

*Supplemental Material to:*

## **Beyond Natural Flavonoids: Exploring Bioisosterism in Design and Synthesis of Influenza Endonuclease Inhibitors**

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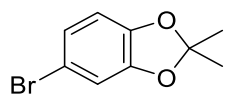
## General information

Unless noted otherwise, all reactions were carried out under argon in oven-dried glassware. Anhydrous solvents were distilled from solvents using agents as indicated and transferred under nitrogen: THF (Na/benzophenone), toluene (Na/benzophenone), MeCN (CaH<sub>2</sub>), and DCM (CaH<sub>2</sub>). Chromatography was performed using flash chromatography Teledyne ISCO CombiFlash NextGen 300+ system with RediSep Rf Gold Silica or RediSep (marked as SiO<sub>2</sub>) Rf Gold Reversed-phase C18 columns (marked as SiO<sub>2</sub>-C<sub>18</sub> or SiO<sub>2</sub>-C<sub>18</sub>-Aq). All starting materials were used as purchased (Merck, Alfa Aesar, TCI, Fluorochem, Combi-Blocks), unless otherwise indicated. All inhibitors were purified using an ECOM compact preparative system TOY18DAD800 (flow rate 15 mL/min; gradient 0 → 60% or 0 → 80% H<sub>2</sub>O (0.1% trifluoroacetic acid)/MeCN in 60 min), with a ProntoSIL 120-10-C18 ace-EPS column, 10 μm, 20 × 250 mm. The purity of compounds and composition of the reaction mixtures were tested on a Waters UPLC-MS Acquity with QDa Mass Detector (flow rate 0.5 mL/min, gradient 0 → 100% H<sub>2</sub>O (0.1% formic acid)/MeCN in 7 min) with an ACQUITY UPLC BEH C18 Column, 130 Å, 1.7 μm, 2.1 mm × 100 mm with 2.1 mm × 5 mm pre-column. The final inhibitors were of at least 95% purity. <sup>1</sup>H-NMR spectra were recorded on Bruker instruments at 401, 500 or 600 MHz; <sup>13</sup>C-NMR spectra were recorded at 101, 126 or 151 MHz. Chemical shifts are provided in δ-scale in ppm; coupling constants *J* are given in Hz. Chemical shifts marked with an asterisk (\*) are visible in two-dimensional NMR spectra (HMBC). FT-IR spectra were recorded using Nicolet iS50 spectrophotometer. Wavenumbers  $\tilde{\nu}$  are provided in cm<sup>-1</sup>. ESI or APCI high resolution mass spectra were recorded using a Thermo Scientific LTQ Orbitrap XL (Termo Fisher Scientific) and EI or CI high resolution mass spectra were recorded using an Agilent 7250 GC/Q-TOF, both controlled by MassLynx software.

## Experimental procedures

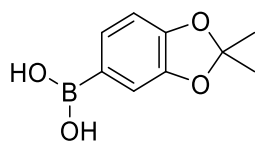
### Synthesis of heterocyclic intermediates

#### *5-Bromo-2,2-dimethylbenzo[d][1,3]dioxole (37):*



Compound was prepared according to according to the published procedure<sup>[1]</sup>. 2,2-Dimethylbenzo[d][1,3]dioxole (1.00 g, 6.66 mmol, 1.0 eq.) was dissolved in DMF (20 mL) followed by an addition of *N*-bromosuccinimide (1.18 g, 6.66 mmol, 1.0 eq.). The reaction mixture was allowed to stir for 16 hours until the starting material was consumed (GC-MS). Then EtOAc (50 mL) was added, and the organic phase was washed with water (3 × 80 mL) and brine (1 × 20 mL). Then the organic phase was dried over anhydrous MgSO<sub>4</sub>, and the solvents were evaporated, and the residue was purified by flash chromatography (SiO<sub>2</sub>, *n*-hexane/EtOAc = 100:0 → 95:5) to afford the desired acetone **37** (1.28 g, 84%). <sup>1</sup>H NMR (401 MHz, CDCl<sub>3</sub>) δ = 6.89 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.86 (d, *J* = 2.0 Hz, 1H), 6.60 (d, *J* = 8.1 Hz, 1H), 1.67 (s, 6H) ppm. <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ = 148.4, 146.8, 123.7, 119.0, 112.4, 112.1, 109.4, 25.8 ppm.

#### *(2,2-Dimethylbenzo[d][1,3]dioxol-5-yl)boronic acid (38):*



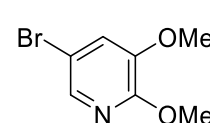
Compound was prepared according to according to the published procedure<sup>[2]</sup>. Compound **37** (2.0 g, 8.73 mmol, 1.0 eq.) was dissolved in anhydrous THF (44 mL) in flame-dried flask under argon atmosphere. The solution was cooled to −78 °C followed by an addition of 2.5M solution of *n*-butyllithium in hexanes (4.2 mL, 10.5 mmol, 1.2 eq.) dropwise at −78 °C under argon atmosphere. Then the reaction mixture was allowed to stir for 1 hour followed by an addition of triisopropyl borate (2.4 mL, 10.5 mmol, 1.2 eq.) dropwise. The reaction mixture was allowed to stir for 1 hour −78 °C and then 16 hours at room temperature. Then the reaction mixture was quenched by water (2.0 mL) followed by an addition of 5% HCl (aq.) until pH = 4 and slurry was dissolved. The aqueous phase was extracted with EtOAc (3 × 50 mL) and combined organic phases was washed with brine (1 × 10 mL) and dried over anhydrous MgSO<sub>4</sub>. The solvents were evaporated, and the residue was purified by flash chromatography (SiO<sub>2</sub>, *n*-hexane/EtOAc = 100:0 → 0:100) to afford the desired boronic acid **38** (1.21 g, 71%). <sup>1</sup>H NMR (401 MHz, CDCl<sub>3</sub>) δ = 7.75 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.53 (d, *J* = 1.2 Hz,

1H), 6.86 (d,  $J = 7.8$  Hz, 1H), 1.73 (s, 6H) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta = 151.4, 147.5, 130.7, 118.2, 114.4, 108.5, 26.1$  ppm.

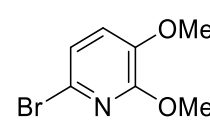
### Bromination of 2,3-dimethoxypyridine:

2,3-Dimethoxypyridine (1.00 g, 7.2 mmol, 1.0 eq.) was dissolved in anhydrous DMF (2.0 mL) followed by an addition of solution of *N*-bromosuccinimide (1.28 g, 7.2 mmol, 1.0 eq.) in anhydrous DMF (1.0 mL). Then the reaction mixture was allowed to stir for 24 hours at room temperature until the starting material was consumed (TLC, UPLC-MS). Then EtOAc (40 mL) was added, and organic phase was washed with water ( $3 \times 100$  mL) and brine ( $1 \times 10$  mL). The organic phase was dried over anhydrous  $\text{MgSO}_4$ . The solvents were evaporated, and the residue was purified by flash chromatography ( $\text{SiO}_2\text{-C}_{18}$ ,  $\text{H}_2\text{O}$  (0.1% TFA)/MeCN = 100:0  $\rightarrow$  30:70) to obtain two fractions. Both were separately concentrated to half of volume and extracted with dichloromethane ( $3 \times 30$  mL), washed with water ( $1 \times 20$  mL) and dried over anhydrous  $\text{MgSO}_4$ . The solvents were evaporated to afford more polar 5-bromo-2,3-dimethoxypyridine (**39**) (628 mg, 40%) and less polar 6-bromo-2,3-dimethoxypyridine (**40**) (707 mg, 45%).

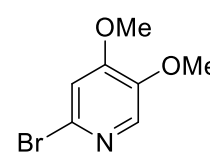
#### 5-Bromo-2,3-dimethoxypyridine (**39**):

  $^1\text{H}$  NMR (401 MHz,  $\text{CDCl}_3$ )  $\delta = 7.76$  (d,  $J = 2.1$  Hz, 1H), 7.12 (d,  $J = 2.1$  Hz, 1H), 3.97 (s, 3H), 3.85 (s, 3H) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta = 153.5, 144.6, 137.4, 120.3, 111.2, 56.1, 54.0$  ppm. MS (ESI)  $m/z$  calcd for  $\text{C}_7\text{H}_9\text{BrNO}_2$   $[\text{M} + \text{H}^+]^+$  217.98, found 217.75.

#### 6-Bromo-2,3-dimethoxypyridine (**40**):

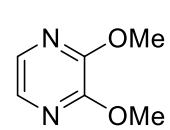
  $^1\text{H}$  NMR (401 MHz,  $\text{CDCl}_3$ )  $\delta = 6.96$  (d,  $J = 8.0$  Hz, 1H), 6.89 (d,  $J = 8.0$  Hz, 1H), 3.98 (s, 3H), 3.82 (s, 3H) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta = 153.9, 143.6, 126.7, 119.9, 119.8, 56.1, 54.5$  ppm. MS (ESI)  $m/z$  calcd for  $\text{C}_7\text{H}_9\text{BrNO}_2$   $[\text{M} + \text{H}^+]^+$  217.98, found 217.78.

#### 2-Bromo-4,5-dimethoxypyridine (**41**):

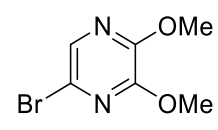
 2-Bromo-5-fluoro-4-iodopyridine (1.21 g, 4.0 mmol, 1.0 eq.) was dissolved in anhydrous DMF (2.5 mL). Then the flask was put into a cold-water bath (approx. 15 °C) and freshly prepared sodium methoxide (540 mg, 10 mmol, 2.5 eq.) was added to the mixture. The reaction mixture was allowed to stir for 2 hours at room temperature under nitrogen atmosphere until starting material was consumed (according to

UPLC-MS ( $\lambda = 254$  nm): ratio (2-bromo-4,5-dimethoxypyridine/2-bromo-4-iodo-5-methoxypyridine) = 27.6:72.4). Then the reaction mixture was quenched by an addition of saturated aqueous solution of  $\text{NH}_4\text{Cl}$  (30 mL) and water (30 mL). Aqueous phase was extracted with EtOAc ( $1 \times 20$  mL). Then the organic phase was washed with water ( $3 \times 50$  mL), brine ( $1 \times 10$  mL) and dried over anhydrous  $\text{MgSO}_4$ . The solvents were evaporated, and the residue was purified by flash chromatography ( $\text{SiO}_2$ , cyclohexane/hexane/EtOAc = 100:0  $\rightarrow$  0:100) to afford the desired 2-bromo-4,5-dimethoxypyridine (**41**) (242 mg, 28%).  $^1\text{H}$  NMR (401 MHz,  $\text{CDCl}_3$ )  $\delta$  = 7.80 (s, 1H), 6.88 (s, 1H), 3.87 (s, 6H) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  = 156.5, 145.9, 133.8, 132.6, 110.5, 56.7, 56.3 ppm. HRMS (EI)  $m/z$  calcd for  $\text{C}_7\text{H}_8\text{BrNO}_2$   $[\text{M}]^{+}$  216.9733, found 216.9731.

*2,3-Dimethoxypyrazine (42):*

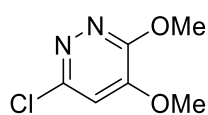
 2,3-Dichloropyrazine (1.50 g, 10.1 mmol, 1.0 eq.) was dissolved in anhydrous methanol (10 mL). Then the solution was cooled to  $0^\circ\text{C}$  by ice/water bath and freshly prepared sodium methoxide (4.91 g, 90.1 mmol, 9.0 eq.) was added in one portion. Then the reaction mixture was allowed to stir for 30 minutes at  $0^\circ\text{C}$  and then for 2 hours at room temperature under nitrogen atmosphere until the starting material was consumed (TLC, UPLC-MS). Solvent was evaporated and residual solids were suspended in water (50 mL). Aqueous phase was extracted with dichloromethane ( $3 \times 40$  mL) and combined organic phases were washed with brine ( $1 \times 10$  mL) dried over anhydrous  $\text{MgSO}_4$ . The solvents were evaporated to afford the desired 2,3-dimethoxypyrazine (**42**) (1.12 g, 84%).  $^1\text{H}$  NMR (401 MHz,  $\text{CDCl}_3$ )  $\delta$  = 7.61 (s, 2H), 4.00 (s, 6H) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  = 150.7 (2C), 132.1 (2C), 54.0 (2C) ppm. MS (ESI)  $m/z$  calcd for  $\text{C}_6\text{H}_9\text{N}_2\text{O}_2$   $[\text{M} + \text{H}^+]^+$  141.06, found 141.07.

*5-Bromo-2,3-dimethoxypyrazine (43):*

 Compound **42** (280 mg, 2.0 mmol, 1.0 eq.) was dissolved in anhydrous DMF (2.8 mL) followed by an addition of *N*-bromosuccinimide (498 mg, 2.8 mmol, 1.4 eq.). Then the reaction mixture was allowed to stir for 4 days at room temperature until the starting material was consumed (TLC, UPLC-MS). Then EtOAc (20 mL) was added, and organic phase was washed with water ( $3 \times 50$  mL). The organic phase was dried over anhydrous  $\text{MgSO}_4$ . The solvents were evaporated to afford the desired 5-bromo-2,3-dimethoxypyrazine (**43**) (107 mg, 24%).  $^1\text{H}$  NMR (401 MHz,  $\text{CDCl}_3$ )  $\delta$  = 7.69 (s, 1H), 4.01 (s,

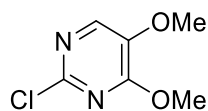
3H), 3.98 (s, 3H) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  = 149.7, 149.6, 133.0, 124.9, 54.9, 54.5 ppm. MS (ESI)  $m/z$  calcd for  $\text{C}_6\text{H}_8\text{BrN}_2\text{O}_2$   $[\text{M} + \text{H}^+]^+$  218.98, found 218.76.

*6-Chloro-3,4-dimethoxypyridazine (44):*



Compound was prepared according to the published procedure<sup>[3]</sup>. 3,4,6-Trichloropyridazine (2.00 g, 10.9 mmol, 1.0 eq.) was dissolved in anhydrous methanol (150 mL). Then the solution was cooled to 0°C by ice/water bath and freshly prepared sodium methoxide (1.76 g, 32.7 mmol, 2.0 eq.) was added. Then the reaction mixture was allowed to stir for 48 hours at room temperature under nitrogen atmosphere until conversion of starting material stayed unchanged (according to UPLC-MS ( $\lambda$  = 254 nm): ratio (product/starting material) = 30.1:69.9). Solvent was evaporated and residual solids were dissolved in EtOAc (40 mL). Organic phase was washed with saturated aqueous solution of  $\text{NH}_4\text{Cl}$  ( $3 \times 40$  mL) and brine ( $1 \times 20$  mL) dried over anhydrous  $\text{MgSO}_4$ . The solvents were evaporated, and the residue was purified by flash chromatography ( $\text{SiO}_2$ , cyclohexane/EtOAc = 100:0  $\rightarrow$  70:30) to afford the desired 6-chloro-3,4-dimethoxypyridazine (**44**) (286 mg, 15%).  $^1\text{H}$  NMR (401 MHz,  $\text{CDCl}_3$ )  $\delta$  = 6.76 (s, 1H), 4.14 (s, 3H), 3.94 (s, 3H) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  = 157.1, 151.2, 149.9, 56.4, 55.5 ppm. MS (ESI)  $m/z$  calcd for  $\text{C}_6\text{H}_8\text{ClN}_2\text{O}_2$   $[\text{M} + \text{H}^+]^+$  175.03, found 175.15.

*2-Chloro-4,5-dimethoxypyrimidine (45):*

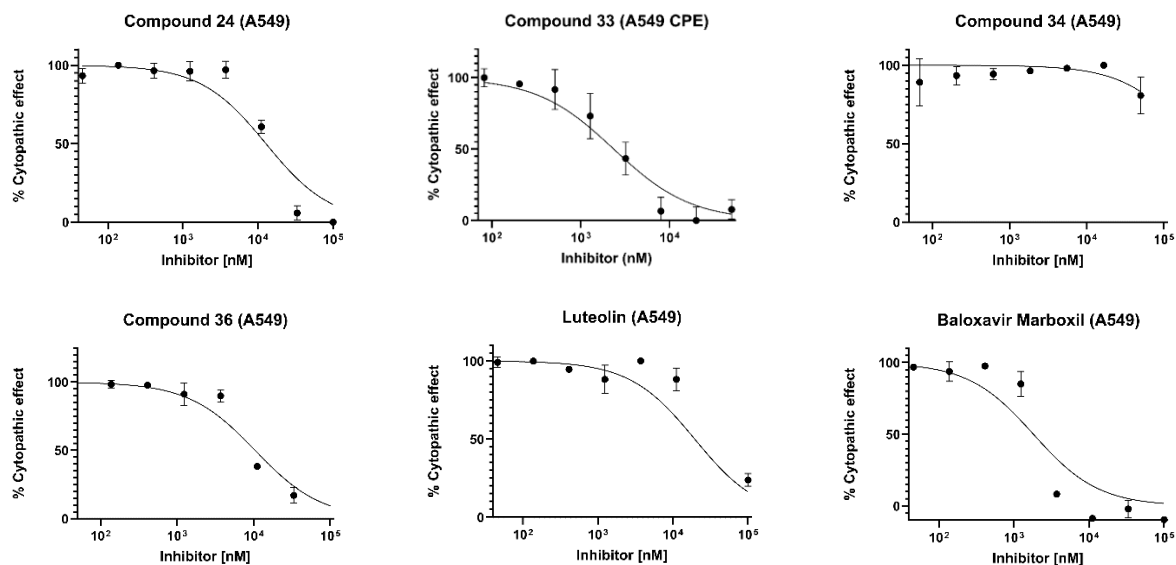


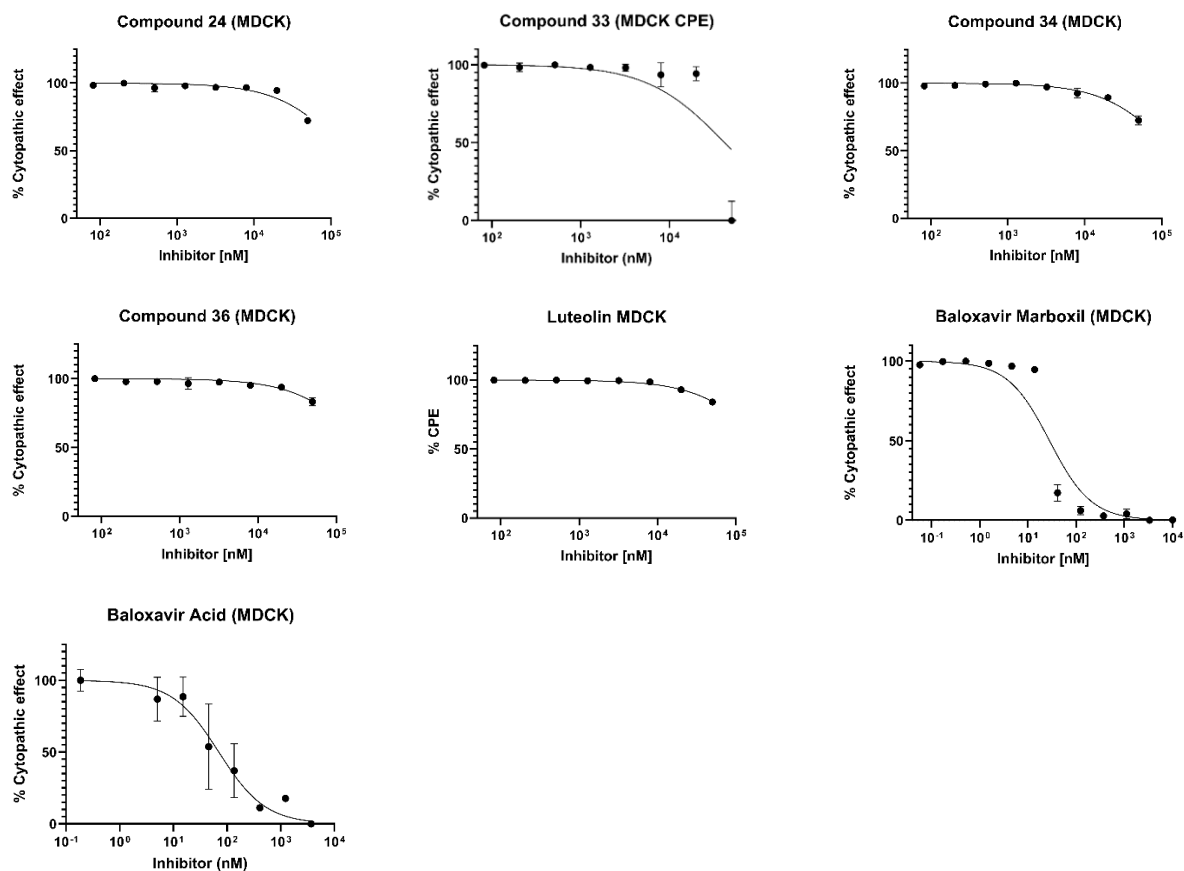
Compound was prepared according to the published procedure<sup>[3]</sup>. 2,4-Dichloro-5-methoxypyrimidine (1.00 g, 5.59 mmol, 1.0 eq.) was dissolved in methanol (21 mL) followed by an addition of potassium carbonate (772 mg, 5.59 mmol, 1.0 eq.) Then the reaction mixture was allowed to stir for 16 hours at room temperature under nitrogen atmosphere until the starting material was consumed (TLC, UPLC-MS). Solvent was evaporated and residual solids were suspended in EtOAc (50 mL). Organic phase was washed with water ( $2 \times 50$  mL), brine ( $1 \times 10$  mL) and dried over anhydrous  $\text{MgSO}_4$ . The solvents were evaporated to afford the desired 2-chloro-4,5-dimethoxypyrimidine (**45**) (801 mg, 82%).  $^1\text{H}$  NMR (401 MHz,  $\text{CDCl}_3$ )  $\delta$  = 7.87 (s, 1H), 4.06 (s, 3H), 3.91 (s, 3H) ppm.  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  = 161.3, 150.1, 141.8, 138.2, 56.7, 55.2 ppm. MS (ESI)  $m/z$  calcd for  $\text{C}_6\text{H}_8\text{ClN}_2\text{O}_2$   $[\text{M} + \text{H}^+]^+$  175.03, found 175.09.

## Anti-Influenza A H1N1 California effect of compounds, cytopathic effect (CPE) detection

Anti-Influenza A H1N1 California activity of selected compounds was tested in MDCK cells (20,000 cells/100  $\mu$ L), and A549 cells (25,000 cells/100  $\mu$ L) in 96-well plate. Compounds were added to the cells, and after one hour, cells were infected with Influenza A H1N1 California virus (MOI 0.002 for MDCK cells and MOI 0.02 for A549 cells). Infection was carried out in the Influenza growth medium (DMEM high glucose, no serum, 10 mM HEPES, 0.125% BSA Fraction, 1  $\mu$ g/mL TPCK-Trypsin, Penicillin/Streptomycin)

Cells were incubated for 3 days (MDCK cells) or 4 days (A549) in 37°C, 5% CO<sub>2</sub>. After incubation, cytopathic effect (CPE) was analysed by XTT colorimetric assay. 50  $\mu$ L of 50:1 mixture of XTT labelling reagent (1 mg/mL) and PMS electron-coupling reagent (0.383 mg/mL) was added to the wells and incubated for 4 hours at 37 °C in 5% CO<sub>2</sub>. Formation of orange formazan dye was measured in EnVision plate reader. The experiments were performed in biological triplicates, and the resulting data were analysed using GraphPad Prism 9.0 software (GraphPad Software, San Diego, CA, USA).

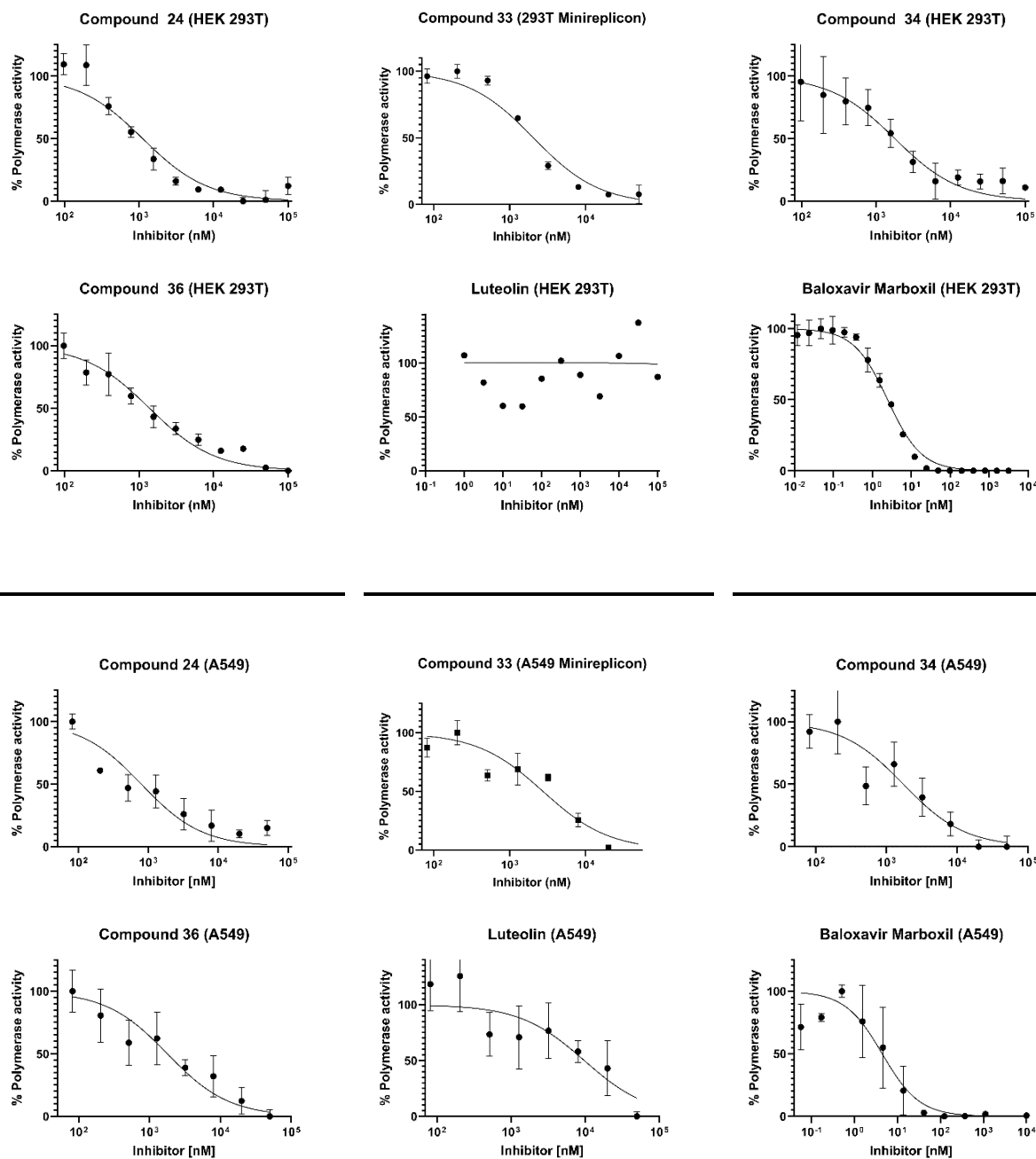


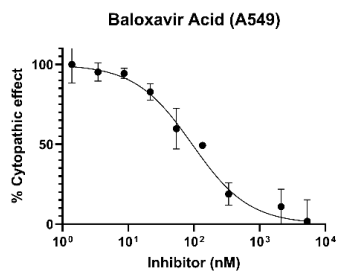


## Minireplicon assay

To determine the effect of the tested compounds on polymerase activity in cell, we used a minireplicon assay adapted as described. All plasmids for the minireplicon assay were kindly provided by Prof. Yoshihiro Kawaoka, University of Wisconsin, Madison, USA. HEK293T cells were seeded in a 96-well plate at a concentration of  $3 \times 10^5$  cells per well in 100  $\mu$ L of DMEM complete (10% FBS, Penicillin/Streptomycin) medium. Alternatively, A549 cells were seeded in a 96-well plate at a concentration of  $3 \times 10^5$  cells per well in 100  $\mu$ L of DMEM complete (10% FBS, Penicillin/Streptomycin) medium. The cells were co-transfected with a set of plasmids encoding the three polymerase subunits and the viral nucleoprotein (pcDNA-PB1, pcDNA-PB2, pcDNA-PA, pcDNA-NP, all sequences originating from influenza A/WSN/33 H1N1 strain), and with an influenza virus-specific RNA polymerase I-driven firefly luciferase reporter plasmid (pPolII-Flu-ffLuc), using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, USA). To minimize transfection variability, the plasmid pGL4.74 (Promega, Madison, USA) encoding the sequence for *Renilla* luciferase was used as an internal control. Cells were harvested two days after

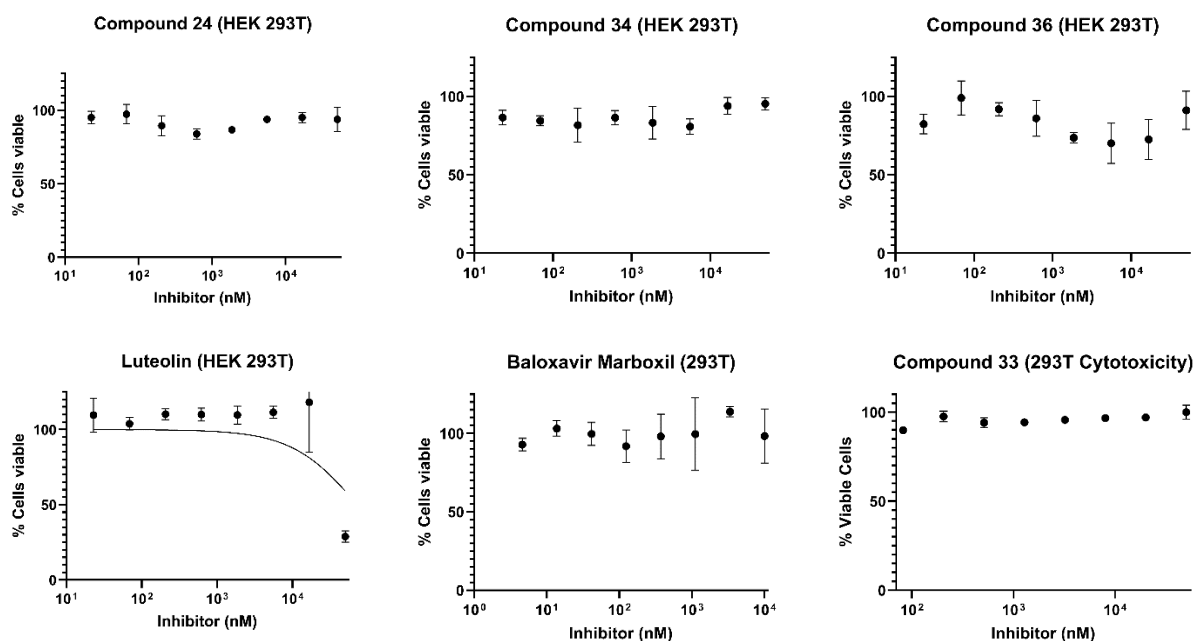
transfection and incubation with peptides, and luciferase expression was determined using Dual-Glo® Luciferase Assay System (Promega, Madison, USA) as per the supplied protocol. The experiments were performed in biological triplicates, and the resulting data were analysed using GraphPad Prism 9.0 software (GraphPad Software, San Diego, CA, USA).

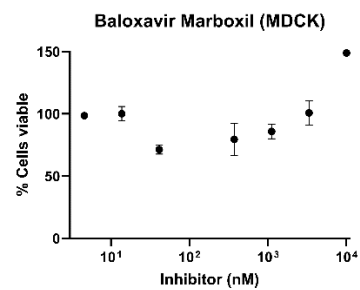
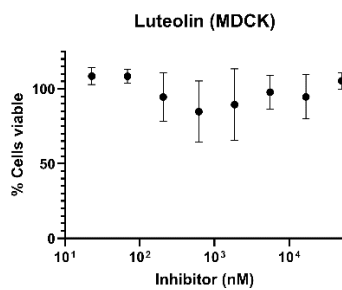
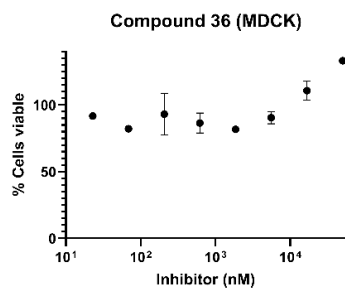
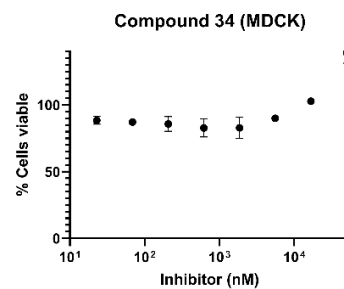
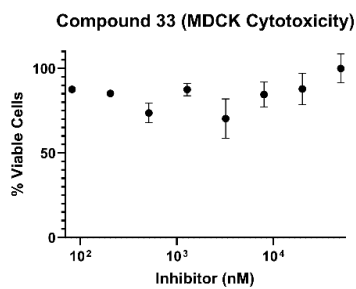
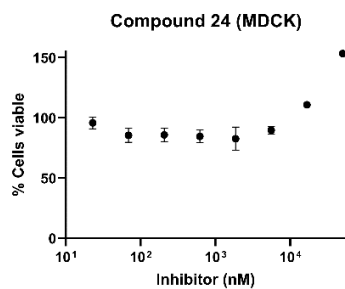
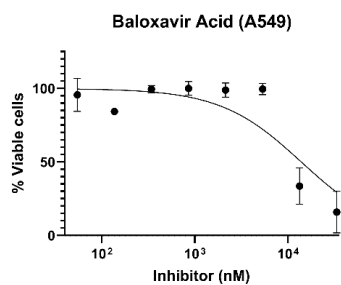
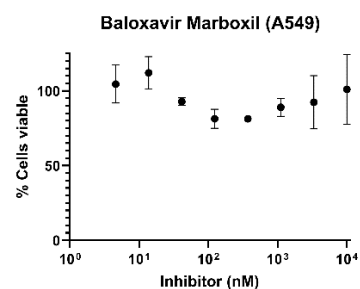
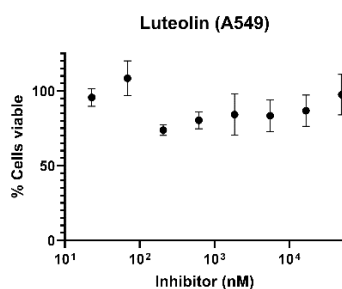
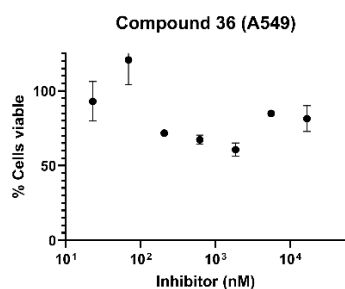
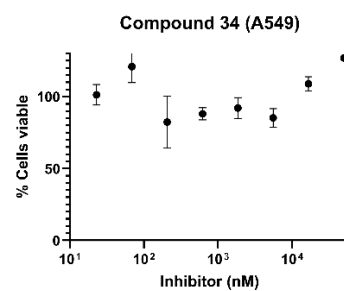
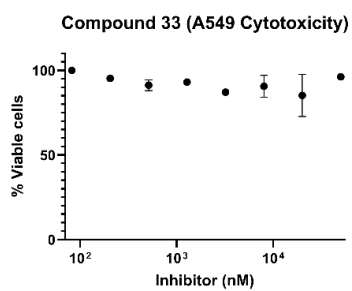
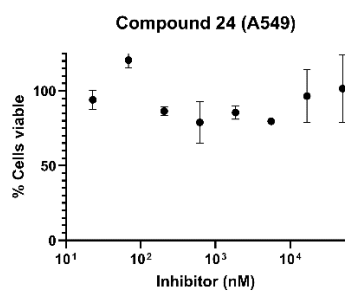


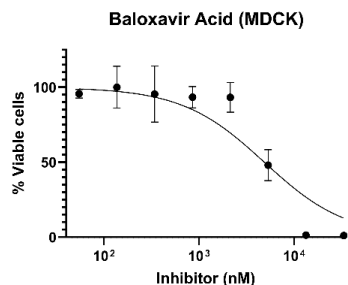


## Cytotoxicity assay

The cytotoxic concentrations that reduced target cell viability by 50% ( $CC_{50}$ ) were determined by incubating serial dilutions of each tested compound and control compounds with the selected cell cultures. HEK 293T cells were seeded at  $3 \times 10^3$  cells per well in 100  $\mu$ l of DMEM complete medium. The following day the tested compounds were added to the corresponding well and the cells were incubated for 48 hours at 37 °C in 5%  $CO_2$ . After incubation, cell viability was analysed by XTT colorimetric assay. 50  $\mu$ l of 50:1 mixture of XTT labelling reagent (1 mg/mL) and PMS electron-coupling reagent (0.383 mg/mL) were added to the wells and incubated for 4 hours at 37 °C in 5%  $CO_2$ . The formation of orange formazan dye was measured on a Tecan Spark plate reader (Tecan Life Sciences, USA). The experiments were performed in biological triplicates, and the resulting data were analyzed using GraphPad Prism 10.0 software (GraphPad Software, San Diego, CA, USA).







## AlphaScreen Assay

AlphaScreen experiments were performed using a Perkin Elmer Enspire plate reader in 96-well ProxiPlates. Biotinylated L-742.001 derivative was captured on Streptavidin-coated donor beads (Perkin Elmer). Separately, GST-PA-Nter fusion protein was bound to GSH-coated acceptor beads (Perkin Elmer). These solutions were incubated for 60 min at room temperature in the dark and subsequently mixed and incubated for an additional 120 min. In experiments screening for endonuclease inhibitors, compounds were mixed with both types of beads prior to the 120-min incubation. The optimal concentrations of biotinylated L-742.001 derivative and GST-PA-Nter were 15 nM and 50 nM, respectively. The concentrations of donor and acceptor beads were 5 µg/mL each in a 50 µL reaction volume. All experiments were performed in AlphaScreen reaction buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% Tween20, 1 mM MnCl<sub>2</sub>, 10 mM MgCl<sub>2</sub>, and 1 mM 2-mercaptoethanol).

## Crystallographic data collection and refinement statistics

Supplementary **Table S1** – Diffraction data collection and refinement statistics. The data in parentheses refer to the highest-resolution shell for data collection statistic.

| NPA in complex with compound <b>36</b>    |                                               |
|-------------------------------------------|-----------------------------------------------|
| Wavelength (Å)                            | 0.9184                                        |
| Space group                               | <i>P</i> 6 <sub>4</sub> 22                    |
| Cell parameters (Å, °)                    | 73.93, 73.93, 128.16,<br>90.00, 90.00, 120.00 |
| Resolution range (Å)                      | 50.00–2.46 (2.61–2.46)                        |
| Number of unique reflections              | 14236 (2289)                                  |
| Multiplicity                              | 20.9 (20.7)                                   |
| Completeness (%)                          | 99.9 (99.9)                                   |
| R <sub>merge</sub> <sup>a</sup>           | 0.27 (5.16)                                   |
| CC <sub>(1/2)</sub> (%)                   | 99.8 (32.1)                                   |
| Average I/σ(I)                            | 11.93 (0.64)                                  |
| Wilson B (Å <sup>2</sup> )                | 66.35                                         |
| Refinement statistics                     |                                               |
| Resolution range (Å)                      | 45.3–2.5 (2.57–2.5)                           |
| No. of reflection in working set          | 7306 (527)                                    |
| No. of reflection in the test set         | 385 (28)                                      |
| R <sub>work</sub> value (%) <sup>b</sup>  | 0.227 (0.345)                                 |
| R <sub>free</sub> value (%) <sup>c</sup>  | 0.258 (0.370)                                 |
| RMSD bond length (Å)                      | 0.004                                         |
| RMSD angle (°)                            | 1.024                                         |
| Number of atoms in AU                     | 1486                                          |
| Number of protein atoms in AU             | 1437                                          |
| Number of water molecules in AU           | 25                                            |
| Mean ADP value (Å <sup>2</sup> )          | 58.05                                         |
| Ramachandran plot statistics <sup>d</sup> |                                               |
| Residues in favored regions (%)           | 93.89                                         |
| Residues in allowed regions (%)           | 6.11                                          |
| PDB code                                  | <b>8PPX</b>                                   |

<sup>a</sup> R<sub>merge</sub> = (|I<sub>hkl</sub> - ⟨I⟩|)/I<sub>hkl</sub>, where the average intensity ⟨I⟩ is taken over all symmetry equivalent measurements and I<sub>hkl</sub> is the measured intensity for any given reflection

<sup>b</sup> R-value =  $\|F_o| - |F_c|\|/|F_o|$ , where  $F_o$  and  $F_c$  are the observed and calculated structure factors, respectively.

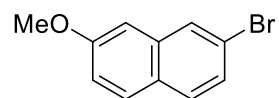
<sup>c</sup>  $R_{\text{free}}$  is equivalent to R-value but is calculated for 5% of the reflections chosen at random and omitted from the refinement process.<sup>[4]</sup>

<sup>d</sup> As determined by Molprobit.<sup>[5]</sup>

## Literature

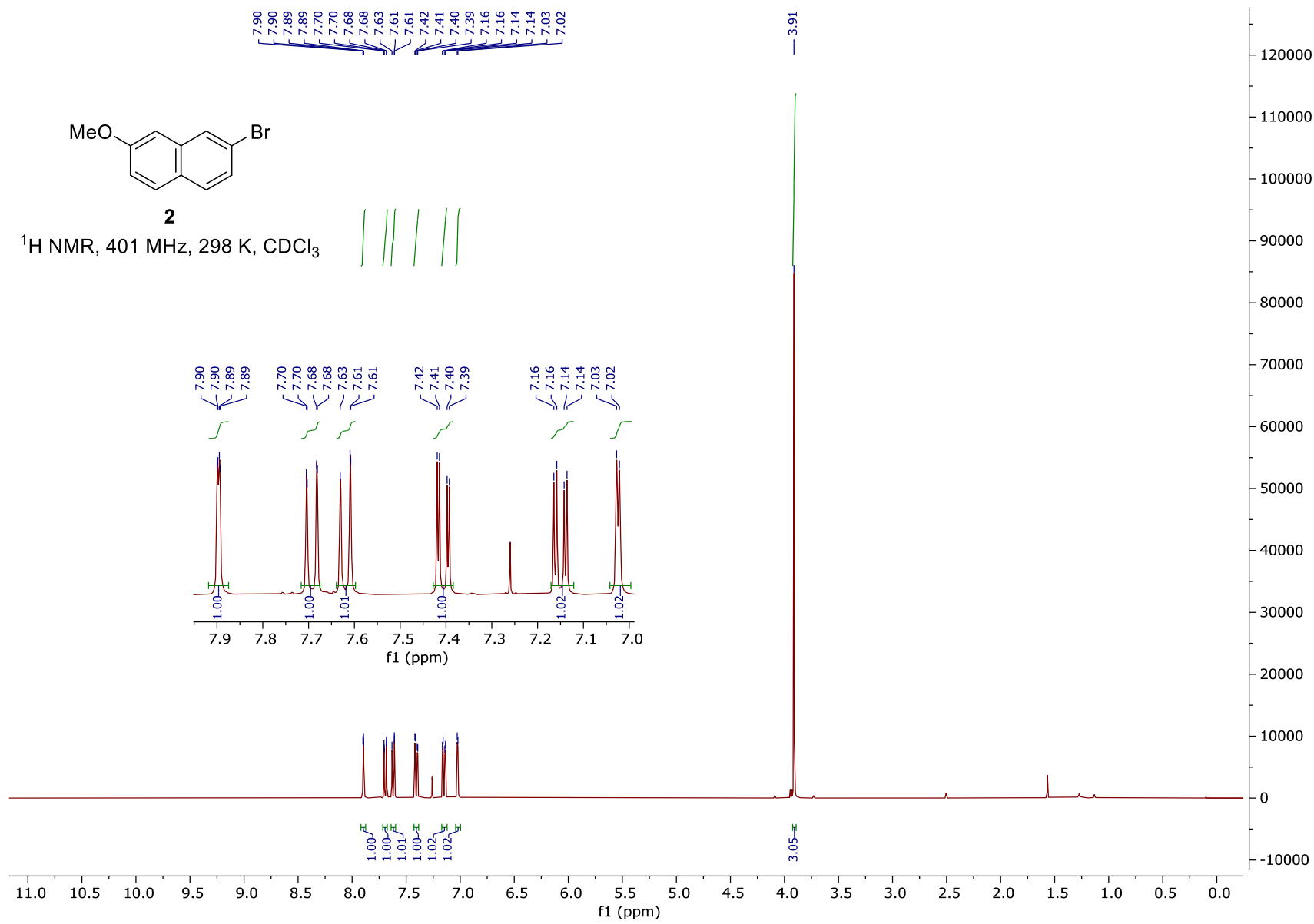
- [1] J.-F. Delhomel, E. Perspicace, Z. Majd, P. Parroche, R. Walczak, *N-{[2-(Piperidin-1-Yl)Phenyl](Phenyl)Methyl}-2-(3-Oxo-3,4-Dihydro-2H-1,4-Benzoxazin-7-Yl)Acetamide Derivatives and Related Compounds as Ror-Gamma Modulators for Treating Autoimmune Diseases*, **2018**, WO2018138362A1.
- [2] P. C. Meltzer, M. McPhee, B. K. Madras, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4133–4137.
- [3] E. J. Lavoie, J. D. Bauman, A. Parhi, H. Y. Sagong, D. Patel, E. Arnold, K. Das, S. K. Vijayan, *Therapeutic Hydroxypyridinones, Hydroxypyrimidinones and Hydroxypyridazinones*, **2014**, WO2014043252A2.
- [4] G. N. Murshudov, P. Skubák, A. A. Lebedev, N. S. Pannu, R. A. Steiner, R. A. Nicholls, M. D. Winn, F. Long, A. A. Vagin, *Acta Crystallogr. D Biol. Crystallogr.* **2011**, *67*, 355–367.
- [5] V. B. Chen, W. B. Arendall, J. J. Headd, D. A. Keedy, R. M. Immormino, G. J. Kapral, L. W. Murray, J. S. Richardson, D. C. Richardson, *Acta Crystallogr. D Biol. Crystallogr.* **2010**, *66*, 12–21.

