Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

Probing the furanose conformation in the 2'-5'strand of *iso*DNA:RNA duplexes by freezing nucleoside conformations

Namrata Erande, Anita D. Gunjal, Moneesha Fernandes and Vaijayanti A. Kumar*

Organic Chemistry Division, National Chemical Laboratory, Pashan Road, Pune 411008, India
va.kumar@ncl.res.in

Content

General information:

All the non-aqueous reactions were carried out under the inert atmosphere of Nitrogen/ Argon and the chemicals used were of laboratory or analytical grade. All solvents used were dried and distilled according to standard protocols. TLCs were carried out on pre-coated silica gel GF254 sheets (Merck 5554). Column chromatographic separations were performed using silica gel 60-120 mesh (Merck) or 200- 400 mesh (Merck) and using the solvent systems EtOAc/Pet ether and MeOH/DCM. ^{1}H and ^{13}C NMR spectra were obtained using Bruker AC-200, AC-400 or NMR spectrometers. The chemical shifts are reported in delta (δ) values and referred to internal standard TMS for ^{1}H. Mass spectra were recorded on a Finnigan-Matt mass spectrometer. Oligomers were analyzed by RP HPLC (Varian/Waters) on a C18 column using an increasing gradient of acetonitrile in 0.1N triethylammonium acetate, pH 7.0, and characterized by MALDI-TOF mass spectrometry. The MALDI-TOF spectra were recorded on Voyager-De-STR (Applied Biosystems) MALDI-TOF instrument and the matrix used for analysis was THAP (2',4',6'-trihydroxyacetophenone). DNA oligomers were synthesized on a Bioautomation Mer-Made 4 synthesizer using standard β-cyanoethyl phosphoramidite chemistry.

Experimental procedures for compounds Ia, IIa, IIIa, IVa (R_1=DMT, R_2=H)

General procedure for the synthesis of Ia-IVa (R_1=DMT, R_2=H):

Synthesis of 1-(5'-O-dimethoxytrityl-3'-deoxy-3'-fluoro-β-D-xylofuranocyl) uracil **IVa** :

A mixture of compound 3'-deoxy-3'-xylofluorouridine **IV** (R_1=R_2=H), (0.200g, 0.81mmol), 4, 4'-dimethoxytrityl chloride, (DMTCl) (0.827g, 2.4mmol), and 4-dimethylaminopyridine (DMAP) catalytic amount was dissolved in pyridine (5mL). The reaction mixture was stirred at room temperature for 12h. The pyridine was removed under vacuum. The residue was dissolved in ethyl acetate (100mL), washed with saturated aq. $NaHCO_3$ (2 X 50ml) and saturated aqueous NaCl (2X30mL). The ethyl acetate layer was dried over Na_2SO_4 filtered and evaporated to dryness. The crude product was purified by silica gel (neutralized with Et_3N) column chromatography using EtOAC / Pet ether (8:2) to offer the title compound **IV$_a$**, (70%, white foam). Compounds **I, II** and **III** were likewise converted to their respective dimethoxytrityl derivatives **Ia, IIa, IIIa** respectively.

Spectral data for Compounds IIa, IIIa, IVa (R_1= DMT, R_2 = H), IIb, IIIb, IVb (R_1= DMT, R_2= $P(OCH_2CH_2CN)$ $N-CH(CH_3)_2$ $CH(CH_3)_2$),

1. **5'-O-dimethoxytrityl-2'-hydroxy-3',6'-anhydro-uridine IIa**

^{1}H NMR ($CDCl_3$, 50 MHz): δ 3.18-3.34 (m, 2H), 3.79 (s, 6H), 4.16-4.17 (d, 1H), 4.33-4.44 (m,2H), 5.71 (d,1H, J = 8.1Hz) 5.77 (s, 1H), 6.83-6.86 (m,4H), 7.27-7.51 (m, 9H) 8.30 (d,1H, J = 8.1Hz), 10.16 (bs, 1H). **^{13}C NMR ($CDCl_3$, 50 MHz):** δ 55.2, 72.2, 75.1, 80.1, 85.3, 86.7, 88.0, 96.0, 101.3, 113.3-113.4, 123.8, 127.1, 127.8, 128.1, 129.9, 135.8, 136.0, 136.0, 140.6, 144.9, 149.6, 150.8 158.8, 164.2. **Mass**: mass calculated for $C_{31}H_{30}N_2O_8$ +Na^+ 581.1900, observed mass m/z 581.08 M+ 23(Na).

2. **1-(5'-O-dimethoxytrityl-3'-deoxy-3'-fluoro-β-D-ribofuranosyl) uracil IIIa**

^{1}H NMR ($CDCl_3$,200MHz): δ 3.39-3.54 (m, 2H). 3.78 (s, 6H), 4.36-4.45 (m, 2H), 4.94-5.23 (dd, 1H, $J_{3',F}$ = 54.3 and $J_{3',4'}$ = 2.0Hz), 5.45 (d,1H, $J_{6,5}$ = 8.0Hz), 6.15 (d,1H, $J_{1,2}$=6.5Hz), 6.84 (m, 4H), 7.22-7.33 (m, 9H),7.71 (d, 1H, $J_{5,6}$= 8.0Hz) **^{13}C NMR ($CDCl_3$,50 MHz,):** δ 55.2, 62.7, 74.8, 82.2, 87.4, 90.0, 93.6, 103.0, 113.3, 127.2, 128.0, 129.9,134.8,139.7, 143.8, 151.2, 158.7, 163.4. **Mass:** mass calculated for $C_{30}H_{29}FN_2O_7$ +Na^+ 571.1856, observed mass m/z 571.15 M+ 23(Na).

3. **1-(5'-O-dimethoxytrityl-3'-deoxy-3'-fluoro-β-D-xylofuranosyl) uracil IVa**

^{1}H NMR ($CDCl_3$,200MHz): ^{1}H NMR ($CDCl_3$): δ: 3.53 (m, 2H). 3.80 (s, 6H), 4.40 (d, 1H), 4.51-4.73 (m, 1H), 4.86-5.10 (dd, 1H, $J_{3',F}$ = 50.7 and $J_{3',4'}$ = 3.1Hz), 5.63 (d, 1H, J_{65} = 8.2 Hz), 6.87(m, 4H) 5.81(s, 1H), 7.26-7.35(m, 9H), 7.46 (d, 1H, $J_{5,6}$= 8.2Hz), **^{13}C NMR($CDCl_3$,50MHz):** δ 55.1, 60.3, 78.4, 82.8, 86.5, 93.0, 96.4, 101.7, 113.1, 126.9,

127.8, 128.0, 130.0, 135.5, 139.8, 144.3, 150.9, 158.5, 164.1. **Mass:** mass calculated for $C_{30}H_{29}FN_2O_7$ +Na^+ 571.1856, observed mass m/z 571.37 M+ 23(Na).

4. **5'-*O*-dimethoxytrityl-3'-*O*,5'-*C*-methylene-3'-xylo-uridine-2'-*O*-(2-cyanoethoxy)-*N,N*-diisopropylphosphoramidite IIb**

   ^{31}P NMR ($CDCl_3$, 50 MHz): δ 150.7, 152.0. **Mass:** mass calculated for $C_{40}H_{47}N_4O_9P$ +Na^+ 781.2978 observed mass m/z 781.23 M+ 23(Na).

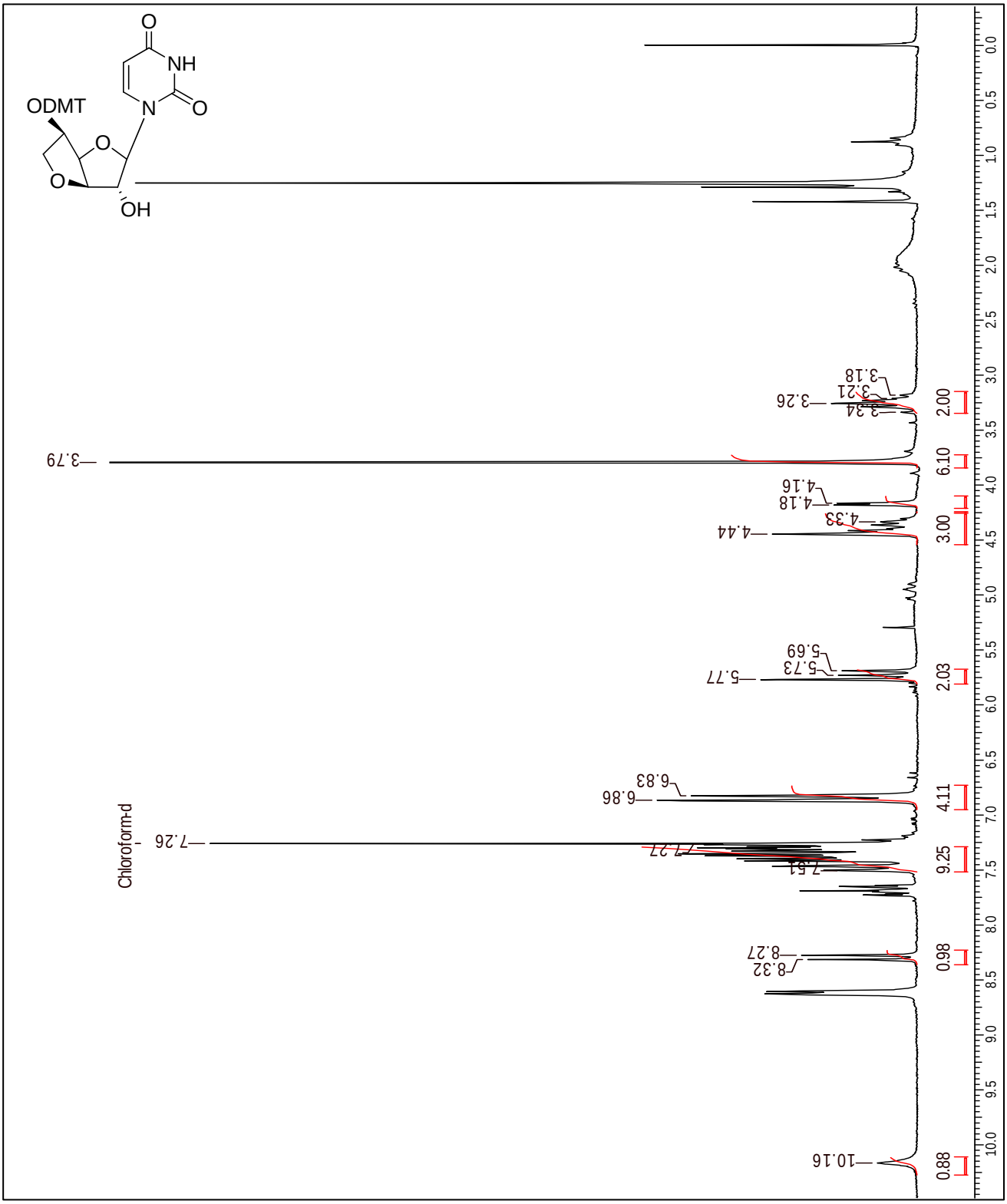
5. **5'-O-(4, 4-dimethoxy)trityl-3-deoxy-3-ribofluoro-2-O-(2-cyanoethyl-N,N diisopropylphosphoramidite) uridine IIIb**

   ^{31}P NMR ($CDCl_3$): δ 151.4, 152.5. **Mass:** mass calculated for $C_{39}H_{46}FN_4O_8P$ +Na^+ 771.2935, observed mass m/z 771.35 M+ 23(Na).

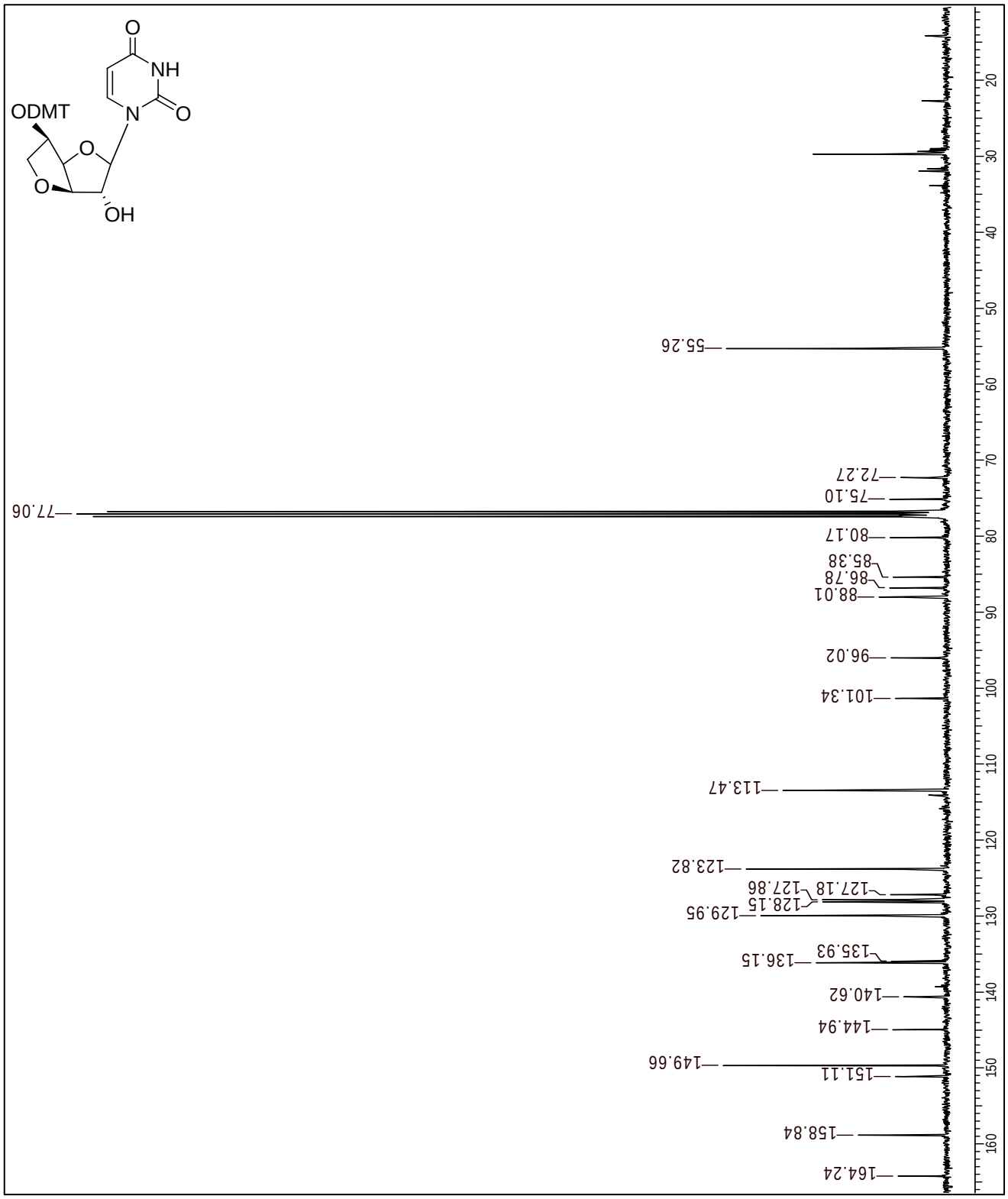
6. **1-(5'-O-dimethoxytrityl-3'-deoxy-3'-fluoro-2'-cyanoethyl-phosphoramidite-β-D-xylofuranosyl)uracil IVb**

   ^{31}P NMR (50MHz, $CDCl_3$): δ 151.4, 153.5. **Mass:** mass calculated for $C_{39}H_{46}FN_4O_8P$ +Na^+ 771.2935, observed mass m/z 771.33 M+ 23(Na).

