

Supporting Information

A Highly HDAC6–Selective Inhibitor Acts as a Fluorescent Probe

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Table of Contents

1.	Photophysical characterization.....	S2
2.	Fluorescence microscopy imaging	S4
3.	Spectroscopic titration.....	S5
4.	¹ H and ¹³ C NMR spectra	S6

1. Photophysical characterization

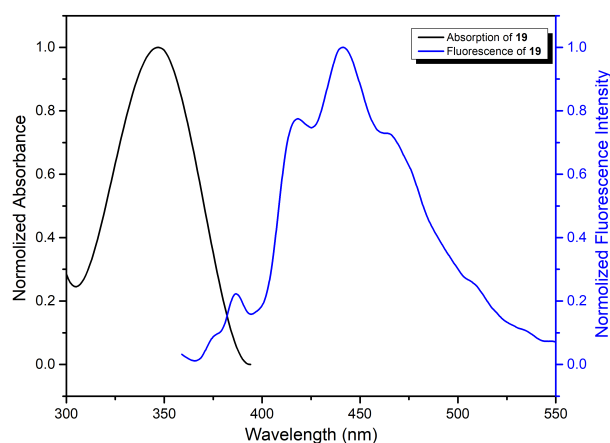


Figure S1. Absorption and fluorescence spectra of **19** in DMSO (5 μM). The excitation wavelength was 362 nm for the fluorescence spectrum.

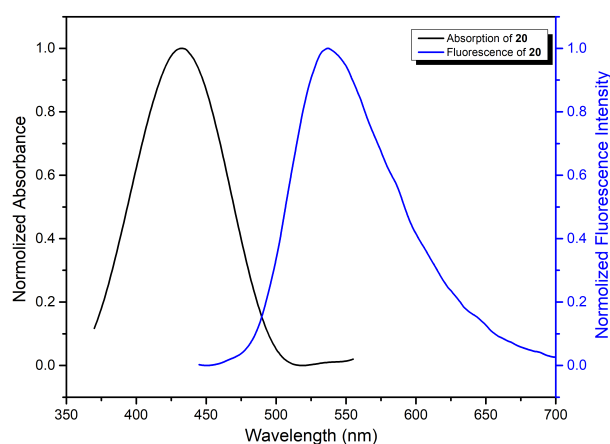


Figure S2. Absorption and fluorescence spectra of **20** in DMSO (15 μM). The excitation wavelength was 430 nm for the fluorescence spectrum.

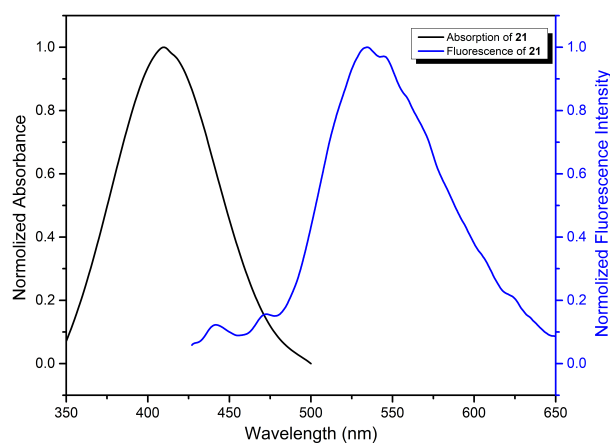


Figure S3. Absorption and fluorescence spectra of **21** in DMSO (15 μM). The excitation wavelength was 412 nm for the fluorescence spectrum.

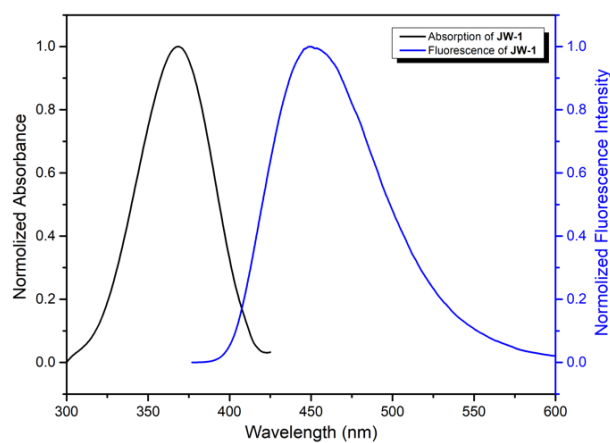


Figure S4. Absorption and fluorescence spectra of **22 (JW-1)** in DMSO (15 μ M). The excitation wavelength was 362 nm for fluorescence spectrum. $\lambda_{\text{ex}} = 364$ nm; $\lambda_{\text{em}} = 449$ nm; Stokes shift = 85

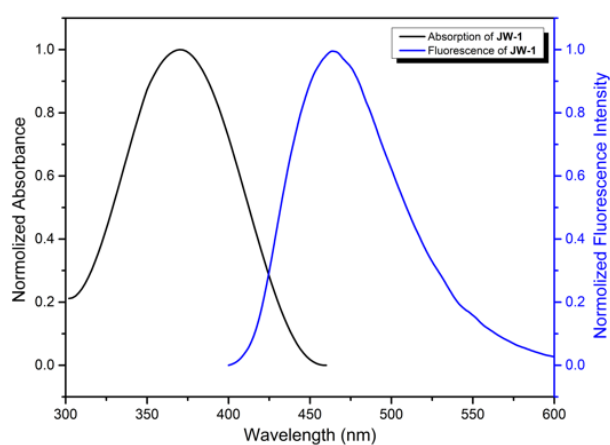


Figure S5. Absorption and fluorescence spectra of **22 (JW-1)** (10 μ M) in 10 mM phosphate buffer containing 0.1% DMSO (pH 7.4). The excitation wavelength was 376 nm for fluorescence spectrum. $\lambda_{\text{ex}} = 370$ nm; $\lambda_{\text{em}} = 463$ nm; Stokes shift = 93

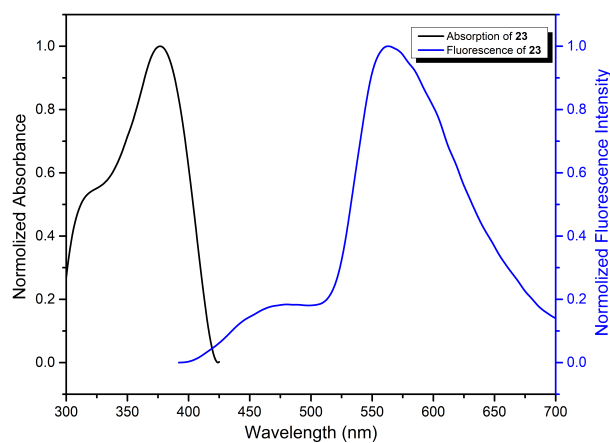


Figure S6. Absorption and fluorescence spectra of **23** in DMSO (20 μ M). The excitation wavelength was 377 nm for fluorescence spectrum.

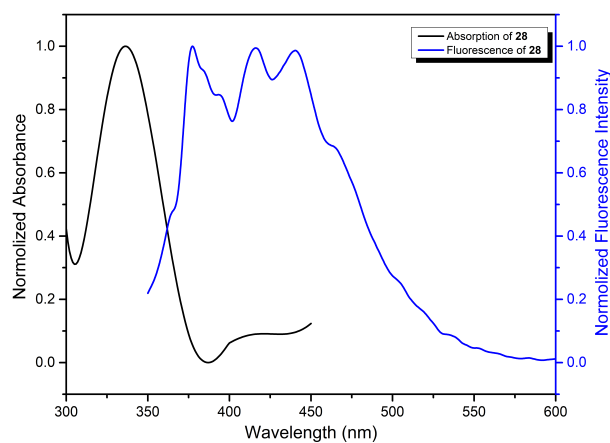


Figure S7. Absorption and fluorescence spectra of **28** in DMSO (12 μ M). The excitation wavelength was 337 nm for the fluorescence spectrum.

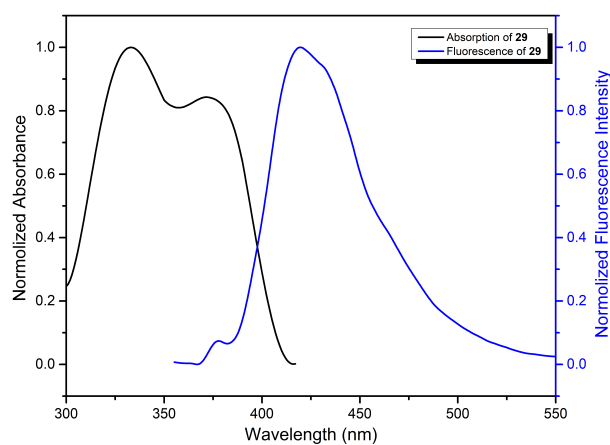


Figure S8. Absorption and fluorescence spectra of **29** in DMSO (20 μ M). The excitation wavelength was 338 nm for the fluorescence spectrum.

2. Fluorescence microscopy imaging

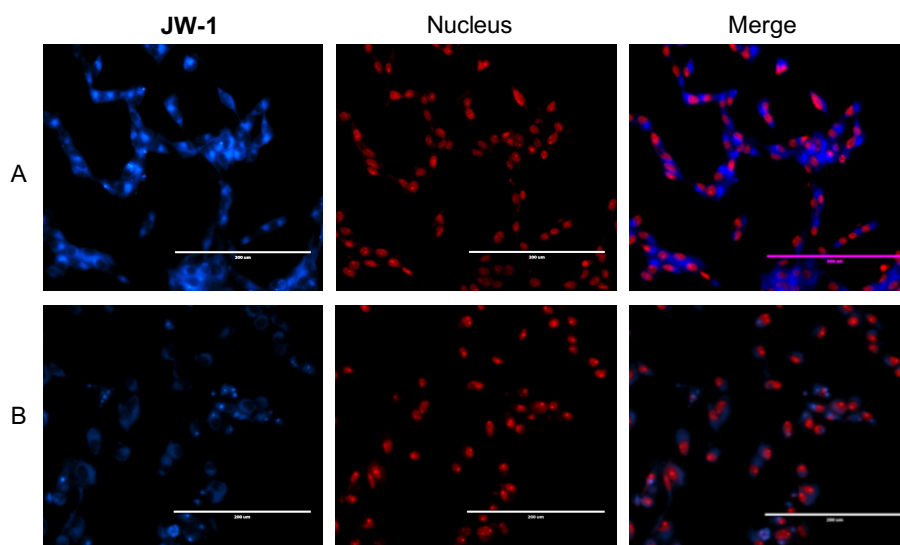


Figure S9. Fluorescence microscopy images of MDA-MB-231 cells incubated with (A) 20 μ M **JW-1** for 4 h or (B) 4.0 μ M **JW-1** for 16 h. Cells were co-stained with propidium iodide to visualize their nuclei (red). Scale bar: 200 μ m.

3. Spectroscopic titration

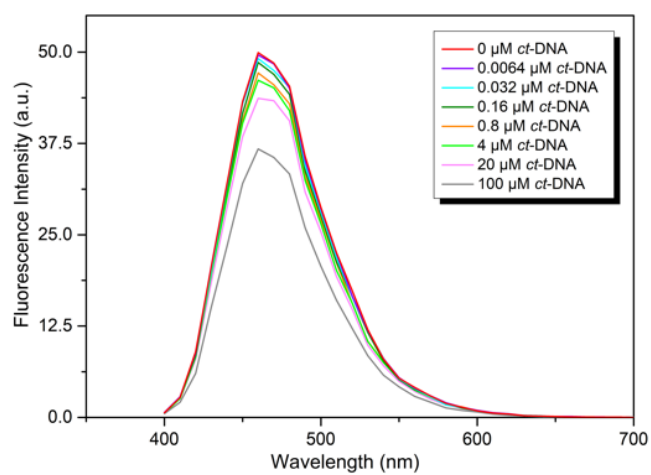
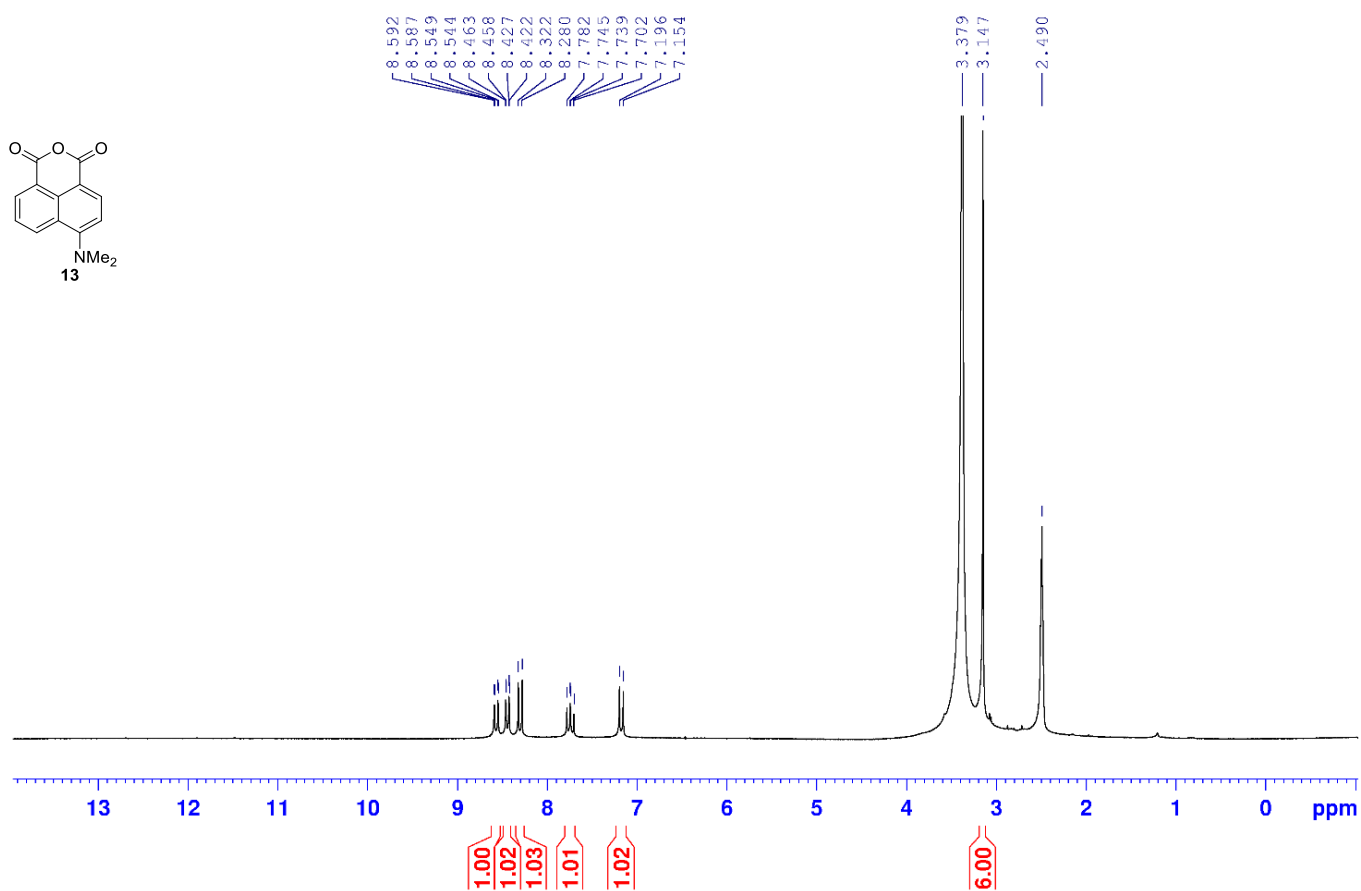


