Supporting Information

Synthesis and Evaluation of (*S*)-5′-*C*-Aminopropyl and (*S*)-5′-*C*-Aminopropyl-2′-arabinofluoro Modified DNA Oligomers for Novel RNase H-dependent Antisense Oligonucleotides

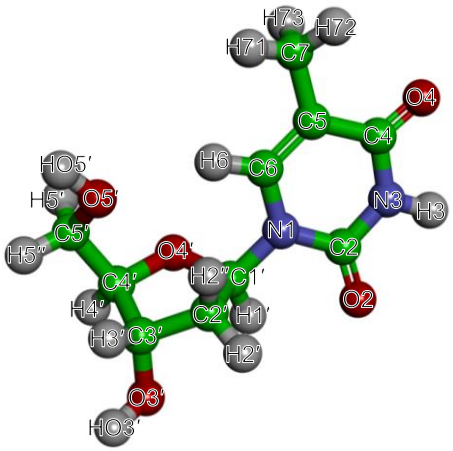
Yujun Zhou[a], Ryohei Kajino[a], Seiichiro Ishii[e], Kenji Yamagishi[e] and Yoshihito Ueno[a, b, c, d]

[a]Graduate School of Natural Science and Technology, [b]Faculty of Applied Biological Sciences, [c]United Graduate School of Agricultural Science, and [d]Center for Highly Advanced Integration of Nano and Life Sciences (G-CHAIN), Gifu University, 1-1 Yanagido, Gifu, 501-1193, Japan.

[e]Department of Chemical Biology and Applied Chemistry, College of Engineering, Nihon University, 1 Nakagawara, Tokusada, Tamuramachi, Koriyama, Fukushima 963-8642, Japan

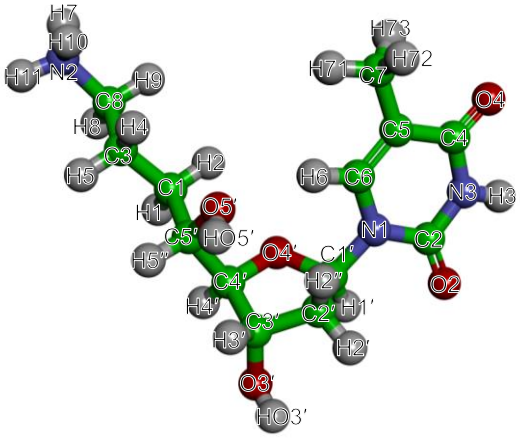
Contents

a.



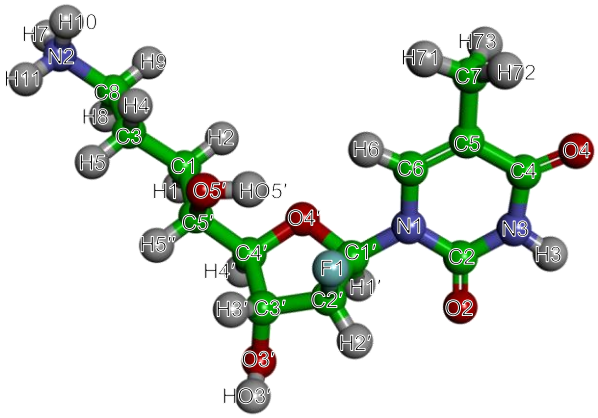
Atom name	Atom type	RESP charge	Atom name	Atom type	RESP charge
O5'	oh	-0.642377	H1'	h2	0.183207
HO5'	ho	0.445969	N1	n	0.004881
C5'	c3	-0.026110	C6	cc	-0.258929
H5''	h1	0.085899	H6	h4	0.277171
H5'	h1	0.085899	C5	cd	-0.015884
C4'	c3	0.167490	C7	c3	-0.226761
C3'	c3	0.131625	H73	hc	0.078192
C2'	c3	-0.085285	H72	hc	0.078192
H2''	hc	0.056570	H71	hc	0.078192
H2'	hc	0.056570	C4	c	0.604130
H3'	h1	0.089949	O4	o	-0.561408
O3'	oh	-0.670856	N3	n	-0.505706
HO3'	ho	0.440276	H3	hn	0.345687
H4'	h1	0.110996	C2	c	0.606967
O4'	os	-0.394698	O2	o	-0.597377
C1'	c3	0.057527			

b.



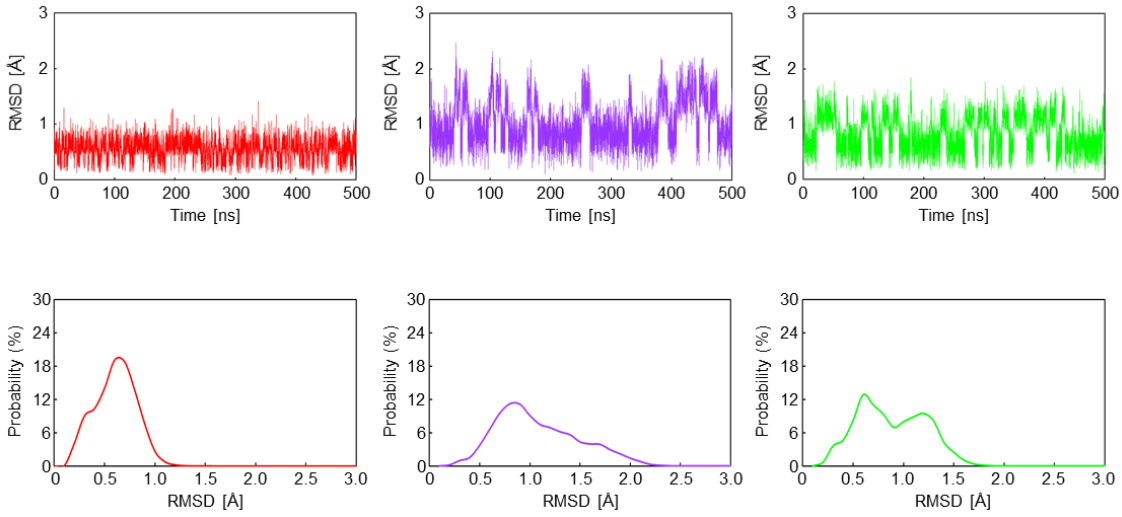
Atom name	Atom type	RESP charge	Atom name	Atom type	RESP charge
N2	n4	-0.262310	H2"	hc	0.030480
H7	hn	0.296620	H2'	hc	0.030480
H10	hn	0.296620	H3'	h1	0.055066
H11	hn	0.296620	H4'	h1	0.141936
C8	c3	0.056368	O4'	os	-0.396941
H8	hx	0.087808	C1'	c3	0.108014
H9	hx	0.087808	H1'	h2	0.175389
C3	c3	0.031312	N1	n	-0.016848
H4	hc	0.035624	C6	cc	-0.231780
H5	hc	0.035624	H6	h4	0.266459
C1	c3	-0.116672	C5	cd	-0.020402
H1	hc	0.070456	C7	c3	-0.253122
H2	hc	0.070456	H73	hc	0.088682
C5'	c3	0.027808	H72	hc	0.088682
O5'	oh	-0.628030	H71	hc	0.088682
HO5'	ho	0.437222	C4	c	0.585963
H5"	h1	0.083525	O4	o	-0.537039
C4'	c3	0.098860	N3	n	-0.453277
C3'	c3	0.174422	H3	hn	0.344928
O3'	oh	-0.681593	C2	c	0.542066
HO3'	ho	0.455432	O2	o	-0.562935
C2'	c3	-0.028466			

c.



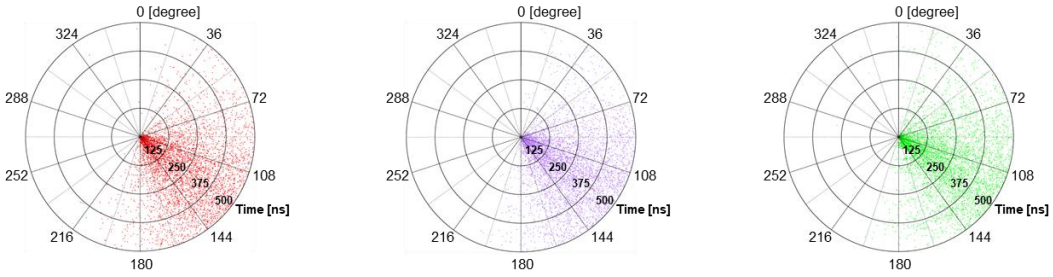
Atom name	Atom type	RESP charge	Atom name	Atom type	RESP charge
N2	n4	-0.320336	F1	f	-0.225659
H11	hn	0.312967	H2'	h1	0.146457
H10	hn	0.312967	H3'	h1	0.114231
H7	hn	0.312967	H4'	h1	0.164414
C8	c3	0.047498	O4'	os	-0.349118
H9	hx	0.093169	C1'	c3	0.041278
H8	hx	0.093169	H1'	h2	0.183224
C3	c3	0.002191	N1	n	0.018101
H5	hc	0.049590	C6	cc	-0.281532
H4	hc	0.049590	H6	h4	0.266824
C1	c3	-0.053607	C5	cd	0.004856
H2	hc	0.048251	C7	c3	-0.252306
H1	hc	0.048251	H73	hc	0.088667
C5'	c3	0.030046	H72	hc	0.088667
O5'	oh	-0.622083	H71	hc	0.088667
HO5'	ho	0.429055	C4	c	0.626134
H5''	h1	0.094626	O4	o	-0.542153
C4'	c3	0.057593	N3	n	-0.537816
C3'	c3	0.090972	H3	hn	0.365852
O3'	oh	-0.649012	C2	c	0.619609
HO3'	ho	0.454943	O2	o	-0.586622
C2'	c3	0.075419			

Figure S1. Molecular Modeling: the reference structure, atom types and calculated RESP charges for each nucleoside. The reference structures were obtained from an energy minimization calculation. (a) Thymidine. (b) (*S*)-5′-*C*-aminopropyl-thymidine (**A3**). (c) (*S*)-5′-*C*-aminopropyl-2′-β-fluoro-thymidine (**A4**).



Thymidine **A3** **A4**

Figure S2. Molecular Modeling: the changes (up) and the distributions (down) of time-dependent RMSD (Å) of each nucleoside



Thymidine **A3** **A4**

Figure S3. Molecular Modeling: pseudorotational phase angle (*P*).

The radial axes are the times with the origin as 0 ns and increasing outward to 500 ns. The angular axes represent the torsion angles with ranges from 0 to 2π in clockwise direction.

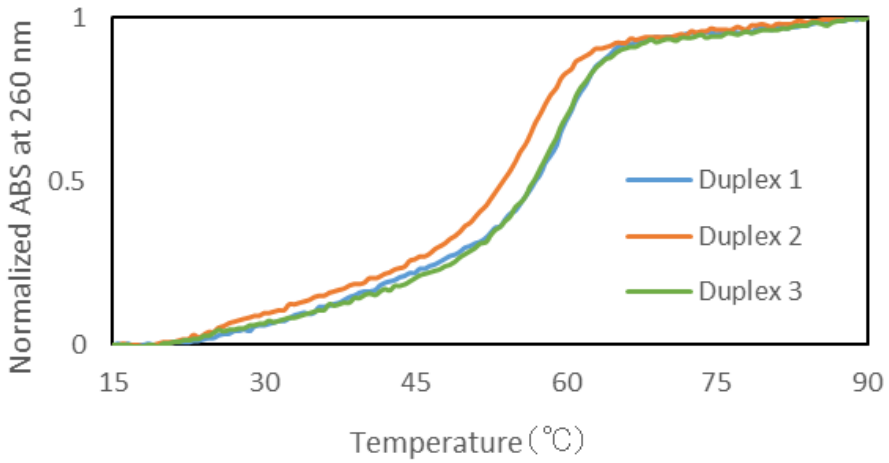
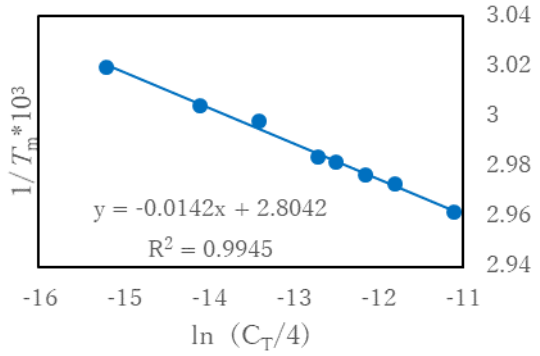
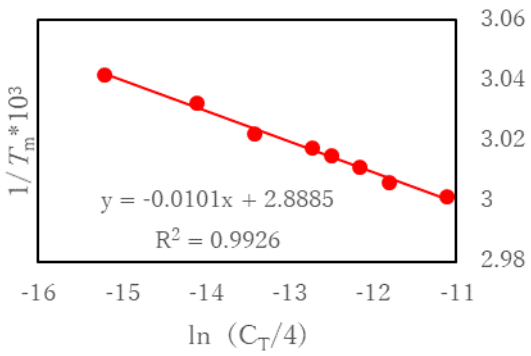


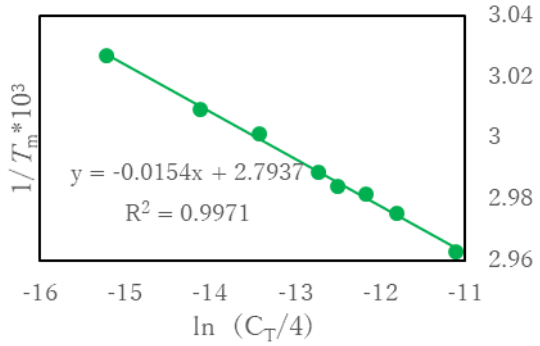
Figure S4. Thermal Stability of Duplexes: the UV melting profiles of duplex 1-3.



Duplex **1**



Duplex **2**



Duplex **3**

Figure S5. Thermal Stability of Duplexes: the graphical data of $1/T_m$ vs ln (C_T/4)

plots

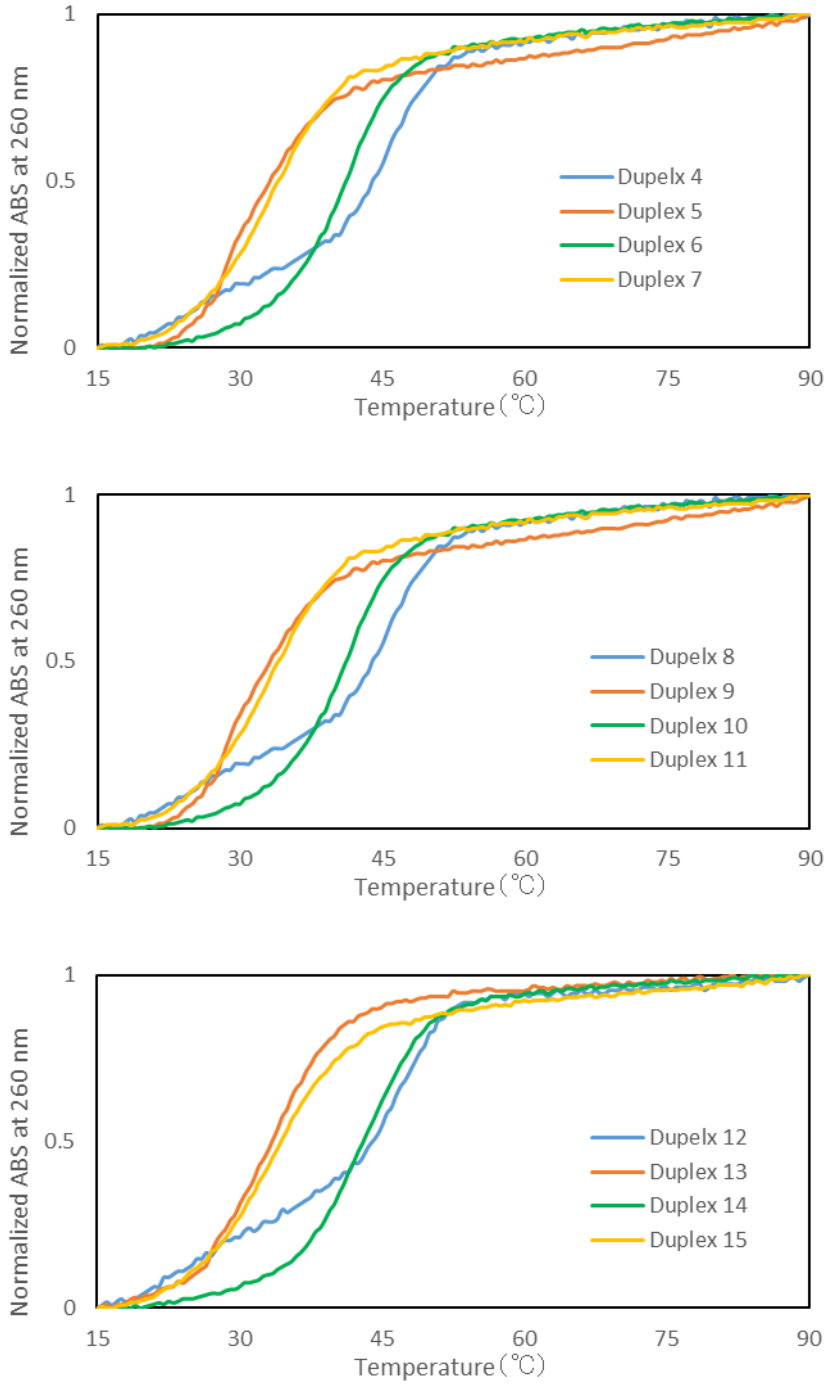


Figure S6. Thermal Stability of Duplexes: the UV melting profiles of duplex 4-15

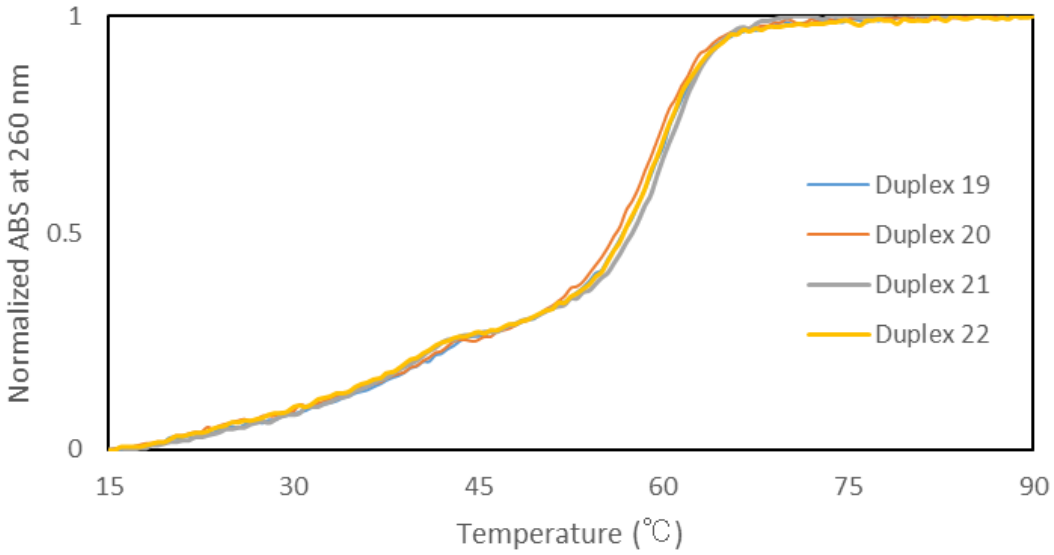


Figure S7. Thermal Stability of Duplexes: the UV melting profiles of duplex 19-22.

Table S1. Thermal Stability of Duplexes: the sequences and T_m values of duplex 4-15.

Abbreviation of DNA/RNA duplex	Abbreviation of DNA or RNA	Sequence[a]	T_m (°C)[b]	Δ T_m (°C)[c]
Duplex **4**	DNA **4**	5' - d(GGCTATAATGTCG) - 3'	47.4	–
	RNA **2**	5'- r(CGACAUUAUAGCC) -3'		
Duplex **5**	DNA **4**	5' - d(GGCTATAATGTCG) - 3'	35.9	-11.5
	RNA **3**	5'- r(CGACAUUUUAGCC) -3'		
Duplex **6**	DNA **4**	5' - d(GGCTATAATGTCG) - 3'	42.1	-5.3
	RNA **4**	5'- r(CGACAUUGUAGCC) -3'		
Duplex **7**	DNA **4**	5' - d(GGCTATAATGTCG) - 3'	33.6	-13.8
	RNA **5**	5'- r(CGACAUUCUAGCC) -3'		
Duplex **8**	DNA **5**	5' – d(GGCTATAATGTCG) – 3'	47.1	–
	RNA **2**	5'- r(CGACAUUAUAGCC) -3'		
Duplex **9**	DNA **5**	5' – d(GGCTATAATGTCG) – 3'	31.3	-15.8
	RNA **3**	5'- r(CGACAUUUUAGCC) -3'		
Duplex **10**	DNA **5**	5' - d(GGCTATAATGTCG) - 3'	41.1	-6.0
	RNA **4**	5'- r(CGACAUUGUAGCC) -3'		
Duplex **11**	DNA **5**	5' - d(GGCTATAATGTCG) - 3'	32.4	-14.7
	RNA **5**	5'- r(CGACAUUCUAGCC) -3'		
Duplex **12**	DNA **6**	5' - d(GGCTATAATGTCG) - 3'	47.3	–
	RNA **2**	5'- r(CGACAUUAUAGCC) -3'		
Duplex **13**	DNA **6**	5' - d(GGCTATAATGTCG) - 3'	34.0	-13.3
	RNA **3**	5'- r(CGACAUUUUAGCC) -3'		
Duplex **14**	DNA **6**	5' - d(GGCTATAATGTCG) - 3'	43.3	-4.0
	RNA **4**	5'- r(CGACAUUGUAGCC) -3'		
Duplex **15**	DNA **6**	5' – d(GGCTATAATGTCG) – 3'	32.7	-14.6
	RNA **5**	5'- r(CGACAUUCUAGCC) -3'		

[a]Underlined letters denote mismatched base pairs. T(red) and T(green) denote (*S*)-5'-*C*-Aminopropyl-thymidine **(A3)** and (*S*)-5'-*C*-Aminopropyl-2'-β-fluoro-thymidine (**A4**), respectively. [b]The T_m value were

measured in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl. The concentrations the duplexes were 3 μM. [c]ΔT_m represents [T_m (duplex $_{fully\ matched}$)- T_m (duplex$_{mismatched}$)].

Table S2. Thermal Stability of Duplexes: the sequences and T_m values of duplex 1, 16-19.

Abbreviation of DNA/RNA duplex	Abbreviation of DNA or RNA	Sequence[a]	T_m (°C)[b]	ΔT_m (°C)[c]
Duplex **1**	DNA **1**	5'- d(TCTTTCTCTTTCCCTT) -3'	60.0	–
	RNA **1**	5'- r(AAGGGAAGAGAAAGA) -3'		
Duplex **19**	DNA **10**	5'- d(TCTTTCTCTTTCCCTT) -3'	60.1	+0.1
	RNA **1**	5'- r(AAGGGAAGAGAAAGA) -3'		
Duplex **20**	DNA **11**	5'- d(TCTTTCTCTTTCCCTT) -3'	59.1	-0.9
	RNA **1**	5'- r(AAGGGAAGAGAAAGA) -3'		
Duplex **21**	DNA **12**	5'- d(TCTTTCTCTTTCCCTT) -3'	60.3	+0.3
	RNA **1**	5'- r(AAGGGAAGAGAAAGA) -3'		
Duplex **22**	DNA **13**	5'- d(TCTTTCTCTTTCCCTT) -3'	59.5	-0.5
	RNA **1**	5'- r(AAGGGAAGAGAAAGA) -3'		

[a] T(green) denotes (*S*)-5'-*C*-Aminopropyl-2'-β-fluoro-thymidine (**A4**). [b]The T_m value were measured in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl. The concentrations of the duplexes were 3 μM. All measurements were carried out three times, and the data are shown as an average value. [c]ΔT_m represents [T_m (duplex **19-22**)- T_m (duplex **1**)].

(a)

Abbreviation of DNA/RNA duplex	Abbreviation of DNA or RNA	Sequence[a]
Duplex **23**	DNA **10**	5'- d(TCTTTCTCTTTCCCTT) -3'
	RNA **6**	5'- F - r(AAGGGAAGAGAAAGA) -3'
Duplex **24**	DNA **11**	5'- d(TCTTTCTCTTTCCCTT) -3'
	RNA **6**	5'- F - r(AAGGGAAGAGAAAGA) -3'
Duplex **25**	DNA **12**	5'- d(TCTTTCTCTTTCCCTT) -3'
	RNA **6**	5'- F - r(AAGGGAAGAGAAAGA) -3'
Duplex **26**	DNA **13**	5'- d(TCTTTCTCTTTCCCTT) -3'
	RNA **6**	5'- F - r(AAGGGAAGAGAAAGA) -3'

[a]F(orange) and T(green) denote fluorescein and (*S*)-5'-*C*-Aminopropyl-2'-β-fluoro-thymidine (**A4**), respectively.

