Supporting Information

Synthesis and Structure-Activity Relationships of Benzothienothiazepinone Inhibitors of Protein Kinase D

Karla Bravo-Altamirano,[†] Kara M. George,[†] Marie-Céline Frantz,[†] Courtney R. LaValle,[‡] Manuj Tandon,[‡] Stephanie Leimgruber,[‡,§] Elizabeth R. Sharlow,[‡,§] John S. Lazo,[‡,§] Q. Jane Wang,[‡,§] and Peter Wipf*,[†,§]

[†]Department of Chemistry, [‡]Department of Pharmacology and Chemical Biology, and [§]Drug Discovery Institute, University of Pittsburgh, Pittsburgh PA 15260, United States of America

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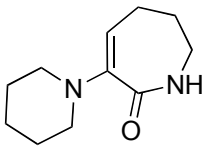
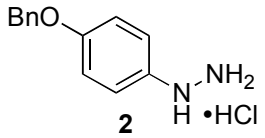
General information. All moisture- and air-sensitive reactions were performed using syringe-septum cap techniques under an inert atmosphere (N_2 or argon) in glassware that was dried in an oven at 140 °C for at least 2 h prior to use. Reactions carried out at a temperature below 0 °C employed a CO_2/acetone bath. All reagents and solvents were used as received unless otherwise specified. Triethylamine, *N*,*N*-dimethylaniline, and pyridine were distilled over CaH_2. THF and Et_2O were distilled over sodium/benzophenone ketyl. DCM and toluene were purified using an alumina column filtration system. Anhydrous MeOH and Et_2O were purchased from Acros Organics and Fisher Scientific, respectively. Anhydrous DMF was purchased from Acros Organics or distilled and stored over 4Å molecular sieves. Analytical thin-layer chromatography (TLC) was performed on pre-coated SiO_2 60 F_{254} plates (250 μm layer thickness) available from Merck. Visualization was accomplished by UV irradiation at 254 nm and/or by staining with Vaughn's reagent (4.8 g $(NH_4)_6Mo_7O_{24}$•$4H_2O$ and 0.2 g $Ce(SO_4)_2$•$4H_2O$ in 100 mL of a 3.5 N H_2SO_4 solution), a $KMnO_4$ solution (1.5 g $KMnO_4$ and 1.5 g K_2CO_3 in 100 mL of a 0.1% NaOH solution), a ninhydrin solution (2 g ninhydrin in 100 mL EtOH), a PMA solution (5 g phosphomolybdic acid in 100 mL EtOH), or a *p*-anisaldehyde solution (2.5 mL *p*-anisaldehyde, 2 mL AcOH and 3.5 mL conc. aq. H_2SO_4 in 100 mL EtOH). Preparative thin-layer chromatography was performed on pre-coated SiO_2 GF (UV254) 1000 microns (20 x 20 cm) plates available from Analtech. Flash column chromatography was performed using SiO_2 60 (particle size 0.040–0.055 mm, 230–400 mesh, or Silicycle SiliaFlash® P60, 40-63 μm). Melting points were determined on a Meltemp capillary melting point apparatus fitted with a Fluke 51 II digital thermometer. Infrared spectra were recorded on a Smiths IdentifyIR ATR spectrometer or a Perkin Elmer Spectrum 100 FT-IR spectrometer using the Universal ATR Sampling Accessory for both oil and solid compounds. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance 300, 400 or 600 instrument at 300/75 MHz, 400/100 MHz or 600/150 MHz, respectively. Chemical shifts were reported in parts per million (ppm) as referenced to residual solvent. ^{1}H NMR spectra are tabulated as follows: chemical shift, multiplicity (app = apparent, b = broad, s = singlet, d = doublet, t = triplet, q = quartet, quint = quintuplet, sext, = sextuplet, m = multiplet), number of protons, coupling constant(s). ^{13}C NMR were obtained using a proton-decoupled pulse sequence and are tabulated by observed peak. Mass spectra were obtained on a Waters Autospec double focusing mass spectrometer (EI) or a Waters Q-Tof mass spectrometer (ESI), at the University of Pittsburgh Mass Spectrometry facility.



1

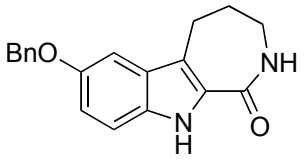
3-Piperidin-1-yl-1,5,6,7-tetrahydroazepin-2-one (1).[1] To a solution of ε-caprolactam (15.2 g, 133 mmol) in $CHCl_3$ (400 mL) cooled to 0-5 °C was added PCl_5 (55.2 g, 265 mmol) over the course of 30 min, followed by addition of anhydrous ZnI_2 (1.53 g, 4.79 mmol) under N_2. The reaction mixture was slowly allowed to reach room temperature as Br_2 (42.4 g, 265 mmol) was added dropwise over the course of 30 min. The mixture was stirred at room temperature for 6 h and then poured into ice-water (300 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 100 mL) and the combined organic layers were washed with 0.50 M aq. $NaHSO_3$ (3 x 200 mL) and brine (1 x 400 mL), dried ($MgSO_4$) and concentrated to yield a yellow solid residue. The solid was suspended in water, filtered, and washed with water and Et_2O to give 3,3-dibromoazepan-2-one (27.5 g, 76%) as a white solid: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.91 (bs, 1 H), 3.39 (app dd, 2 H, J = 10.3, 5.8 Hz), 2.77-2.72 (m, 2 H), 2.02-1.94 (m, 2 H), 1.73-1.67 (m, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 168.7, 69.7, 46.2, 42.8, 28.6, 28.5; HRMS (ESI) *m/z* calcd for $C_6H_9Br_2NONa$ (M+Na) 291.8949, found 291.8973.

A solution of 3,3-dibromoazepan-2-one (15.7 g, 57.9 mmol) in piperidine (140 mL) was heated at reflux for 4.5 h under N_2. The solution was then allowed to reach room temperature and washed with 0.50 M aq. $NaHSO_3$ (200 mL). The aqueous phase was extracted with $CHCl_3$ (3 x 100 mL) and the combined organic layers were washed with brine (1 x 300 mL), dried ($MgSO_4$) and concentrated to afford a brown, oily solid, that crystallized upon standing. The resulting solid was suspended in water, filtered, and washed with water and Et_2O to give **1** (10.3 g, 91%) as a white solid: IR (ATR, neat) 3193, 2950, 2935, 2923, 2855, 1655, 1605 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.51 (bs, 1 H), 5.06 (t, 1 H, *J* = 7.6 Hz), 3.22 (q, 2 H, *J* = 6.5 Hz), 2.78 (app t, 4 H, *J* = 5.3 Hz), 2.15 (q, 2 H, *J* = 7.2 Hz), 1.76 (app quint, 2 H, *J* = 6.8 Hz), 1.69-1.62 (m, 4 H), 1.54-1.48 (m, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.5, 147.6, 105.4, 50.1, 39.5, 30.2, 25.5, 24.5, 21.5; HRMS (EI) *m/z* calcd for $C_{11}H_{18}N_2O$ 194.1419, found 194.1422.



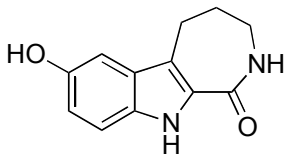
2

(4-(Benzyloxy)phenyl)hydrazine hydrochloride (2).[2] Note: The reaction mixture and all added solutions were maintained at 0 °C during this procedure. 4-Benzyloxyaniline hydrochloride (3.00 g, 12.5 mmol) was added to conc. aq. HCl (25 mL) and stirred for 10 min at 0 °C, followed by dropwise addition of a solution of $NaNO_2$ (852 mg, 12.3 mmol) in water (6 mL) over the course of 15 min. The mixture was stirred for an additional 15 min-period and then a solution of $SnCl_2$ (6.40 g, 33.1 mmol) in conc. aq. HCl (7.5 mL) was added dropwise. The reaction mixture was stirred for 1 h and filtered to yield an off-white precipitate, which was washed with water and triturated with Et_2O, to yield **2** (3.01 g, 96%): IR (ATR, neat) 3232, 2906 (br), 2693, 1568, 1508, 1242, 1177 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 10.11 (bs, 3 H), 7.44-7.40 (m, 2 H), 7.40-7.35 (m, 2 H), 7.33-7.29 (m, 1 H), 7.01-6.93 (m, 4 H), 5.05 (s, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 153.7, 139.1, 137.3, 128.5, 128.4, 128.3, 127.9, 127.7, 127.5, 117.1, 116.9, 115.5, 115.3, 69.5; HRMS (EI) *m/z* calcd for $C_{13}H_{14}N_2O$ 214.1106, found 214.1110.



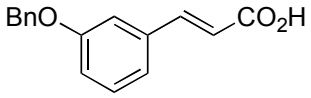
3

3,4,5,10-Tetrahydro-7-benzyloxy-azepino[3,4-*b*]indol-1(2*H*)-one (3). A mixture of **2** (632 mg, 2.52 mmol) and **1** (390 mg, 2.01 mmol) in anhydrous EtOH (3 mL) and H_2SO_4 (0.30 mL) was heated at reflux for 5 h. The reaction was allowed to reach room temperature and the resulting black solid was filtered, washed with water and Et_2O, preadsorbed on SiO_2 and purified by chromatography on SiO_2 (7:3, DCM/acetone) to yield **3** (328 mg, 53%) as a light orange solid: IR (ATR, neat) 3227, 3194, 3033, 2920, 1623, 1543, 1478, 1453, 1276, 1197 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 11.03 (s, 1 H), 7.95 (bs, 1 H), 7.50-7.42 (d, 2 H), 7.41-7.33 (m, 2 H), 7.32-7.29 (m, 2 H), 7.11 (bs, 1 H), 6.97-6.91 (m, 1 H), 5.10 (s, 2 H), 3.38-3.34 (m, 2 H), 2.96 (bs, 2 H), 2.02 (bs, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.1, 152.2, 137.6, 131.2, 128.4, 127.8, 127.7, 127.6, 116.4, 115.6, 113.0, 102.2, 69.7, 41.6, 26.8, 25.4; HRMS (EI) *m/z* calcd for $C_{19}H_{18}N_2O_2$ 306.1368, found 306.1366.



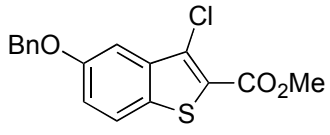
kb-NB123-57

3,4,5,10-Tetrahydro-7-hydroxy-azepino[3,4-*b*]indol-1(2*H*)-one (kb-NB123-57). To a solution of **3** (30.0 mg, 0.0979 mmol) in MeOH (4 mL) was added ammonium formate (100 mg, 1.59 mmol) and 10% Pd/C (20.0 mg, 0.0188 mmol), and the reaction mixture was heated at reflux for 1.5 h under N_2. After cooling to room temperature, the mixture was filtered through Celite® and the filtrate was concentrated under reduced pressure to yield a solid residue, which was dissolved in a minimum amount of MeOH, preadsorbed on SiO_2 and purified by chromatography on SiO_2 (1:1, DCM/acetone) to yield **kb-NB123-57** (18.9 mg, 89%) as a light orange solid: IR (ATR, neat) 3362, 3276, 1600, 1545, 1484, 1362 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 10.82 (s, 1 H), 8.78 (s, 1 H), 7.86 (t, 1 H, J = 4.5 Hz), 7.19 (d, 1 H, J = 8.7 Hz), 6.79 (d, 1 H, J = 1.8 Hz), 6.73 (dd, 1 H, J = 8.7, 2.3 Hz), 3.30-3.22 (m, 2 H), 2.91 (t, 2 H, J = 6.3 Hz), 2.07-1.94 (m, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.2, 150.5, 130.5, 128.1, 127.6, 115.7, 115.2, 112.7, 102.8, 41.6, 26.9, 25.4; HRMS (EI) m/z calcd for $C_{12}H_{12}N_2O_2$ 216.0899, found 216.0898.



4

(*E*)-3-(3-(Benzyloxy)phenyl)prop-2-enoic acid (4).[3] To a stirred suspension of 3-hydroxycinnamic acid (5.00 g, 30.5 mmol) in EtOH (100 mL) was added 1 M aq. NaOH (65 mL) and the reaction mixture was stirred for 5 min, then treated with benzyl bromide (3.72 mL, 31.1 mmol) and stirred for 14 h at room temperature under N_2. The mixture was concentrated under reduced pressure to yield a white solid, which was suspended in water (400 mL) and acidified with conc. aq. HCl. The mixture was filtered and the resulting solid was washed with water and Et_2O, then dried under high vacuum to give **4** (7.10 g, 92%) as a white solid: IR (ATR, neat) 3400-2500 (br), 1691, 1629, 1577, 1260 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.49 (d, 1 H, J = 16.0 Hz), 7.46 (app d, 2 H, J = 7.4 Hz), 7.39 (t, 2 H, J = 7.6 Hz), 7.35-7.29 (m, 3 H), 7.22 (d, 1 H, J = 7.6 Hz), 7.03 (dd, 1 H, J = 8.2, 2.3 Hz), 6.56 (d, 1 H, J = 16.0 Hz), 5.14 (s, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 168.1, 158.7, 142.7, 137.0, 136.1, 130.0, 128.5, 128.0, 127.9, 121.2, 120.9, 116.8, 113.7, 69.3; HRMS (EI) m/z calcd for $C_{16}H_{14}O_3$ 254.0943, found 254.0950.

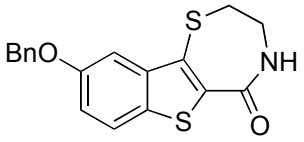


5

Methyl 5-(benzyloxy)-3-chlorobenzo[*b*]thiophene-2-carboxylate (5). To a mixture of **4** (8.80 g, 34.6 mmol) in chlorobenzene (50 mL), anhydrous pyridine (0.26 mL, 3.29 mmol) and anhydrous DMF (2.60 mL) was added dropwise $SOCl_2$ (12.6 mL, 173 mmol) at room temperature. The reaction mixture was heated at 120 °C for 22 h under N_2. The solution was concentrated by rotary evaporation and traces of pyridine were removed by azeotropic distillation with toluene. The resulting brown oil was dried under high vacuum overnight to yield a brown solid, which was suspended in Et_2O, filtered and dried under high vacuum to yield 3-chloro-5-(benzyloxy)-benzo[*b*]thiophene-2-carbonyl chloride (5.87

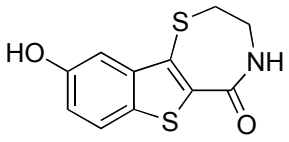
g). Upon cooling the filtrate, a second crop of product (1.25 g) was collected by filtration (7.12 g, 61% combined yield). Representative experimental data are as follows: Mp 138-143 °C (lit Mp 139-142 °C);[3] IR (ATR, neat) 1745, 1602, 1483, 1284, 1158 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 8.01 (d, 1 H, J = 8.8 Hz), 7.50 (d, 2 H, J = 7.3 Hz), 7.44 (d, 1 H, J = 2.3 Hz), 7.41 (t, 2 H, J = 7.3 Hz), 7.37-7.33 (m, 2 H), 5.25 (s, 2 H); MS (EI) *m/z* 338 (90), 336 (M^+, 100), 303 (40), 301 ($[M–Cl]^+$, 88).

To a suspension of the precursor 3-chloro-5-(benzyloxy)-benzo[*b*]thiophene-2-carbonyl chloride (516 mg, 1.53 mmol) in anhydrous MeOH (30 mL) was added anhydrous Et_3N (0.43 mL, 3.06 mmol) and the reaction mixture was heated at reflux for 12 h under N_2. The solution was concentrated under reduced pressure and the residue was purified by chromatography on (SiO_2, hexanes to 9:1, hexanes/EtOAc) to yield **5** (392 mg, 77%) as a light yellow solid. Representative experimental data are as follows: IR (ATR, neat) 1682, 1602, 1509, 1305, 1192 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 8.03 (d, 1 H, J = 8.9 Hz), 7.50 (d, 2 H, J = 7.6 Hz), 7.46 (s, 1 H), 7.41 (t, 2 H, J = 7.6 Hz), 7.39-7.33 (m, 2 H), 5.25 (s, 2 H), 3.89 (s, 3 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 160.8, 157.3, 137.3, 136.6, 130.6, 128.5, 128.0, 127.9, 126.4, 125.2, 124.7, 120.3, 105.4, 69.7, 52.8; HRMS (EI) *m/z* calcd for $C_{17}H_{13}ClO_3S$ 332.0274, found 332.0268.



6

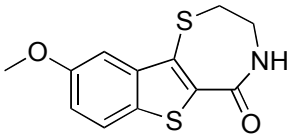
3,4-Dihydro-9-benzyloxy-[1]benzothieno[2,3-*f*]-1,4-thiazepin-5(2*H*)-one (6). To a solution of **5** (390 mg, 1.17 mmol) in anhydrous DMF (8 mL) was added cysteamine•HCl (533 mg, 4.69 mmol) and DBU (1.42 mL, 9.38 mmol) at room temperature under N_2. The reaction mixture was stirred at room temperature for 1.5 h and heated to 70 °C for 12 h, then diluted with EtOAc (15 mL) and washed with 2 M aq. HCl (15 mL) to give a white precipitate, which was filtered, triturated with water and Et_2O, and dried under high vacuum to yield **6** (260 mg) as a white solid. The layers of the filtrate were separated and the aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (1 x 100 mL), dried ($MgSO_4$) and concentrated to give a yellow precipitate, which was suspended in hexanes/EtOAc (1:1), filtered, triturated with Et_2O and dried under high vacuum to yield a second crop of **6** (90.0 mg) as an off-white solid. The overall amount obtained was 350 mg (87%). Representative experimental data are as follows: Mp 247-249 °C; IR (ATR, neat) 3165, 3037, 1650, 1500, 1282 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 8.47 (t, 1 H, J = 5.6 Hz), 7.90 (d, 1 H, J = 8.8 Hz), 7.49 (d, 2 H, J = 7.5 Hz), 7.40 (t, 2 H, J = 7.7 Hz), 7.36-7.32 (m, 1 H), 7.29-7.27 (m, 1 H), 7.26-7.23 (m, 1 H), 5.20 (s, 2 H), 3.64-3.60 (m, 2 H), 3.41-3.37 (m, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 165.1, 156.4, 139.4, 136.9, 133.3, 131.2, 128.5, 127.9, 127.8, 123.8, 118.0, 105.8, 69.6, 42.4, 33.4; HRMS (EI) m/z calcd for $C_{18}H_{15}NO_2S_2$ 341.0544, found 341.0543.



kb-NB142-70

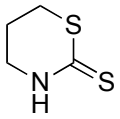
3,4-Dihydro-9-hydroxy-[1]benzothieno[2,3-*f*]-1,4-thiazepin-5(2*H*)-one (kb-NB142-70). To a solution of **6** (250 mg, 0.732 mmol) in anhydrous DCM (20 mL) at –20 °C was added a 1.0 M solution of BBr_3 in DCM (1.10 mL, 1.10 mmol)

under N_2. The reaction mixture was stirred at –20 °C for 0.5 h, then slowly warmed to 0 °C and stirred for 2 h. The solution was warmed to room temperature, diluted with DCM (20 mL) and poured into ice-water (30 mL) to give a white precipitate, which was filtered, triturated with water and DCM and dried under high vacuum to yield kb-NB142-70 (172 mg, 93%) as a light green solid. Representative experimental data are as follows: Mp 218-220 °C (dec., dark brown), 235-238 °C (dec., melts); IR (ATR, neat) 3269, 1633, 1597, 1496, 1432, 1197 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 9.73 (s, 1 H), 8.42 (t, 1 H, J = 5.5 Hz), 7.77 (d, 1 H, J = 8.7 Hz), 7.11 (d, 1 H, J = 1.9 Hz), 7.01 (dd, 1 H, J = 8.7, 1.6 Hz), 3.64-3.59 (m, 2 H), 3.40-3.36 (m, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 165.2, 155.3, 139.6, 132.8, 129.3, 127.l, 123.6, 117.8, 107.0, 42.5, 33.3; HRMS (EI) m/z calcd for $C_{11}H_9NO_2S_2$ 251.0075, found 251.0080.



kb-NB165-09

3,4-Dihydro-9-methoxy-[1]benzothieno[2,3-*f*]-1,4-thiazepin-5(2*H*)-one (kb-NB165-09).[4] To a solution of **kb-NB142-70** (30.0 mg, 0.119 mmol) in anhydrous DMF (1 mL) was added K_2CO_3 (165 mg, 1.19 mmol) followed by MeI (8.0 μL, 0.128 mmol). The reaction mixture was stirred at room temperature for 12 h, excess K_2CO_3 was filtered off and the solution was concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography on SiO_2 (1000 μm, EtOAc to 10% MeOH in EtOAc) to yield **kb-NB165-09** (29.0 mg, 92%) as a white solid: Mp 202-204 °C (lit. 209-209.5 °C);[4] IR (ATR, neat) 3156, 3018, 2916, 1633, 1499, 1403, 1284 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 8.47 (t, 1 H, J = 5.3 Hz), 7.89 (d, 1 H, J = 8.3 Hz), 7.19-7.15 (m, 2 H), 3.84 (s, 3 H), 3.65-3.60 (m, 2 H), 3.42-3.38 (m, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 165.2, 157.4, 139.4, 133.4, 130.9, 127.7, 123.8, 117.5, 104.4, 55.4, 42.4, 33.4; HRMS (EI) *m/z* calcd for $C_{12}H_{11}NO_2S_2$ 265.0231, found 265.0232.

