

Design and synthesis of a novel fluorescent benzo[g]imidazo[4,5-c]quinoline nucleoside for monitoring base-pair-induced protonation with cytosine: Distinguishing cytosine *via* changes in the intensity and wavelength of fluorescence

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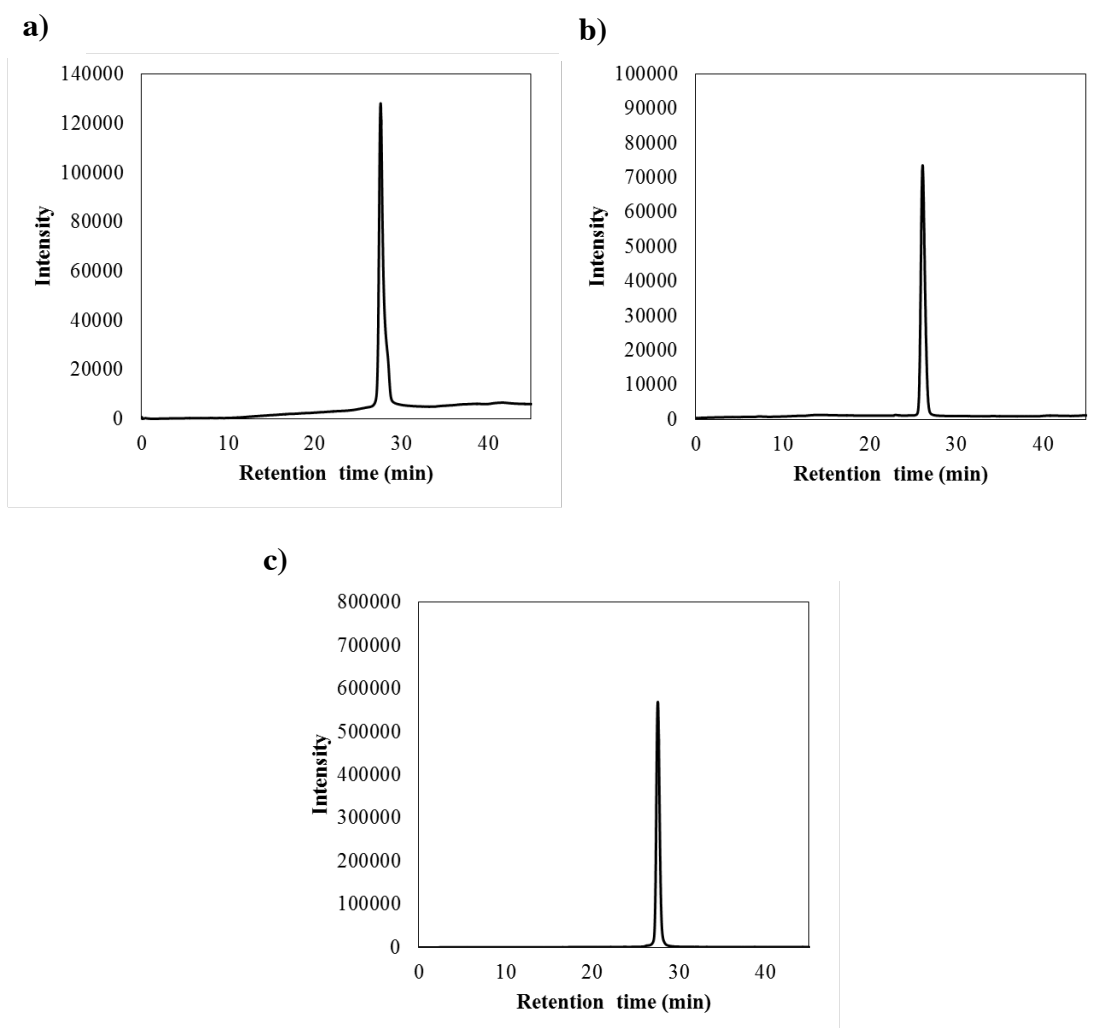


Figure S1. HPLC profiles determined at 260 nm of single-stranded oligonucleotides. (a) ODN1^(BIQ A), (b) ODN2^(BIQ A), and (c) Probe_(BRCA1). HPLC analysis was performed on a CHEMCOBOND 5-ODS-H column (10 × 150 nm) eluted with 50mM ammonium formate buffer containing acetonitrile. Gradient: from 3 to 20 % acetonitrile at a flow rate 2.0 ml/min over 45min.

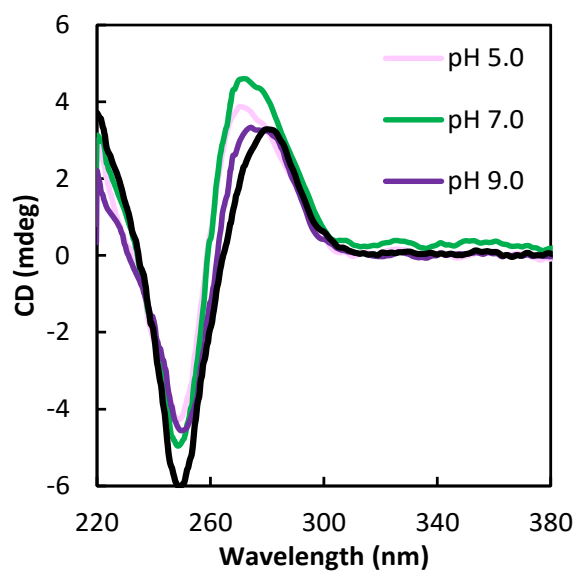


Figure S2. CD spectra of ODN1(^{BIQ}A) hybridized with complementary strand cODN1(C) at various pH values. Various buffer solutions with 5 μ M ODN were used to access range of pH values: 0.1 M NaCl and 10 mM sodium acetate (pH = 5.0), 10 mM sodium phosphate (pH = 7.0), and 10 mM ammonium (pH = 9.0). Black line indicates unmodified duplex ODN1(A)/cODN1(T) at pH = 7.0.

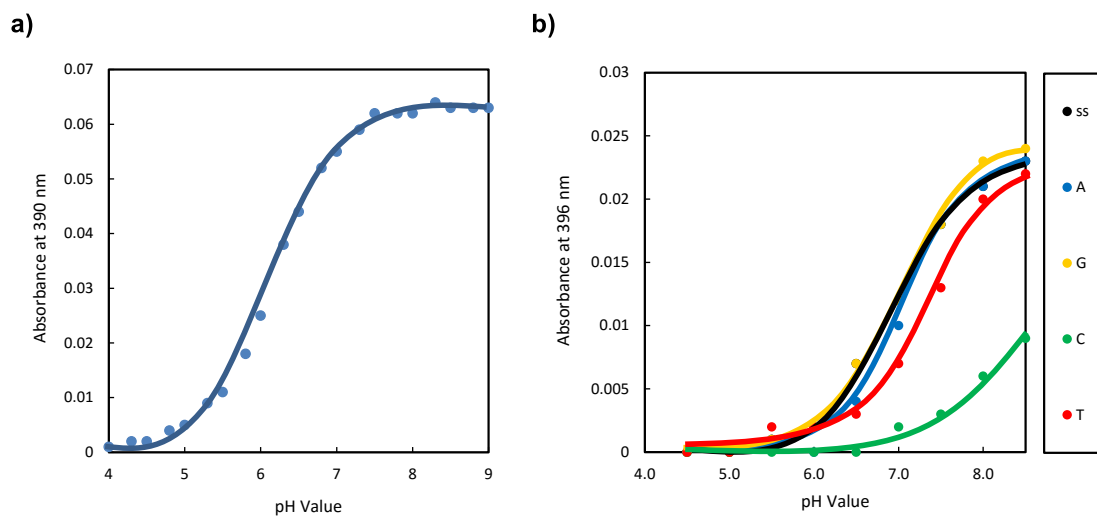


Figure S3. Plots of the absorbance of (a) BIQA monomer ($10 \mu\text{M}$, 390 nm) and (b) BIQA -containing ODNs ($\text{ssODN1}(\text{BIQA})$, $\text{ODN1}(\text{BIQA})/\text{cODN1}(\text{N})$, $\text{N} = \text{A, G, T, and C}$, $5 \mu\text{M}$, 396 nm) as a function of pH.