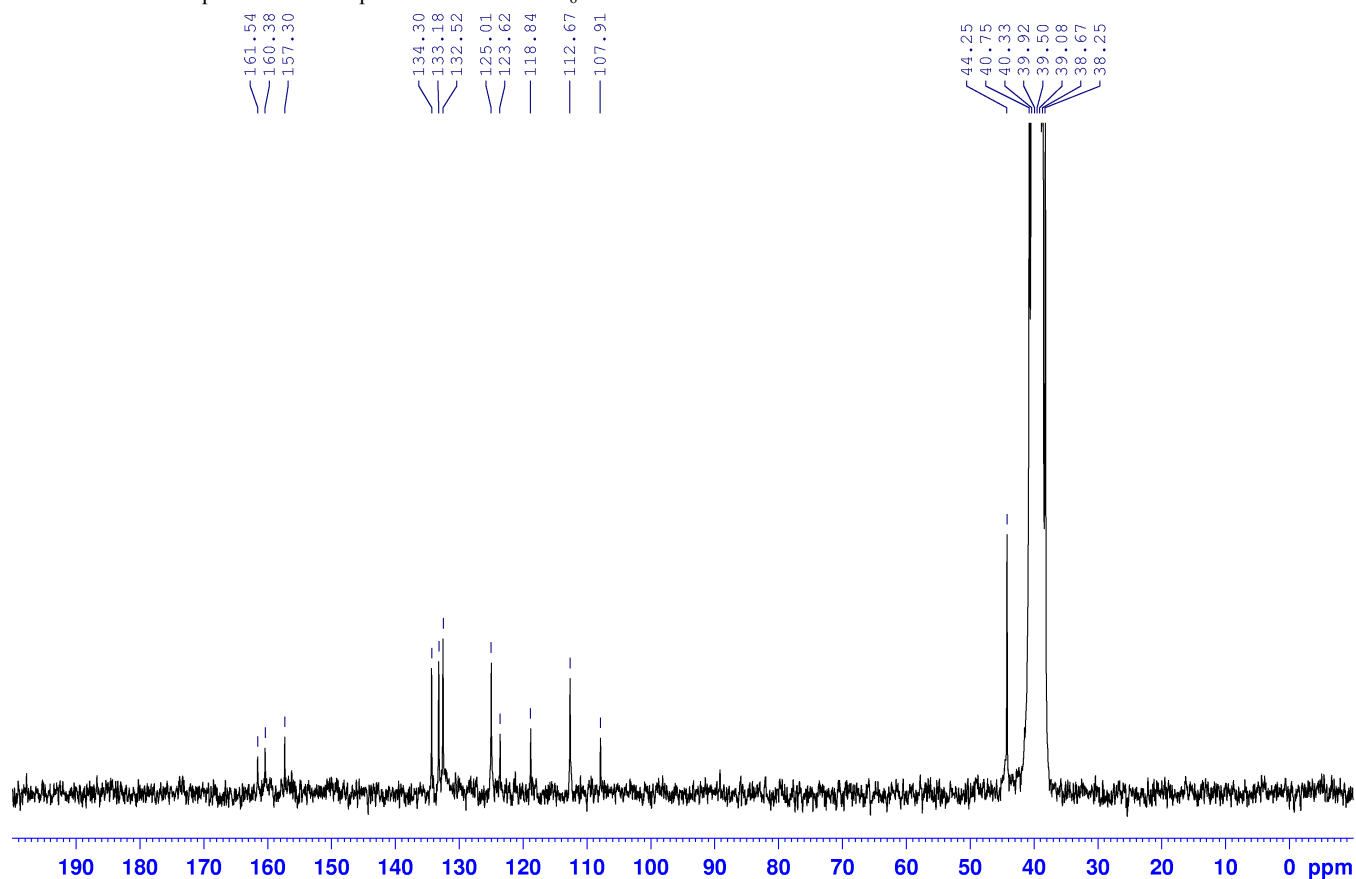
Figure S10. Fluorescence spectra of **JW-1** (4 μM) in the presence of increasing concentration of *ct*-DNA (0–100 μM) in 10 mM phosphate buffer containing 0.05% DMSO (pH 7.4).

4. ^1H and ^{13}C NMR spectra

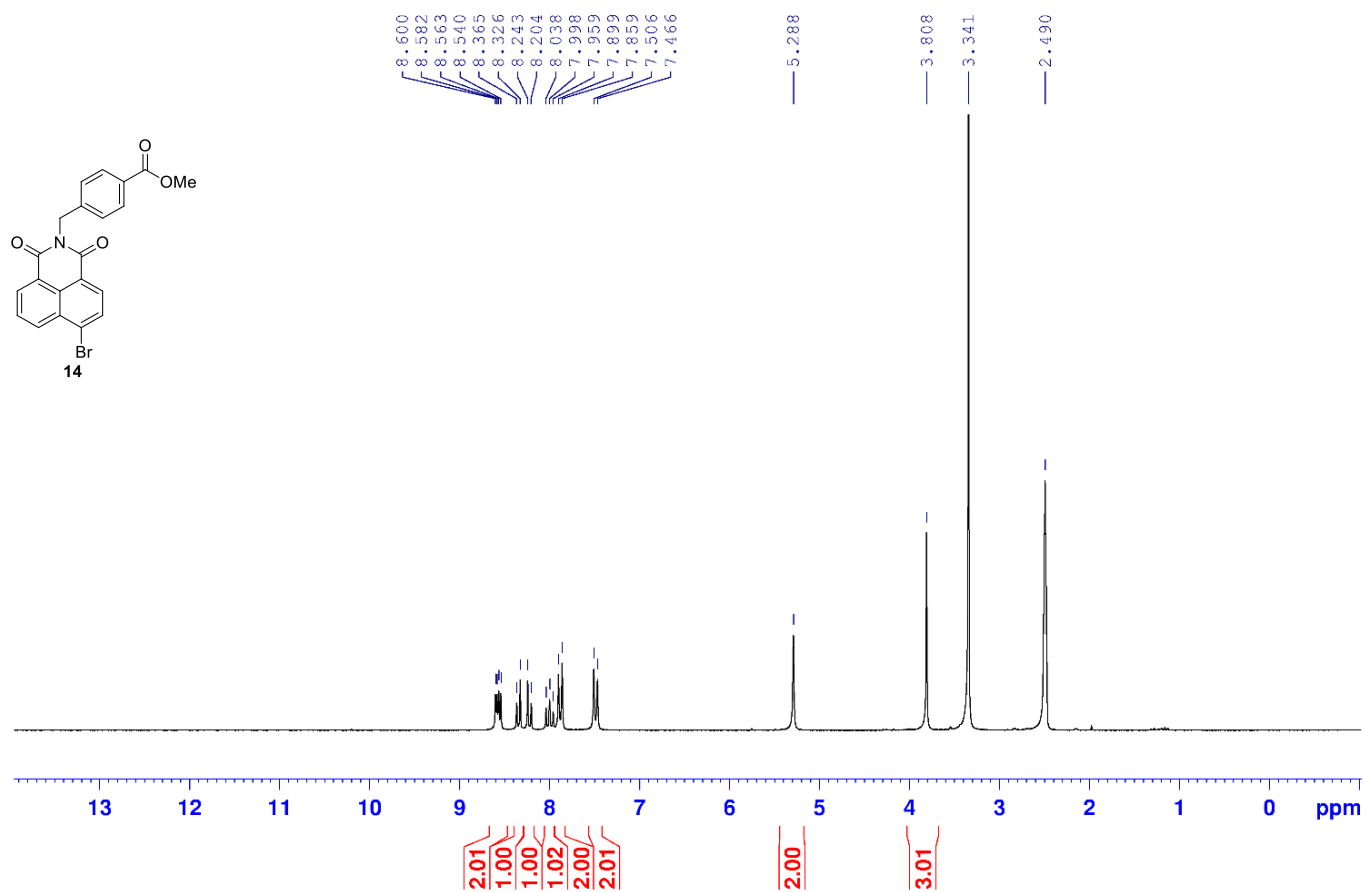
200 MHz ^1H NMR Spectrum of Compound **13** in $\text{DMSO}-d_6$



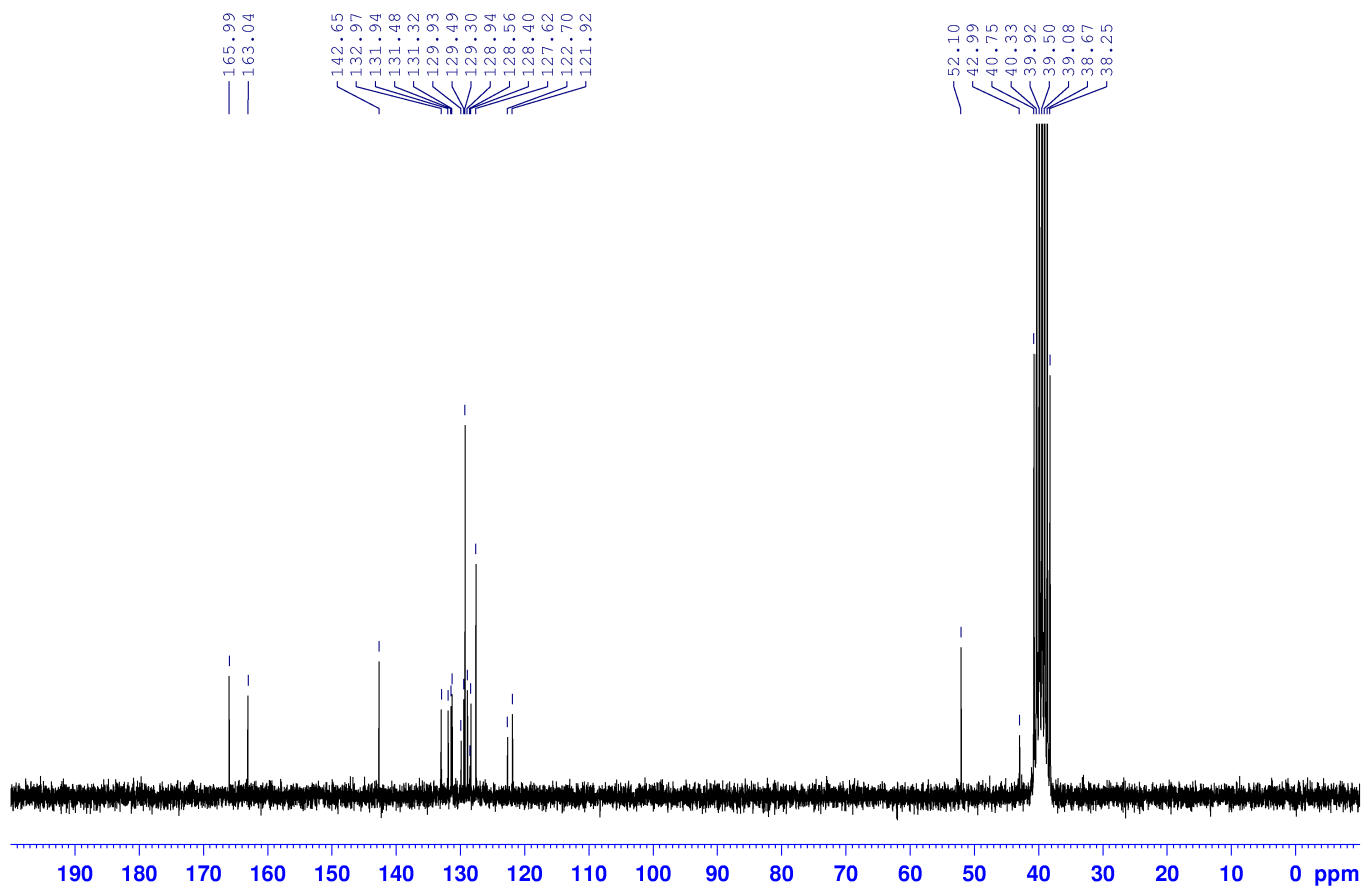
50 MHz ^{13}C NMR Spectrum of Compound **13** in $\text{DMSO}-d_6$



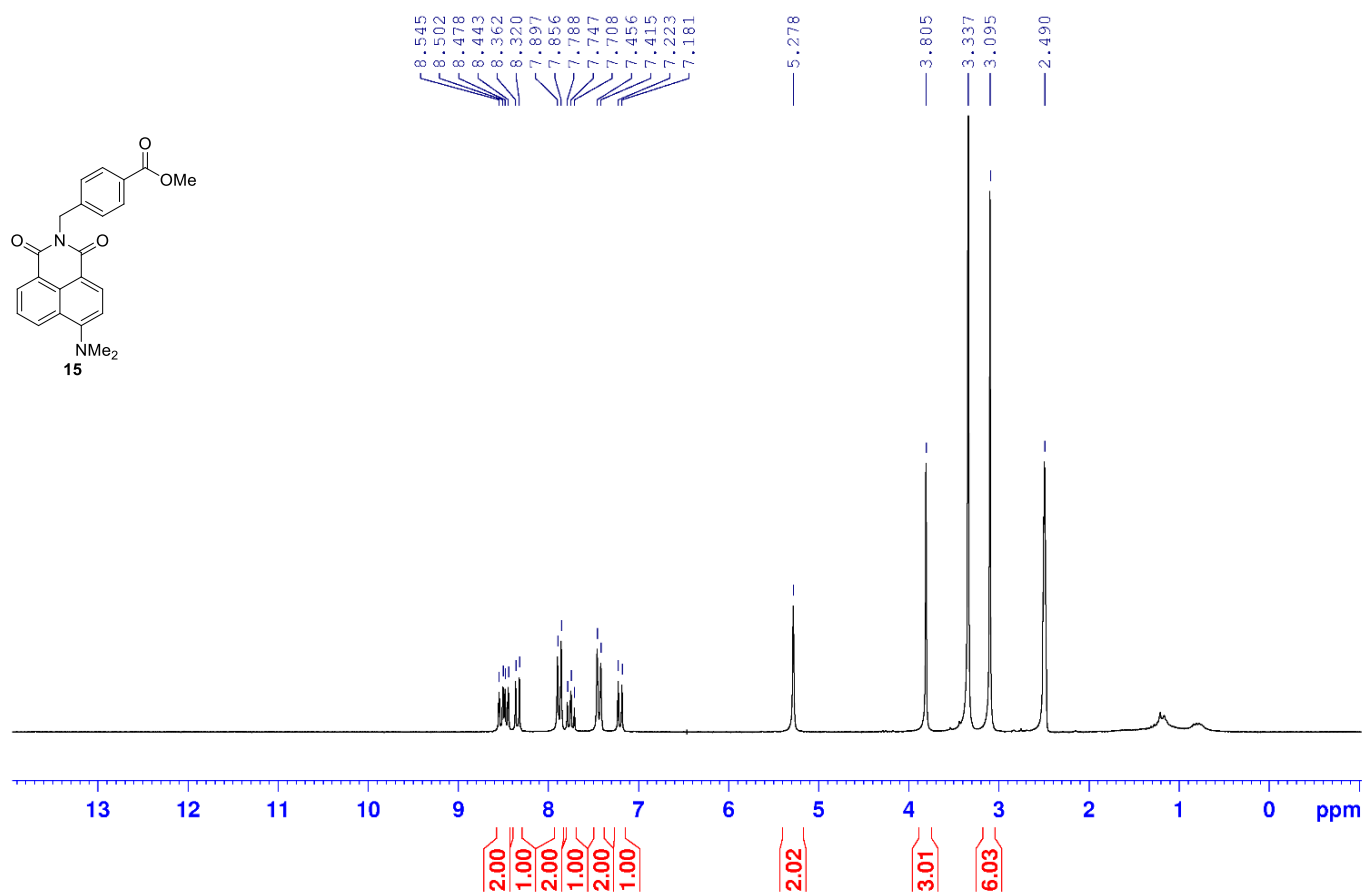
200 MHz ^1H NMR Spectrum of Compound **14** in $\text{DMSO}-d_6$



