Supporting Information

Design, Synthesis, Radiolabeling Mechanism, and Modeling Study of [^{18}F] and [^{11}C] N-Benzyl-isatin Sulfonamide Analogs for Imaging Caspase-3/7 Activation in Apoptosis

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General methods and materials All chemicals were obtained from standard commercial sources and used without further purification. All reactions were carried out by standard air-free and moisture-free techniques under an inert argon atmosphere with dry solvents unless otherwise stated. Flash column chromatography was conducted using Scientific Adsorbents, Inc. silica gel, 60A, "40 Micron Flash" (32-63 μm). Melting points were uncorrected. Routine ^{1}H NMR spectra were recorded at 300 MHzr. All chemical shifts were reported as a part per million (ppm) downfield from tetramethylsilane (TMS). All coupling constants (*J*) are given in Hertz (Hz). Splitting patterns are typically described as follows: s, singlet; d, doublet; t, triplet; m, multiplet. ^{19}F NMR spectra were recorded at 282.2 MHz, and chemical shifts are reported as Hz upfield from an external $CFCl_3$ standard. High resolution ^{13}C NMR was recorded at 150.9 MHz. ESI/MS was performed on a Waters ZQ 4000 single quadrupole mass spectrometer equipped with an electrospray ionization (ESI) LC-MS interface. High performance liquid chromatography (HPLC) was performed with an ultraviolet detector operating at 251 nm and a well-scintillation NaI (Tl) detector and associated electronics for radioactivity detection. Alltech Econosil C18 250 × 10 mm semi-preparative column and Altech Altima C18 250 × 4.6 mm analytical column were used for preparation and analysis respectively. N-benzyl isatin **21** was synthesized according to literature procedure (Marti, C.; Carreira, E. M. *J. Am. Chem. Soc.* **2005**, *127*, 11505-11515.)

$H_2{}^{18}O$ was purchased from Rotem Industries (Israel). [^{18}F]Fluoride was produced in Washington University by the $^{18}O(p,n)^{18}F$ reaction through proton irradiation of enriched (95%) ^{18}O water using RDS111 cyclotron. Materials were heated using a custom-designed microwave cavity, model 420BX (Micro-Now Instruments, Skokie, IL). Screw-cap test tubes used for microwave heating were purchased from Fisher Scientific (Pyrex No. 9825). Classic C-18 Sep-

Pak cartridges were purchased from Waters Corporation. For the TLC analyses, EM Science Silica Gel 60 F_{254} TLC plates were purchased from Fisher Scientific (Pittsburgh, PA). Radio-TLC was accomplished using a Bioscan 200 imaging scanner (Bioscan, Inc., Washington, DC). Radioactivity was counted with a Beckman Gamma 8000 counter containing a NaI crystal (Beckman Instruments, Inc., Irvine, CA). $[^{11}C]CH_3I$ was produced from $[^{11}C]CO_2$ using a GE PETtrace MeI Microlab. $[^{11}C]CO_2$ was produced at the Cyclotron Facility of Washington University School of Medicine using a JSW BC-16/8 cyclotron by irradiating a gas target of 0.2% O_2 in N_2 for 15–30 min with a 40 μA beam of 16 MeV protons. $[^{11}C]CO_2$ was converted to $[^{11}C]CH_3I$ by the GE PETtrace MeI Microlab using a nickel catalyst [Shimalite-Ni (reduced), Shimadzu, Japan P.N.221-27719] in the presence of H_2 at 360^{o}C and followed by reaction with iodine in the gas phase at 690^{o}C. $[^{11}C]CH_3I$ was delivered in the gas phase with helium approximately 12 min following the end of bombardment.

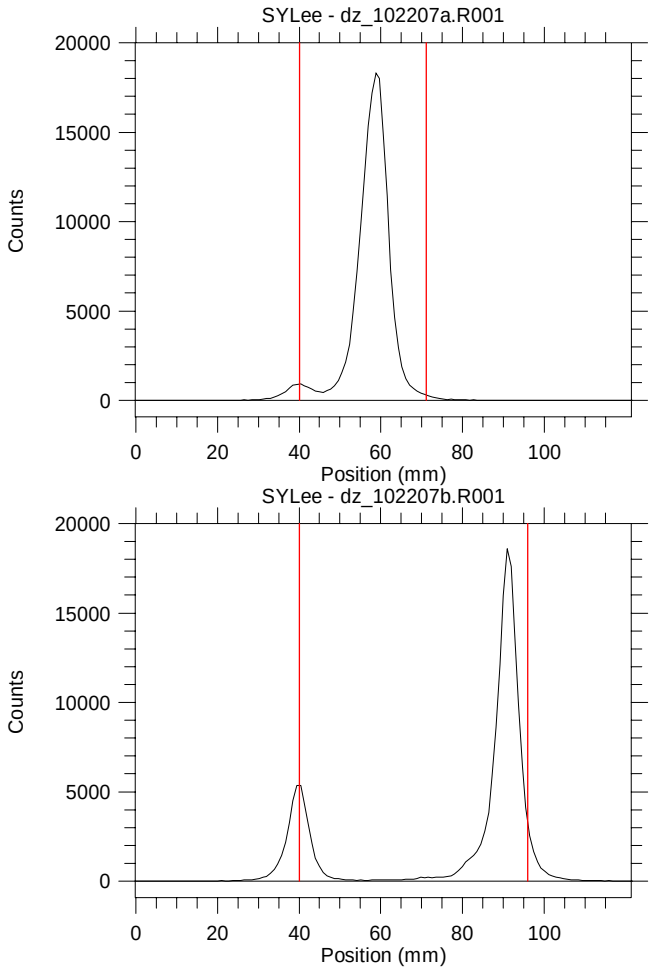

Figure S**1**. RadioTLCs of the reaction mixture of radiosynthesis of [^{18}F] **2**.

Above: Precursor **7a**, [^{18}F]fluoride, K_{222}, K_2CO_3, DMSO, Microwave;

Bottom: 1 N HCl, Microwave.

Silica Gel TLC place; solvent: 20% MeOH, 80% CH_2Cl_2.

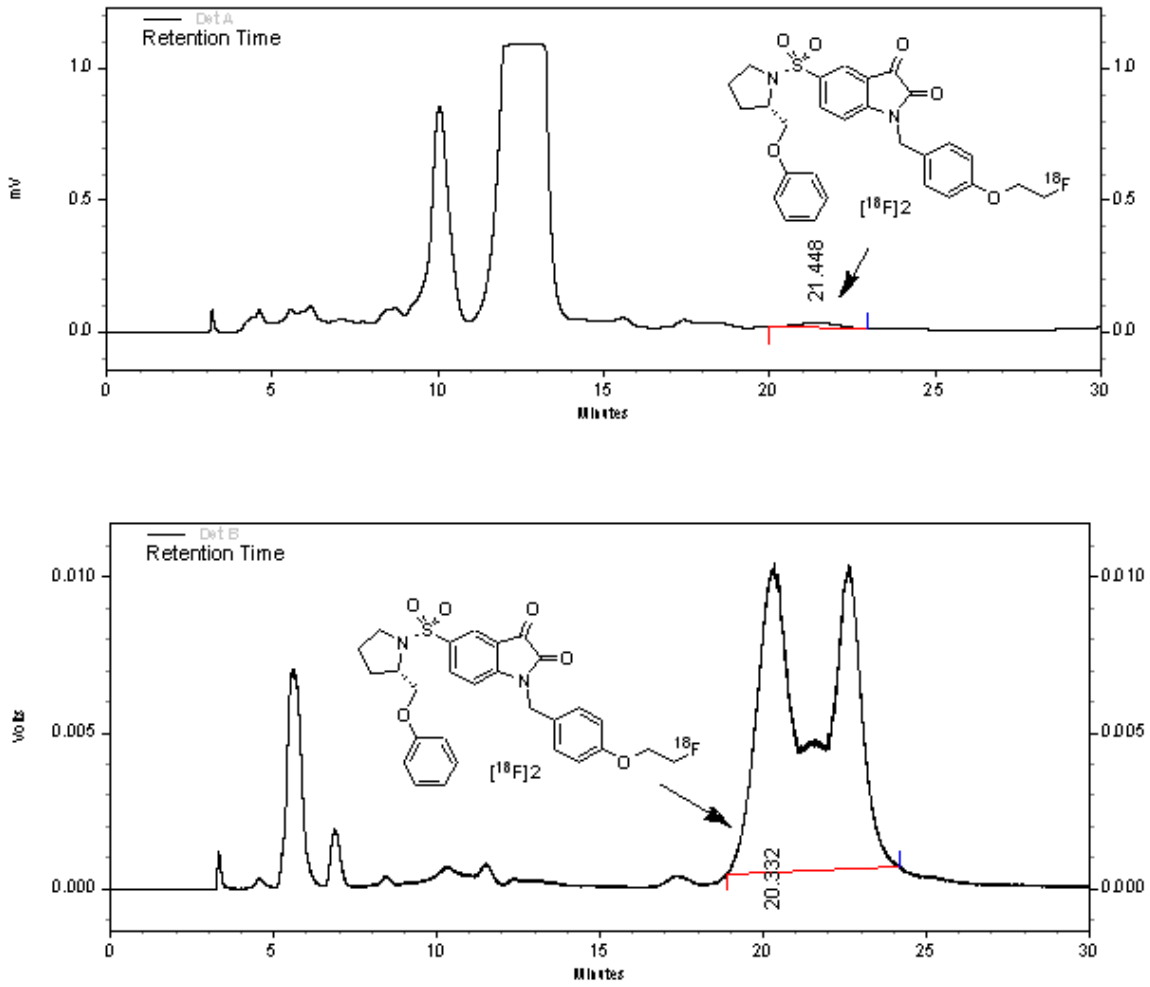

Figure S**2**. Typical HPLC chromatograms for [^{18}F]**2** purification

Above: UV absorbance at 251 nm;

Bottom: radioactivity (The “rabbit” ears are due to saturation of the radioactive detector)

Alltech Econosil 250×10 mm, 10μ; 4.0 mL/min, 251 nm, 800 psi; 24% Acetonitrile, 44% methanol, 32% Ammonium formate buffer (pH = 4.5)

