Supporting Information

Application of 4′-*C*-α-aminoethoxy-2′-*O*-methyl-5-propynyl-uridine for antisense therapeutics

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Keywords: Antisense oligonucleotides, RNase H, 4′-Aminoethoxy, 2′-Methoxy, 5-Propynyl

Contents

ONs	Sequence[a]	$[M-H]^-$	*m/z*
TC-Nat.	5'-TCT TTC TCT TTC CCT T-3'	4712.78	4713.77
TC-m.	5'-TCT TmC TCT mTC CCm T-3'	4979.92	4981.88
TC-p.	5'-TCT TpC TCT pTC CCp T-3'	5051.92	5052.90
TC-mm-m	5'-TCT TTC mCT TTC CCT T-3'	4801.83	4802.91
TC-mm-p	5'-TCT TTC pCT TTC CCT T-3'	4825.83	4827.02
TC-Nat.-F	5'-F-TCT TTC TCT TTC CCT T-3'	5250.91	5251.70
TC-m-F	5'-F-TCT TmC TCT mTC CCm T-3'	5519.05	5519.09
TC-p-F	5'-F-TCT TpC TCT pTC CCp T-3'	4712.78	4713.77
KRAS-pos	5'-G·C·T·A·T·T·A·G·G·A·G·T·C·T·T·T-3'	5321.49	5325.32
KRAS-m-1	5'-G·C·m·A·T·T·A·G·G·A·G·T·C·T·T·T-3'	5630.79	5634.80
KRAS-m-2	5'-G·C·T·A·m·T·A·G·G·A·G·T·C·T·T·T-3'	5459.61	5463.50
KRAS-m-3	5'-G·C·T·A·T·T·A·G·G·A·G·m·C·T·T·T-3'	5424.59	5428.48
KRAS-m-4	5'-G·C·T·A·T·T·A·G·G·A·G·T·C·m·T·T-3'	5367.53	5371.38
KRAS-p-1	5'-G·C·p·A·T·T·A·G·G·A·G·T·C·T·T·T-3'	5424.59	5429.21
KRAS-p-2	5'-G·C·T·A·p·T·A·G·G·A·G·T·C·T·T·T-3'	5367.53	5371.49
KRAS-p-3	5'-G·C·T·A·T·T·A·G·G·A·G·p·C·T·T·T-3'	5424.59	5427.11
KRAS-p-4	5'-G·C·T·A·T·T·A·G·G·A·G·T·C·p·T·T-3'	5367.53	5372.01
cRNA1	5'-aag gga aag aga aag a-3'	5298.86	5300.66
cRNA2	5'-F-aag gga aag aga aag a-3'	5836.99	5837.57

Table 1. The sequences of all oligonucleotides used in this study.

[a]Capital letters denote DNAs while small letters denote RNAs. The red m and p denote the 4′-*C*-α-aminoethoxy-2′-*O*-methyl-5-methyl-uridine ($4AEo^mU$) and the 4′-*C*-α-aminoethoxy-2′-*O*-methyl-5-propynyl-uridine ($4AEo^pU$), respectively. Underlined letters denote LNAs. F denote fluorescein. Black dots denote the phosphorothioate (PS) linkages. The cRNAs for base-mismatching test were prepared in previous study [32].

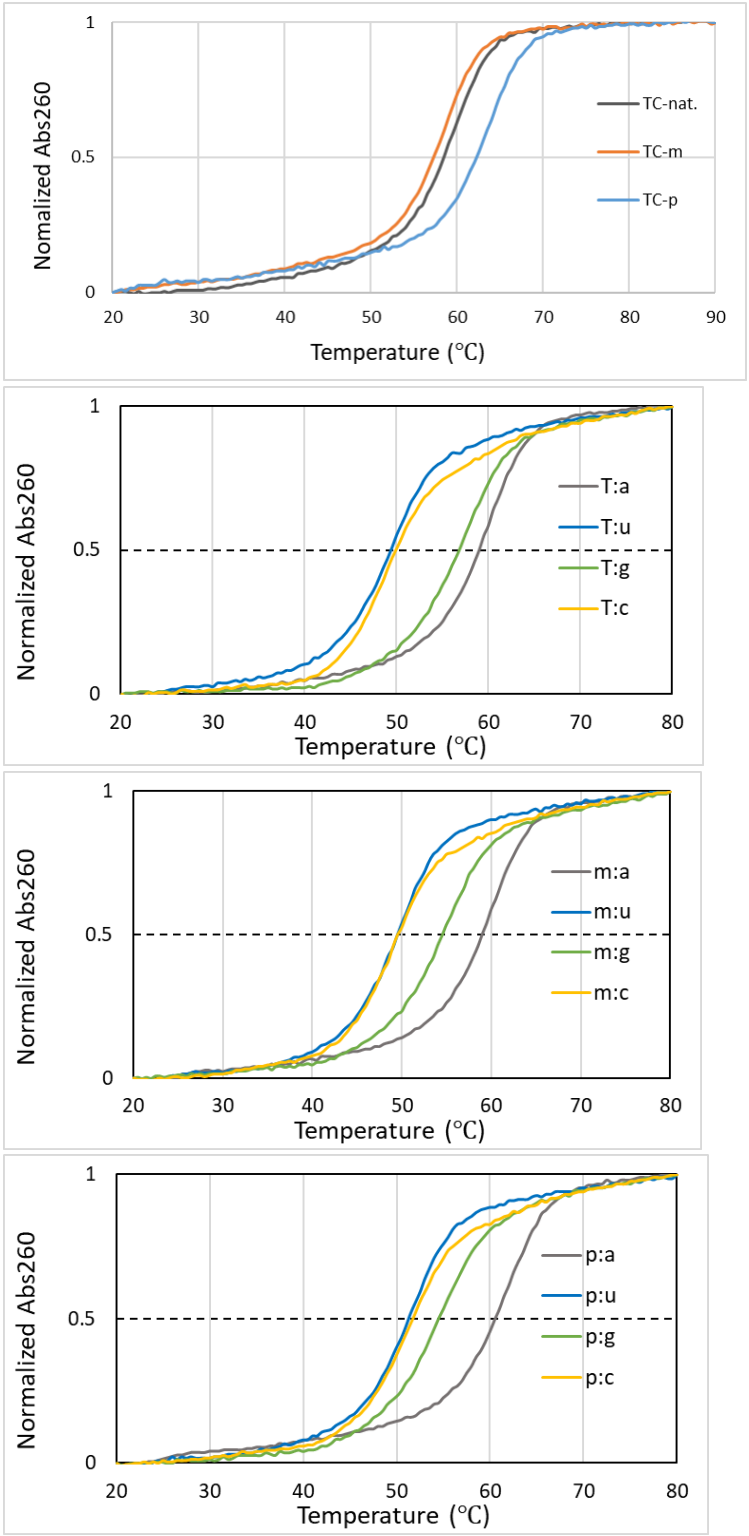
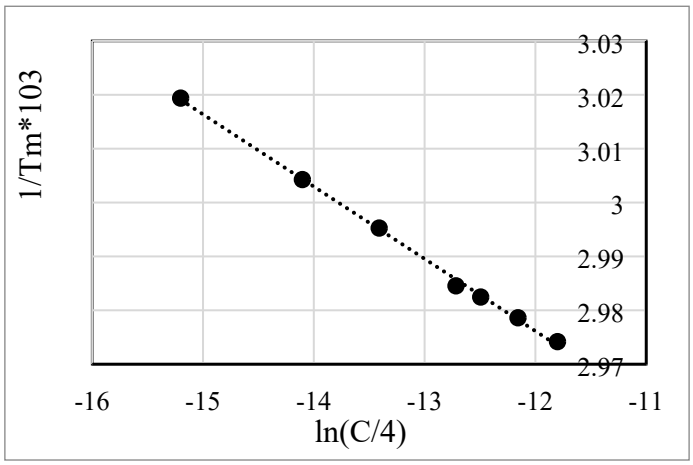
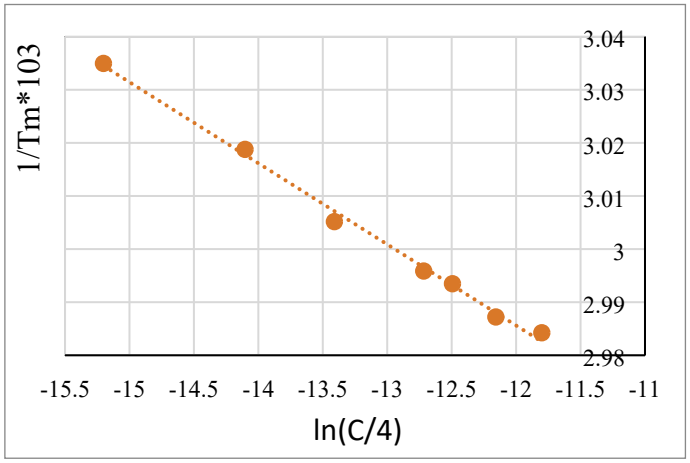


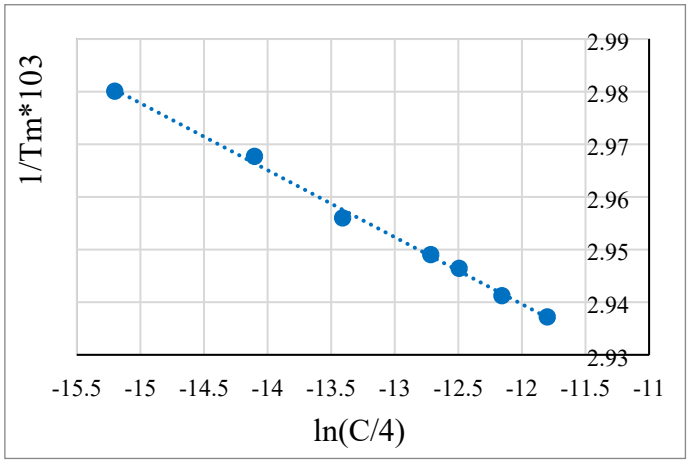
Figure S1. The UV melting profiles of DNA/RNA duplexes in this study.



(TC-Nat.)



(TC-m)



(TC-p)

Figure S2. The graphical data of $1/T_m$ vs ln ($C_T/4$) plots.

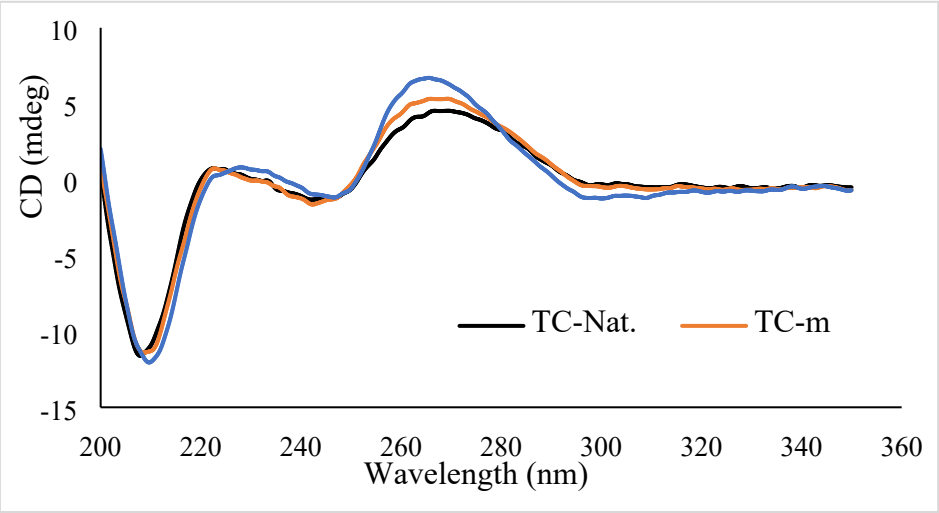


Figure S3. CD spectrum of DNA/RNA duplexes in this study.