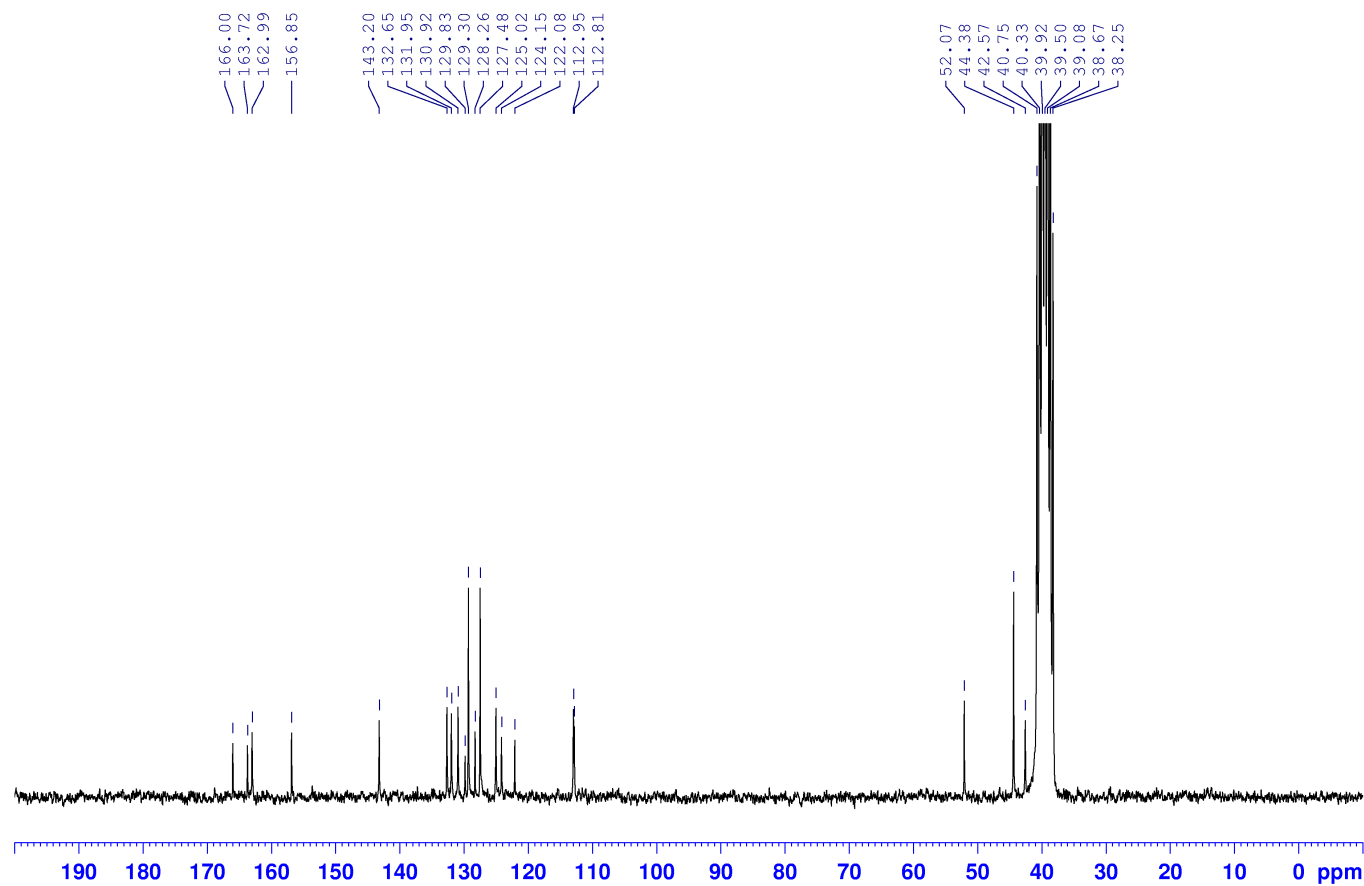
50 MHz ^{13}C NMR Spectrum of Compound **14** in $\text{DMSO}-d_6$



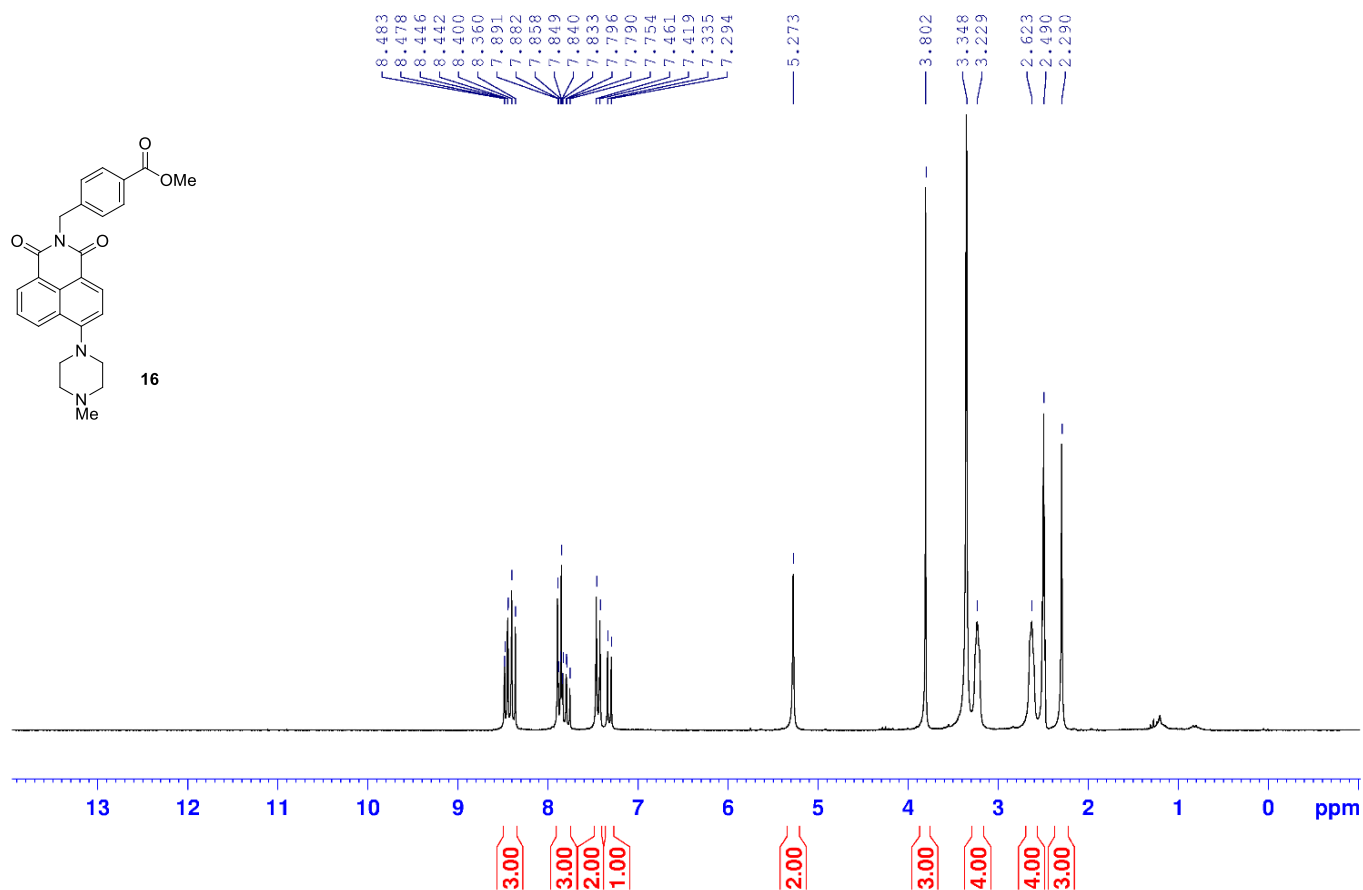
200 MHz ^1H NMR Spectrum of Compound **15** in $\text{DMSO}-d_6$



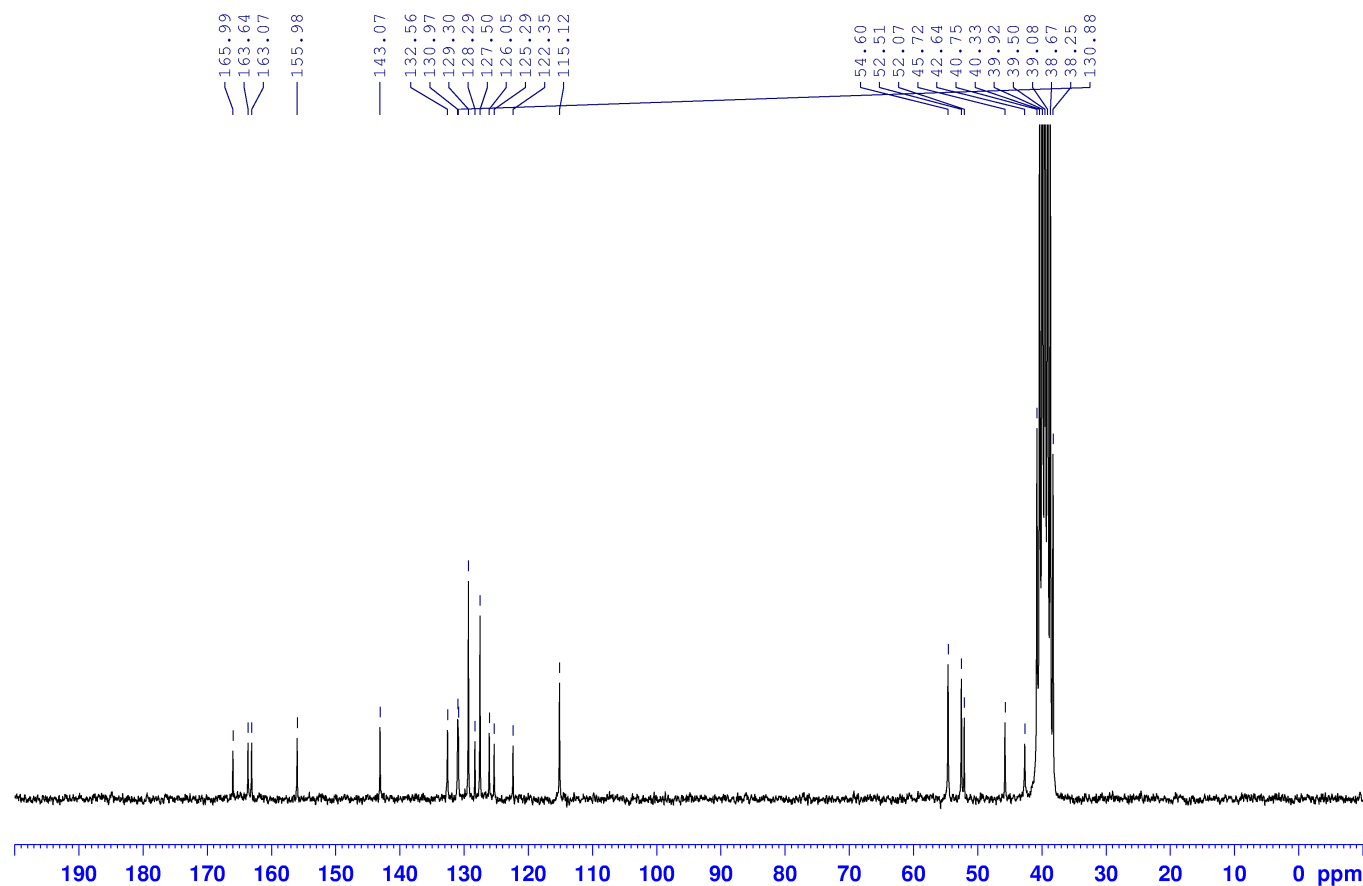
50 MHz ^{13}C NMR Spectrum of Compound **15** in $\text{DMSO}-d_6$



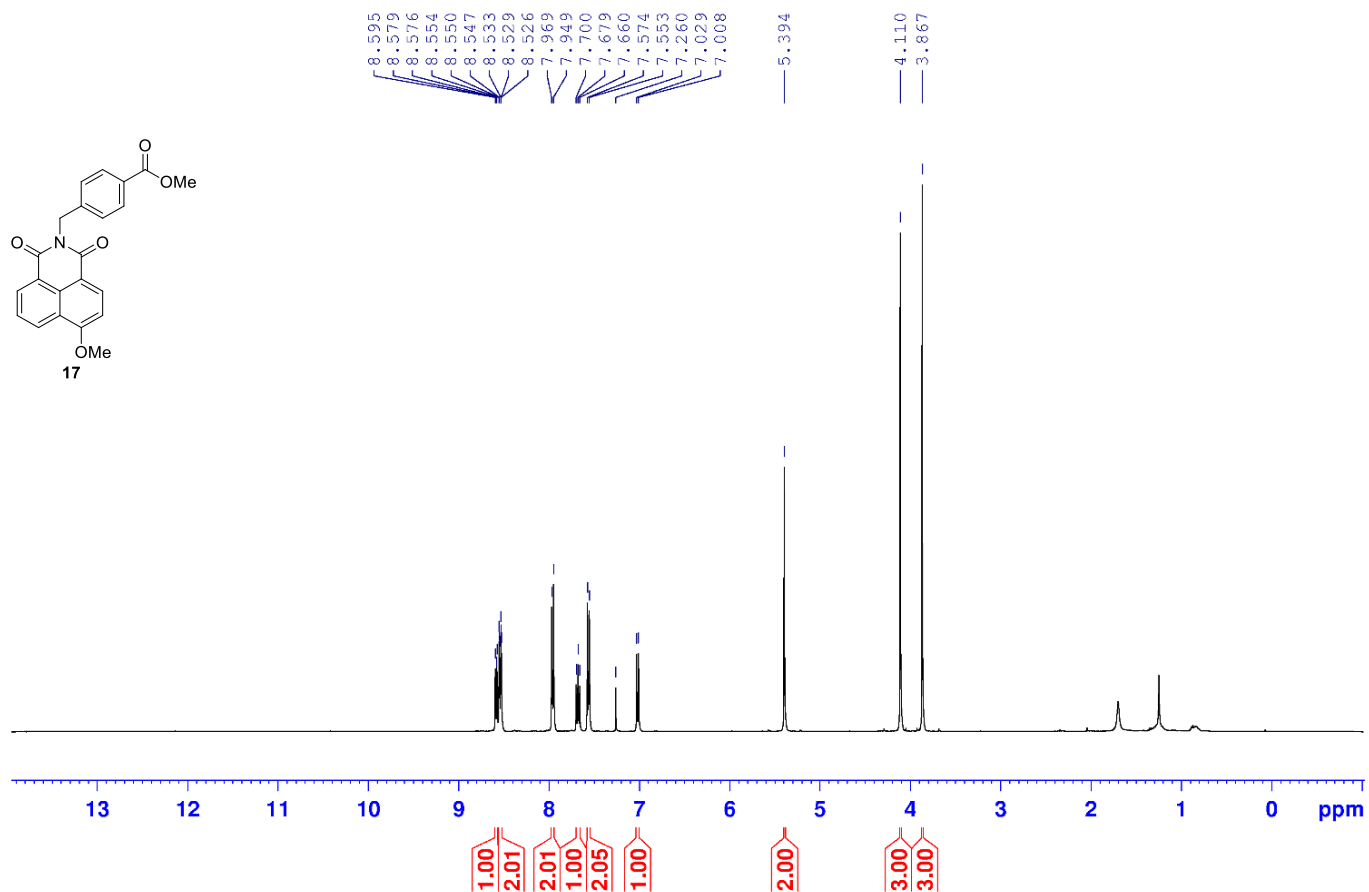
200 MHz ^1H NMR Spectrum of Compound **16** in $\text{DMSO}-d_6$



