Electronic Supplementary Material (ESI) for Green Chemistry.

Electronic Supporting Information

Polymerizable Phosphoramidites with Acid-Cleavable Linker for Eco-Friendly Synthetic Oligodeoxynucleotide Purification

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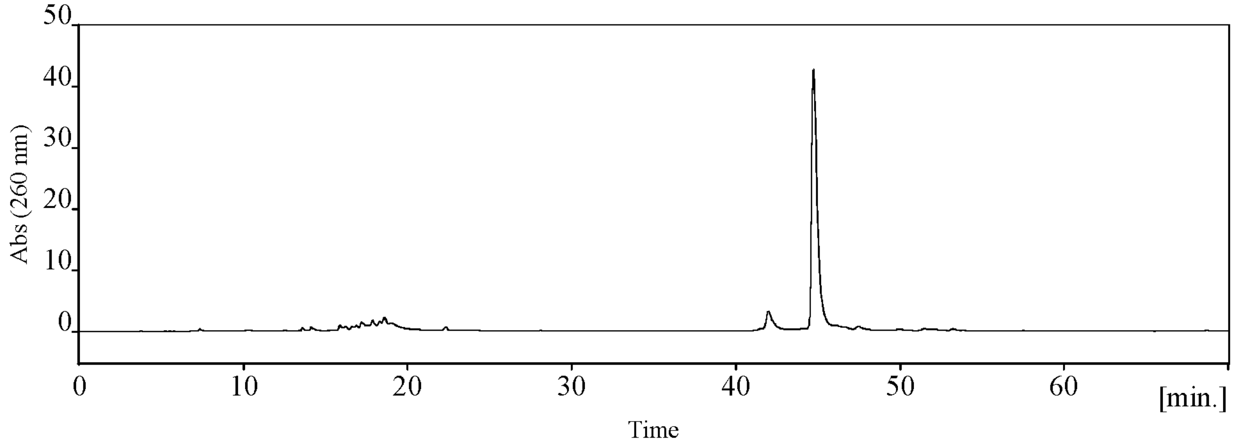
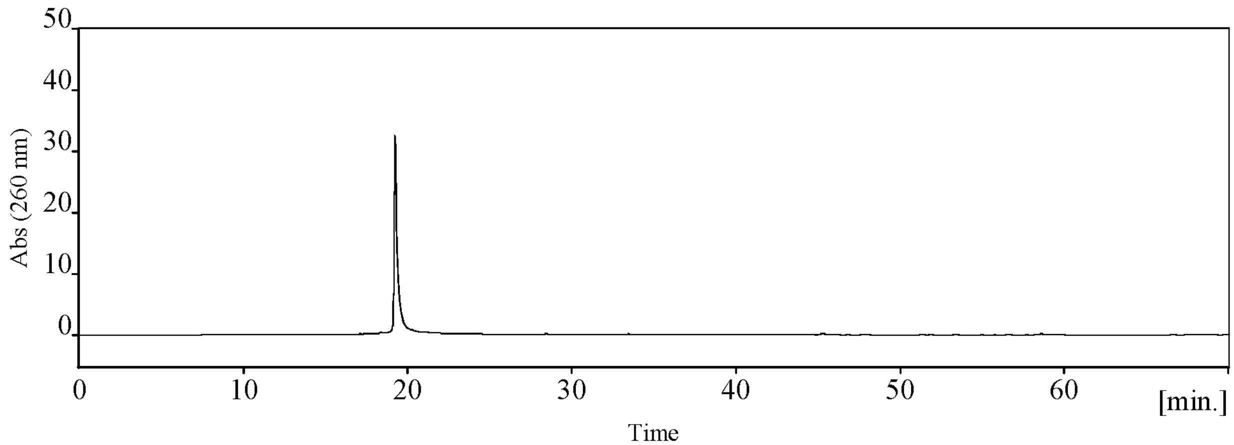
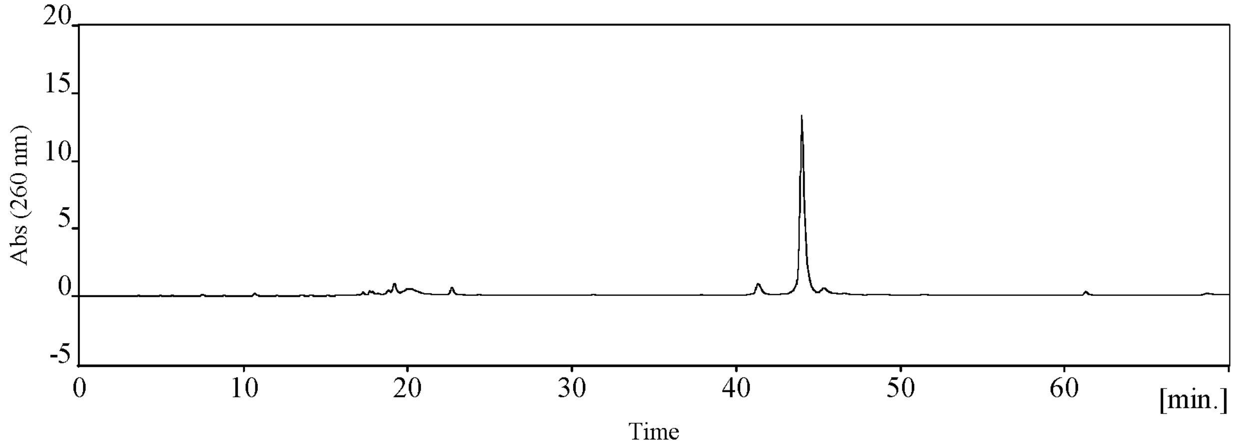


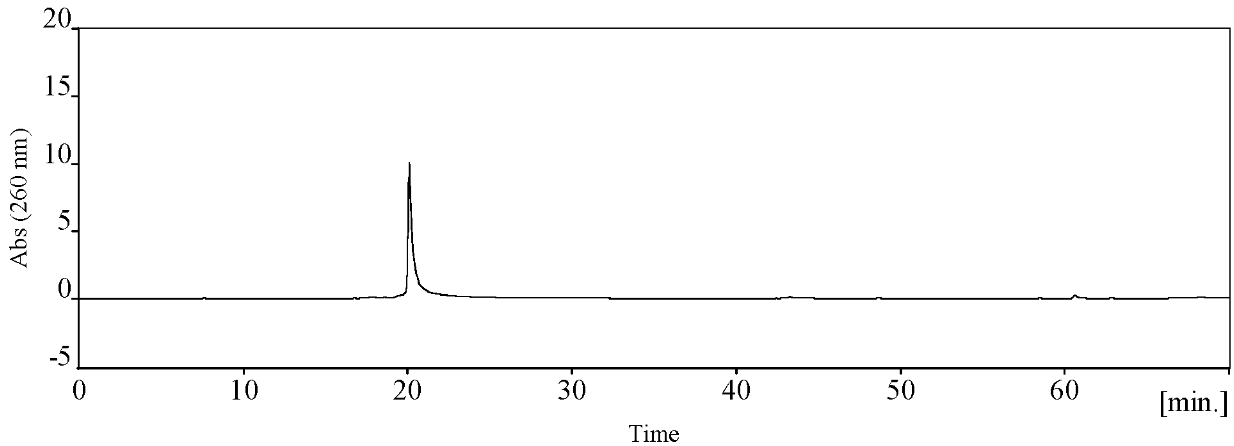
Crude RP HPLC profile of the 20-mer ODN **7**.



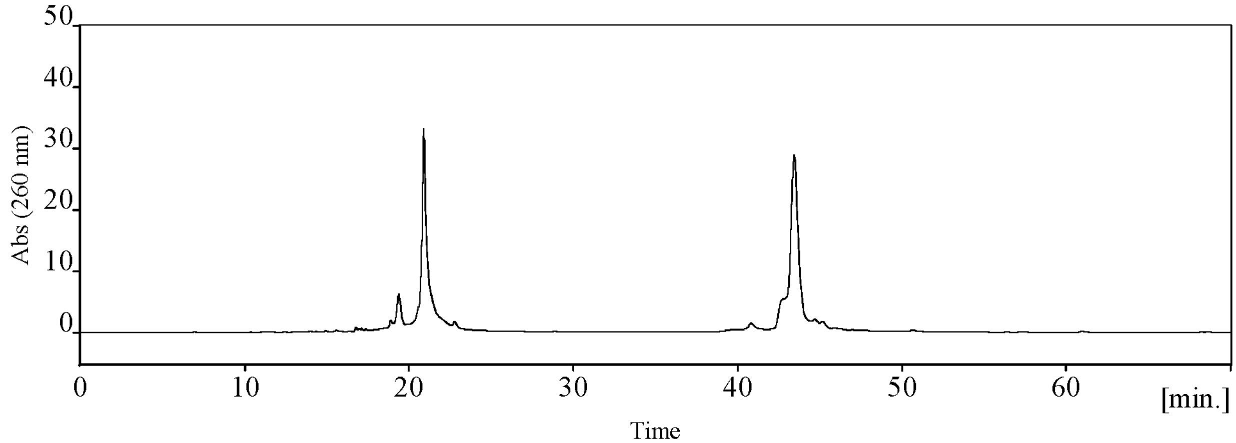
Pure RP HPLC profile of the 20-mer ODN **7**.



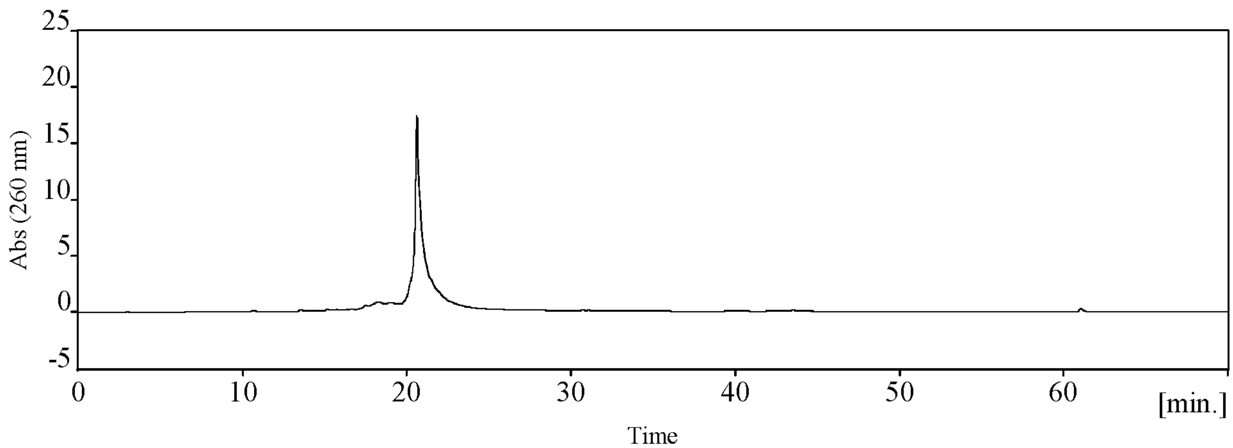
Crude RP HPLC profile of the 31-mer ODN **8**.



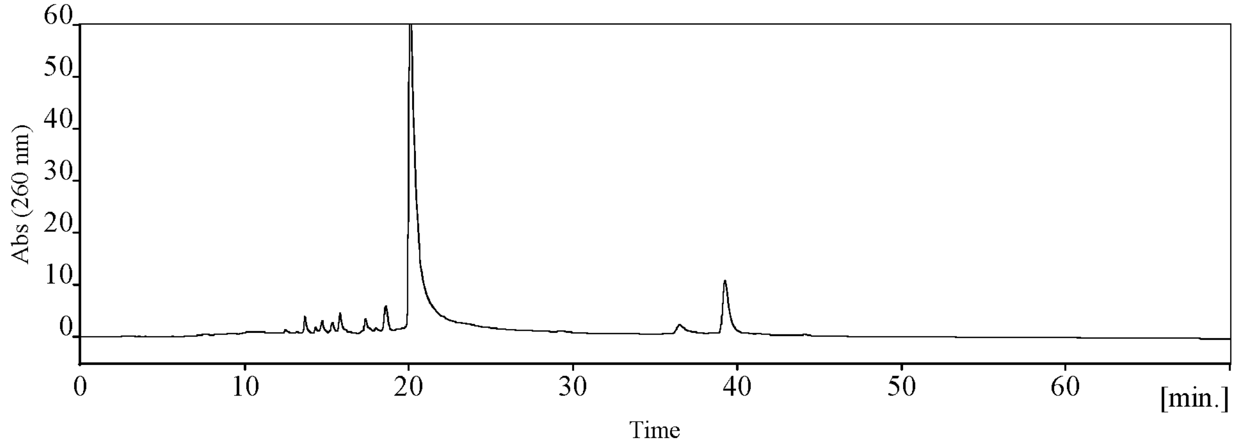
Pure RP HPLC profile of the 31-mer ODN **8**.



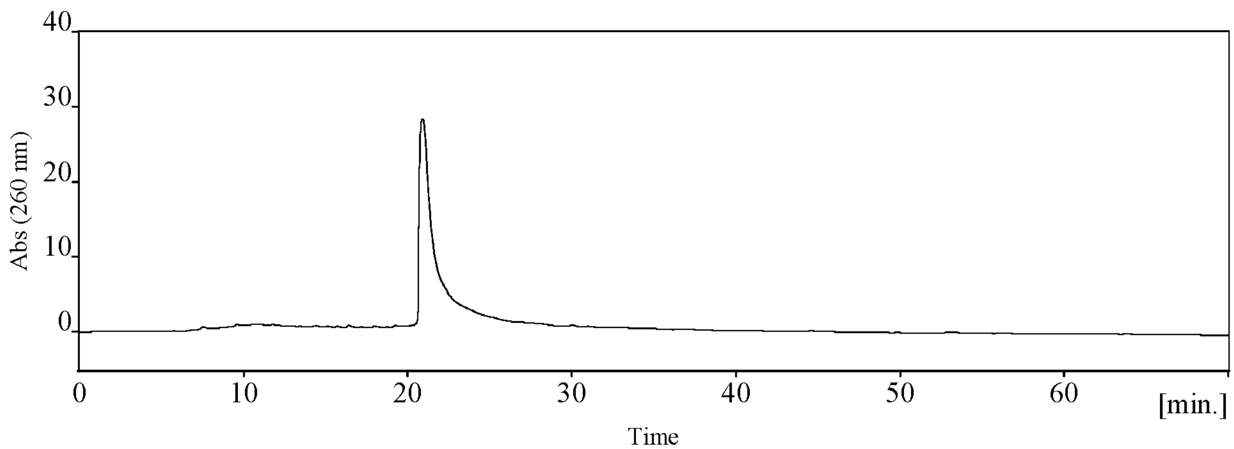
Crude RP HPLC profile of 37-mer ODN **9**.



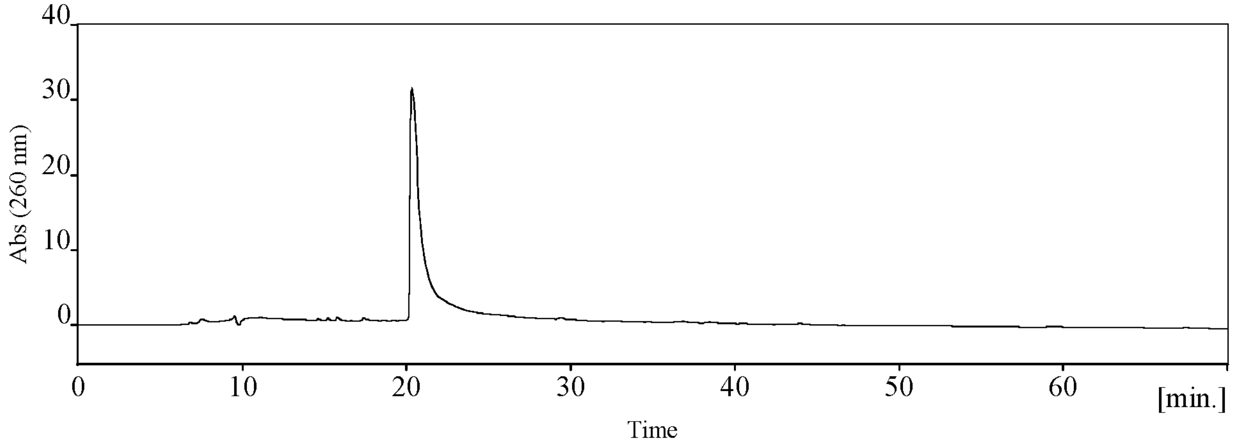
Pure RP HPLC profile of 37-mer ODN **9**



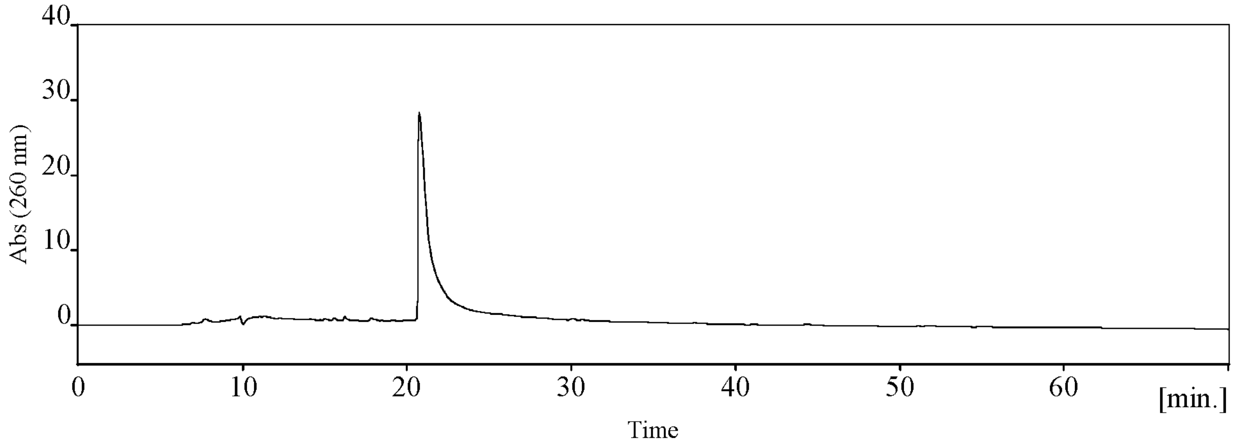
Crude RP HPLC profile of 43-mer ODN **10**.



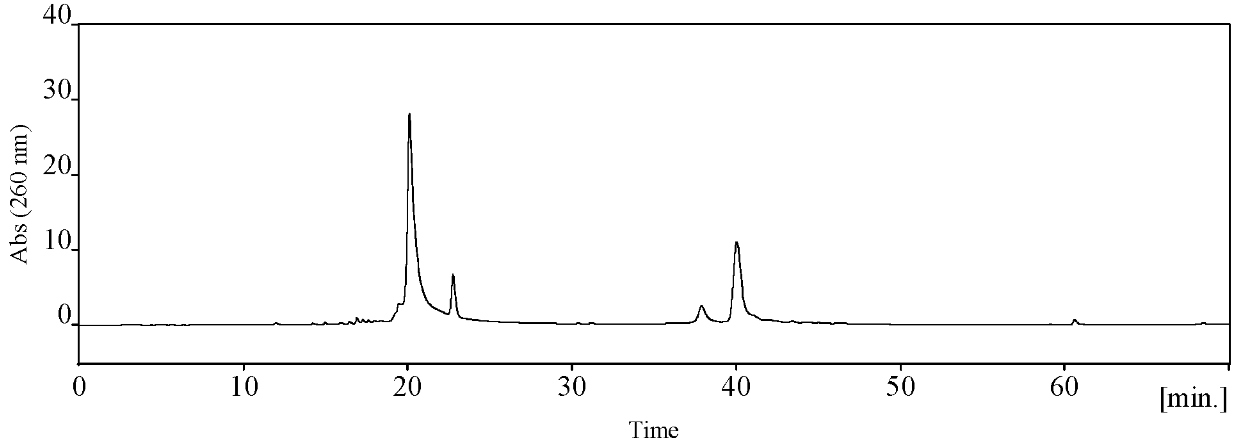
Pure RP HPLC profile of 43-mer ODN **10**.



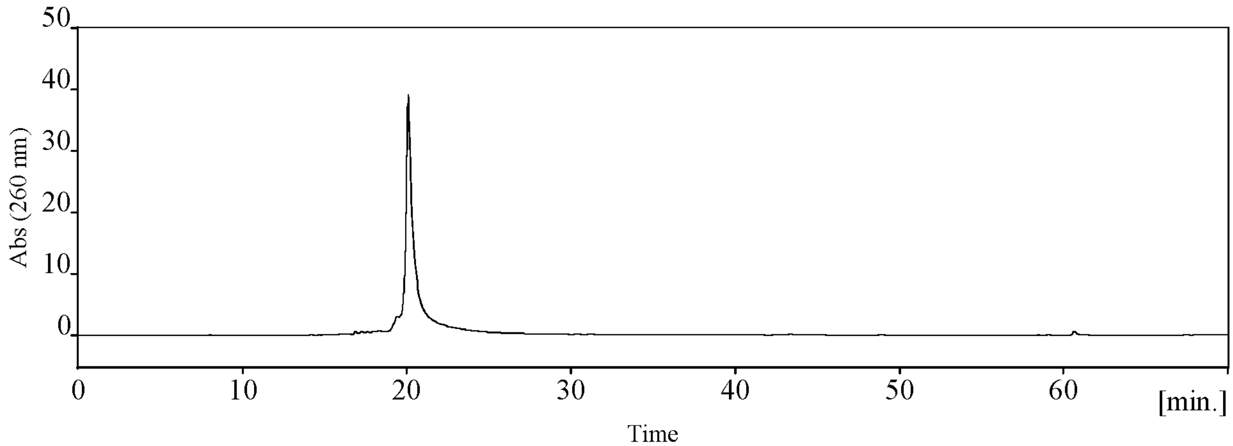
Pure RP HPLC profile of 43-mer ODN **10**. The reagent for cleaving ODN from polyacrylamide gel was changed from 80% AcOH to 0.1 mM Et_3NHCl at pH 3.0.



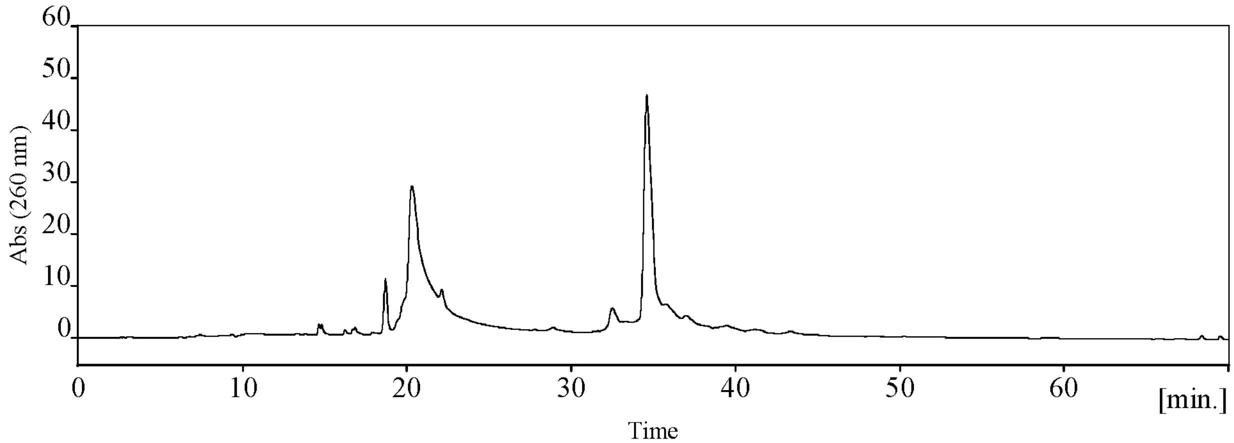
Pure RP HPLC profile of 43-mer ODN **10**. The reagent for cleaving ODN from polyacrylamide gel was changed from 80% AcOH to 10 mM $Et_3NHO_2CCF_3$ at pH 3.0.



