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# Supporting Information

for

## Synthesis of novel profluorescent nitroxides as dual luminescent-paramagnetic active probes

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## 1. Electron paramagnetic resonance

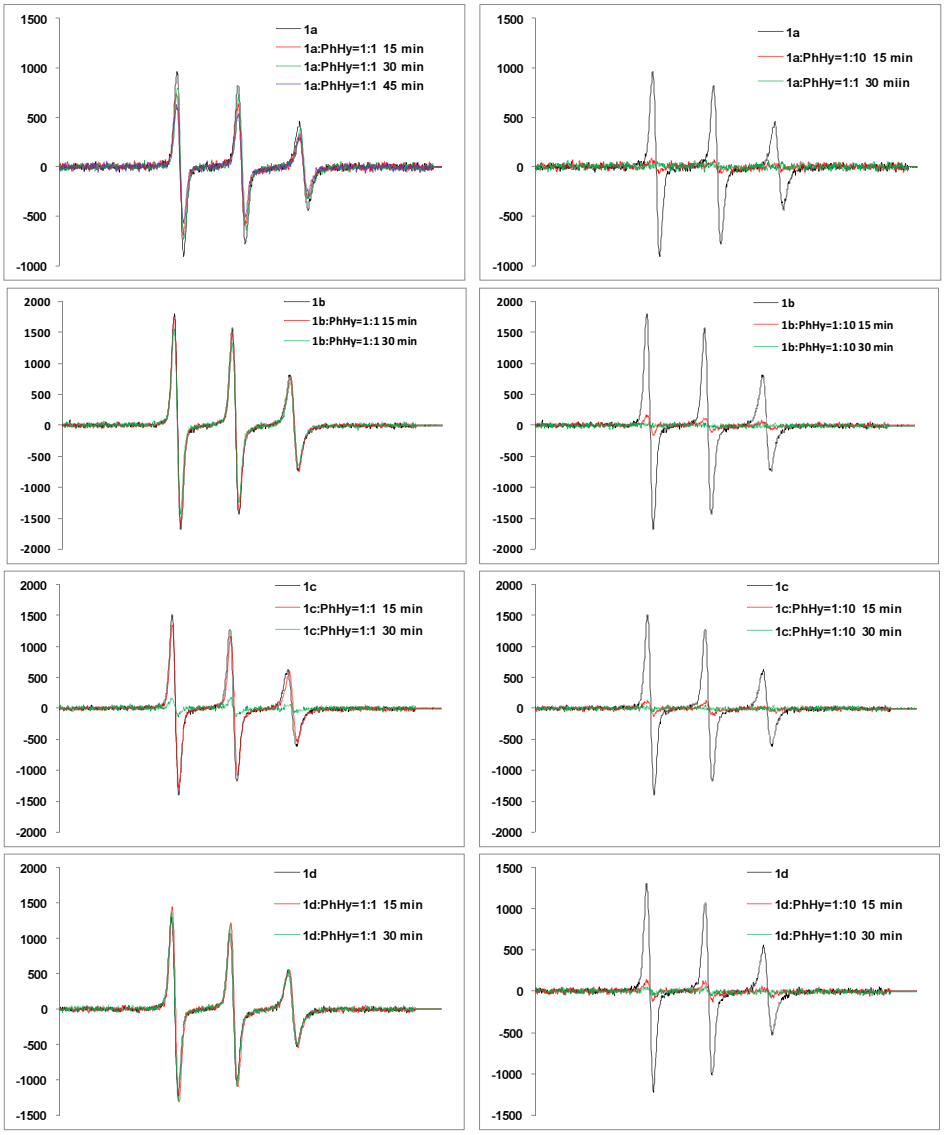



**Figure S1** Left: EPR spectra of compounds **1a-d** after treatment with phenylhydrazine in a 1:1 ratio at different reaction times. Right: Left: EPR spectra of compounds **1a-d** after treatment with phenylhydrazine in a 1:10 ratio at different reaction times. Experiments were conducted in 20% DMSO in HEPES 0,01M pH= 7.5, at a concentration of 5 x $10^{-5}$M

EPR spectra were recorded on a Jeol Jes FA 100 apparatus. Aqueous solutions of free radicals at $10^{-4}$ M concentration were charged into capillary glass tubes and the spectra were recorded at room temperature. The following settings were used: frequency 8.99 GHz, field 322 mT, sweep width 10 mT, sweep time 120 s, time constant 30 ms, gain 200, modulation frequency 100 kHz, modulation width 0.1 mT.

**2. Fluorescence spectroscopy**

**a) Fluorescence spectra of compounds 5a-d.** Fluorescence spectra were recorded with a Cary Eclipse fluorimeter. The spectra were recorded in 1 cm quartz luminescence cells 5 nm excitation and emission slits were used for all measurements. Absorption spectra for quantum yields determination were recorded with a UV-3100 spectrometer. Stock solutions of compounds were prepared in DMSO at $10^{-3}$ mol $L^{-1}$ and diluted to $5\times10^{-7}$ mol $L^{-1}$ in HEPES buffer ($10^{-2}$ mol $L^{-1}$ pH = 7.4). Quantum yields were determined using a solution of quinine sulphate in 0.5 mol $L^{-1}$ aqueous $H_2SO_4$ as standard for quantum yield determination.

**b) Fluorescence spectra of 1a-d and fluorescence spectra of compounds 1a-d incubated with a reducing agent.** Fluorescence spectra were recorded with a Thermo Scientific Varioskan Flash spectral scanning multimode reader. The spectra were recorded in suitable plates using 5 nm excitation and emission slits were used for all measurements. Stock solutions of compounds were prepared in DMSO at $10^{-3}$ mol $L^{-1}$, diluted to $5\times10^{-5}$ mol $L^{-1}$ in $10^{-2}$ mol $L^{-1}$ pH = 7.4 HEPES buffer and incubated for different times, before reading the fluorescence, with a solution of sodium ascorbate $5\times10^{-5}$ mol $L^{-1}$ in HEPES buffer ($10^{-2}$ mol $L^{-1}$ pH = 7.4).

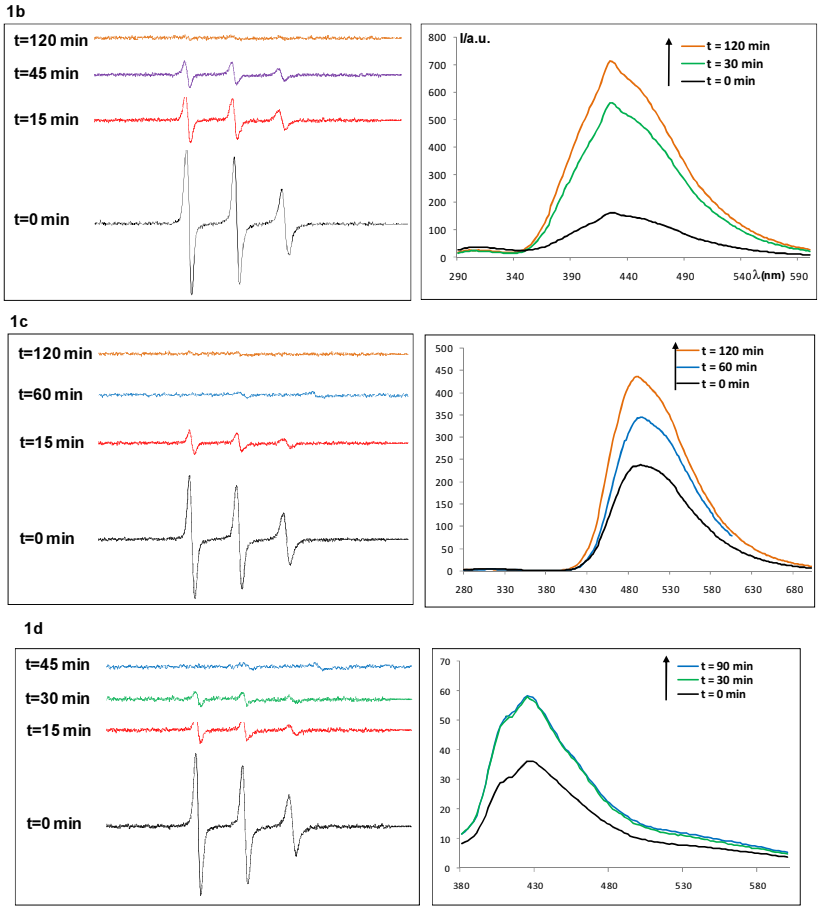



**Figure S2** Left: EPR spectra of compounds **1b-d** after treatment with sodium ascorbate. Right: Emission spectra of compounds **1b-d** after treatment with sodium ascorbate. Experiments were conducted in 20% DMSO in HEPES 0,01M pH= 7.5, at a concentration of 5 x $10^{-5}$M

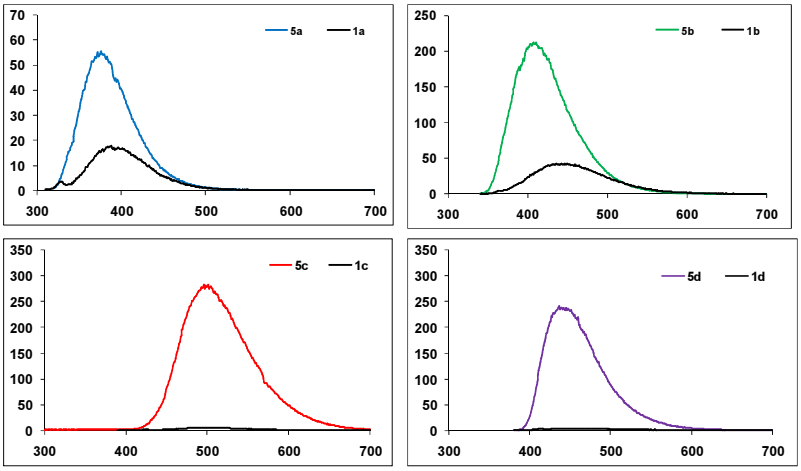



**Figure S3** Solution emission spectra of the nitroxides **1a-d** (black lines) compared to their precursor carboxy derivatives **5a-d** (coloured lines) recorded in 15% DMSO in HEPES 0.01 M pH= 7.51, at a concentration of $5x10^{-7}$ M, using $\lambda_{ex}$=296 nm for **1a**, $\lambda_{ex}$=319 nm for **1b**, $\lambda_{ex}$=372 nm for **1c**, $\lambda_{ex}$=370 nm for **1d**

**c) Response of 1a to sodium ascorbate.** Fluorescence spectra were recorded with a Thermo Scientific Varioskan Flash spectral scanning multimode reader. The spectra were recorded in suitable plates using 5 nm excitation and emission slits were used for all measurements. Stock solution of compound was prepared in DMSO at $10^{-3}$ mol $L^{-1}$ and aliquots of 50 µL were incubated for 30 and 45 minutes, respectively, before reading, with solutions of sodium ascorbate $10^{-3}$ mol $L^{-1}$ in HEPES buffer ($10^{-2}$ mol $L^{-1}$ pH = 7.4) in ratios from 0 to 2 (8 points) to a final volume of 1 mL with 5 x $10^{-5}$ mol $L^{-1}$ final concentration of the organic compound.

**d) Fluorescence spectra of compound 1d in various solvents and of compound 1d in various solvents incubated with a reducing agent.** Fluorescence spectra were recorded with a Thermo Scientific Varioskan Flash spectral scanning multimode reader. The spectra were recorded in suitable plates using 5 nm excitation and emission slits were used for all measurements.. Absorption spectra for quantum yields determination were recorded with a Jasco V-630 spectrophotometer, using 10 mm quartz cell. Stock solutions of compound were prepared in $CHCl_3$ ($2.68\times10^{-3}$ mol $L^{-1}$), $CH_3CN$ ($2\times10^{-4}$ mol $L^{-1}$), $CH_3OH$ ($2\times10^{-3}$ mol $L^{-1}$), DMSO ($10^{-4}$ mol $L^{-1}$), diluted to $5\times10^{-5}$ mol $L^{-1}$ using the corresponding solvent and incubated for 30 minutes, before reading the absorbance, with a solution of phenylhydrazine $10^{-1}$ mol $L^{-1}$ in the corresponding solvent, in a ratio **1d**:phenyldrazine=1:10. Quantum yields were determined using a solution of quinine sulphate in 0.5 mol $L^{-1}$ aqueous $H_2SO_4$ as standard for quantum yield determination.

e) **The solid state emission spectra** were recorded on a JASCO FP 8300 spectrofluorometer.

