

Supporting Information

Synthesis and evaluation of self-calibrating ratiometric viscosity sensors

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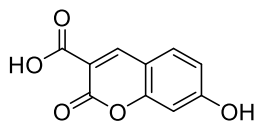
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General Procedures

All reagents were purchased at highest commercial quality and used without further purification except where noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation below 45 °C at approximately 20 mmHg. All non-aqueous reactions were carried out under anhydrous conditions. Yields refer to chromatographically and spectroscopically (¹H NMR, ¹³C NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Dynamic Adsorbents, Inc. silica gel plates (60F-254) and visualized under UV light and/or developed by dipping in solutions of 10% ethanolic phosphomolybdic acid (PMA) and applying heat. Dynamic Adsorbents, Inc. silica gel (60, particle size 0.040-0.063 mm) was used for flash chromatography. NMR spectra were recorded on the Varian Mercury 400, 300 and/or Unity 500 MHz instruments and calibrated using the residual non-deuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. High resolution mass spectra (HRMS) were recorded on a VG 7070 HS mass spectrometer under electron spray ionization (ESI) or electron impact (EI) conditions.

Synthesis of 7-Hydroxy-3-carboxycoumarin (**5**).

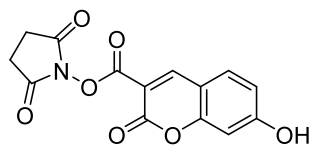


5

To a round bottom flask, diethyl malonate (3.6 mL, 23.71 mmol) was added to a solution of 2,5-Dihydroxybenzaldehyde (2.0g, 14.48 mmol) in ethanol (100 mL). Piperidine (2.4 mL, 24.30mmol) was added to the resulting solution. The reaction was heated to reflux overnight and upon completion it was concentrated under reduced pressure and purified via flash chromatography (10% MeOH-DCM). The purified product (2.47g, 10.14 mmol) and HCl (37%, 4.7 mL) in water (47 mL) was refluxed overnight. The suspension was cooled to room temperature and the precipitate was isolated by filtration. The filtrate

was washed with cold water and ethanol and dried under vacuum to yield **5** (0.865 g, 57%). **5**: beige solid; ^1H NMR (400 MHz, DMSO- d_6) δ 8.66 (s, 1H), 7.73 (d, 1H, $J = 8.6$ Hz), 6.83 (dd, 1H, $J = 2.0$ Hz, $J = 8.6$ Hz), 6.72 (d, 1H, $J = 2.0$ Hz); ^{13}C NMR (100 MHz, DMSO- d_6) δ 164.3, 164.0, 157.6, 157.1, 149.5, 132.1, 114.1, 112.5, 110.7, 101.9; HRMS calc for $\text{C}_{10}\text{H}_6\text{O}_5\text{Na}$ ($\text{M}+\text{Na}$) $^+$ 229.0107 found 229.0106.

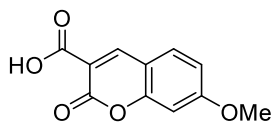
Synthesis of 7-Hydroxy-3-carboxycoumarin, succinimidyl ester (**8**)



8

To a round bottom flask, N-hydroxysuccinimide (115 mg, 1.12 mmol) and EDC·HCL (192 mg, 1.23 mmol) were added to a stirred suspension of 7-Hydroxy-3-carboxycoumarin (**5**) (230 mg, 1.15 mmol) in DMF (10 mL) at room temperature. The reaction was stirred overnight and upon completion, it was quenched with a 10% citric acid solution (10 ml) and washed with ethyl acetate (3 x 30 ml). The product was isolated by filtration (310 mg, 92%). **8**: pale yellow solid; ^1H NMR (400 MHz, DMSO- d_6) δ 9.01 (s, 1H), 7.87 (d, 1H, $J = 8.7$ Hz), 6.90 (dd, 1H, $J = 2.1$ Hz, $J = 8.7$ Hz), 6.77 (d, 1H, $J = 2.1$ Hz), 2.88 (s, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 170.4, 165.9, 158.7, 158.1, 155.7, 152.8, 133.3, 114.6, 110.5, 106.3, 102.0, 25.5; HRMS calc for $\text{C}_{14}\text{H}_9\text{NO}_7\text{Na}$ ($\text{M}+\text{Na}$) $^+$ 326.0271 found 326.0273.

Synthesis of 7-Methoxy-3-carboxycoumarin (**6**)

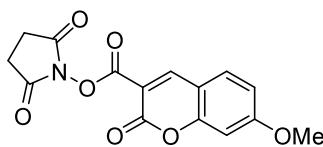


6

To a round bottom flask, Meldrum's Acid (1.00 g, 6.57 mmol) was added to a solution of 2-hydroxy-4-methoxybenzaldehyde (0.947 g, 6.57 mmol) in ethanol (5 ml). Piperidine (10 μl , 0.101 mmol) was then added to the resulting solution and stirred for 20 minutes at

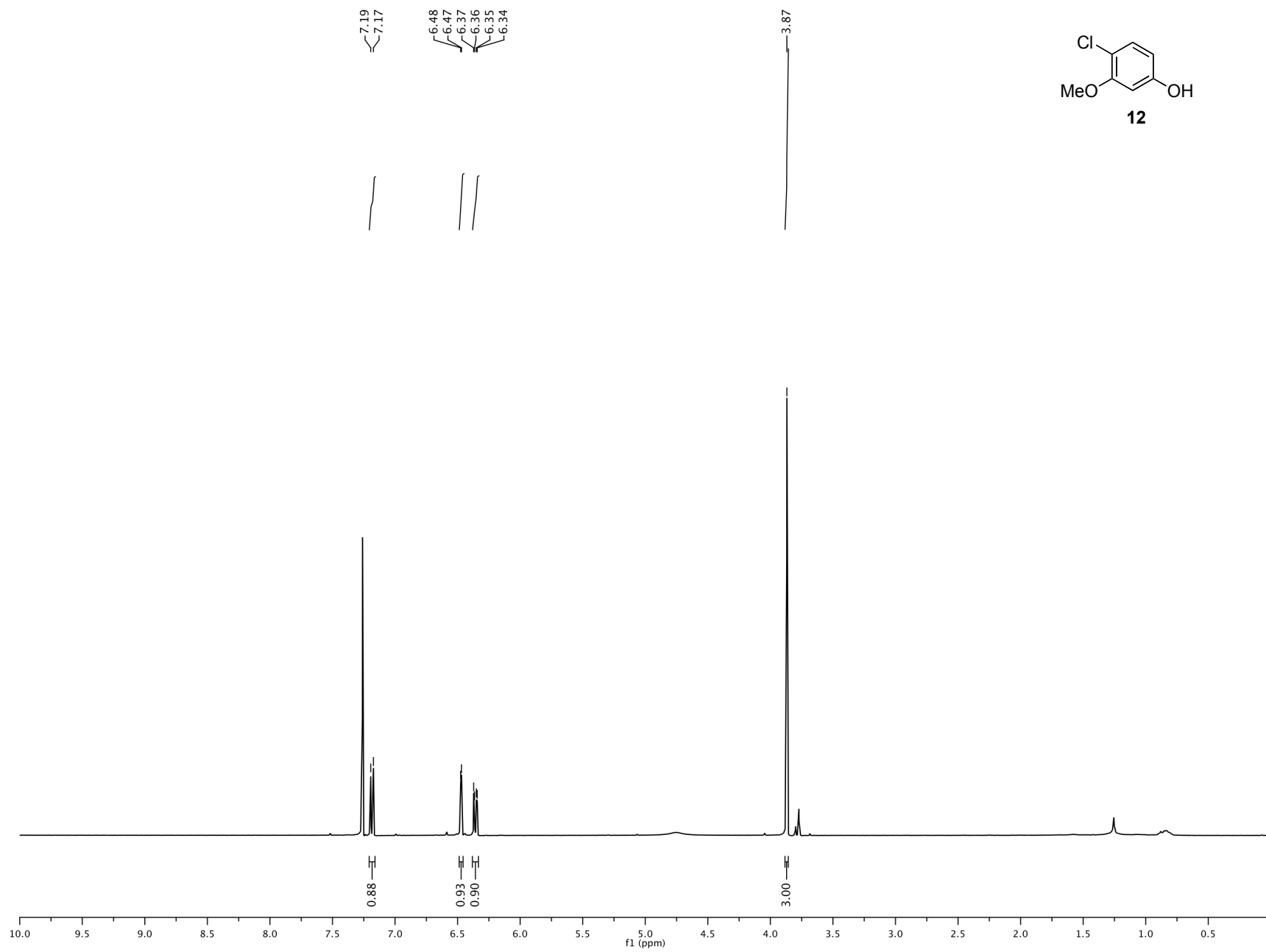
room temperature. The reaction was heated to reflux overnight. Upon completion, the reaction was cooled to 0°C and the product was filtered out and washed with cold ethanol to yield **6** (1.09 g, 75%). **6**: off-white solid; ¹H NMR (400 MHz, DMSO-d₆) δ 8.71 (s, 1H), 7.82 (d, 1H, J = 8.6 Hz), 7.03 (d, 1H, J = 2.4 Hz), 7.00 (dd, 1H, J = 2.4 Hz, J = 8.6 Hz), 3.88 (s, 3H); ¹³C NMR (100 MHz, DMSO-d₆) δ 164.7, 164.2, 157.2, 156.9, 149.1, 131.6, 113.8, 113.3, 111.6, 100.3, 56.3; HRMS calc for C₁₁H₈O₅Na (M+Na)⁺ 243.0264 found 243.0265.

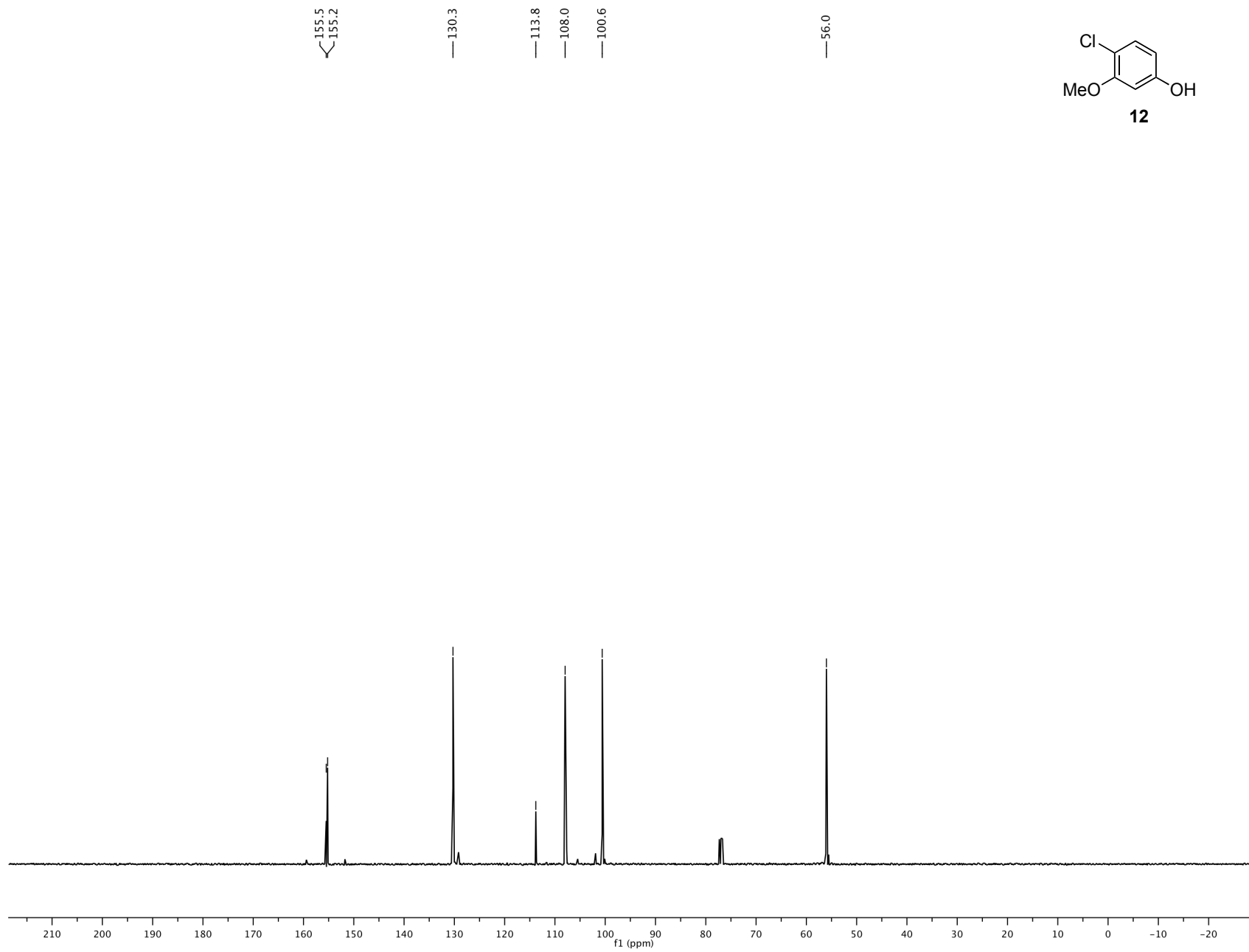
Synthesis of 7-Methoxy-3-carboxycoumarin, succinimidyl ester (**9**)

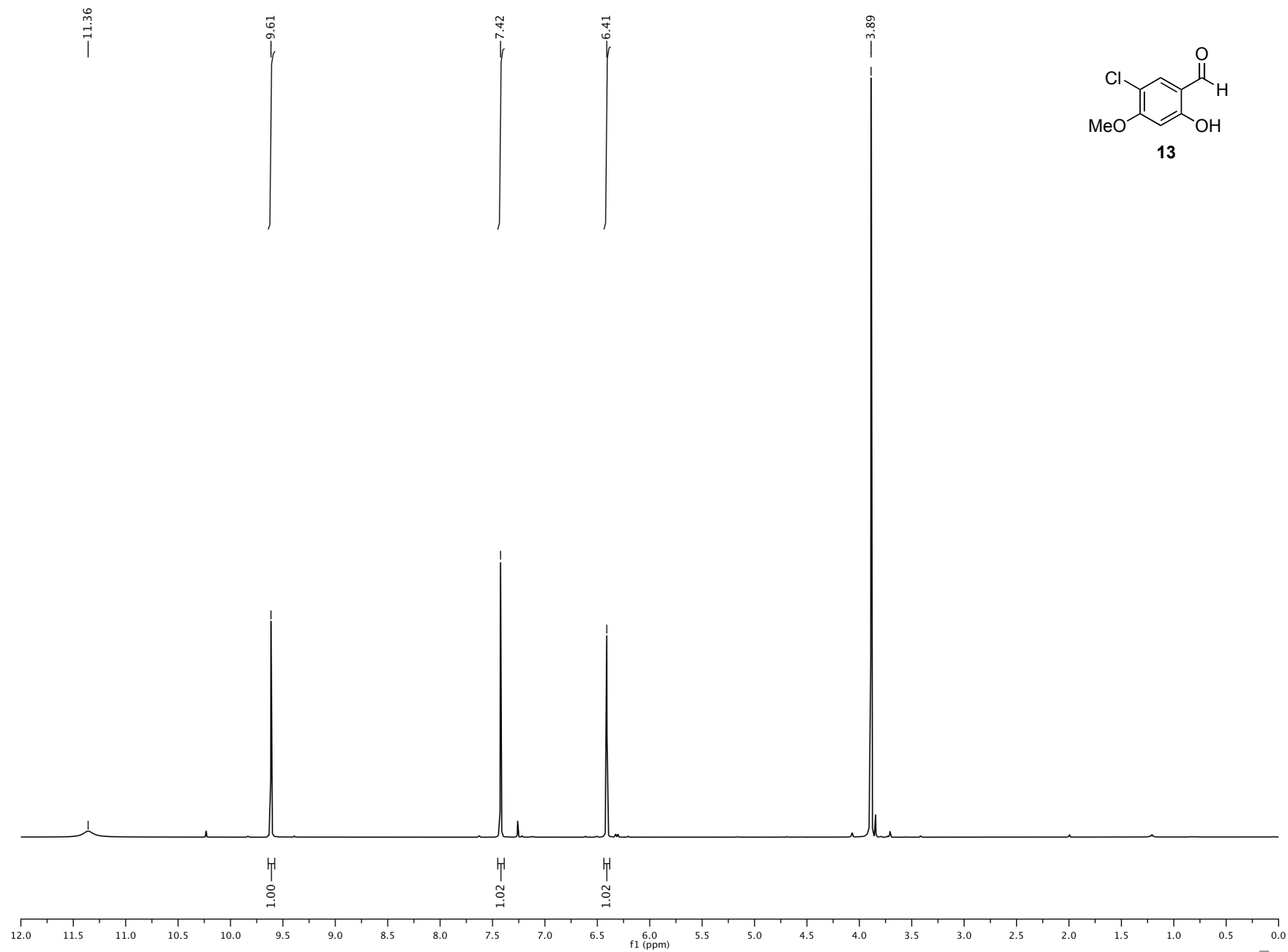


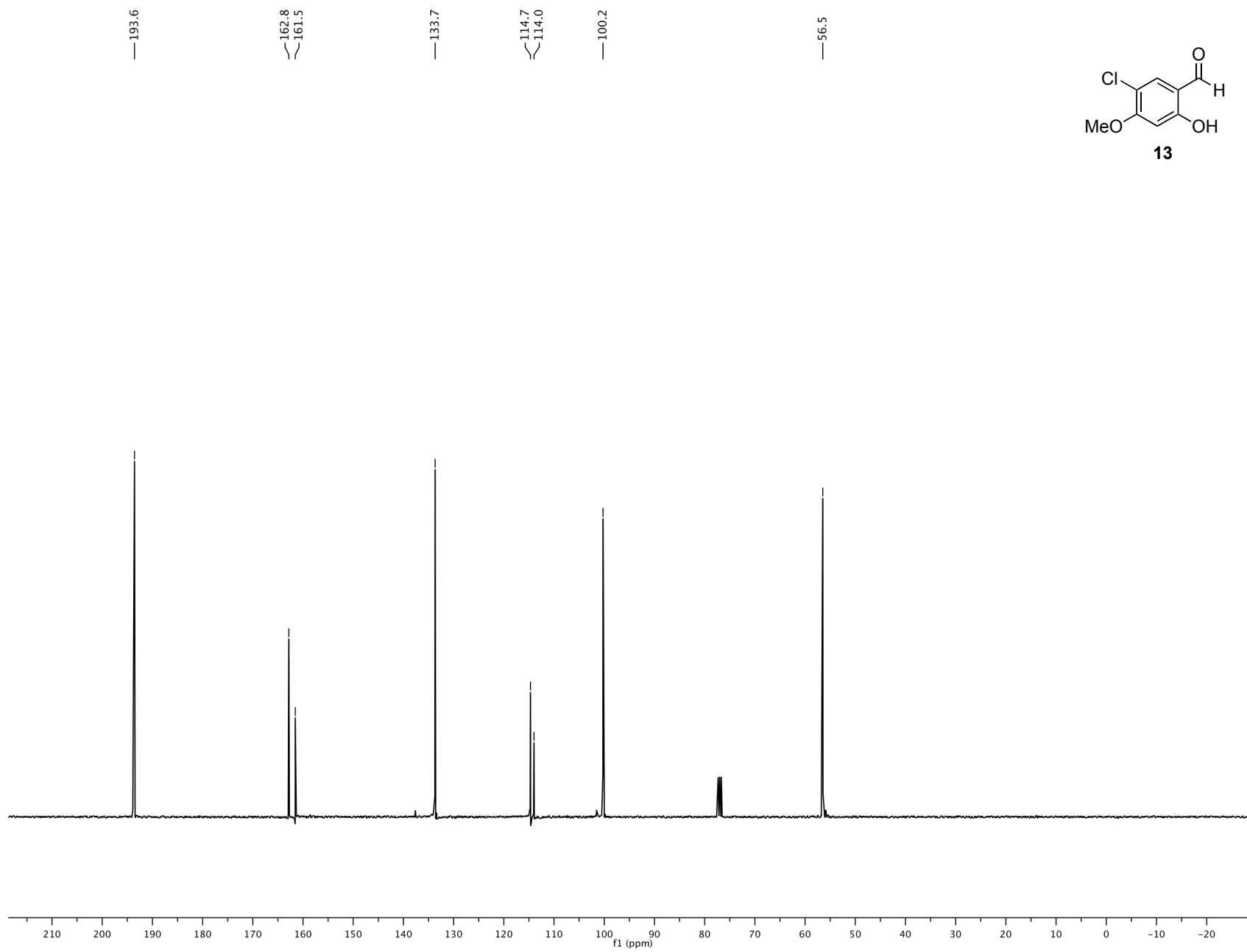
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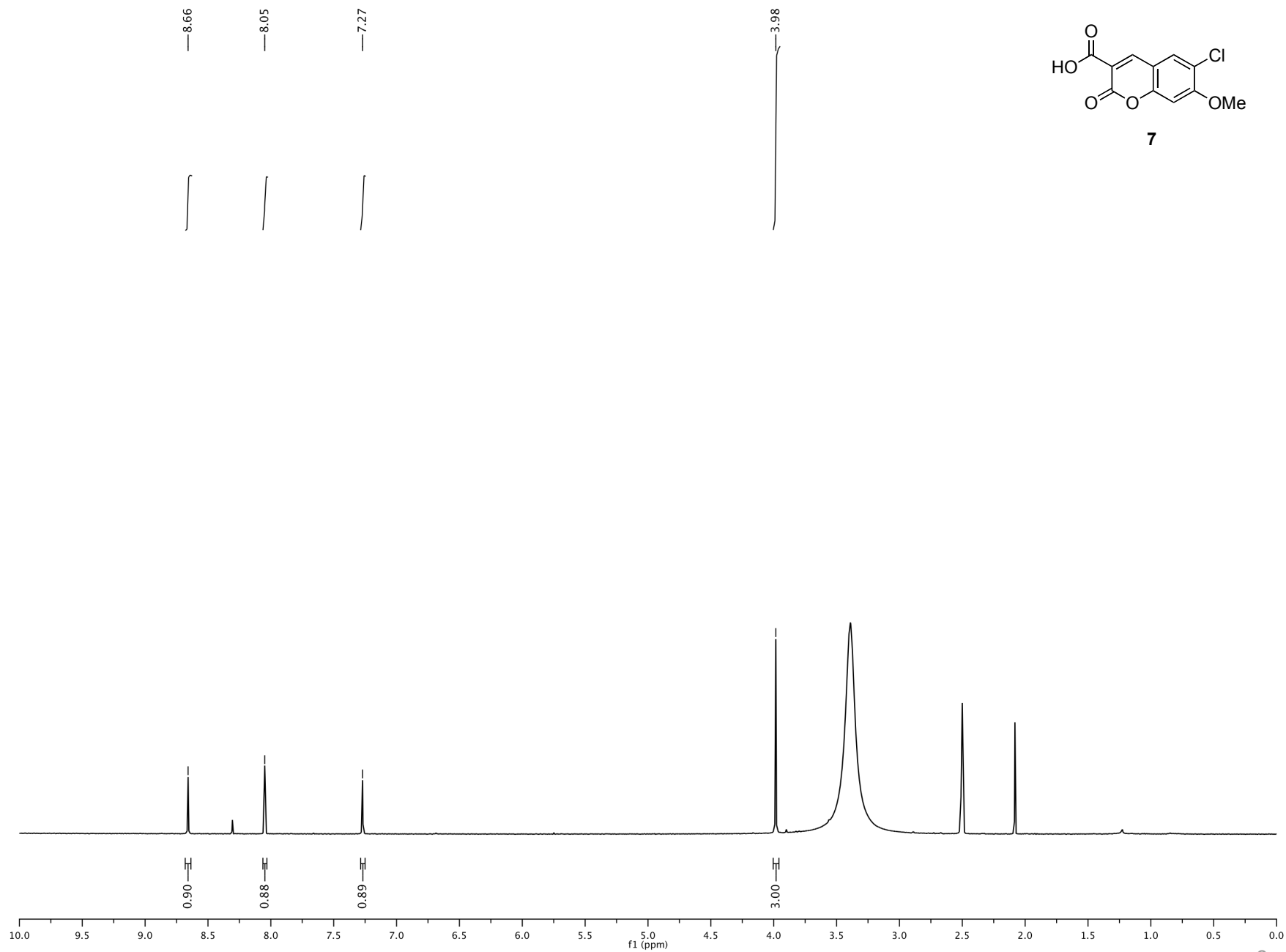
To a round bottom flask, N-hydroxysuccinimide (196 mg, 1.70 mmol) and EDC·HCL (249 mg, 1.30 mmol) were added to a stirred suspension of 7-Methoxy-3-carboxycoumarin (**6**) (375 mg, 1.70 mmol) in DMF (10 mL) at room temperature. The reaction was stirred overnight at 40°C. Upon completion, the reaction was quenched with a 10% citric acid solution (10 ml) and washed with ethyl acetate (3 x 30 ml). The product was isolated by filtration (545 mg, 100%). **9**: off-white solid; ¹H NMR (400 MHz, DMSO-d₆) δ 9.08 (s, 1H), 7.97 (d, 1H, J = 8.7 Hz), 7.11 (d, 1H, J = 2.4 Hz), 7.07 (dd, 1H, J = 2.4 Hz, J = 8.7 Hz), 3.93 (s, 3H), 2.89 (s, 4H); ¹³C NMR (100MHz, DMSO-d₆) δ 170.3, 166.3, 158.6, 158.0, 155.5, 152.7, 132.7, 113.9, 111.4, 107.7, 100.5, 56.5, 25.6; HRMS calc for C₁₅H₁₁NO₇Na (M+Na)⁺ 340.0428 found 340.0430.