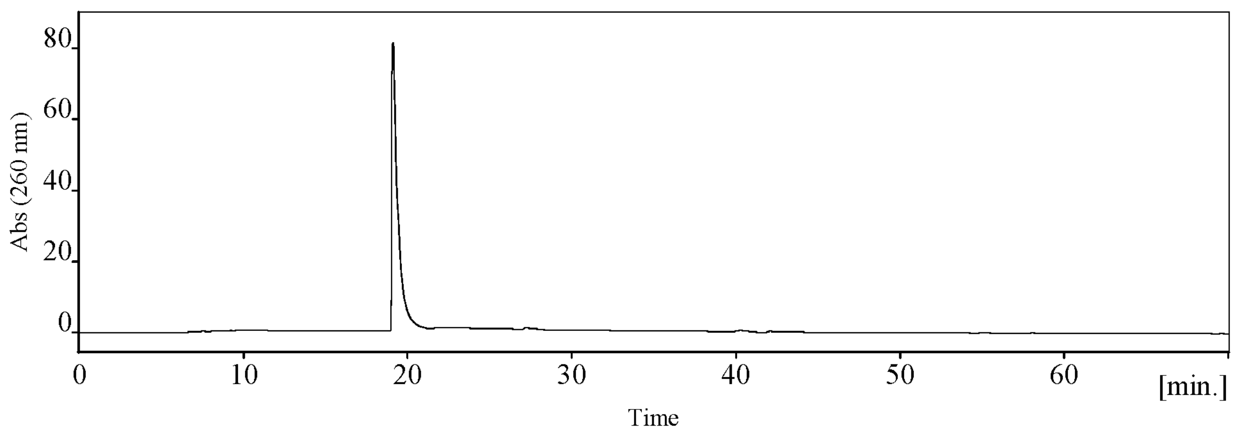
Crude RP HPLC profile of the 61-mer ODN **11**.



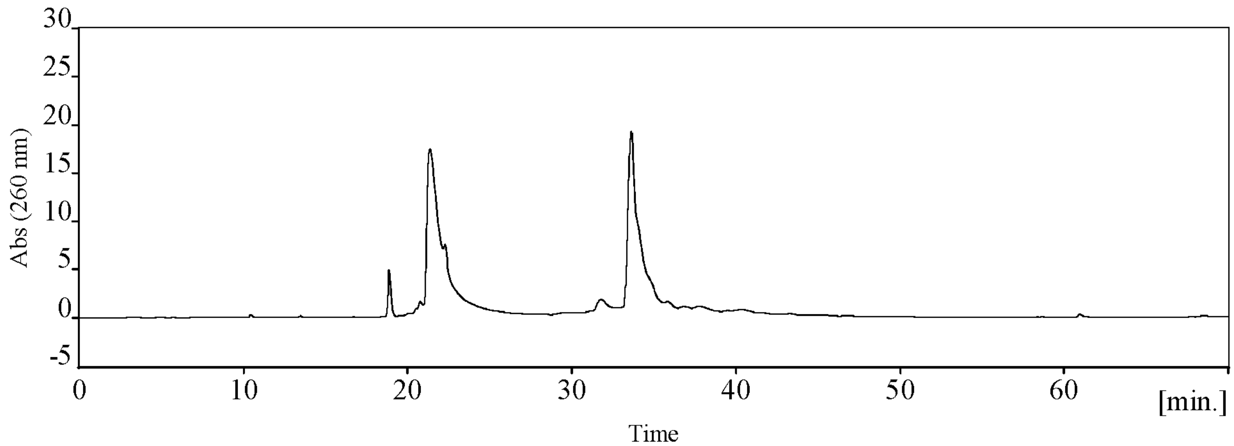
Pure RP HPLC profile of the 61-mer ODN **11**.



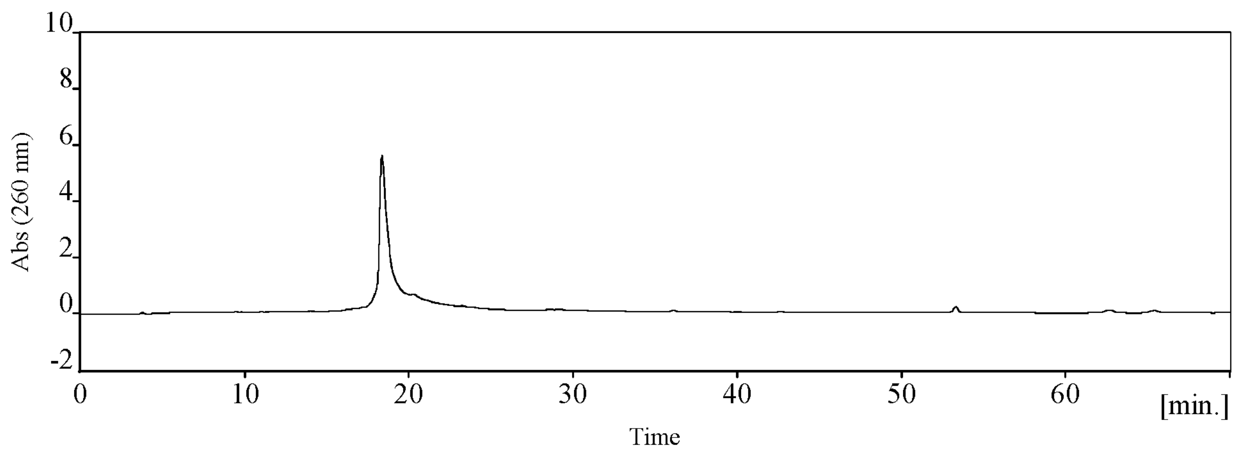
Crude RP HPLC profile of the 81-mer ODN **12**.



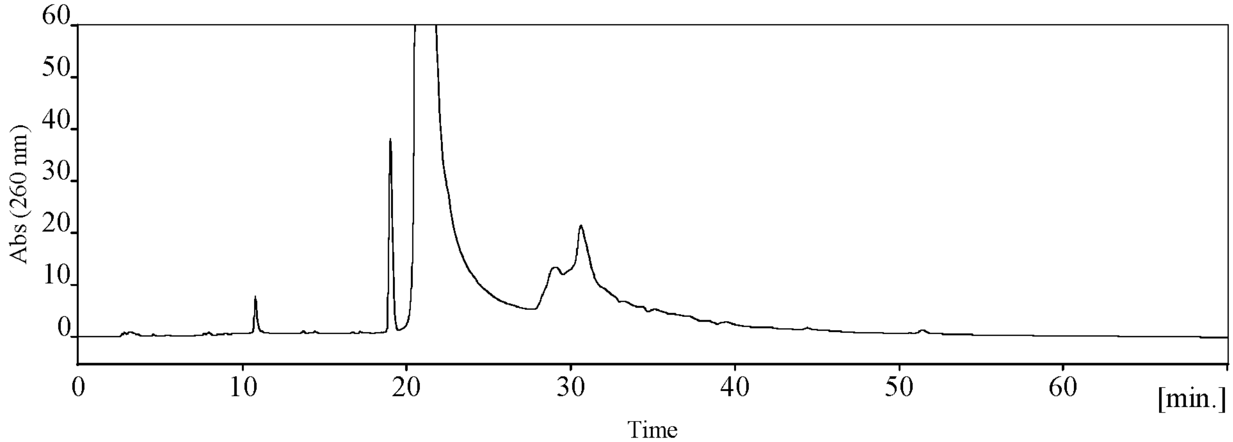
Pure RP HPLC profile of the 81-mer ODN **12**.



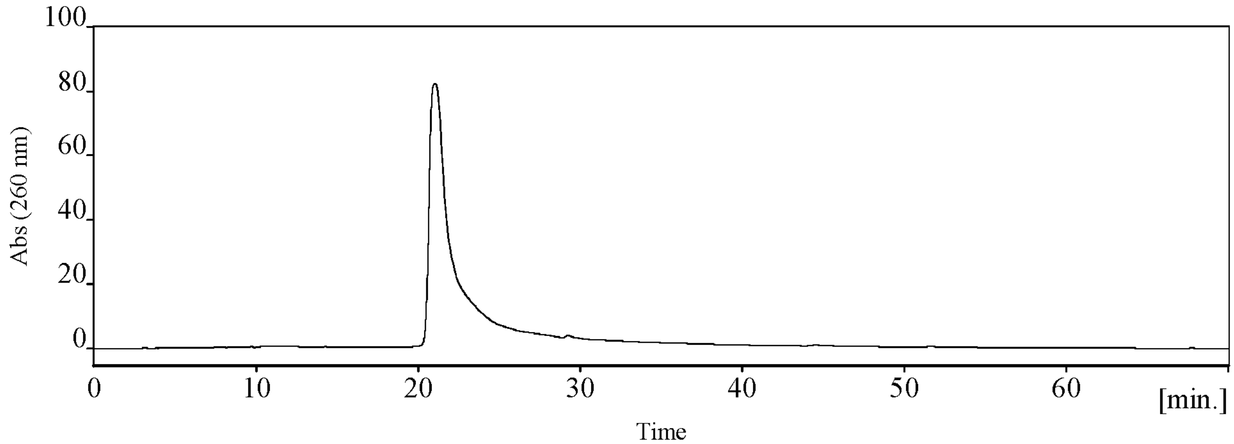
Crude RP HPLC profile of the 151-mer ODN **13**.



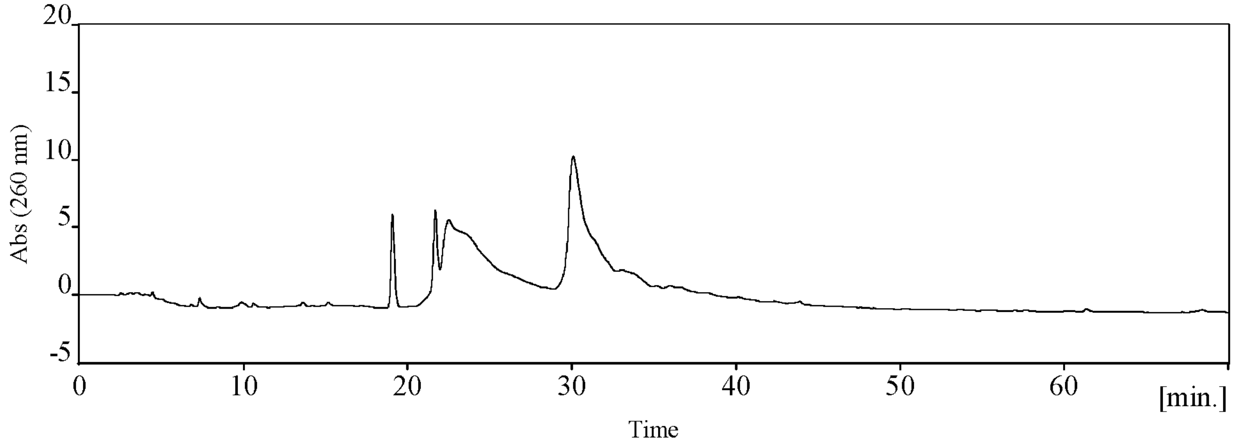
Pure RP HPLC profile of the 151-mer ODN **13**.



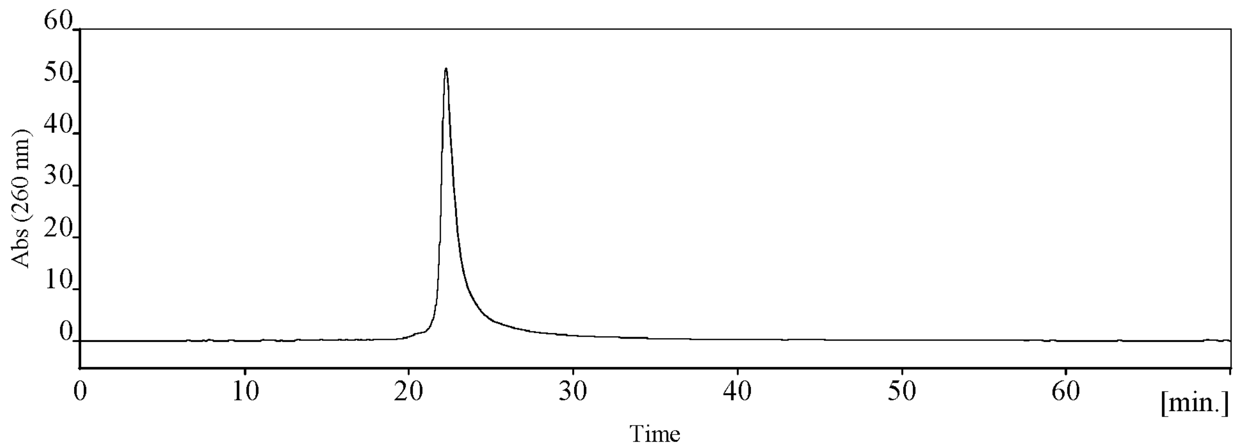
Crude RP HPLC profile of the 196-mer ODN **14**.



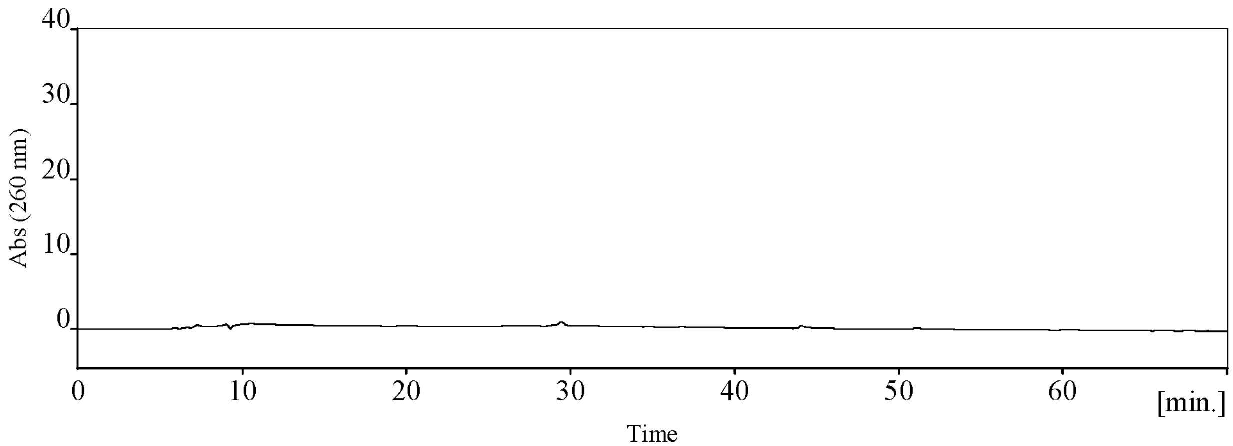
Pure RP HPLC profile of the 196-mer ODN **14**.



Crude RP HPLC profile of the 197-mer ODN **15**.

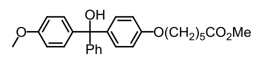


Pure RP HPLC profile of the 197-mer ODN **15**.

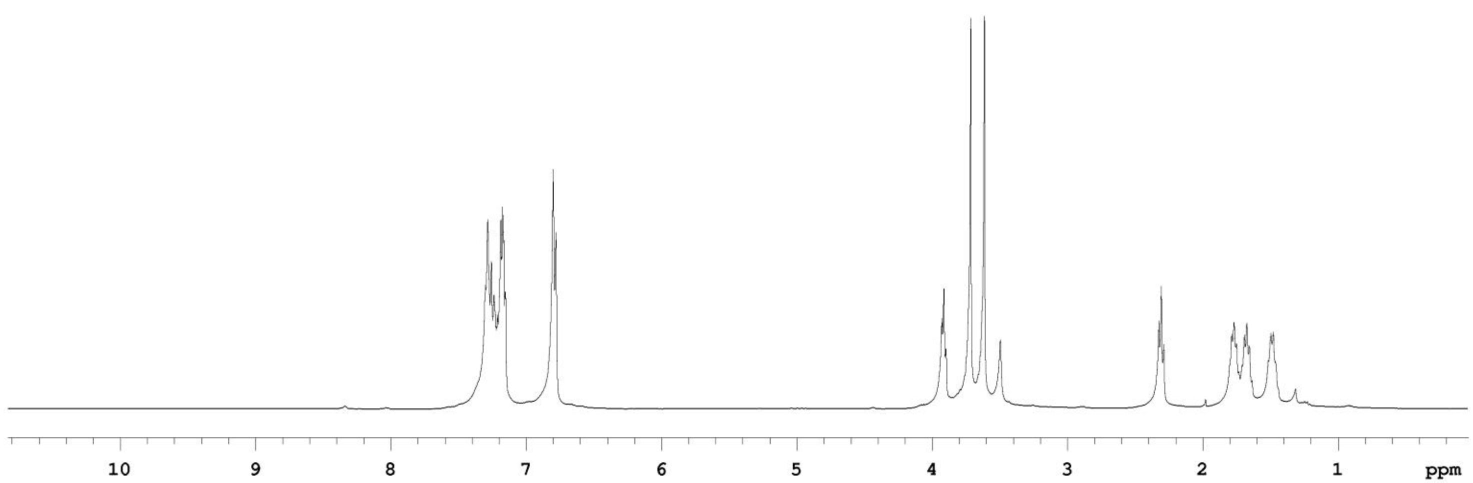


The RP HPLC profile of a blank control experiment. The procedure for the purification of ODN **15** was followed except that no crude DNA was introduced. The final residue was dissolved in 30 µl water and 20 µl was injected into HPLC. The result shows that the purification procedure does not introduce any UV active impurity.

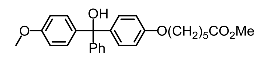
^{1}H NMR of compound **3** in $CDCl_3$ at 400 MHz



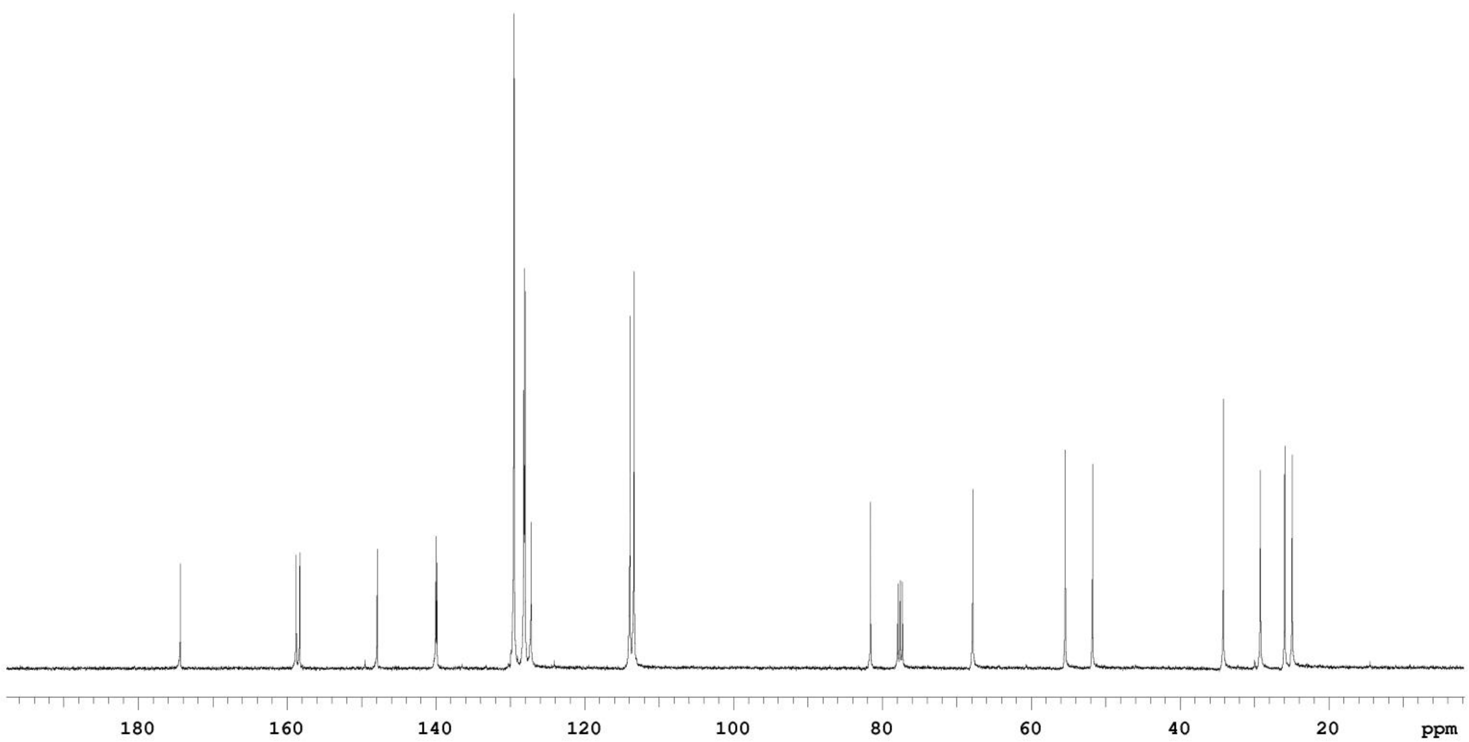
3



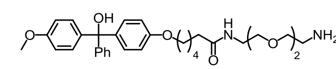
^{13}C NMR of compound **3** in $CDCl_3$ at 100 MHz



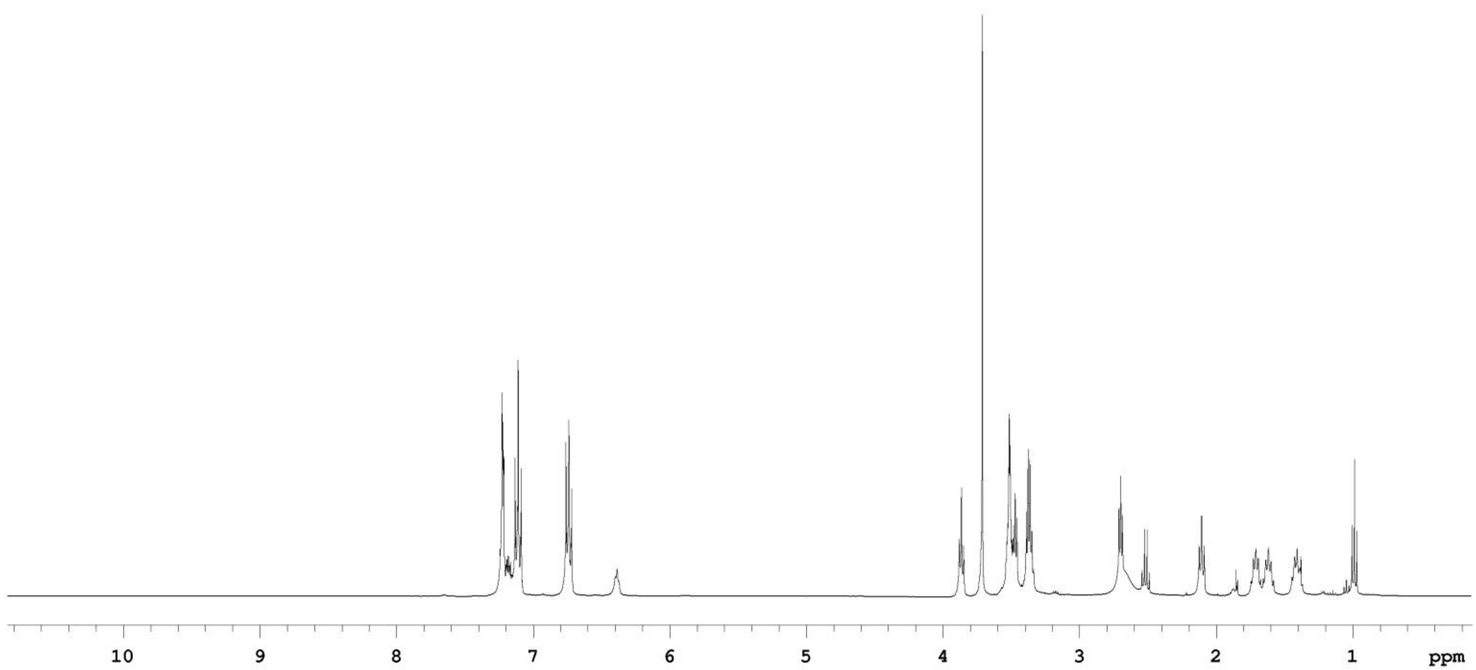
3



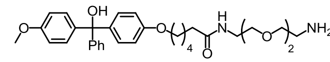
^{1}H NMR of compound **4** in $CDCl_3$ at 400 MHz



