Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Synthesis of 8-aza-3,7-dideaza-2'-deoxyadenosines possessing a new adenosine skeleton as an environmentally sensitive fluorescent nucleoside for monitoring DNA minor groove

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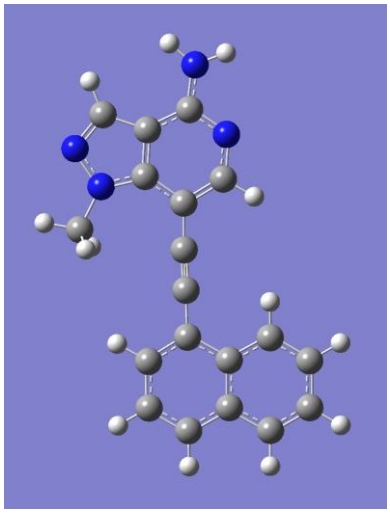
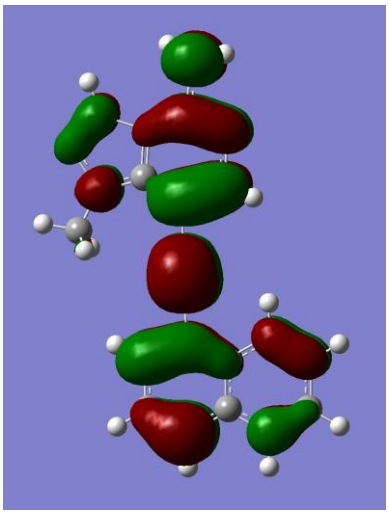
N^9-methylated **^{3n7z}A** (ground state)



N^9-methylated **^{3n7z}A** (HOMO)



N^9-methylated **^{3n7z}A** (LUMO)

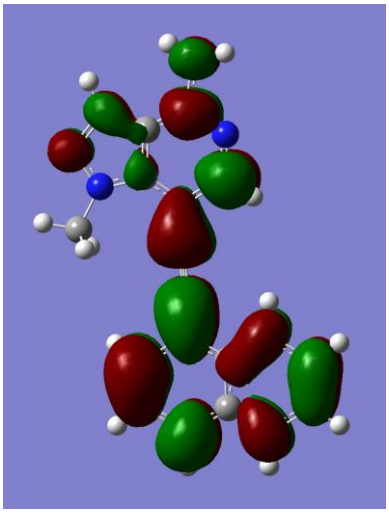


Figure S1. Optimized structure of N^9-methylated **^{3n7z}A** calculated at the DFT(B3LYP)/6-31G* level.

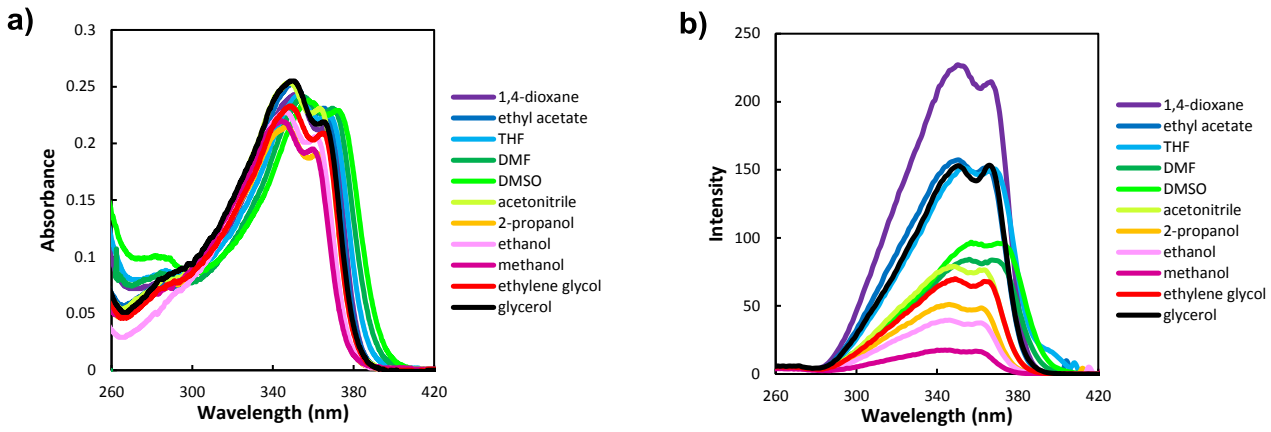


Figure S2. (a) Absorption and (b) excitation spectra of ^{3n7z}**A** (**2**) in various solvents. All measurements were performed at a concentration of 10 μM.

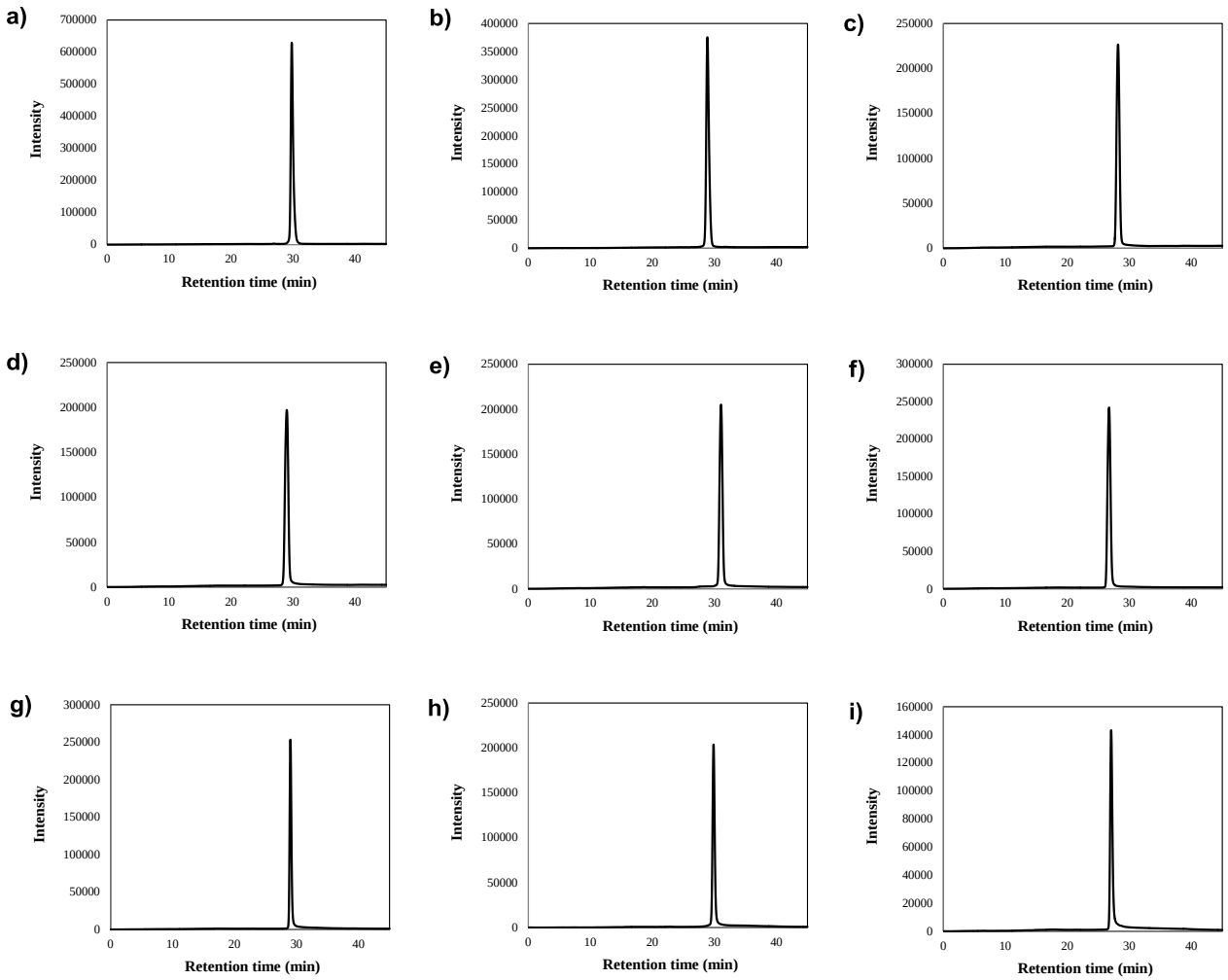


Figure S3. HPLC profiles determinded at 260 nm of single-stranded oligonucleotides. (a) ODN1(^{3n7z}**A**), (b) ODN2(^{3n7z}**A**), (c) ODN3(^{3n7z}**A**), (d) ODN4(^{3n7z}**A**), (e) ODN5(^{3n7z}**A**), (f) ODN6(^{3n7z}**A**), (g) Probe$_{(JAK2)}$, (h) Probe$_{(MTHFR)}$, and (i) Probe$_{(DRD2)}$. HPLC analysis was performed on a CHEMCOBOND 5-ODS-H columm (10 × 150 nm) eluted with 50 mM ammonium formate buffer containing acetonitrile. Gradient: from 3 to 20 % acetonitrile at a flow rate 2.0 ml/min over 45 min.

Table 1. MALDI-TOF mass spectral data for the ODNs

ODNs	Sequences	MALDI-TOF mass	
		calcd. $[M + H]^+$	found $[M + H]^+$
ODN1	5'-d(CGCAAT$^{\mathbf{3n7z}}$**A**TAACGC)-3'	4077.85	4078.10
ODN2	5'-d(CGCAAC$^{\mathbf{3n7z}}$**A**CAACGC)-3'	4047.83	4048.14
ODN3	5'-d(CGCAAT$^{\mathbf{3n7z}}$**A**CAACGC)-3'	4062.84	4062.09
ODN4	5'-d(CGCAAC$^{\mathbf{3n7z}}$**A**TAACGC)-3'	4062.84	4063.77
ODN5	5'-d(CGCACT$^{\mathbf{3n7z}}$**A**TCACGC)-3'	4029.80	4029.21
ODN6	5'-d(CGCAGT$^{\mathbf{3n7z}}$**A**TGACGC)-3'	4109.85	4109.83
$Probe_{(JAK2)}$	5'-d(CCACAGA$^{\mathbf{3n7z}}$**A**ACATACT)-3'	4664.25	4664.62
$Probe_{(MTHFR)}$	5'-d(GAAATCG$^{\mathbf{3n7z}}$**A**CTCCCGC)-3'	4672.22	4672.20
$Probe_{(DRD2)}$	5'-d(GGCTGTC$^{\mathbf{3n7z}}$**A**GGAGTGC)-3'	4799.28	4799.34

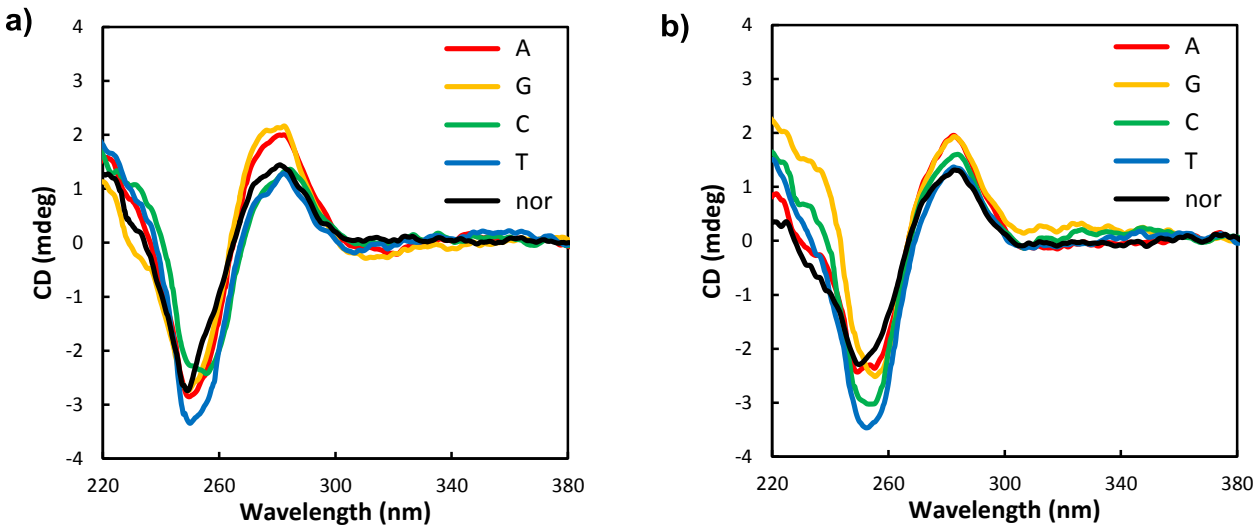


Figure S4. CD spectra of (a) 2.5 μM ODN1(**^{3n7z}A**) hybridized with complementary strand 2.5 μM cODN1(**N**) and (b) 2.5 μM ODN2(**^{3n7z}A**) hybridized with complementary strand 2.5 μM cODN2(**N**) (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt). "nor" denotes the corresponding unmodified duplex: a) ODN1(A)/cODN1(T), b) ODN2(A)/cODN2(T).

