

## Supporting Information

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

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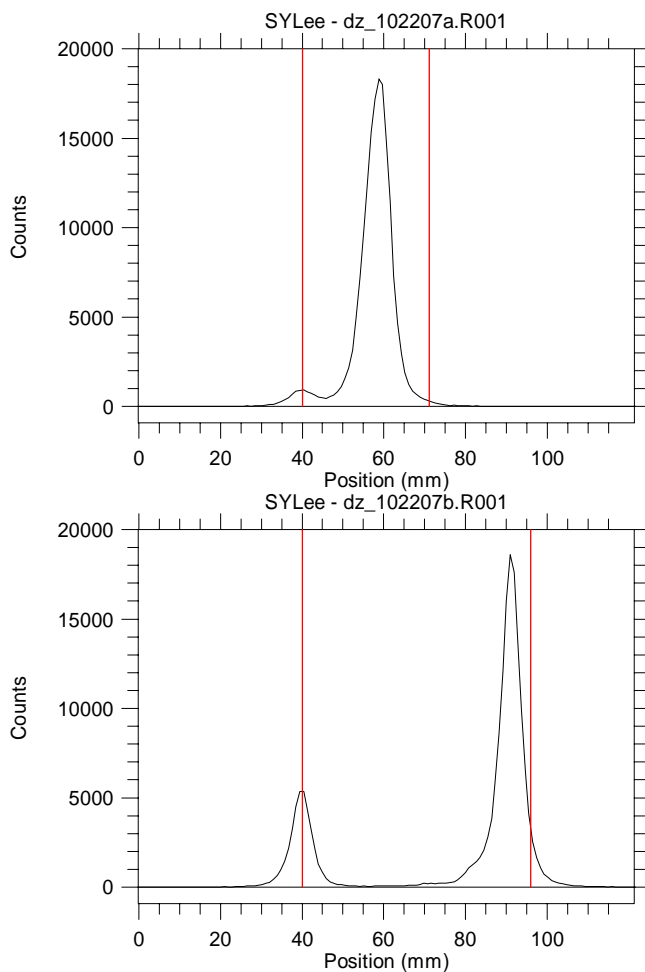
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**General methods and materials** All chemicals were obtained from standard commercial sources and used without further purification. All reactions were carried out by standard air-free and moisture-free techniques under an inert argon atmosphere with dry solvents unless otherwise stated. Flash column chromatography was conducted using Scientific Adsorbents, Inc. silica gel, 60A, "40 Micron Flash" (32-63  $\mu\text{m}$ ). Melting points were uncorrected. Routine  $^1\text{H}$  NMR spectra were recorded at 300 MHz. 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.  $^{19}\text{F}$  NMR spectra were recorded at 282.2 MHz, and chemical shifts are reported as Hz upfield from an external  $\text{CFCl}_3$  standard. High resolution  $^{13}\text{C}$  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 (TI) detector and associated electronics for radioactivity detection. Alltech Econosil C18  $250 \times 10$  mm semi-preparative column and Alltech Altima C18  $250 \times 4.6$  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.)

$\text{H}_2^{18}\text{O}$  was purchased from Rotem Industries (Israel).  $[^{18}\text{F}]\text{Fluoride}$  was produced in Washington University by the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  reaction through proton irradiation of enriched (95%)  $^{18}\text{O}$  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<sub>254</sub> 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). [<sup>11</sup>C]CH<sub>3</sub>I was produced from [<sup>11</sup>C]CO<sub>2</sub> using a GE PETtrace MeI Microlab. [<sup>11</sup>C]CO<sub>2</sub> 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<sub>2</sub> in N<sub>2</sub> for 15–30 min with a 40 μA beam of 16 MeV protons. [<sup>11</sup>C]CO<sub>2</sub> was converted to [<sup>11</sup>C]CH<sub>3</sub>I by the GE PETtrace MeI Microlab using a nickel catalyst [Shimalite-Ni (reduced), Shimadzu, Japan P.N.221-27719] in the presence of H<sub>2</sub> at 360°C and followed by reaction with iodine in the gas phase at 690°C. [<sup>11</sup>C]CH<sub>3</sub>I was delivered in the gas phase with helium approximately 12 min following the end of bombardment.

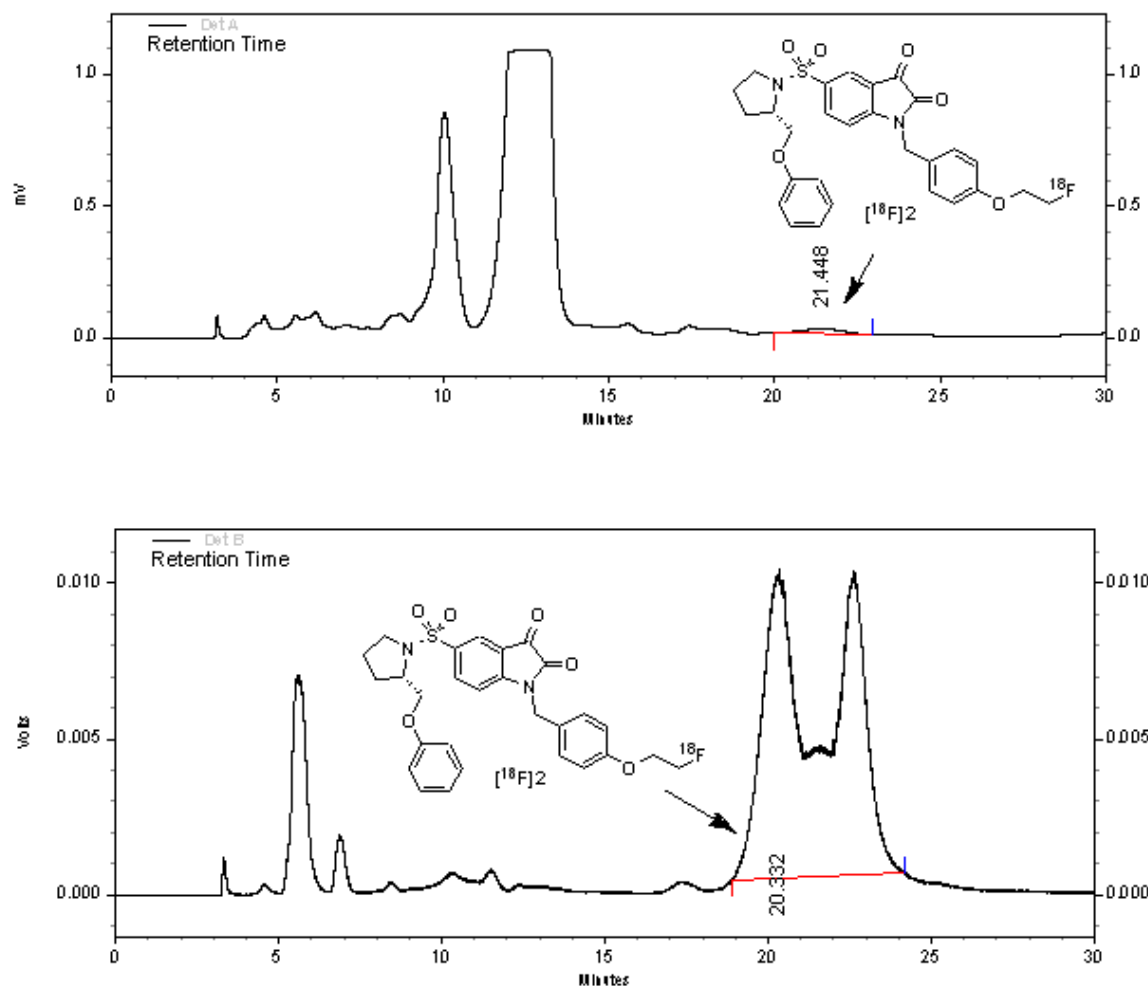


**Figure S1.** RadioTLCs of the reaction mixture of radiosynthesis of [ $^{18}\text{F}$ ] **2**.

Above: Precursor **7a**, [ $^{18}\text{F}$ ]fluoride,  $\text{K}_{222}$ ,  $\text{K}_2\text{CO}_3$ , DMSO, Microwave;

Bottom: 1 N HCl, Microwave.

Silica Gel TLC plate; solvent: 20% MeOH, 80%  $\text{CH}_2\text{Cl}_2$ .

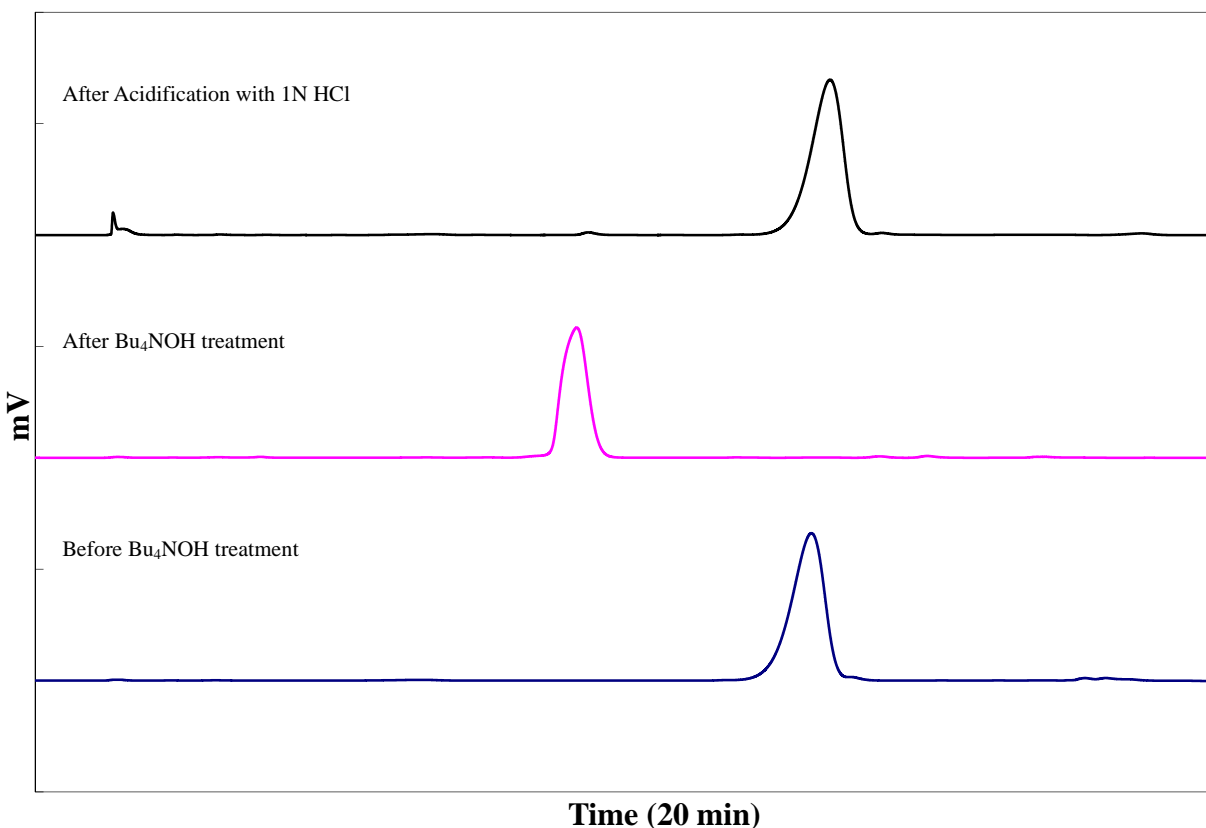


**Figure S2.** Typical HPLC chromatograms for  $[^{18}\text{F}]\mathbf{2}$  purification

Above: UV absorbance at 251 nm;