(b)

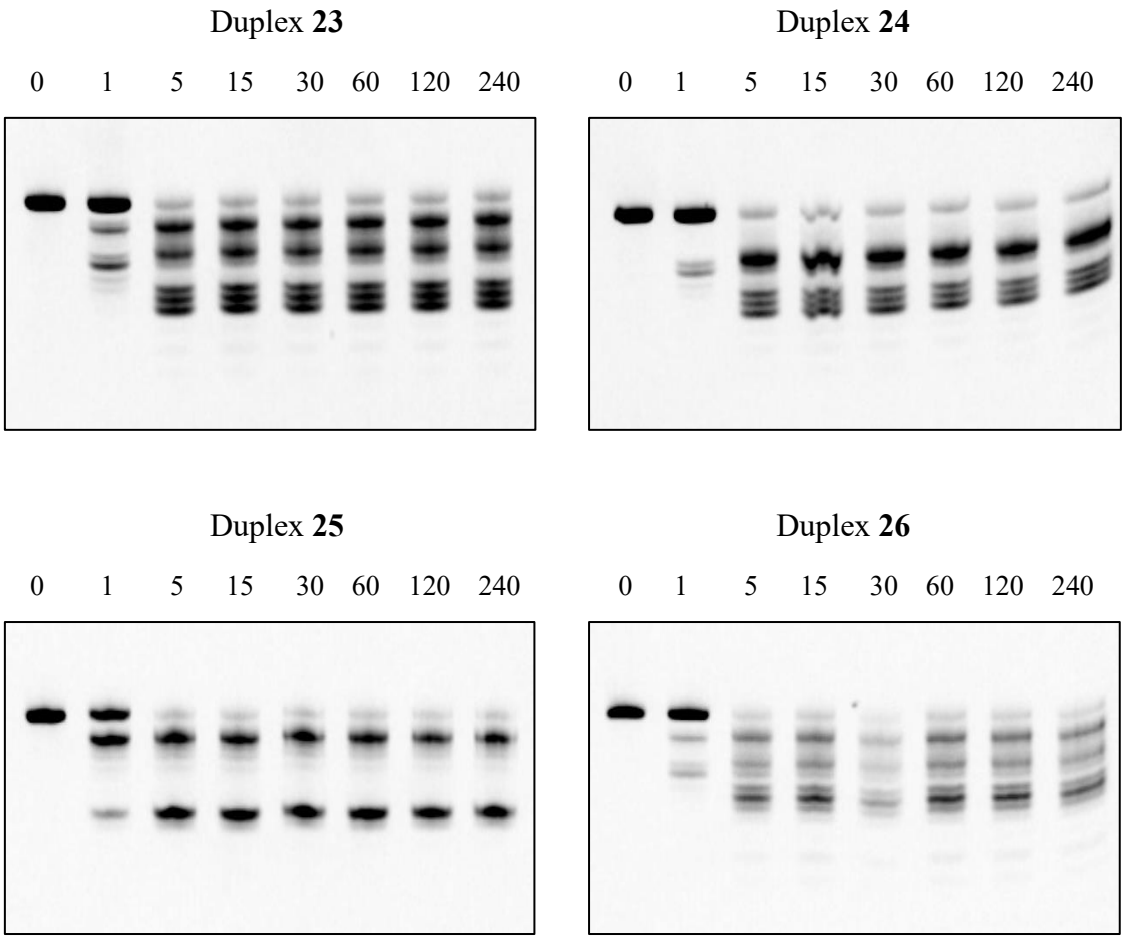
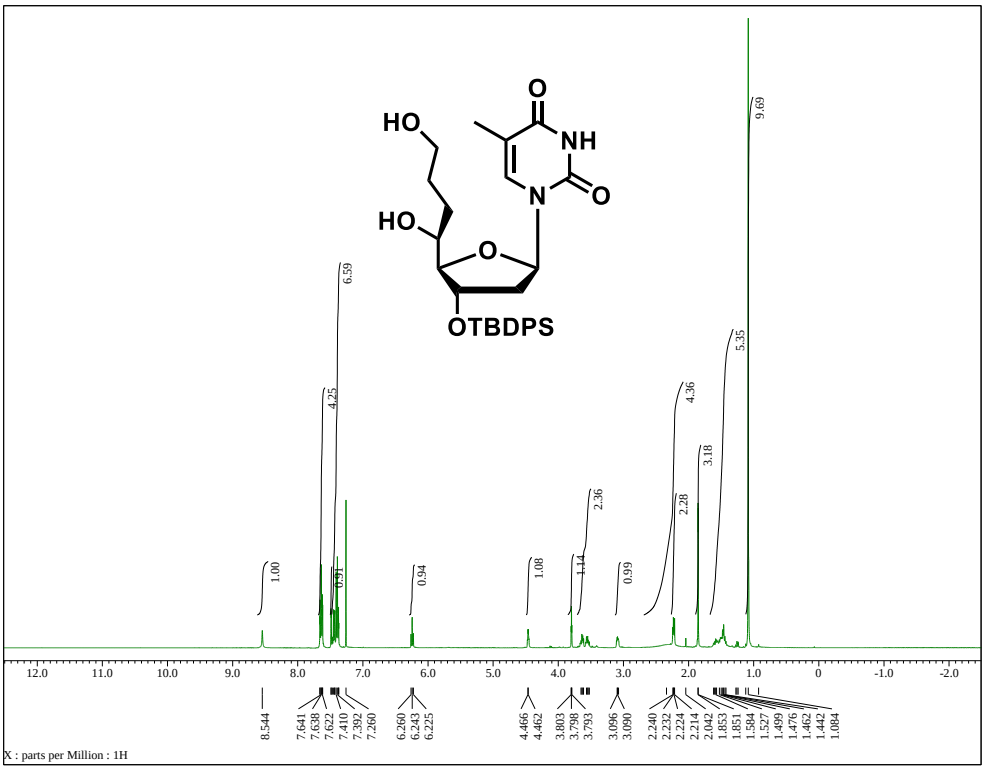


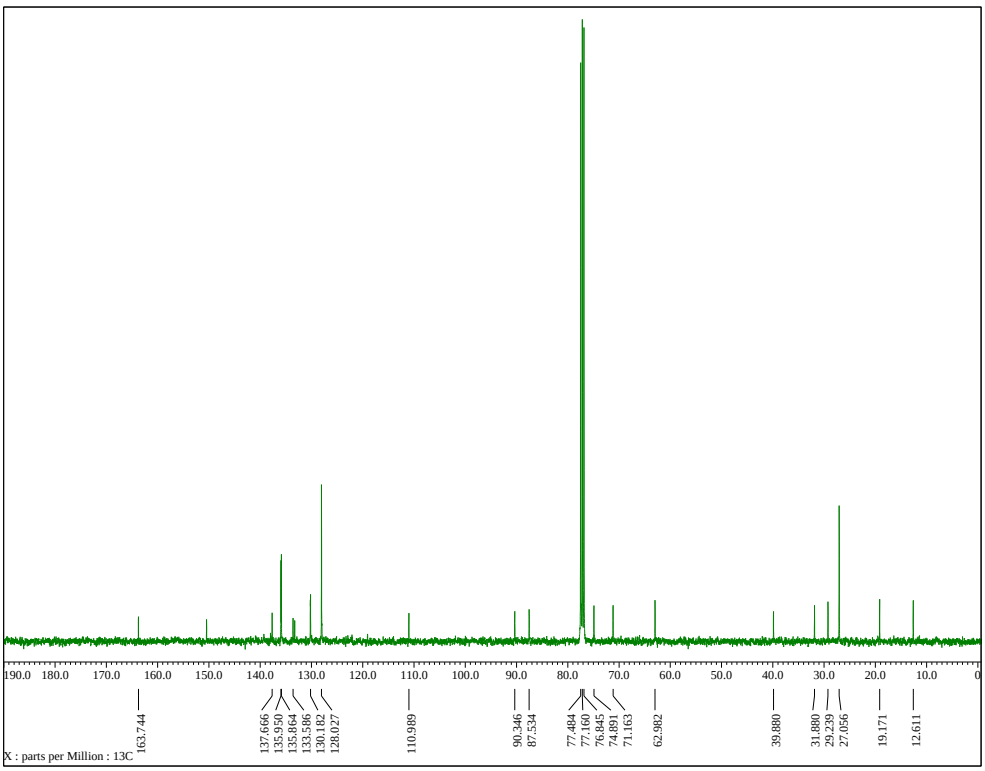
Figure S8. RNase H-mediated cleavage assays: the sequences of duplex 20-23 (a) and the images of PAGE analysis (b). PAGE analysis of DNA/RNA duplexes treated in a buffer containing 50 mM Tris–HCl (pH 8.0), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, and RNase H from *E.coli* . The duplexes **23-26** were incubated in a buffer containing 50 mM Tris–HCl (pH 8.0), 75 mM KCl, 3 mM $MgCl_2$ and 10 mM dithiothreitol, and then diluted RNase H solution (60 unit/L in H_2O) was added, and subsequently the mixture was incubated at 37℃ for the required time. The reaction mixtures at various incubation time (0, 1, 5, 15, 30, 60, 120, 240 min) were analyzed by 20% PAGE.

NMR spectra (^{1}H, ^{13}C, ^{19}F, ^{31}P and NOESY)