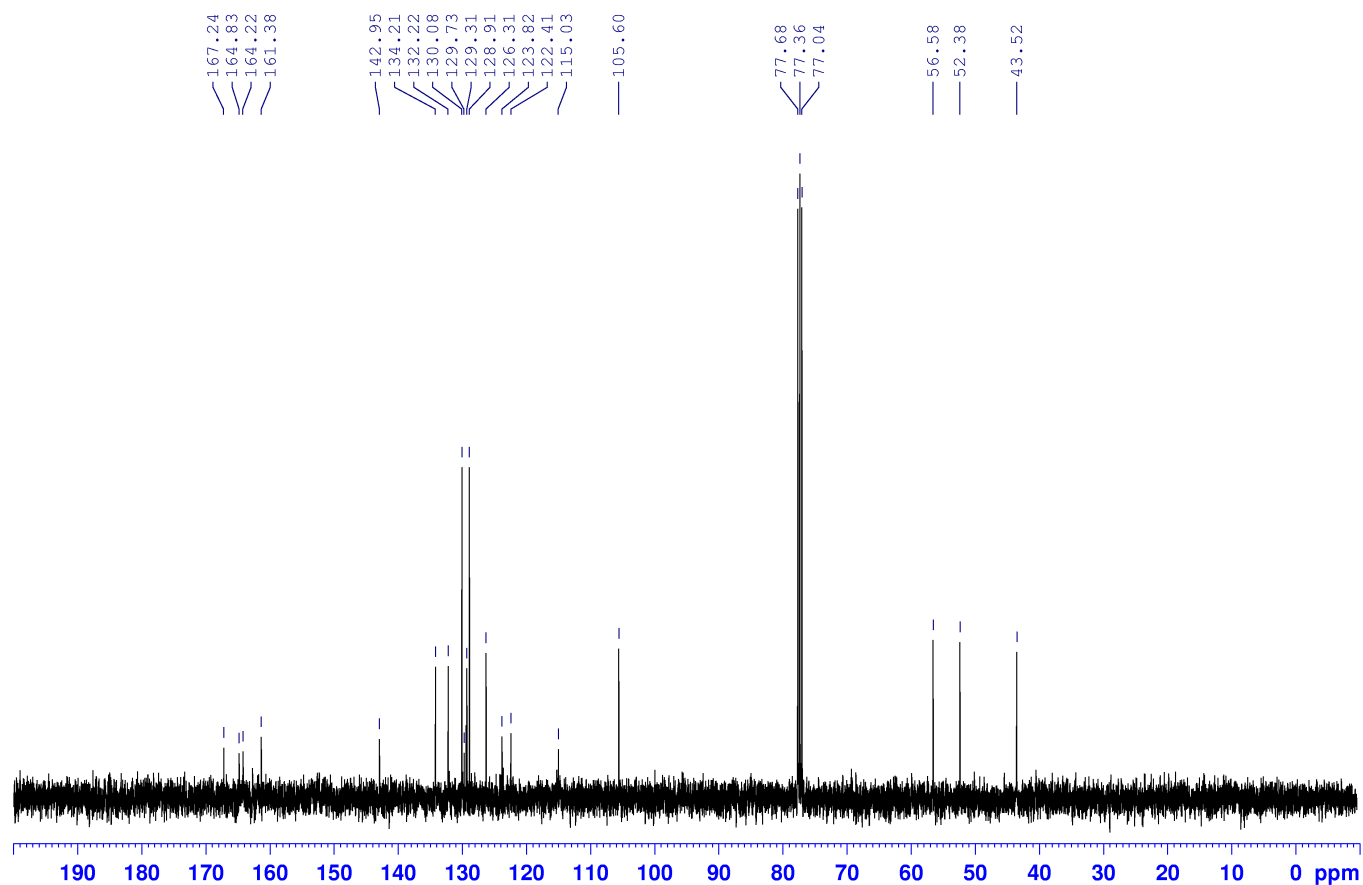
50 MHz ^{13}C NMR Spectrum of Compound **16** in $\text{DMSO}-d_6$



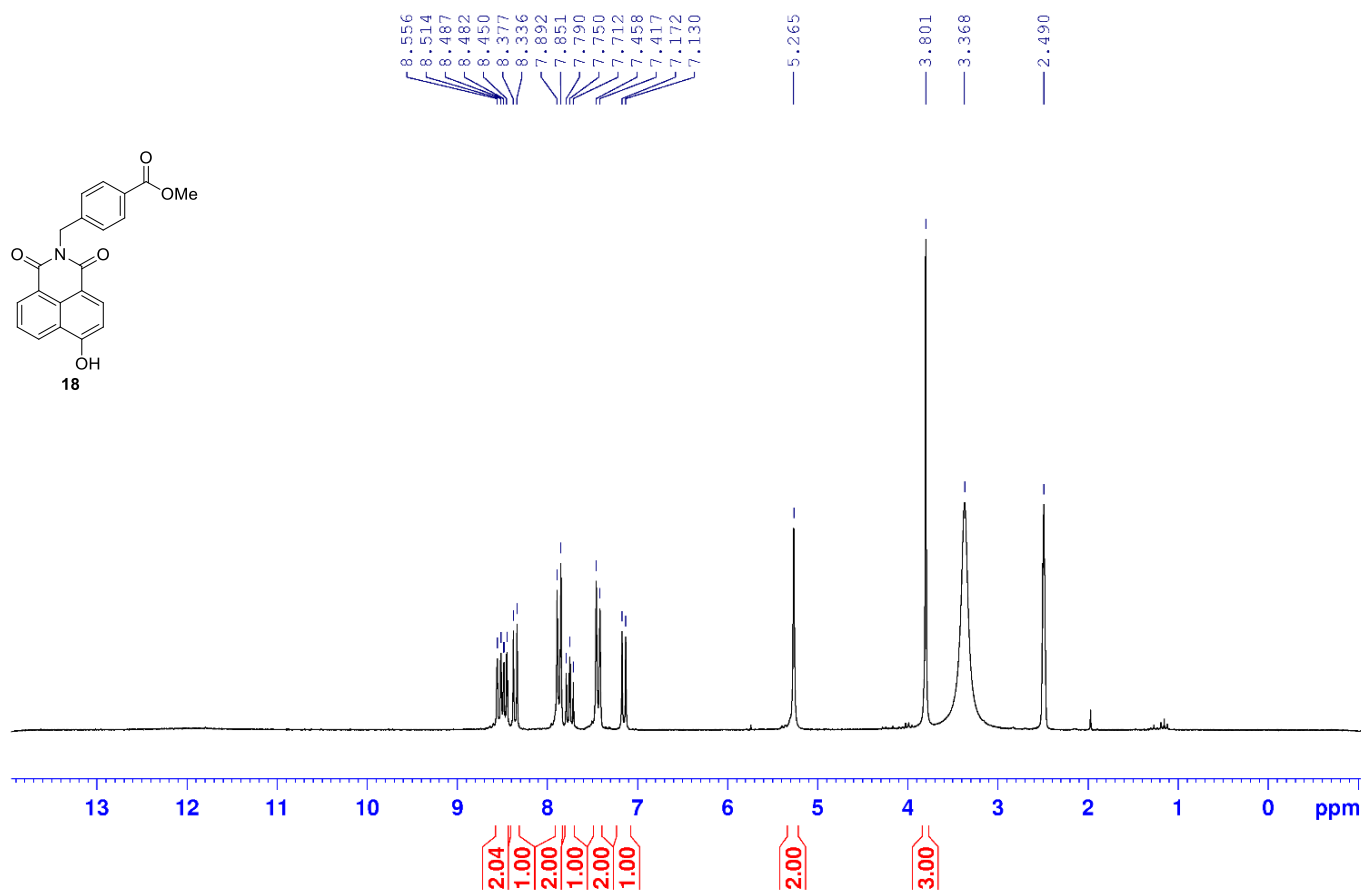
400 MHz ^1H NMR Spectrum of Compound **17** in CDCl_3



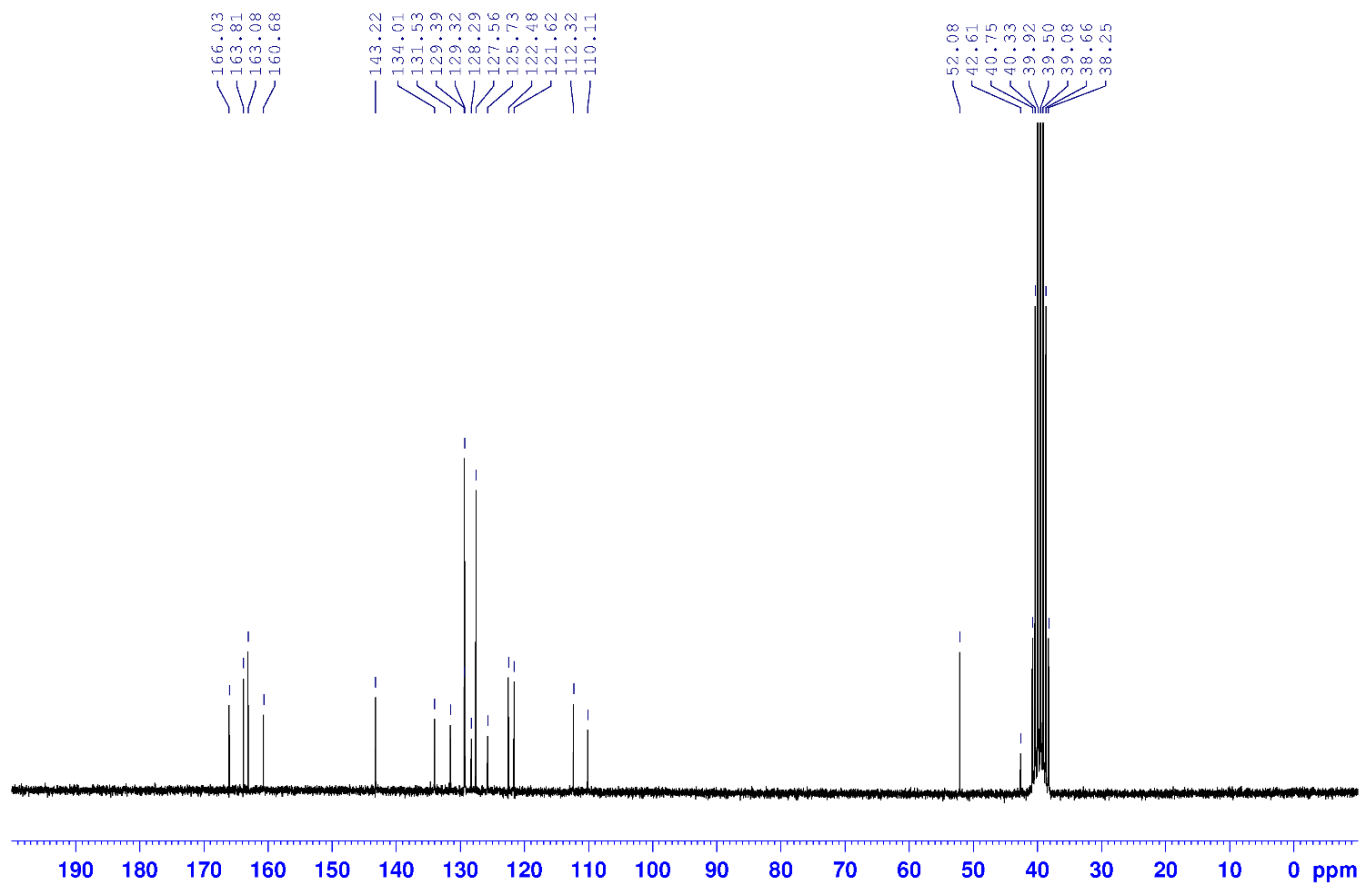
100 MHz ^{13}C NMR Spectrum of Compound **17** in CDCl_3



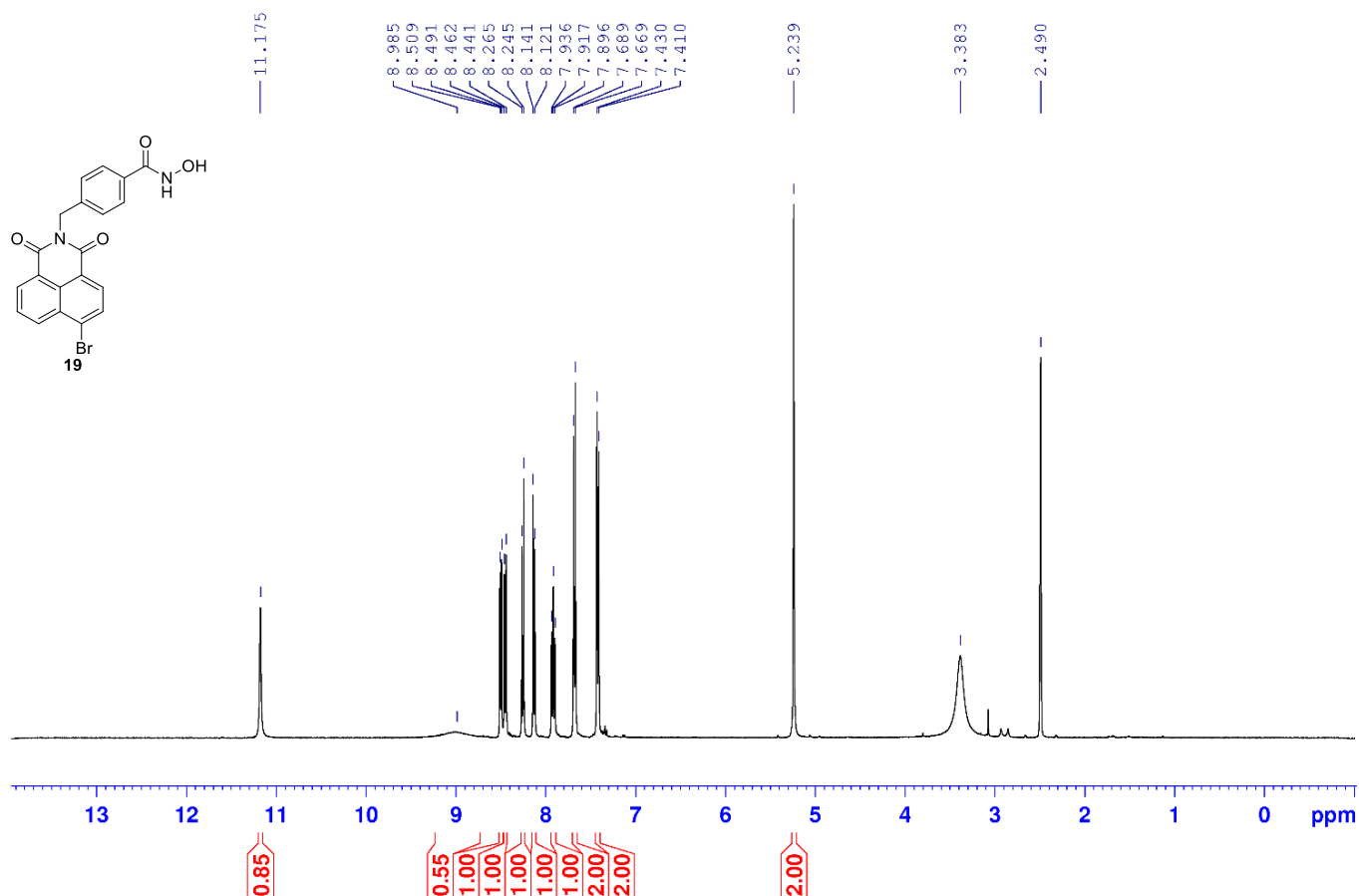
200 MHz ^1H NMR Spectrum of Compound **18** in $\text{DMSO}-d_6$