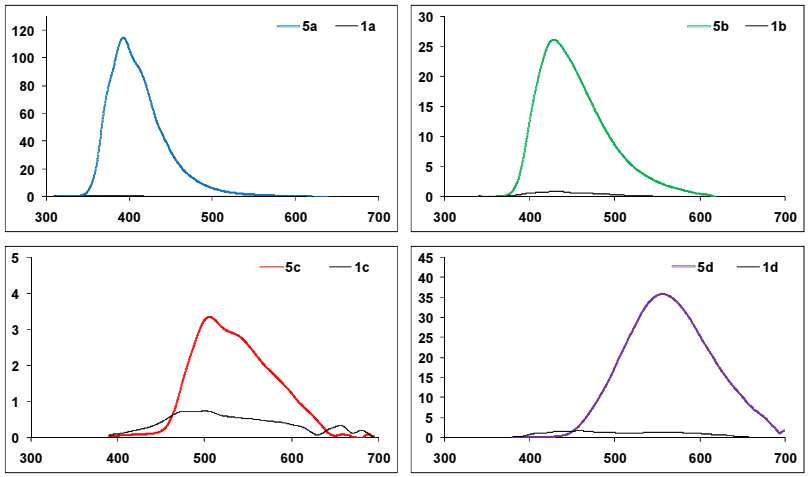



**Figure S4** Solid state emission spectra of the nitroxides **1a-d** (black lines) and their precursor carboxy derivatives **5a-d** (coloured lines) recorded using $\lambda_{ex}$=295 nm for **1a**, $\lambda_{ex}$=318 nm for **1b**, $\lambda_{ex}$=370 nm for **1c**, $\lambda_{ex}$=360 nm for **1d.**

## 3. Cyclic voltammetry

Cyclic voltammetry was performed in dichloromethane solution using tetrabutylammonium hexafluorophosphate 0.10M as supporting electrolyte. All solutions were deaerated by bubbling with a dry argon streamprior to each experiment. Experiments were carried out in a one-compartment cell equipped with platinum electrodes and saturated calomel reference electrode (SCE) with a Biologic SP-150 potentiostat with positive feedback compensation.

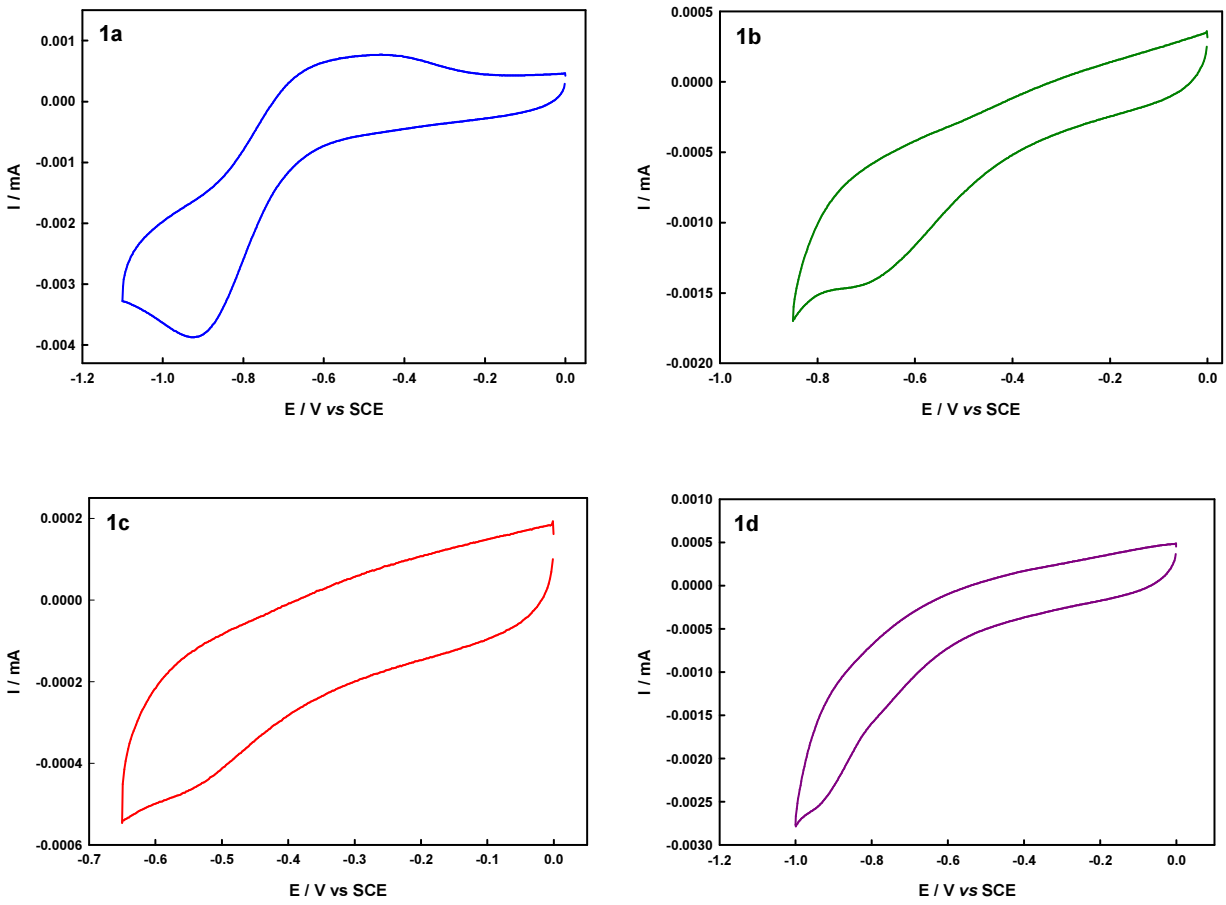



**Figure S5**. Cyclic voltammograms of compounds **1** in 0.1 M $Bu_4NPF_6/CH_2Cl_2$, Pt electrodes, scan rate = 100 mV $s^{-1}$.

## 4. $^1$H NMR spectra (selection - reduced with $PhNHNH_2$) of compound 1d in $CDCl_3$ and DMSO-$d_6$

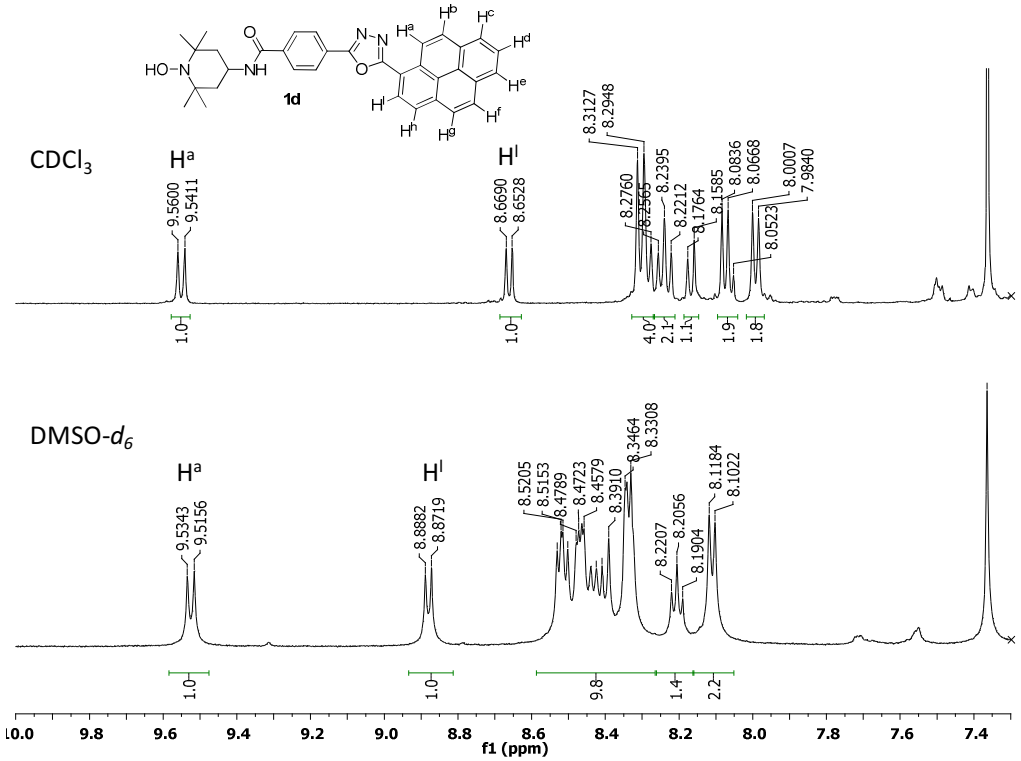
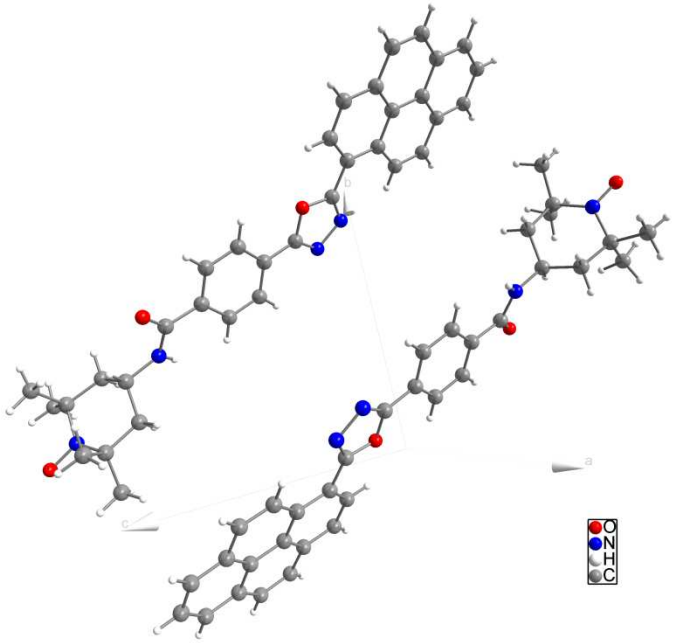



**Figure S6** Fragments of the NMR spectra of compound **1d** (reduced with phenylhydrazine) in $CDCl_3$ and DMSO-$d_6$

## 5. Single crystal X-Ray Diffraction - the elementary cell of compound 1d containing two molecules head to tail oriented



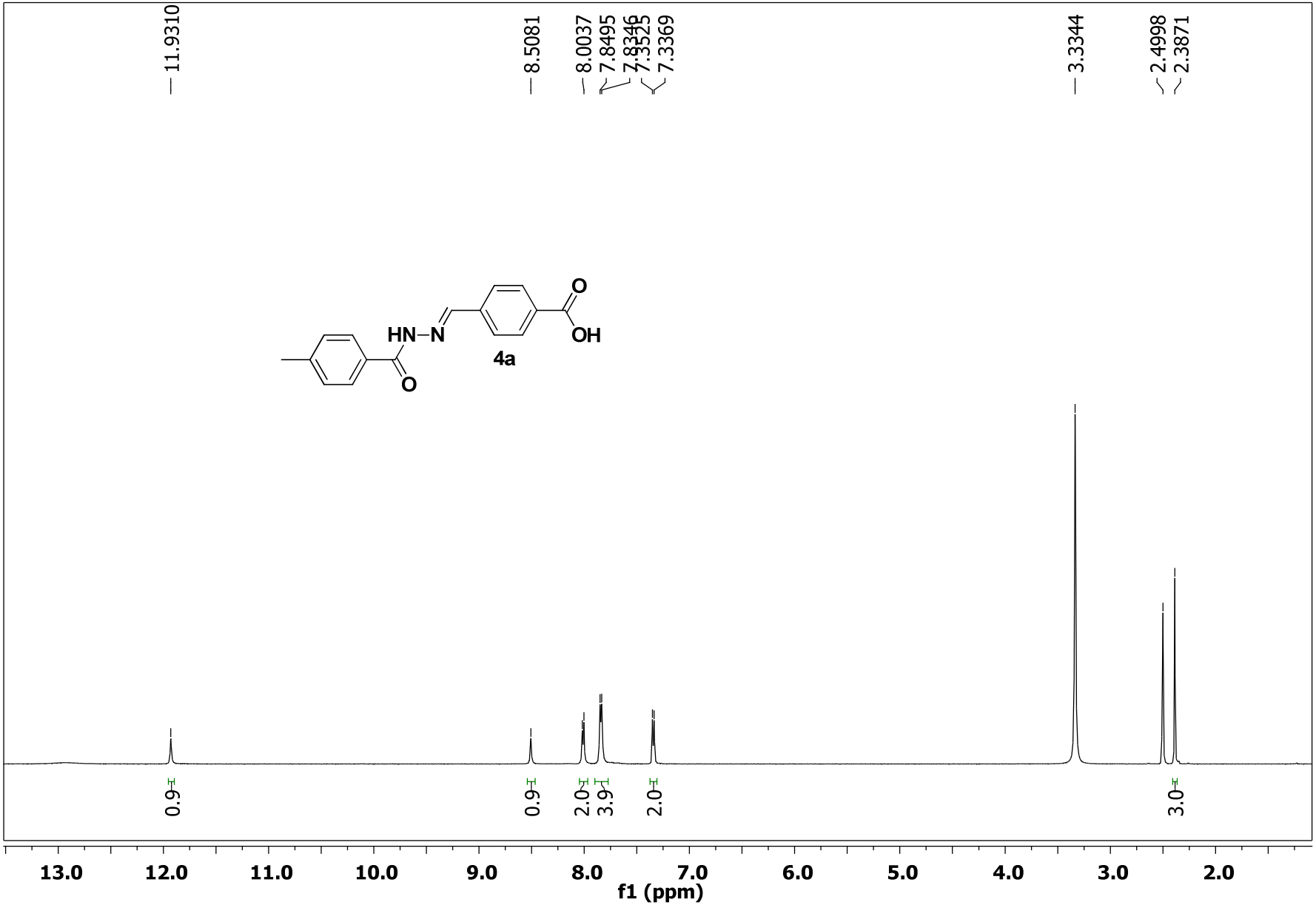
## Table S1. Sample and crystal data for 1d