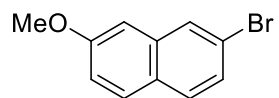
# <sup>1</sup>H, <sup>13</sup>C and APT NMR spectra



**2**

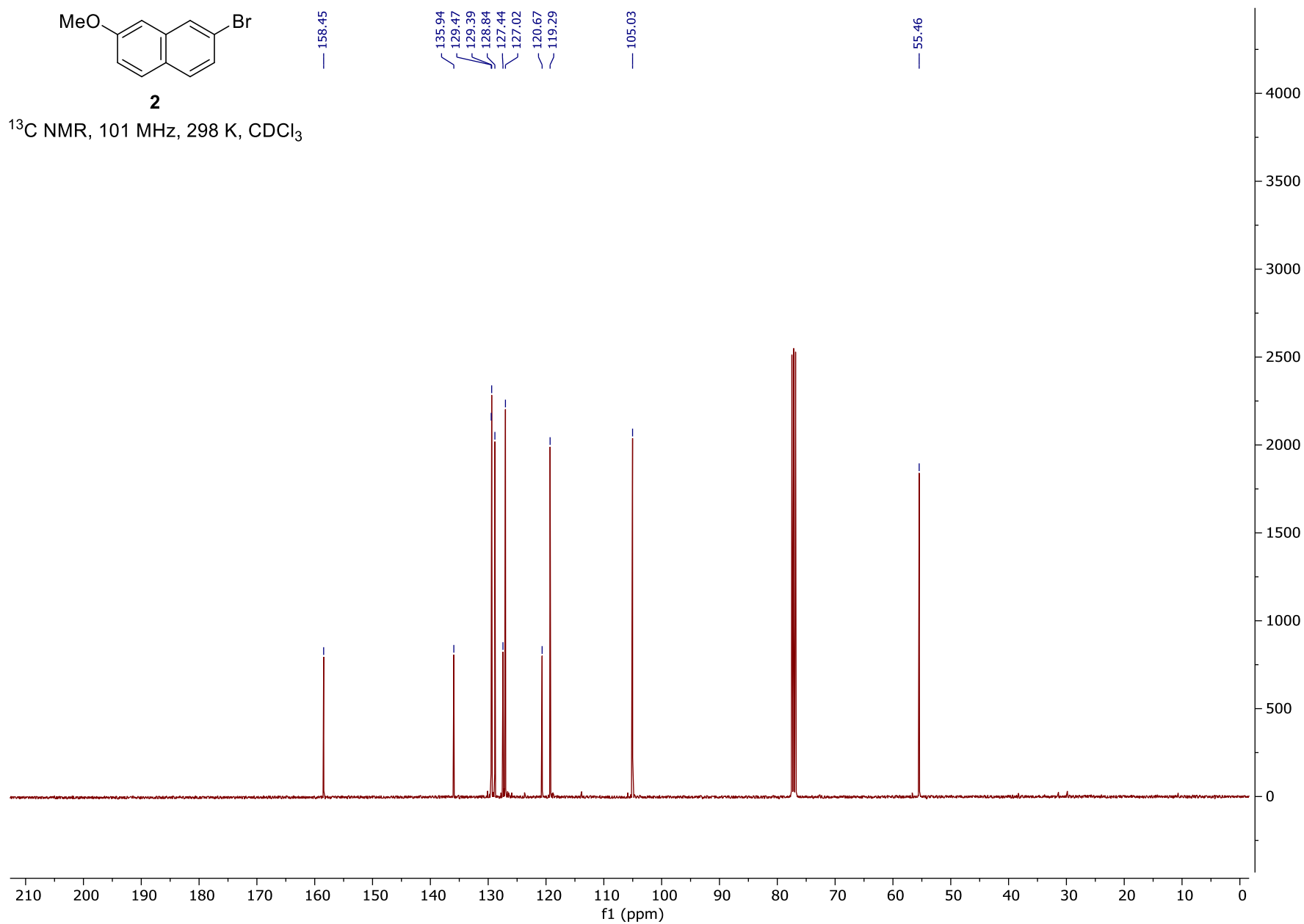
<sup>1</sup>H NMR, 401 MHz, 298 K, CDCl<sub>3</sub>

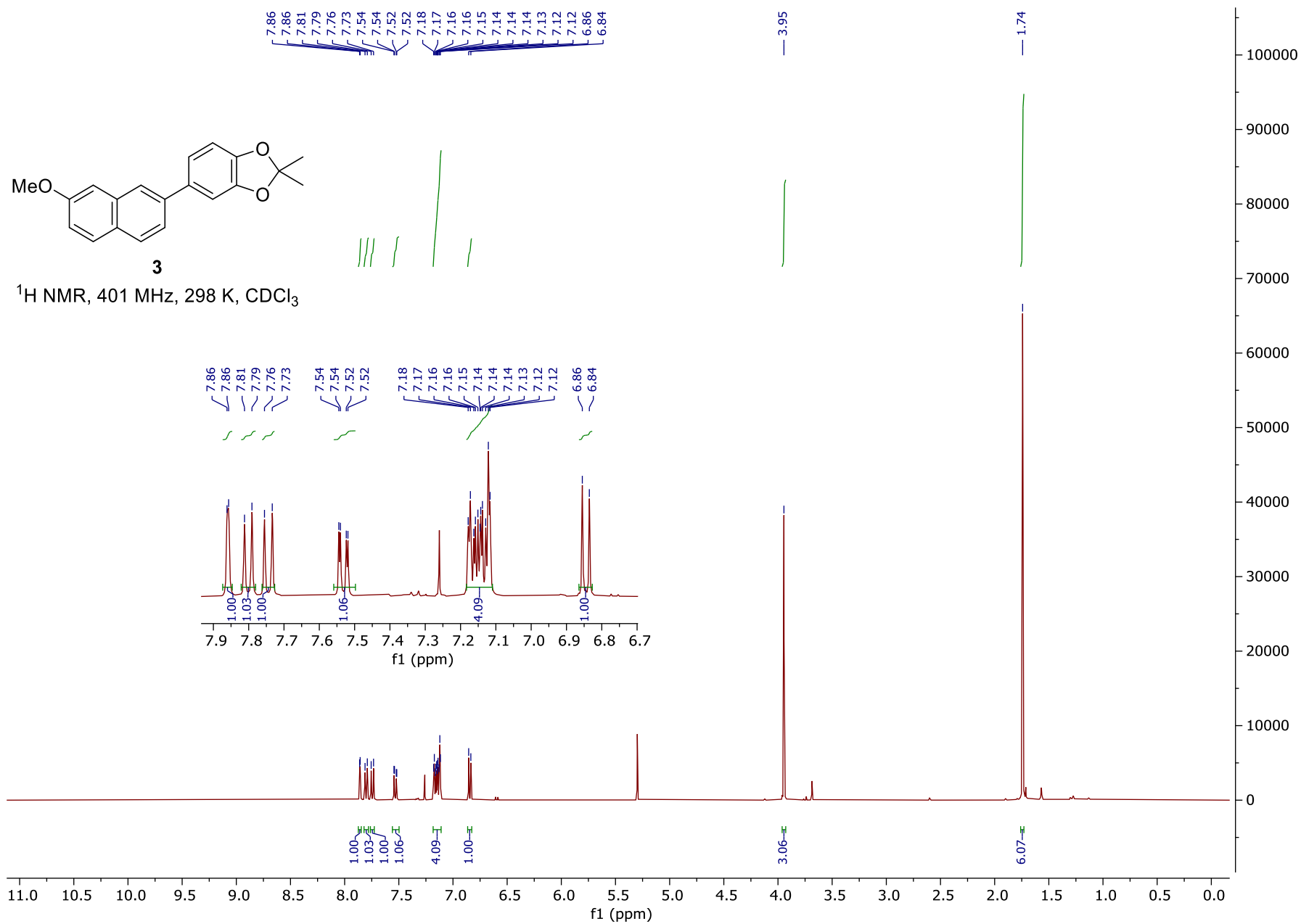


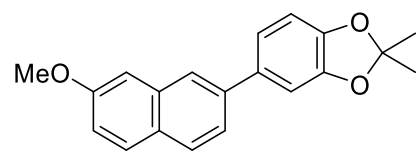


**2**

$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

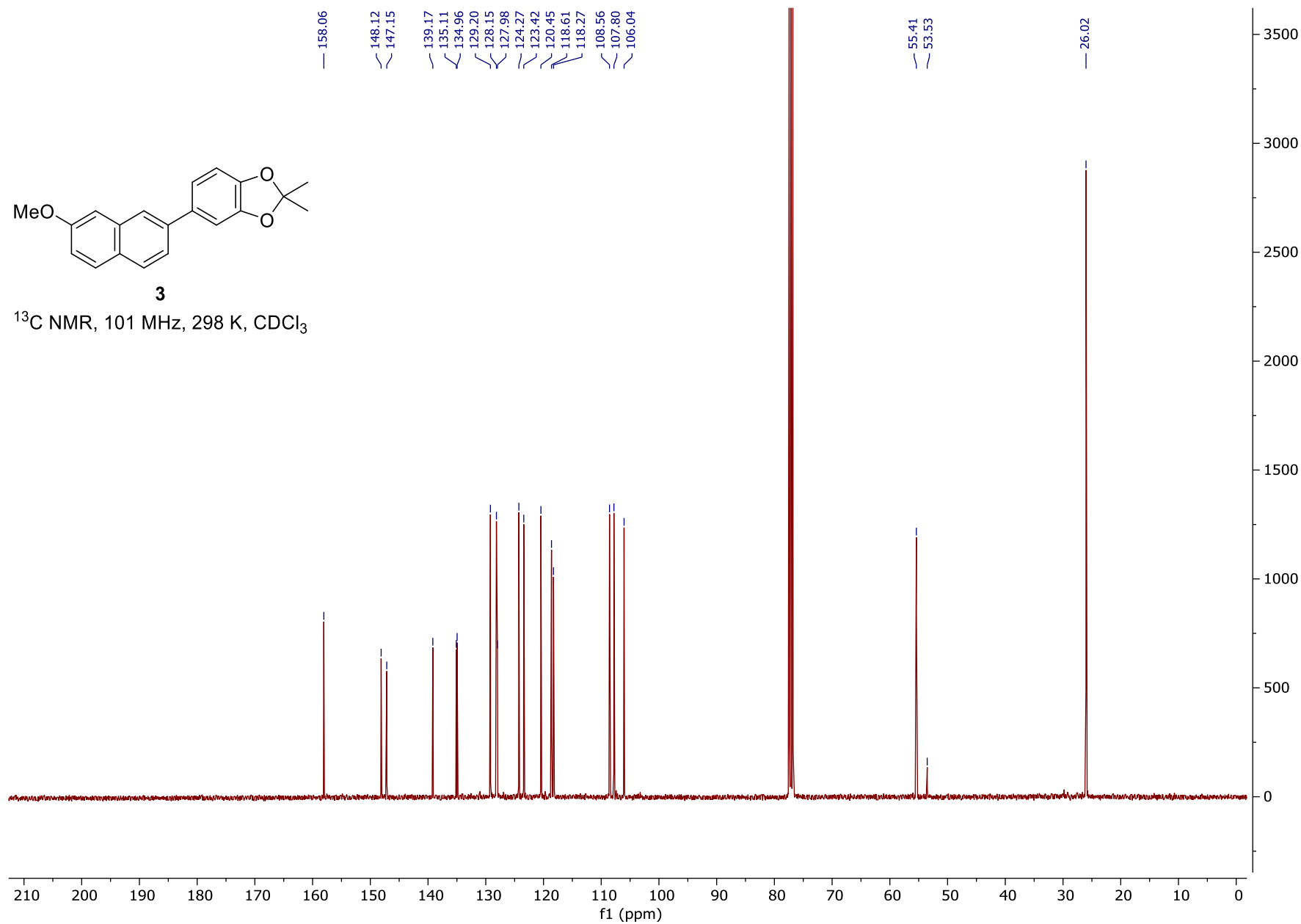


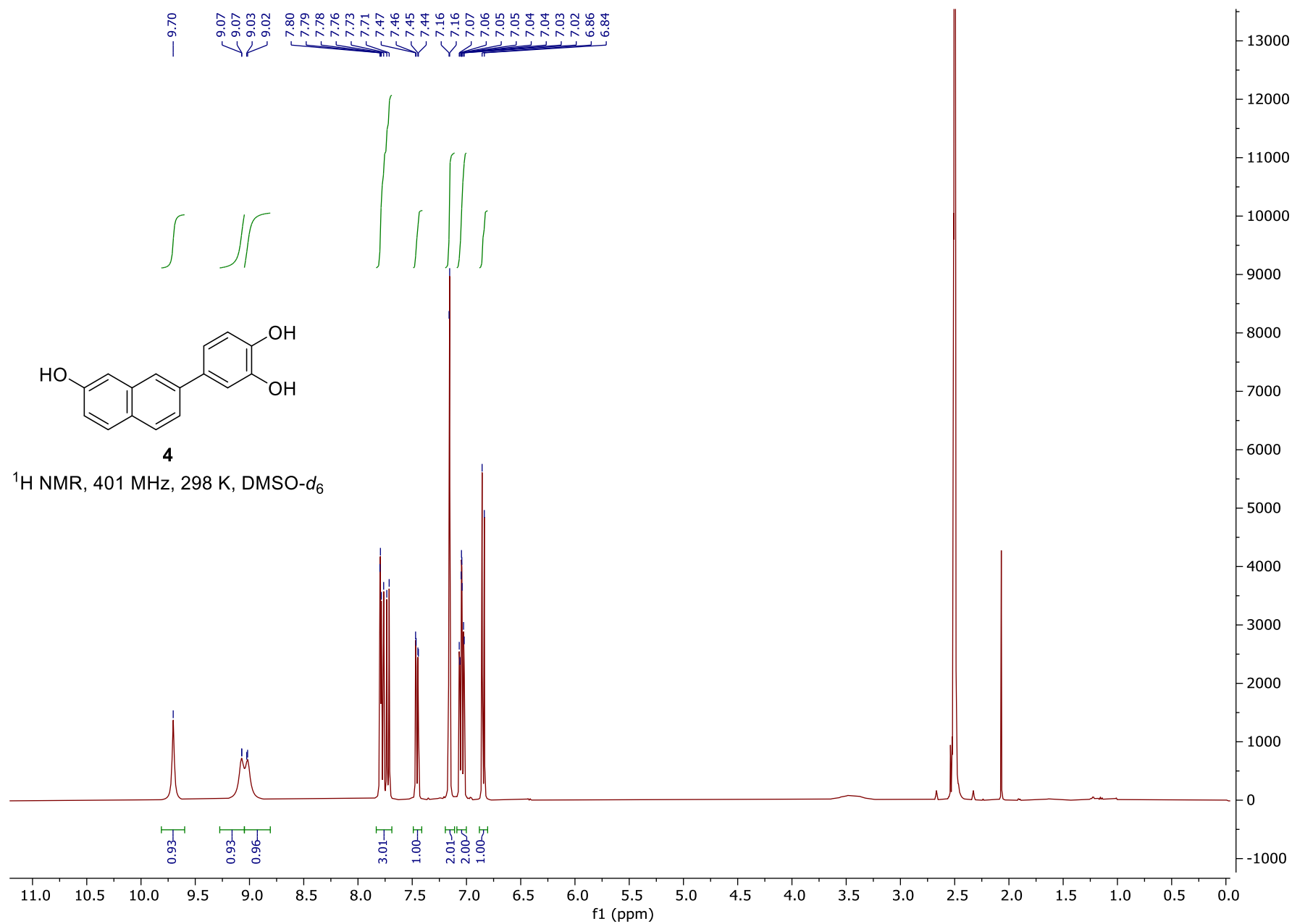


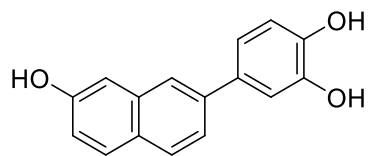


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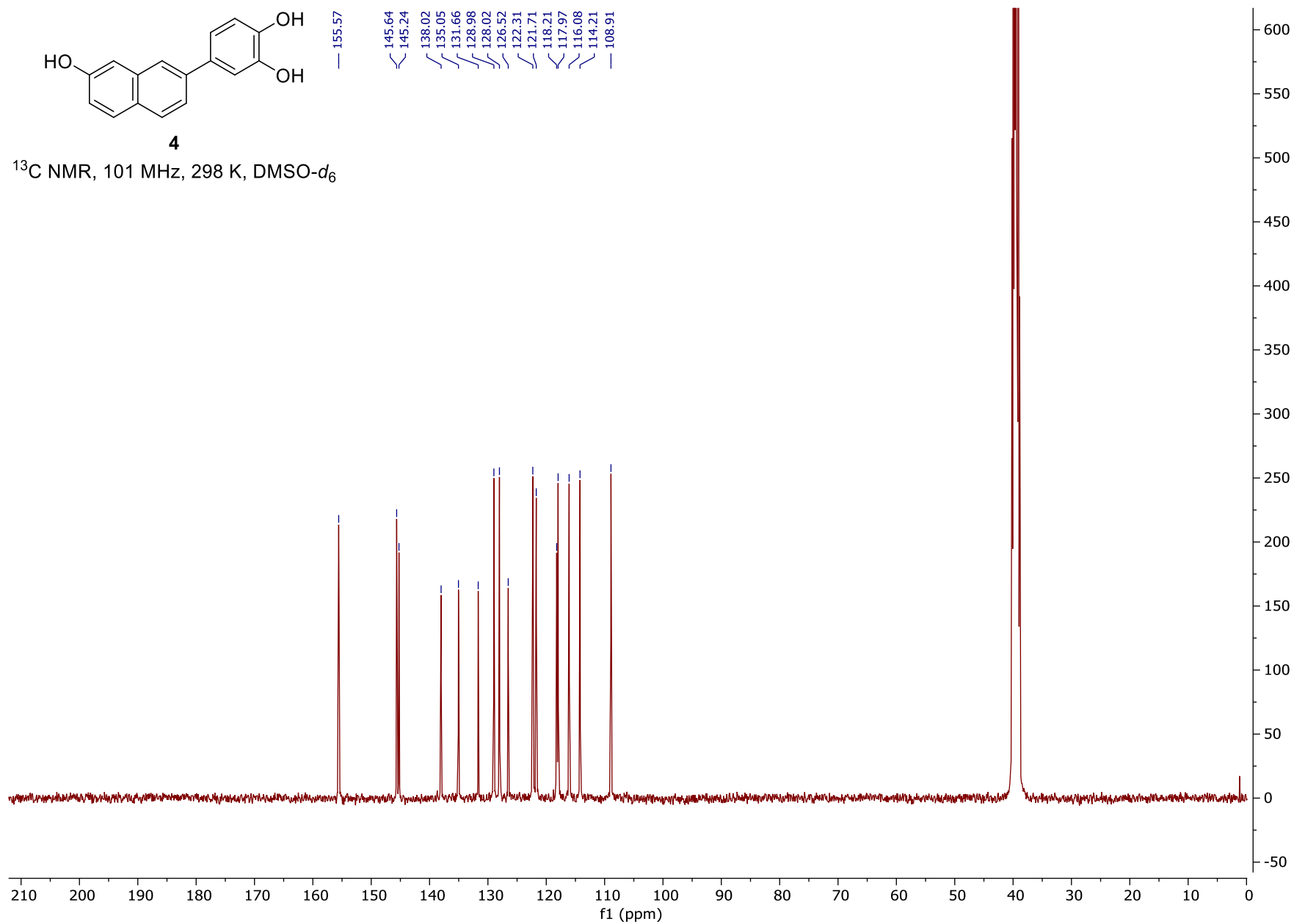


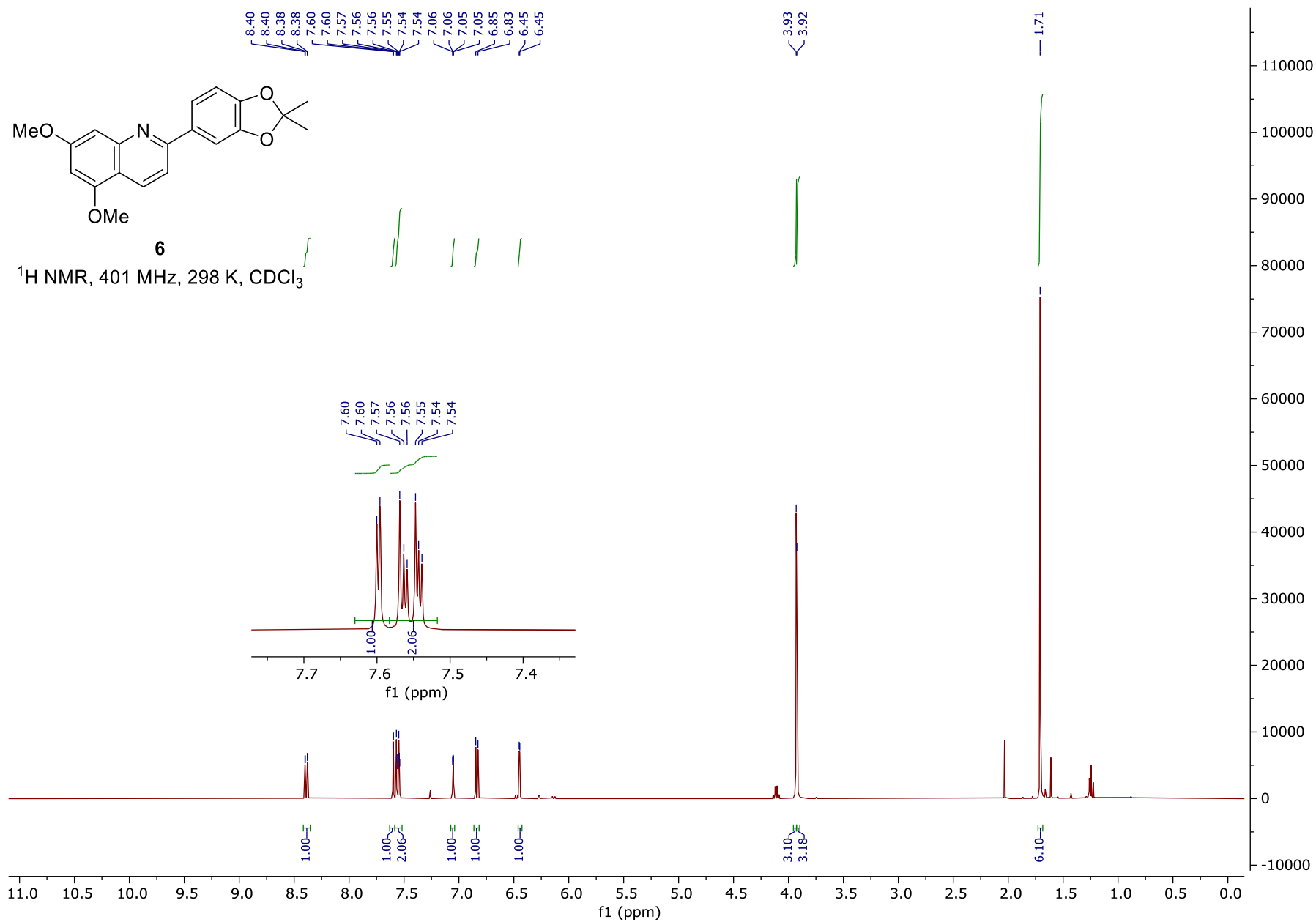


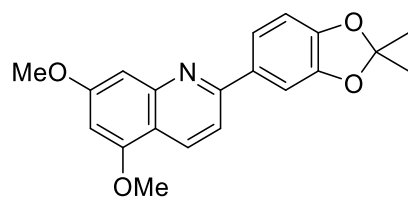


**4**

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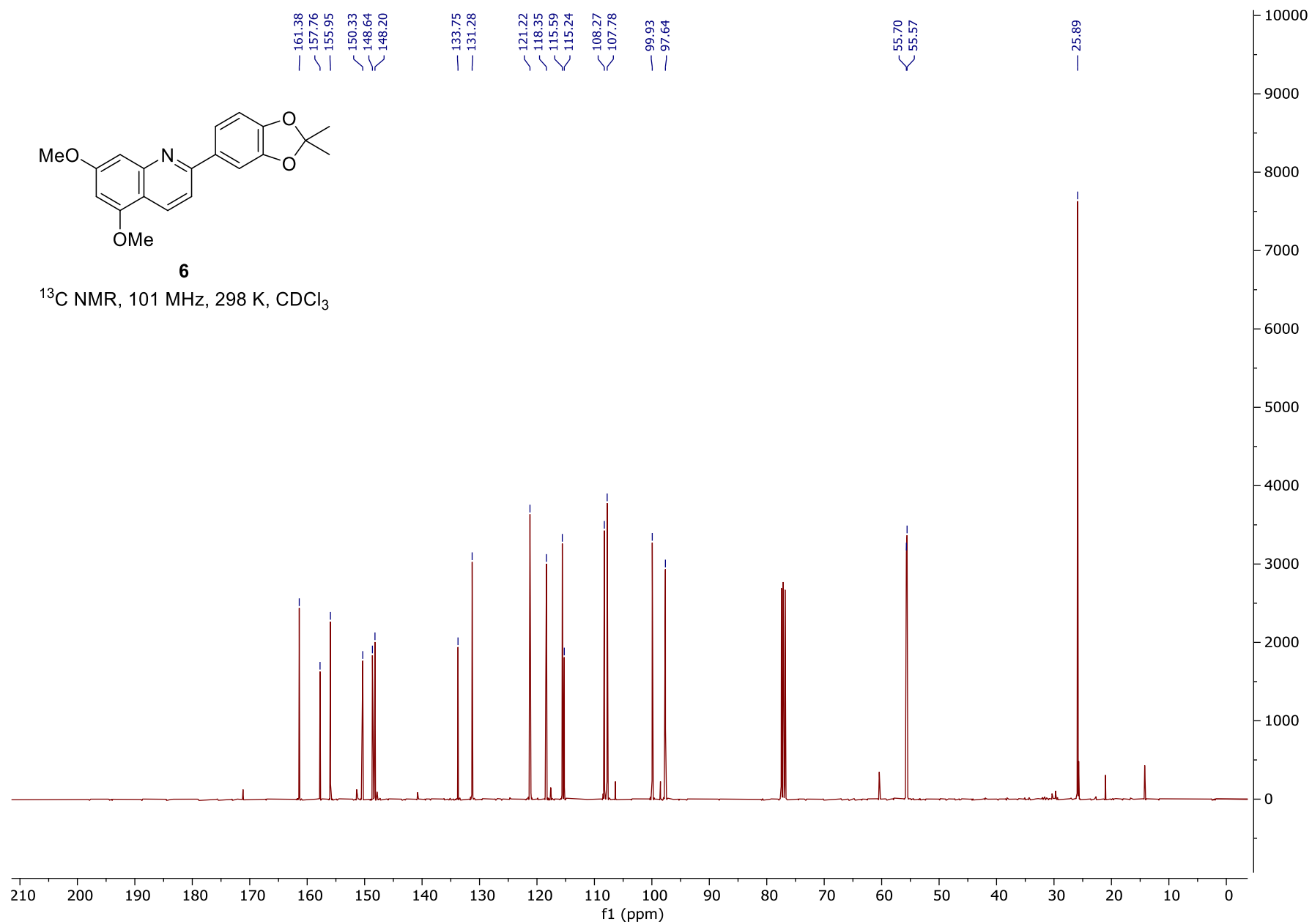


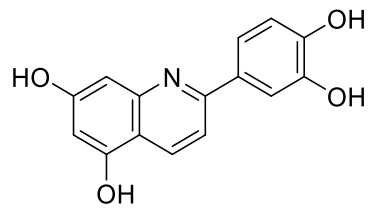




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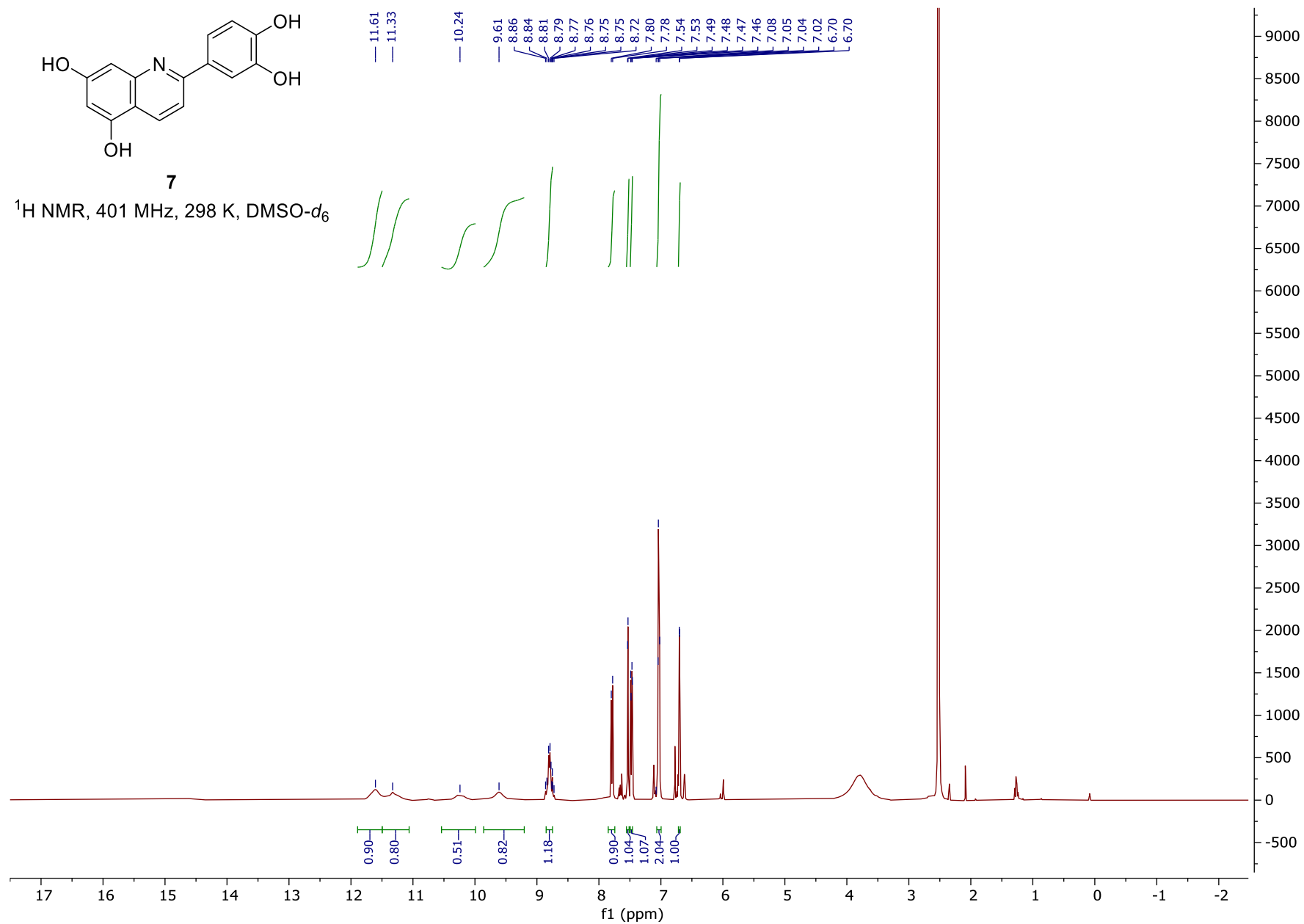
$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

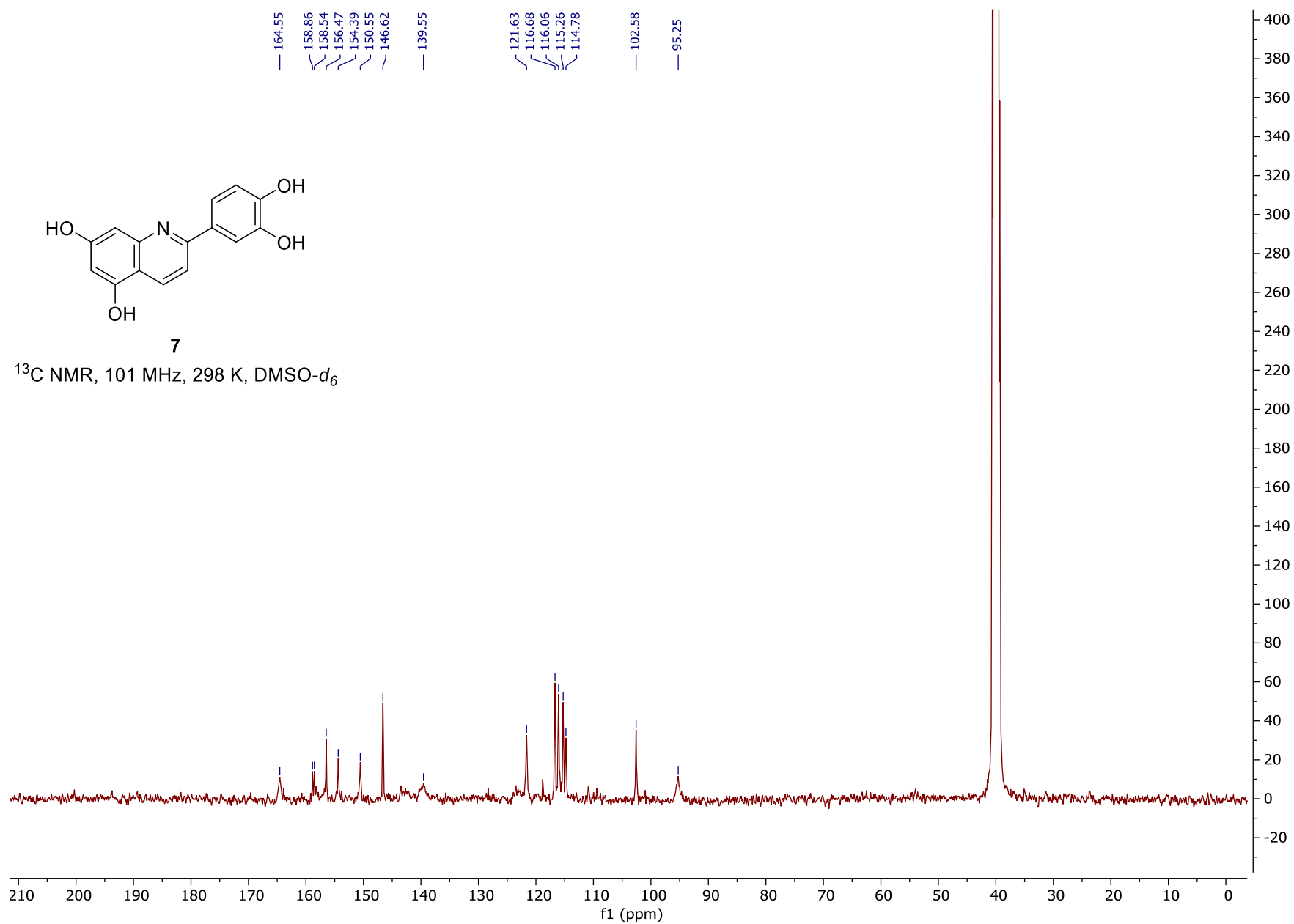


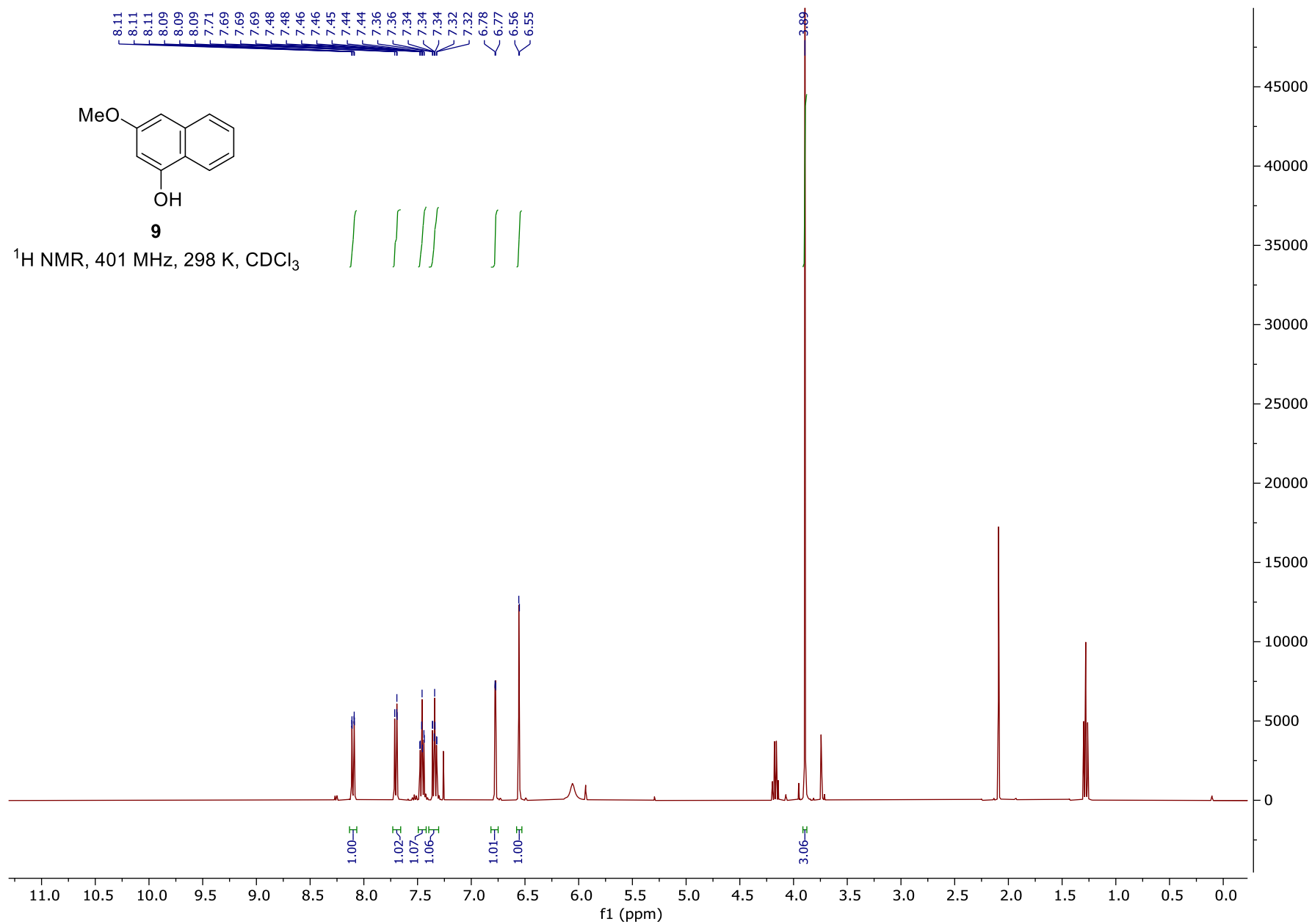


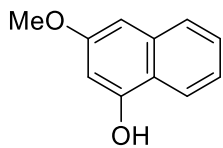
7

<sup>1</sup>H NMR, 401 MHz, 298 K, DMSO-*d*<sub>6</sub>



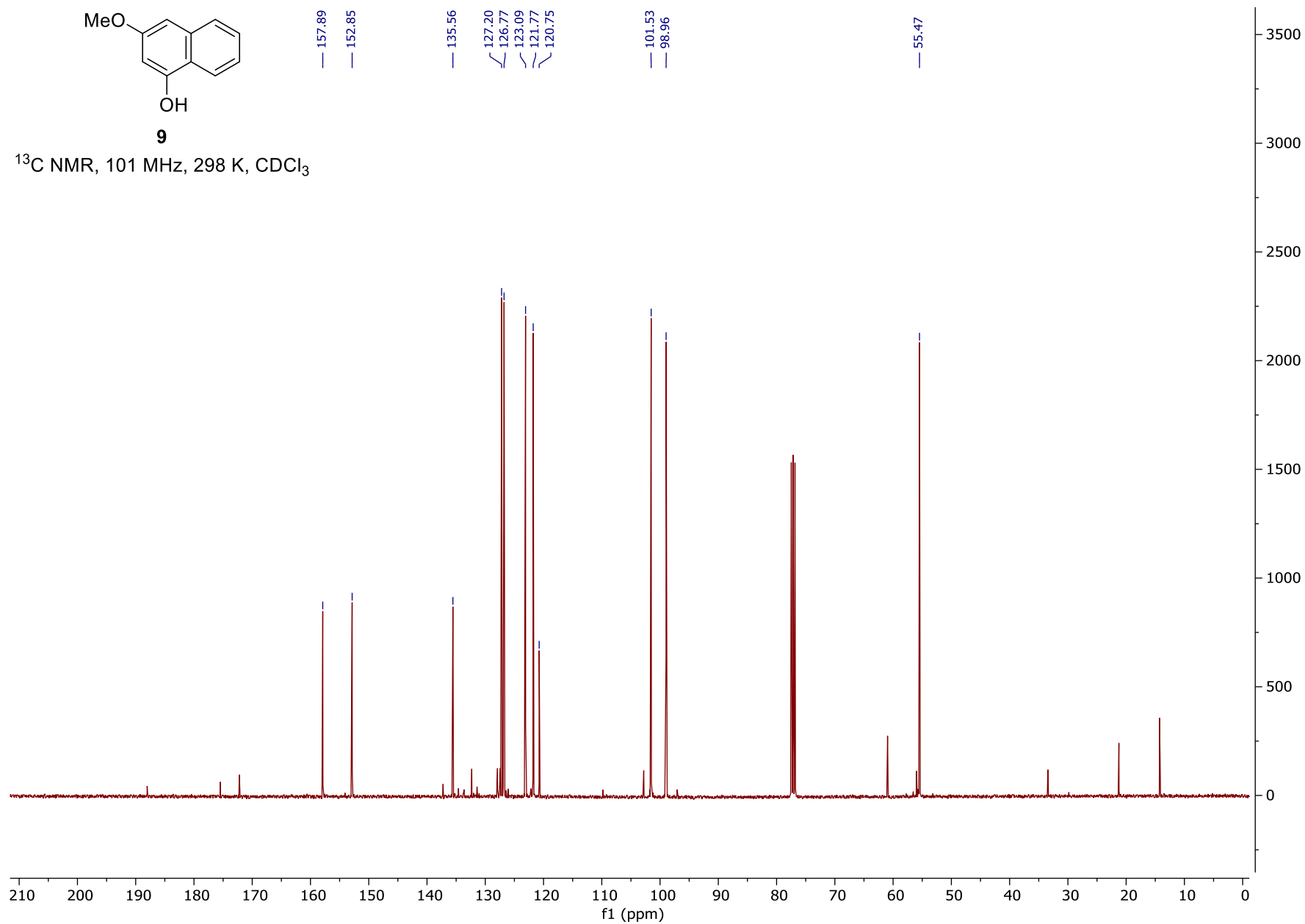


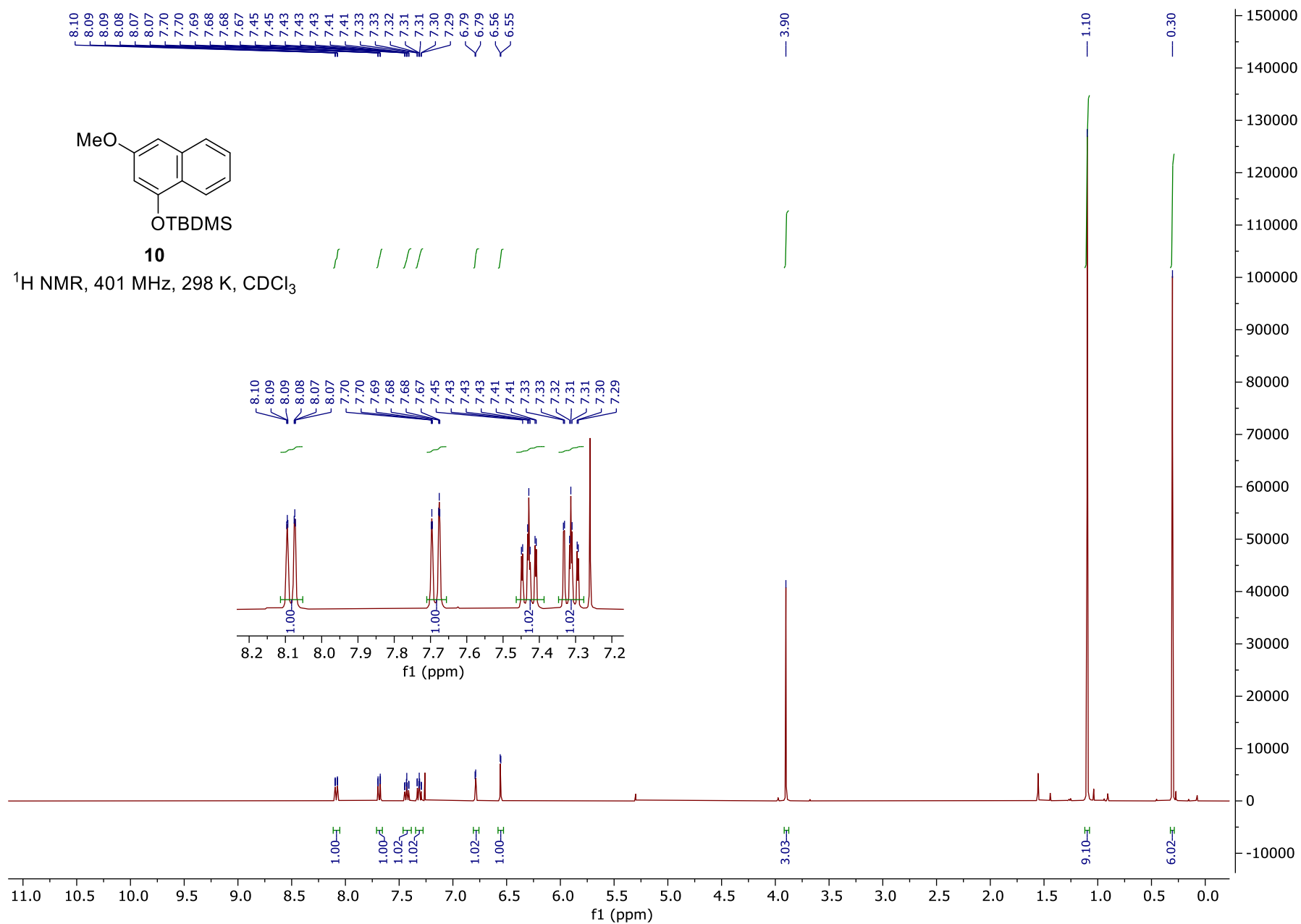


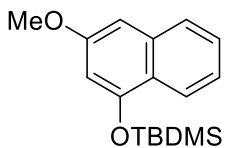


**9**

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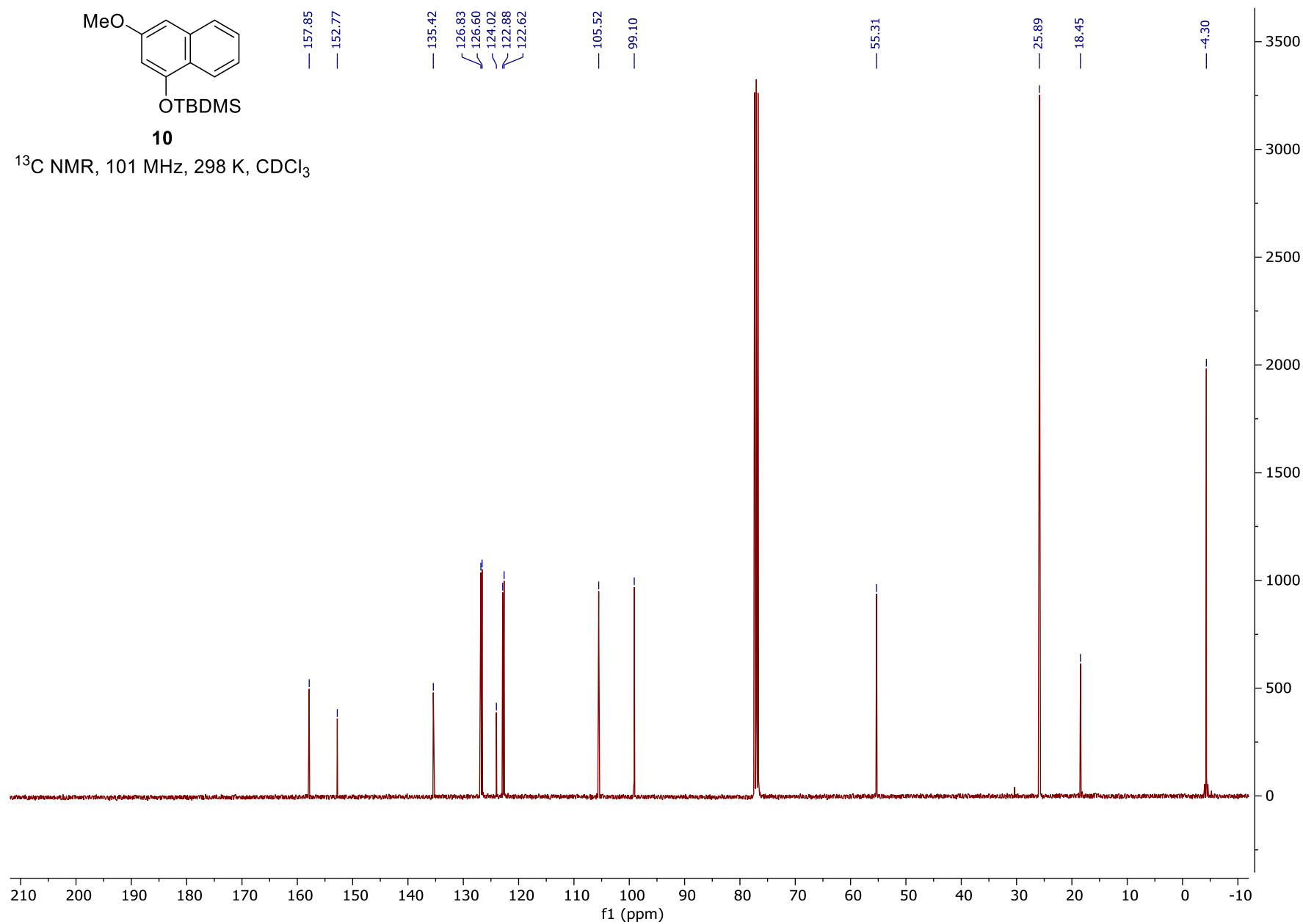


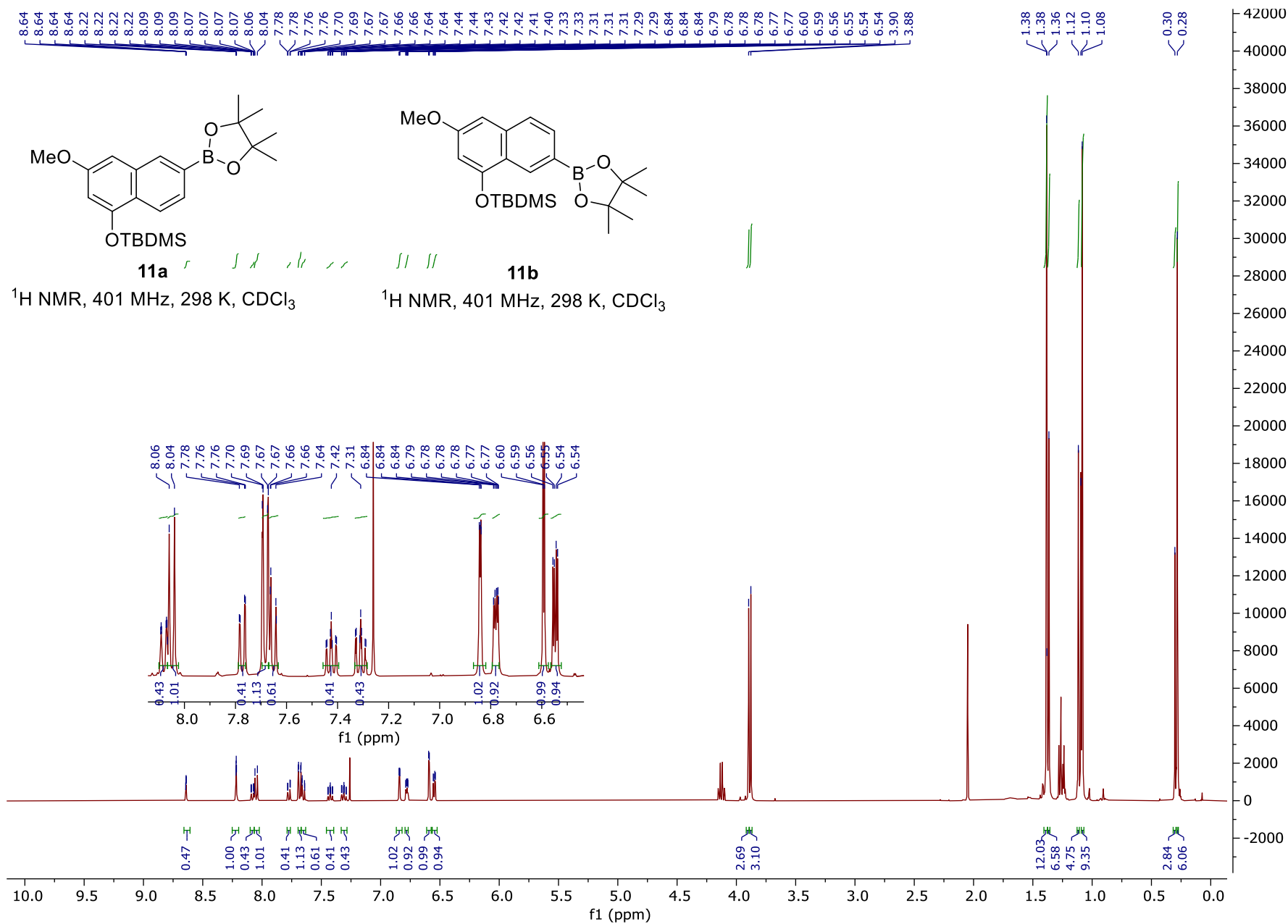


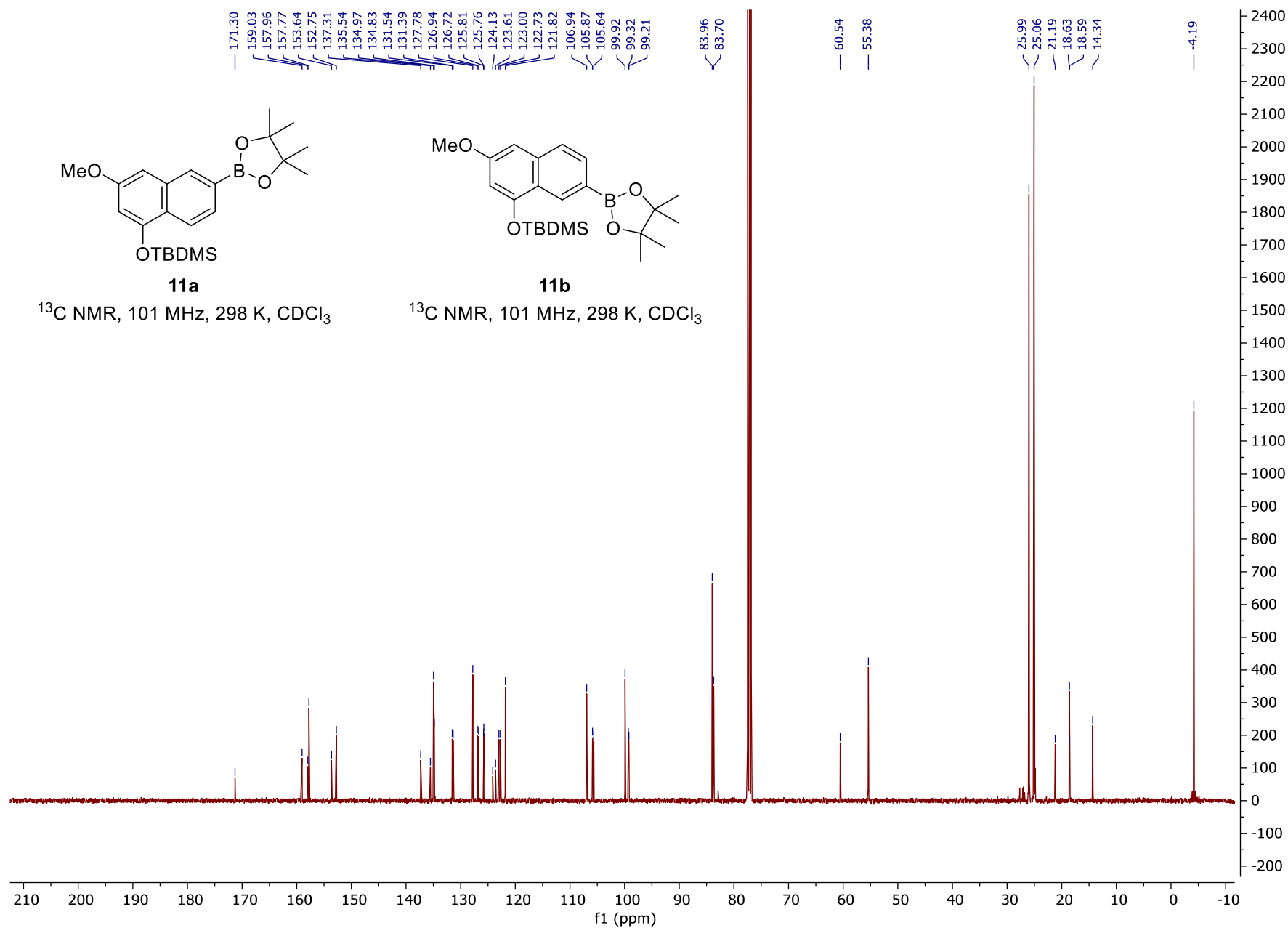


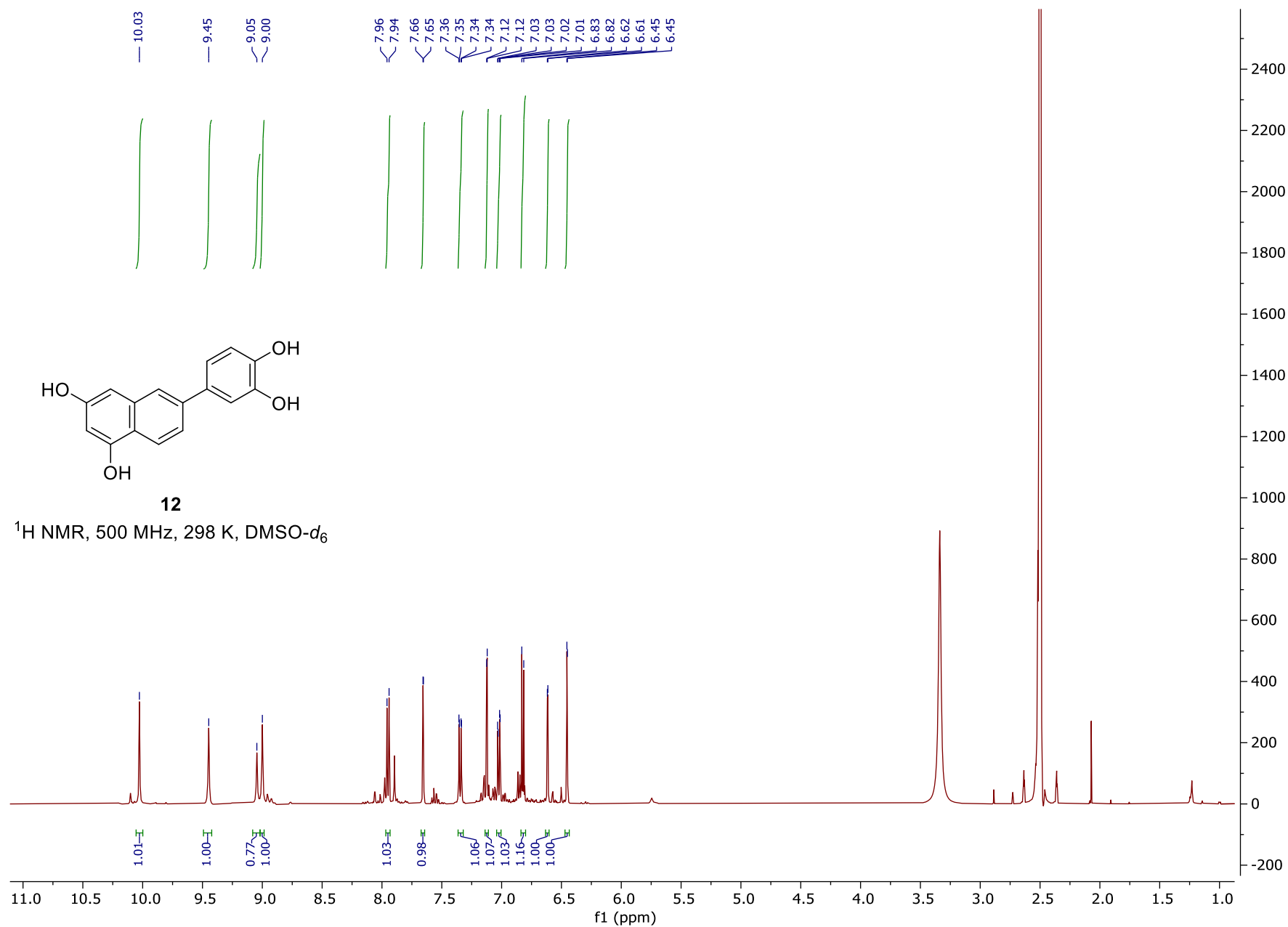
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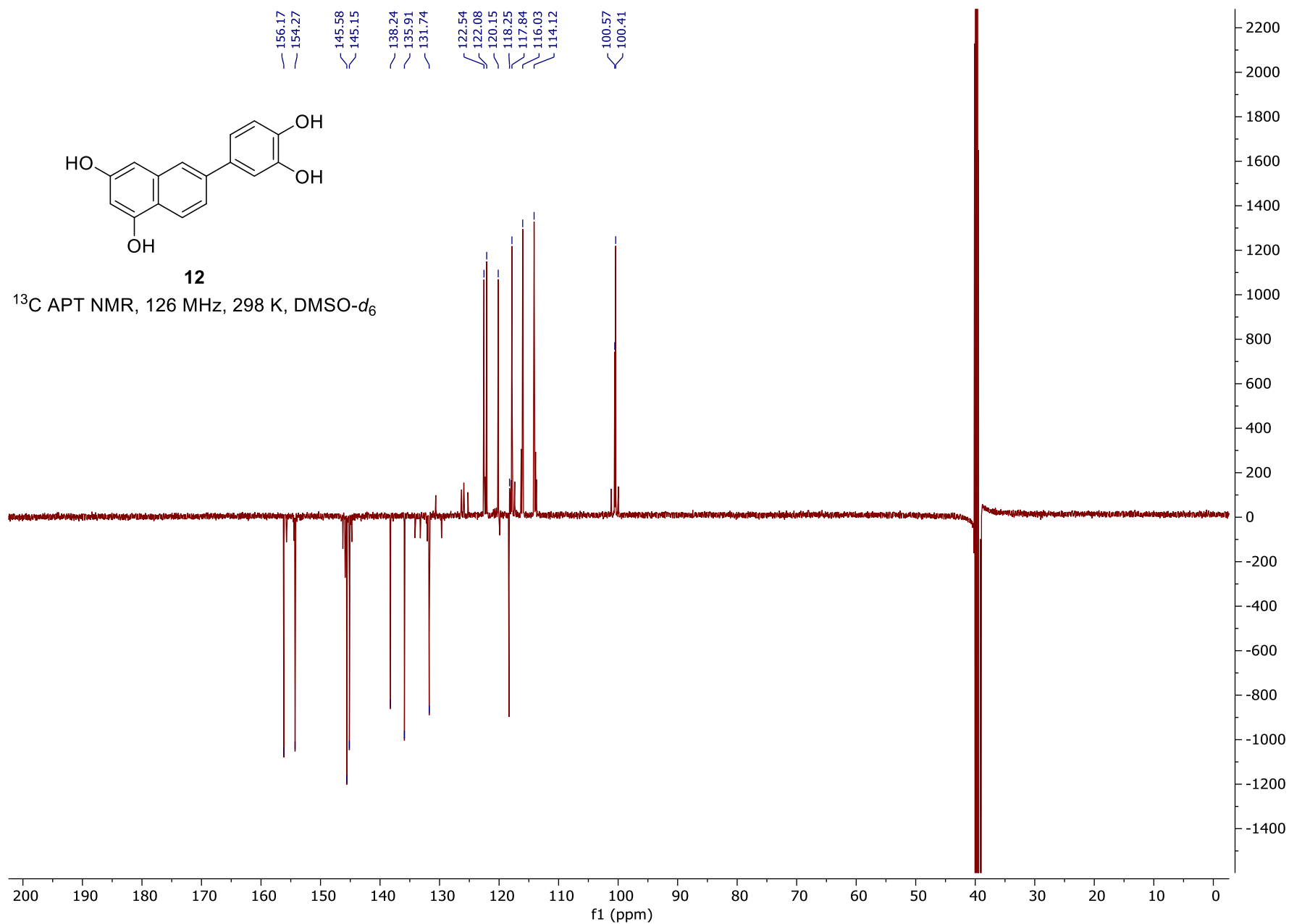
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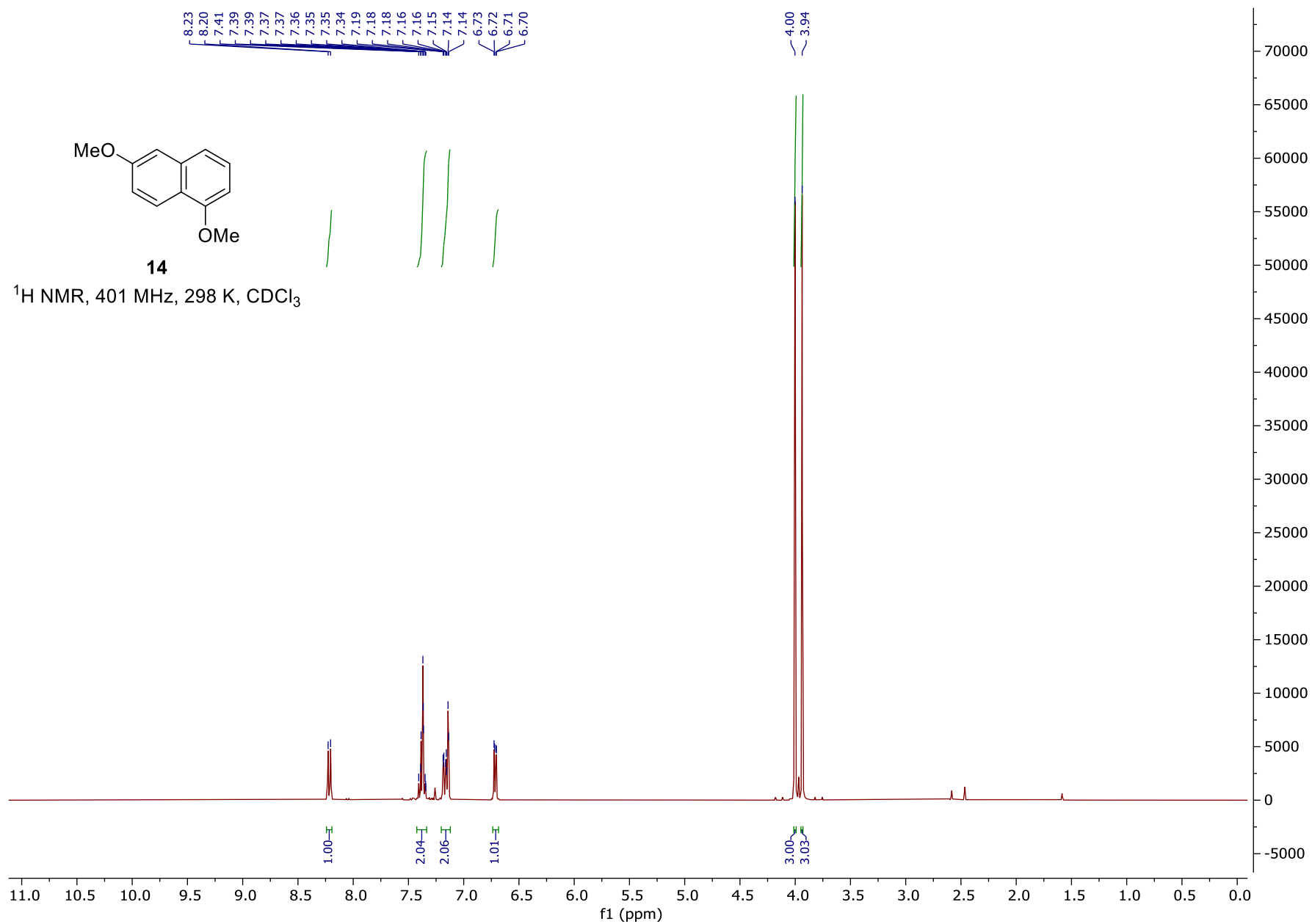