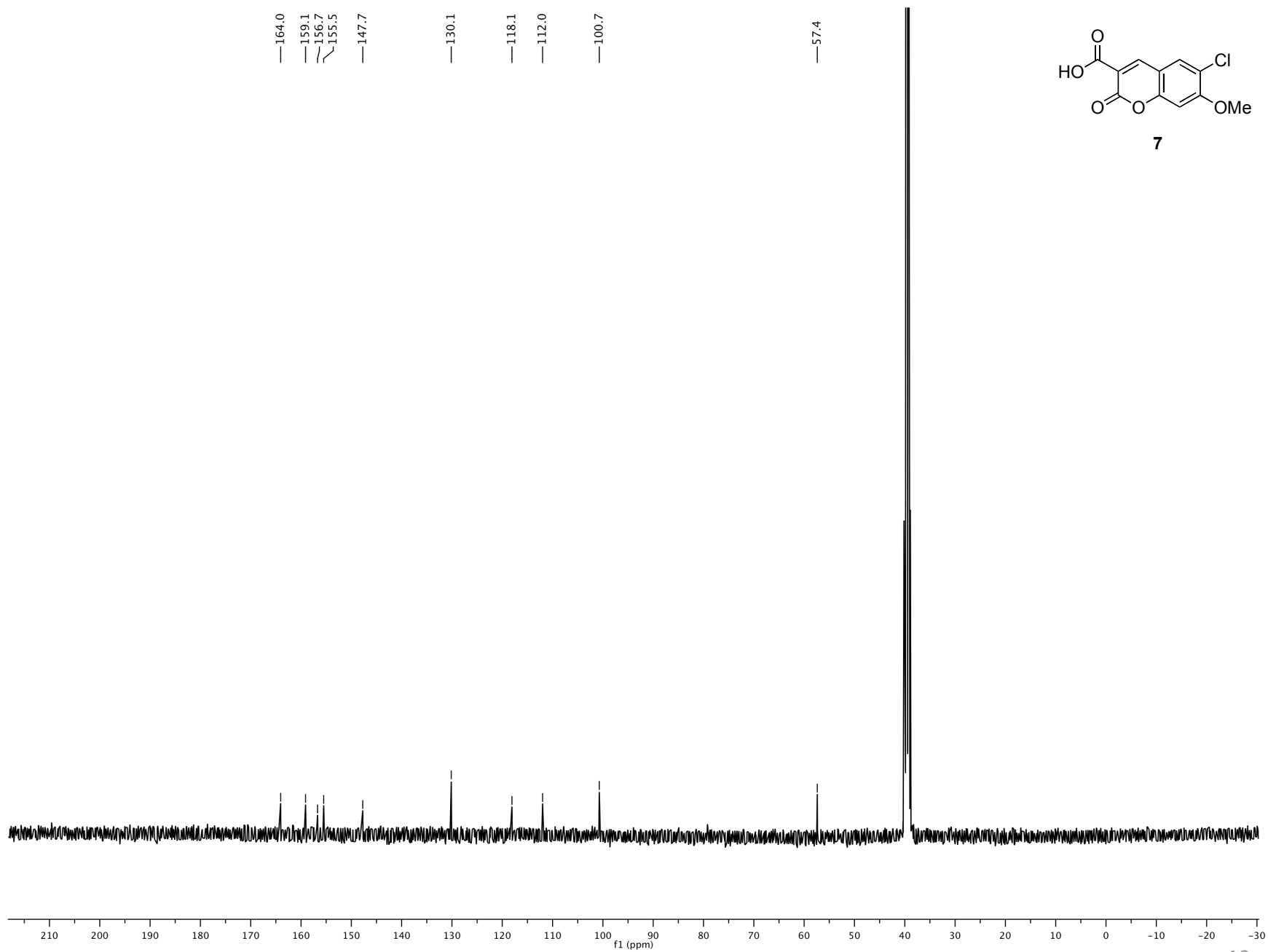


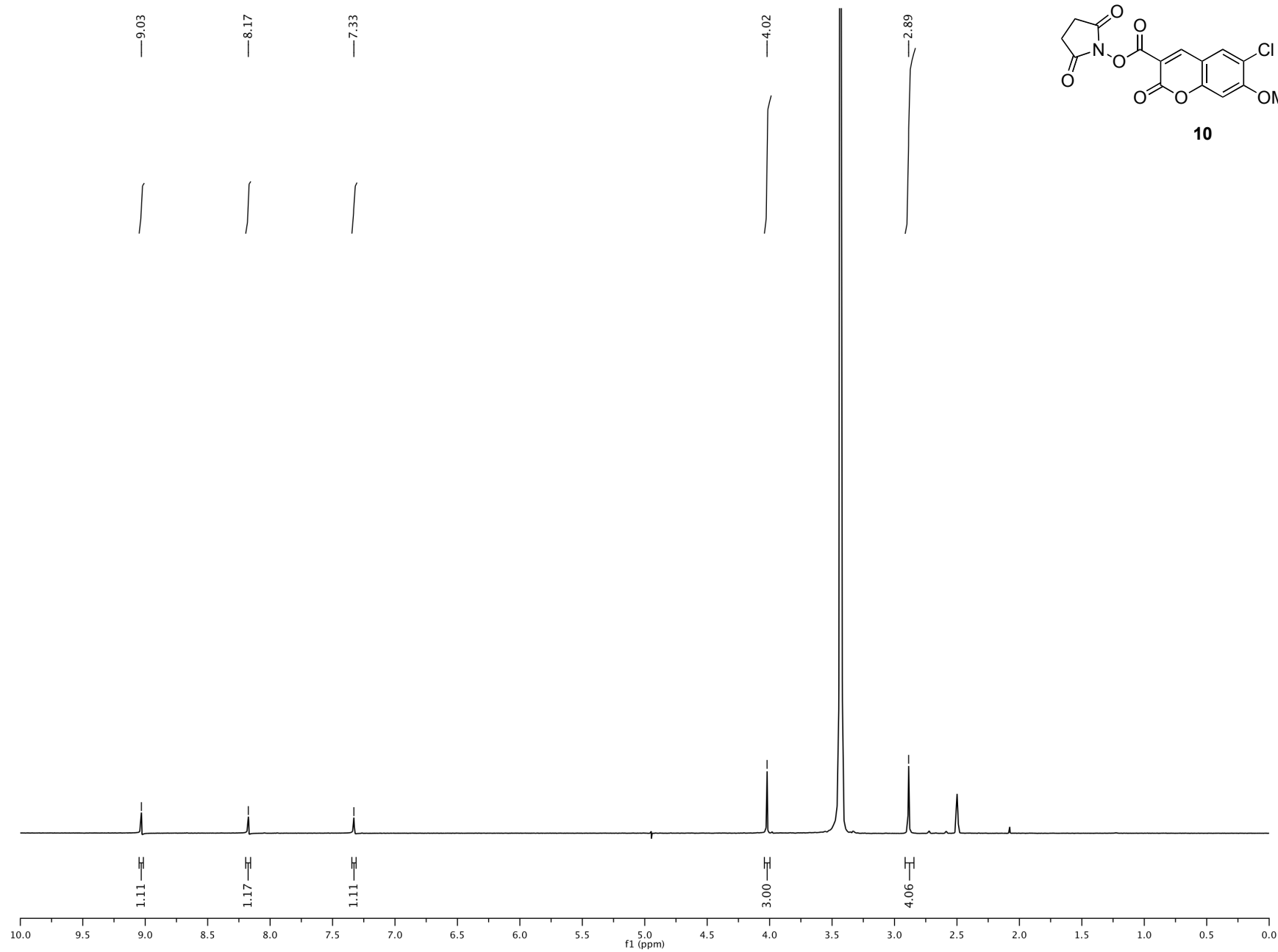


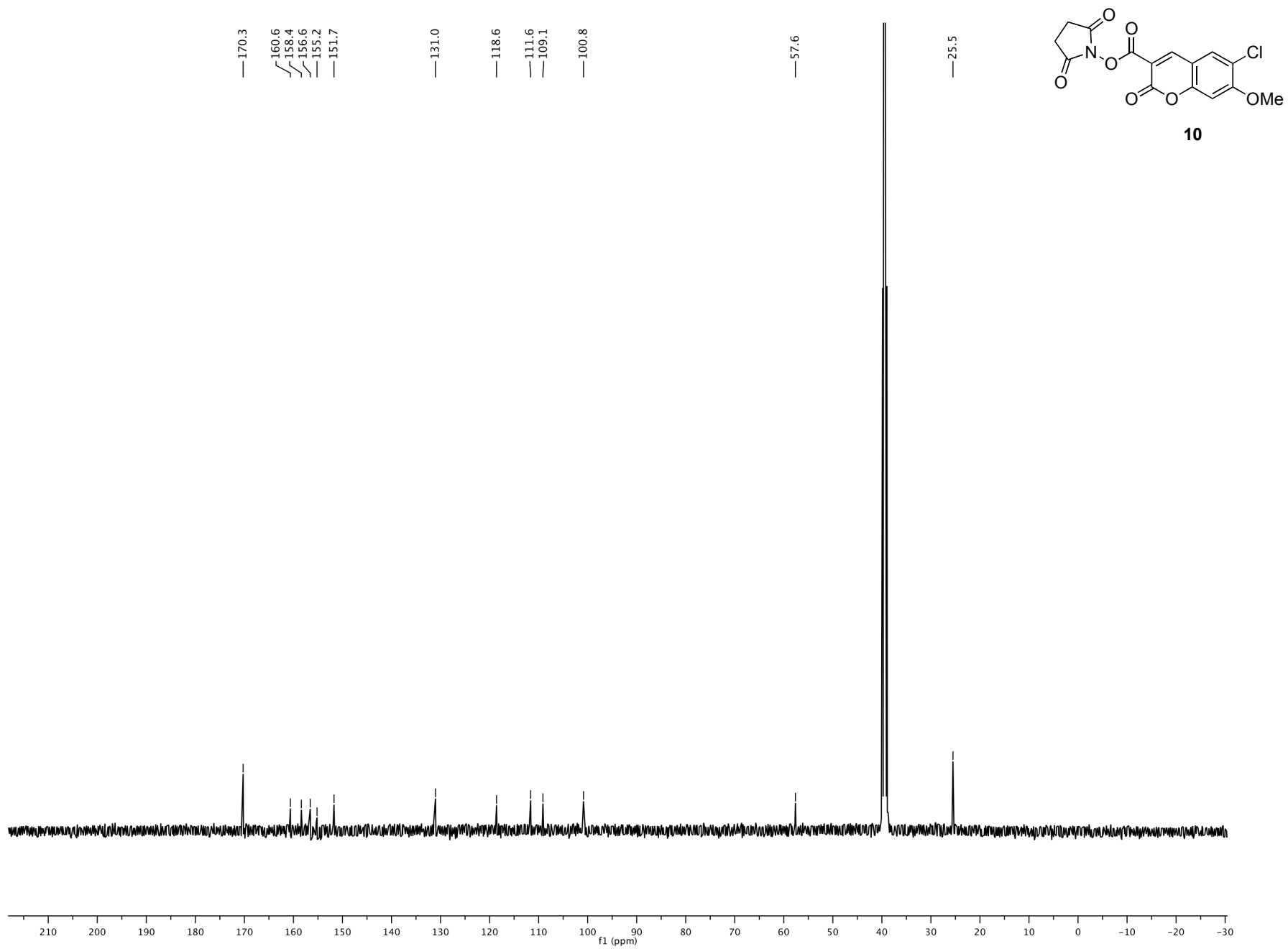


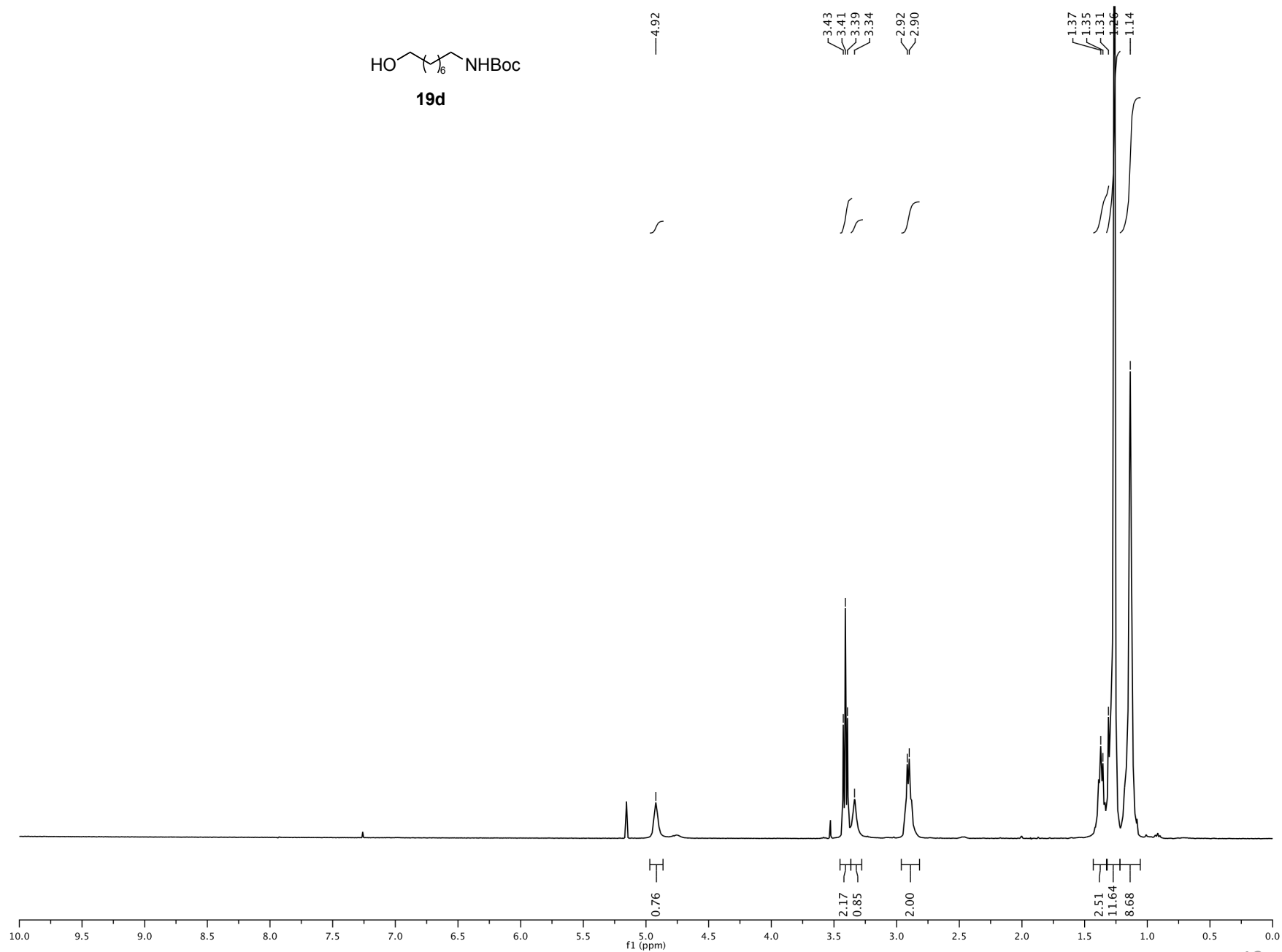
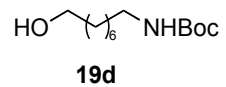


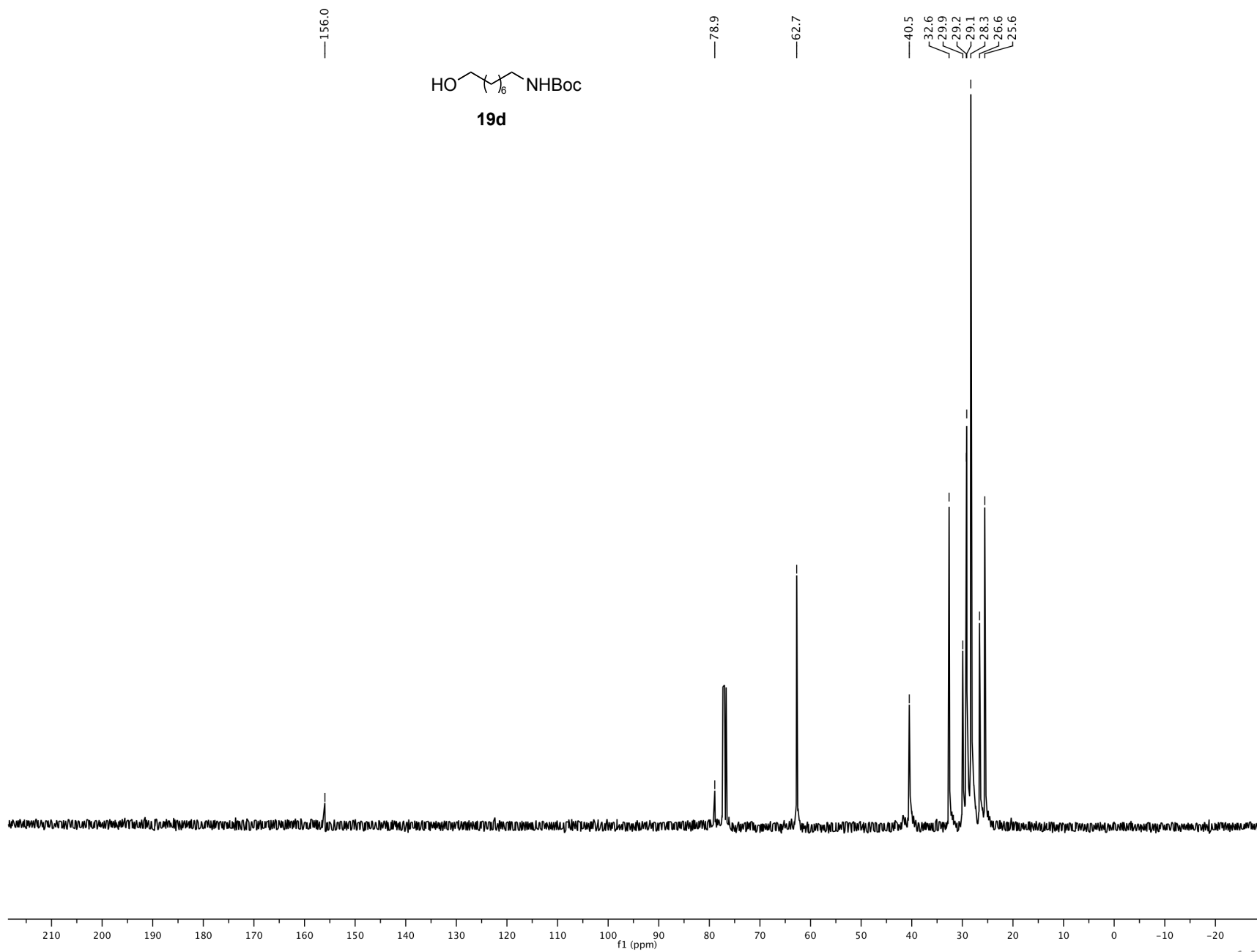


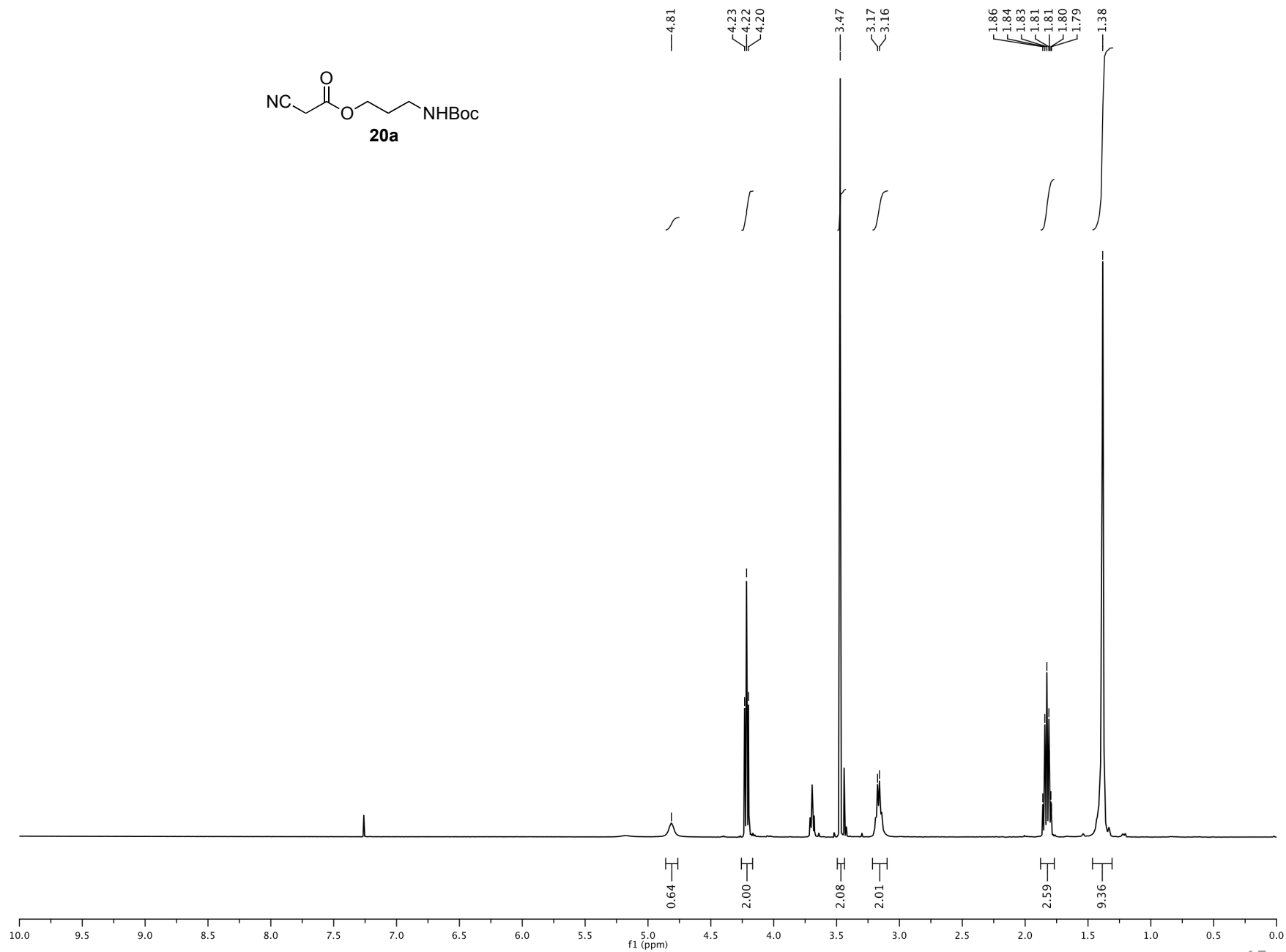
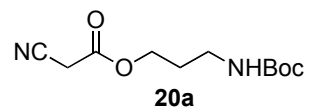




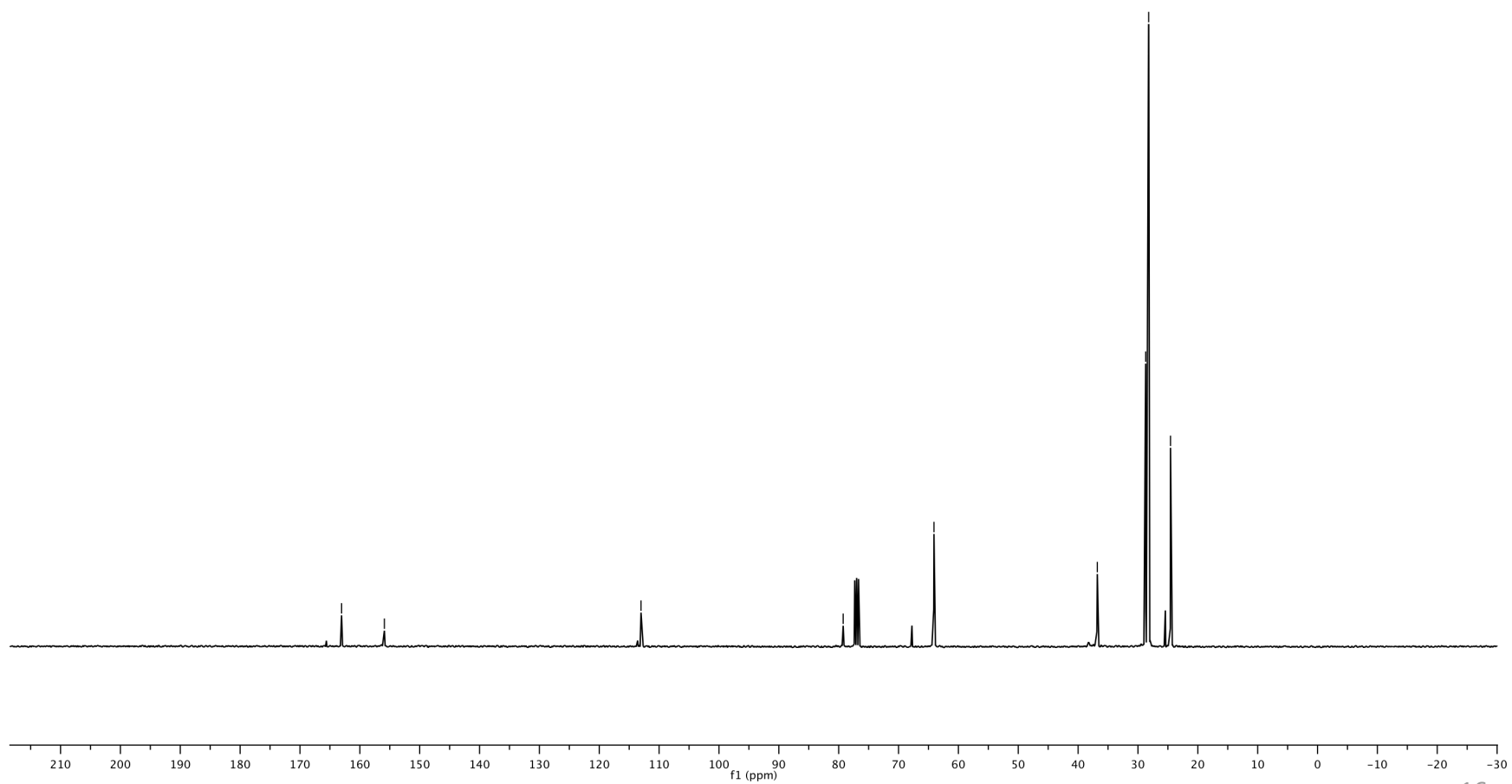
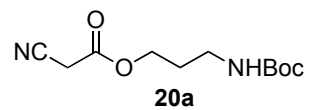


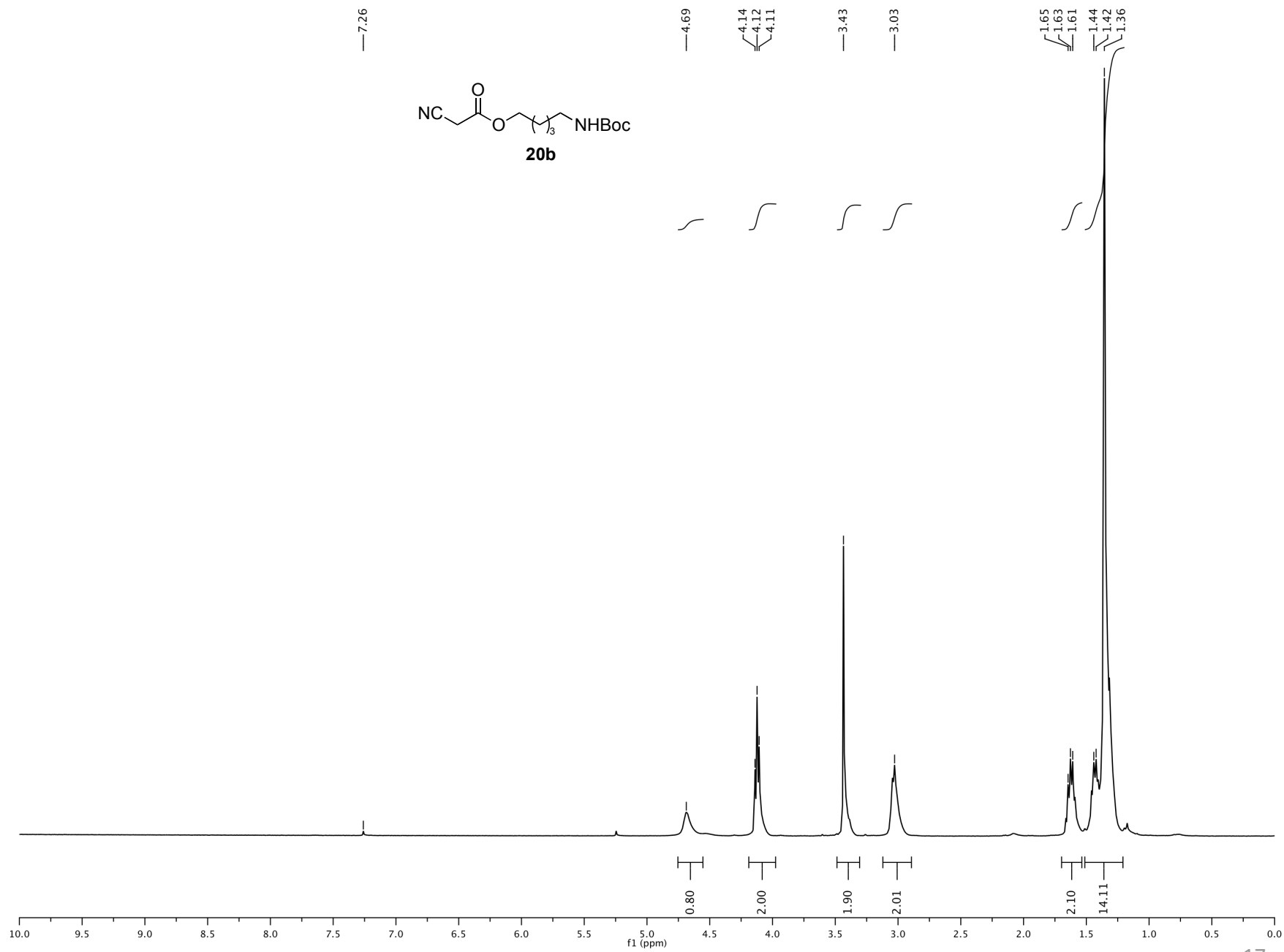


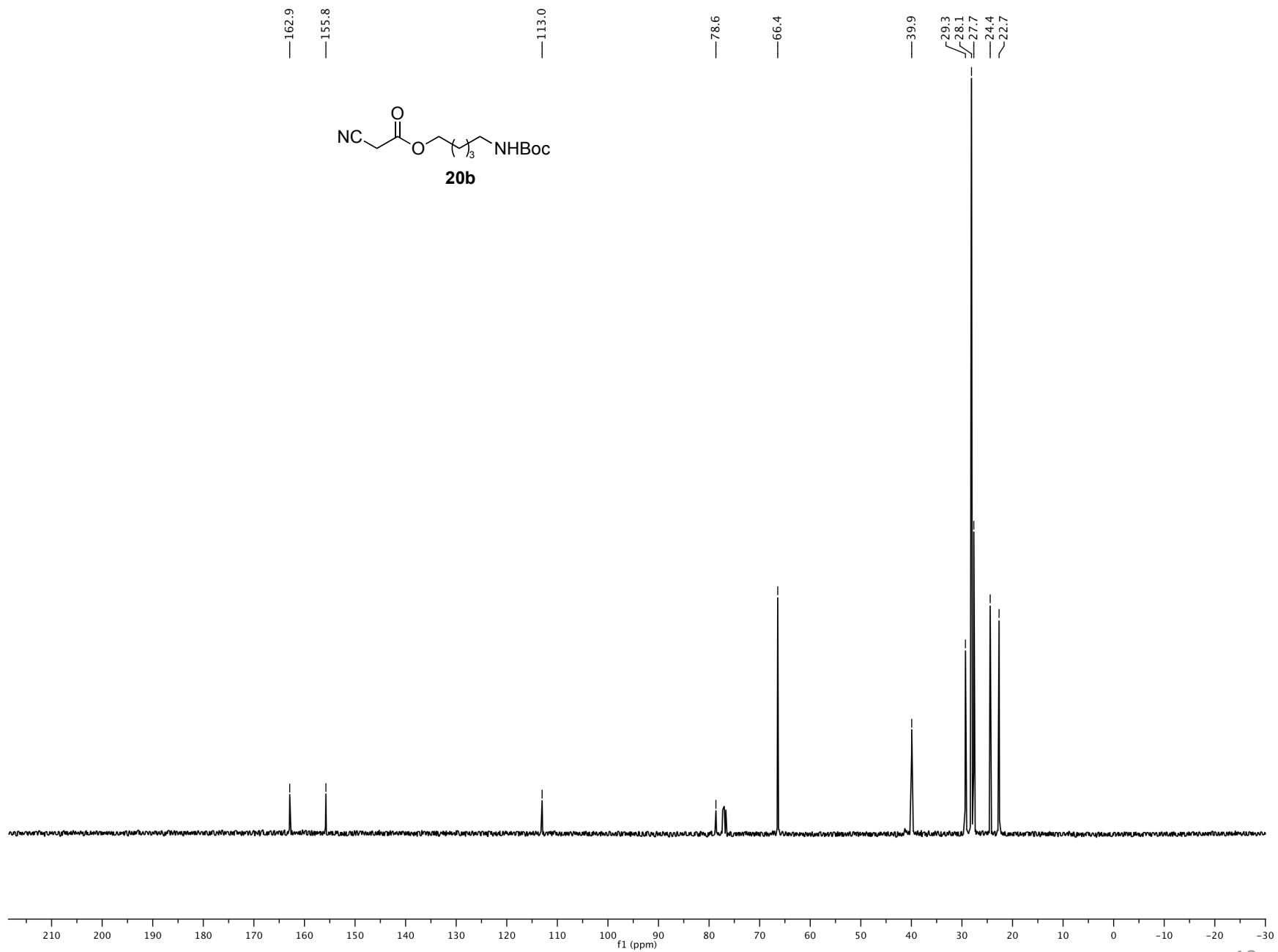


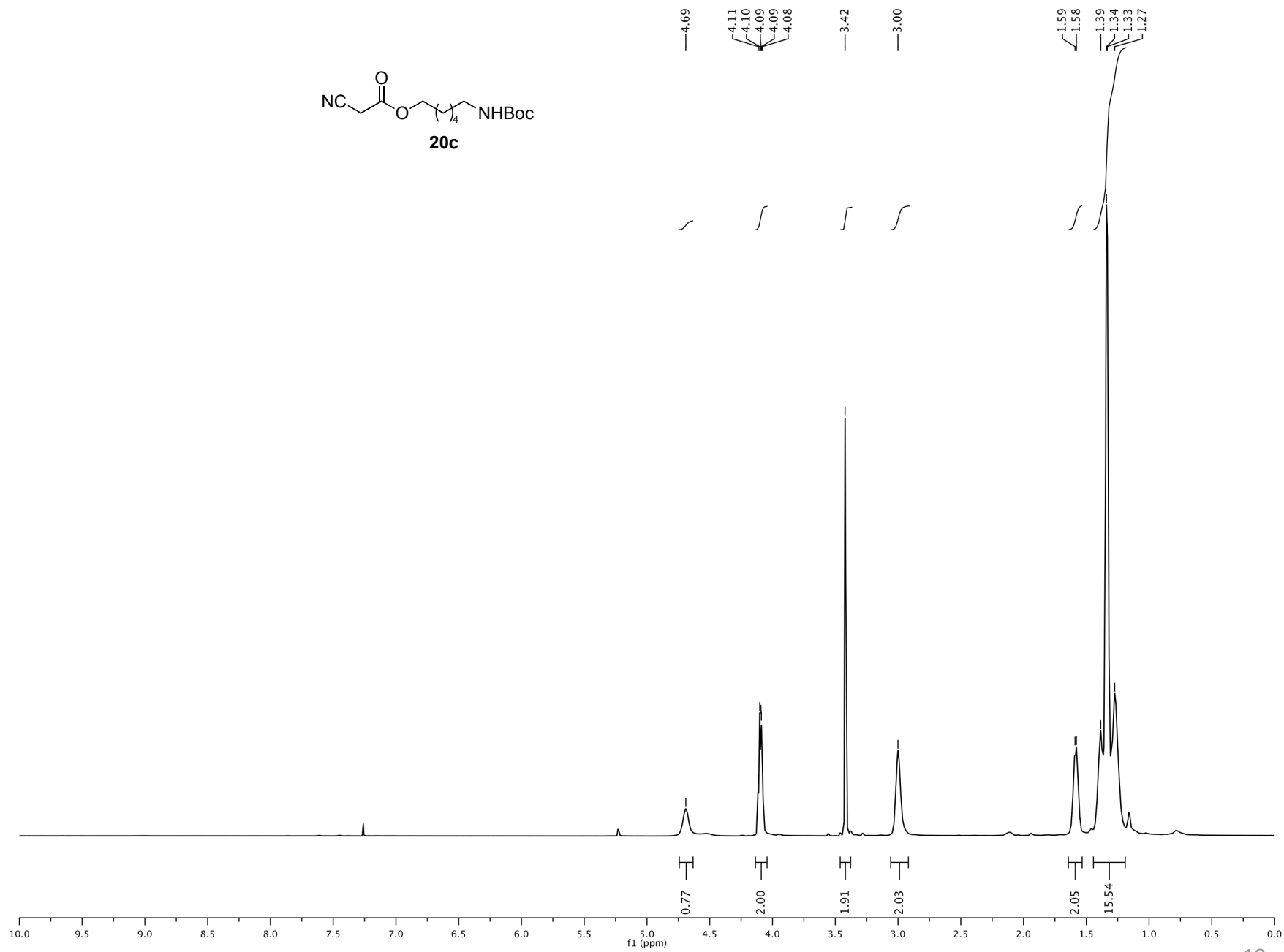
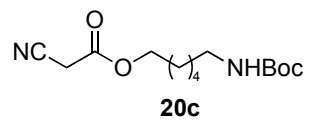


