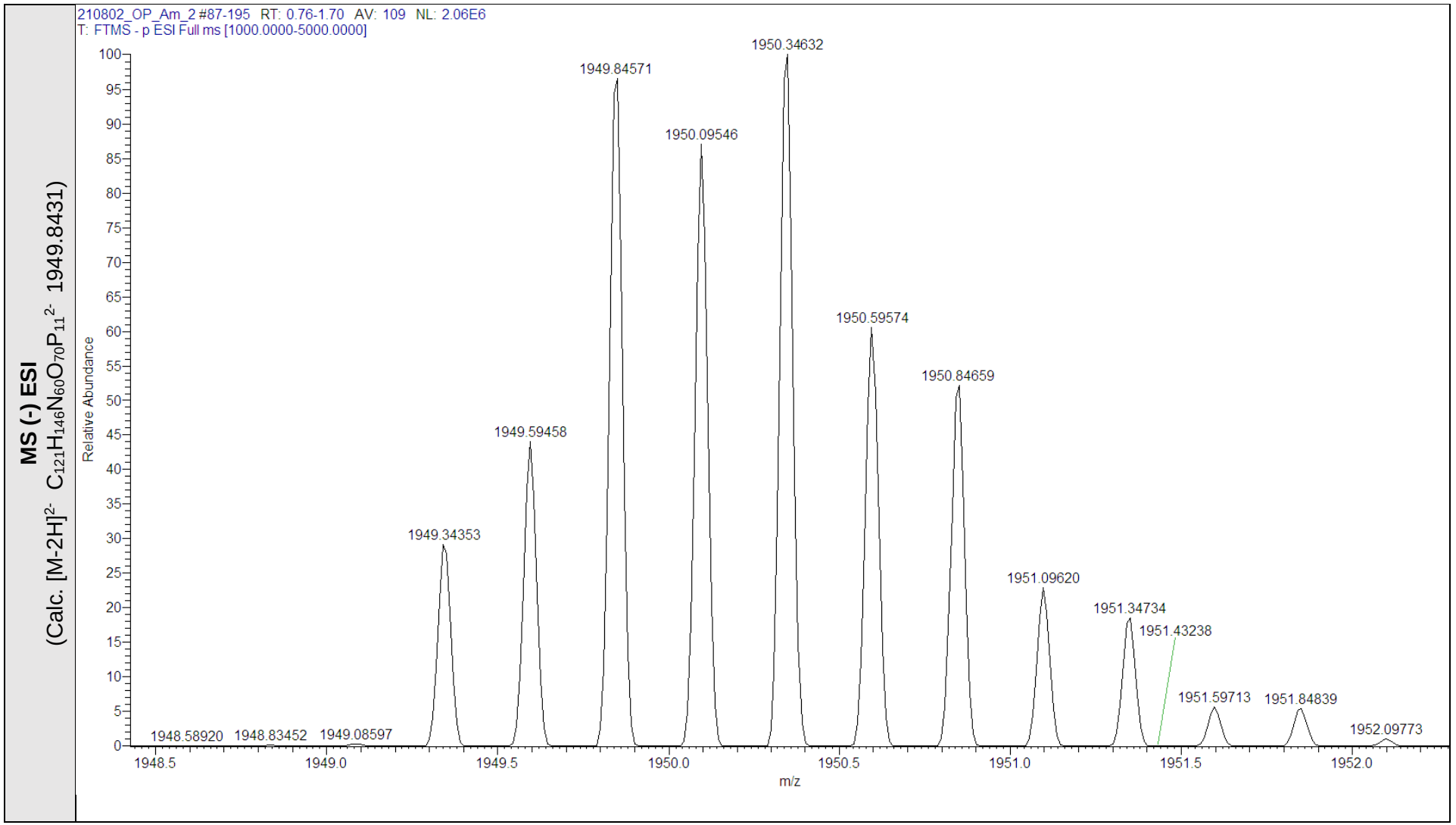
Chemical structure	
RP HPLC	


MS (-) ESI
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210802_OP_Am_2 #87-195 RT: 0.76-1.70 AV: 109 NL: 2.06E6
T: FTMS - p ESI Full ms [1000.0000-5000.0000]
Relative Abundance
m/z
1948.58920
1948.83452
1949.08597
1949.34353
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1950.34632
1950.59574
1950.84659
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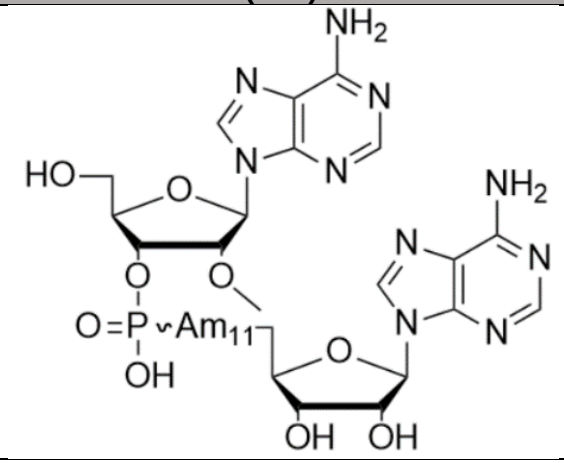
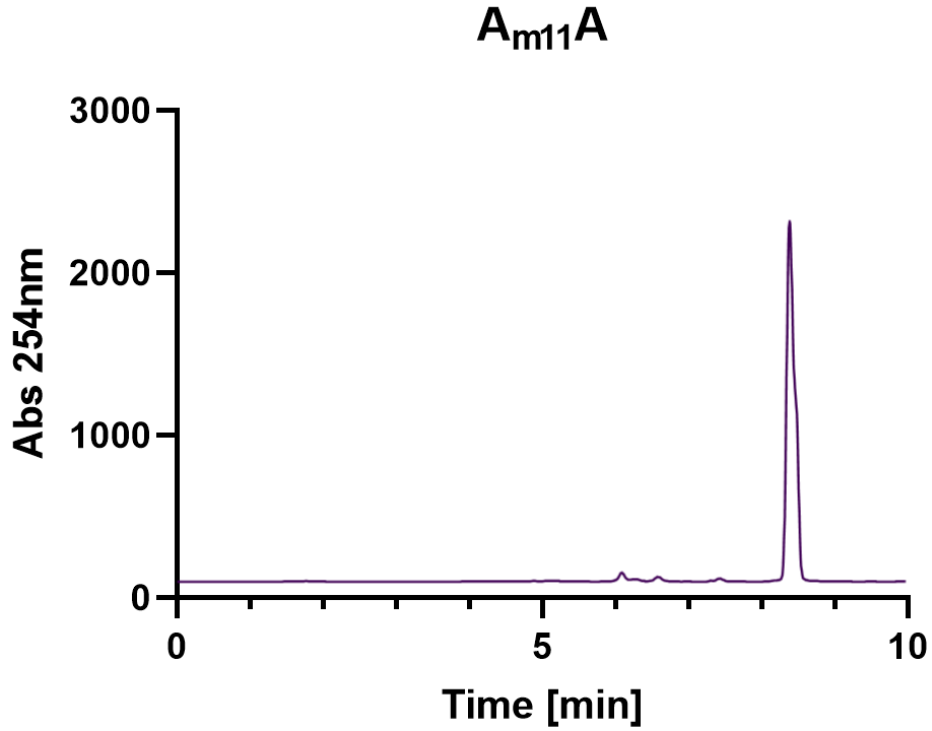
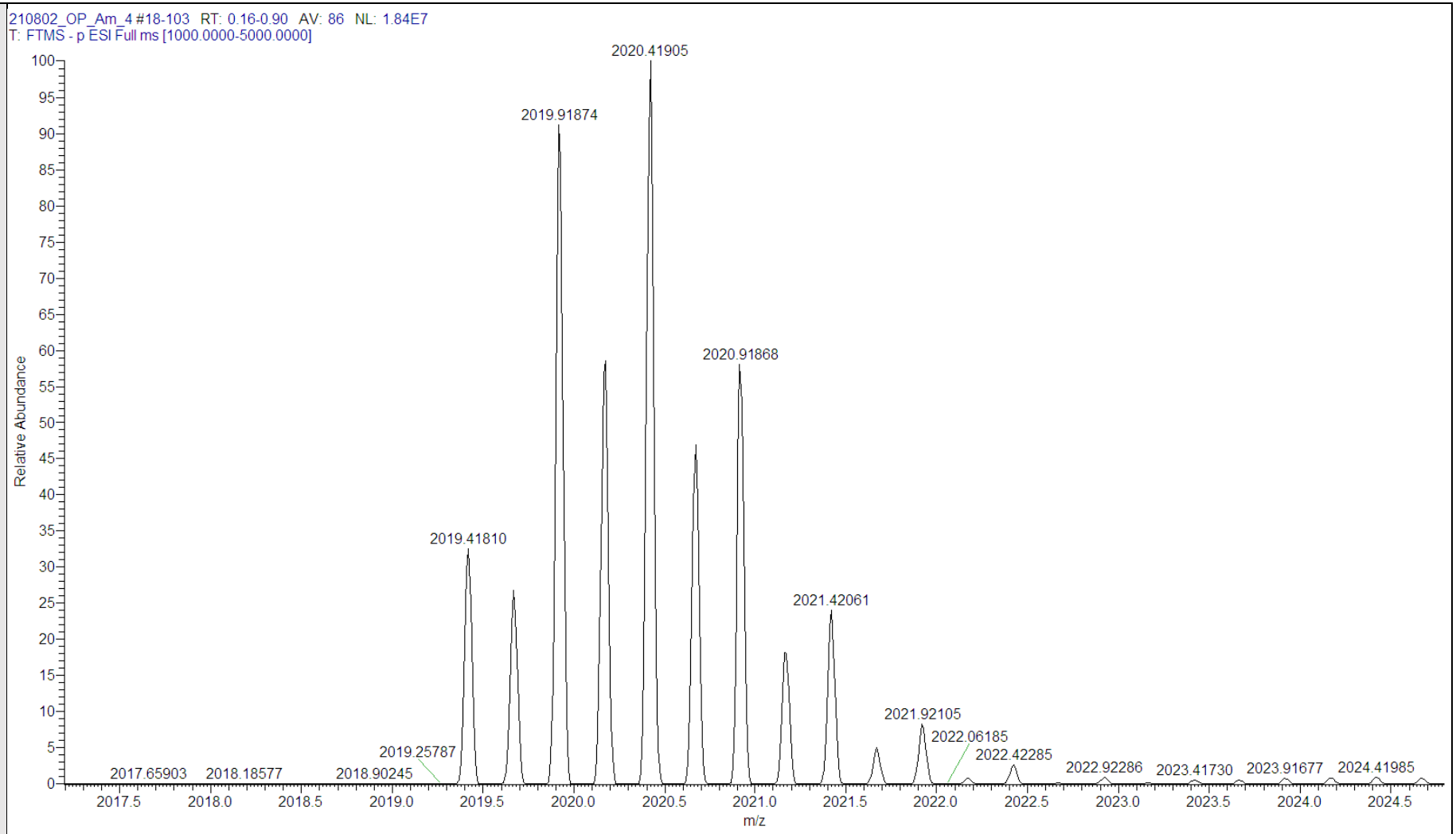
1952.09773

$A_{m11}A$ (A_m4)	
Chemical structure	
RP HPLC	

MS (-) ESI

(Calc. $[M-2H]^{2-}$ $C_{131}H_{166}N_{60}O_{70}P_{11}^{2-}$ 2019.92135)



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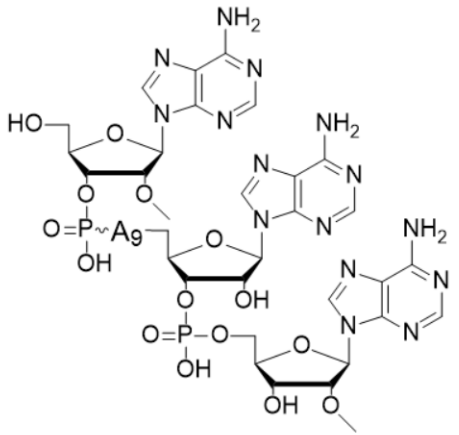
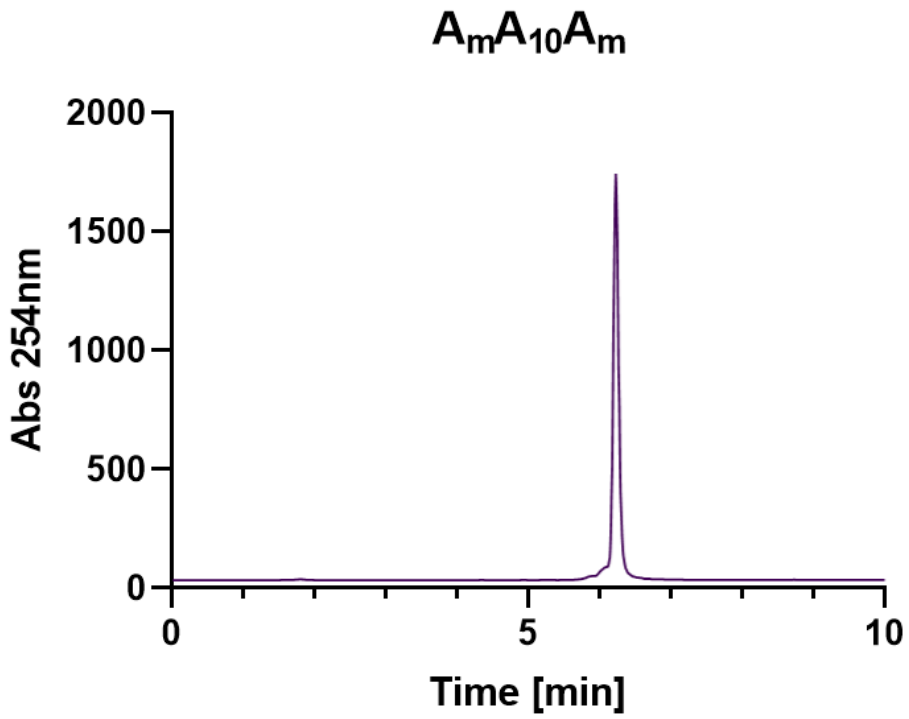
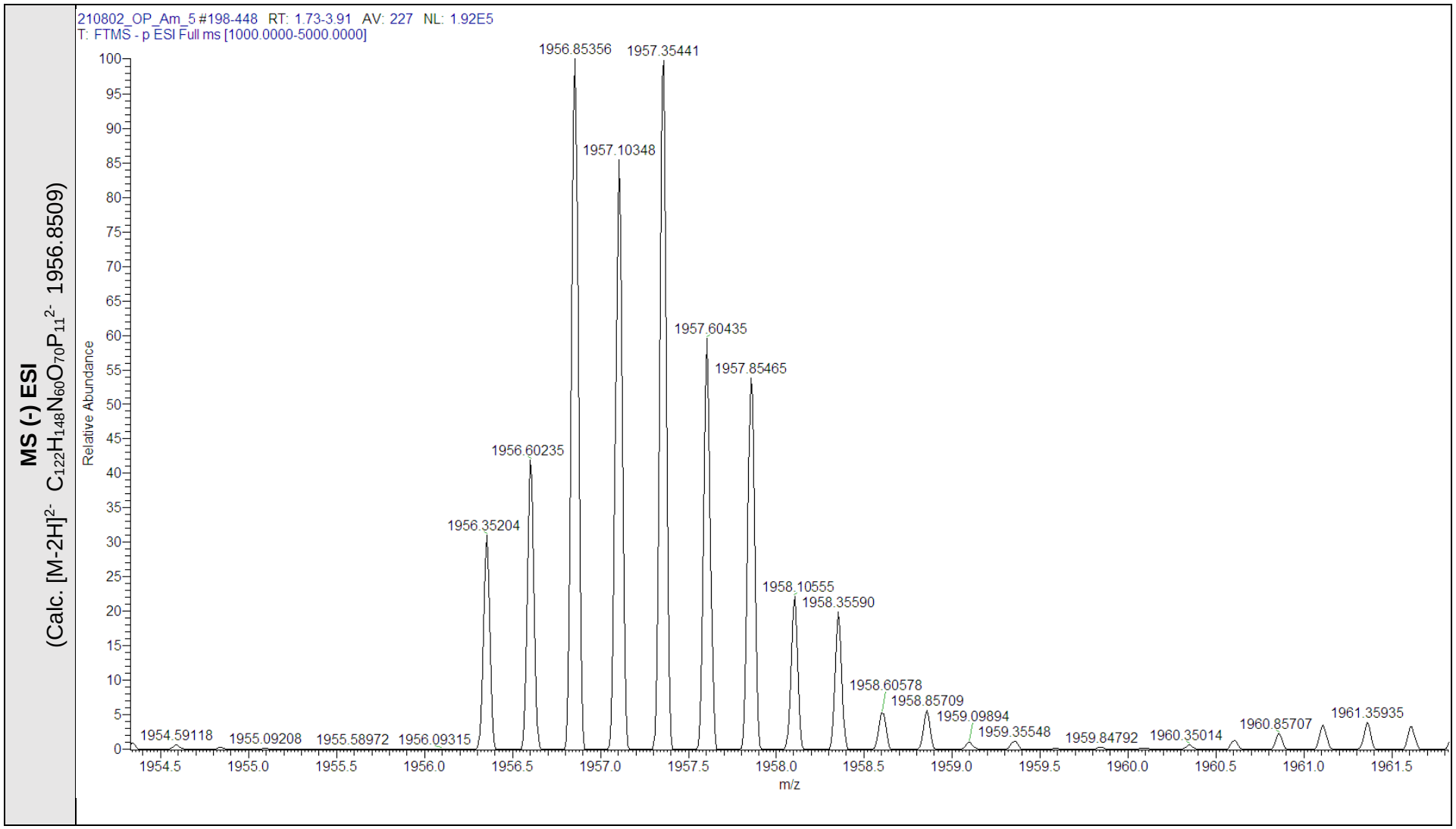
Chemical structure	
RP HPLC	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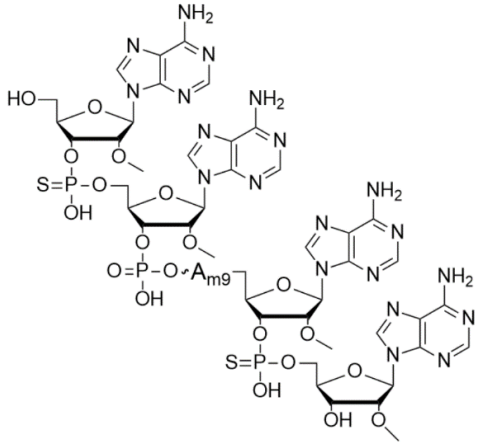
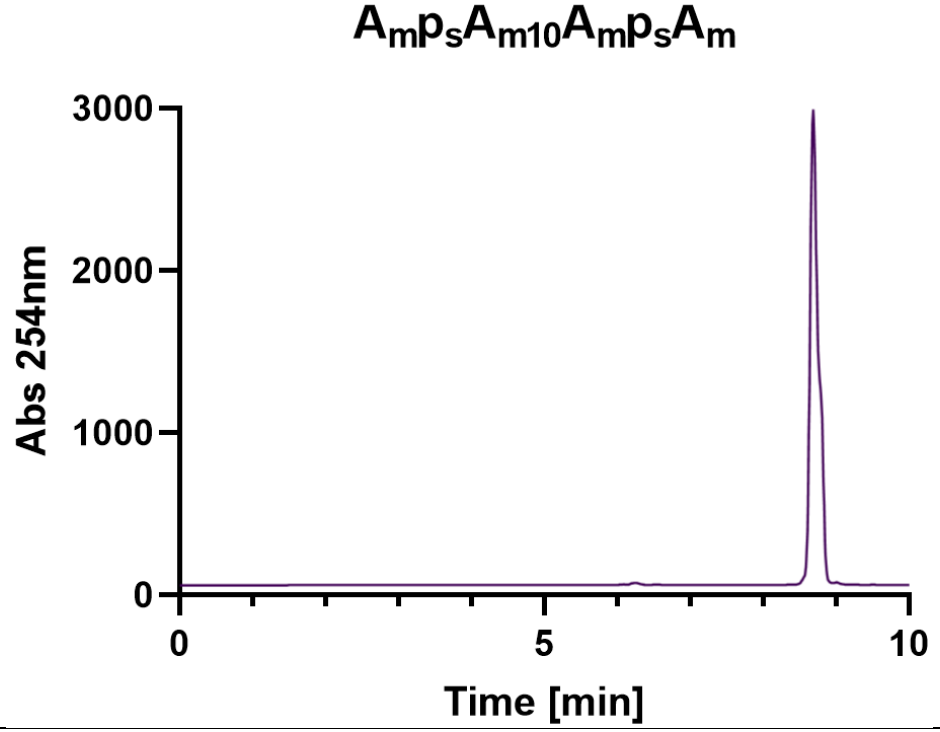
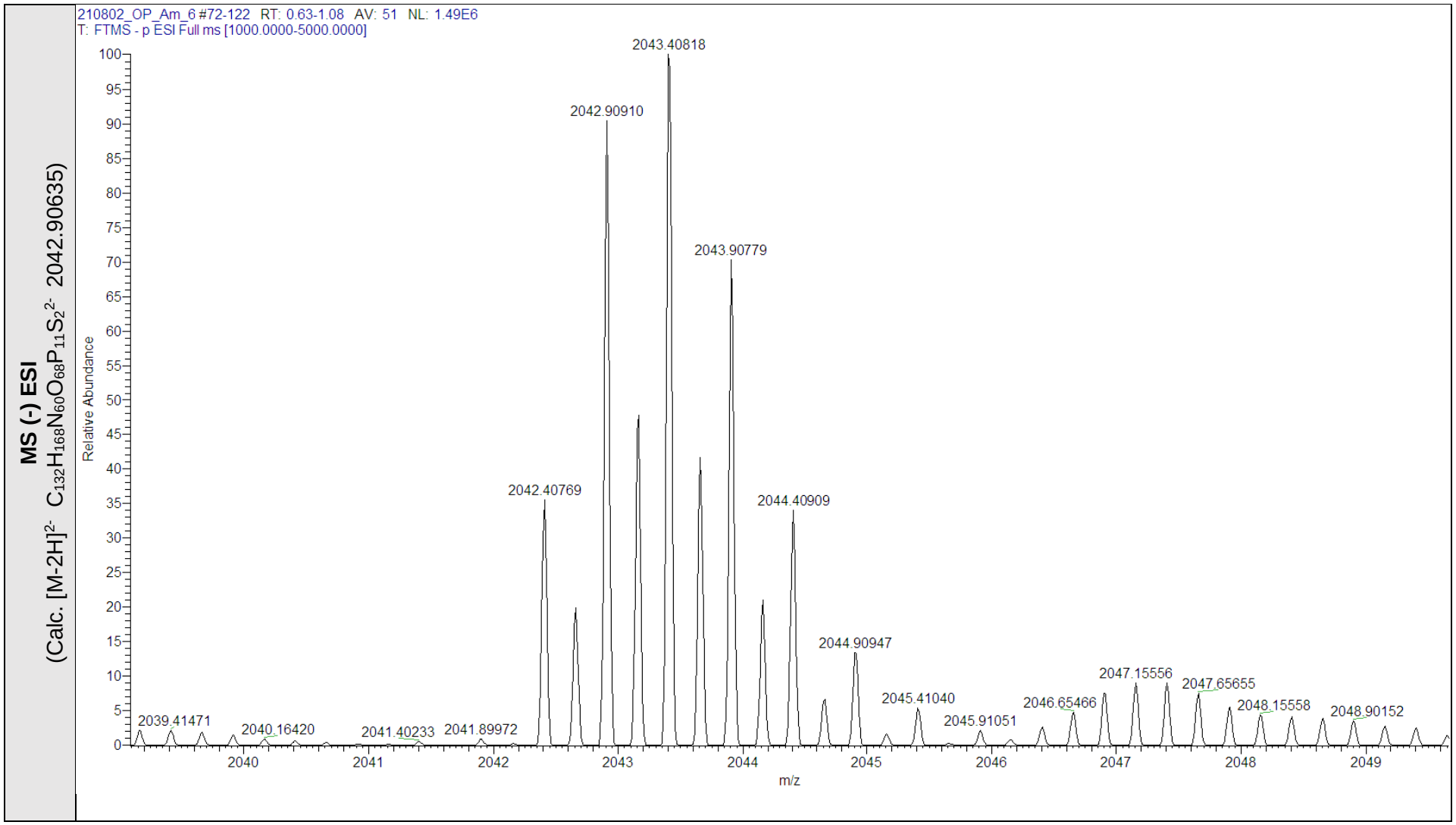
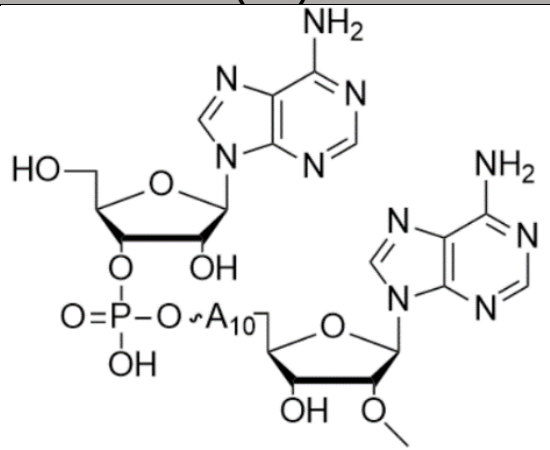
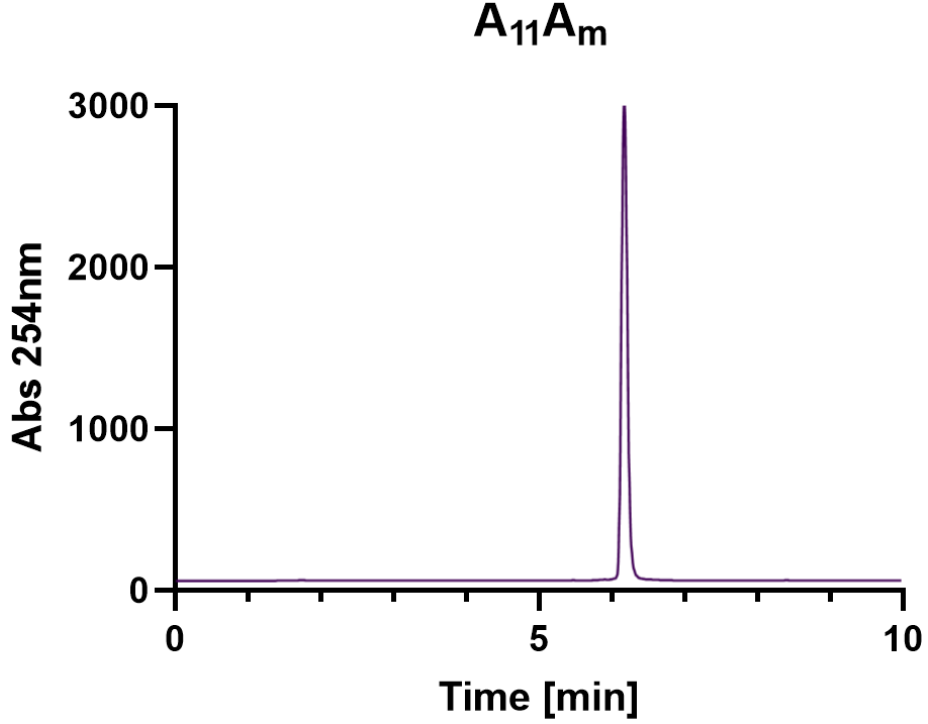
T: FTMS - p ESI Full ms [1000.0000-5000.0000]
Relative Abundance
m/z
1956.85356
1957.35441
1957.10348
1957.60435
1957.85465
1956.60235
1956.35204
1958.10555
1958.35590
1958.60578
1958.85709
1959.09894
1959.35548
1959.84792
1960.35014
1960.85707
1961.35935
1954.59118
1955.09208
1955.58972
1956.09315

