

Electronic Supplementary Information.

Photolabile *N*-hydroxypyrid-2(1*H*)-one derivatives of guanine nucleosides: a new method for independent guanine radical generation.

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Experimental

General

¹H NMR and ¹³C NMR spectra were recorded on a Varian Mercury 200 MHz or 400 MHz spectrometers, in deuterated solvents by using the residual solvent peak as internal standard. FT-IR spectra were recorded on a Perkin-Elmer 841 or 1760X Fourier transform infrared spectrometer using solutions in CHCl₃. UV spectra were recorded on a Varian Cary 50 Conc. TLC was carried out on SiO₂ (silica gel F₂₅₄). Flash chromatography was carried out on SiO₂ (silica gel 60, SDS, 230-400 mesh ASTM). For reactions conducted under inert atmosphere, flasks were flame dried and purged with dry argon until cool.

Mass spectrometry measurements

Electrospray Ionization (ESI) mass spectrometry analyses of the nucleoside derivatives together with those of the modified oligonucleotides were conducted on a Finnigan AQA quadrupole, an LCQ Ion Trap (ThermoFinnigan, San Jose, CA) or a Finnigan LTQ-FT spectrometer. Samples were diluted in a solution of AcCN and water (50/50, v/v) that contained either 0.1% formic acid (positive mode) or 1% NH₃ (negative mode) at a concentration of 10 μmol/L. The samples were introduced into the electrospray ion source by a syringe pump at a flow rate of 10 μL/min.

Nanosecond laser flash photolysis

For excitation at 308 nm, an excimer laser with a Xe-HCl-Ne mixture was used. The single

pulses were ca. 17 ns duration and the energy was 200 mJ output at the source. For both systems, a pulsed Lo255 Oriel (Stratford, CT) xenon lamp was employed as the detecting light source. The laser flash photolysis apparatus consisted of the pulsed laser, the Xe lamp, a 77200 Oriel monochromator, an Oriel photomultiplier tube (PMT) system made up of a 77348 side-on PMT tube, 70680 PMT housing and a 70705 PMT power supply. The output signal from the oscilloscope (TDS-640A Tektronix, Köln, Germany) was transferred to a personal computer.

Concentrations for the guanosine derivatives and the reference compound were *ca.* 5×10^{-5} M and 9×10^{-5} M, respectively. These values ensured an absorbance of ~ 0.4 in the laser cell at the excitation wavelength. Each sample received only one shot; then fresh solutions were used for a new measurement. The spectra were recorded at intervals of 20 nm. When different thiols were added to the solution of the guanosine derivatives, their concentrations ranged from equimolar to 50 times for the case of tert-butylthiol and glutathione. In one experiment, isopropanol was used both as hydrogen donor and as solvent. For the measurements of quenching by oxygen, solutions were air-equilibrated or bubbled with oxygen.

Photolysis of **2c**

Photolysis experiments were performed with a variable intensity Xenon arc lamp (150 W) equipped with a UV-A filter. Solutions were prepared and irradiated in pyrex glass vials with a 4.5 mW/cm^2 local light intensity. The reaction mixture containing **2c** (1.0 mM) in water in a pyrex glass vial was irradiated for 30 min either under argon or under aerobic conditions at room temperature. The photolysis progress was followed by reverse phase HPLC a Zorbax SB-C18 column (4.6 x 150 mm), eluted in triethylammonium acetate buffer (20 mM, pH 7) with a 0-25% acetonitrile non linear gradient over 30 min with a flow rate of 1.0 mL/min (detection at 254 nm) as well as with LC-ESI-MS carried out on a Alltima HP C-18 column (2.1 x 150 mm), eluted with a solvent mixture of 0.1% TFA, 0-25% acetonitrile/ 30 min at a flow rate of 0.2 mL/min (detection at 254 nm).

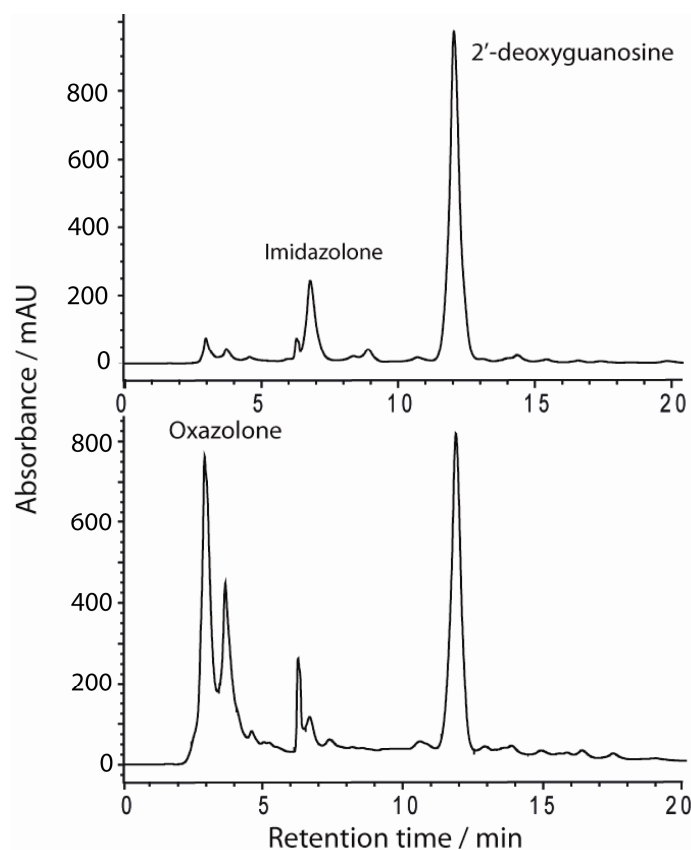


Fig. S1 HPLC chromatogram showing the photolysis of **2c** after 20 min (upper) and after subsequent incubation for 24 h at 37°C.

Photolysis of **8d**

The reaction mixture containing **8d** (1.0 mM) in water in a pyrex glass vial was irradiated for 25 min under aerobic conditions at room temperature. The photoirradiated mixture was incubated for 24 h at room temperature in the dark and subsequently analyzed by reverse phase HPLC carried out on a Zorbax SB-C18 column (4.6 x 150 mm), eluted in triethylammonium acetate buffer (20 mM, pH 7) with a 0-25% acetonitrile non linear gradient over 30 min with a flow rate of 1.0 mL/min (detection at 254 nm) as well as with LC-ESI-MS carried out on a Alltima HP C-18 column (2.1 x 150 mm), with detection at 254 nm and elution with a solvent mixture of 0.1% TFA, 0-25% acetonitrile/30 min at a flow rate of 0.2 mL/min.

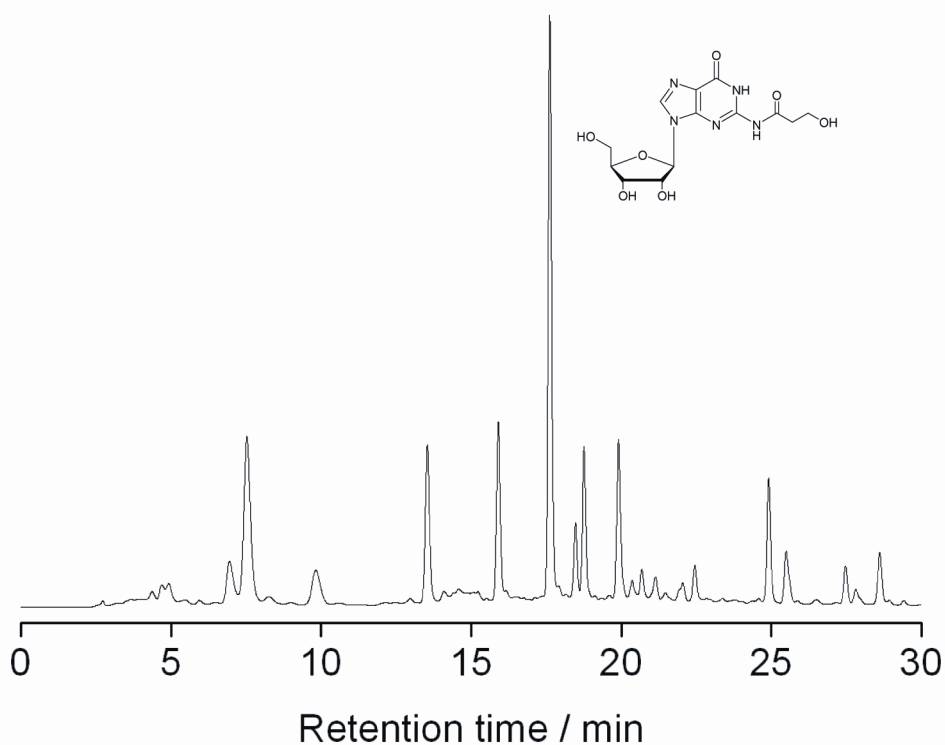


Fig. S2 HPLC chromatogram showing the photolysis of **8d** after 25 min and subsequent overnight incubation at ambient temperature.

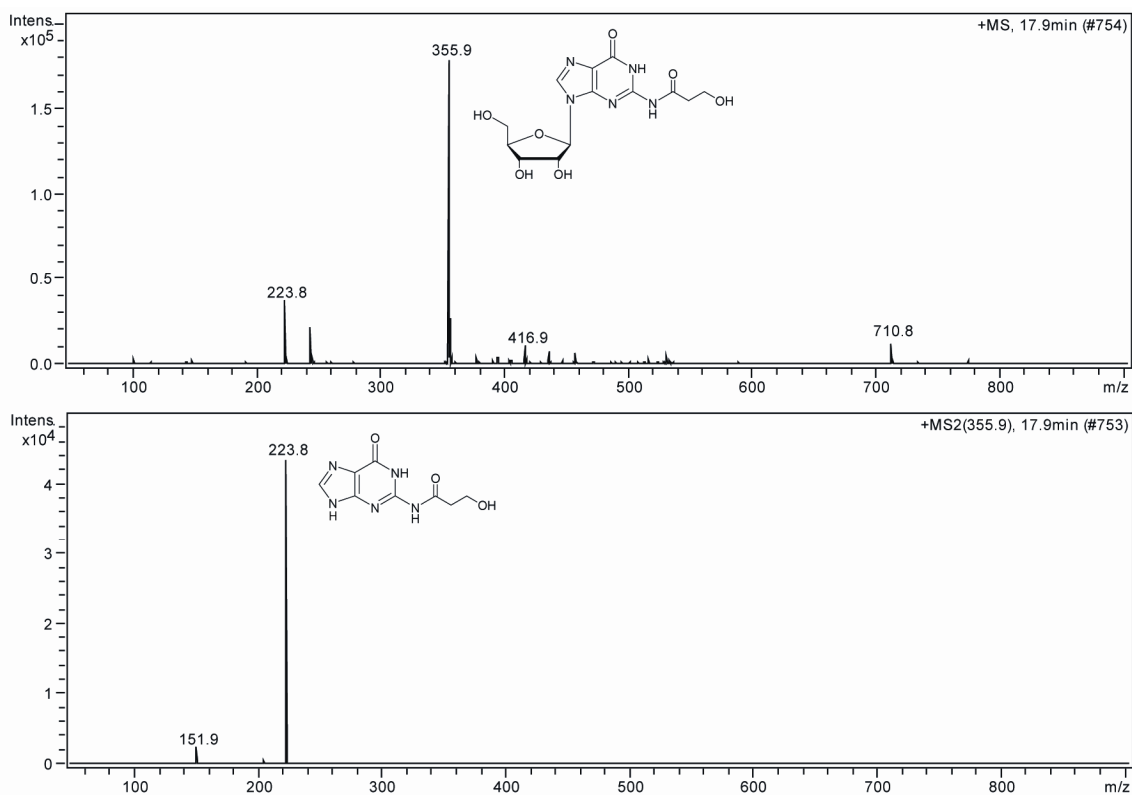
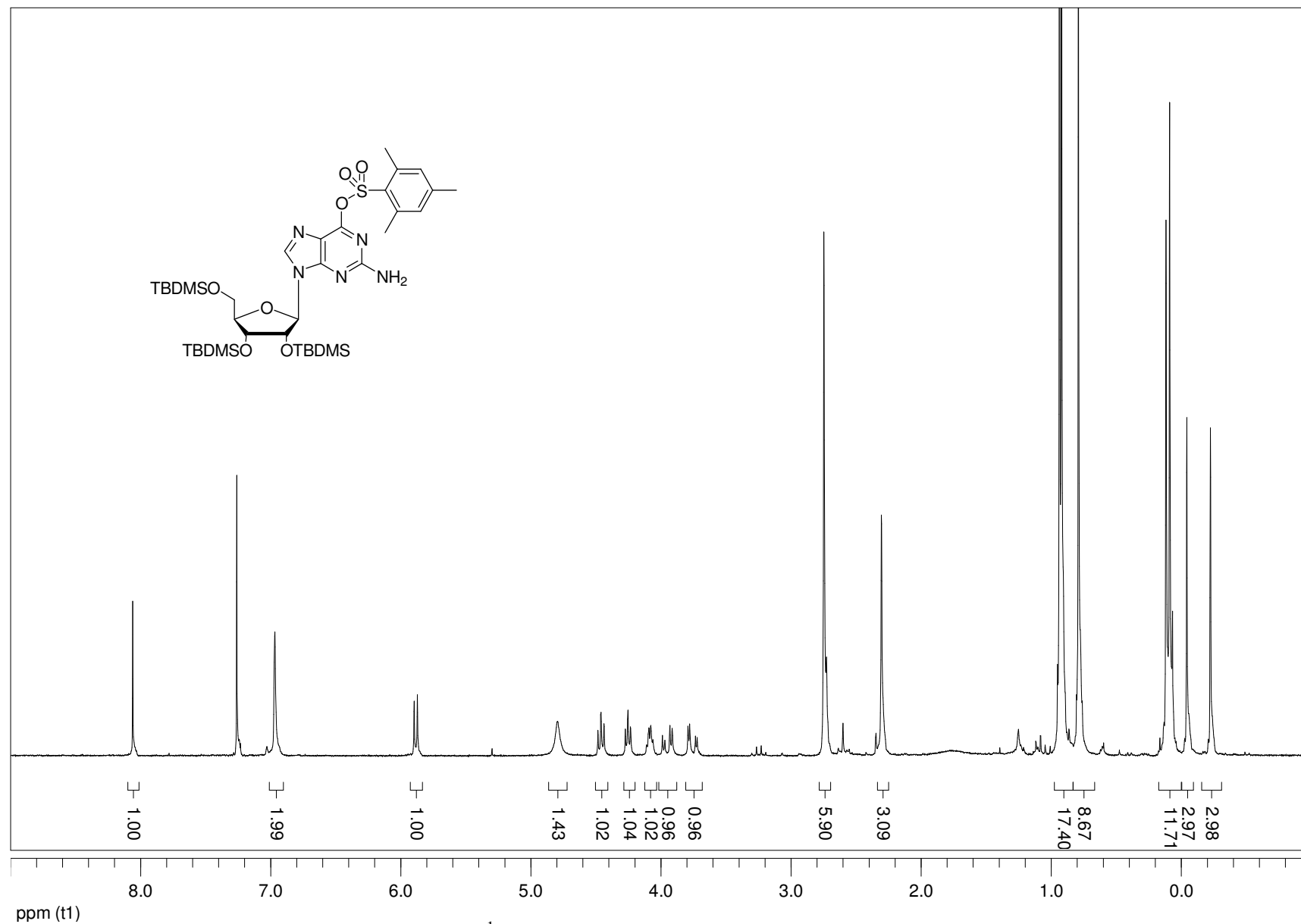
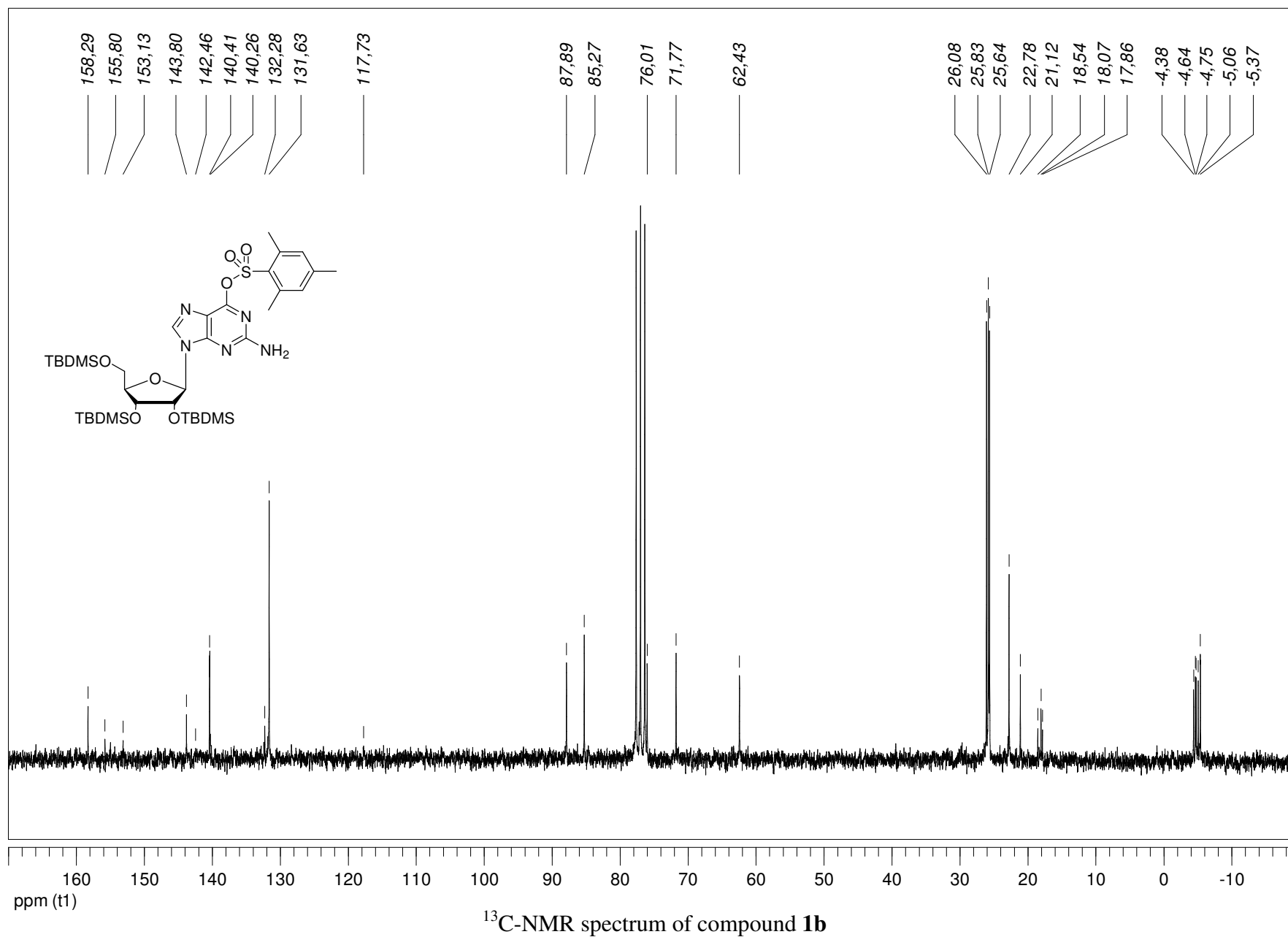
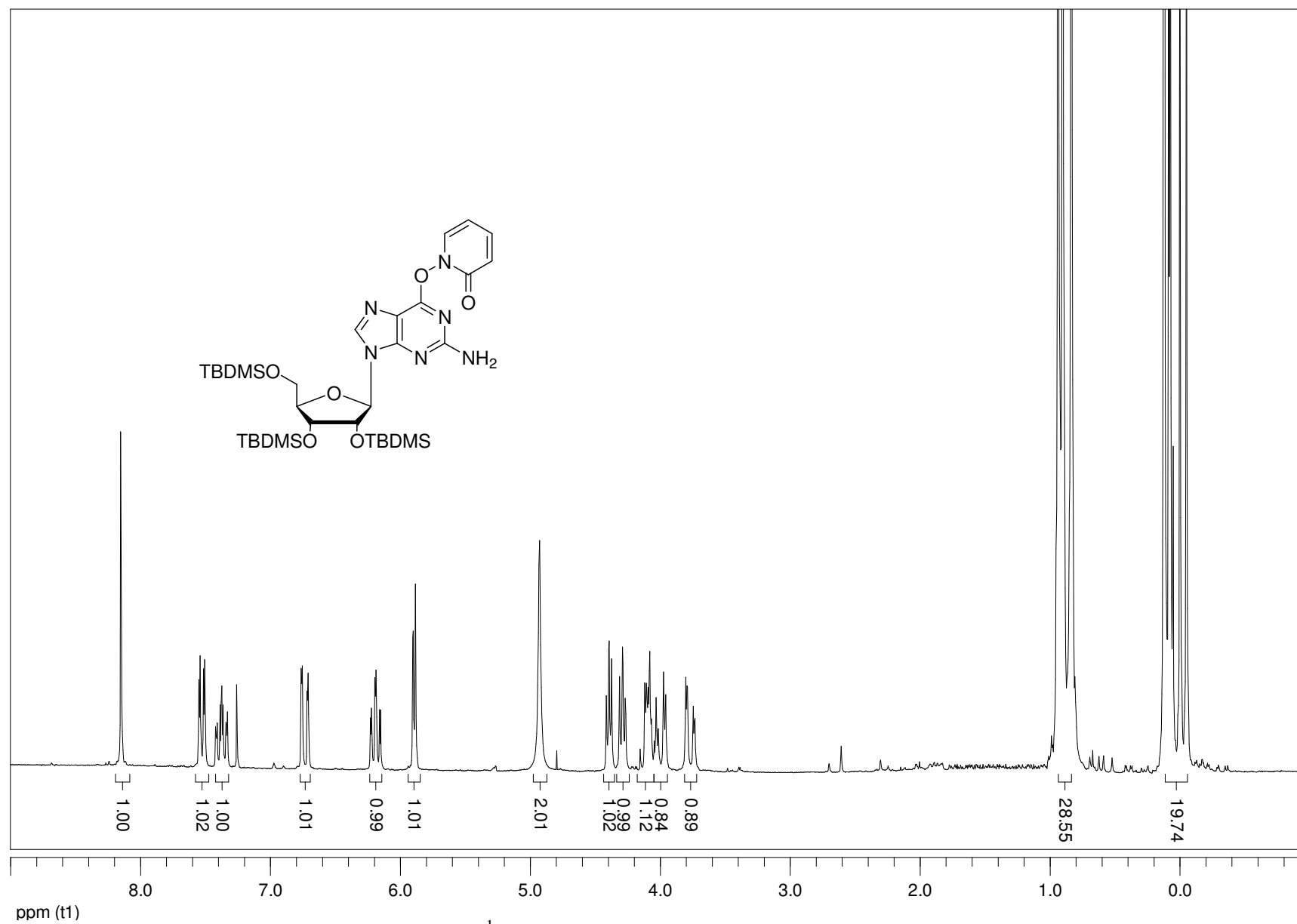