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

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



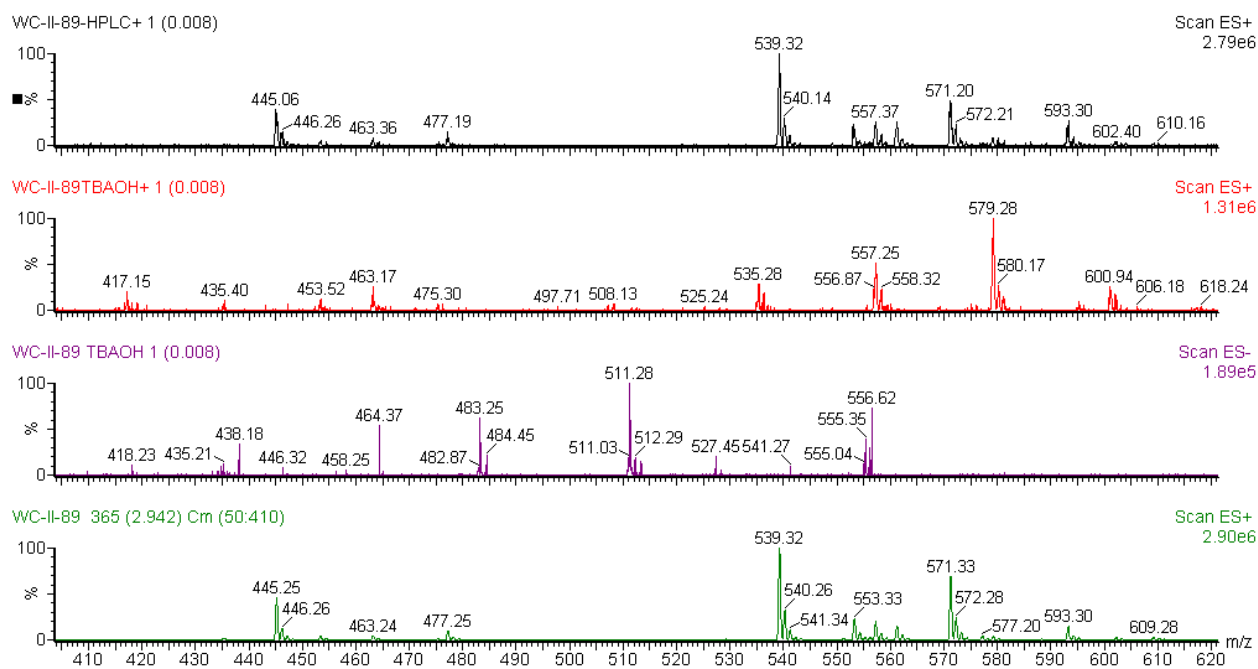
**Figure S3.** HPLC chromatograms of isatin analogue **2** (bottom), isatinate **17** (middle) and isatin **2** recycled from isatinate **17** (top).

HPLC condition: Alltech Altima C18 250 × 4.6 mm 10 $\mu$ , 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<sub>4</sub>NOH treatment: 20  $\mu$ L 1000 ppm **2** injected

Bu<sub>4</sub>NOH treatment: 0.1 mg **2** in 100  $\mu$ L acetonitrile treated with 2  $\mu$ L 1 M Bu<sub>4</sub>NOH/H<sub>2</sub>O, 20  $\mu$ L 1000 ppm injected

1 N HCl treatment: 100  $\mu$ L 1 N HCl, 1 hour at ambient temperature, 40  $\mu$ L 500 ppm injected.



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

Isatin and isatin recycled from isatin were purified by HPLC (see above)



19F OBSERVE  
STANDARD PARAMETERS

Pulse Sequence: s2pul

Solvent: DMSO  
Ambient temperature  
File: isatin\_TBAF\_F  
Mercury-300 "m300"

PULSE SEQUENCE

Relax. delay 4.000 sec  
Pulse 19.5 degrees  
Acq. time 0.300 sec  
Width 50000.0 Hz  
64 repetitions

OBSERVE F19, 282.3941399 MHz

DATA PROCESSING

Line broadening 0.3 Hz  
FT size 32768  
Total time 1 hr, 16 min, 2 sec

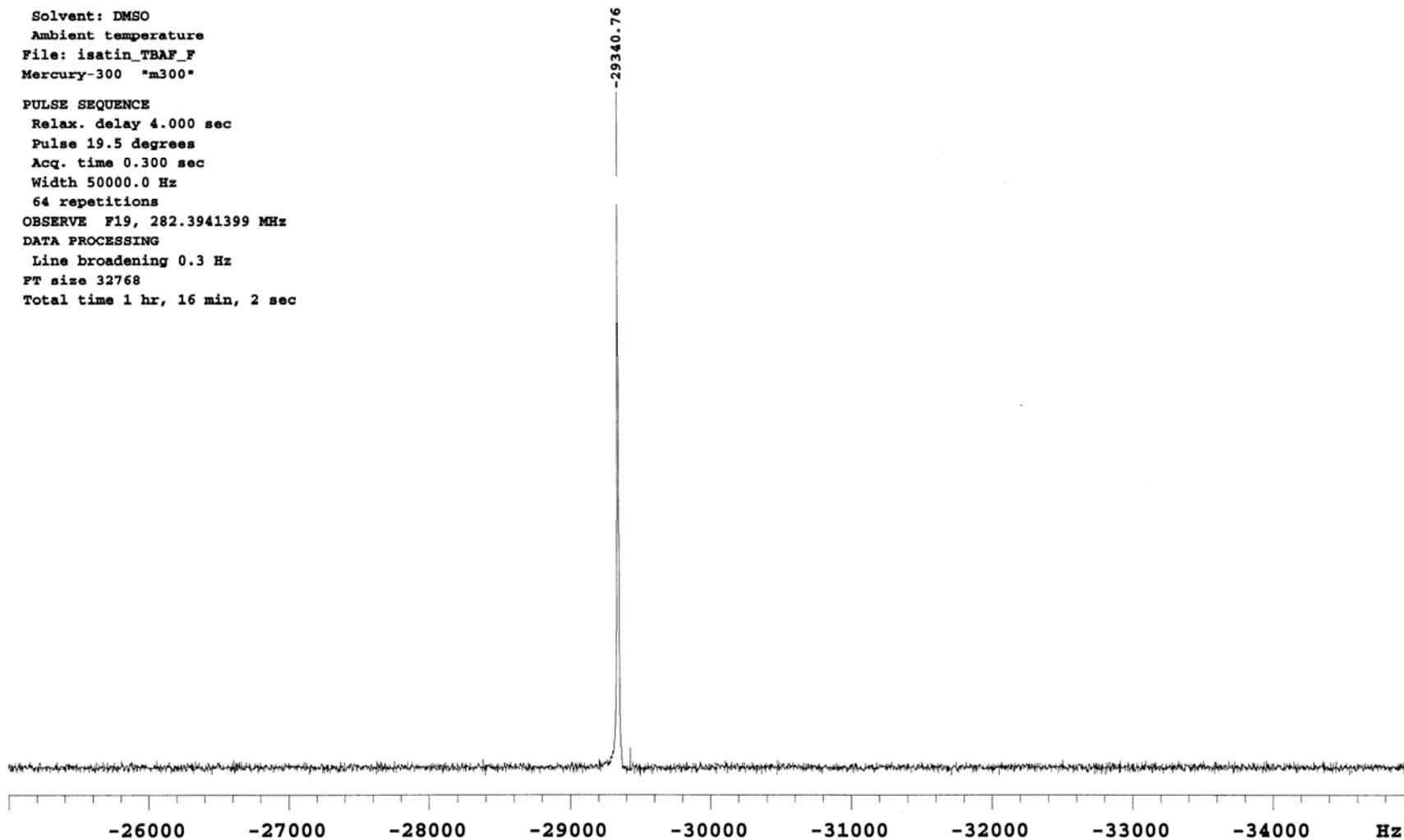


Figure S5. 282 MHz <sup>19</sup>F NMR spectrum (DMSO-d<sub>6</sub>) of Bu<sub>4</sub>NF

19F OBSERVE  
STANDARD PARAMETERS  
  
Pulse Sequence: s2pul  
  
Solvent: DMSO  
Ambient temperature  
File: isatin\_plustBAF300ul\_F  
Mercury-300 "m300"  
  
PULSE SEQUENCE  
Relax. delay 4.000 sec  
Pulse 19.5 degrees  
Acq. time 0.300 sec  
Width 50000.0 Hz  
64 repetitions  
OBSERVE F19, 282.3941399 MHz  
DATA PROCESSING  
Line broadening 0.3 Hz  
FT size 32768  
Total time 1 hr, 16 min, 2 sec