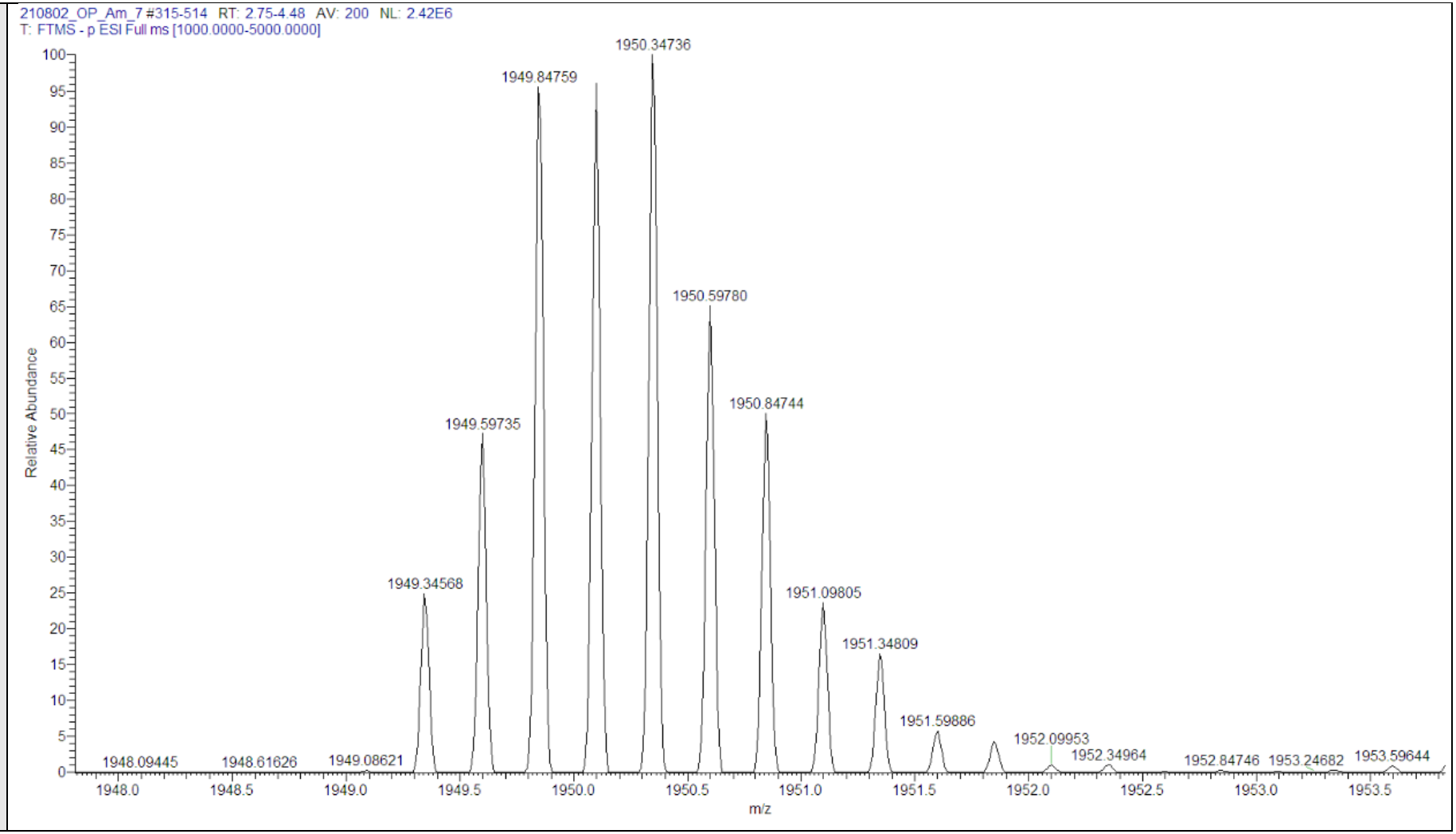
$A_mp_sA_{m10}Ap_sA_m$ (A_m6)	
Chemical structure	
RP HPLC	

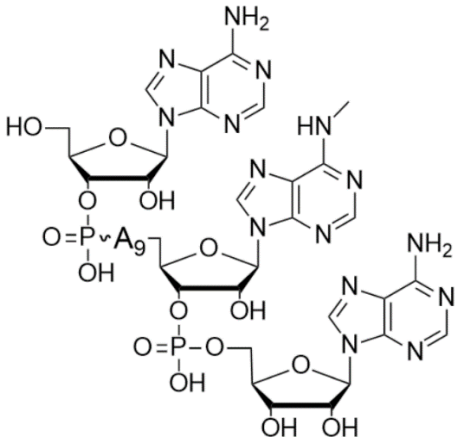
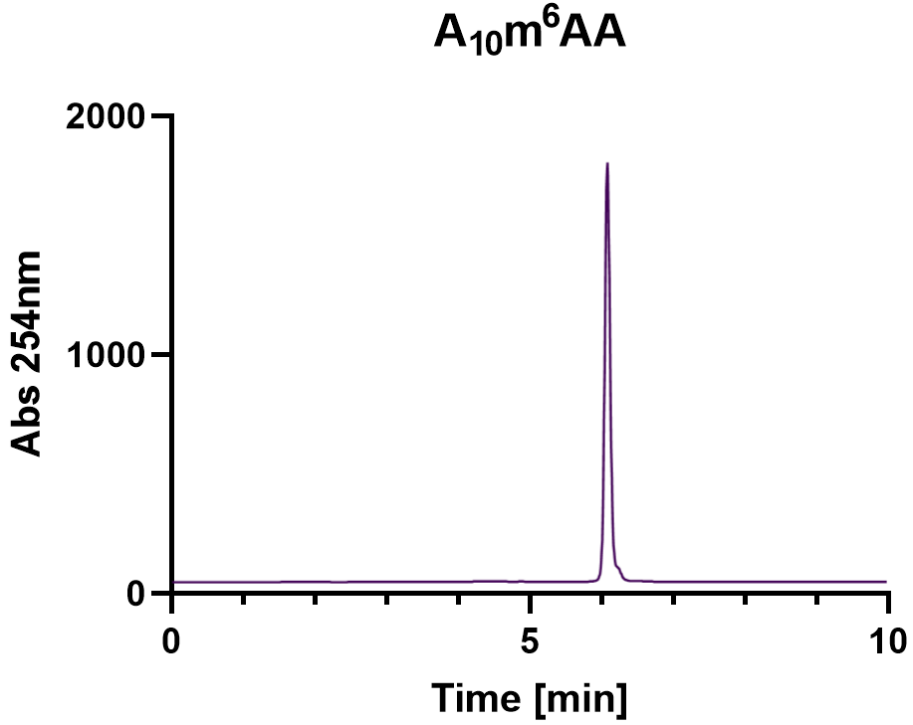

MS (-) ESI
(Calc. [M-2H]2- C132H168N60O68P11S92- 2042.90635)
210802_OP_Am_6 #72-122 RT: 0.63-1.08 AV: 51 NL: 1.49E6
T: FTMS - p ESI Full ms [1000.0000-5000.0000]
Relative Abundance
m/z
2043.40818
2042.90910
2043.90779
2042.40769
2044.40909
2044.90947
2045.41040
2045.91051
2046.65466
2047.15556
2047.65655
2048.15558
2048.90152
2039.41471
2040.16420
2041.40233
2041.89972

$A_{11}A_m$ (A_m7)	
Chemical structure	
RP HPLC	

MS (-) ESI

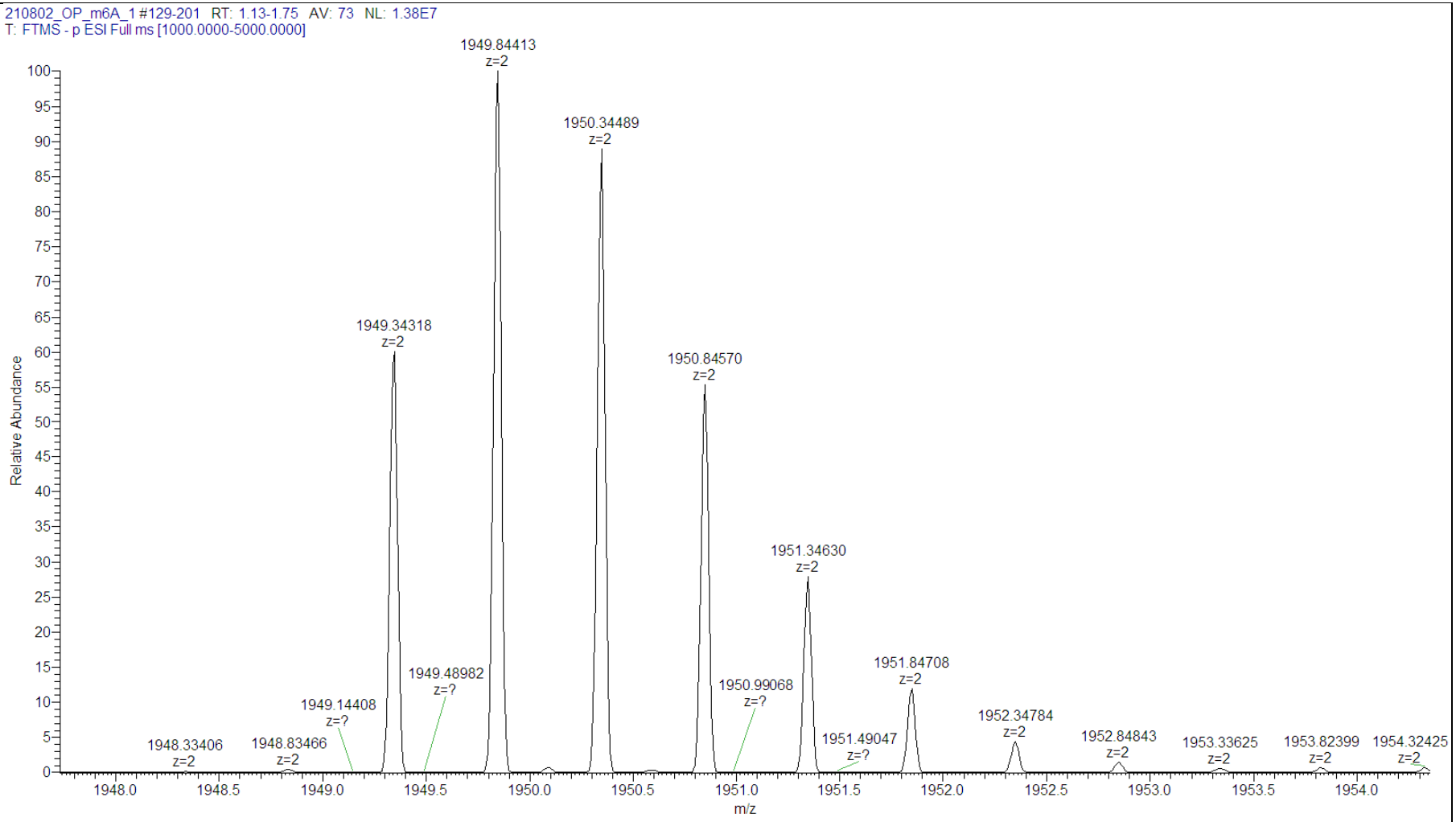
(Calc. $[M-2H]^{2-}$ $C_{121}H_{146}N_{60}O_{70}P_{11}^{2-}$ 1949.8431)

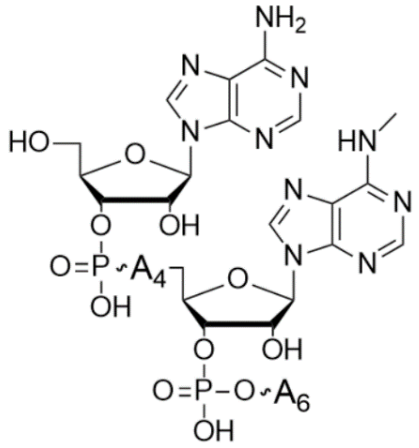
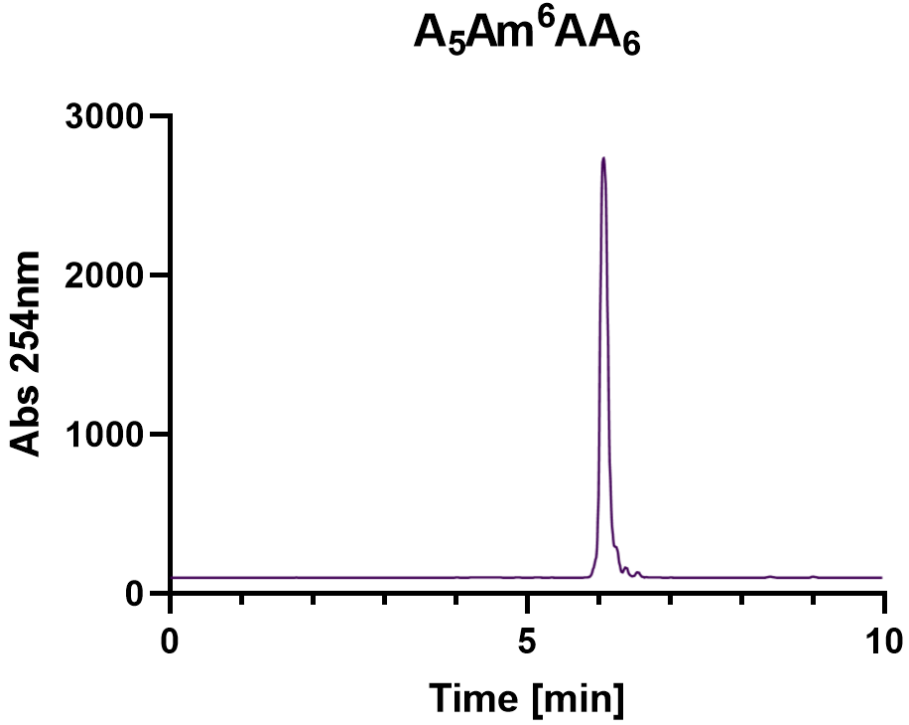


$A_{10}m^6AA$ (m^6A1)	
Chemical structure	
RP HPLC	

MS (-) ESI

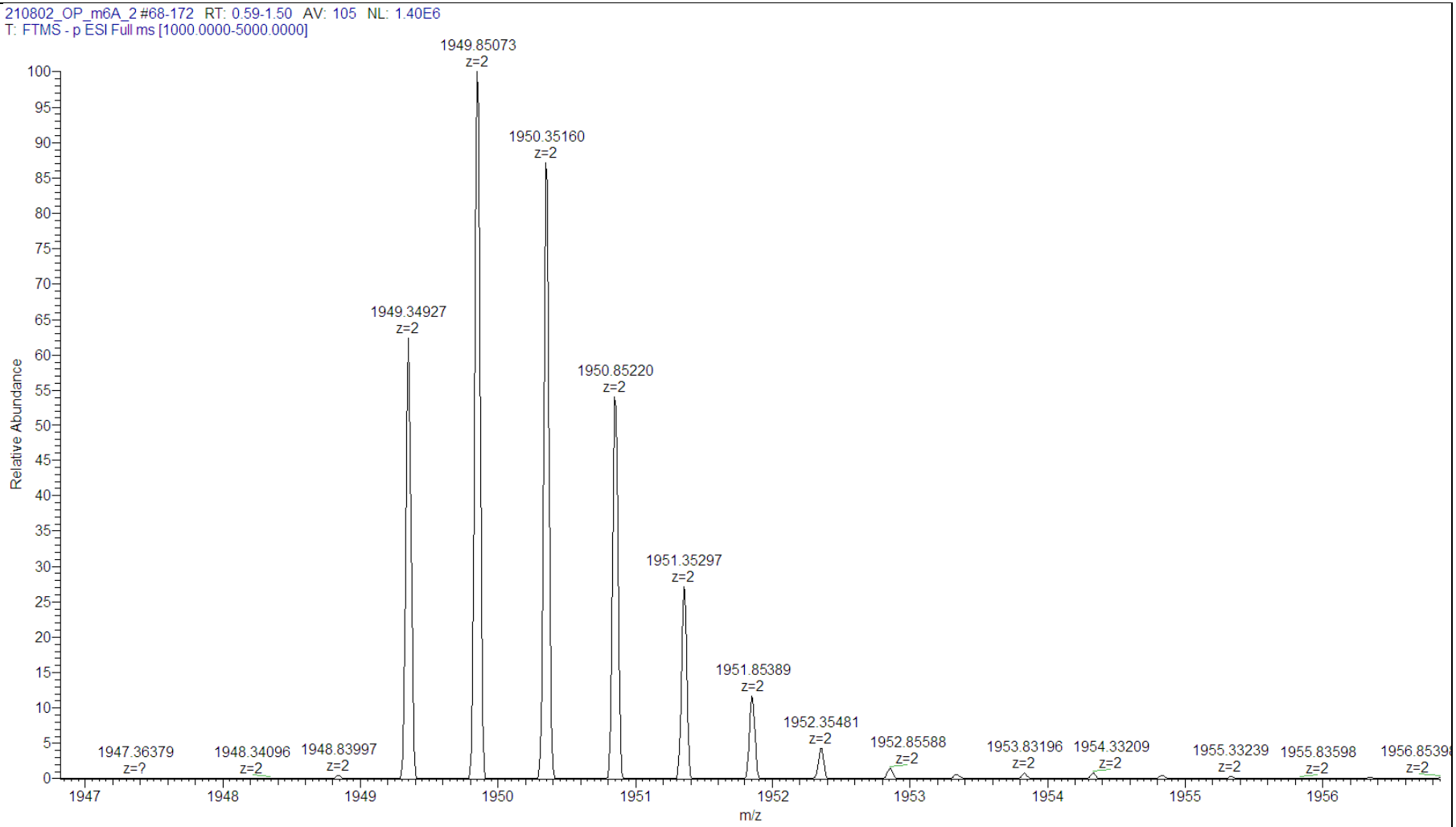
(Calc. $[M-2H]^{2-}$ $C_{121}H_{146}N_{60}O_{70}P_{11}^{2-}$ 1949.8431)

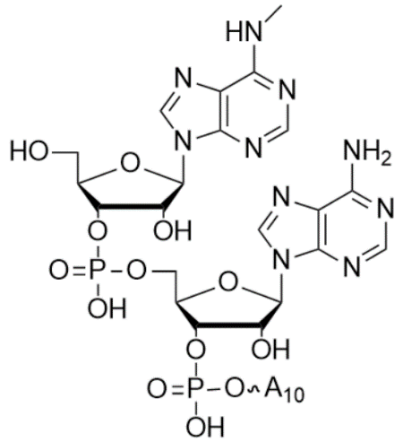
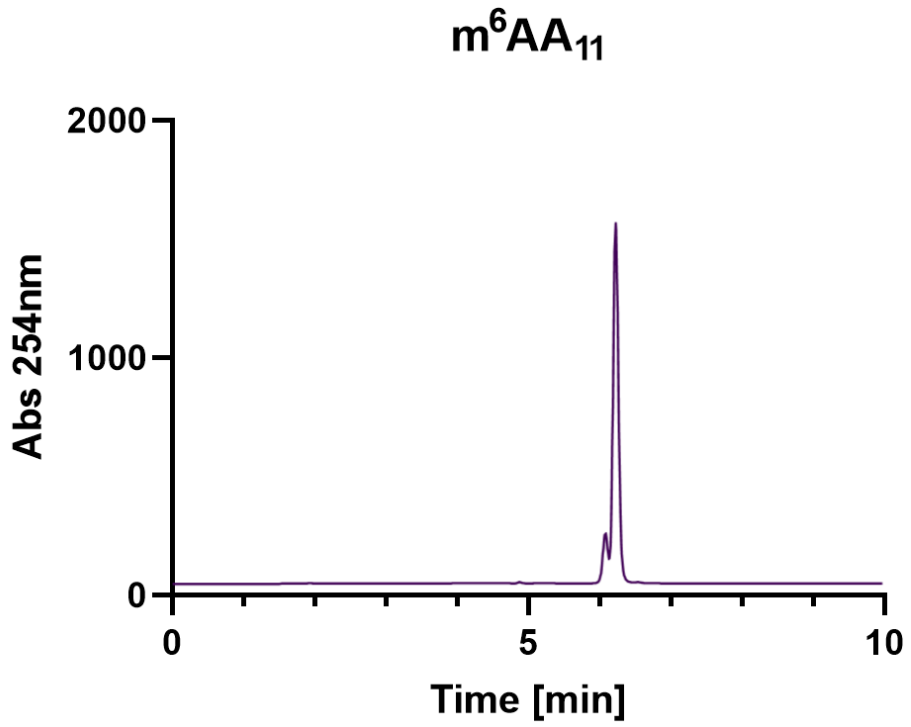