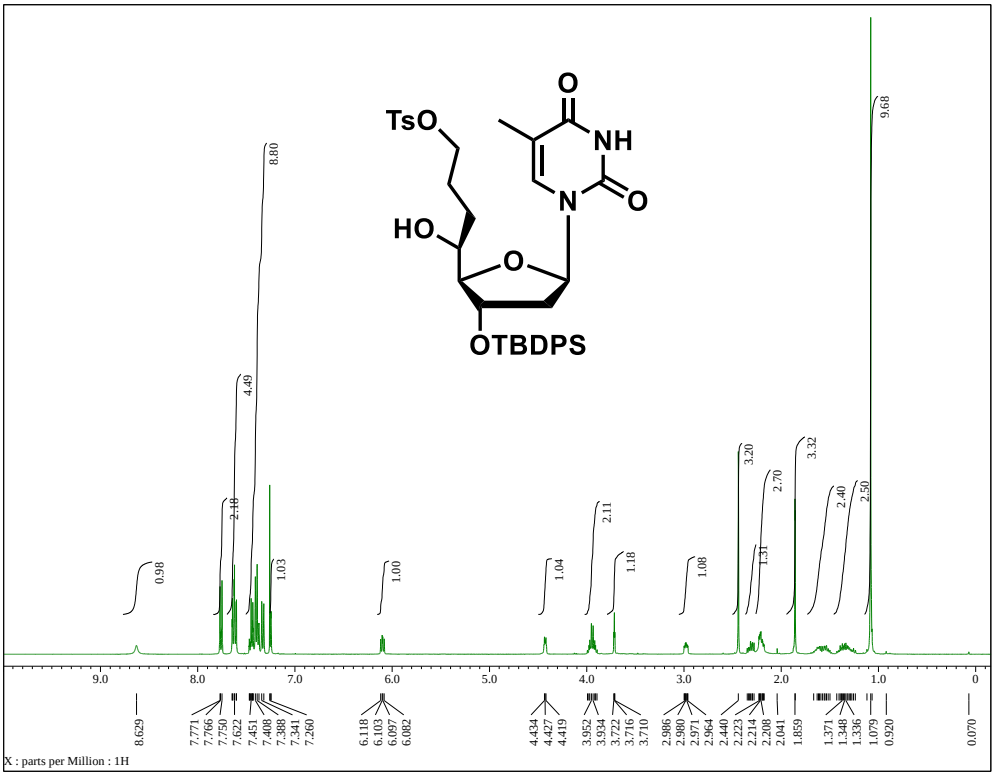
^{1}H NMR spectrum of compound **2**



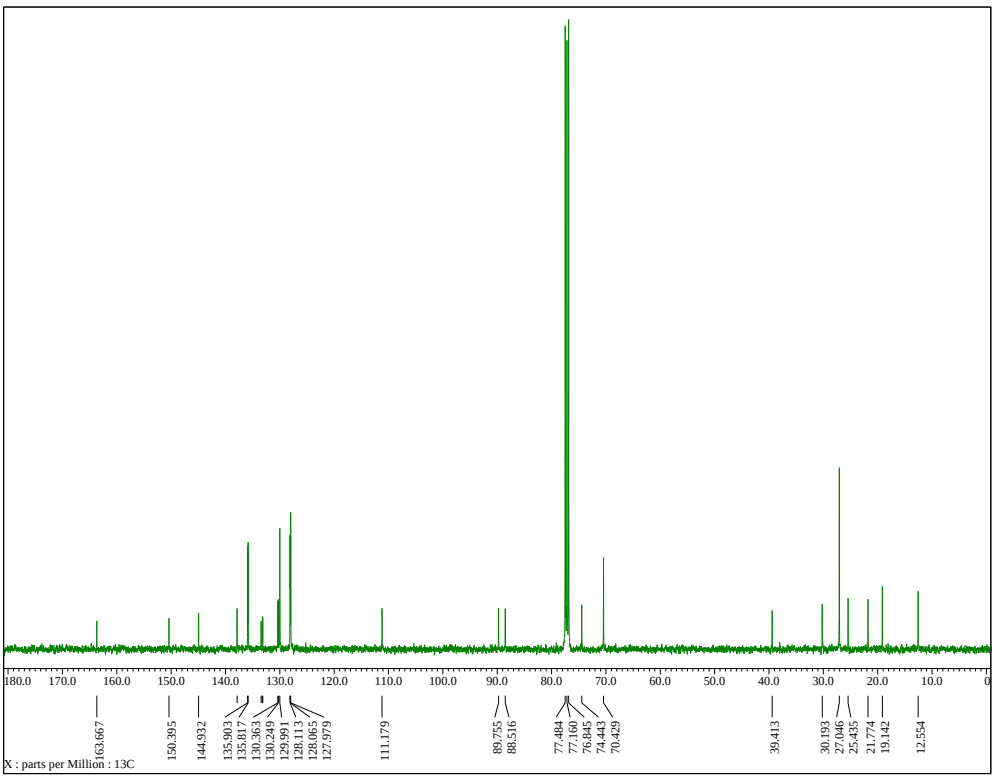
^{13}C NMR spectrum of compound **2**



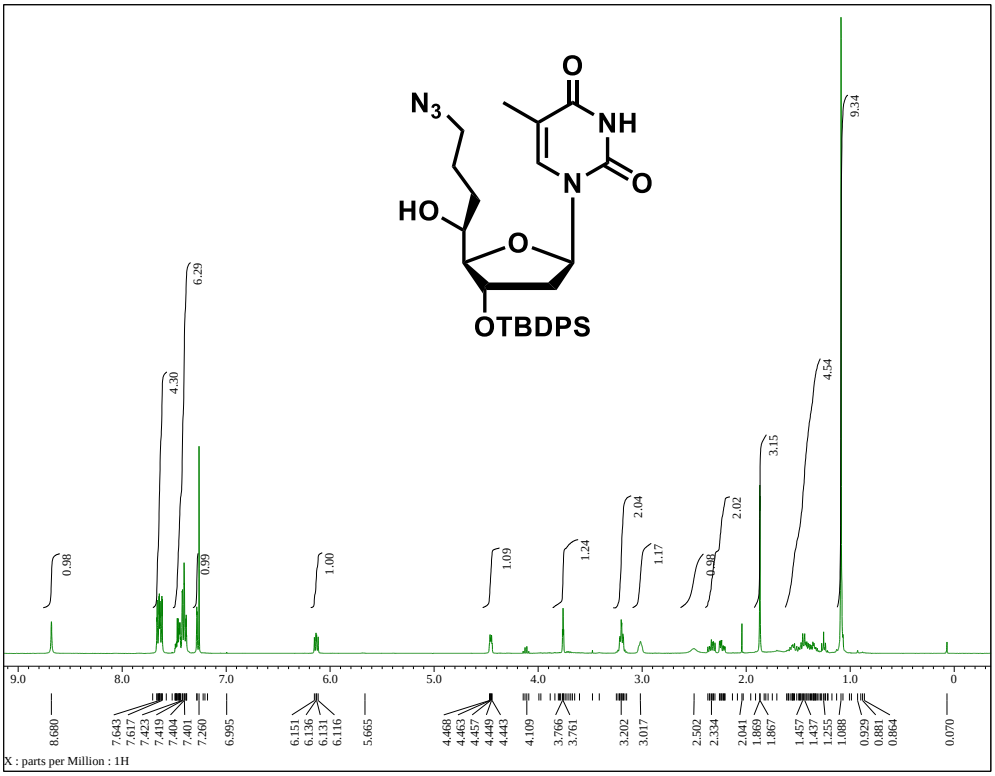
^{1}H NMR spectrum of compound **3**



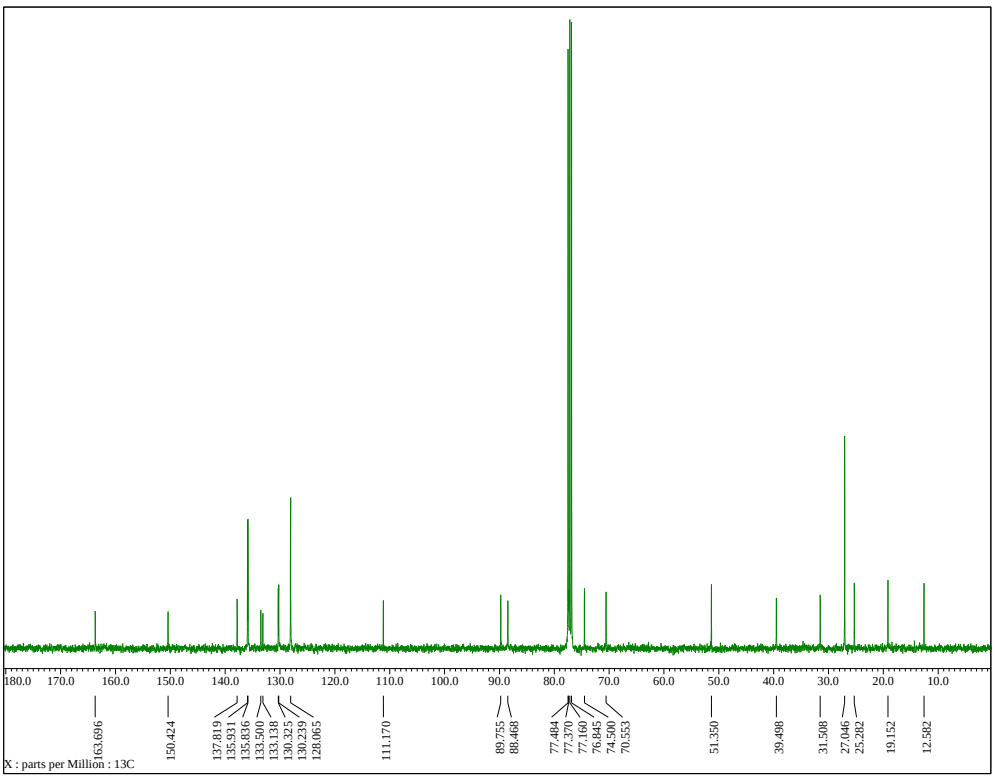
^{13}C NMR spectrum of compound **3**



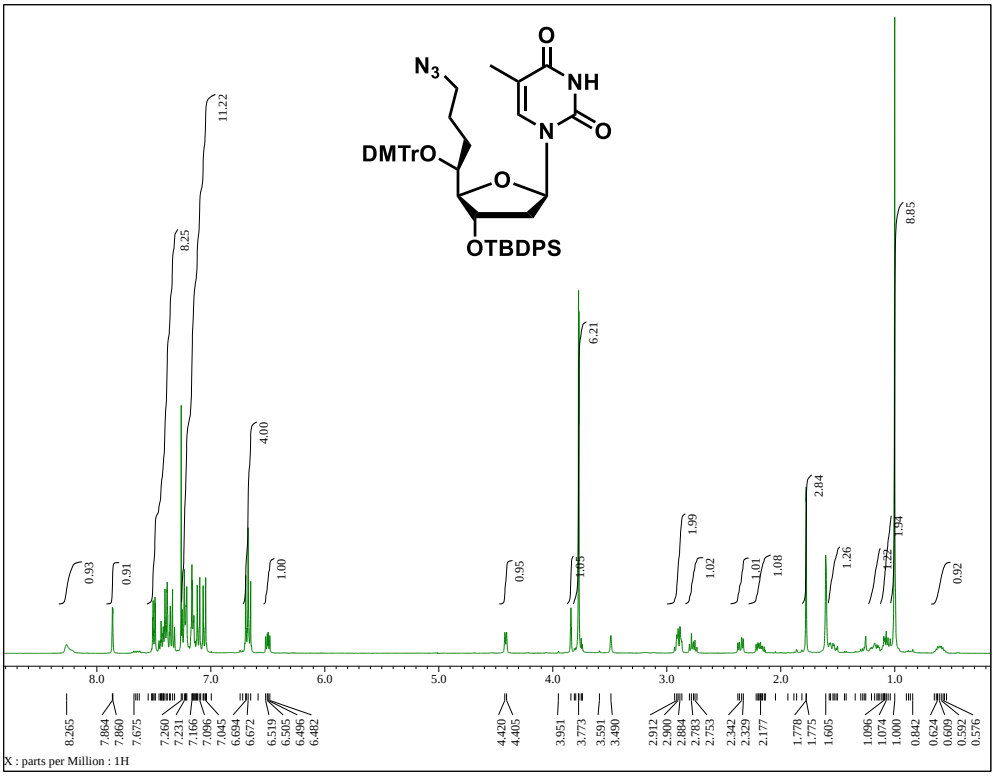
^{1}H NMR spectrum of compound **4**



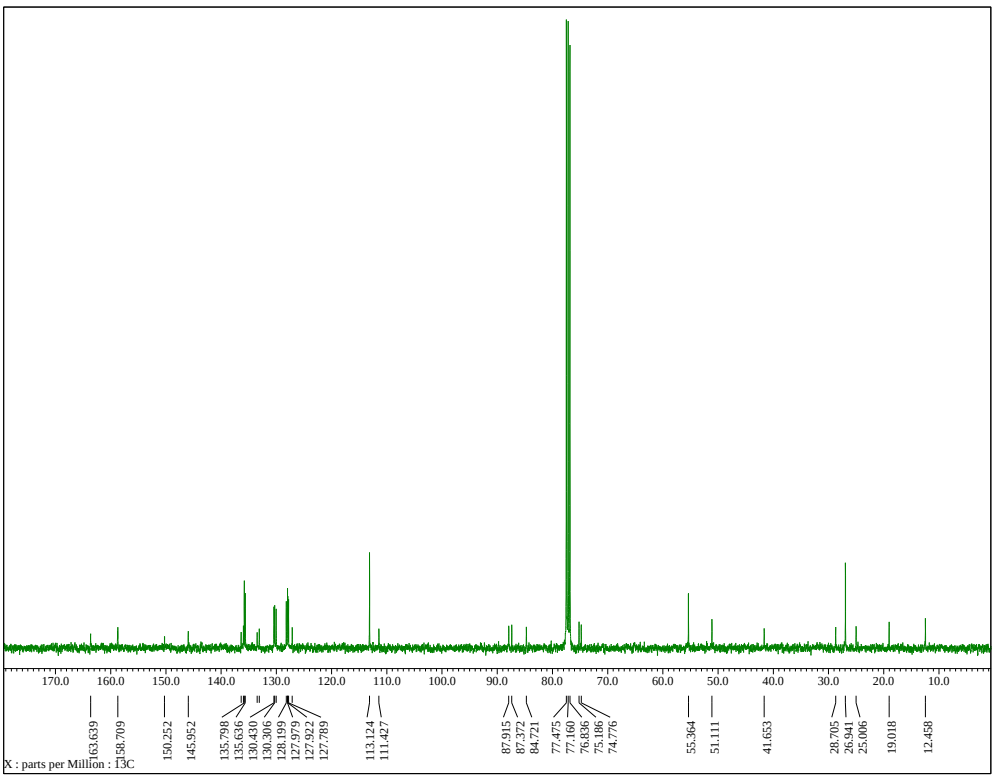
^{13}C NMR spectrum of compound **4**



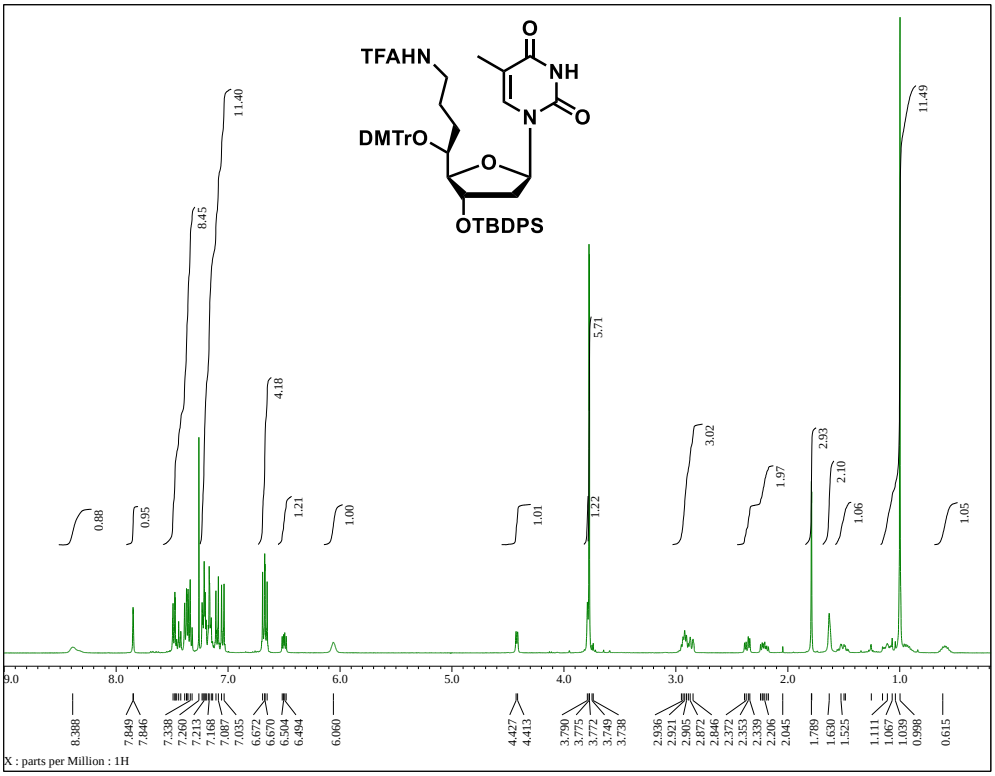
^{1}H NMR spectrum of compound **5**



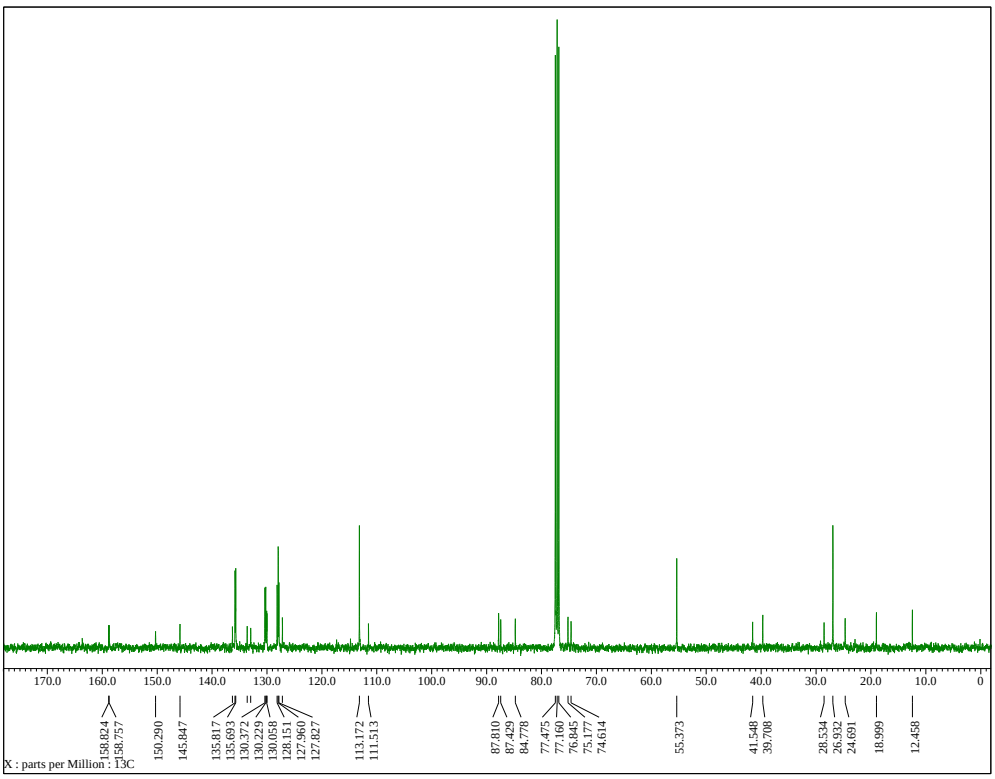
^{13}C NMR spectrum of compound **5**



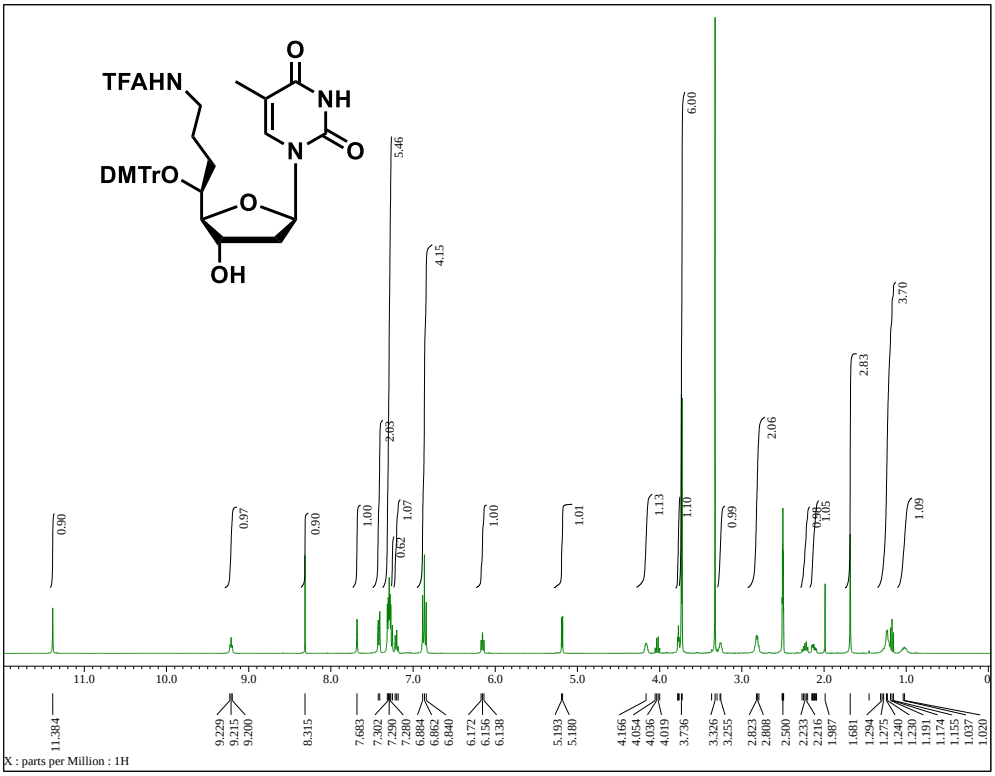
^{1}H NMR spectrum of compound **6**



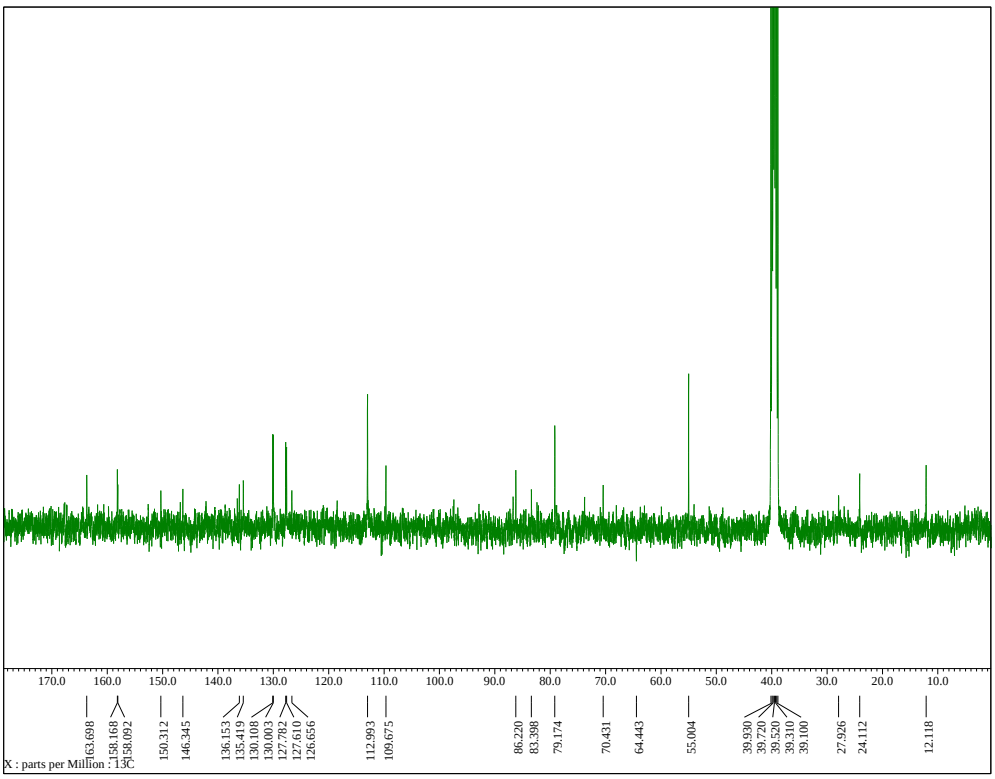
^{13}C NMR spectrum of compound **6**



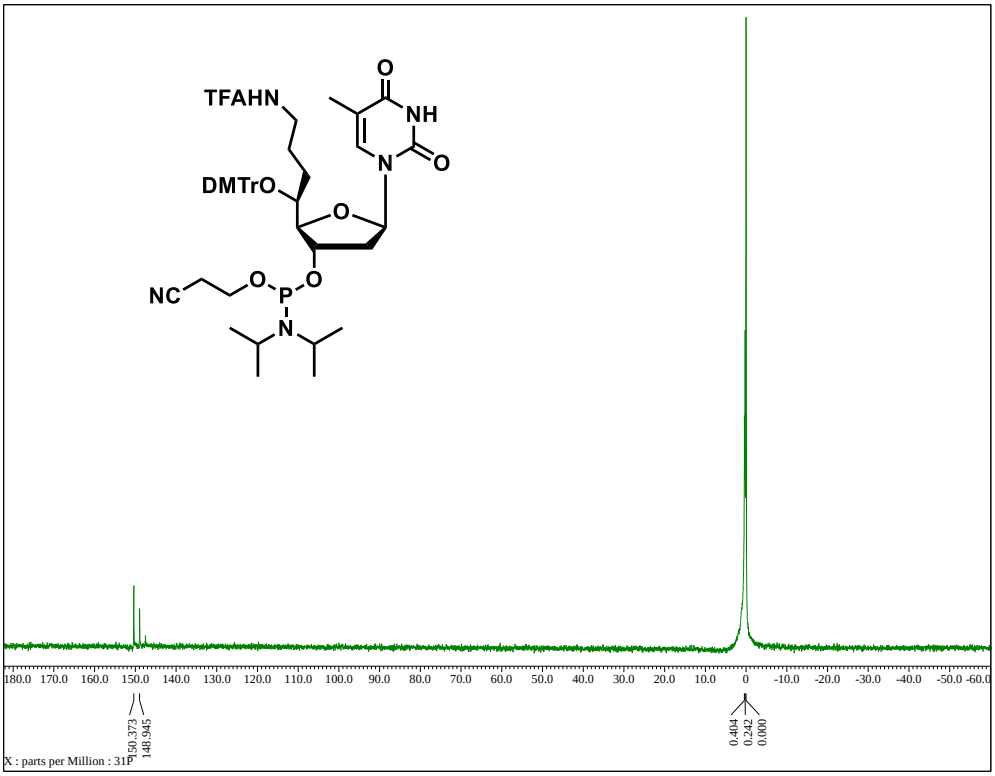