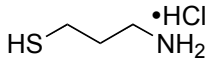


7

1,3-Thiazinane-2-thione (7).[5] To a solution of 3-amino-1-propanol (7.61 mL, 100 mmol) in CCl_4 (20 mL) at 0 °C was added dropwise chlorosulfonic acid (6.70 mL, 101 mmol) through an addition funnel. *Warning*: Reaction is very exothermic! The reaction mixture was stirred at room temperature overnight and then concentrated under reduced pressure to yield a solid residue, which was suspended in MeOH (40 mL), filtered, triturated with MeOH, and dried under high vacuum to yield 3-aminopropyl hydrogen sulfate (12.5 g, 80%) as a white powder:[5] IR (ATR, neat) 3128, 3069, 2979, 1198, 1172, 925 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 7.65 (bs, 3 H), 3.81 (t, 2 H, J = 6.1 Hz), 2.86 (app dq, 2 H, J = 12.8, 6.0 Hz), 1.83-1.77 (m, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 62.9, 36.6, 27.2.

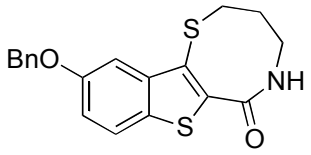
To a suspension of the precursor 3-aminopropyl hydrogen sulfate (12.0 g, 77.3 mmol) and CS_2 (5.60 mL, 92.8 mmol) in 50% aq. (*v*/*v*) EtOH (33 mL) at 0 °C was slowly added a solution of NaOH (6.80 g, 170 mmol) in 50% aq. (*v*/*v*) EtOH (15.0 mL). The reaction mixture was heated at reflux for 40 min and then cooled down to room temperature, resulting in the formation of off-white crystals, which were filtered, washed with ice-cold water and dried under high vacuum to yield the first crop of **7** (7.85 g). Upon cooling the filtrate to 0 °C, more crystals formed, and they were filtered, washed with ice-cold water and dried under high vacuum to yield the second crop of **7** (0.790 g). The overall amount of **7** was 8.64 g

(84%) obtained as an off-white crystalline solid: IR (ATR, neat) 3128, 3039, 2917, 1539, 1326 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 10.22 (s, 1 H), 3.29 (t, 2 H, J = 4.9 Hz), 2.97-2.93 (m, 2 H), 1.99-1.94 (m, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 191.7, 43.5, 29.7, 20.2; MS (EI) m/z 133 (M^+, 100), 134 (6), 135 (9); HRMS (EI) m/z calcd for $C_4H_7NS_2$ 133.0020, found 133.0016.



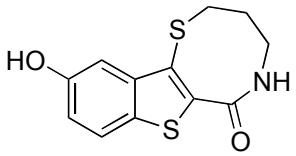
8

3-Aminopropanethiol hydrochloride (8).[5] A suspension of **7** (7.70 g, 57.8 mmol) in conc. aq. HCl (50 mL) was heated at reflux for 2 weeks under N_2. After cooling to room temperature, the residual aq. HCl was removed by vacuum distillation (P = 5 mmHg) under mild heating (60 °C) for 1 h. The solid residue was suspended in a degassed solution of Et_2O/EtOH (9:1, 50 mL) and stirred for 10 min at room temperature under argon, then poured into a coarse fritted Schlenk filter under argon and thoroughly washed with a degassed solution of Et_2O/EtOH (9:1), to give a white solid, which was dried in the Schlenk filter under high vacuum overnight to yield the first crop of solid as a mixture of **8** and the corresponding disulfide (4.35 g). A second crop of solid was collected by filtration into a regular coarse fritted funnel under air to yield pure disulfide (38.0 mg). The overall yield based on mass recovery was 64% (4.73 g) of a white, highly hygroscopic solid that requires storage in a vacuum dessicator containing drierite: Spectroscopic data for crop 1 [disulfide (57%) + **8** (43%)]: IR (ATR, neat) 2894 (br), 2965 (br), 1607, 1493 cm^{-1}; *major product (disulfide)*: ^1H NMR (D_2O, 600 MHz) δ 3.14-3.10 (m, 2 H), 2.81 (t, 2 H, J = 7.1 Hz), 2.14-2.07 (m, 2 H); ^{13}C NMR (D_2O, 150 MHz) δ 40.9, 36.5, 28.8; *minor product (**8**)*: ^1H NMR (D_2O, 600 MHz) δ 3.14-3.10 (m, 2 H), 2.63 (t, 2 H, J = 7.0 Hz), 2.00-1.94 (m, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 40.9, 33.4, 23.4.



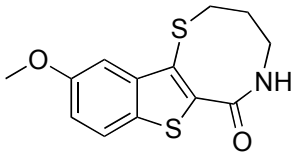
9

2,3,4,5-Tetrahydro-10-benzyloxybenzo[*b*]thieno[2,3-*f*]-1,5-thiazocin-6-one (9). To a solution of **5** (300 mg, 0.901 mmol) in anhydrous, degassed DMF (7.5 mL) was added hydrochloride salt **8** (1.15 g, 3.88 mmol, 43% purity) followed by degassed DBU (1.50 mL, 9.93 mmol) under argon. The reaction mixture was stirred at room temperature for 2 h, then warmed to 70 °C and stirred for 18 h. After cooling to room temperature, the solution was diluted with EtOAc (30 mL) and washed with 2 M aq. HCl (30 mL). The aqueous phase was extracted with EtOAc (3 x 30 mL) and the combined organic layers were washed with brine (1 x 150 mL), dried ($MgSO_4$) and concentrated to yield a yellow oil, which was dried under high vacuum to remove residual DMF. The residue was preloaded on SiO_2 and purified by chromatography on SiO_2 (hexanes to 5% MeOH in EtOAc) to give a yellow solid, which was suspended in Et_2O/MeOH (9:1), filtered, triturated with Et_2O and dried under high vacuum to yield **9** (133 mg, 42%) as a white solid: Mp 198-199 °C; IR (ATR, neat) 3162, 3033, 2937, 1644, 1619, 1600, 1497, 1384, 1274, 1193 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 8.02 (bs, 1 H), 7.90-7.84 (m, 1 H), 7.51-7.45 (m, 2 H), 7.43-7.37 (m, 2 H), 7.36-7.31 (m, 1 H), 7.26-7.19 (m, 2 H), 5.20 (s, 2 H), 3.50-3.43 (m, 2 H), 3.30-3.24 (m, 2 H), 1.92-1.89 (m, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.8, 156.4, 138.7, 136.9, 130.3, 128.5, 128.5, 127.9, 127.7, 127.4, 123.6, 117.3, 105.8, 69.6, 30.5, 27.4; MS (EI) m/z, 355 (M^+, 100), 356 (23), 357 (12); HRMS (EI) m/z calcd for $C_{19}H_{17}NO_2S_2$ 355.0701, found 355.0689.



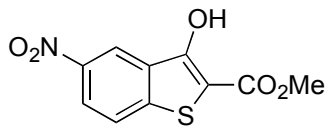
kb-NB165-92

2,3,4,5-Tetrahydro-10-hydroxybenzo[*b*]thieno[2,3-*f*]-1,5-thiazocin-6-one (kb-NB165-92). To a suspension of **9** (54.0 mg, 0.152 mmol) in anhydrous DCM (5 mL) at –20 °C was added a 1 M solution of BBr_3 in DCM (0.30 mL, 0.300 mmol) under N_2. The reaction mixture was stirred at –20 °C for 30 min, warmed to 0 °C and stirred for 1 h, and finally warmed to room temperature and stirred for another 30 min. The solution was diluted with DCM (5 mL) and quenched with cold water (10 mL), resulting in the formation of a white solid, which was filtered, triturated with water, DCM and Et_2O, and dried under high vacuum to yield **kb-NB165-92** (23.5 mg) as an off-white solid. The filtrate was concentrated and purified by preparative thin-layer chromatography on SiO_2 (1000 μm, 5% to 15% MeOH in DCM) to yield **kb-NB165-92** (13.5 mg). The overall amount of **kb-NB165-92** was 37.0 mg (92%) obtained as an off-white solid: Mp 139-142 °C; IR (ATR, neat) 3256 (br), 3169 (br), 1615, 1492, 1444, 1182 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 9.71 (s, 1 H), 7.97 (t, 1 H, J = 7.8 Hz), 7.74 (d, 1 H, J = 8.6 Hz), 7.09 (s, 1 H), 6.98 (d, 1 H, J = 8.6 Hz), 3.50-3.43 (m, 2 H), 3.30-3.23 (m, 2 H), 1.92-1.85 (m, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.9, 155.3, 139.0, 128.4, 127.0, 123.4, 117.1, 106.8, 30.5, 27.3; MS (EI) m/z 265 (M^+, 100), 266 (15), 267 (11); HRMS (EI) m/z calcd for $C_{12}H_{11}NO_2S_2$ 265.0231, found 265.0230.



kb-NB184-02

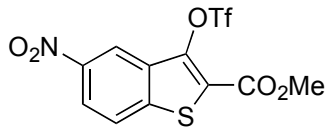
2,3,4,5-Tetrahydro-10-methoxybenzo[*b*]thieno[2,3-*f*]-1,5-thiazocin-6-one (kb-NB184-02). To a solution of **kb-NB165-92** (19.0 mg, 0.0716 mmol) in anhydrous DMF (1.5 mL) was added K_2CO_3 (0.100 g, 0.724 mmol) followed by MeI (4.5 μL, 0.0720 mmol). The reaction mixture was stirred at room temperature overnight, quenched with water (15 mL) and extracted with EtOAc (15 mL). The aqueous phase was further extracted with EtOAc (3 x 15 mL) and the combined organic layers were washed with brine (1 x 50 mL), dried ($MgSO_4$), concentrated and purified by preparative thin-layer chromatography on SiO_2 (1000 μm, 5% to 15% MeOH in EtOAc) to yield **kb-NB184-02** (15.5 mg, 77%) as a white solid: Mp 185-188 °C; IR (ATR, neat) 3152, 3026, 2939, 1636, 1498, 1395, 1209 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 8.02 (t, 1 H, J = 7.1 Hz), 7.87 (d, 1 H, J = 9.4 Hz), 7.16-7.13 (m, 2 H), 3.84 (s, 3 H), 3.50-3.44 (m, 2 H), 3.30-3.26 (m, 2 H), 1.92-1.87 (m, 2 H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.8, 157.4, 138.7, 130.1, 127.4, 123.6, 116.8, 104.3, 55.4, 30.5, 27.4; MS (EI) m/z 279 (M^+, 100), 280 (16); HRMS (EI) m/z calcd for $C_{13}H_{13}NO_2S_2$ 279.0388, found 279.0379.



10

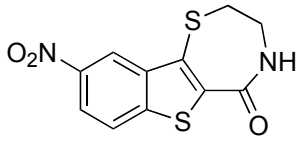
Methyl 3-hydroxy-5-nitrobenzo[*b*]thiophene-2-carboxylate (10). Methyl thioglycolate (2.6 mL, 27.3 mmol) was added to a solution of methyl 2-chloro-5-nitrobenzoate (5.00 g, 22.7 mmol) in dry MeOH (100 mL), followed by addition of

Et_3N (9.6 mL, 68.2 mmol) with stirring over a period of *ca.* 5 min. The resulting yellow solution was vigorously stirred at room temperature under N_2, becoming rapidly red then dark red. A precipitate formed after 30 min. The mixture was heated to 40-50 °C and stirred for 4 h, then poured into a stirred mixture of ice and 1 N aq. HCl (300 mL). The resulting pale yellow precipitate was filtered, rinsed with water and dried by forming an azeotrope with toluene. Recrystallization from toluene (*ca.* 200 mL) afforded **10** (5.08 g, 88%) as a yellow cristalline powder. Representative experimental data are as follows: Mp 221.4-222.0 °C (softening point: 220 °C, toluene); IR (ATR, neat) 3241 (br), 3083, 2963, 1674, 1596, 1582, 1540, 1504, 1437, 1355, 1338, 1318, 1195, 1167, 1139, 1094, 1079, 1049, 978, 915, 911, 828, 783, 770, 738, 671 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.21 (bs, 1 H), 8.80 (d, 1 H, J = 2.1 Hz), 8.31 (dd, 1 H, J = 9.0, 2.4 Hz), 8.21 (d, 1 H, J = 9.0 Hz), 3.86 (s, 3 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.2, 155.2, 145.2, 143.4, 132.0, 125.1, 122.4, 118.7, 107.3, 52.6; MS (EI) *m/z* 253 (M^+, 36), 221 ([M–MeOH]$^+$, 100), 119 (60); HRMS (EI) *m/z* calcd for $C_{10}H_7NO_5S$ 253.0045, found 253.0040.



11

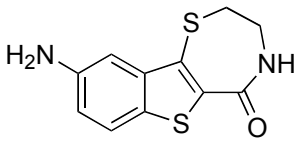
Methyl 5-nitro-3-(trifluoromethylsulfonyloxy)benzo[*b*]thiophene-2-carboxylate (11). To a suspension of **10** (500 mg, 1.97 mmol) in dry DCM (10 mL) at 0 °C were added DMAP (12.3 mg, 0.0987 mmol), Et_3N (0.39 mL, 2.76 mmol) and Tf_2O (0.47 mL, 2.76 mmol). The resulting solution was stirred at room temperature under argon for 2 h. The reaction mixture was then quenched with sat. aq. $NaHCO_3$ and extracted twice with DCM. The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Chromatography of the residue on SiO_2 (8:2 to 7:3, hexanes/EtOAc) afforded **11** (705 mg, 93%) as a pale yellow cristalline powder: Mp 106.7-107.0 °C (softening point: 106.0 °C); IR (ATR, neat) 3098, 2960, 1722, 1603, 1581, 1538, 1517, 1420, 1405, 1344, 1316, 1279, 1230, 1210, 1150, 1124, 1111, 1088, 1062, 1034, 966, 954, 919, 904, 848, 828, 811, 781, 768, 757, 740, 732, 656 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.74 (d, 1 H, J = 1.5 Hz), 8.43 (dd, 1 H, J = 9.0, 2.1 Hz), 8.04 (d, 1 H, J = 9.0 Hz), 4.05 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.1, 146.7, 142.3, 140.0, 131.2, 127.0, 124.7, 122.7, 118.8 (q, J = 319.0 Hz), 118.3 (d, J = 1.2 Hz), 53.6; MS (EI) *m/z* 385 (M^+, 28), 252 (99), 196 (100), 68 (76); HRMS (EI) *m/z* calcd for $C_{11}H_6F_3NO_7S_2$ 385.9538, found 385.9534.



12

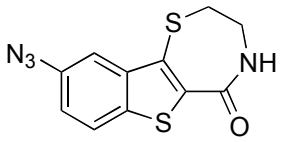
3,4-Dihydro-9-nitro-[1]benzothieno[2,3-*f*]-1,4-thiazepin-5(2*H*)-one (12). To a solution of triflate **11** (632 mg, 1.64 mmol) in dry DMF (9.2 mL) were added cysteamine•HCl (745 mg, 6.56 mmol) and then DBU (2.0 mL, 13.1 mmol). The resulting dark red mixture was stirred at room temperature under argon for 1.5 h. The slurry was then heated at 70 °C for 13 h, then diluted with EtOAc and 2 N aq. HCl was added. The resulting yellow mixture was filtered, and the solid boiled in toluene, filtered immediately over a hot filter, rinsed with toluene and dried to afford **12** (231 mg, 50%, 67% b.r.s.m.) as a yellow powder. An additional 105 mg (25%) of **10** was recovered from the filtrate. Representative experimental data for **12** are as follows: Mp 294-296 °C (dec.); IR (ATR, neat) 3262, 3150, 3020, 2915, 1640, 1597, 1567, 1515, 1495, 1467, 1454, 1439, 1418, 1402, 1346, 1327, 1286, 1258, 1245, 1232, 1189, 1139, 1096, 1081, 1016, 965, 923, 885, 878, 829,

811, 734 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.71 (bt, 1 H, J = 5.4 Hz), 8.53 (t, 1 H, J = 1.5 Hz), 8.32 (d, 2 H, J = 1.5 Hz), 3.73-3.63 (m, 2 H), 3.52-3.43 (m, 2 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.4, 145.2, 144.8, 138.2, 135.6, 129.4, 124.5, 120.9, 118.1, 42.3, 33.4; MS (EI) *m/z* 280 (M^+, 100); HRMS (EI) *m/z* calcd for $C_{11}H_8N_2O_3S_2$ 279.9976, found 279.9974.



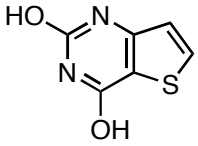
13

3,4-Dihydro-9-amino-[1]benzothieno[2,3-*f*]-1,4-thiazepin-5(2*H*)-one (13). To a suspension of **12** (250 mg, 0.892 mmol) in degassed EtOH (9 mL) was added $SnCl_2$ (1.73 g, 8.92 mmol). The resulting suspension was heated at reflux for 5 h under argon, then quenched with 2.5 N aq. NaOH and extracted with hot 8:2 $CHCl_3$/*i*-PrOH (9 x 50 mL). The combined organic layers were washed with water (2x), brine (2x), dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford crude **13** (158 mg, 71%) as a dark orange powder. A sample (*ca.* 30 mg) was further purified by chromatography on SiO_2 (5:5 hexanes/EtOAc to EtOAc) to afford pure **13** (28.2 mg) as a yellow powder: Mp 198.6-199.0 °C; IR (ATR, neat) 3370, 3254, 3146, 3008, 2915, 1623, 1599, 1556, 1491, 1454, 1430, 1403, 1346, 1333, 1312, 1286, 1243, 1204, 1184, 1129, 1083, 975, 887, 837, 798, 766, 749, 719, 691, 677, 663 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.58 (dd, 1 H, J = 8.8, 0.4 Hz), 7.15 (dd, 1 H, J = 2.4, 0.4 Hz), 6.98 (dd, 1 H, J = 8.8, 2.0 Hz), 3.77-3.71 (m, 2 H), 3.42-3.37 (m, 2 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 169.3, 147.0, 141.5, 132.4, 130.8, 130.7, 124.0, 119.7, 108.4, 44.3, 35.1; MS (EI) *m/z* 250 (M^+, 100); HRMS (EI) *m/z* calcd for $C_{11}H_{10}N_2OS_2$ 250.0235, found 250.0225.



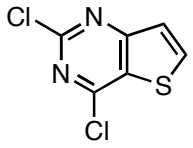
mcf292-08

3,4-Dihydro-9-azido-[1]benzothieno[2,3-*f*]-1,4-thiazepin-5(2*H*)-one (mcf292-08). To a suspension of crude aniline **13** (50.0 mg, 0.200 mmol) in MeCN (0.5 mL) were added *t*-BuONO (53 µL, 0.399 mmol) and then $TMSN_3$ (45 µL, 0.320 mmol) dropwise at 0 °C. The reaction mixture was stirred at room temperature in the dark for 1.5 h, then diluted in $CHCl_3$ (*ca.* 60 mL) and stirred with 2.5 N aq. NaOH (*ca.* 50 mL) for 1 h. The layers were then separated, the organic layer washed with water (20 mL), dried (K_2CO_3), filtered and concentrated *in vacuo*. Chromatography of the residue on SiO_2 ($CHCl_3$ to 98:2 $CHCl_3$/MeOH) afforded a fraction that was dissolved in $CHCl_3$ and stirred with 2.5 N aq. NaOH for 2 h. The layers were then separated, and the organic layer was stirred with 2.5 N aq. NaOH for 1 h. The procedure was repeated 4 times (1 x 30 min and 3 x 15 min stirring). The layers were then separated, the organic layer was washed with water, dried (K_2CO_3), filtered and concentrated *in vacuo* to yield **mcf292-08** (29.4 mg, 53%, 38% over 2 steps, 96% purity estimated by ^1H NMR) as a beige powder: Mp 193 °C (dec.); IR (ATR, neat) 3260, 3154, 3016, 2922, 2115, 1631, 1592, 1495, 1467, 1441, 1422, 1400, 1340, 1284, 1252, 1234, 1215, 1198, 1144, 1113, 975, 889, 835, 809, 792, 751, 721 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.53 (bt, 1 H, J = 5.6 Hz), 8.06 (d, 1 H, J = 8.8 Hz), 7.39 (d, 1 H, J = 1.6 Hz), 7.31 (dd, 1 H, J = 8.6, 2.2 Hz), 3.68-3.60 (m, 2 H), 3.45-3.38 (m, 2 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.9, 139.4, 136.9, 135.2, 134.2, 127.7, 124.6, 119.2, 112.0, 42.4, 33.4; MS (EI) *m/z* 276 (M^+, 14), 248 ($[M–N_2]^+$, 50), 68 (100); HRMS (EI) *m/z* calcd for $C_{11}H_8N_4OS_2$ 276.0140, found 276.0137.