$A_5m^6AA_6$ (m^6A2)	
Chemical structure	
RP HPLC	

MS (-) ESI

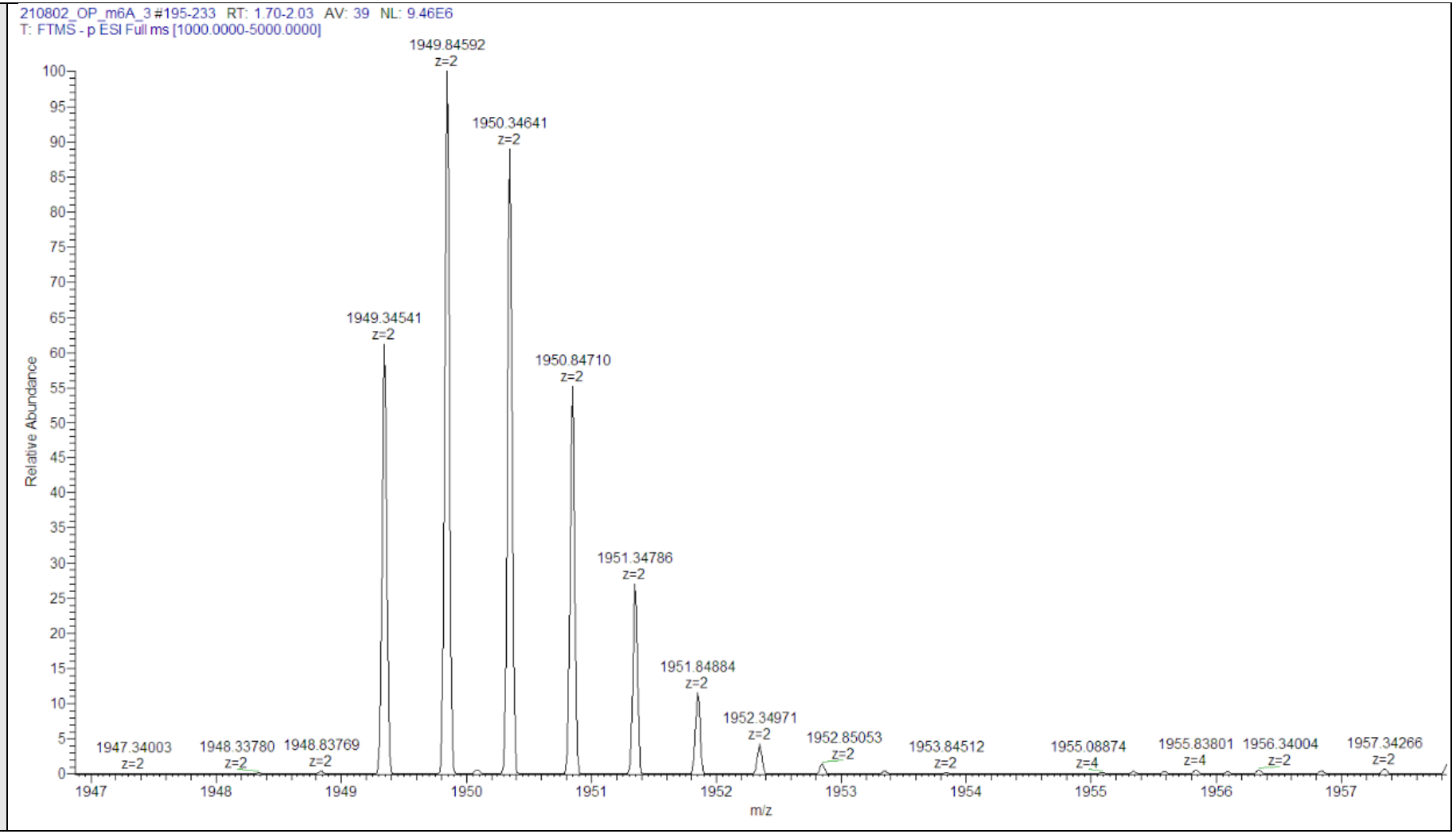
(Calc. $[M-2H]^{2-}$ $C_{121}H_{146}N_{60}O_{70}P_{11}^{2-}$ 1949.8431)

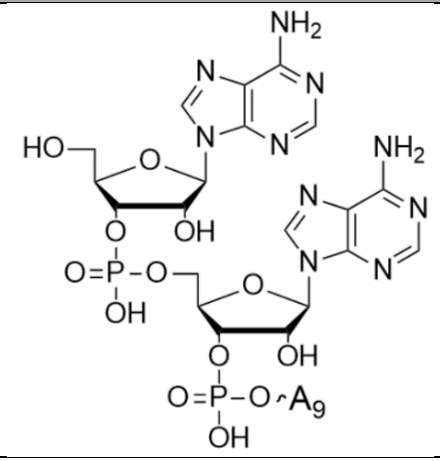
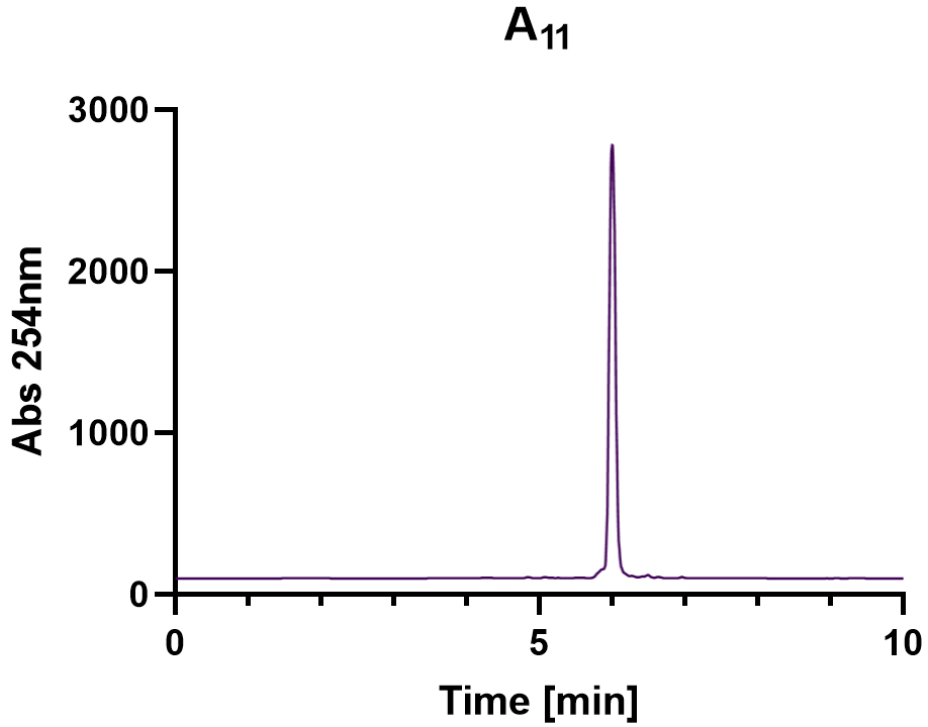


m^6A-A_{11} (m^6A3)	
Chemical structure	
RP HPLC	

MS (-) ESI

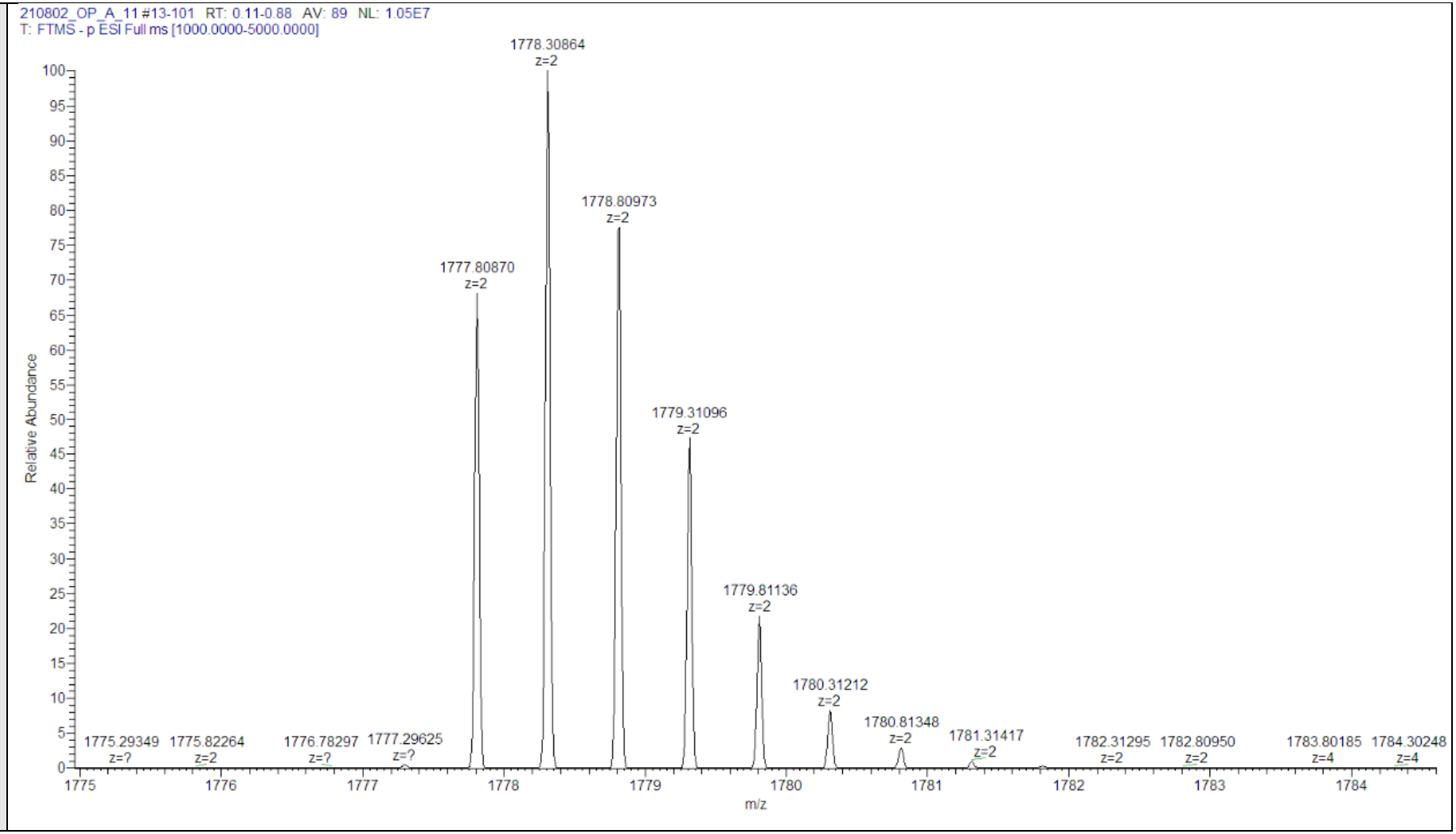
(Calc. $[M-2H]^{2-}$ $C_{121}H_{146}N_{60}O_{70}P_{11}^{2-}$ 1949.8431)

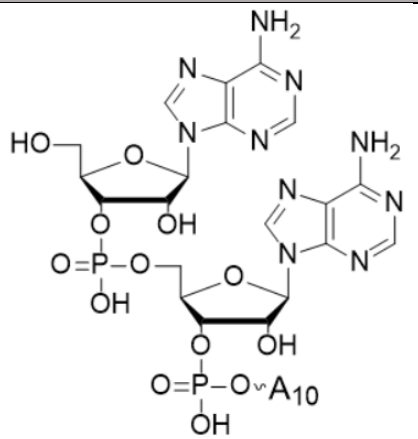
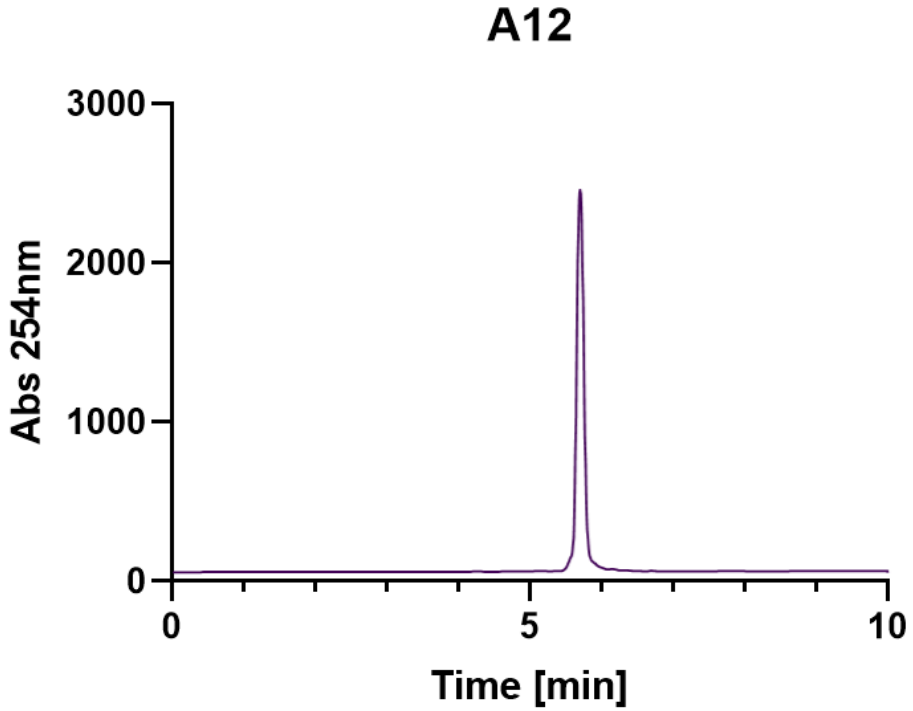


A_{11}	
Chemical structure	
RP HPLC	

MS (-) ESI

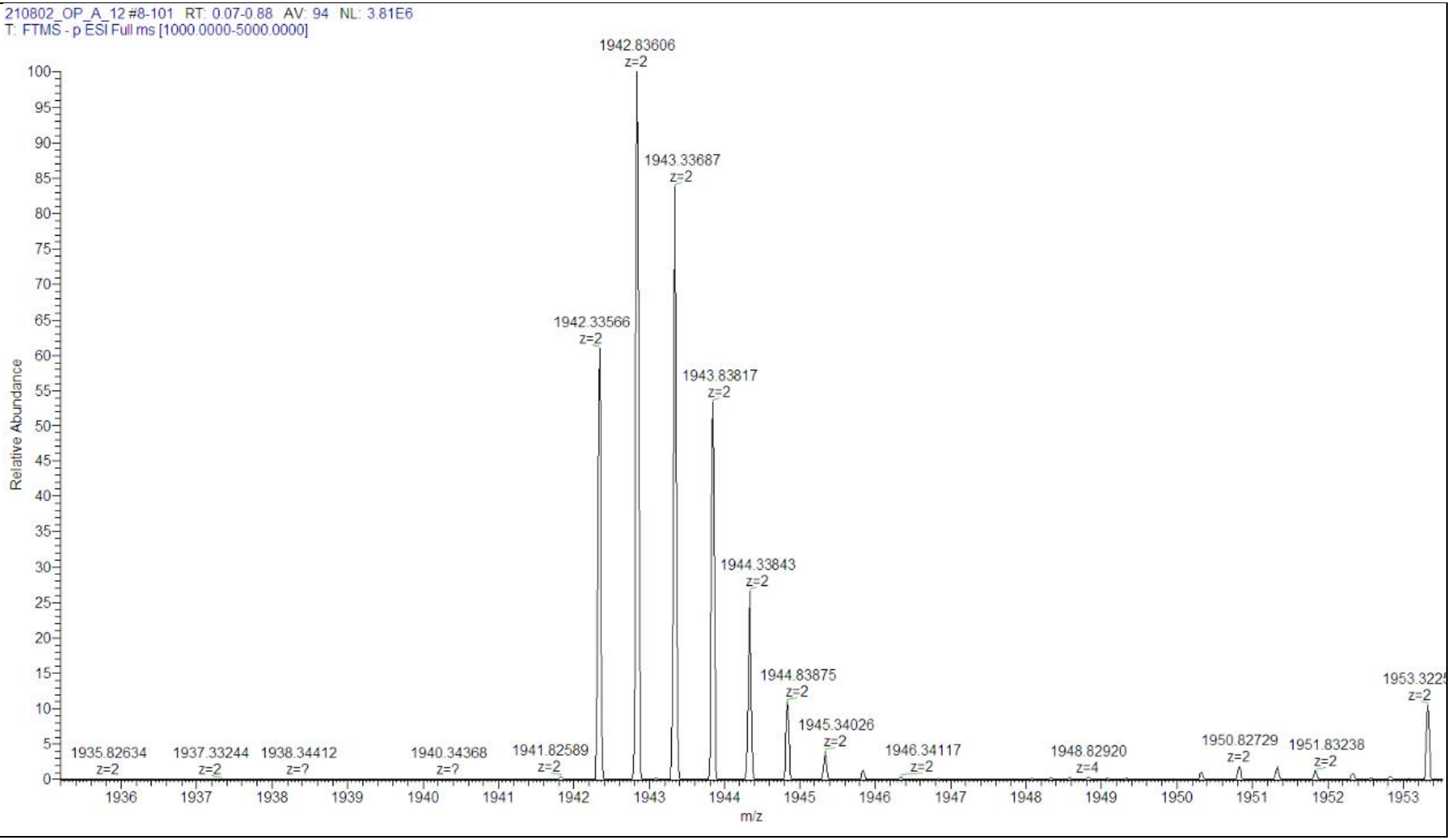
(Calc. $[M-2H]^{2-}$ $C_{110}H_{132}N_{55}O_{64}P_{10}^{2-}$ 1778.30585)

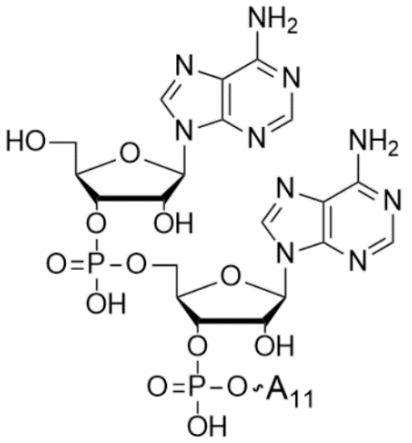
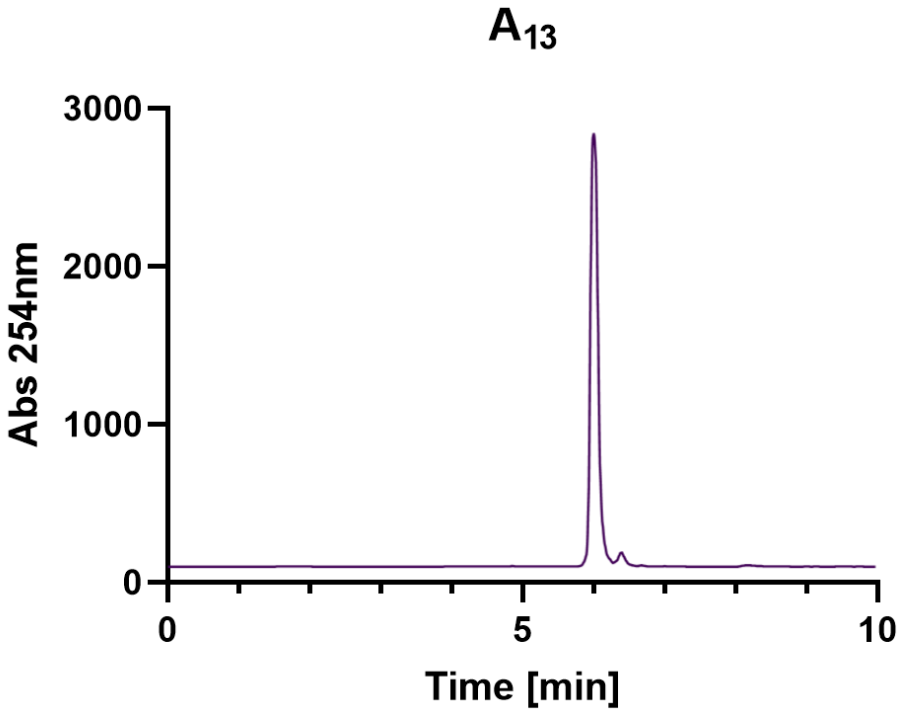


A_{12}	
Chemical structure	
RP HPLC	

MS (-) ESI

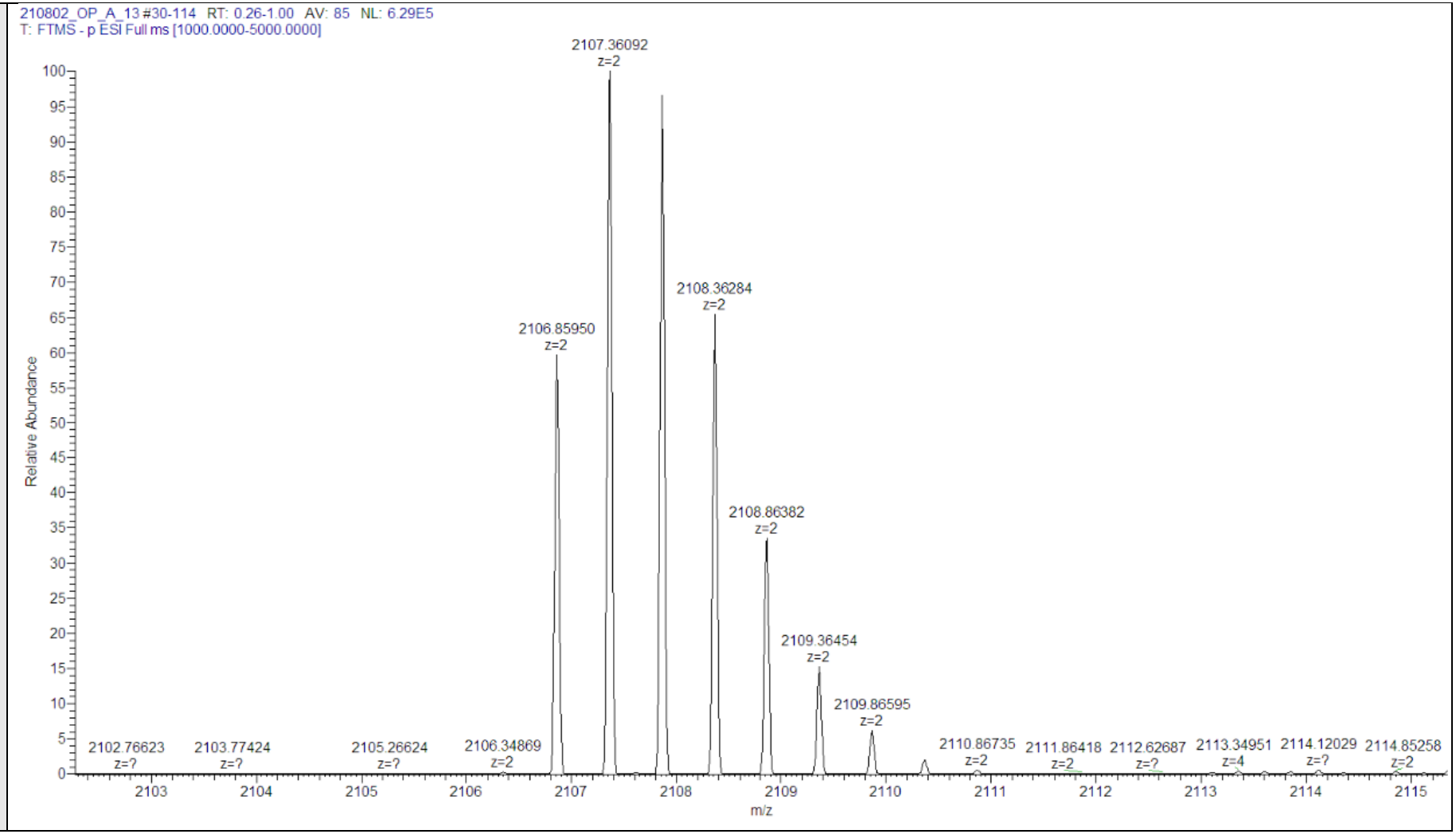
(Calc. $[M-2H]^{2-}$ $C_{120}H_{144}N_{60}O_{70}P_{11}^{2-}$ 1942.835)