^{1}H NMR spectrum of compound **7**



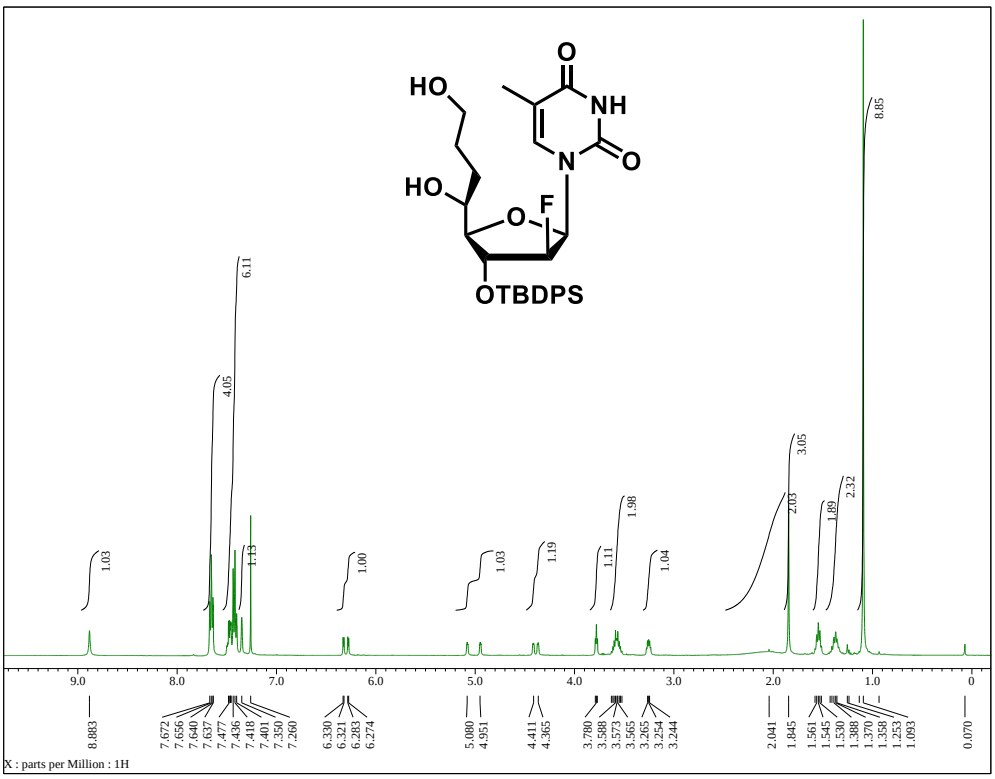
^{13}C NMR spectrum of compound **7**



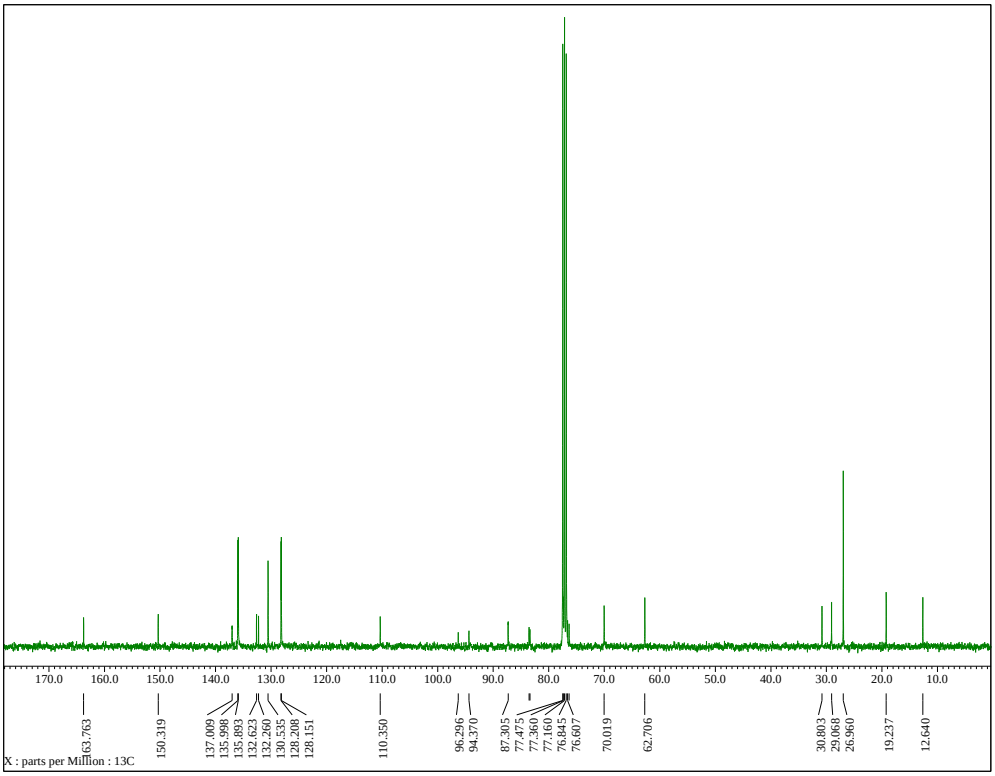
^{31}P NMR spectrum of compound **8**



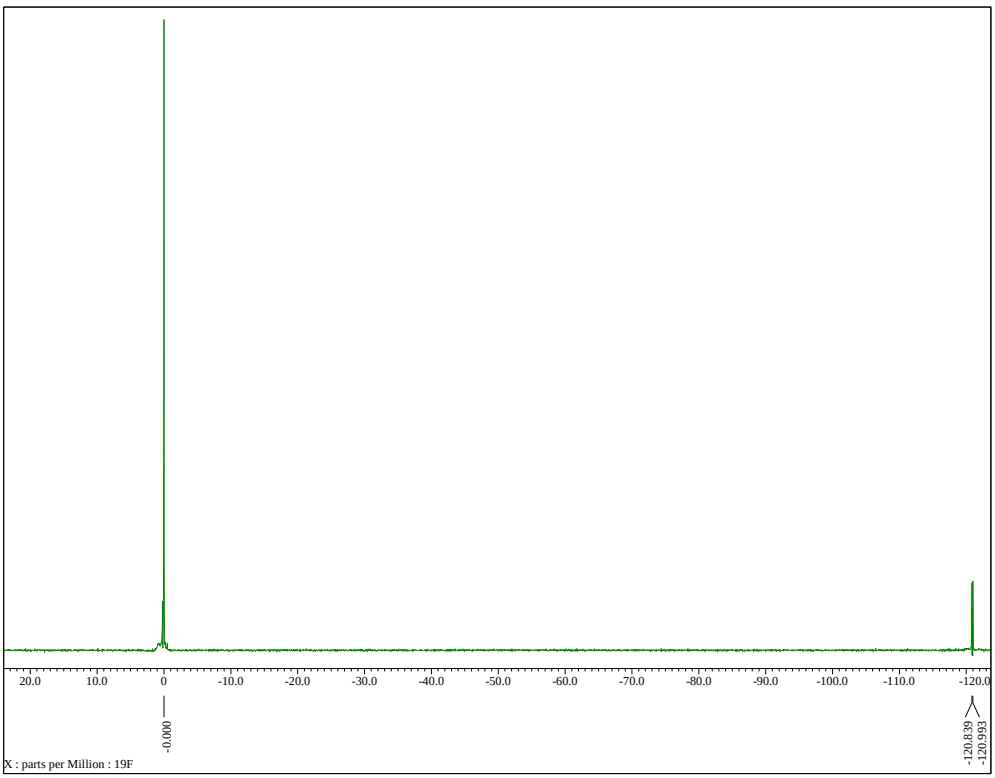
^{1}H NMR spectrum of compound **13**



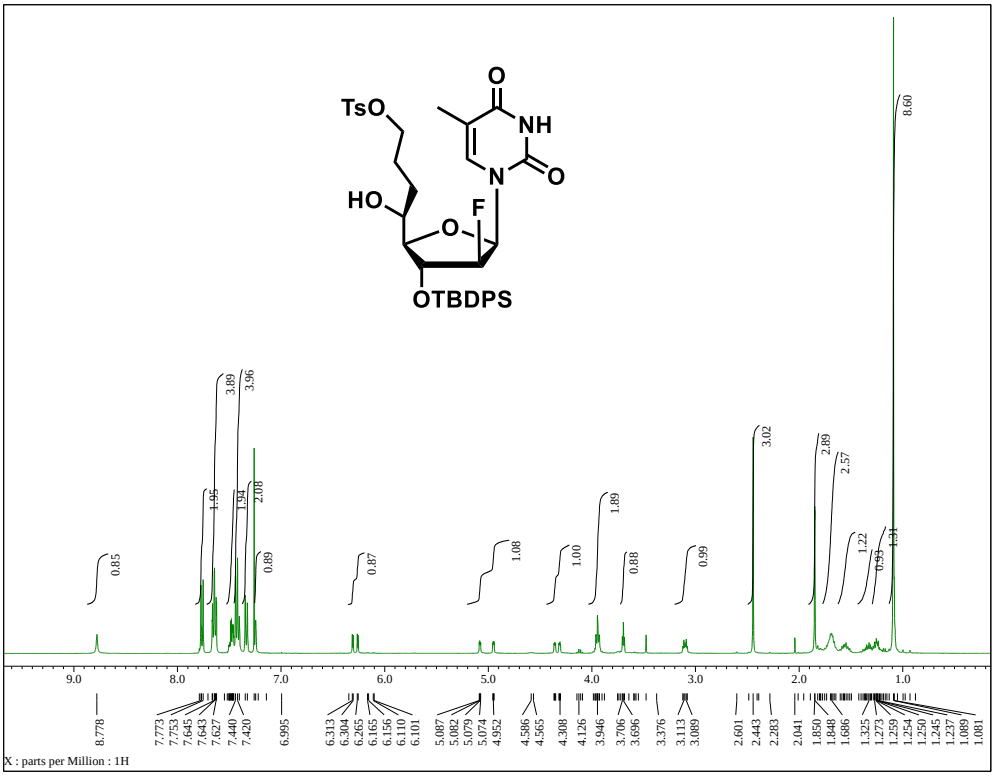
^{13}C NMR spectrum of compound **13**



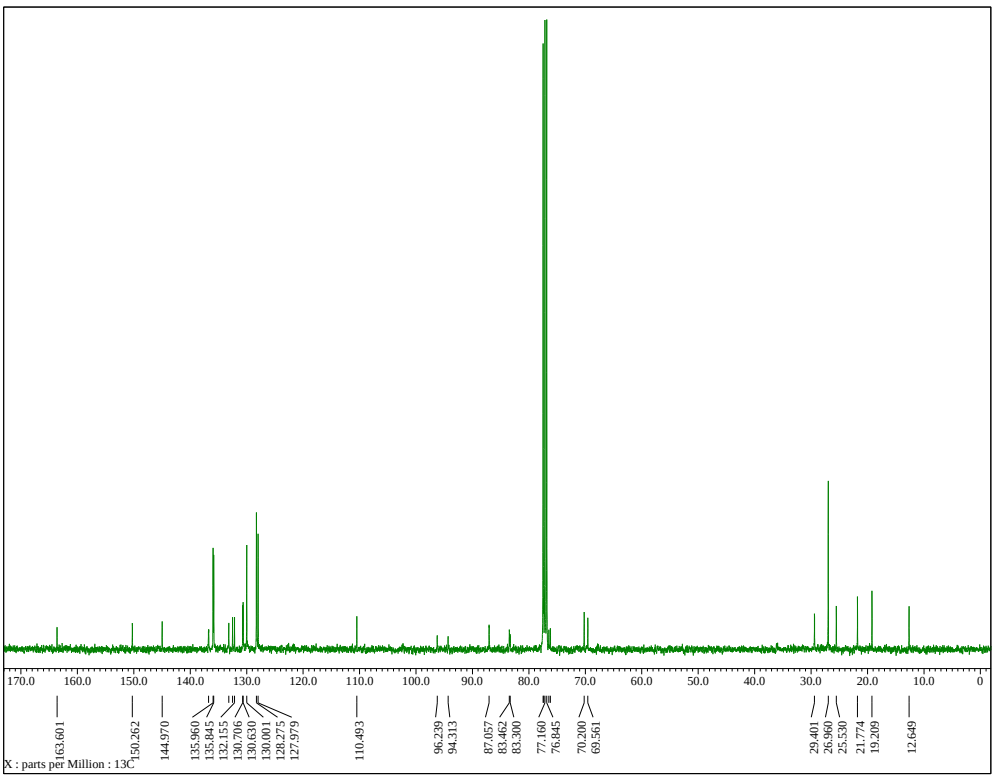
^{19}F NMR spectrum of compound **13**



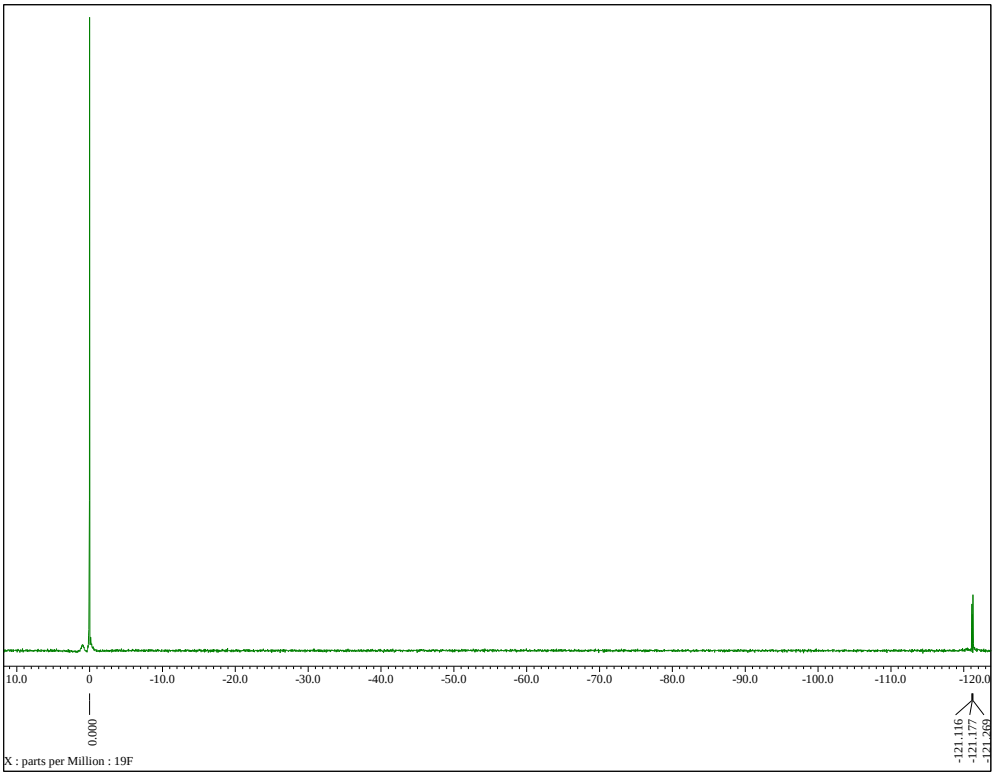
^{1}H NMR spectrum of compound **14**



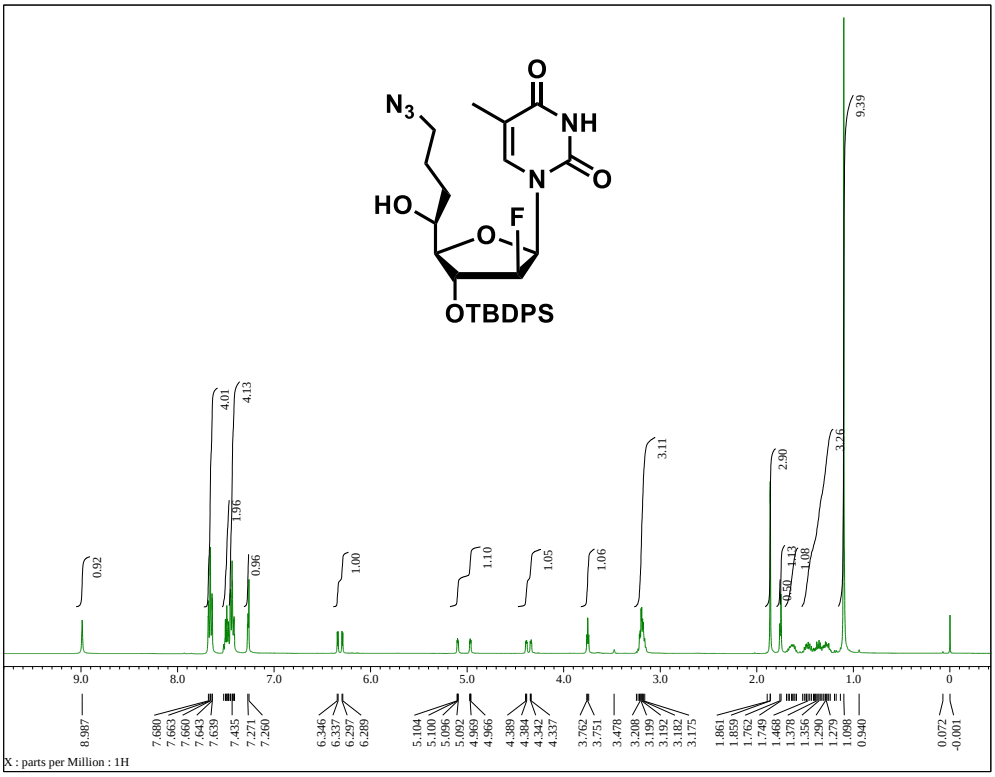
^{13}C NMR spectrum of compound **14**



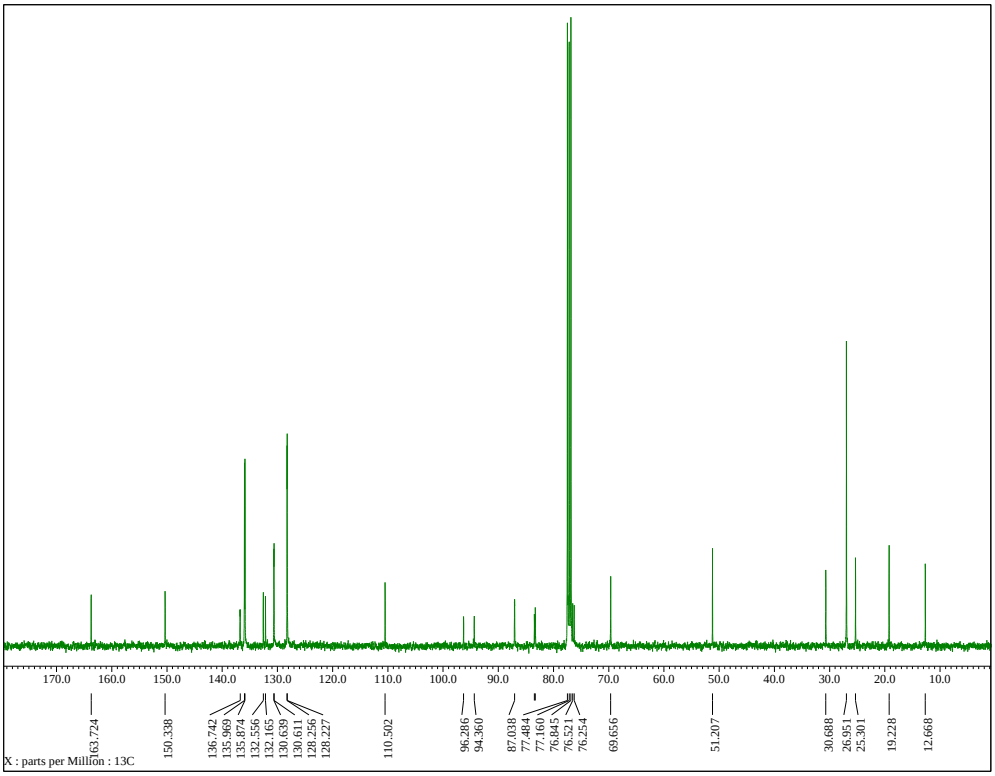
^{19}F NMR spectrum of compound **14**



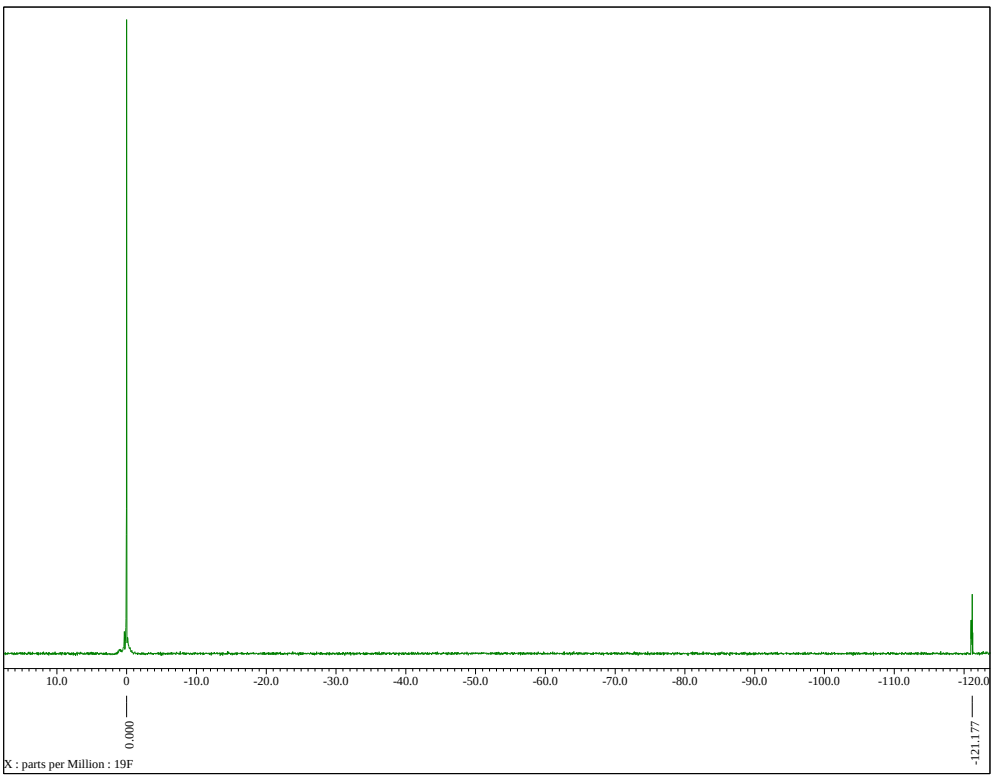
^{1}H NMR spectrum of compound **15**



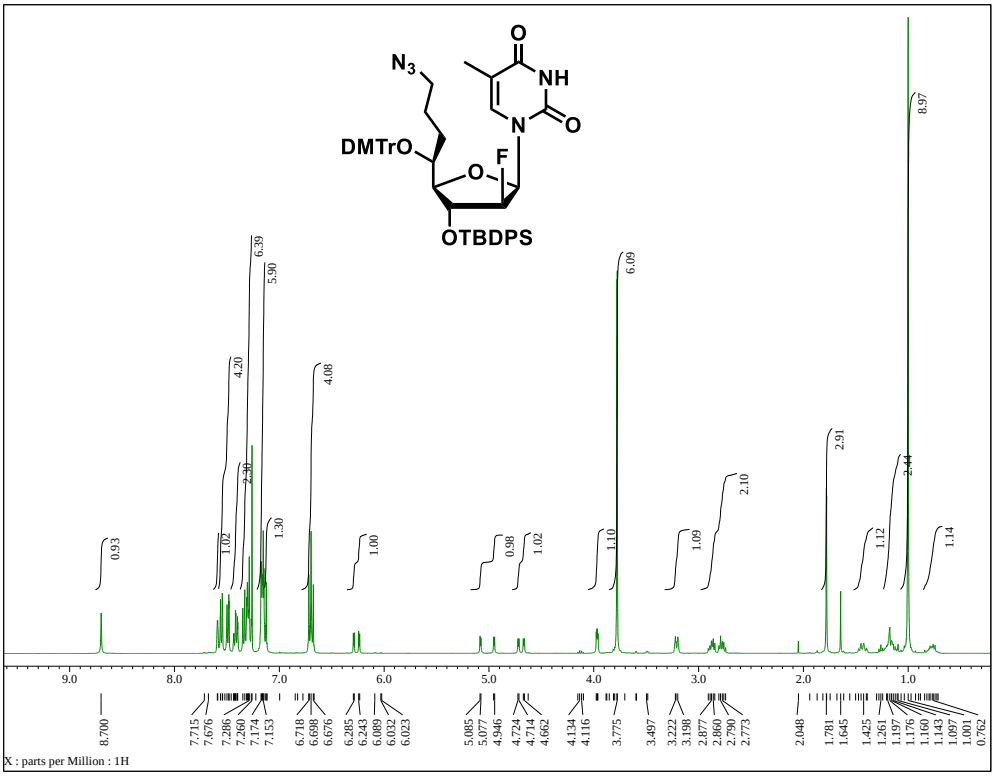
^{13}C NMR spectrum of compound **15**



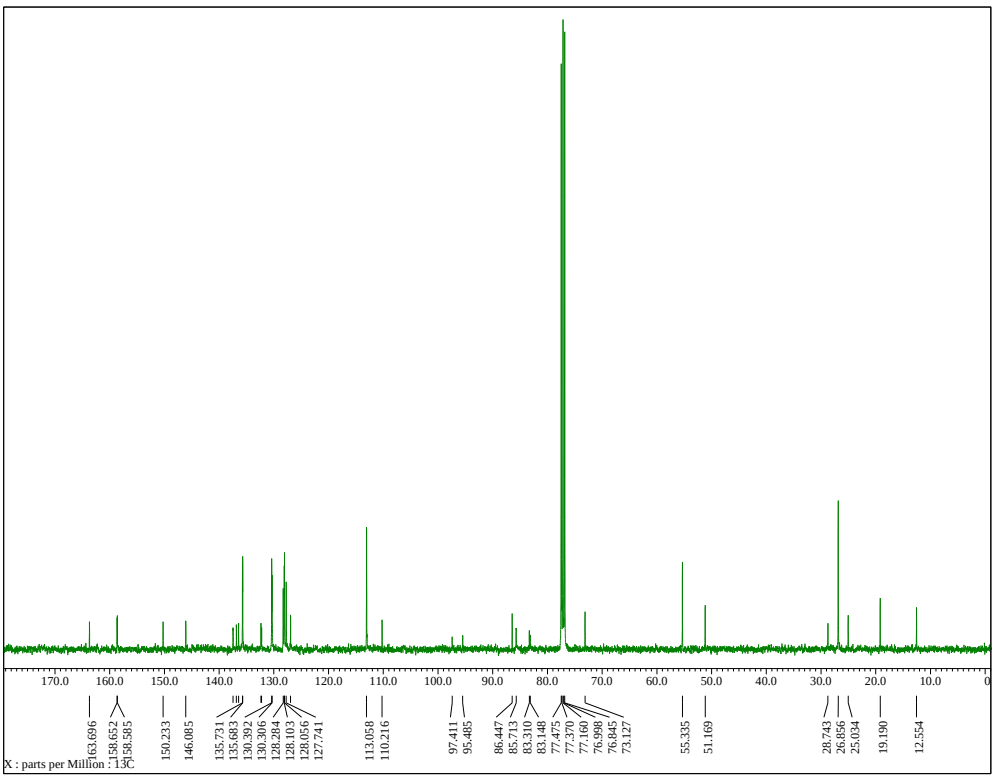