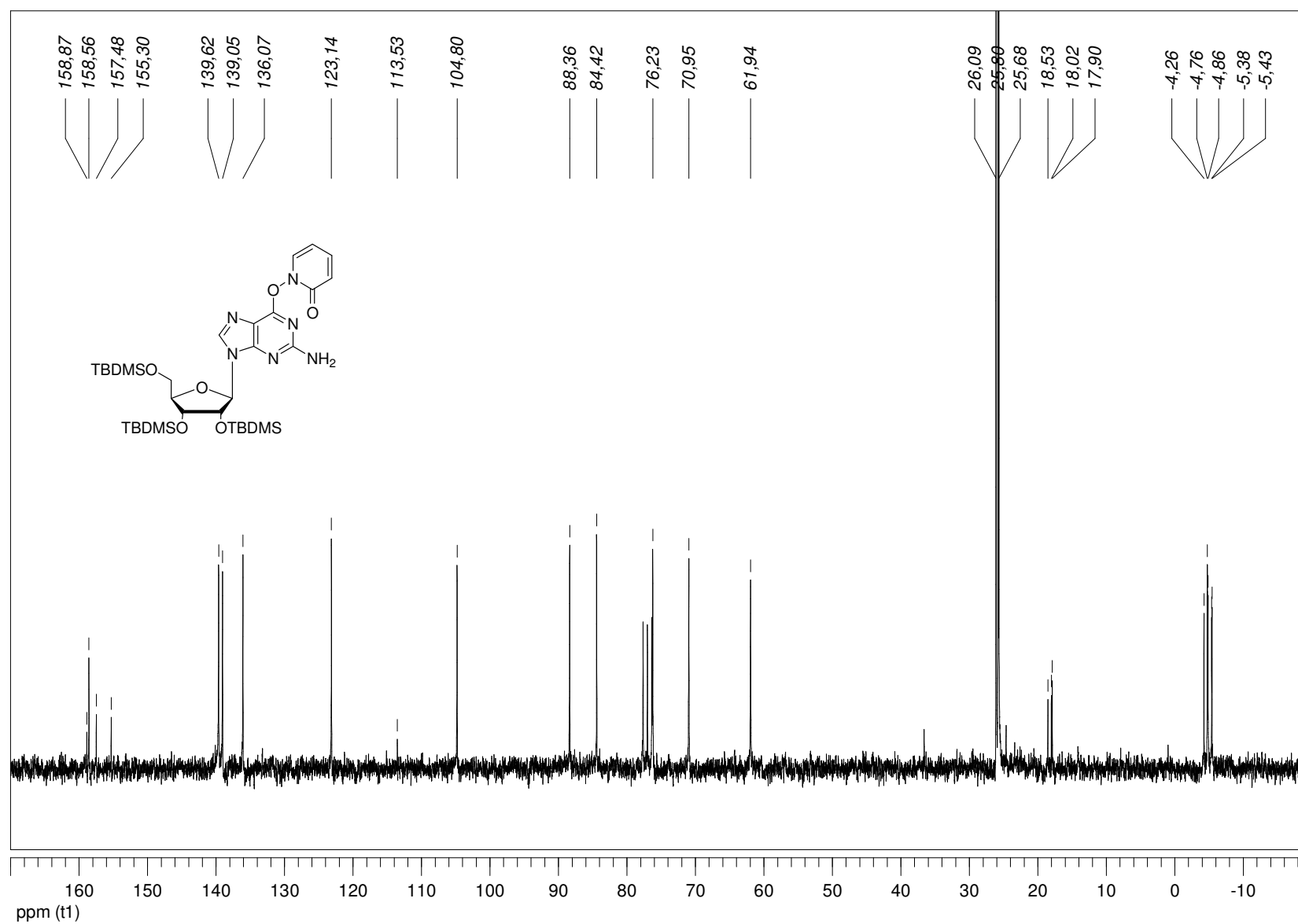
Fig. S3 LC-MS (upper) and LC-MS/MS (lower) analysis of the peak at 17.9 min (**14d**).



