

Supplementary Information for:

# Esterase-Sensitive Prodrugs of a Potent Bisubstrate Inhibitor of Nicotinamide N-Methyltransferase (NNMT) Display Cellular Activity

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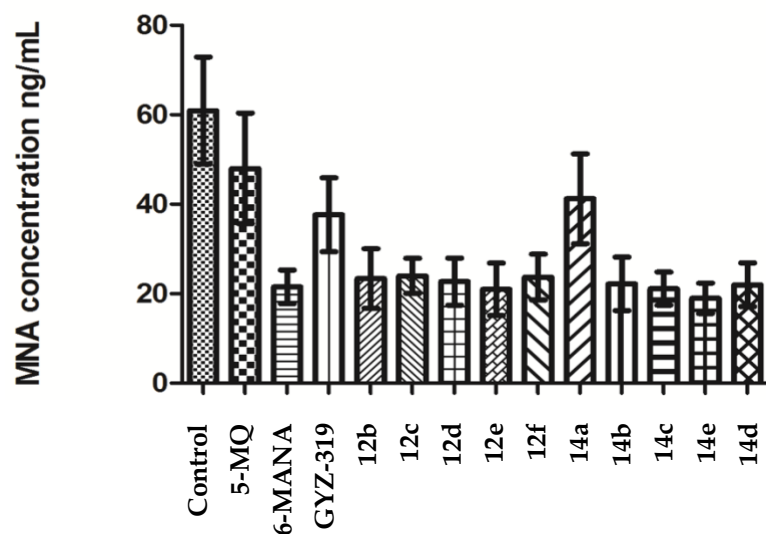
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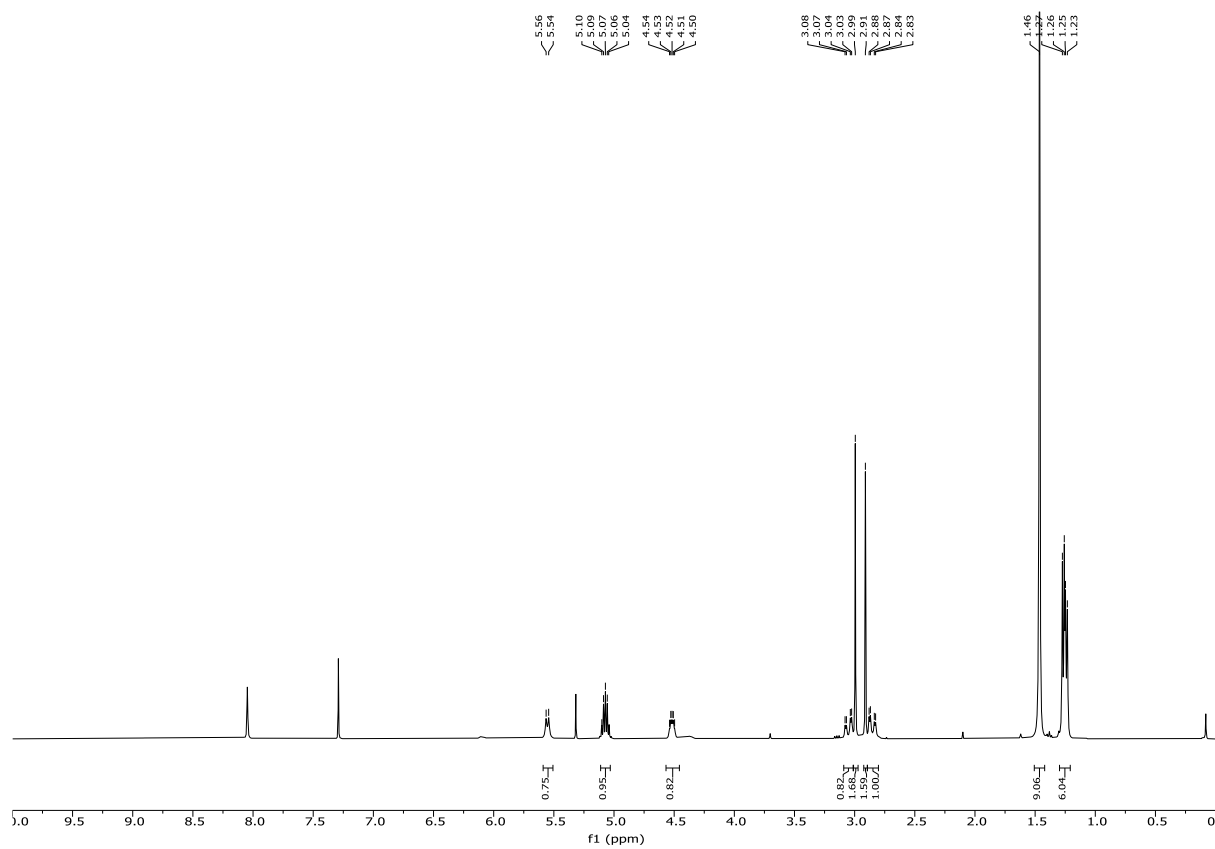
## Cellular MNA data



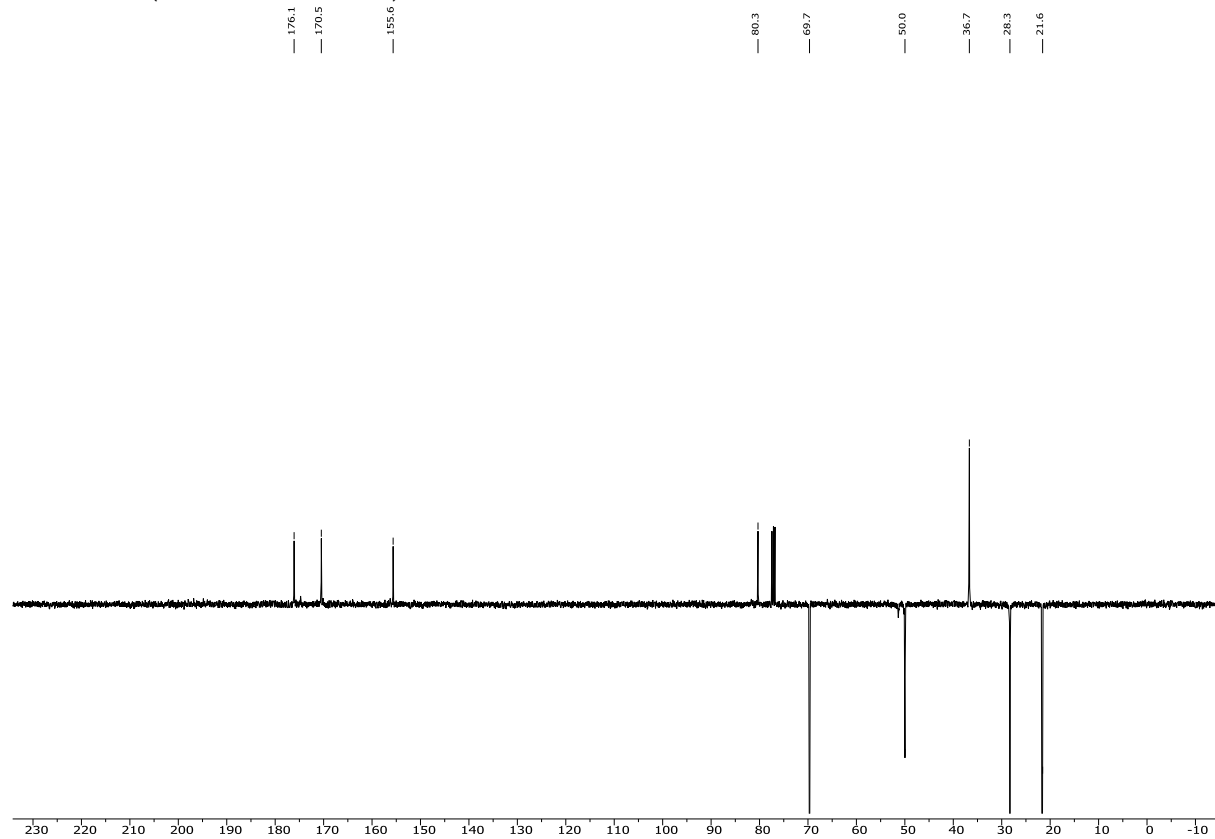
**Figure S1.** 1-methylnicotinamide (MNA) concentrations in endothelial HMEC-1 cells after 24-hour incubation with 10  $\mu$ M of reference compounds 5-amino-methylquinolinium (5-MQ), 6-methylamino-nicotinamide (6-MANA), parent compound GYZ-319 or prodrug compounds **12b-f** and **14a-e**.

# NMR spectra

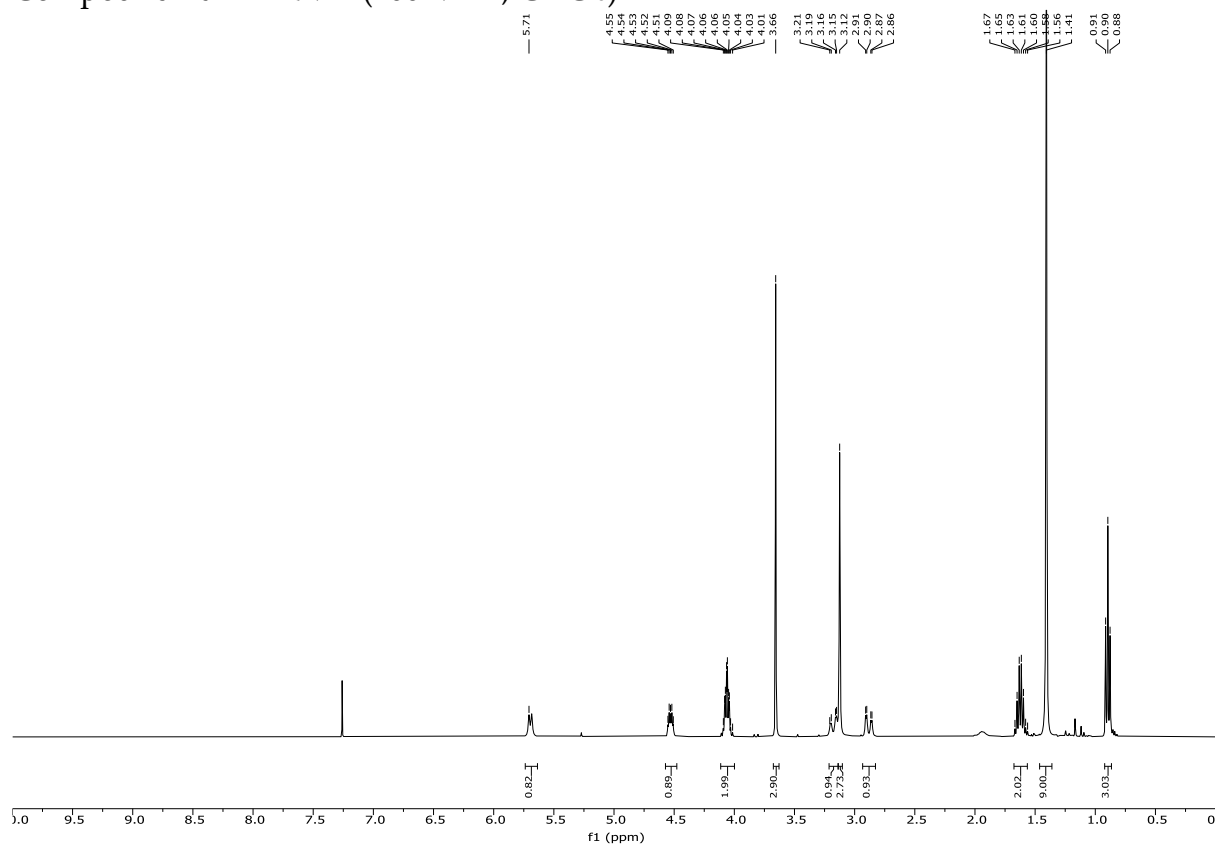
Compound **3e**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



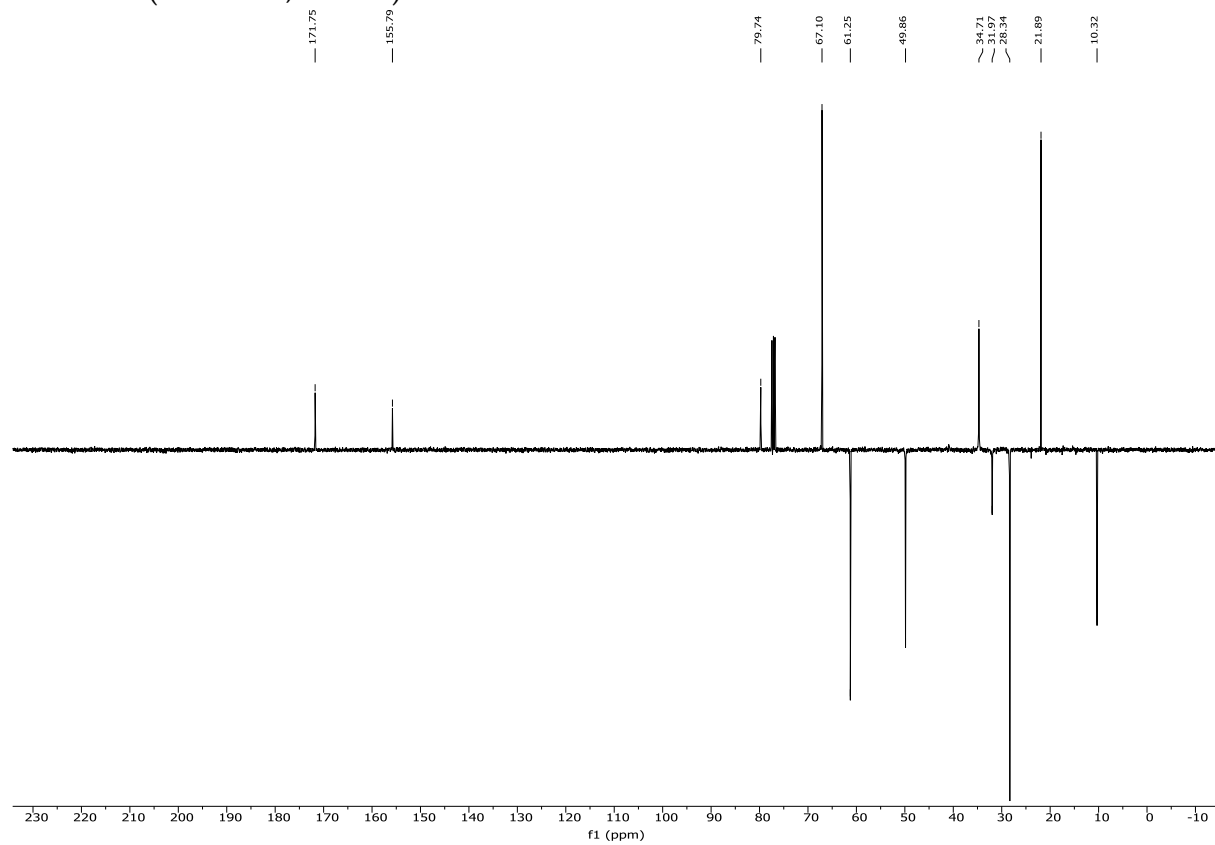
$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )



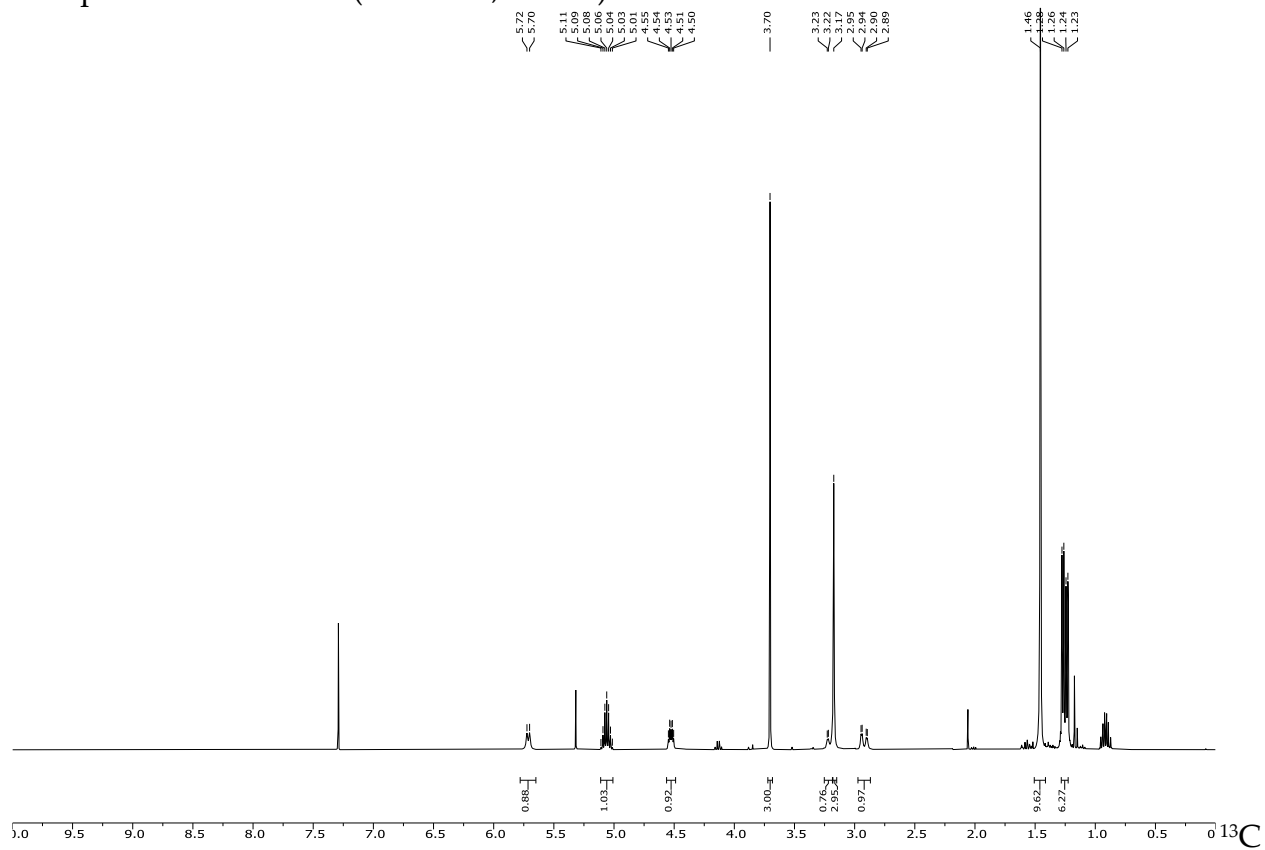
Compound **4d**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



