

Supplementary Information
Potent Antifungal Synergy of Phthalazinone and Isoquinolones with Azoles Against *C. albicans*

Aaron D. Mood,^{†, #} Ilandari Dewage Udara Anulal Premachandra,^{†, #} Stanley Hiew,[†] Fuqiang Wang,[‡] Kevin A. Scott,[†] Nathan J. Oldenhuis,[†] Haoping Liu,[‡] David L. Van Vranken^{*, †}

[†] 1102 Natural Sciences 2, Department of Chemistry, University of California, Irvine, CA 92697-2025

[‡] Department of Biological Chemistry, University of California, Irvine, CA 92697-1700

Contents:

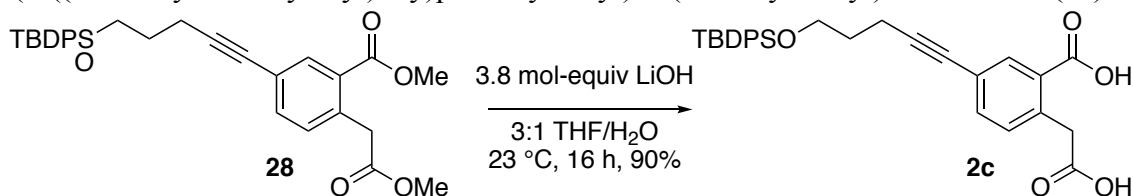
1. General Experimental Procedures	1
2. Synthetic Procedures and Characterization	2-27
3. Biological Evaluations	27-32
4. Calculated Physicochemical Properties of Analogues 15 and 24	32
5. References	33-34
6. NMR Spectra	35-127

1. General Experiment Procedure:

NMR spectral data were recorded at 25 °C using a Bruker 500 MHz spectrometer. The NMR data are reported as follows: chemical shifts in ppm are calibrated to tetramethylsilane (0 ppm), or residual solvents signals of CDCl₃ (7.26 ppm) or DMSO-*d*₆ (2.50 ppm), multiplicity (br = broad, app = apparent, s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet), coupling constants (Hz), and integration. Analytical thin layer chromatography (TLC) was performed using EMD Reagents 0.25 mm silica gel 60-F plates. Silica gel chromatography was performed using Silicycle silica gel (40–63 μm). All reactions were carried out under an atmosphere of nitrogen. Reactions were carried out at ambient temperature unless otherwise indicated. Unless otherwise noted, all reagents were commercially obtained and, where appropriate, purified prior to use. Amines were distilled from calcium hydride. THF and CH₂Cl₂ were dried by filtration through alumina according to the procedure of Grubbs and coworkers.¹ The purity of compounds tested for antifungal activity was determined by reversed-phase high-performance liquid chromatography using a Waters Symmetry C8 column (4.6 x 5.50 mm). A mixture of H₂O (with 0.1% TFA) and MeOH (50-85% MeOH) was used as eluent.

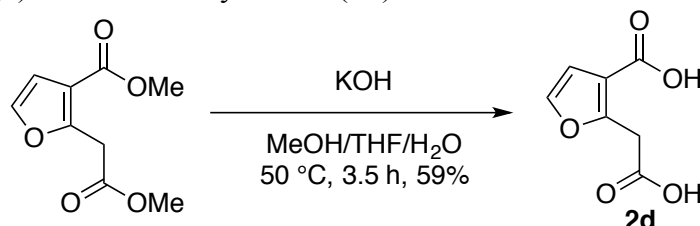
2. Synthetic Procedures and Characterization:

5-(5-((*tert*-Butyldimethylsilyl)oxy)pent-1-yn-1-yl)-2-(carboxymethyl)benzoic acid (**2c**):



Carboxylic acid **2c** was synthesized from bis(methylester) **28** (*vide infra*, 23-24) according to a procedure developed by Selwood.² A round bottom flask equipped with stir bar was charged with alkyne **28** (0.73 g, 1.4 mmol, 1.0 mol-equiv). THF (13 mL) and 3.9 mL of aqueous LiOH (1.4 M, 5.3 mmol, 3.8 mol-equiv) were added to the flask via syringe. The reaction progress was monitored by TLC, which indicated complete consumption of **28** after 16 h. Next, 40 mL of 1 N HCl and 65 mL of EtOAc were added to the reaction mixture. The mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried with Na₂SO₄, and concentrated *in vacuo* to yield dicarboxylic acid **2c** (0.62 g, 1.2 mmol, 90%) as a brown solid. $R_f = 0.5$ (17:3 CH₂Cl₂/MeOH). ¹H NMR (500 MHz, DMSO-*d*₆) δ 13.2–12.0 (br s, 2H), 7.82 (s, 1H), 7.66–7.61 (m, 4H), 7.46–7.36 (m, 7H), 7.30 (d, *J* = 7.8 Hz, 1H), 3.93 (s, 2H), 3.79 (t, *J* = 6.0 Hz, 2H), 2.57 (t, *J* = 7.0 Hz, 2H), 1.81 (quin, *J* = 6.6 Hz, 2H), 1.01 (s, 9H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 172.2, 167.6, 136.3, 135.0, 134.5, 134.1, 133.1, 133.0, 132.8, 129.9, 127.9, 121.9, 90.7, 79.7, 62.0, 30.9, 26.7, 18.8, 15.2; HRMS (ESI): *m/z* calculated for C₃₀H₃₁O₅Si [M-H]⁻ 499.1941, found 499.1953.

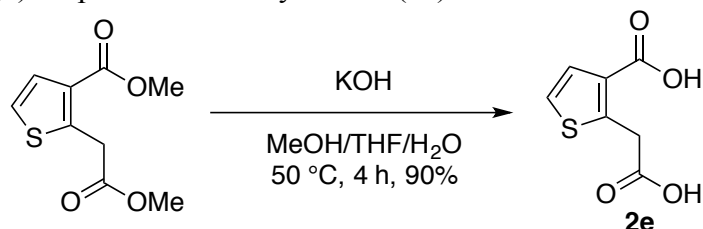
2-(Carboxymethyl)furan-3-carboxylic acid (**2d**):



Dicarboxylic acid **2d** was synthesized as previously reported by Ozer *et. al.* with minor modification.³ A 50 mL round bottom flask equipped with stir bar was charged with methanol (15 mL), THF (15 mL), H₂O (1 mL), and KOH (0.80 g, 14 mmol, 2.8 mol-equiv), then stirred until the KOH was dissolved. To the solution was added the bis(methylester) starting material (1.0 g, 5.0 mmol, 1.0 mol-equiv), which was synthesized as previously reported.⁴ The flask was sealed with a rubber septum and submerged in a 50 °C oil bath. After 2 h, a white precipitate was observed, and the reaction was allowed to stir for an additional 1.5 h. The flask was removed from the oil bath and cooled in an ice-water bath for 15 min. The precipitate was collected by filtration and washed with ethyl acetate (5 mL). The precipitate was dissolved in H₂O (10 mL) and acidified to pH = 2 with 1 N HCl (aq). The mixture was extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na₂SO₄, and concentrated *in vacuo*. The product was further dried under high vacuum to obtain dicarboxylic acid **2d** as a white solid (0.50 g, 3.0 mmol, 59%). ¹H

NMR (500 MHz, DMSO- d_6) δ 12.67 (br, 2H), 7.65 (d, $J = 2.0$ Hz, 1H), 6.67 (d, $J = 2.0$ Hz, 1H), 3.98 (s, 2H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 169.9, 164.4, 154.8, 142.3, 115.5, 110.8, 33.3; HRMS (ESI): m/z calculated for $\text{C}_7\text{H}_6\text{O}_5\text{Na}$ $[\text{M}+\text{Na}]^+$ 193.0113, found 193.0111.

2-(Carboxymethyl)thiophene-3-carboxylic acid (**2e**):



Dicarboxylic acid **2e** was synthesized by adapting a previously reported procedure by Ozer *et. al.*³ which obtained **2e** as a minor product. A 50 mL round bottom flask equipped with stir bar was charged with methanol (15 mL), THF (15 mL), H₂O (1 mL), and KOH (0.80 g, 14 mmol, 2.8 mol-equiv), then stirred until the KOH dissolved. To the solution was added the bis(methylester) starting material (1.1 g, 5.0 mmol, 1.0 mol-equiv), which was synthesized as previously reported.⁴ The flask was sealed with a rubber septum and submerged in a 50 °C oil bath. After 4 h, the flask was cooled to room temperature. The reaction mixture was diluted with 50 mL H₂O and acidified to pH = 2 with 1 N HCl (aq). The mixture was extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na₂SO₄, and concentrated *in vacuo*. The product was further dried under high vacuum to obtain dicarboxylic acid **2e** as a pale-yellow solid (0.84 g, 4.51 mmol, 90%). ^1H NMR (500 MHz, DMSO- d_6) δ 12.57 (br, 2H), 7.41(d, $J = 5.4$ Hz, 1H), 7.34 (d, $J = 5.4$ Hz, 1H), 4.14 (s, 2H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 171.2, 164.3, 144.4, 130.2, 128.9, 123.9, 34.1; HRMS (ESI): m/z calculated for $\text{C}_7\text{H}_6\text{O}_4\text{SNa}$ $[\text{M}+\text{Na}]^+$ 208.9884, found 208.9882.

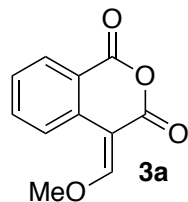
General procedure for the synthesis of enol ethers (**3a-e**):



Enol ethers **3a-e** were synthesized according to a procedure developed by Wolfbeis.⁵ An oven-dried round bottom flask equipped with stir bar was charged with dicarboxylic acid (1.0 mol-equiv), trimethyl orthoformate (1.3 mol-equiv), and acetic anhydride (to obtain a 2.2 M solution of the dicarboxylic acid). The flask was fitted with a reflux condenser. The reaction mixture was heated at reflux and monitored by TLC for disappearance of the dicarboxylic acid. Upon consumption of the dicarboxylic acid, the reaction mixture was cooled to room temperature. The resulting solid was filtered using a Büchner funnel, washed with cold carbon tetrachloride and dried under vacuum to obtain the enol ether as a solid, which was used without further purification. The stereochemistry of the alkene in **3a** was determined by NOESY. No NOE signal was

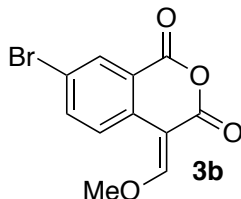
detected between the alkene proton and the proximal proton of the benzo ring. The stereochemistry of all other enol ethers was assigned by analogy to **3a**.

Example: (*E*)-4-(Methoxymethylene)isochromane-1,3-dione (**3a**):



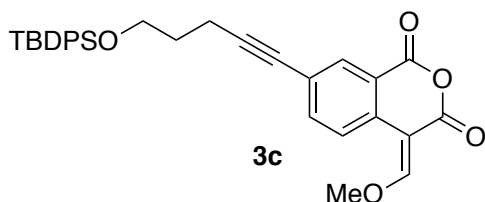
An oven-dried round bottom flask equipped with stir bar was charged with homophthalic acid (15 g, 83 mmol, 1.0 mol-equiv), trimethyl orthoformate (11 mL, 108 mmol, 1.3 mol-equiv), and acetic anhydride (38 mL). The flask was fitted with a reflux condenser. The reaction mixture was heated at reflux for 1.5 h and monitored by TLC for disappearance of homophthalic acid. Upon consumption of homophthalic acid, the reaction mixture was cooled to room temperature. The resulting solid was filtered with cold carbon tetrachloride (3 x 20 mL) in a Büchner funnel and dried under vacuum to obtain enol ether **3a** (11 g, 53 mmol, 64%) as a white solid. $R_f = 0.9$ (1:9 MeOH/CH₂Cl₂). ¹H NMR (500 MHz, CDCl₃) δ 8.23 – 8.21 (m, 2H), 8.05 (s, 1H), 7.67 (t, $J = 7.9$ Hz, 1H), 7.41 (t, $J = 7.5$, 1H), 4.26 (s, 1H).

(*E*)-7-Bromo-4-(methoxymethylene)isochromane-1,3-dione (**3b**):



Using the general procedure for enol ether synthesis outlined above, 5-Bromohomophthalic acid (1.5 g, 5.8 mmol, 1.0 mol-equiv) was used to give enol ether **3b** (0.94 g, 3.3 mmol, 58%) as a white solid. $R_f = 0.2$ (1:1 EtOAc/Hex). (¹H NMR (500 MHz, CDCl₃) δ 8.23 (s, 1H), 8.15 – 8.13 (m, 2H), 7.95 (dd, $J = 8.7, 2.3$ Hz, 1H), 4.30 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 169.5, 161.8, 160.2, 137.9, 132.8, 131.7, 127.5, 121.2, 119.5, 99.4, 65.4; HRMS (ESI): m/z calculated for C₁₁H₇BrO₄Na [M+Na]⁺ 304.9425, found 304.9418.

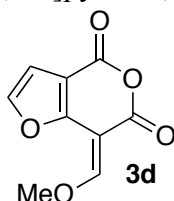
(*E*)-7-(5-((*tert*-Butyldiphenylsilyl)oxy)pent-1-yn-1-yl)-4(methoxymethylene)isochromane-1,3-dione (**3c**):



Using the general procedure for enol ether synthesis outlined above, dicarboxylic acid **2c** (0.48 g, 0.95 mmol, 1.0 mol-equiv) was used to give enol ether **3c** (0.13 g, 0.24 mmol, 25%) as a white solid. $R_f = 0.9$ (8.5:1.5 CH₂Cl₂/MeOH). ¹H NMR (600 MHz,

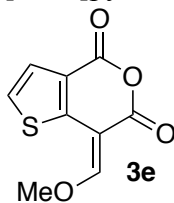
CDCl₃) δ 8.21 (s, 1H), 8.14 (d, J = 8.4 Hz, 1H), 8.04 (s, 1H), 7.69-7.67 (m, 4H), 7.59 (dd, J = 8.4, 1.8 Hz, 1H), 7.41-7.40 (m, 2H), 7.38-7.36 (m, 4H), 4.26 (s, 3H), 3.81 (t, J = 5.7 Hz, 2H), 2.59 (d, J = 7.2 Hz, 2H), 1.86 (d, J = 6.3 Hz, 2H), 1.07 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 167.1, 137.9, 135.6, 133.8, 133.6, 129.7, 127.7, 125.9, 123.8, 64.9, 62.3, 31.4, 26.9, 19.3, 16.1; HRMS (ESI): m/z calculated for C₃₂H₃₂NaO₅SiNa [M+Na]⁺ 547.1917, found 547.1911.

(*E*)-7-(Methoxymethylene)-4*H*-furo[3,2-*c*]pyran-4,6(7*H*)-dione (**3d**):



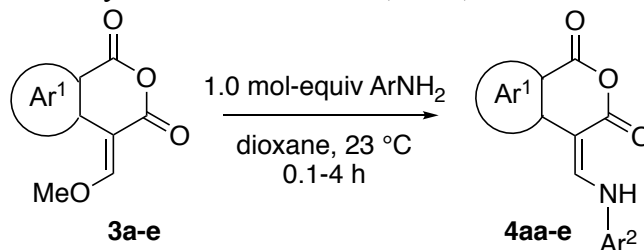
An oven dried 5 mL round bottom flask equipped with a stir bar was charged with dicarboxylic acid **2d** (500 mg, 3.0 mmol, 1 mol-equiv), acetic anhydride (1.3 mL, 14.2 mmol, 5 mol-equiv), and trimethyl orthoformate (0.40 mL, 3.6 mmol, 1.2 mol-equiv). The flask was equipped with a reflux condenser, and submerged in a 110 °C oil bath. The reaction was monitored by TLC (95:5 CH₂Cl₂/MeOH). After 2 h, additional trimethyl orthoformate (0.3 mL) was added. After 2.5 h total of heating, the reaction was complete by TLC. The flask was removed from the oil bath and cooled to room temperature. The resulting precipitate was collected by filtration and washed with the cold carbon tetrachloride (20 mL). The precipitate was dried under high vacuum to obtain enol ether **3d** as a light brown solid (400 mg, 2.1 mmol, 69%). ¹H NMR (500 MHz, CDCl₃) δ 7.86 (s, 1H), 7.51 (d, J = 2.0, 1H), 6.86 (d, J = 2.0, 1H), 4.25 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 165.0, 161.6, 156.8, 156.1, 144.8, 109.0, 108.3, 97.8, 65.1; HRMS (ESI): m/z calculated for C₉H₆O₅Na [M+Na]⁺ 217.0113, found 217.011.

(*E*)-7-(Methoxymethylene)-4*H*-thieno[3,2-*c*]pyran-4,6(7*H*)-dione (**3e**):



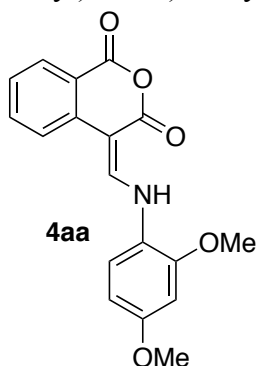
An oven dried 5 mL round bottom flask equipped with stir bar was charged with dicarboxylic acid **2e** (750 mg, 4.0 mmol, 1 mol-equiv), acetic anhydride (1.9 mL, 20 mmol, 5 mol-equiv), and trimethyl orthoformate (0.90 mL, 8.1 mmol, 2 mol-equiv). The flask was equipped with a reflux condenser, and the flask submerged in a 110 °C oil bath. After 2 h, the flask was removed from the oil bath and cooled to room temperature. The resulting precipitate was collected by filtration. The precipitate was dried under high vacuum to obtain enol ether **3e** as a purple solid (415 mg, 2.0 mmol, 49%) and used without further purification. ¹H NMR (500 MHz, CDCl₃) δ 7.92 (s, 1H), 7.54 (d, J = 5.3, 1H), 7.33 (d, J = 5.4, 1H), 4.29 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.6, 161.9, 156.4, 144.3, 126.5, 125.7, 122.6, 102.1, 64.5; HRMS (ESI): m/z calculated for C₉H₆O₄SNa [M+Na]⁺ 232.9884, found 232.9879.

General procedure for the synthesis of enamines (**4aa-e**):



Enamines **4aa-e** were synthesized according to a procedure developed by Wolfbeis⁵ An oven-dried round bottom flask equipped with stir bar was charged with the enol ether starting material (1.0 mol-equiv). Next, dioxane was added to the reaction flask to obtain an enol ether concentration of 0.5 M. A separate oven-dried round bottom flask equipped with stir bar was charged with the substituted aniline (1.0 mol-equiv). Dioxane was added to the flask to obtain a 0.5 M solution of the aniline. The aniline solution was transferred via syringe to the flask containing the enol ether solution. The reaction was monitored until enol ether was no longer detected by TLC. The resulting solid was filtered in a Büchner funnel. The solid was washed with cold dioxane and dried under vacuum to obtain the enamine, which was used without further purification. The stereochemistry of the alkene in **4ab** was determined by NOESY, which indicated an NOE signal between the alkene proton and the proximal proton of the benzo ring. The stereochemistry of all other enamines was assigned by analogy to **4ab**.

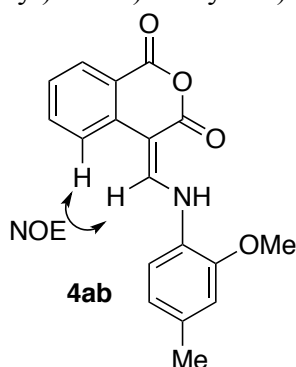
Example: (*Z*)-4-(((2,4-Dimethoxyphenyl)amino)methylene)isochromane-1,3-dione (**4aa**):



An oven-dried round bottom flask equipped with stir bar was charged with enol ether **3a** (0.50 g, 2.5 mmol, 1.0 mol-equiv). Next, dioxane (5.0 mL) was added to the reaction flask. A separate oven-dried round bottom flask equipped with stir bar was charged with 2,4-dimethoxyaniline (0.38 g, 2.5 mmol, 1.0 mol-equiv) and dioxane (5.0 mL) to obtain a homogeneous solution. The solution was transferred to the flask containing **3a** via syringe. The reaction was monitored until **3a** was no longer detected by TLC (10 min). Over this period, a green precipitate formed, which was collected by filtration using a Büchner funnel. The solid was washed with cold dioxane (45 mL) to give enamine **4aa** (0.53 g, 1.6 mmol, 70%). $R_f = 0.4$ (1:1:0.05 EtOAc/Hex/Et₃N). ¹H

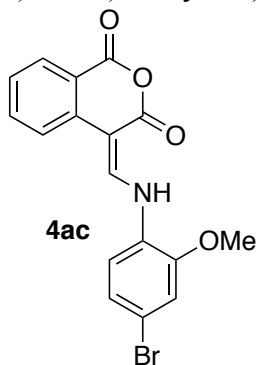
NMR (500 MHz, CDCl₃) δ 11.64 (d, *J* = 13.2 Hz, 1H), 8.41 (d, *J* = 13.8 Hz, 1H), 8.20 (d, *J* = 8.1 Hz, 1H), 7.61 (t, *J* = 7.3 Hz, 1H), 7.55 (d, *J* = 8.1 Hz, 1H), 7.27–7.21 (m, 2H), 6.56–6.54 (m, 2H), 3.94 (s, 3H), 3.84 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.5, 161.9, 158.6, 150.7, 144.2, 137.5, 135.0, 131.0, 124.9, 121.9, 117.7, 117.3, 116.3, 104.8, 99.5, 91.1, 56.1, 55.7; HRMS (ESI): *m/z* calculated for C₁₈H₁₅NO₅Na [M+Na]⁺ 348.0848, found 348.0854.

(*Z*)-4-(((2-Methoxy-4-methylphenyl)amino)methylene)isochromane-1,3-dione (**4ab**):



Using the general procedure for enamine formation outlined above, enol ether **3a** (0.50 g, 2.5 mmol, 1.0 mol-equiv) was used to give enamine **4ab** (0.27 g, 0.86 mmol, 35%) as a green solid. *R_f* = 0.5 (1:1 EtOAc/Hex). ¹H NMR (500 MHz, CDCl₃) δ 11.68 (d, *J* = 13.5 Hz, 1H), 8.46 (d, *J* = 13.6, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 8.1 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H), 6.80 (s, 1H), 3.95 (s, 3H), 2.38 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.5, 161.8, 149.3, 143.9, 137.4, 136.5, 135.0, 131.1, 125.7, 125.0, 121.7, 117.8, 117.4, 114.9, 112.5, 91.5, 56.0, 21.5; HRMS (ESI): *m/z* calculated for C₁₈H₁₅NO₄Na [M+Na]⁺ 332.0899, found 348.0907.

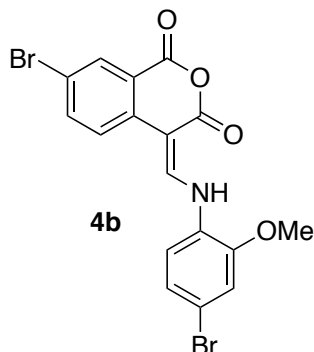
(*Z*)-4-(((4-Bromo-2-methoxyphenyl)amino)methylene)isochromane-1,3-dione (**4ac**):



Using the general procedure for enamine formation outlined above, enol ether **3a** (0.50 g, 2.5 mmol, 1.0 mol-equiv) was used to give enamine **4ac** (0.97 g, 2.6 mmol, 70%) as a green solid. *R_f* = 0.5 (1:1:0.05 EtOAc/Hex/Et₃N). ¹H NMR (500 MHz, CDCl₃) δ 11.65 (d, *J* = 13.3 Hz, 1H), 8.43 (d, *J* = 13.5 Hz, 1H), 8.21 (d, *J* = 8.4 Hz, 1H), 7.64 (td, *J* = 7.5 Hz, 1.3, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.30 (t, *J* = 7.8 Hz, 1H), 7.18 (br s, 2H), 7.11 (s, 1H), 3.98 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.5, 161.5, 149.8, 143.1, 136.9,

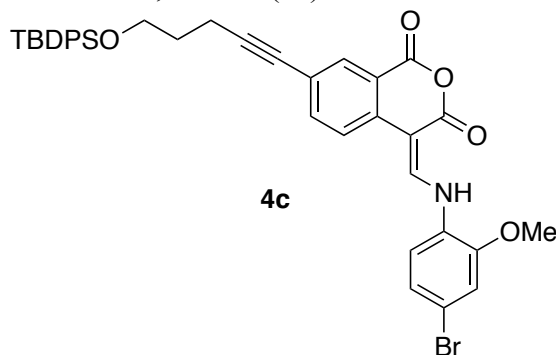
135.2, 131.2, 127.5, 125.5, 124.2, 118.4, 118.0, 117.6, 115.8, 115.2, 92.7, 56.4; HRMS (ESI): m/z calculated for $C_{17}H_{12}BrNO_4Na$ $[M+Na]^+$ 395.9847, found 395.9850.

(*Z*)-7-Bromo-4-(((4-bromo-2-methoxyphenyl)amino)methylene)isochromane-1,3-dione (**4b**):



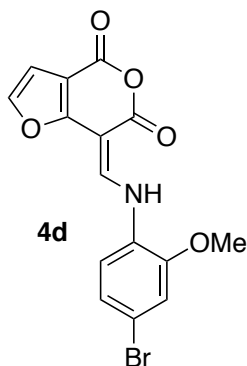
Using the general procedure for enamine formation outlined above, enol ether **3b** (0.75 g, 2.6 mmol, 1.0 mol-equiv) was used to give enamine **4b** (1.1 g, 2.3 mmol, 90%) as an orange solid. $R_f = 0.5$ (1:1 EtOAc/Hex). 1H NMR (500 MHz, $DMSO-d_6$) δ 11.70 (d, $J = 13.2$, 1H), 9.06 (d, $J = 9.9$, 1H), 8.17 – 7.88 (m, 4H), 7.37 – 7.27 (m, 2H), 3.97 (s, 3H); ^{13}C NMR (125 MHz, $DMSO-d_6$) δ 162.8, 160.3, 149.1, 145.9, 137.4, 136.7, 131.3, 127.0, 123.8, 122.8, 118.7, 117.6, 117.4, 117.2, 115.1, 91.7, 56.9; HRMS (ESI): m/z calculated for $C_{17}H_{11}Br_2NO_4Na$ $[M+Na]^+$ 473.8953, found 473.8943.

(*Z*)-4-(((4-Bromo-2-methoxyphenyl)amino)methylene)-7-(5-((*tert*-butyldiphenylsilyl)oxy)pent-1-yn-1-yl)isochromane-1,3-dione (**4c**):



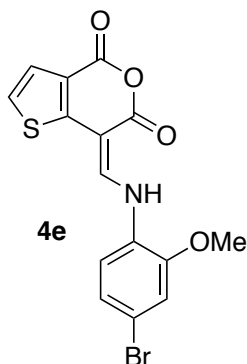
Using the general procedure for enamine formation outlined above, enol ether **3c** (0.11 g, 0.20 mmol, 1.0 mol-equiv) was used to give enamine **4c** (0.08 g, 0.11 mmol, 57%) as a green solid. $R_f = 0.8$ (3:7 EtOAc/Hex). 1H NMR (600 MHz, $CDCl_3$) δ 8.39 (d, $J = 13.8$, 1H), 8.19 (d, $J = 1.2$, 1H), 7.69 (d, $J = 6.6$, 4H), 7.56 (dd, $J = 8.4$, 1.8, 1H), 7.48–7.47 (m, 1H), 7.42–7.36 (m, 6H), 7.18 (s, 2H), 7.12 (s, 1H), 3.98 (s, 3H), 3.81 (t, $J = 6.0$, 2H), 2.59 (t, $J = 7.2$, 2H), 1.86 (t, $J = 6.6$, 2H), 1.07 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.9, 143.2, 137.8, 135.6, 134.1, 133.8, 129.7, 127.7, 127.4, 124.2, 121.5, 118.6, 118.0, 115.9, 115.3, 67.2, 62.4, 56.5, 31.5, 26.9, 19.3, 16.0; HRMS (ESI): m/z calculated for $C_{38}H_{36}BrNO_5SiNa$ $[M+Na]^+$ 716.1444, found 716.1458.

(*Z*)-7-(((4-Bromo-2-methoxyphenyl)amino)methylene)-4*H*-furo[3,2-*c*]pyran-4,6(7*H*)-dione (**4d**):



An oven dried 5 mL round bottom flask equipped with stir bar was charged with enol ether **3d**, (200 mg, 1.0 mmol, 1 mol-equiv), 4-bromo-2-methoxyaniline (230 mg, 1.1 mmol, 1.1 mol-equiv), and dioxanes (4 mL). The reaction was stirred for 2 h at room temperature. The resulting yellow-brown precipitate was collected by filtration and washed with cold dioxanes (10 mL). The precipitate was further purified by silica gel chromatography using chloroform as eluent. Combined fractions were concentrated *in vacuo* and dried under high vacuum to obtain enamine **4d** as a bright yellow solid (290 mg, 0.80 mmol, 80%). ¹H NMR (500 MHz, CDCl₃) δ 11.36 (d, *J*=13.7, 1H), 8.25 (d, *J*=13.8, 1H), 7.35 (d, *J*=7.4, 1H), 7.20 – 7.15 (m, 2H), 7.13 (s, 1H), 6.82 (d, *J*=2.1, 1H), 3.99 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.6, 160.8, 156.5, 149.8, 142.6, 141.3, 126.8, 124.3, 119.0, 115.8, 115.2, 108.4, 104.4, 89.8, 56.5; HRMS (ESI): *m/z* calculated for C₁₅H₁₀BrNO₅Na [M+Na]⁺ 385.9640, found 385.9646.

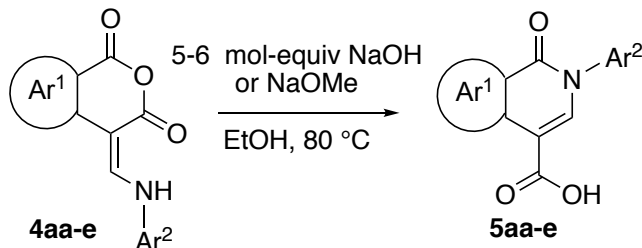
(*Z*)-7-(((4-Bromo-2-methoxyphenyl)amino)methylene)-4*H*-thieno[3,2-*c*]pyran-4,6(7*H*)-dione (**4e**):



An oven dried 5 mL round bottom flask equipped with stir bar was charged with enol ether **3e**, (230 mg, 1.1 mmol, 1 mol-equiv), 4-bromo-2-methoxyaniline (245 mg, 1.2 mmol, 1.1 mol-equiv), and dioxanes (4.5 mL). The reaction was stirred for 2 h at room temperature. The resulting yellow-brown precipitate was collected by filtration, then purified by silica gel chromatography using chloroform as eluent. Combined column fractions were concentrated *in vacuo* and dried under high vacuum to obtain enamine **4e** as a bright yellow solid (250 mg, 0.65 mmol, 60%). ¹H NMR (500 MHz, CDCl₃) δ 11.46 (d, *J*=13.3, 1 H), 8.07 (d, *J*=13.4, 1 H), 7.43 (d, *J*=5.3, 1H), 7.18 (dd, *J*=8.5, 1.7, 1H), 7.15 (d, *J*=8.5, 1H), 7.12 (d, *J*=1.7, 1H), 7.03 (d, *J*=5.3, 1H), 3.98 (s, 3H); ¹³C NMR

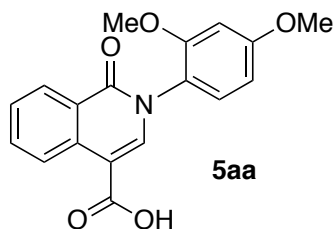
(125 MHz, CDCl₃) δ 163.3, 156.5, 151.6, 149.8, 142.6, 126.9, 126.4, 124.2, 120.9, 120.1, 118.8, 115.8, 115.2, 93.9, 56.5; HRMS (ESI): m/z calculated for C₁₅H₁₀BrNO₄SNa [M+Na]⁺ 401.9412, found 401.9407.

General procedure for the synthesis of isoquinolone carboxylic acids (**5aa-e**):



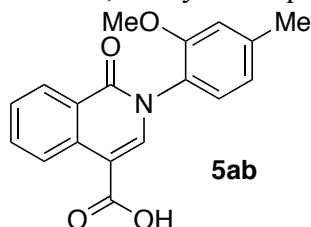
Isoquinolone carboxylic acids **5aa-e** were synthesized according to a procedure developed by Patil *et al.*⁶ An oven-dried round bottom flask equipped with stir bar was charged with the corresponding enamine **4aa-e** (1.0 mol-equiv) and anhydrous NaOH (5.0 mol-equiv). Next, EtOH was added to the flask and the flask was fitted with a reflux condenser. The reaction mixture was heated at reflux for 1.5 h. The reaction was monitored by TLC for disappearance of the enamine. Upon consumption of the enamine, the reaction mixture was cooled to room temperature. Then, the resulting mixture was adjusted to a pH of 1-2 with 3 M HCl. The resulting solid was filtered with cold water in a Büchner funnel and dried under vacuum to obtain the isoquinolone carboxylic acids **5aa-e**.

Example: 2-(2,4-Dimethoxyphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**5aa**):



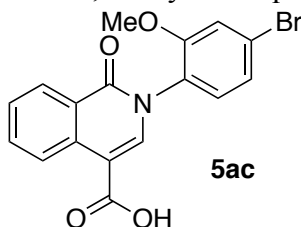
An oven-dried round bottom flask equipped with stir bar was charged with enamine **4aa** (1.5 g, 4.6 mmol, 1.0 mol-equiv) and anhydrous NaOH (0.91 g, 23 mmol, 5.0 mol-equiv). Next, EtOH (18 mL) was added to the flask and the flask was fitted with a reflux condenser. The reaction mixture was heated at reflux for 1.5 h. The reaction was monitored by TLC for disappearance of the enamine. Upon consumption of the enamine, the reaction mixture was cooled to room temperature. Then, the resulting mixture was adjusted to a pH of 1-2 with 3 M HCl. The resulting solid was filtered with cold water (3 x 20 mL) using a Büchner funnel to give isoquinolone carboxylic acid **5aa** (1.1 g, 3.5 mmol, 75%) as a purple solid. R_f = 0.6 (4:1 CH₂Cl₂/MeOH). ¹H NMR (125 MHz, DMSO-*d*₆) δ 12.86 (s, 1H), 8.86 (d, J = 8.4 Hz, 1H), 8.28 (d, J = 7.2 Hz, 1H), 8.02 (s, 1H), 7.85 (td, J = 7.7 Hz, 1.3, 1H), 7.60 (t, J = 7.7 Hz, 1H), 7.35 (d, J = 8.7 Hz, 1H), 6.78 (d, J = 2.6 Hz, 1H), 6.66 (d, J = 2.6 Hz, 1H), 6.64 (d, J = 2.7 Hz, 1H), 3.84 (s, 3H), 3.75 (s, 3H); ¹³C NMR (500 MHz, DMSO-*d*₆) δ 166.3, 161.0, 160.9, 155.1, 142.0, 134.5, 133.2, 129.3, 127.7, 127.2, 125.2, 125.2, 122.0, 105.7, 105.1, 99.4, 56.0, 55.6; HRMS (ESI): m/z calculated for C₁₈H₁₅NO₅Na [M+Na]⁺ 348.0848, found 348.0858.

2-(2-Methoxy-4-methylphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**5ab**):



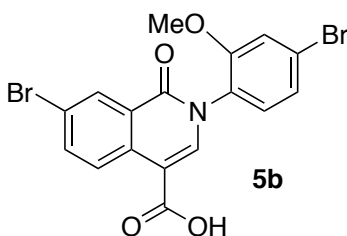
Using the general procedure for the synthesis of isoquinolone carboxylic acids outlined above, enamine **4ab** (0.20 g, 0.81 mmol, 1.0 mol-equiv) was used to give isoquinolone carboxylic acid **5ab** (0.12 g, 0.38 mmol, 47%) as a brown solid. $R_f = 0.6$ (9:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$). $^1\text{H NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.86 (br s, 1H), 8.86 (d, $J = 8.3$ Hz, 1H), 8.29 (d, $J = 8.0$ Hz, 1H), 8.02 (s, 1H), 7.85 (t, $J = 7.8$ Hz, 1H), 7.61 (t, $J = 7.6$ Hz, 1H), 7.31 (d, $J = 7.8$ Hz, 1H), 7.08 (s, 1H), 6.91 (d, $J = 7.9$ Hz, 1H), 3.75 (s, 3H), 2.41 (s, 3H); $^{13}\text{C NMR}$ (125 MHz, $\text{DMSO-}d_6$) δ 166.3, 160.9, 153.8, 141.7, 140.5, 134.5, 133.3, 128.4, 127.7, 127.3, 126.4, 125.2, 125.16, 121.1, 113.2, 105.7, 55.8, 21.3; HRMS (ESI): m/z calculated for $\text{C}_{18}\text{H}_{15}\text{NO}_4\text{Na}$ $[\text{M}+\text{Na}]^+$ 332.0899, found 348.0904.

2-(4-Bromo-2-methoxyphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**5ac**):



Using the general procedure for synthesis of isoquinolone carboxylic acids outlined above, enamine **4ac** (0.23 g, 0.61 mmol, 1.0 mol-equiv) was used to give isoquinolone carboxylic acid **5ac** (0.18 g, 0.47 mmol, 77% yield) as a brown solid. $R_f = 0.3$ (9:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$). $^1\text{H NMR}$ (500 MHz, $\text{DMSO-}d_6$) δ 12.90 (s, 1H), 8.85 (d, $J = 8.4$ Hz, 1H), 8.28 (d, $J = 8.0$ Hz, 1H), 8.06 (s, 1H), 7.86 (t, $J = 7.7$ Hz, 1H), 7.61 (t, $J = 7.7$ Hz, 1H), 7.47–7.43 (m, 2H), 7.31 (dd, $J = 8.2, 1.6$ Hz, 1H), 3.79 (s, 3H); $^{13}\text{C NMR}$ (125 MHz, $\text{DMSO-}d_6$) δ 166.2, 160.7, 155.1, 141.1, 134.5, 133.4, 133.4, 130.6, 128.2, 127.7, 127.4, 125.3, 125.0, 123.6, 123.0, 116.0, 106.1, 56.6; HRMS (ESI): m/z calculated for $\text{C}_{17}\text{H}_{12}\text{BrNO}_4\text{Na}$ $[\text{M}+\text{Na}]^+$ 395.9847, found 395.9842.

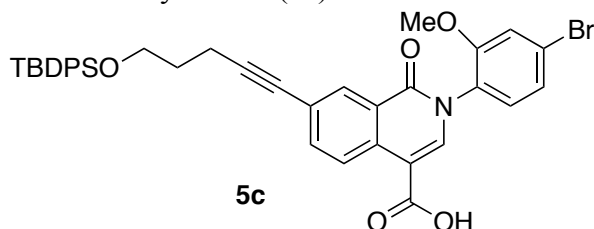
7-Bromo-2-(4-bromo-2-methoxyphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**5b**):



Using the general procedure for the synthesis of isoquinolone carboxylic acids outlined above, enamine **4b** (0.5 g, 1.1 mmol, 1.0 mol-equiv) was used to give

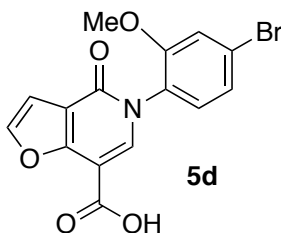
isoquinolone carboxylic acid **5b** as a green solid. The crude material was purified by flash chromatography using CH₂Cl₂/MeOH to give **5b** (0.30 g, 0.38 mmol, 61%) as a green solid. *R_f* = 0.1 (3:2 EtOAc/Hex). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.87 (d, *J* = 8.5, 1H), 8.35 (s, 1H), 8.07 – 8.02 (m, 2H), 7.47 – 7.43 (m, 2H), 7.31 (d, *J* = 8.3, 1H) 3.79 (s, 3H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 166.5, 160.1, 155.5, 142.2, 136.8, 134.0, 131.0, 130.2, 128.33, 128.31, 127.2, 124.1, 123.7, 120.9, 116.5, 106.3, 57.0; HRMS (ESI): *m/z* calculated for C₁₇H₁₁Br₂NO₄Na [M+Na]⁺ 473.8953, found 473.8947.

2-(4-Bromo-2-methoxyphenyl)-7-(5-((*tert*-butyldiphenylsilyl)oxy)pent-1-yn-1-yl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**5c**):



Using the general procedure for the synthesis of isoquinolone carboxylic acids outlined above, enamine **4c** (57.5 mg, 0.08 mmol, 1.0 mol-equiv) was used to give isoquinolone carboxylic acid **5c** as a green solid. The crude material was purified by flash chromatography using CH₂Cl₂/MeOH to give **5c** (51.0 mg, 0.07 mmol, 92%) as a white solid. *R_f* = 0.3 (3:7 EtOAc/Hex). ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.81 (d, *J* = 8.4, 1H), 8.18 (s, 1H), 8.06 (s, 1H), 7.77 (d, *J* = 8.4, 1H), 7.64–7.63 (m, 4H), 7.47–7.40 (m, 8H), 7.32 (d, *J* = 7.8, 1H), 3.82–3.80 (m, 5H), 2.61 (t, *J* = 6.6, 2H), 1.84 (t, *J* = 6.3, 2H), 1.01 (s, 9H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 166.6, 161.1, 155.5, 142.2, 135.5, 133.6, 131.0, 130.3, 128.4, 125.5, 124.1, 123.7, 116.5, 106.3, 62.5, 57.0, 31.3, 27.2, 19.3, 15.7; HRMS (ESI): *m/z* calculated for C₃₈H₃₆BrNO₅SiNa [M+Na]⁺ 716.1444, found 716.1438.

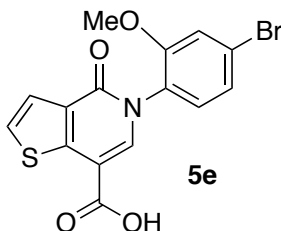
5-(4-Bromo-2-methoxyphenyl)-4-oxo-4,5-dihydrofuro[3,2-*c*]pyridine-7-carboxylic acid (**5d**):



A 10 mL round bottom flask equipped with stir bar was charged with sodium methoxide (180 mg, 3.3 mmol, 6 mol-equiv), ethanol (6 mL) and enamine **4d** (200 mg, 0.55 mmol, 1 mol-equiv). The flask was equipped with a condenser and the mixture heated at refluxed for 21 h. The mixture was cooled to room temperature and dissolved in 10 mL H₂O. The mixture was adjusted to pH = 2 with 1 N HCl (aq). The resulting white precipitate was collected by filtration, washed with H₂O (20 mL), and dried under high vacuum to obtain isoquinolone carboxylic acid **5d** as a white solid (160 mg, 0.44 mmol, 80%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 13.14 (br, 1H), 8.07 (d, *J* = 2.0, 1H), 8.01 (s, 1H), 7.46 (d, *J* = 1.9, 1H), 7.39 (d, *J* = 8.3, 1H), 7.30 (dd, *J* = 8.3, 2.0, 1H), 7.06 (d, *J* = 2.0, 1H), 3.79 (s, 3H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 163.2, 157.8, 156.1, 155.1, 145.5,

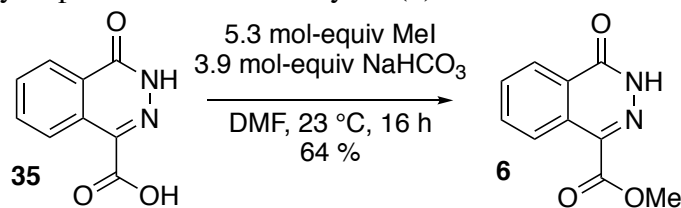
141.4, 130.6, 127.8, 123.4, 123.0, 115.8, 115.2, 107.1, 101.6, 56.4; HRMS (ESI): m/z calculated for $C_{15}H_{10}BrNO_5Na$ $[M+Na]^+$ 385.9640, found 385.9642.

5-(4-Bromo-2-methoxyphenyl)-4-oxo-4,5-dihydrothieno[3,2-*c*]pyridine-7-carboxylic acid (**5e**):



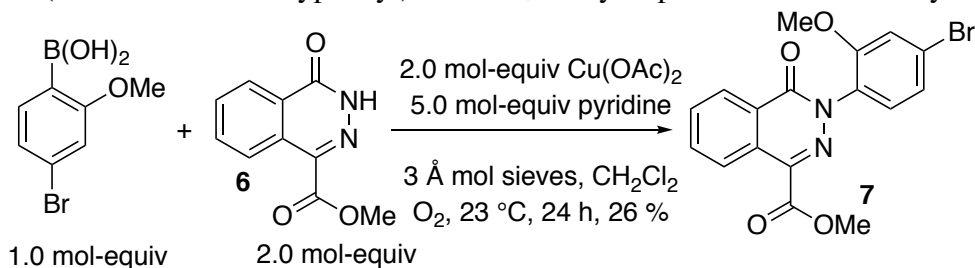
A 10 mL round bottom flask equipped with stir bar was charged with sodium methoxide (128 mg, 2.4 mmol, 6 mol-equiv), ethanol (5 mL) and enamine **4e** (150 mg, 0.39 mmol, 1 mol-equiv). The flask was equipped with a condenser and refluxed for 22 h. Then, an additional 3 mL of ethanol was added to replace ethanol lost to evaporation, and refluxed for an additional 6 h. The reaction mixture was cooled to room temperature and dissolved in 10 mL H_2O . The mixture was adjusted to pH = 2 with 1 N HCl (aq). The resulting light-brown precipitate was collected by filtration, washed with H_2O (20 mL). The precipitate was purified by silica gel chromatography using EtOAc. Combined column fractions were concentrated *in vacuo* and dried under high vacuum to obtain isoquinolone carboxylic acid **5e** as a white solid (79 mg, 0.21 mmol, 54%). 1H NMR (500 MHz, DMSO- d_6) δ 13.35 (br, 1H), 8.06 (s, 1H), 7.78 (d, $J = 5.4$, 1H), 7.55 (d, $J = 5.4$, 1H), 7.46 (d, $J = 1.9$, 1H), 7.42 (d, $J = 8.3$, 1H), 7.31 (dd, $J = 8.3, 1.9$, 1H), 3.79 (s, 3H); ^{13}C NMR (500 MHz, DMSO- d_6) δ 165.2, 157.3, 155.0, 145.0, 139.5, 130.5, 129.1, 128.8, 127.8, 123.9, 123.4, 123.0, 115.8, 106.0, 56.4; HRMS (ESI): m/z calculated for $C_{15}H_{10}BrNO_4SNa$ $[M+Na]^+$ 401.9412, found 401.9425.

Methyl 4-oxo-3,4-dihydrophthalazine-1-carboxylate (**6**):



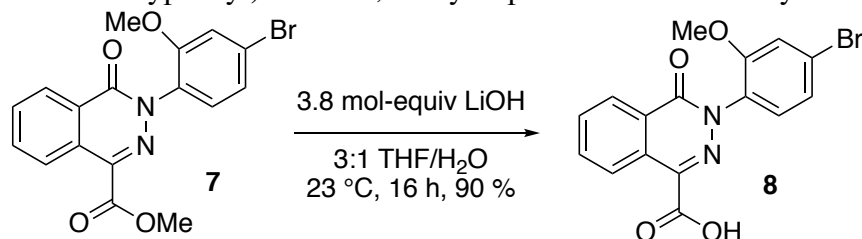
Phthalazinone methyl ester **6** was synthesized by adapting a procedure developed by Brittain and Wood.⁷ An oven-dried round bottom flask equipped with stir bar was charged with phthalazinone carboxylic acid **35** (0.10 g, 0.53 mmol, 1.0 mol-equiv), $NaHCO_3$ (0.17 g, 2.1 mmol, 3.9 mol-equiv), DMF (2.3 mL), and methyl iodide (0.17 mL, 2.8 mmol, 5.3 mol-equiv). The reaction mixture was stirred for 16 h. Upon consumption of **35**, the reaction mixture was diluted with water (7 mL) and the resulting gray solid was collected by filtration using a Büchner funnel. The solid was washed with cold water (3 x 5 mL) to give phthalazinone methyl ester **6** (0.069 g, 0.34 mmol, 64%) as a gray solid. $R_f = 0.5$ (3:2 EtOAc/Hex). 1H NMR (500 MHz, $CDCl_3$) δ 8.76 (d, $J = 8.3$ Hz, 1H), 8.47 (d, $J = 7.9$ Hz, 1H), 7.91 (t, $J = 7.3$ Hz, 1H), 7.83 (t, $J = 7.6$ Hz, 1H), 4.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.6, 160.4, 136.3, 134.5, 132.2, 128.4, 128.0, 126.9, 126.8, 53.2; HRMS (ESI): m/z calculated for $C_{10}H_8N_2O_3Na$ $[M+Na]^+$ 227.0433, found 227.0440.

Methyl 3-(4-bromo-2-methoxyphenyl)-4-oxo-3,4-dihydrophthalazine-1-carboxylate (**7**):



Phthalazinone methyl ester **7** was synthesized by adapting a procedure developed by Su and co-workers.⁸ A flame-dried round bottom flask equipped stir bar was charged with amide **6** (0.050 g, 0.24 mmol, 1.0 mol-equiv), 4-bromo-2-methoxyboronic acid (0.11 g, 0.49 mmol, 2.0 mol-equiv), Cu(OAc)₂ (0.089 g, 0.49 mmol, 2.0 mol-equiv), and 3 Å mol sieves (1.2 g). Next, CH₂Cl₂ (3.9 mL) and pyridine (0.10 mL, 1.2 mmol, 5.0 mol-equiv) were added to the reaction mixture. The flask was connected to a drying tube, which allowed atmospheric oxygen to enter the flask. After 24 h, the reaction mixture was filtered through celite and the filtrate concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/Hex) to afford phthalazinone methyl ester **7** (0.025 g, 0.064 mmol, 26%) as a white solid. *R_f* = 0.6 (1:1 EtOAc/Hex). ¹H NMR (500 MHz, CDCl₃) δ 8.66 (d, *J* = 8.3 Hz, 1H), 8.50 (d, *J* = 7.9 Hz, 1H), 7.91 (t, *J* = 7.9 Hz, 1H), 7.83 (t, *J* = 7.9, 1H), 7.28 – 7.22 (m, 2H) 7.19 (d, *J* = 1.6 Hz, 1H), 3.99 (s, 3H), 3.80 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.8, 159.1, 155.5, 136.4, 134.1, 132.2, 129.9, 129.6, 128.12, 128.06, 127.6, 126.6, 124.3, 124.1, 116.1, 56.5, 53.2; HRMS (ESI): *m/z* calculated for C₁₇H₁₃BrN₂O₄ [M]⁺ 388.0059, found 388.0050.

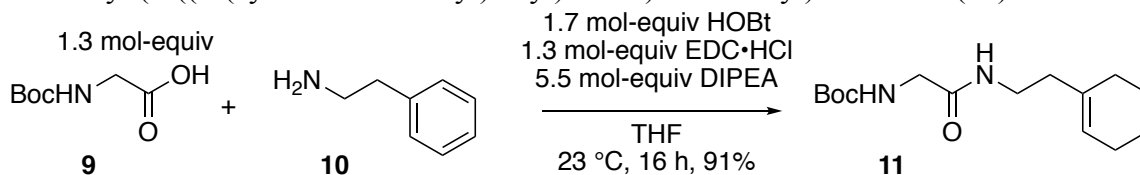
3-(4-Bromo-2-methoxyphenyl)-4-oxo-3,4-dihydrophthalazine-1-carboxylic acid (**8**):



Carboxylic acid **8** was synthesized according to a procedure developed by Selwood.² A round bottom flask equipped with stir bar was charged with phthalazinone methyl ester **7** (0.024 g, 0.061 mmol, 1.0 mol-equiv). THF (0.60 mL) and 0.17 mL of aqueous LiOH (1.4 M, 0.23 mmol, 3.8 mol-equiv) was added to the flask via syringe. The reaction progress was monitored by TLC, which indicated complete consumption of **7** after stirring overnight. 1.7 mL of 1 N HCl and 2.8 mL of EtOAc were added to the reaction mixture. The mixture was extracted with EtOAc (3 x 7 mL). The combined organic layers were washed with brine and dried over Na₂SO₄. The solution was concentrated *in vacuo* to yield phthalazinone carboxylic acid **8** (0.021 g, 0.055 mmol, 90%) as a light yellow solid. *R_f* = 0.3 (4:1 CH₂Cl₂/MeOH). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.55 (d, *J* = 8.2, 1H), 8.33 (d, *J* = 7.8, 1H), 8.03 (t, *J* = 8.1 Hz, 1H), 7.94 (t, *J* = 7.8, 1H), 7.46 – 7.43 (m, 2H), 7.31 (dd, *J* = 8.3, 1.9, 1H), 3.78 (s, 3H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 164.4, 158.1, 155.3, 137.4, 134.3, 132.4, 130.6, 129.6, 127.45, 127.39,

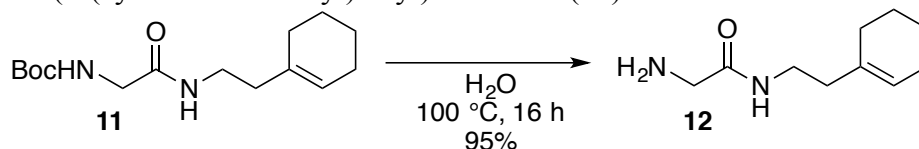
126.65, 126.58, 123.6, 123.0, 115.8, 56.4; HRMS (ESI): m/z calculated for $C_{16}H_{10}BrN_2O_4$ $[M-H]^-$ 372.9824, found 372.9834.

tert-Butyl 2-((2-(cyclohex-1-en-1-yl)ethyl)amino)-2-oxoethyl)carbamate (**11**):



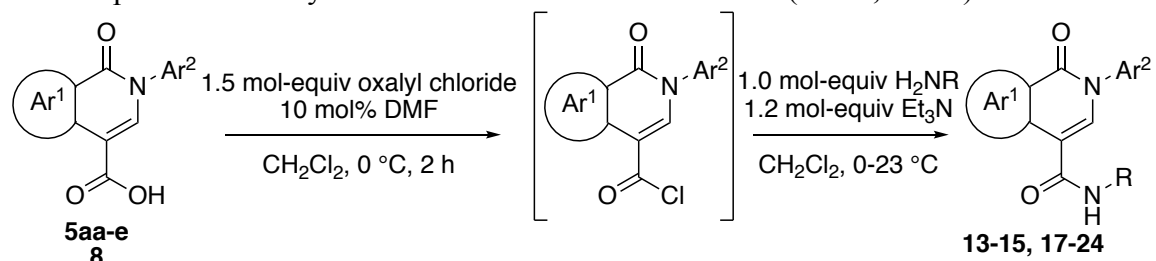
Amide **11** was synthesized according to a procedure developed by Awuah *et. al.*⁹ An oven-dried round bottom flask equipped with stir bar was charged with Boc-glycine **9** (5.5 g, 31 mmol, 1.3 mol-equiv), HOBt (5.3 g, 40 mmol, 1.7 mol-equiv), and EDC·HCl (6.0 g, 31 mmol, 1.3 mol-equiv). THF (120 mL) was added to the reaction vessel followed by amine **10** (3.3 mL, 24 mmol, 1.0 mol-equiv). While stirring, DIPEA (23 mL, 130 mmol, 5.5 mol-equiv) was added to the reaction vessel via syringe. The reaction progress was monitored by TLC, which indicated complete consumption of **10** after 16 h. The reaction mixture was diluted with saturated NH_4Cl and extracted with EtOAc (3 x 360 mL). The combined organic layers were washed with brine, dried with Na_2SO_4 , and concentrated *in vacuo* to afford a white solid and purified with flash chromatography (EtOAc/Hex) to yield amide **11** (6.2 g, 22 mmol, 91%) as a white solid. R_f = 0.8 (1:1 EtOAc/Hex). 1H NMR (500 MHz, $CDCl_3$) δ 6.18 (s, 1H), 5.44 (s, 1H), 5.25 (s, 1H), 3.74 (d, J = 5.7 Hz, 2H), 3.31 (q, J = 5.9 Hz, 2H), 2.10 (t, J = 6.8 Hz, 2H), 1.97 – 1.89 (m, 4H), 1.62 – 1.50 (m, 4H), 1.43 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.3, 156.1, 134.5, 123.8, 80.2, 44.4, 37.6, 37.2, 28.4, 28.0, 25.3, 22.9, 22.4; HRMS (ESI): m/z calculated for $C_{15}H_{26}N_2O_3Na$ $[M+Na]^+$ 305.1841, found 305.1833.

2-Amino-*N*-(2-(cyclohex-1-en-1-yl)ethyl)acetamide (**12**):



Amine **12** was synthesized by adapting a procedure developed by Zinelaabidine *et. al.*¹⁰ A round bottom flask equipped with stir bar was charged with Boc-protected amine **11** (1.5 g, 5.3 mmol, 1.0 mol-equiv) and water (110 mL). The flask was fitted with a condenser and heated at reflux while monitoring for consumption of **11** by TLC. After 16 h, the reaction mixture was cooled to room temperature. The aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were concentrated *in vacuo* to give amine **12** (0.91 g, 5.0 mmol, 95%) as a yellow oil, which solidified upon storage at -20 °C. 1H NMR (500 MHz, $CDCl_3$) δ 7.21 (br s, 1H), 5.48 (s, 1H), 3.38–3.34 (m, 4H), 2.15 (t, J = 6.7 Hz, 2H), 2.0–1.94 (m, 4H), 1.65–1.51 (m, 4H), 1.42 (br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 134.7, 123.4, 44.8, 37.7, 36.8, 28.0, 25.3, 22.9, 22.4; HRMS (ESI): m/z calculated for $C_{10}H_{18}N_2ONa$ $[M+Na]^+$ 205.1317, found 205.1312.

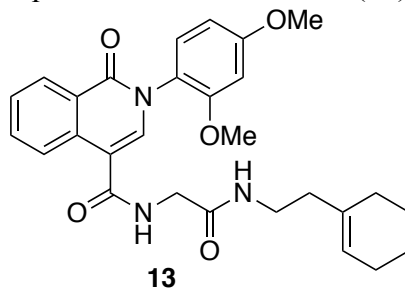
General procedure for synthesis of amides via acid chlorides (**13-15**, **17-24**):



Amides **13-15** and **17-24** were synthesized by adapting procedures developed by Berghasuen *et. al.*¹¹ and Liu *et. al.*¹² A flame-dried round bottom flask equipped with stir bar was charged with the corresponding carboxylic acid **5aa-e** or **8** (1.0 mol-equiv). Next, CH_2Cl_2 was added to the flask and the reaction mixture was cooled to 0 °C in an ice-water bath. Oxalyl chloride (1.5 mol-equiv) and DMF (0.1 mol-equiv) were added sequentially. The reaction mixture was stirred at 0 °C for 2 h to obtain a clear solution. The solution was concentrated *in vacuo* to yield the corresponding acid chloride, which was used without purification.

A separate flame-dried round bottom flask equipped with stir bar was charged with the corresponding amine (1.0 mol-equiv), CH_2Cl_2 , and Et_3N (1.2 mol-equiv). The flask was cooled to 0 °C in an ice-water bath. Next, the acid chloride was dissolved in CH_2Cl_2 and transferred dropwise to the amine solution over 10 min. The reaction vessel was warmed to room temperature, and monitored by TLC for consumption of the acid chloride (0.5-2 h). The reaction mixture was washed with K_2CO_3 (aq, 10 wt%) and extracted with CH_2Cl_2 . The combined organic layers were washed with brine, dried over Na_2SO_4 , and concentrated *in vacuo*. The crude material was purified by flash chromatography to obtain the corresponding amide **13-15**, **17**, or **19-24**. Amide **18** was obtained by TBDPS-deprotection of the crude product, followed by purification using chromatography.

Example: *N*-(2-((2-(Cyclohex-1-en-1-yl)ethyl)amino)-2-oxoethyl)-2-(2,4-dimethoxyphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxamide (**13**):

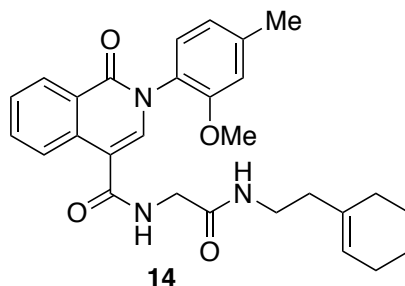


A flame-dried round bottom flask equipped with stir bar was charged with isoquinolone carboxylic acid **5aa** (0.050 g, 0.15 mmol, 1.0 mol-equiv) and CH_2Cl_2 (2.0 mL). The reaction mixture was cooled to 0 °C in an ice-water bath. Subsequently, oxalyl chloride (20 μL , 0.23 mmol, 1.5 mol-equiv) and DMF (1.2 μL , 0.015 mmol, 0.1 mol-equiv) were added sequentially. The reaction mixture was stirred at 0 °C for 2 h to obtain a clear solution. The solution was concentrated *in vacuo* to yield the acid chloride.