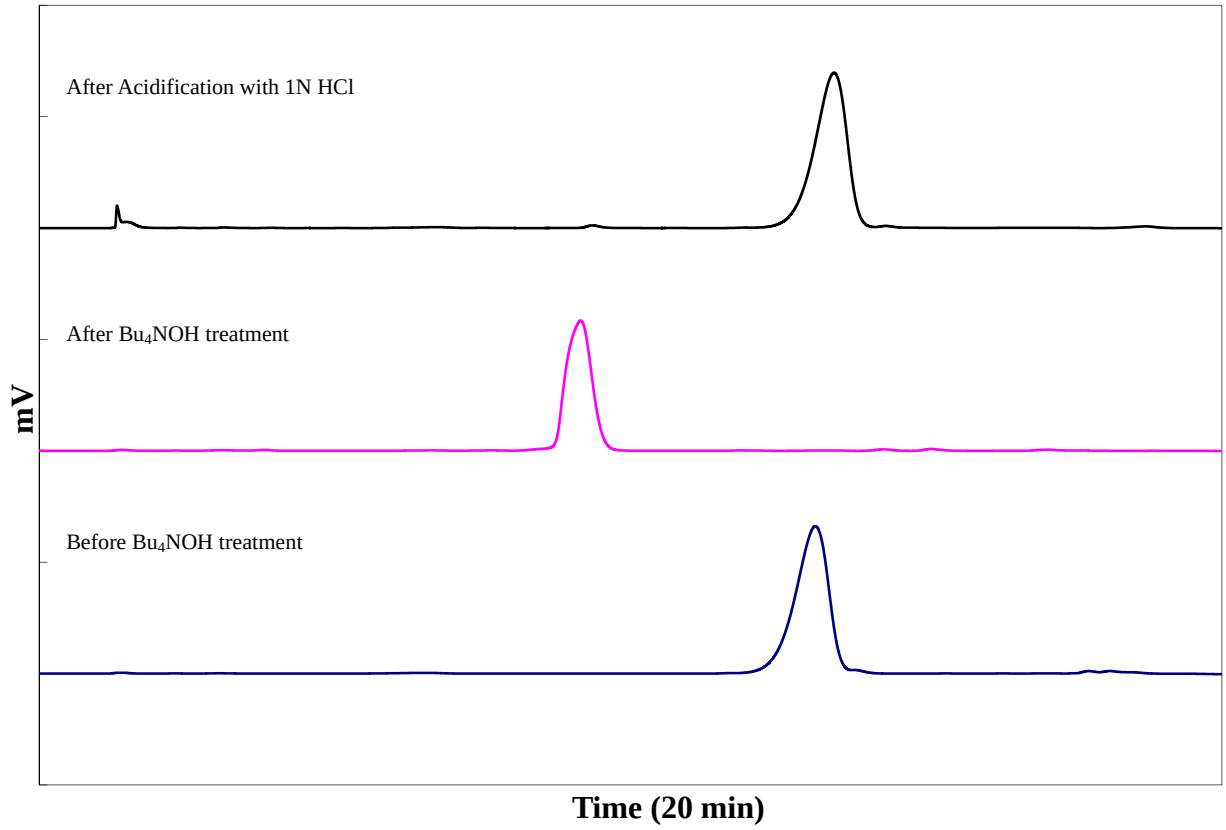

Figure S**3**. HPLC chromatograms of isatin analogue **2** (bottom), isatinate **17** (middle) and isatin **2** recyclized from isatinate **17** (top).

HPLC condition: Alltech Altima C18 250 × 4.6 mm 10µ, gradient: 25% Acetonitrile, 40% ammonium formate buffer (pH = 4.5), 35% Methanol to 35% Acetonitrile, 20% ammonium formate buffer (pH = 4.5), 45% Methanol over 15 min, 1.0 mL/min, 251 nm.

Before Bu_4NOH treatment: 20 µL 1000 ppm **2** injected

Bu_4NOH treatment: 0.1 mg **2** in 100 µL acetonitrile treated with 2 µL 1 M Bu_4NOH/H_2O, 20 µL 1000 ppm injected

1 N HCl treatment: 100 µL 1 N HCl, 1 hour at ambient temperature, 40 µL 500 ppm injected.

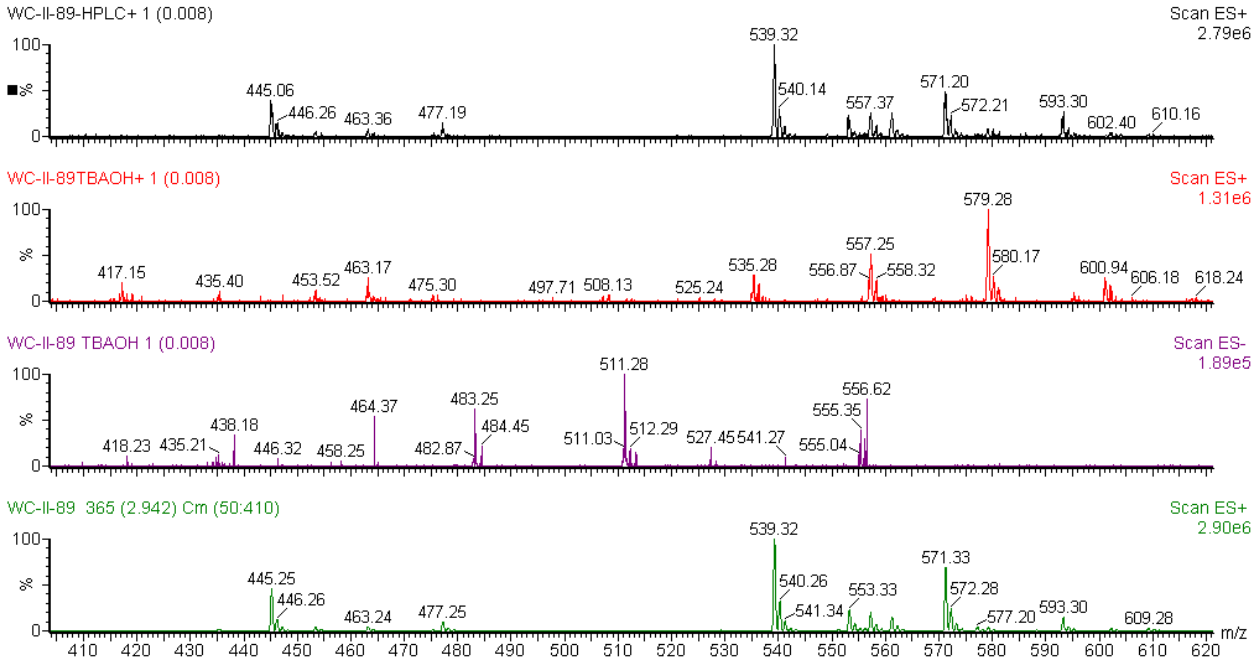

Figure S4. ESI/MS of isatin analogue **2** (bottom), isatinate **17** (middle, negative and positive) and isatin **2** recyclized from isatinate **17** (top).

Isatinate and isatin recyclized from isatinate were purified by HPLC (see above)