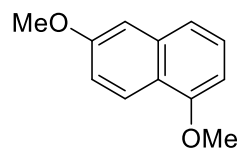






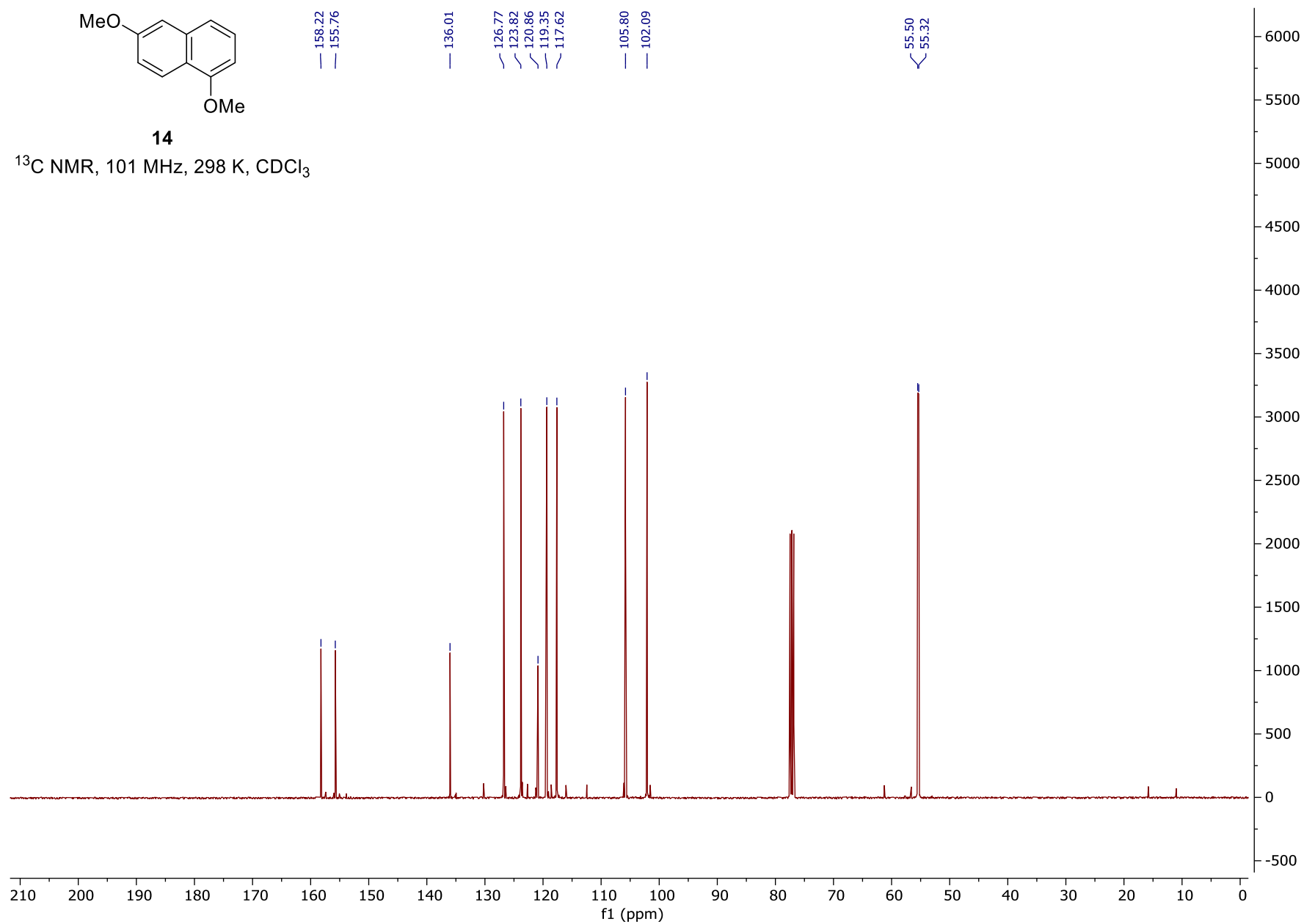


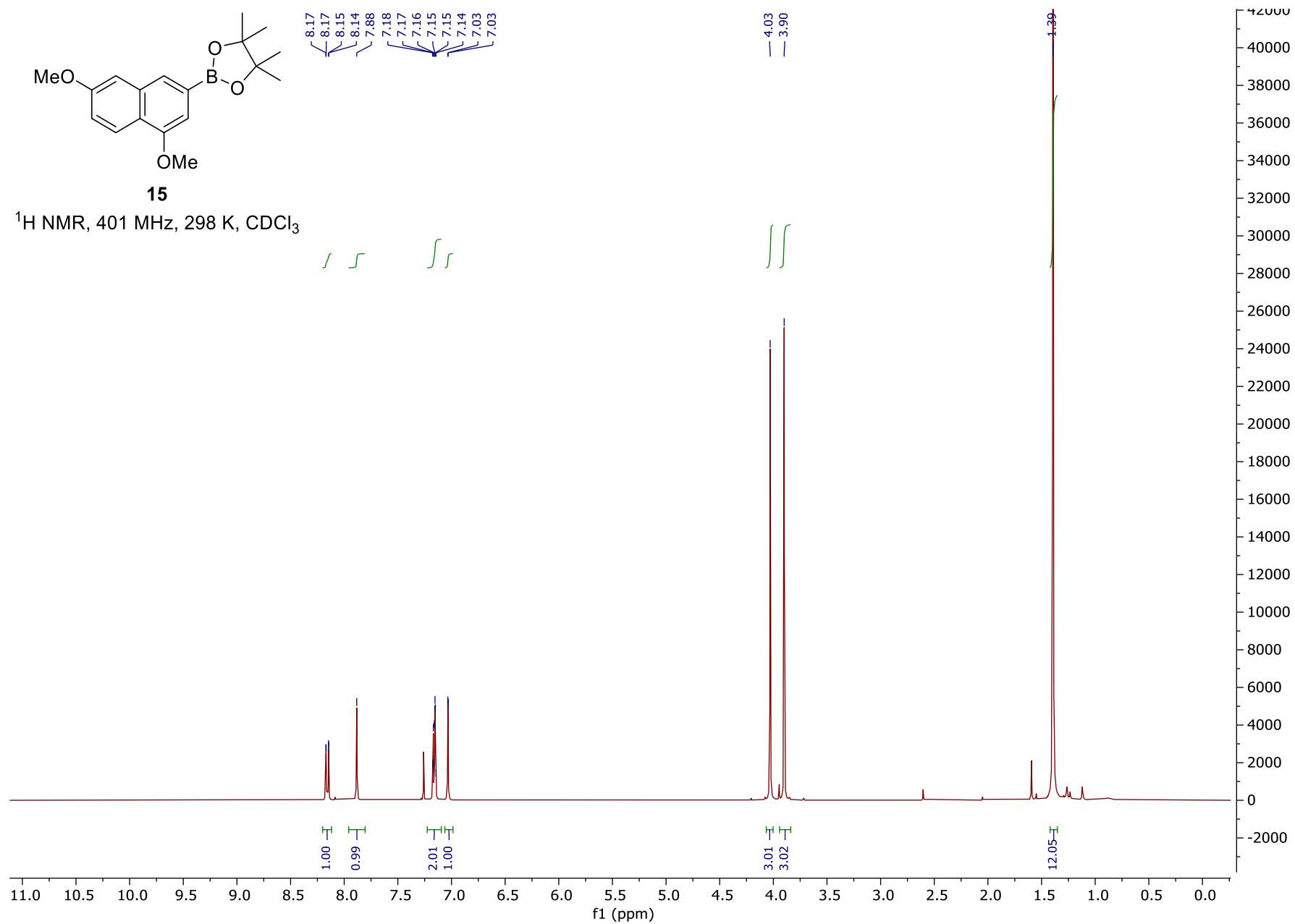


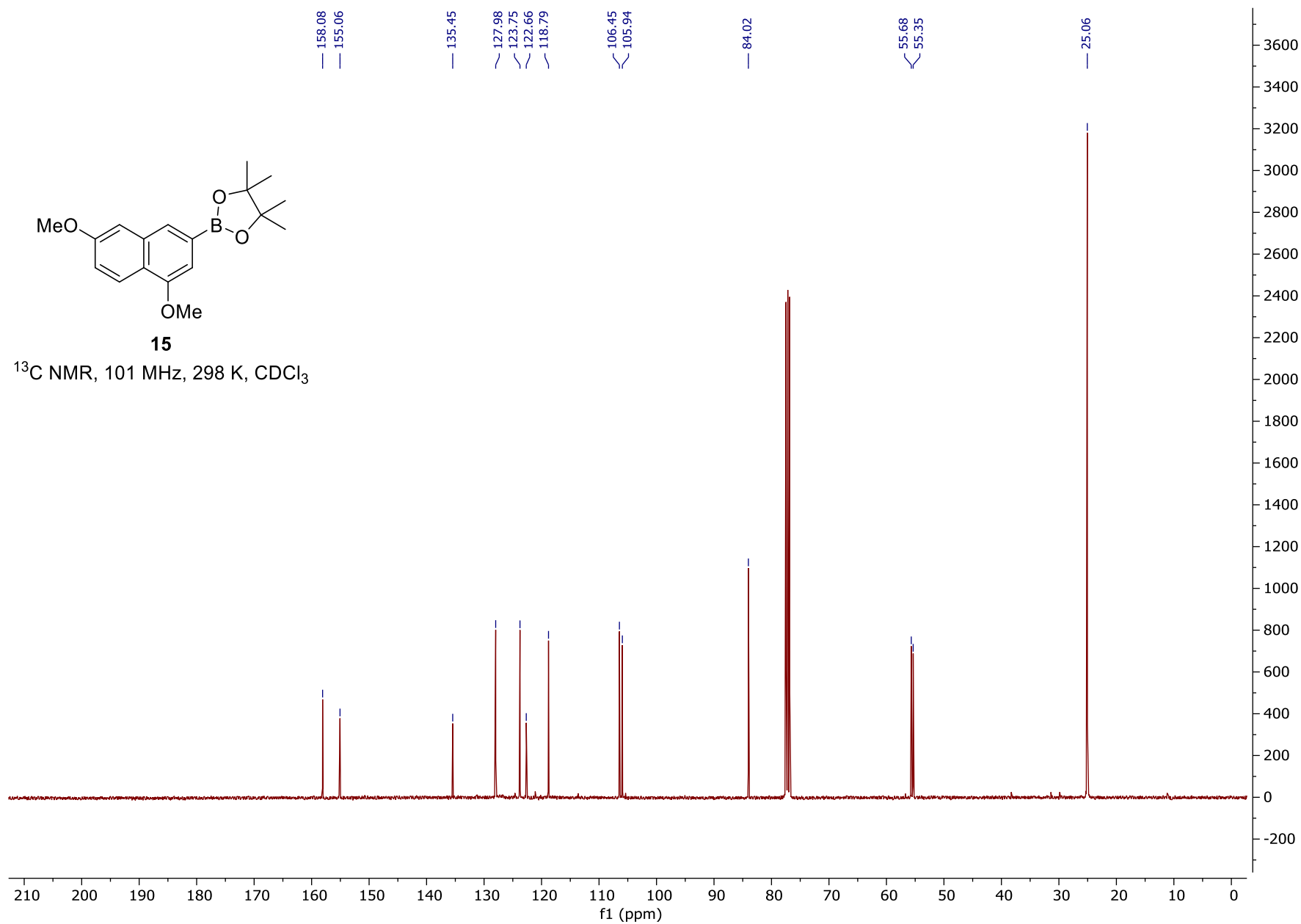


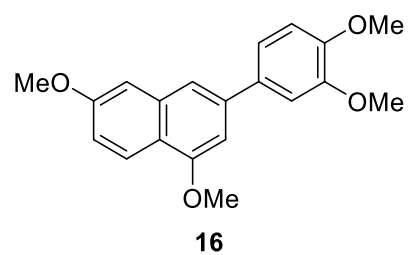
**14**

$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

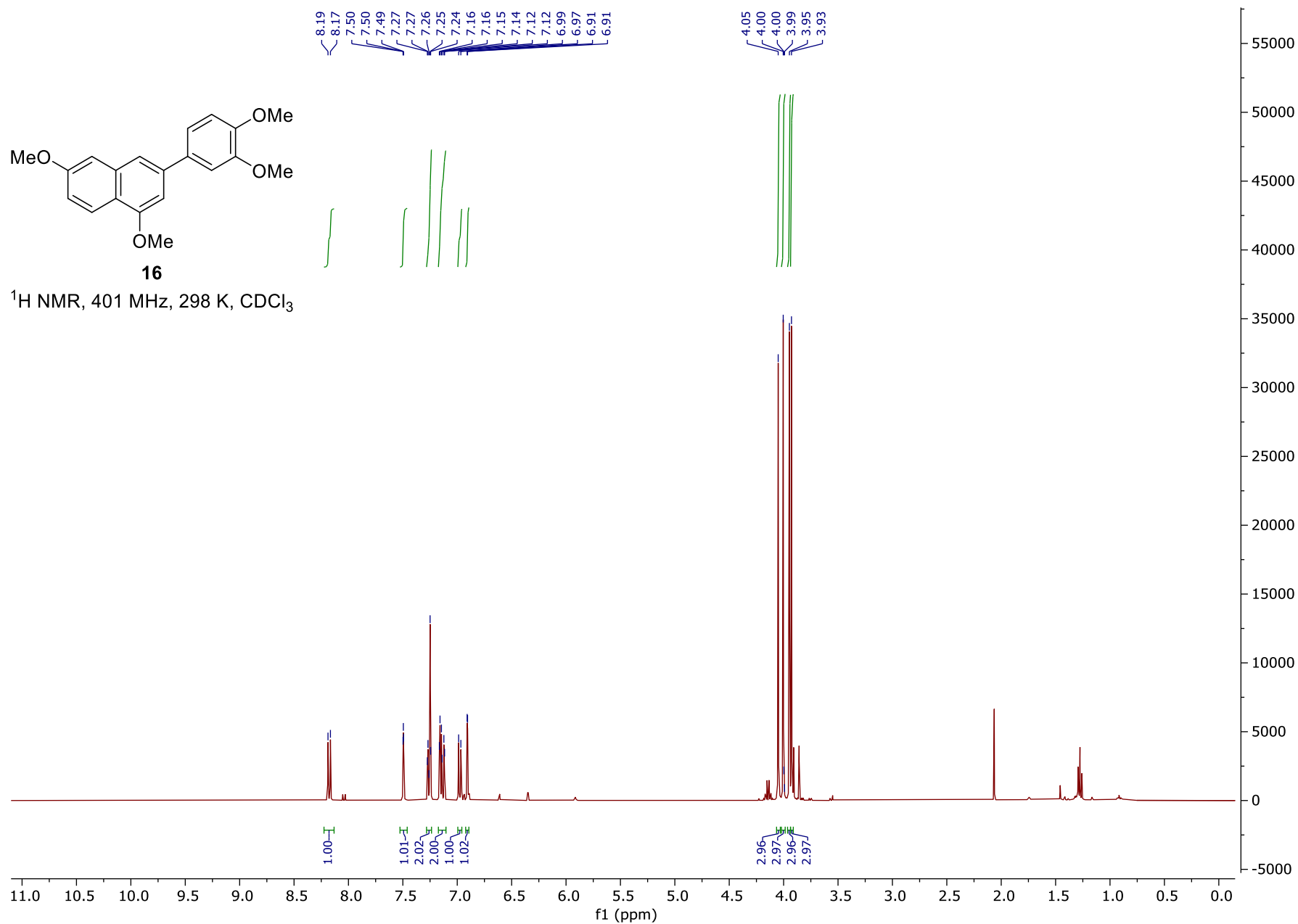


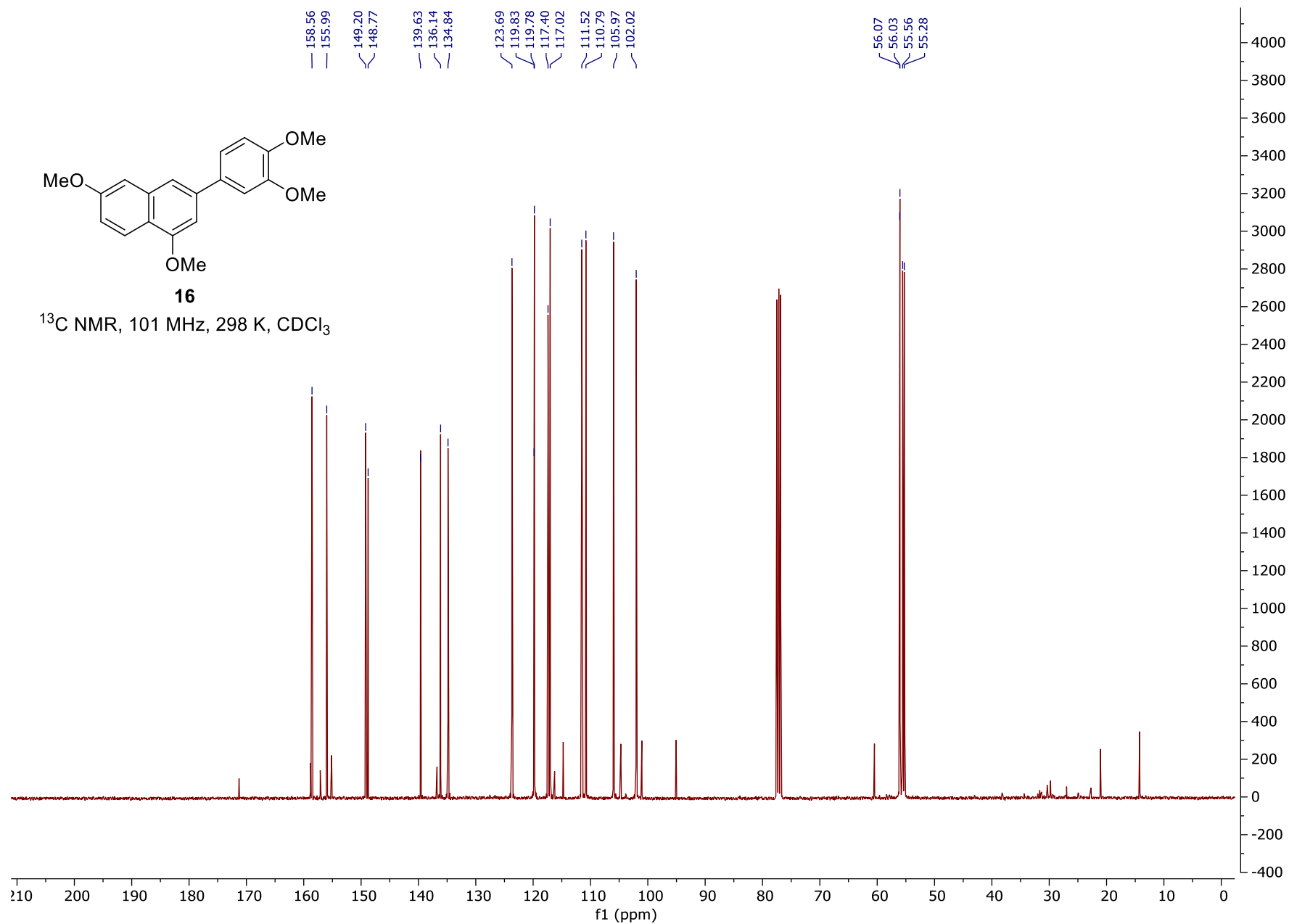


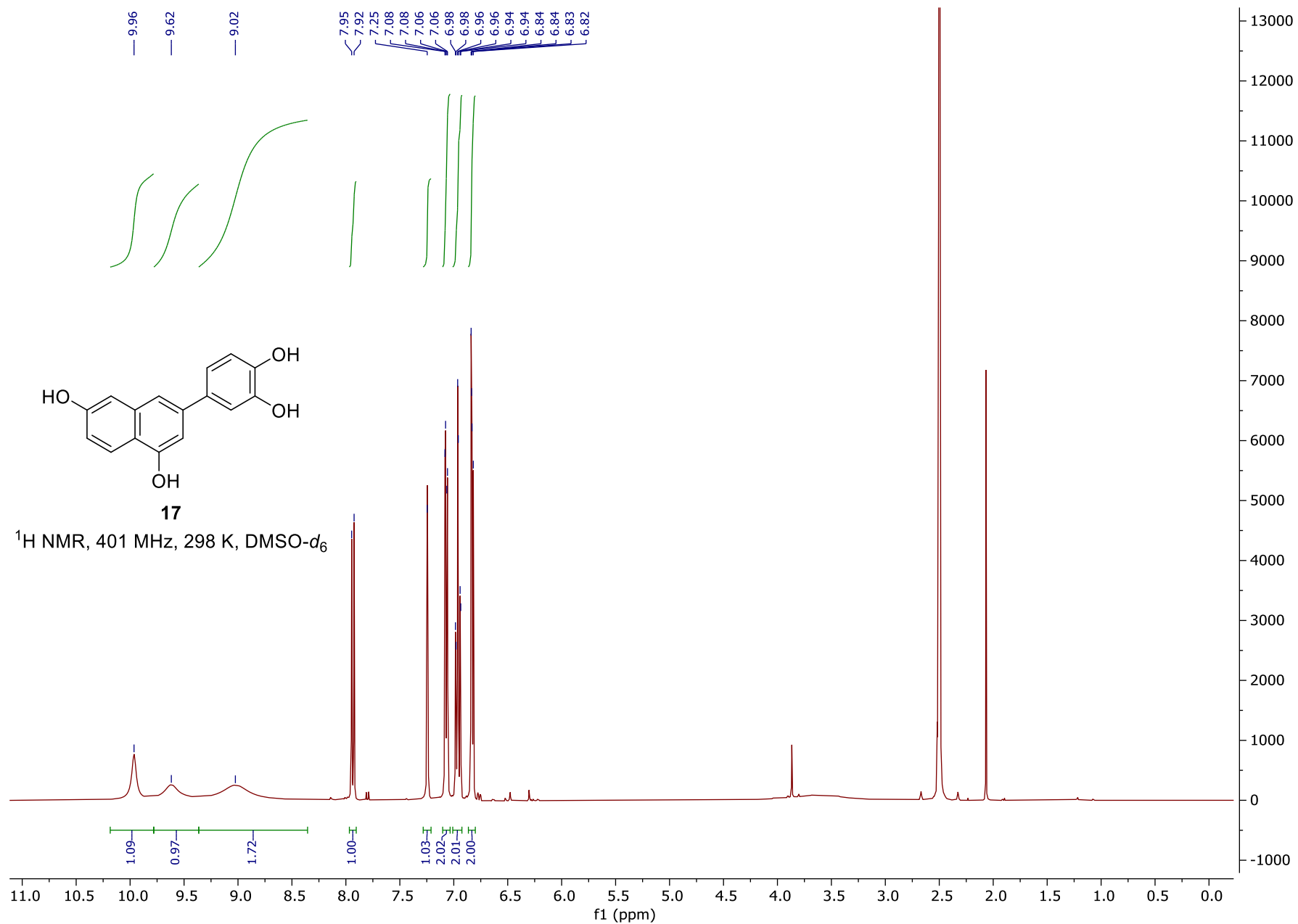


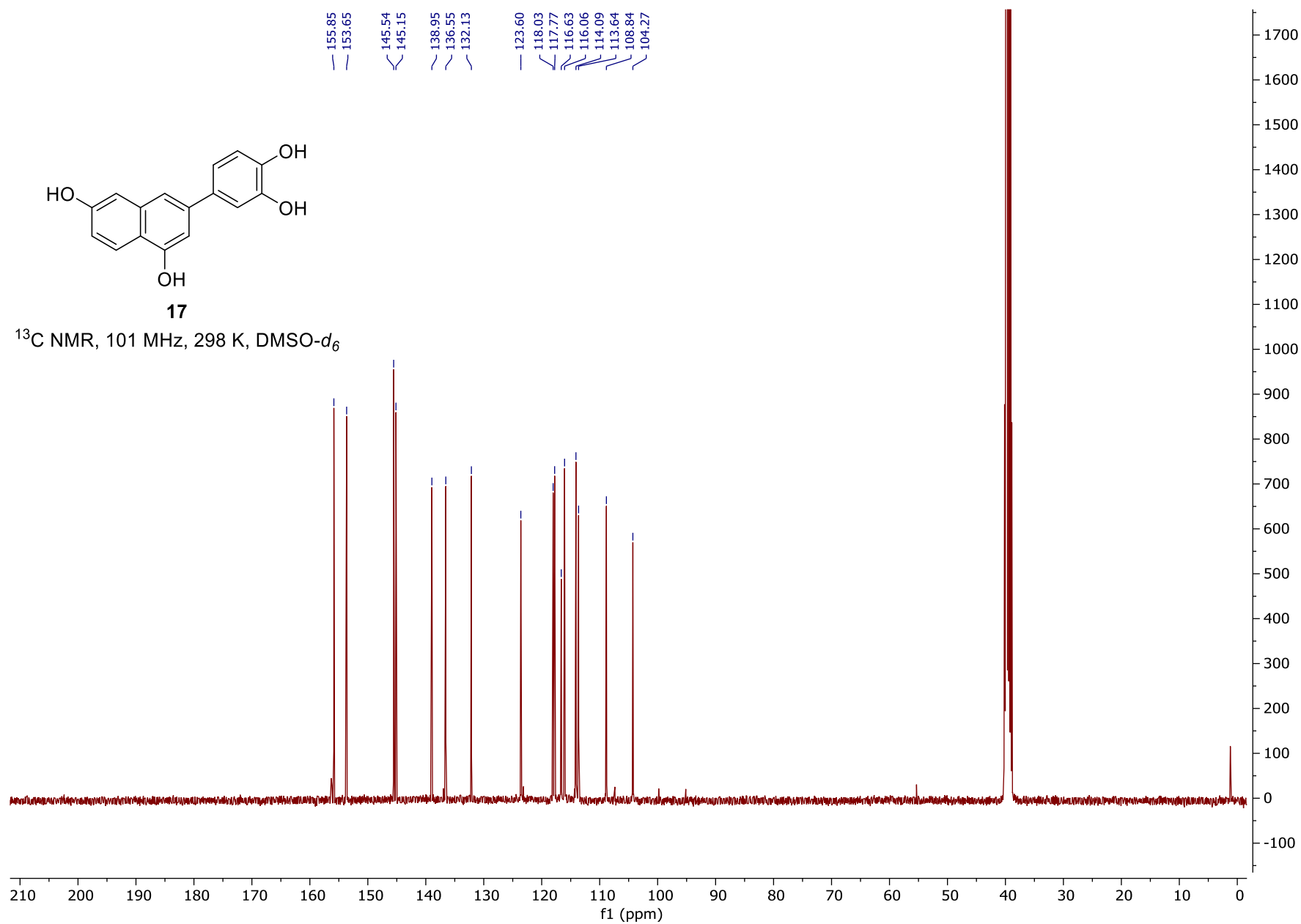


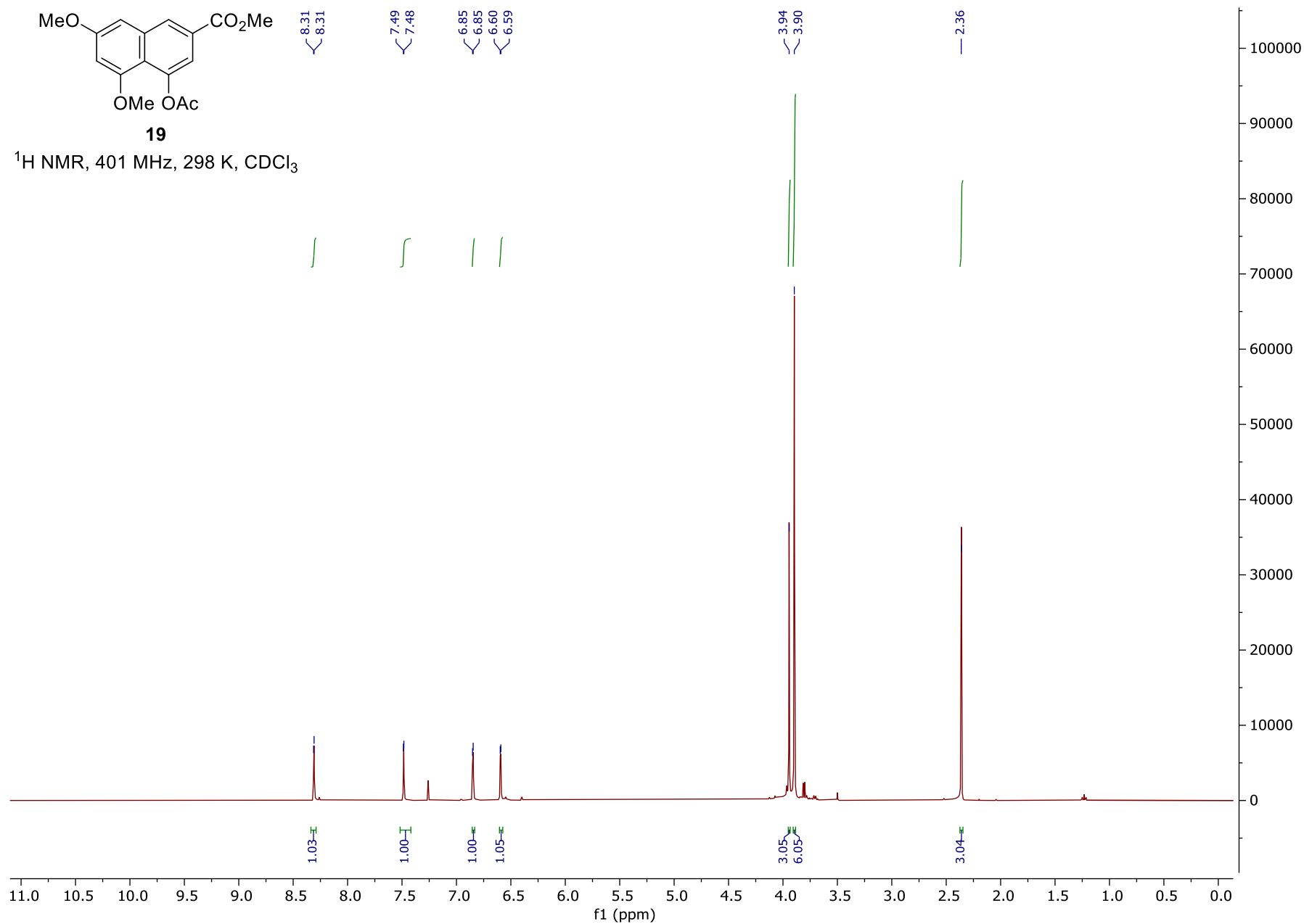
$^1\text{H}$  NMR, 401 MHz, 298 K,  $\text{CDCl}_3$