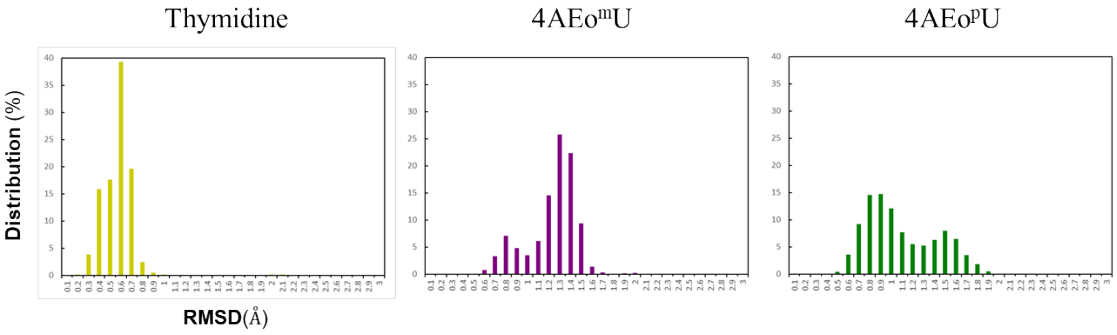


Figure S4. The changes of time-dependent RMSD (Å) of each nucleoside.

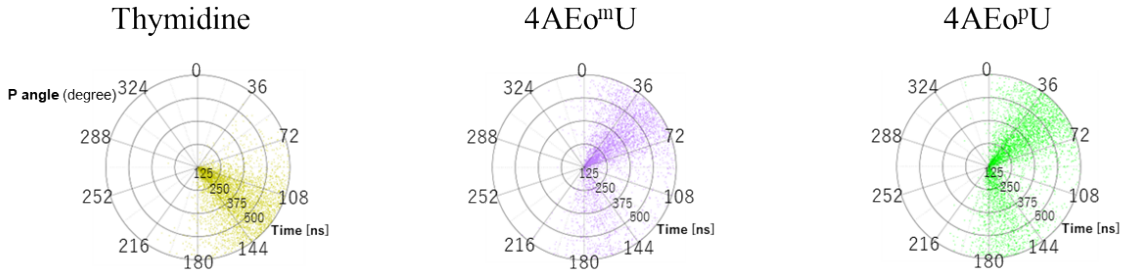


Figure S5. Pseudorotational phase angle (*P*) of each nucleoside.

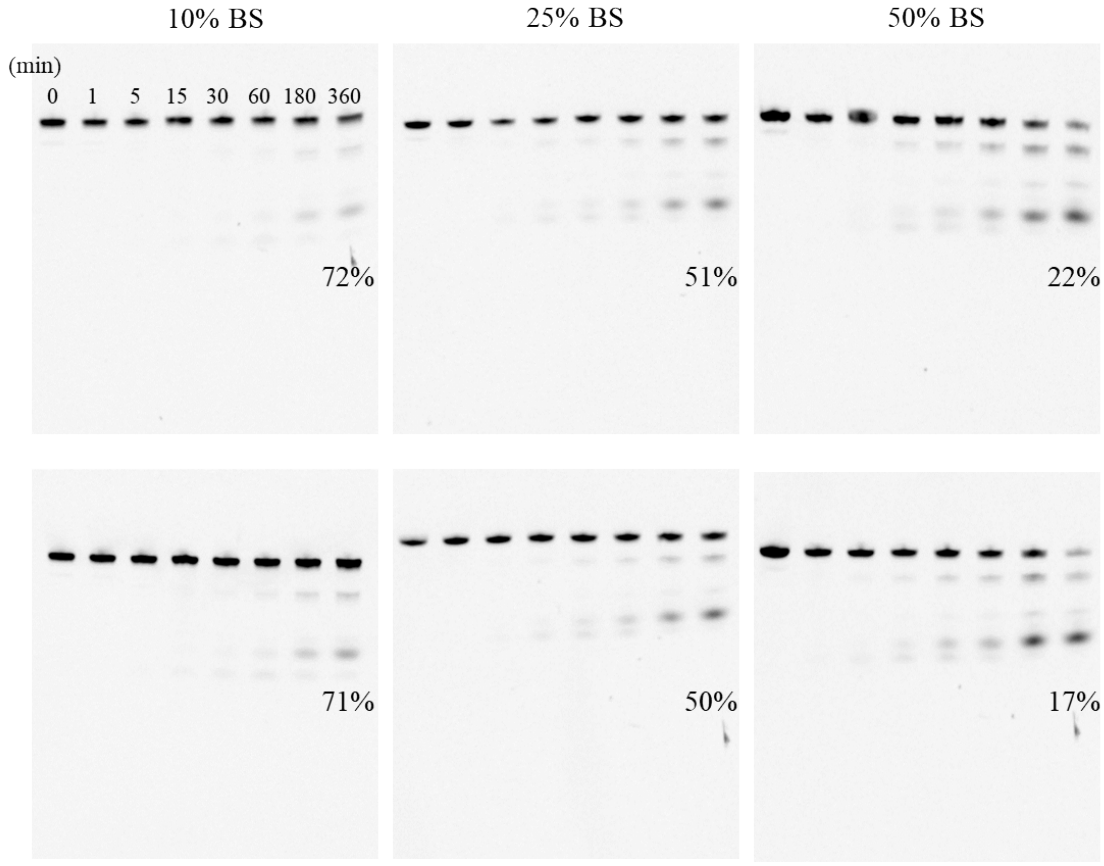
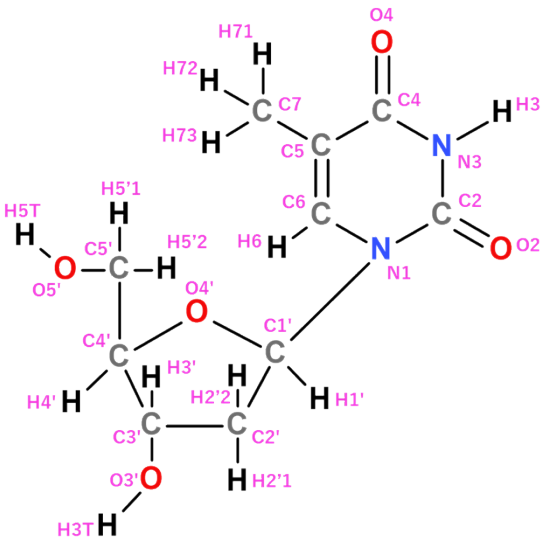
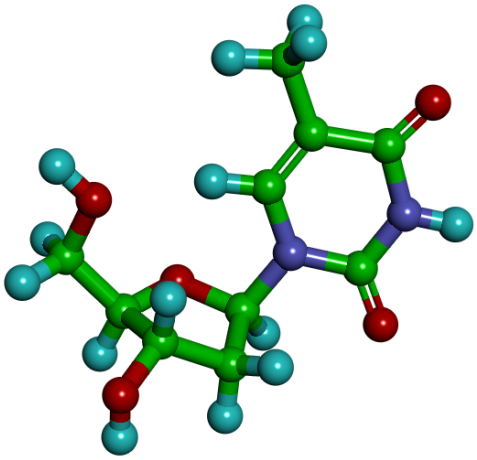


Figure S6. PAGE analysis of single-stranded oligomers treated with bovine serum at various concentrations: TC-m-F (up) and TC-p-F (down).

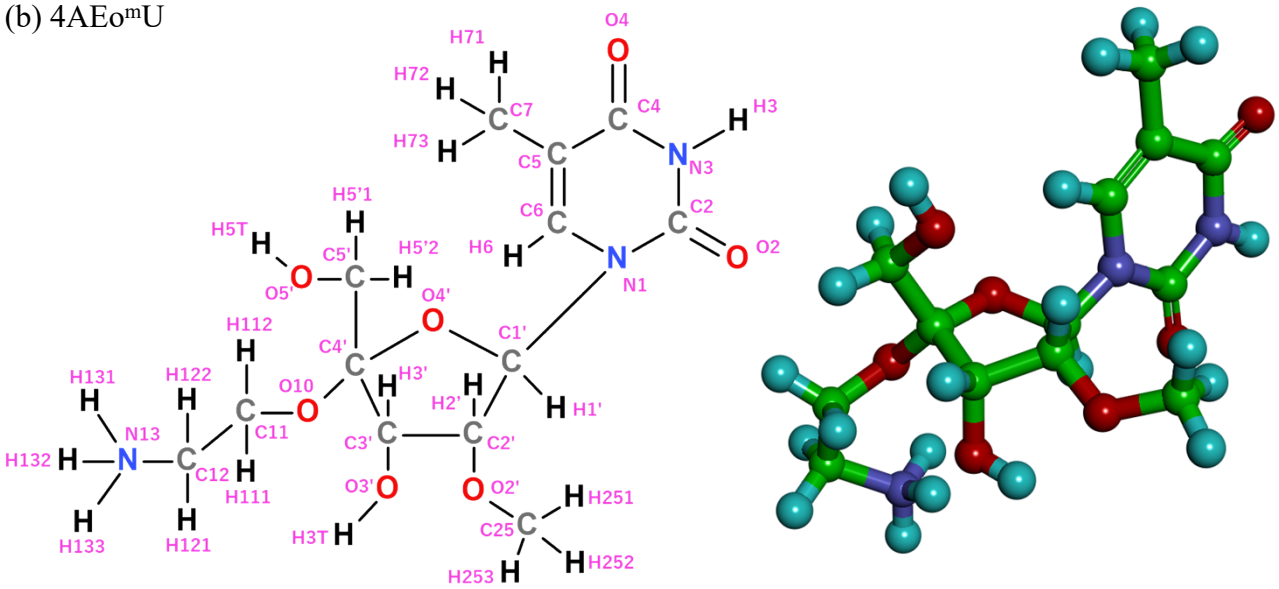
(a)Thymidine





Atom name	Atom type	RESP charge	Atom name	Atom type	RESP charge
O2	o	-0.632498	H3T	ho	0.425732
C2	c	0.793243	H3'	h1	0.046292
N3	n	-0.726469	C4'	c3	0.187230
H3	hn	0.397873	H4'	h1	0.007755
C4	c	0.771164	C5'	c3	0.115366
O4	o	-0.597090	H5'1	h1	0.045585
C5	cd	-0.070669	H5'2	h1	0.045585
C7	c3	-0.329531	O5'	oh	-0.703396
H71	hc	0.105895	H5T	ho	0.463505
H72	hc	0.105895			
H73	hc	0.105895			
C6	cc	-0.192145			
H6	h4	0.265353			
N1	n	-0.200708			
C1'	c3	0.414366			
O4'	os	-0.478007			
H1'	h2	0.056209			
C2'	c3	-0.463805			
H2'1	hc	0.129227			
H2'2	hc	0.129227			
C3'	c3	0.526271			
O3'	oh	-0.743351			