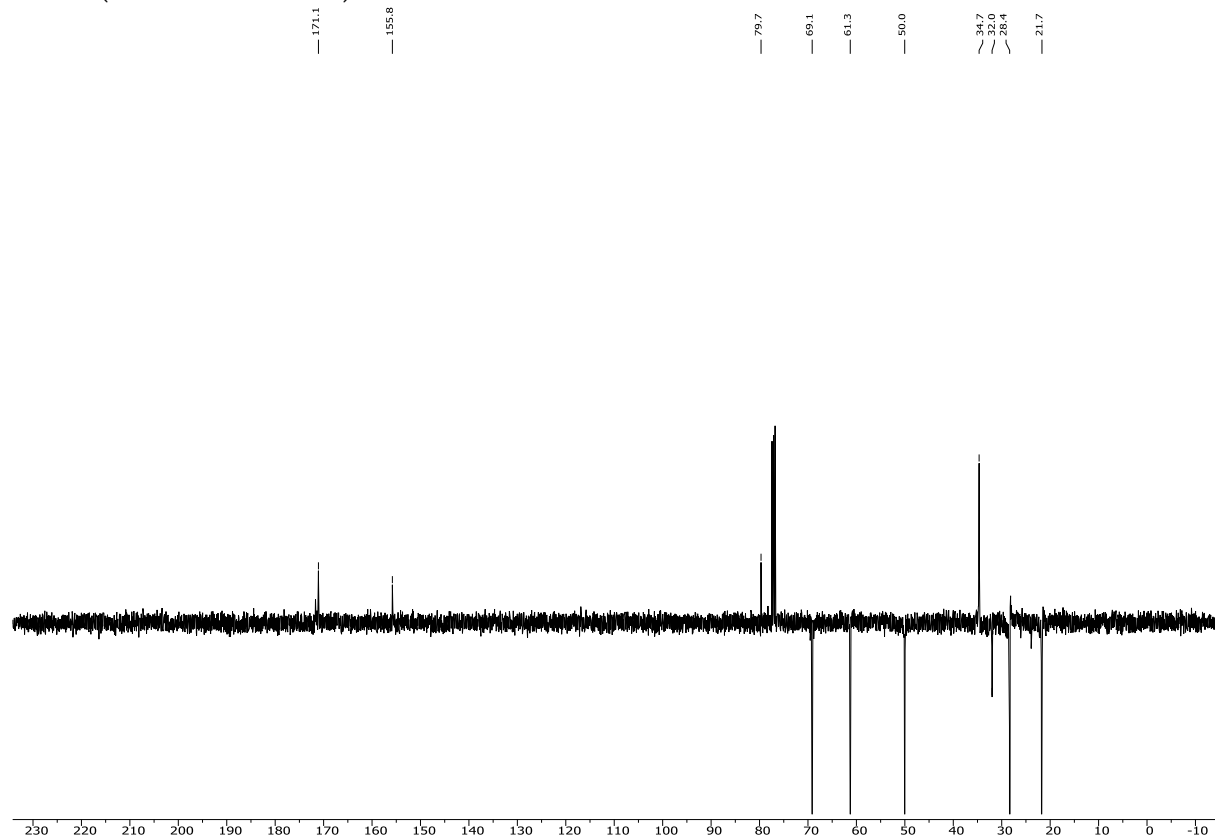
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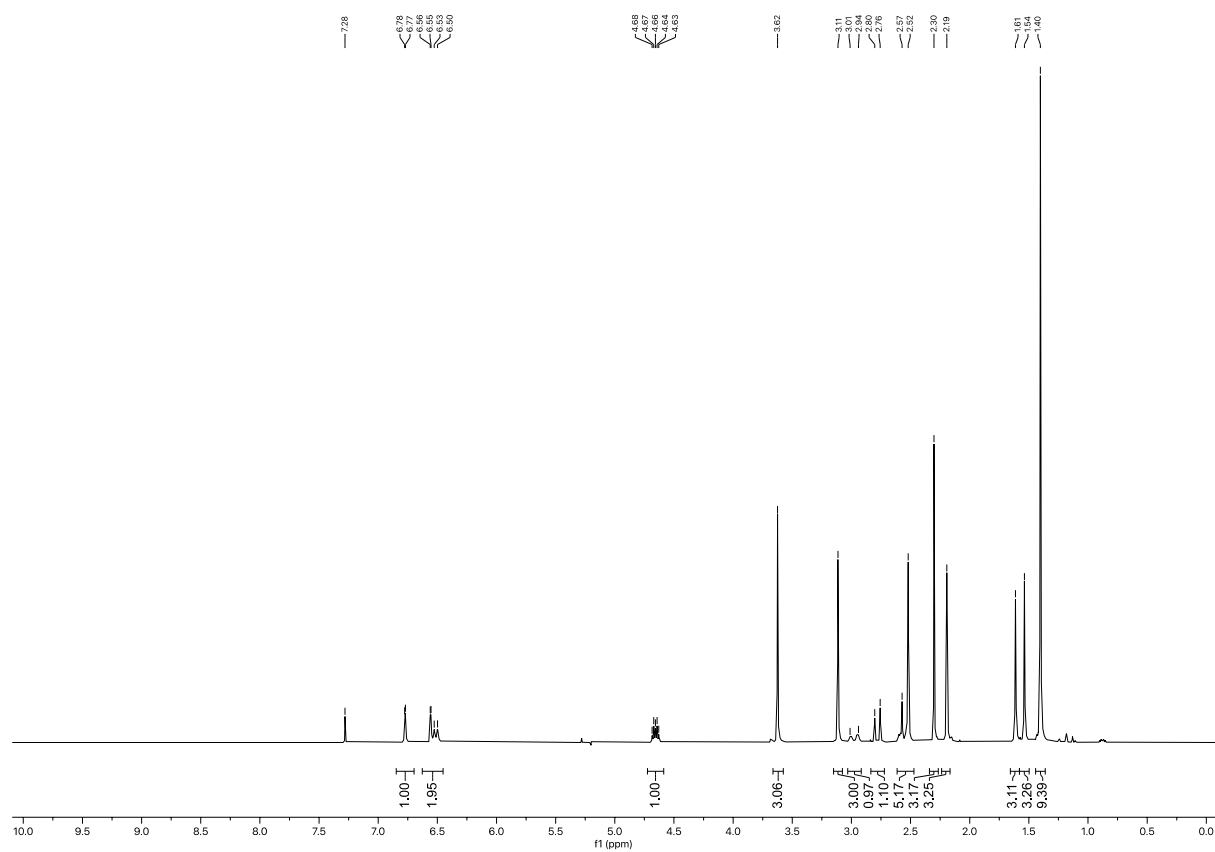
Compound **4e**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



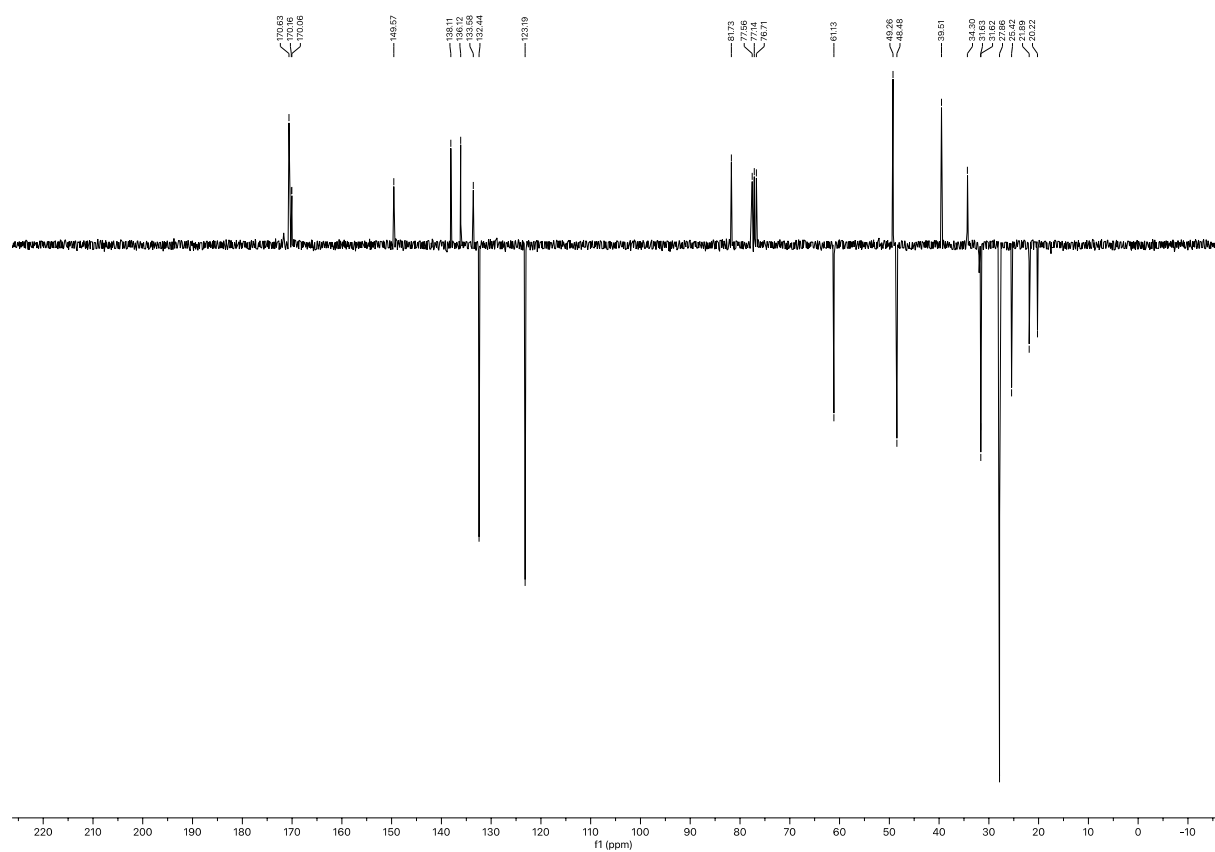
$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )



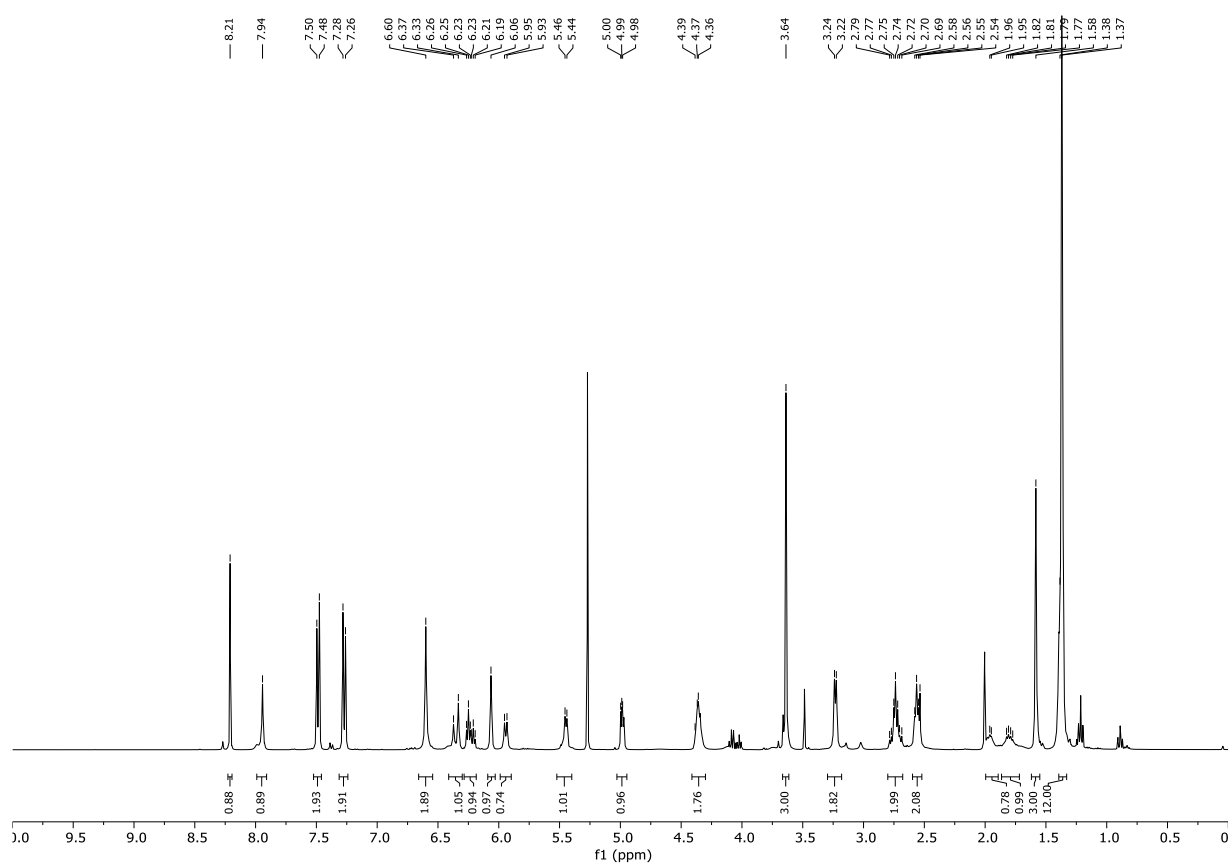
Compound **8a**  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )