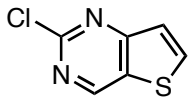
14

Thieno[3,2-*d*]pyrimidine-2,4-diol (14). To a solution of methyl 3-aminothiophene-2-carboxylate (5.00 g, 31.8 mmol) in glacial AcOH (35.0 mL) and H_2O (31 mL) was added KOCN (8.06 g, 95.4 mmol) in H_2O (18.0 mL) dropwise. The resulting slurry was stirred at room temperature for 20 h, and filtered. The solid was placed in a flask, flushed with N_2, treated with 2 N aq. NaOH (85 mL), and stirred at room temperature for 3 h. The slurry was filtered to remove any undissolved material. The solution was acidified with conc. aq. HCl until a pH of 5-6 was obtained. The precipitate was filtered and the solid was dried at 50 °C to provide **14** (3.49 g, 65%) as a white solid: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.56 (s, 1 H), 11.21 (s, 1 H), 8.05 (d, 1 H, J = 5.3 Hz), 6.90 (d, 1 H, J = 5.3 Hz).



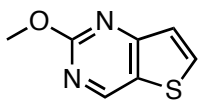
15

2,4-Dichlorothieno[3,2-*d*]pyrimidine (15). To a solution of **14** (500 mg, 2.97 mmol) and *N*,*N*-dimethylaniline (0.29 mL, 2.23 mmol) in MeCN (2.5 mL) cooled to 0 °C was slowly added $POCl_3$ (1.4 mL, 14.9 mmol). The purple slurry was heated to 80-85 °C and stirred for 48 h. A second portion of $POCl_3$ (1.0 mL) was added after 24 h. The resulting clear purple solution was poured into ice and water and stirred for 5 min. The slurry was filtered, and the solid was dried at 45 °C. The solid was dissolved in EtOAc, washed with sat. aq. $NaHCO_3$, and stirred with activated charcoal. The solution was filtered through Celite® and concentrated to provide **15** (482 mg, 79%) as a yellow solid: Mp 138.8-139.3 °C (H_2O); IR (ATR, neat) 3066, 3088, 1545, 1508, 1307, 1204, 798 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.70 (d, 1 H, J = 5.4 Hz), 7.74 (d, 1 H, J = 5.4 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.6, 154.8, 154.7, 142.4, 129.3, 124.1; HRMS (EI) *m/z* calcd for $C_6H_2N_2SCl_2$ 203.9316, found 203.9312.



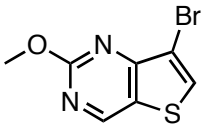
16

2-Chlorothieno[3,2-*d*]pyrimidine (16). To a solution of **15** (44.0 mg, 0.022 mmol) and $NaHCO_3$ (27.0 mg, 0.32 mmol) in EtOH (2.0 mL) was added 10% Pd/C (8.90 mg, 20% by wt). The suspension was stirred at room temperature under an atmosphere of H_2 for 23 h. A second portion of 10% Pd/C (8.90 mg, 20% by wt) was added after 12 h. The reaction mixture was filtered through Celite® with EtOAc washings. The filtrate was washed with H_2O/brine (4:1), dried ($MgSO_4$), and concentrated under reduced pressure to provide **16** (33.0 mg, 90%) as white solid: Mp 164.9-165.5 °C (EtOAc); IR (ATR, neat) 3105, 3051, 2924, 1543, 1515, 1456, 1420, 1334, 1349, 1301, 1159, 794 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.50 (s, 1 H), 8.64 (d, 1 H, J = 5.4 Hz), 7.64 (d, 1 H, J = 5.4 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 162.7, 156.3, 155.3, 142.2, 130.1, 122.9; MS (EI) *m/z* 170 (M^+, 100), 135 (72); HRMS (EI) *m/z* calcd for $C_6H_3N_2SCl$ 169.9705, found 169.9700.



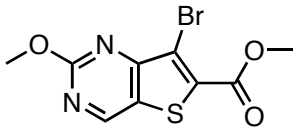
17

2-Methoxythieno[3,2-*d*]pyrimidine (17). To a solution of **16** (146 mg, 0.86 mmol) in MeOH (20 mL) was added NaOMe (130 mg, 2.41 mmol). The solution was heated at reflux for 37 h. An additional 1.4 equiv of NaOMe (65.0 mg) was added after 24 h (Note: The reaction was complete in 7 h with comparable yields when 4.2 equiv of NaOMe were added at the start of the reaction). The reaction mixture was cooled to room temperature, quenched with 1 N aq. HCl (2.0 mL), and extracted with DCM (4 x 10 mL). The combined organic layers were washed with H_2O (10 mL), dried ($MgSO_4$), and concentrated under reduced pressure to provide **17** (125 mg, 88%) as an off-white solid: Mp 167.0-168.5 °C (DCM); IR (ATR, neat) 3071, 3025, 2917, 1558, 1528, 1478, 1379, 1295, 1249, 1031, 796, 677 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 9.30 (d, 1 H, J = 0.6 Hz), 8.45 (d, 1 H, J = 5.4 Hz), 7.47 (dd, 1 H, J = 5.4, 0.7 Hz), 3.96 (s, 3 H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.4, 162.4, 154.8, 139.9, 124.8, 122.9, 54.6; MS (EI) *m/z* 166 (M^+, 29), 84 (100); HRMS (EI) *m/z* calcd for $C_7H_6N_2OS$ 166.0201, found 166.0201.



18

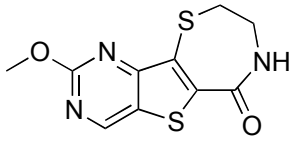
7-Bromo-2-methoxythieno[3,2-*d*]pyrimidine (18). To a reaction vial containing **17** (100 mg, 0.602 mmol) and AcOH (1.5 mL) under an atmosphere of N_2 was added Br_2 (0.093 mL, 1.81 mmol). The reaction vial was sealed and heated to 70 °C for 24 h. The mixture was cooled to room temperature, quenched with sat. aq. $NaHCO_3$, and extracted with EtOAc. The combined organic layers were washed with sat. aq. $Na_2S_2O_3$, sat. aq. $NaHCO_3$, and brine, dried ($MgSO_4$) and concentrated under reduced pressure to give a white solid. The solid was adsorbed onto SiO_2 and purified by chromatography on SiO_2 (1:20 EtOAc/hexanes, 1:10 EtOAc/hexanes, 3:20 EtOAC/hexanes, 100% EtOAc) to provide **18** (58.3 mg, 40%) as a white solid: Mp 115.3-115.9 °C (EtOAc); IR (ATR, neat) 3090, 3019, 2956, 1567, 1524, 1474, 1463, 1370, 1312, 1271 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 9.34 (s, 1 H), 8.61 (s, 1 H), 4.01 (s, 3 H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.9, 158.4, 155.8, 136.7, 124.0, 106.8, 54.8; HRMS (EI) *m/z* calcd for $C_7H_5N_2OSBr$ 243.9306, found 243.9304.



19

Methyl 7-bromo-2-methoxythieno[3,2-*d*]pyrimidine-6-carboxylate (19). To a reaction vial containing **18** (40.0 mg, 0.16 mmol) and THF (0.6 mL) cooled to –55 to –60 °C under an atmosphere of argon was added TMPMgCl•LiCl[6] (0.17 mL, 0.22 mmol) dropwise. The white slurry became a clear yellow solution after the addition of TMPMgCl•LiCl and was stirred for 2 h at –55 to –60 °C, turning into a pale yellow slurry at the end of this time. Methyl cyanoformate (0.016 mL, 0.20 mmol) in THF (0.10 mL) was added dropwise at –50 °C and the solution was stirred for 2 h while warming to 0 °C.

The pale yellow slurry turned pale yellow-orange as it warmed to 0 °C. The reaction was quenched at 0 °C with sat. aq. NH_4Cl (0.5 mL). The mixture was diluted with EtOAc and the organic layer was washed with sat. aq. NH_4Cl (2 x 5 mL). The combined aqueous layers were extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with brine (5 mL), dried ($MgSO_4$), and concentrated under reduced pressure to provide **19** (48.0 mg, 97%) as a yellow solid: Mp 180.9-181.4 °C (EtOAc); IR (ATR, neat) 2956, 2915, 2848, 1735, 1569, 1472, 1382, 1213, 1031, 788 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 9.49 (s, 1 H), 4.04 (s, 3 H), 3.95 (s, 3 H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 164.1, 160.6, 158.3, 157.4, 136.3, 124.8, 113.9, 55.0, 53.3; MS (EI) *m/z* 302 (M^+, 100), 274 (45); HRMS (ESI) *m/z* calcd for $C_9H_8BrN_2O_3S$ (M+H) 302.9439, found 302.9418.



kmg-NB4-23

2-Methoxy-7*H*,8*H*,9*H*-1,4-thiazepino[7',6'-5,4]thiopheno[3,2-*d*]pyrimidin-6-one (kmg-NB4-23). To a solution of **19** (41.0 mg, 0.14 mmol) in DMF (1.3 mL) under an atmosphere of N_2 was added cysteamine•HCl (63.0 mg, 0.54 mmol) in one portion and DBU (0.17 mL, 1.1 mmol) dropwise. The reaction mixture turned dark blue upon addition of DBU and after stirring for 20 min, the mixture was a pale purple colored slurry. The reaction mixture was stirred at room temperature for 1.5 h, and then heated to 70 °C for 9 h 50 min. The resulting yellow slurry was diluted with EtOAc, washed with 2 N aq. HCl, and filtered (H_2O and EtOAc washings). Residual DMF was removed by azeotropic distillation with heptane to provide **kmg-NB4-23** (25.0 mg, 68%) as a pale yellow solid: Mp 308 °C (dec.); IR (ATR, neat) 3260, 3153, 3015, 1636, 1554, 1495, 1467, 1374, 1269, 1353, 1323 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 9.36 (s, 1 H), 8.70 (t, 1 H, J = 5.4 Hz), 3.98 (s, 1 H), 3.68 (app dd, 2 H, J = 6.0 Hz), 3.40-3.36 (m, 2 H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 164.2, 163.1, 159.6, 155.9, 138.5, 129.7, 124.1, 54.8, 42.7, 31.9; HRMS (ESI) *m/z* calcd for $C_{10}H_{10}N_3O_2S_2$ (M+H) 268.0214, found 268.0237.

***In vitro* radiometric PKD kinase assays.** *In vitro* radiometric PKD kinase assays were conducted as previously described.[7] Briefly, 50 ng recombinant human PKD1 (Biomol International, Plymouth Meeting, PA), PKD2 (SignalChem, Richmond, BC, Canada), or 75 ng PKD3 (Enzo Life Sciences) was incubated at 30°C for 10 min with a reaction mixture containing 2.5 μg syntide-2 (Sigma), 70 μM ATP, and 1 μCi γ-^{32}P-ATP (PerkinElmer, Boston, MA) in kinase buffer (50 mM Tris-HCl pH 7.5, 4 mM $MgCl_2$, and 10 mM β-mercaptoethanol). 25 μL of the reaction mixture was then spotted on Whatman P81 filter paper (Whatman Inc., Clifton, NJ), and filter papers were washed three times with 0.5% phosphoric acid, then counted using a Beckman LS6500 multi-purpose scintillation counter.

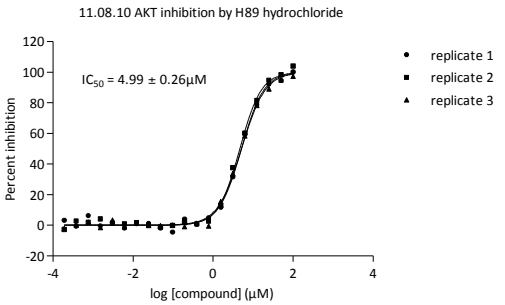
***In vitro* radiometric PKC and CAMKIIα kinase assays.** *In vitro* radiometric kinase assays to assess the specificity of the PKD inhibitor analogs were performed as previously described.[7] Briefly, for PKC assays, 50 ng of recombinant human PKCα, PKCβI, or PKCδ (SignalChem) were incubated with 5 μg MBP4-14, 0.25 mg/mL BSA, 0.1 mg/mL PC/PS (80%/20%), 1 μM PDBu, 20 μM ATP, and 1 μCi γ-^{32}P-ATP in kinase buffer with the indicated concentrations of inhibitor for 10 min at 30°C. For CAMKIIα activity assays, 50 ng CAMKIIα (SignalChem) was pre-incubated with 0.5 mM $CaCl_2$ and 30 ng/μL calmodulin for 10 min on ice before being added to a reaction mixture containing 2.5 μg syntide-2, 70 μM ATP, and 1 μCi γ-^{32}P-ATP in kinase buffer. Reactions were then incubated with the indicated concentrations of inhibitor for 10 min at 30°C. Following incubation, 25 μL of the reaction mixture was spotted onto Whatman P81 filter paper. The filter papers were then washed three times with 0.5% phosphoric acid, and incorporation of γ-^{32}P-ATP into the substrate was determined using a Beckman LS6500 multi-purpose scintillation counter.

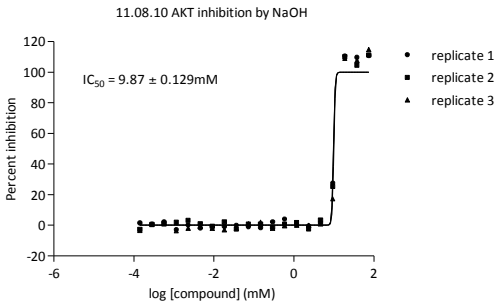
Determination of cellular IC_{50} for PKD1 inhibition. Inhibition of PKD1 in cells was determined as previously described.[7] LNCaP cells were pre-treated with PKD inhibitors for 45 min at various concentrations, and then stimulated with 10 nM phorbol 12-myristate 13-acetate (PMA) for 20 min. Cells were then collected and lysed in lysis buffer containing 200 mM Tris-HCl, pH 7.4, 100 μM 4-(2-aminoethyl) benzenesulfonyl fluoride, 1 mM EGTA, and 1% Triton X-100. Cell lysates were probed by Western blot analysis using primary antibodies targeting p-S916-PKD1 (Millipore), p-S744/748-PKD1, PKD1 (Cell Signaling Technology), or GAPDH. Densitometry analysis of visualized bands was used to determine the cellular IC_{50} values for PKD1 inhibition.

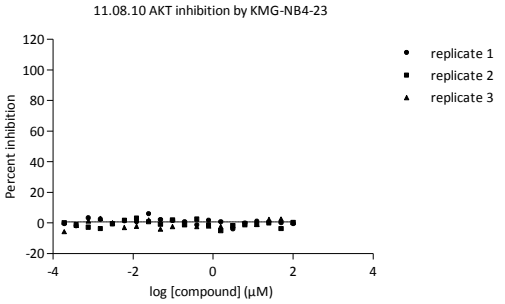
Statistical analysis. GraphPad Prism V software was used to determine statistical significance. Each assay was repeated two or three times with triplicate determination at each point. A p value of < 0.05 was considered significant.

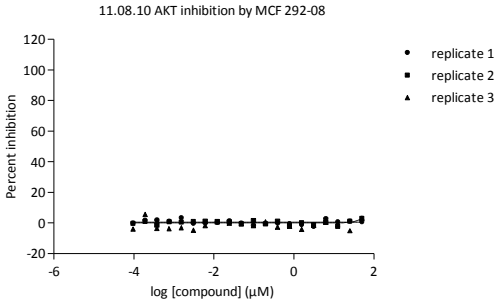
IMAP-based kinase counterscreening assays. Automated, HTS formatted IMAP-based AKT FP, and PLK1 and CAK TR-FRET assays were used to assess the specificity of the PKD analogs as previously described.[7] For the PLK2 IMAP TR-FRET assay, PLK2 kinase reactions were generated by the stepwise addition of 3 times concentrations of substrate/ATP (1650 nM/105 μM), analog, and PLK2 enzyme (1.02 milliunits/μL). PLK2 kinase reactions were incubated for 150 min at room temperature and stopped with the addition IMAP binding reagent supplemented with terbium. Assay plates were then incubated overnight. Data capture was as previously described.[7] PLK2 substrate (FAM-LKKLTRRASFSGQ) was obtained from Molecular Devices (Sunnyvale, CA). H-89 was used as a positive inhibitory control compound in the assays. A 10 point concentration range of each compound used to determine IC_{50} values. The maximum concentration was either 50 or 100 μM, depending on the solubility of each individual compounds. Each experiment was assayed in triplicate and data is represented as average $IC_{50} \pm$ SD. The IC_{50} determinations for each analog evaluated in the IMAP (PLK2, PLK1, CAK, and AKT) formats were conducted within the linear range of the captured signal readout.

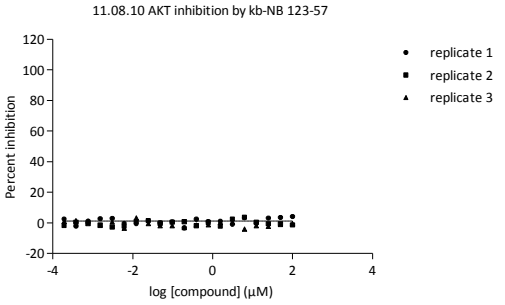
Kinase Counterscreens: AKT

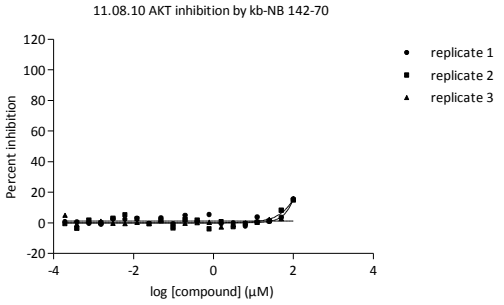


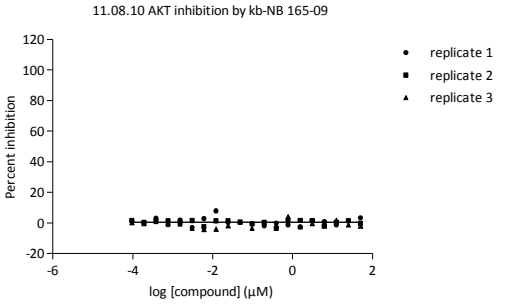


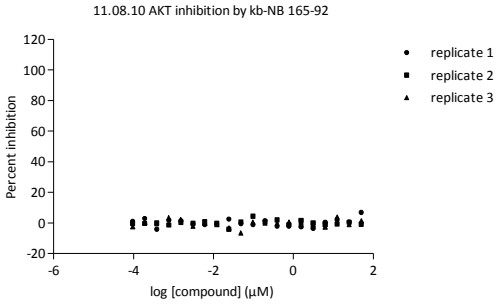


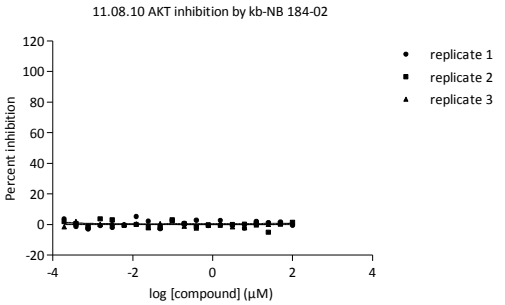




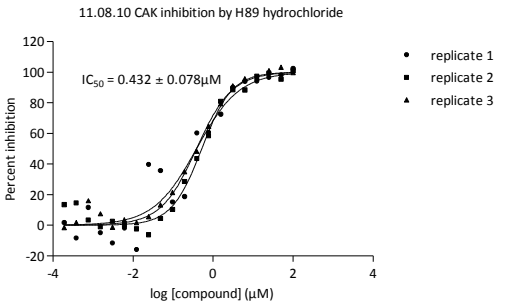


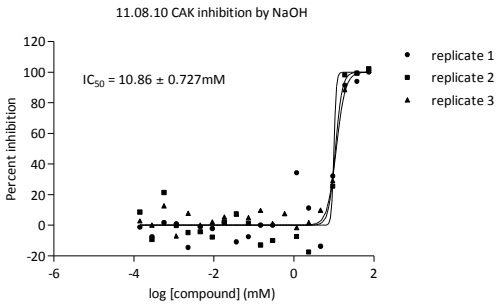


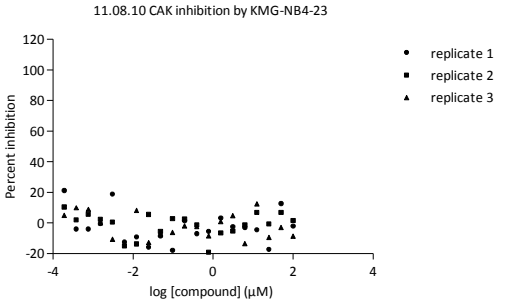


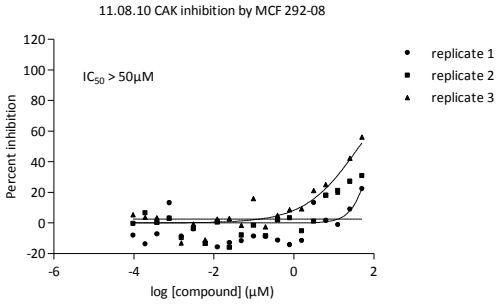


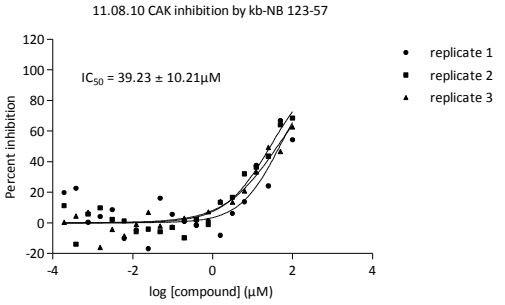
Kinase Counterscreens: CAK (CDK7)