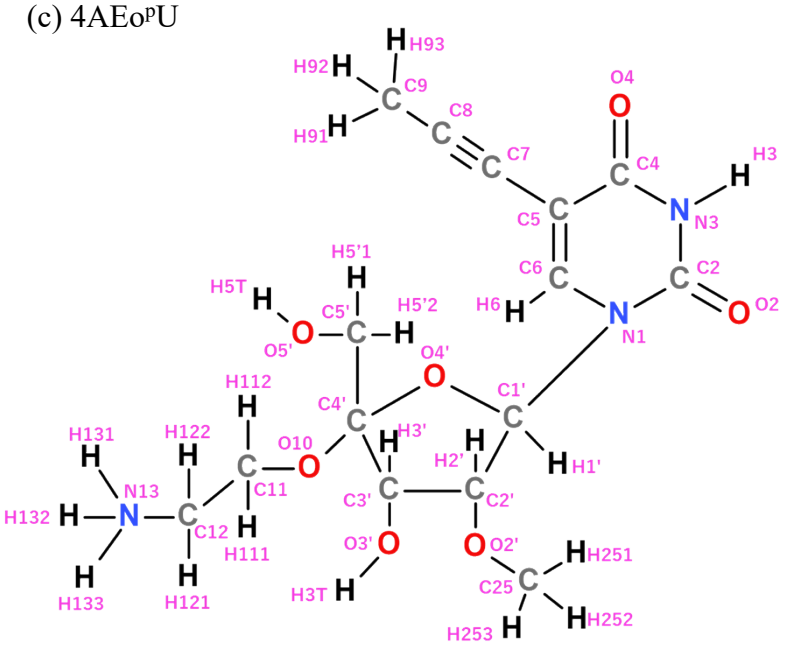
(b) 4AEo^{m}U

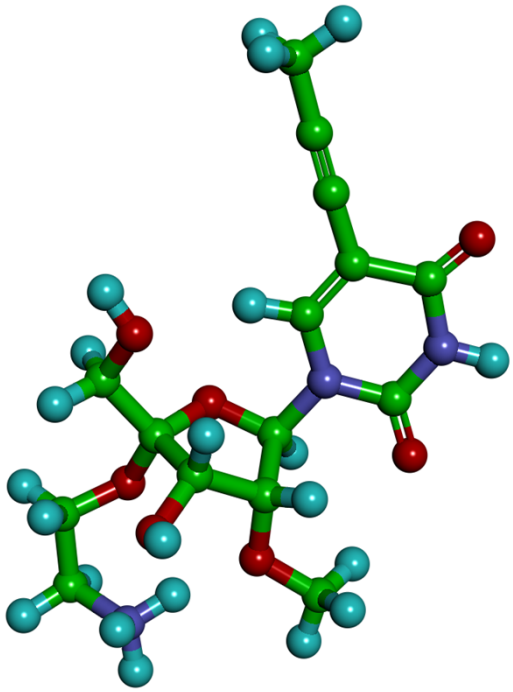


Atom name	Atom type	RESP charge	Atom name	Atom type	RESP charge
O2	o	-0.627573	C3'	c3	0.194429
C2	c	0.814088	O3'	oh	-0.655476
N3	n	-0.743745	H3T	ho	0.422985
H3	hn	0.411288	H3'	h1	0.099814
C4	c	0.774280	C4'	c3	0.397484
O4	o	-0.560321	C5'	c3	0.071495
C5	cd	-0.029080	O5'	oh	-0.760414
C7	c3	-0.383170	H5T	ho	0.501195
H71	hc	0.131254	H5'1	h1	0.089834
H72	hc	0.131254	H5'2	h1	0.089834
H73	hc	0.131254	O10	os	-0.423908
C6	cc	-0.262349	C11	c3	0.218866
H6	h4	0.267977	H111	h1	0.026149
N1	n	-0.134063	H112	h1	0.026149
C1'	c3	0.277225	C12	c3	-0.001046
O4'	os	-0.421061	H121	hx	0.108387
H1'	h2	0.095923	H122	hx	0.108387
C2'	c3	-0.052774	N13	n4	-0.364088
O2'	os	-0.333044	H131	hn	0.329086
C25	c3	-0.044824	H132	hn	0.329086
H251	h1	0.088272	H133	hn	0.329086

H252	h1	0.088272
H253	h1	0.088272
H2'	h1	0.155314

(c) 4AEo^{P}U



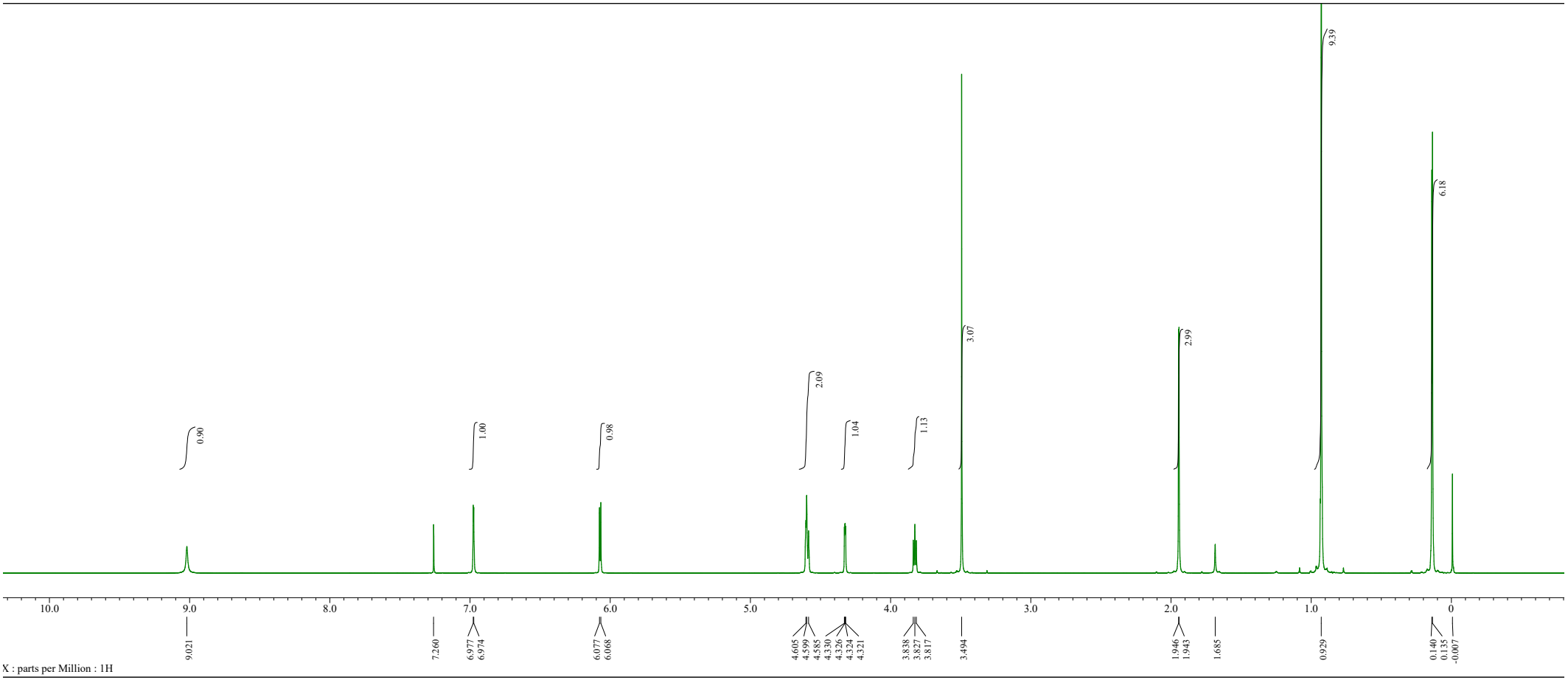


Atom name	Atom type	RESP charge	Atom name	Atom type	RESP charge
C9	c3	-0.265152	H253	h1	0.140156
H91	hc	0.114692	H2'	h1	0.146678
H92	hc	0.114692	C3'	c3	0.377801
H93	hc	0.114692	O3'	oh	-0.705015
C8	c1	0.154207	H3T	ho	0.463512
C7	ch	-0.402377	H3'	h1	0.053246
C5	cd	0.033503	C4'	c3	0.298573
C6	cc	-0.108650	C5'	c3	0.131048
H6	h4	0.239967	O5'	oh	-0.691739
C4	c	0.793449	H5T	ho	0.470579
O4	o	-0.546271	H5'1	h1	0.071127
N3	n	-0.781446	H5'2	h1	0.071127
H3	hn	0.426030	O10	os	-0.383798
C2	c	0.810720	C11	c3	0.265775
O2	o	-0.632116	H111	h1	0.016884
N1	n	-0.187852	H112	h1	0.016884

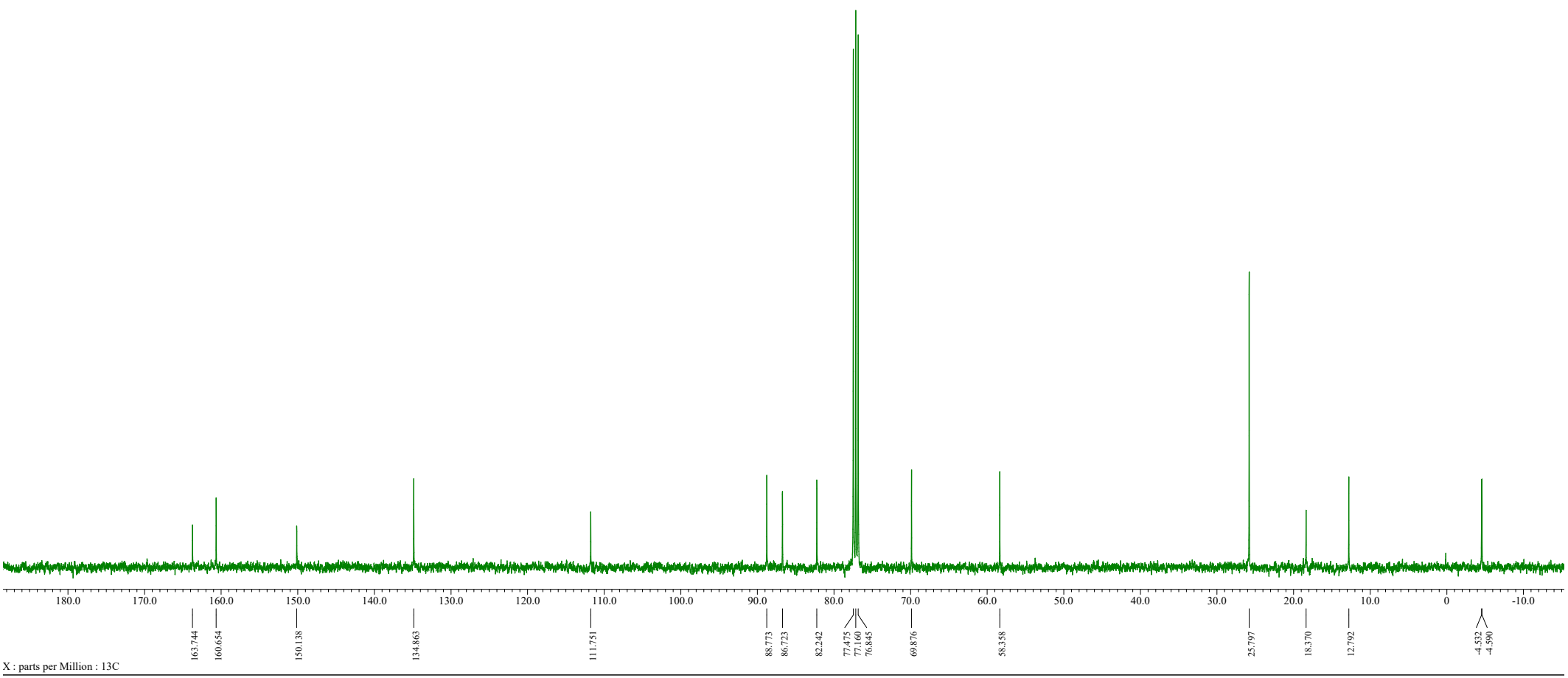
C1'	c3	0.233131	C12	c3	-0.086019
O4'	os	-0.385815	H121	hx	0.127501
H1'	h2	0.141355	H122	hx	0.127501
C2'	c3	-0.152767	N13	n4	-0.324466
O2'	os	-0.294486	H131	hn	0.307884
C25	c3	-0.210827	H132	hn	0.307884
H251	h1	0.140156	H133	hn	0.307884
H252	h1	0.140156			

Figure S7. The reference structure atom types and calculated RESP charges for each nucleoside: (a) thymidine, (b) 4AEomU and (c) 4AEopU.