^{19}F NMR spectrum of compound **15**



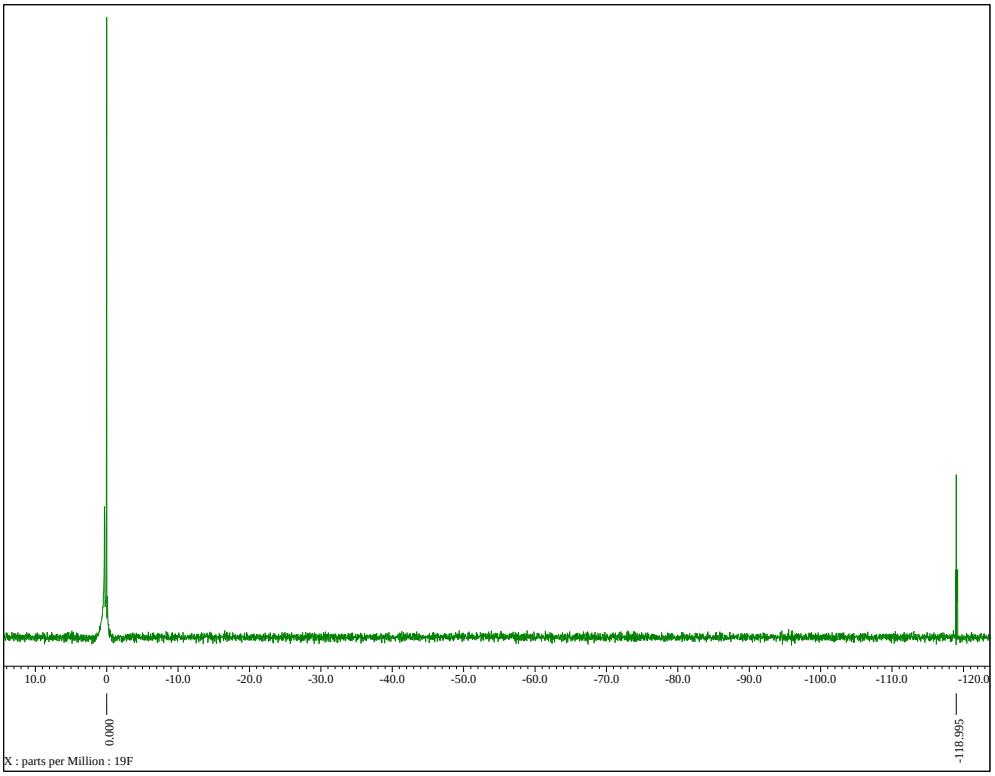
^{1}H NMR spectrum of compound **16**



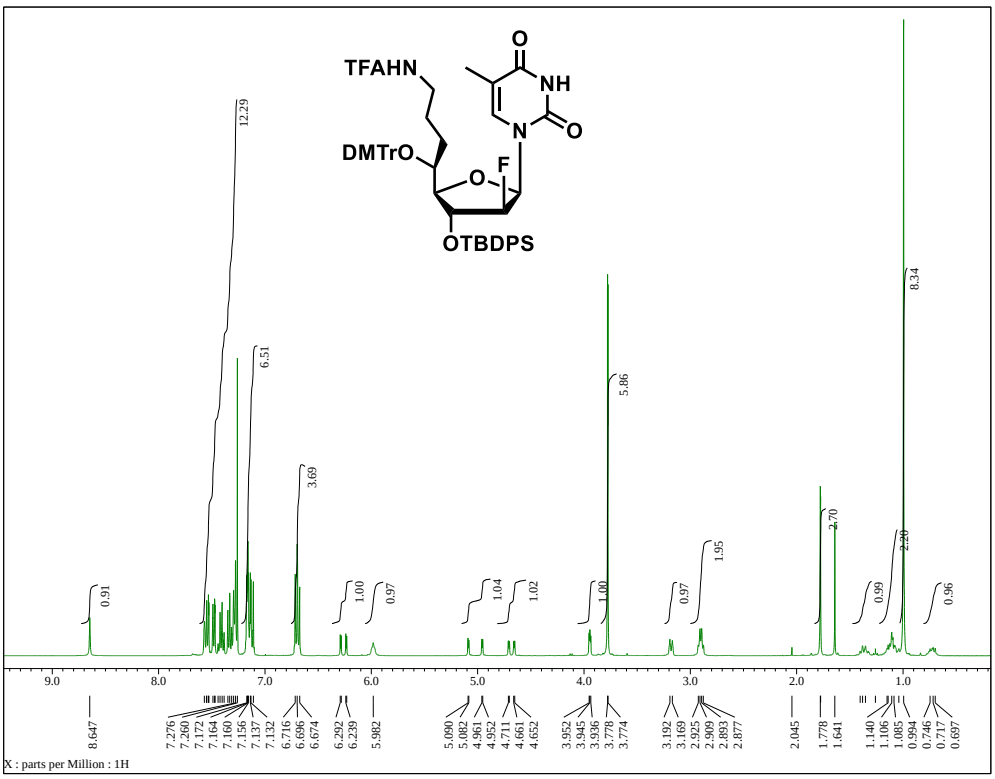
^{13}C NMR spectrum of compound **16**



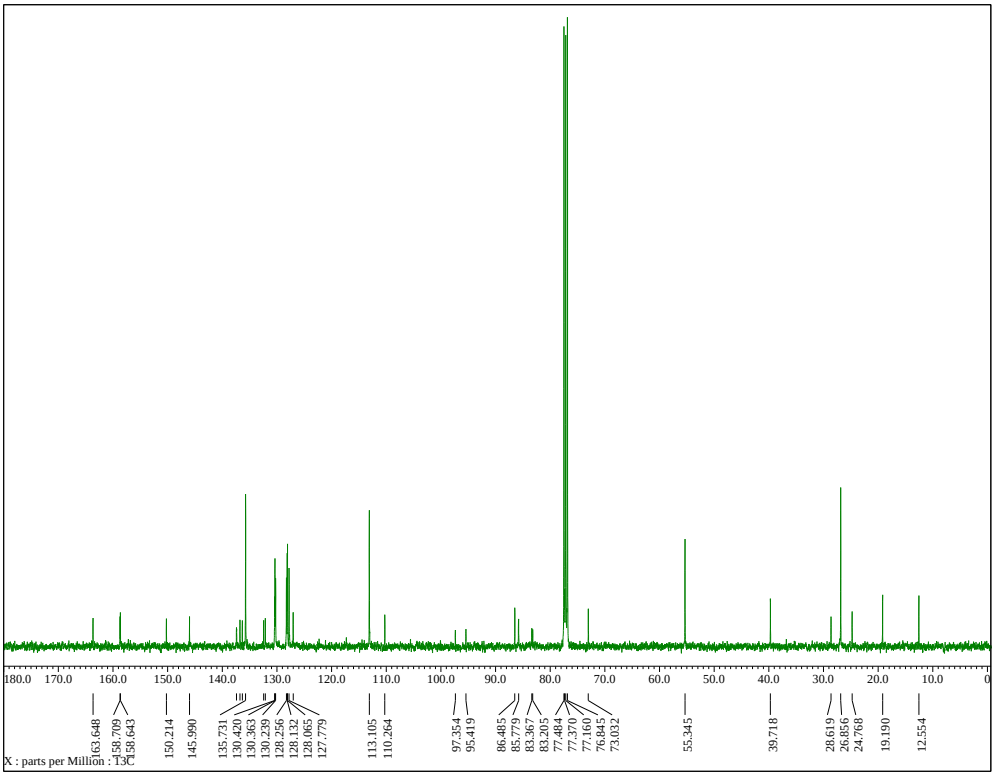
^{19}F NMR spectrum of compound **16**



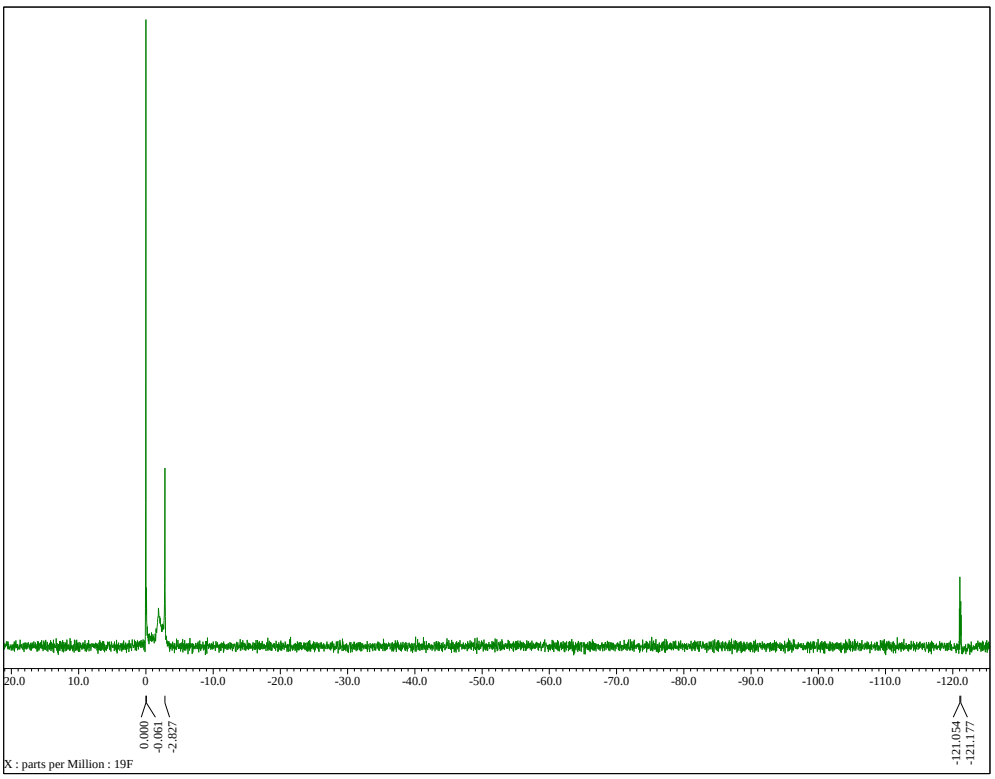
^{1}H NMR spectrum of compound **17**



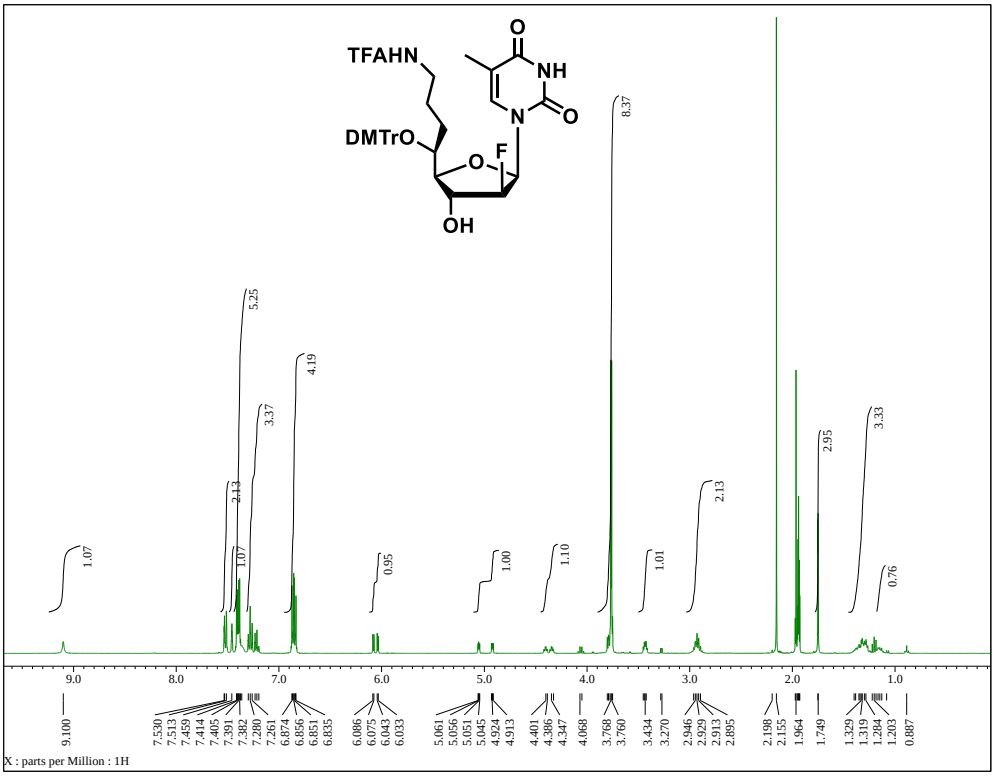
^{13}C NMR spectrum of compound **17**



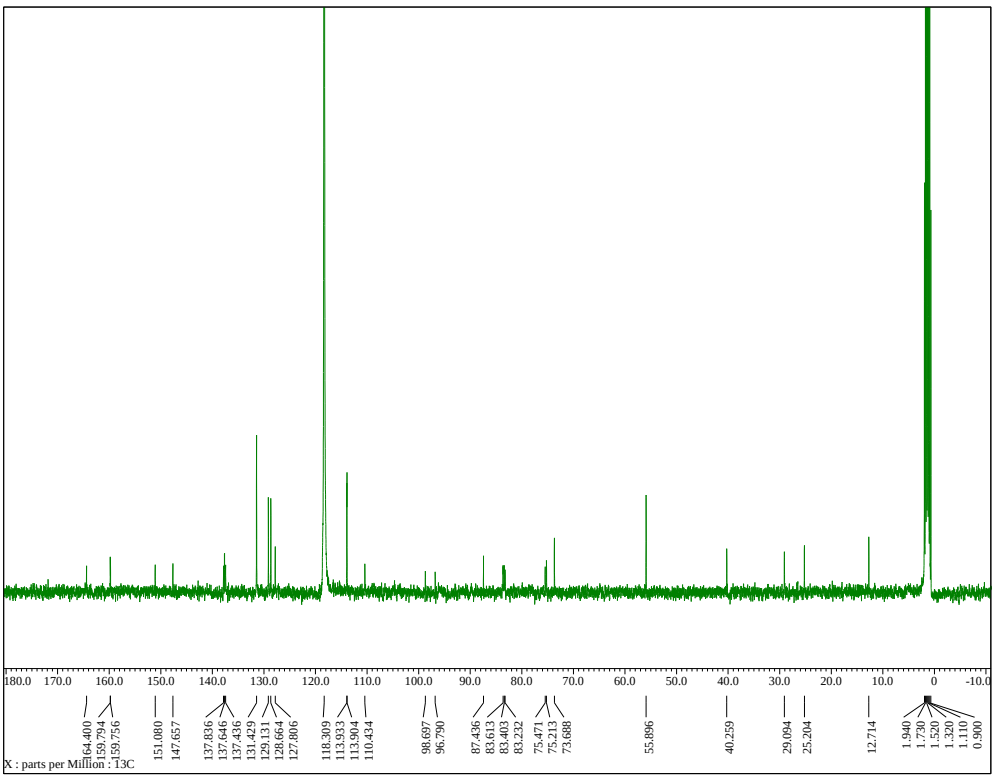
^{19}F NMR spectrum of compound **17**



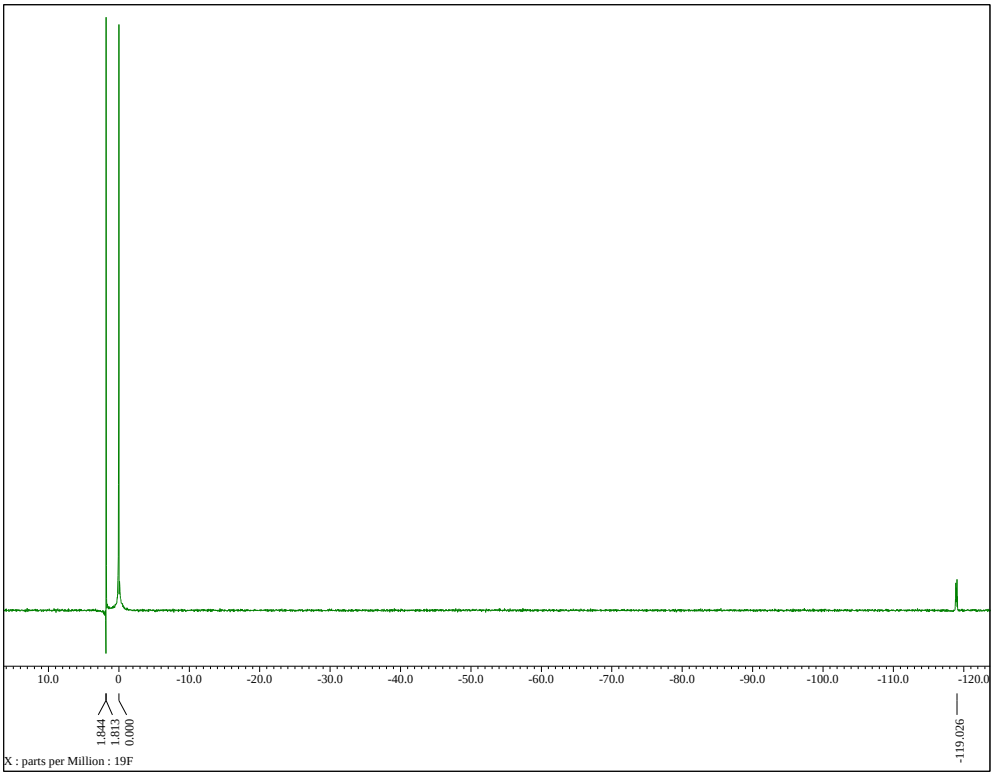
^{1}H NMR spectrum of compound **18**



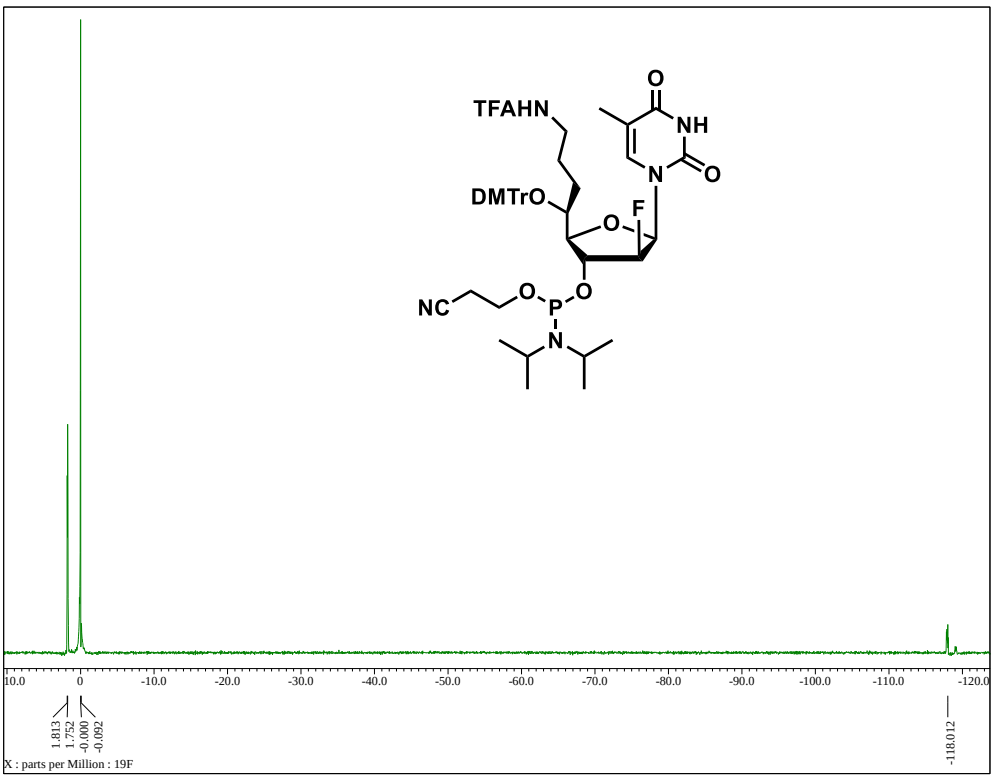
^{13}C NMR spectrum of compound **18**



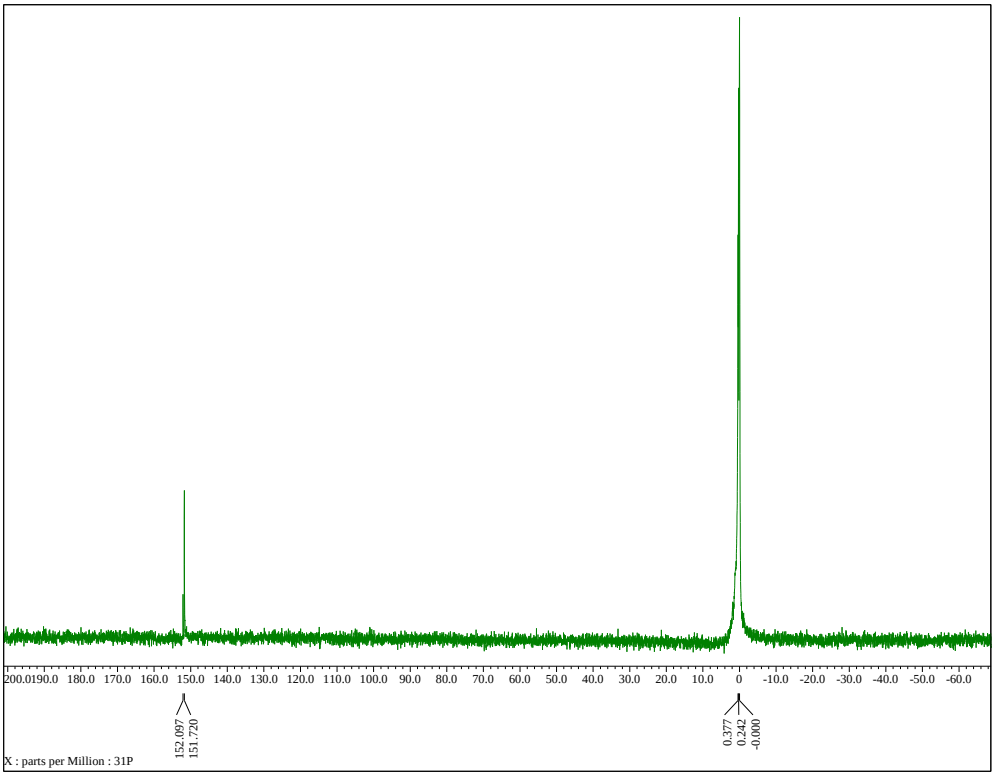
^{19}F NMR spectrum of compound **18**



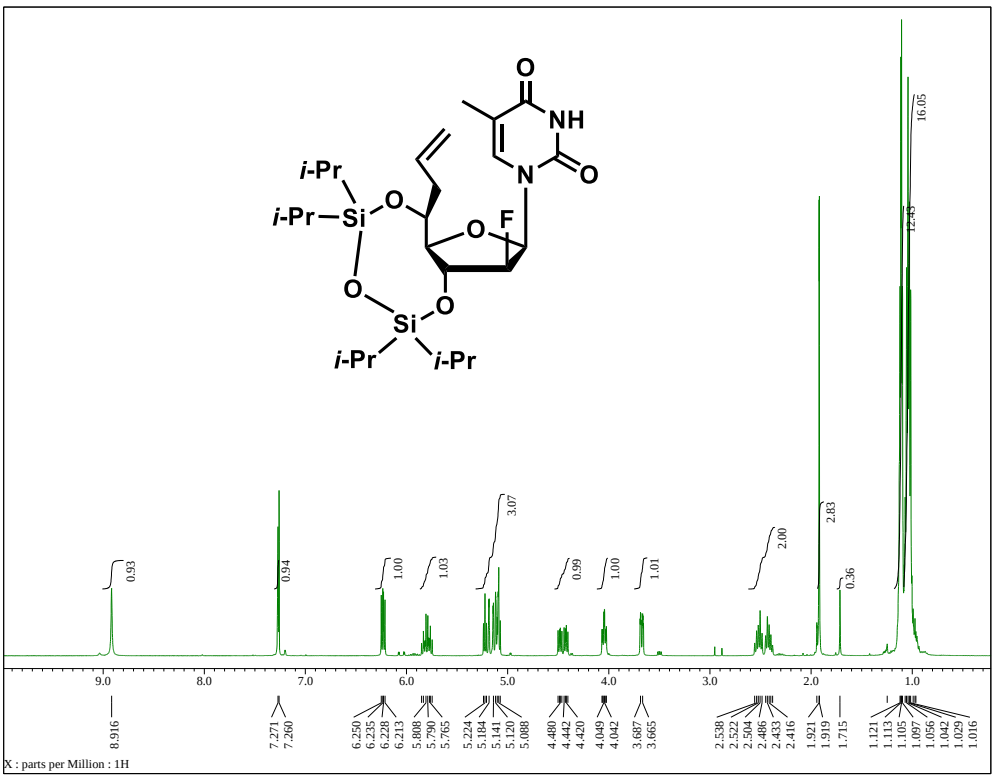
^{19}F NMR spectrum of compound **19**