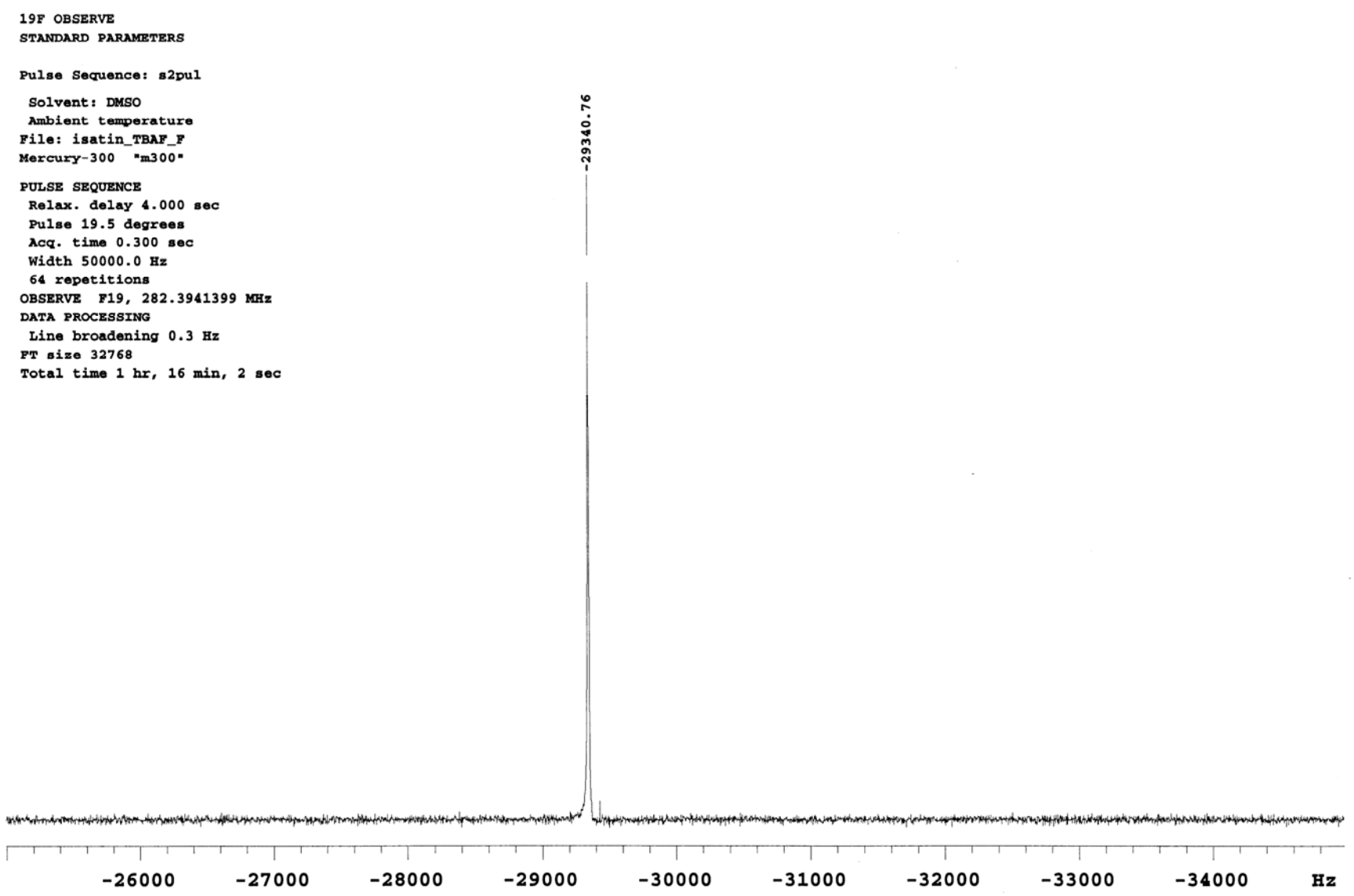

Figure S5. 282 MHz ^{19}F NMR spectrum (DMSO-d6) of Bu_4NF

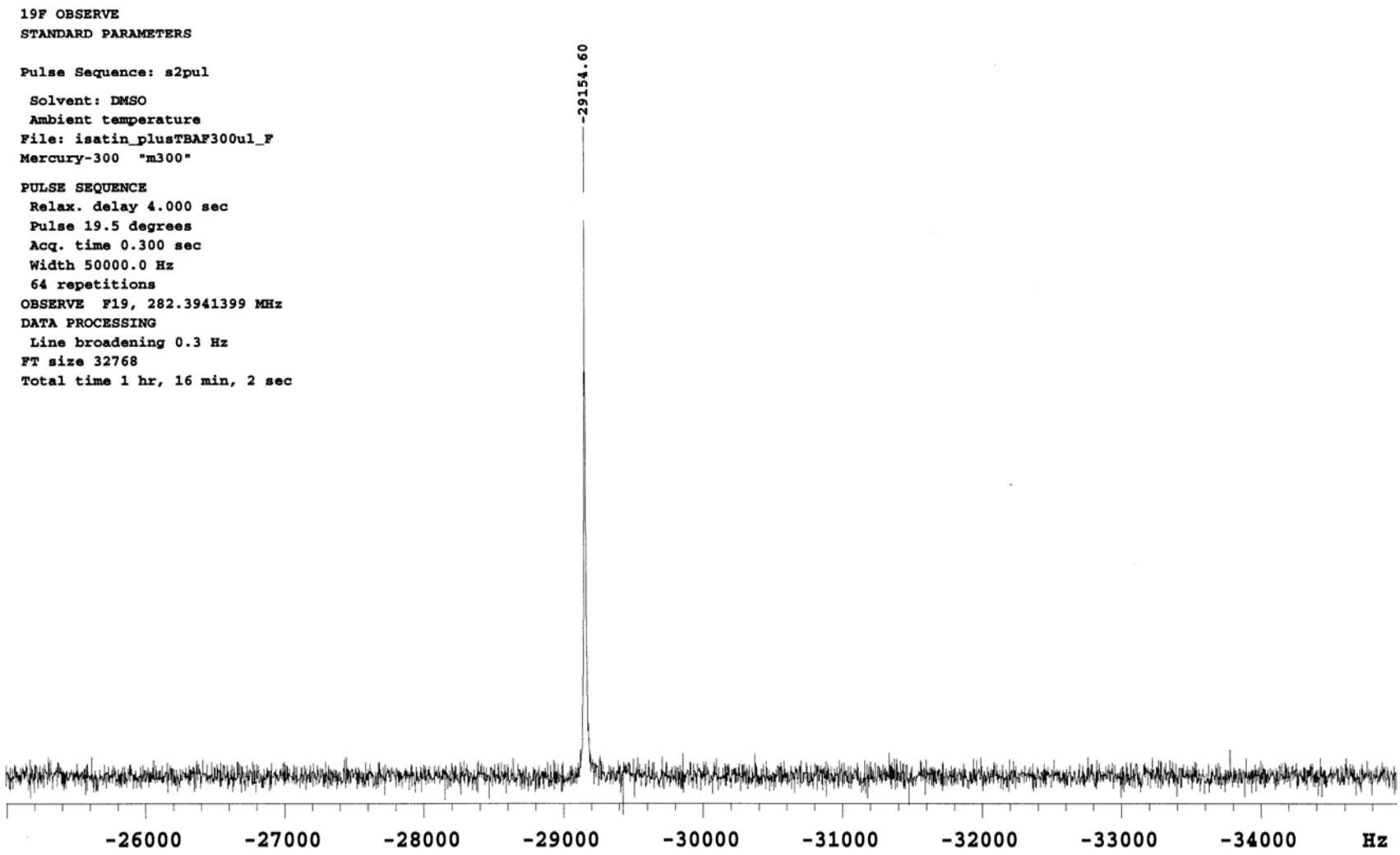

Figure S6. 282 MHz ^{19}F NMR spectrum (DMSO-d6) of Bu_4NF with addition of N-benzyl isatin **21**

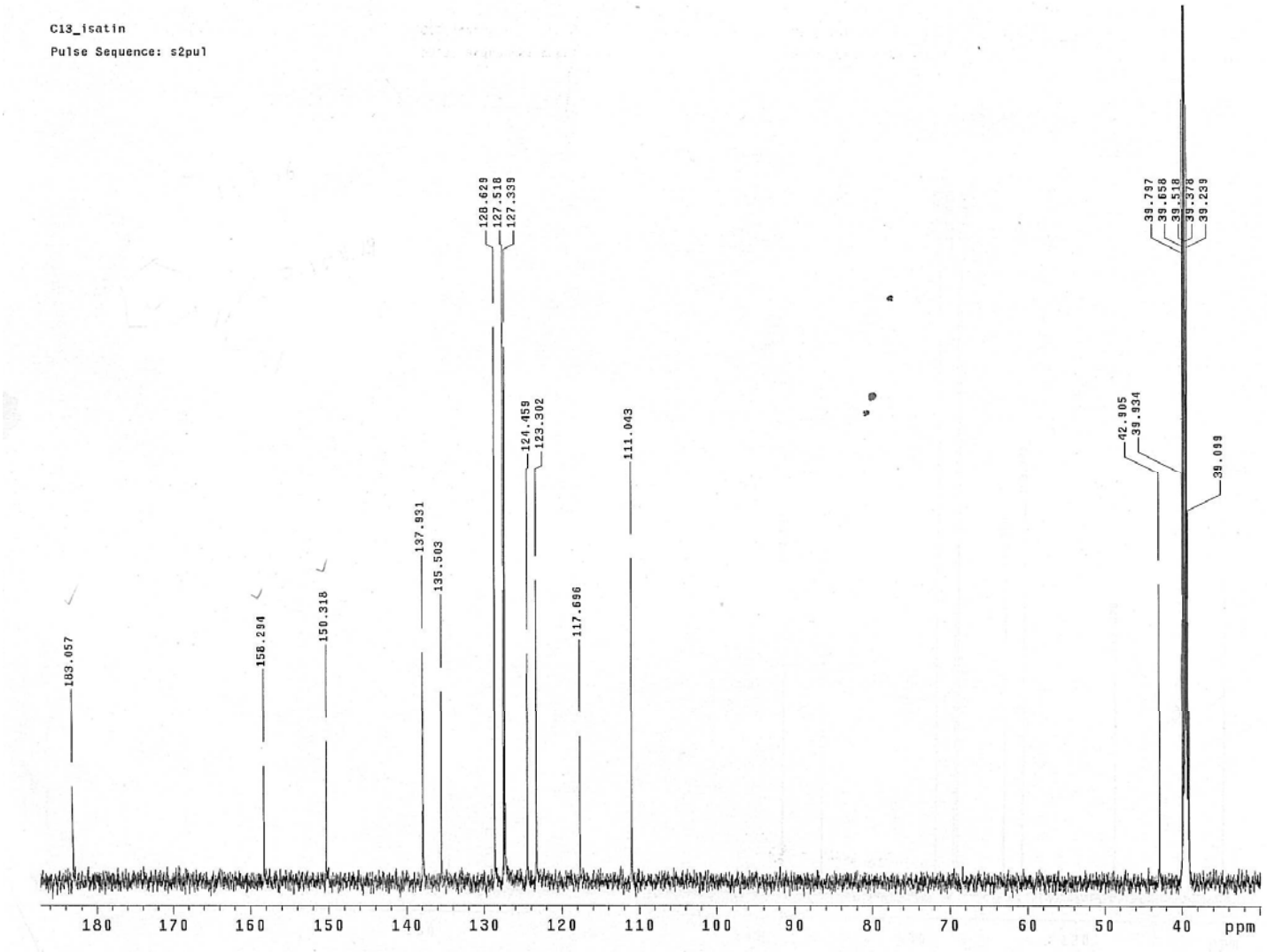

Figure S7. 150 MHz ^{13}C NMR (DMSO-d6) Spectrum of N-benzyl isatin **21**.