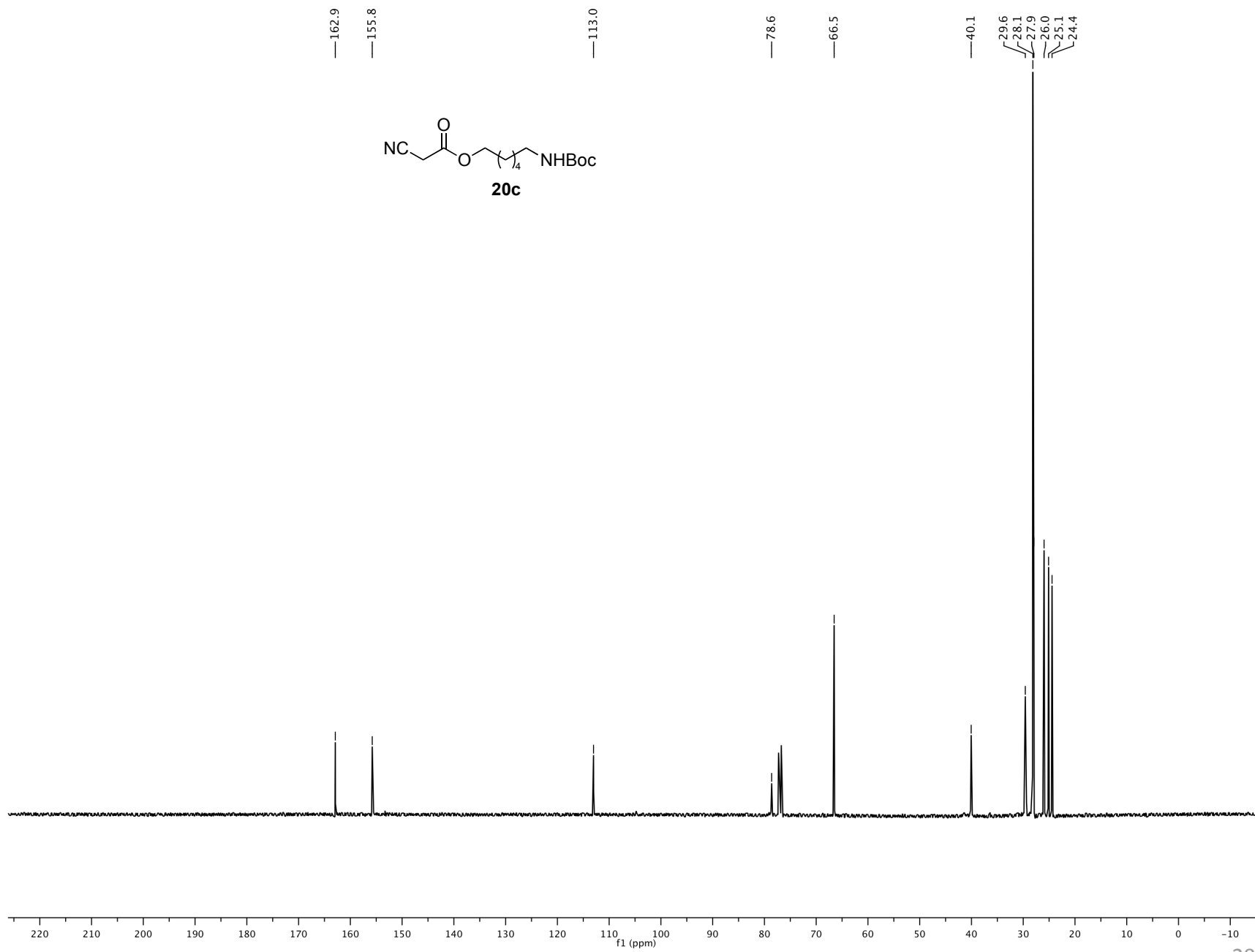
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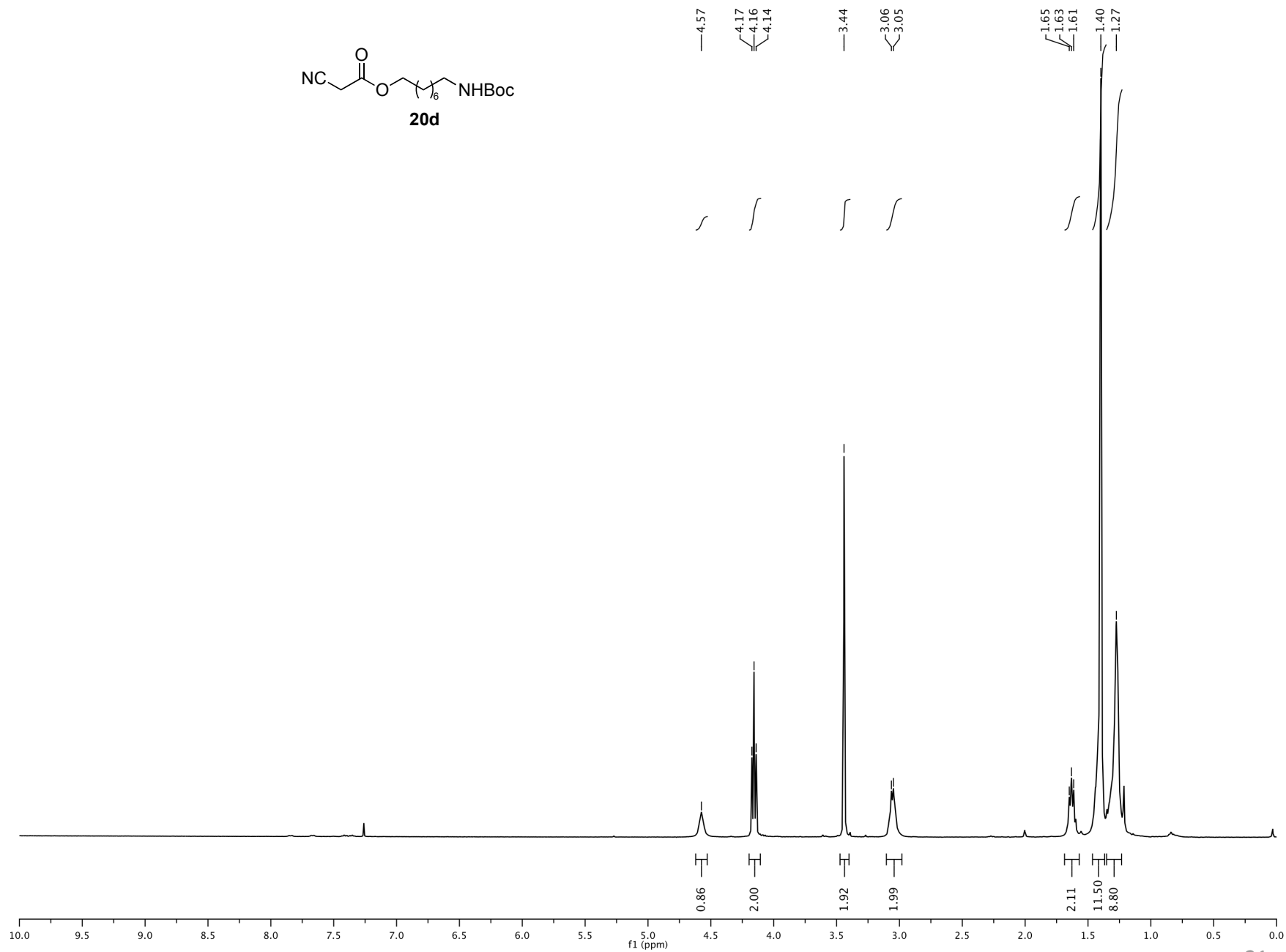
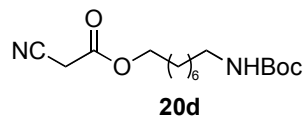