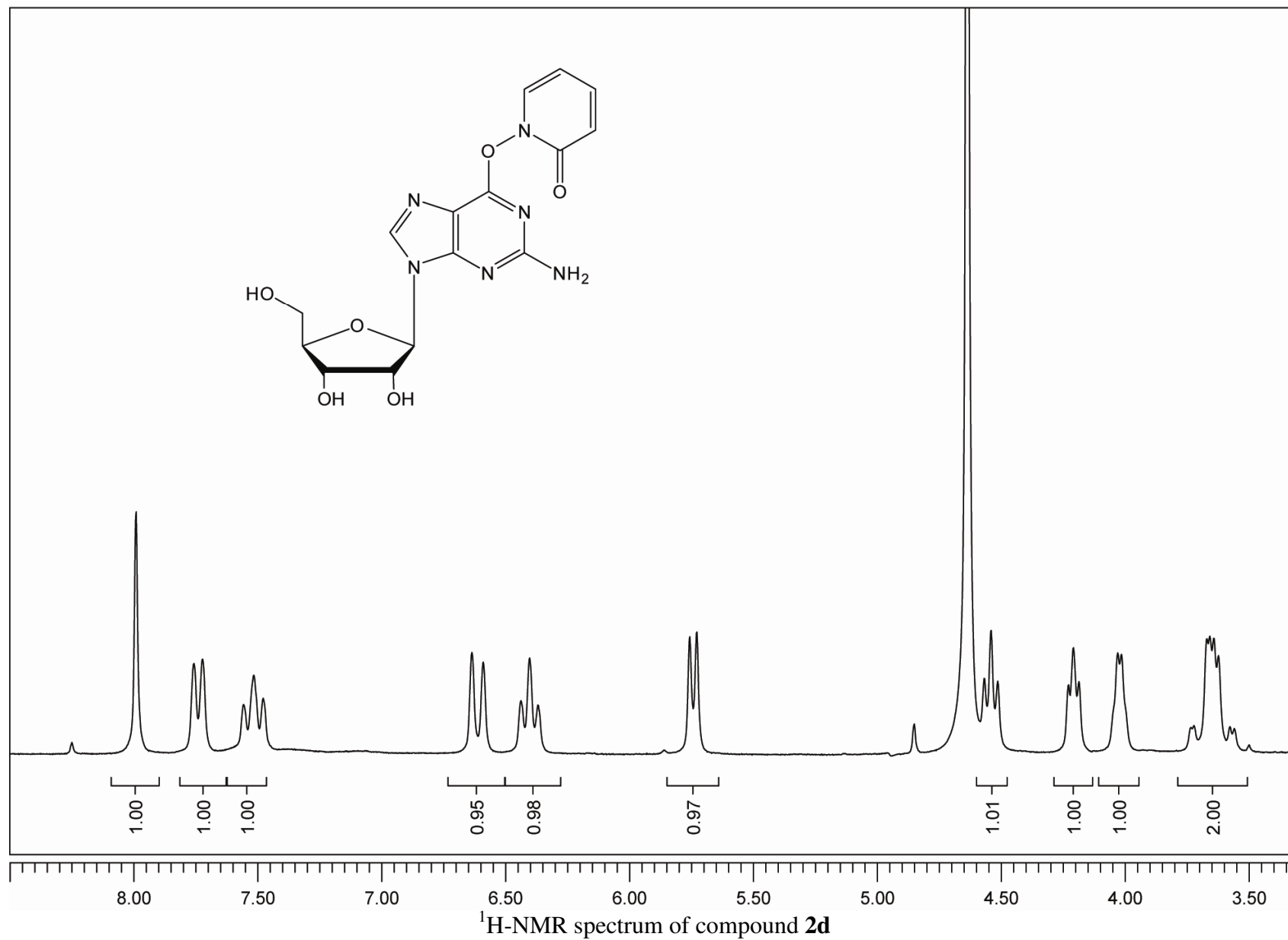


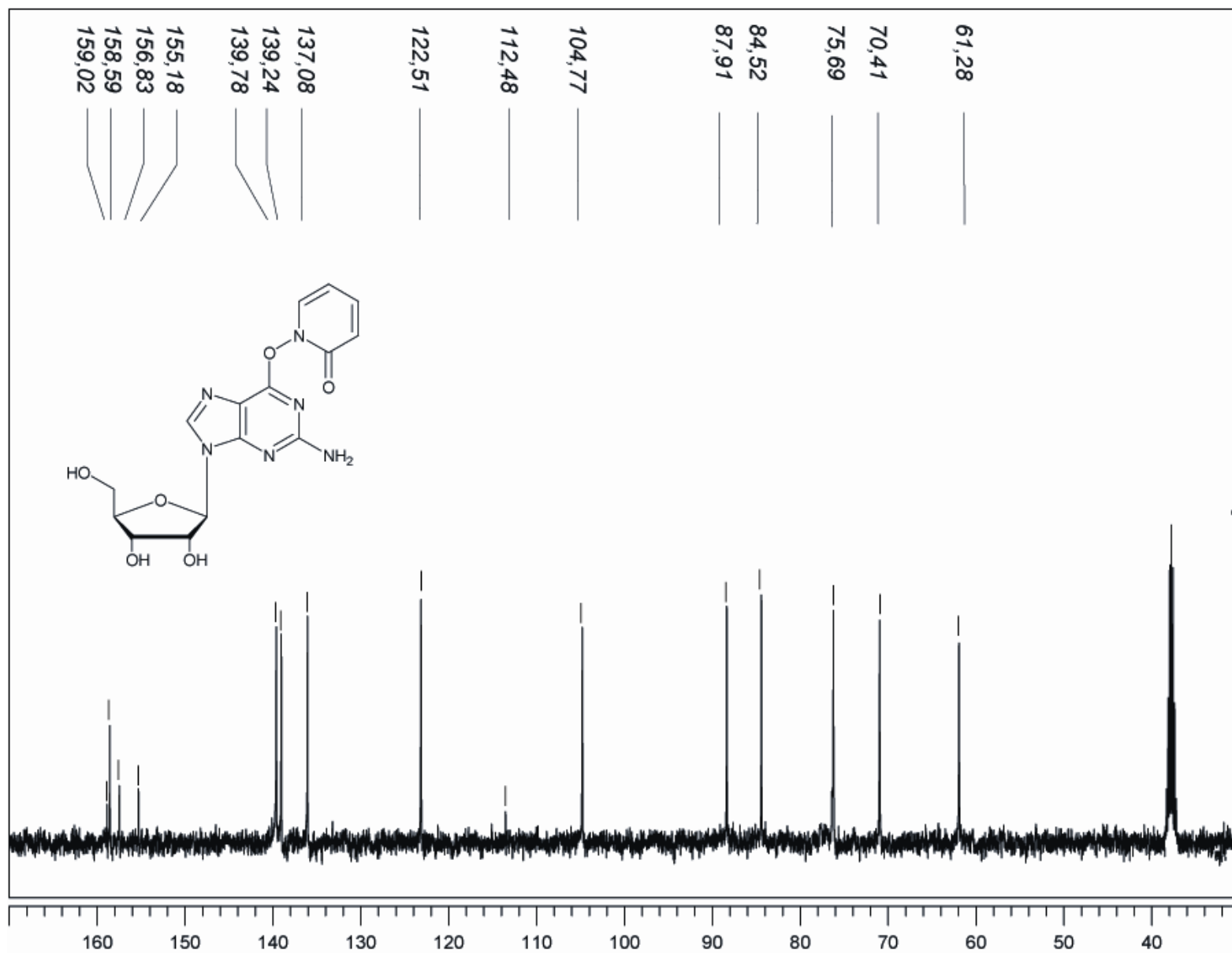


¹H-NMR spectrum of compound **2b**

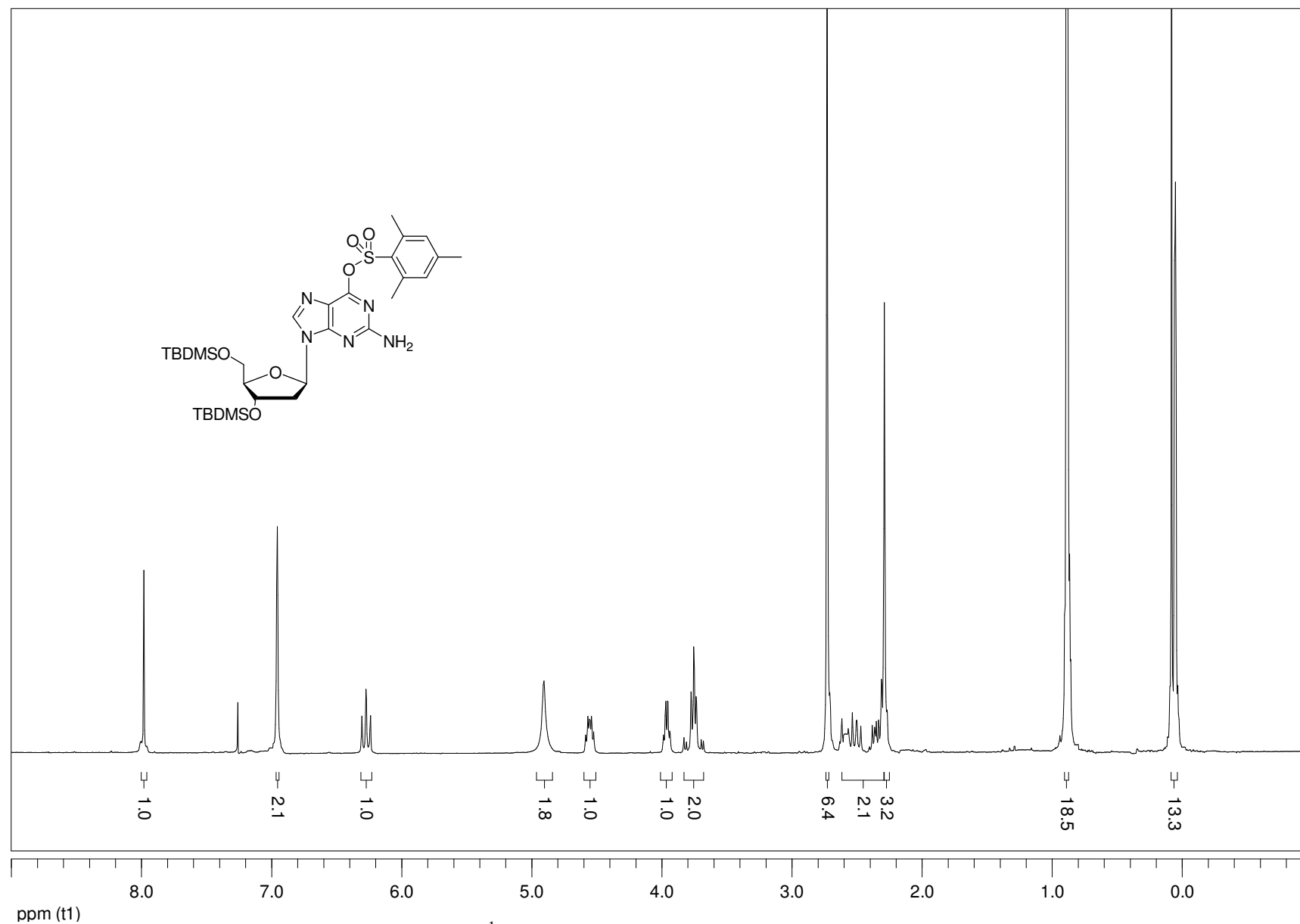


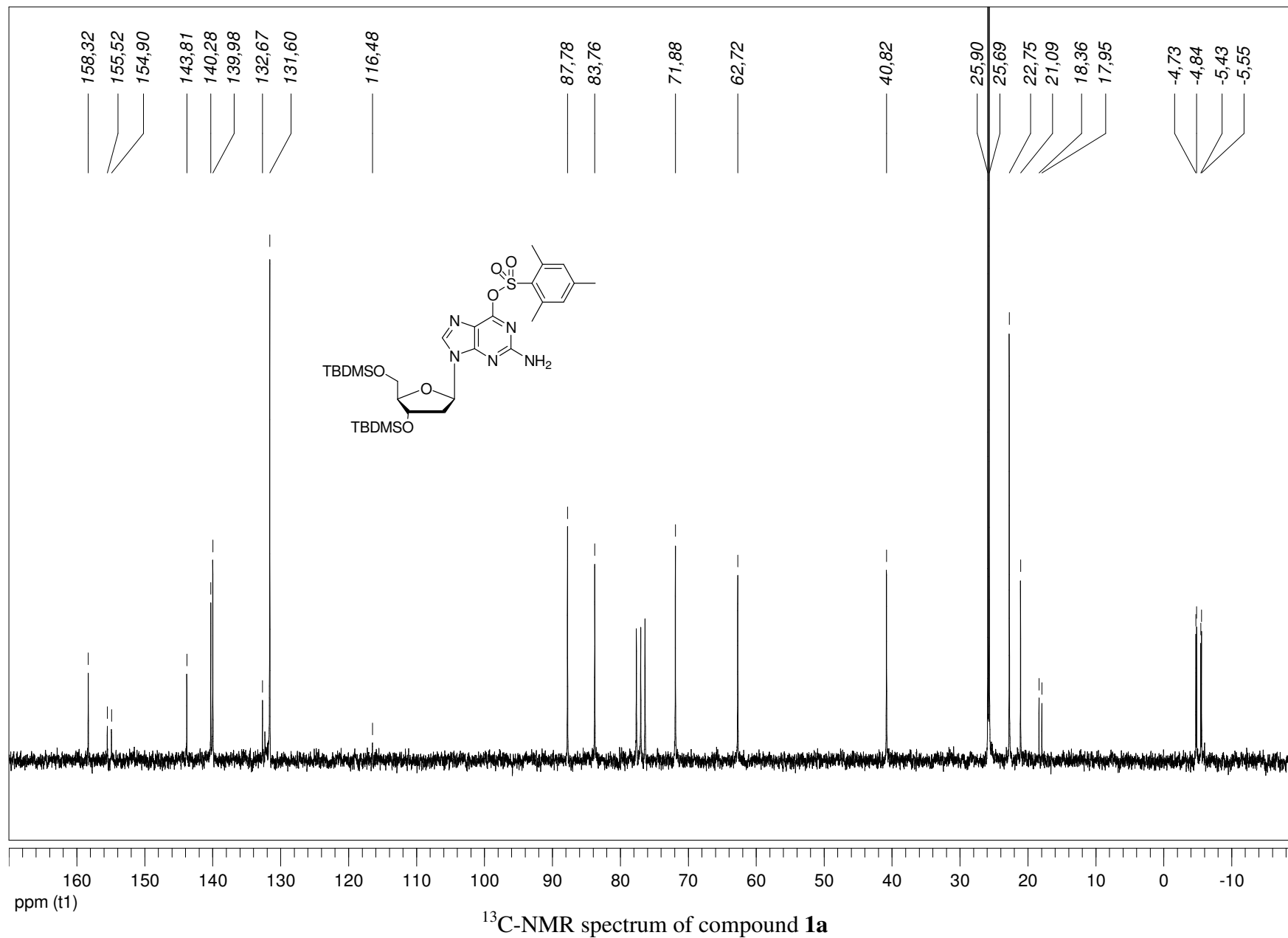
^{13}C -NMR spectrum of compound **2b**

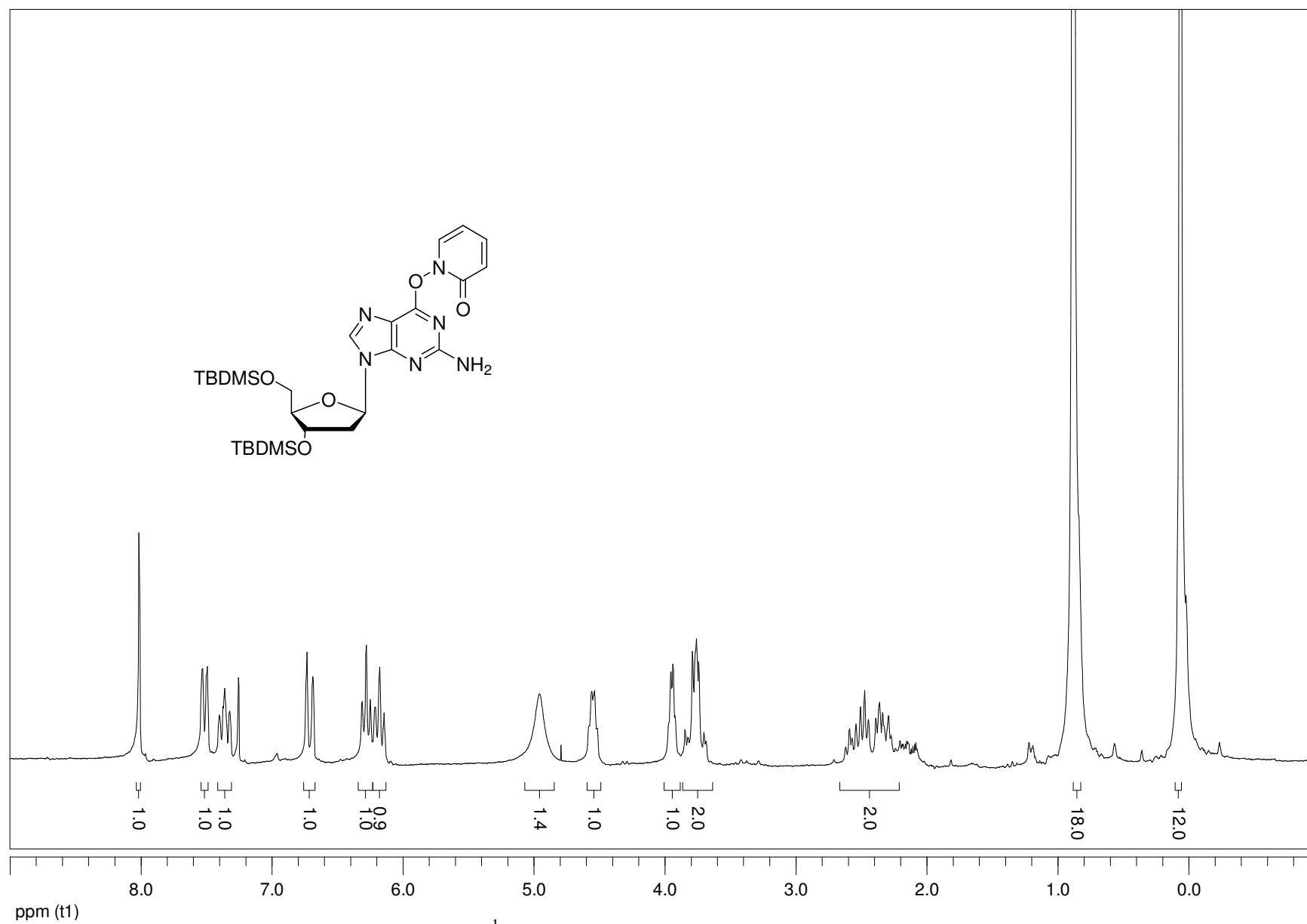