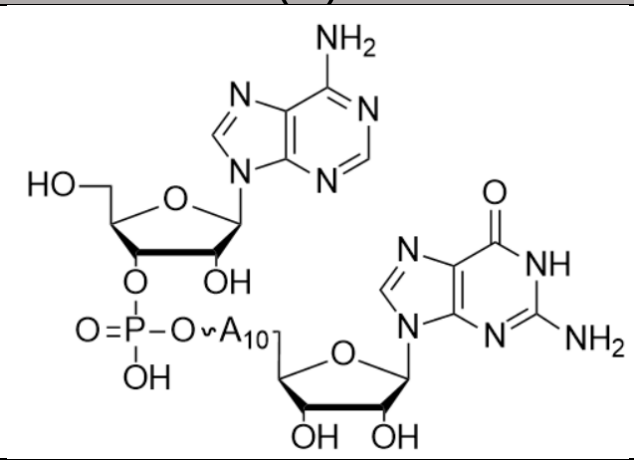
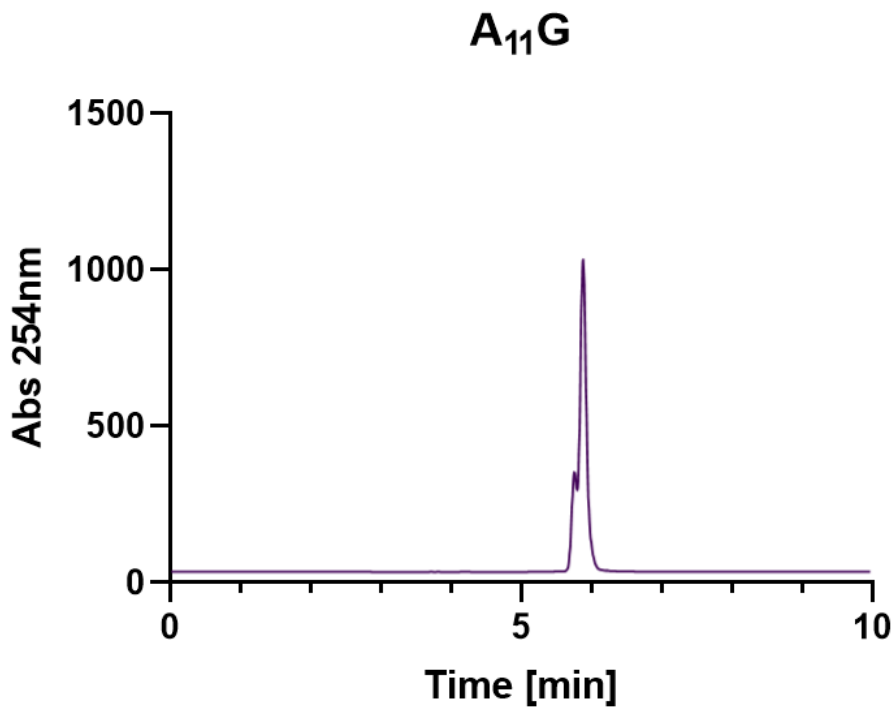


A_{13}	
Chemical structure	
RP HPLC	

MS (-) ESI

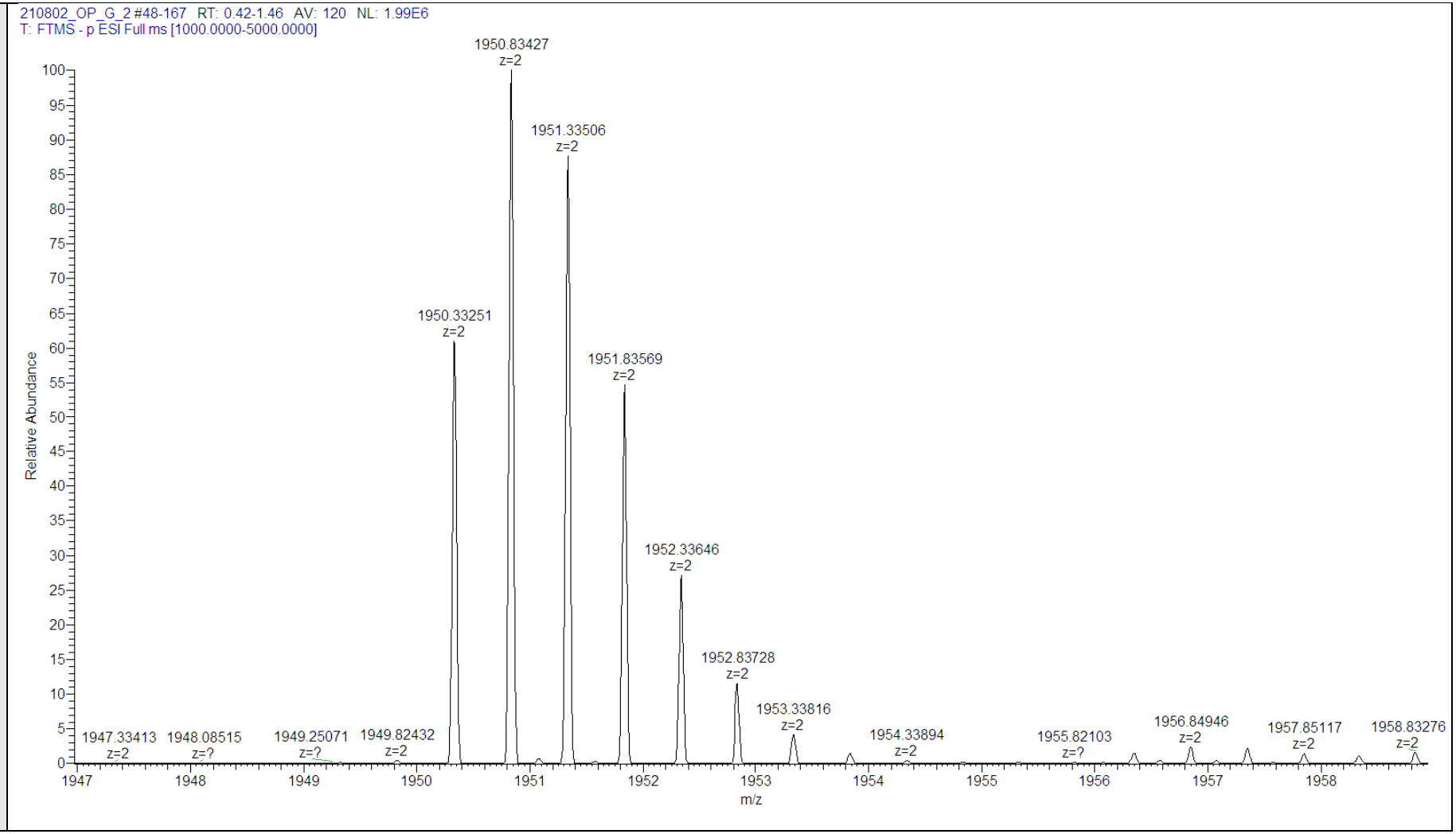
(Calc. $[M-2H]^{2-}$ $C_{130}H_{156}N_{65}O_{76}P_{12}^{2-}$ 2107.36155)

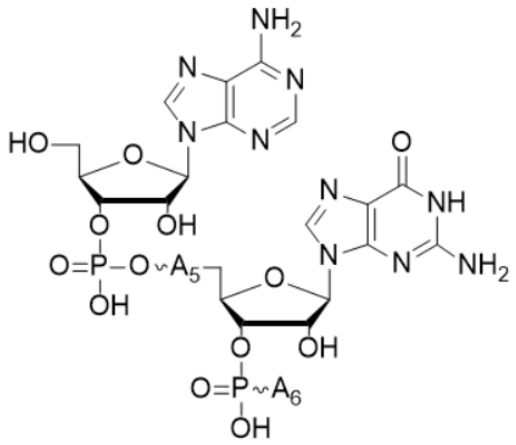
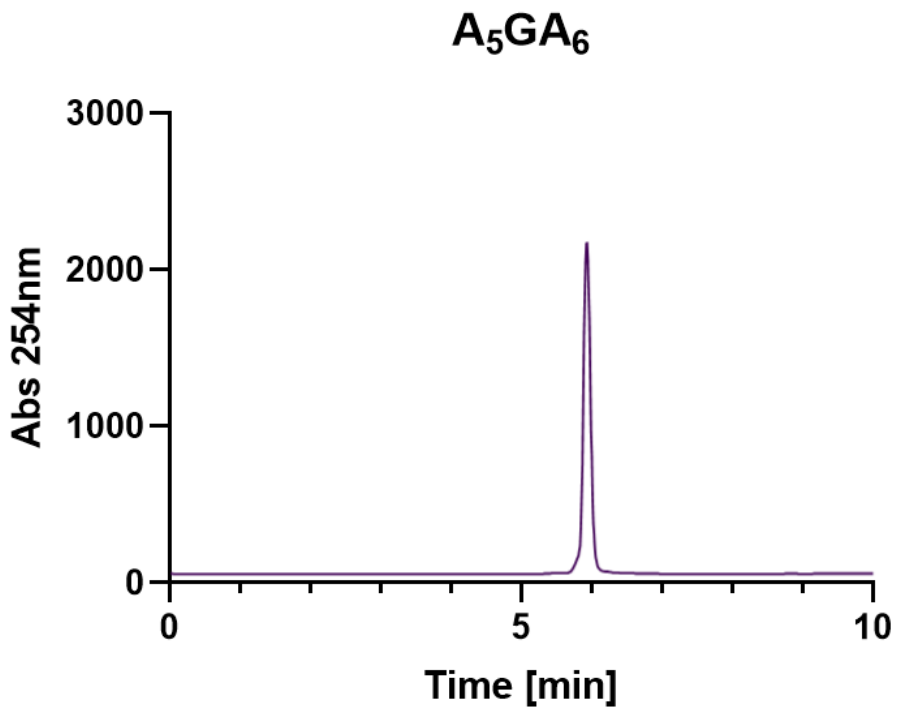


$A_{11}G$ (G1)	
Chemical structure	
RP HPLC	

MS (-) ESI

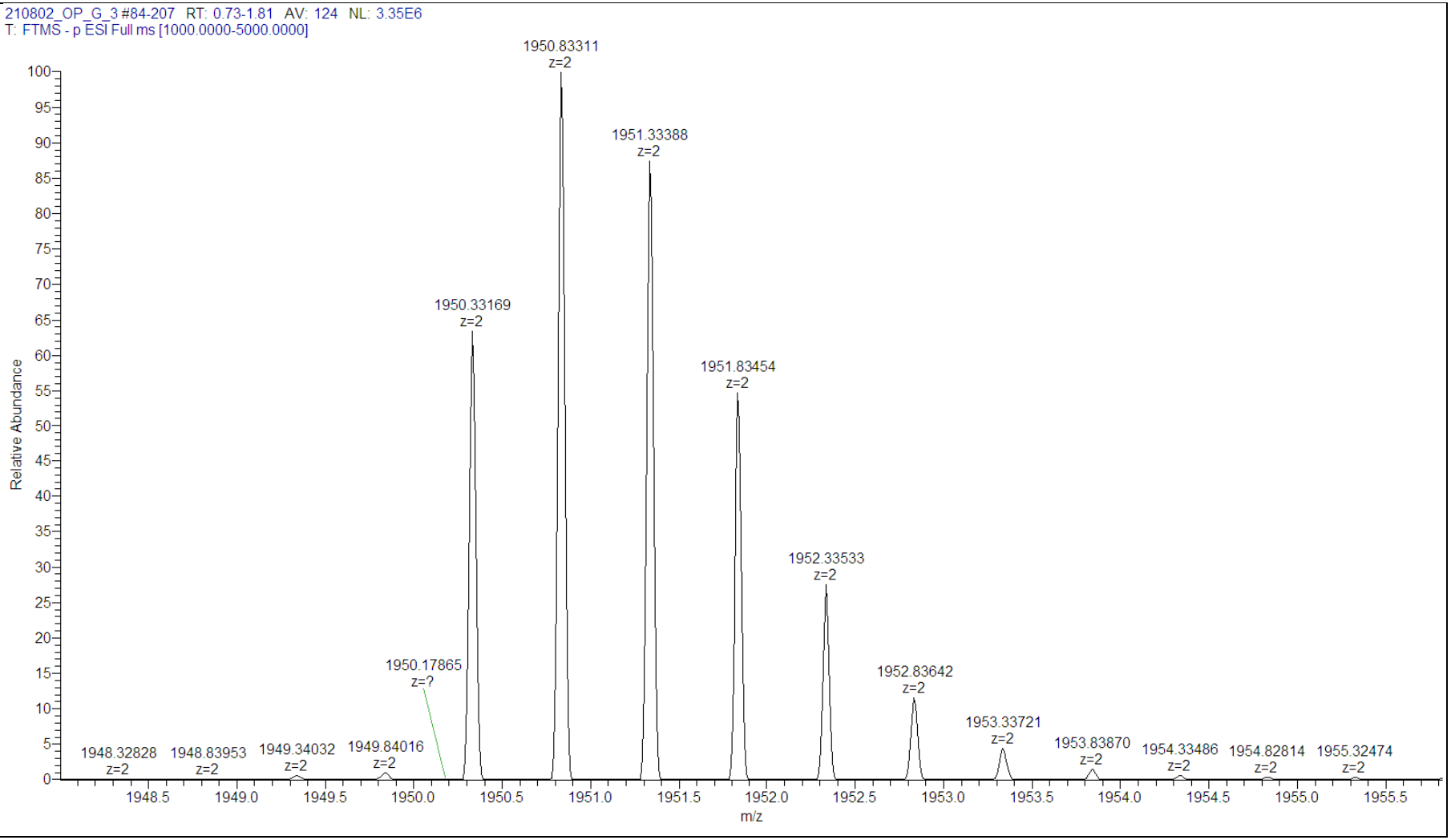
(Calc. $[M-2H]^{2-}$ $C_{120}H_{144}N_{60}O_{71}P_{11}^{2-}$ 1950.83275)

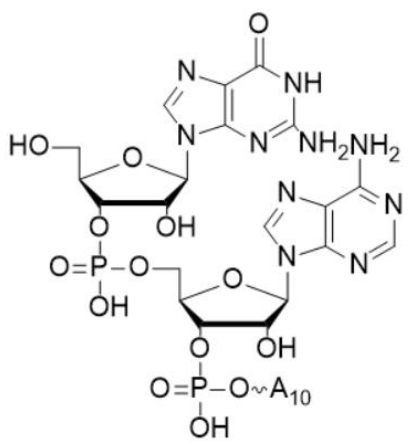
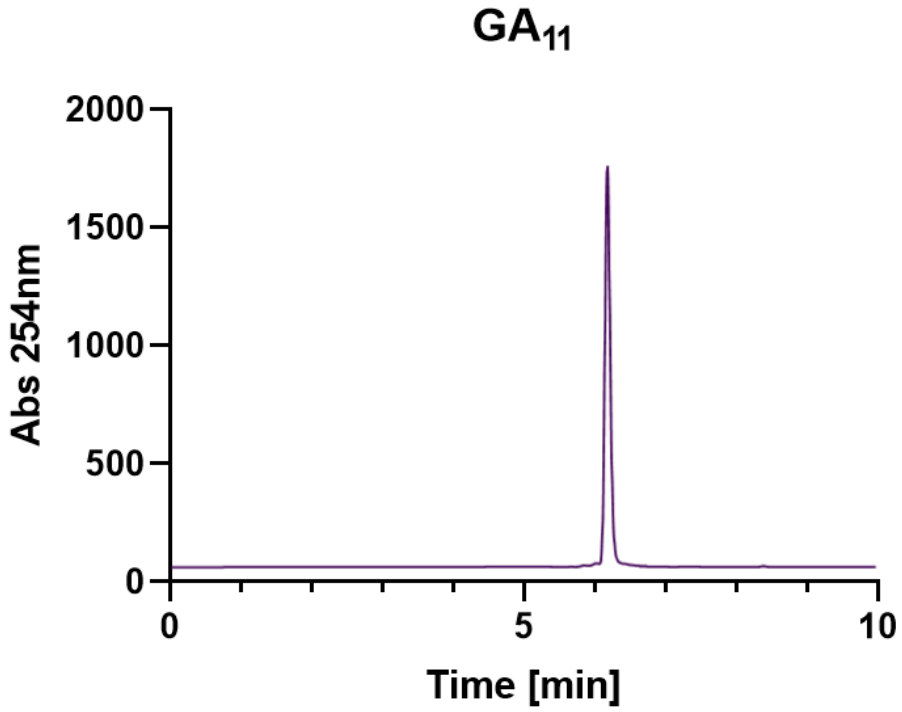


A_5GA_6 (G2)	
Chemical structure	
RP HPLC	

MS (-) ESI

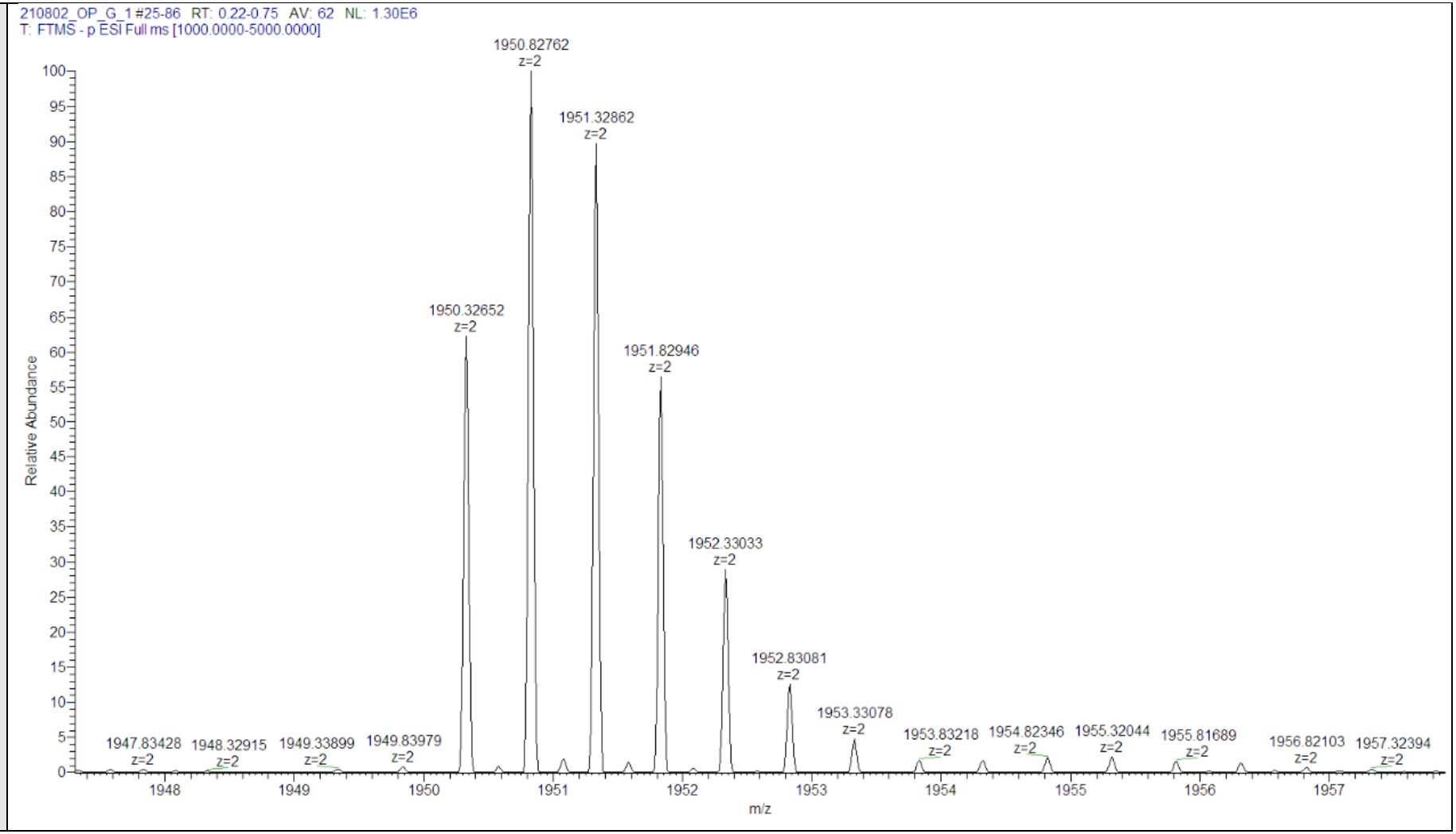
(Calc. $[M-2H]^{2-}$ $C_{120}H_{144}N_{60}O_{71}P_{11}^{2-}$ 1950.83275)

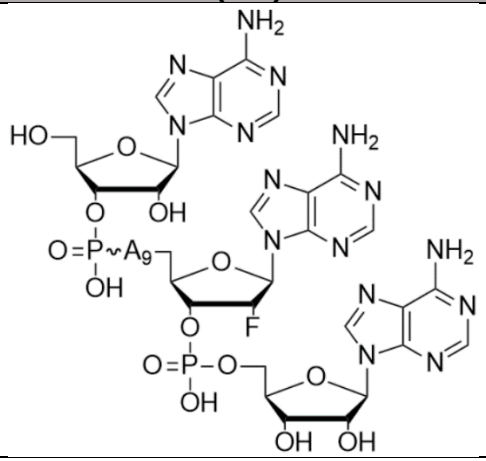
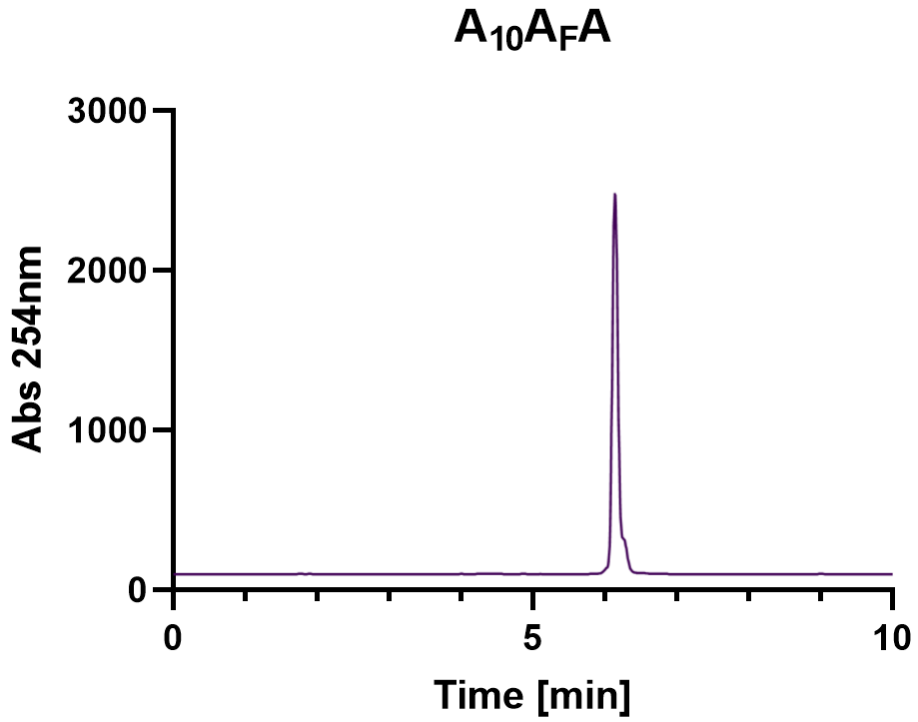


GA_{11} (G3)	
Chemical structure	
RP HPLC	

MS (-) ESI

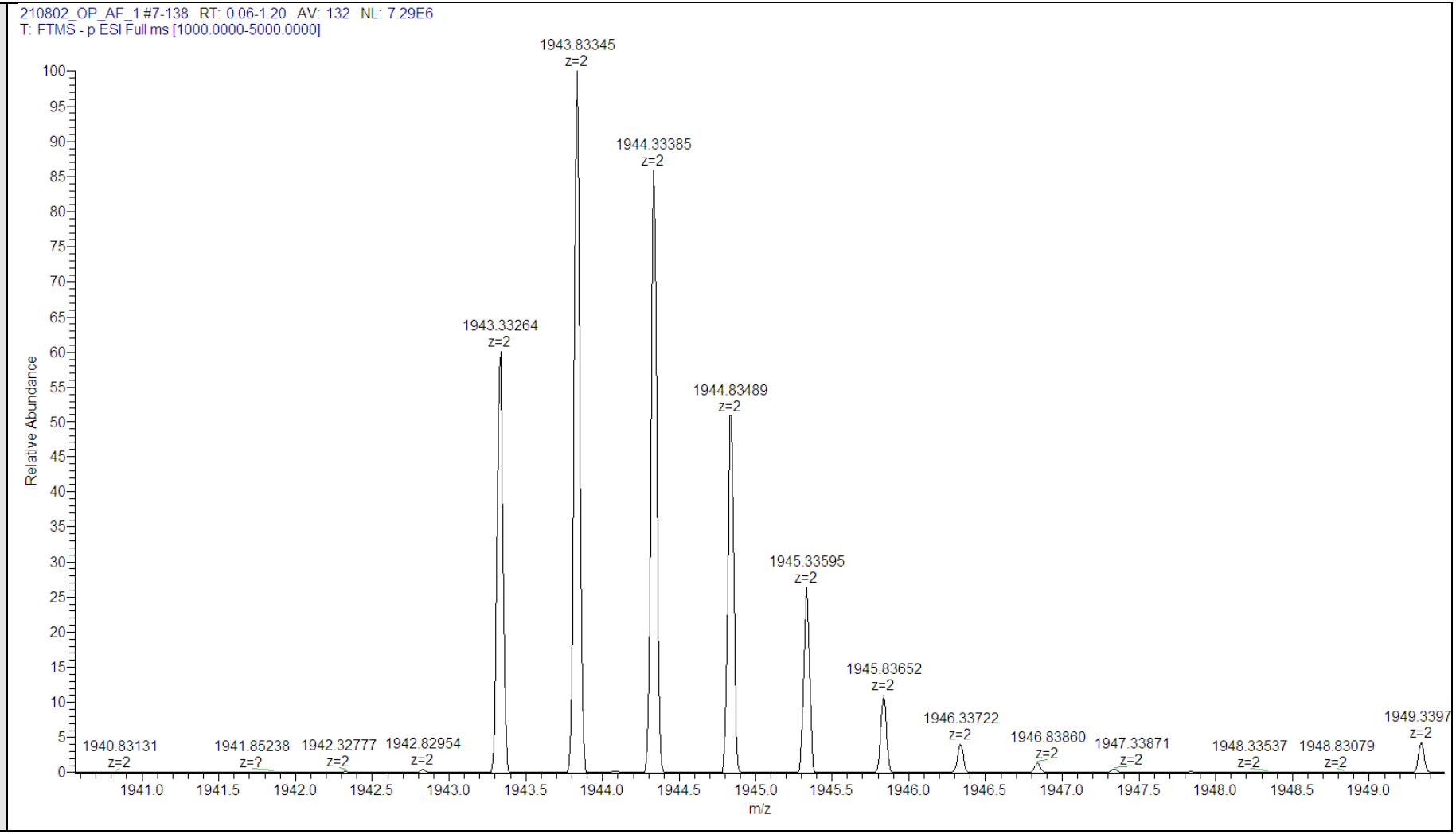
(Calc. $[M-2H]^{2-}$ $C_{120}H_{144}N_{60}O_{71}P_{11}^{2-}$ 1950.83275)