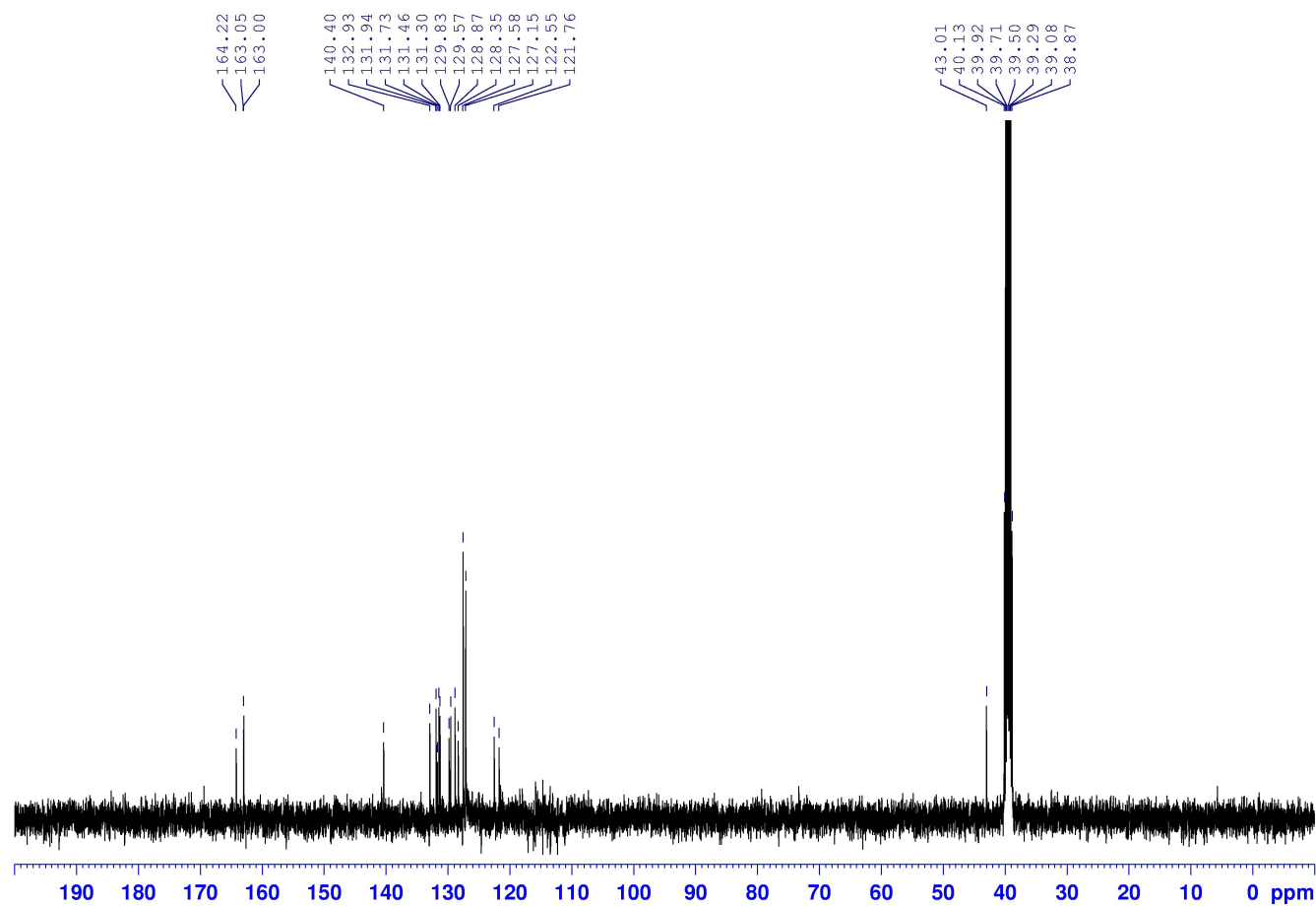
50 MHz ^{13}C NMR Spectrum of Compound **18** in $\text{DMSO}-d_6$



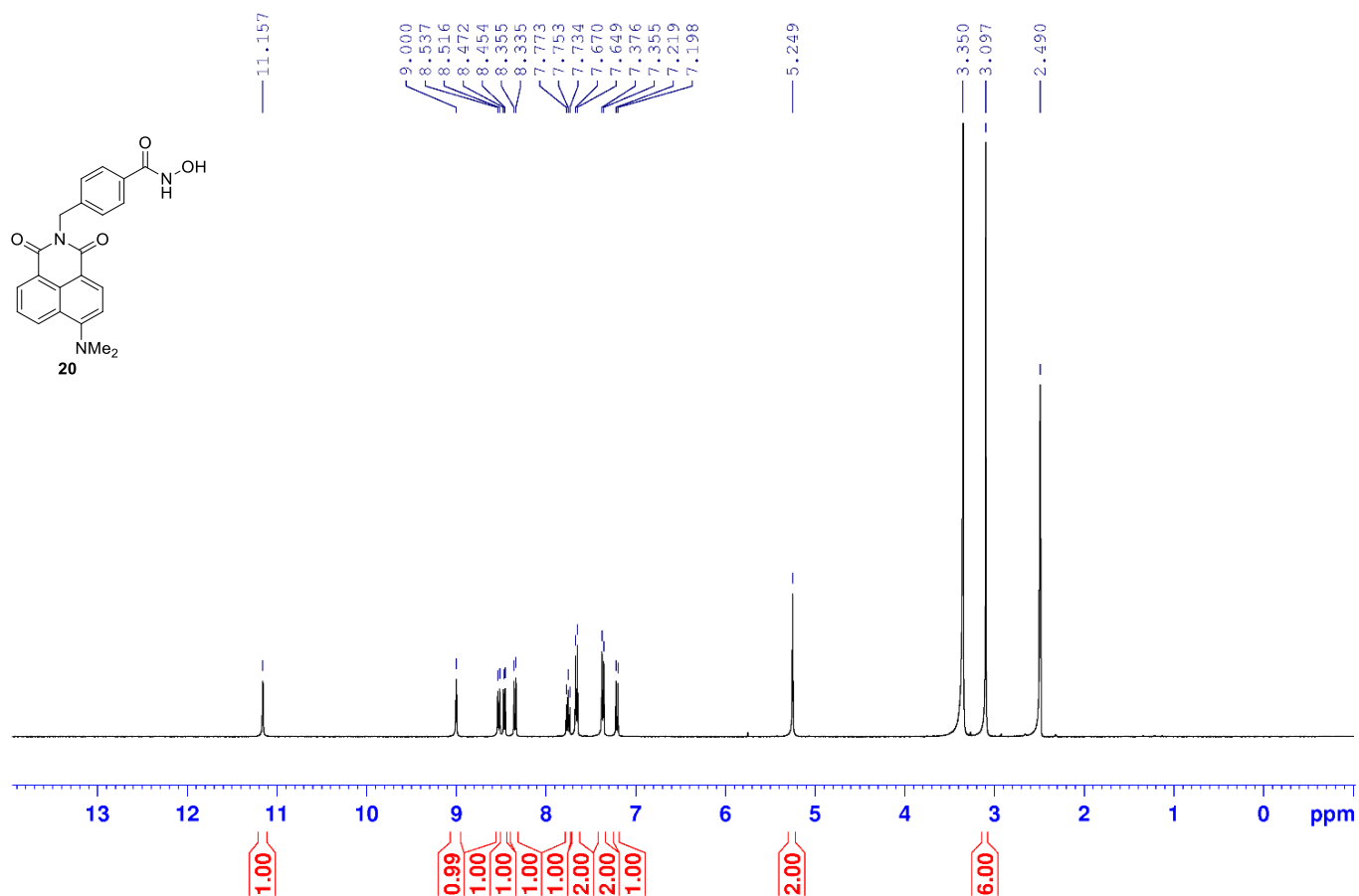
400 MHz ^1H NMR Spectrum of Compound **19** in $\text{DMSO}-d_6$



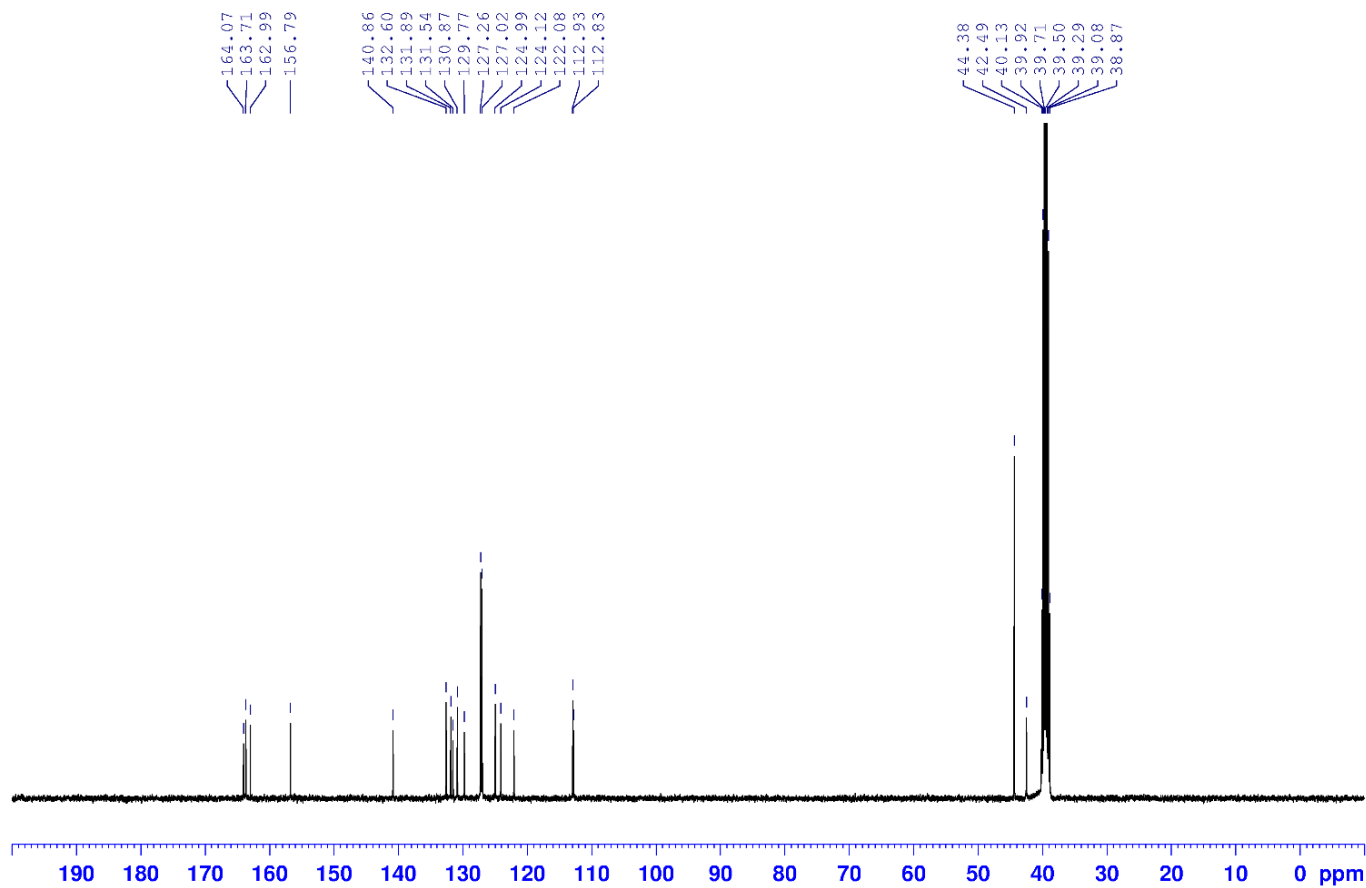
100 MHz ^{13}C NMR Spectrum of Compound **19** in $\text{DMSO}-d_6$



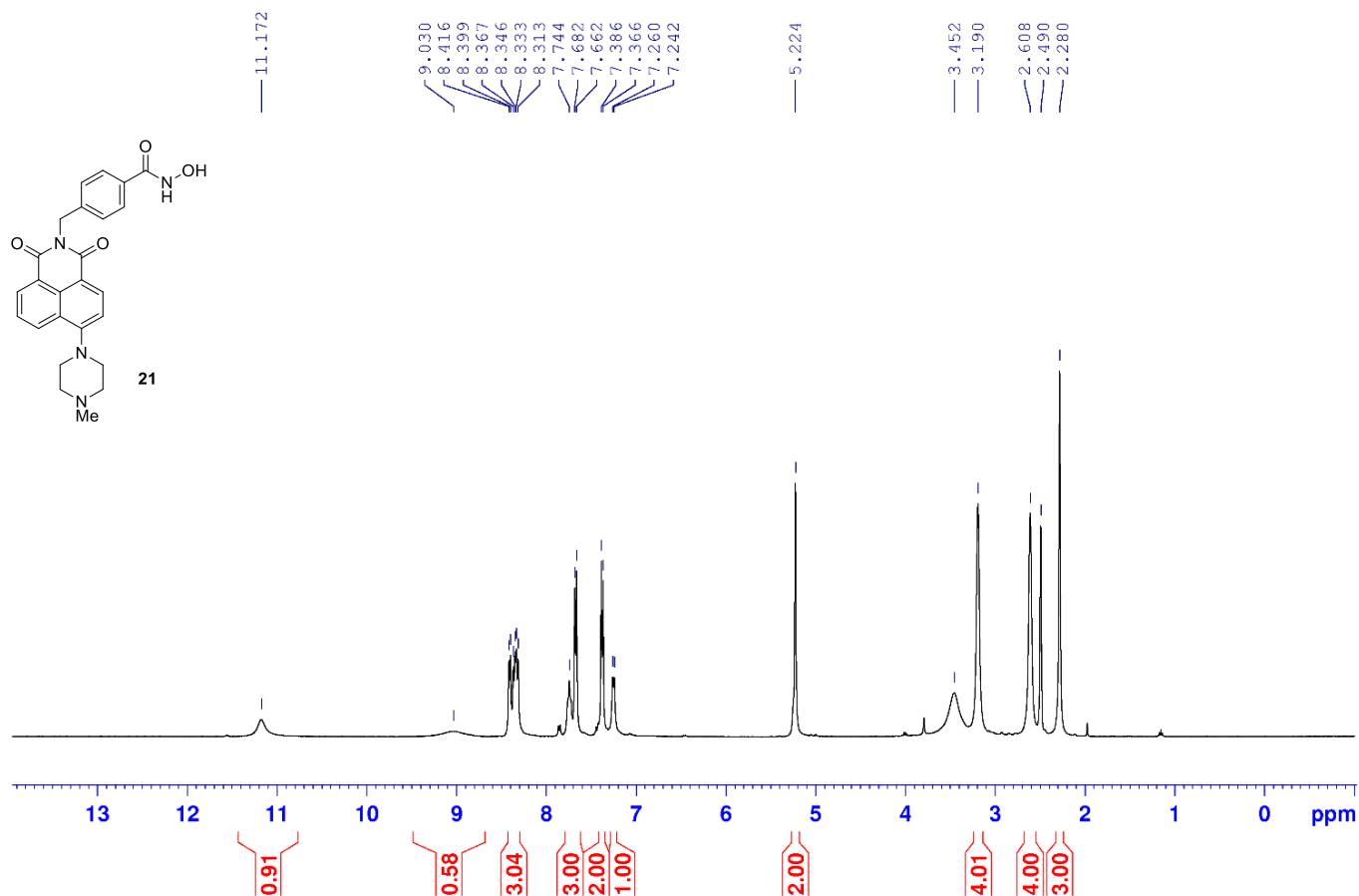
400 MHz ^1H NMR Spectrum of Compound **20** in $\text{DMSO-}d_6$