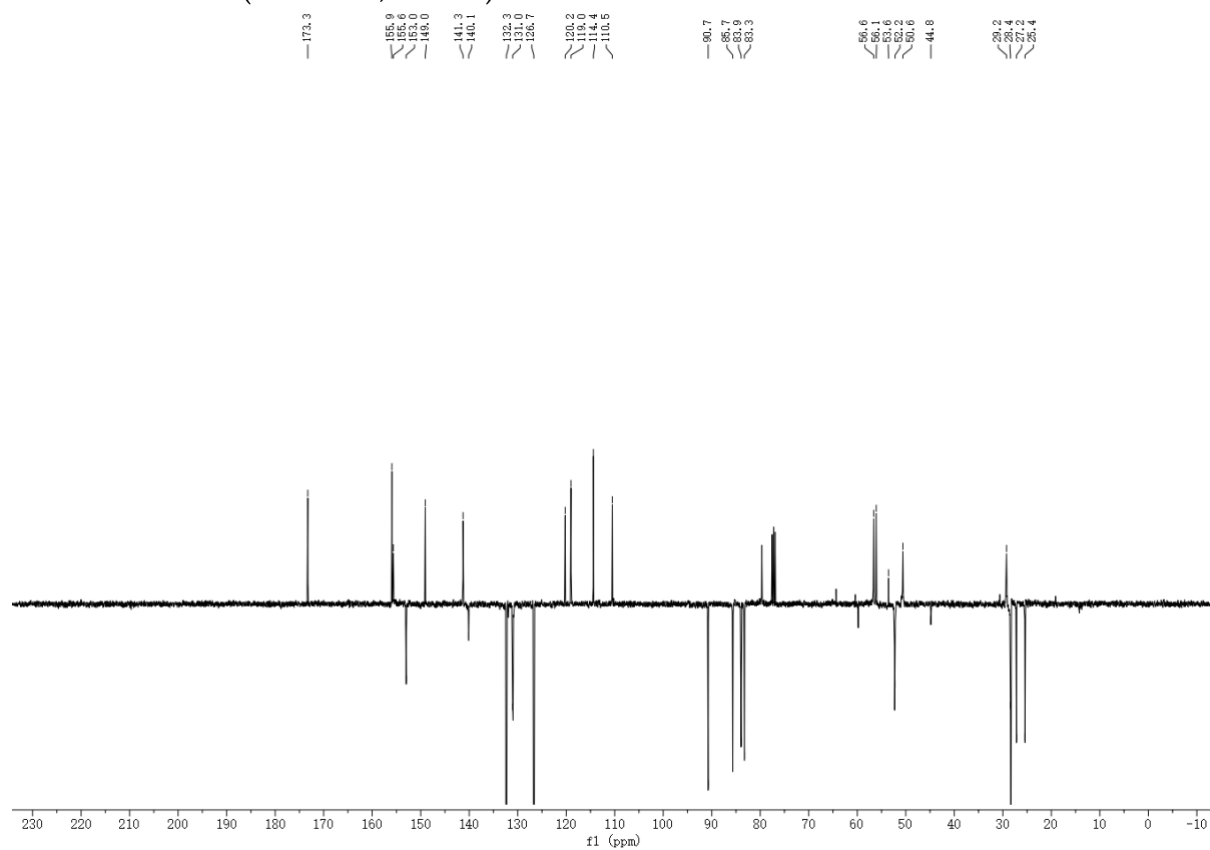
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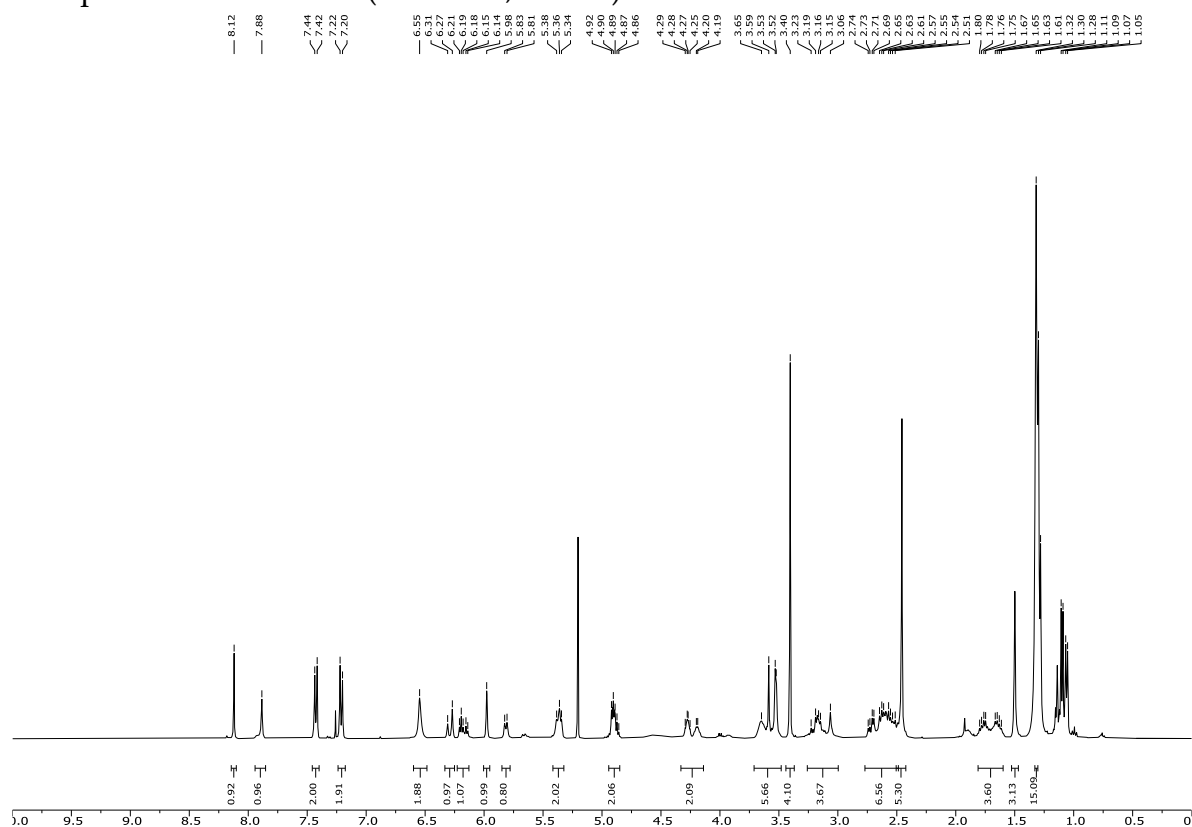
Compound **11b**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



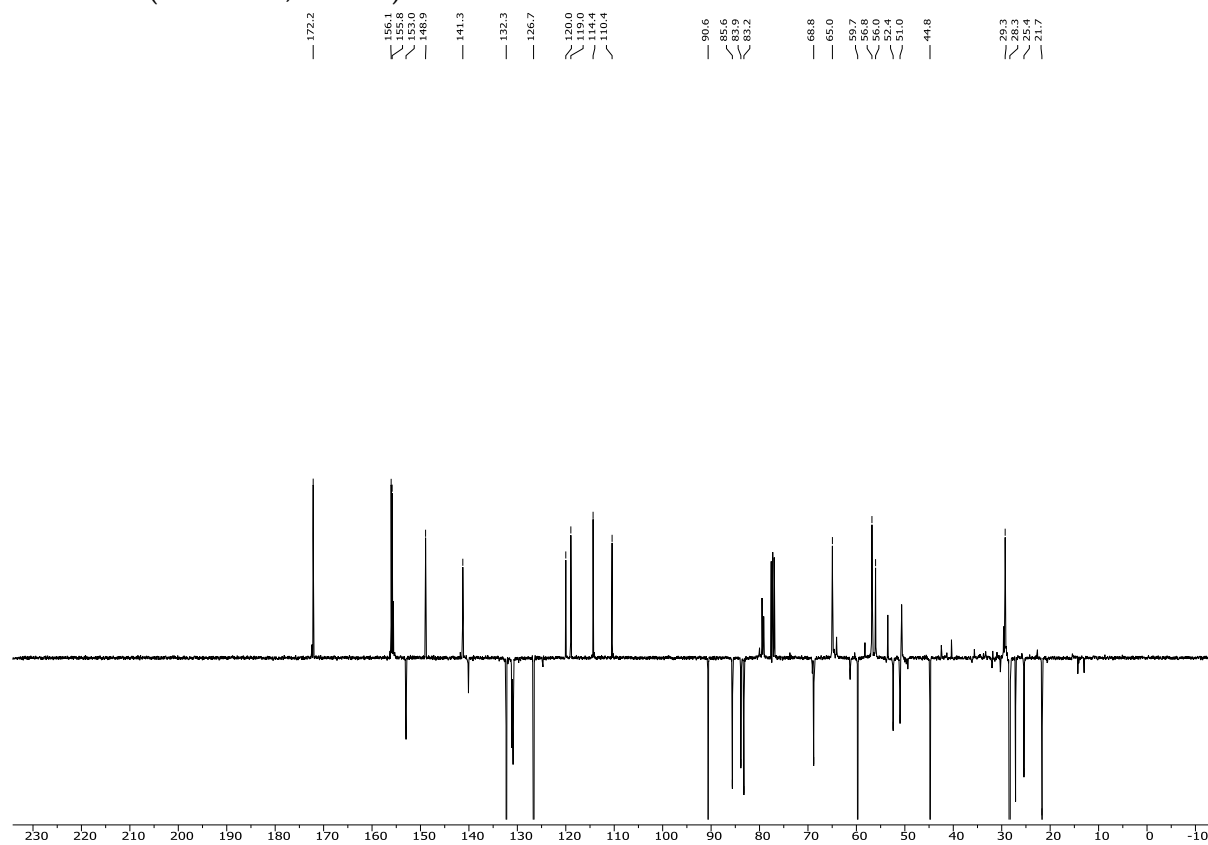
$^{13}\text{C}$  NMR  $\text{CDCl}_3$  (101 MHz,  $\text{CDCl}_3$ )



Compound **11c**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )

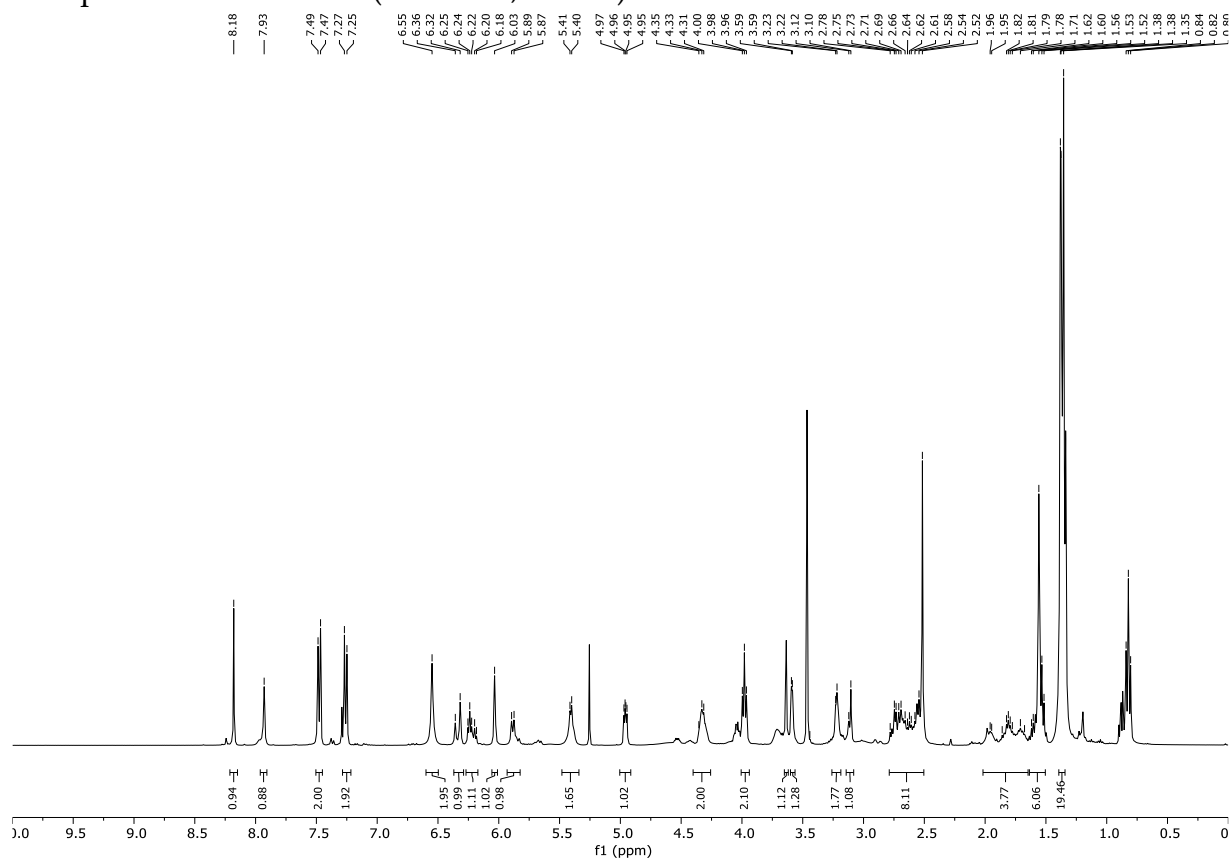


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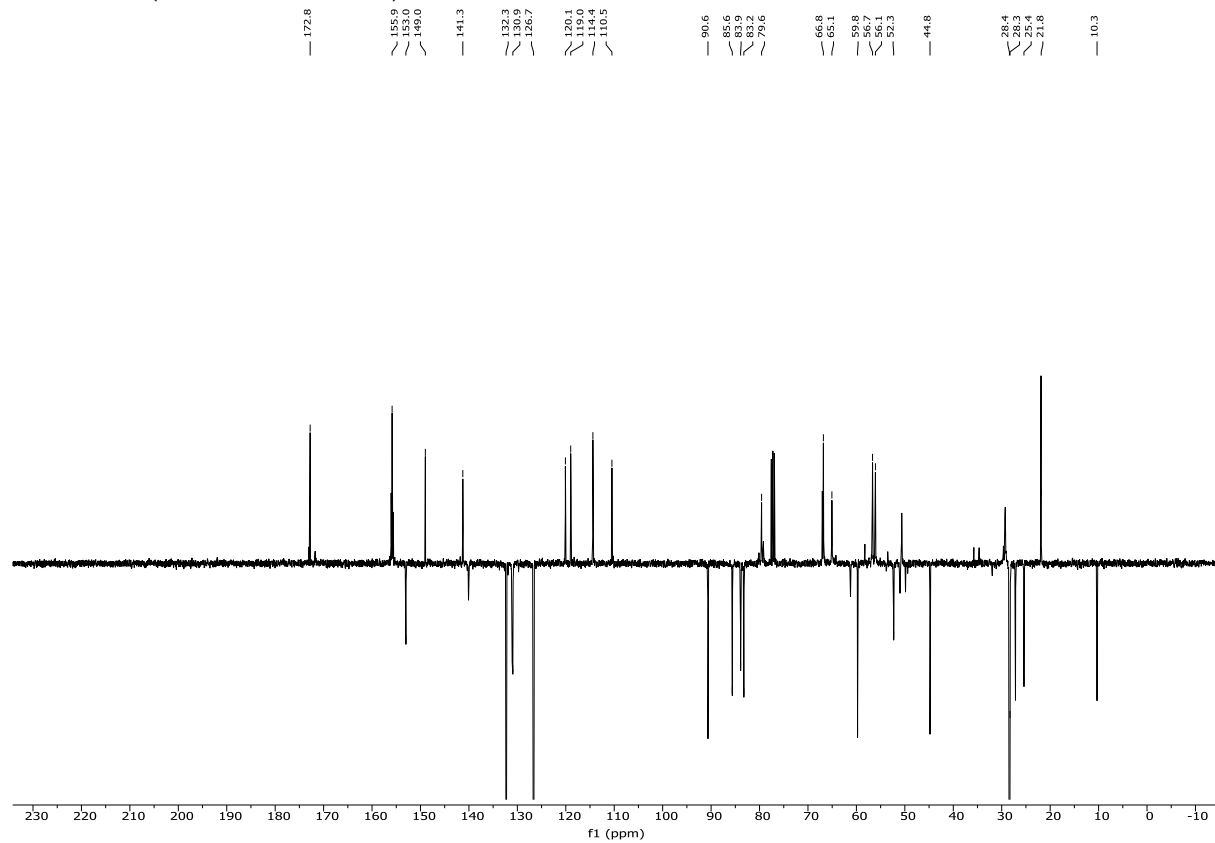




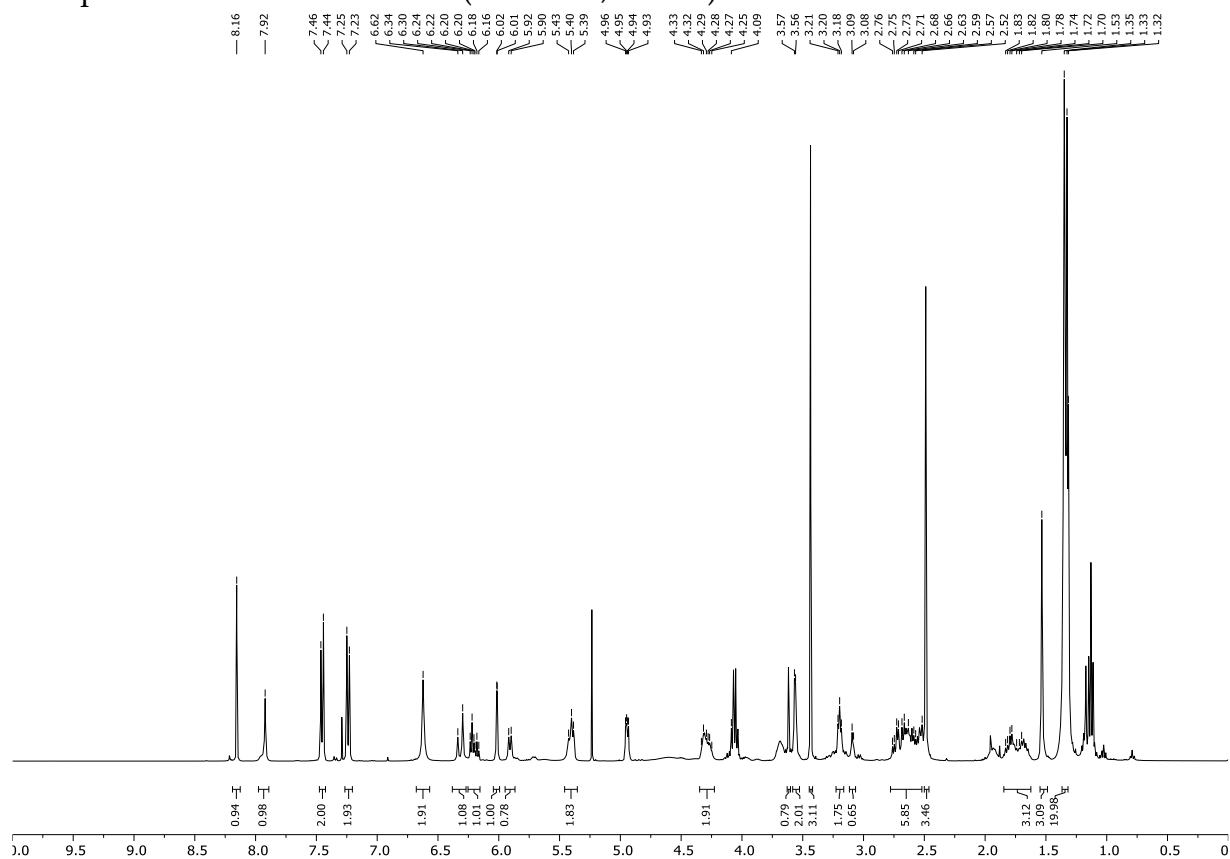
Compound **11d**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