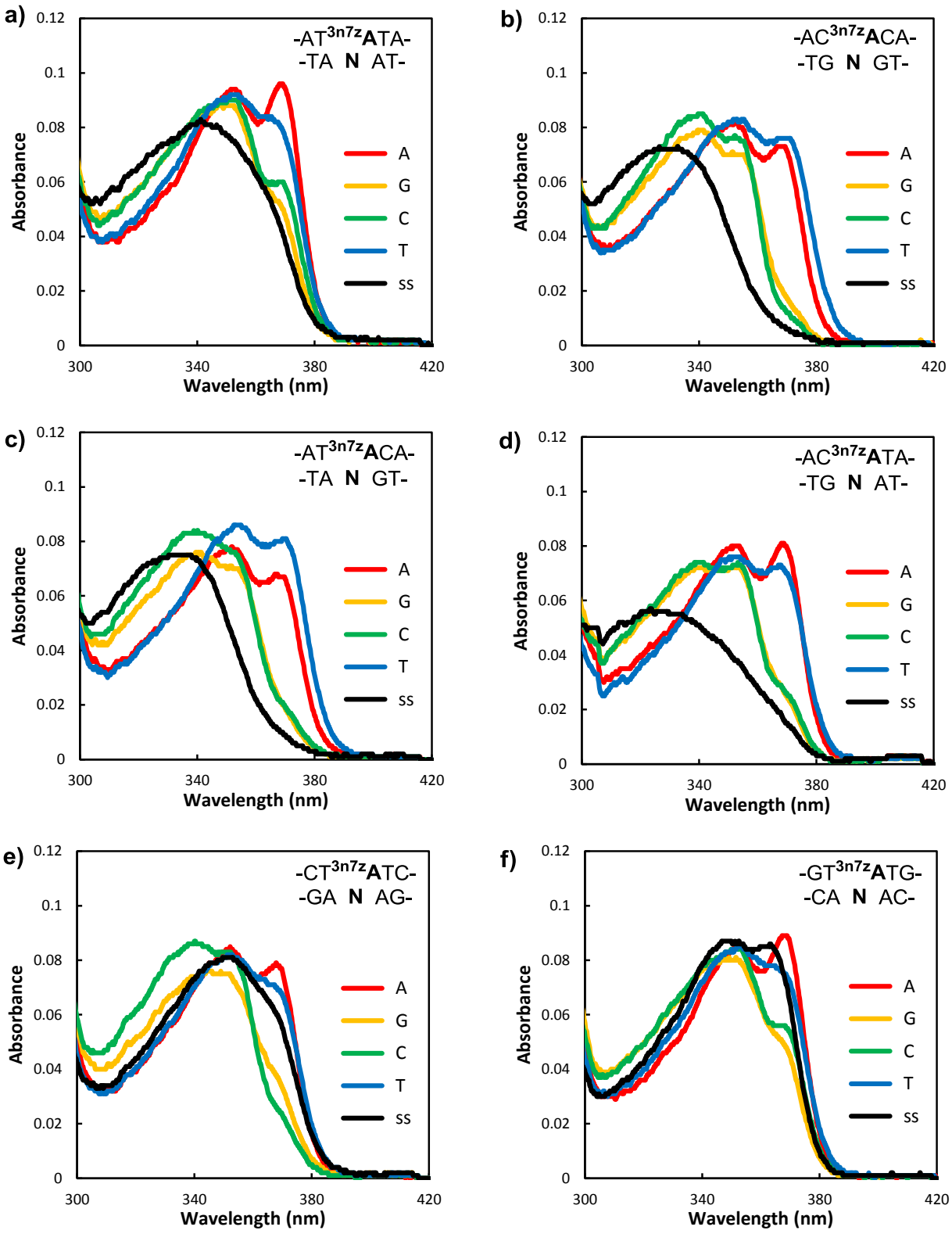


Figure S5. Absorption spectra of 2.5 μM (a) ODN1(^{3n7z}**A**), (b) ODN2(^{3n7z}**A**), (c) ODN3(^{3n7z}**A**), (d) ODN4(^{3n7z}**A**), (e) ODN5(^{3n7z}**A**), and (f) ODN6(^{3n7z}**A**) hybridized with their complementary cODN1(**N**) - cODN6(**N**)(50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt). "ss" denotes single-stranded ODN(^{3n7z}**A**). ODN and cODN concentrations both amounted to 2.5 μM.

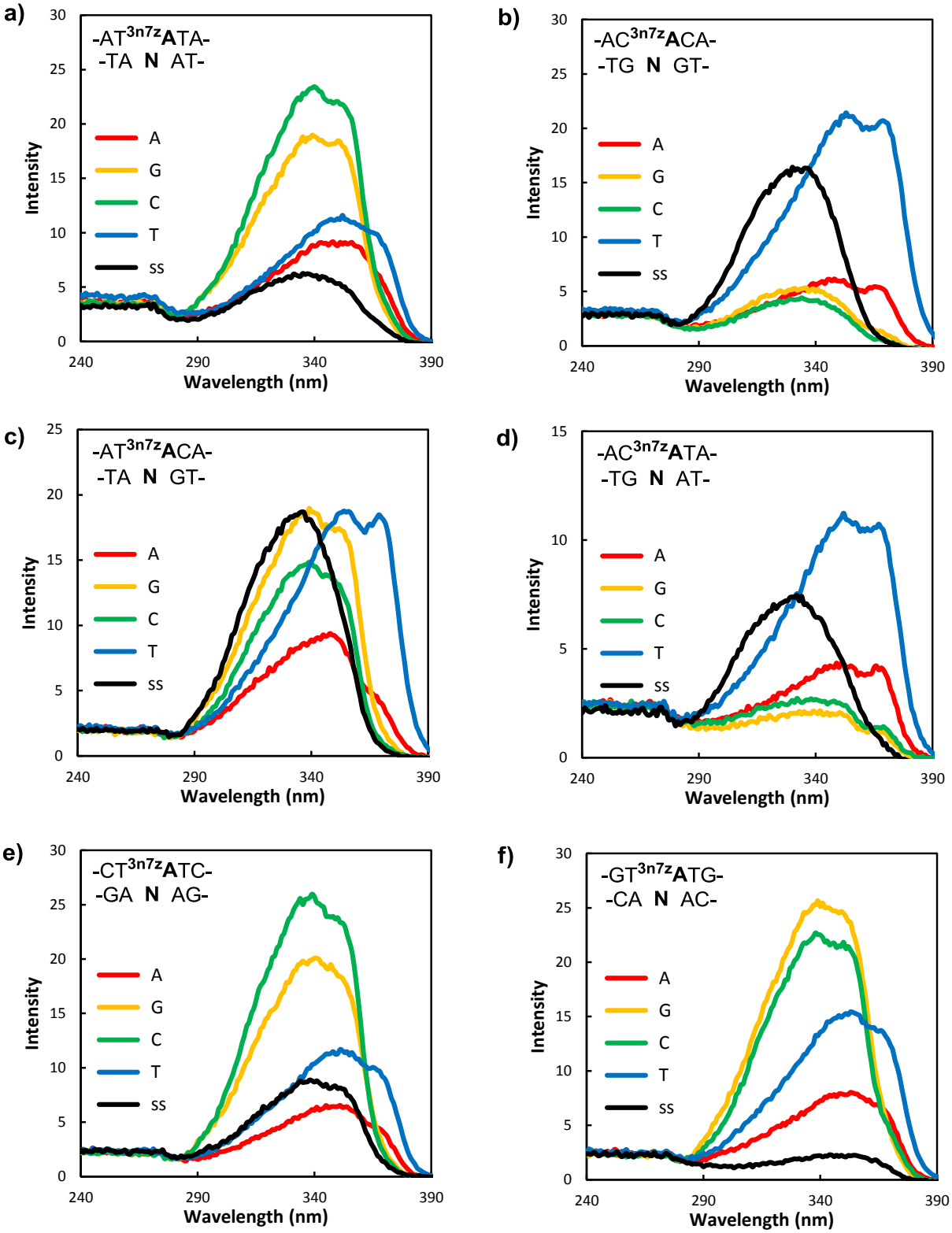


Figure S6. Excitation spectra of 2.5 μM (a) ODN1(^{3n7z}**A**), (b) ODN2(^{3n7z}**A**), (c) ODN3(^{3n7z}**A**), (d) ODN4(^{3n7z}**A**), (e) ODN5(^{3n7z}**A**), and (f) ODN6(^{3n7z}**A**) hybridized with their complementary cODN1(**N**) - cODN6(**N**)(50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt). "ss" denotes single-stranded ODN (^{3n7z}**A**). ODN and cODN concentrations both amounted to 2.5 μM.

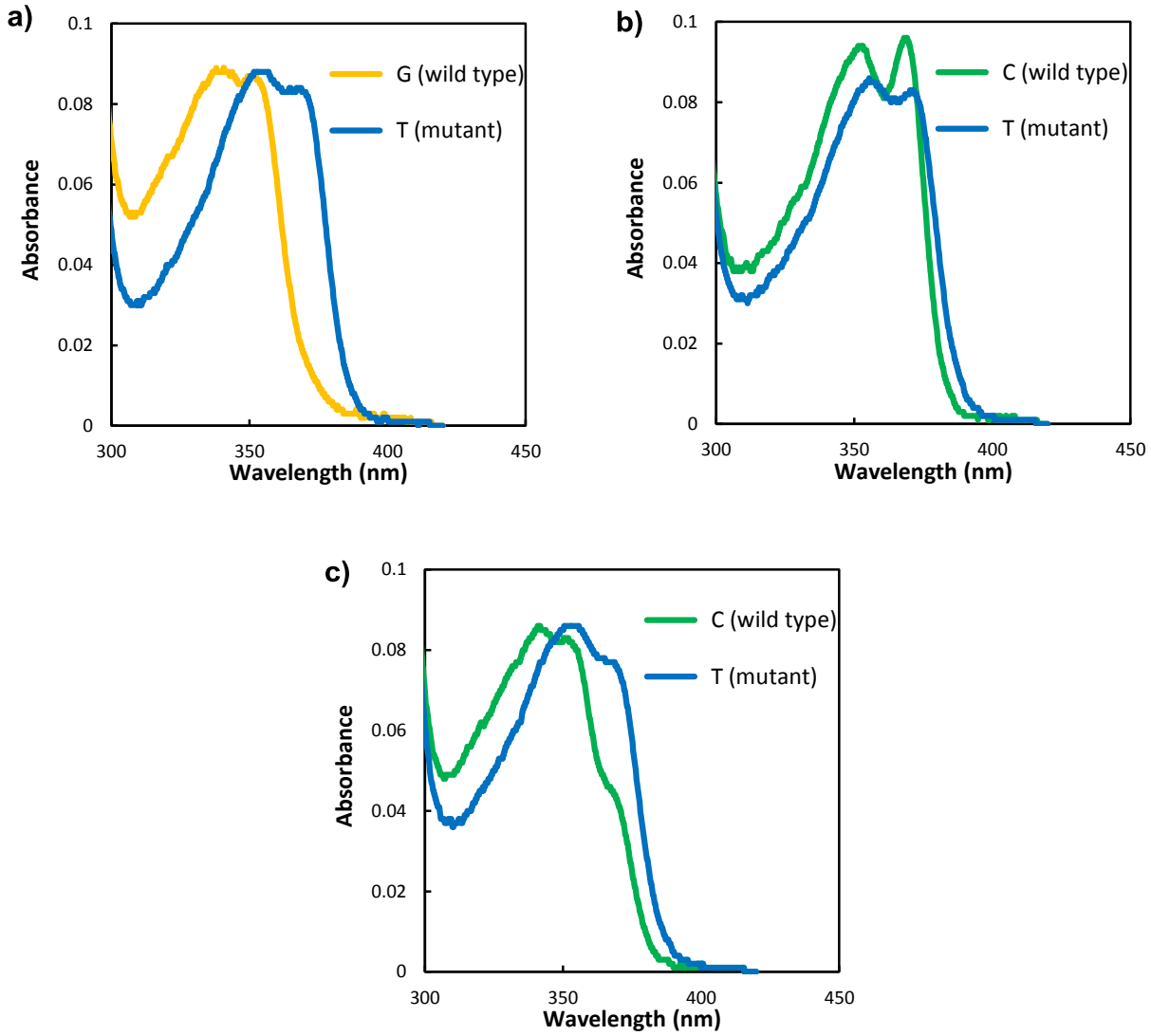


Figure S7. Absorption spectra of (a) **Probe$_{(JAK2)}$** (2.5 μM) hybridized with complementary strand **JAK2(G)** or **JAK2(T)** (2.5 μM), (b) **Probe$_{(MTHFR)}$** (2.5 μM) hybridized with **MTHFR(C)** or **MTHFR(T)** (2.5 μM), and (c) **Probe$_{(DRD2)}$** (2.5 μM) hybridized with **DRD2(C)** or **DRD2(T)** (2.5 μM). The duplexes were measured in 50 mM sodium phosphate, 0.1 M sodium chloride (pH 7.0) at room temparture.