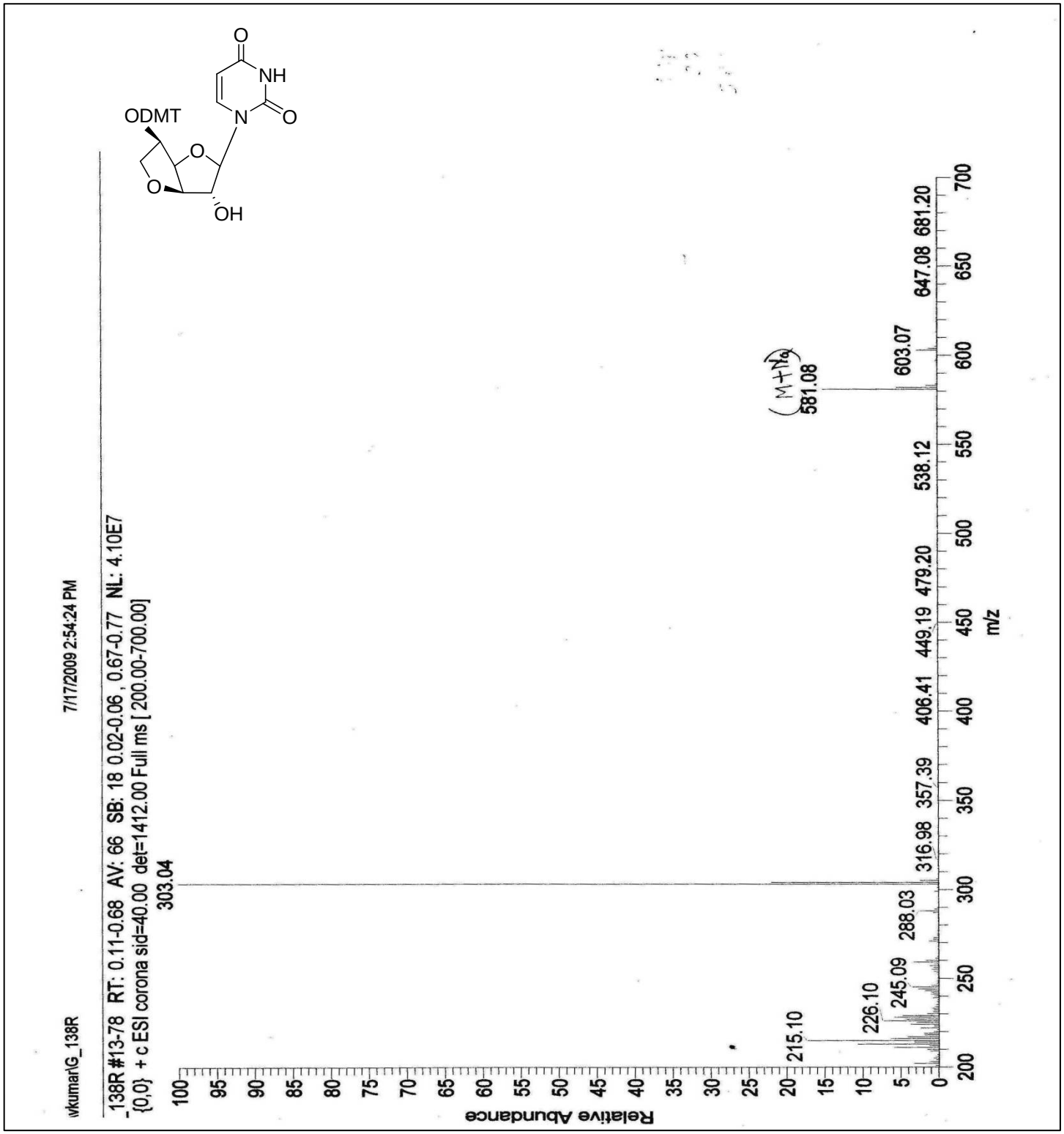
^{1}H NMR ($CDCl_3$, 200MHz) Compound IIa



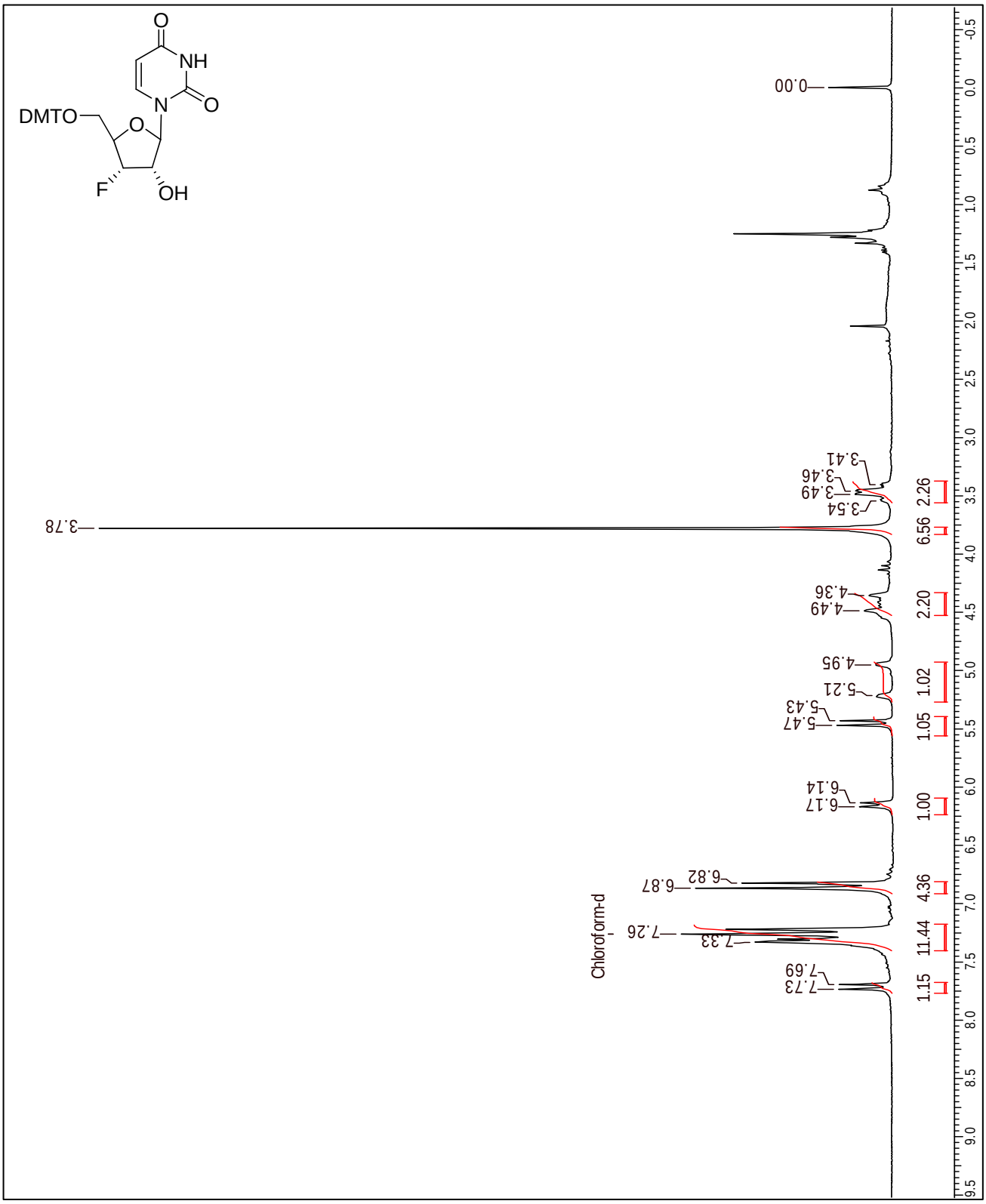
^{13} C NMR ($CDCl_3$, 50MHz) Compound IIa



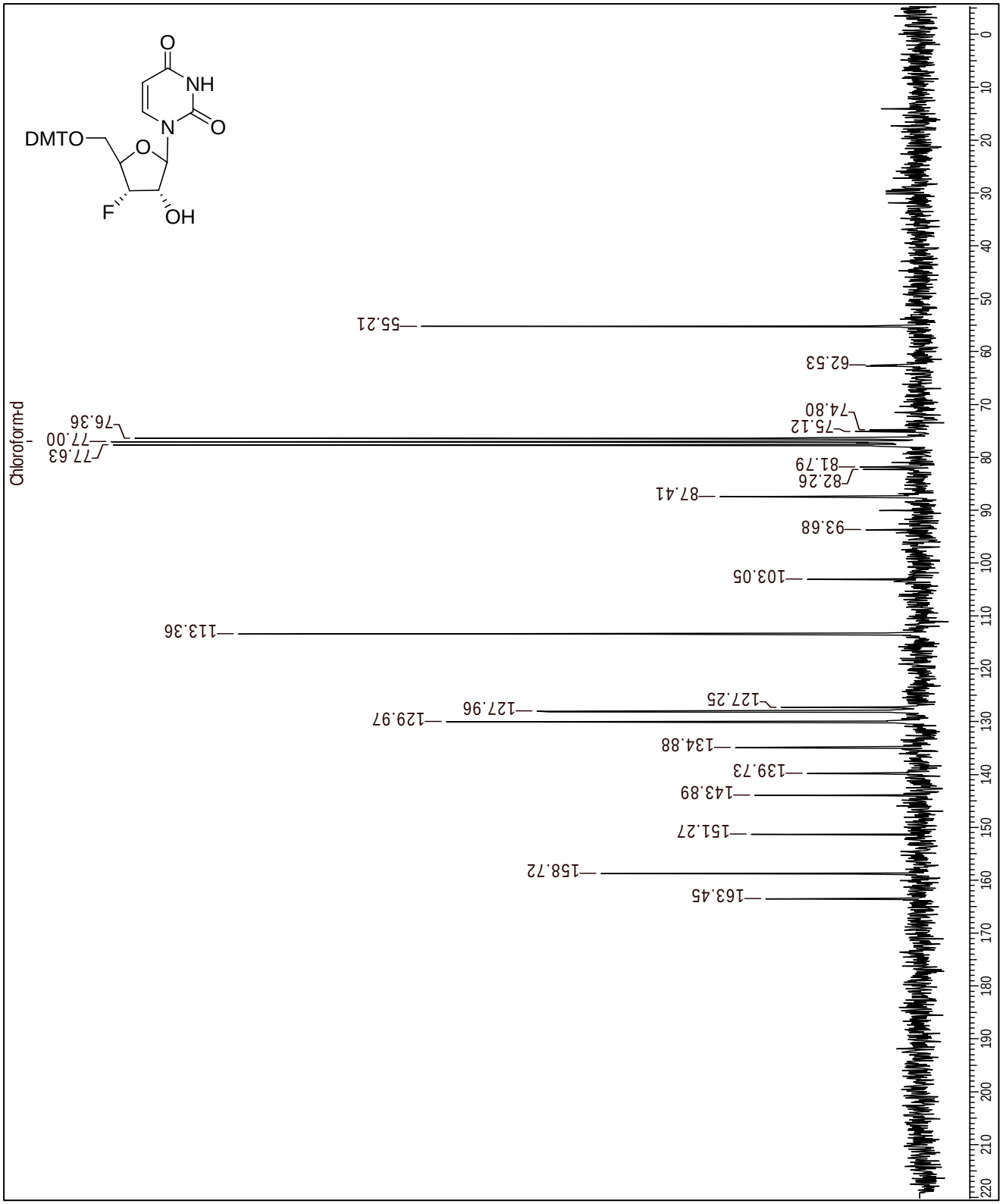
Mass spectrum of Compound IIa



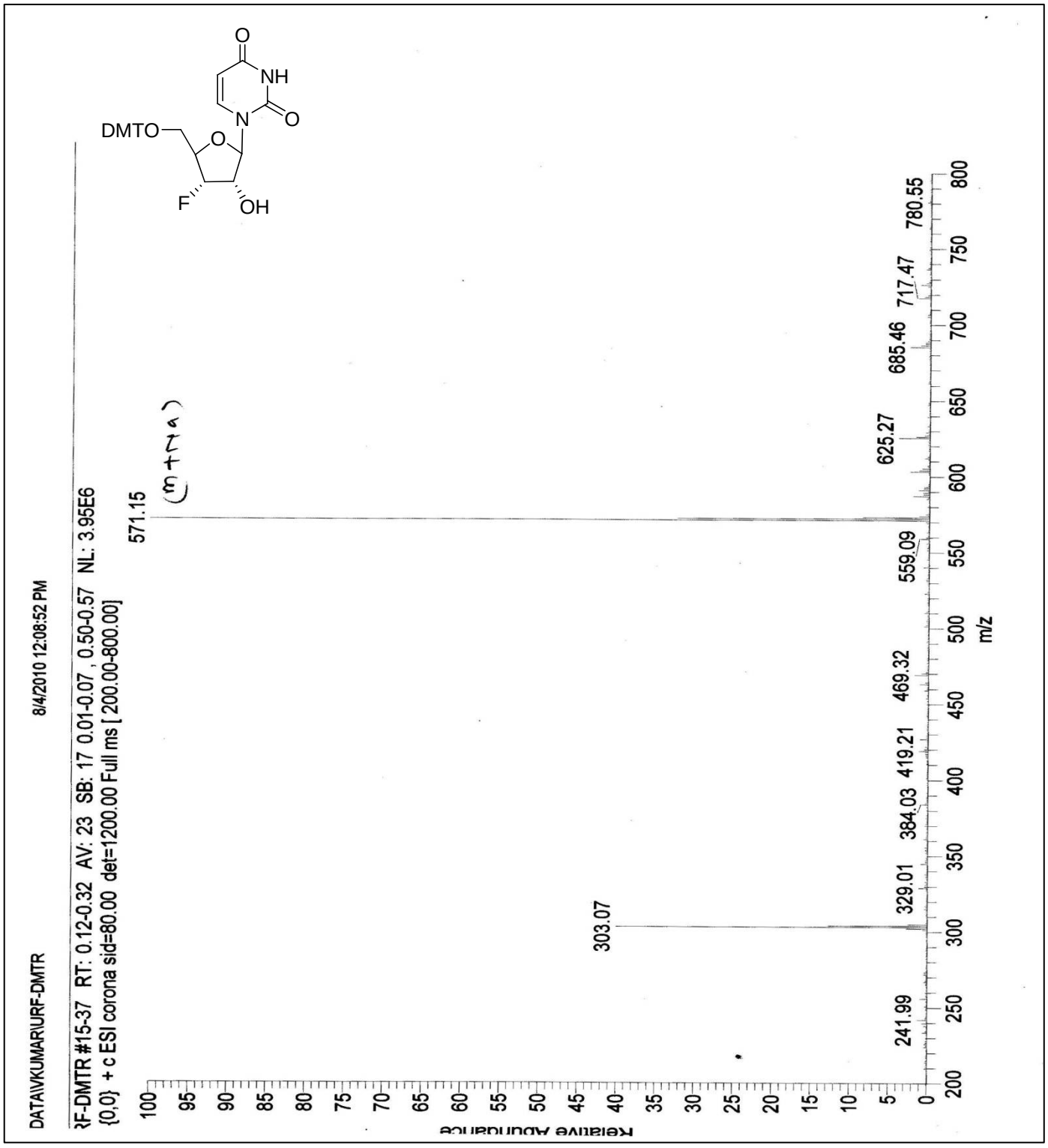
^{1}H NMR ($CDCl_3$, 200MHz) Compound IIIa



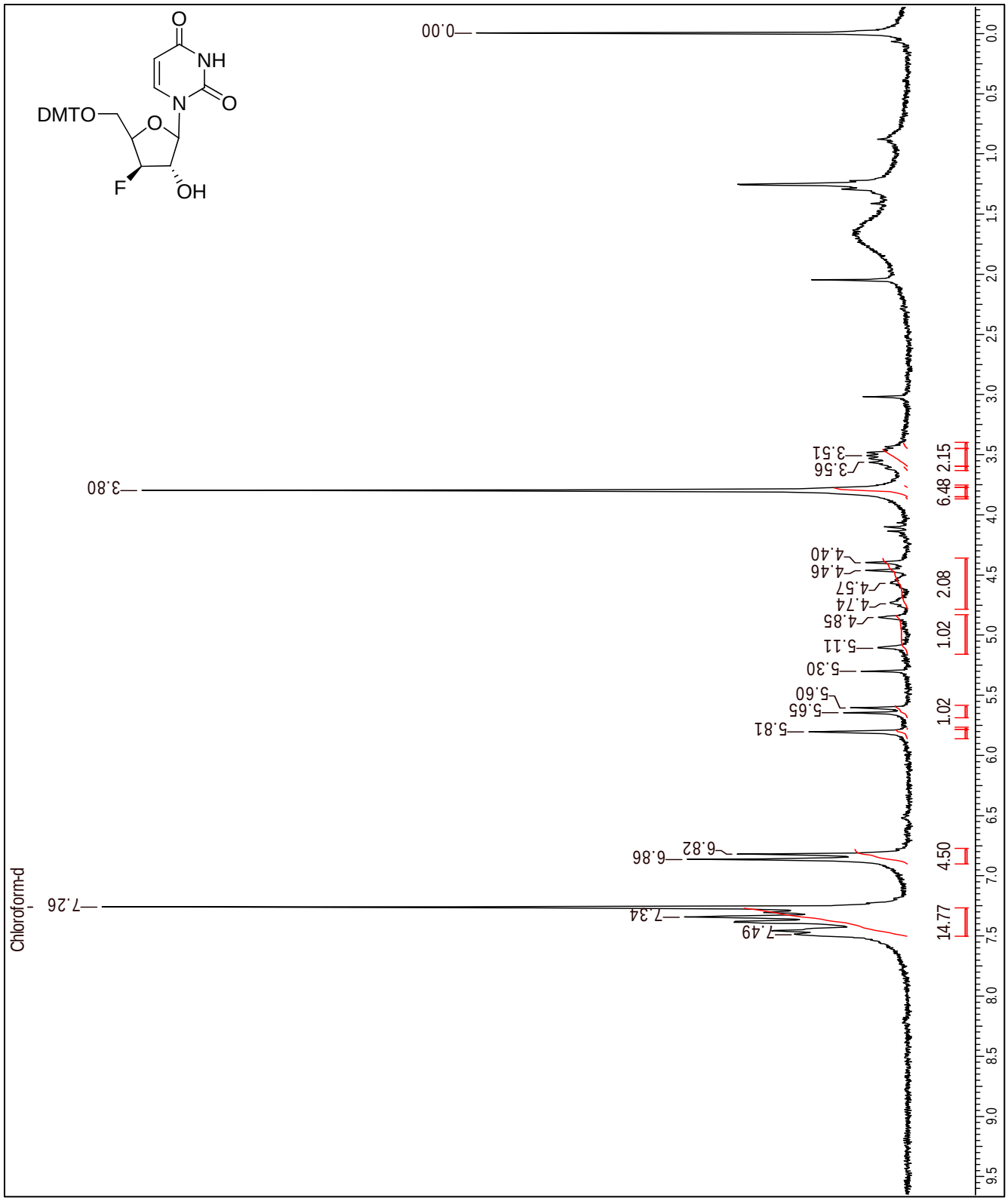
^{13}C NMR ($CDCl_3$, 50MHz) Compound IIIa