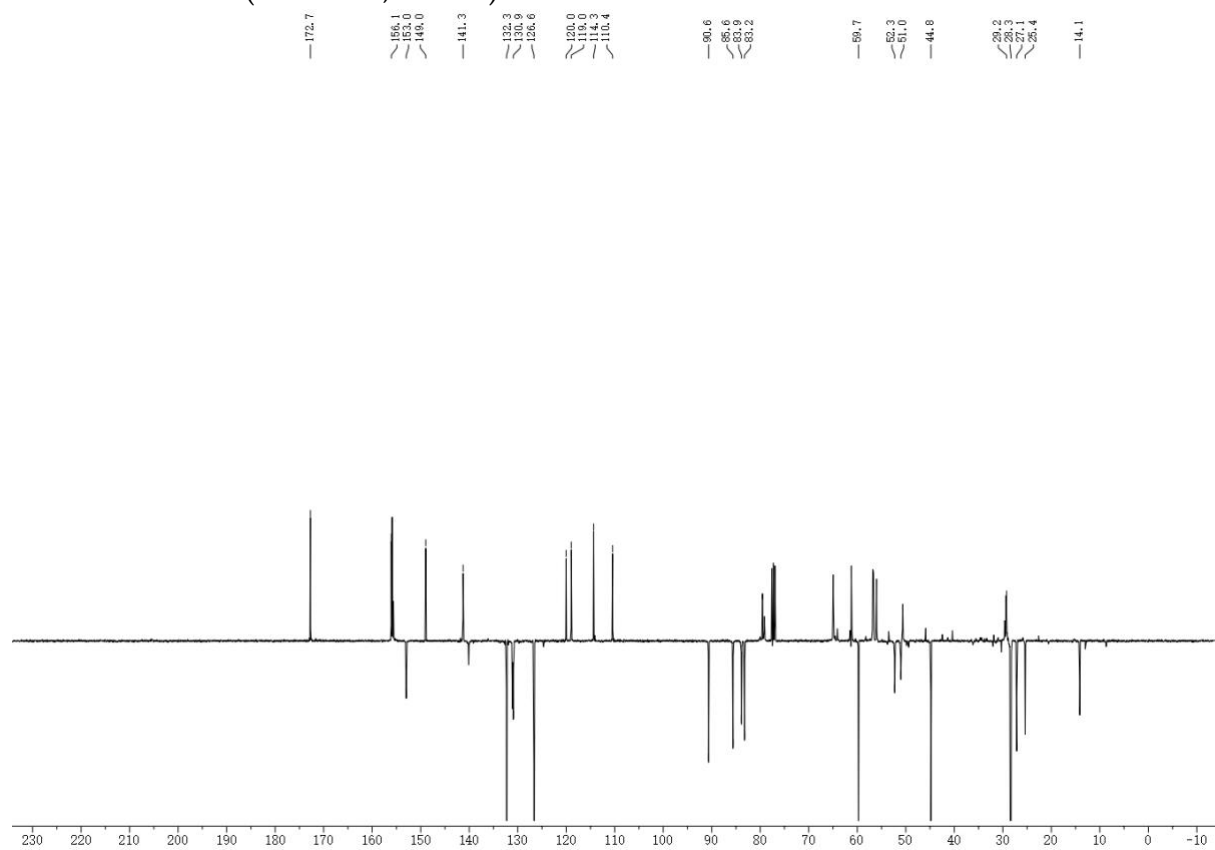
$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )



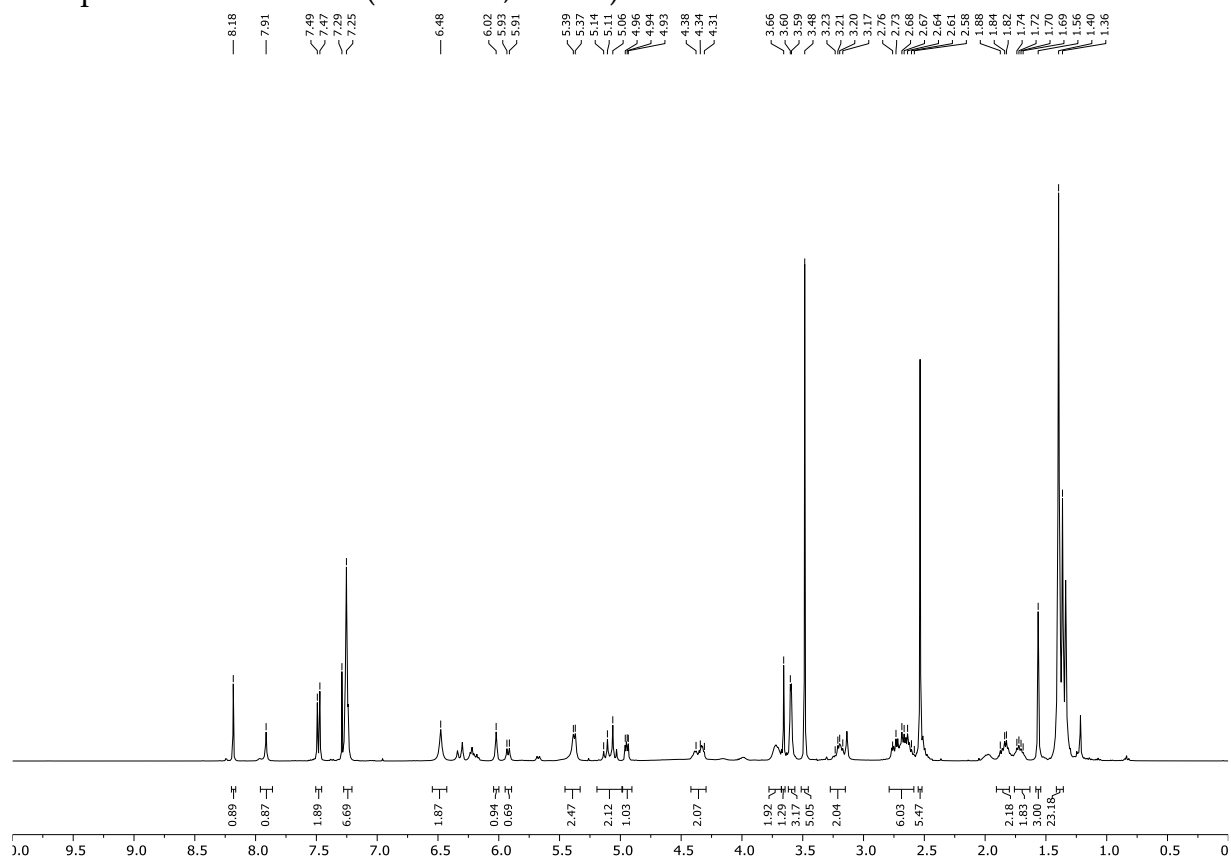
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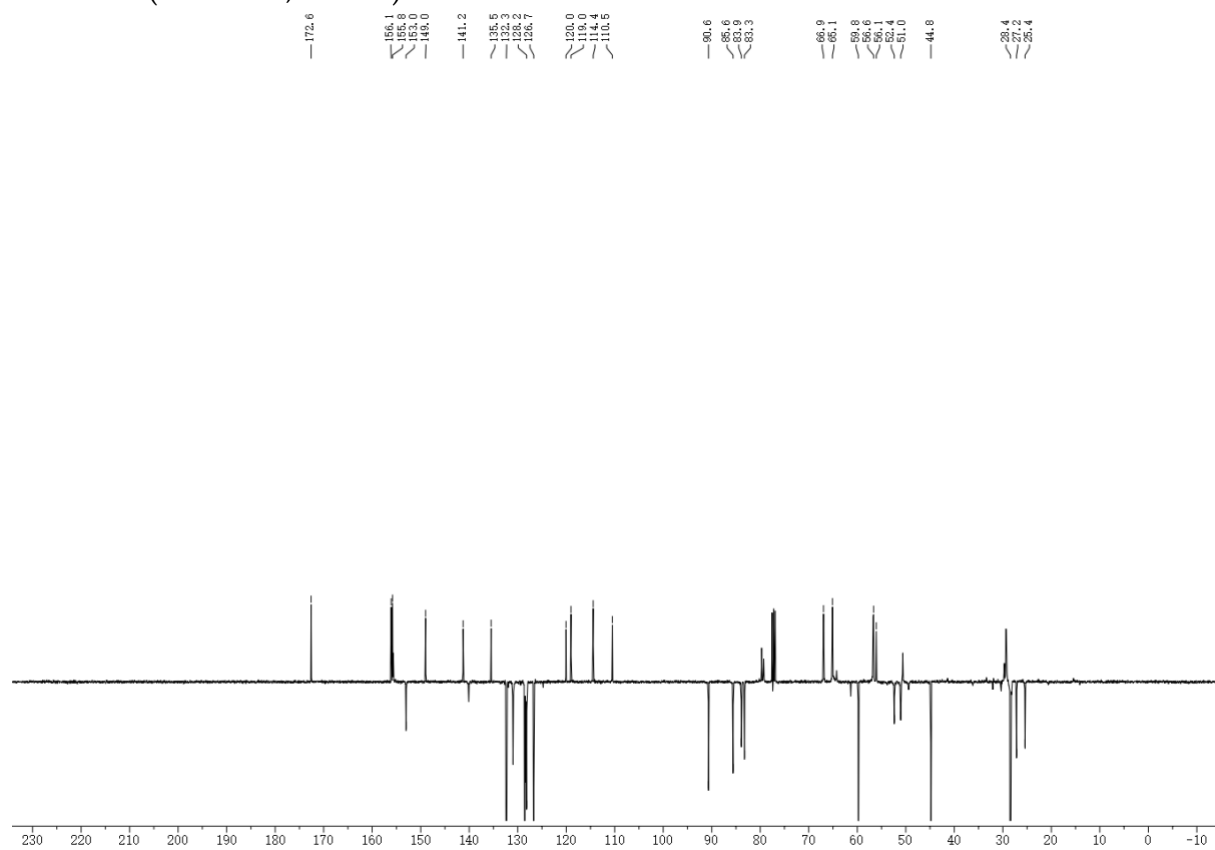
$^{13}\text{C}$  NMR  $\text{CDCl}_3$  (101 MHz,  $\text{CDCl}_3$ )



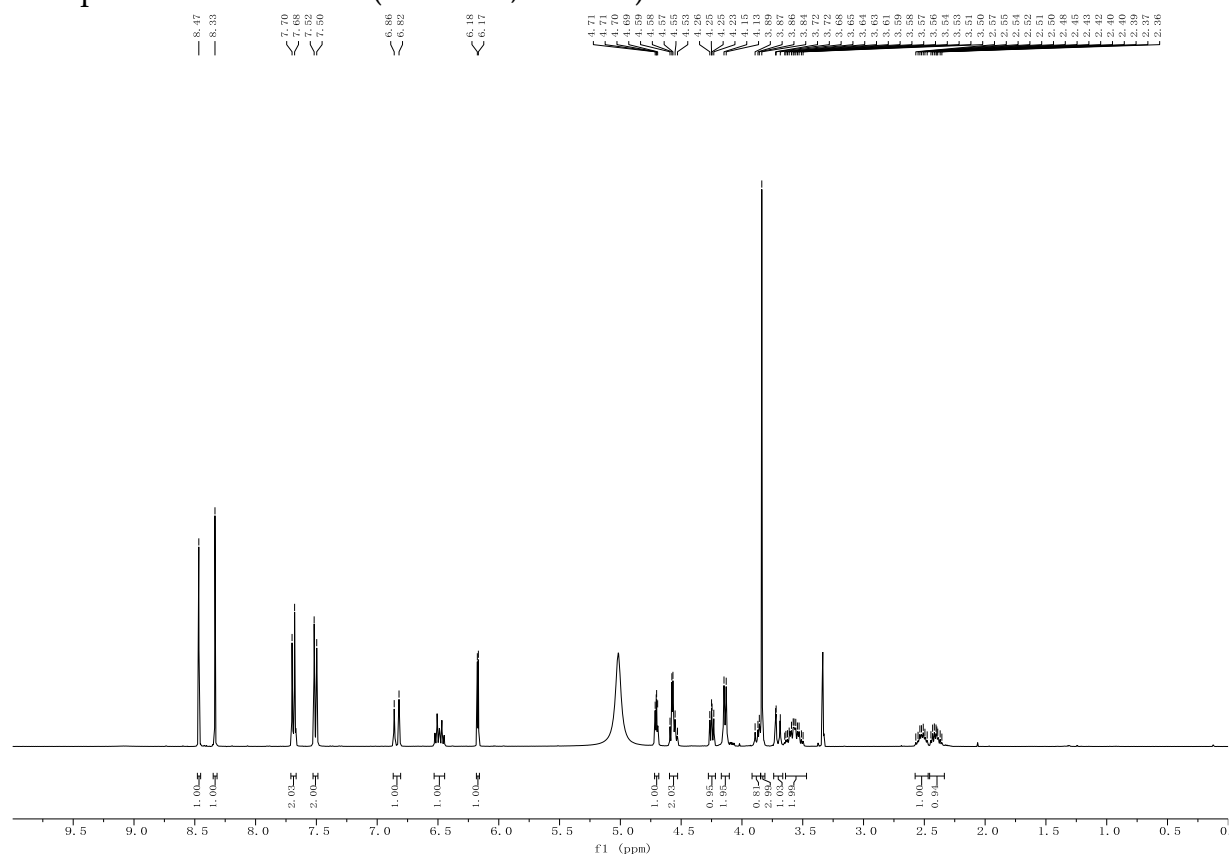
Compound **11f**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



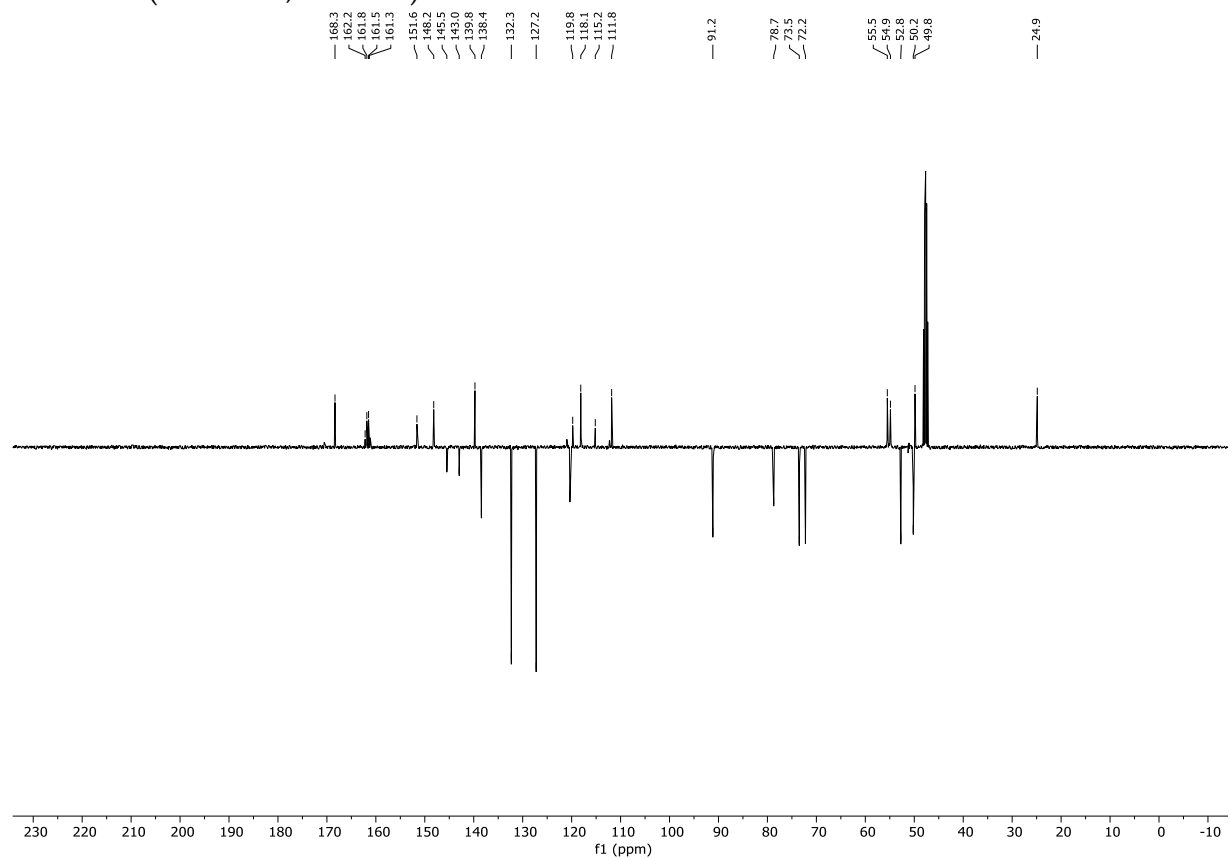
$^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ )