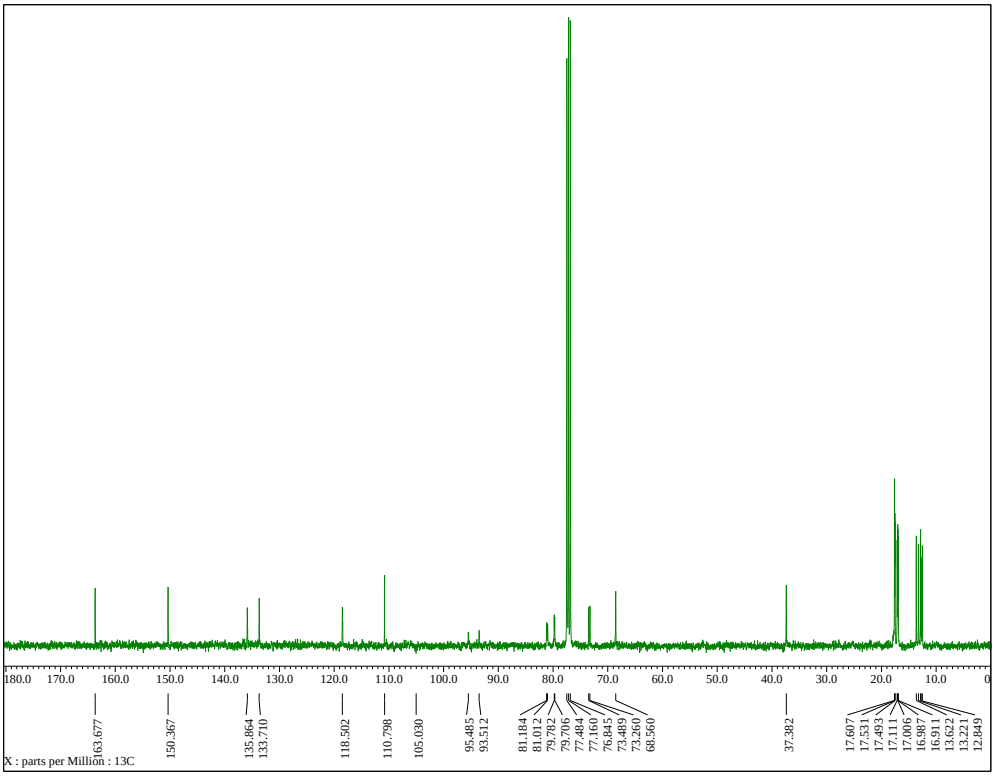
^{31}P NMR spectrum of compound **19**



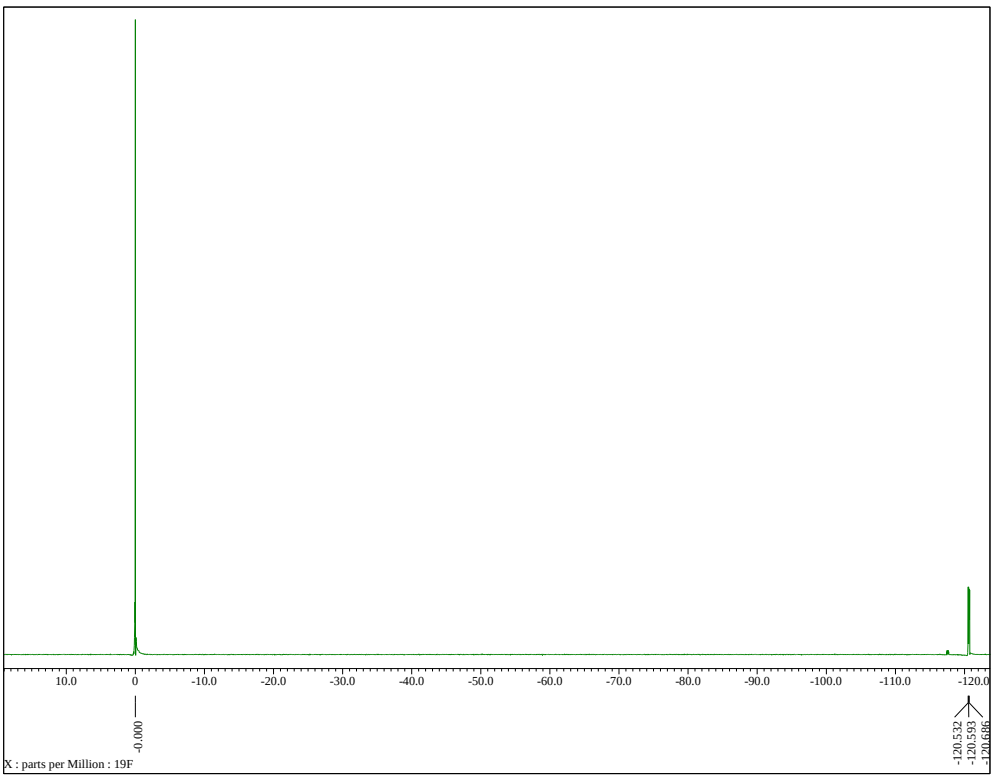
^{1}H NMR spectrum of compound **20**



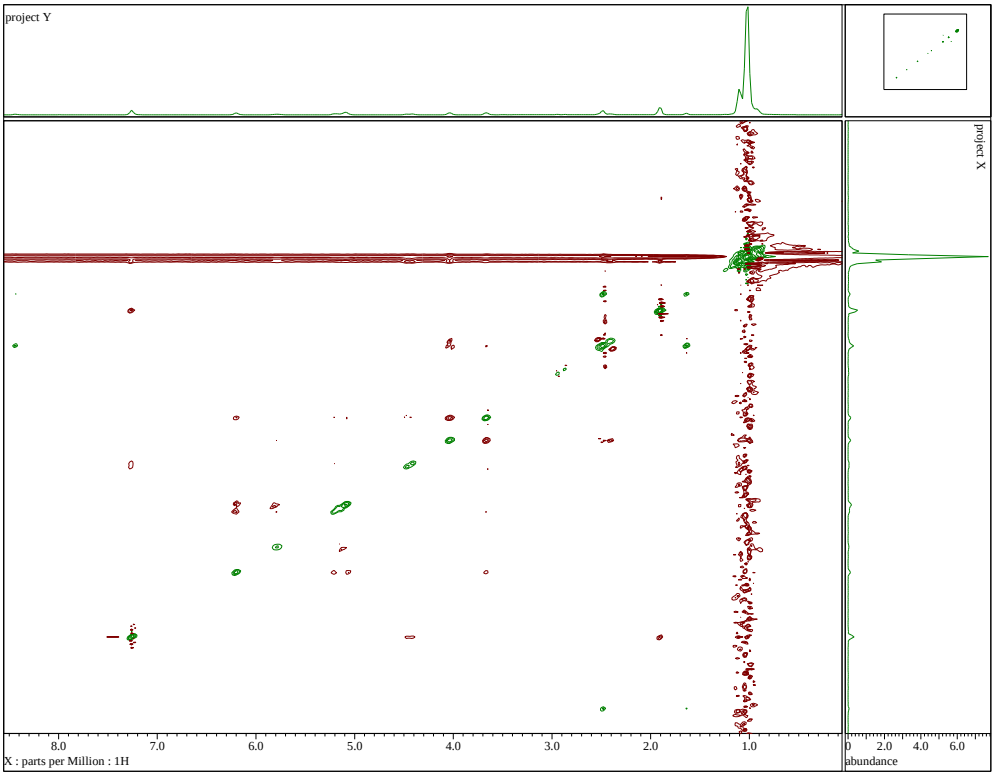
^{13}C NMR spectrum of compound **20**



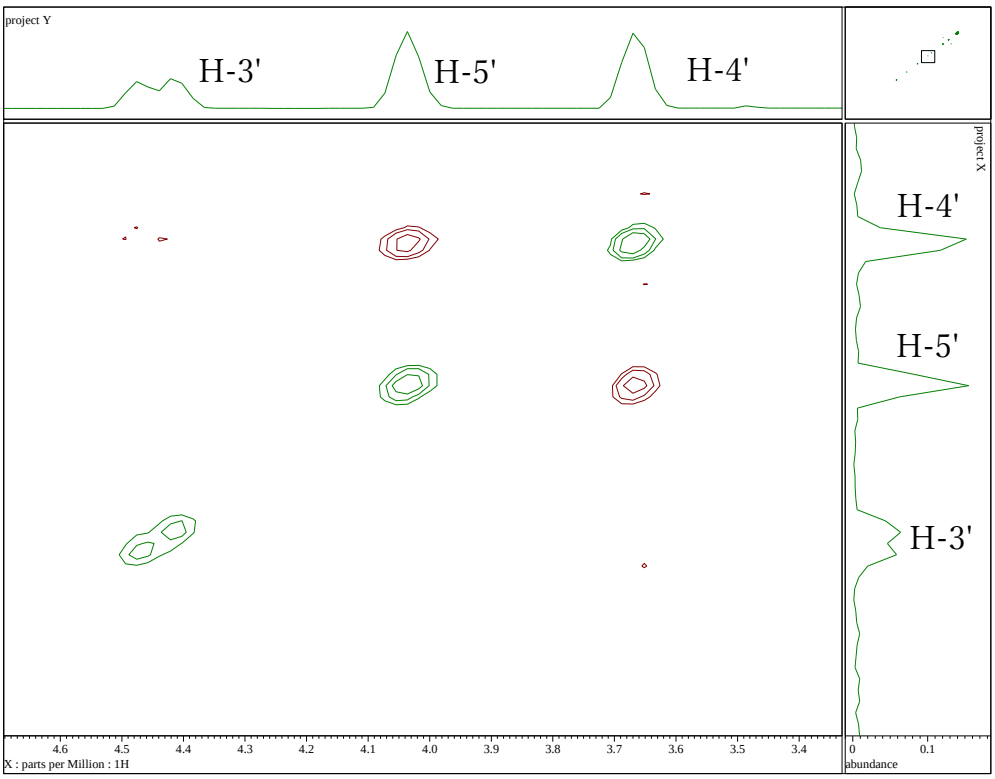
^{19}F NMR spectrum of compound **20**



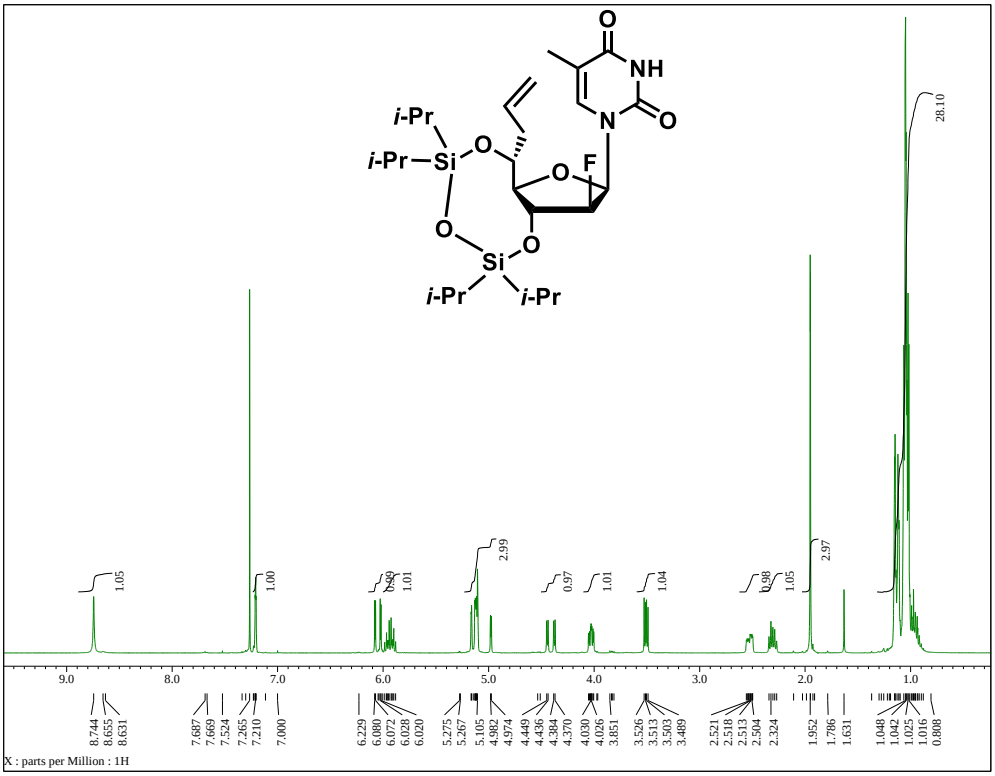
^{1}H NOESY spectrum of compound **20**



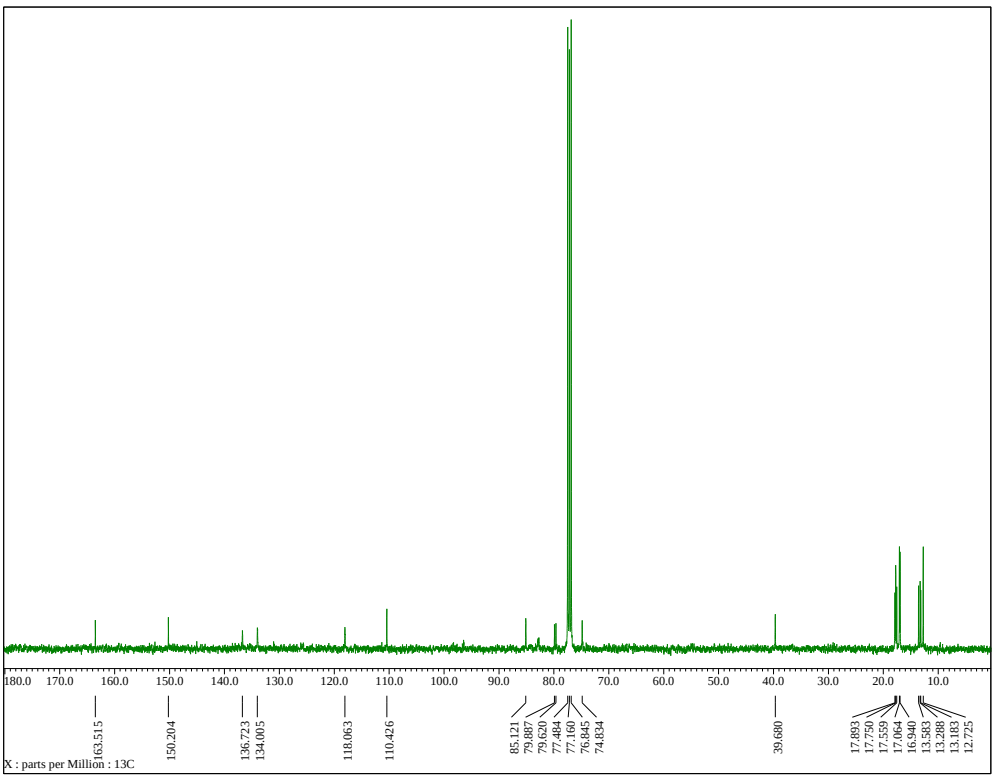
(Enlargement of H-3', H-4' and H-5')



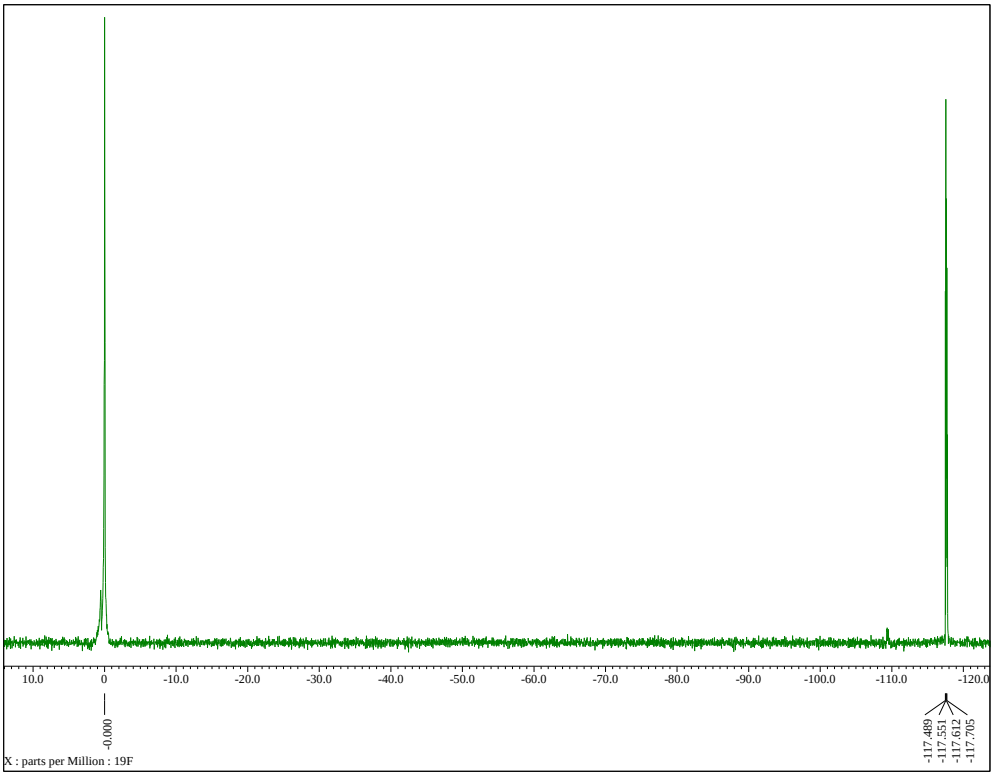
^{1}H NMR spectrum of compound **21**



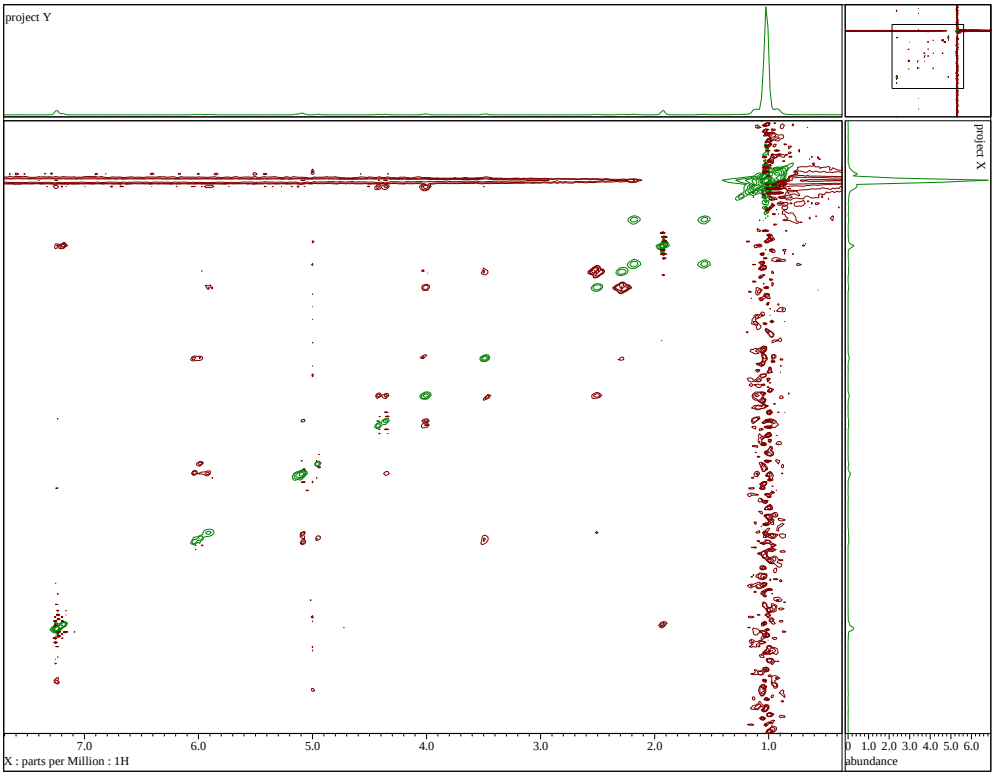
^{13}C NMR spectrum of compound **21**



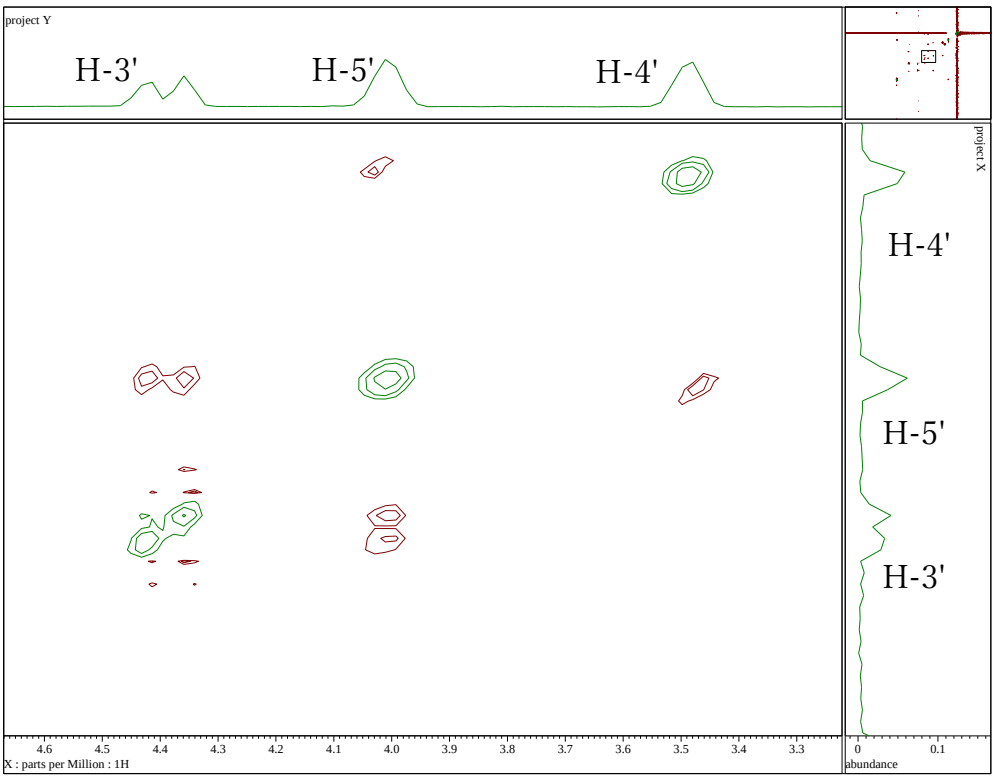
^{19}F NMR spectrum of compound **21**



^{1}H NOESY spectrum of compound **24**

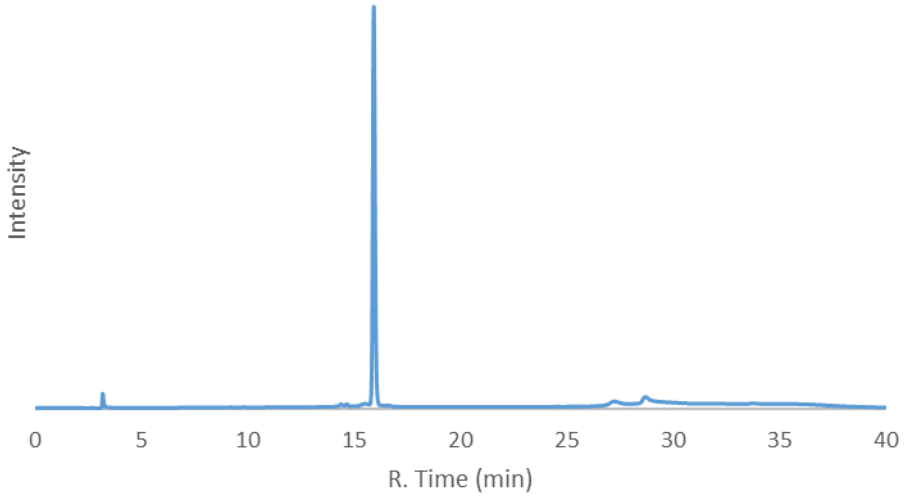


(Enlargement of H-3', H-4' and H-5')

