

Complementation of Biotransformations with Chemical C-H Oxidation: Copper-Catalyzed Oxidation of Tertiary Amines in Complex Pharmaceuticals

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Supporting Online Material

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A. General methods

A.1. Biotransformations

A.1.a. Solutions

Substrates solutions: all substrates were added to the culture as stock solutions with a concentration of 4 mg/mL in MeCN. HPLC samples containing oxcarbazepine **67**, naproxen **68**, phthalazine **1**, and their respective metabolites were analyzed by LC/MS-MS analyses with assistance from the Novartis separations laboratory.

For the preparation of growth media, see reference 30. The biomass was produced accordingly to reference 31 and references therein.

Buffer solutions:

PSE-buffer

100 mM KH ₂ PO ₄	13.61 g/L
+ titrate with NaOH to adjust the pH of the solution	
0.5 M D-(+) sucrose	171.2 g/L
0.5 mM EDTA dihydrate	1.8 mg/mL

EDTA (cell permeabilizer)

EDTA dihydrate (100 mg/mL) was dissolved in deionized water and the pH was adjusted to 7.5. The solution was then filtered under sterile conditions and then stored at room temperature. It was added to the biotransformation reaction at final concentration of 5 mg/mL (250 μ L of the stock solution).

NADPH solution

NADPH (50 mg/mL) was dissolved in deionized water just before use, followed by a filtration under sterile conditions. The NADPH stock solution was added in 1.5 equiv. with respect to the substrate in the conversion assay (15 μ L of stock solution to each conversion assay).

NADPH regenerating system

NADP⁺ (8.6 mg) was dissolved in deionized water (500 μ L) in an Eppendorf® tube. 1 M sodium formate (500 μ L, 68.06 g/L) and FDH (160 μ L, 60 U/mL) were then added and the resulting mixture was incubated at 30 °C in the Eppendorf® mixer over 10 min. The NADPH regenerating system was freshly prepared before use and 500 μ L were added to each assay before starting the conversion assay.

A.1.b. Assays:

Determination of the cell density

The determination of the cell density was performed by measuring the optical density (OD) of the cell broth with a spectrophotometer (Shimadzu, UV-1601). The absorbance of the sample at 600 nm was measured against the absorbance of a reference containing the growth medium only. The value obtained was kept below 1.0 in order to stay in the linear range of the cell density/OD relation.

Determination of substrate conversion

The conversion of each substrate was analyzed by HPLC. The standard procedure entailed: An aliquot of the reaction mixture (500 μ L) was sampled out and quenched with AcCN (500 μ L) in an Eppendorf® tube. The Eppendorf® tubes were then put into an Eppendorf® mixer and agitated for 15 minutes. The tubes were centrifuged in a Heraeus Sepatech Biofuge 15 at 14000 rpm for 2 minutes. The supernatant was recovered and analyzed using an Agilent HPLC (1100 series, diode array detector - DAD) by injecting 10 μ L onto an octadecyl (C₁₈) reverse-phase HPLC column (Chromolith Performance 100-4.6mm with LiChrospher 100 RP-18 5 μ m particle size, Merck KgaA, Darmstadt, Germany). After HPLC analysis, the conversion yield was determined by comparison of the substrate and the metabolite peak areas detected at 220 nm. Before each analysis, the column was conditioned for 5 minutes with the starting elution mixture and at the end of each analysis the column was washed with 100 % AcCN for one minute.

A.2. Chemistry

When suitable, reactions were carried out in oven-dried glassware and under a nitrogen atmosphere using 8cc vials fitted with Teflon caps. Chromatographic purification of products was accomplished using neutral silica gel (Sigma, Activity grade I, type WN-3: neutral) HPLC reverse phase under basic conditions (see further down). Nuclear magnetic resonance (NMR) spectra were acquired on a Bruker Avance 400 spectrometer (400 MHz) unless stated otherwise. All ¹H NMR spectra are reported in parts per million (ppm) and were measured relative to the solvent signals at 7.26 ppm (CHCl₃) or 2.52 ppm (Me₂SO). All ¹³C NMR spectra were also reported in parts per million (ppm) and measured relative to the solvent signals at 77.1 ppm (CHCl₃) or at 40.4 ppm (Me₂SO). Data for ¹H NMR are described as follow: chemical shift (δ in ppm), multiplicity (s: singlet; d: doublet; t: triplet; q: quartet; quint: quintuplet; m: multiplet; br: broad signal), integration, coupling constant J (Hz). Data for ¹³C NMR spectra are described in terms of chemical shift (δ in ppm). All tested compounds were 95% purity (unless stated otherwise) as determined by LC/ESI-MS Data recorded using an Agilent 6220 mass spectrometer with electrospray ionization source and Agilent 1200 liquid chromatograph. The mass accuracy of the system has been

found to be < 5 ppm. When determined, crude ^1H NMR yields were obtained using dimethyl terephthalate (Aldrich, 99+ %) as an external standard. In practice, a known amount of dimethyl terephthalate in chloroform (100 mg/mL) was added to the reaction mixture following the quench with water.

B. Other compounds selected for the comparative study

Oxcarbazepine **67**, a generic anticonvulsant and mood stabilizing drug,¹⁰ naproxen **68**, a non-steroidal anti-inflammatory drug (NSAID),¹¹ and phthalazine **1** (see main text),⁹ were selected for the biotransformation/chemical C-H oxidation comparative study (Figure 1).

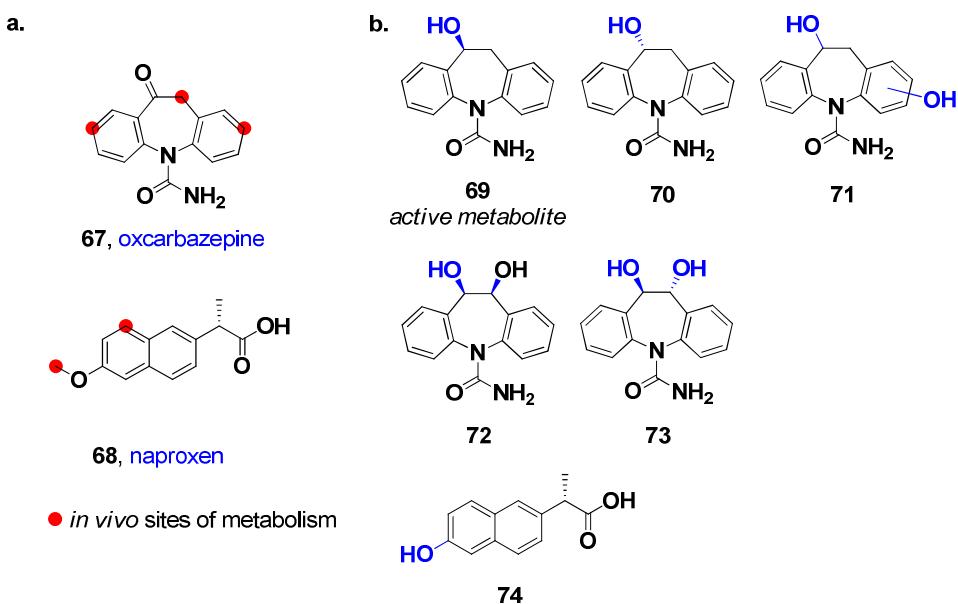


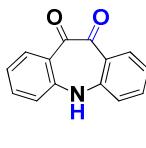
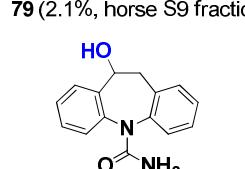
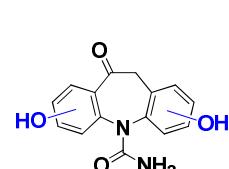
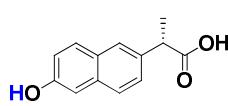
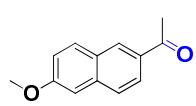
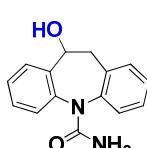
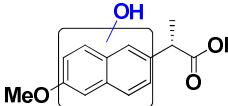
Figure 1. Selected compounds for biotransformation/chemical C-H oxidation comparative study; **a.** MetaSite prediction of metabolic soft spots; **b.** Known clinical phase I metabolites.

B.1. Analytical screen on oxcarbazepine and naproxen

Applying the biotransformation toolbox to oxcarbazepine **67**, three biotransformation products were formed in small amounts in the whole-cell biooxidation studies as established by UPLC-MS/MS (**75-77**) (Table 1). Benzylic oxidation product **75** (5.2%, CYP2C18), mono-aromatic hydroxylation product **76** (2.3%, CYP3A4), and decarbamoylation product **77** (0.6%, CYP3A5) were afforded. In an attempt to access the known clinical metabolites directly (Figure 1b), we also screened **67** with S9 fractions of 10 animal sources. This time, **78**, which may correspond to **69** or **70**, was observed in 57.4% along with 2.1% of a new mono-aromatic hydroxylated product **79** using horse S9 fraction.

When naproxen (**68**) was submitted to a similar protocol, only two minor biotransformation products were observed after the full biocatalyst screening; namely, *O*-

dealkylation product **74** (bacterially-expressed CYP102A7) and naphthol **80** (CYP3A4) were obtained in 8% and 2.4% yield respectively. Unfortunately, **80** could not be scaled up to support NMR structural elucidation studies and build MetaSite correlation. However, in the case of oxcarbazepine and naproxen, with the exceptions of **74** and perhaps **69/70**, biotransformation approaches did not directly yield the known clinical metabolites (Figure 1b).

drug	biotransformation product (yield, biocatalyst)	chemical C-H oxidation product (yield) ^a
67	 75 (5.2%, 2C18)	 81 (26%, Phl(OAc)_2) ^b
	 76 (2.3%, 3A4)	 82 (4%, $\text{FeSO}_4, \text{O}_2$) ^c
	 79 (2.1%, horse S9 fraction)	 83 (< 2%, $\text{FeSO}_4, \text{O}_2$) ^c
68	 77 (0.6%, 3A5)	 84 (26%, KMnO_4) ^d
	 78 = 69 or 70 (57.4%, horse S9 fraction)	
	 80 (2.4%, 3A4)	

^a Average of at least two isolated yields; ^b Phl(OAc)_2 (2 equiv.), AcOH , 100 °C, 2-16 h; ^c FeSO_4 (1 equiv.), Mn(OAc)_2 (1.5 equiv.), EDTA (1 equiv.), ascorbic acid (7 equiv.), O_2 , acetate buffer pH 4.5, 40-60 °C, 48 h; ^d KMnO_4 (5 equiv.), $t\text{-BuOH}$, 40 °C, 16 h.

Table 1. Comparative study between bio- and chemo-transformations using oxcarbazepine **67** and naproxen **68**.

B.2. Chemical C-H oxidations on oxcarbazepine and naproxen

The chemical conditions screened on the drugs are reported in “section C.2.”.

We started with oxcarbazepine (**67**), which contained a ketone and urea functionalities both potentially capable of directing the catalyst towards proximal C-H bonds (Table 1). Unfortunately, the directed C-H oxidation procedures described in section C.2.^{3,20b-e,20g-i,20n} did not deliver any sp^2 -hydroxylation product.³² Instead, acetoxylation of the benzylic C-H bond was accomplished (**81**, 26%) under the condition developed by Sanford et al albeit, in this case, the metal was not required.³³ The C-H borylation reaction also failed.^{20d,e} Other catalysts such as the Udenfriend system³ led to the formation of decarbamoylated product **82** (4%). In addition, formation of a complex mixture of aromatic hydroxylated products was observed using the Udenfriend system (**83**, <2%). The above same set of methods proved inefficient when applied to naproxen; except for the formation of **84** which was observed upon treatment of **68** with KMnO_4 , no other products were isolated. Finally, application of the

previously mentioned sp^3 -hydroxylation catalysts to **67** and **68** also failed to deliver the targeted products in all cases.

*The results discussed above and in the main text for phthalazine **1** served to illustrate the inability of the classical and recently developed C-H oxidation methods to effect oxidation of complex pharmaceutical compounds.*

C. Reactions conditions & results

C.1. Biotransformations (analytical screen)

All drugs (oxcarbazepine **67**, naproxen **68**, and phthalazine **1**) were incubated with various CYPs and S9 fractions. Monitoring was carried out after 1, 20, and 48 h for the CYPs and 6 h and 21 h for the S9 fractions. The proportion of metabolites formed is mentioned for each CYP enzyme/ S9 fractions used. The conversion of starting drug is also mentioned (Table 2-7).

Analytical conversion assay using rhCYP

A suspension of *E. coli* cells expressing the selected CYP isoform was diluted in PSE buffer to a constant wet biomass (100 mg/mL). The media solution (50 μ L) consisting of: DL-isocitrate (60 g/L), glucose (40 g/L), and lactose (40 g/L) in PSE buffer and the pre-dissolved substrate (12.5 μ L, 4 mg/mL) were then added to 500 μ L of cell suspension diluted as described before (Ethanol (50 μ L) was added to CYP 3A4 and 3A4b5). The Eppendorf cup was closed with an oxygen-permeable cotton stopper to ensure aeration (steristopper no. 10 from Herenz, Hamburg, Germany) and was then incubated in a temperature-controlled Eppendorf mixer at 30 °C and 1100 rpm (Eppendorf-Vaudaux- AG, Schönenbuch, Switzerland). The biotransformations were monitored after 4 h and 20 h when CYP expressed in *E. coli* were used and after 48 h for bacterial CYP. Monitoring was carried out by transferring a sample (100 μ L) of the solution into a 0.5 mL Eppendorf cup containing MeCN (100 μ L). The resulting mixture was shaken in a temperature-controlled Eppendorf mixer at (30 °C, 1100 rpm, 10 min) before being centrifuged (2 min, Eppendorf 5424R centrifuge). The supernatant was analyzed by HPLC and UPLC-MS/MS.

Analytical conversion assay using S9 fractions

The S9 fractions were prepared as described below (see reference 34).

A 1:1 mixture (mass ratio) of the liver and a 0.9 % sodium chloride solution is homogenized using a potter-Teflon-homogenizer before being centrifuged at 4 °C first at 6700 x g for 5 minutes and then at 17700 x g for 10 minutes. The supernatant homogenate is collected and frozen at –80 °C until use.

To an empty Eppendorf cup was added 0.1 M PSE pH 7.5 (200 μ L), the drug pre-dissolved in DMSO (10 μ L, 10 mM; final concentration of the drug in the assay: 0.22 mM), the selected S9 fraction (200 μ L), and the NADPH / isocitrate dehydrogenase (IDH) regeneration system (50 μ L, 10:1 ratio). The biotransformations were monitored after 6 h and 21 h by transferring a sample (100 μ L) of the solution into a 0.5 mL Eppendorf cup containing MeCN (100 μ L). The resulting mixture was shaken in a temperature-controlled Eppendorf mixer at (30 °C, 1100 rpm, 10 min) before being centrifugated (2 min, Eppendorf 5424R centrifuge). The supernatant was analyzed by HPLC and UPLC-MS/MS.

Preparative biotransformation with phthalazine 1

Phthalazine **1** (30 mg) pre-dissolved in MeCN (7.5 mL) was added to a mixture of media solution (150 mL, see analytical screen) and PSE buffer (150 mL). CYP3A4b5 expressed in *E. coli* (15 mL) was then added followed by ethanol (7.5 mL, representing 5% of the total volume). The biotransformations were monitored after 1 h by transferring a sample (200 μ L) of the solution into a 0.5 mL Eppendorf cup containing MeCN (200 μ L). The resulting mixture was shaken in a temperature-controlled Eppendorf mixer at (30 °C, 1100 rpm, 10 min) before being centrifugated (2 min, Eppendorf 5424R centrifuge). The supernatant was analyzed by HPLC and UPLC-MS/MS. An Amberlite® XAD® polymeric adsorbent resin (5 g) was added to the cultures and gently stirred with a mechanical stirrer (30 min) until no more substrates were detectable in solution (*via* HPLC analysis). The suspension was filtered and the drug / metabolites were extracted from the XAD with MeCN (100 mL) using gentle stirring (30 min). The extraction procedure was repeated as needed, and after decantation and separation of the phases, the combined organic layers was dried over Na_2SO_4 and evaporated to dryness *in vaccuo*. The crude was purified by HPLC to generate 5 metabolites. See main body of the publication.

		products of biotransformation			
		75	76	77	78
retentention time (min):		9.3	7.6	6.0	9.1
entry	CYP (% conversion)	after 1 h incubation			
1	3A4 (5)	2.1	2.3	0.1	-
2	3A4b5 (3)	0.9	1.5	0.6	-
3	3A5 (1)	0.6	0.2	0.2	-
4	1A1 (2)	1.8	0.2	-	-
5	1A2 (1)	1.2	0.2	-	-
6	1B1 (3)	2.8	-	-	-
7	2A6 (1)	1.0	-	-	-
8	2B6 (1)	1.0	-	-	-
9	2C8 (1)	1.3	-	-	-
10	2C9 (2)	2.5	-	-	-
11	2C18 (5)	5.2	-	-	-
12	2C19 (3)	2.8	-	-	-
13	2D6 (2)	1.5	-	-	-
14	2E1 (2)	2.0	-	-	-
15	4A11 (1)	1.0	-	-	-
16	Host (0)	-	-	-	-
after 20 h incubation					
17	3A4 (1.3)	1.3	-	-	-
18	3A4b5 (1)	0.3	-	0.5	-
19	3A5 (2)	1.1	-	0.6	-
20	1A1 (1.3)	1.3	-	-	-
21	1A2 (1.2)	1.2	-	-	-
22	1B1 (0)	-	-	-	-
23	2A6 (0)	-	-	-	-
24	2B6 (0)	-	-	-	-
25	2C8 (0)	-	-	-	-
26	2C9 (1)	0.9	-	-	-
27	2C18 (0)	-	-	-	-
28	2C19 (1)	-	-	-	0.7
29	2D6 (0.7)	0.7	-	-	-
30	2E1 (1.3)	1.3	-	-	-
31	4A11 (1.1)	1.1	-	-	-
32	Host (0)	1.3	-	-	-
microbial CYP		after 20 h incubation			
33	105D1 (0)	-	-	-	-
34	107D4 (0)	-	-	-	-
35	102A1 (0)	-	-	-	-
36	102A7 (0)	-	-	-	-
37	109B1 (0)	-	-	-	-
after 48 h incubation					
38	105D1 (0.4)	-	-	0.4	-
39	107D4 (0.3)	-	-	0.3	-
40	102A1 (0)	-	-	-	-
41	102A7 (0)	-	-	-	-
42	109B1 (0)	-	-	-	-

Table 2. Analytical screen using CYP on oxcarbazepine **67**

		products of biotransformation		
		75	78	79
retentention time (min):		9.3	9.1	8.6
entry	CYP (% conversion)		after 6 h incubation	
1	rabbit (32)		0.1	31.6
2	rat (4)		0.1	3.5
3	horse (60)		0.1	57.4
4	foal (38)		0.2	36.9
5	dog (9)		0.5	6.7
6	bovine (24)		0.1	22.6
7	pig (7)		0.1	6.6
8	sheep (12)		0.2	10.3
9	mouse (1)		0.2	0.7
10	poultry (2)		0.6	1.2
after 21 h incubation				
11	rabbit (42)		0.5	-
12	rat (0)		-	-
13	horse (37.3)		0.2	36.6
14	foal (47.2)		0.2	47.0
15	dog (0)		-	-
16	bovine (31)		-	28.5
17	pig (9.1)		-	9.1
18	sheep (16)		0.2	14.8
19	mouse (0)		-	-
20	poultry (1.4)		0.3	1.1

Table 3. Analytical screen using S9 fractions on oxcarbazepine **67**

For oxcarbazepine **67**, the results were analyzed with the following systems:

UPLC: Waters UPLC Acquity with PDA detector; Column: Waters HSS T3 C18; 1.8 μ m; 1.0 \times 150 mm; column temperature: 40 °C; mobile phase: A = H₂O/TFA 100:0.02, B = ACN/TFA 100:0.02; flow: 0.1 ml/min; Gradient (%B): 0 min 3% B, 12 min 40% B, 14 – 15.5 min 95% B; injection: 2 μ l (as received); detection: 220 nm (DAD 200 – 350 nm).

MS: Thermo LTQ Velos with h-ESI (+) inlet; Scan range: *m/z* 150 - 500 (5 μ -scans); normalized collision energy: 35% CID; isolation width: 2.8 Da, w/o wideband activation.

		products of biotransformation	
		74	80
retentention time (min):		8.7	10.7
entry		CYP (% conversion)	
1	3A4 (2.4)	-	2.4
2	3A4b5 (0)	-	-
3	3A5 (0)	-	-
4	1A1 (0)	-	-
5	1A2 (0)	-	-
6	1B1 (3.2)	3.2	-
7	2A6 (0)	-	-
8	2B6 (0)	-	-
9	2C8 (0)	-	-
10	2C9 (0)	-	-
11	2C18 (0)	-	-
12	2C19 (0)	-	-
13	2D6 (0)	-	-
14	2E1 (0)	-	-
15	4A11 (0)	-	-
16	Host (0)	-	-
after 1 h incubation			
17	3A4 (0)	-	-
18	3A4b5 (0)	-	-
19	3A5 (0)	-	-
20	1A1 (2)	1.7	-
21	1A2 (0)	-	-
22	1B1 (4.3)	4.3	-
23	2A6 (0)	-	-
24	2B6 (0)	-	-
25	2C8 (0)	-	-
26	2C9 (0)	-	-
27	2C18 (1)	0.9	-
28	2C19 (0)	-	-
29	2D6 (0)	-	-
30	2E1 (0)	-	-
31	4A11 (0)	-	-
32	Host (0)	-	-
microbial CYP		after 20 h incubation	
33	105D1 (0)	-	-
34	107D4 (0)	-	-
35	102A1 (0)	-	-
36	102A7 (8)	8	-
37	109B1 (0)	-	-
after 48 h incubation			
38	105D1 (0)	-	-
39	107D4 (2)	-	-
40	102A1 (0)	-	-
41	102A7 (0)	-	-
42	109B1 (0)	-	-

Table 4. Analytical screen using CYP on naproxen **68**

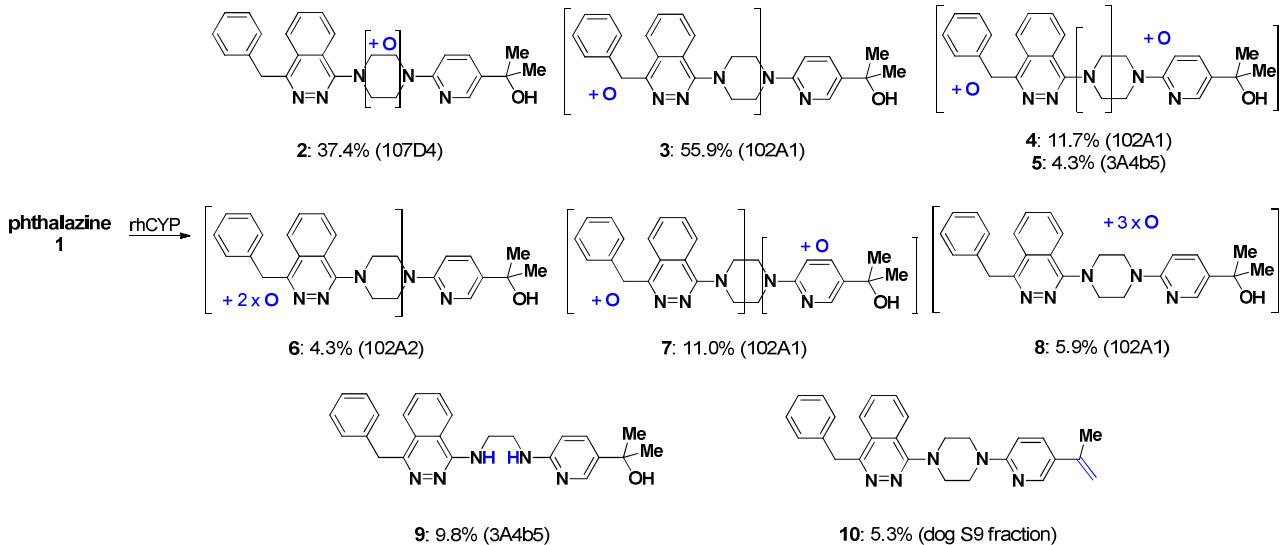
		products of biotransformation	
		74	80
retentention time (min):		8.7	10.7
entry		S9 fraction (% conversion)	
1	rabbit (2)	1.6	-
2	rat (0)	-	-
3	horse (2)	1.8	-
4	foal (0)	-	-
5	dog (0)	-	-
6	bovine (0)	-	-
7	pig (0)	-	-
8	sheep (0)	-	-
9	mouse (0)	-	-
10	poultry (0)	-	-
after 21 h incubation			
11	rabbit (0)	-	-
12	rat (0)	-	-
13	horse (2)	1.6	-
14	foal (0)	-	-
15	dog (0)	-	-
16	bovine (0)	-	-
17	pig (0)	-	-
18	sheep (0)	-	-
19	mouse (0)	-	-
20	poultry (0)	-	-

Table 5. Analytical screen using S9 fractions on naproxen **68**

For naproxen **68**, the results were analyzed with the following systems:

UPLC: Waters UPLC Acuity with PDA detector; column: Waters HSS T3 C18; 1.8 μ m; 1.0 x 150 mm; column temperature: 40 °C; Mobile phase: A = H₂O/TFA 100:0.02, B = ACN/TFA 100:0.02; flow: 0.1 ml/min; Gradient (%B): 0 min 5% B, 1 min 15% B, 16 min 55% B, 18 – 19 min 95% B; injection: 5 μ l (as received); detection: 220 nm (DAD 200 – 350 nm).

MS: Thermo LTQ Velos with h-ESI (+) inlet; Scan range: *m/z* 150 - 500 (5 μ -scans); normalized collision energy: 35% CID; isolation width: 2.8 Da, w/o wideband activation.



		products of biotransformation								
		2	3	4	5	6	7	8	9	10
retentention time (min):		10.7	11.9	9.2	9.4	10.2	10.5	7.8	12.7	16.5
	rhCYP (% conversion)	after 4 h incubation								
1	3A4 (41.2)	16.8	7.5	0.4	0.3	-	-	-	4.8	4.6
2	3A4b5 (71.8)	25.7	11.2	6.0	4.3	-	0.6	-	6.5	1.1
3	3A5 (47.9)	26.0	6.3	0.7	0.2	-	-	-	6.9	1.3
4	1A1 (24.6)	4.6	5.1	-	-	-	-	-	4.2	5.3
5	1A2 (22.6)	6.4	3.2	-	-	-	-	-	4.5	4.0
6	1B1 (22.6)	7.9	2.7	-	-	-	-	-	4.6	3.5
7	2A6 (24.1)	4.4	3.5	-	-	-	-	-	3.3	5.2
8	2B6 (16.9)	4.1	2.4	-	-	-	-	-	3.8	2.8
9	2C8 (23.2)	5.9	3.8	-	-	-	-	-	4.2	3.5
10	2C9 (23.6)	10.0	2.6	-	-	-	-	-	3.9	2.6
11	2C18 (26)	10.9	2.6	-	-	-	-	-	4.2	2.9
12	2C19 (23.5)	7.5	3.0	-	-	-	-	-	4.6	3.9
13	2D6 (20.1)	7.9	2.3	-	-	-	-	-	3.8	2.7
14	2E1 (21.1)	8.4	2.7	-	-	-	-	-	3.9	2.6
15	4A11 (20.9)	7.0	2.9	-	-	-	-	-	4.0	3.2
16	Host (19.3)	4.6	3.0	-	-	-	-	-	4.2	3.2
		after 20 h incubation								
17	3A4 (48.6)	18.4	8.1	0.7	0.5	-	-	-	7.0	5.1
18	3A4b5 (76.4)	22.8	11.4	6.1	3.9	-	-	-	9.8	0.7
19	3A5 (51.8)	25.2	9.7	1.2	0.4	-	-	-	5.4	2.1
20	1A1 (26.2)	3.8	5.2	0.1	-	-	-	-	4.0	6.2
21	1A2 (22)	7.7	3.1	-	-	-	-	-	4.7	1.8
22	1B1 (30.3)	9.4	3.4	0.1	0.5	-	-	-	5.5	5.3
23	2A6 (26.6)	4.2	3.4	-	-	-	-	-	3.9	7.1
24	2B6 (25.9)	2.8	3.6	-	-	-	-	-	4.7	7.6
25	2C8 (25.7)	6.1	4.7	-	-	-	-	-	4.4	3.5
26	2C9 (22)	5.0	2.8	-	-	-	-	-	4.8	3.7
27	2C18 (4.5)	1.2	0.8	-	-	-	-	-	0.8	1.3

28	2C19 (29.8)	7.7	3.6	-	-	-	-	-	5.8	6.4
29	2D6 (27.7)	5.7	3.5	-	-	-	-	-	4.7	6.7
30	2E1 (25.7)	8.1	3.3	-	-	-	-	-	4.6	4.6
31	4A11 (27)	5.8	3.6	-	-	-	-	-	4.7	6.6
32	Host (26.5)	5.2	3.1	-	-	-	-	-	4.6	6.7
microbial CYP		after 20 h incubation								
33	105D1 (26.8)	3.7	3.9	-	-	-	-	-	4.7	7.6
34	107D4 (51.6)	35.7	1.8	1.0	-	-	-	-	6.9	2.4
35	102A1 (72.4)	0.6	55.4	4.6	-	1.3	8.8	-	-	0.6
36	102A7 (29.2)	3.9	4.0	-	-	-	-	-	5.3	6.5
37	109B1 (27.4)	5.3	3.9	-	-	-	-	-	4.9	6.4
after 48 h incubation										
38	105D1 (20.1)	9.2	1.3	-	-	-	-	-	2.5	2.4
39	107D4 (45.7)	20.3	2.3	0.7	-	-	-	-	9.8	5.2
40	102A1 (83.9)	2.8	26.8	11.7	-	4.3	11.0	5.9	0.5	0.9
41	102A7 (24.1)	4.5	2.9	0.1	-	-	-	-	3.0	5.9
42	109B1 (26.9)	4.4	3.2	-	-	-	-	-	4.5	7.0

Table 6. Analytical screen using CYP on phthalazine **1** (only metabolites formed in >2% are shown); MS/MS experiments was used to refine the areas of oxidations.

		products of biotransformation									
		+ 0				+ 2 O			+ 3 O - 2H	open piperazine	olefin
entry	S9 origin (% conversion)	after 6 h incubation									
		M ₁	M ₂	M ₃	M ₄	M ₅	M ₆	M ₇	M ₈	M ₉	M ₁₀
retention time (min):		10.7	11.7	10.3	11.3	9.4	9.2	8.3	11.4	12.3	16.5
1	control (8.9)	0.8	1.0	-	-	-	-	-	1.0	0.5	1.7
2	rabbit (54.7)	9.2	17.4	0.6	2.1	1.8	2.6	0.2	2.1	1.1	2.9
3	rat (13.3)	1.8	1.6	0.2	0.2	0.1	0.0	0.0	1.6	0.6	2.3
4	horse (17.3)	2.7	2.8	0.6	0.4	0.5	0.2	0.9	1.4	0.9	2.4
5	foal (25.7)	3.5	3.1	0.7	0.2	0.7	0.1	1.2	2.4	1.5	5.0
6	dog (39.6)	8.0	5.1	2.8	0.9	1.4	0.4	0.1	4.5	3.3	5.3
7	bovine (34.5)	7.8	5.2	1.6	1.1	1.8	1.0	1.9	2.0	0.7	3.0
8	pig (20.7)	3.9	3.1	0.4	0.2	0.8	0.2	0.2	2.1	1.0	3.3
9	sheep (36.9)	2.5	11.3	1.9	0.9	2.6	0.8	3.5	1.1	0.3	2.5
10	mouse (21.3)	4.2	3.9	0.7	1.1	0.3	0.4	0.4	1.4	0.4	2.5
11	poultry (18.1)	2.0	-	0.1	0.2	0.0	0.1	0.0	2.7	1.1	3.9

Table 7. Analytical screen using S9 fractions phthalazine **1** (only metabolites formed in >2% are shown)

Notes:

- Over 15 other metabolites were also detected in small amount (< 2%, not shown), which were accounted for the rest of converted phthalazine. These metabolites include compound **12** as well as other mono, bis, and tris-oxidation products containing either hydroxyl or carbonyl functionalities or both.
- The structures of **M₁₋₆** were not investigated further.

- Despite a slight shift in retention time, LC-MS/MS analysis confirmed that **M₁**, **M₂**, **M₉**, and **M₁₀** correlated with metabolites **2**, **3**, **9**, and **10** respectively.

For phthalazine **1**, the results were analyzed with the following systems:

UPLC: Waters UPLC Acuity with PDA detector; column: Waters Acuity UPLC HSS T3; 1.7 μ m; 1.0 \times 150 mm; column temperature: 60 °C; Mobile phase: A = H₂O + 0.02% TFA, B = AcN + 0.02% TFA; flow: 0.1 ml/min; gradient (%B): 1 min 3% B, 16 min 35% B 17 min 98 % B 20 min 98% B; equilibration time: 7 min; injection: 2 μ l (as received); detection: PDA (210 – 350 nm).

MS: LTQ Velos, Thermo-Scientific, San Jose; Scan range: m/z 150 - 500 (5 μ -scans); MS_n experiments: data dependent scan: MS2: isolation width 1.6 Da; collision energy 40%; wideband activation MS3: isolation width 2.0 Da; collision energy 40%; wideband activation.

C.2. Chemistry

The reactions conditions investigated for the drugs **1**, **67**, and **68** are listed below. The references corresponding to these methodologies can be found in the main text.²⁰ The procedures were followed as described in the original publications. In addition, when no products would be obtained, minor variations were also attempted (temperature increase, reaction time, solvents, equivalents) to see if any product could be formed.

- Cu(OAc)₂ (10 mol%), O₂ (balloon), AcOH:Ac₂O (1:1), 120 °C, 16 h.^{20b}
- AuCl₃ (10 mol%), PhI(OAc)₂ (2 equiv.), DCE, 80 °C, 2-18 h.^{20c}
- FeSO₄ (1 equiv.), Mn(OAc)₂ (1.5 equiv.), EDTA (1 equiv.), ascorbic acid (7 equiv.), O₂, acetate buffer pH 4.5, 40-60 °C, 2-48 h.³
- [Fe(S,S)-PDP] (15 mol%), AcOH (1.5 equiv.), 30% H₂O₂ (3.6 equiv.), MeCN, r.t., 2-18 h.^{2a}
- KMnO₄ (1-5 equiv.), various solvents (e.g. *t*-BuOH, DCM), r.t. to 40 °C, 2-24 h.^{20f}
- Pd(OAc)₂ (5 mol%), PhI(OAc)₂ (2 equiv.) or oxone (2 equiv.), AcOH or MeCN or MeOH, 50-100 °C.^{20h,i}
- Pd(OAc)₂ (10 mol%), O₂ (balloon), benzoquinone (1 equiv.), KOAc (2 equiv.), DMA, 120 °C.^{20g}
- [Ir(OMe)(cod)]₂ or [IrCl(cod)]₂ (1.5-20 mol%), B₂pin₂ (1-2 equiv.) / dtbpy (40 mol%), THF or heptane, 60 °C.^{20d,e}
- K₂PtCl₄ (5 mol%), CuCl₂ (7 equiv.), H₂O, 100-160 °C, 24 h.^{20j}
Note: Condition tested on naproxen only.
- RuCl₃.H₂O (5 mol%), KBrO₃ (3 equiv.), pyridine (0.1 equiv.), MeCN:H₂O:MeOAc (various ratios), 60 °C, 2-18 h.^{20k}
- MeReO₃ (20 mol%), H₂O₂ (25 equiv.), *t*-BuOH, 40-60 °C, 2-18 h.^{20l}
- Ag(II) picolinate (2.5 equiv.), H₂O:TFA (10:1), 50 °C, 2-18 h.^{20m}
- [NO(SO₃)₂]K₂, various solvents (e.g. acetone, THF) / phosphate buffer (various ratios).²⁰ⁿ

D. Cu-mediated oxidation

D.1. General procedure

Procedure using molecular oxygen:

The substrates were dissolved in DMSO (0.1 M) and CuI (20 mol%) was added to the solution in one portion under atmosphere of oxygen (balloon). The resulting mixture was heated to 120 °C over 8 to 16 h under vigorous stirring. The reaction was monitored by LCMS and was quenched with water and extracted with EtOAc (x 3). The combined organic layers were washed with water (x 5), dried over MgSO₄, and in vaccuo. The crudes were purified by HPLC reverse phase under basic or acidic conditions.

Procedure under air:

The substrates were dissolved in DMSO (0.3 M) and CuI (20 mol%) was added to the solution in one portion at room temperature. The reactions were heated to 120 °C under vigorous stirring over 16 h under air (without balloon). The reaction was monitored by LCMS and was quenched with water and extracted with EtOAc (x 3). The combined organic layers were washed with water (x 5), dried over MgSO₄, and in vaccuo. The crudes were purified by HPLC reverse phase under basic or acidic conditions.

D.2. General notes

- Amine substrates were free-based prior use since the presence of acids appeared to impact yields and product stability in some instances.
- Oxygen was bubbled through DMSO for 15-20 minutes prior use.
- In some instances (of product solubility issues), the crude mixtures were quenched with H₂O, filtered, and purified directly by HPLC reverse phase under basic or acidic conditions.
- Reactions that stopped or with a slow conversion under air (closed vials, no balloons) could be carried out in opened-vials (no caps) to reach full conversion.

D.3. Human metabolites of the generic drugs

When the human metabolites were not known, MetaSite was used to predict the site of metabolism (i.e. compounds **28** and **32**). For the human metabolites of the following generic drugs, see: chloropheniramine,³⁵ aripiprazole,³⁶ trazodone,³⁷ and levofloxacin.³⁸

E. References

The references below continue the numbering of the main body of the publication.

- 30 Kittelmann, M.; Laumen, K.; Lütz, S.; Kuhn, A.; Riepp, A.; Kuehnoel, J.; Fredenhagen, A.; Oberer, L.; Ghisalba, O. *Practical Methods for Biocatalysis and Biotransformations* (Wiley, John Wiley & Sons Ltd, England, Vol. 2, 138-147, 2012).
- 31 Hanlon, S. P.; Friedberg, T.; Wolf, C. R.; Ghisalba, O.; Kittelmann, M. *Modern Biooxidation*, (Wiley-VCH; Weinheim, Germany, 233-252, 2007).
- 32 For a review on transition-metal-catalyzed hydroxylation of arenes see: (a) Alonso, D. A.; Nájera, C.; Pastor, I. M.; Yus, M. *Chem. Eur. J.* **2010**, *16*, 5274-5284; For a review on enzymatic hydroxylation of arenes see: (b) Ullrich, R.; Hofrichter, M. *Cell. Mol. Life Sci.* **2007**, *64*, 271-293.
- 33 This result is analogous to older reports on the α -acetoxylation/-hydroxylation of benzoketones using hypervalent iodine: (a) Mizukami, F.; Ando, M.; Tanaka, T.; Imamura, J. *Bull. Chem. Soc. Jap.* **1978**, *51*, 335-336; (b) Moriarty, R. M.; Berglund, B. A.; Penmasta, R. *Tetrahedron Lett.* **1992**, *33*, 6065-6068; (c) For a perspective article on organohypervalent iodine chemistry, see: Moriarty, R. M. *J. Org. Chem.* **2005**, *70*, 2893-2903.
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- 36 Wood, M. D.; Scott, C.; Clarke, K.; Westaway, J.; Davies, C. H.; Reavill, C.; Hill, M.; Rourke, C.; Newson, M.; Jones, D. N. C.; Forbes, I. T.; Gribble, A. *Eur. J. Pharmacol.* **2006**, *546*, 88-94.
- 37 Rotzinger, S.; Fang, J.; Baker, J. B. *Drug Metab. Disp.* **1998**, *26*, 572-575.
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F. Products characterization

F.1. Mini-prep biotransformation (using rhCYP3A4b5)

In vitro incubation of 30 mg phthalazine **1** with rhCYP3A4b5 was conducted to isolate two major metabolites (**11** and **12**) on milligram scale *via* preparative HPLC/UV (see further down for characterization details). A mixture of other metabolites collected in this purification was analyzed by capillary HPLC/MS (Figure 4 and 5) and further purified for micro-scale isolation and full structural identification. The identification of metabolites was performed by interpretation of the product ion spectra obtained by HPLC/MSⁿ experiments. A range of 6-7 metabolites were detected with some in the lower microgram range, which was sufficient for an isolation using a micro-preparative HPLC-SPE/MS. Three metabolites could be isolated (**13**, **14**, and **15** also referred below as m456a, m456b, and m472c): two monohydroxy metabolites and one dihydroxy metabolite of phthalazine **1** were resulting in 40-200 µg of the respective metabolite at a purity of >90% based on HPLC/MS data. Structure elucidation was conducted *via* NMR analysis by different homo- and heteronuclear 2D NMR experiments and determination of ¹H and ¹³C chemical shifts. Representative HPLC/MS chromatograms (+EIC: Extracted Ion Chromatogram; +BPC: Base Peak Chromatogram), +ES product ion spectra and NMR spectra (¹H ROESY, ¹H NMR) are shown below.

The conditions used for the Isolation and characterization of metabolites **13**, **14**, and **15** are mentioned below.

<i>Analytical method</i>	Micro-preparative HPLC/MS		
<i>Mass Spectrometry</i>	LTQ XL	FC	Programmed time cutoff
<i>Preparative method</i>	MicroPrep-HPLC-SPE/MS (MetID, MAP/BS)		
<i>Sample preparation</i>	<ul style="list-style-type: none"> • Filtration through 0.45 µm PVDF membrane and centrifugation at 4000 g for 15 min at +10°C of CYP3A4b5 sample (5.5 mL) • Evaporation of organic layer under vacuum and N₂ stream at +40°C for 40 min (Cyclone) • Centrifugation of the residue after mixing at 20000 g for 10 min at +10°C • Pre-dilution of the supernatant sample 1:2 in H₂O • Solid Phase Extraction on Oasis HLB 200 mg sorbent (Waters) by conditioning with 4 mL MeOH; equilibration with 4 mL H₂O; sample load (2x cartridges, 8 mL); wash with 2 x 4 mL H₂O/MeOH (95/5) and elution with 4 mL MeOH/AcN (50/50) • Evaporation of extracted sample to dryness under vacuum and N₂ stream at +40°C for 40 min (Cyclone) • Reconstitution in 0.5 mL H₂O/MeOH (85/15) 		
<i>MS</i>	LTQ XL (Thermo Fisher), +ESI FS 200-1000 amu/sec, CID fragmentation, norm. coll. energy : 40%, Wide Band Activation for MS/MS exp.		

<i>HPLC column / temp.</i>	XBridge BEH130 C18 3.5 μ m, 4.6 x 150 mm (Waters) / +30°C
<i>Mobile phases</i>	H_2O , + 1% HCOOH (A) AcN/MeOH (50/50) (B)
<i>Gradient</i>	5-95% B / 16 min at 0.7 mL/min separation step: 25-45% B / 5-15 min at 0.5 mL/min (Prominence-UFLC, Shimadzu)
<i>LC injection</i>	20 μ L loop injection: n= 30 (SIL-20AC, Shimadzu)
<i>Post column dilution</i>	H_2O at 2.0 mL/min (Prominence-UFLC, Shimadzu)
<i>Trapping material</i>	Oasis HLB 5 μ m, 4.6 x 20 mm (Waters)
<i>Switching system</i>	Pre-conditioned with MeOH and equilibrated with H_2O at 2 mL/min for 3 min respectively Divert valve 6-port bi-position (time cutoff) Selector valve 8-port multi-position (trapping)
<i>Fraction collection</i>	Multi-trapping from programmed time cutoff window
<i>Analyte work-up</i>	Desorption of isolated metabolites from each trapping columns by: <ul style="list-style-type: none"> • Wash of acid with H_2O at 1 mL/min for 10 min • Flush with N_2 flow for 10 min at 6 bars • Elution with 4 mL AcN/MeOH (50/50) in a glass-tube • Evaporation to dryness under vacuum and N_2 stream at +40°C for 60 min (Cyclone) in a 1.5 mL pre-cleaned high recovery screw glass-vial (National Scientific) The dried vials were filled with Argon, closed with a parafilm and delivered to AS/NMR laboratory
<i>Fraction control</i>	Capillary HPLC/MS ^a (MetID, MAP/BS) Check of purity and amount of isolated metabolites
<i>MS</i>	LTQ XL (Thermo Fisher), +CSI (Captive Spray) CID fragmentation, nomn. coll. energy : 40%, Wide Band Activation for MS/MS exp.
<i>HPLC column / temp.</i>	Reprosil Pur ODS3 C18 3 μ m, 0.3 x 150 mm (Maisch) / +40°C
<i>Mobile phases</i>	$\text{H}_2\text{O}/\text{AcN}$ (95/5), + 0.1% HCOOH + 0.02% TFA (A) $\text{H}_2\text{O}/\text{AcN}/\text{MeOH}$ (5/47.5/47.5), + 0.1% HCOOH + 0.02%TFA (B)
<i>Gradient</i>	5-95% B / 25 min at 4.5 μ L/min (Chorus-220)
<i>LC injection</i>	2 μ L loop injection (LC-PAL, CTC Analytics)
<i>Structure elucidation</i>	NMR analysis (AS/NMR)
<i>NMR</i>	Avance 600 with 1.7mm TXI micro-cryoprobe (Bruker)
<i>Solvent</i>	DMSO- D_6
<i>Experiments</i>	¹³ C HMBC, ¹³ C HSQC, TOCSY, ROESY, ¹ H

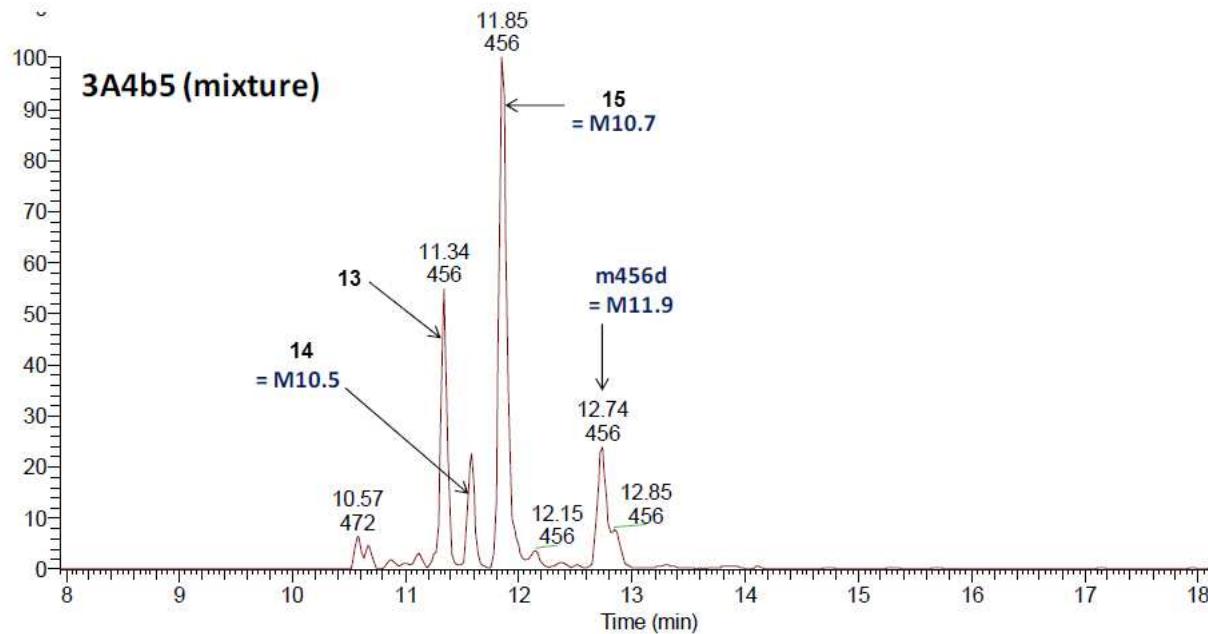
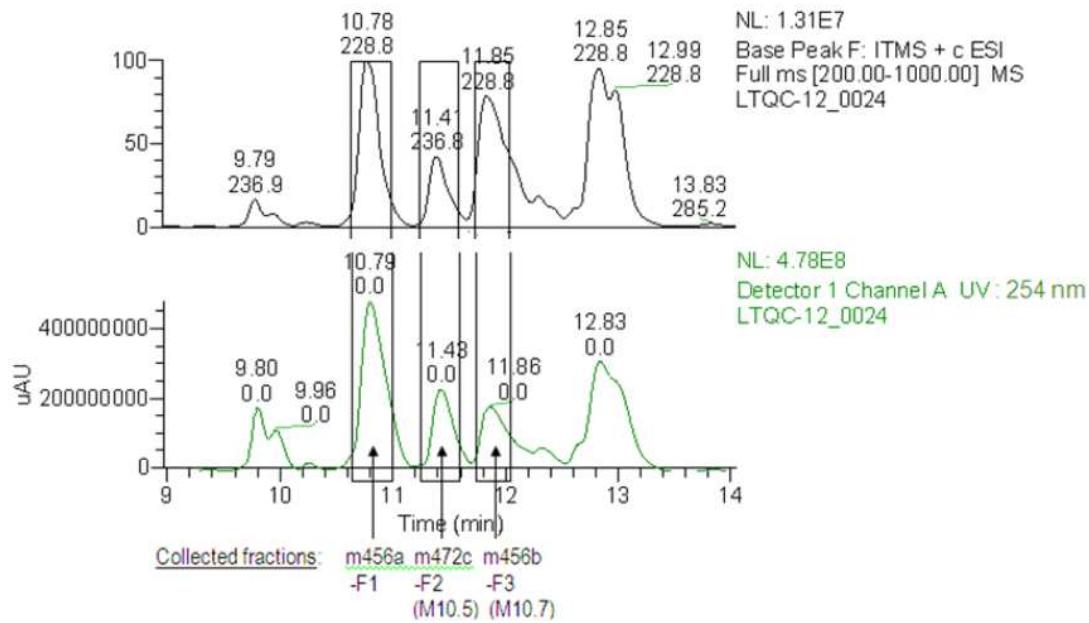


Figure 2. +EIMS chromatogram of a mixture of metabolites analyzed by capillary HPLC/MS



Notes: Ions detected at m/z 228.8 and 236.8 in the BPMS chromatogram correspond to the predominant doubled charged ions $[M+2H]^{2+}$ of respective metabolites (m/z 456 and m/z 475) observed in the MS full scan

Figure 3. +BPMS/UV chromatogram of the bioreaction mixture and isolation of **13**, **14**, and **15** via preparative HPLC-SPE/MS.