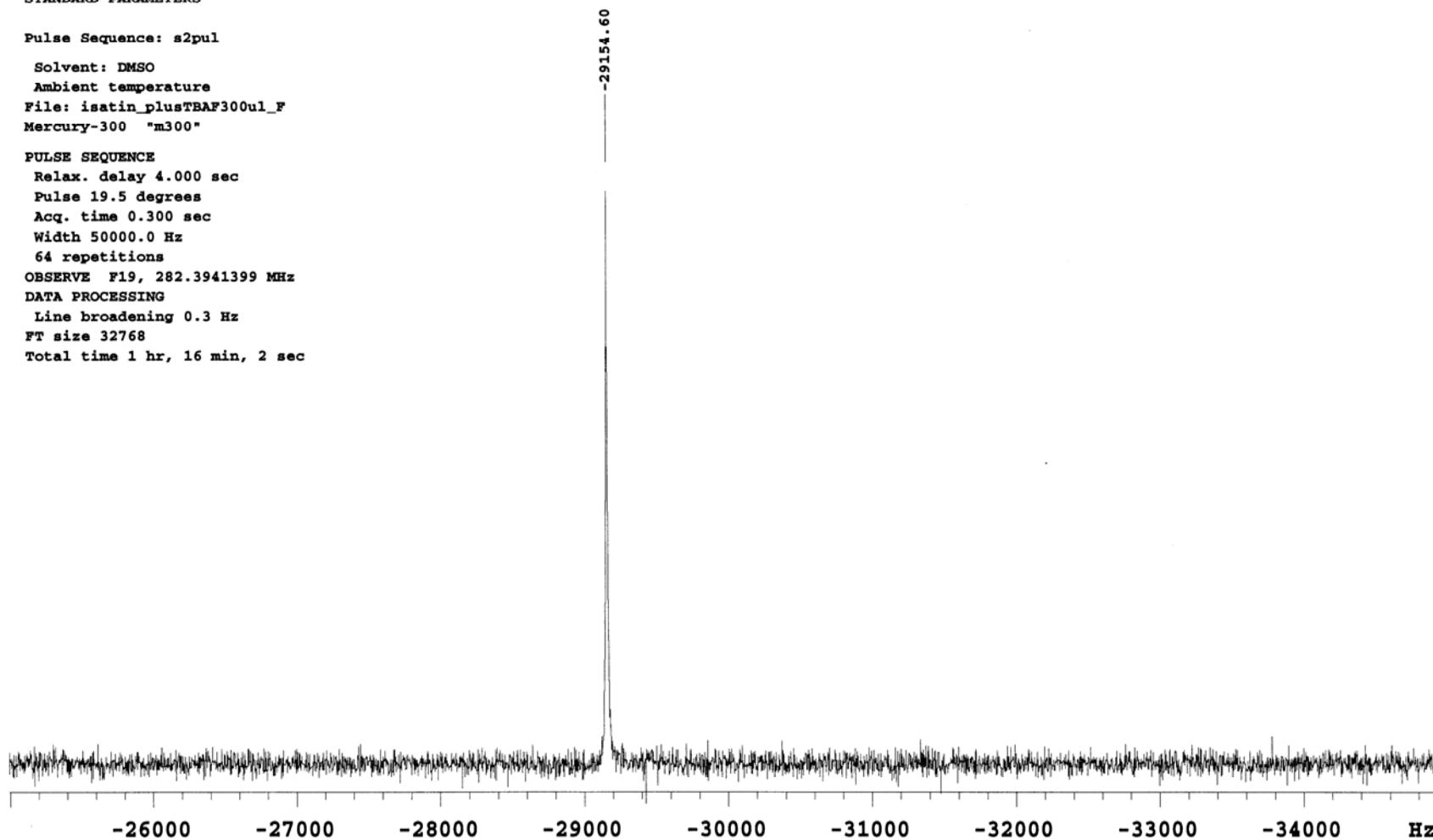


Figure S6. 282 MHz <sup>19</sup>F NMR spectrum (DMSO-d<sub>6</sub>) of Bu<sub>4</sub>NF with addition of N-benzyl isatin **21**

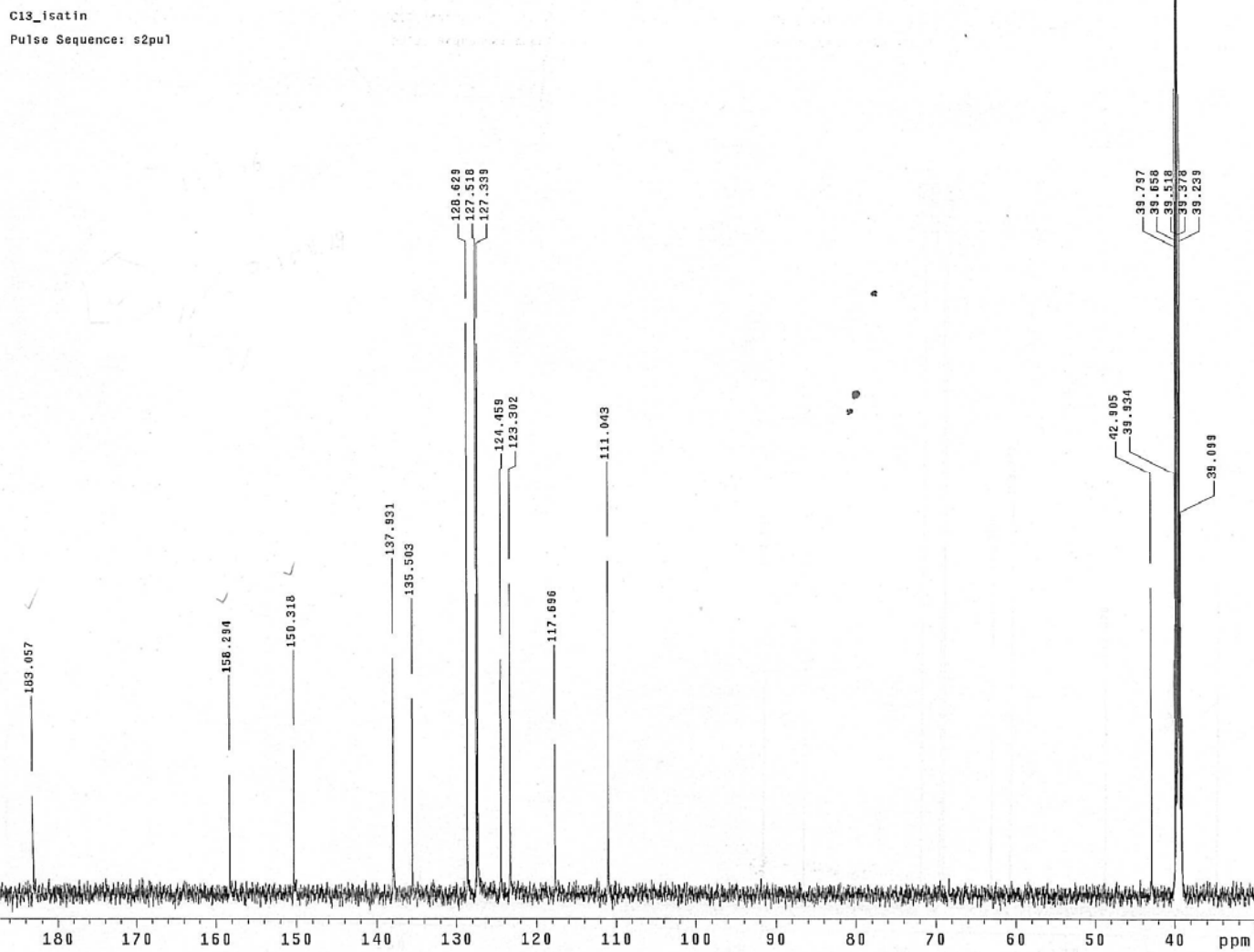
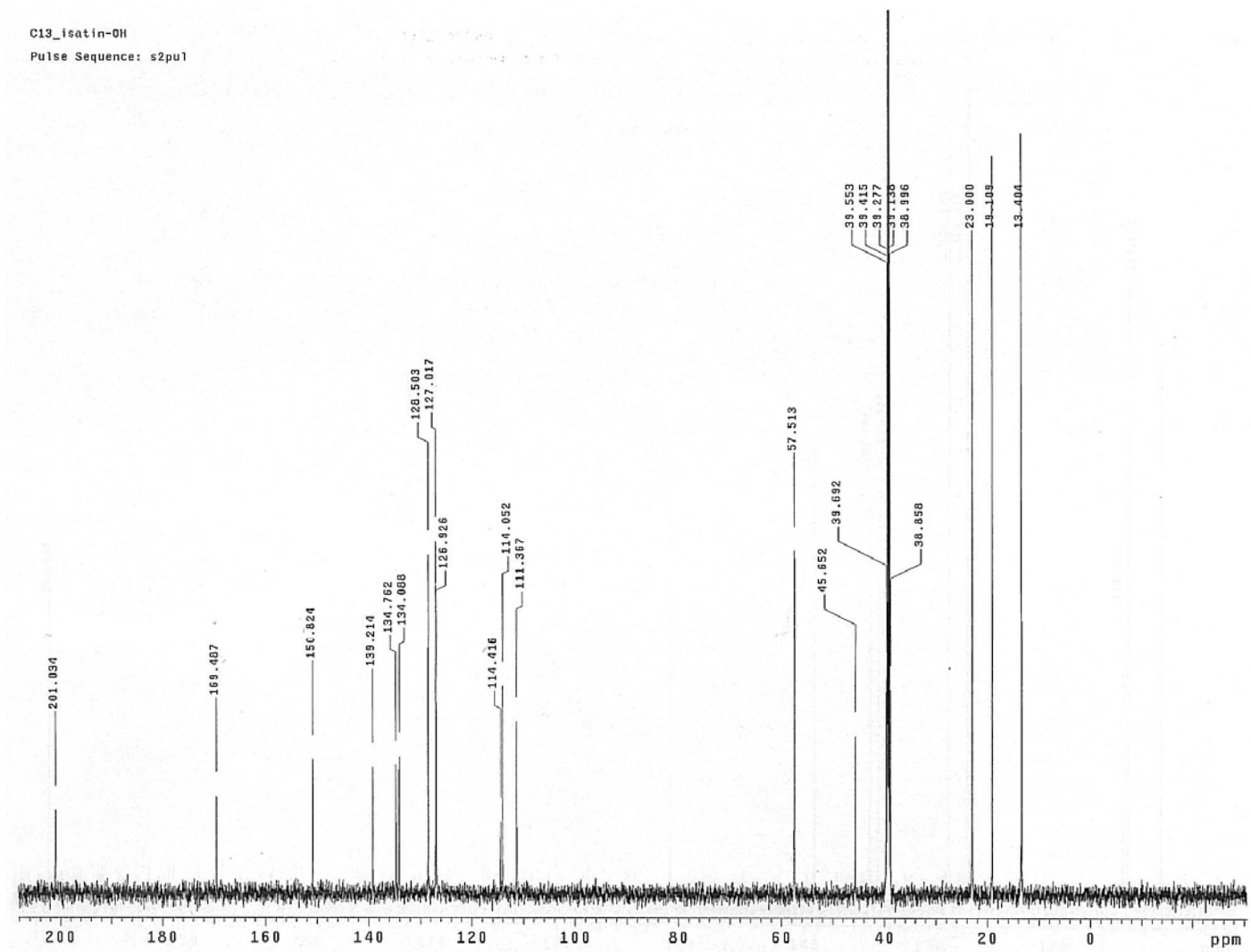
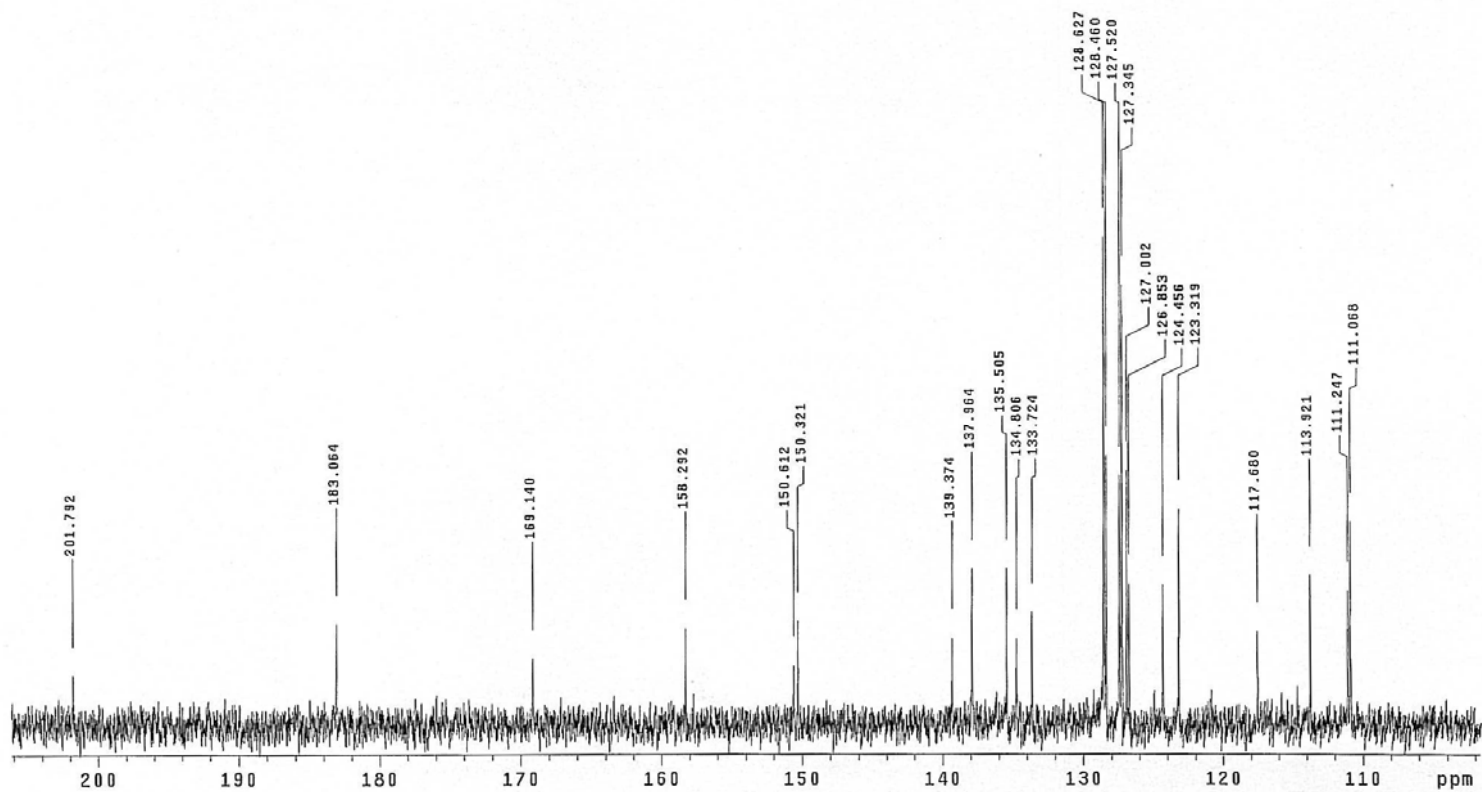