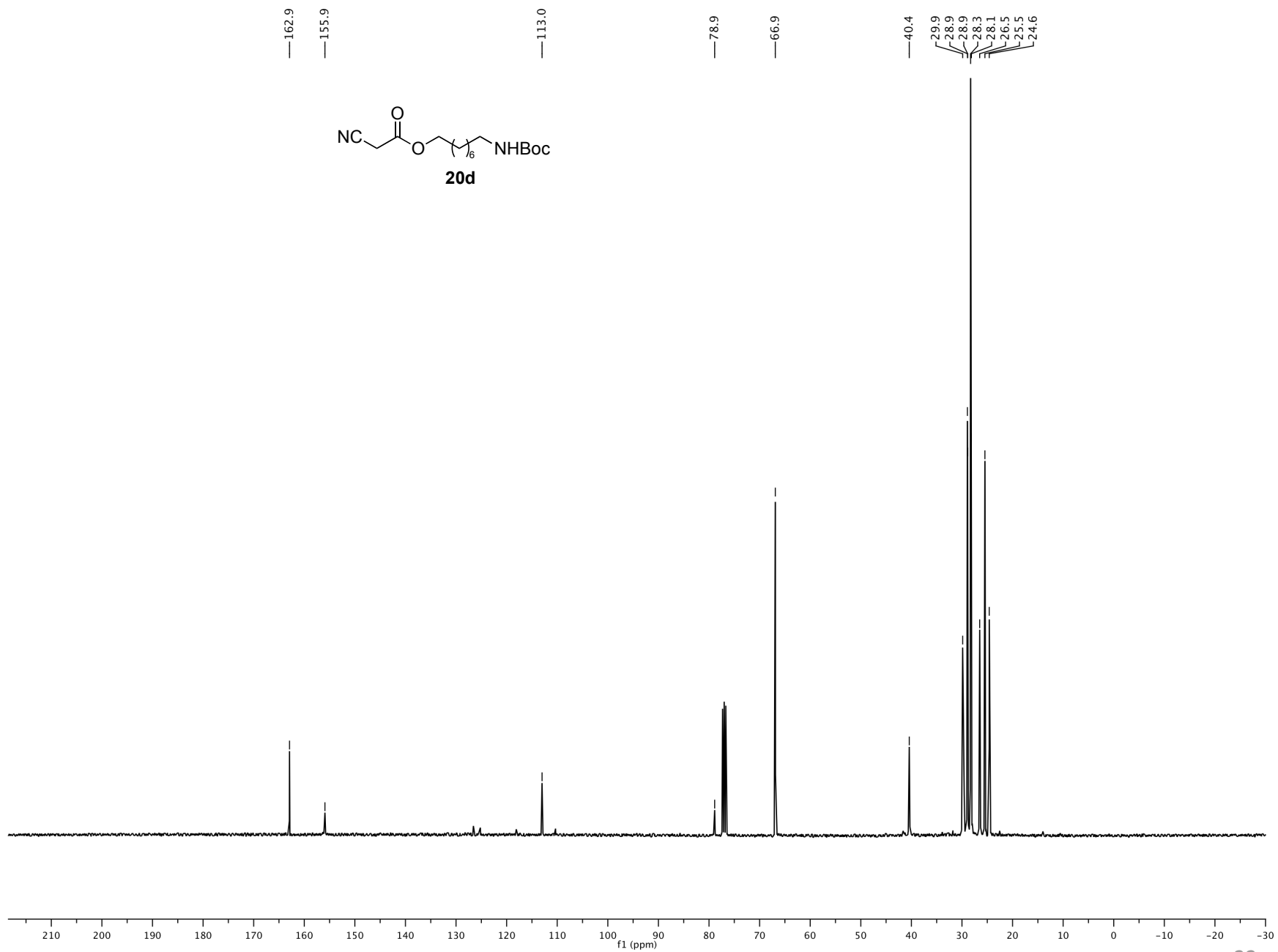


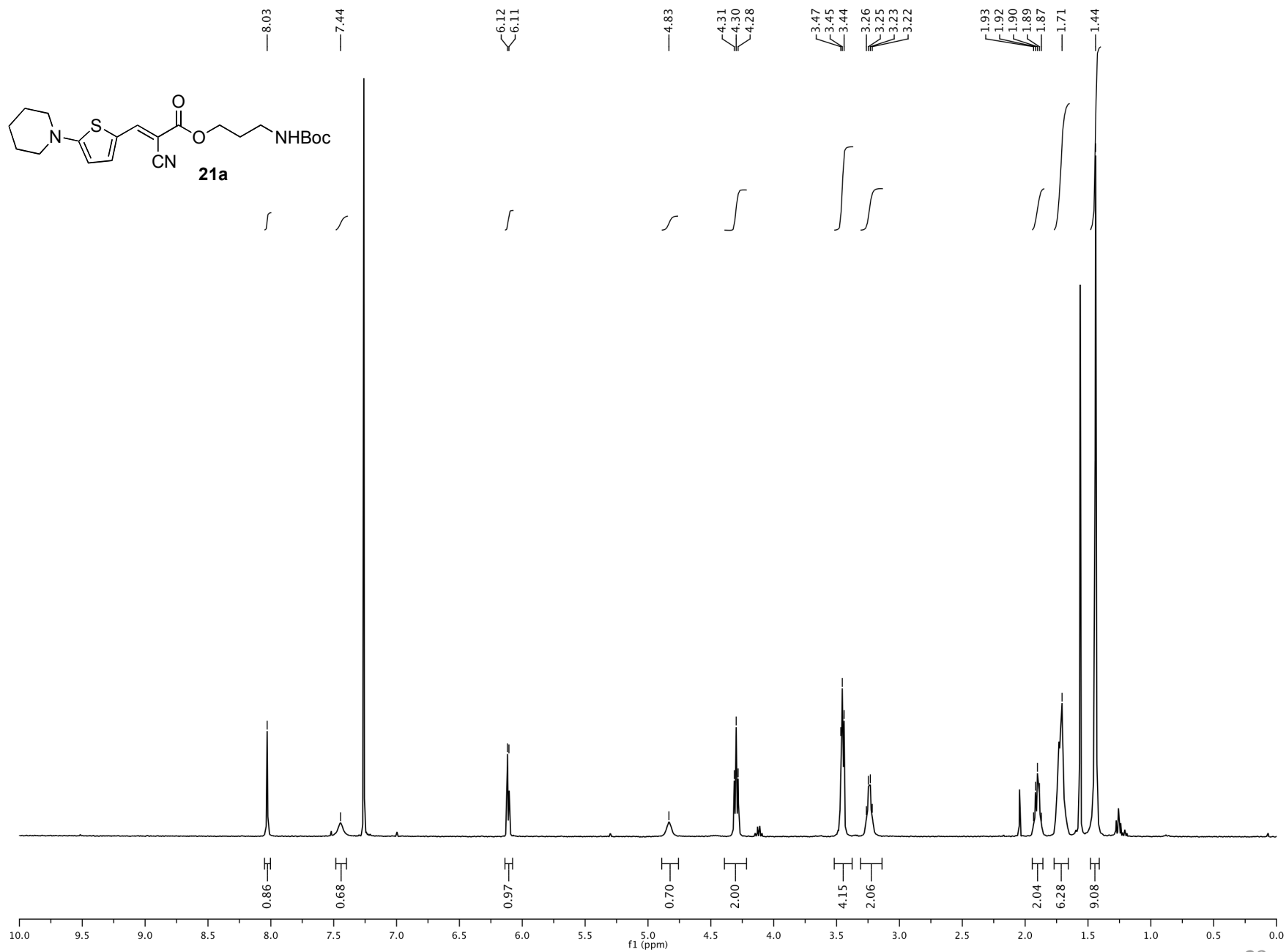


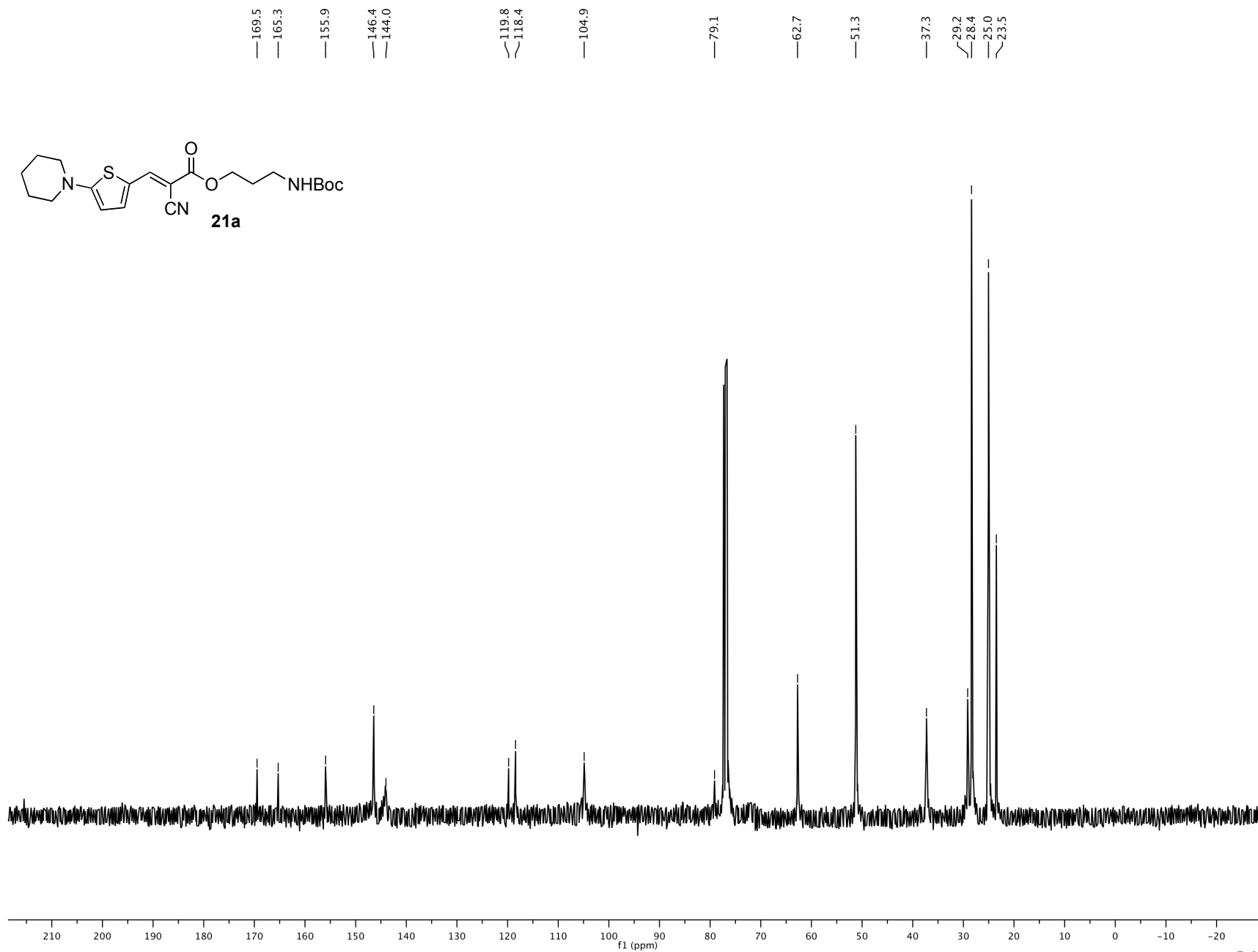


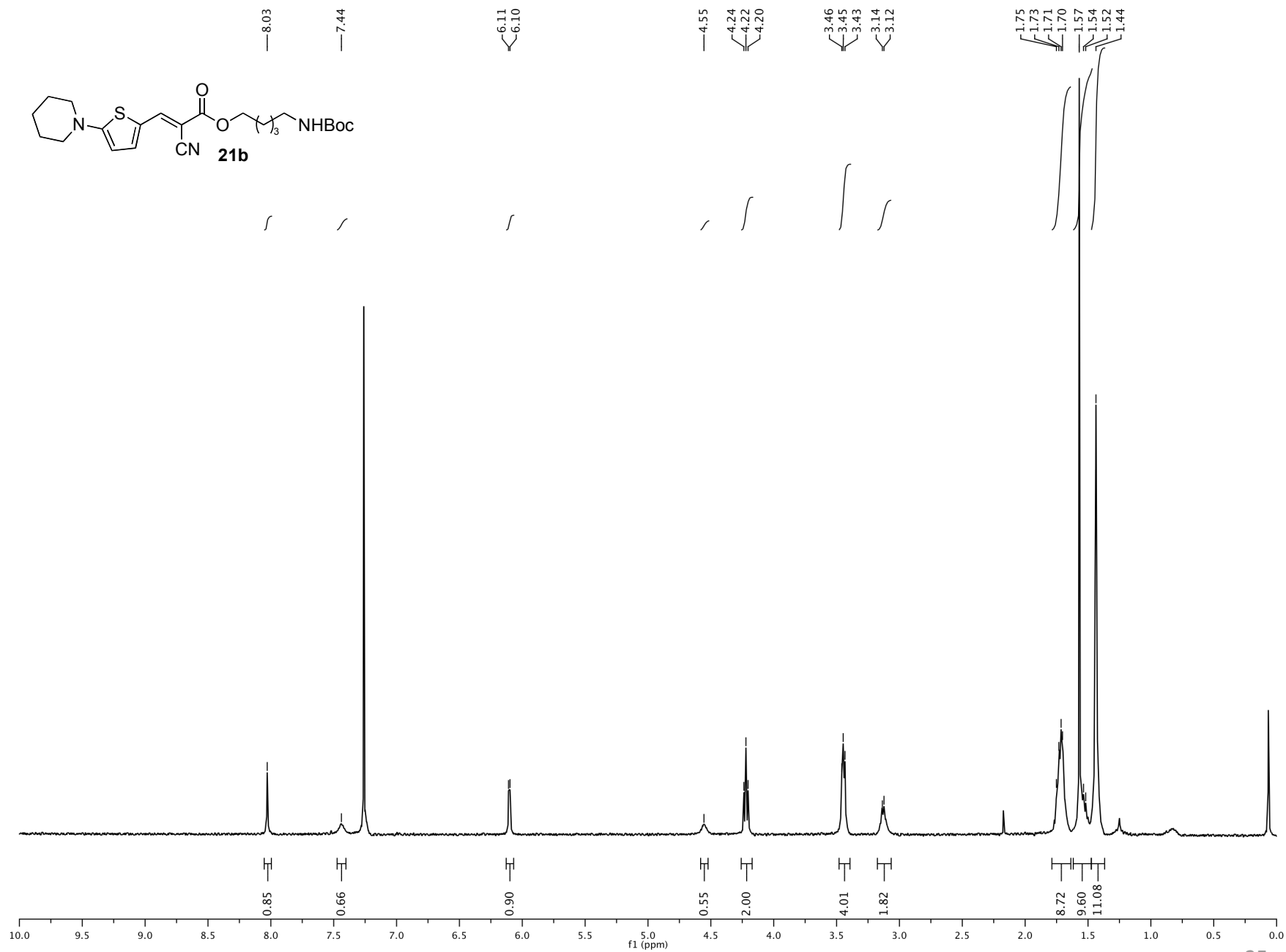


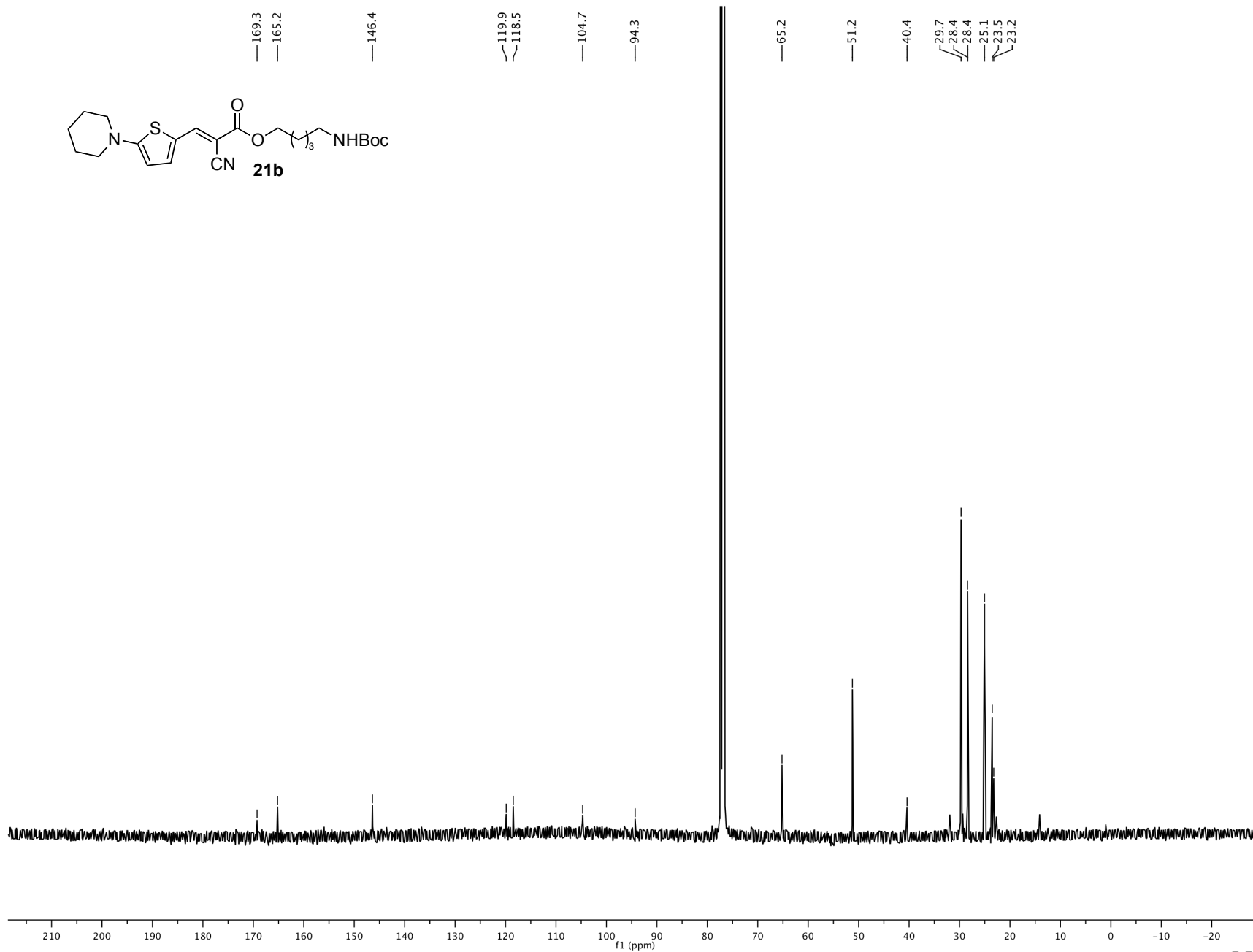


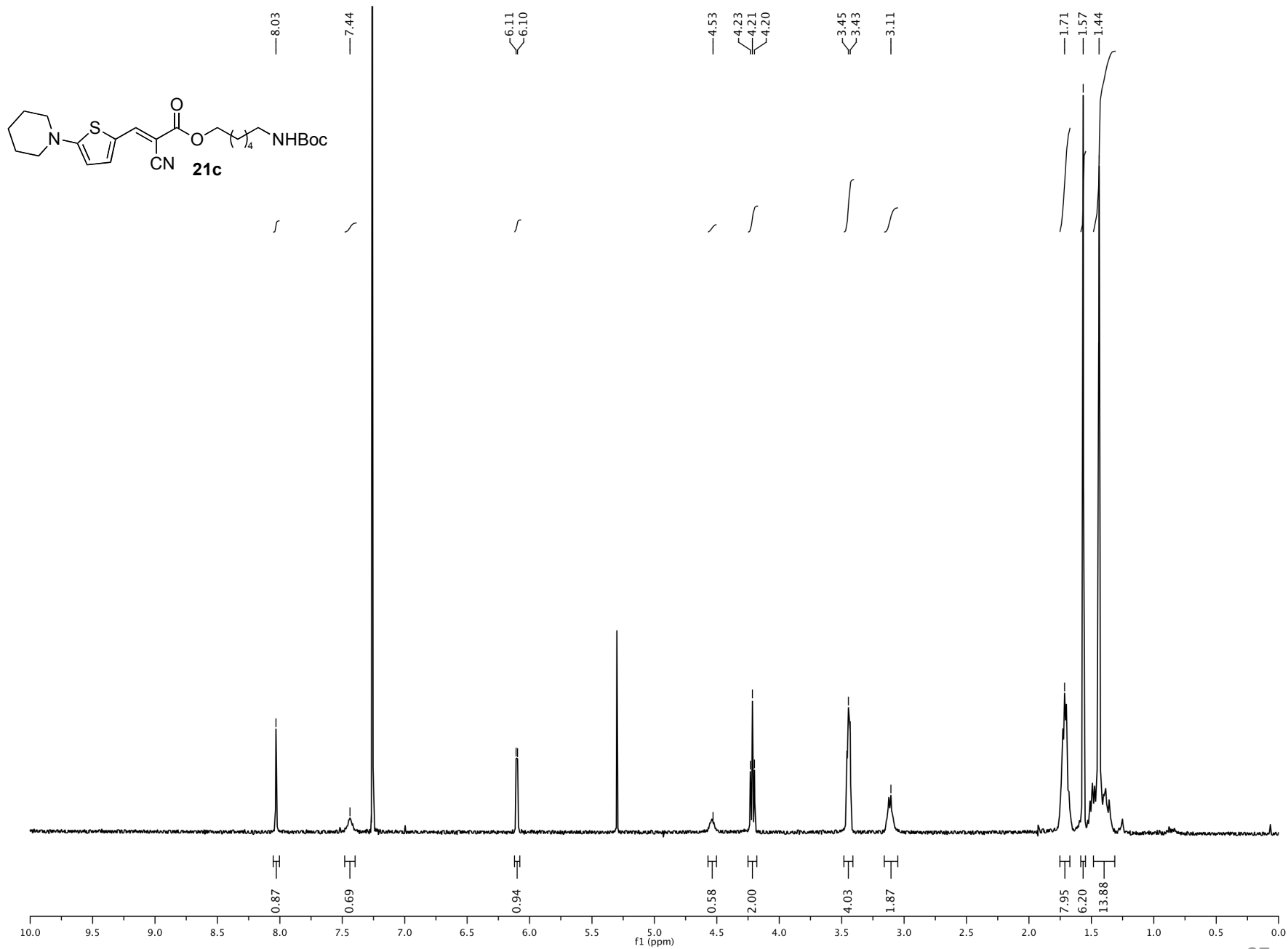


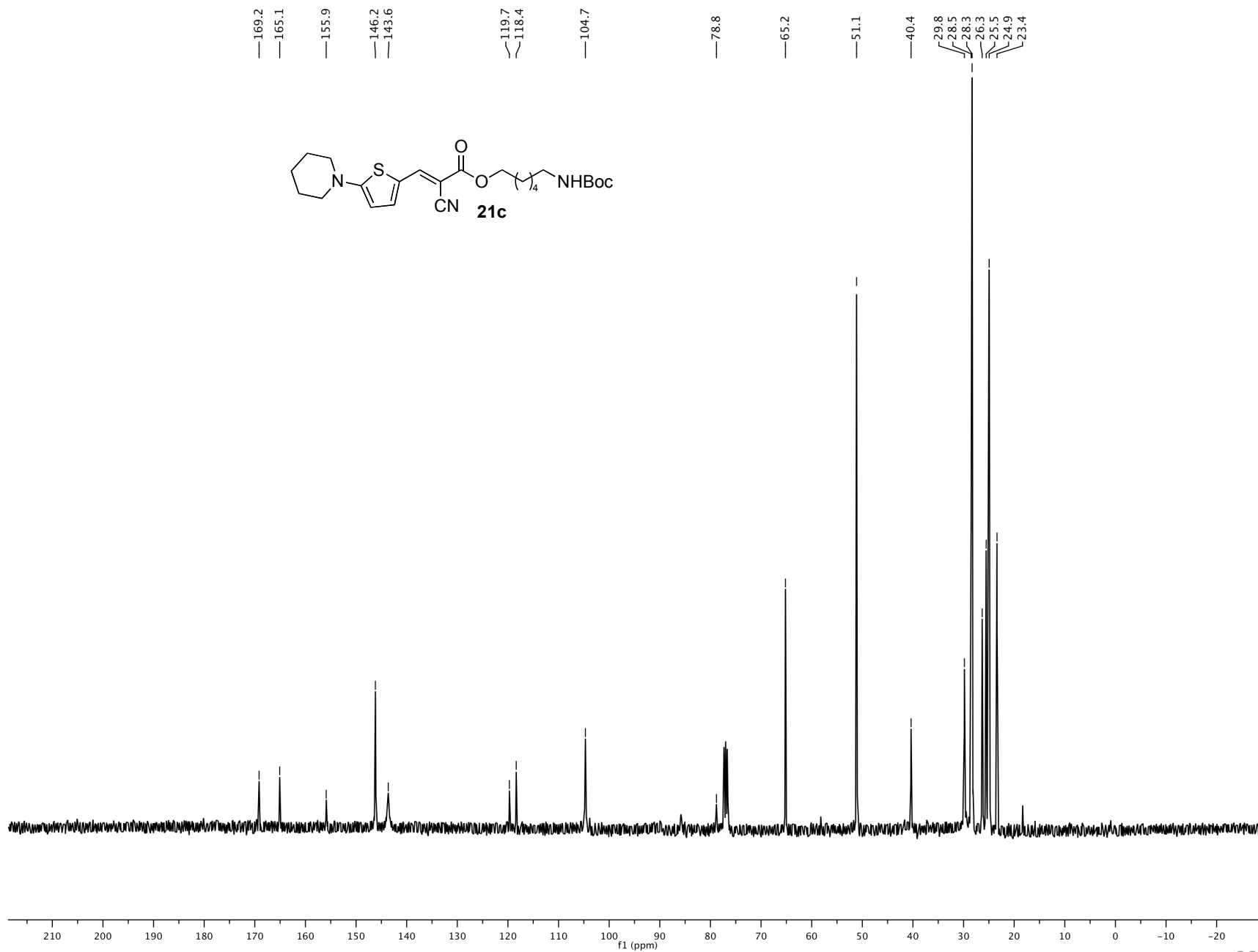


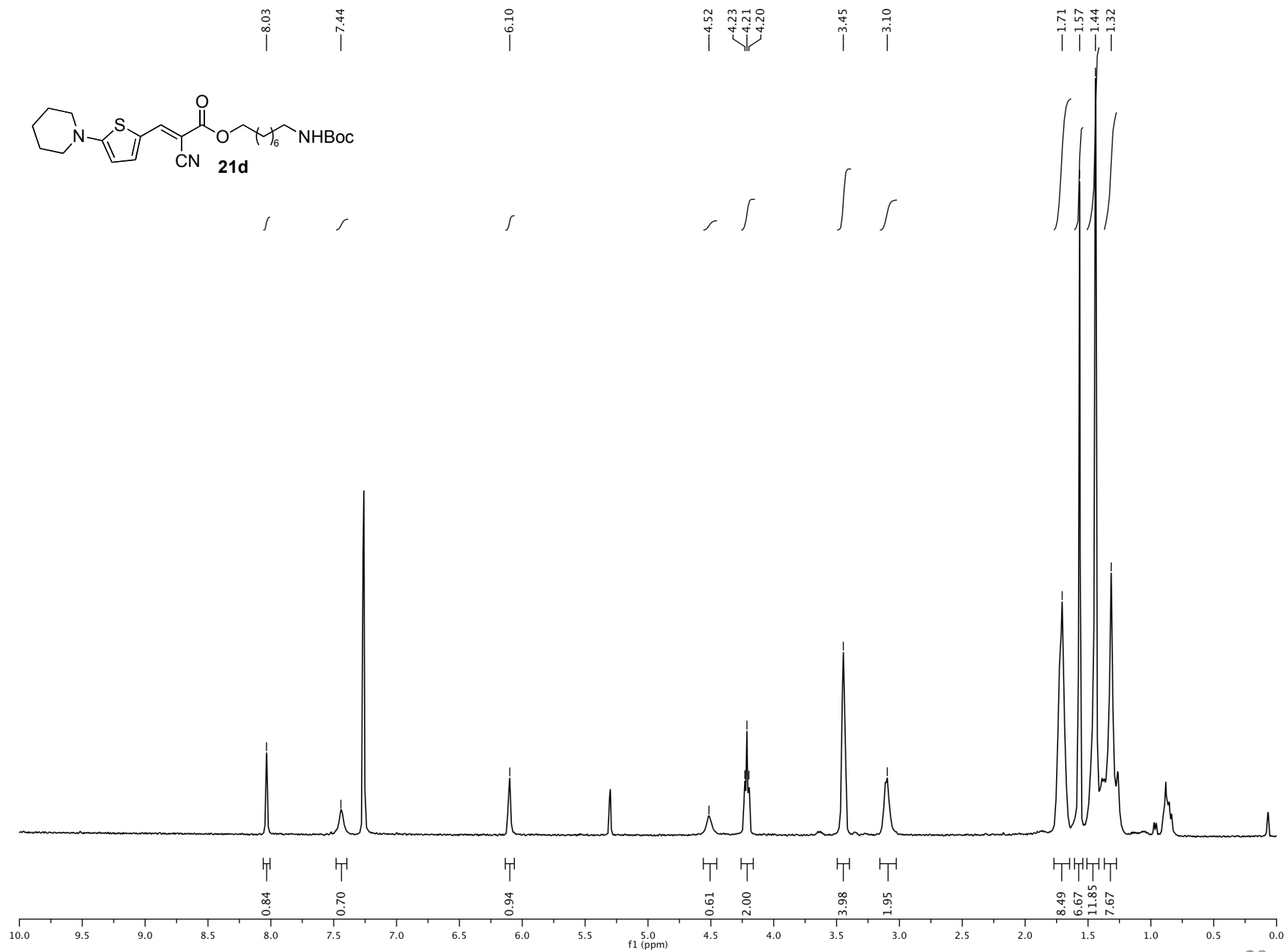


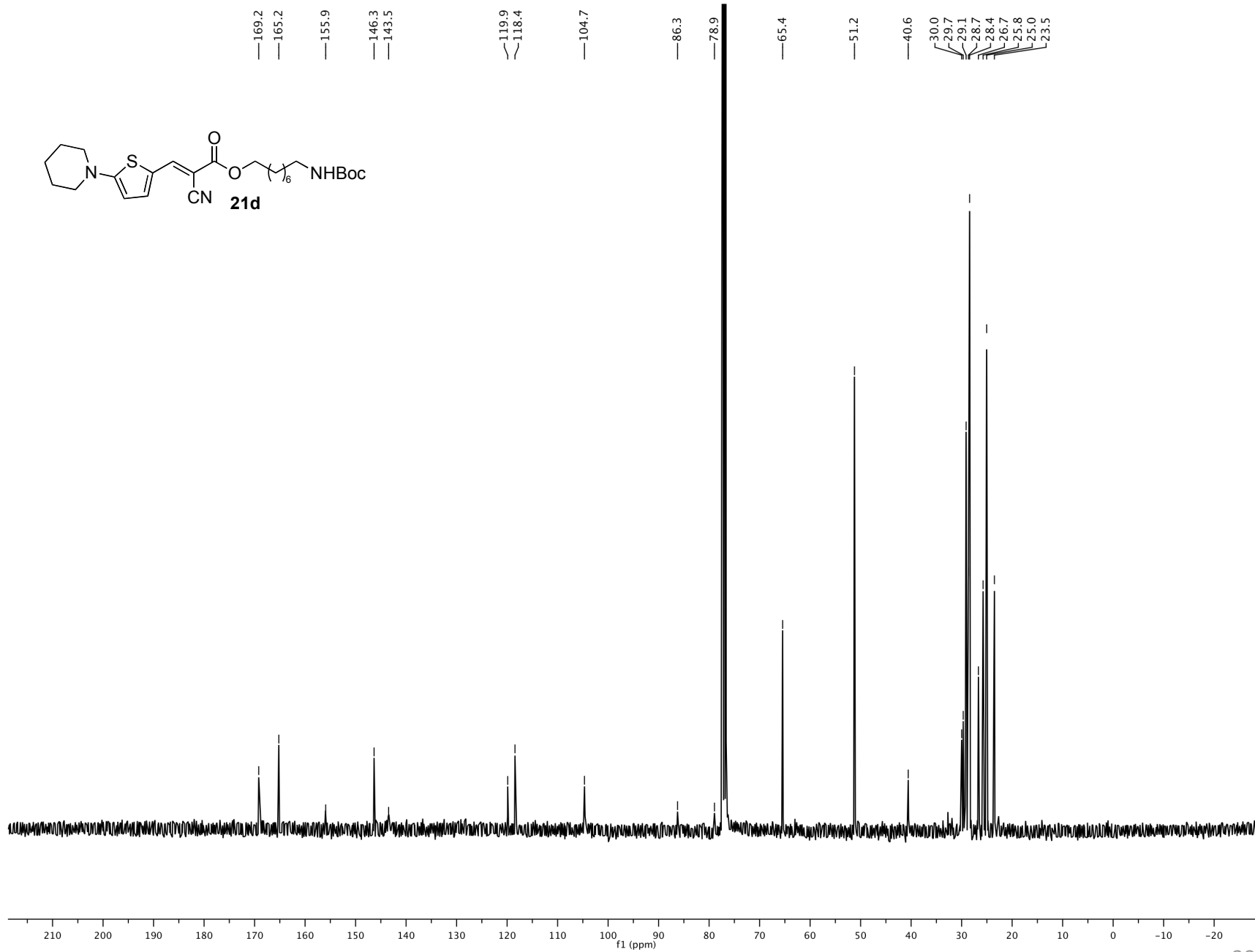


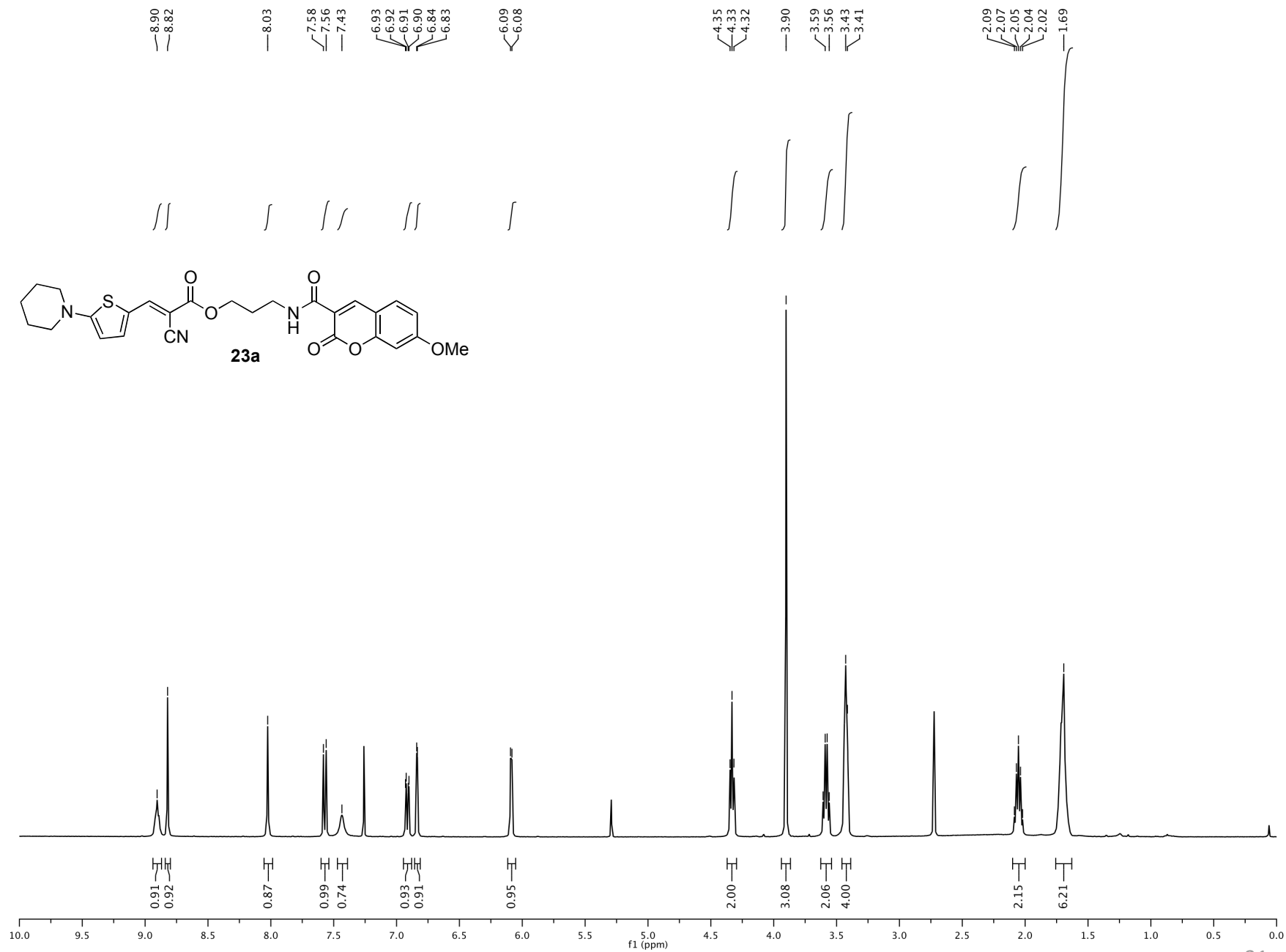


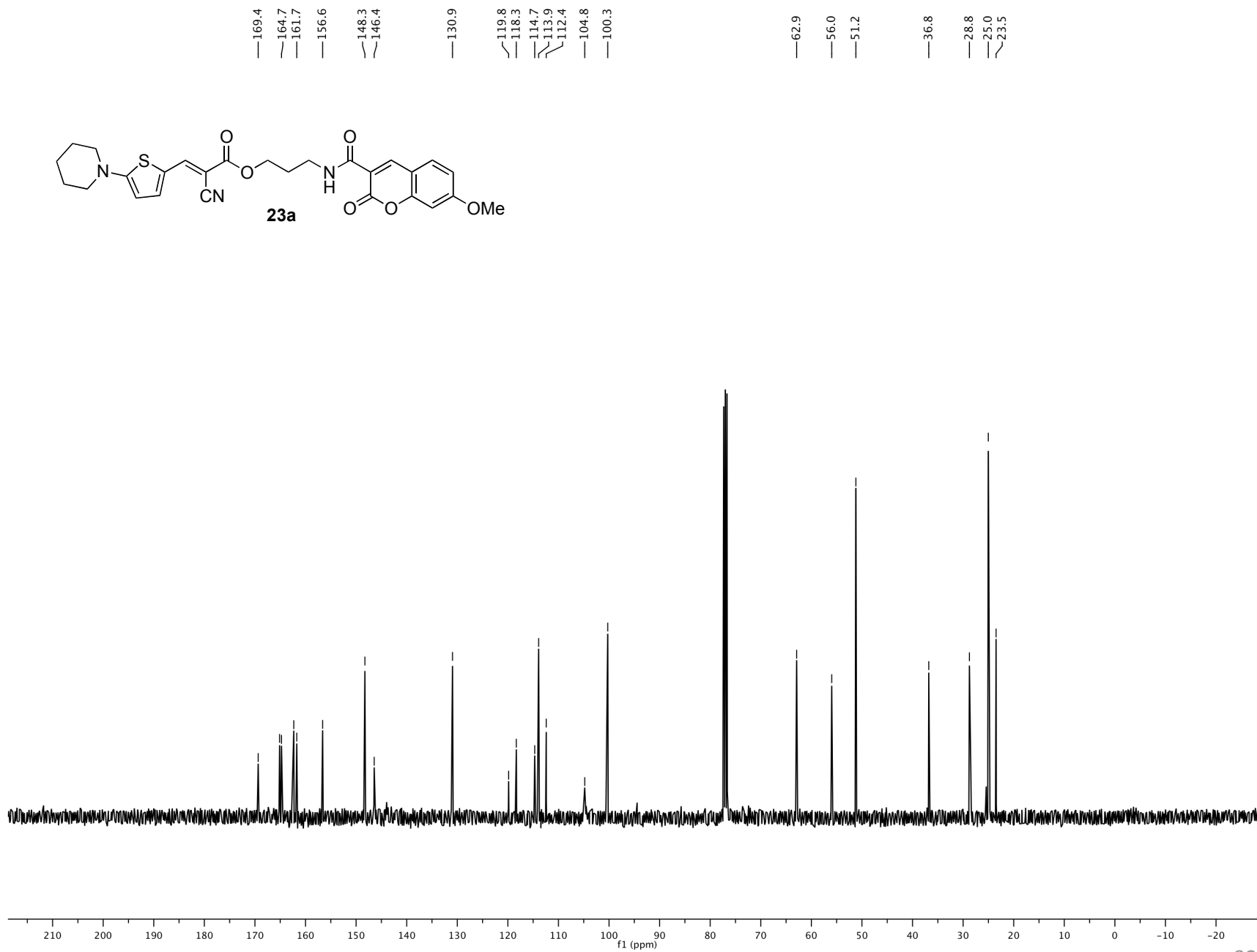


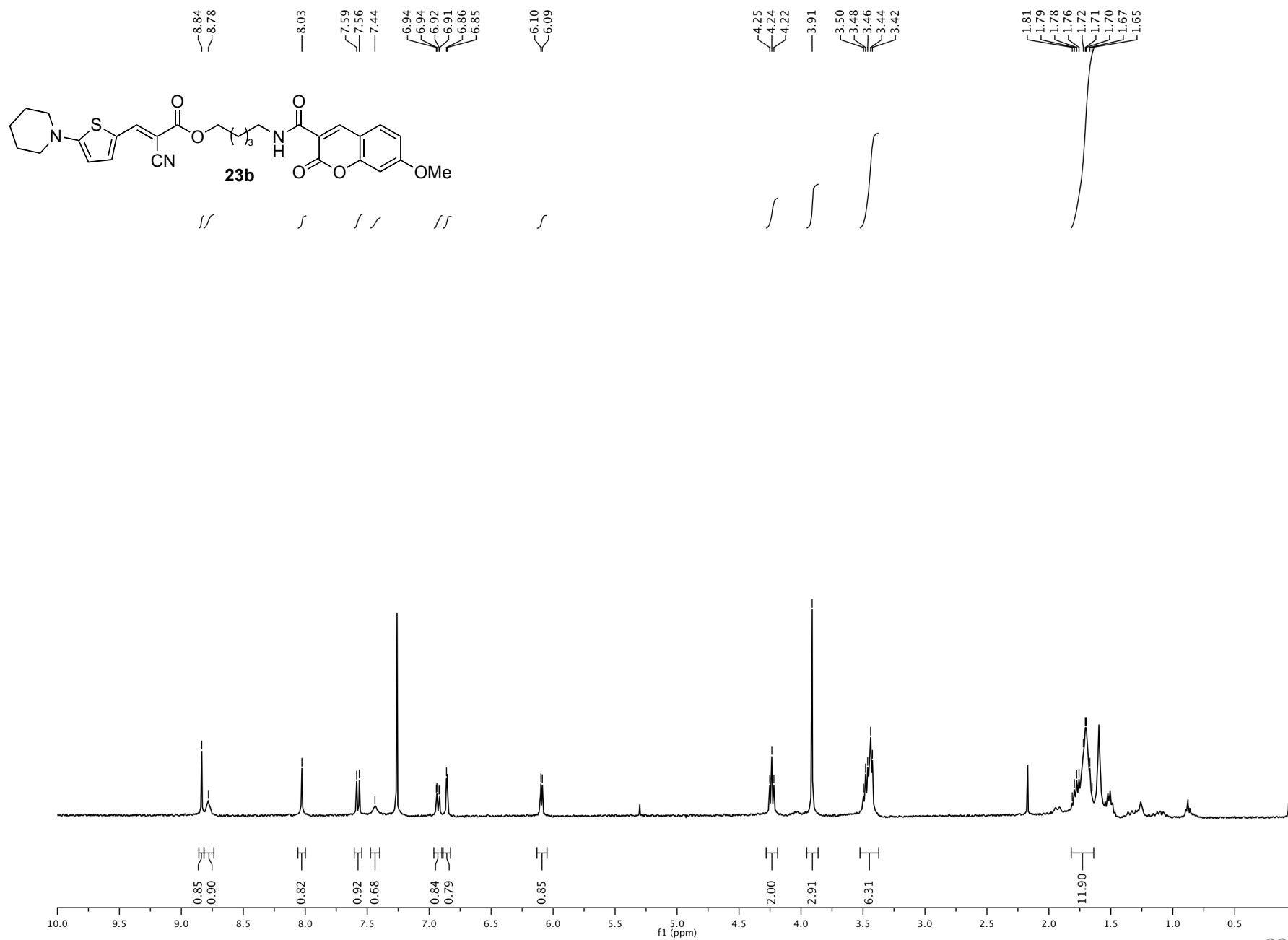


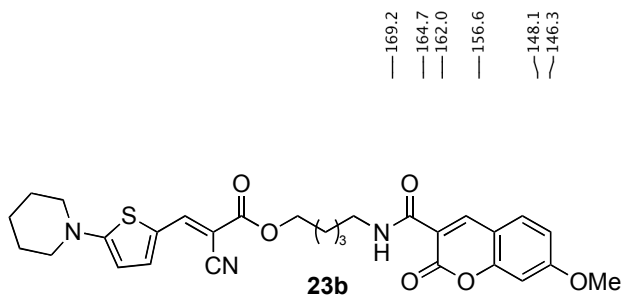












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—156.6

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—130.9

—118.3

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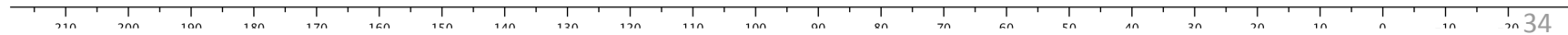
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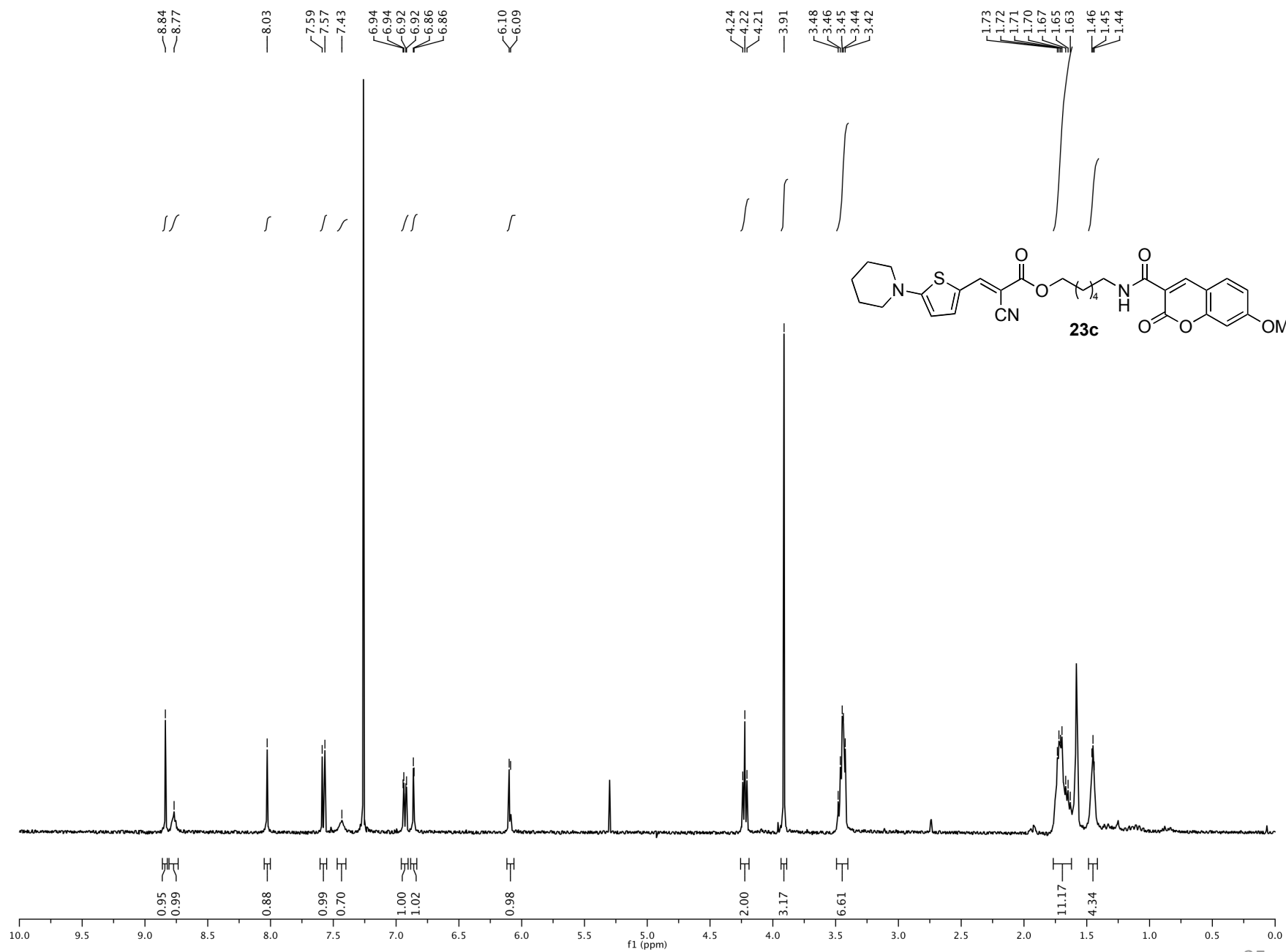
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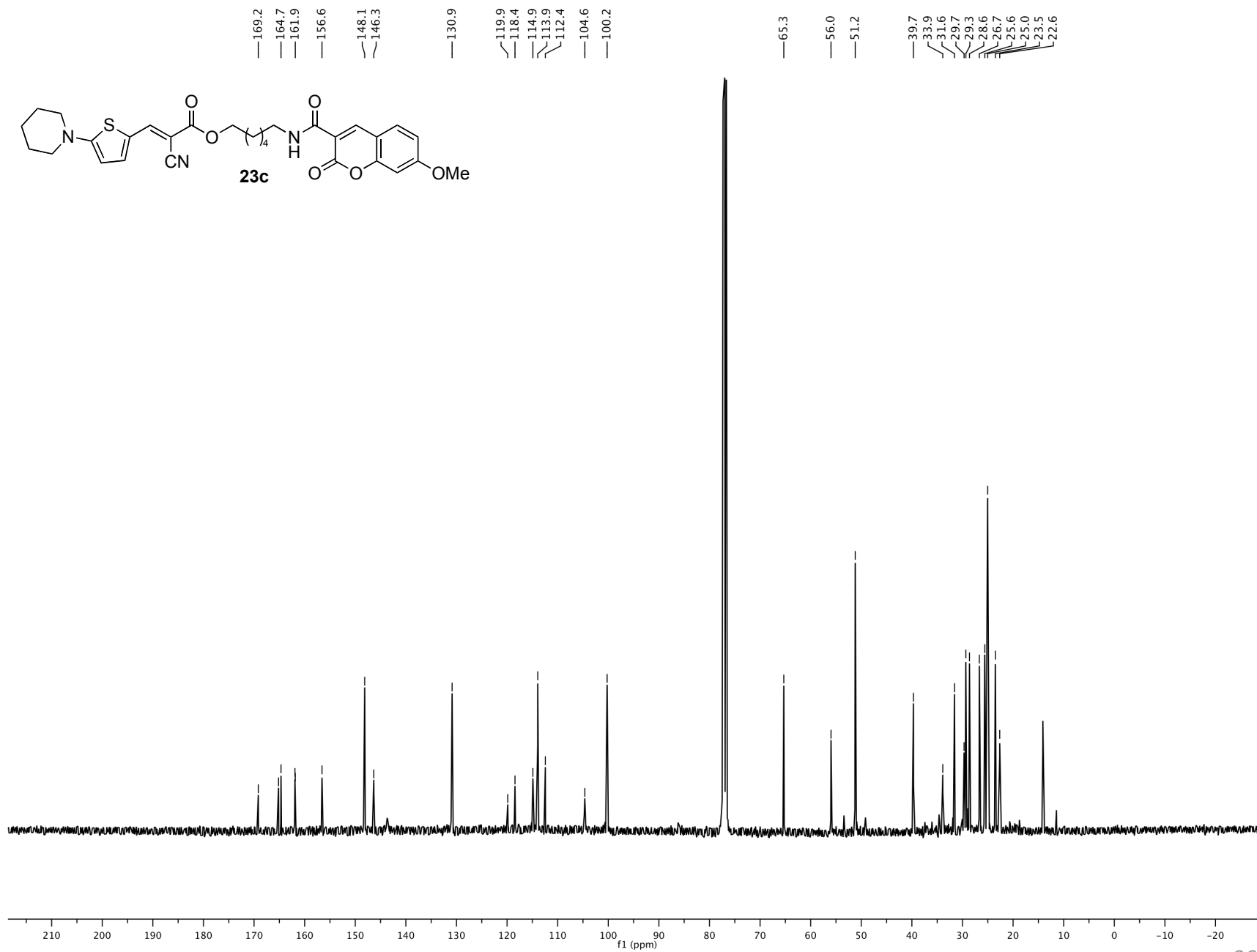