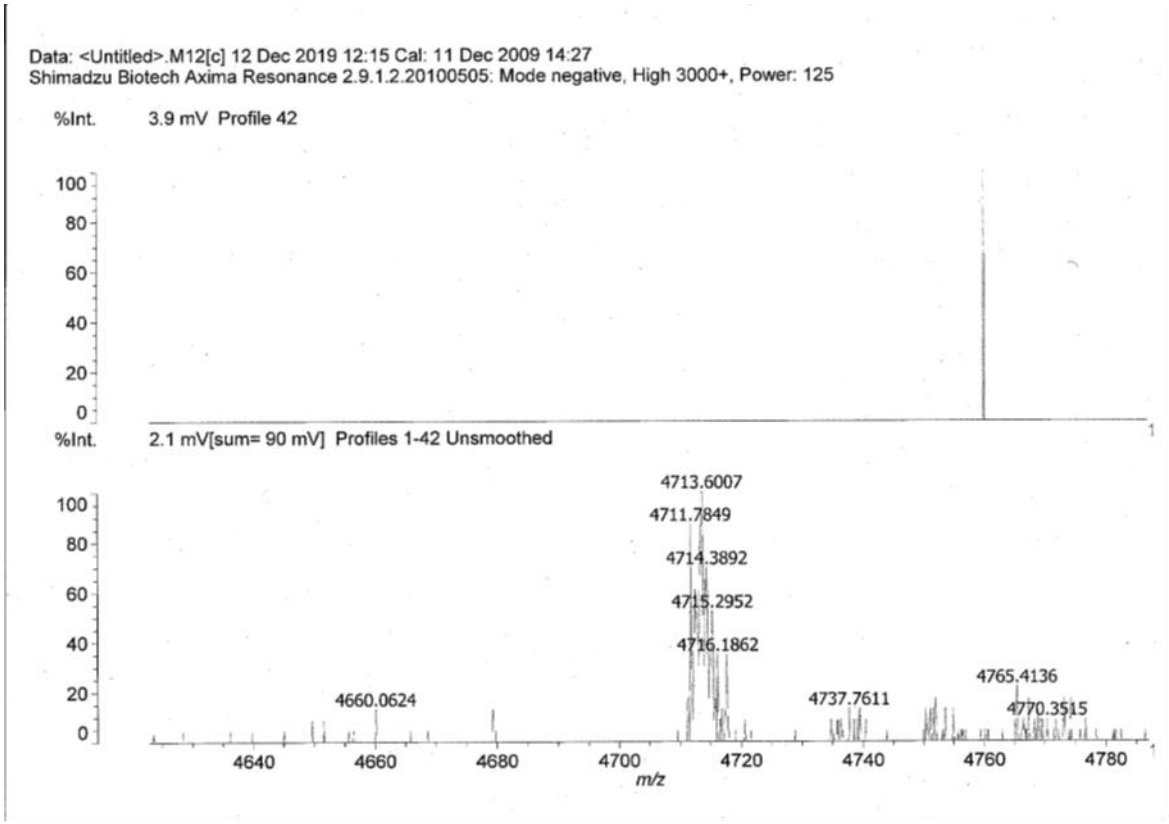


Reverse-phase HPLC spectra and MALDI-TOF/MS spectra

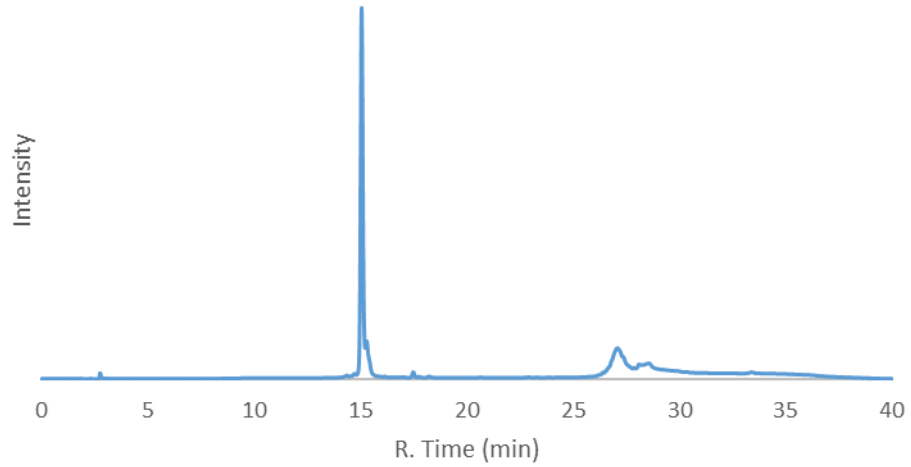
Reverse-phase HPLC spectra of DNA **1**



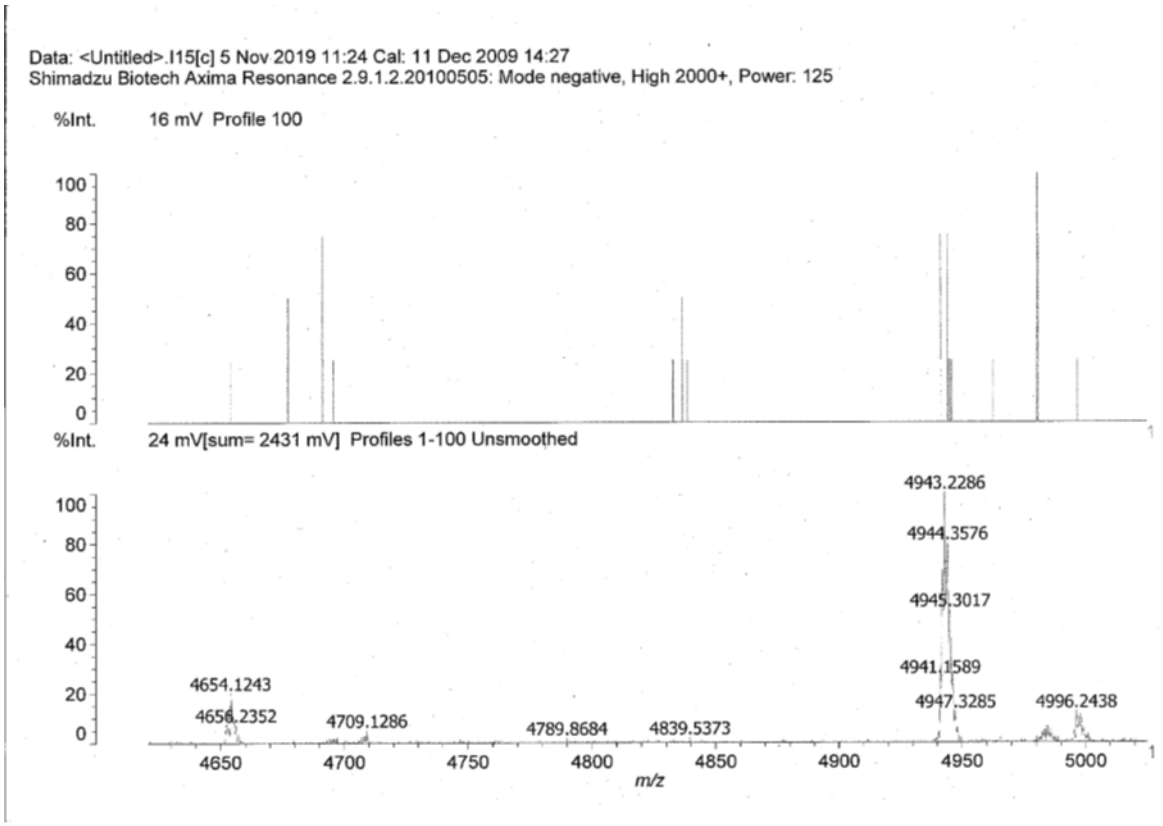
MALDI-TOF/MS spectra of DNA **1**



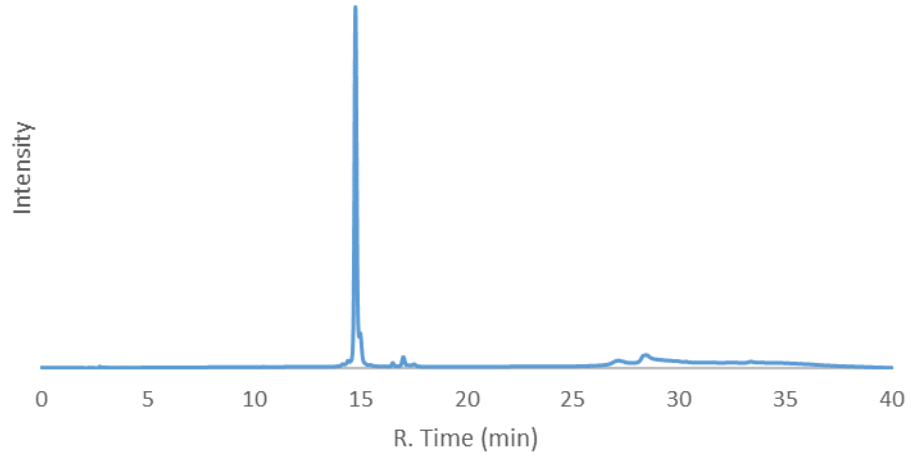
Reverse-phase HPLC spectra of DNA **2**



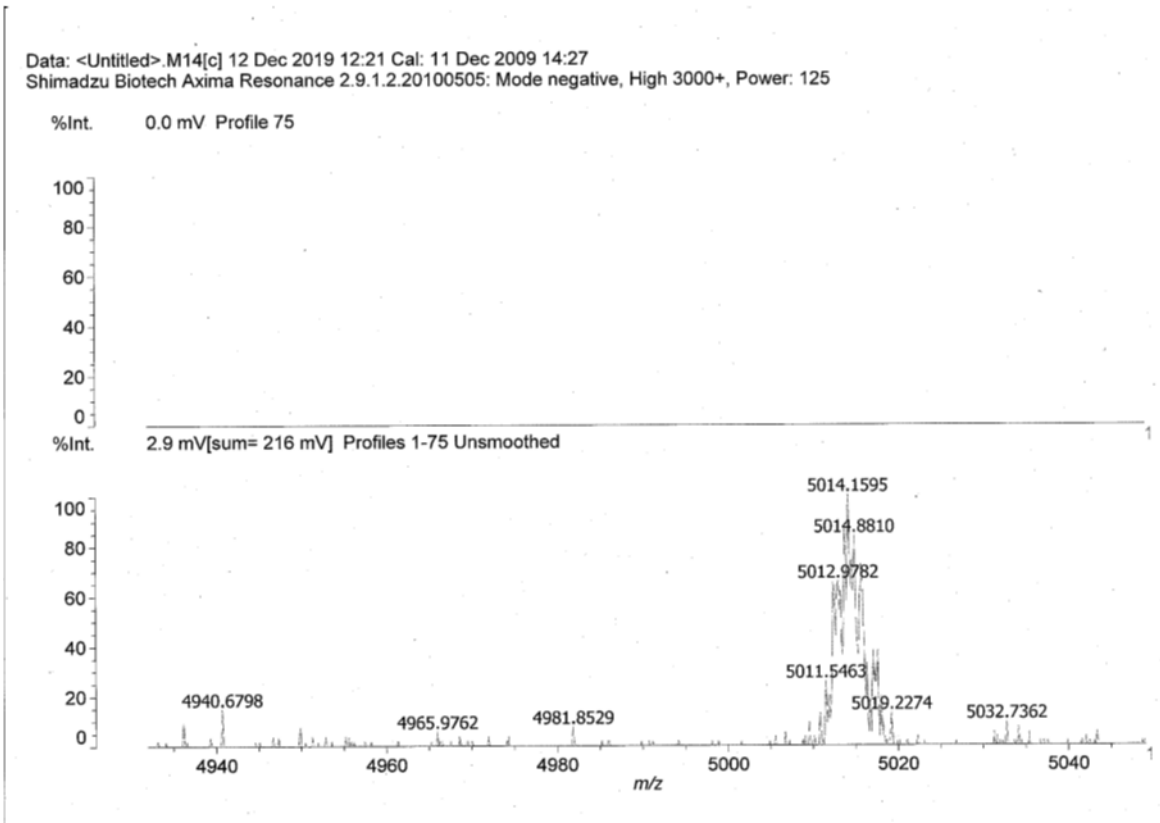
MALDI-TOF/MS spectra of DNA **2**



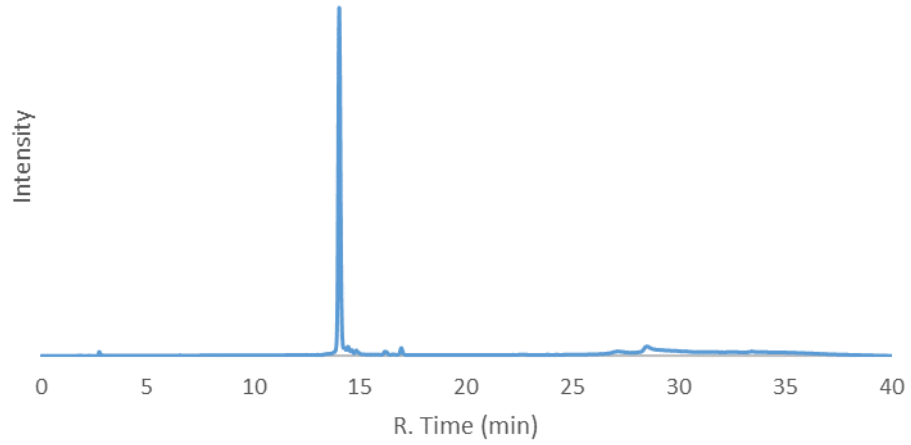
Reverse-phase HPLC spectra of DNA **3**



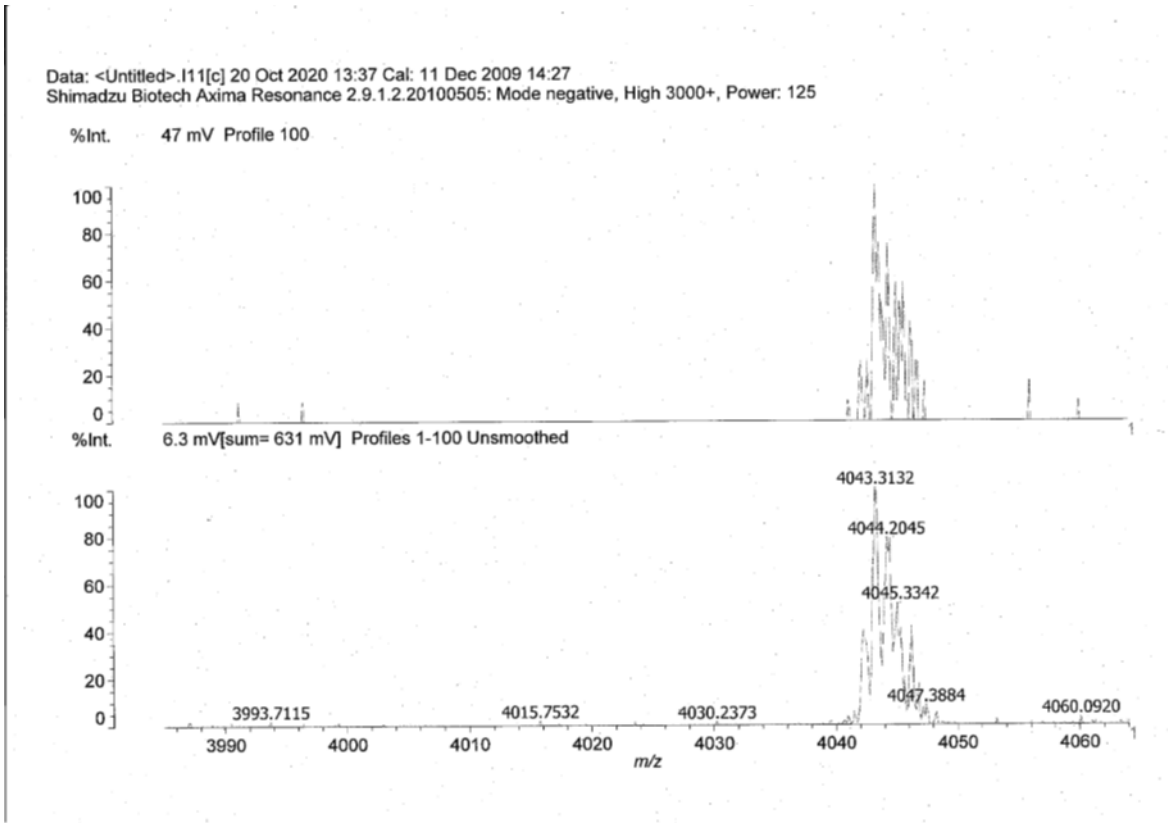
MALDI-TOF/MS spectra of DNA **3**



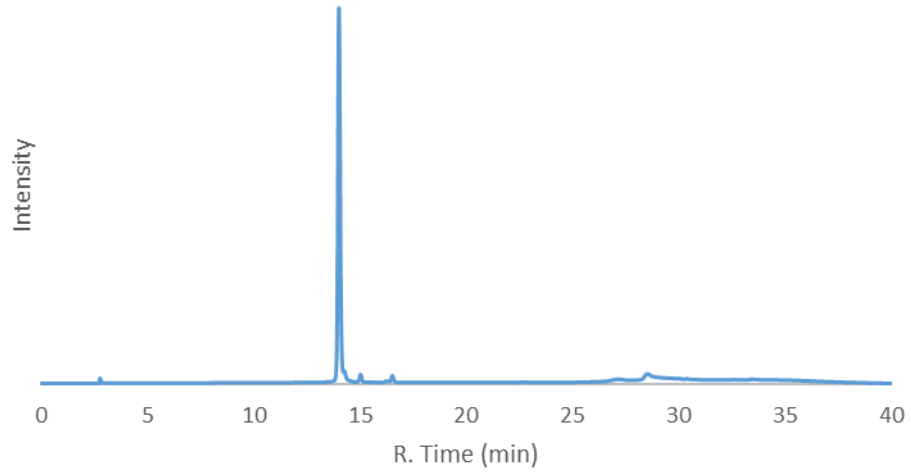
Reverse-phase HPLC spectra of DNA **5**



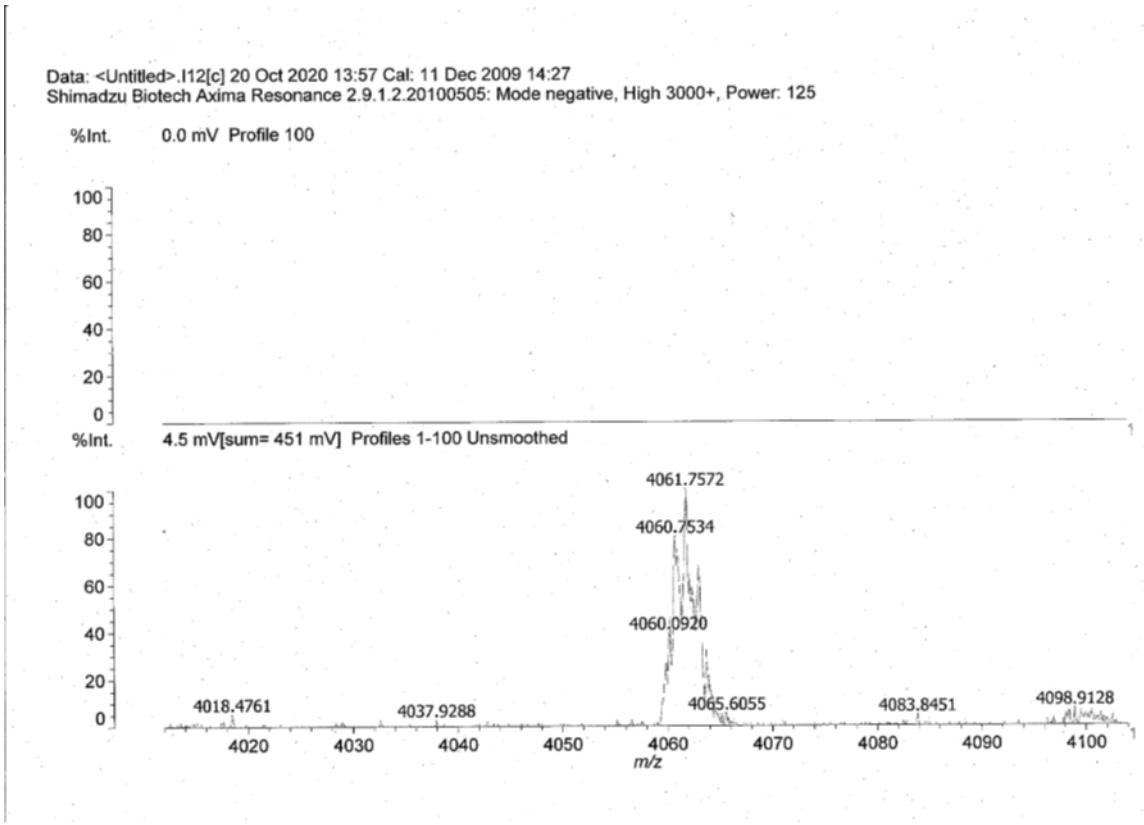
MALDI-TOF/MS spectra of DNA **5**



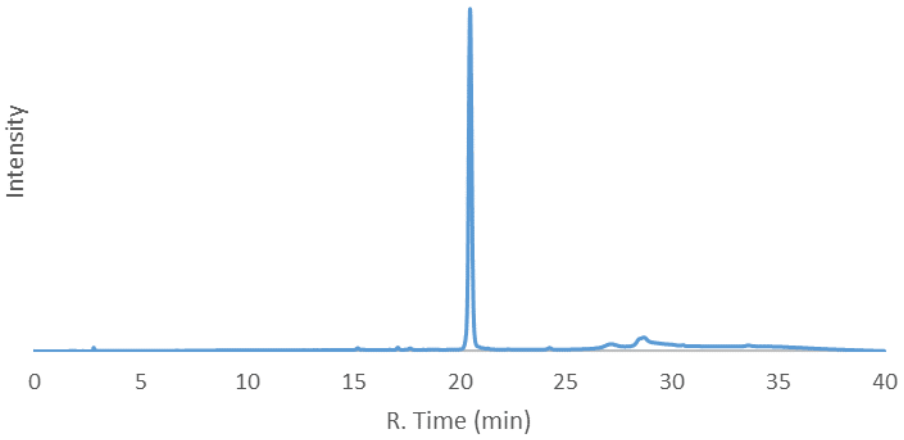
Reverse-phase HPLC spectra of DNA **6**



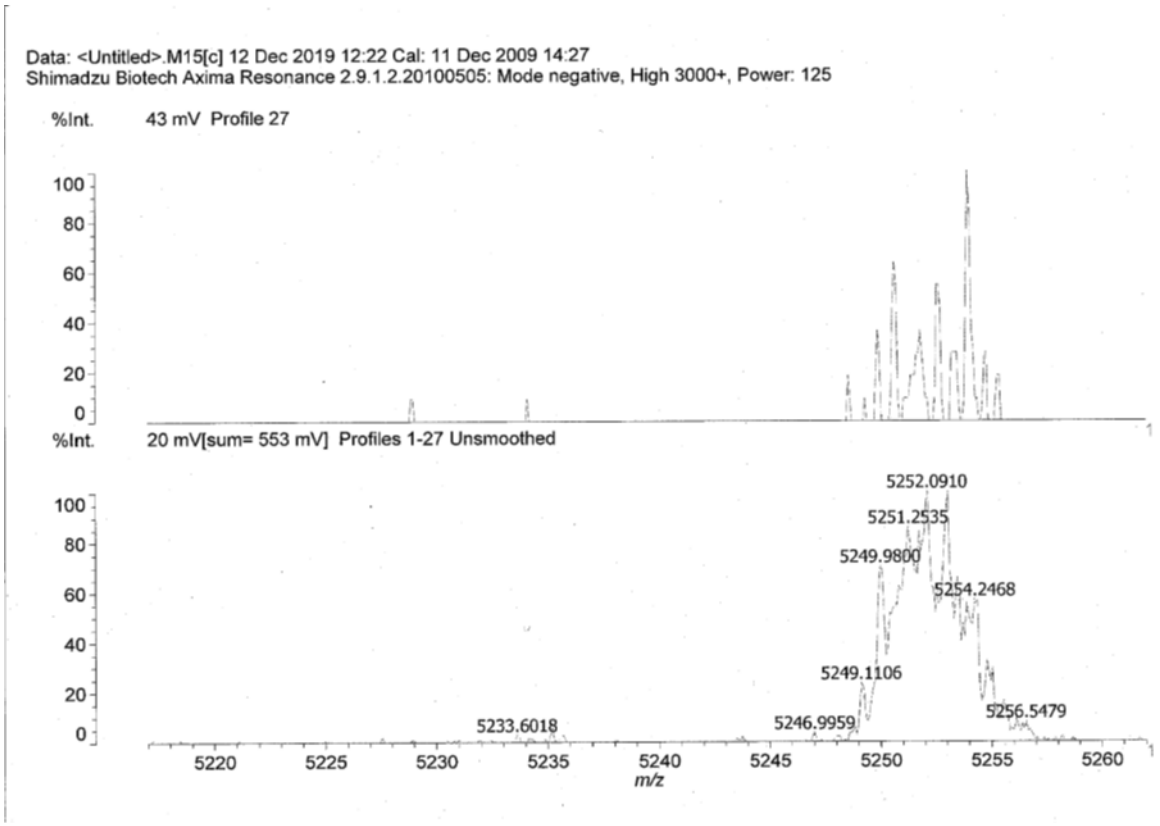
MALDI-TOF/MS spectra of DNA **6**



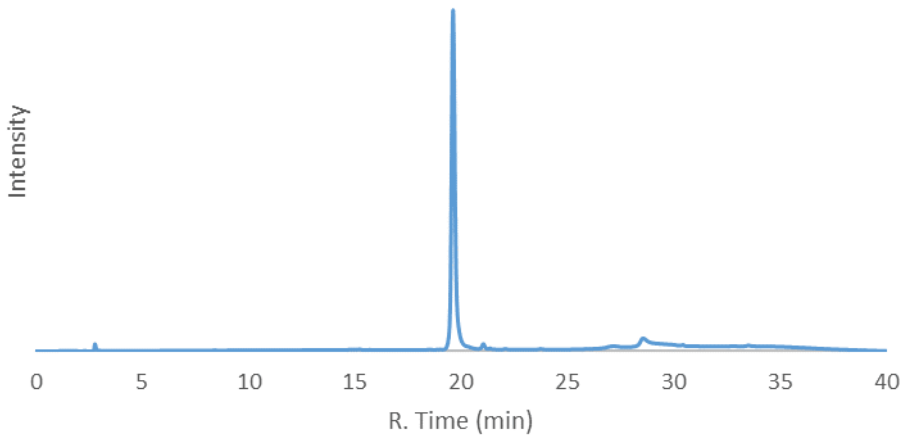
Reverse-phase HPLC spectra of DNA **7**



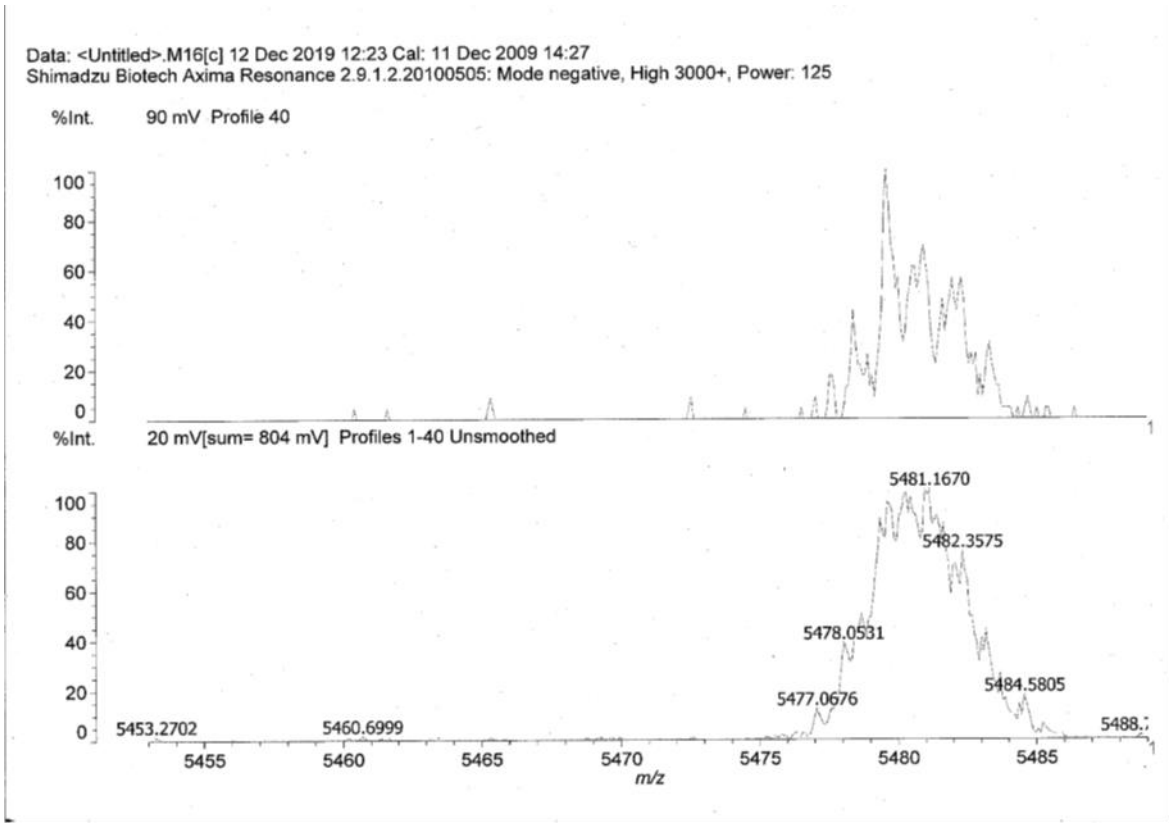
MALDI-TOF/MS spectra of DNA **7**



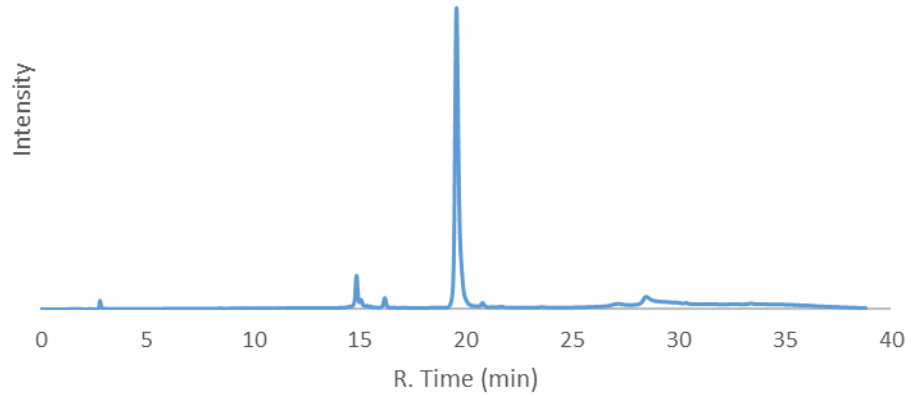