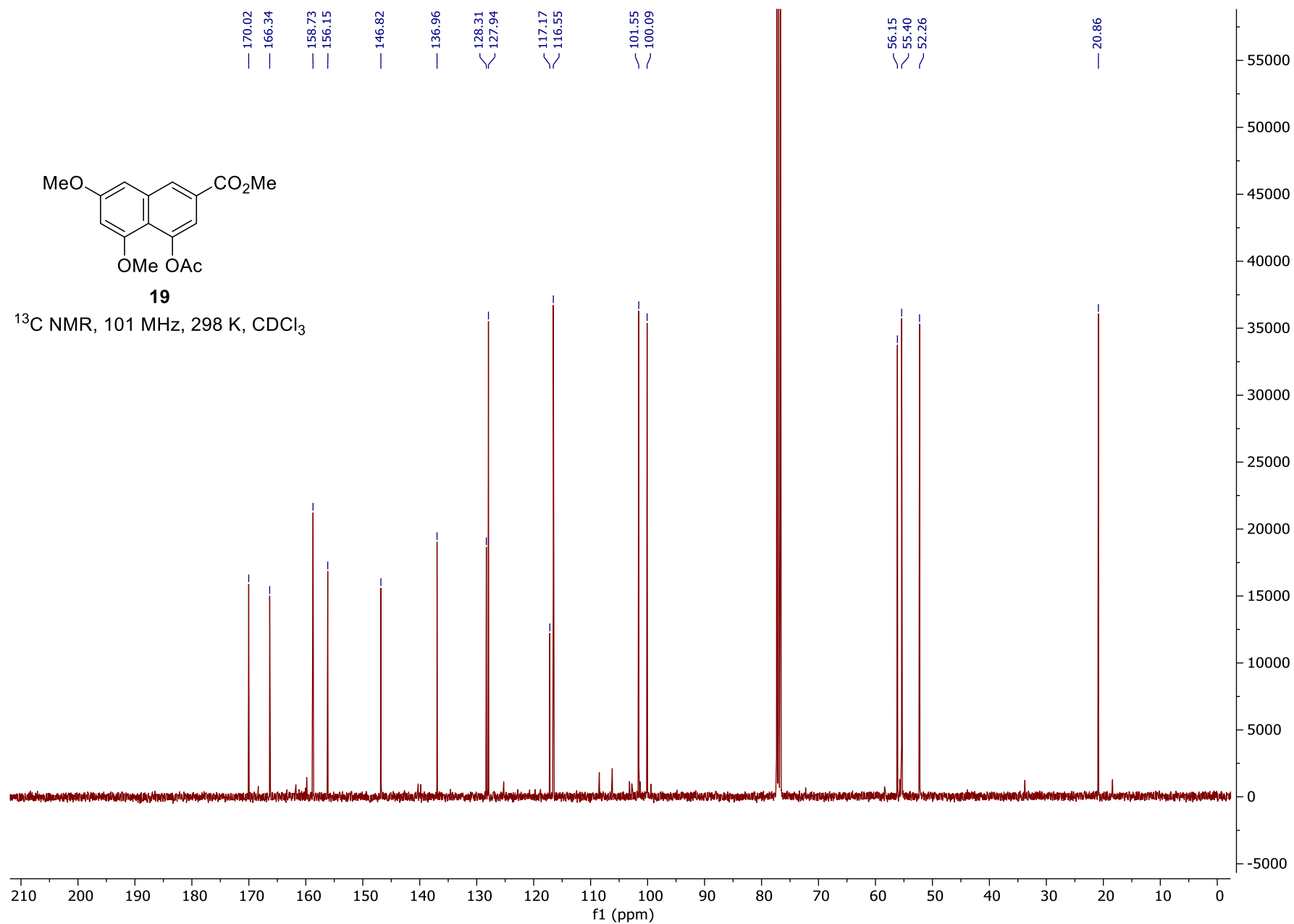


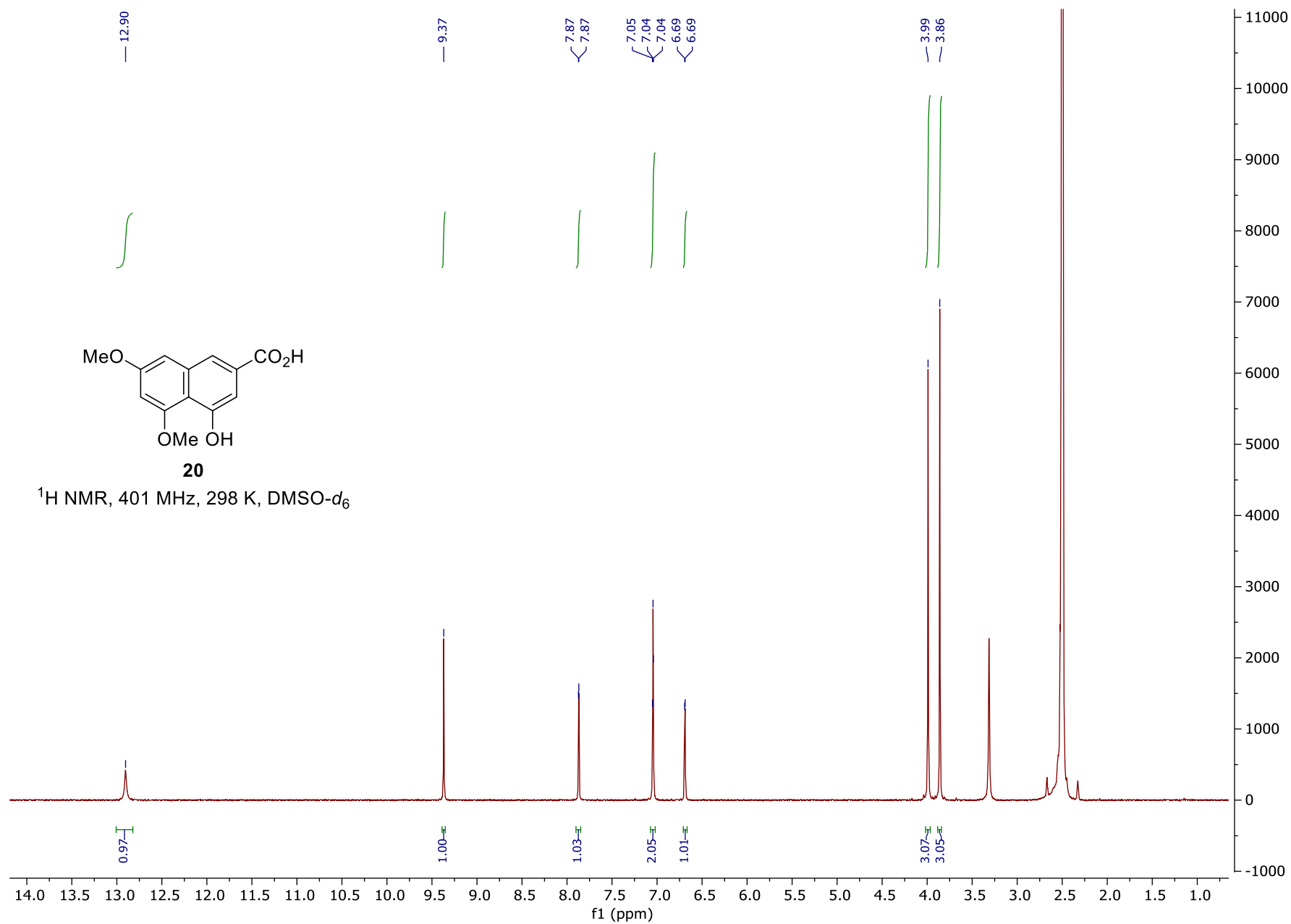


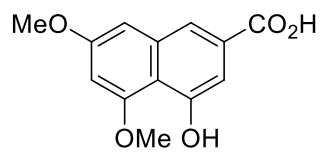








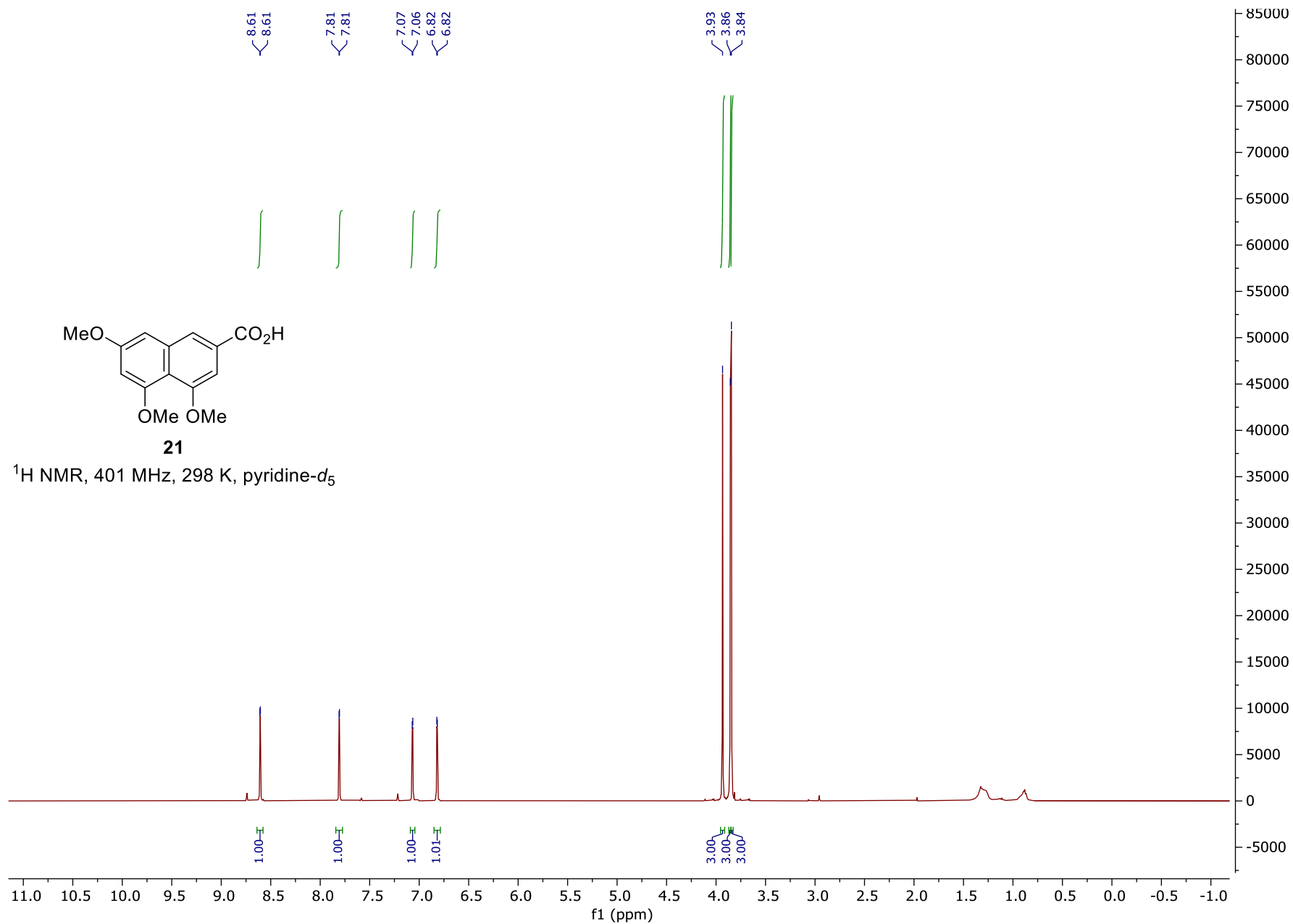


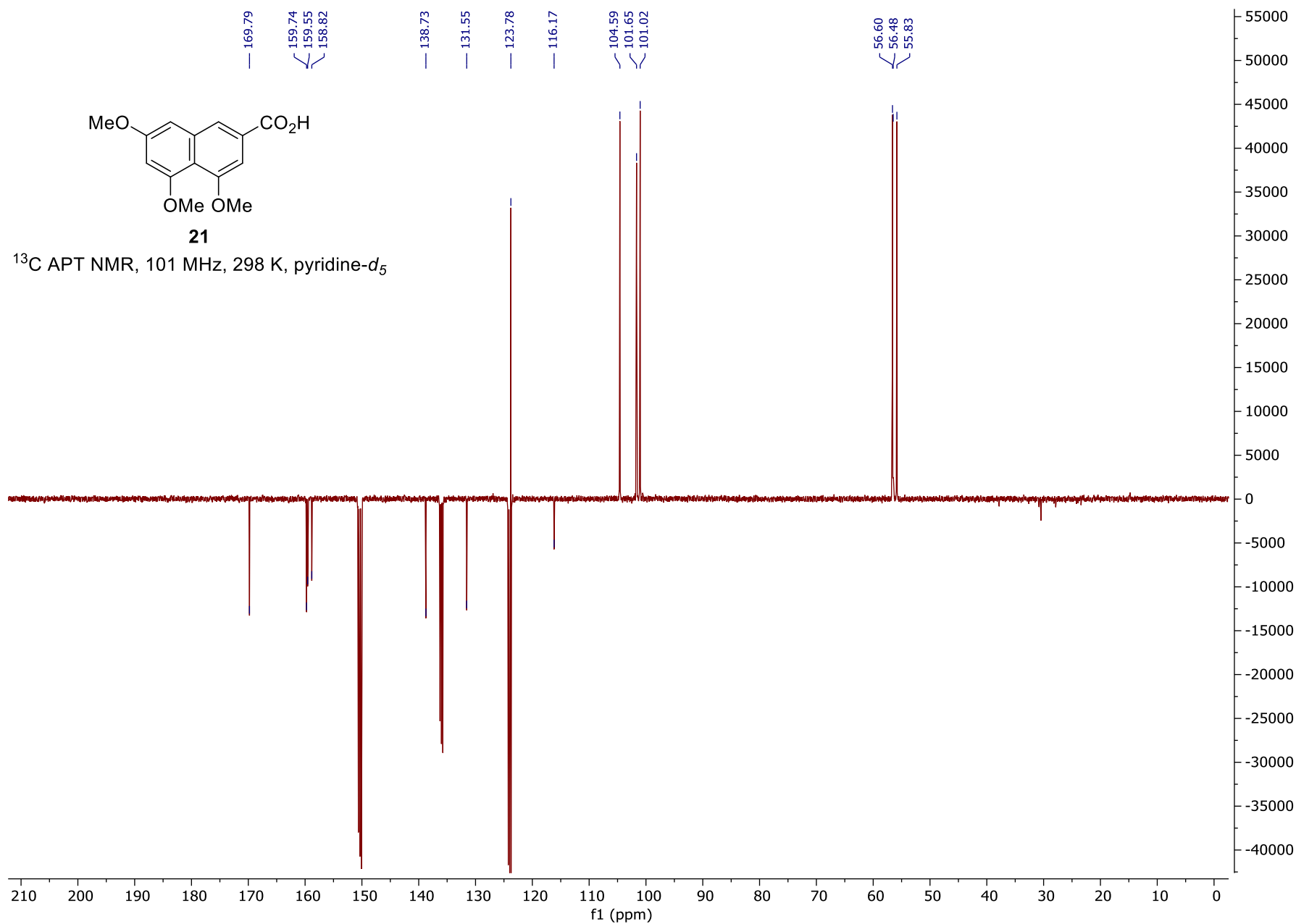


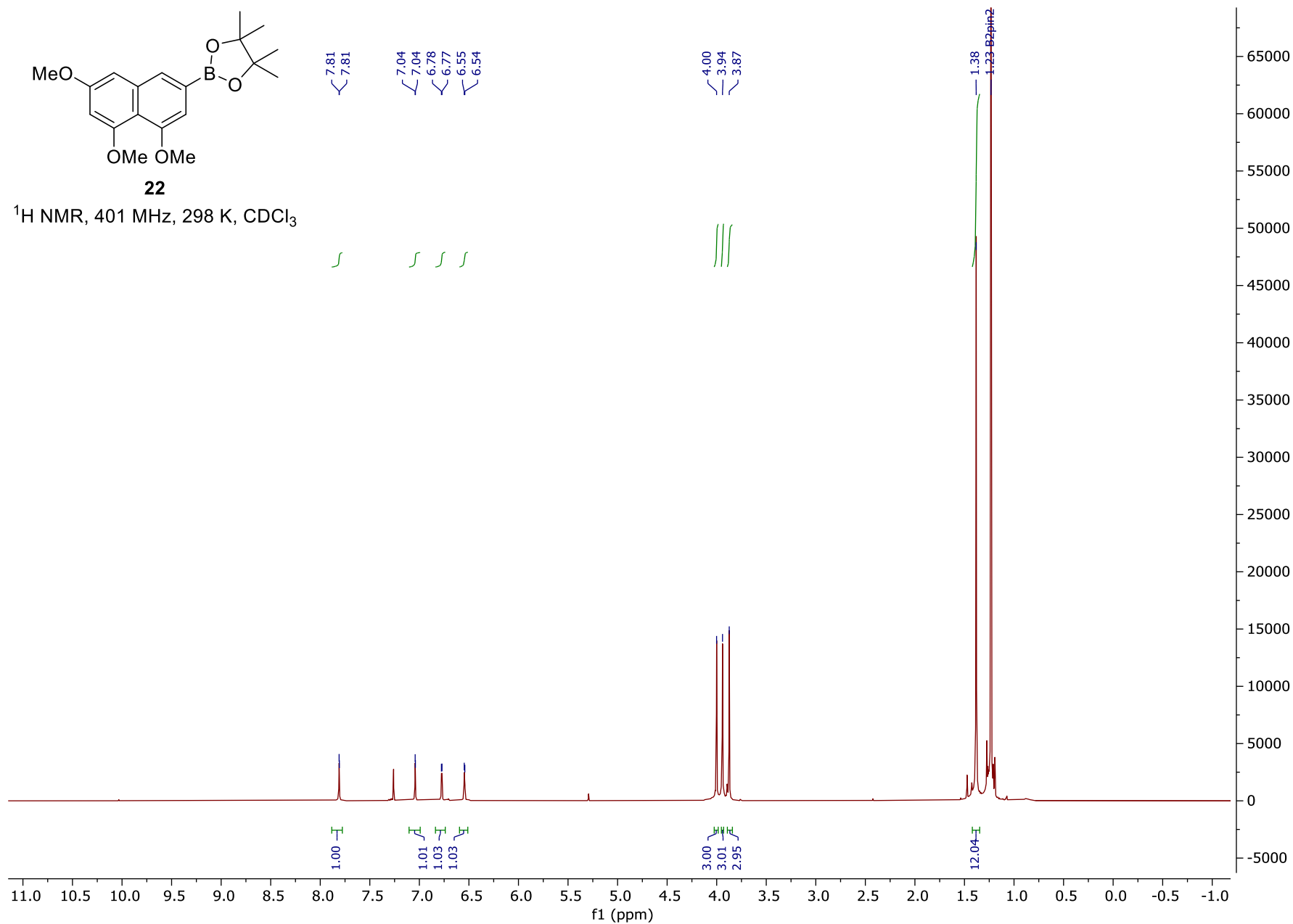
**20**

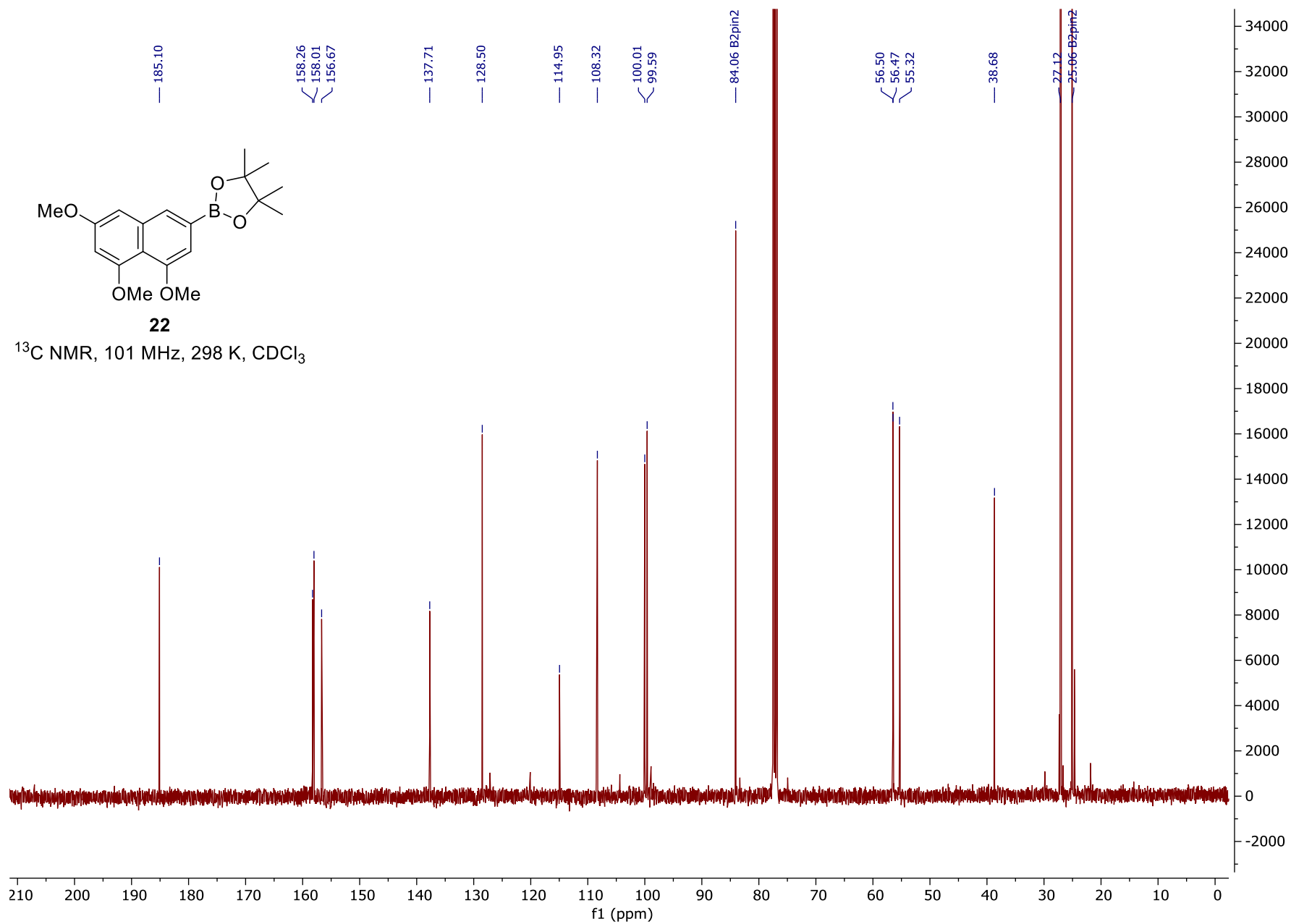
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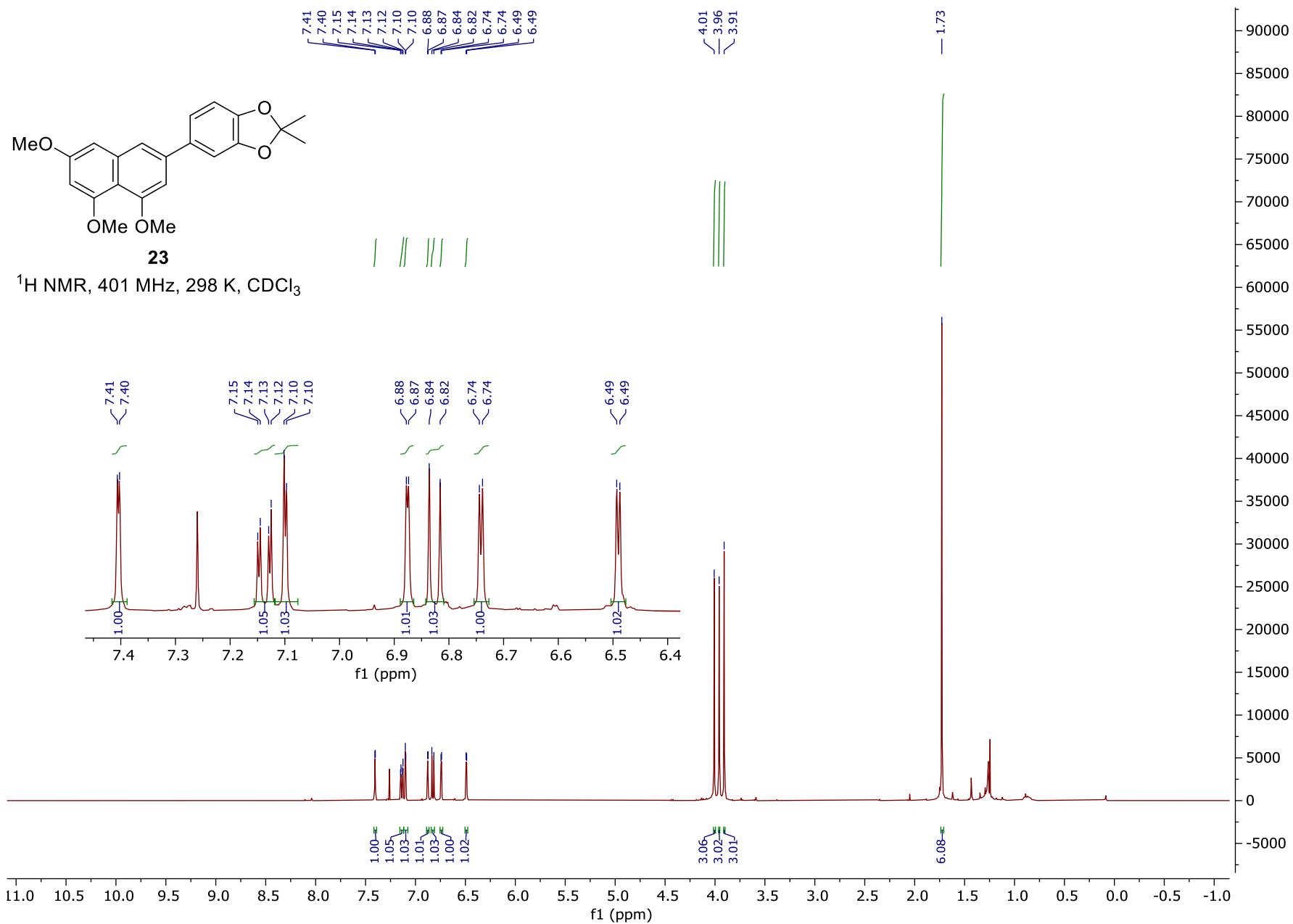


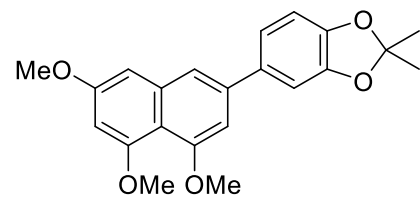






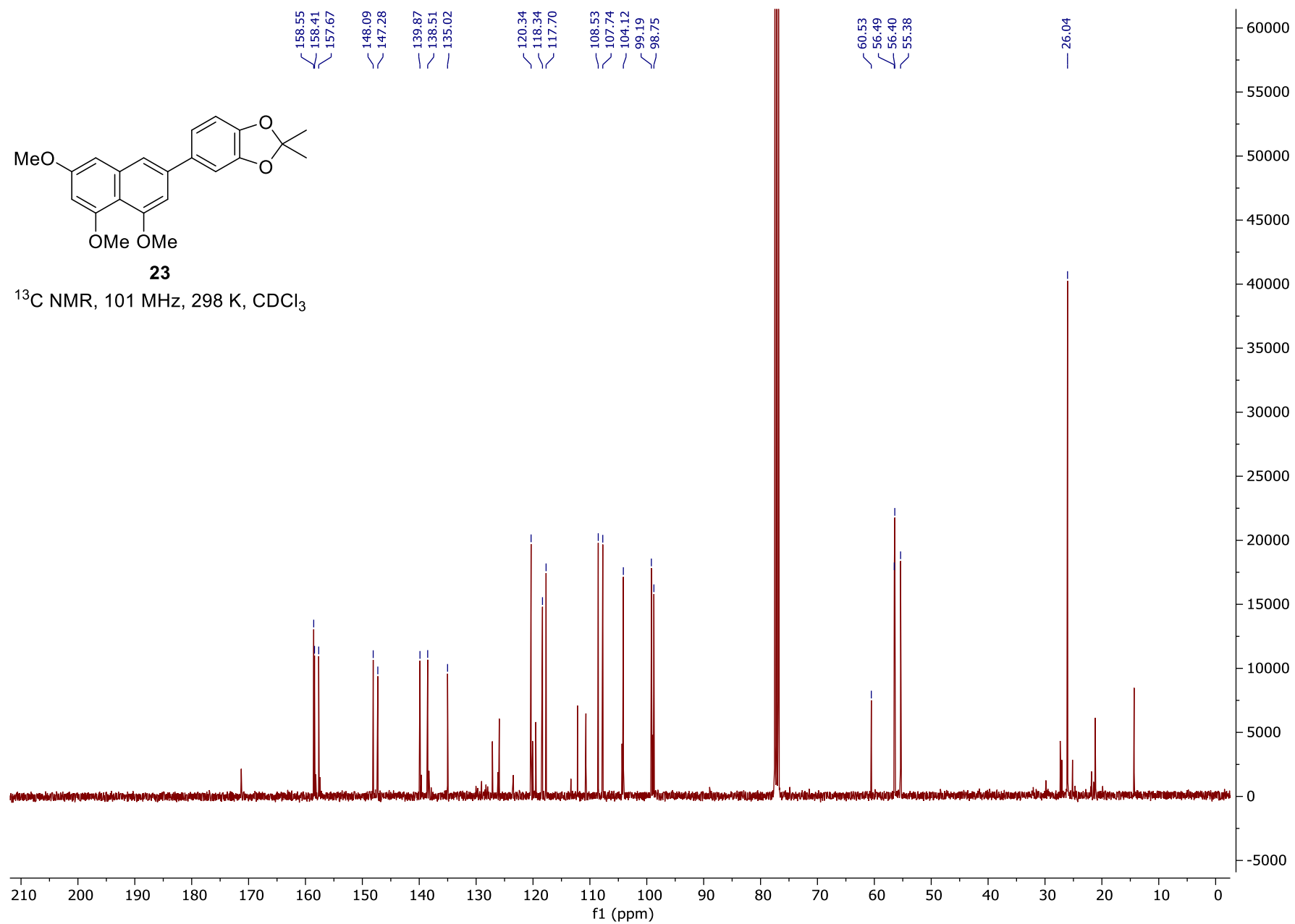


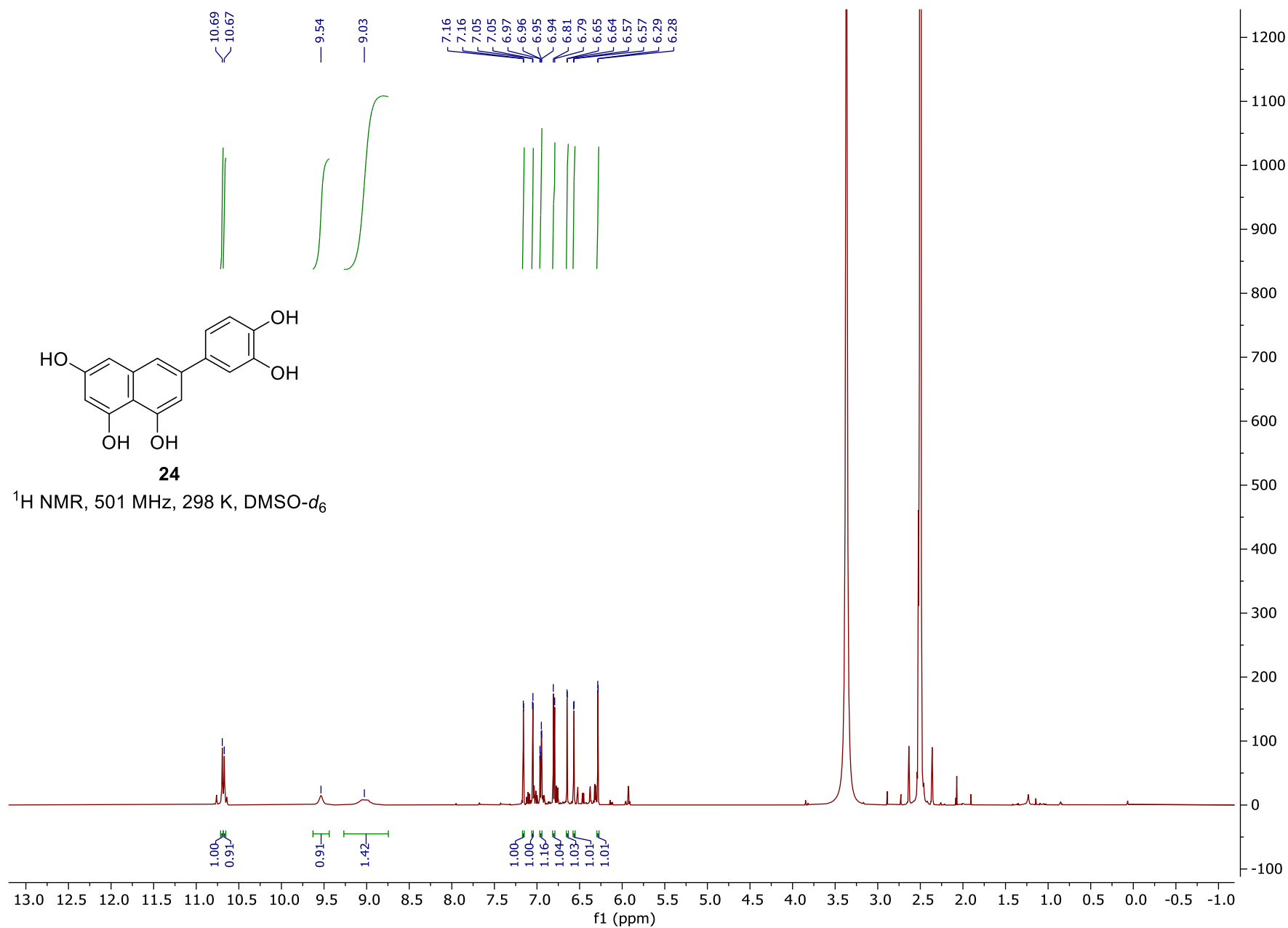


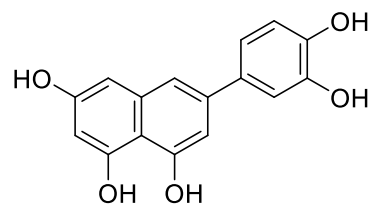


**23**

$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

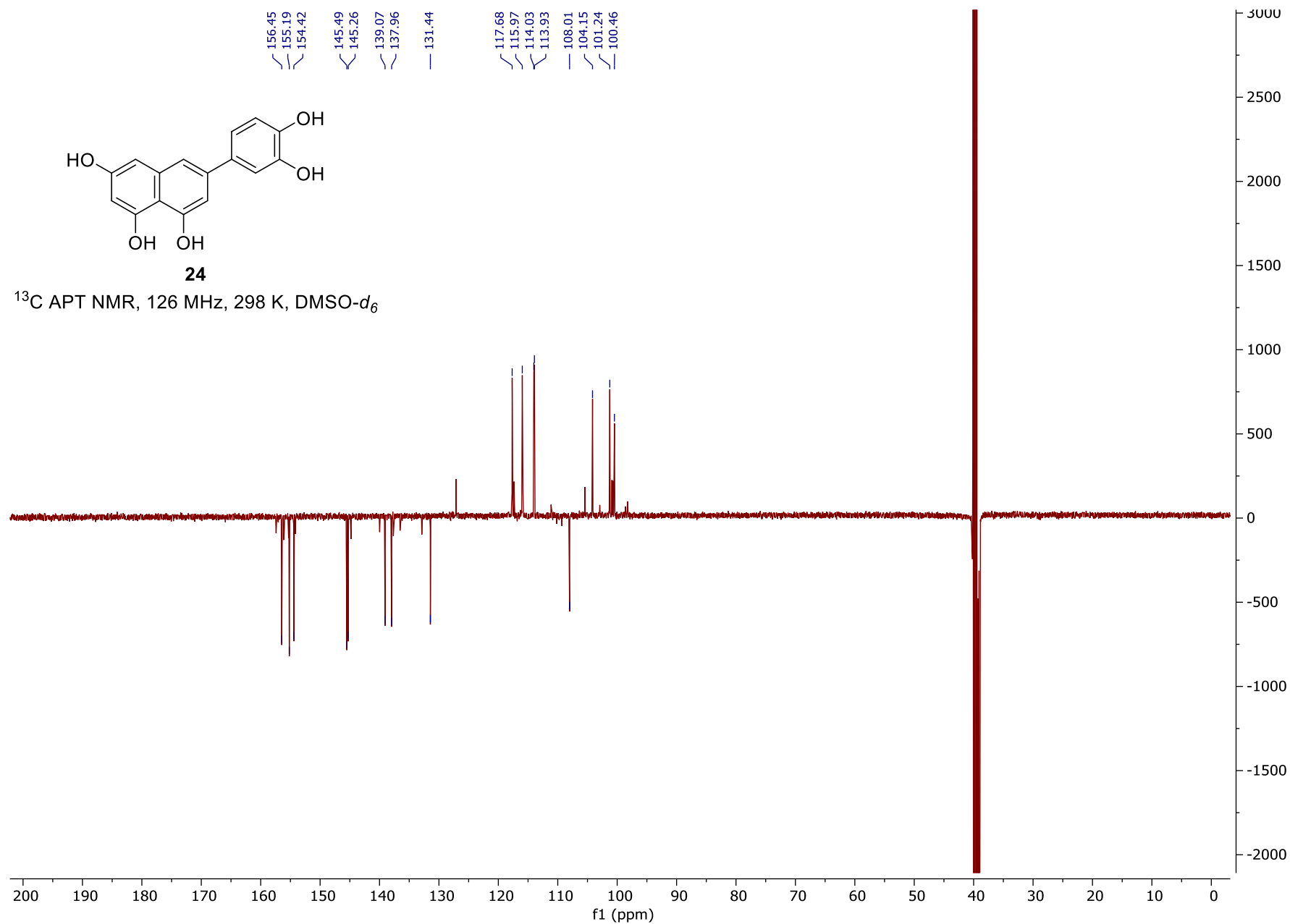


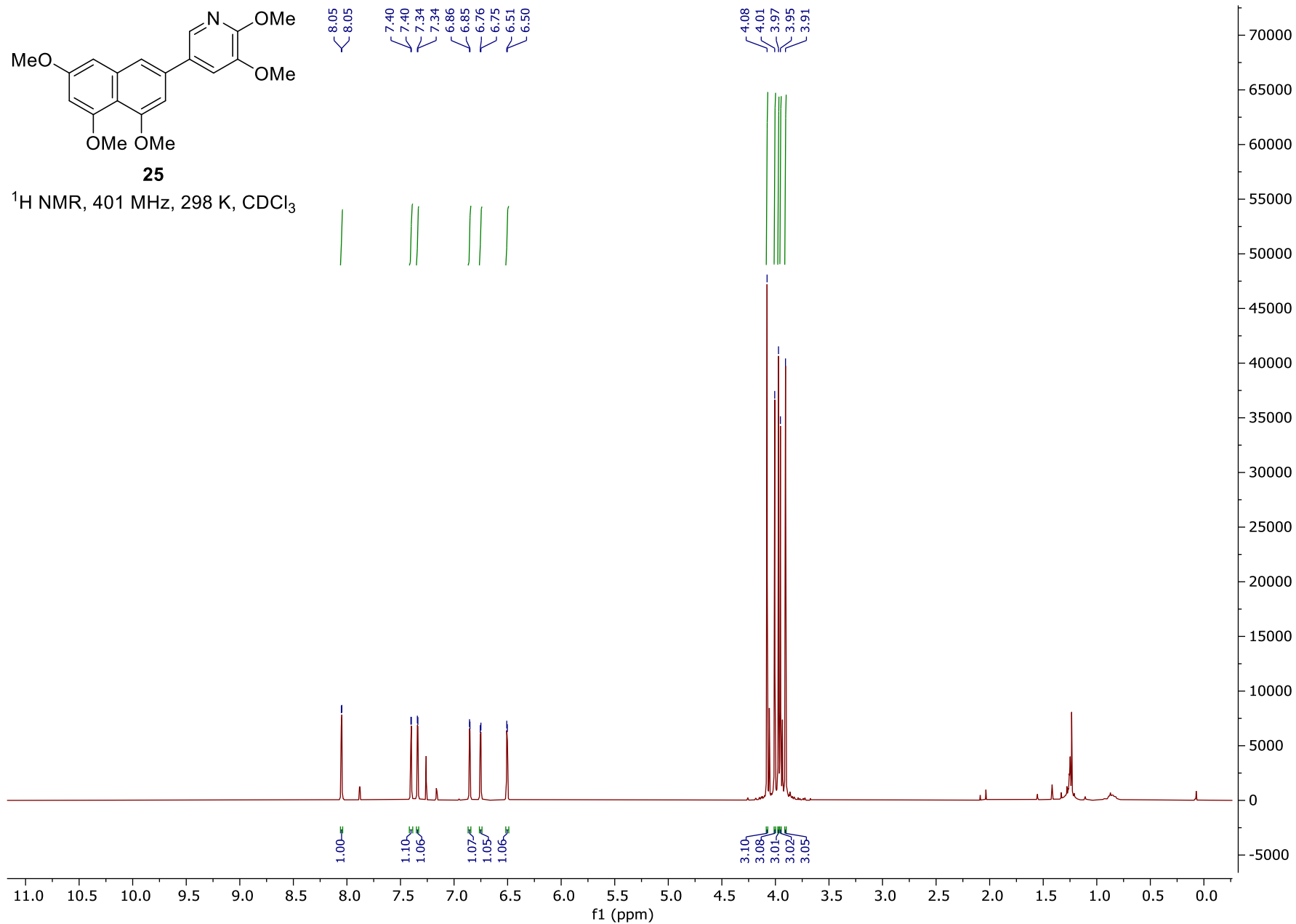


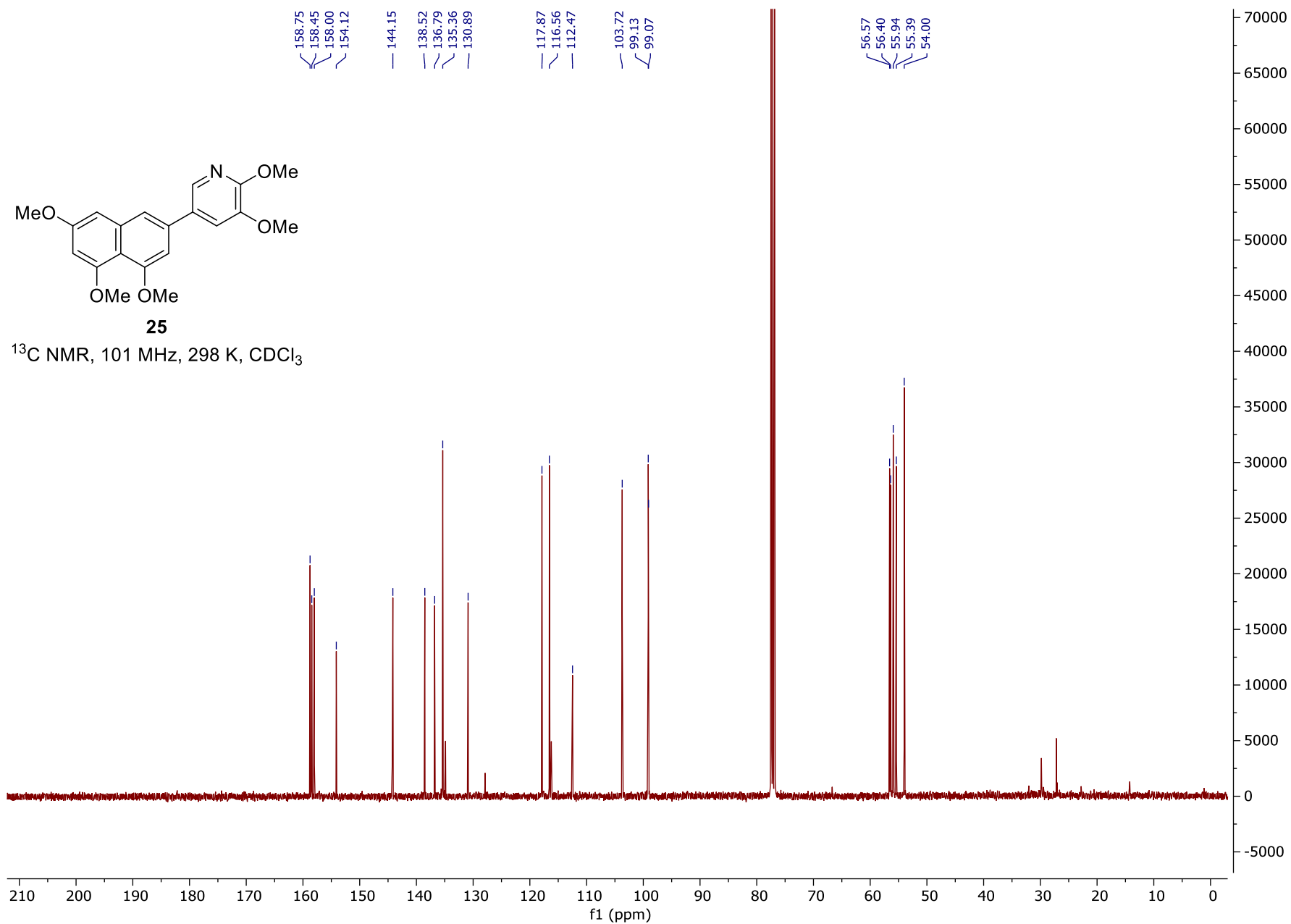


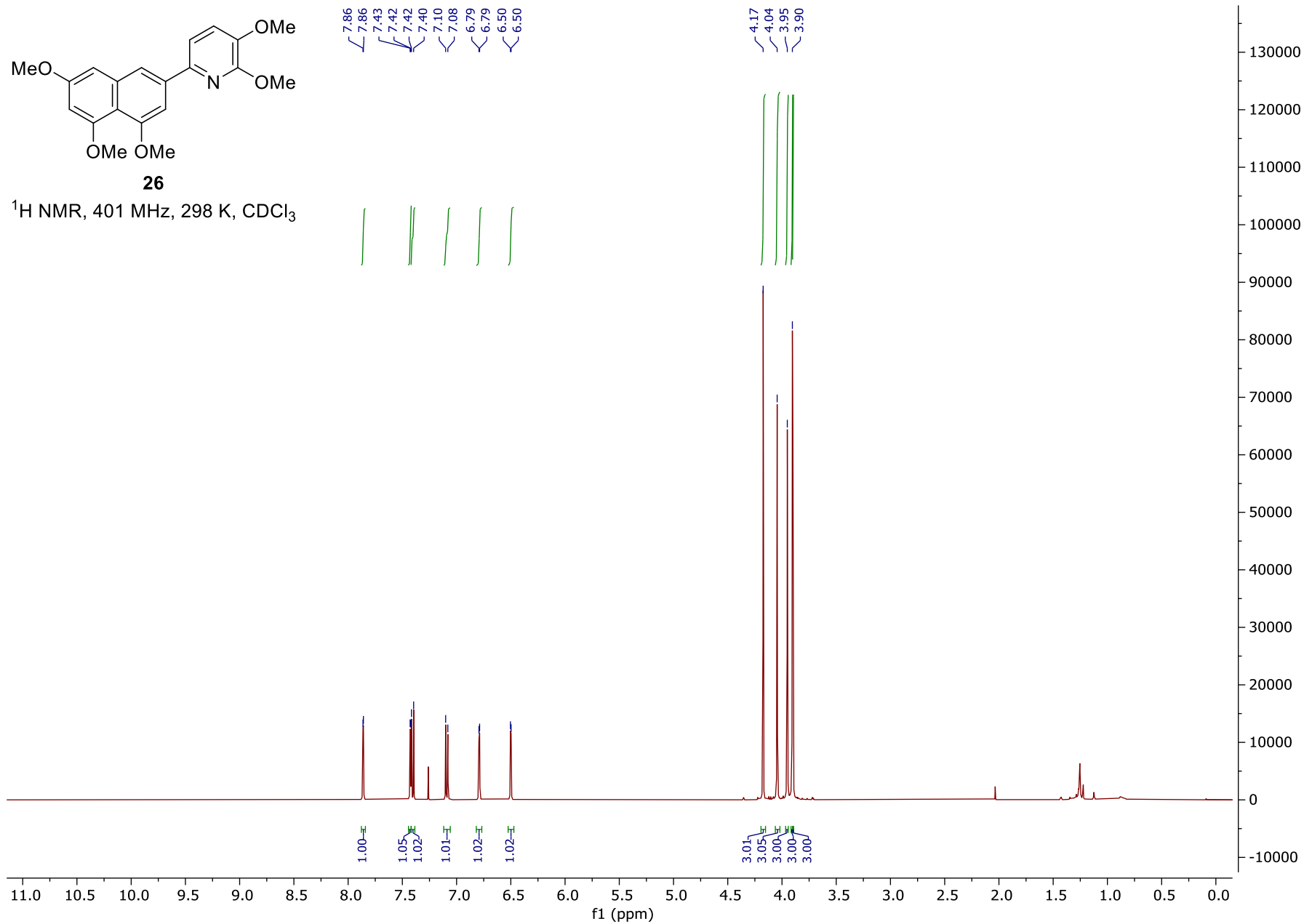
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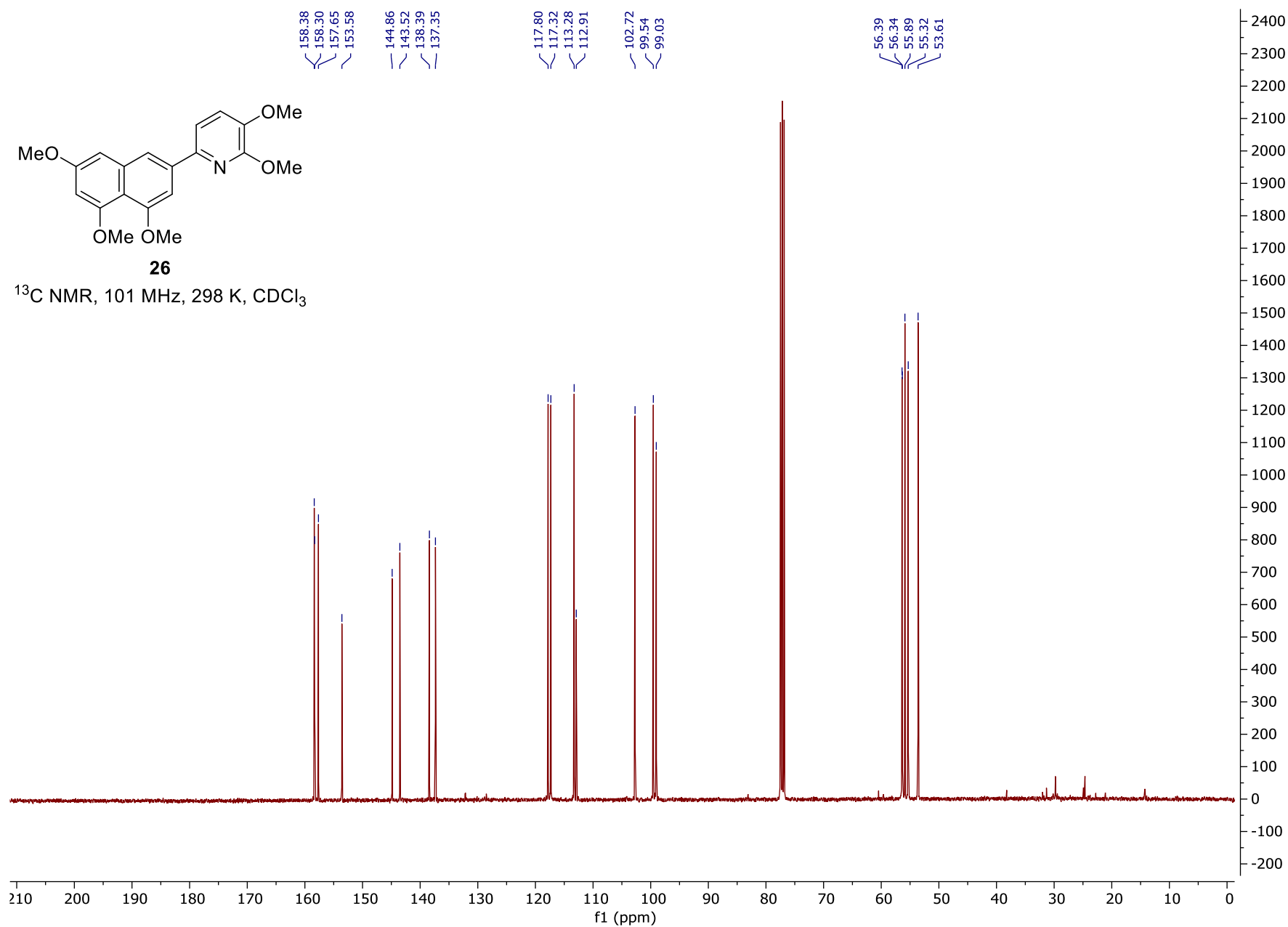
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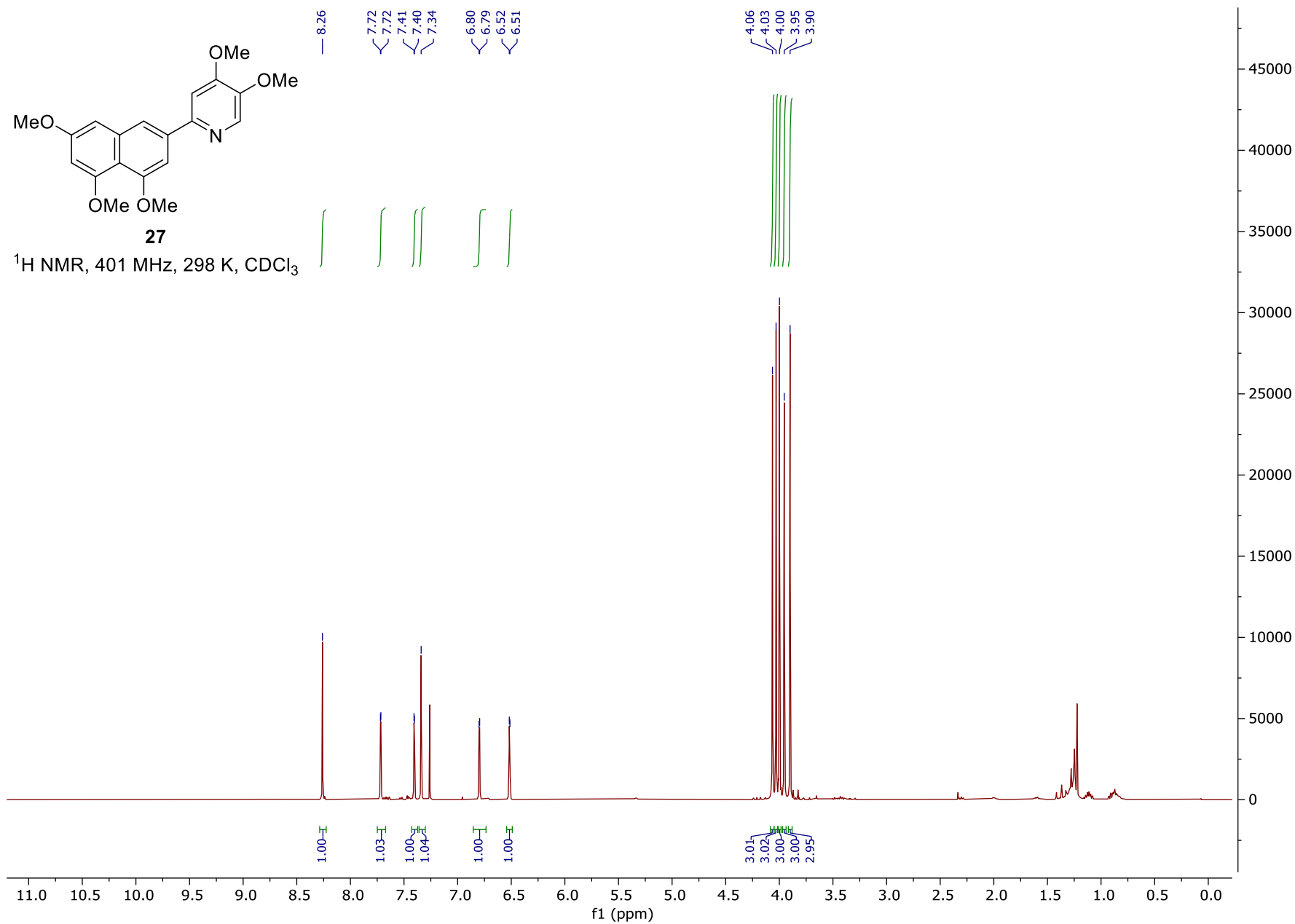


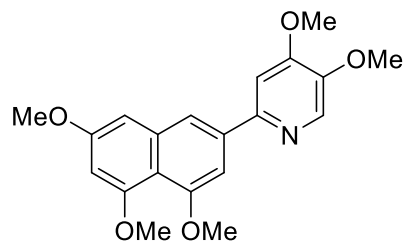






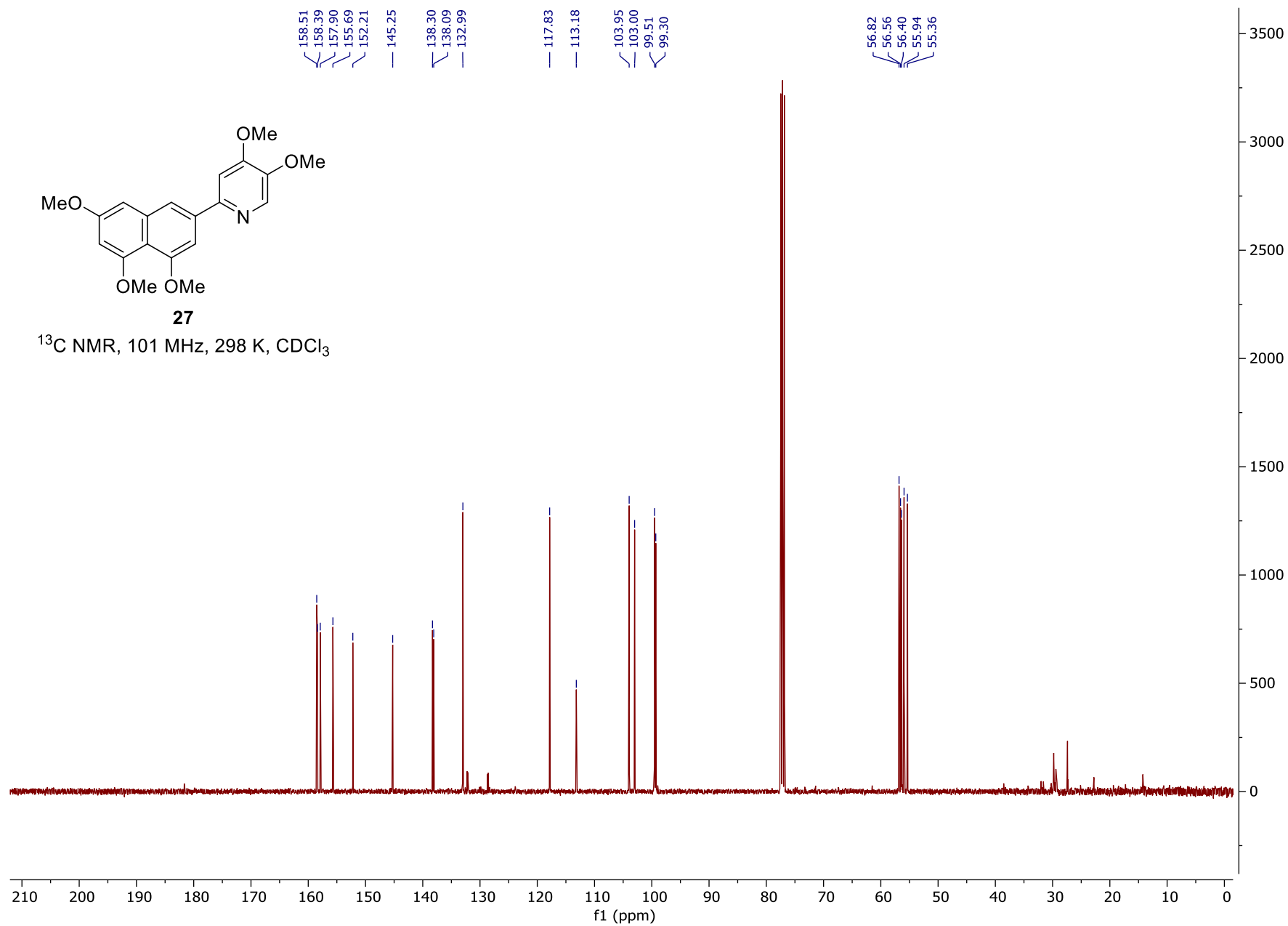


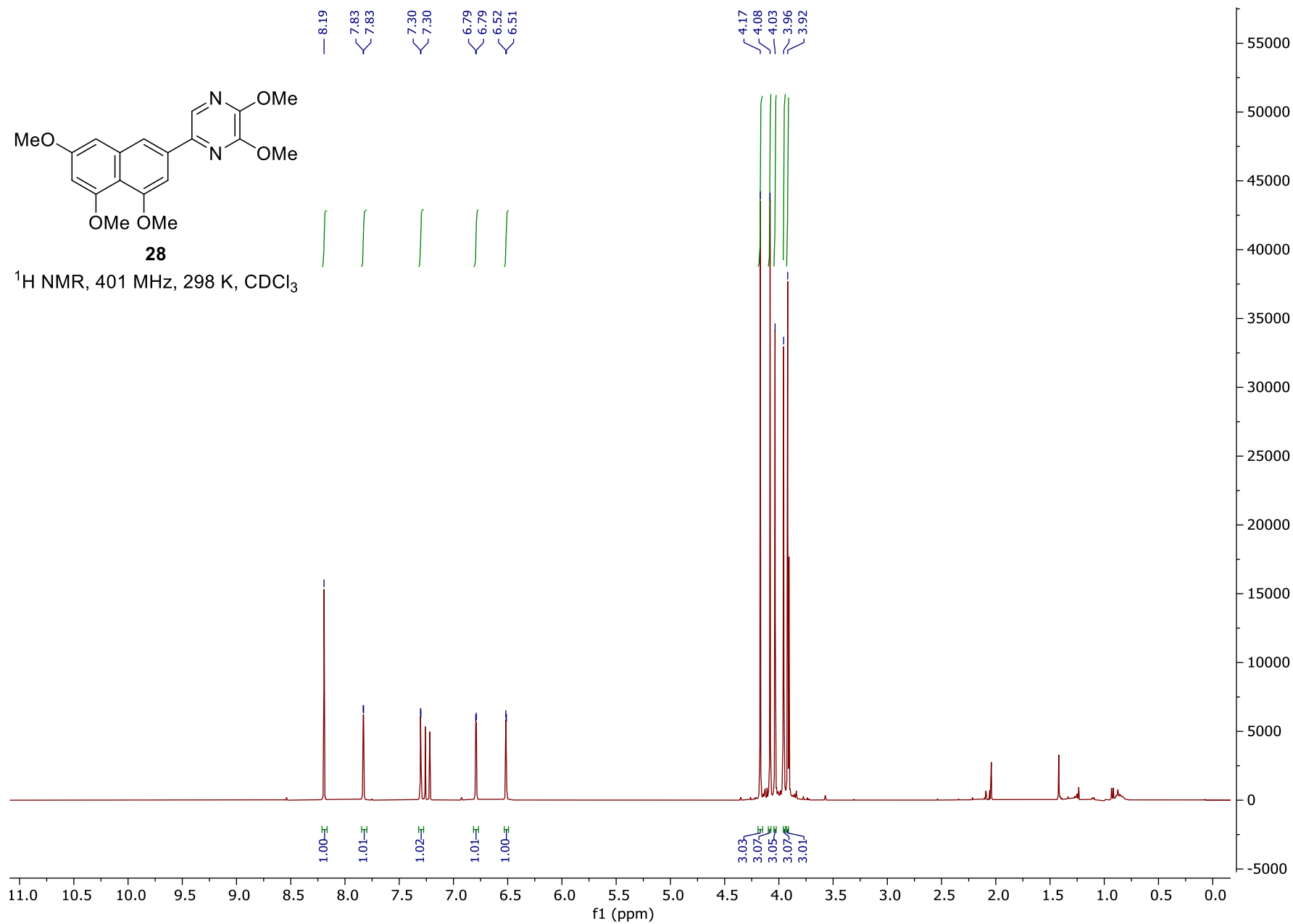


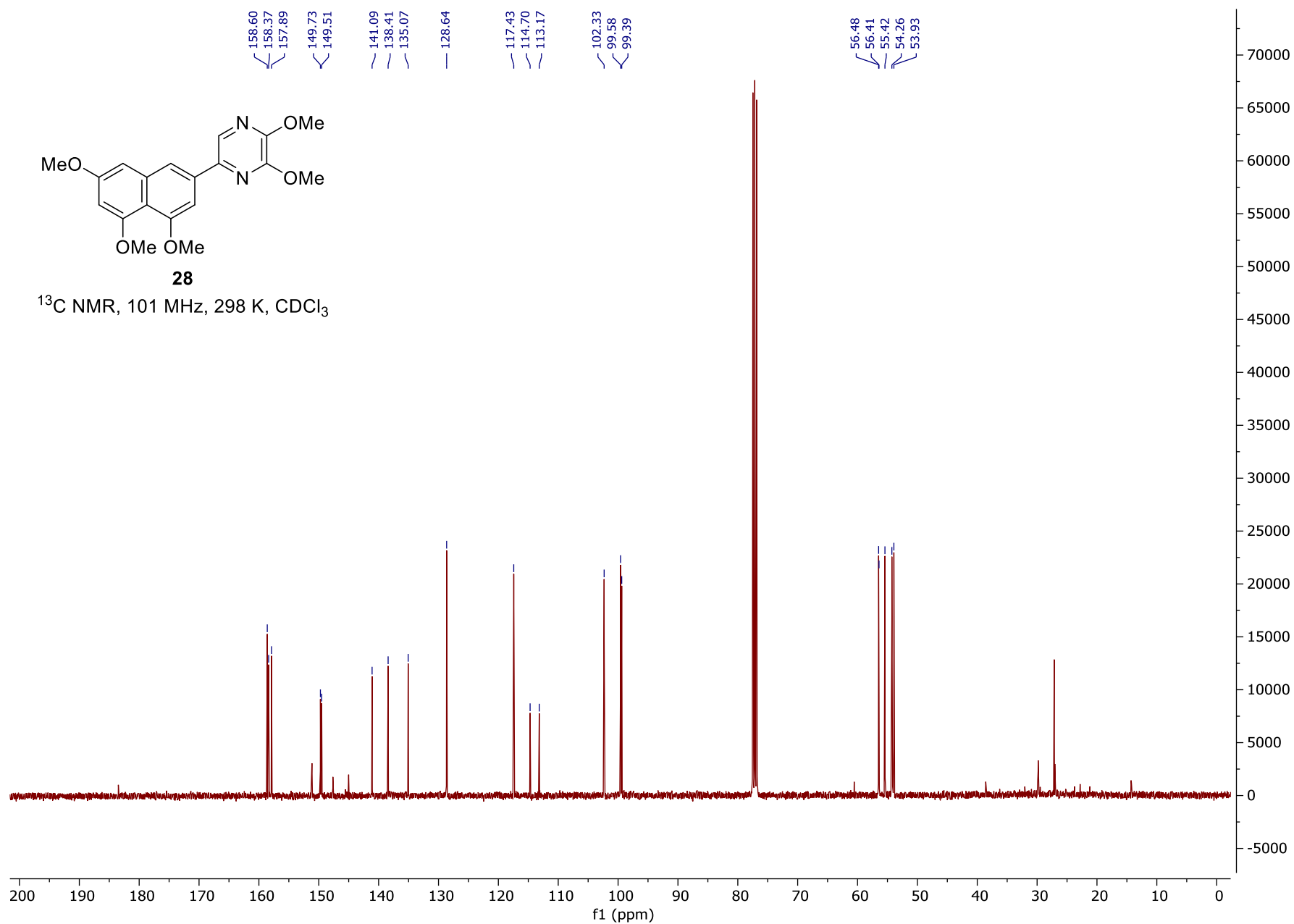


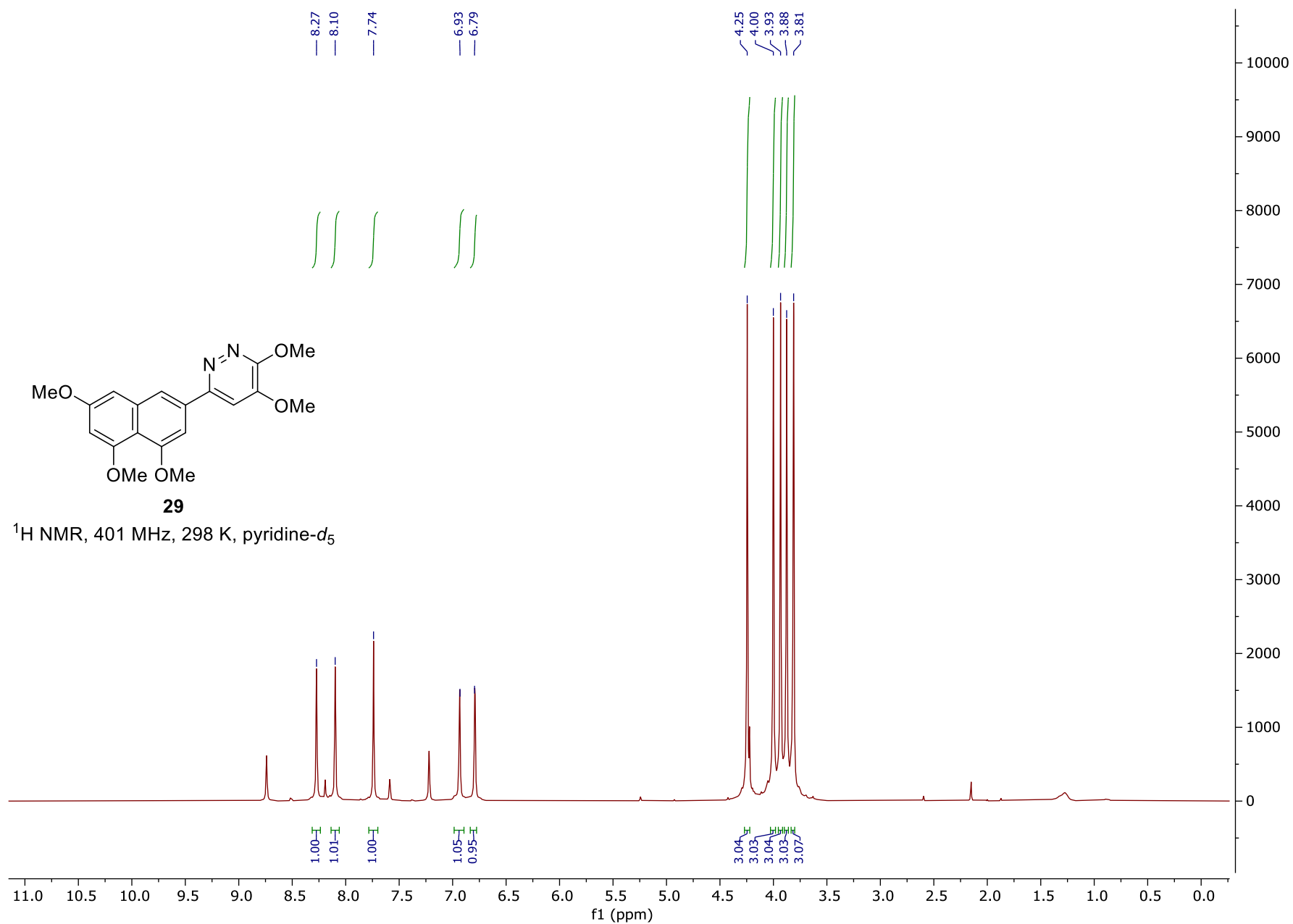
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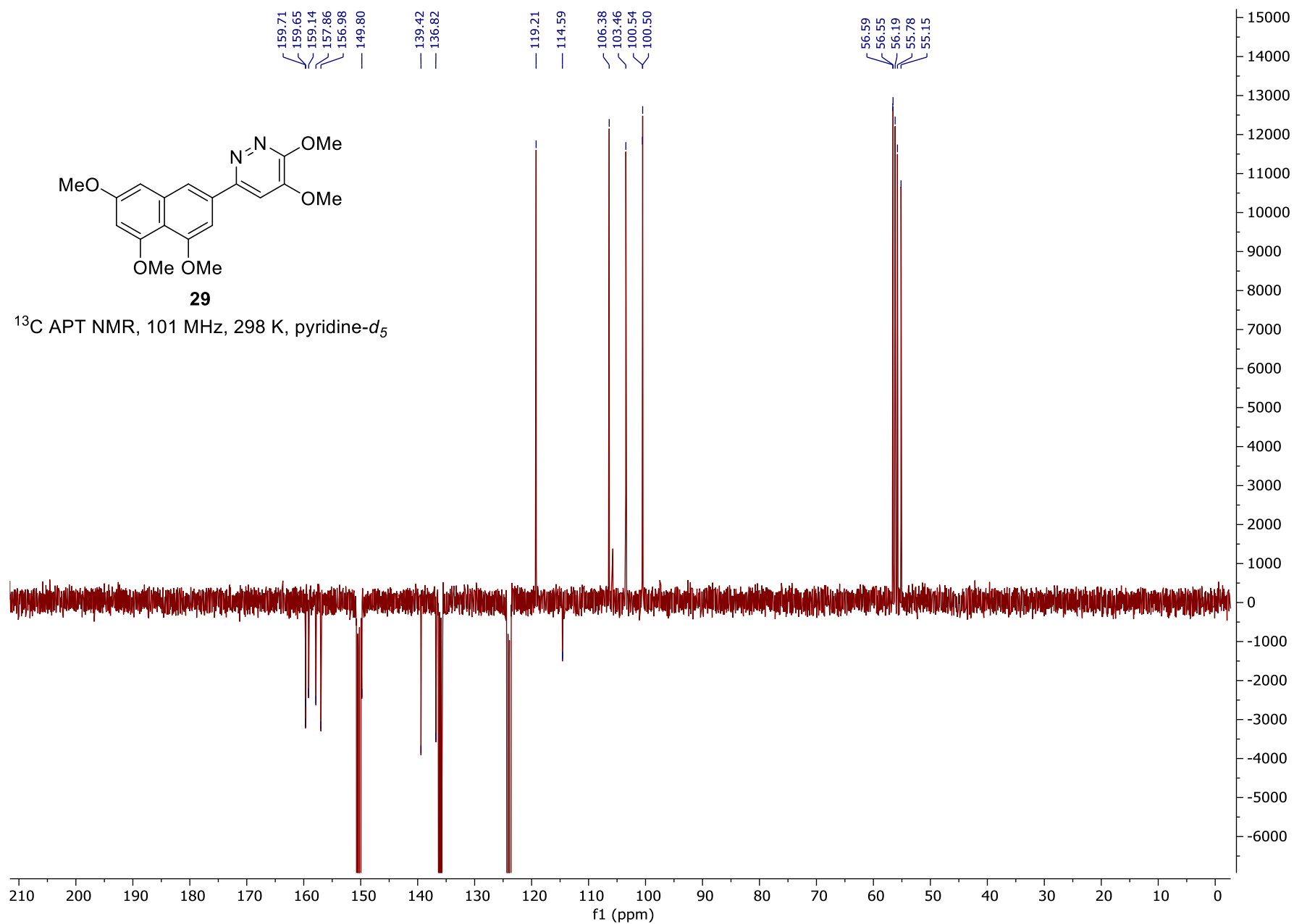
$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