A separate flame-dried round bottom flask equipped with stir bar was charged with amine **12** (0.028 g, 0.15 mmol, 1.0 mol-equiv), CH_2Cl_2 (4.0 mL) and Et_3N (0.025

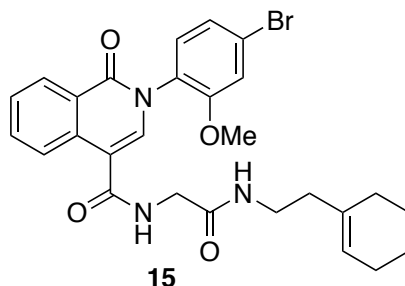
mL, 0.18 mmol 1.2 mol-equiv). The flask was cooled to 0 °C in an ice-water bath. Next, the acid chloride was dissolved in CH₂Cl₂ (6.0 mL) and injected dropwise via syringe into the reaction mixture over 10 min. The reaction vessel was warmed to room temperature. The reaction mixture was stirred until the acid chloride was no longer detected by TLC (0.5 h). Upon consumption of the acid chloride, the reaction mixture was washed with K₂CO₃ (aq, 10 wt%) and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, and concentrated *in vacuo* to afford a purple solid. The solid was purified by flash chromatography (CH₂Cl₂/Et₂O) to afford amide **13** (0.074 g, 0.15 mmol, 98%) as a white solid. *R_f* = 0.2 (6:4 EtOAc/Hex). ¹H NMR (500 MHz, CDCl₃) δ 8.47 (d, *J* = 7.9 Hz, 1H), 8.23 (d, *J* = 8.3 Hz, 1H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.51 (s, 1H), 7.17 (d, *J* = 8.7 Hz, 1H), 6.84 (t, *J* = 4.9 Hz, 1H), 6.57 (d, *J* = 2.6 Hz, 1H), 6.53 (dd, *J* = 9.6, 2.5 Hz, 1H), 6.06 (t, *J* = 5.3 Hz, 1H), 5.46 (s, 1H), 4.08 (d, *J* = 5.0 Hz, 2H), 3.85 (s, 3H), 3.75 (s, 3H), 3.56 (q, *J* = 5.6 Hz, 2H), 2.14 (t, *J* = 7.0 Hz, 2H), 1.96–1.90 (m, 4H), 1.62–1.50 (m, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 168.4, 168.8, 162.0, 161.4, 155.5, 135.7, 134.4, 134.2, 133.1, 129.3, 128.8, 127.6, 126.3, 124.8, 124.0, 122.4, 112.8, 104.7, 99.8, 56.0, 55.8, 43.6, 37.6, 37.5, 28.0, 25.3, 22.9, 22.4; HRMS (ESI): *m/z* calculated for C₂₈H₃₁N₃O₅Na [M+Na]⁺ 512.2161, found 512.2150; HPLC purity: 99.5%.

N-(2-((2-(Cyclohex-1-en-1-yl)ethyl)amino)-2-oxoethyl)-2-(2-methoxy-4-methylphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxamide (**14**):



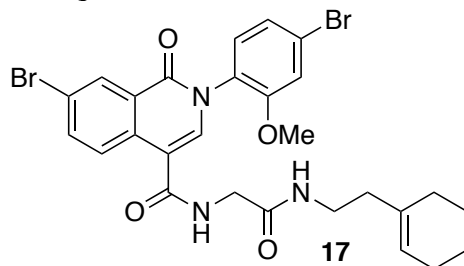
Isoquinolone carboxylic acid **5ab** (0.051 g, 0.17 mmol, 1.0 mol-equiv) and amine **12** (0.031 g, 0.17 mmol, 1.0 mol-equiv) were reacted following the general procedure for the synthesis of amides outlined above. The crude material was purified by flash chromatography using CH₂Cl₂/Et₂O to give amide **14** (0.075 g, 0.16 mmol, 96%) as a white solid. *R_f* = 0.2 (6:4 EtOAc/Hex). ¹H NMR (500 MHz, CDCl₃) δ 8.47 (d, *J* = 8.0 Hz, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 7.72 (t, *J* = 7.1 Hz, 1H), 7.53 (t, *J* = 8.0 Hz, 1H), 7.51 (s, 1H), 7.13 (d, *J* = 8.3 Hz, 1H), 6.86 – 6.83 (m, 3H), 6.08 (br s, 1H), 5.45 (s, 1H), 4.07 (d, *J* = 5.0 Hz, 2H), 3.77 (s, 3H), 3.35 (q, *J* = 6.7 Hz, 2H), 2.41 (s, 3H), 2.13 (t, *J* = 6.8 Hz, 2H), 1.97 – 1.86 (m, 4H), 1.62 – 1.49 (m, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 168.4, 166.8, 161.9, 154.3, 141.0, 135.5, 134.4, 134.2, 133.1, 128.8, 128.4, 127.6, 126.6, 126.3, 124.8, 124.0, 121.7, 113.1, 112.8, 55.9, 43.6, 37.6, 37.5, 28.0, 25.3, 22.9, 22.4, 21.9; HRMS (ESI): *m/z* calculated for C₂₈H₃₁N₃O₄Na [M+Na]⁺ 496.2212, found 496.2229; HPLC purity: 99.6%.

2-(4-Bromo-2-methoxyphenyl)-*N*-(2-((2-(cyclohex-1-en-1-yl)ethyl)amino)-2-oxoethyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxamide (**15**):



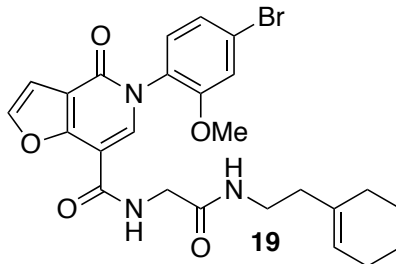
Isoquinolone carboxylic acid **5ac** (0.059 g, 0.15 mmol, 1.0 mol-equiv) and amine **12** (0.029 g, 0.16 mmol, 1.0 mol-equiv) were reacted following the general procedure for the synthesis of amides outlined above. The crude material was purified by flash chromatography using CH₂Cl₂/Et₂O to give amide **15** (0.078 g, 0.14 mmol, 95%) as a white solid. $R_f = 0.2$ (6:4 EtOAc/Hex). ¹H NMR (500 MHz, CDCl₃) δ 8.46 (d, $J = 8.1$ Hz, 1H), 8.22 (d, $J = 8.1$ Hz, 1H), 7.73 (t, $J = 7.6$ Hz, 1H), 7.55 (t, $J = 7.5$ Hz, 1H), 7.46 (s, 1H), 7.19–7.14 (m, 3H), 6.89 (br s, 1H), 6.05 (br s, 1H), 5.46 (s, 1H), 4.08 (d, $J = 4.8$ Hz, 2H), 3.79 (s, 3H), 3.35 (q, $J = 6.2$ Hz, 2H), 2.14 (t, $J = 6.72$ Hz, 2H), 1.97–1.90 (m, 4H), 1.62–1.50 (m, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 168.4, 166.6, 161.6, 155.3, 134.7, 134.4, 134.2, 133.3, 130.1, 128.8, 128.3, 127.9, 126.2, 124.9, 124.2, 124.1, 123.9, 116.1, 113.3, 56.4, 43.5, 37.57, 37.56, 28.0, 25.3, 22.9, 22.4; HRMS (ESI): m/z calculated for C₂₇H₂₈BrN₃O₄Na [M+Na]⁺ 560.1161, found 560.1158; HPLC purity: 98.3%.

7-Bromo-2-(4-bromo-2-methoxyphenyl)-*N*-(2-((2-(cyclohex-1-en-1-yl)ethyl)amino)-2-oxoethyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxamide (**17**):



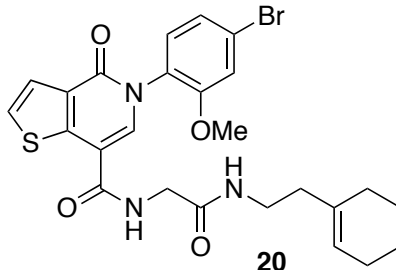
Isoquinolone carboxylic acid **5b** (0.069 g, 0.15 mmol, 1.0 mol-equiv) and amine **12** (0.028 g, 0.15 mmol, 1.0 mol-equiv) were reacted following the general procedure for the synthesis of amides outlined above. The crude material was purified by flash chromatography using CH₂Cl₂/MeOH to give amide **17** (0.092 g, 0.15 mmol, >99%) as a yellow solid. $R_f = 0.3$ (6:4 EtOAc/Hex). ¹H NMR (500 MHz, CDCl₃) δ 8.56 (d, $J = 2.2$ Hz, 1H), 8.15 (d, $J = 8.8$, 1H), 7.80 (dd, $J = 8.8, 2.2$ Hz, 1H), 7.48 (s, 1H), 7.21 – 7.14 (m, 3H), 6.93 (t, $J = 4.5$ Hz, 1H), 5.93 (br s, 1H), 5.46 (br s, 1H), 4.05 (d, $J = 4.03$ Hz, 2H), 3.79 (s, 3H), 3.35 (q, $J = 5.5$ Hz, 2H), 2.14 (t, $J = 6.7$ Hz, 2H) 1.98 – 1.90 (m, 4H) 1.64 – 1.52 (m, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 168.2, 166.1, 160.3, 155.1, 136.3, 134.9, 134.2, 132.9, 131.2, 129.9, 127.9, 127.5, 126.8, 124.2, 124.10, 124.05, 122.9, 116.1, 112.6, 56.3, 43.3, 37.47, 37.46, 27.9, 25.3, 22.8, 22.3; HRMS (ESI): m/z calculated for C₂₇H₂₇Br₂N₃O₄Na [M+Na]⁺ 638.0266, found 638.0270; HPLC purity: 97.1%.

5-(4-Bromo-2-methoxyphenyl)-*N*-(2-((2-(cyclohex-1-en-1-yl)ethyl)amino)-2-oxoethyl)-4-oxo-4,5-dihydrofuro[3,2-*c*]pyridine-7-carboxamide (**19**):



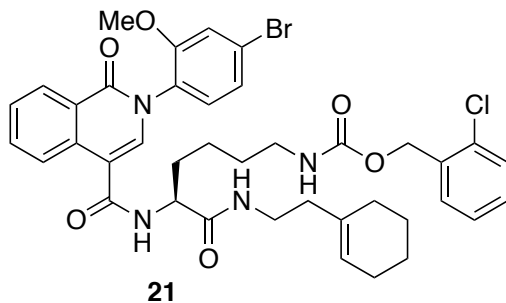
Isoquinolone carboxylic acid **5d** (70 mg, 0.19 mmol, 1.0 mol-equiv) and amine **12** (83 mg, 0.45 mmol, 2.4 mol-equiv) were reacted following the general procedure for the synthesis of amides outlined above. The crude material was purified by flash chromatography using ethyl acetate to obtain amide **19** (83 mg, 0.16 mmol, 82%) as a white solid. ^1H NMR (500 MHz, CDCl_3) δ 8.07 (s, 1H), 7.83 (t, $J = 4.7$ Hz, 1H), 7.68 (d, $J = 1.8$ Hz, 1H), 7.22 (dd, $J = 8.3$ Hz, 1.9 Hz, 1H), 7.20 (d, $J = 2.0$ Hz, 1H), 7.14 (d, $J = 8.2$ Hz, 1H), 6.06-5.90 (m, 1H), 5.44 (s, 1H), 4.16 (d, $J = 5.1$ Hz, 2H), 3.79 (s, 3H), 3.38 (q, $J = 6.5$ Hz, 2H), 2.15 (t, $J = 6.7$ Hz, 2H), 1.98-1.84 (m, 4H), 1.63-1.55 (m, 2H), 1.54-1.45 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 168.3, 162.0, 158.7, 155.9, 155.2, 144.0, 140.7, 134.5, 130.0, 128.0, 124.3, 124.2, 124.1, 116.1, 115.6, 108.5, 103.5, 56.4, 43.8, 37.6, 37.5, 28.0, 25.3, 22.9, 22.4; HRMS (ESI): m/z calculated for $\text{C}_{25}\text{H}_{26}\text{BrN}_3\text{O}_5\text{Na}$ $[\text{M}+\text{Na}]^+$ 550.0953, found 550.0948; HPLC purity: 98.9%.

5-(4-Bromo-2-methoxyphenyl)-*N*-(2-((2-(cyclohex-1-en-1-yl)ethyl)amino)-2-oxoethyl)-4-oxo-4,5-dihydrothieno[3,2-*c*]pyridine-7-carboxamide (**20**):



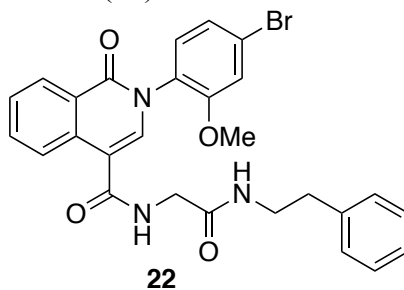
Isoquinolone **5e** (70 mg, 0.19 mmol, 1.0 mol-equiv) and amine **12** (83 mg, 0.45 mmol, 2.4 mol-equiv) were reacted following the general procedure for the synthesis of amides outlined above. The crude material was purified by flash chromatography using 50:50 EtOAc/Hex followed by 75:25 EtOAc/Hex to obtain amide **20** (83 mg, 0.16 mmol, 82%) as a white solid. ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.67 (t, $J = 5.8$ Hz, 1H), 8.33 (s, 1H), 7.84 (t, $J = 5.6$ Hz, 1H), 7.73 (d, $J = 5.4$ Hz, 1H), 7.51 (d, $J = 5.5$ Hz, 1H), 7.50 (d, $J = 2.0$ Hz, 1H), 7.42 (d, $J = 8.3$ Hz, 1H), 7.35 (dd, $J = 8.3$ Hz, 1.9 Hz, 1H), 5.42-5.31 (br, 1H), 3.86-3.74 (m, 5H), 3.12 (q, $J = 6.8$ Hz, 2H), 2.05-1.96 (t, $J = 7.3$ Hz, 2H), 1.93-1.83 (m, 4H), 1.56-1.49 (m, 2H), 1.49-1.42 (m, 2H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 168.5, 163.9, 157.2, 155.3, 145.2, 135.7, 134.8, 130.7, 129.4, 129.3, 128.5, 123.6, 123.5, 123.0, 122.0, 115.8, 108.7, 56.5, 42.4, 37.5, 37.2, 27.7, 24.6, 22.4, 21.9. HRMS (ESI): m/z calculated for $\text{C}_{25}\text{H}_{26}\text{BrN}_3\text{O}_4\text{SNa}$ $[\text{M}+\text{Na}]^+$ 566.0725, found 566.0711; HPLC purity: 99.3%.

2-Chlorobenzyl (*S*)-5-(2-(4-bromo-2-methoxyphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxamido)-6-((2-(cyclohex-1-en-1-yl)ethyl)amino)-6-oxohexyl)carbamate (**21**):



Isoquinolone carboxylic acid **5ac** (0.058 g, 0.16 mmol, 1.0 mol-equiv) and amine **33** (0.068 g, 0.16 mmol, 1.0 mol-equiv) were reacted following the general procedure for the synthesis of amides outlined above. The crude material was purified by flash chromatography using EtOAc/Hex to give amide **21** (0.097 g, 0.12 mmol, 80%) as a white solid. $R_f = 0.6$ (6:4 EtOAc/Hex). $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 8.44 (d, $J = 7.9$ Hz, 1H), 8.17 (d, $J = 8.1$ Hz, 1H), 7.72 (t, $J = 7.3$ Hz, 1H), 7.53 (t, $J = 7.7$ Hz, 1H), 7.41 (s, 1H), 7.35–7.32 (m, 2H), 7.26–7.13 (m, 5H), 6.80 (d, $J = 7.8$ Hz, 1H), 6.15 (t, $J = 5.6$ Hz, 1H), 5.44 (s, 1H), 5.12 (q, $J = 9.3$ Hz, 2H), 4.95 (s, $J = 5.1$ Hz, 1H), 4.57 (q, $J = 6.5$ Hz, 1H), 3.78 (s, 3H), 3.42–3.34 (m, 1H), 3.31–3.25 (m, 1H), 3.20 (q, $J = 6.5$ Hz, 2H), 2.13 (t, $J = 6.5$ Hz, 2H), 1.98–1.86 (m, 5H), 1.88–1.70 (m, 1H), 1.62–1.39 (m, 8H); $^{13}\text{C NMR}$ (125 MHz, CDCl_3) δ 171.3, 166.3, 161.5, 156.5, 155.3, 134.43, 134.37, 134.27, 134.17, 133.6, 133.3, 130.1, 129.8, 129.6, 129.5, 128.8, 128.3, 127.8, 127.0, 126.1, 124.8, 124.2, 124.0, 116.1, 113.6, 64.1, 56.3, 53.4, 40.5, 37.6, 37.5, 32.7, 29.7, 28.0, 25.4, 22.9, 22.7, 22.4; HRMS (ESI): m/z calculated for $\text{C}_{39}\text{H}_{42}\text{BrClN}_4\text{O}_6\text{Na}$ $[\text{M}+\text{Na}]^+$ 799.1874, found 799.1873; HPLC purity: 98.1%.

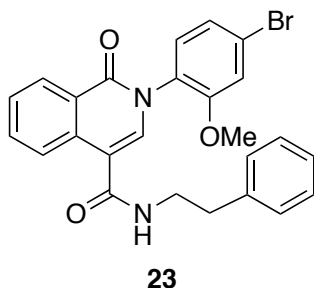
2-(4-Bromo-2-methoxyphenyl)-1-oxo-N-(2-oxo-2-(phenethylamino)ethyl)-1,2-dihydroisoquinoline-4-carboxamide (**22**):



Isoquinolone carboxylic acid **5ac** (0.059 g, 0.16 mmol, 1.0 mol-equiv) and amine **31** (0.029 g, 0.16 mmol, 1.0 mol-equiv) were reacted following the general procedure for the synthesis of amides outlined above. The crude material was purified by flash chromatography using EtOAc/Hex to give amide **22** (0.087 g, 0.16 mmol, >99%) as a white solid. $R_f = 0.3$ (6:4 EtOAc/Hex). $^1\text{H NMR}$ (500 MHz, $\text{DMSO}-d_6$) δ 8.58 (t, $J = 5.6$ Hz, 1H), 8.33 (d, $J = 8.8$ Hz, 1H), 8.27 (d, $J = 8.1$ Hz, 1H), 8.05 (t, $J = 5.5$ Hz, 1H), 7.81 (t, $J = 8.1$ Hz, 1H), 7.71 (s, 1H), 7.60 (t, $J = 7.5$ Hz, 1H), 7.48 (d, $J = 1.6$ Hz, 1H), 7.40 (d, $J = 8.2$ Hz, 1H), 7.35 (dd, $J = 8.2$ Hz, 1.8, 1H), 7.26 (t, $J = 7.6$ Hz, 2H), 7.22–7.16 (m,

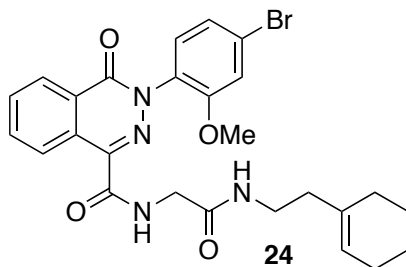
3H), 3.82–3.78 (m, 5H), 3.3 (q, $J = 7.0$ Hz, 2H), 2.72 (t, $J = 7.6$ Hz, 2H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 168.8, 165.8, 160.2, 155.4, 139.4, 135.3, 134.6, 133.0, 130.6, 128.7, 128.3, 127.5, 127.4, 126.1, 125.3, 125.1, 123.6, 122.8, 115.9, 112.0, 79.2, 56.5, 42.4, 40.3, 35.2 HRMS (ESI): m/z calculated for $\text{C}_{27}\text{H}_{24}\text{BrN}_3\text{O}_4\text{Na}$ $[\text{M}+\text{Na}]^+$ 556.0848, found 556.0844; HPLC purity: 98.0%.

2-(4-Bromo-2-methoxyphenyl)-1-oxo-N-phenethyl-1,2-dihydroisoquinoline-4-carboxamide (**23**):



Isoquinolone carboxylic acid **5ac** (0.058 g, 0.16 mmol, 1.0 mol-equiv) and phenethylamine (0.019 g, 0.16 mmol, 1.0 mol-equiv) were reacted following the general procedure for the synthesis of amides outlined above. The crude material was purified by flash chromatography using EtOAc/Hex to give amide **23** (0.067 g, 0.14 mmol, 92%) as a white solid. $R_f = 0.8$ (6:4 EtOAc/Hex). ^1H NMR (500 MHz, CDCl_3) δ 8.42 (d, $J = 8.1$, 1H), 8.0 (d, $J = 8.2$, 1H), 7.7 (t, $J = 7.3$, 1H), 7.52 (t, $J = 7.8$, 1H), 7.30–7.25 (m, 3H), 7.25–7.15 (m, 5H), 7.11 (d, $J = 8.3$, 1H), 5.94 (t, $J = 5.5$, 1H), 3.76–3.70 (m, 5H), 2.95 (t, 6.9, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 166.3, 161.4, 155.2, 138.7, 134.1, 133.9, 133.2, 130.0, 128.9, 128.8, 128.6, 128.3, 127.7, 126.7, 126.1, 124.7, 124.1, 123.8, 116.0, 114.4, 56.2, 41.0, 35.5; HRMS (ESI): m/z calculated for $\text{C}_{25}\text{H}_{21}\text{BrN}_2\text{O}_3\text{Na}$ $[\text{M}+\text{Na}]^+$ 499.0633, found 499.0637; HPLC purity: 98.9%.

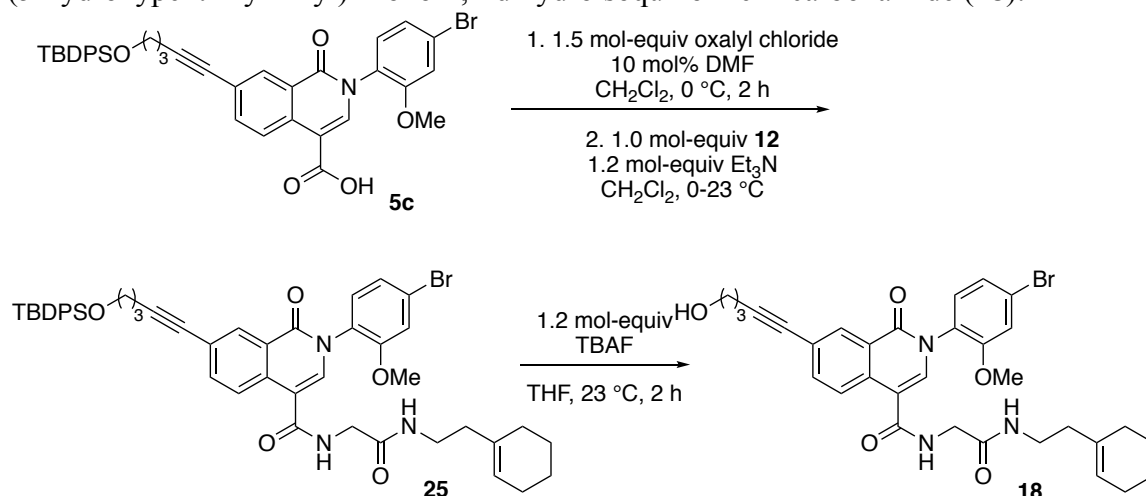
3-(4-Bromo-2-methoxyphenyl)-*N*-(2-((2-cyclohex-1-en-1-yl)ethyl)amino)-2-oxoethyl)-4-oxo-3,4-dihydrophthalazine-1-carboxamide (**24**):



Phthalazinone carboxylic acid **8** (0.021 g, 0.055 mmol, 1.0 mol-equiv) and amine **12** (0.0099 g, 0.055 mmol, 1.0 mol-equiv) were reacted following the general procedure for the synthesis of amides outlined above. The crude material was purified by flash chromatography using $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$ to give amide **24** (0.020 g, 0.047 mmol, 67%) as a white solid. $R_f = 0.5$ (4:1 EtOAc/Hex). ^1H NMR (500 MHz, CDCl_3) δ 9.13 (d, $J = 8.2$ Hz, 1H), 8.49 (d, $J = 7.8$, 1H), 7.89 (t, $J = 8.1$ Hz, 1H), 7.83 (t, $J = 7.8$ Hz, 1H), 7.74 (t, $J = 7.0$ Hz, 1H), 7.26 – 7.21 (m, 3H), 6.00 (br s, 1H), 5.42 (br s, 1H), 4.05 (d, $J = 5.7$ Hz, 2H), 3.81 (s, 3H), 3.34 (q, $J = 5.9$ Hz, 2H), 2.12 (t, $J = 6.7$ Hz, 2H), 1.88 – 1.87 (m, 4H),

1.57 – 1.56 (m, 2H), 1.48 – 1.47 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 168.1, 163.8, 159.1, 155.4, 136.5, 134.4, 134.0, 132.2, 129.7, 129.4, 128.3, 128.0, 127.6, 127.4, 124.12, 124.10; 124.0, 116.0, 56.3, 43.4, 37.5, 37.2, 27.8, 25.2, 22.8, 22.3; HRMS (ESI): m/z calculated for $\text{C}_{26}\text{H}_{27}\text{BrN}_4\text{O}_4\text{Na}$ $[\text{M}+\text{Na}]^+$ 561.1113, found 561.1127; HPLC purity: 97.8%.

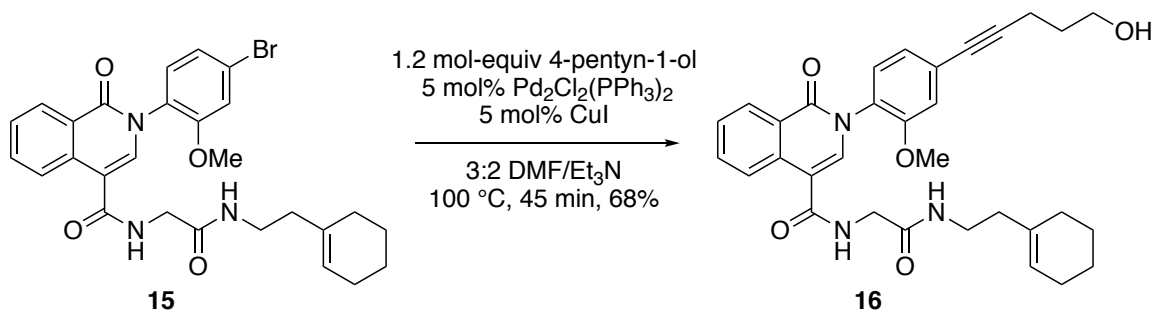
2-(4-Bromo-2-methoxyphenyl)-*N*-(2-((2-(cyclohex-1-en-1-yl)ethyl)amino)-2oxoethyl)-7-(5-hydroxypent-1-yn-1-yl)-1-oxo-1,2-dihydroisoquinoline-4-carboxamide (**18**):



Isoquinolone carboxylic acid **5c** (0.045 g, 0.053 mmol, 1.0 mol-equiv) and amine **12** (0.0095 g, 0.053 mmol, 1.0 mol-equiv) were reacted following the general procedure for the synthesis of amides outlined above. The crude material **25** was used in subsequent TBDPS deprotection without further purification.

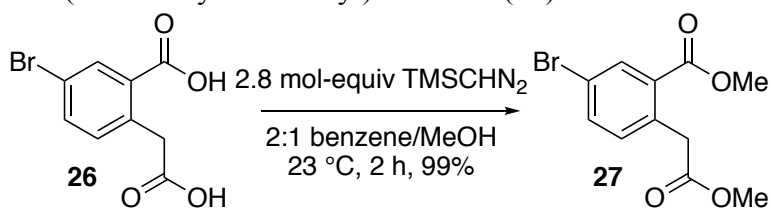
A flame-dried round bottom flask equipped with stir bar was charged with TBDPS-protected alcohol **25** (0.045 g, 0.053 mmol, 1.0 mol-equiv) and THF (0.2 mL, 0.3 M with respect to **25**). Next, TBAF (0.06 mL, 0.06 mmol, 1.2 mol-equiv) was added and the reaction mixture was stirred at 23 °C for 2 h. The resulting solution was clear. Upon consumption of **25**, the reaction mixture was concentrated *in vacuo* to yield a yellow oil. The crude material was purified by flash chromatography (Hex/EtOAc) to afford alcohol **18** (0.018 g, 0.03 mmol, 56%) as a white solid. R_f = 0.7 (7:3 EtOAc/Hex). ^1H NMR (600 MHz, CDCl_3) δ 8.44 (d, J = 1.8 Hz, 1H), 8.14 (d, J = 9.0 Hz, 1H), 7.67 (dd, J = 8.4, 1.8 Hz, 1H), 7.44 (s, 1H), 7.19-7.14 (m, 3H), 6.91-6.90 (m, 1H), 6.01-5.99 (m, 1H), 5.46 (s, 1H), 4.06 (d, J = 4.8 Hz, 2H), 3.82 (t, J = 6.0 Hz, 2H), 3.78 (s, 3H), 3.36-3.33 (m, 2H), 2.57 (t, J = 7.2 Hz, 2H), 2.18 (s, 1H), 2.14 (t, J = 6.6 Hz, 2H), 1.97–1.96 (m, 2H), 1.90–1.86 (m, 4H), 1.62–1.60 (m, 2H), 1.55–1.52 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 168.3, 166.3, 160.8, 155.1, 135.9, 134.9, 134.3, 133.1, 131.6, 129.9, 128.0, 125.9, 124.9, 124.2, 124.0, 123.9, 123.5, 116.1, 112.9, 91.6, 80.4, 61.6, 56.3, 53.8, 43.4, 37.5, 31.8, 31.3, 29.8, 29.3, 27.9, 25.2, 22.8, 22.3, 16.1; HRMS (ESI): m/z calculated for $\text{C}_{32}\text{H}_{34}\text{BrN}_3\text{O}_5\text{Na}$ $[\text{M}+\text{Na}]^+$ 642.1580, found 642.1574; HPLC purity: 98.4%.

N-2-((2-(Cyclohex-1-en-1-yl)ethyl)amino)-2-oxoethyl)-2-(4-(5-hydroxypent-1-yn-1-yl)-2-methoxyphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxamide (**16**):



Alkynylated derivative **16** was synthesized by adapting a procedure developed by Reddy *et al.*¹³ A flame-dried round bottom flask was charged with aryl bromide **15** (0.051 g, 0.095 mmol, 1.0 mol-equiv). Next, 3:2 DMF/Et₃N (1 mL) and 4-pentyn-1-ol (0.010 mL, 0.11 mmol, 1.2 mol-equiv) were added to the reaction flask via syringe and the reaction mixture was stirred for 3 min. The flask was briefly unsealed. CuI (0.90 mg, 0.0046 mmol, 5 mol%) and Pd₂Cl₂(PPh₃)₂ (3.3 mg, 0.0046 mmol, 5 mol%) were added. The flask was fitted with a reflux condenser and heated at reflux until aryl bromide **15** appeared consumed by TLC (45 min). The reaction mixture was cooled to room temperature and diluted with CH₂Cl₂ (10 mL). The organic layer was washed with water (5 x 10 mL) followed by brine, dried over Na₂SO₄, and concentrated *in vacuo* to give a red solid. The crude material was purified by flash chromatography (EtOAc/Hex) to yield alkynylated derivative **16** (0.035 g, 0.065 mmol, 68%) as a white solid. *R_f* = 0.2 (4:1 EtOAc/Hex). ¹H NMR (500 MHz, CDCl₃) δ 8.46 (d, *J* = 8.3 Hz, 1H), 8.22 (d, *J* = 8.2 Hz, 1H), 7.73 (t, *J* = 7.8 Hz, 1H), 7.54 (t, *J* = 8.1 Hz, 1H), 7.49 (s, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 7.06–7.04 (m, 2H), 6.93 (t, *J* = 5.0 Hz, 1H), 6.12 (t, *J* = 5.1 Hz, 1H), 5.45 (s, 1H), 4.07 (d, *J* = 5.4 Hz, 2H), 3.82 (t, *J* = 6.6 Hz, 2H), 3.77 (s, 3H), 3.35 (q, *J* = 5.9 Hz, 2H), 2.56 (t, *J* = 7.2, 2H), 2.14 (t, *J* = 6.9 Hz, 2H), 1.98–1.70 (m, 7H), 1.63–1.49 (m, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 168.4, 166.7, 161.6, 154.3, 135.0, 134.4, 134.2, 133.2, 128.8, 128.73, 128.67, 127.8, 126.24, 126.18, 124.9, 124.4, 124.0, 115.4, 113.1, 91.0, 80.6, 61.8, 56.1, 43.6, 37.55, 37.53, 31.40, 28.0, 25.3, 22.9, 22.4, 16.1; HRMS (ESI): *m/z* calculated for C₃₂H₃₅N₃O₅Na [M+Na]⁺ 564.2474, found 564.2480; HPLC purity: 96.3%.

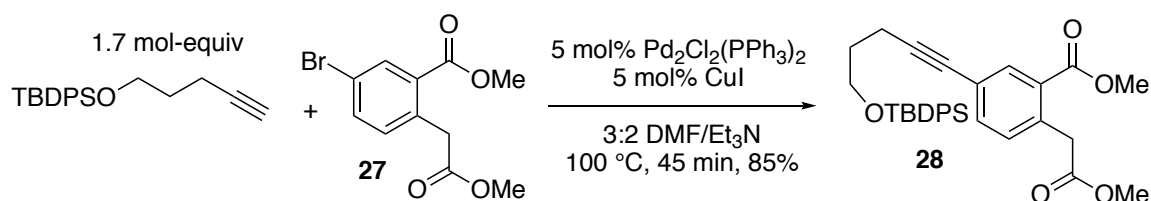
Methyl-5-bromo-2-(2-methoxy-2-oxoethyl)benzoate (**27**):



Bis(methylester) **27** was synthesized by adapting a procedure developed by Ochoa-Puentes *et al.*¹⁴ An oven-dried round bottom flask equipped with stir bar was charged with dicarboxylic acid **26** (0.99 g, 3.9 mmol, 1.0 mol-equiv). Benzene (20 mL) and MeOH (10 mL) were added to the reaction flask and stirred until a homogeneous solution was obtained. TMSCHN₂ (2.0 M in Et₂O, 5.5 mL, 11 mmol, 2.8 mol-equiv) was added dropwise to the reaction flask via syringe over 2 h. The mixture was monitored for

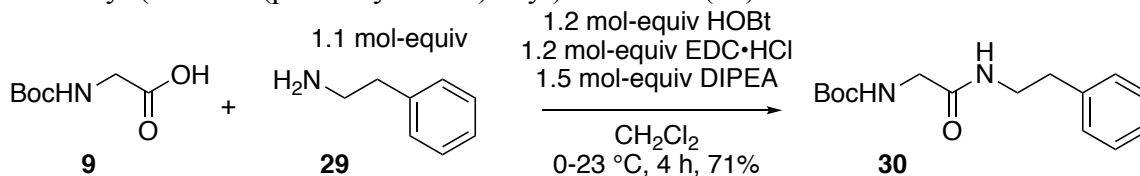
consumption of **26** by TLC. The reaction mixture was concentrated *in vacuo* to afford a pale yellow oil. The crude material was purified by flash chromatography (EtOAc/Hex) to yield bis(methylester) **27** (1.1 g, 3.8 mmol, 99%) as a clear oil. $R_f = 0.9$ (9:1 CH₂Cl₂/MeOH). ¹H NMR (500 MHz, CDCl₃) δ 8.15 (s, 1H), 7.61 (d, $J = 8.2$ Hz, 1H), 7.14 (d, $J = 8.2$ Hz, 1H), 3.97 (s, 2H), 3.88 (s, 3H), 3.70 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 171.6, 166.3, 135.4, 135.1, 134.1, 134.0, 131.4, 121.3, 52.5, 52.2, 40.0; HRMS (ESI): m/z calculated for C₁₁H₁₁O₄Na [M+Na]⁺ 308.9738, found 308.9739.

Methyl-5-(5-((tert-butyldimethylsilyl)oxy)pent-1-yn-1-yl)-2-(2-methoxy-2-oxoethyl)benzoate (**28**):



Alkynylated derivative **28** was synthesized by adapting a procedure developed by Reddy *et. al.*¹³ A flame-dried round bottom flask equipped with stir bar was charged with bis(methylester) **27** (0.75 g, 2.6 mmol, 1.0 mol-equiv). DMF (18 mL), Et₃N (12 mL), and tert-butylpent-4-ynyloxydiphenylsilane (1.4 g, 4.3 mmol, 1.7 mol-equiv) were added to the reaction flask via syringe. The reaction mixture was stirred for 3 min. The flask was briefly unsealed. CuI (0.025 g, 0.13 mmol, 5 mol%) and Pd₂Cl₂(PPh₃)₂ (0.092 g, 0.13 mmol, 5 mol%) were added. The flask was fitted with a reflux condenser and heated at reflux for 45 min. The reaction mixture was monitored by TLC for consumption of **27**. The reaction mixture was cooled to room temperature and diluted with CH₂Cl₂ (50 mL). The organic layer was washed with water (5 x 50 mL) followed by brine, dried over Na₂SO₄, and concentrated *in vacuo* to afford a red oil. The crude material was purified by flash chromatography (EtOAc/Hex) to yield alkyne **28** (1.2 g, 2.2 mmol, 85%) as a red oil. $R_f = 0.5$ (1:4 EtOAc/Hex). ¹H NMR (500 MHz, CDCl₃) δ 8.02 (d, $J = 1.7$ Hz, 1H), 8.03–8.00 (m, 4H), 7.45–7.35 (m, 7H), 7.2 (d, $J = 8.1$ Hz, 1H), 3.99 (s, 2H), 3.87 (s, 3H), 3.81 (t, $J = 6.1$ Hz, 2H), 3.70 (s, 3H), 2.57 (t, $J = 7.2$ Hz, 2H), 1.85 (quin, $J = 7.2$ Hz, 2H), 1.06 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 171.9, 167.1, 135.7, 135.24, 135.22, 134.3, 133.9, 132.4, 129.74, 129.71, 127.8, 123.7, 91.3, 79.7, 62.5, 52.3, 52.2, 40.4, 31.6, 27.0, 19.4, 16.1; HRMS (ESI): m/z calculated for C₃₂H₃₆O₅SiNa [M+Na]⁺ 551.2230, found 551.2228.

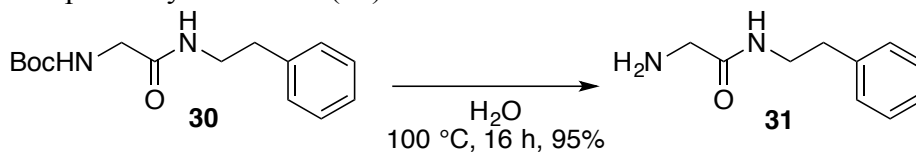
tert-Butyl (2-oxo-2-(phenethylamino)ethyl)carbamate (**30**):



Amide **30** was synthesized by adapting a procedure developed by Liu *et. al.*¹⁵ An oven-dried round bottom flask equipped with stir bar was charged with Boc-glycine **9** (0.64 g, 3.7 mmol, 1.0 mol-equiv) and HOBt (0.59 g, 4.3 mmol, 1.2 mol-equiv). CH₂Cl₂

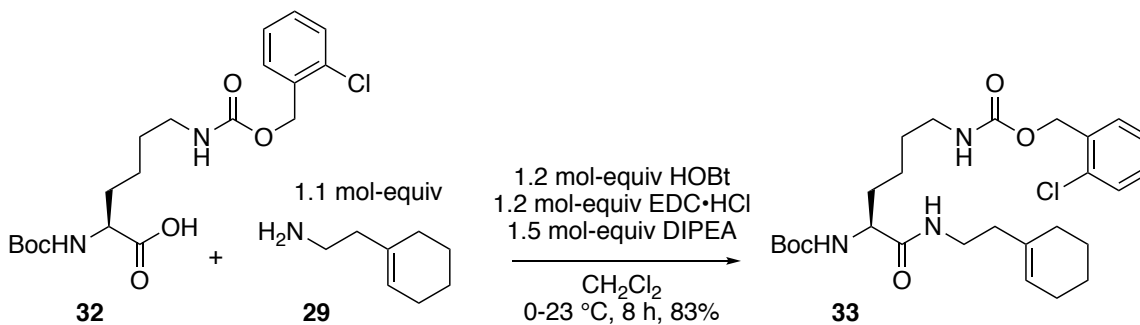
(90 mL) was added to the reaction vessel followed by phenethylamine **29** (0.50 mL, 3.97 mmol, 1.1 mol-equiv). The reaction vessel was cooled to 0 °C with an ice-water bath. The flask was briefly unsealed and EDC•HCl (0.83 g, 4.33 mmol, 1.2 mol-equiv) was added. DIPEA (0.95 mL, 5.6 mmol, 1.5 mol-equiv) was then added to the reaction vessel via syringe and the mixture was stirred for 1 h. The reaction vessel was warmed to room temperature and stirred until Boc-amine **9** appeared consumed by TLC (4 h). The reaction mixture was washed with saturated NaHCO₃ and extracted with CH₂Cl₂ (3 x 100 mL). The combined organic layers were washed with brine, dried with Na₂SO₄, and concentrated *in vacuo* to afford a yellow oil. The crude material was purified by flash chromatography (EtOAc/Hex) to yield amide **30** (0.72 g, 2.6 mmol, 71%) as a yellow oil. *R_f* = 0.57 (17:3 CH₂Cl₂/MeOH). ¹H NMR (500 MHz, CDCl₃) δ 7.31 (t, *J* = 7.3 Hz, 2H), 7.25–7.18 (m, 3H), 6.12 (s, 1H), 5.09 (s, 1H), 3.74 (d, *J* = 5.7 Hz, 2H), 3.54 (q, *J* = 6.6 Hz, 2H), 2.82 (t, *J* = 7.1 Hz, 2H), 1.43 (s, 9H); HRMS (ESI): *m/z* calculated for C₁₅H₂₂N₂O₃Na [M+Na]⁺ 301.1528, found 301.1531.

2-Amino-*N*-phenethylacetamide (**31**):



Amine **31** was synthesized by adapting a procedure developed by Zinelaabidine.¹⁰ A round bottom flask equipped with stir bar was charged with Boc-protected amine **30** (0.25 g, 0.89 mmol, 1 mol-equiv). Next, water (20 mL) was added and the flask was fitted with a condenser. The reaction mixture was heated at reflux and monitored by TLC for disappearance of **30** (16 h). The reaction mixture was cooled to room temperature and extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layers were concentrated *in vacuo* to obtain amine **31** (0.91 g, 0.85 mmol, 95%) as a yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 7.34–7.18 (m, 6H), 3.55 (q, *J* = 6.7 Hz, 2H), 3.32 (s, 2H), 2.84 (t, *J* = 7.1 Hz, 2H), 1.40 (s, 2H); HRMS (ESI): *m/z* calculated for C₁₀H₁₄N₂ONa [M+Na]⁺ 201.1004, found 201.1002.

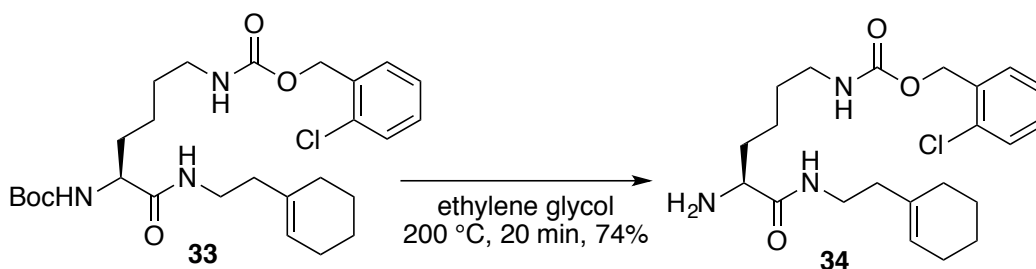
tert-Butyl-(2-chlorobenzyl)-(6-((2-(cyclohex-1-en-1-yl)ethyl)amino)-6-oxohexane-1,5-diyl)(*S*)-dicarbamate (**33**):



Amide **33** was synthesized by adapting a procedure developed by Liu *et al.*¹⁵ An oven-dried round bottom flask equipped with stir bar was charged with Boc-amino acid

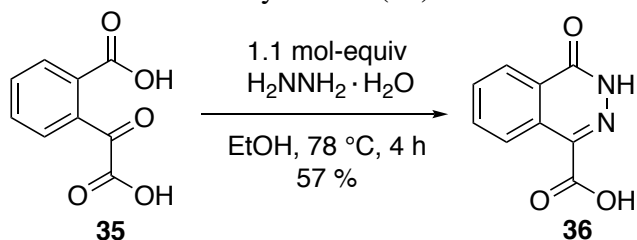
derivative **32** (1.5 g, 3.6 mmol, 1.0 mol-equiv) and HOBt (0.59 g, 4.3 mmol, 1.2 mol-equiv). CH₂Cl₂ (90 mL) was added to the reaction vessel followed by amine **29** (0.55 mL, 3.98 mmol, 1.1 mol-equiv). The reaction vessel was cooled to 0 °C with an ice-water bath. The flask was briefly unsealed and EDC•HCl (0.83 g, 4.3 mmol, 1.2 mol-equiv) was added. DIPEA (0.95 mL, 5.6 mmol, 1.5 mol-equiv) was then added to the reaction vessel via syringe and the mixture was stirred for 1 h. The reaction vessel was warmed to room temperature and stirred until Boc-amine **32** appeared consumed by TLC (8 h). The reaction mixture was washed with saturated NaHCO₃ and extracted with CH₂Cl₂ (3 x 100 mL). The combined organic layers were washed with brine, dried with Na₂SO₄, and concentrated *in vacuo* to afford a yellow oil. The crude material was purified by flash chromatography (EtOAc/Hex) to yield amide **33** (1.6 g, 3.0 mmol, 83%) as a white solid. R_f = 0.2 (9:1 CH₂Cl₂/MeOH). ¹H NMR (500 MHz, CDCl₃) δ 7.41 – 7.40 (m, 1H), 7.37 – 7.35 (m, 1H), 7.26 – 7.24 (m, 2H), 6.10 (br s, 1H), 5.44 (s, 1H), 5.22 – 5.20 (m, 2H), 5.13 (br s, 1H), 4.97 (br s, 1H), 4.01 (br s, 1H), 3.36 – 3.25 (m, 2H), 3.19 (q, *J* = 6.4 Hz, 2H), 2.11 (t, *J* = 6.7 Hz, 2H), 1.98 (br s, 2H), 1.90 (br s, 2H), 1.83 (br s, 1H), 1.61 – 1.52 (m, 7H), 1.42 (s, 9H), 1.38 – 1.35 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 171.9, 156.4, 155.9, 134.6, 134.4, 133.6, 129.8, 129.6, 129.4, 127.0, 123.8, 80.1, 64.0, 54.5, 40.6, 37.7, 37.3, 32.3, 29.6, 28.4, 28.0, 25.3, 22.9, 22.6, 22.4; HRMS (ESI): *m/z* calculated for C₂₇H₄₀ClN₃O₅Na [M+Na]⁺ 544.2554, found 544.2558.

2-Chlorobenzyl (*S*)-(5-amino-6-((2-(cyclohex-1-en-1-yl)ethyl)amino)-6-oxohexyl)carbamate (**34**):



Amine **34** was synthesized by adapting a procedure developed by Martin *et al.*¹⁶ An oven-dried round bottom flask equipped with stir bar was charged with the Boc-protected amine **33** (0.66 g, 1.3 mmol, 1 mol-equiv). Ethylene glycol (28 mL) was added and the flask was fitted with a condenser. The reaction mixture was heated at reflux and monitored by TLC for disappearance of the Boc-protected amine **33** (20 min). The reaction mixture was cooled to room temperature and diluted with CH₂Cl₂ (50 mL). Next, the organic layer was washed with water (5 x 100 mL) followed by brine, dried over Na₂SO₄, and concentrated *in vacuo* to give amine **34** (0.39 g, 0.96 mmol, 74%) as a yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 7.43–7.35 (m, 2H), 7.26–2.20 (m, 3H), 5.45 (s, 1H), 5.25–5.17 (m, 3H), 4.92 (br s, 1H), 3.34–3.29 (m, 2H), 3.21 (q, *J* = 6.5 Hz, 2H), 2.13 (t, *J* = 6.8 Hz, 2H), 2.02–1.90 (m, 4H), 1.85–1.78 (m, 1H), 1.64–1.39 (m, 11H); ¹³C NMR (125 MHz, CDCl₃) δ 174.8, 156.4, 134.8, 134.5, 133.6, 129.8, 129.6, 129.4, 127.0, 123.6, 64.0, 55.2, 40.9, 37.9, 37.0, 34.8, 29.9, 28.0, 25.4, 23.0, 22.9, 22.5; HRMS (ESI): *m/z* calculated for C₂₂H₃₂ClN₃O₃H [M+H]⁺ 422.2210, found 422.2216.

4-Oxo-3,4-dihydrophthalazine-1-carboxylic acid (**35**):



Phthalazinone carboxylic acid **36** was synthesized by adapting a procedure developed by Geneste *et. al.*¹⁷ An oven-dried round bottom flask equipped with stir bar was charged with dicarboxylic acid **35** (0.30 g, 1.4 mmol, 1.0 mol-equiv), EtOH (6.4 mL), and hydrazine hydrate (0.073 mL, 1.6 mmol, 1.1 mol-equiv). The flask was fitted with a condenser. The mixture was heated at reflux and monitored by TLC for disappearance of **35** (4 h). The mixture was cooled to room temperature and the resulting gray solid was collected via filtration using a Büchner funnel. The solid was washed with cold EtOH (3 x 3 mL) to obtain **36** (0.16 g, 0.82 mmol, 57%). $R_f = 0.2$ (1:4 MeOH/CH₂Cl₂). ¹H NMR (500 MHz, DMSO-*d*₆) δ 12.93 (br s, 1H), 8.49 (d, $J = 8.2$ Hz, 1H), 8.26 (d, $J = 7.8$ Hz, 1H), 7.94 (t, $J = 8.1$ Hz, 1H), 7.86 (t, $J = 7.8$ Hz, 1H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 165.3, 159.6, 138.8, 133.7, 131.7, 128.0, 127.5, 126.6, 125.8; HRMS (ESI): m/z calculated for C₉H₇N₂O₃ [M+H]⁺ 191.0457, found 191.0462.

3. Biological evaluations:

Strains, media, and compounds: The *C. albicans* strain HLY4123 was used to assay analogues for antifungal activity in the presence of azole drugs. HLY4123 carries a GFP reporter for ERG3 expression and was constructed by plasmid transformation of the commonly used *C. albicans* strain CAI4. Selected resistant fungal strains¹⁸ with different mechanisms of drug resistance were obtained from Dr. David Rogers (Department of Clinical Pharmacy, University of Tennessee Health Sciences Center, Memphis, Tennessee, USA). *C. neoformans var. grubii* H99¹⁹ and *C. glabrata* BG2²⁰ were also tested. The strains were cultured at 30 °C under constant shaking (200 rpm) in synthetic complete (SC) medium containing 2% glucose. The stock solution of fluconazole (Sigma–Aldrich, USA) was prepared in sterile H₂O. Stock solutions of isavuconazole and analogues **1** and **13-24** were prepared in DMSO.

Dose–response curves for analogues tested against HLY4123 C. albicans with and without fluconazole: *C. albicans* was grown in SC medium overnight and then diluted to an effective OD₆₀₀ of 0.0625. Serial ten-fold dilutions of analogues **1** and **13-24** (15 mM - 0.0015 mM) were prepared in DMSO in 1.5 mL Eppendorf tubes. To each well in columns B, C, and D (triplicate analysis) of a 24-well Falcon plate (4 columns x 6 rows) was added 2.5 μL of a 0.1 mg/mL fluconazole solution. No azole was added to column A, which was used to assay the anti-fungal activity of analogues **1** and **13-24** in the absence of azole. To every well of the plate was added 1 mL of cells in SC medium, to achieve a final azole concentration of 0.25 $\mu\text{g}/\text{mL}$ in columns B, C, and D.

Next, a given analogue was added at varying concentration to rows two through six. To every well of row 2 was added 2 μL of a given 15 mM analogue solution, to achieve a concentration of 30 μM in each well. Addition of 2 μL of analogue solutions was continued for rows 3, 4, 5, and 6, using 1.5 mM, 0.15 mM, and 0.015 mM, and

0.0015 mM DMSO solutions, respectively. Rows 3, 4, 5, and 6 therefore had a final analogue concentration of 3 μ M, 0.3 μ M, 0.03 μ M, and 0.003 μ M, respectively. After both azole and analogue were added to the cells, every well was mixed thoroughly by repeated pipetting.

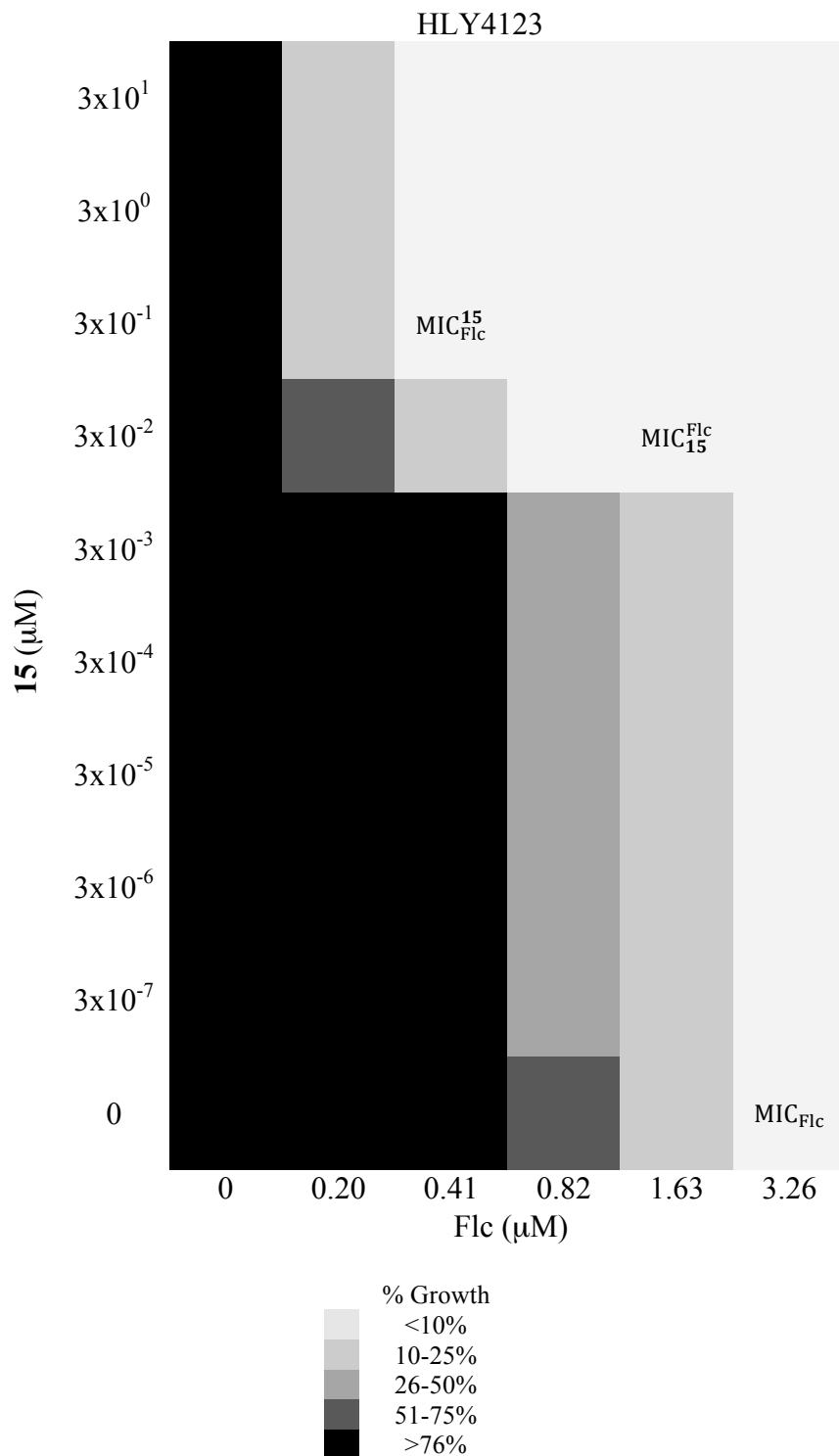
The plates were incubated in a rotary shaker/incubator at 30 °C for 16 h. After incubation, the contents of each well were re-suspended with a micropipette. A 50 μ L aliquot was added to a polystyrene cuvette and diluted with 950 μ L deionized water (20-fold dilution). The contents of the cuvettes were re-suspended immediately before measuring the absorbance at $\lambda = 600$ nm (OD_{600}) for cell densities. EC_{50} values were determined by fitting to a standard curve using the Excel-based tool ED50PLUS v. 1.0 (Mario H. Vargas).

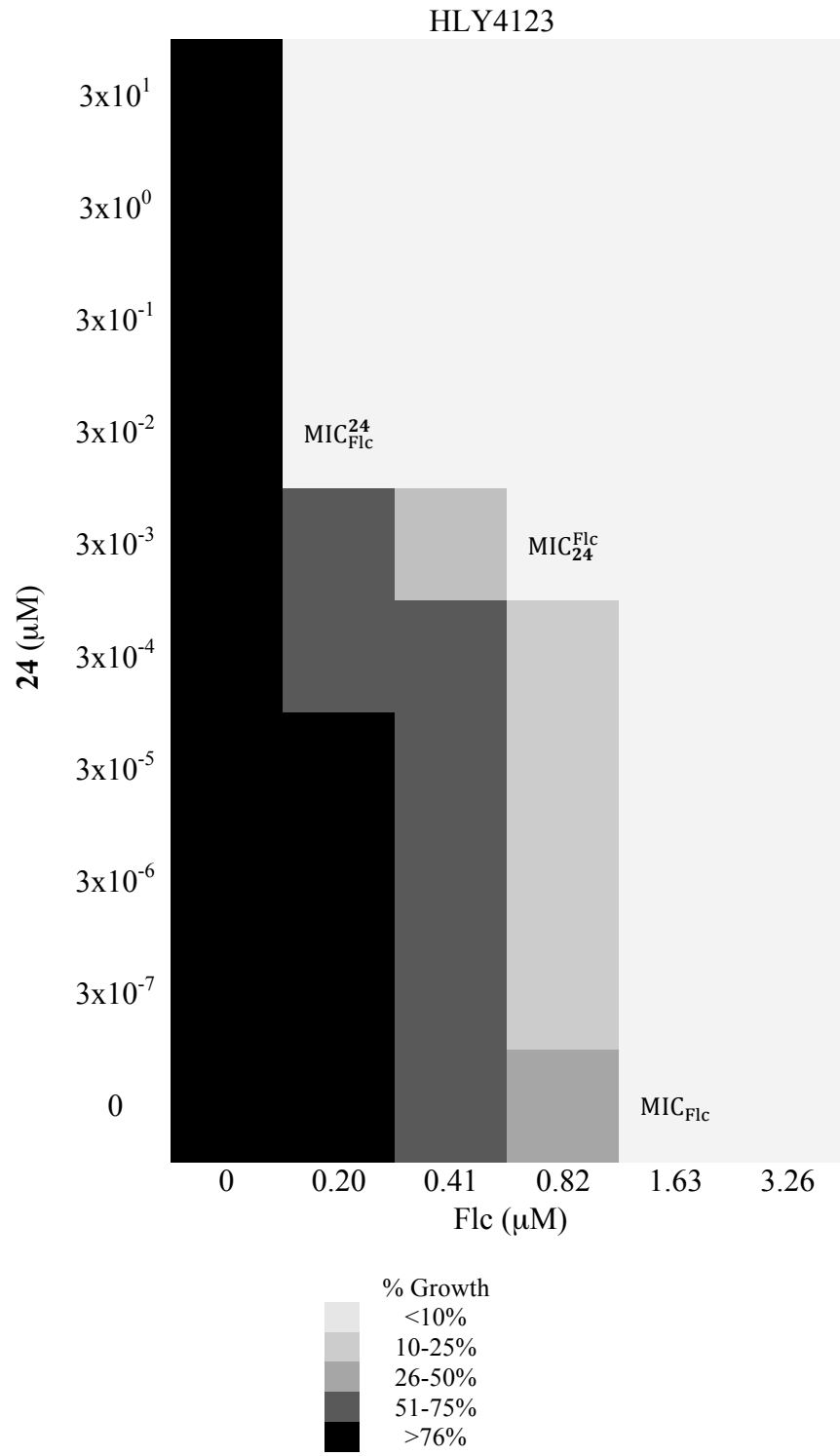
Determination of MIC_{90} values by checkerboard assay: Checkerboard assays were carried out using four 24-well plates (6 rows x 4 columns), to create an effective 96-well plate (12 rows x 8 columns). *C. albicans* was grown in SC medium overnight and then diluted to an effective OD_{600} of 0.0625. Serial tenfold dilutions of the analogues **15** or **24** were prepared in DMSO (15 mM – 0.015 nM) in 1.5 mL Eppendorf tubes. Serial twofold dilutions of fluconazole (0.5 mg/mL - 0.03125 mg/mL) were prepared in sterile H₂O in 1.5 mL Eppendorf tubes.

To each well was added 2 μ L of a given analogue DMSO solution and 2 μ L of the fluconazole solution. Concentrations of analogues in rows 1–6 varied from 30 μ M to 0.3 nM. Concentrations of analogues in rows 7–11 varied from 3 nM to 0.0003 nM. Rows 5 and 7 both had analogue concentrations of 3 nM and rows 6 and 8 both had analogue concentrations of 0.3 nM to normalize cell densities between plates. No analogue was added to the last row 12. Concentrations of fluconazole in columns A–D varied from 1 μ g/mL – 0.125 μ g/mL. Concentrations of fluconazole in columns E–G varied from 0.25 μ g/mL – 0.0625 μ g/mL. Columns D and E both had analogue concentrations of 0.25 μ g/mL to normalize cell densities between plates. No fluconazole was added to the final column H. After both azole and analogue were added to the cells, every well was mixed thoroughly by repeated pipetting.