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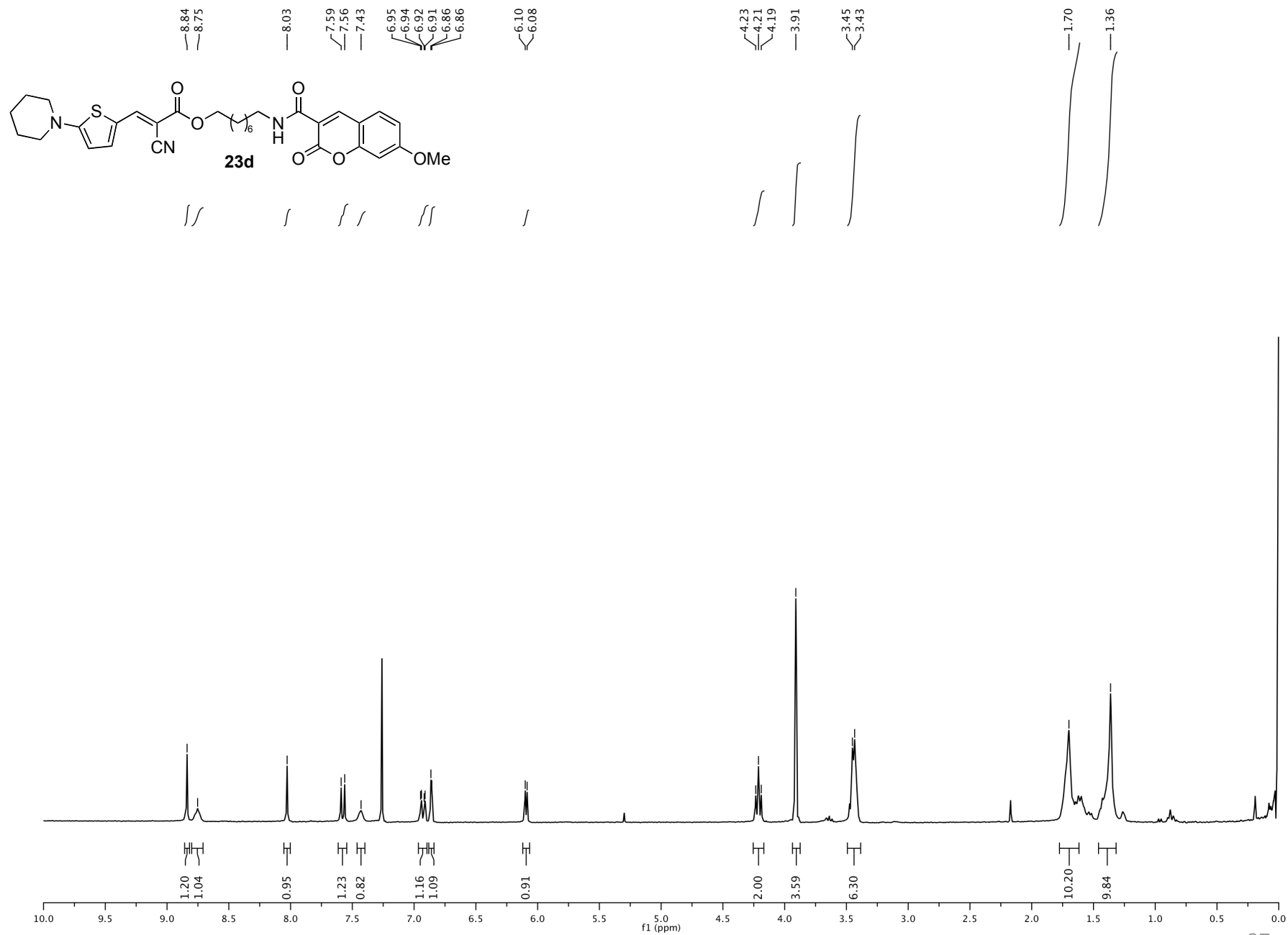
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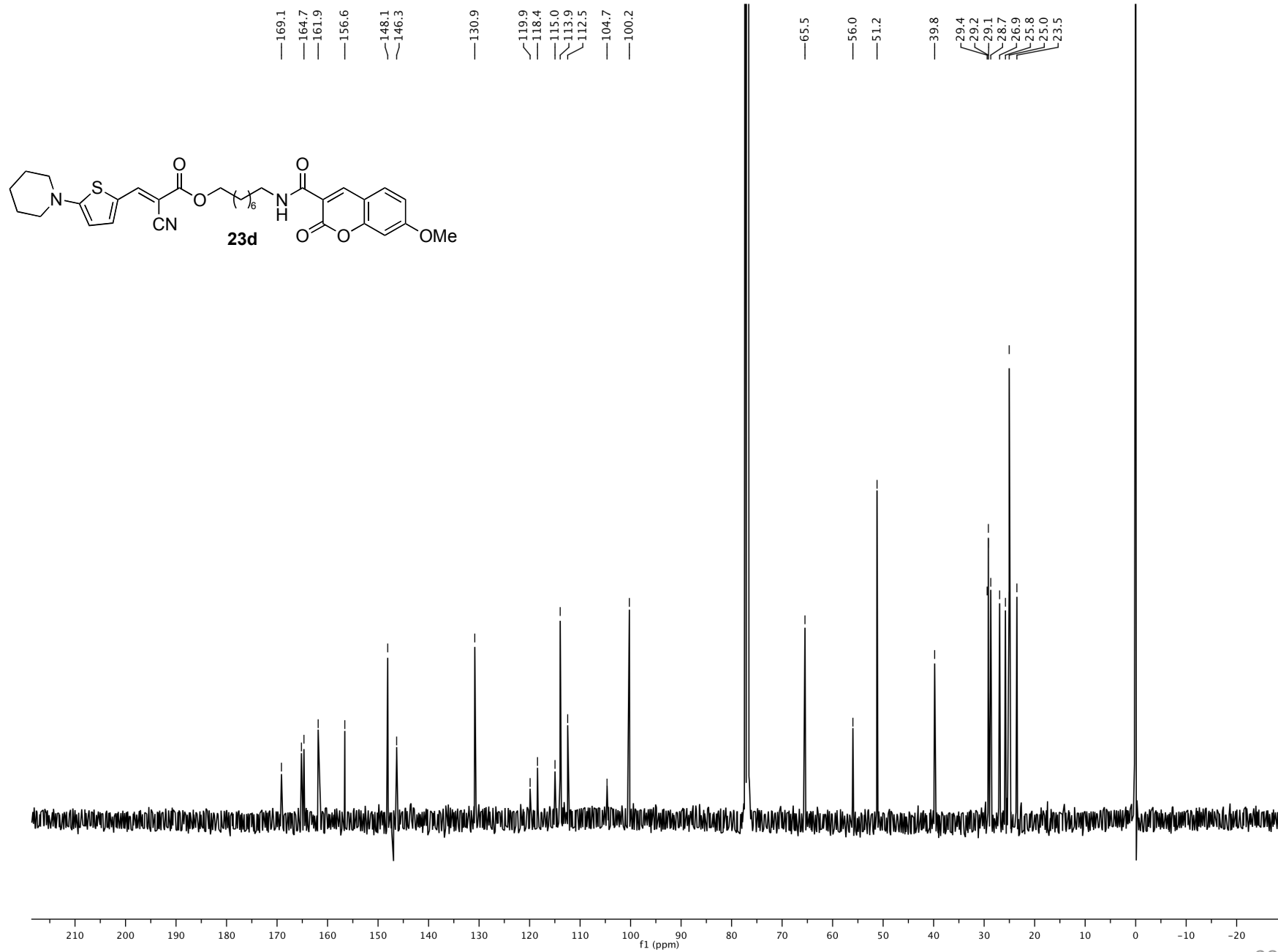
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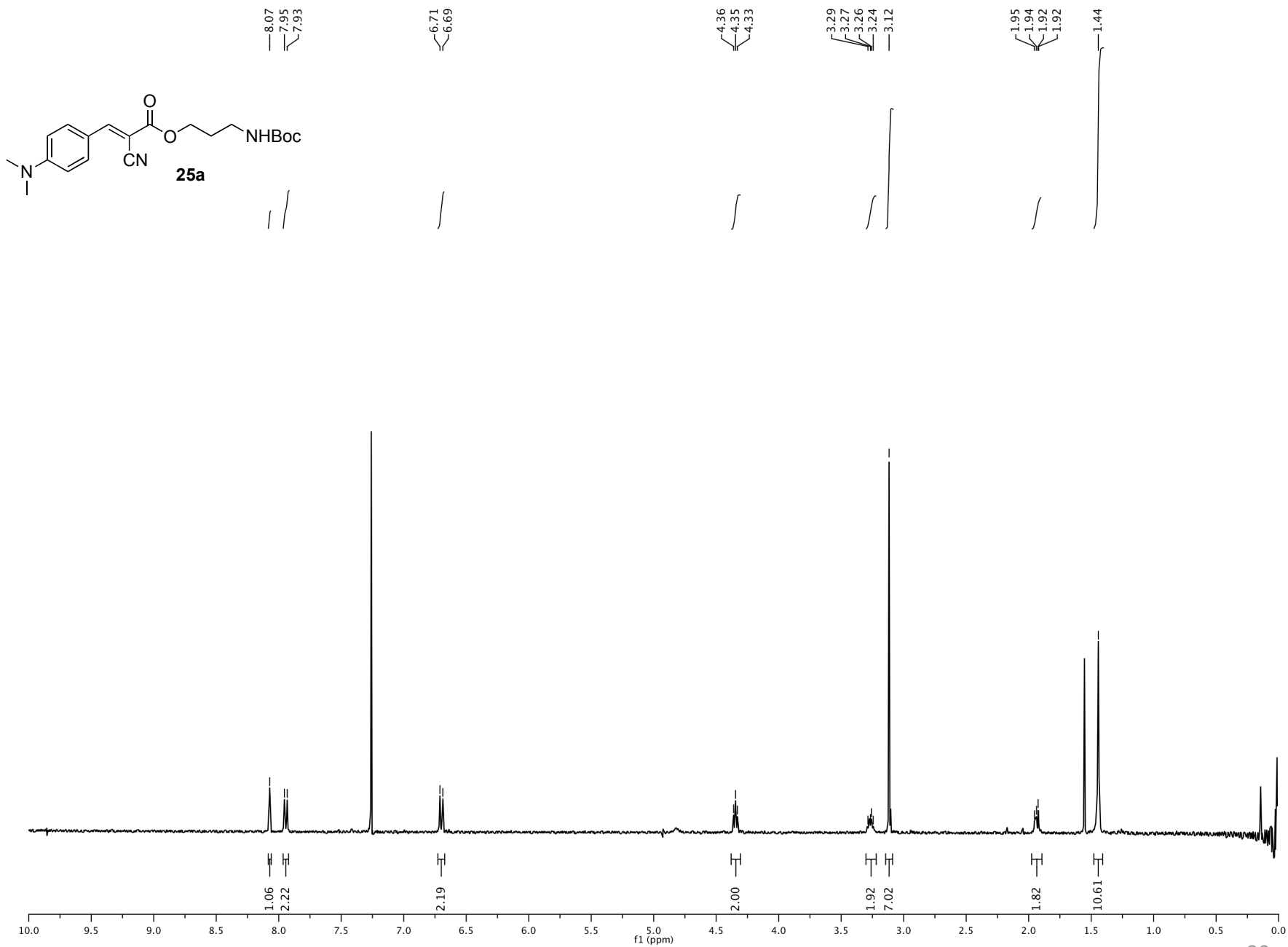


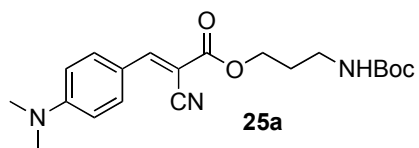












—164.4

—155.9

—154.8

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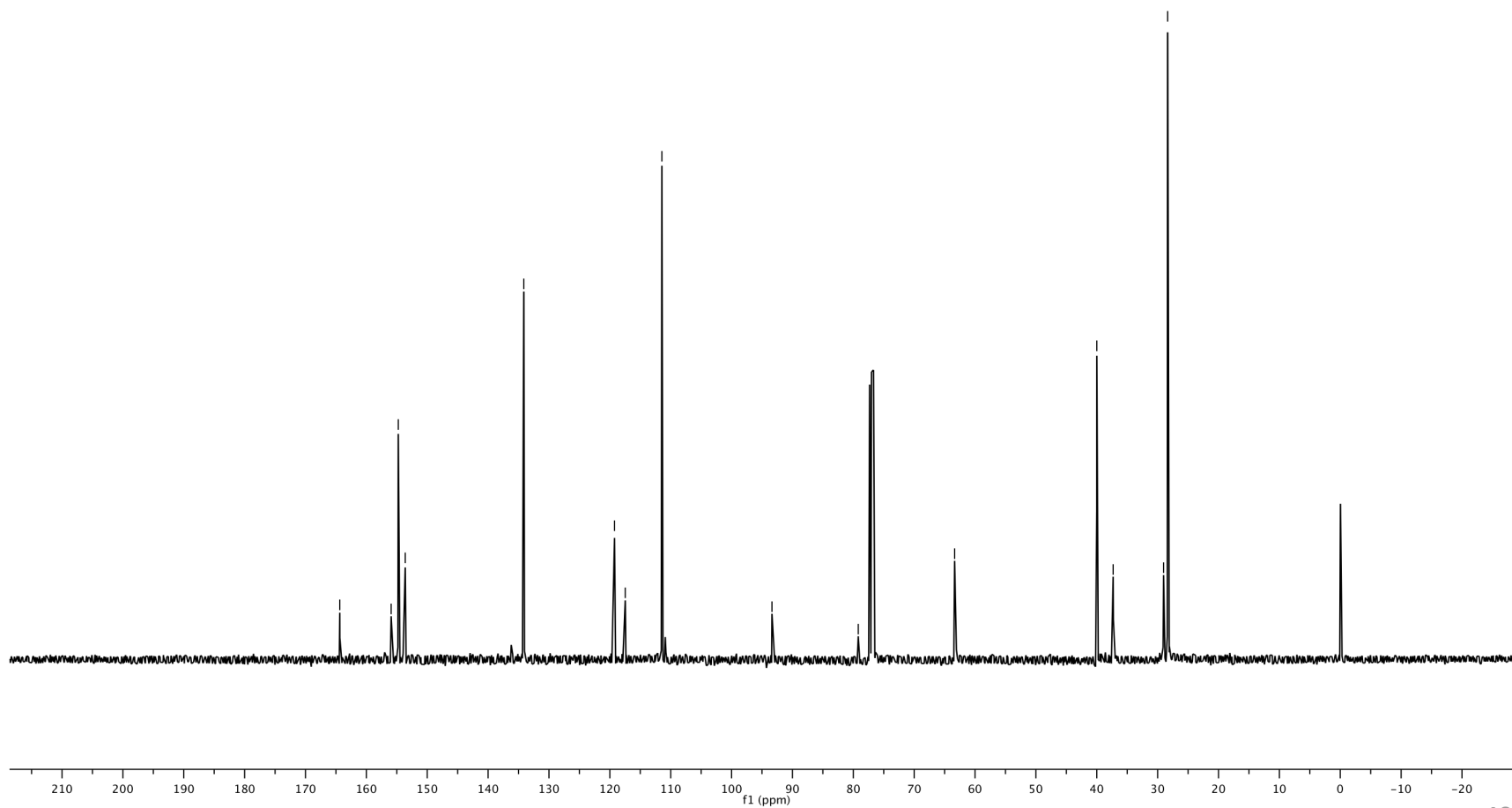
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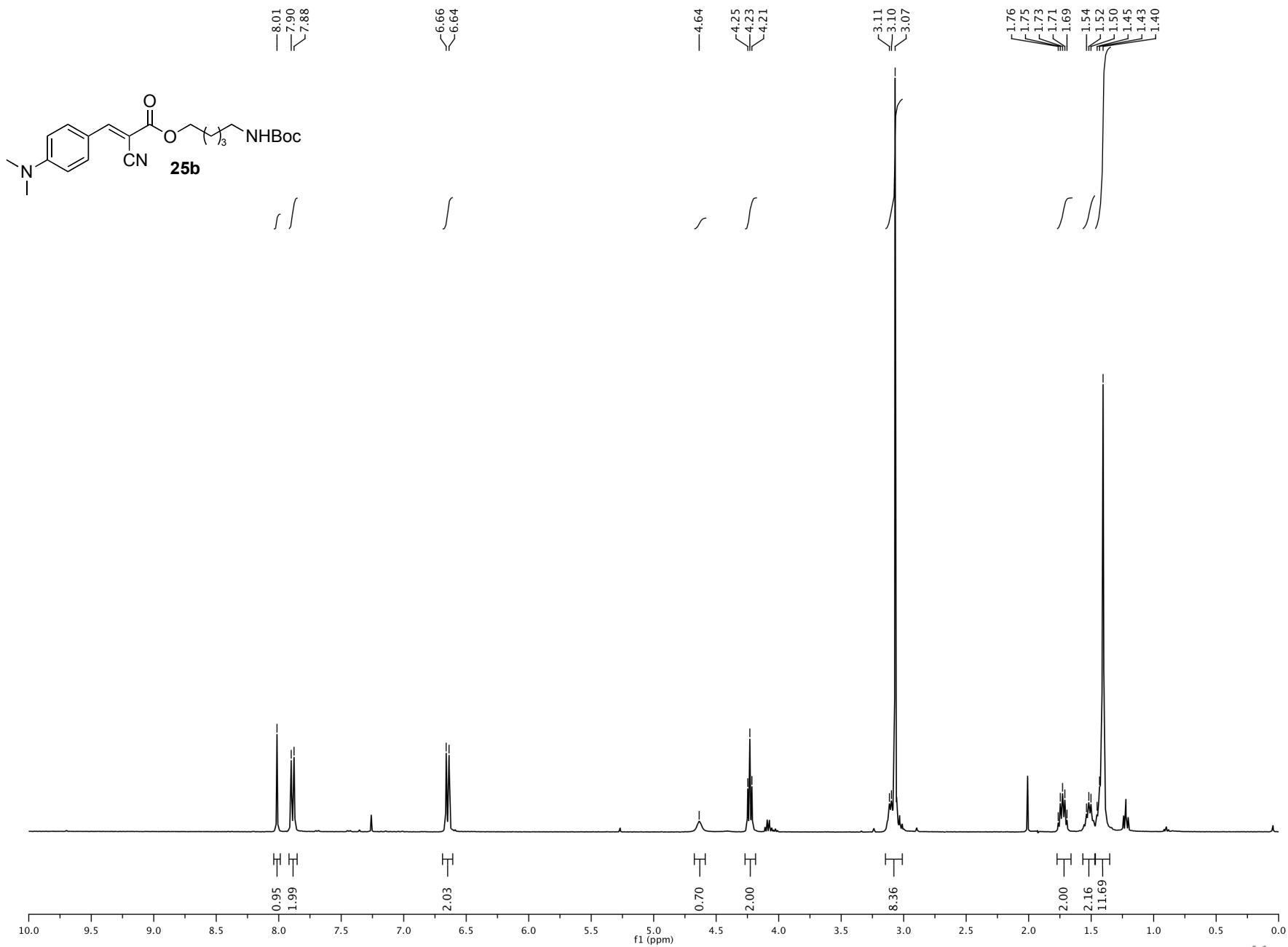
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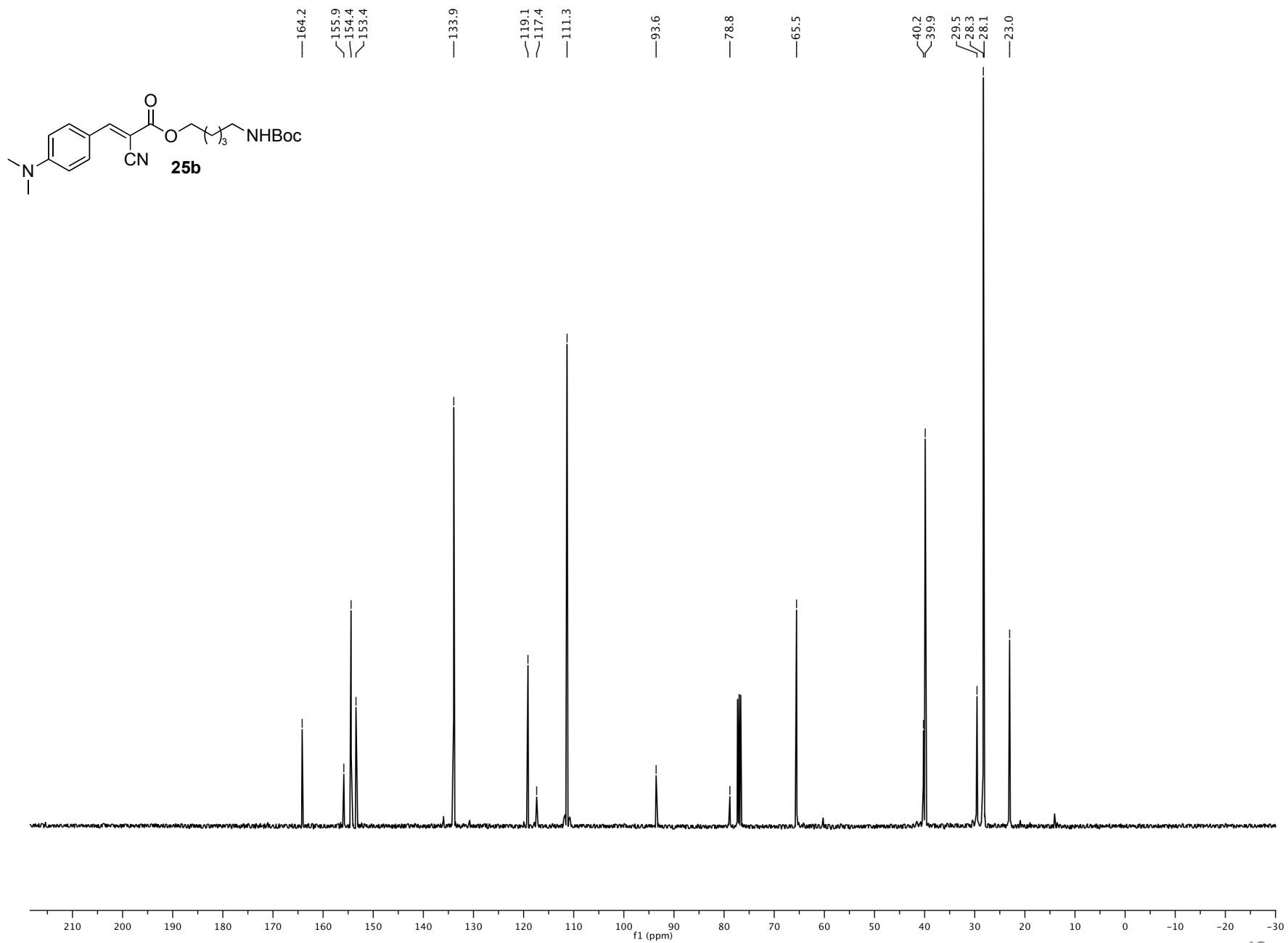
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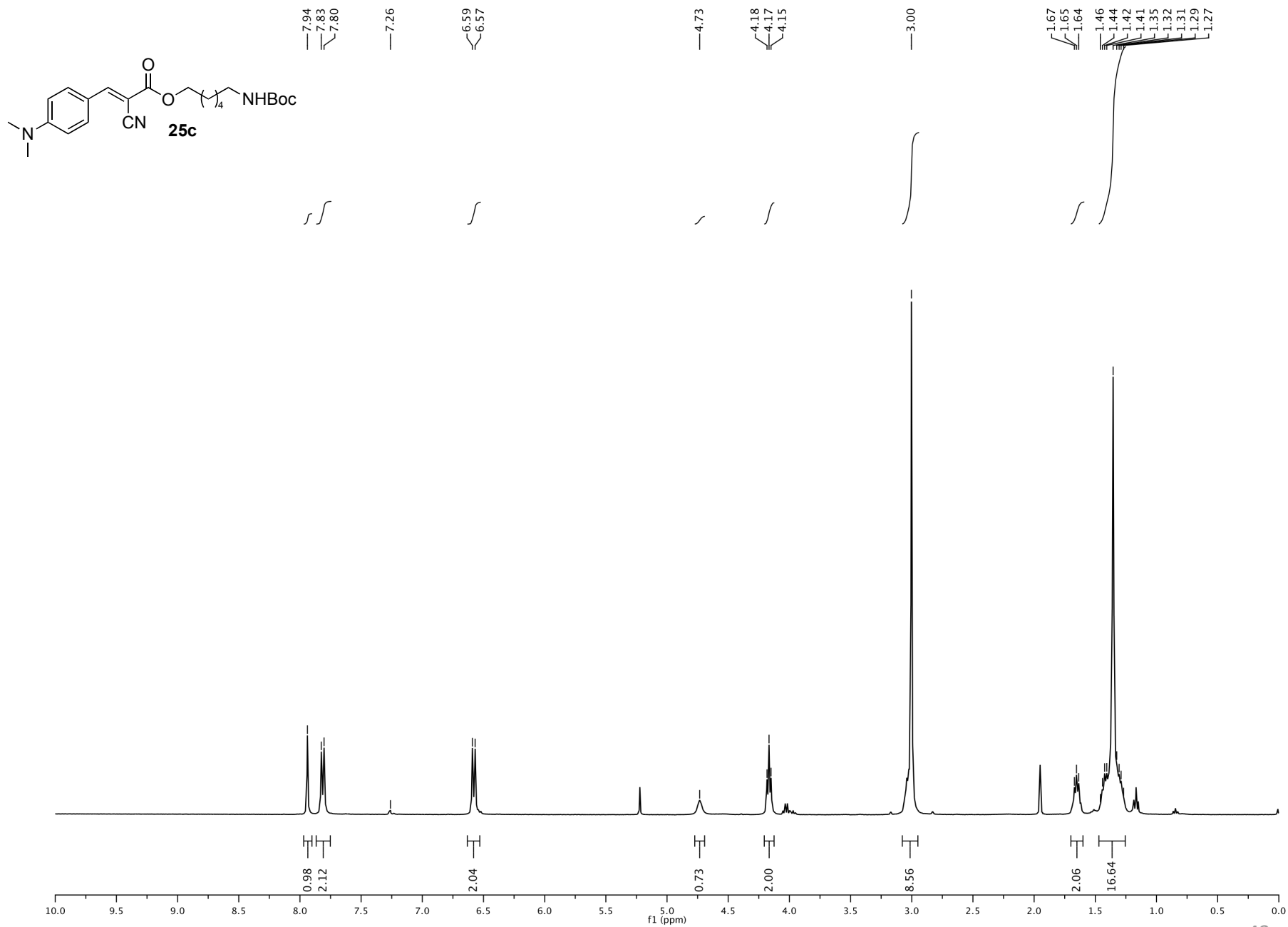
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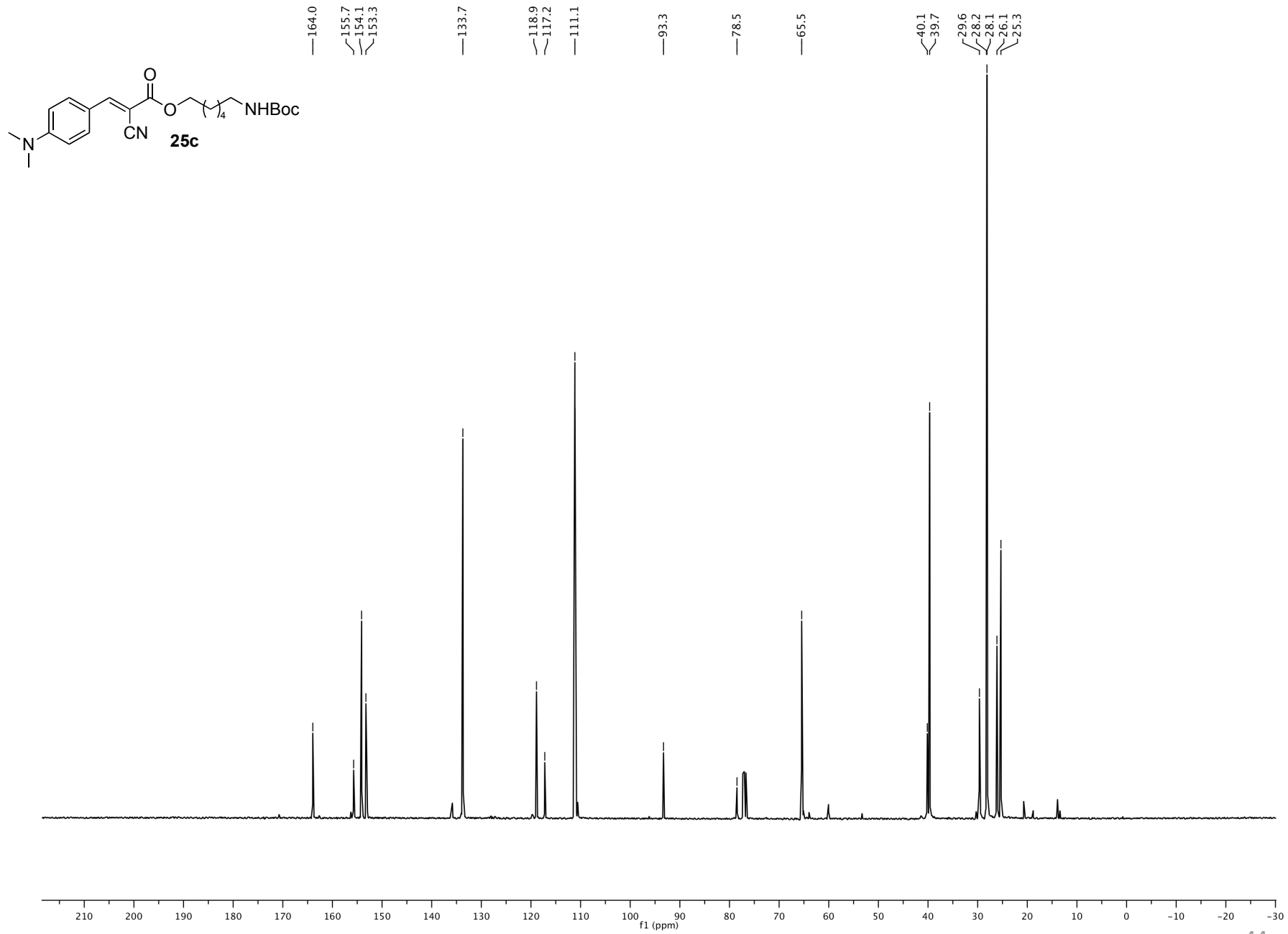
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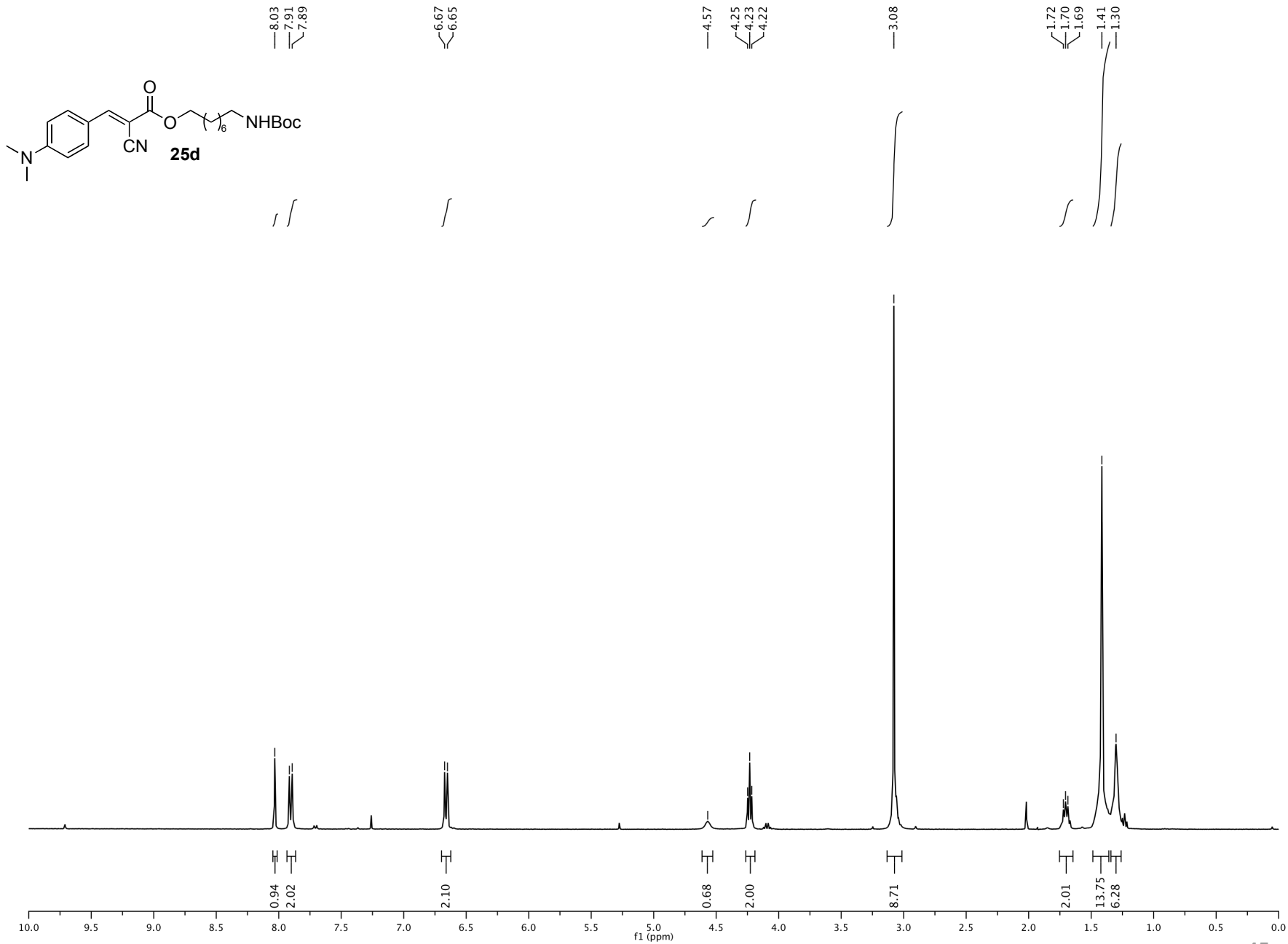