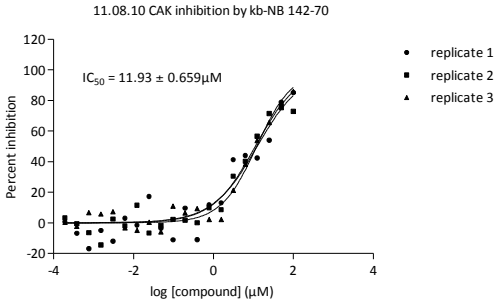


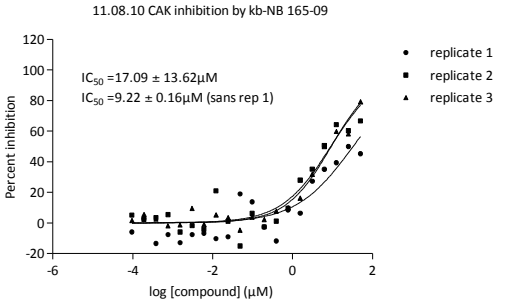


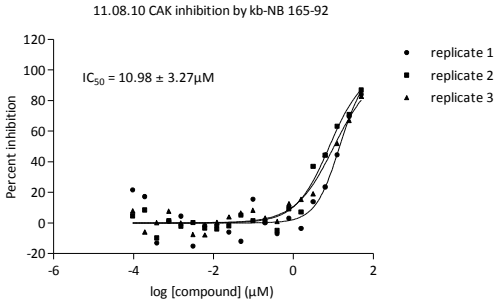


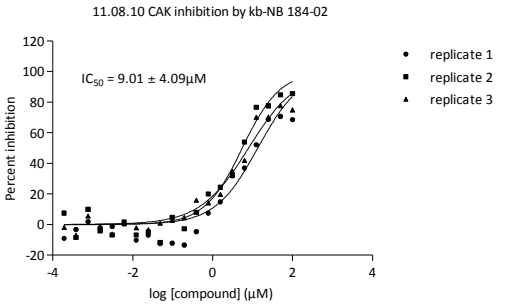




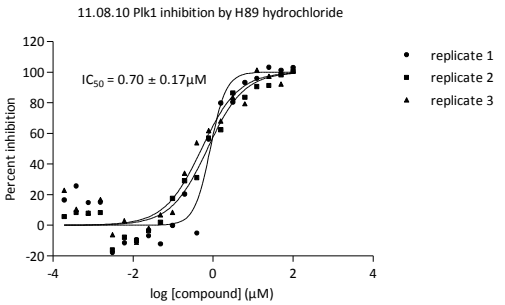


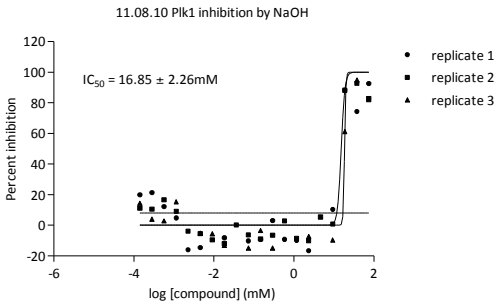


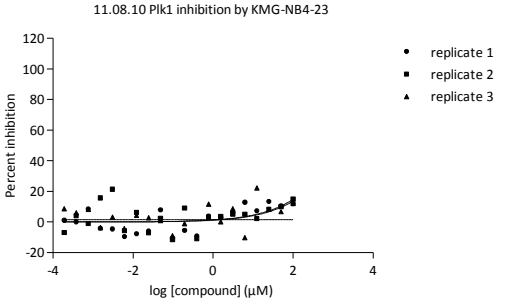


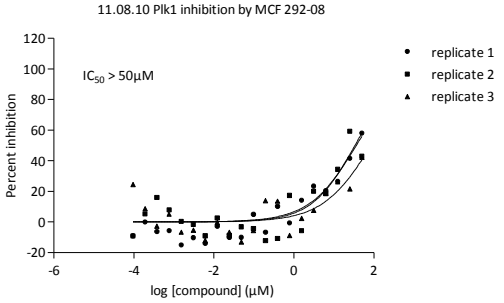


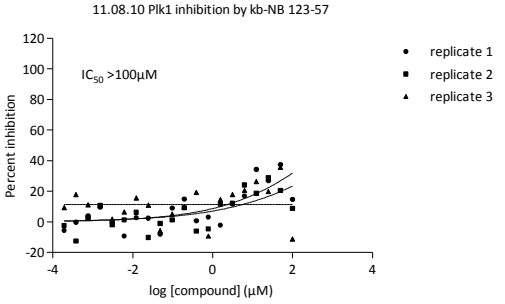
Kinase Counterscreens: PLK1

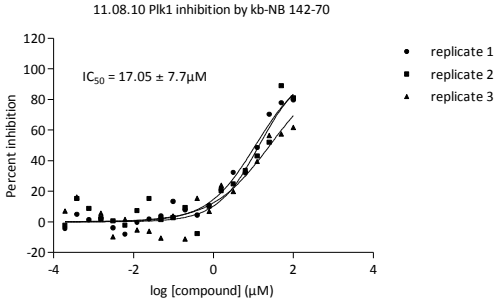


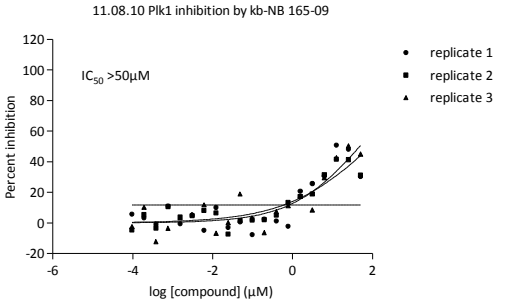


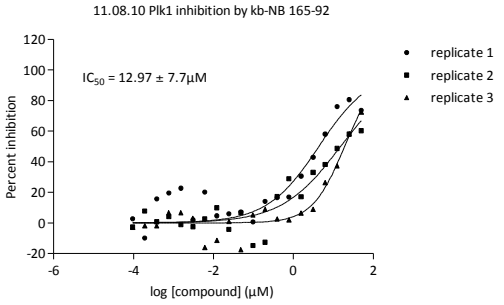


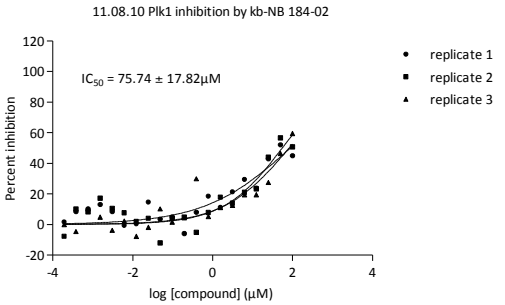




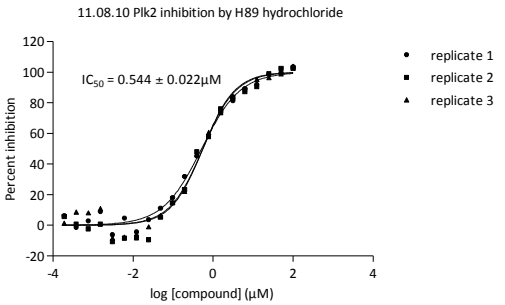


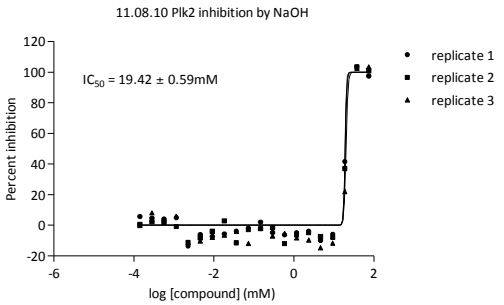


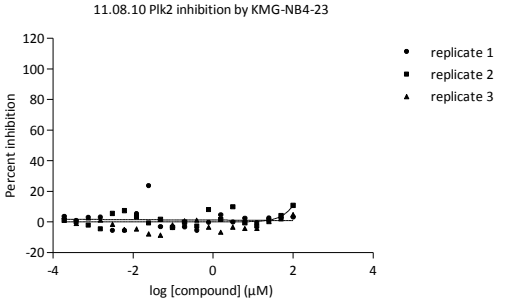


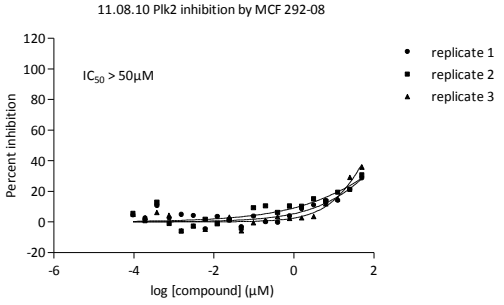


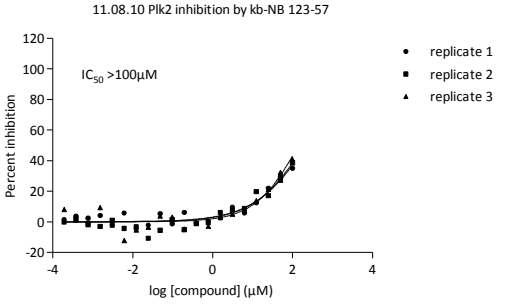
Kinase Counterscreens: PLK2

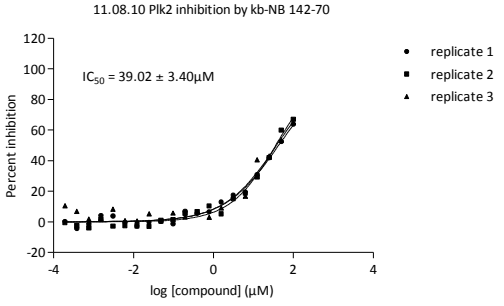


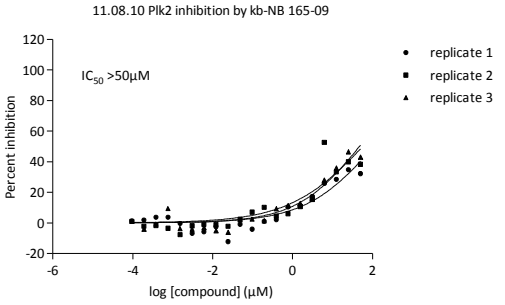


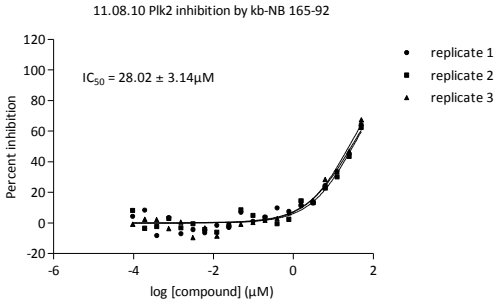


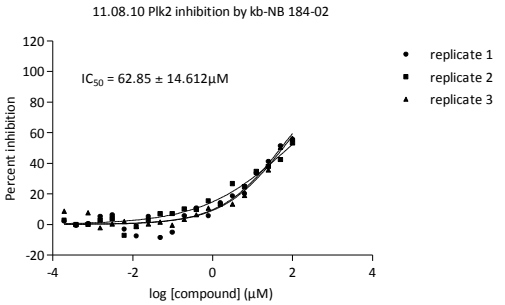












Kinase Counterscreens: Summary for AKT, CAK, PLK1, and PLK2

11.08.10						
IMAP counterscreens						
AKT	rep 1	rep 2	rep 3		average	stdev
H89	5.142	4.689	5.152		4.994	0.264
NaOH	9.777	9.824	10.02		9.874	0.129
KMG-NB4-23					not inhibitory	
MCF 292-08					not inhibitory	
Kb-NB 142-70					not inhibitory	
Kb-NB 165-09					not inhibitory	
Kb-NB 123-57					not inhibitory	
Kb-NB 184-02					not inhibitory	
Kb-NB 165-92					not inhibitory	
Plk1						
H89	0.8546	0.7277	0.5185		0.700	0.170
NaOH		15.26	18.45		16.855	2.256
KMG-NB4-23					not inhibitory	
MCF 292-08	36.3	33.59	85.64		51.843	29.300
Kb-NB 142-70	11.31	14.03	25.82		17.053	7.713
Kb-NB 165-09					>50	
Kb-NB 123-57					>100	
Kb-NB 184-02	89.03	55.49	82.71		75.743	17.822
Kb-NB 165-92	4.369	15.29	19.24		12.966	7.703
Plk2						
H89	0.5186	0.5573	0.5559		0.544	0.022
NaOH	18.98	19.18	20.1		19.420	0.597
KMG-NB4-23					not inhibitory	
MCF 292-08					>50	
Kb-NB 142-70	42.83	36.28	37.96		39.023	3.402
Kb-NB 165-09	108.4	57.98	47.51		>50	
Kb-NB 123-57					>100	
Kb-NB 184-02	51.31	79.28	57.96		62.850	14.612
Kb-NB 165-92	28.47	30.9	24.68		28.017	3.135
CAK						
H89	0.3875	0.5226	0.3864		0.432	0.078
NaOH	10.98	10.08	11.52		10.860	0.727
KMG-NB4-23					not inhibitory	
MCF 292-08	100.4	0	43.3		47.900	50.358
Kb-NB 142-70	12.46	11.19	12.13		11.927	0.659
Kb-NB 165-09	32.81	9.112	9.337		17.086	13.618
Kb-NB 123-57	47.68	27.88	42.14		39.233	10.215
Kb-NB 184-02	13.52	5.537	7.97		9.009	4.092
Kb-NB 165-92	14.47	7.995	10.48		10.982	3.267
red = tested only to 50µM [highest]						
all other compounds testd to 100µM						

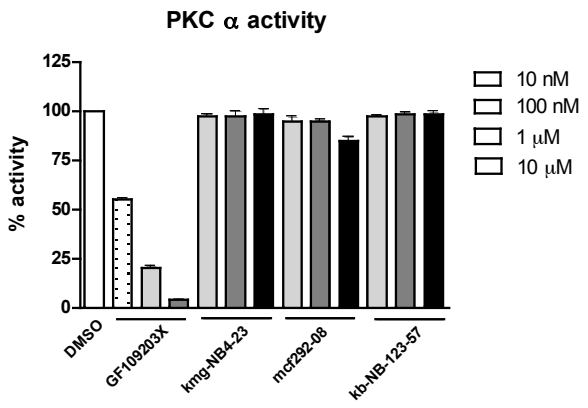
IC_{50} (µM)(Ave ± SD)

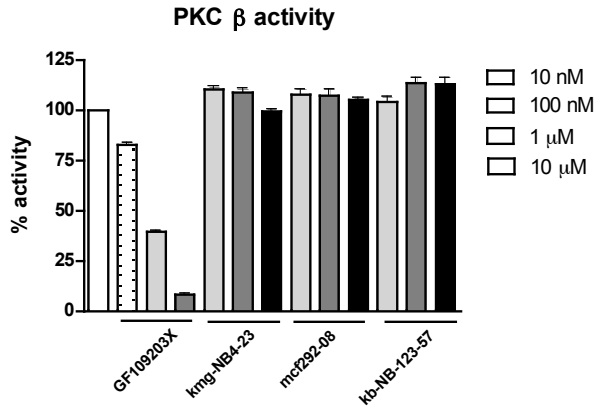
Analog	AKT	PLK1	PLK2	CAK(CDK7)
KMG-NB4-23	>100	>100	>100	>100
MCF 292-08	>50	>50	>50	>50
Kb-NB-142-70	>100	17 ± 7.7	39 ± 3.4	12 ± 0.6
Kb-NB 165-09	>50	>50	>50	17.1 ± 13.6
Kb-NB-123-57	>100	>100	>100	39.2 ± 10.2
Kb-NB-184-02	>100	>50	>50	9 ± 4.1
Kb-NB-165-92	>50	13 ± 7.7	28 ± 3.1	11 ± 3.3

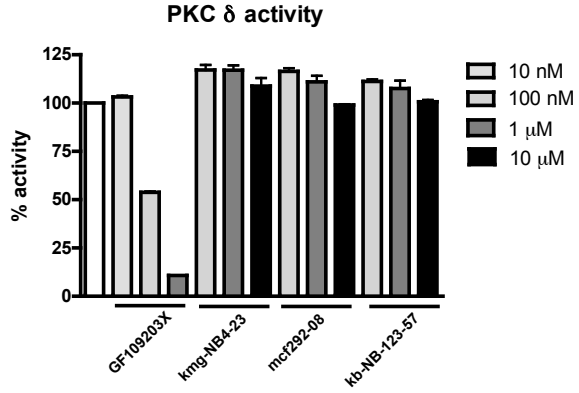
Kinase Counterscreens: Selectivity analysis for kmg-NB4-23, mcf292-08 and kb-NB123-57

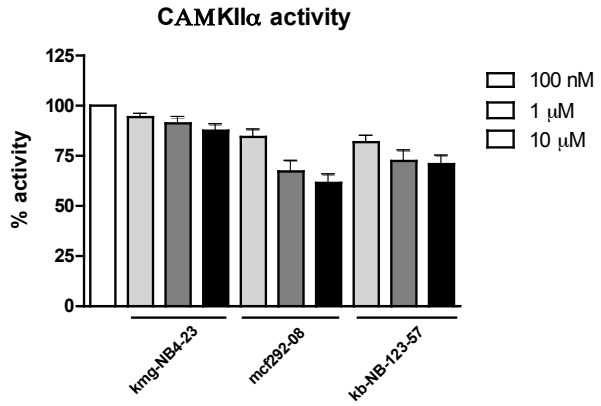
Inhibition of PKCa, PKCbI, PKCd and CAMKIIa by **kmg-NB4-23**, **mcf292-08** and **kb-NB123-57** was determined at 100 nM, 1 uM and 10 uM concentrations. In the PKC assays, the potent PKC inhibitor **GF109203X** was used as a control.