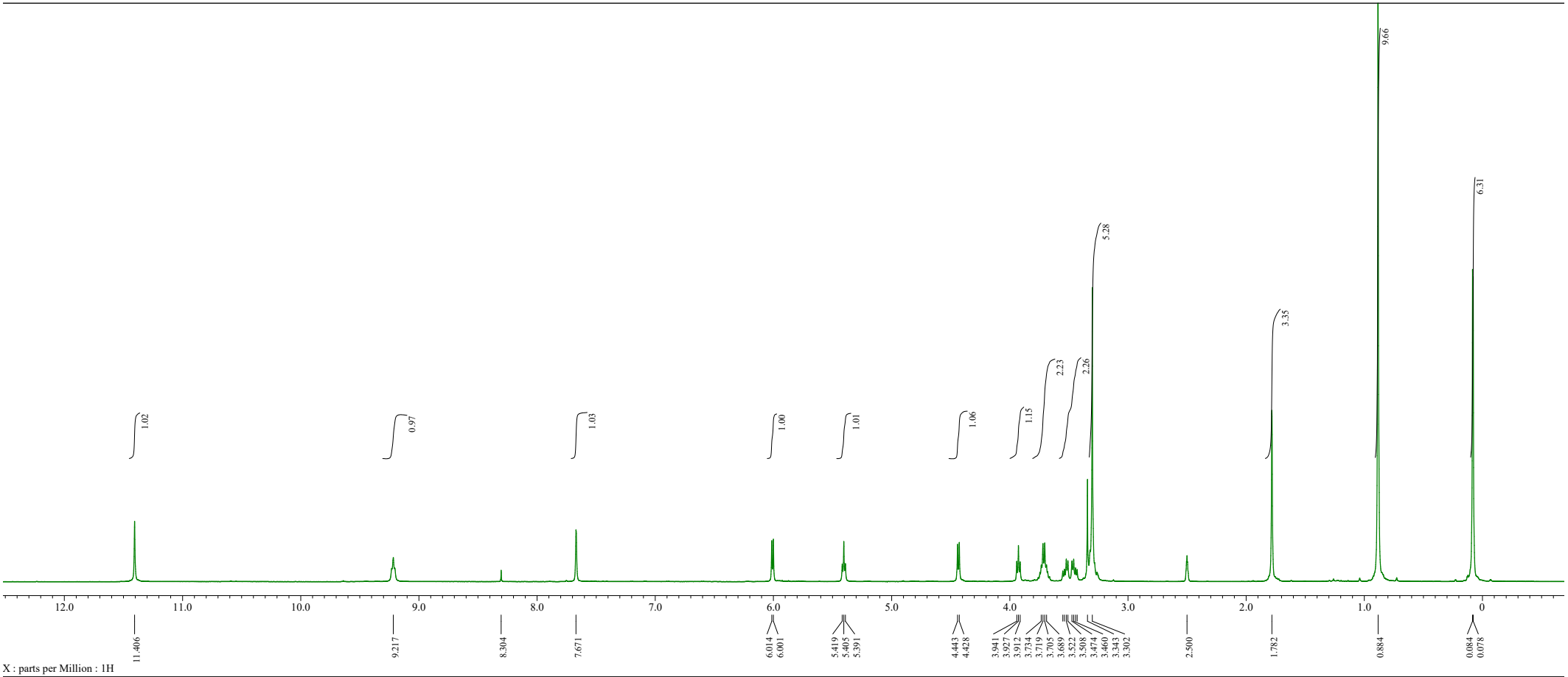
^{1}H NMR spectrum of compound **4**



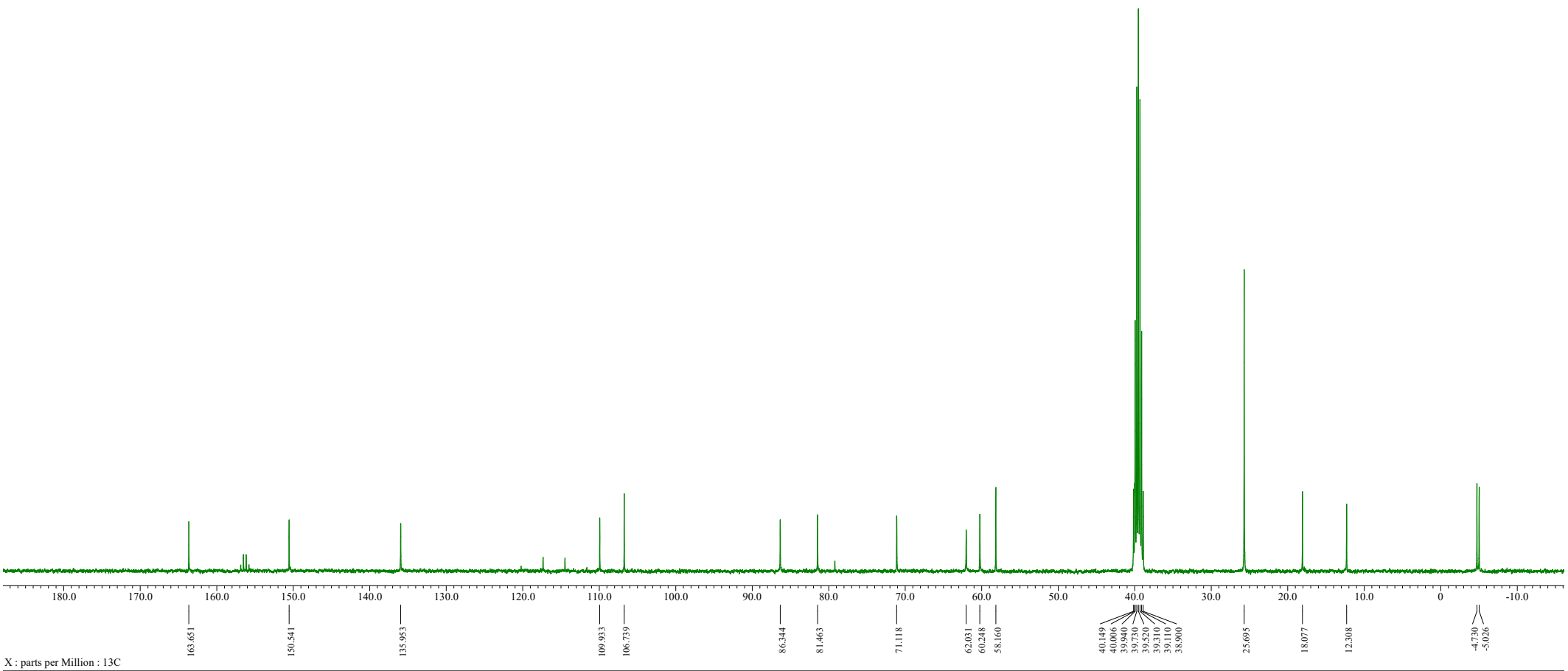
^{13}C NMR spectrum of compound **4**



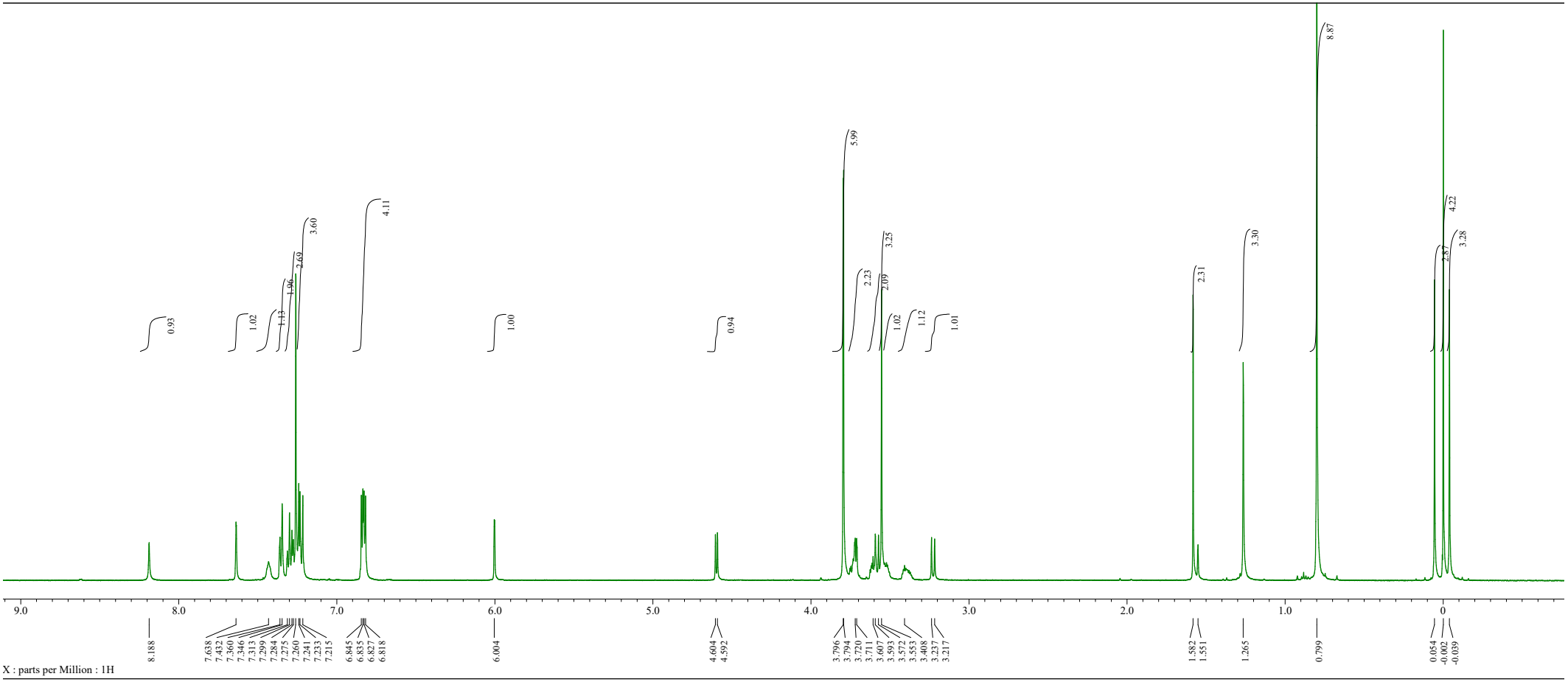
^{1}H NMR spectrum of compound **6**



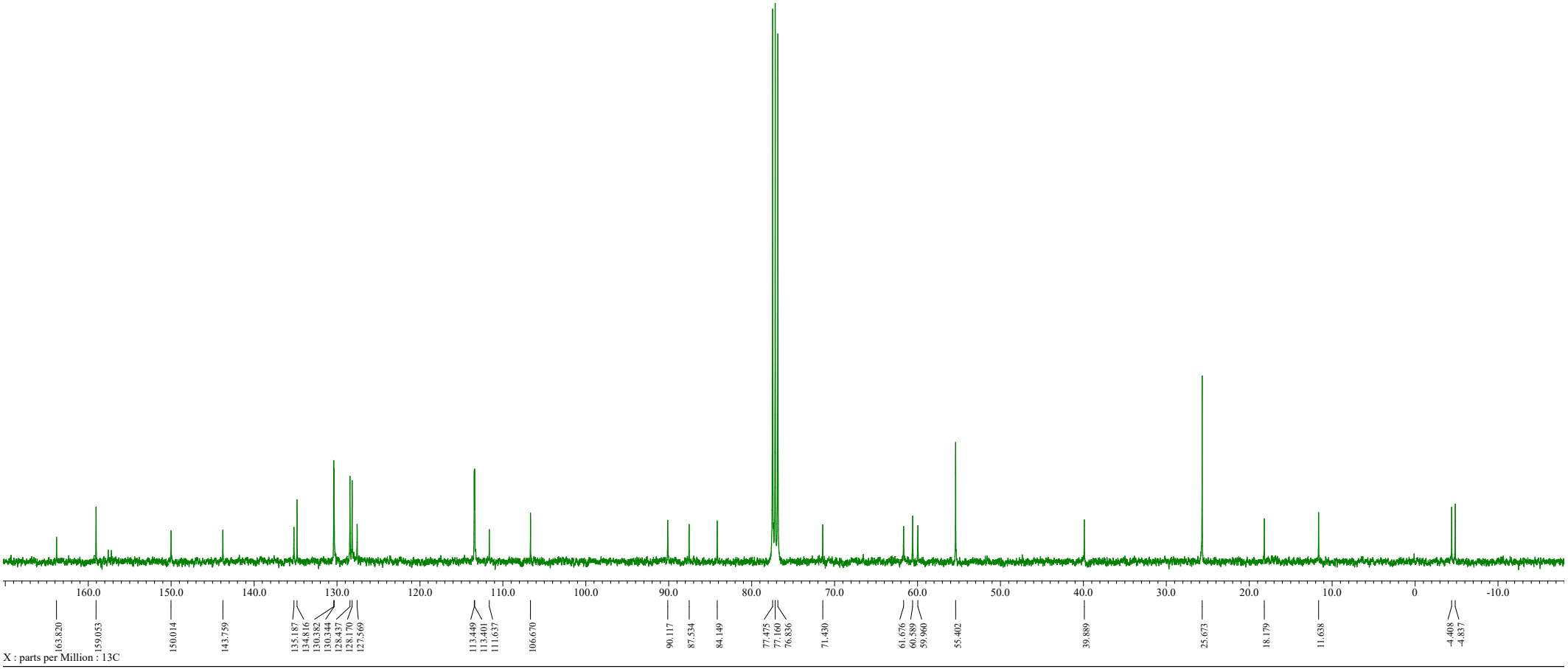
^{13}C NMR spectrum of compound **6**