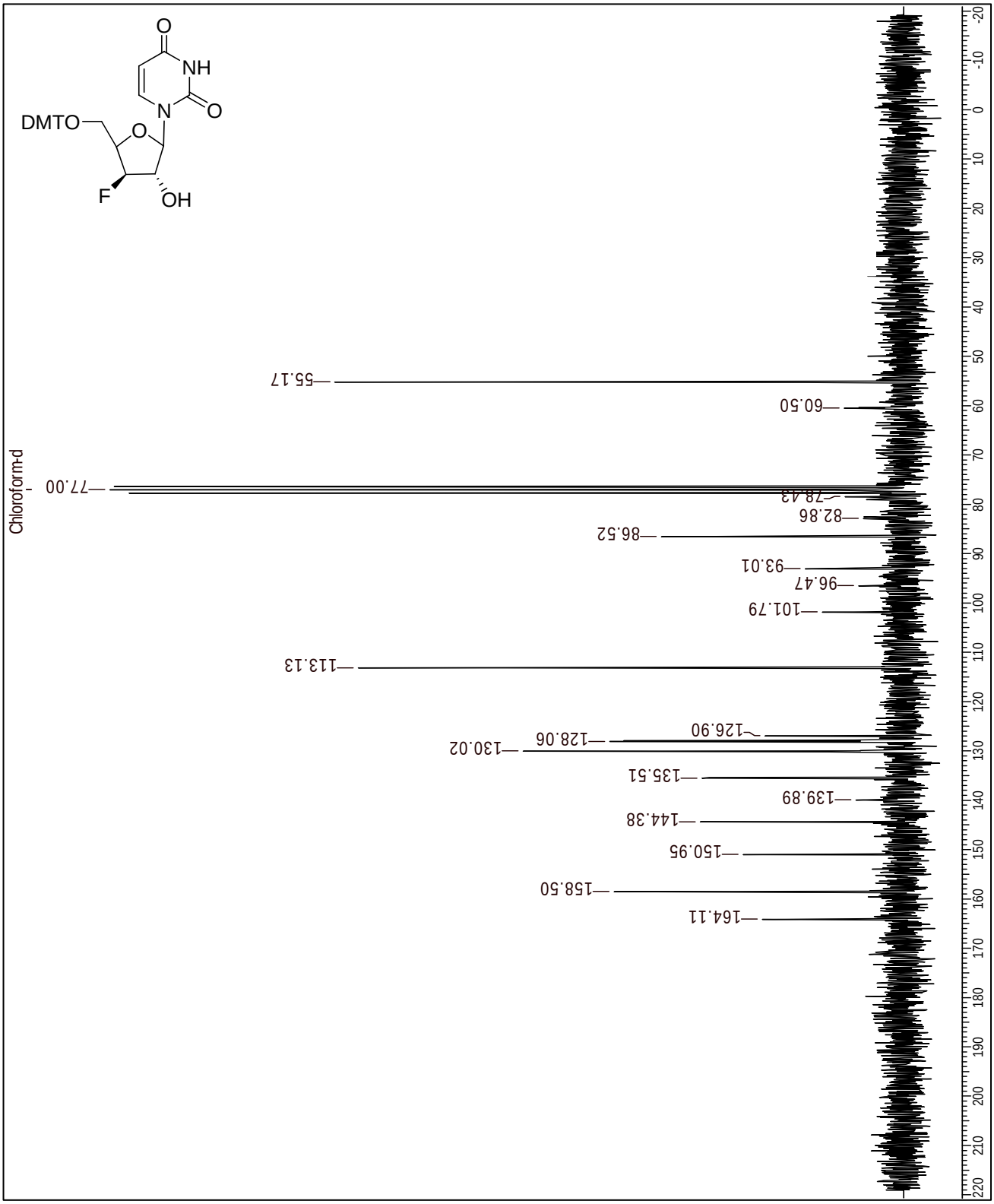
Mass spectrum of Compound IIIa



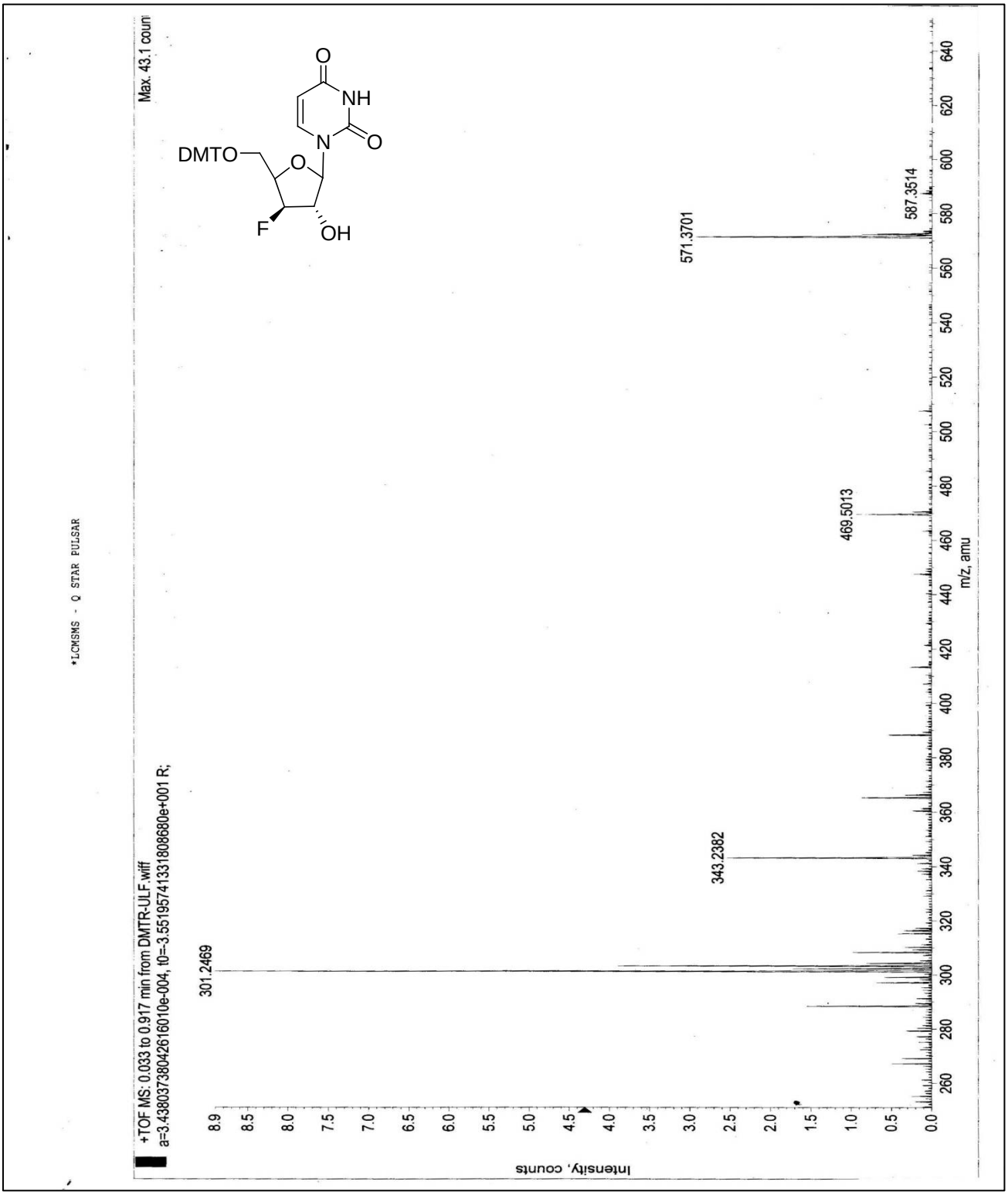
^{1}H NMR ($CDCl_3$, 200MHz) Compound IVa



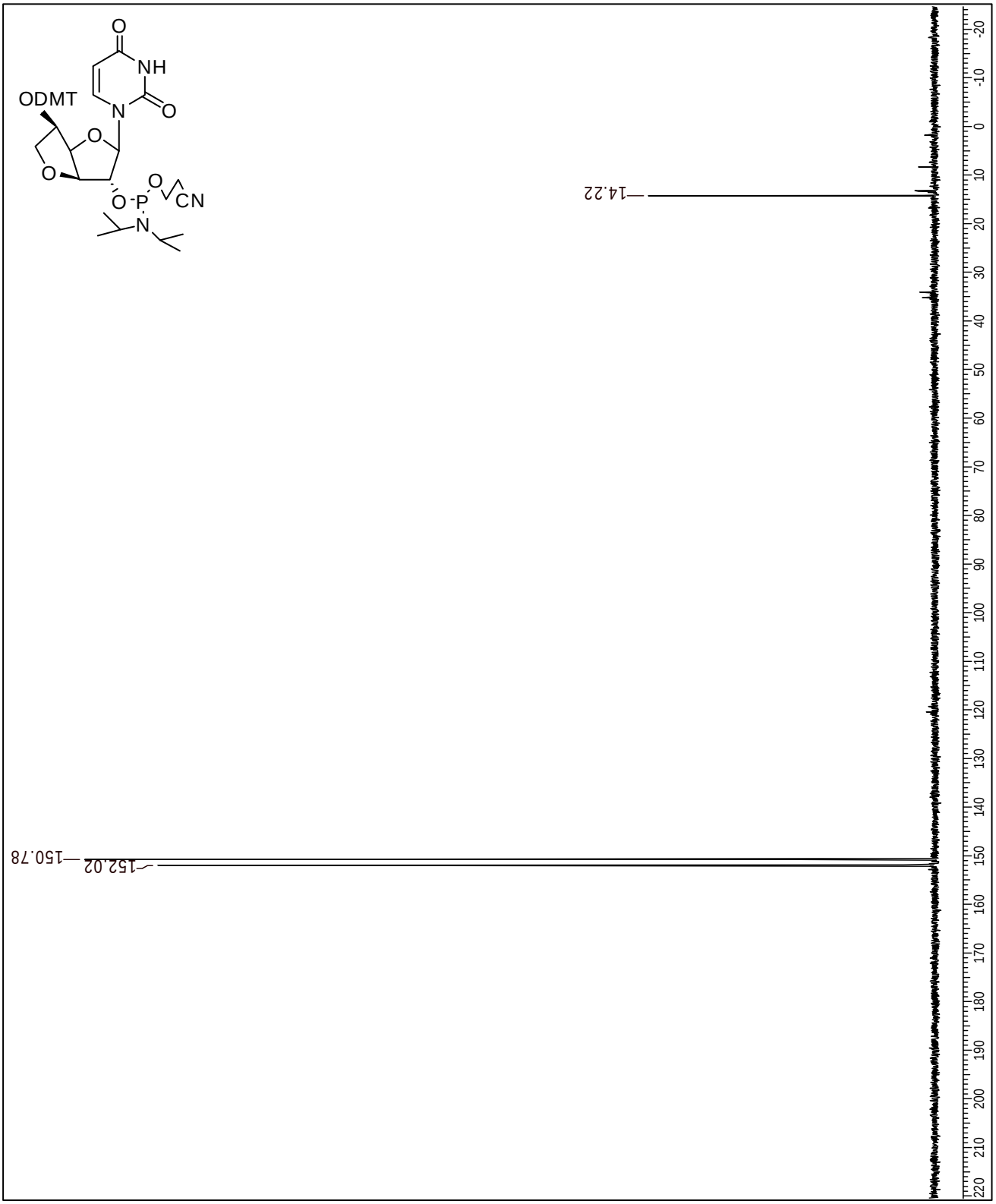
^{13}C NMR ($CDCl_3$, 50MHz) Compound IVa



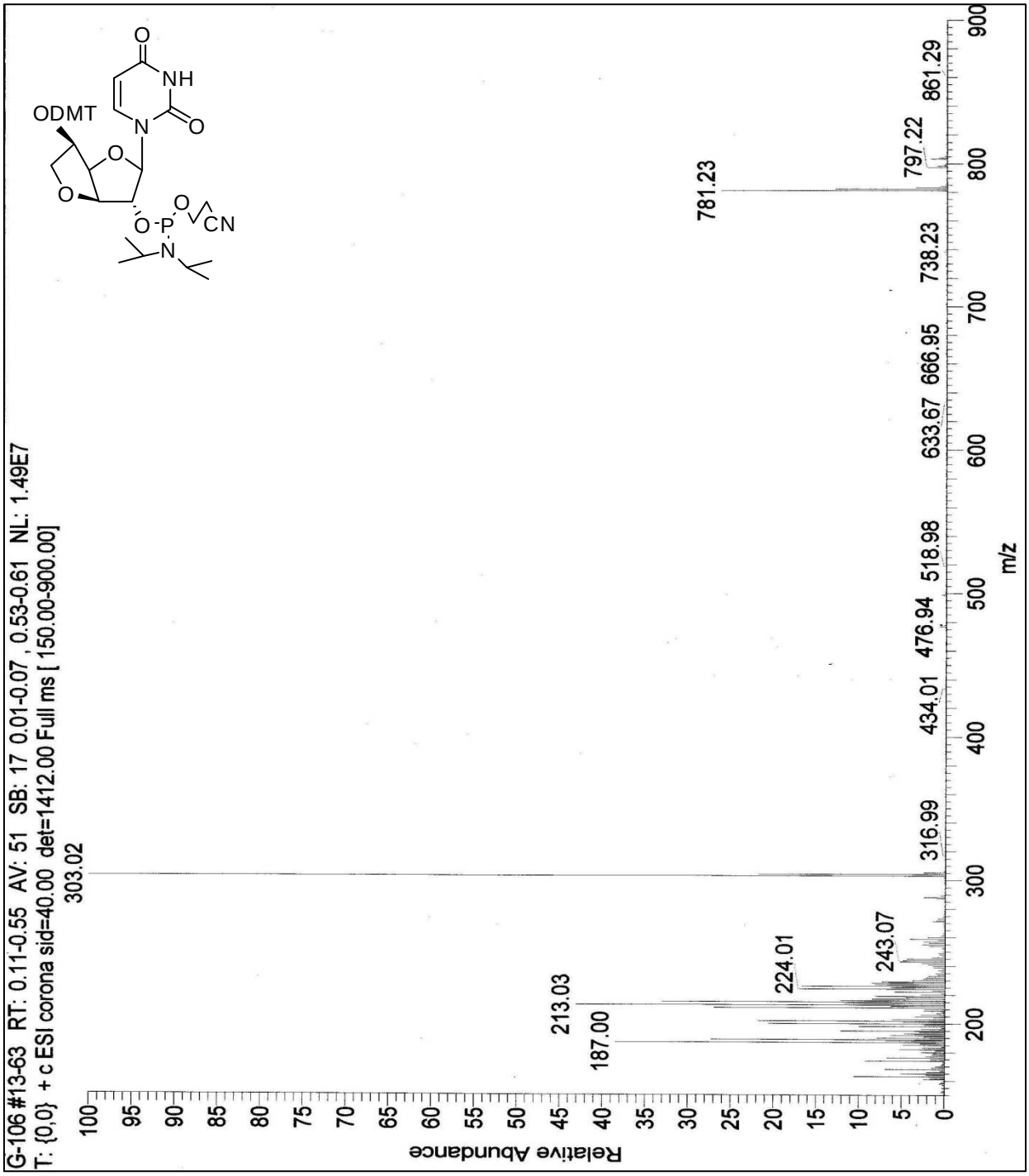
Mass spectrum of Compound IVa



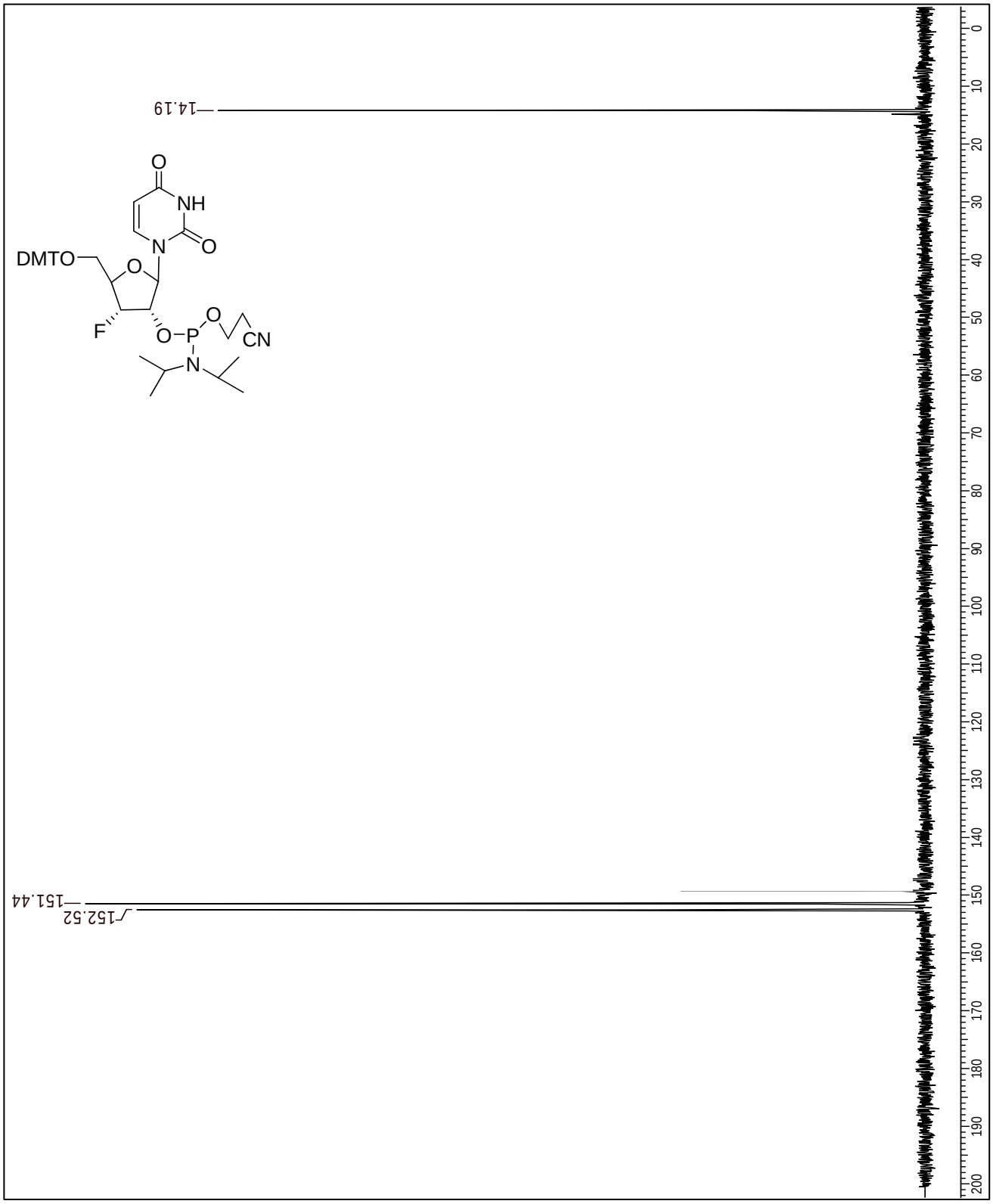
^{31} P NMR ($CDCl_3$, 50MHz) Compound IIb