Figure S7. 150 MHz  $^{13}\text{C}$  NMR (DMSO- $d_6$ ) Spectrum of N-benzyl isatin **21**.

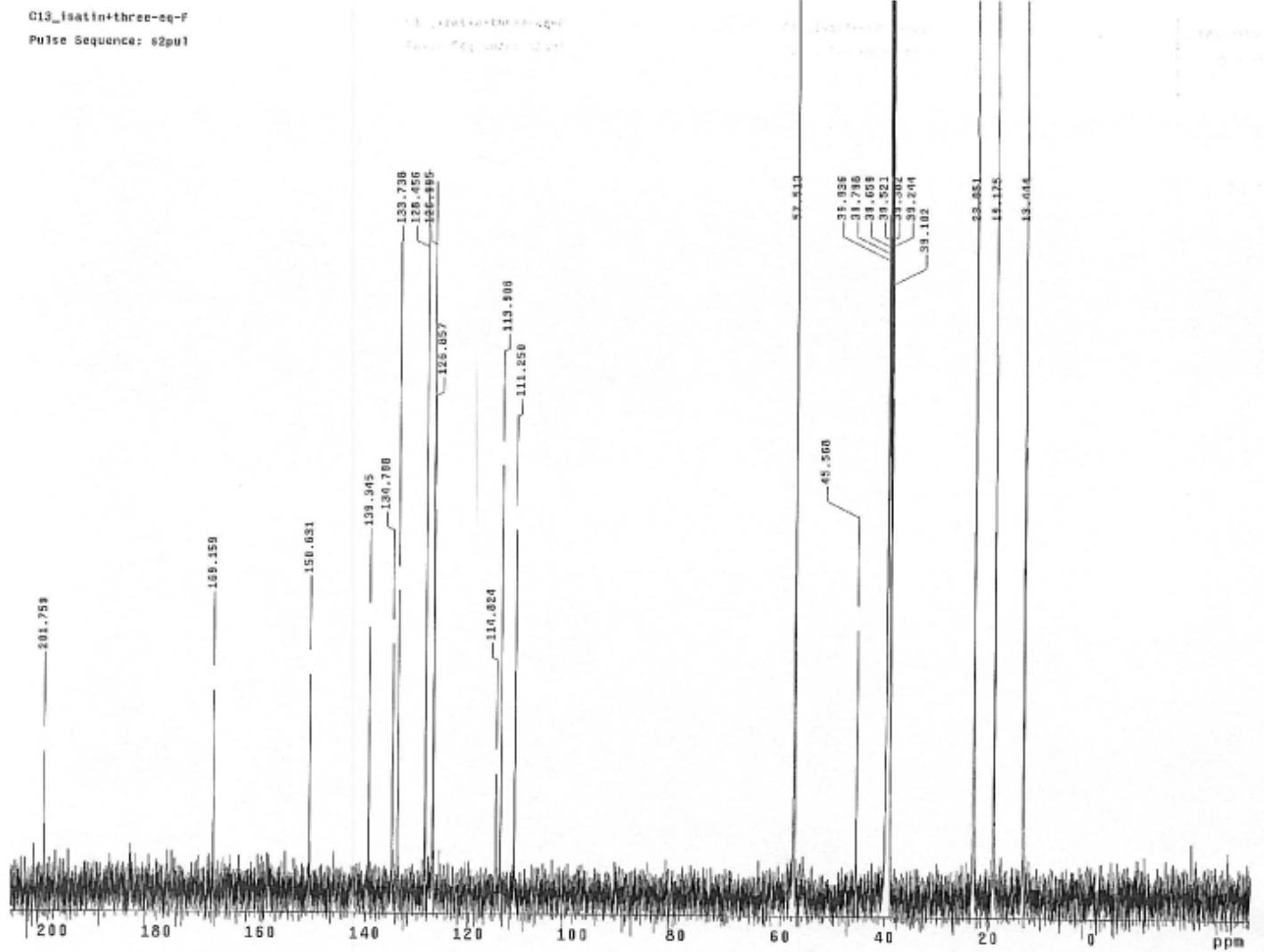


**Figure S8.** 150 MHz  $^{13}\text{C}$  NMR Spectrum (DMSO- $d_6$ ) of N-benzyl isatin **21** treated with 1M  $\text{Bu}_4\text{NOH}/\text{H}_2\text{O}$  (10 mg **21** in 0.6 mL DMSO- $d_6$ )

C13\_isatin+one-eq-F  
Pulse Sequence: s2pu1



**Figure S9.** 150 MHz <sup>13</sup>C NMR Spectrum (DMSO-d<sub>6</sub>) of N-benzyl isatin **21** treated with 1 equivalent Bu<sub>4</sub>NF/H<sub>2</sub>O (10 mg **21** in 0.6 mL DMSO-d<sub>6</sub>)



**Figure S10.** 150 MHz  $^{13}\text{C}$  NMR Spectrum (DMSO- $d_6$ ) of N-benzyl isatin **21** treated with 3 equivalents  $\text{Bu}_4\text{NF}/\text{H}_2\text{O}$  (10 mg **21** in 0.6 mL DMSO- $d_6$ )

C13\_WC2-89\_OMs  
Pulse Sequence: s2pu1  
Solvent: DMSO  
Temp. 25.0 C / 298.1 K  
User: 1-14-87  
File: C13\_WC2-89\_OMs  
INNOVA-600 "khan"  
  
Relax. delay 1.000 sec  
Pulse 86.5 degrees  
Acq. time 1.300 sec  
Width 29996.3 Hz  
344 repetitions  
OBSERVE C13, 150.8063061 MHz  
DECOUPLE H1, 599.7479451 MHz  
Power 44 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 2.0 Hz  
FT size 131072  
Total time 641 hr, 30 min, 5 sec