The plates were incubated in a rotary shaker/incubator at 30 °C for 16 h. The contents of each well were re-suspended with a micropipette. A 50 μ L aliquot was added to a polystyrene cuvette and diluted with 950 μ L deionized water (20-fold dilution). The contents of the cuvettes were re-suspended immediately before measuring the absorbance at $\lambda = 600$ nm (OD_{600}). MIC_{90} values were determined as the lowest drug concentrations (alone or in combination) that inhibited fungal growth by 90% compared to wells containing no analogue.

Plots of checkerboard assays for compounds **15** and **24**: MIC_{Flc} is the MIC_{90} for fluconazole in the absence of **15** or **24**. MIC_{Flc}^{15} and MIC_{Flc}^{24} are the lowest MIC_{90} for fluconazole in the presence of **15** or **24**. MIC_{15}^{Flc} and MIC_{24}^{Flc} are the lowest MIC_{90} for **15** or **24** in the presence of fluconazole. The MIC_{90} for both **15** and **24** in the absence of fluconazole was above the solubility limit ($30\mu M$).





Determination of EC₅₀ values for analogues 15 and 24 tested against resistant C. albicans strains

Part I: Determination of fluconazole MIC₅₀ values for resistant C. albicans strains: Selected resistant *C. albicans* isolates (CaCi-17, 26, 33, 36, and 45) were obtained from Dr. David Rogers.¹⁸ MIC₅₀ values were re-measured in our own laboratory by incubating each isolate with fluconazole concentrations between 512 ug/mL to 8 ug/mL. After incubation, the MIC₅₀ was determined by absorption at $\lambda = 600$ nm (see above). MIC values agreed with those provided for Rogers except for strains CaCi-17 and CaCi-45 (12 μ g/mL vs. 32 μ g/mL and 96 μ g/mL vs. 128 μ g/mL).

**Measured Fluconazole MIC₅₀ Values for Resistant
C. albicans Clinical Isolates**

Isolate	Fluconazole MIC ₅₀ (μ g/mL)
17	12
26	32
33	64
36	64
45	96

Part II: Determination of EC₅₀ values for analogues 15 and 24:

The aforementioned isolates were grown in SC medium overnight and then diluted to an effective OD₆₀₀ of 0.0625. Serial ten-fold dilutions of analogues **15** and **24** (15 mM-0.0015 mM) were prepared in DMSO in 1.5 mL Eppendorf tubes. To each well in columns B, C, and D (triplicate analysis) of a 24-well Falcon plate (4 columns x 6 rows) was added 6.0 μ L for CaCi-17, 16.0 μ L for CaCi-26, 32.0 μ L for CaCi-33, 32.0 μ L for CaCi-36, and 48.0 μ L for CaCi-45 of a 2.0 mg/mL fluconazole solution. No azole was added to column A, which was used to assay the anti-fungal activity of analogues **15** and **24** in the absence of azole. To every well of the plate was added 1 mL of cells in SC medium, to achieve a final azole concentration of 12 μ g/mL for CaCi-17, 32 μ g/mL for CaCi-26, 64 μ g/mL for CaCi-33, 64 μ g/mL for CaCi-36, 96 μ g/mL for CaCi-45 in columns B, C, and D.

Next, a given analogue was added at varying concentration to rows two through six. To every well of row 2 was added 2 μ L of a given 15 mM analogue solution, to achieve a concentration of 30 μ M in each well. Addition of 2 μ L of analogue solutions was continued for rows 3, 4, 5, and 6, using 1.5 mM, 0.15 mM, and 0.015 mM, and 0.0015 mM DMSO solutions, respectively. Rows 3, 4, 5, and 6 therefore had a final analogue concentration of 3 μ M, 0.3 μ M, 0.03 μ M, and 0.003 μ M, respectively. After both azole and analogue were added to the cells, every well was mixed thoroughly by repeated pipetting.

The plates were incubated in a rotary shaker/incubator at 30 °C for 16 h. After incubation, the contents of each well were re-suspended with a micropipette. A 50 μ L aliquot was added to a polystyrene cuvette and diluted with 950 μ L deionized water (20-fold dilution). The contents of the cuvettes were re-suspended immediately before

measuring the absorbance at $\lambda = 600$ nm (OD_{600}) for cell densities. EC_{50} values were determined by fitting to a standard curve using the Excel-based tool ED50PLUS v. 1.0 (Mario H. Vargas).

**Antifungal Activities of Analogues 15 and 24
against Resistant Isolates of *C. albicans* in the
Presence of Fluconazole**

Isolate	[Flu] ^b	EC_{50} ^a	
		15	24
17	12	74 ± 10	39 ± 3
26	32	11 ± 1	17 ± 2
33	64	371 ± 23	72 ± 6
36	64	32 ± 4	20 ± 2
45	96	170 ± 20	65 ± 15

^a EC_{50} (nM) expressed as arithmetic mean ± S.D. of three independent experiments. ^bMeasured MIC_{50} values for fluconazole alone; EC_{50} values were determined at these concentrations of fluconazole.

*Determination of EC_{50} values for analogues 15 and 24 tested against *C. glabrata* and *C. neoformans**

C. glabrata BG2 and *C. neoformans* var. *grubii* H99 were assayed in the same manner as described above for resistant *C. albicans* isolates, except the final concentrations of fluconazole used was 4.0 μ g/mL for *C. neoformans* and 128 μ g/mL for *C. glabrata*, corresponding to MIC_{50} values measured in our laboratory for these species.

4. Calculated Physicochemical Properties of Analogues 15 and 24^a

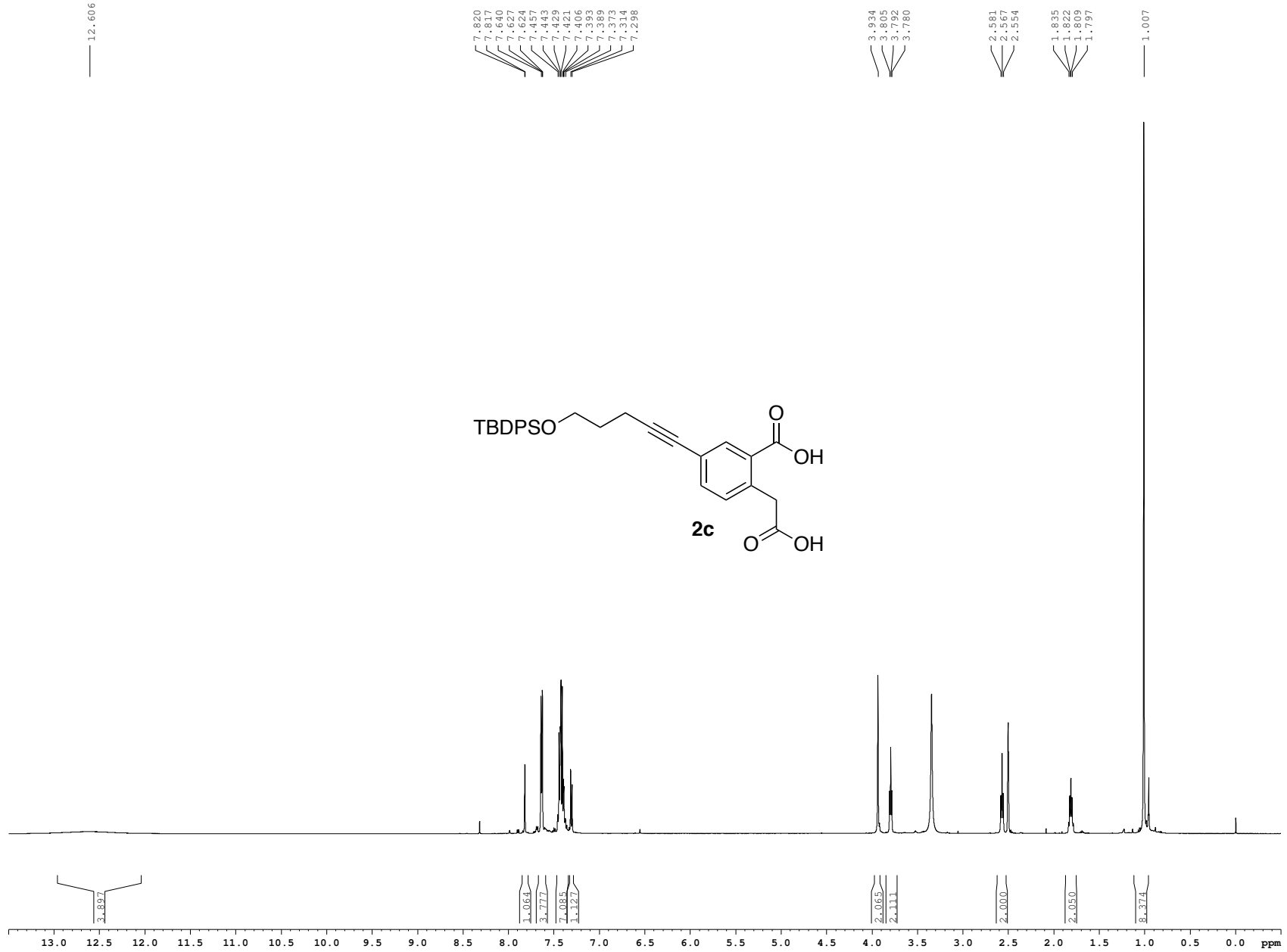
Ligand	Desirable	15	24
MW	≤460	538.4	539.4
logP	-4 to 4.2	4.13	4.2
logD	0 to 3	3.69	3.67
tPSA	≤120	89.4	102.3
Fraction sp ³	0.15-0.80	0.30	0.31
Rotatable bonds	≤10	8	8
HBD	≤5	2	2
HBA	≤9	7	8
Oral Bioavail.		Good	Good
Phospholipidosis		NonInducer	NonInducer

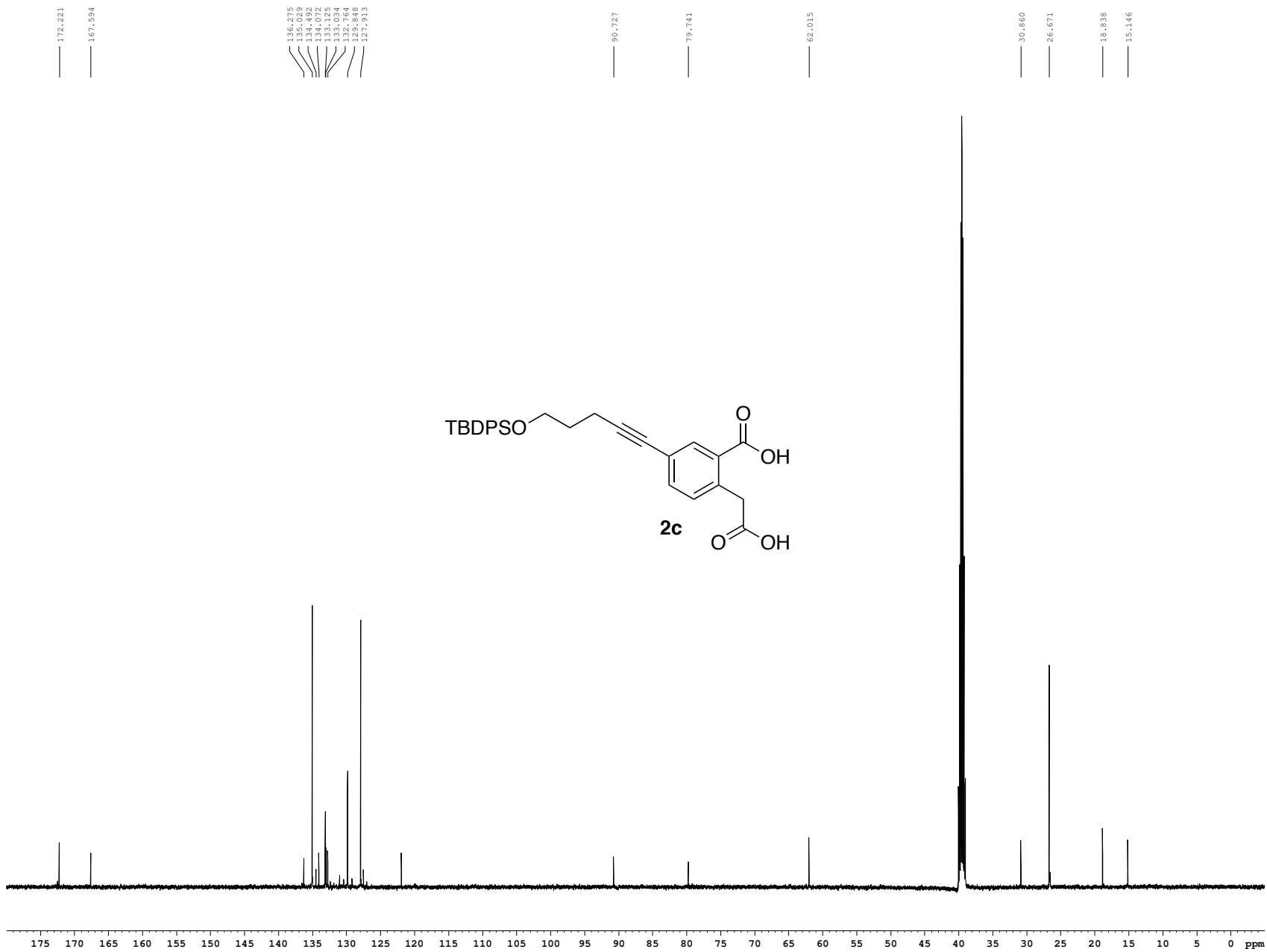
^aCalculated with FAF-Drugs3

5. References:

- ¹ Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. Safe and Convenient Procedure for Solvent Purification. *Organometallics*. 1996, *15*, 1518–1520.
- ² Selwood, D. “Benzamide derivatives useful in the treatment of muscular disorders and pain and for controlling spasticity and tremors.” WO Patent 201 582 938, 2015.
- ³ Ozer, M. S.; Koza, G.; Sahin, E.; Balci, M. Furo- and thieno-fused 1,3-diazepine-4,6-diones. *Tetrahedron Lett.* **2013**, *54*, 6553–6556.
- ⁴ Koza, G.; Balci, M. A novel class of compounds: synthesis of 5,5'-carbonyl-bis(5,6-dihydro-4H-furo- and thieno-[2,3-*c*]pyrrol-4-ones). *Tetrahedron* **2011**, *67*, 8679-8685.
- ⁵ Wolfbeis, O. Synthesen von Fluoreszenzfarbstoffen, 11 über die Umlagerung von Alkoxymethylen- und Aminomethylenhomophthalsäureanhydriden zu Isocumarinen bzw. Isochinolinonen. *Liebigs Ann. Chem.* **1981**, *5*, 819–827.
- ⁶ Patil, C. D.; Sadana, A.; Deodhar, S. Syntheses of 2-substituted-1,2-dihydro-1-oxo-4-isoquinoline-carboxamides and 4-amidoximino-2-substituted-1,2-dihydro-1-oxoisoquinolines as antimicrobial agents. *J. Indian Chem. Soc.* **1990**, *67*, 654–656.
- ⁷ Brittain, D.; Wood, R. Phthalazin-4-ylacetic acid derivatives. U.S. Patent 4 251 528, 1981.
- ⁸ Su, W.; Dai, G.; Xiao, K.; Jia, H.; Venable, J.; Bembenek, S. Novel Heteroaryl and Heterocycle Compounds, Compositions, and Methods. WO Patent 201 415 675, 2014
- ⁹ Awuah, E.; Ma, E.; Hoegl, A. Vong, K.; Habib, E.; Auclair, K. Exploring structural motifs necessary for substrate binding in the active site of *Escherichia coli* pantothenate kinase. *Bioorg. Med. Chem.* 2014, *22*, 3083–3090.
- ¹⁰ Zinelaabidine, C.; Souad, O.; Zoubir, J.; Malika, B.; Nour-Eddine, A. A Simple and Efficient Green Method for the Deprotection of N-Boc in Various Structurally Diverse Amines under Water-mediated Catalyst-free Conditions. *Int. J. Chem.* **2012**, *4*, 73–79.
- ¹¹ Berghausen, J.; Buschmann, N.; Furet, P.; Gessier, F.; Hergovich Lisztwan, J.; Holzer, P.; Jacoby, E.; Kallen, J.; Masuya, K.; Pissot Soldermann, C.; Ren, H.; Stutz, S. Isoquinolinone and quinazolinone derivatives as MDM2 and MDM4 inhibitors and their preparation and use for the treatment of diseases. PCT Int. Appl. 2 011 076 786, 2011.
- ¹² Liu, Z.; Wang, R.; Guo, R.; Hu, J.; Li, R.; Zhao, Y.; Gong, P. Design, synthesis and biological evaluation of novel 6,7-disubstituted-4-phenoxyquinoline derivatives bearing 4-oxo-3,4-dihydrophthalazine-1-carboxamide moieties as c-Met kinase inhibitors. *Bioorg. Med. Chem.* **2014**, *22*, 3642–3653.
- ¹³ Reddy, T.; Reddy, G.; Reddy, L.; Jammula, S.; Lingappa, Y.; Kapavarapu, R.; Meda, C.; Parsa, K.; Pal, M. Montmorillonite K-10 mediated green synthesis of cyano pyridines: Their evaluation as potential inhibitors of PDE4. *Eur. J. Med. Chem.* **2012**, *48*, 265–274.
- ¹⁴ Ochoa-Puentes, C.; Bauer, S.; Kuhnle, M.; Bernhardt, G.; Buschauer, A.; König, B. Benzanilide–Biphenyl Replacement: A Bioisosteric Approach to Quinoline Carboxamide-Type ABCG2 Modulators. *ACS Med. Chem. Lett.* **2013**, *4*, 393–396.
- ¹⁵ Liu, B.; Yi, W.; Zhang, J.; Liu, Q.; Liu, Y.; Fan, S.; Yu, X. Synthesis and gene transfection activity of cyclen-based cationic lipids with asymmetric acyl-cholesteryl hydrophobic tails. *Org. Biomol. Chem.* **2014**, *12*, 3484–3492.

-
- ¹⁶ Martin, L.; Coello, M.; Reyes, B.; Rodriguez, V.; Garranzo, G.; Murcia, P.; Francesch, S.; Sanchez, S.; Fernandez, R. Antitumoral dihydropyran-2-one compounds. WO Patent 2 007 144 423, 2007.
- ¹⁷ Geneste, H.; Ochse, M.; Drescher, K.; Dinges, J.; Jakob, C. Heterocyclic Carboxamides Useful As Inhibitors of Phosphodiesterase Type 10A. U.S. Patent 2 013 116 229, 2013.
- ¹⁸ Flowers, S.; Barker, K.; Berkow, E.; Toner, G.; Chadwick, S.; Gyax, S.; Morschhauser, J.; Rogers, D. Gain-of-Function Mutations in *UPC2* Are a Frequent Cause of *ERG11* Upregulation in Azole-Resistant Clinical Isolates of *Candida albicans*. *Eukaryotic Cell* **2012**, *11*, 1289–1299.
- ¹⁹ Basso, L.; Gast, C.; Bruzual, I.; Wong, B. Identification and properties of plasma membrane azole efflux pumps from the pathogenic fungi *Cryptococcus gattii* and *Cryptococcus neoformans*. *J. Antimicrob Chemother* **2015**, *70*, 1396–1407.
- ²⁰ Cormack, B. P.; Falkow, S. Efficient homologous and illegitimate recombination in the opportunistic yeast pathogen *Candida glabrata*. *Genetics* **1999**, *151*, 979–987.



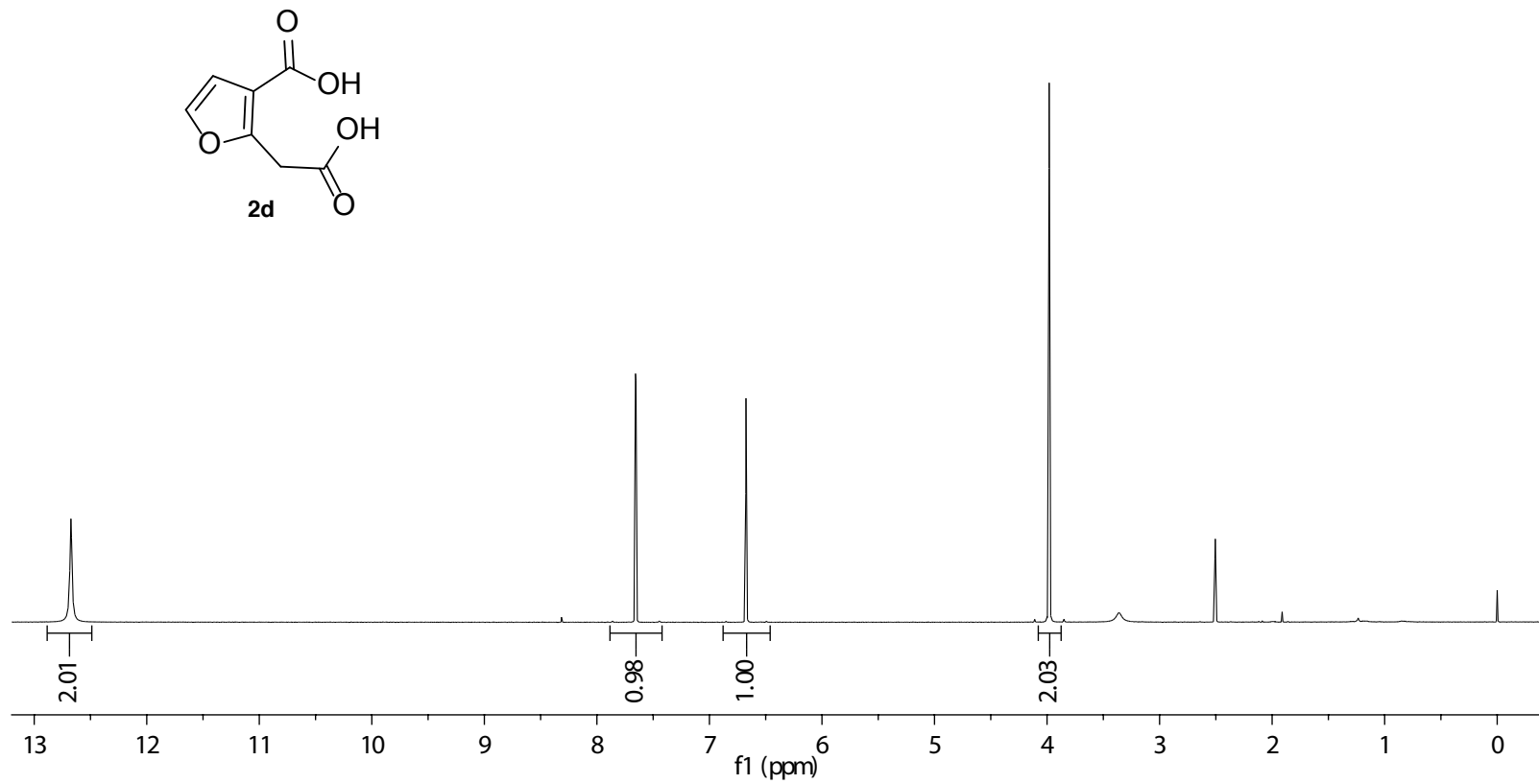
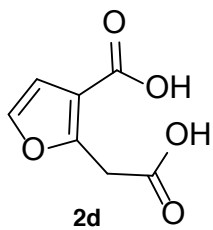


—12.67

<7.66
<7.65

<6.68
<6.67

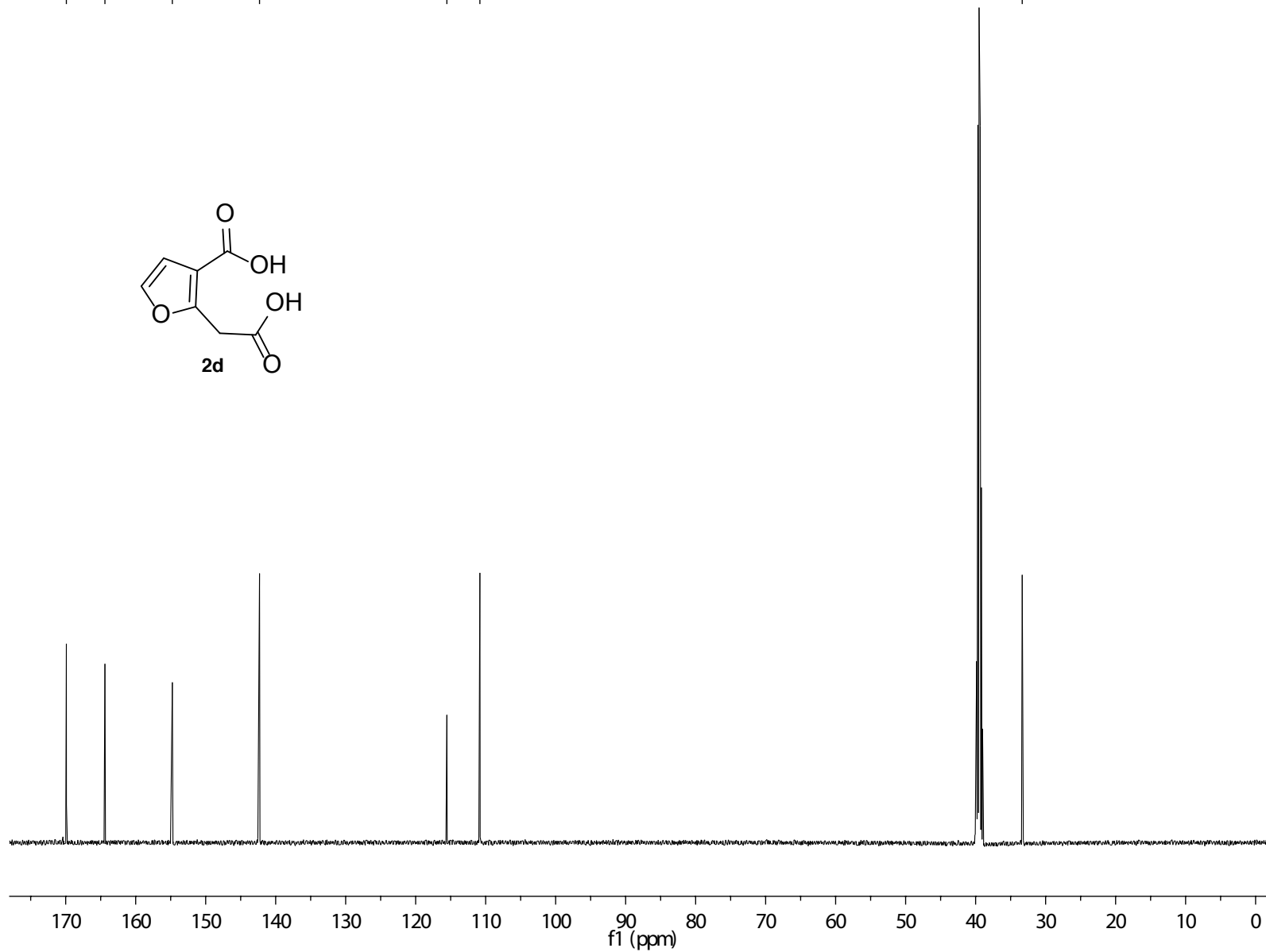
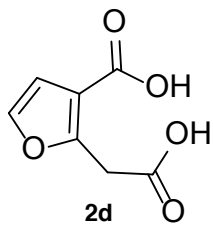
—3.98



—169.90
—164.39
—154.76
—142.31

—115.54
—110.82

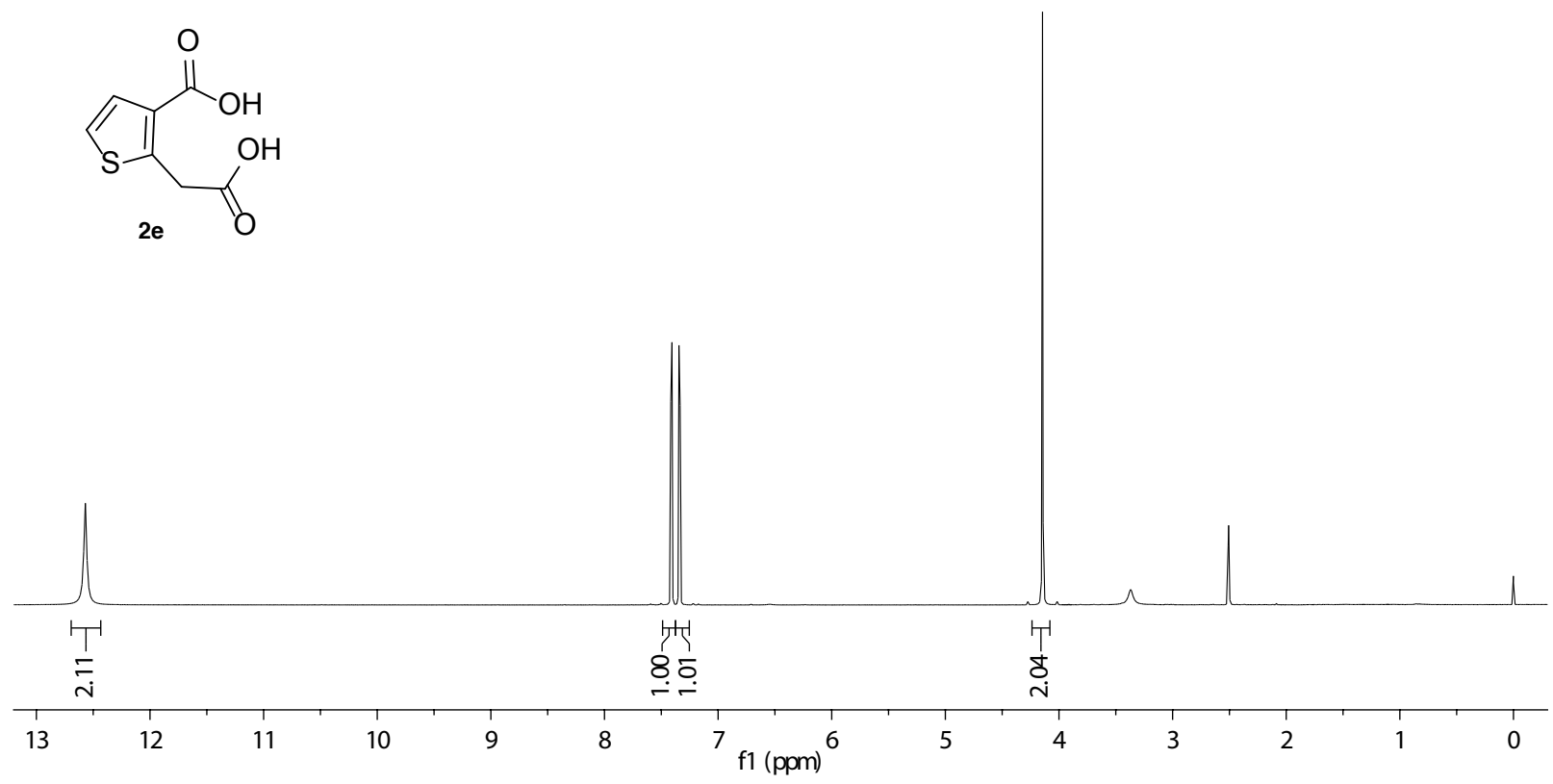
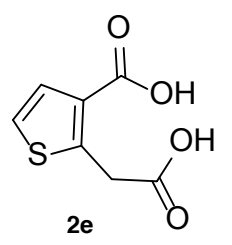
—33.34



—12.57

7.42
7.41
7.34
7.33

—4.14



—171.20

—164.26

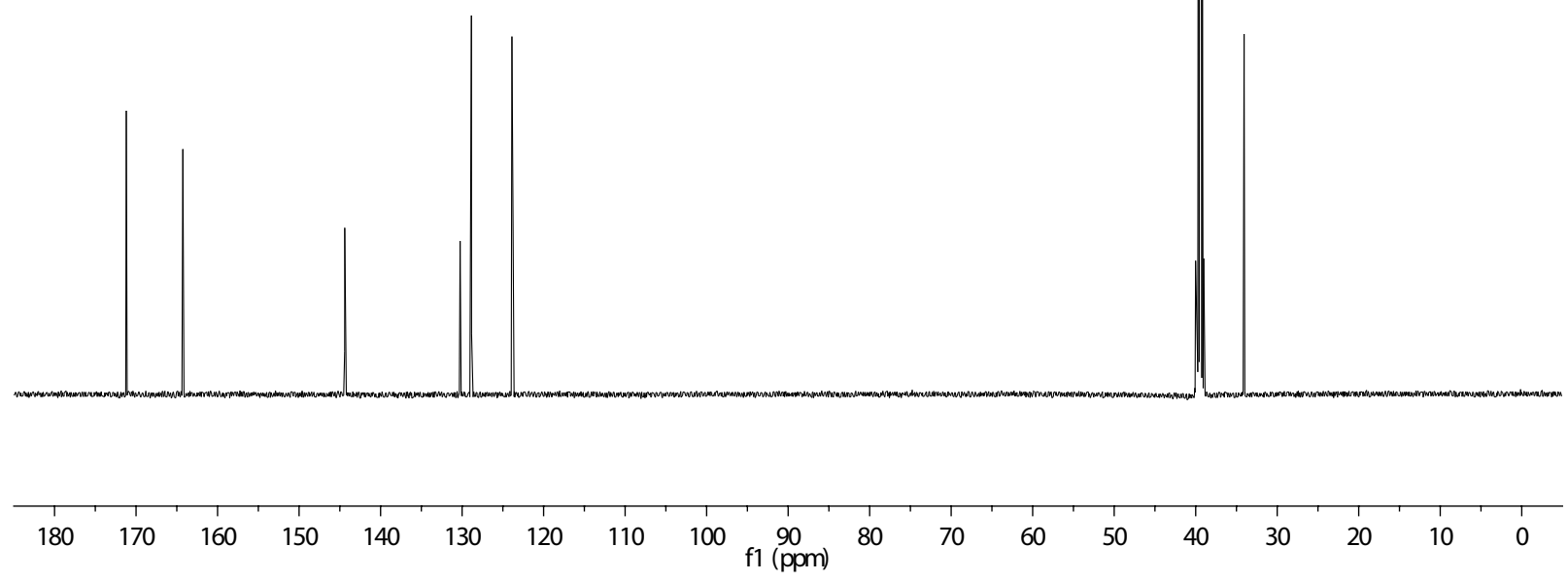
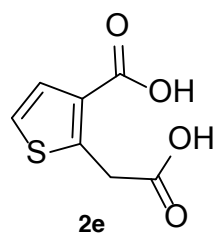
—144.40

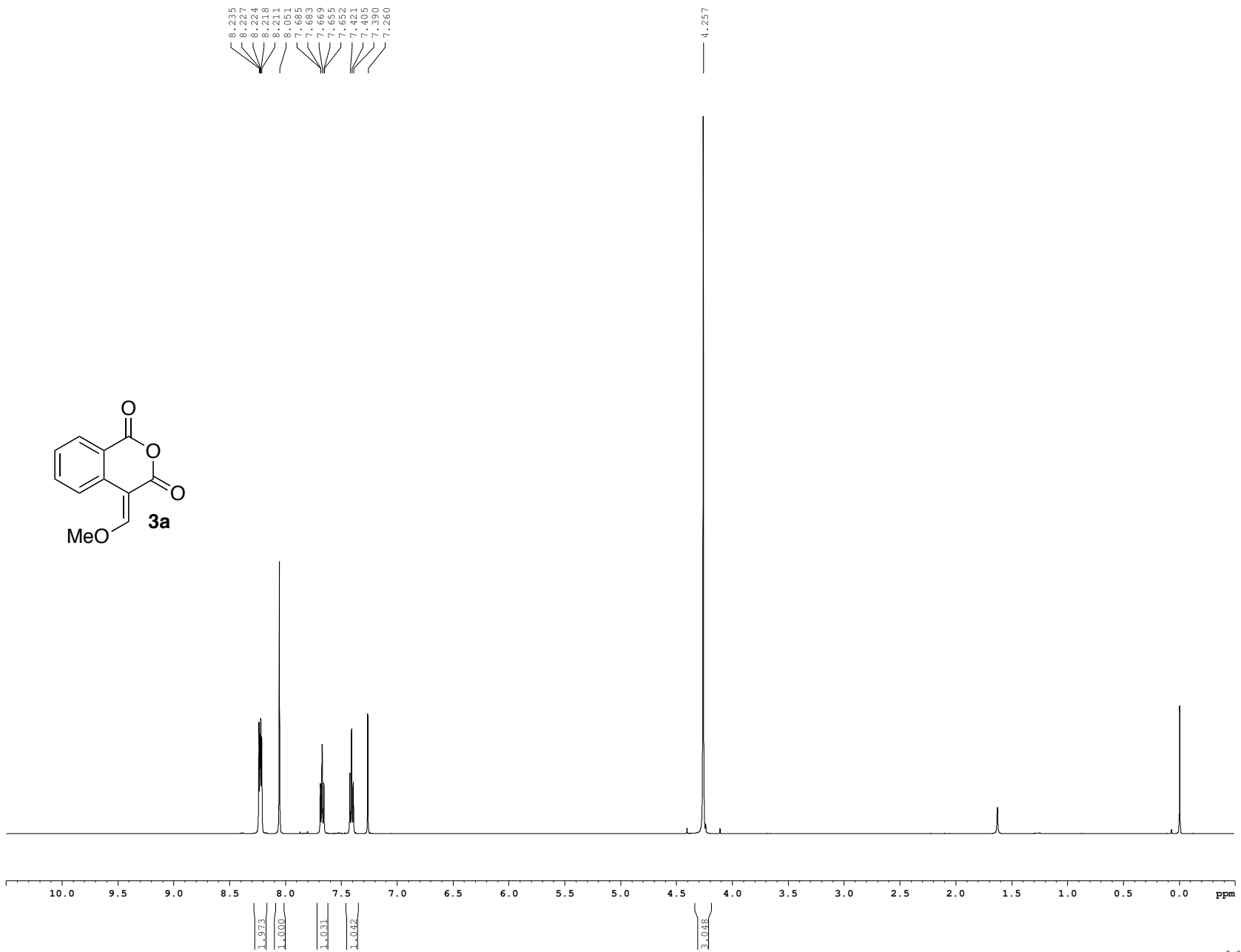
—130.24

—128.86

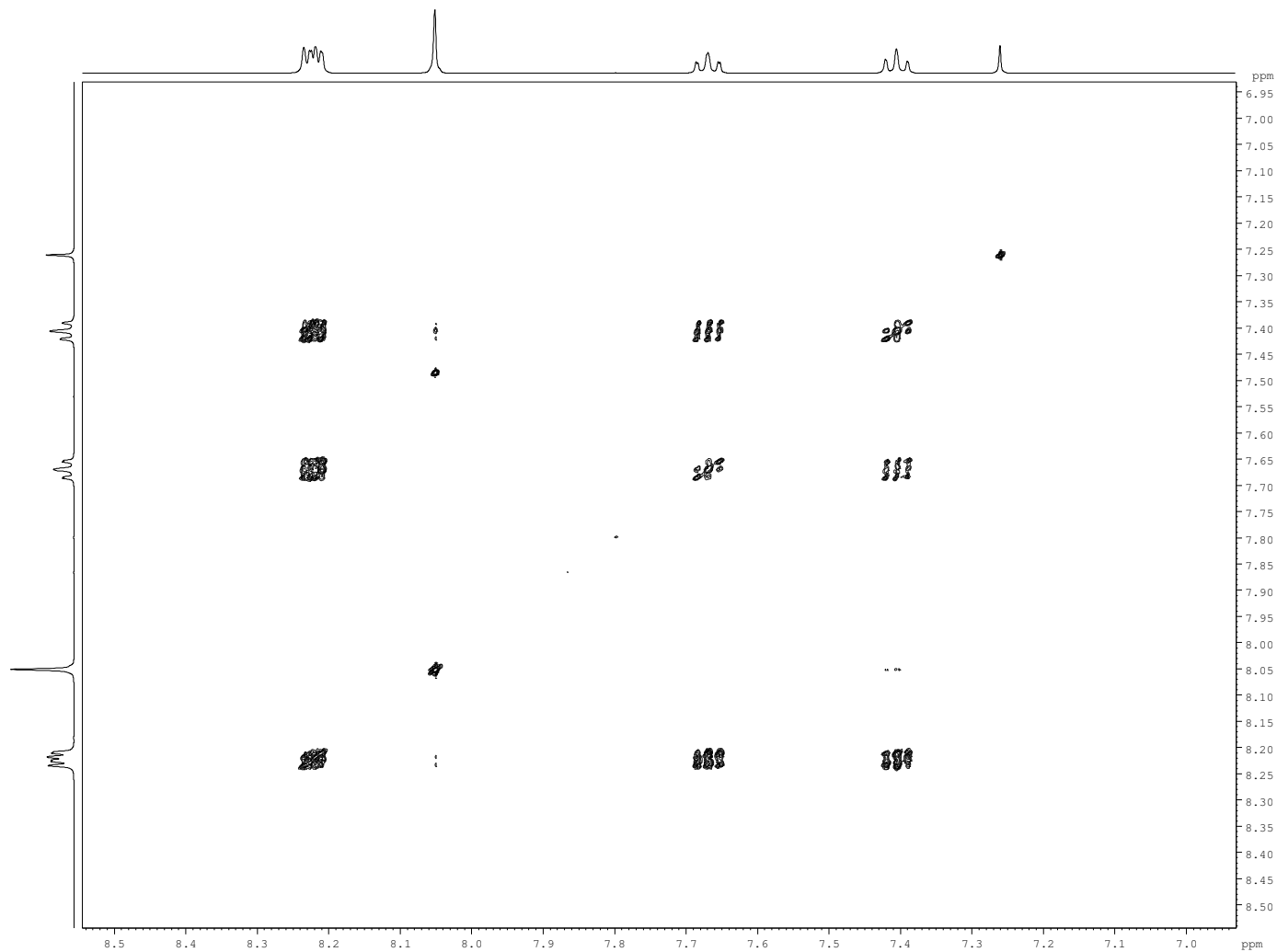
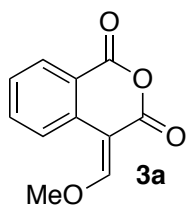
—123.89

—34.06

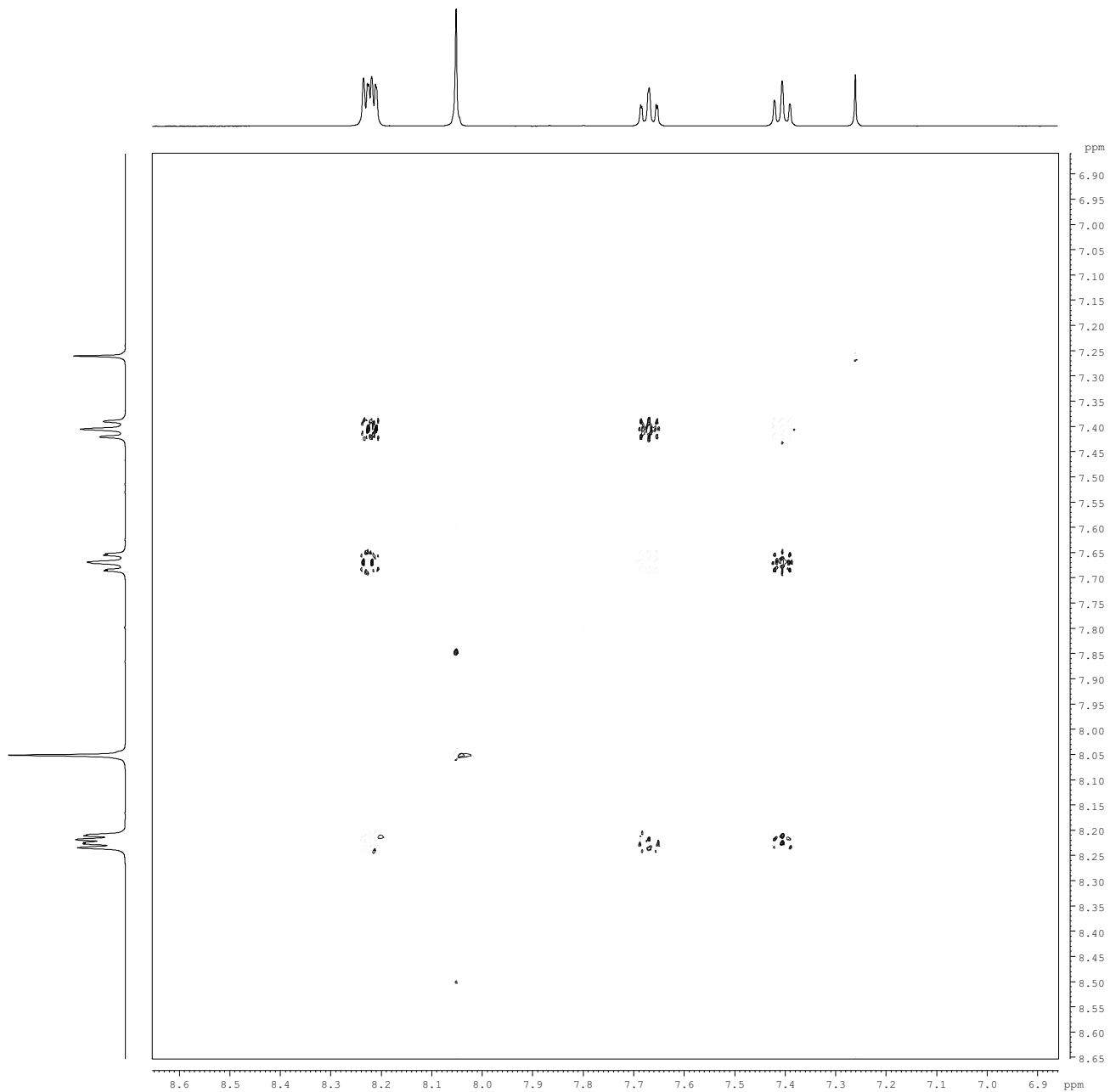
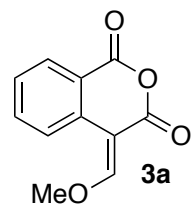


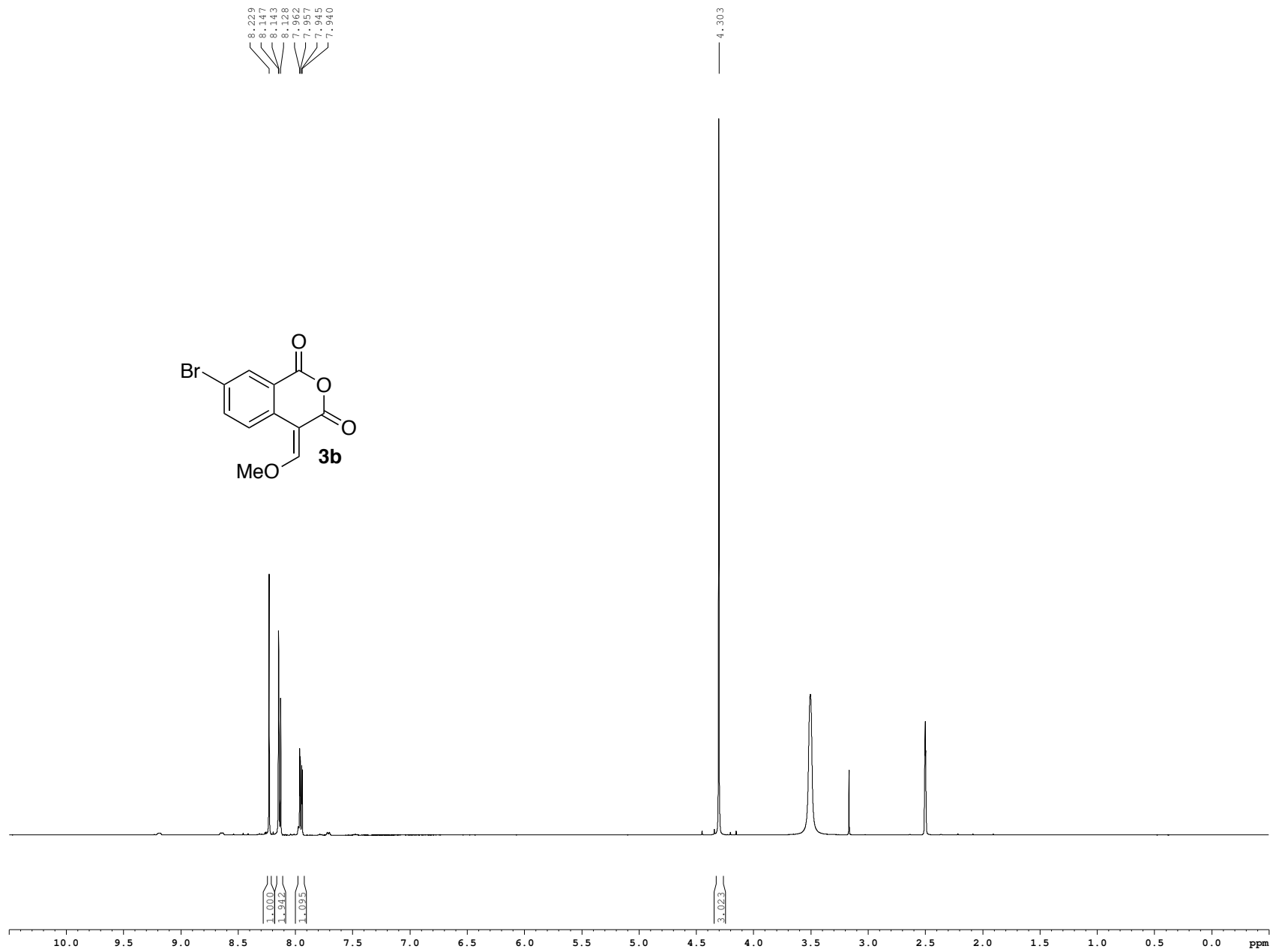
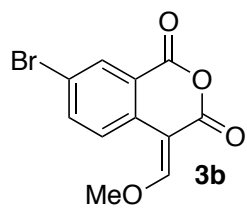


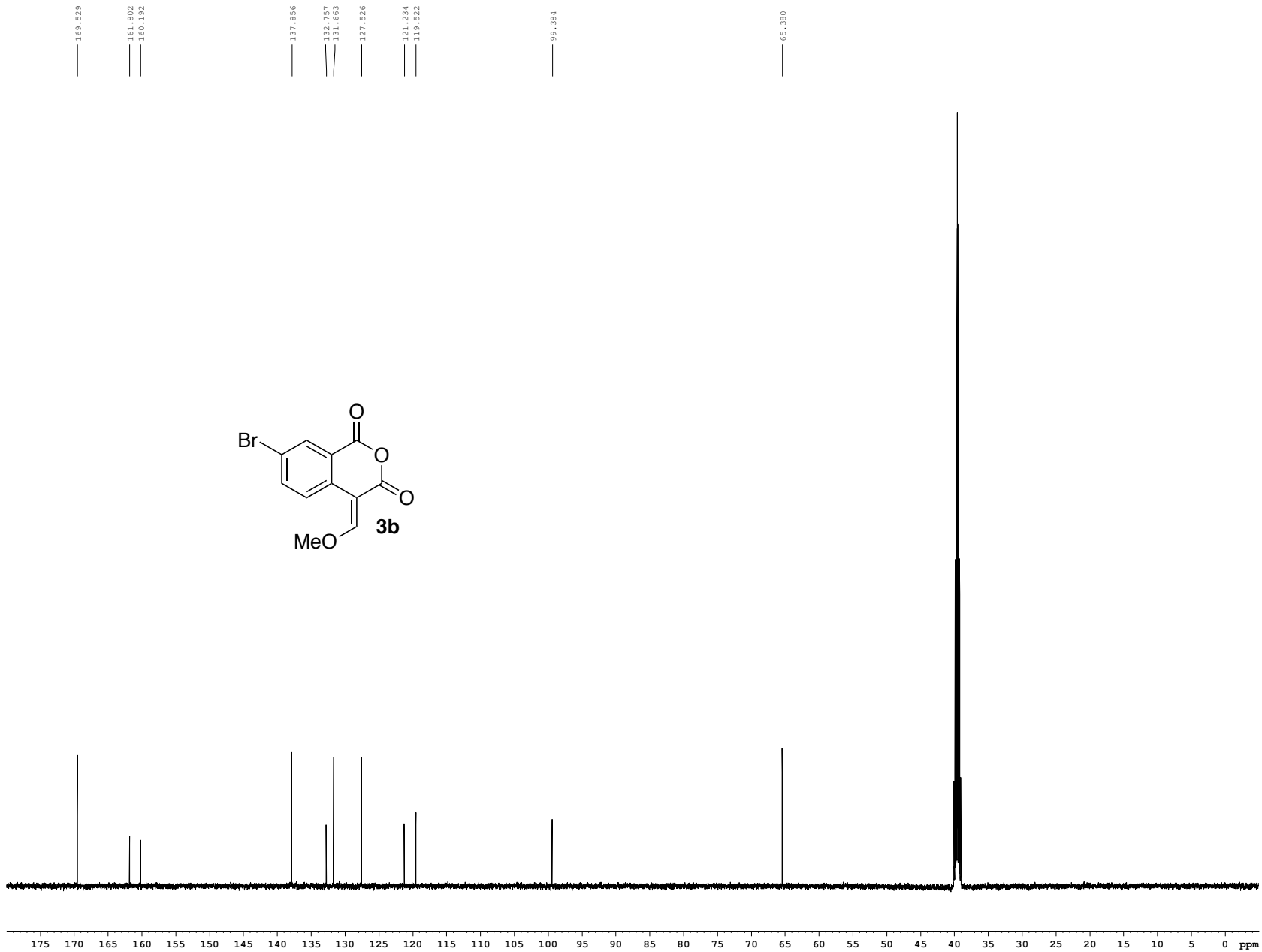
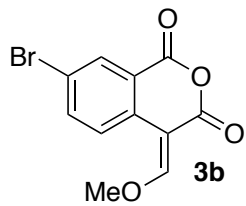
COSY

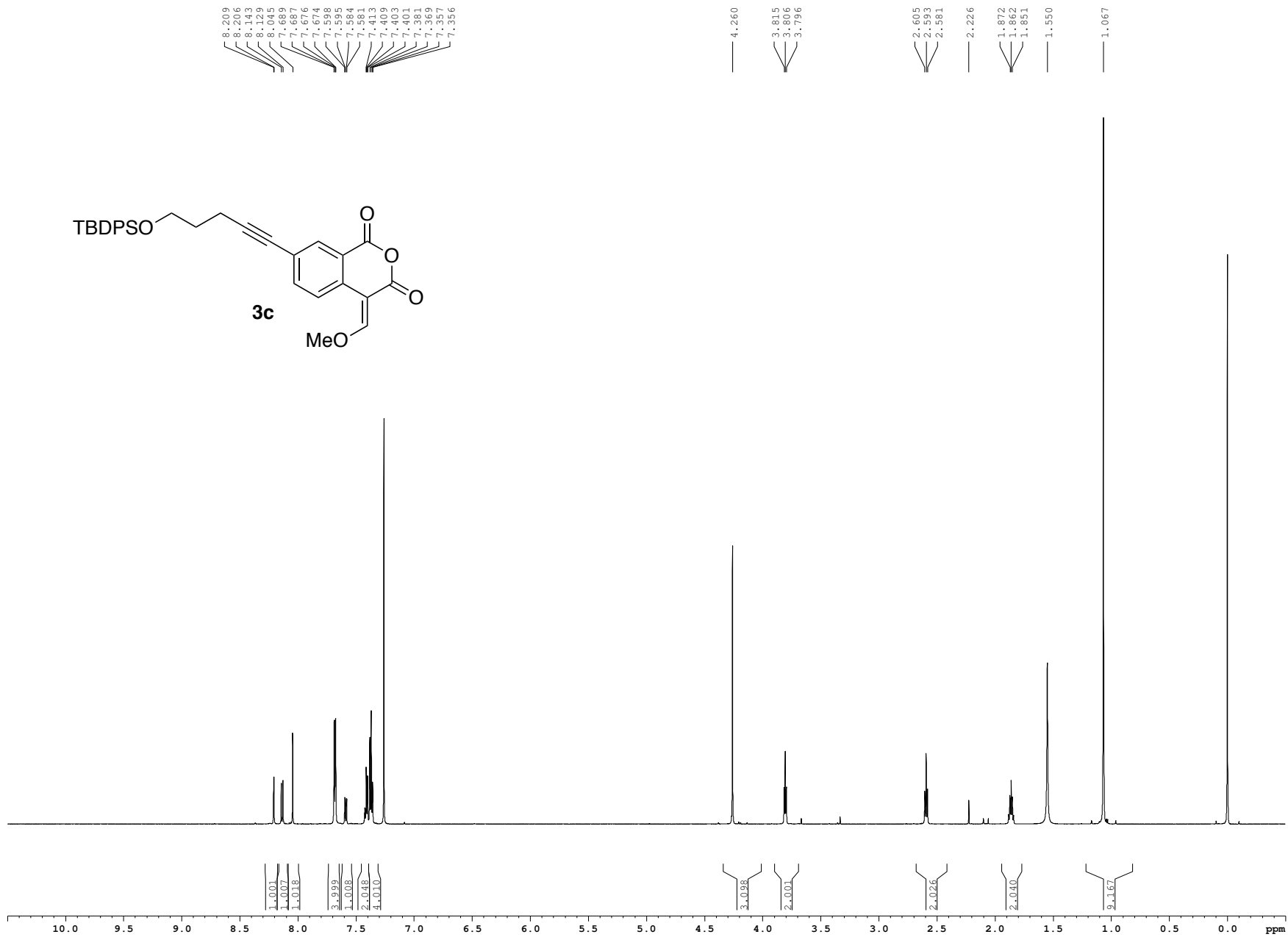


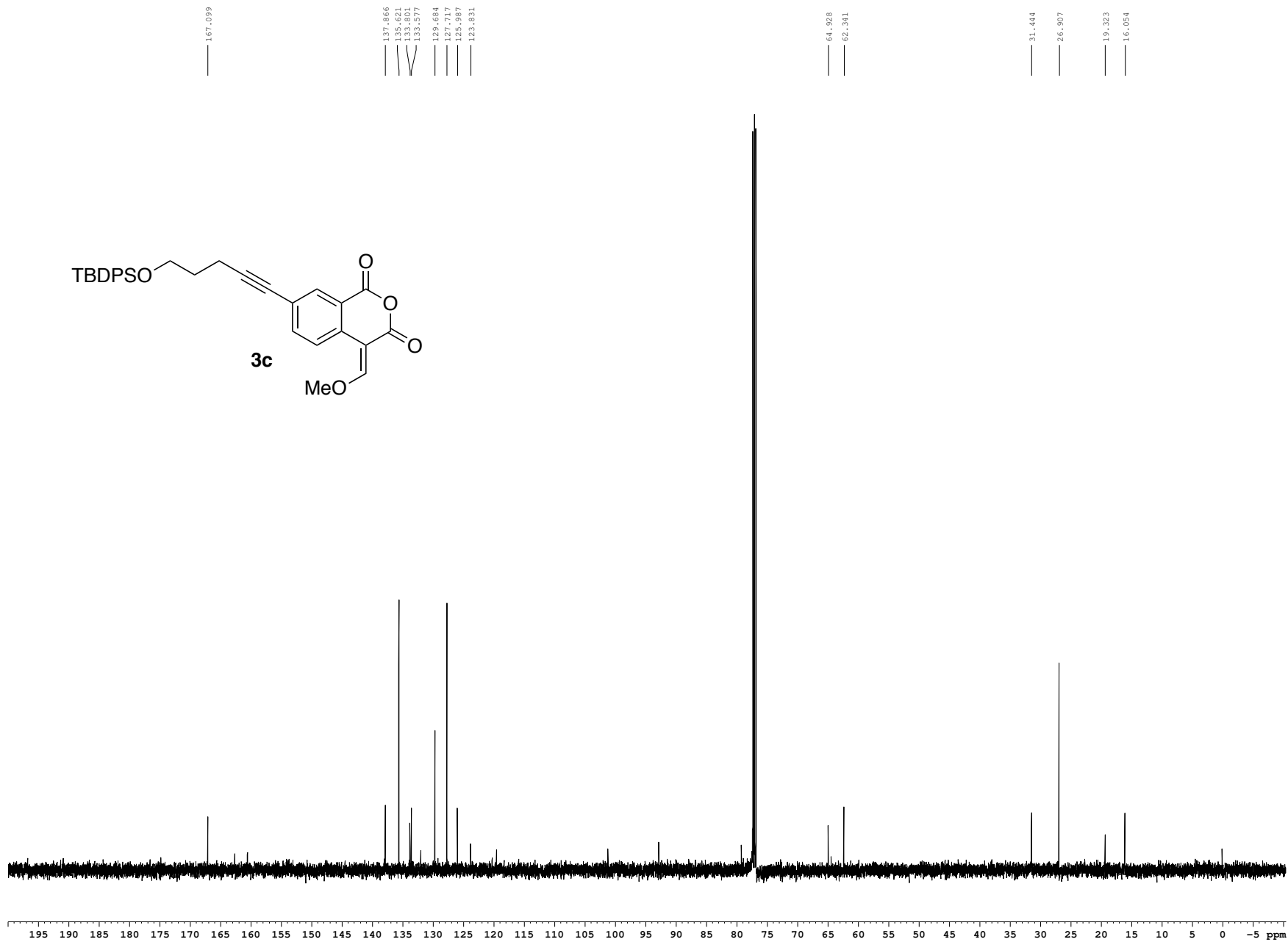
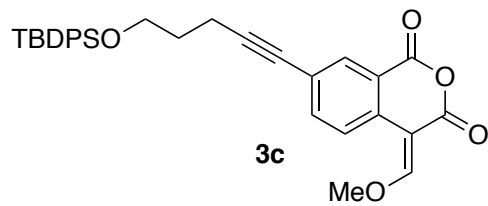
NOESY