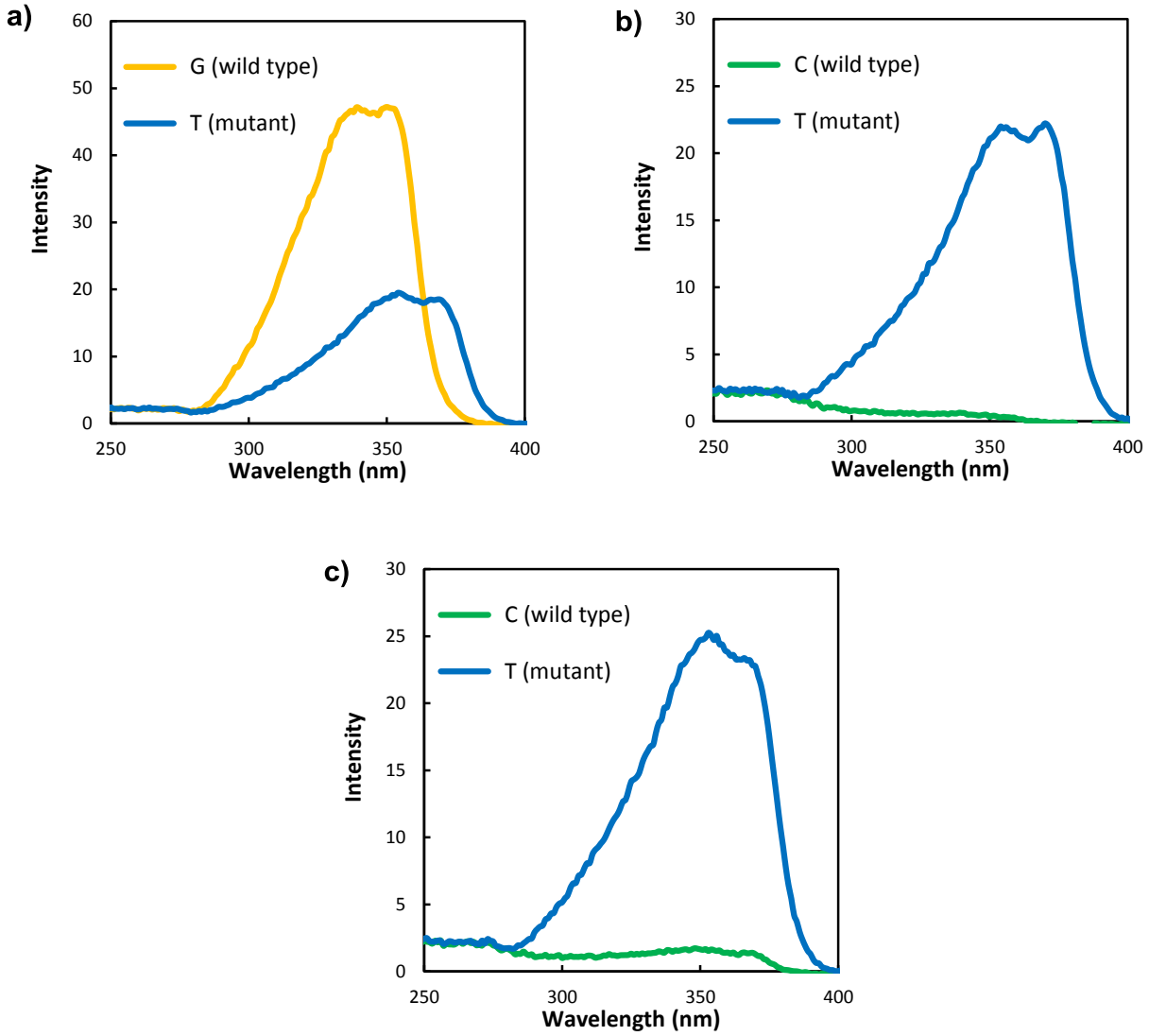


Figure S8. Excitation spectra of (a) **Probe$_{(JAK2)}$** (2.5 μM) hybridized with complementary strand **JAK2(G)** or **JAK2(T)** (2.5 μM), (b) **Probe$_{(MTHFR)}$** (2.5 μM) hybridized with **MTHFR(C)** or **MTHFR(T)** (2.5 μM), and (c) **Probe$_{(DRD2)}$** (2.5 μM) hybridized with **DRD2(C)** or **DRD2(T)** (2.5 μM). The duplexes were measured in 50 mM sodium phosphate, 0.1 M sodium chloride (pH 7.0) at room temparture.

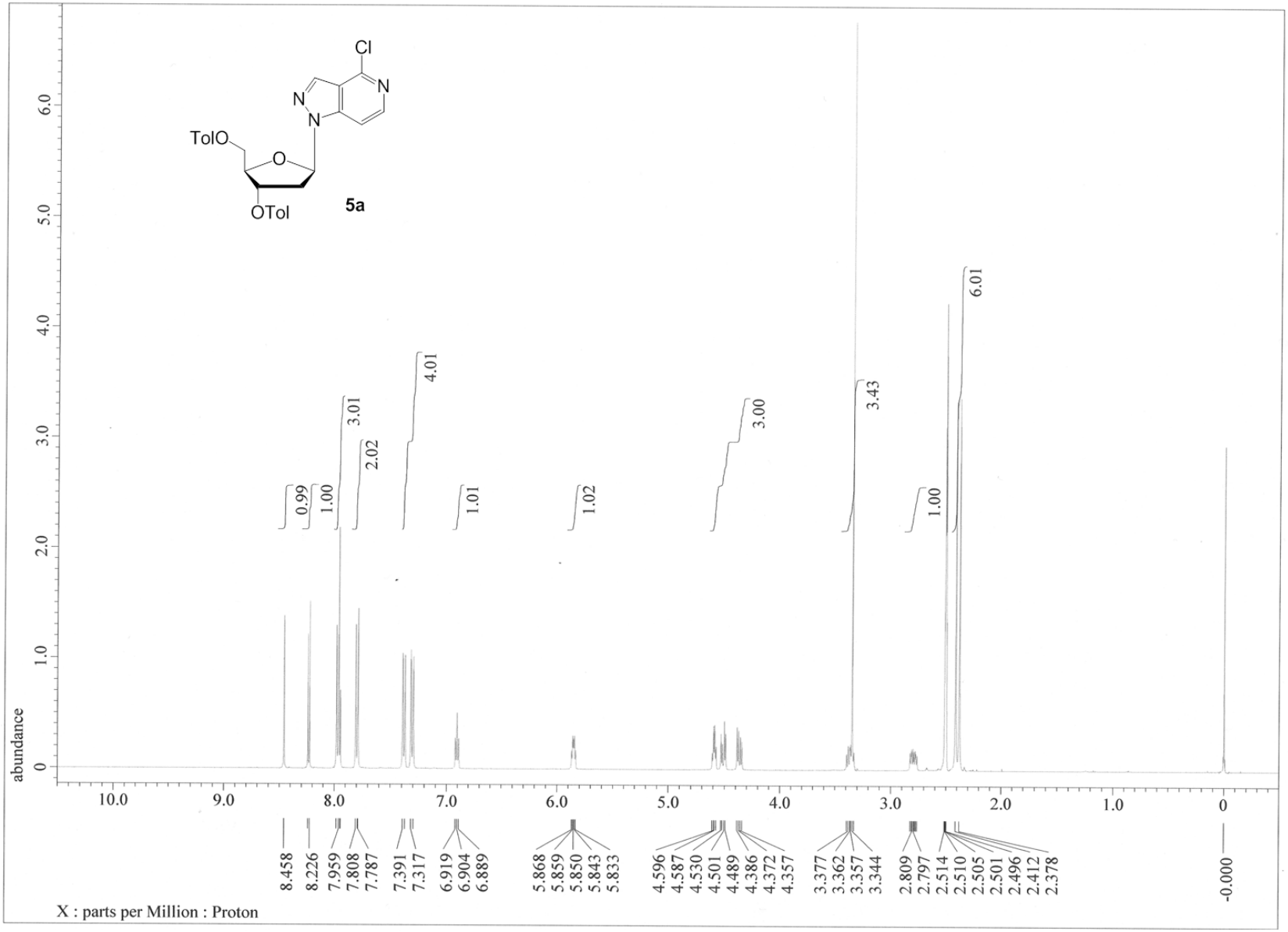


Figure S9. ^{1}H-NMR spectra of compound **5a** (DMSO-d_6).

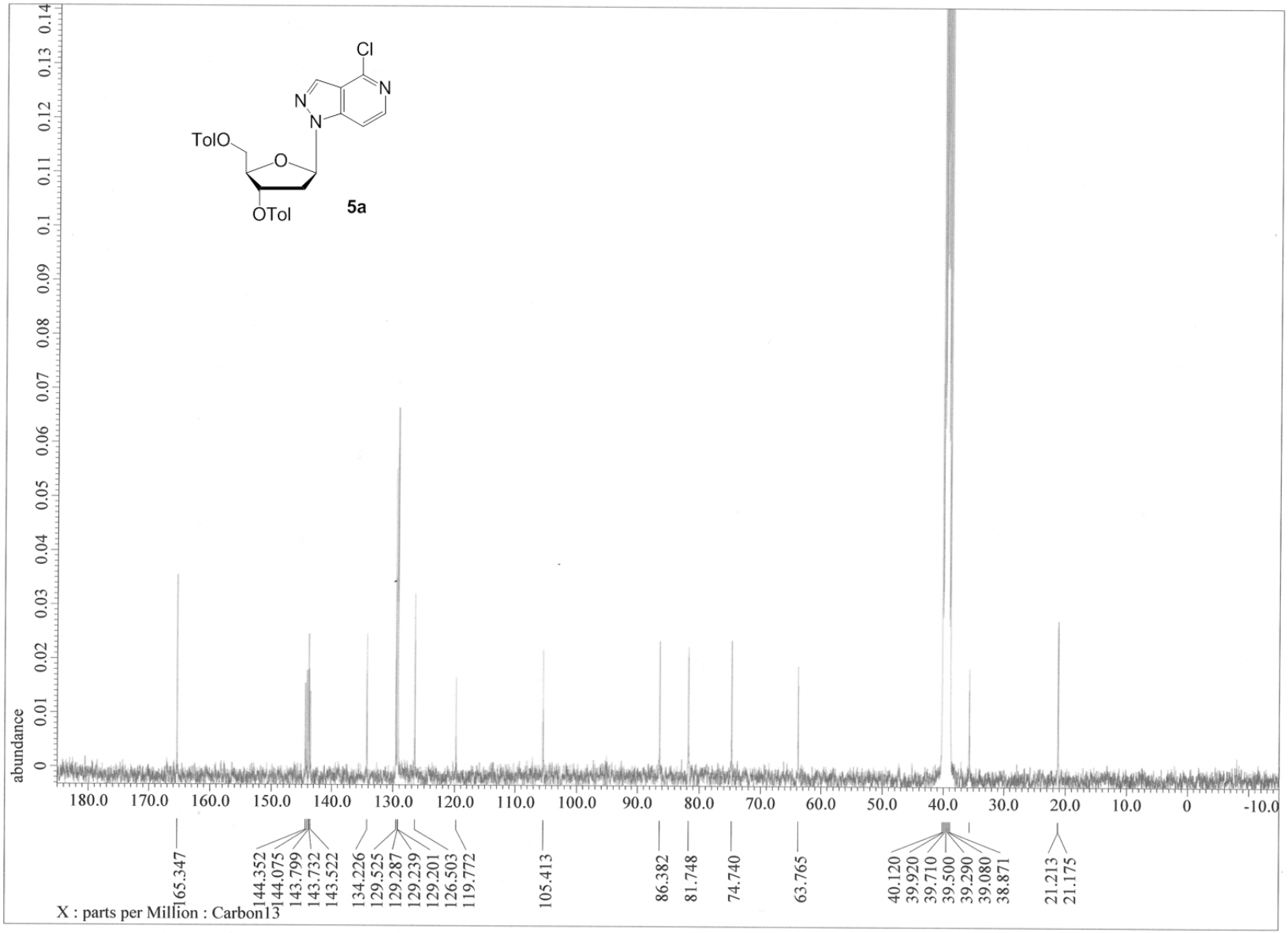


Figure S10. ^{13}C-NMR spectra of compound **5a** (DMSO-d_6).

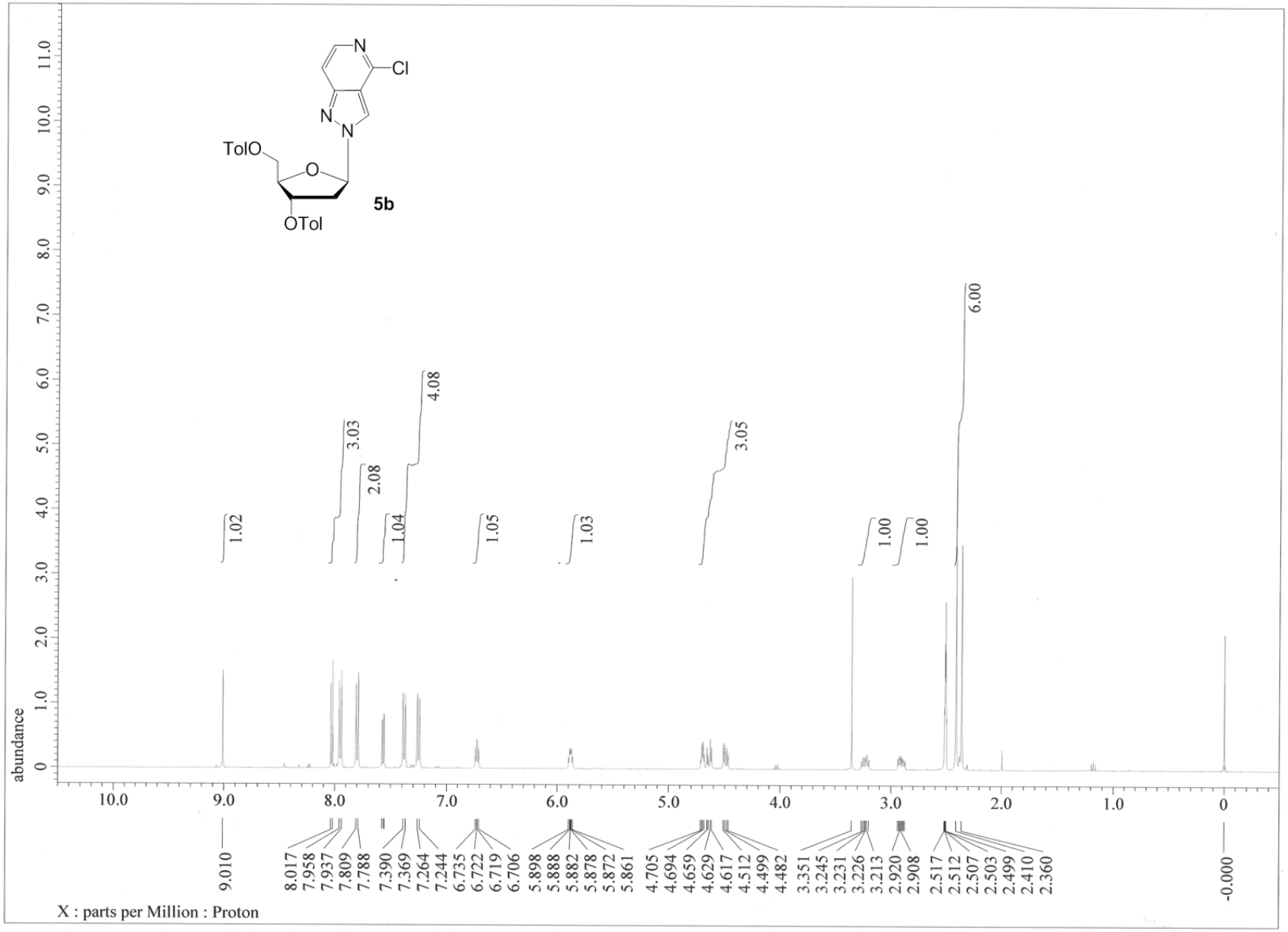


Figure S11. ^{1}H-NMR spectra of compound **5b** (DMSO-d_6).