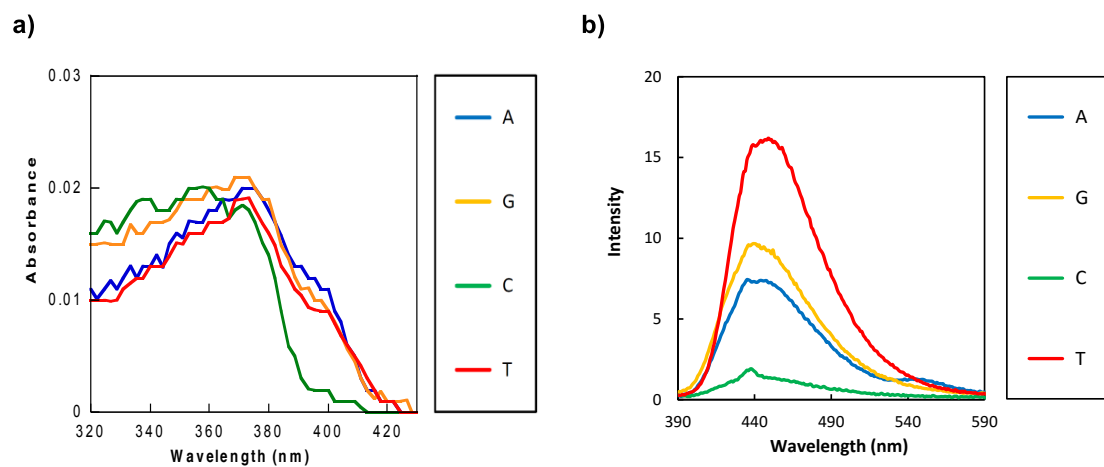


Figure S4. (a) Absorption and (b) fluorescence spectra of ODN2(**BIQA**) hybridized with cODN2(N) at pH = 8.5. Measurements were made in buffer with 0.1 M NaCl and 10 mM ammonium; concentration of ODN was 5 μ M.

Table S1. Thermal melting temperatures (T_m) and photophysical properties of oligonucleotides^a

ODNs	T_m ($^{\circ}$ C)	$\lambda_{\max}^{\text{abs}}$ (nm)	$\lambda_{\max}^{\text{em}}$ (nm)	Φ_F^b
ODN2(BIQA)	-	363, 399*	442	0.126
ODN2(BIQA)/cODN2(A)	61.2	371, 398*	435	0.028
/cODN2(G)	60.4	369, 398*	440	0.033
/cODN2(T)	63.9	371, 400*	449	0.059
/cODN2(C)	64.6	341, 355, 371	438	0.006
ODN2(A)/cODN2(T)	63.3	-	-	-

* shoulder

^a ODNs (5 μ M) were measured in buffer with 0.1 M NaCl and 10 mM ammonium (pH 8.5) at r.t.

^b Fluorescence quantum yields were calculated according to ref.12

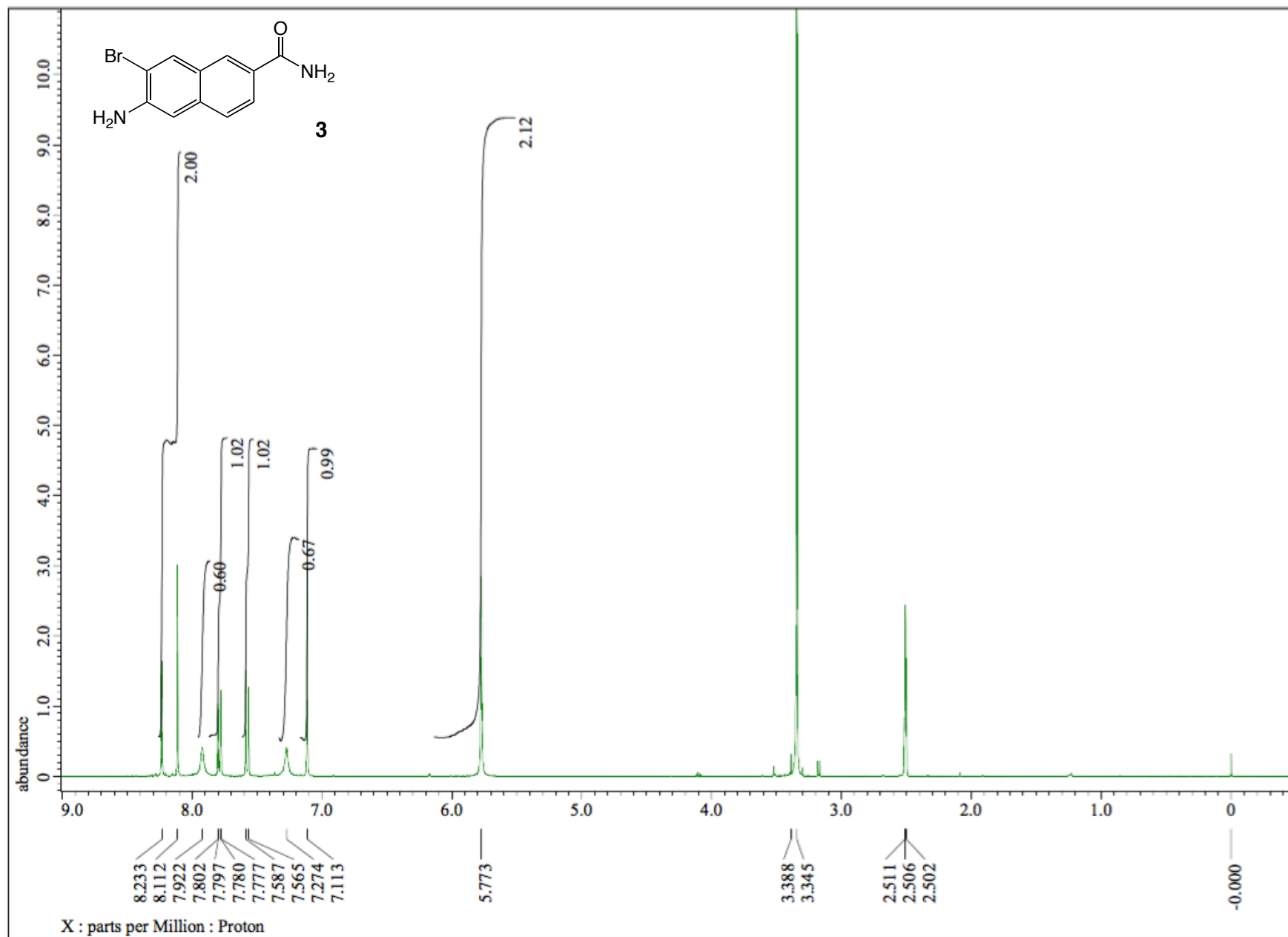


Figure S5. ¹H-NMR spectrum of compound 3 (DMSO-*d*₆)