| | | |
|---|---|---|
| **Identification code** | CA104 | |
| **Chemical formula** | $C_{34}H_{31}N_4O_3$ | |
| **Formula weight** | 543.63 g/mol | |
| **Temperature** | 293(2) K | |
| **Wavelength** | 0.71073 Å | |
| **Crystal size** | 0.092 x 0.125 x 0.354 mm | |
| **Crystal system** | triclinic | |
| **Space group** | P -1 | |
| **Unit cell dimensions** | a = 7.990(3) Å | α = 94.821(10)° |
| | b = 12.475(4) Å | β = 94.404(9)° |
| | c = 14.539(5) Å | γ = 108.372(9)° |
| **Volume** | 1362.3(8) $Å^3$ | |
| **Z** | 2 | |
| **Density (calculated)** | 1.325 $g/cm^3$ | |
| **Absorption coefficient** | 0.086 $mm^{-1}$ | |
| **F(000)** | 574 | |

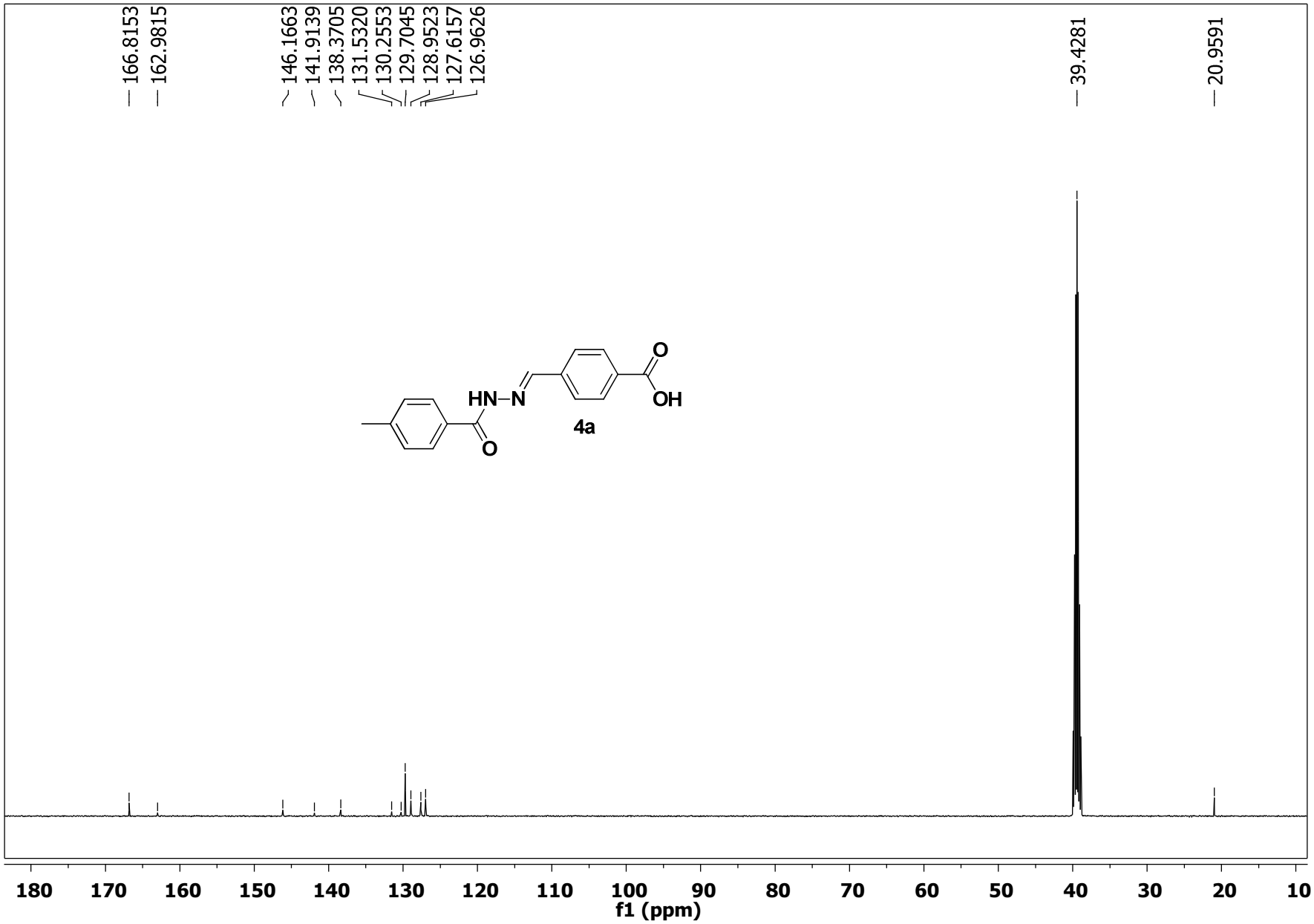
## Table S2. Data collection and structure refinement for 1d

| | | |
|---|---|---|
| **Theta range for data collection** | 2.70 to 25.87° | |
| **Index ranges** | -9<=h<=9, -15<=k<=15, -17<=l<=17 | |
| **Reflections collected** | 43403 | |
| **Independent reflections** | 5258 [R(int) = 0.0668] | |
| **Coverage of independent reflections** | 99.6% | |
| **Absorption correction** | Numerical Mu From Formula | |
| **Max. and min. transmission** | 0.9920 and 0.9700 | |
| **Refinement method** | Full-matrix least-squares on $F^2$ | |
| **Refinement program** | SHELXL-2014/7 (Sheldrick, 2014) | |
| **Function minimized** | $\Sigma\, w(F_o^2 - F_c^2)^2$ | |
| **Data / restraints / parameters** | 5258 / 0 / 374 | |
| **Goodness-of-fit on $F^2$** | 1.057 | |
| **Final R indices** | 3530 data; I>2σ(I) | R1 = 0.0452, wR2 = 0.1194 |
| | all data | R1 = 0.0810, wR2 = 0.1385 |
| **Weightingscheme** | $w=1/[\sigma^2(F_o^2)+(0.0711P)^2+0.1369P]$ where $P=(F_o^2+2F_c^2)/3$ | |
| **Largest diff. peak and hole** | 0.383 and -0.253 $eÅ^{-3}$ | |
| **R.M.S. deviation from mean** | 0.056 $eÅ^{-3}$ | |

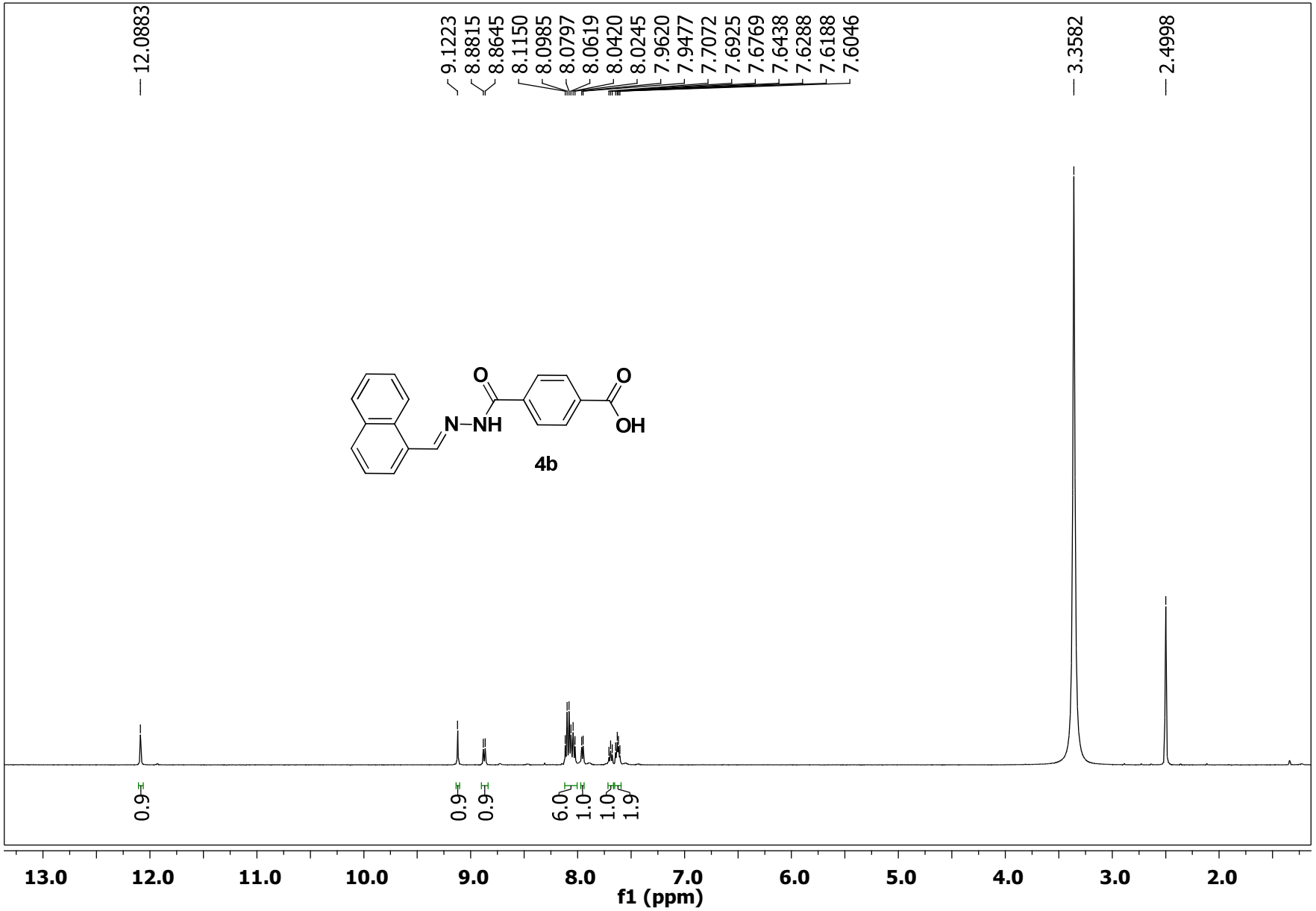
# Figure S 7 $^{1}$H NMR spectrum of compound 4a