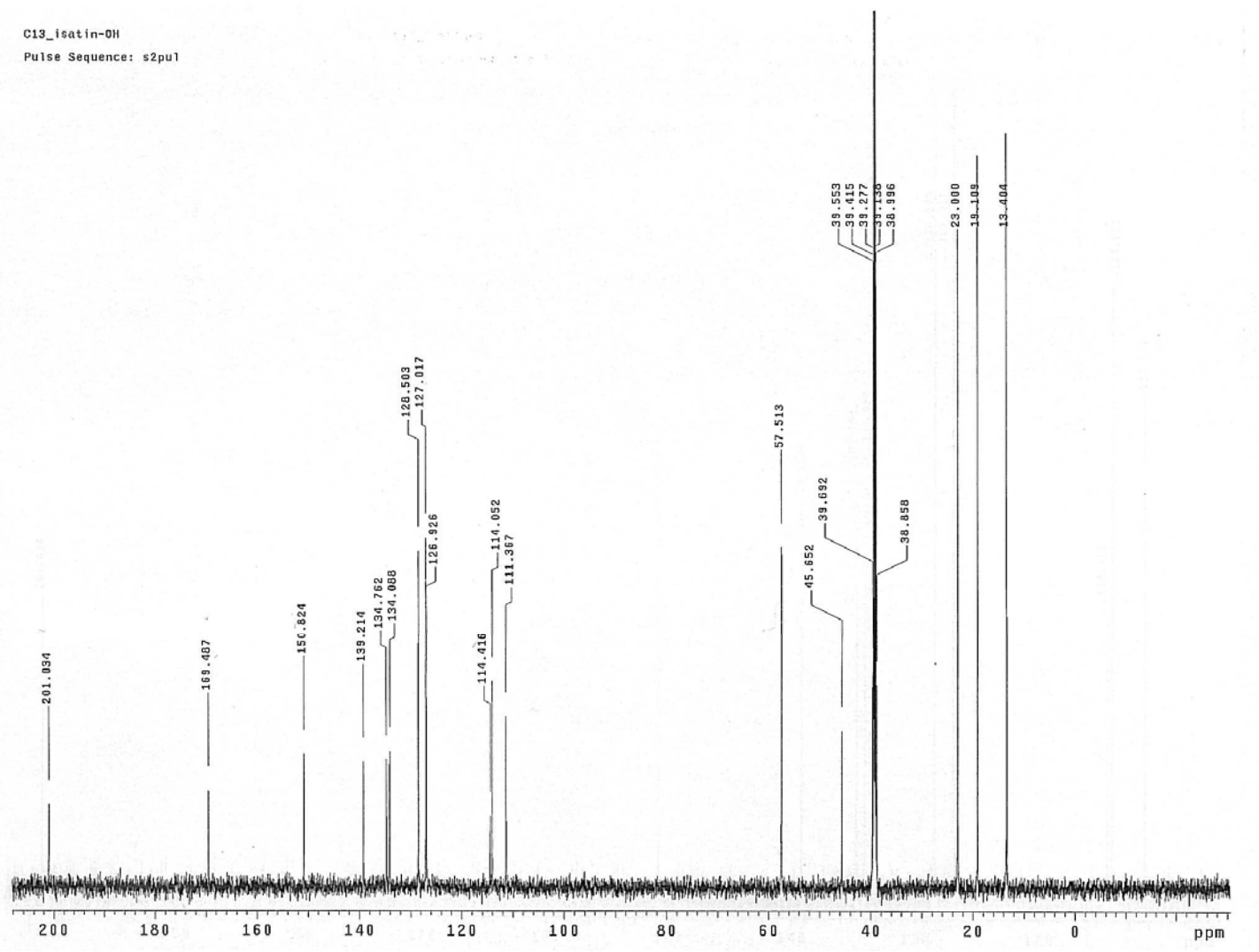

Figure S8. 150 MHz ^{13}C NMR Spectrum (DMSO-d6) of N-benzyl isatin **21** treated with 1M Bu_4NOH/H_2O (10 mg **21** in 0.6 mL DMSO-d6)

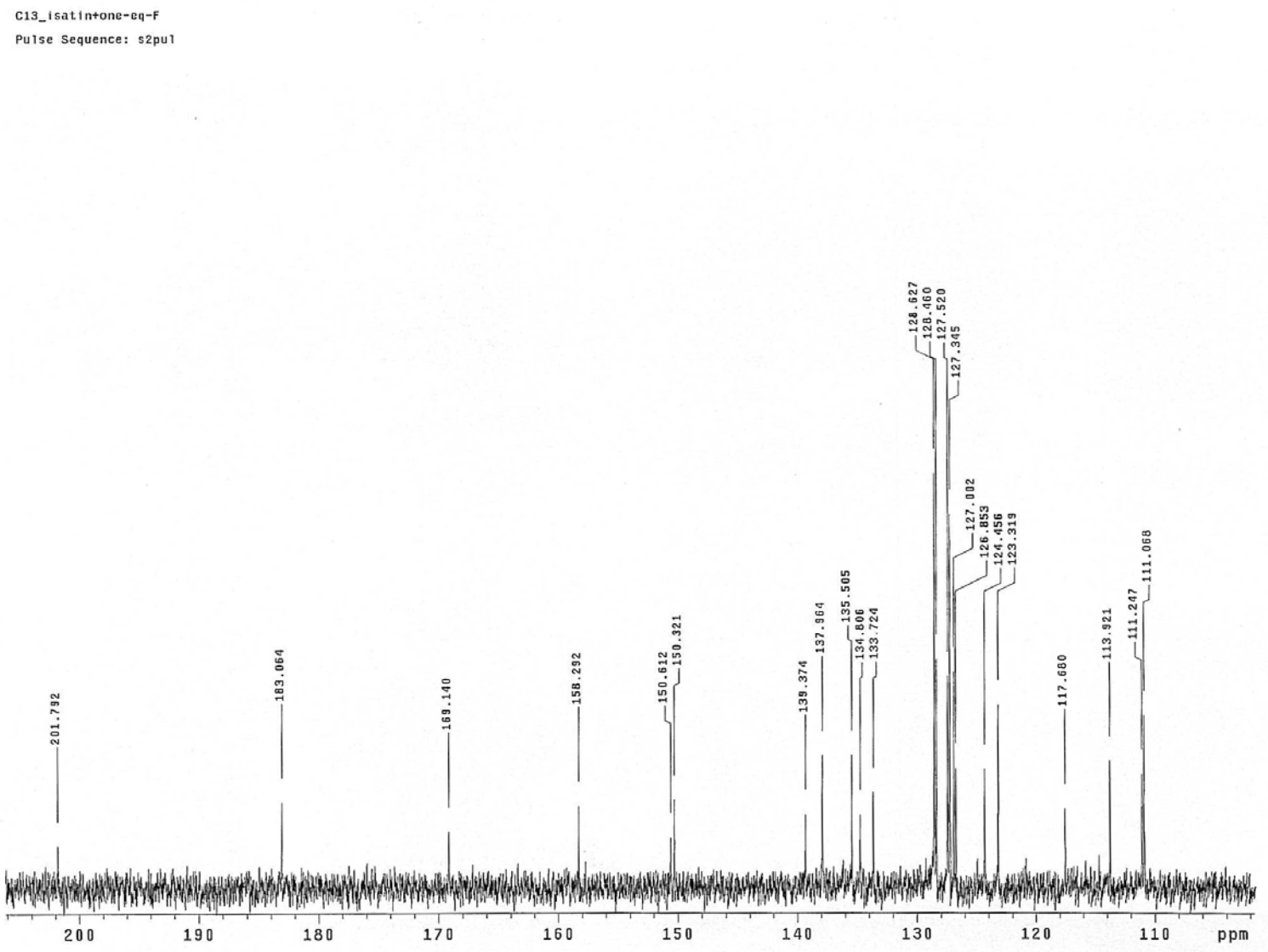

Figure S9. 150 MHz ^{13}C NMR Spectrum (DMSO-d6) of N-benzyl isatin **21** treated with 1 equivalent Bu_4NF/H_2O (10 mg **21** in 0.6 mL DMSO-d6)

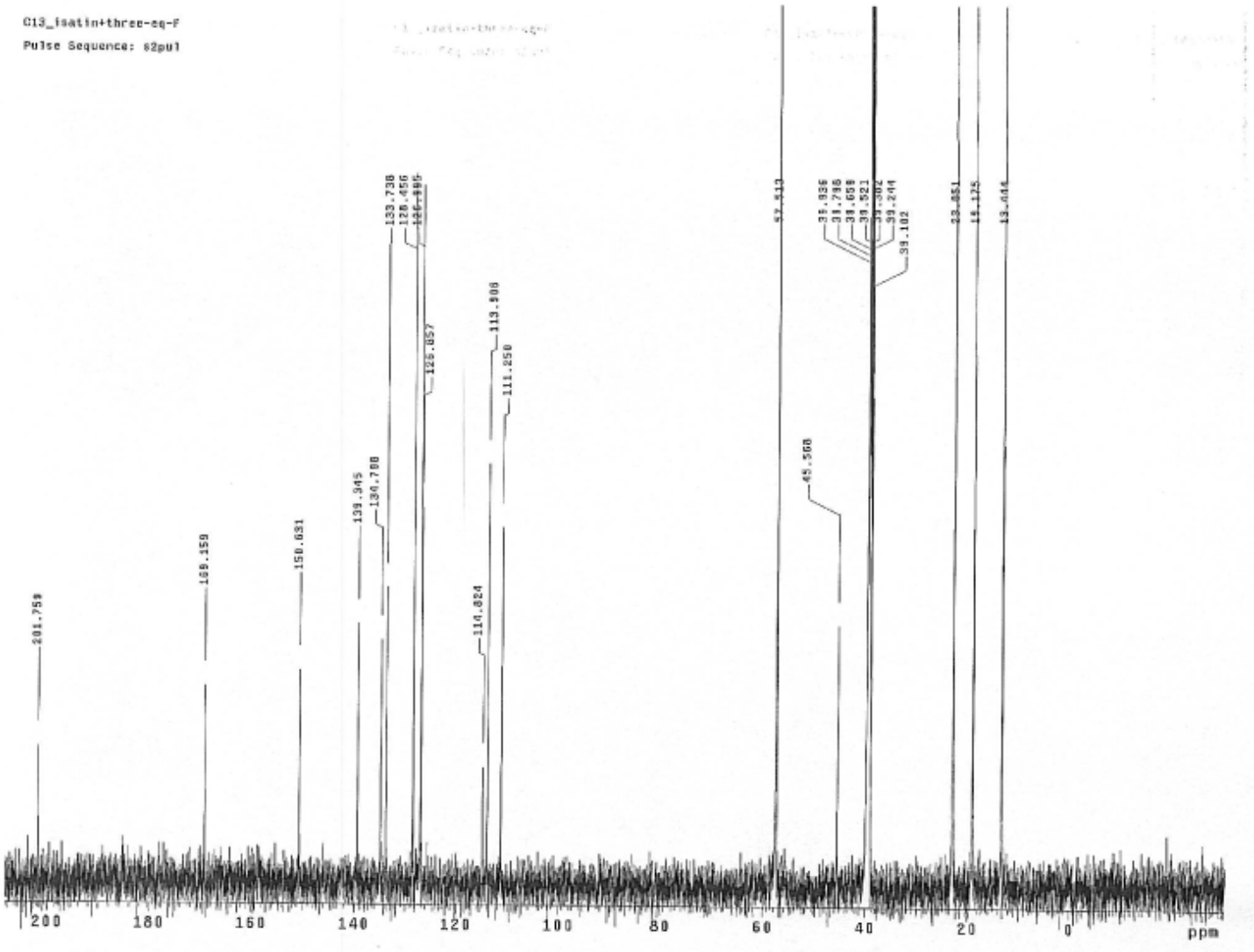

Figure S10. 150 MHz ^{13}C NMR Spectrum (DMSO-d6) of N-benzyl isatin **21** treated with 3 equivalents Bu_4NF/H_2O (10 mg **21** in 0.6 mL DMSO-d6)