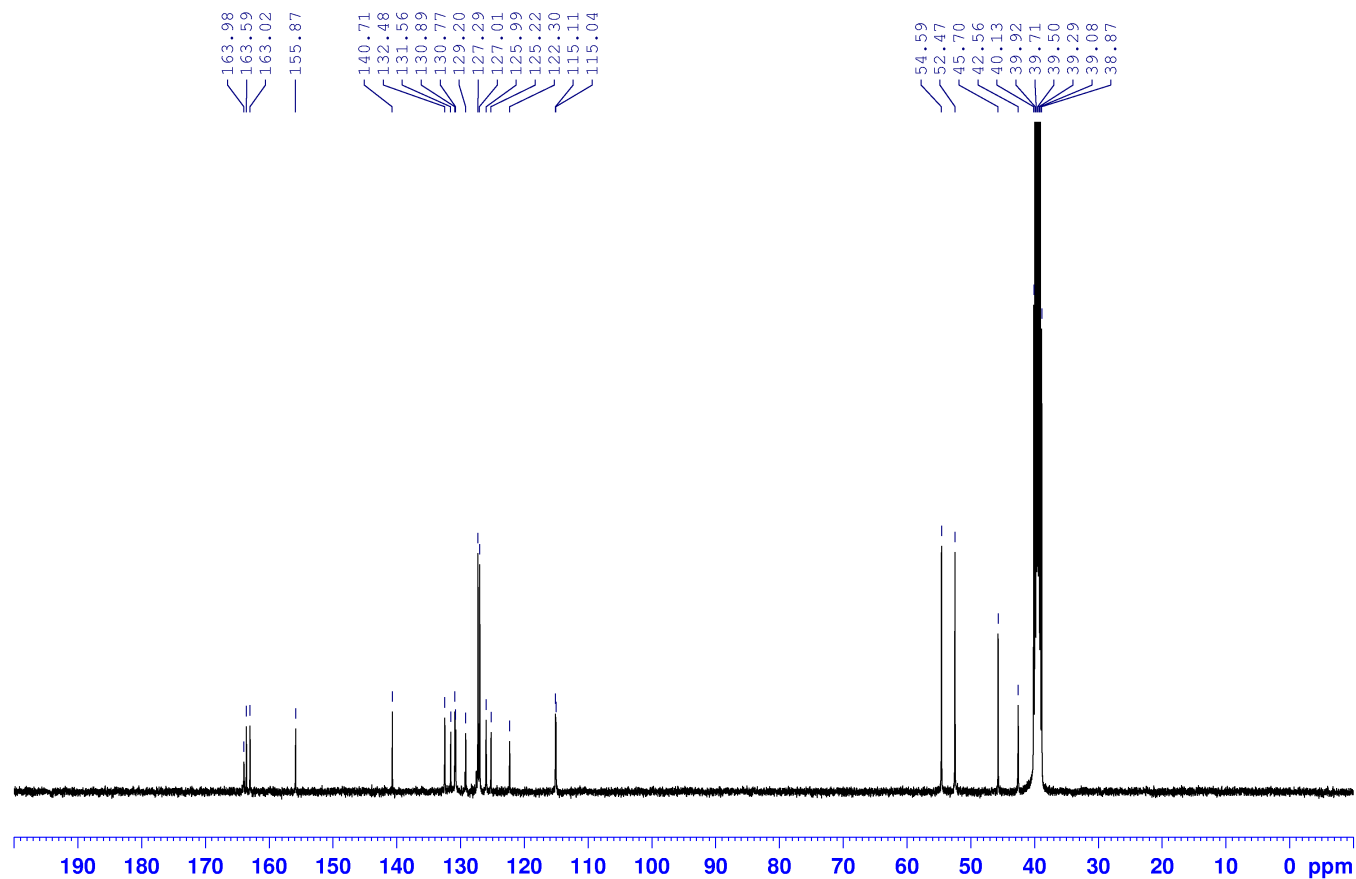
100 MHz ^{13}C NMR Spectrum of Compound **20** in $\text{DMSO-}d_6$



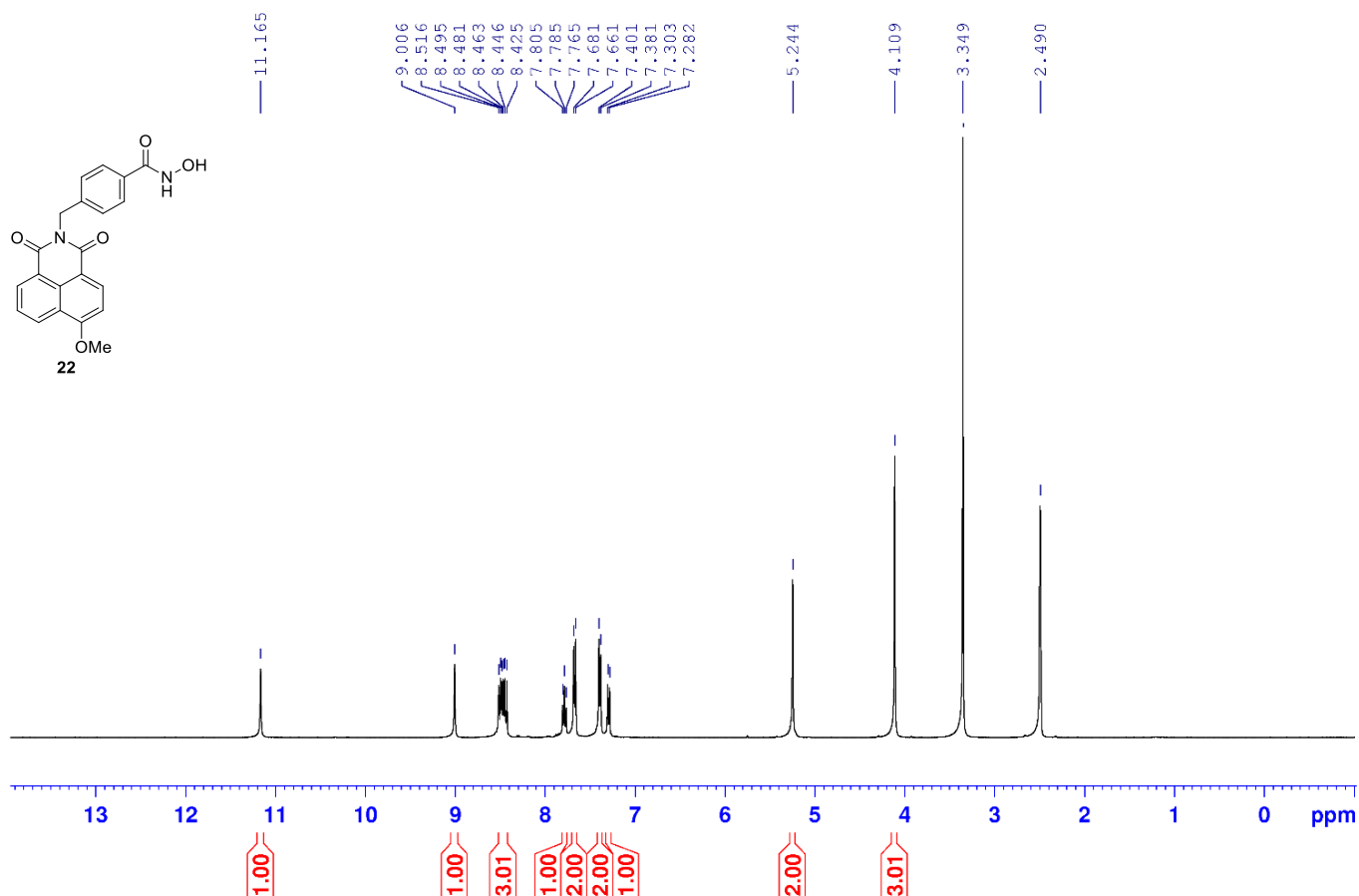
400 MHz ^1H NMR Spectrum of Compound **21** in $\text{DMSO}-d_6$



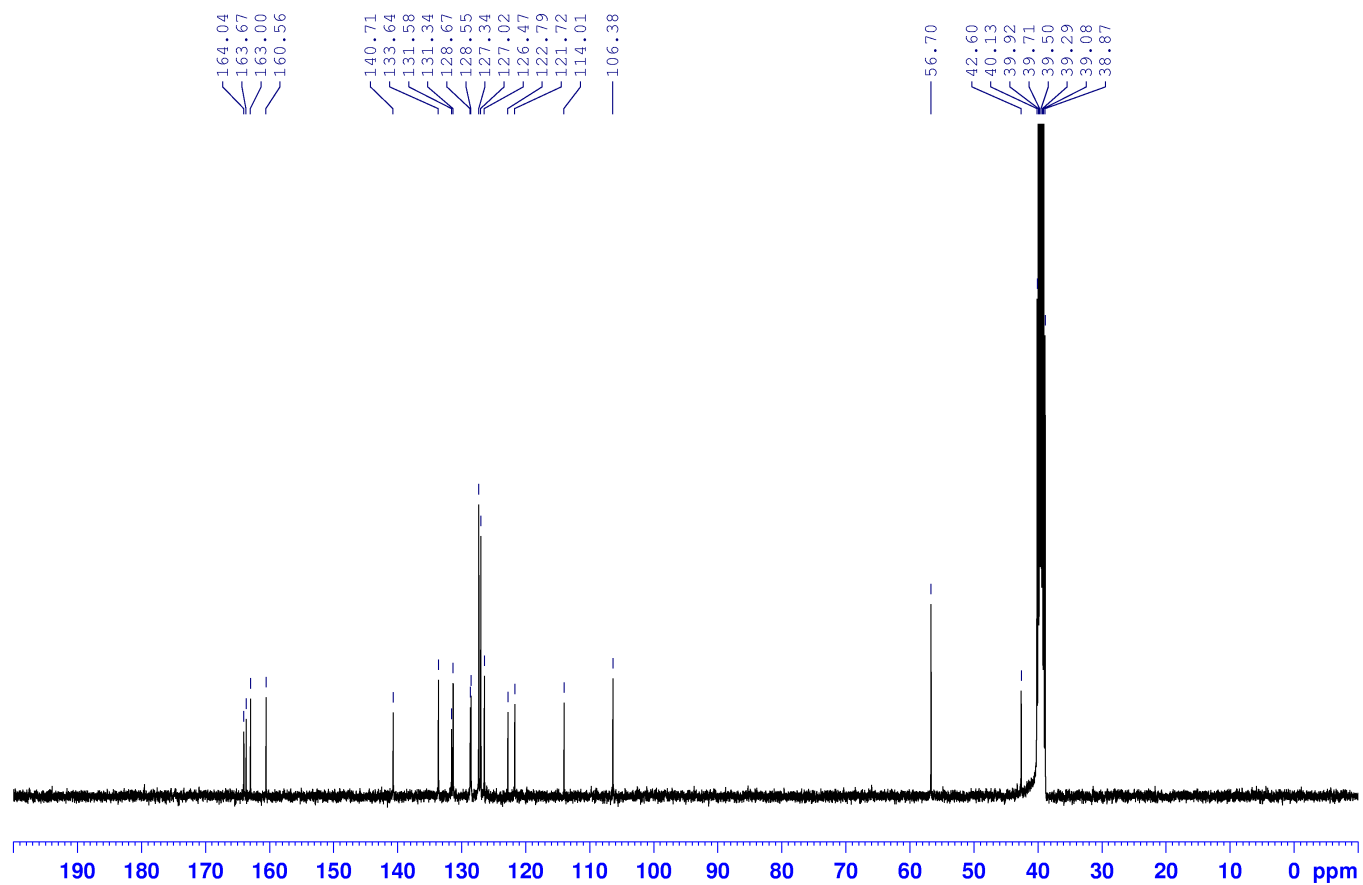
100 MHz ^{13}C NMR Spectrum of Compound **21** in $\text{DMSO}-d_6$



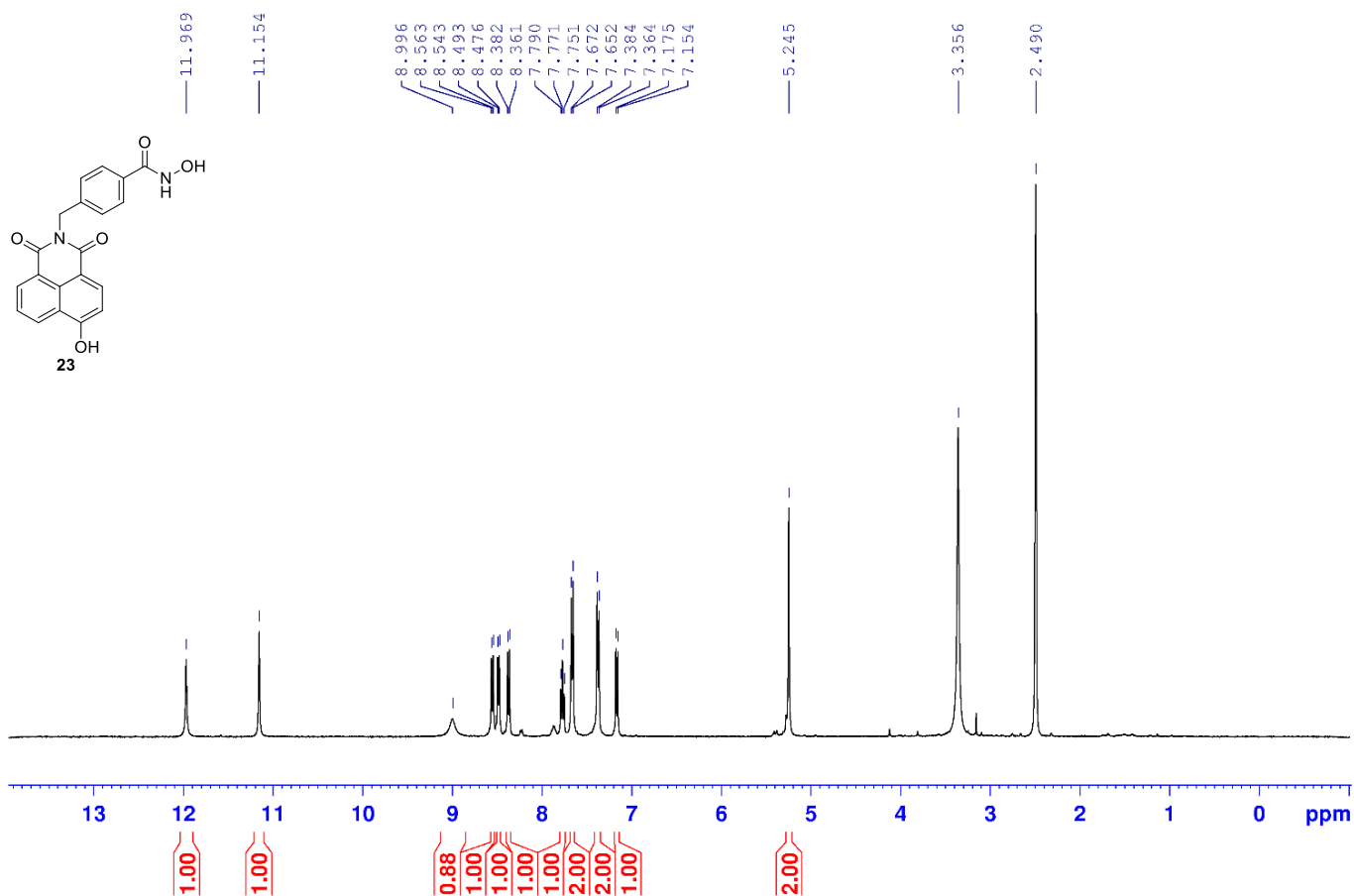
400 MHz ^1H NMR Spectrum of Compound **22** (**JW-1**) in $\text{DMSO-}d_6$