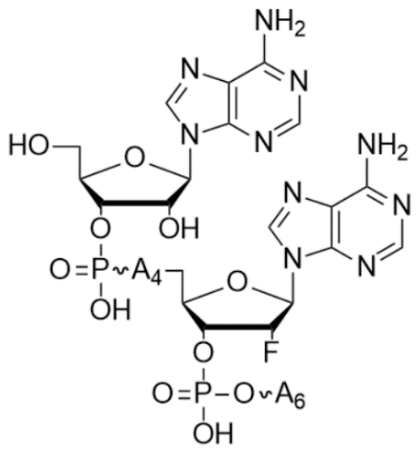
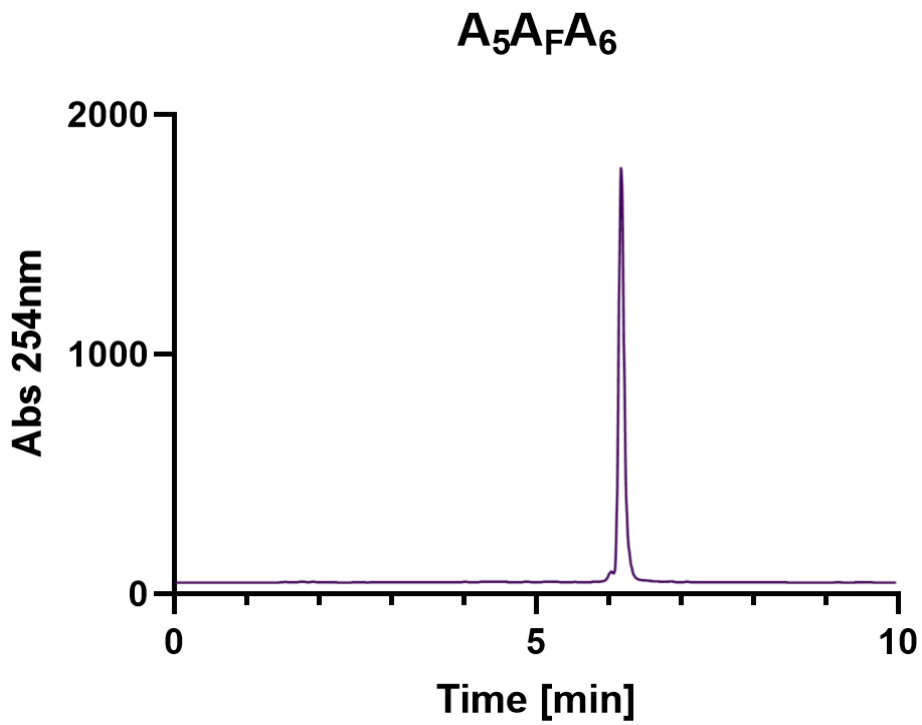


$A_{10}A_FA$ (A_F1)	
Chemical structure	
RP HPLC	

MS (-) ESI

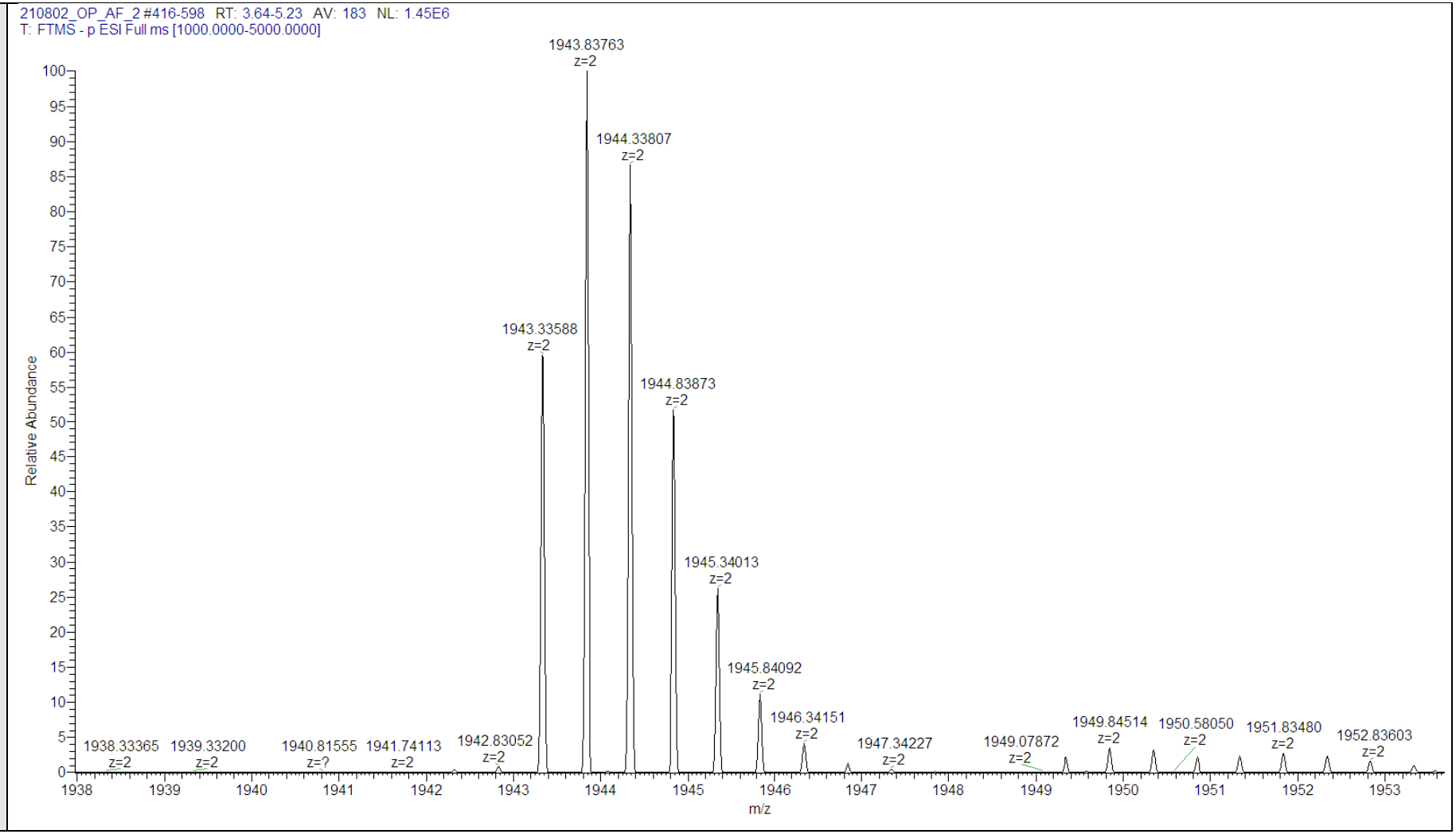
(Calc. $[M-2H]^{2-}$ $C_{120}H_{143}FN_{60}O_{69}P_{11}^{2-}$ 1943.8331)

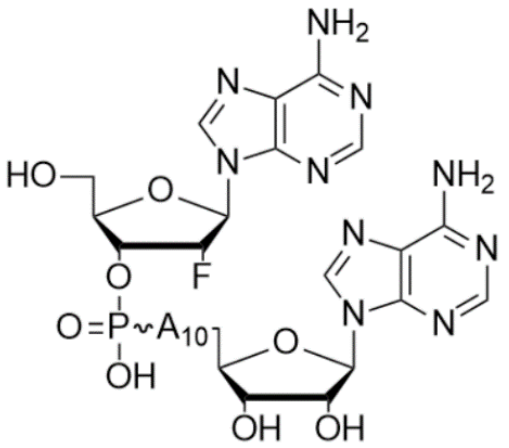
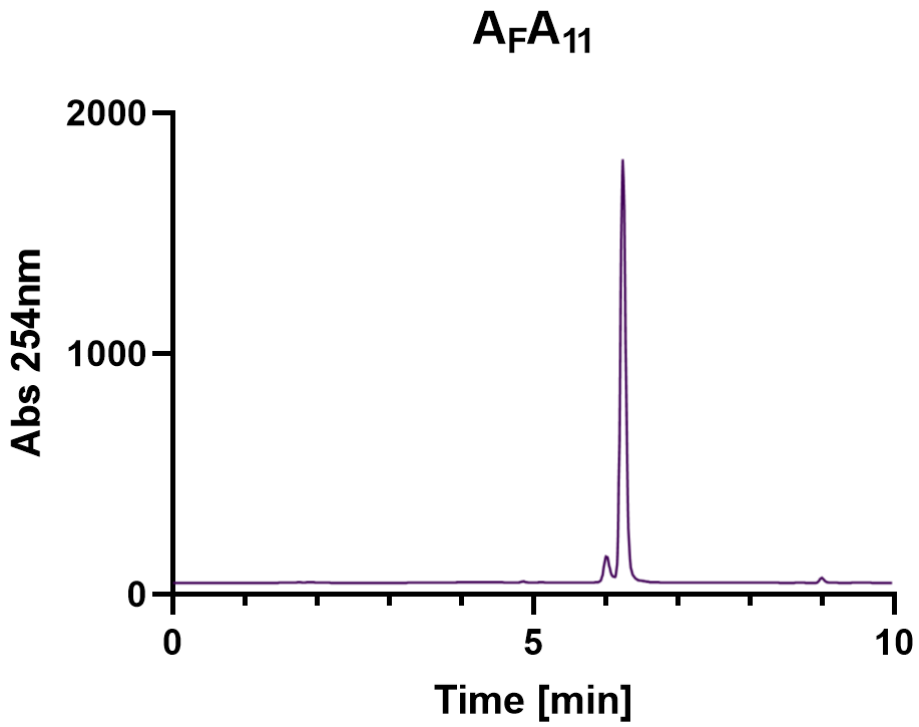


$A_5A_FA_6$ (A_F2)	
Chemical structure	
RP HPLC	

MS (-) ESI

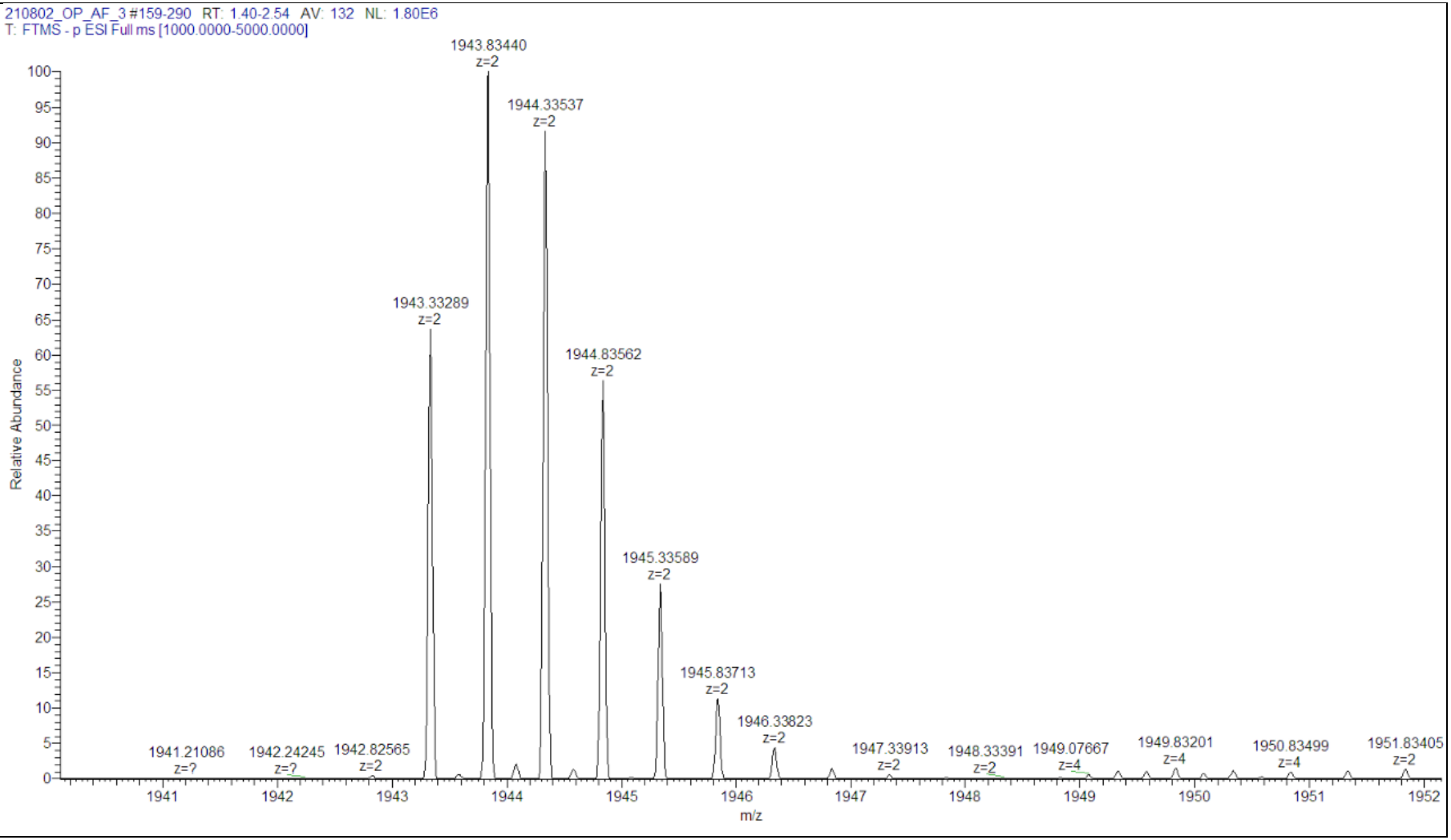
(Calc. $[M-2H]^{2-}$ $C_{120}H_{143}FN_{60}O_{69}P_{11}^{2-}$ 1943.8331)

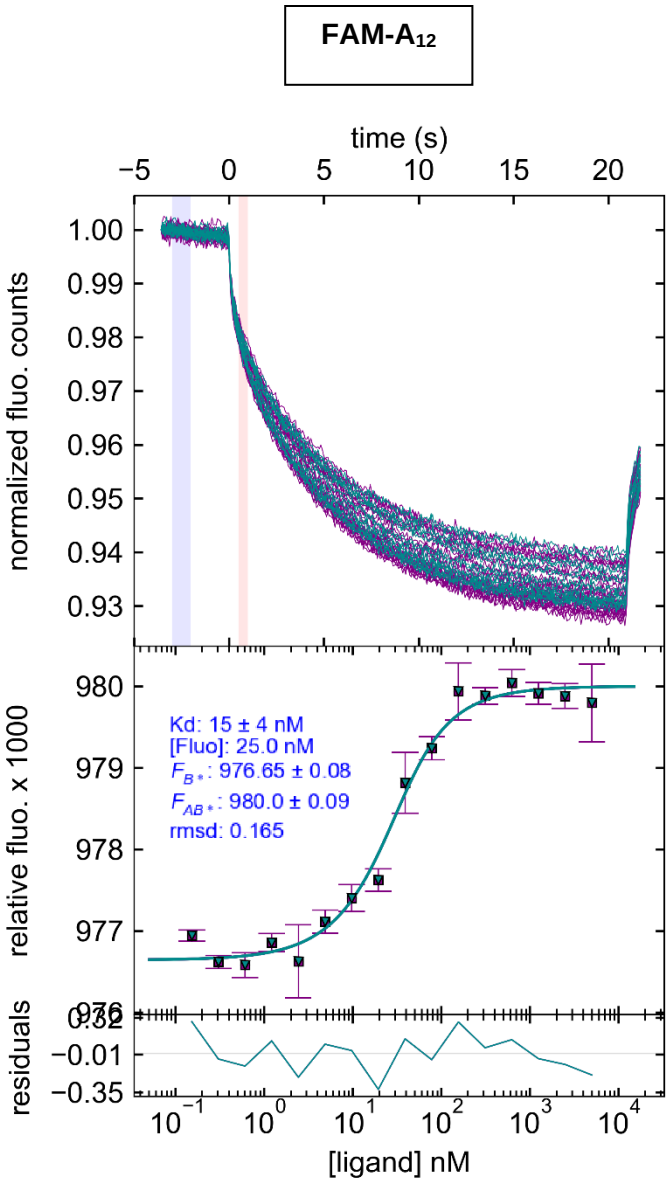


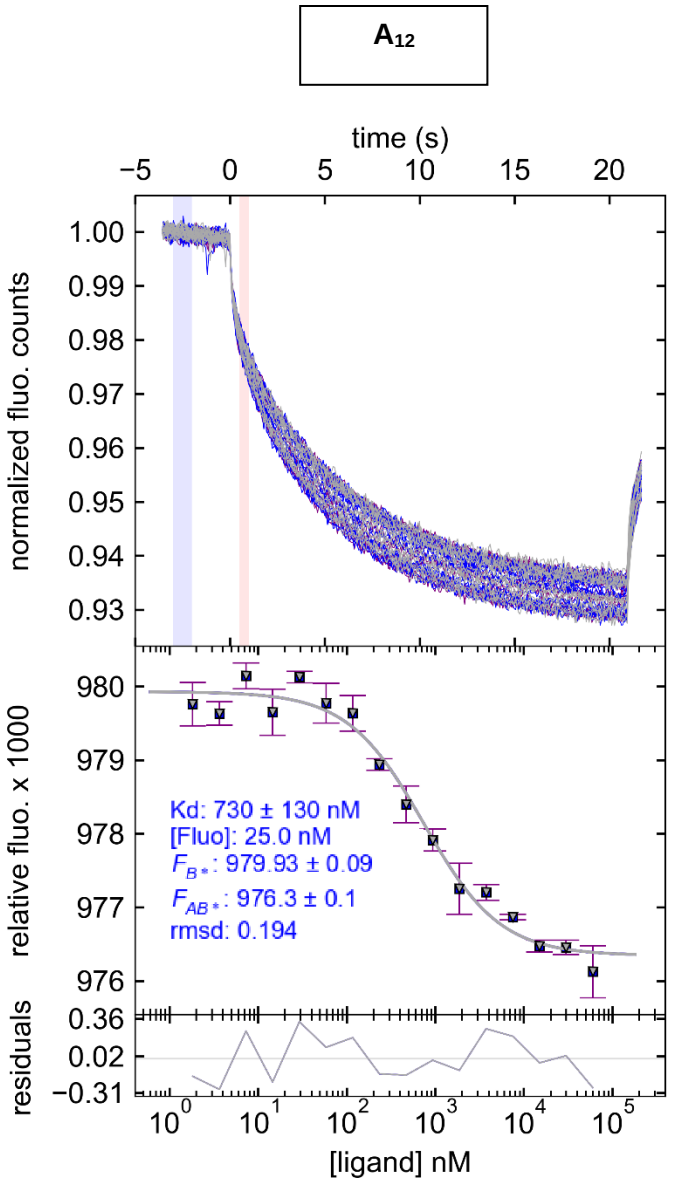
A_FA_{11} (A_F3)	
Chemical structure	
RP HPLC	

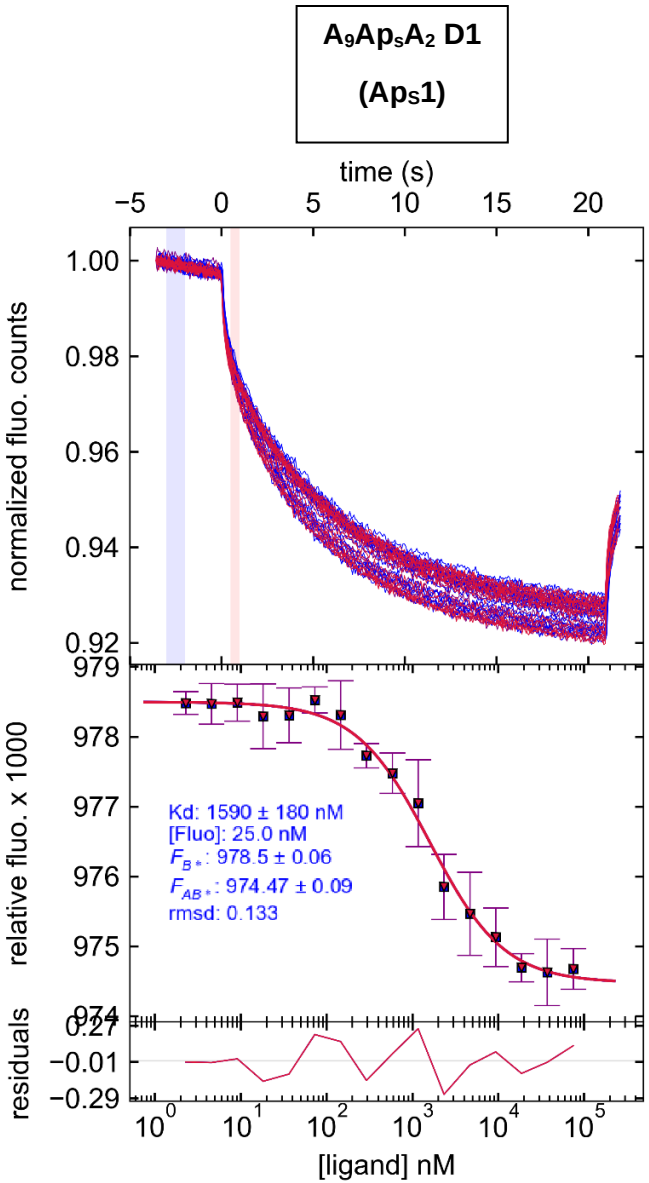
MS (-) ESI

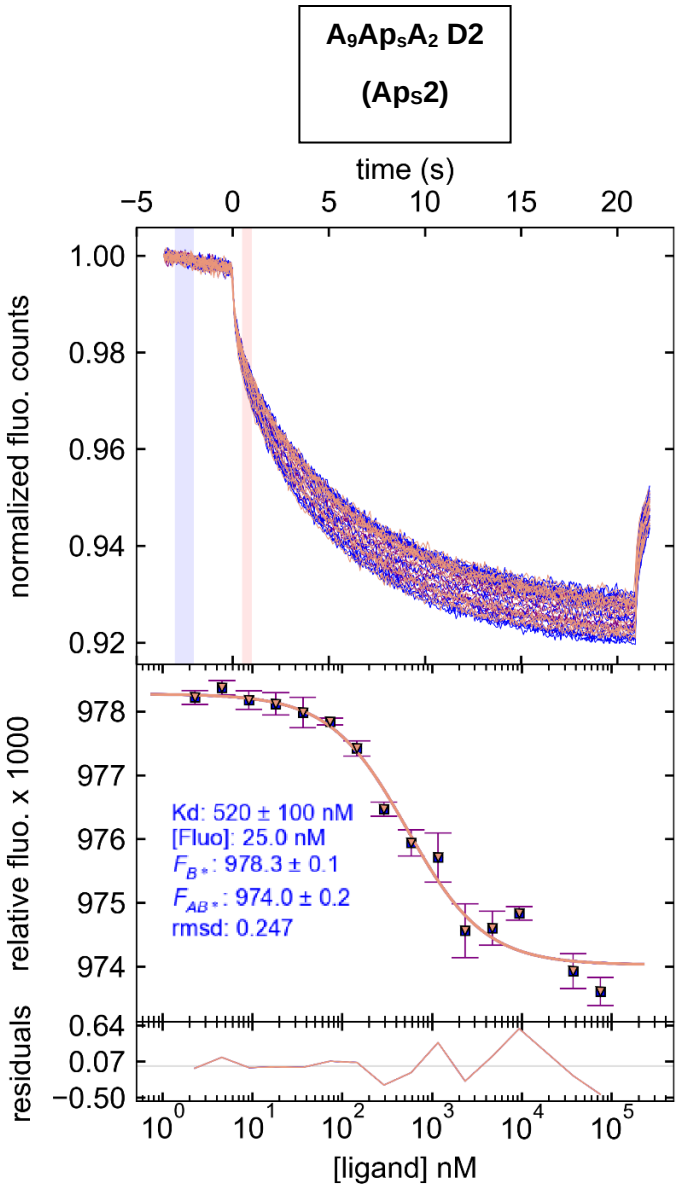
(Calc. $[M-2H]^{2-}$ $C_{120}H_{143}FN_{60}O_{69}P_{11}^{2-}$ 1943.8331)