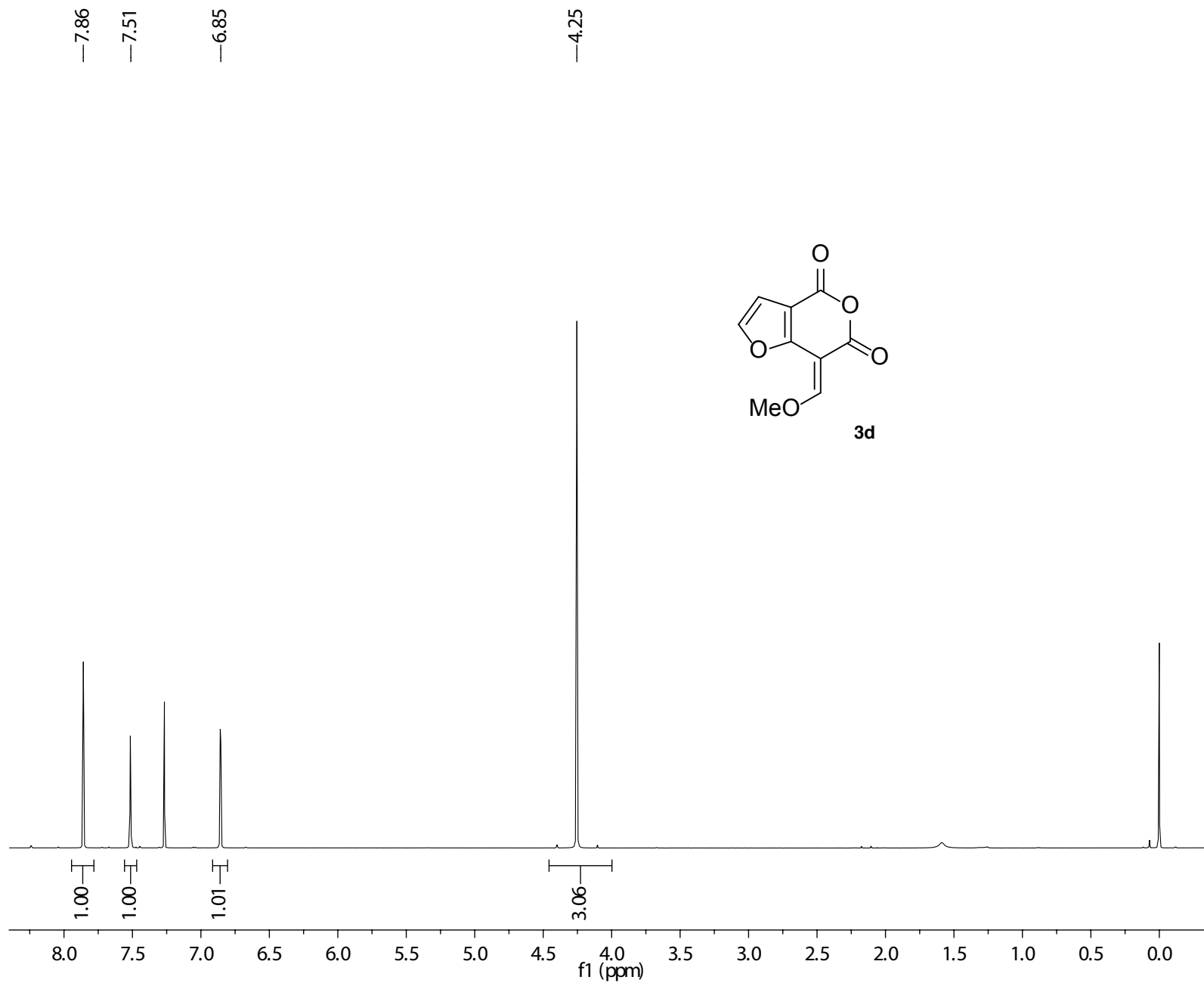


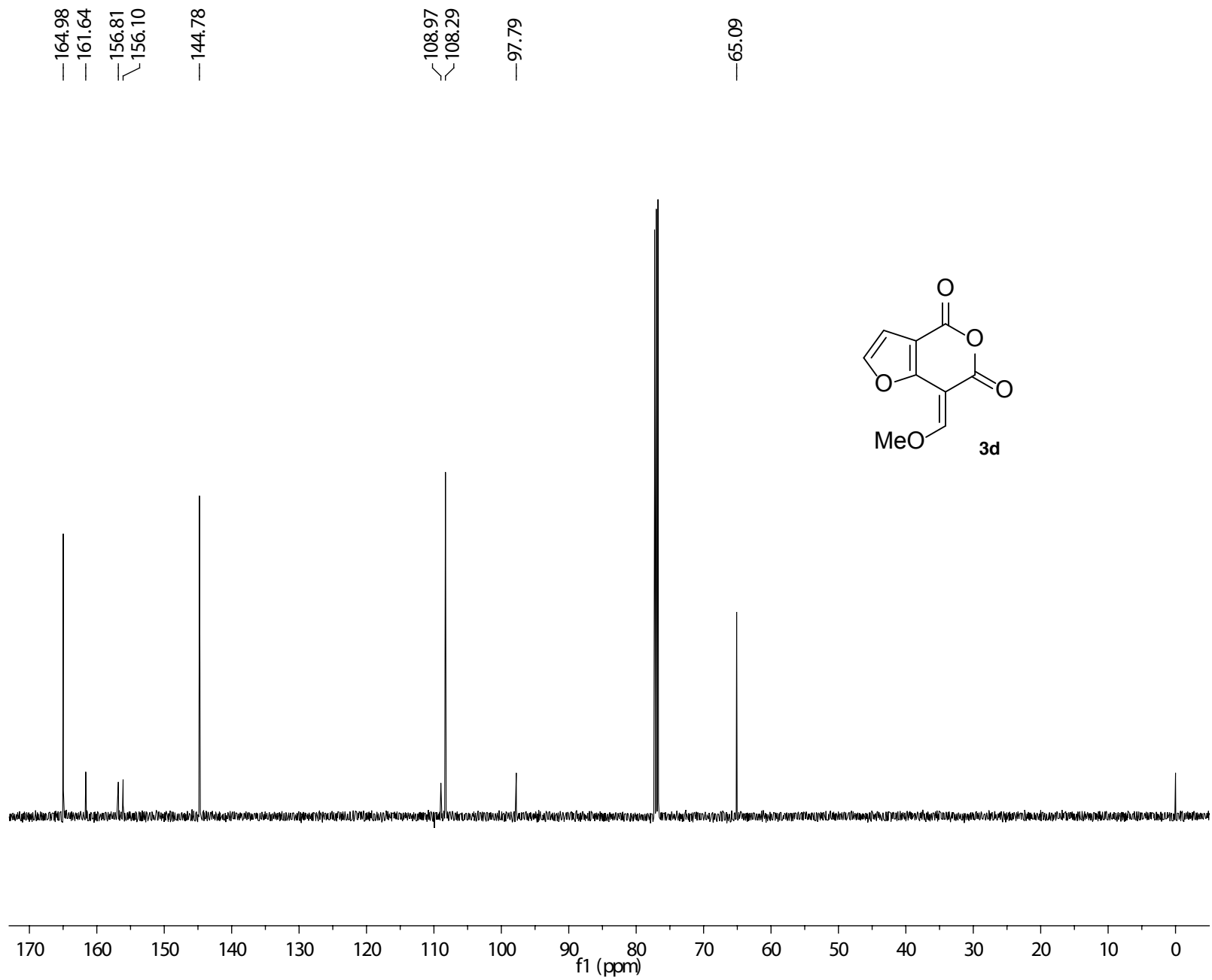






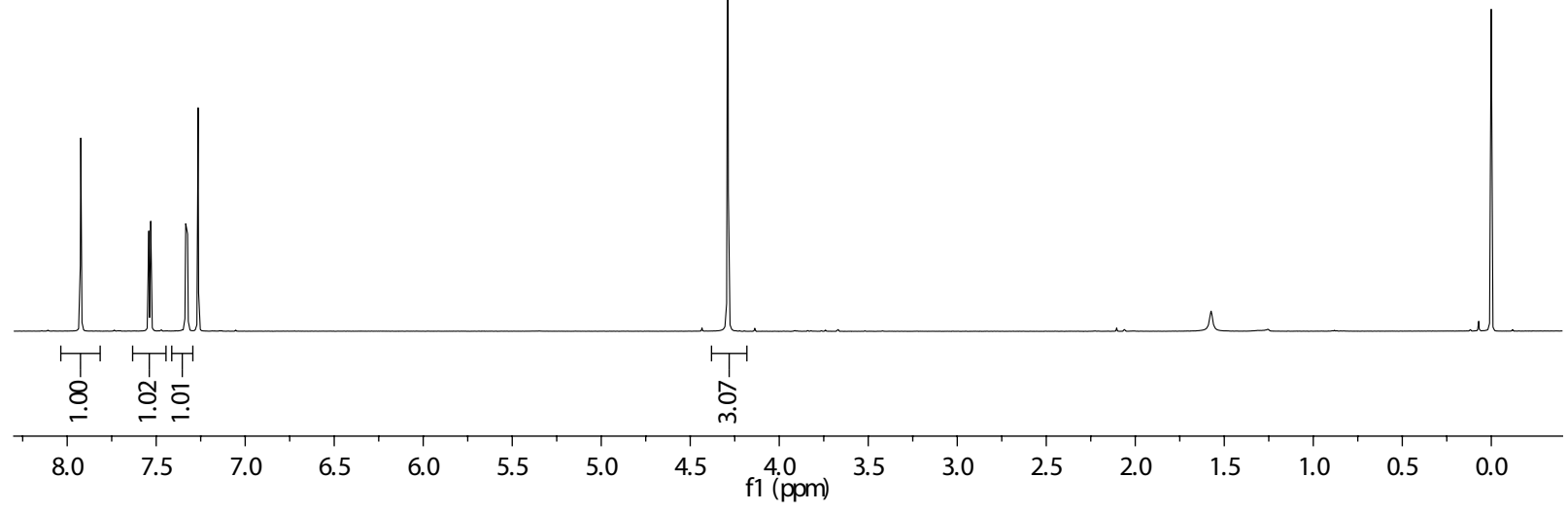




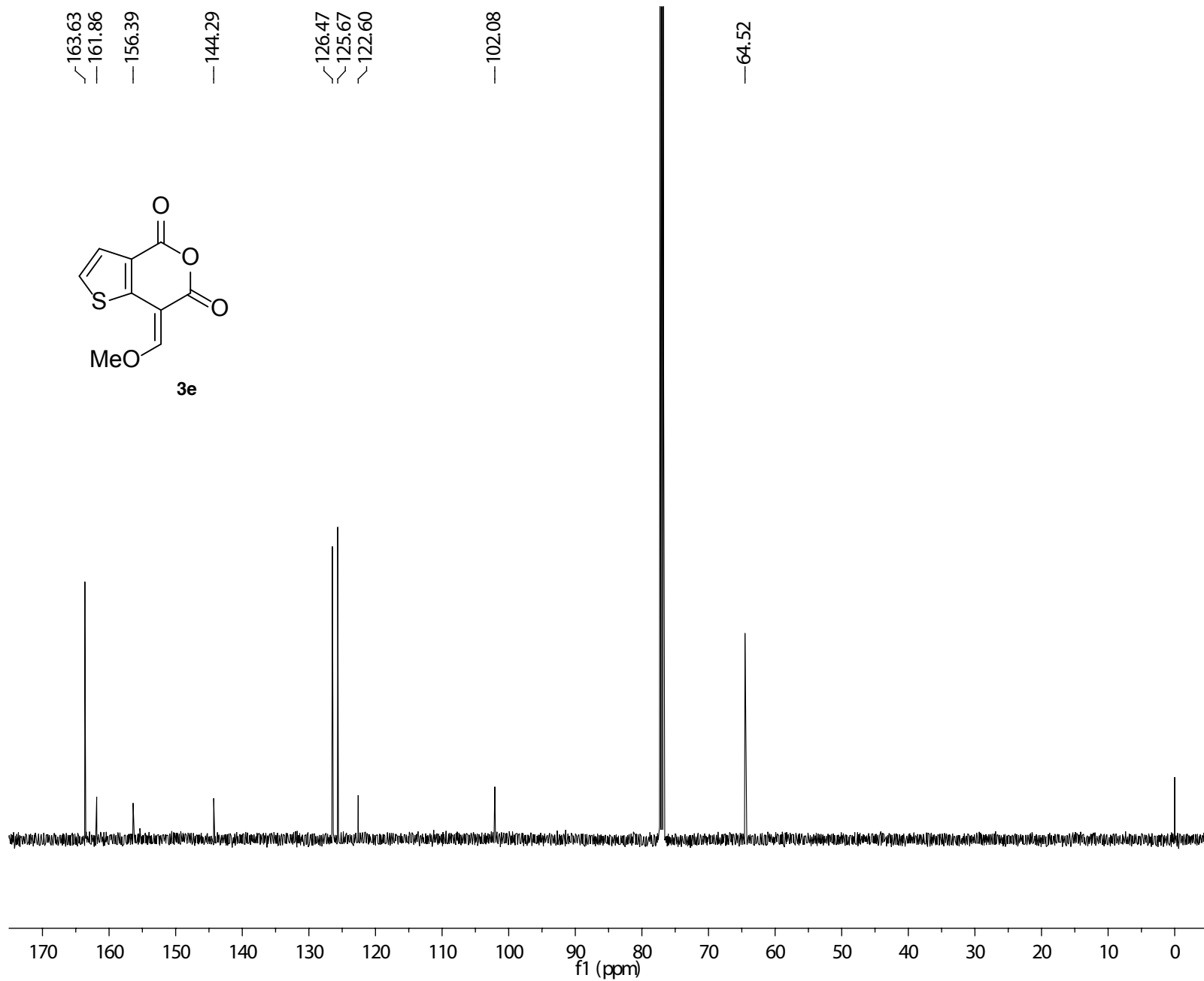
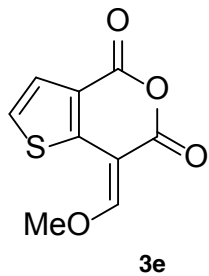


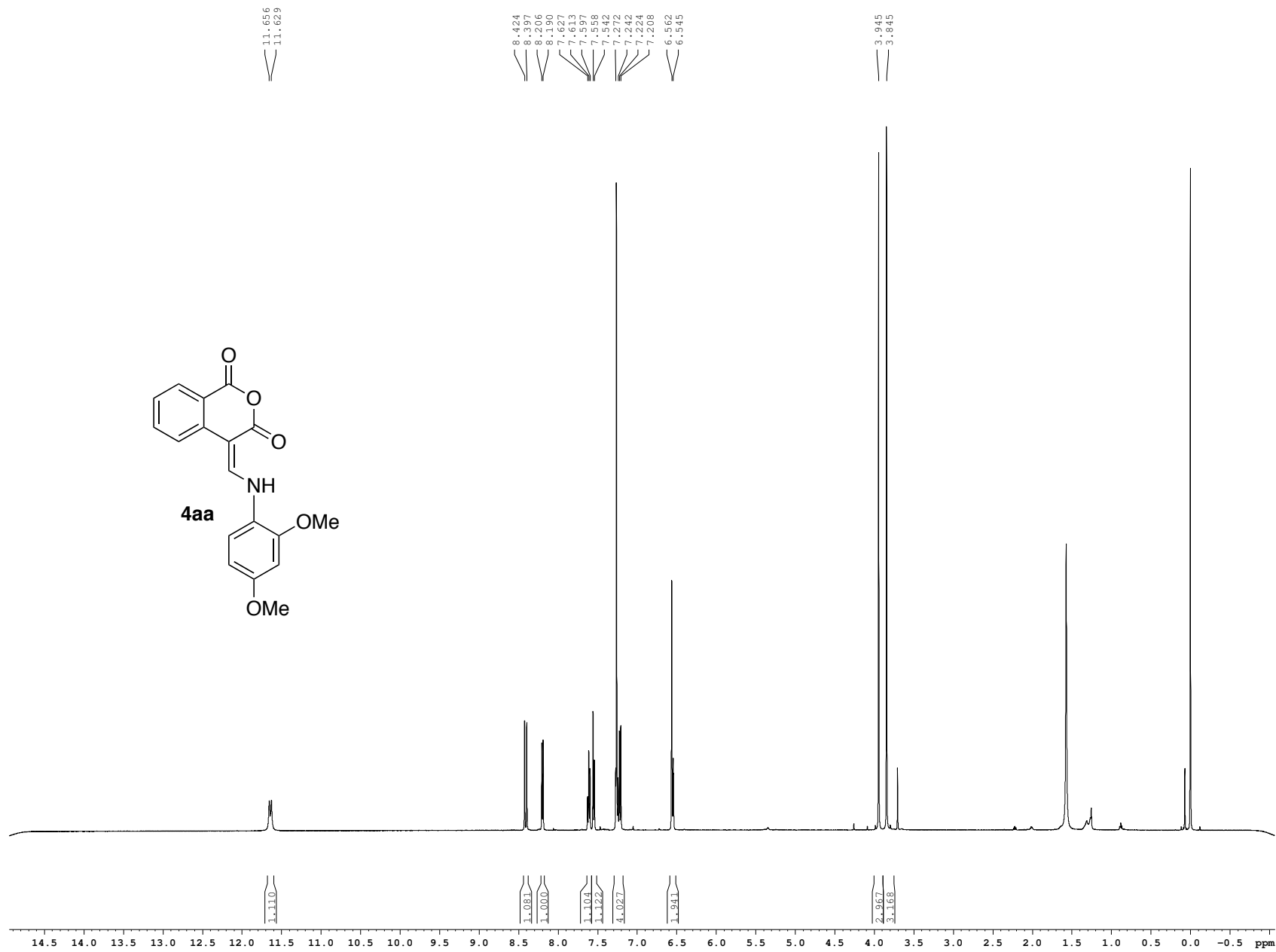
—7.92
—7.53
—7.33

—4.29



163.63
161.86
156.39
144.29
126.47
125.67
122.60
102.08
64.52





163.526
161.897
158.576

150.727

144.255

137.501

135.002

131.063

124.866

121.913

117.723

117.282

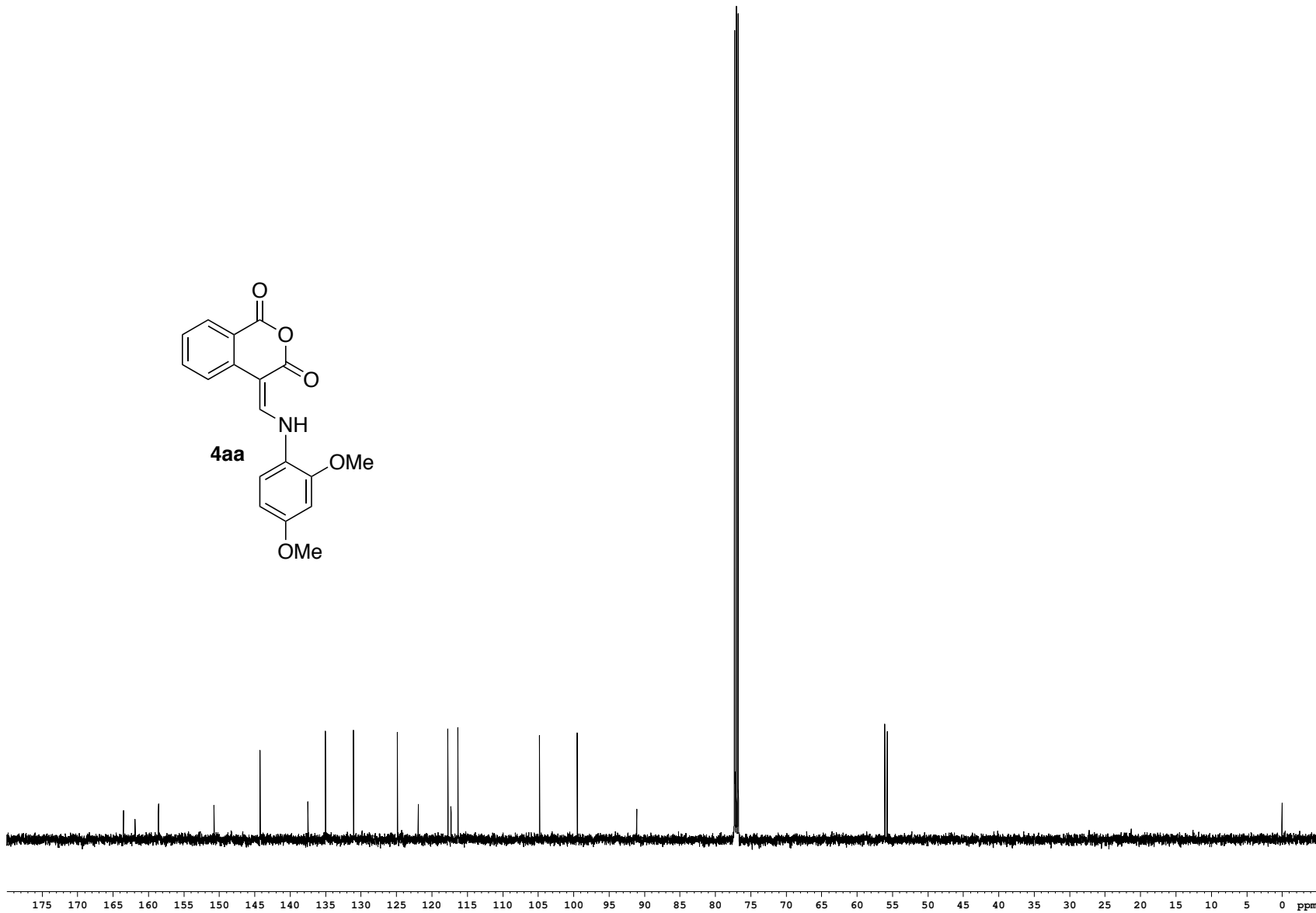
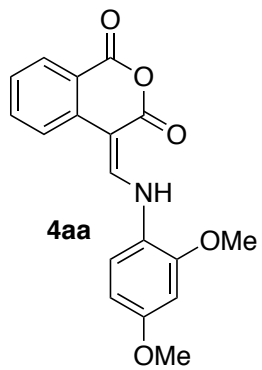
116.329

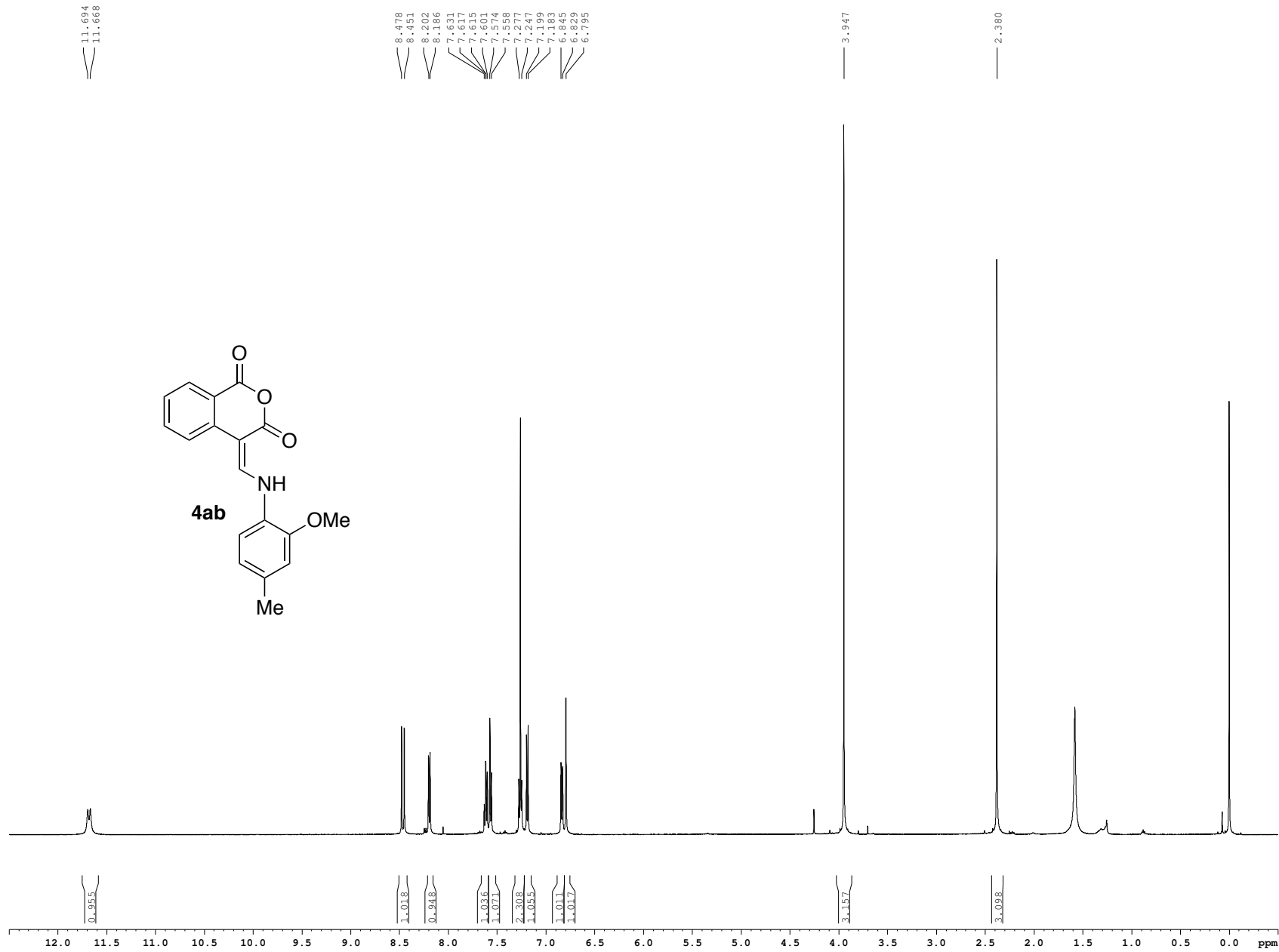
104.799

99.481

91.081

56.059
55.702





163.485
161.857

149.302

143.844

137.422
136.507
135.029

131.067

125.713
125.013

121.657

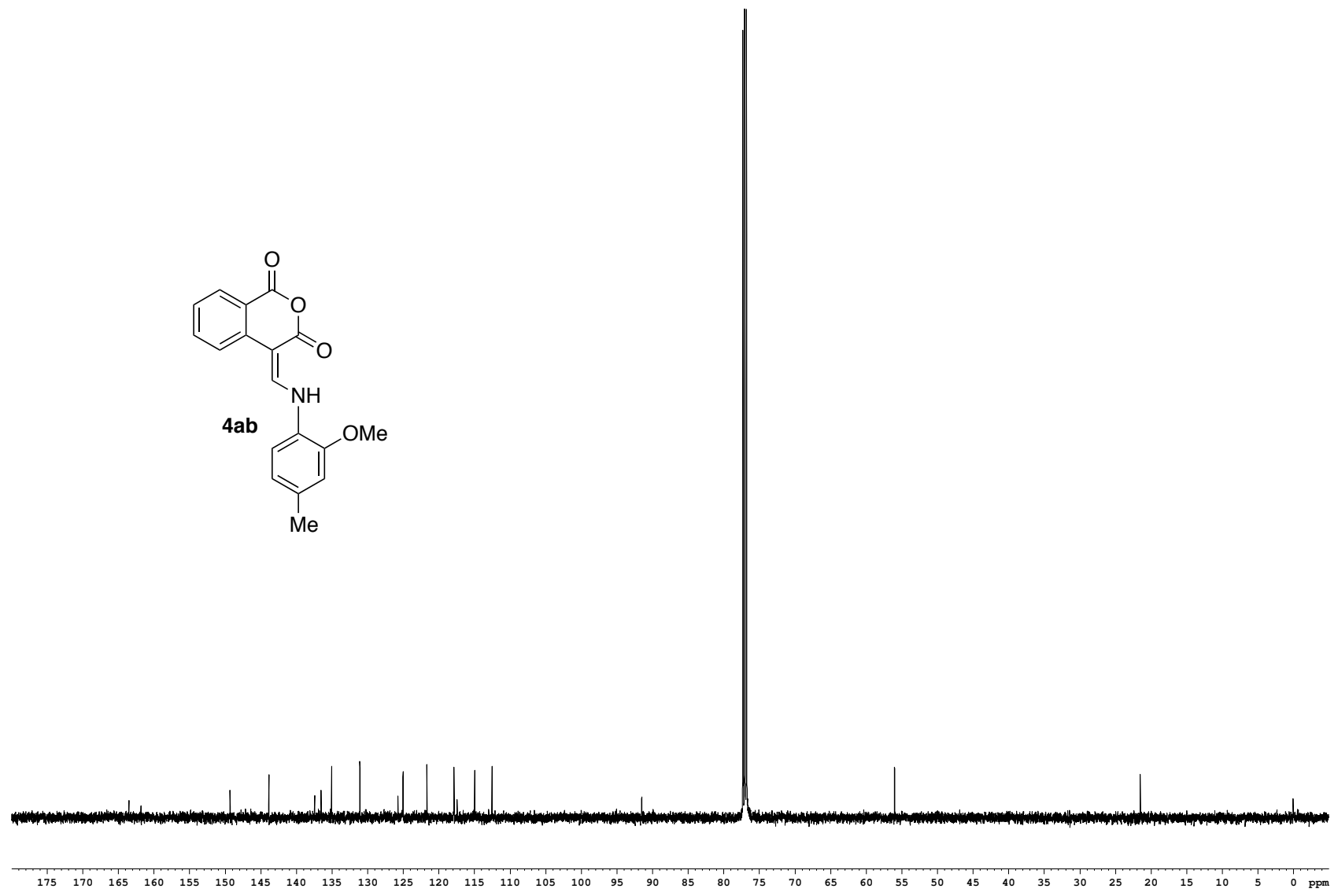
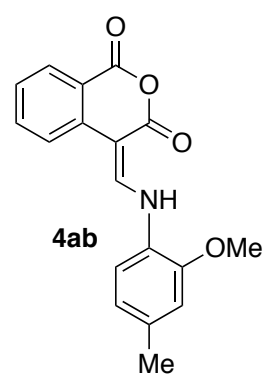
117.842
117.410

114.933
112.501

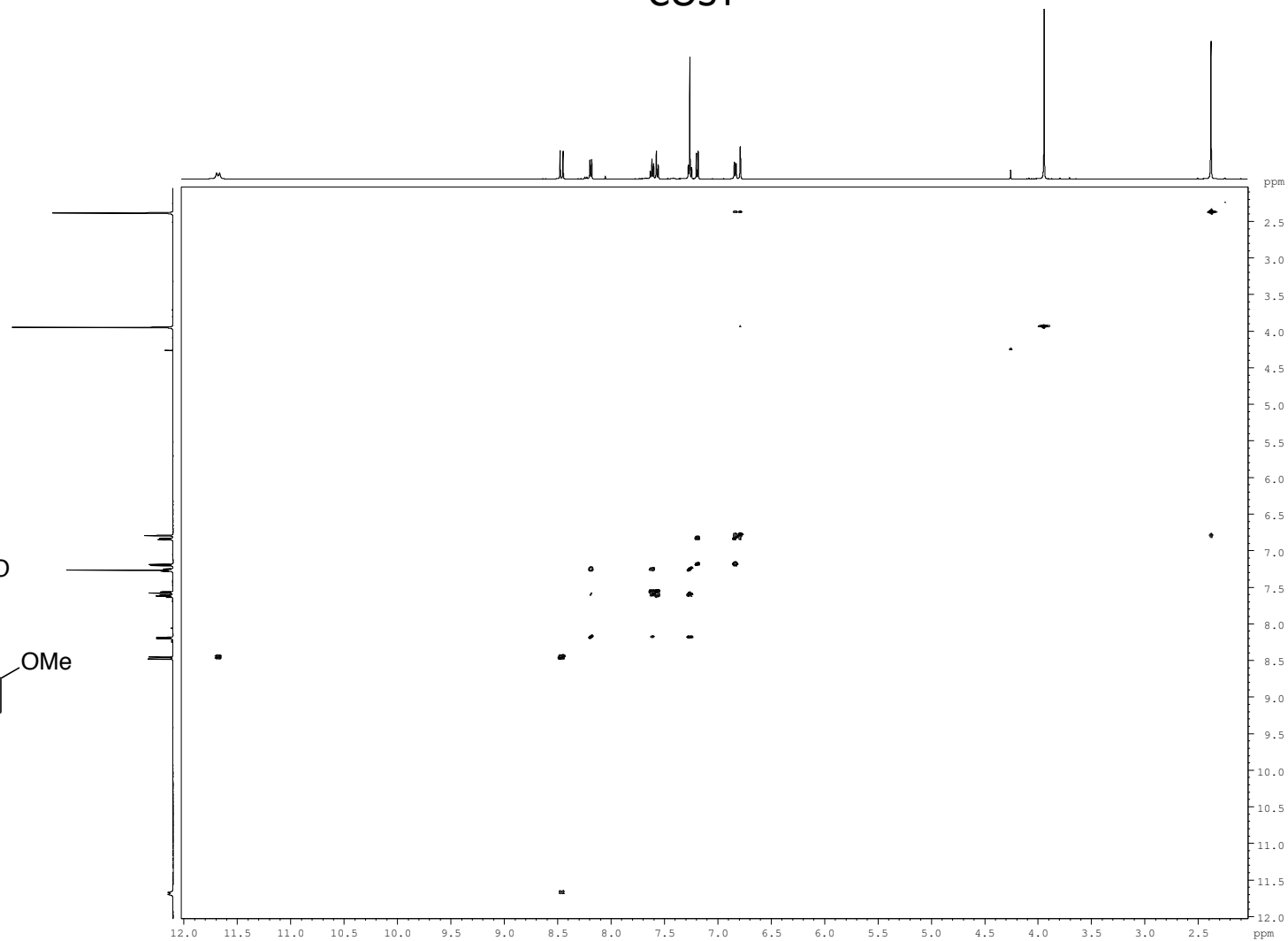
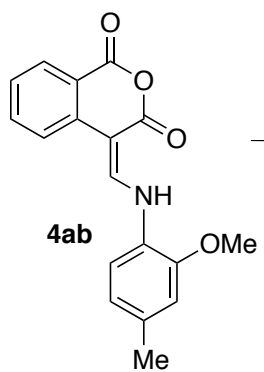
91.494

55.979

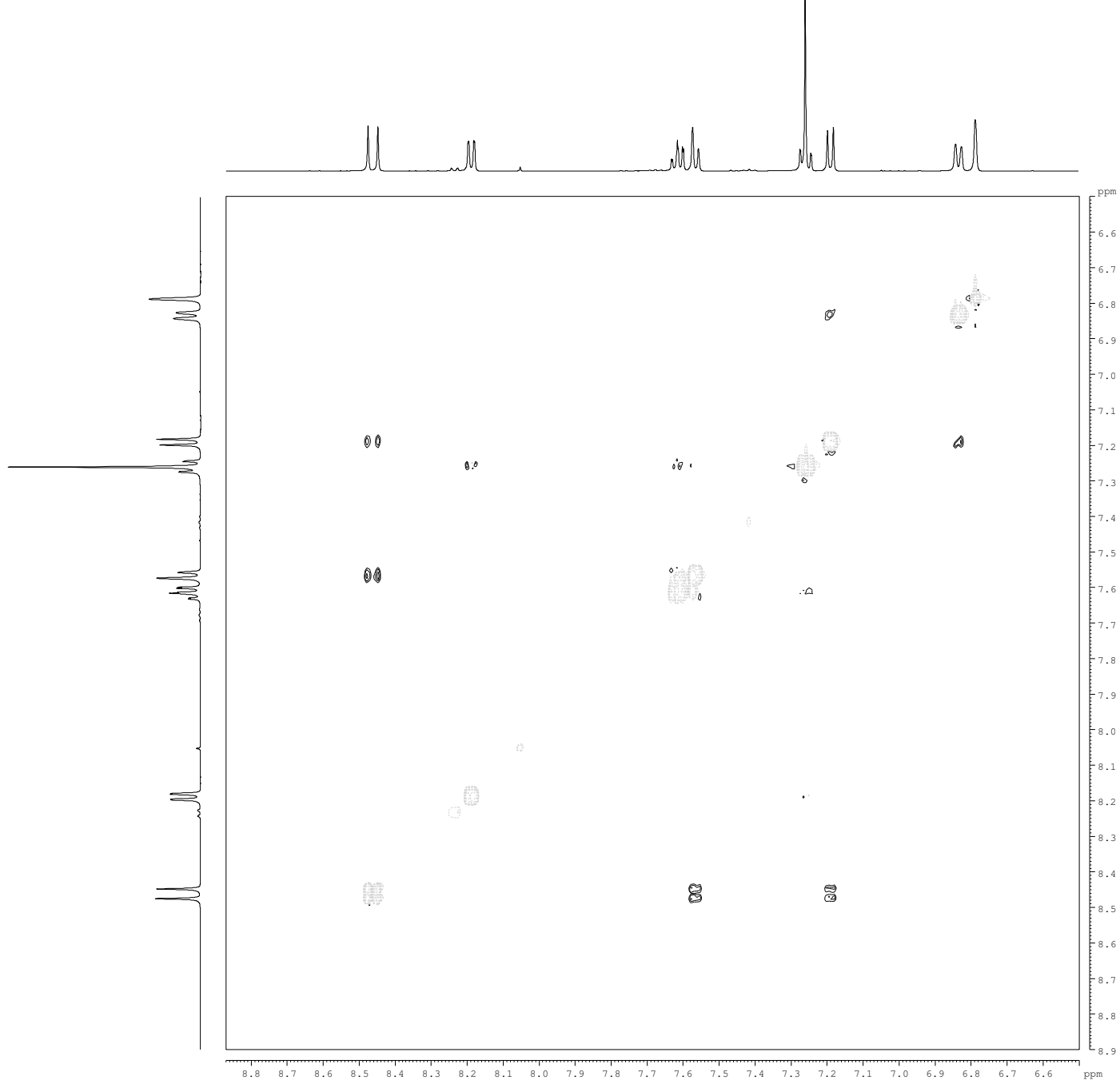
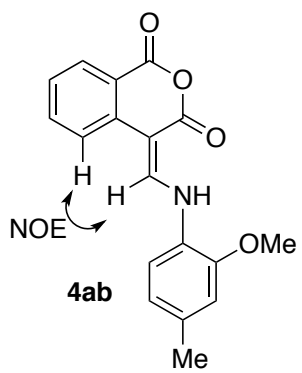
21.483

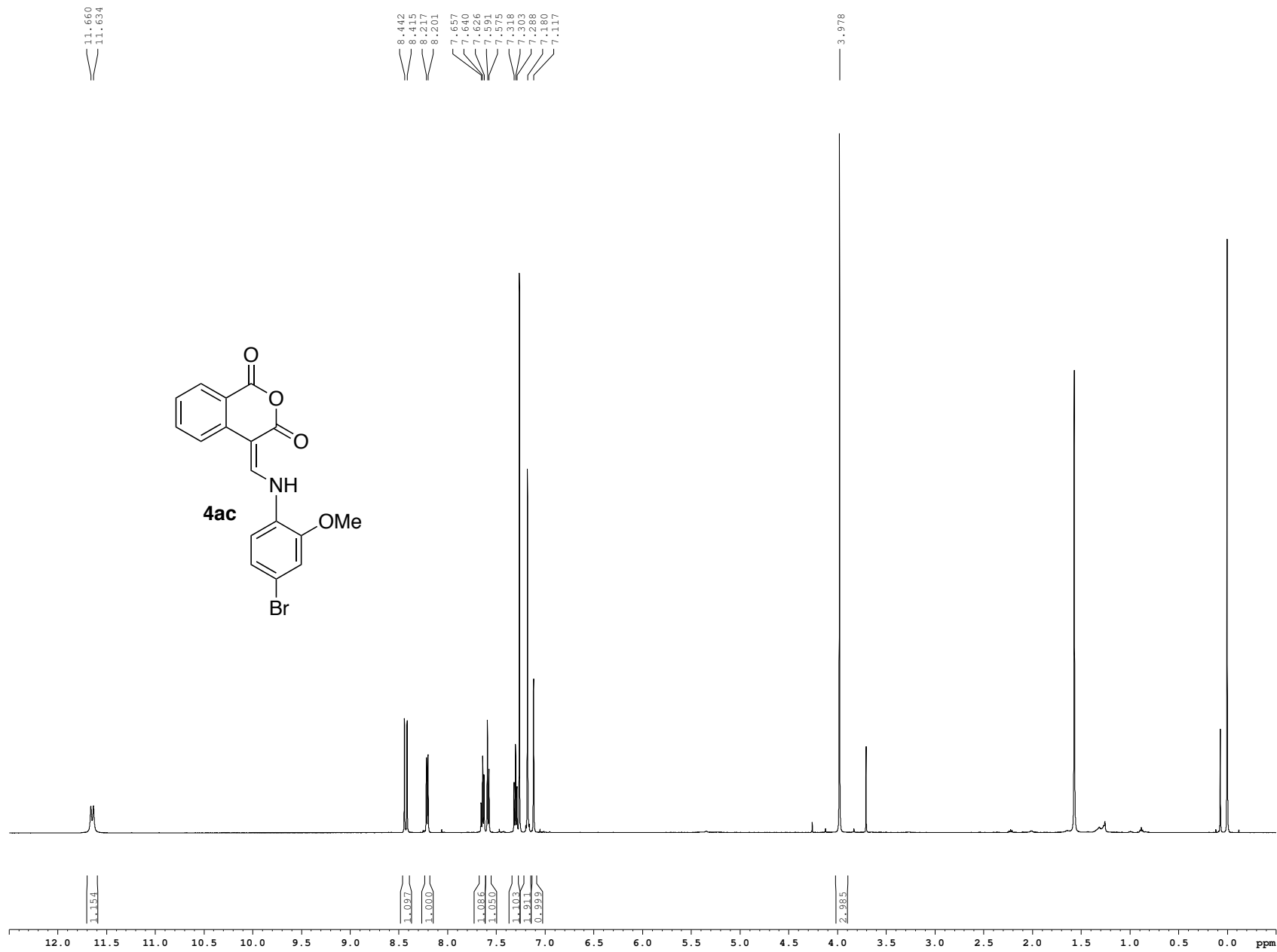


COSY



NOESY





163.519
161.490

149.824

143.059

136.862

135.149

131.158

127.515

125.519

124.156

118.418

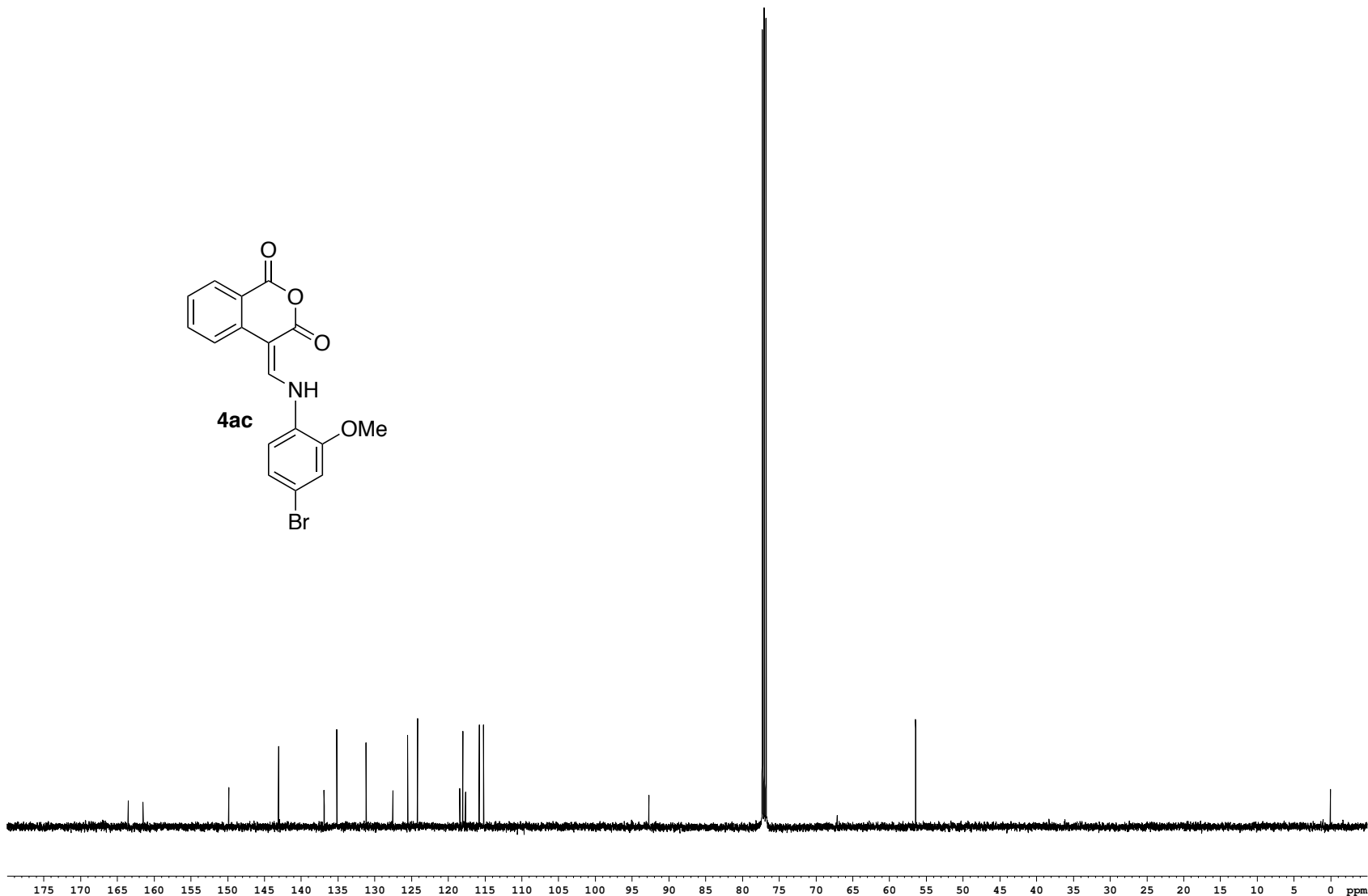
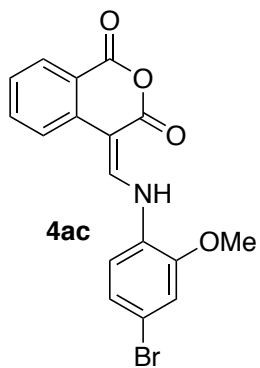
118.007