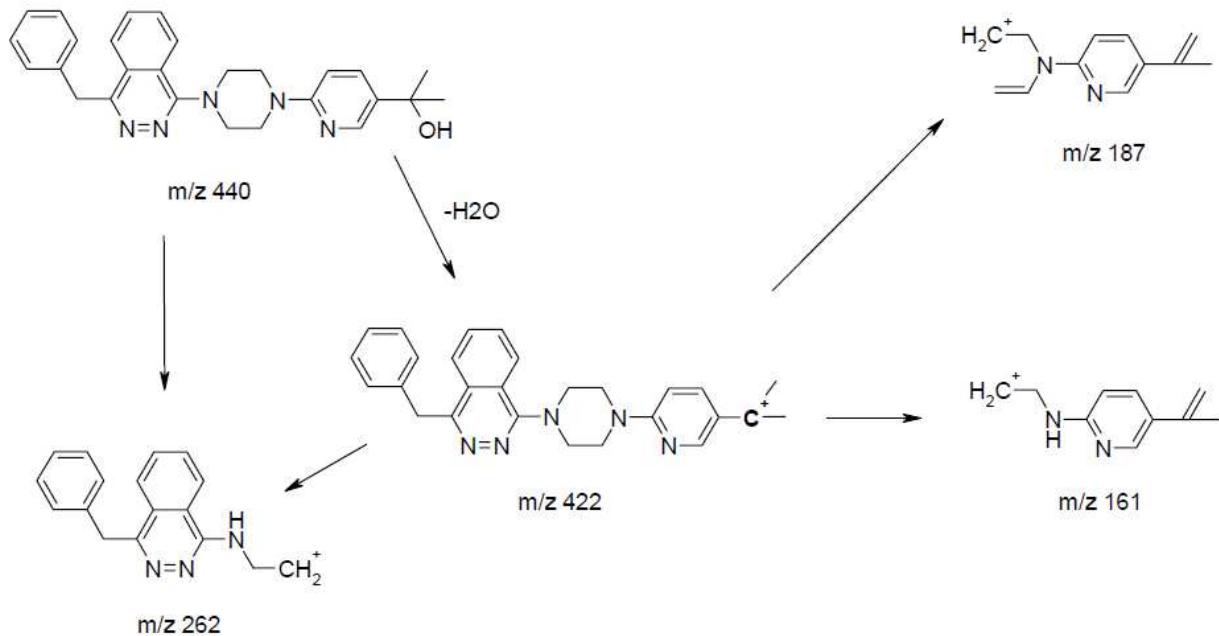
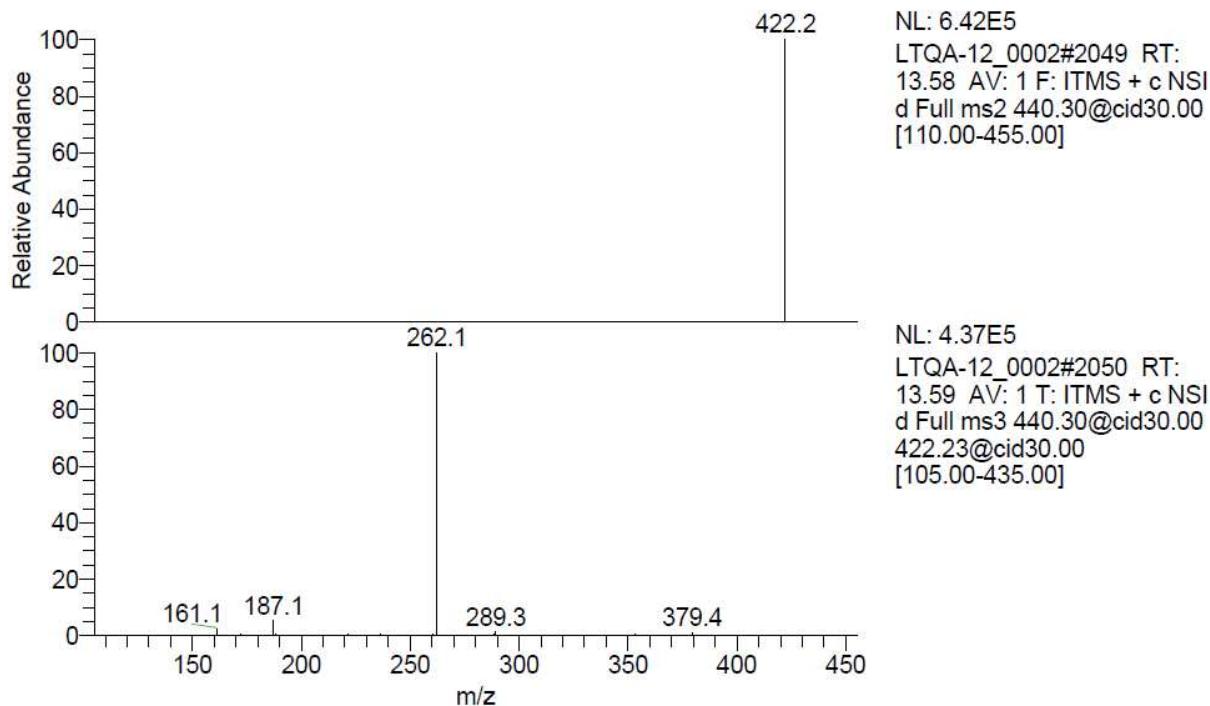


Figure 4. +ESMS ion spectrum for parent phthalazine **1** ($M+H^+$ 456 m/z) and proposed MS^n fragmentations.

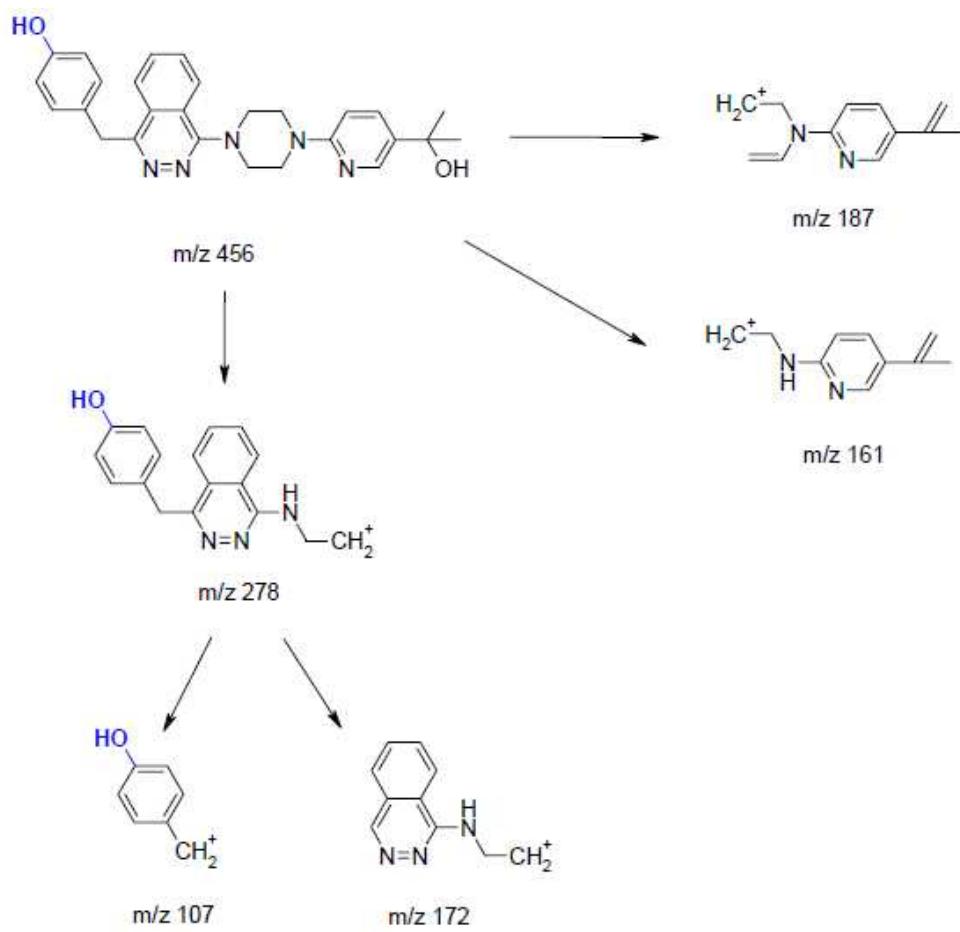
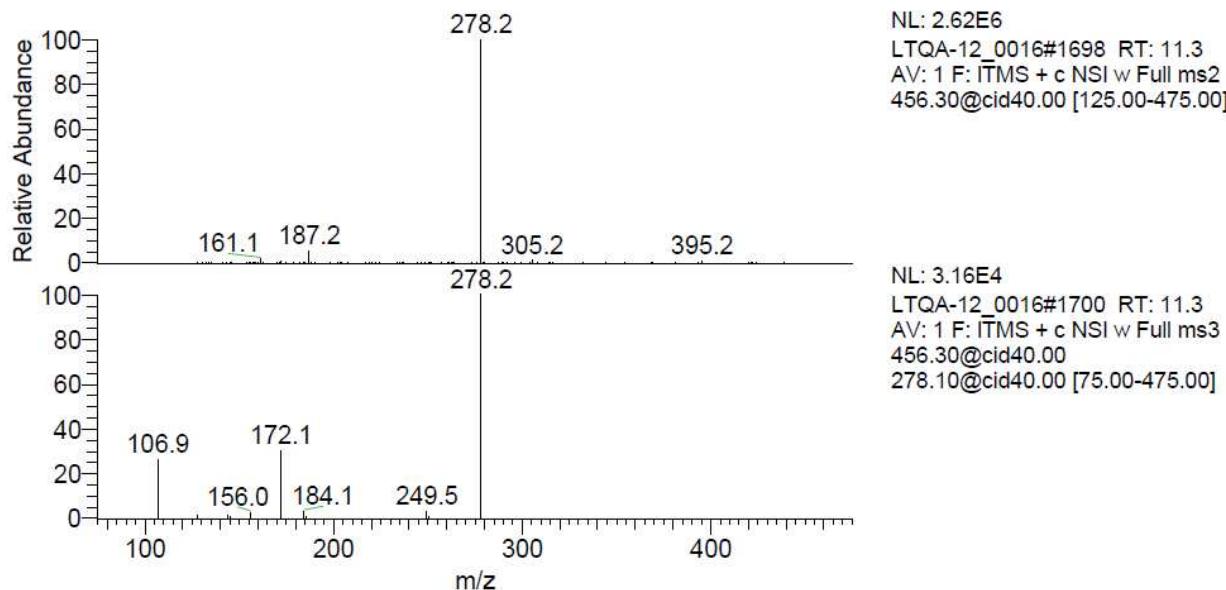


Figure 5. +ESMS ion spectrum for metabolite **13** ($M+H^+$ 456 m/z) and proposed MS^n fragmentations.

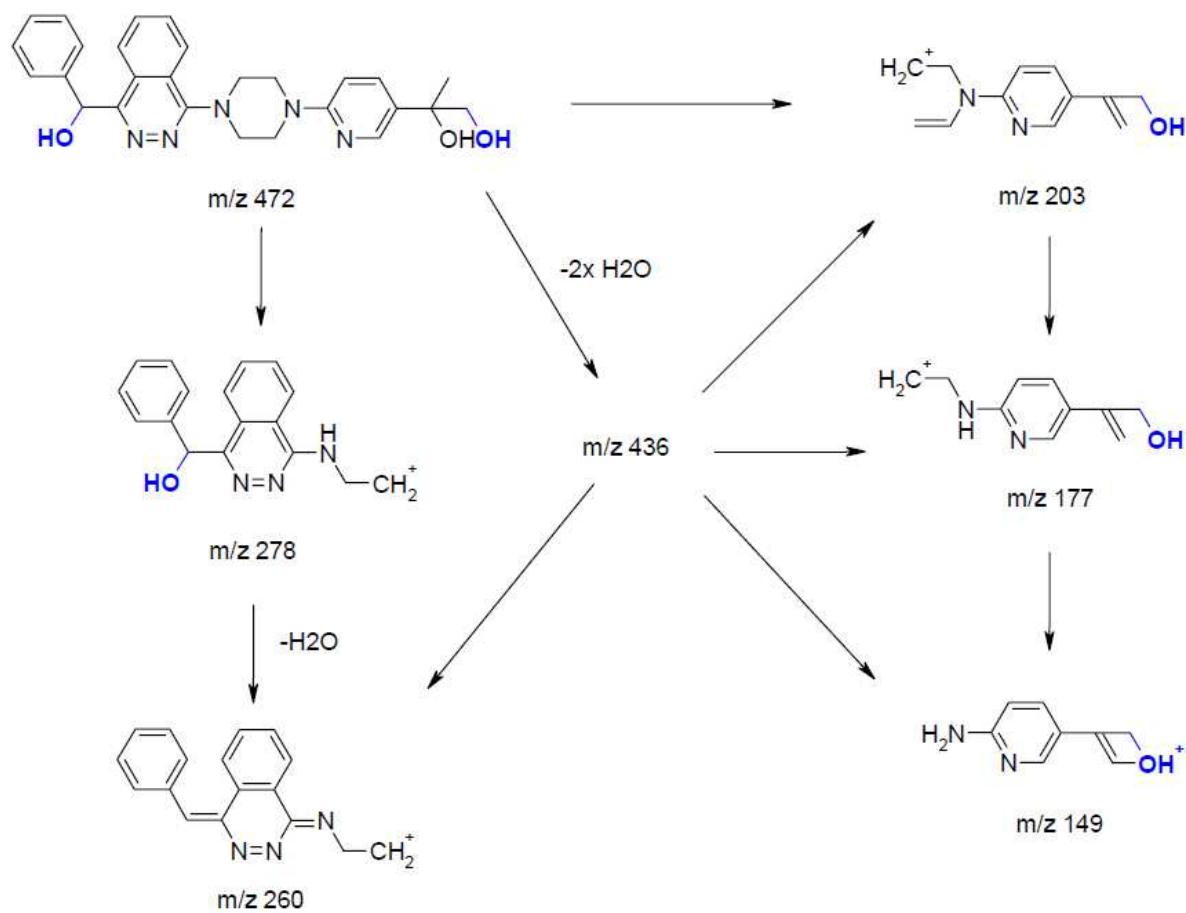
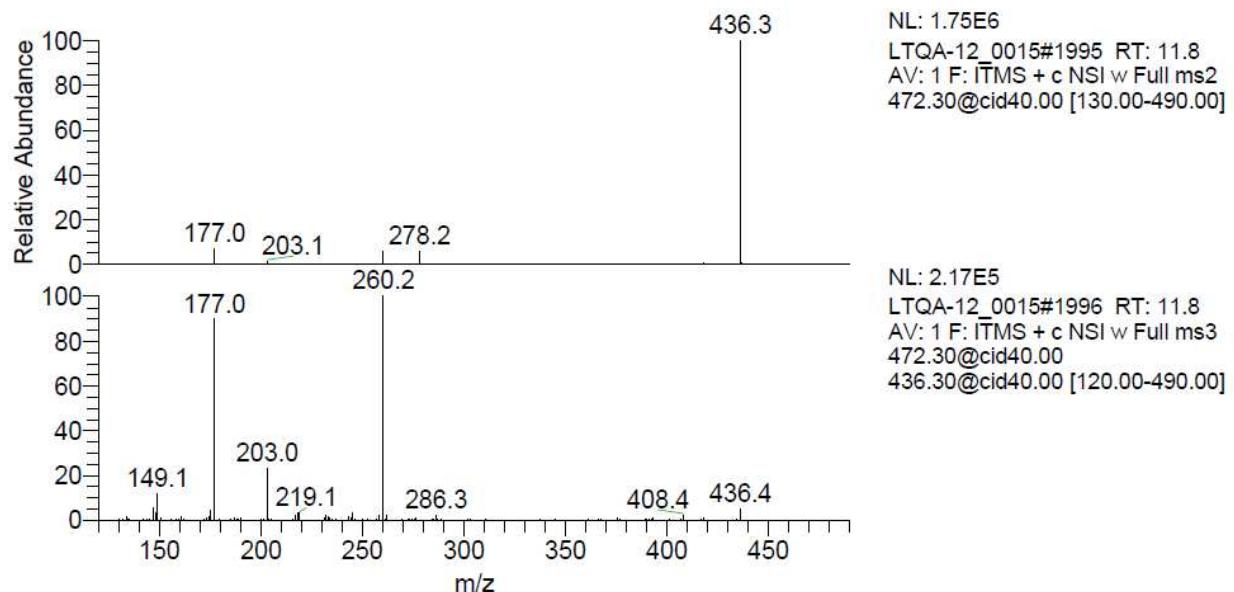


Figure 6. +ESMS ion spectrum for metabolite **14** ($M+H^+$ 472 m/z) and proposed MS^n

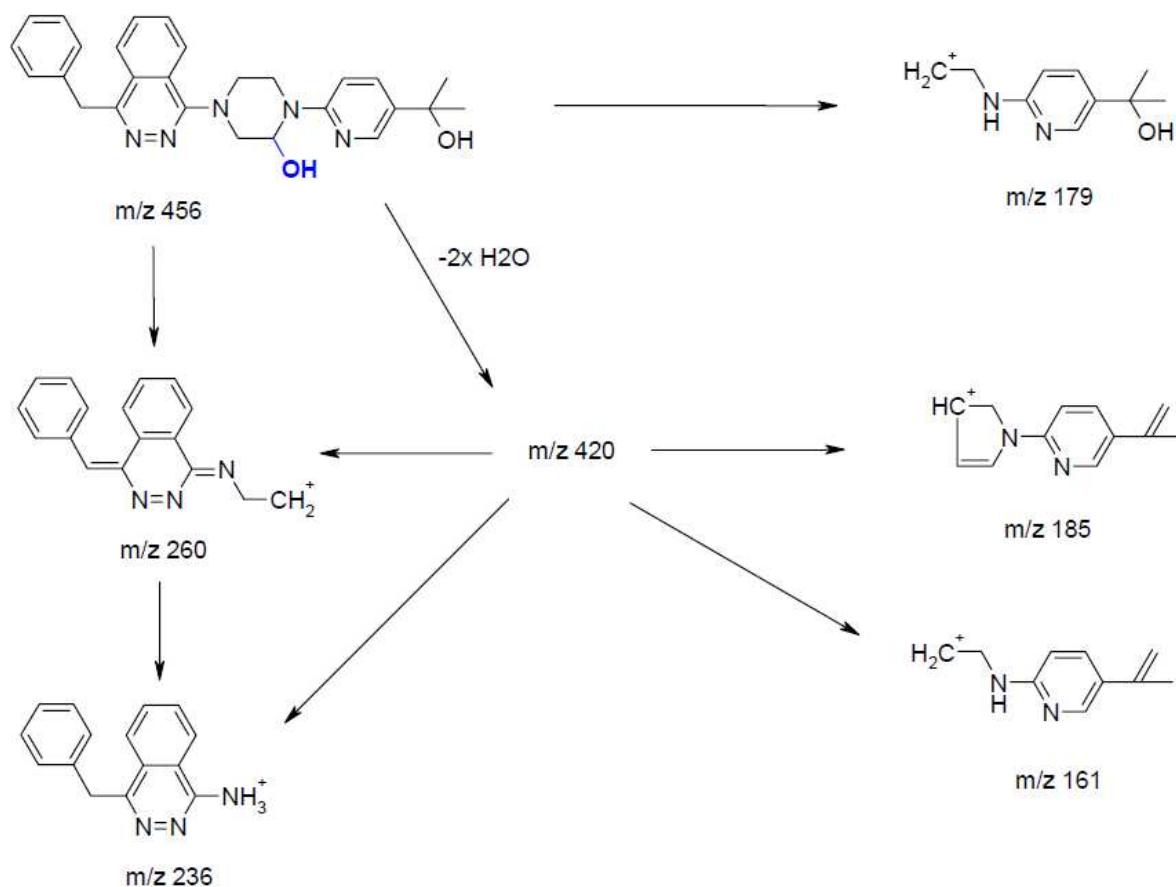
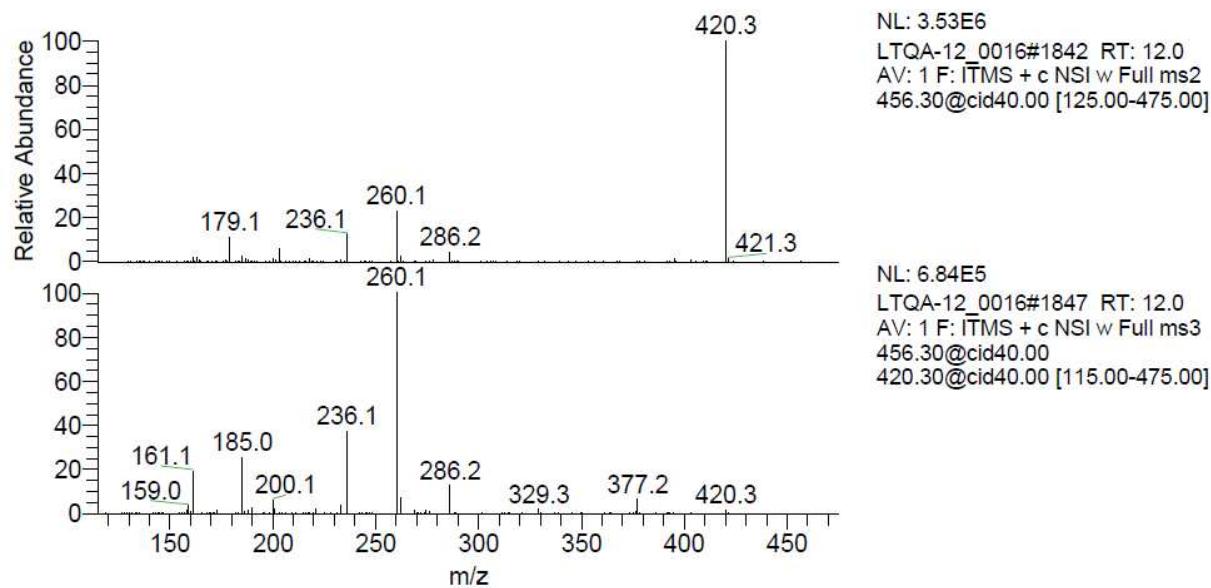


Figure 7. +ESMS ion spectrum for metabolite **15** ($M+H^+$ 456 m/z) and proposed MS^n fragmentations.

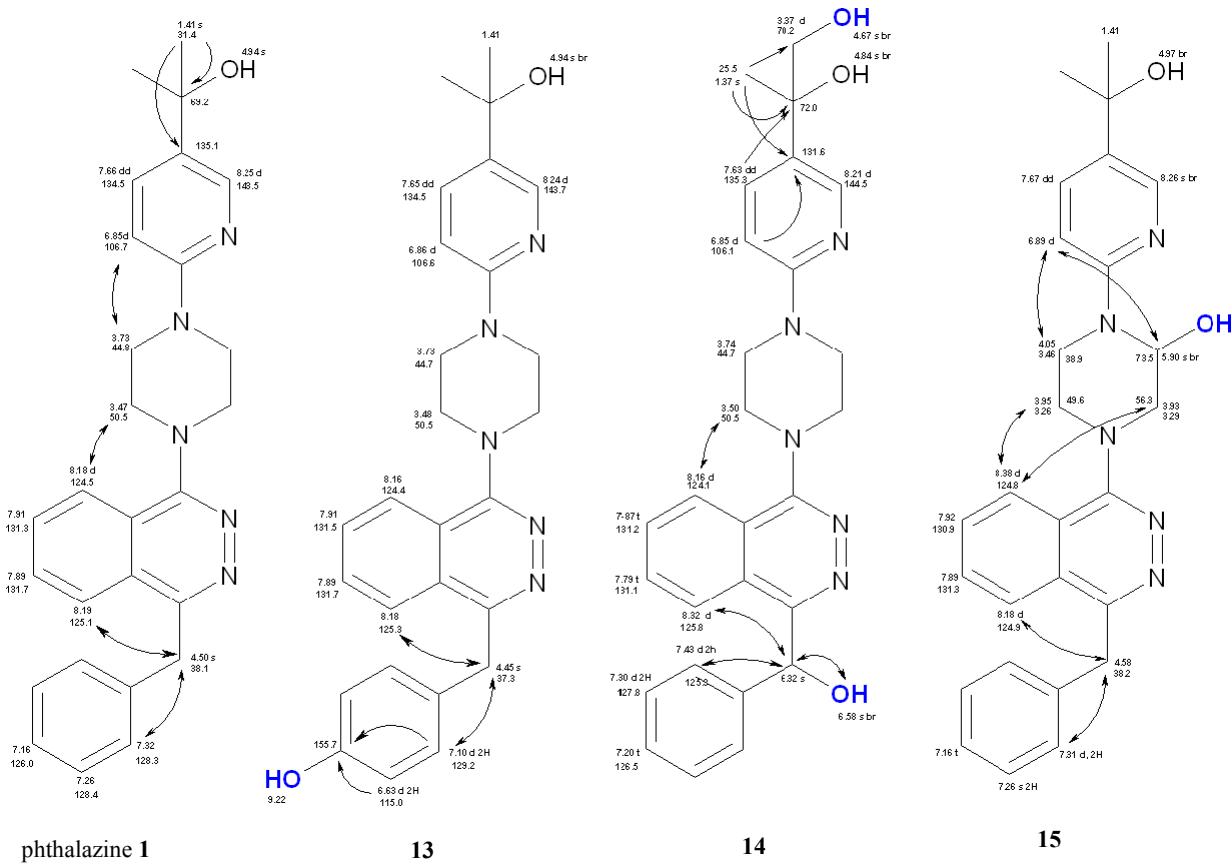
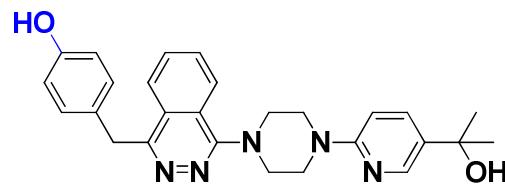
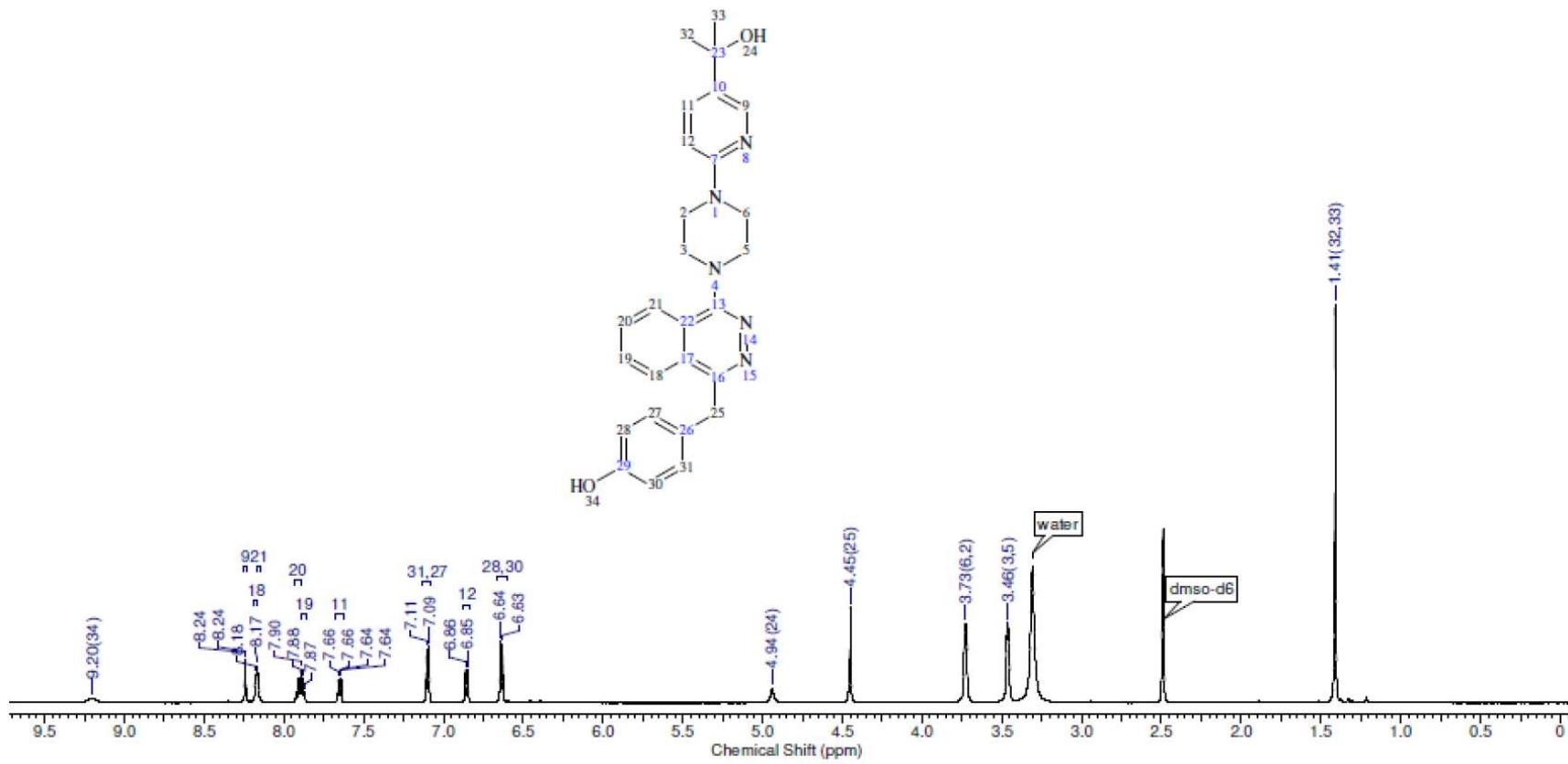
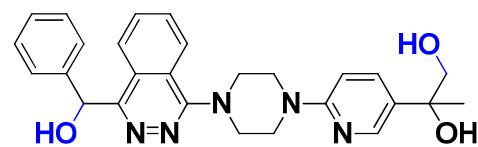


Figure 8. ^1H ROESY chemical shifts and correlations of NMR spectra for phthalazine **1** and **13**, **14**, and **15**.

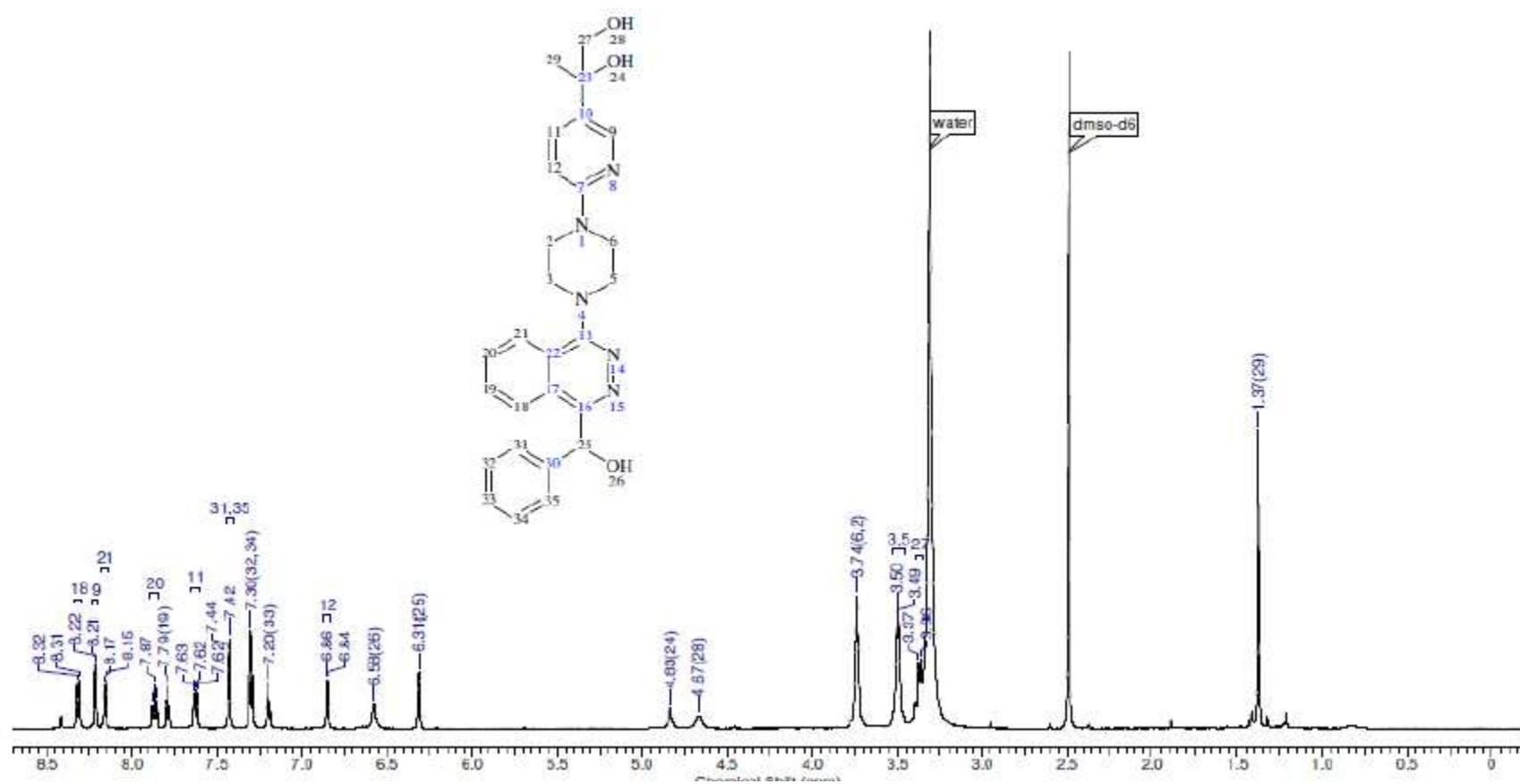


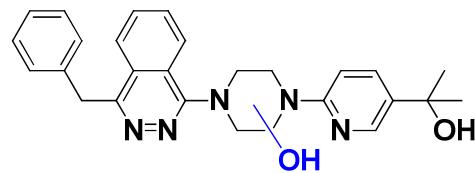
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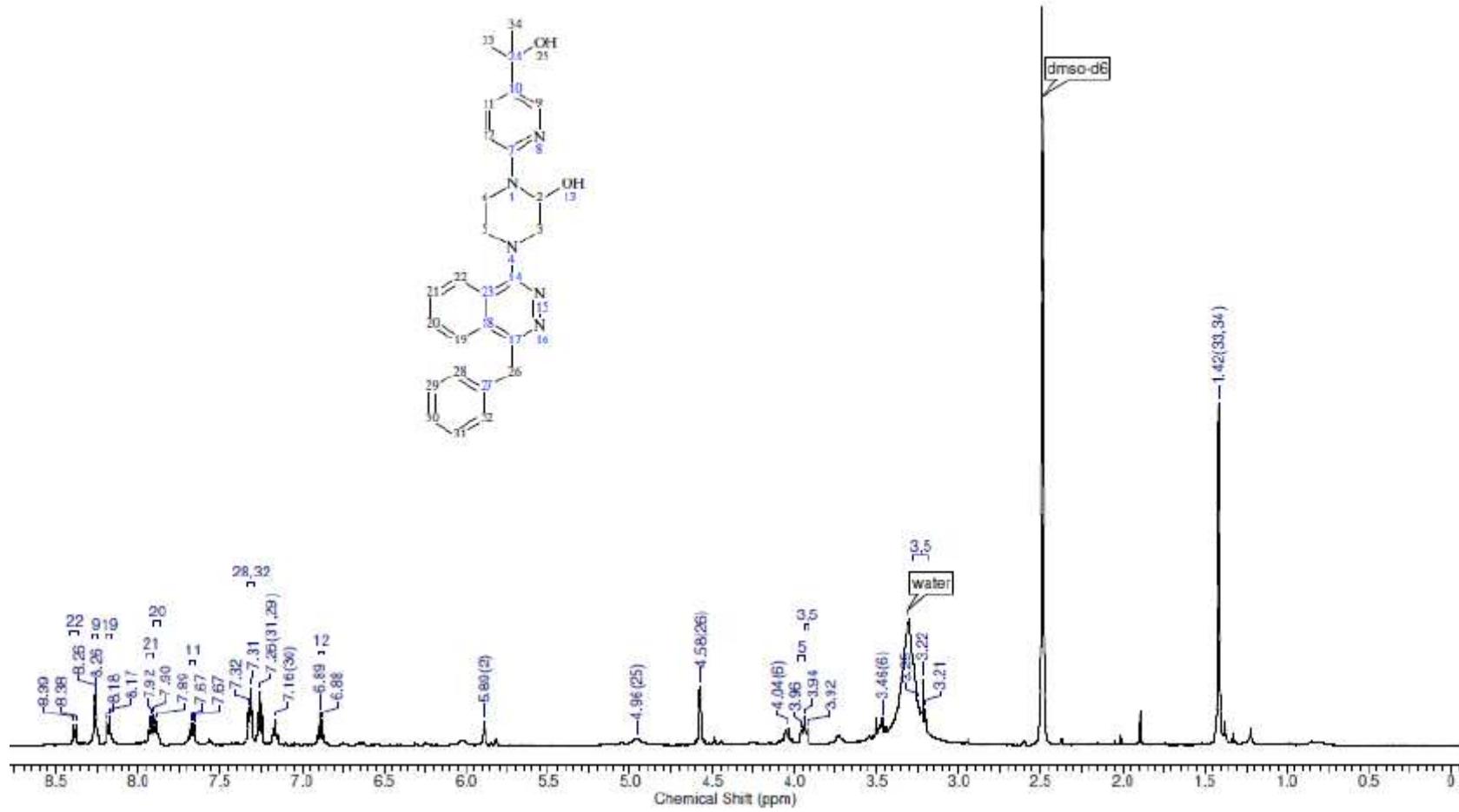


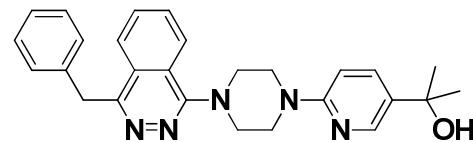
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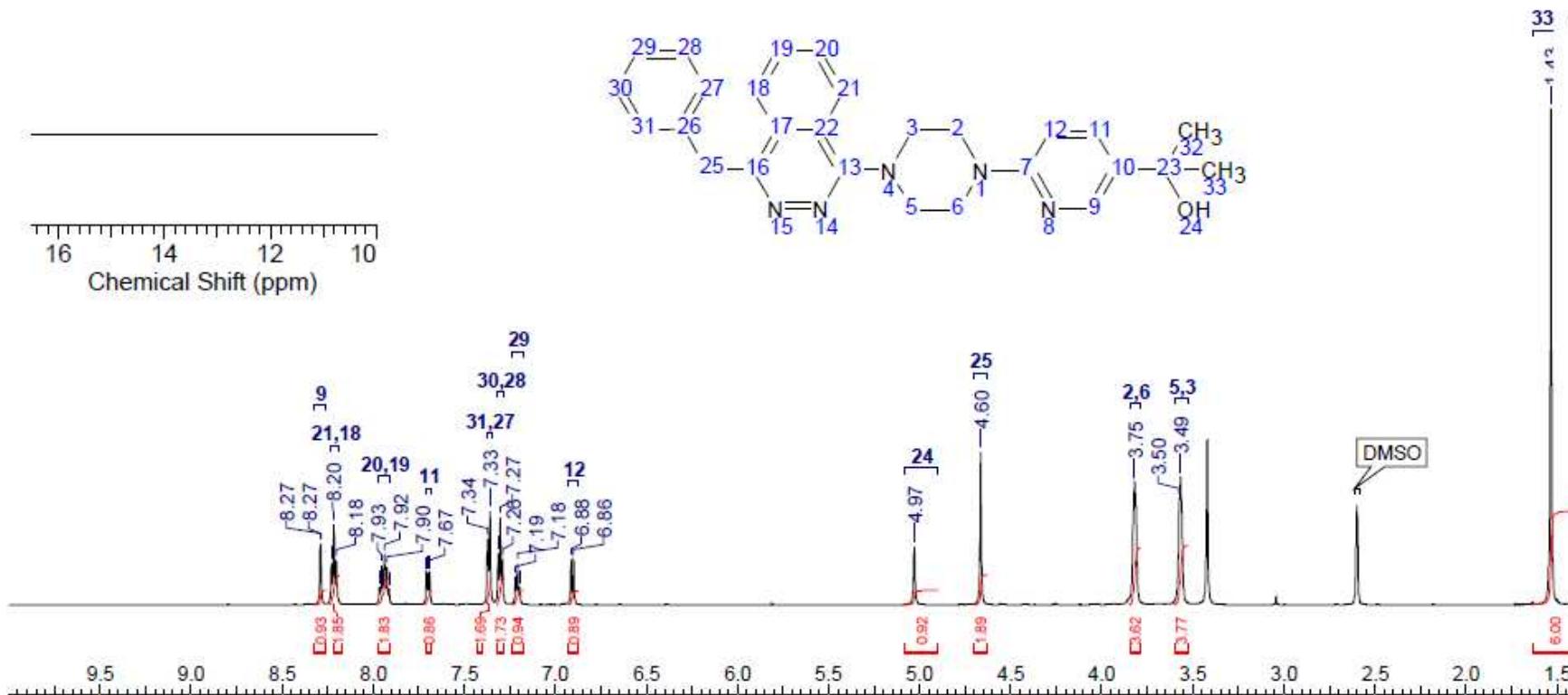


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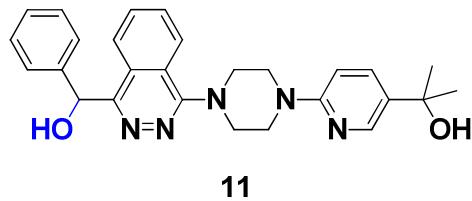




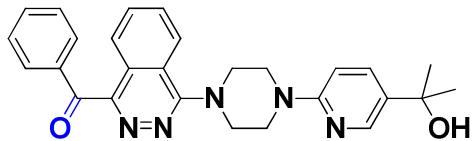
1



Characterization of **11** and **12**:

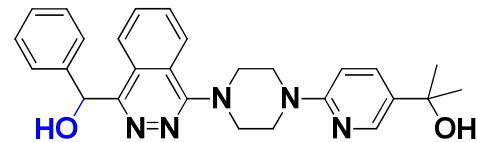


Yield: 32% (10 mg, isolated from the biotransformation mini-preparative scale), pale yellow solid; ^1H NMR (600.13 MHz, $\text{DMSO}-d_6$) δ 8.34 (d, J = 12.0 Hz, 1H); 8.26 (s, 1H), 8.18 (d, J = 12.0 Hz, 1H), 7.89 (app t, J = 7.7 Hz, 1H), 7.82 (app t, J = 7.7 Hz, 1H), 7.67 (d, J = 12.0 Hz, 1H), 7.45 (d, J = 12.0 Hz, 2H), 7.32 (app t, J = 6.0 Hz, 2H), 7.22 (app t, J = 6.0 Hz, 1H), 6.88 (d, J = 12.0 Hz, 1H), 6.60 (bs, 1H, -OH), 6.34 (s, 1H), 4.97 (bs, 1H, -OH), 3.76 (s, 4H), 3.52 (s, 4H), 1.43 (s, 6H); ^{13}C NMR (100.61 MHz, $\text{DMSO}-d_6$) δ 160.31, 158.27, 157.40, 144.34, 143.61, 135.55, 135.09, 131.97, 131.78, 128.62, 127.30, 126.67, 126.34, 126.12, 125.04, 121.95, 106.91, 75.36, 69.80, 51.21, 45.48, 32.26; HRMS: $(\text{M}+\text{H}^+)$ calcd. 456.2400; found 456.2395; the structure was further confirmed by ^{13}C HSQC, ROESY, COSY, and ^{13}C HMBC experiments.



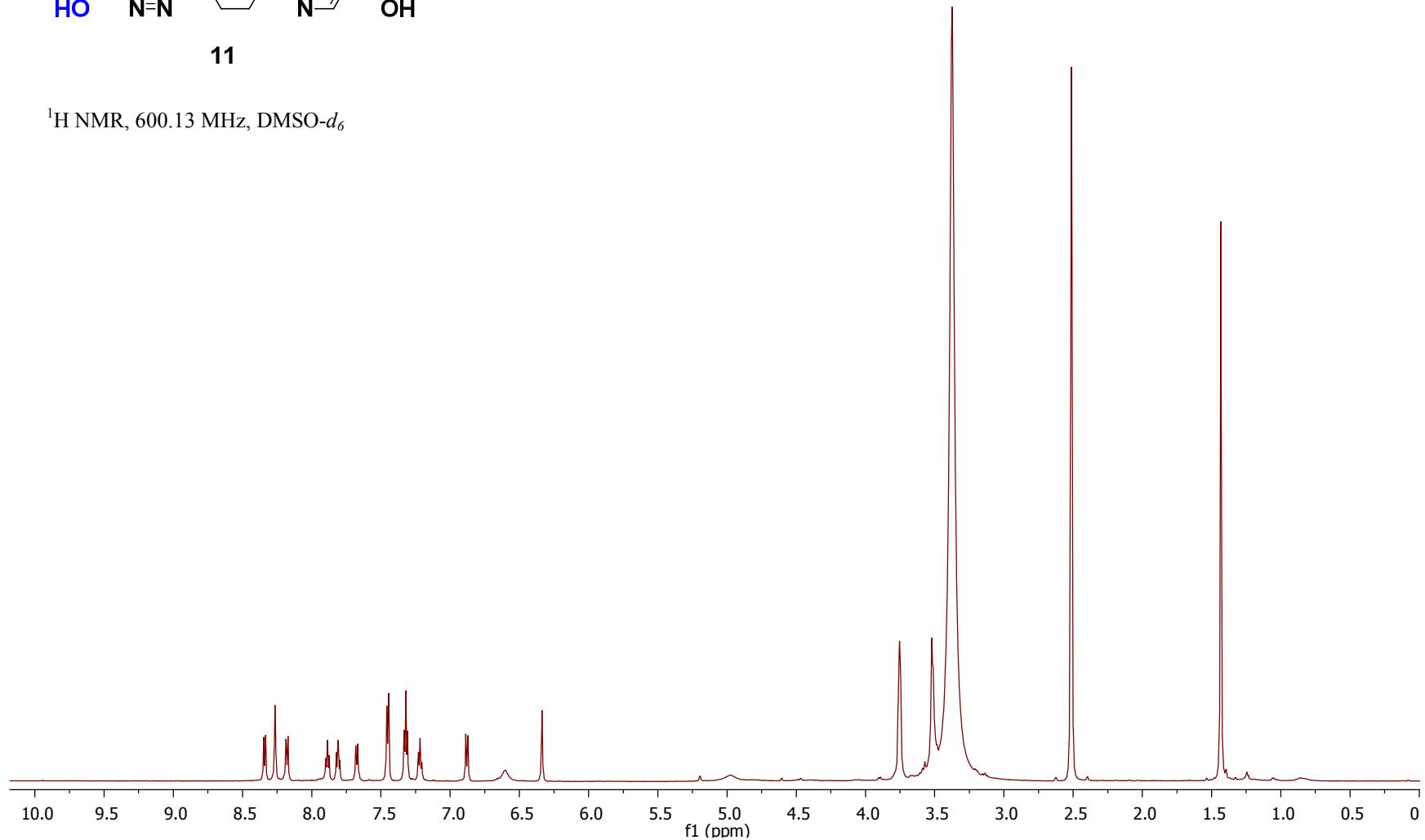
12

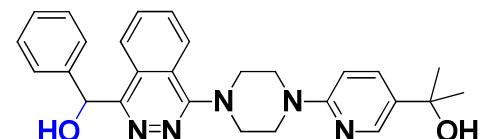
Yield: 7% (2.1 mg, isolated from the biotransformation mini-preparative scale), pale yellow solid, ^1H NMR: δ (600.13 MHz, $\text{DMSO}-d_6$) = 8.30 (d, J = 7.6 Hz, 1H); 8.26 (s, 1H), 8.16 (d, J = 7.0 Hz, 1H), 8.04 (dd, J = 7.6 Hz, 7.4 Hz, 1H), 8.00 (dd, J = 7.4 Hz, 7.0 Hz, 1H), 7.95 (d, J = 7.2 Hz, 2H), 7.73 (app t, J = 7.0 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.58 (dd, J = 7.2 Hz, 7.0 Hz, 2H), 6.89 (d, J = 8.0 Hz, 1H), 3.78 (bs, 4H), 3.72 (bs, 4H), 1.42 (s, 6H); ^{13}C NMR: δ_{C} (100.61 MHz, $\text{DMSO}-d_6$) = 193.64, 160.29, 158.19, 152.17, 144.34, 137.05, 135.58, 135.12, 134.39, 133.31, 132.77, 131.05, 129.06, 126.86, 125.64, 125.42, 120.74, 106.93, 70.24, 50.84, 45.39, 32.26; HRMS: $(\text{M}+\text{H}^+)$ calcd. 454.2243; found 454.2241; the structure was further confirmed by ^{13}C HSQC, ROESY, COSY, and ^{13}C HMBC experiments.