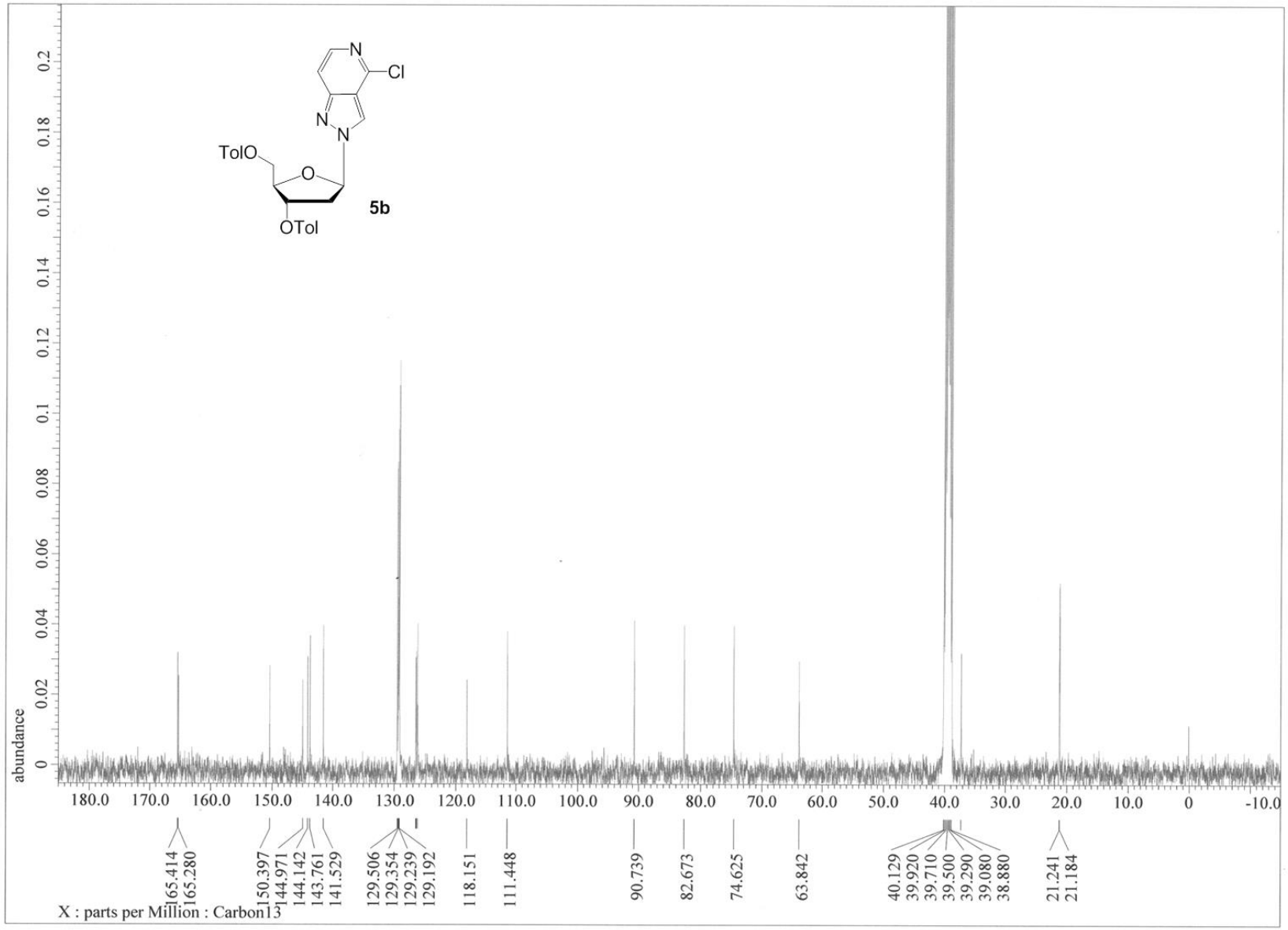


Figure S12. ^{13}C-NMR spectra of compound **5b** (DMSO-d_6).

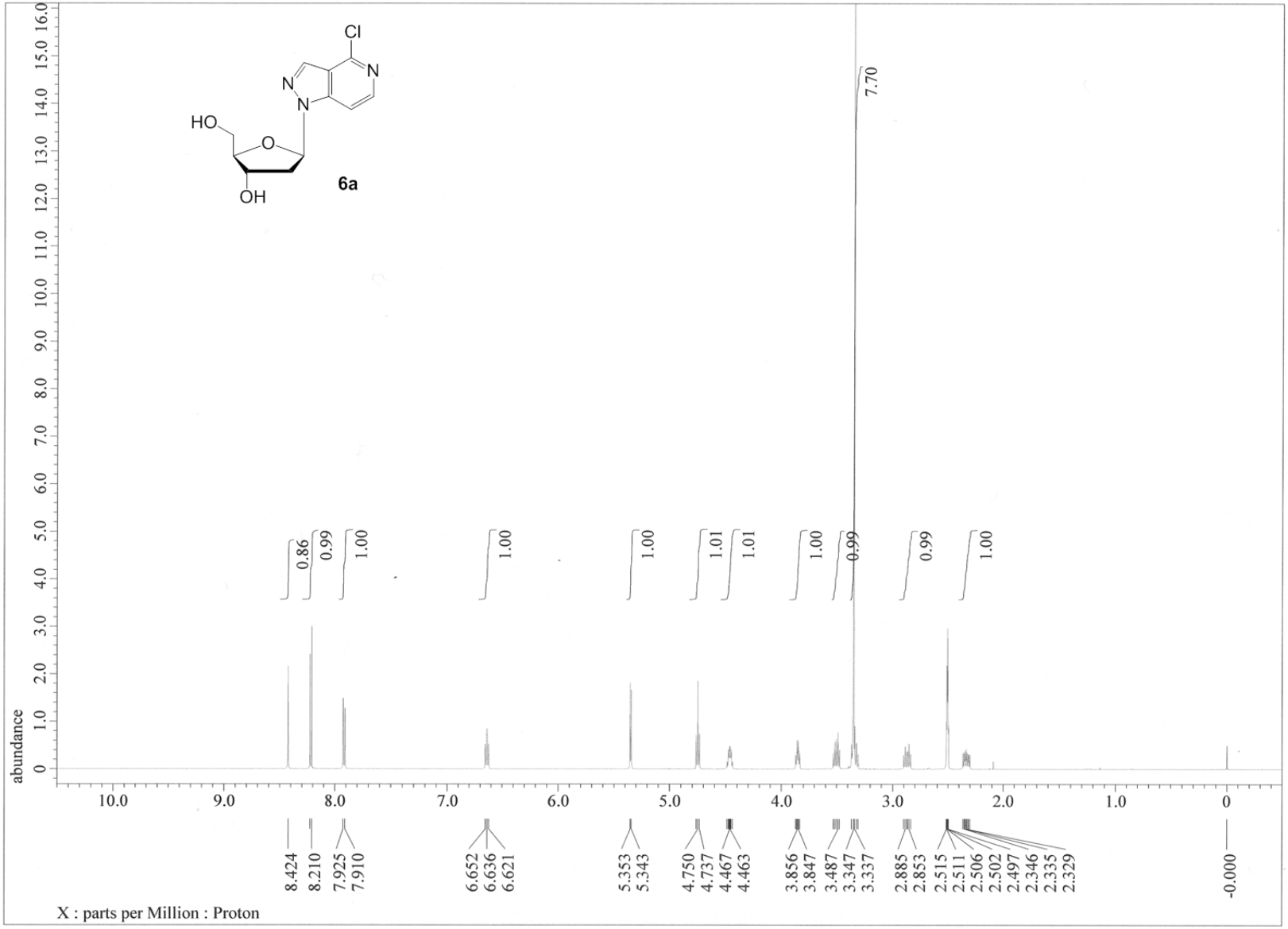


Figure S13. ^{1}H-NMR spectra of compound **6a** (DMSO-d_6).

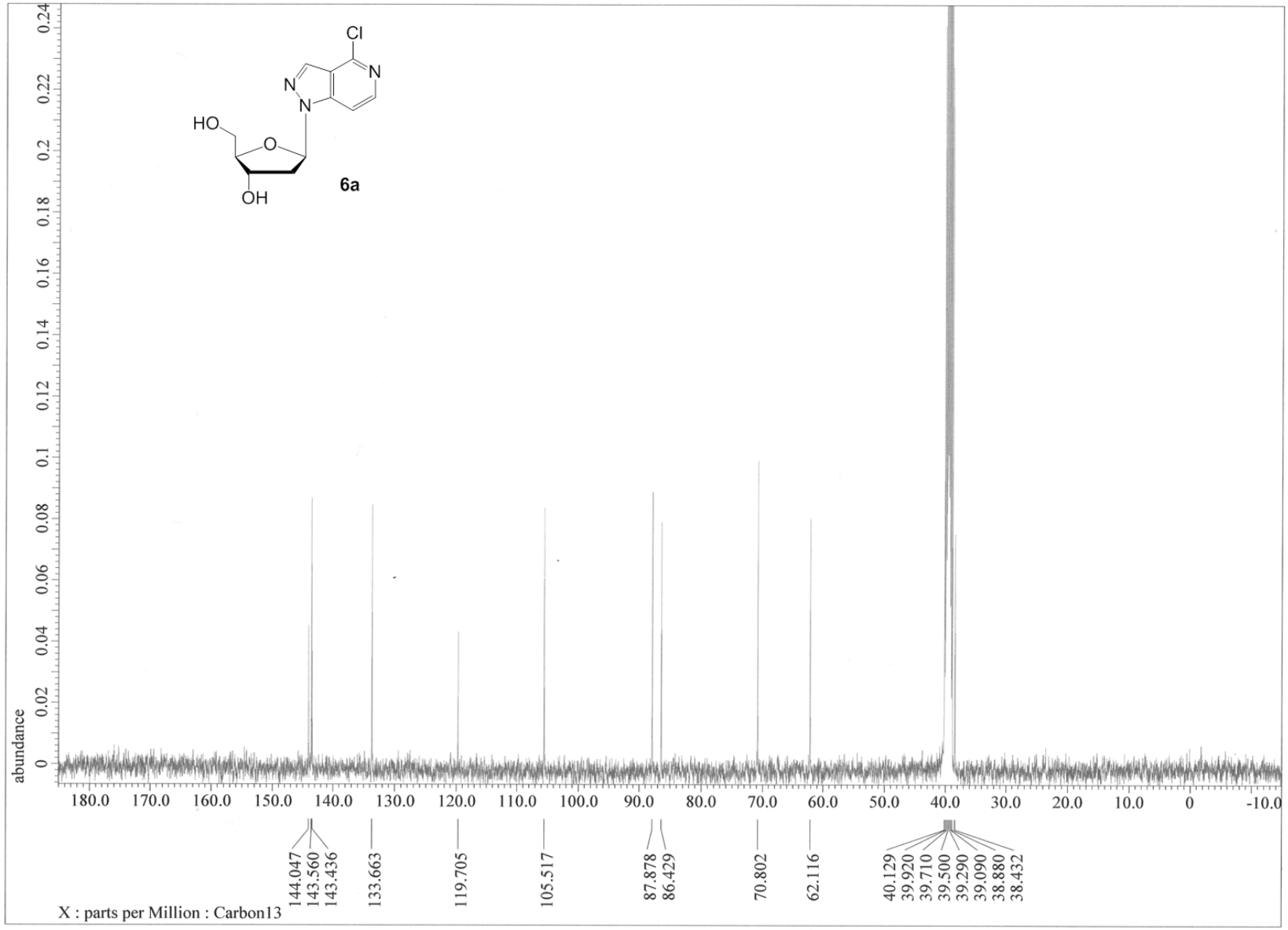


Figure S14. ^{13}C-NMR spectra of compound **6a** (DMSO-d_6).

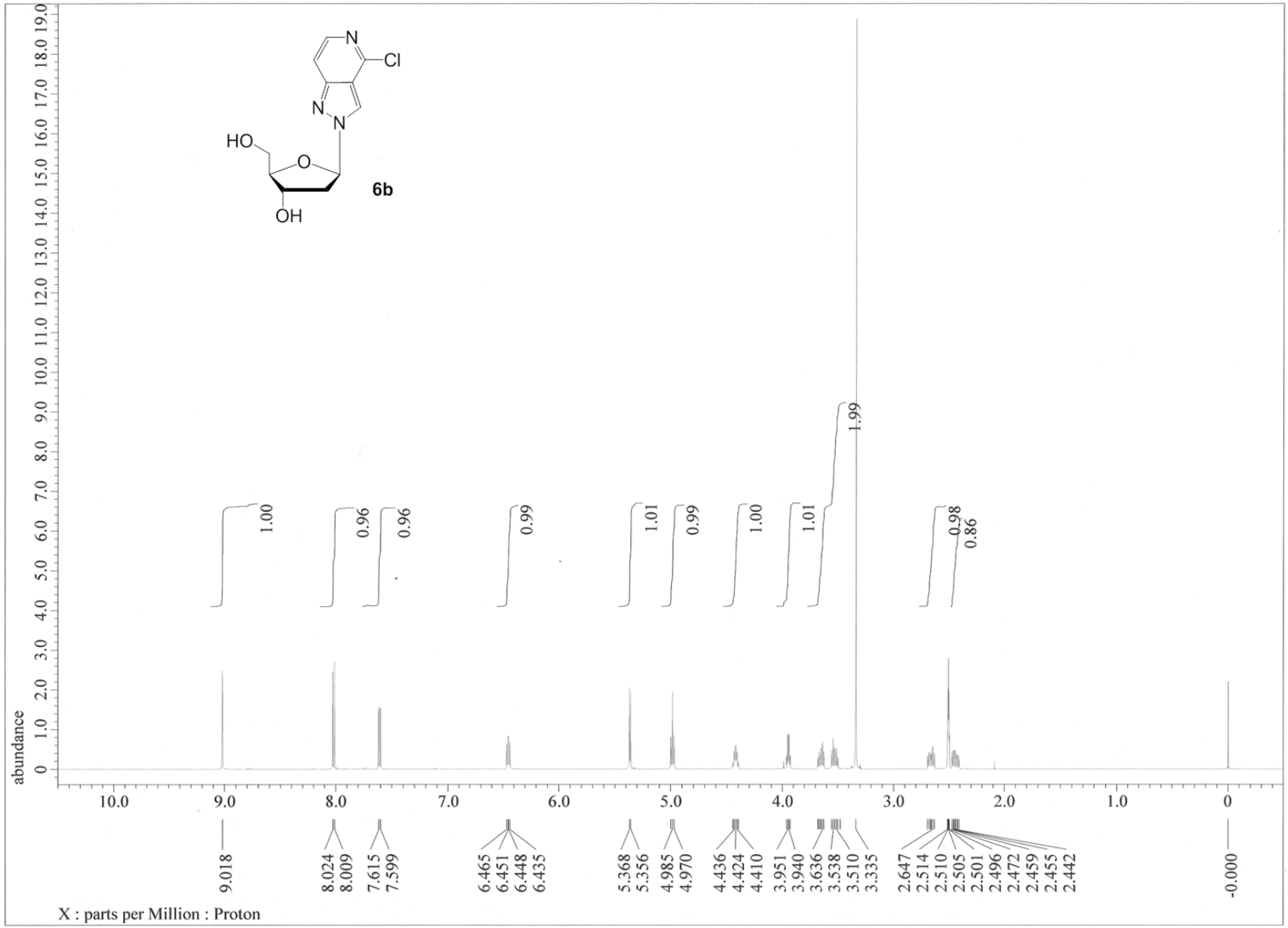


Figure S15. ^{1}H-NMR spectra of compound **6b** (DMSO-d_6).