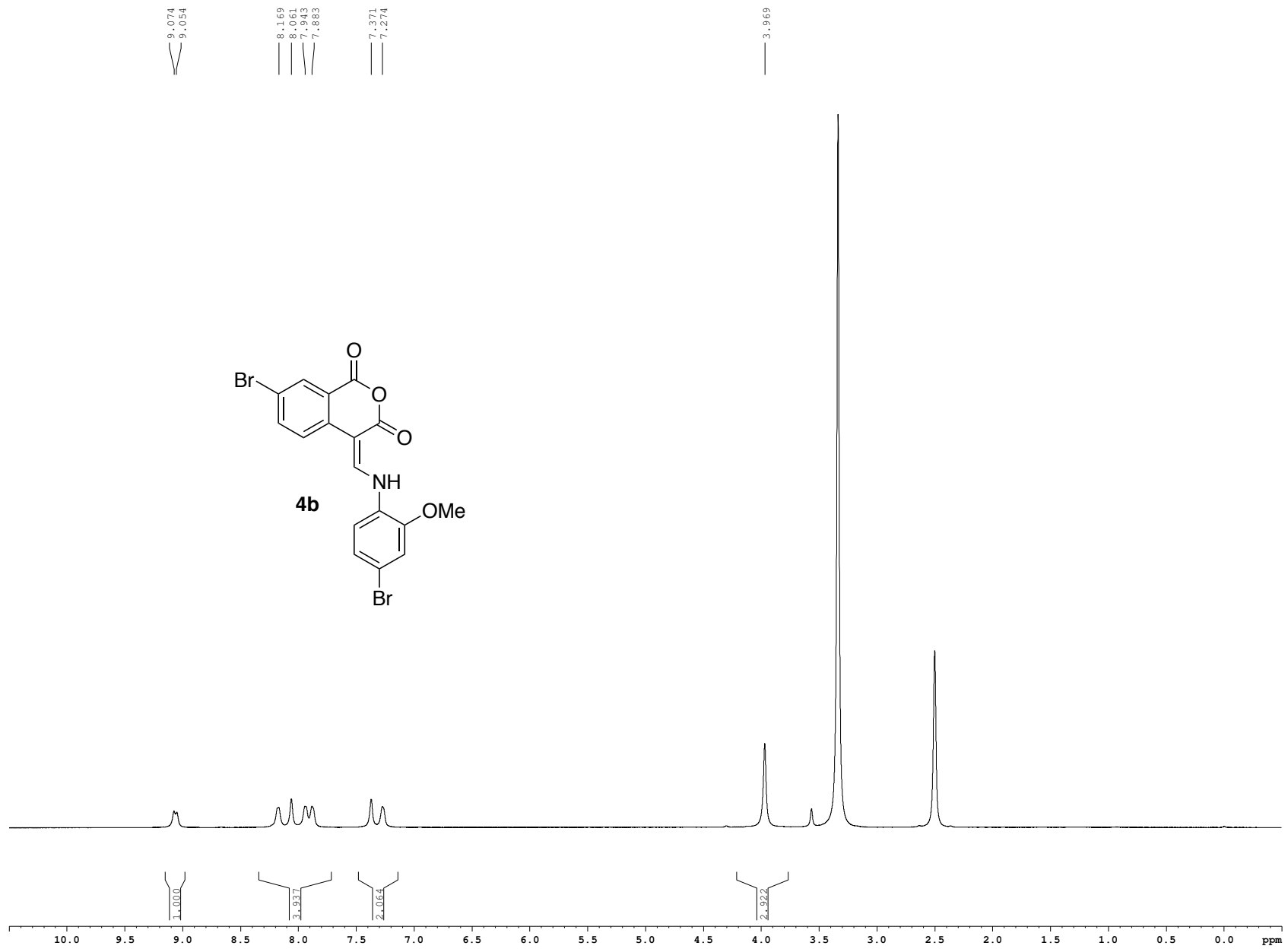
117.623

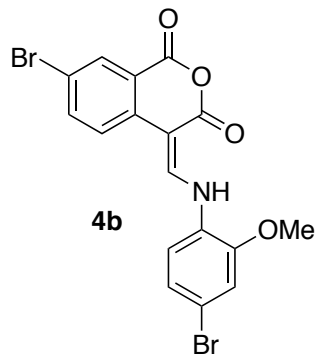
115.180

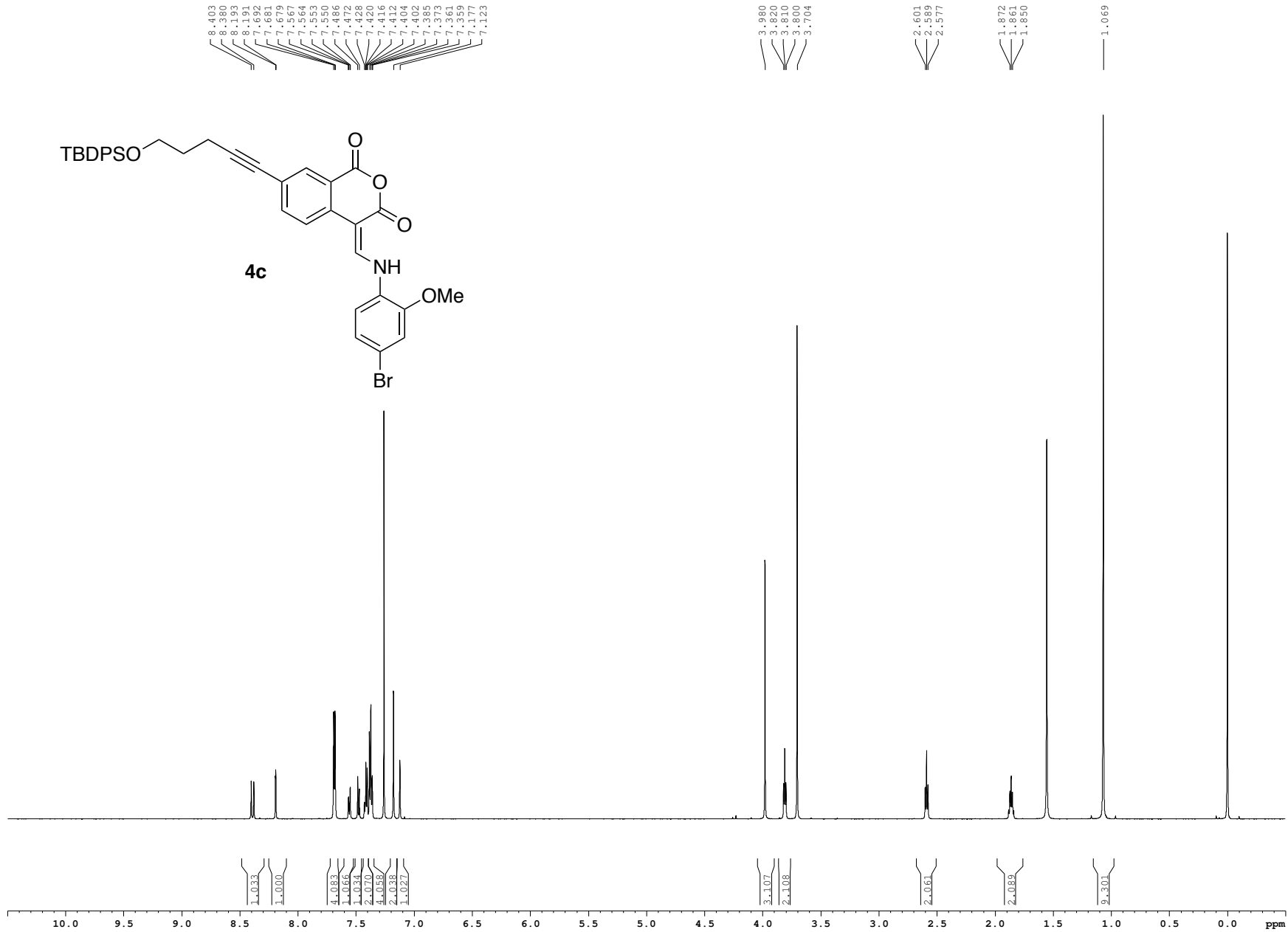
92.691

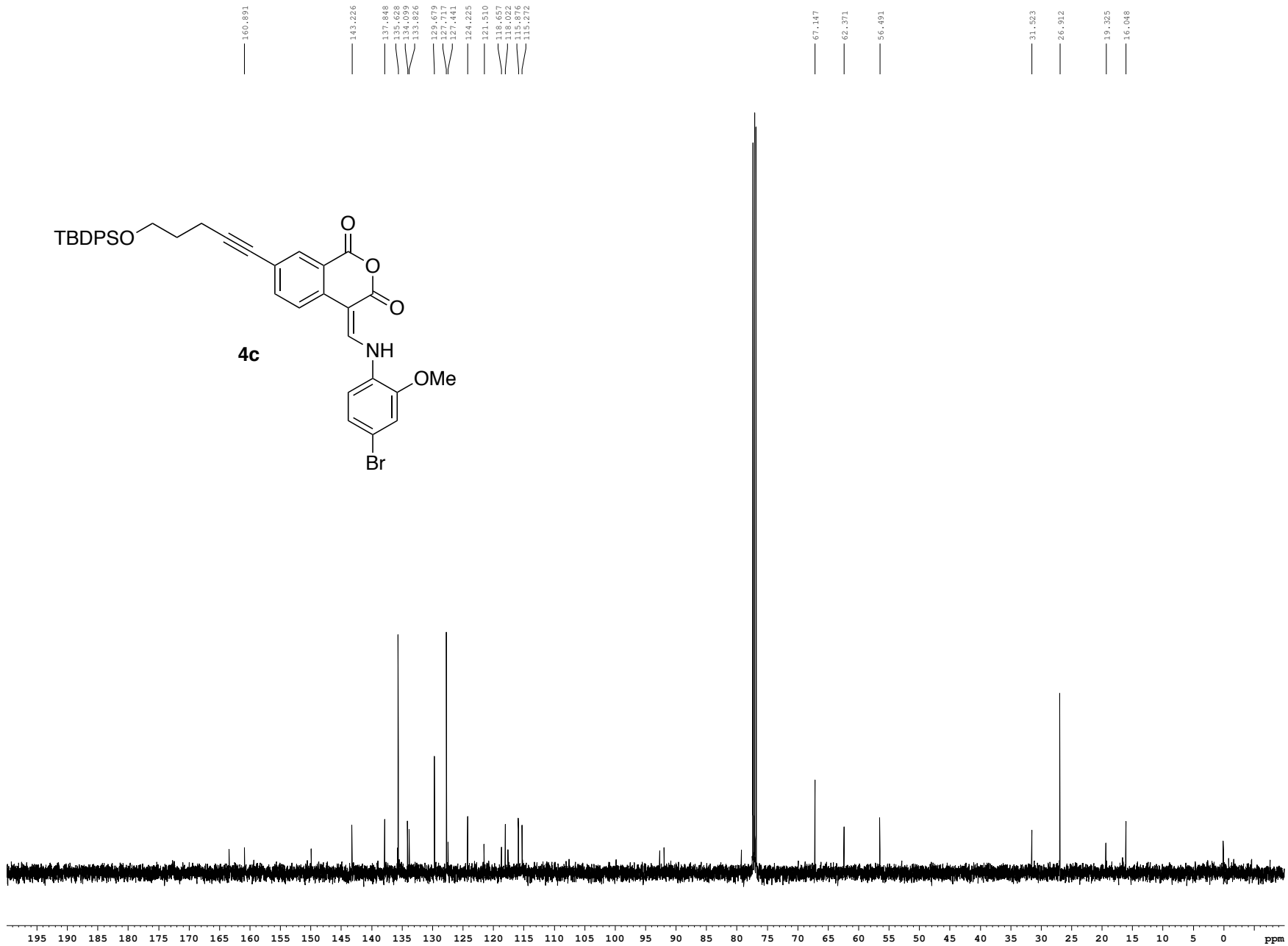
56.418

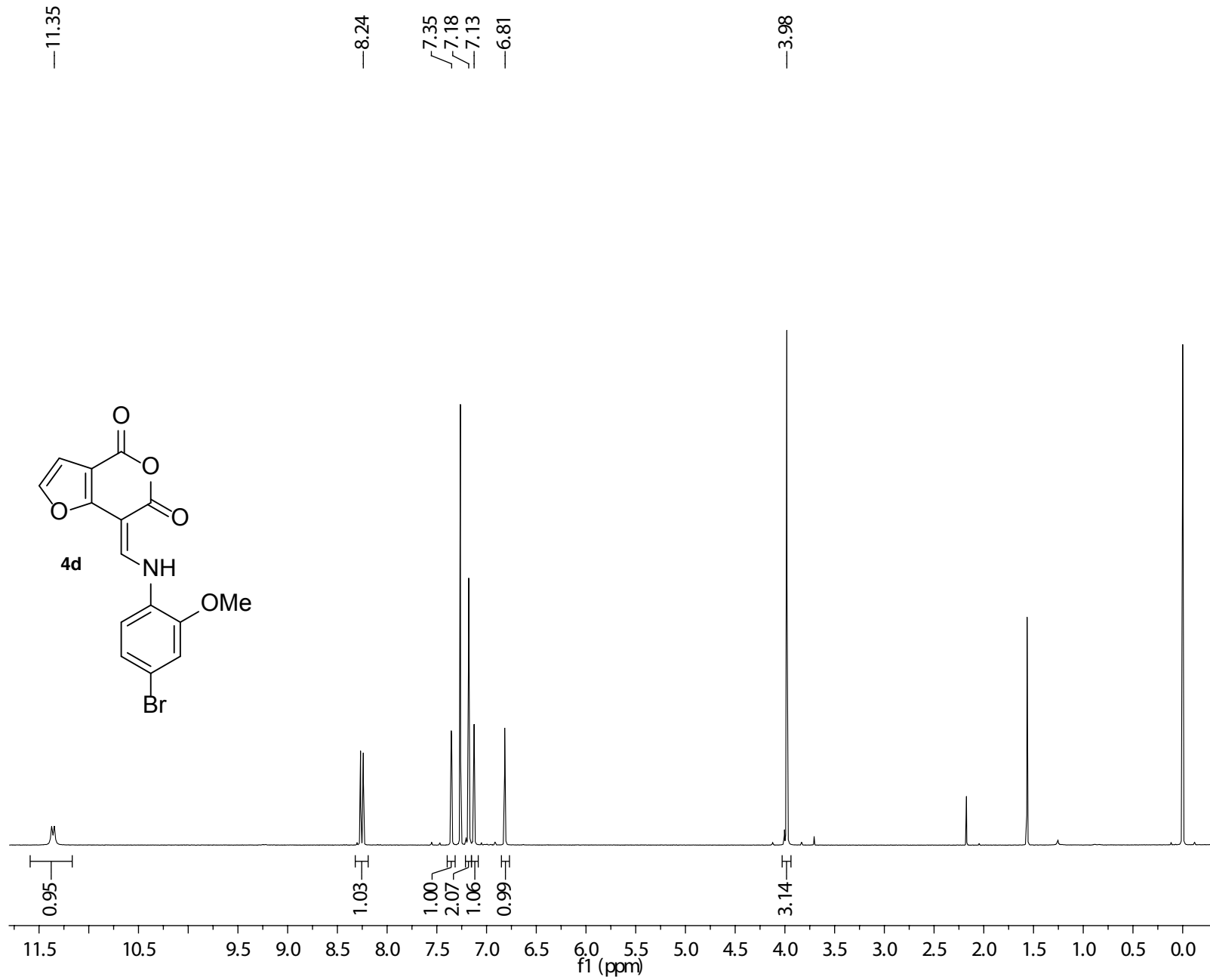


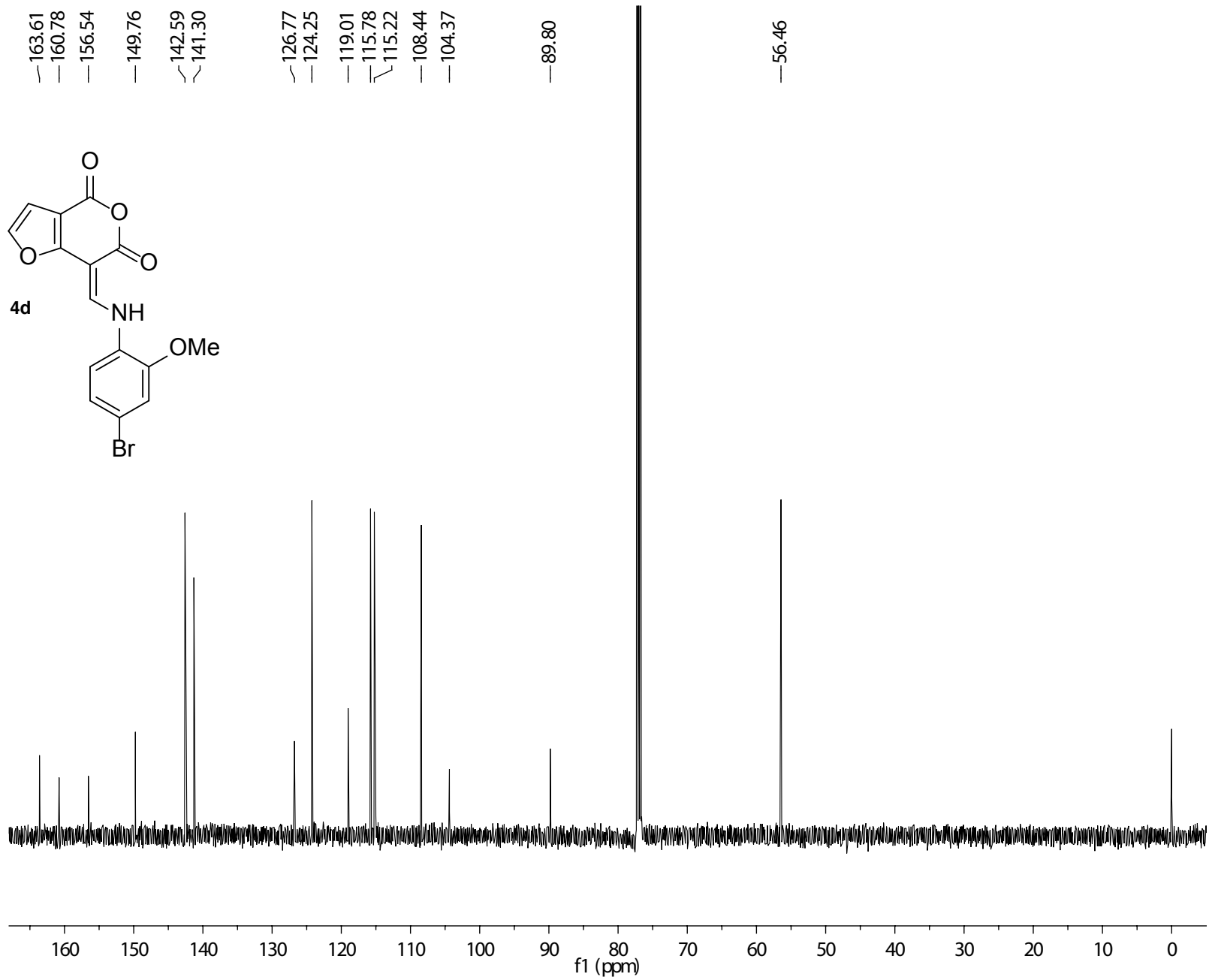


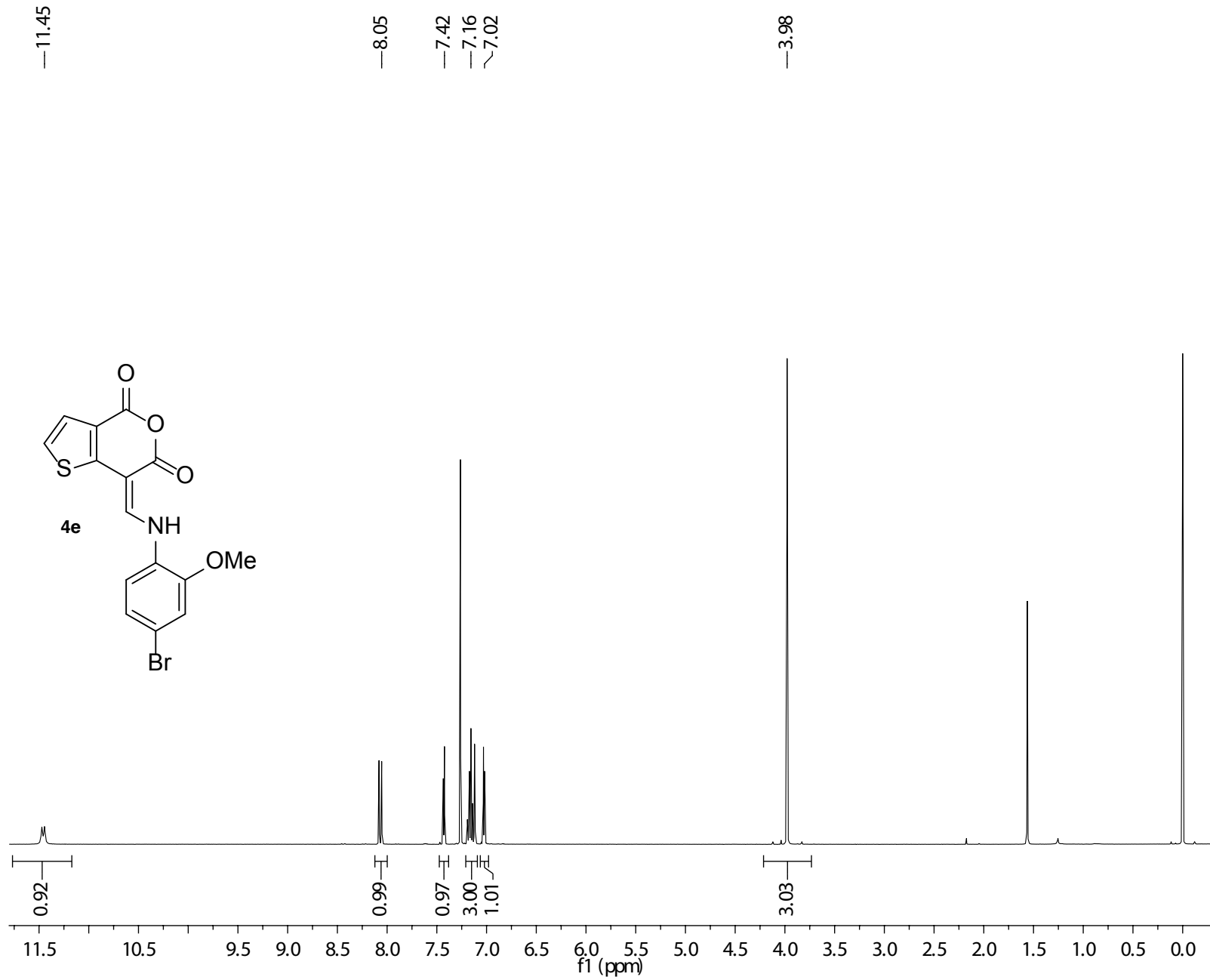


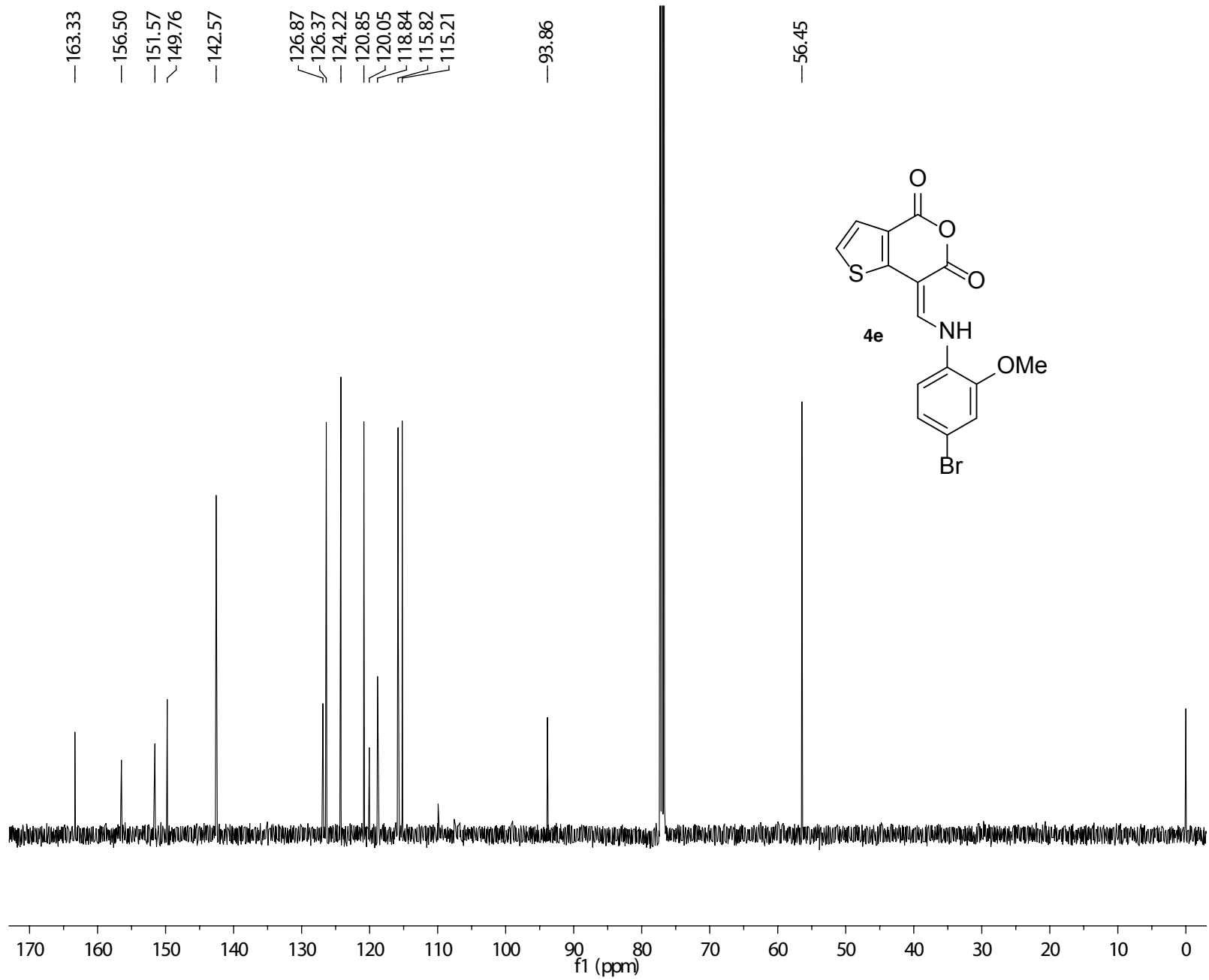


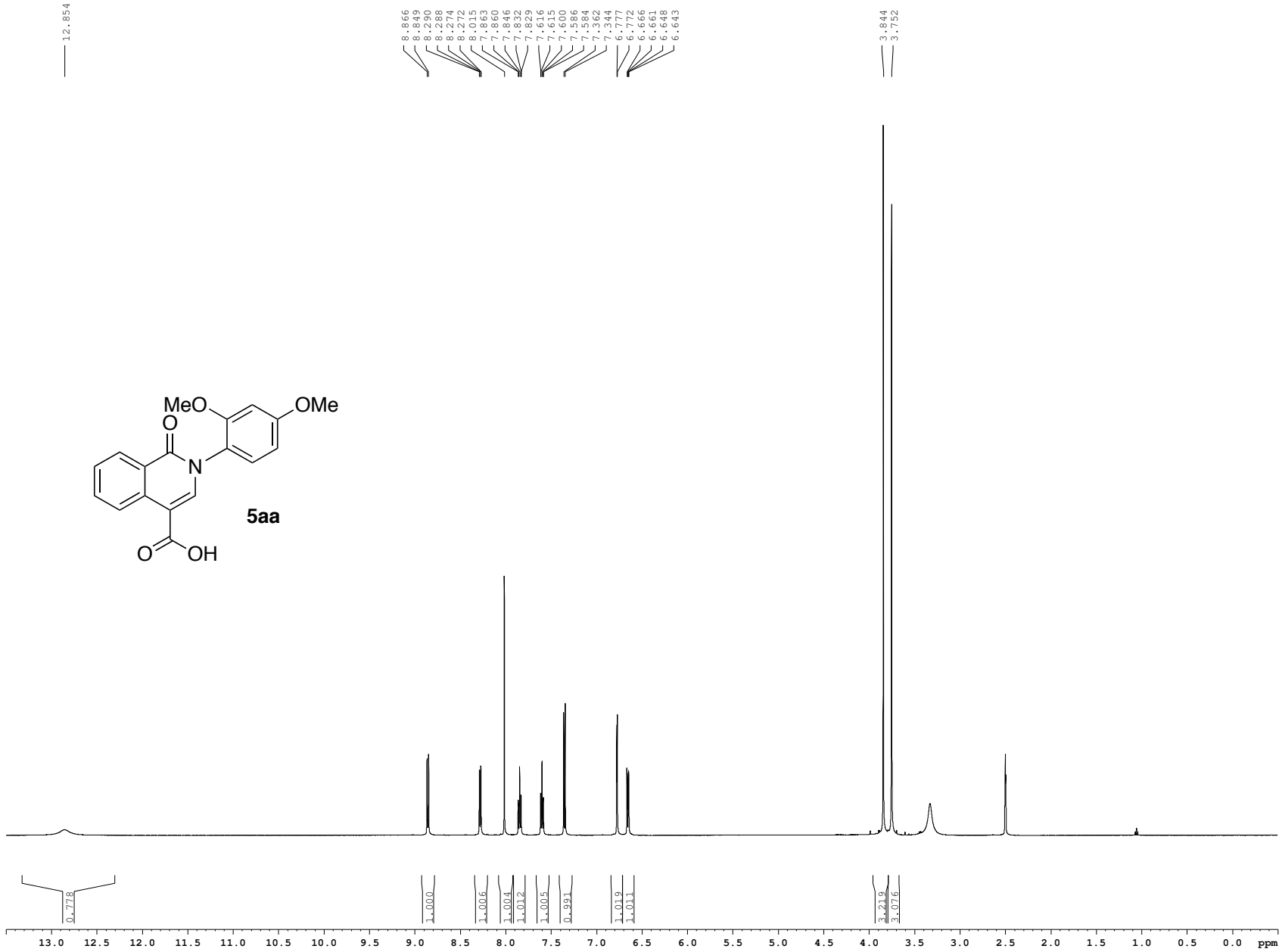












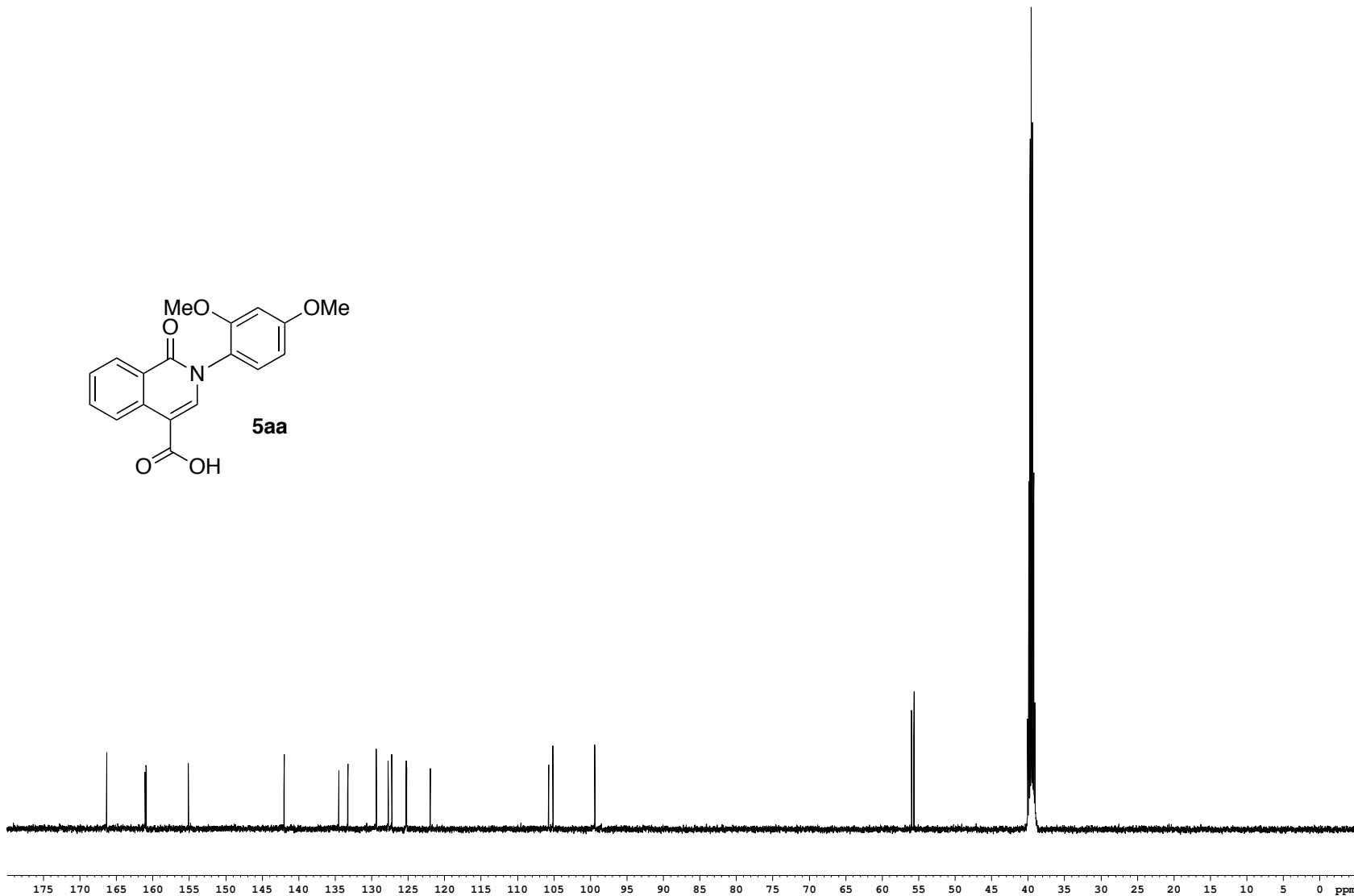
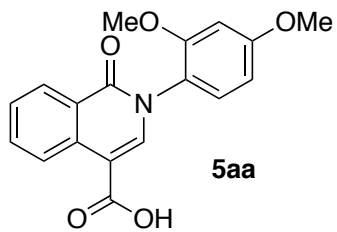
166.317
161.040
160.902
155.117

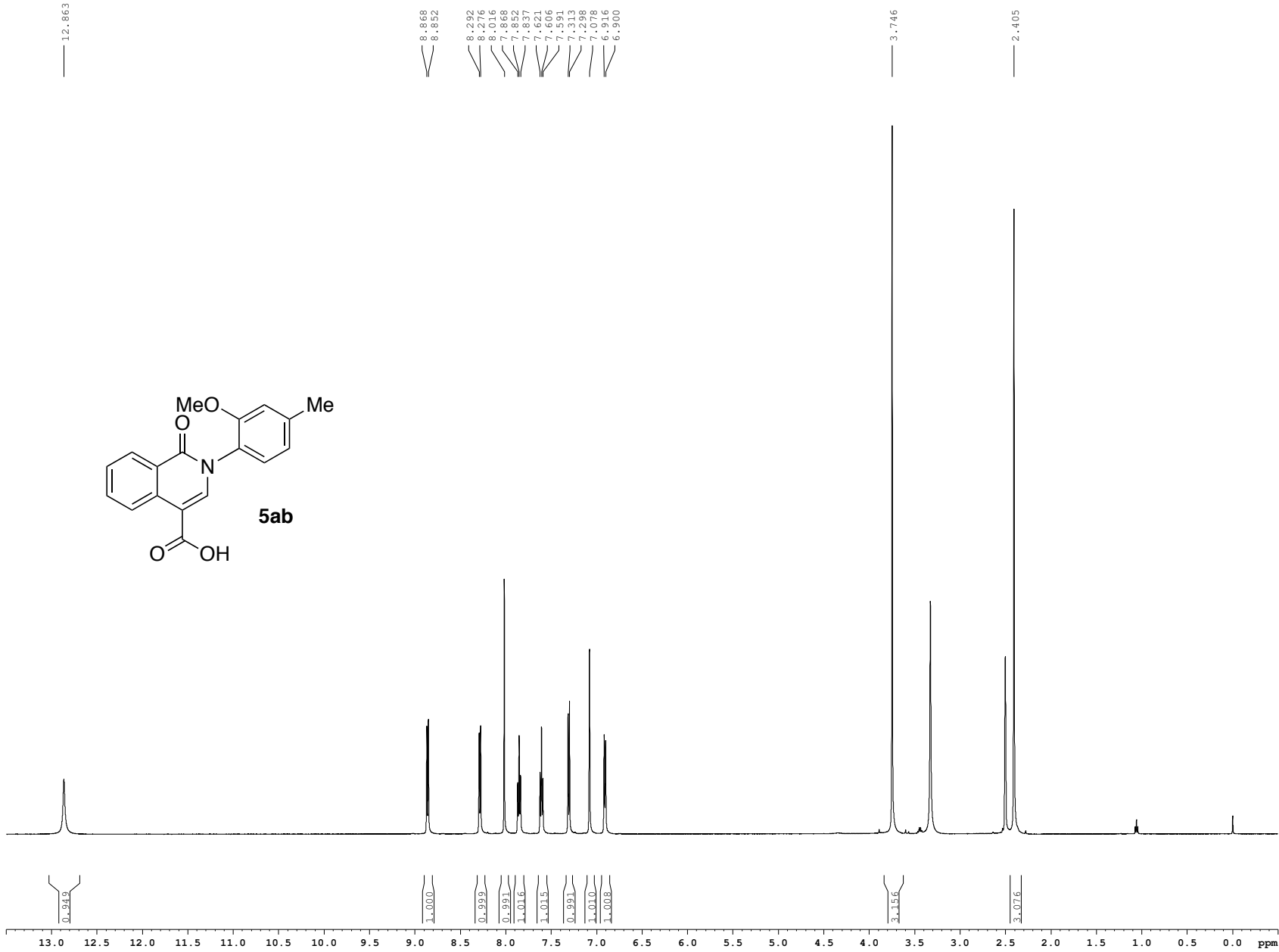
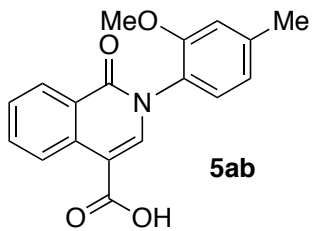
141.980

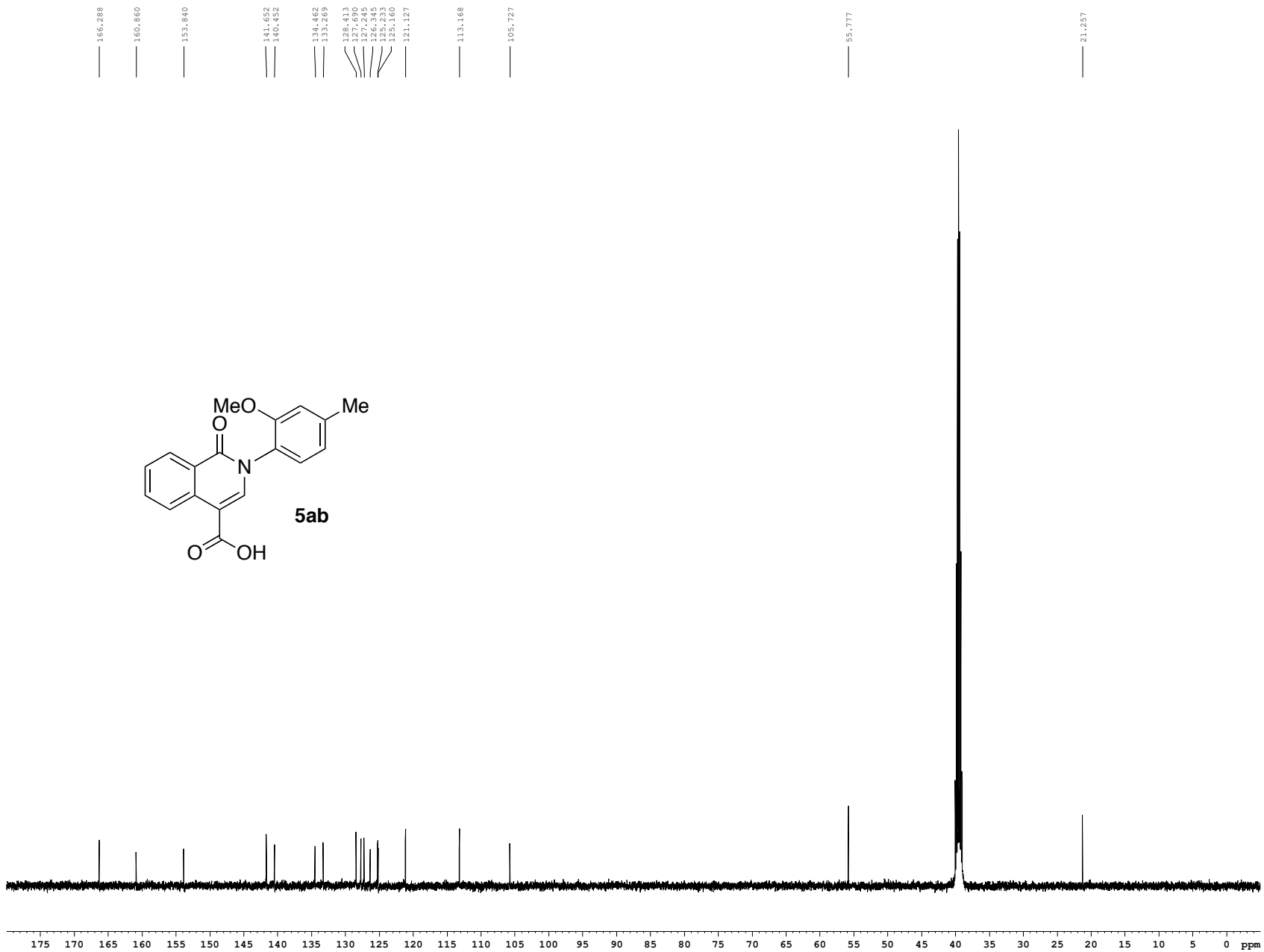
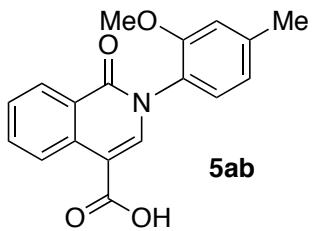
134.486
133.242
129.340
127.705
127.216
125.229
125.167
121.944

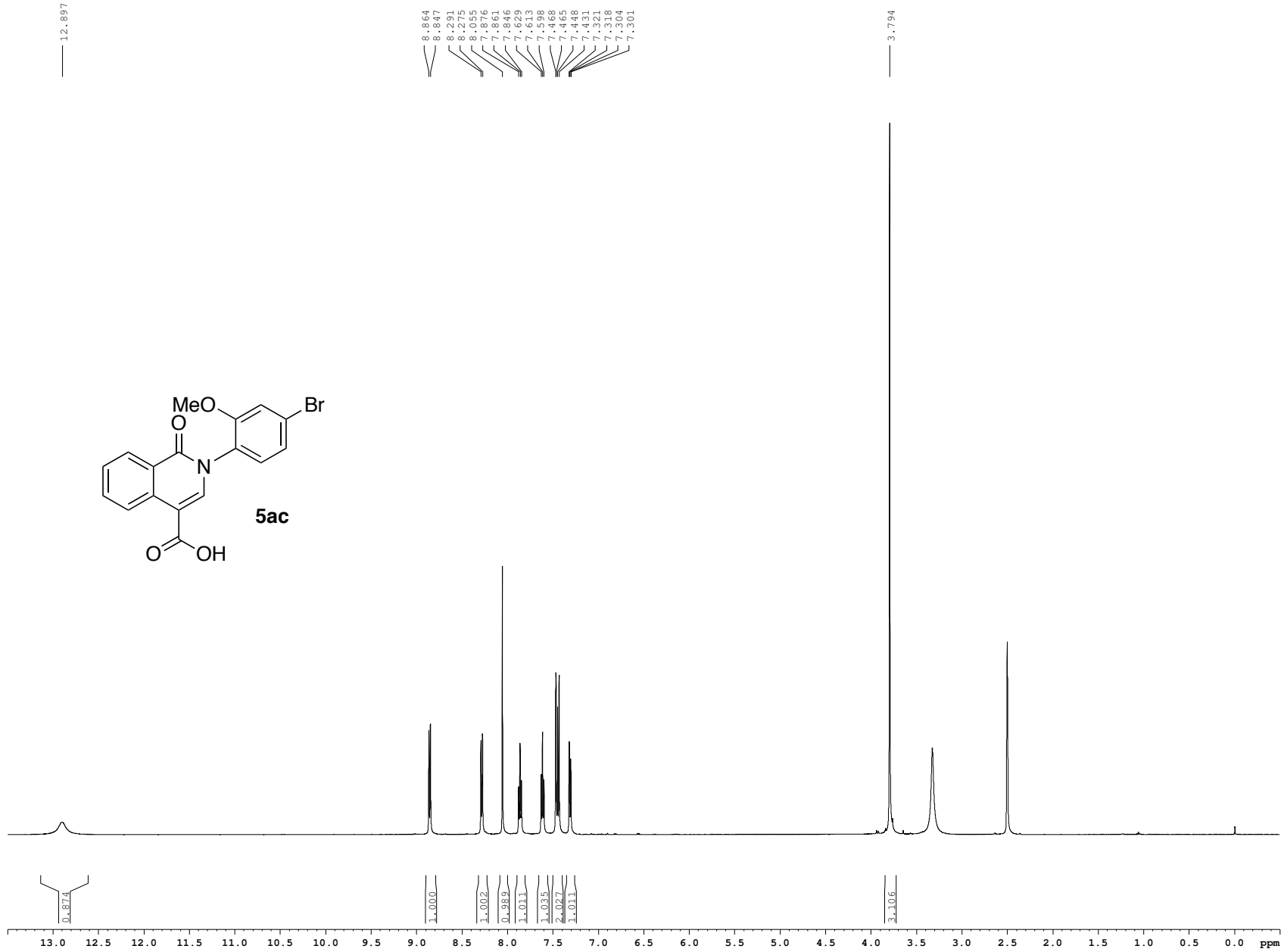
105.698
105.126
99.377

55.947
55.601









166.248

160.676

155.131

141.103

134.456

133.413

130.585

128.181

127.951

125.294

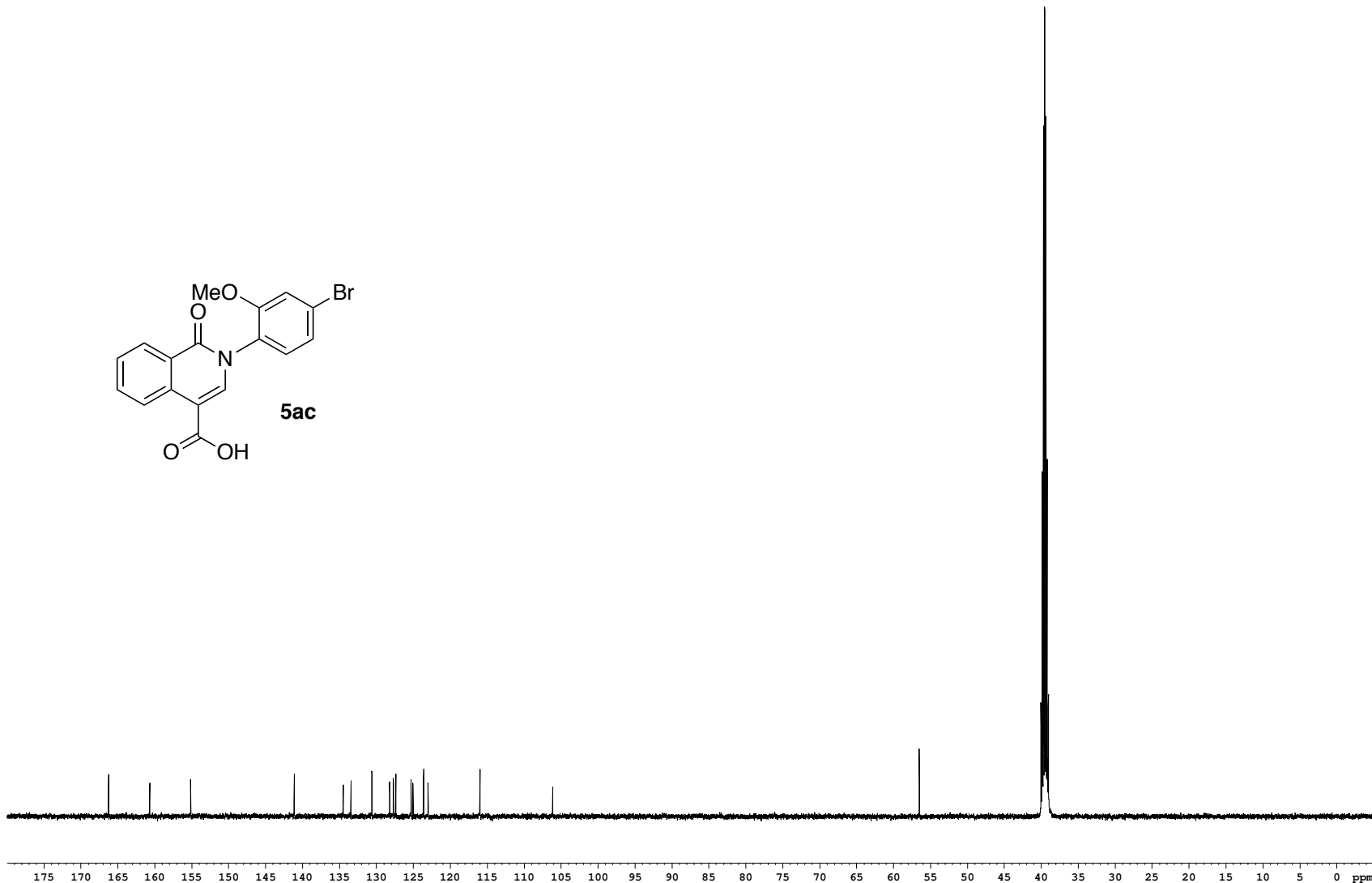
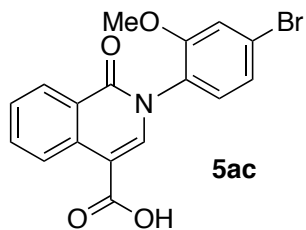
125.029

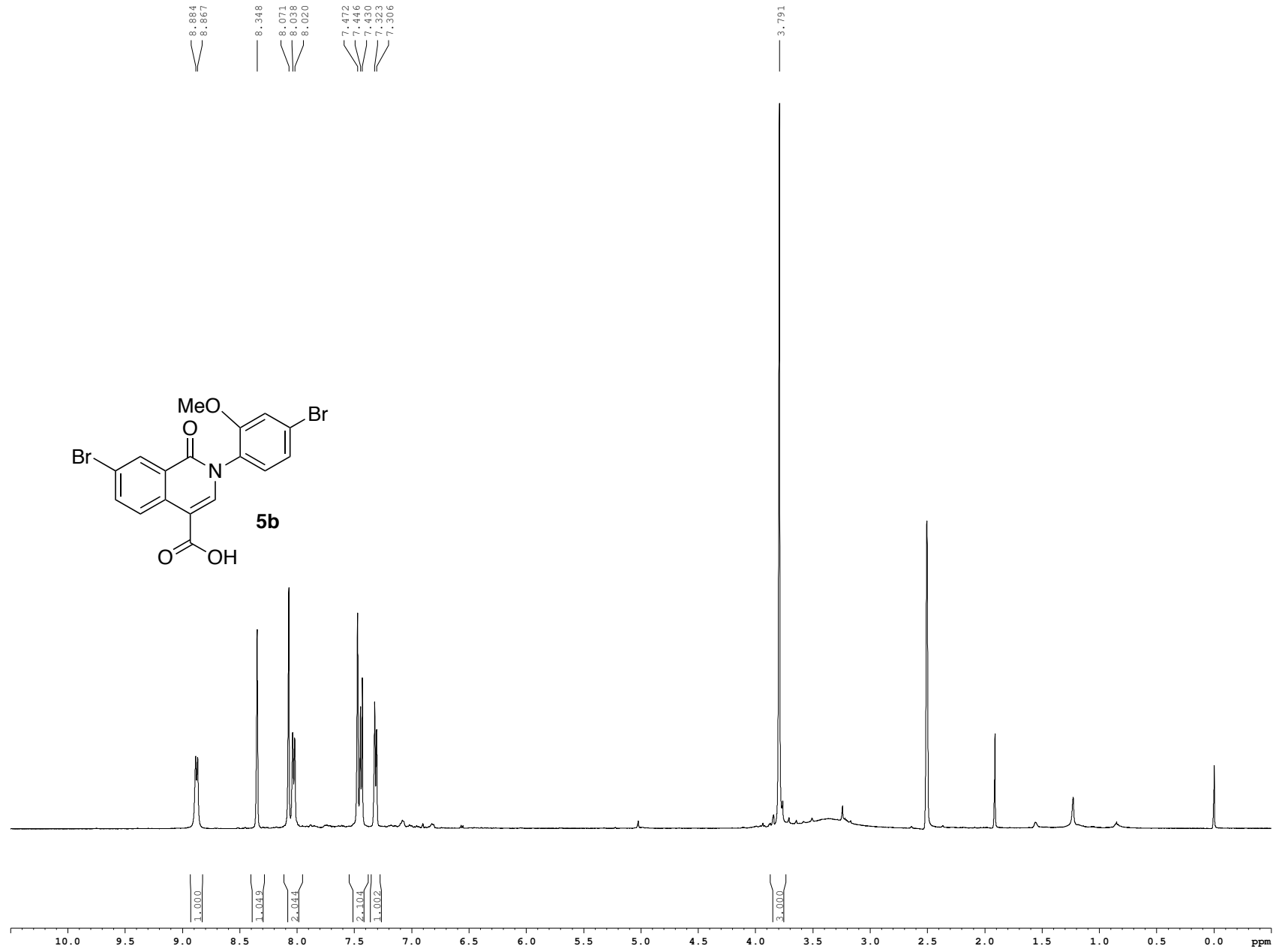
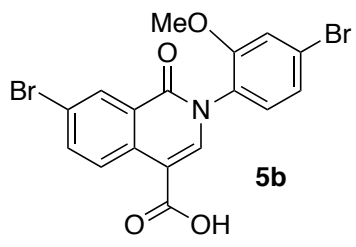
122.984

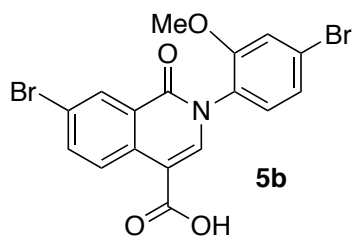
115.954

106.109

56.487



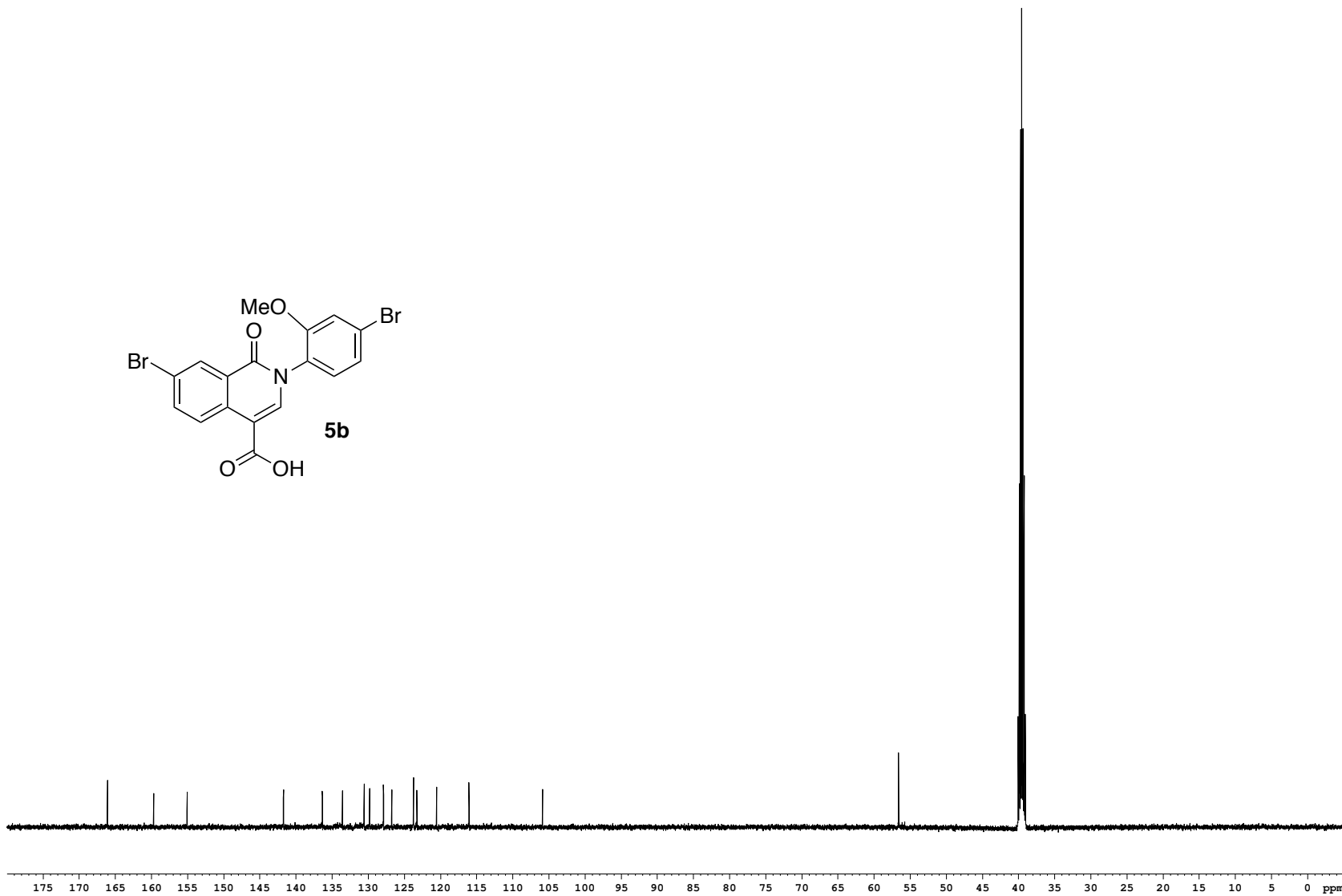


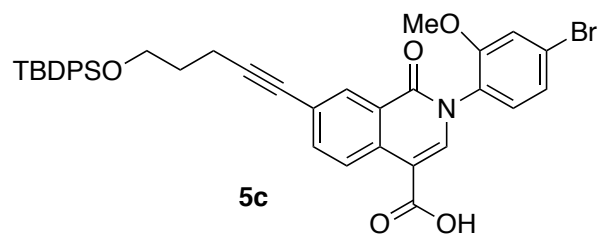


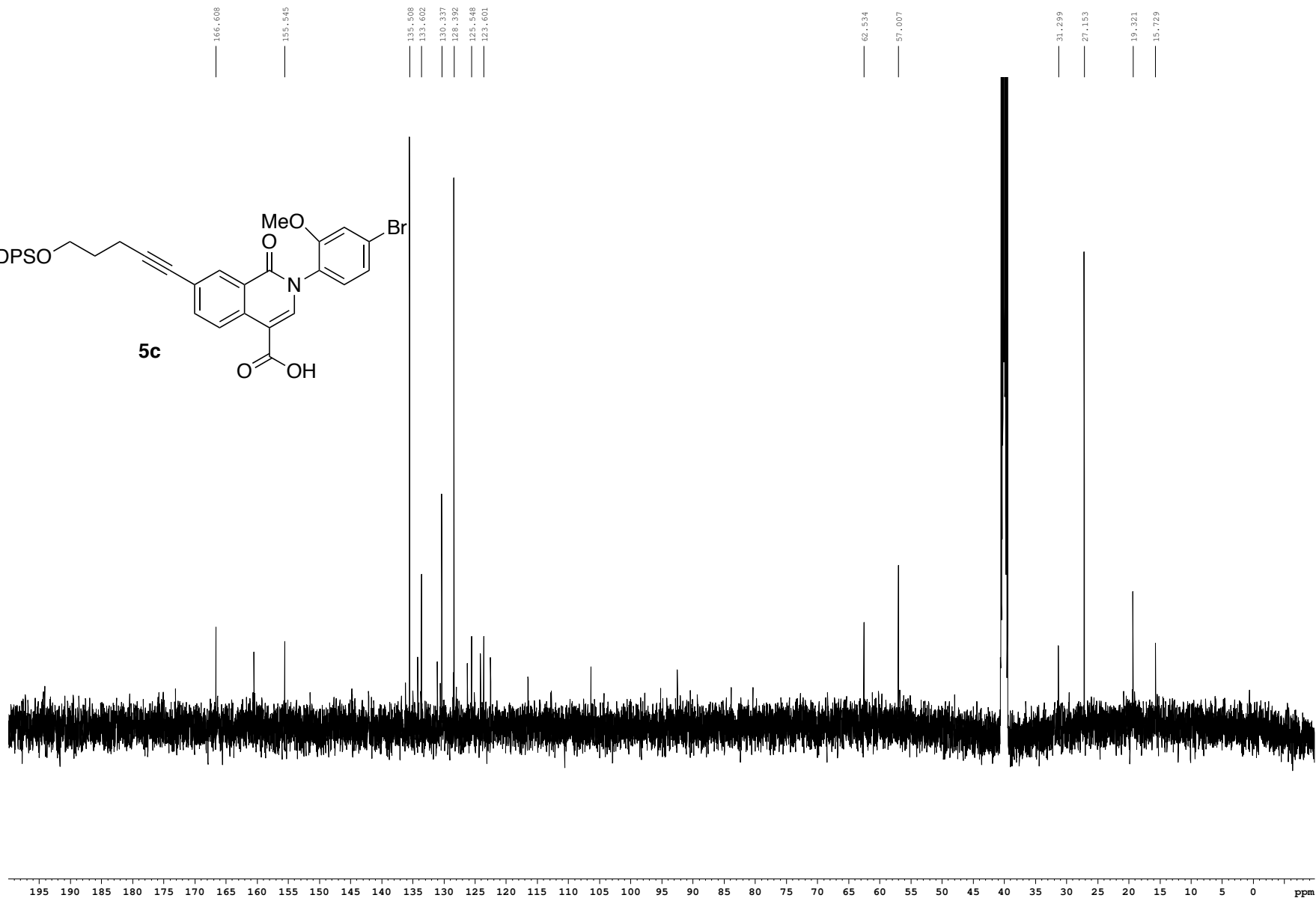
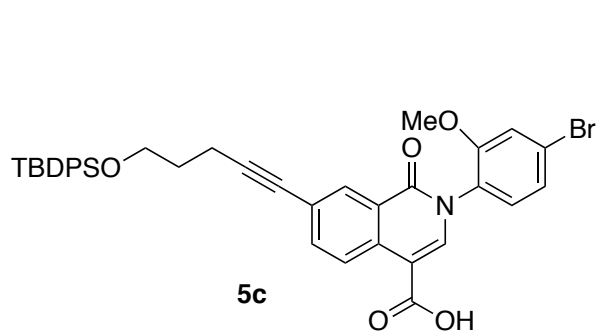
166.515
160.094
155.496
142.151
136.769
133.981
130.984
130.210
128.332
127.166
124.126
123.678
120.941
116.467

106.278

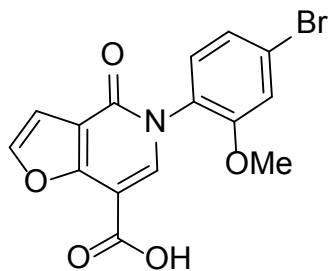
57.011



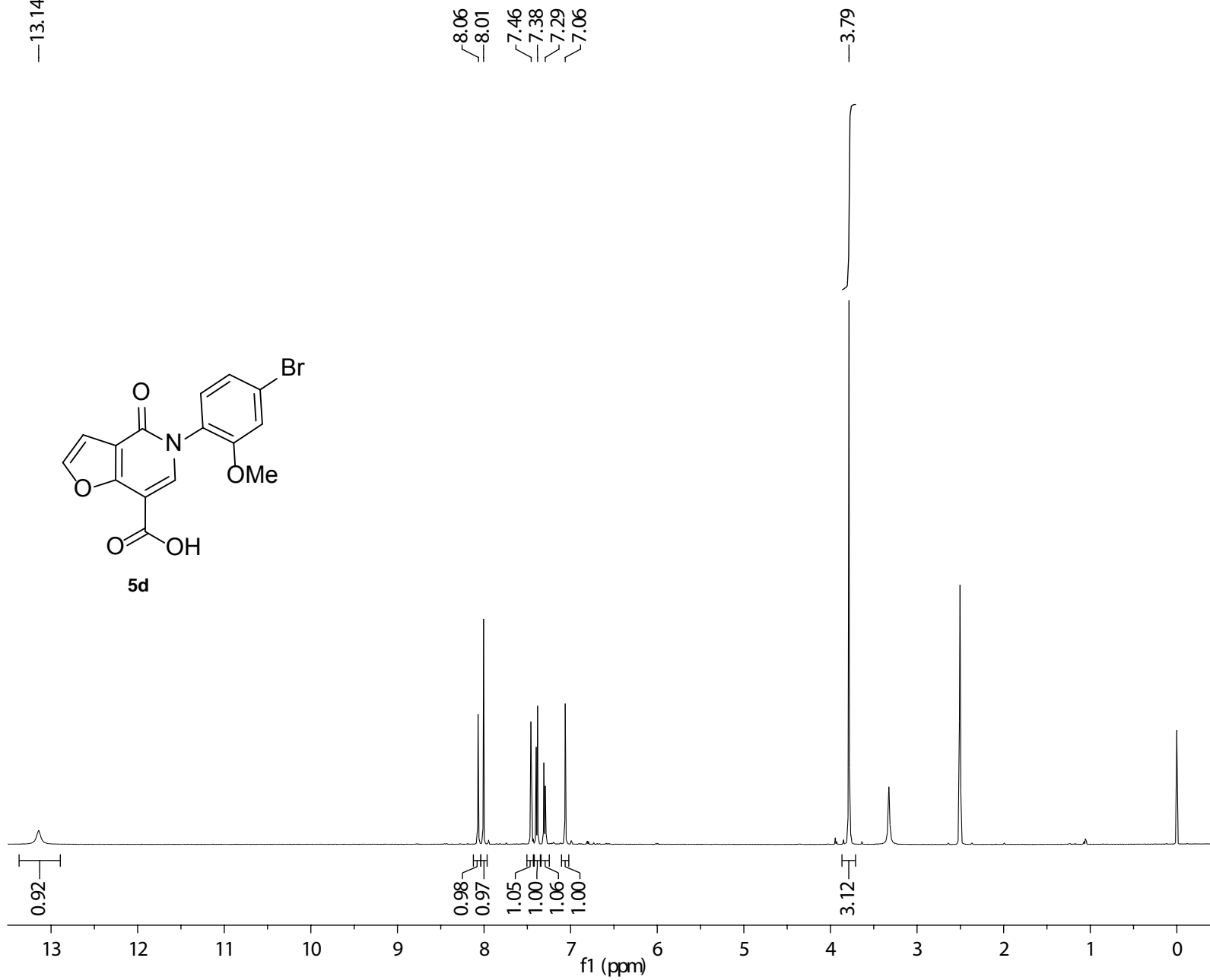


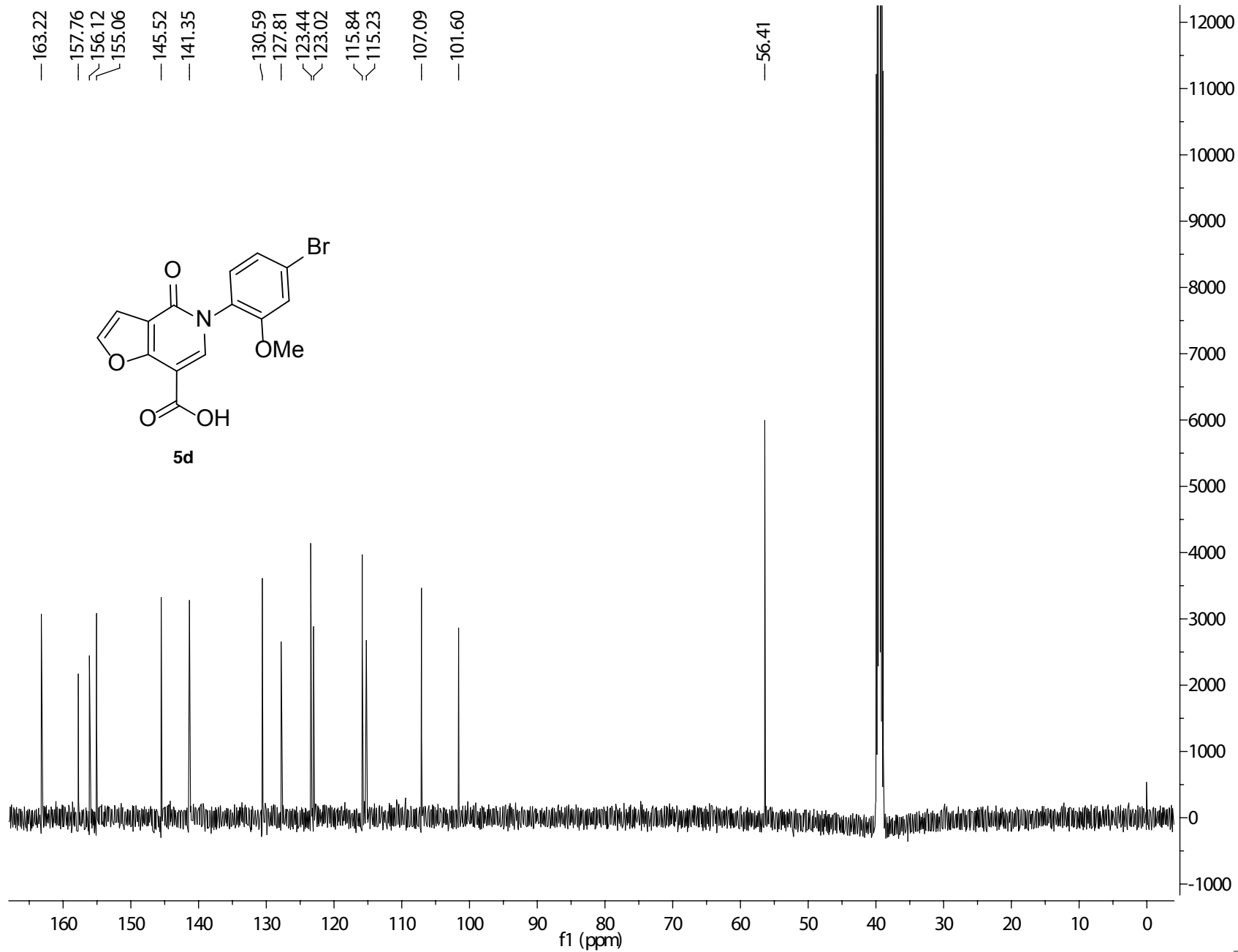


—13.14



5d



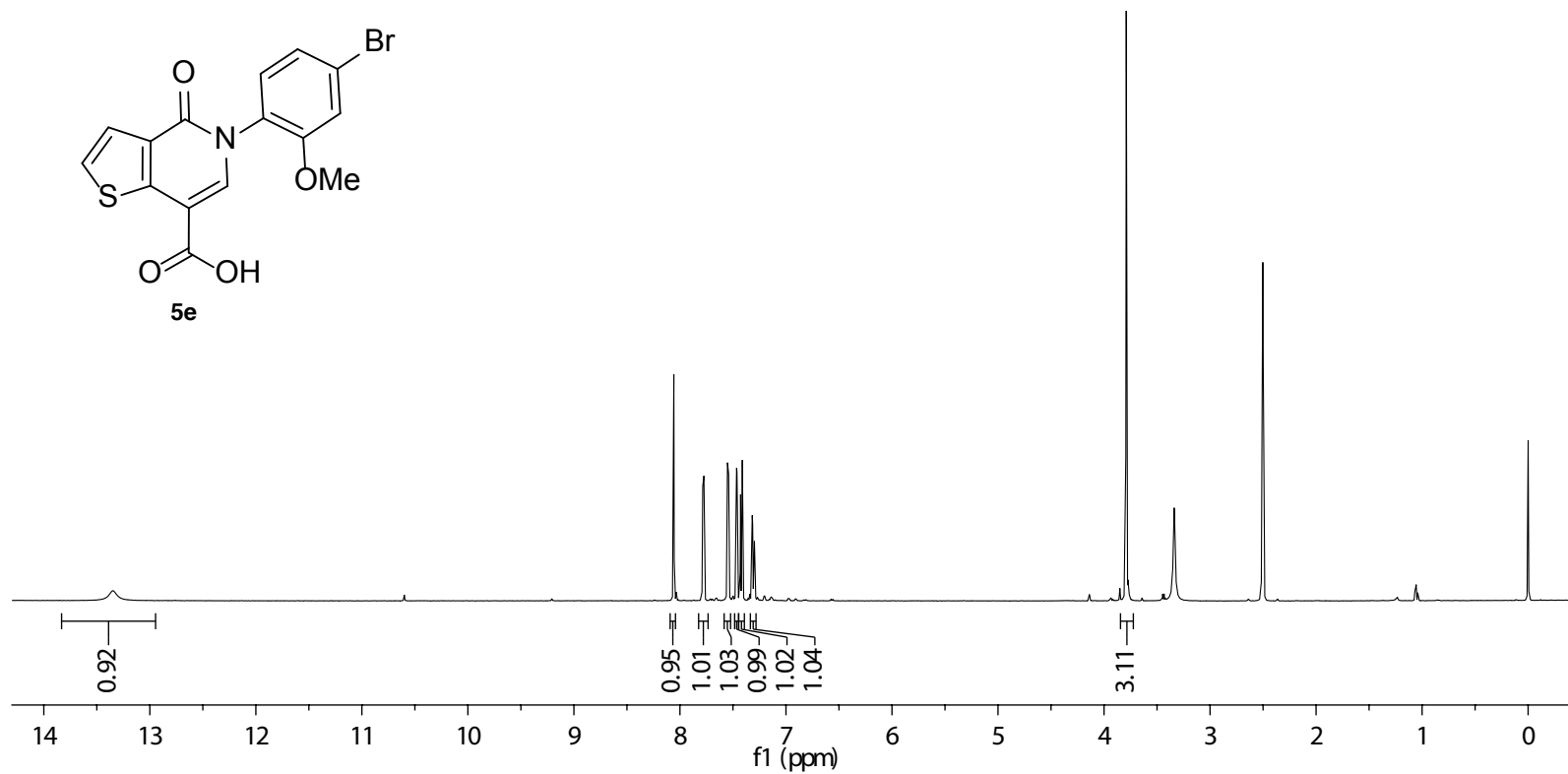
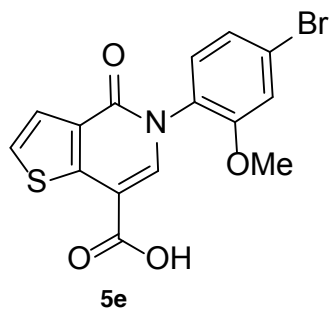