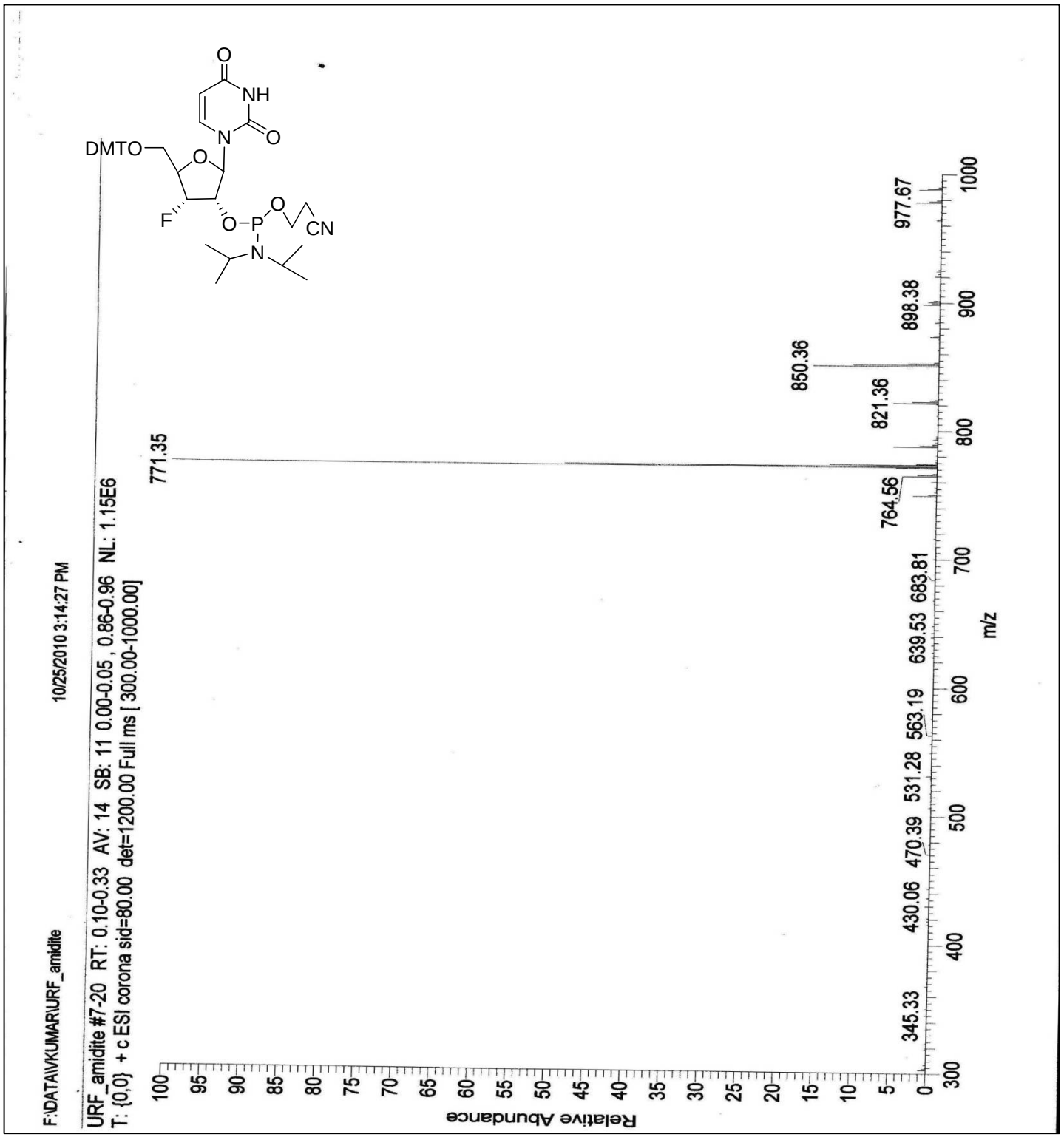
Mass spectrum of Compound IIb



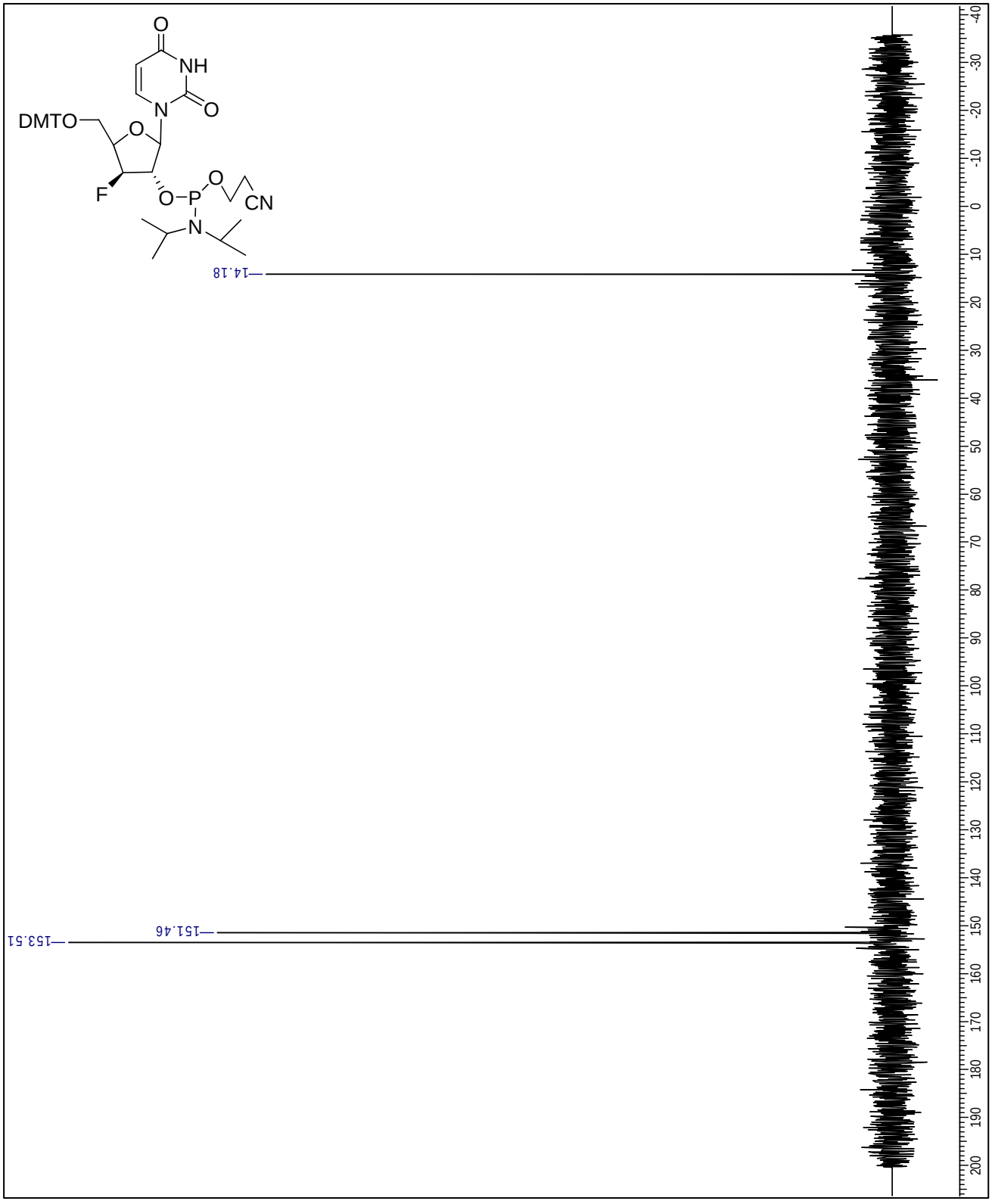
^{31} P NMR ($CDCl_3$, 50MHz) Compound IIIb



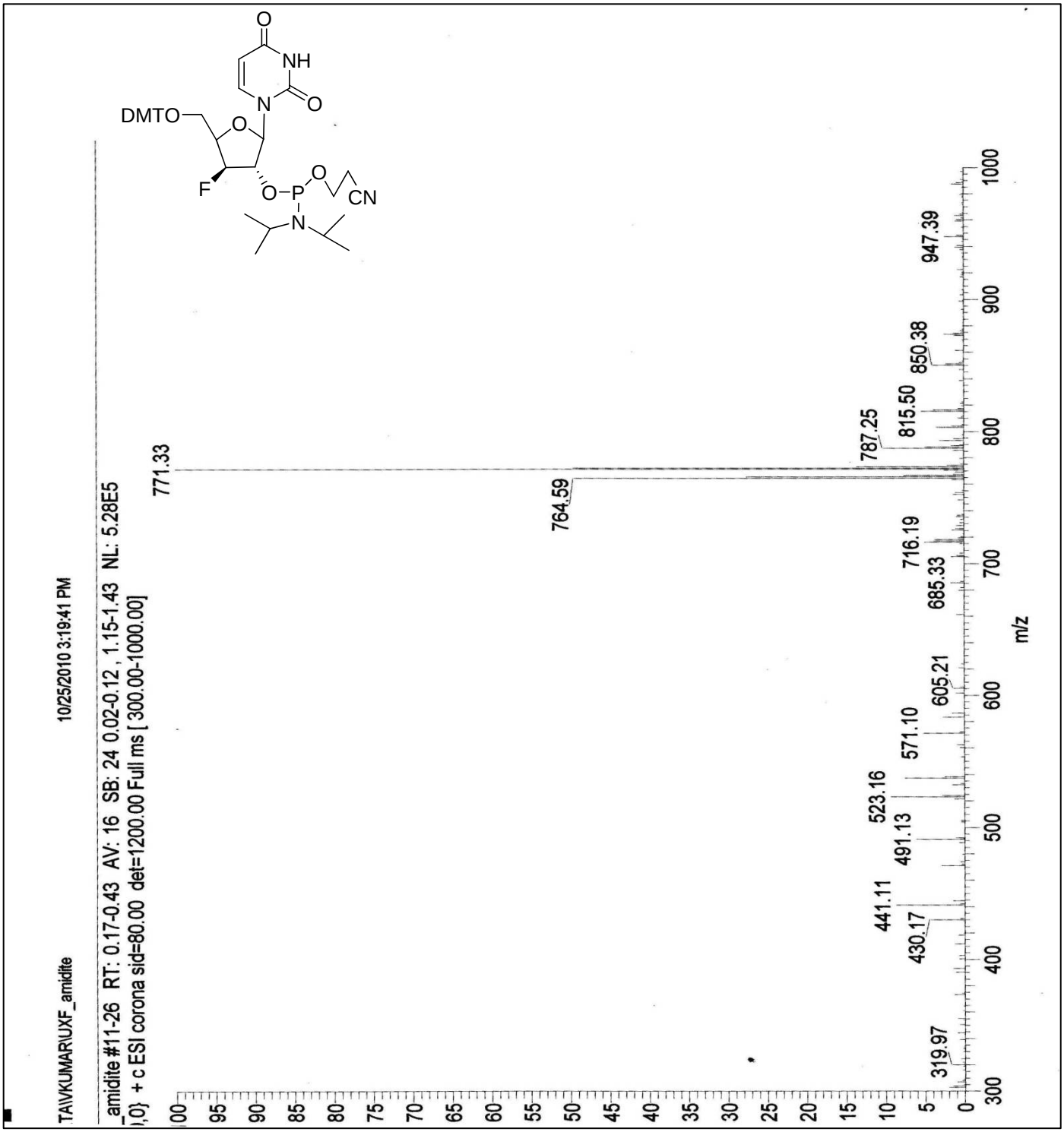
Mass spectrum of Compound IIIb



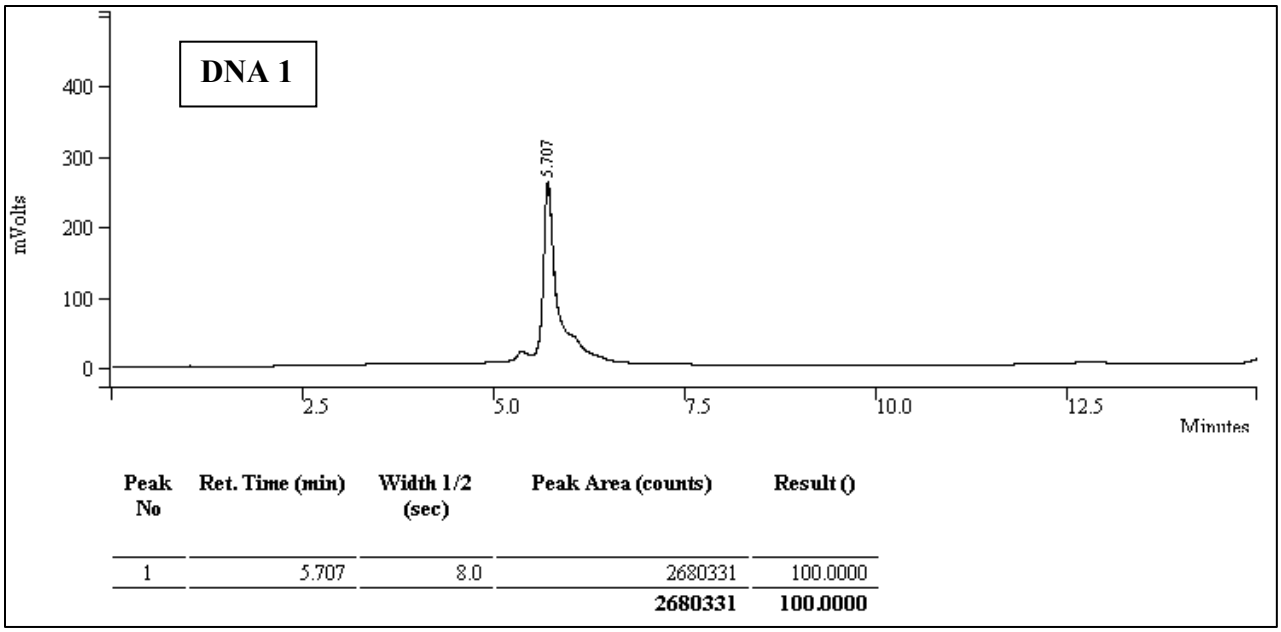
^{31} P NMR ($CDCl_3$, 50MHz) Compound IVb



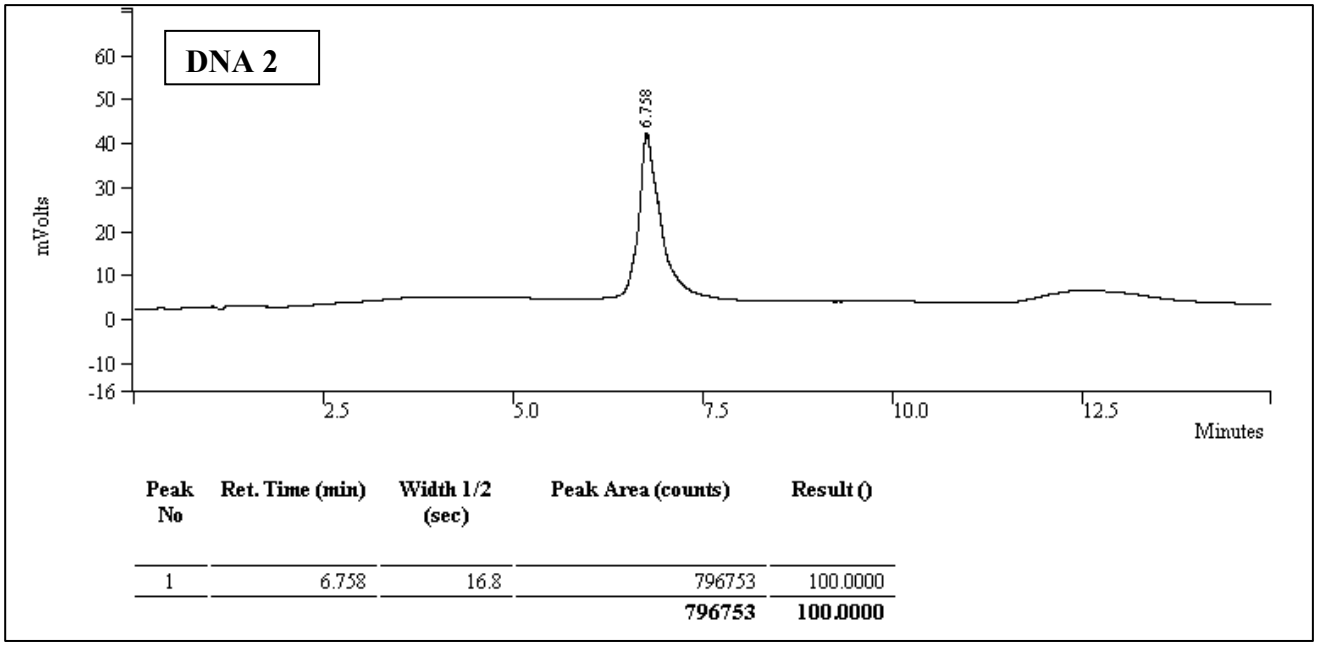
Mass spectrum of Compound IVb



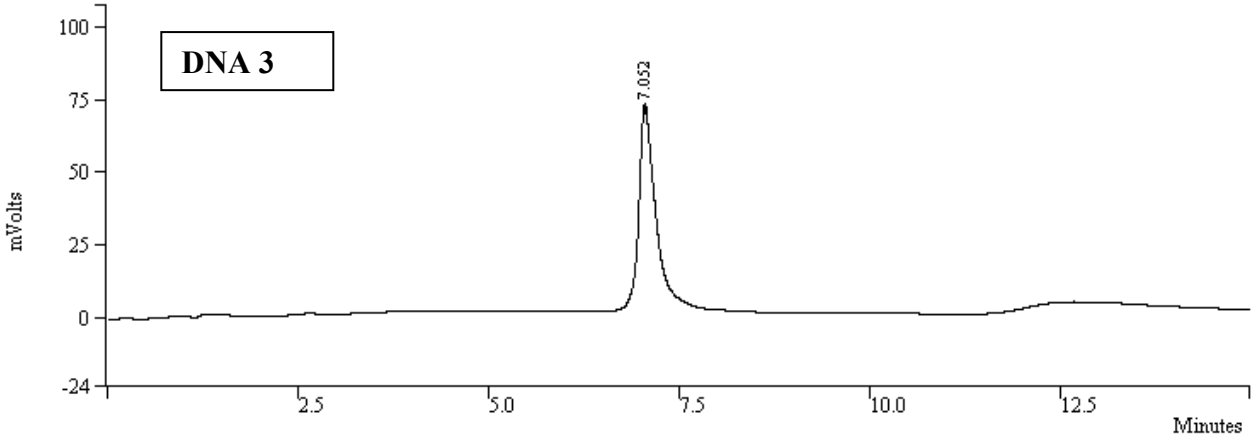
HPLC chromatograms of synthesized oligomers