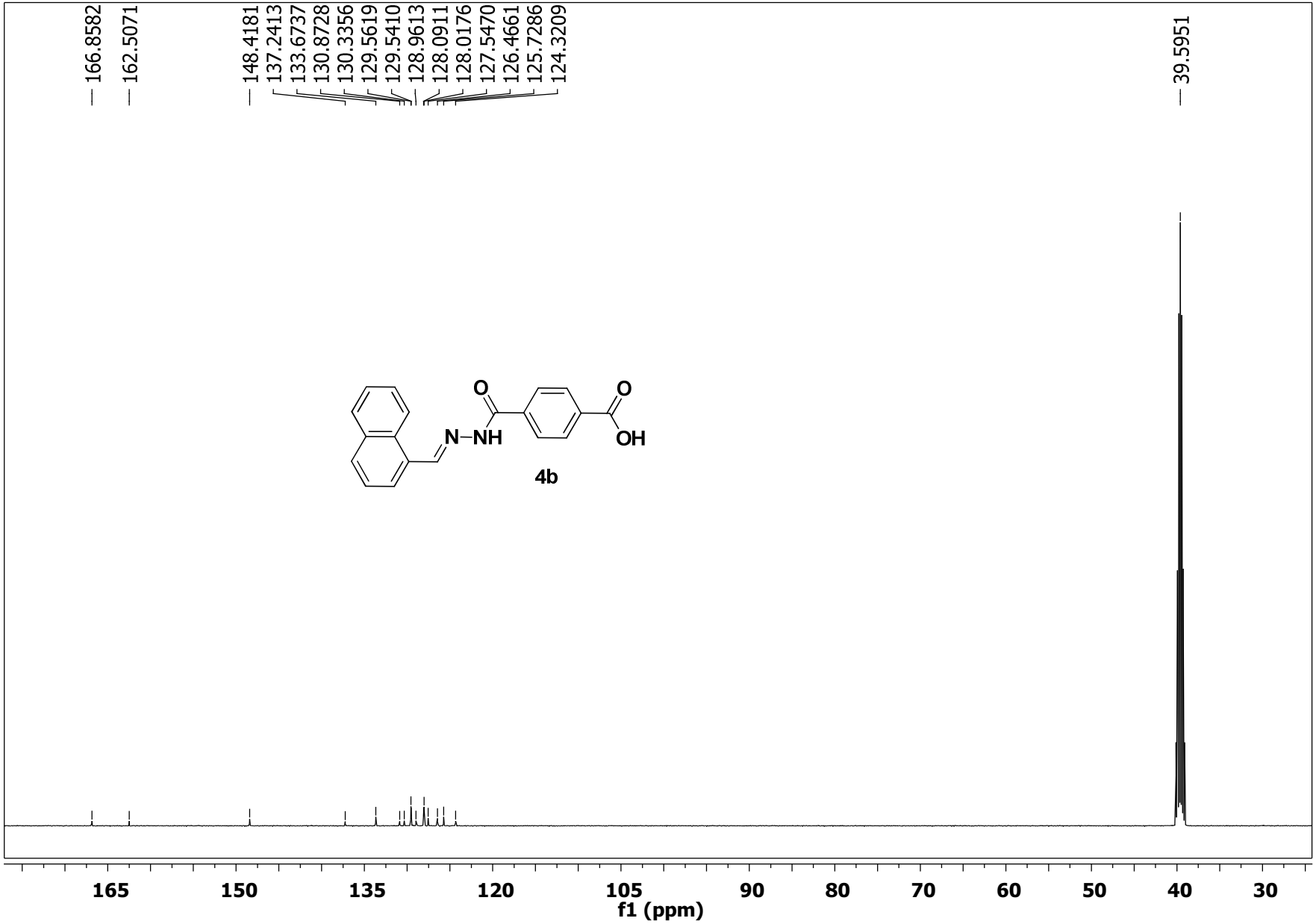
# Figure S 8 $^{13}$C NMR spectrum of compound 4a



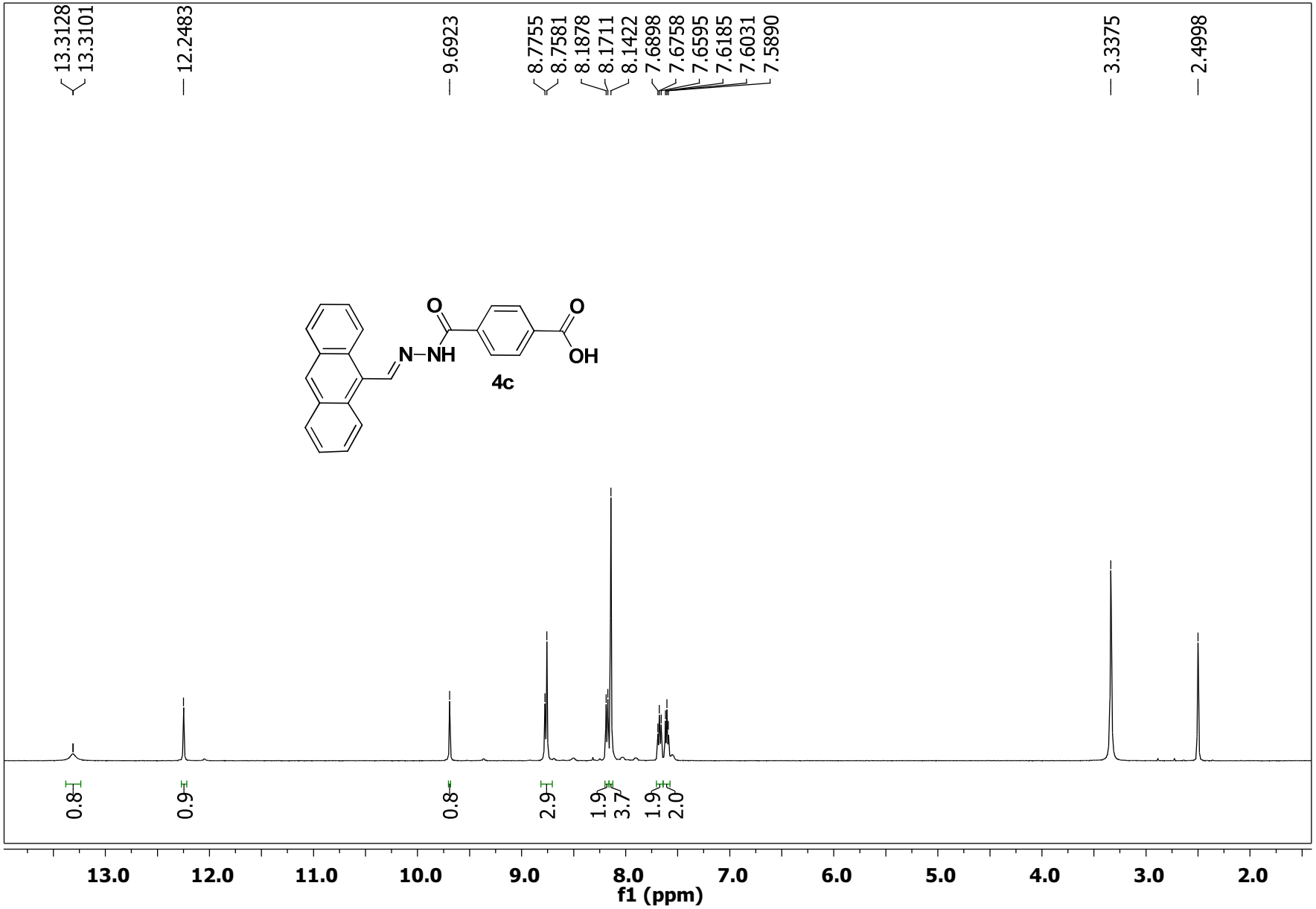
# Figure S 9 $^{1}$H NMR spectrum of compound 4b



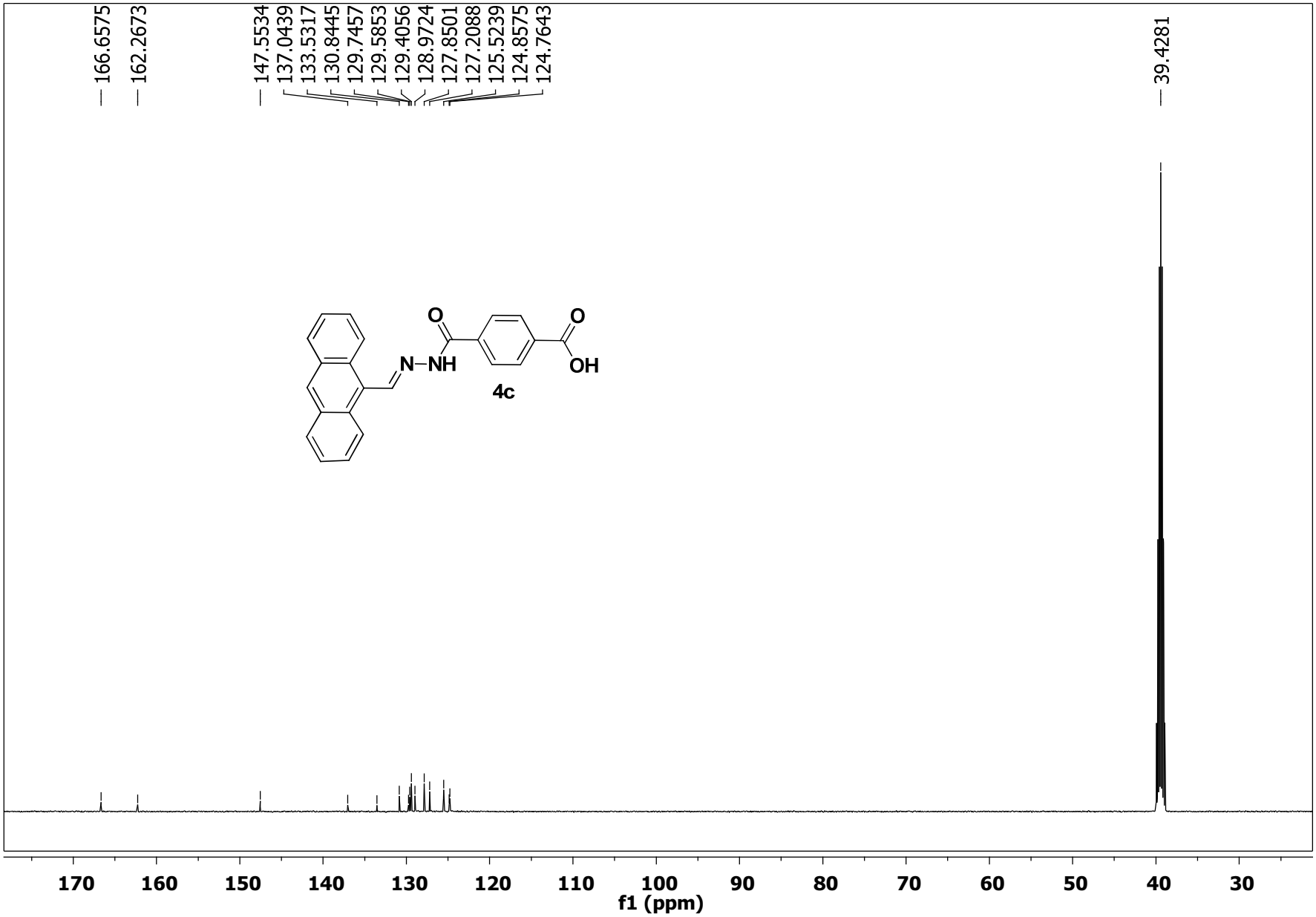
# Figure S 10 $^{13}$C NMR spectrum of compound 4b