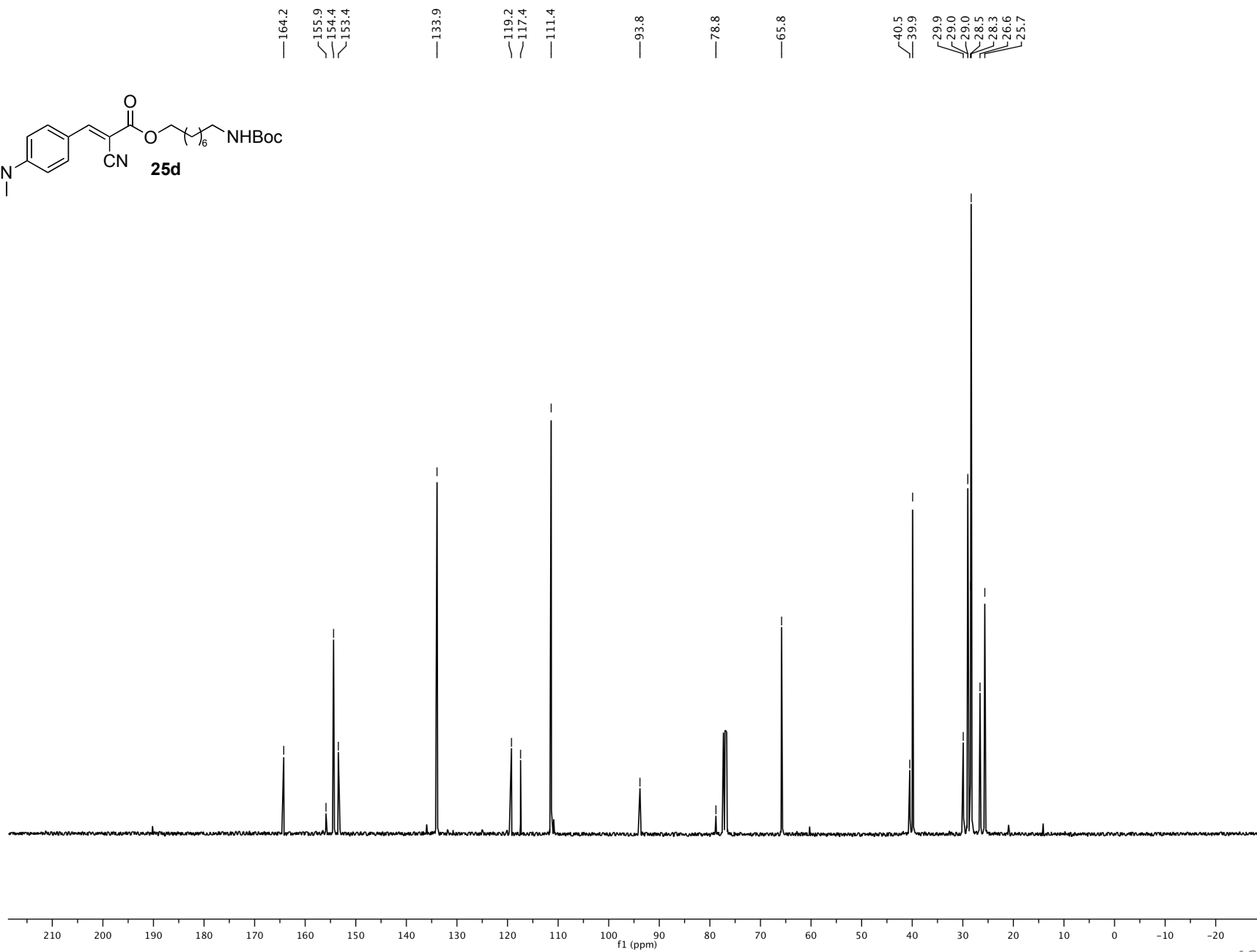
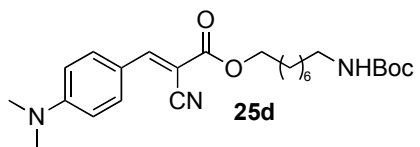