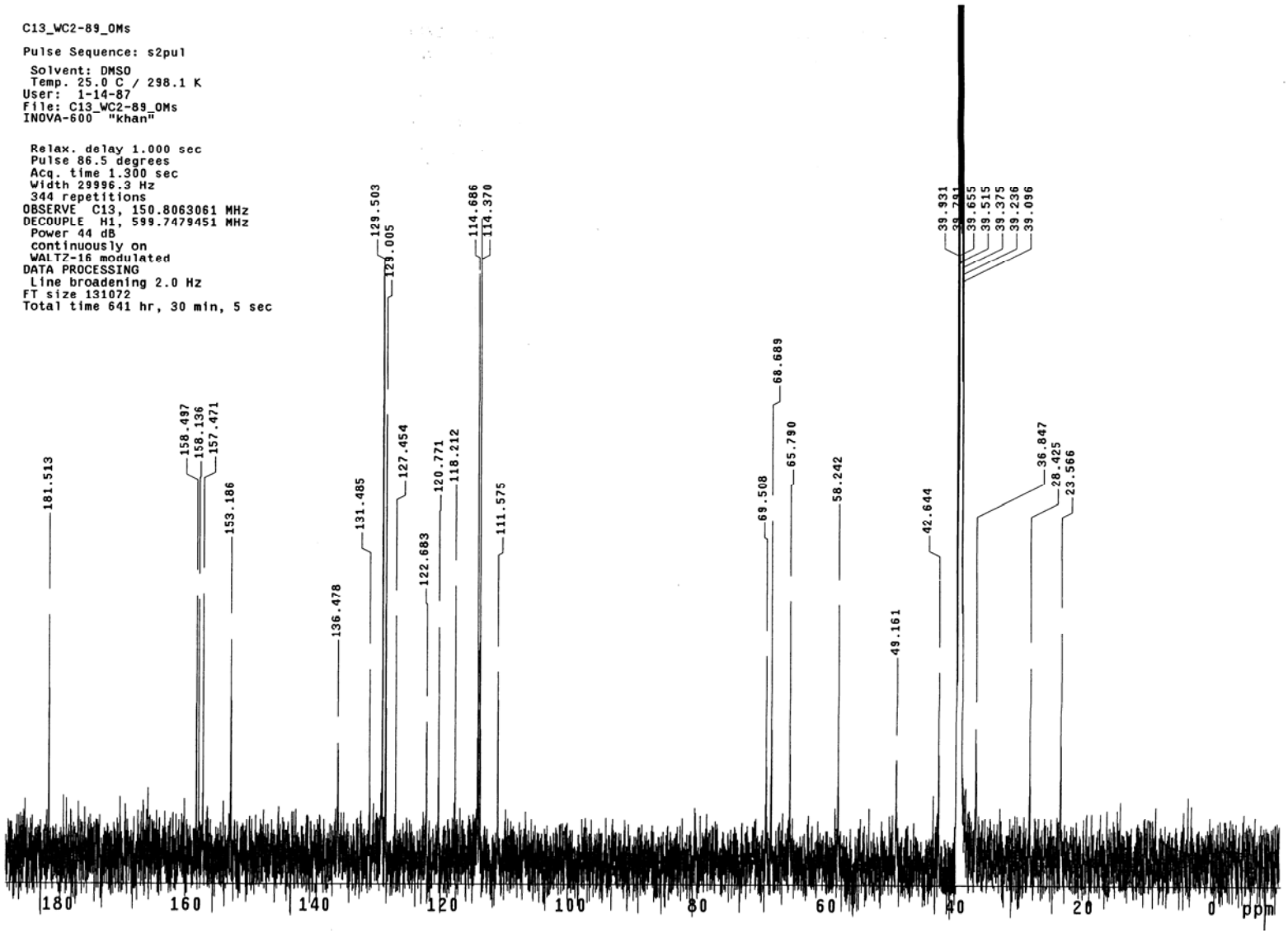

Figure S11. 150 MHz ^{13}C NMR spectrum (DMSO-d6) of **7a (**10 mg **7a** in 0.6 mL DMSO-d6)

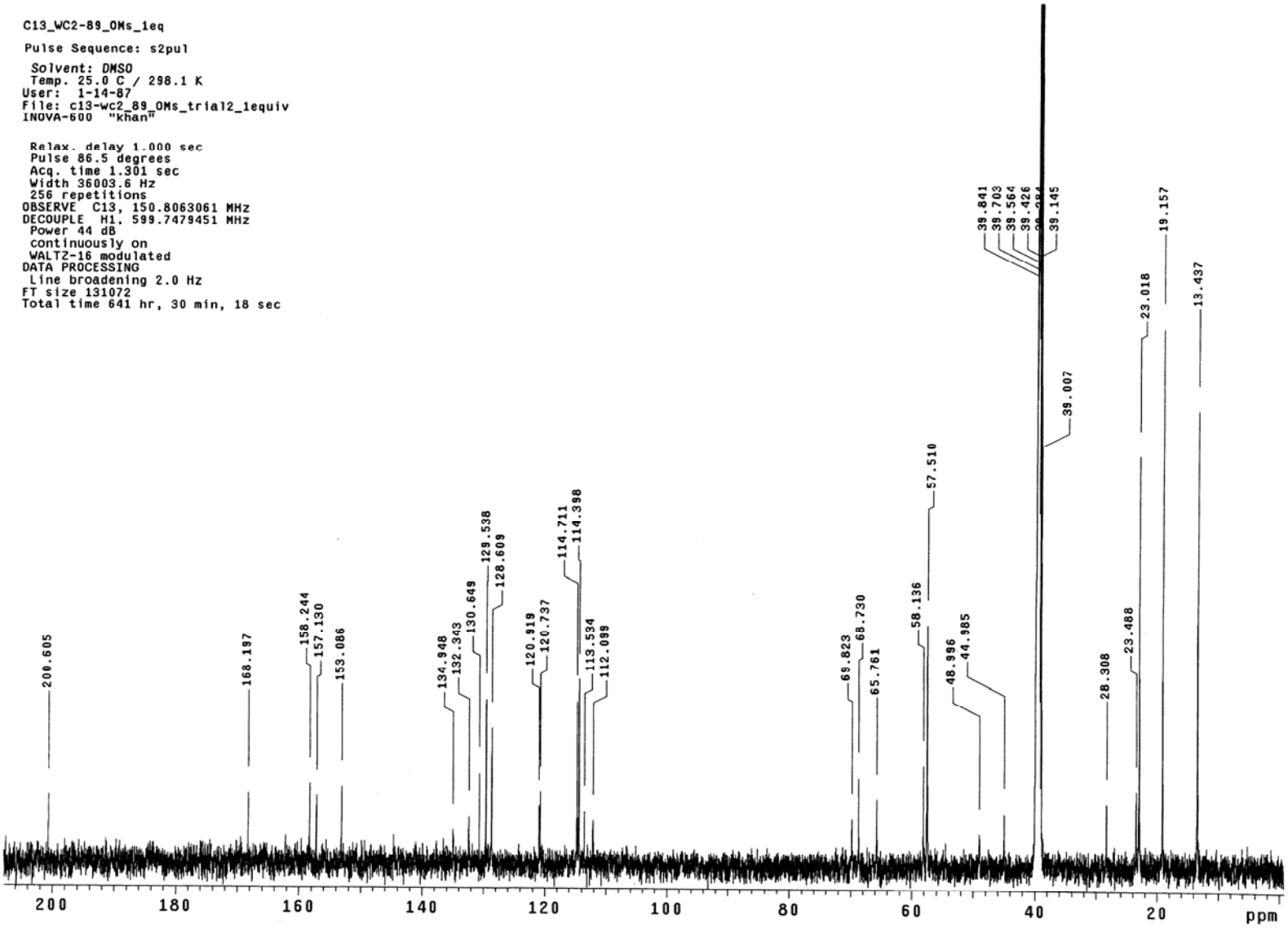

Figure S12. 150 MHz ^{13}C NMR spectrum (DMSO-d6) of ring-opened **7a** (10 mg **7a** in 0.6 mL DMSO-d6 treated with 20 µL 1M Bu_4NOH/H_2O)