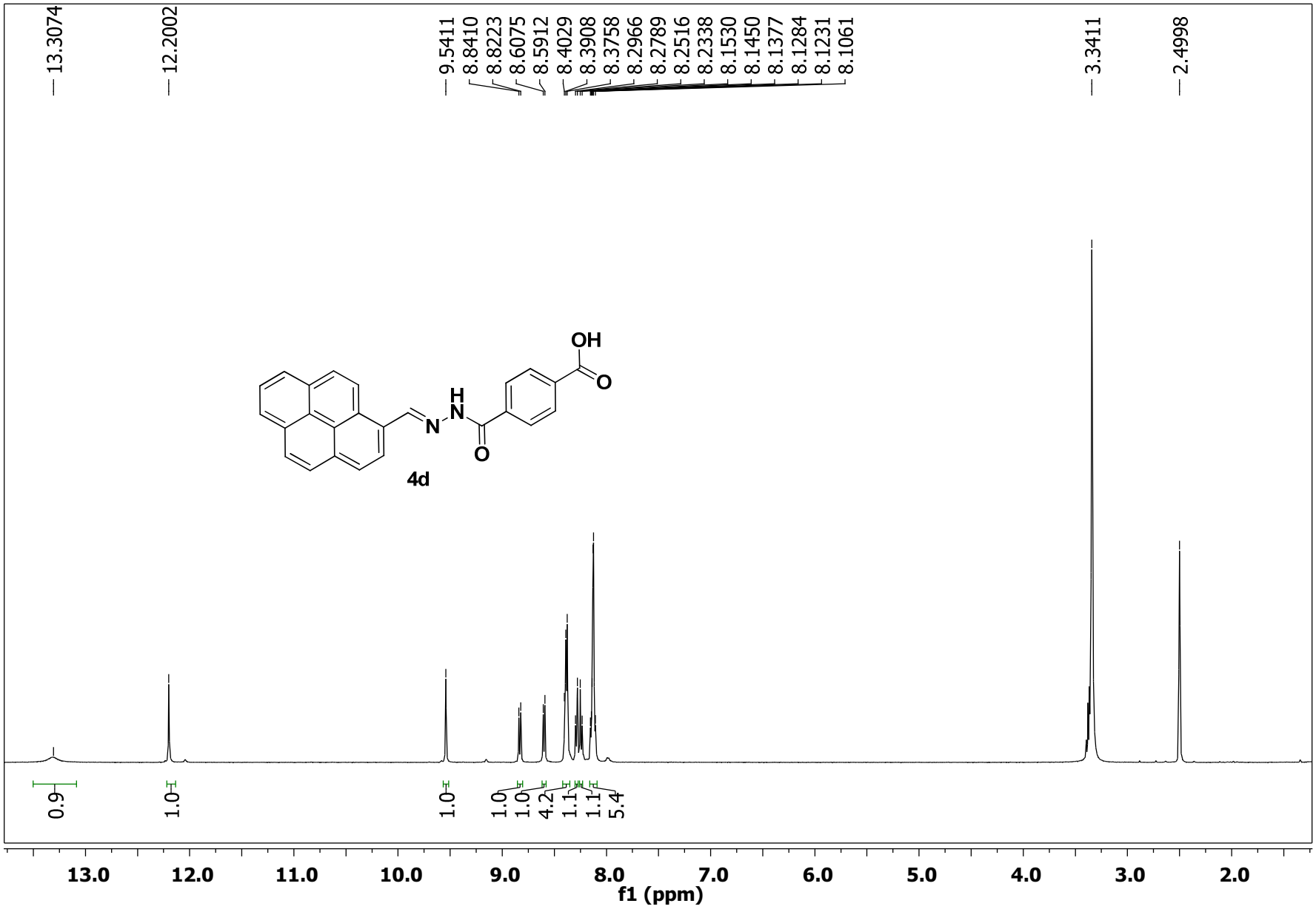
# Figure S 11 $^{1}$H NMR spectrum of compound 4c



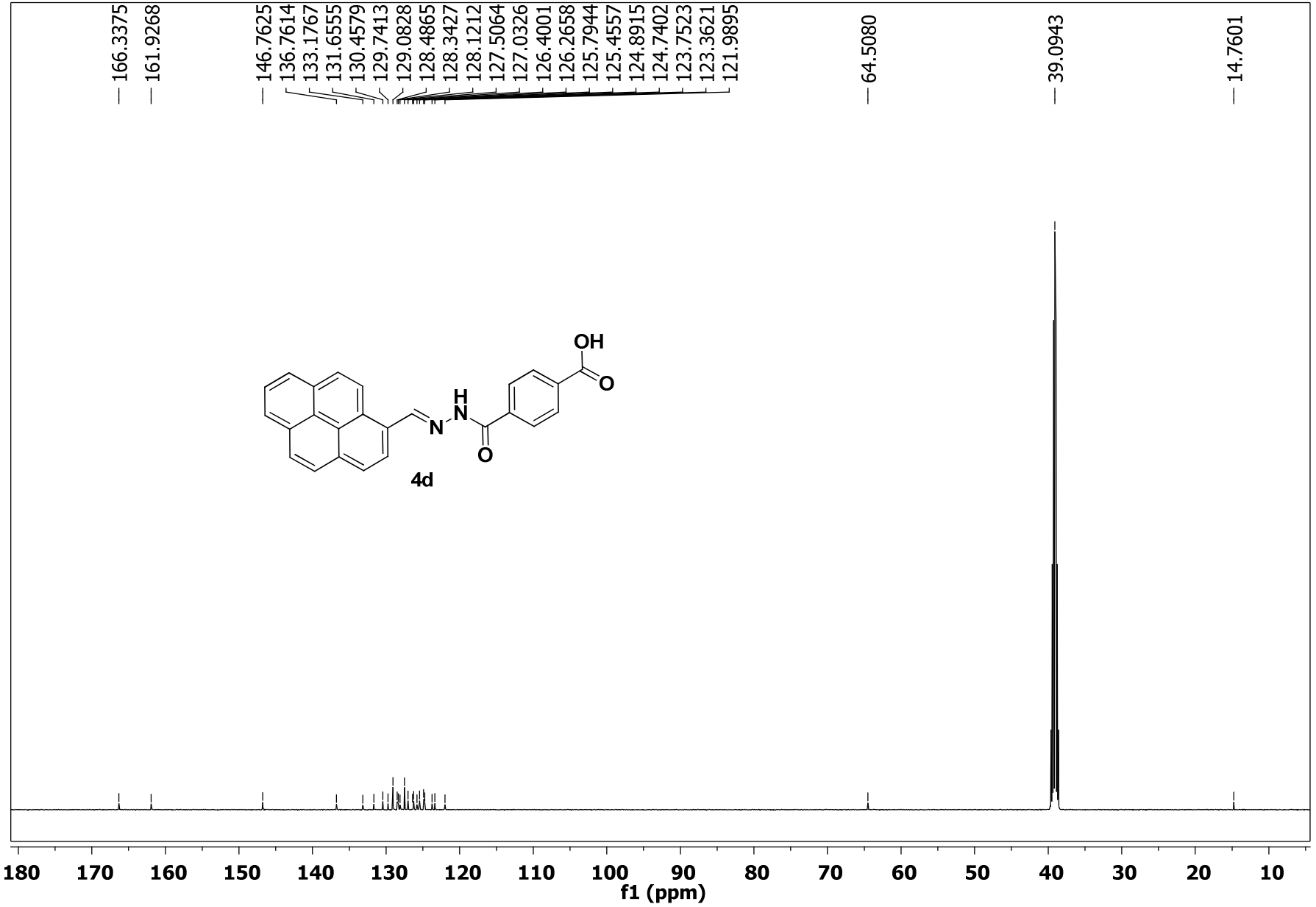
# Figure S 12 $^{13}$C NMR spectrum of compound 4c



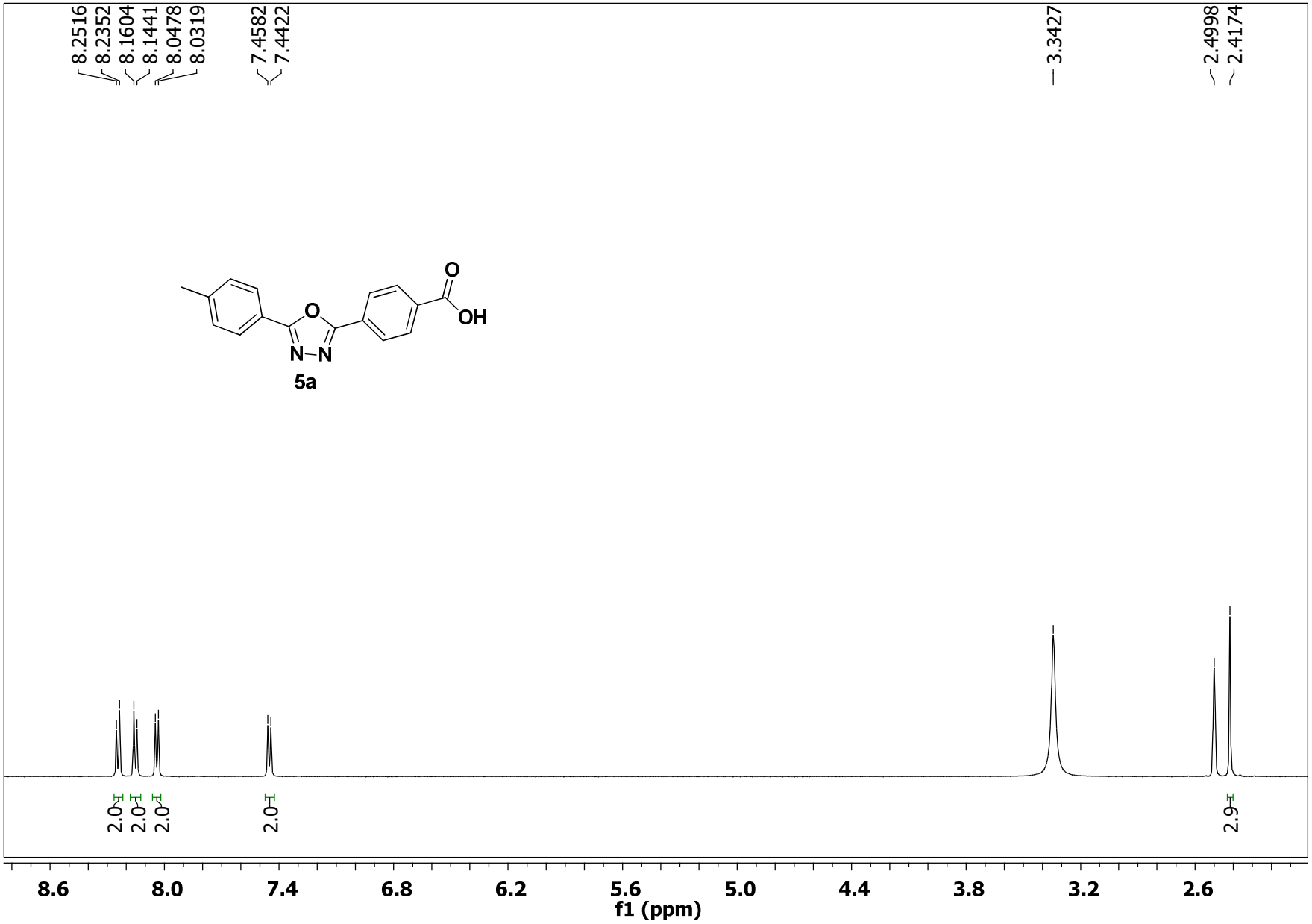
# Figure S 13 $^{1}$H NMR spectrum of compound 4d