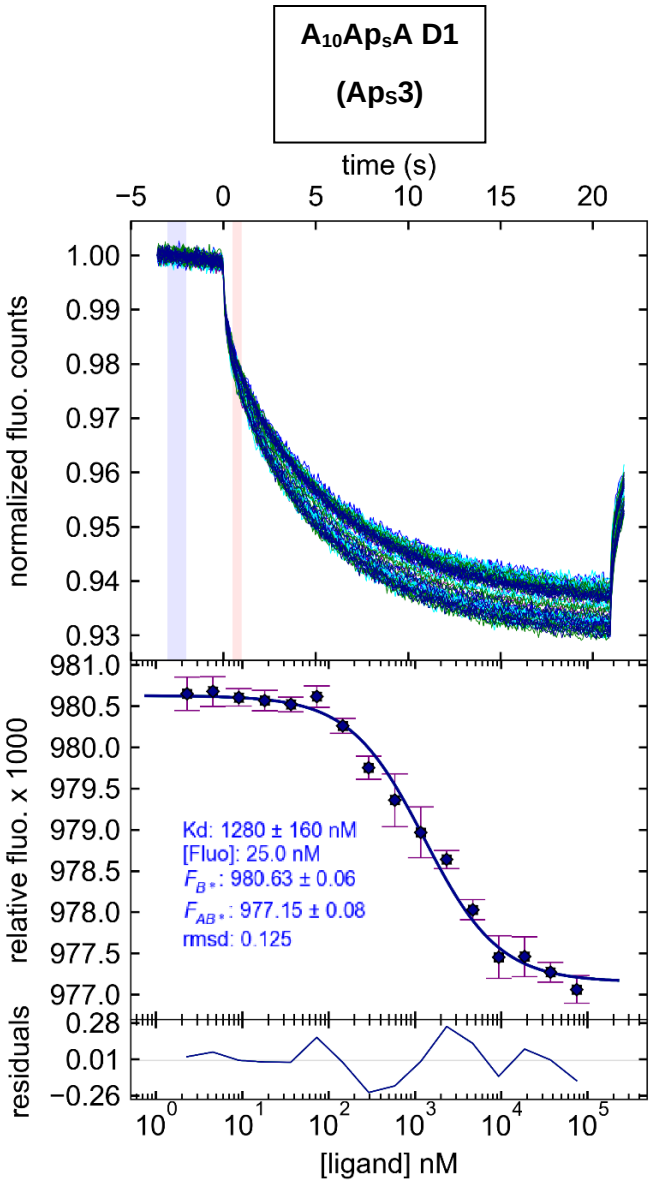


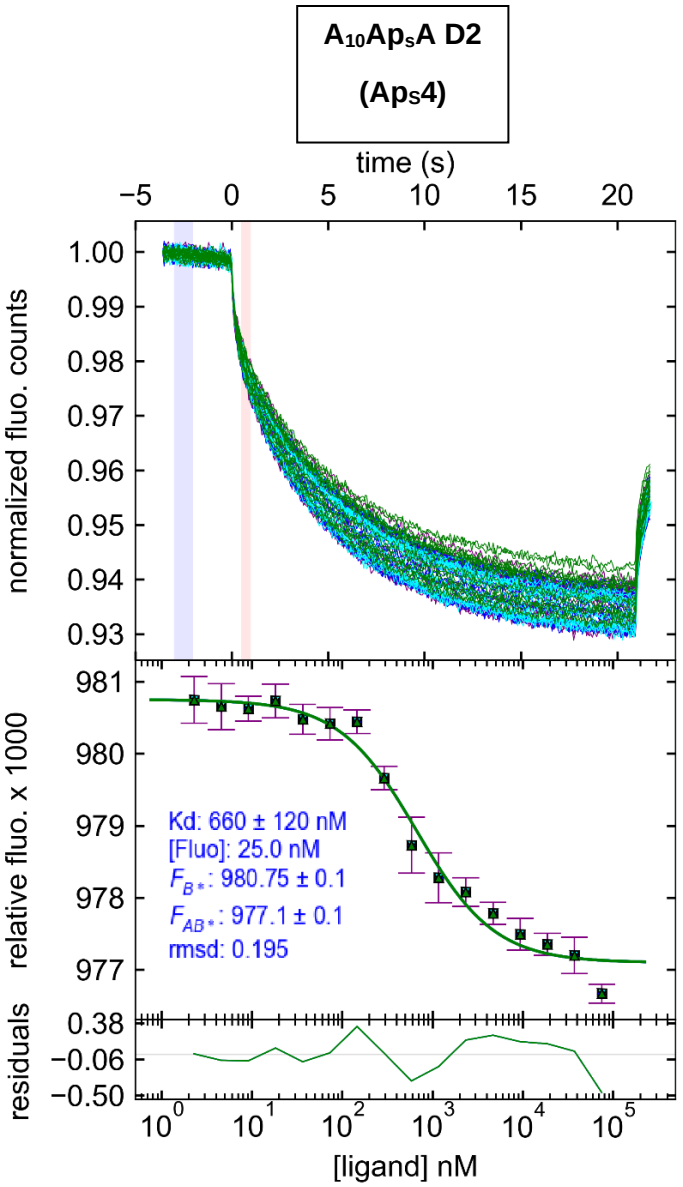

FAM-A12
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 15 ± 4 nM
[Fluo]: 25.0 nM
F_B*: 976.65 ± 0.08
F_AB*: 980.0 ± 0.09
rmsd: 0.165
[ligand] nM

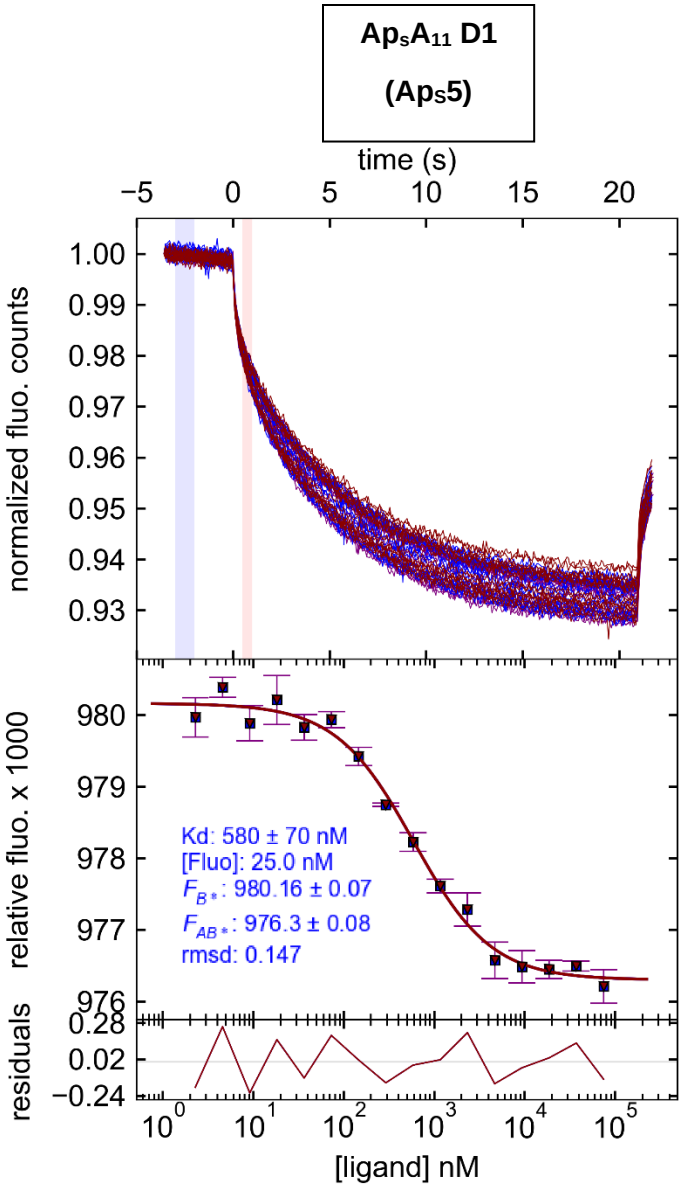

A12
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 730 ± 130 nM
[Fluo]: 25.0 nM
F_B*: 979.93 ± 0.09
F_AB*: 976.3 ± 0.1
rmsd: 0.194
[ligand] nM

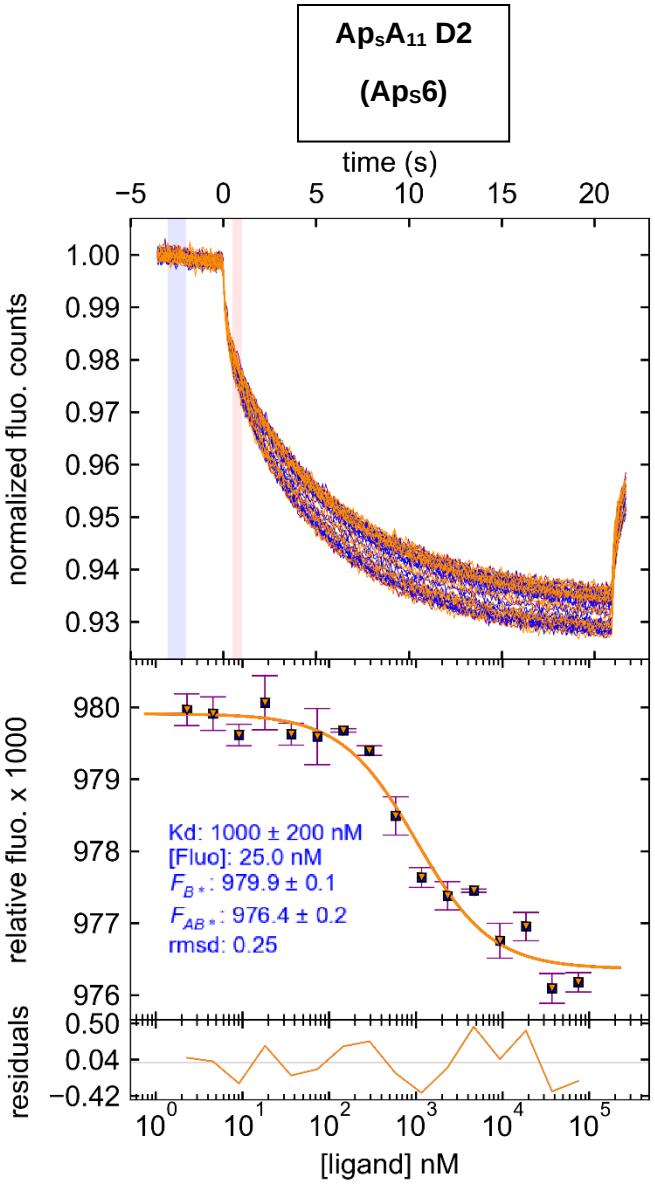

A9ApsA2 D1
(Aps1)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 1590 ± 180 nM
[Fluo]: 25.0 nM
FB*: 978.5 ± 0.06
FAB*: 974.47 ± 0.09
rmsd: 0.133
[ligand] nM

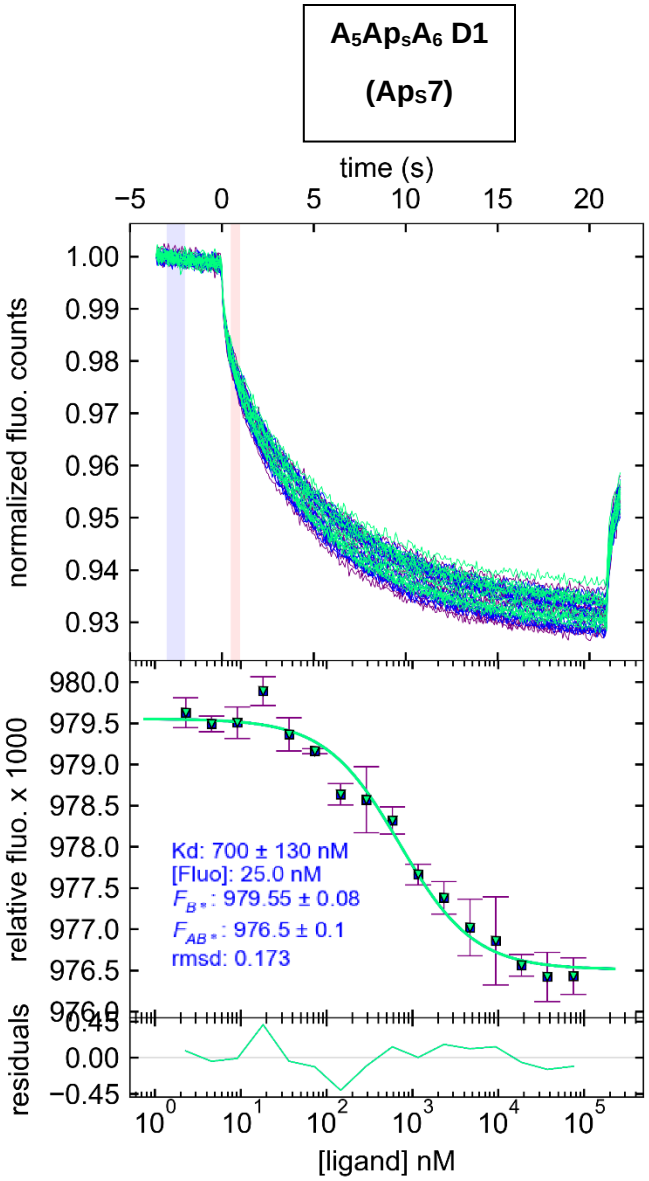

A9ApsA2 D2
(Aps2)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 520 ± 100 nM
[Fluo]: 25.0 nM
FB*: 978.3 ± 0.1
FAB*: 974.0 ± 0.2
rmsd: 0.247
[ligand] nM

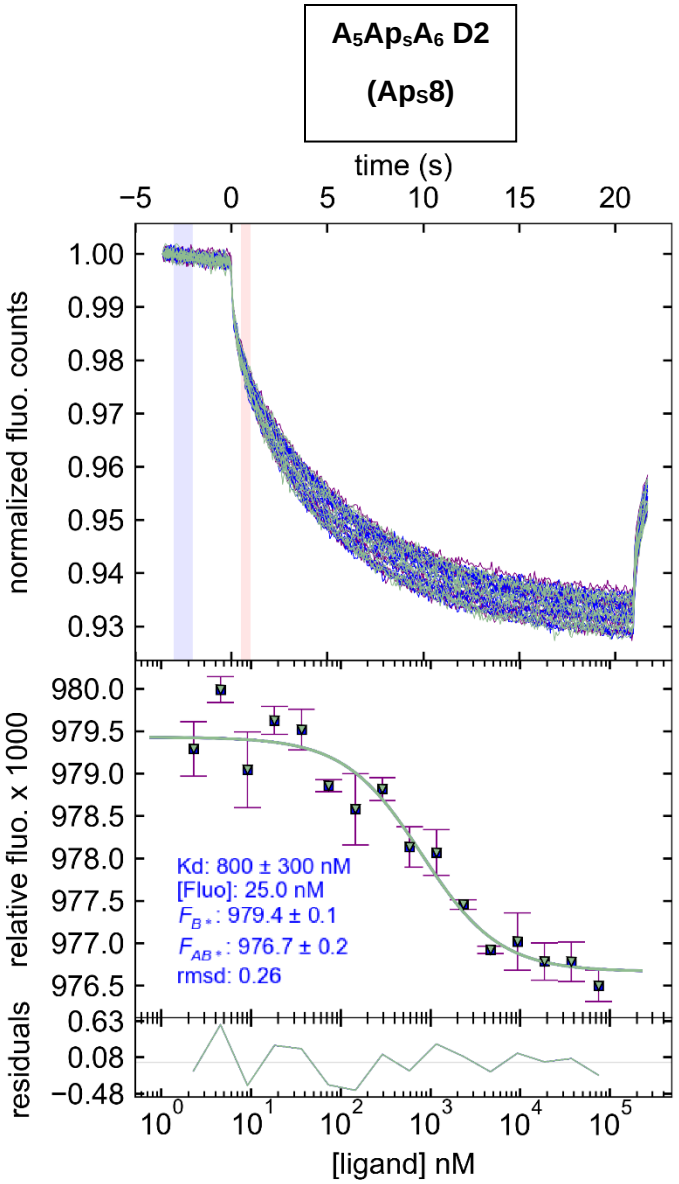

A10ApsA D1
(Aps3)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 1280 ± 160 nM
[Fluo]: 25.0 nM
FB*: 980.63 ± 0.06
FAB*: 977.15 ± 0.08
rmsd: 0.125
[ligand] nM

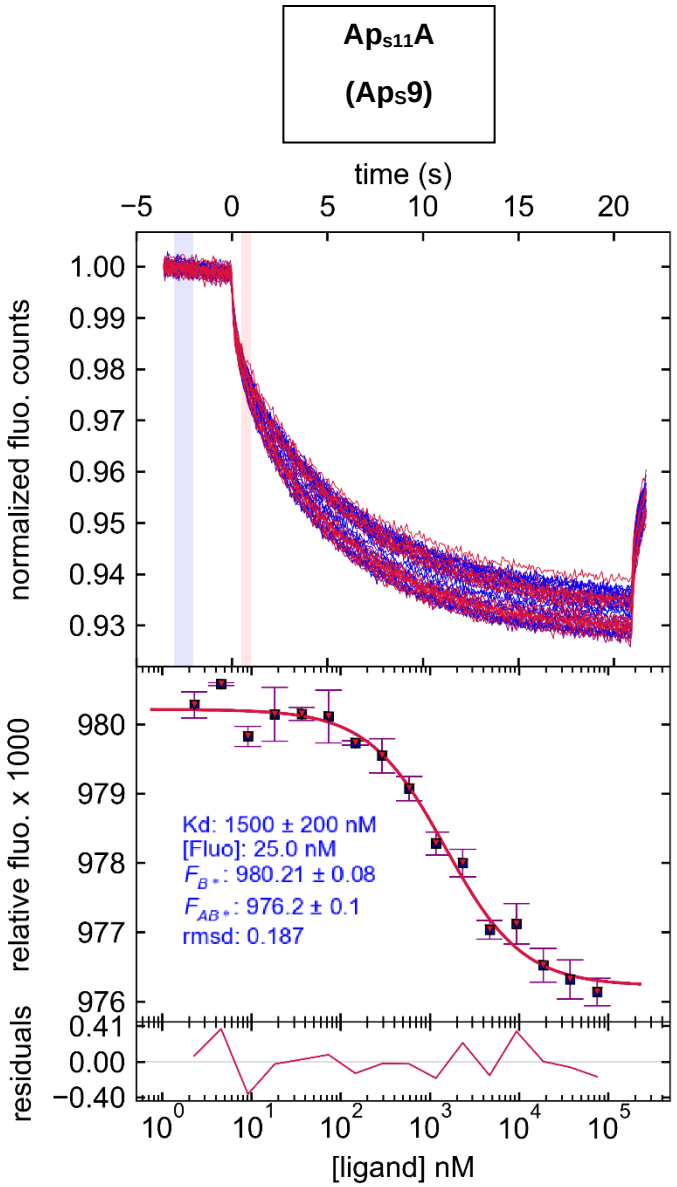

A10ApsA D2
(Aps4)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 660 ± 120 nM
[Fluo]: 25.0 nM
FB*: 980.75 ± 0.1
FAB*: 977.1 ± 0.1
rmsd: 0.195
[ligand] nM

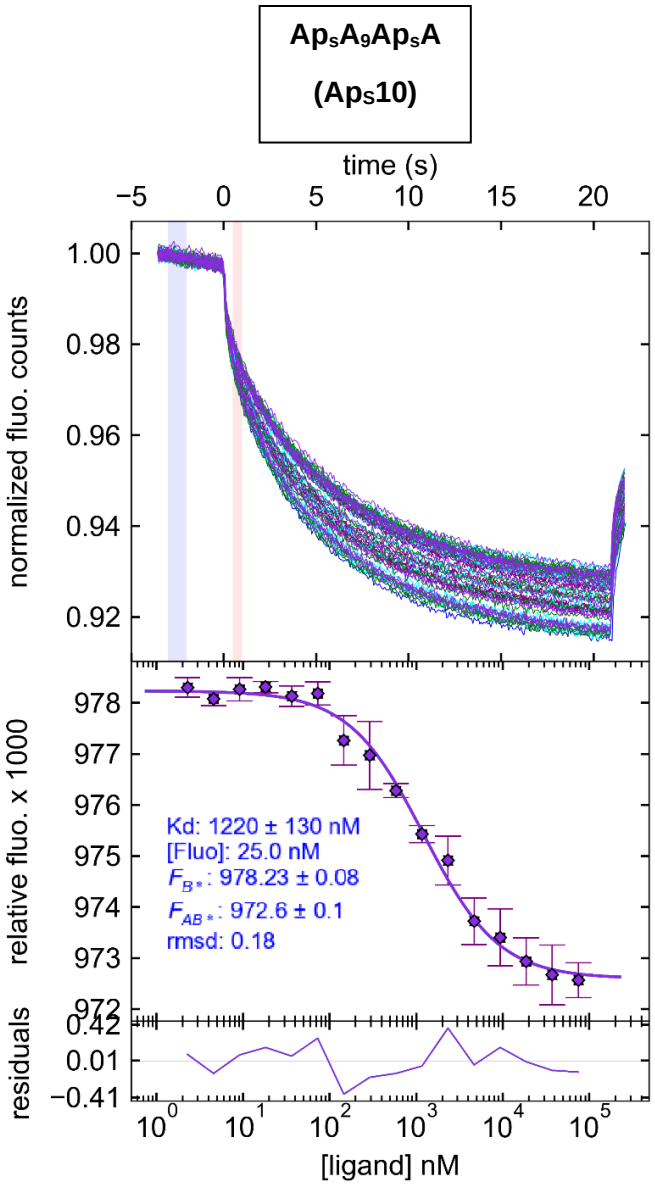

ApsA11 D1
(Aps5)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 580 ± 70 nM
[Fluo]: 25.0 nM
F_{B*}: 980.16 ± 0.07
F_{AB*}: 976.3 ± 0.08
rmsd: 0.147
[ligand] nM

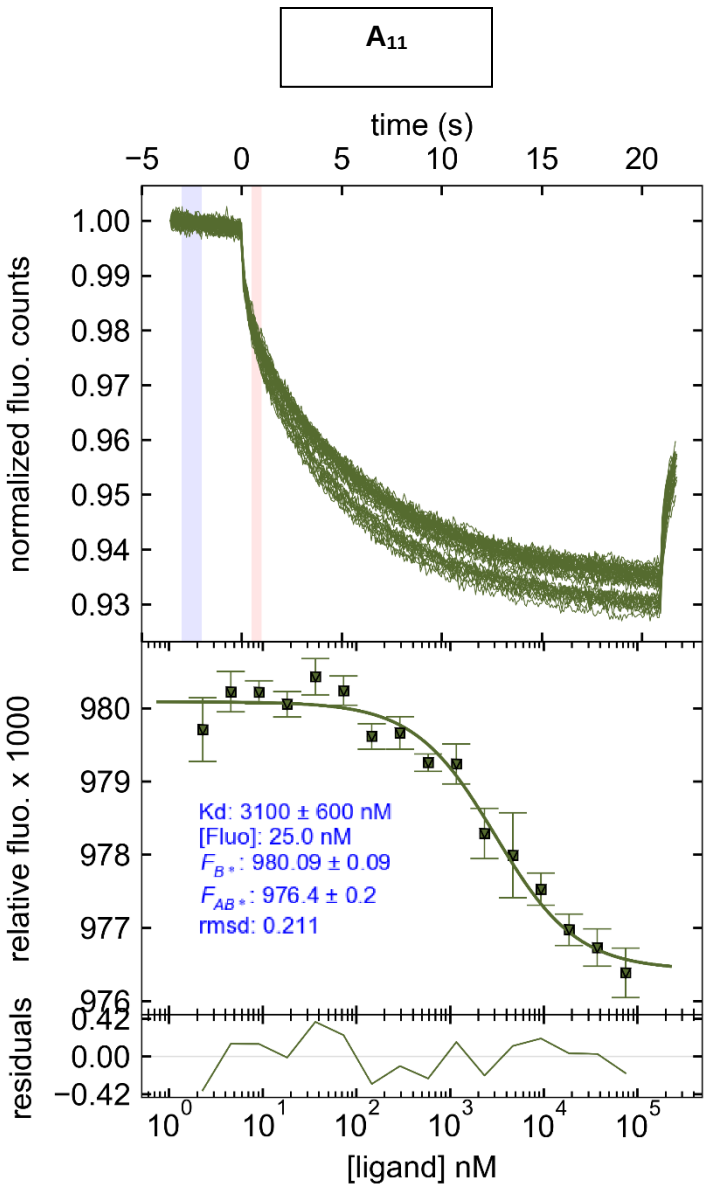

ApsA11 D2
(Aps6)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 1000 ± 200 nM
[Fluo]: 25.0 nM
F_{B*}: 979.9 ± 0.1
F_{AB*}: 976.4 ± 0.2
rmsd: 0.25
[ligand] nM