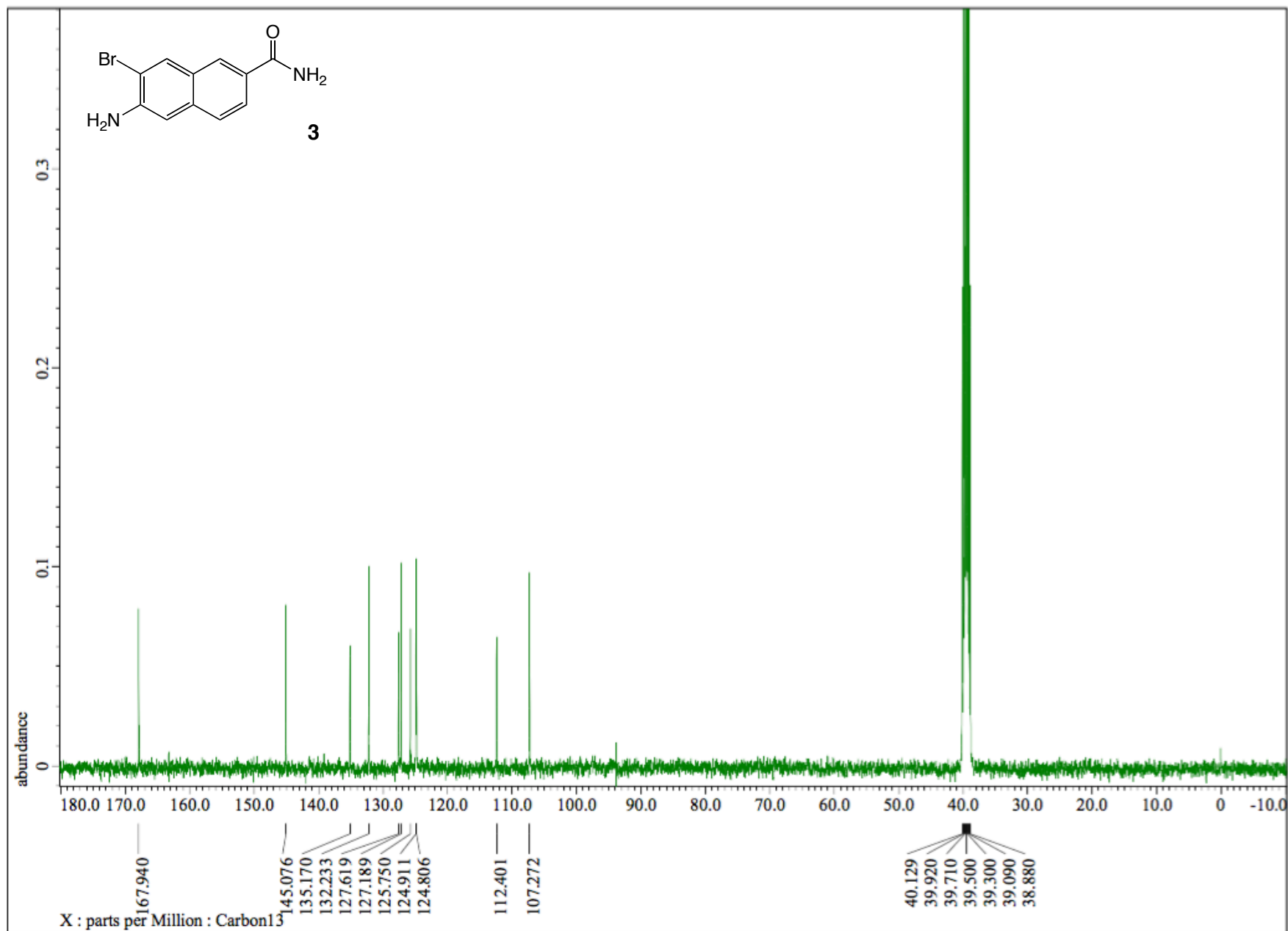


Figure S6. ¹³C-NMR spectrum of compound 3 (DMSO-*d*₆)

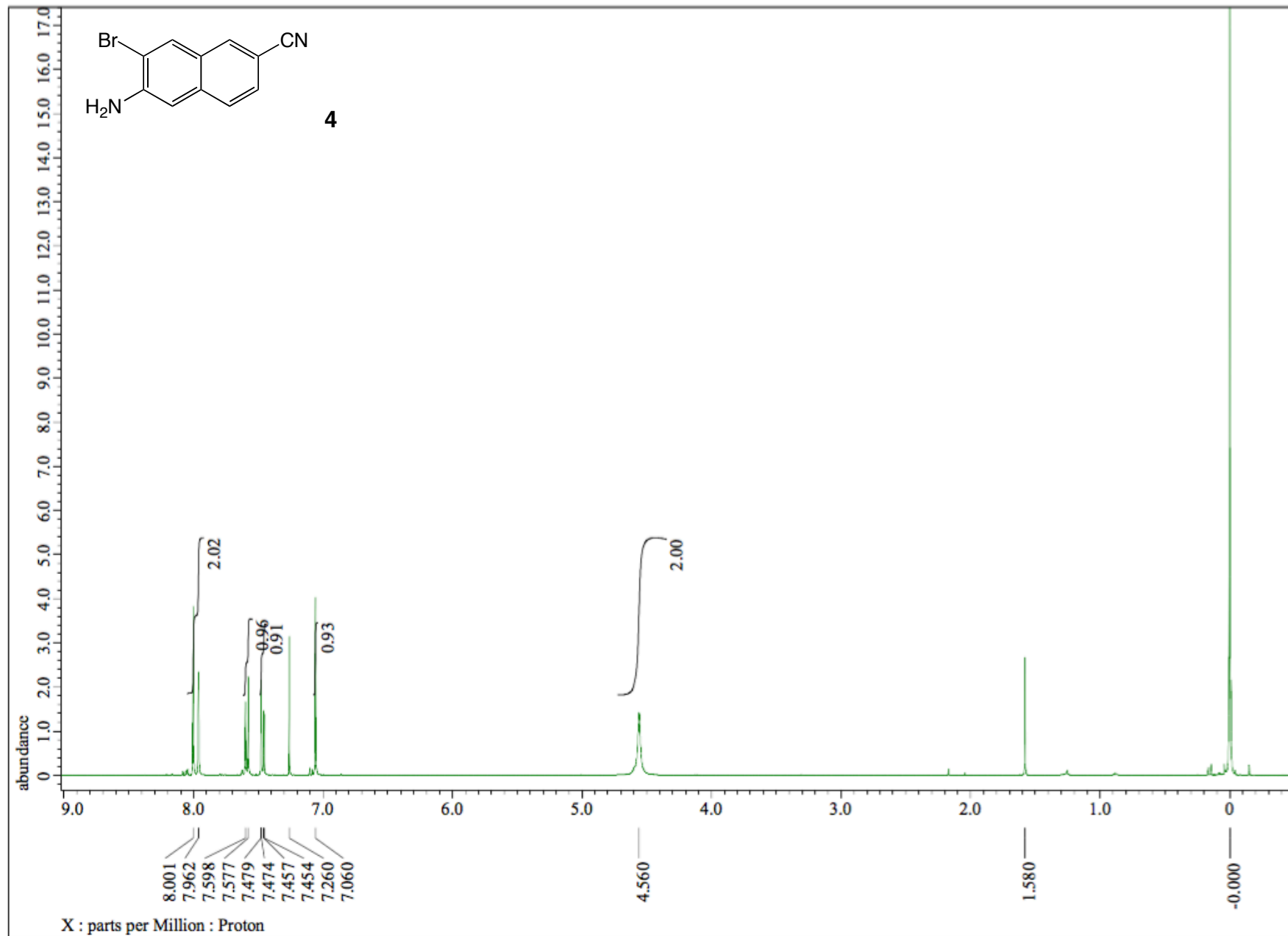


Figure S7. $^1\text{H-NMR}$ spectrum of compound 4 (CDCl_3)