11

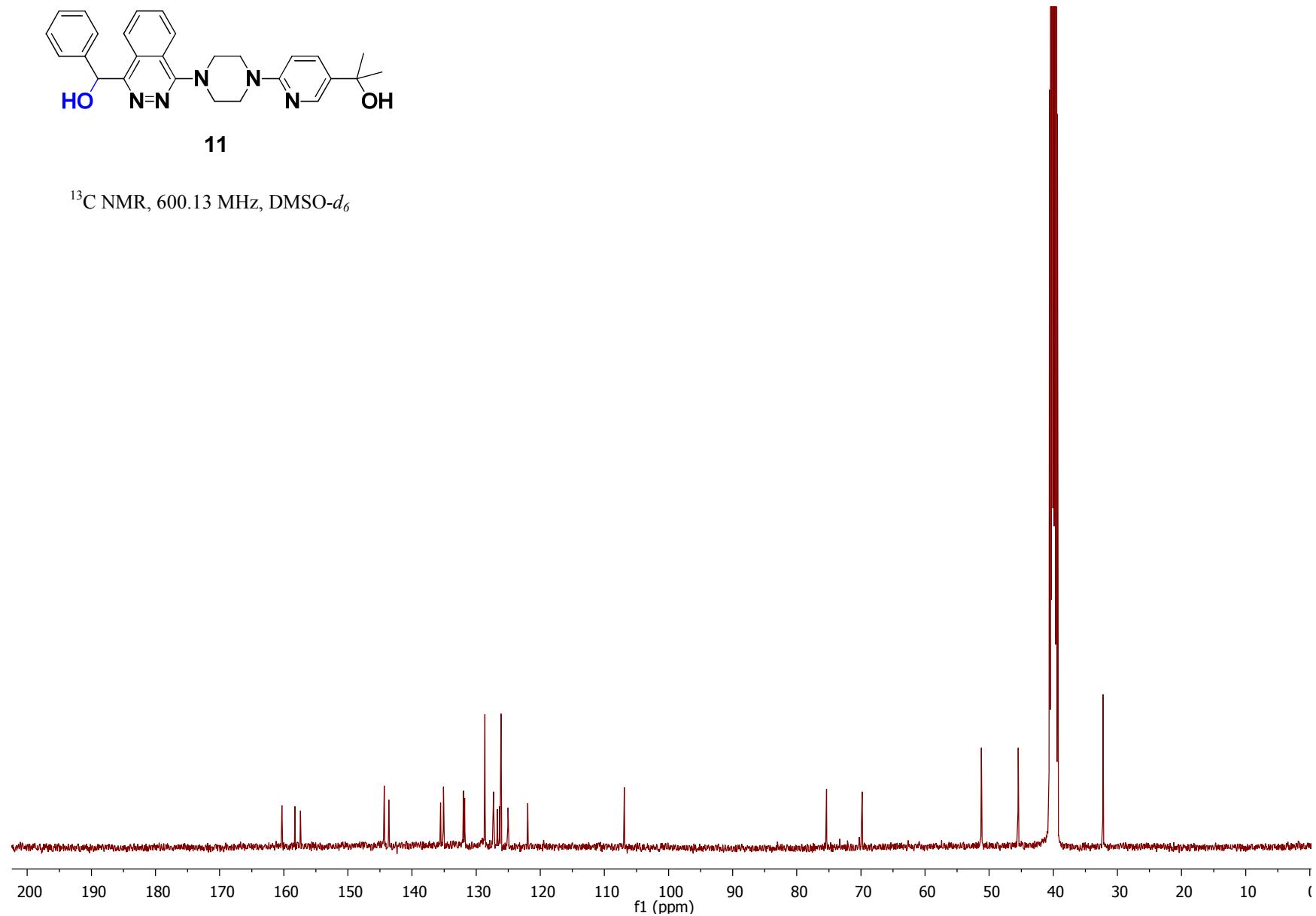
^1H NMR, 600.13 MHz, $\text{DMSO}-d_6$

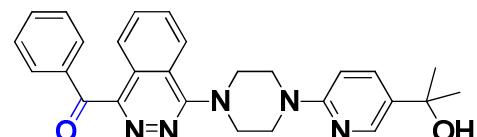




11

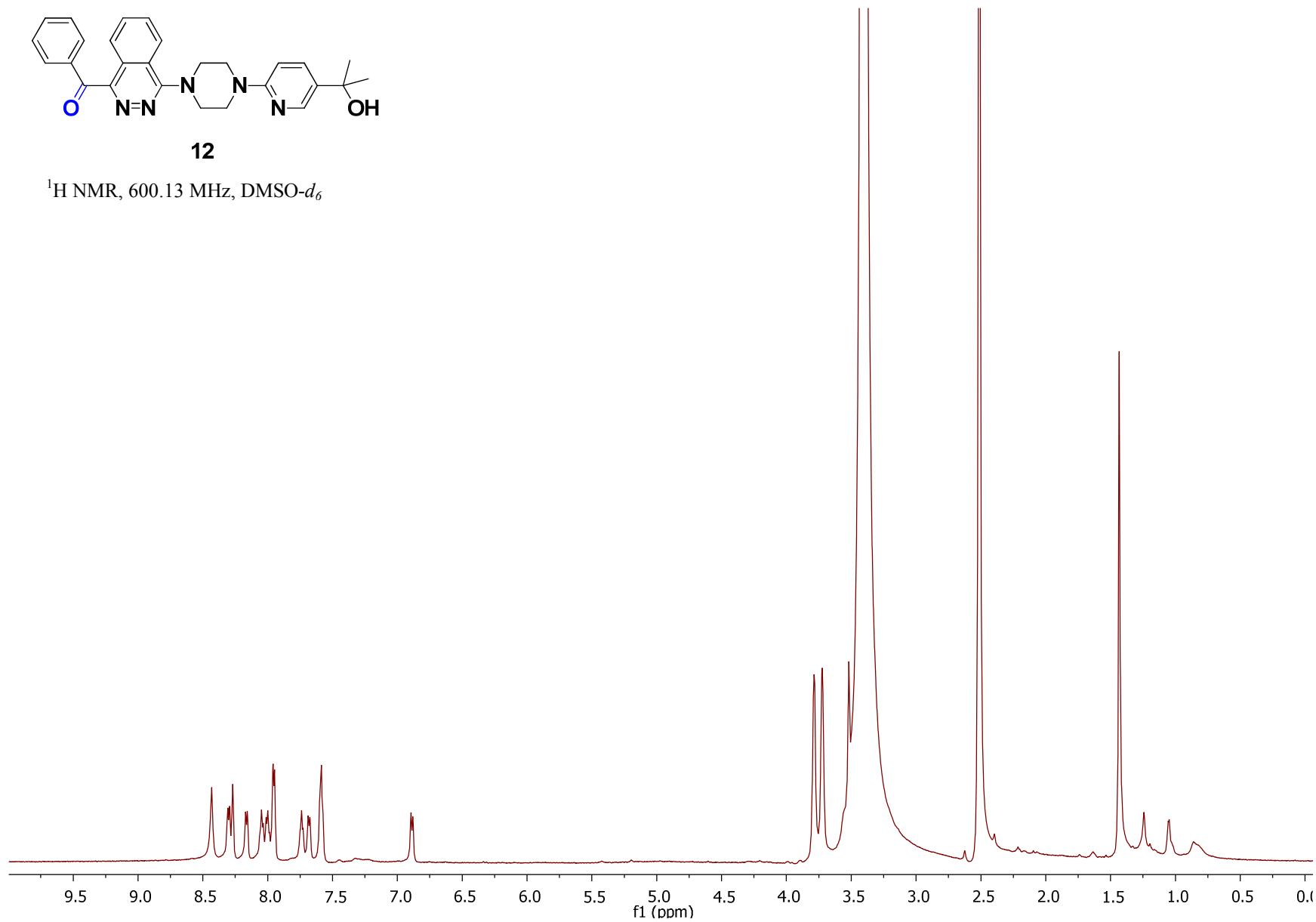
^{13}C NMR, 600.13 MHz, $\text{DMSO}-d_6$

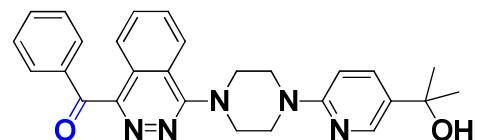




12

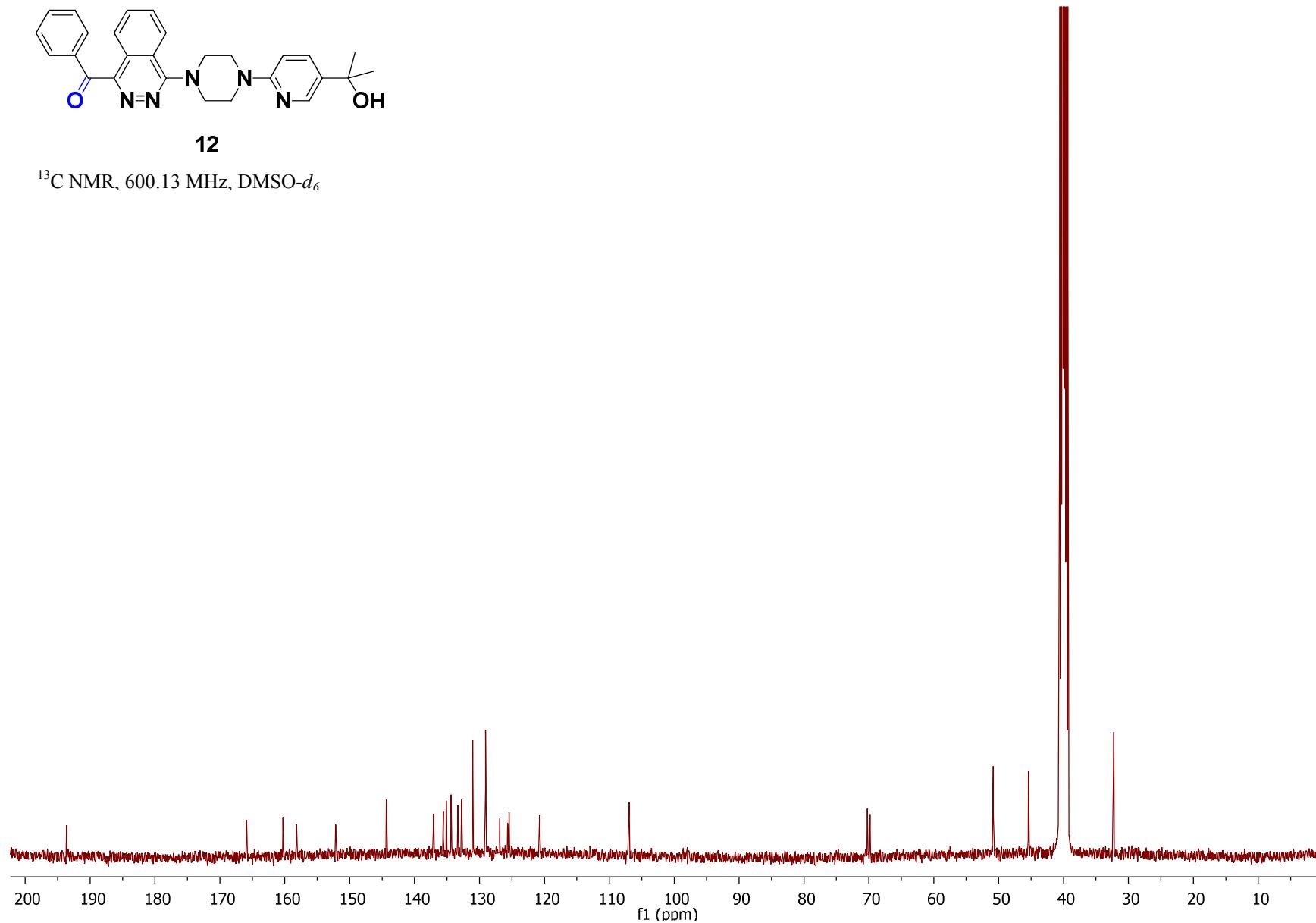
^1H NMR, 600.13 MHz, $\text{DMSO}-d_6$



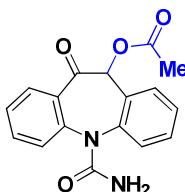


12

^{13}C NMR, 600.13 MHz, $\text{DMSO}-d_6$



F.2.Chemistry



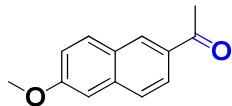
81

Procedure: oxcarbazepine (375.0 mg, 1.5 mmol) was dissolved in AcOH (10 mL, 0.149 M) and PhI(OAc)₂ (1 equiv., 479.0 mg, 1.5 mmol) was added. The resulting mixture was heated to 50 °C for 16 h at which point full conversion was achieved. The solution was diluted with water (10 mL) and extracted with a mixture of CHCl₃/i-PrOH (5:1; 3 × 100 mL). The combined organic layers was washed with aqueous NaHCO₃ (20 mL × 5) before being dried over MgSO₄, filtered, and concentrated in vaccuo. The resulting slurry was purified by HPLC reverse phase under basic condition to provide pure acetoxylated oxcarbazepine **81** (124.2 mg); yield: 27%; light yellow solid; ¹H NMR (400 MHz, CDCl₃) δ 8.16 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.75 (dd, *J* = 8.0, 0.9 Hz, 1H), 7.67 (dd, *J* = 7.6, 1.3 Hz, 1H), 7.62 (ddd, *J* = 7.5, 7.5, 1.8 Hz, 1H), 7.53 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.45 (ddd, *J* = 7.6, 7.5, 1.5 Hz, 1H), 7.42 – 7.33 (m, 2H), 6.88 (s, 1H), 4.95 (bs, 2H, □NH₂), 2.39 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 186.44, 169.42, 155.18, 142.57, 138.66, 134.44, 133.52, 130.97, 129.55, 129.32, 128.57, 128.01, 127.76, 127.18, 125.73, 77.26, 20.79; HRMS: (M+H⁺) calcd. 311.1026; found 311.1034.



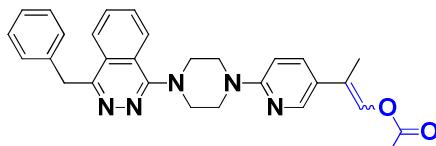
82

Procedure: oxcarbazepine (18.1 mg, 72.0 μ mol) and MeReO₃ (20 mol%, 3.58 mg, 14.0 μ mol) were suspended in *t*-BuOH (717 μ L, 0.1 M) before 30% aqueous H₂O₂ (25 equiv., 183 μ L, 1.79 mmol) was added dropwise at room temperature. The resulting mixture was heated to 40 $^{\circ}$ C for 16 h. The solution was diluted with water (500 μ L) and extracted with a mixture of CHCl₃ / *i*-PrOH (5:1; 3 \times 2 mL). The combined organic layers was dried over MgSO₄, filtered, and concentrated *in vacuo*. The crude mixture was purified by HPLC reverse phase under basic condition to provide pure urea-deprotected diketone **82** (4.3 mg); yield: 27%; orange solid; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.78 (s, 1H, -NH), 7.84 (dd, *J* = 8.1, 1.6 Hz, 2H), 7.70 (ddd, *J* = 8.4, 6.9, 1.6 Hz, 2H), 7.56 (dd, *J* = 8.4, 1.6 Hz, 2H), 7.20 (ddd, *J* = 8.1, 6.9, 1.0 Hz, 2H); ¹³C NMR (101 MHz, DMSO) δ 188.08, 140.86, 134.66, 130.08, 121.73, 120.26, 119.86; HRMS: (M+H⁺) calcd. 224.0712; found 224.0704.



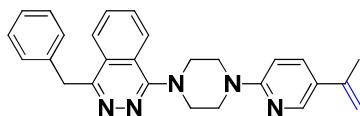
84

Procedure: naproxen (33.7 mg, 0.1 mmol) was dissolved in DCM (1 mL) and KMnO₄ (2 equiv., 46.3 mg, 0.3 mmol) was added. The resulting mixture was stirred for 24 h, at which point the reaction had stopped with no further conversion of starting material (~50%) observed (note: addition of more KMnO₄ or longer stirring time with heating did not improve conversion). The solution was diluted with water (2 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers was washed with brine (5 mL) before being dried over MgSO₄, filtered, and concentrated *in vacuo*. The resulting white solid was purified by silica flash column chromatography to provide pure methyl ketone **84** (12.0 mg); yield: 26%; white solid; ¹H NMR (400 MHz, MeOD) δ 8.53 (d, *J* = 1.7 Hz, 1H), 8.00 (dd, *J* = 8.7, 1.7 Hz, 1H), 7.95 (d, *J* = 8.8 Hz, 1H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.32 (d, *J* = 2.5 Hz, 1H), 7.24 (dd, *J* = 8.7, 2.5 Hz, 1H), 3.97 (s, 3H), 2.71 (s, 3H); ¹³C NMR (101 MHz, MeOD) δ 200.40, 161.53, 139.04, 133.70, 132.27, 131.63, 129.32, 128.26, 125.34, 120.79, 106.85, 55.97, 26.58; HRMS: (M+H⁺) calcd. 201.0910; found 201.0905.



16

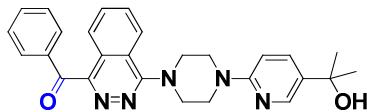
Yield: 18% (isolated), light beige solid; ¹H NMR (400 MHz, MeOD) δ 8.28 (d, *J* = 8.1 Hz, 1H), 8.19 (d, *J* = 2.2 Hz, 1H), 8.18 (dd, *J* = 8.1 Hz, 1.3 Hz, 1H), 7.95 (ddd, *J* = 8.2, 7.1, 1.4 Hz, 1H), 7.88 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.70 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.46 (q, *J* = 1.5 Hz, 1H), 7.34 – 7.24 (m, 4H), 7.23 – 7.15 (m, 1H), 6.95 (d, *J* = 8.8 Hz, 1H), 4.65 (s, 2H), 3.89 – 3.82 (m, 4H), 3.66 – 3.57 (m, 4H), 2.22 (s, 3H), 2.09 (d, *J* = 1.5 Hz, 3H); ¹³C NMR (101 MHz, MeOD) δ 169.62, 161.72, 160.29, 157.32, 145.48, 139.9, 136.56, 133.36, 132.47, 132.21, 129.74, 129.56, 129.05, 127.70, 127.06, 126.18, 126.01, 123.54, 119.98, 108.87, 52.25, 46.65, 39.85, 20.55, 13.30; HRMS: (M+H⁺) calcd. 480.2400; found 480.2389.



10

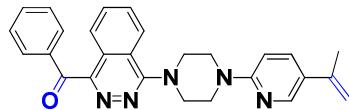
Isolated as a side product under various reaction conditions; light orange solid; ¹H NMR (400 MHz, CDCl₃) δ 8.28 (d, *J* = 2.6 Hz, 1H), 8.04 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 8.3 Hz, 1H), 7.74 – 7.62 (m, 2H), 7.59 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.28 (d, *J* = 1.8 Hz, 2H), 7.19 (app t, *J* = 7.5 Hz, 2H), 7.10 (dd, *J* = 8.4, 6.1 Hz, 1H), 6.65 (d, *J* = 8.8 Hz, 1H), 5.21 (s, 1H), 4.91 (s, 1H), 4.55 (s,

2H), 3.80 – 3.69 (m, 4H), 3.58 – 3.56 (m, 4H), 2.05 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 159.83, 158.80, 155.52, 145.06, 140.26, 138.77, 134.69, 131.53, 131.05, 128.71, 128.62, 127.68, 126.52, 126.51, 125.42, 124.72, 121.97, 110.33, 106.57, 51.02, 45.39, 39.87, 21.53; HRMS: $(\text{M}+\text{H}^+)$ calcd. 422.2345; found 422.2351.



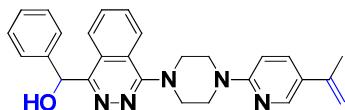
12

Yield: 24% (isolated), light green solid; ^1H NMR (400 MHz, CDCl_3) δ 8.38 – 8.32 (m, 2H), 8.18 – 8.13 (m, 1H), 8.09 (d, J = 7.1 Hz, 2H), 7.92 – 7.83 (m, 2H), 7.71 (dd, J = 8.8, 2.6 Hz, 1H), 7.61 (app. t, J = 7.4 Hz, 1H), 7.49 (app. t, J = 7.7 Hz, 2H), 6.74 (d, J = 8.6 Hz, 1H), 3.83 (s, 8H), 1.63 – 1.53 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 193.29, 160.32, 158.46, 151.82, 144.29, 136.86, 134.73, 134.07, 133.52, 132.39, 131.60, 131.11, 128.37, 127.51, 126.09, 124.56, 121.22, 106.78, 71.17, 50.78, 45.39, 31.56; HRMS: $(\text{M}+\text{H}^+)$ calcd. 454.2243; found 454.2235.



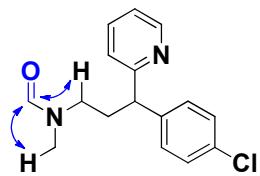
17

Yield: 60% (isolated), light green solid; ^1H NMR (400 MHz, CDCl_3) δ 8.38 – 8.35 (m, 2H), 8.18 – 8.14 (m, 1H), 8.09 (dd, J = 8.4, 1.4 Hz, 2H), 7.94 – 7.82 (m, 2H), 7.68 (dd, J = 8.8, 2.5 Hz, 1H), 7.62 (app. t, J = 7.4 Hz, 1H), 7.49 (app. t, J = 7.7 Hz, 2H), 6.73 (dd, J = 8.8, 0.8 Hz, 1H), 5.29 (s, 1H), 5.00 – 4.97 (app. p, J = 1.5 Hz, 1H), 3.85 – 3.73 (m, 8H), 2.12 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 193.22, 160.27, 158.63, 151.82, 145.10, 140.23, 136.87, 134.76, 133.52, 132.39, 131.60, 131.11, 128.37, 127.53, 126.71, 126.10, 124.55, 121.22, 110.47, 106.58, 50.75, 45.28, 21.53; HRMS: $(\text{M}+\text{H}^+)$ calcd. 436.2137; found 436.2127.



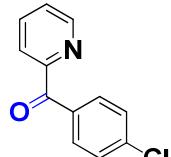
18

Yield: 5% (isolated), white solid; ^1H NMR (400 MHz, CDCl_3) δ 8.28 (d, J = 2.5 Hz, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.80 (d, J = 8.2 Hz, 1H), 7.73 (app t, J = 7.6 Hz, 1H), 7.66 (d, J = 7.7 Hz, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.35 (d, J = 6.8 Hz, 2H), 7.25 (app. t, J = 7.3 Hz, 2H), 7.21 – 7.20 (m, 1H), 6.68 (d, J = 9.0 Hz, 1H), 6.25 (s, 1H), 5.83 (s, 1H, –OH), 5.23 (s, 1H), 4.92 (s, 1H), 3.82 – 3.76 (m, 4H), 3.62-3.58 (m, 4H), 2.06 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 161.03, 158.72, 154.03, 145.08, 142.37, 140.23, 134.75, 131.87, 131.55, 128.89, 128.23, 127.68, 126.74, 126.21, 125.02, 124.56, 121.94, 110.48, 106.62, 72.56, 51.05, 45.38, 21.52; HRMS: $(\text{M}+\text{H}^+)$ calcd. 438.2288; found 438.2285.



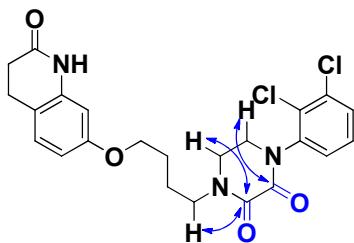
20 *hmbc correlation*

Yield: 76% (isolated as a 2:1 mixture of two rotamers); colorless oil; rotamer 1 (major): ^1H NMR (400 MHz, CDCl_3) δ 8.58 (d, J = 6.6 Hz, 1H), 7.81 (s, 1H, $\square\text{CHO}$), 7.57 (app. t, J = 7.8 Hz, 1H), 7.33 – 7.22 (m, 4H), 7.17 – 7.05 (m, 2H), 3.96 (t, J = 7.8 Hz, 1H), 3.19 (t, J = 6.8 Hz, 2H), 2.87 (s, 3H), 2.25 (m, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 162.75, 161.53, 149.60, 141.07, 136.82, 132.76, 129.23, 128.97, 123.27, 121.93, 49.38, 47.71, 32.73, 29.39; rotamer 2 (minor): ^1H NMR (400 MHz, CDCl_3) δ 8.58 (d, J = 6.6 Hz, 1H), 7.95 (s, 1H, $\square\text{CHO}$), 7.57 (app. t, J = 7.8 Hz, 1H), 7.33 – 7.22 (m, 4H), 7.17 – 7.05 (m, 2H), 4.04 (t, J = 7.7 Hz, 1H), 3.29 (t, J = 7.4 Hz, 2H), 2.88 (s, 3H), 2.59 (m, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 162.52, 162.19, 149.37, 141.61, 136.66, 132.46, 129.25, 128.75, 123.17, 121.74, 50.56, 43.05, 34.61, 31.75; HRMS: $(\text{M}+\text{H}^+)$ calcd. 289.1108; found 289.1117.



21

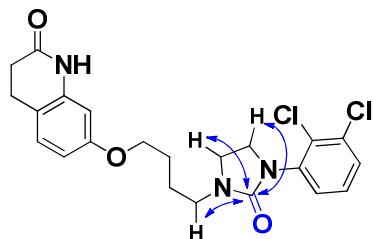
Modified procedure: AcOH (1 equiv.); yield: 25% (isolated); white solid; ^1H NMR (400 MHz, CDCl_3) δ 8.72 (dt, J = 4.5, 1.3 Hz, 1H), 8.12 – 8.03 (m, 3H), 7.92 (td, J = 7.8, 1.7 Hz, 1H), 7.51 (ddd, J = 7.6, 4.8, 1.3 Hz, 1H), 7.47 (d, J = 8.6 Hz, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 192.52, 154.65, 148.55, 139.44, 137.25, 134.55, 132.52, 128.51, 126.48, 124.74; HRMS: $(\text{M}+\text{H}^+)$ calcd. 218.0373; found 218.0376.



23 *hmbc correlation*

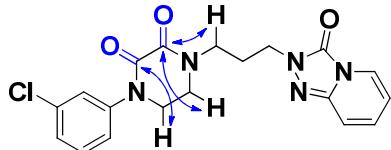
Modified procedure: TEMPO (5 equiv.), CuI (1 equiv.); yield: 38% (isolated), 31% in the absence of TEMPO; white solid; ^1H NMR (400 MHz, CDCl_3) δ 8.99 (s, 1H, $\square\text{NH}$), 7.48 (dd, J = 7.6, 2.0 Hz, 1H), 7.32 – 7.22 (m, 2H), 7.03 (d, J = 8.3 Hz, 1H), 6.51 (dd, J = 8.3, 2.4 Hz, 1H),

6.44 (d, $J = 2.4$ Hz, 1H), 3.99 (t, $J = 5.4$ Hz, 2H), 3.95 – 3.77 (m, 3H), 3.69 – 3.57 (m, 3H), 2.88 (app dd, $J = 8.4, 6.5$ Hz, 2H), 2.61 (app dd, $J = 8.5, 6.5$ Hz, 2H), 1.92 – 1.76 (m, 4H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.76, 158.39, 157.17, 156.68, 139.55, 138.23, 134.46, 130.66, 130.63, 128.74, 128.22, 127.40, 115.93, 108.65, 102.19, 67.24, 47.44, 47.35, 44.96, 31.10, 26.35, 24.59, 23.80; HRMS: $(\text{M}+\text{H}^+)$ calcd. 476.1144; found 476.1144.



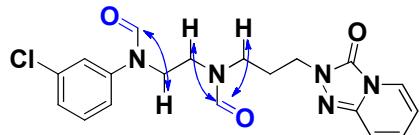
24 *hmbc correlation*

Yield: 15% (isolated); white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.74 (s, 1H, $\square\text{NH}$), 7.32 (dd, $J = 7.9, 1.7$ Hz, 1H), 7.21 (dd, $J = 7.9, 1.7$ Hz, 1H), 7.15 (app t, $J = 7.9$ Hz, 1H), 6.96 (d, $J = 8.3$ Hz, 1H), 6.45 (dd, $J = 8.3, 2.4$ Hz, 1H), 6.26 (d, $J = 2.4$ Hz, 1H), 3.92 (t, $J = 6.0$ Hz, 2H), 3.69 (app dd, $J = 9.0, 6.7$ Hz, 2H), 3.47 (app dd, $J = 9.0, 6.7$ Hz, 2H), 3.30 (t, $J = 6.8$ Hz, 2H), 2.81 (app dd, $J = 8.5, 6.4$ Hz, 2H), 2.53 (app dd, $J = 8.5, 6.4$ Hz, 2H), 1.83 – 1.62 (m, 4H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.44, 159.16, 158.51, 139.18, 138.13, 133.93, 131.40, 129.04, 128.73, 127.77, 127.64, 115.76, 108.71, 101.96, 67.34, 44.76, 43.60, 42.76, 31.08, 26.21, 24.60, 23.96; HRMS: $(\text{M}+\text{H}^+)$ calcd. 448.1195; found 448.1184.



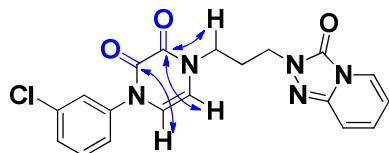
26 *hmbc correlation*

Yield: 10% (isolated); white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.69 (dt, $J = 7.0, 1.3$ Hz, 1H), 7.30 (t, $J = 2.0$ Hz, 1H), 7.27 (d, $J = 7.9$ Hz, 1H), 7.24 – 7.16 (m, 2H), 7.09 – 7.00 (m, 2H), 6.46 (ddd, $J = 7.2, 5.4, 2.0$ Hz, 1H), 4.03 (t, $J = 6.6$ Hz, 2H), 3.93 (app dd, $J = 6.7, 4.6$ Hz, 2H), 3.71 (app dd, $J = 6.7, 4.5$ Hz, 2H), 3.54 (t, $J = 6.8$ Hz, 2H), 2.19 (tt, $J = 6.8$ Hz, 6.8 Hz 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 157.47, 156.52, 148.67, 141.78, 141.75, 134.73, 130.22, 130.19, 127.35, 124.71, 123.71, 122.73, 115.39, 110.83, 47.66, 45.42 ($\times 2$), 43.24, 26.50; HRMS: $(\text{M}+\text{H}^+)$ calcd. 400.1176; found 400.1167.



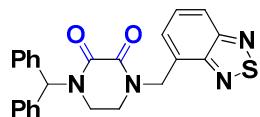
27 *hmhc correlation*

Bis-formamide **27** was obtained only once out of three experiments; yield: 16% (isolated as a mixture of two rotamers: 1:1.6 ratio); light white translucide solid; rotamer 1 (major): ¹H NMR (400 MHz, CDCl₃) δ 8.41 (s, 1H, □CHO), 8.12 (s, 1H, □CHO), 7.78 (ddd, *J* = 6.9, 5.5, 1.3 Hz, 1H), 7.36 (dd, *J* = 8.1, 4.6 Hz, 1H), 7.30 (m, 1H), 7.28 (s, 1H), 7.18 (ddd, *J* = 8.2, 2.6, 2.1 Hz, 1H), 7.15 – 7.07 (m, 2H), 6.54 (m, 1H), 4.04 (t, *J* = 6.5 Hz, 2H), 3.96 (t, *J* = 6.3 Hz, 2H), 3.57 (t, *J* = 6.4 Hz, 2H), 3.42 (t, *J* = 7.0 Hz, 2H), 2.18-2.11 (m, 2H); ¹³C NMR (101 MHz, CDCl₃) δ 163.54, 162.27, 148.61, 142.92, 141.41, 135.34, 130.85, 130.21, 127.08, 123.76, 123.44, 121.47, 115.36, 110.77, 45.03, 42.71, 42.64, 40.20, 27.88; rotamer 2 (minor): ¹H NMR (400 MHz, CDCl₃) δ 8.39 (d, 1H, □CHO), 7.89 (s, 1H, □CHO), 7.76 (ddd, *J* = 6.9, 5.5, 1.3 Hz, 1H), 7.39 (dd, *J* = 8.2, 4.2 Hz, 1H), 7.32 (m, 1H), 7.28 (s, 1H), 7.20 (dd, *J* = 2.2, 1.0 Hz, 1H), 7.15 – 7.03 (m, 2H), 6.52 (m, 1H), 4.03-3.96 (m, 2H), 3.54 (t, *J* = 6.2 Hz, 2H), 3.36 (t, *J* = 7.0 Hz, 1H), 2.14-2.06 (m, 2H); ¹³C NMR (101 MHz, CDCl₃) δ 162.89, 162.29, 148.55, 141.78, 141.67, 135.74, 131.13, 130.03, 127.54, 123.80, 123.77, 121.88, 115.38, 110.65, 44.69, 43.25, 43.23, 39.59, 26.49; HRMS: (M+H⁺) calcd. 402.1333; found 402.1320.



85 *hmhc correlation*

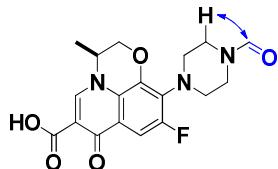
Yield: 3% (isolated); white solid; ¹H NMR (400 MHz, CDCl₃) δ 7.70 (dt, *J* = 7.1, 1.3 Hz, 1H), 7.39 – 7.27 (m, 3H), 7.24 (dt, *J* = 7.5, 1.8 Hz, 1H), 7.16 – 7.08 (m, 2H), 6.51 (d, *J* = 6.2 Hz, 1H), 6.47 (ddd, *J* = 7.3, 5.6, 1.8 Hz, 1H), 6.31 (d, *J* = 6.2 Hz, 1H), 4.12 (t, *J* = 6.5 Hz, 2H), 3.92 (t, *J* = 6.5 Hz, 2H), 2.35 (tt, *J* = 6.5 Hz, 6.5 Hz, 2H); ¹³C NMR (101 MHz, CDCl₃) δ 156.04, 155.01, 148.78, 141.84, 140.12, 135.06, 130.51, 130.28, 128.92, 126.07, 124.03, 123.73, 115.42, 113.89, 112.71, 110.88, 46.60, 42.98, 27.40; HRMS: (M+H⁺) calcd. 398.1010; found 398.1020.



29

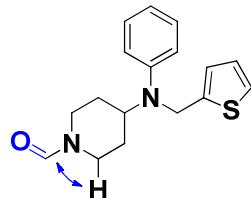
Modified procedure: TEMPO (2 equiv.); yield: 23% (isolated), white solid; ¹H NMR (400 MHz, CDCl₃) δ 7.89 (d, *J* = 8.8 Hz, 1H), 7.64 (d, *J* = 6.7 Hz, 1H), 7.52 (dd, *J* = 8.8, 6.8 Hz, 1H), 7.38 – 7.24 (m, 6H), 7.14 (dd, *J* = 7.4, 1.9 Hz, 4H), 7.04 (s, 1H), 5.06 (s, 2H), 3.69 (app dd, *J* = 6.9, 4.6

Hz, 2H), 3.21 (app dd, J = 6.9, 4.6 Hz, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 157.71, 157.62, 155.04, 154.29, 137.36, 129.91, 129.73, 128.78, 128.61, 128.55, 128.06, 121.37, 61.14, 47.76, 45.61, 41.26; HRMS: $(\text{M}+\text{H}^+)$ calcd. 429.1385; found 429.1377.



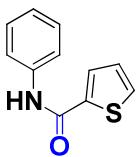
31 *hmbc correlation*

Modified procedure: addition of TEMPO (2 equiv.); yield: 8% (isolated), light yellow solid; ^1H NMR (400 MHz, CDCl_3) δ 14.84 (bs, 1H, $-\text{CO}_2\text{H}$), 8.63 (s, 1H), 8.13 (s, 1H, $\square\text{CHO}$), 7.76 (d, J_{H-F} = 11.9 Hz, 1H), 4.56-4.50 (m, 1H), 4.48 (dd, J = 11.4, 2.3 Hz, 1H), 4.38 (dd, J = 11.4, 2.4 Hz, 1H), 3.71 (t, J = 5.1 Hz, 2H), 3.53 (m, 2H), 3.43-3.26 (m, 4H), 1.63 (d, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 177.18 (d, $^4J_{C-F}$ = 3.0 Hz), 166.99, 161.02, 156.21 (d, $^1J_{C-F}$ = 245.1 Hz), 144.75, 139.81 (d, 3J = 6.5 Hz), 132.23 (d, $^2J_{C-F}$ = 14.5 Hz), 124.65, 121.41 (d, $^3J_{C-F}$ = 9.6 Hz), 108.16, 105.49 (d, $^2J_{C-F}$ = 24.2 Hz), 68.32, 55.53, 51.37 (d, $^4J_{C-F}$ = 4.3 Hz), 50.28 (d, $^4J_{C-F}$ = 3.6 Hz), 46.43, 40.87, 18.38; HRMS: $(\text{M}+\text{H}^+)$ calcd. 376.1309; found 376.1306.



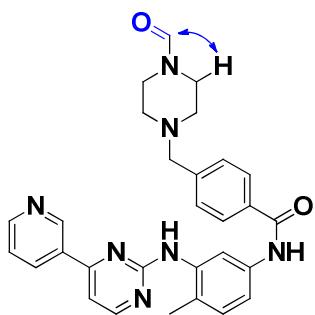
33 *hmbc correlations*

Yield: 24% (isolated); white / colorless wax; ^1H NMR (400 MHz, CDCl_3) δ 8.02 (s, 1H, $-\text{CHO}$), 7.22 (dd, J = 8.8, 7.2 Hz, 2H), 7.15 (dd, J = 8.8, 1.4 Hz, 1H), 6.95 – 6.89 (m, 2H), 6.85 (d, J = 7.2, 1.4 Hz, 2H), 6.79 (dd, J = 7.4, 7.4 Hz, 1H), 4.57 (s, 2H), 4.55 – 4.51 (m, 1H), 3.93 (tt, J = 11.8, 3.6 Hz, 1H), 3.72 (ddt, J = 13.5, 4.7, 2.2 Hz, 1H), 3.18 (td, J = 12.9, 2.9 Hz, 1H), 2.68 (td, J = 12.9, 2.9 Hz, 1H), 2.08 – 1.95 (m, 2H), 1.68 – 1.52 (m, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 160.73, 148.24, 144.81, 129.29, 126.88, 123.91, 123.90, 118.41, 114.72, 56.20, 45.94, 45.53, 39.27, 30.47, 28.80; HRMS: $(\text{M}+\text{H}^+)$ calcd. 301.1375; found 301.1385.



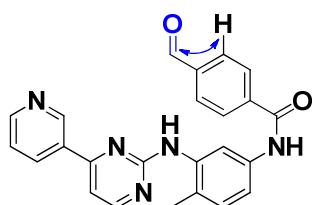
34

Yield: 4% (isolated); white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.70 (bs, 1H, $\square\text{NH}$), 7.66 (dd, J = 3.8, 1.2 Hz, 1H), 7.63 (dd, J = 8.5, 1.2 Hz, 2H), 7.58 (dd, J = 4.9, 1.2 Hz, 1H), 7.40 (dd, J = 8.5, 7.3 Hz, 2H), 7.22 – 7.13 (m, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 159.95, 139.13, 137.49, 130.85, 129.17, 128.54, 127.89, 124.72, 120.21; HRMS: $(\text{M}+\text{H}^+)$ calcd. 204.0483; found 204.0487.



36 *hmhc correlation*

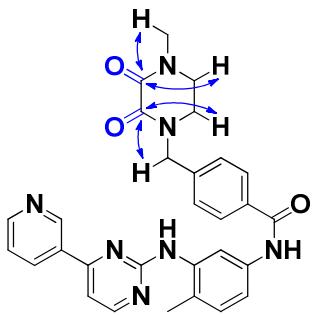
Yield: 6% (isolated); light yellow solid; ^1H NMR (400 MHz, CDCl_3) δ 9.27 (s, 1H), 8.71 (s, 1H), 8.60 (d, J = 2.3 Hz, 1H), 8.52 (d, J = 5.0 Hz, 1H), 8.49 (m, 1H), 8.10 (s, 1H, $-\text{NH}$), 8.03 (s, 1H, $-\text{CHO}$), 7.87 (d, J = 7.9 Hz, 2H), 7.45 (m, 3H), 7.33 (dd, J = 8.2, 2.3 Hz, 1H), 7.22 (d, J = 8.2 Hz, 1H), 7.18 (d, J = 5.0 Hz, 1H), 7.09 (s, 1H, $-\text{NH}$), 3.60 (s, 2H), 3.59 (d, J = 5.3 Hz, 2H), 3.40 (t, J = 5.0 Hz, 2H), 2.47 (t, J = 5.0 Hz, 2H), 2.44 (t, J = 5.3 Hz, 2H), 2.36 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 165.36, 162.74, 160.77, 160.55, 159.04, 151.47, 148.52, 141.66, 137.78, 136.62, 134.94, 134.27, 132.66, 130.81, 129.22, 127.25, 124.27, 123.76, 115.34, 113.11, 108.39, 62.36, 53.41, 52.32, 45.60, 39.92, 17.74; HRMS: $(\text{M}+\text{H}^+)$ calcd. 508.2461; found 508.2461.



37 *hmhc correlation*

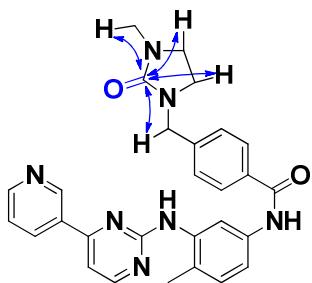
Yield: 8% (isolated), 4% on 1 g scale; light yellow solid; ^1H NMR (400 MHz, CDCl_3) δ 10.07 (s, 1H, $-\text{CHO}$), 9.27 (s, 1H), 8.70 (s, 1H), 8.65 (d, J = 4.6 Hz, 1H), 8.52 (d, J = 5.2 Hz, 1H), 8.50 (d,

$J = 7.9$ Hz, 1H), 8.28 (s, 1H, \square NH), 8.04 (d, $J = 8.0$ Hz, 2H), 7.96 (d, $J = 8.0$ Hz, 2H), 7.44 (dd, $J = 7.9, 4.6$ Hz, 1H), 7.34 (d, $J = 8.0$ Hz, 1H), 7.22 (d, $J = 8.0$ Hz, 1H), 7.18 (d, $J = 5.2$ Hz, 1H), 7.13 (s, 1H, \square NH), 2.37 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 191.54, 164.53, 162.49, 160.28, 158.89, 150.65, 147.89, 140.25, 138.29, 137.74, 136.17, 135.72, 132.92, 130.91, 130.02, 127.86, 124.54, 124.05, 115.44, 113.06, 108.45, 17.77; HRMS: $(\text{M}+\text{H}^+)$ calcd. 410.1617; found 410.1609.



38 *hmhc correlation*

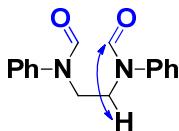
Yield: 13% (isolated); light yellow solid; ^1H NMR (400 MHz, CDCl_3) δ 9.27 (d, $J = 2.5$ Hz, 1H), 8.69 (dd, $J = 4.4, 1.7$ Hz, 1H), 8.63 (d, $J = 2.2$ Hz, 1H), 8.50 (d, $J = 5.2$ Hz, 1H), 8.47-8.49 (m, 1H), 8.38 (s, 1H, --NH), 7.88 (d, $J = 8.2$ Hz, 2H), 7.43 (dd, $J = 8.2, 4.4$ Hz, 1H), 7.39 – 7.32 (m, 3H), 7.20 (d, $J = 8.2$ Hz, 1H), 7.16 (d, $J = 5.2$ Hz, 1H), 7.10 (s, 1H, --NH), 4.69 (s, 2H), 3.52 – 3.47 (m, 2H), 3.47 – 3.42 (m, 2H), 3.08 (s, 3H), 2.35 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 165.17, 162.61, 160.49, 159.00, 157.47, 157.44, 151.25, 148.37, 139.24, 137.68, 136.66, 135.14, 135.02, 132.70, 130.76, 128.41, 127.87, 124.40, 123.83, 115.55, 113.27, 108.33, 50.35, 46.30, 43.58, 35.09, 17.76; HRMS: $(\text{M}+\text{H}^+)$ calcd. 522.2254; found 522.2252.



38 *hmhc correlation*

Yield: 3% (isolated), light yellow solid; ^1H NMR (400 MHz, CDCl_3) δ 9.24 (s, 1H), 8.69 (d, $J = 5.0$ Hz, 1H), 8.61 (d, $J = 2.2$ Hz, 1H), 8.50 – 8.54 (m, 1H), 8.51 (d, $J = 5.2$ Hz, 1H), 8.36 (s, 1H, --NH), 7.86 (d, $J = 8.2$ Hz, 2H), 7.42 (dd, $J = 8.0, 5.0$ Hz, 1H), 7.36 (m, 1H), 7.34 (d, $J = 8.2$ Hz, 2H), 7.20 (d, $J = 8.0$ Hz, 1H), 7.17 (d, $J = 5.2$ Hz, 1H), 7.08 (s, 1H, --NH), 4.39 (s, 2H), 3.30 (app dd, $J = 8.7, 6.0$ Hz, 2H), 3.18 (app dd, $J = 8.7, 6.0$ Hz, 2H), 2.83 (s, 3H), 2.35 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 165.47, 162.71, 161.45, 160.59, 159.06, 151.49, 148.51, 141.28, 137.71,

136.78, 134.97, 134.37, 132.67, 130.74, 128.20, 127.53, 124.33, 123.77, 115.50, 113.33, 108.31, 48.18, 45.00, 42.23, 31.46, 17.74; HRMS: (M+H⁺) calcd. 494.2304; found 494.2296.



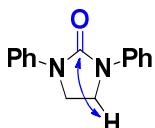
41 *hmbc correlation*

Yield: 32% (by ¹H NMR using 1,1,2,2-tetrachloroethane as standard), 21% (isolated); light pink solid; ¹H NMR (400 MHz, CDCl₃) δ 8.35 (s, 2H, –CHO), 7.40 (dd, *J* = 8.1, 7.4 Hz, 4H), 7.31 (app t, *J* = 7.4 Hz, 2H), 7.08 (d, *J* = 8.1 Hz, 4H), 4.08 (s, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 162.68, 140.68, 129.72, 126.88, 123.51, 43.29; HRMS: (M+H⁺) calcd. 269.1290; found 269.1292.



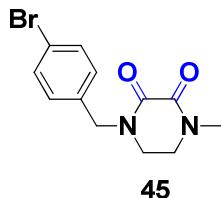
42 *hmbc correlation*

Yield: 30% (by ¹H NMR using 1,1,2,2-tetrachloroethane as standard), 23% (isolated), light pink solid; ¹H NMR (400 MHz, CDCl₃) δ 7.55 – 7.35 (m, 8H), 7.31 (app. tt, *J* = 6.3, 1.7 Hz, 2H), 4.13 (s, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 156.90, 140.75, 129.35, 127.33, 124.48, 48.00; HRMS: (M+H⁺) calcd. 267.1134; found 267.1133.