4



^{13}C NMR of compound **4** in $CDCl_3$ at 100 MHz



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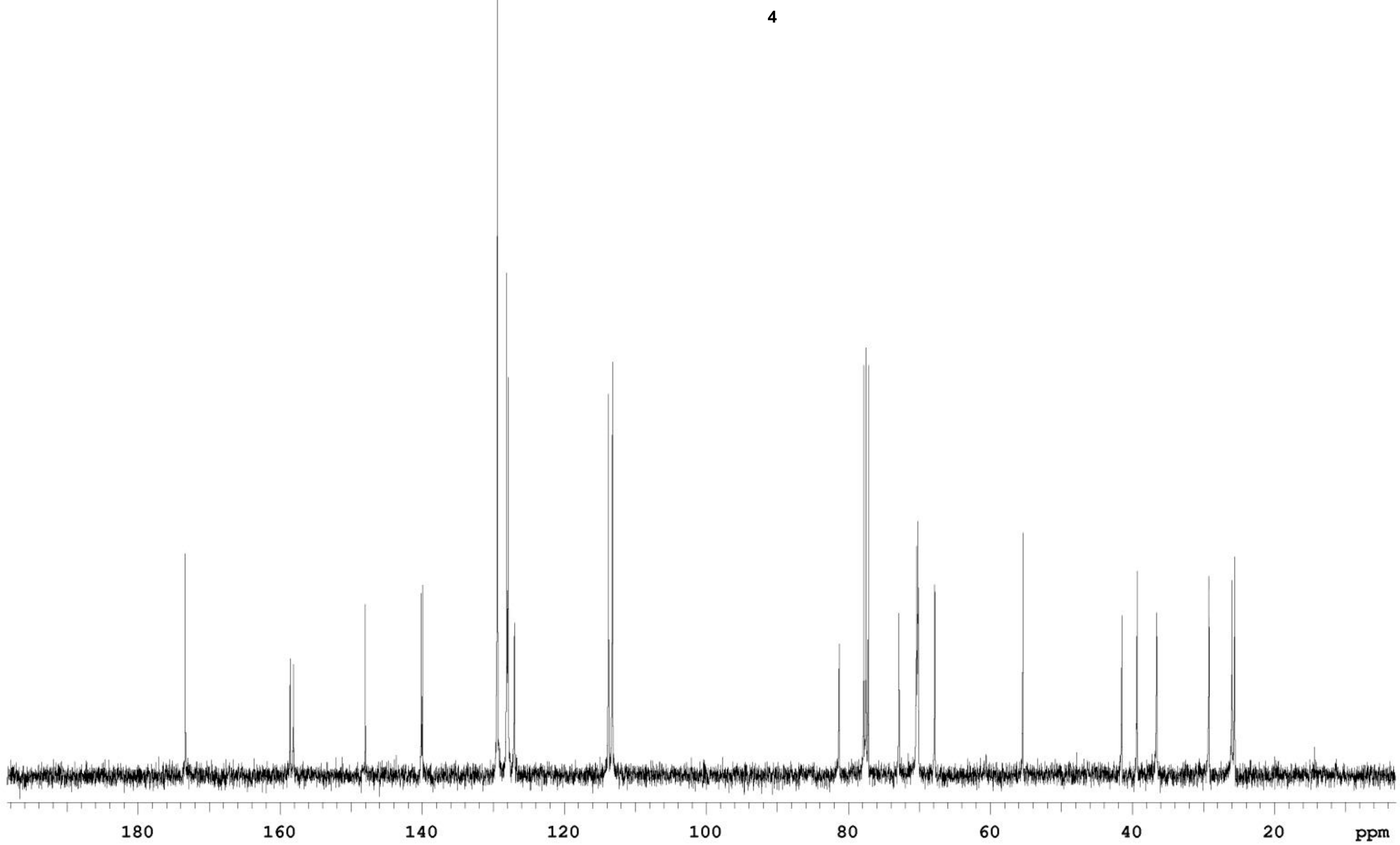
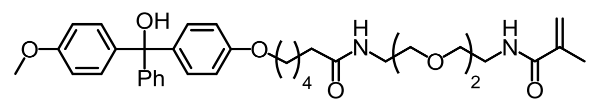


^{1}H NMR of compound **5** in $CDCl_3$ at 400 MHz



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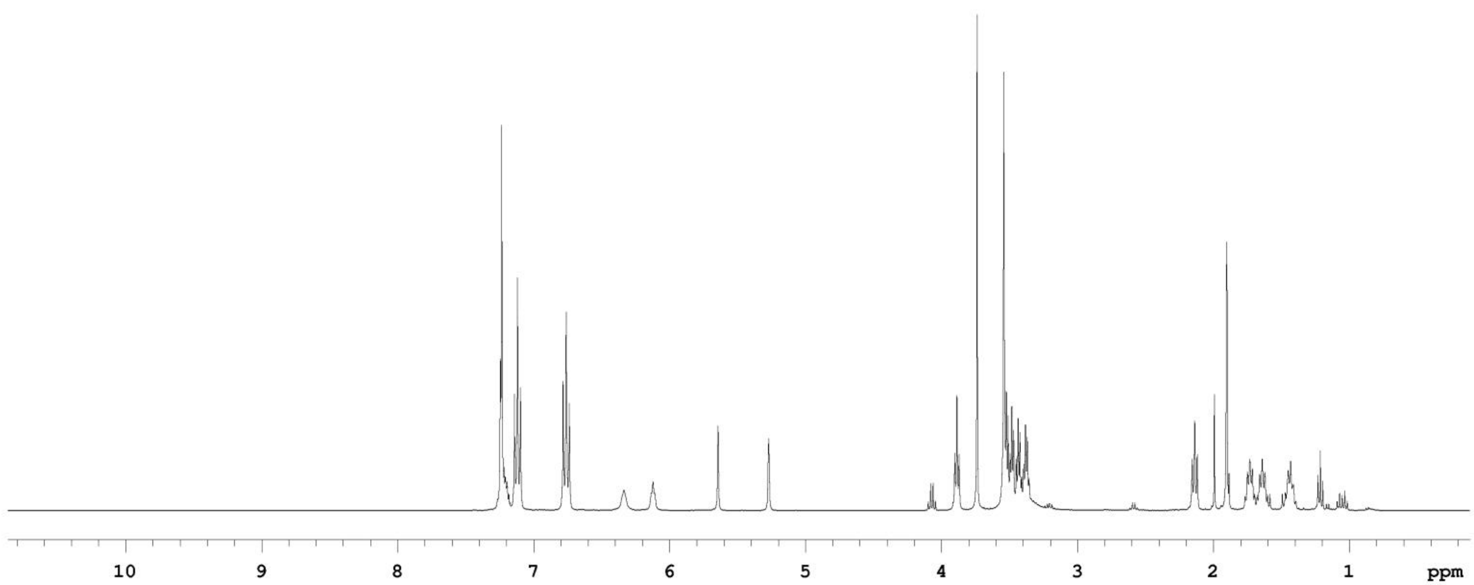
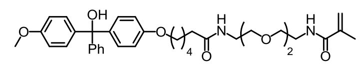


^{13}C NMR of compound **5** in $CDCl_3$ at 100 MHz



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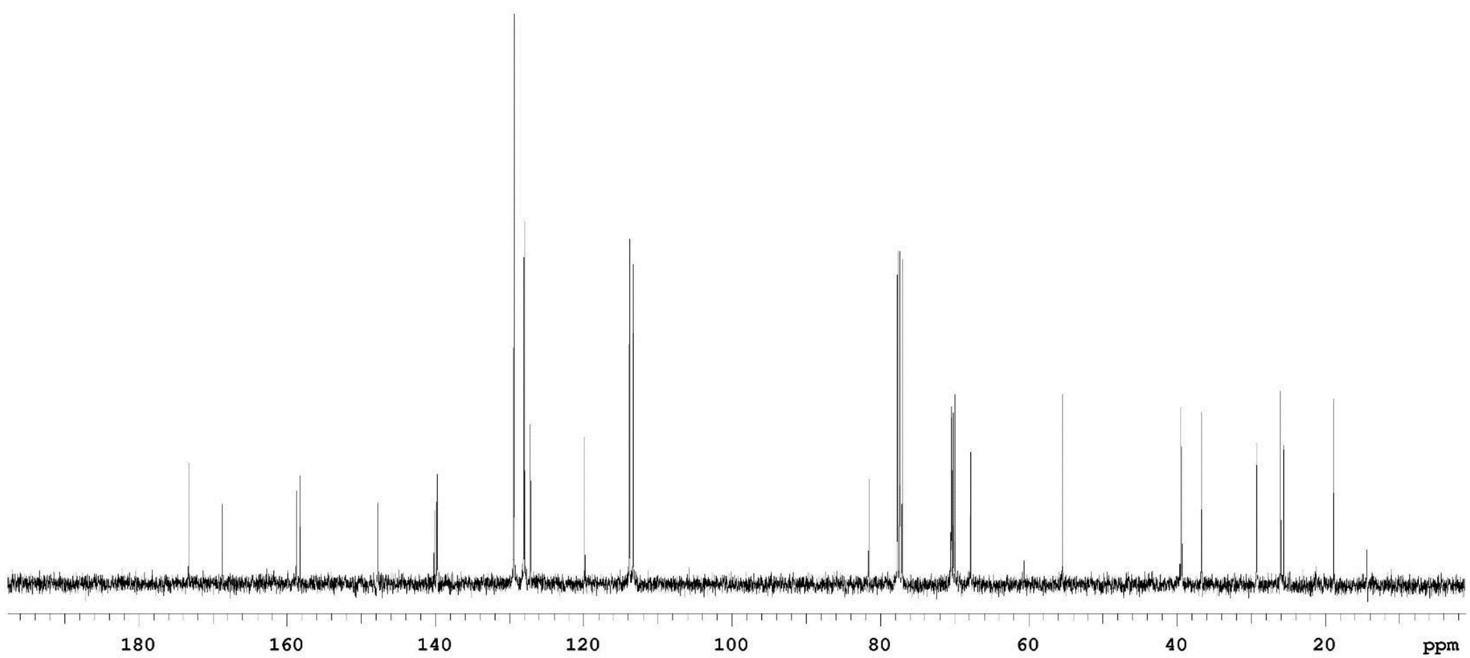
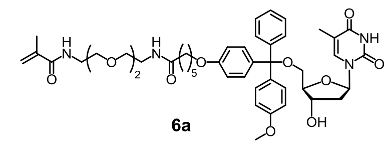
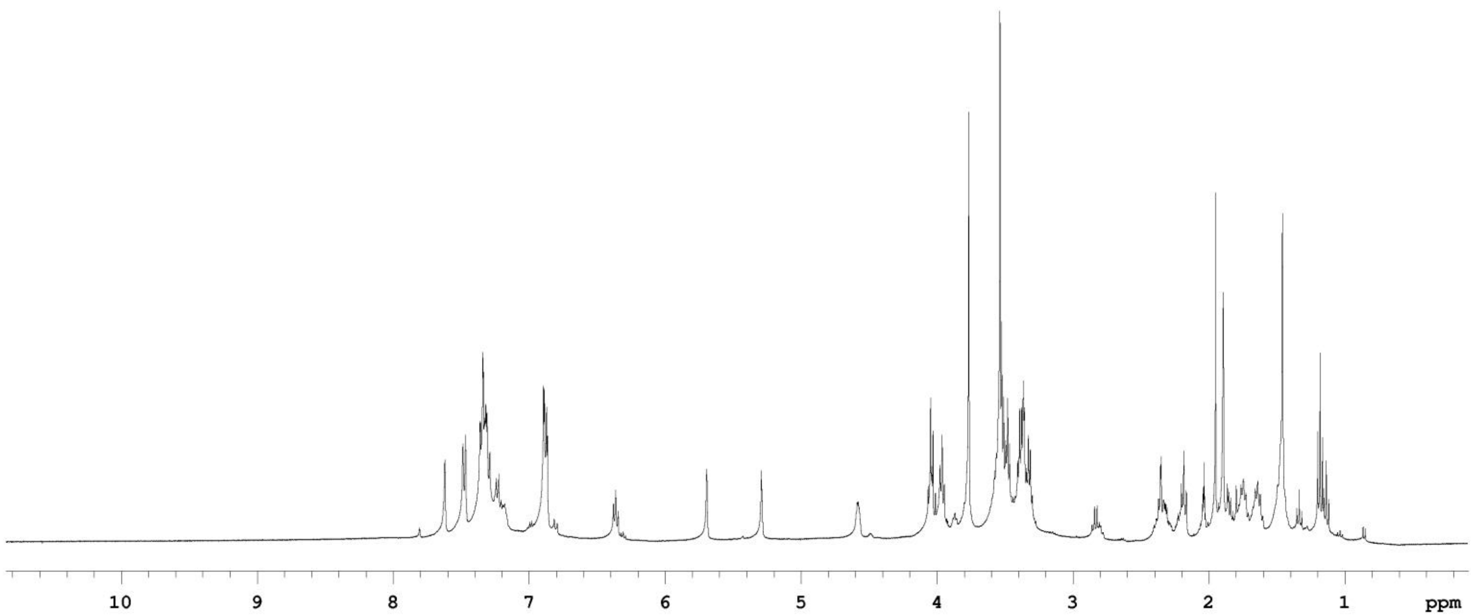
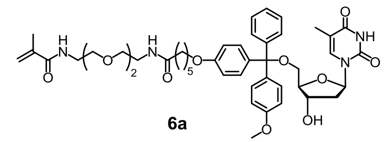


^1H NMR of compound **6a** in acetone-d_6 at 400 MHz

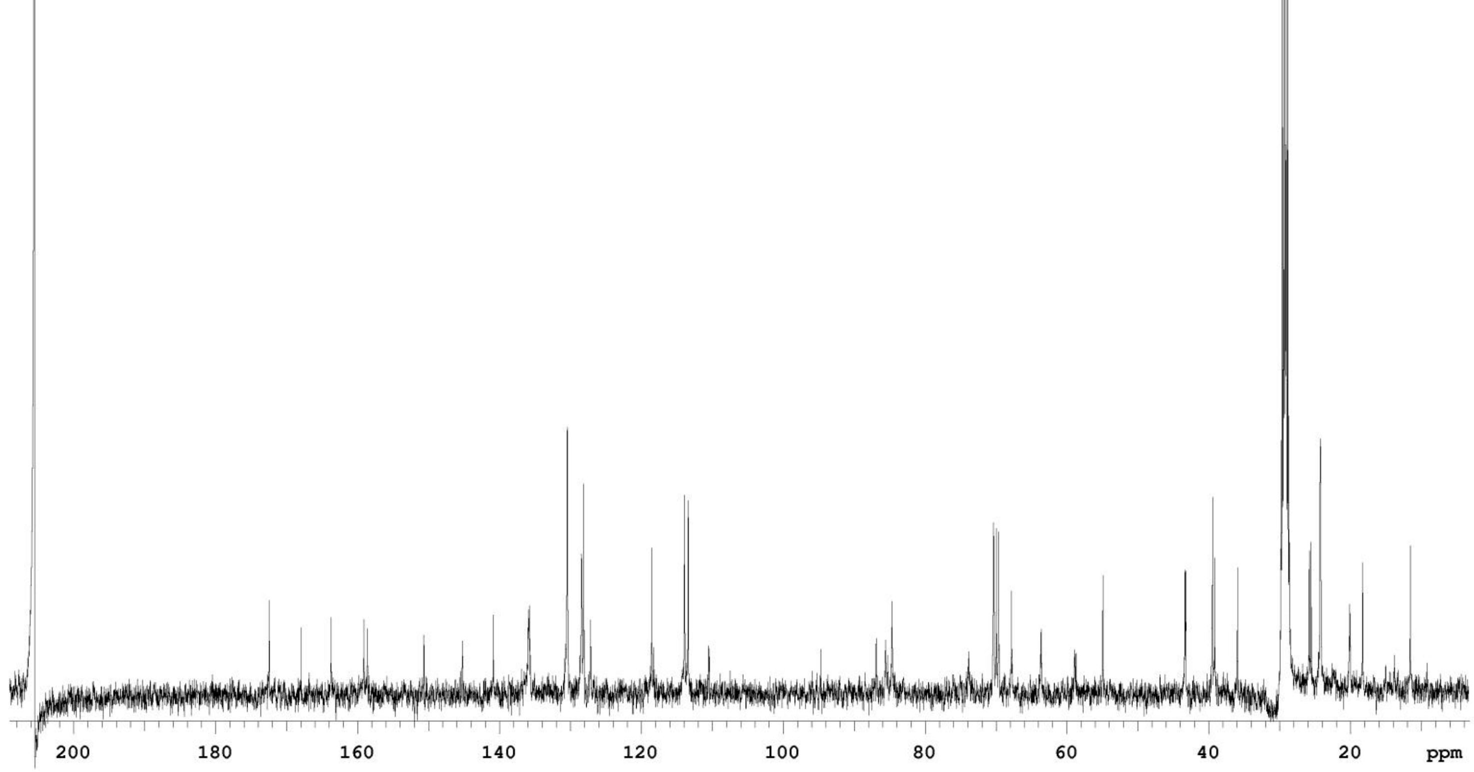




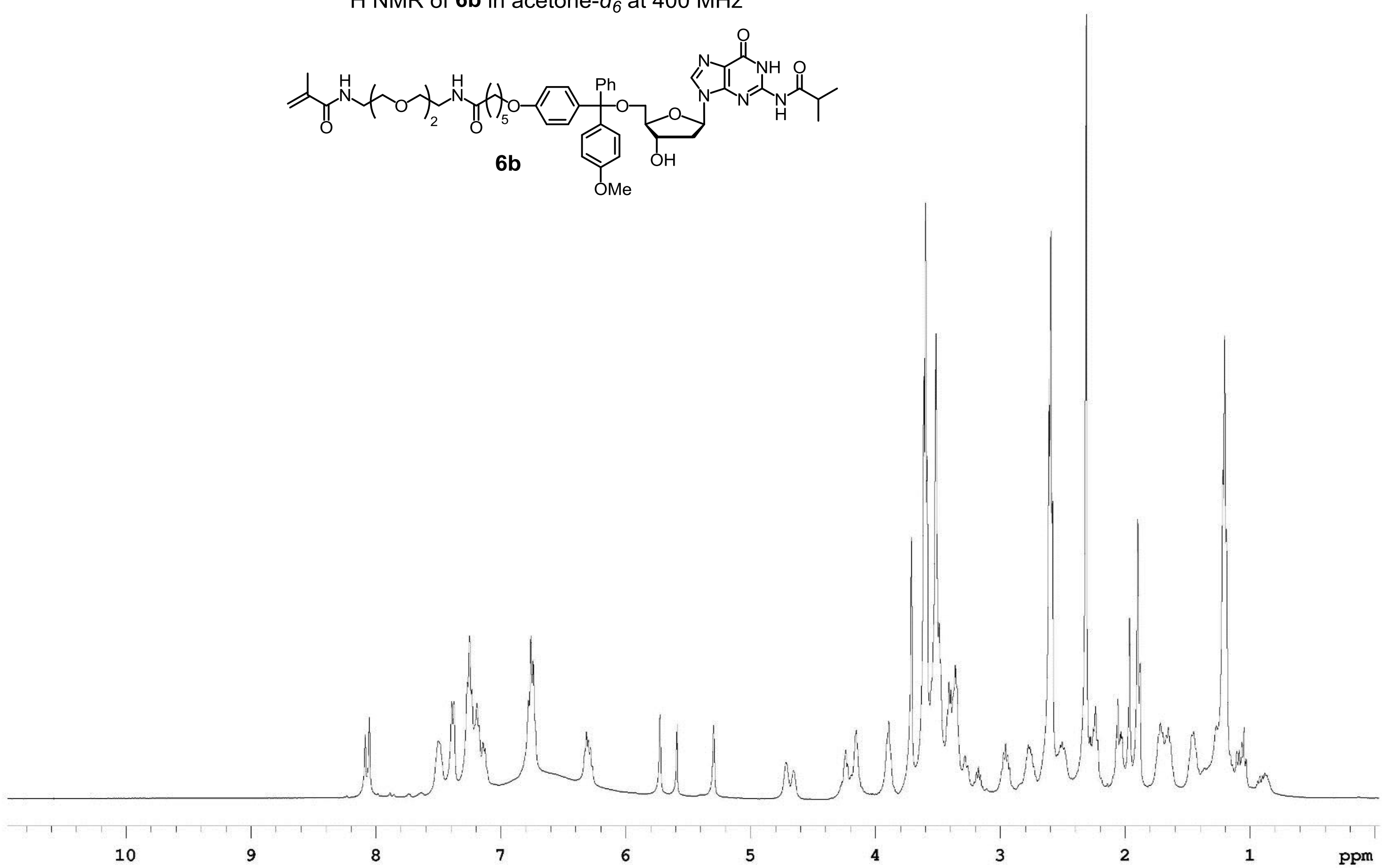
^{13}C NMR of compound **6a** in acetone-d_6 at 100 MHz



6a

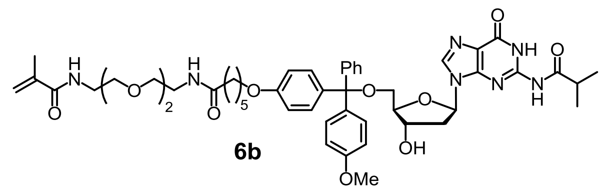


^{1}H NMR of **6b** in acetone-d_6 at 400 MHz

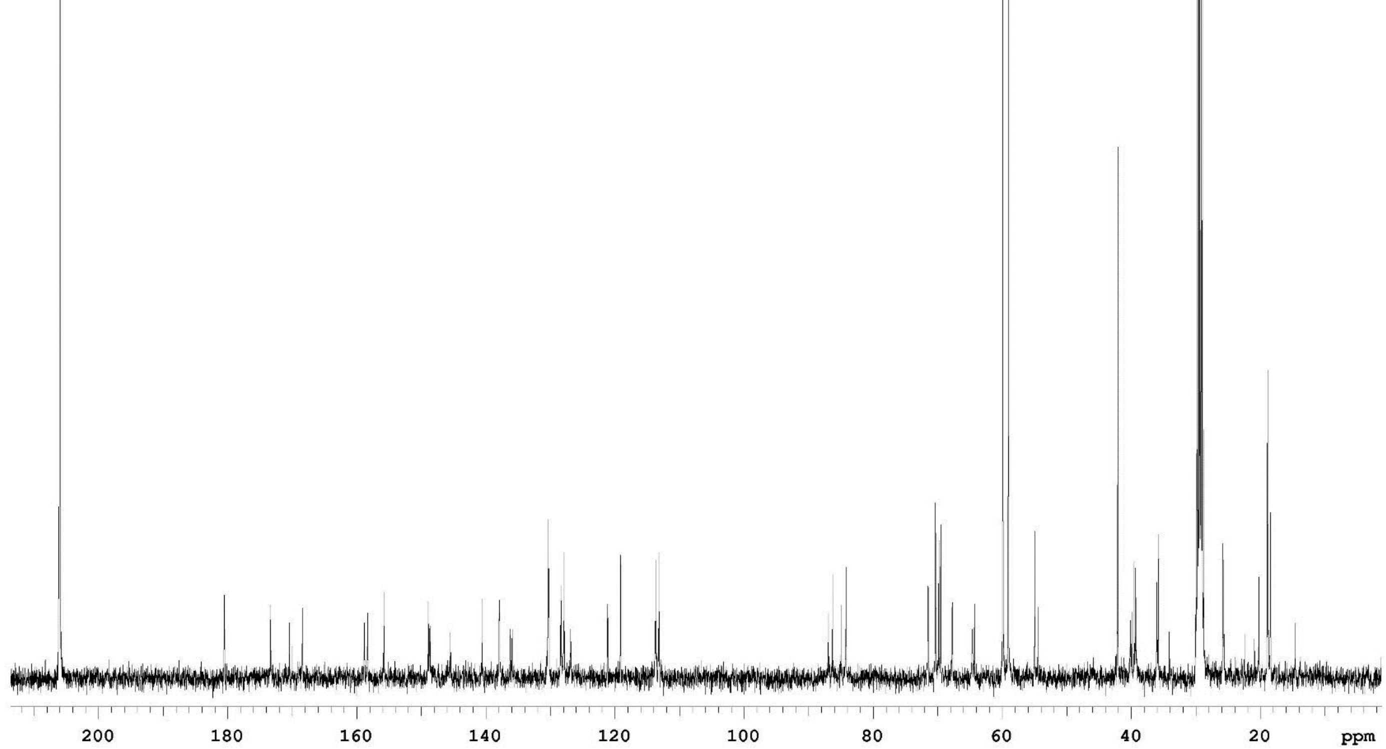


6b

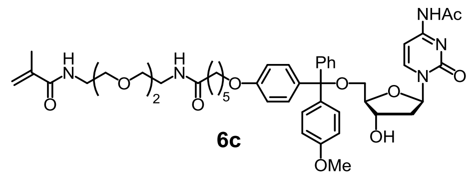
^{13}C NMR of **6b** in acetone-d_6 at 100 MHz