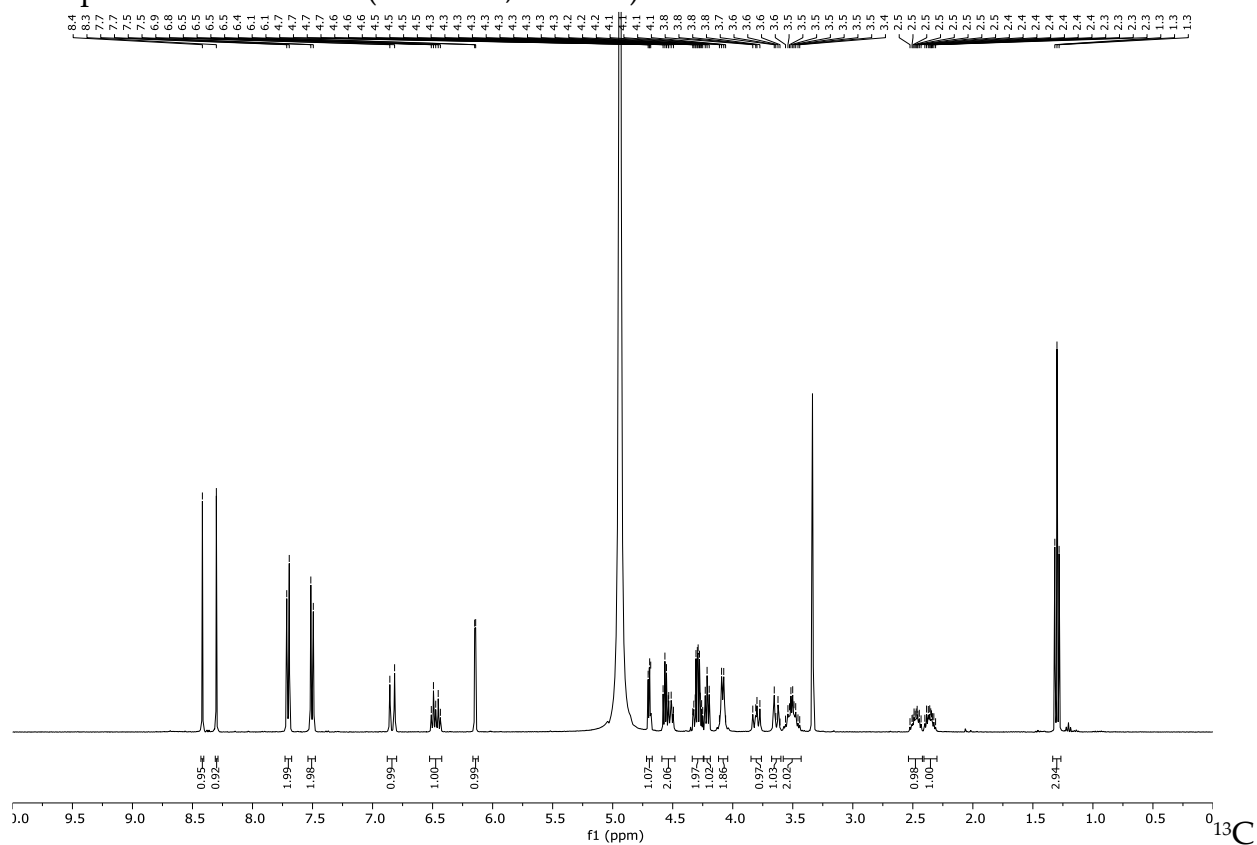
Compound **12b**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )



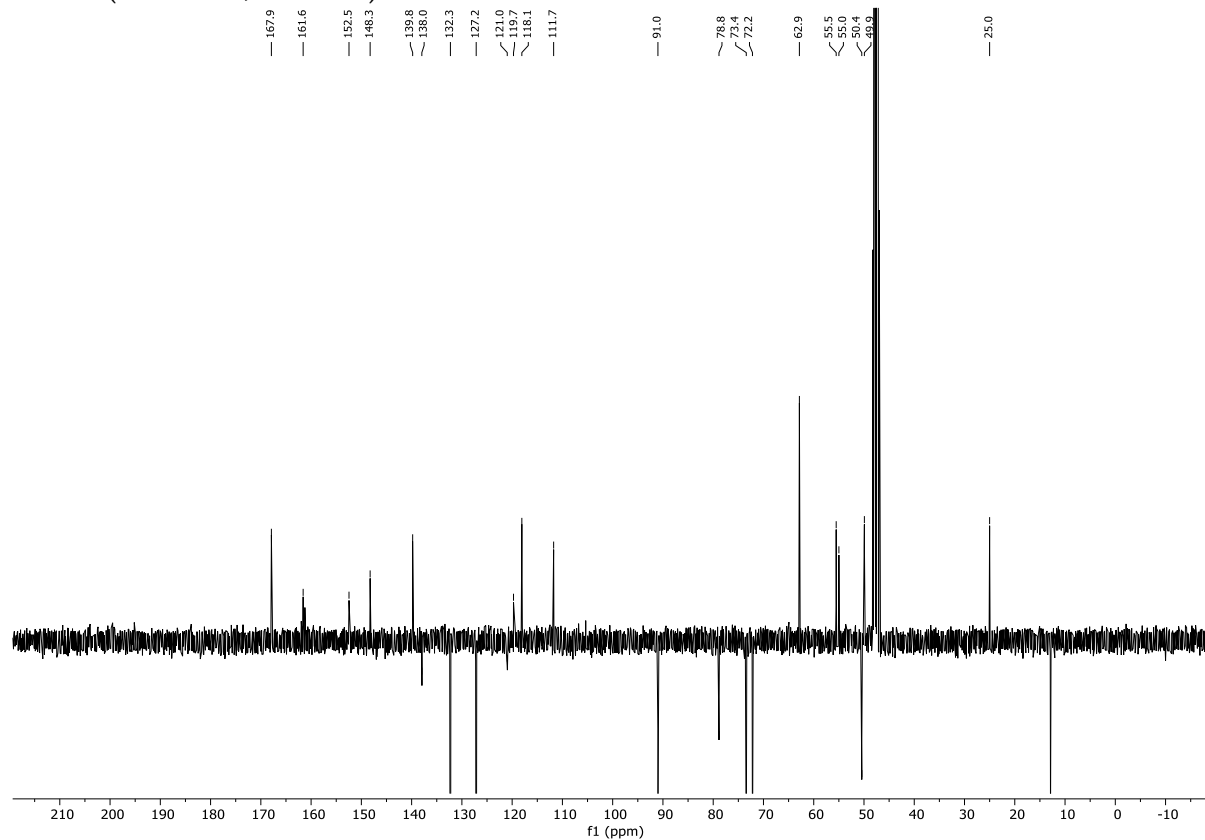
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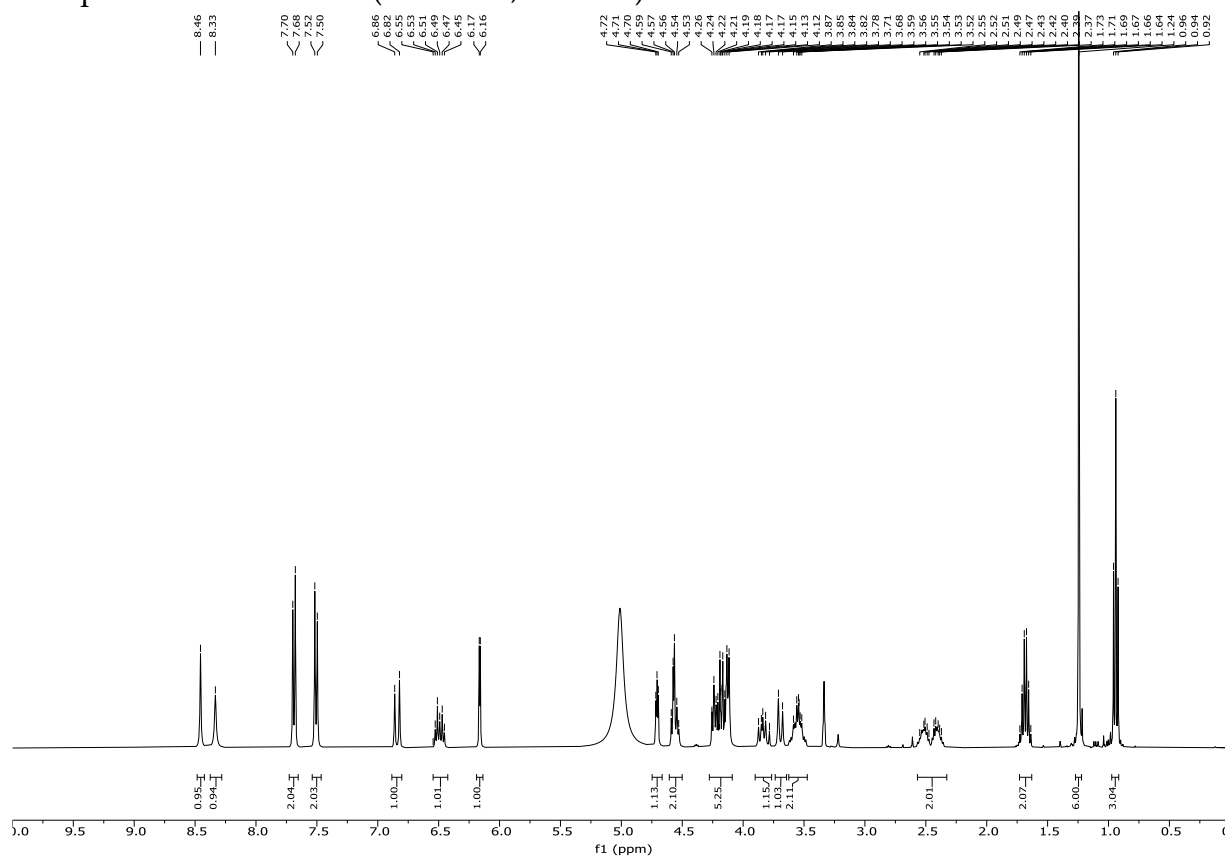
Compound **12c**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )



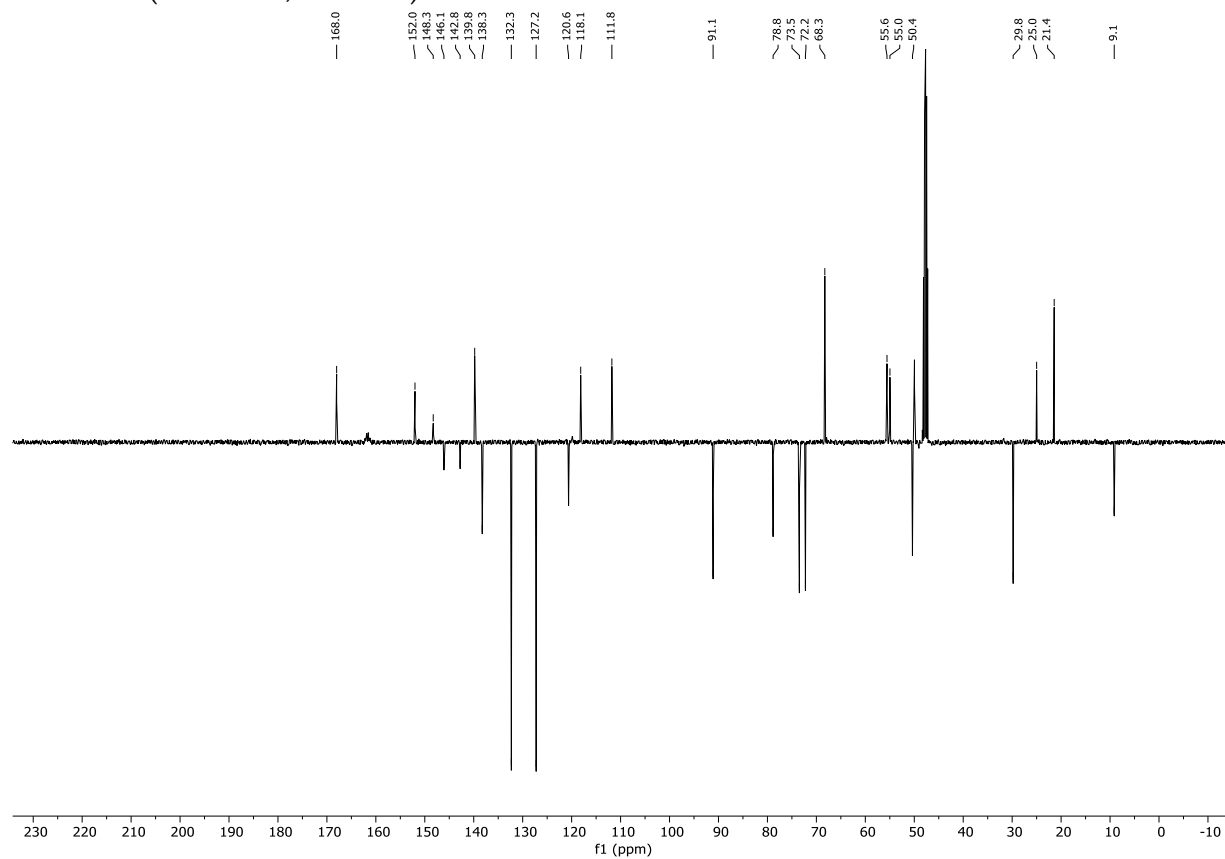
NMR (101 MHz,  $\text{CD}_3\text{OD}$ )



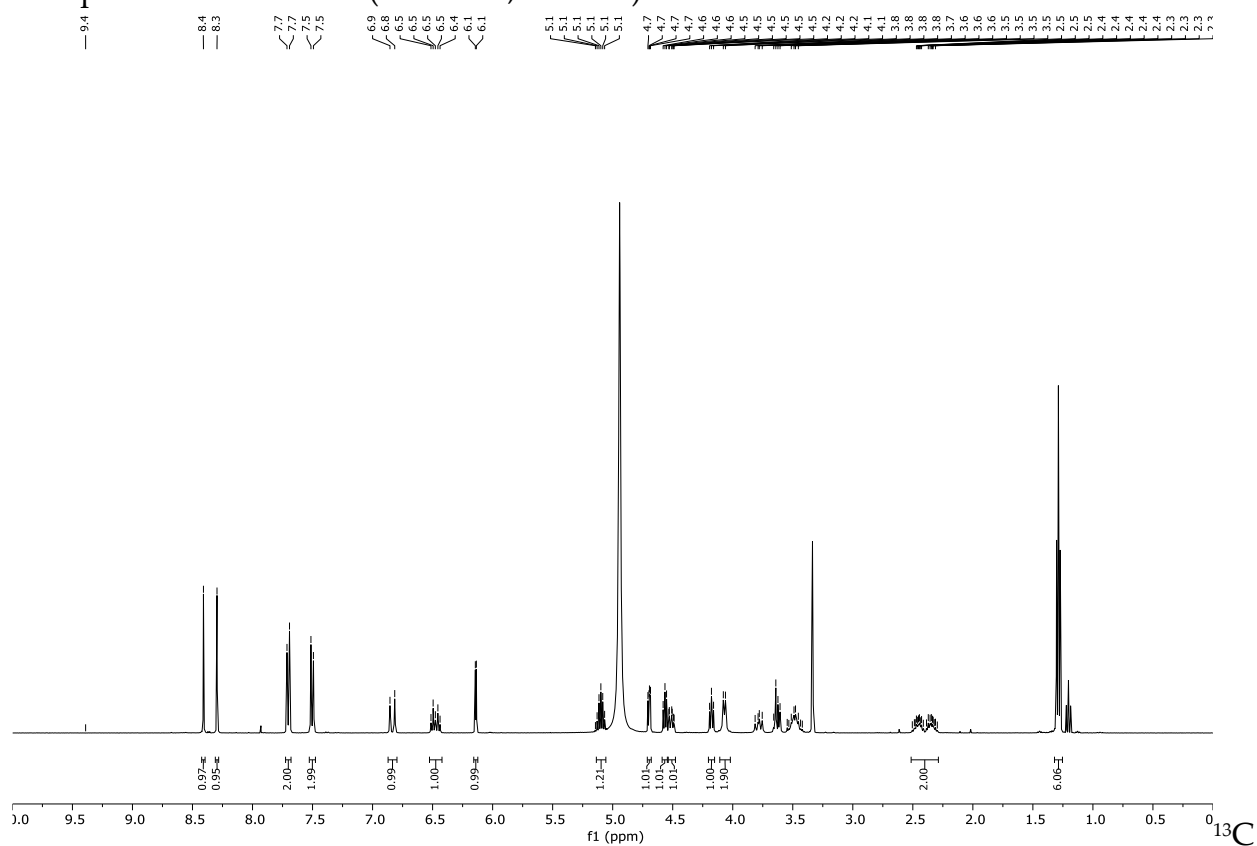
Compound **12d**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )



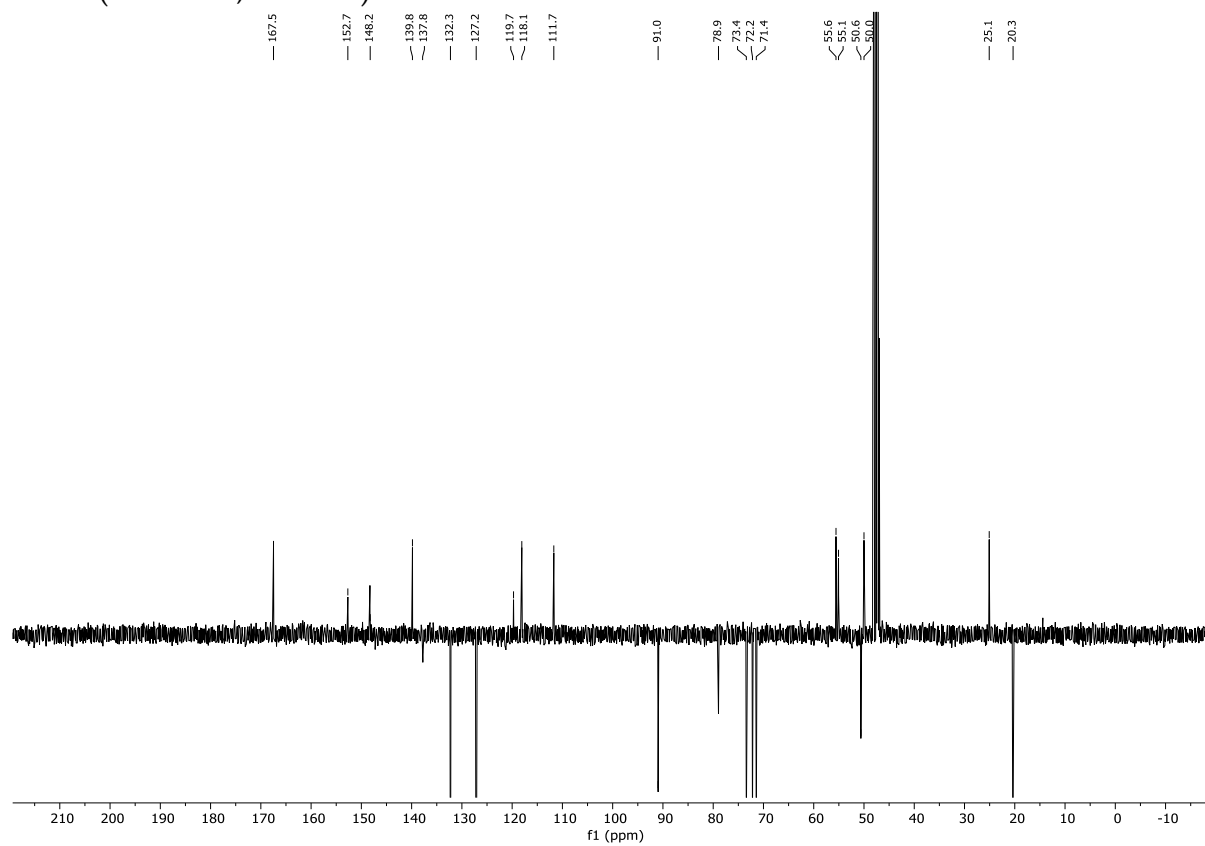
$^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ )