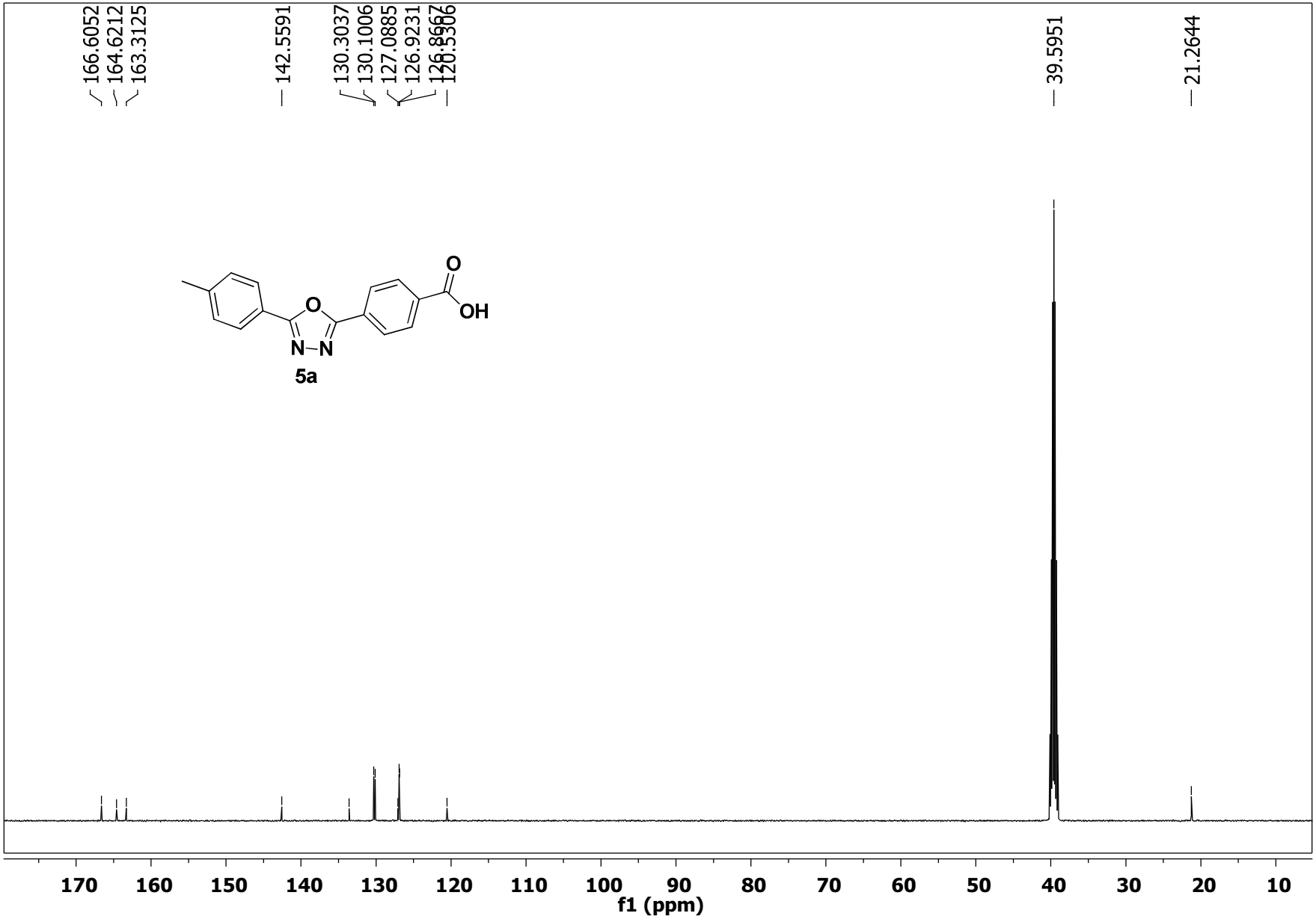
# Figure S 14 $^{13}$C NMR spectrum of compound 4d



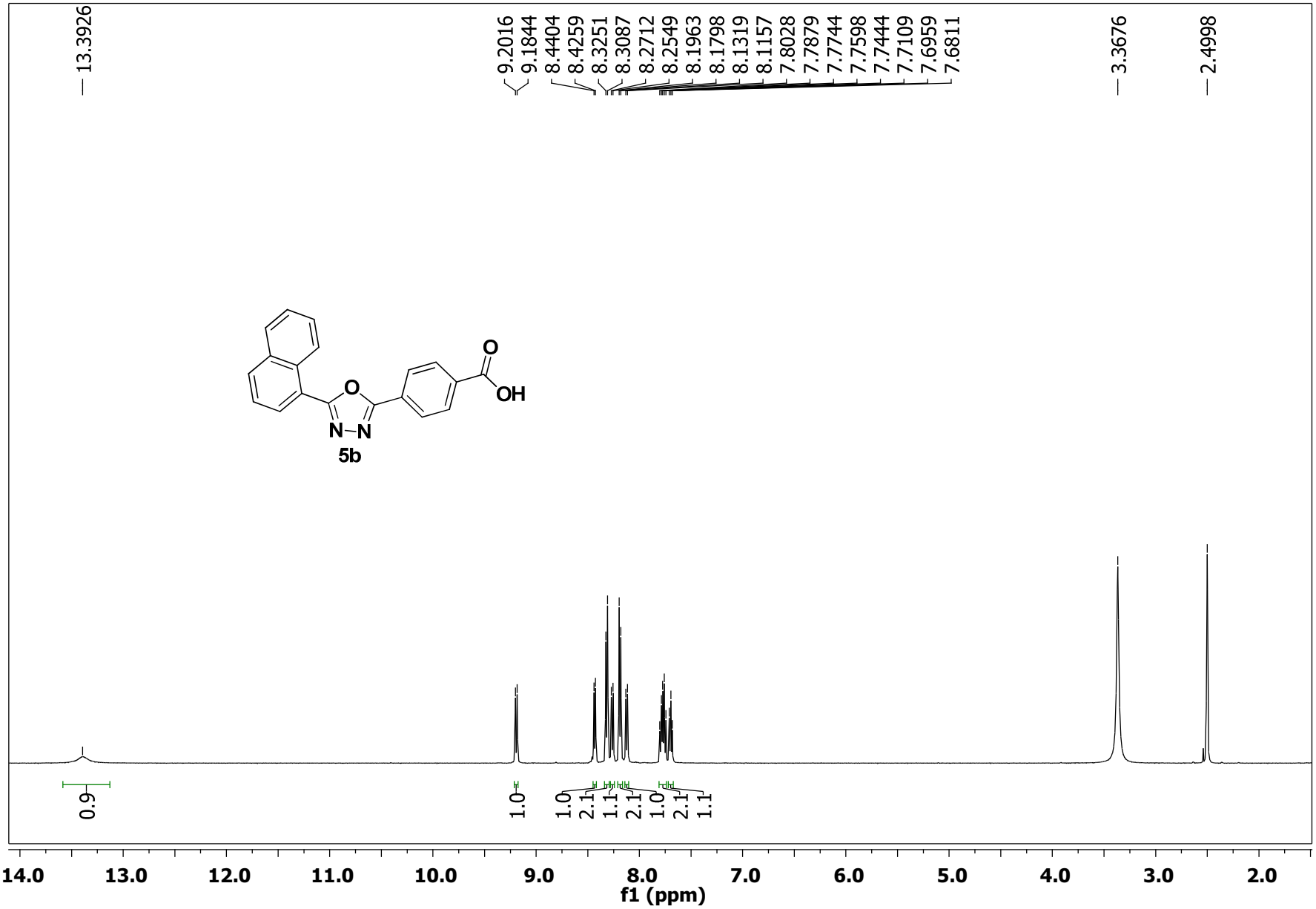
# Figure S 15 $^{1}$H NMR spectrum of compound 5a



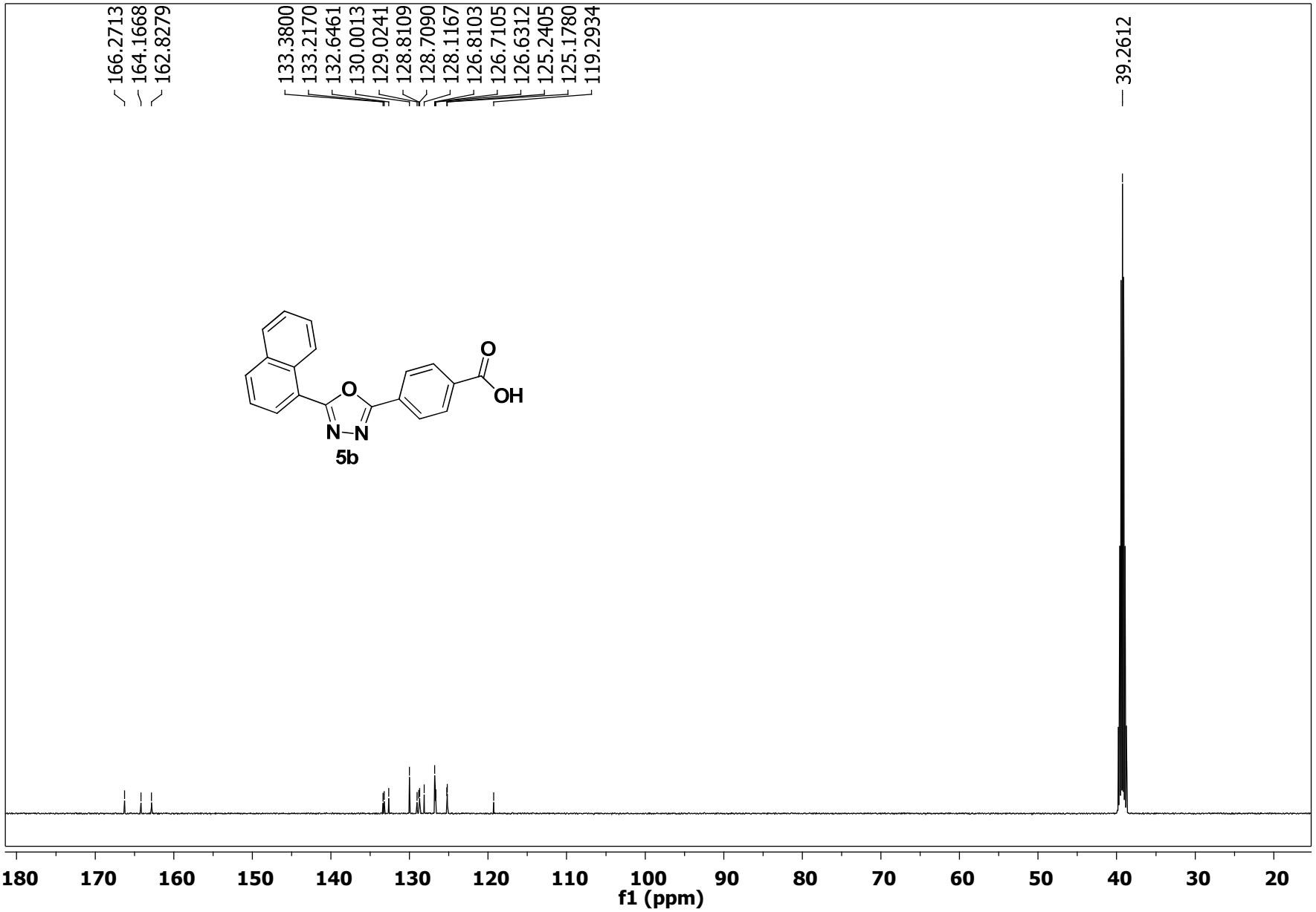
# Figure S 16 $^{13}$C NMR spectrum of compound 5a



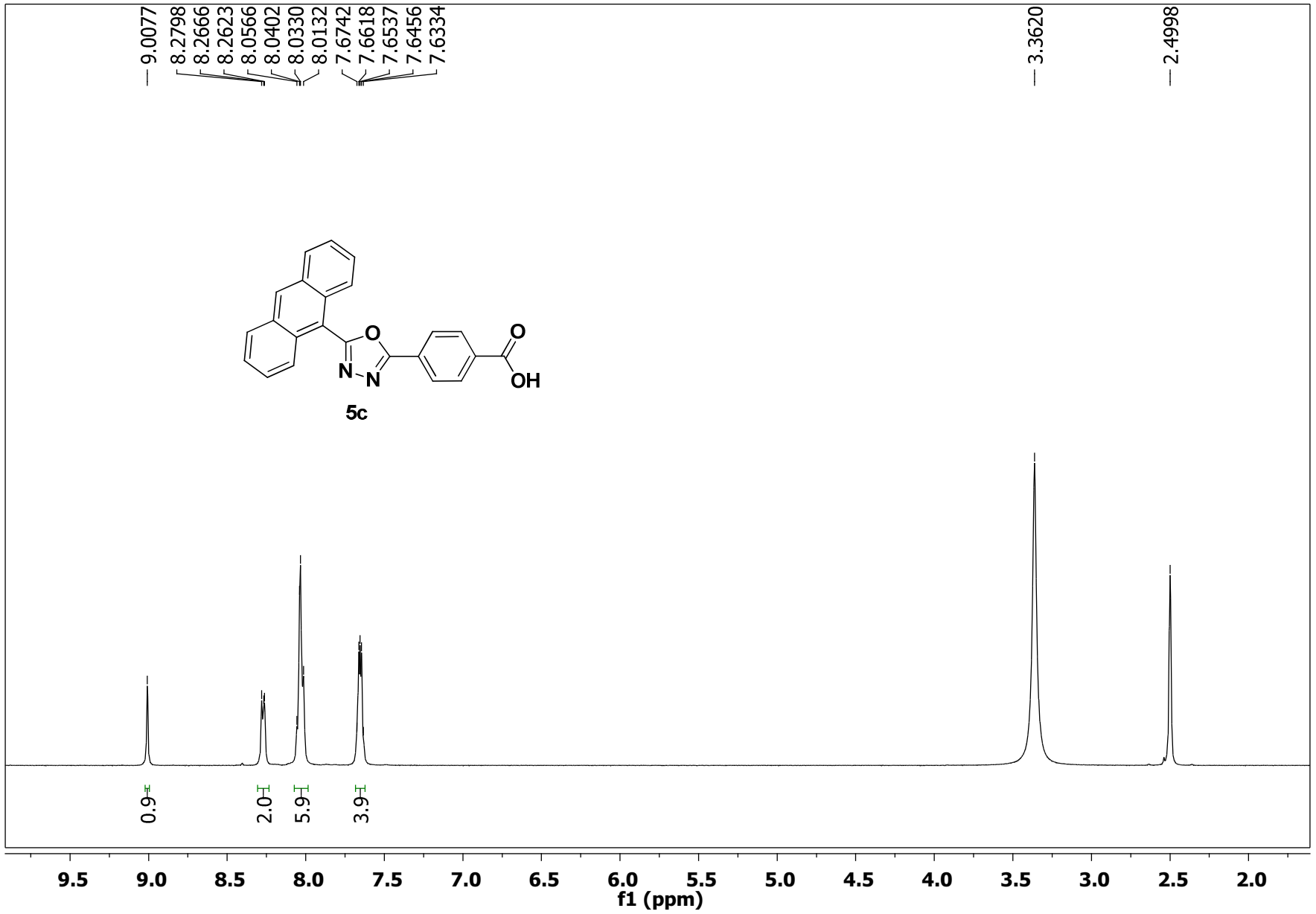
# Figure S 17 $^{1}$H NMR spectrum of compound 5b