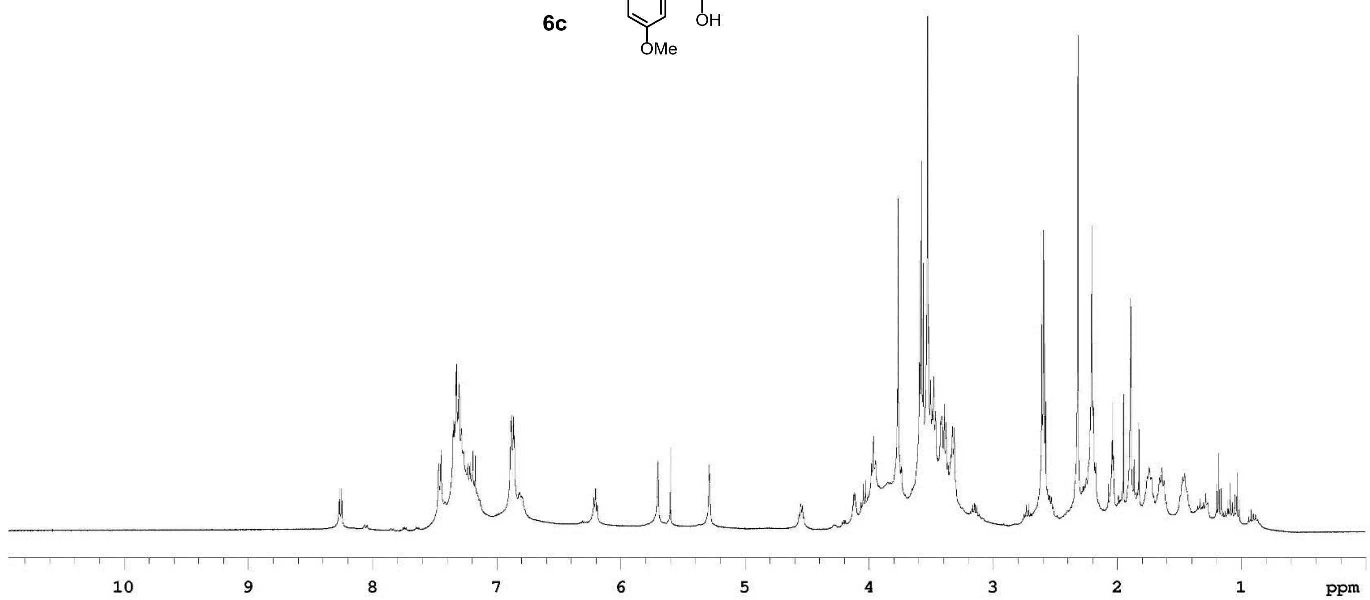
6b

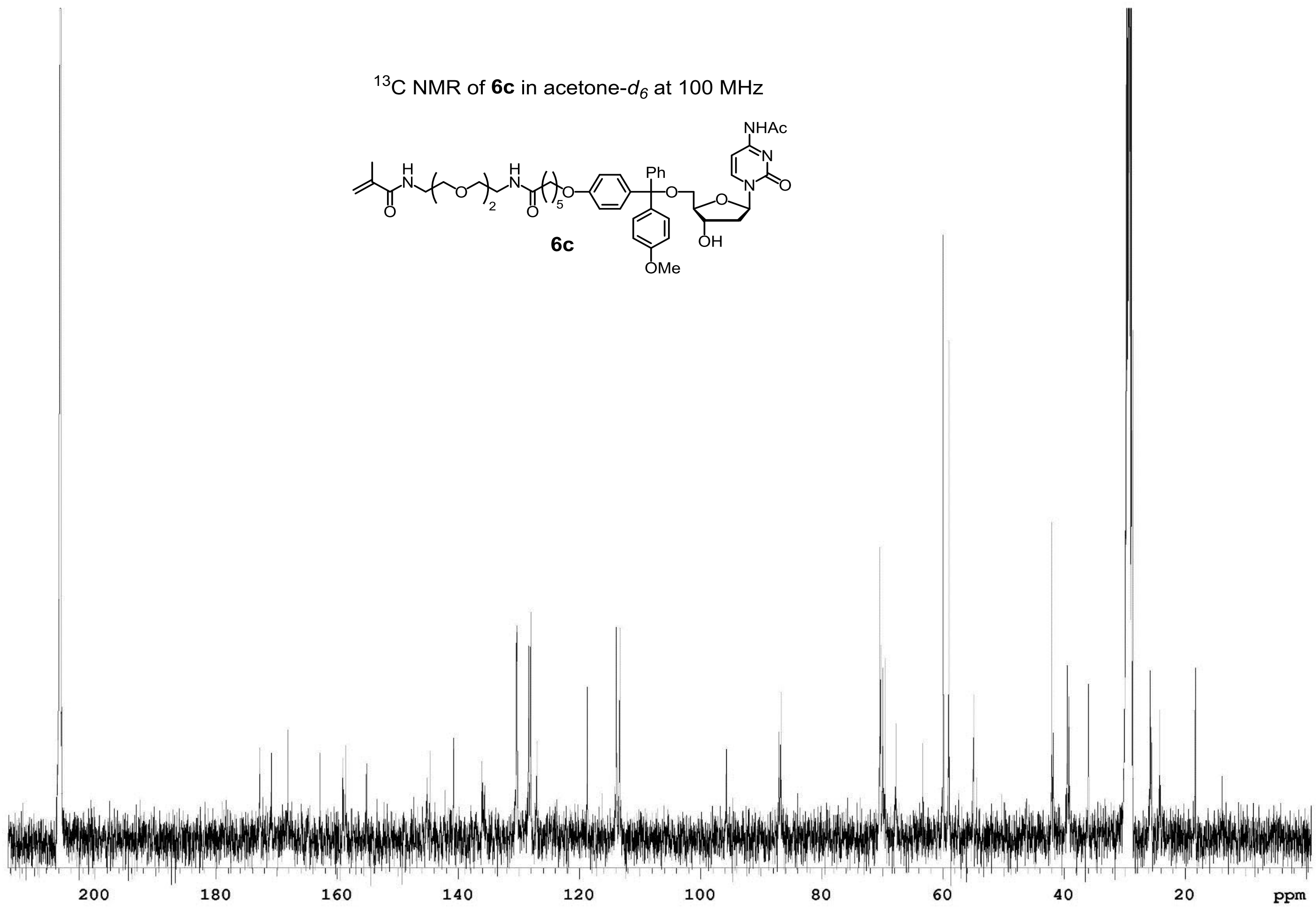


^{1}H NMR of **6c** in acetone-d_6 at 400 MHz



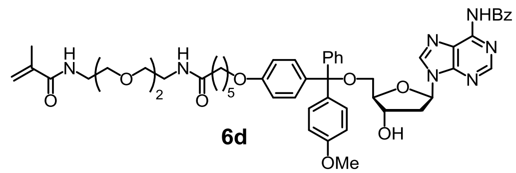
6c

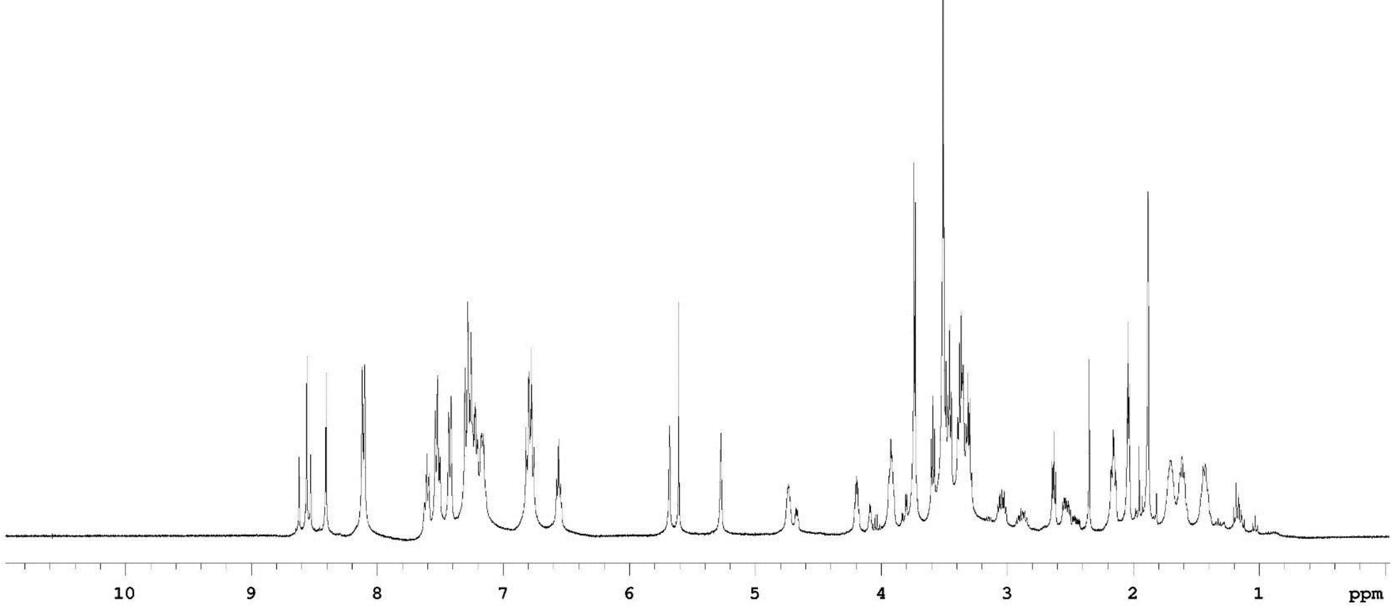




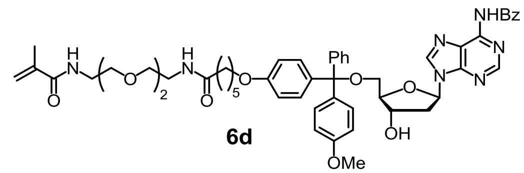
^{13}C NMR of **6c** in acetone-d_6 at 100 MHz

[1]H NMR of **6d** in acetone-d_6 at 400 MHz

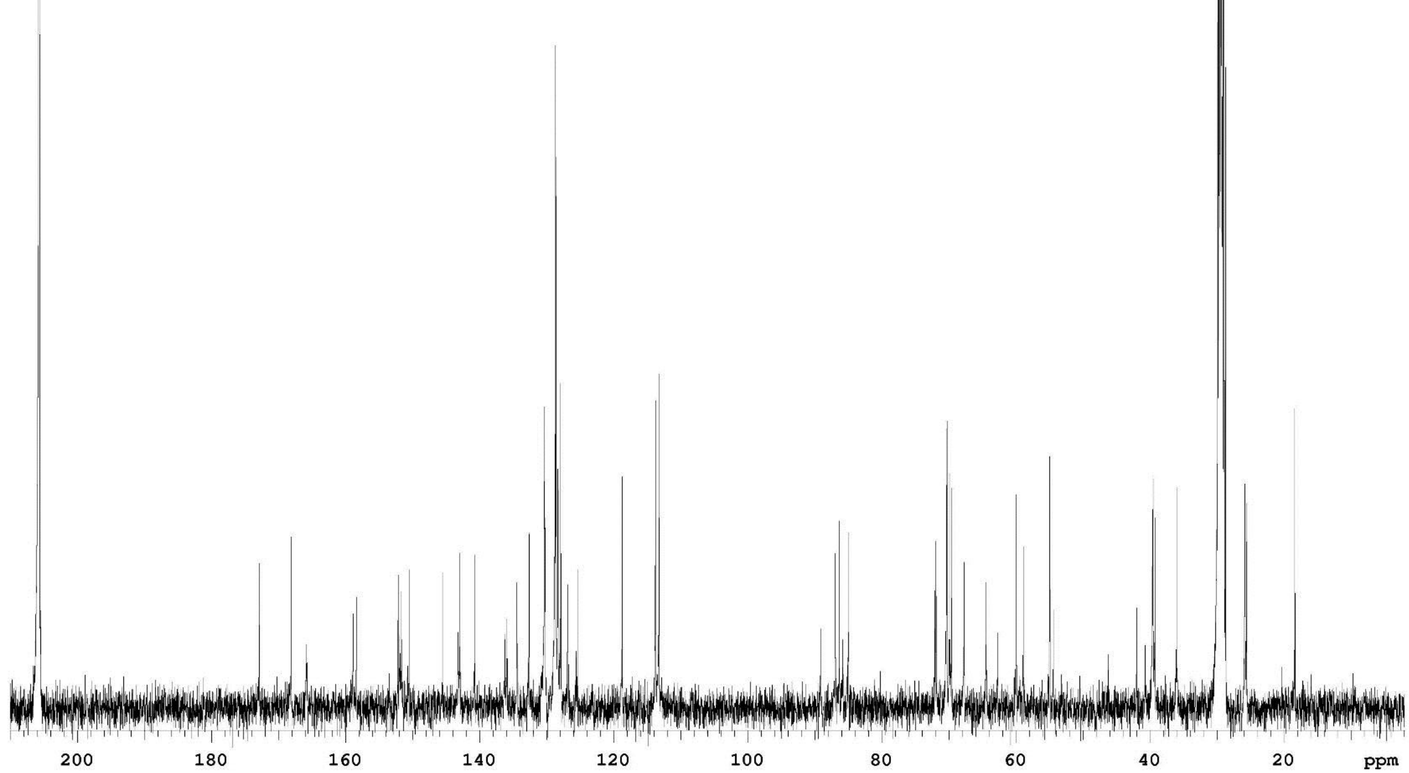




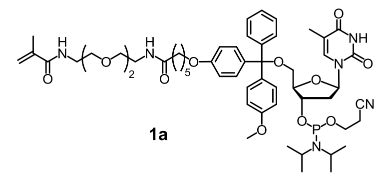
^{13}C NMR of **6d** in acetone-d_6 at 100 MHz



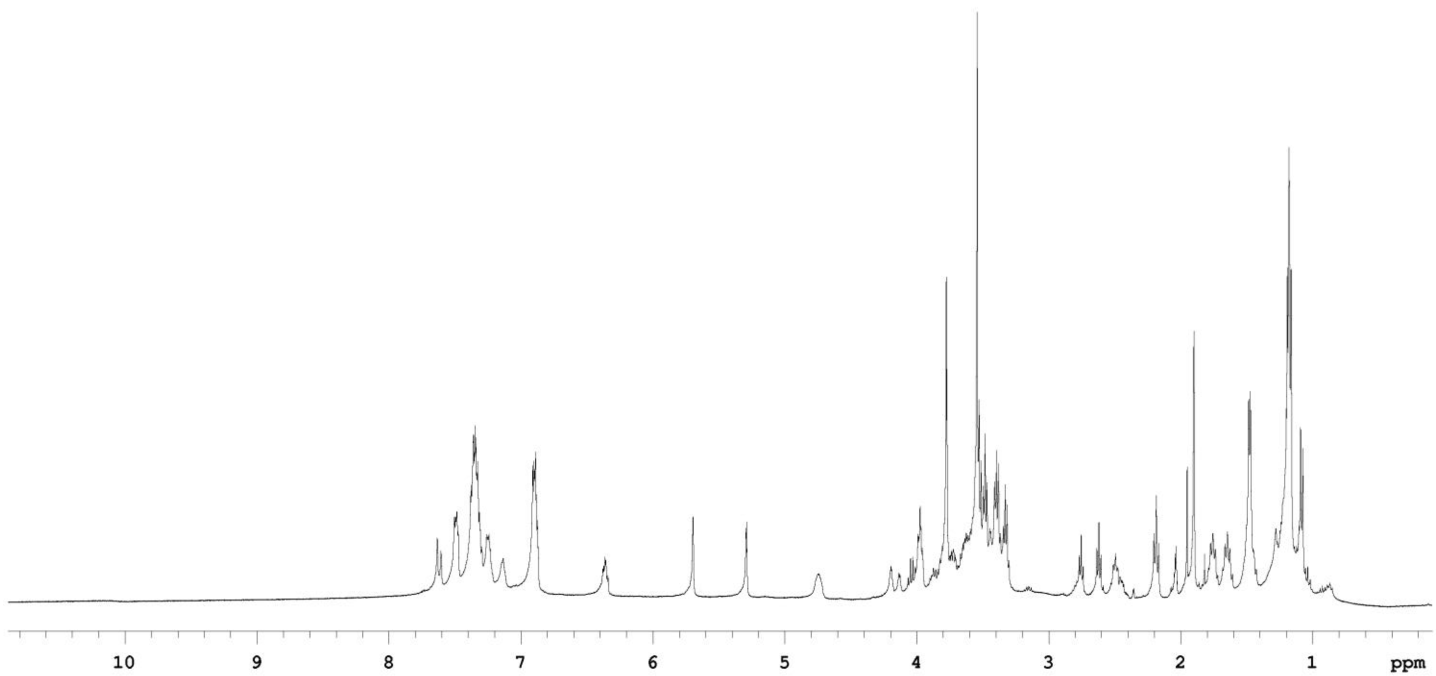
6d



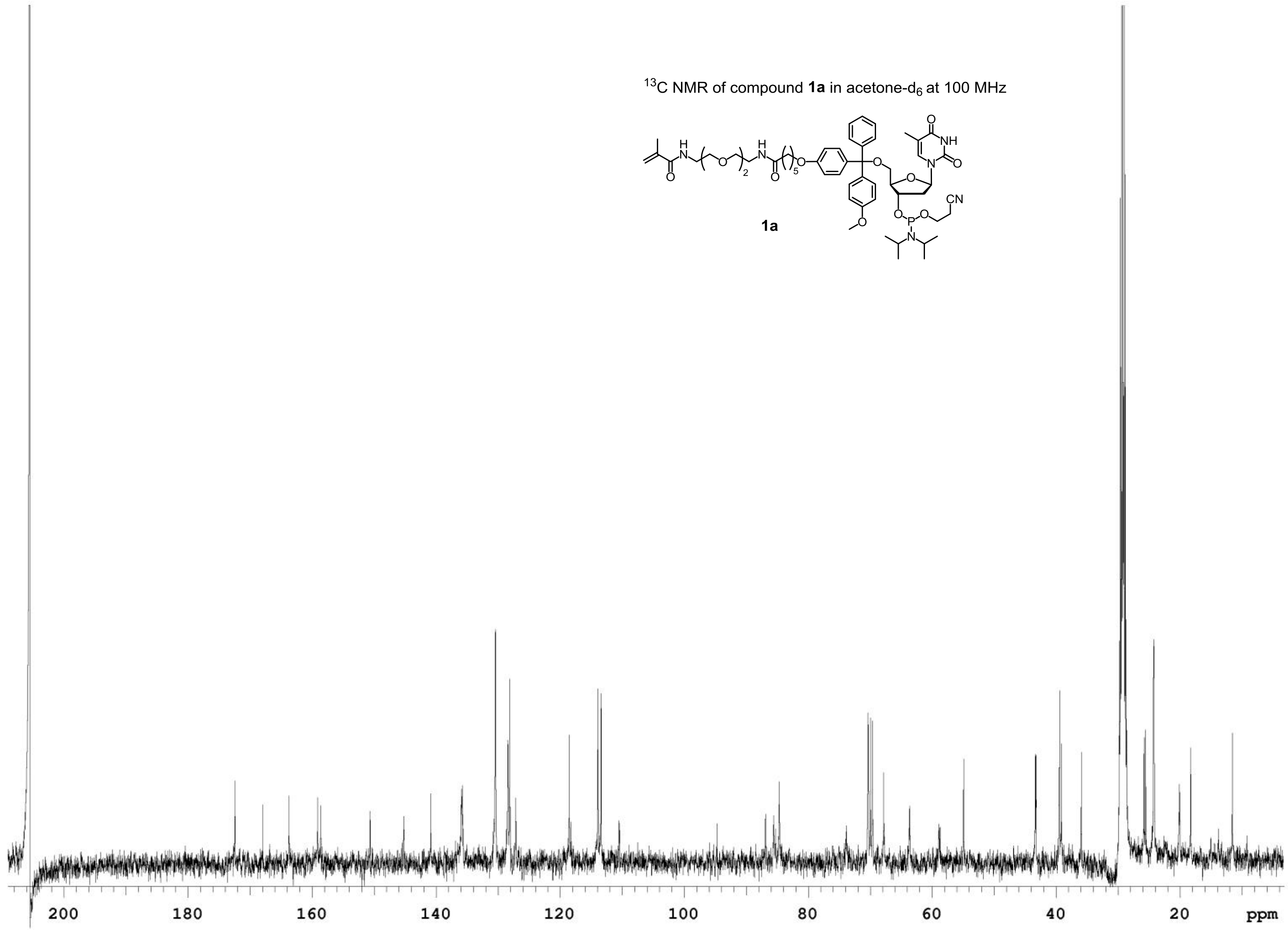
^1H NMR of compound **1a** in acetone-d_6 at 400 MHz