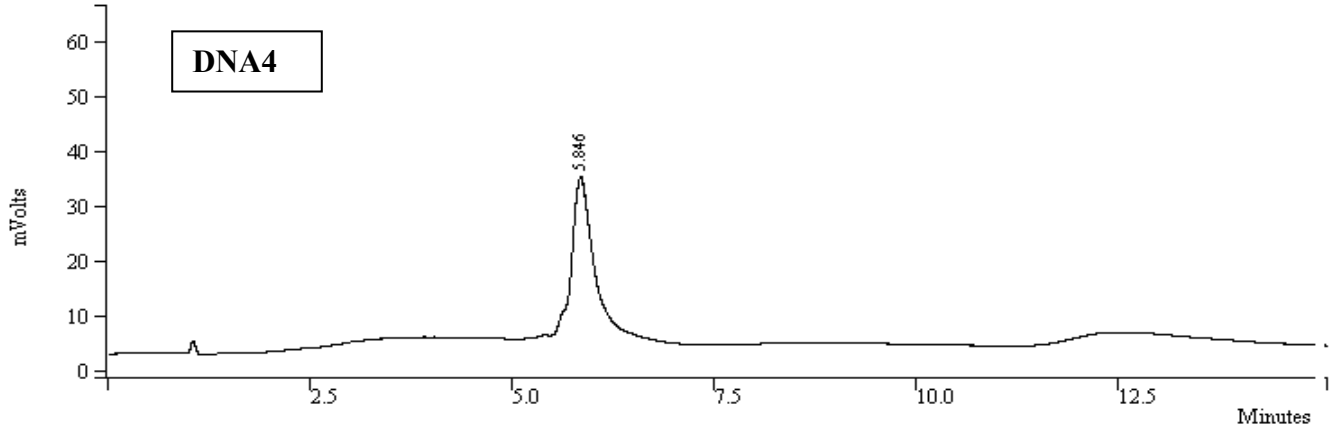
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	5.707	8.0	2680331	100.0000
			2680331	**100.0000**



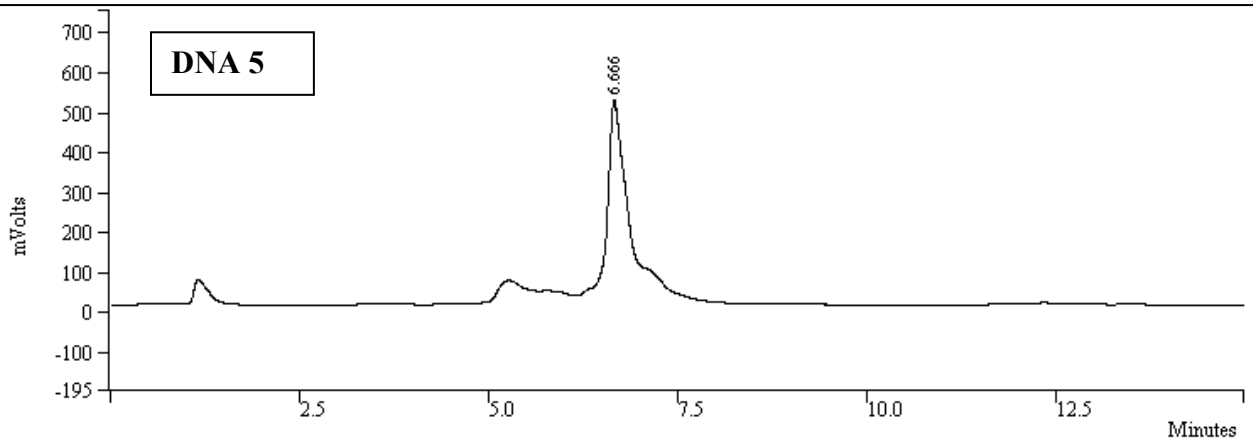
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	6.758	16.8	796753	100.0000
			796753	**100.0000**



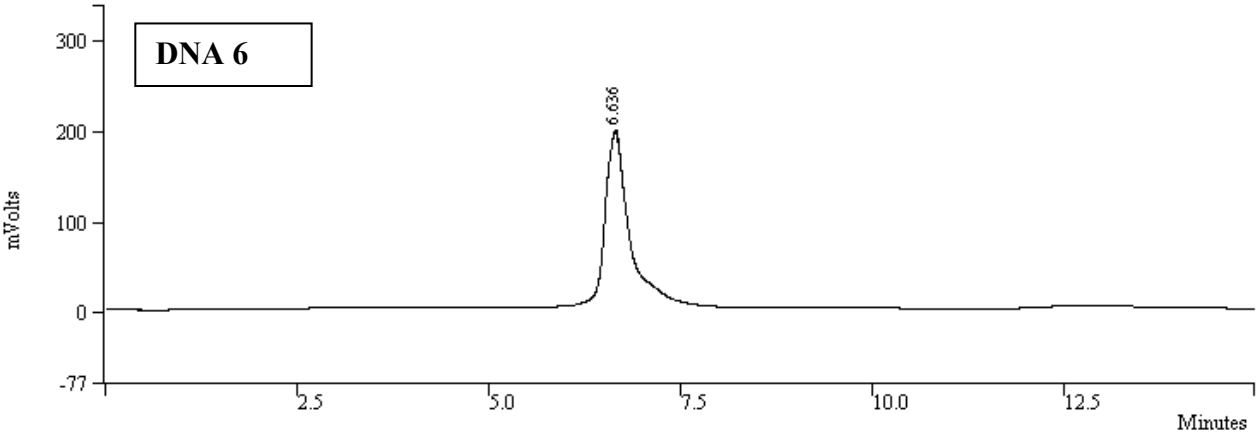
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	7.052	13.1	1183090	100.0000
			1183090	**100.0000**



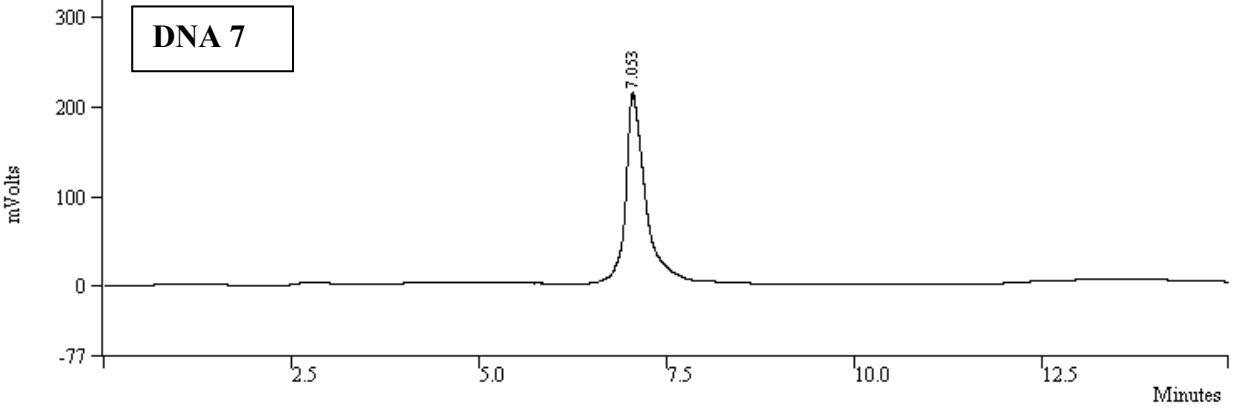
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	5.846	15.7	633568	100.0000
			633568	**100.0000**



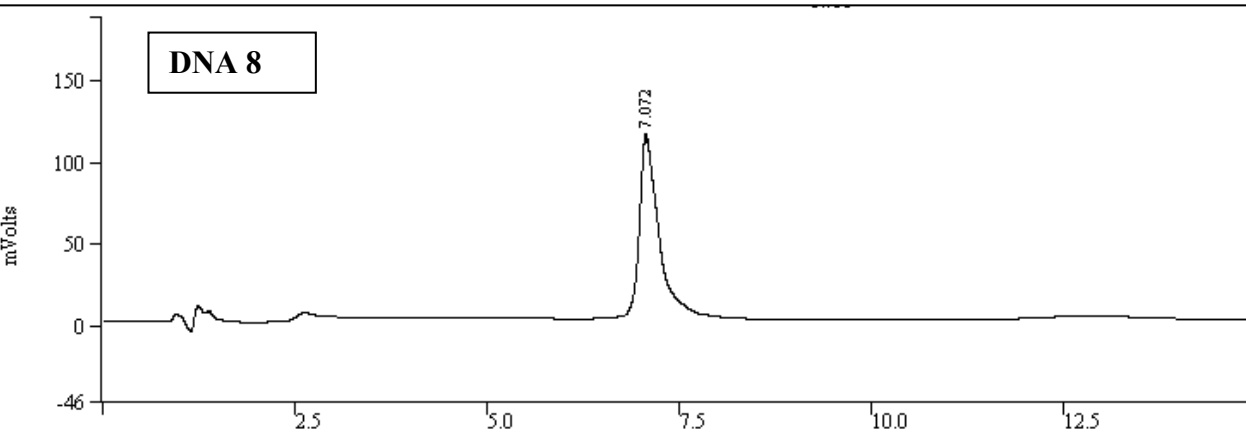
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	6.666	14.7	9957784	100.0000
			9957784	**100.0000**



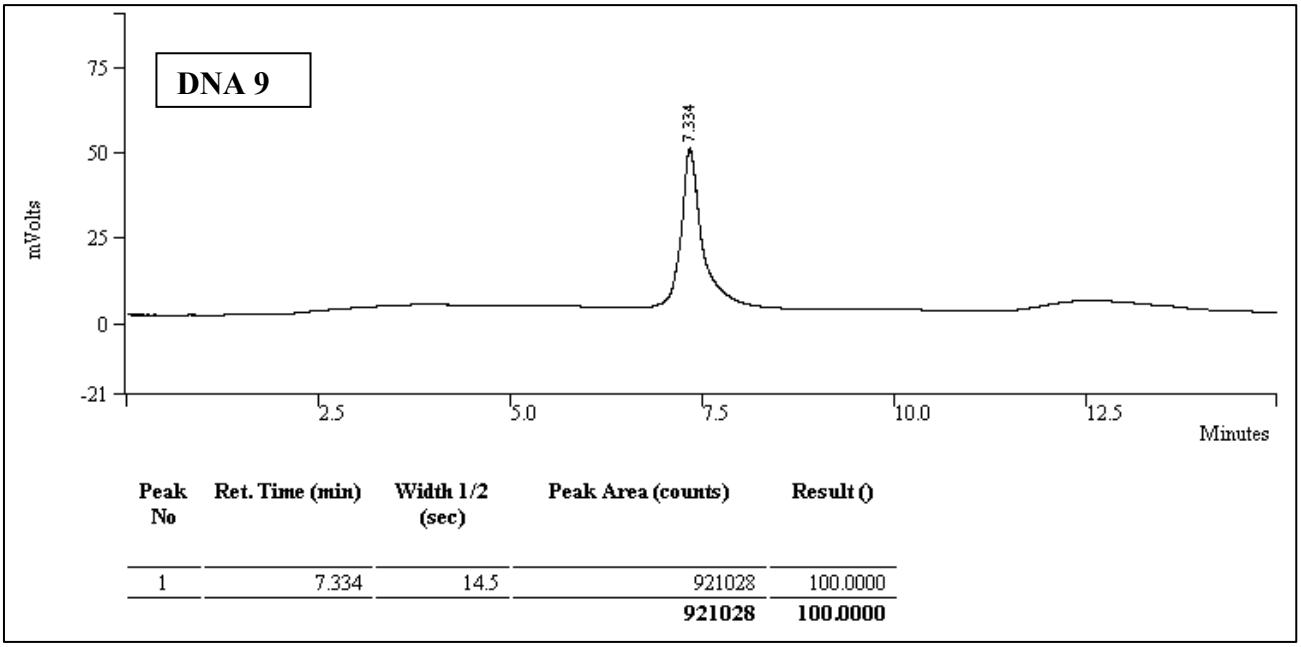
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	6.636	17.6	4517427	100.0000
			4517427	**100.0000**



Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	7.053	14.8	4042004	100.0000
			4042004	**100.0000**

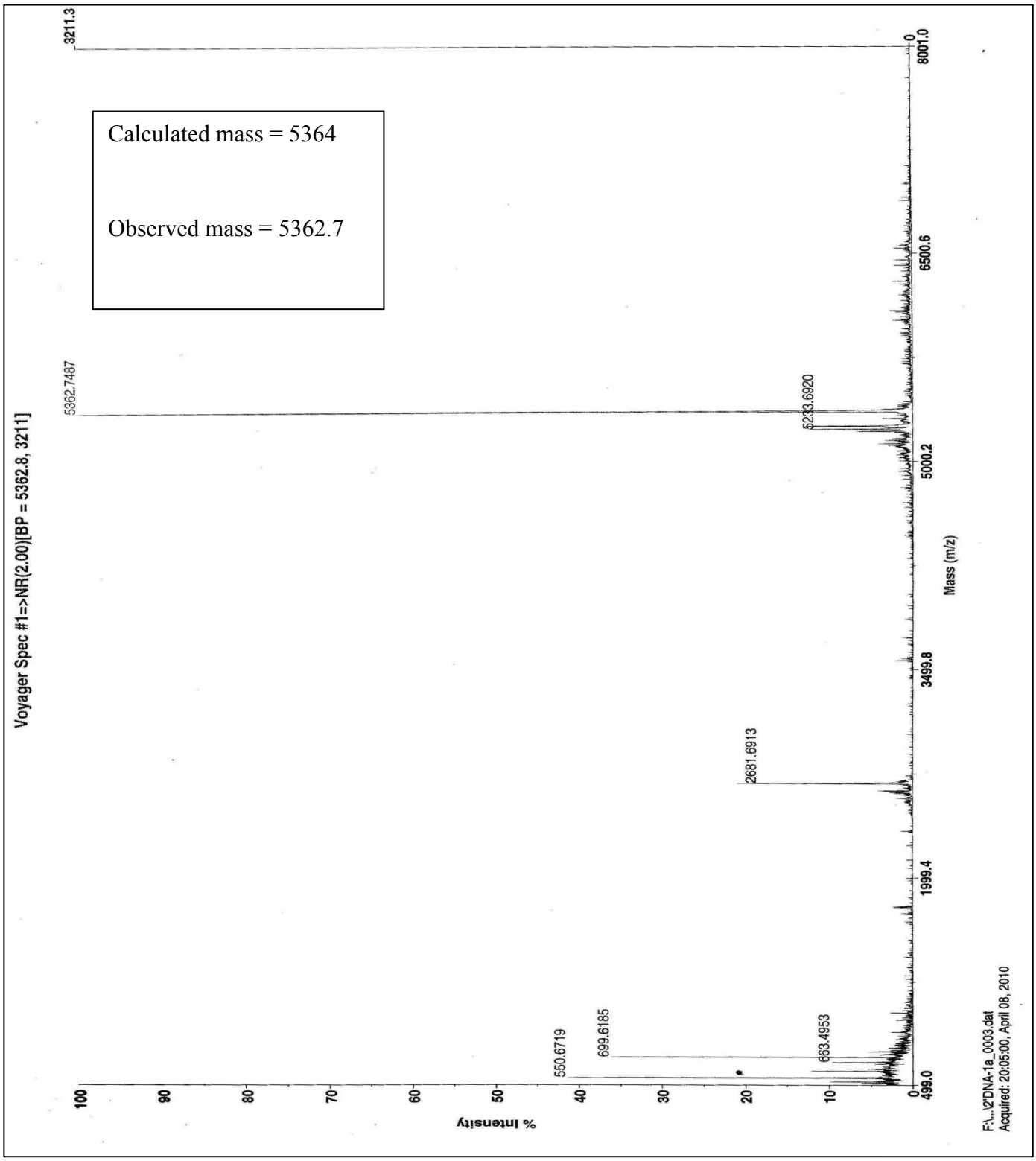


Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	7.072	14.7	2055475	100.0000
			2055475	**100.0000**