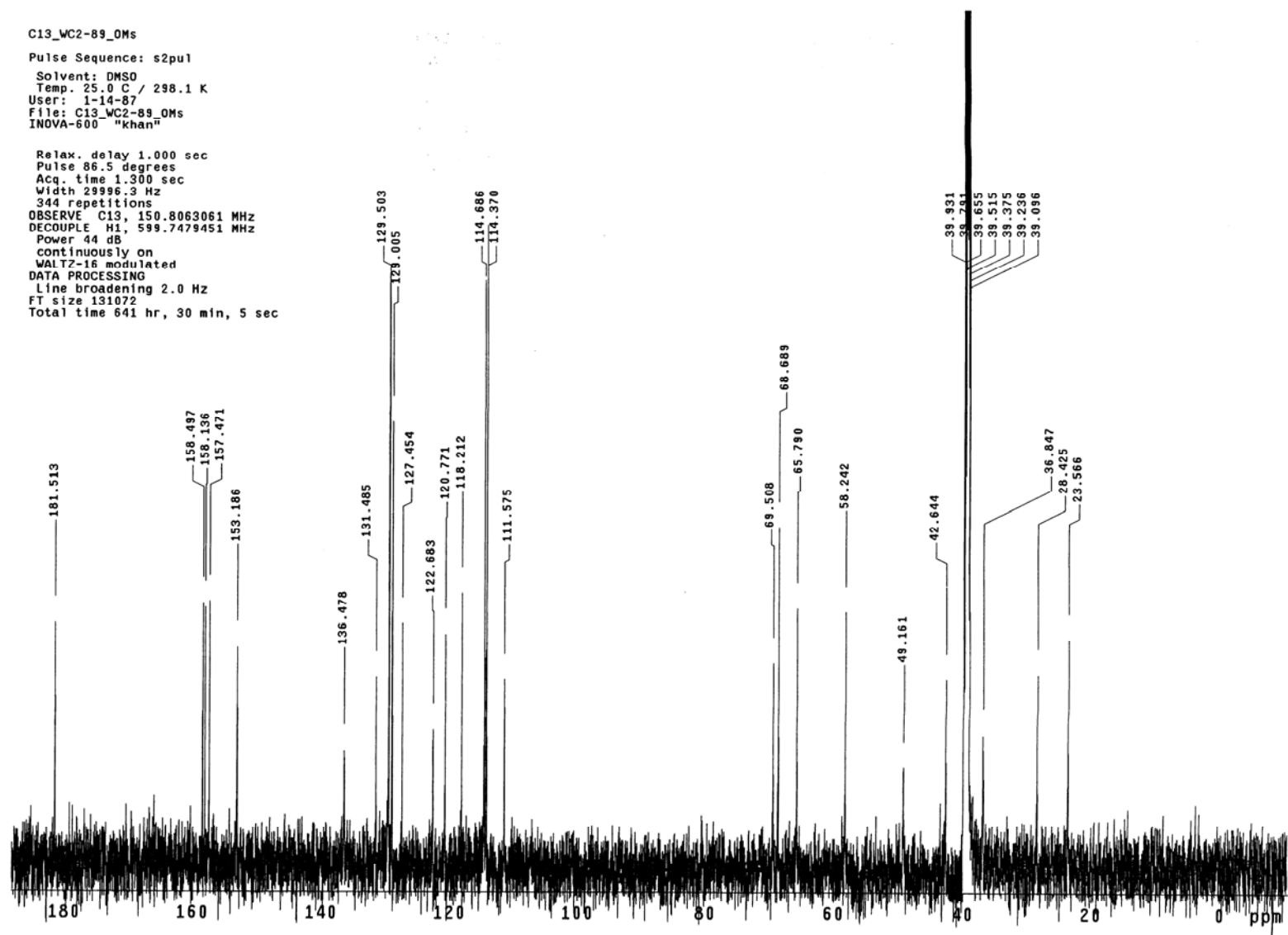
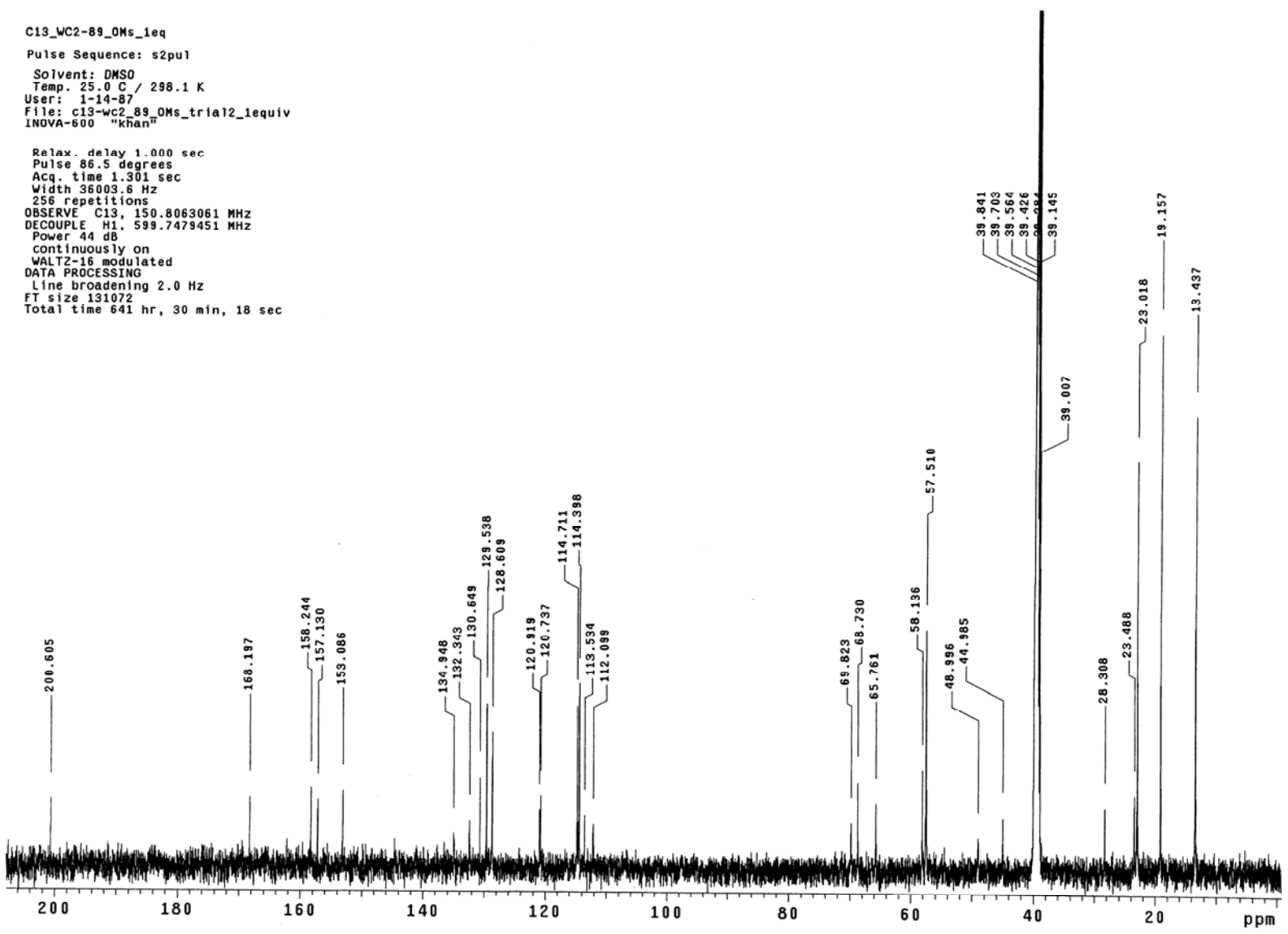


Figure S11. 150 MHz  $^{13}\text{C}$  NMR spectrum (DMSO- $d_6$ ) of **7a** (10 mg **7a** in 0.6 mL DMSO- $d_6$ )



**Figure S12.** 150 MHz  $^{13}\text{C}$  NMR spectrum (DMSO- $d_6$ ) of ring-opened **7a** (10 mg **7a** in 0.6 mL DMSO- $d_6$  treated with 20  $\mu\text{L}$  1M  $\text{Bu}_4\text{NOH}/\text{H}_2\text{O}$ )



C13\_WC2-89  
Pulse Sequence: s2pul  
Solvent: DMSO  
Temp. 25.0 C / 298.1 K  
User: 1-14-87  
File: C13\_WC2-89  
INOVA-600 "khan"  
  
Relax. delay 1.000 sec  
Pulse 86.5 degrees  
Acq. time 1.301 sec  
Width 36003.6 Hz  
112 repetitions  
OBSERVE C13, 150.8063061 MHz  
DECOUPLE H1, 599.7478451 MHz  
Power 44 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 2.0 Hz  
FT size 131072  
Total time 641 hr, 30 min, 18 sec