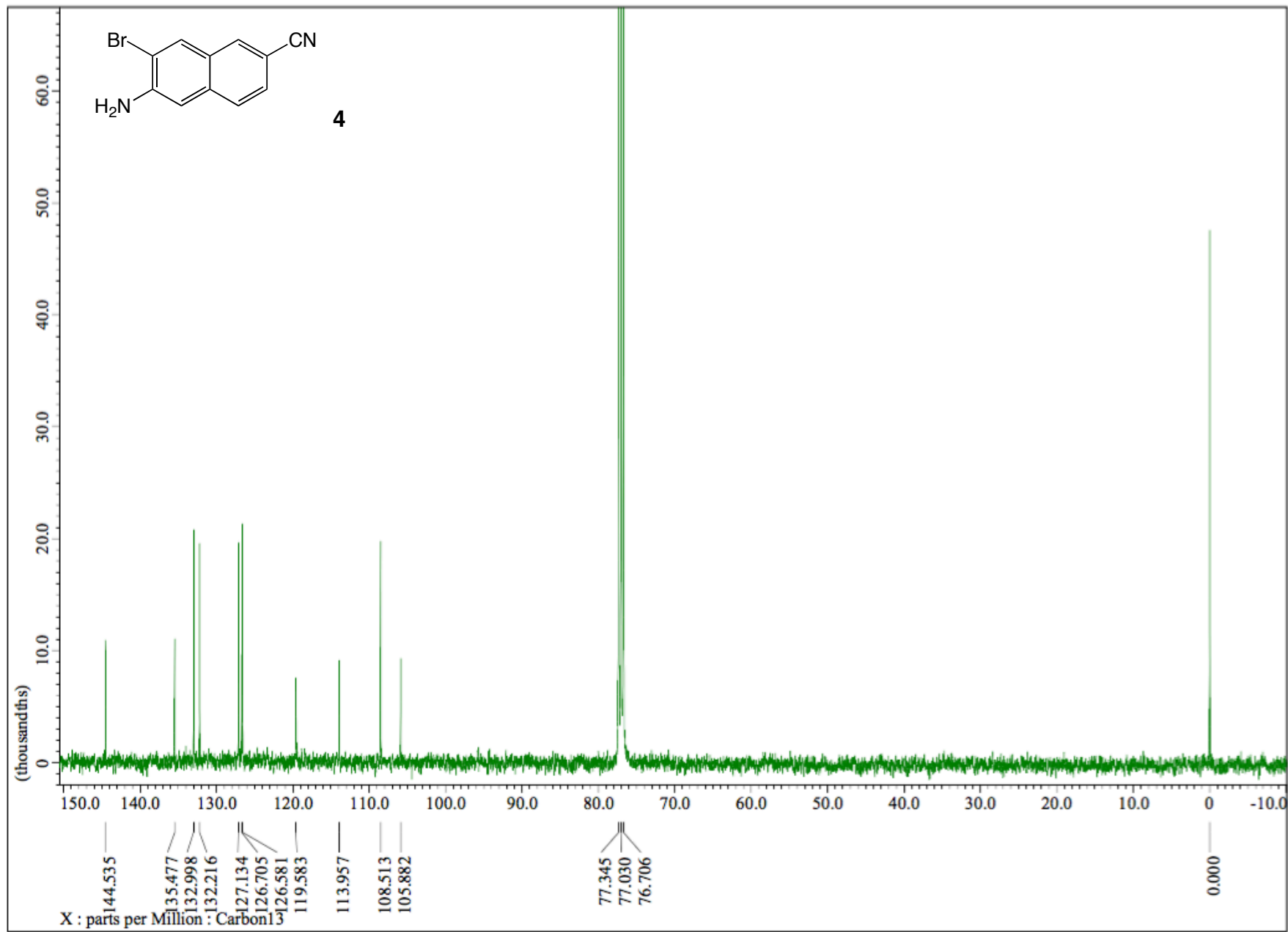


Figure S8. ^{13}C -NMR spectrum of compound **4** (CDCl_3)

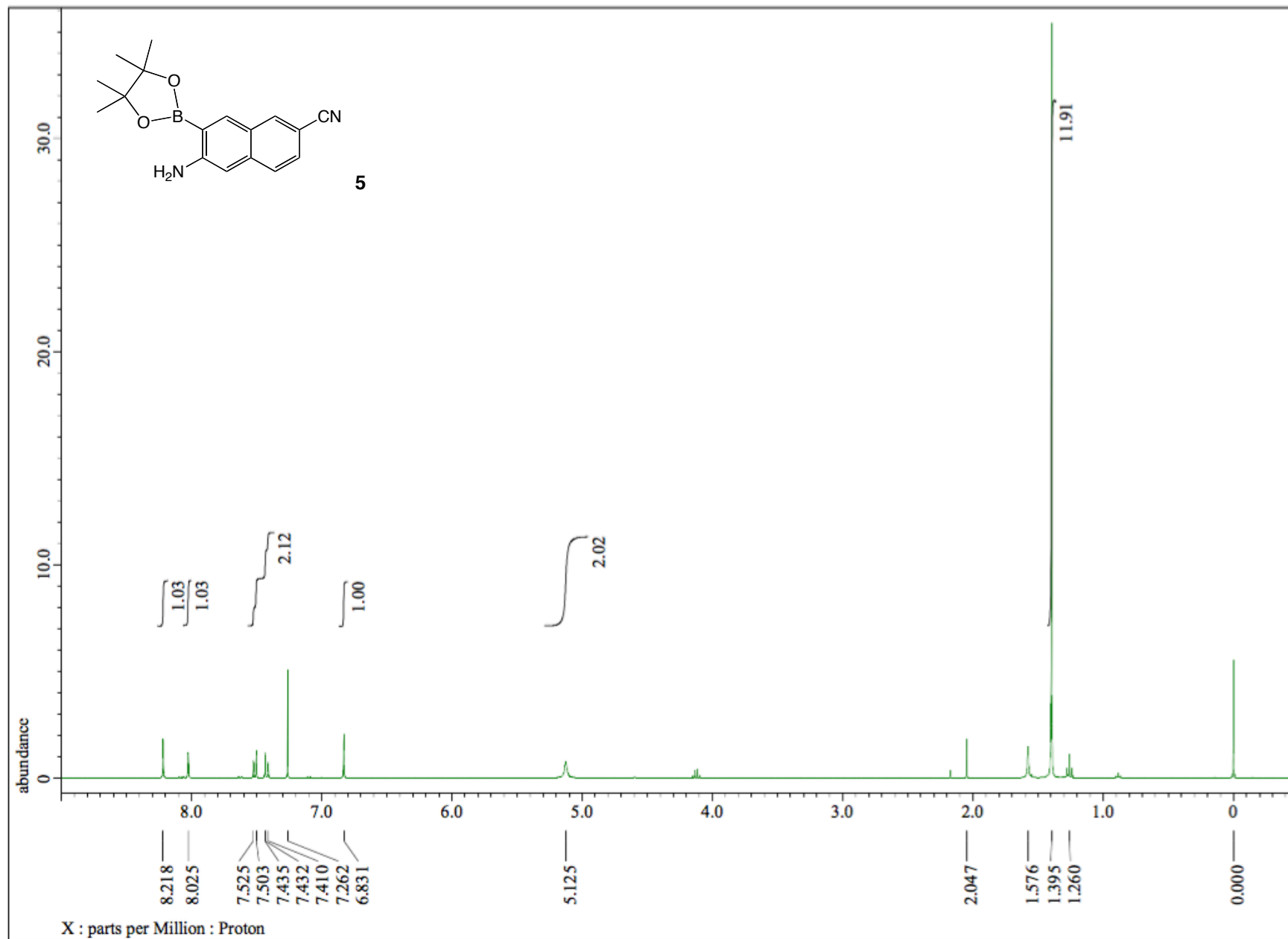


Figure S9. $^1\text{H-NMR}$ spectrum of compound **5** (CDCl_3)