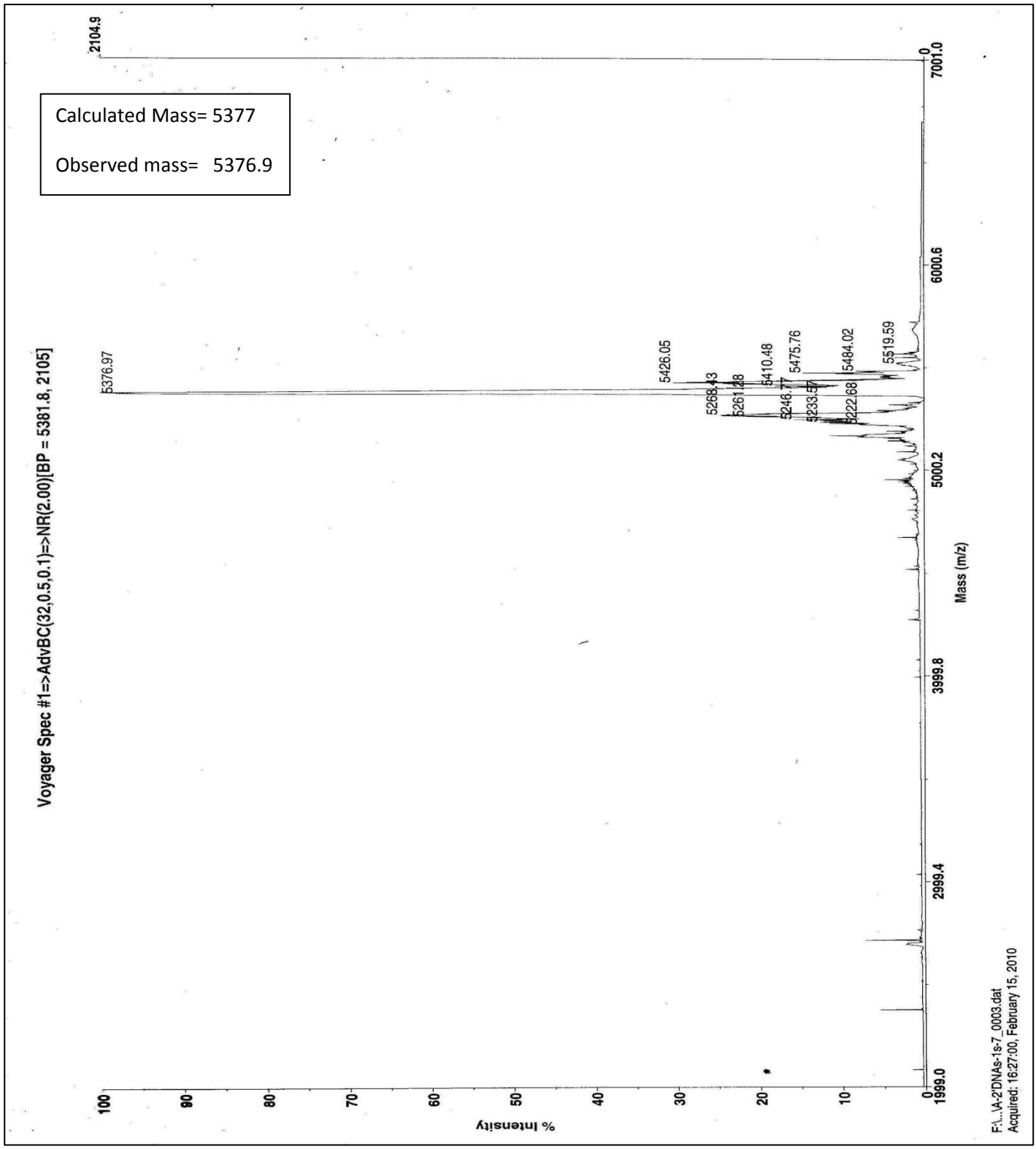


Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	7.334	14.5	921028	100.0000
			921028	**100.0000**