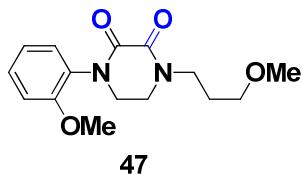


43 *hmbc correlation*

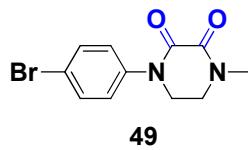
Yield: 19% (isolated) or 18% (isolated) from 1,2-diphenylethylamine; purple solid, ¹H NMR (400 MHz, CDCl₃) δ 7.53 (d, *J* = 8.4 Hz, 4H), 7.35 – 7.26 (m, 4H), 7.02 (app t, *J* = 7.4 Hz, 2H), 3.90 (s, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 155.06, 140.01, 128.92, 123.36, 123.09, 41.99; HRMS: (M+H⁺) calcd. 239.1184; found 239.1184.



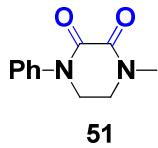
Yield: 51% (isolated); white crystalline solid; ^1H NMR (400 MHz, CDCl_3) δ 7.40 (d, $J = 8.3$ Hz, 2H), 7.11 (d, $J = 8.3$ Hz, 2H), 4.55 (s, 2H), 3.45 – 3.40 (m, 2H), 3.40 – 3.34 (m, 2H), 3.01 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 157.44, 157.38, 134.69, 132.05, 130.08, 122.13, 50.02, 46.33, 43.36, 35.03; HRMS: $(\text{M}+\text{H}^+)$ calcd. 297.0239; found 297.0243.



Yield: 47% (isolated), white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.34 (ddd, $J = 7.9, 6.6, 1.7$ Hz, 1H), 7.27 (dd, $J = 6.6$ Hz, 1.7 Hz, 1H), 7.02 (m, 2H), 3.87 (s, 3H), 3.82 (app dd, $J = 7.3, 3.7$ Hz, 2H), 3.72 (app dd, $J = 7.3, 3.7$ Hz, 2H), 3.65 (t, $J = 7.1$ Hz, 2H), 3.50 (t, $J = 6.4$ Hz, 2H), 3.37 (s, 3H), 2.02 – 1.92 (m, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 157.88, 157.15, 154.02, 129.52, 129.01, 128.47, 121.03, 112.21, 69.98, 58.74, 55.76, 47.58, 45.71, 45.41, 27.48; HRMS: $(\text{M}+\text{H}^+)$ calcd. 293.1501; found 293.1498.

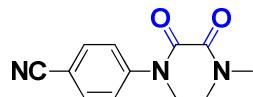


Yield: 43% (isolated); light yellow solid; ^1H NMR (400 MHz, CDCl_3) δ 7.45 (d, $J = 8.7$ Hz, 2H), 7.16 (d, $J = 8.7$ Hz, 2H), 3.89 (dd, $J = 6.5, 4.9$ Hz, 2H), 3.63 (dd, $J = 6.5, 4.9$ Hz, 2H), 3.08 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 157.50, 156.49, 139.71, 132.32, 126.07, 120.53, 47.16, 46.59, 35.18; $(\text{M}+\text{H}^+)$ calcd. 283.0082; found 283.0083.



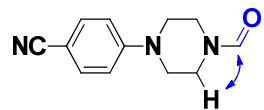
Yield: 23% (isolated); white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.34 (dd, $J = 8.9, 7.4$ Hz, 2H), 7.26 (d, $J = 8.9$ Hz, 2H), 7.20 (app t, $J = 7.4$ Hz, 1H), 3.92 (dd, $J = 8.4, 6.5$ Hz, 2H), 3.63 (dd, $J =$

8.4, 6.5 Hz, 2H), 3.08 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 157.84, 156.62, 140.80, 129.26, 127.21, 124.53, 47.37, 46.70, 35.13;



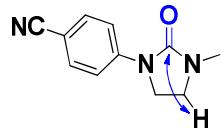
53

Yield: 22% (isolated yield); ^1H NMR (400 MHz, CDCl_3) δ 7.71 (d, $J = 6.9$ Hz, 2H), 7.53 (d, $J = 8.2$ Hz, 2H), 4.04 (app. s, 2H), 3.74 (app. s, 2H), 3.18 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 157.09, 156.41, 144.36, 133.12, 124.45, 118.21, 110.27, 46.79, 46.53, 35.35; HRMS: $(\text{M}+\text{H}^+)$ calcd. 230.0930; found 230.0934.



54 *hmhc correlation*

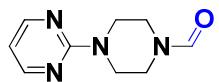
Yield: 20% (isolated); white solid; ^1H NMR (400 MHz, CDCl_3) δ 8.05 (s, 1H, $-\text{CHO}$), 7.46 (d, $J = 8.9$ Hz, 2H), 6.83 (d, $J = 8.9$ Hz, 2H), 3.63 (app dd, $J = 6.3, 4.1$ Hz, 2H), 3.47 (app dd, $J = 6.0, 4.5$ Hz, 2H), 3.29 (app dd, $J = 6.3, 4.1$ Hz, 2H), 3.25 (app dd, $J = 6.0, 4.5$ Hz, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 160.76, 153.06, 133.68, 119.64, 115.23, 101.80, 48.43, 47.27, 44.96, 39.55; HRMS: $(\text{M}+\text{H}^+)$ calcd. 216.1137; found 216.1137.



55 *hmhc correlation*

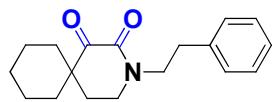
Yield: 17% (isolated); light grey solid; ^1H NMR (400 MHz, CDCl_3) δ 7.67 (d, $J = 8.9$ Hz, 2H), 7.59 (d, $J = 8.9$ Hz, 2H), 3.82 (app dd, $J = 8.6, 6.2$ Hz, 2H), 3.53 (app dd, $J = 8.8, 6.3$ Hz, 2H), 2.92 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 157.18, 144.37, 133.05, 119.41, 116.61, 104.55, 43.63, 41.91, 31.06; HRMS: $(\text{M}+\text{H}^+)$ calcd. 202.0980; found 202.0974.

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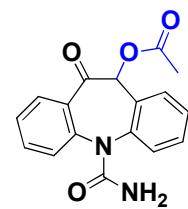
57

Yield: 33% (isolated), colorless wax; ^1H NMR (400 MHz, CDCl_3) δ 8.38 (d, J = 4.8 Hz, 2H), 8.17 (s, 1H, -CHO), 6.60 (app t, J = 4.8 Hz, 1H), 3.98 – 3.93 (m, 2H), 3.93 – 3.87 (m, 2H), 3.72 – 3.62 (m, 2H), 3.53 – 3.44 (m, 2H); ^{13}C NMR (101 MHz, CDCl_3) δ 160.95, 157.77, 157.76, 110.59, 45.43, 44.61, 43.43, 39.99; HRMS: $(\text{M}+\text{H}^+)$ calcd. 193.1089; found 193.1081.



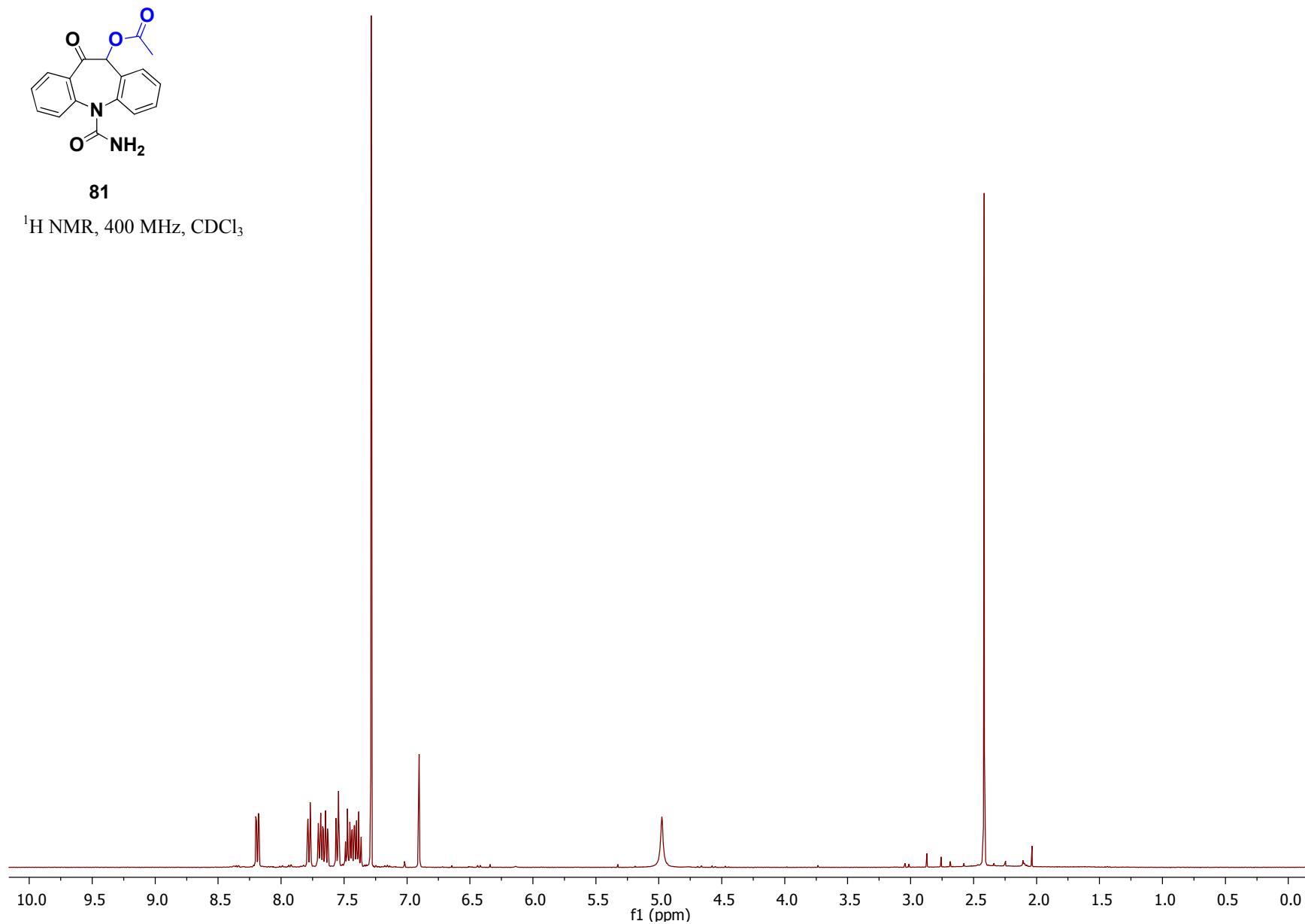
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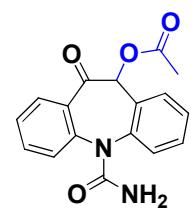
Yield: 19% (isolated), white solid; ^1H NMR (400 MHz, CDCl_3) δ 7.35 – 7.29 (m, 2H), 7.27 – 7.21 (m, 3H), 3.70 (t, J = 7.2 Hz, 2H), 3.26 (t, J = 6.0 Hz, 2H), 2.98 (t, J = 7.2 Hz, 2H), 1.92 (t, J = 6.0 Hz, 2H), 1.74 (m, 2H), 1.60 (m, 2H), 1.42 (m, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 195.98, 158.01, 138.53, 128.85, 128.63, 126.67, 49.78, 45.91, 44.27, 33.11, 30.72, 30.53, 25.59, 21.42; HRMS: $(\text{M}+\text{H}^+)$ calcd. 208.0399; found 208.0400.



81

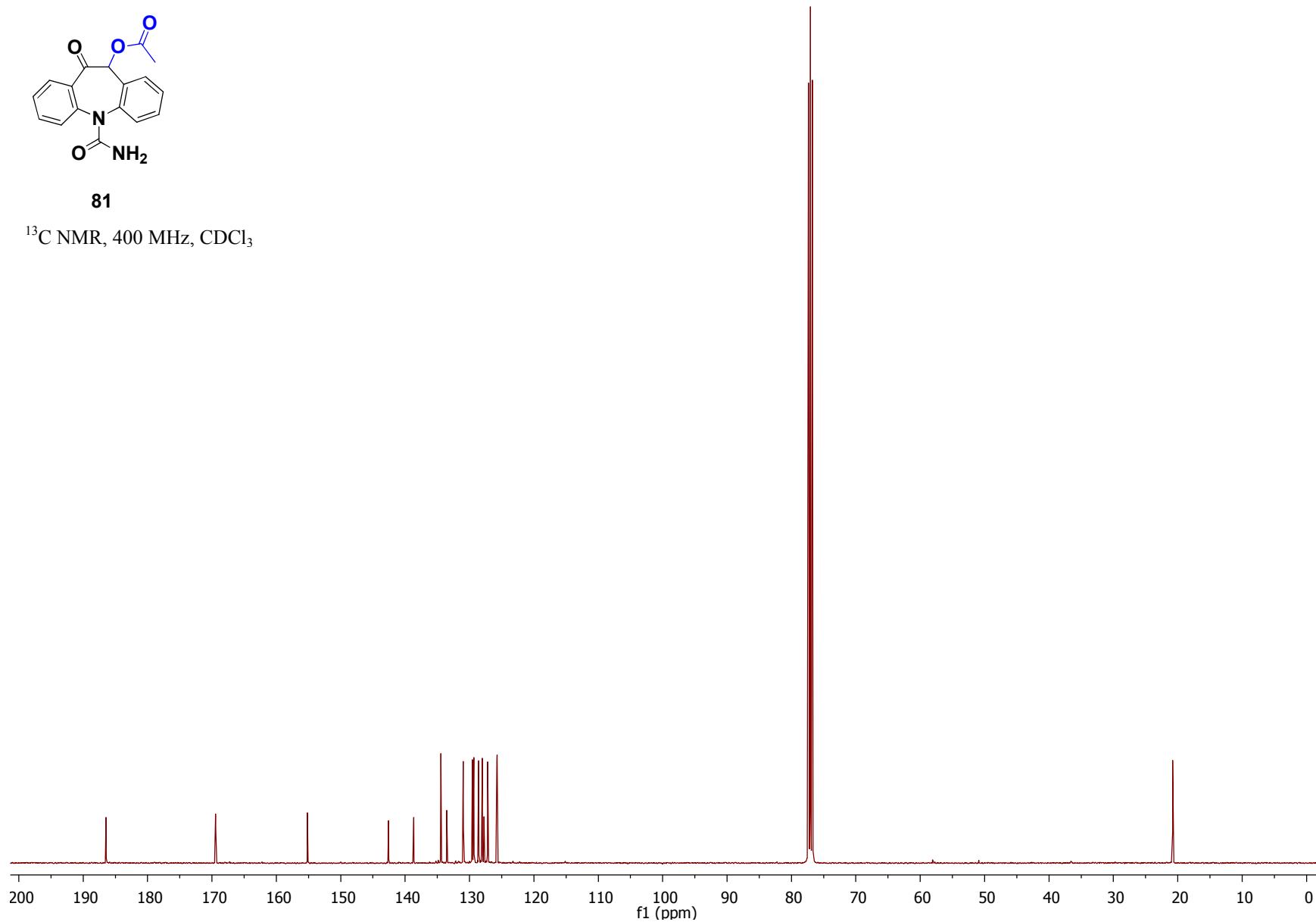
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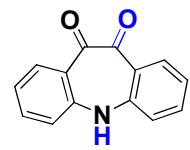




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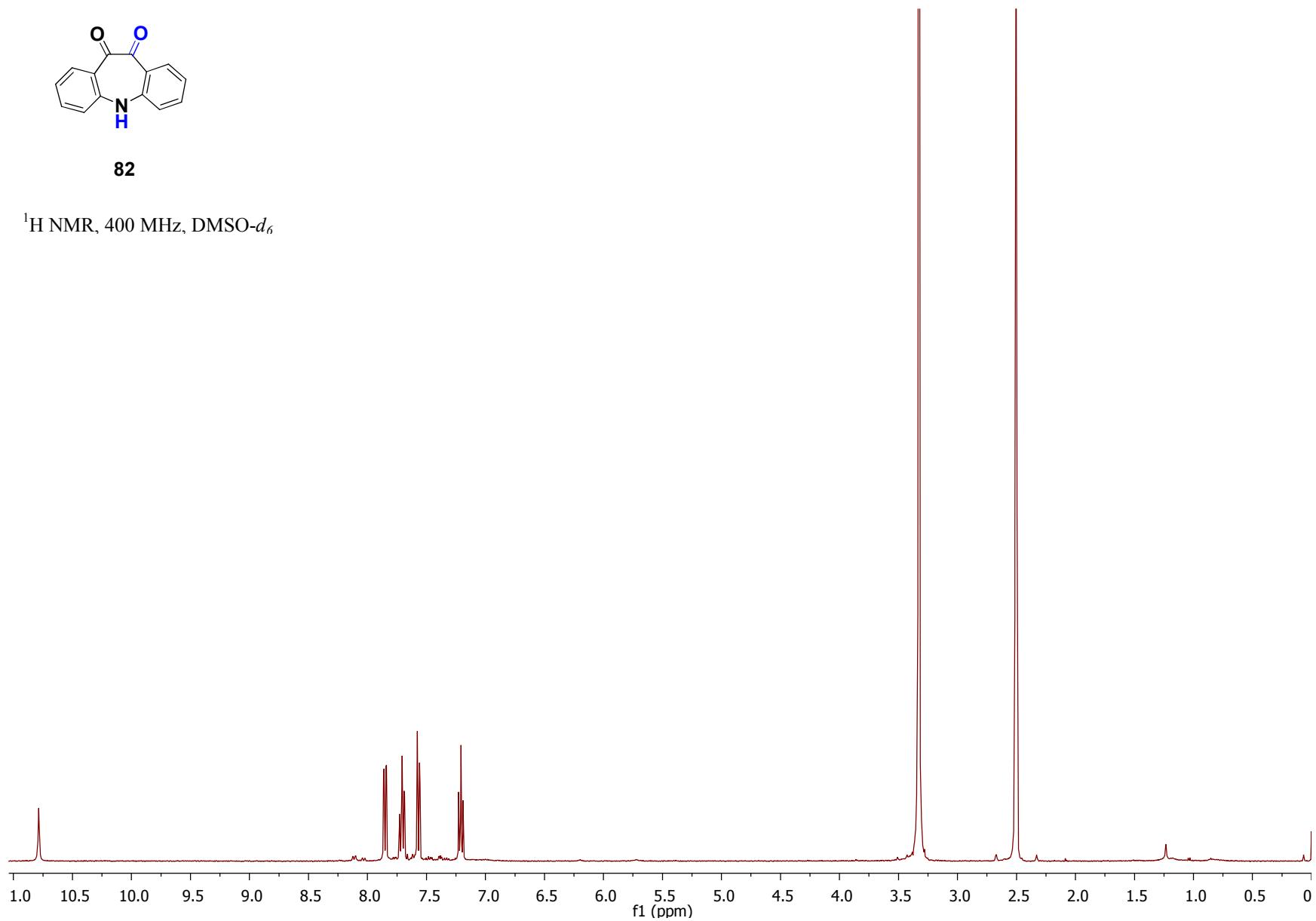
¹³C NMR, 400 MHz, CDCl₃

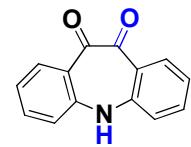




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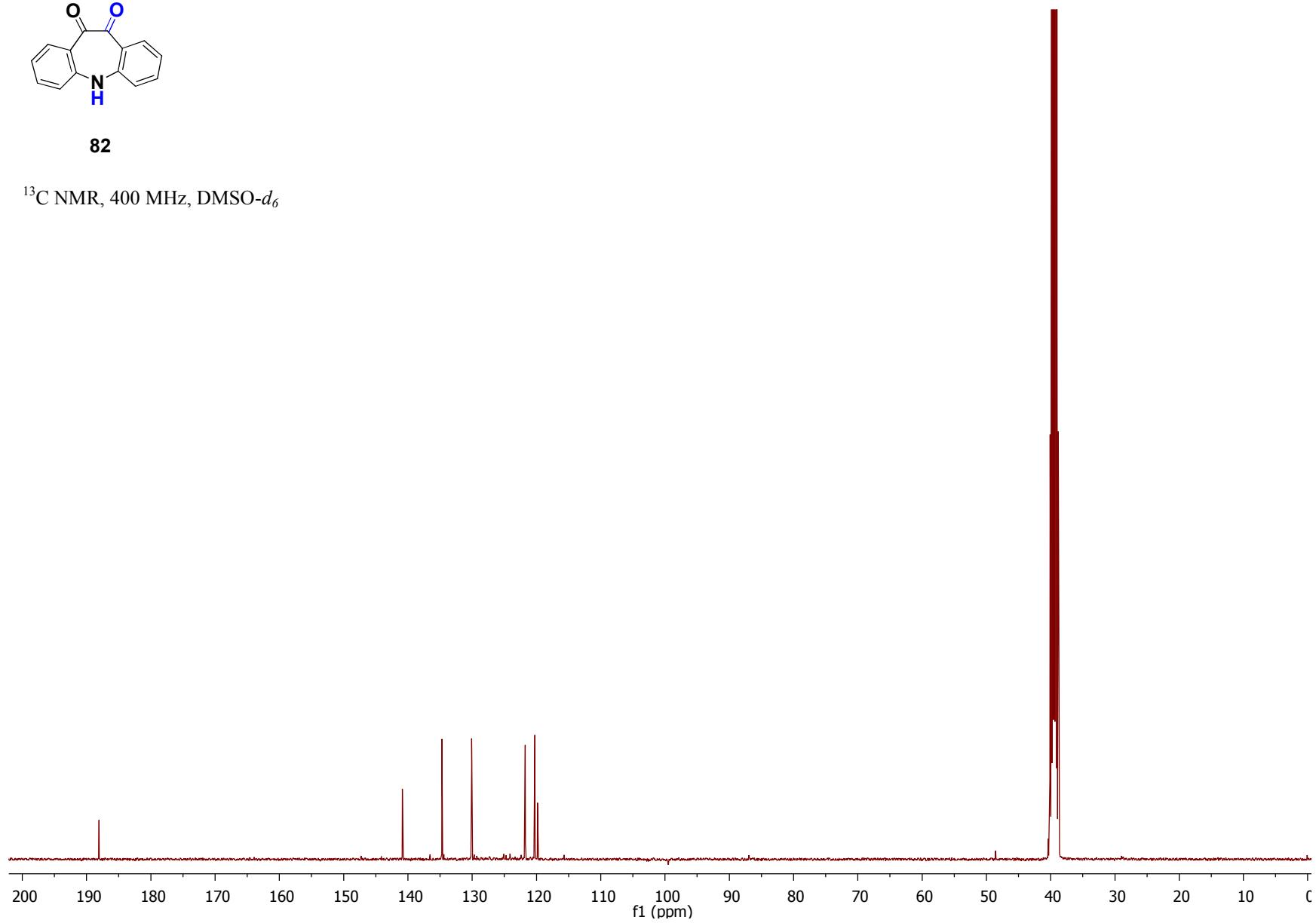
^1H NMR, 400 MHz, $\text{DMSO}-d_6$

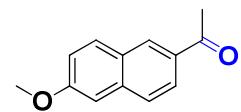




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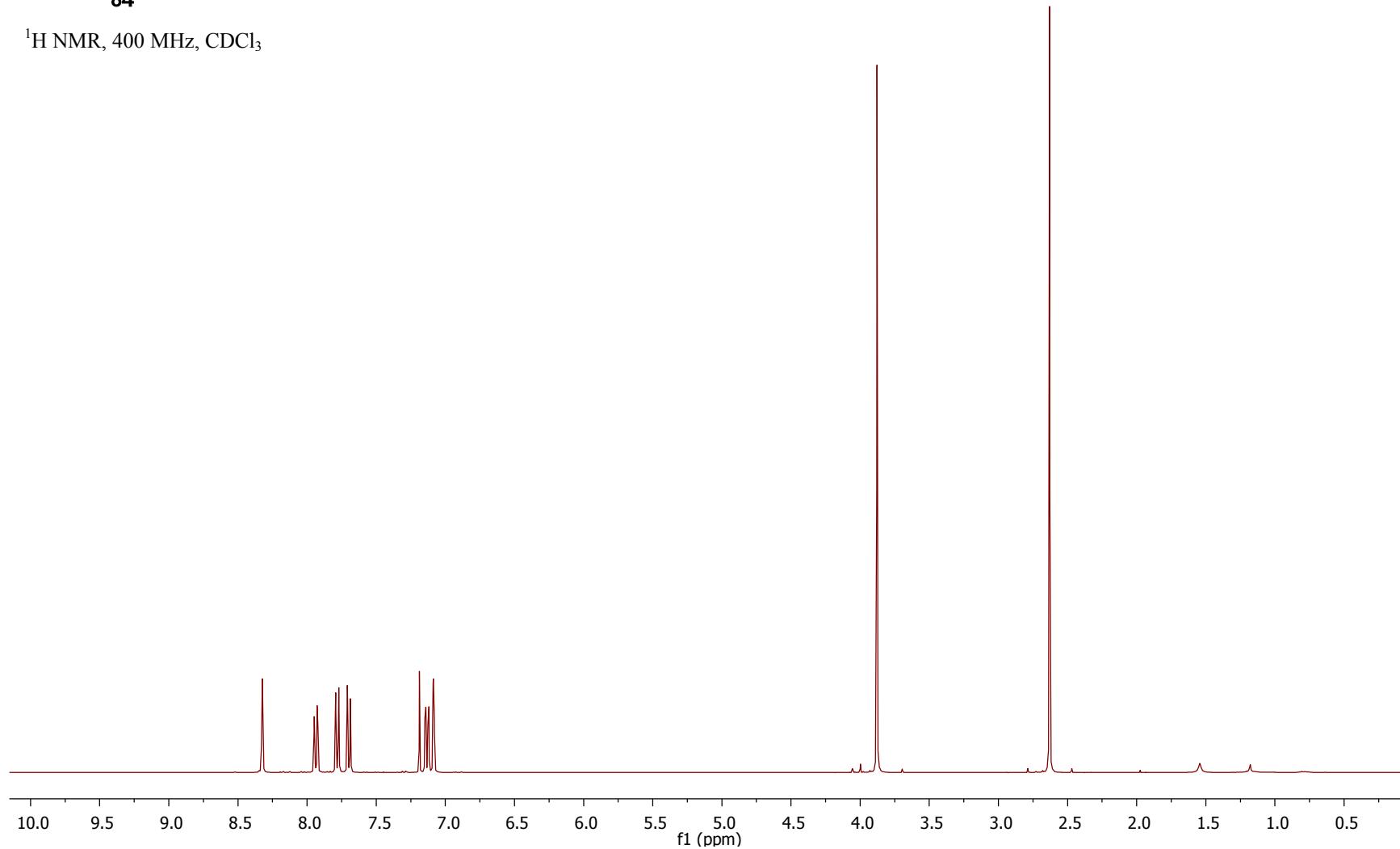
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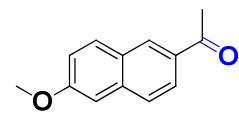




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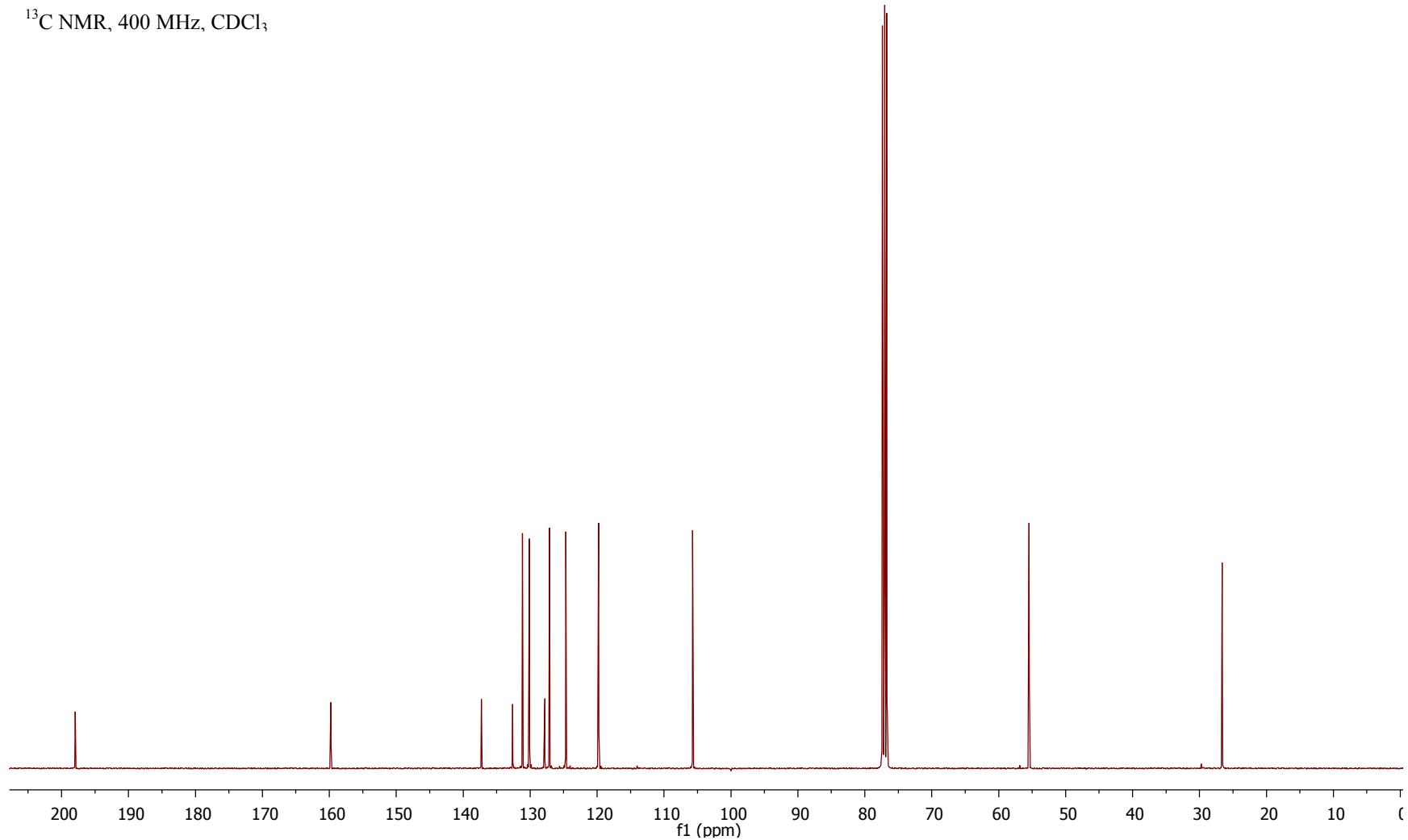
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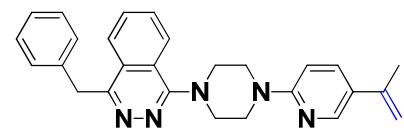




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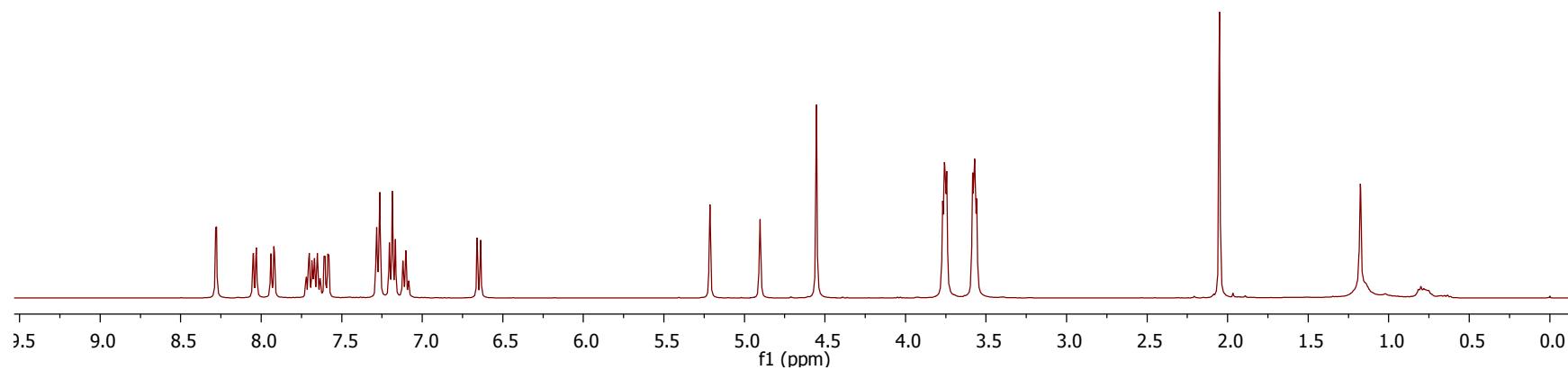
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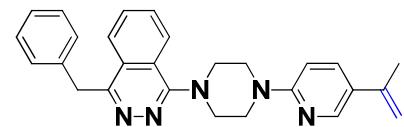




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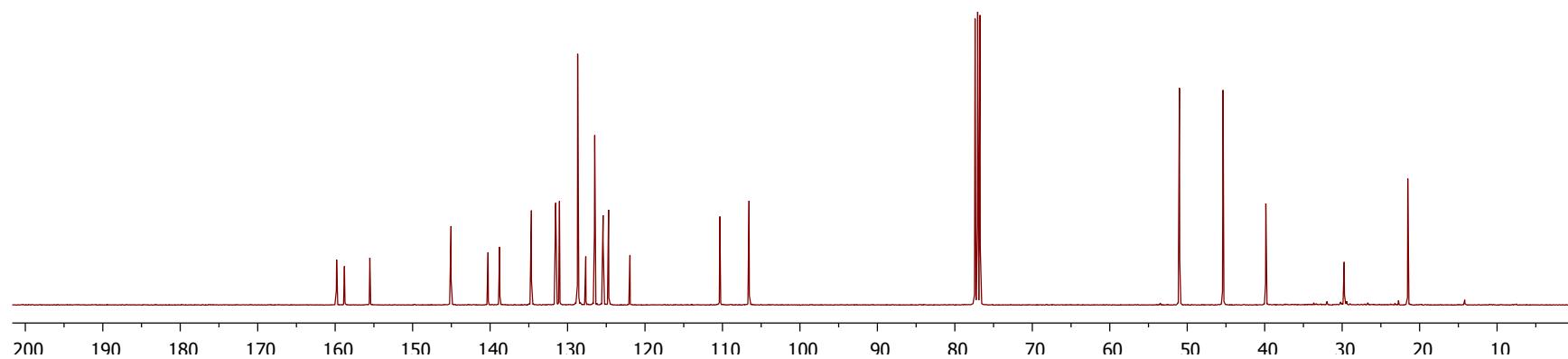
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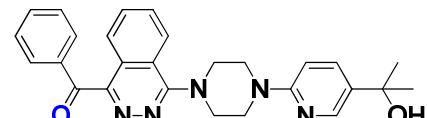




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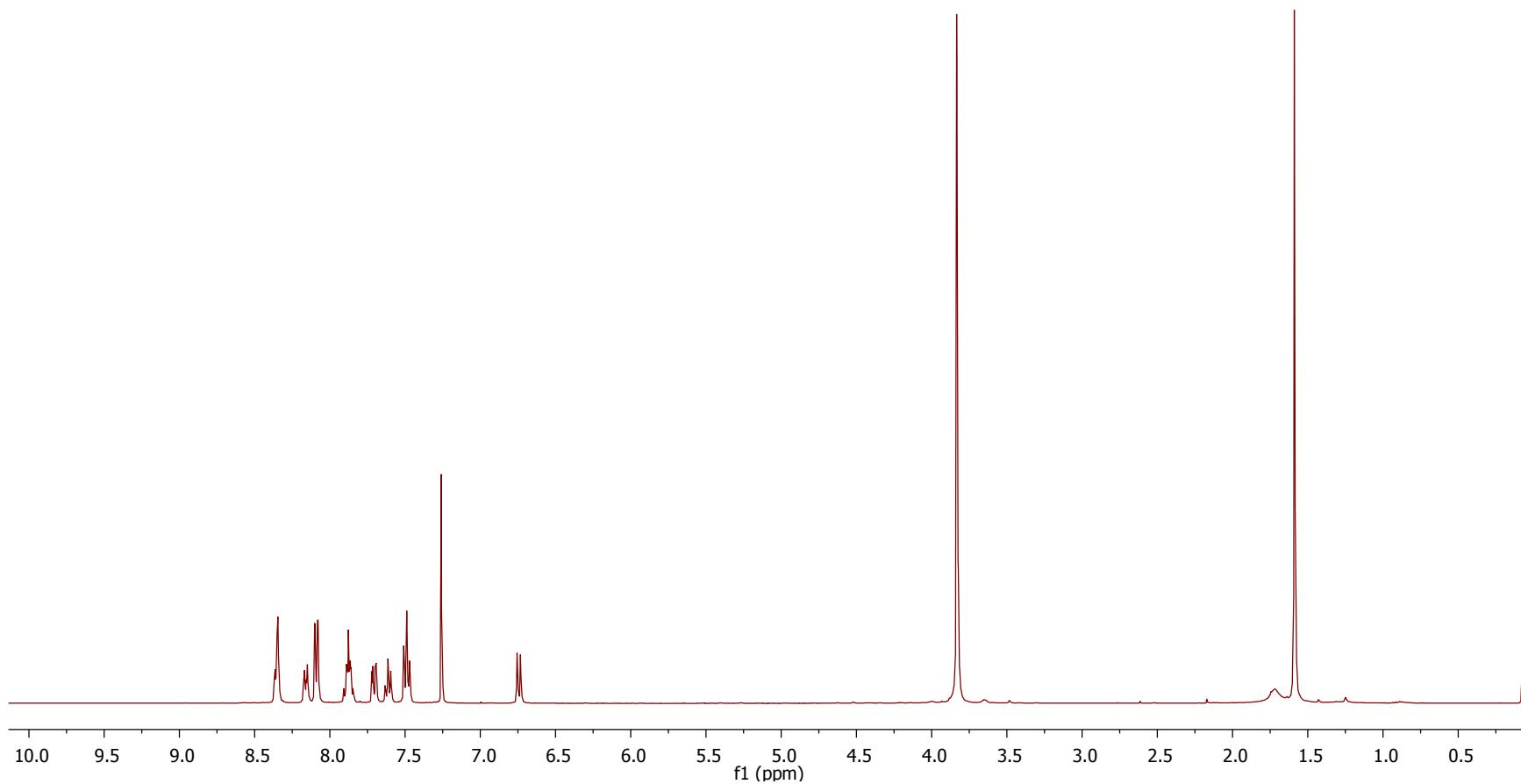
^{13}C NMR, 400 MHz, CDCl_3

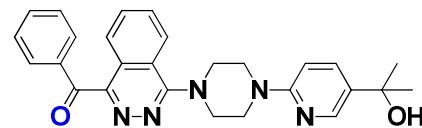




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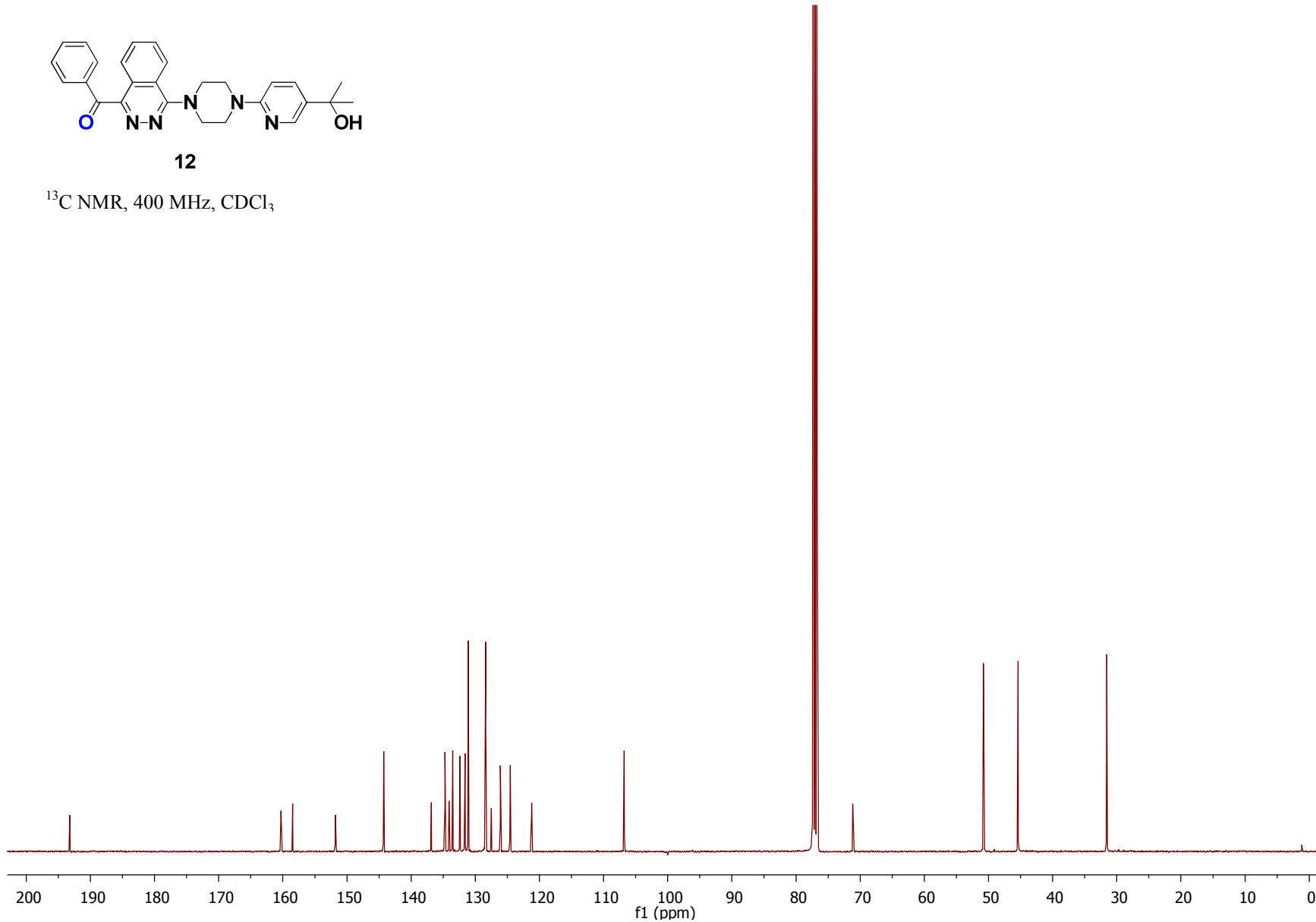
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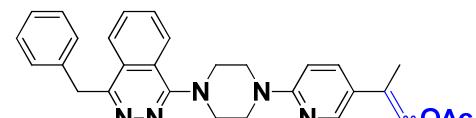




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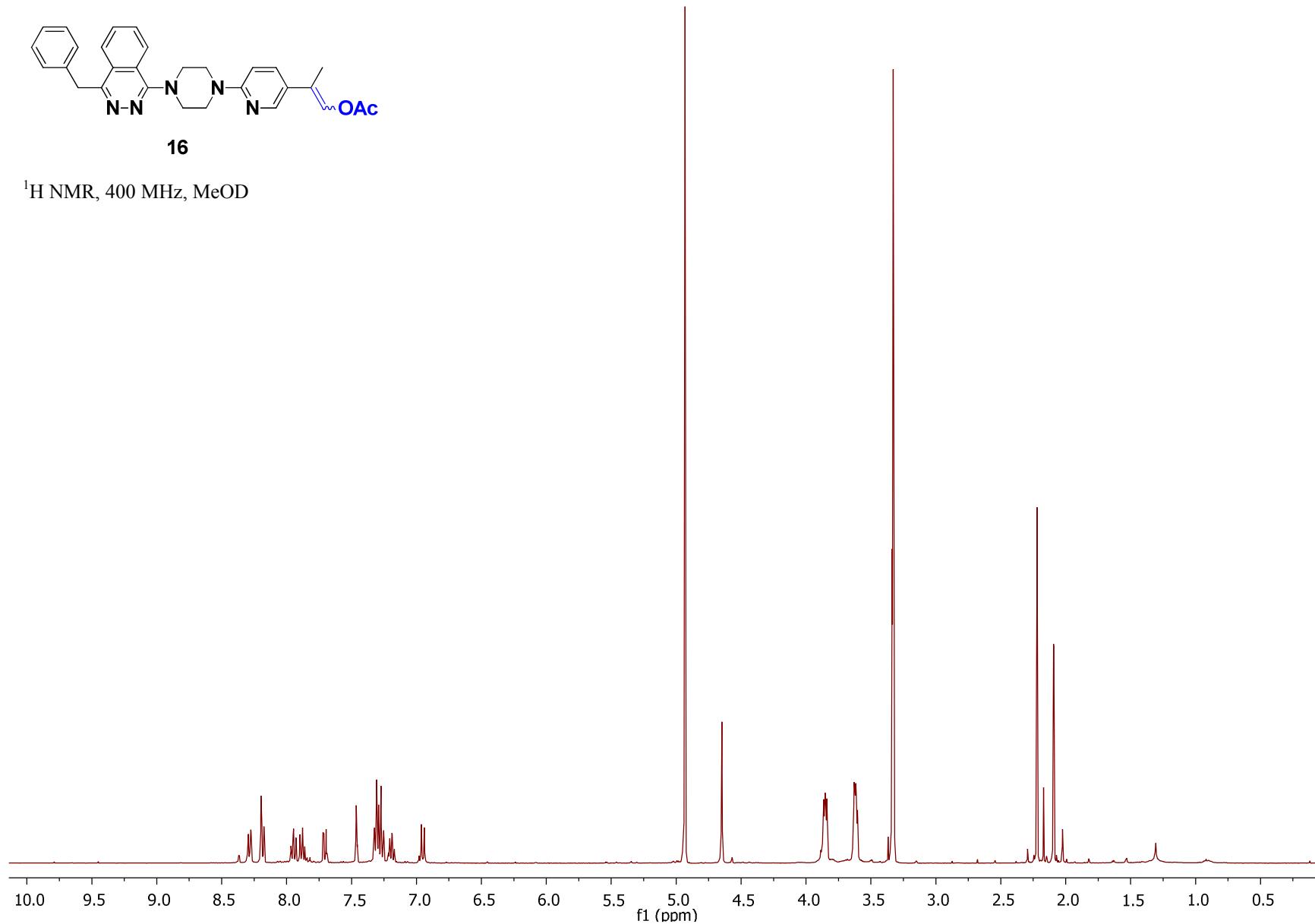
^{13}C NMR, 400 MHz, CDCl_3