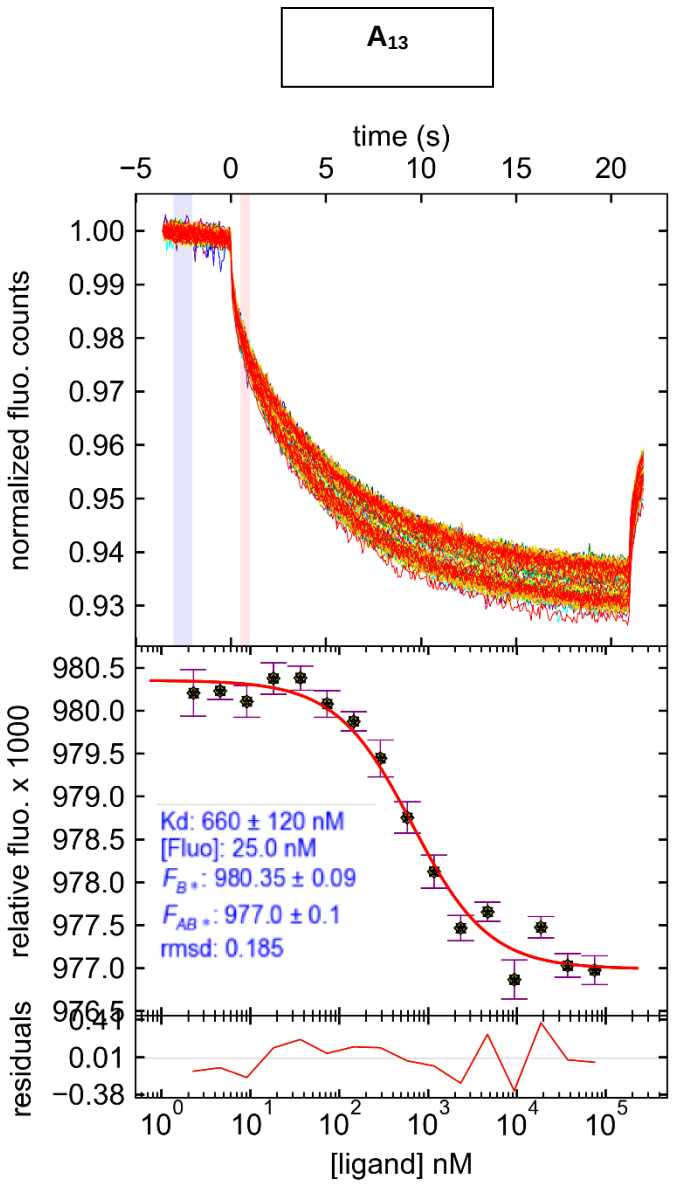

A5ApsA6 D1
(Aps7)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 700 ± 130 nM
[Fluo]: 25.0 nM
FB*: 979.55 ± 0.08
FAB*: 976.5 ± 0.1
rmsd: 0.173
[ligand] nM

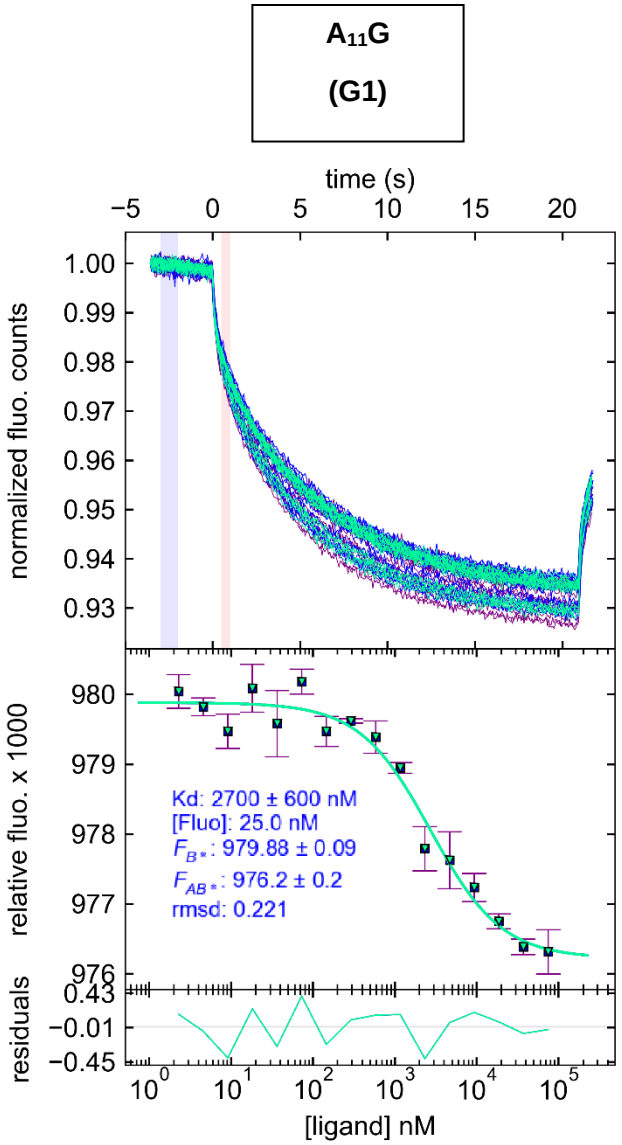

A5ApsA6 D2
(Aps8)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 800 ± 300 nM
[Fluo]: 25.0 nM
FB*: 979.4 ± 0.1
FAB*: 976.7 ± 0.2
rmsd: 0.26
[ligand] nM

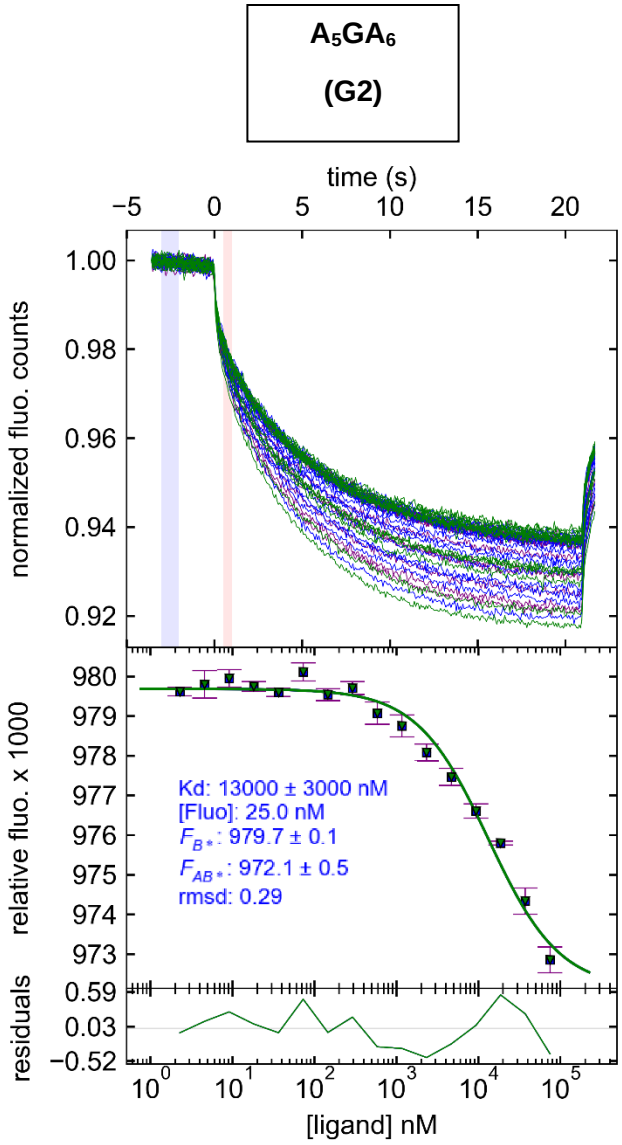

Aps11A
(ApS9)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 1500 ± 200 nM
[Fluo]: 25.0 nM
FB*: 980.21 ± 0.08
FAB*: 976.2 ± 0.1
rmsd: 0.187
[ligand] nM

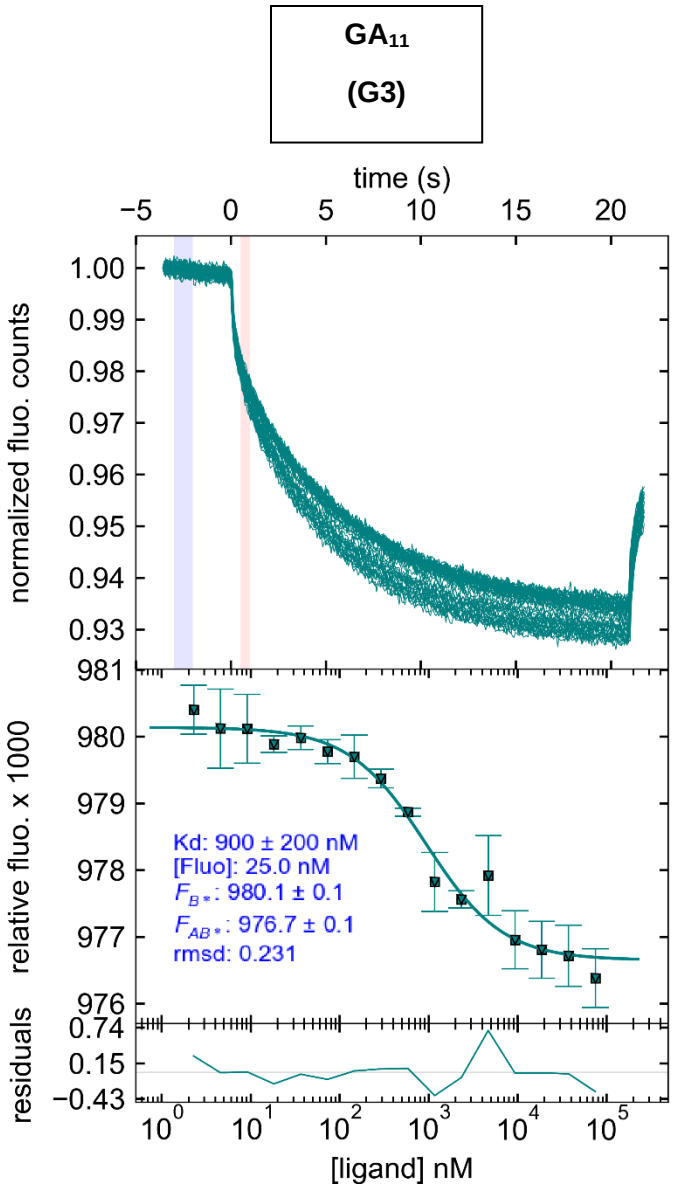

ApsA9ApsA
(ApS10)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 1220 ± 130 nM
[Fluo]: 25.0 nM
FB*: 978.23 ± 0.08
FAB*: 972.6 ± 0.1
rmsd: 0.18
[ligand] nM

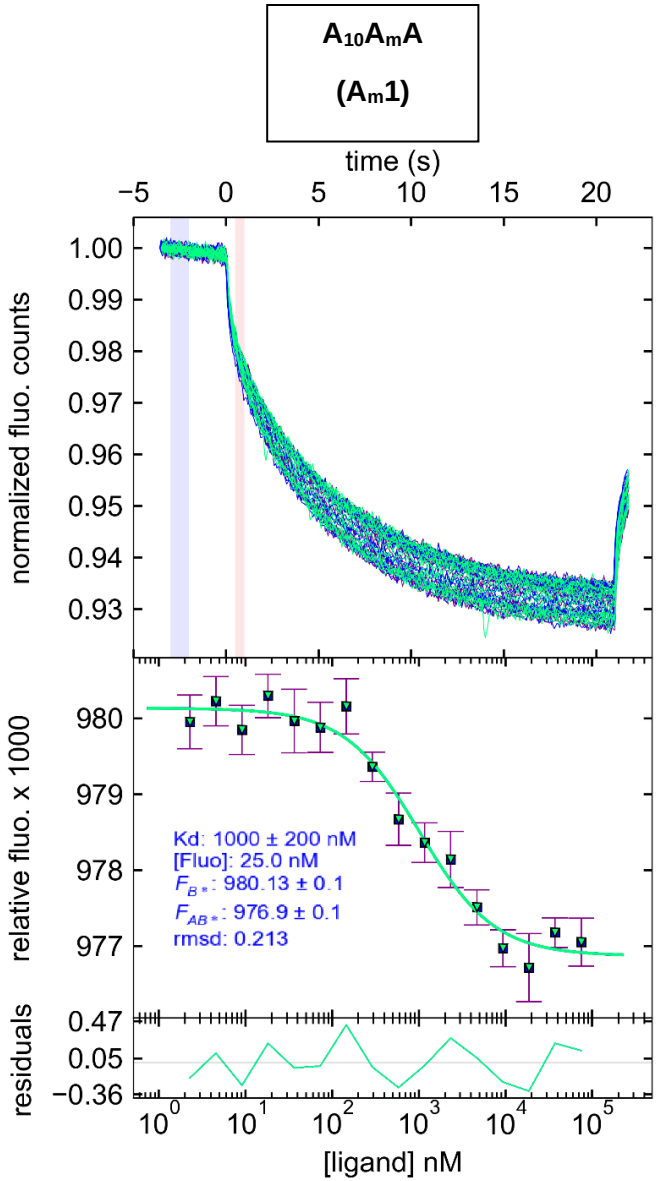

A11
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 3100 ± 600 nM
[Fluo]: 25.0 nM
F_{B*}: 980.09 ± 0.09
F_{AB*}: 976.4 ± 0.2
rmsd: 0.211
[ligand] nM

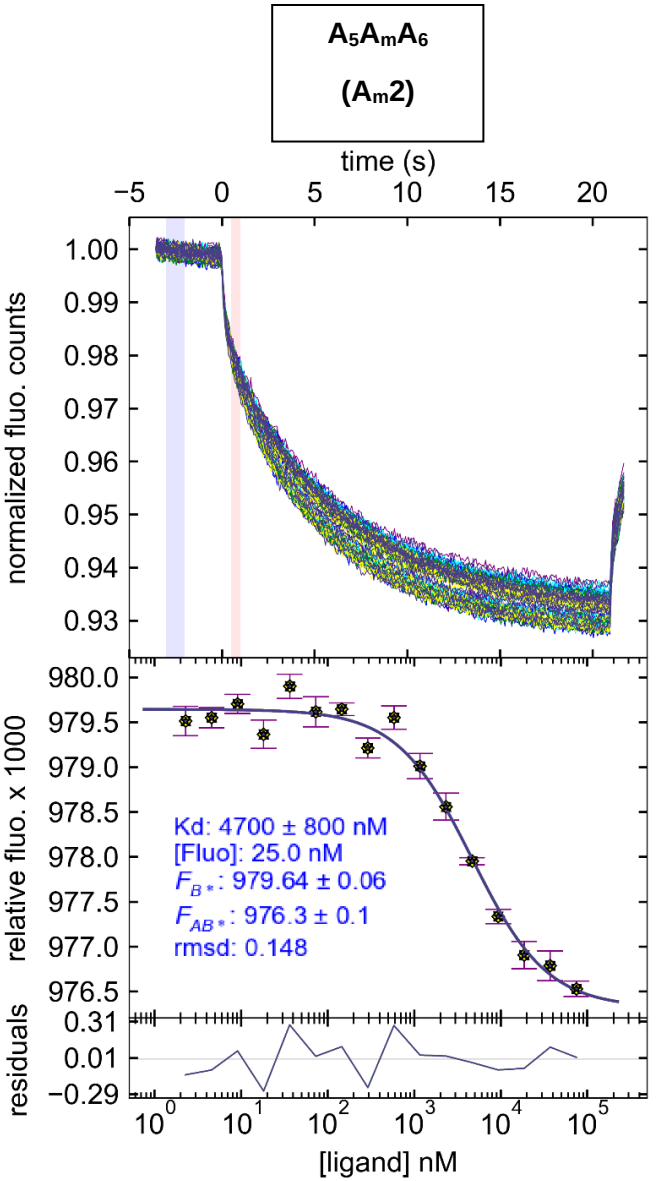

A13
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 660 ± 120 nM
[Fluo]: 25.0 nM
F_{B*}: 980.35 ± 0.09
F_{AB*}: 977.0 ± 0.1
rmsd: 0.185
[ligand] nM

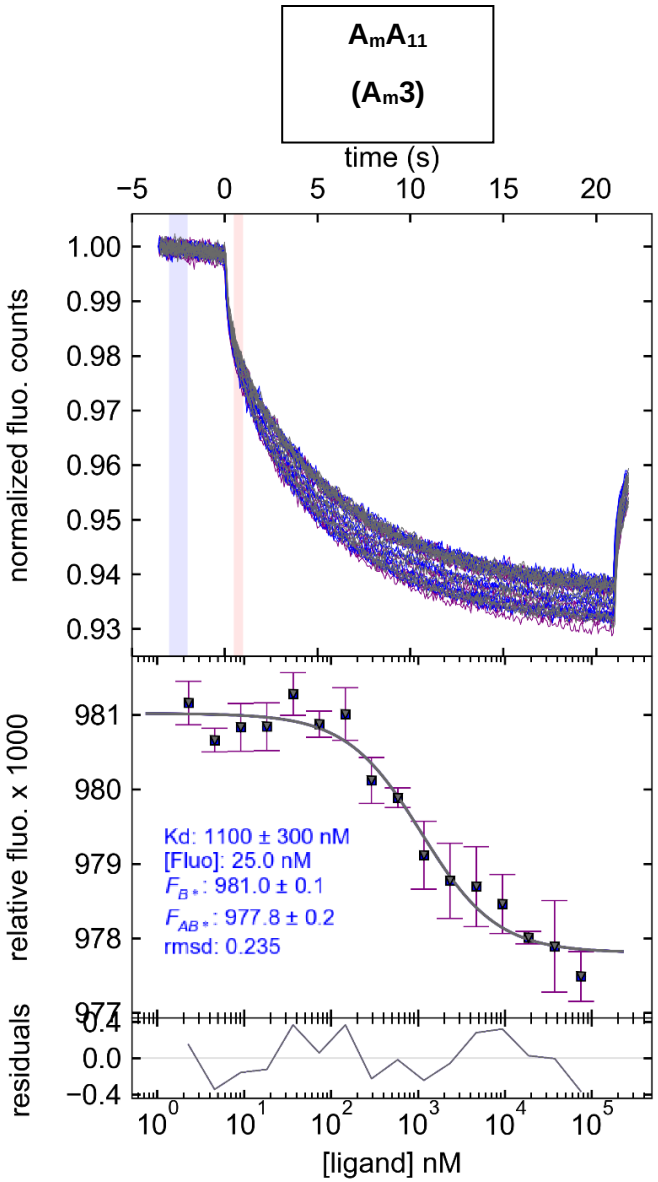

A11G
(G1)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 2700 ± 600 nM
[Fluo]: 25.0 nM
FB*: 979.88 ± 0.09
FAB*: 976.2 ± 0.2
rmsd: 0.221
[ligand] nM

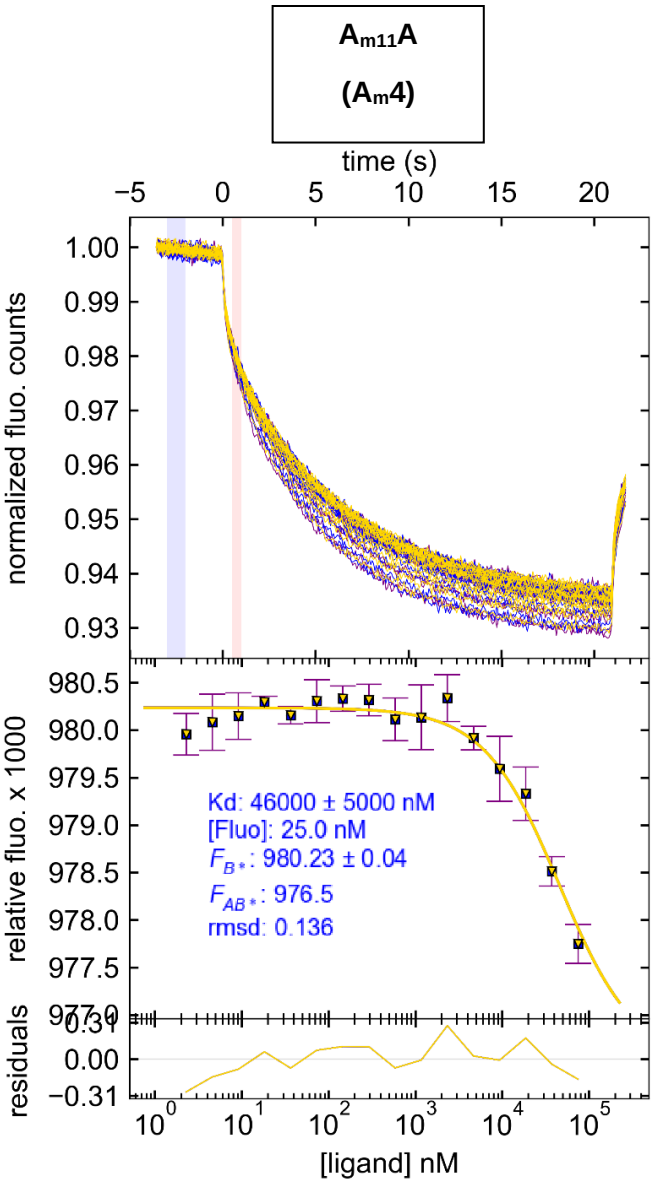

A5GA6
(G2)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 13000 ± 3000 nM
[Fluo]: 25.0 nM
FB*: 979.7 ± 0.1
FAB*: 972.1 ± 0.5
rmsd: 0.29
[ligand] nM

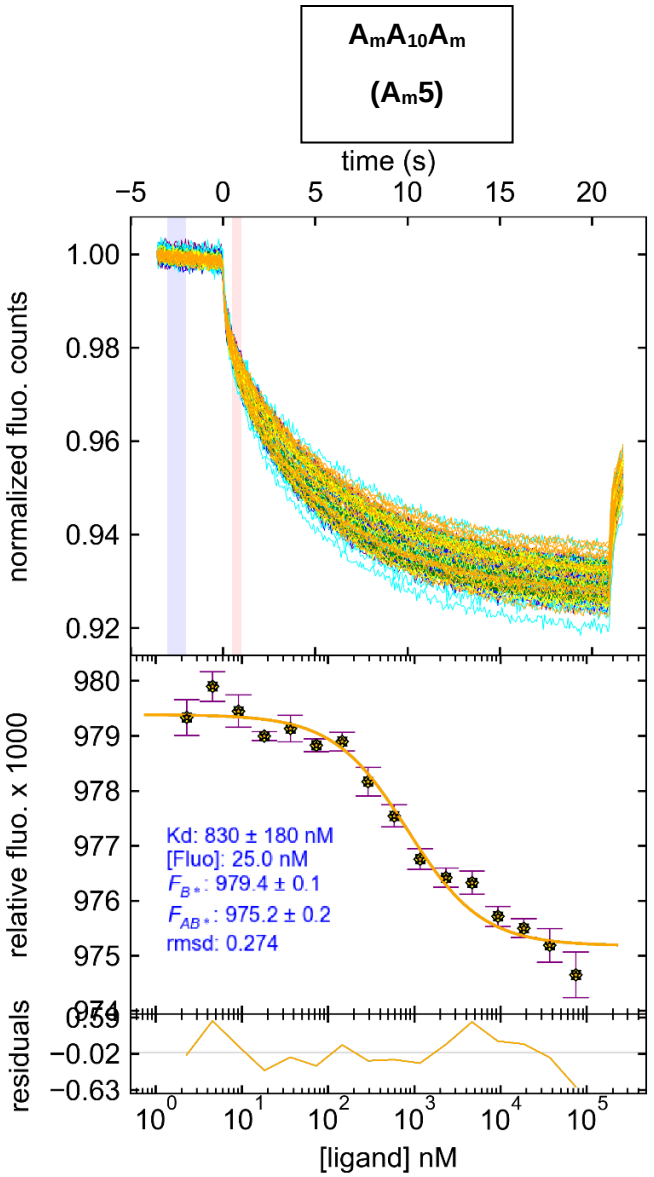

GA11
(G3)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 900 ± 200 nM
[Fluo]: 25.0 nM
F_{B*}: 980.1 ± 0.1
F_{AB*}: 976.7 ± 0.1
rmsd: 0.231
[ligand] nM