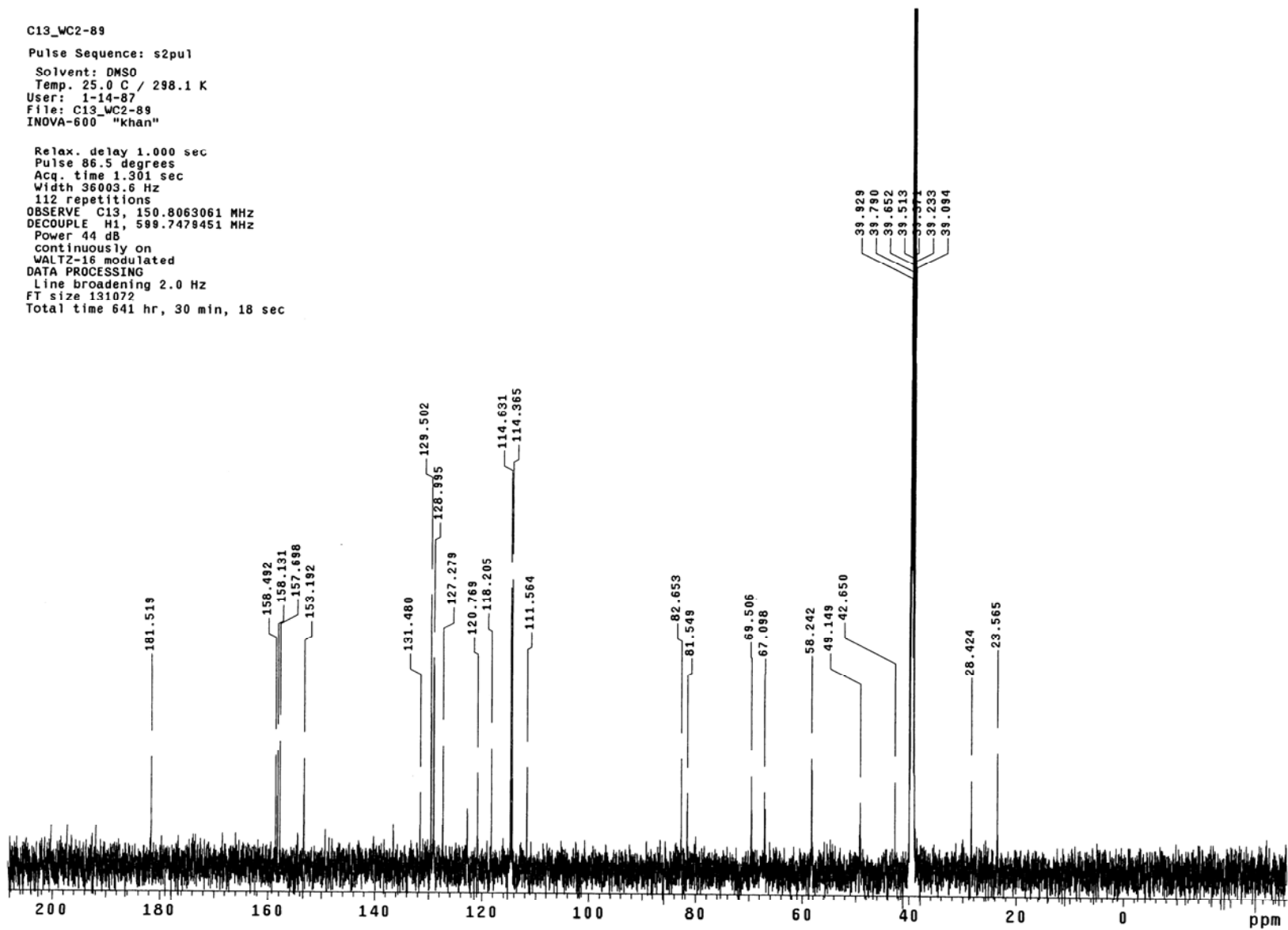
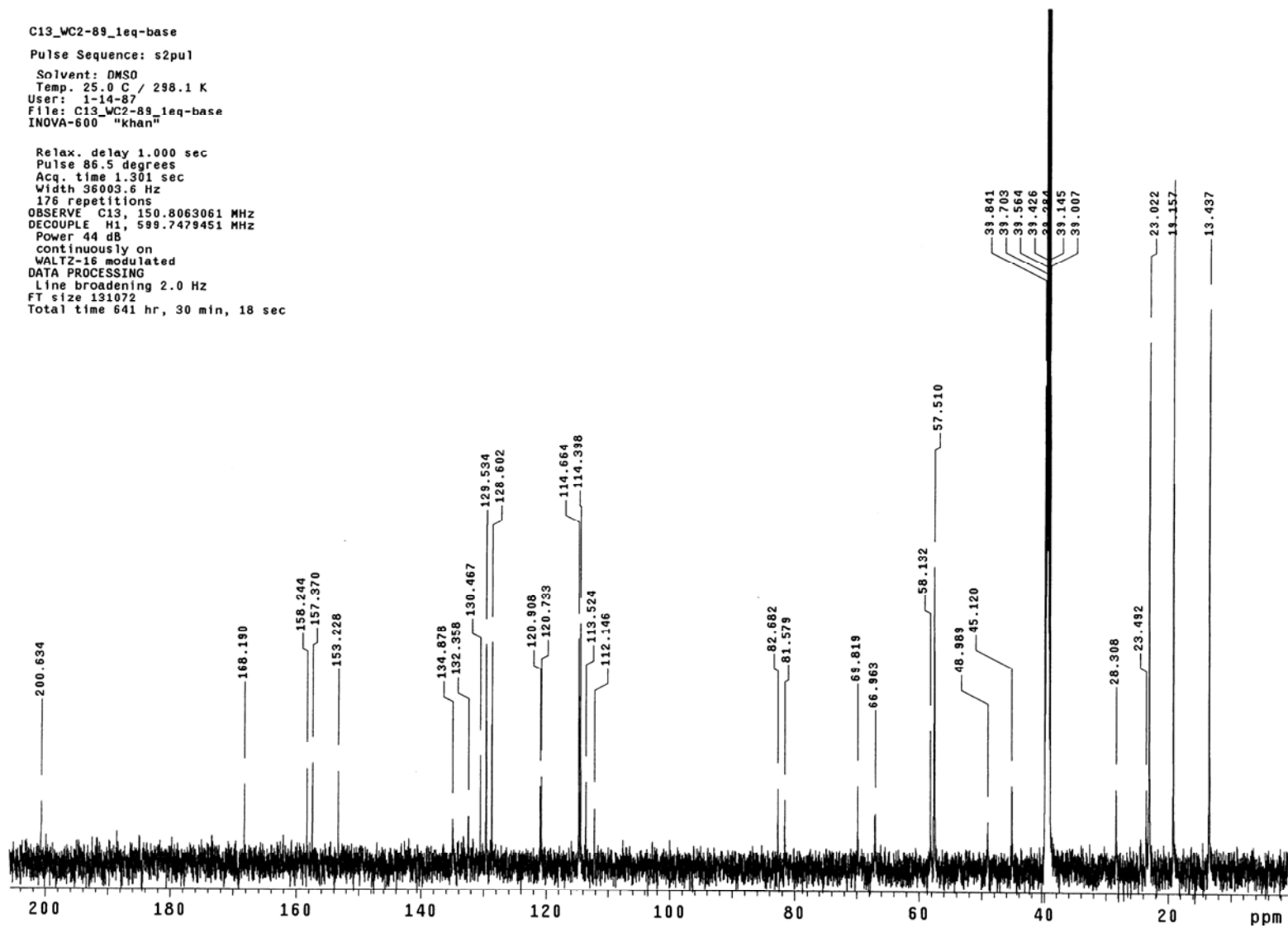
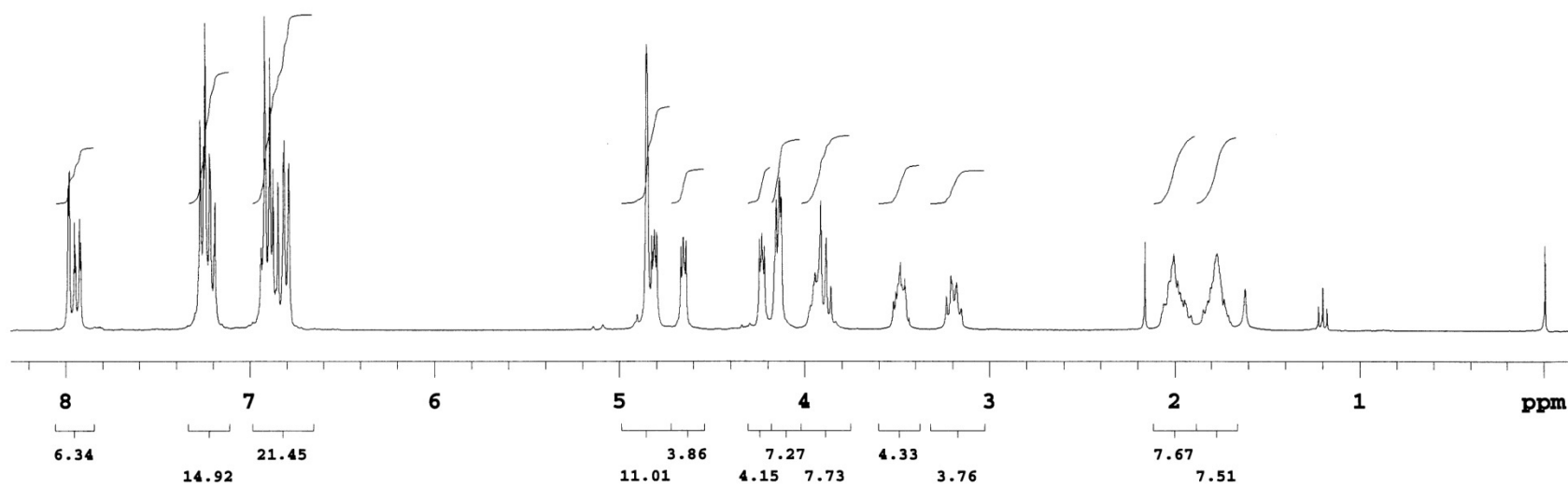


Figure S13. 150 MHz  $^{13}\text{C}$  NMR spectrum (DMSO- $d_6$ ) of **2** (10 mg **2** in 0.6 mL DMSO- $d_6$ )