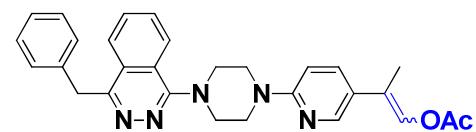




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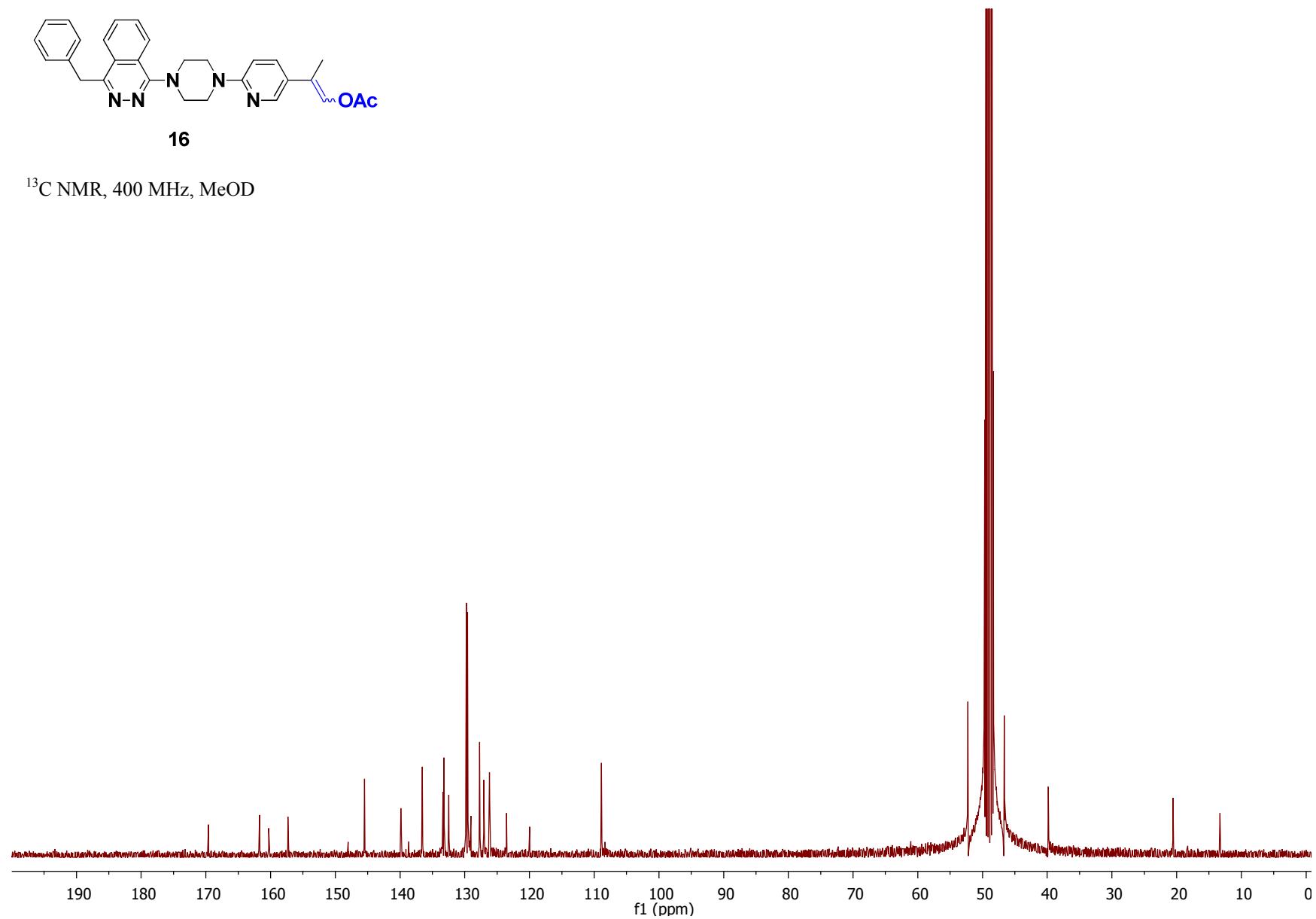
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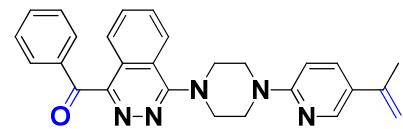




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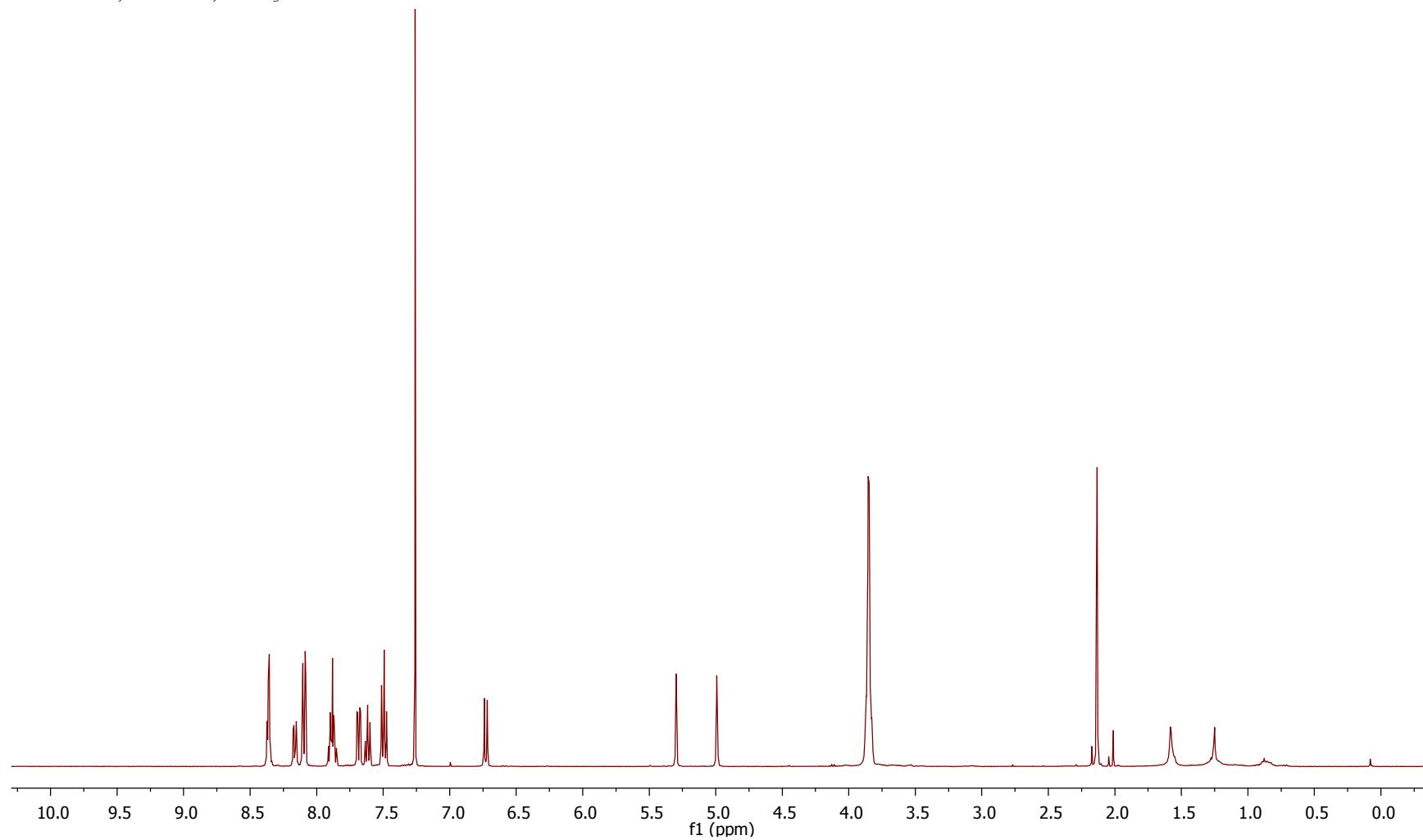
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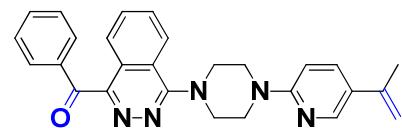




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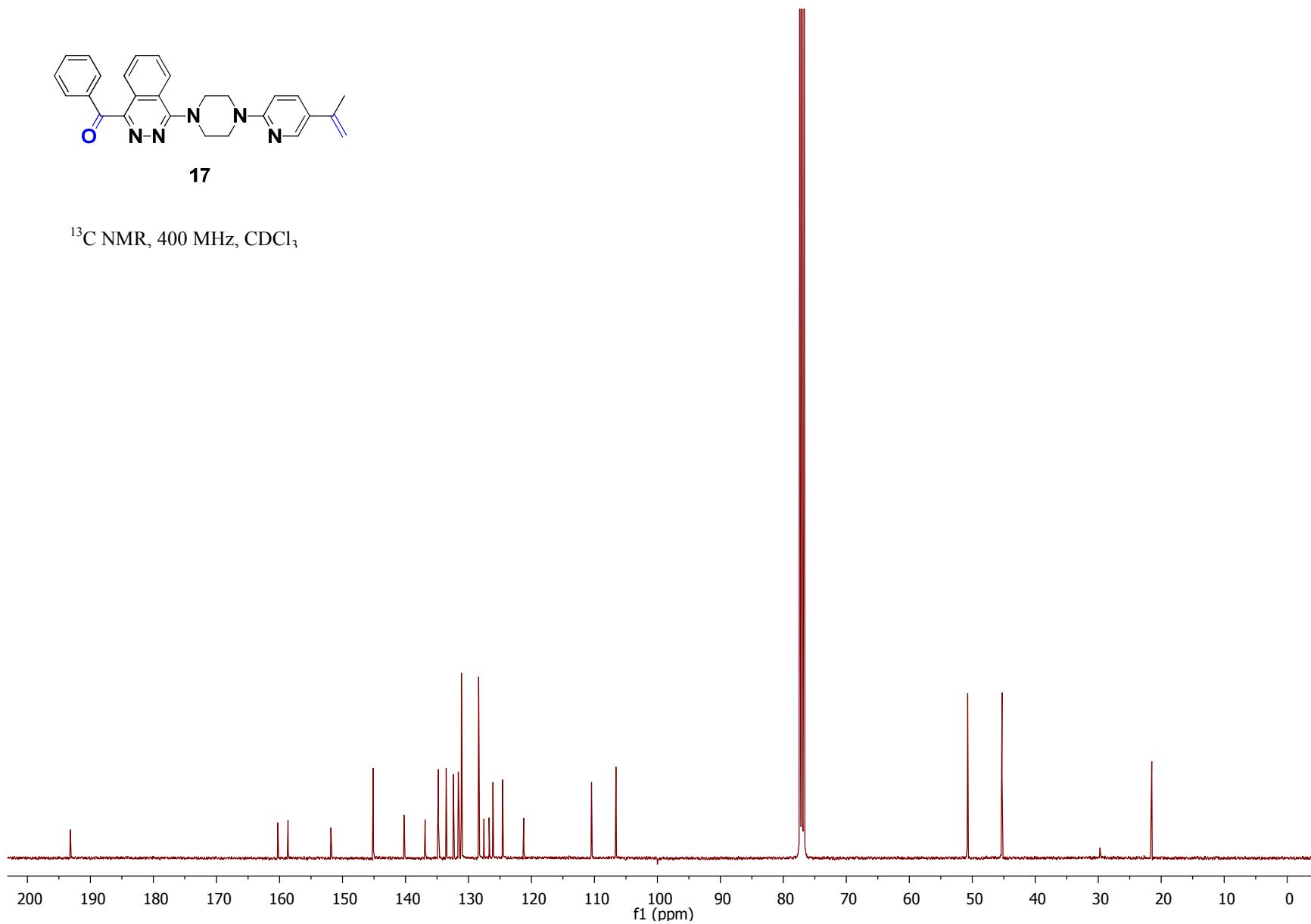
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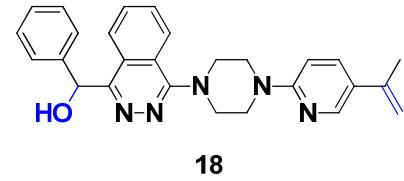




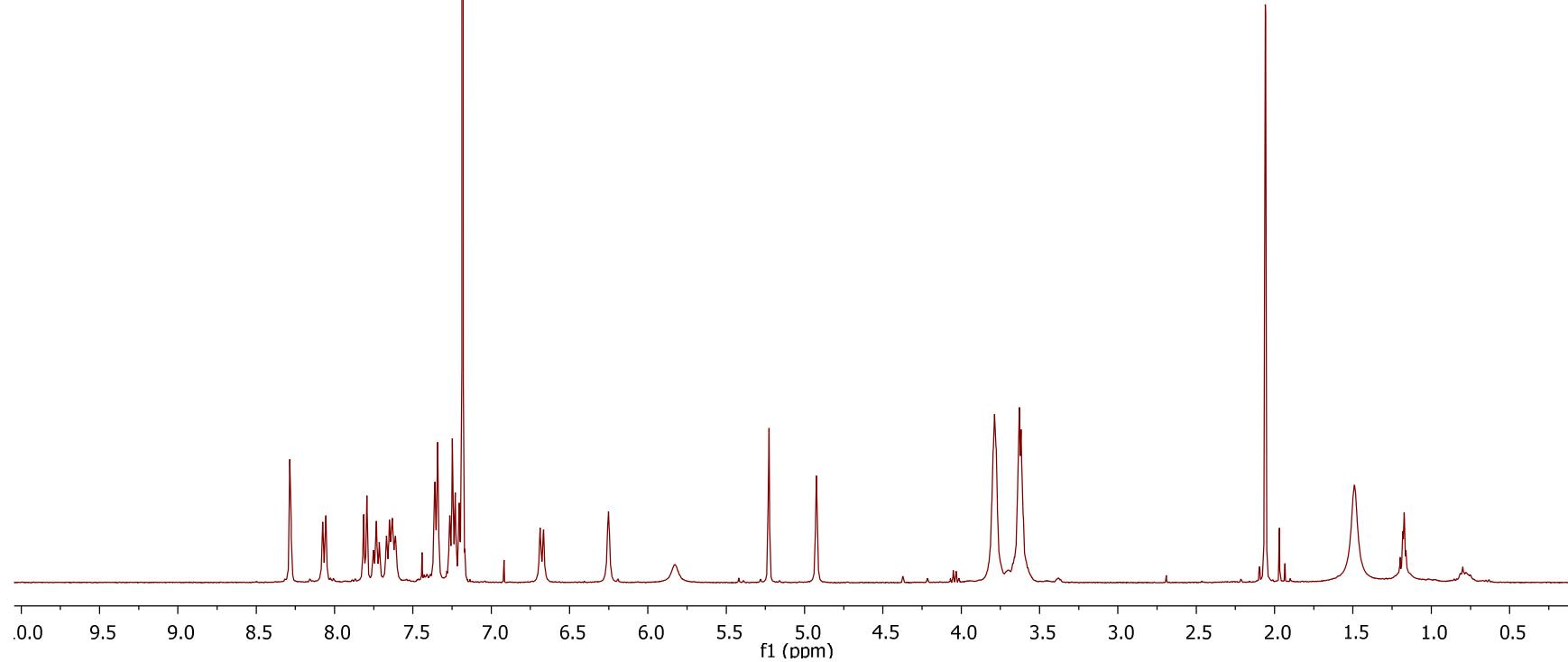
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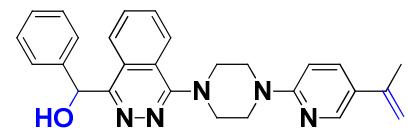
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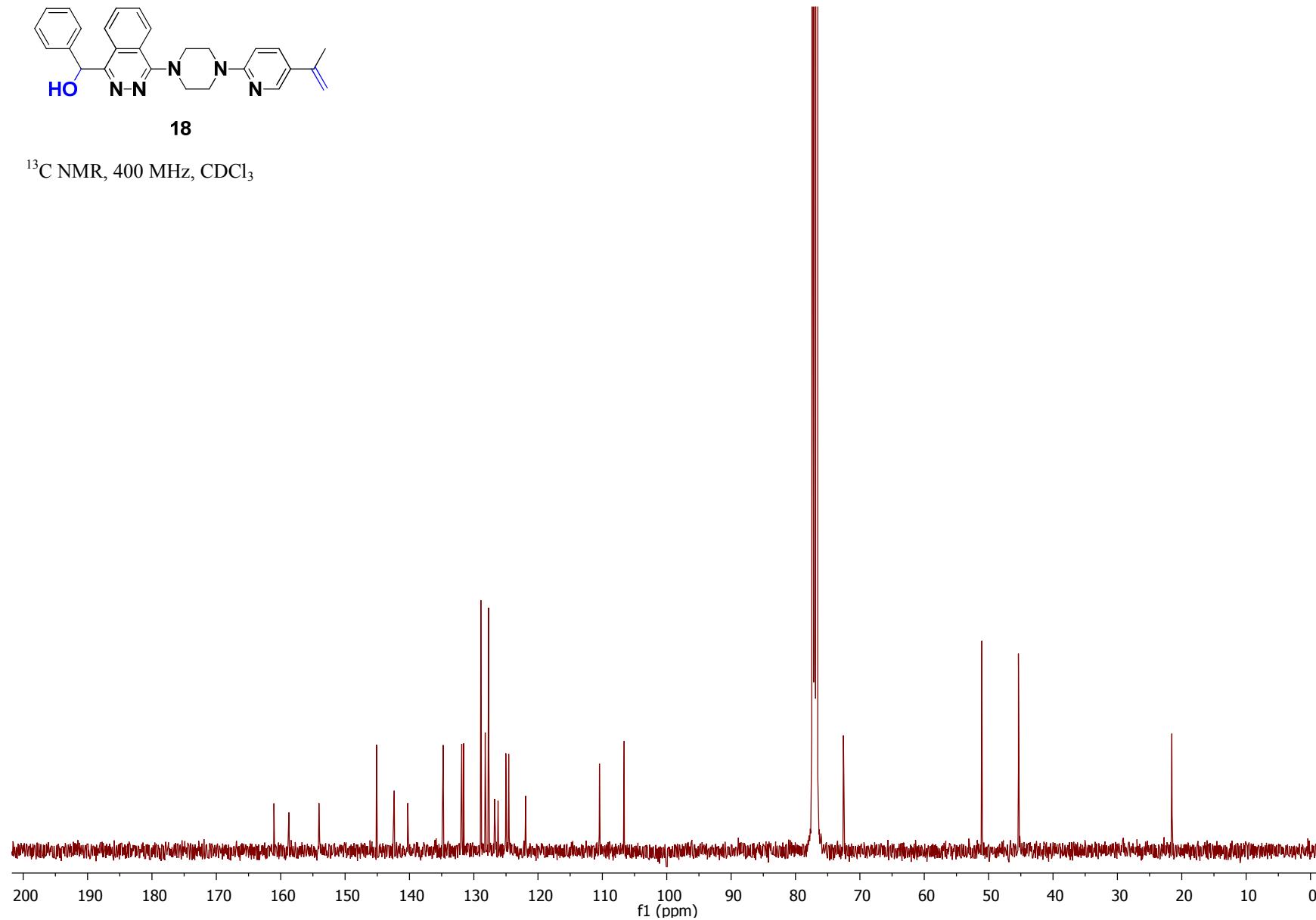
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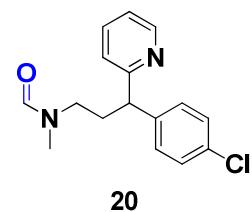




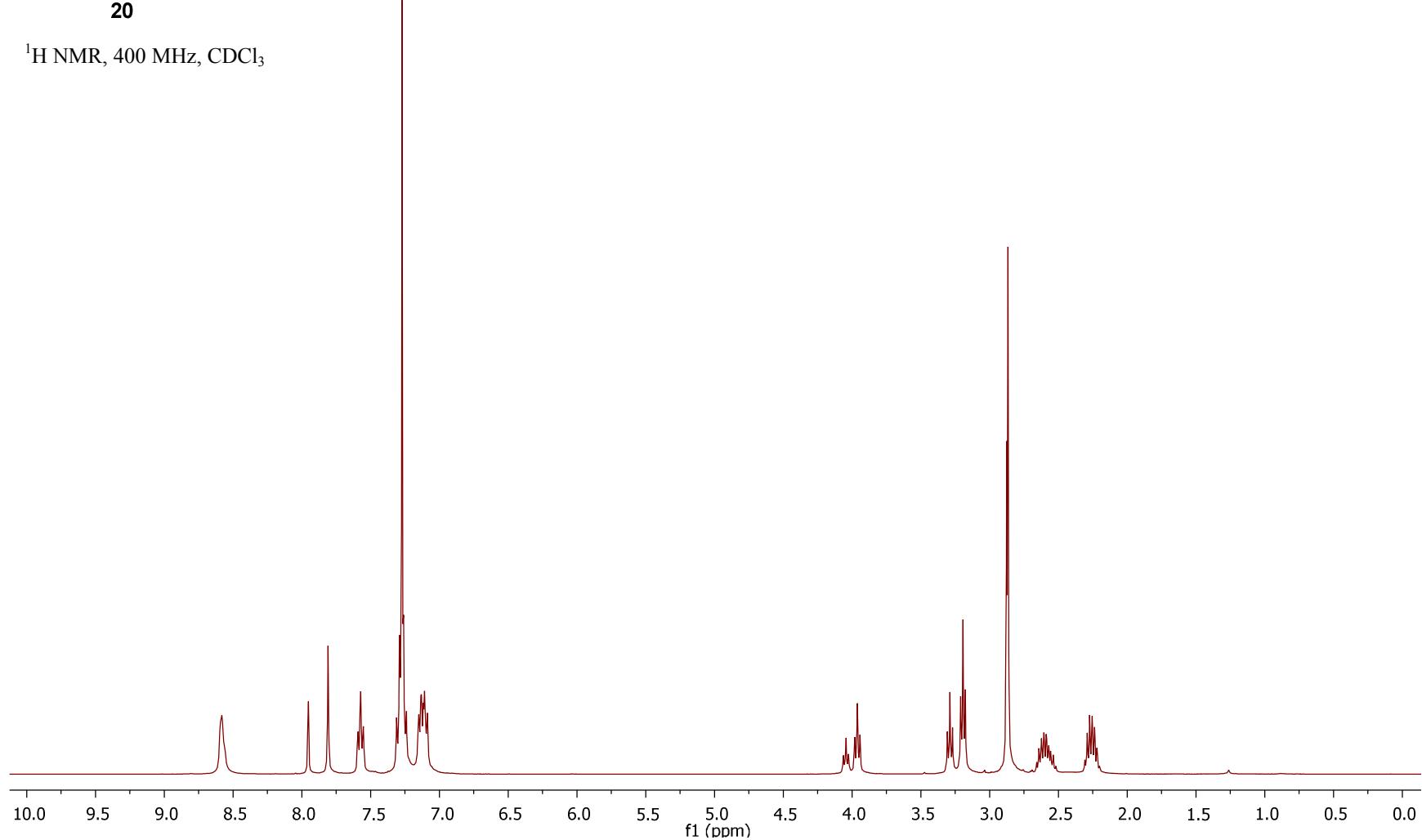
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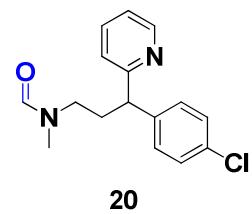
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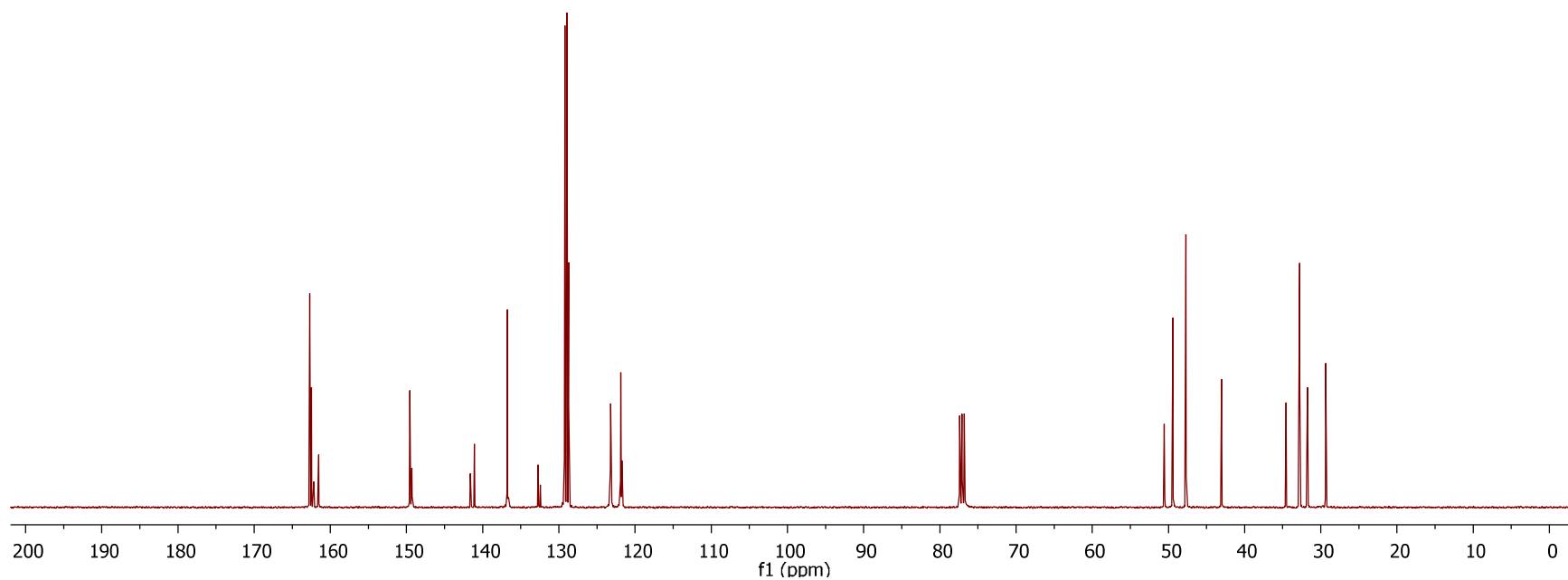


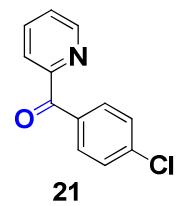
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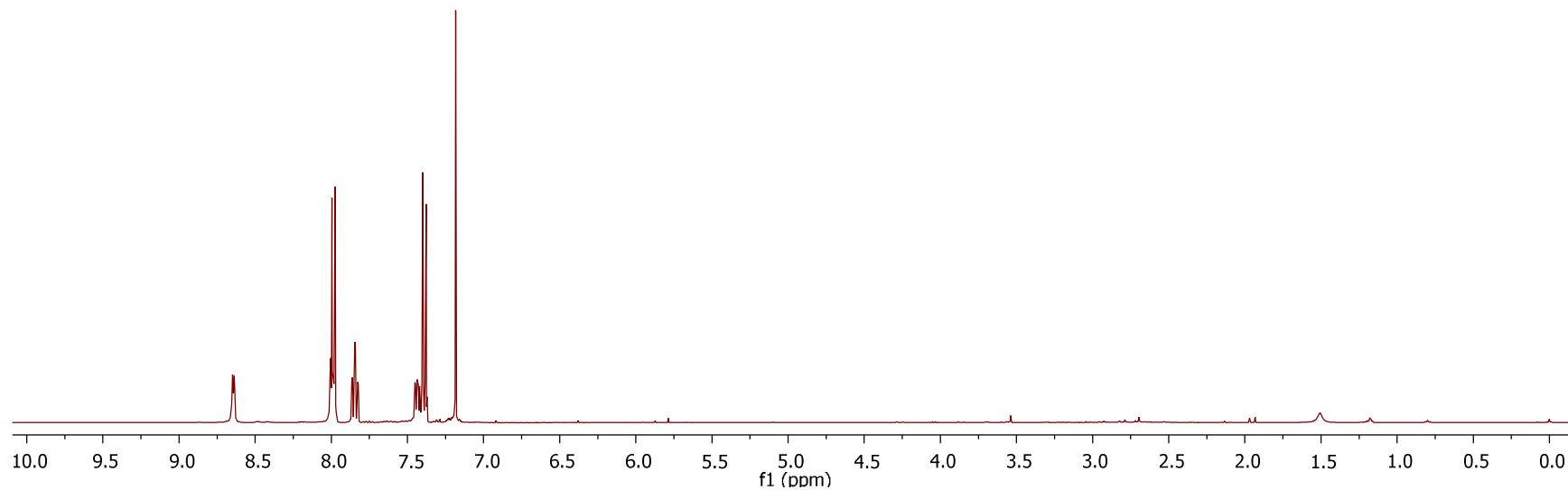


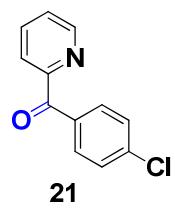
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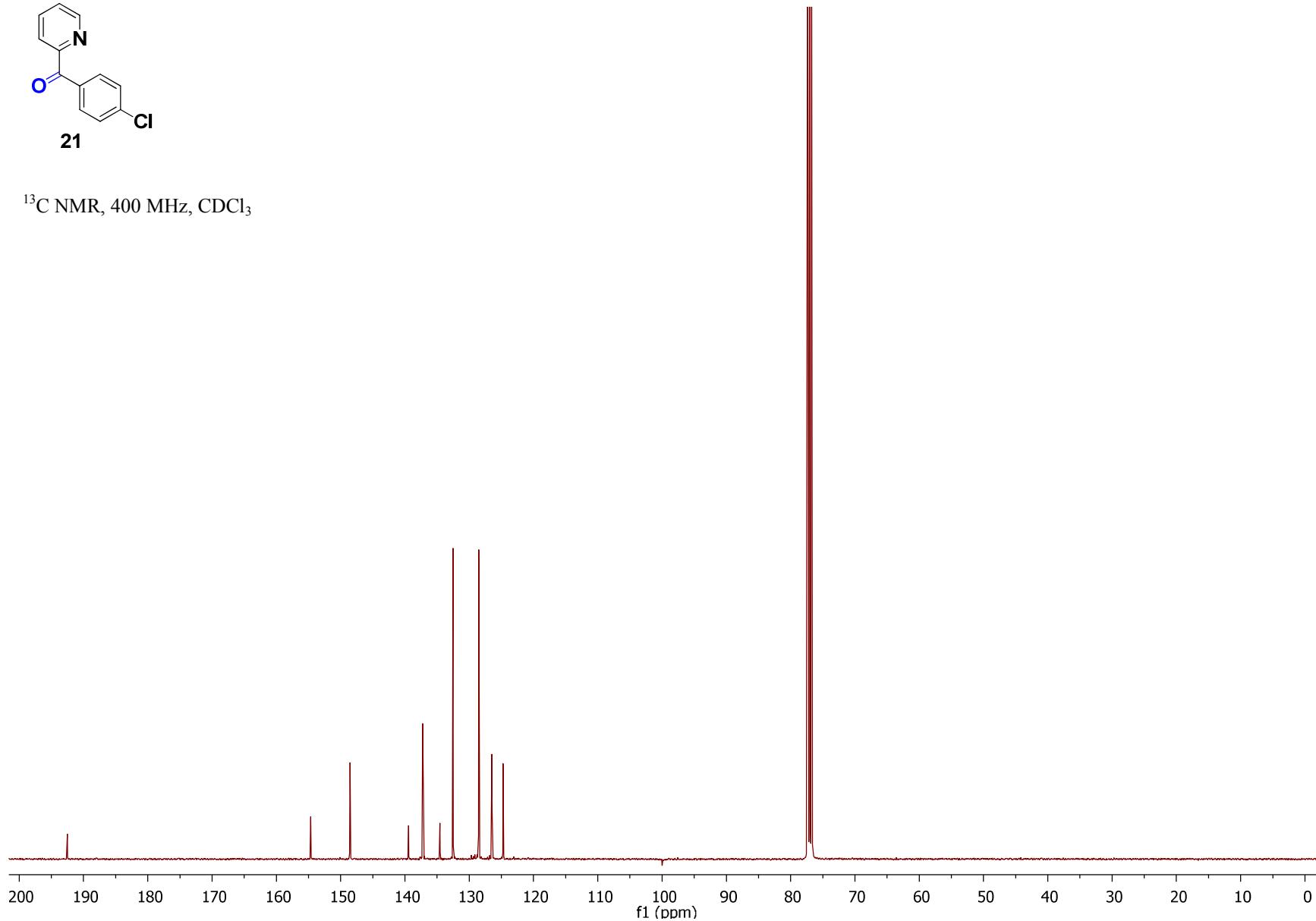


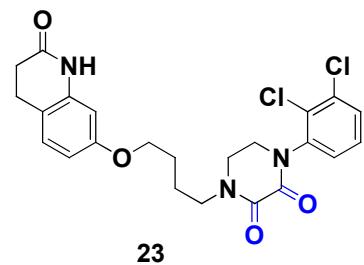
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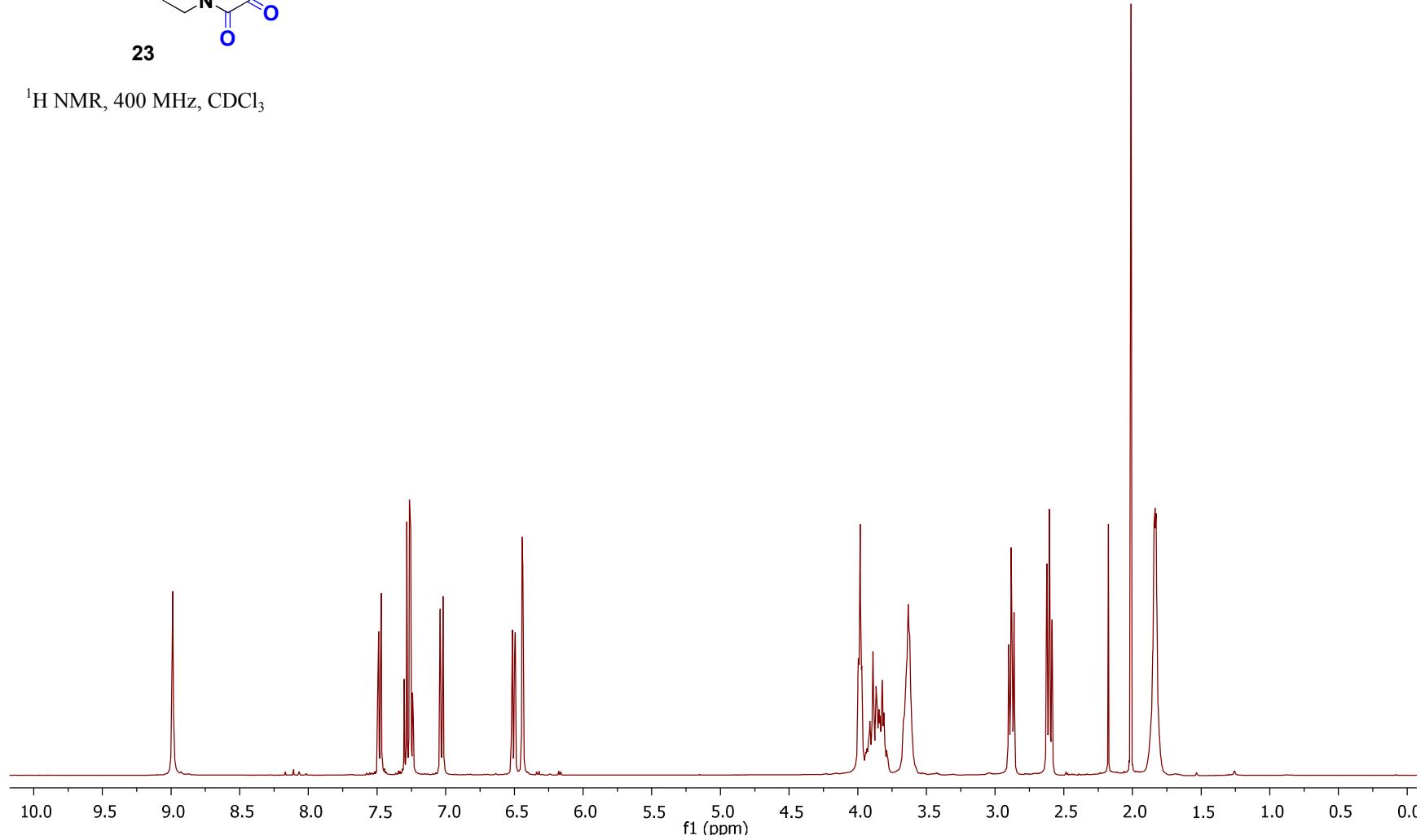


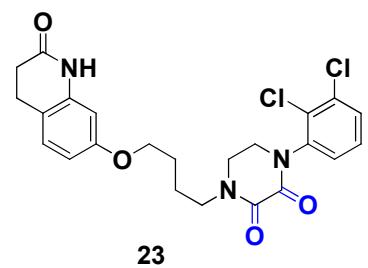
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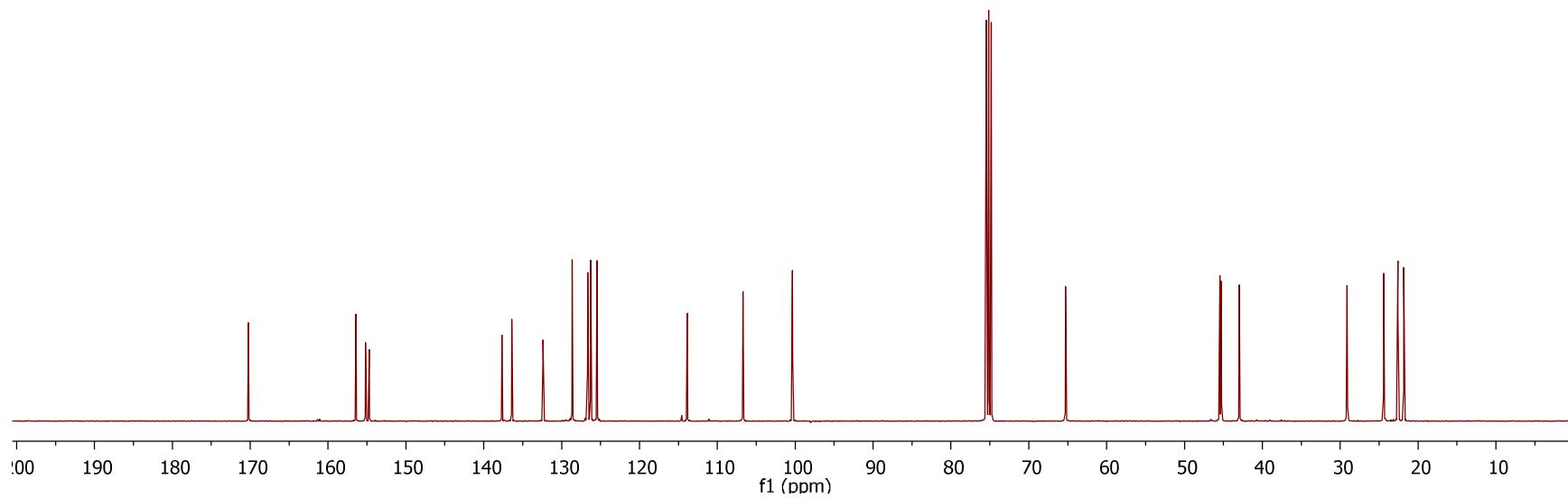


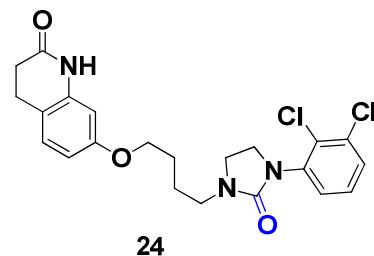
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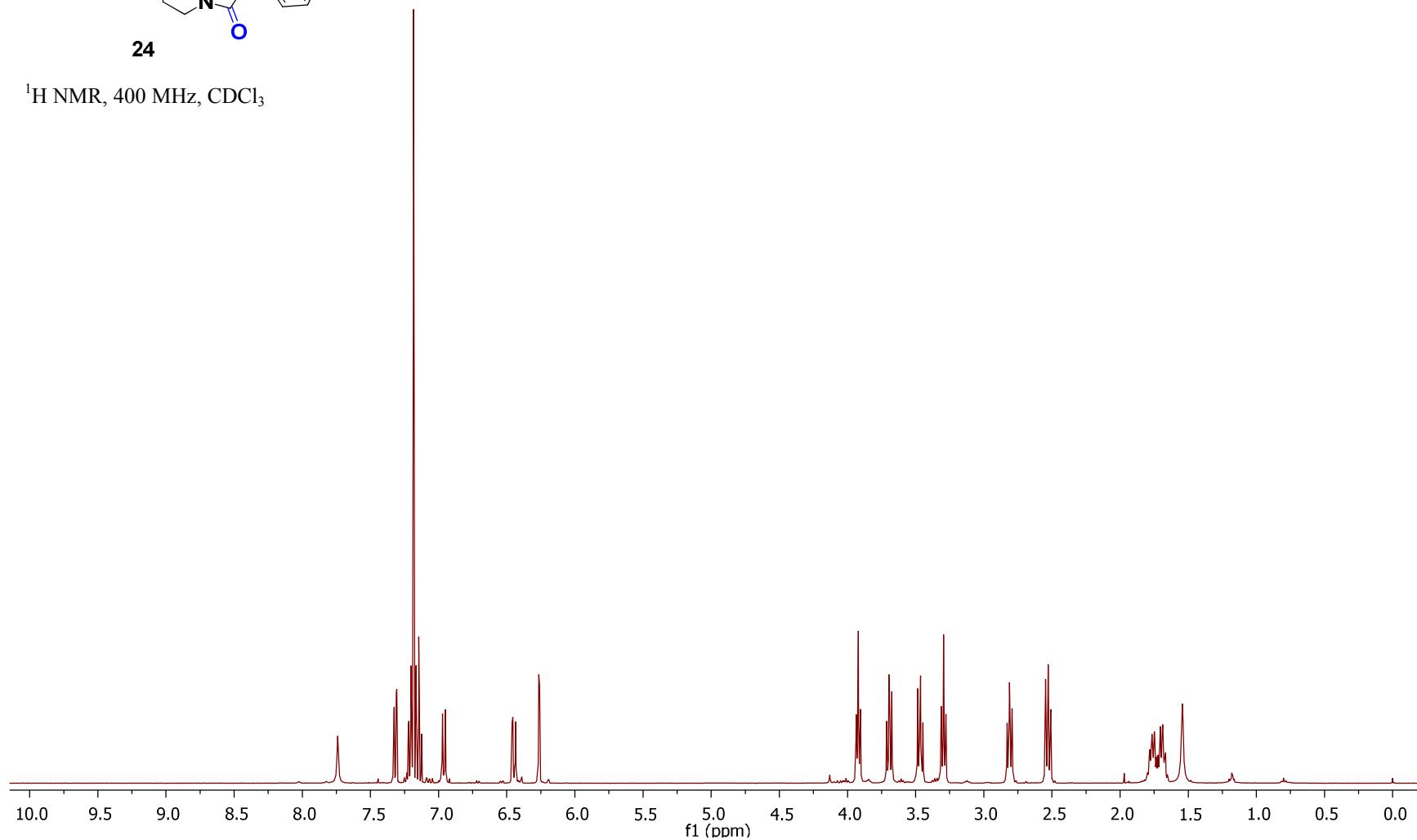


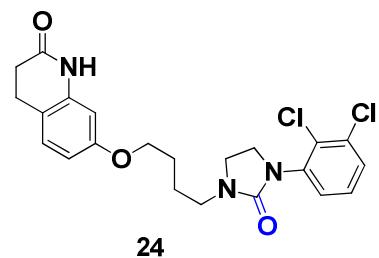
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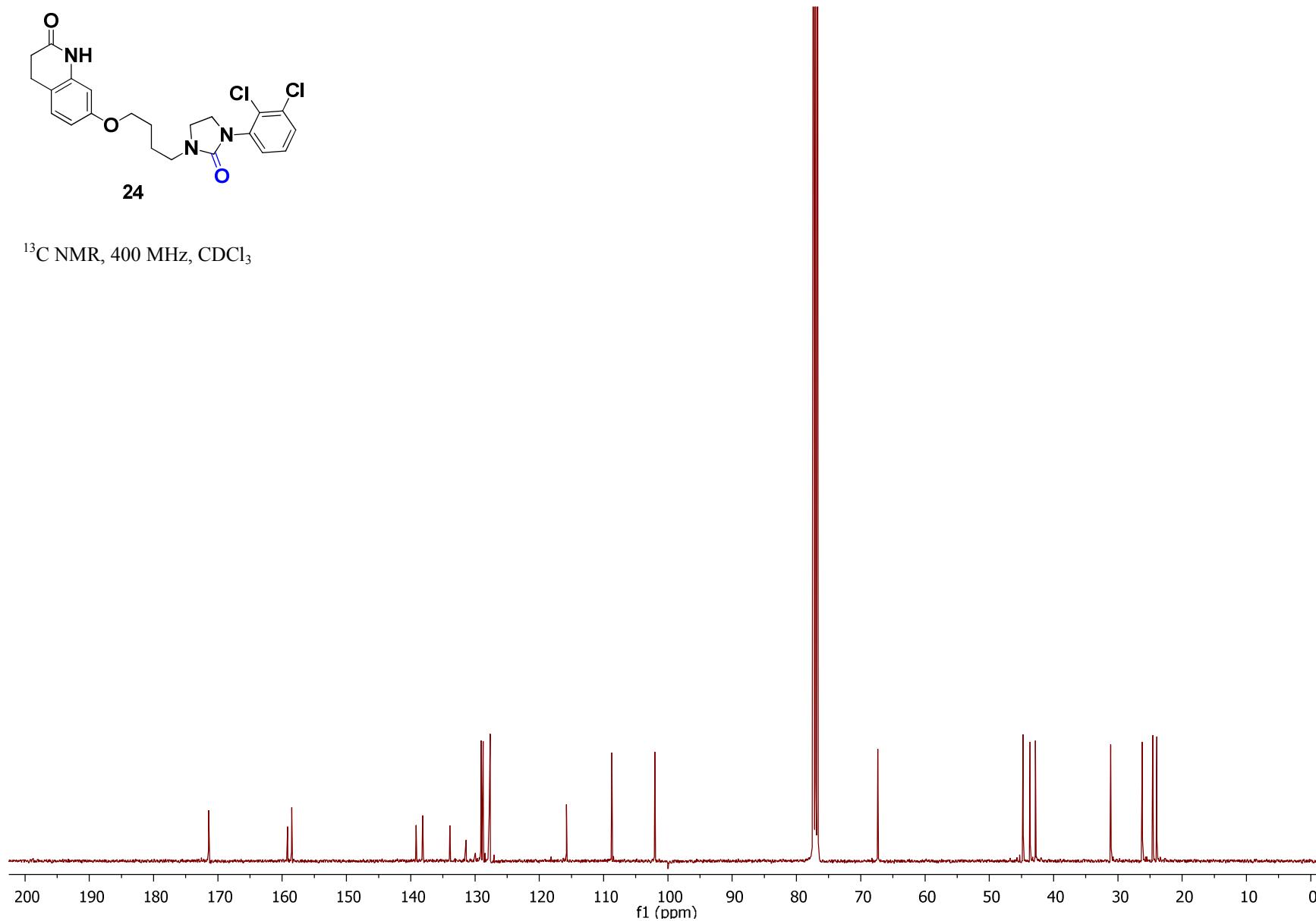


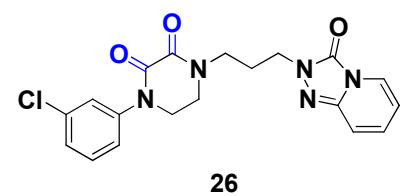
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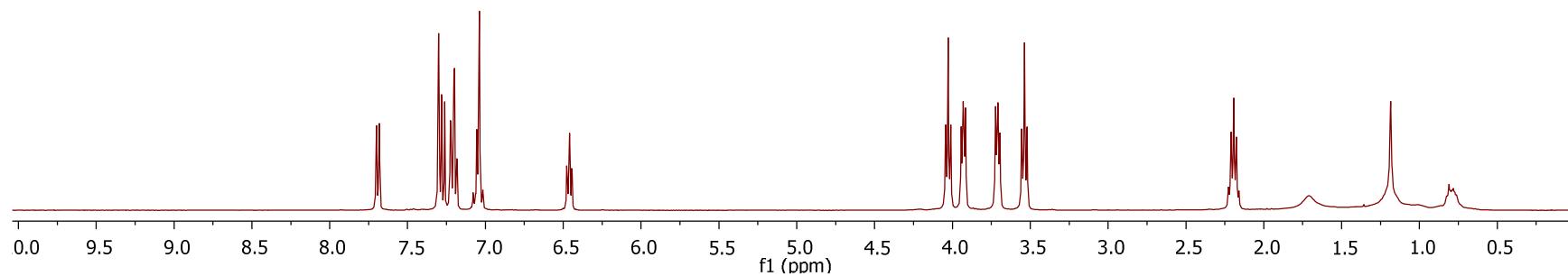