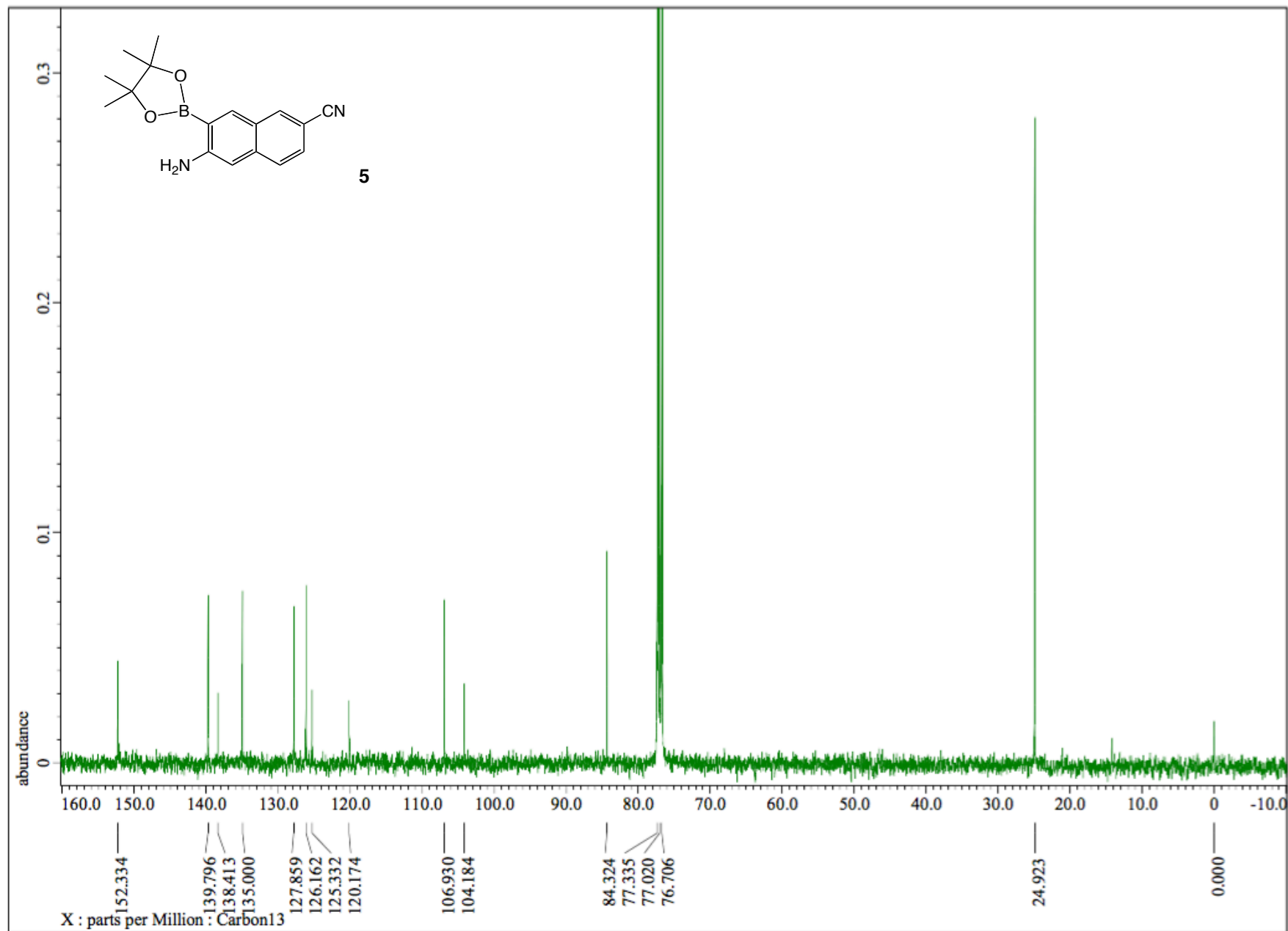


Figure S10. ¹³C-NMR spectrum of compound **5** (CDCl₃)

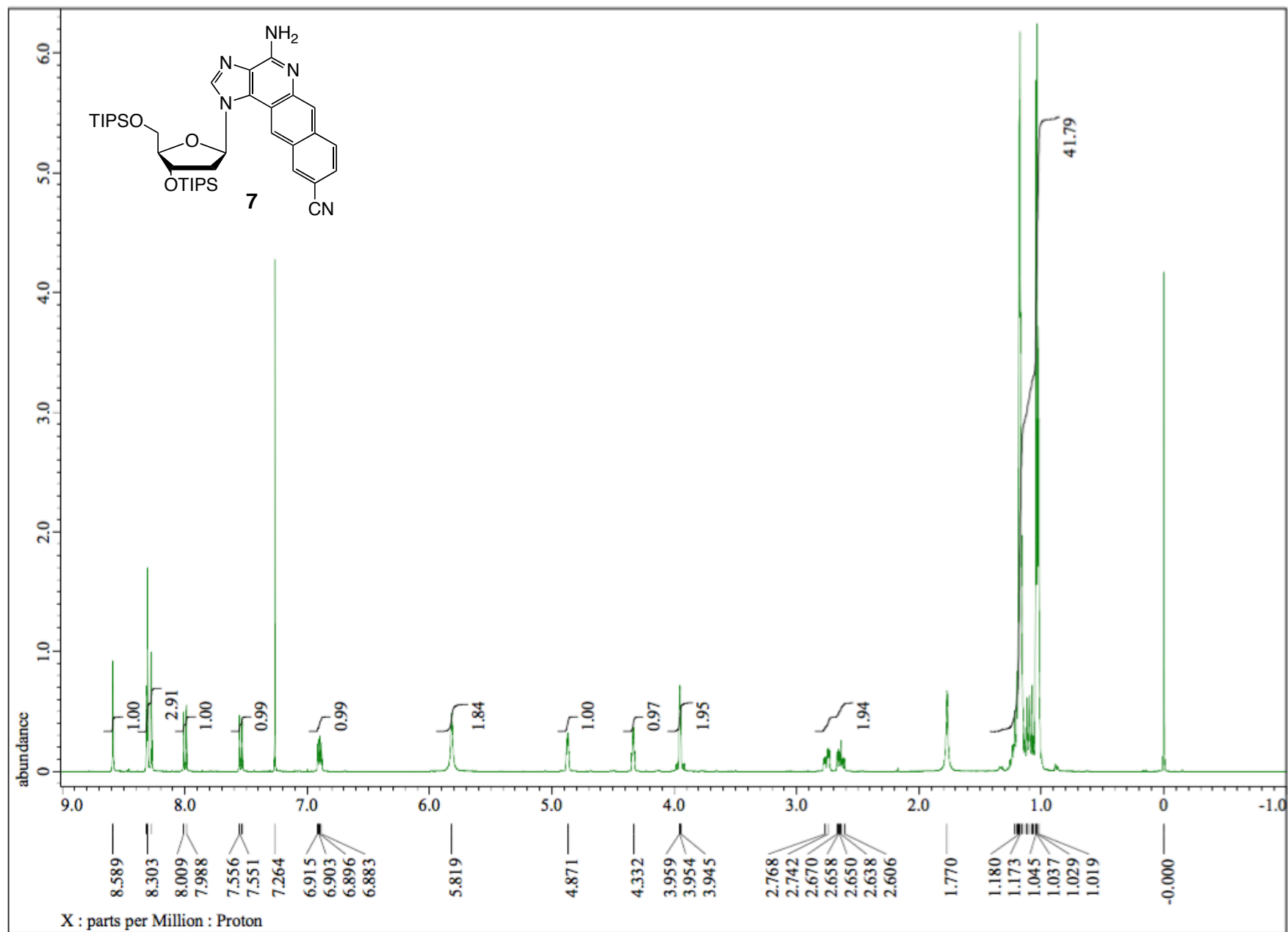


Figure S11. $^1\text{H-NMR}$ spectrum of compound 7 (CDCl₃)

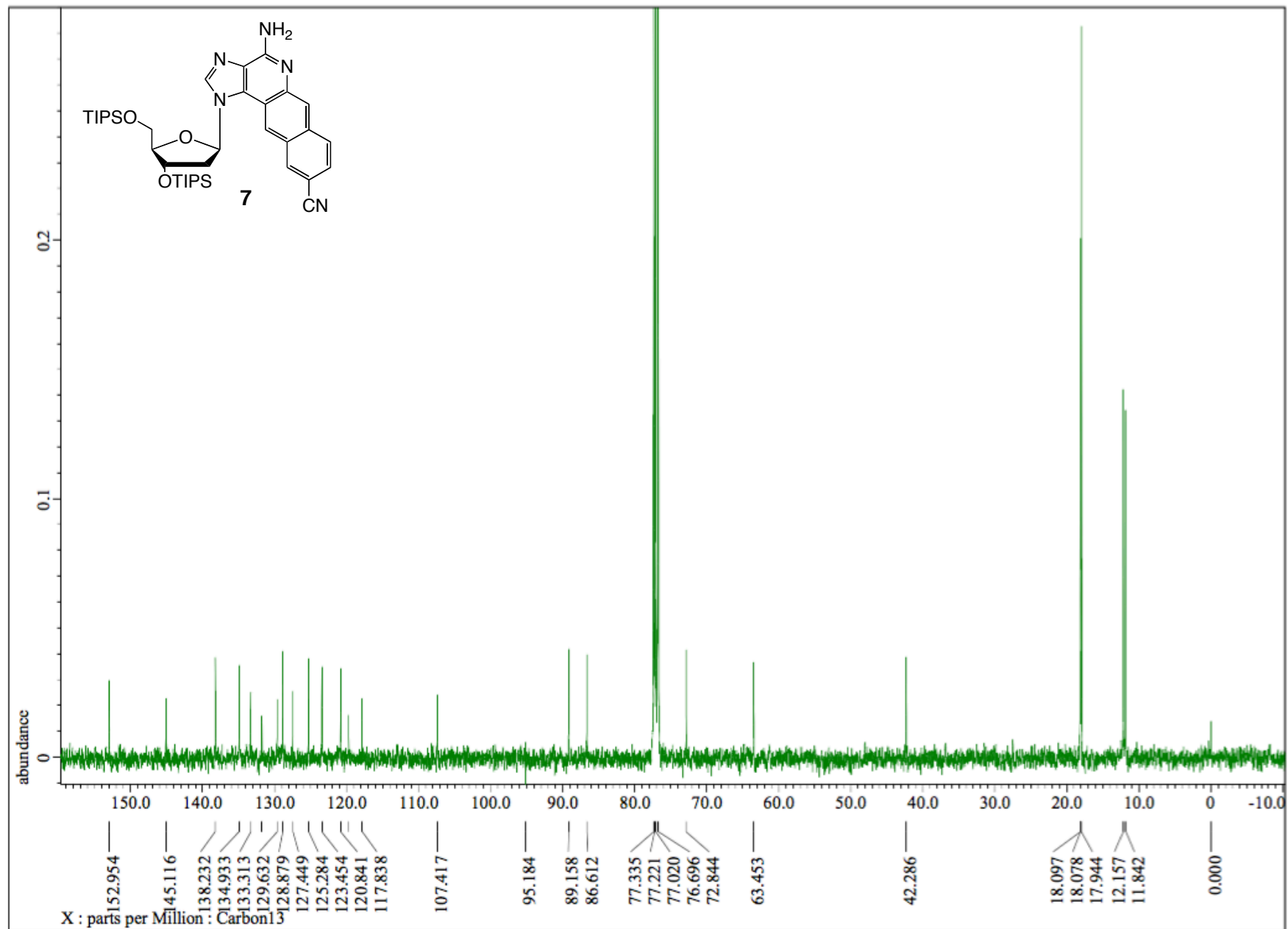


Figure S12. ^{13}C -NMR spectrum of compound 7 (CDCl_3)