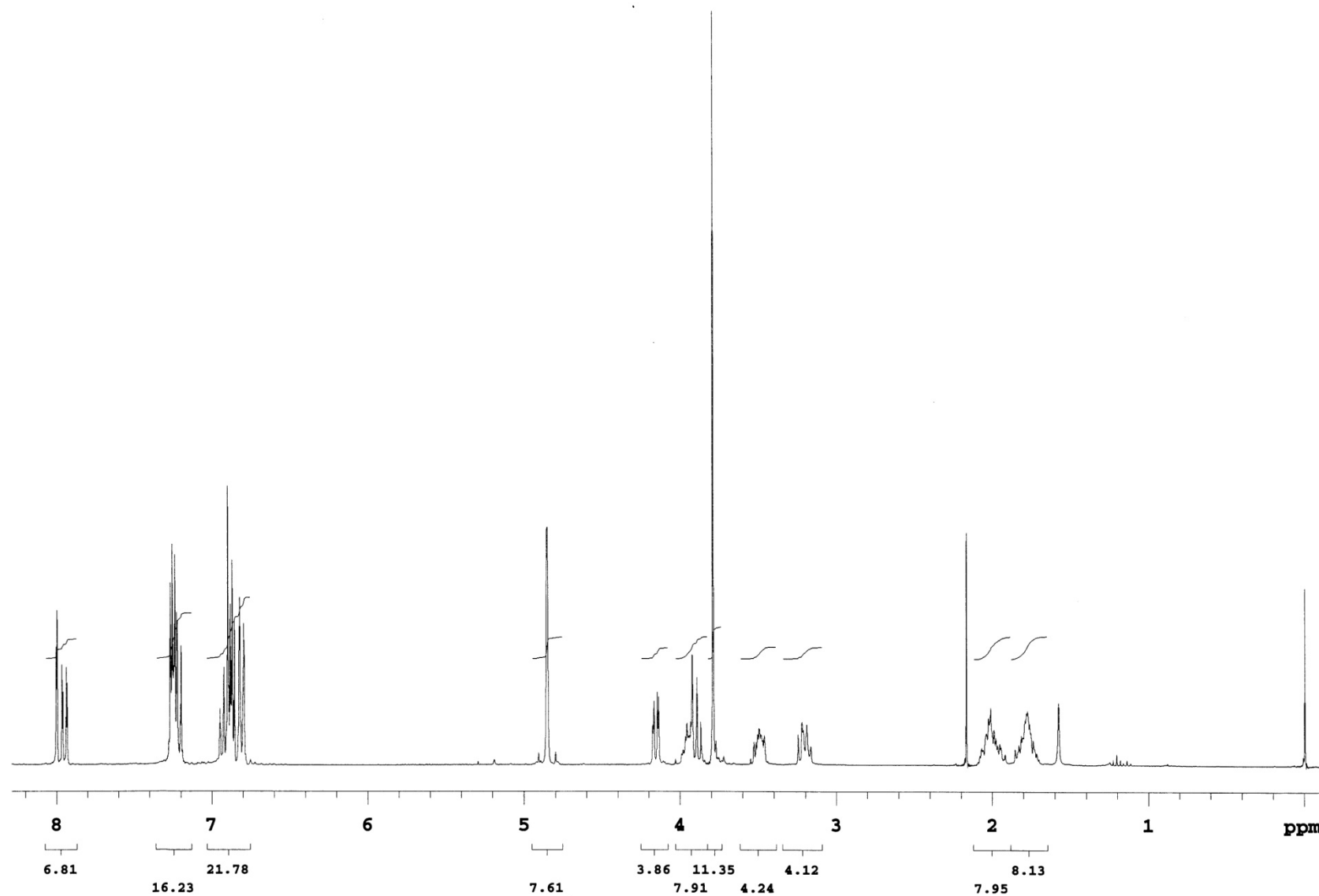
C13\_WC2-89\_1eq-base  
Pulse Sequence: s2pul  
Solvent: DMSO  
Temp: 25.0 C / 298.1 K  
User: 1-14-87  
File: C13\_WC2-89\_1eq-base  
INOVA-600 "khan"  
  
Relax. delay 1.000 sec  
Pulse 86.5 degrees  
Acq. time 1.301 sec  
Width 36003.6 Hz  
176 repetitions  
OBSERVE C13, 150.8063061 MHz  
DECOUPLE H1, 599.7479451 MHz  
Power 44 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 2.0 Hz  
FT size 131072  
Total time 641 hr, 30 min, 18 sec



**Figure S14.** 150 MHz  $^{13}\text{C}$  NMR spectrum (DMSO- $d_6$ ) of ring-opened **2** (10 mg **2** in 0.6 mL DMSO- $d_6$  treated with 20  $\mu\text{L}$  1M  $\text{Bu}_4\text{NOH}/\text{H}_2\text{O}$ )



**Figure S15.** 300 MHz  $^1\text{H}$  NMR spectrum (CDCl<sub>3</sub>) of **2**



**Figure S16.** 300 MHz  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ ) of **4**

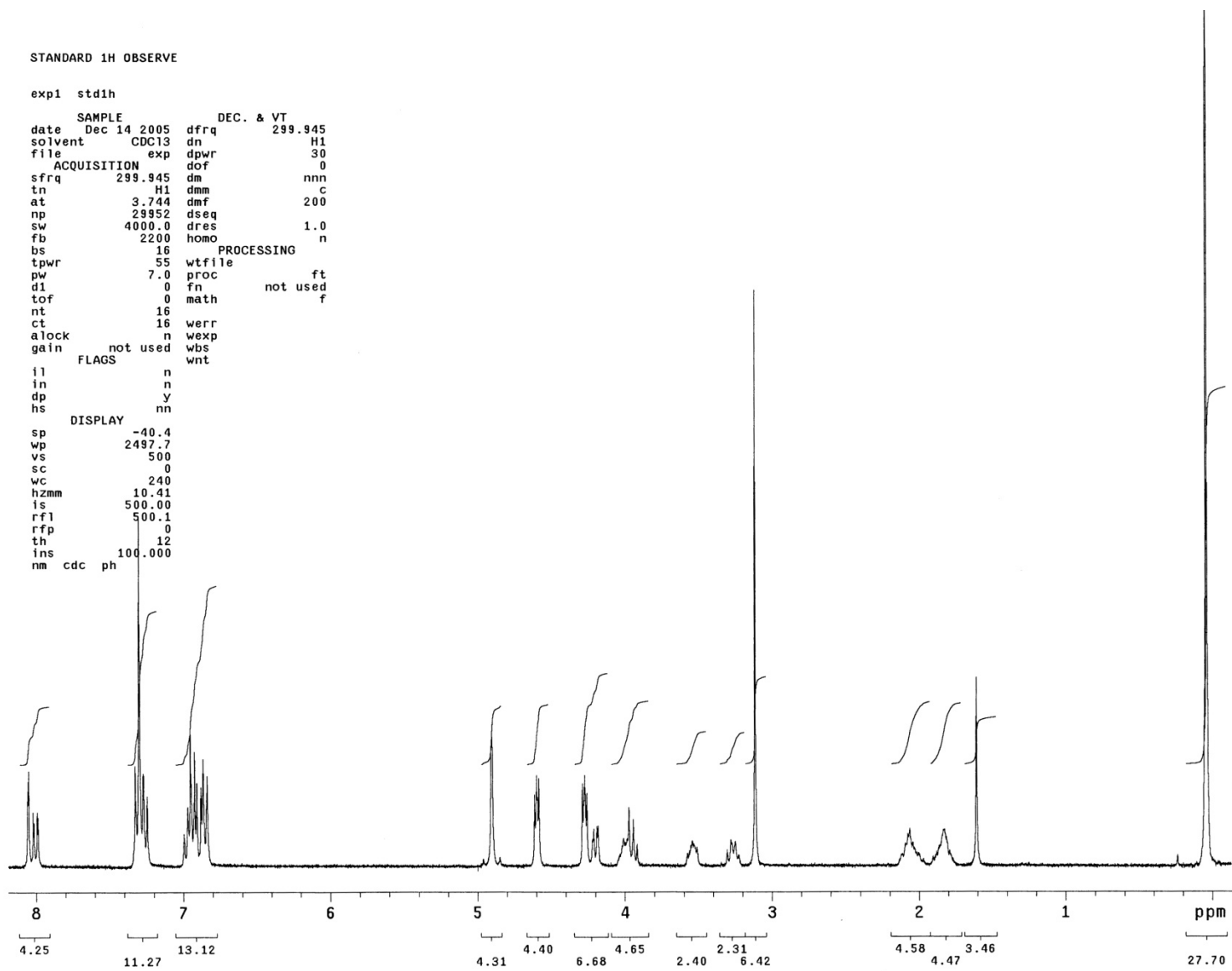
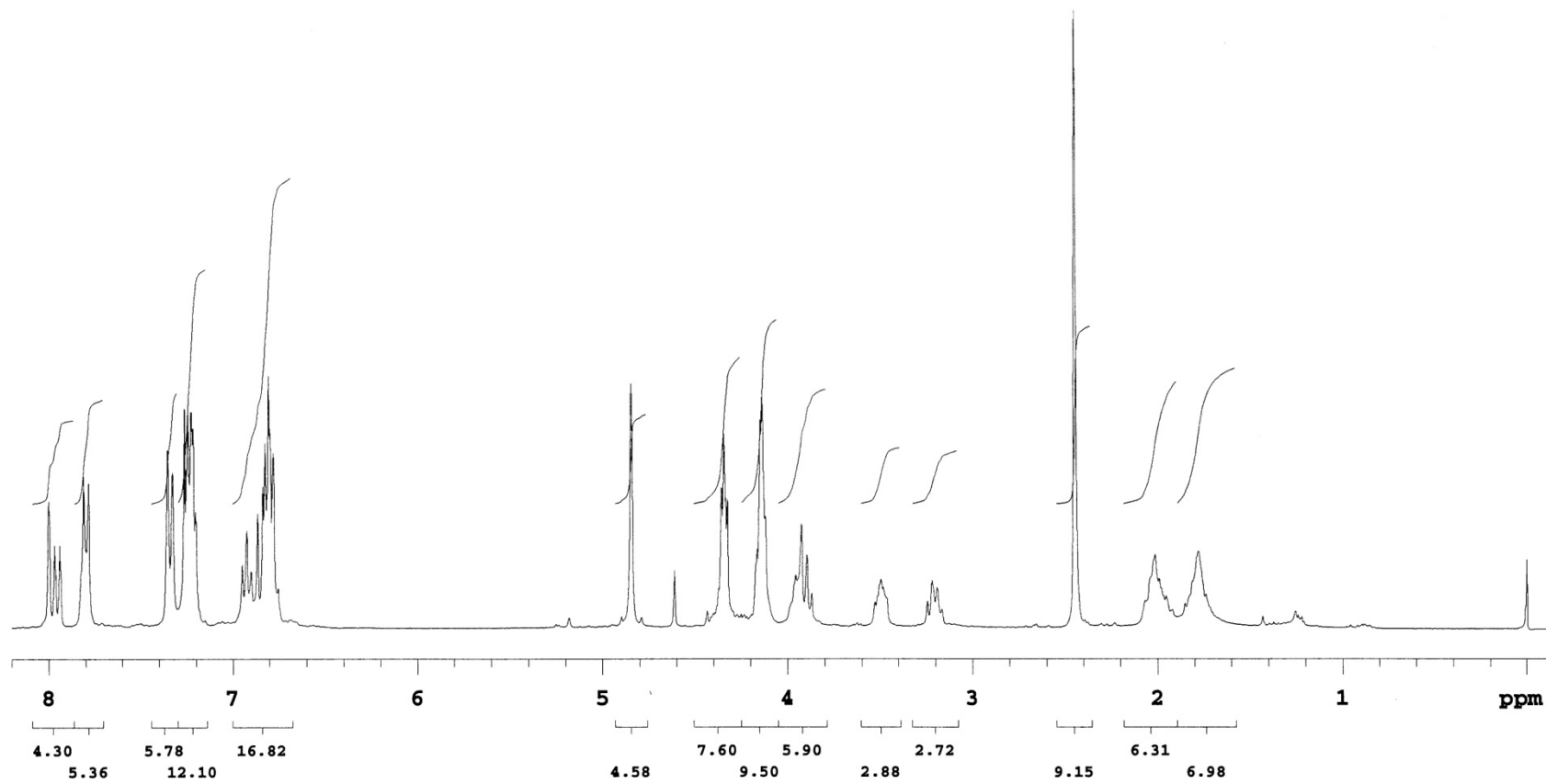