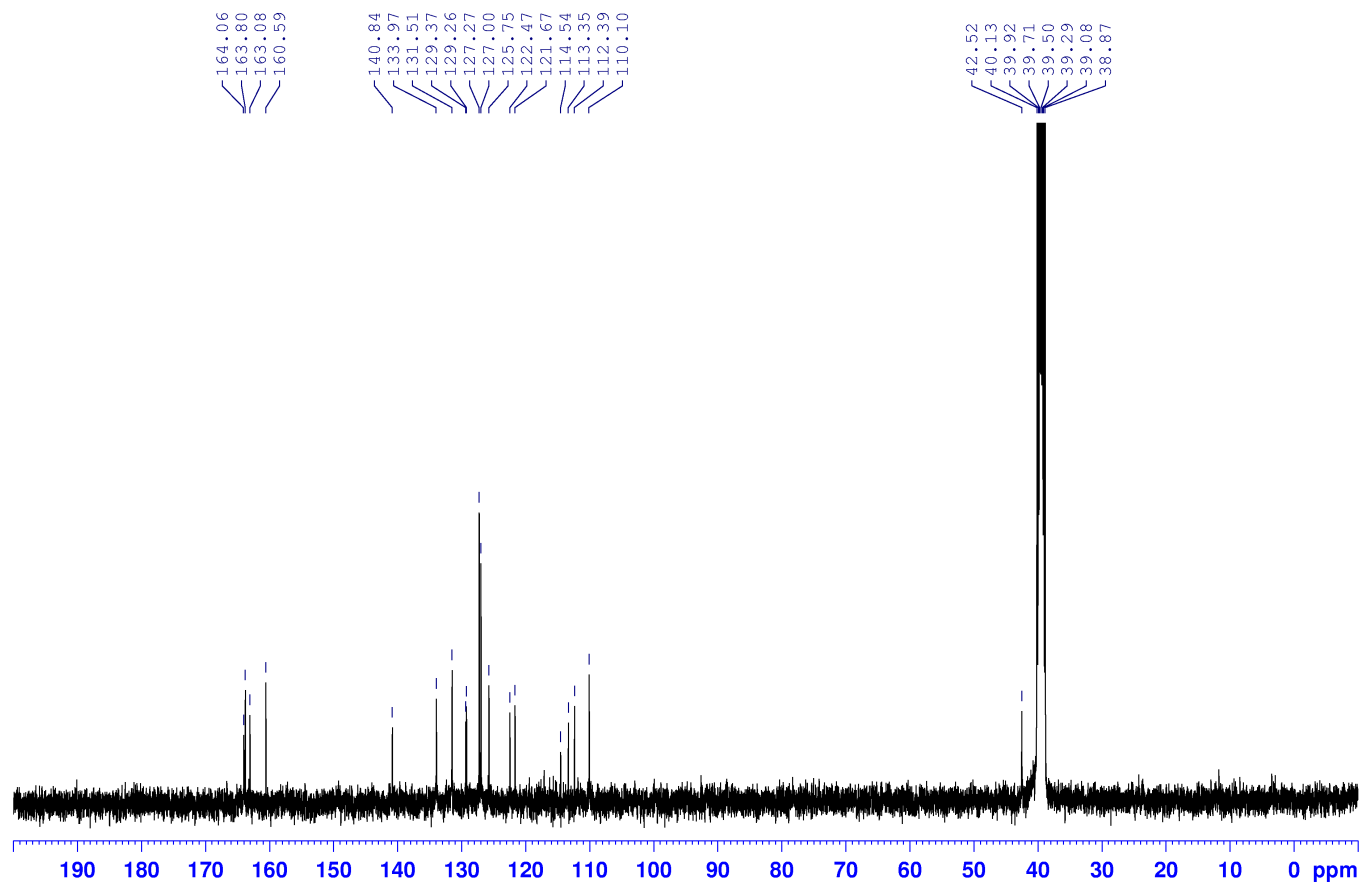
100 MHz ^{13}C NMR Spectrum of Compound **22** (**JW-1**) in $\text{DMSO-}d_6$



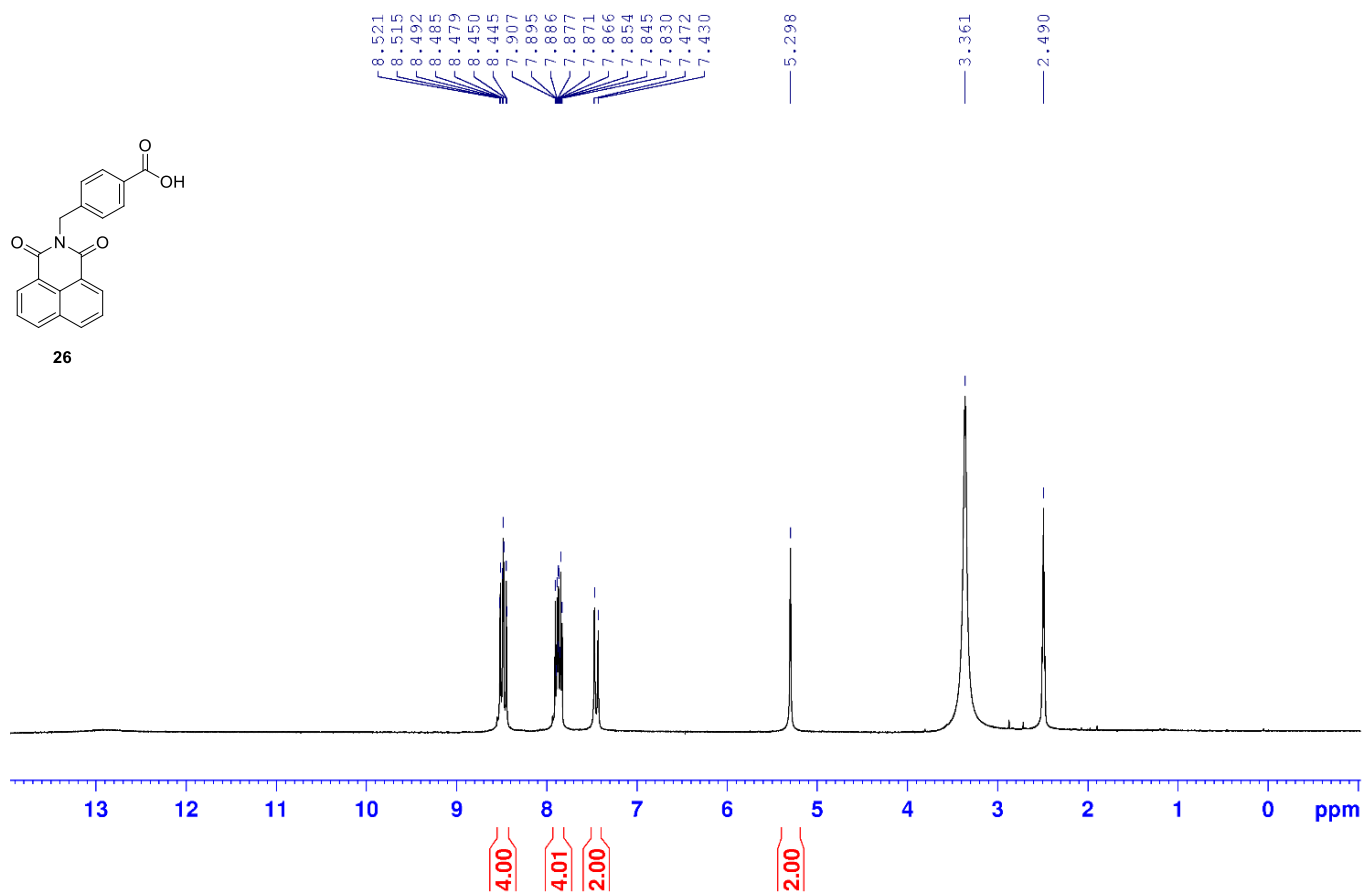
400 MHz ^1H NMR Spectrum of Compound **23** in $\text{DMSO}-d_6$



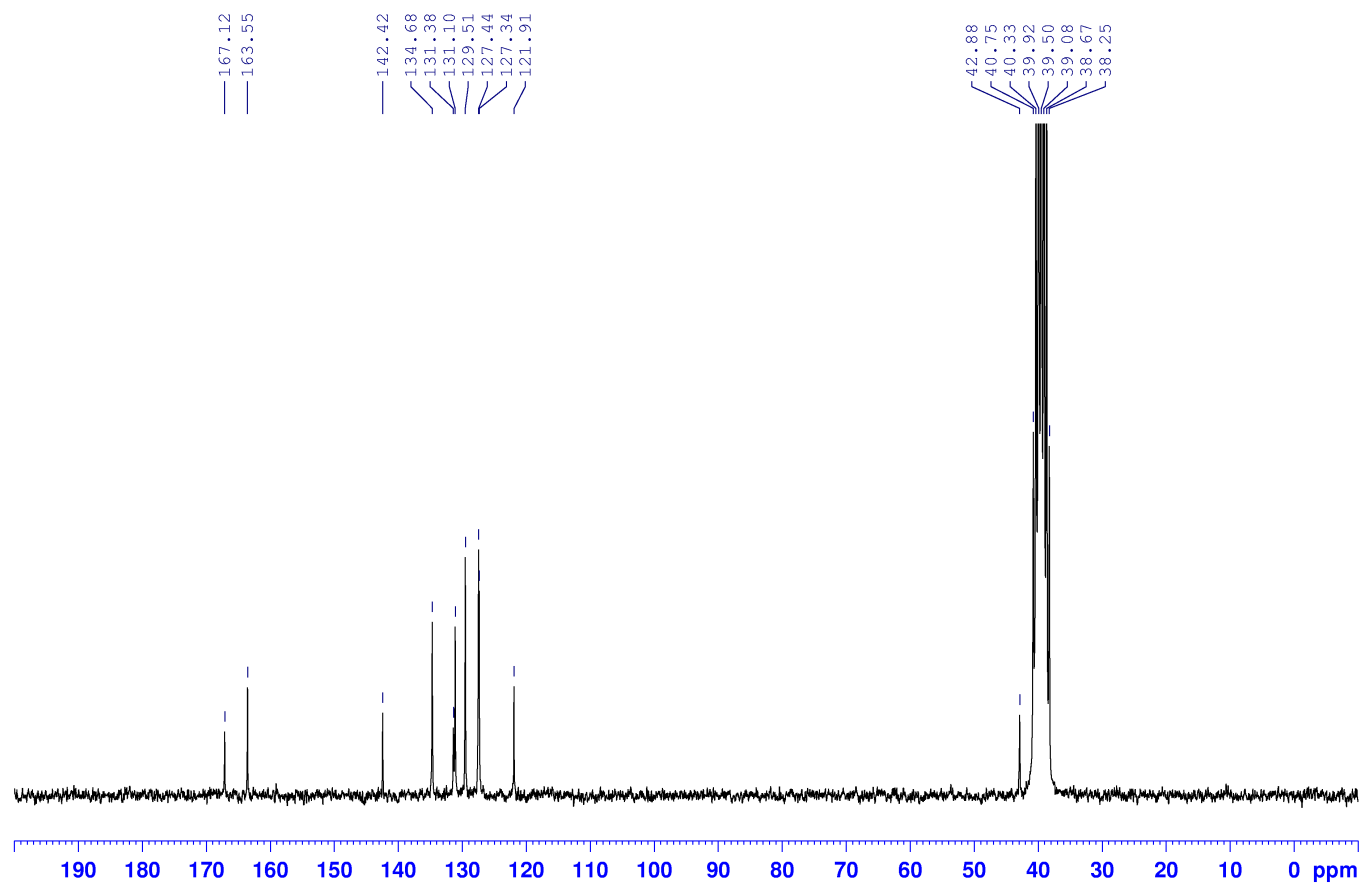
100 MHz ^{13}C NMR Spectrum of Compound **23** in $\text{DMSO}-d_6$