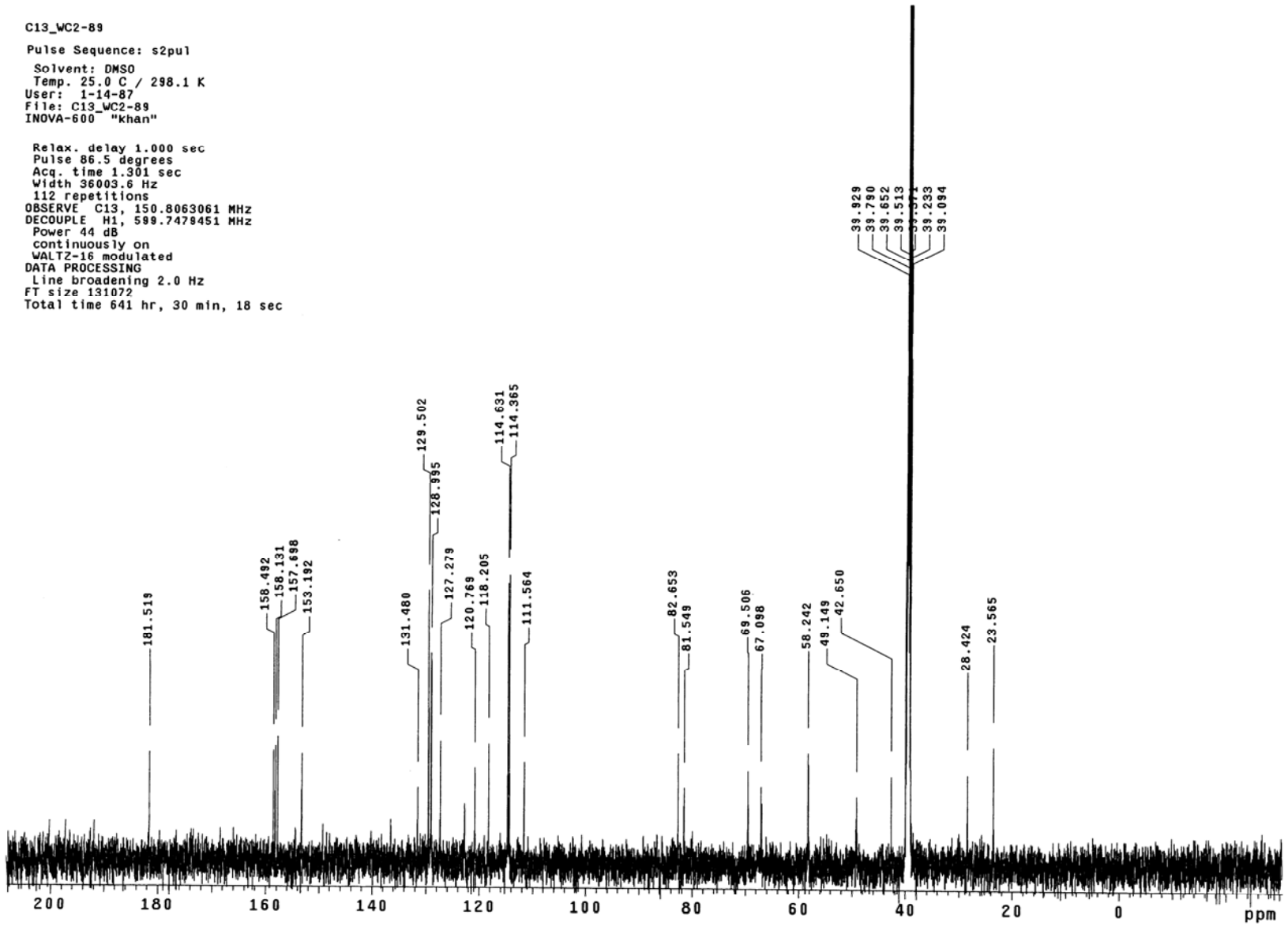

Figure S13. 150 MHz ^{13}C NMR spectrum (DMSO-d6) of **2 (**10 mg **2** in 0.6 mL DMSO-d6)

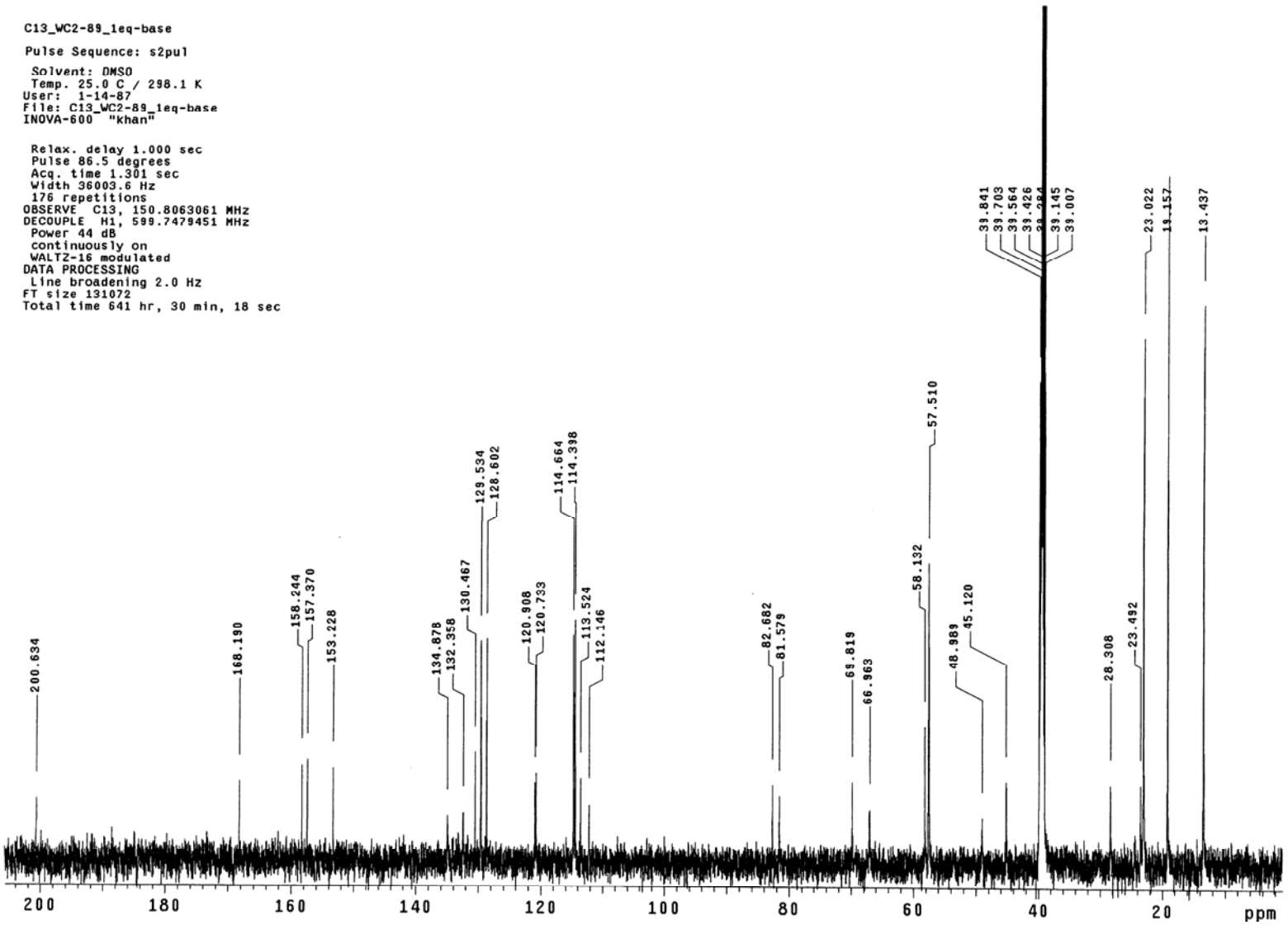

Figure S14. 150 MHz ^{13}C NMR spectrum (DMSO-d6) of ring-opened **2**(10 mg **2** in 0.6 mL DMSO-d6 treated with 20 µL 1M Bu_4NOH/H_2O)