Compound **12e**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )



$^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ )

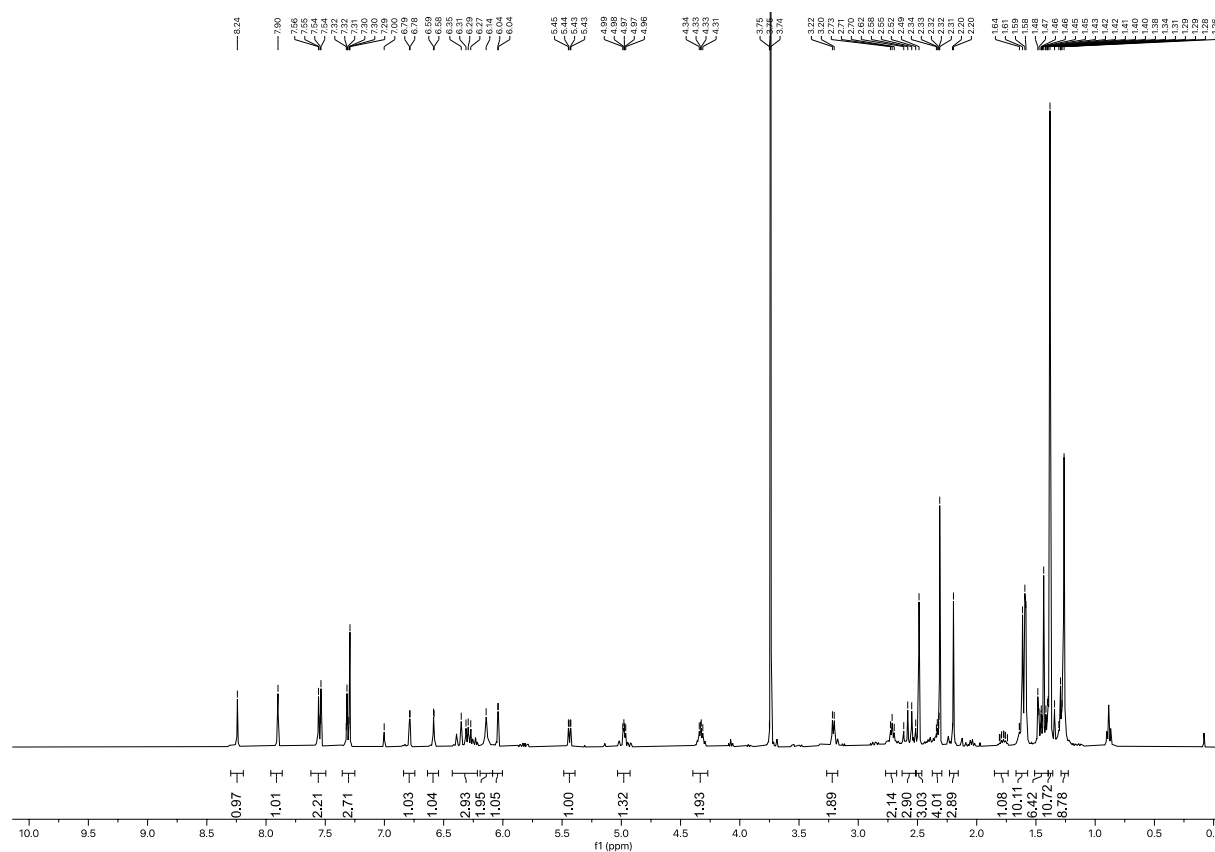


<sup>13</sup>C NMR spectrum of compound 10. The x-axis is labeled 'f1 (ppm)' and ranges from 0.0 to 10.0. The spectrum shows several peaks with corresponding integrations: 0.96, 0.96, 0.99, 4.95, 1.01, 1.02, 1.00, 2.11, 1.02, 2.06, 1.03, 1.90, 0.99, 0.99, 2.02, and 2.00. A list of chemical shifts (delta) is provided on the right side of the spectrum, ranging from 8.4 to 2.3 ppm.

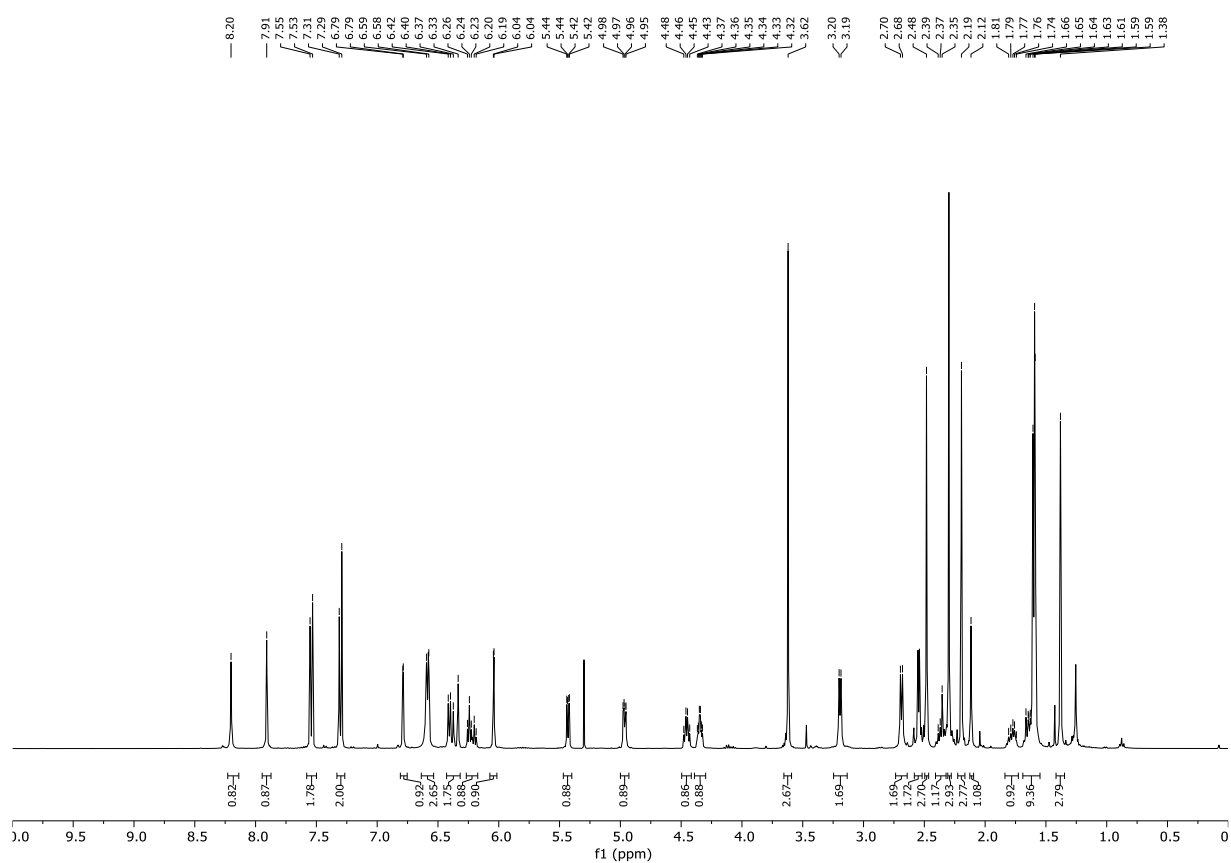
13C NMR spectrum of compound 10. The x-axis is labeled 'f1 (ppm)' and ranges from -10 to 210. The spectrum shows several sharp peaks. Key peaks are labeled with their chemical shifts: 167.9, 152.1, 148.2, 134.7, 132.3, 119.7, 118.1, 111.8, 91.0, 78.8, 73.4, 72.2, 68.2, 55.5, 50.4, 49.9, and 25.0. A large solvent peak is visible at approximately 49.9 ppm.



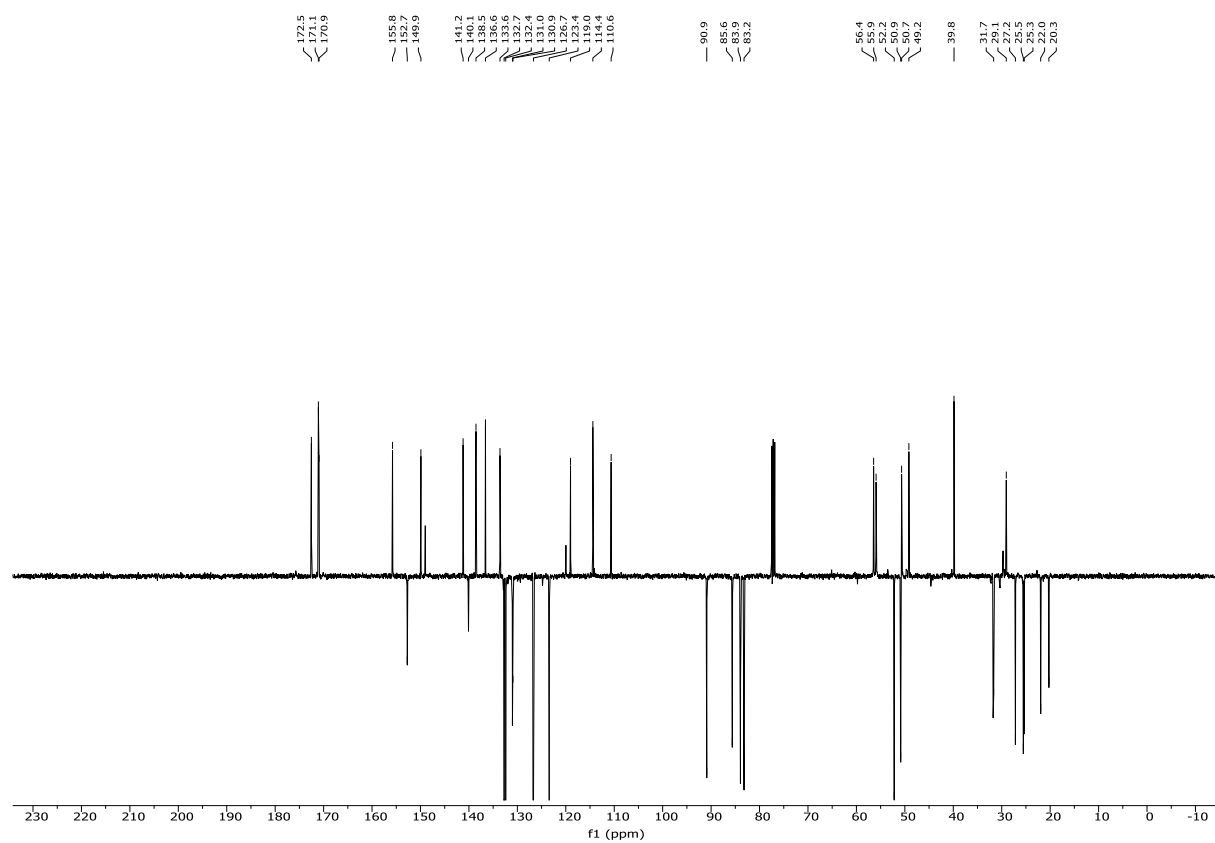
Compound **13a**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



Compound **13b**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )



$^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )



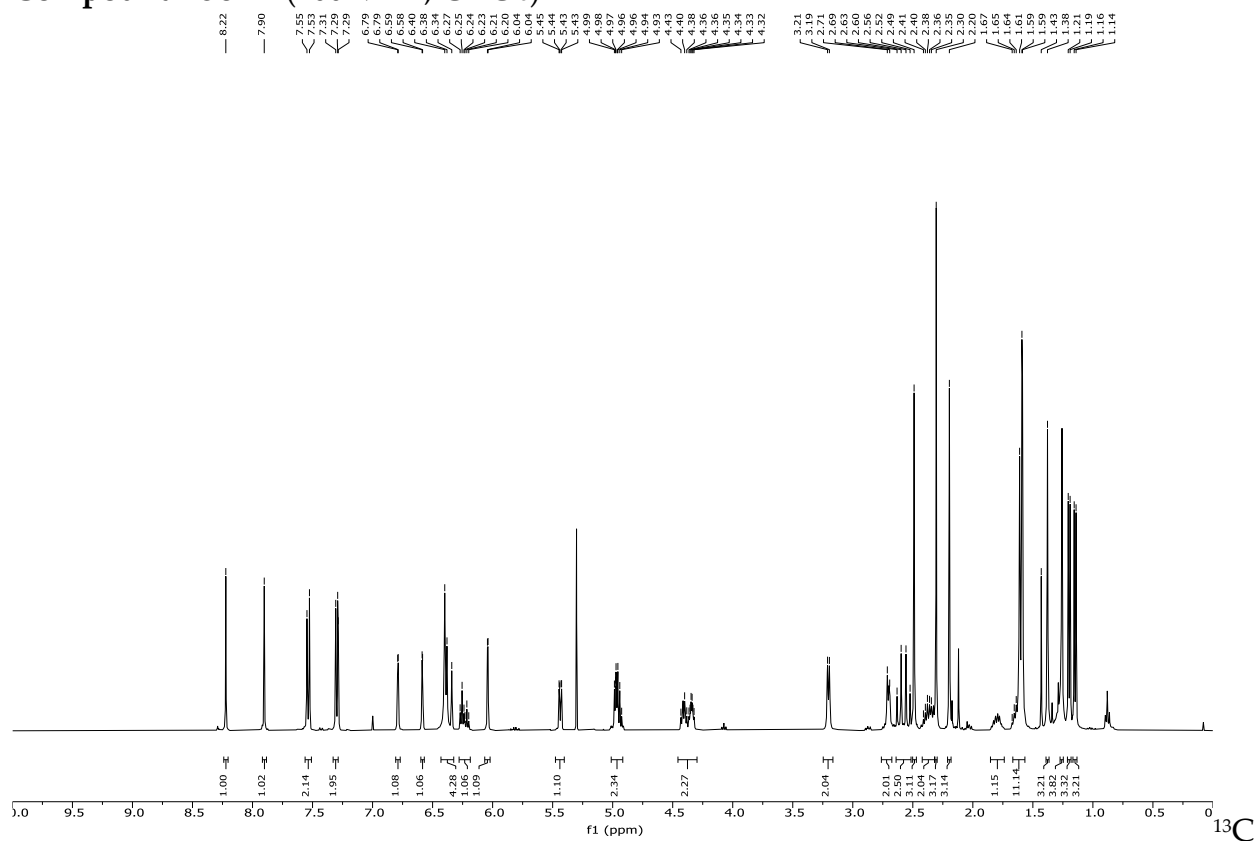
1H NMR spectrum of compound 10a in CDCl<sub>3</sub>. The spectrum shows peaks from 0.91 to 8.22 ppm. Integration values are provided below the baseline for each major peak group. The x-axis is labeled 'f1 (ppm)' and ranges from 10.0 to 0.0.