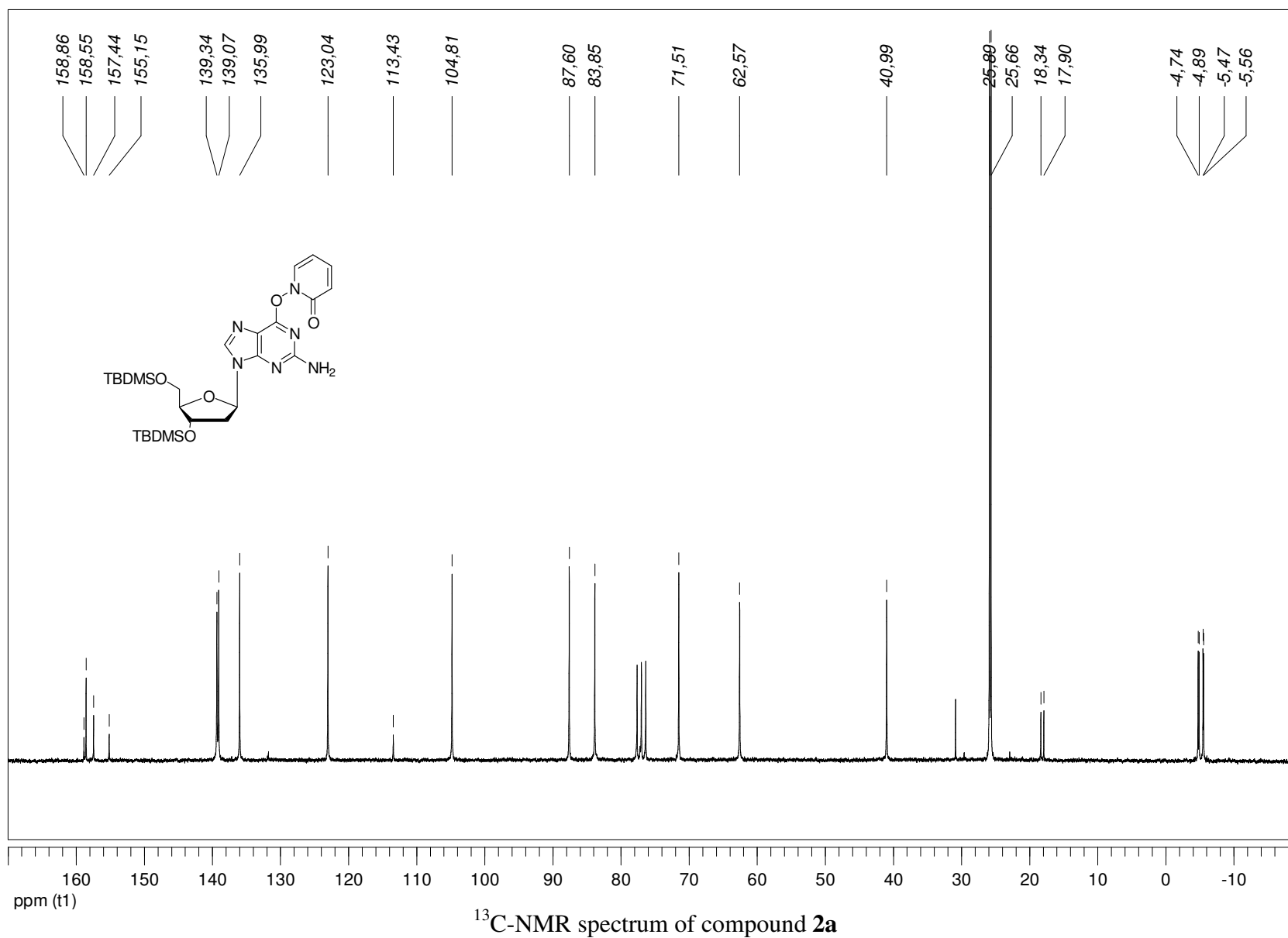
^{13}C -NMR spectrum of compound **2d**

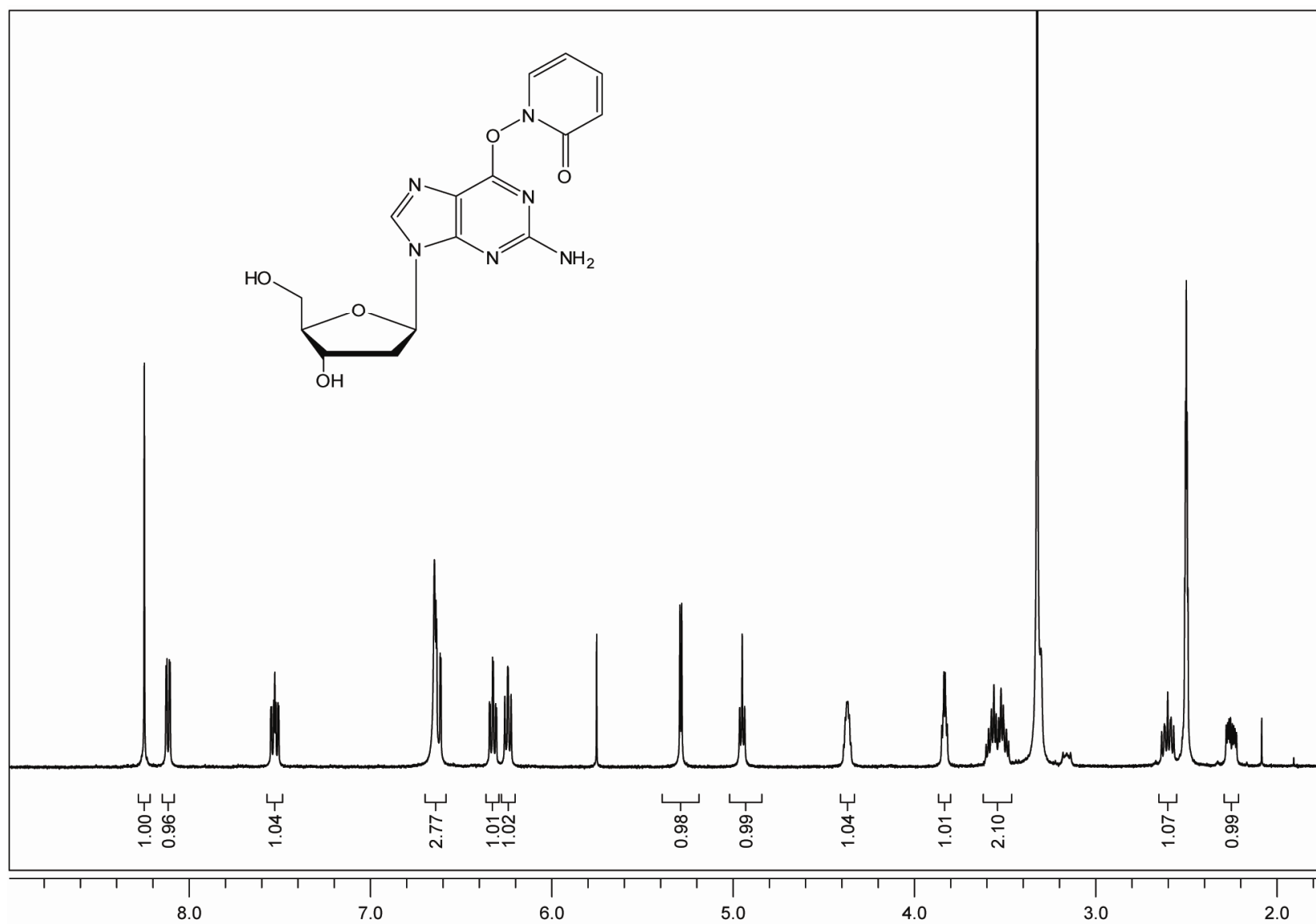




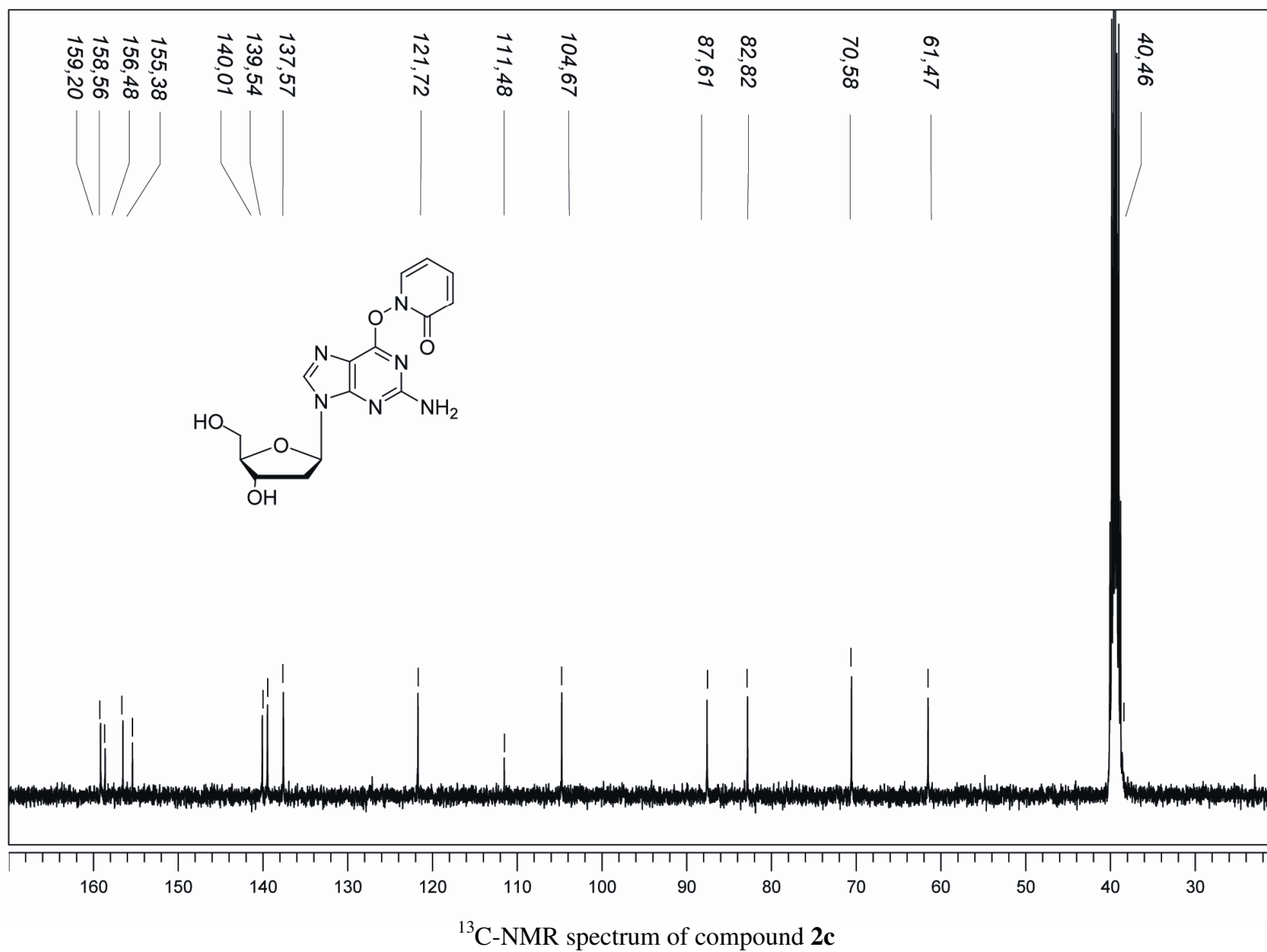


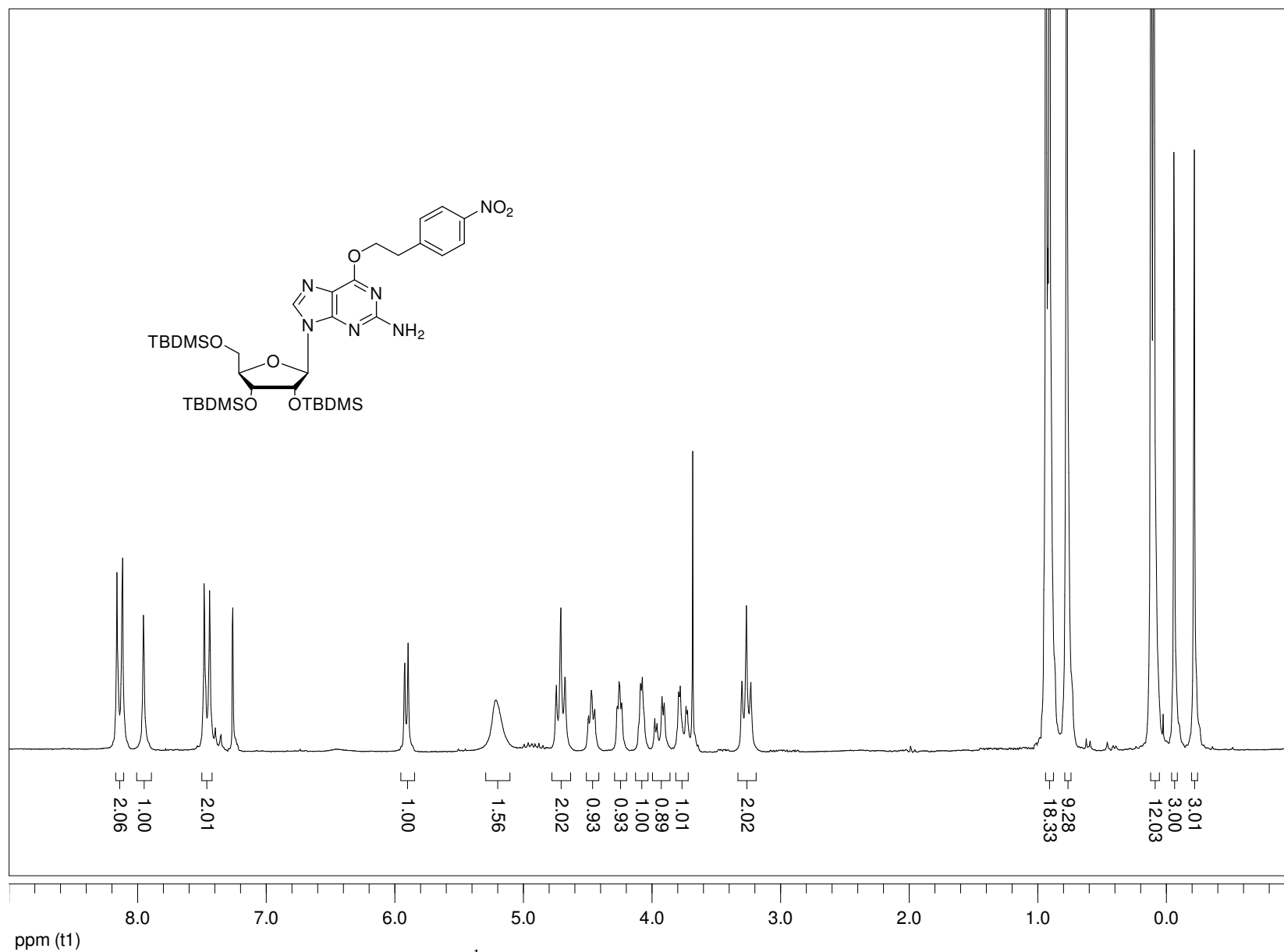
¹H-NMR spectrum of compound **2a**