(Representative data from two independent experiments)



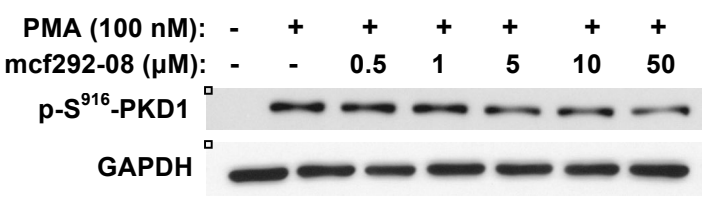


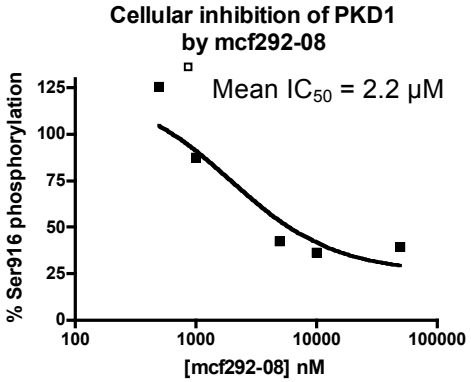


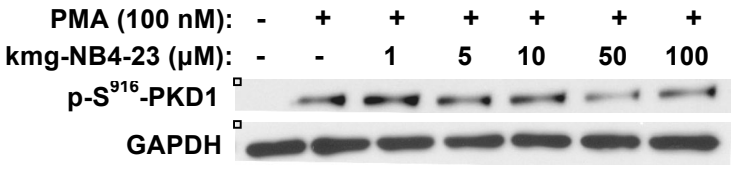


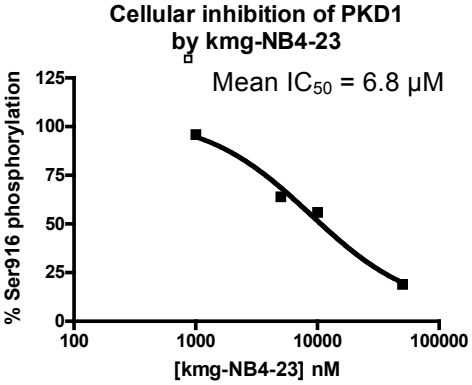
Inhibition of PMA-induced PKD1 S^{916} autophosphorylation by kmg-NB4-23 and mcf292-08 in LNCaP prostate cancer cells

(Representative data from three independent experiments)



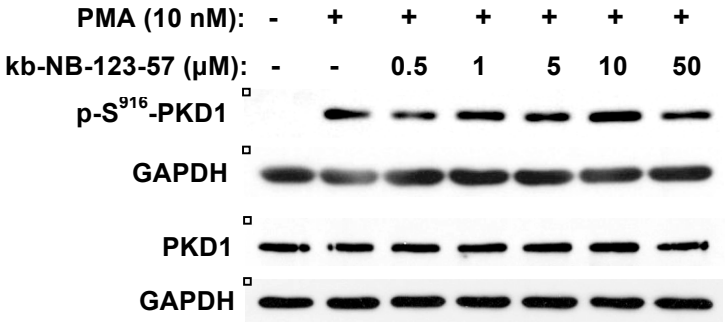






Data demonstrating that kb-NB123-57 did not significantly inhibit PMA-induced PKD1 autophosphorylation at S^{916} (IC_{50} > 50 µM):

(Representative data from three independent experiments)



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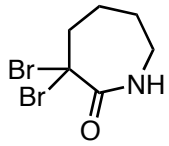
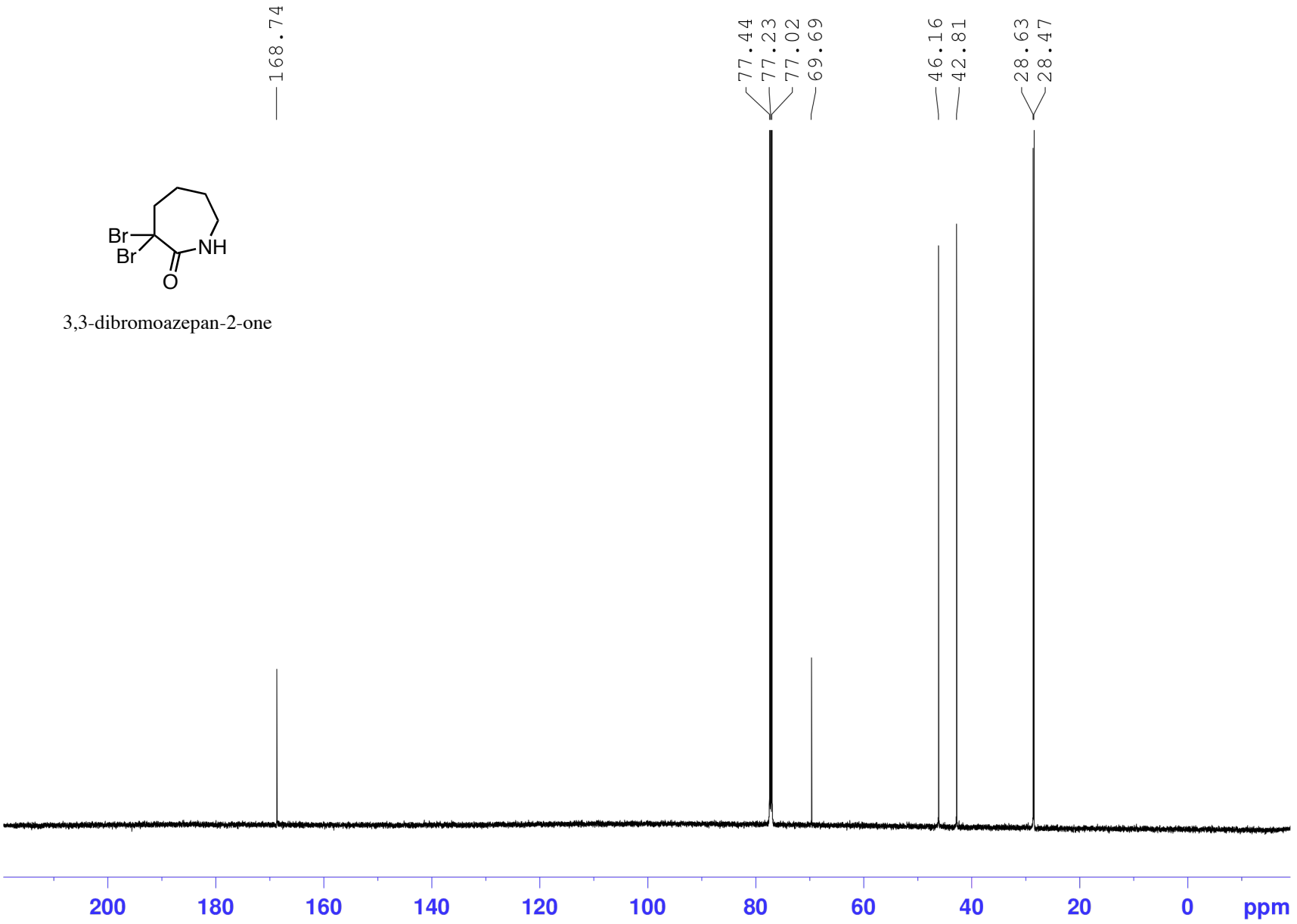
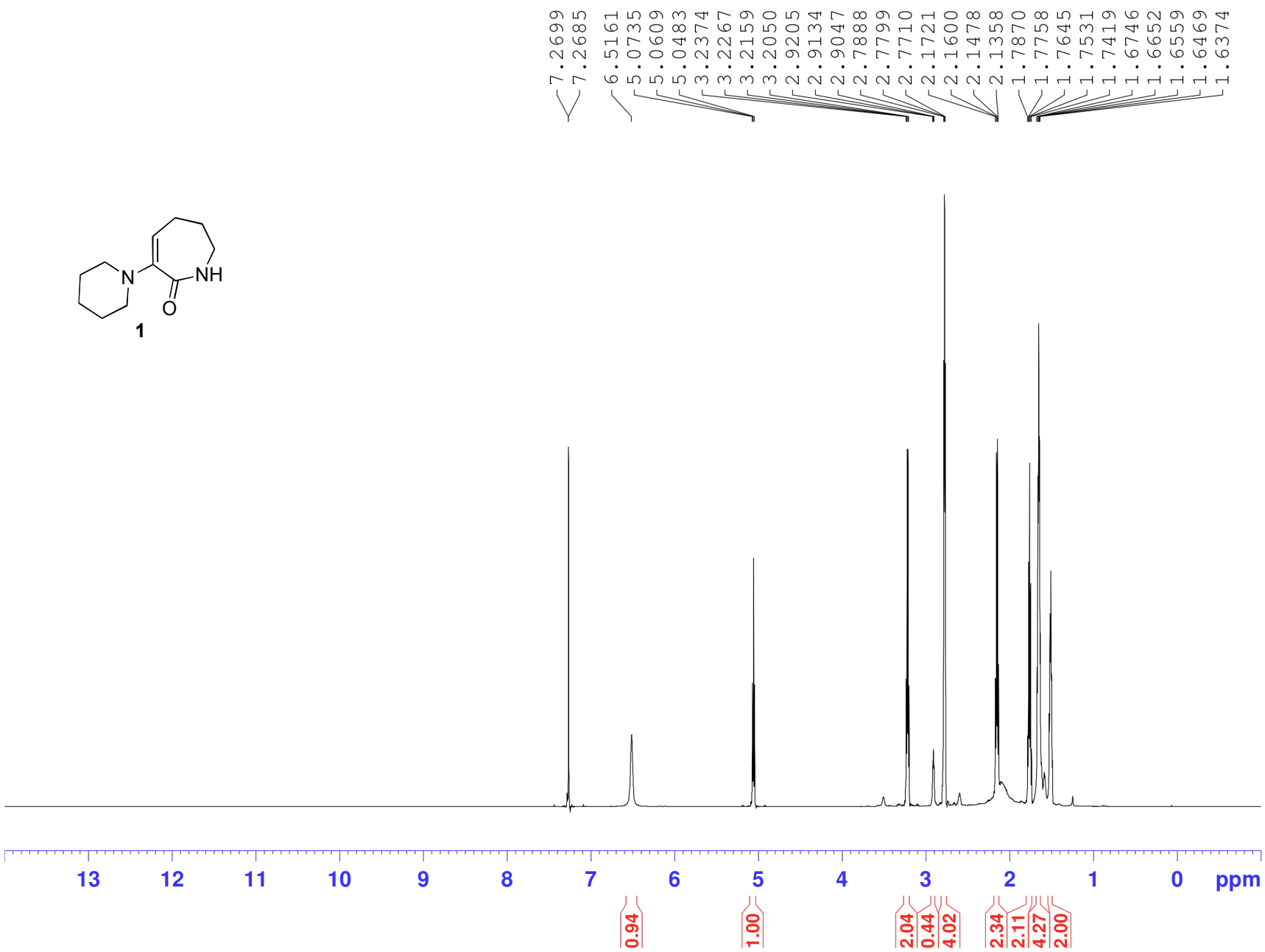