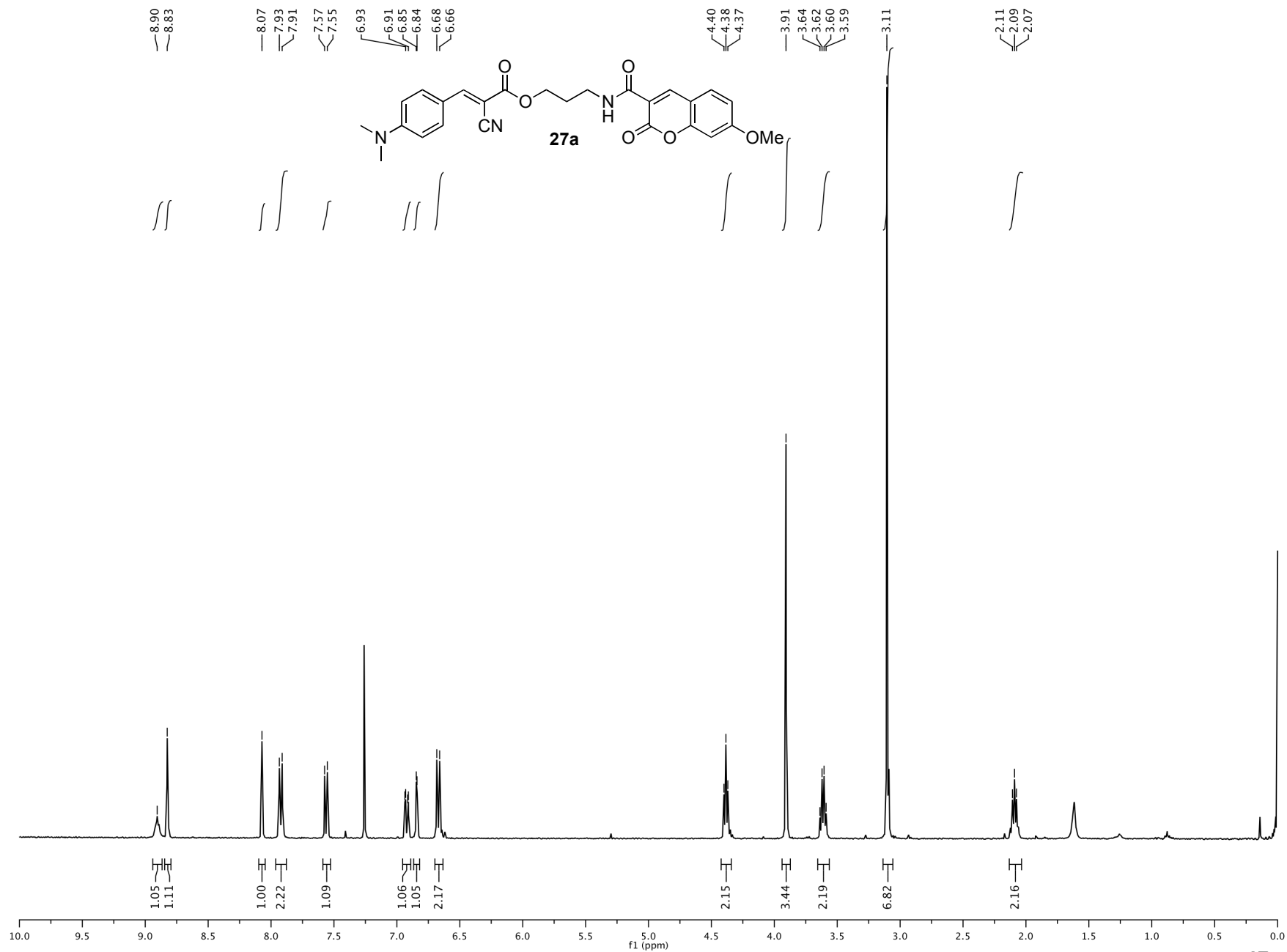


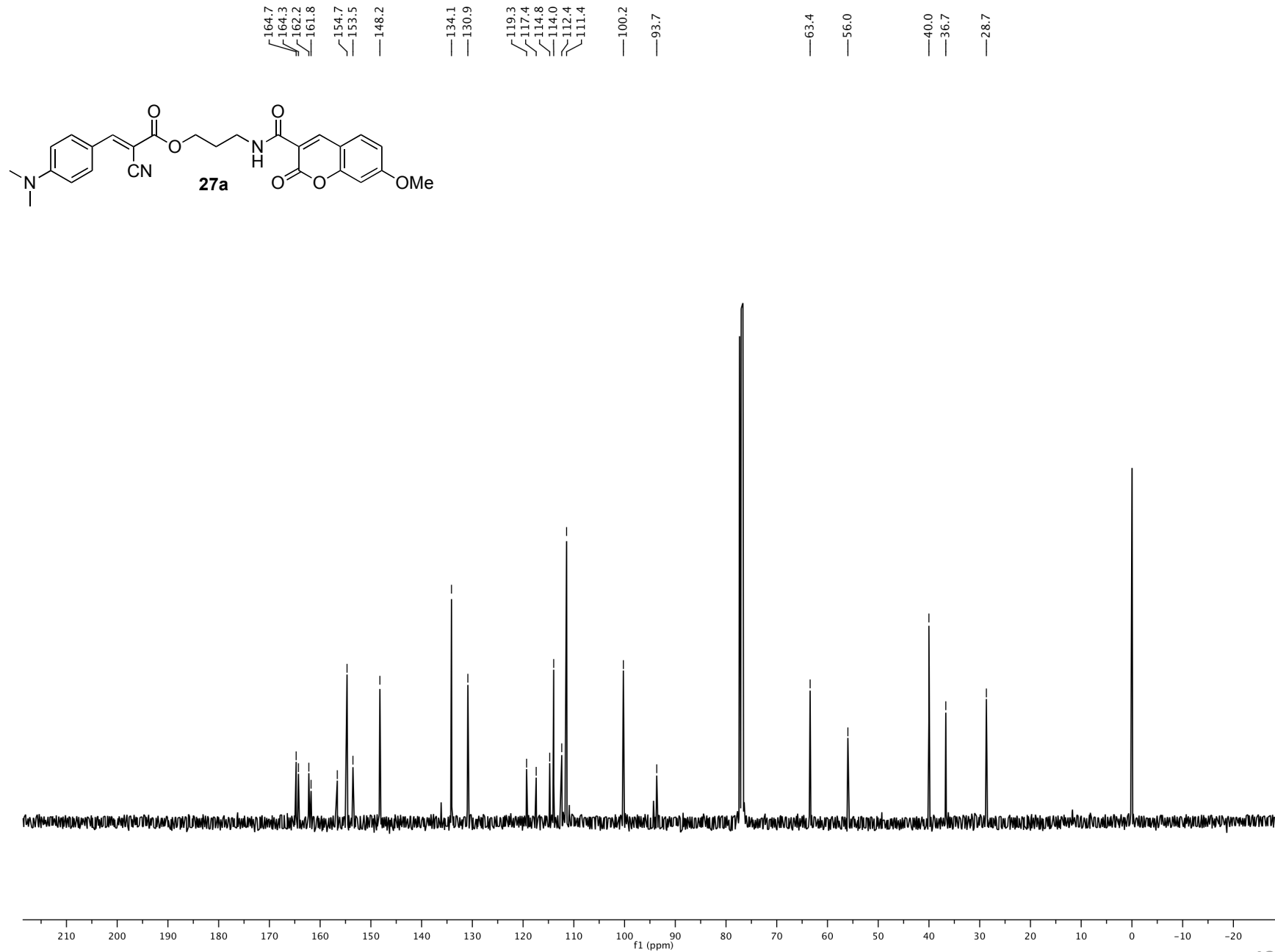


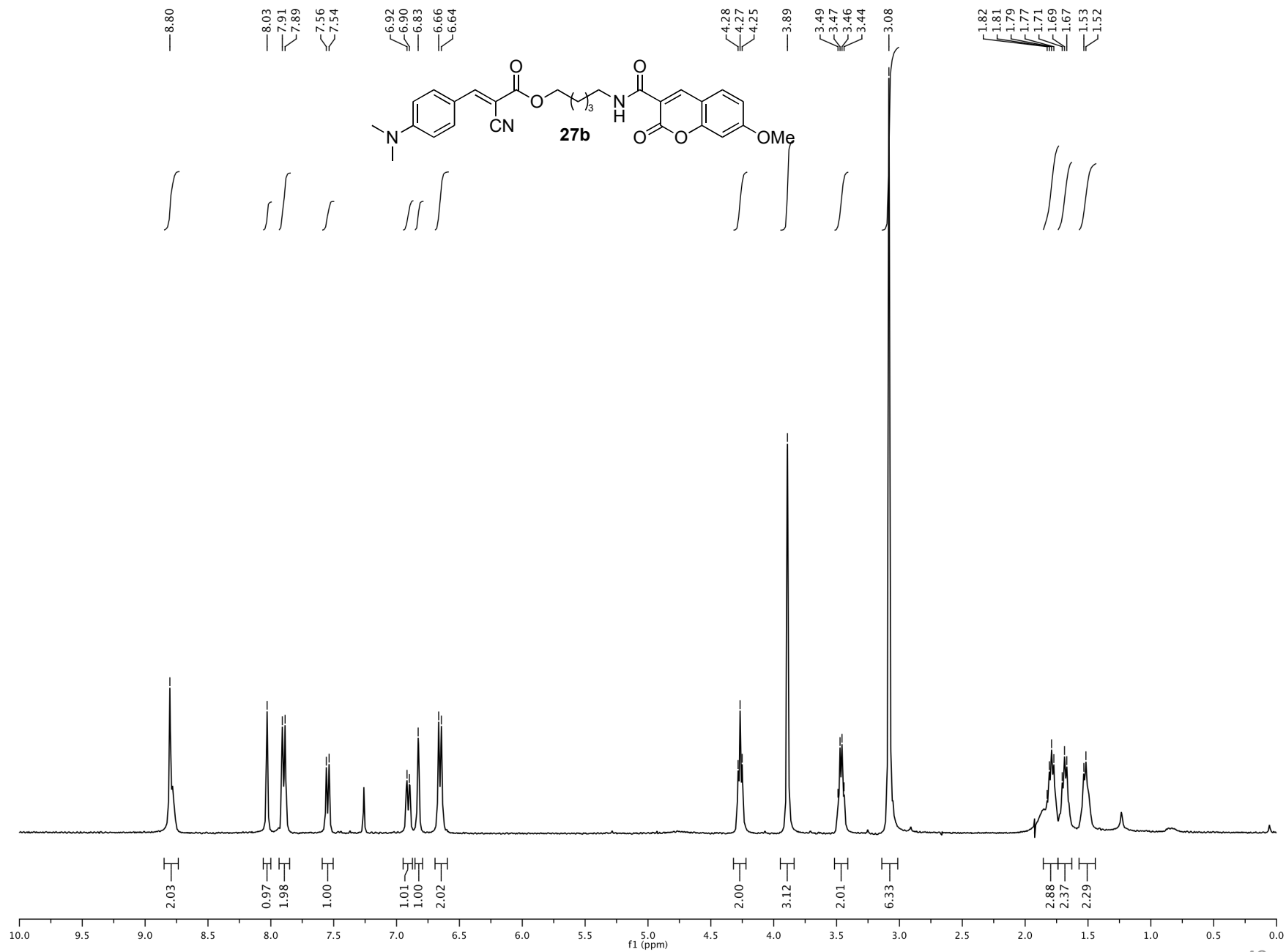


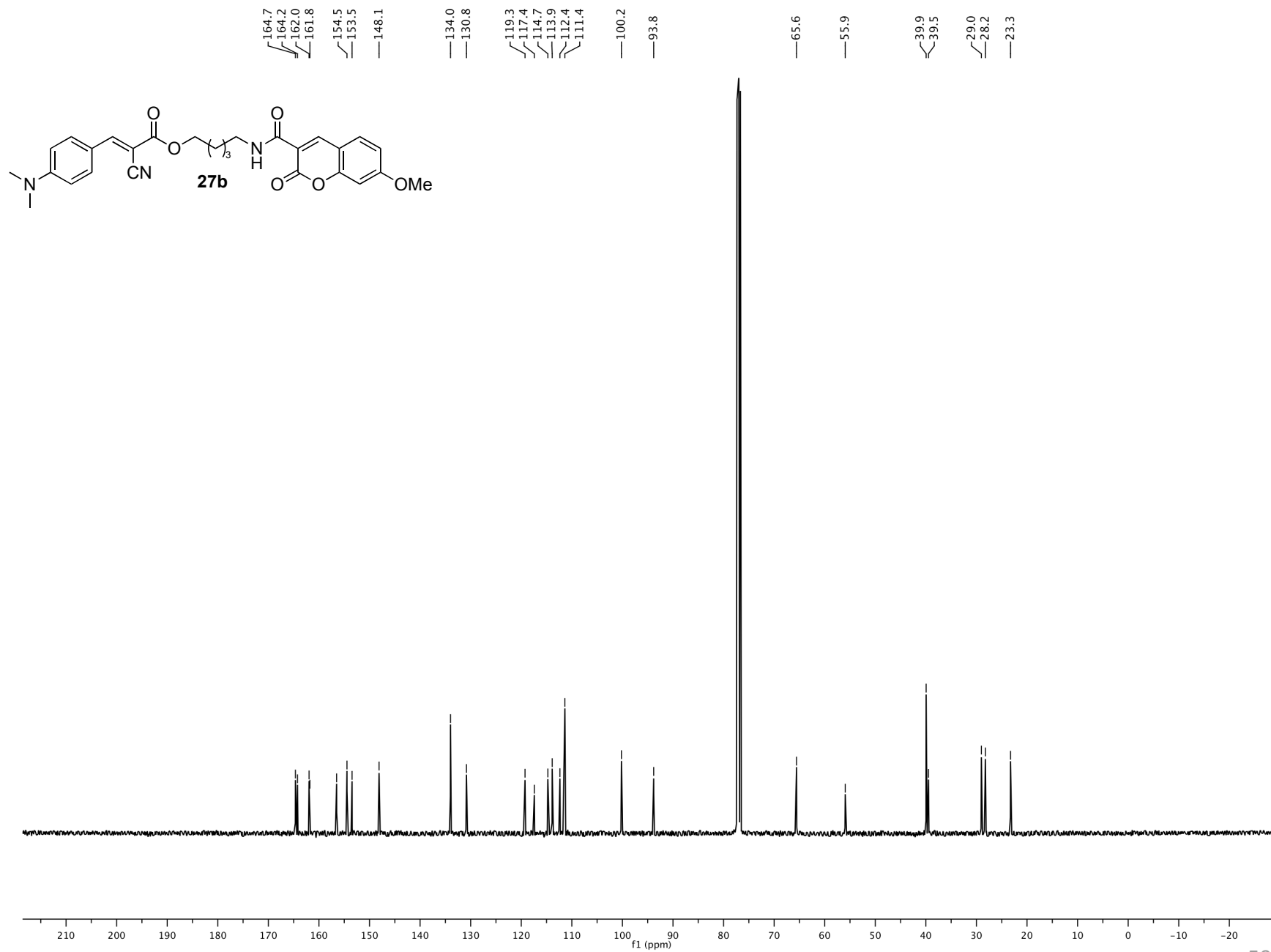


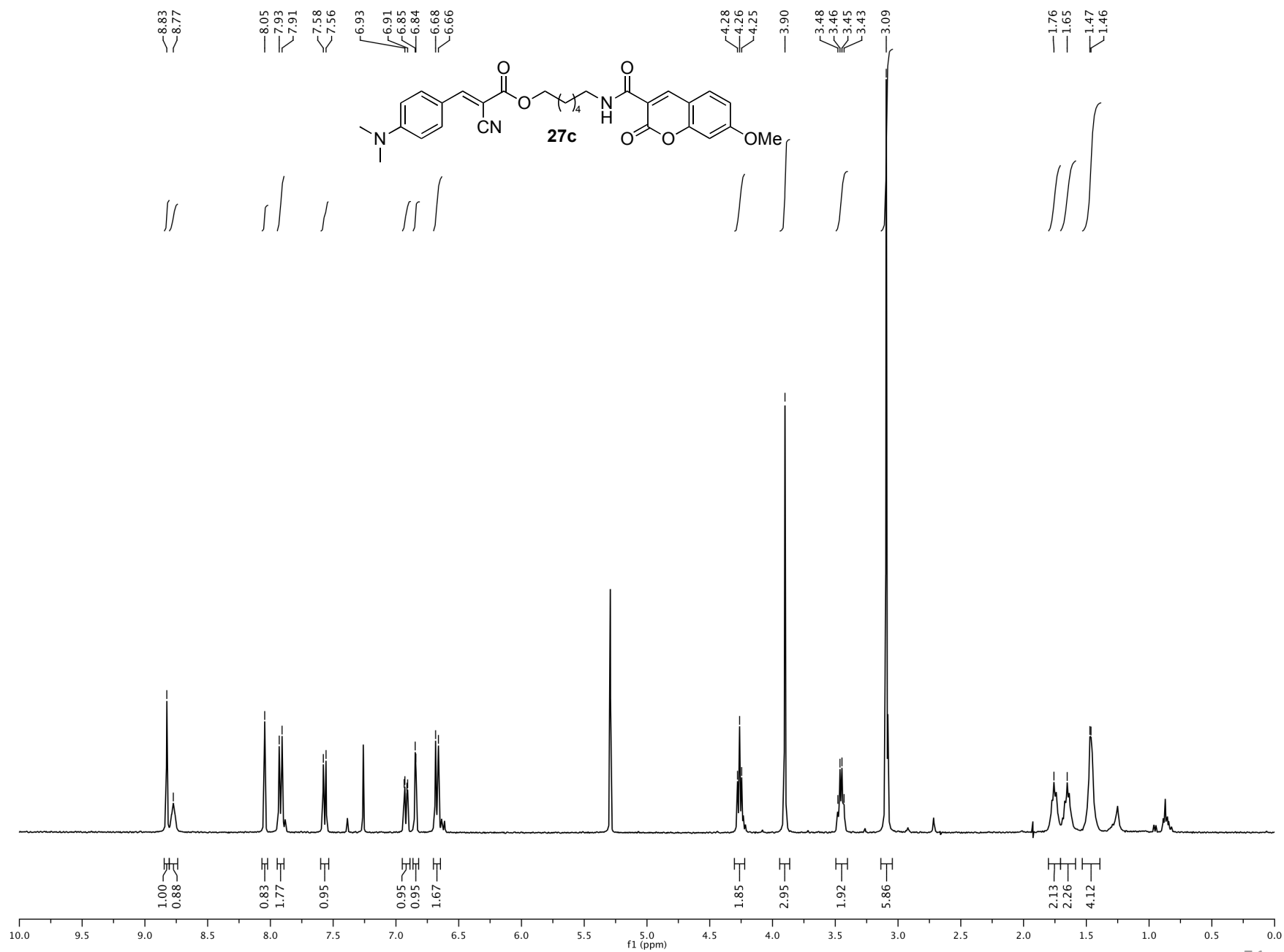


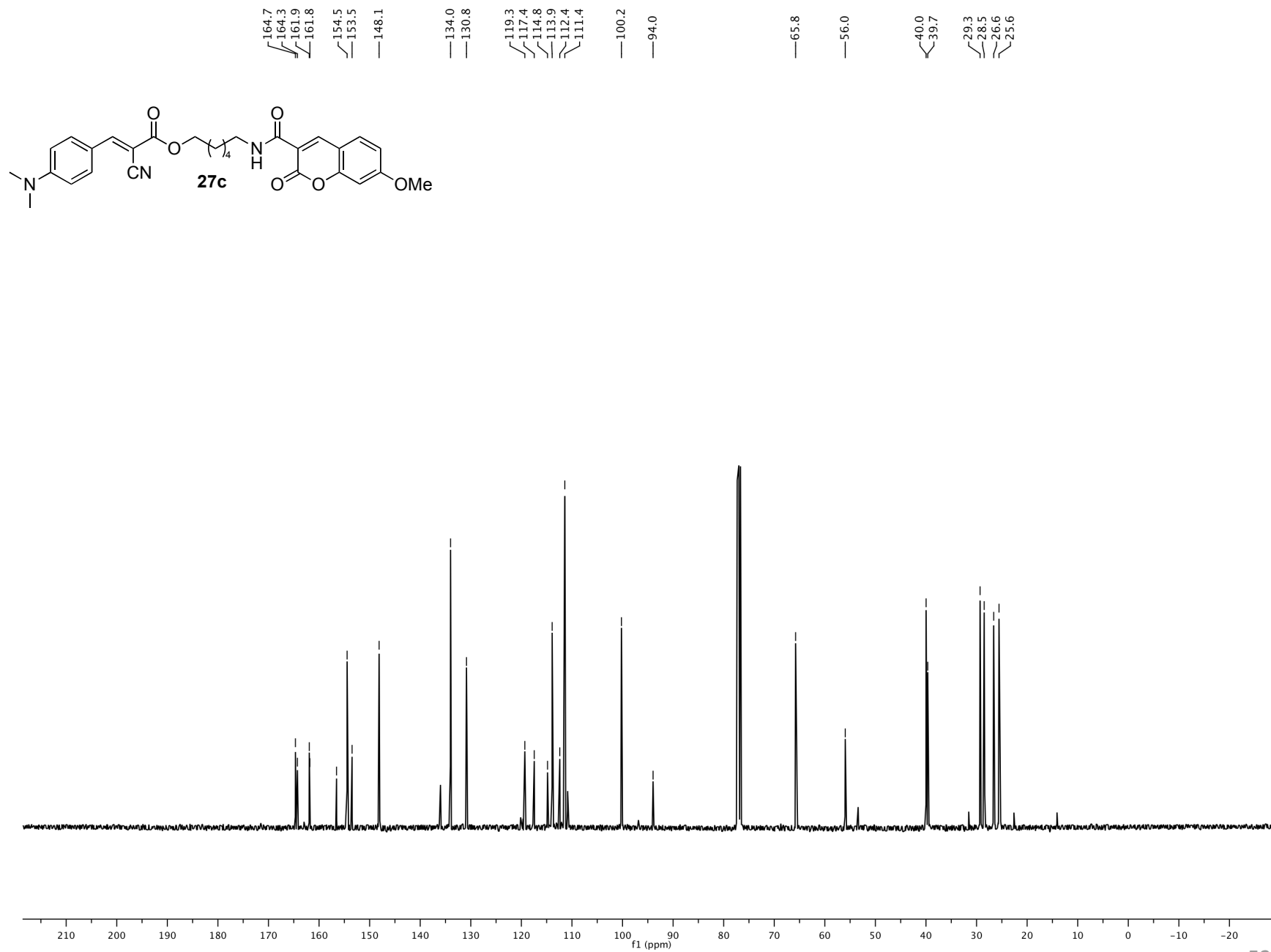


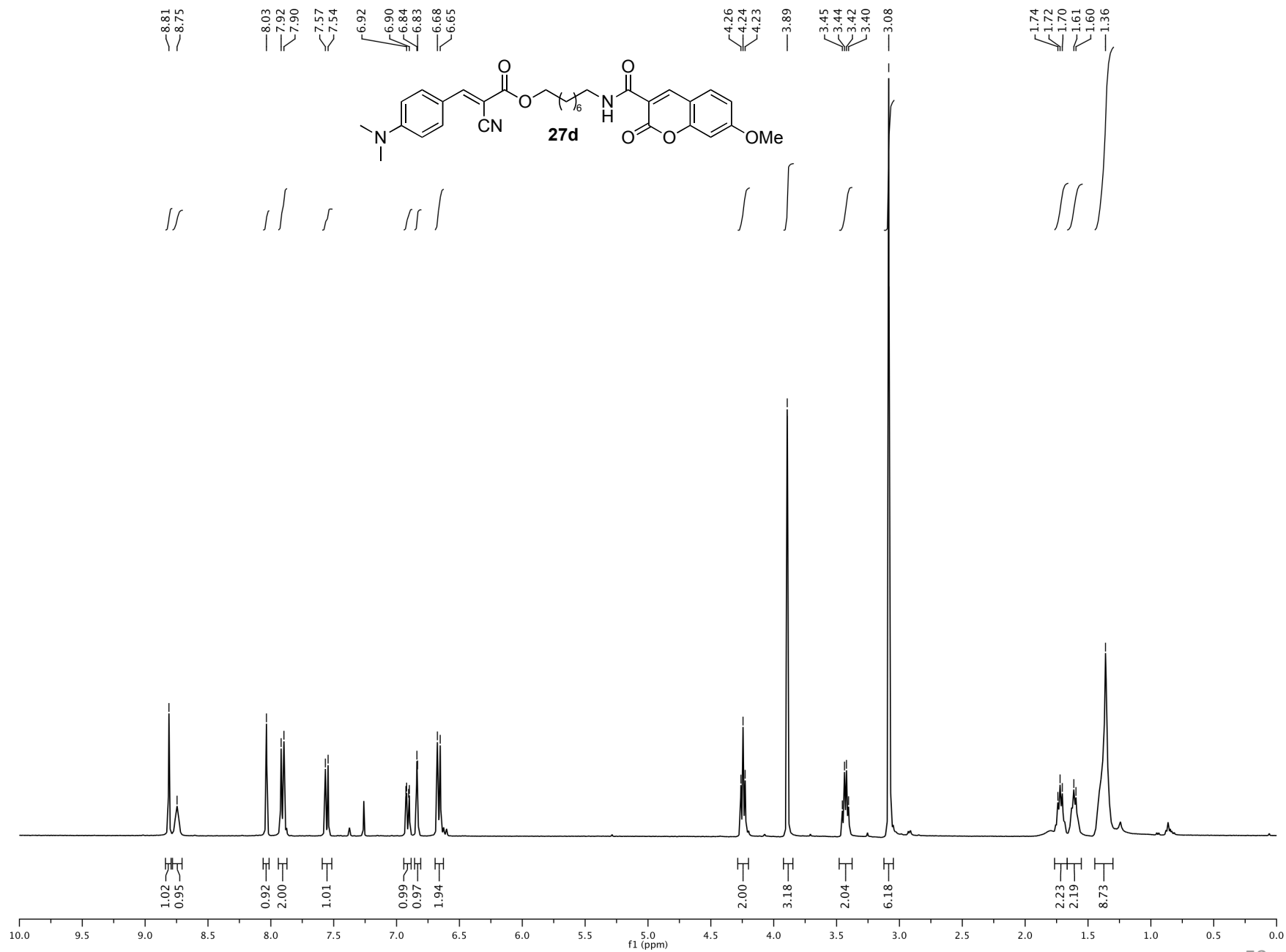


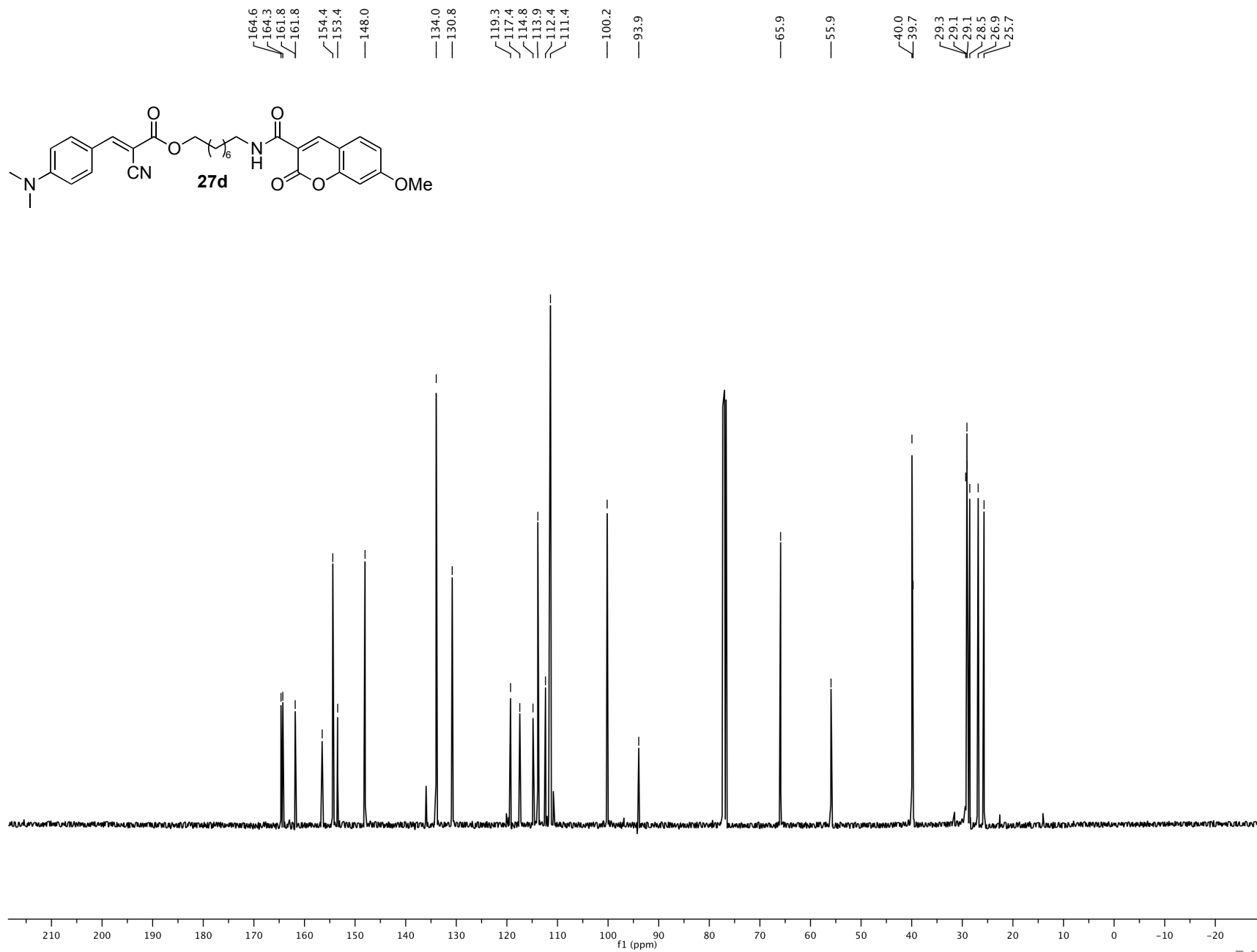


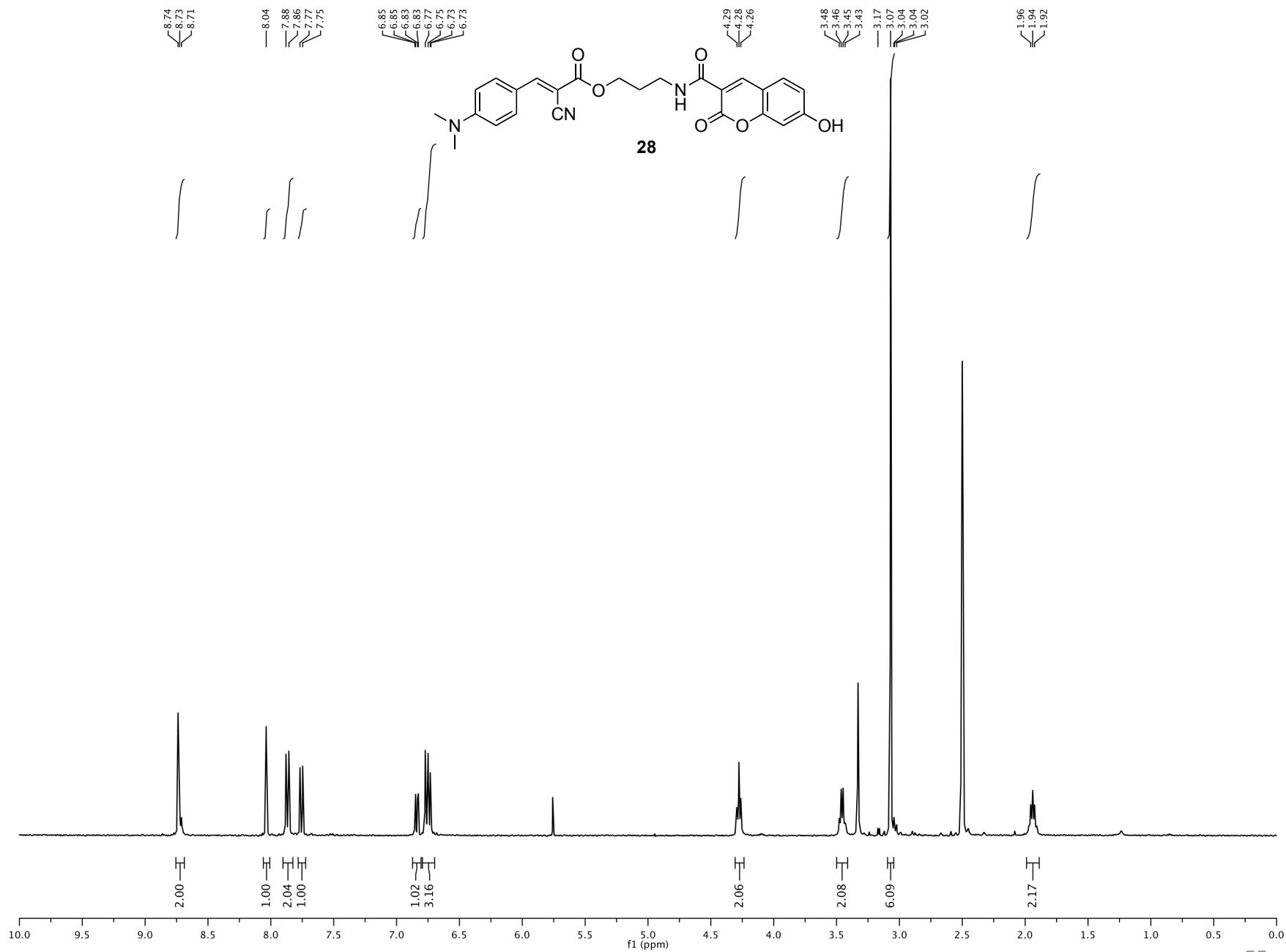


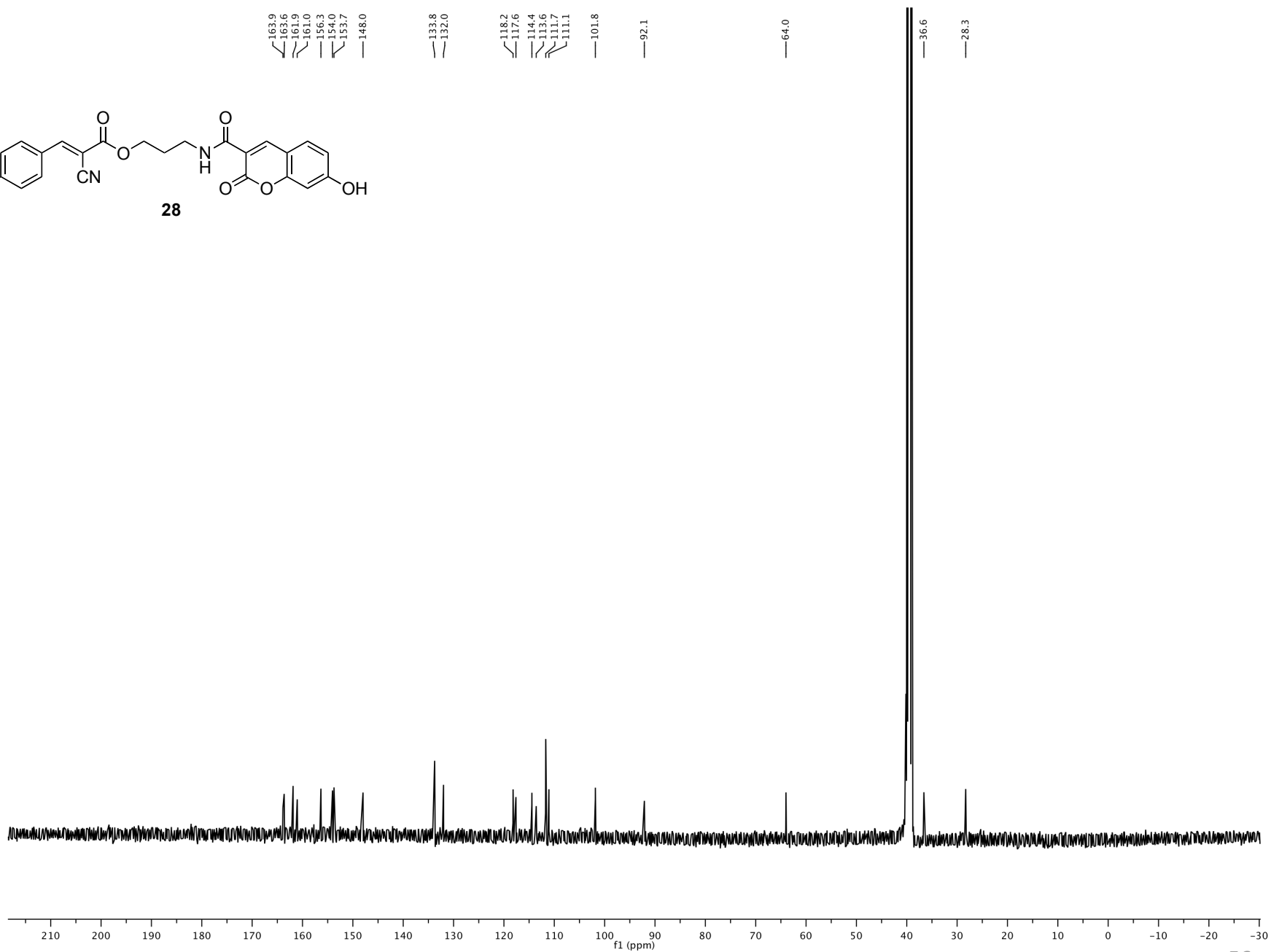
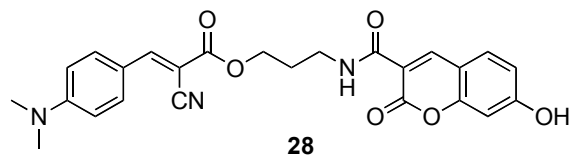


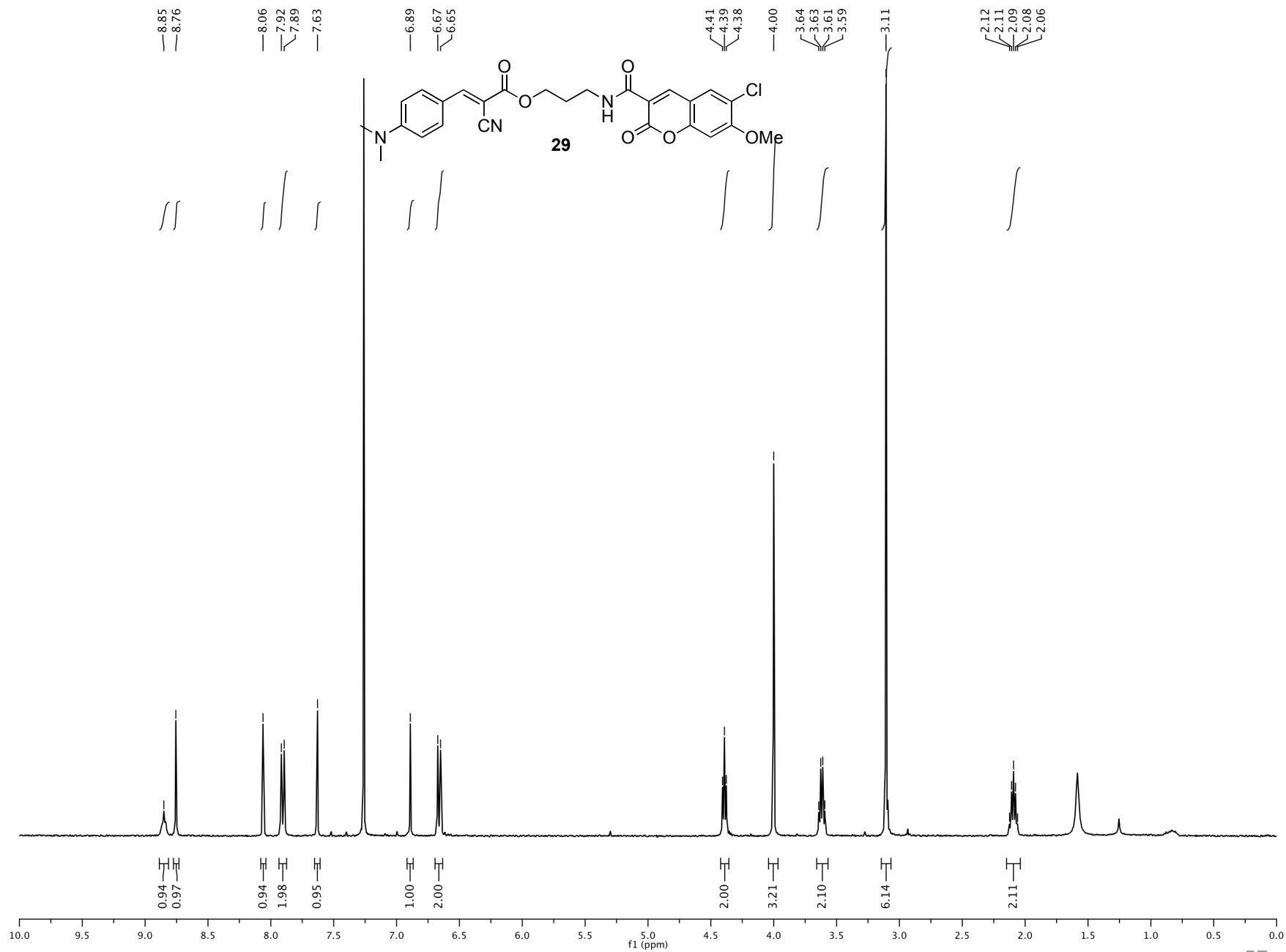


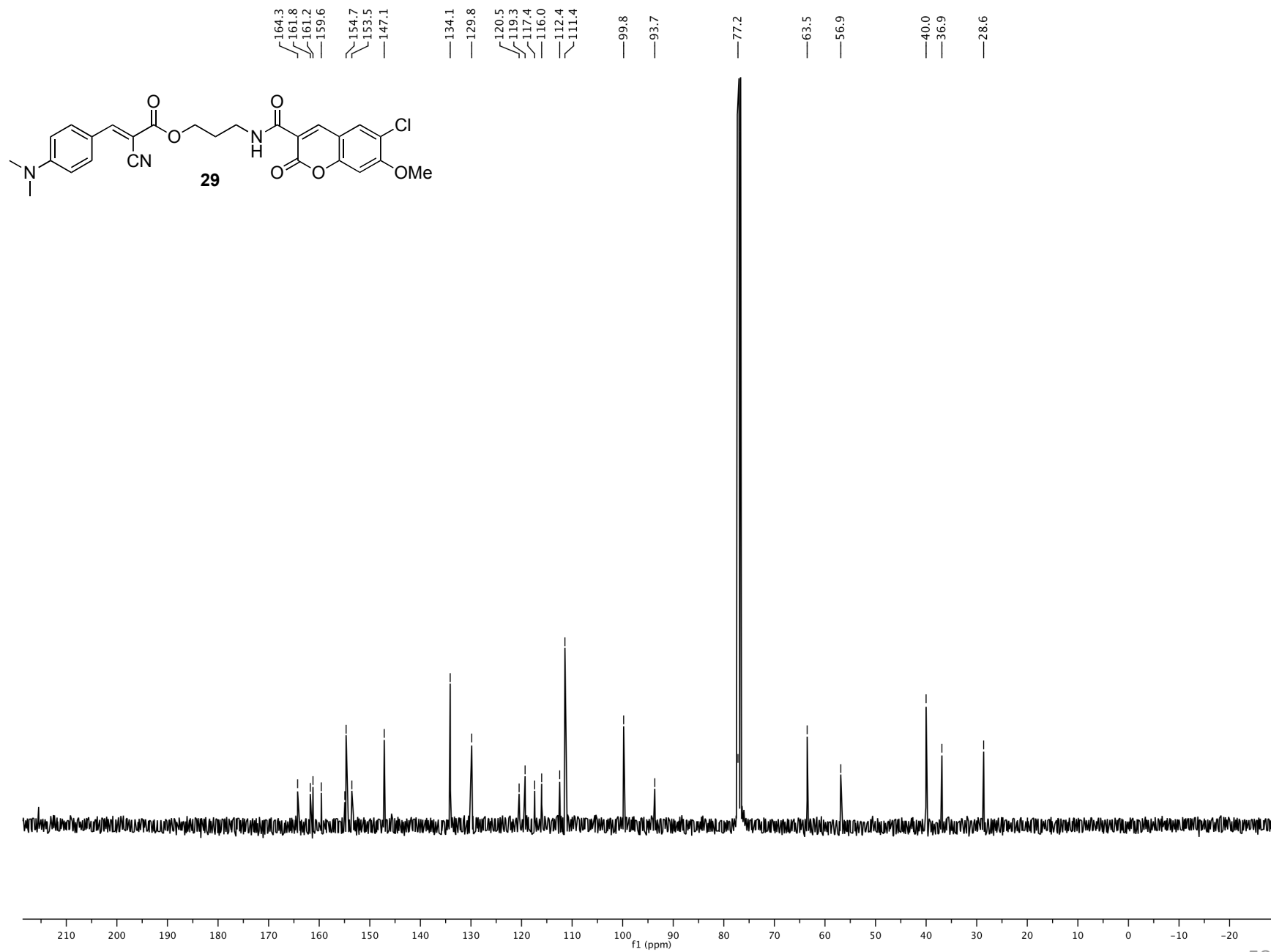


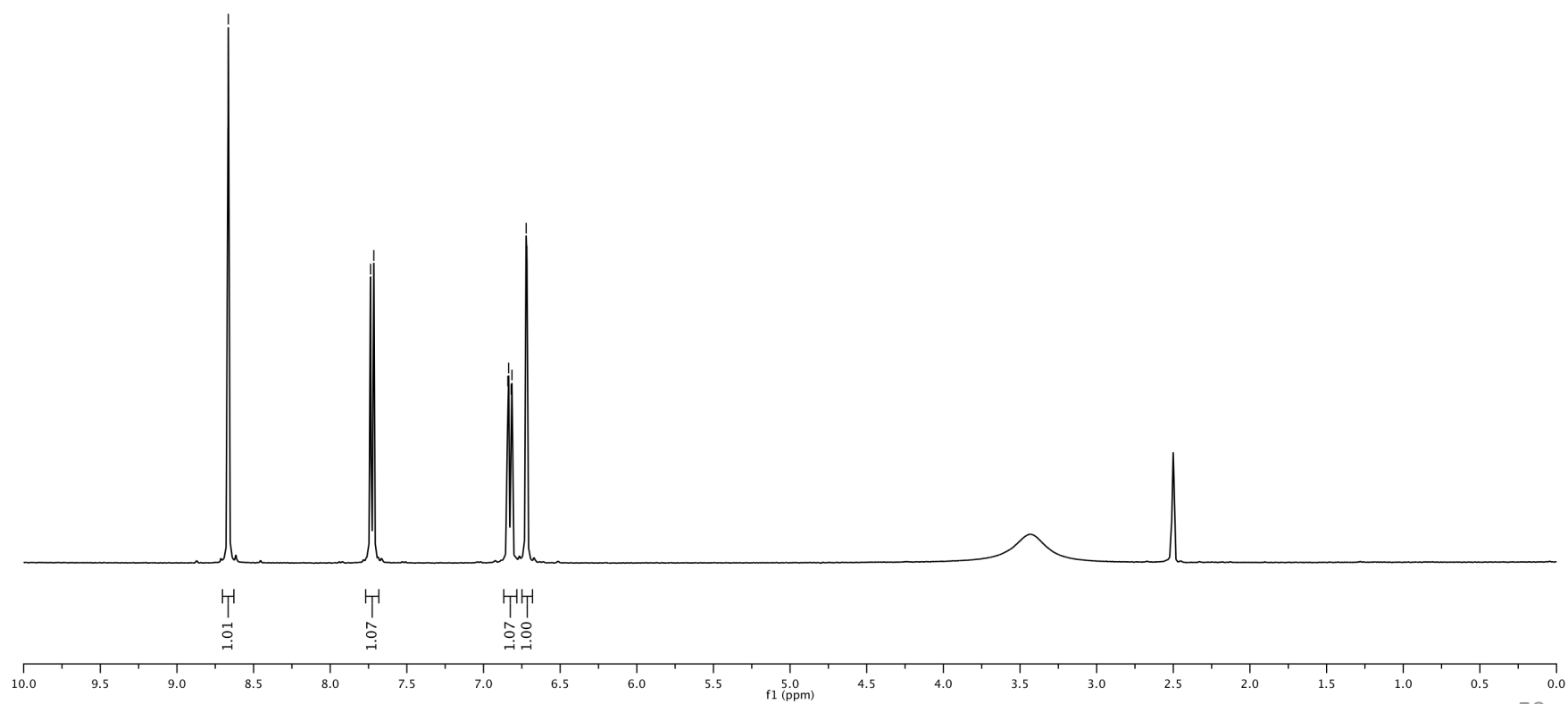
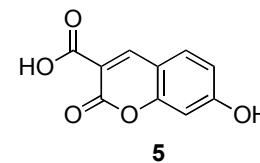
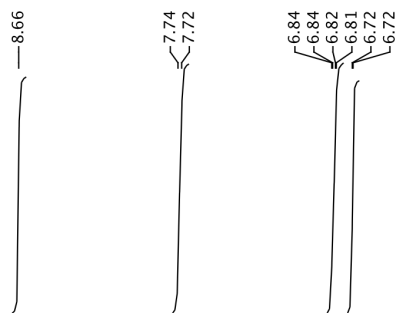