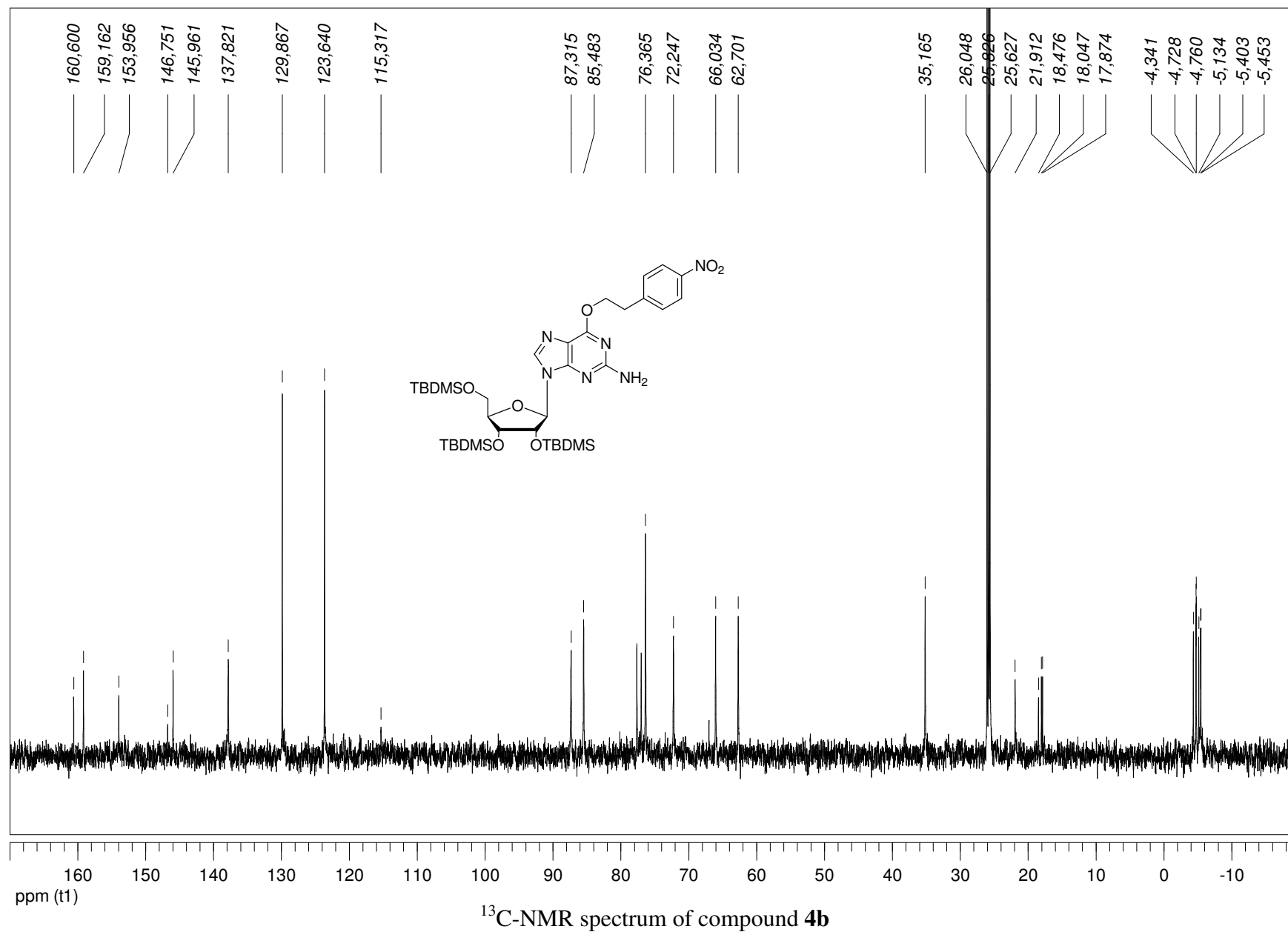


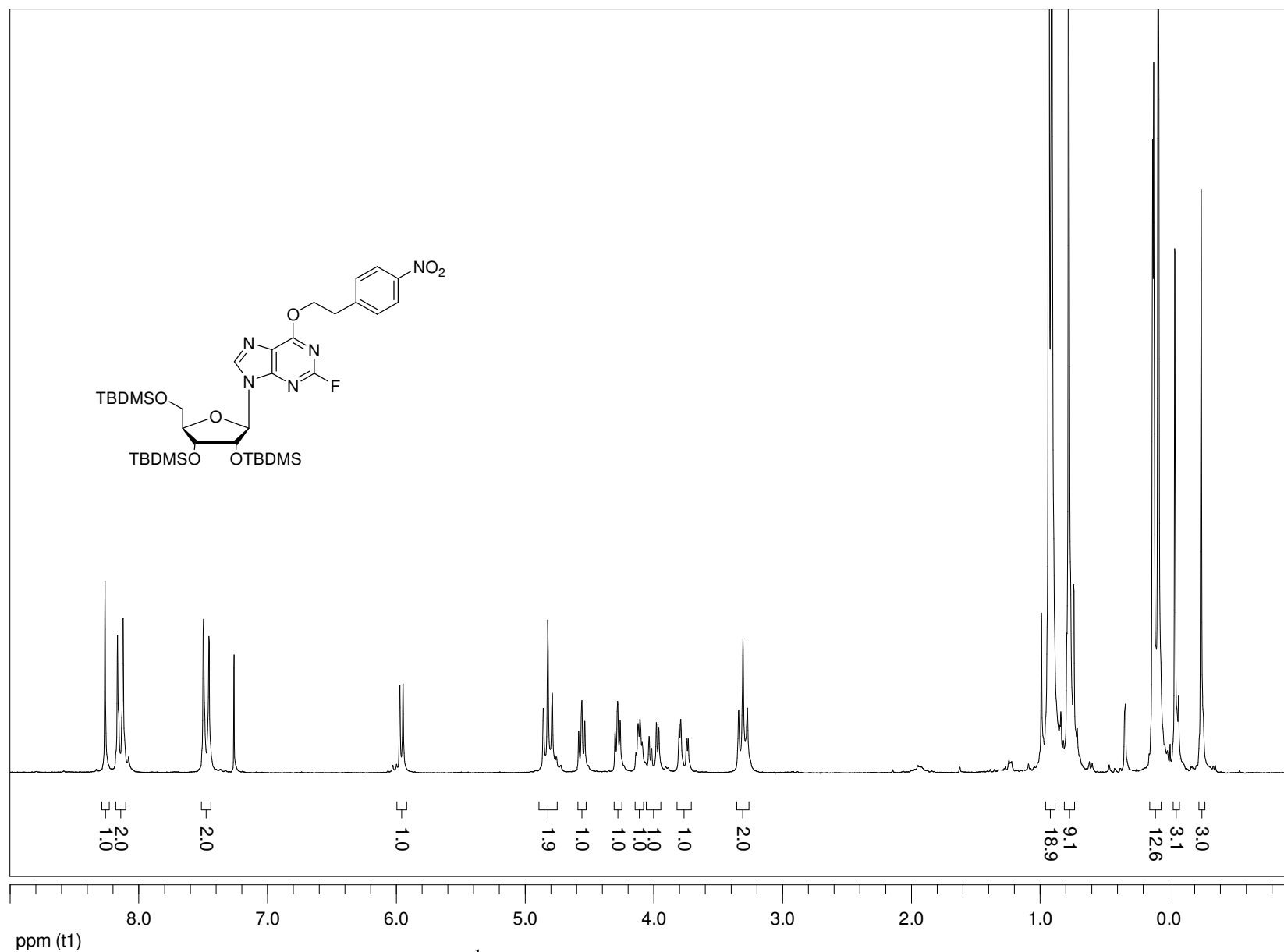
¹H-NMR spectrum of compound **2c**

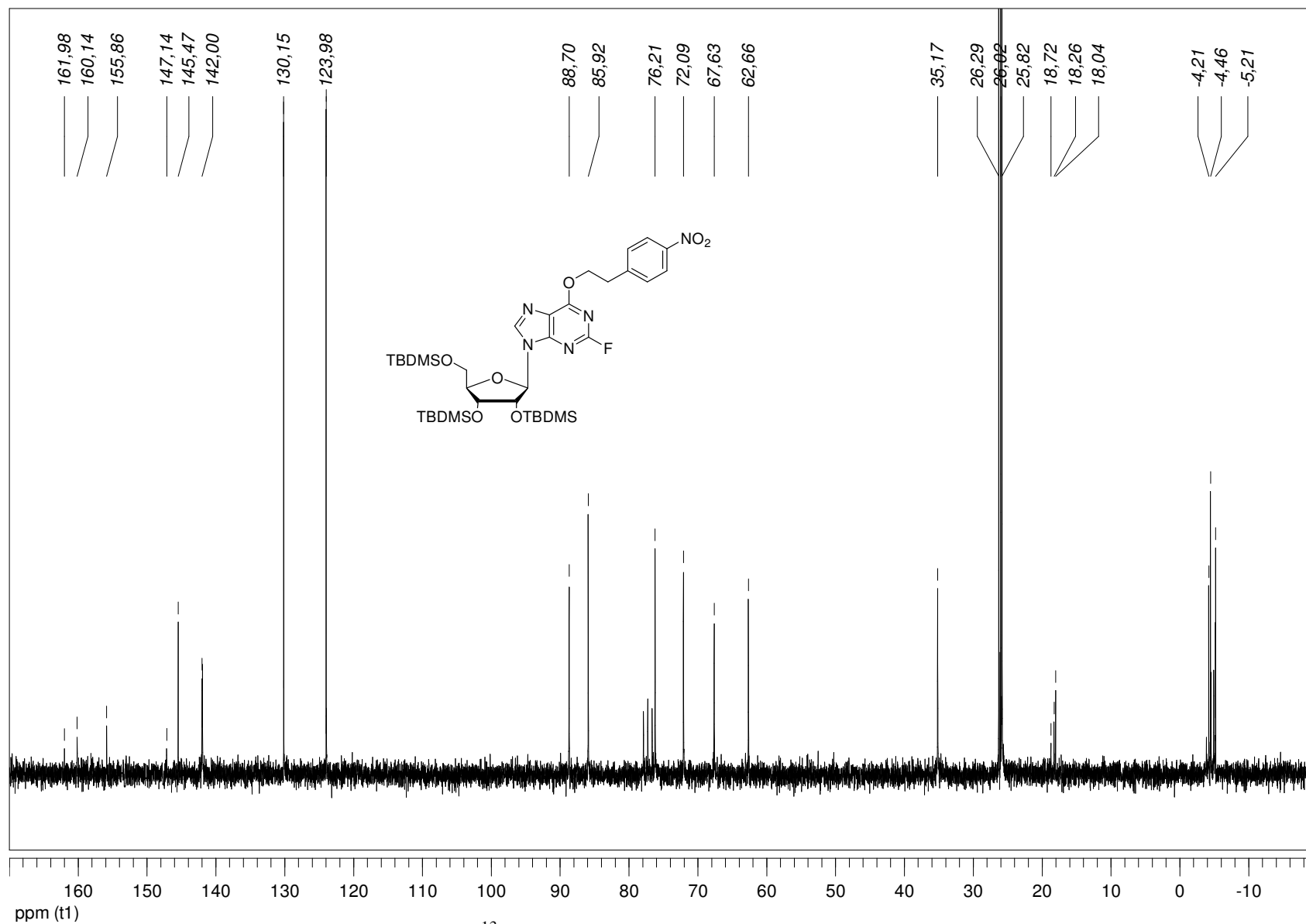




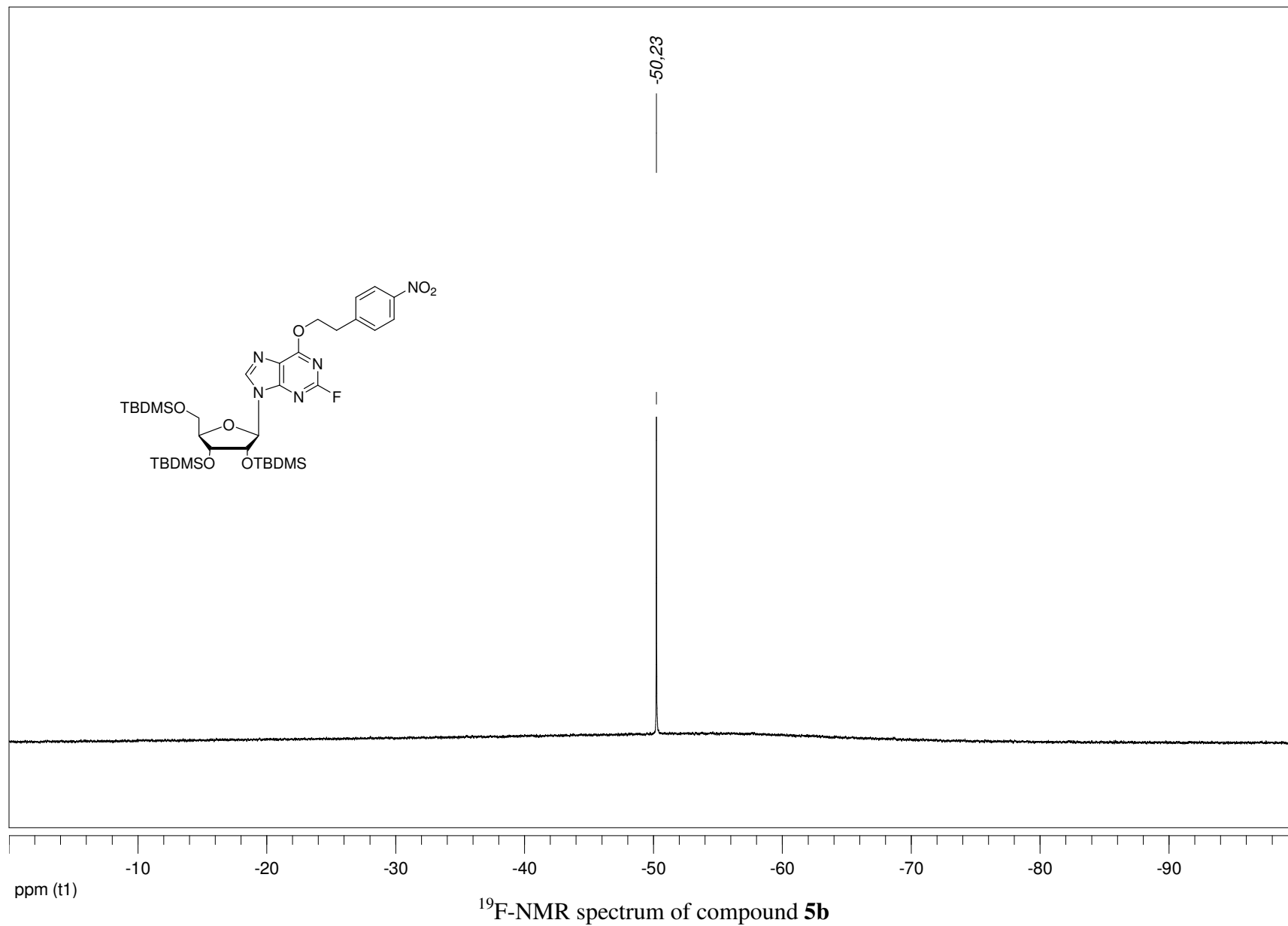
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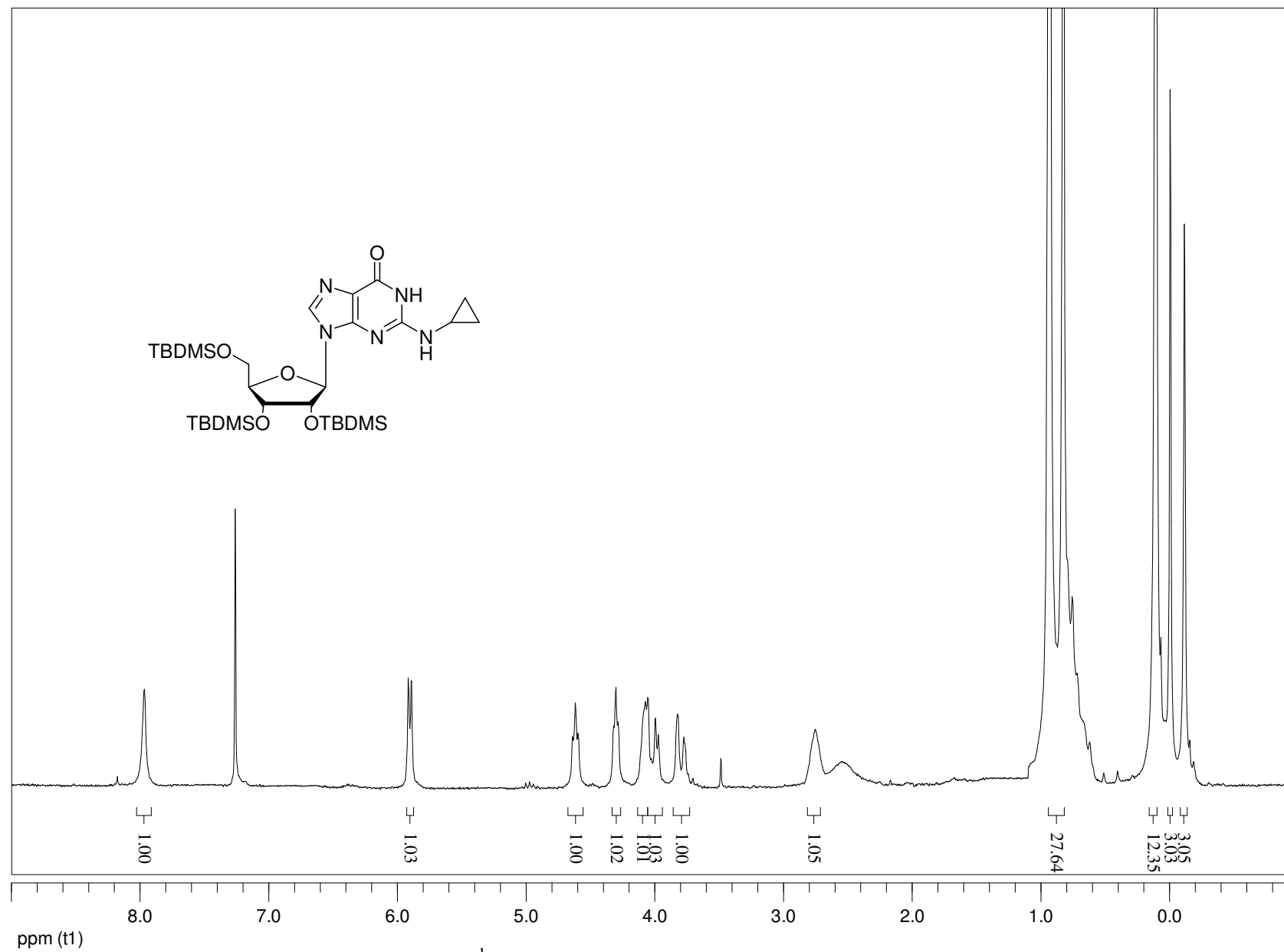