3,3-dibromoazepan-2-one



3,3-dibromoazepan-2-one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2.7888
2.7799
2.7710
2.1721
2.1600
2.1478
2.1358
1.7870
1.7758
1.7645
1.7531
1.7419
1.6746
1.6652
1.6559
1.6469
1.6374
N
NH
O
1
13
12
11
10
9
8
7
6
5
4
3
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ppm
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4.02
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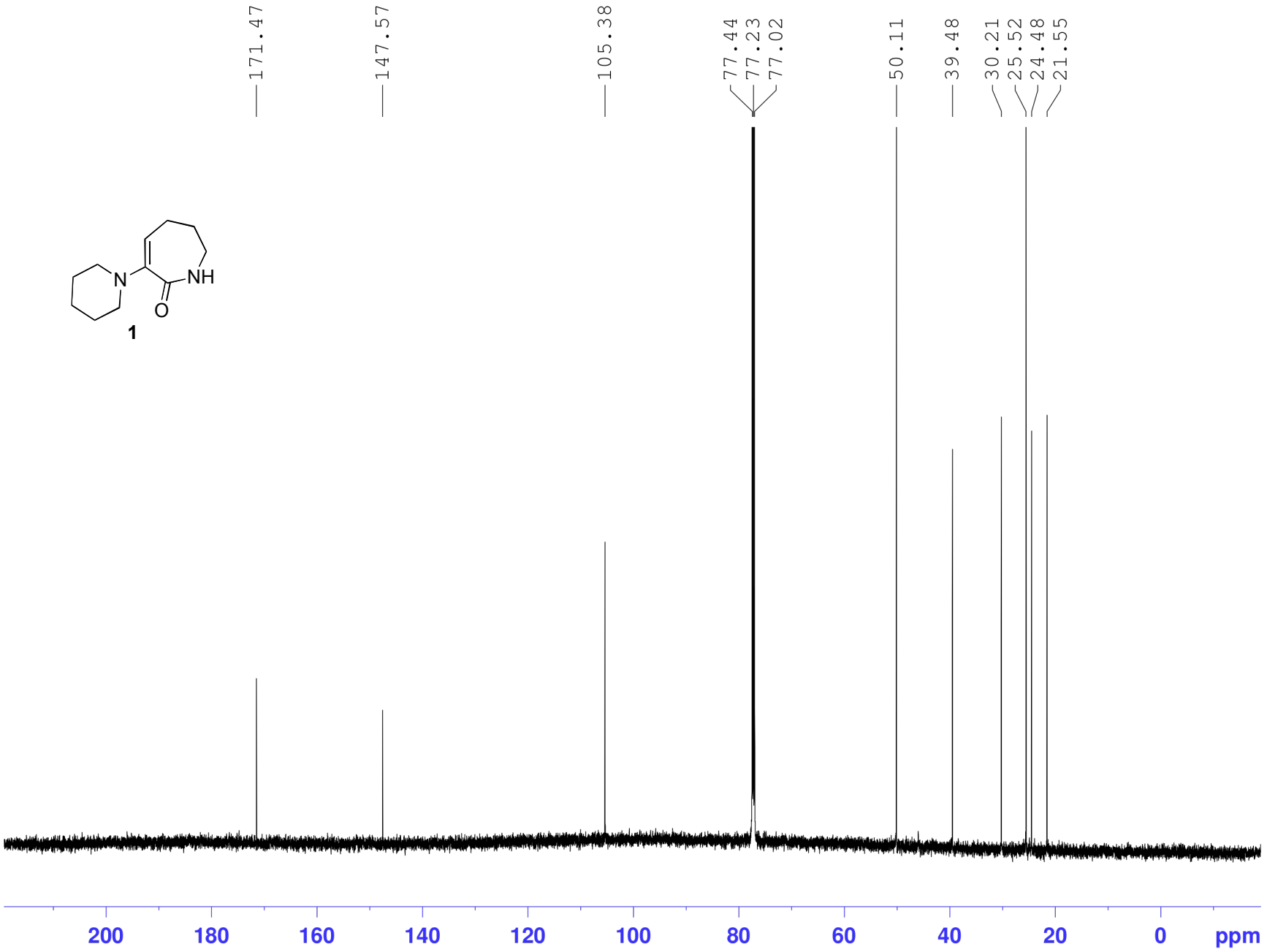
2.00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25.52
24.48
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NH
O
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200
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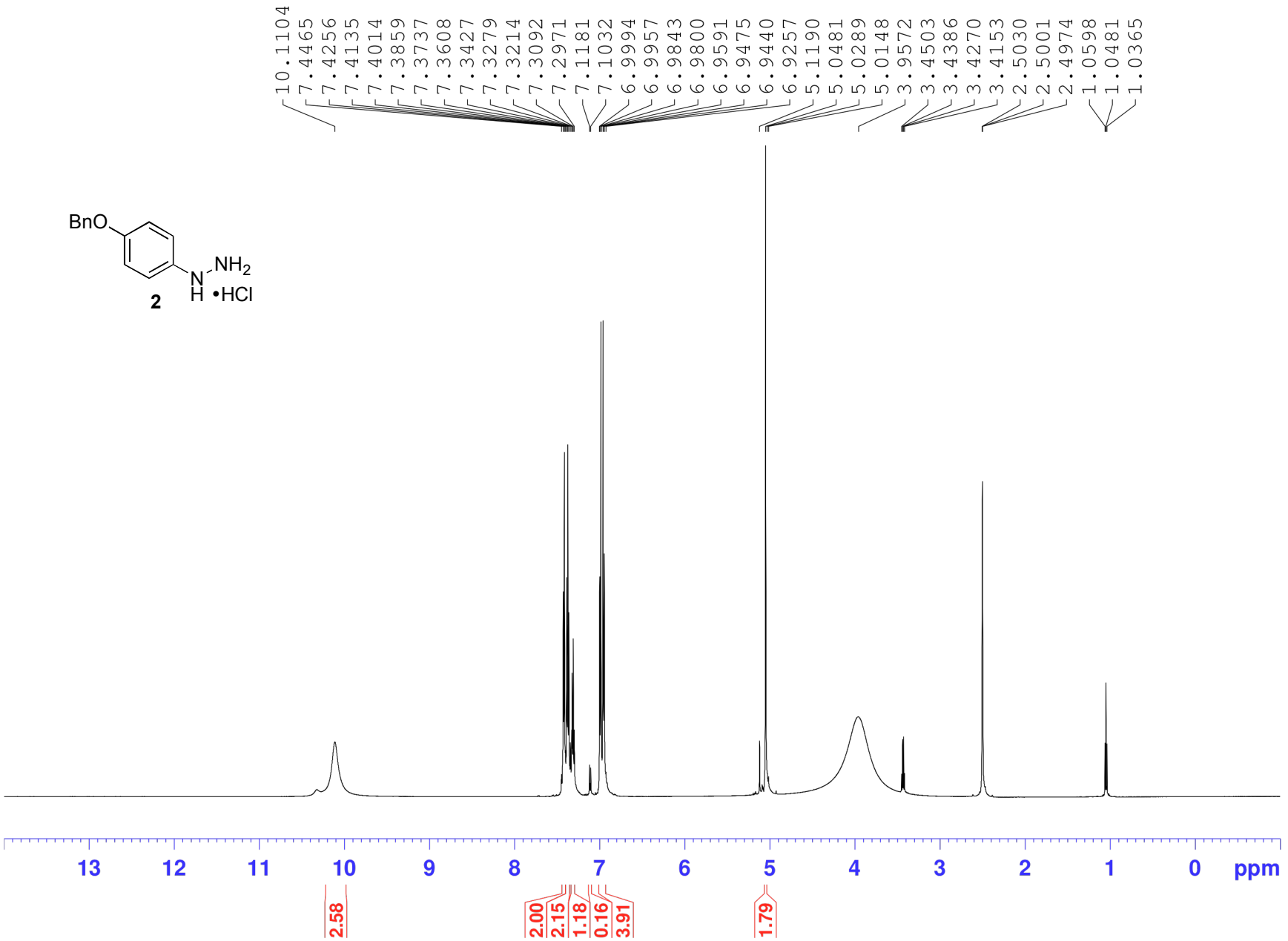
140
120
100
80
60
40
20
0
ppm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7.4135
7.4014
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7.3608
7.3427
7.3279
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7.1032
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6.9957
6.9843
6.9800
6.9591
6.9475
6.9440
6.9257
5.1190
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5.0289
5.0148
3.9572
3.4503
3.4386
3.4270
3.4153
2.5030
2.5001
2.4974
1.0598
1.0481
1.0365
BnO
NH2
N
H •HCl
2
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12
11
10
9
8
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3
2
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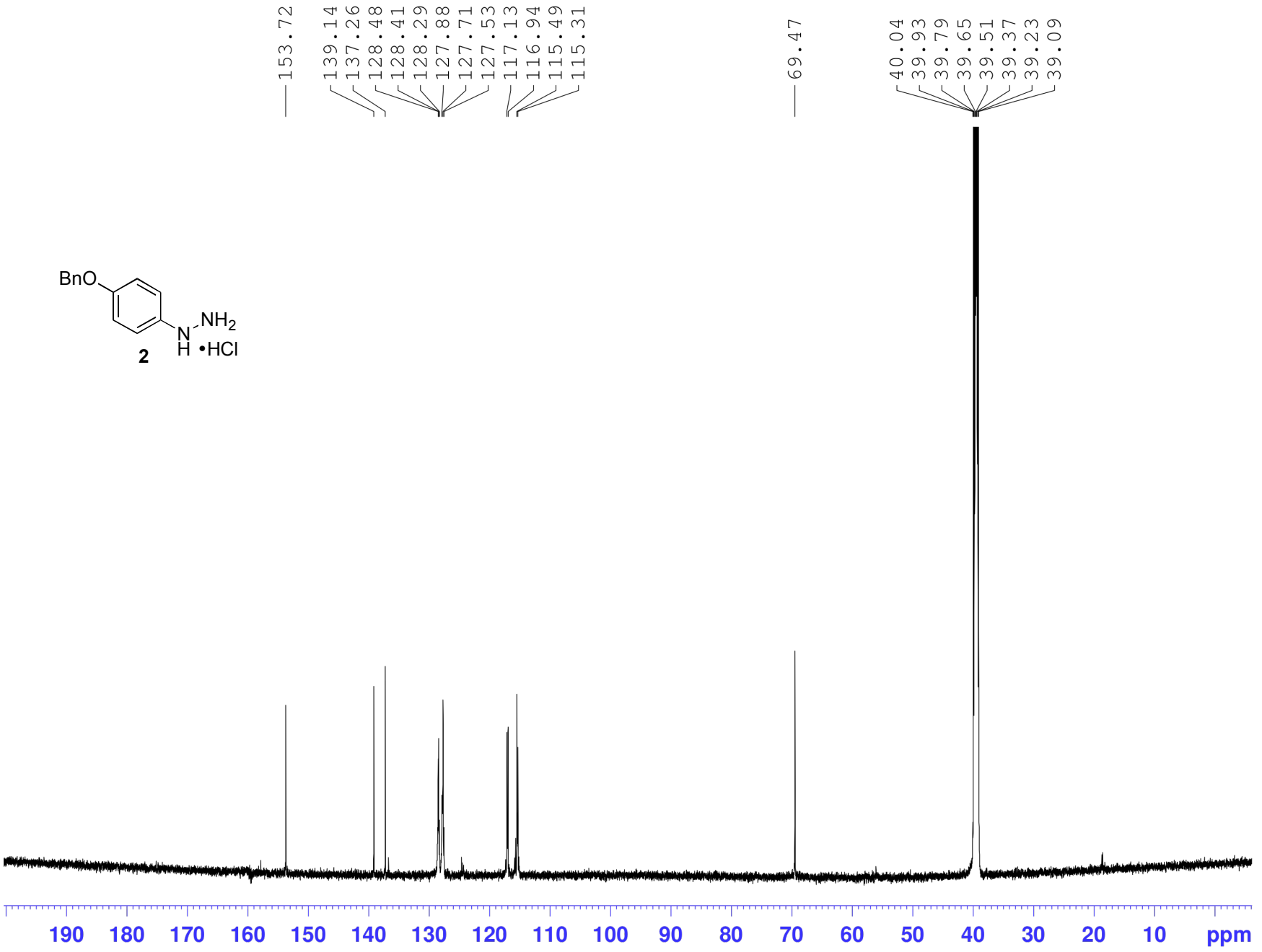
1.79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115.31
69.47
40.04
39.93
39.79
39.65
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39.09
BnO
NH2
N
H •HCl
2
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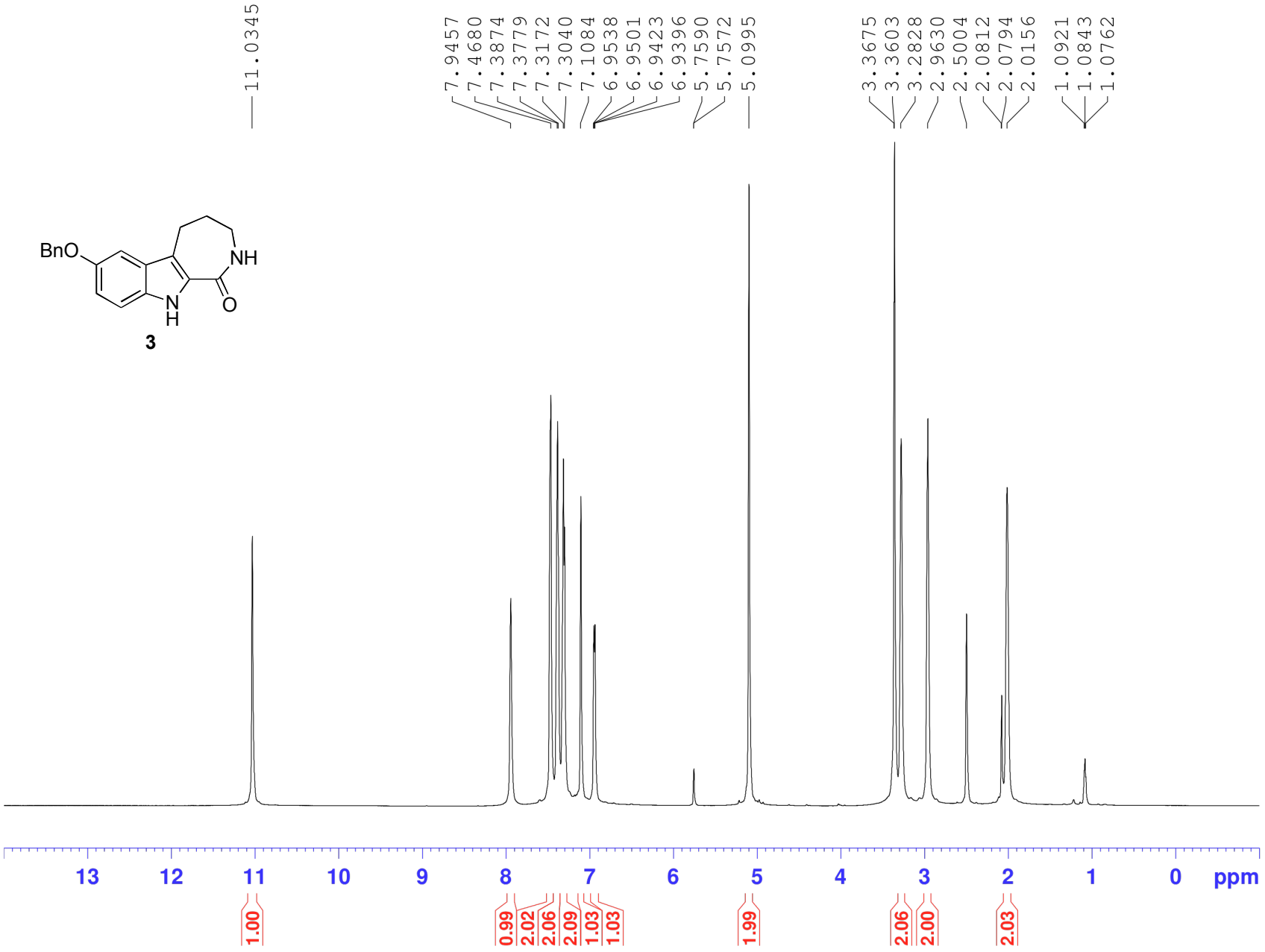
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H
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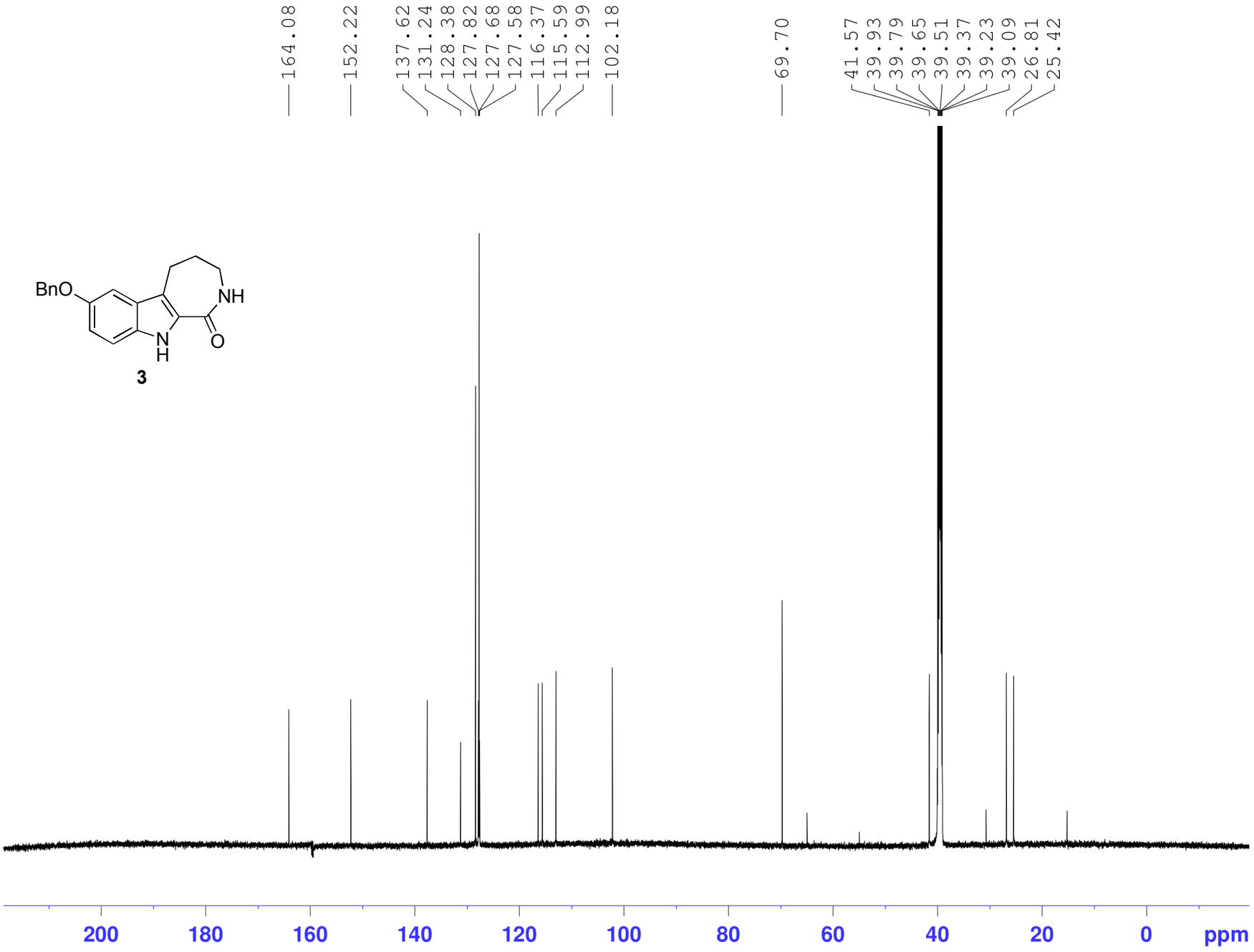
2.03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39.79
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H
O
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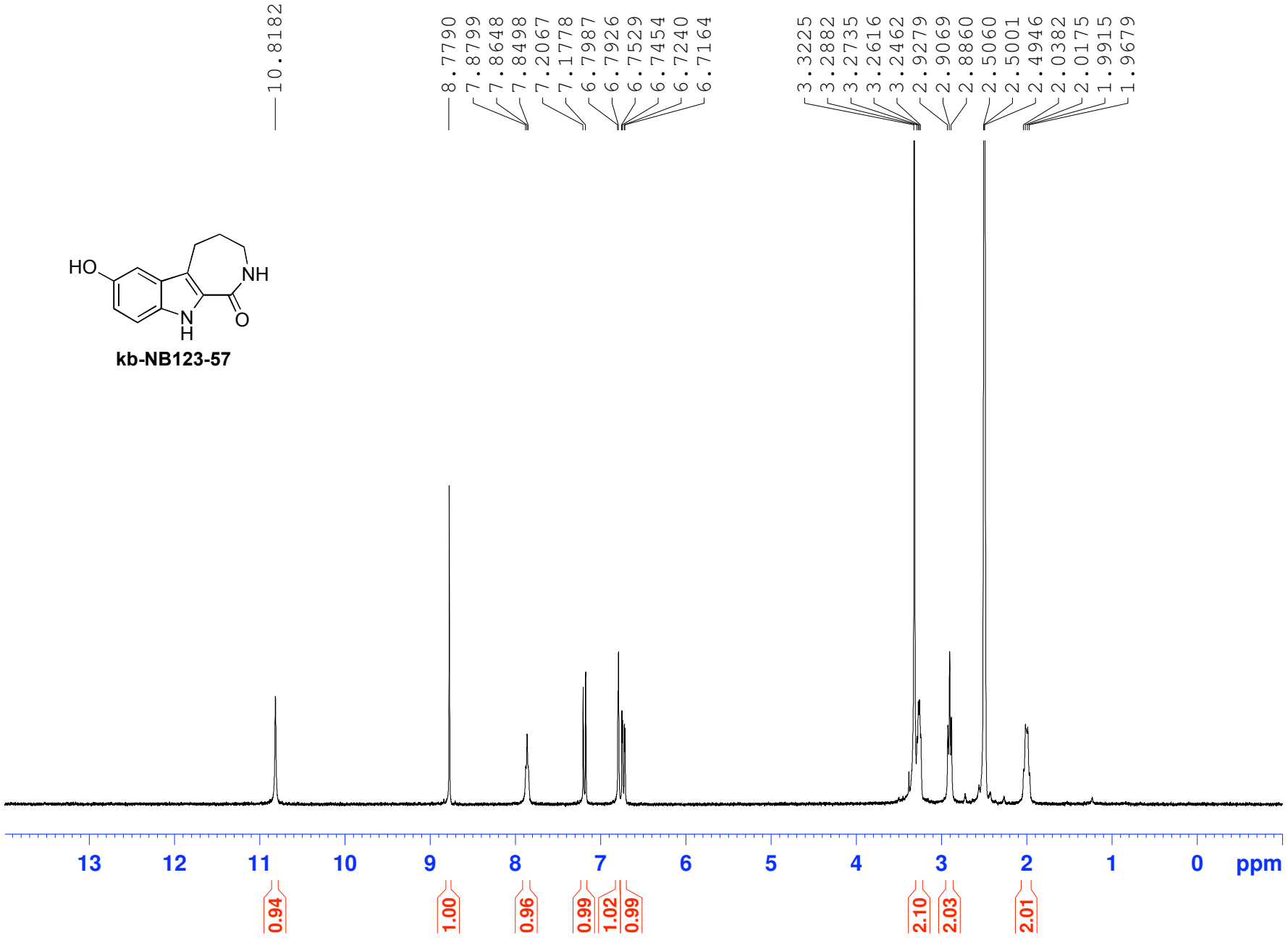
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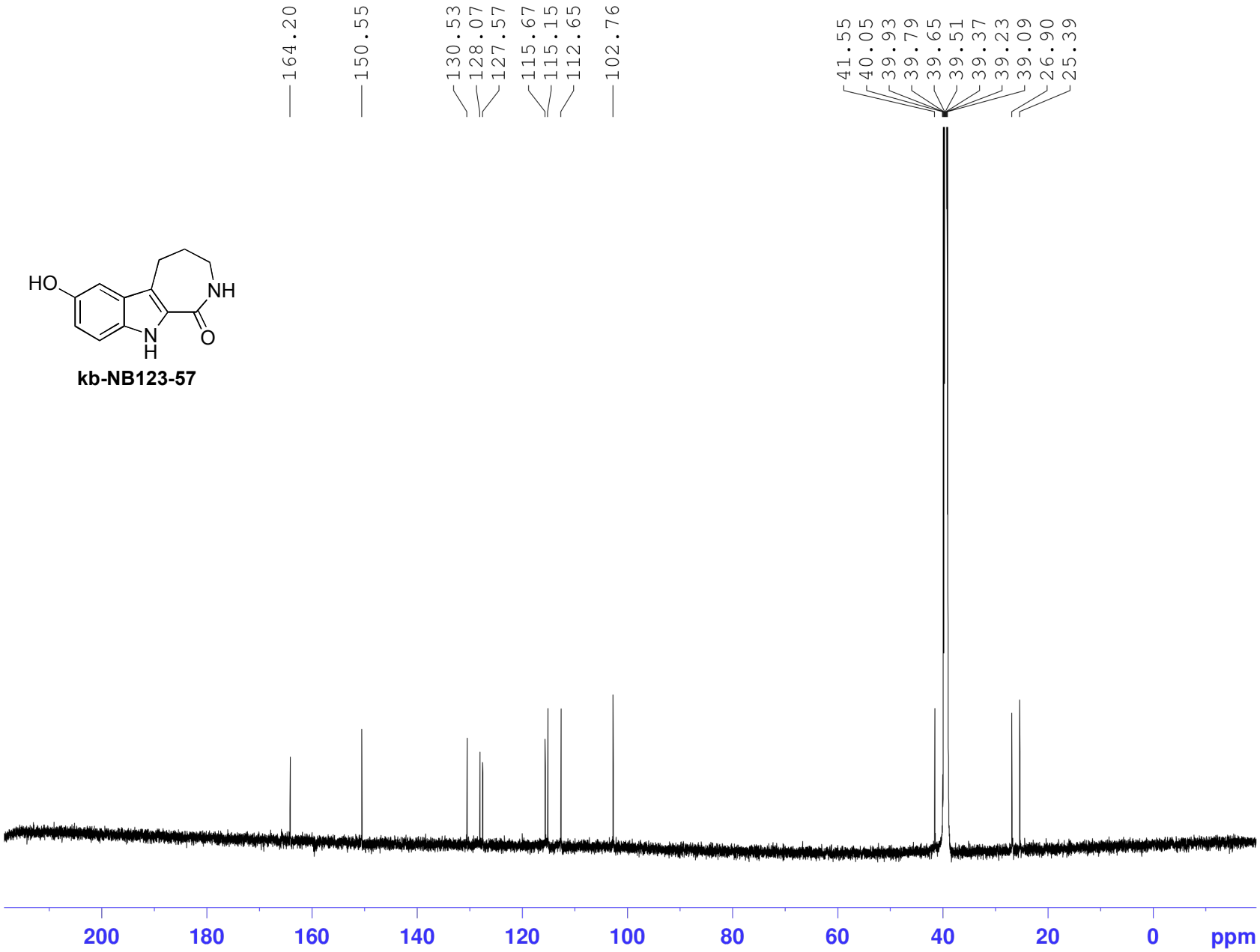
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O
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120
100
80
60
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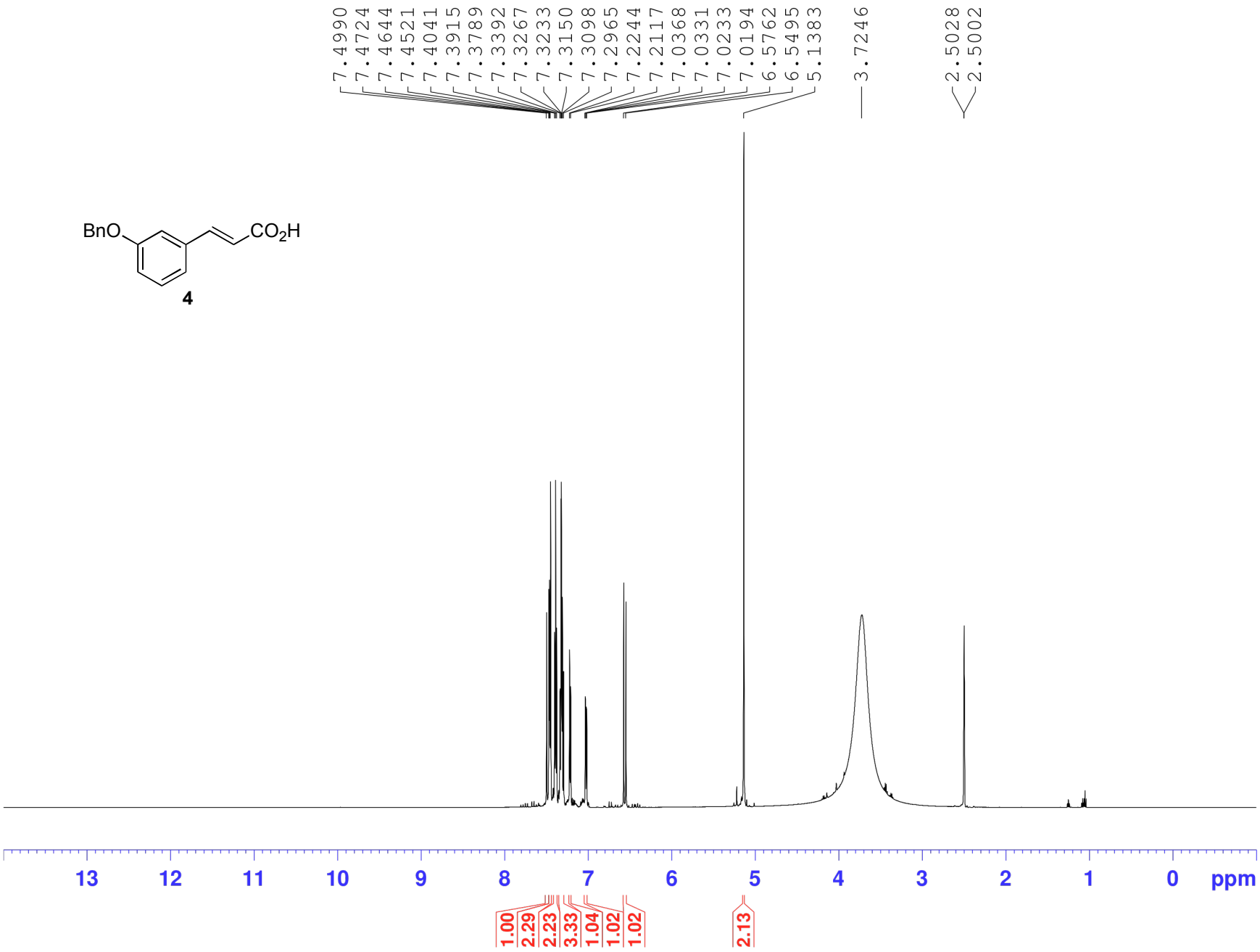
0
ppm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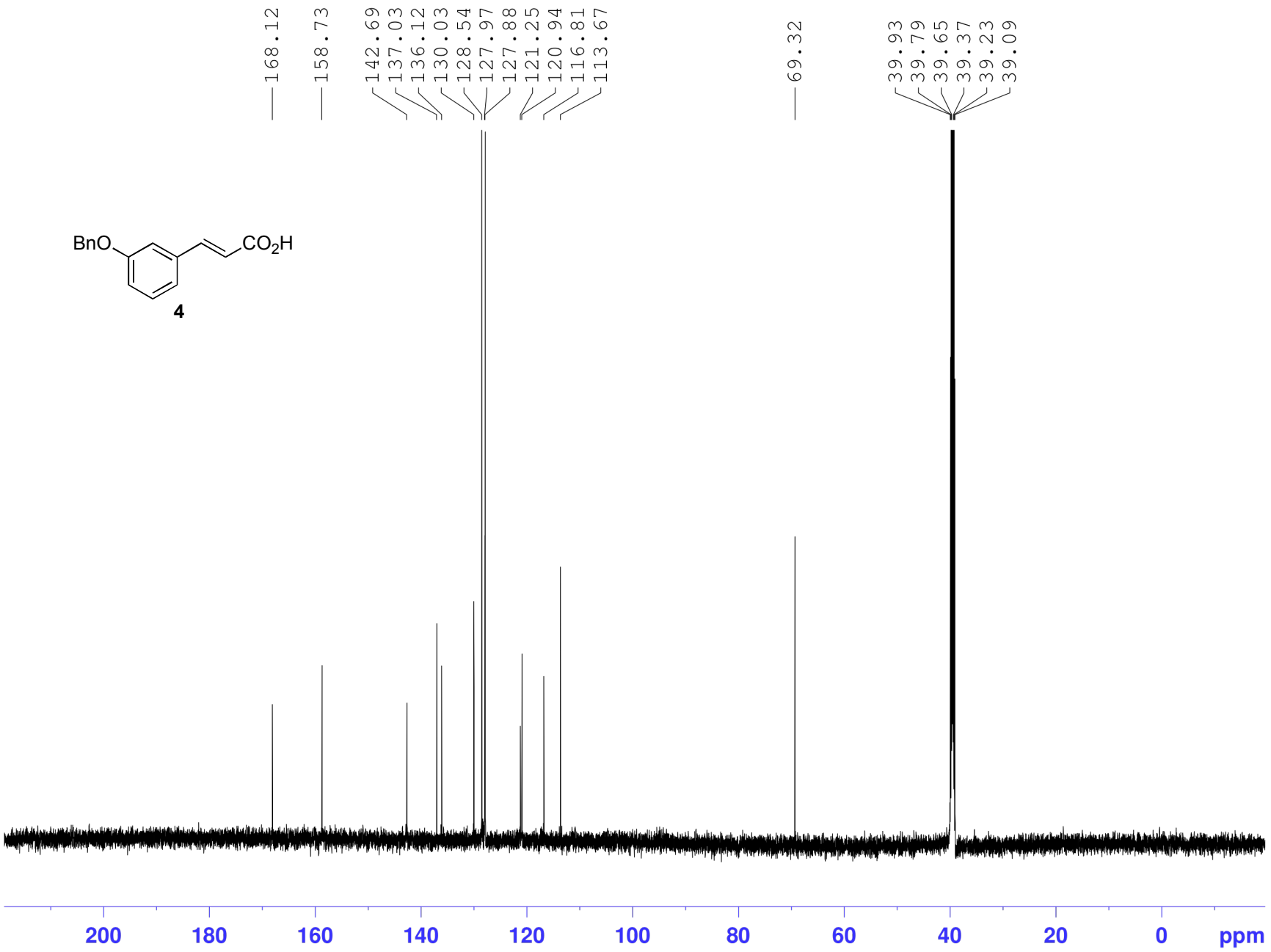
1.02
2.13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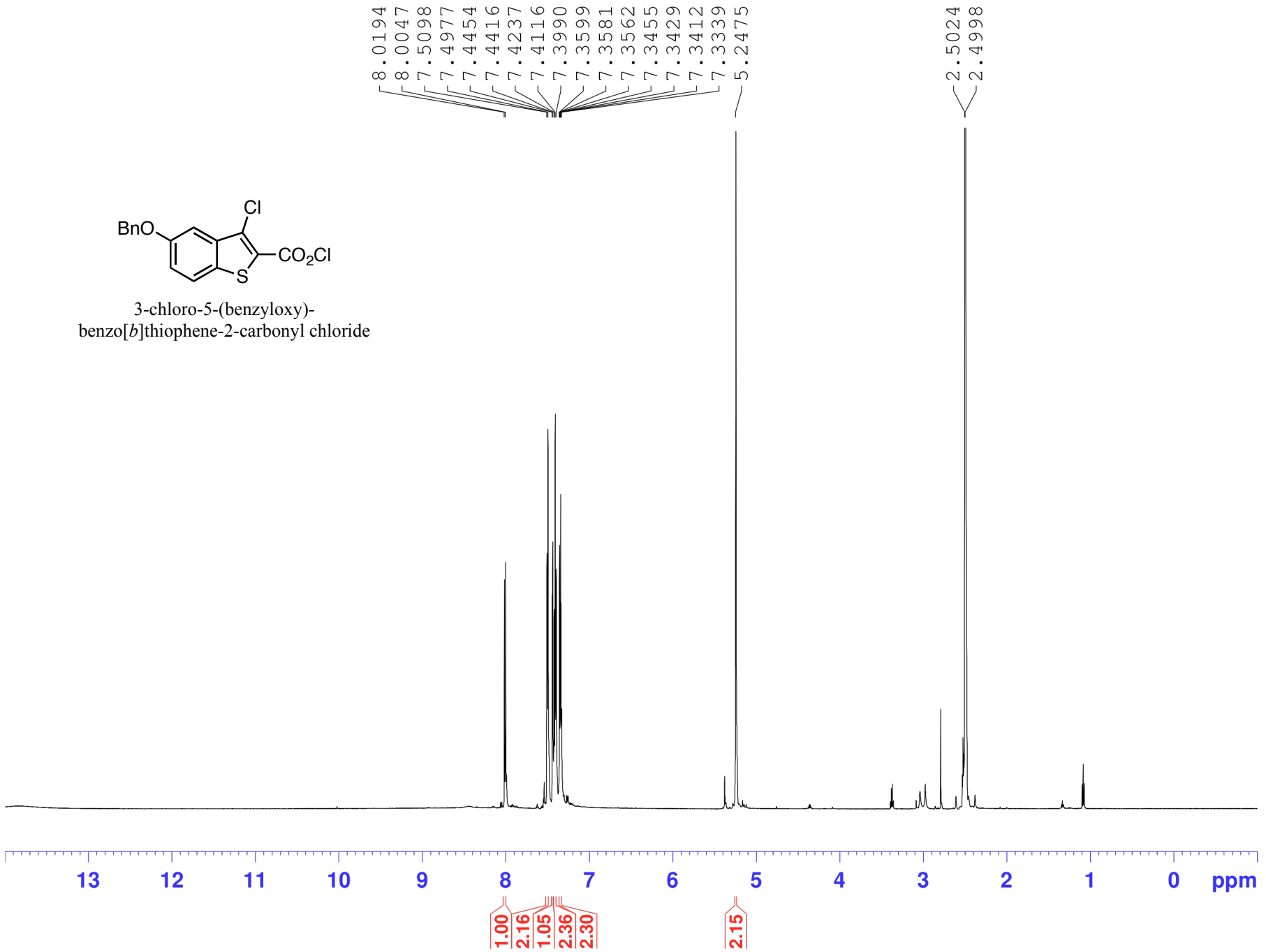
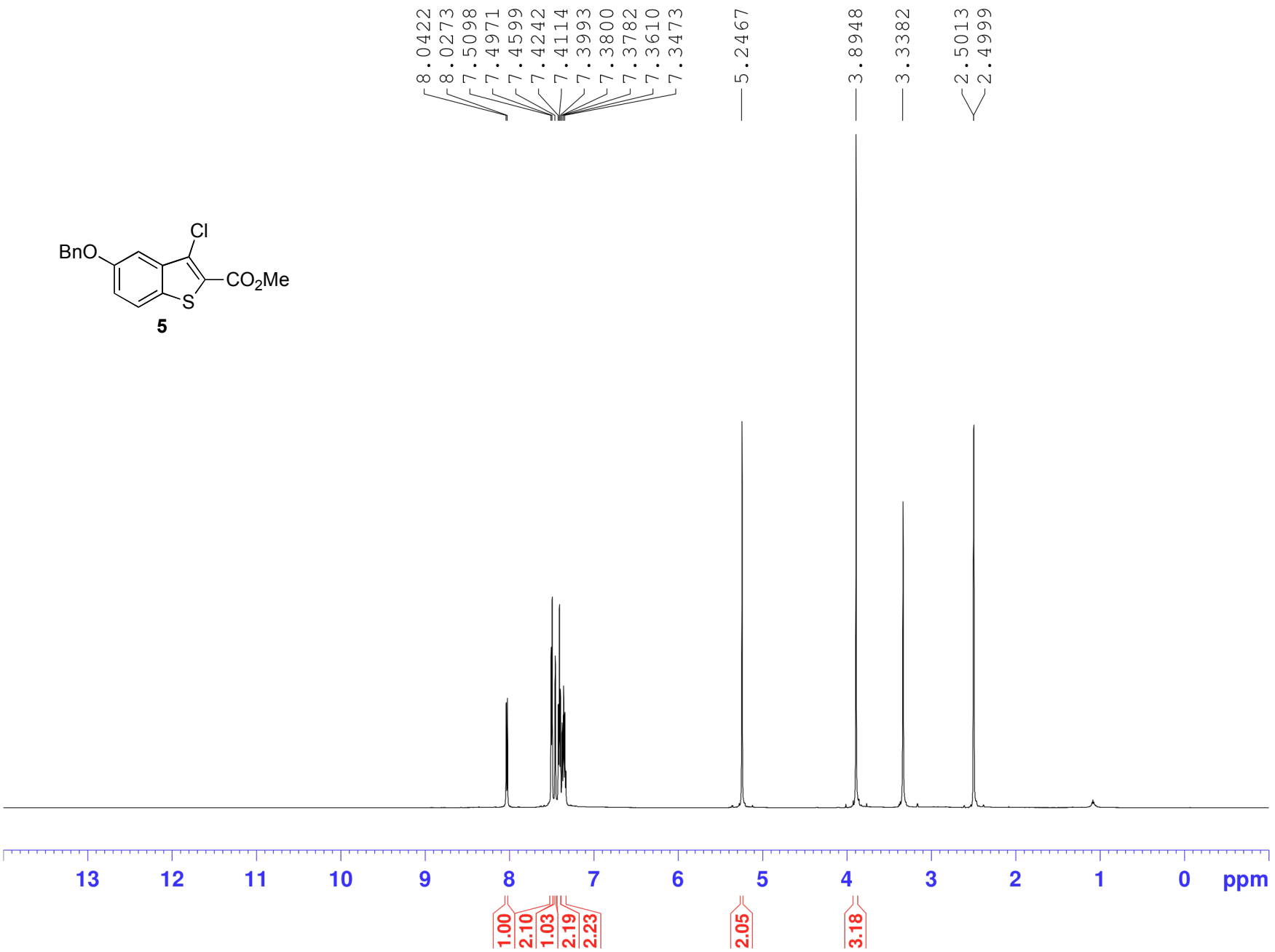
158.73
142.69
137.03
136.12
130.03
128.54
127.97
127.88
121.25
120.94
116.81
113.67
69.32
39.93
39.79
39.65
39.37
39.23
39.09
BnO
CO2H
4
200
180
160
140
120
100
80
60
40
20
0
ppm