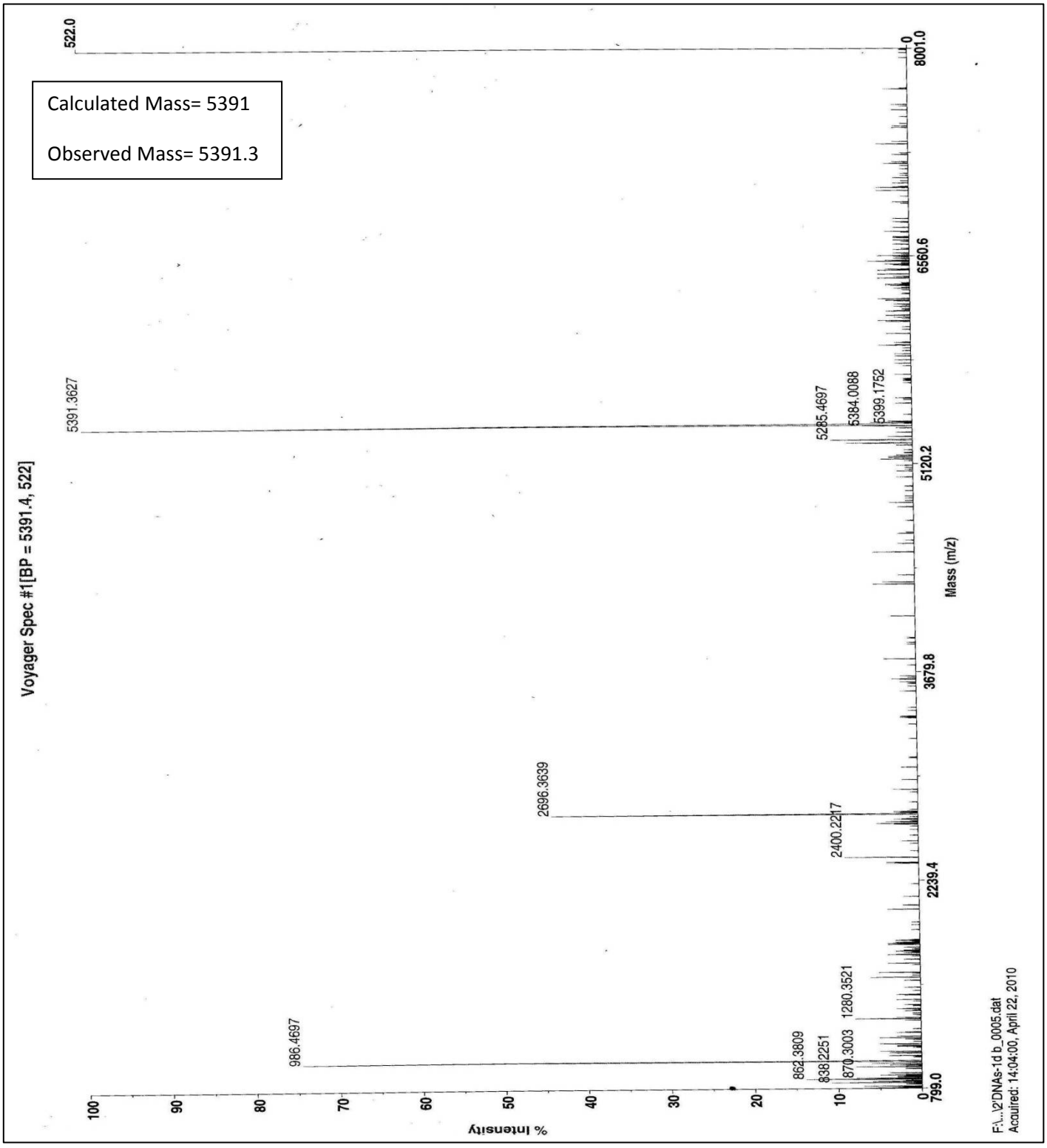
MALDI-TOF spectrum of DNA1



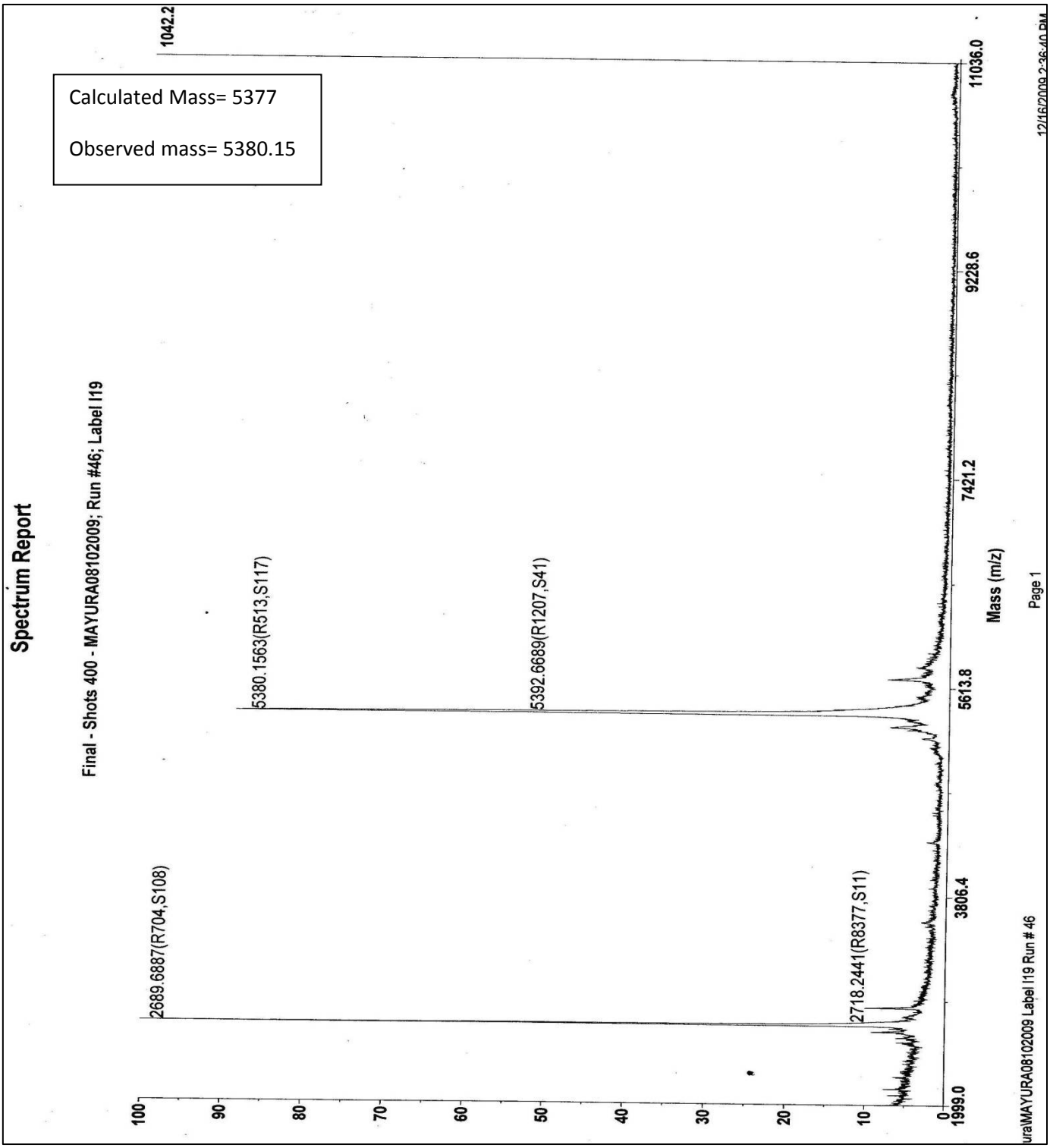
MALDI-TOF spectrum of DNA2



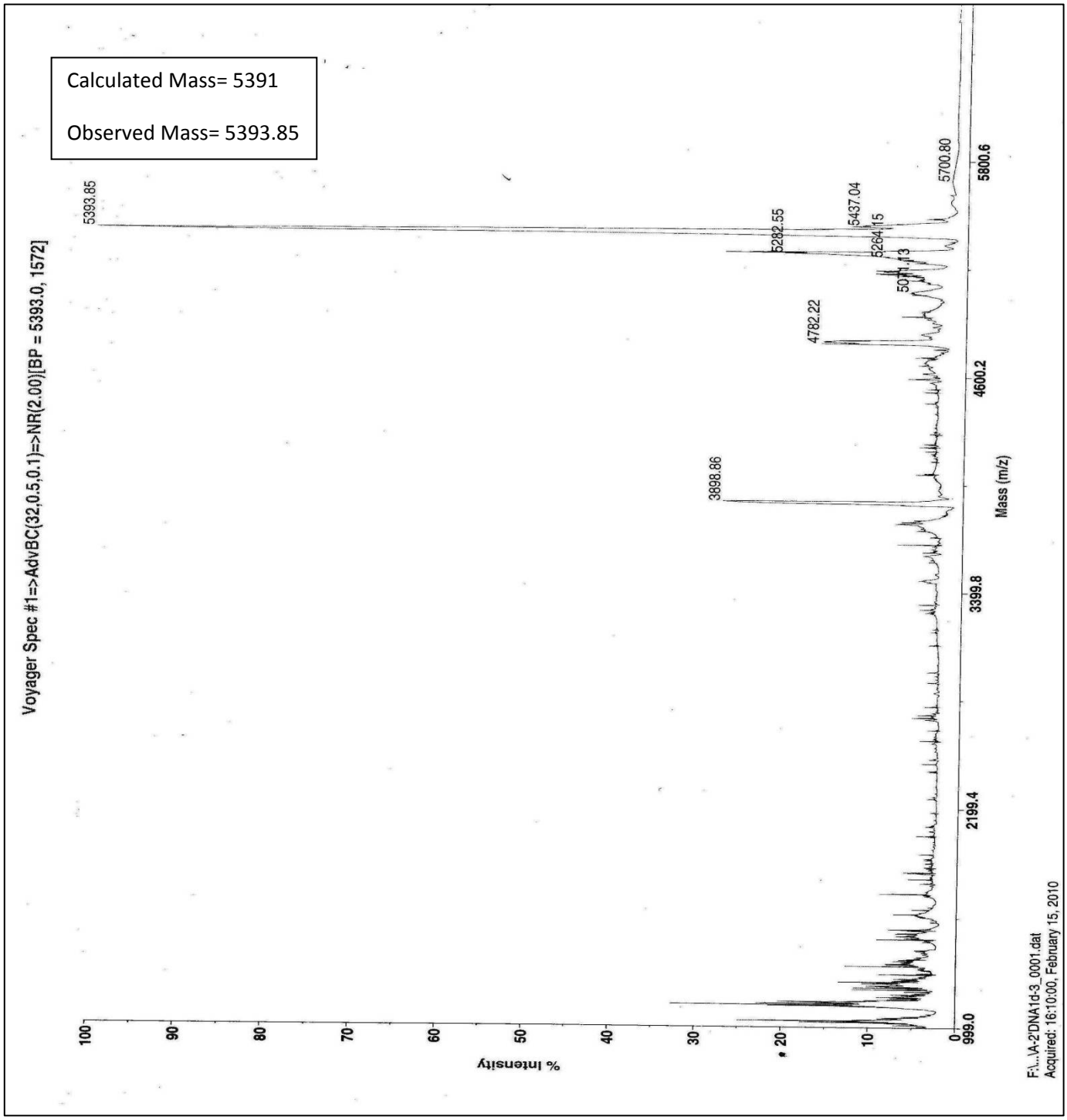
MALDI-TOF spectrum of DNA3



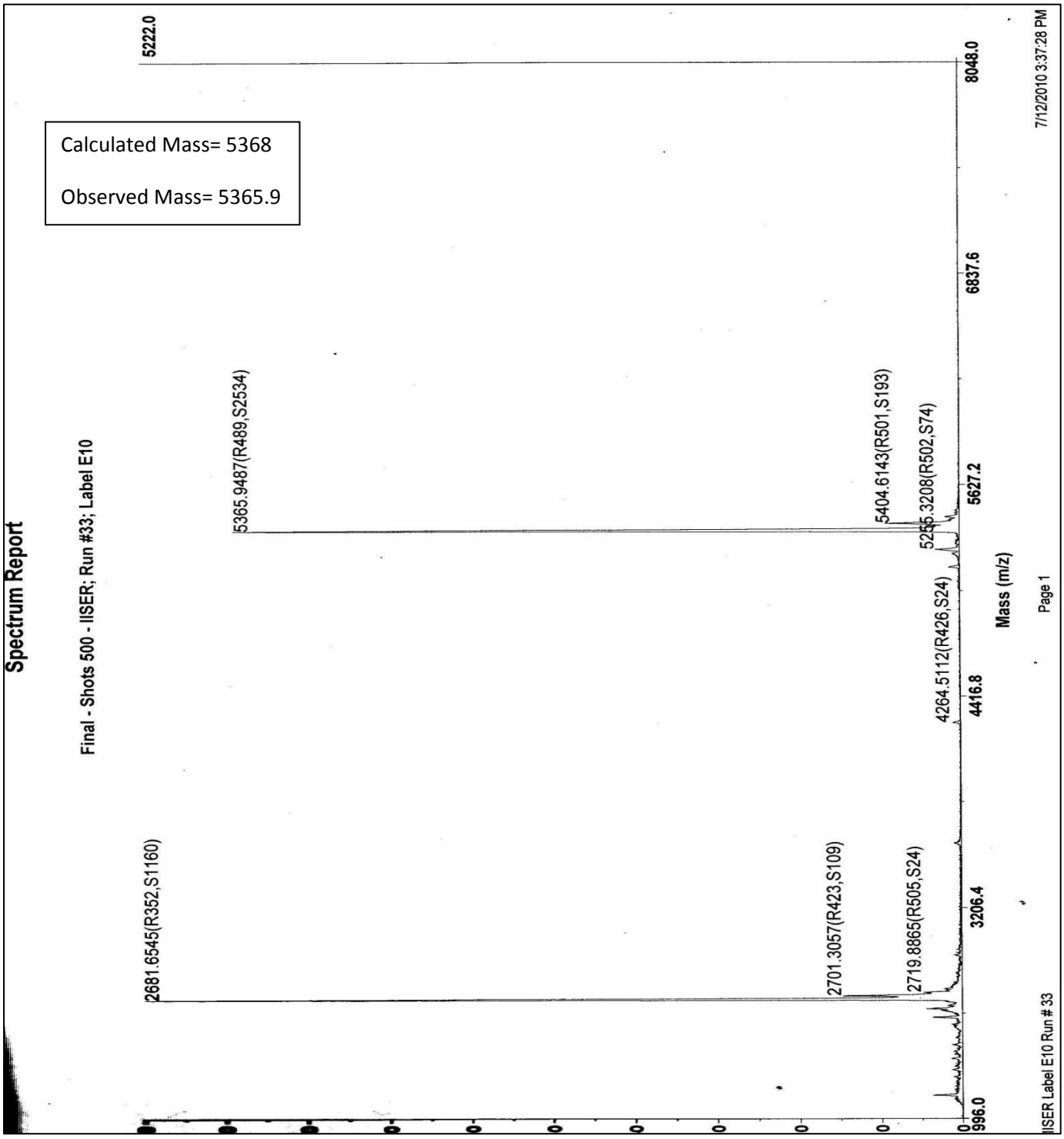
MALDI-TOF spectrum of DNA4



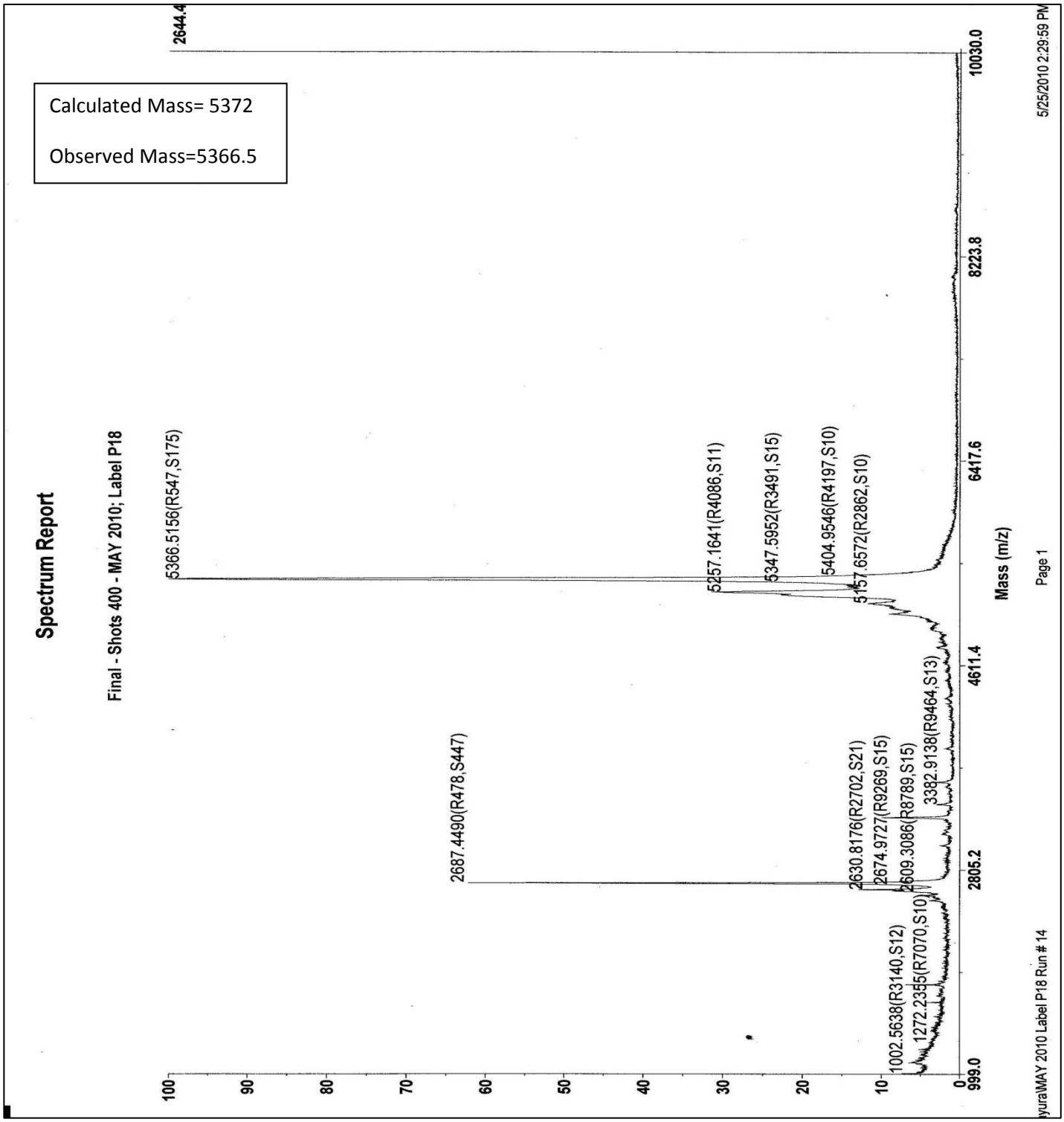
MALDI-TOF spectrum of DNA5



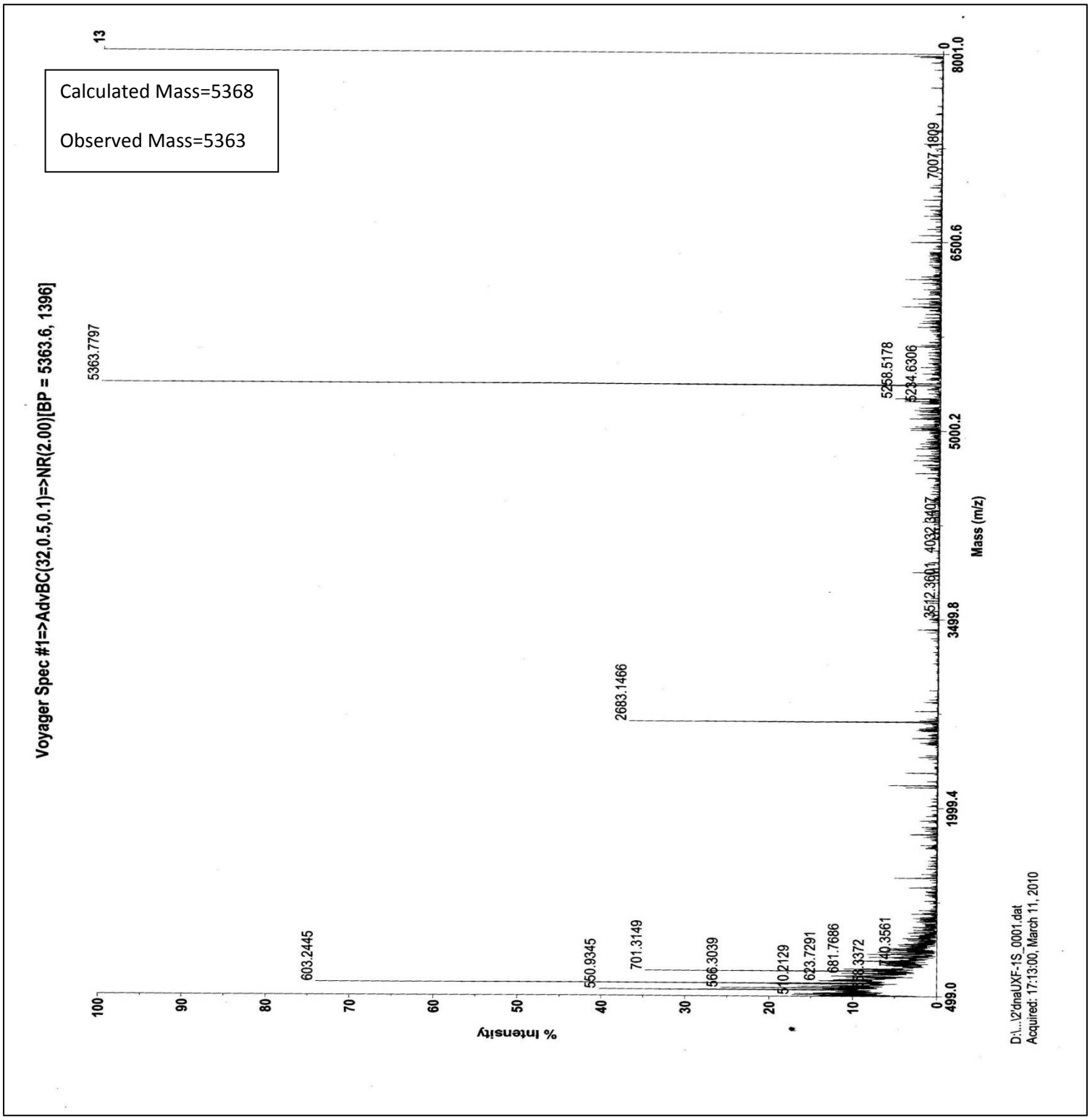
MALDI-TOF spectrum of DNA6



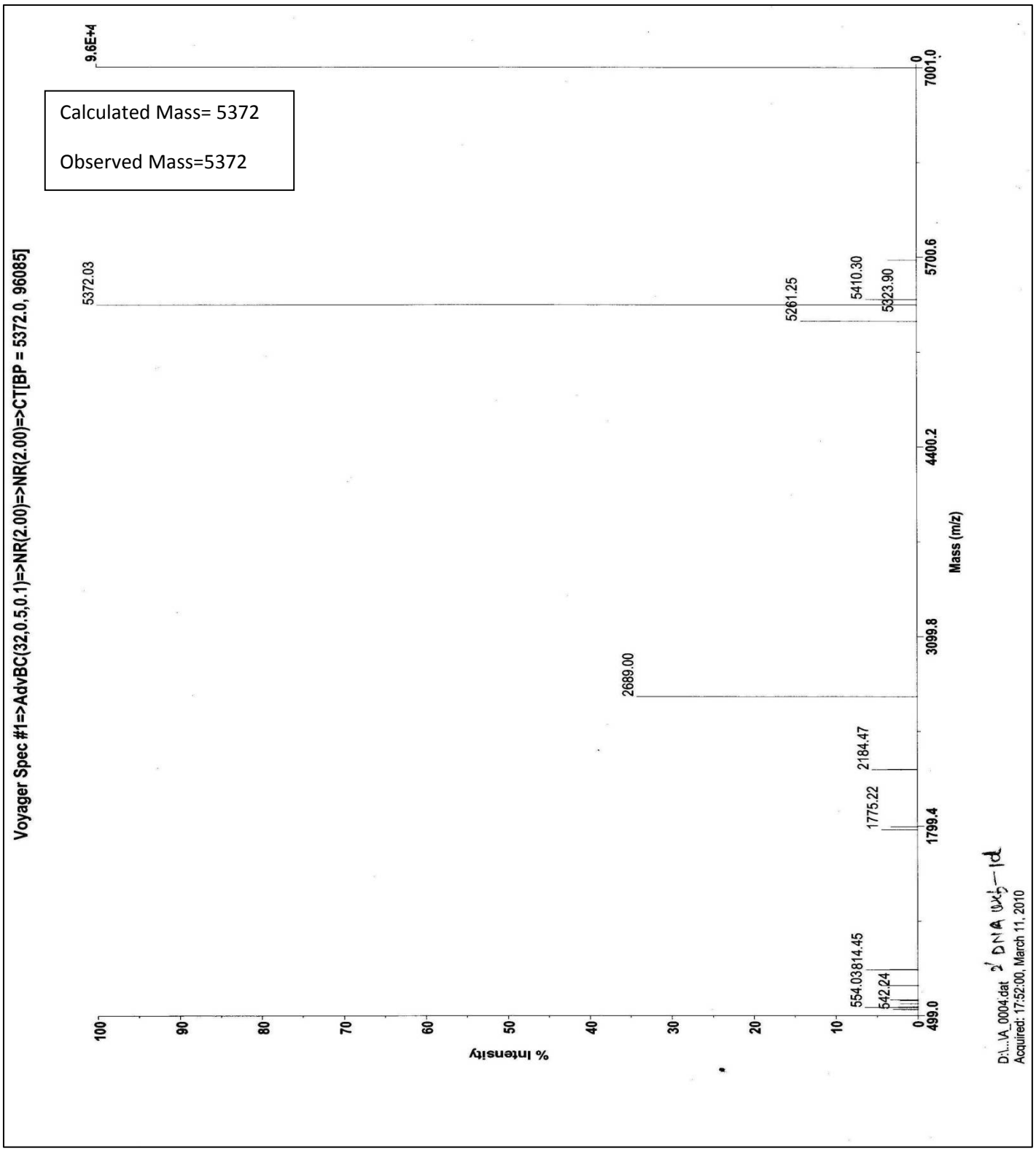
MALDI-TOF spectrum of DNA7



MALDI-TOF spectrum of DNA8



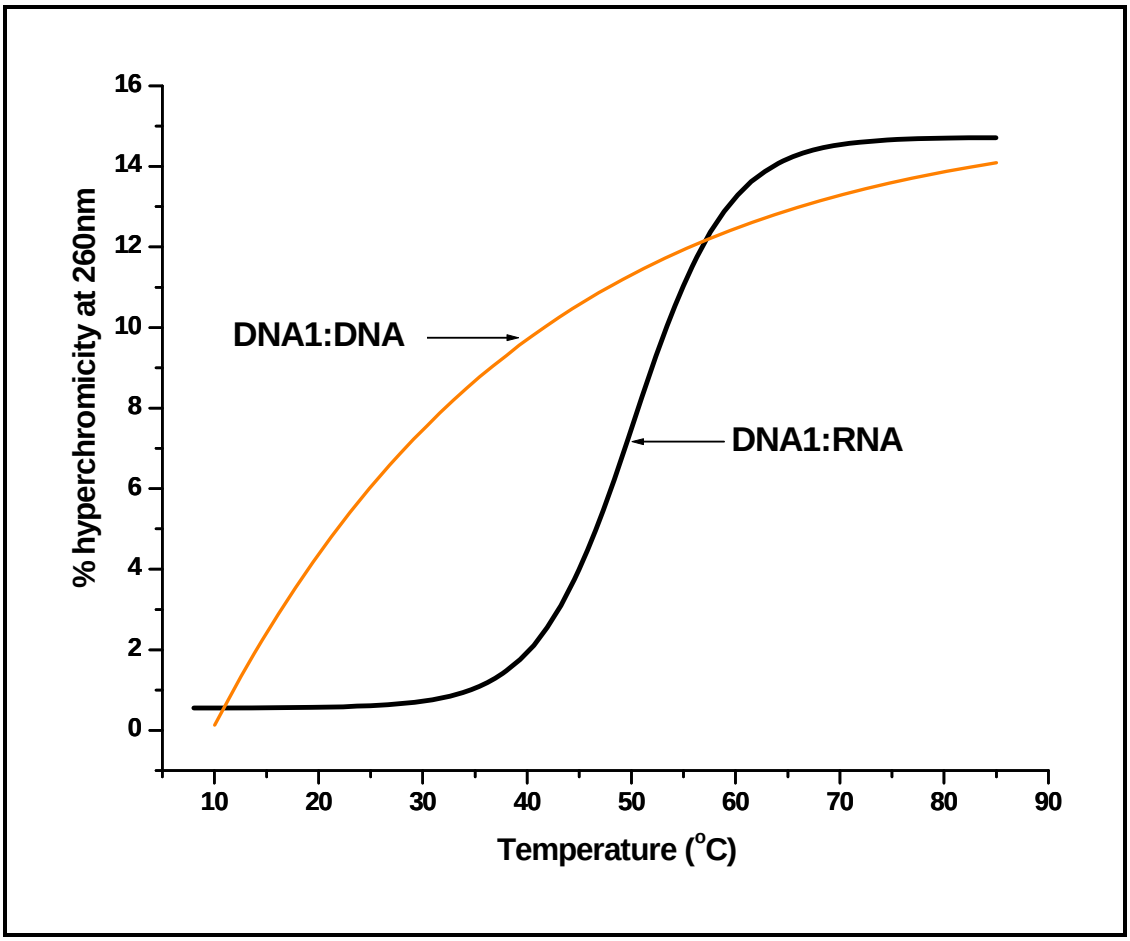
MALDI-TOF spectrum of DNA9



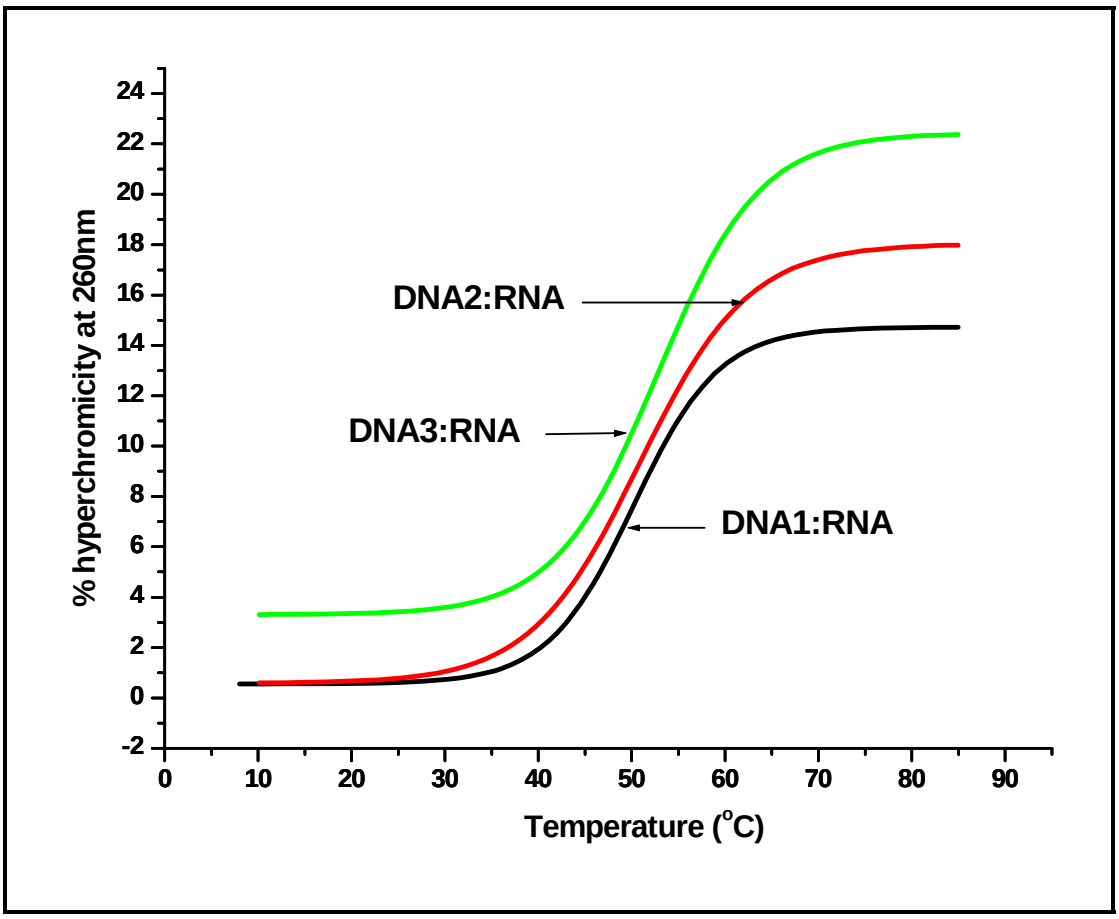
UV-Melting Study

Melting temperatures were obtained from the maxima of the first derivative of the melting curves (A_{260} *vs* temperature) in buffer containing 10mM sodium phosphate, 150mM sodium chloride, pH 7.4 using 1.0μM concentrations of each of the two complementary strands. Each experiment was performed at least thrice.