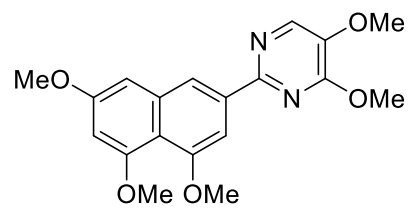






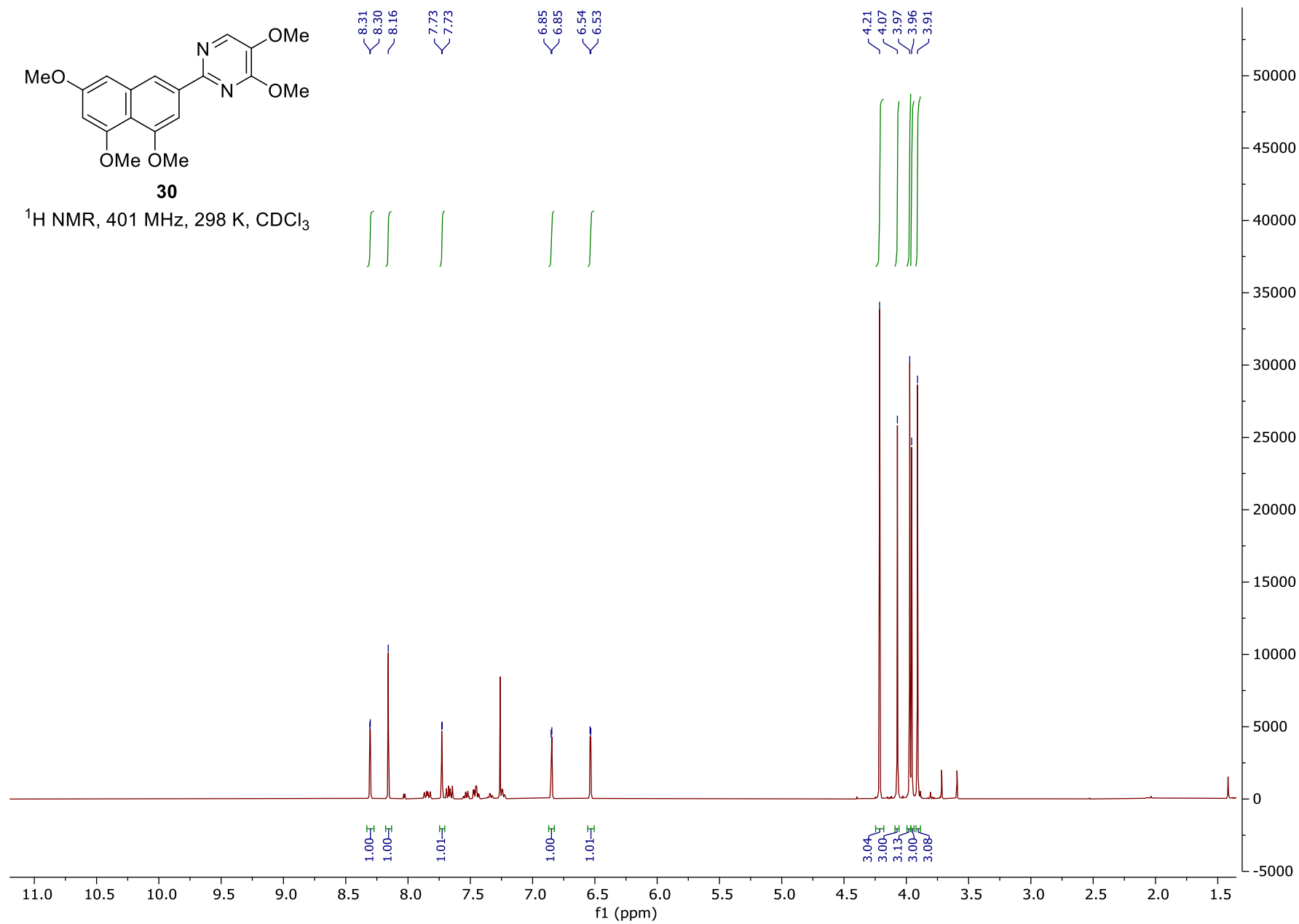


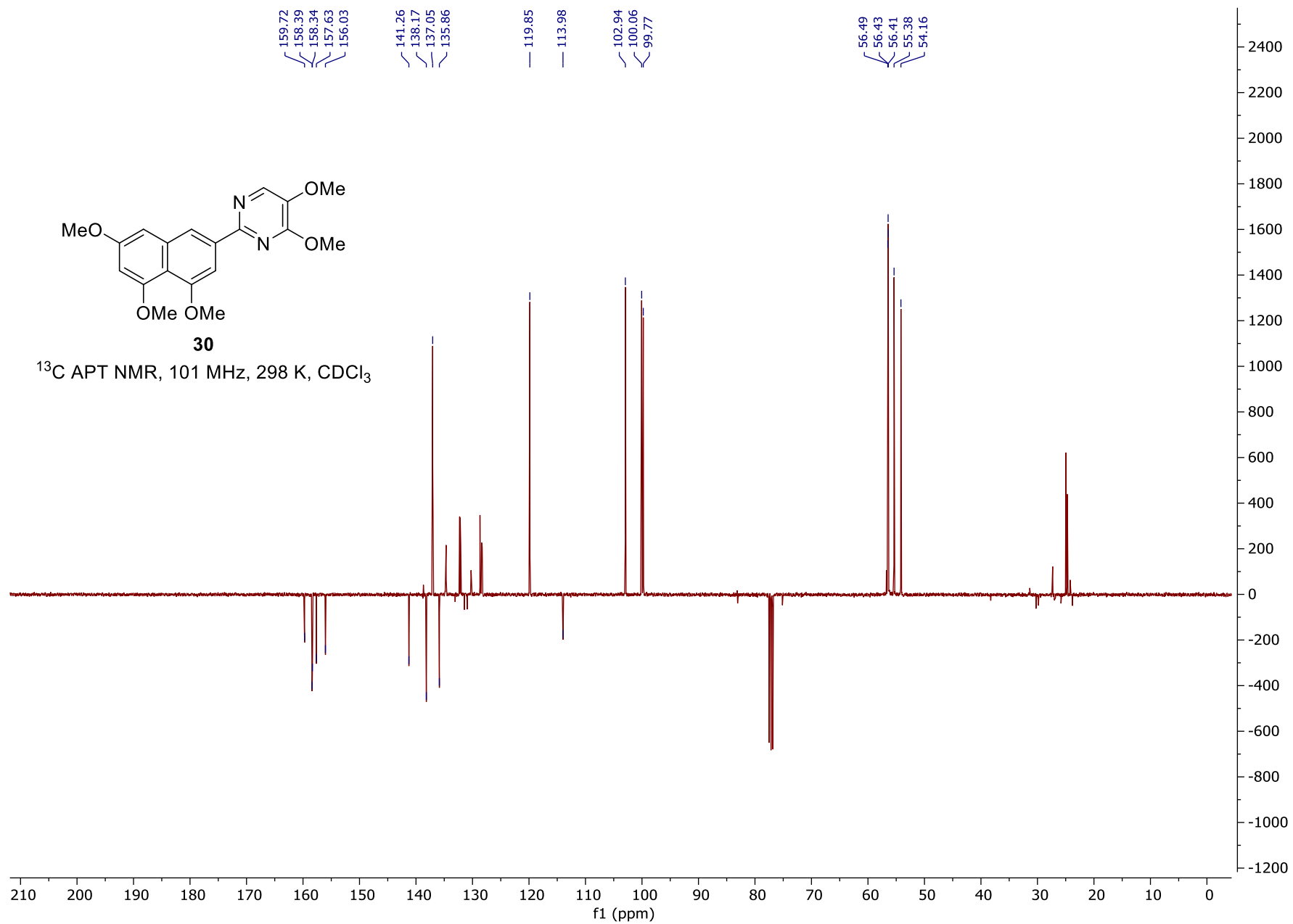


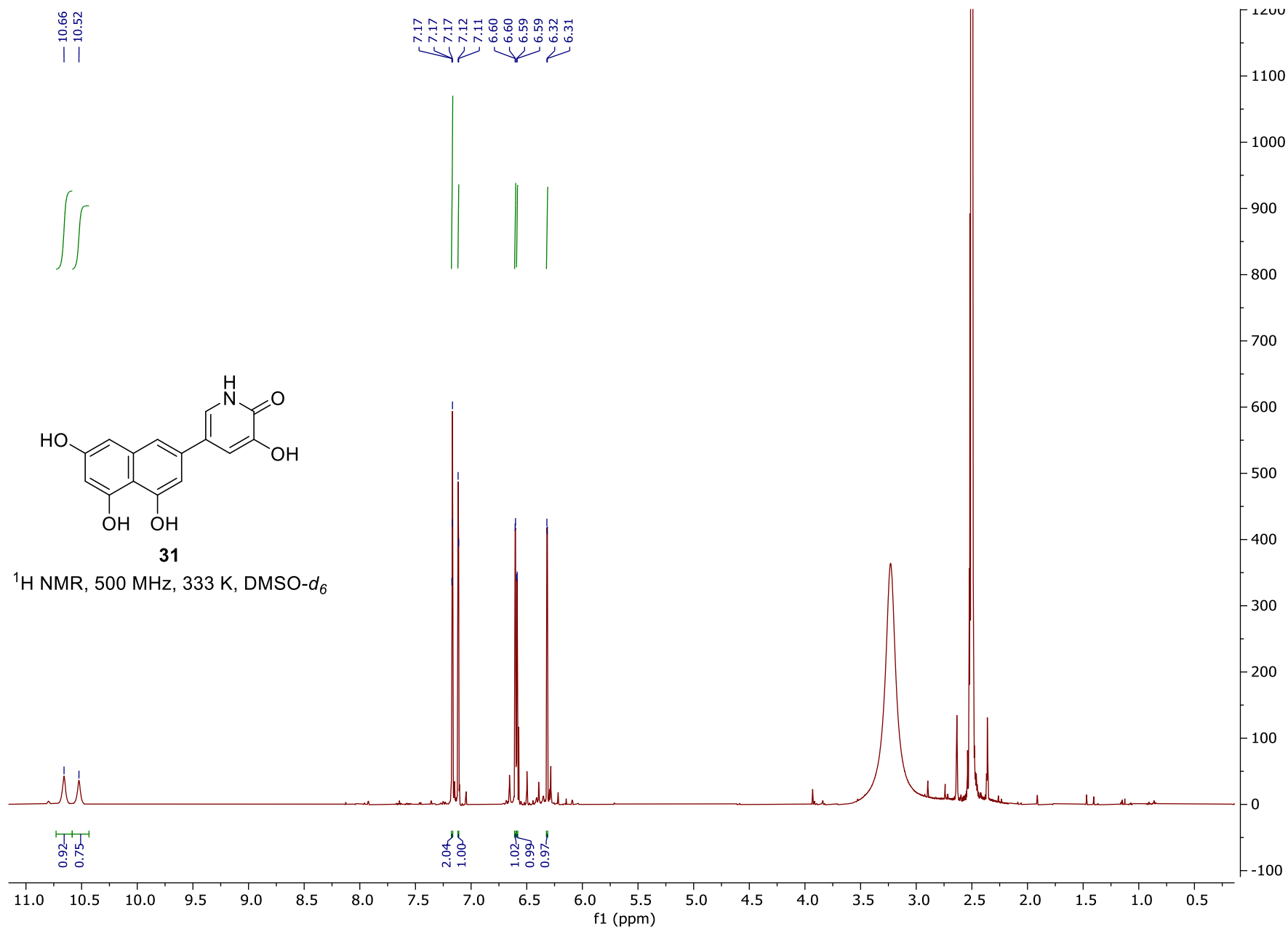


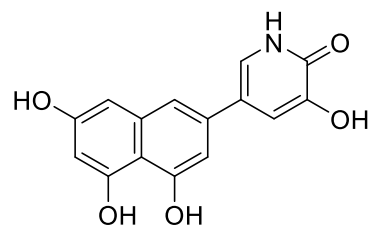
**30**

$^1\text{H}$  NMR, 401 MHz, 298 K,  $\text{CDCl}_3$



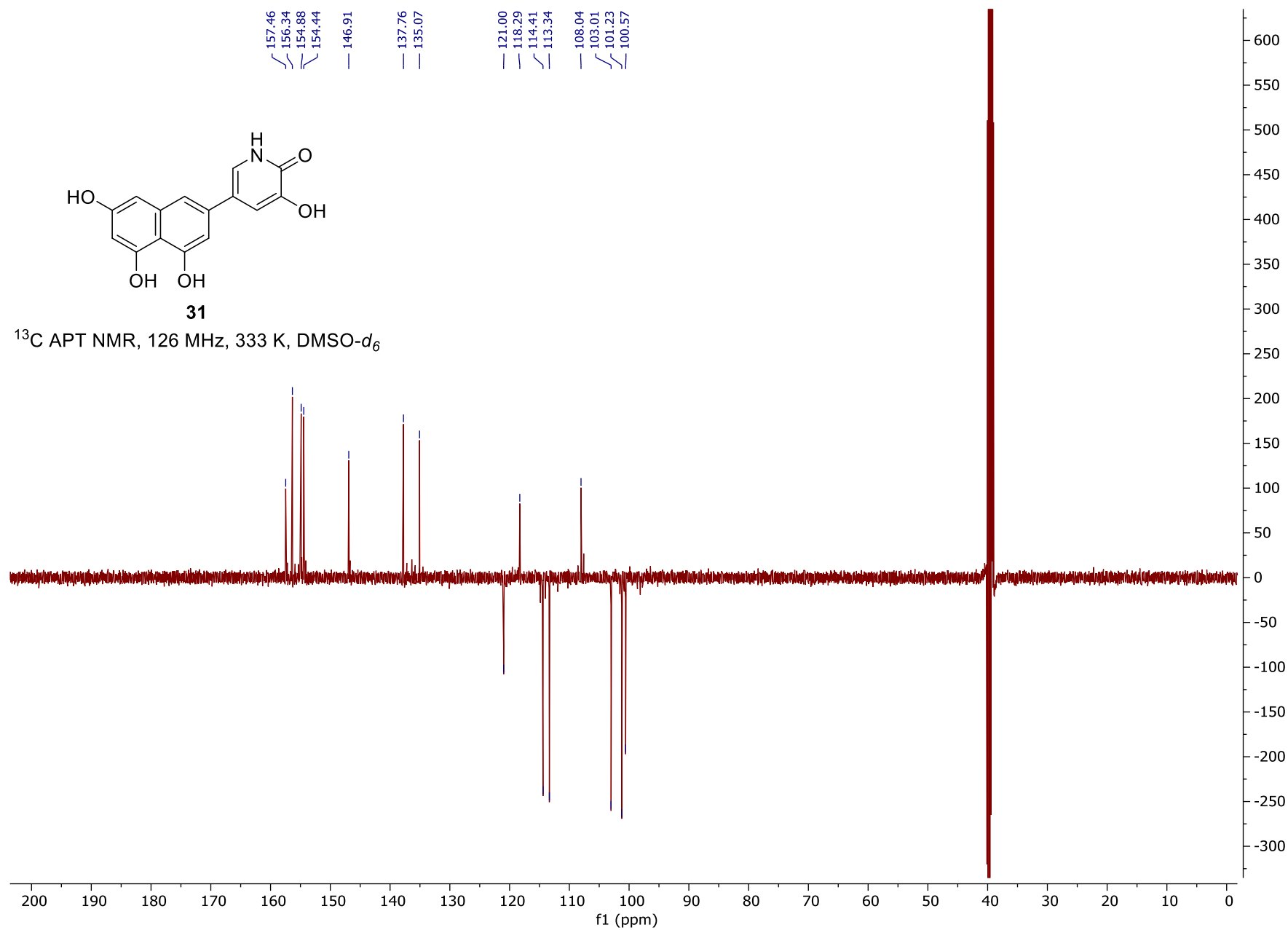


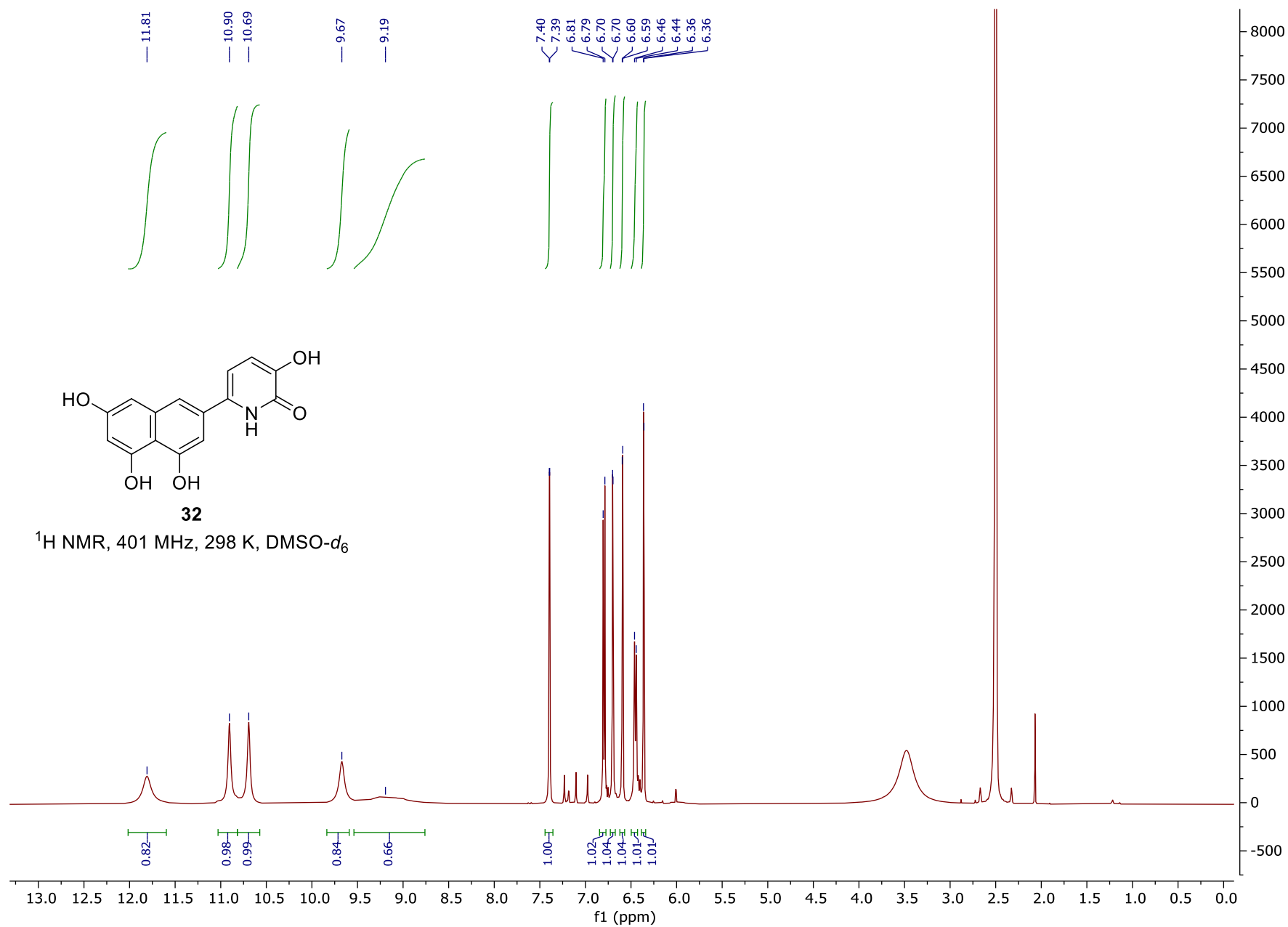


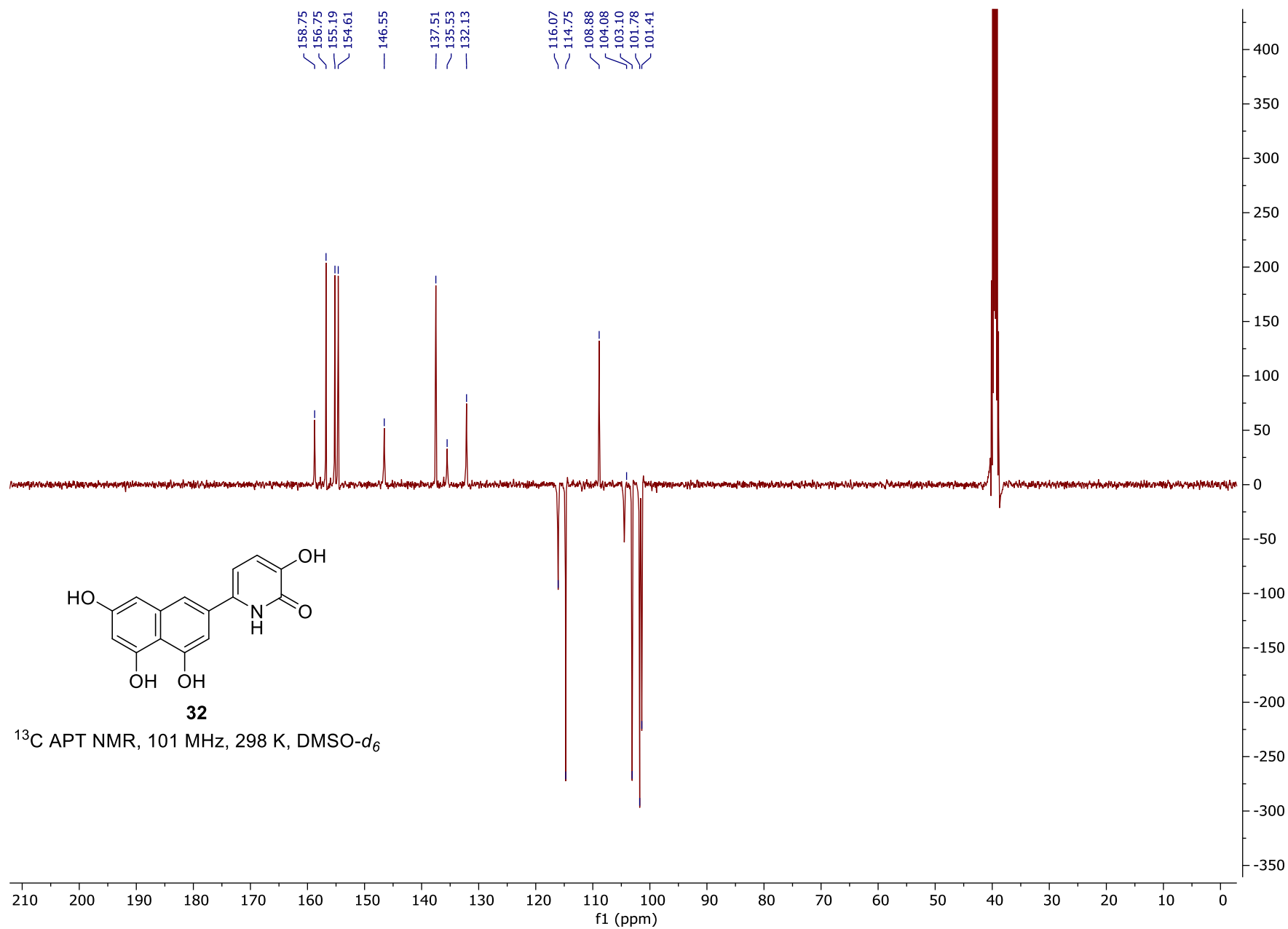


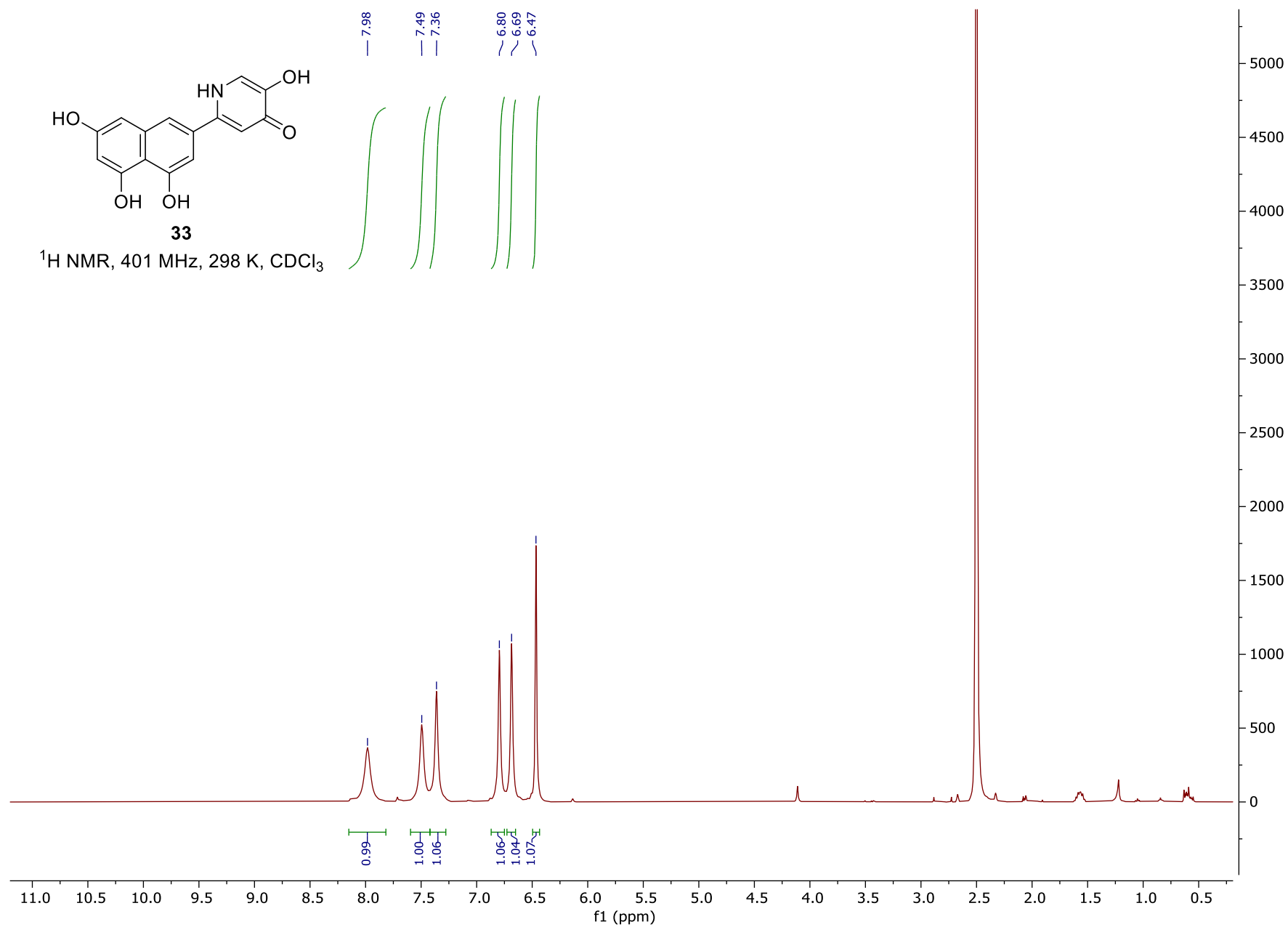
**31**

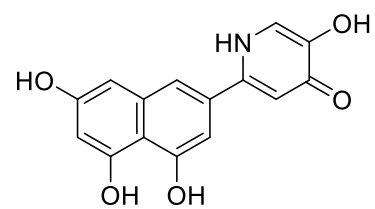
$^{13}\text{C}$  APT NMR, 126 MHz, 333 K,  $\text{DMSO-}d_6$





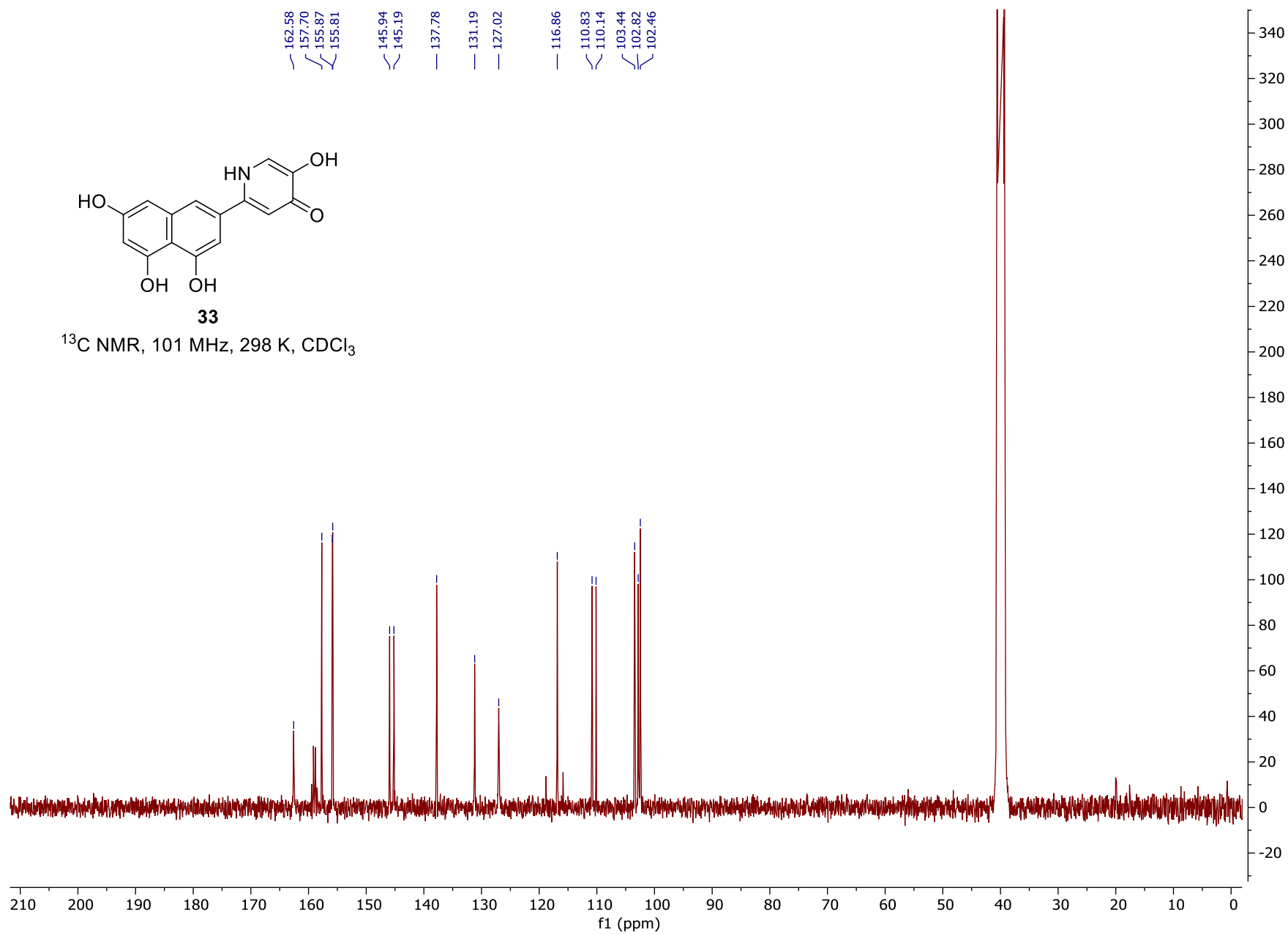


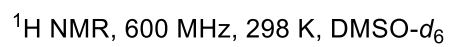


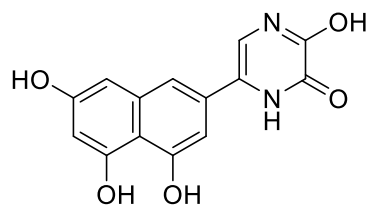


**33**

$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

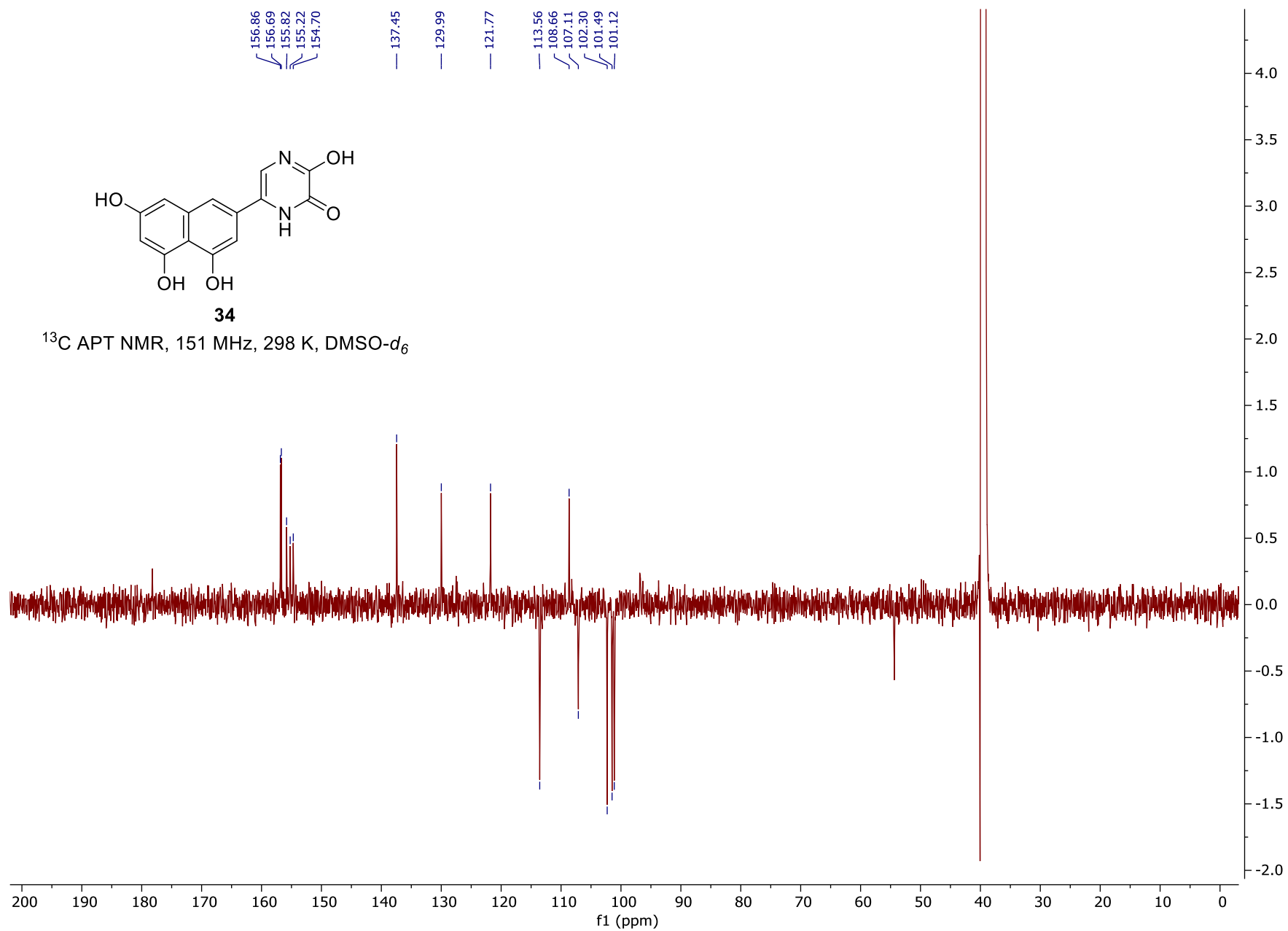


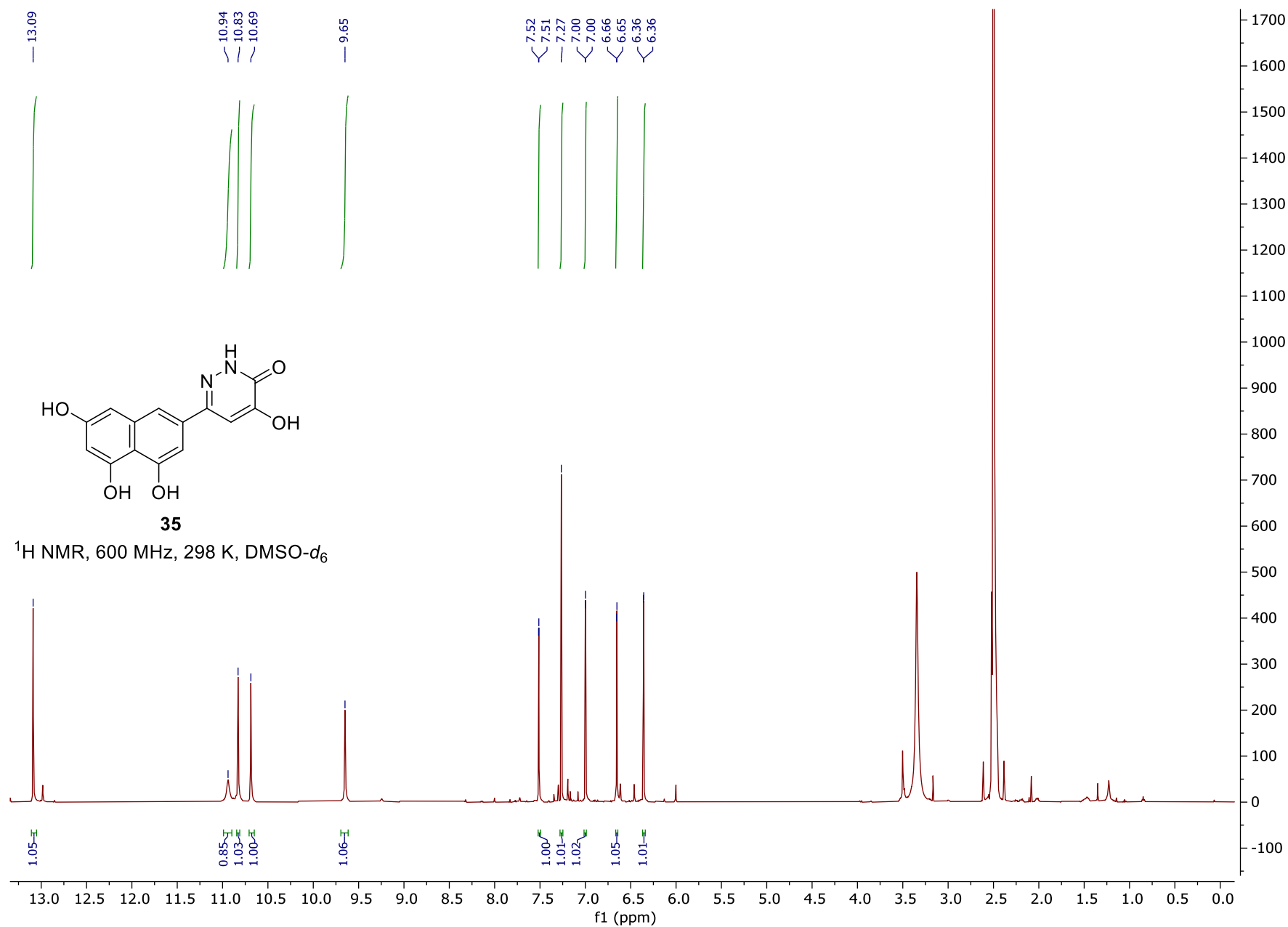


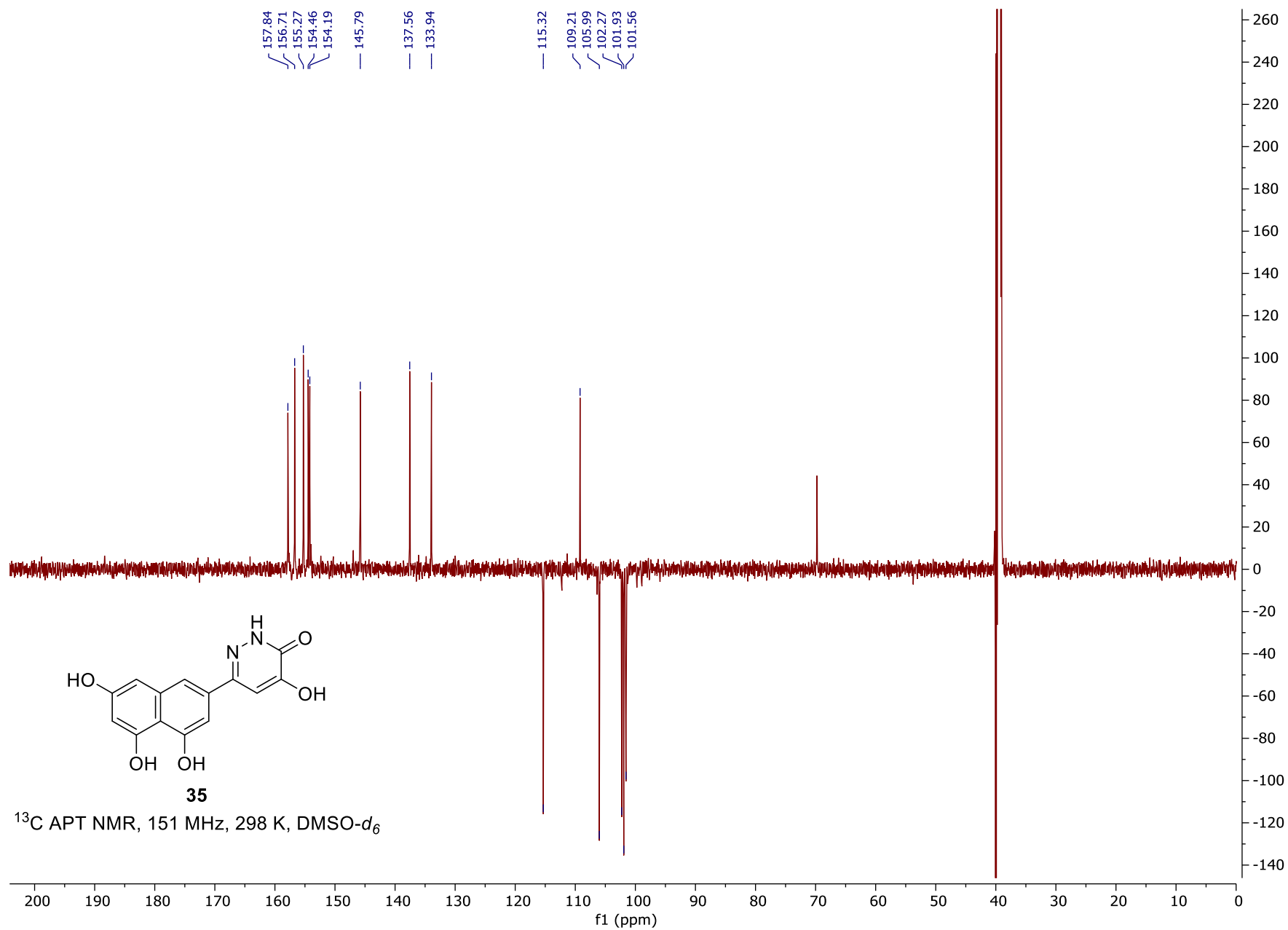


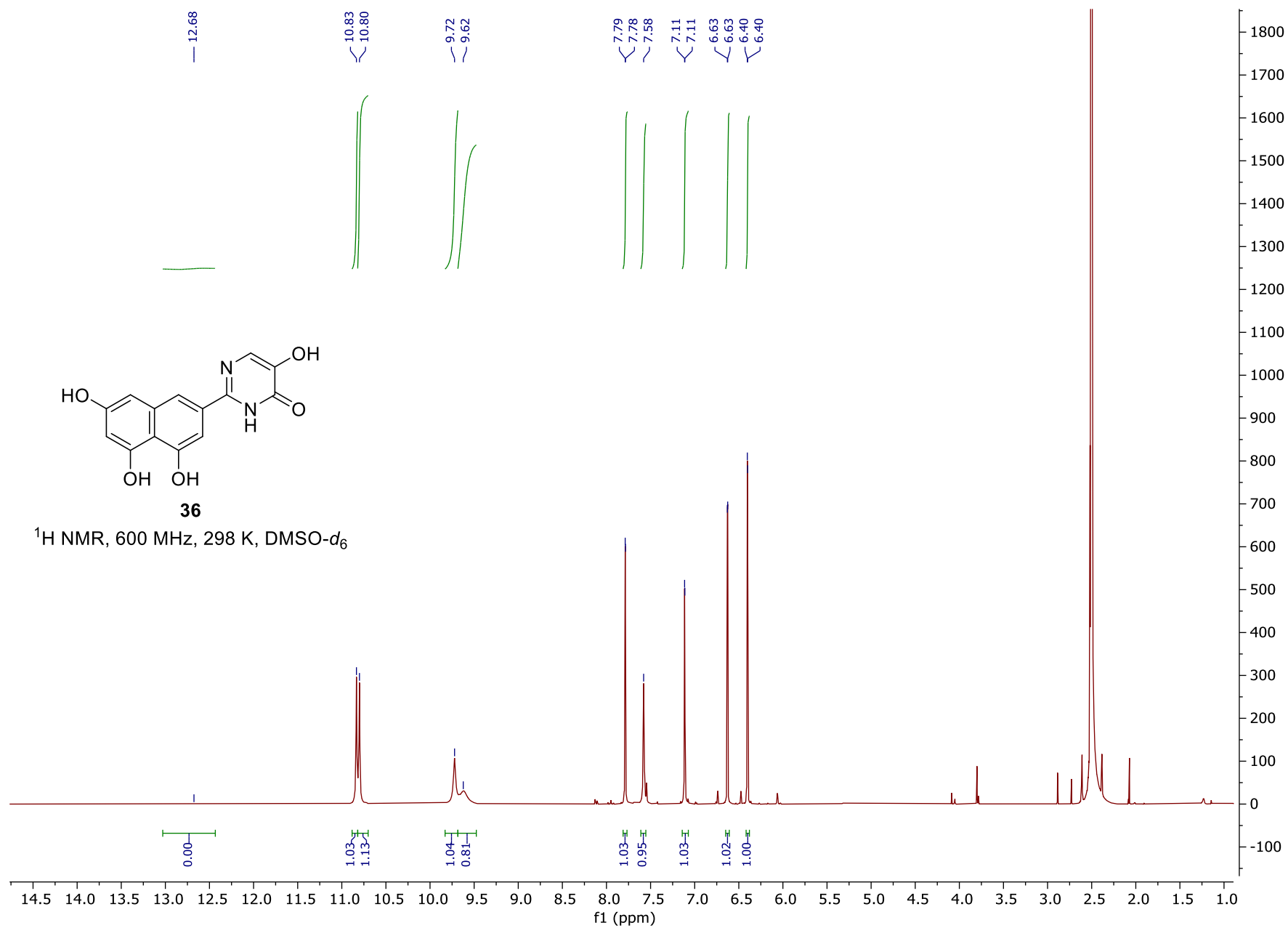
**34**

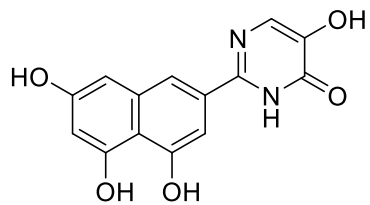
$^{13}\text{C}$  APT NMR, 151 MHz, 298 K,  $\text{DMSO-}d_6$