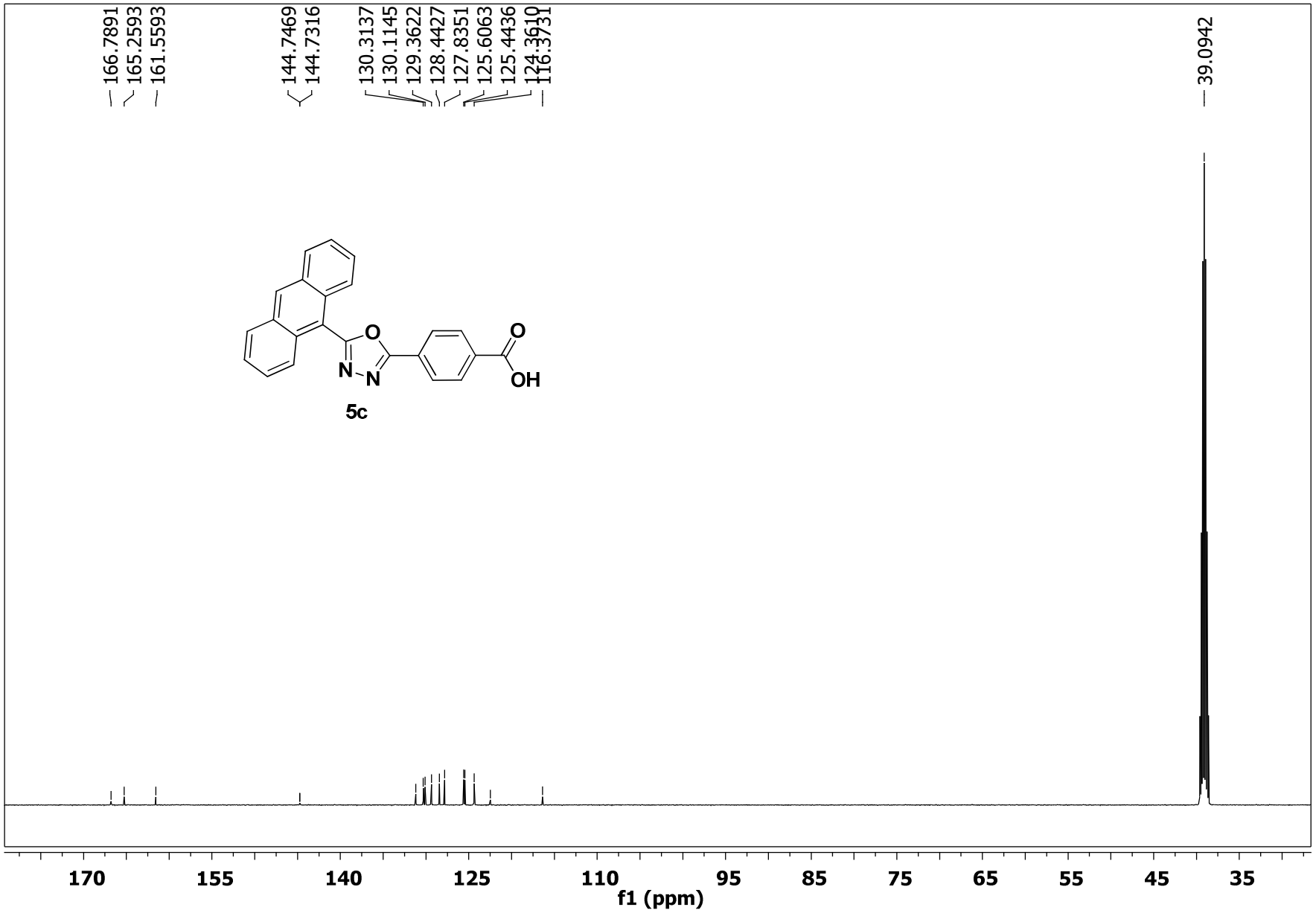
# Figure S 18 $^{13}$C NMR spectrum of compound 5b



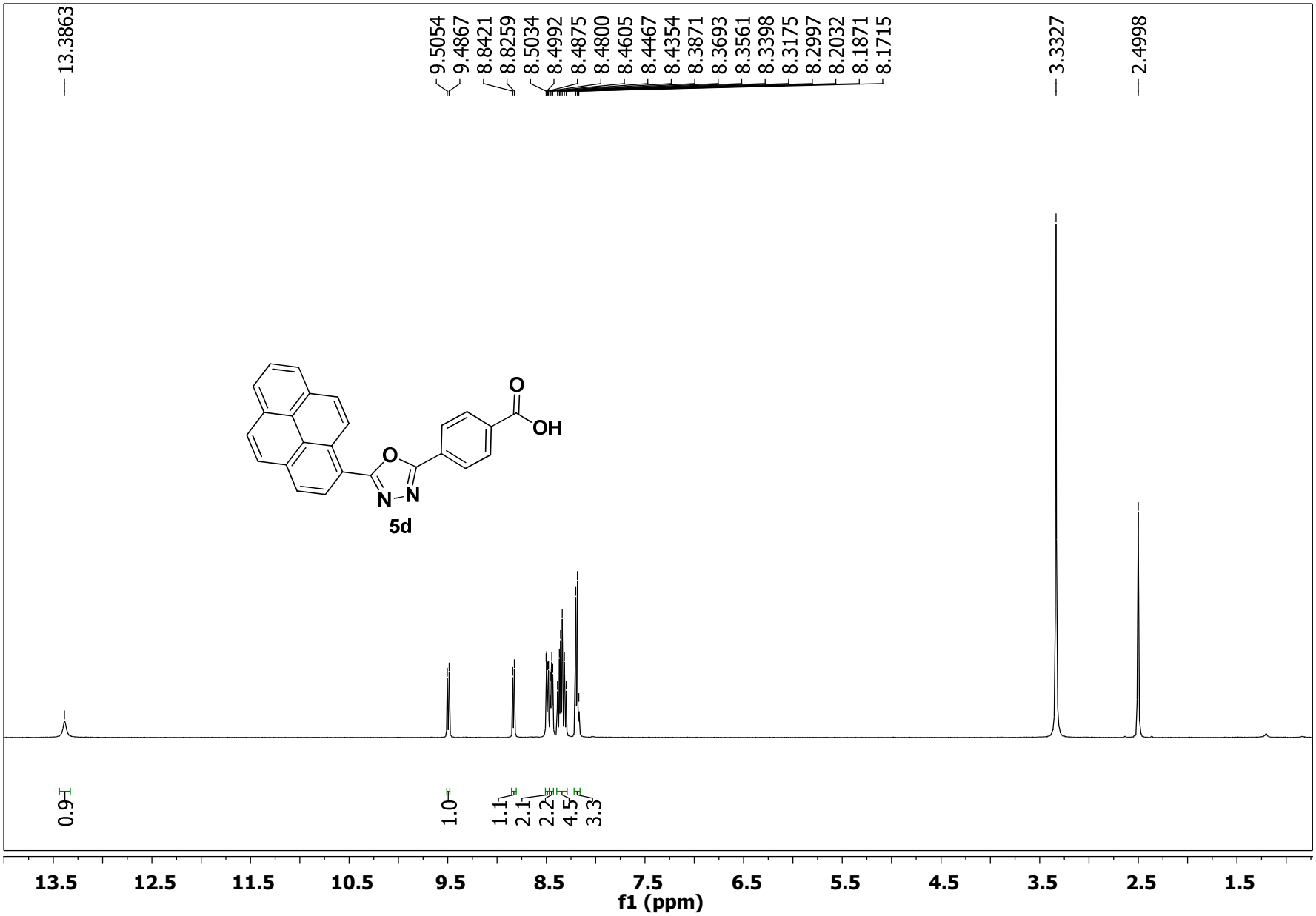
# Figure S 19 $^{1}$H NMR spectrum of compound 5c



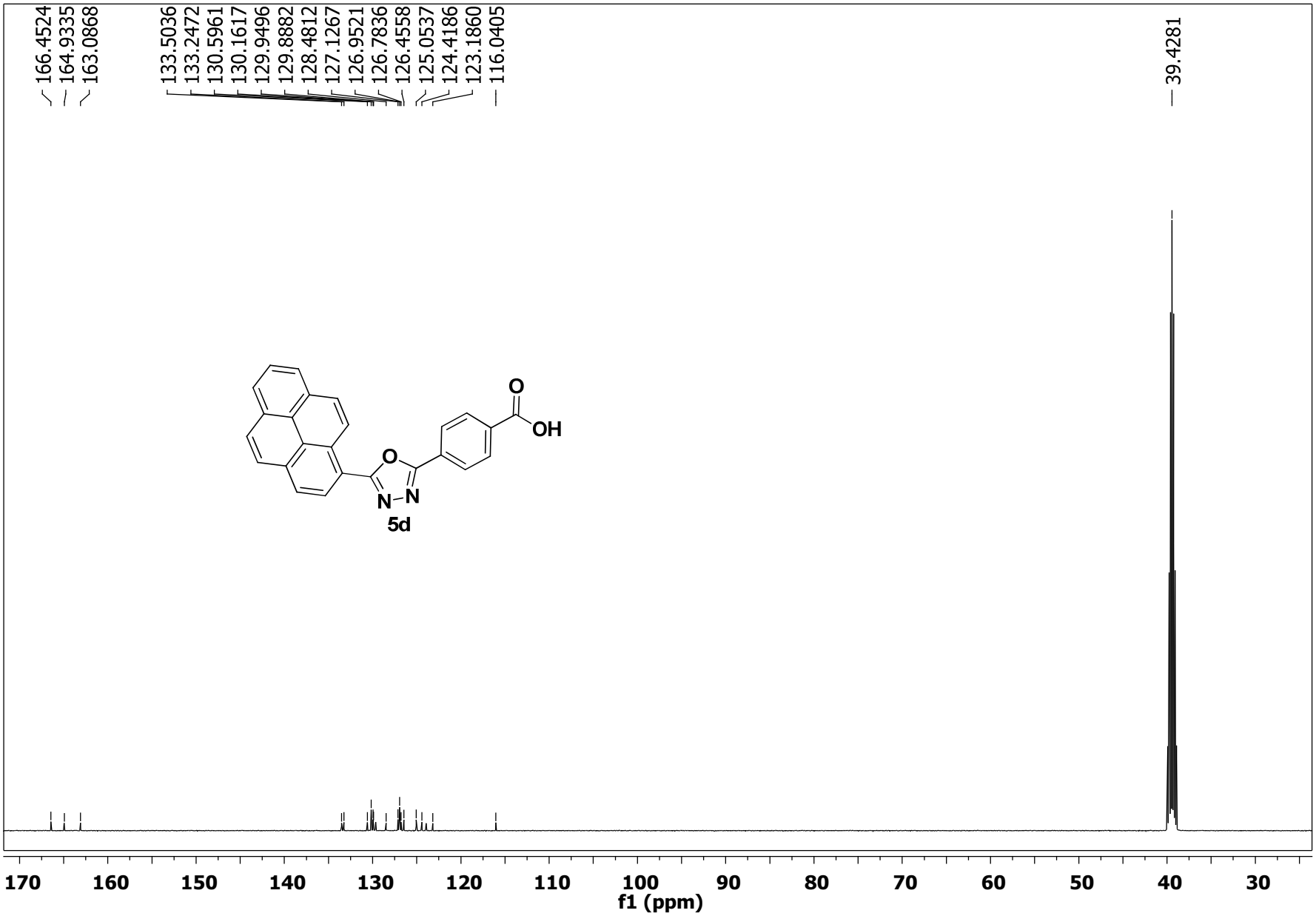
# Figure S 20 $^{13}$C NMR spectrum of compound 5c