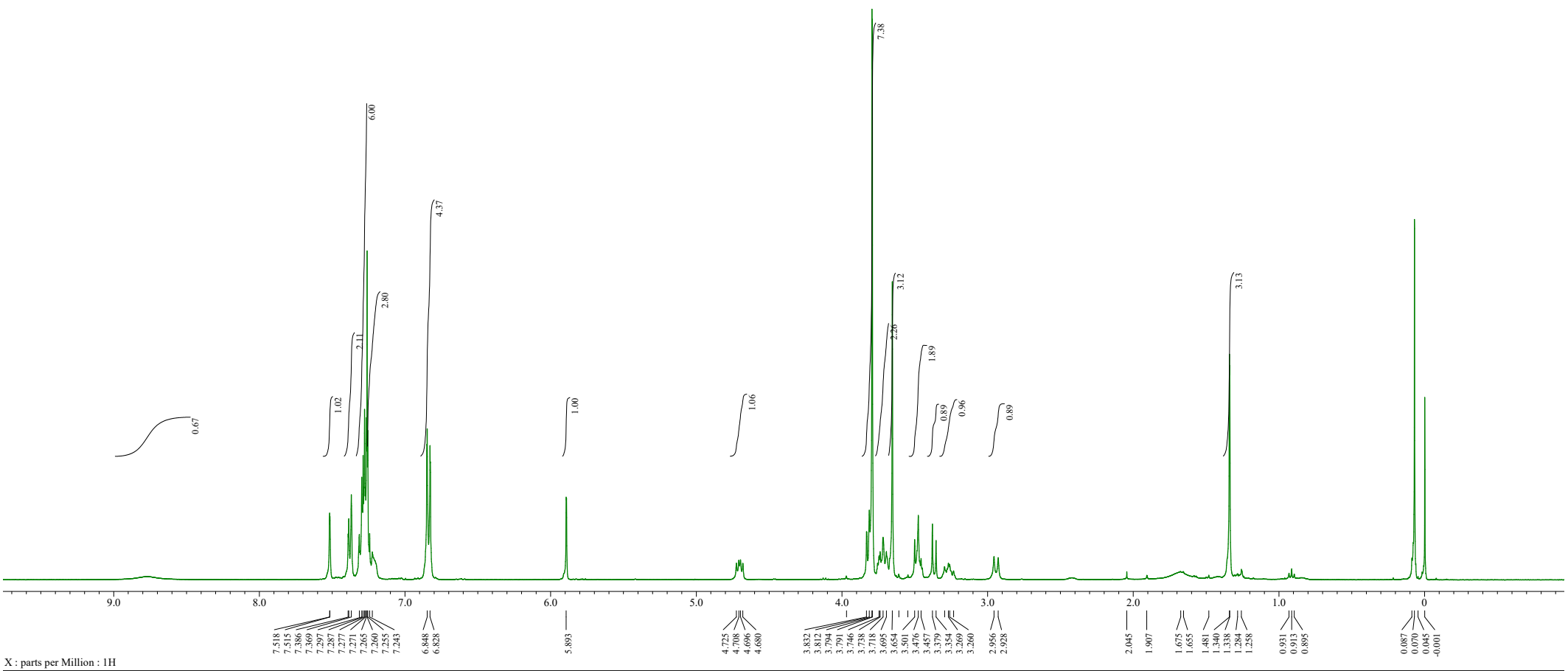
^{1}H NMR spectrum of compound **7**



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

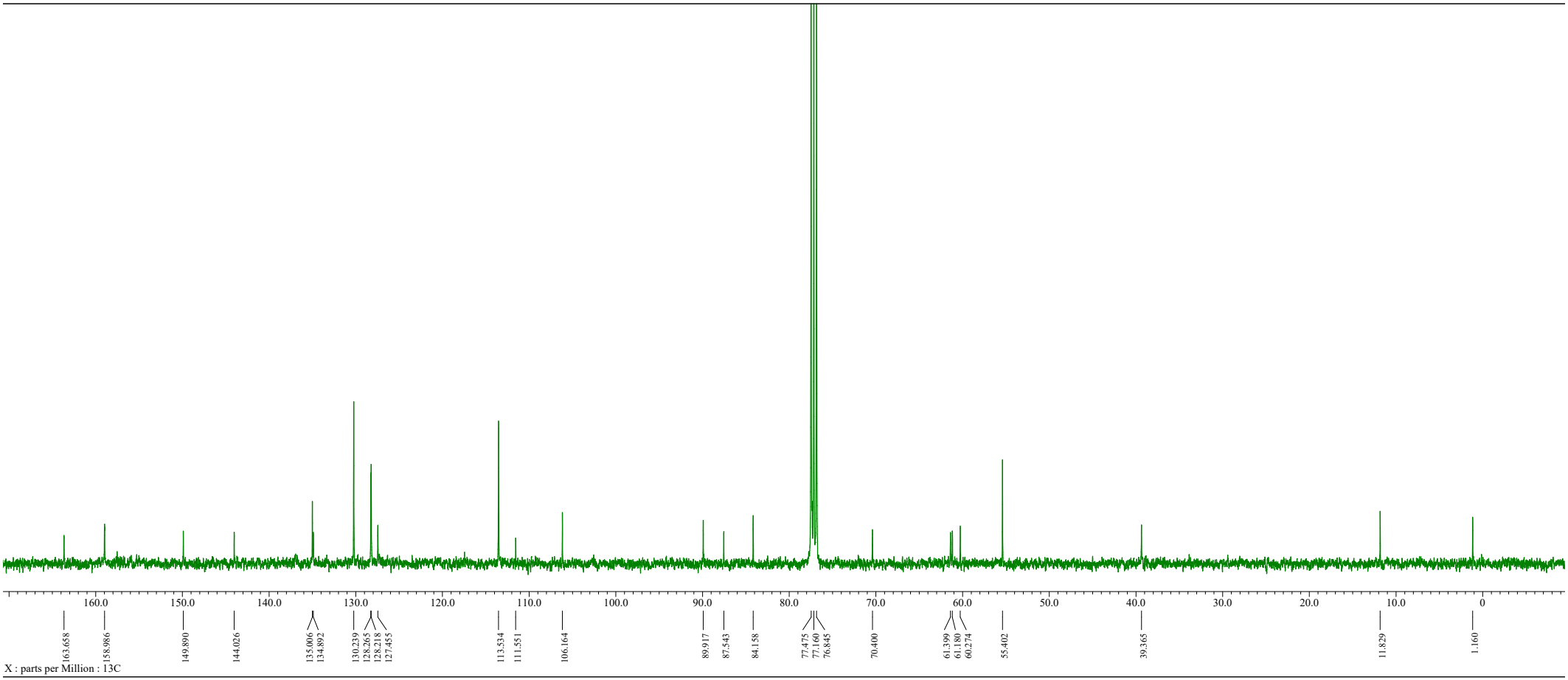


^{1}H NMR spectrum of compound **8**

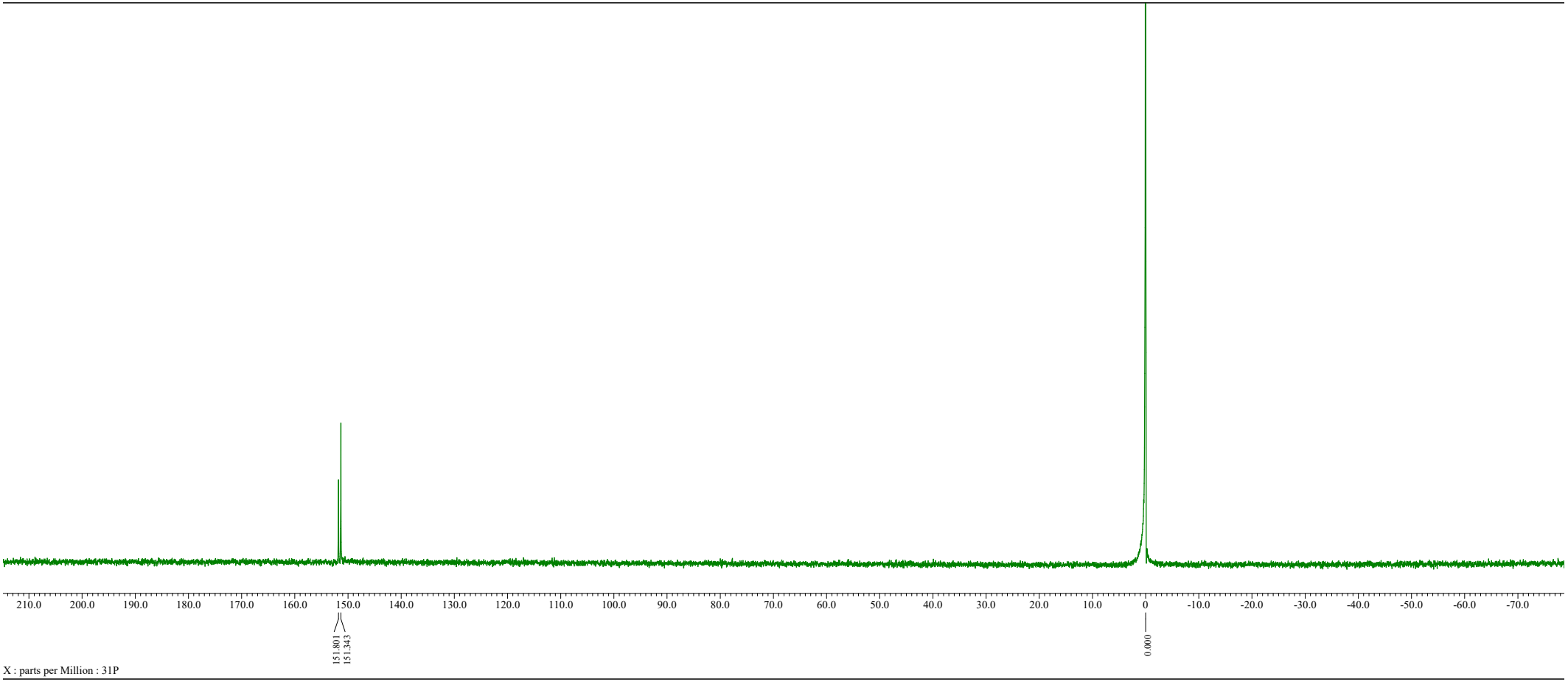


X : parts per Million : 1H

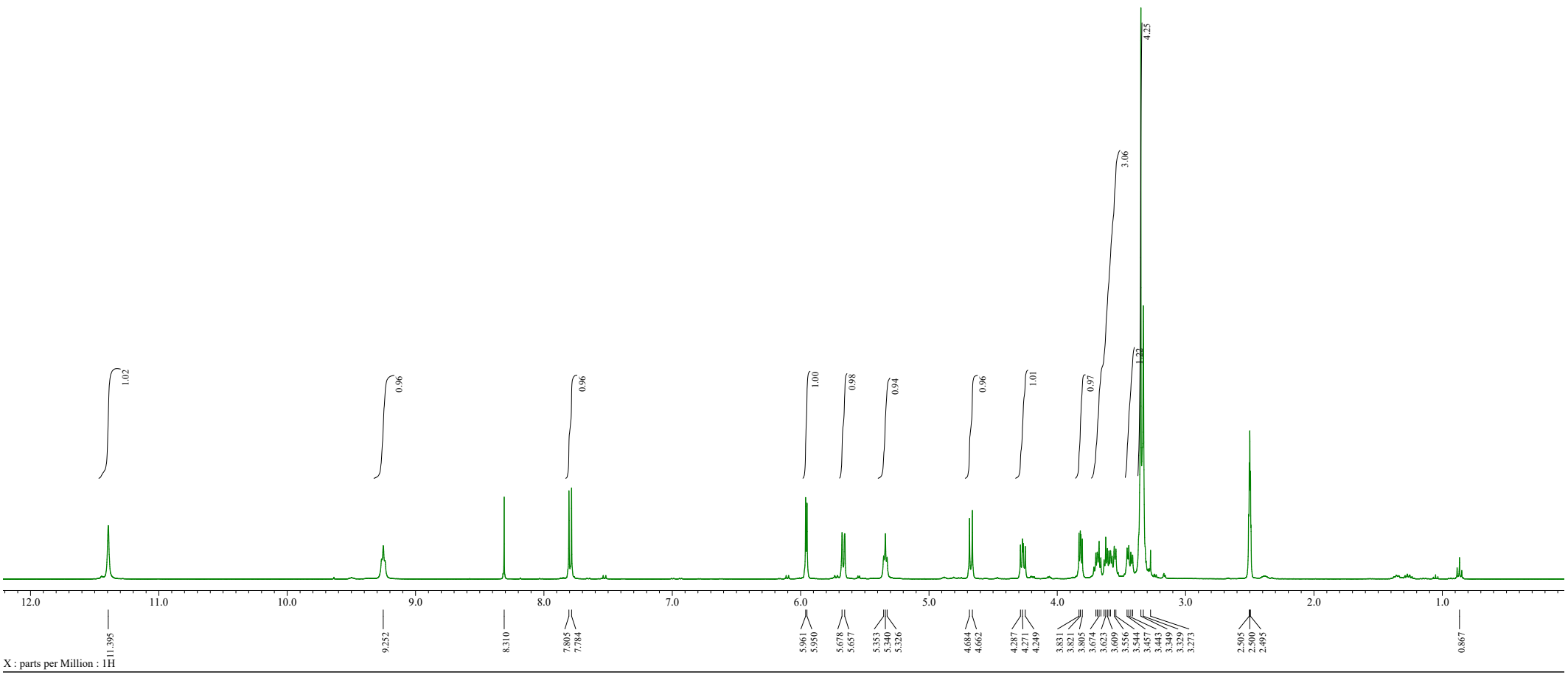