1a

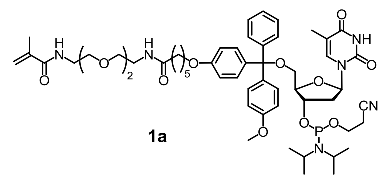


^{13}C NMR of compound **1a** in acetone-d_6 at 100 MHz

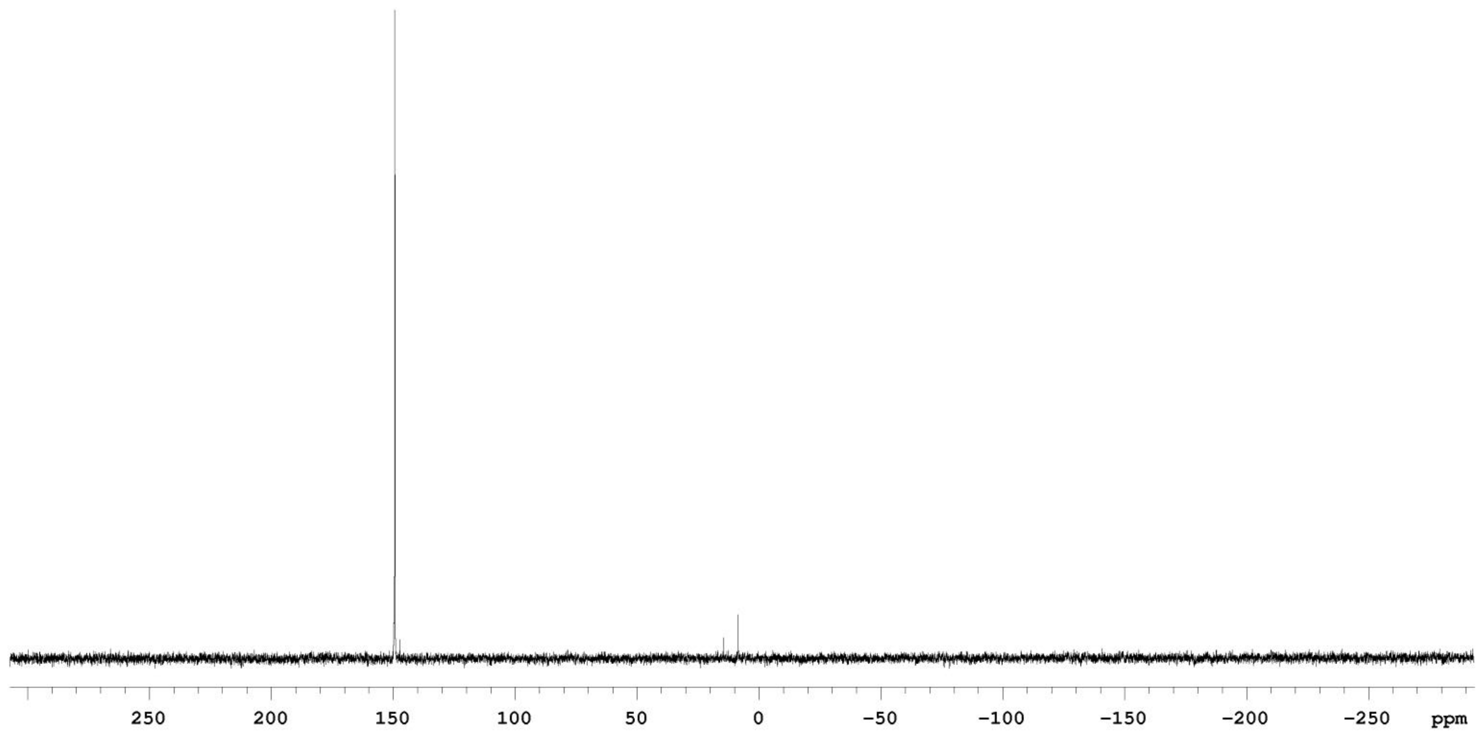


1a

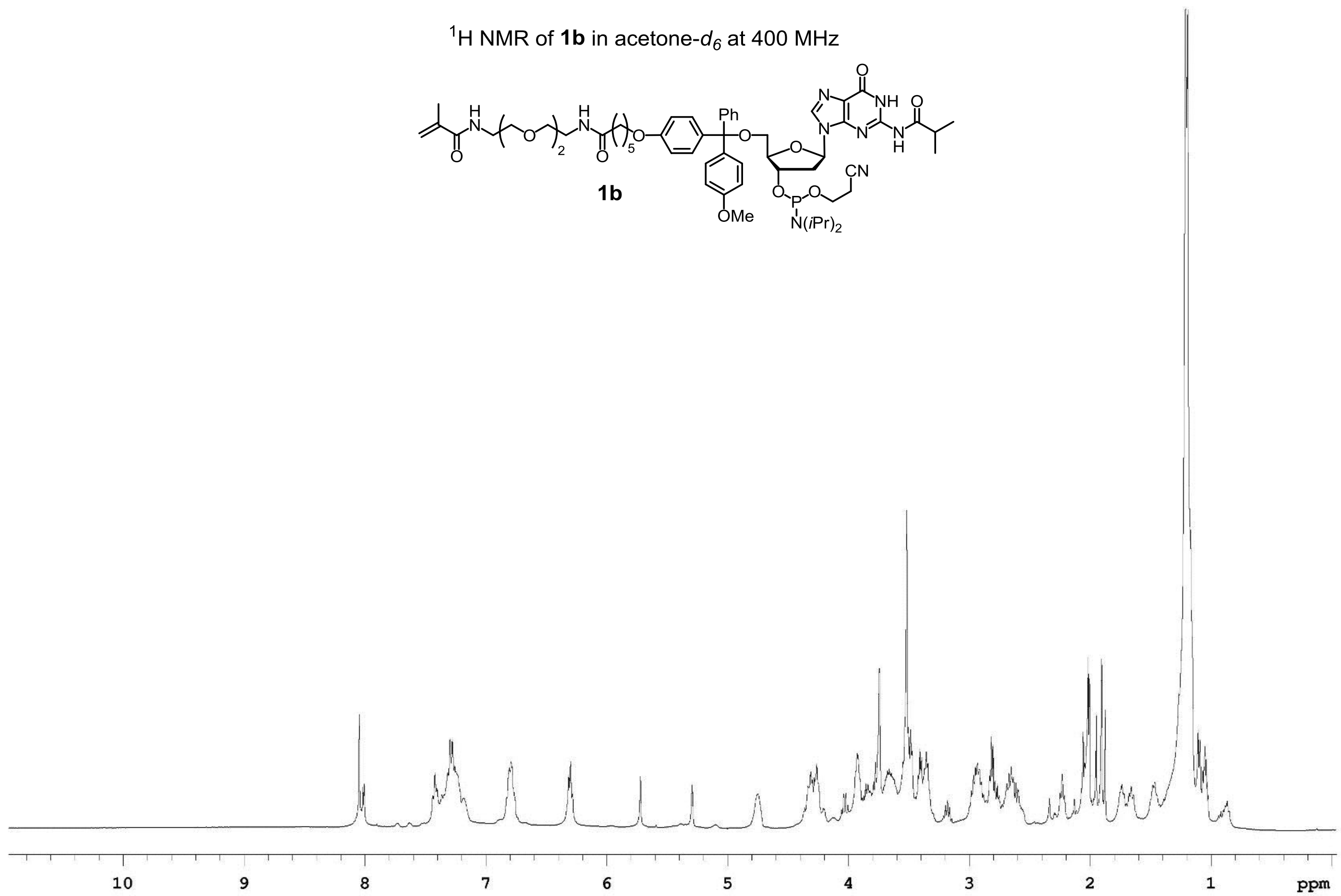
^{31}P NMR of compound **1a** in acetone-d_6 at 162 MHz

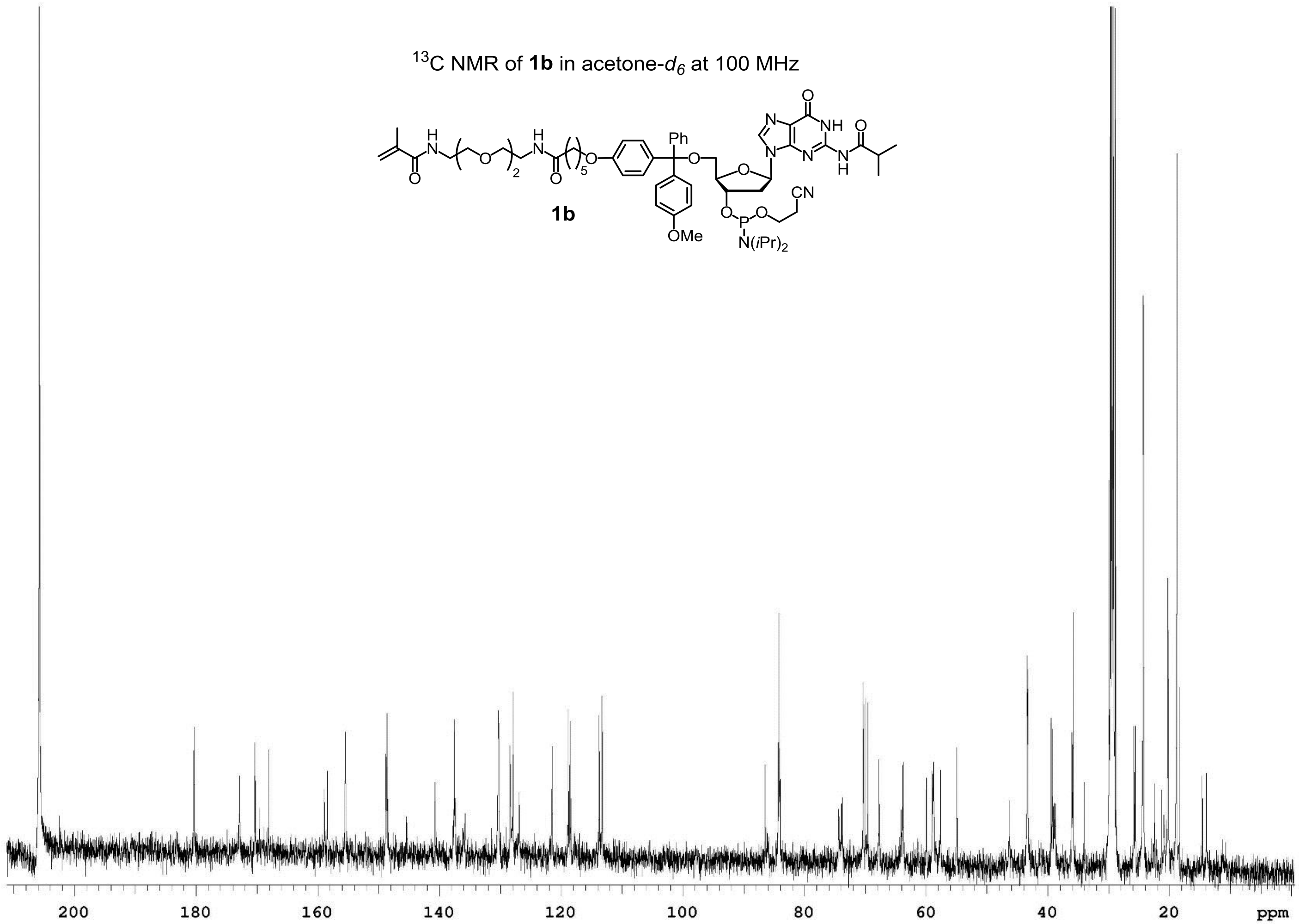


1a

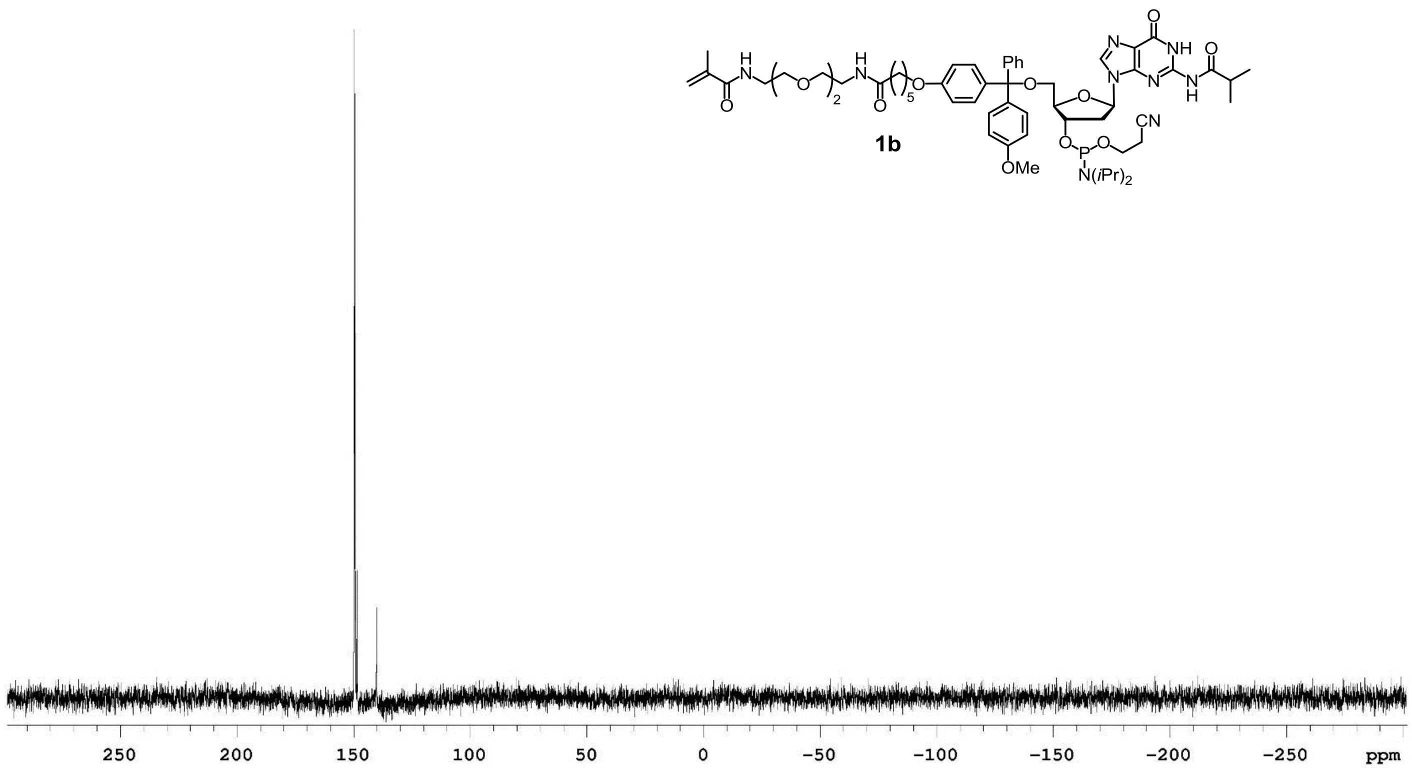


^{1}H NMR of **1b** in acetone-d_6 at 400 MHz

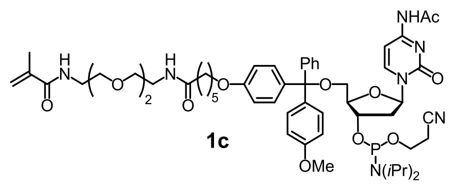



^{13}C NMR of **1b** in acetone-d_6 at 100 MHz
1b
200
180
160
140
120
100
80
60
40
20
ppm

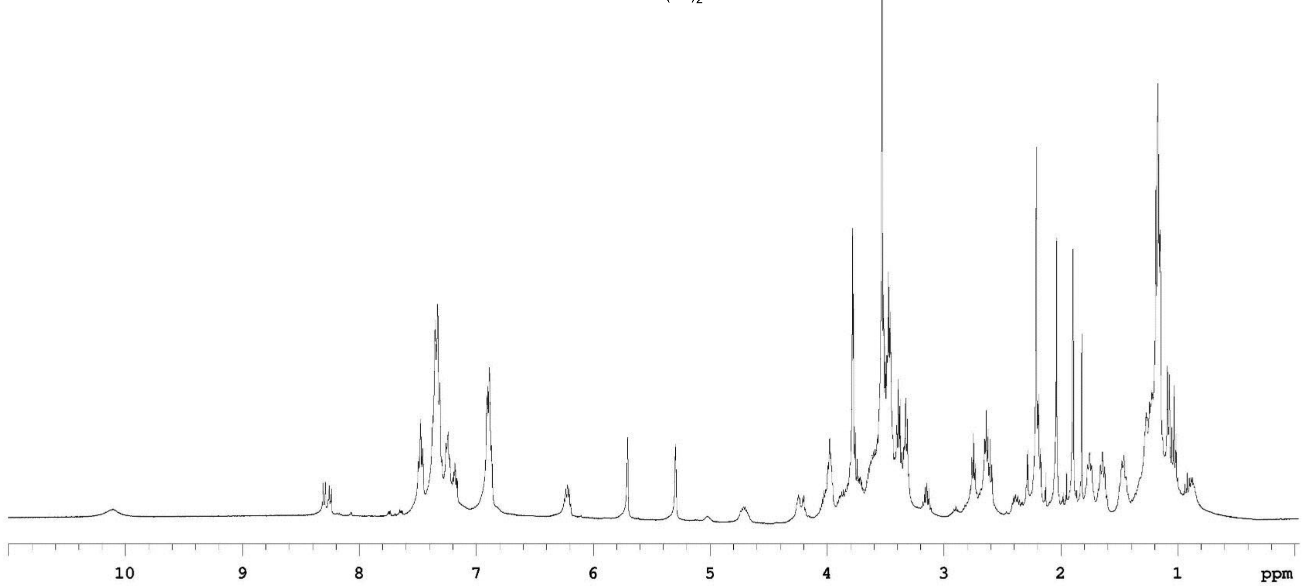
^{31}P NMR of **1b** in acetone-d_6 at 162 MHz



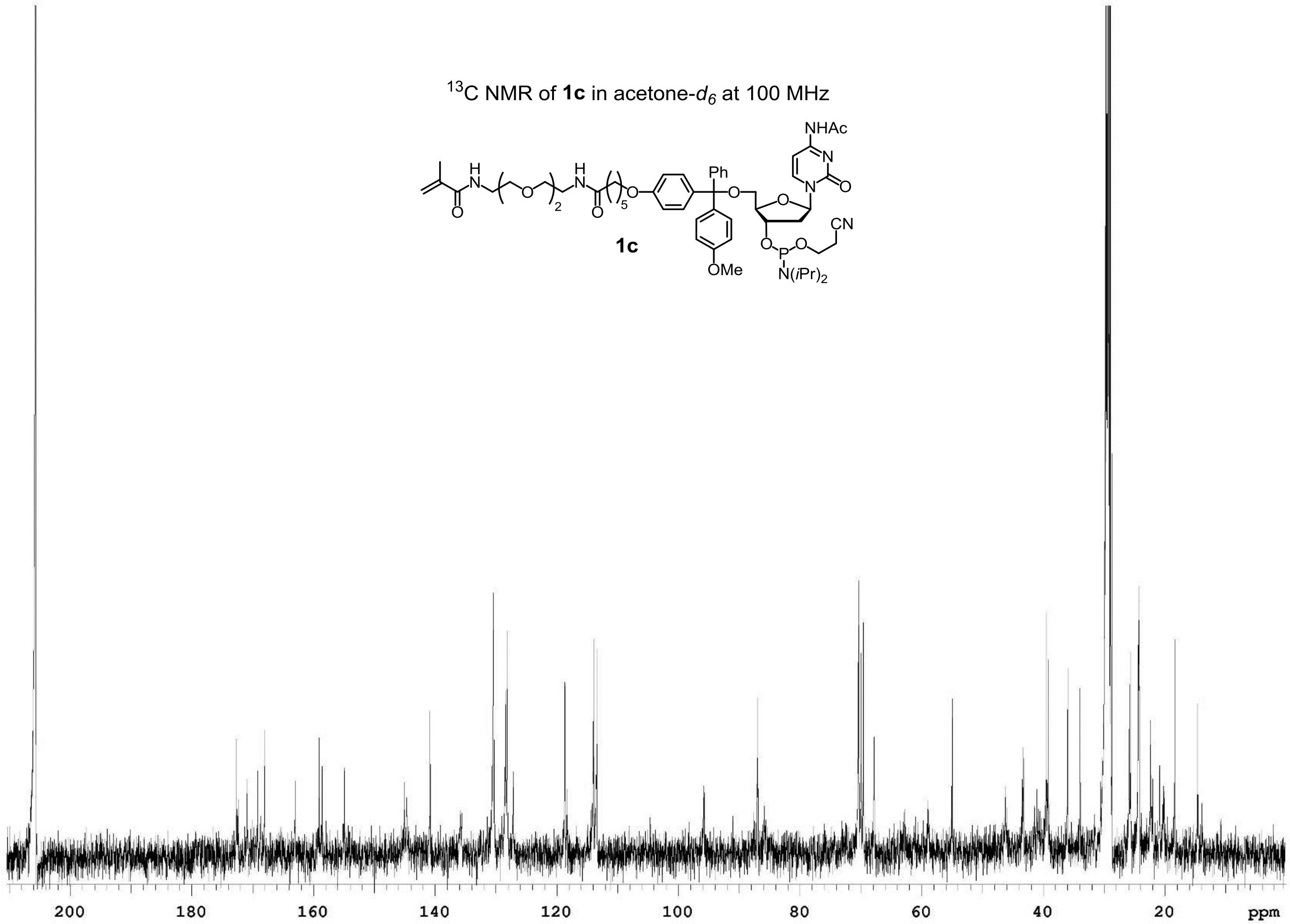
^{1}H NMR of **1c** in acetone-d_6 at 400 MHz



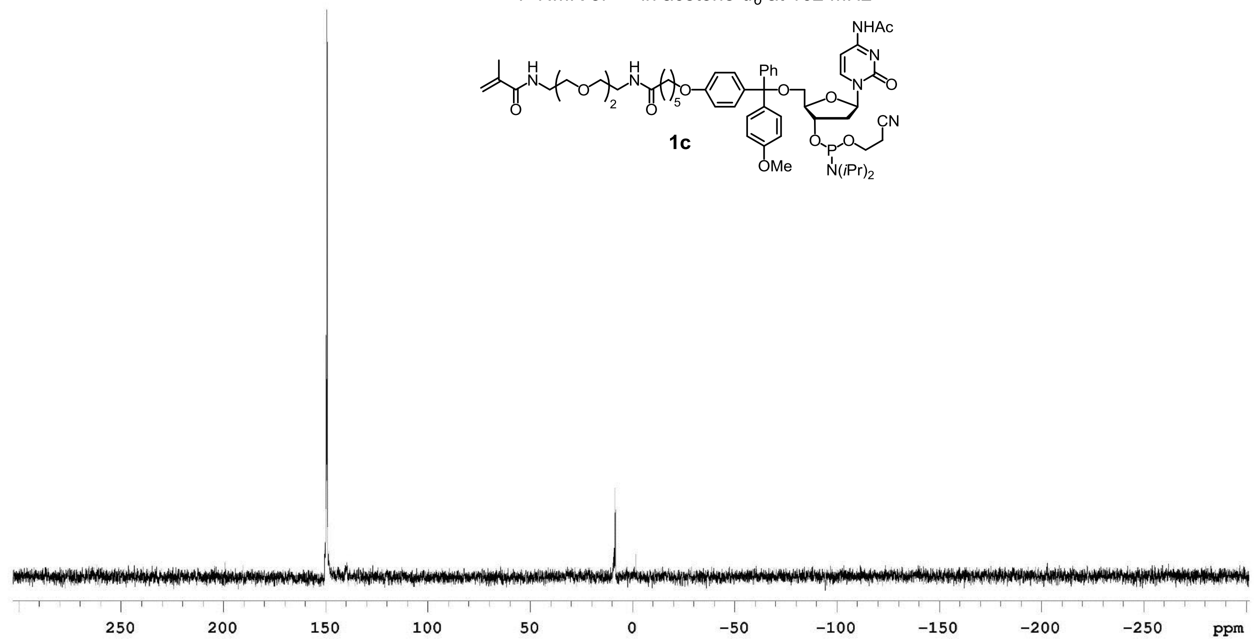
1c



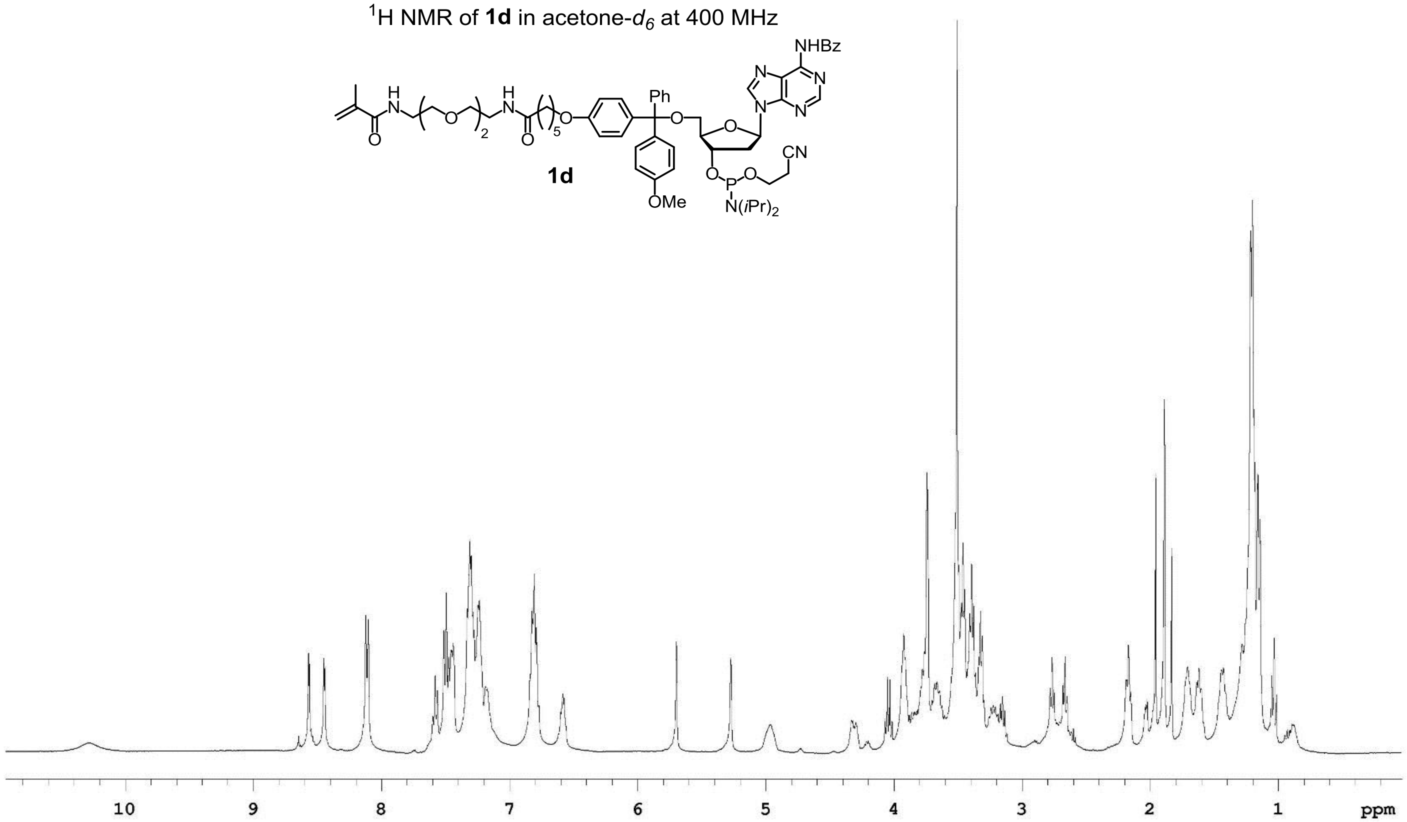
^{13}C NMR of **1c** in acetone-d_6 at 100 MHz