Chemical Shift (ppm)	Integration
8.22	0.91
7.91	1.04
7.58	2.02
7.55	2.60
7.21	1.19
7.20	1.09
6.79	3.88
6.77	0.83
6.75	1.00
6.25	1.00
6.21	0.98
6.00	1.00
5.85	0.97
5.84	1.06
5.83	2.51
5.43	1.80
4.97	1.77
4.96	2.69
4.86	3.30
4.45	5.28
4.43	3.75
4.35	1.05
4.33	0.94
4.13	11.12
4.12	1.10
4.08	3.91
4.06	3.61
3.21	1.77
3.19	2.69
2.71	3.30
2.69	5.28
2.62	3.75
2.59	1.05
2.56	0.94
2.39	11.12
2.34	1.10
2.33	3.91
2.31	3.61
2.17	1.77
2.10	2.69
2.00	3.30
1.66	5.28
1.65	3.75
1.61	1.05
1.59	0.94
1.57	11.12
1.43	1.10
1.37	3.91
1.36	3.61
1.34	1.77
1.30	2.69
1.28	3.30
1.22	5.28
1.19	3.75

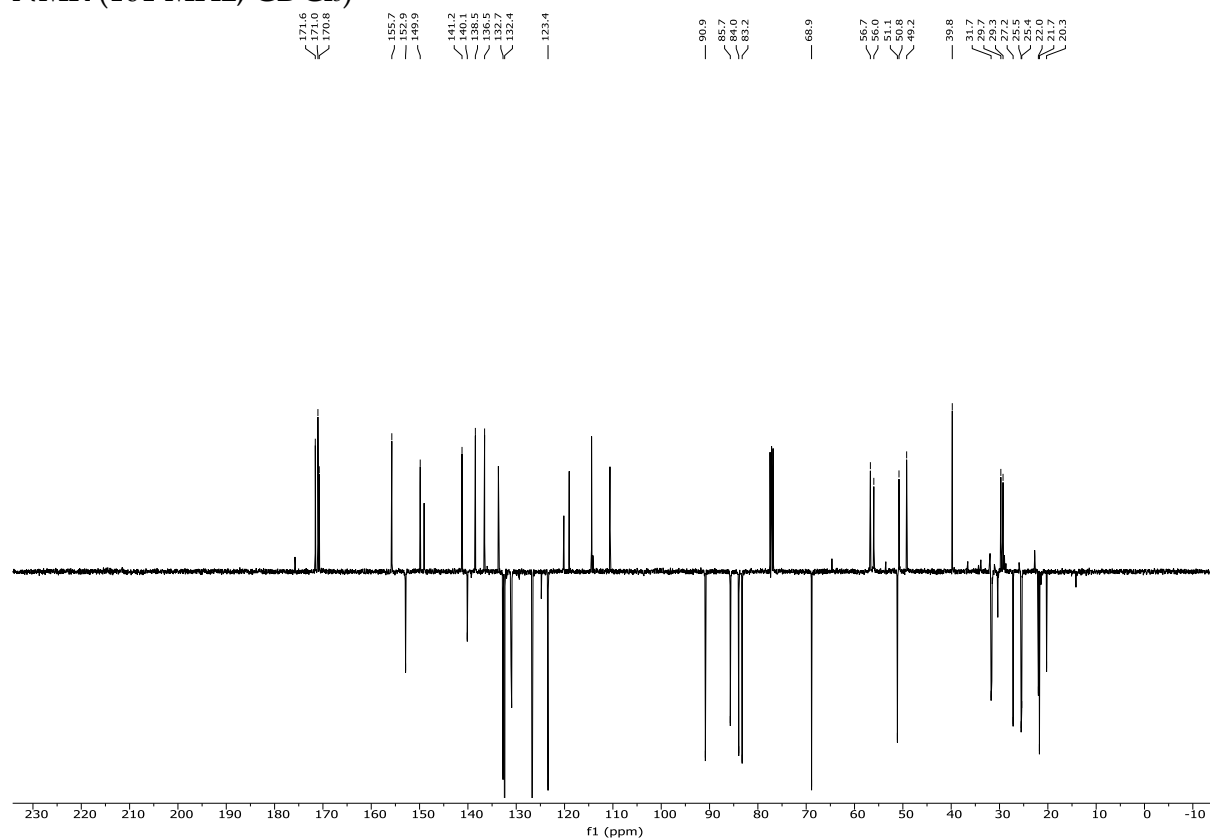
<sup>13</sup>C NMR spectrum (ppm) of compound 10b. The x-axis ranges from -10 to 230 ppm. The spectrum shows several sharp peaks, with the following chemical shift values labeled:

- 173.07, 171.07, 170.86
- 155.73, 155.69, 149.89, 149.06
- 141.22, 138.57, 136.65, 132.47, 132.41, 130.88, 128.68, 124.82, 120.11, 119.04, 114.53, 110.61
- 95.89, 86.05, 83.55, 83.23, 77.48, 77.16, 76.84
- 61.71, 61.23, 56.55, 56.08, 50.73, 49.20
- 39.81, 31.76, 30.56, 29.73, 29.40, 29.37, 25.53, 23.27, 21.96, 20.27
- 14.17

Compound 13e <sup>1</sup>H (400 MHz, CDCl<sub>3</sub>)



NMR (101 MHz, CDCl<sub>3</sub>)



<sup>1</sup>H NMR spectrum of compound 10a in CDCl<sub>3</sub>. The x-axis represents the chemical shift in ppm, ranging from 0.00 to 10.00. The spectrum shows several peaks, with integration values provided below the baseline. A list of chemical shifts (δ) is provided on the right side of the spectrum.

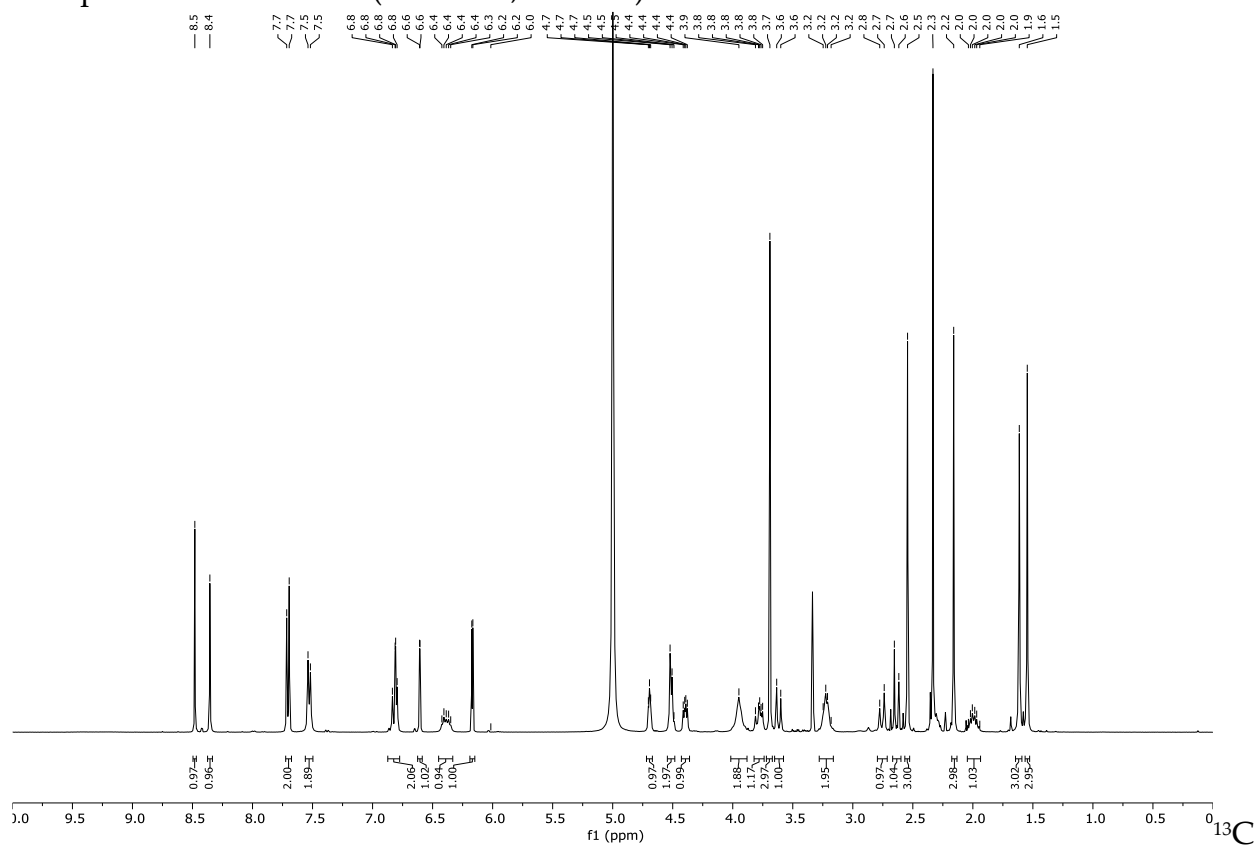
Chemical shifts (δ) listed on the right:

- 8.47, 8.33, 8.12, 8.10, 7.81, 7.56, 6.84, 6.75, 6.74, 6.86, 6.44, 6.02, 6.01, 5.75, 4.60, 4.66, 4.39, 4.36, 3.99, 3.97, 3.90, 3.19, 3.02, 2.95, 2.96, 2.84, 2.82, 2.81, 2.80, 2.79, 2.68, 2.67, 2.47, 2.46, 2.26, 2.25, 2.24, 2.23, 2.22, 2.21, 2.18, 2.16, 2.07, 1.94, 1.91, 1.48, 1.44, 1.43, 1.41.

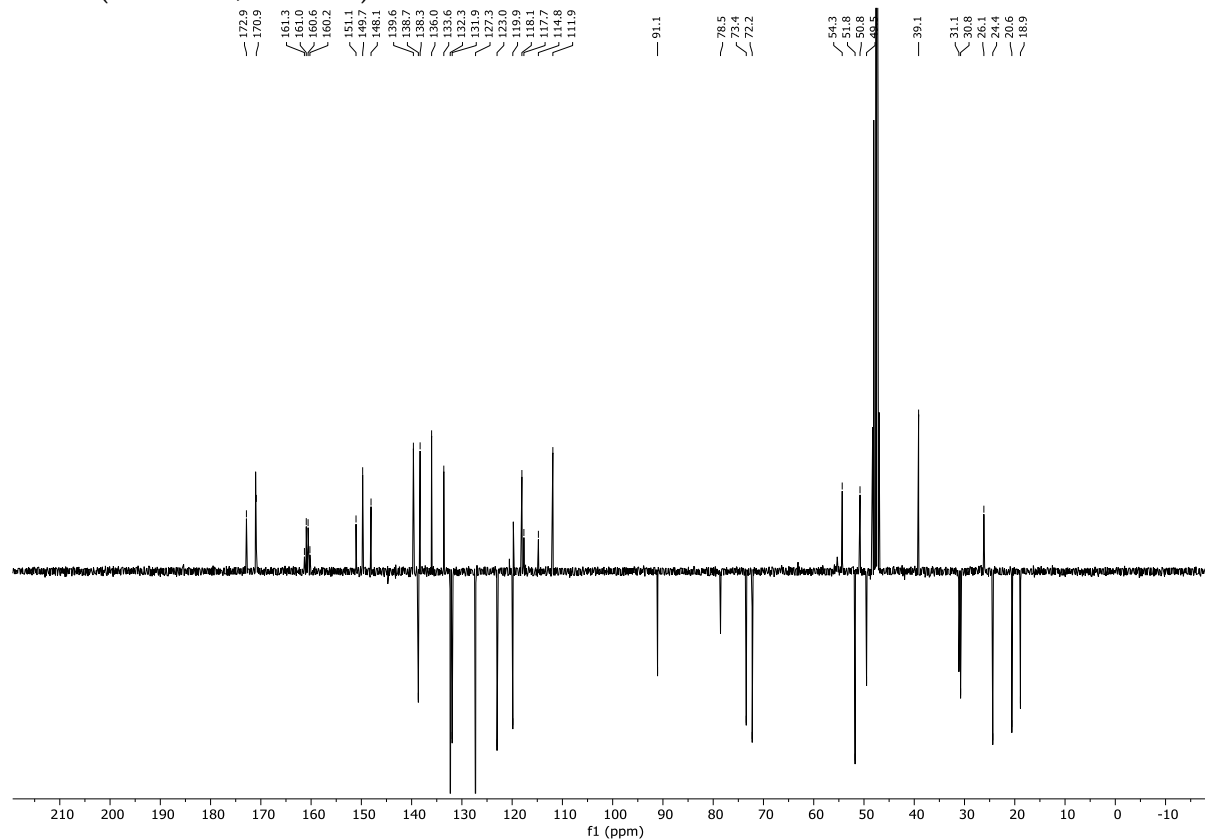
Integration values listed below the baseline:

- 0.91, 0.99, 0.83, 1.26, 2.02, 1.81, 2.01, 1.07, 0.95, 1.00, 1.11, 1.16, 2.17, 2.88, 1.16, 1.12, 15.61, 6.77, 0.89, 5.87.

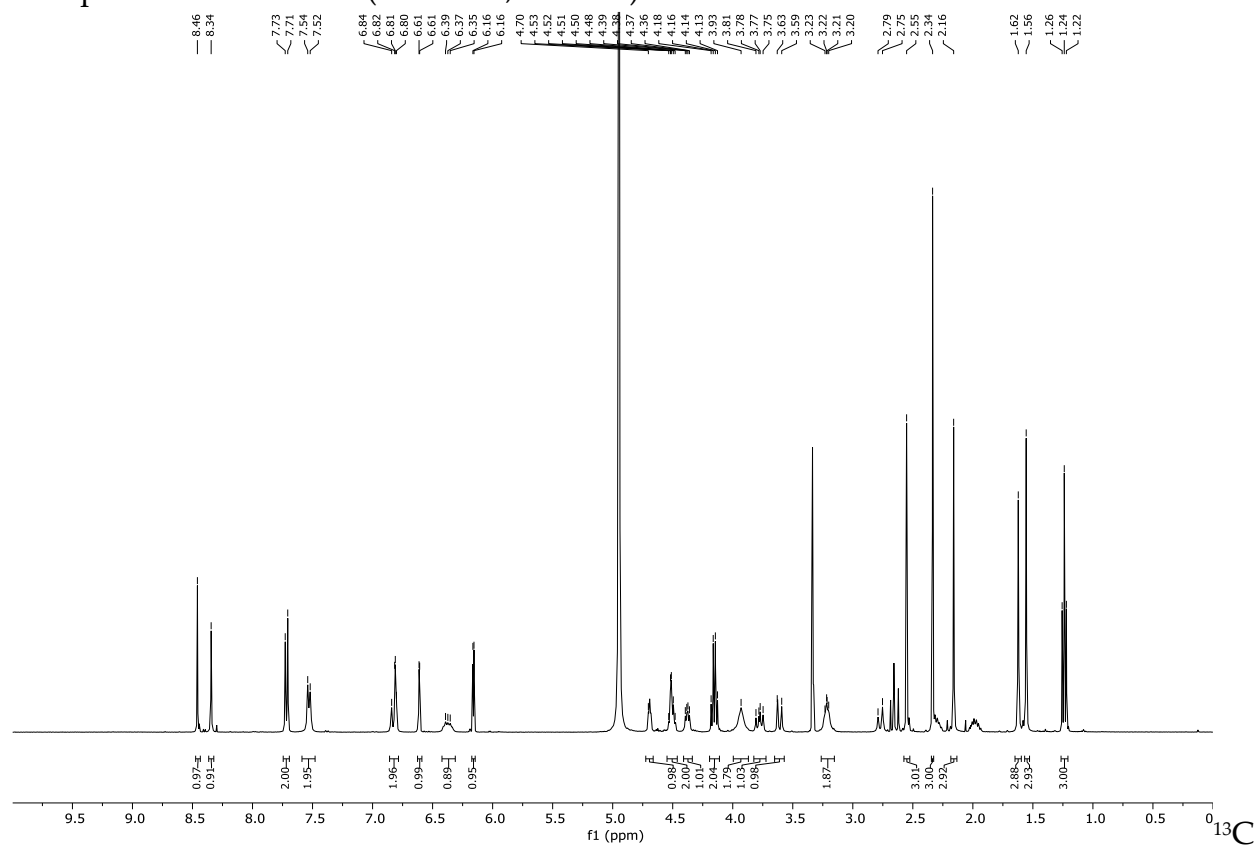
Compound **14b**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )



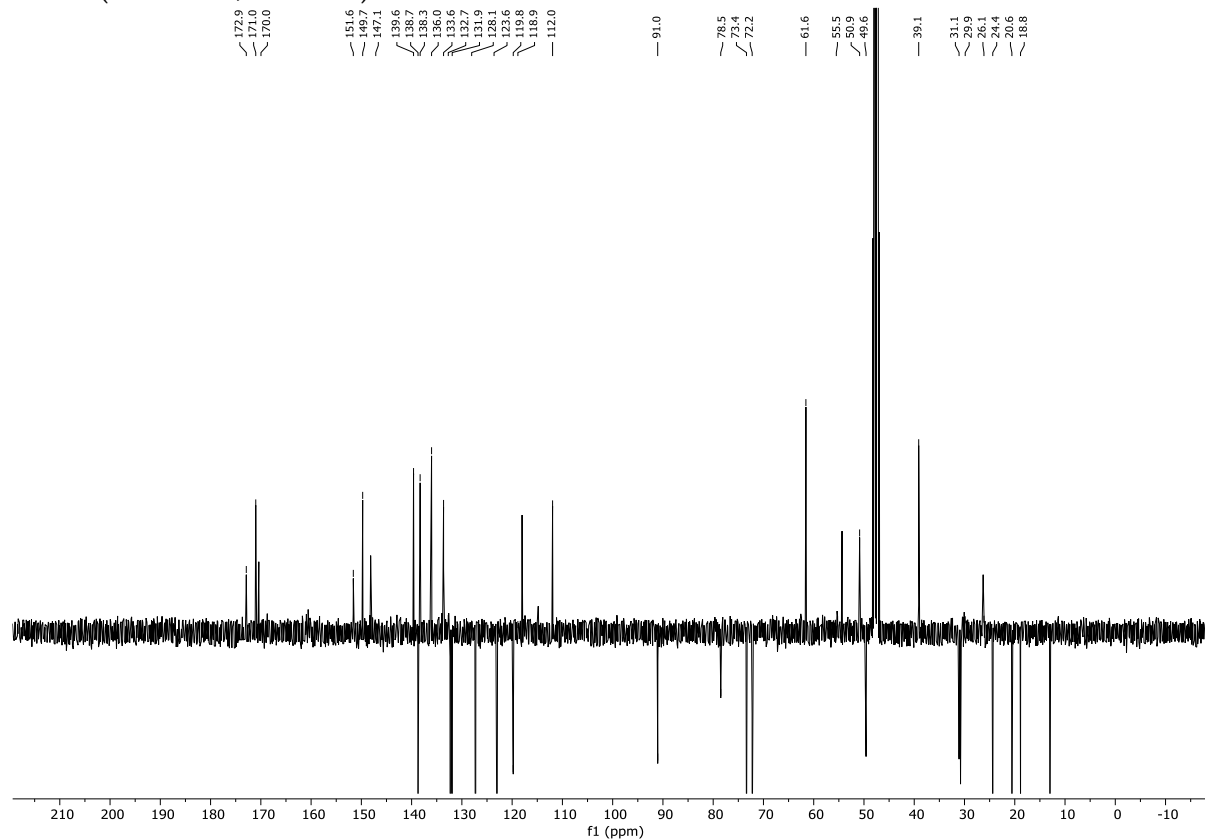
$^{13}\text{C}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )