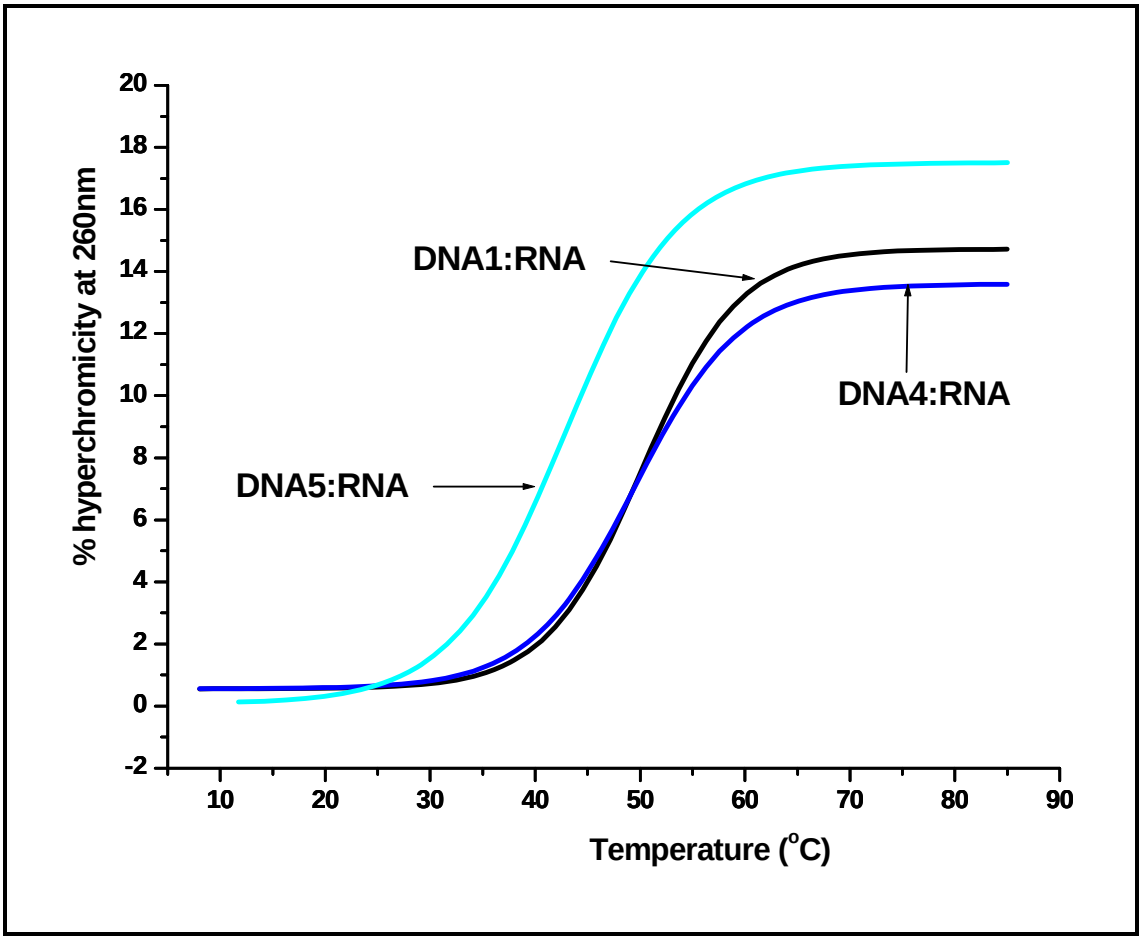
UV-melting plots of complexes of DNA1 with complementary RNA and DNA



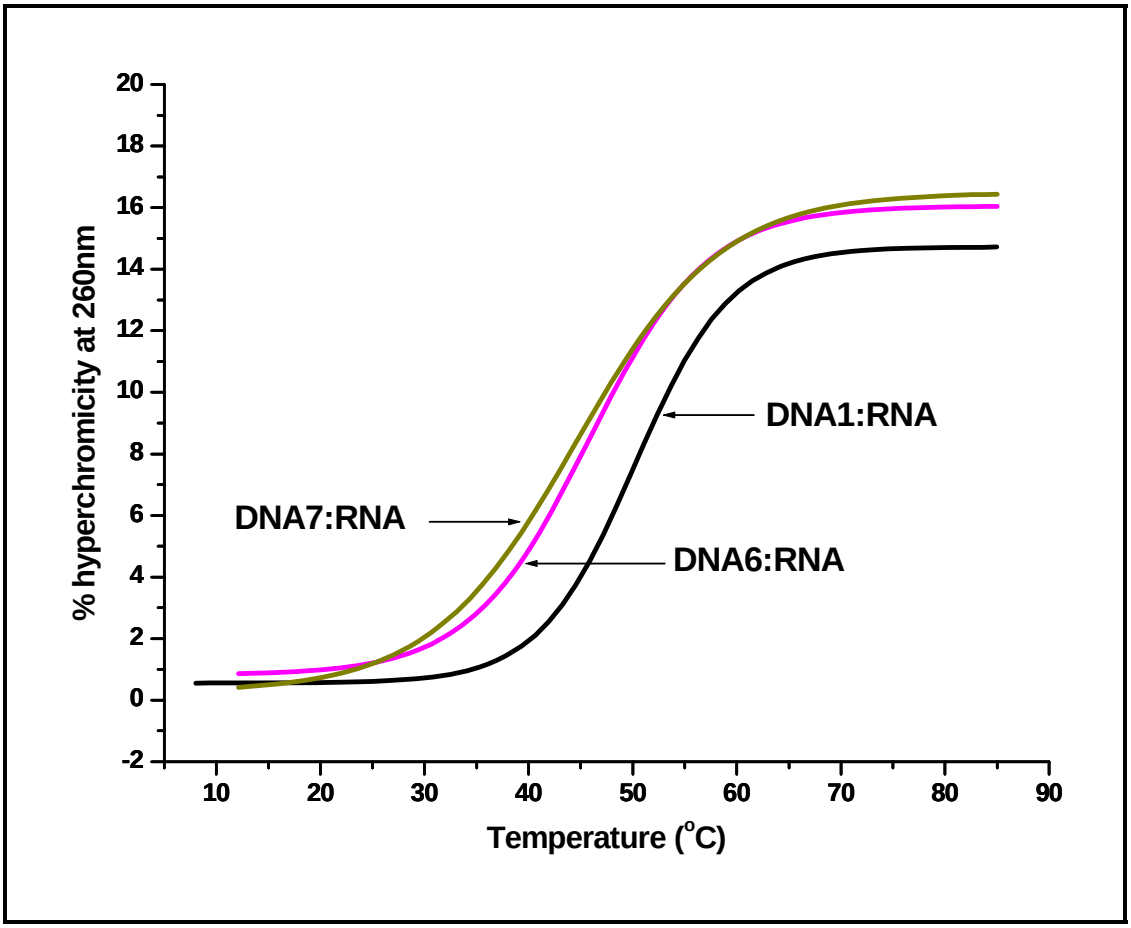
UV-melting plots of complexes of DNA1, DNA2 and DNA3 with complementary RNA



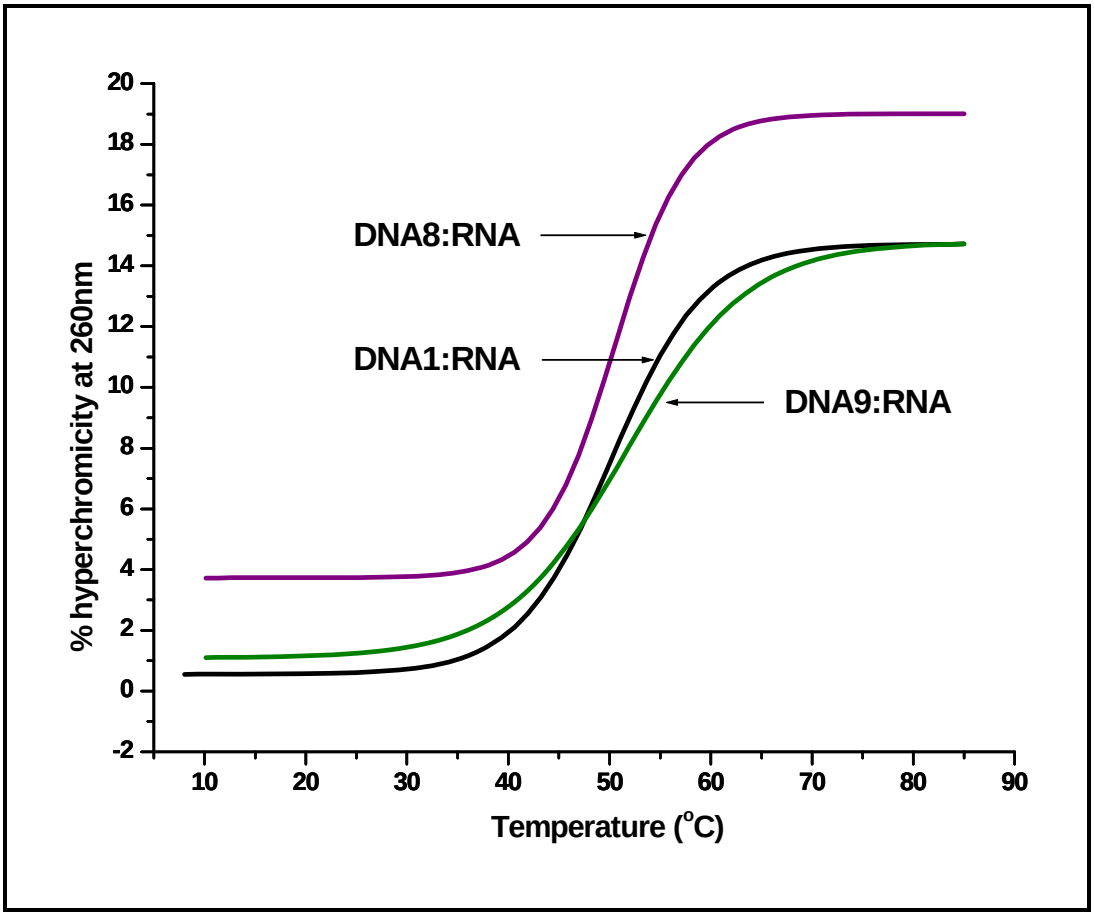
UV-melting plots of complexes of DNA1, DNA4 and DNA5 with complementary RNA



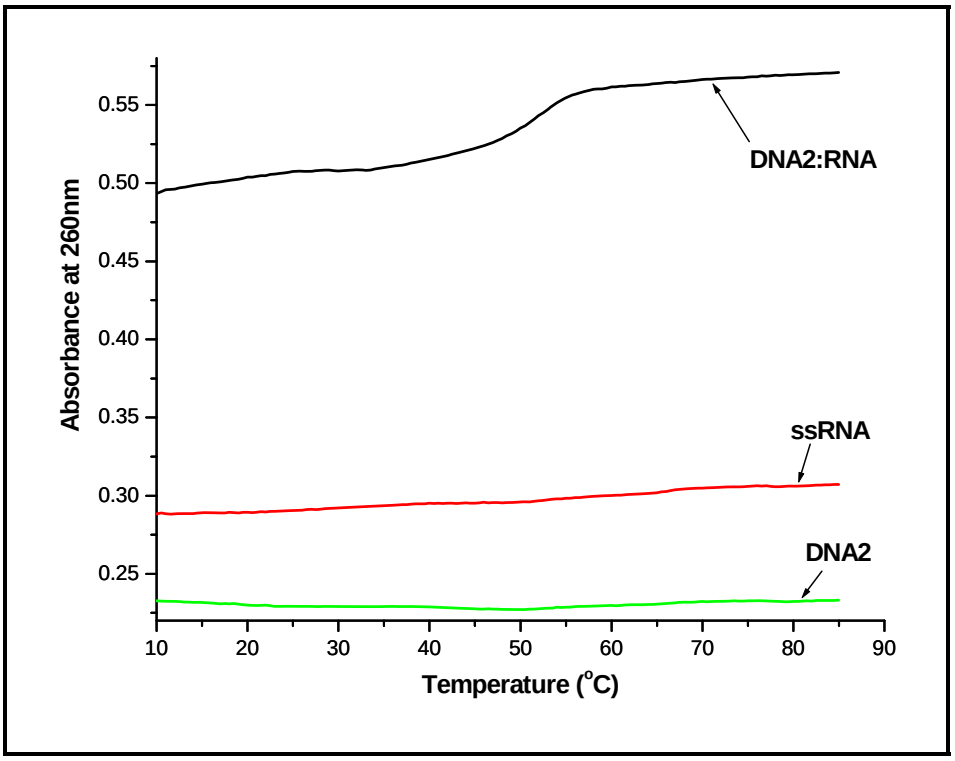
UV-melting plots of complexes of DNA1, DNA6 and DNA7 with complementary RNA



UV-melting plots of complexes of DNA1, DNA8 and DNA9 with complementary RNA



Representative UV-melting and CD plots of Single strand DNA2, Complementary Single strand RNA and Complex of DNA2 with complementary RNA



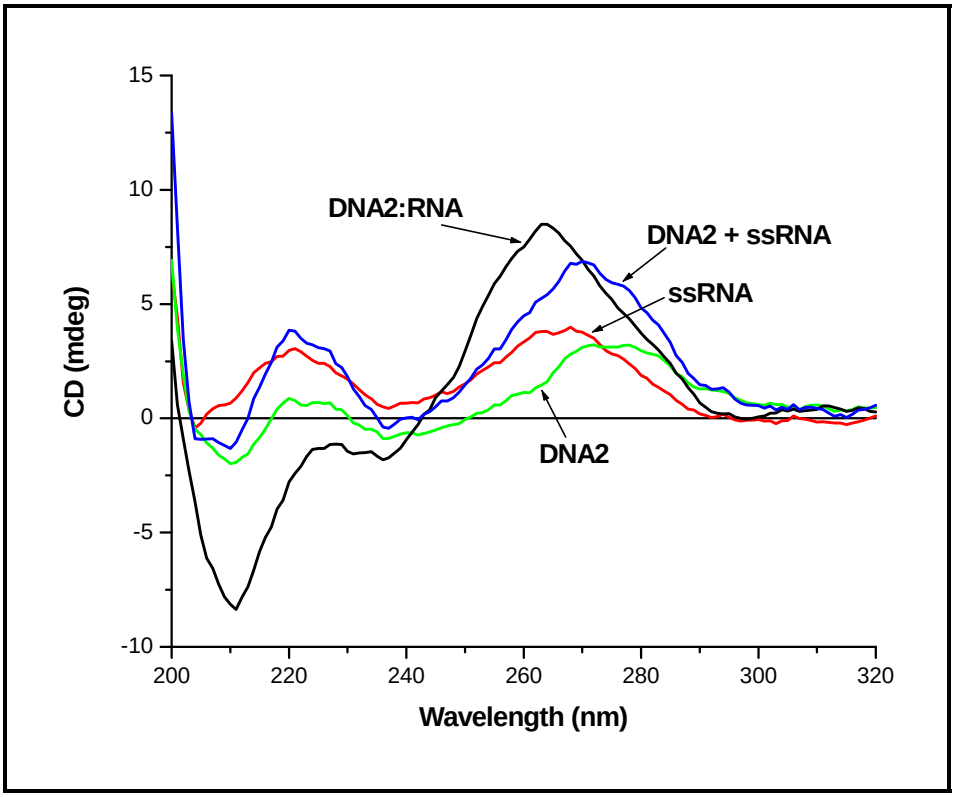


Table S1 Conformational analysis of the nucleosides using H1'-H2' coupling constants from ^{1}H NMR spectra

Nucleoside derivative	H1'-H2' coupling *J* in Hz	%S	Reference
3'-Deoxy uridine	1.2	3	15
S-Locked uridine($\mathbf{U}^{\mathbf{S}}$)	7.5	94	15
N-Locked uridine($\mathbf{U}^{\mathbf{N}}$)	3.5	36	16
3'-ribo-3'-fluoro Uridine($^{\mathbf{r}}\mathbf{U}^{\mathbf{F}}$)	7.7	97	17
3'-xylo-3'-fluoro Uridine($^{\mathbf{X}}\mathbf{U}^{\mathbf{F}}$)	1.0	0.3	18