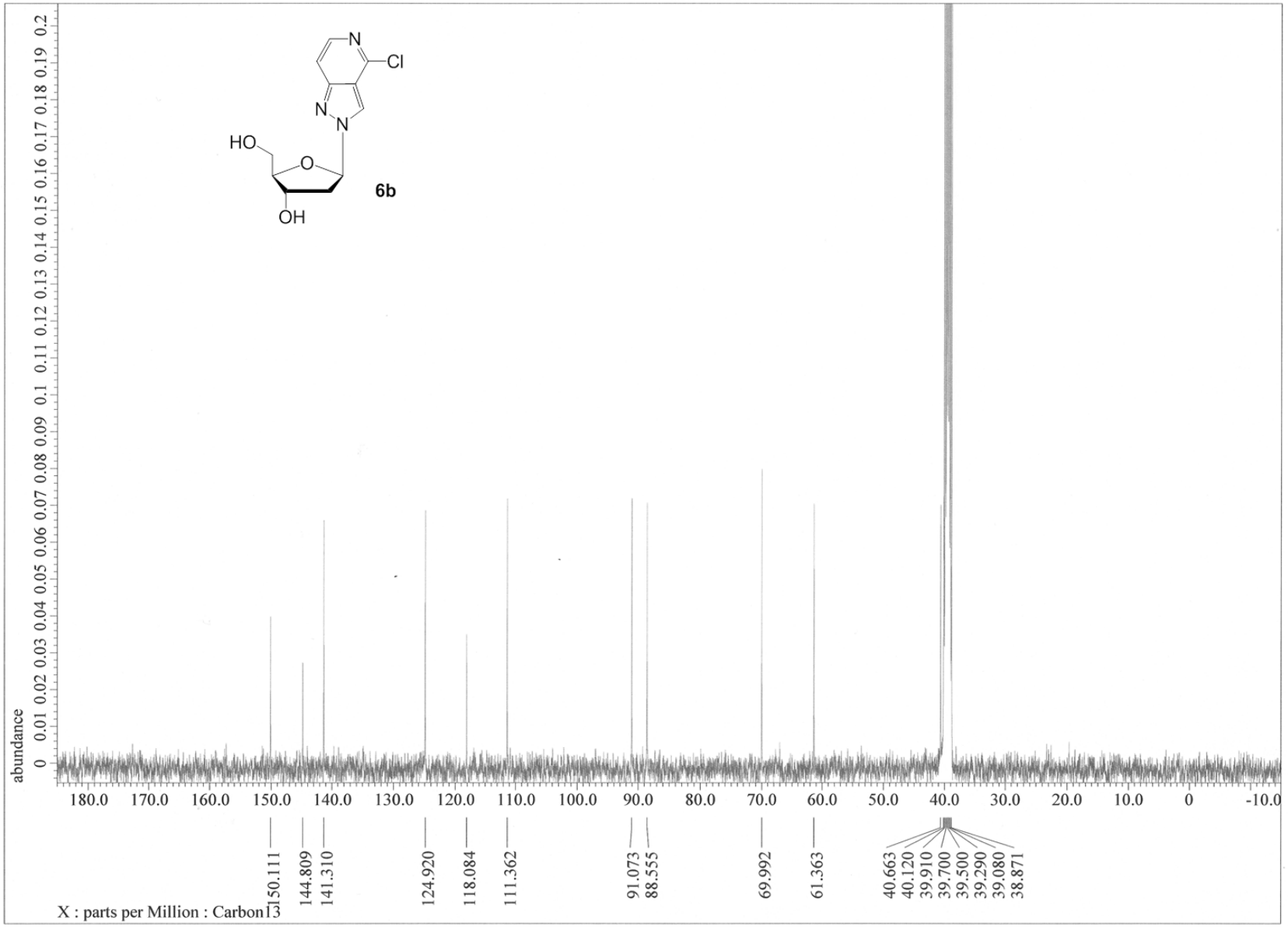


Figure S16. ^{13}C-NMR spectra of compound **6b** (DMSO-d_6).

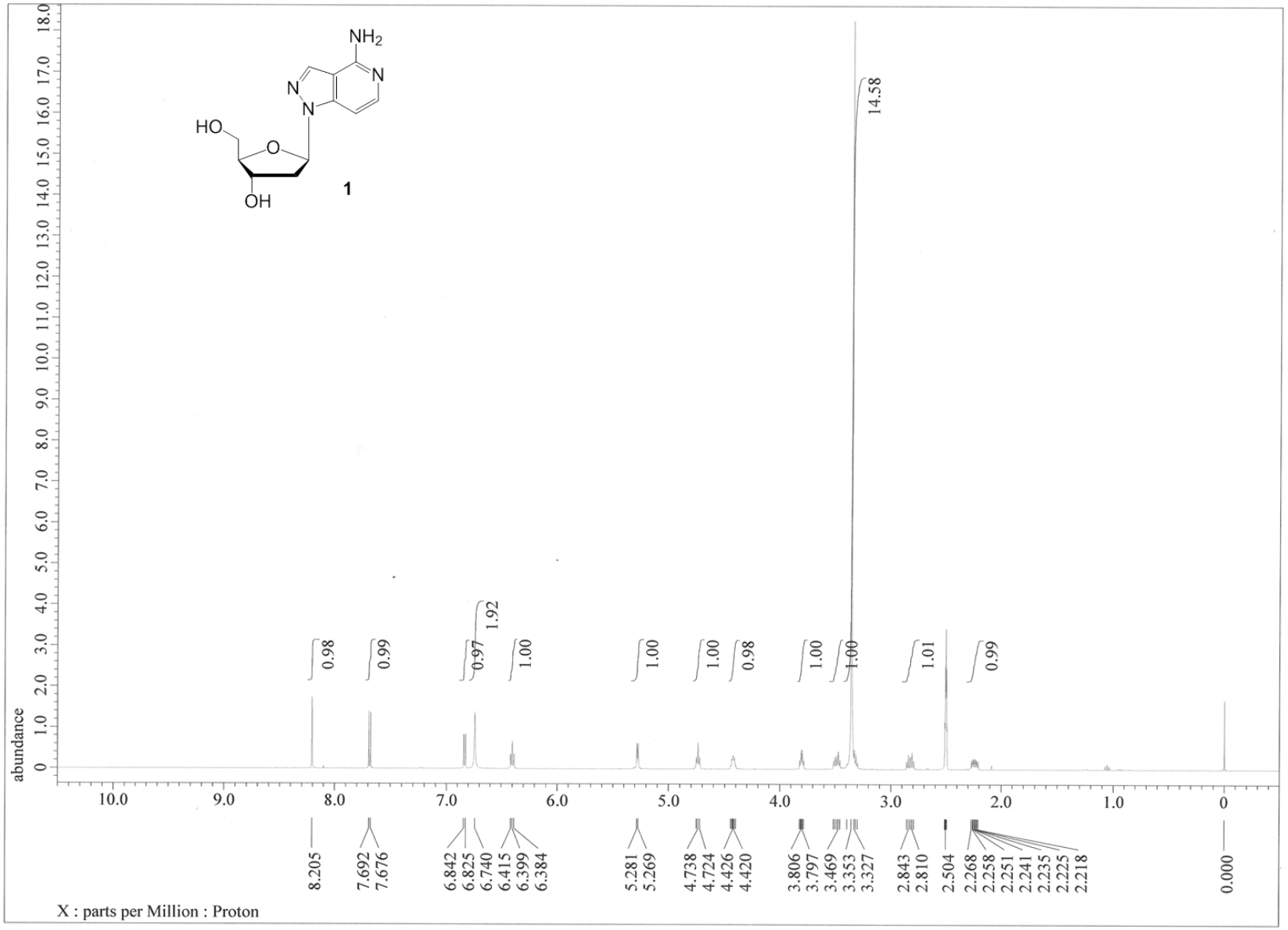


Figure S17. ^{1}H-NMR spectra of compound **1** (DMSO-d_6).

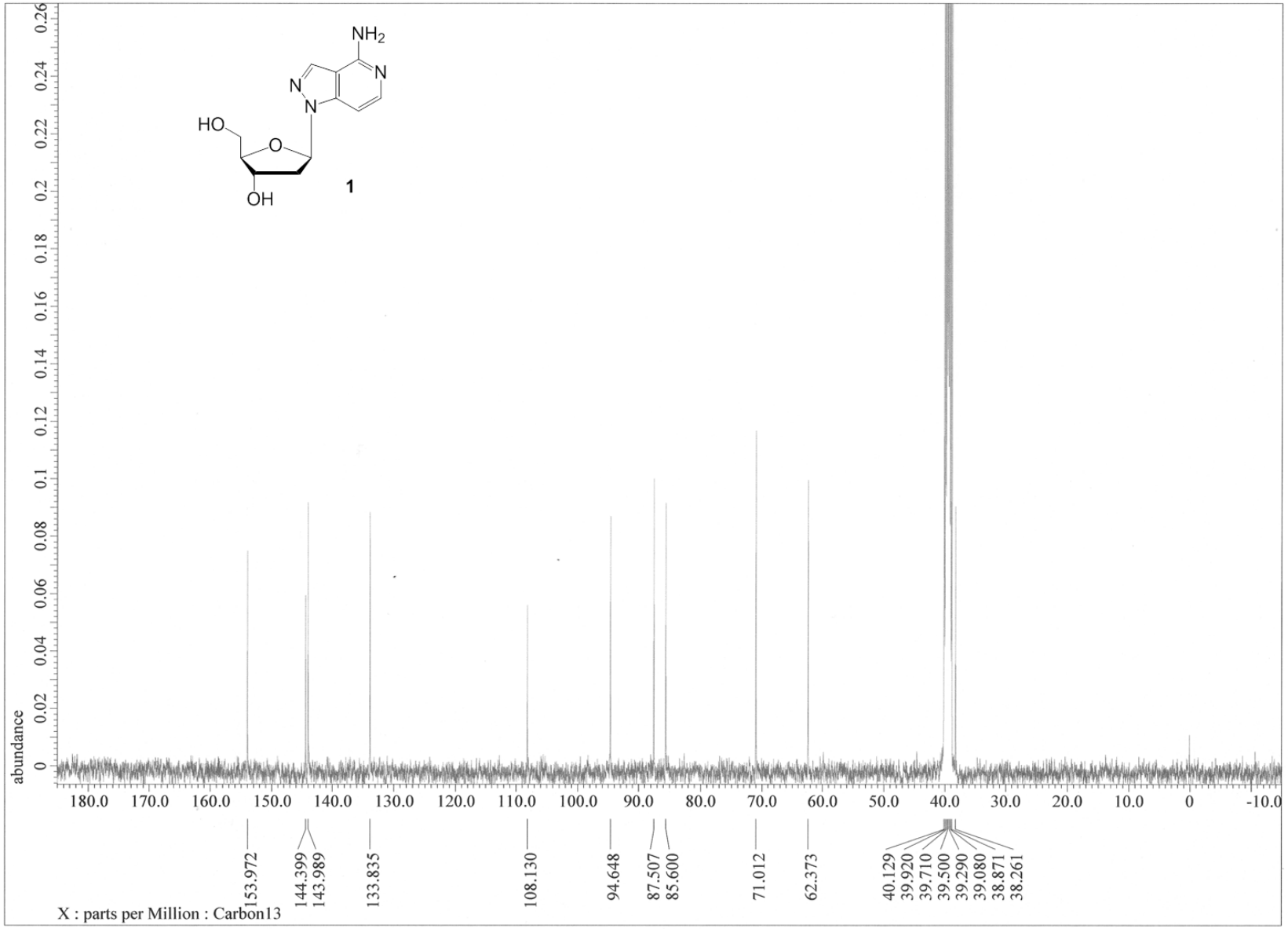


Figure S18. ^{13}C-NMR spectra of compound **1** (DMSO-d_6).