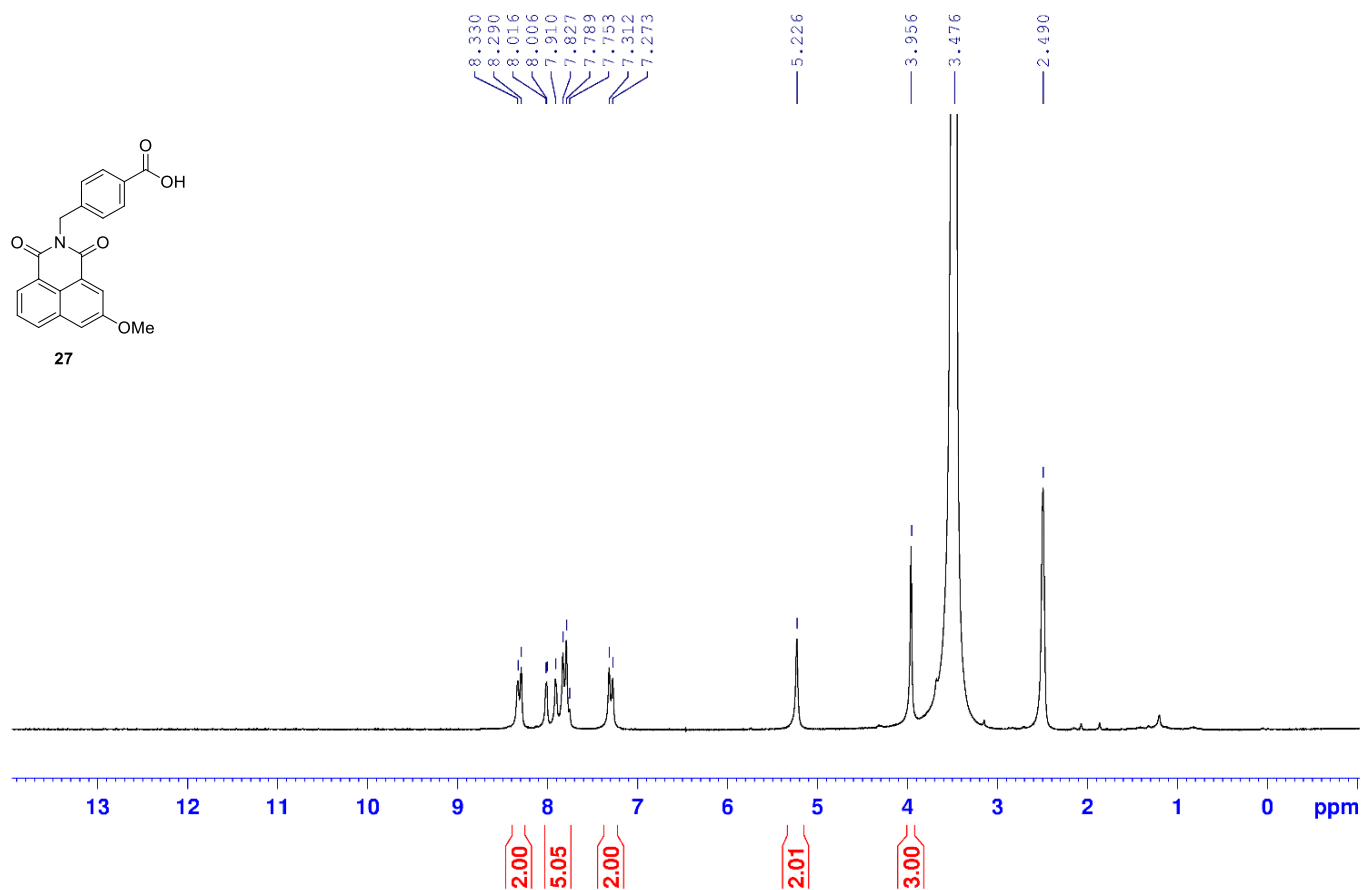
200 MHz ^1H NMR Spectrum of Compound **26** in $\text{DMSO}-d_6$



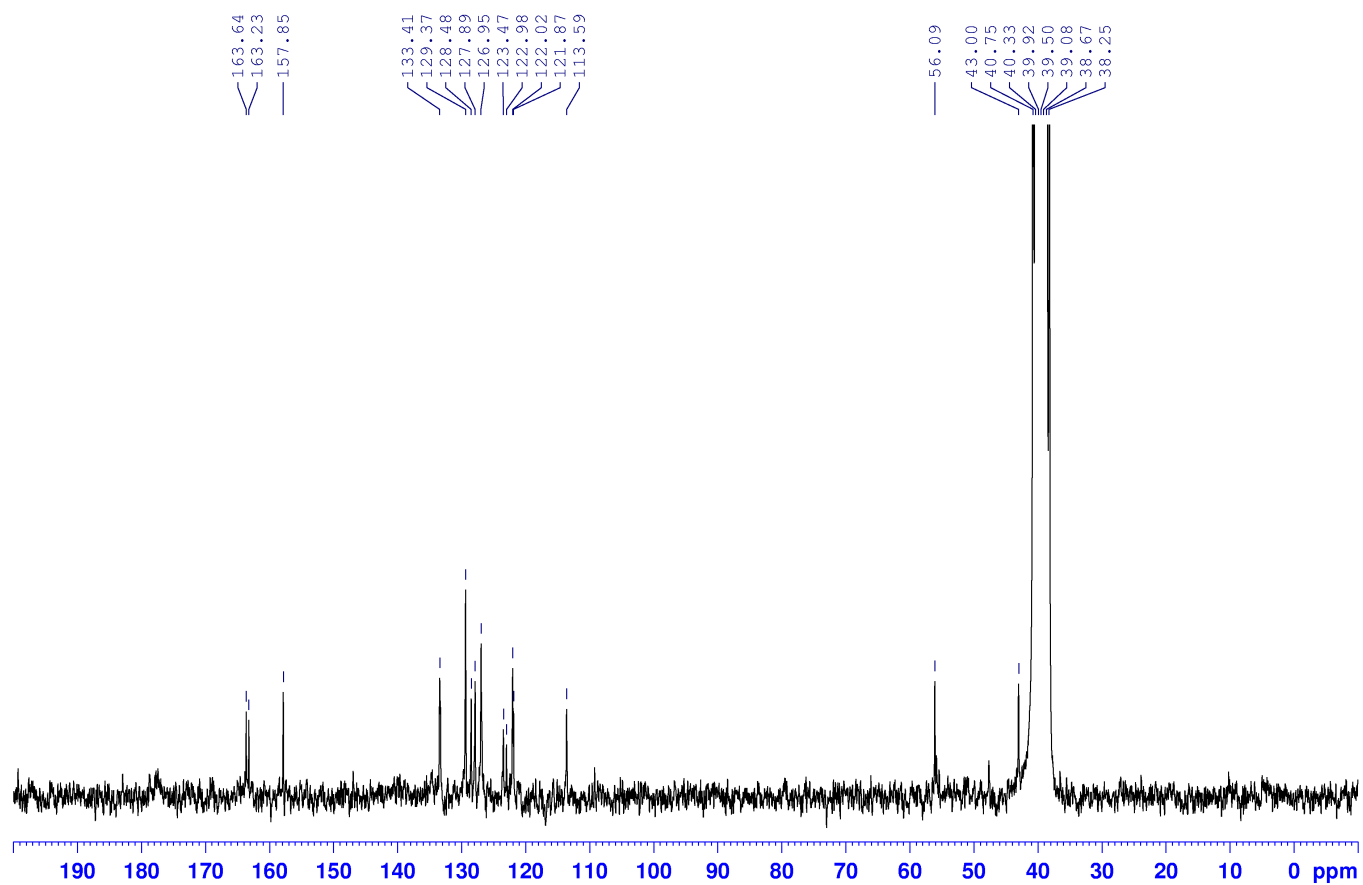
50 MHz ^{13}C NMR Spectrum of Compound **26** in $\text{DMSO}-d_6$



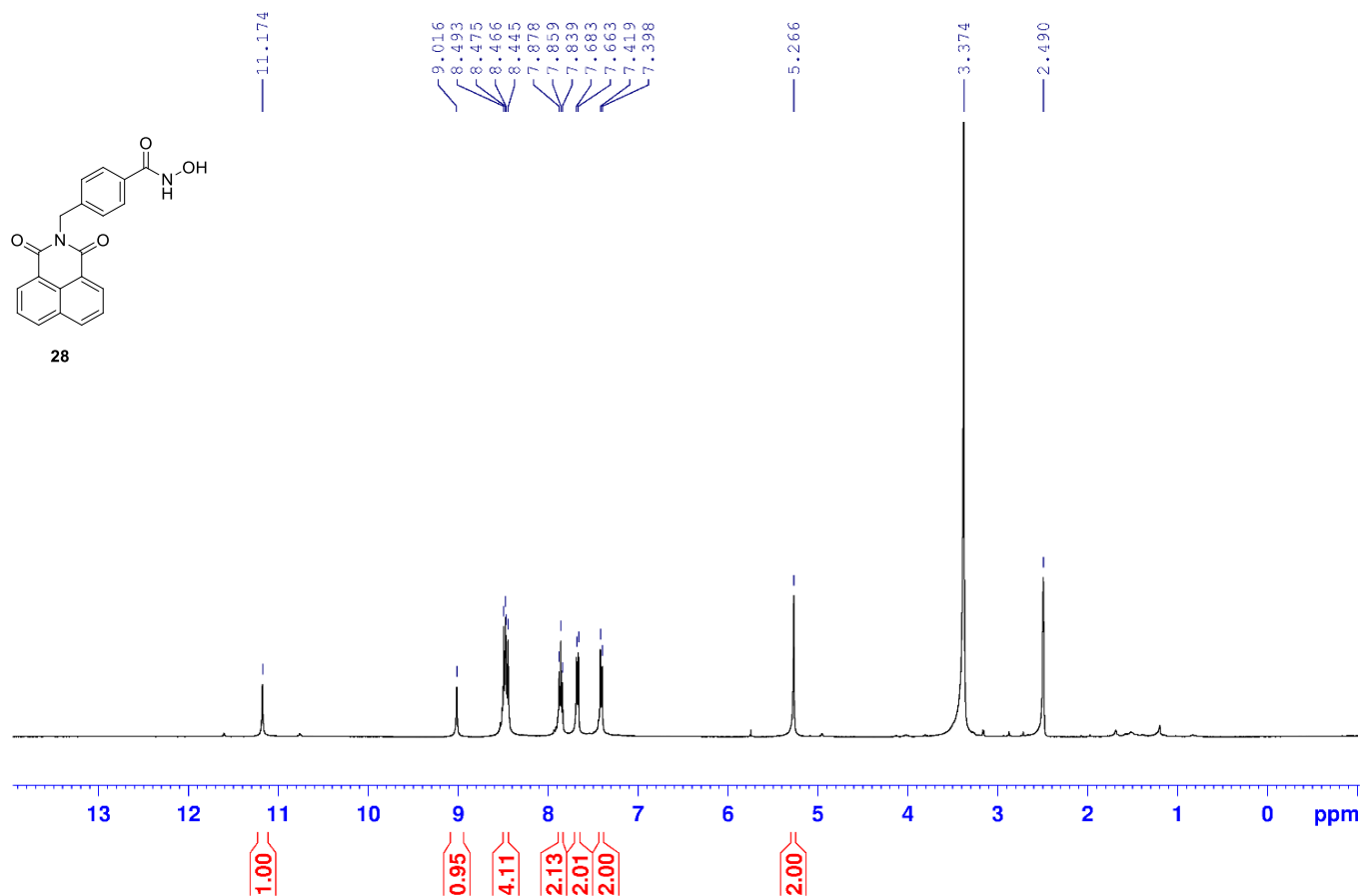
200 MHz ^1H NMR Spectrum of Compound **27** in $\text{DMSO}-d_6$



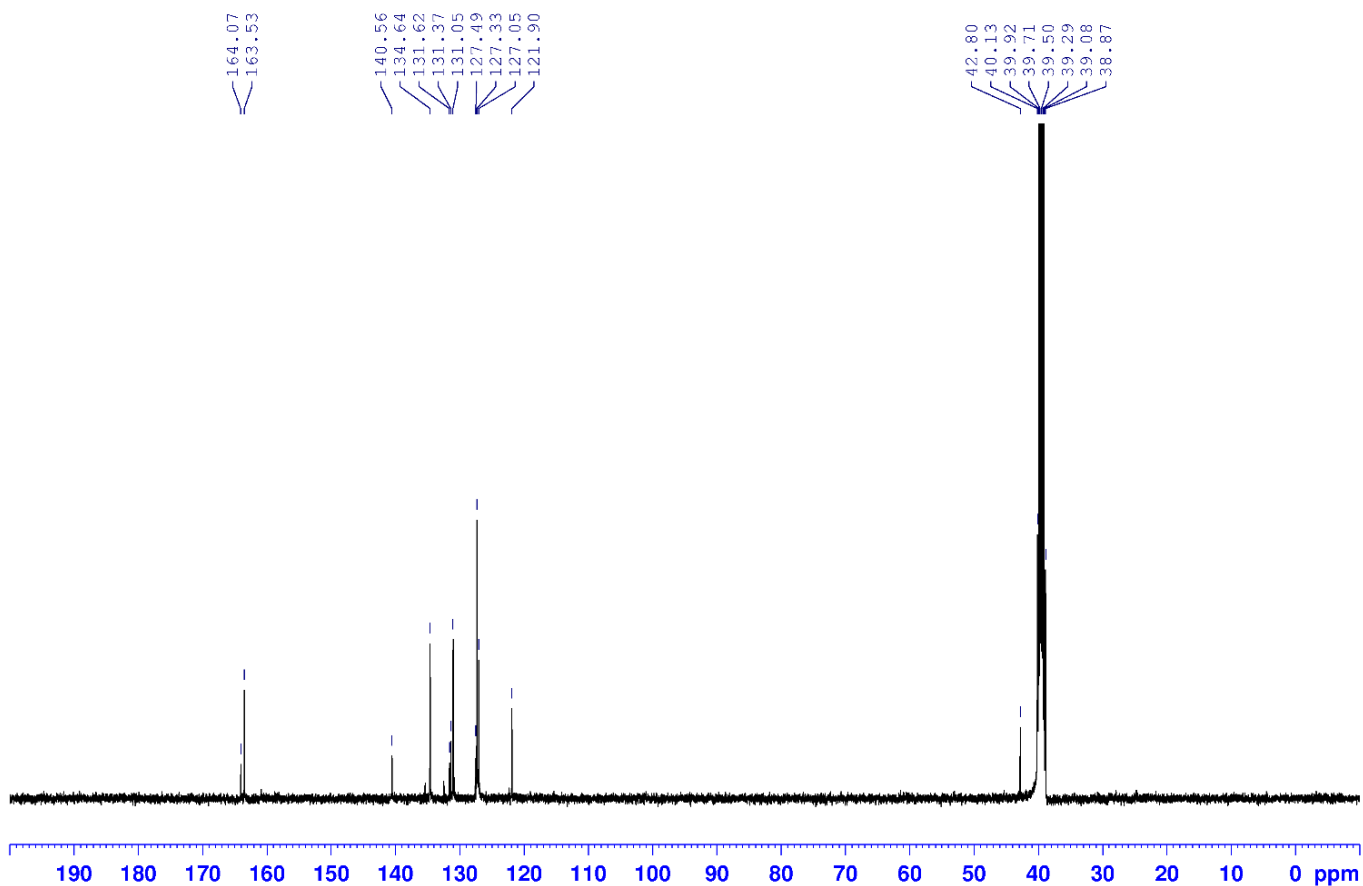
50 MHz ^{13}C NMR Spectrum of Compound **27** in $\text{DMSO}-d_6$



400 MHz ^1H NMR Spectrum of Compound **28** in $\text{DMSO}-d_6$



100 MHz ^{13}C NMR Spectrum of Compound **28** in $\text{DMSO}-d_6$



29

COc1ccc2c(c1)c3ccccc3c(=O)n(Cc4ccc(cc4)C(=O)NO)c2=O

¹H NMR spectrum (CDCl₃) of compound **29**. The spectrum shows peaks in the aromatic region (7.3-9.0 ppm), a singlet at 5.249 ppm, a singlet at 3.966 ppm, a singlet at 3.350 ppm, a singlet at 2.490 ppm, and a small peak at 1.167 ppm. Integration values are provided below the baseline.

Chemical Shift (ppm)	Integration
11.167	1.00
9.006	0.98
8.330	2.00
8.317	0.88
8.314	0.98
8.310	1.00
8.299	2.00
8.017	2.00
8.012	0.98
7.912	1.00
7.908	2.00
7.818	2.00
7.799	2.00
7.779	2.00
7.679	2.00
7.659	2.00
7.411	2.00
7.391	2.00
5.249	2.00
3.966	3.02
3.350	3.02
2.490	3.02
1.167	1.00

¹³C NMR spectrum (CDCl₃) of compound 10. The spectrum displays peaks corresponding to the following chemical shifts (ppm): 164.03, 163.54, 163.13, 157.75, 140.48, 133.29, 133.20, 131.63, 128.35, 127.75, 127.35, 127.03, 123.38, 122.92, 121.89, 121.79, 113.48, 55.98, 42.84, 40.13, 39.92, 39.71, 39.50, 39.29, 39.08, and 38.87.