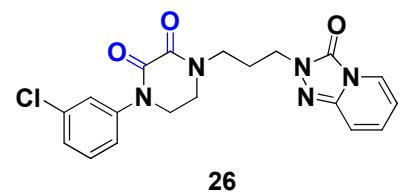
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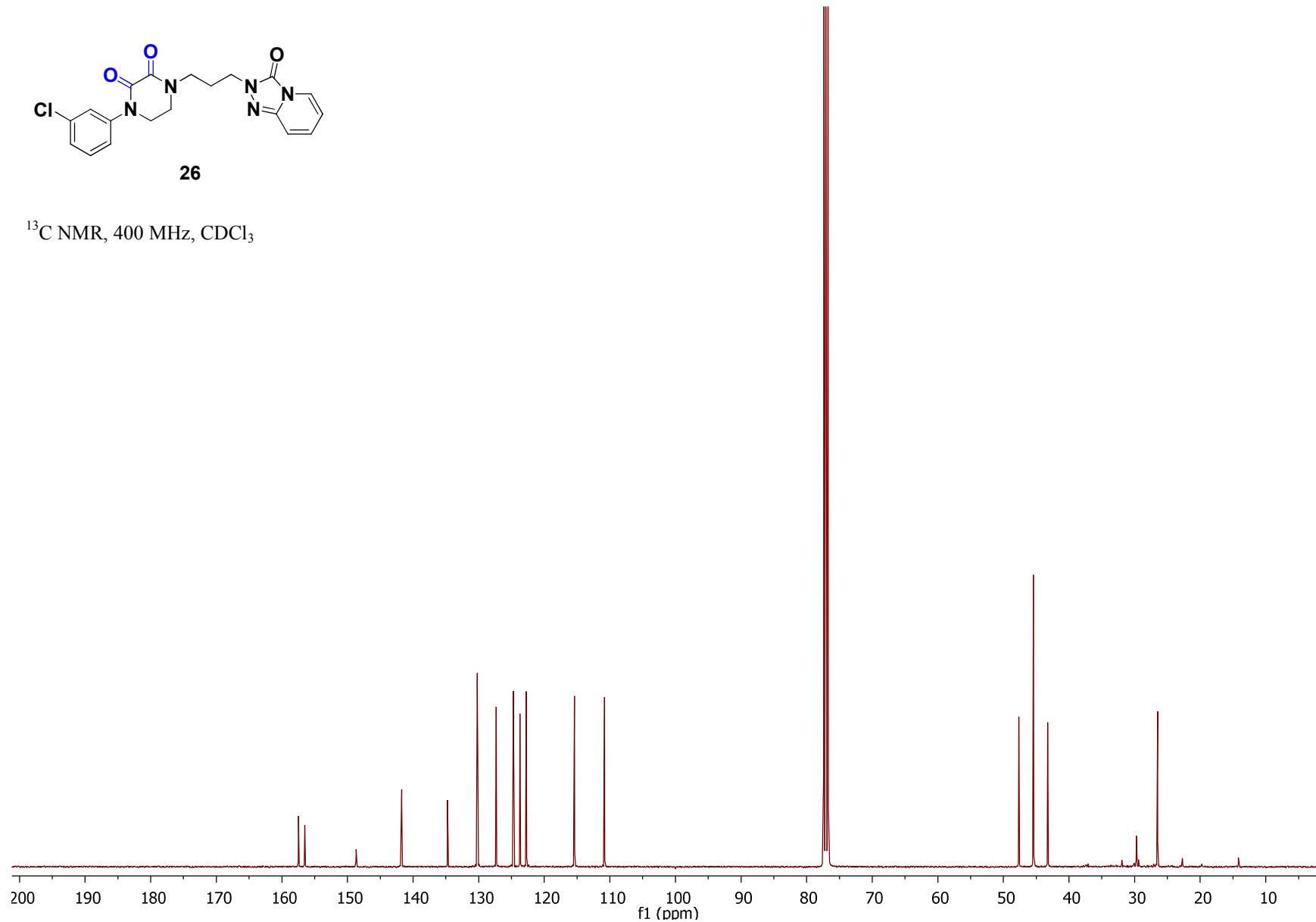


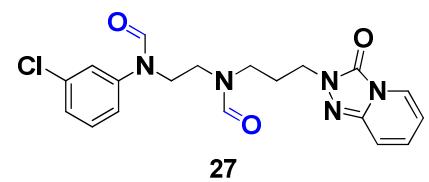
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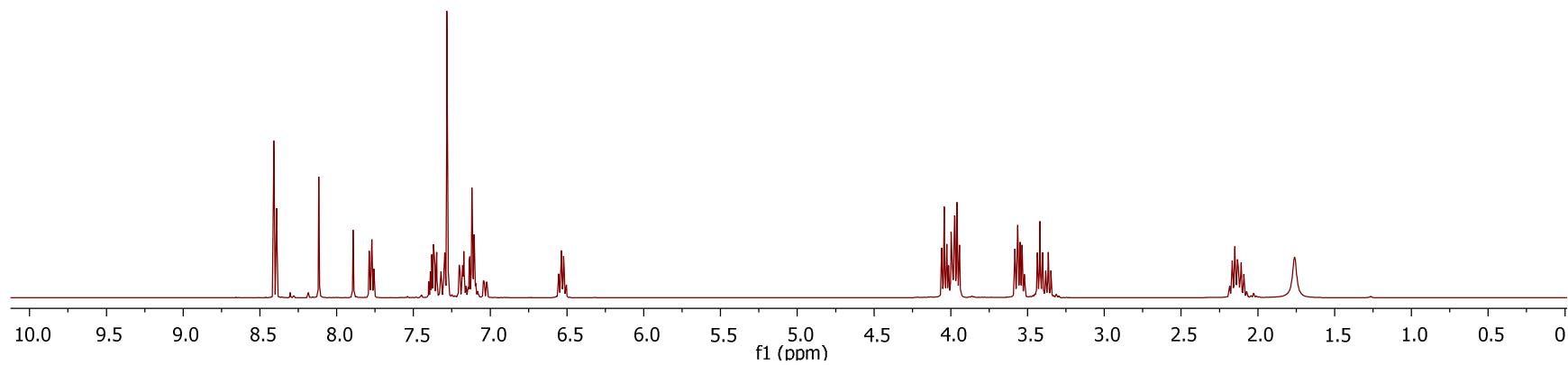


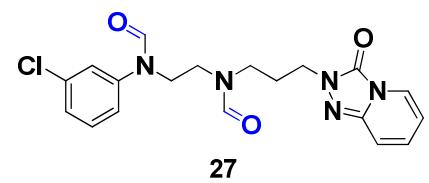
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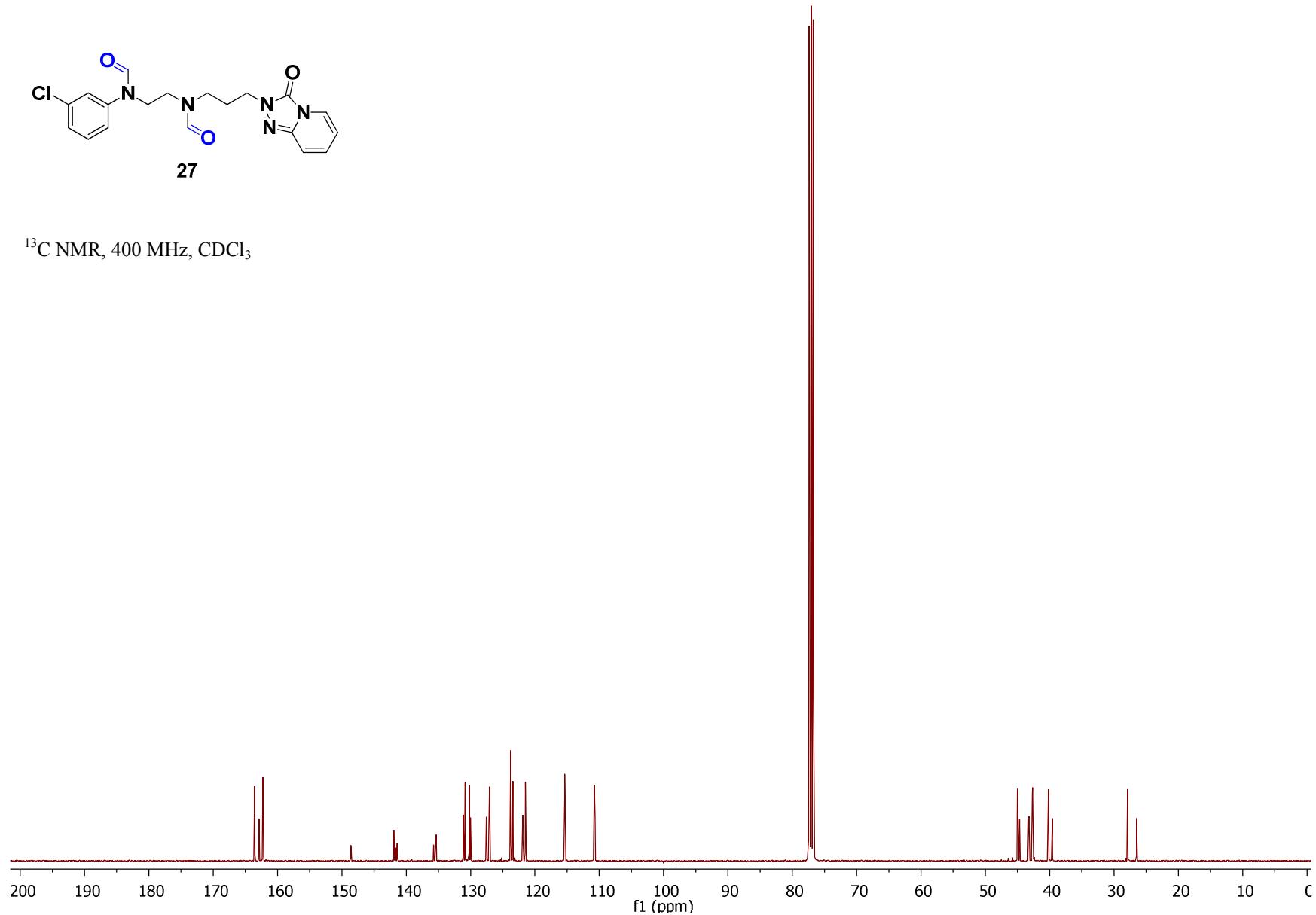


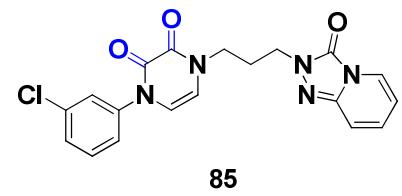
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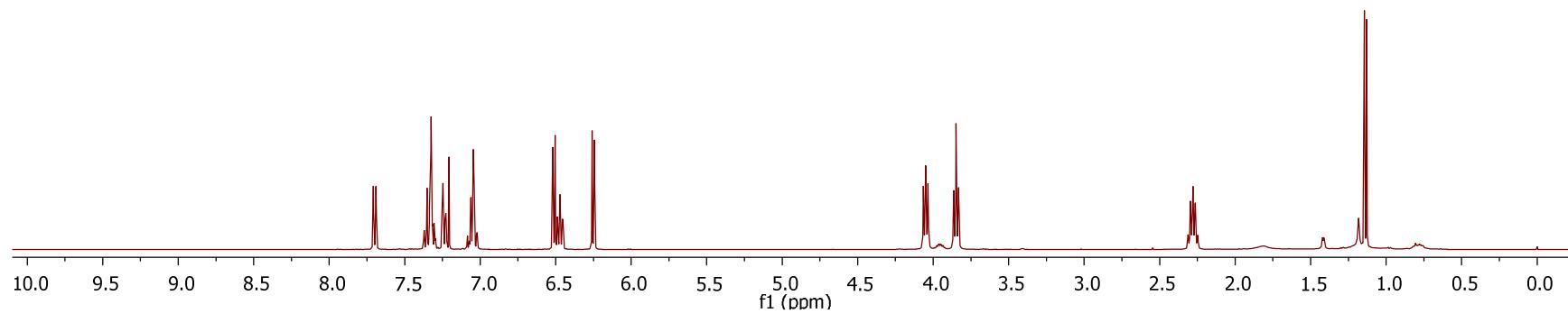


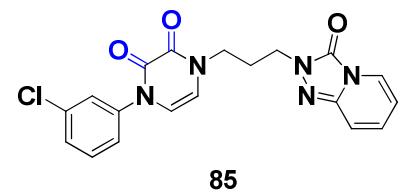
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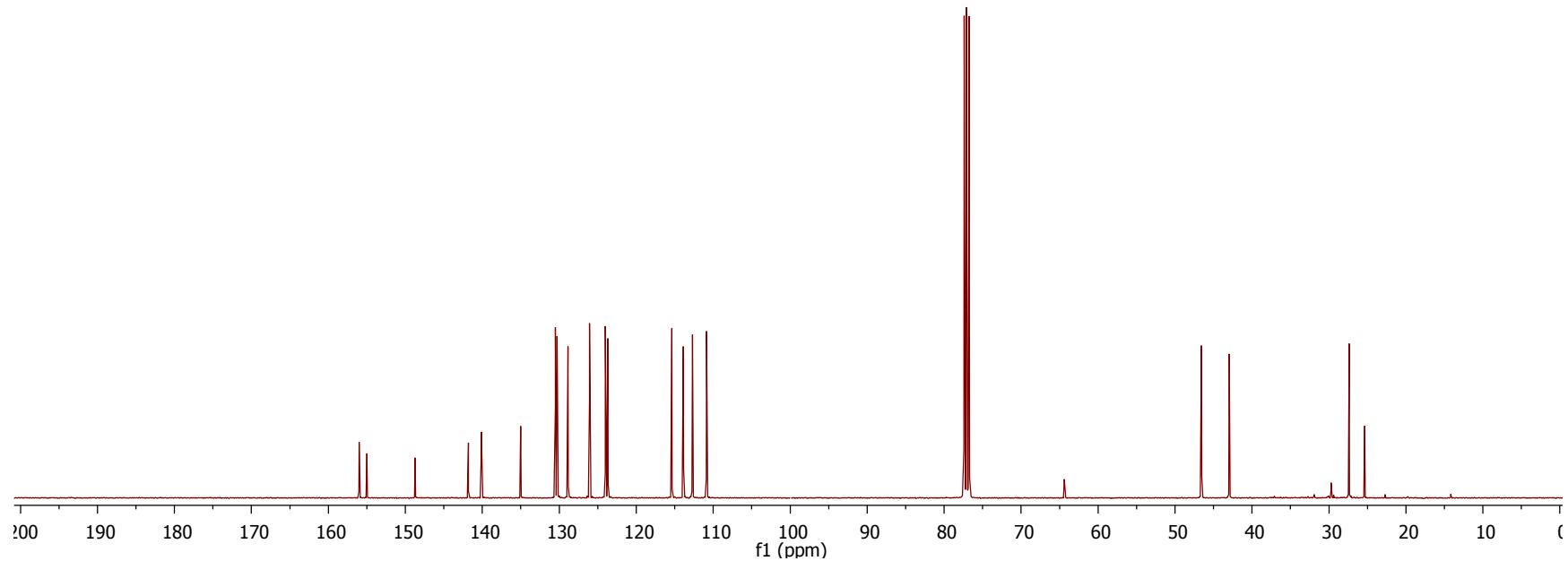
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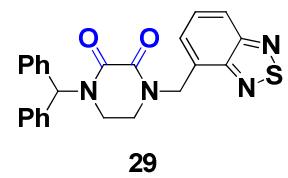




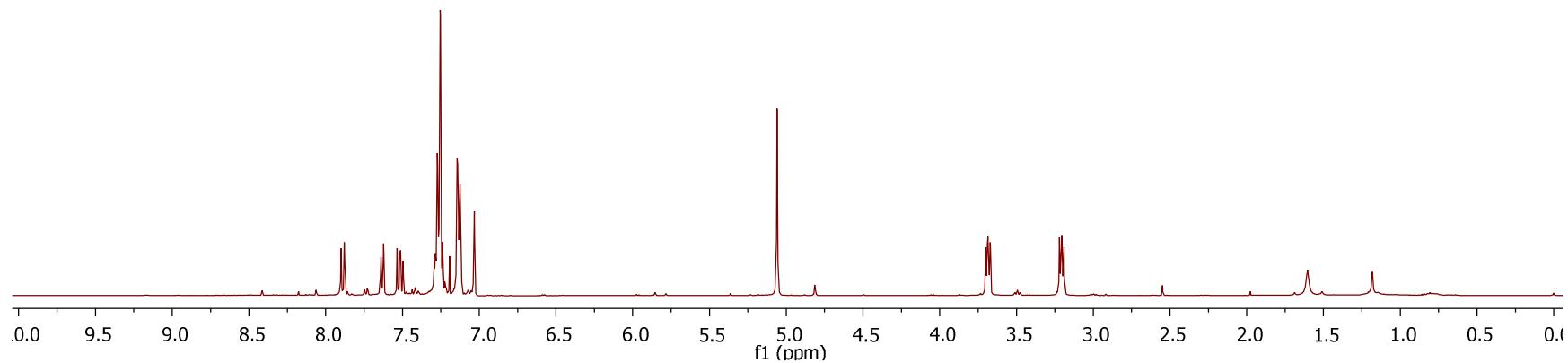
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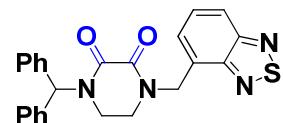
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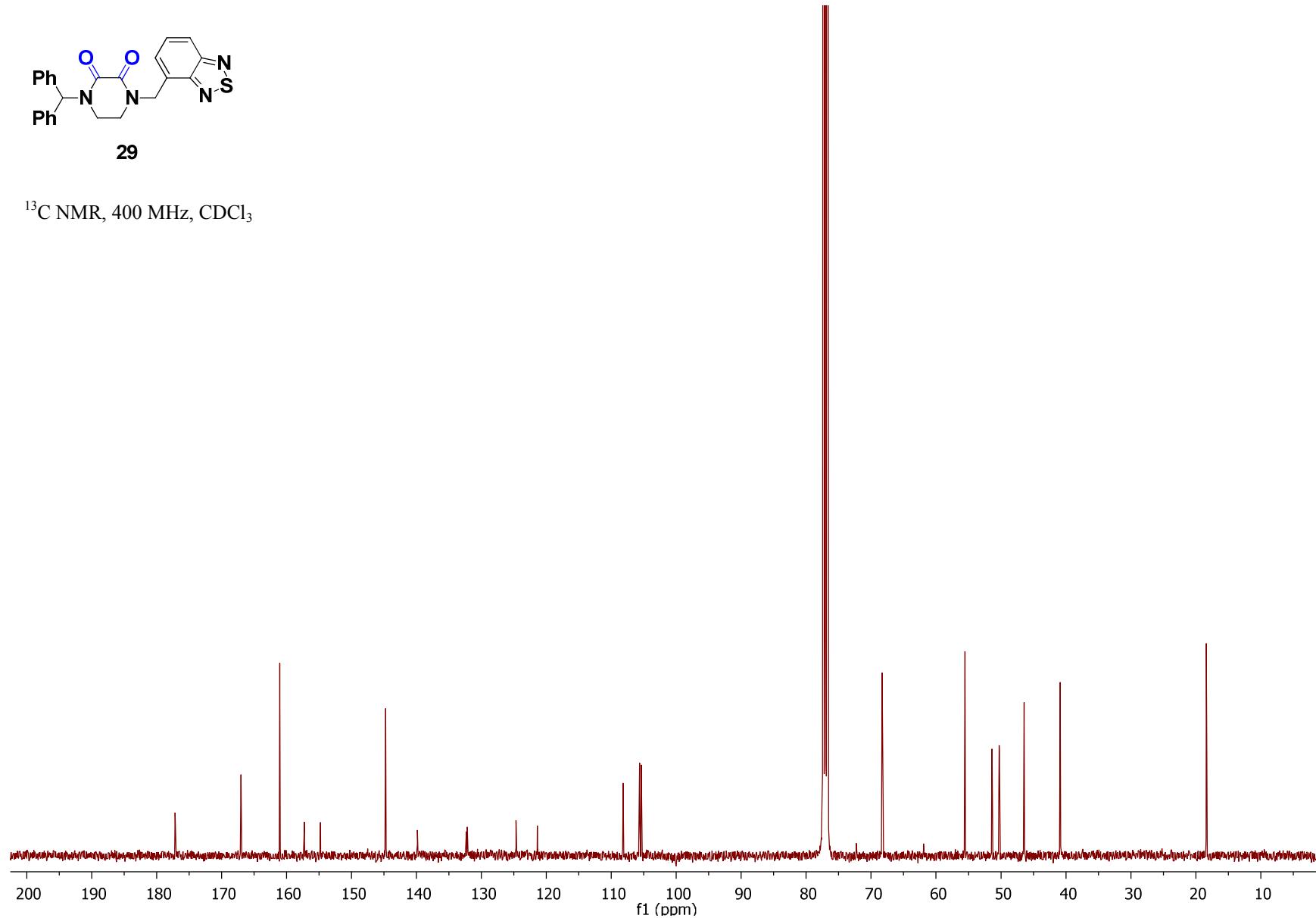
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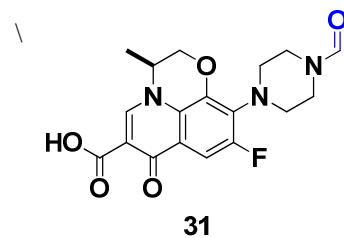




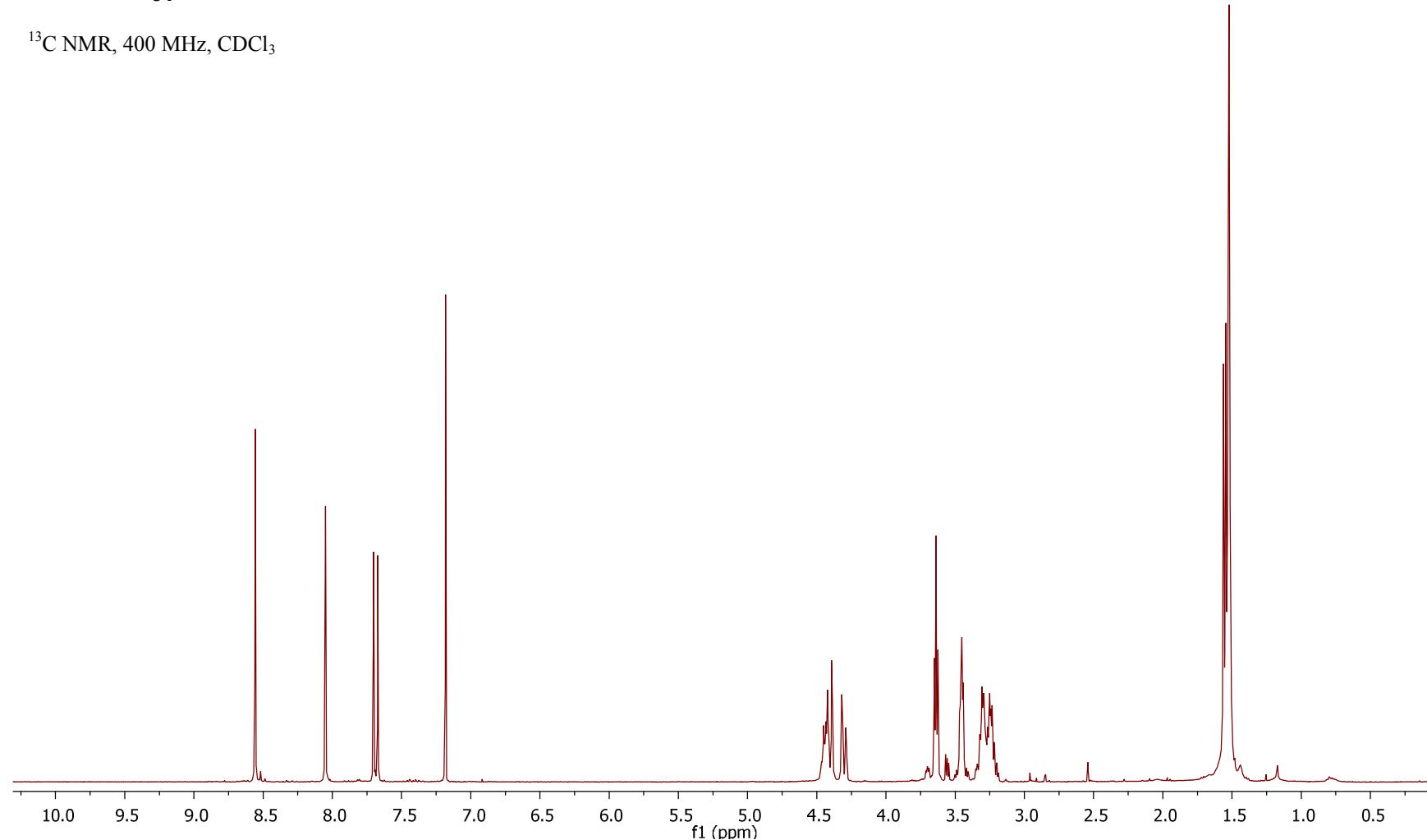
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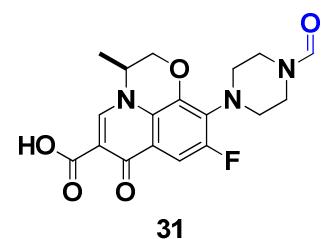
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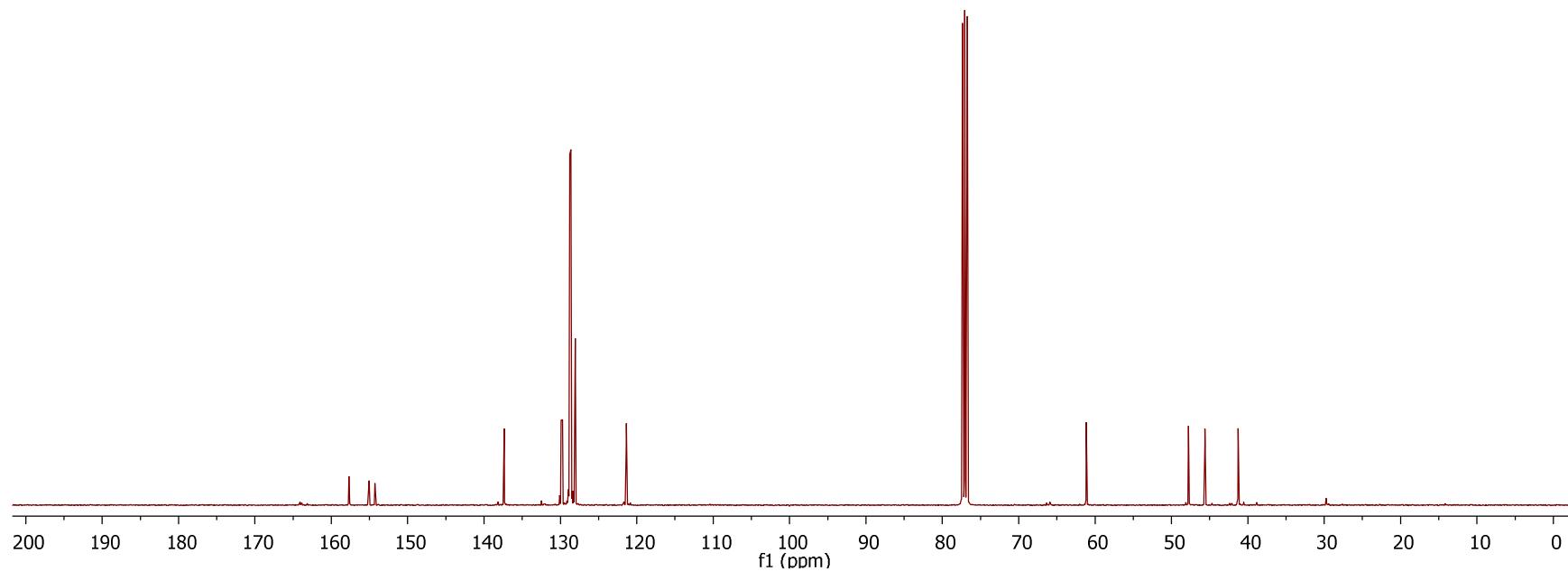
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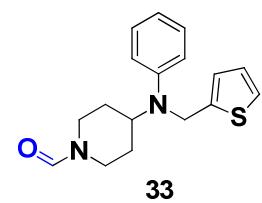




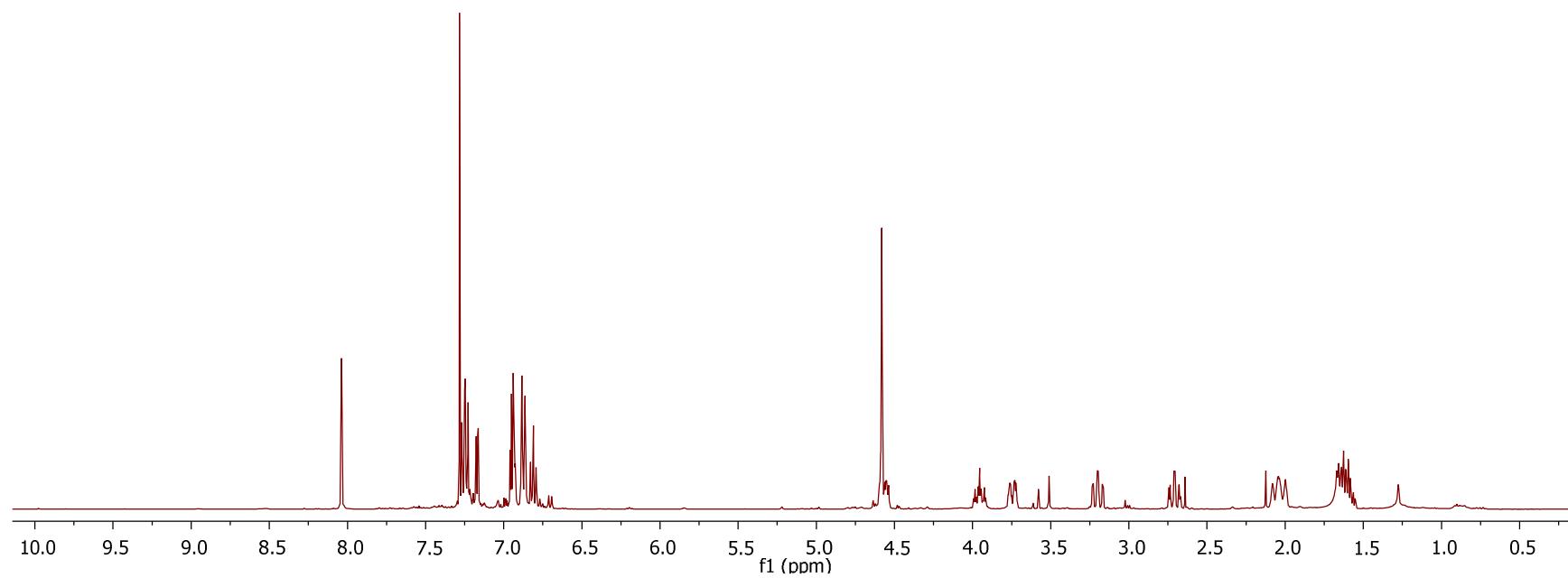
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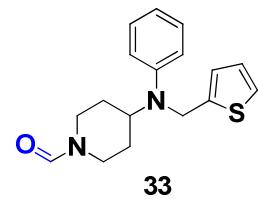
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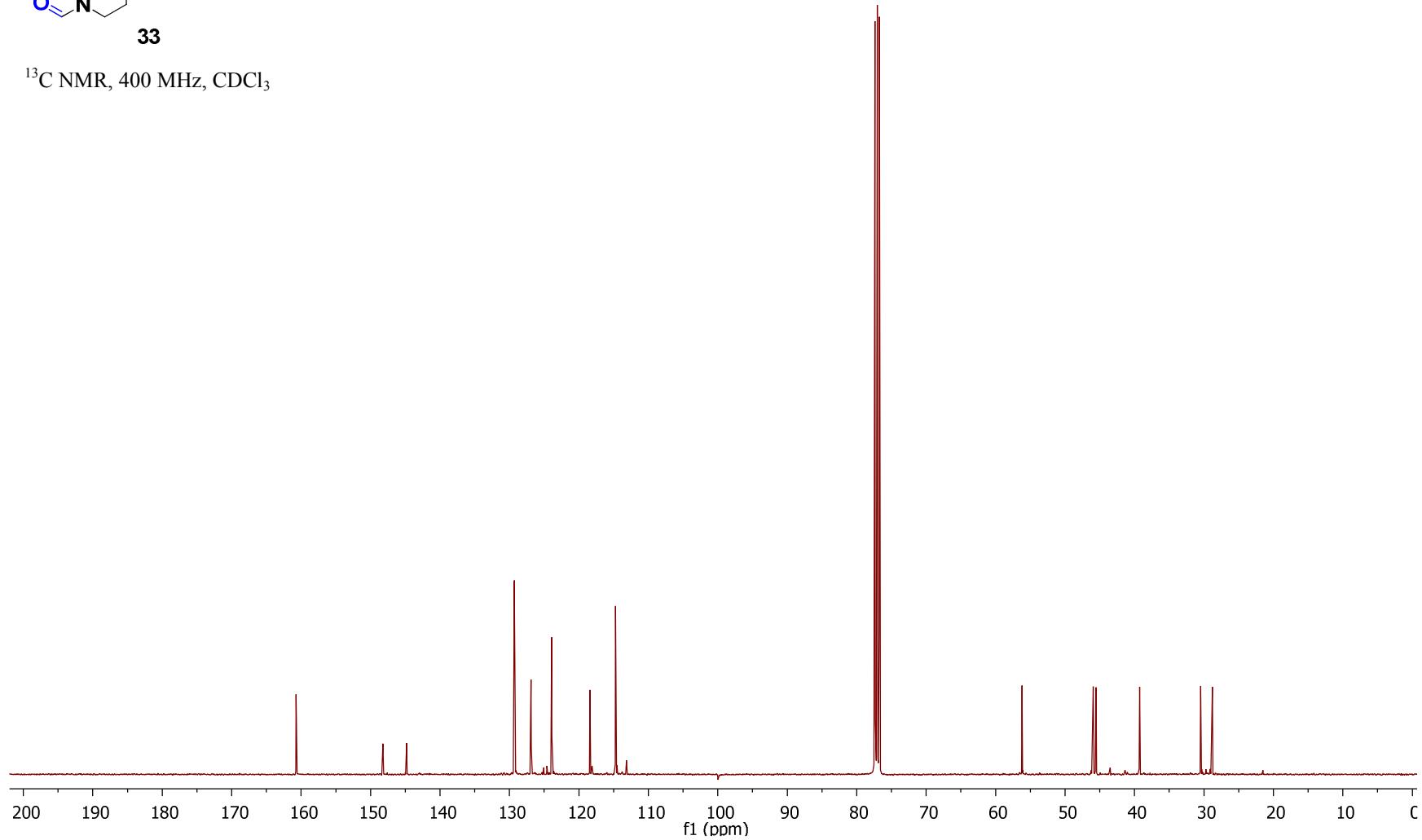
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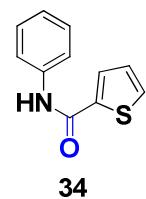




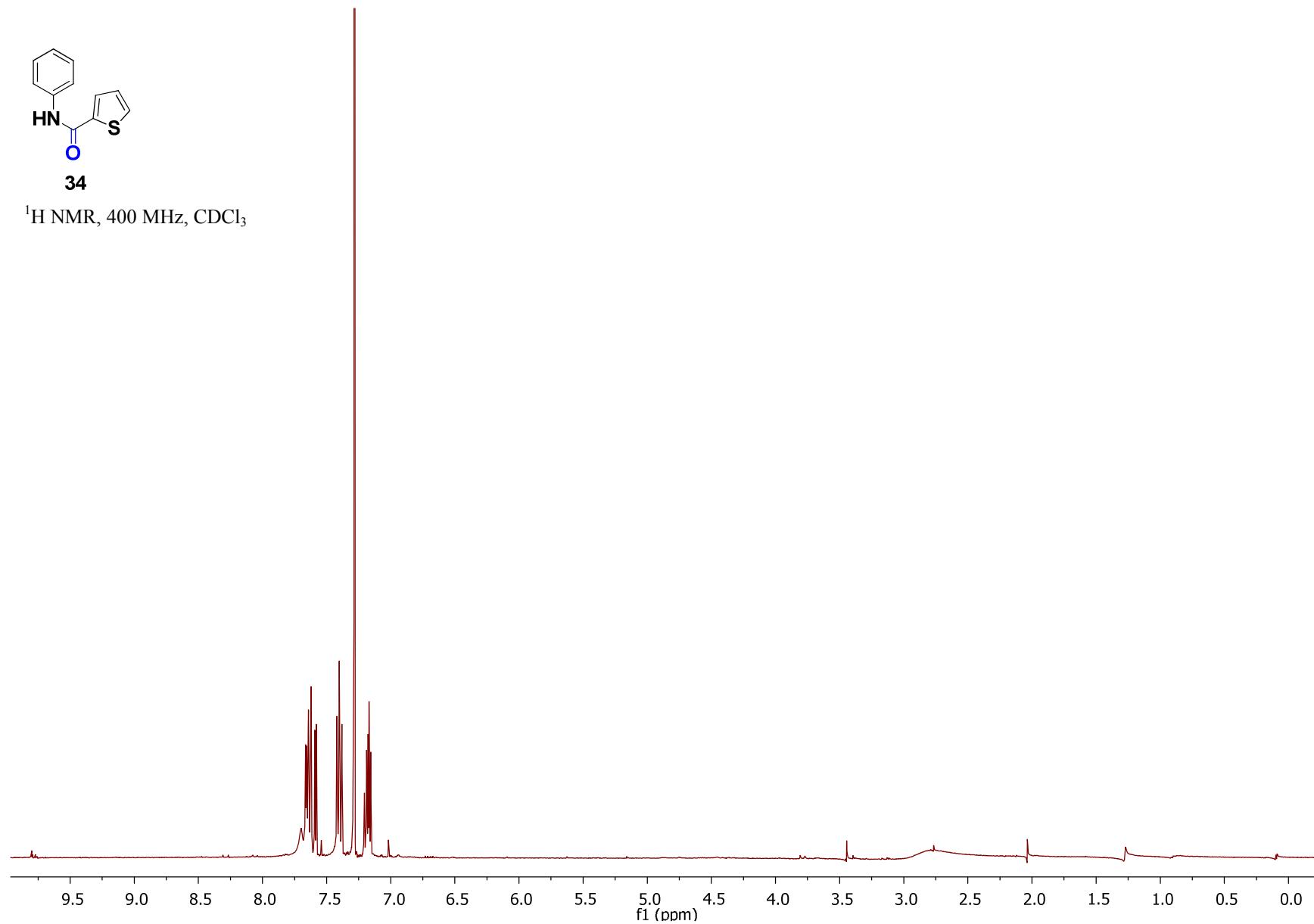
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^{13}C NMR, 400 MHz, CDCl_3



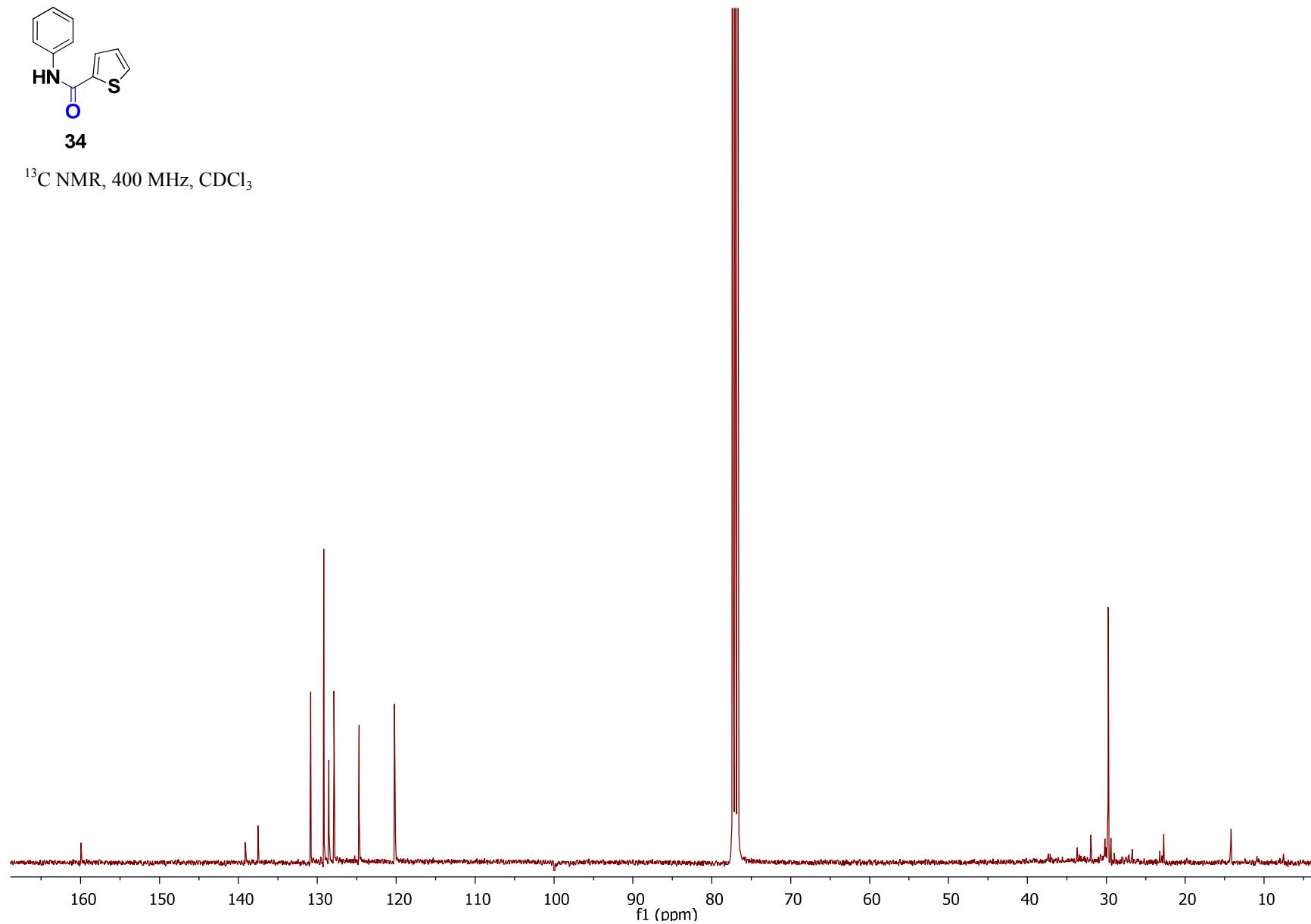


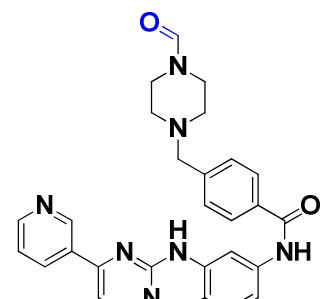
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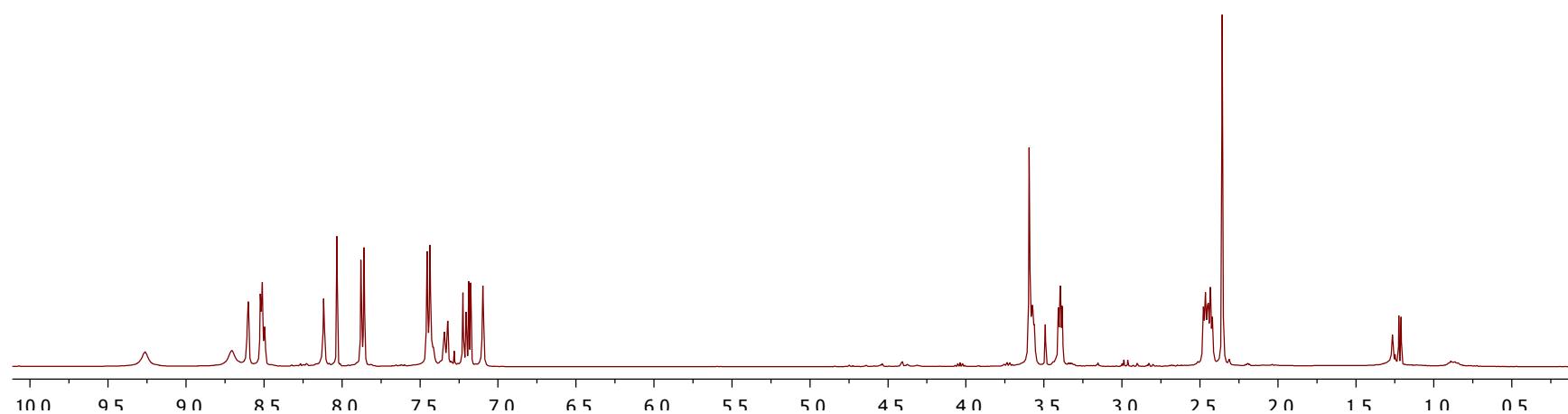
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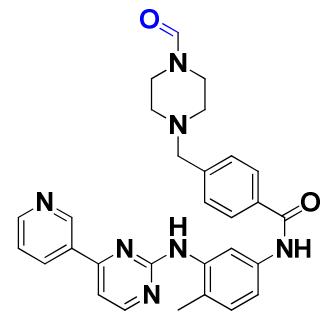




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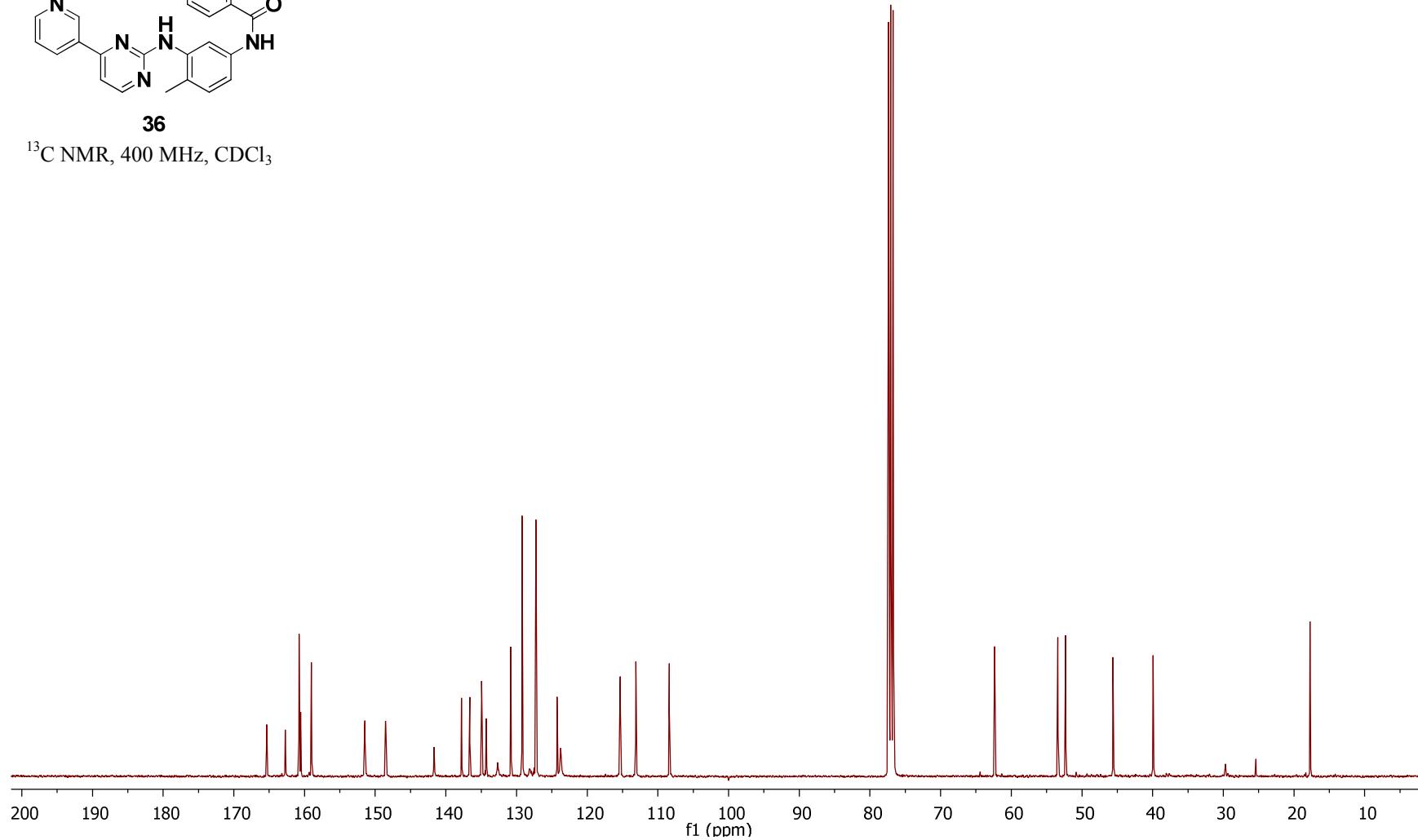
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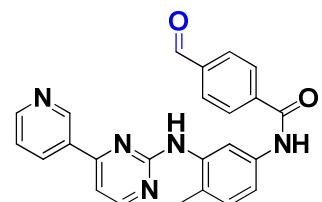