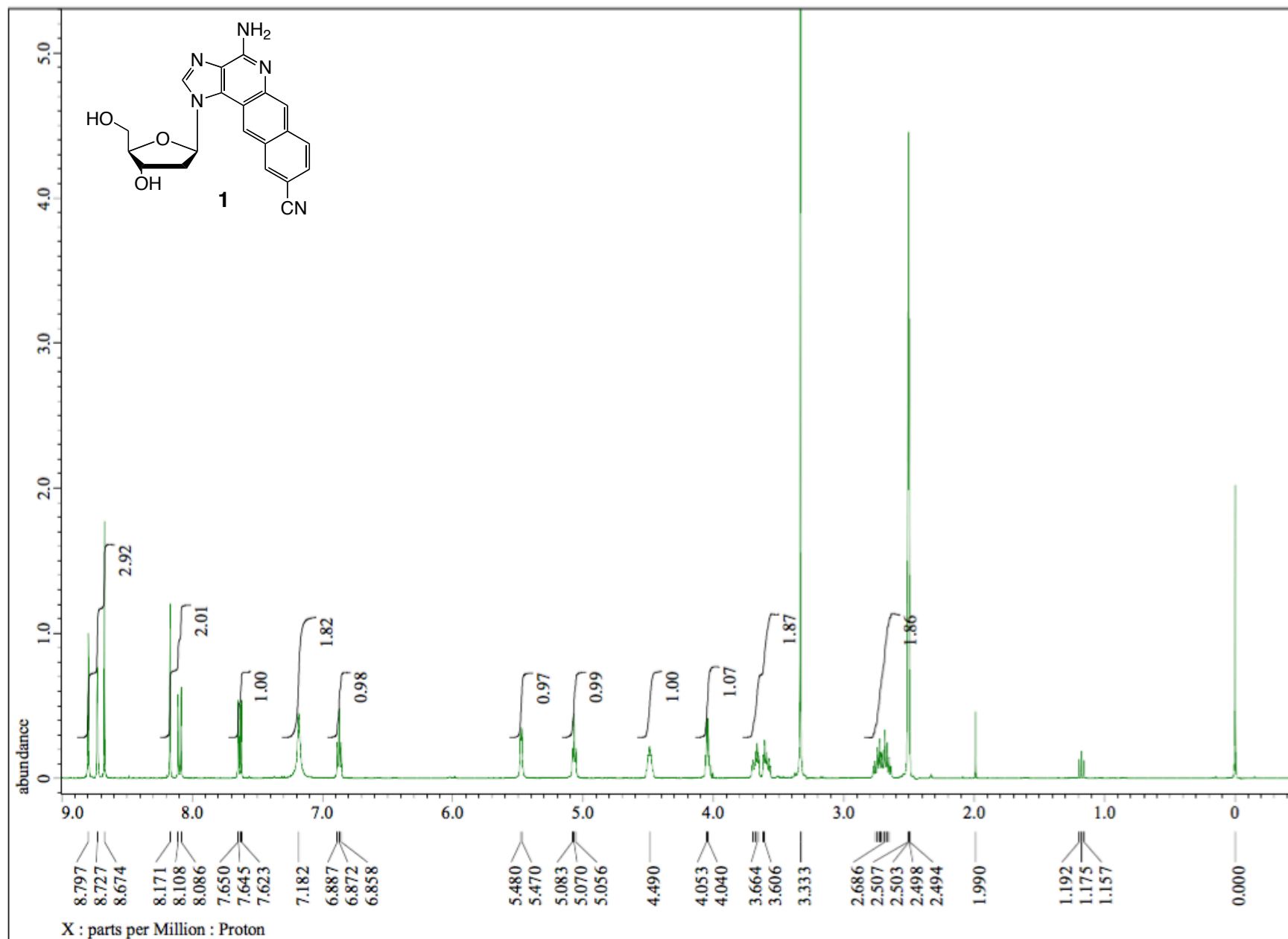


Figure S13. ¹H-NMR spectrum of compound **1** (DMSO-*d*₆)

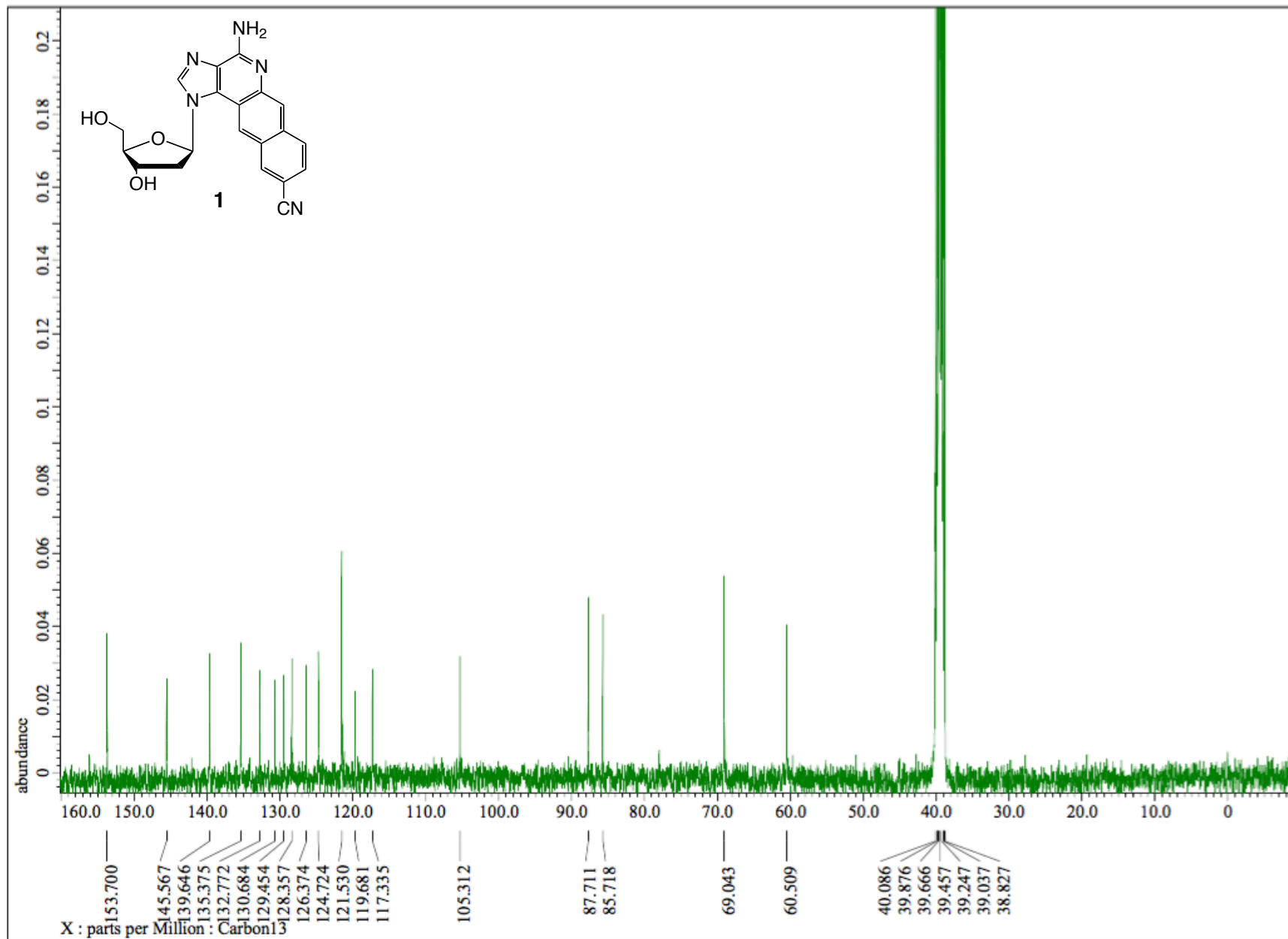


Figure S14. ^{13}C -NMR spectrum of compound **1** (DMSO- d_6)