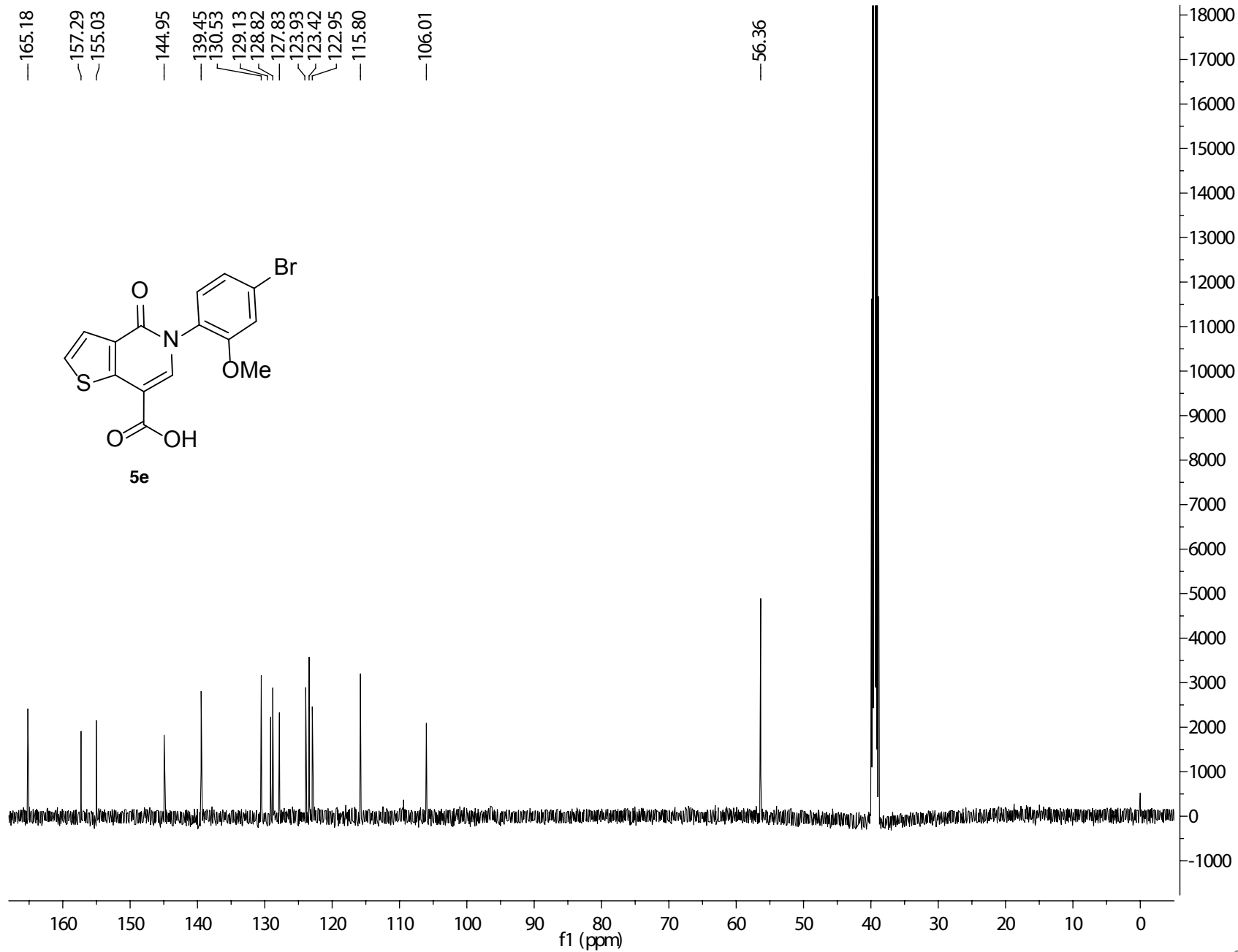
—13.35

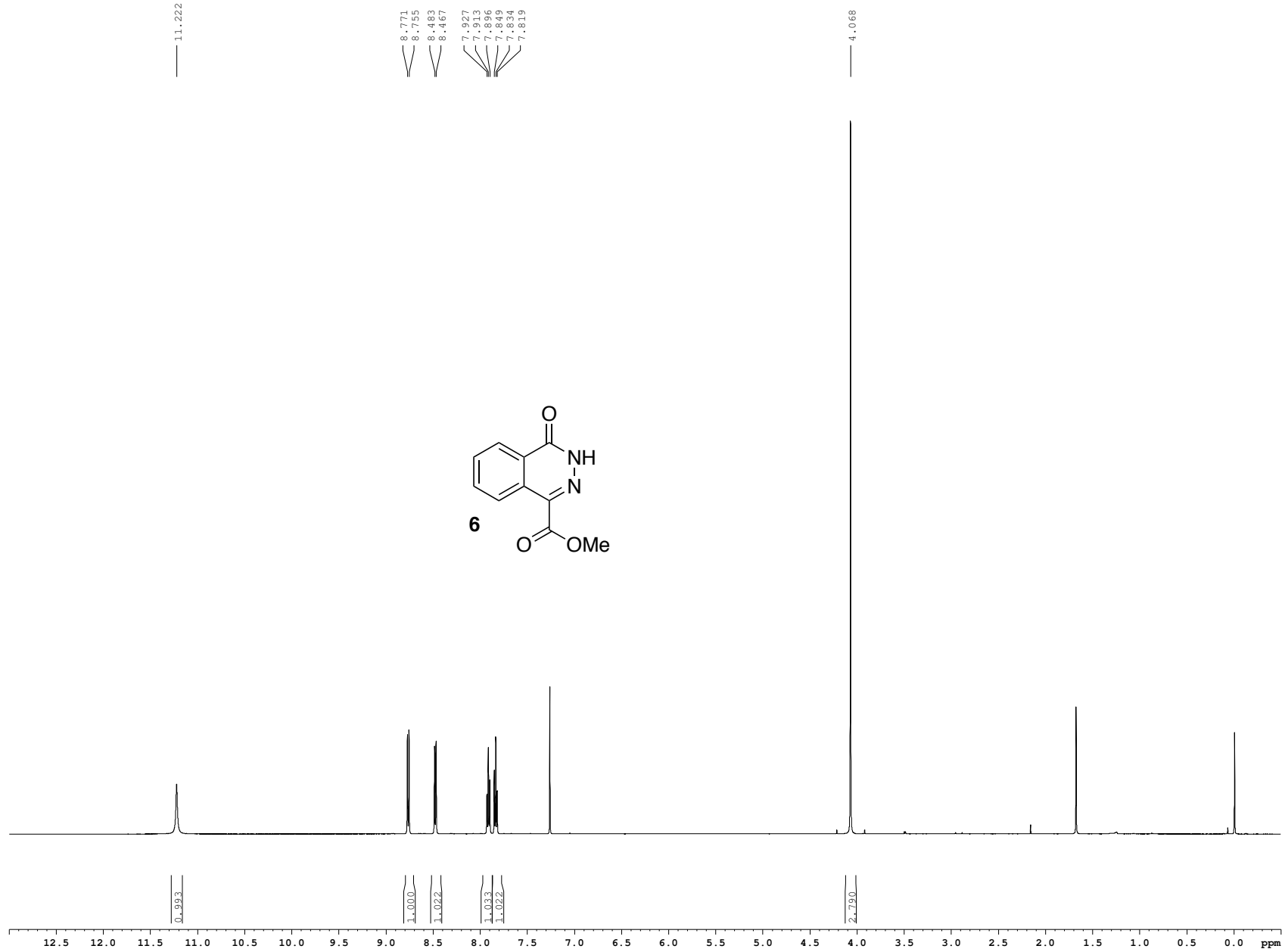
—8.06
—7.77
—7.54
—7.47
—7.43
—7.31

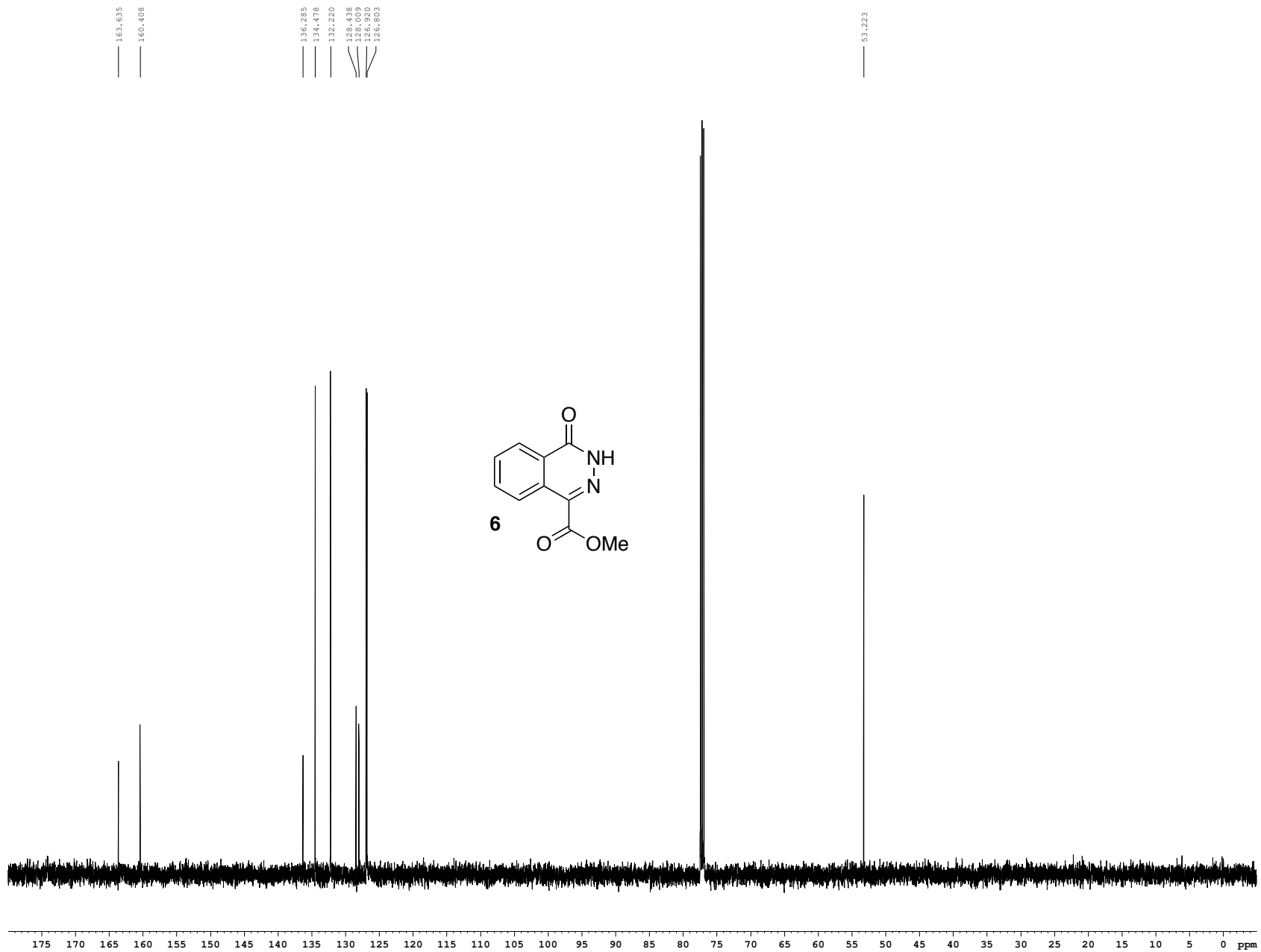
—3.79

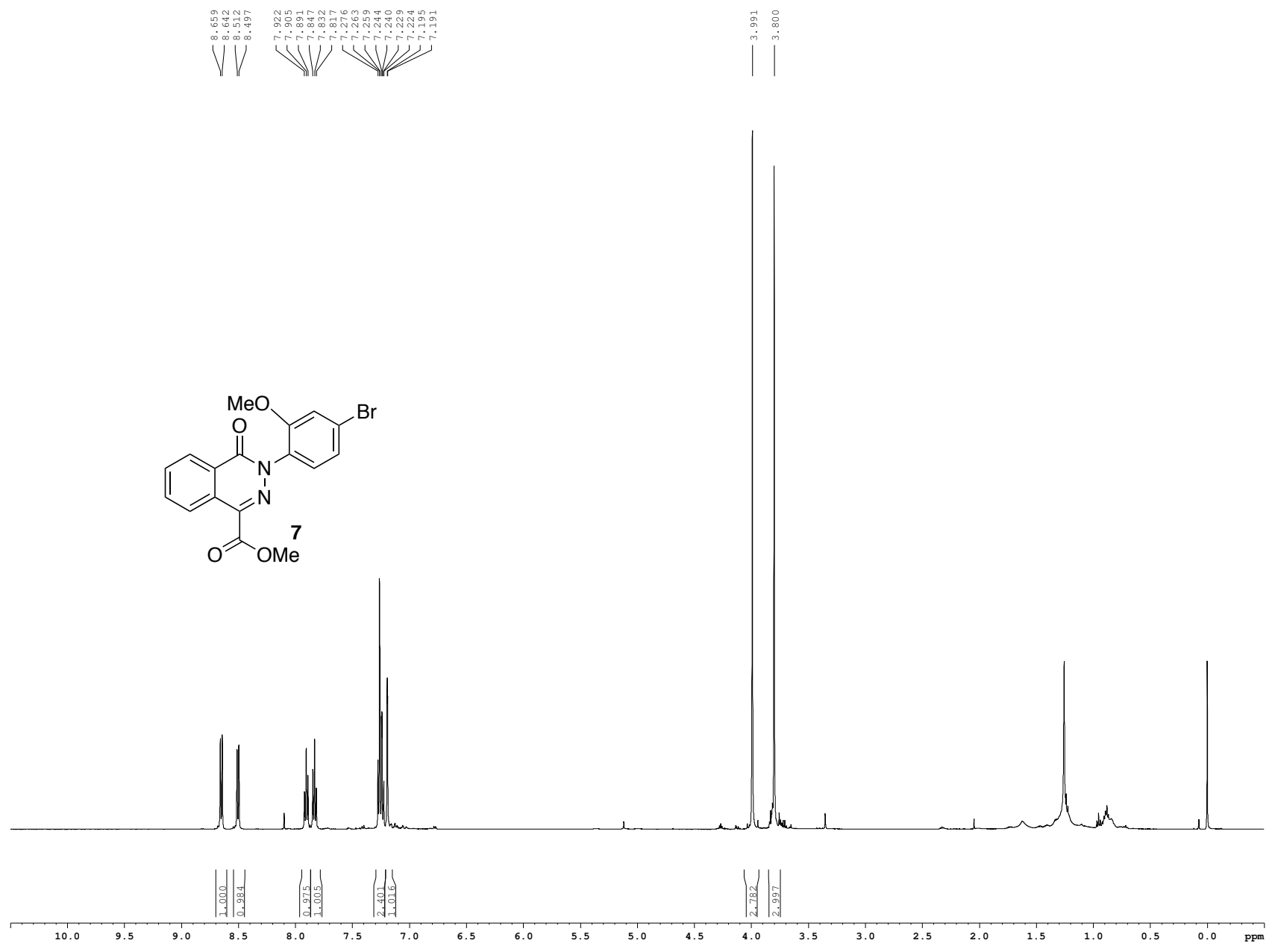
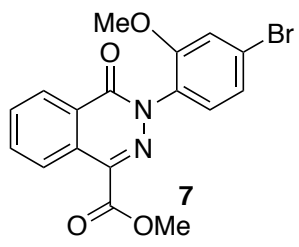
—0.00

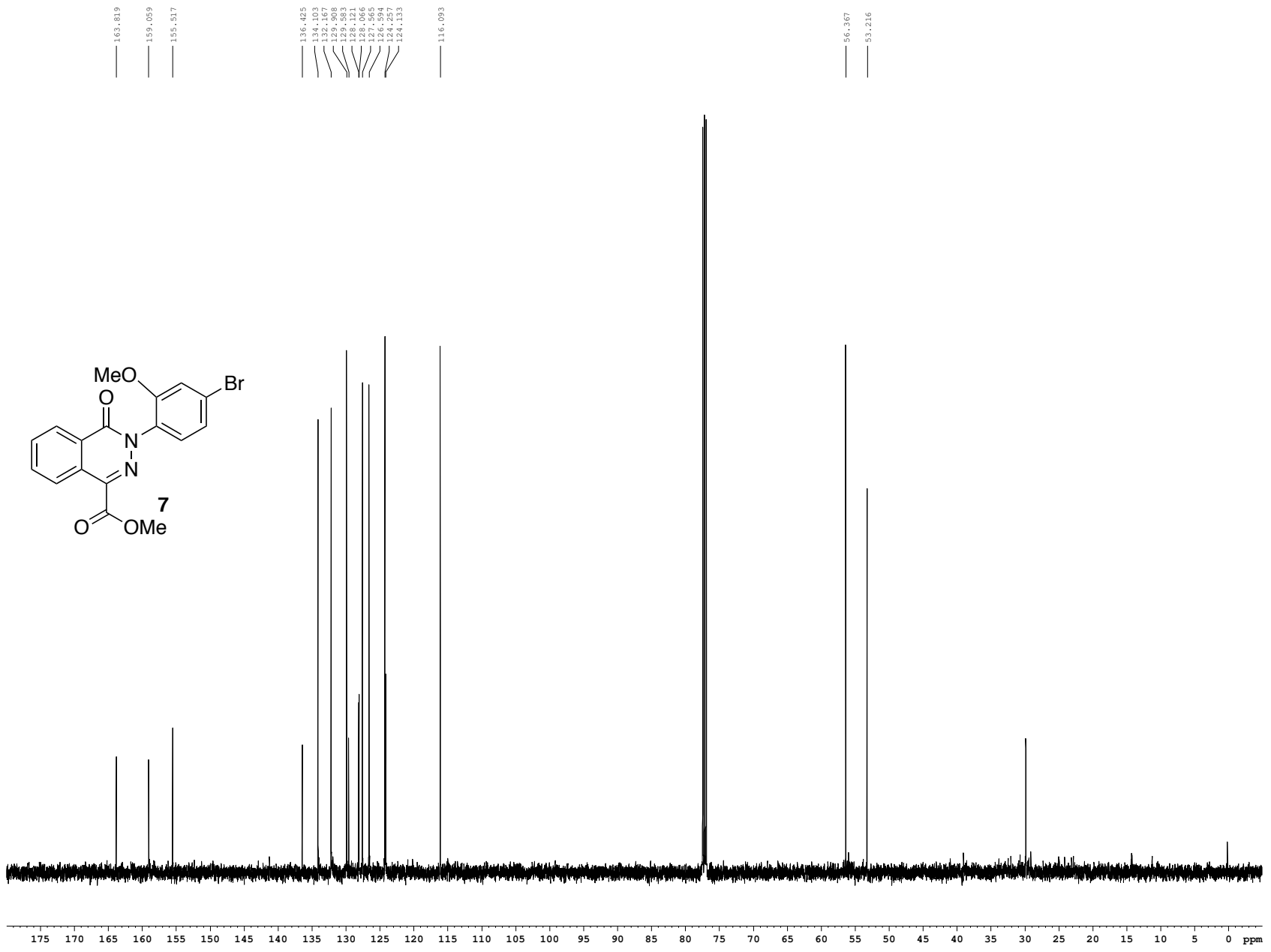
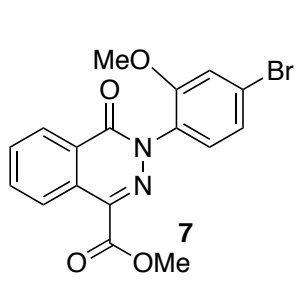


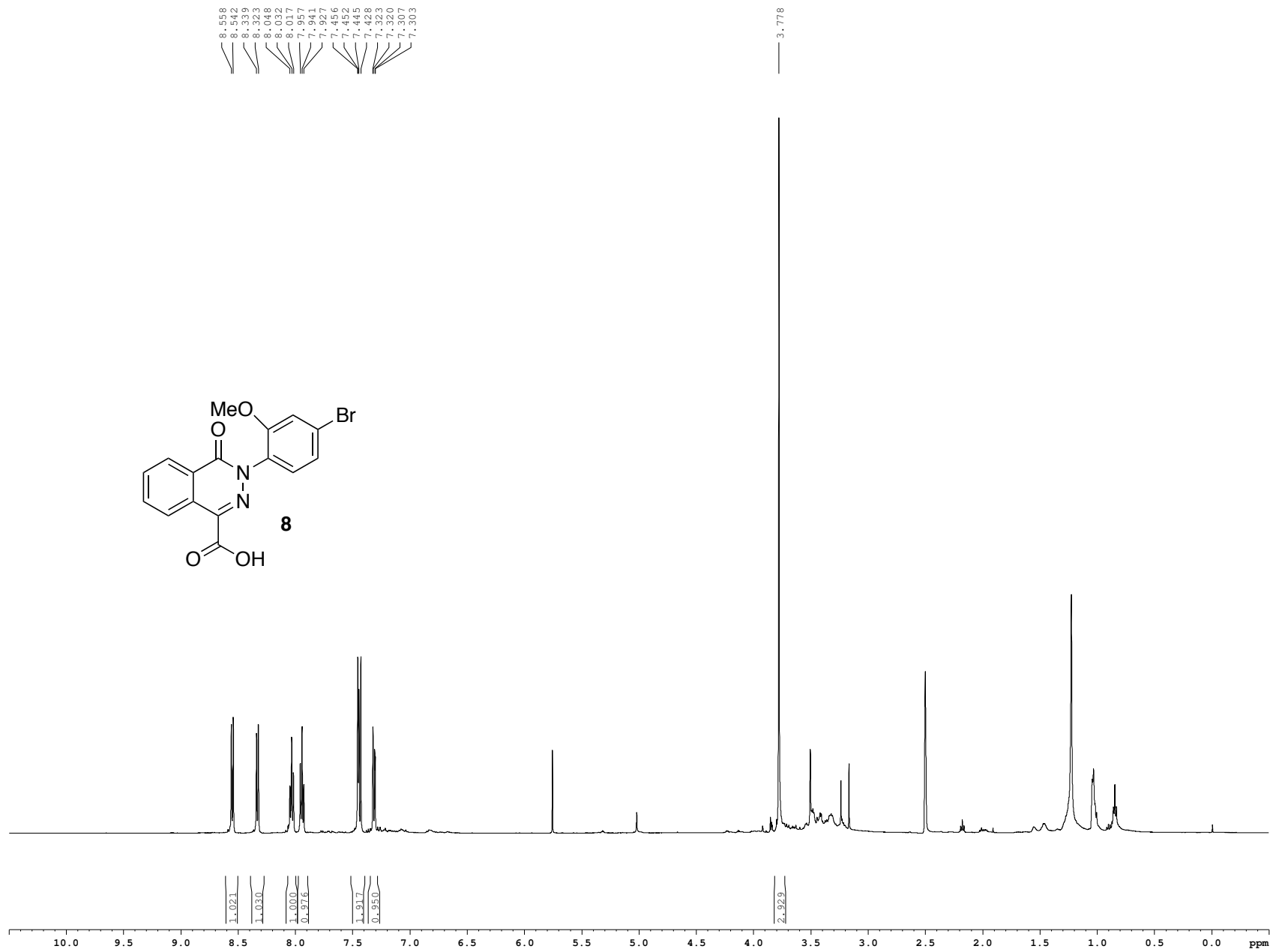
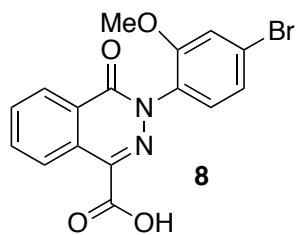


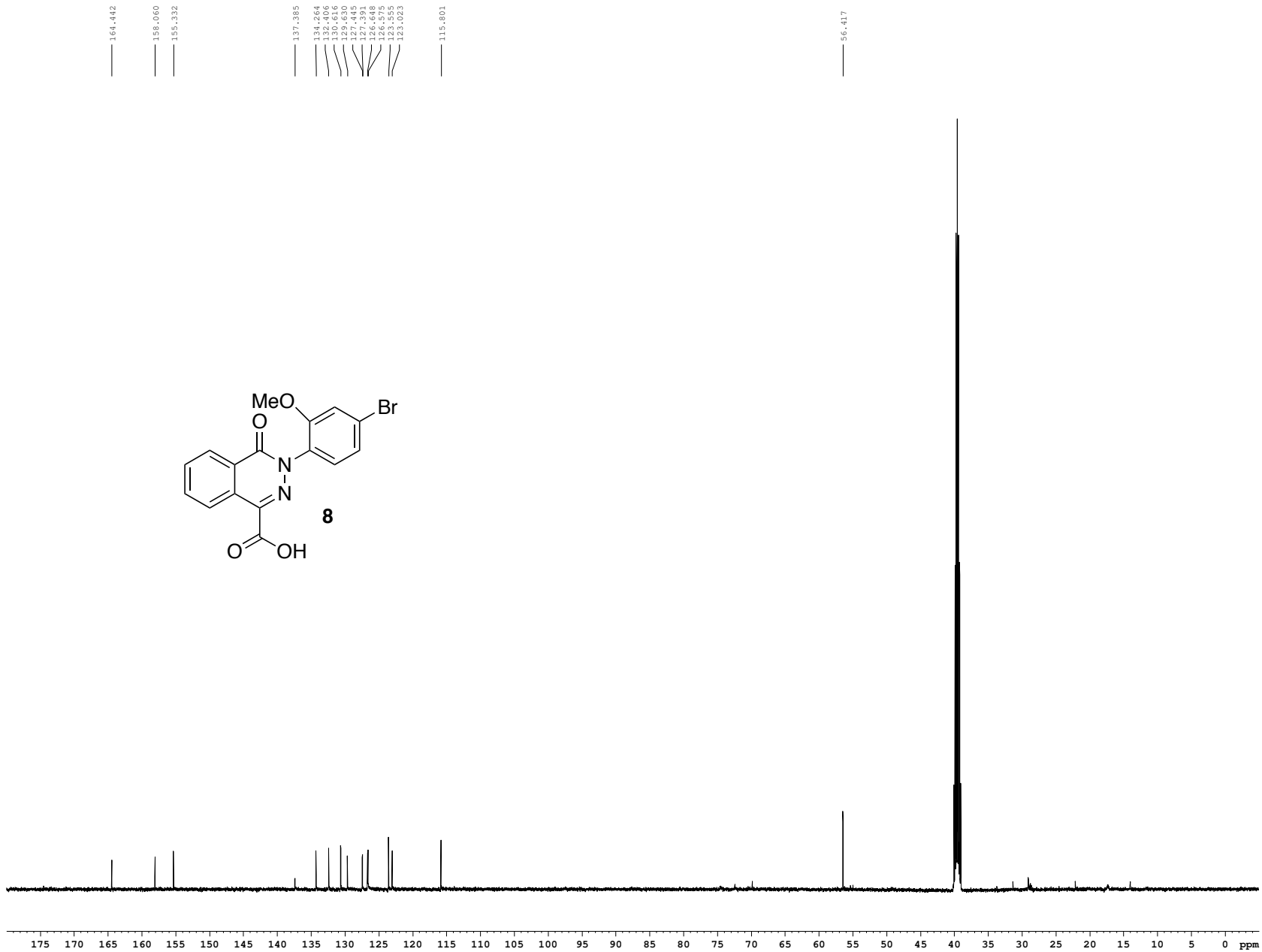
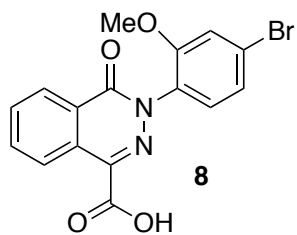


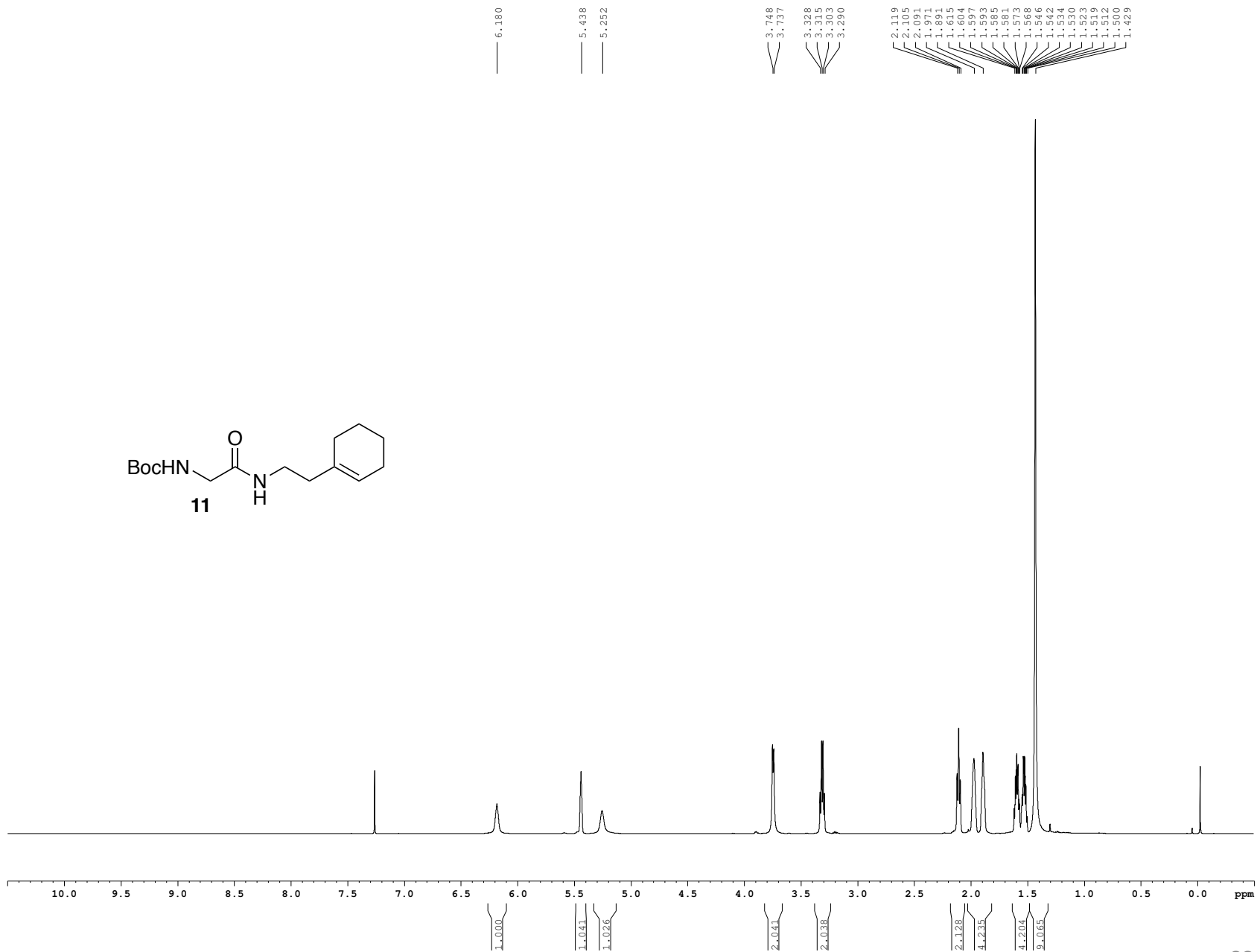
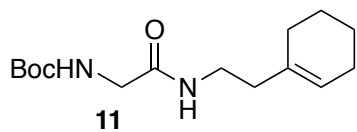


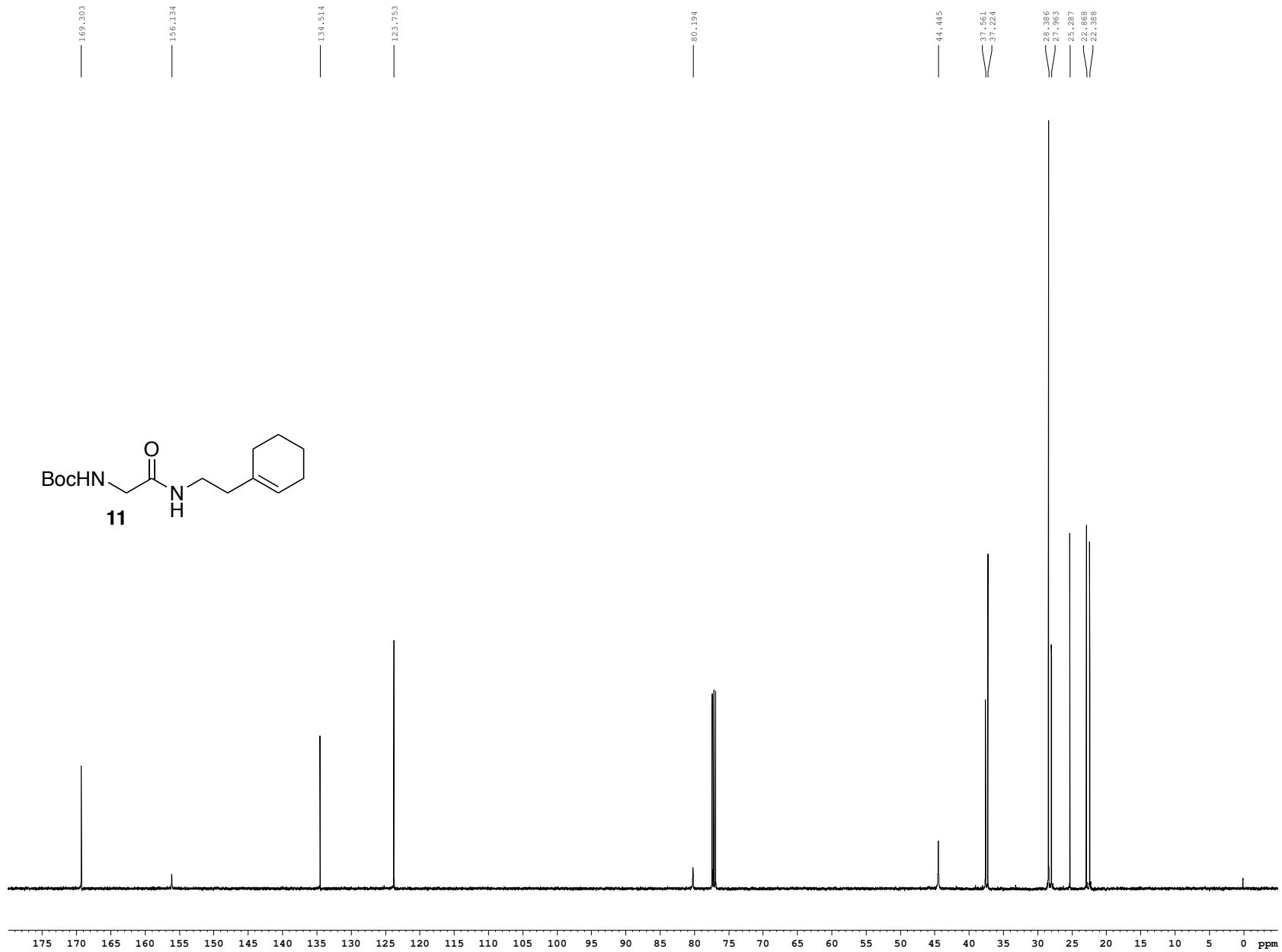
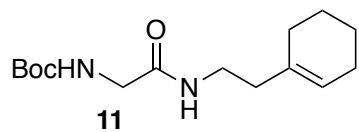


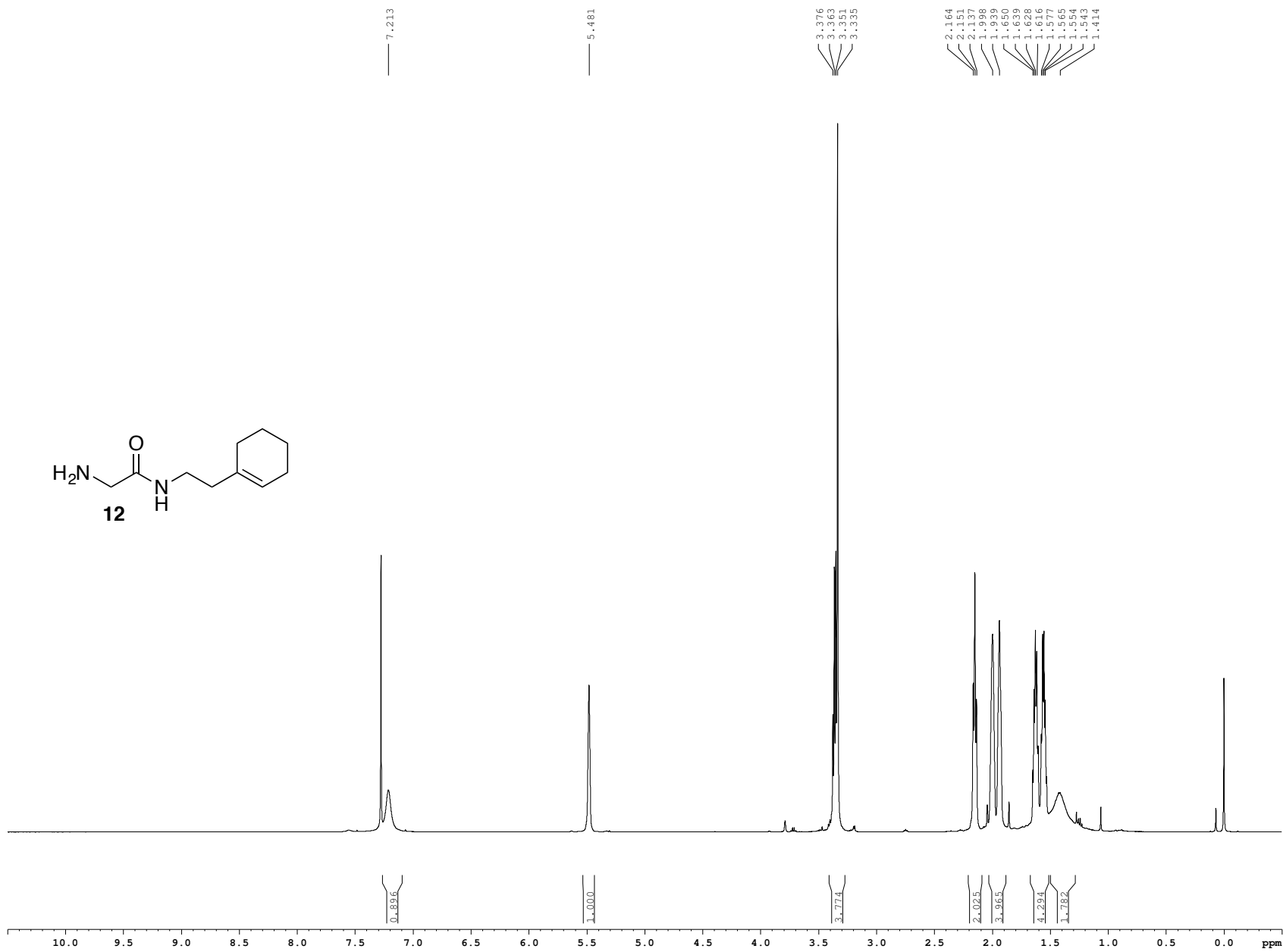
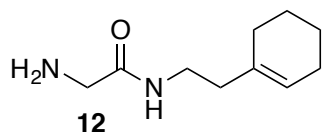


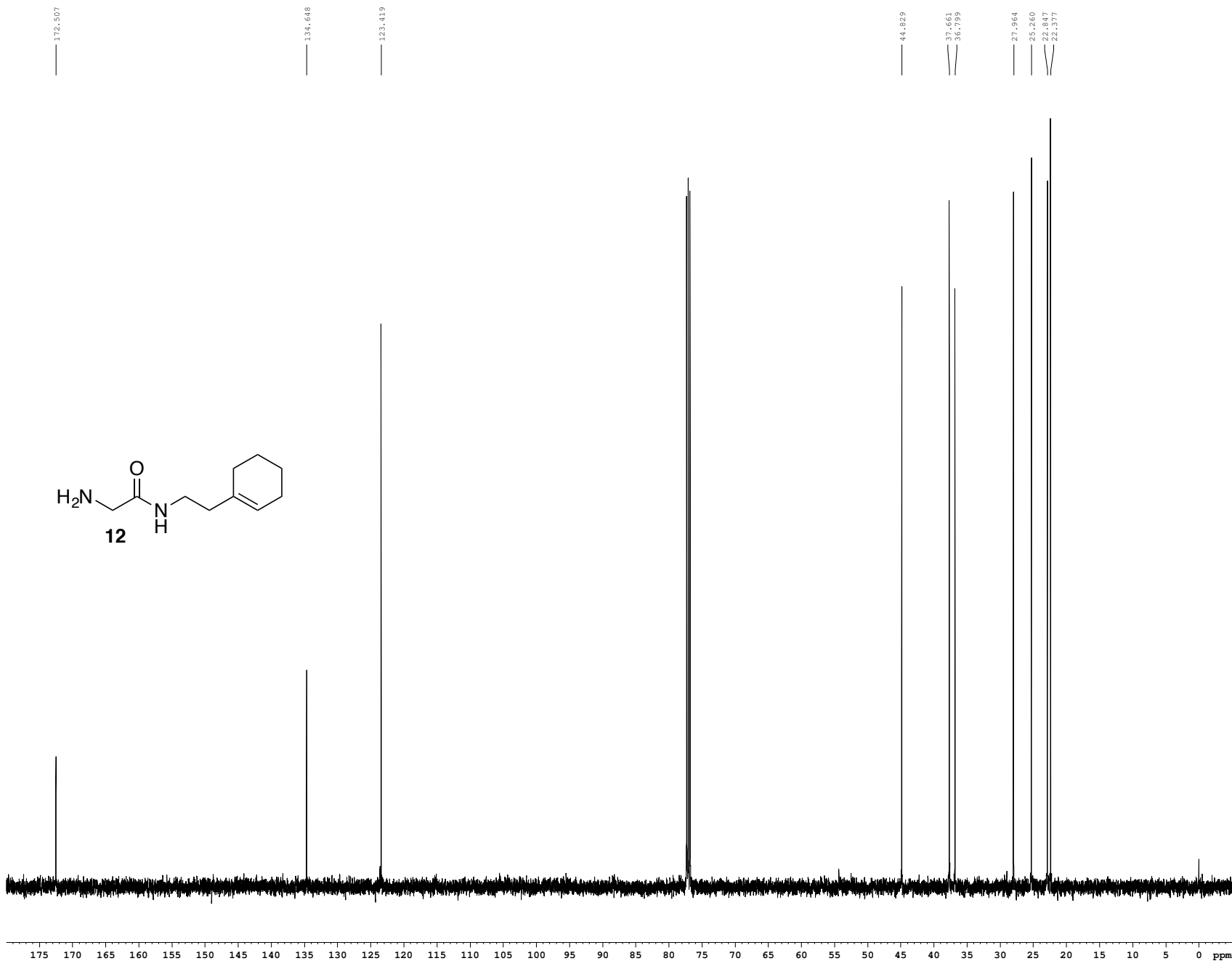
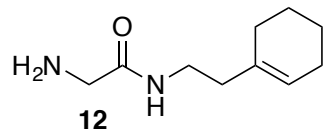


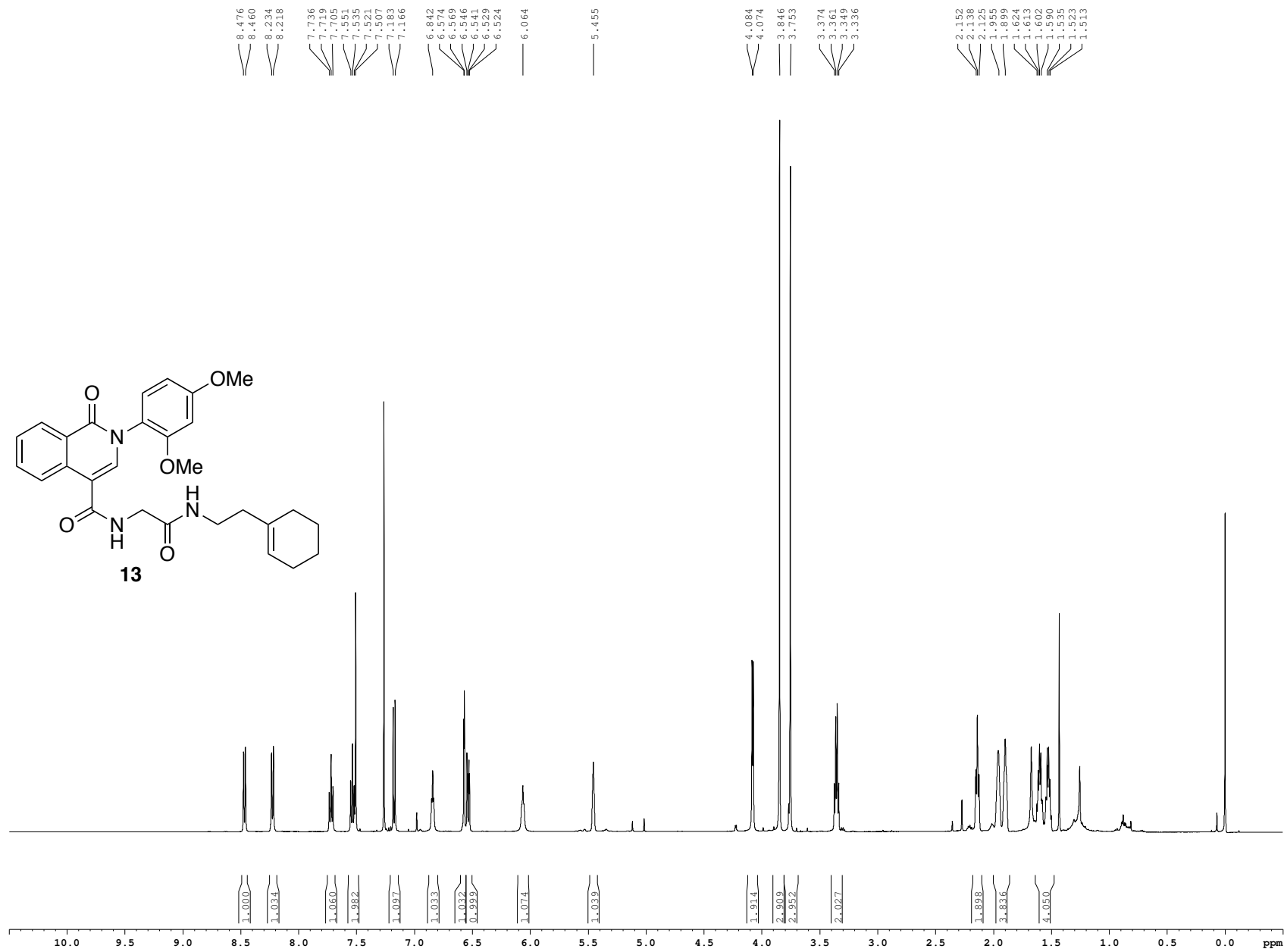
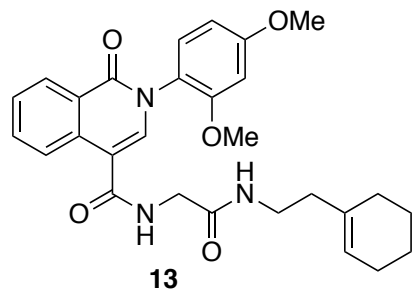












168.438
166.854
162.041
161.405
155.541

135.720
134.966
134.282
133.084
129.275
128.744
127.612
126.799
124.779
123.980
122.362

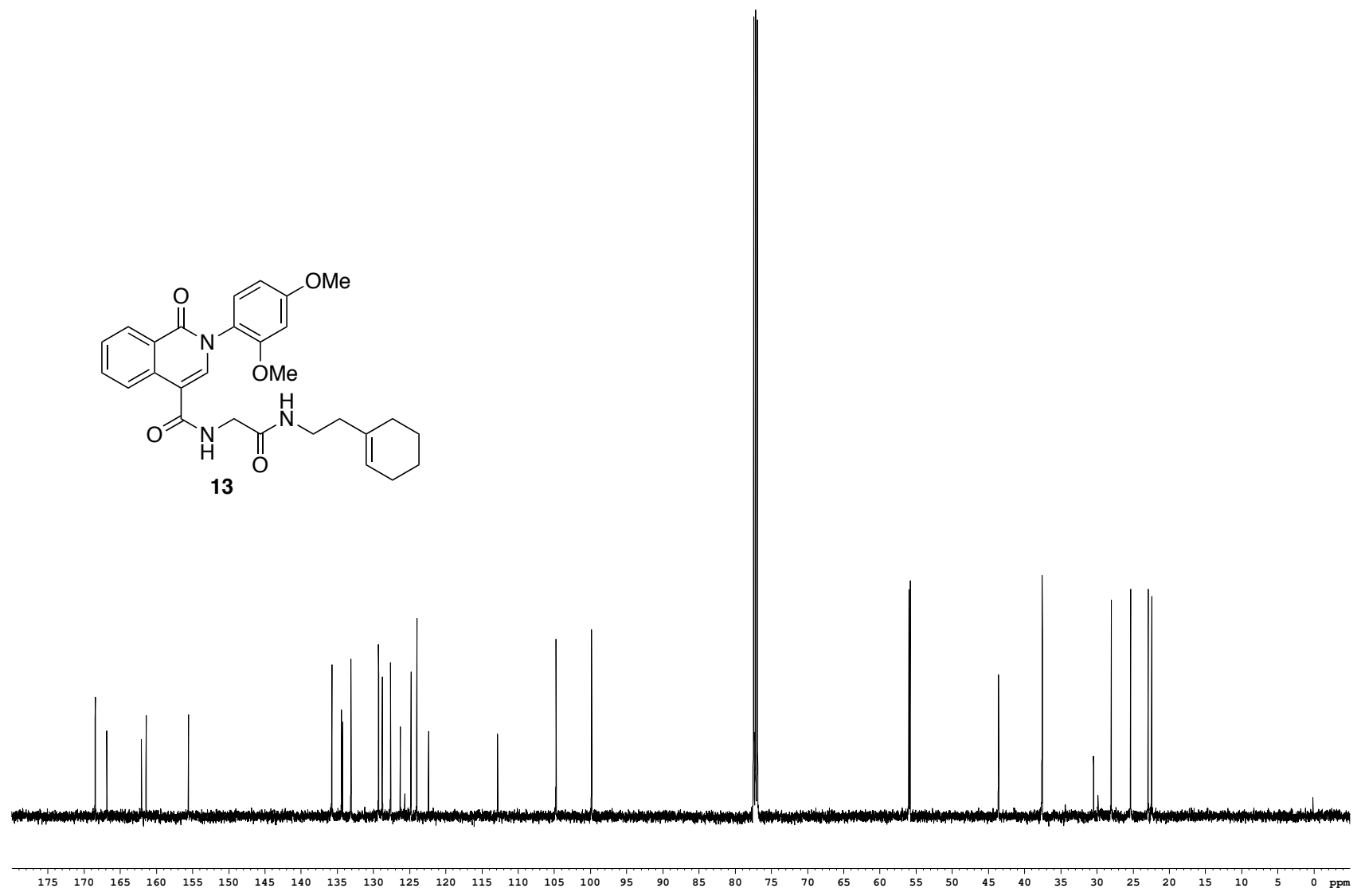
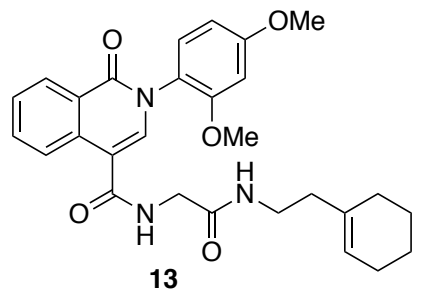
112.817
104.738
99.819

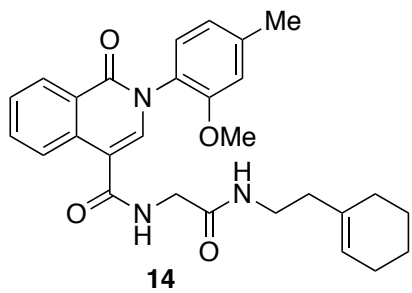
55.950
55.785

43.569

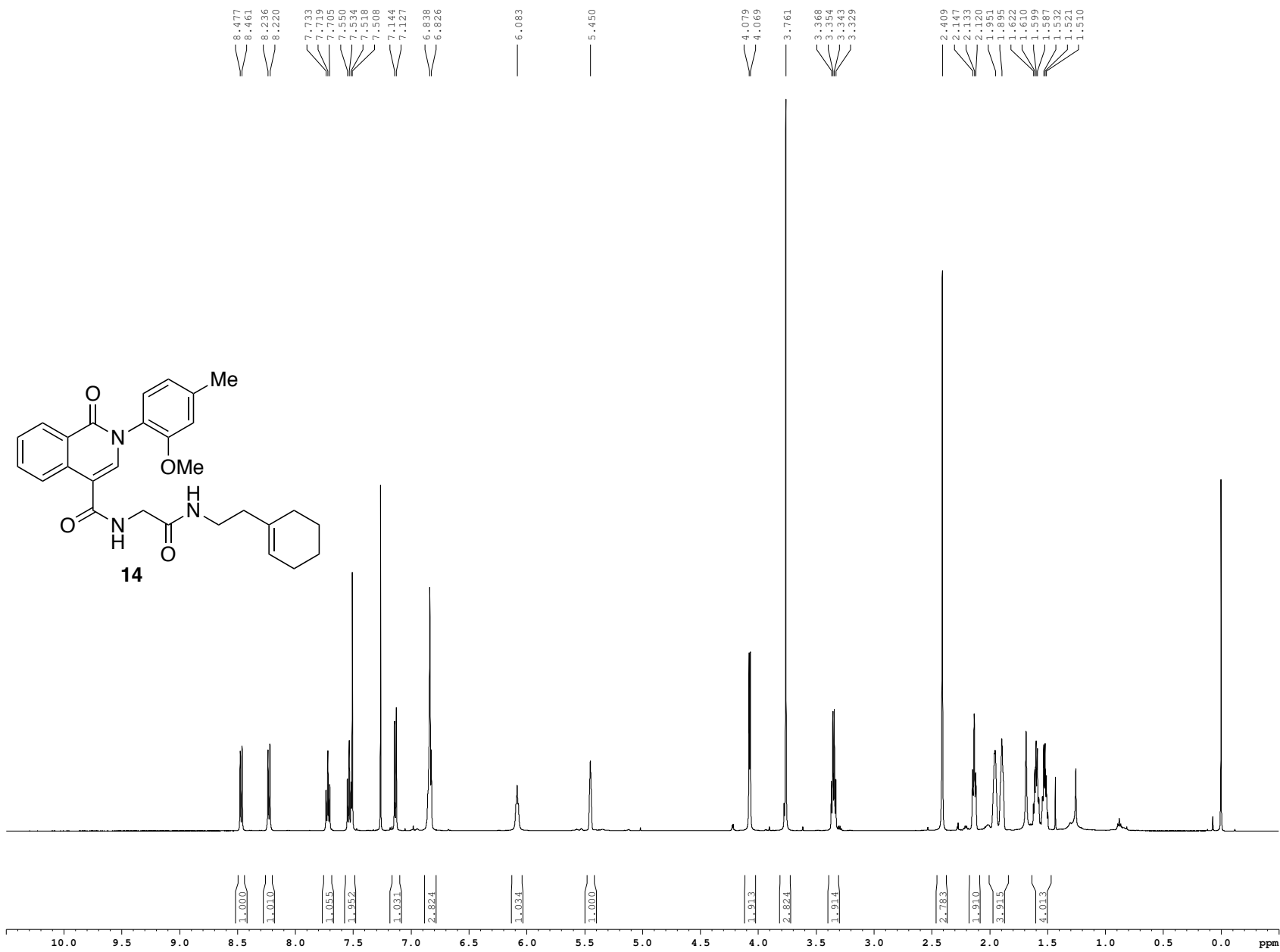
37.552
37.522

27.978
25.304
22.877
22.397





14



166.403
166.630

161.861

154.277

141.018

135.482

134.385

133.084

133.084

128.758

128.434

127.610

126.287

124.784

124.018

121.657

113.119

112.827

55.891

43.570

37.558

37.501

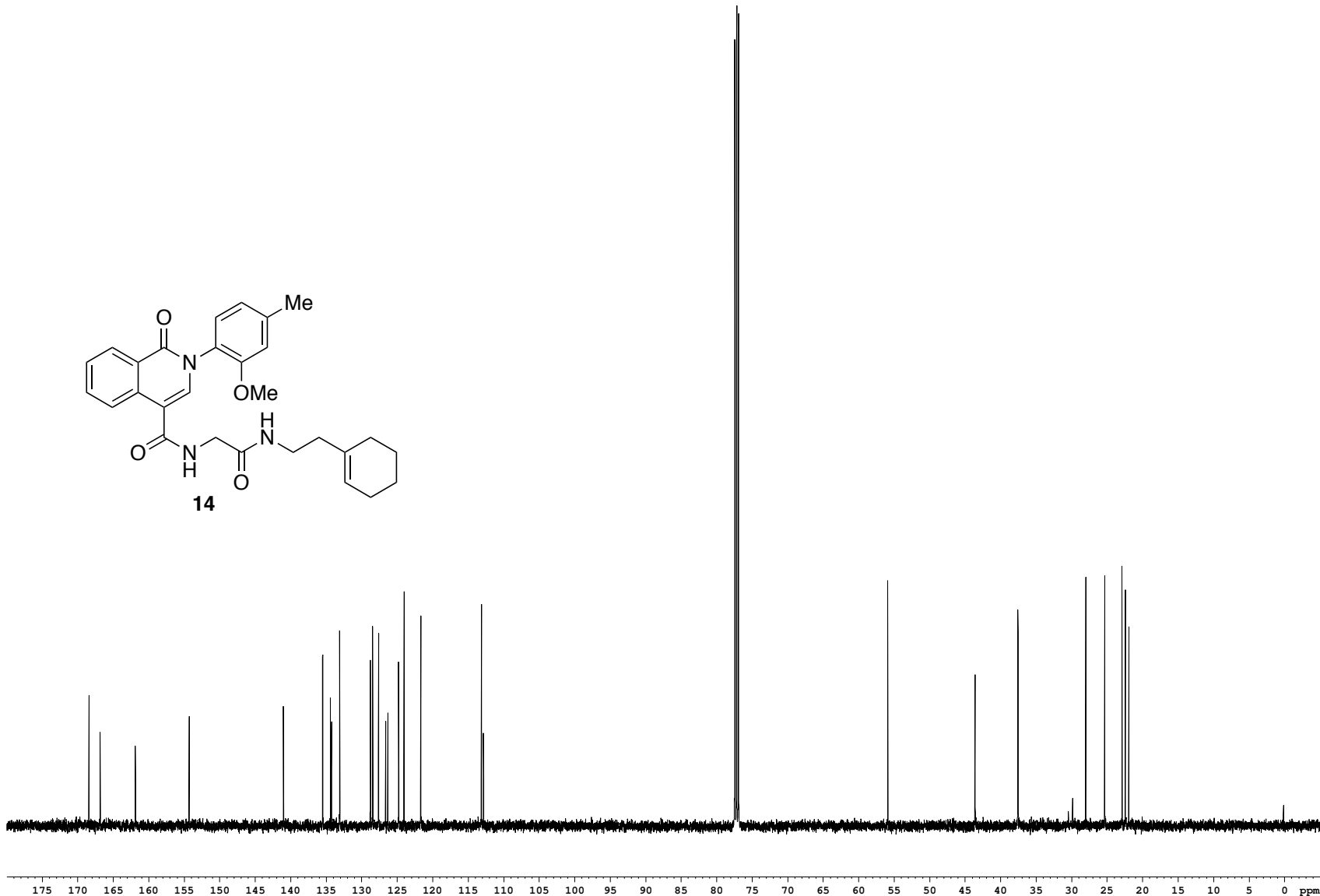
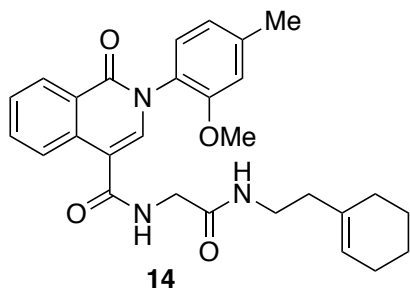
27.978