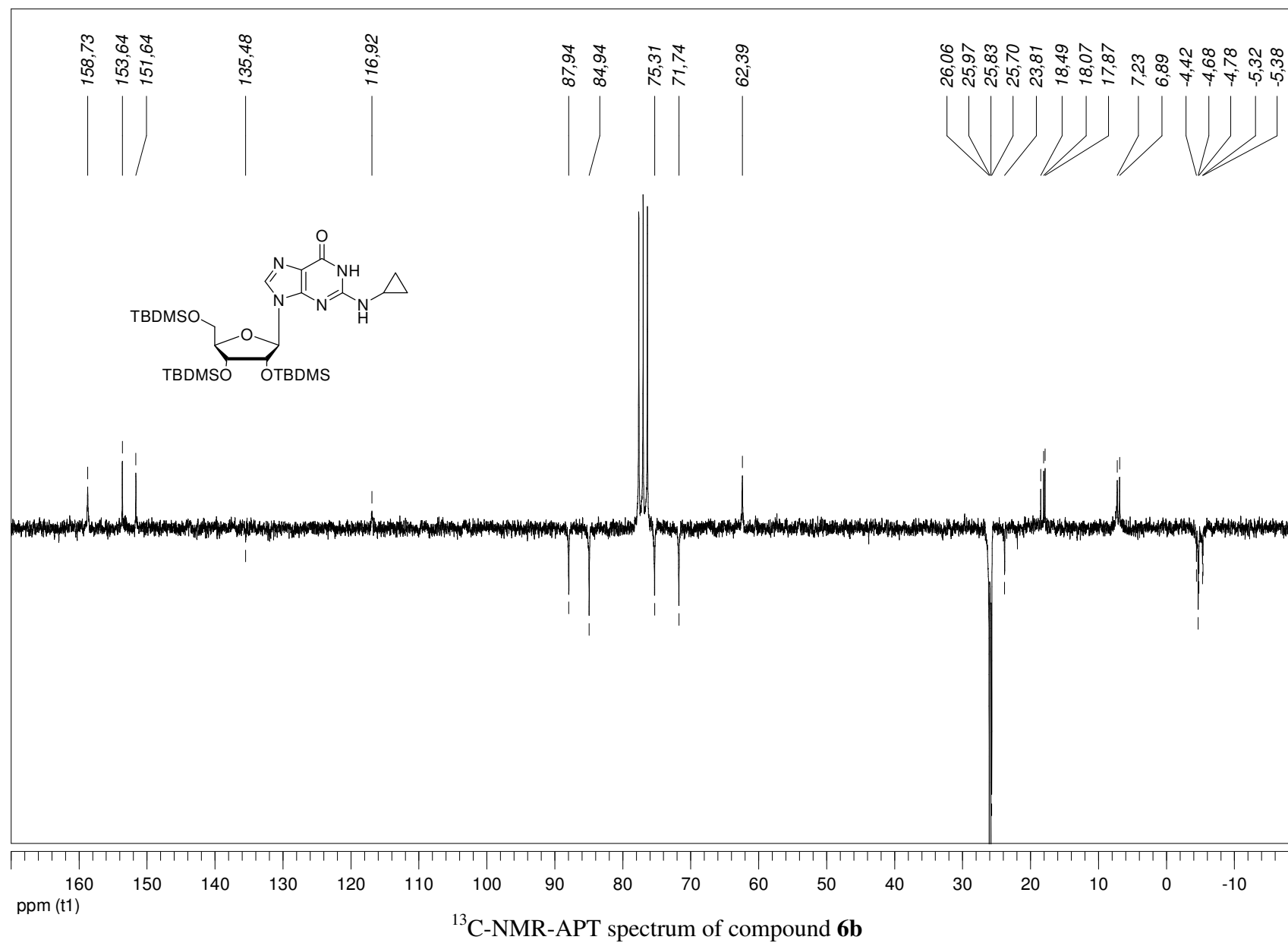


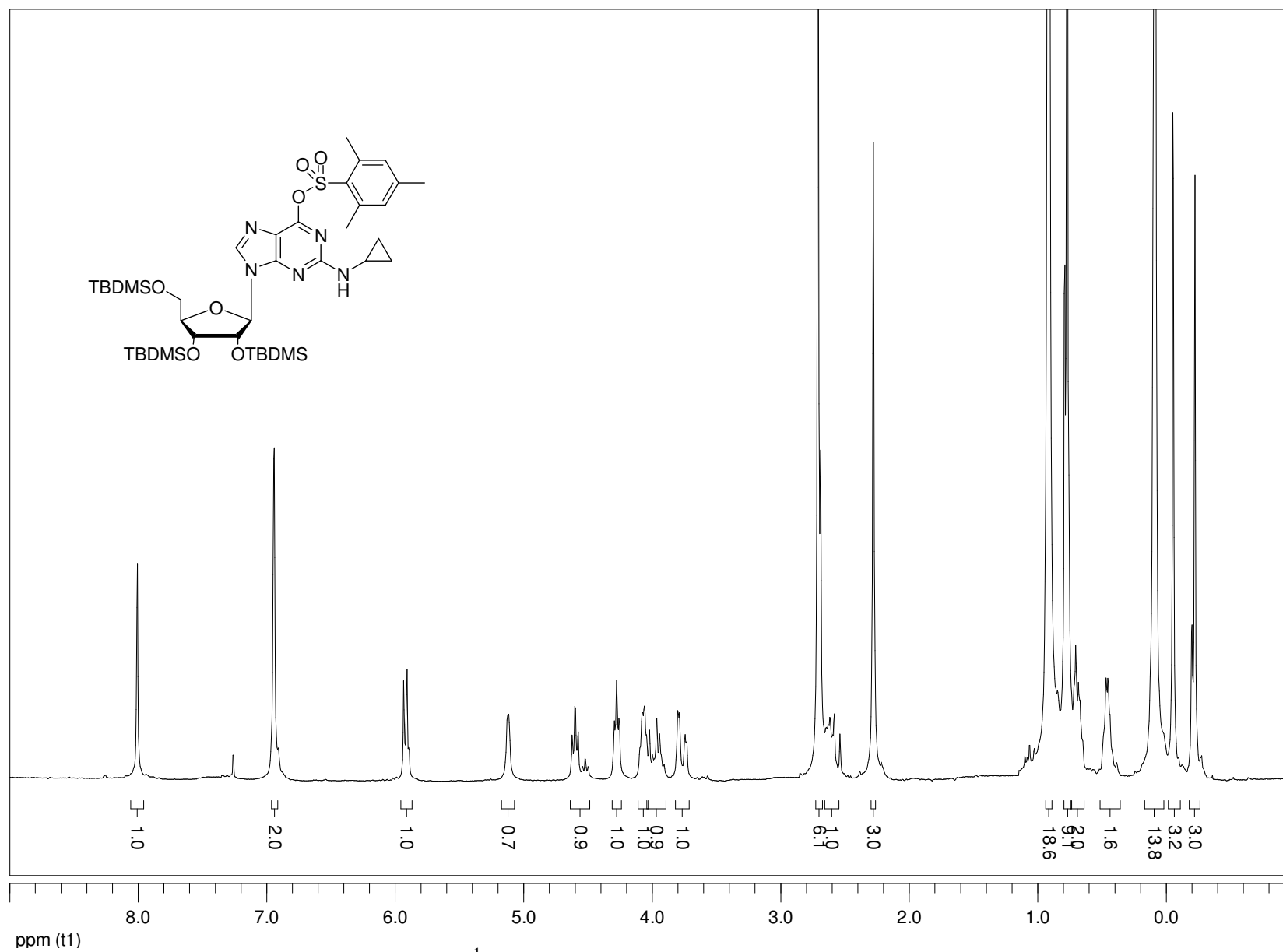


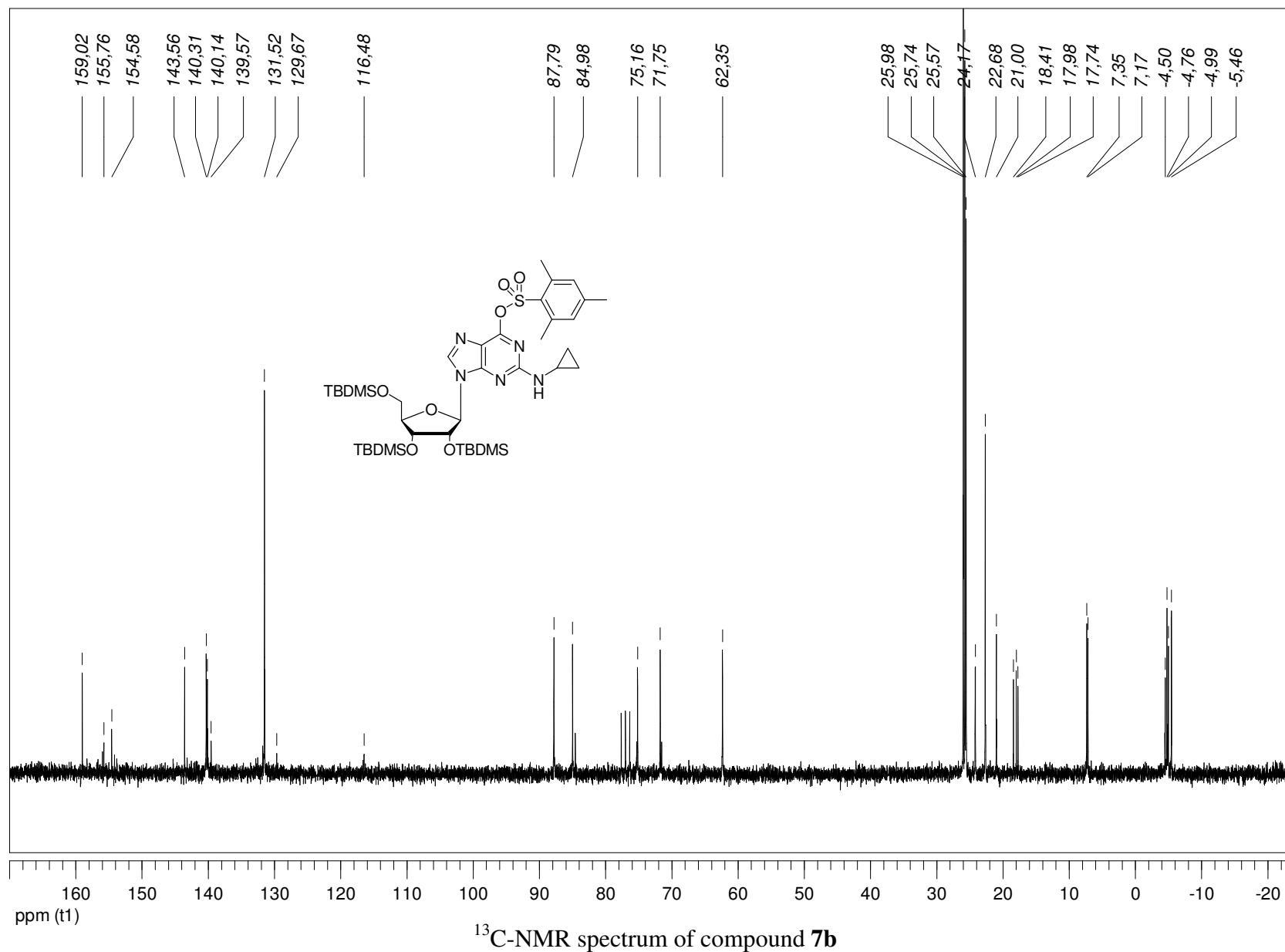
¹³C-NMR spectrum of compound **5b**

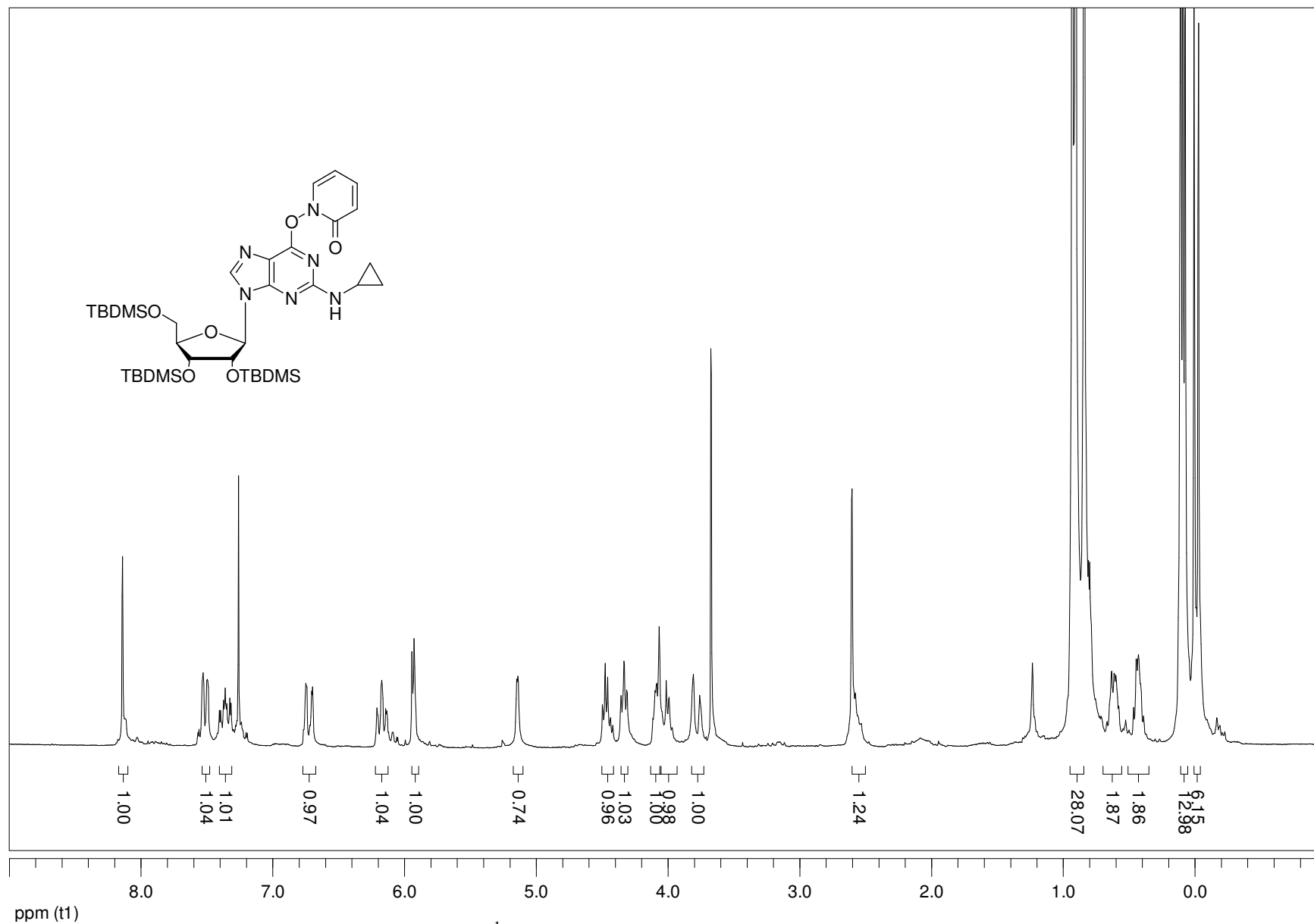




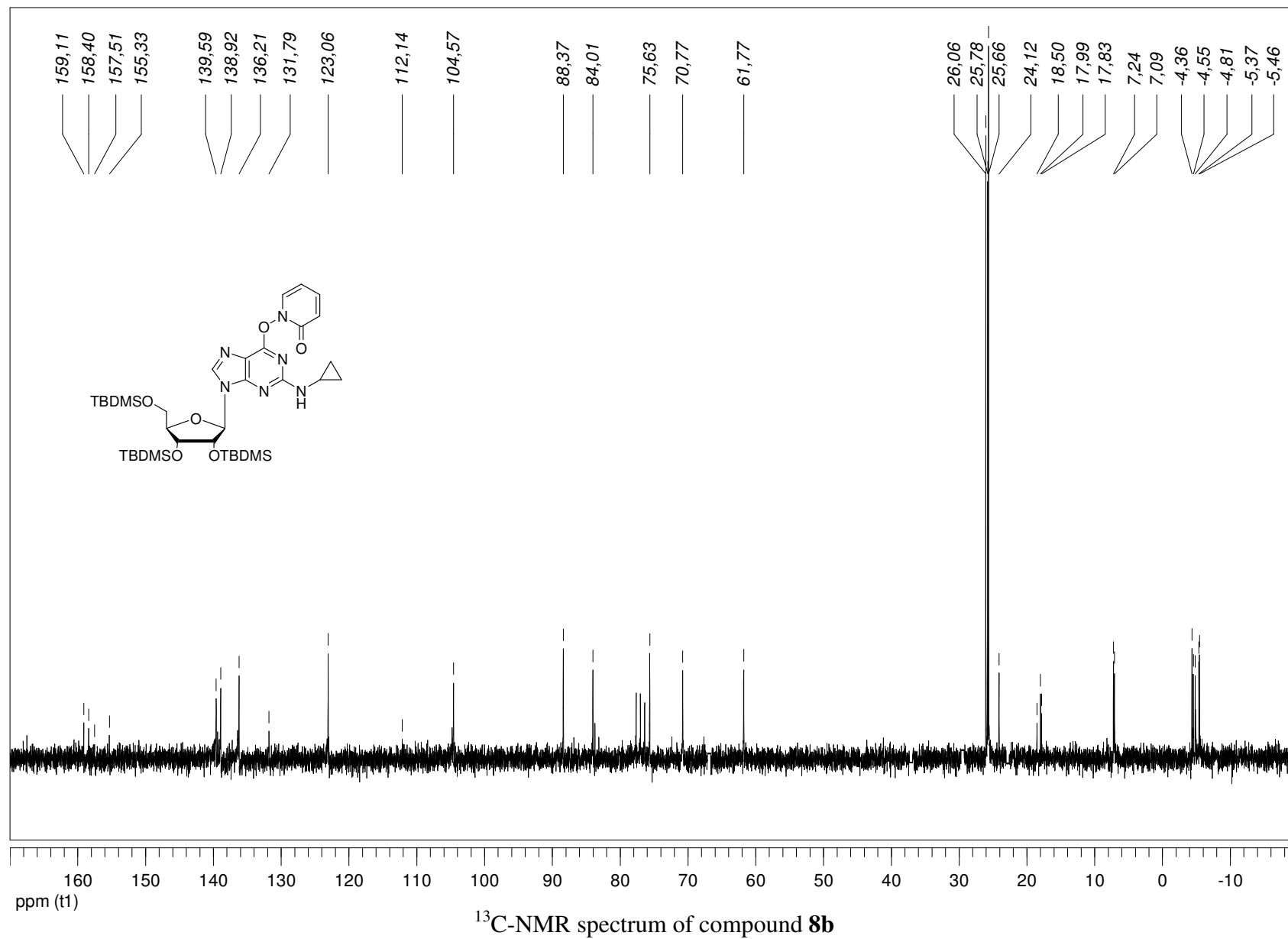