Figure S17. 300 MHz  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ ) of **7a**



**Figure S18.** 300 MHz <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>) of **7b**

STANDARD 1H OBSERVE

exp1 std1h

SAMPLE DEC. & VT  
date Apr 22 2007 dfrq 300.119  
solvent CDCl3 dn H1  
file exp dpwr 43  
ACQUISITION dof 0  
sfrq 300.119 dm nnn  
tn H1 dmm c  
at 1.998 dmf 11500  
np 17984 PROCESSING  
sw 4500.5 wtfile  
fb 2600 proc ft  
bs 16 fn not used  
ss 2  
tpwr 55 werr  
pw 7.0 wexp  
dl 1.000 wbs  
tof 0 wnt wft f ds vp=12-  
nt 16 dscale vsadj aph  
ct 16  
alock n  
gain not used

FLAGS  
il n  
in n  
dp y

DISPLAY  
sp -36.2  
wp 2490.2  
vs 50  
sc 0  
wc 250  
lgmm 9.96  
ls 240.82  
rf1 795.5  
rtp 0  
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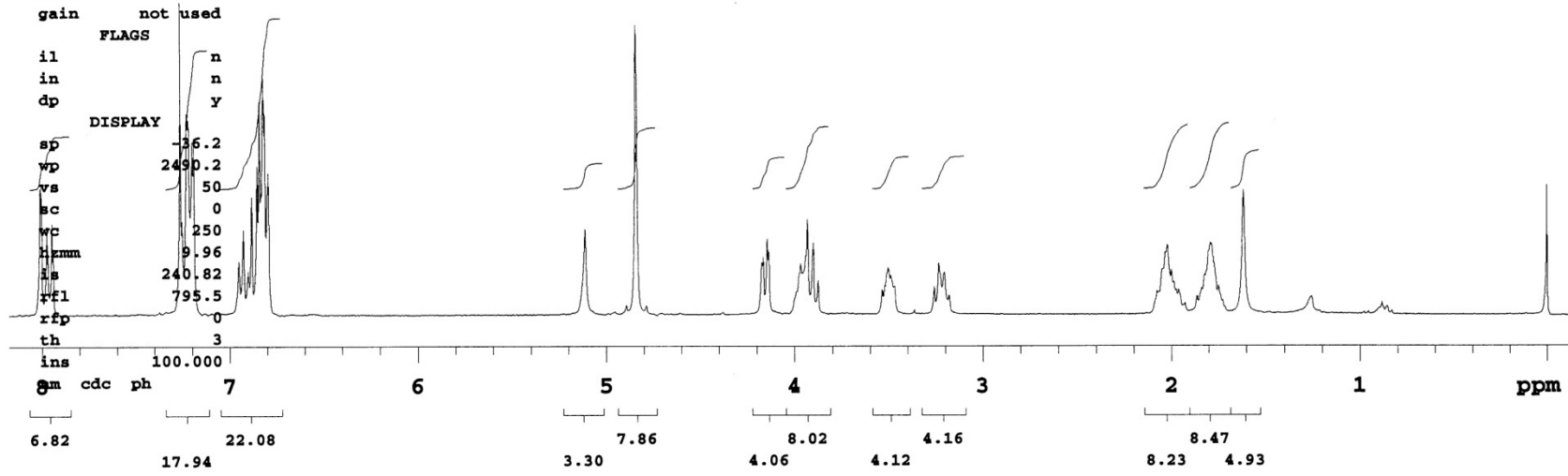
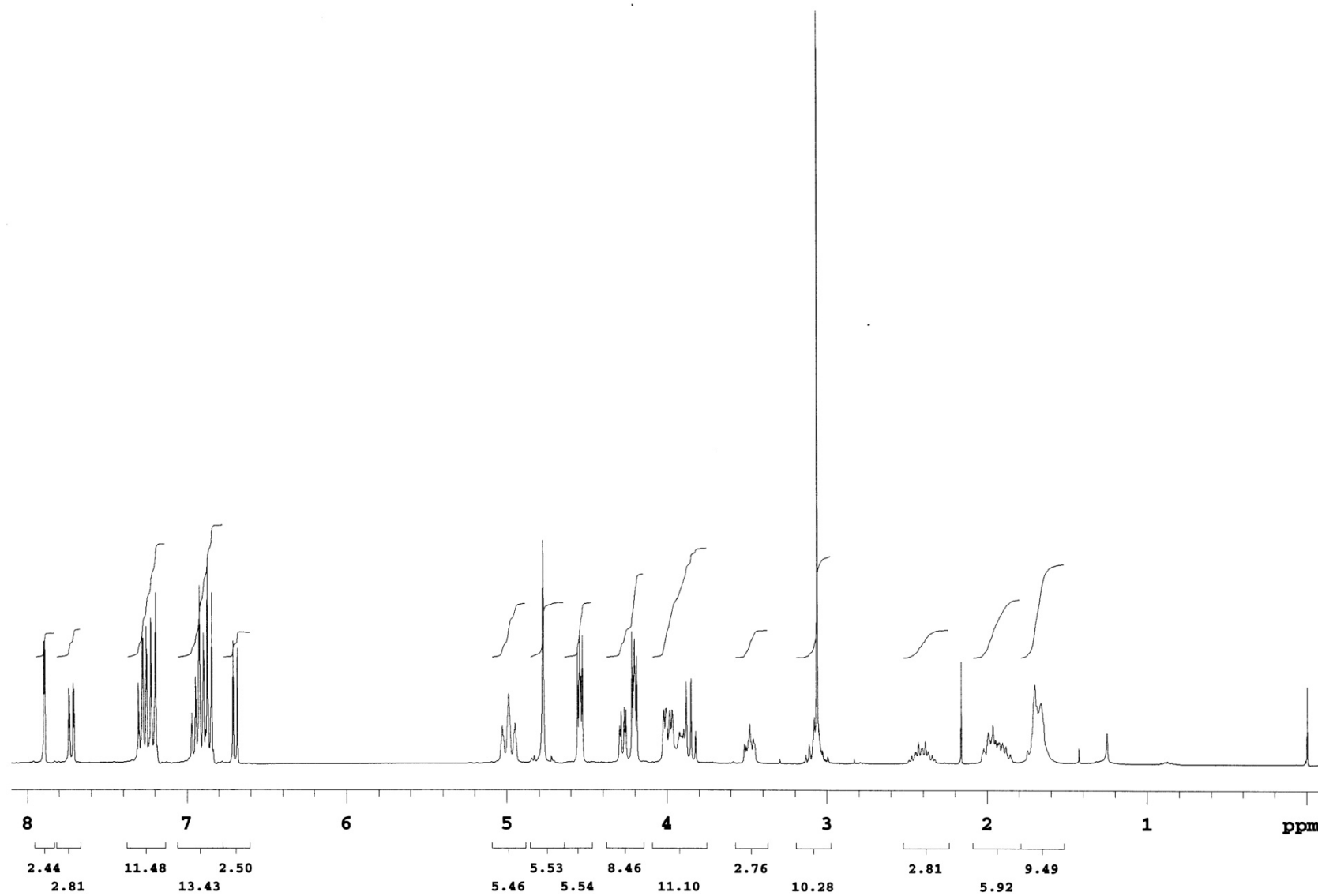
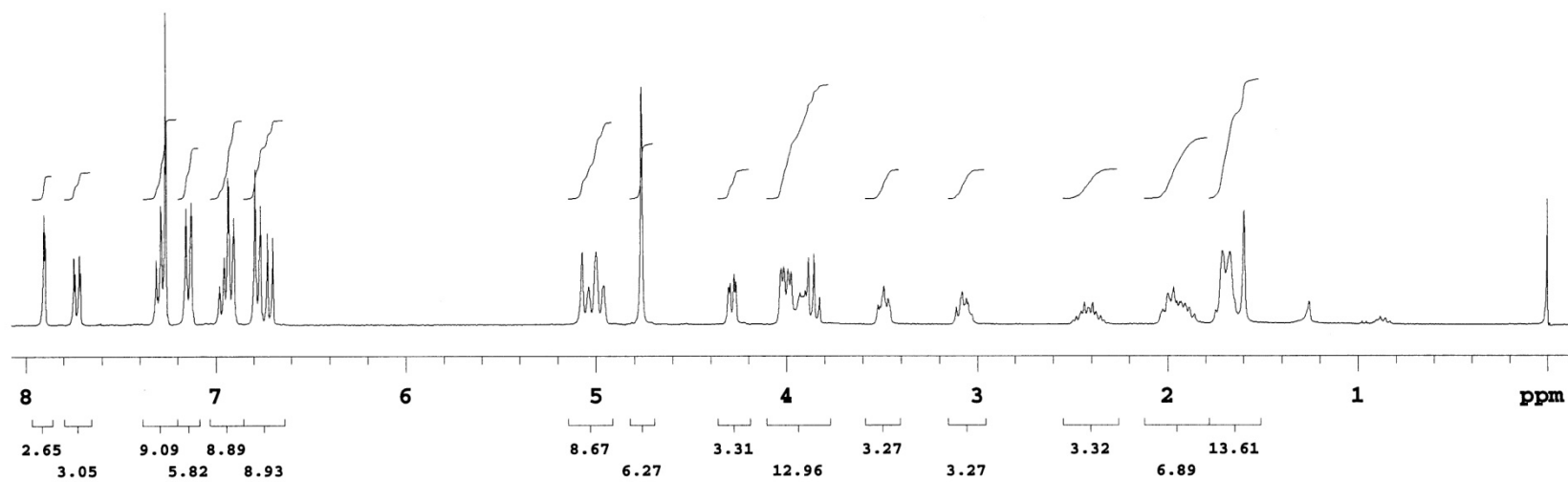


Figure S19. 300 MHz <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>) of **8**



**Figure S20.** 300 MHz  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ ) of **12**





**Figure S21.** 300 MHz  $^1\text{H}$  NMR spectrum ( $\text{CDCl}_3$ ) of **13**