^{13}C NMR spectrum of compound **8**



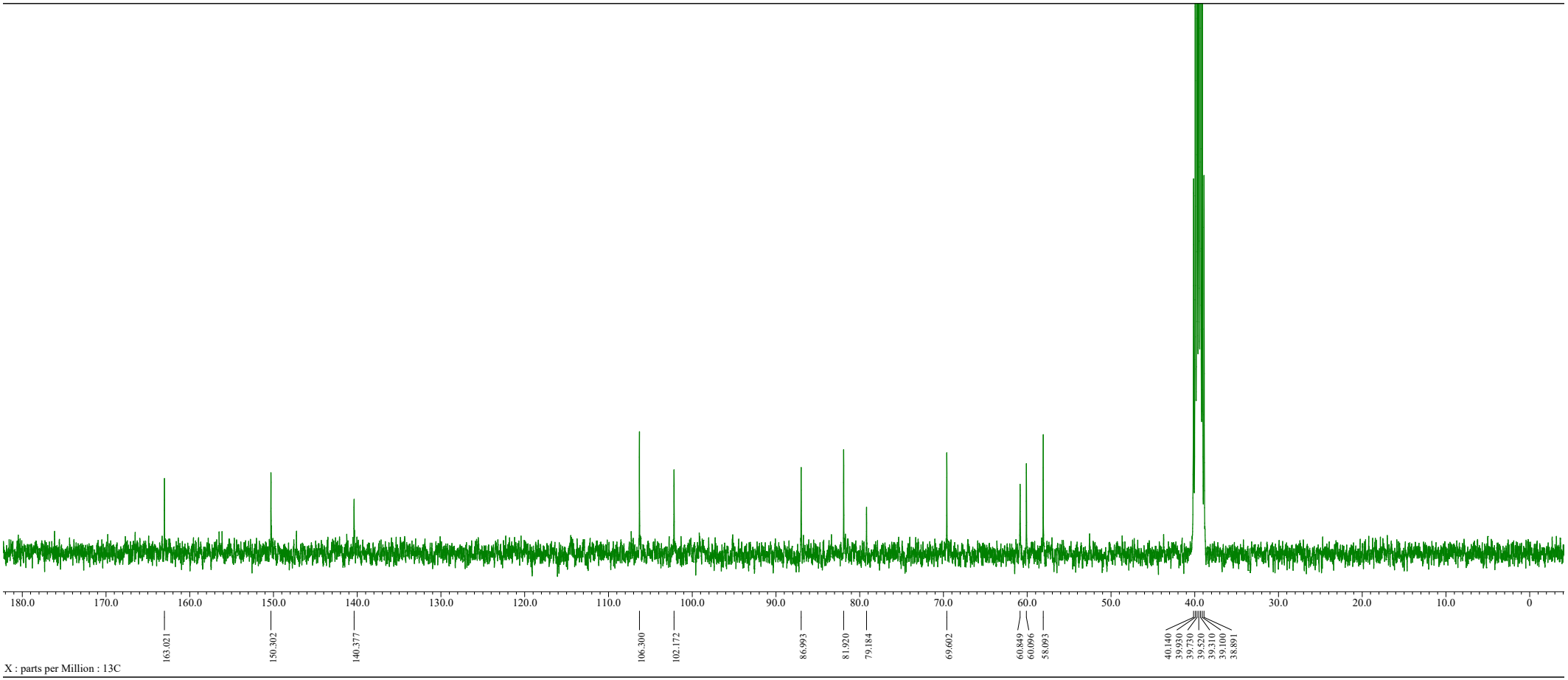
^{31}P NMR spectrum of compound **9**



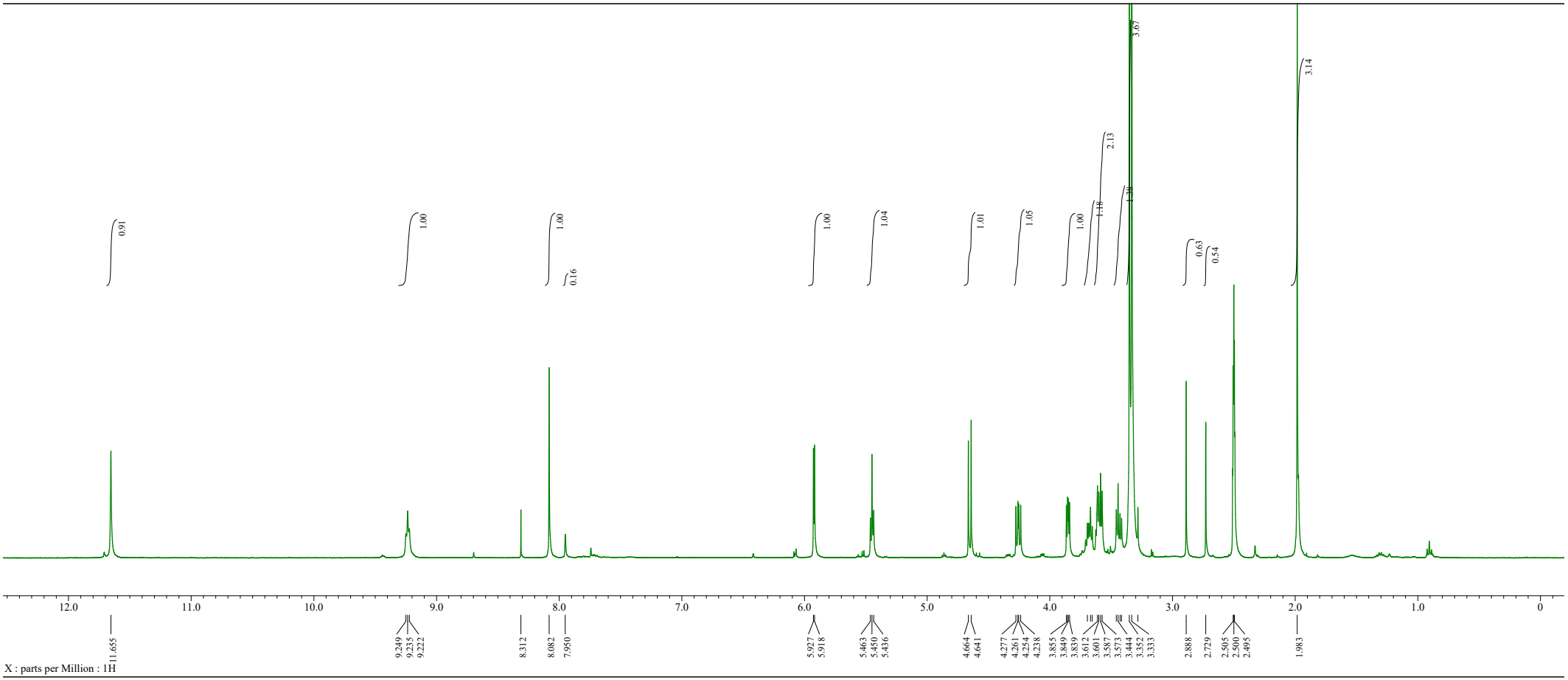
^{1}H NMR spectrum of compound **11**



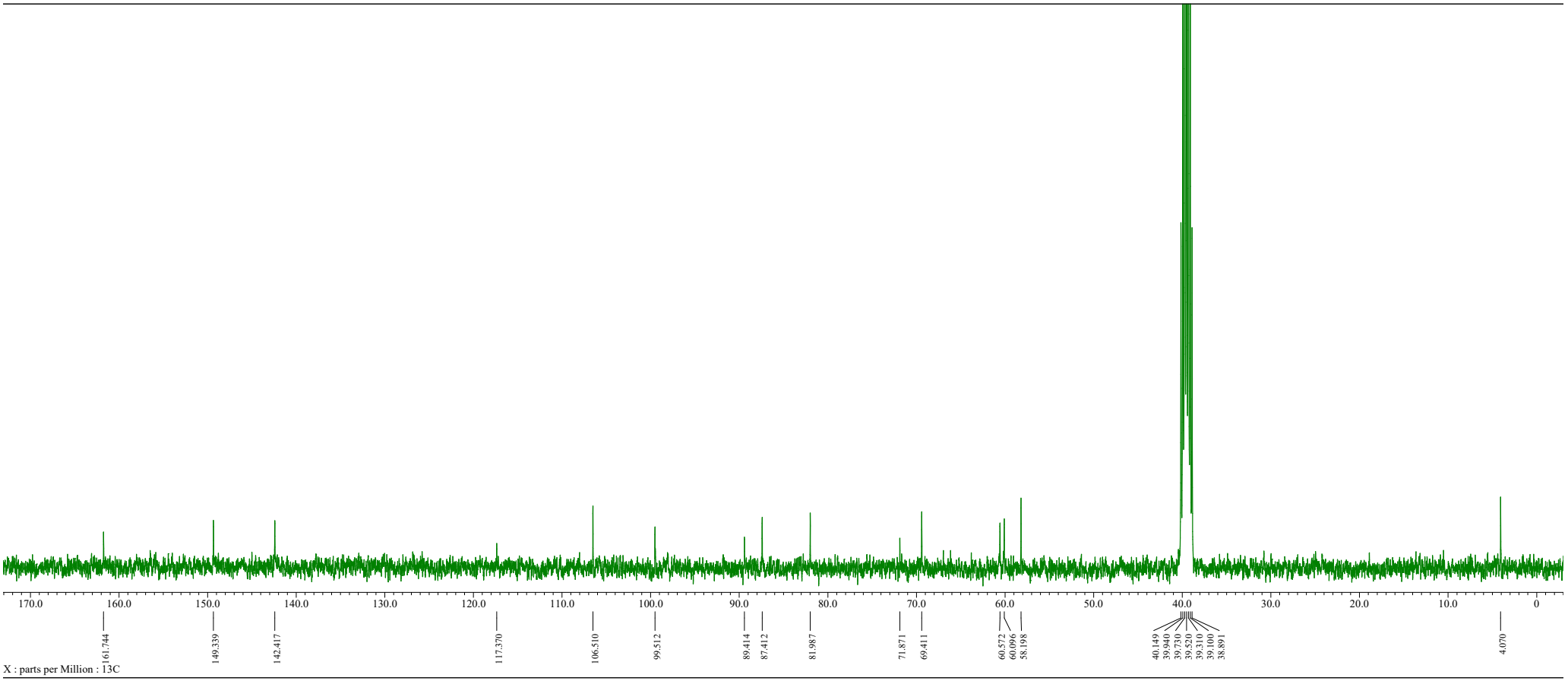
^{13}C NMR spectrum of compound **11**



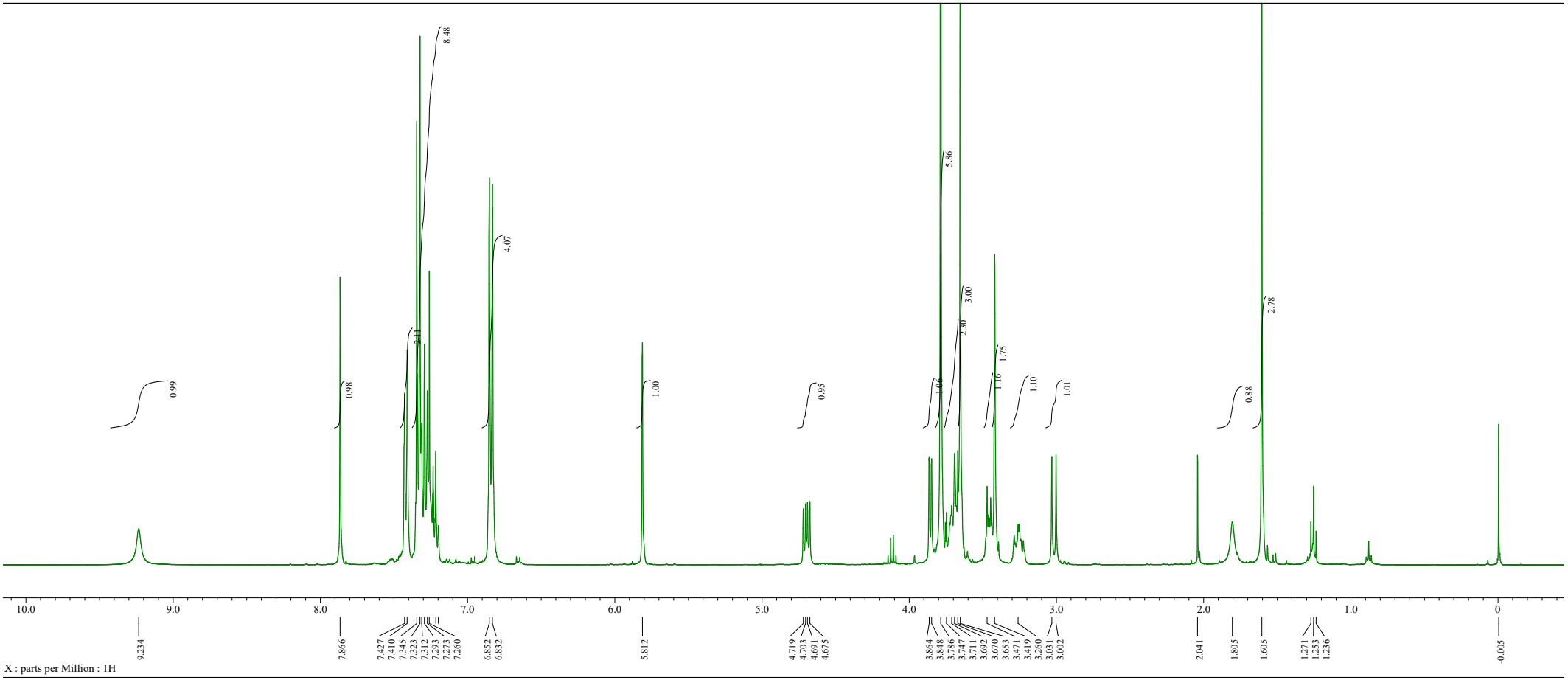
^{1}H NMR spectrum of compound **13**