Reverse-phase HPLC spectra of DNA **8**



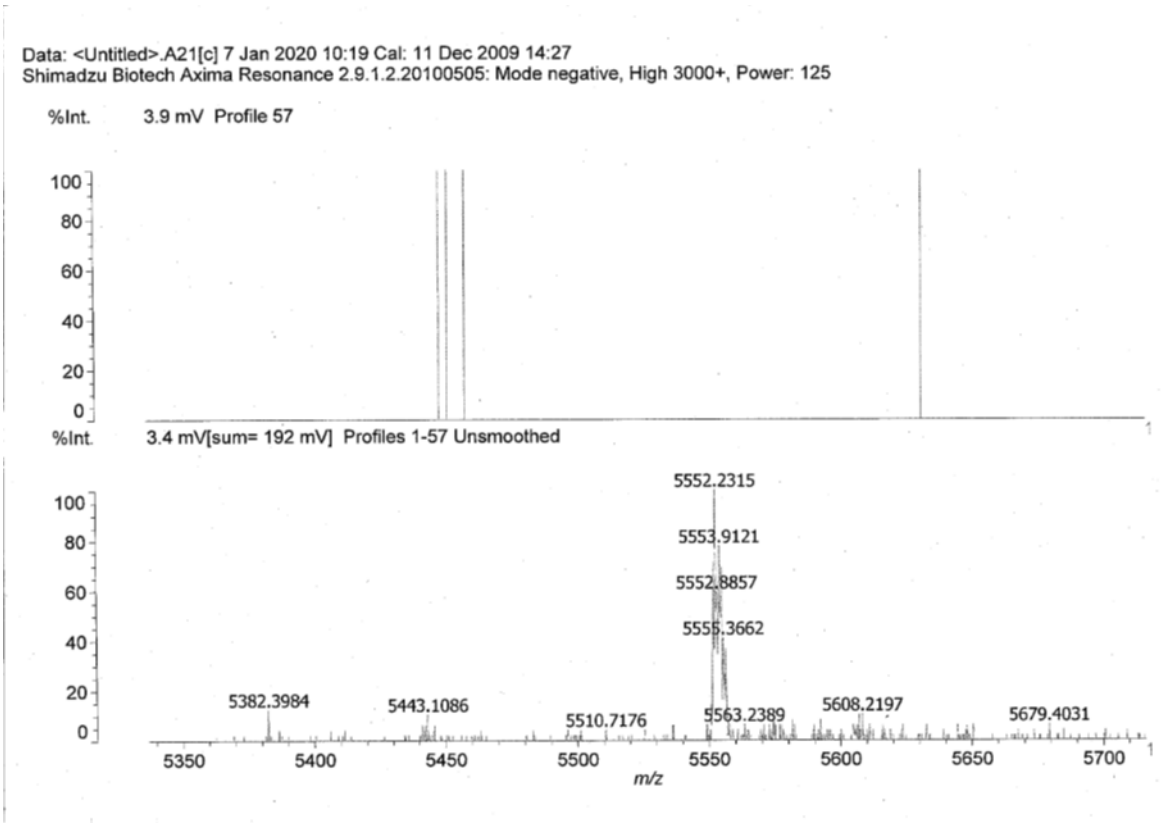
MALDI-TOF/MS spectra of DNA **8**



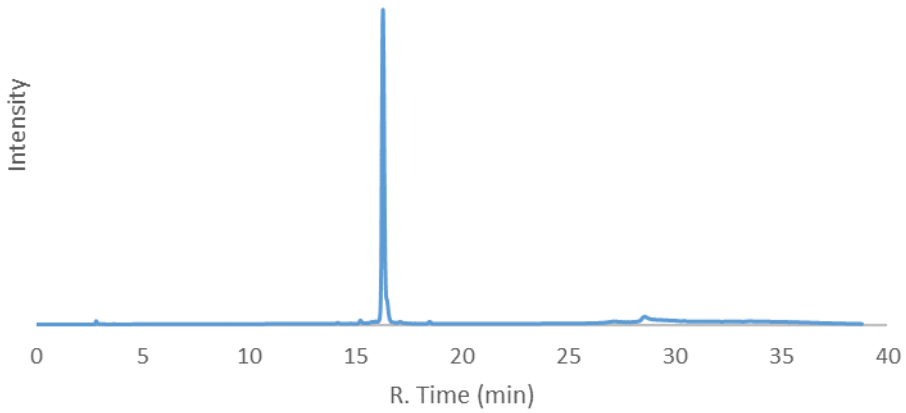
Reverse-phase HPLC spectra of DNA **9**



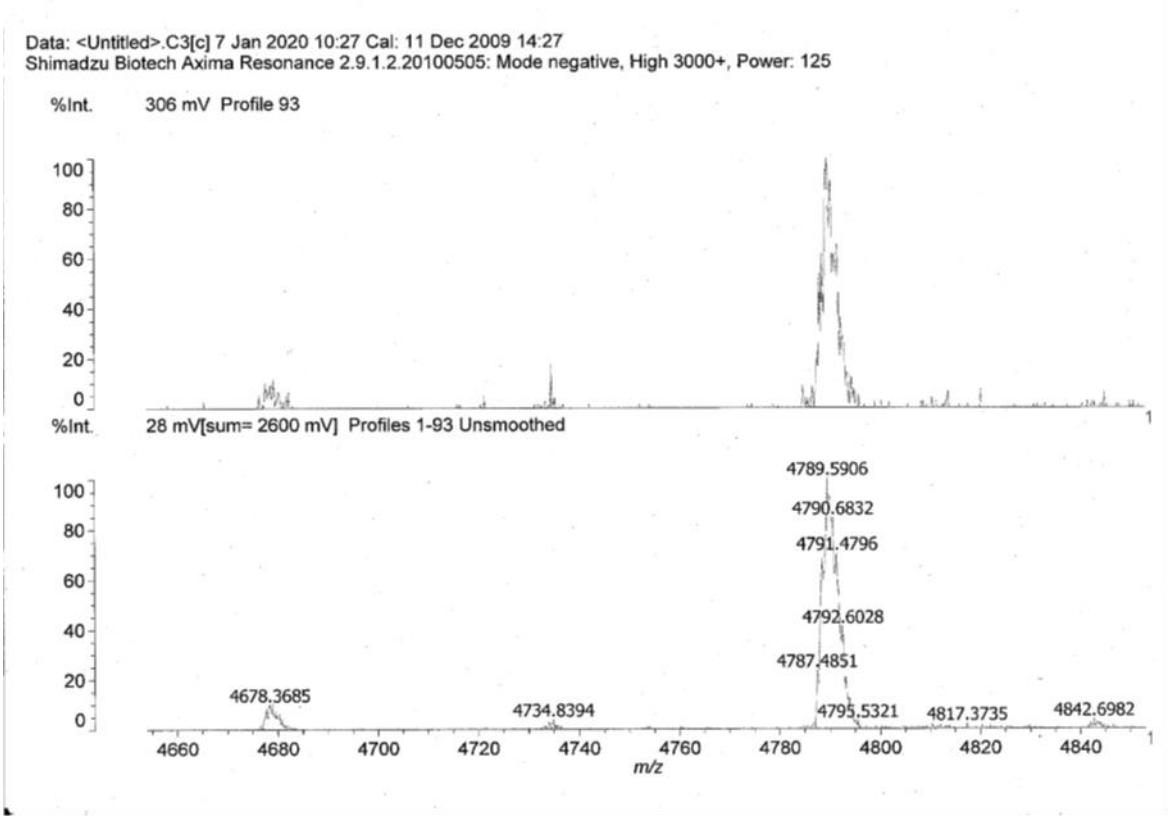
MALDI-TOF/MS spectra of DNA **9**



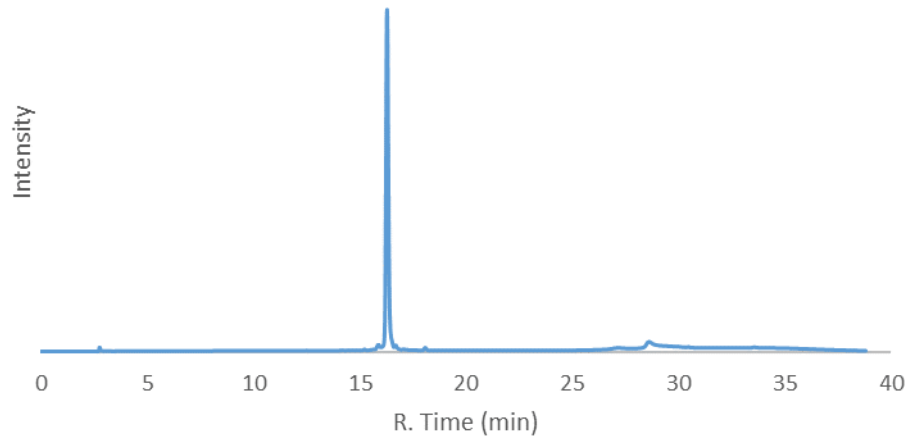
Reverse-phase HPLC spectra of DNA **10**



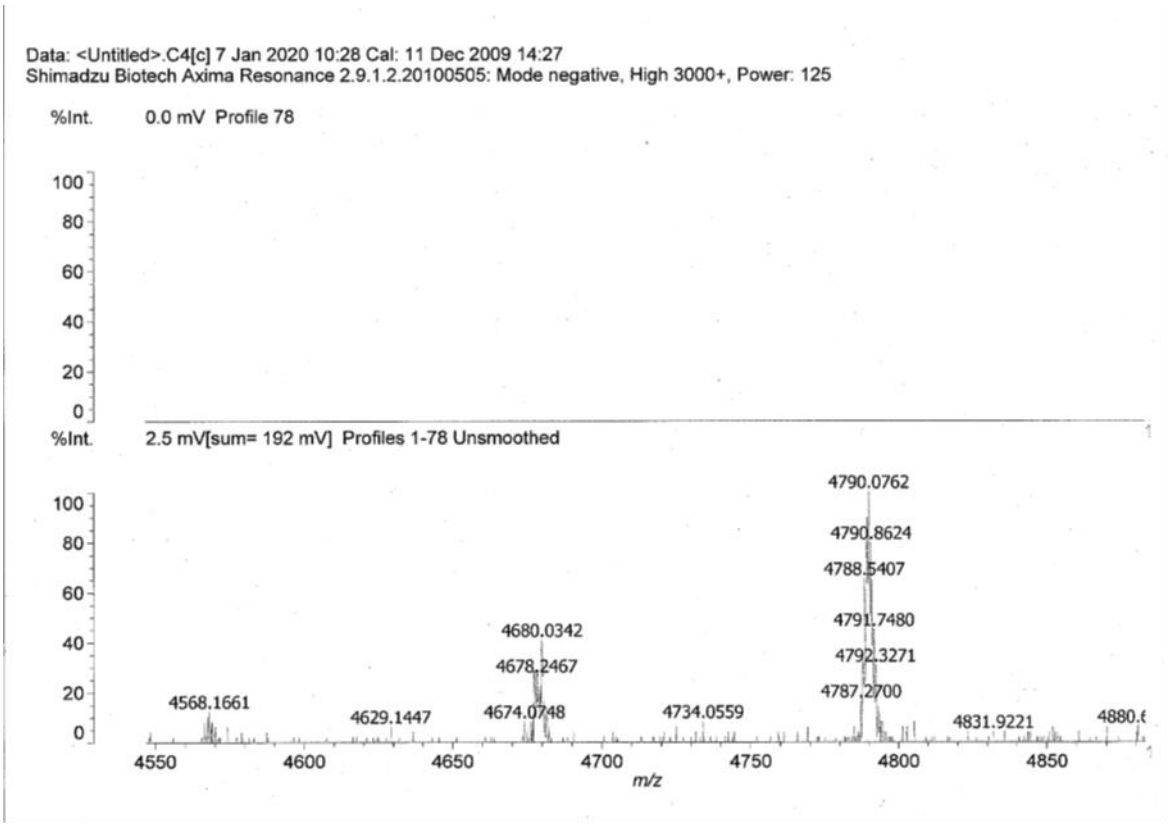
MALDI-TOF/MS spectra of DNA **10**



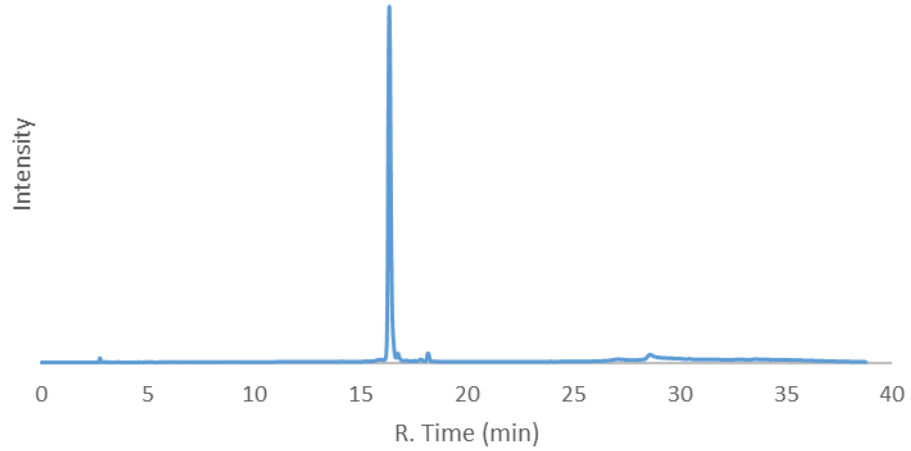
Reverse-phase HPLC spectra of DNA **11**



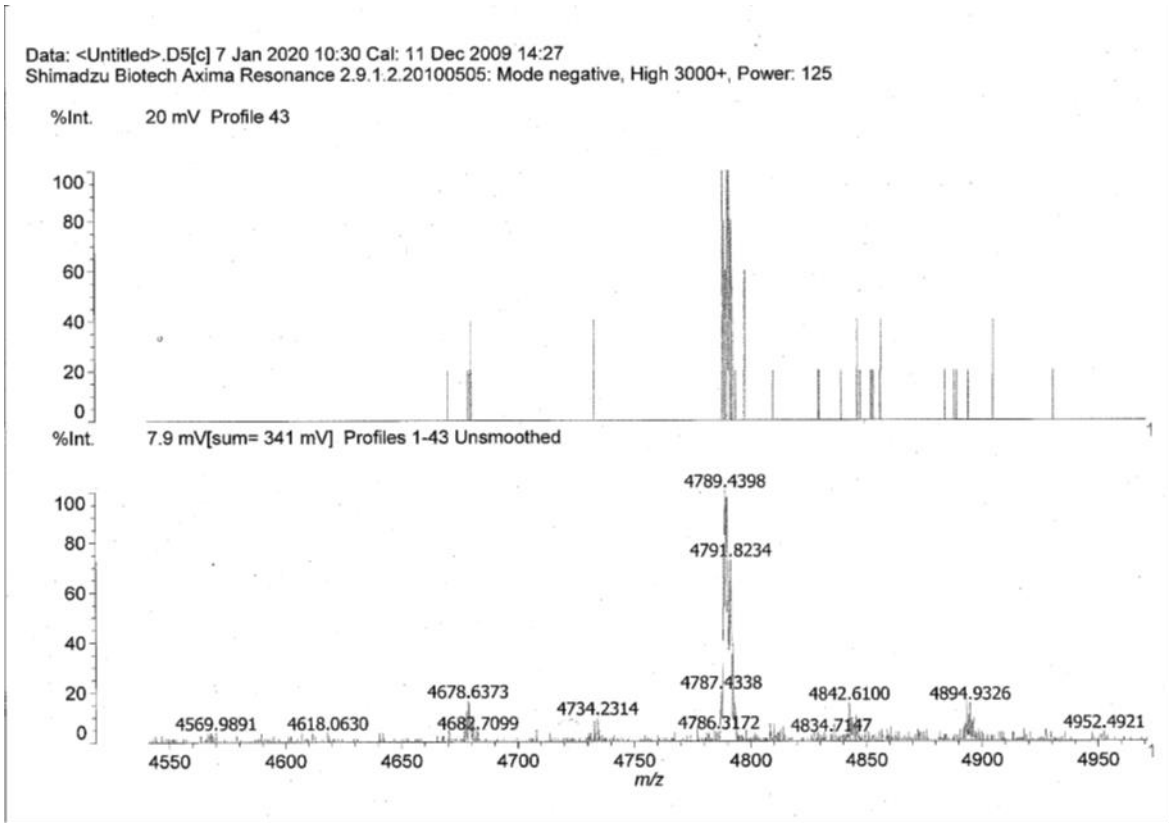
MALDI-TOF/MS spectra of DNA **11**



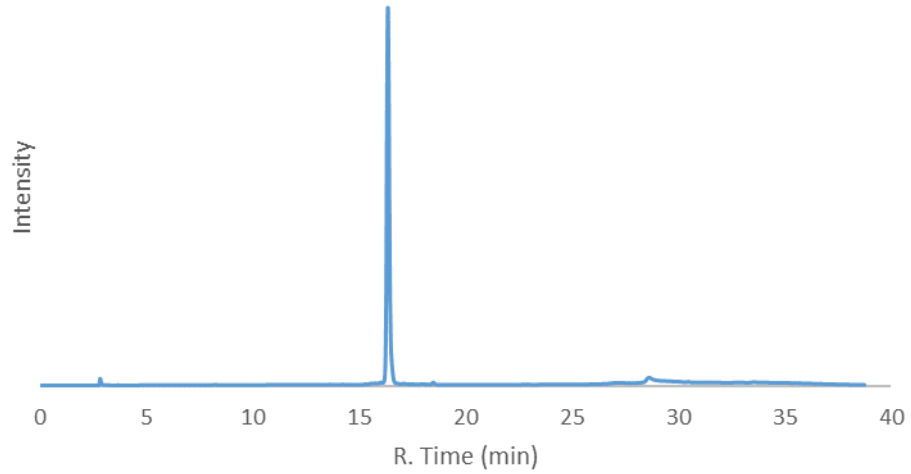
Reverse-phase HPLC spectra of DNA **12**



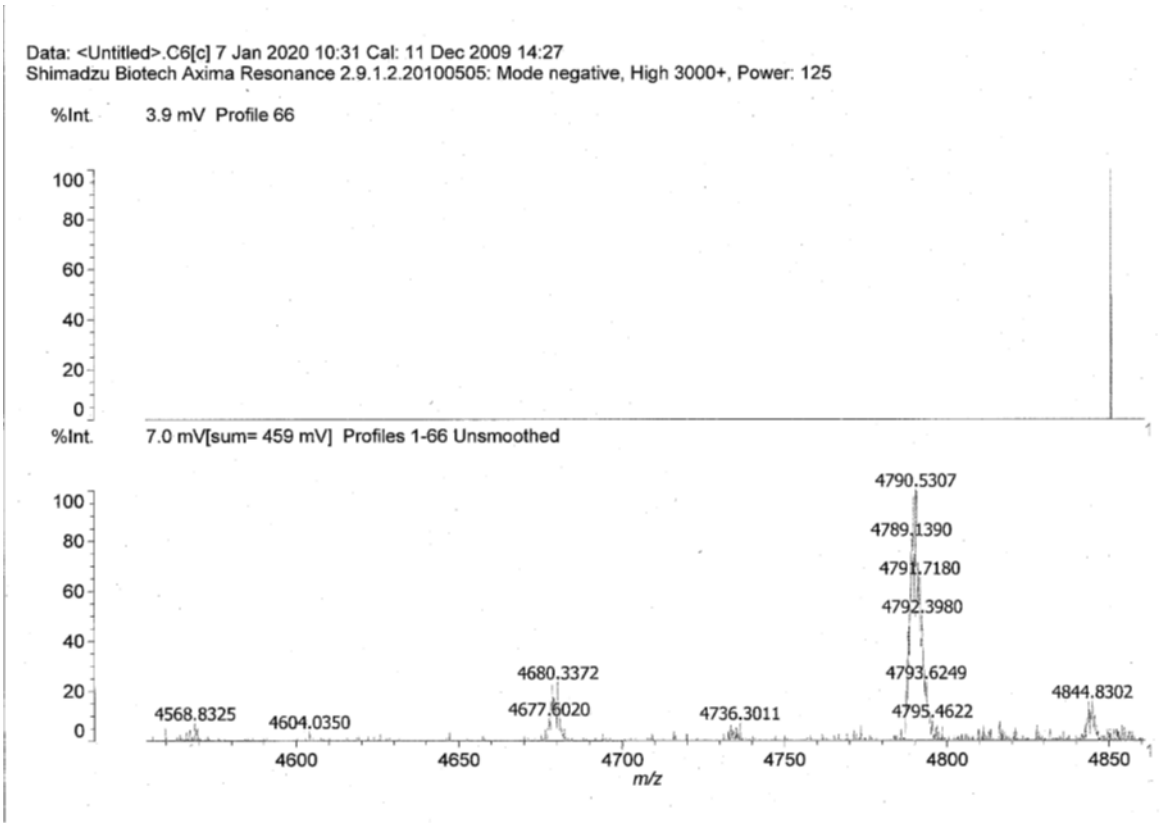
MALDI-TOF/MS spectra of DNA **12**



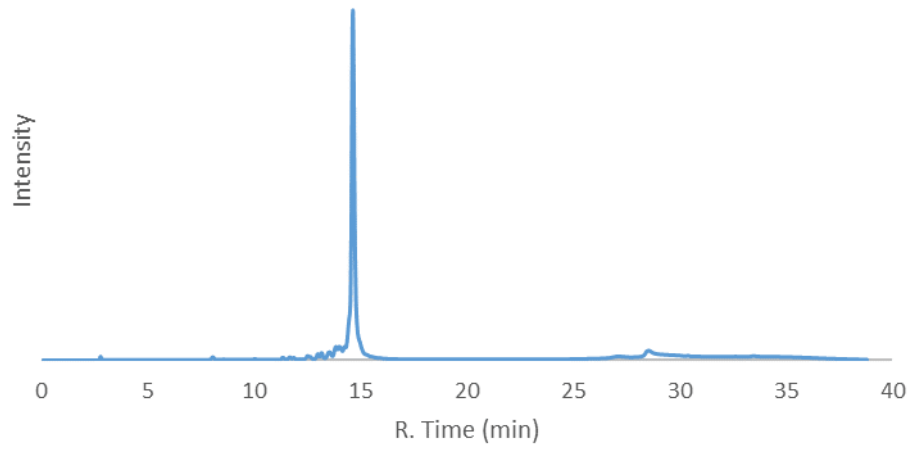
Reverse-phase HPLC spectra of DNA **13**



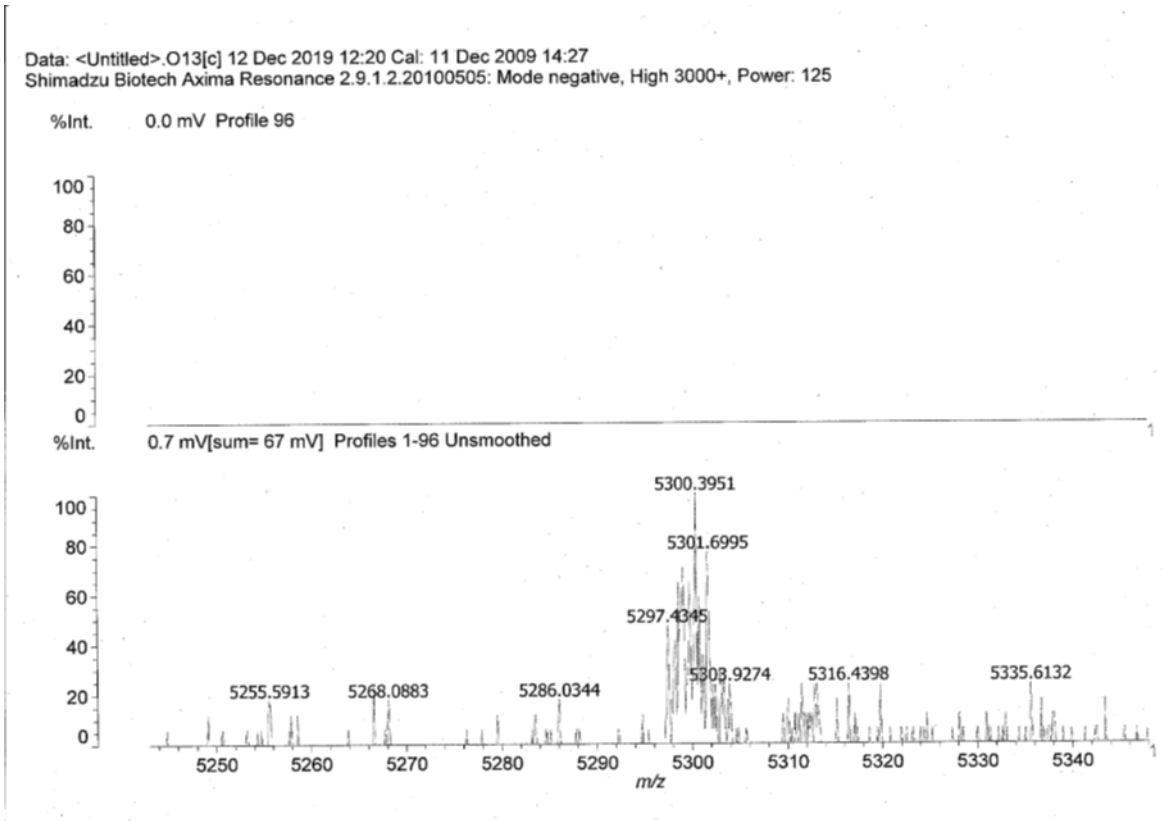
MALDI-TOF/MS spectra of DNA **13**



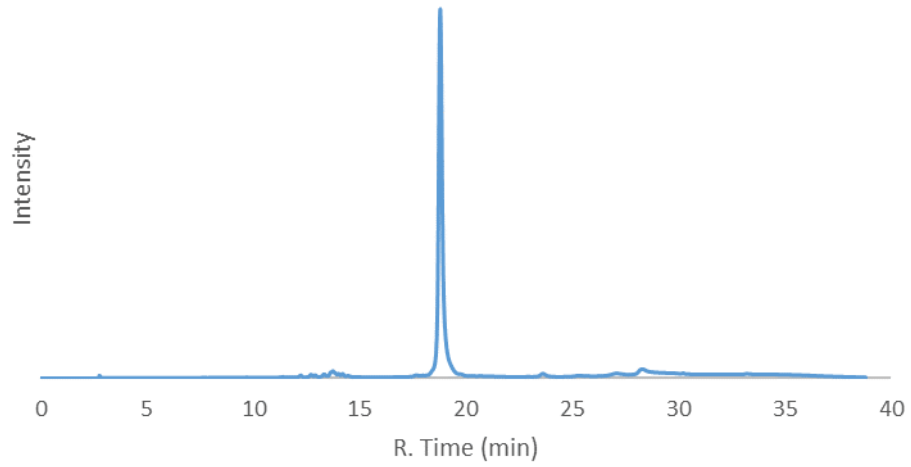
Reverse-phase HPLC spectra of RNA **1**



MALDI-TOF/MS spectra of RNA **1**



Reverse-phase HPLC spectra of RNA **6**



MALDI-TOF/MS spectra of RNA **6**