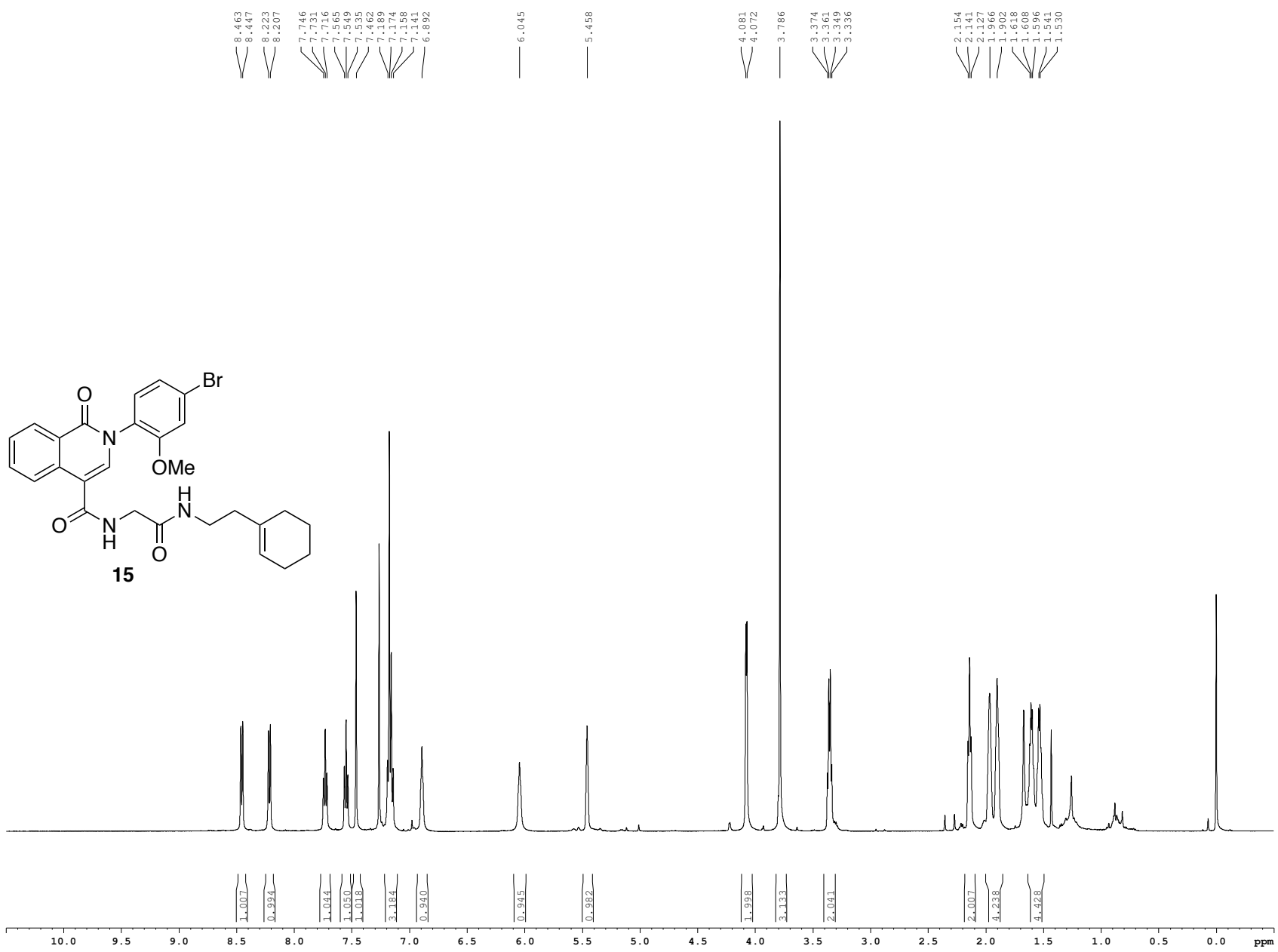
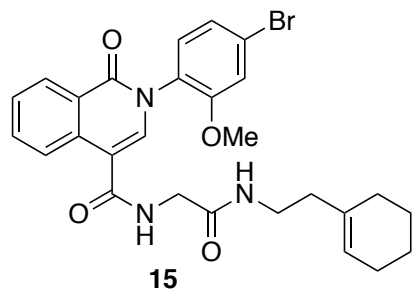
25.311

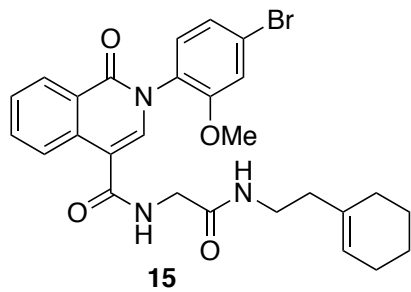
22.884

22.404

21.917







168.344
166.614
161.552
155.316

134.707
134.376
134.172
133.320
132.666
128.764
128.312
127.851
126.862
124.231
124.056
123.937

116.130
113.305

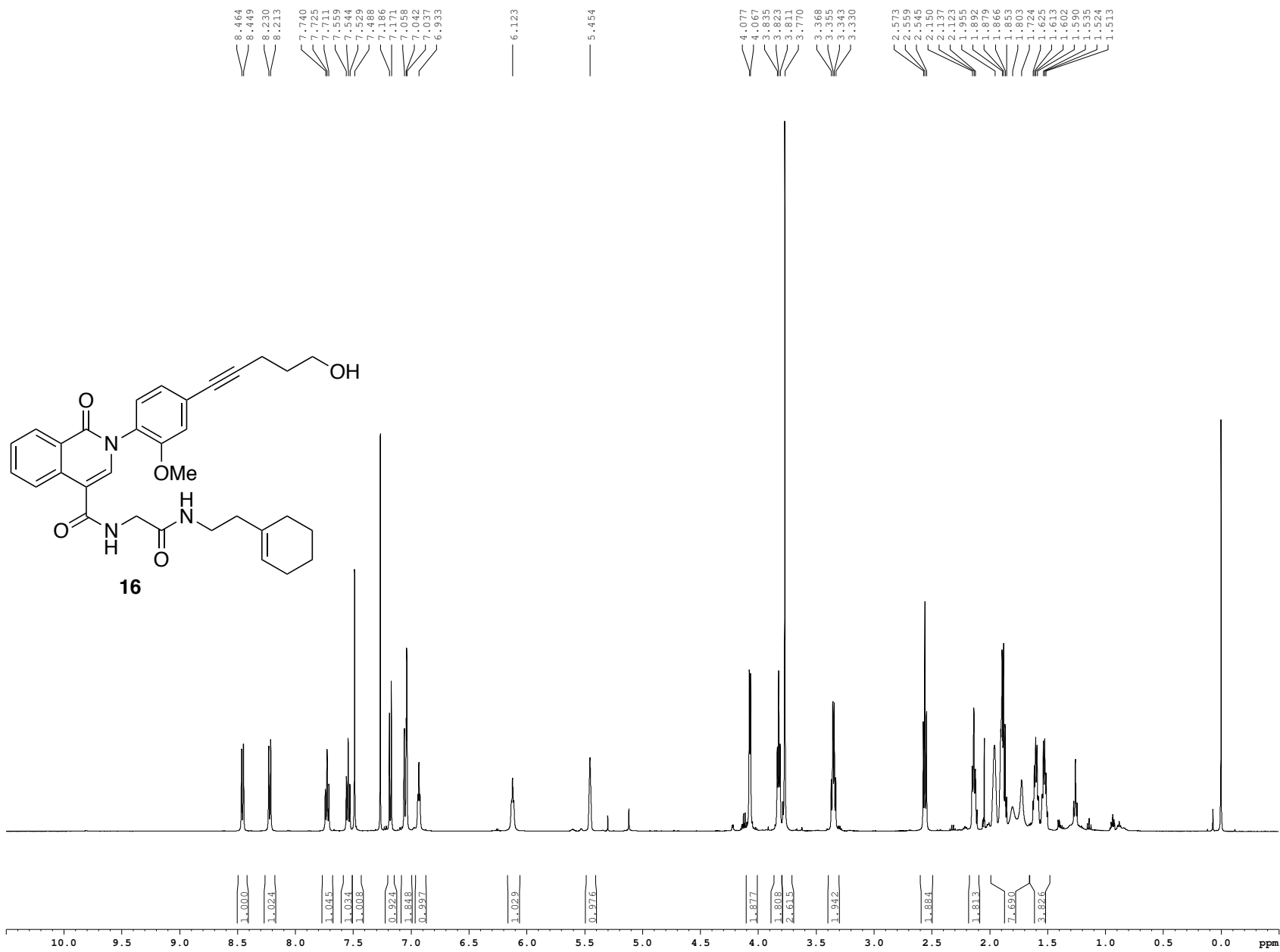
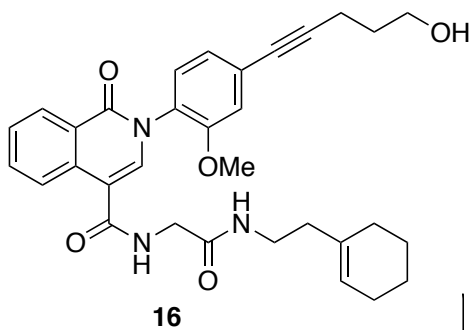
56.359

43.539

37.572
37.557

28.009
25.332
22.897
22.416





166.421
166.727
161.640
154.285

134.945
134.373
134.175
133.242
128.771
128.727
128.666
127.766
126.235
124.844
124.442
124.010

115.351
113.097

90.944

80.547

61.774

56.069

43.563

37.549
37.551

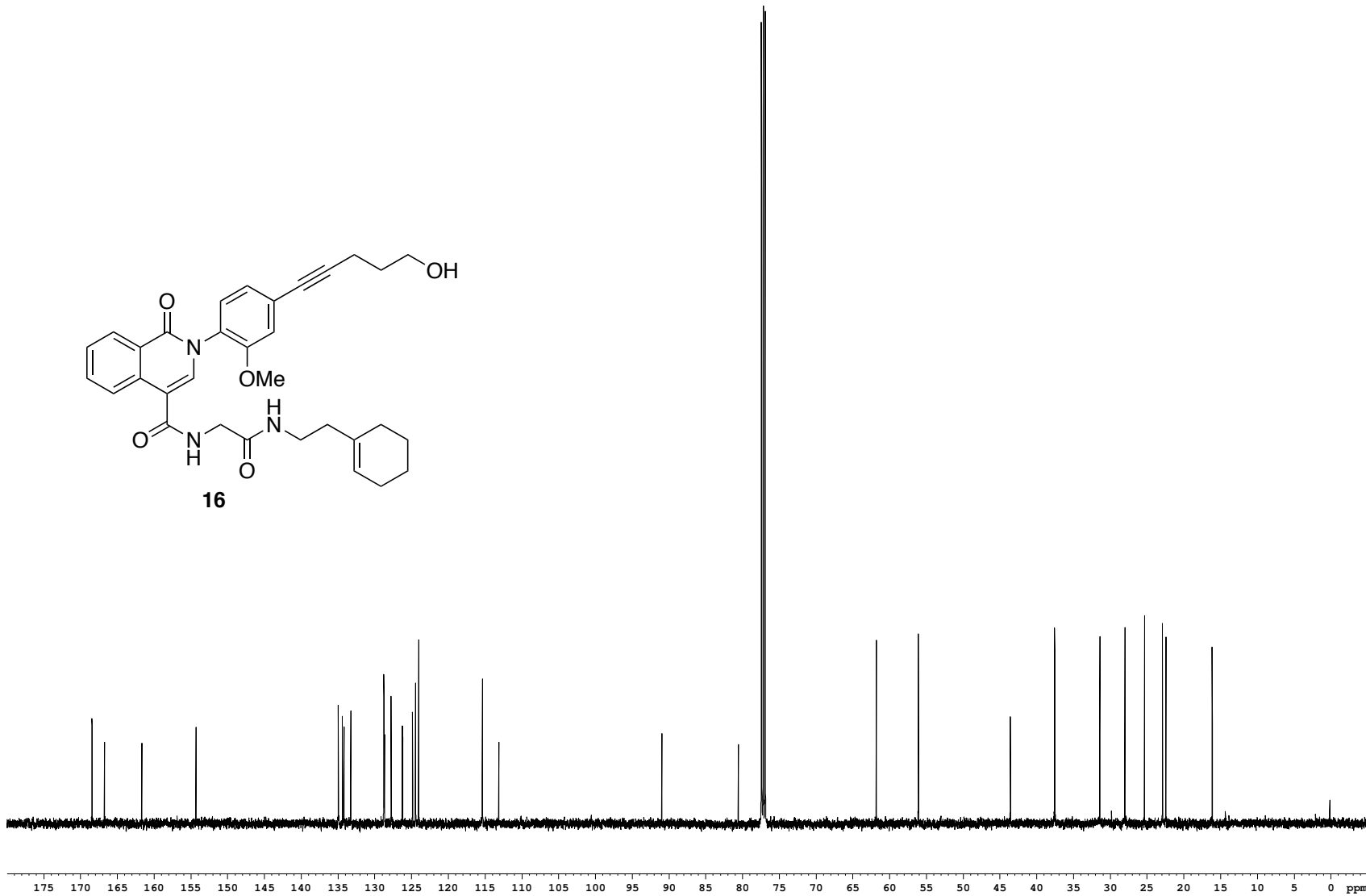
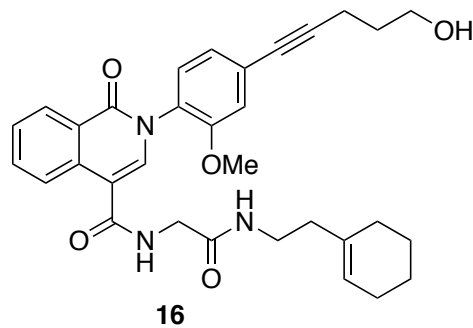
31.396

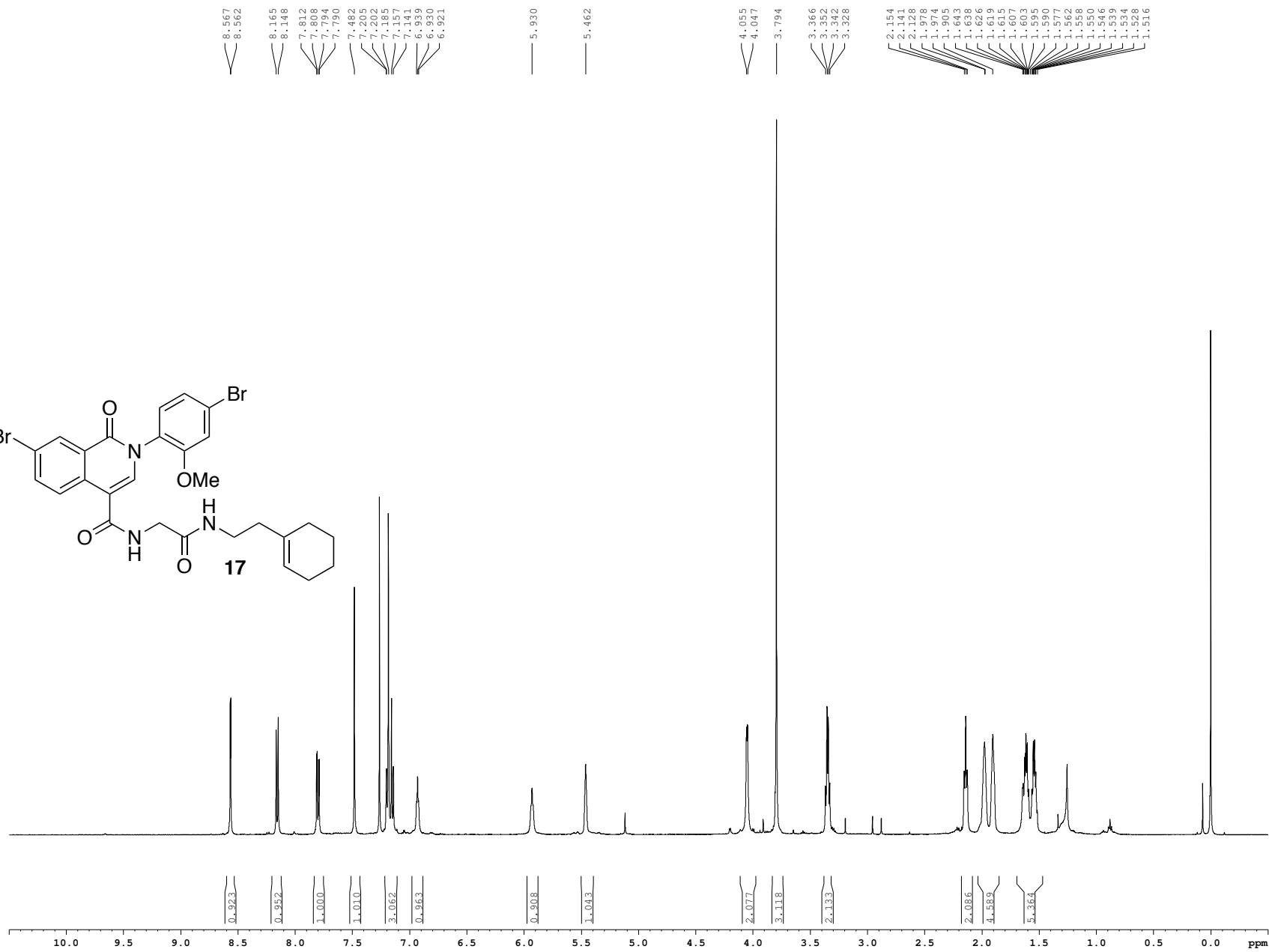
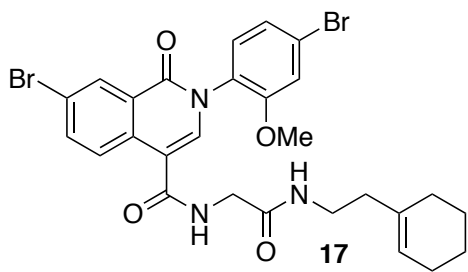
27.981

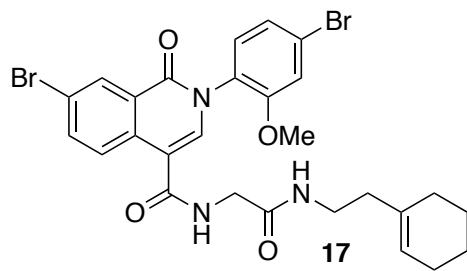
25.313

22.881
22.400

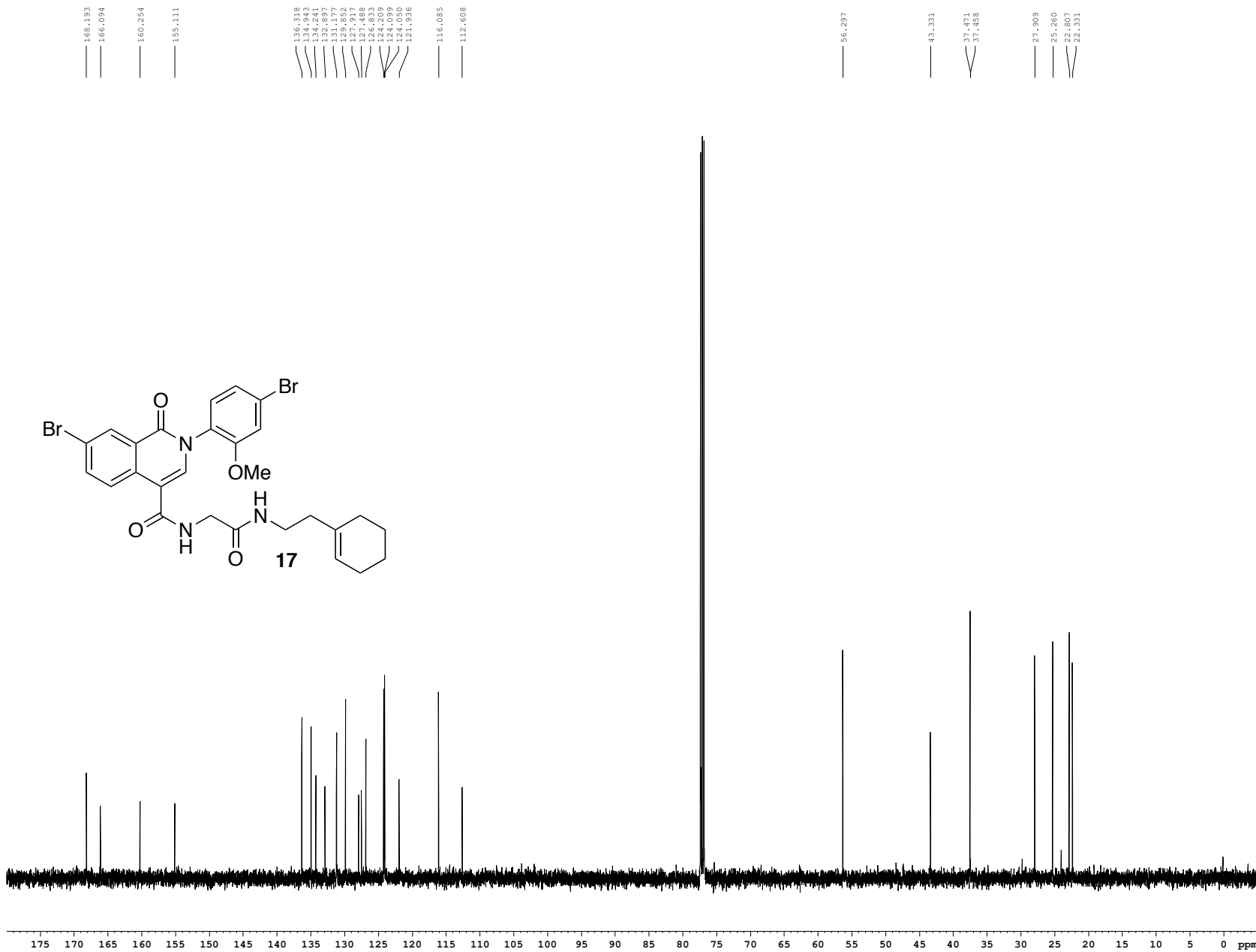
16.115







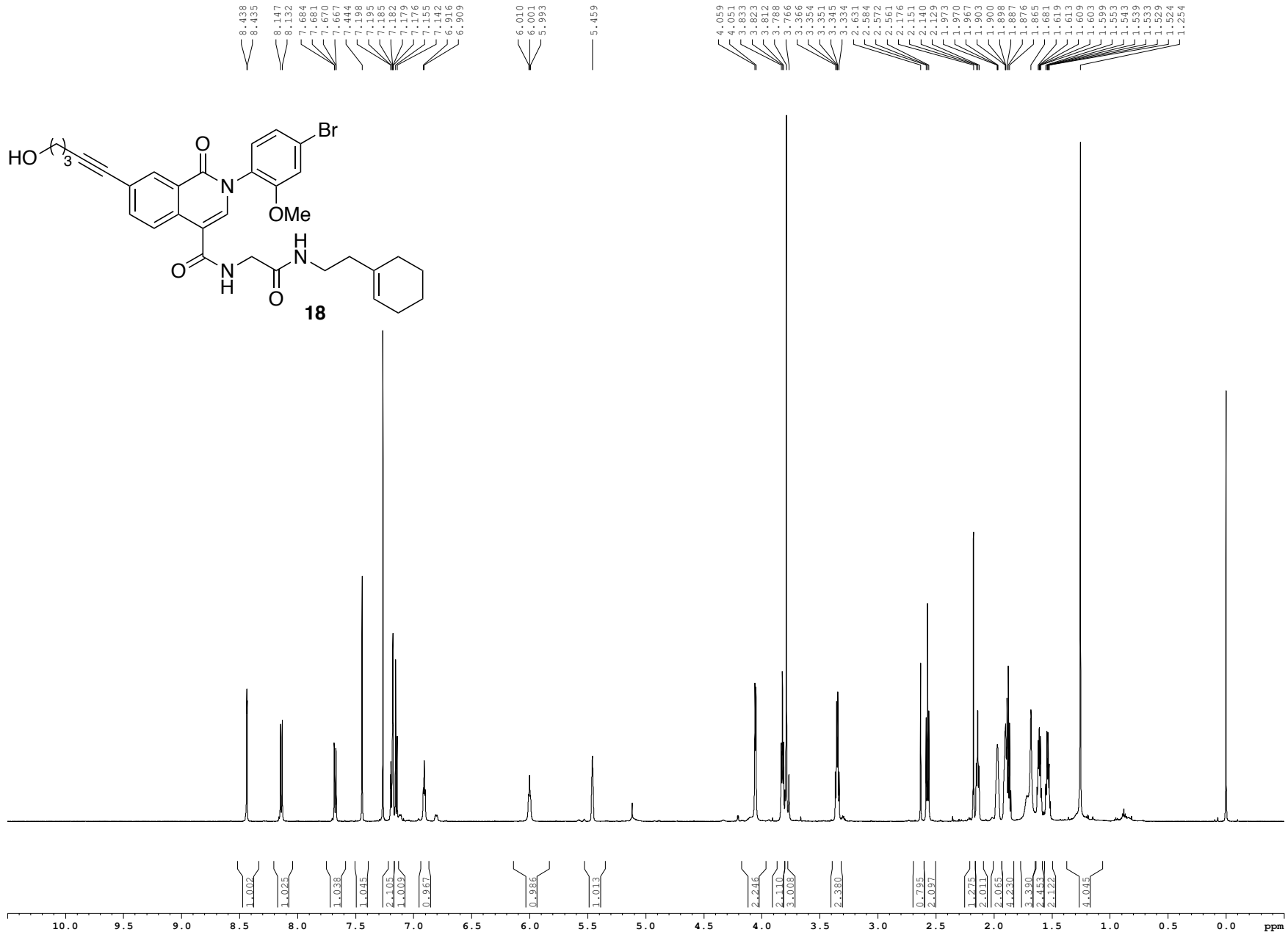
17

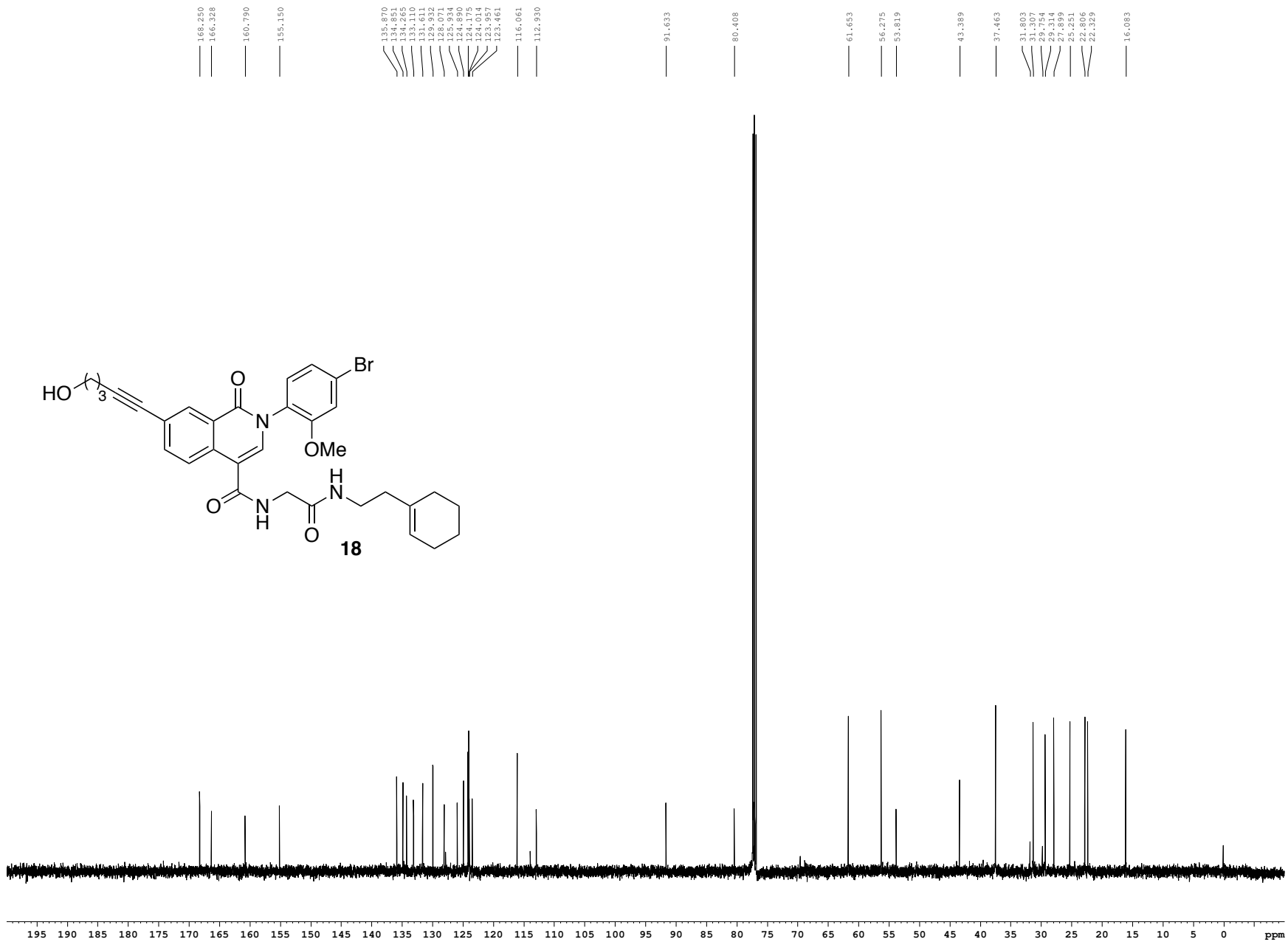


168.193
166.094
160.254
155.111

136.318
134.243
134.241
131.177
129.852
127.217
126.838
124.209
124.050
121.256
116.085
112.608

56.297
43.331
37.471
37.458
27.909
25.260
22.807
22.331





—8.06
—7.86
—7.69

7.23
7.20
7.15
7.09

—6.17

—5.42

—4.17

—3.79

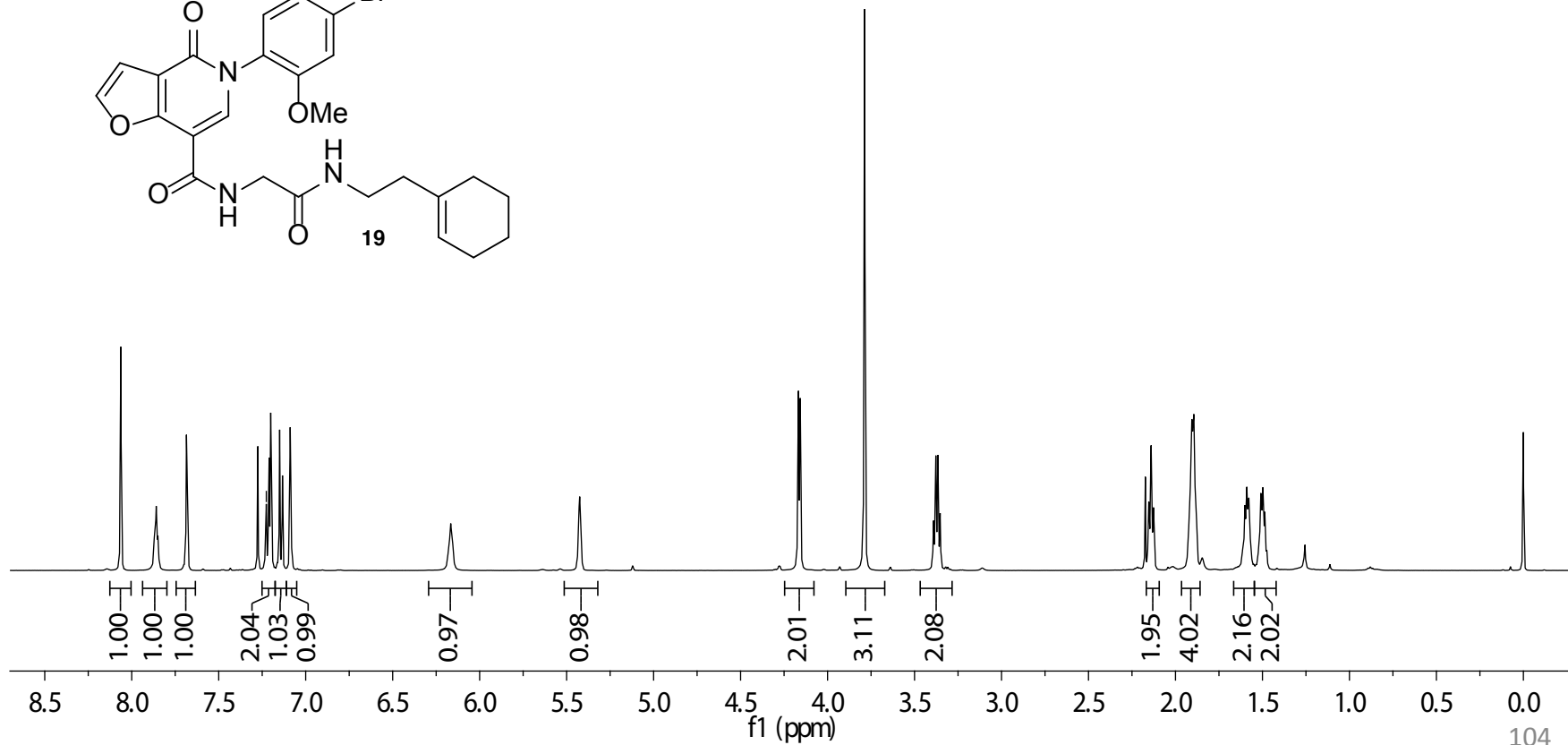
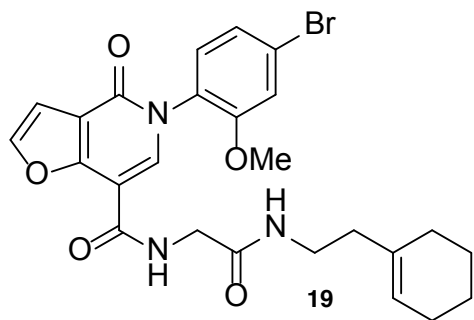
—3.38

—2.14

—1.89

—1.59

1.50



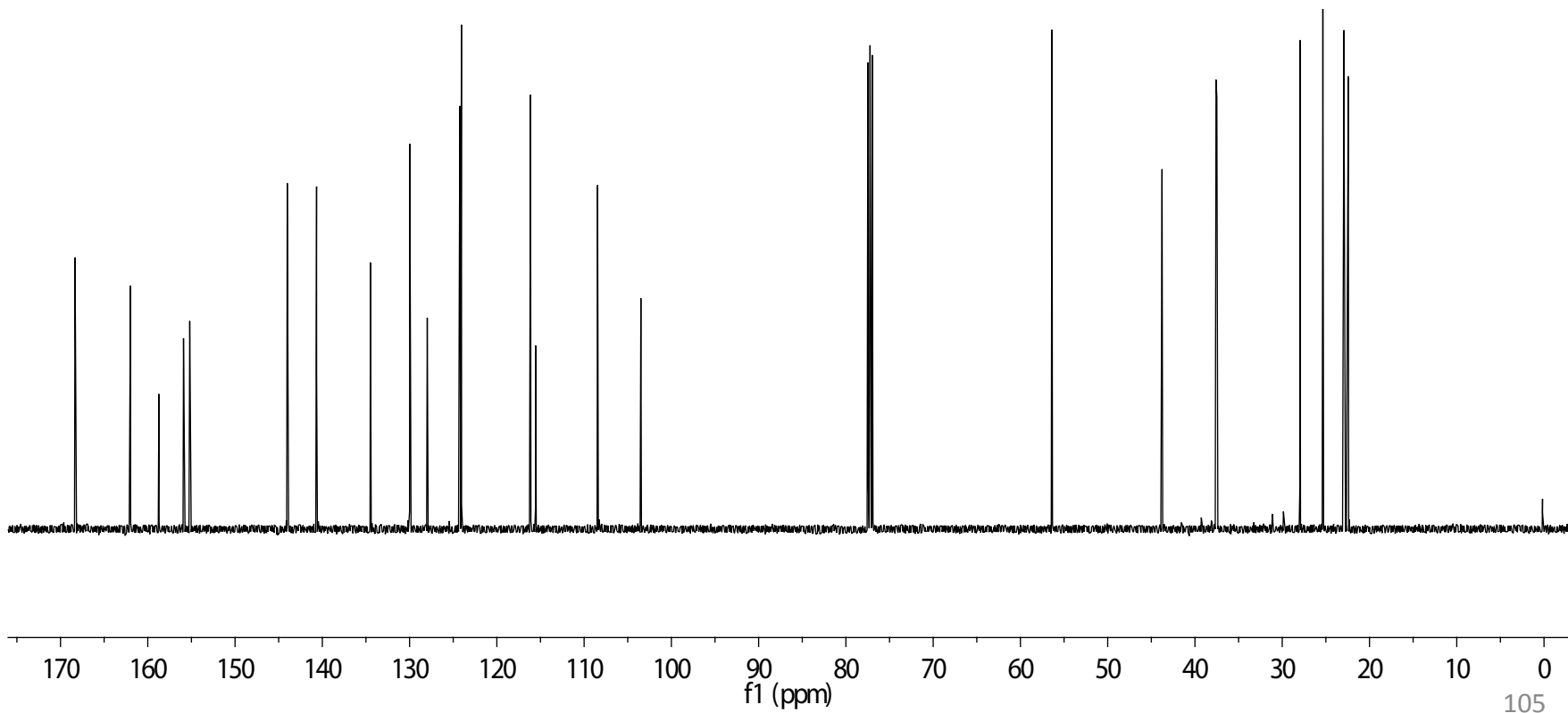
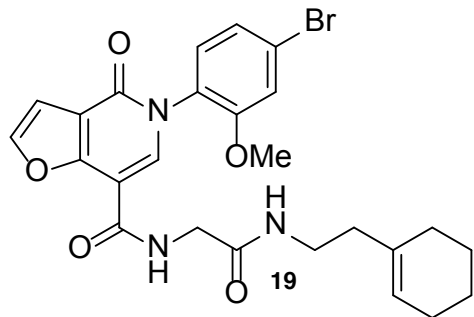
—168.3
—162.0
—158.7
—155.9
—155.2
—144.0
—140.7
—134.5
—130.0
—128.0
—124.3
—124.2
—124.1
—116.1
—115.6
—108.5
—103.5

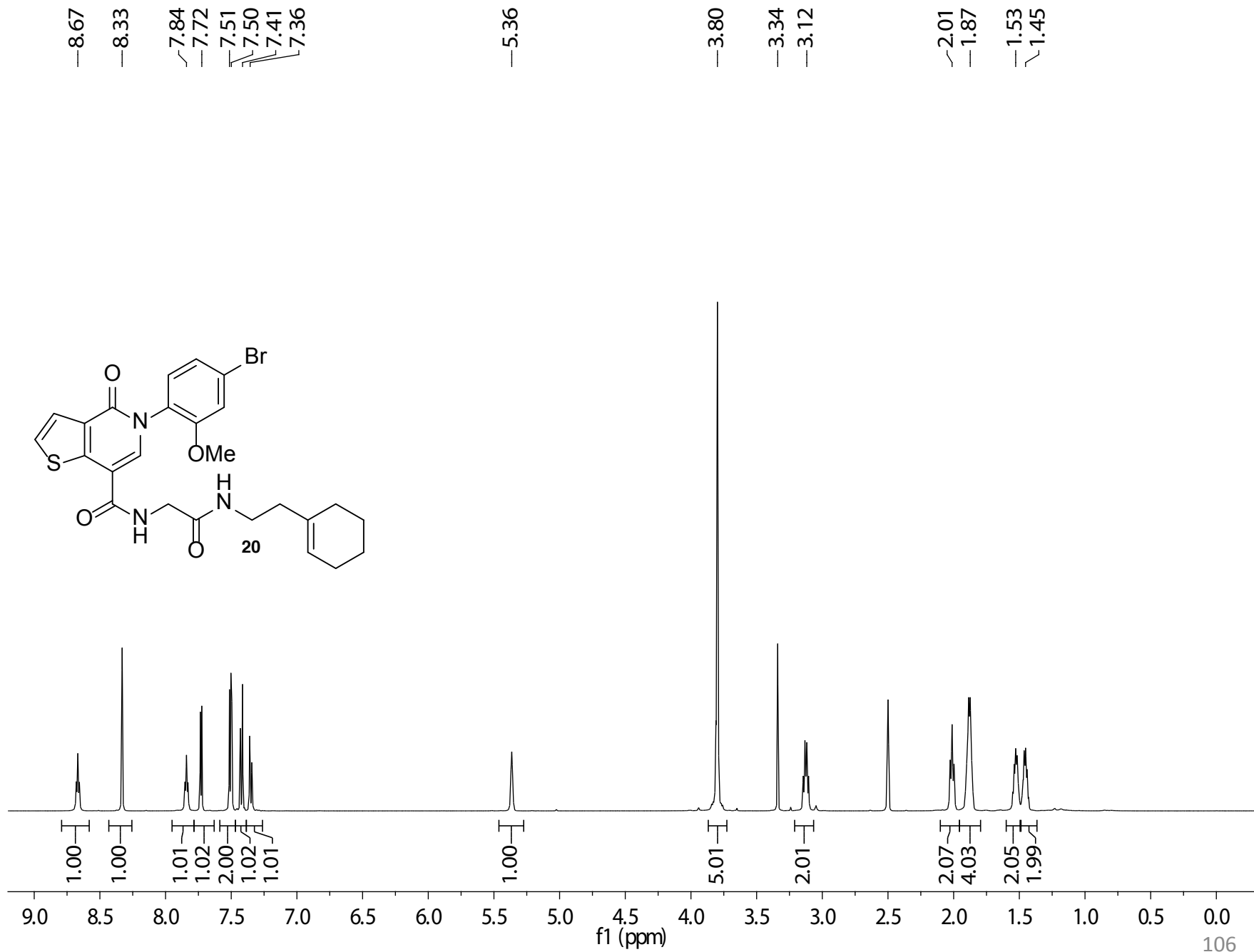
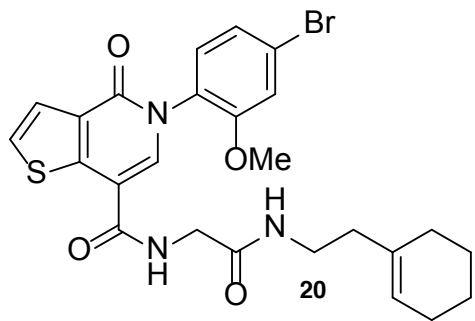
—56.4

—43.8

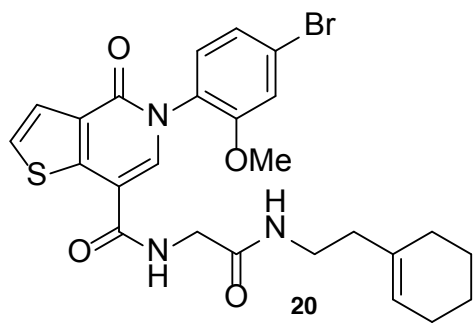
—37.6
—37.5

—28.0
—25.3
—22.9
—22.4

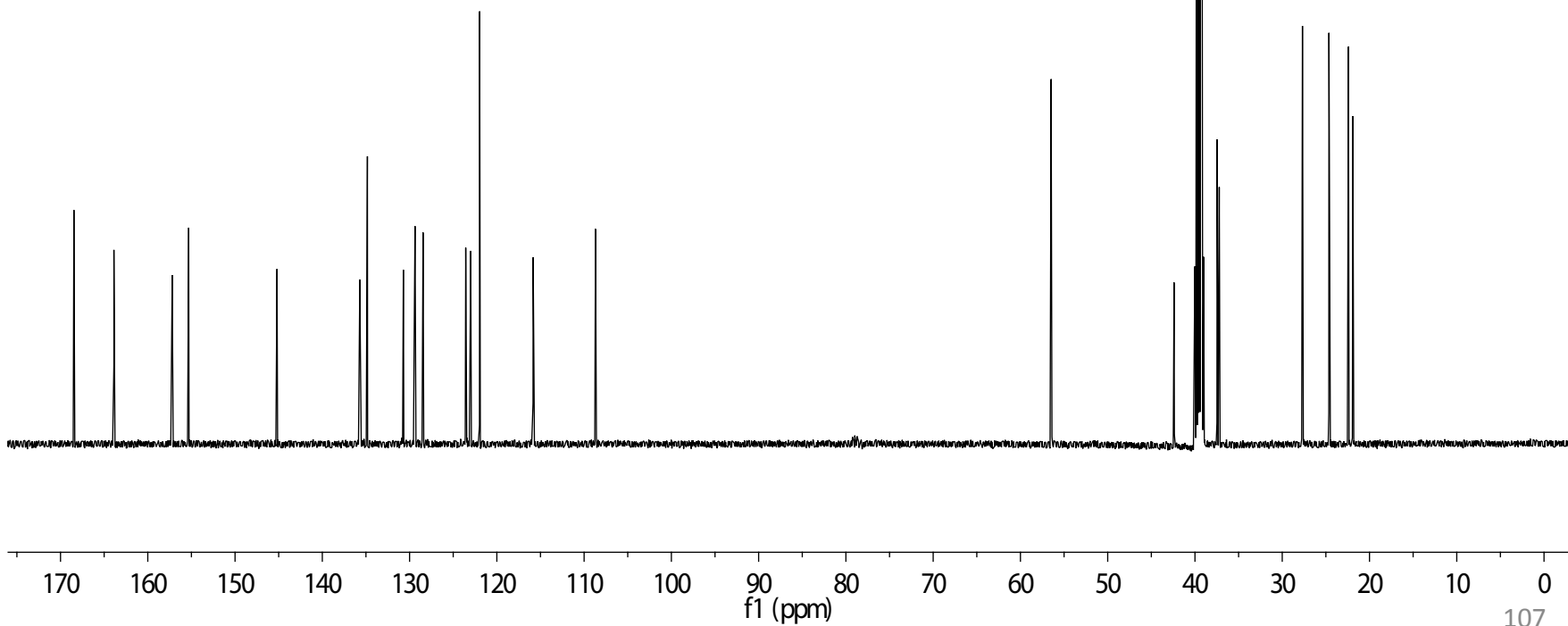


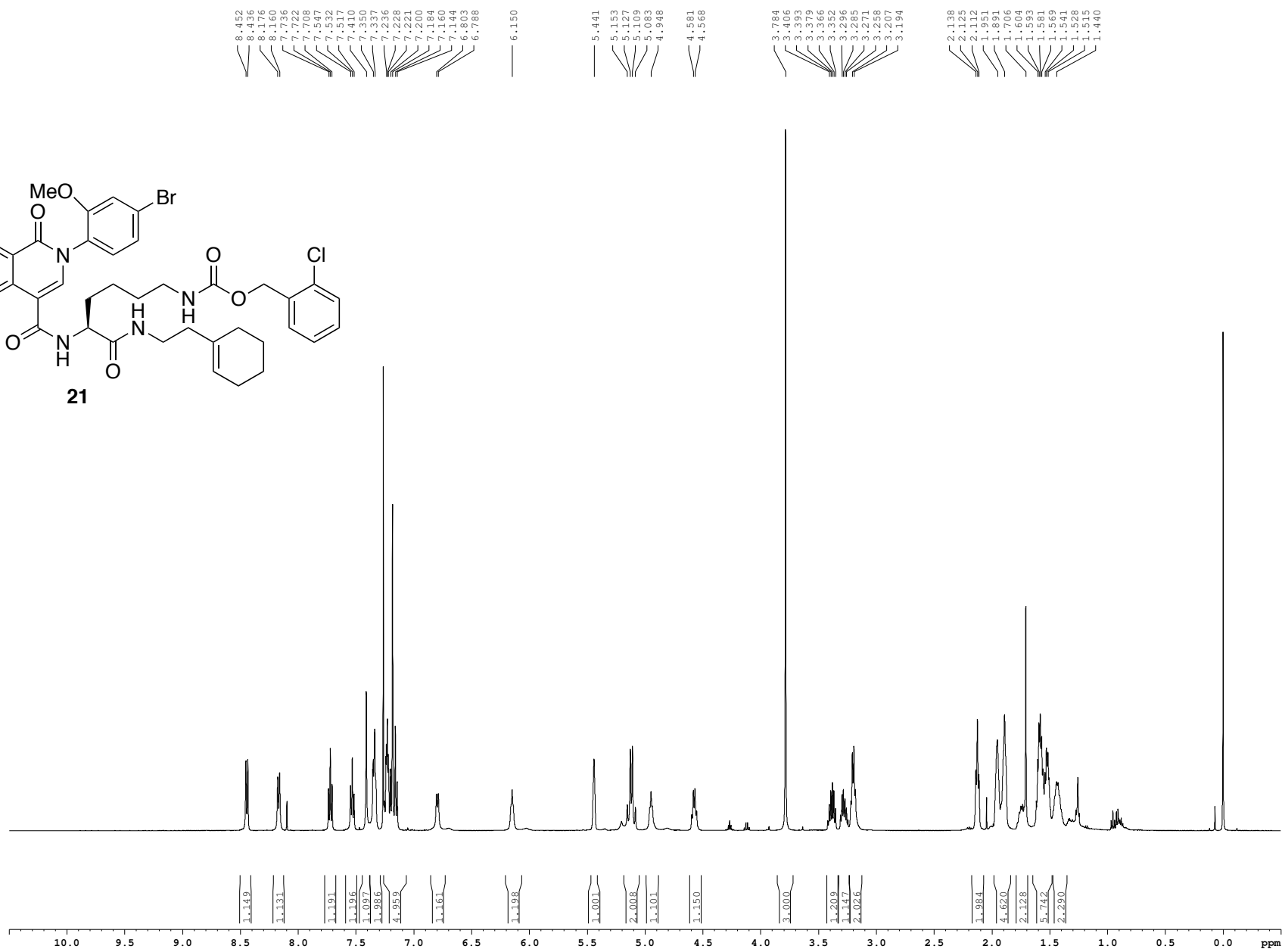
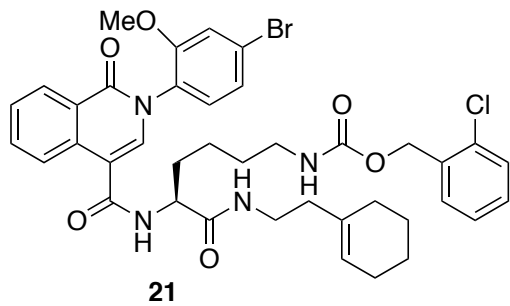


—168.5
—163.9
~157.2
~155.3
—145.2
135.7
134.8
130.7
129.3
—128.5
123.6
—123.0
122.0
~115.8
—108.7



—56.5
—42.4
37.5
37.2
27.7
24.6
22.4
21.9



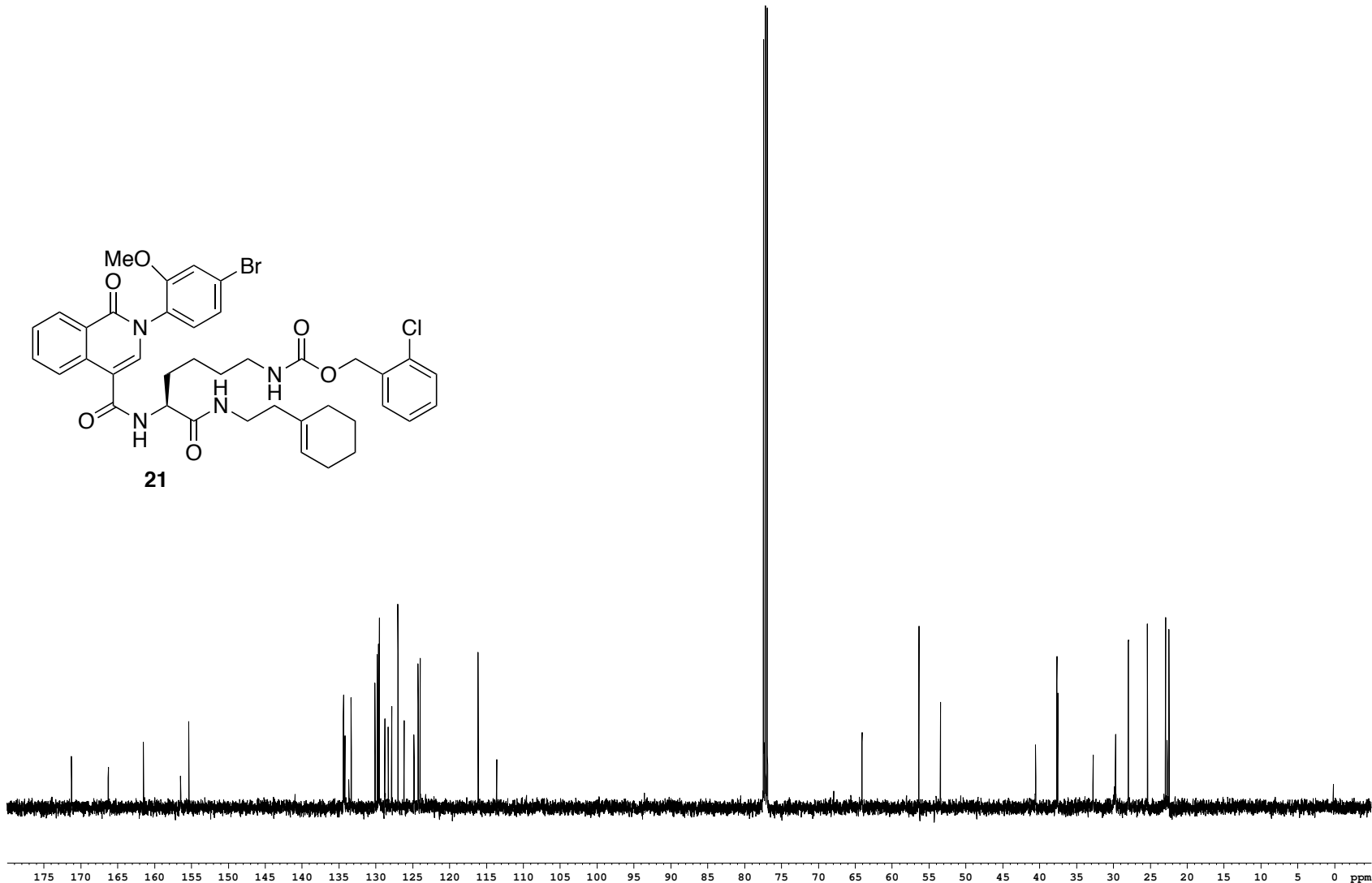
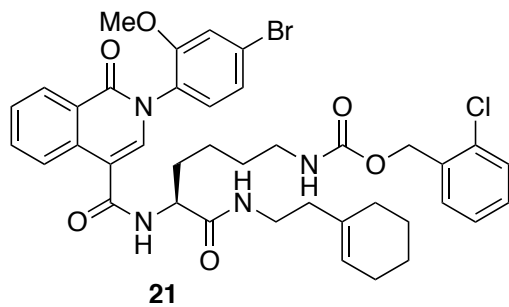


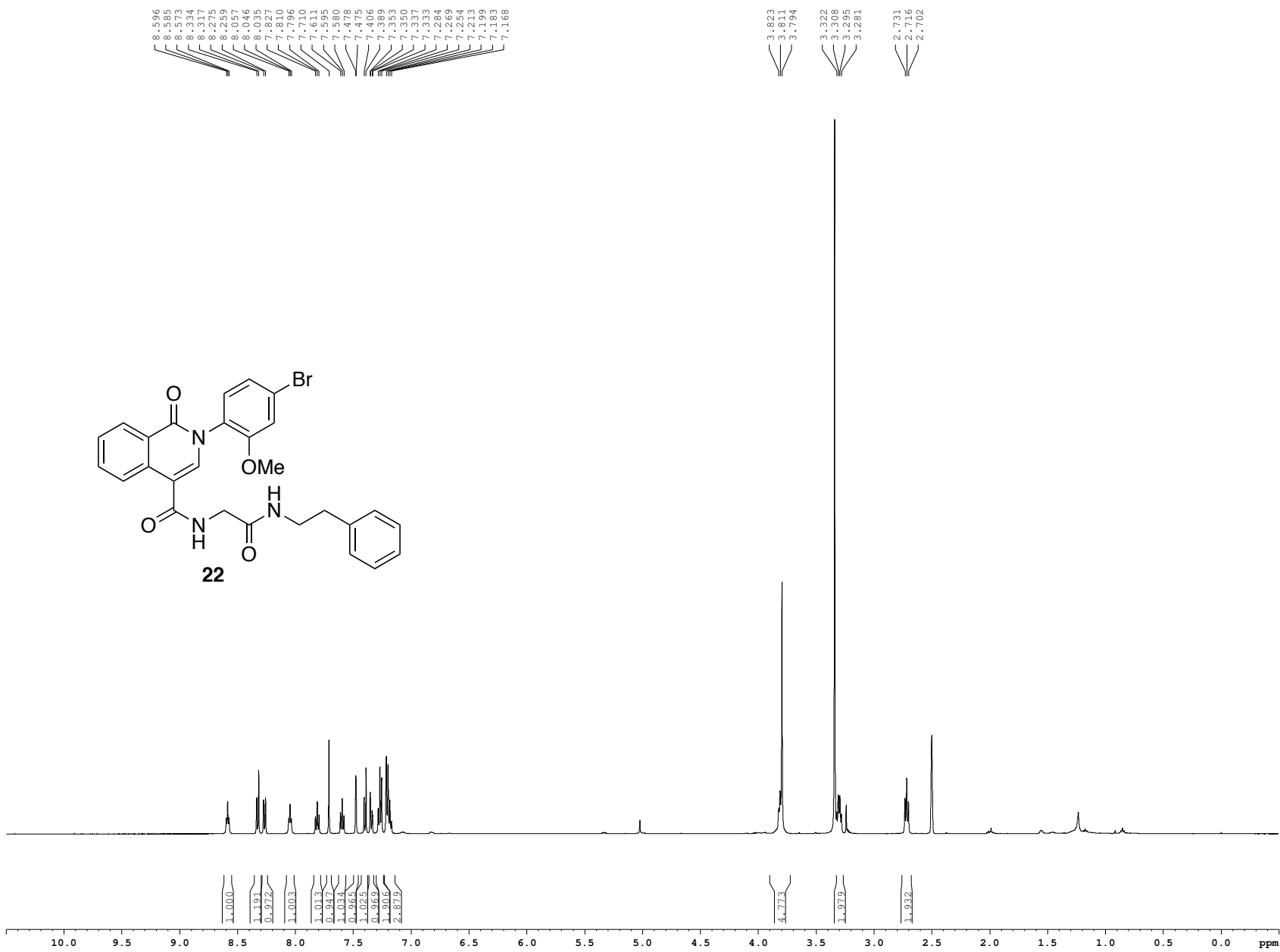
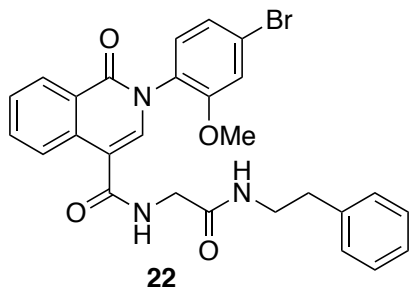
171.255
166.256
161.500
156.474
155.337

134.406
134.365
134.365
134.185
134.185
133.639
133.324
130.991
130.991
129.635
129.518
128.787
128.787
127.929
127.827
126.977
126.138
124.809
124.809
123.957
123.957
116.118
113.587

64.057
56.342
53.400

40.521
37.639
37.505
32.706
29.662
27.944
25.245
22.671
22.423





168.750
165.750
160.240
155.395

139.414
135.294
134.556
132.946
130.640
128.605
127.477
127.360
126.098
125.296
124.128
123.593
122.828

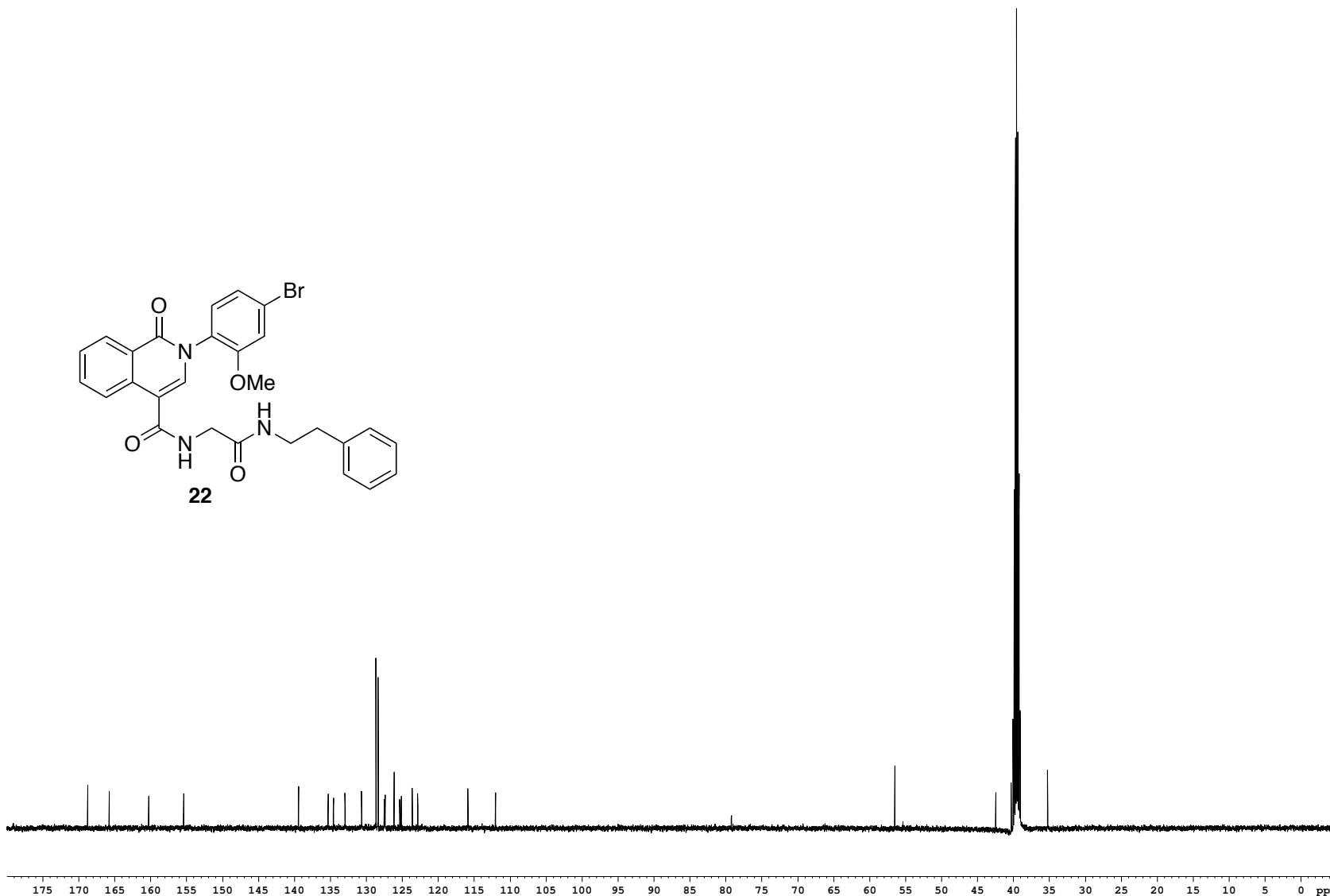
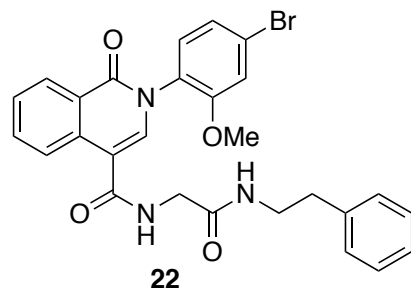
115.857
112.011