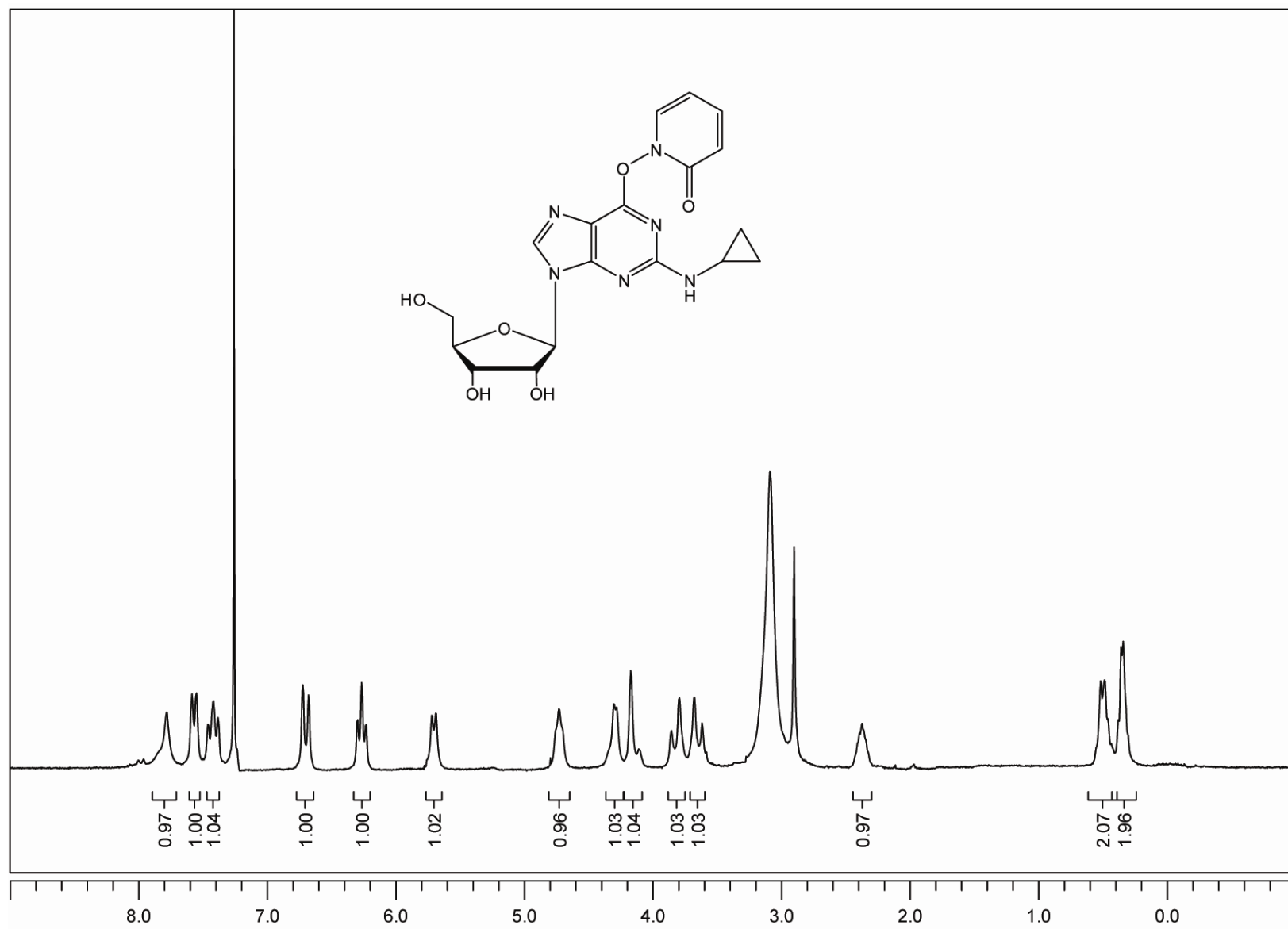




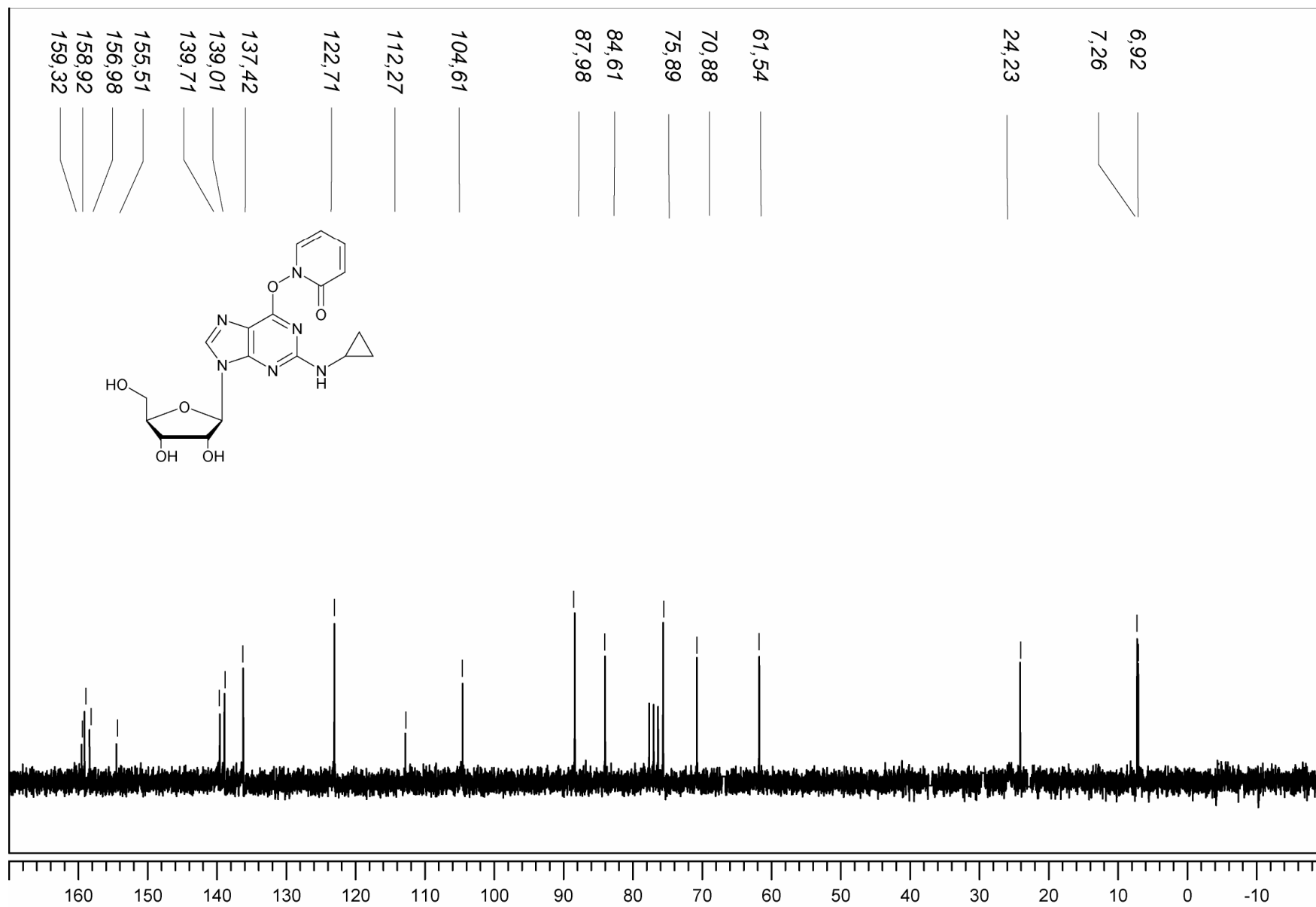


¹H-NMR spectrum of compound **8b**

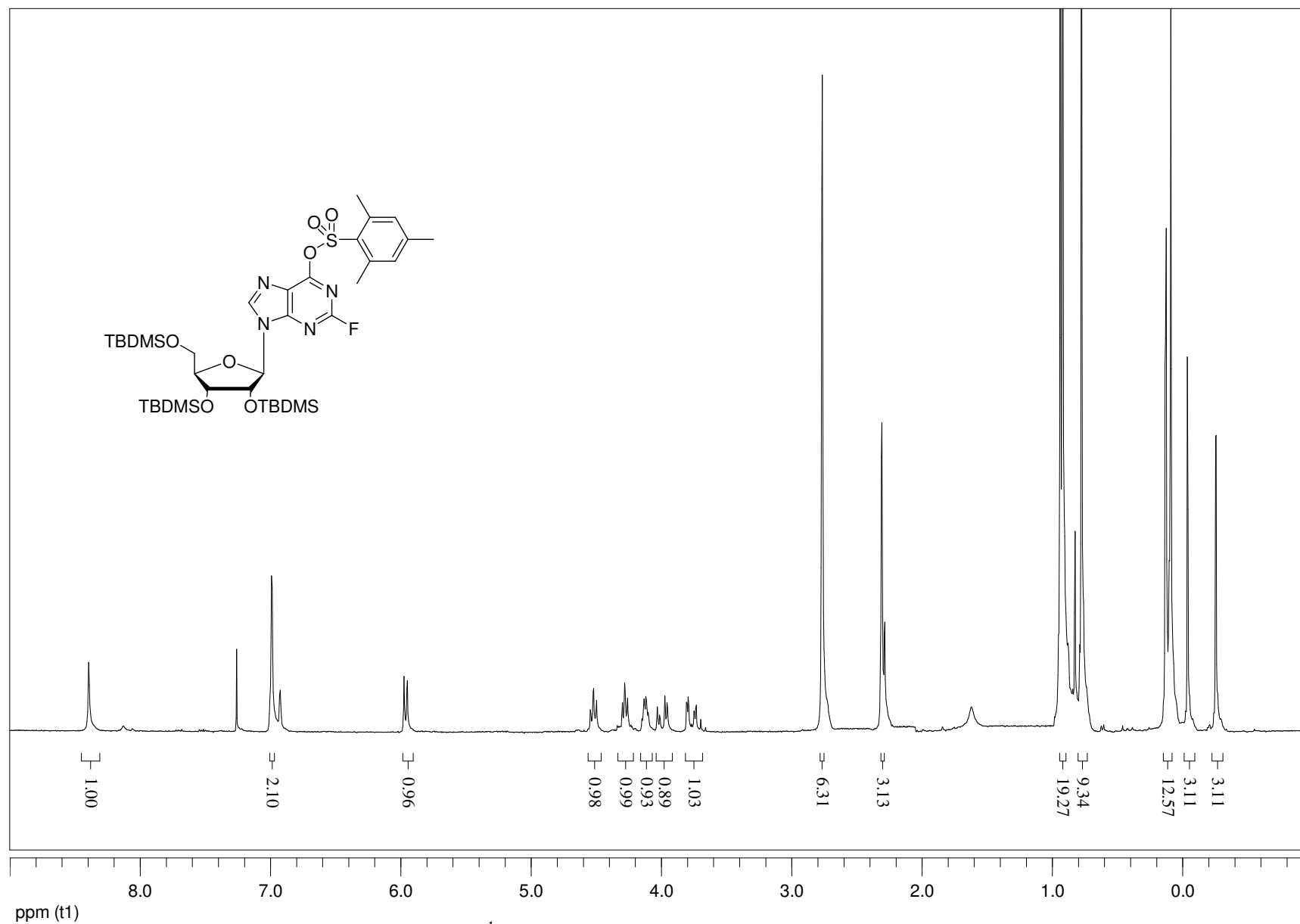


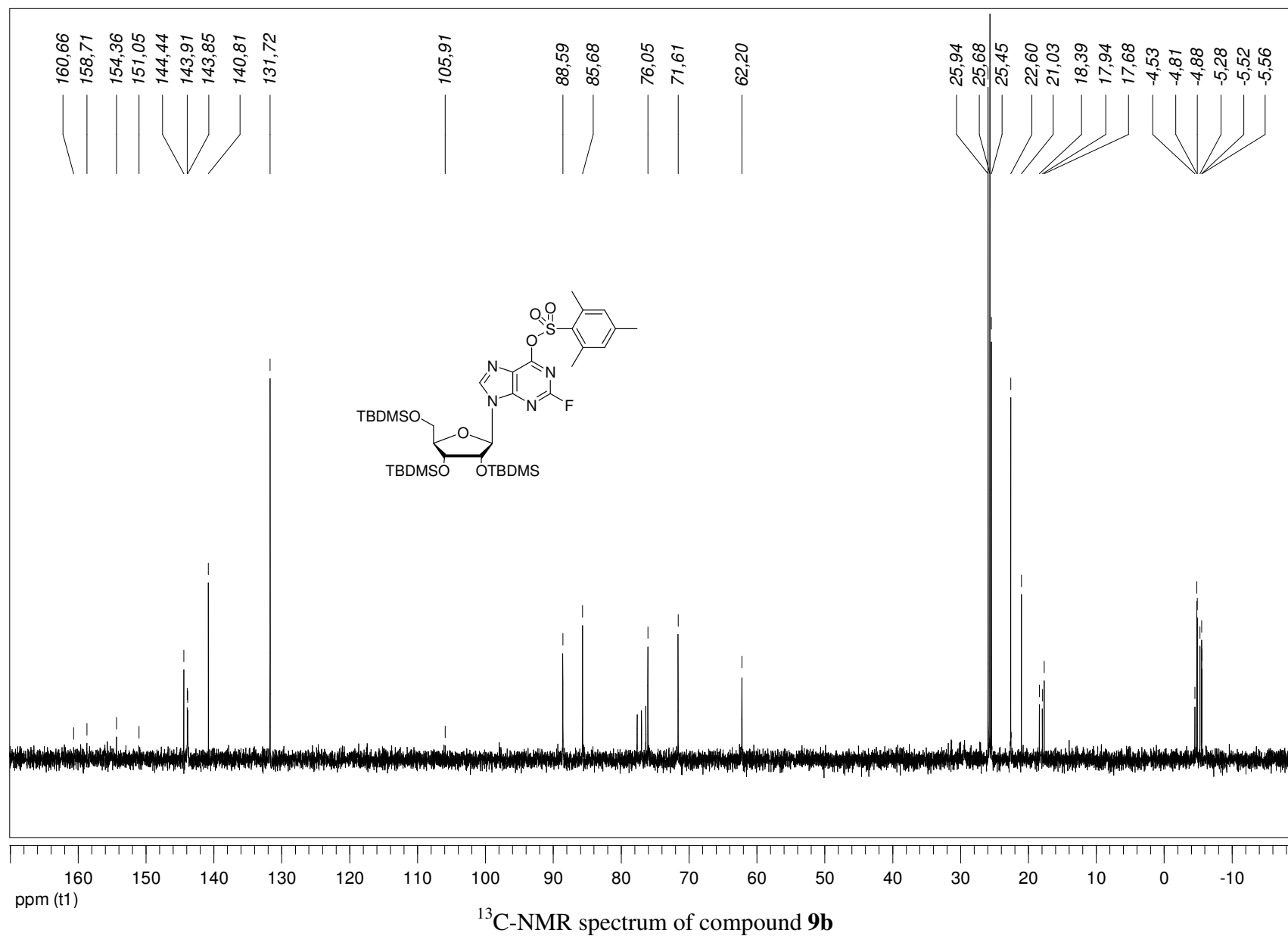


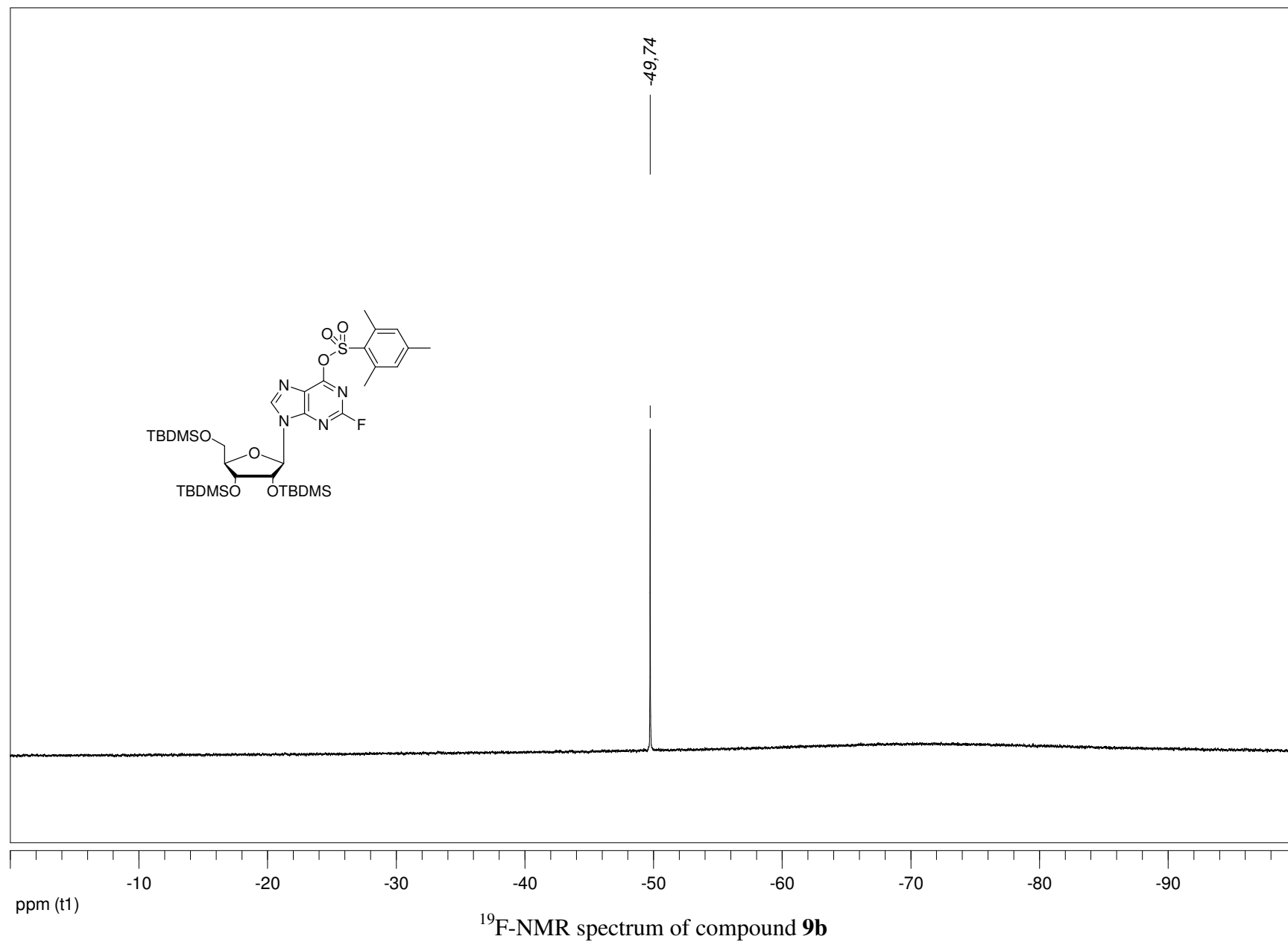
¹H-NMR spectrum of compound **8d**