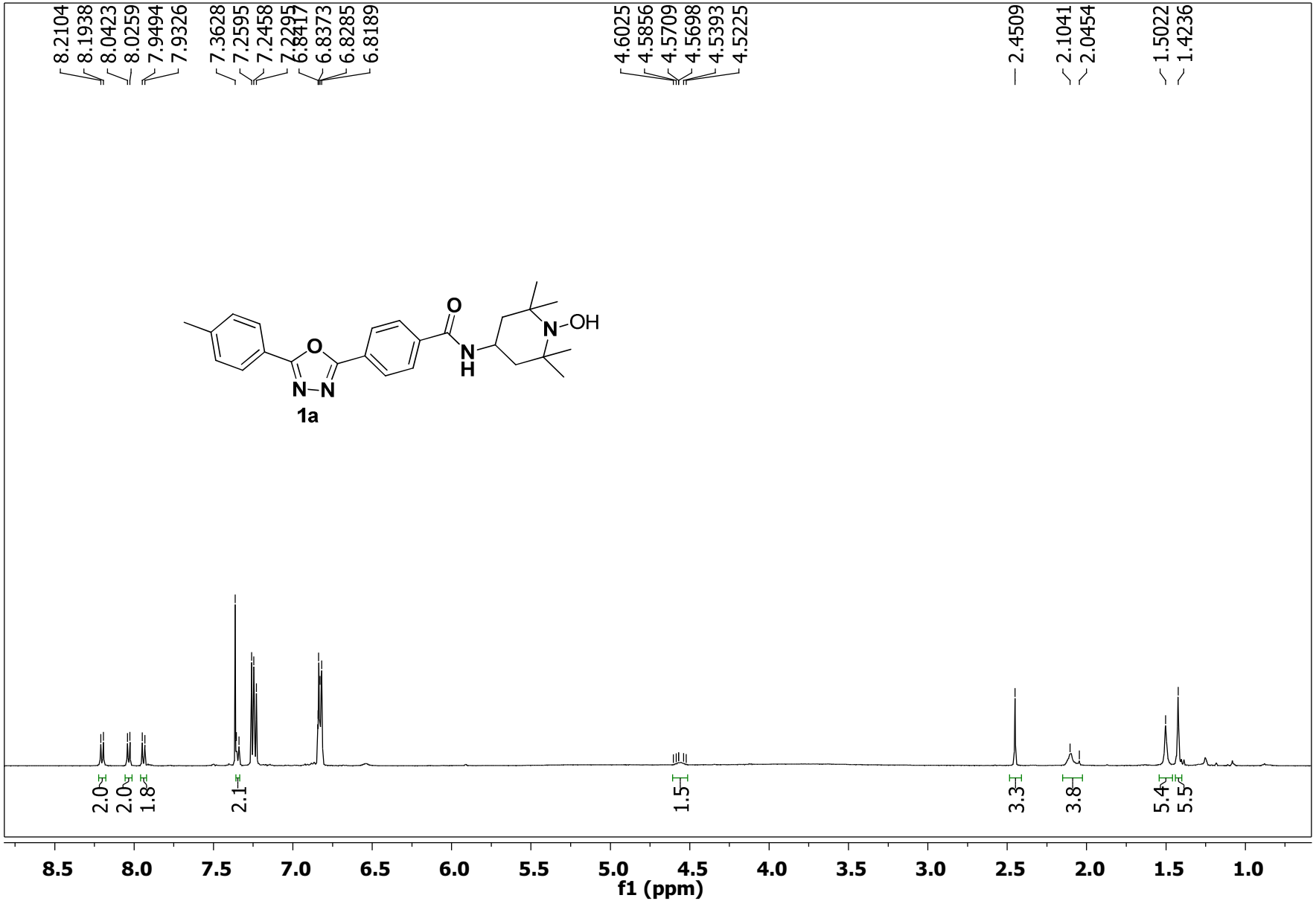
# Figure S 21 $^{1}$H NMR spectrum of compound 5d



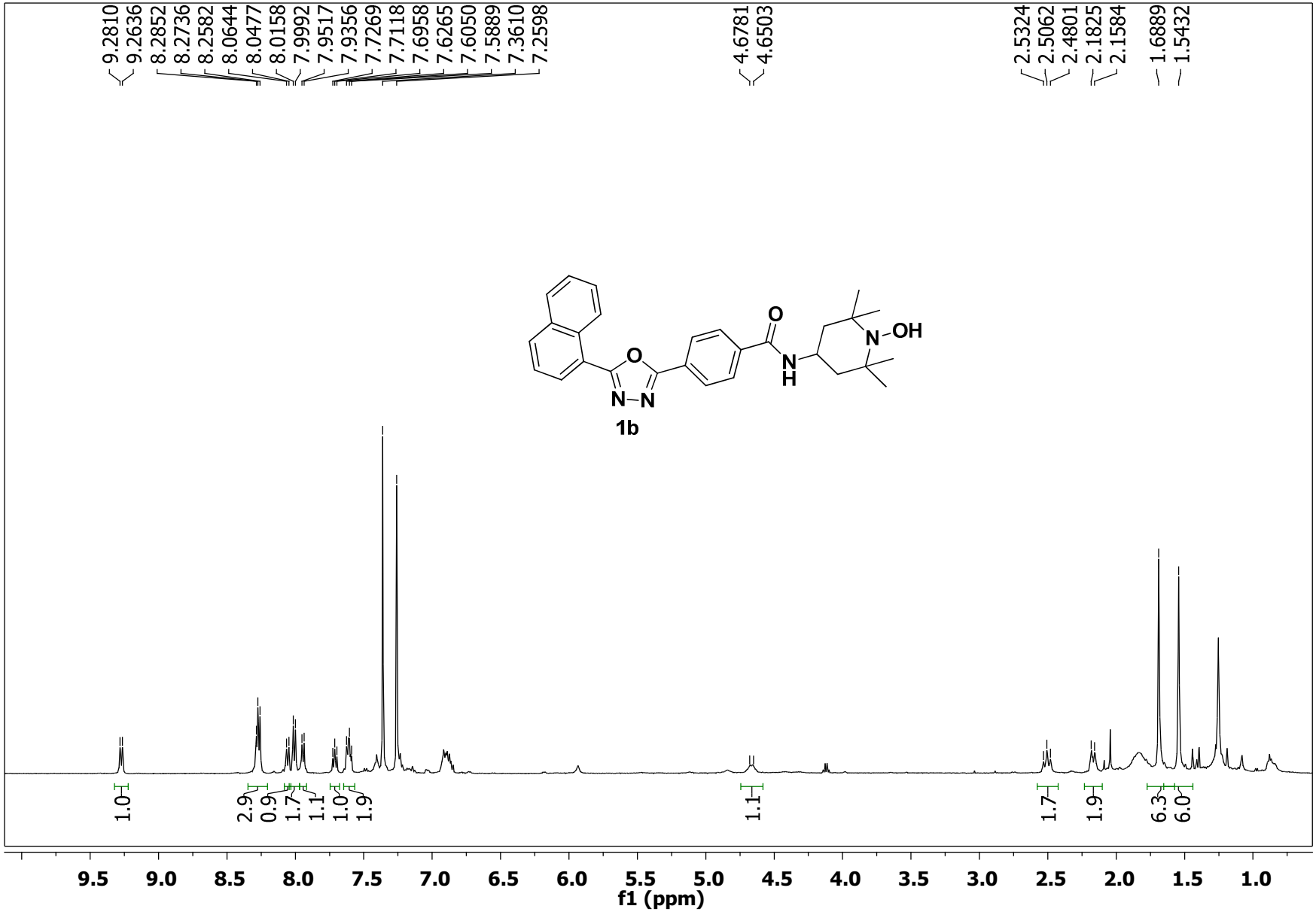
# Figure S 22 $^{13}$C NMR spectrum of compound 5d



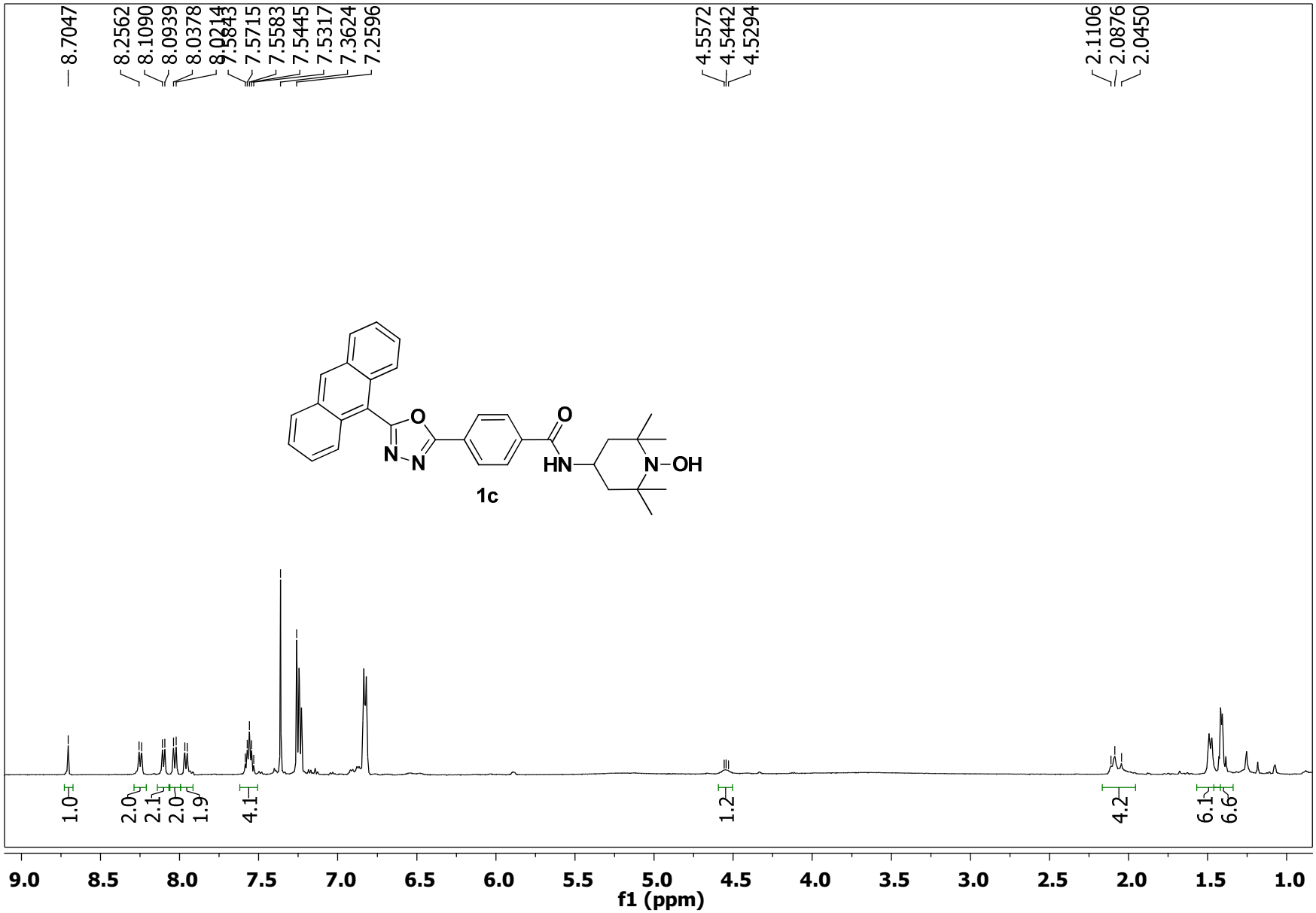
# Figure S 23 $^{1}$H NMR spectrum of compound 1a



# Figure S 24 $^{1}$H NMR spectrum of compound 1b



# Figure S 25 $^{1}$H NMR spectrum of compound 1c



# Figure S 26 $^{1}$H NMR spectrum of compound 1d