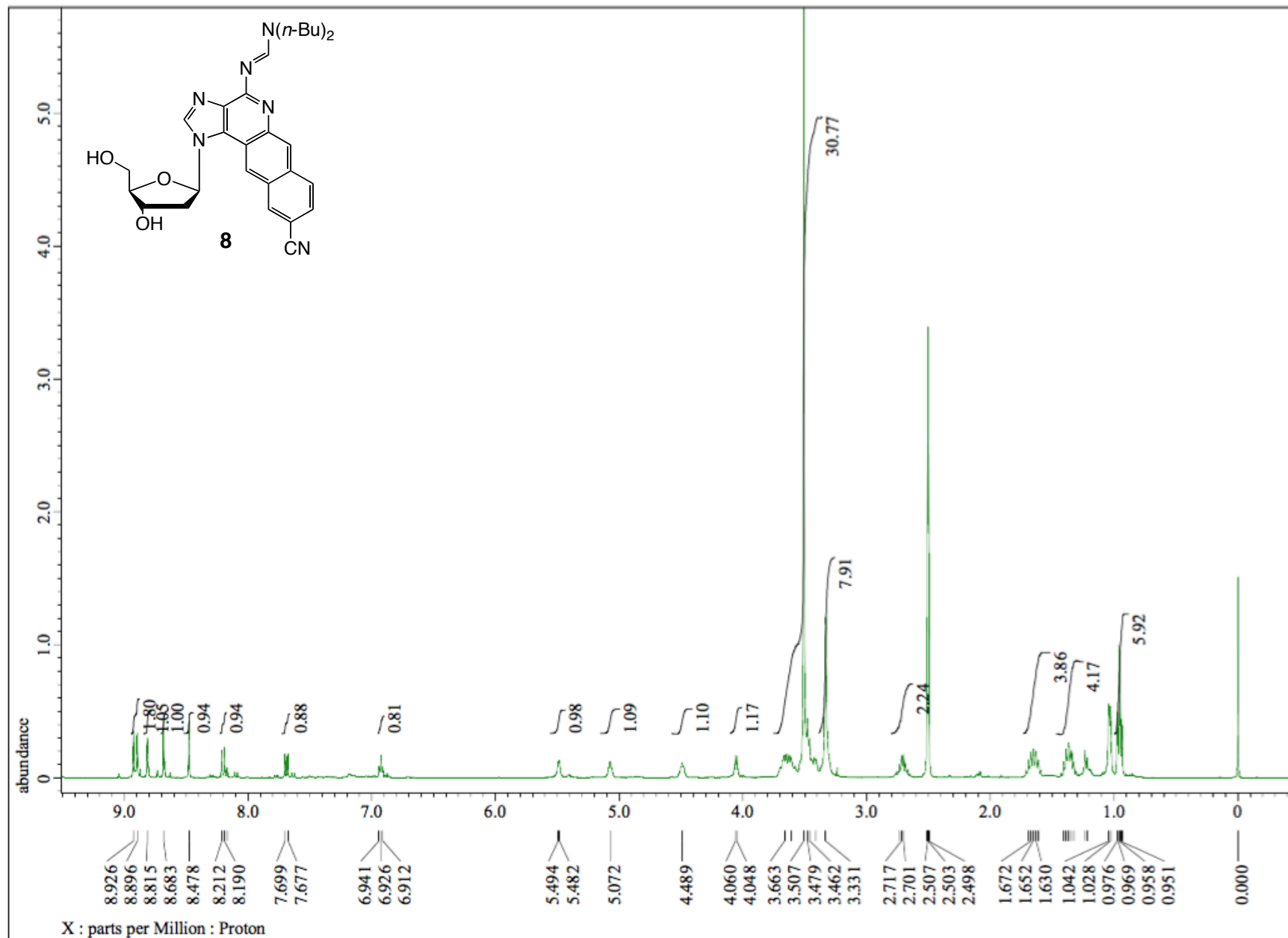


Figure S15. ¹H-NMR spectrum of compound **8** (DMSO-*d*₆)

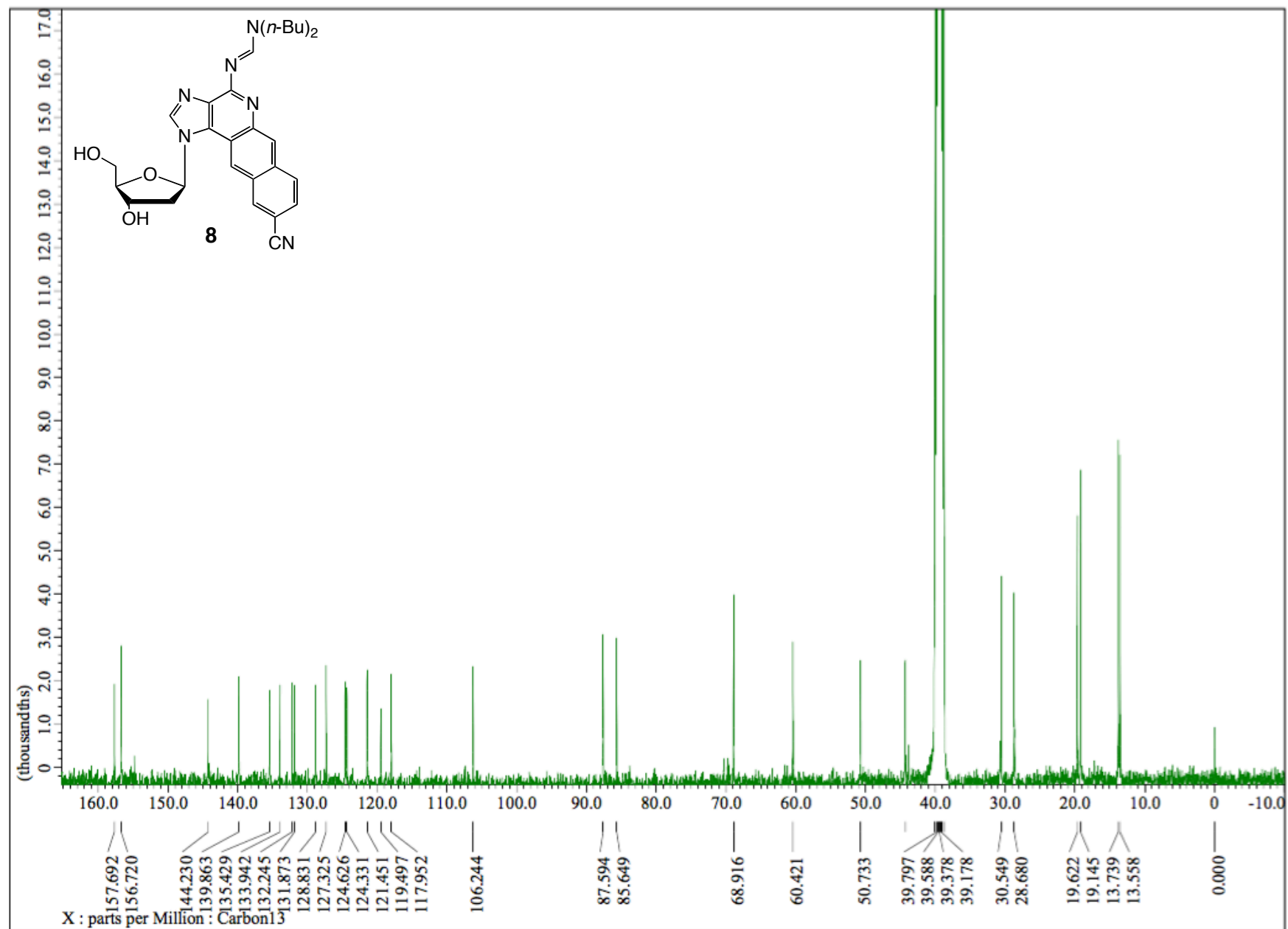


Figure S16. ^{13}C -NMR spectrum of compound **8** (DMSO- d_6)