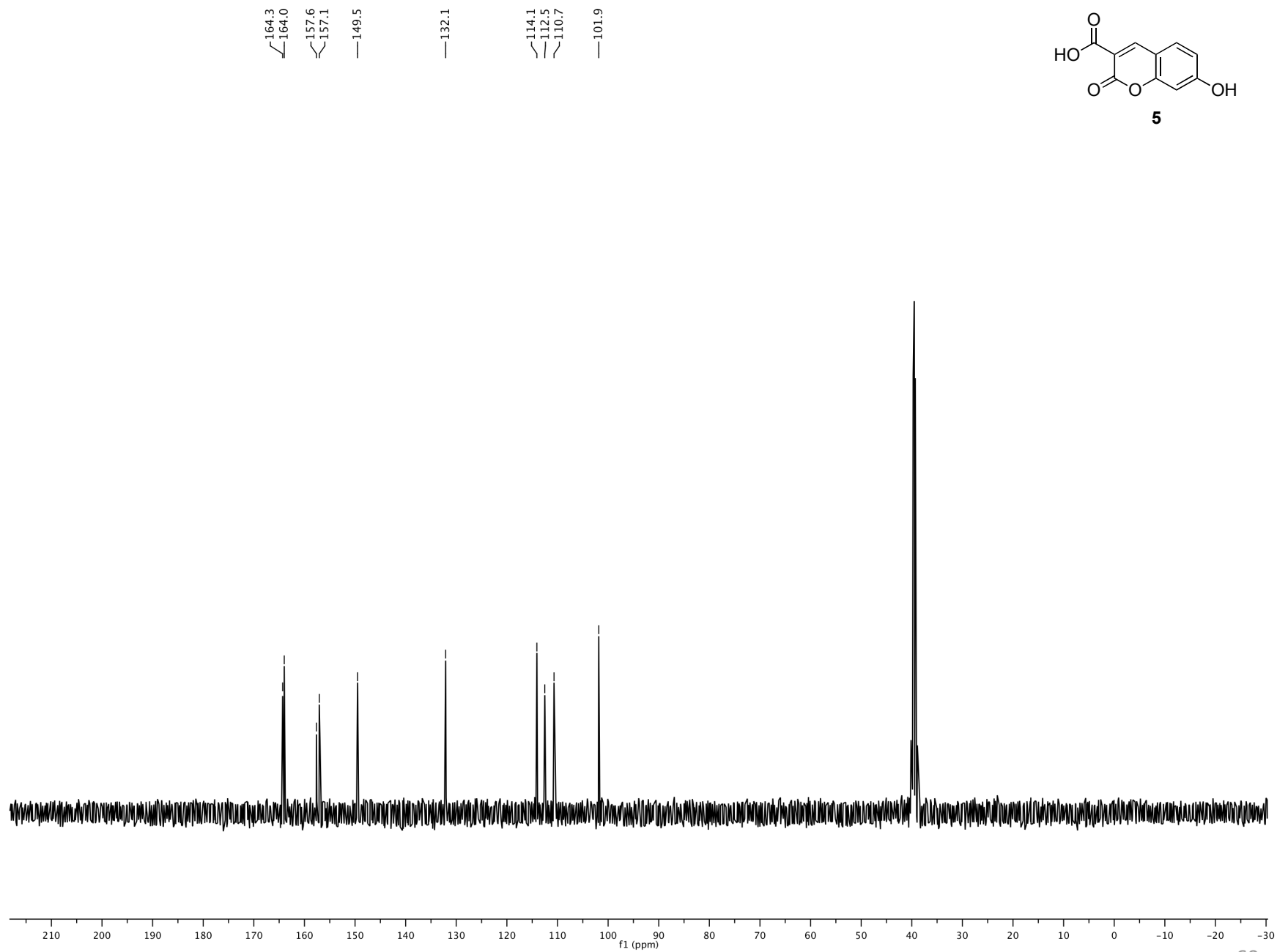


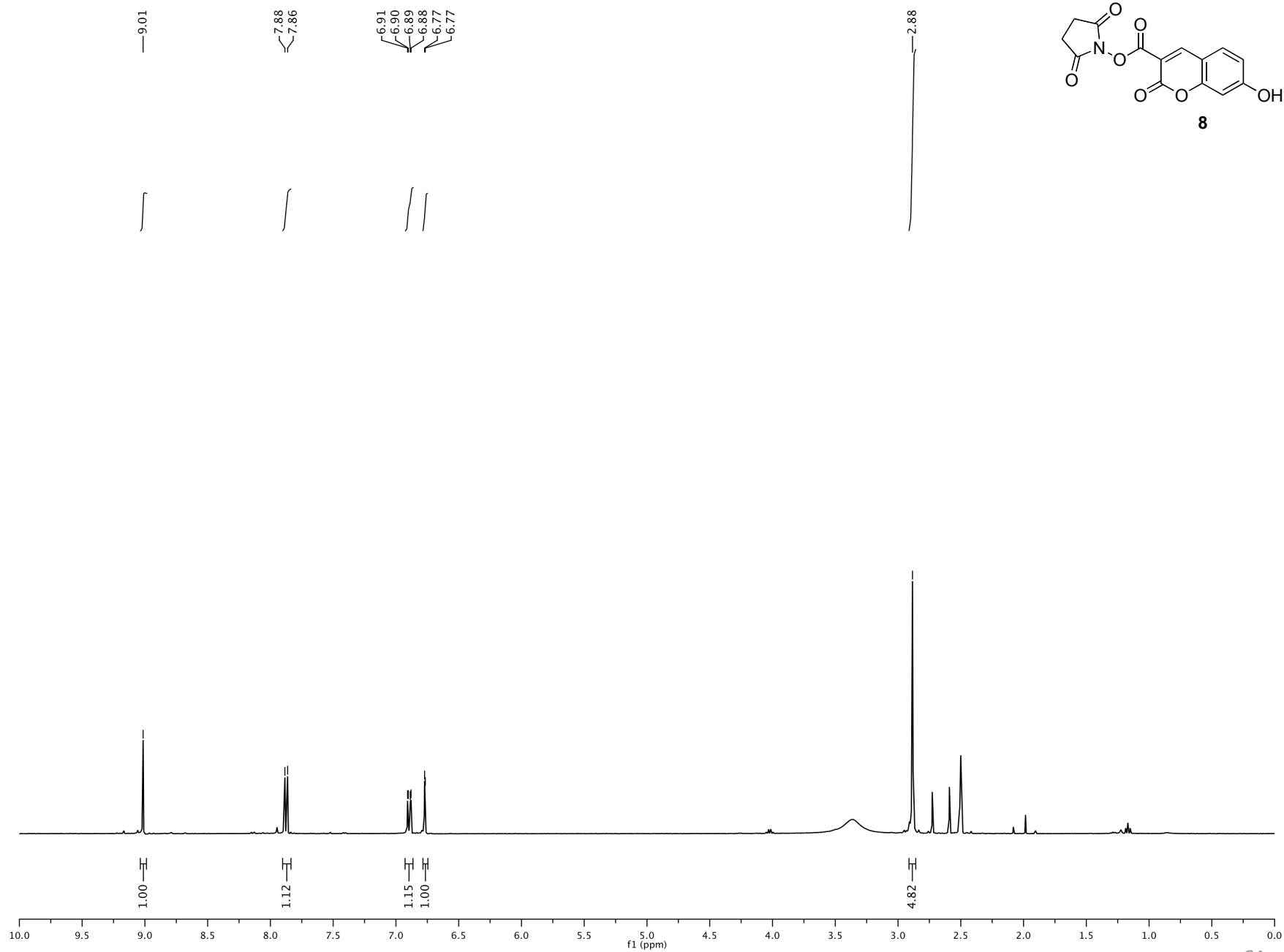


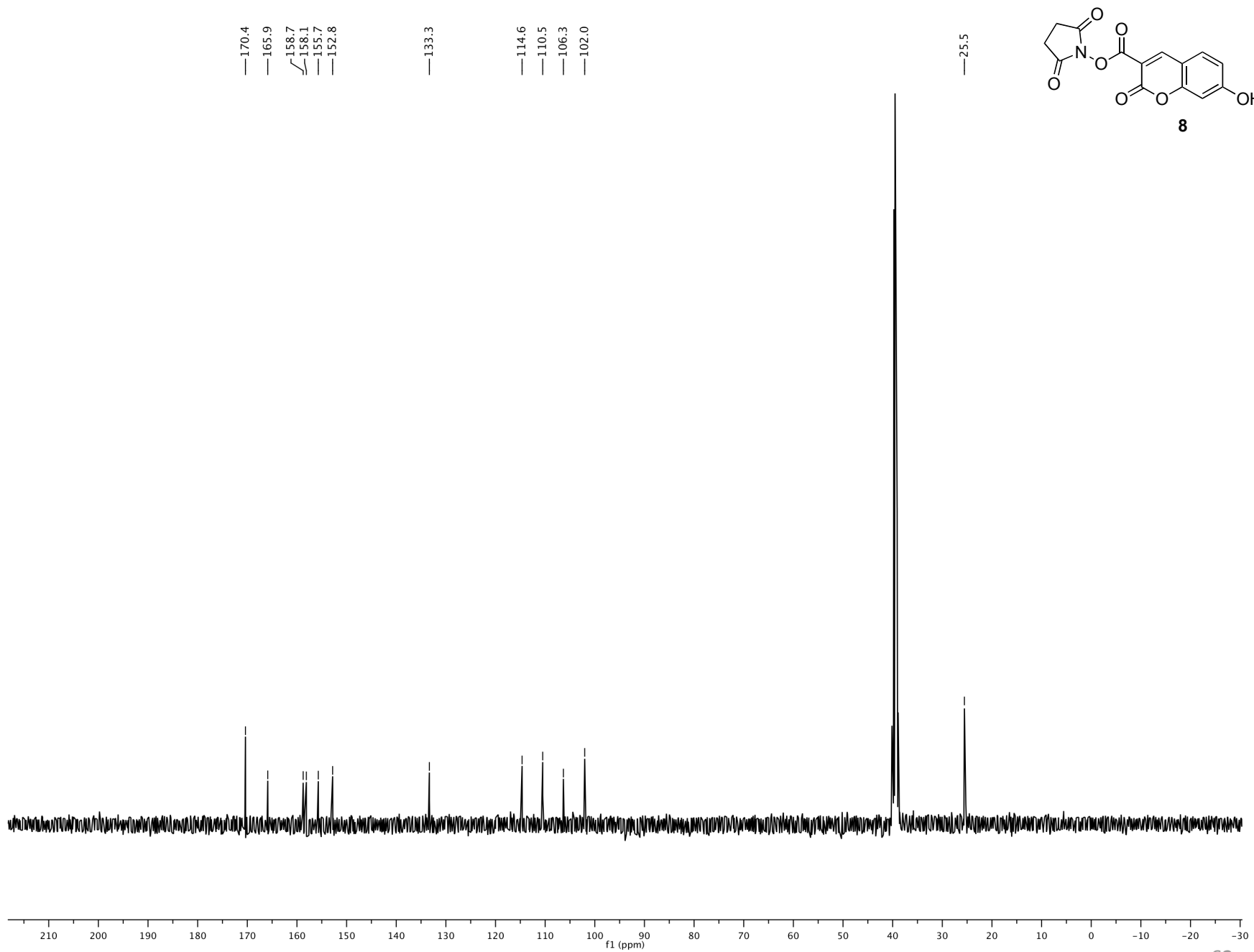


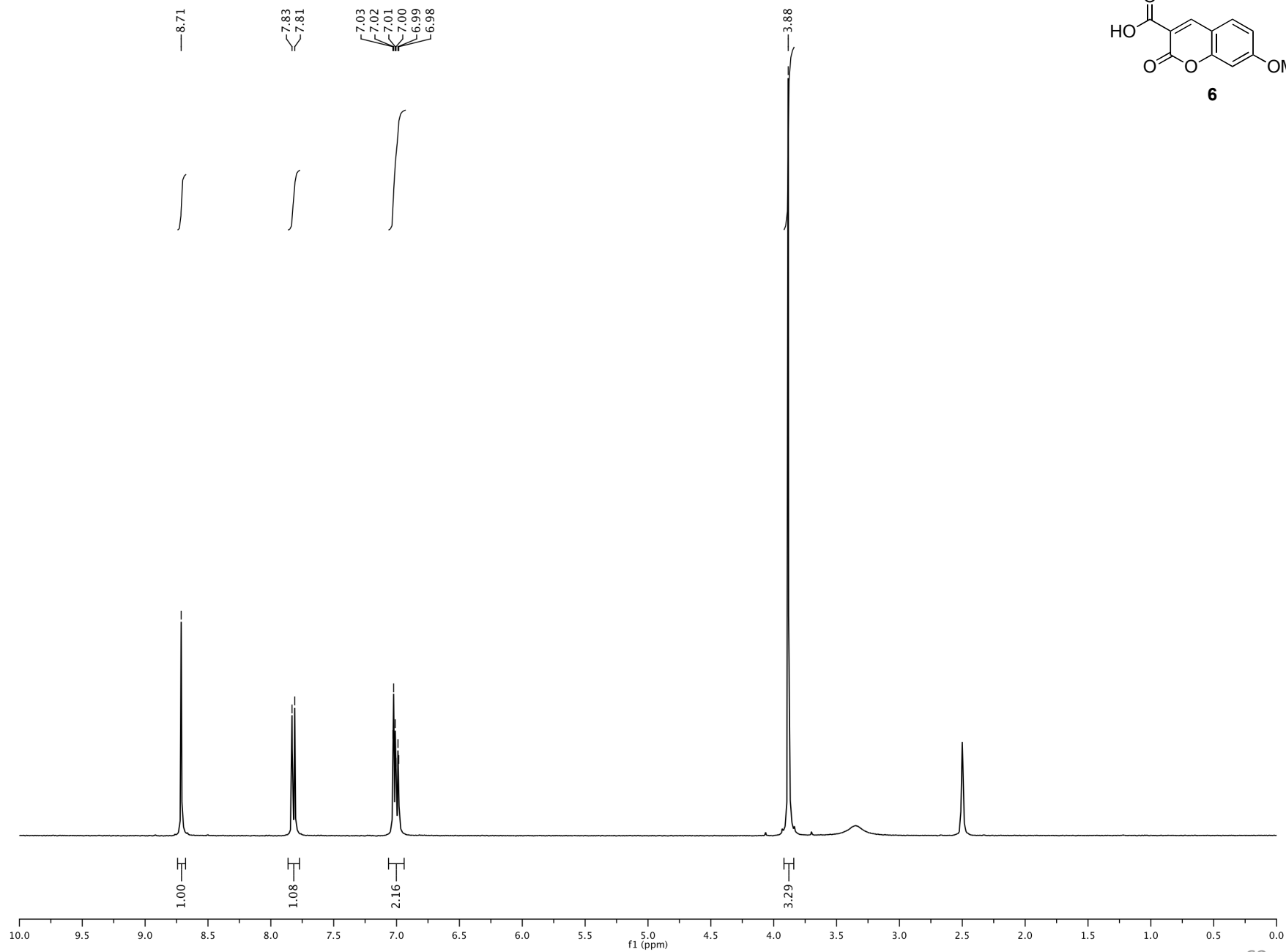
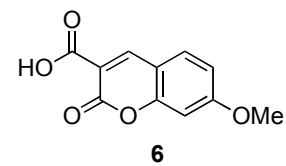


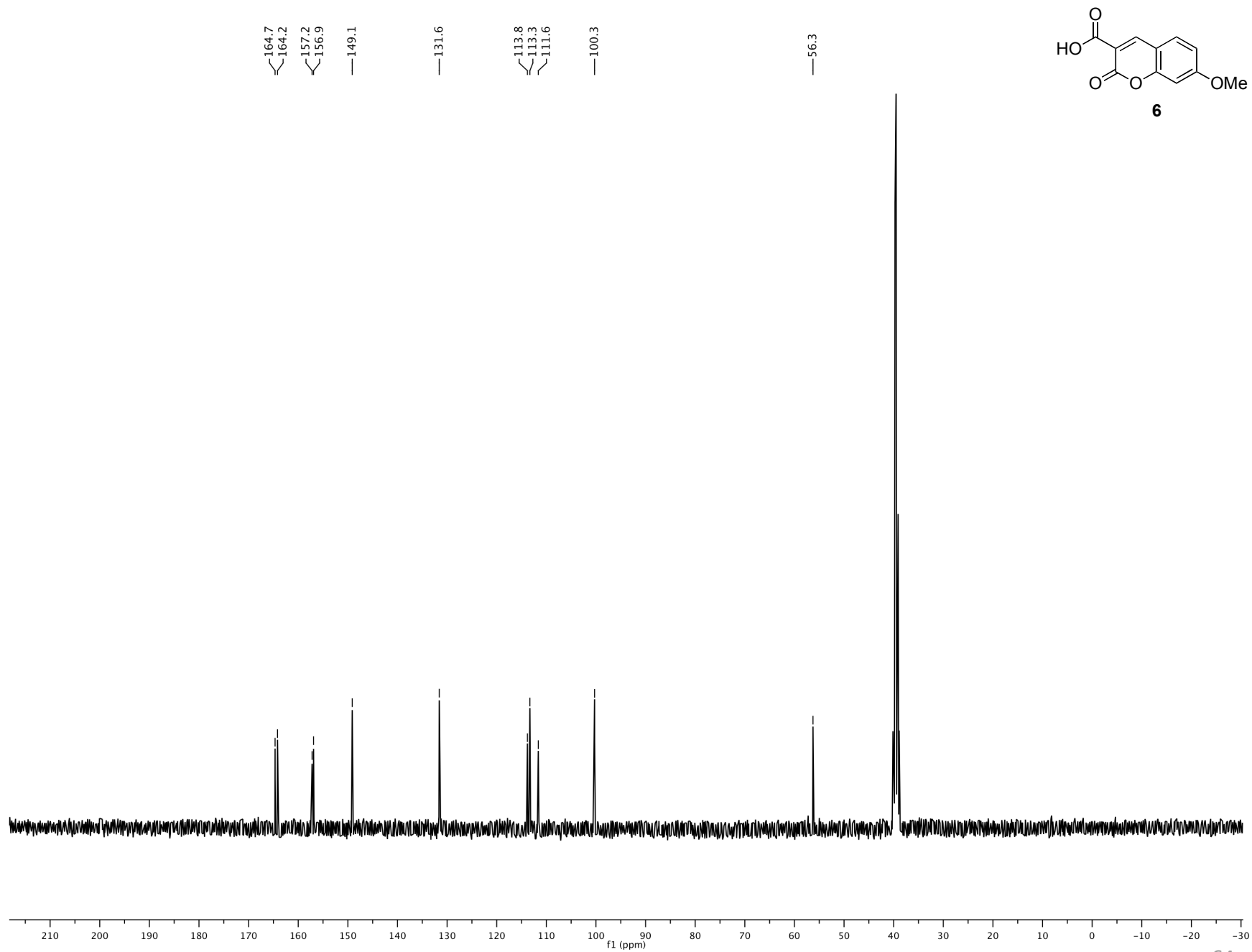


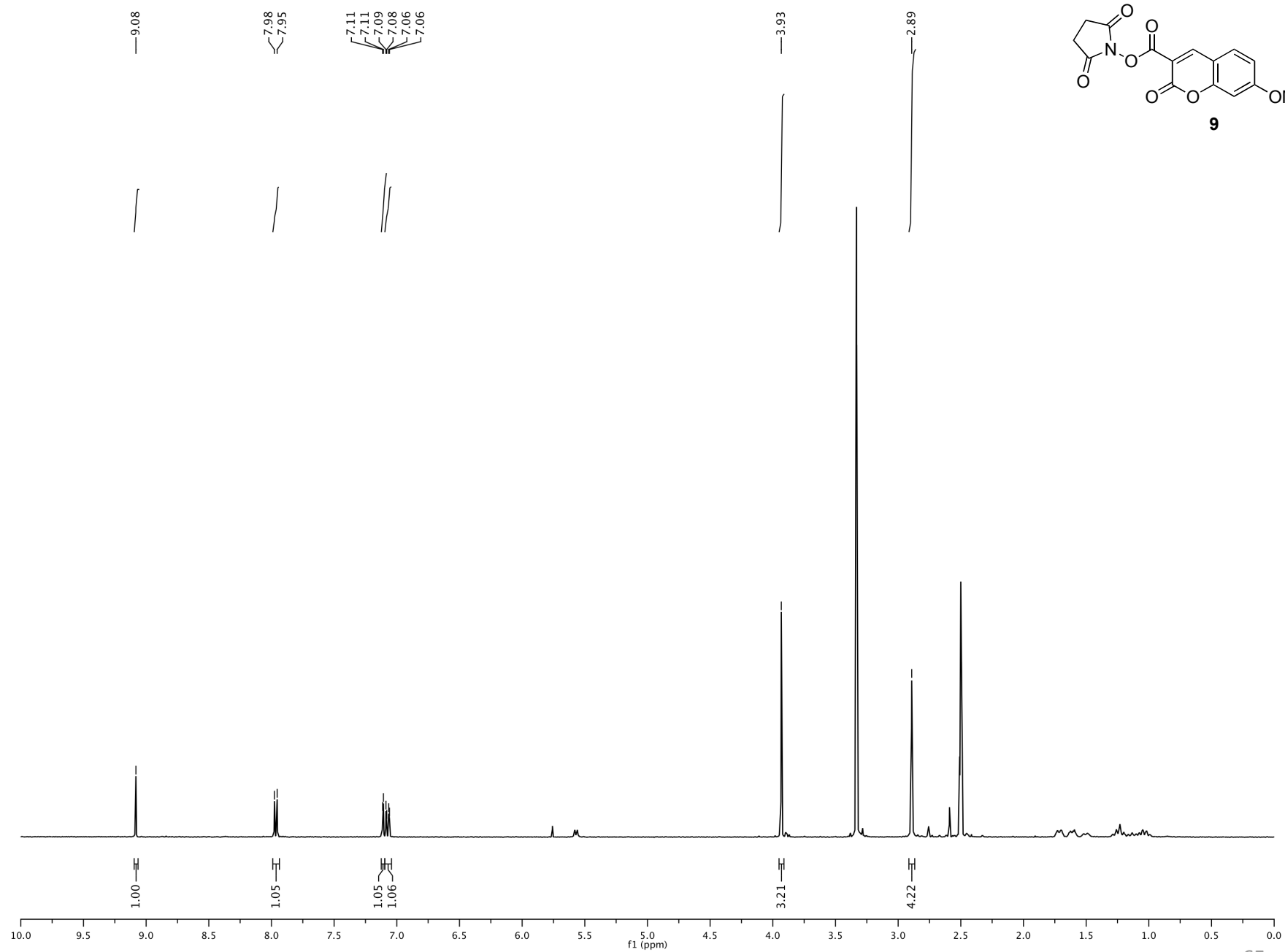


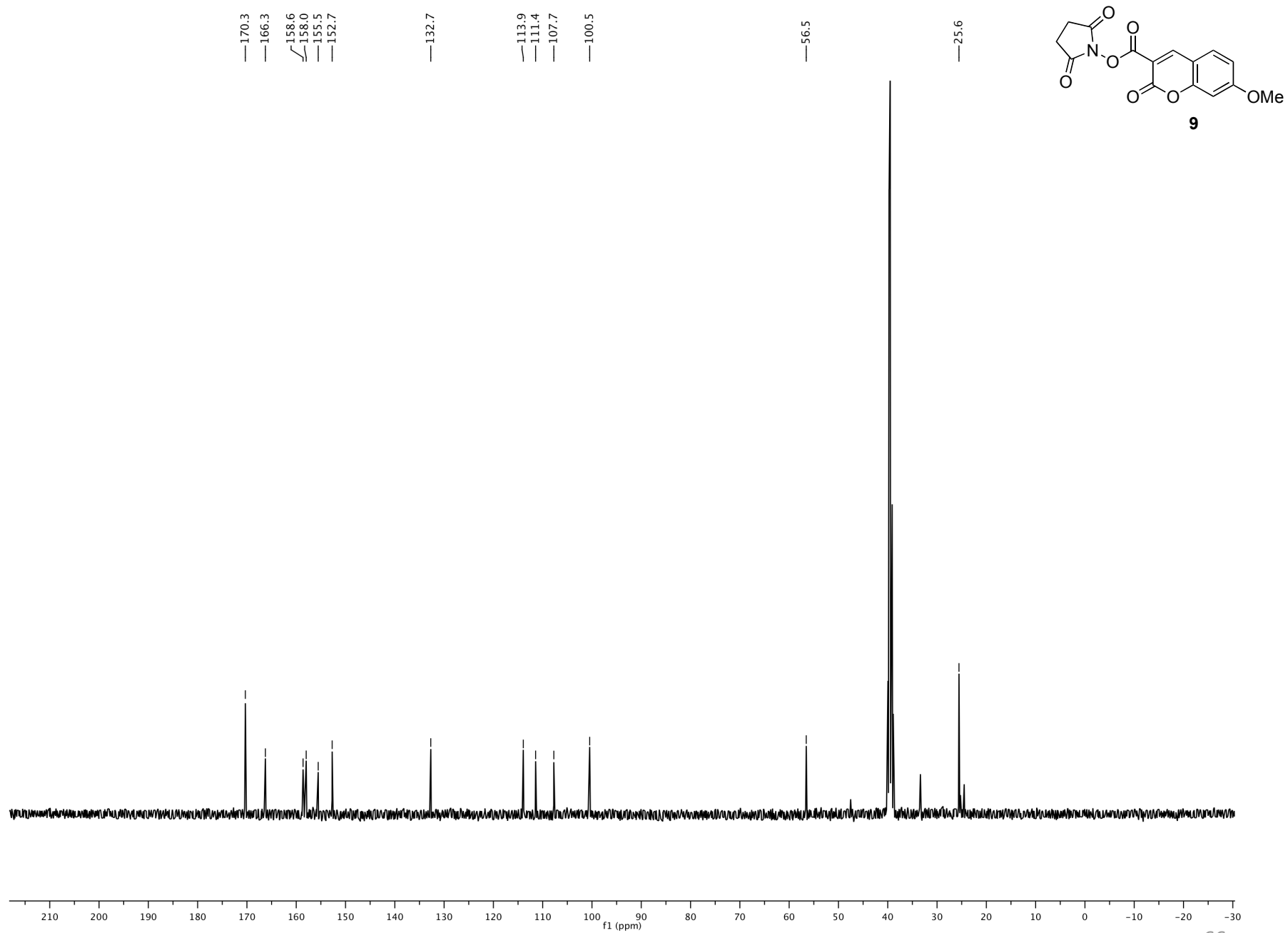


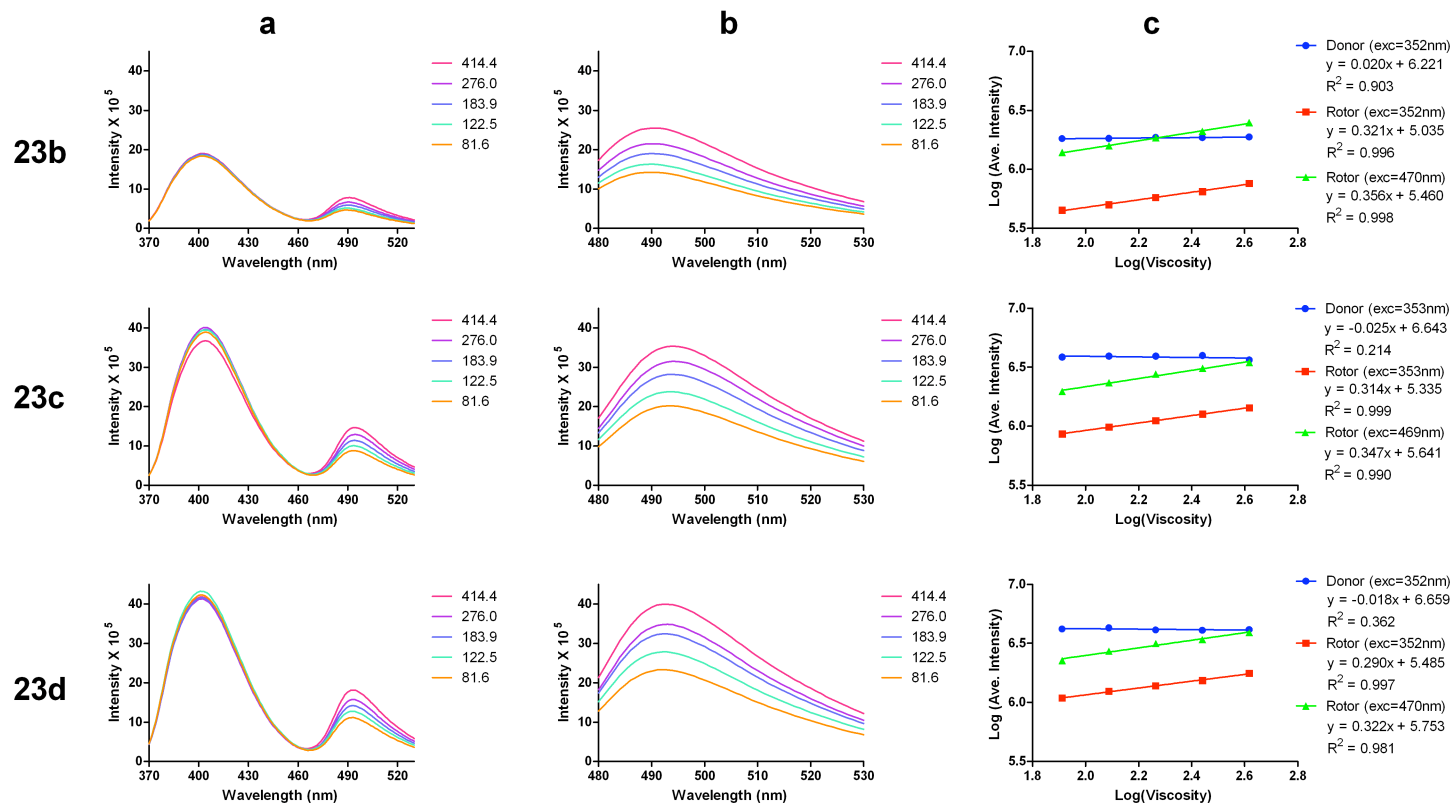




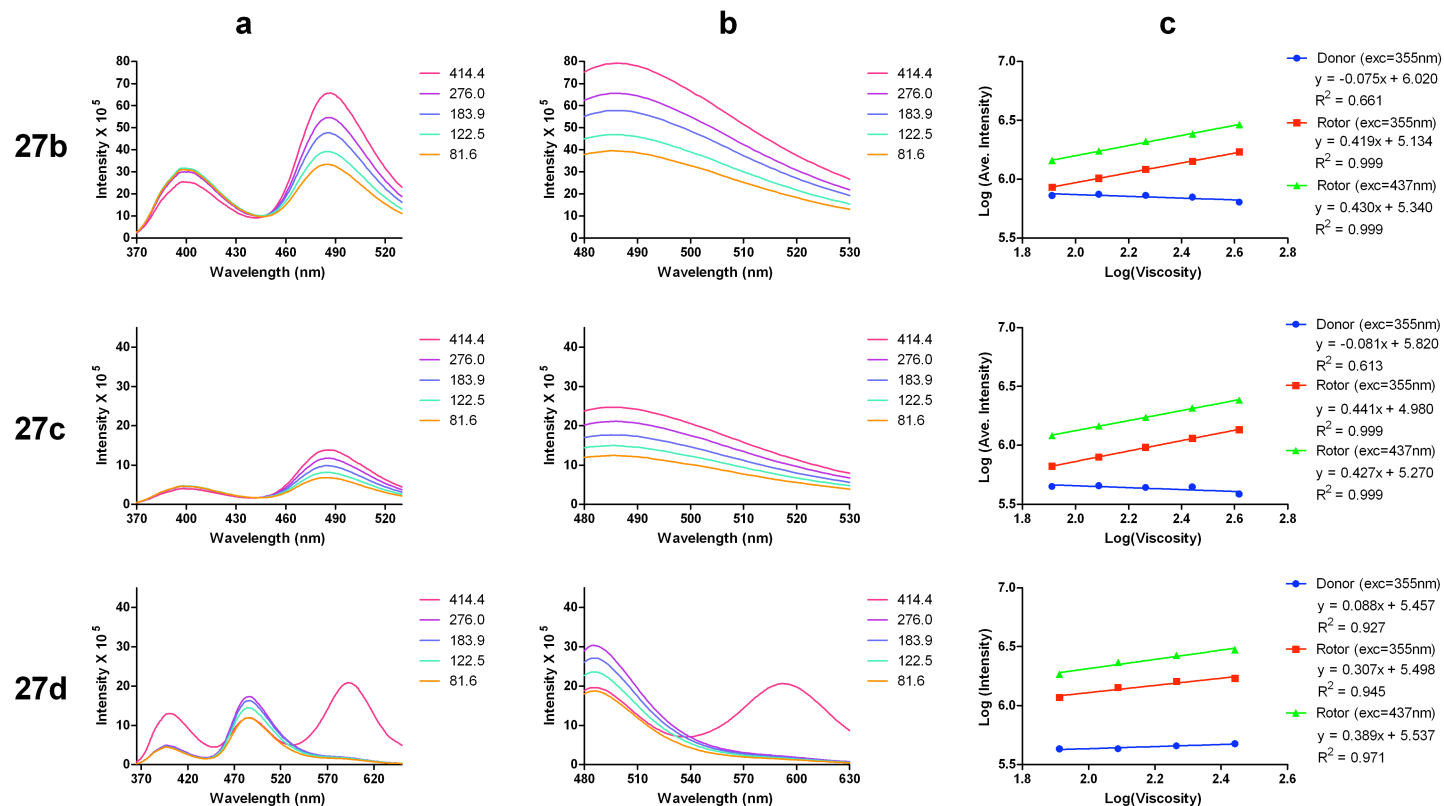




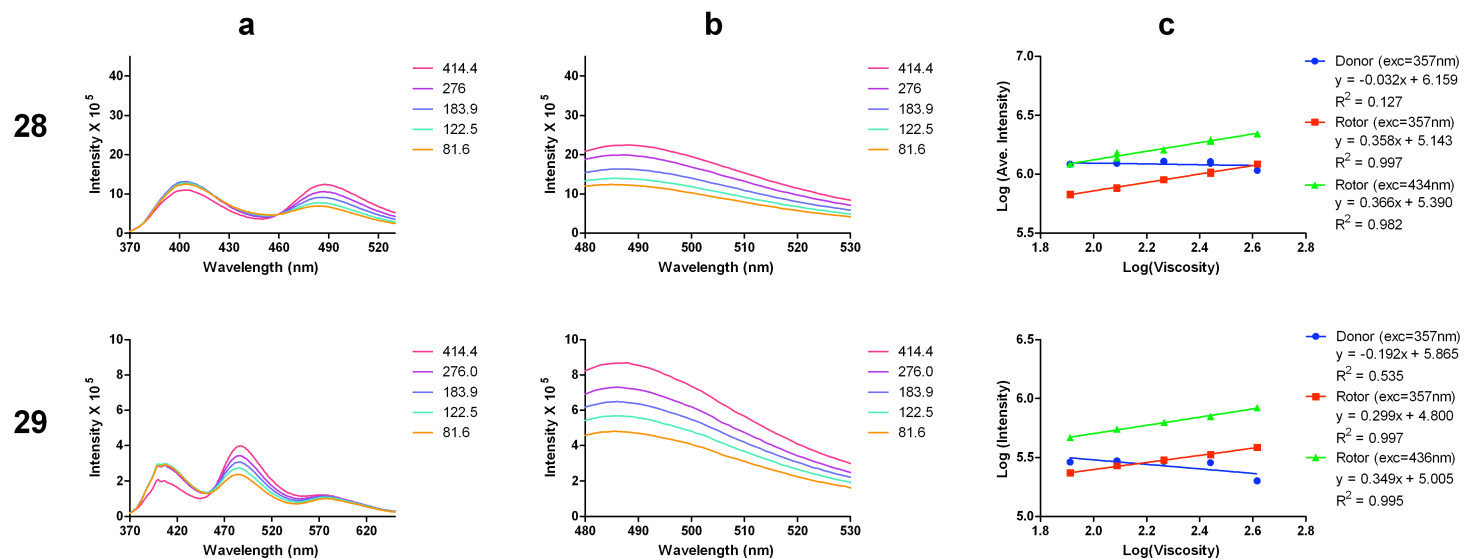




Fluorescent emission spectra and viscosity sensitivity plots of ratiometric rotors **23b**, **23c** and **23d** in ethylene glycol:glycerol mixtures. Fig. a: emission spectra via RET; Fig. b: emission spectra under direct excitation of the rotor; Fig. c: viscosity sensitivity plots



Fluorescent emission spectra and viscosity sensitivity plots of ratiometric rotors **27b**, **27c** and **27d** in ethylene glycol:glycerol mixtures. Fig. a: emission spectra via RET; Fig. b: emission spectra under direct excitation of the rotor; Fig. c: viscosity sensitivity plots



Fluorescent emission spectra and viscosity sensitivity plots of ratiometric rotors **28** and **29** in ethylene glycol:glycerol mixtures. Fig. a: emission spectra via RET; Fig. b: emission spectra under direct excitation of the rotor; Fig. c: viscosity sensitivity plots