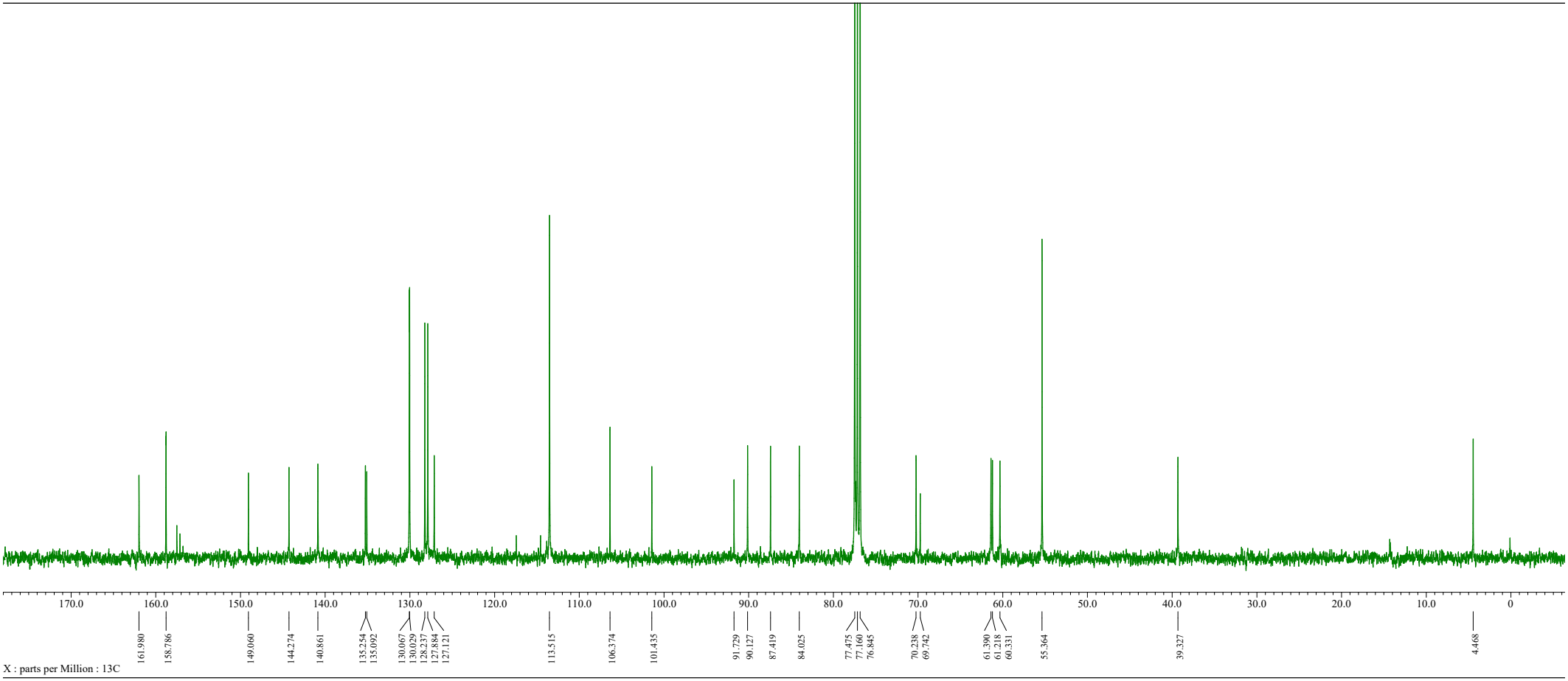
^{13}C NMR spectrum of compound **13**



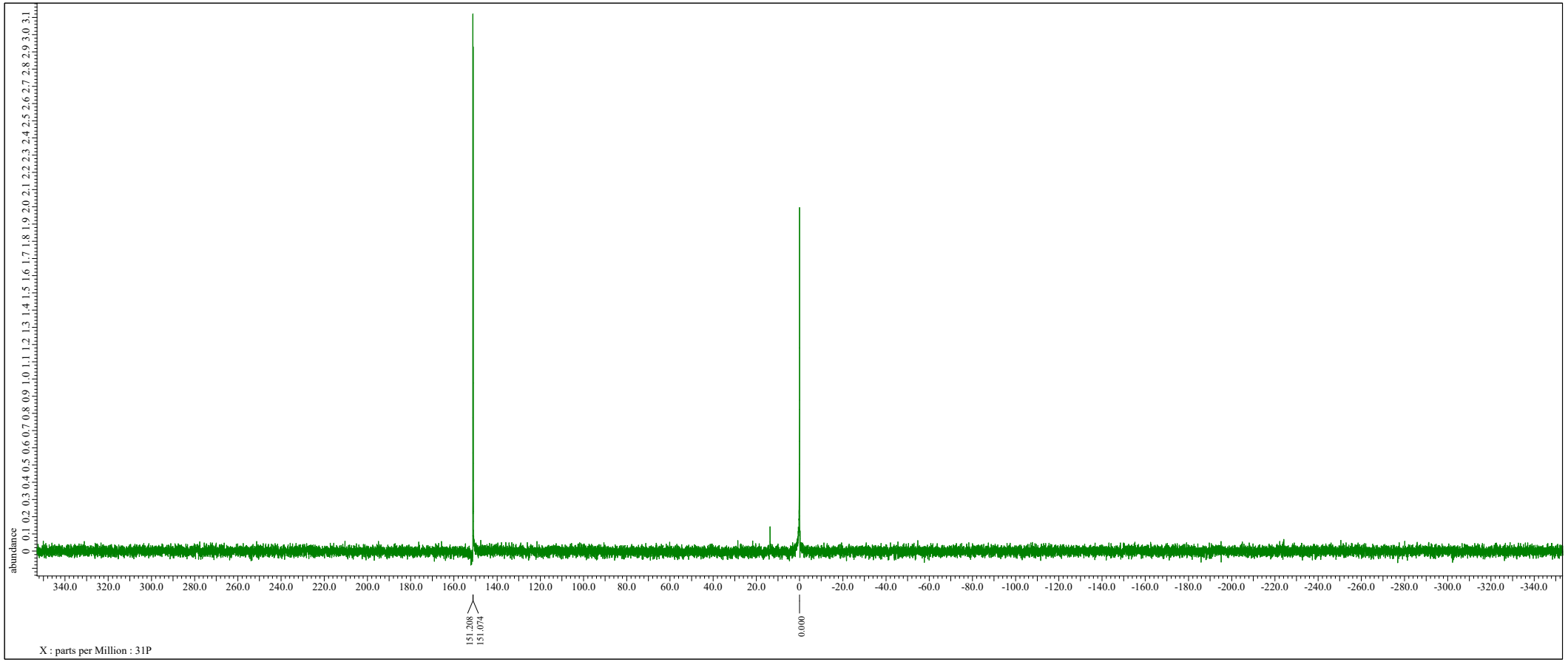
^{1}H NMR spectrum of compound **14**



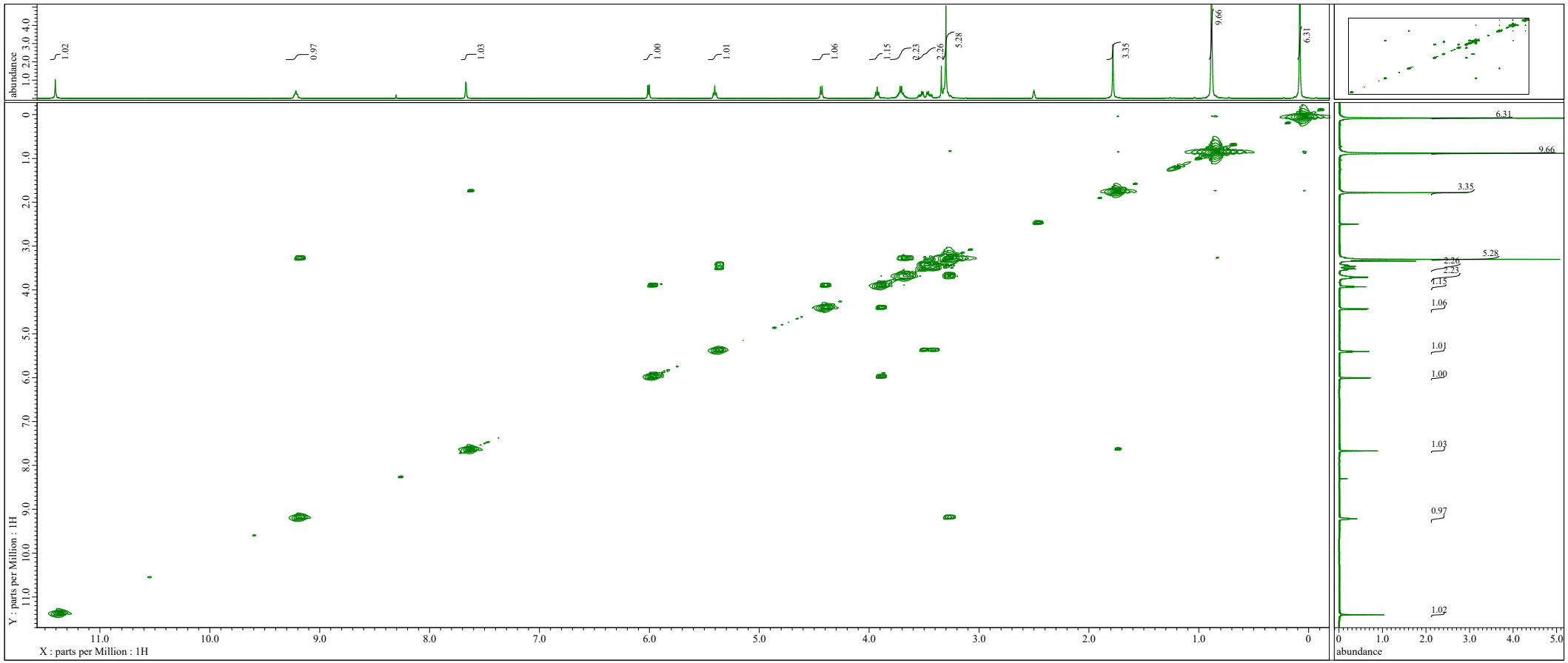
^{13}C NMR spectrum of compound **14**



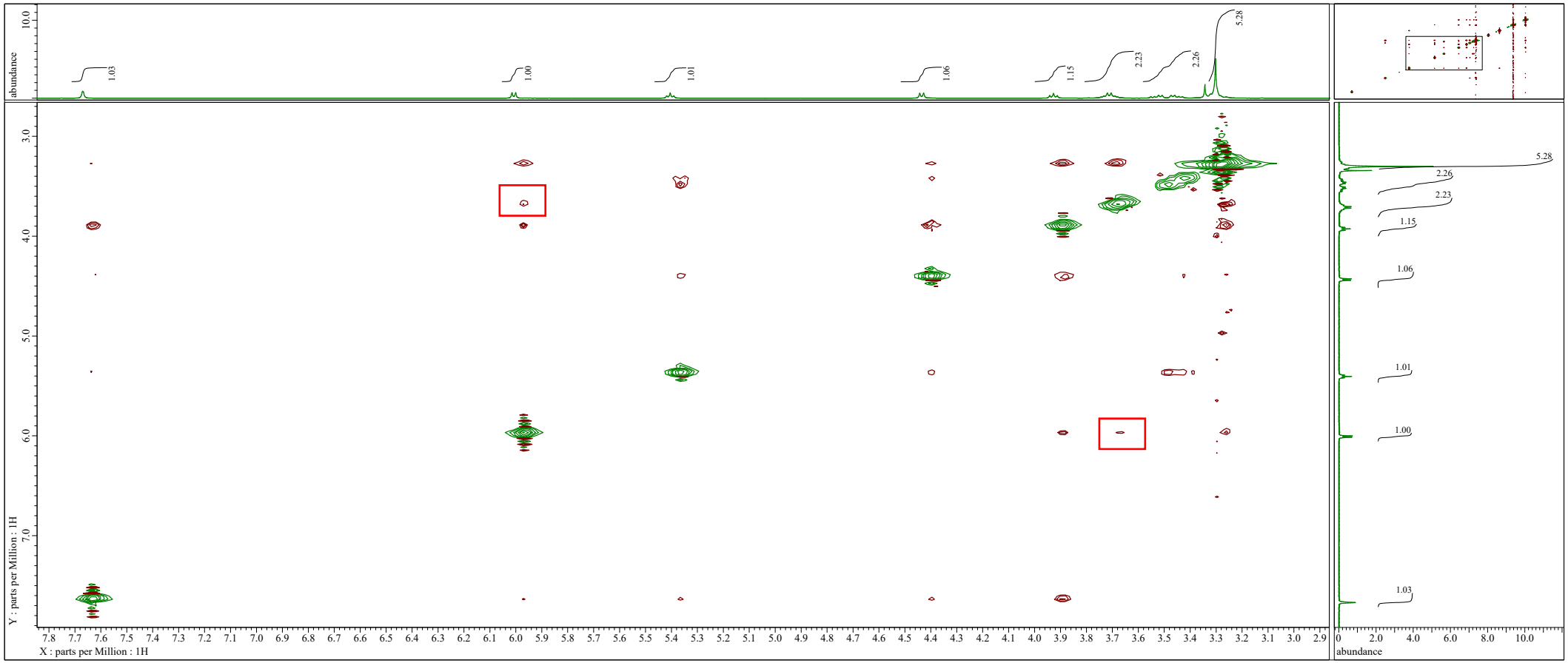
^{31}P NMR spectrum of compound **15**