3-chloro-5-(benzyloxy)-
benzo[*b*]thiophene-2-carbonyl chloride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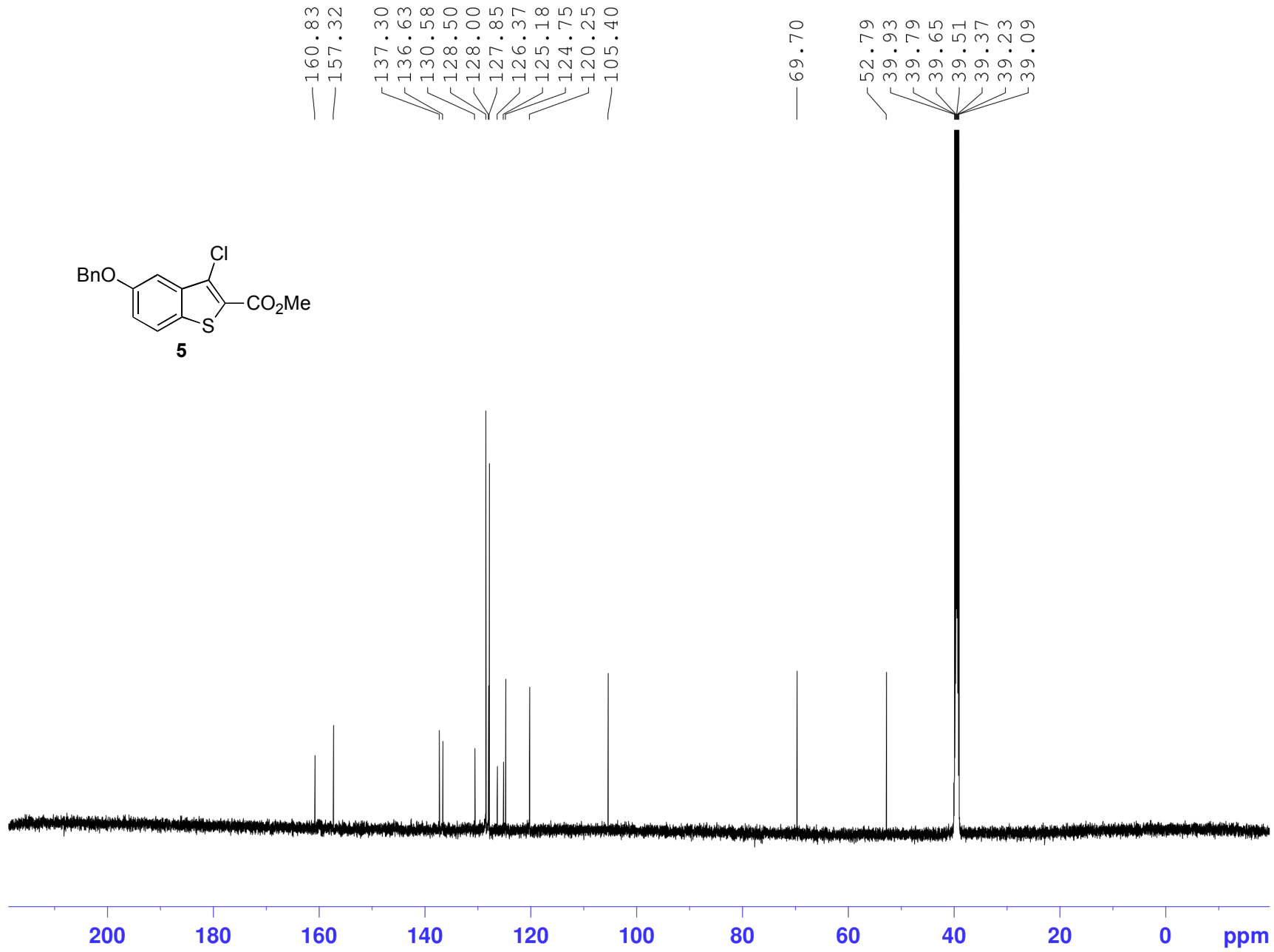
8.0273
7.5098
7.4971
7.4599
7.4242
7.4114
7.3993
7.3800
7.3782
7.3610
7.3473
5.2467
3.8948
3.3382
2.5013
2.4999
Cl
BnO
CO2Me
S
5
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
2.10
1.03
2.19
2.23
2.05
3.18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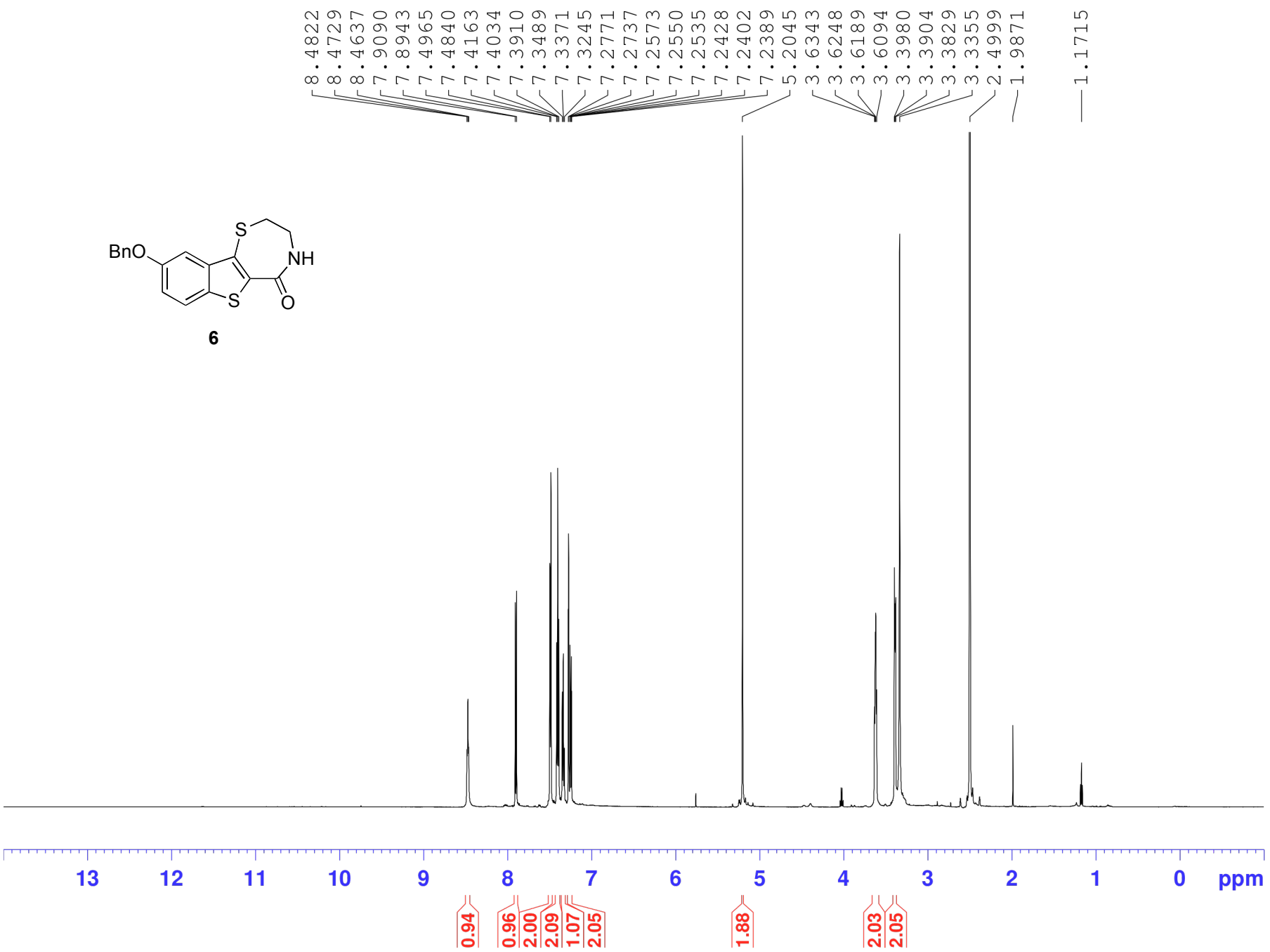
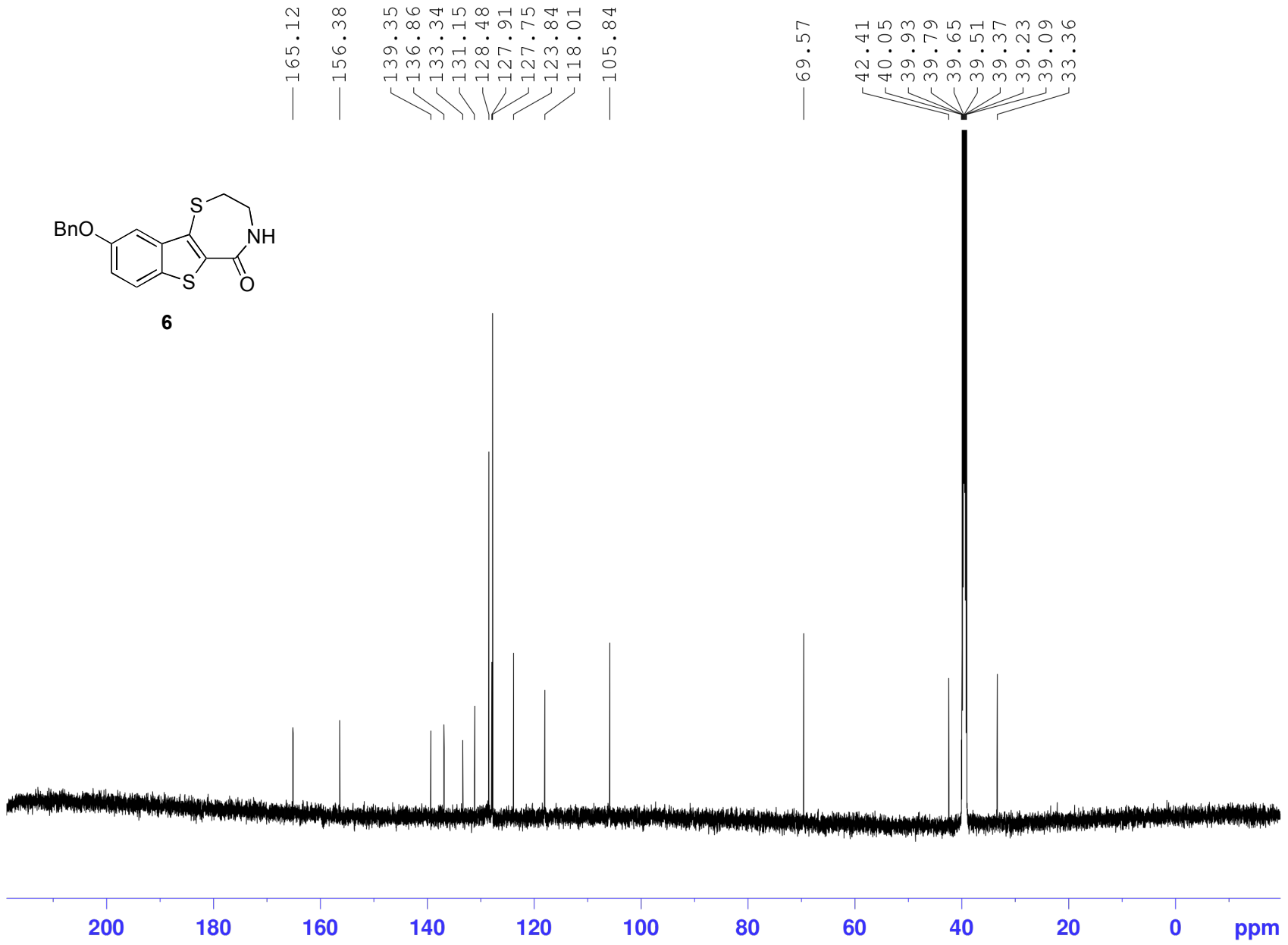
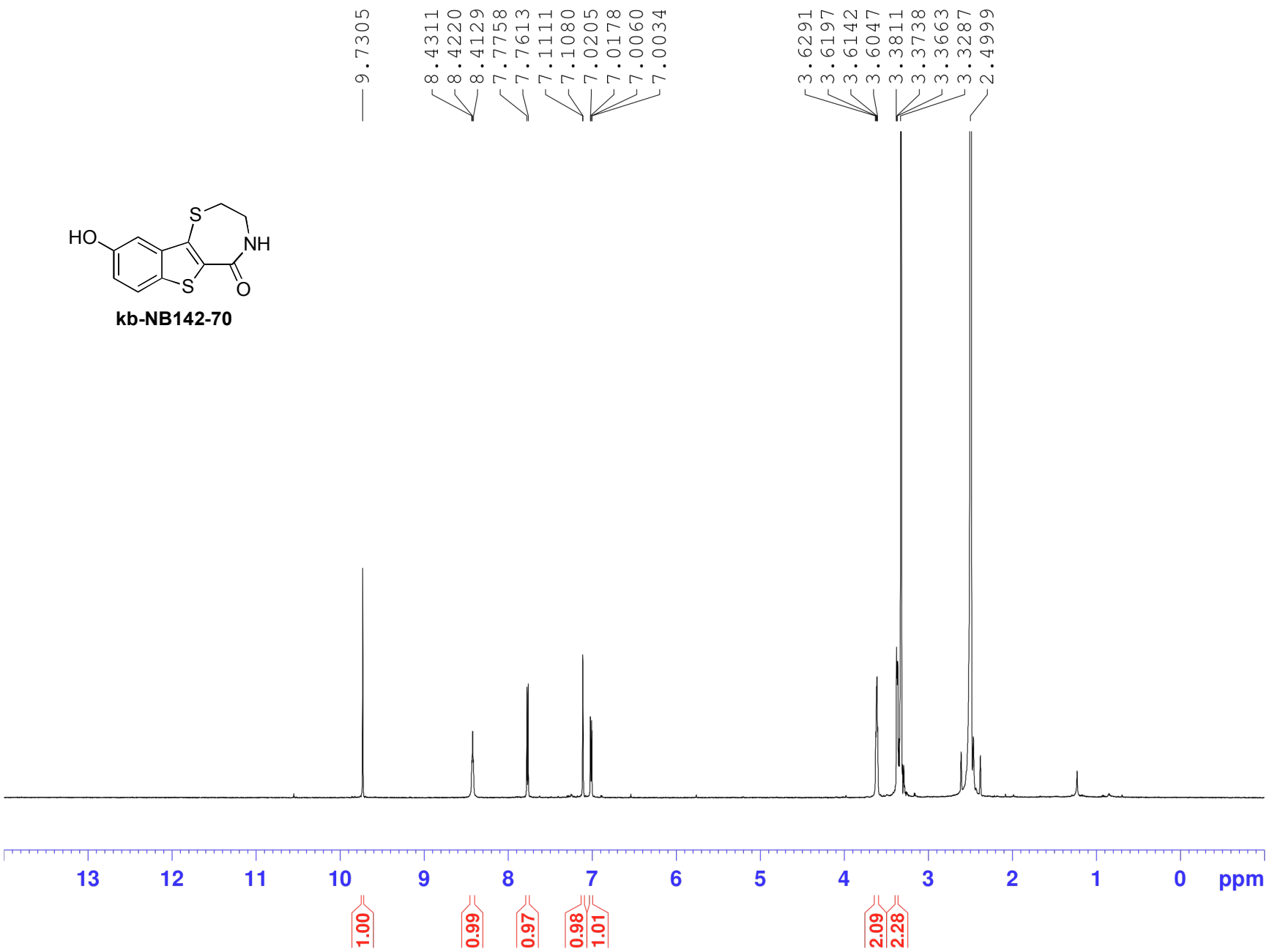
157.32
137.30
136.63
130.58
128.50
128.00
127.85
126.37
125.18
124.75
120.25
105.40
69.70
52.79
39.93
39.79
39.65
39.51
39.37
39.23
39.09
Cl
BnO
CO2Me
S
5
200
180
160
140
120
100
80
60
40
20
0
ppm