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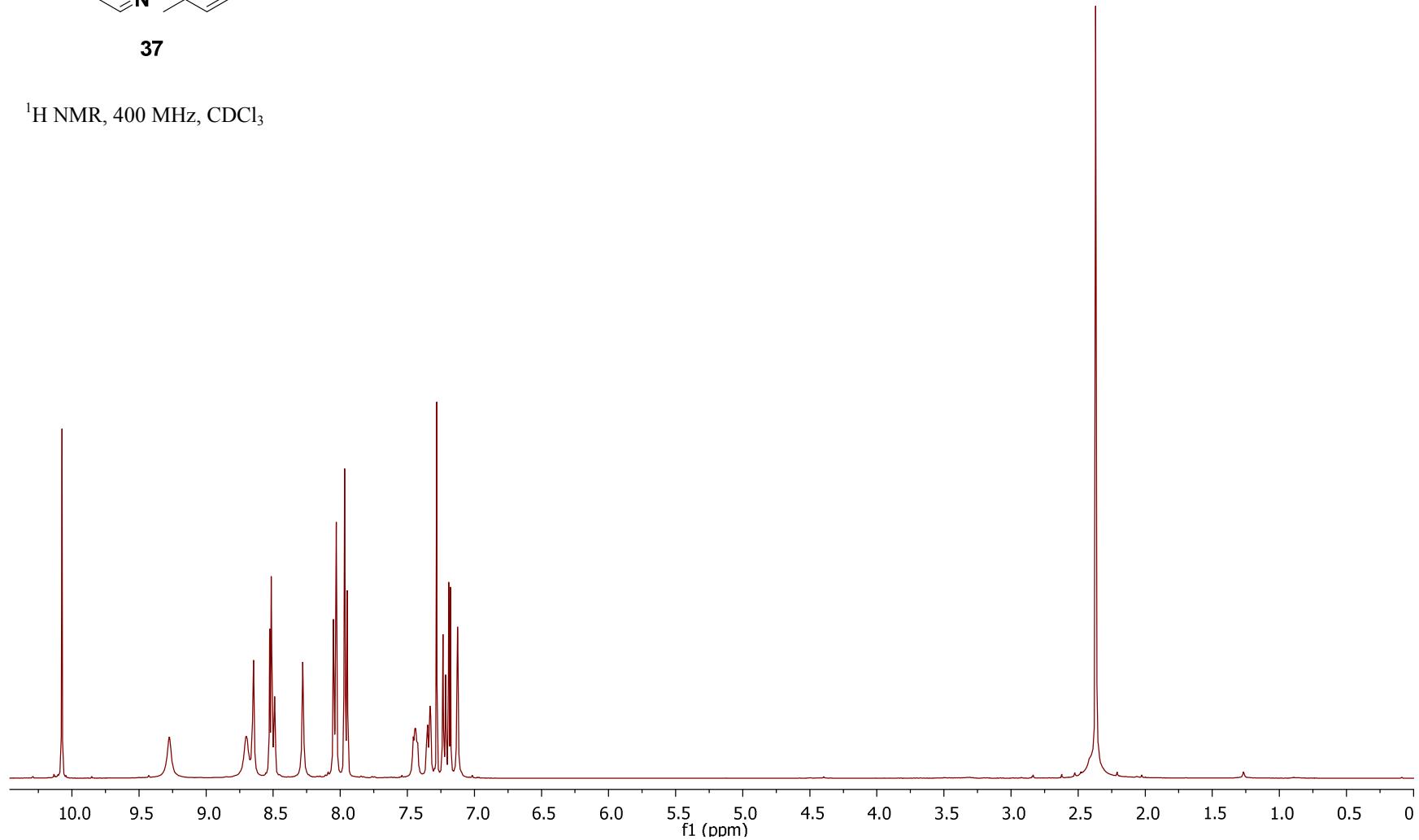
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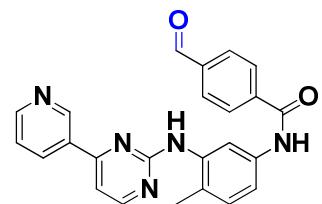




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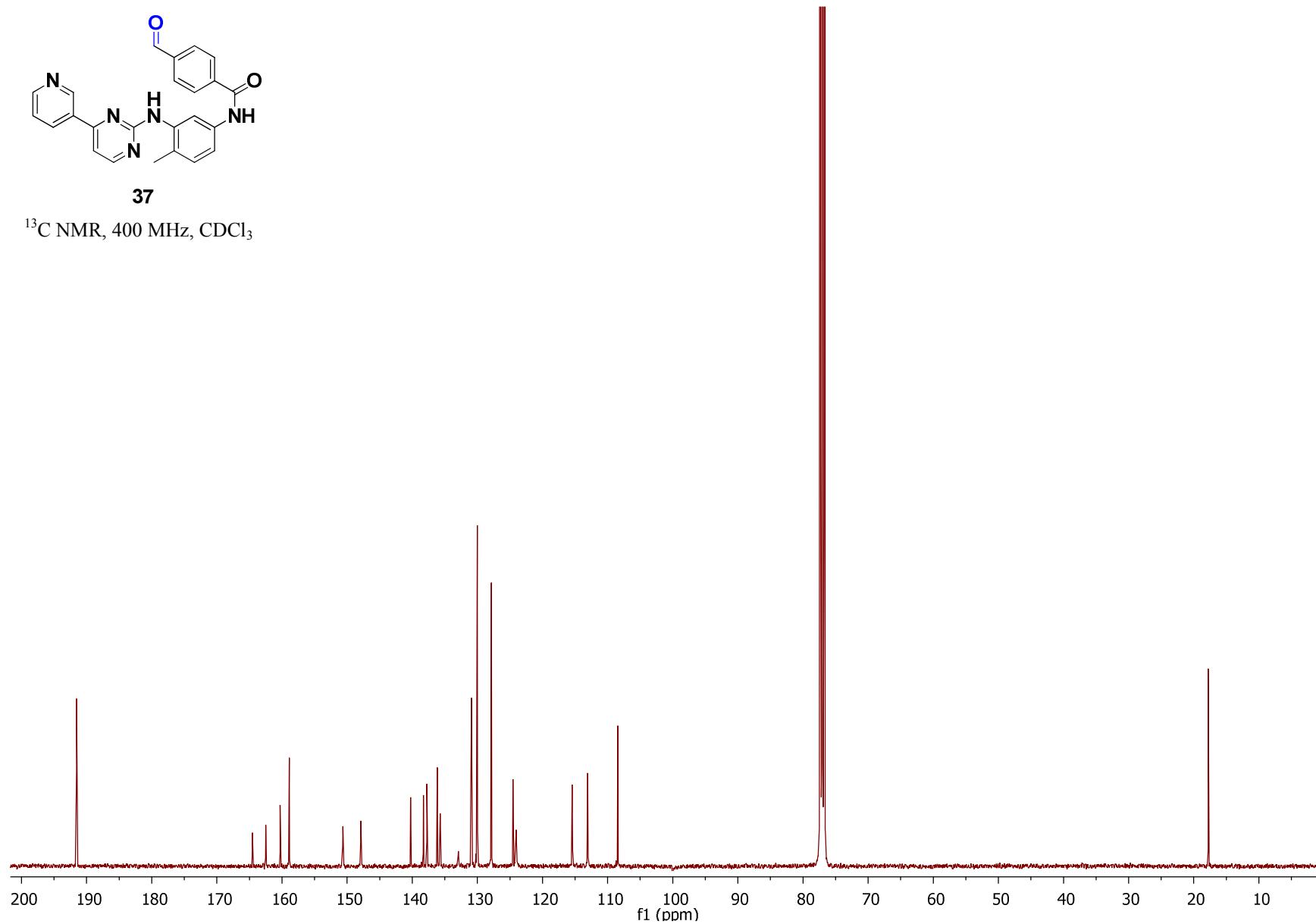
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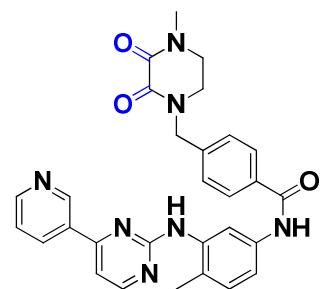




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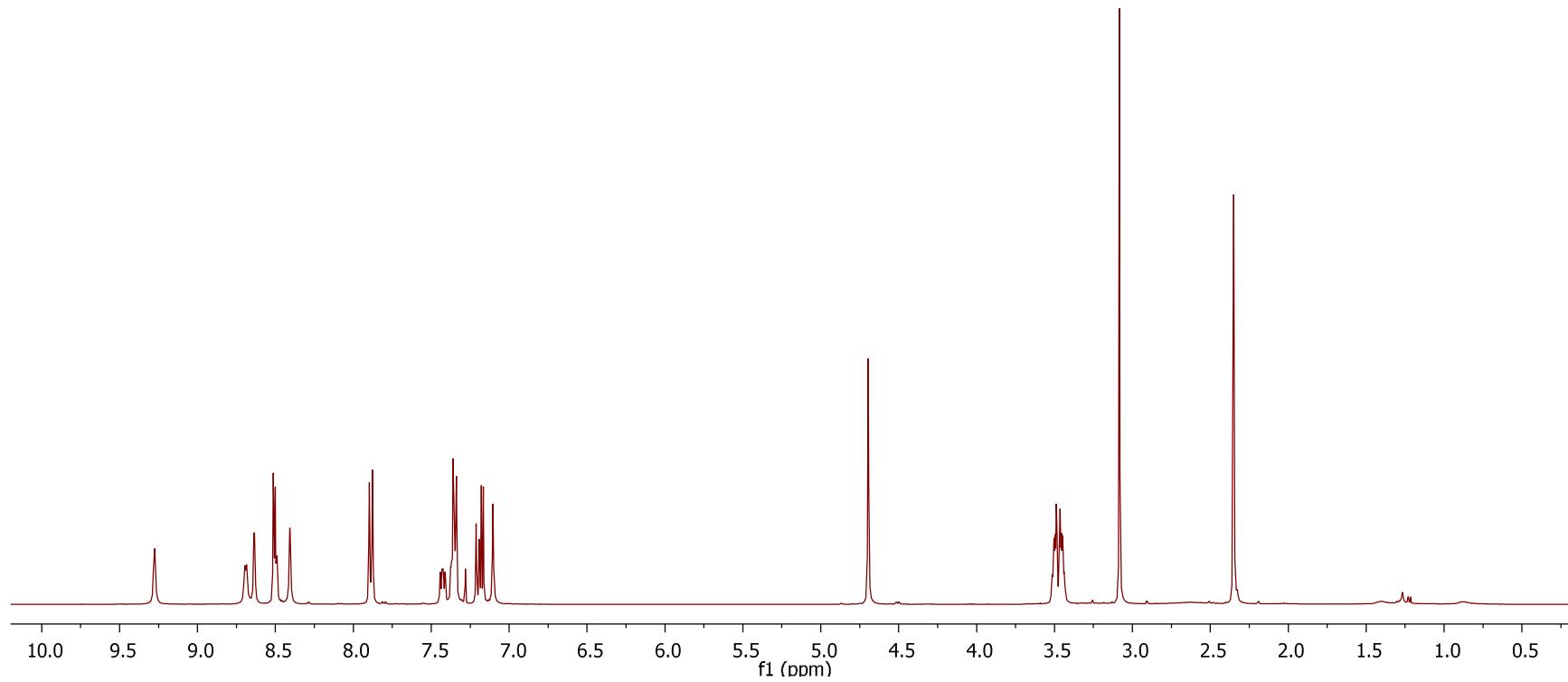
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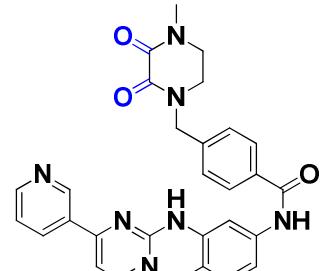




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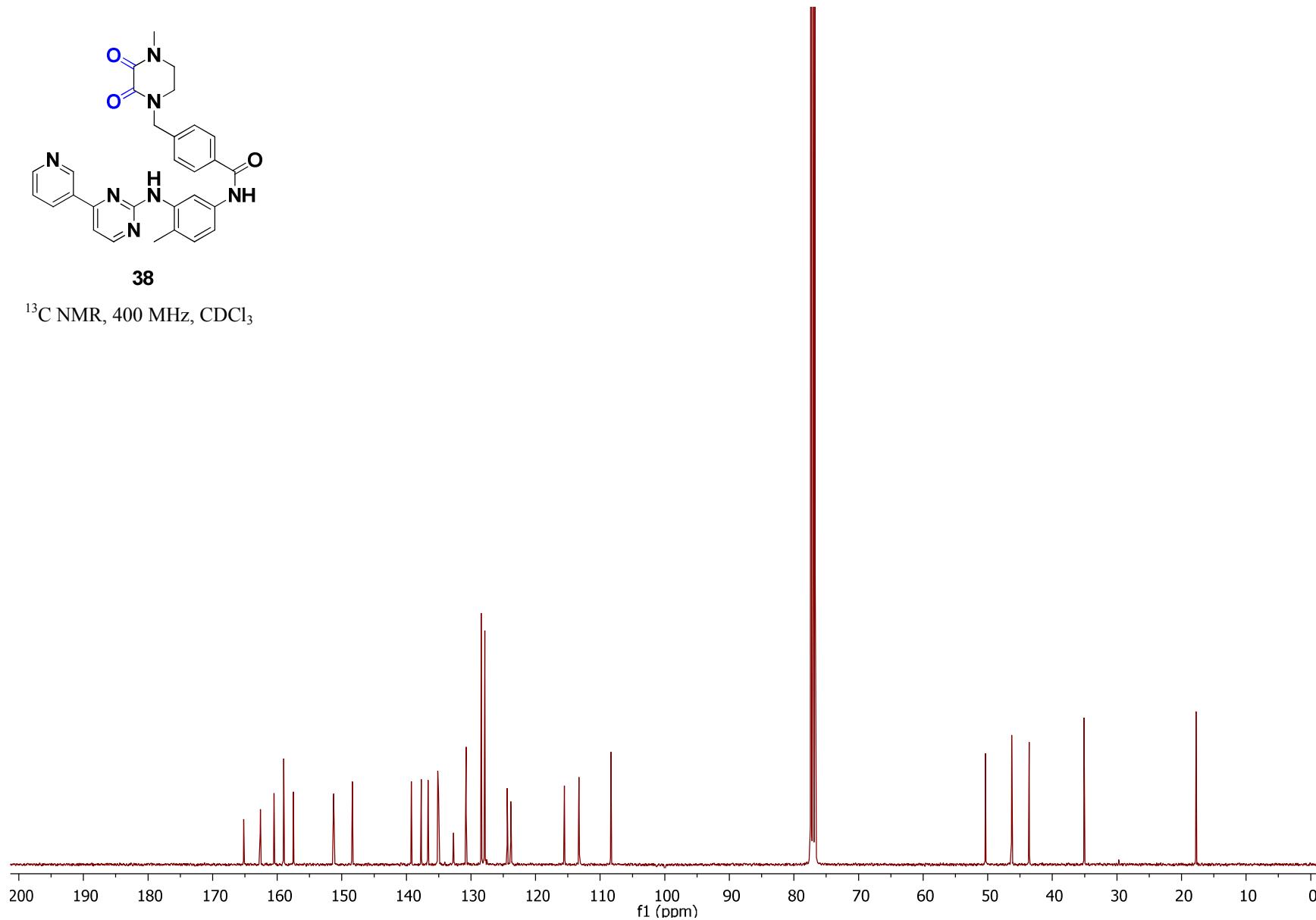
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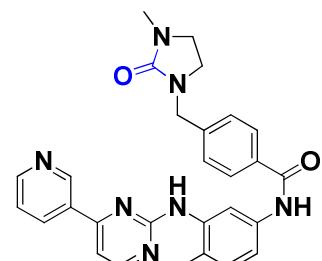




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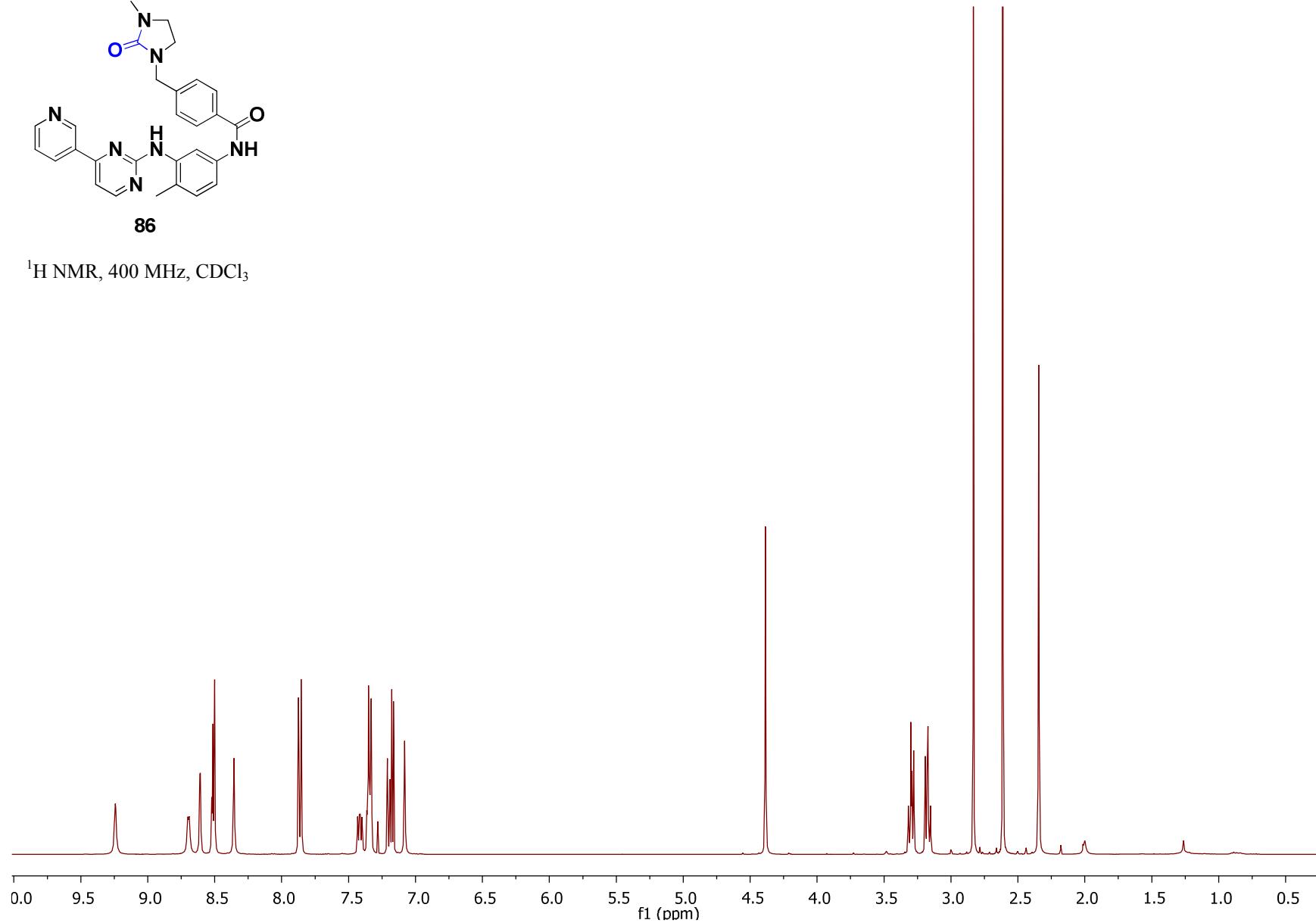
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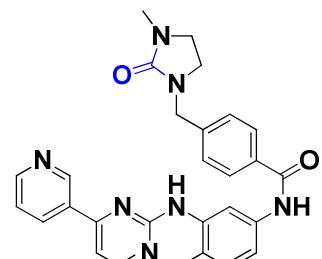




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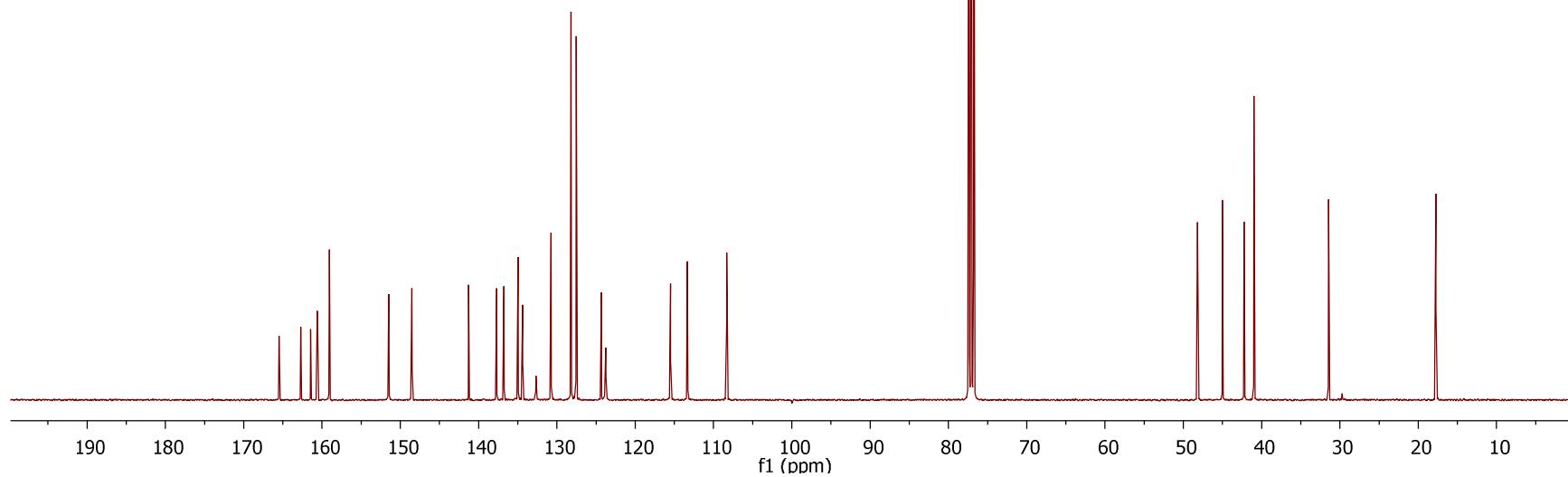
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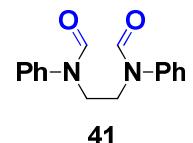




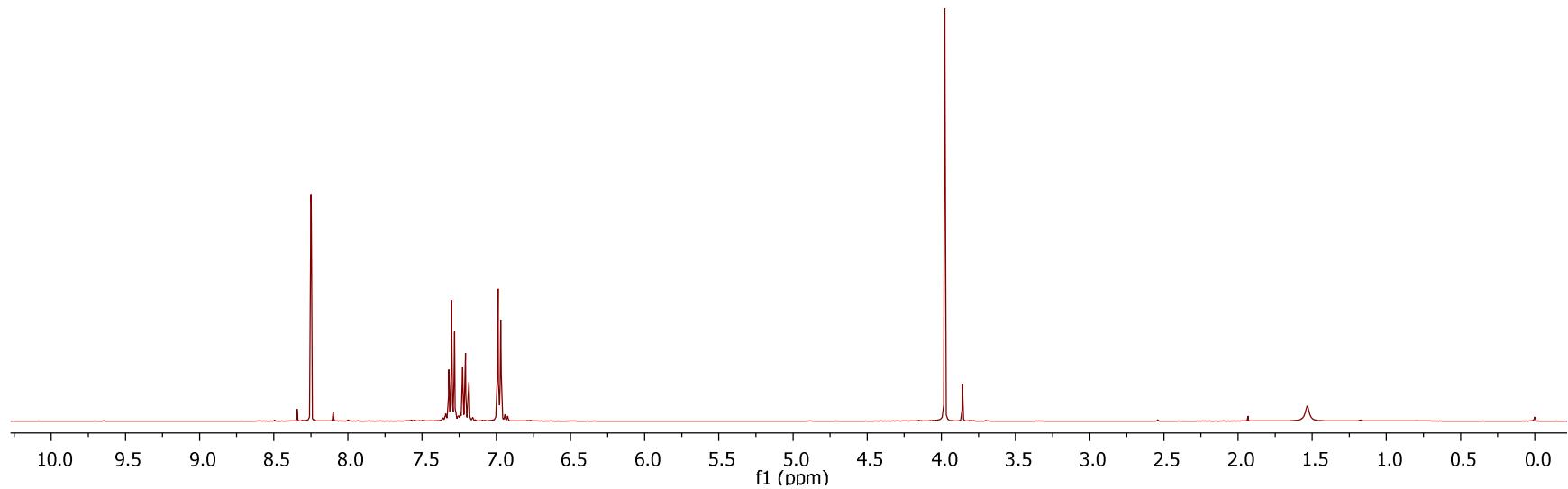
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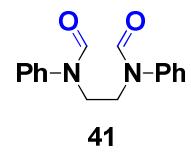
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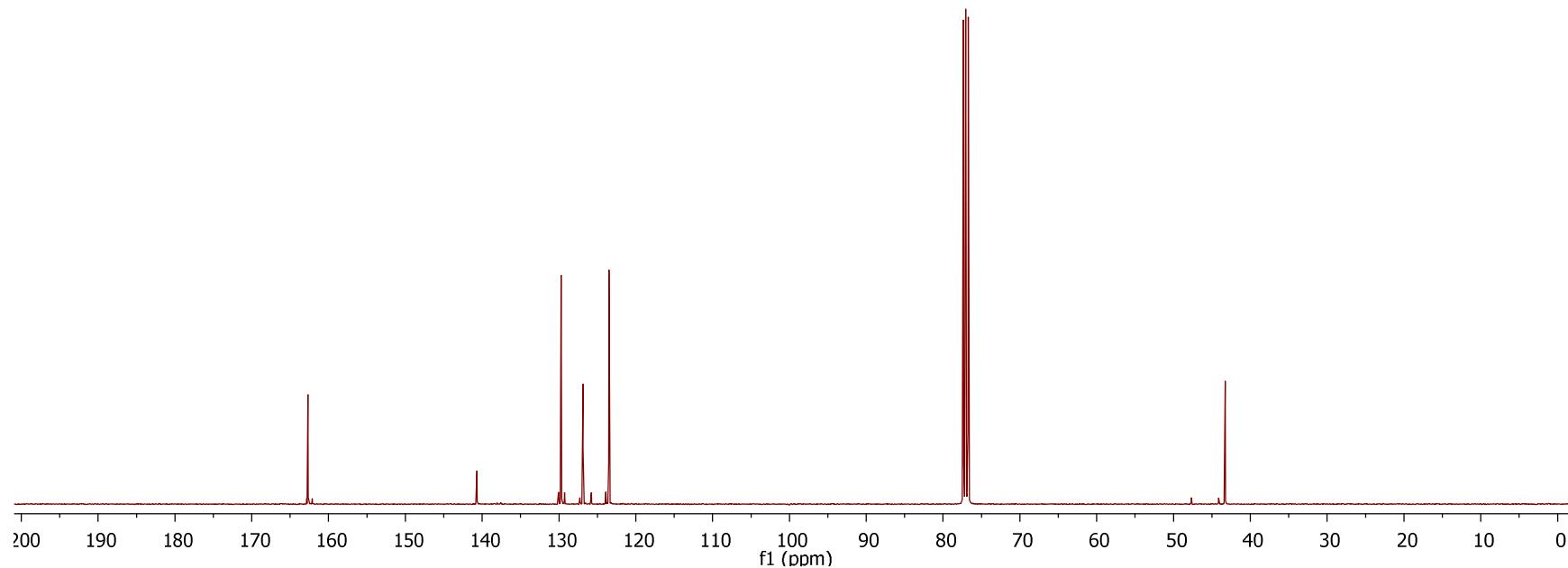


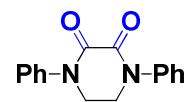
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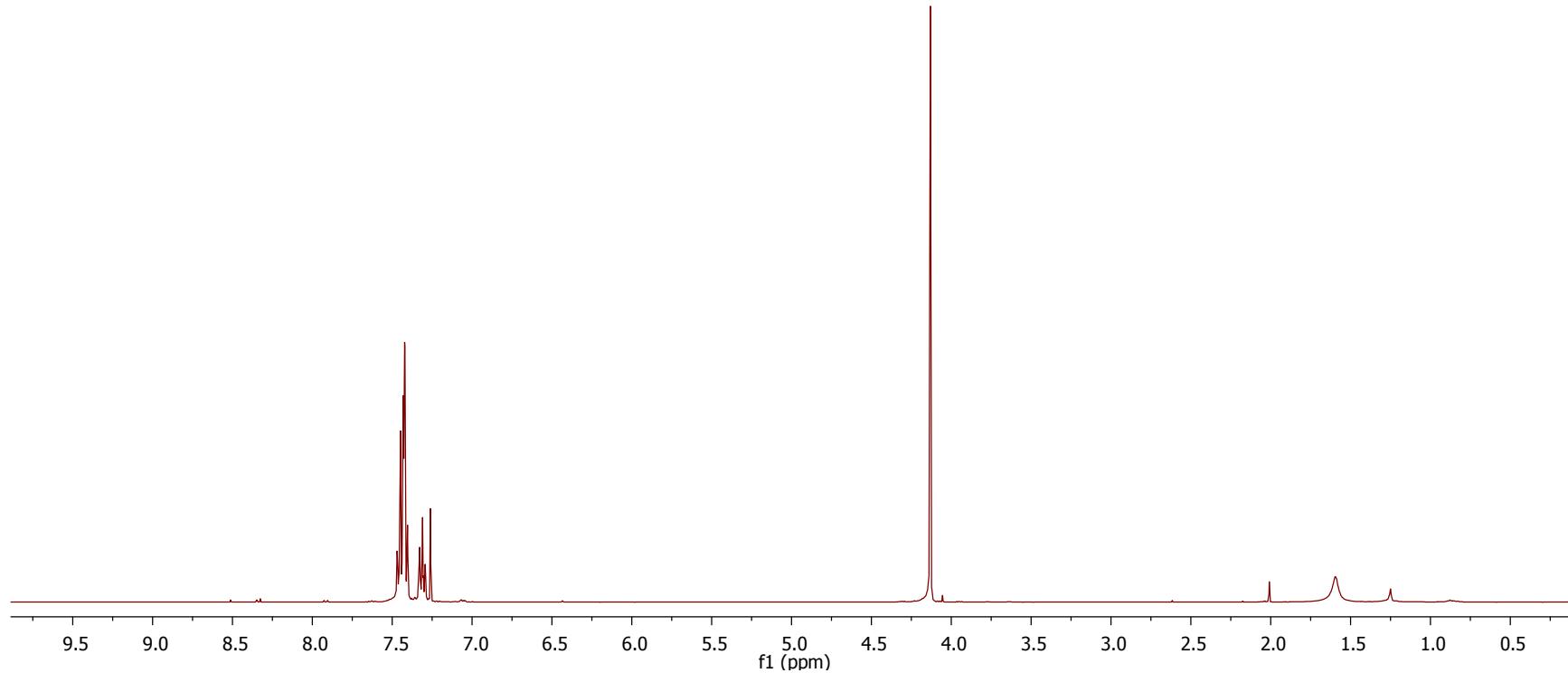
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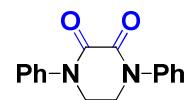




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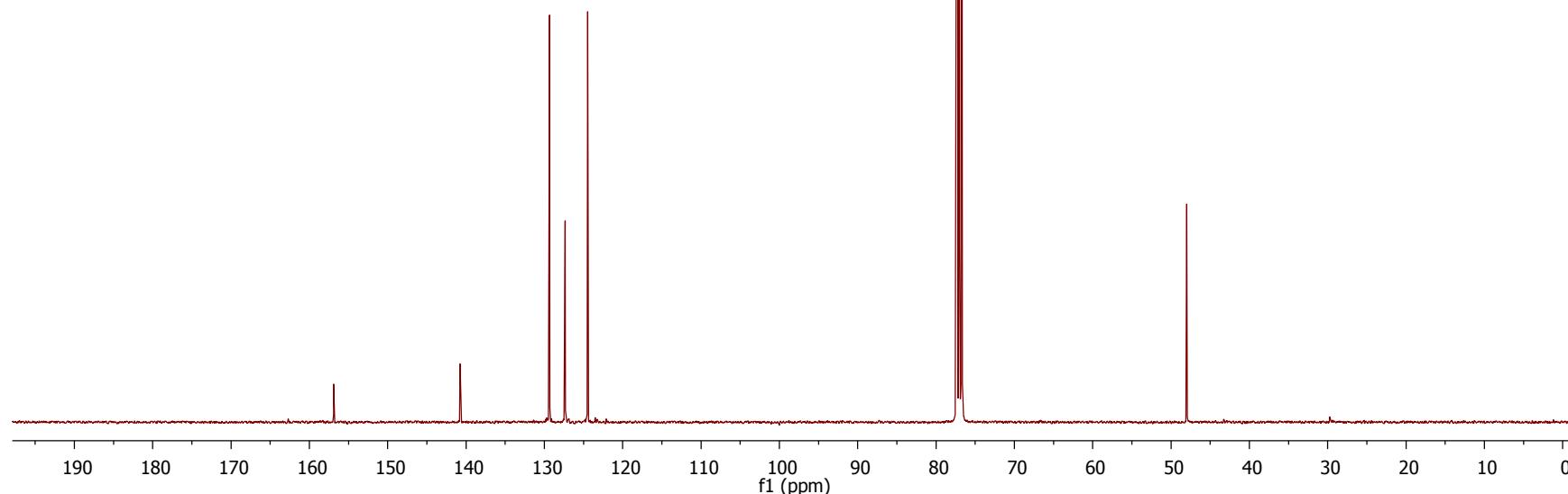
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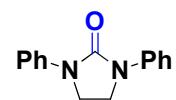




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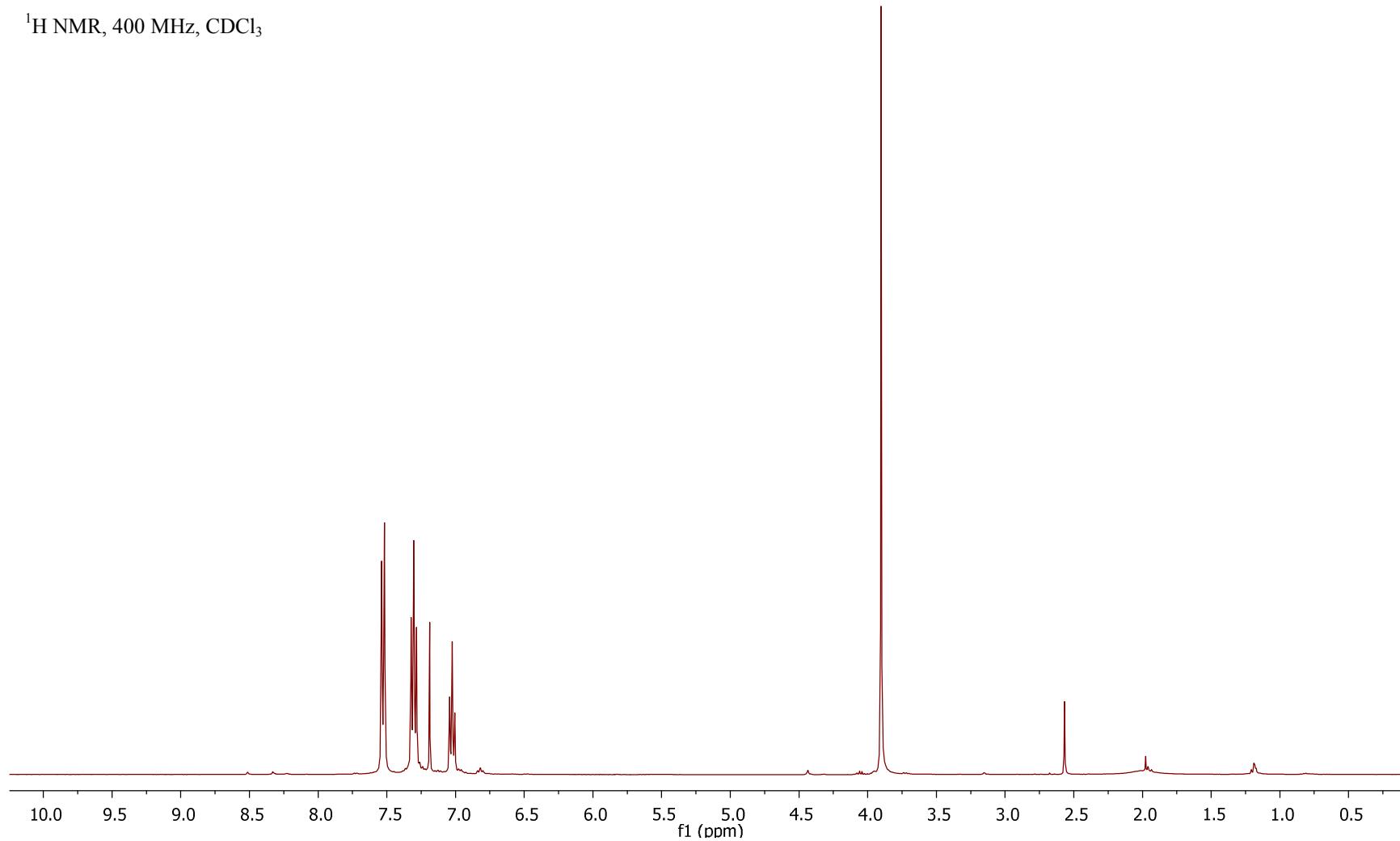
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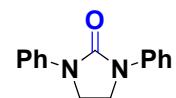




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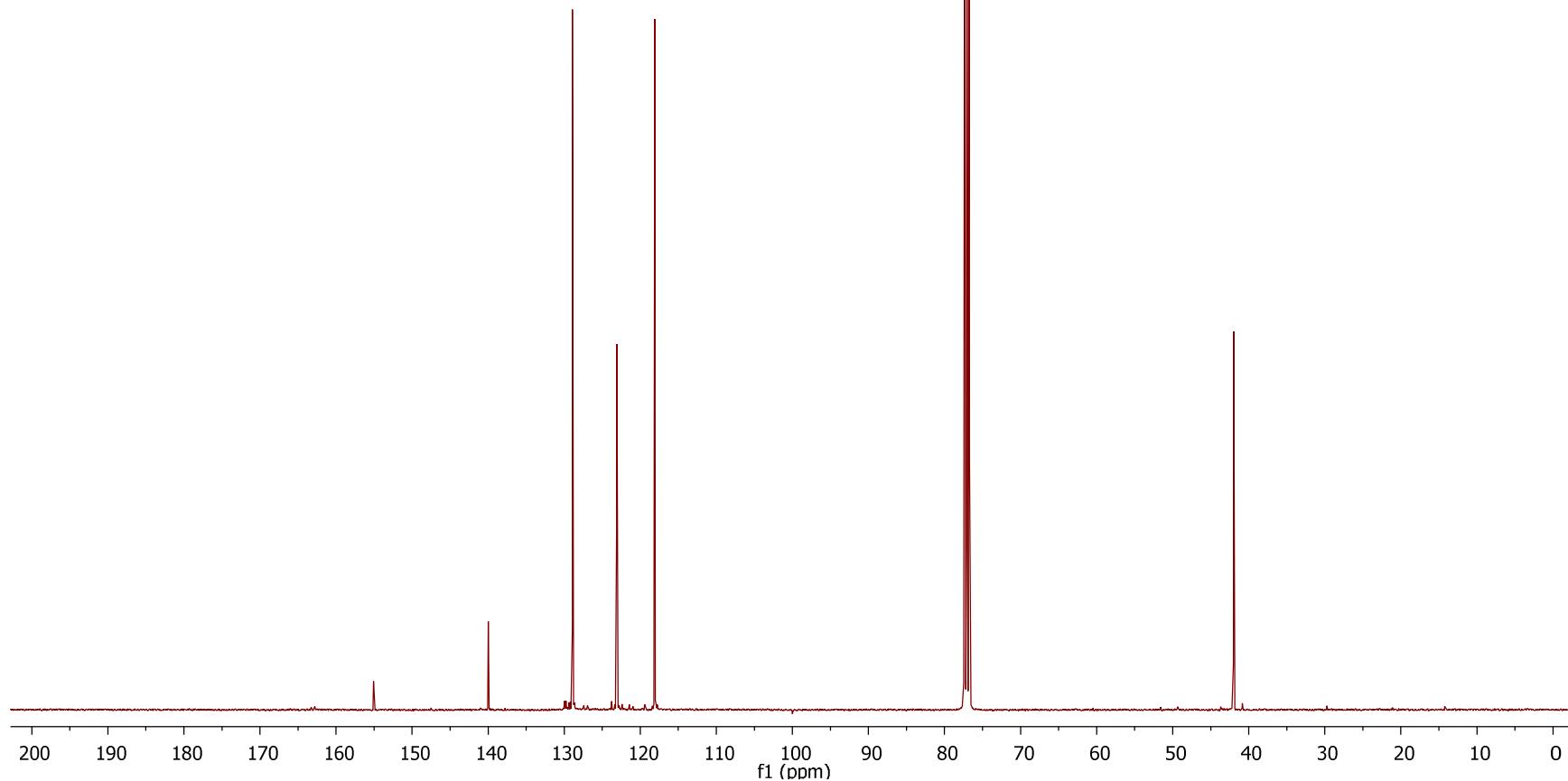
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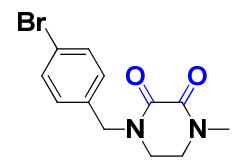




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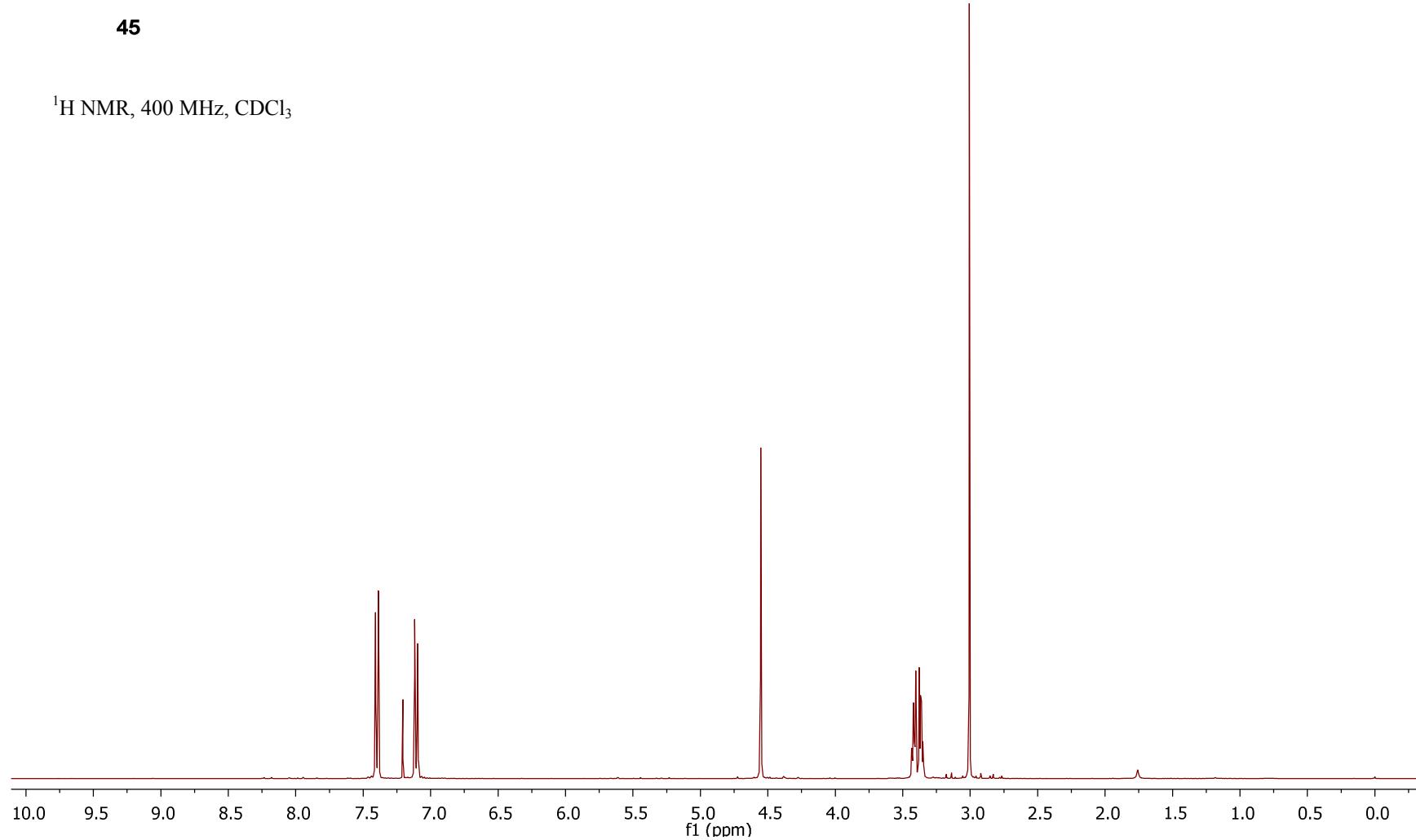
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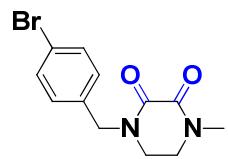




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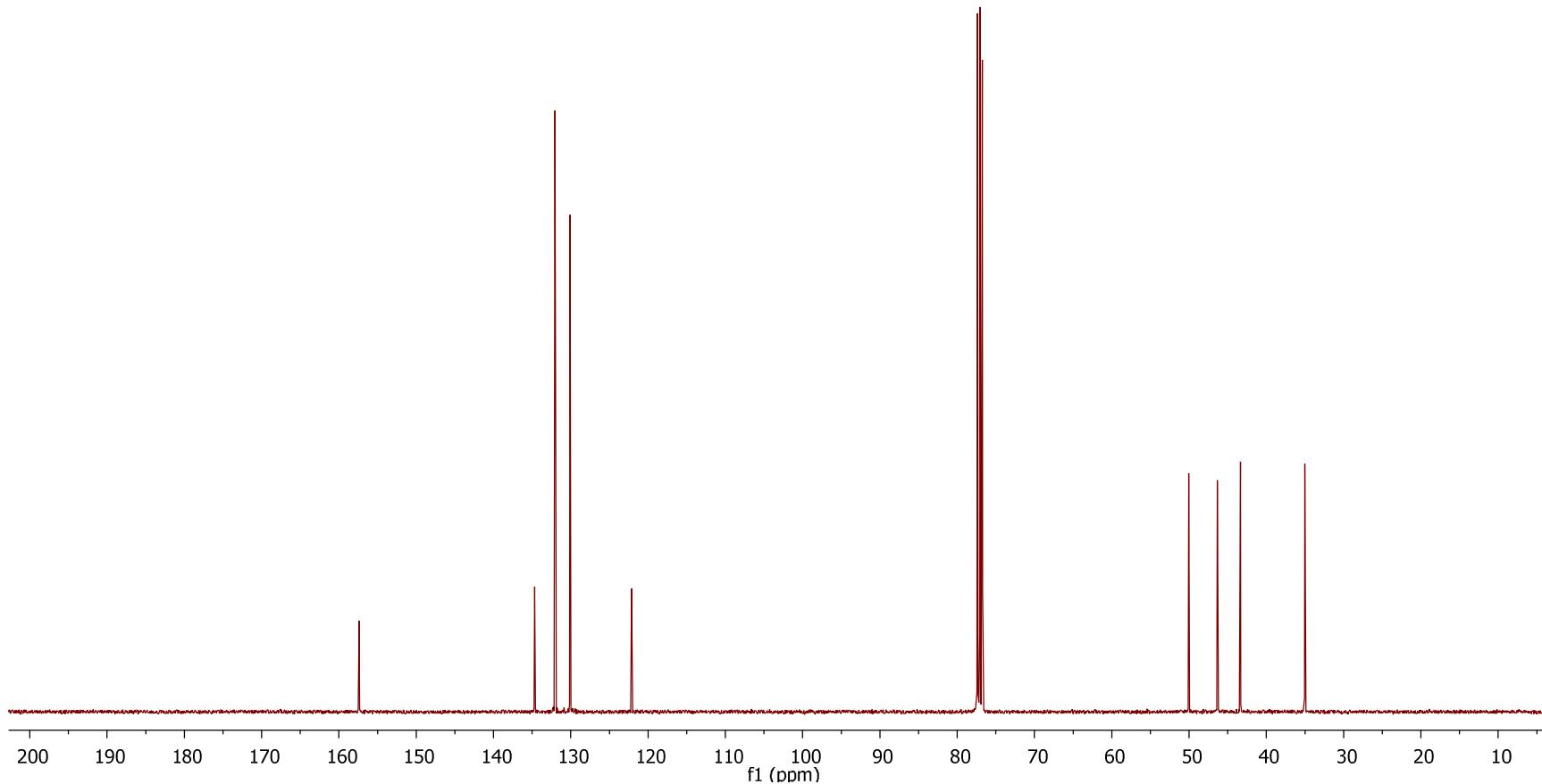
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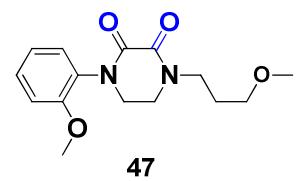