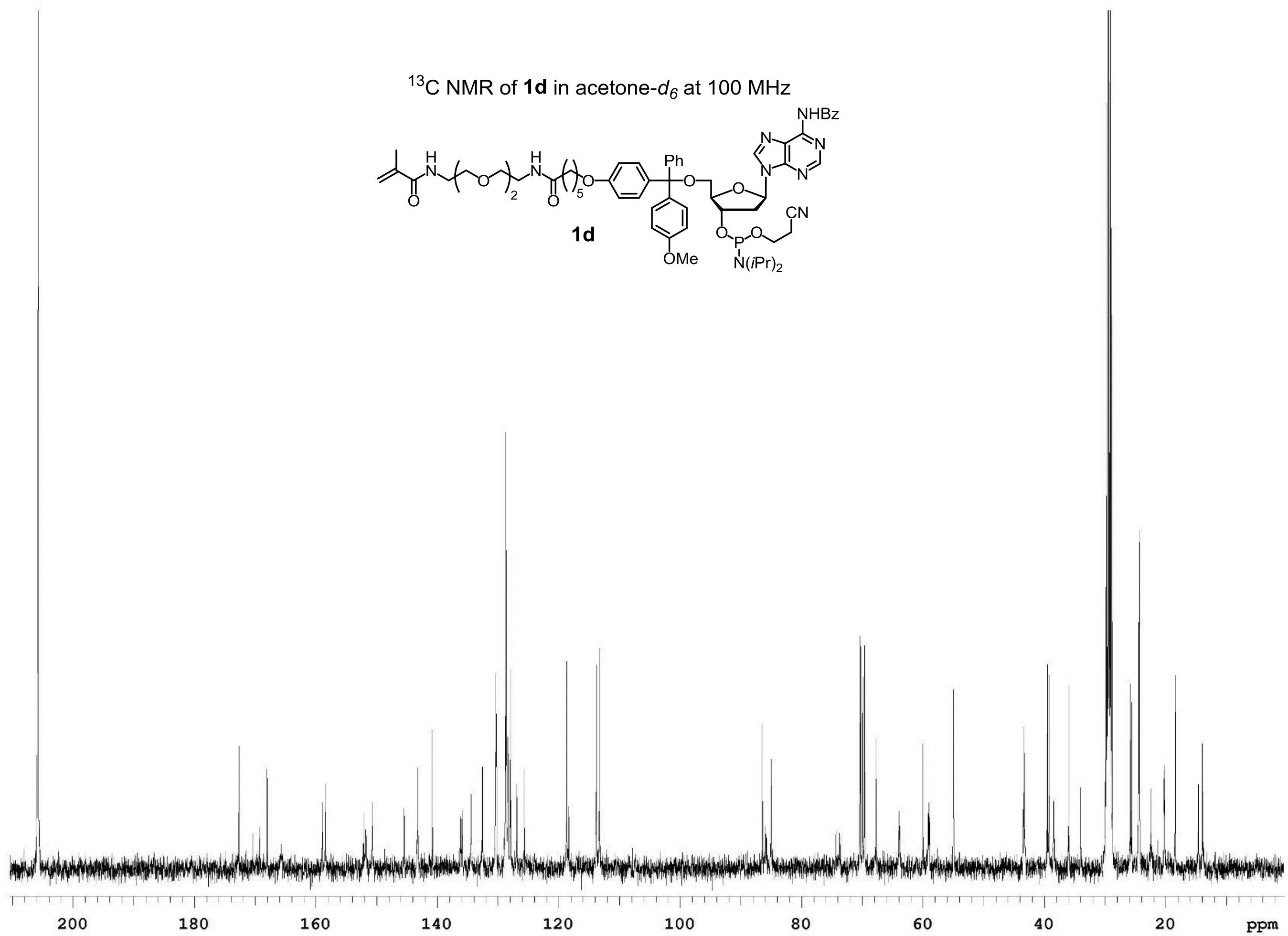


^{31}P NMR of **1c** in acetone-d_6 at 162 MHz



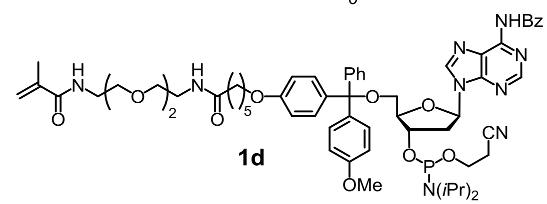
[1]H NMR of **1d** in acetone-d_6 at 400 MHz



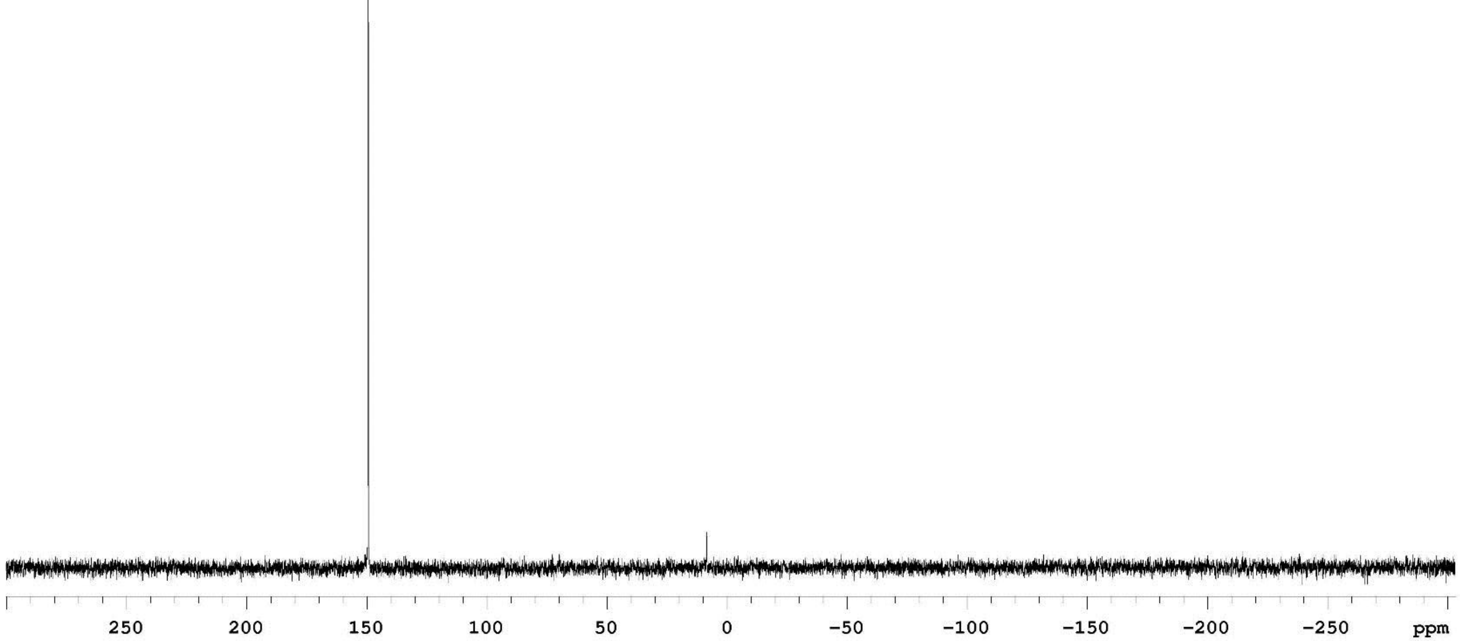


^{13}C NMR of **1d** in acetone-d_6 at 100 MHz
NHBz
Ph
CN
OMe
N(iPr)2
1d
200
180
160
140
120
100
80
60
40
20
ppm

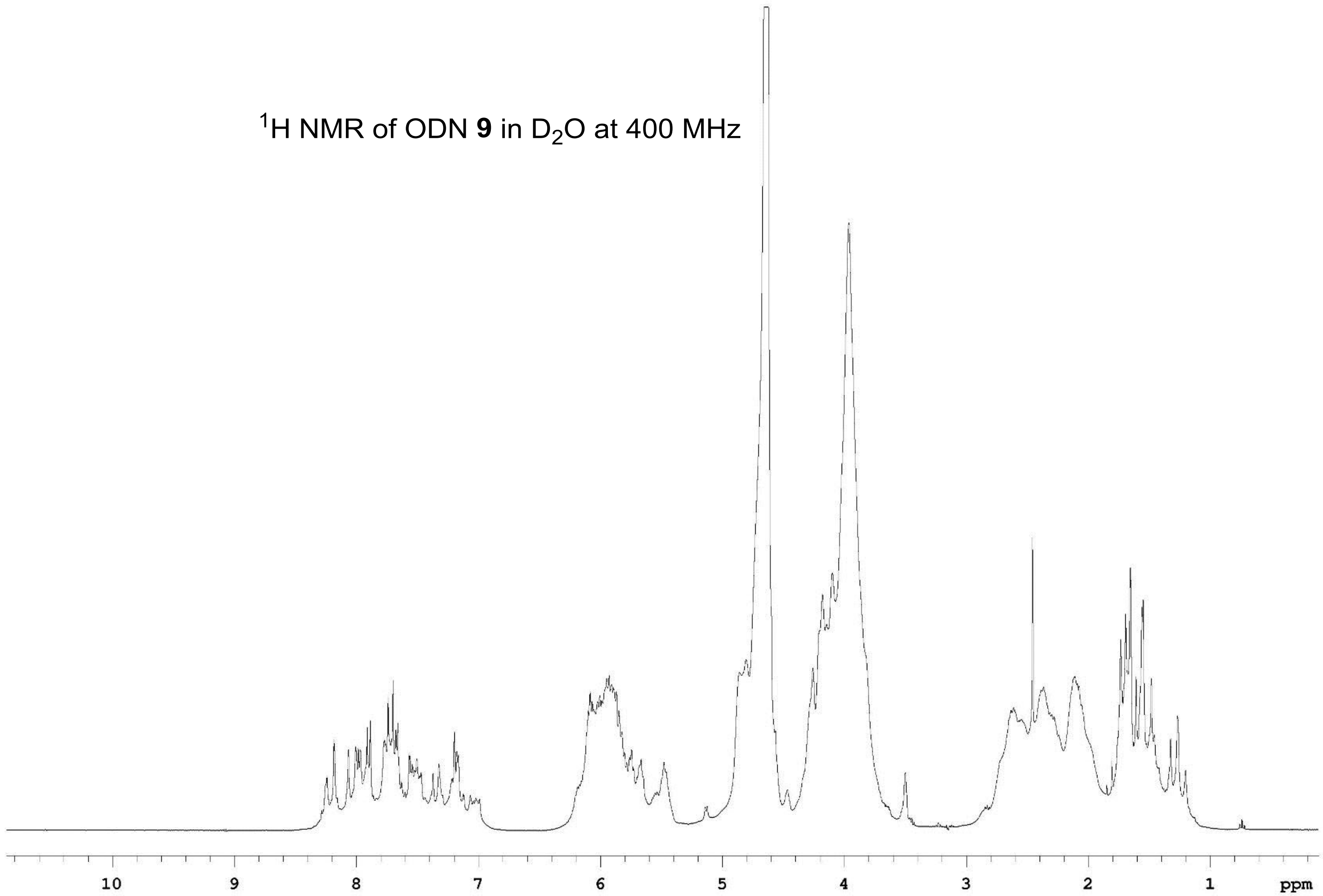
^{31}P NMR of **1d** in acetone-d_6 at 162 MHz



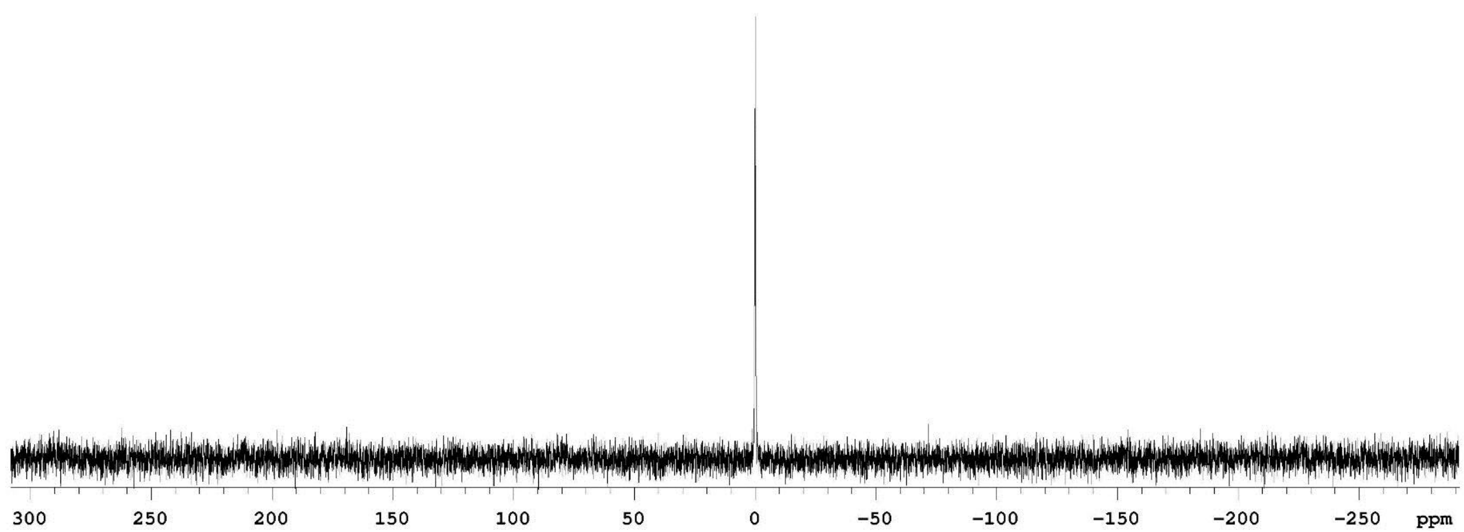
1d

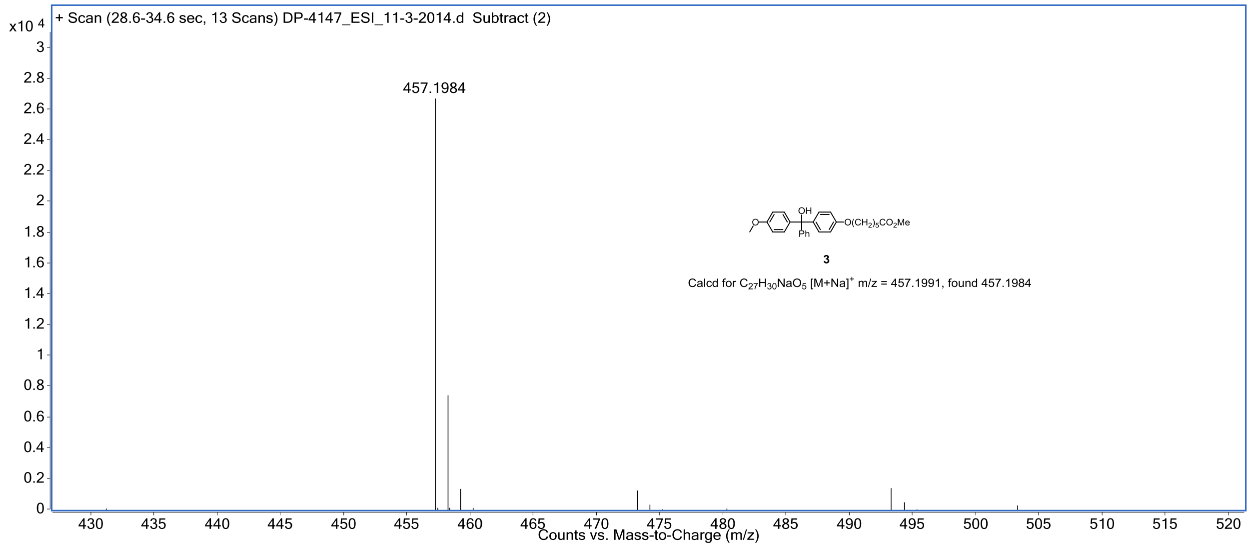


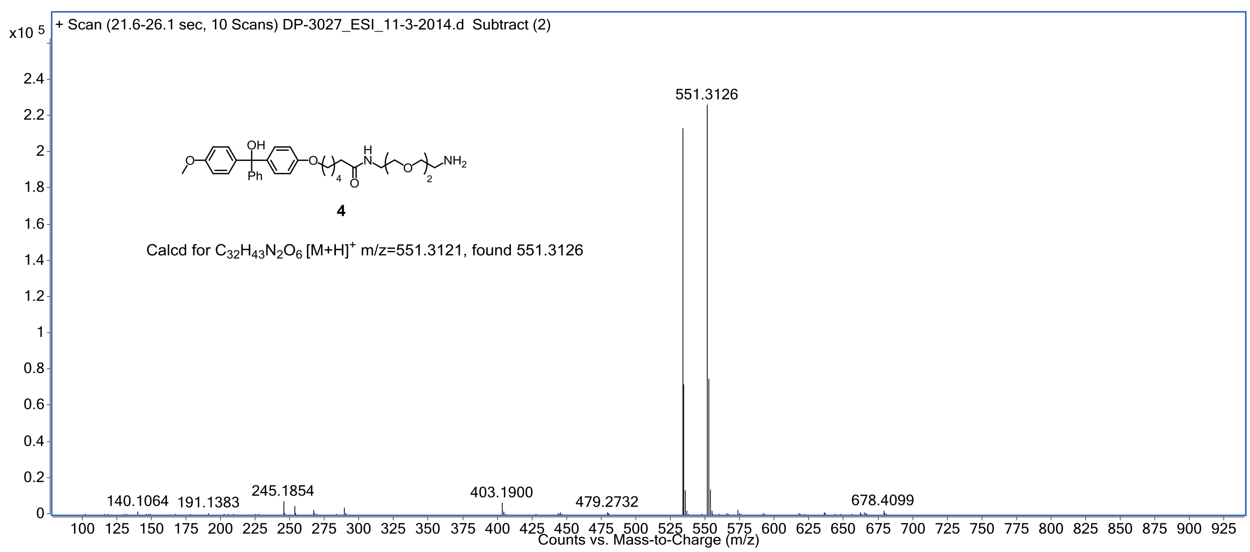
^1H NMR of ODN **9** in D_2O at 400 MHz

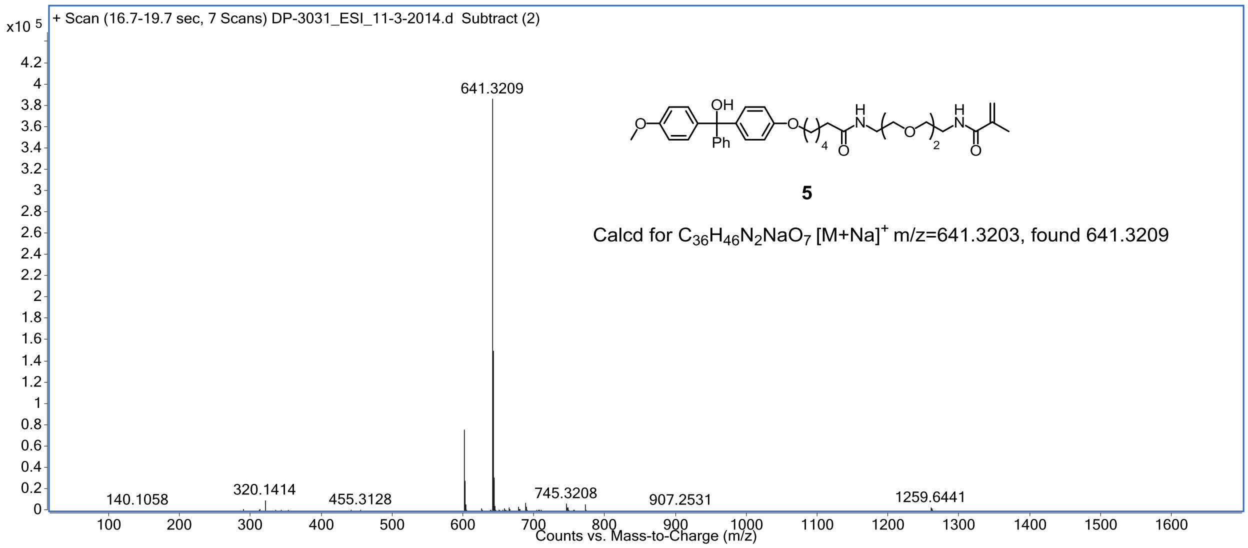


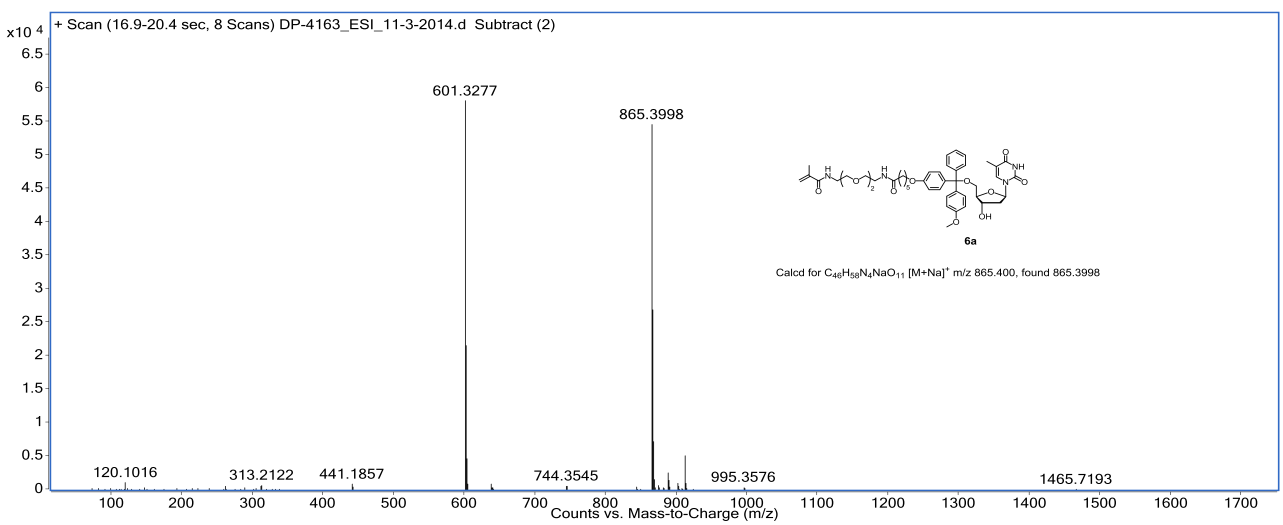
^{31}P NMR of ODN **9** in D_2O at 162 MHz