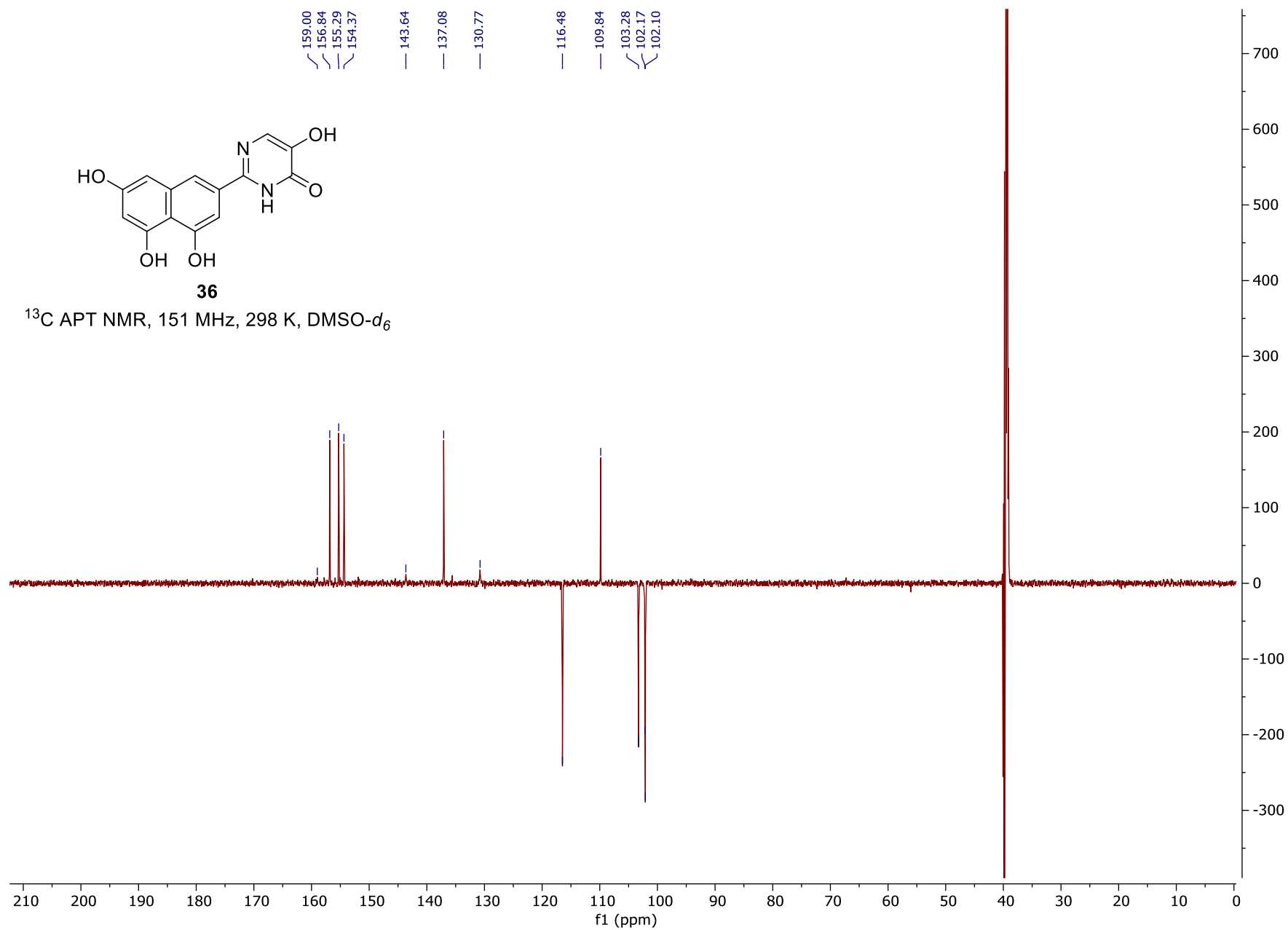


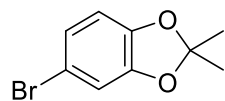




**36**

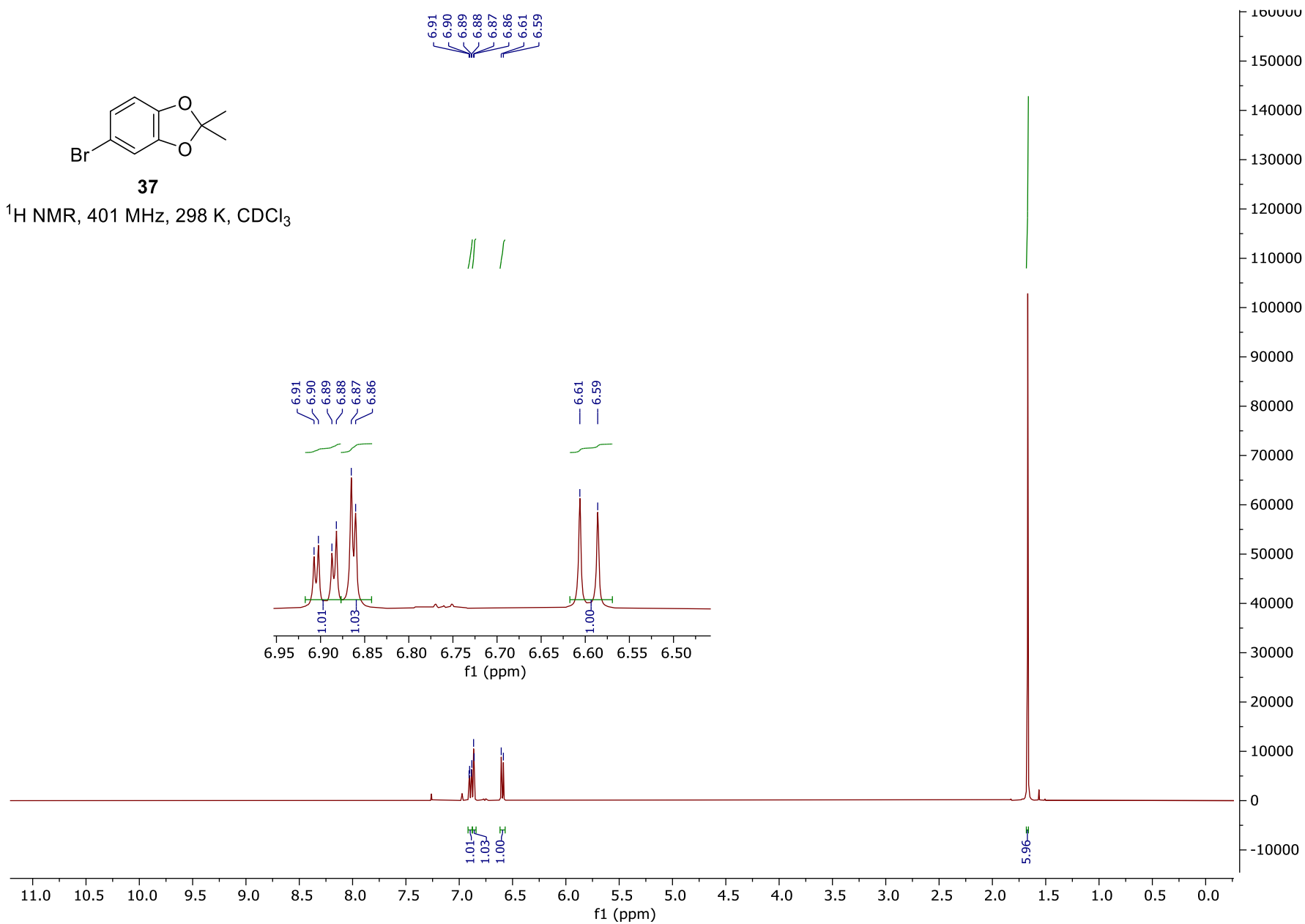
$^{13}\text{C}$  APT NMR, 151 MHz, 298 K,  $\text{DMSO}-d_6$

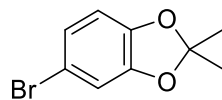




**37**

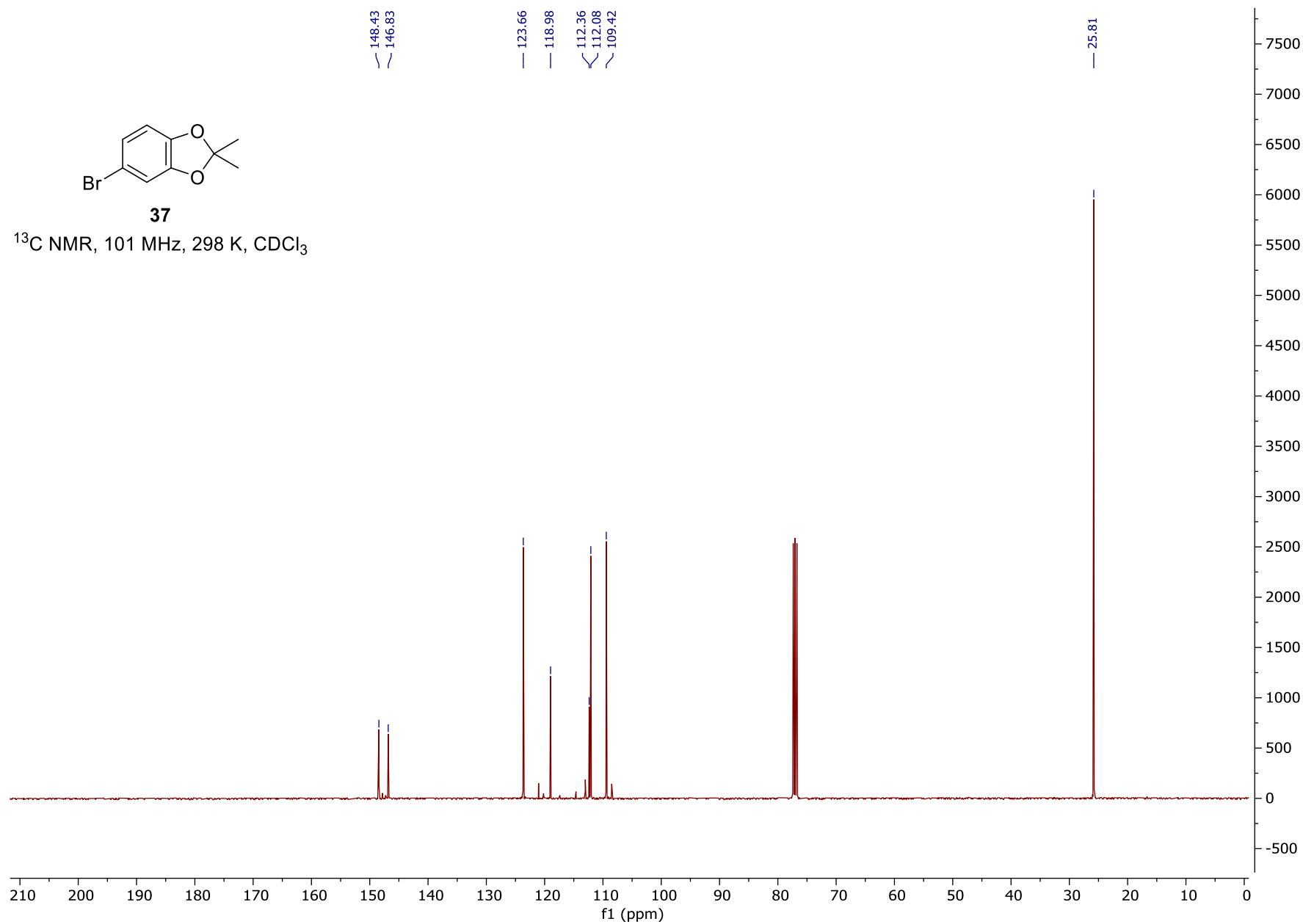
$^1\text{H}$  NMR, 401 MHz, 298 K,  $\text{CDCl}_3$

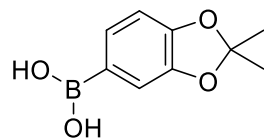




**37**

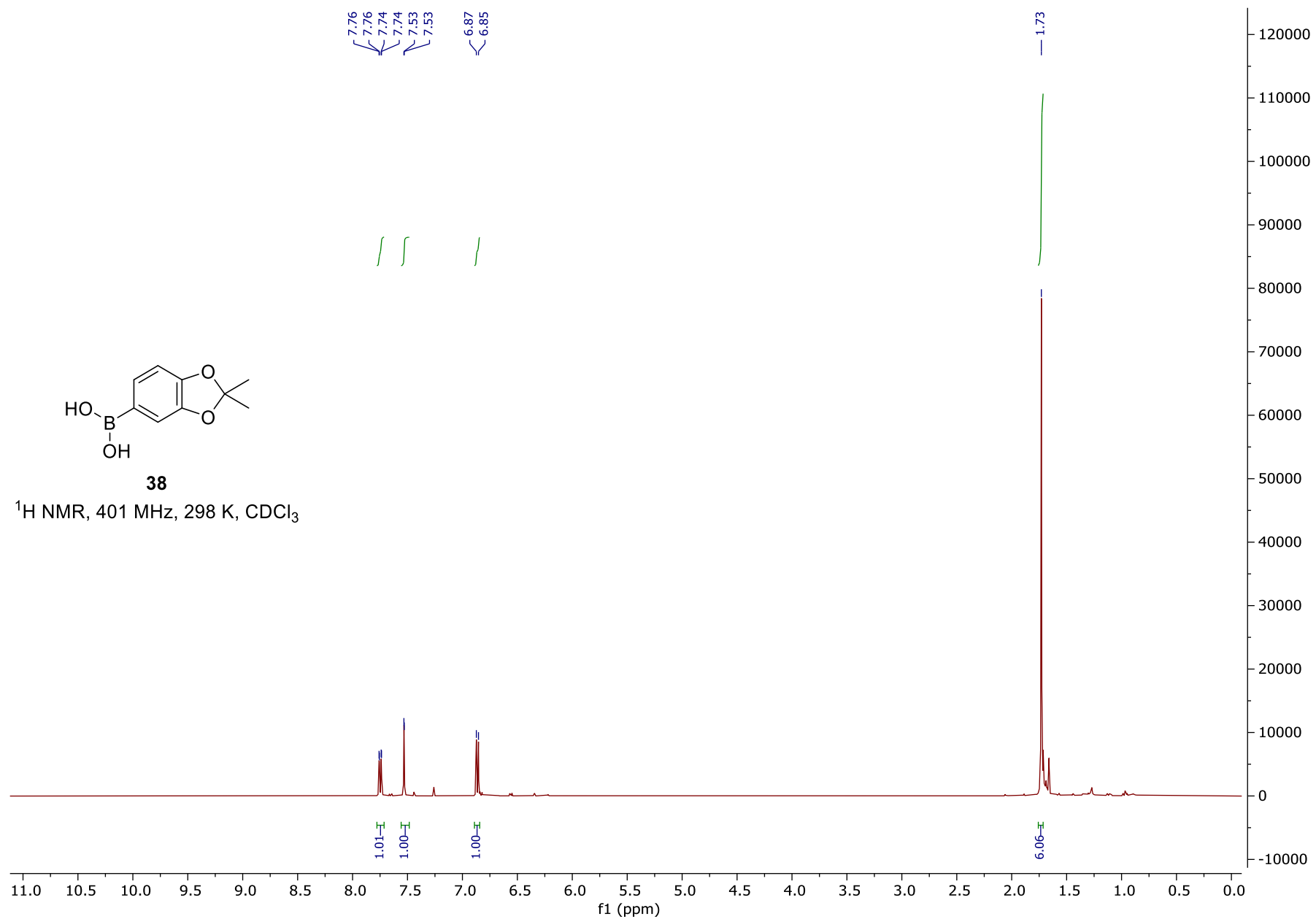
$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

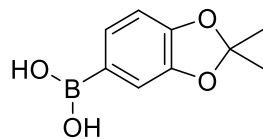




**38**

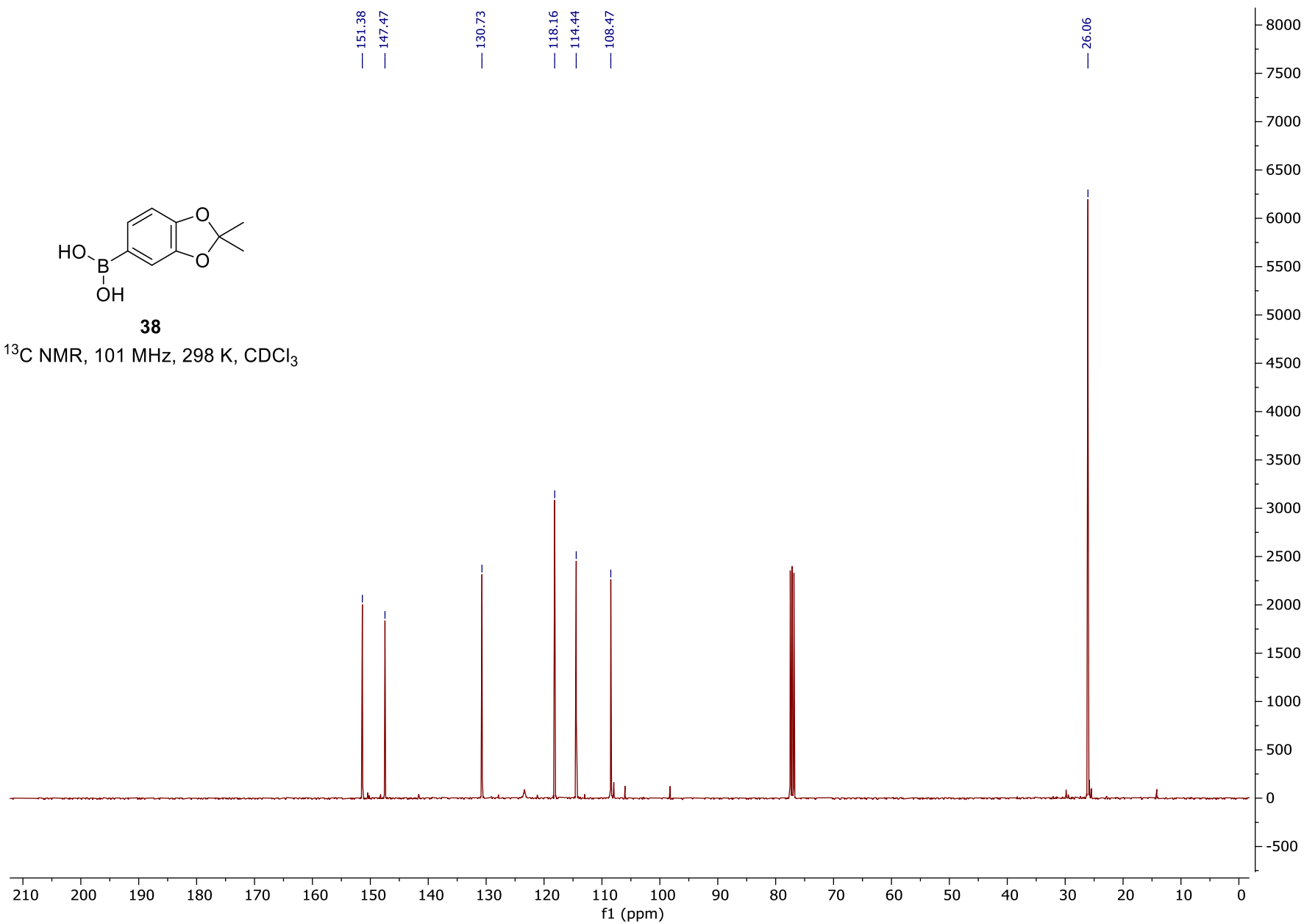
$^1\text{H}$  NMR, 401 MHz, 298 K,  $\text{CDCl}_3$

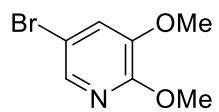




**38**

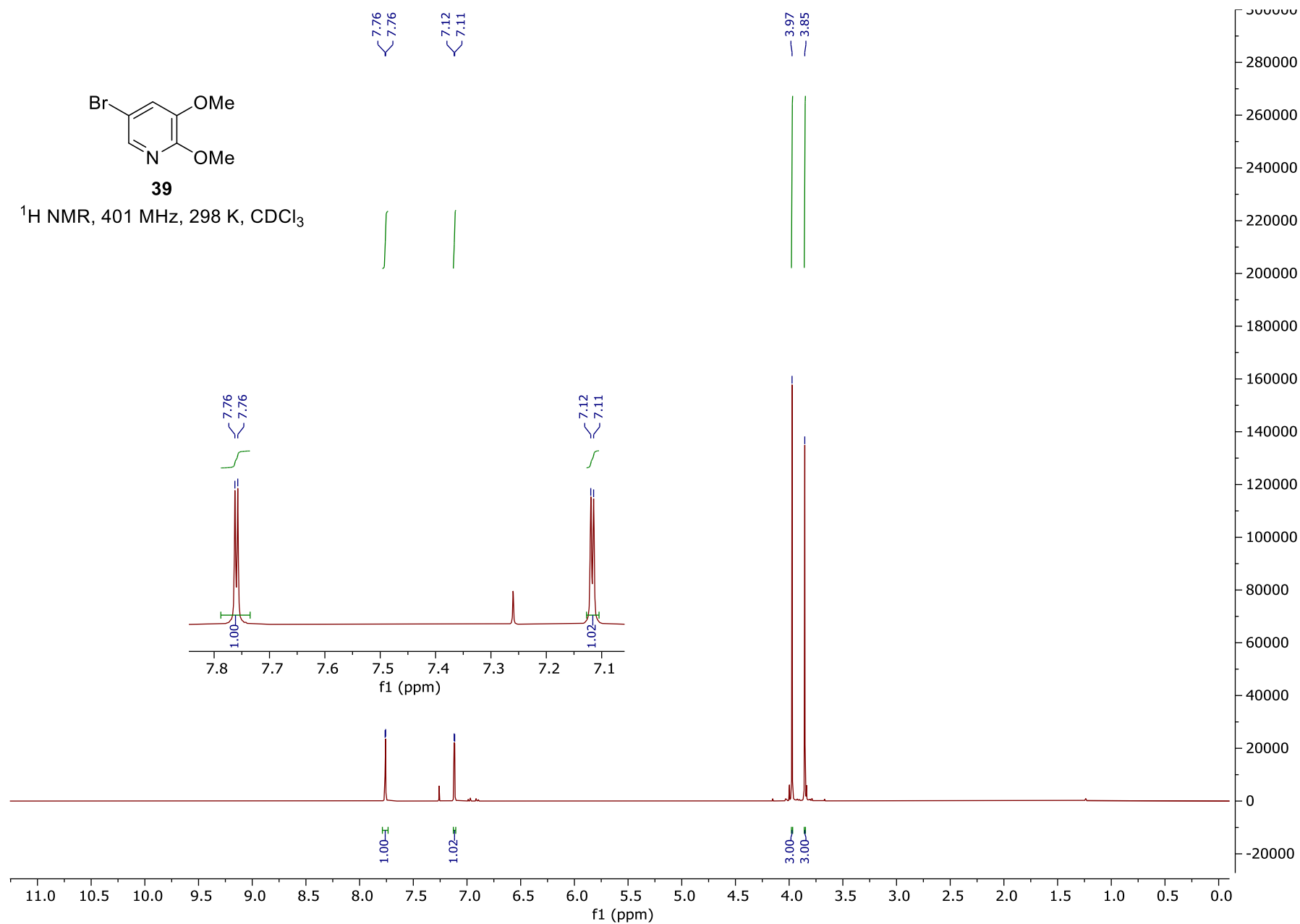
$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

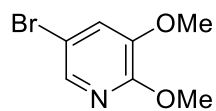




**39**

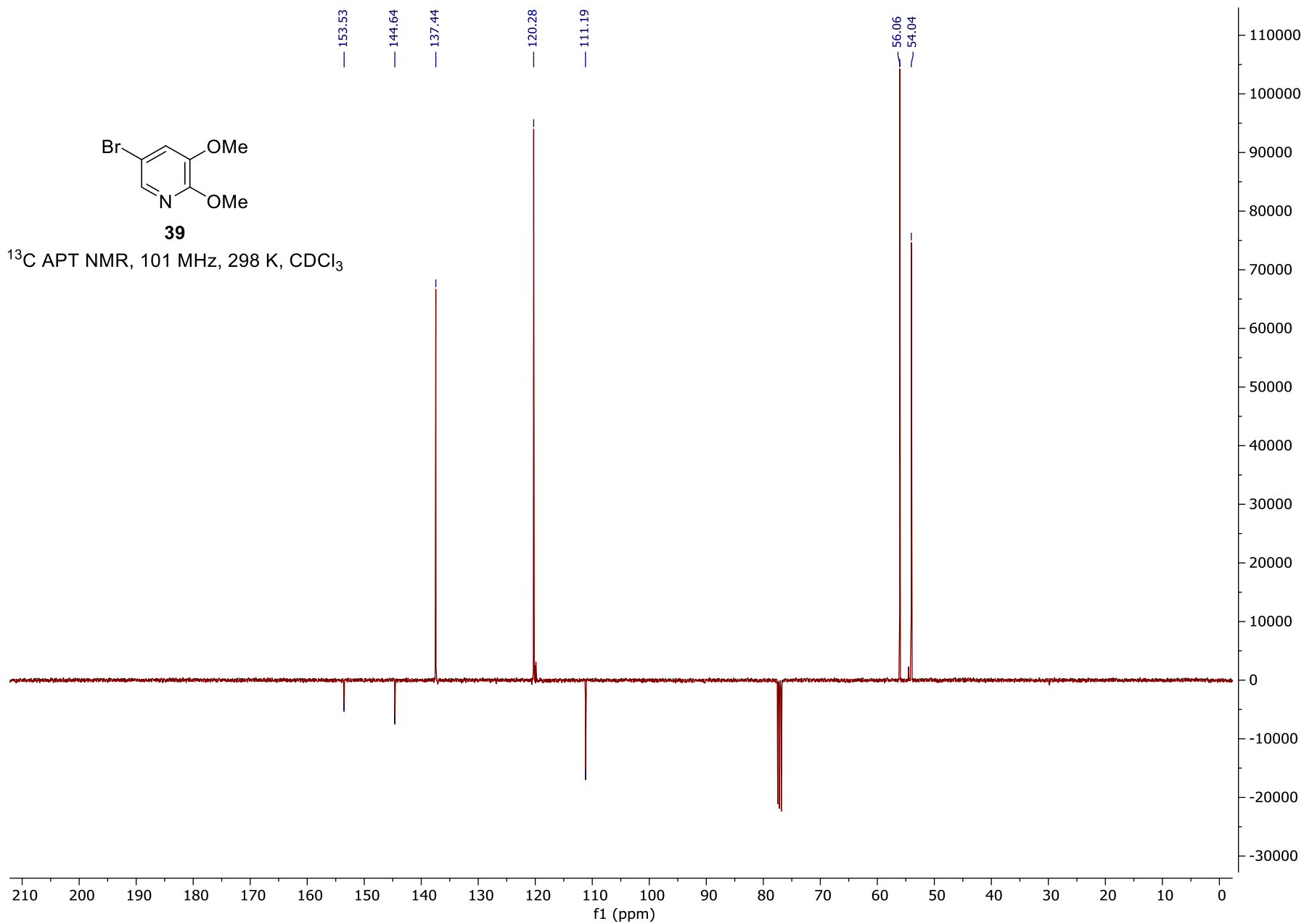
<sup>1</sup>H NMR, 401 MHz, 298 K, CDCl<sub>3</sub>

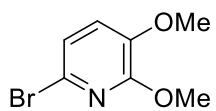




**39**

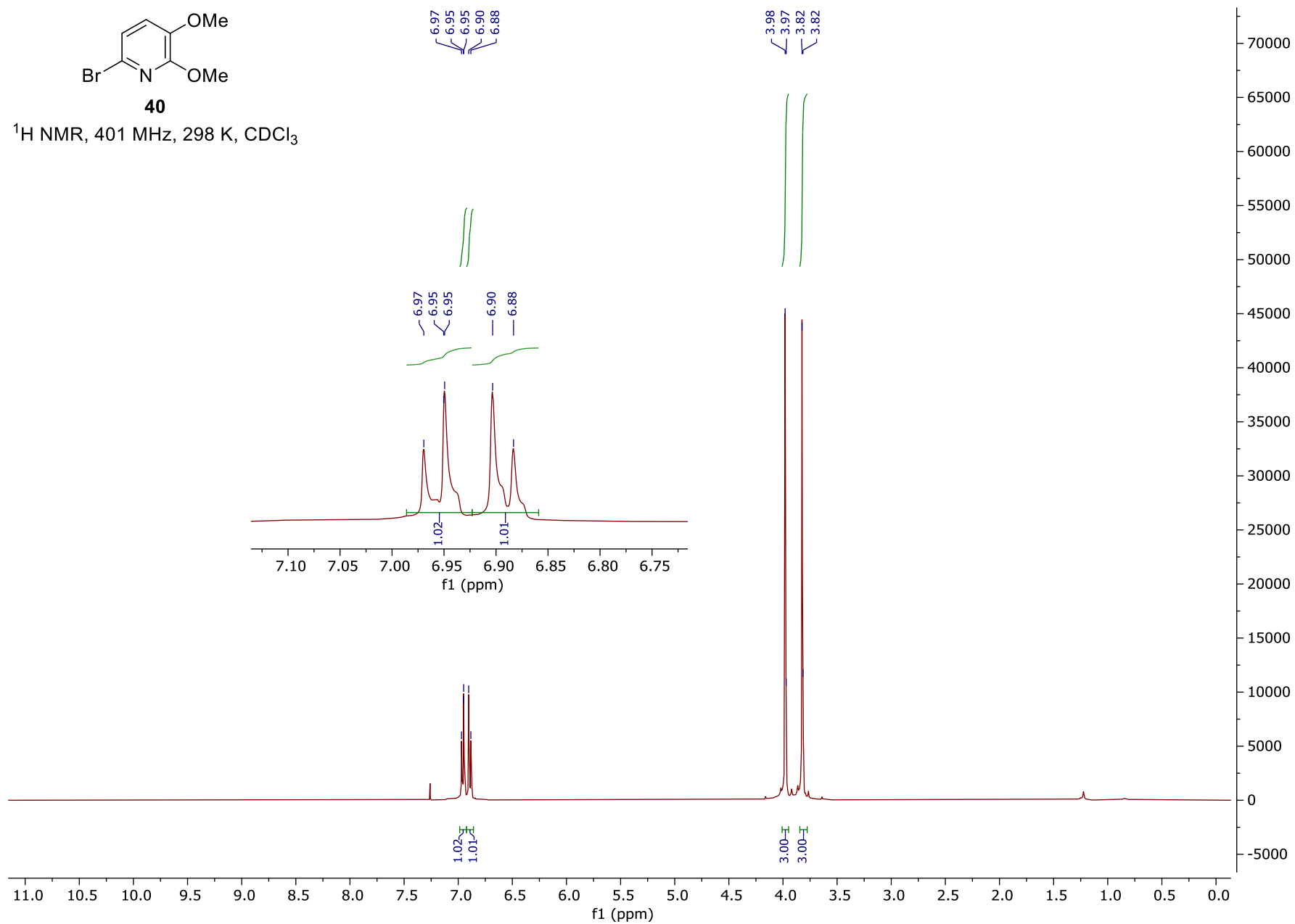
$^{13}\text{C}$  APT NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

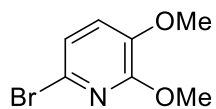




**40**

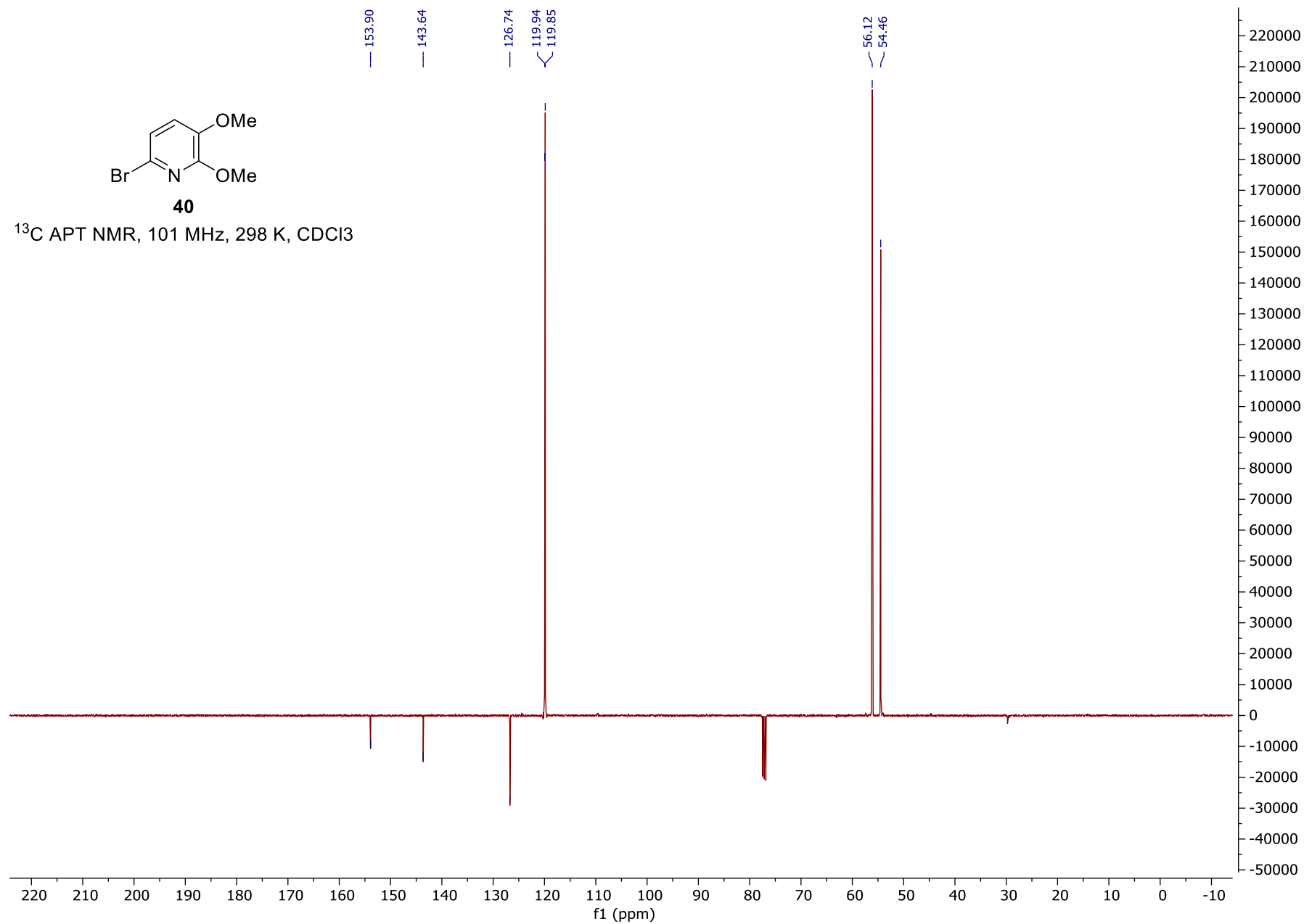
$^1\text{H}$  NMR, 401 MHz, 298 K,  $\text{CDCl}_3$

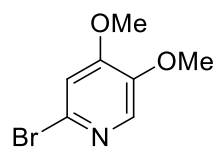




**40**

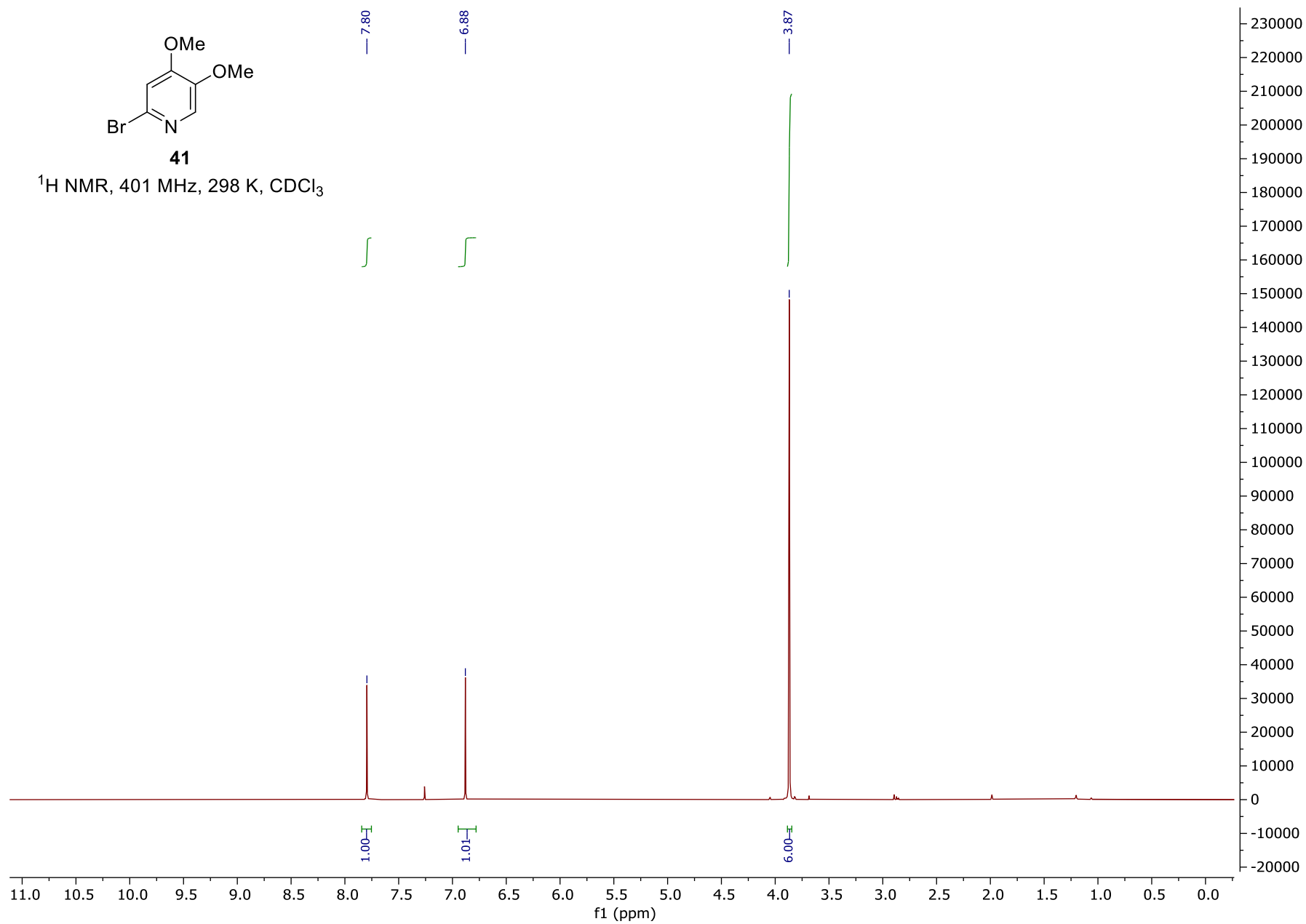
$^{13}\text{C}$  APT NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

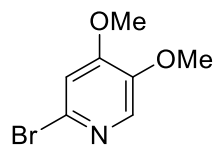




**41**

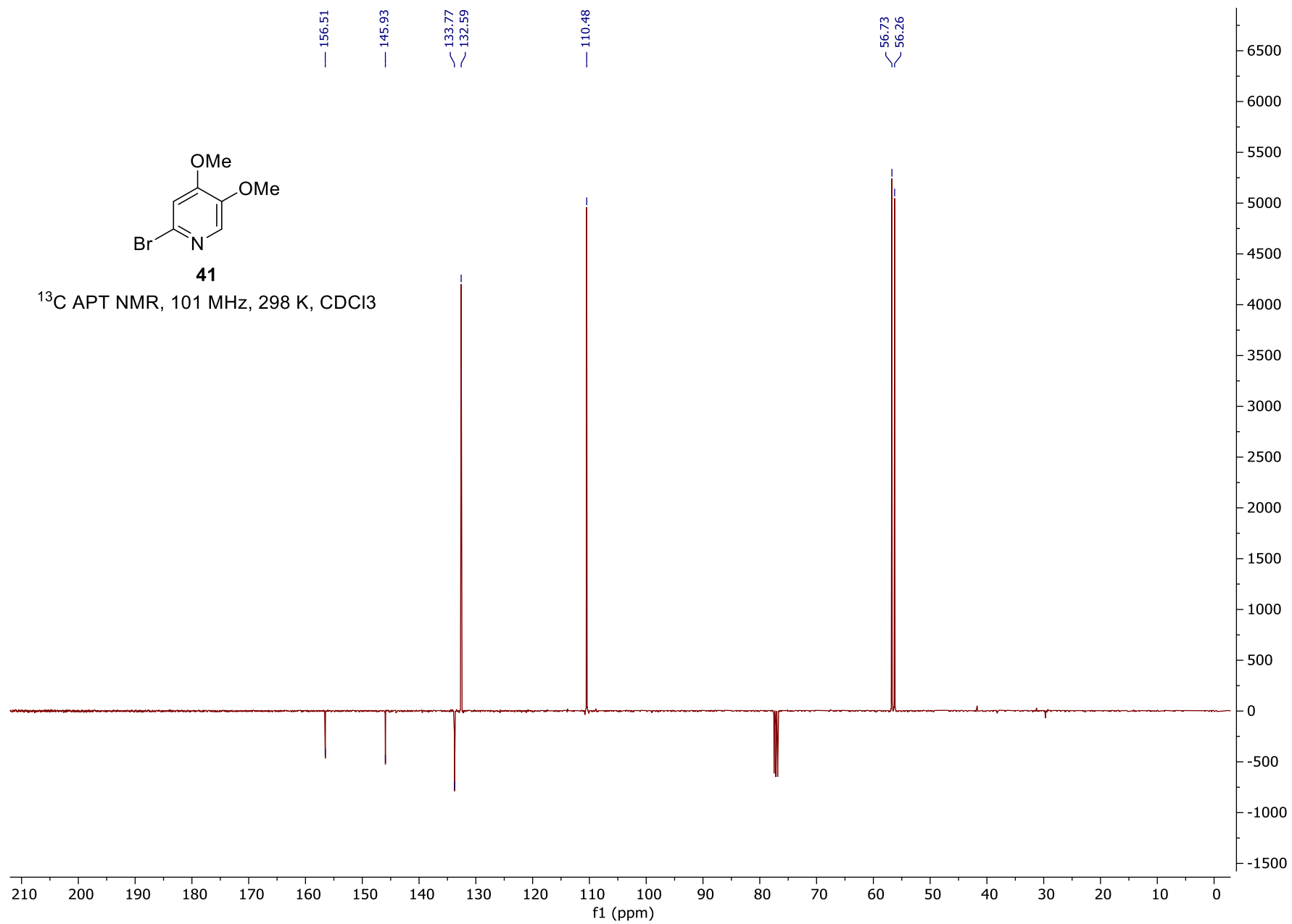
$^1\text{H}$  NMR, 401 MHz, 298 K,  $\text{CDCl}_3$

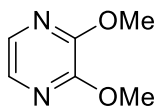




**41**

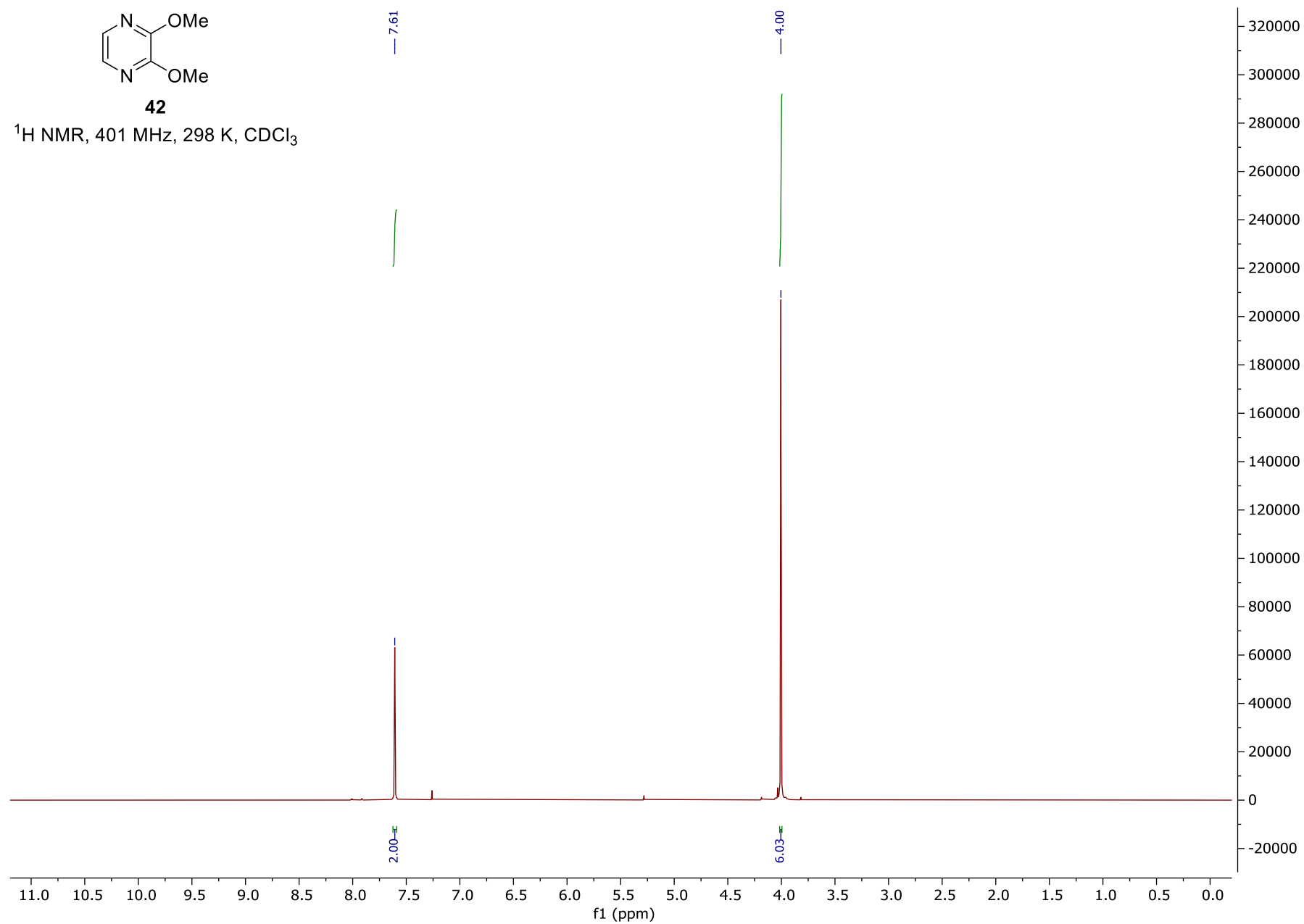
$^{13}\text{C}$  APT NMR, 101 MHz, 298 K,  $\text{CDCl}_3$



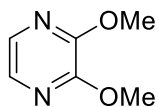


**42**

$^1\text{H}$  NMR, 401 MHz, 298 K,  $\text{CDCl}_3$

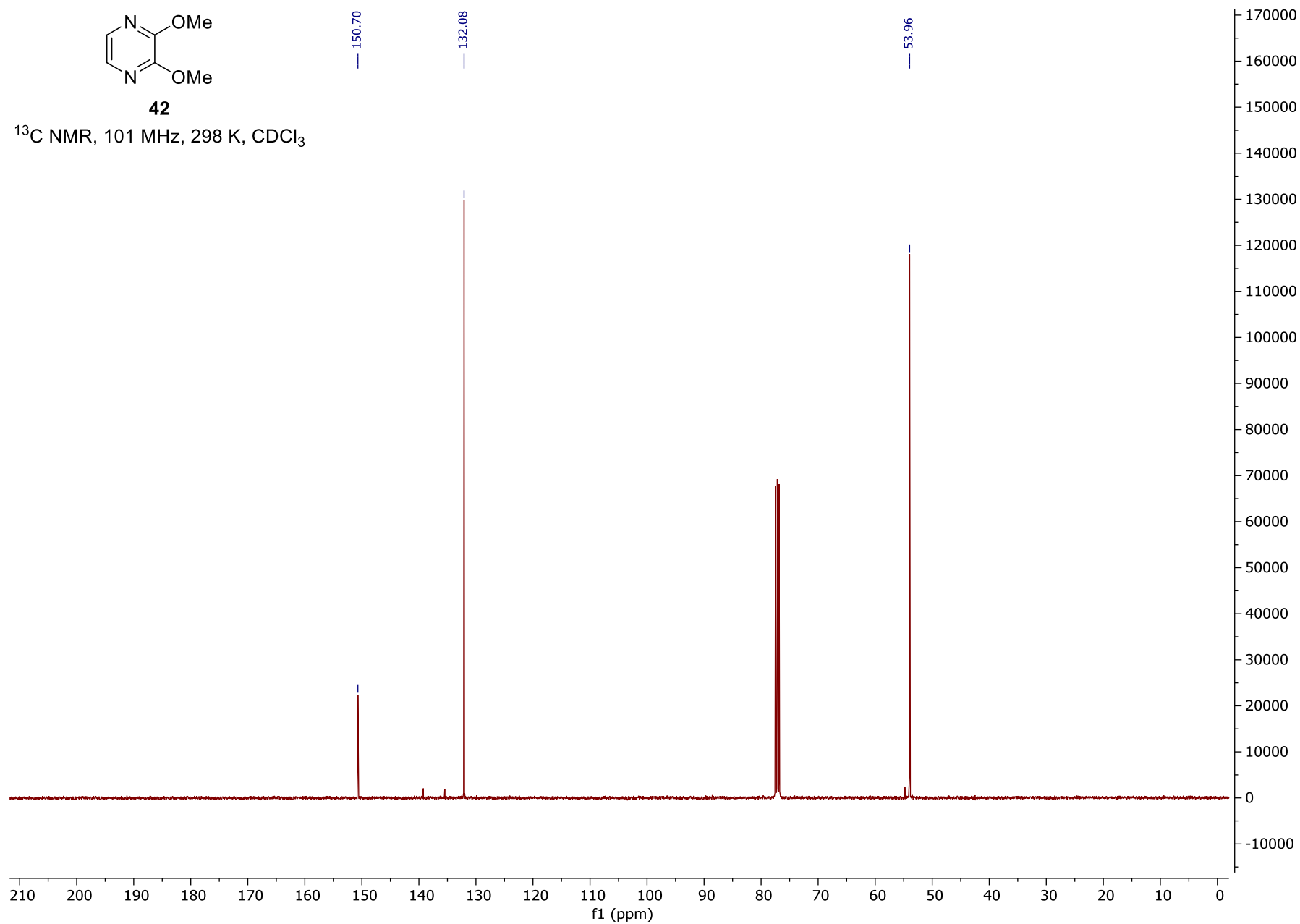


S89

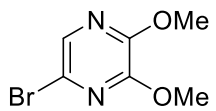


**42**

$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

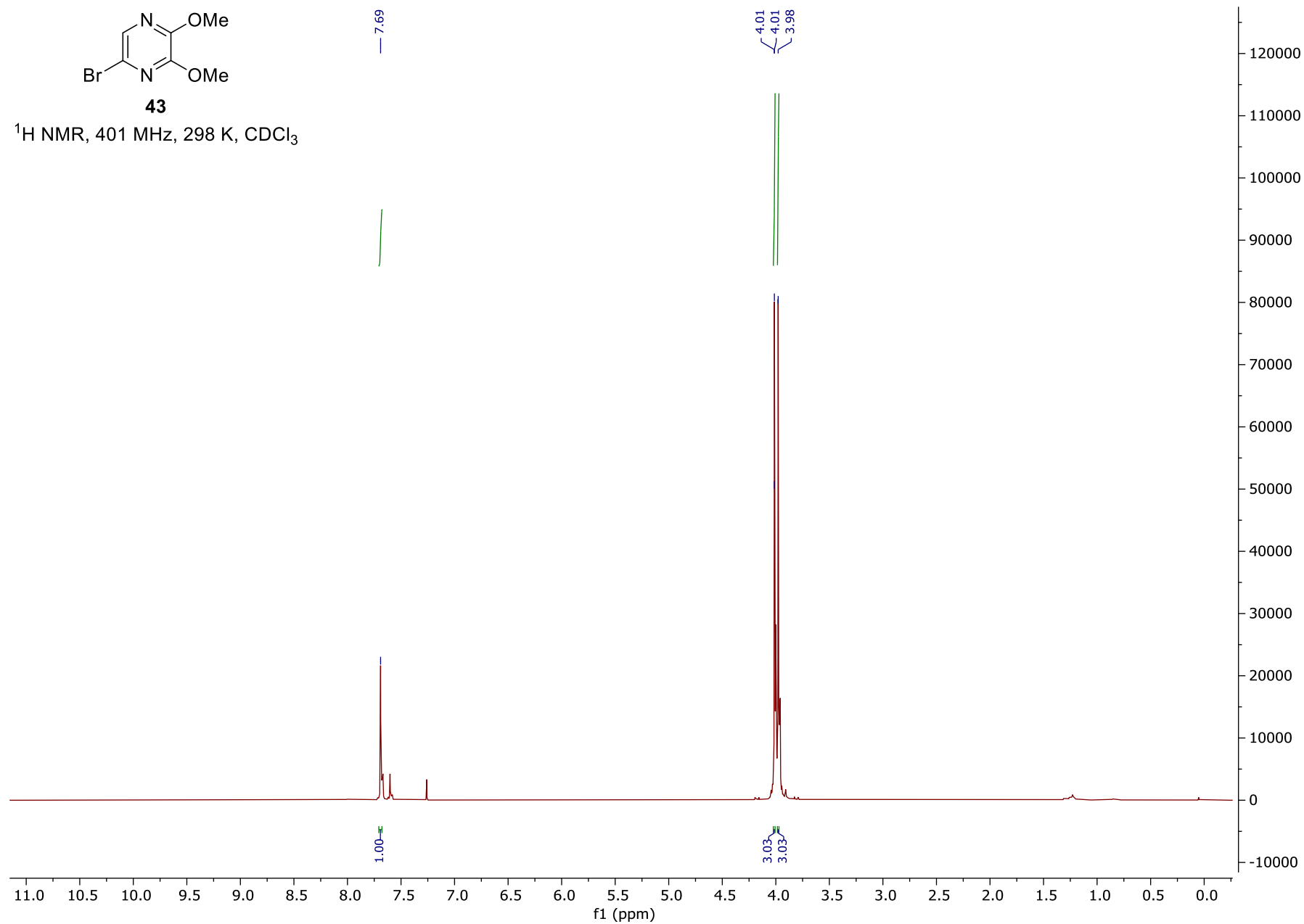


S90

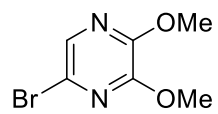


**43**

$^1\text{H}$  NMR, 401 MHz, 298 K,  $\text{CDCl}_3$

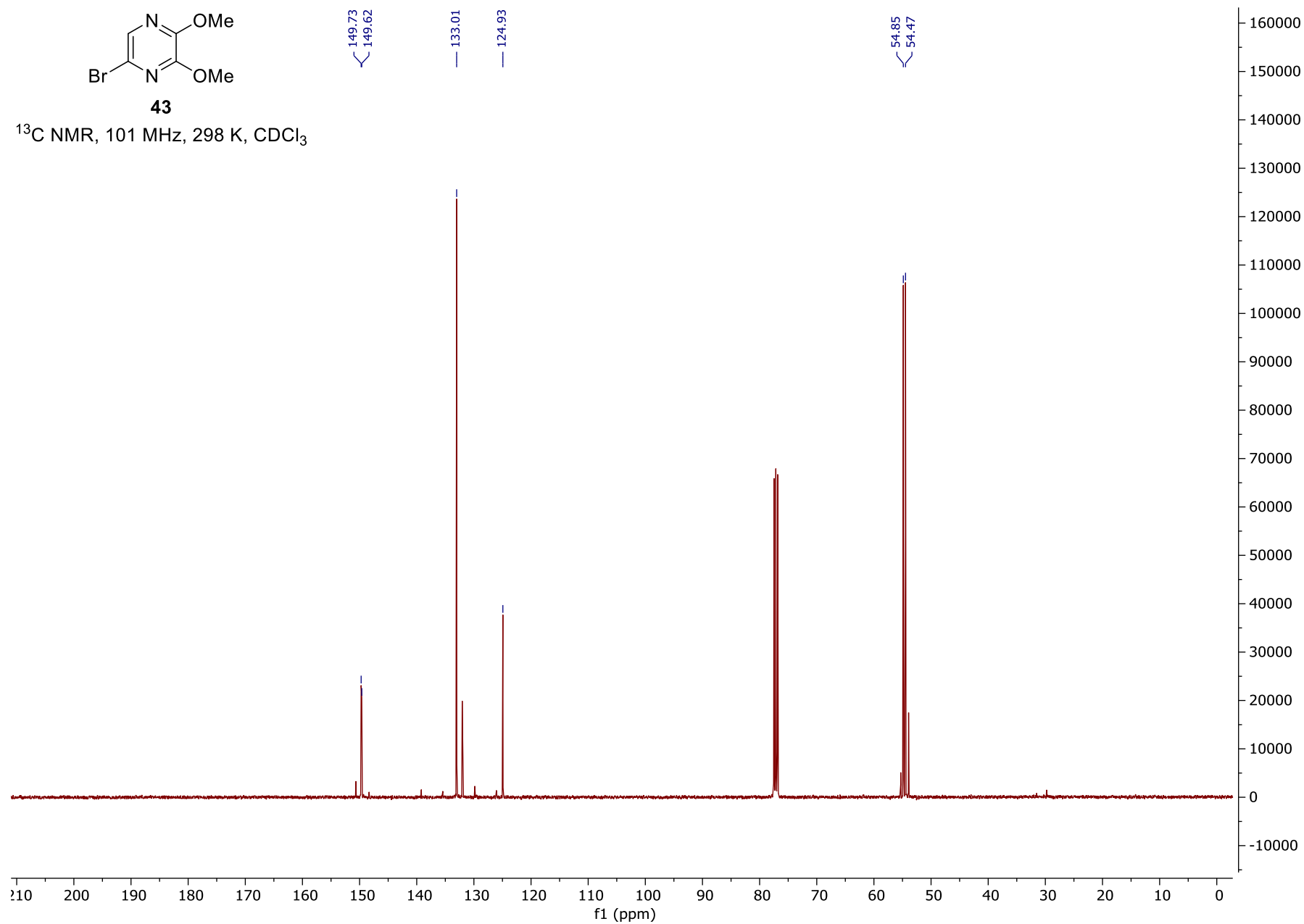


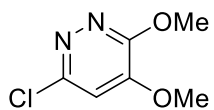
S91



**43**

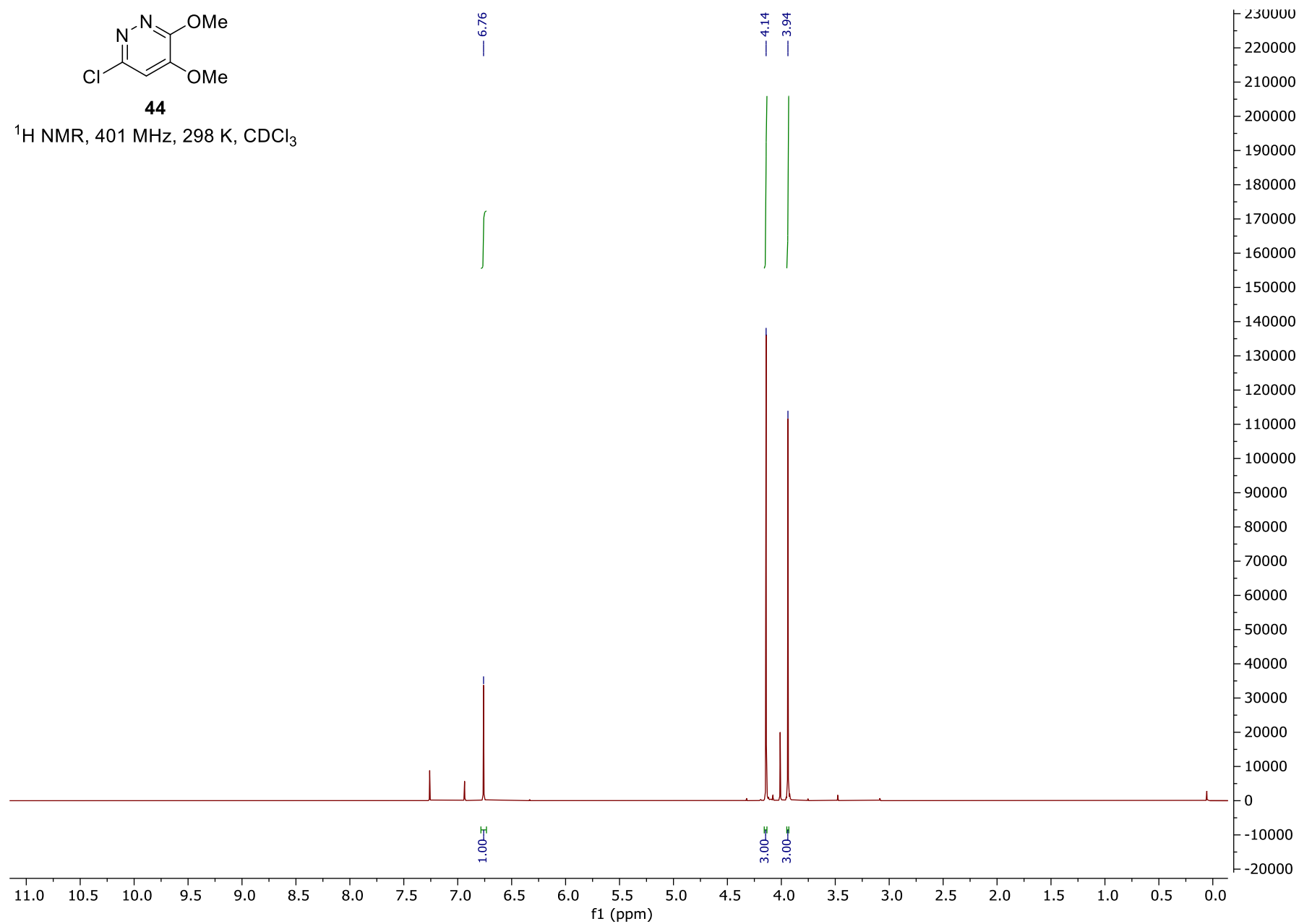
$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$