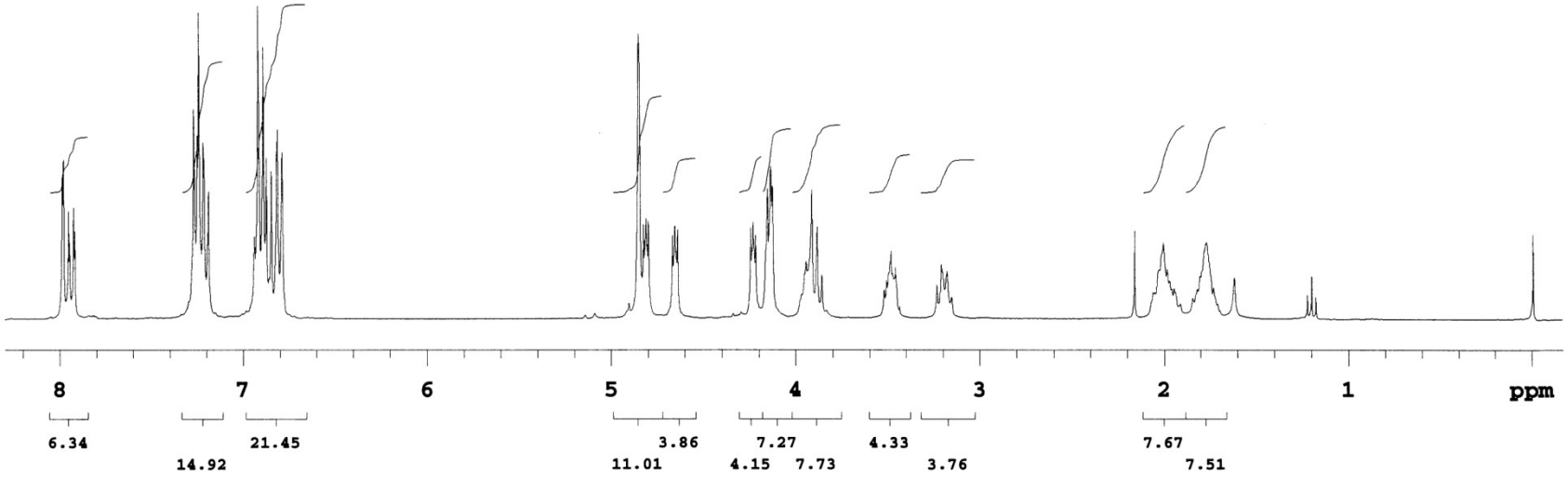

Figure S15. 300 MHz ^{1}H NMR spectrum ($CDCl_3$) of **2**

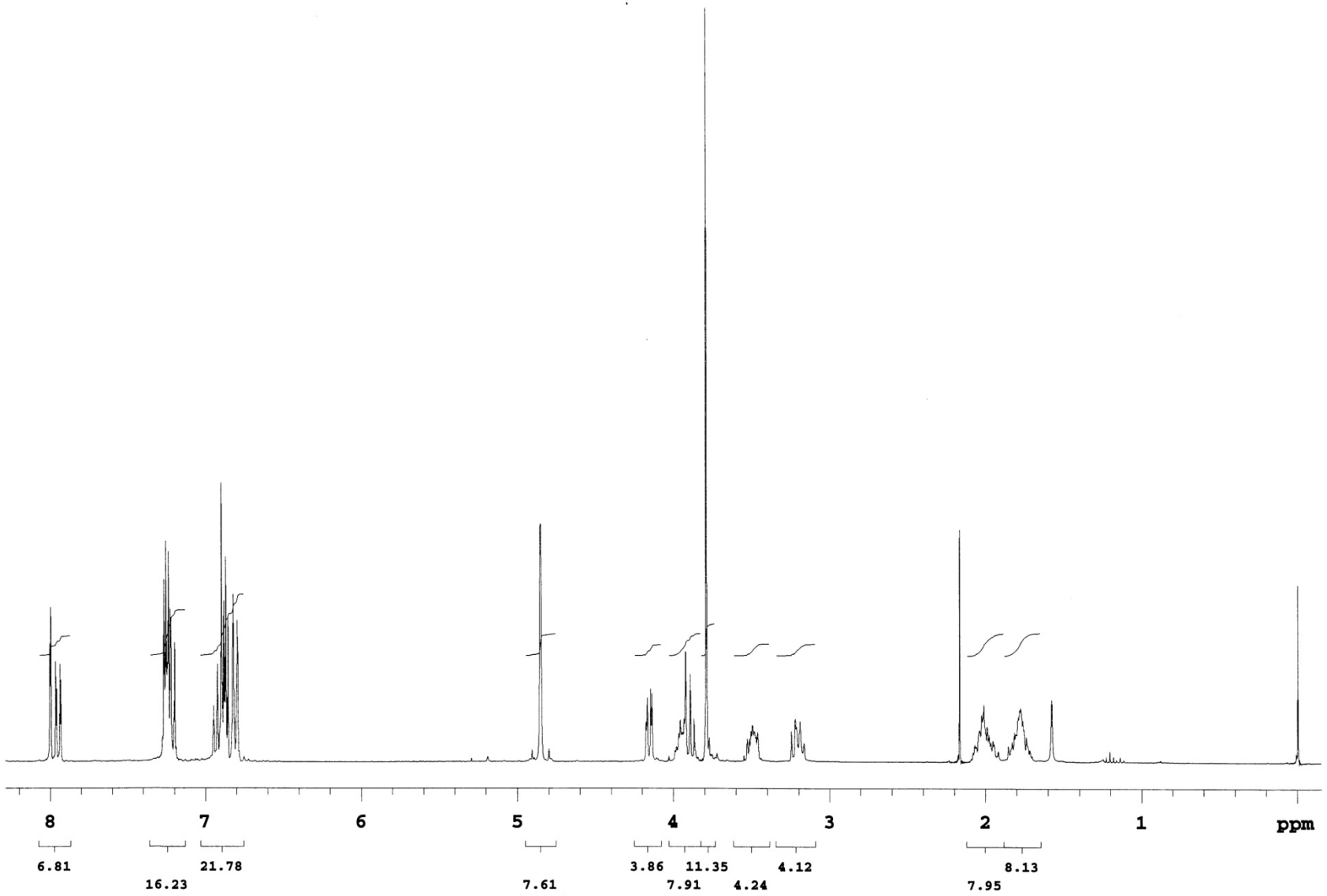

Figure S16. 300 MHz ^{1}H NMR spectrum ($CDCl_3$) of **4**

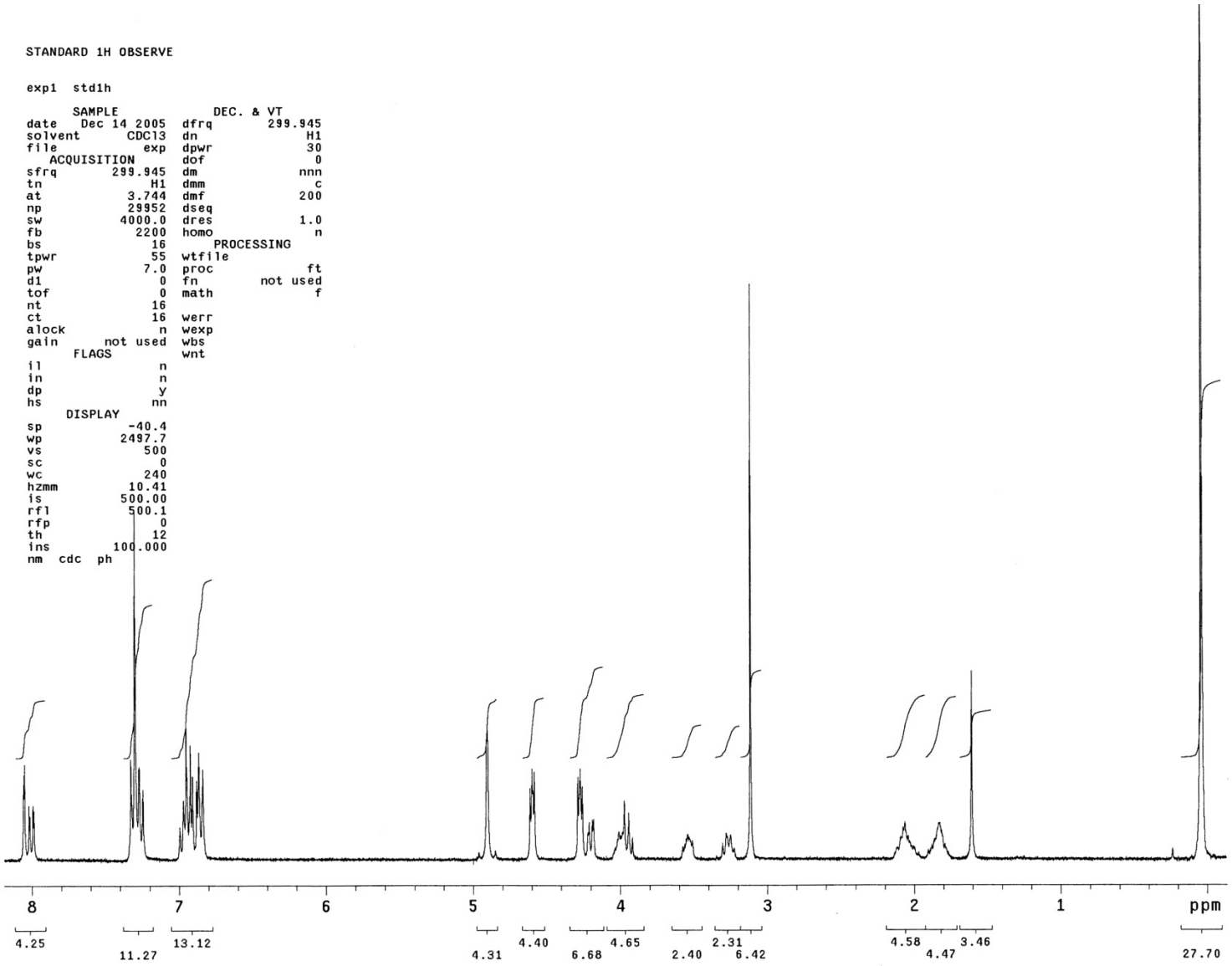

Figure S17. 300 MHz ^{1}H NMR spectrum ($CDCl_3$) of **7a**

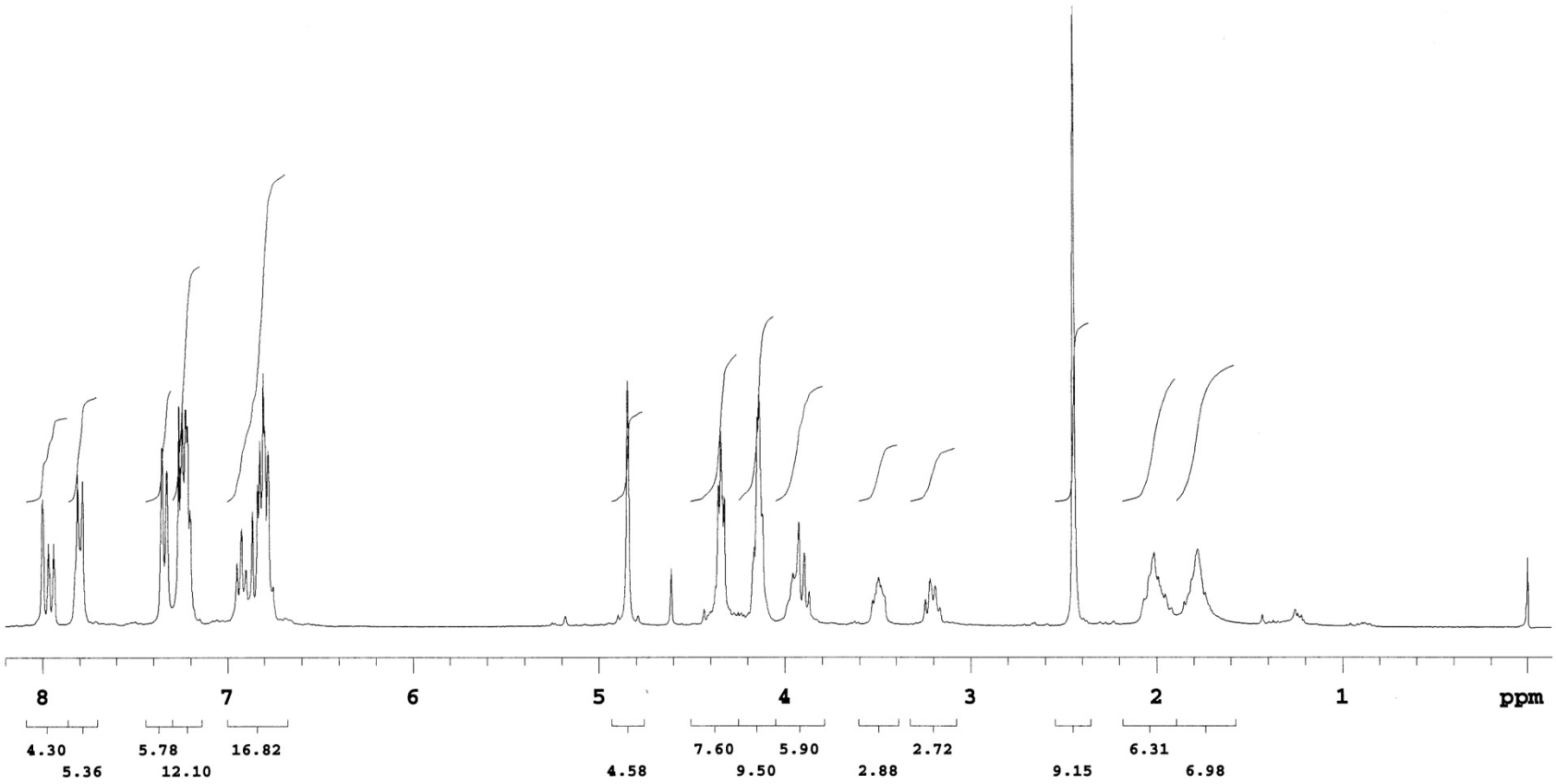