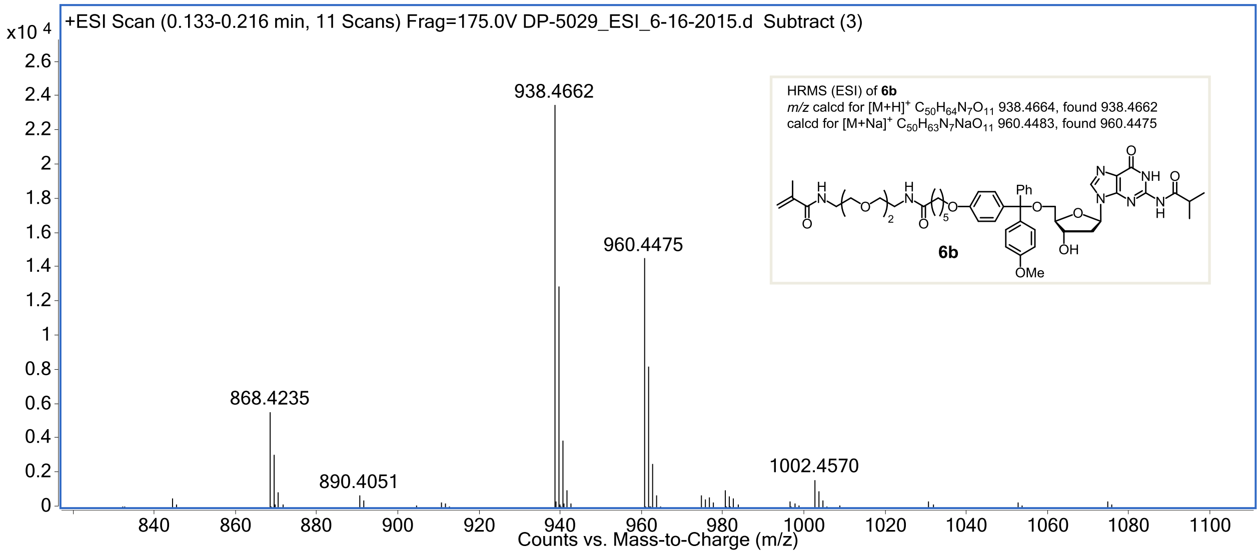


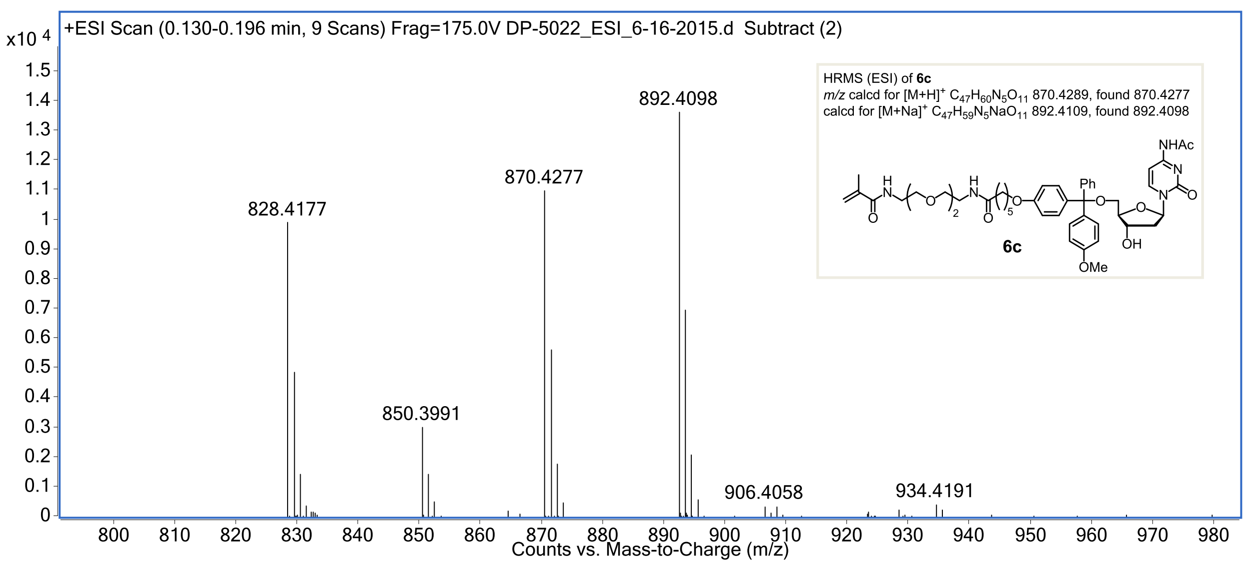

+ Scan (28.6-34.6 sec, 13 Scans) DP-4147_ESI_11-3-2014.d Subtract (2)
x10 4
457.1984
OH
O(CH2)5CO2Me
Ph
3
Calcd for $C_{27}H_{30}NaO_5$ $[M+Na]^+$ m/z = 457.1991, found 457.1984
Counts vs. Mass-to-Charge (m/z)

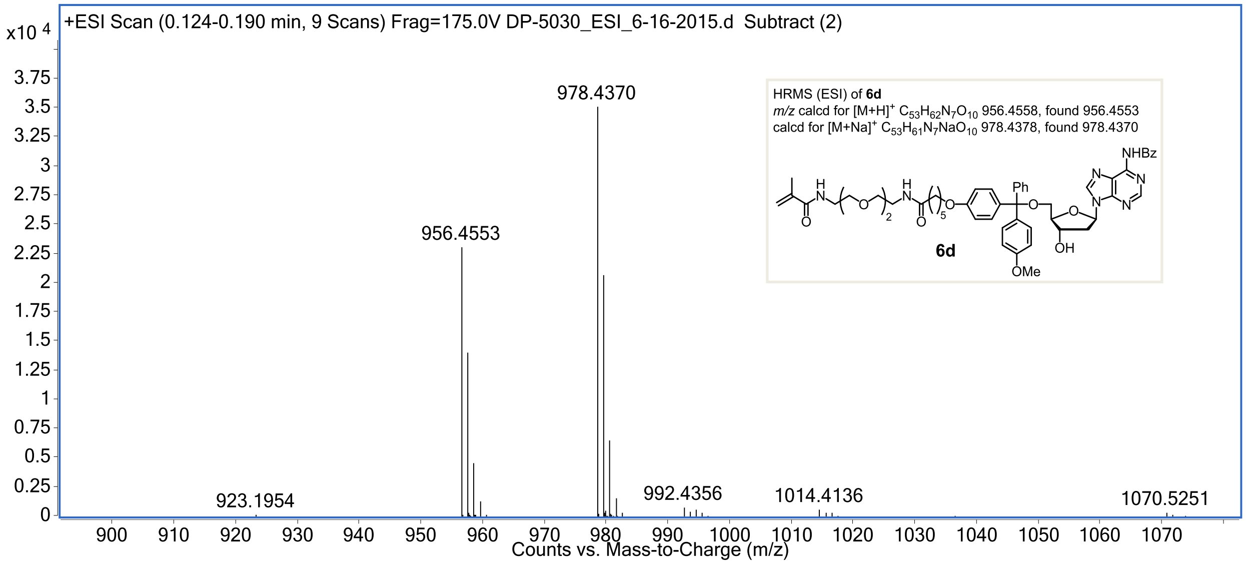

+ Scan (21.6-26.1 sec, 10 Scans) DP-3027_ESI_11-3-2014.d Subtract (2)
x10 5
551.3126
OH
Ph
H
N
4
2
NH2
4
Calcd for $C_{32}H_{43}N_2O_6$ $[M+H]^+$ m/z=551.3121, found 551.3126
140.1064
191.1383
245.1854
403.1900
479.2732
678.4099
Counts vs. Mass-to-Charge (m/z)

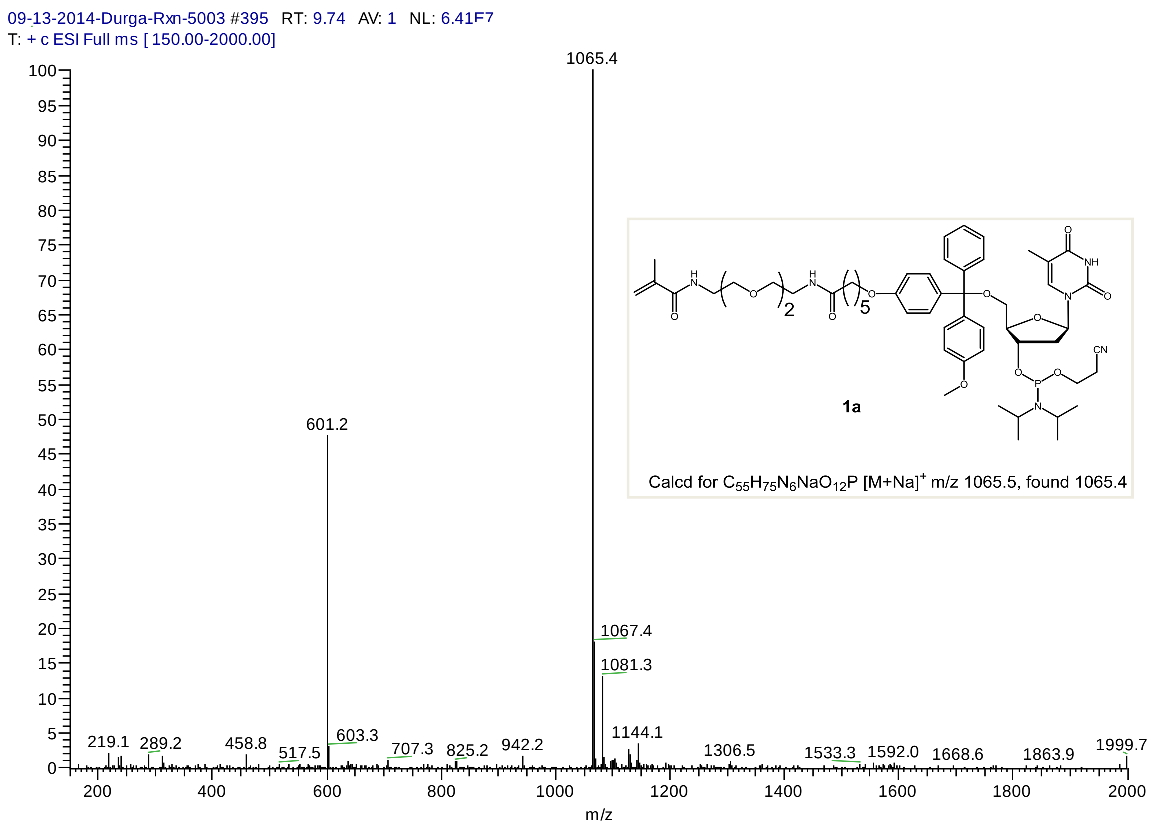

+ Scan (16.7-19.7 sec, 7 Scans) DP-3031_ESI_11-3-2014.d Subtract (2)
x10 5
641.3209
140.1058
320.1414
455.3128
745.3208
907.2531
1259.6441
5
Calcd for $C_{36}H_{46}N_2NaO_7$ $[M+Na]^+$ m/z=641.3203, found 641.3209
Counts vs. Mass-to-Charge (m/z)

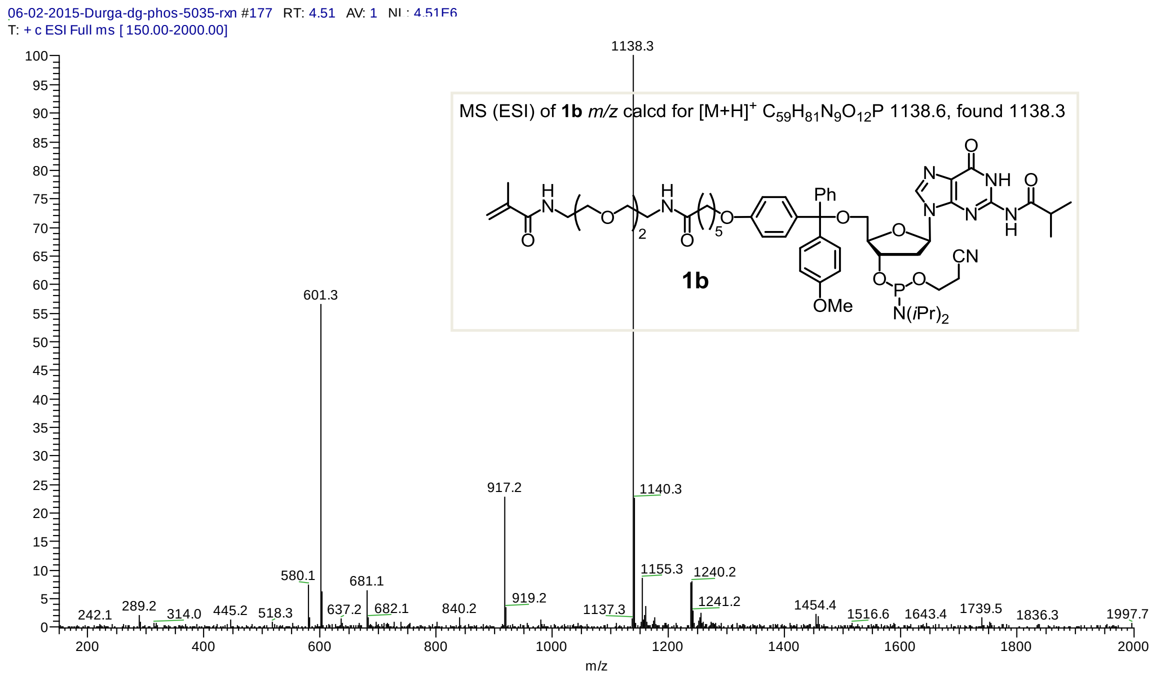

+ Scan (16.9-20.4 sec, 8 Scans) DP-4163_ESI_11-3-2014.d Subtract (2)
x10 4
601.3277
865.3998
120.1016
313.2122
441.1857
744.3545
995.3576
1465.7193
6a
Calcd for $C_{46}H_{58}N_4NaO_{11}$ $[M+Na]^+$ m/z 865.400, found 865.3998
Counts vs. Mass-to-Charge (m/z)

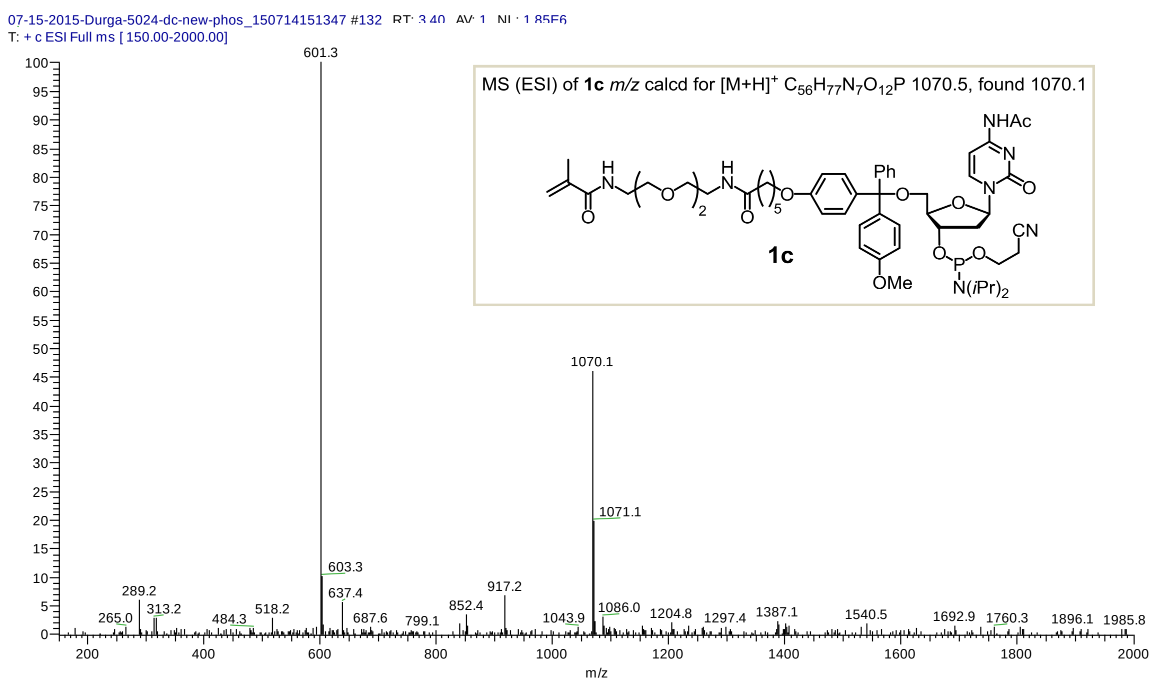

+ESI Scan (0.133-0.216 min, 11 Scans) Frag=175.0V DP-5029_ESI_6-16-2015.d Subtract (3)
x10 4
938.4662
960.4475
868.4235
890.4051
1002.4570
Counts vs. Mass-to-Charge (m/z)
HRMS (ESI) of **6b**
m/z calcd for $[M+H]^+$ $C_{50}H_{64}N_7O_{11}$ 938.4664, found 938.4662
calcd for $[M+Na]^+$ $C_{50}H_{63}N_7NaO_{11}$ 960.4483, found 960.4475
6b

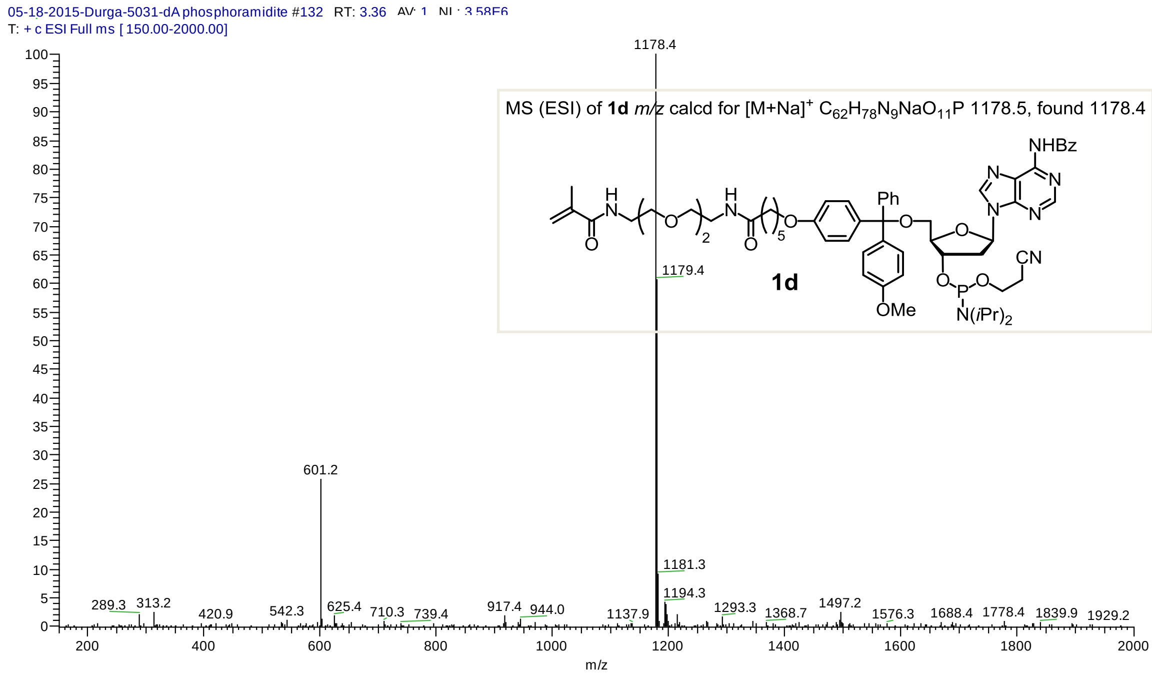

+ESI Scan (0.130-0.196 min, 9 Scans) Frag=175.0V DP-5022_ESI_6-16-2015.d Subtract (2)
x10 4
892.4098
870.4277
828.4177
850.3991
906.4058
934.4191
Counts vs. Mass-to-Charge (m/z)
HRMS (ESI) of **6c**
m/z calcd for $[M+H]^+$ $C_{47}H_{60}N_5O_{11}$ 870.4289, found 870.4277
calcd for $[M+Na]^+$ $C_{47}H_{59}N_5NaO_{11}$ 892.4109, found 892.4098
6c

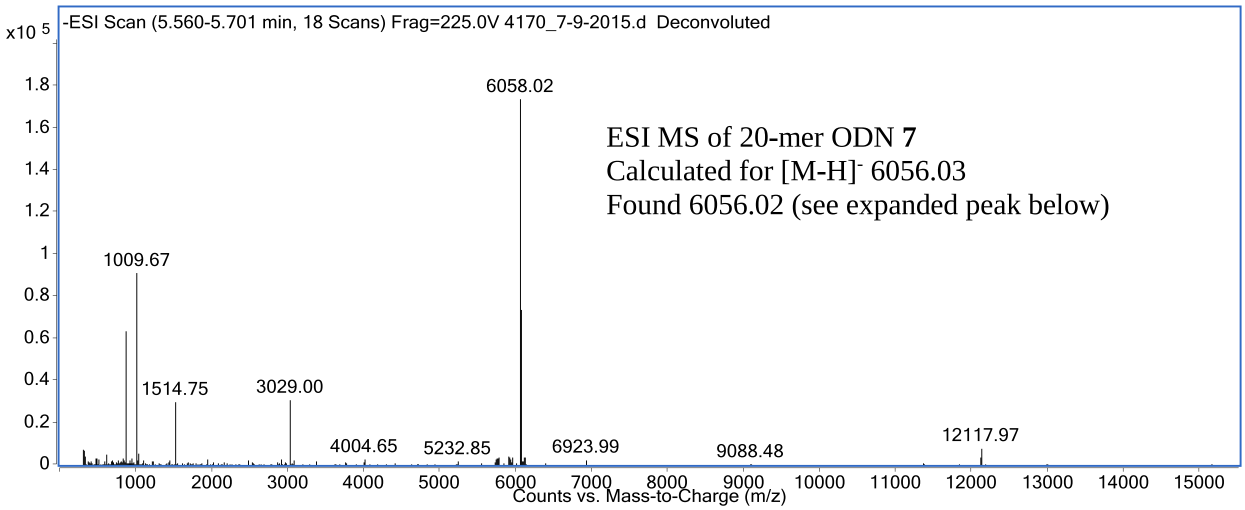

+ESI Scan (0.124-0.190 min, 9 Scans) Frag=175.0V DP-5030_ESI_6-16-2015.d Subtract (2)
x10 4
978.4370
956.4553
923.1954
992.4356
1014.4136
1070.5251
Counts vs. Mass-to-Charge (m/z)
HRMS (ESI) of 6d
m/z calcd for [M+H]+ C53H62N7O10 956.4558, found 956.4553
calcd for [M+Na]+ C53H61N7NaO10 978.4378, found 978.4370
6d