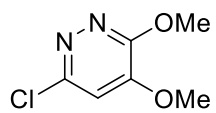


**44**

$^1\text{H}$  NMR, 401 MHz, 298 K,  $\text{CDCl}_3$

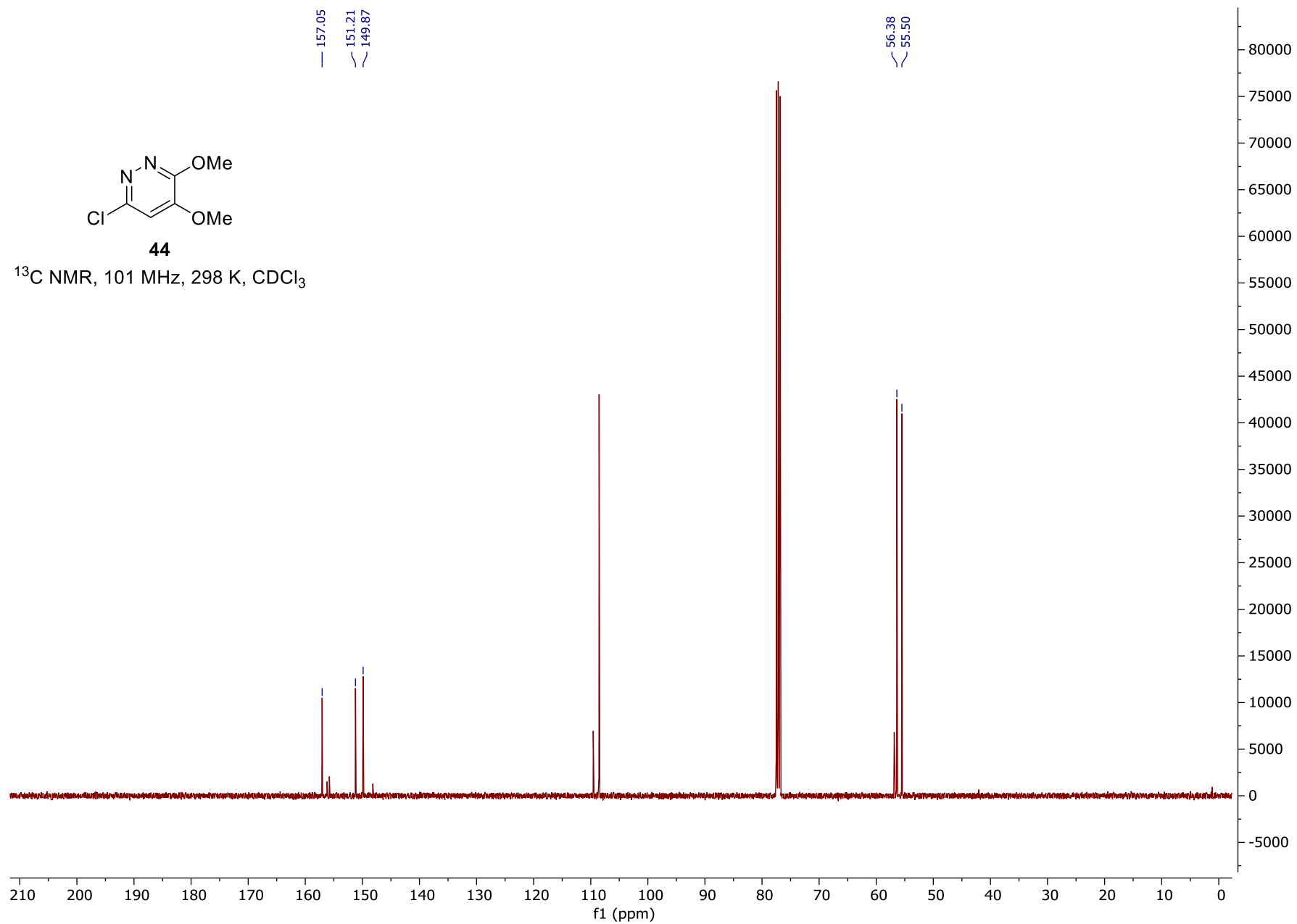


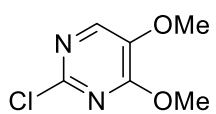
S93



**44**

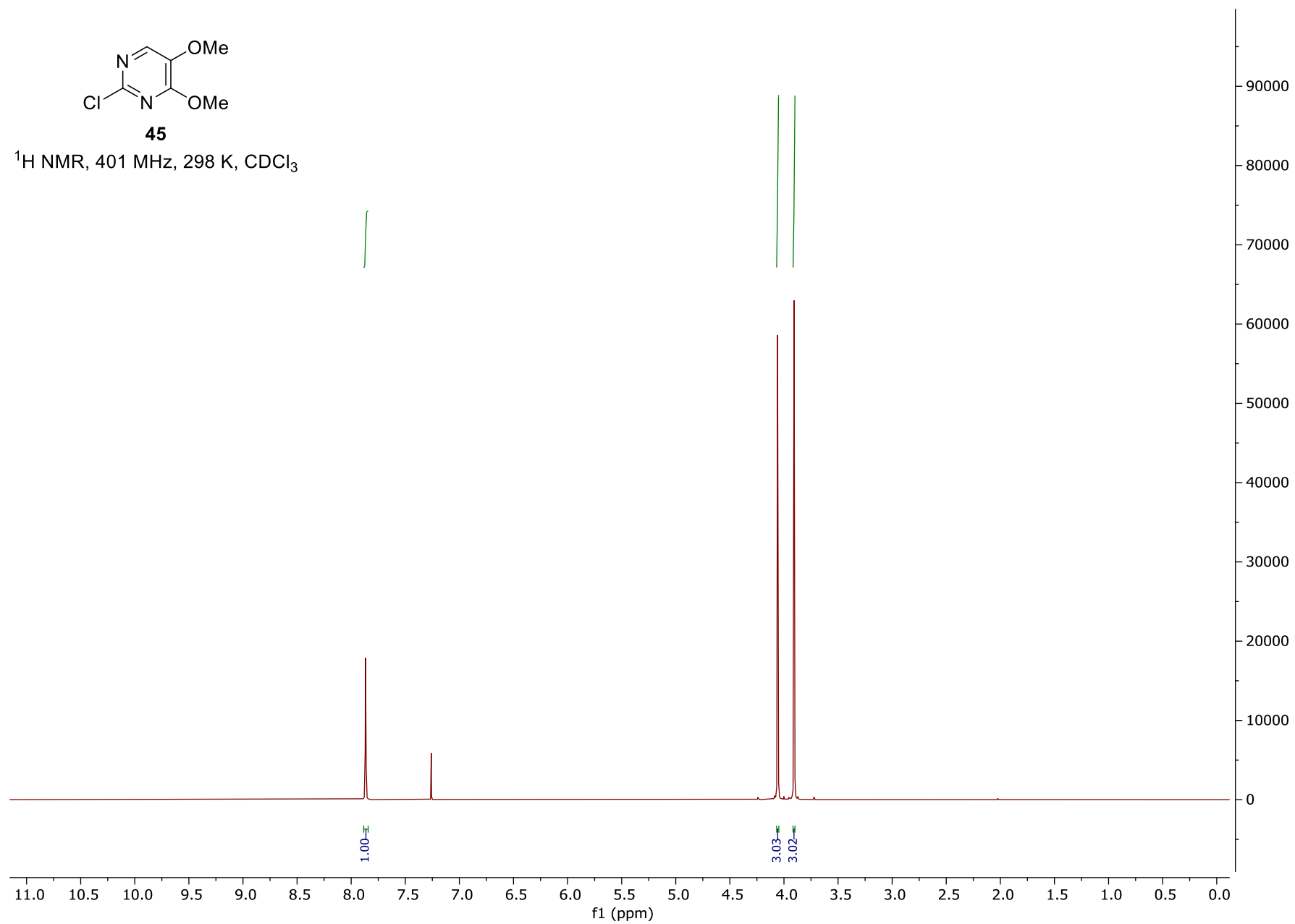
$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$



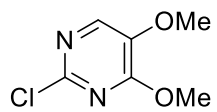


**45**

$^1\text{H}$  NMR, 401 MHz, 298 K,  $\text{CDCl}_3$

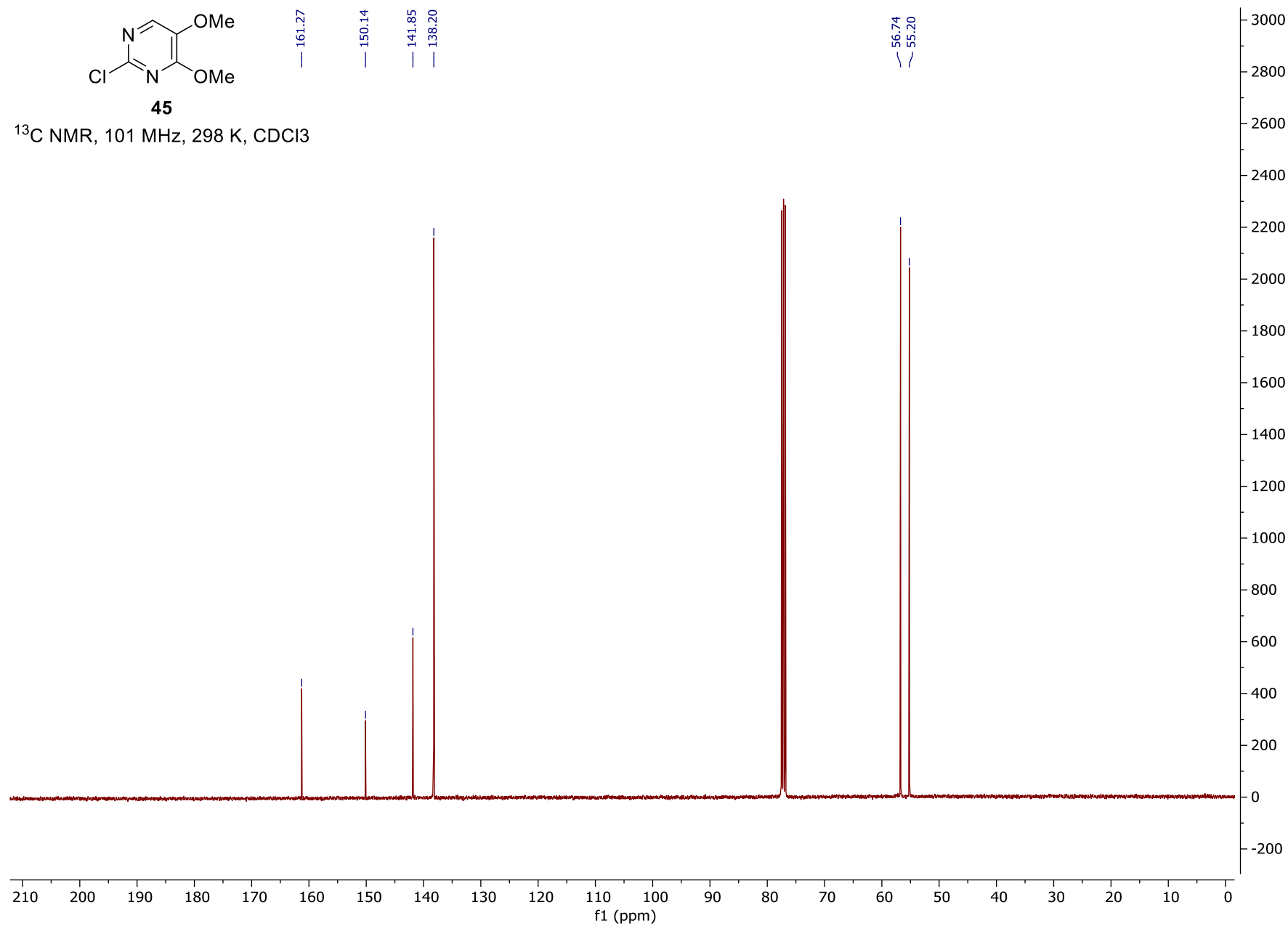


S95

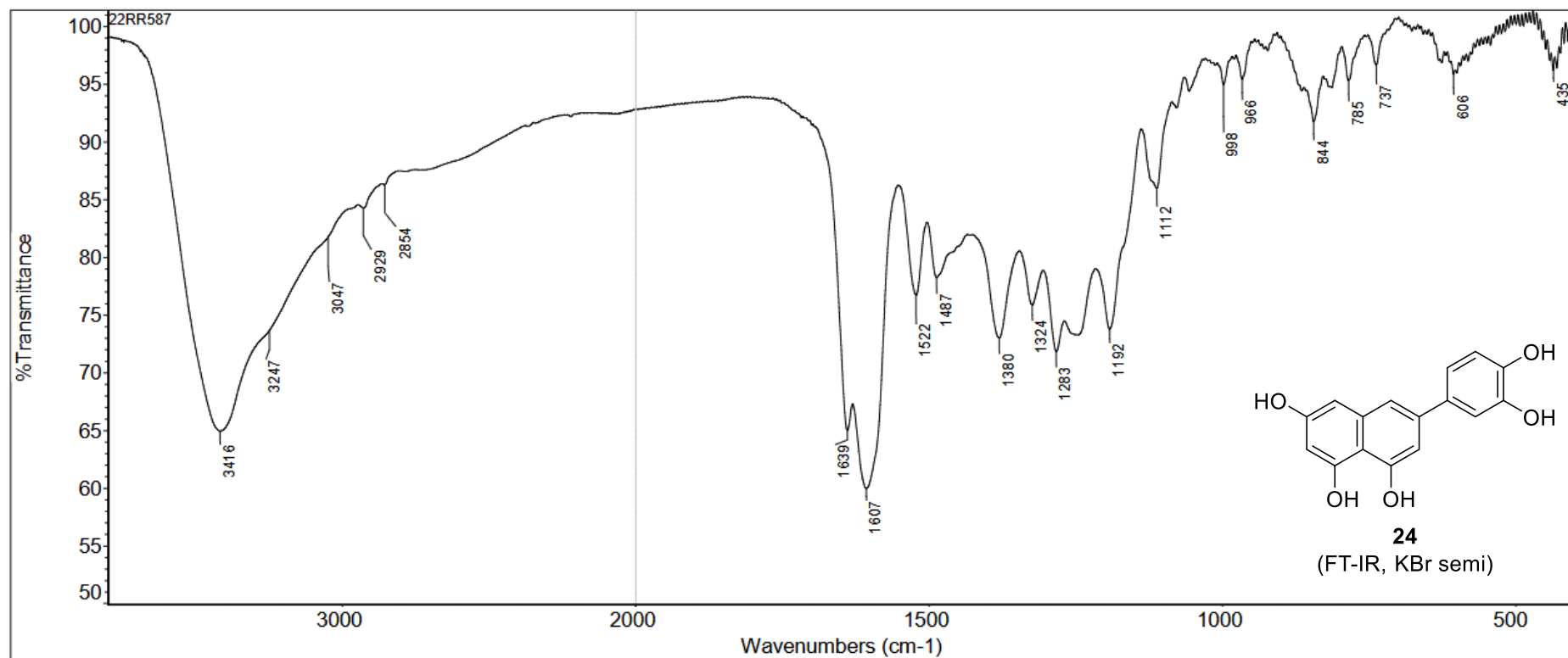


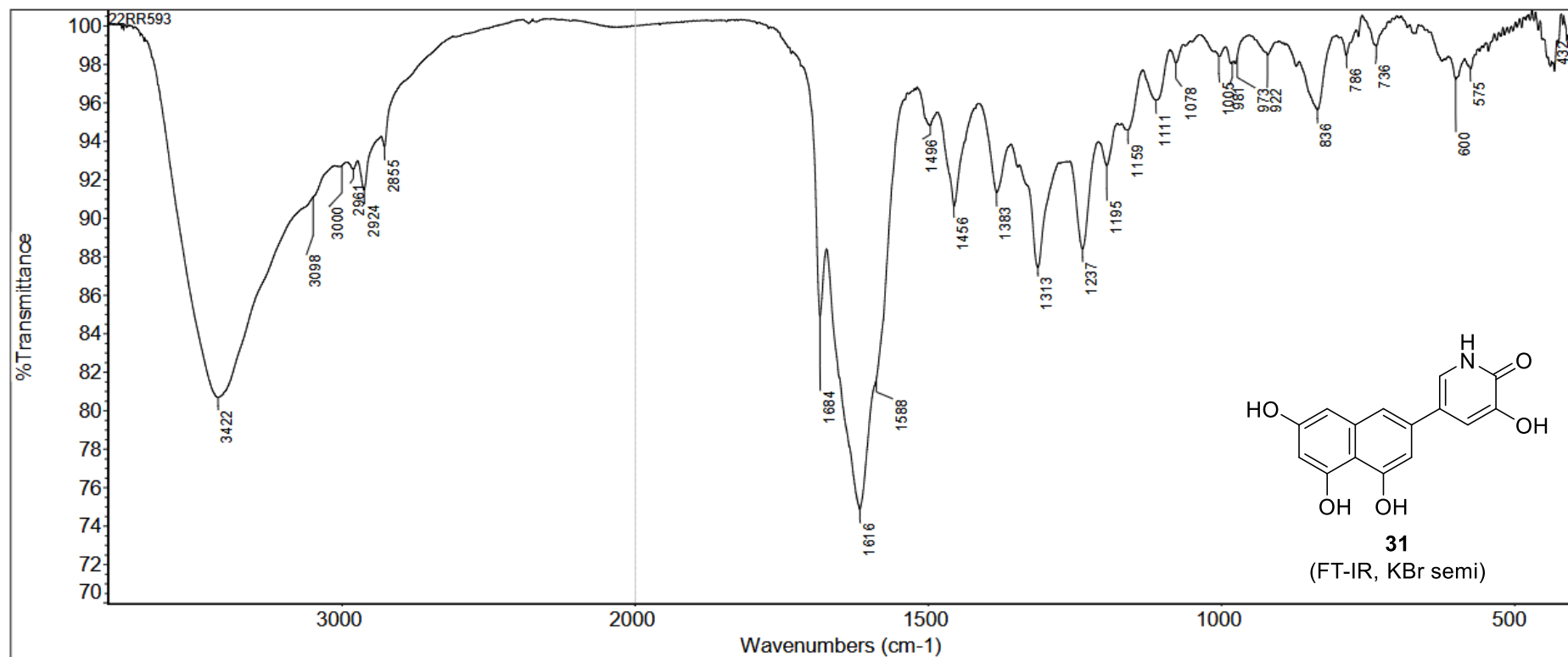
**45**

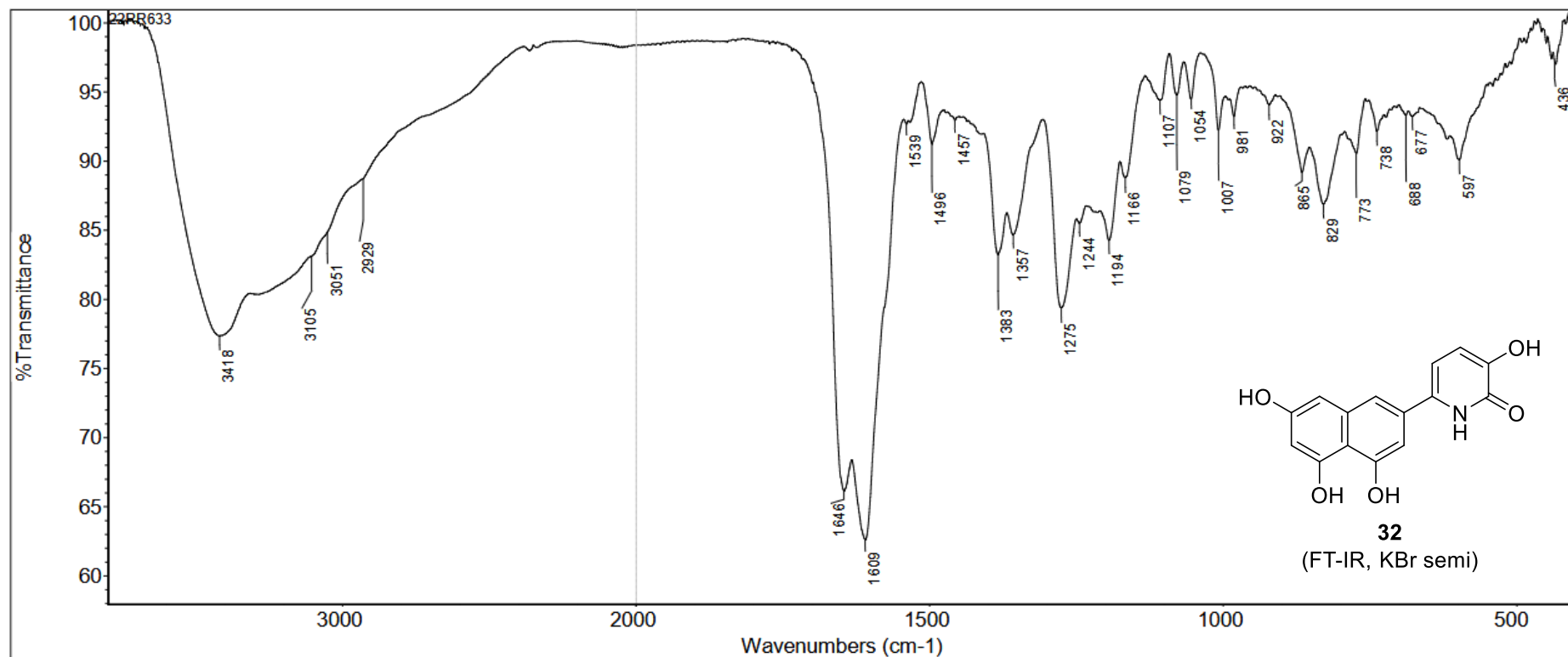
$^{13}\text{C}$  NMR, 101 MHz, 298 K,  $\text{CDCl}_3$

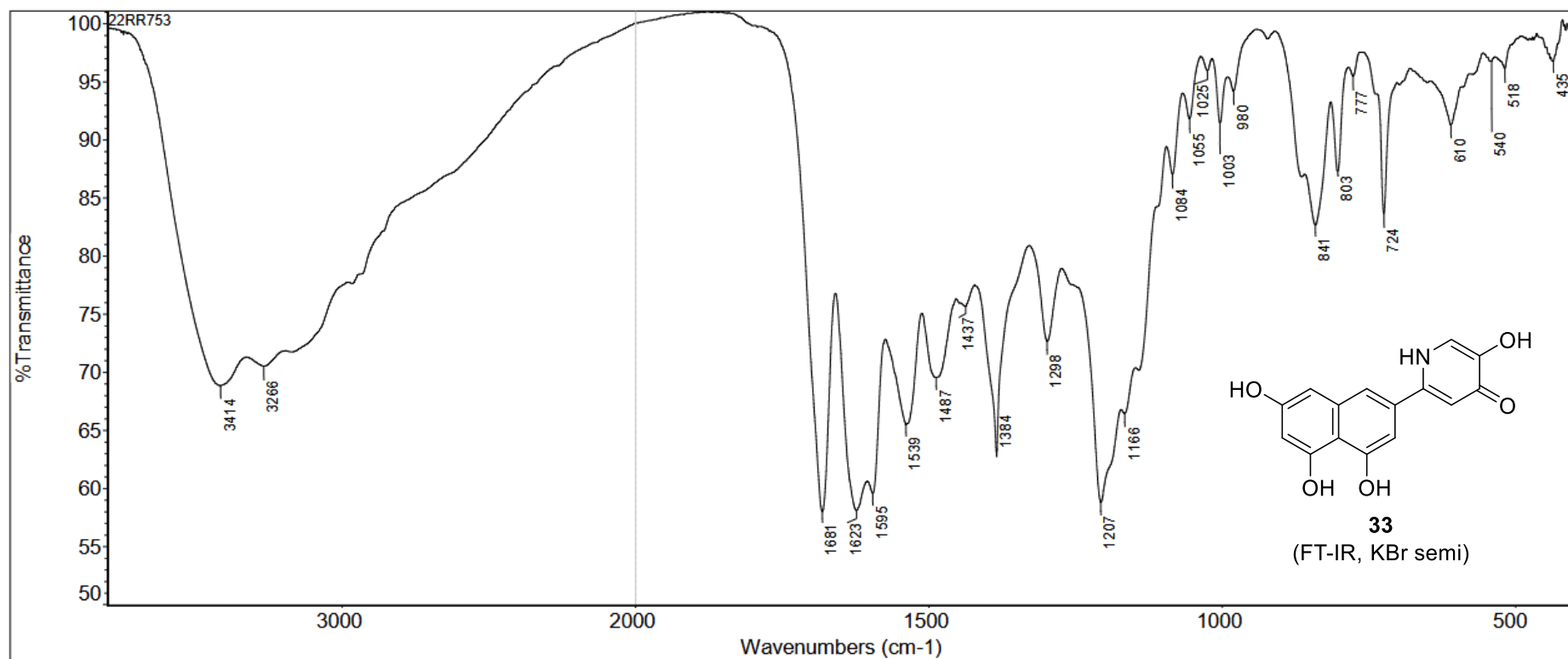


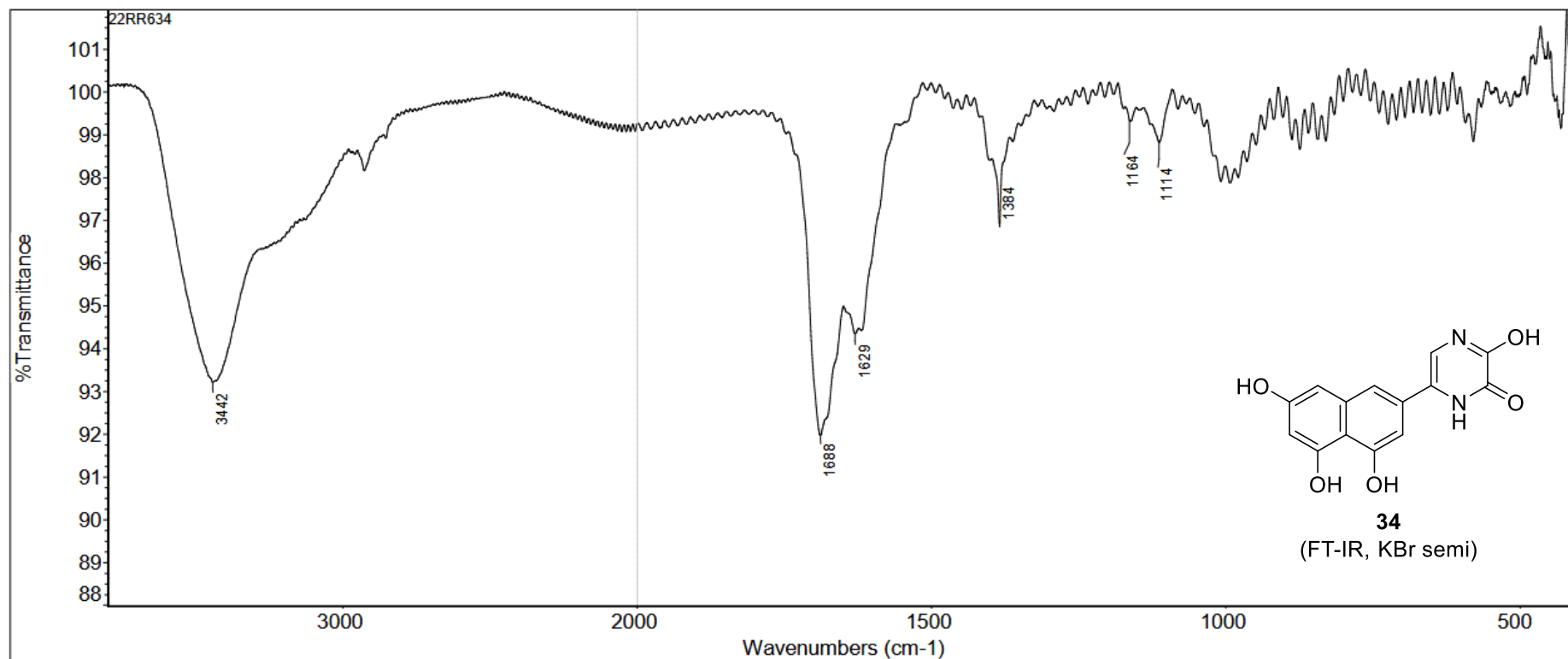
## IR spectra of pseudoflavonoids

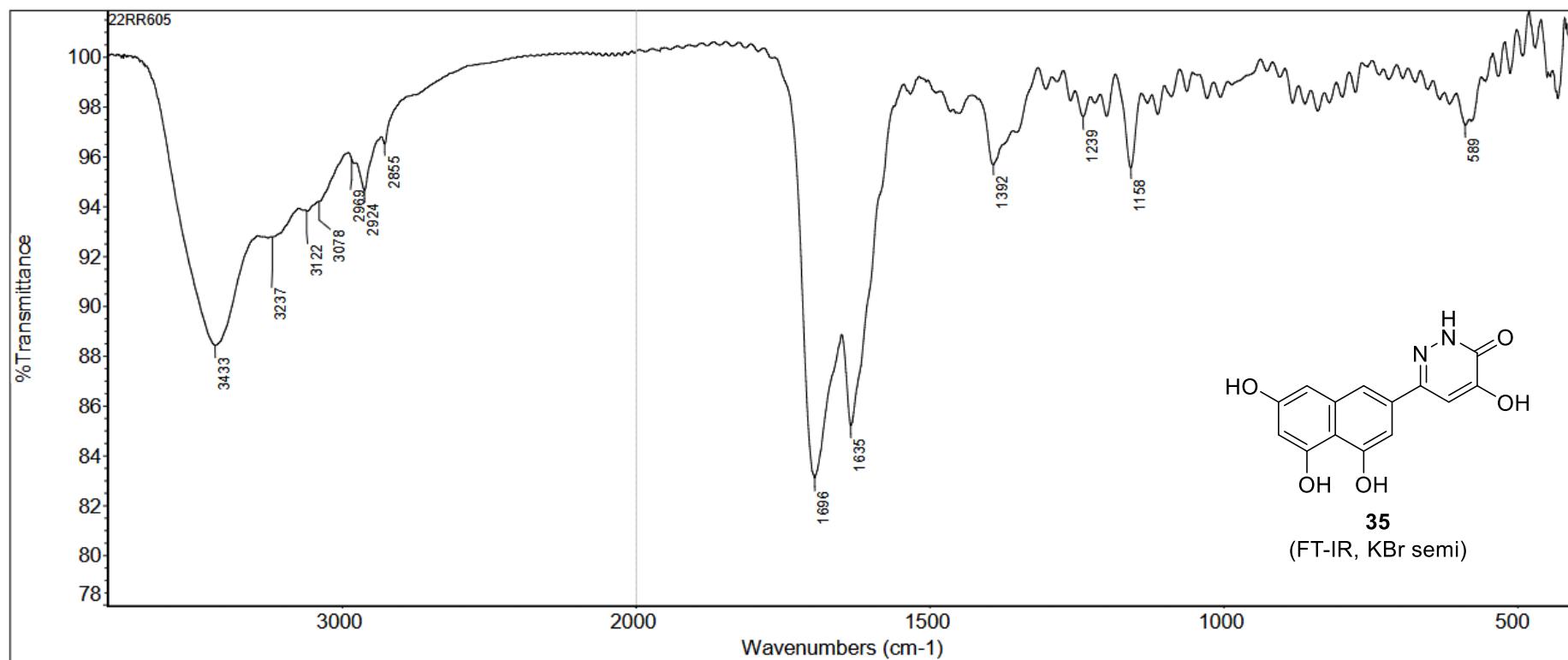


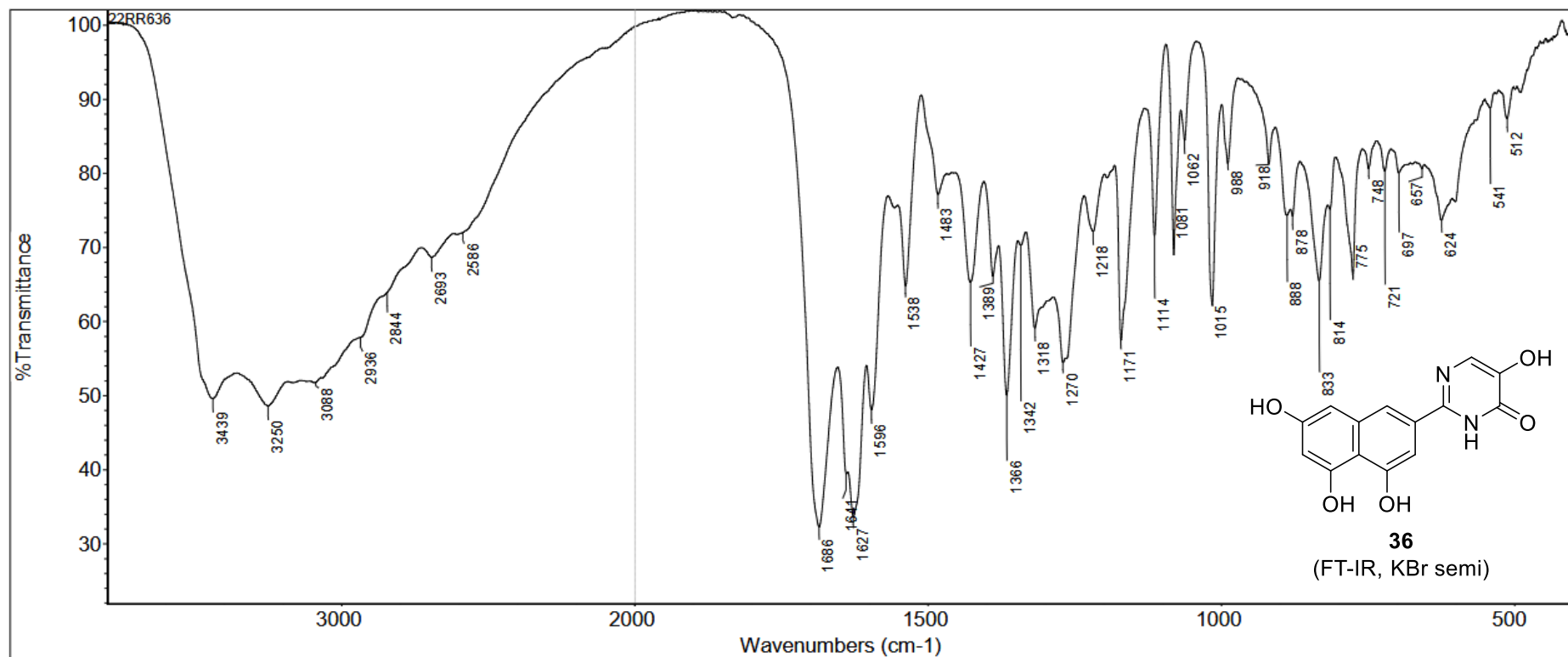












# HRMS spectra

X:\SERVISHR\2021\zari\_2021\apci\RR427

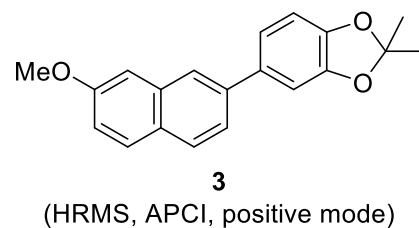
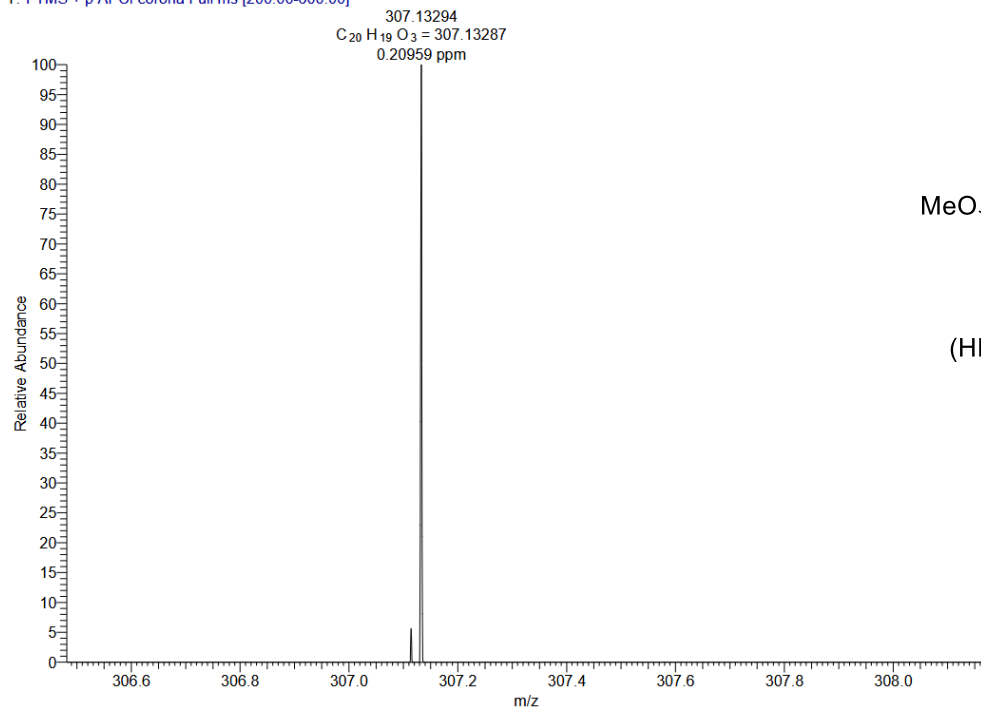
09/07/21 12:01:16

RR427

APCI

RR427 #123-126 RT: 3.35-3.43 AV: 4 SB: 27 0.13-0.41, 0.76-1.17 NL: 3.93E4

T: FTMS + p APCI corona Full ms [200.00-600.00]



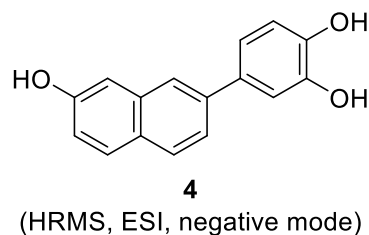
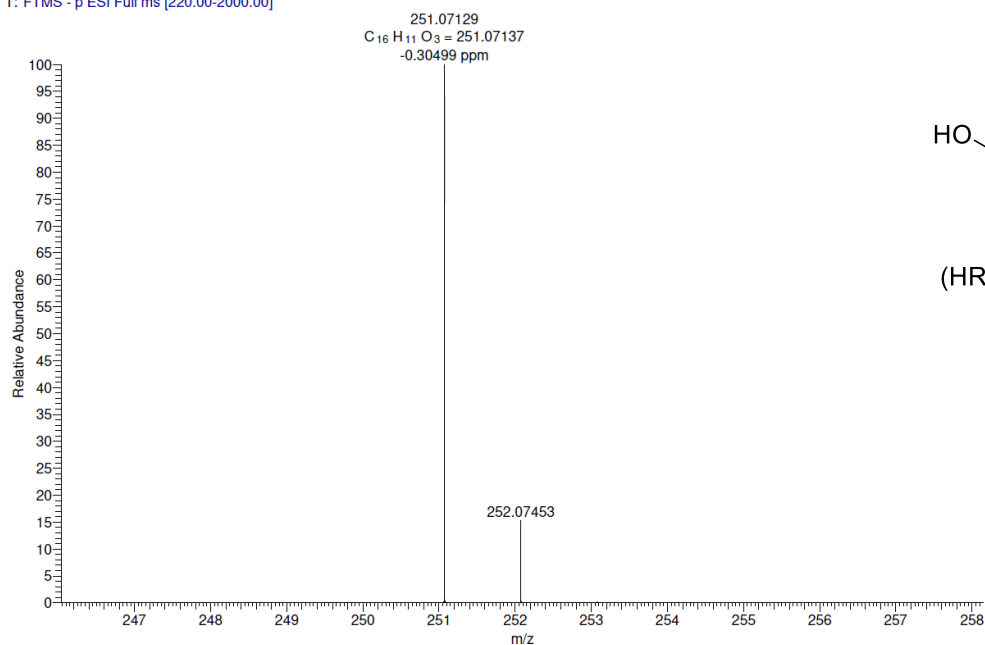
011021\_servisHR\_neg\_2

10/01/21 09:41:08

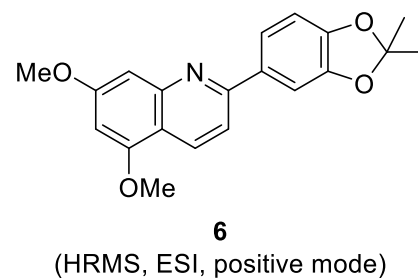
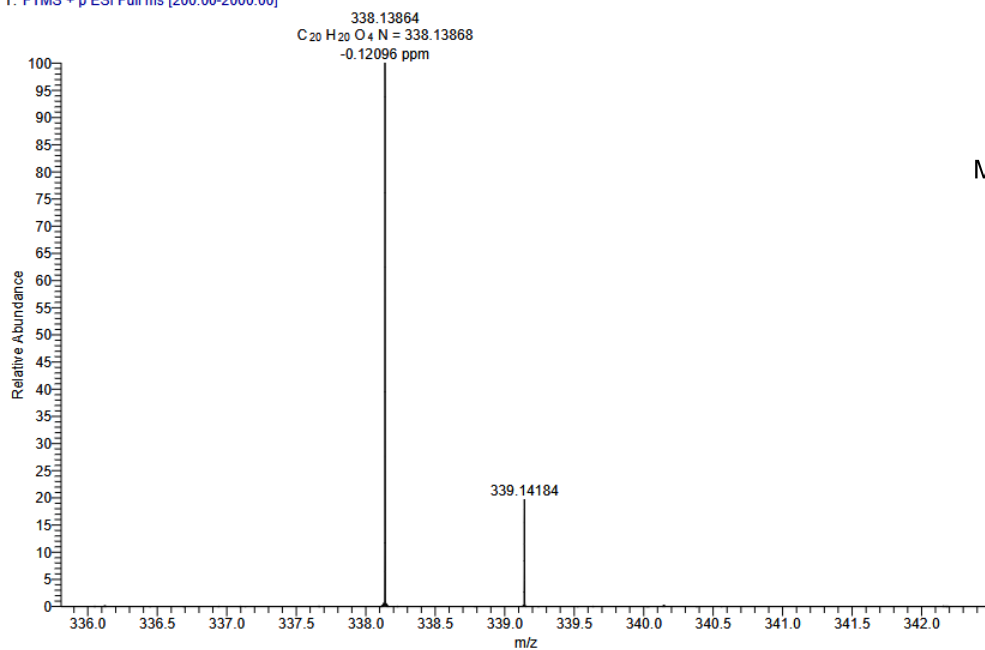
Heiberger, RR428

011021\_servisHR\_neg\_2 #42-48 RT: 2.38-2.73 AV: 7 NL: 8.06E5

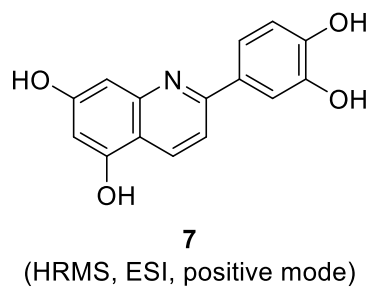
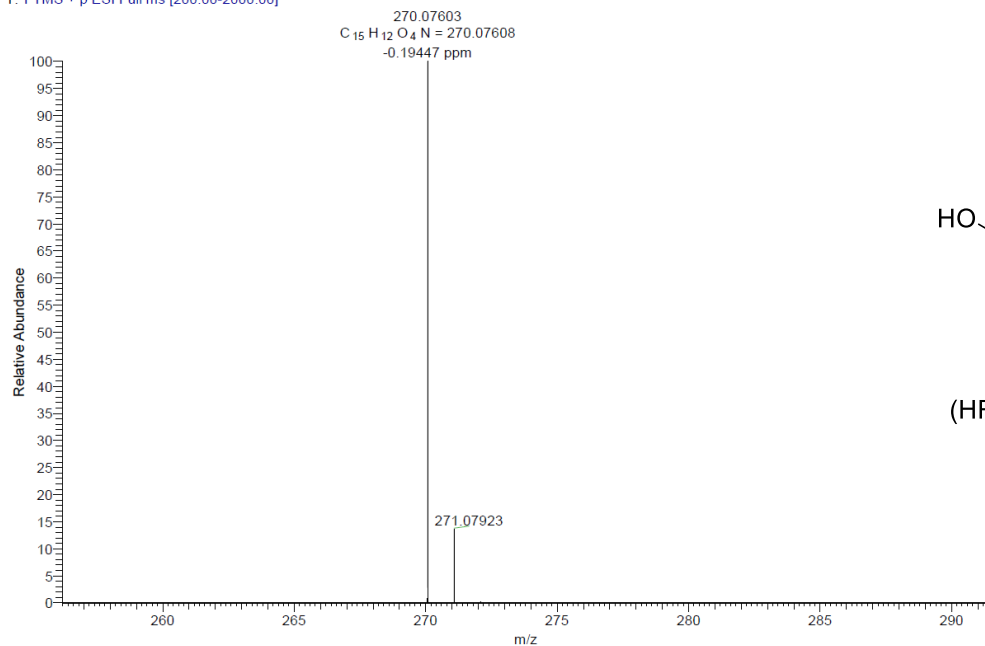
T: FTMS - p ESI Full ms [220.00-2000.00]



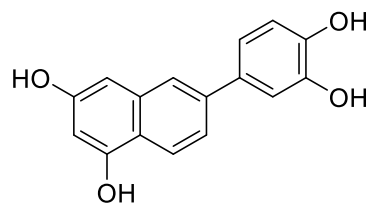
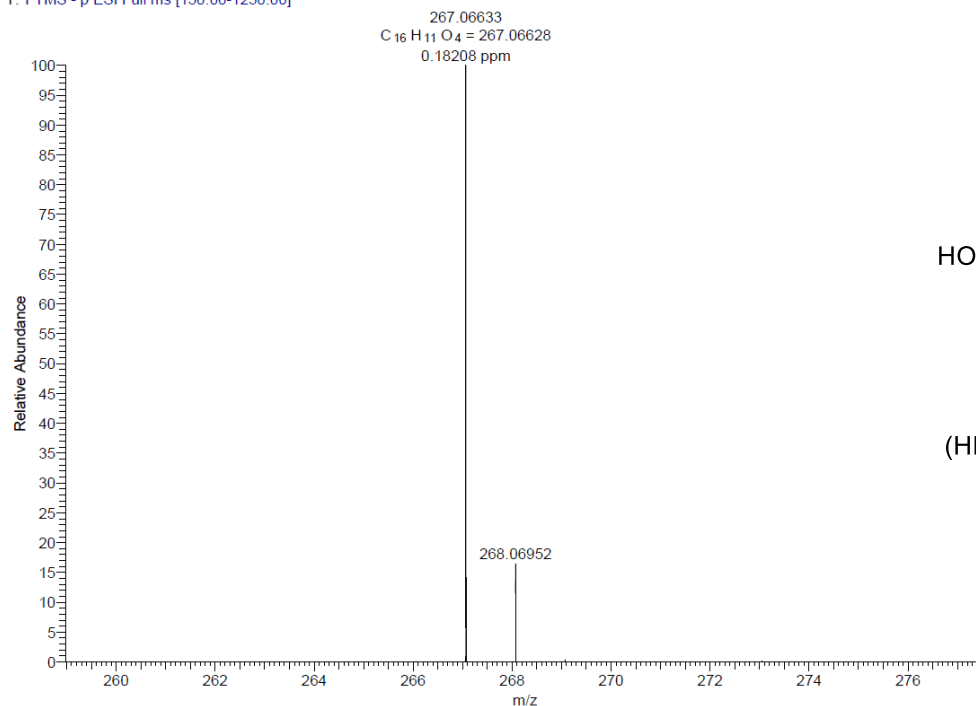
020921\_servisHR\_2 #98-101 RT: 2.62-2.70 AV: 4 NL: 9.38E6  
T: FTMS + p ESI Full ms [200.00-2000.00]



110921\_servisHR\_26 #87-95 RT: 2.32-2.54 AV: 9 NL: 9.33E6  
T: FTMS + p ESI Full ms [200.00-2000.00]

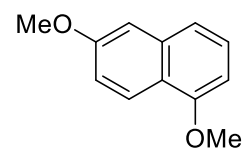
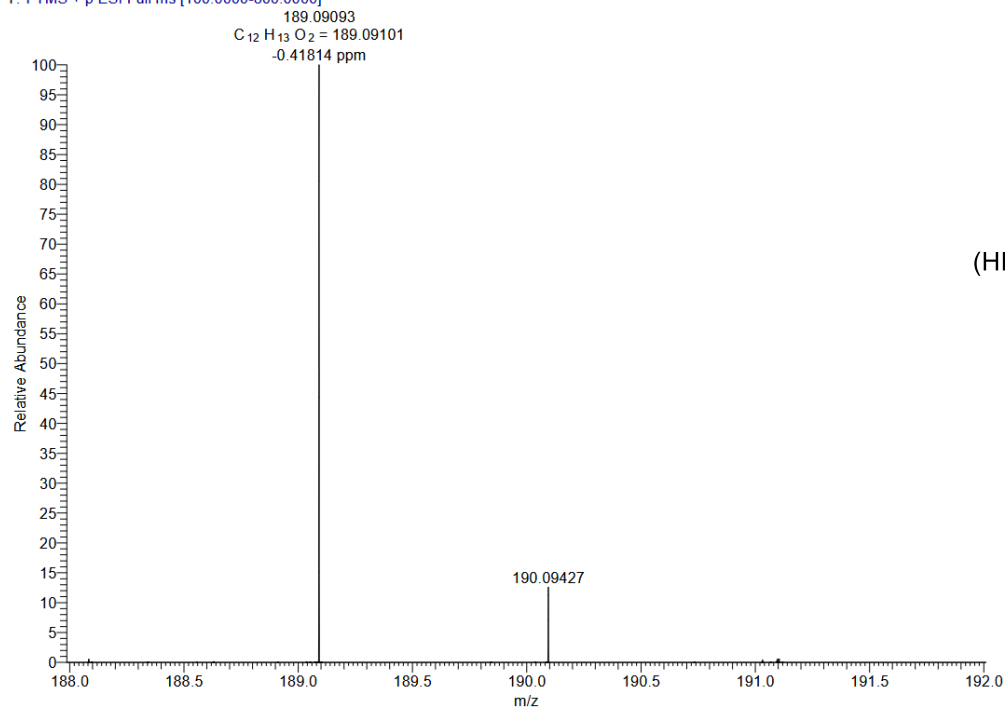