Figure S18. 300 MHz ^{1}H NMR spectrum ($CDCl_3$) of **7b**

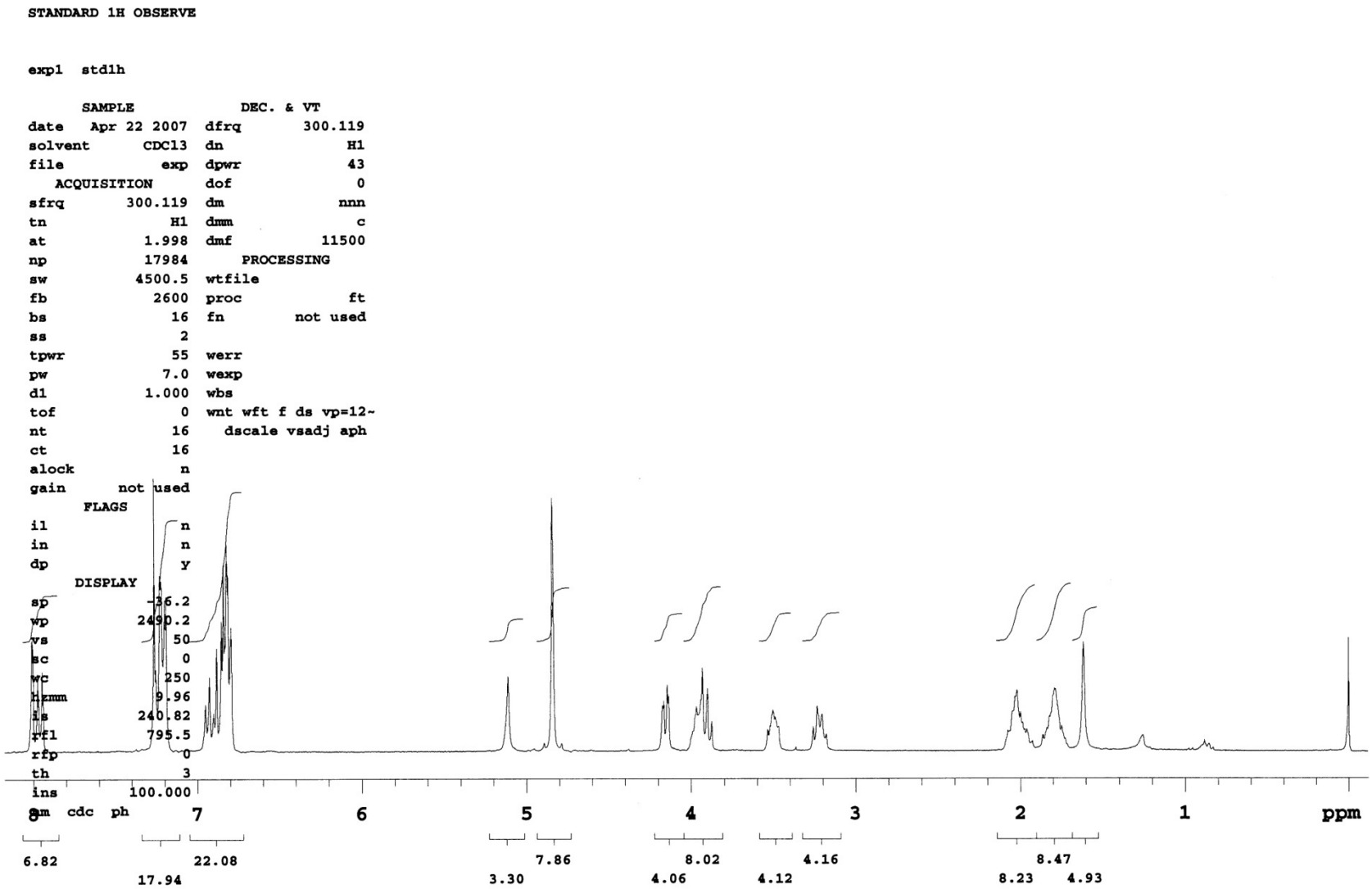

Figure S19. 300 MHz ^{1}H NMR spectrum ($CDCl_3$) of **8**

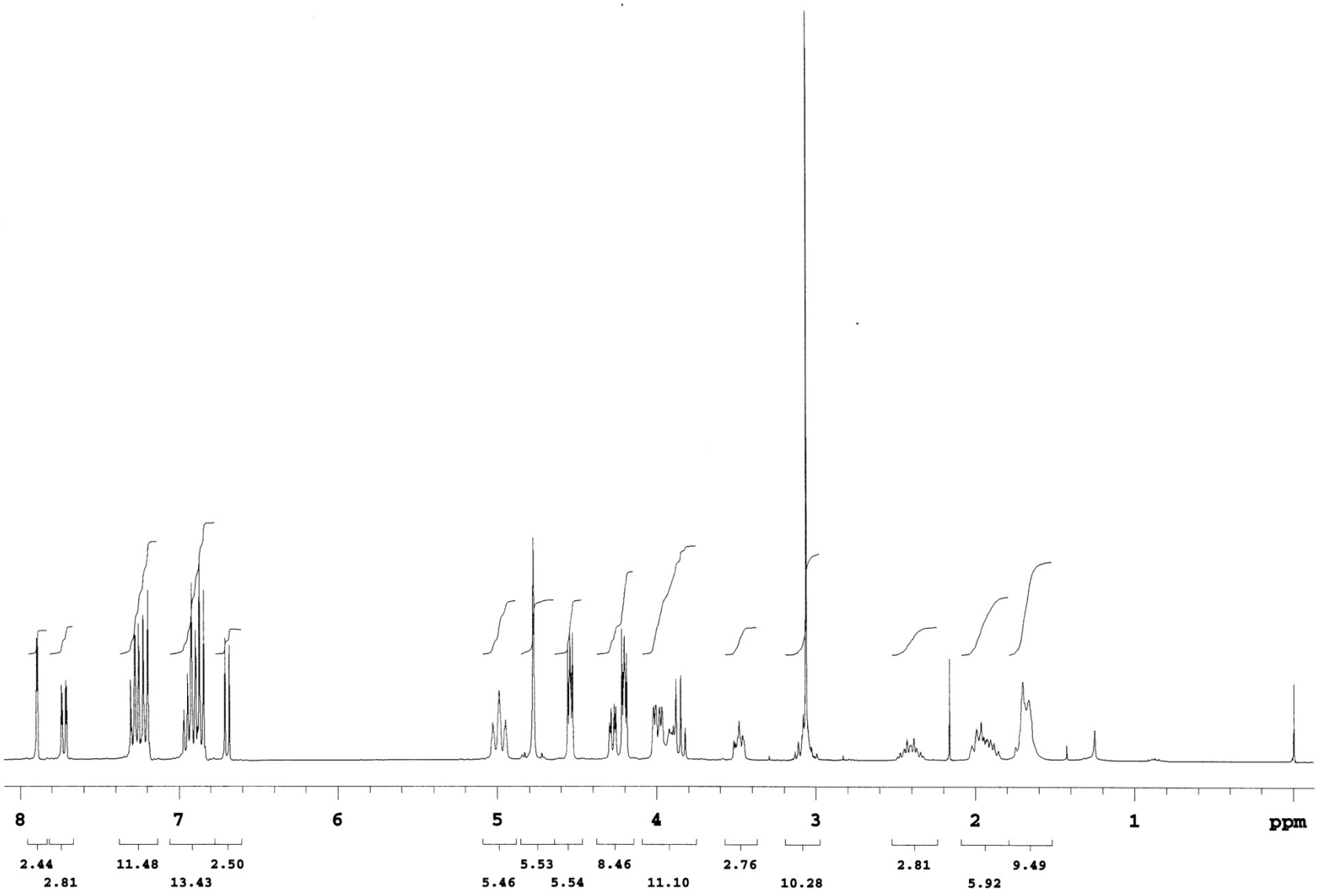

Figure S20. 300 MHz ^{1}H NMR spectrum ($CDCl_3$) of **12**

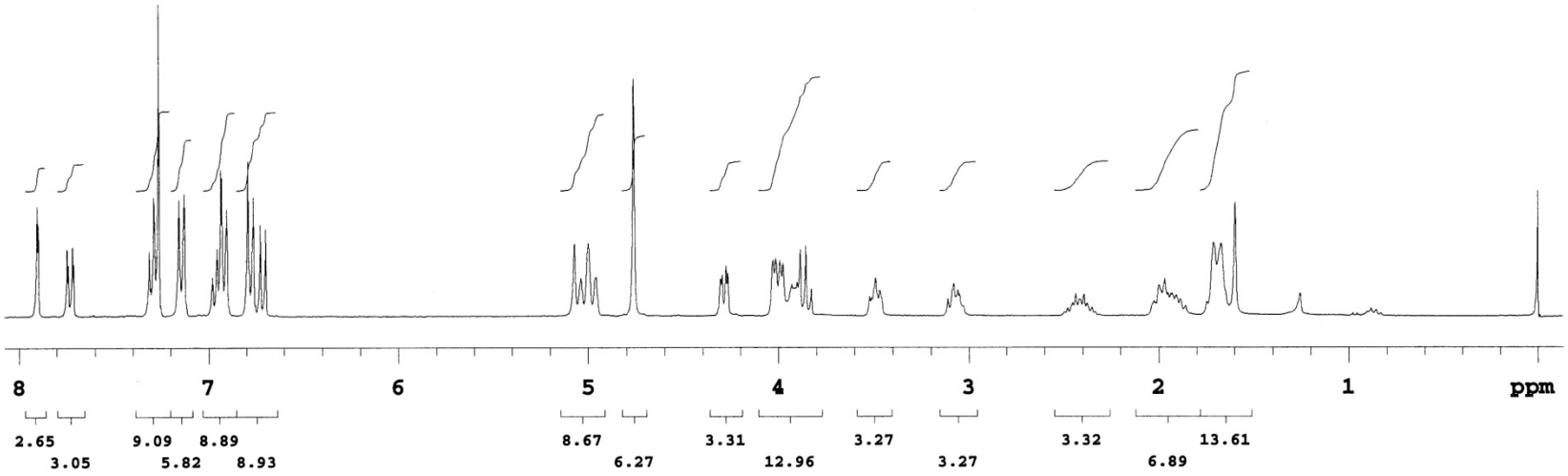

Figure S21. 300 MHz ^{1}H NMR spectrum ($CDCl_3$) of **13**