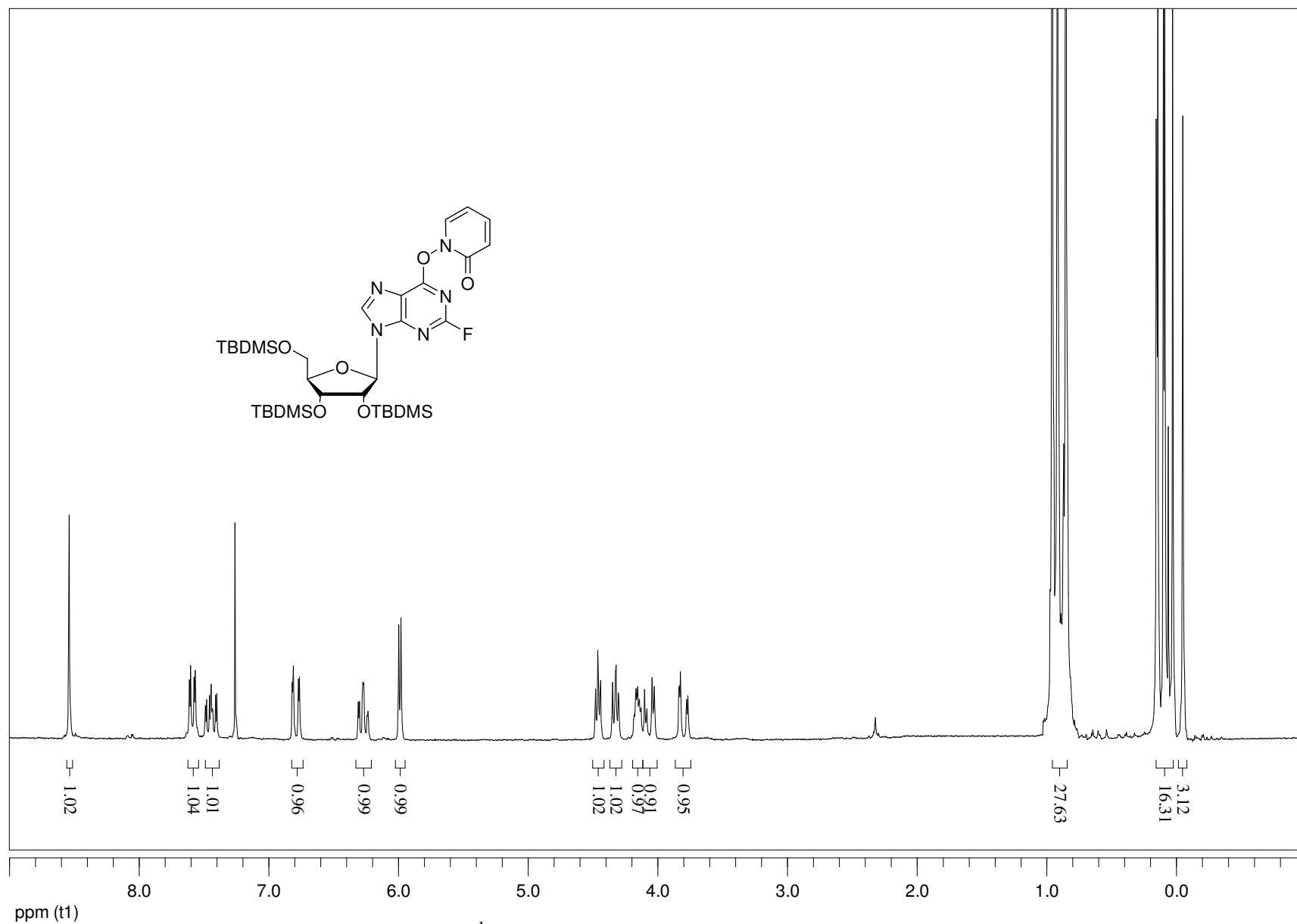


¹³C-NMR spectrum of compound **8d**

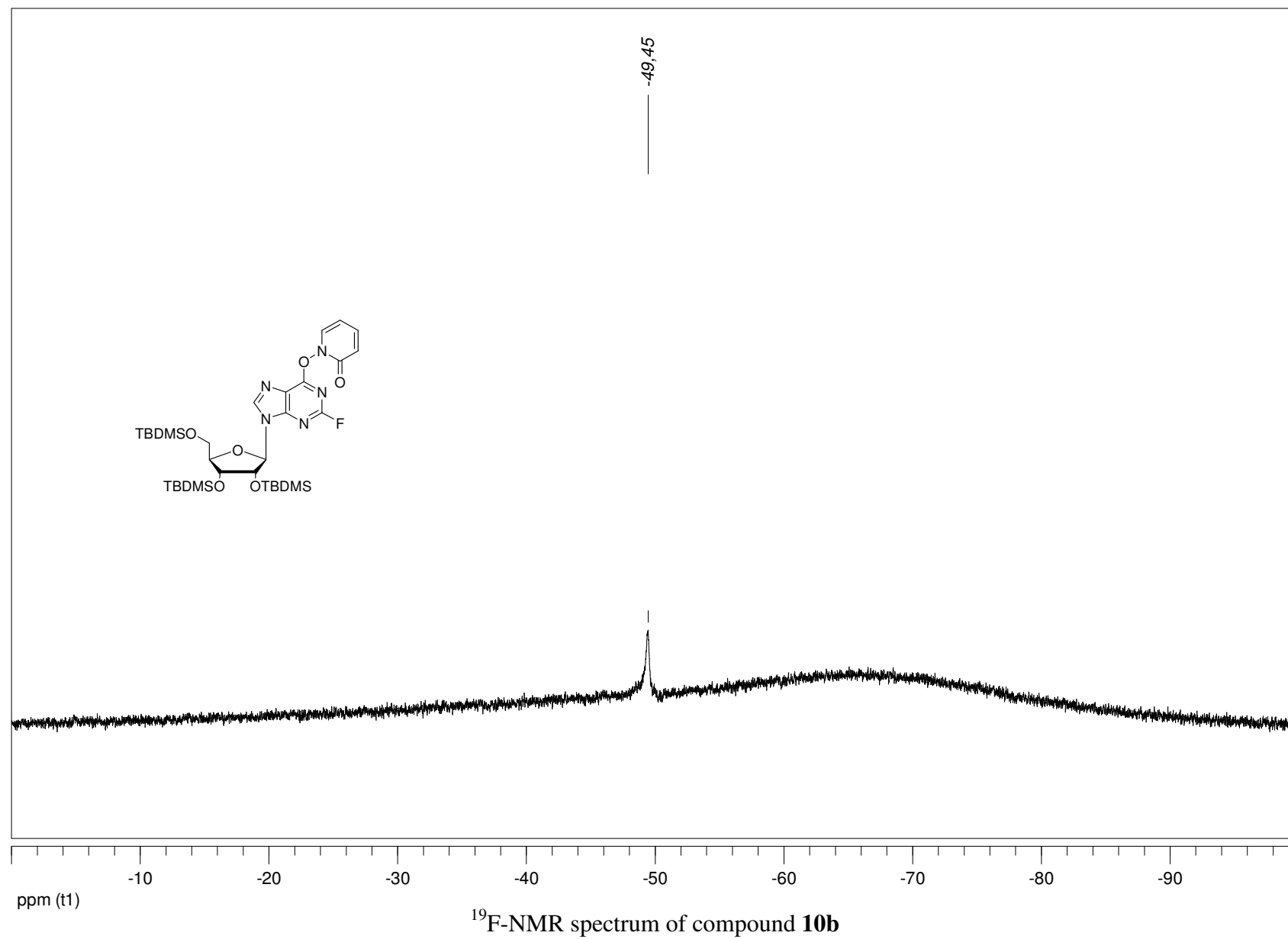


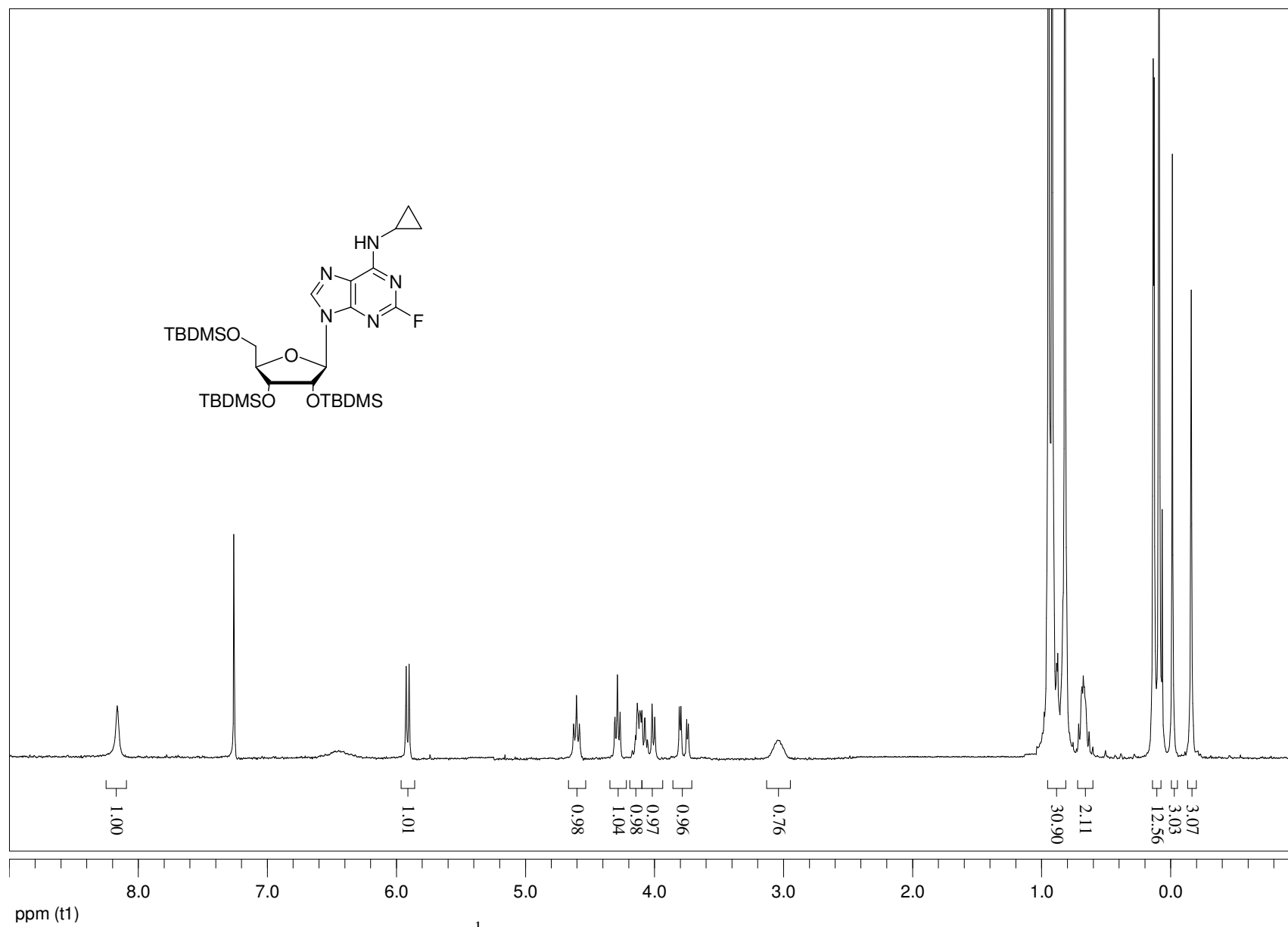






¹H-NMR spectrum of compound **10b**





^1H -NMR spectrum of compound **11b**