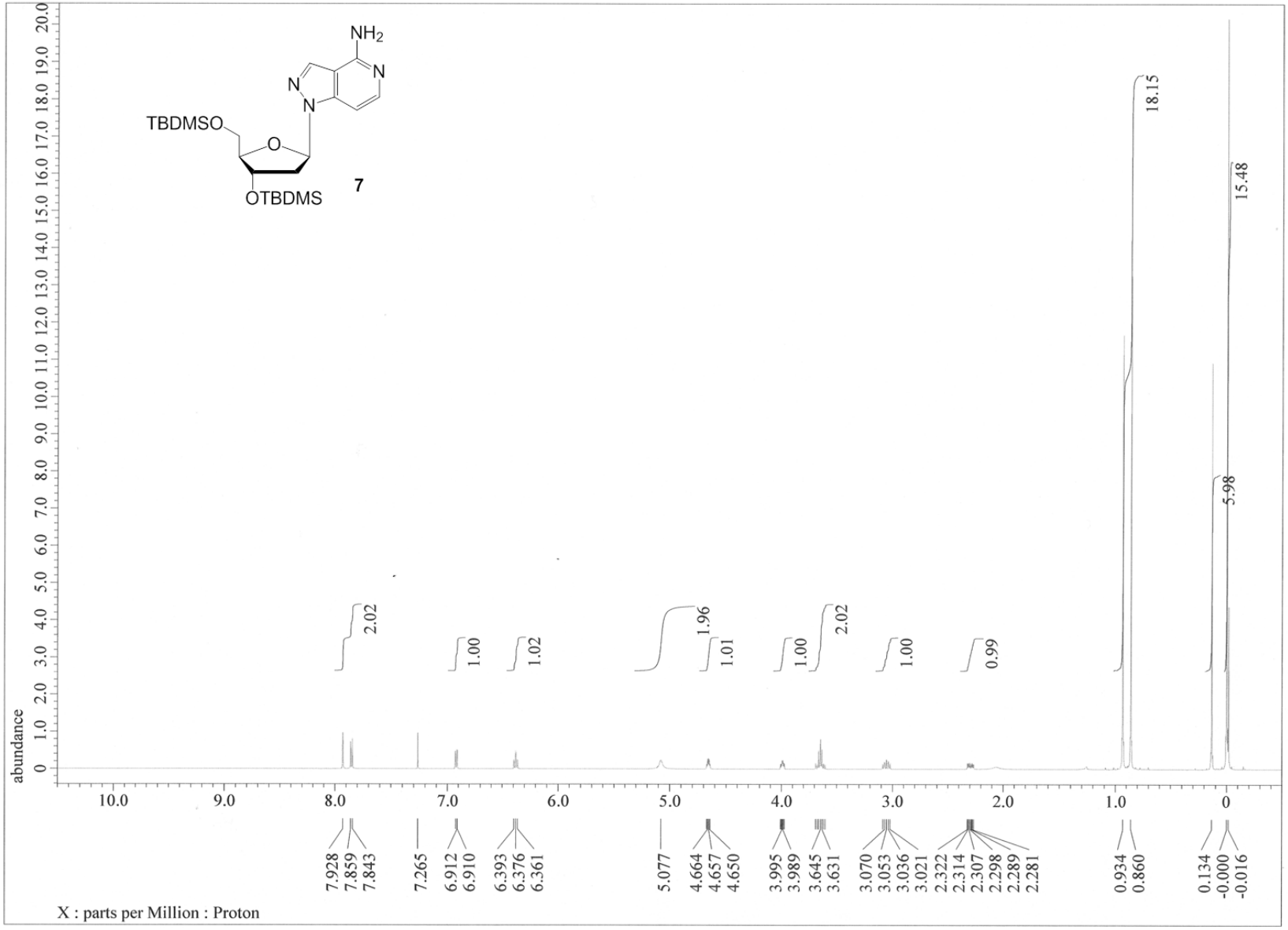


Figure S19. ^{1}H-NMR spectra of compound **7** ($CDCl_3$).

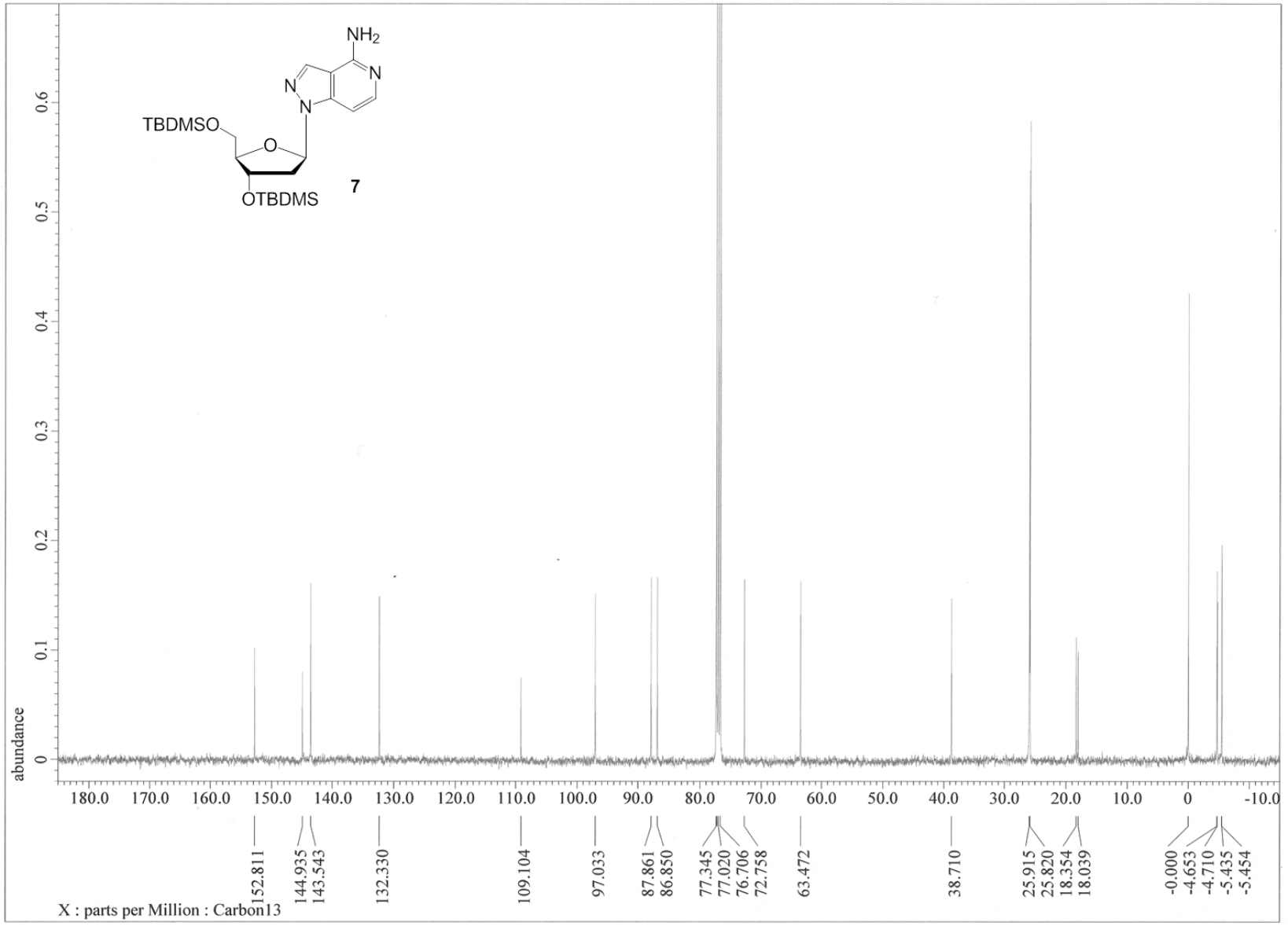


Figure S20. ^{13}C-NMR spectra of compound **7** ($CDCl_3$).

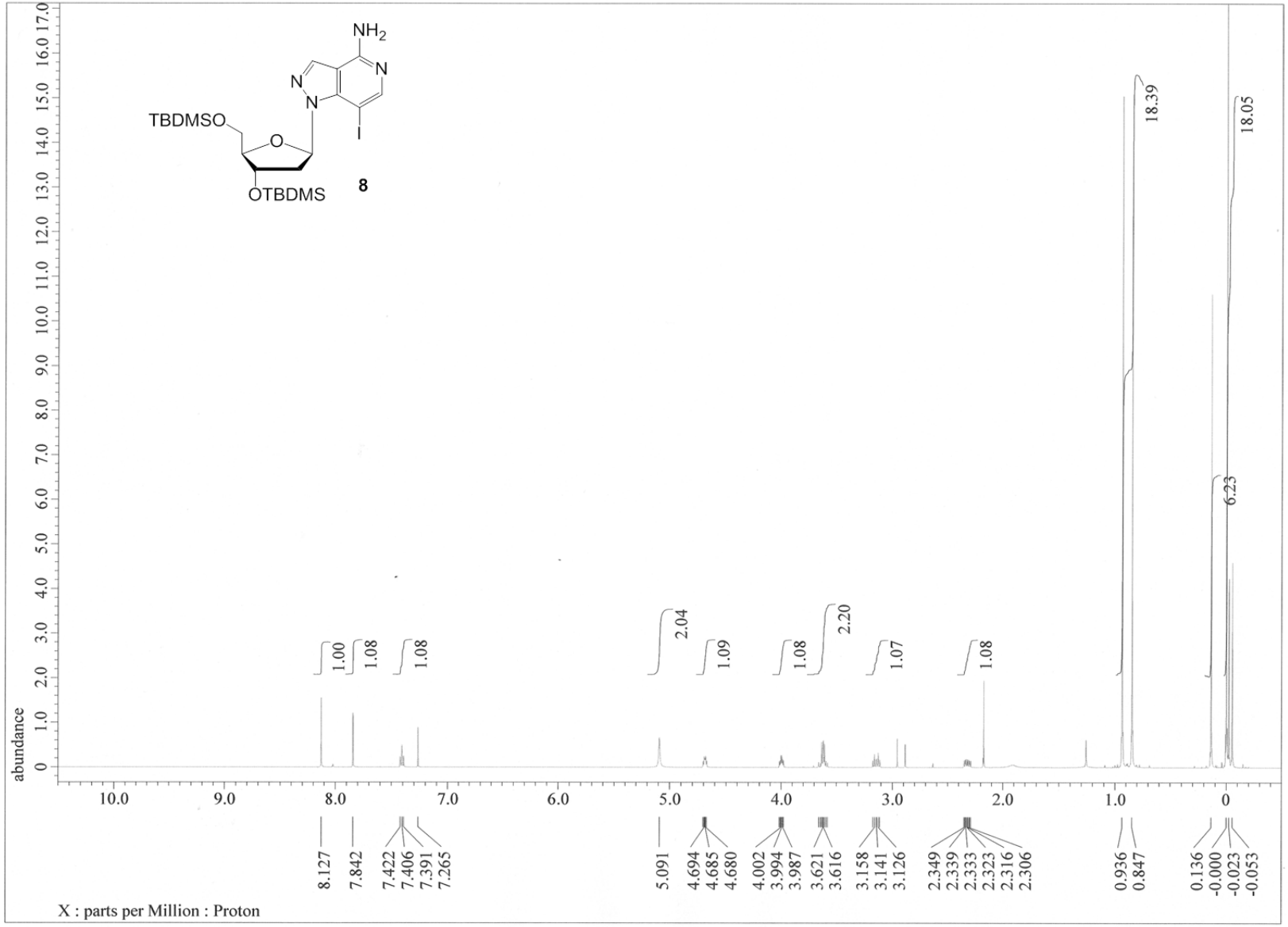


Figure S21. ^{1}H-NMR spectra of compound **8** ($CDCl_3$).

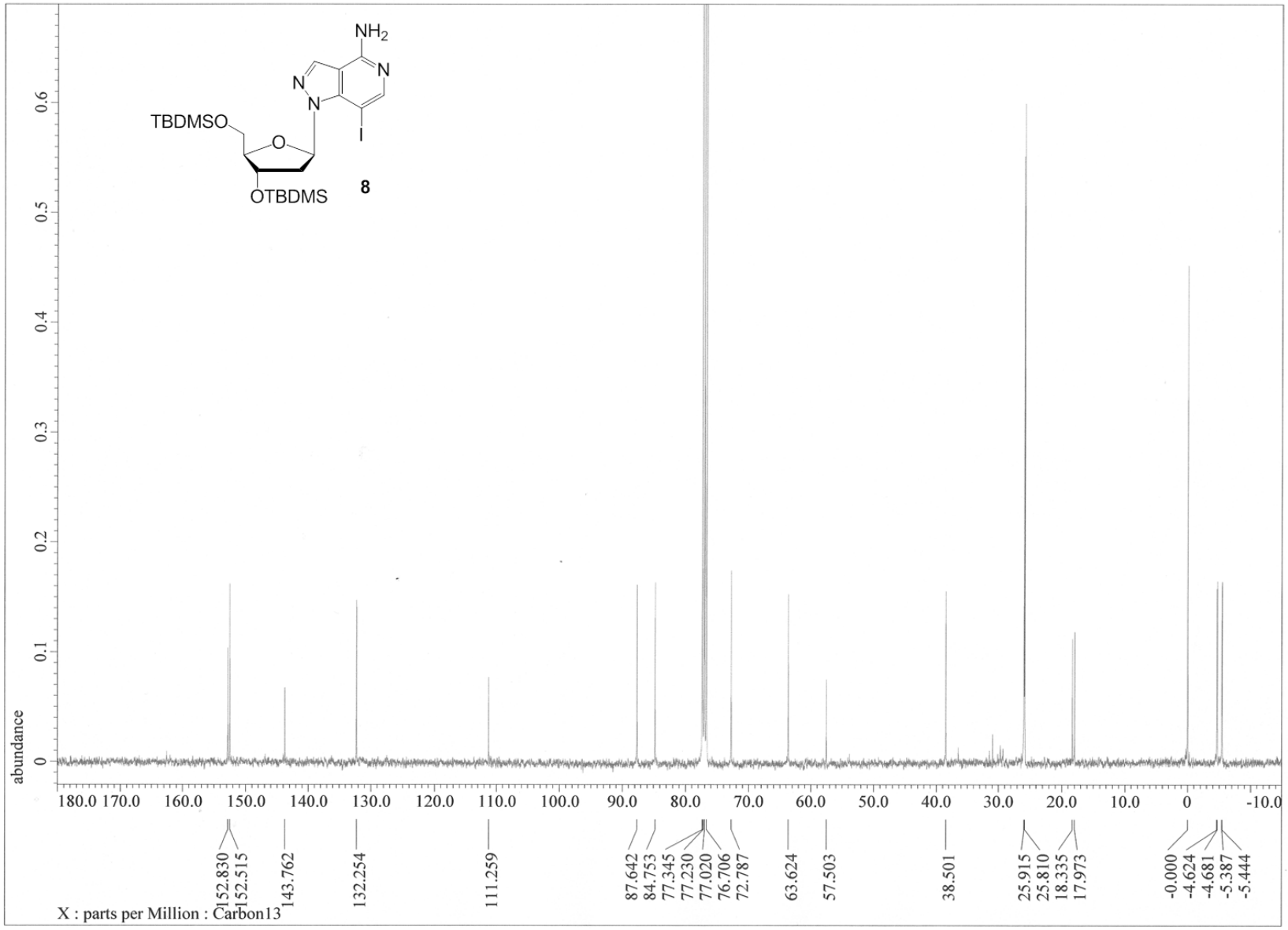


Figure S22. ^{13}C-NMR spectra of compound **8** ($CDCl_3$).