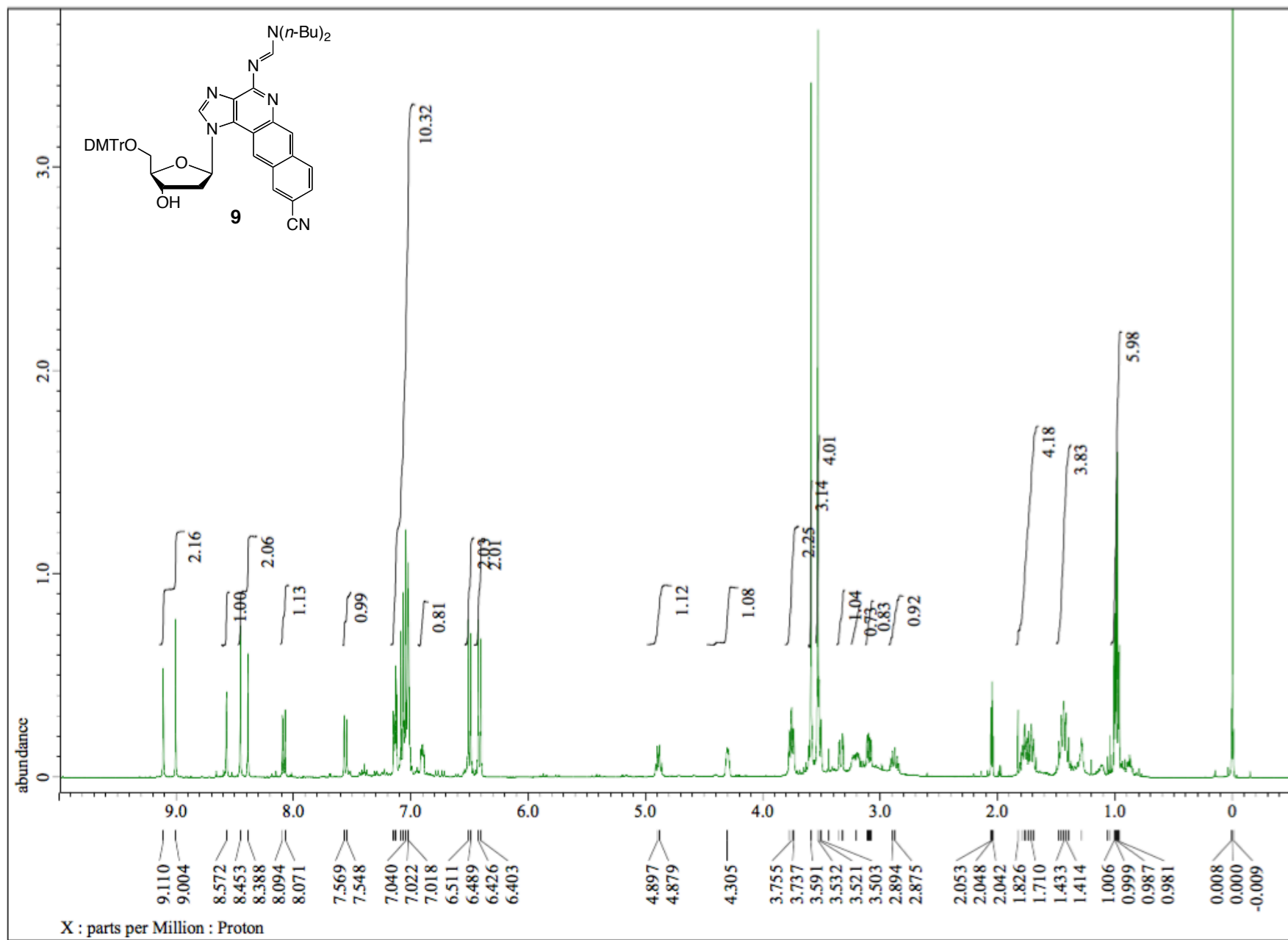


Figure S17. ¹H-NMR spectrum of compound **9** (acetone-d₆)

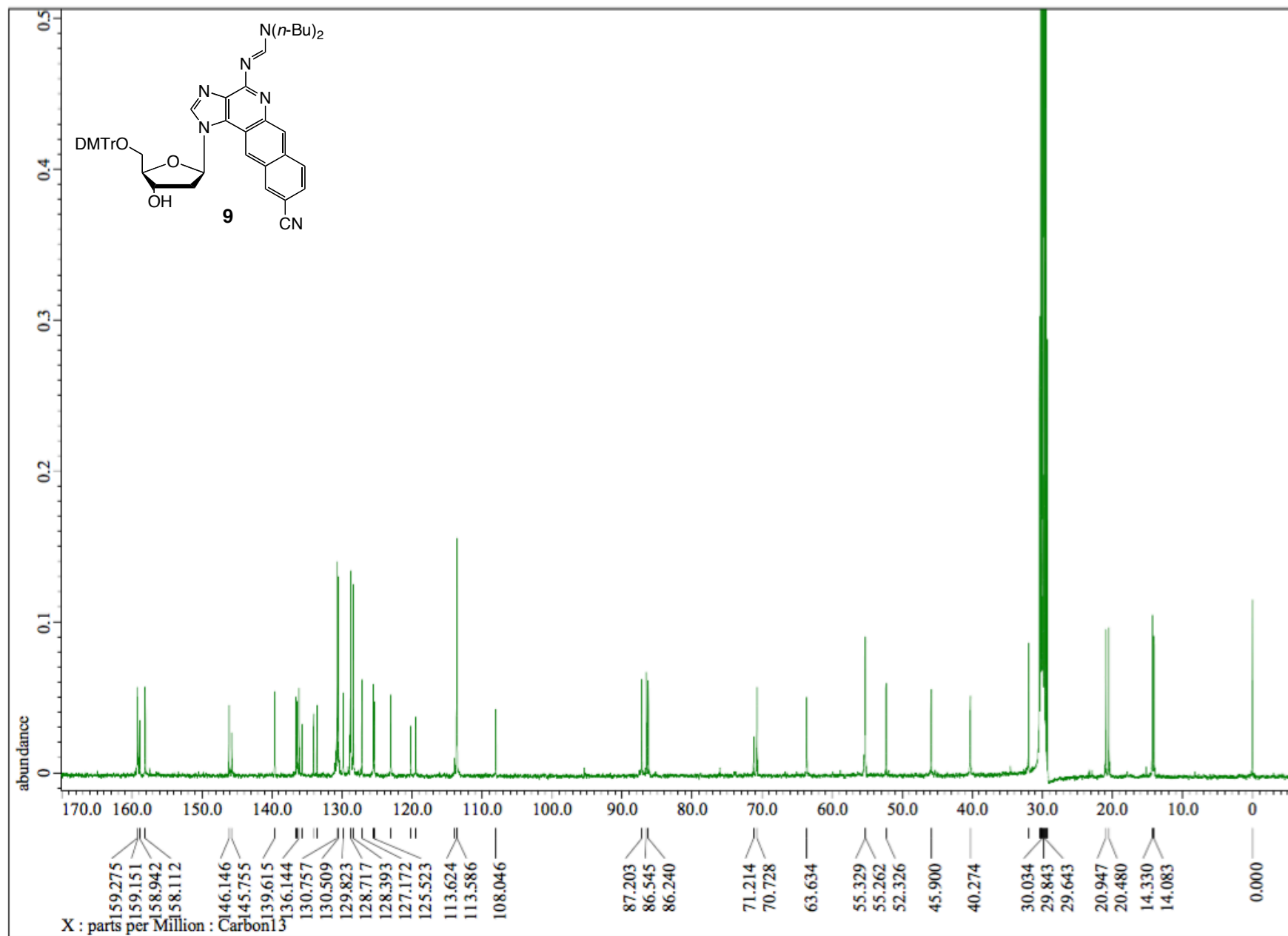


Figure S18. ¹³C-NMR spectrum of compound **9** (acetone-*d*₆)