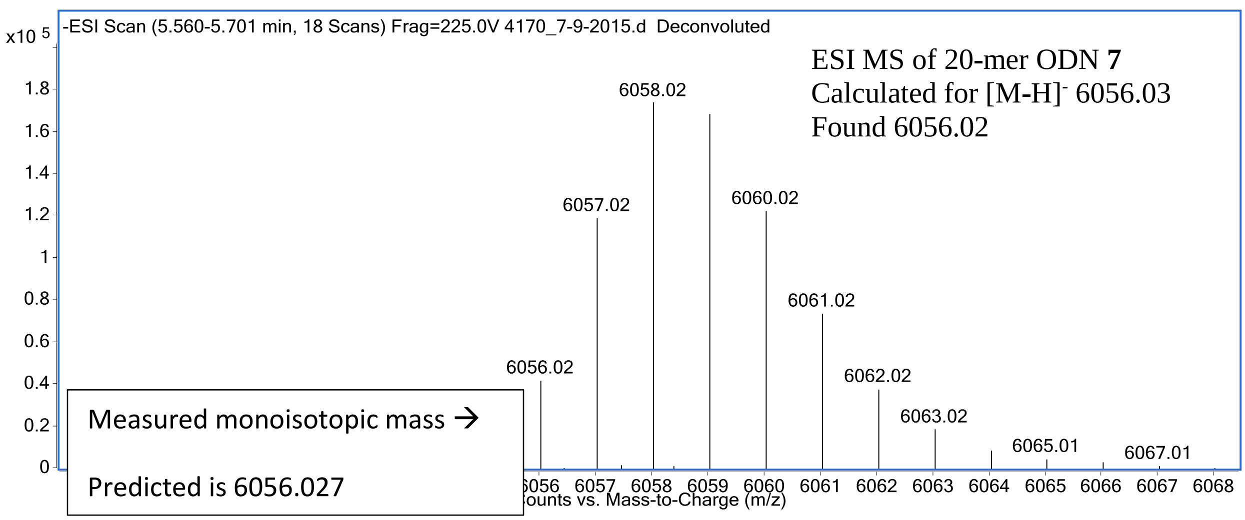

09-13-2014-Durga-Rxn-5003 #395 RT: 9.74 AV: 1 NL: 6.41F7
T: + c ESI Full ms [150.00-2000.00]
1065.4
601.2
1067.4
1081.3
1144.1
219.1
289.2
458.8
517.5
603.3
707.3
825.2
942.2
1306.5
1533.3
1592.0
1668.6
1863.9
1999.7
m/z
1a
Calcd for C55H75N6NaO12P [M+Na]+ m/z 1065.5, found 1065.4

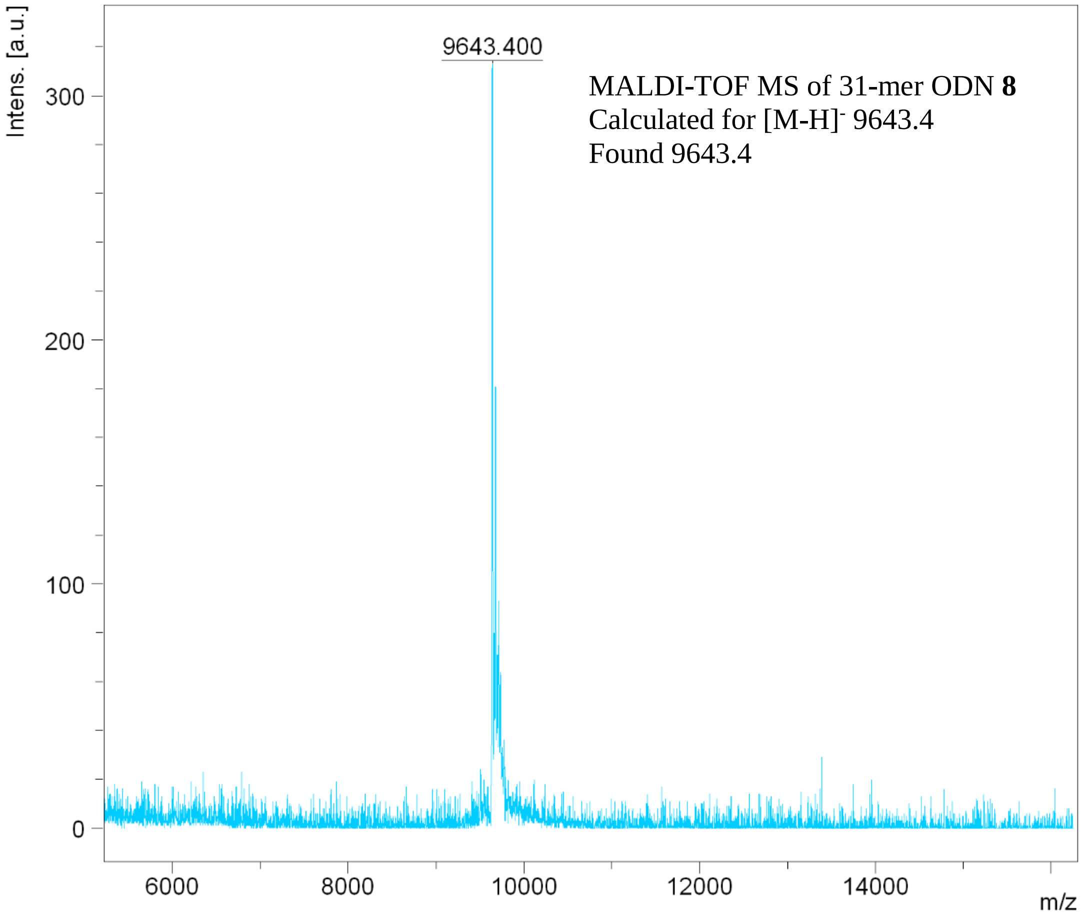

06-02-2015-Durga-dg-phos-5035-rxn #177 RT: 4.51 AV: 1 NL: 4.51E6
T: + c ESI Full ms [150.00-2000.00]
MS (ESI) of 1b m/z calcd for [M+H]+ C59H81N9O12P 1138.6, found 1138.3
1b
1138.3
601.3
917.2
1140.3
580.1
681.1
1155.3
1240.2
1241.2
919.2
242.1
289.2
314.0
445.2
518.3
637.2
682.1
840.2
1137.3
1454.4
1516.6
1643.4
1739.5
1836.3
1997.7
m/z


07-15-2015-Durga-5024-dc-new-phos_150714151347 #132 RT: 3.40 AV: 1 NL: 1.85E6
T: + c ESI Full ms [150.00-2000.00]
MS (ESI) of 1c m/z calcd for [M+H]+ C56H77N7O12P 1070.5, found 1070.1
1c
601.3
1070.1
1071.1
603.3
637.4
289.2
917.2
852.4
265.0
313.2
484.3
518.2
687.6
799.1
1043.9
1086.0
1204.8
1297.4
1387.1
1540.5
1692.9
1760.3
1896.1
1985.8
m/z

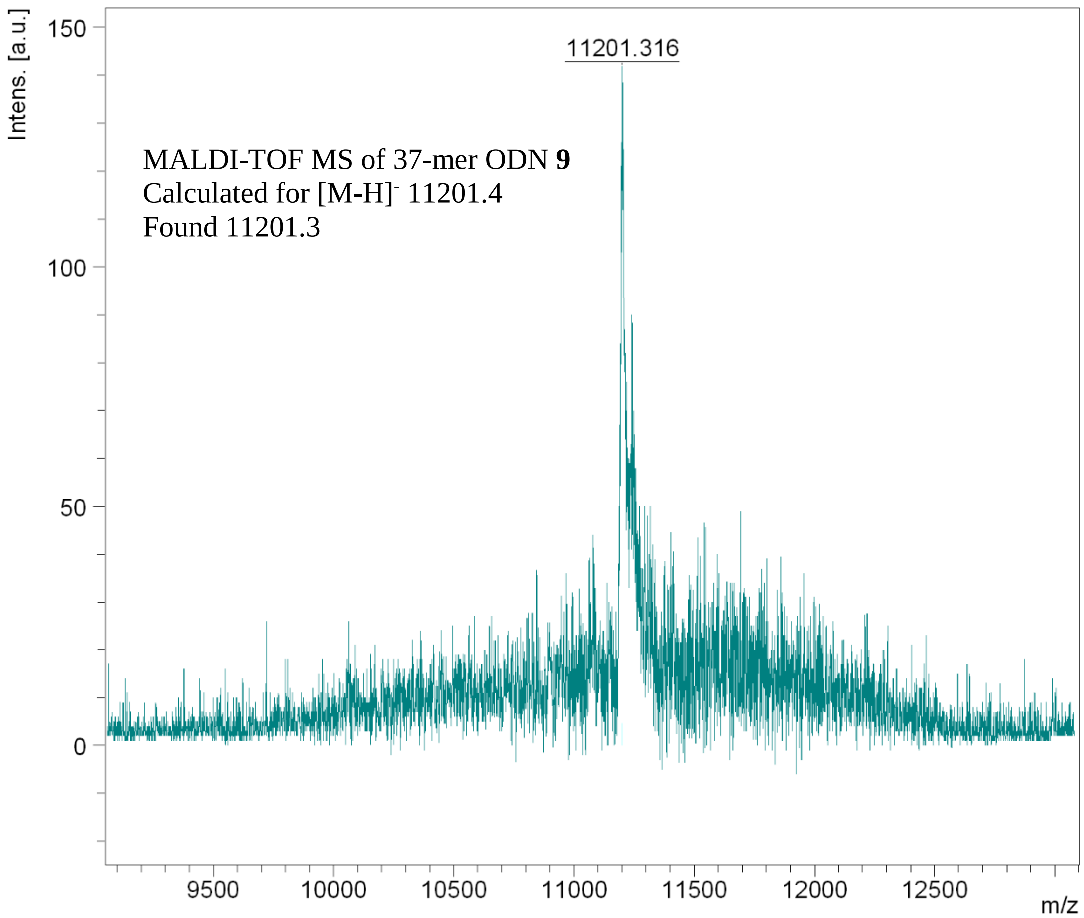

05-18-2015-Durga-5031-dA phosphoramidite #132 RT: 3.36 AV: 1 NL: 3.58E6
T: + c ESI Full ms [150.00-2000.00]
MS (ESI) of 1d m/z calcd for [M+Na]+ C62H78N9NaO11P 1178.5, found 1178.4
1d
1178.4
1179.4
601.2
1181.3
1194.3
m/z

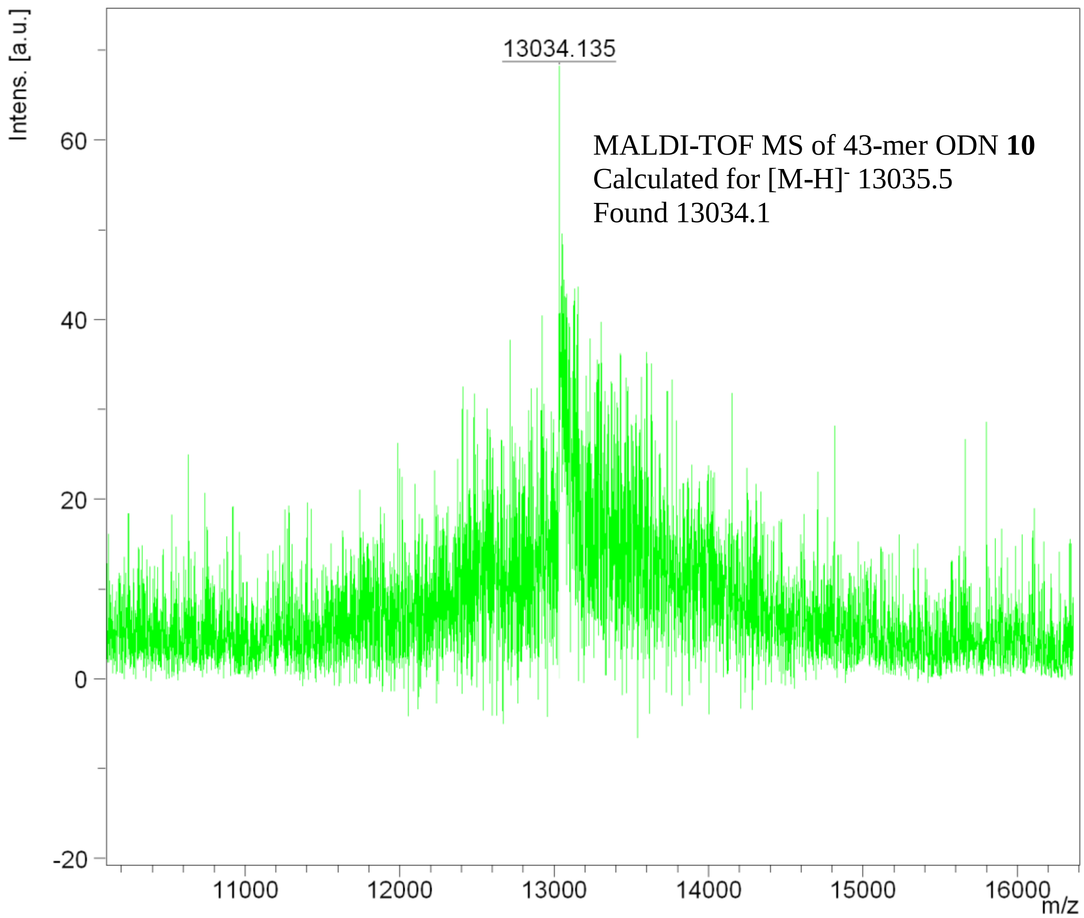

-ESI Scan (5.560-5.701 min, 18 Scans) Frag=225.0V 4170_7-9-2015.d Deconvoluted
x10 5
6058.02
1009.67
1514.75
3029.00
4004.65
5232.85
6923.99
9088.48
12117.97
Counts vs. Mass-to-Charge (m/z)
ESI MS of 20-mer ODN **7**
Calculated for [M-H]- 6056.03
Found 6056.02 (see expanded peak below)

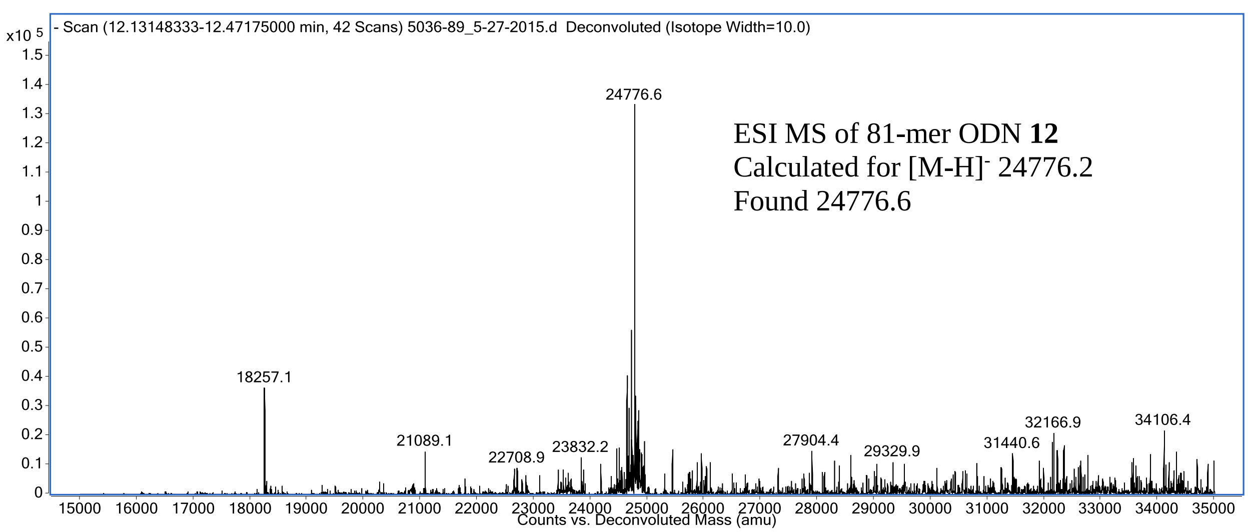

-ESI Scan (5.560-5.701 min, 18 Scans) Frag=225.0V 4170_7-9-2015.d Deconvoluted
x10 5
ESI MS of 20-mer ODN **7**
Calculated for [M-H]- 6056.03
Found 6056.02
6058.02
6057.02
6060.02
6061.02
6056.02
6062.02
6063.02
6065.01
6067.01
Measured monoisotopic mass →
Predicted is 6056.027
Counts vs. Mass-to-Charge (m/z)



9643.400
MALDI-TOF MS of 31-mer ODN **8**
Calculated for [M-H]- 9643.4
Found 9643.4
Intens. [a.u.]
300
200
100
0
6000
8000
10000
12000
14000
m/z


Intens. [a.u.]
11201.316
MALDI-TOF MS of 37-mer ODN **9**
Calculated for [M-H]$^-$ 11201.4
Found 11201.3
150
100
50
0
9500
10000
10500
11000
11500
12000
12500
m/z


MALDI-TOF MS of 43-mer ODN **10**
Calculated for [M-H]$^-$ 13035.5
Found 13034.1
13034.135
Intens. [a.u.]
60
40
20
0
-20
11000
12000
13000
14000
15000
16000
m/z


- Scan (12.13148333-12.47175000 min, 42 Scans) 5036-89_5-27-2015.d Deconvoluted (Isotope Width=10.0)
ESI MS of 81-mer ODN **12**
Calculated for [M-H]$^-$ 24776.2
Found 24776.6
x10 5
24776.6
18257.1
21089.1
22708.9
23832.2
27904.4
29329.9
31440.6
32166.9
34106.4
Counts vs. Deconvoluted Mass (amu)

Protocol:

Purification of Synthetic Oligodeoxynucleotide by Polymerizing Full-Length Sequences

PRINCIPLE

During oligodeoxynucleotide (ODN) synthesis, the failure sequences are capped with Ac_2O and full-length sequences are tagged with the polymerizable methacryl phosphoramidite **1a**-**c** or **1d** depending on the last nucleotide on the 5'-end. After cleavage and deprotection, the crude contains the full-length sequences (the product) with a polymerizable group, failure sequences and small molecules. In purification, the full-length sequences are copolymerized into a polyacrylamide gel. Failure sequences and small molecules are washed away. The product is cleaved and extracted, and precipitated with *n*BuOH.

MATERIALS

Polymerizable tagging methacrylamidite **1a**-**c** or **1d** (**1a**, MW 1043.2, 52.2 mg, 50 µmol; **1b**, MW 1138.3, 56.9 mg, 50 µmol; **1c**, MW 1070.2, 53.5 mg, 50 µmol; **1d**, MW 1156.3, 57.8 mg, 50 µmol; the amount may be enough for two 1 µmol synthesis/purification)
Polymerization mix (**PM**, *N*,*N*-dimethylacrylamide, MW 99.1, d 0.96, 218 mg, 227 µl, 2.21 mmol; *N*,*N'*-methylenebis(acrylamide), MW 154.2, 17 mg, 110 µmol; cross-linking ratio 20:1)
Polymerization initiator A (**PIA**, ammonium persulfate, MW 228.2, 10 mg, 44 µmol)
Polymerization initiator B (**PIB**, *N*,*N*,*N'*,*N'*-tetramethylethylenediamine, MW 116.2, d 0.78, 15.5 mg, 20 µl, 132 µmol)
Centrifugal filter unit

PROTOCOL

*ODN synthesis using **1a-c** or **1d** to tag full-length sequences*

1. Set up ODN synthesis on a synthesizer (ABI-394 is used as example) as usual using Ac_2O or Pac_2O for capping with the exceptions describe below. Do not use amidite solutions that are stored for more than two weeks.
2. Add 500 µl dry acetonitrile to **1a**-**c** or **1d** (0.1 M). Shake gently to dissolve.
3. Attach the **1a**-**c** or **1d** solution to the 5^{th} amidite position. Minimize exposure of the solution to air.
4. Create a new cycle by modifying the standard 1 µmol cycle. Copy the standard cycle to a new file (for < 1 µmol synthesis, copy the corresponding standard cycle). After the coupling steps and before the capping steps, insert a 180 sec (or slightly longer) waiting step and set the step active only for base 5.
5. When editing sequence, add base 5 at the 5′-end (the sequence will have dT, dG, dC or dA at the 5′-end).
6. Start synthesis using the new cycle. Select 5′-DMTr on. Perform cleavage and deprotection as usual (conc. NH_4OH, 55 °C, 10-15 h; or conc. $NH_4OH/MeNH_2$ 1/1, rt, ≥3 h).

7. Transfer the ODN solution into a 1.5 ml centrifuge tube with a pipette. Add ~100 µl (~570 µmol) DIEA, and evaporate volatiles. The residue is the crude ODN with full-length sequences being tagged with **1a-c** or **1d**.
8. The tag **1a-c** or **1d** can also be incorporated with standard synthesis cycle. In this case, only need to put base 5 at the 5′-end during sequence editing. There is no need to edit cycle. If convenient, hold the coupling step for base 5 for 180 sec, which may increase yield. Remember not to remove 5′-DMTr.

Polymerize full-length sequences

9. Add 340 µl ddH$_2$O (for >80-mer, use 7 M urea instead) to **PM**. Vortex and spin briefly.
10. Add 190 µl ddH$_2$O to **PIA**. Vortex and spin briefly.
11. Add 190 µl ddH$_2$O to **PIB**. Vortex and spin briefly.
12. Add 50 µl (no need to change volume with scales of $\leq$ 1 µmol and oligo length) ddH$_2$O (for >80-mer, use 7 M urea instead) to crude ODN. Vortex and spin briefly. If desired, inject ~1 µl into RP HPLC for analysis.
13. Add 12 µl (for x µmol n-mer oligo, use $x \times n \times 0.6$ µl but not less than 12 µl) **PM** solution. Vortex and spin briefly.
14. Add **PIA** and **PIB** (5 µl each irrespective of scale and ODN length), vortex (~1 sec) and spin (~5 sec) immediately; and immediately transfer the mixture into the centrifugal filter unit over filter before polymerization occurs. Try to transfer all contents by sucking with a pipette slowly. Deposit contents to the center by pushing the pipette slowly to avoid splashing. If polymerization occurs before transfer, transferring the gel to the filter tube is acceptable too.
15. Cap the filter unit and let stand for 1 h at room temperature.

Washing away failure sequences and other impurities

16. Loosen the gel that sticks to the bottom using a spatula.
17. Spin for ~15 sec to separate supernatant (if any) from gel.
18. Add ~250 µl 20% NaOAc (or 10% piperidine), wait for ~3 min, and spin. Discard filtrate. Repeat 6 times.
19. Add ~250 µl 5% Et$_3$N, wait for ~3 min, and spin. Discard filtrate. Repeat 3 times.
20. Add ~250 µl ddH$_2$O, no wait, and spin. Discard filtrate.

Cleave and extract full-length sequences

21. Add minimum 80% AcOH to cover the gel (~100 µl), wait for 5 min, spin. Repeat 3 times.
22. Add minimum ddH$_2$O to cover gel (~100 µl), wait for ~3 min, spin. Repeat 5 times.
23. Evaporate the combined filtrates to dryness.

Precipitation (optional)

24. Add 100 µl (or less) conc. NH_4OH into the tube. Cap, and vortex and spin to dissolve.
25. Add 900 µl (or less, keep the v/v ratio of *n*BuOH/NH_4OH at 9) *n*BuOH via a pipette. Close the cap.

26. Vortex the tube for ~20 sec and then centrifuge at ~14K for ~2 min.
27. Remove the supernatant with a pipette, evaporate residue solvents, the solid is pure ODN.
28. Add 49 µl ddH_2O. Vortex and spin briefly. Inject ~1 µl into RP HPLC for purity. Divide peak area by that in step 12 (only work well when the peak is resolved by HPLC) for recovery yield.
29. Evaporate solution to dryness to recover ODN.

Notes

- OND purity is dependent on quality of synthesis, which is similar to trityl-on RP HPLC, fluorous affinity purification, RP cartridge purification, and other affinity purification techniques involving selective tagging of full-length sequences.
- The method cannot remove deletion sequences, therefore efficient capping and detritylation are important.
- The method cannot remove sequences grown from CPG directly instead of from the first nucleoside, therefore pre-capping CPG before synthesis may be desirable.
- The method cannot remove n+1 sequences. Therefore whenever possible, avoid over-extending coupling time because that may cause premature detritylation and generate n+1 sequences.
- We suggest using CPG with 2K Å pore size and pre-capping CPG before synthesis for 20 min for the synthesis of ODNs longer than 100-mer. For shorter ODNs, use standard conditions.
- If purification results are unsatisfactory, replacing reagents with fresh ones for ODN synthesis or heating the product in conc. NH_4OH for additional deprotection will most likely solve the problem.
- The method cannot be used for the purification of ODNs that have 5'-modification. However, our catching failure sequences by polymerization method is suited to do this (*Open Org. Chem. J.*, **2014**, *8*, 15-18).