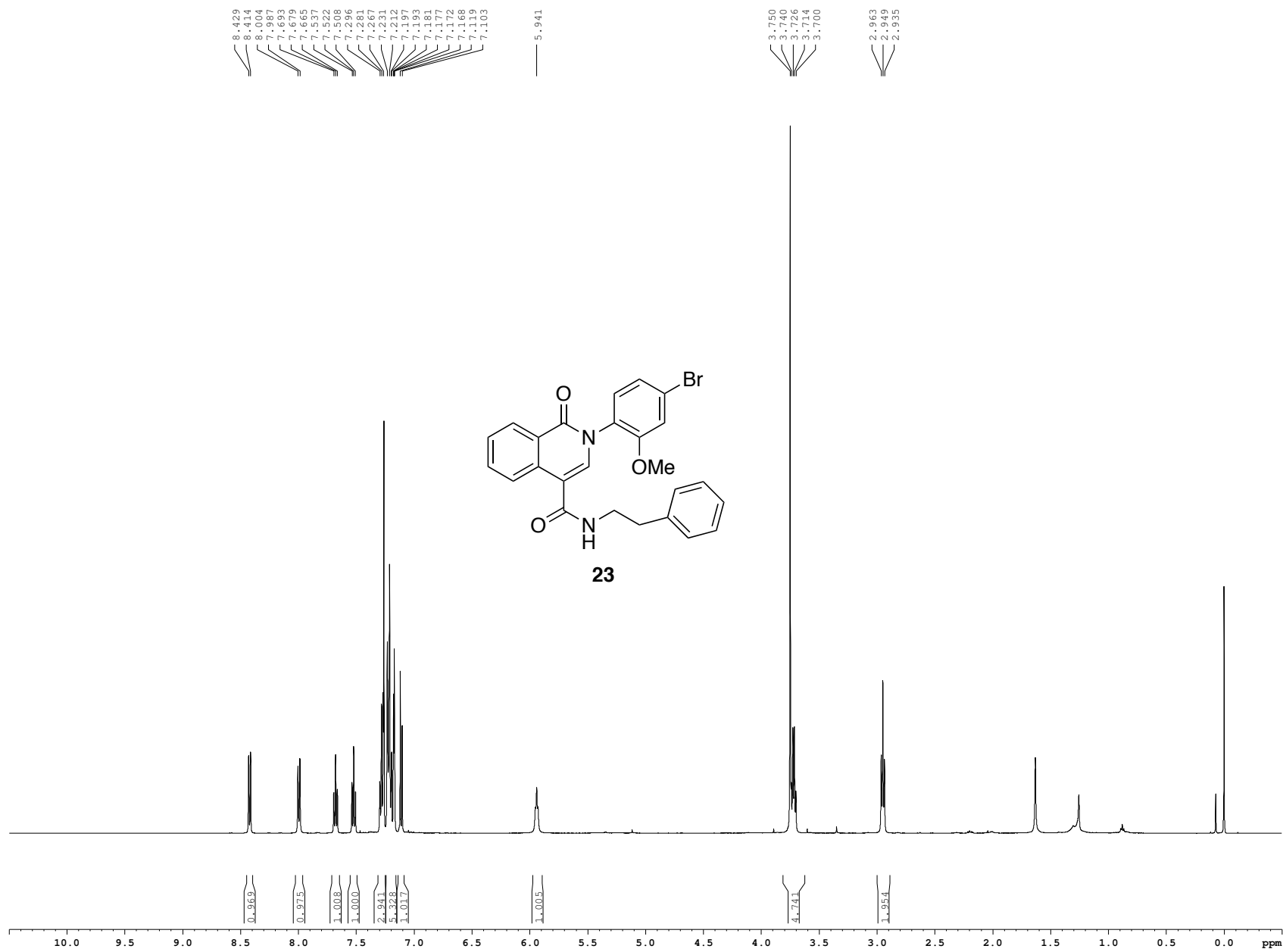
79.193

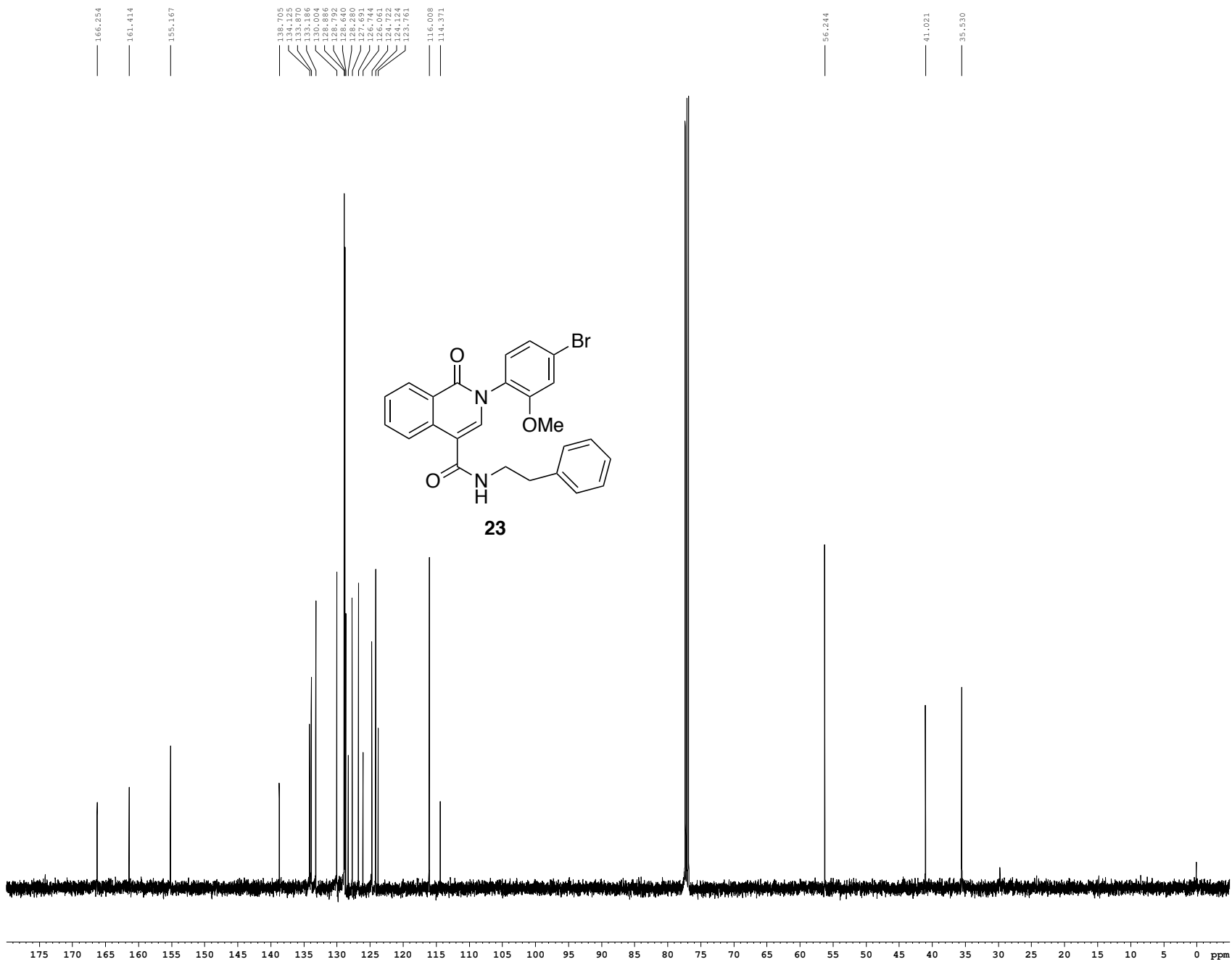
56.456

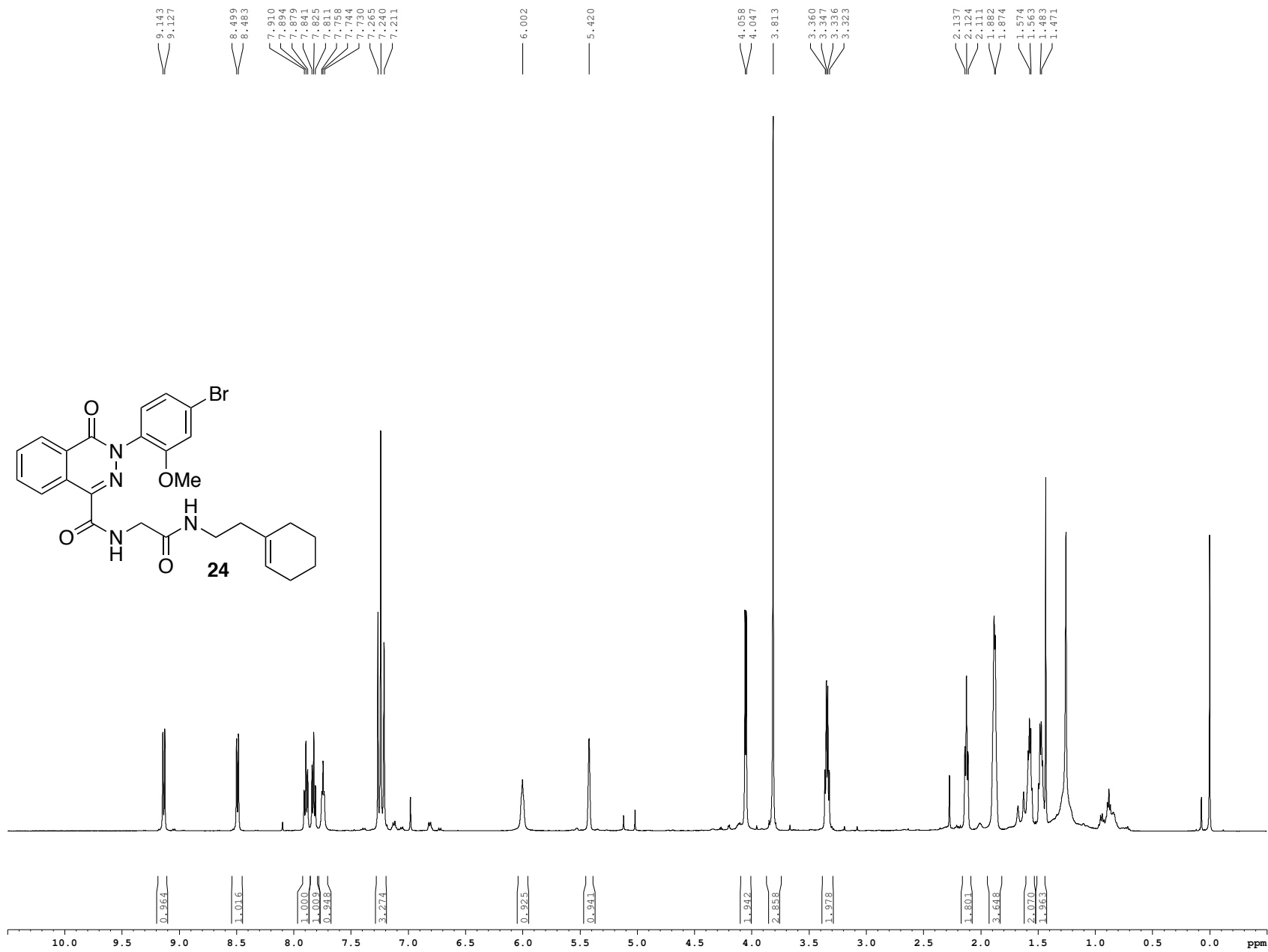
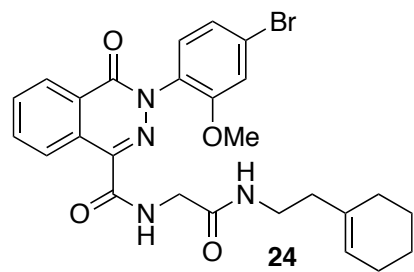
42.416
40.280

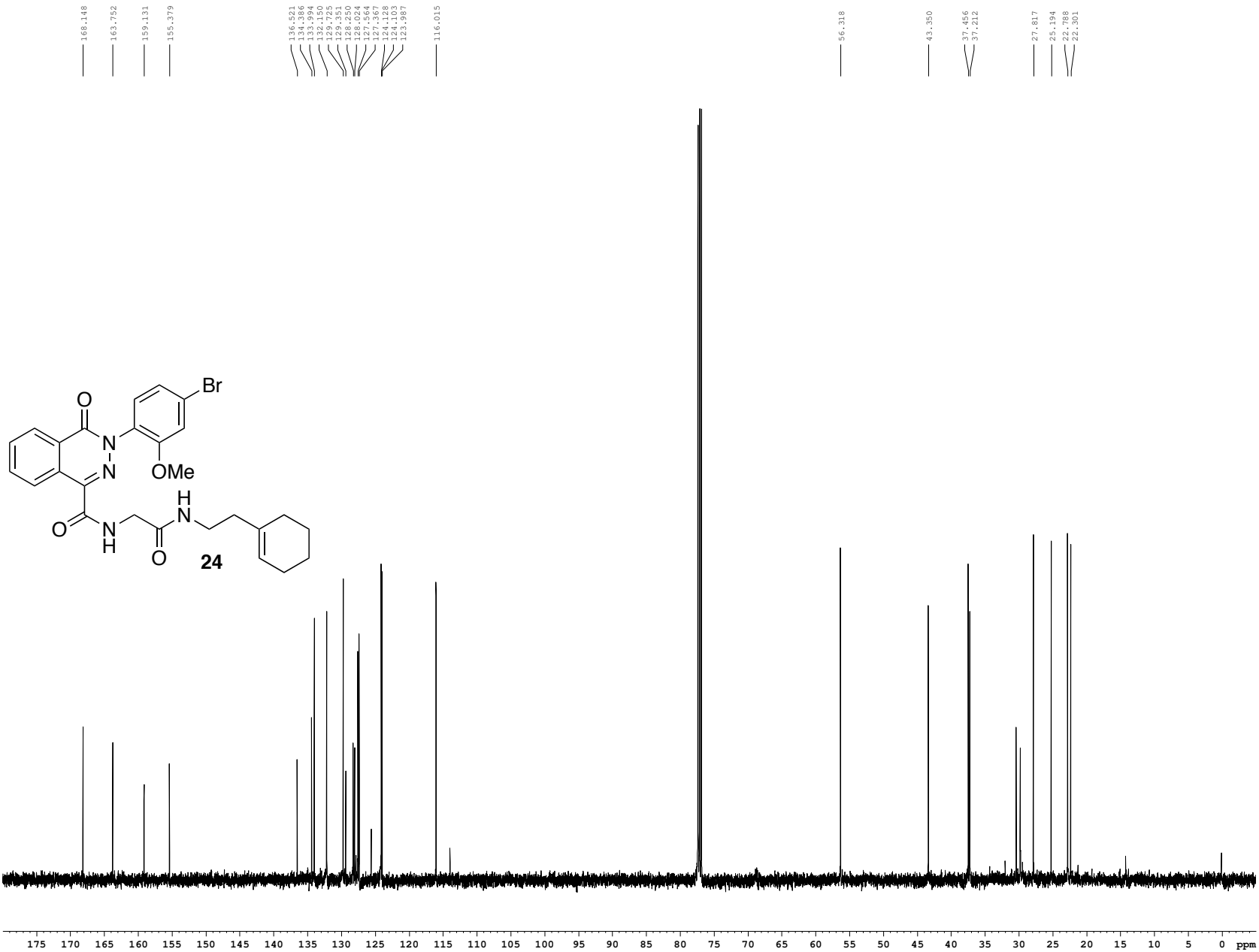
35.206

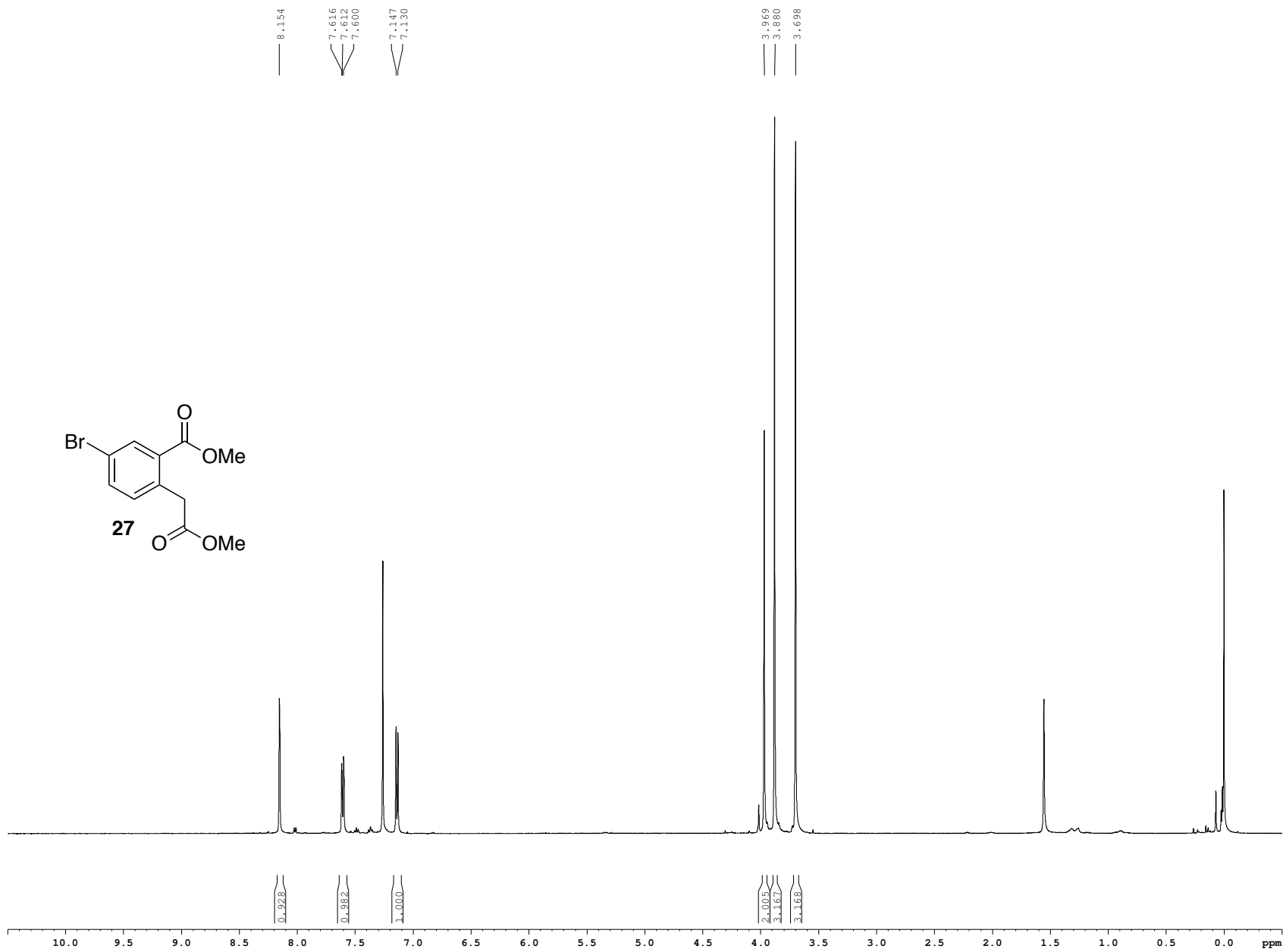
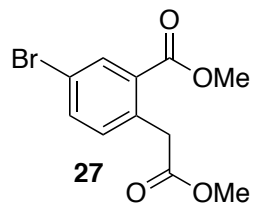


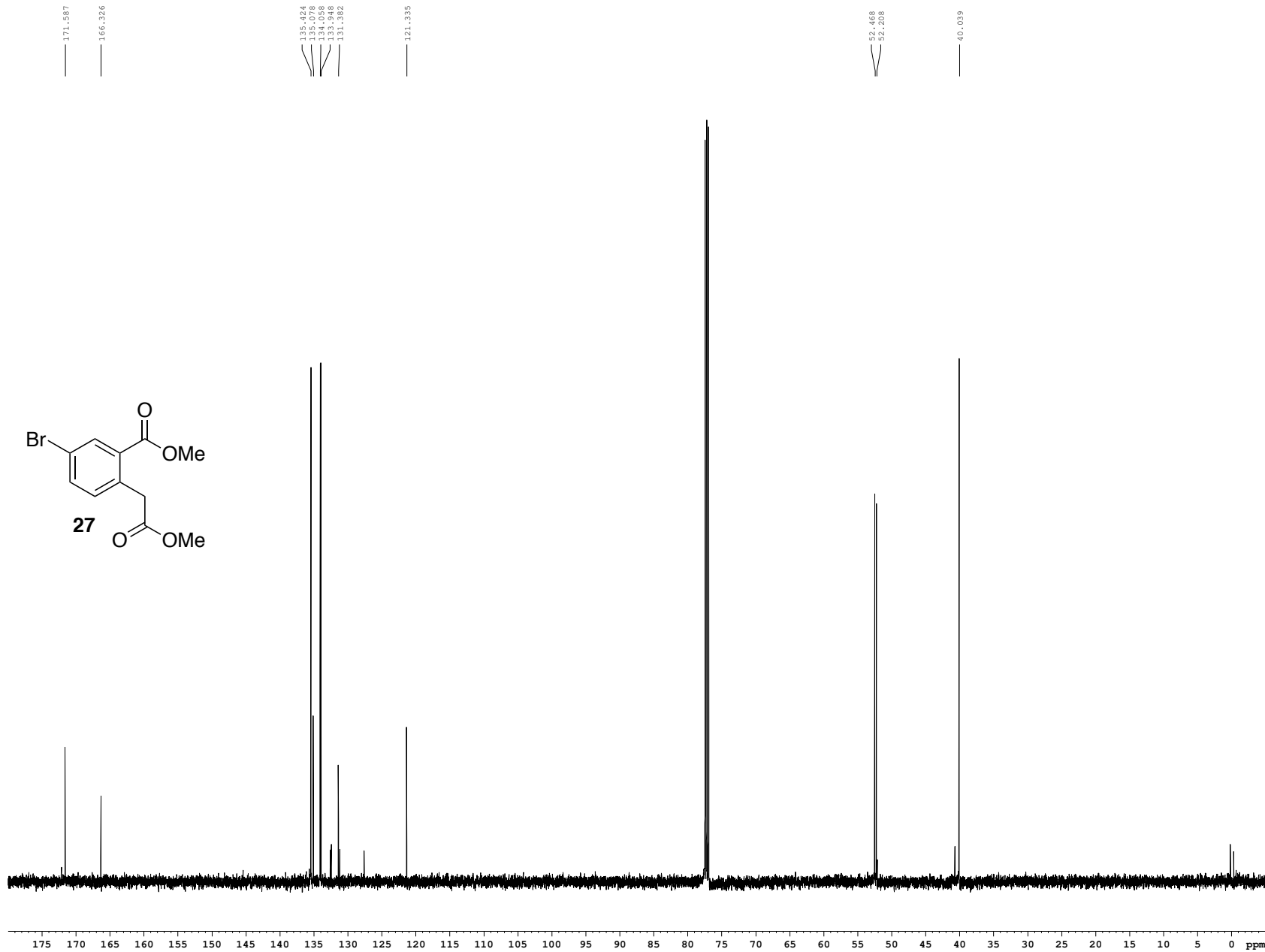
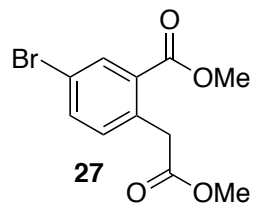


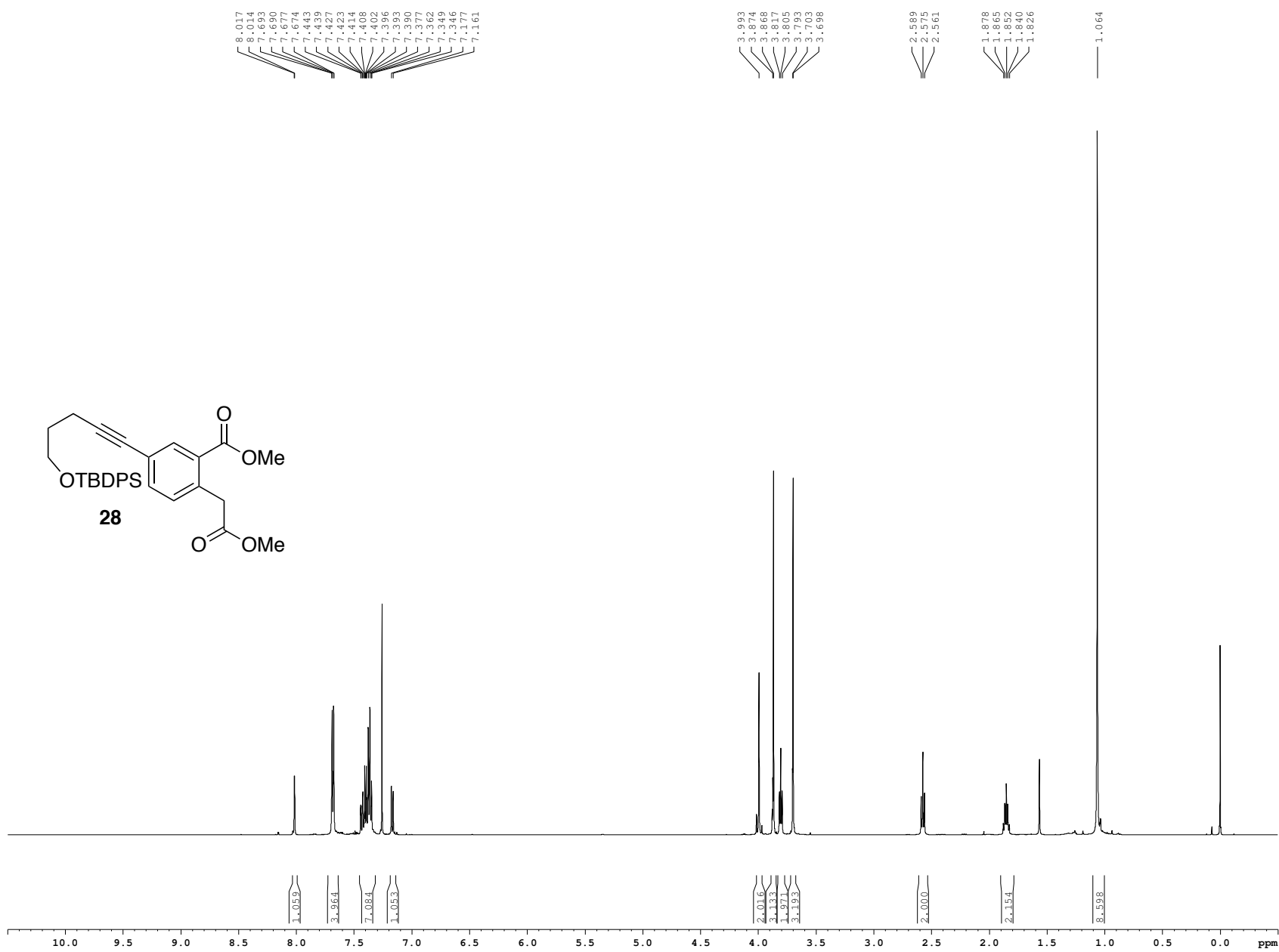
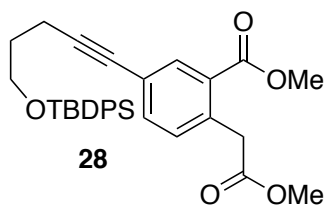












171.877
167.109

135.712
135.223
134.301
133.943
132.866
129.707
127.796
123.699

91.263

79.705

62.462

52.260
52.156

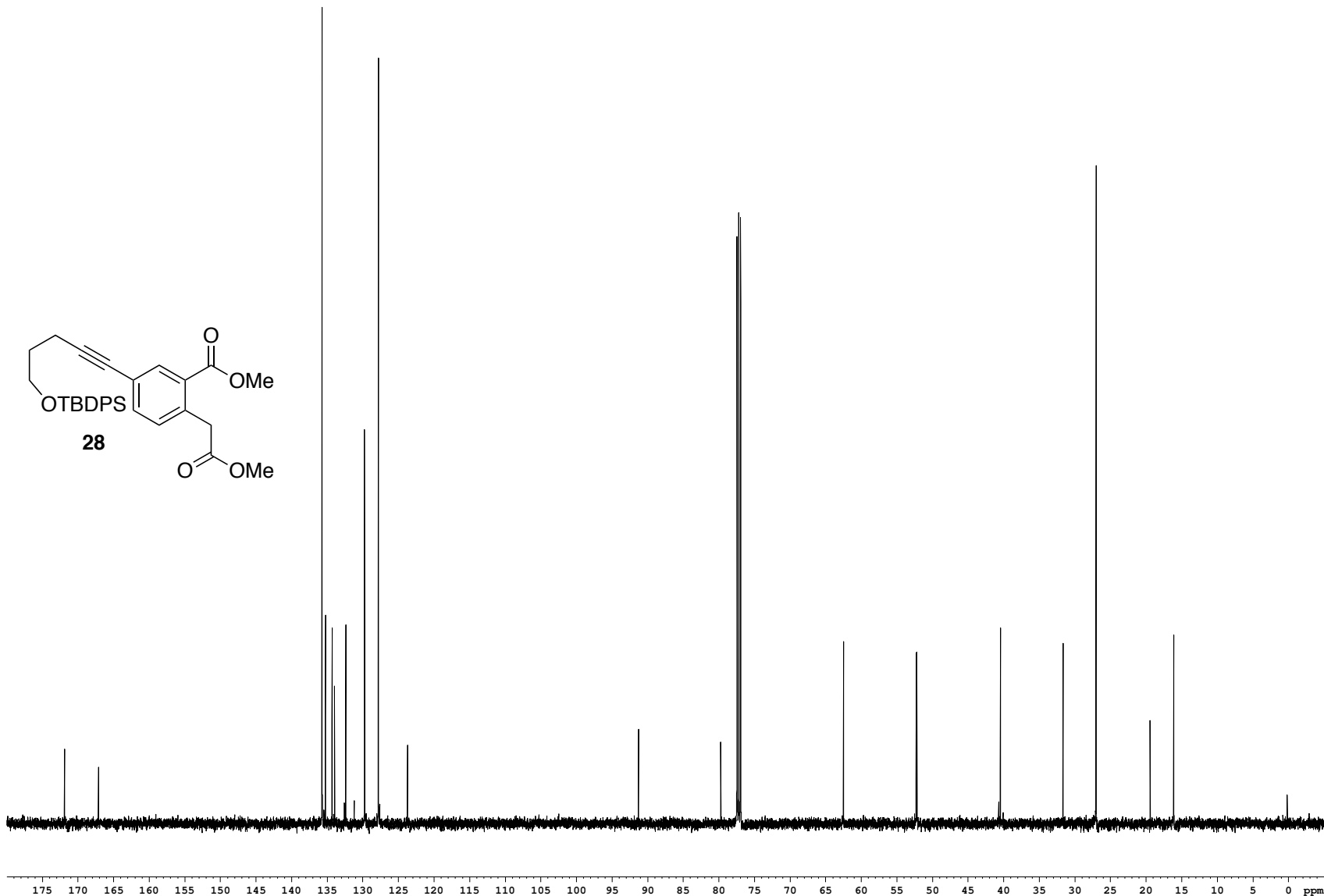
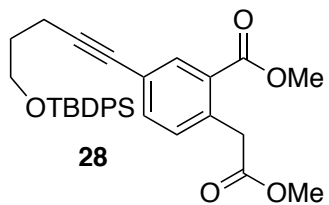
40.430

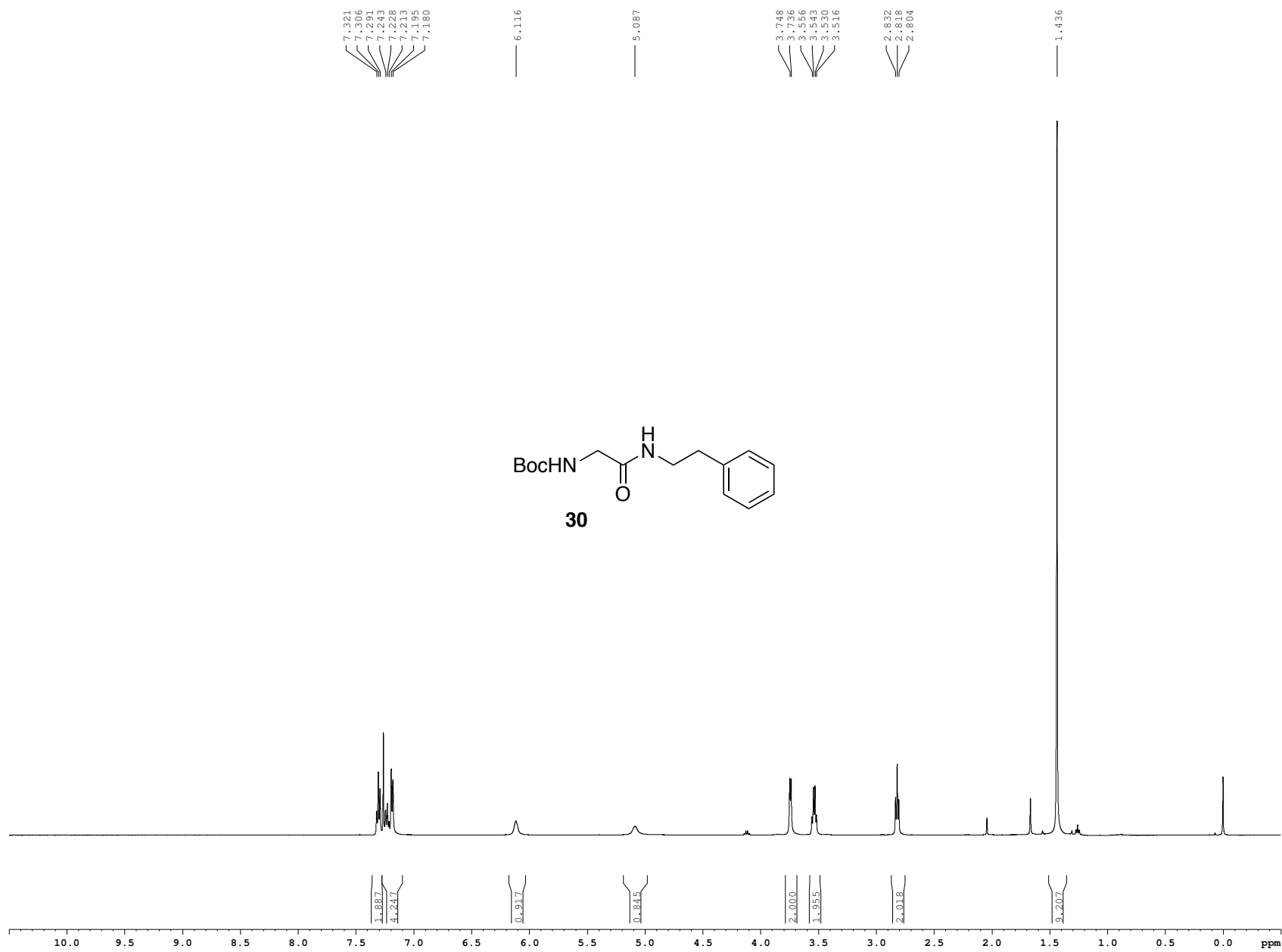
31.628

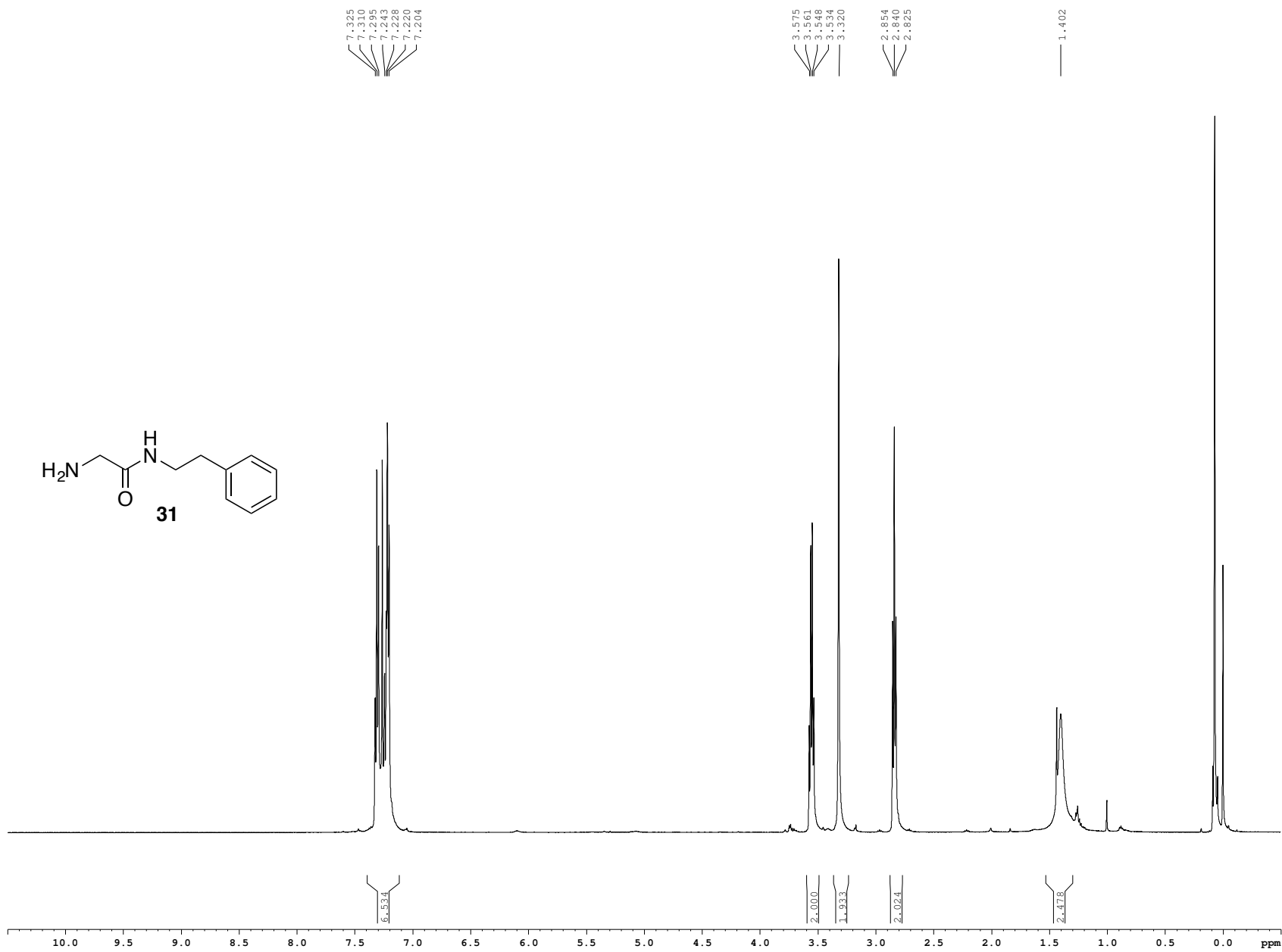
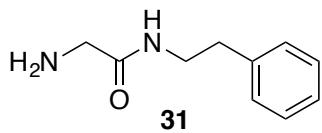
26.997

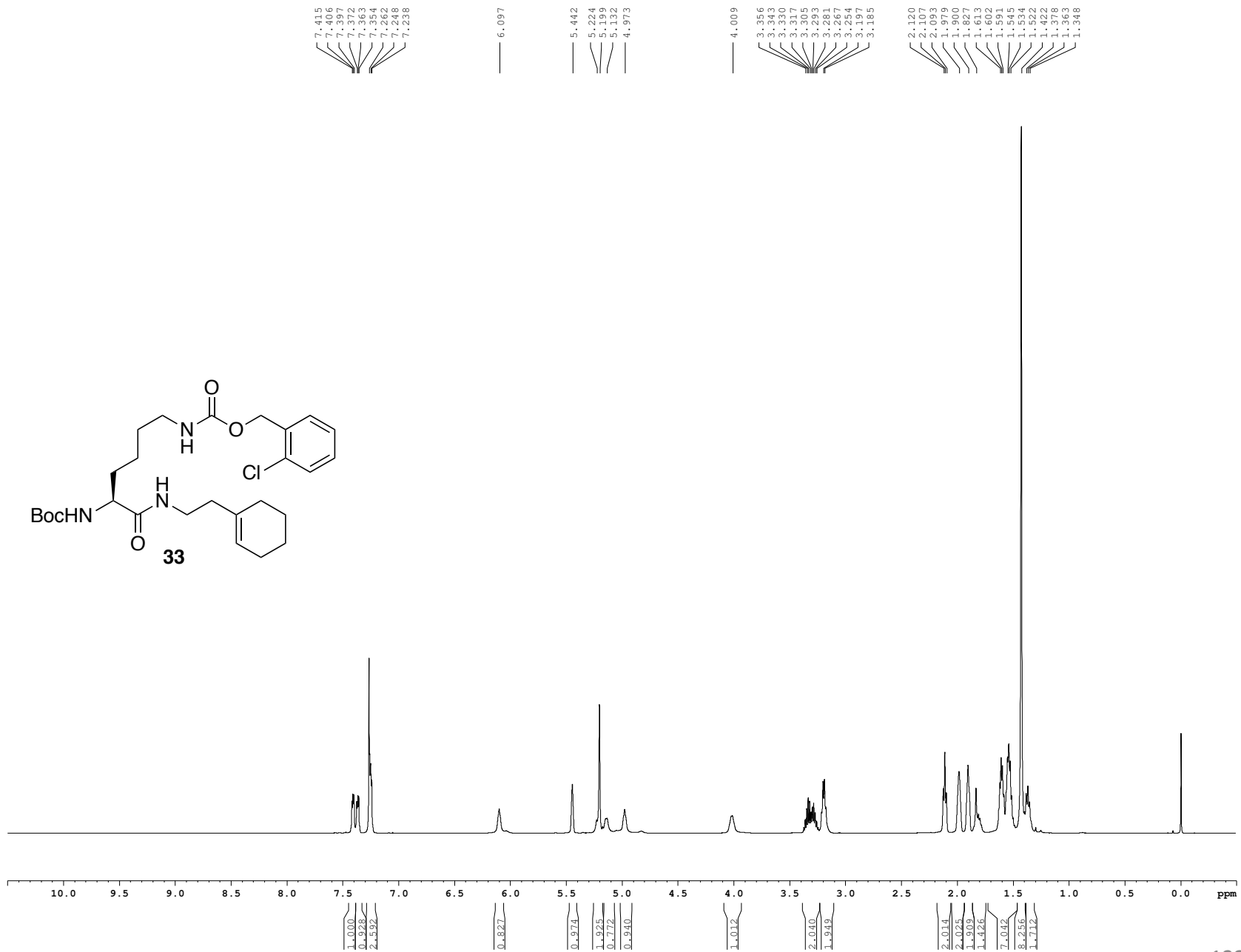
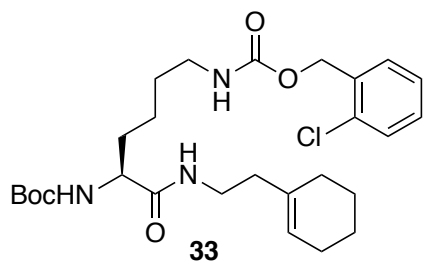
19.413

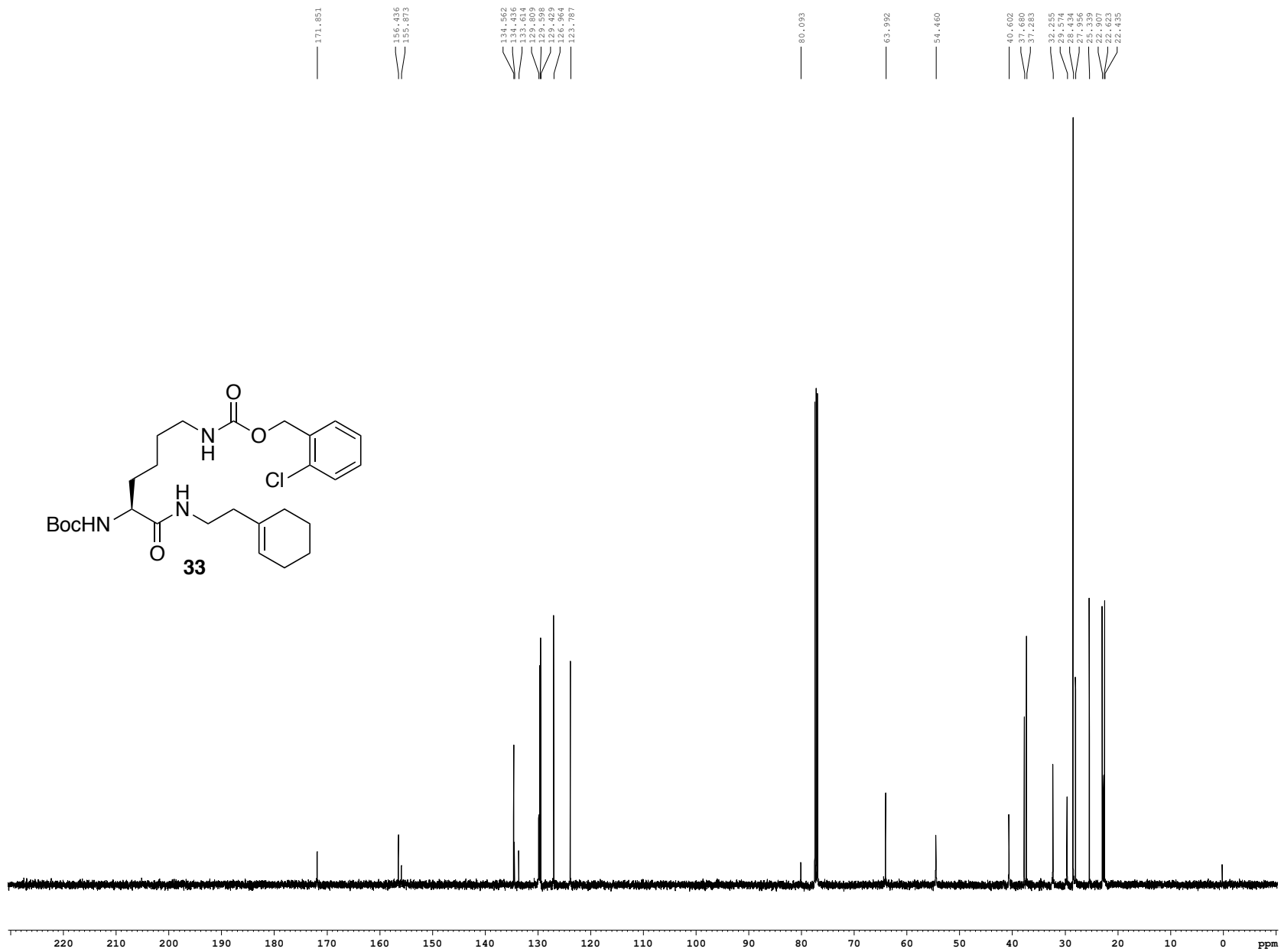
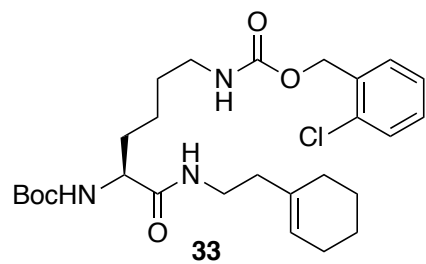
16.074

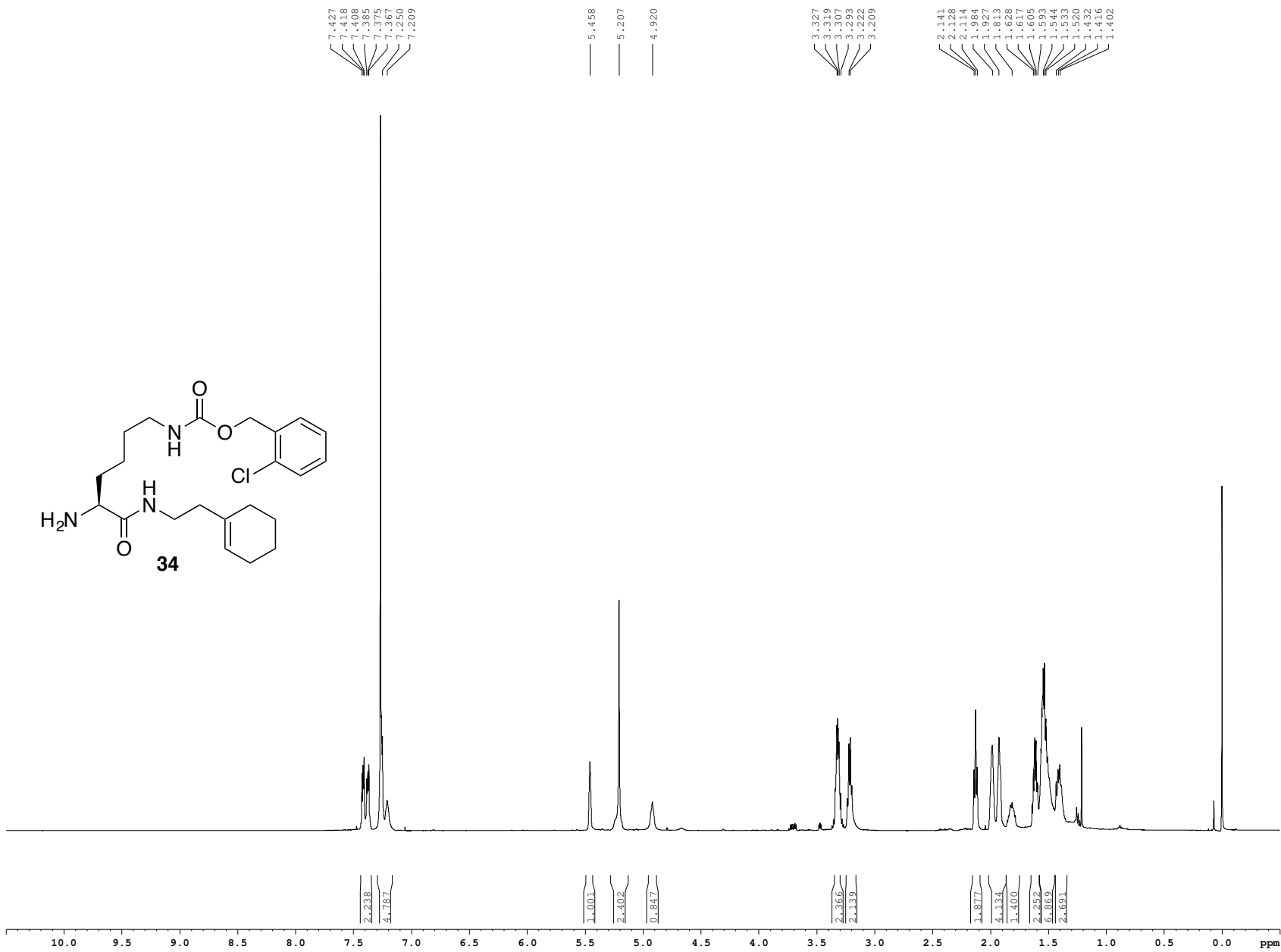
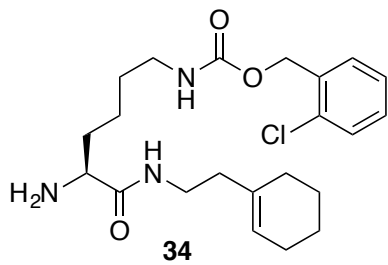


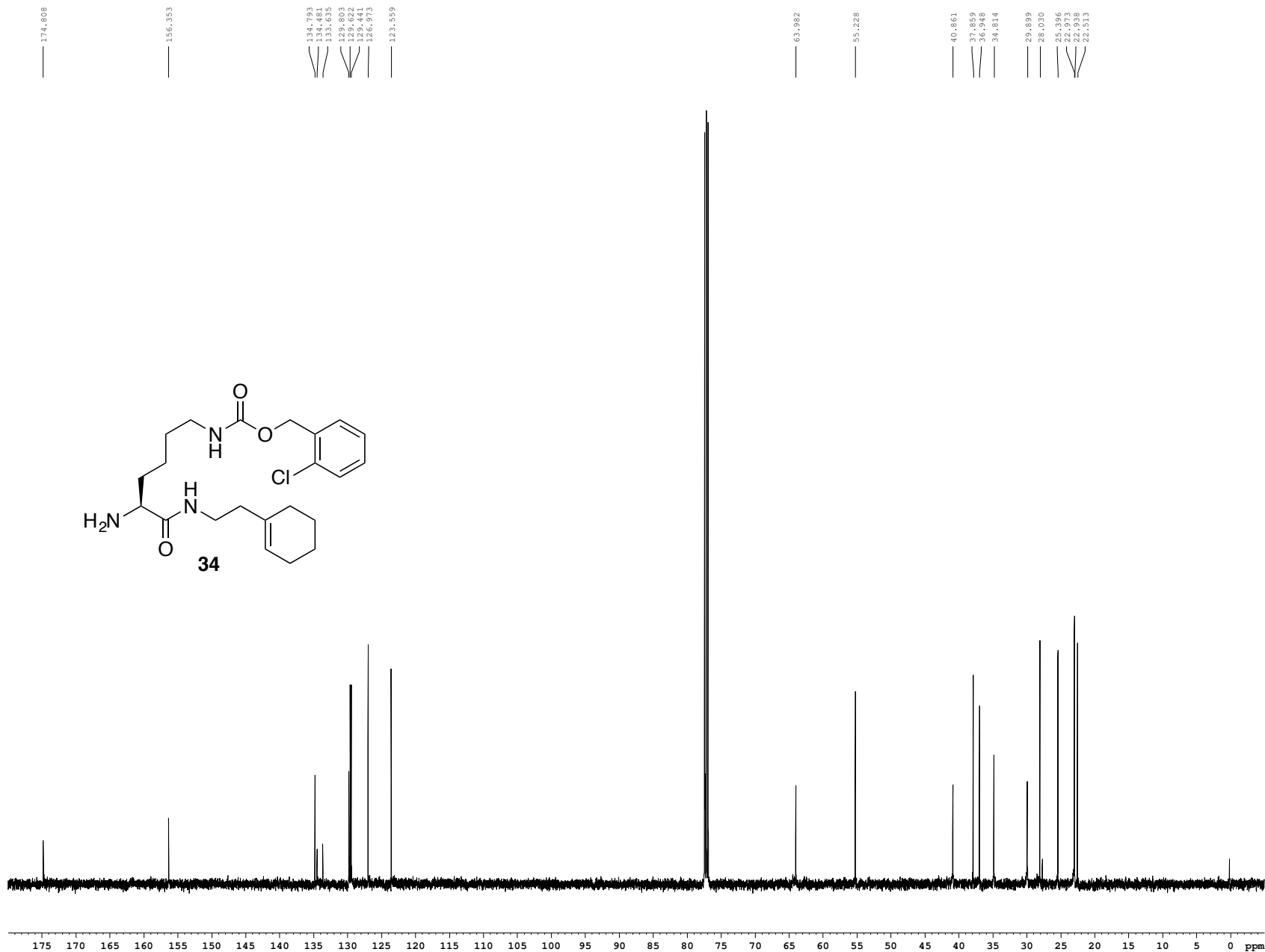
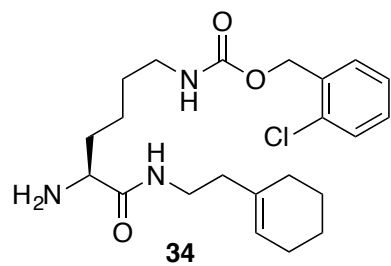




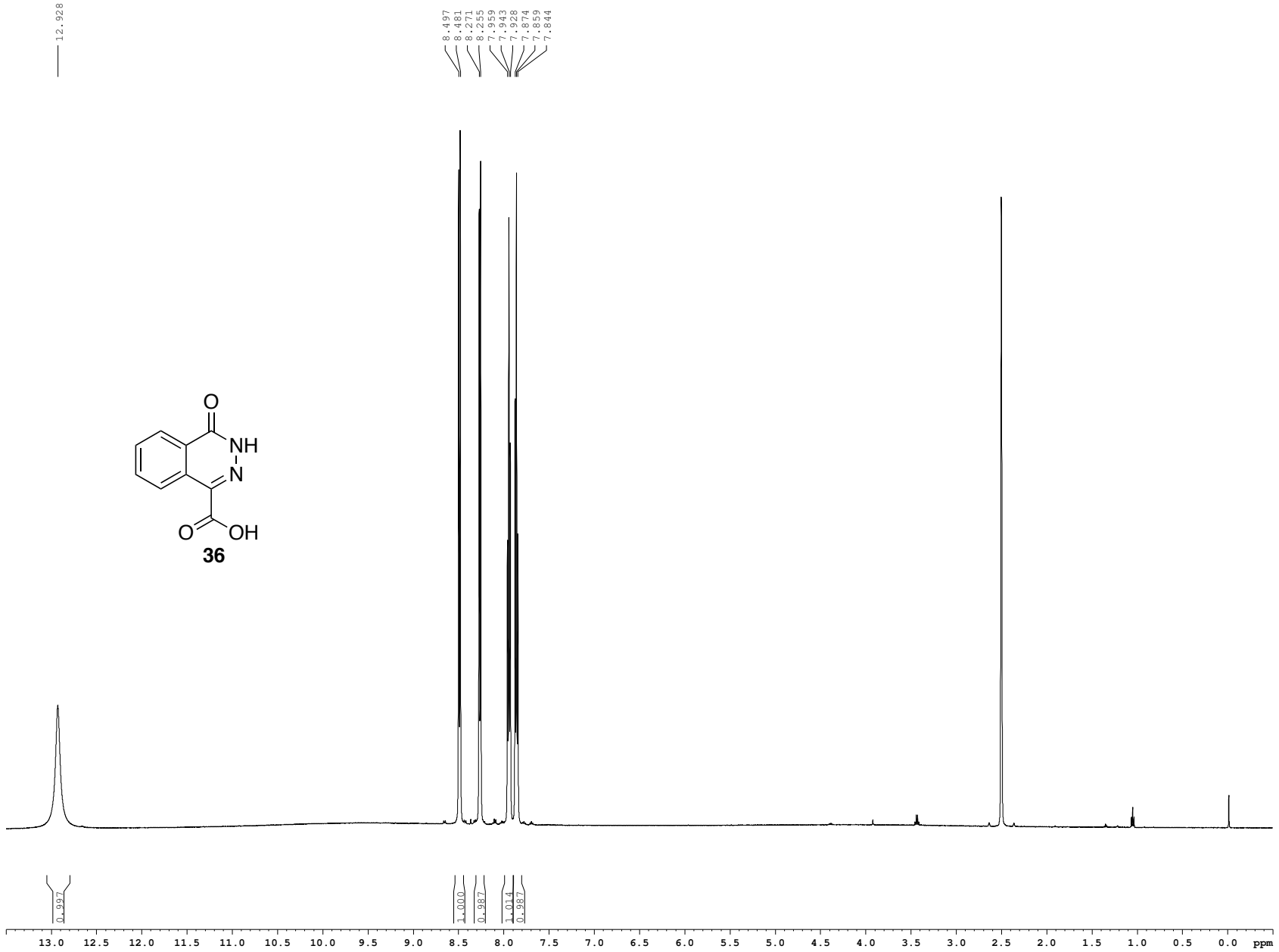
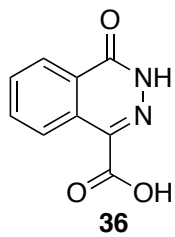








12.928



165.312

159.556

138.763

133.743

131.650

127.970

127.529

126.973