Figure S23. ^{1}H-NMR spectra of compound **9** (DMSO-d_6).

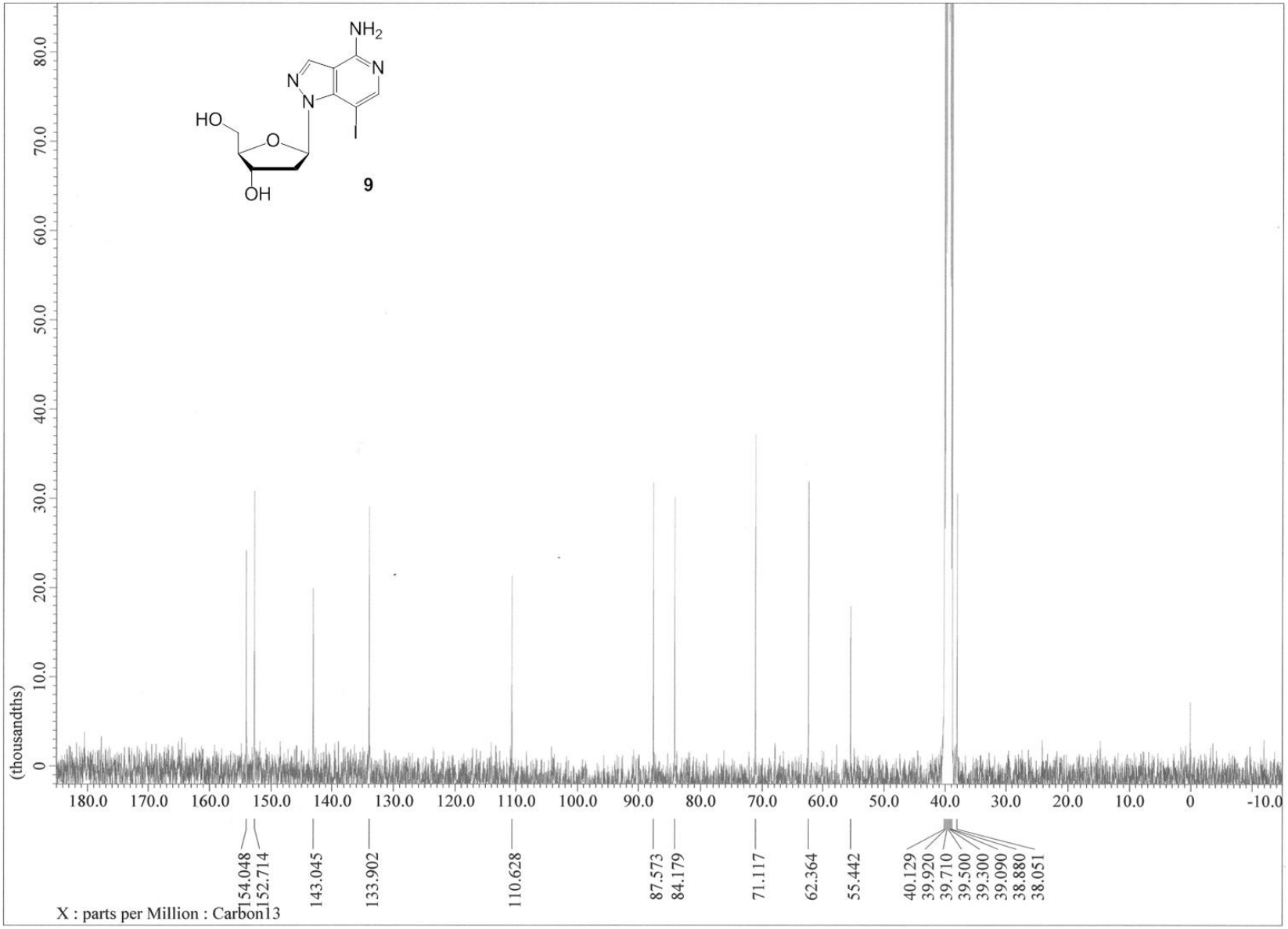


Figure S24. ^{13}C-NMR spectra of compound **9** (DMSO-d_6).

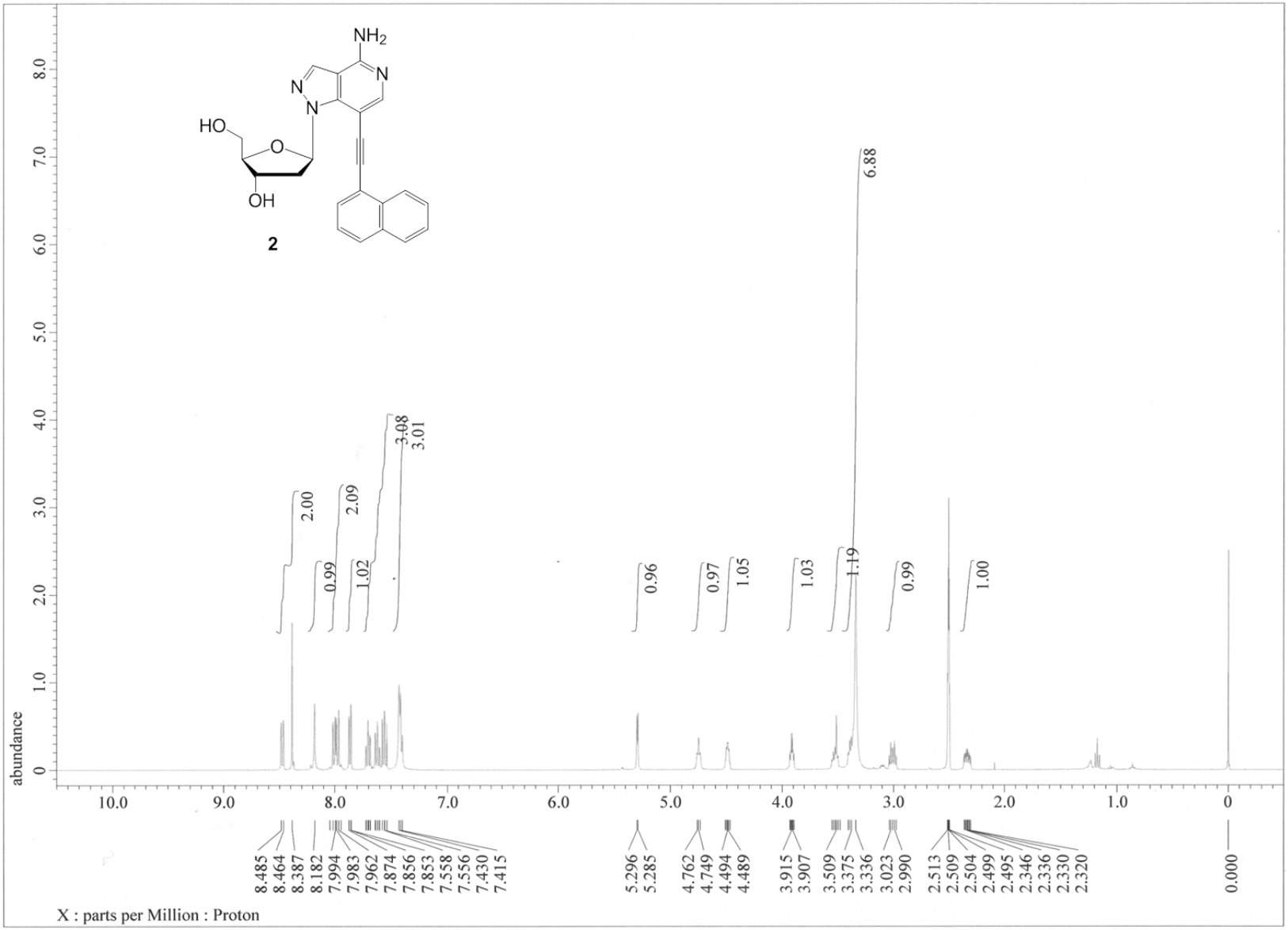


Figure S25. ^{1}H-NMR spectra of compound **2** (DMSO-d_6).

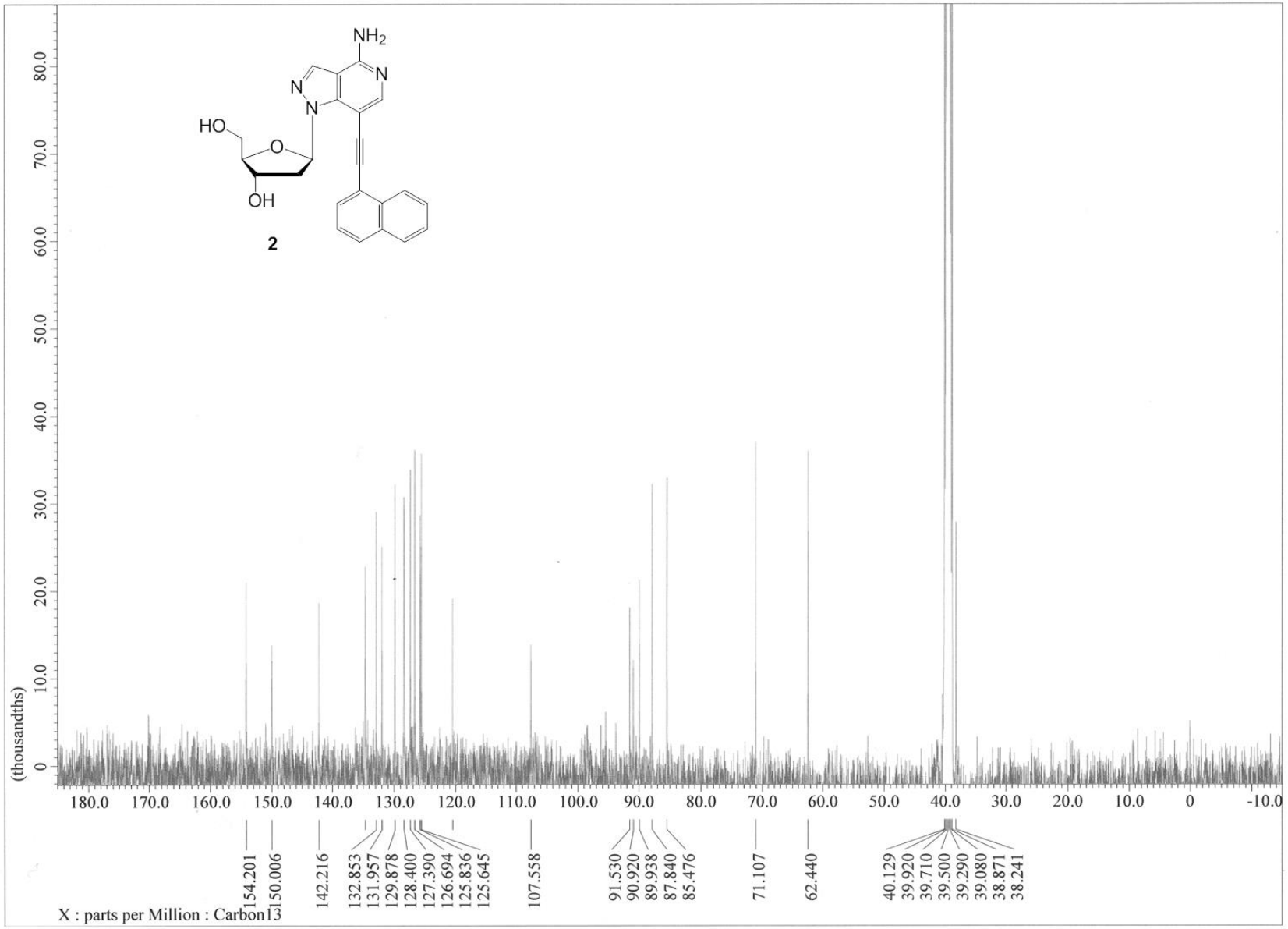


Figure S26. ^{13}C-NMR spectra of compound **2** (DMSO-d_6).