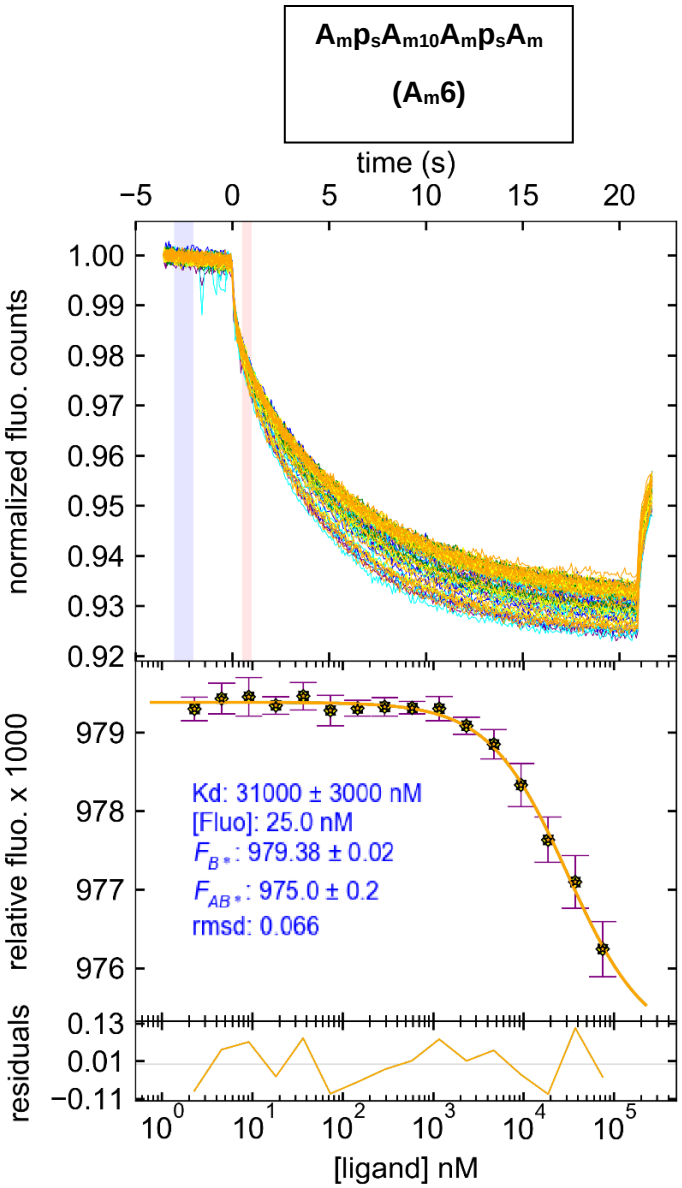

A10AmA
(Am1)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 1000 ± 200 nM
[Fluo]: 25.0 nM
FB*: 980.13 ± 0.1
FAB*: 976.9 ± 0.1
rmsd: 0.213
[ligand] nM

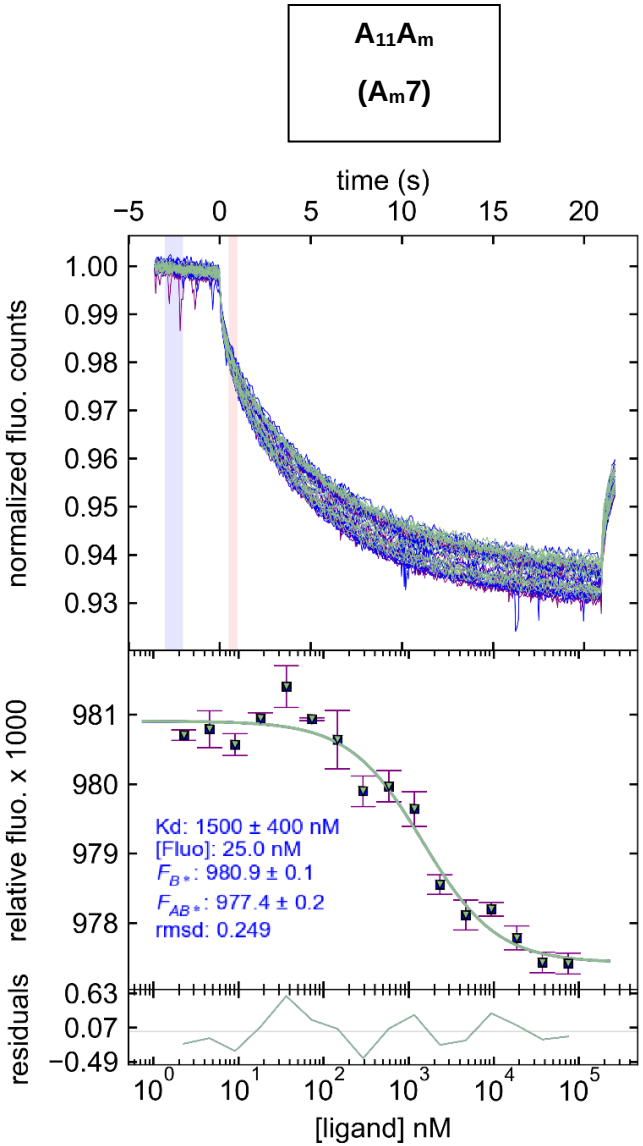

A5AmA6
(Am2)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 4700 ± 800 nM
[Fluo]: 25.0 nM
FB*: 979.64 ± 0.06
FAB*: 976.3 ± 0.1
rmsd: 0.148
[ligand] nM

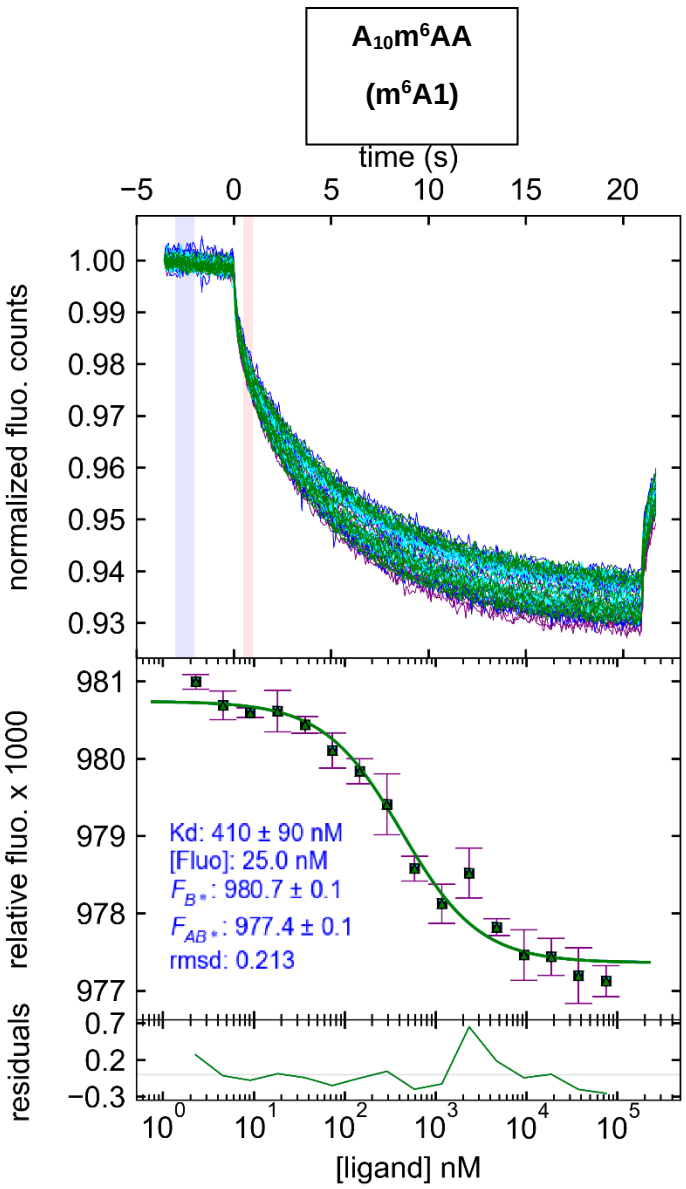

A_mA_{11}
(A_m3)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 1100 ± 300 nM
[Fluo]: 25.0 nM
F_{B*}: 981.0 ± 0.1
F_{AB*}: 977.8 ± 0.2
rmsd: 0.235
[ligand] nM

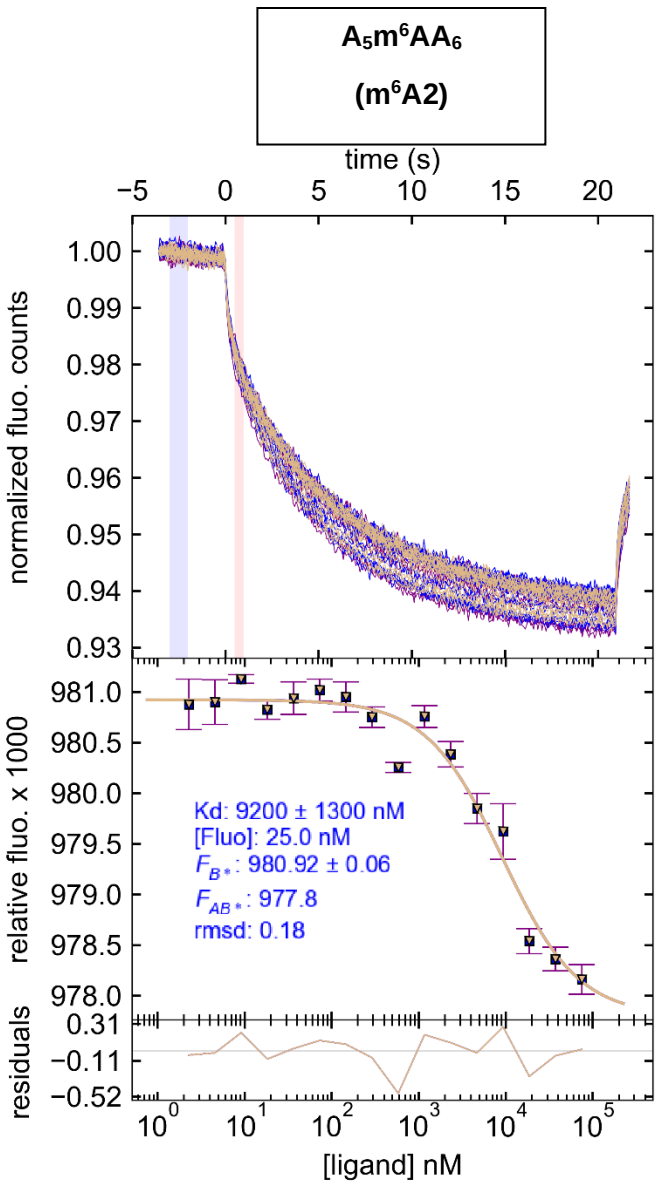

$A_{m11}A$
(A_m4)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 46000 ± 5000 nM
[Fluo]: 25.0 nM
F_{B*}: 980.23 ± 0.04
F_{AB*}: 976.5
rmsd: 0.136
[ligand] nM

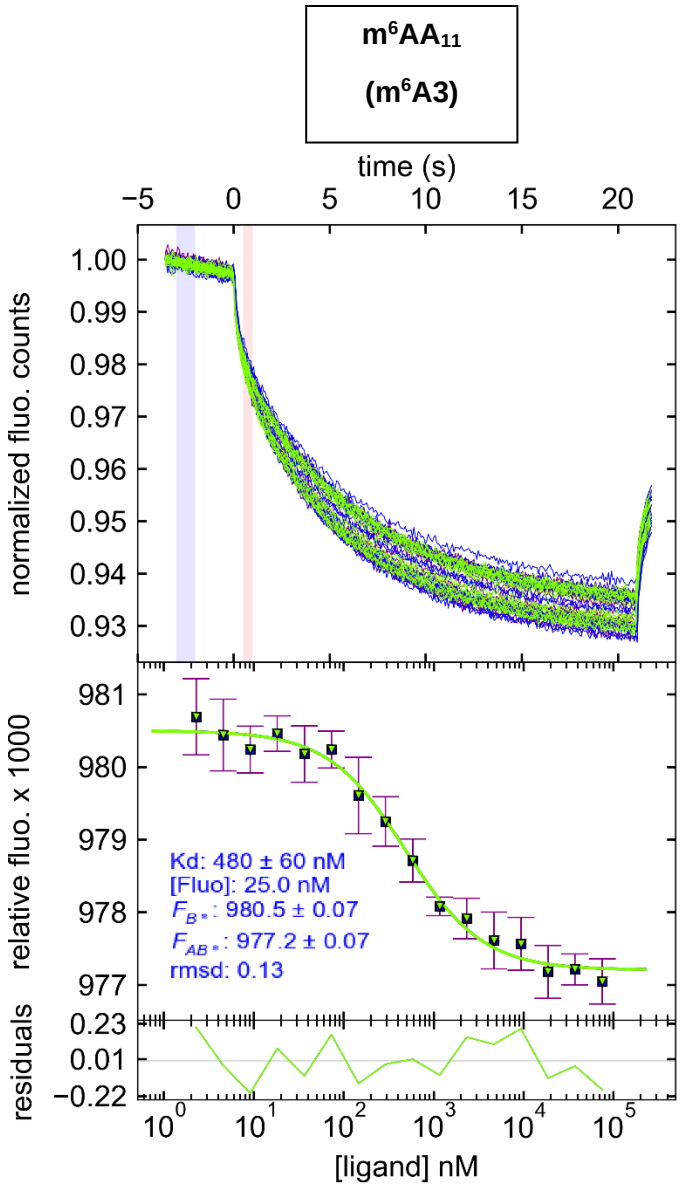

AmA10Am
(Am5)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 830 ± 180 nM
[Fluo]: 25.0 nM
FB*: 979.4 ± 0.1
FAB*: 975.2 ± 0.2
rmsd: 0.274
[ligand] nM

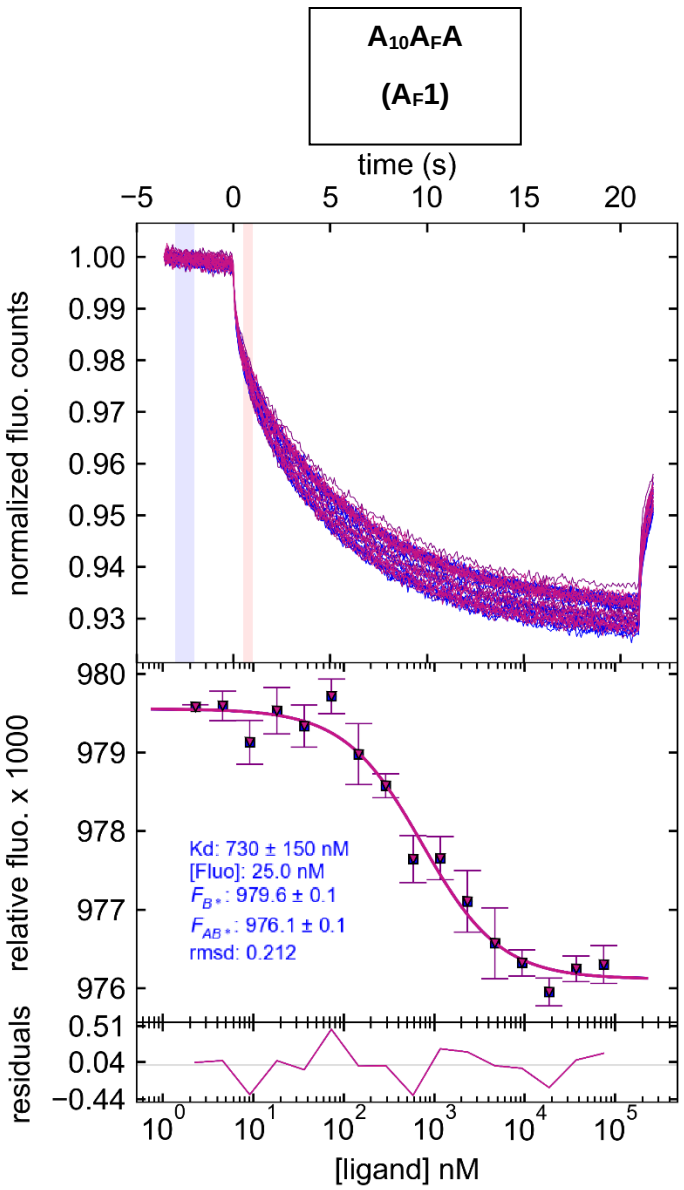

AmpsAm10AmpsAm
(Am6)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 31000 ± 3000 nM
[Fluo]: 25.0 nM
FB*: 979.38 ± 0.02
FAB*: 975.0 ± 0.2
rmsd: 0.066
[ligand] nM

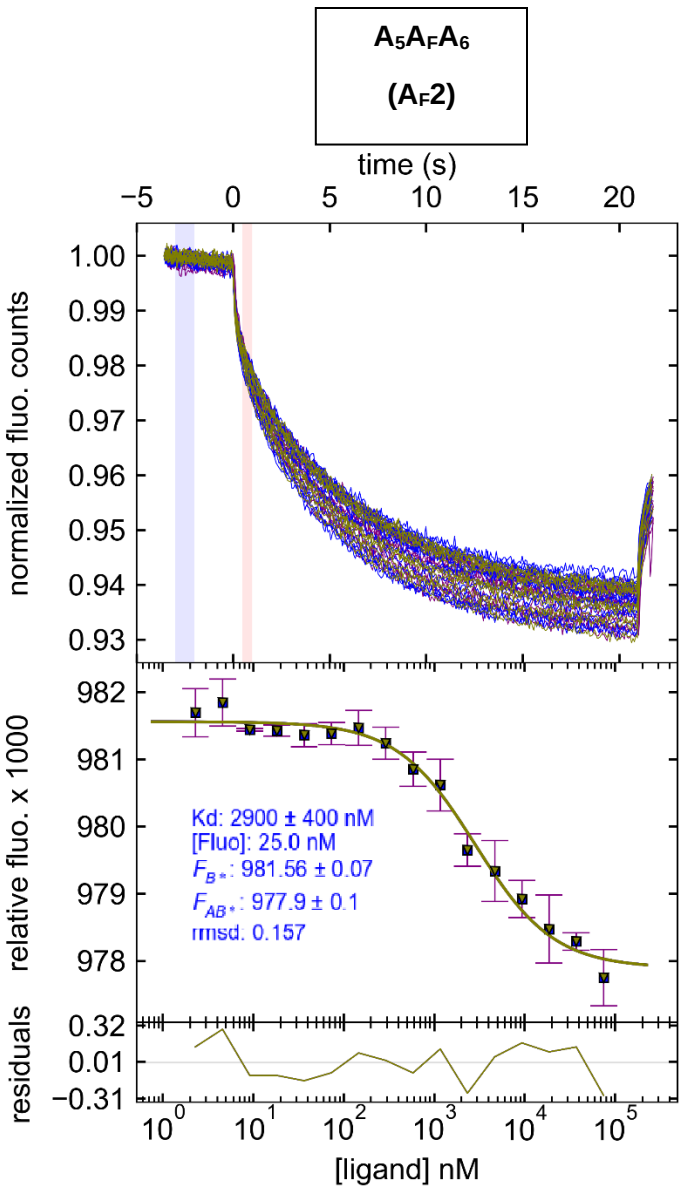

A11Am
(Am7)
time (s)
−5
0
5
10
15
20
normalized fluo. counts
1.00
0.99
0.98
0.97
0.96
0.95
0.94
0.93
relative fluo. x 1000
981
980
979
978
Kd: 1500 ± 400 nM
[Fluo]: 25.0 nM
F_{B*}: 980.9 ± 0.1
F_{AB*}: 977.4 ± 0.2
rmsd: 0.249
residuals
0.63
0.07
−0.49
10^0
10^1
10^2
10^3
10^4
10^5
[ligand] nM

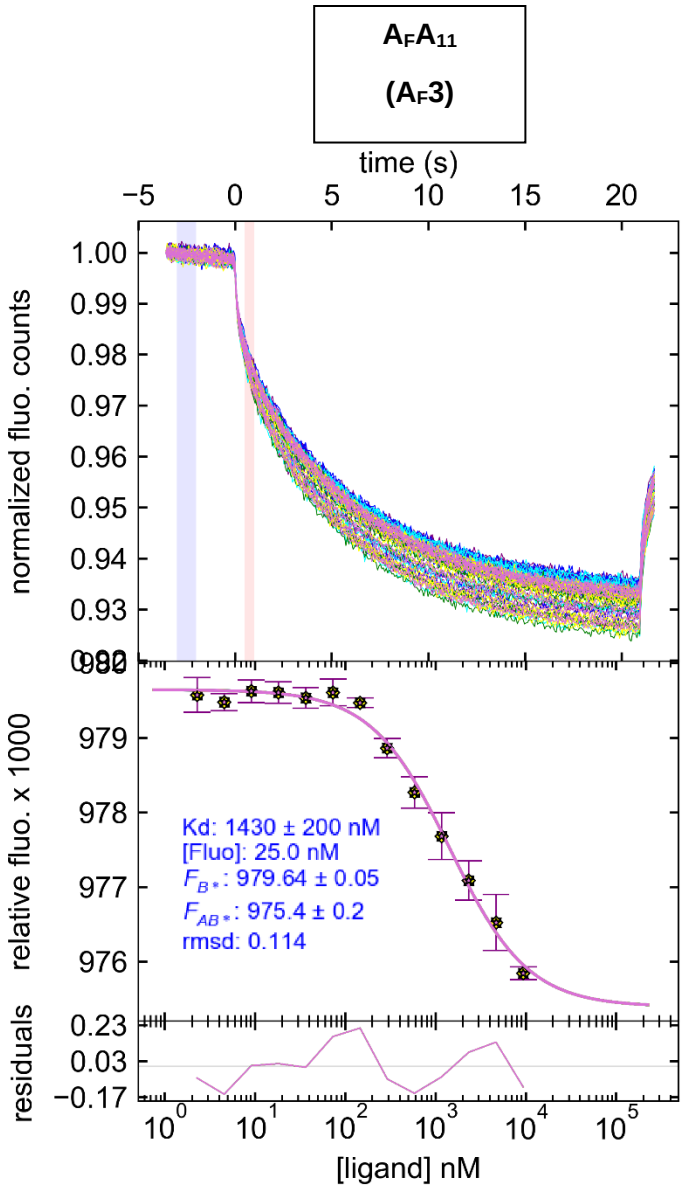

A10m6AA
(m6A1)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 410 ± 90 nM
[Fluo]: 25.0 nM
FB*: 980.7 ± 0.1
FAB*: 977.4 ± 0.1
rmsd: 0.213
[ligand] nM


A5m6AA6
(m6A2)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 9200 ± 1300 nM
[Fluo]: 25.0 nM
FB*: 980.92 ± 0.06
FAB*: 977.8
rmsd: 0.18
[ligand] nM


m6AA11
(m6A3)
time (s)
−5
0
5
10
15
20
normalized fluo. counts
1.00
0.99
0.98
0.97
0.96
0.95
0.94
0.93
relative fluo. x 1000
981
980
979
978
977
Kd: 480 ± 60 nM
[Fluo]: 25.0 nM
F_{B*}: 980.5 ± 0.07
F_{AB*}: 977.2 ± 0.07
rmsd: 0.13
residuals
0.23
0.01
−0.22
10^0
10^1
10^2
10^3
10^4
10^5
[ligand] nM


A10AFA
(AF1)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 730 ± 150 nM
[Fluo]: 25.0 nM
FB*: 979.6 ± 0.1
FAB*: 976.1 ± 0.1
rmsd: 0.212
[ligand] nM


A5AFA6
(AF2)
time (s)
normalized fluo. counts
relative fluo. x 1000
residuals
Kd: 2900 ± 400 nM
[Fluo]: 25.0 nM
FB*: 981.56 ± 0.07
FAB*: 977.9 ± 0.1
rmsd. 0.157
[ligand] nM


A_FA_{11}
(A_F3)
time (s)
−5
0
5
10
15
20
normalized fluo. counts
1.00
0.99
0.98
0.97
0.96
0.95
0.94
0.93
relative fluo. x 1000
979
978
977
976
Kd: 1430 ± 200 nM
[Fluo]: 25.0 nM
F_{B*}: 979.64 ± 0.05
F_{AB*}: 975.4 ± 0.2
rmsd: 0.114
residuals
0.23
0.03
−0.17
10^0
10^1
10^2
10^3
10^4
10^5
[ligand] nM