230414\_servisHR\_38 #36-37 RT: 2.03-2.09 AV: 2 NL: 1.82E6  
T: FTMS - p ESI Full ms [150.00-1250.00]

**12**

(HRMS, ESI, negative mode)

240430\_XservisHR+ESI\_12 #354 RT: 2.23 AV: 1 NL: 2.06E7  
T: FTMS + p ESI Full ms [100.0000-800.0000]

**14**

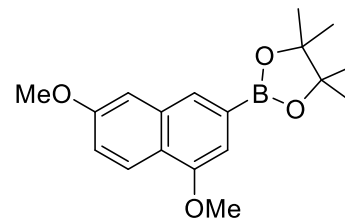
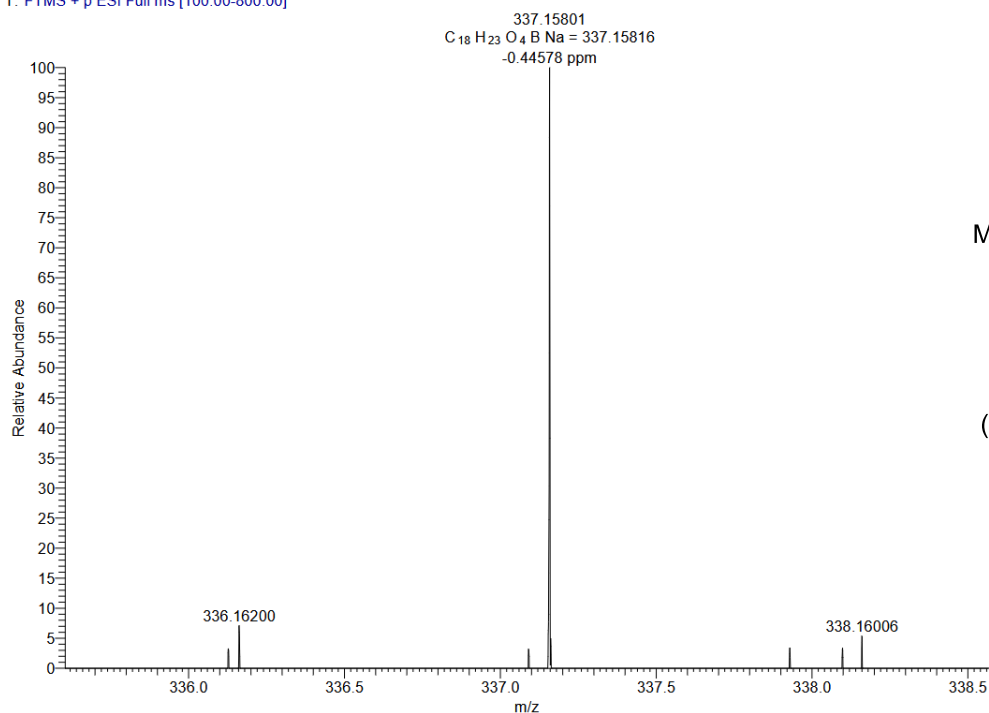
(HRMS, ESI, positive mode)

100821\_servisHK\_2\_210811093444

08/11/21 09:34:44

Reiberger, RR403

100821\_servisHR\_2\_210811093444 #80-82 RT: 2.14-2.19 AV: 3 SB: 9 0.06-0.28 NL: 8.81E4  
T: FTMS + p ESI Full ms [100.00-800.00]

**15**

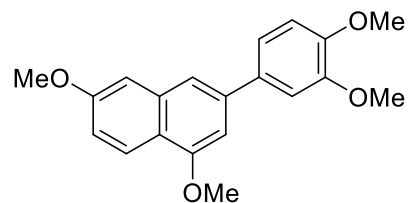
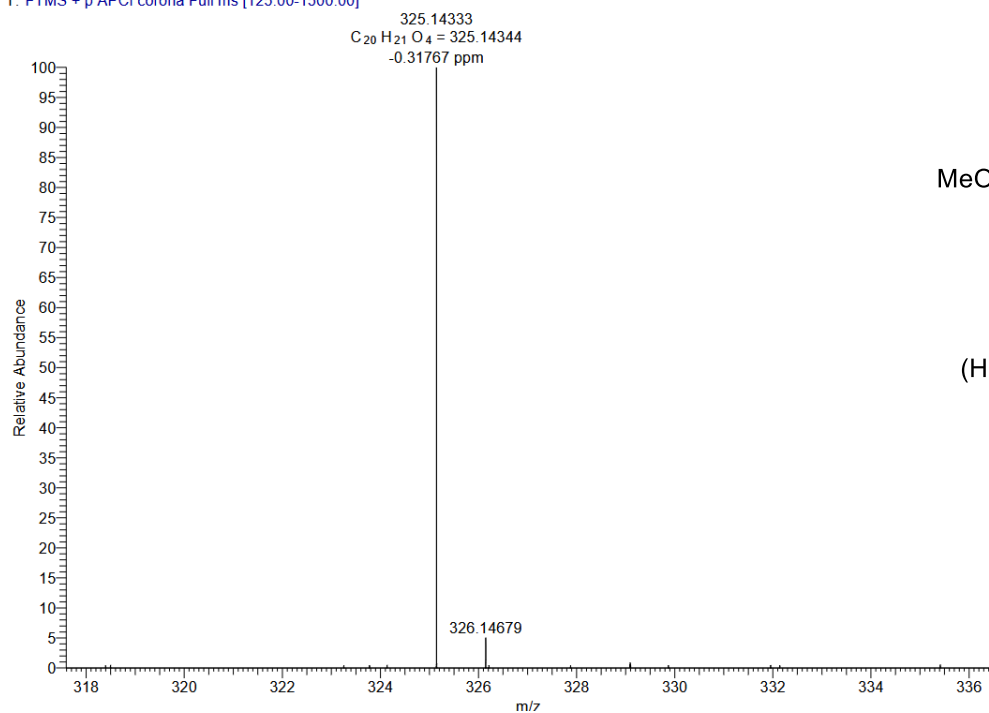
(HRMS, ESI, positive mode)

X:\SERVISHR\2021\srpen\_2021\apci\RR407

08/19/21 14:24:54

RR407

APCI  
RR407 #30-31 RT: 0.80-0.83 AV: 2 SB: 9 0.64-0.66, 0.01-0.17 NL: 1.01E6  
T: FTMS + p APCI corona Full ms [125.00-1500.00]

**16**

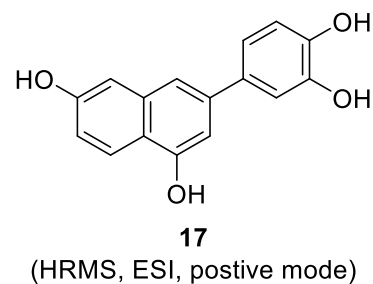
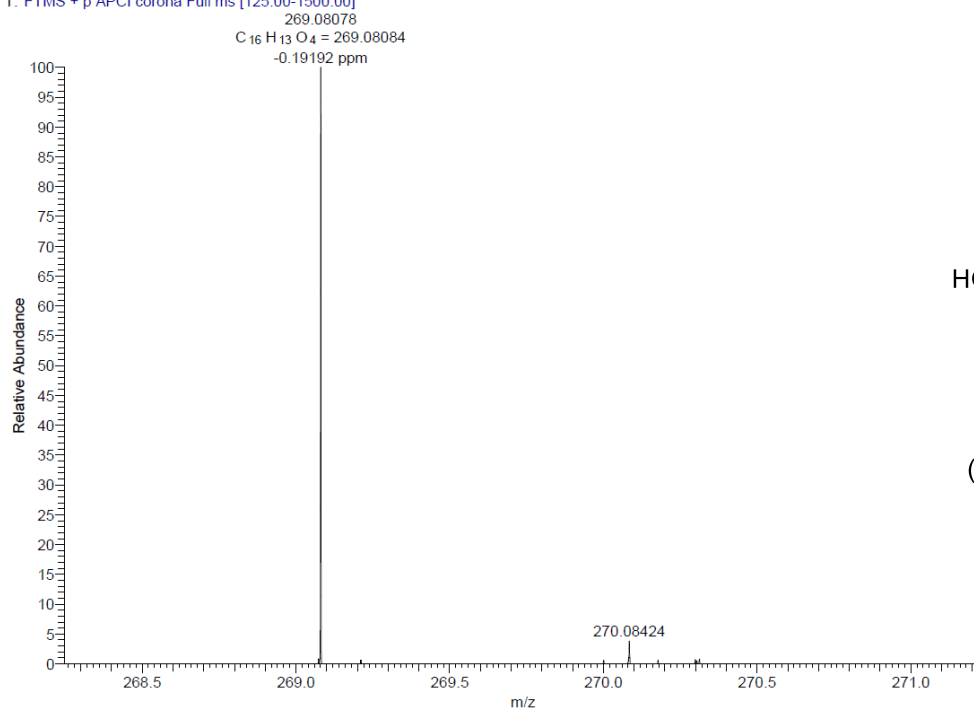
(HRMS, APCI, positive mode)

X:\SERVISHR\2021\srpen\_2021\apci\RR409  
APCI

08/19/21 14:27:29

RR409

RR409 #67-68 RT: 1.81-1.84 AV: 2 SB: 17 0.09-0.36, 0.42-0.55 NL: 7.69E5  
T: FTMS + p APCI corona Full ms [125.00-1500.00]

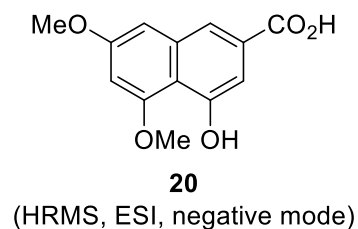
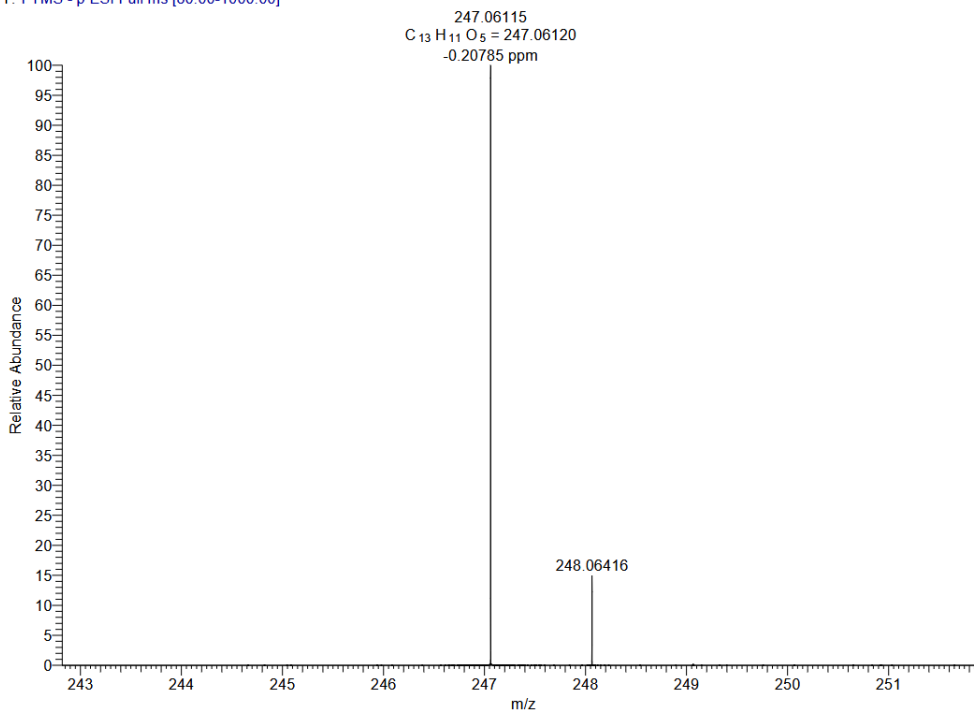


X:\SERVISHR\2020\020622\_servisHR\_neg\_11

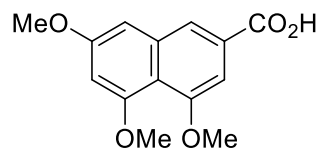
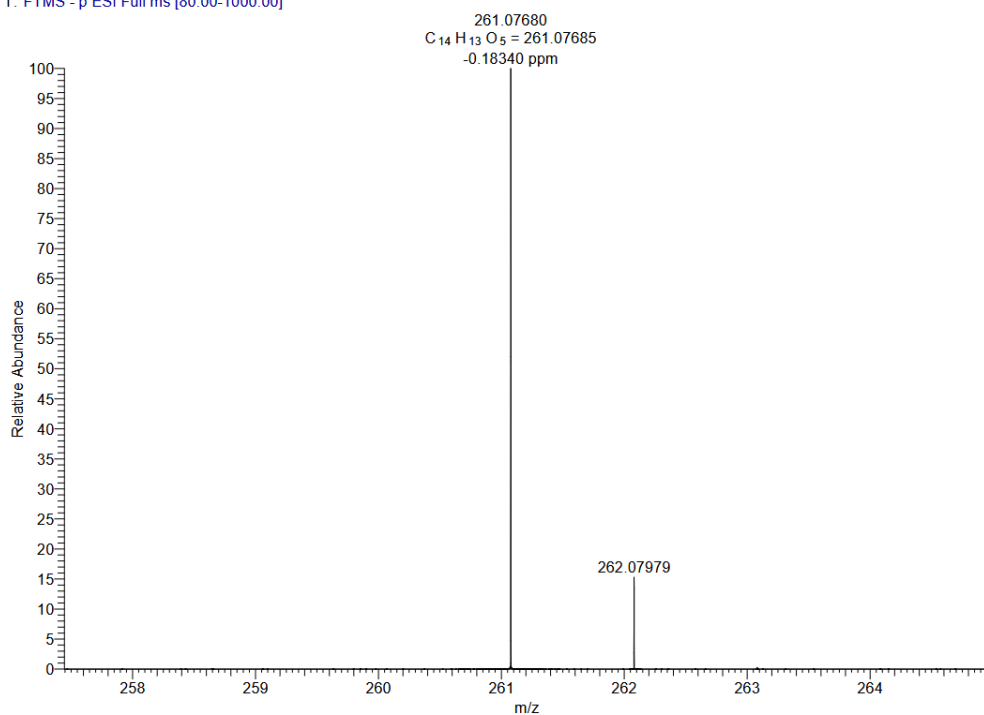
06/02/22 10:49:59

RR534

020622\_servisHR\_neg\_11 #37-39 RT: 2.09-2.20 AV: 3 NL: 9.02E6  
T: FTMS - p ESI Full ms [80.00-1000.00]

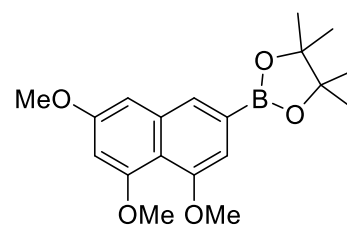
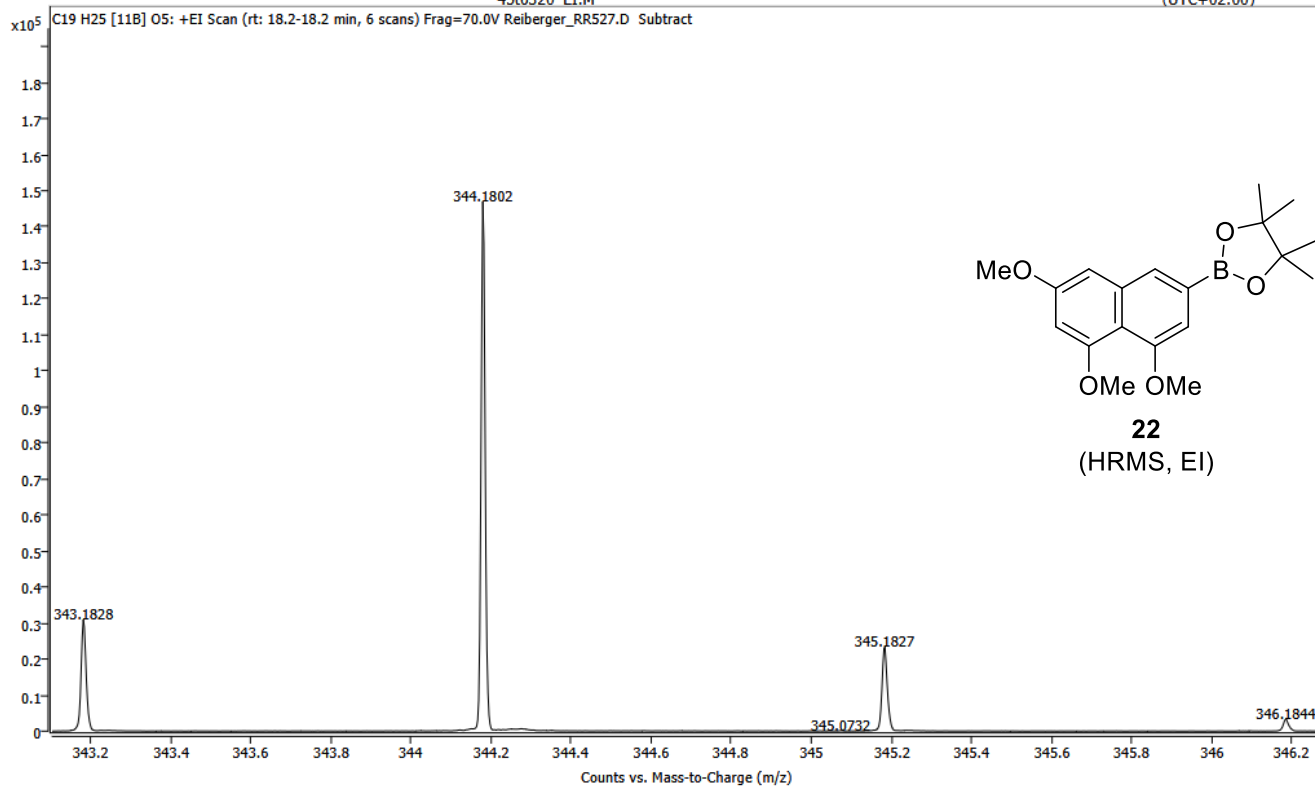


020622\_servisHR\_neg\_12 #37-41 RT: 2.09-2.32 AV: 5 NL: 9.04E6  
T: FTMS - p ESI Full ms [80.00-1000.00]

**21**

(HRMS, ESI, negative mode)

| Name           | Reiberger_RR527   | Rack Pos.    | Instrument                  | GCQTOF  | Operator                        |
|----------------|-------------------|--------------|-----------------------------|---------|---------------------------------|
| Inj. Vol. (ul) | 1                 | Plate Pos.   | IRM Status                  | Success |                                 |
| Data File      | Reiberger_RR527.D | Method (Acq) | 20220127_24min_from Comment |         | Acq. Time (Local)               |
|                |                   |              | 45to320 EI.M                |         | 5/2/2022 2:53:55 PM (UTC+02:00) |

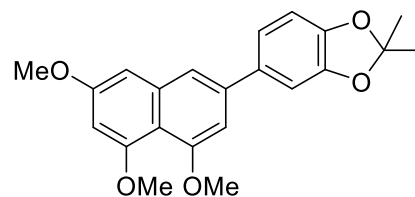
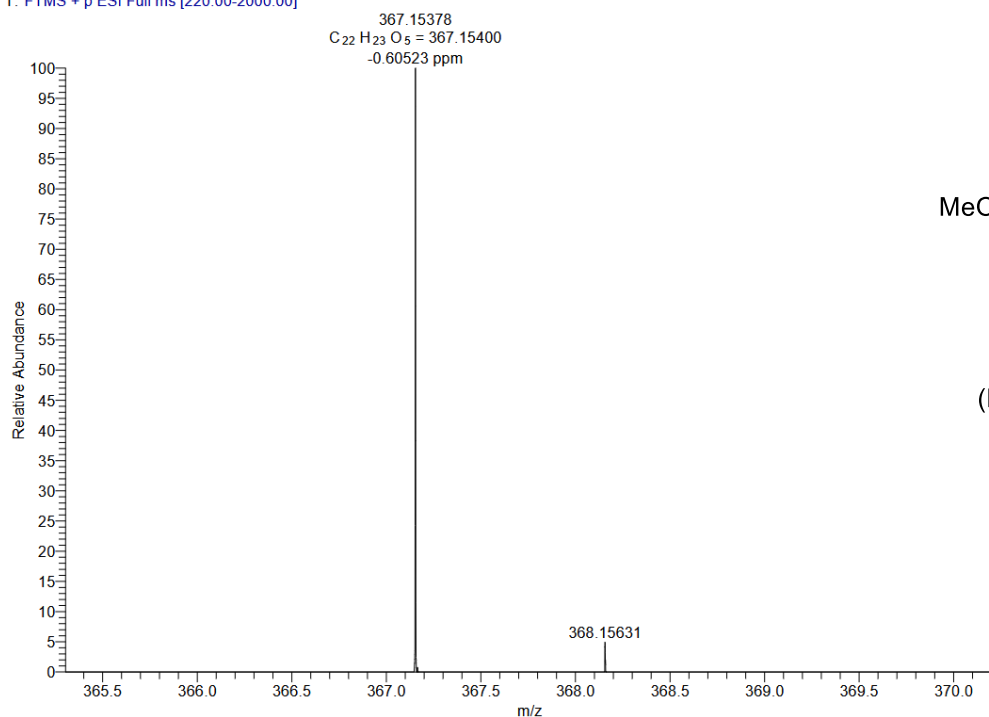
**22**

(HRMS, EI)

020622\_servisHR\_7\_220603093521

06/03/22 09:35:21

RR545

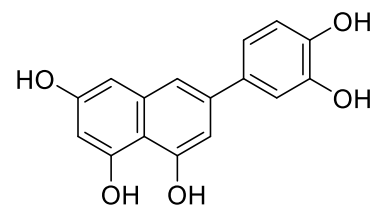
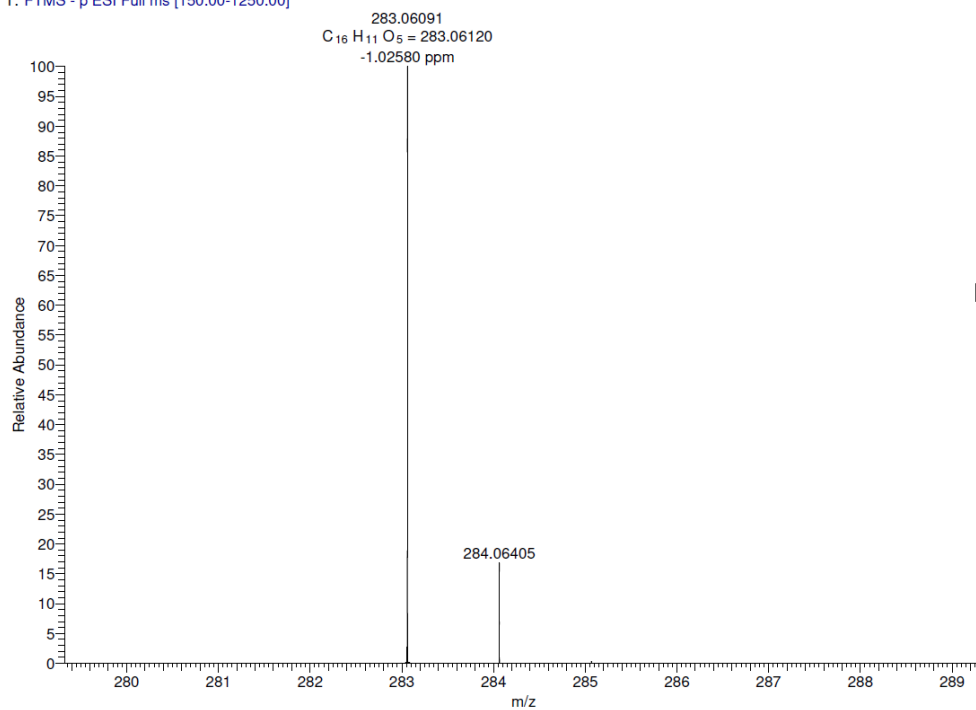
020622\_servisHR\_7\_220603093521 #101 RT: 2.71 AV: 1 NL: 1.16E6  
T: FTMS + p ESI Full ms [220.00-2000.00]**23**

(HRMS, ESI, positive mode)

061022\_servisHR\_neg\_21

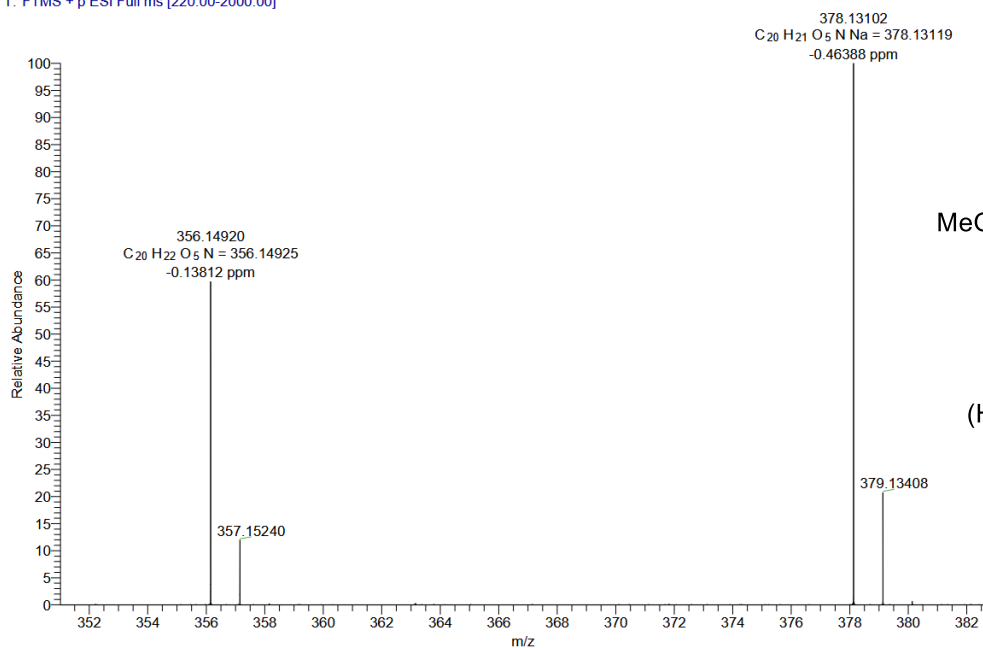
10/06/22 11:43:07

Reiberger RR587

061022\_servisHR\_neg\_21 #42-43 RT: 2.38-2.44 AV: 2 SB: 10<sup>-5</sup> 0.45-0.97 NL: 2.44E6  
T: FTMS - p ESI Full ms [150.00-1250.00]**24**

(HRMS, ESI, negative mode)

290922\_servisHR\_11 #84-85 RT: 2.24-2.27 AV: 2 NL: 2.01E7  
T: FTMS + p ESI Full ms [220.00-2000.00]

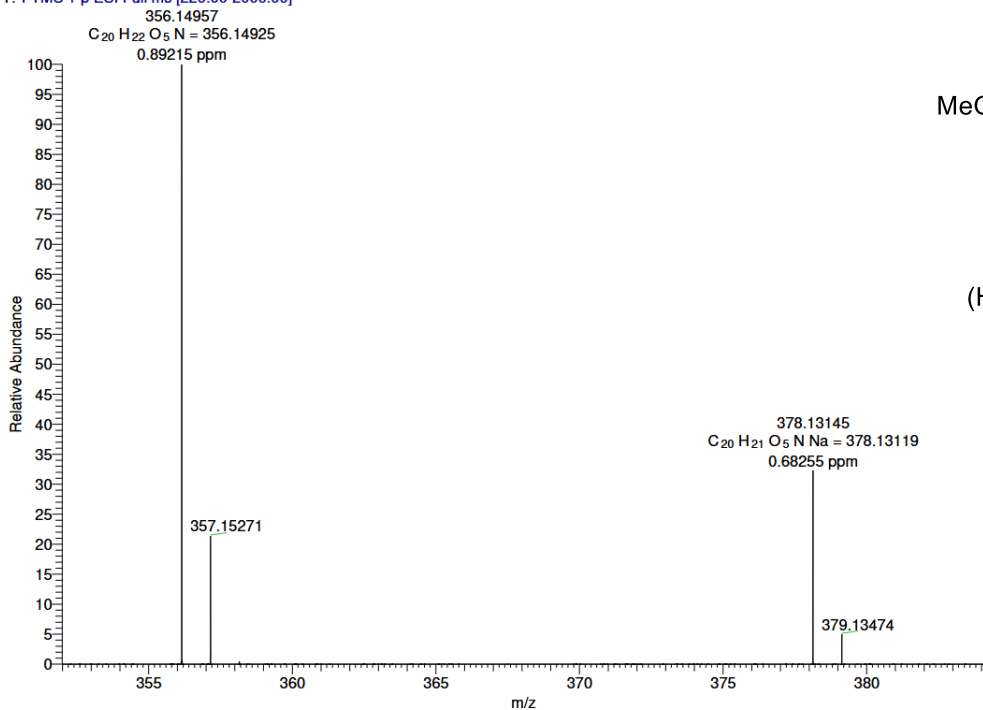


040123\_servisHR\_6

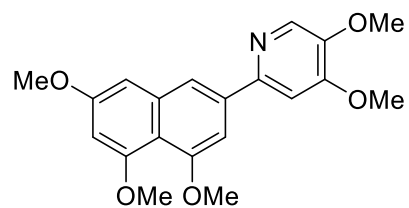
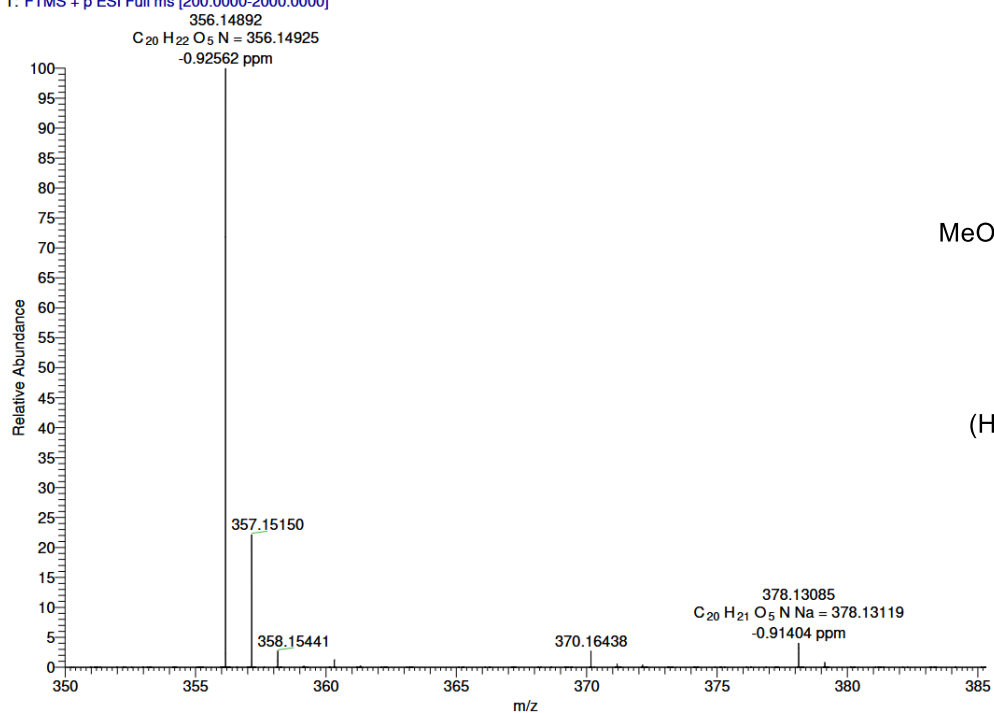
01/04/23 09:35:05

RR629

040123\_servisHR\_6 #132-141 RT: 3.73-3.99 AV: 10 SB: 20 0.44-0.99 NL: 1.34E6  
T: FTMS + p ESI Full ms [220.00-2000.00]

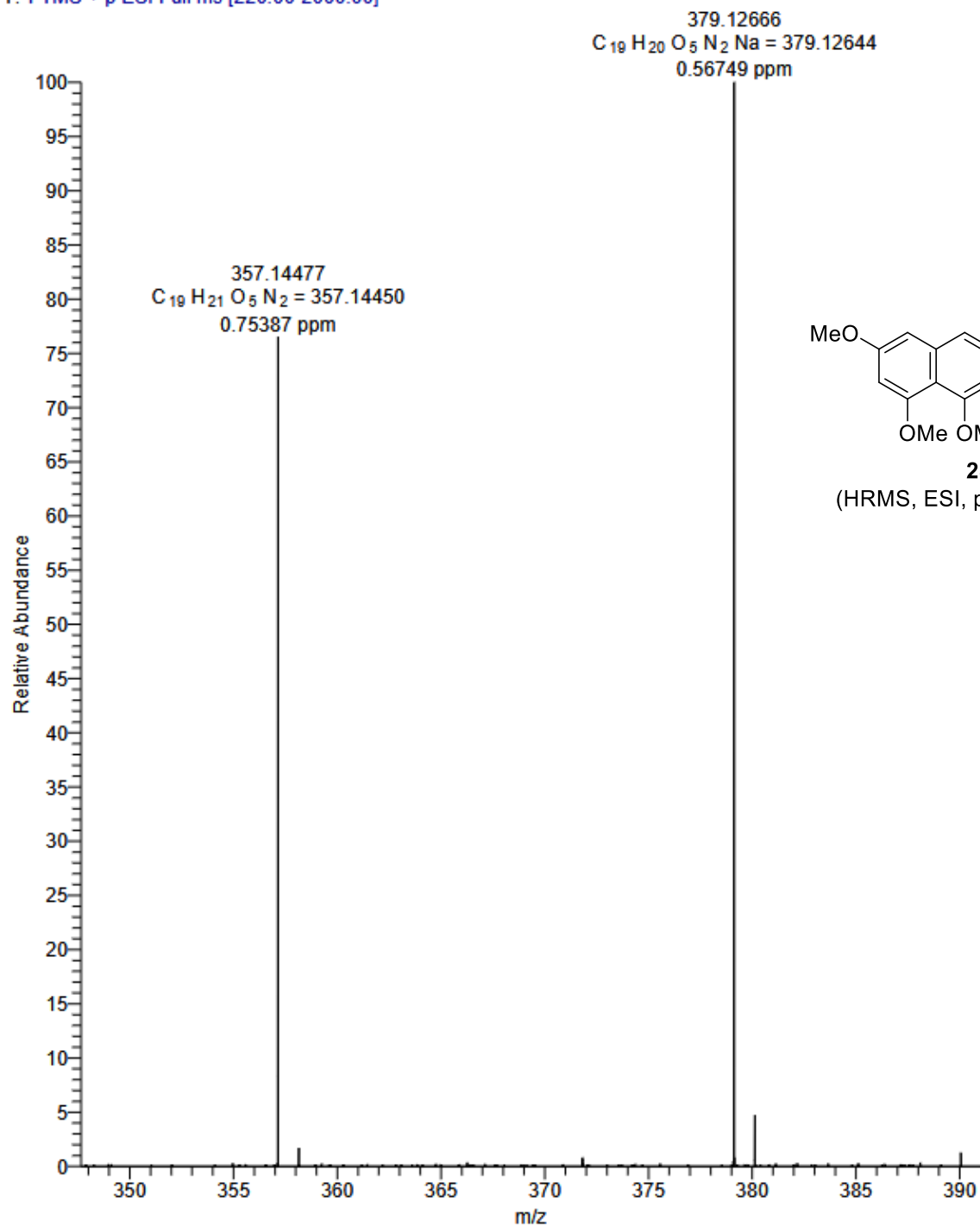


230818\_XservisHR+ESI\_05 #542-566 RT: 3.23-3.37 AV: 25 SB: 91 0.45-1.00 NL: 8.43E7  
T: FTMS + p ESI Full ms [200.0000-2000.0000]

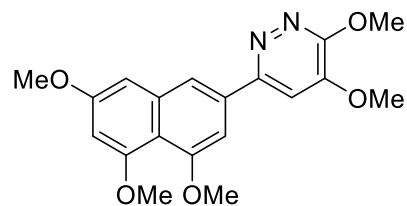
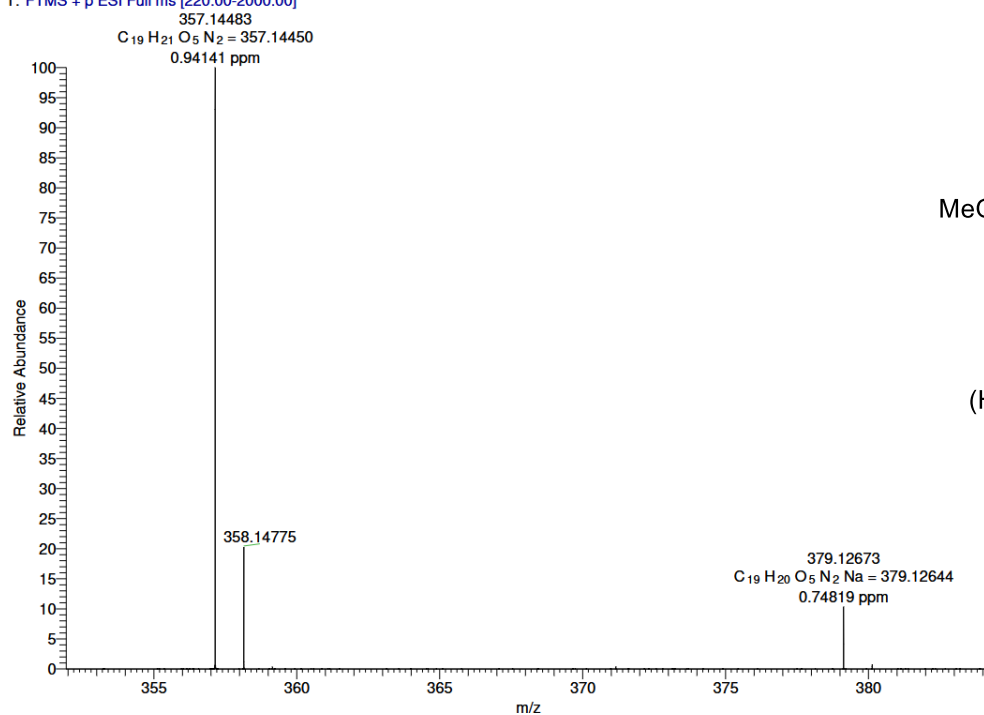
**27**

(HRMS, ESI, positive mode)

031022\_servisHR\_13 #87-91 RT: 2.32-2.43 AV: 5 SB: 46 0.25-0.93, 1.89-2.40 NL: 9.14E5  
T: FTMS + p ESI Full ms [220.00-2000.00]

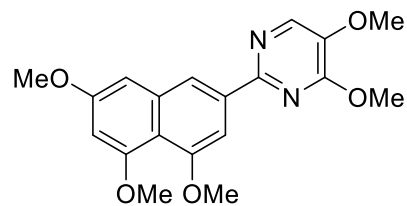
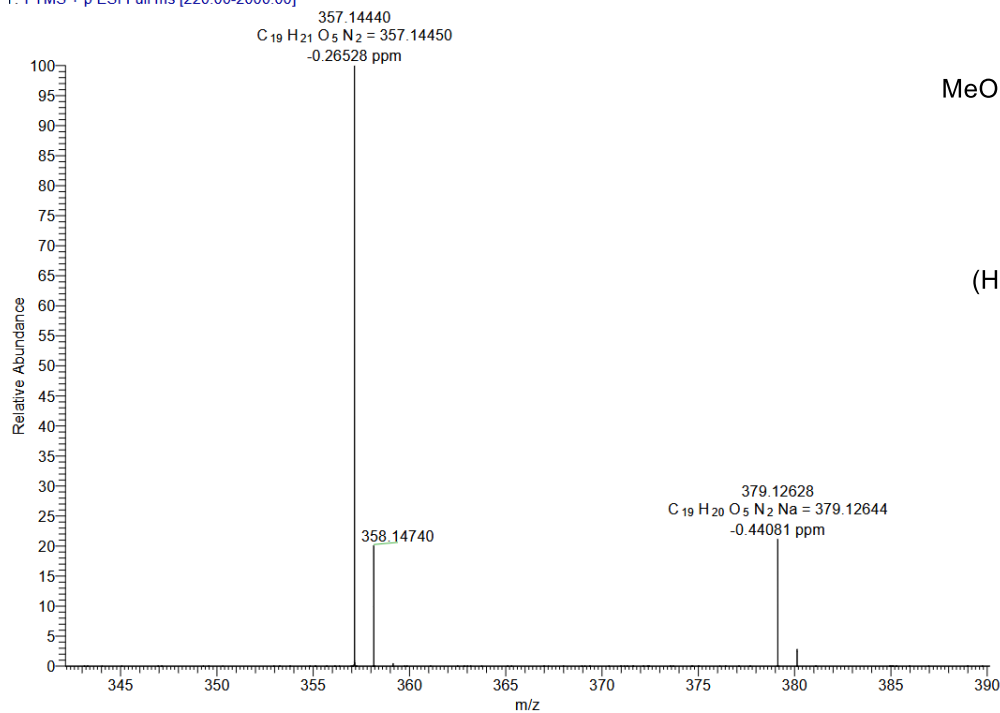


181022\_servisHR\_17 #86-91 RT: 2.40-2.55 AV: 6 SB: 20 0.44-0.99 NL: 2.74E6  
T: FTMS + p ESI Full ms [220.00-2000.00]

**29**

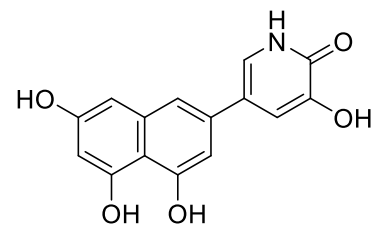
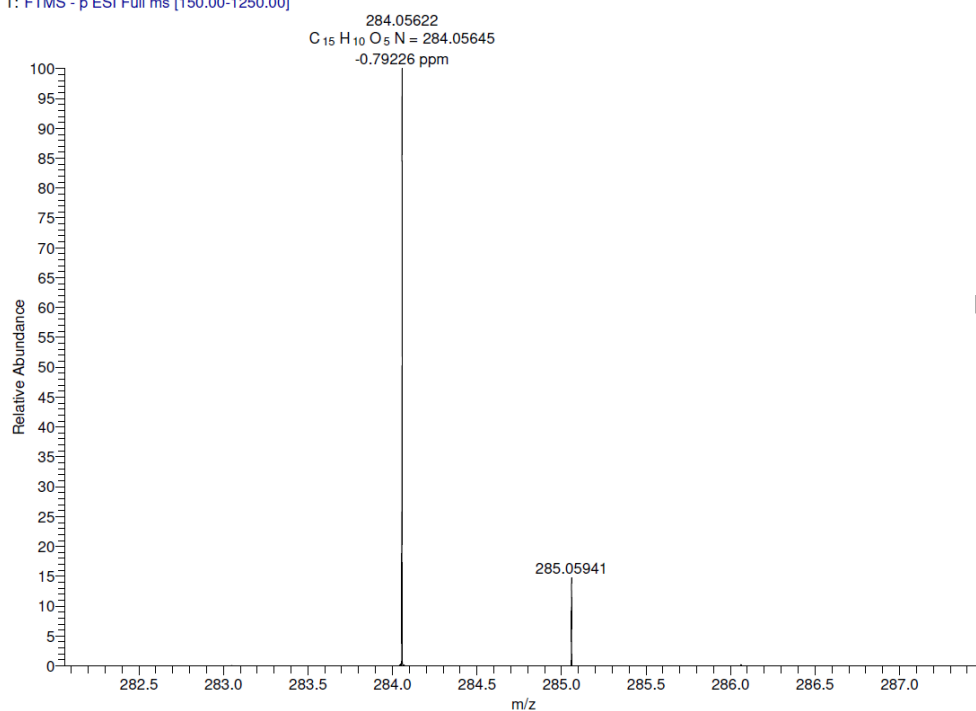
(HRMS, ESI, positive mode)

110123\_servisHR\_5 #142-143 RT: 3.92-3.95 AV: 2 SB: 23 0.24-0.50, 0.18-0.53 NL: 3.87E6  
T: FTMS + p ESI Full ms [220.00-2000.00]

**30**

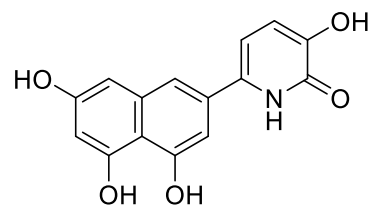
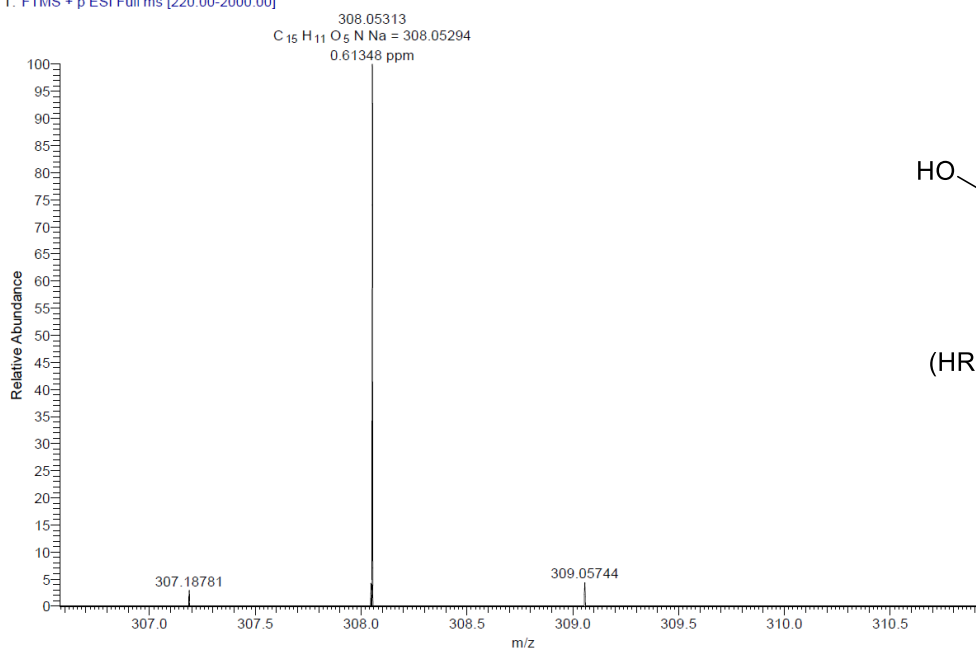
(HRMS, ESI, positive mode)

201022\_servisHR\_neg\_4\_221020142847 #39-41 RT: 2.21-2.32 AV: 3 SB: 10 0.45-0.98 NL: 7.31E5  
T: FTMS - p ESI Full ms [150.00-1250.00]

**31**

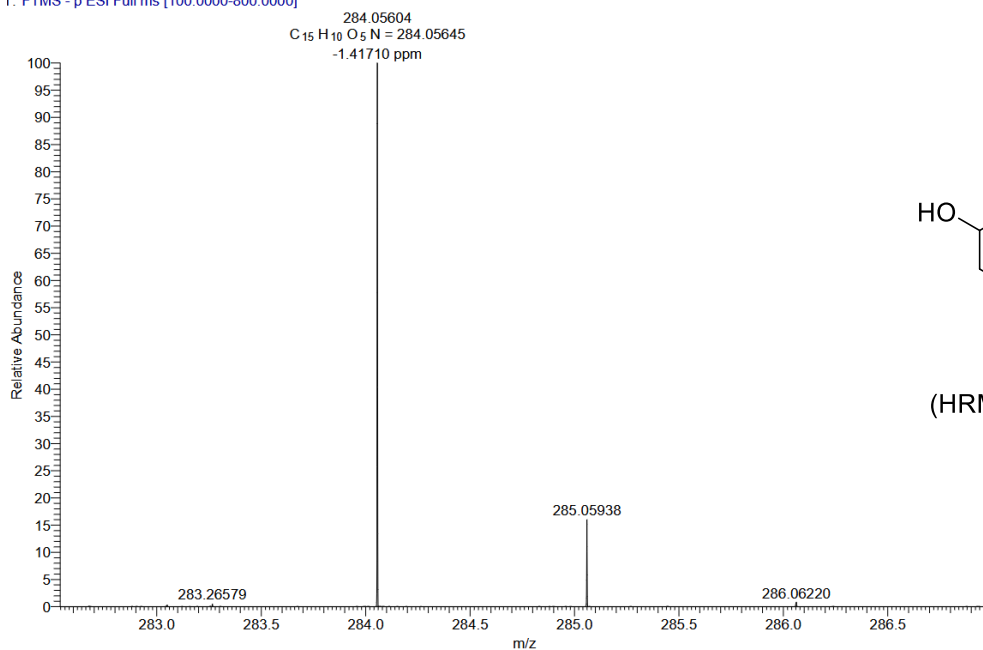
(HRMS, ESI, negative mode)

130123\_servisHR\_22 #75 RT: 2.11 AV: 1 SB: 21 0.79-0.99, 0.84-1.19 NL: 3.49E4  
T: FTMS + p ESI Full ms [220.00-2000.00]

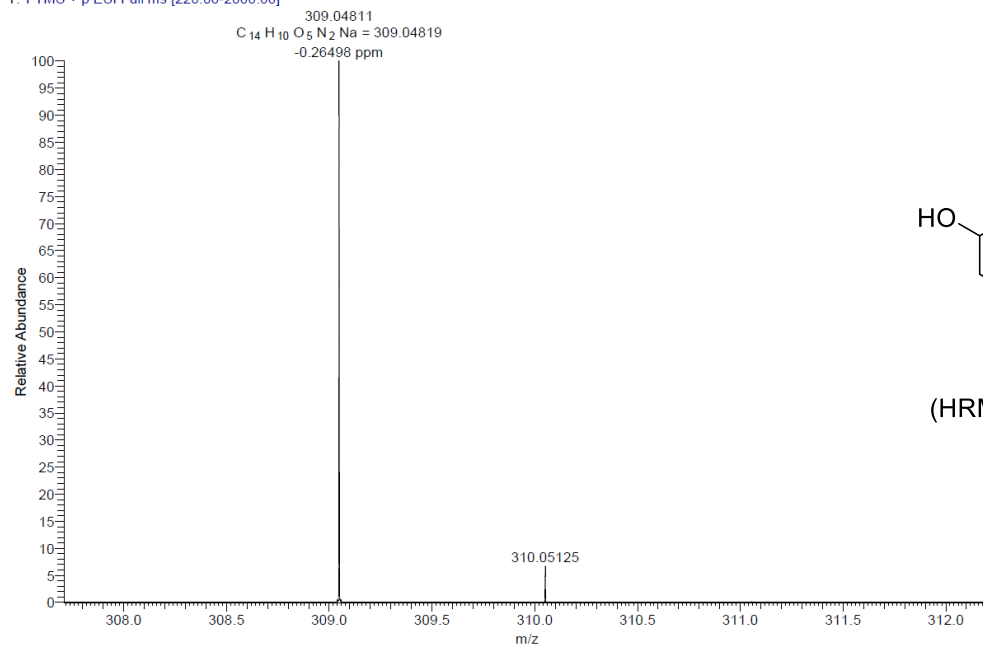
**32**

(HRMS, ESI, positive mode)

230823\_XservisHR\_negESI\_06 #528-530 RT: 3.28-3.29 AV: 3 SB: 81 0.14-0.30, 0.16-0.48 NL: 1.53E7  
T: FTMS - p ESI Full ms [100.0000-800.0000]

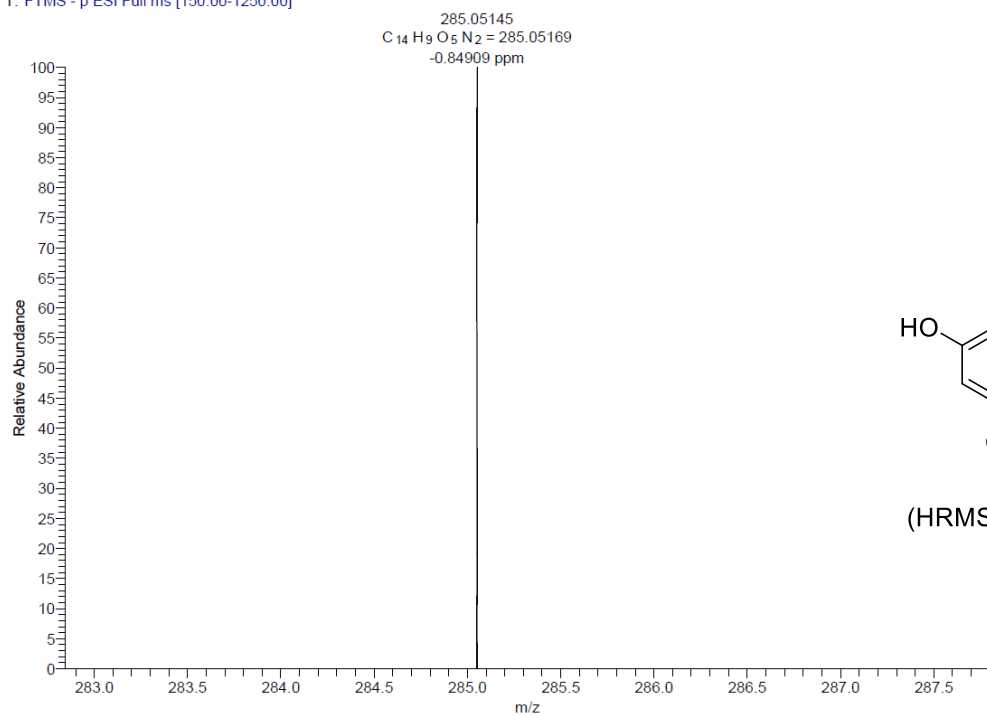


160123\_servisHR\_16 #84-89 RT: 2.36-2.50 AV: 6 SB: 30 0.50-0.89, 0.47-0.87 NL: 4.63E5  
T: FTMS + p ESI Full ms [220.00-2000.00]



091122\_servisHR\_neg\_3 #50 RT: 2.85 AV: 1 NL: 6.44E3

T: FTMS - p ESI Full ms [150.00-1250.00]



170123\_servisHR\_31\_230117151215 #98 RT: 2.76 AV: 1 SB: 58 0.59-1.27, 0.53-1.45 NL: 7.76E5

T: FTMS + p ESI Full ms [60.00-800.00]