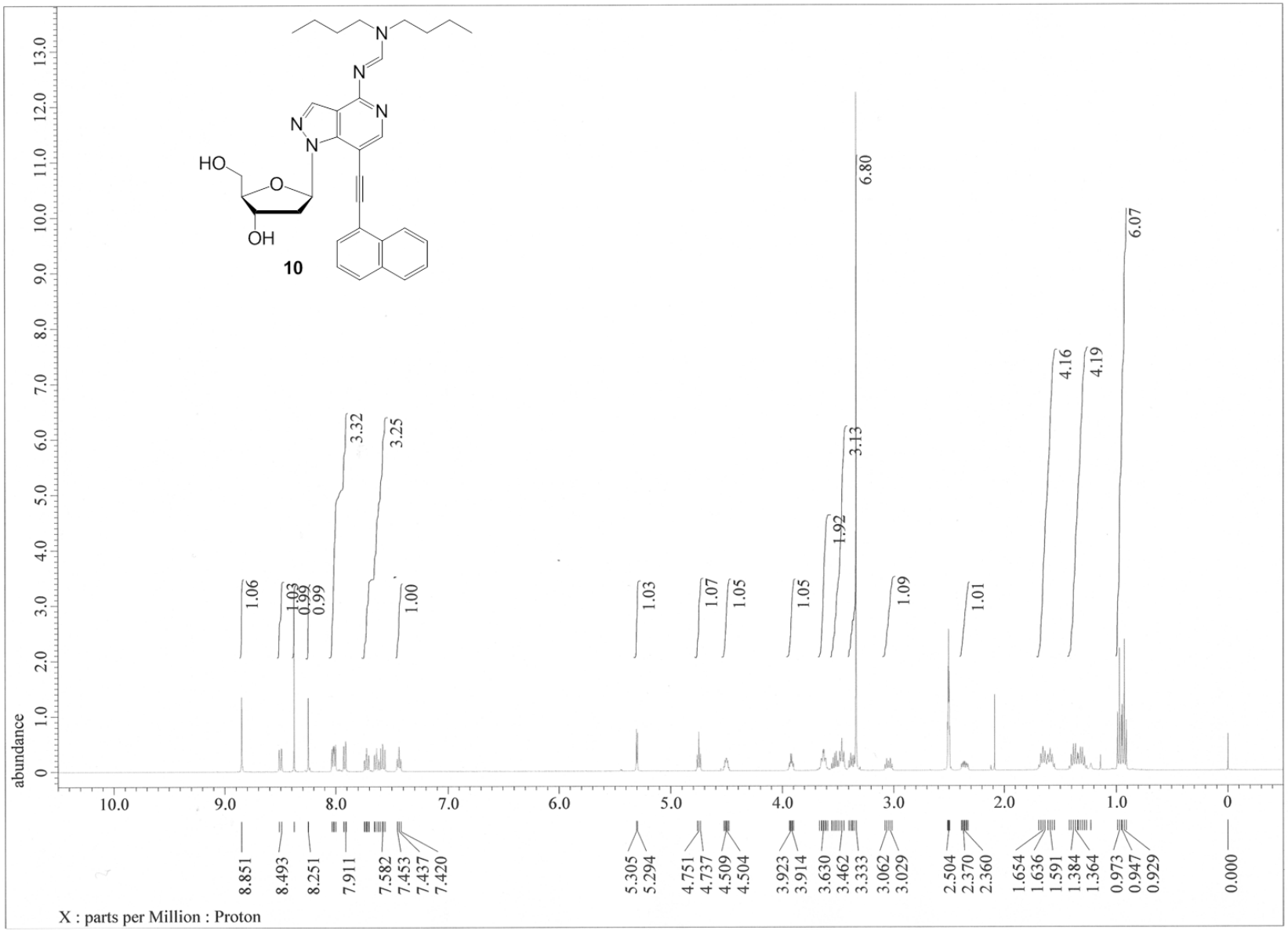


Figure S27. ^{1}H-NMR spectra of compound **10** (DMSO-d_6).

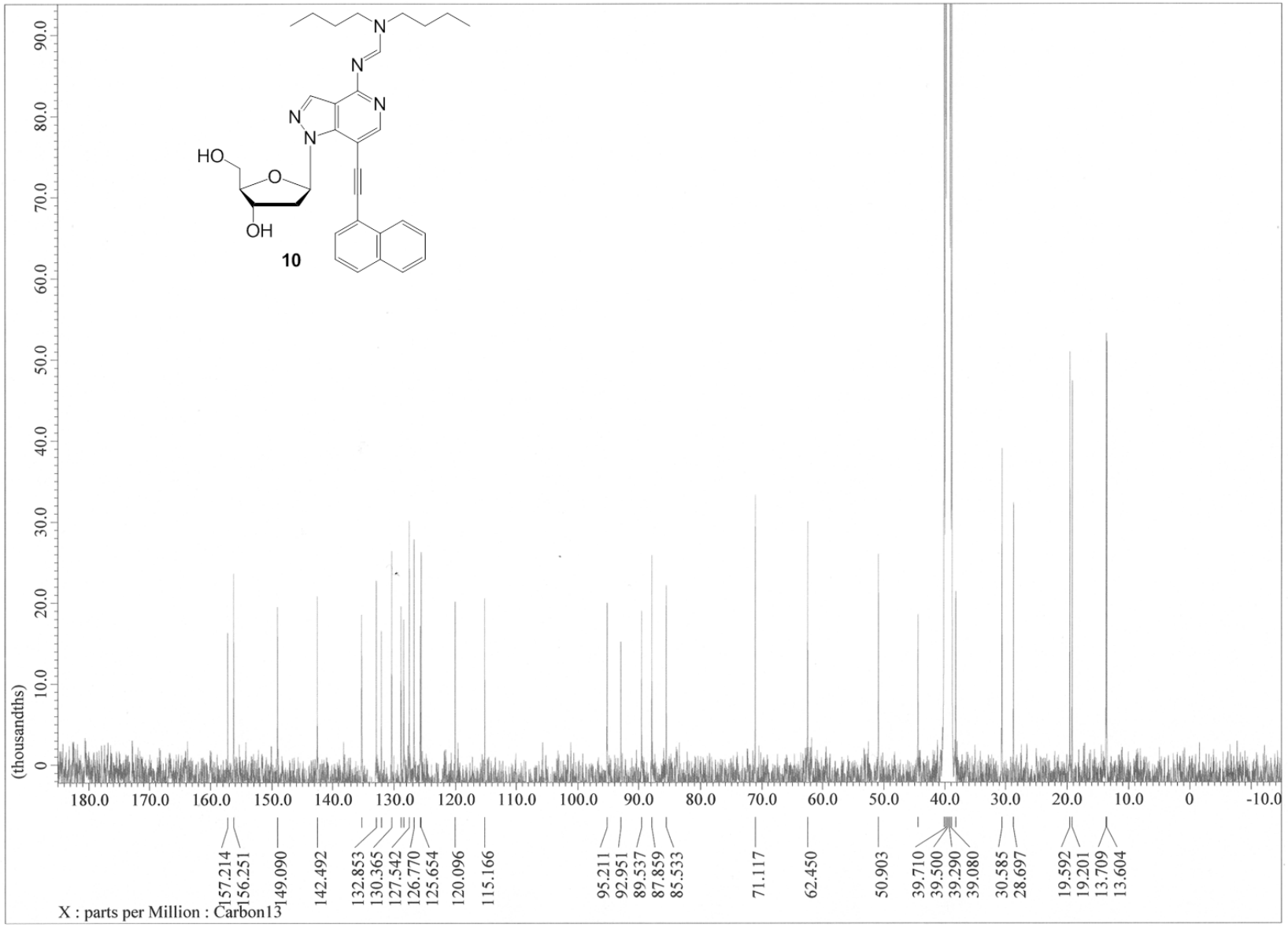


Figure S28. ^{13}C-NMR spectra of compound **10** (DMSO-d_6).

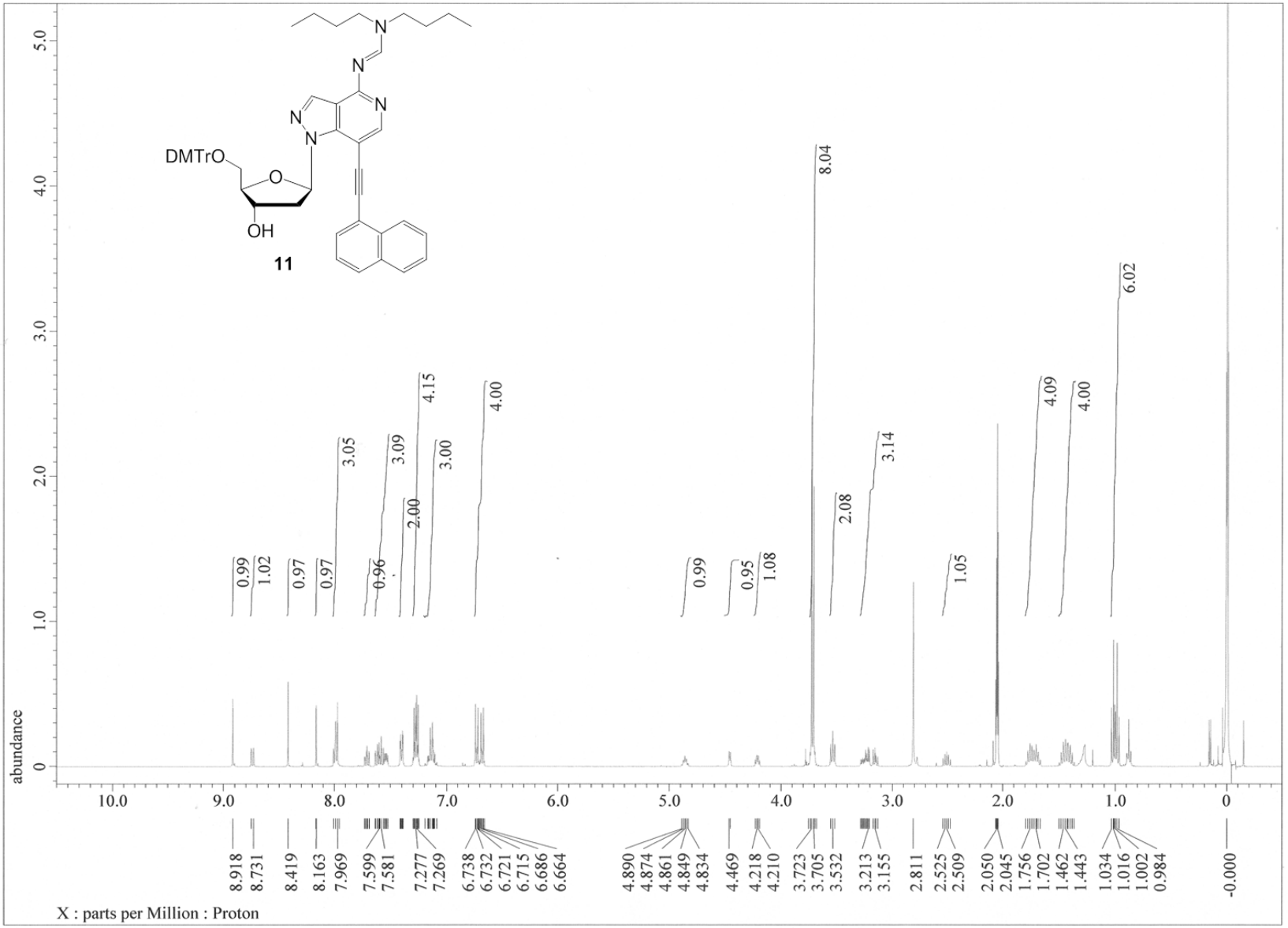


Figure S29. ^{1}H-NMR spectra of compound **11** (Acetone-d_6).

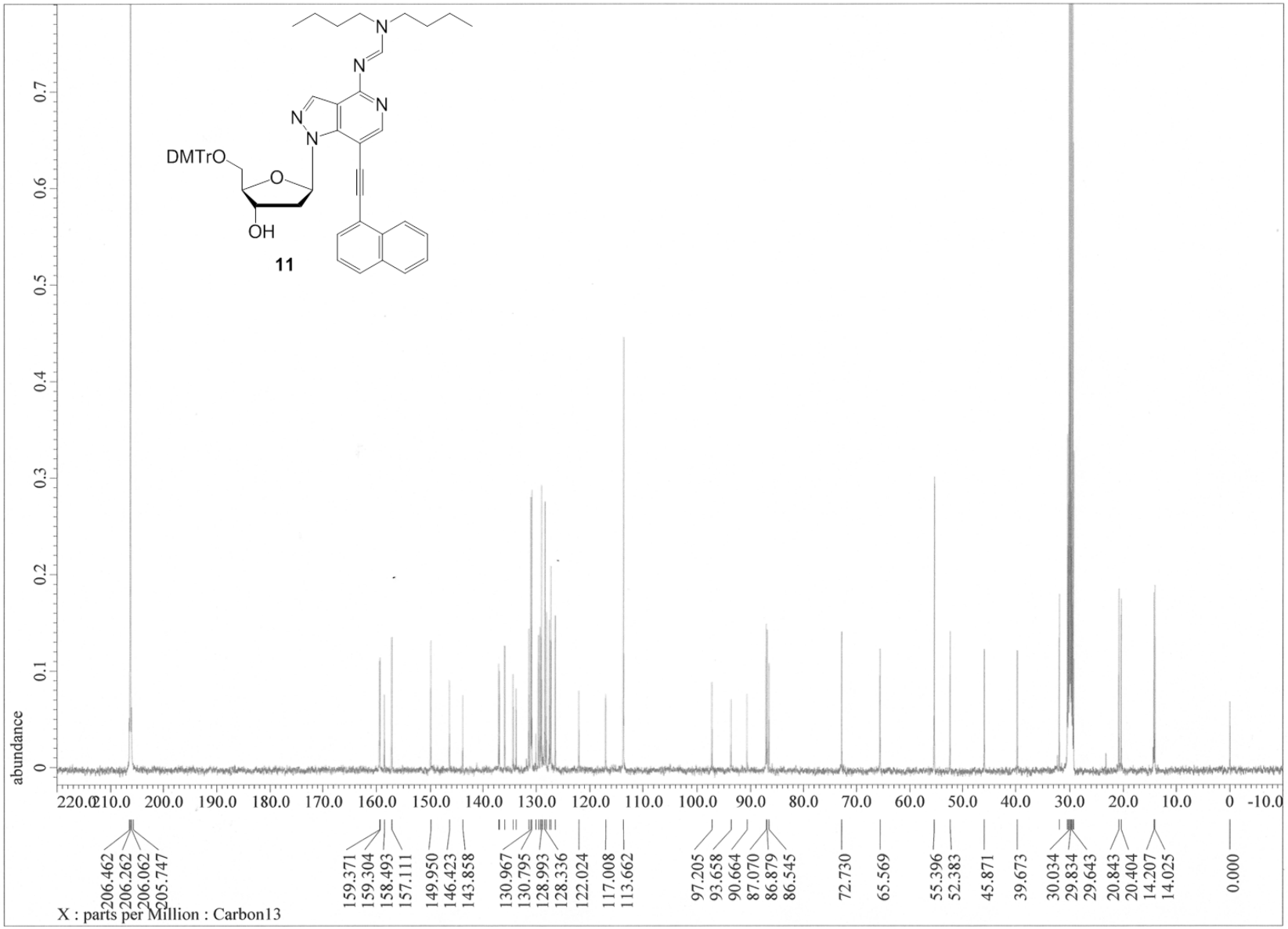


Figure S30. ^{13}C-NMR spectra of compound **11** (Acetone-d_6).