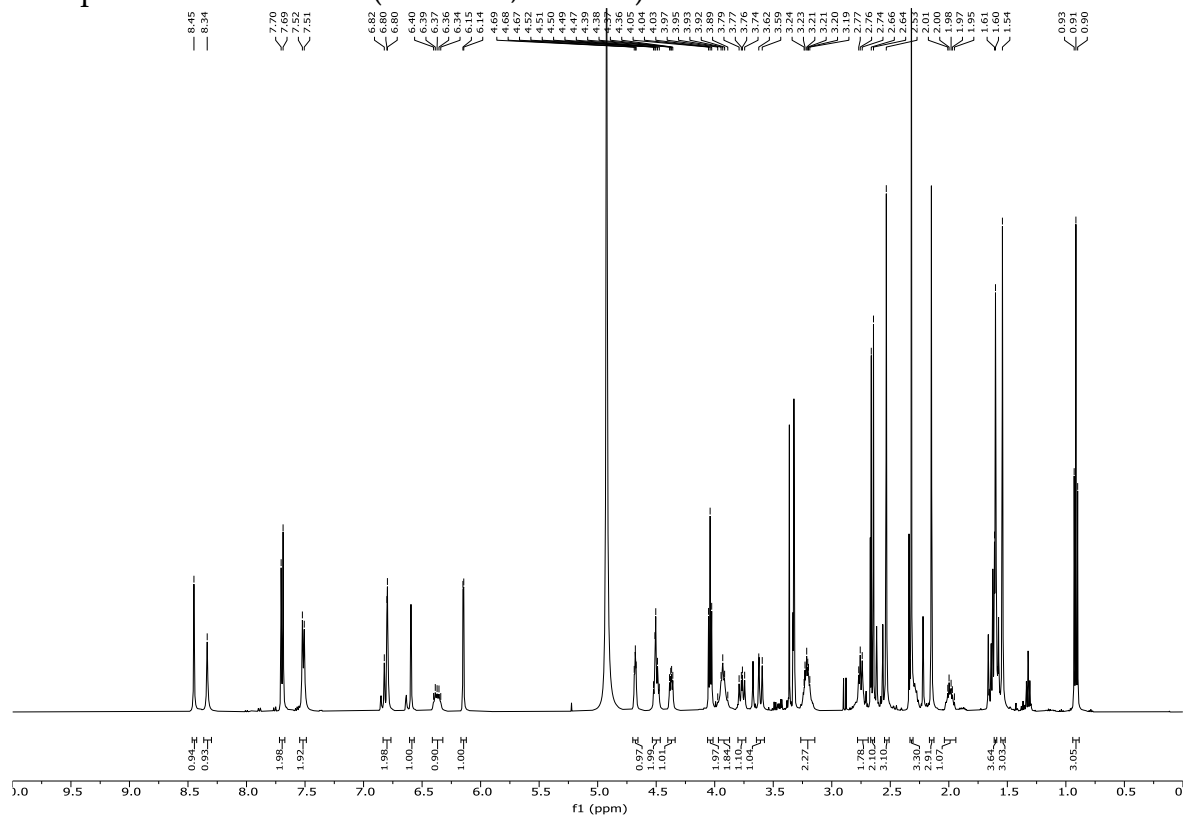
Compound **14c**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )



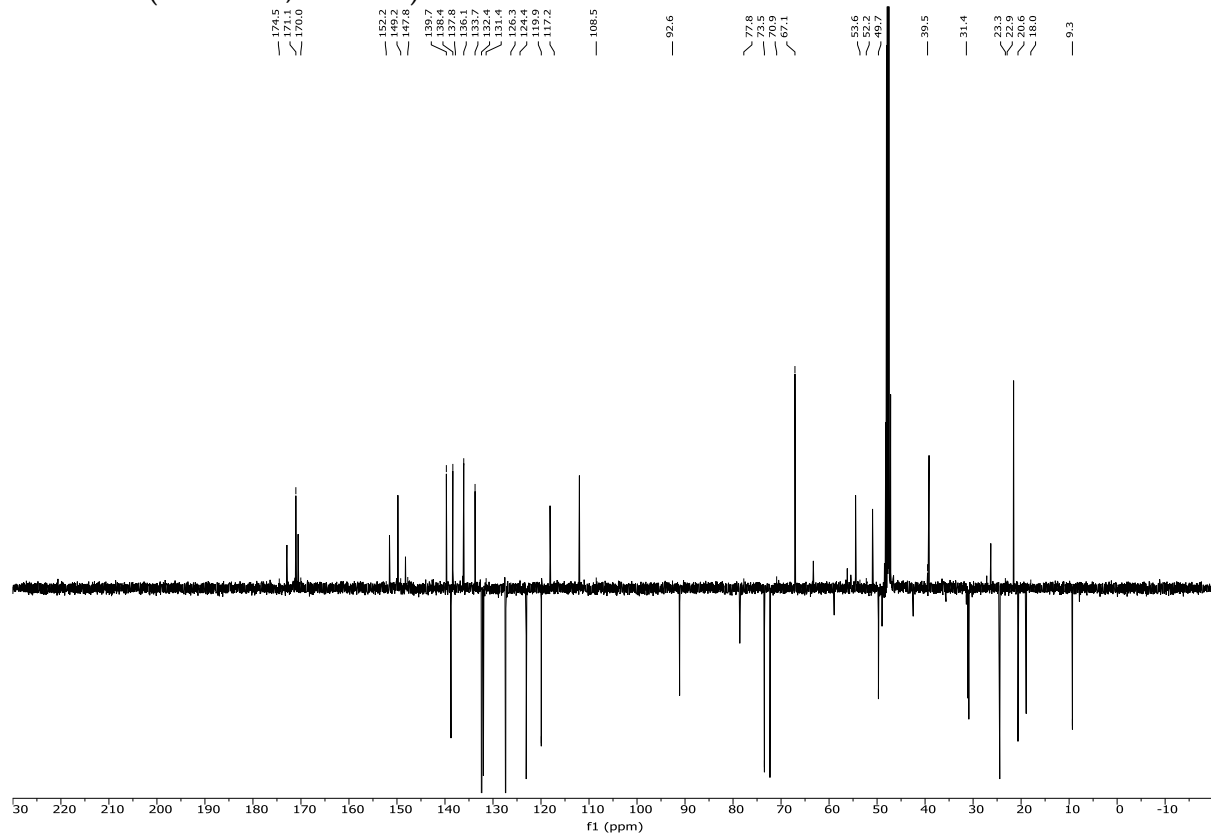
NMR (400 MHz,  $\text{CD}_3\text{OD}$ )



Compound **14d**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )

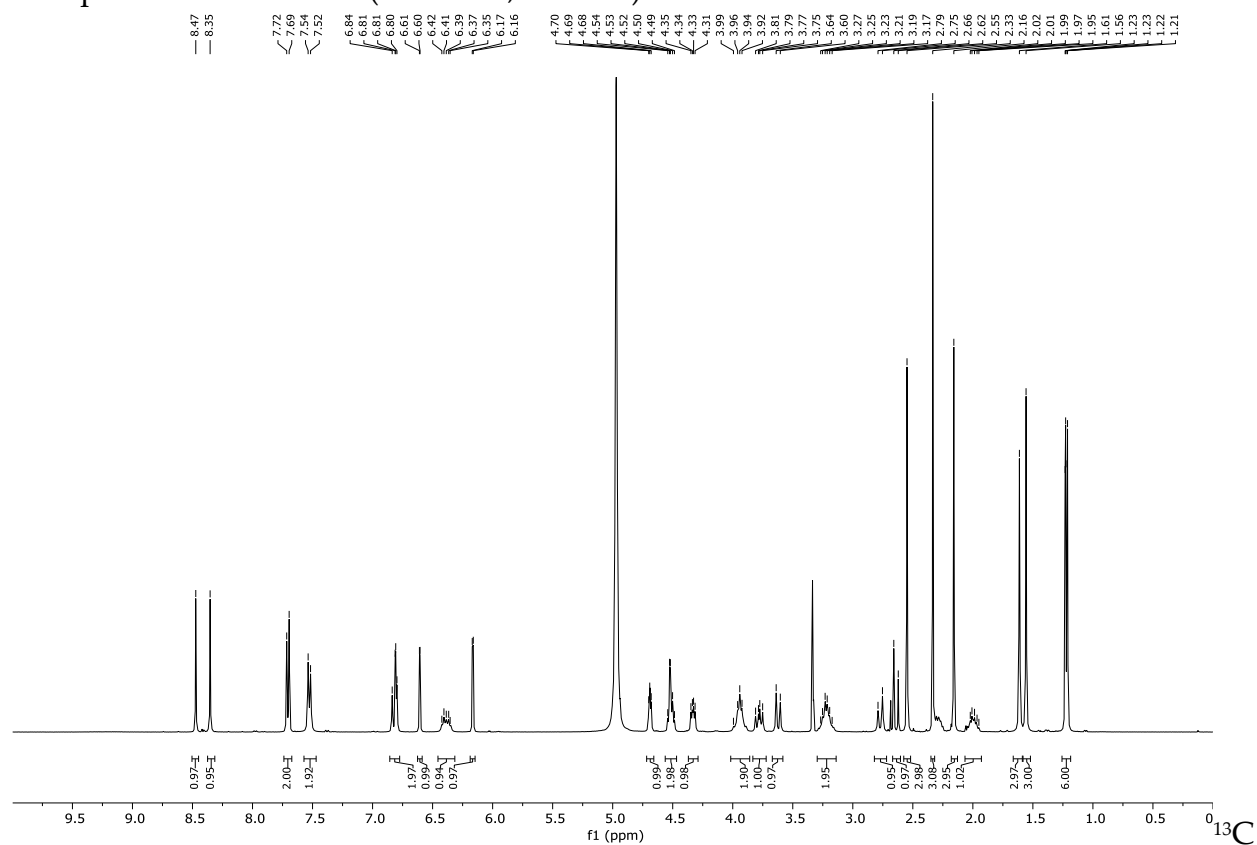


$^{13}\text{C}$  NMR (126 MHz,  $\text{CD}_3\text{OD}$ )

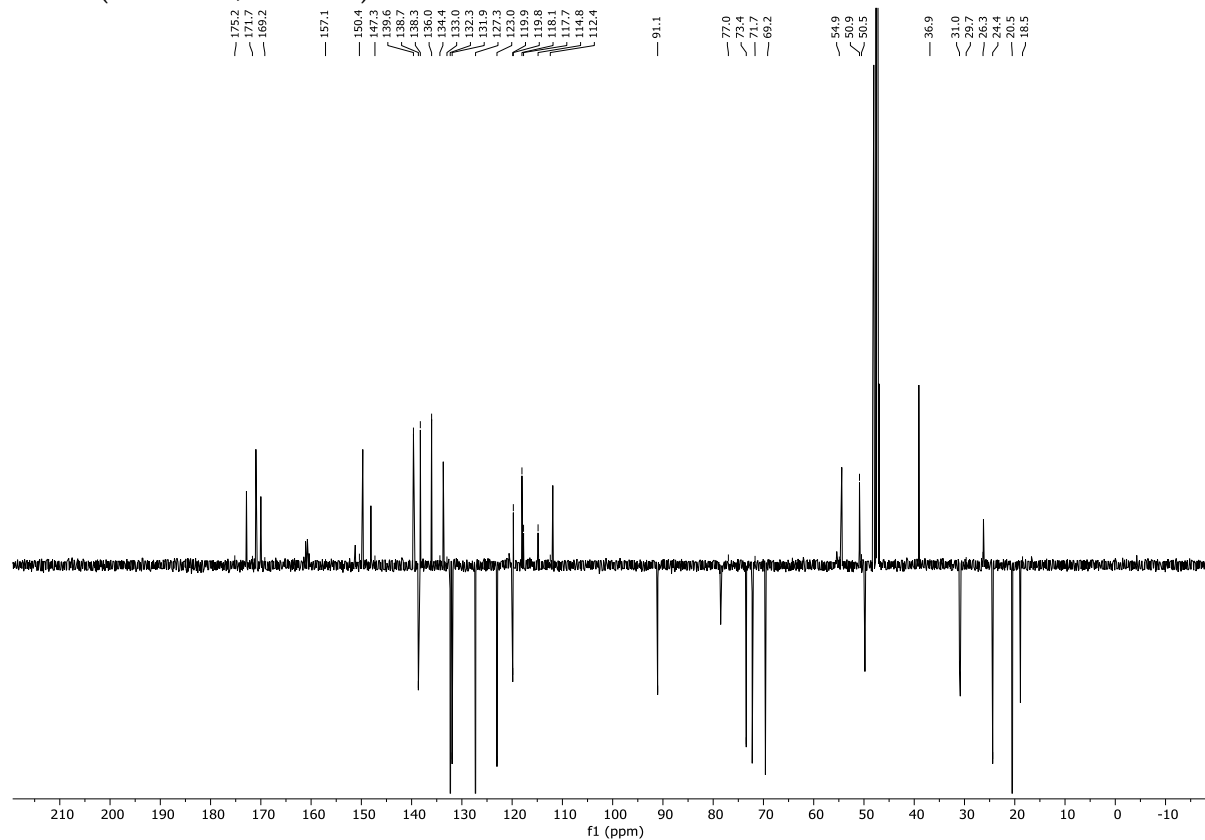




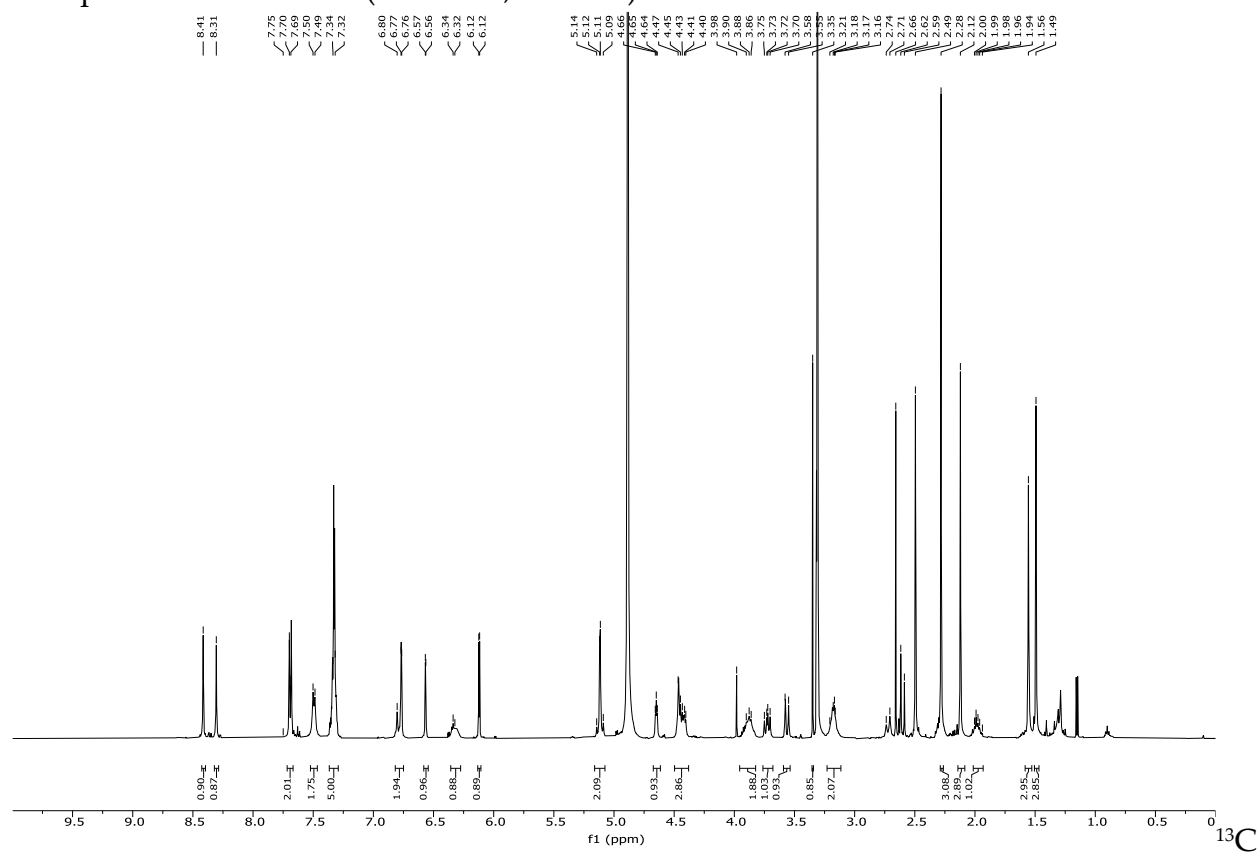
Compound **14e**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )



NMR (101 MHz,  $\text{CD}_3\text{OD}$ )



Compound **14f**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )



NMR (126 MHz,  $\text{CD}_3\text{OD}$ )