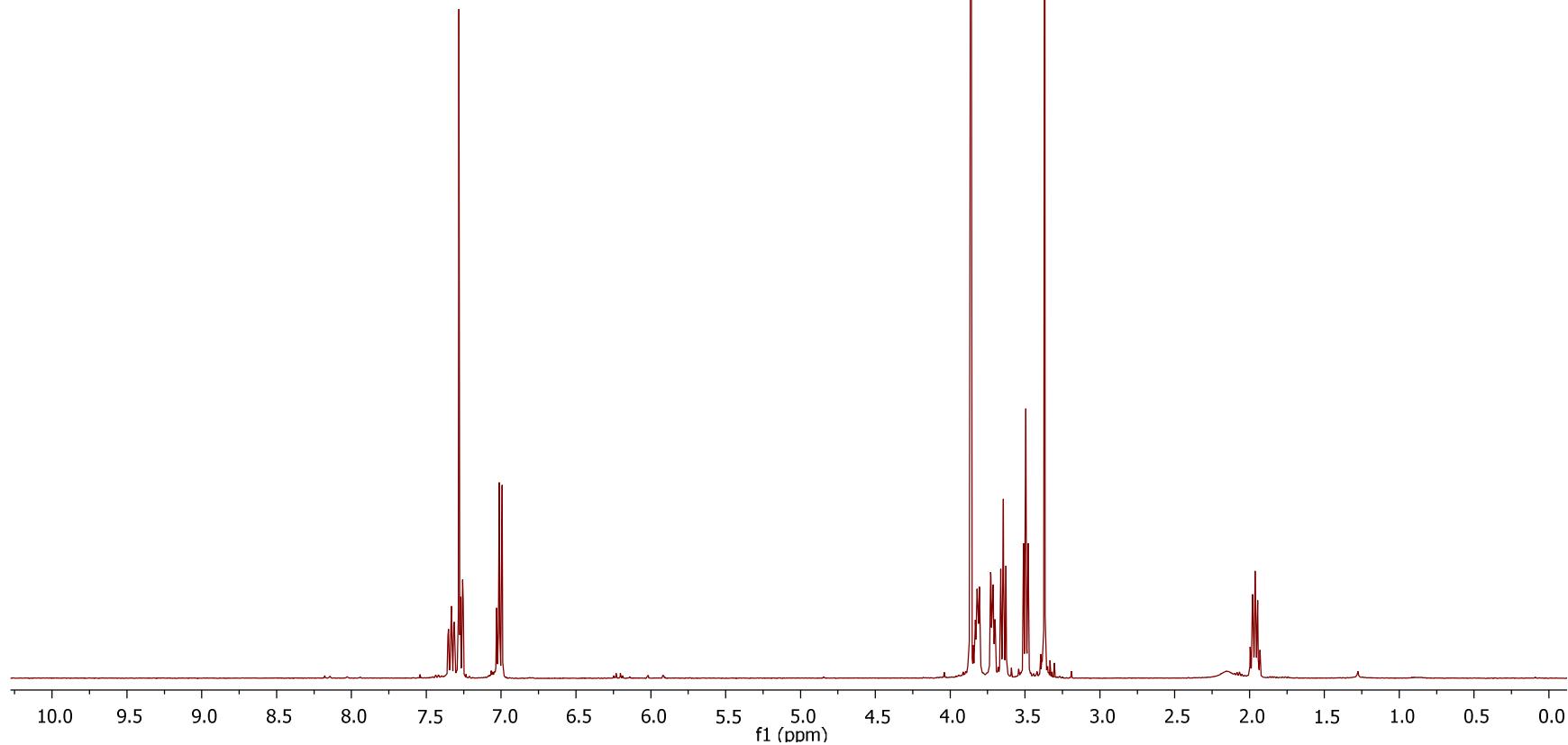
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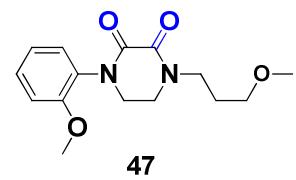
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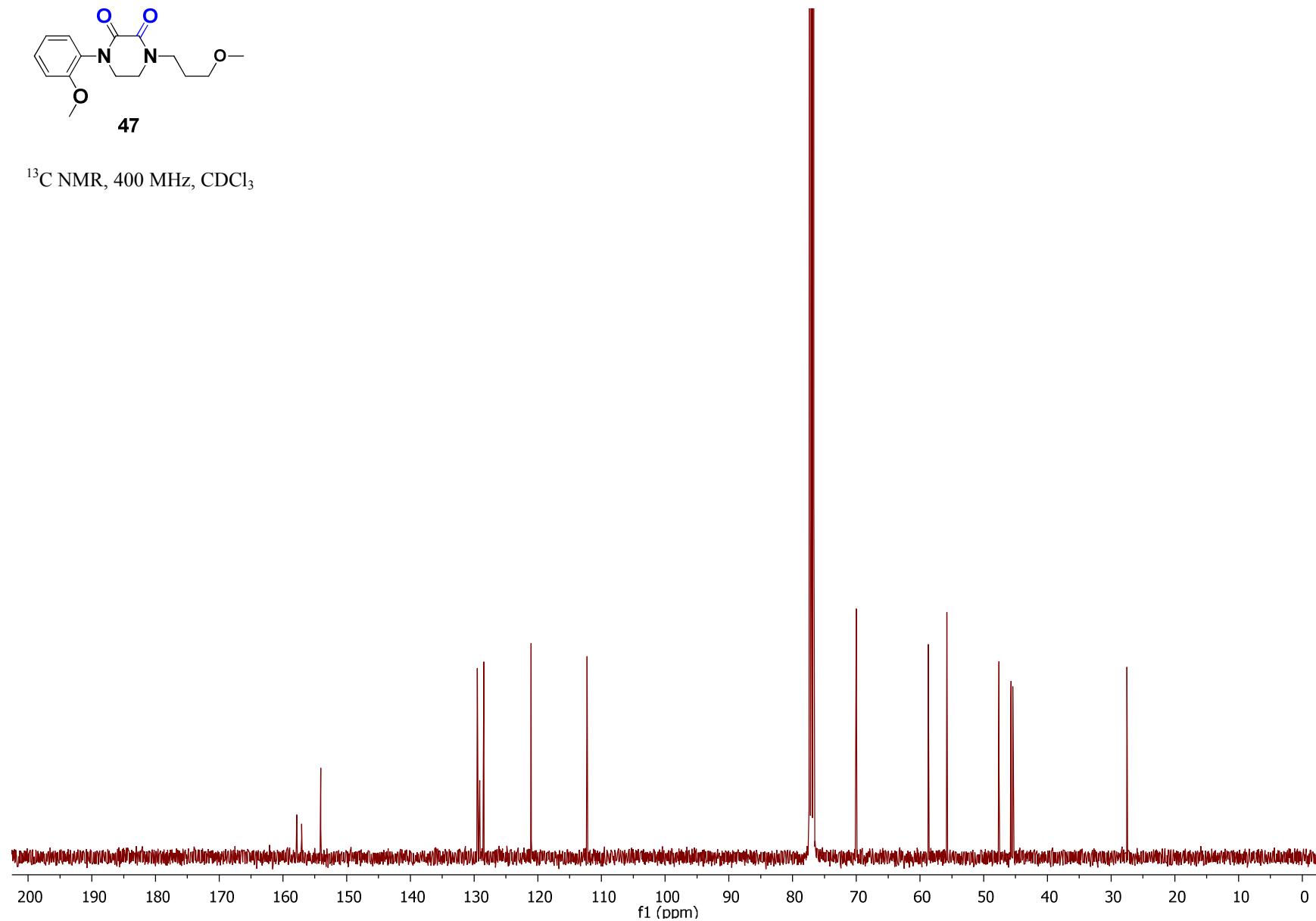


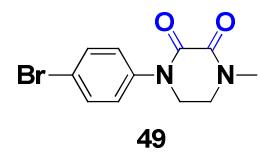
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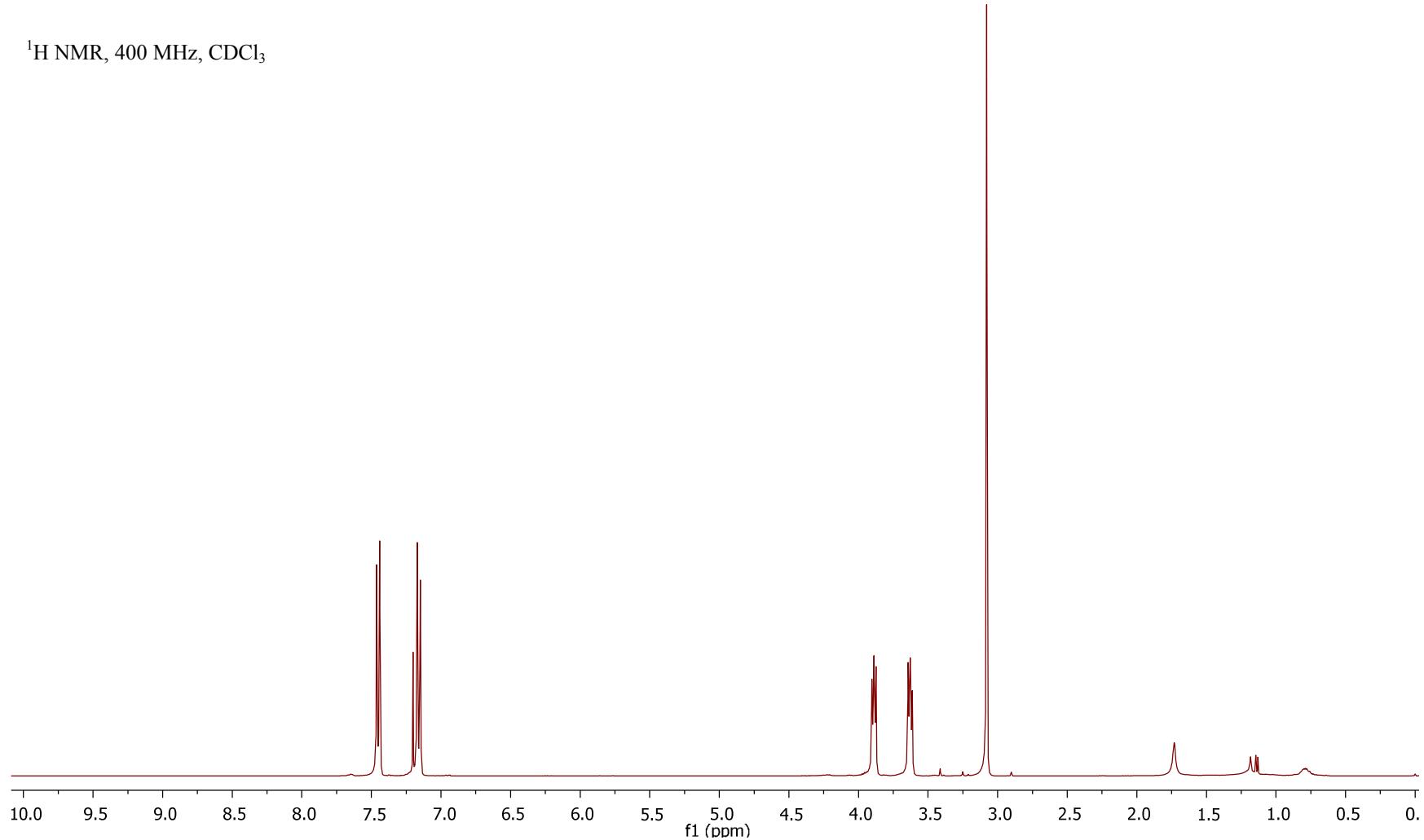


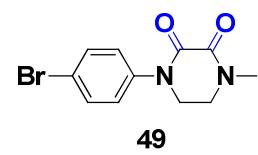
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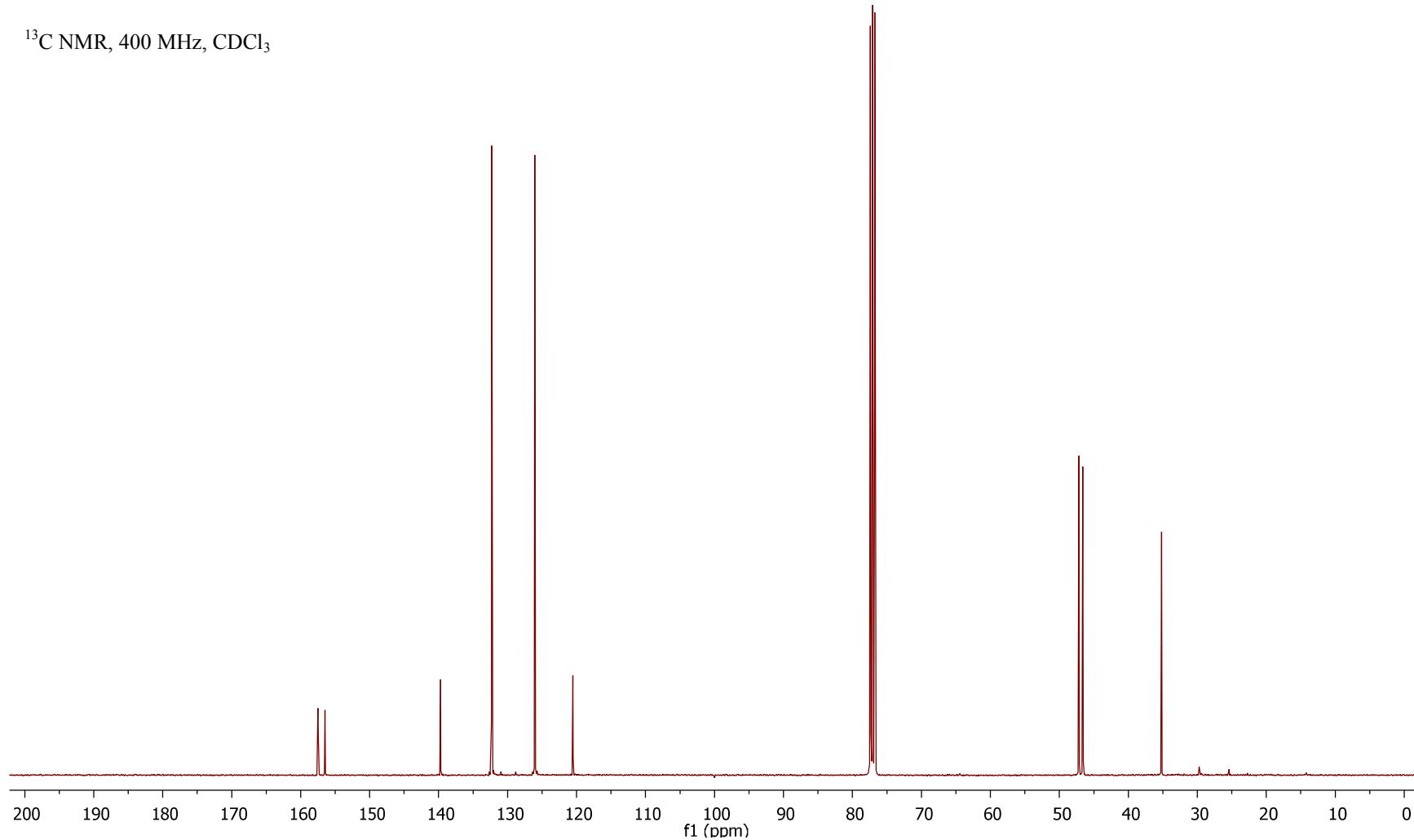


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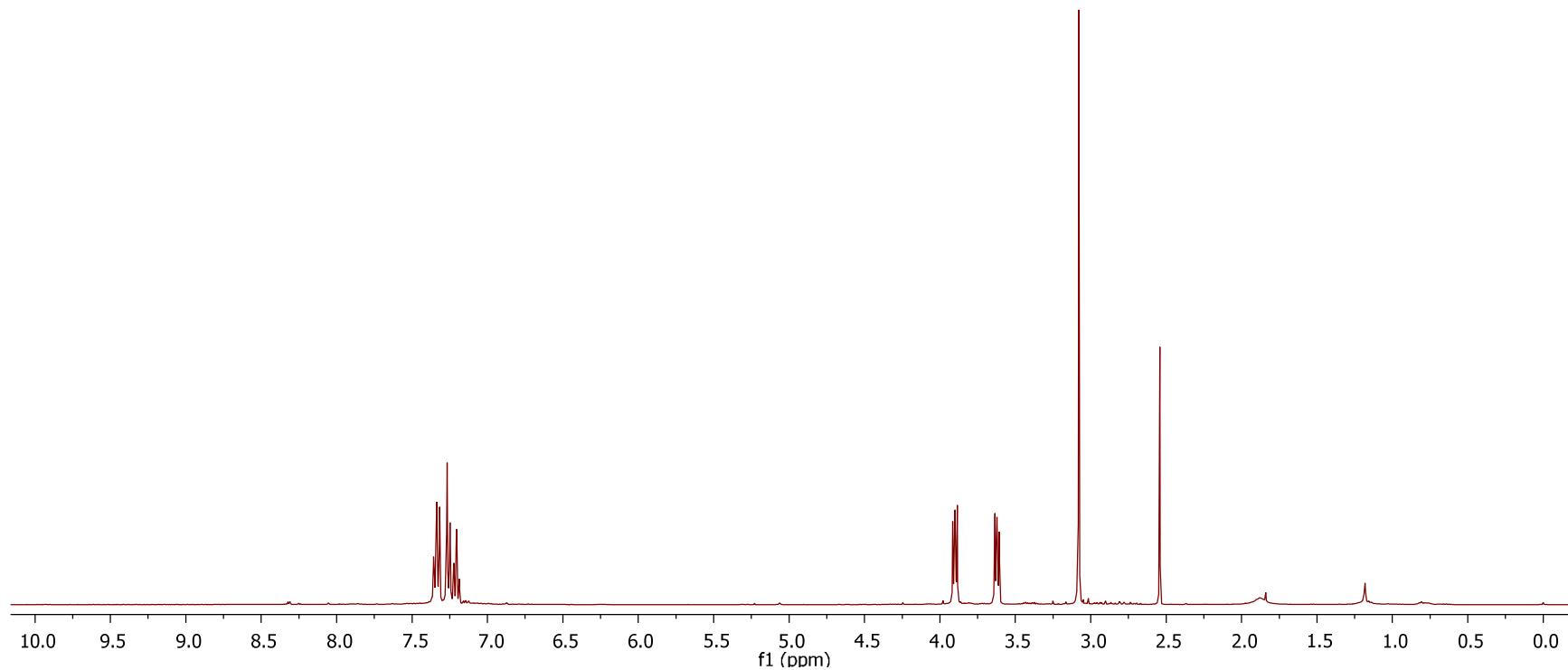
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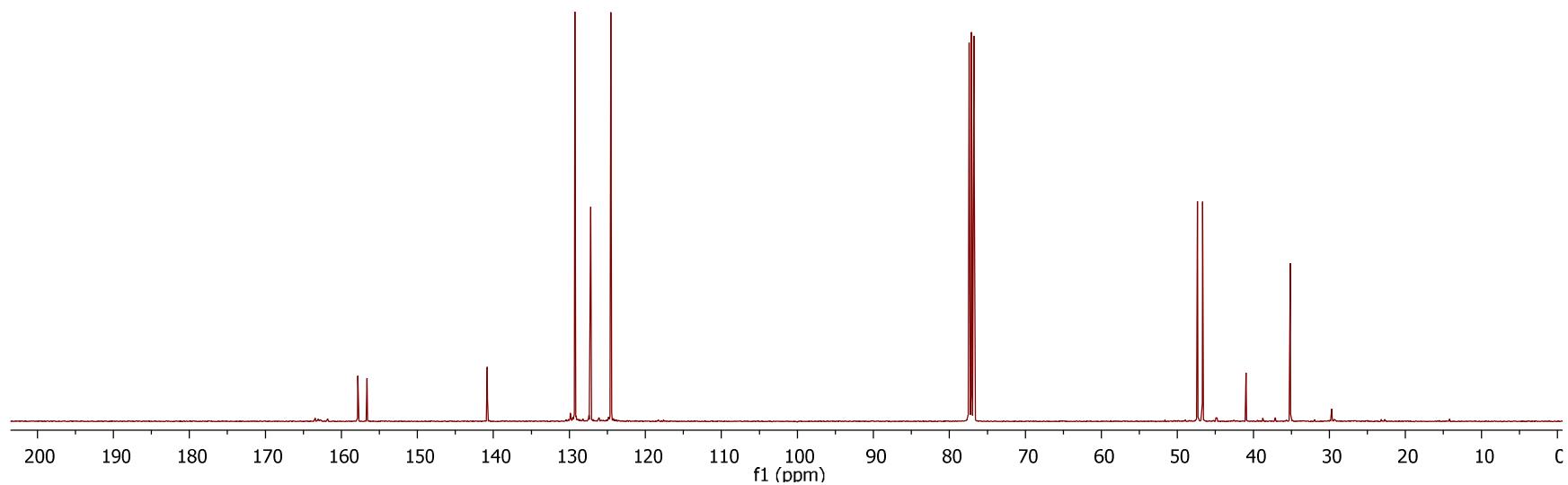
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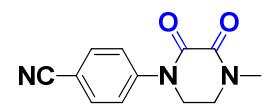




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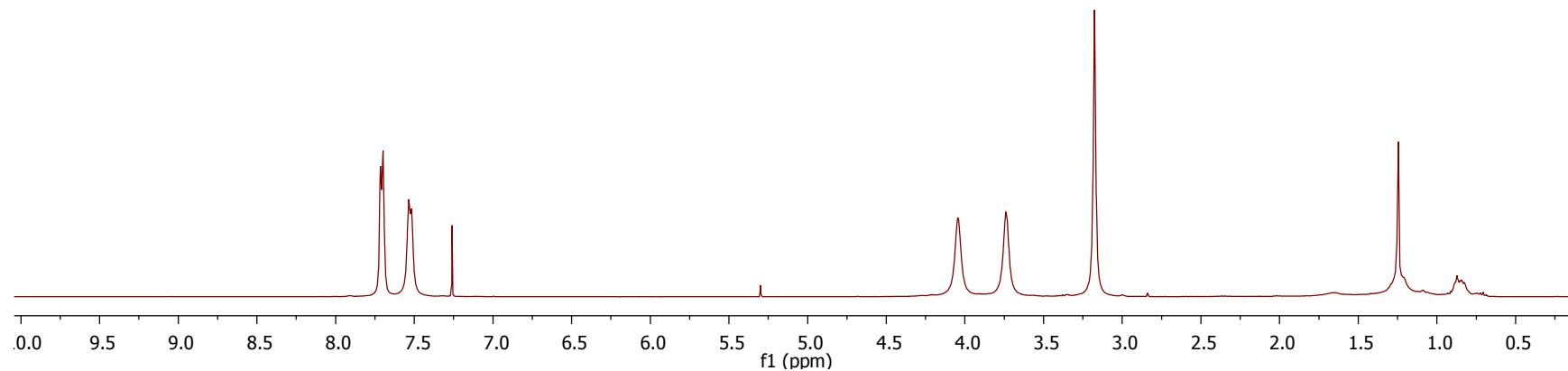
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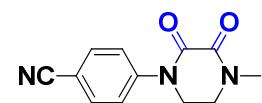




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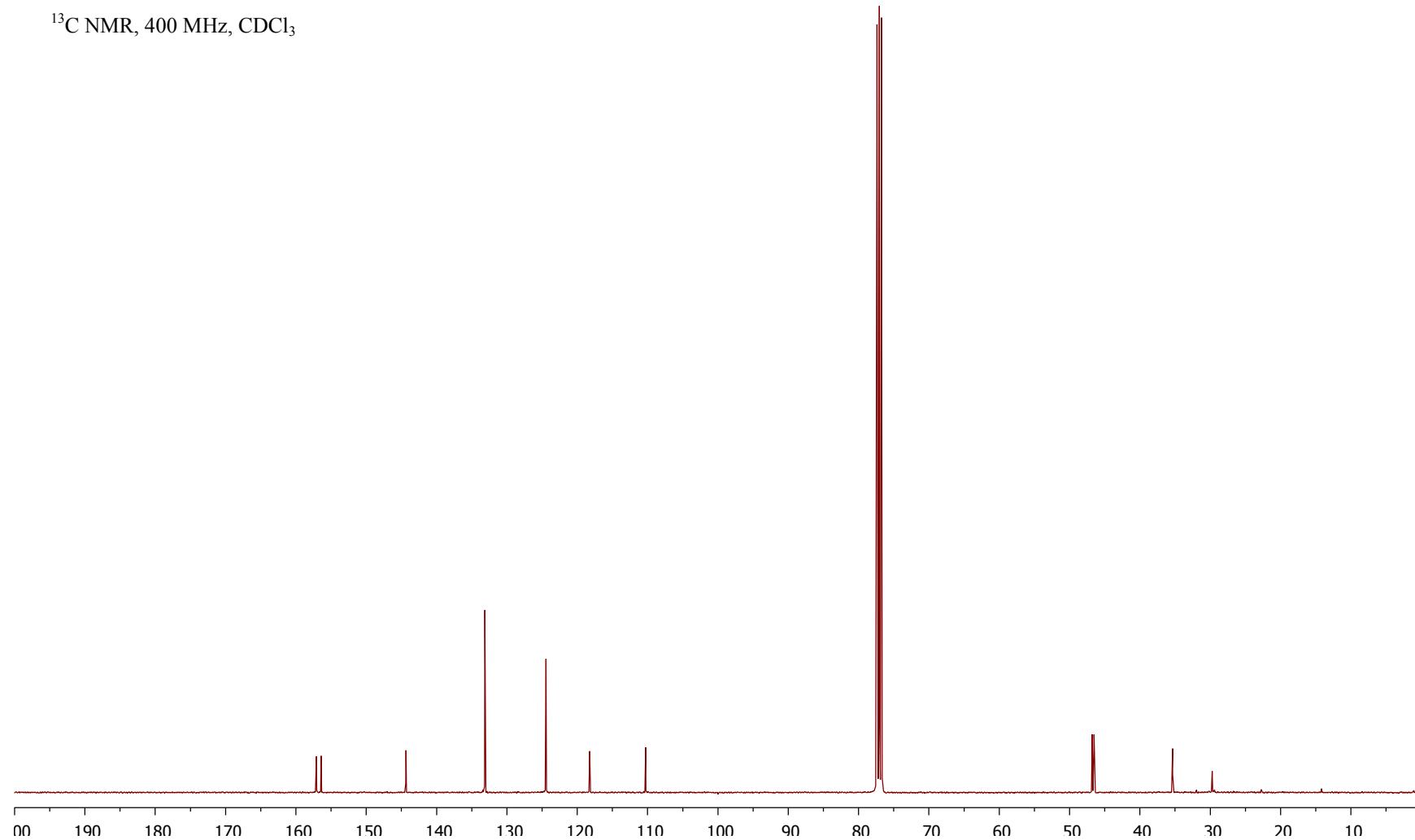
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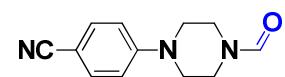




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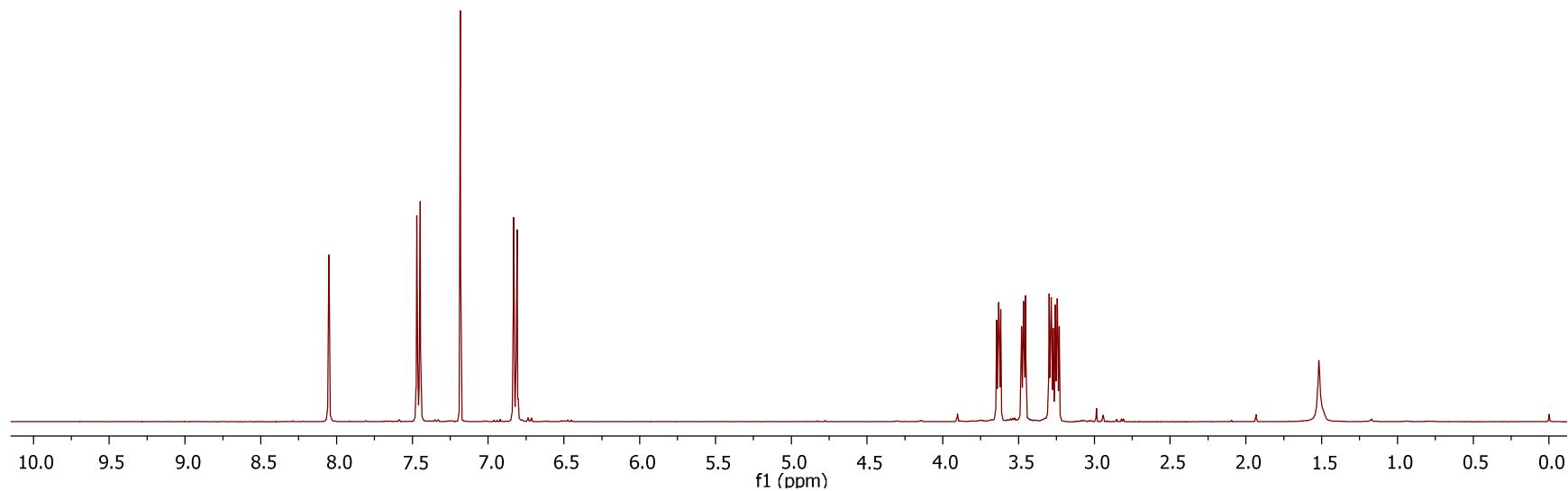
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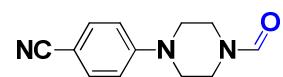




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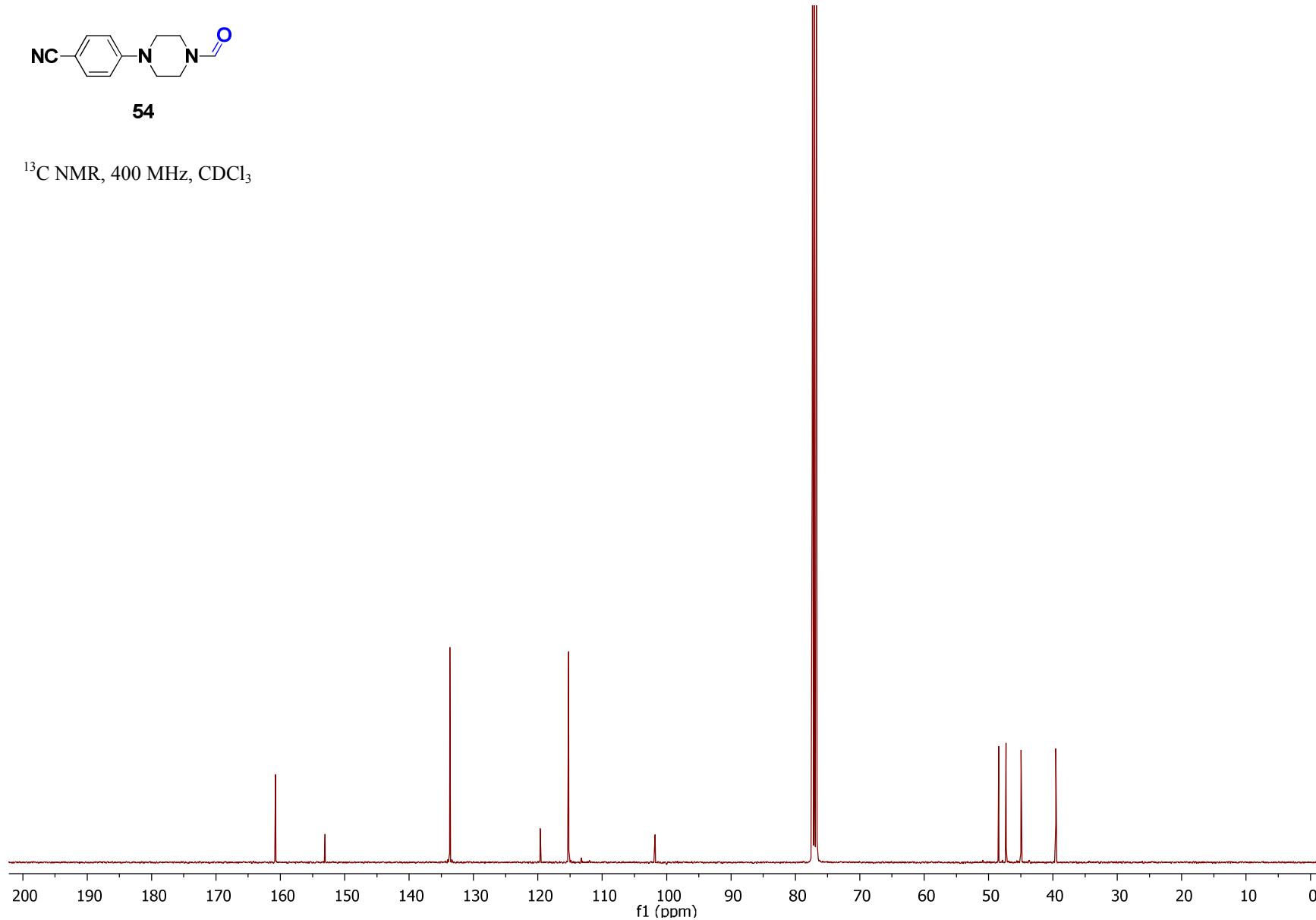
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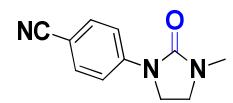




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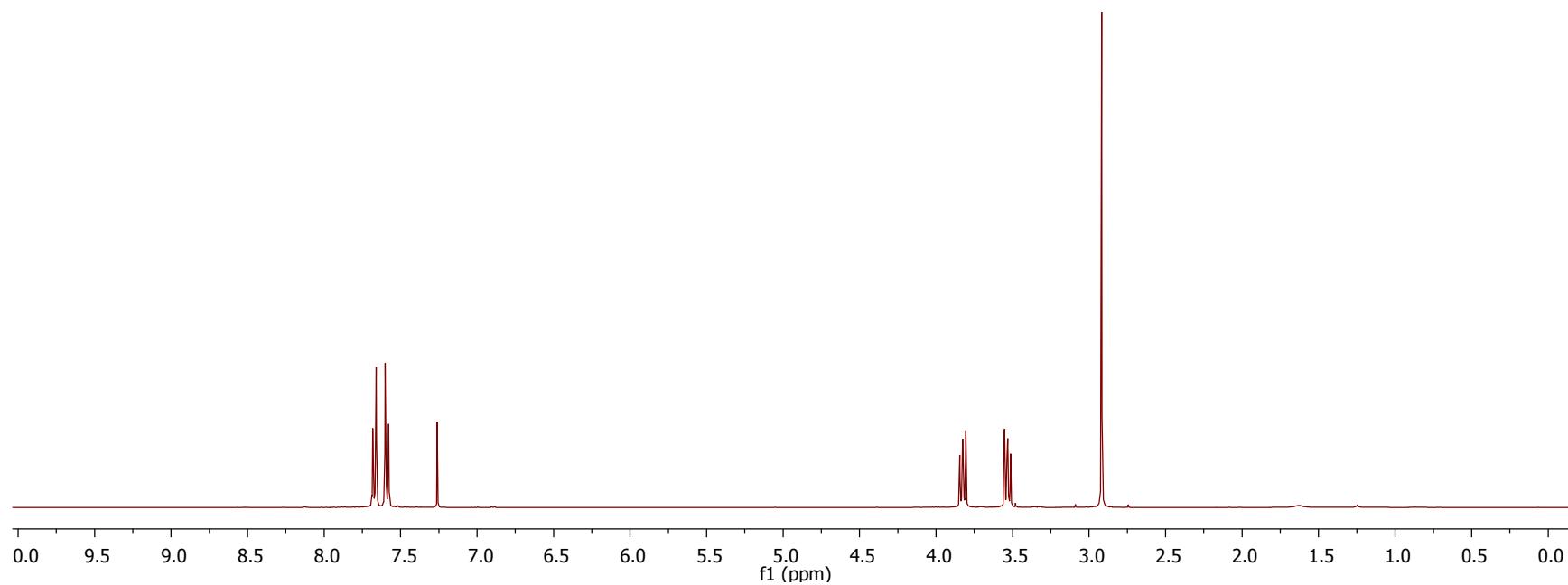
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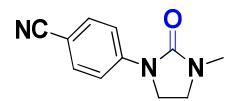




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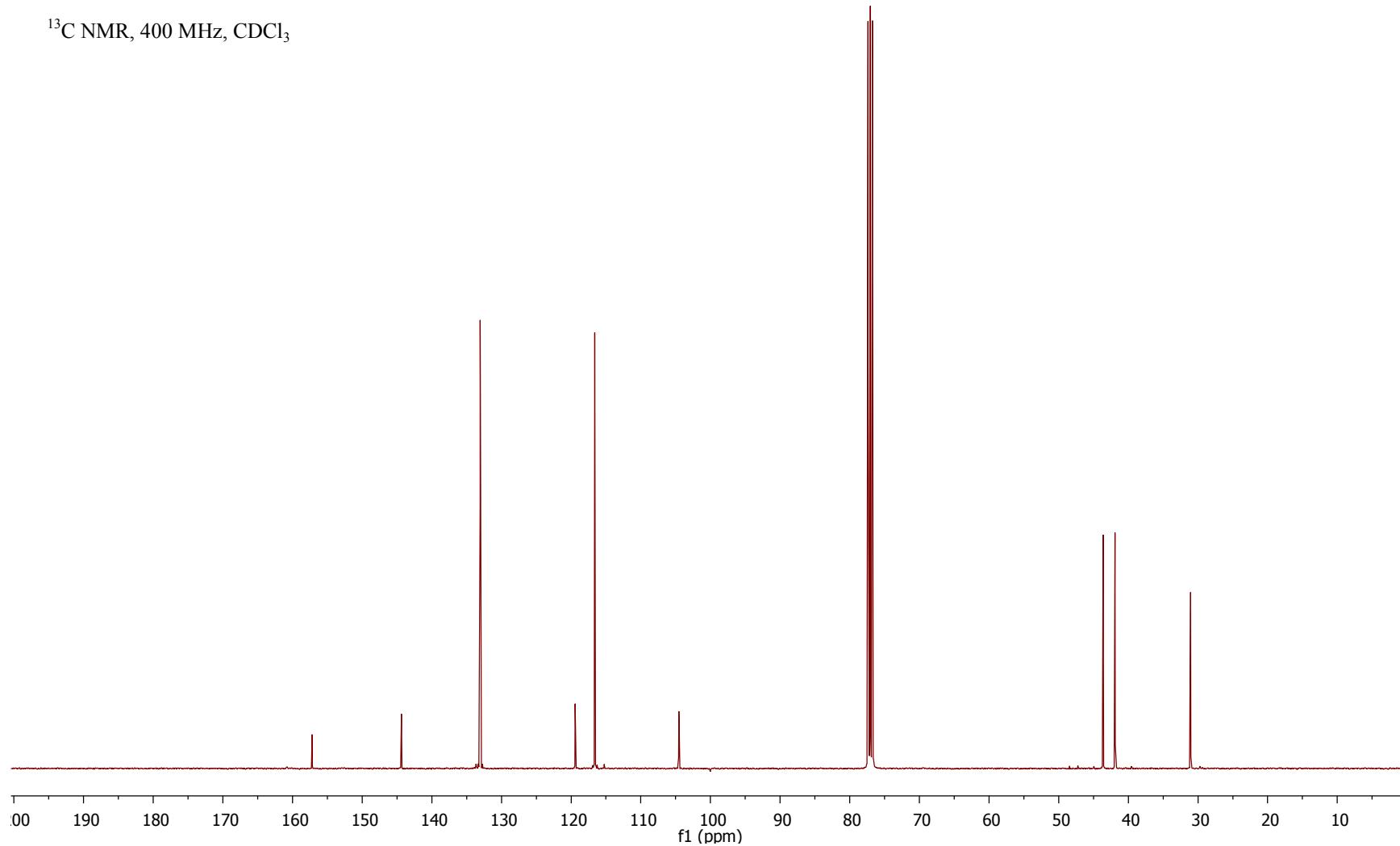
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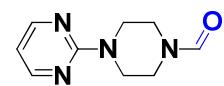




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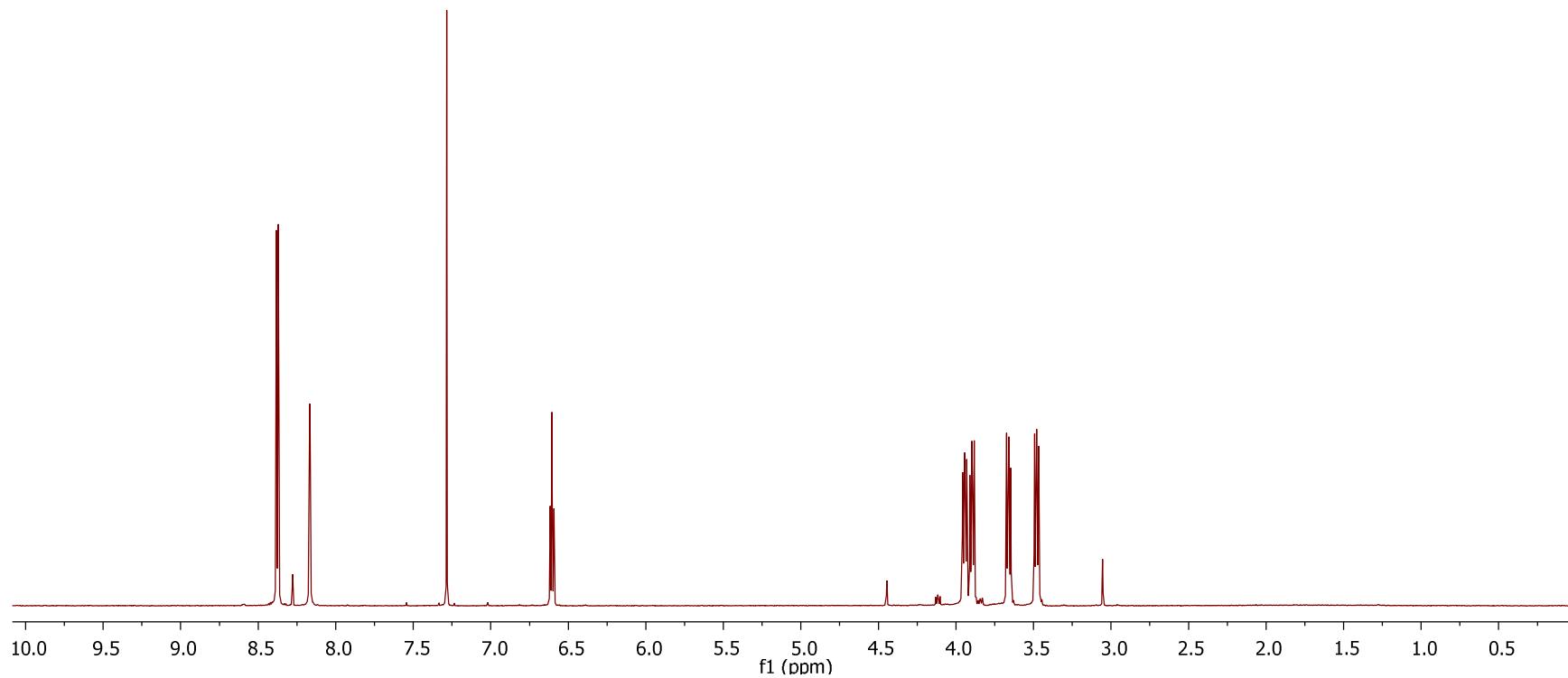
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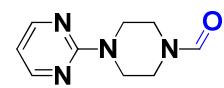




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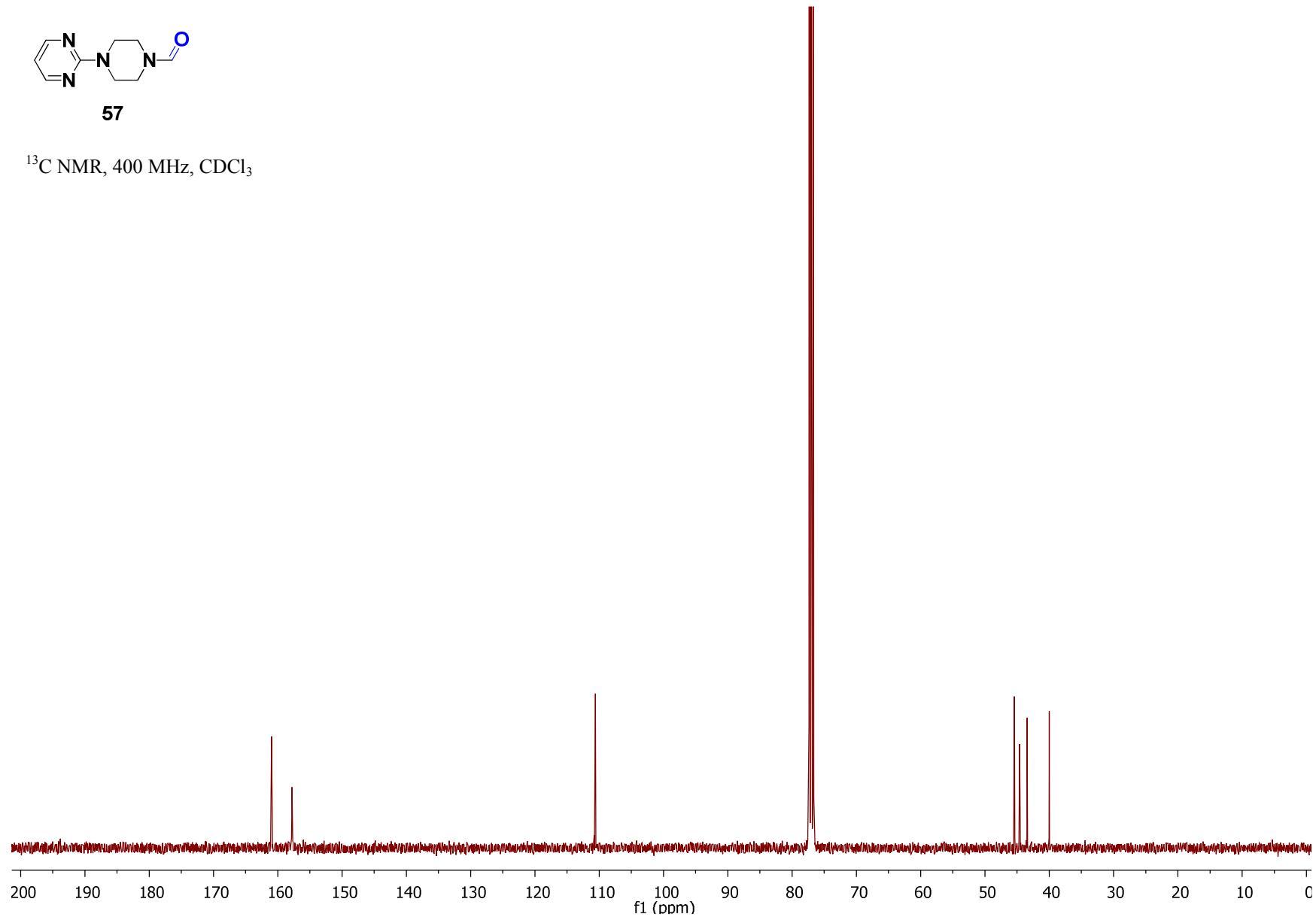
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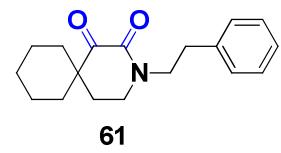




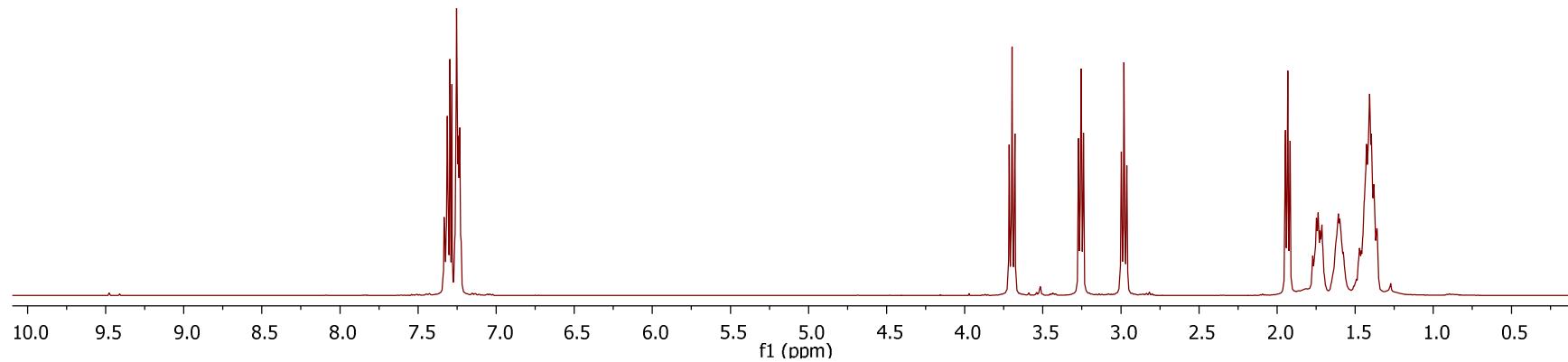
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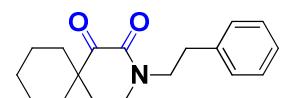
^{13}C NMR, 400 MHz, CDCl_3





¹H NMR, 400 MHz, CDCl₃





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^{13}C NMR, 400 MHz, CDCl_3