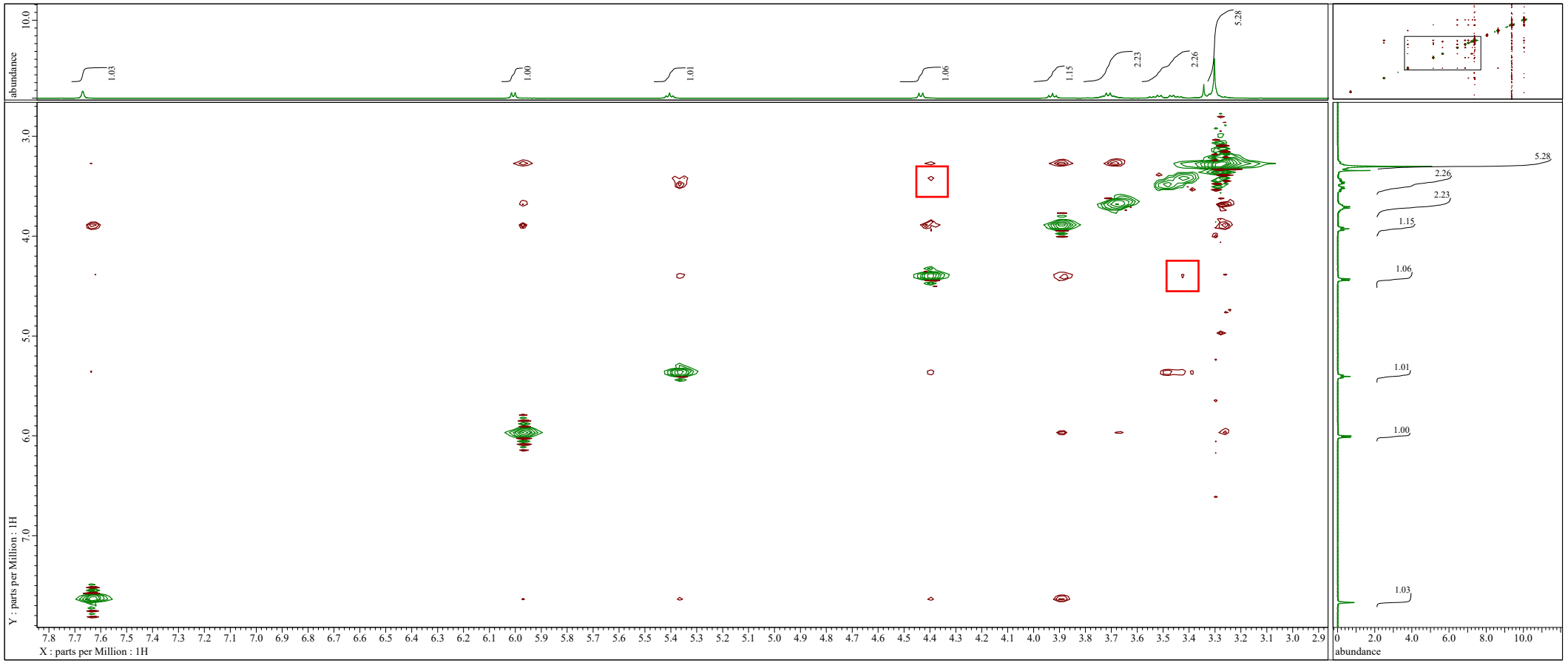
^{1}H-^{1}H COSY spectrum of compound **6**



^{1}H-^{1}H NOESY spectrum of compound **6** (pick-up of H1′-H6′ correlation)



^{1}H-^{1}H NOESY spectrum of compound **6** (pick-up of H3′-H5′ correlation)



^{1}H-^{1}H NOESY spectrum of compound **6** (pick-up of H3′-OH5′ correlation)