8.4822
8.4729
8.4637
7.9090
7.8943
7.4965
7.4840
7.4163
7.4034
7.3910
7.3489
7.3371
7.3245
7.2771
7.2737
7.2573
7.2550
7.2535
7.2428
7.2402
7.2389
5.2045
3.6343
3.6248
3.6189
3.6094
3.3980
3.3904
3.3829
3.3355
2.4999
1.9871
1.1715
BnO
S
NH
O
S
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.94
0.96
2.00
2.09
1.07
2.05
1.88
2.03
2.05

6


165.12
156.38
139.35
136.86
133.34
131.15
128.48
127.91
127.75
123.84
118.01
105.84
69.57
42.41
40.05
39.93
39.79
39.65
39.51
39.37
39.23
39.09
33.36
BnO
S
NH
O
S
200
180
160
140
120
100
80
60
40
20
0
ppm

6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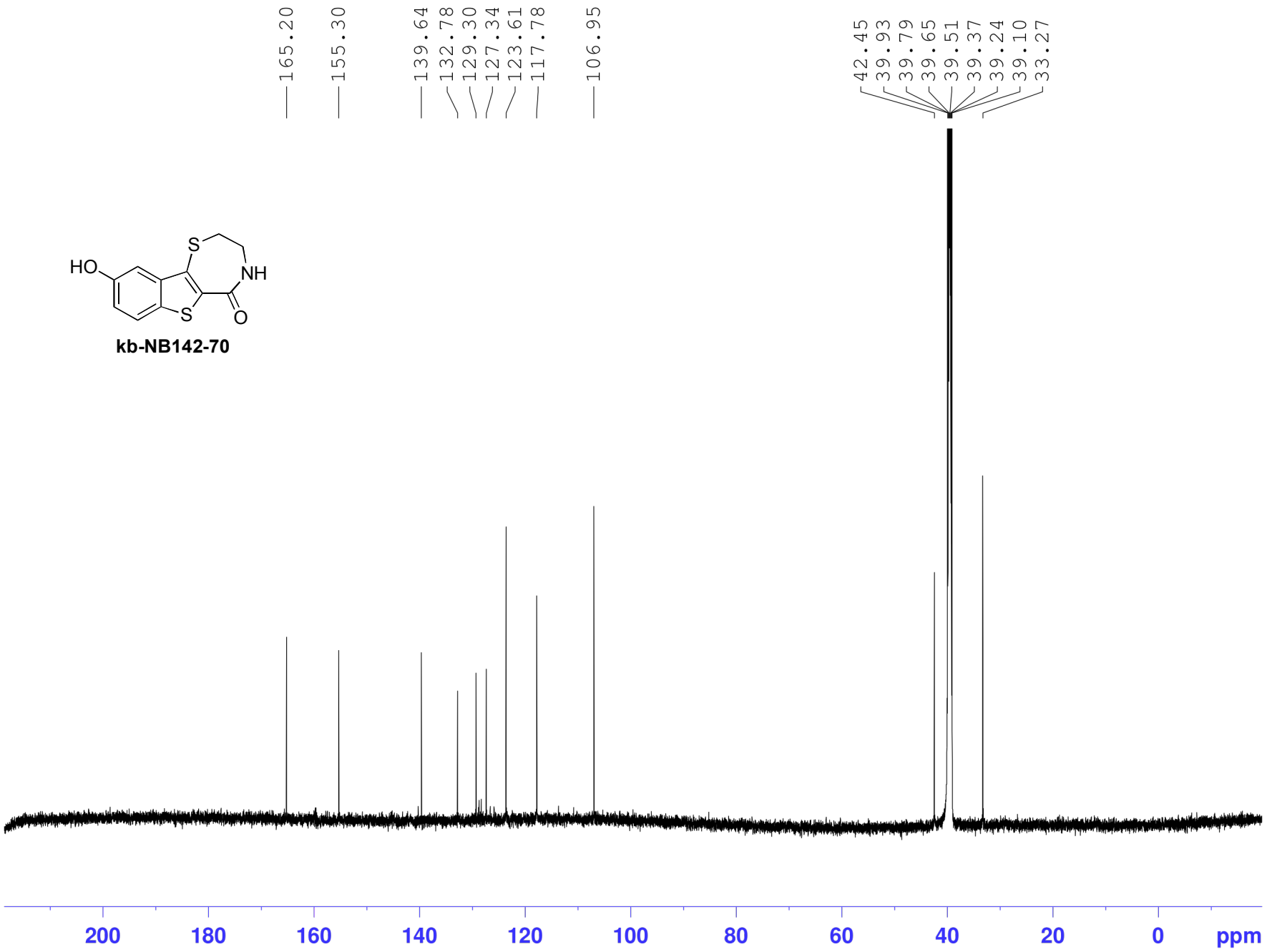
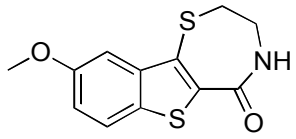
8.4311
8.4220
8.4129
7.7758
7.7613
7.1111
7.1080
7.0205
7.0178
7.0060
7.0034
3.6291
3.6197
3.6142
3.6047
3.3811
3.3738
3.3663
3.3287
2.4999
HO
S
NH
S
O
kb-NB142-70
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.99
0.97
0.98
1.01
2.09
2.28


165.20
155.30
139.64
132.78
129.30
127.34
123.61
117.78
106.95
42.45
39.93
39.79
39.65
39.51
39.37
39.24
39.10
33.27
HO
S
NH
S
O
kb-NB142-70
200
180
160
140
120
100
80
60
40
20
0
ppm

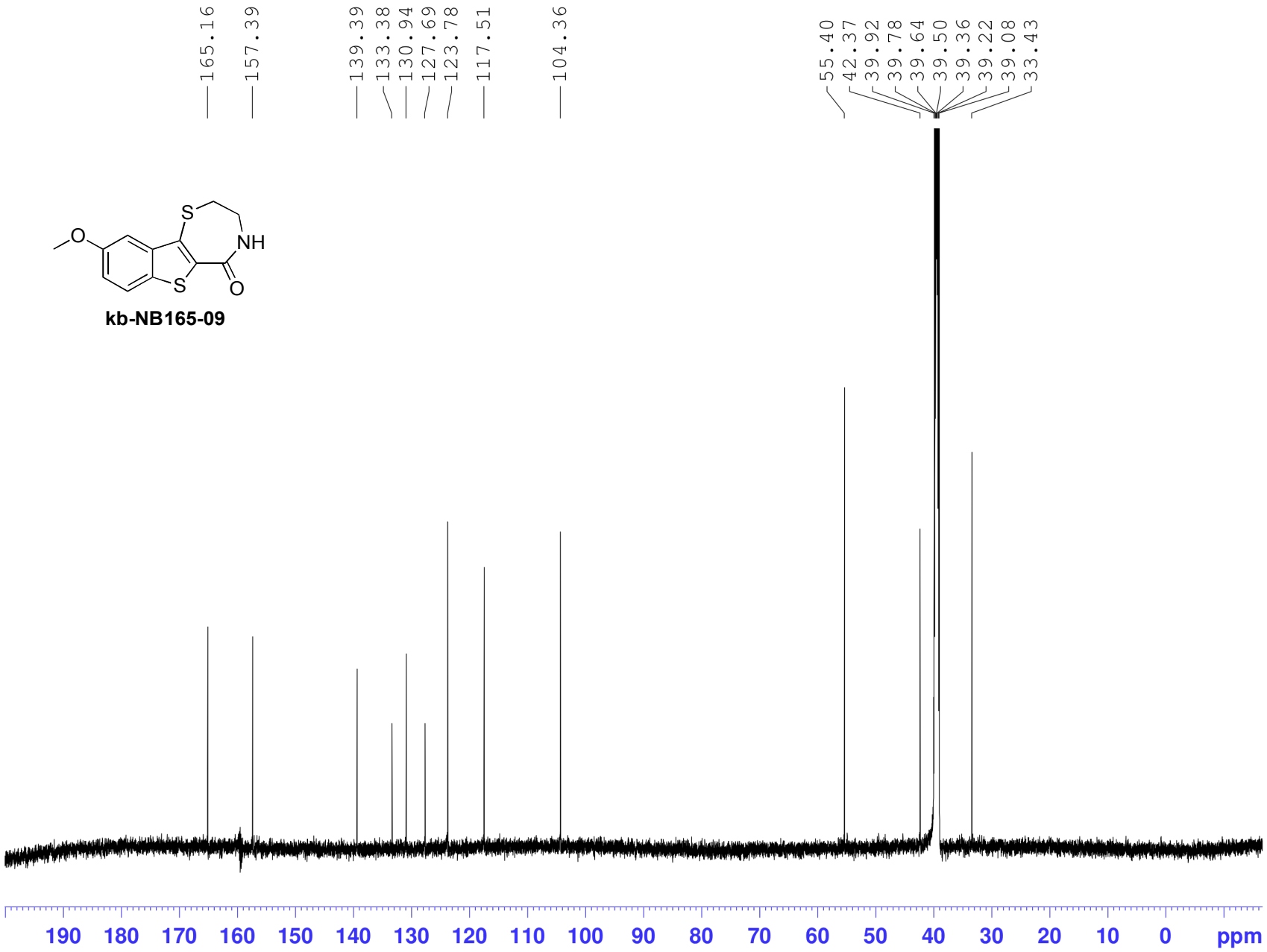


kb-NB165-09

8.4824
8.4735
8.4646
7.8991
7.8852
7.1788
7.1652
7.1613
5.7587
3.8415
3.6377
3.6281
3.6221
3.6127
3.4044
3.4014
3.3967
3.3892
3.3306
2.5000

13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm

1.00 1.00 2.01 2.94 2.03 1.98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139.39
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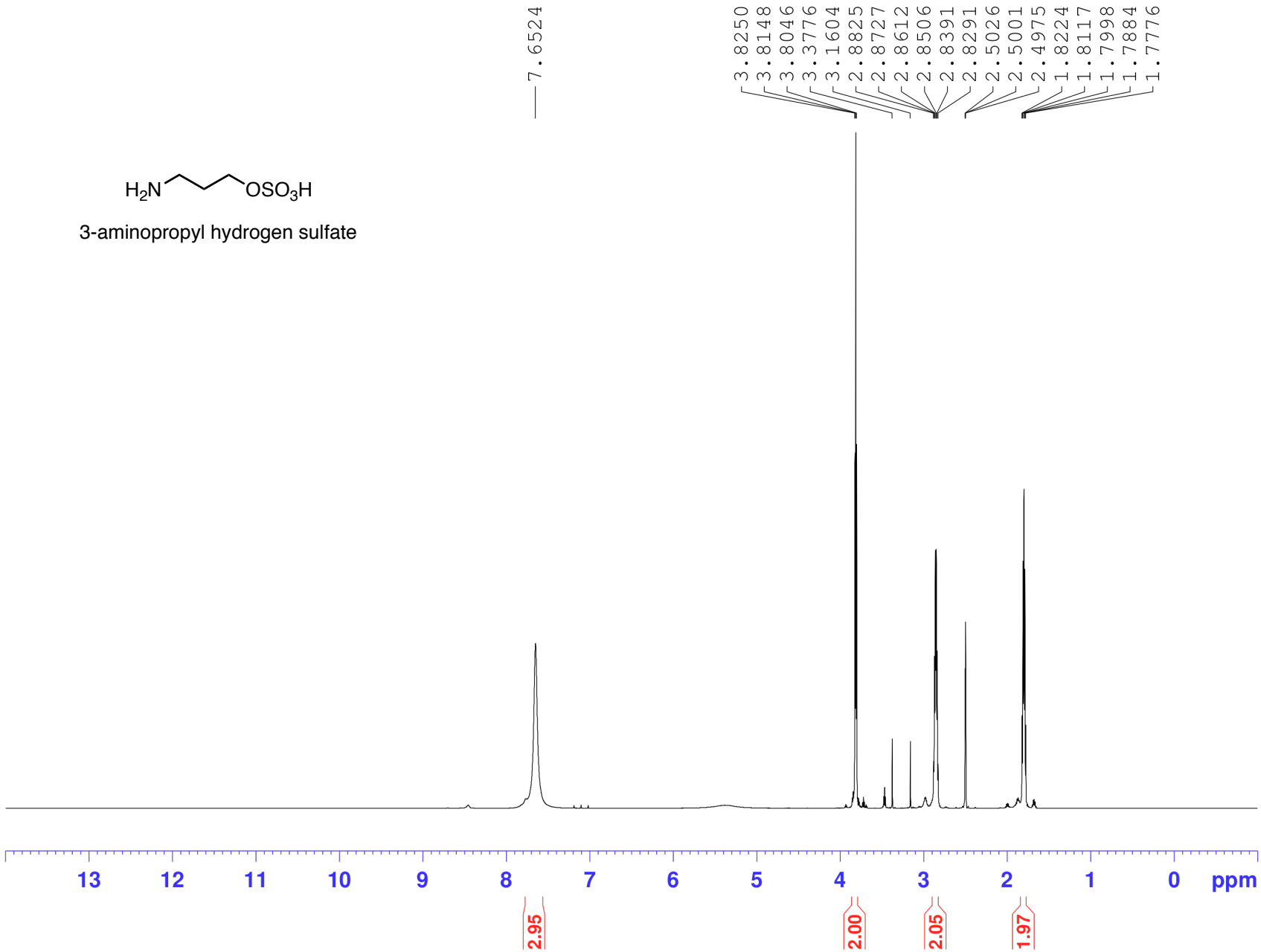
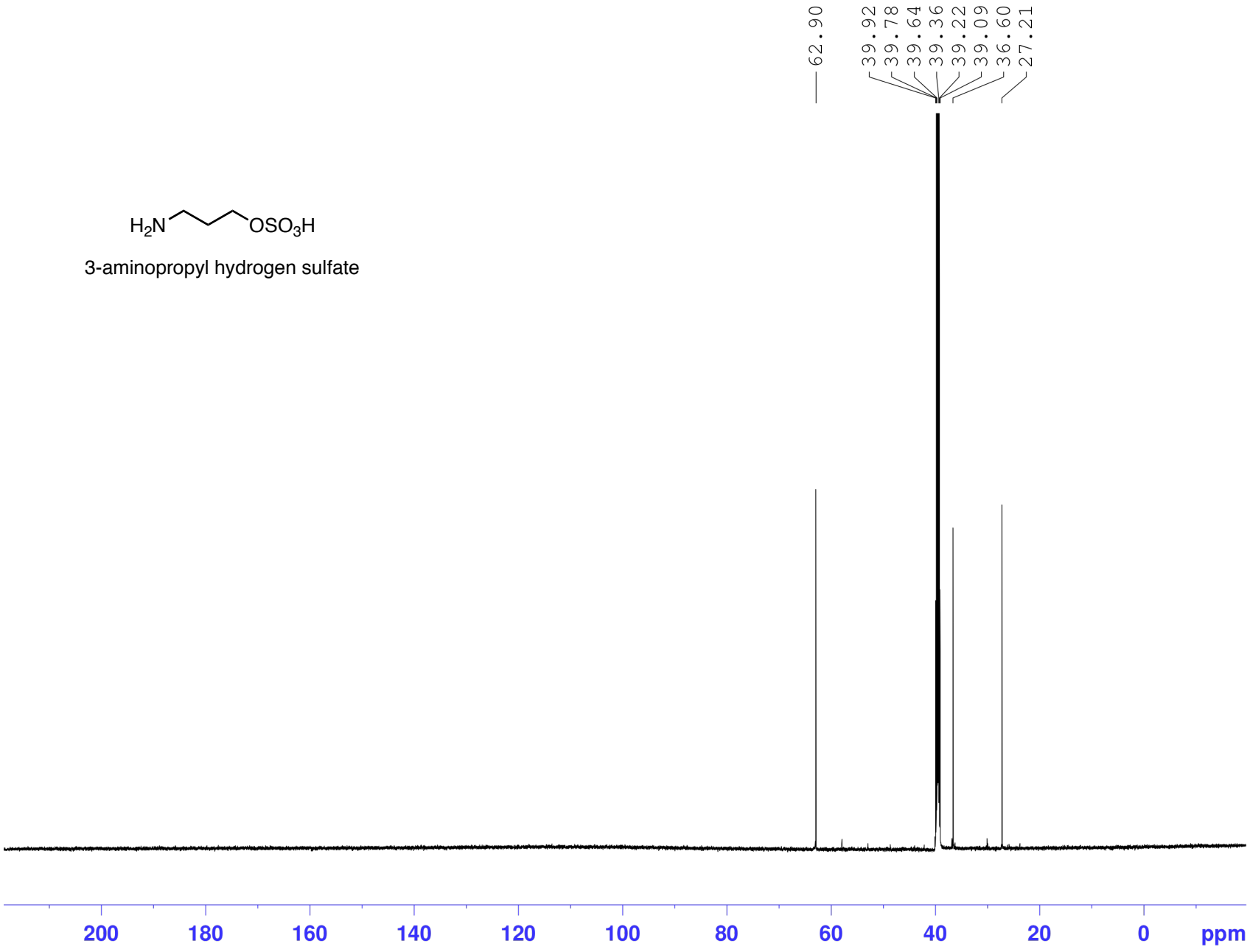
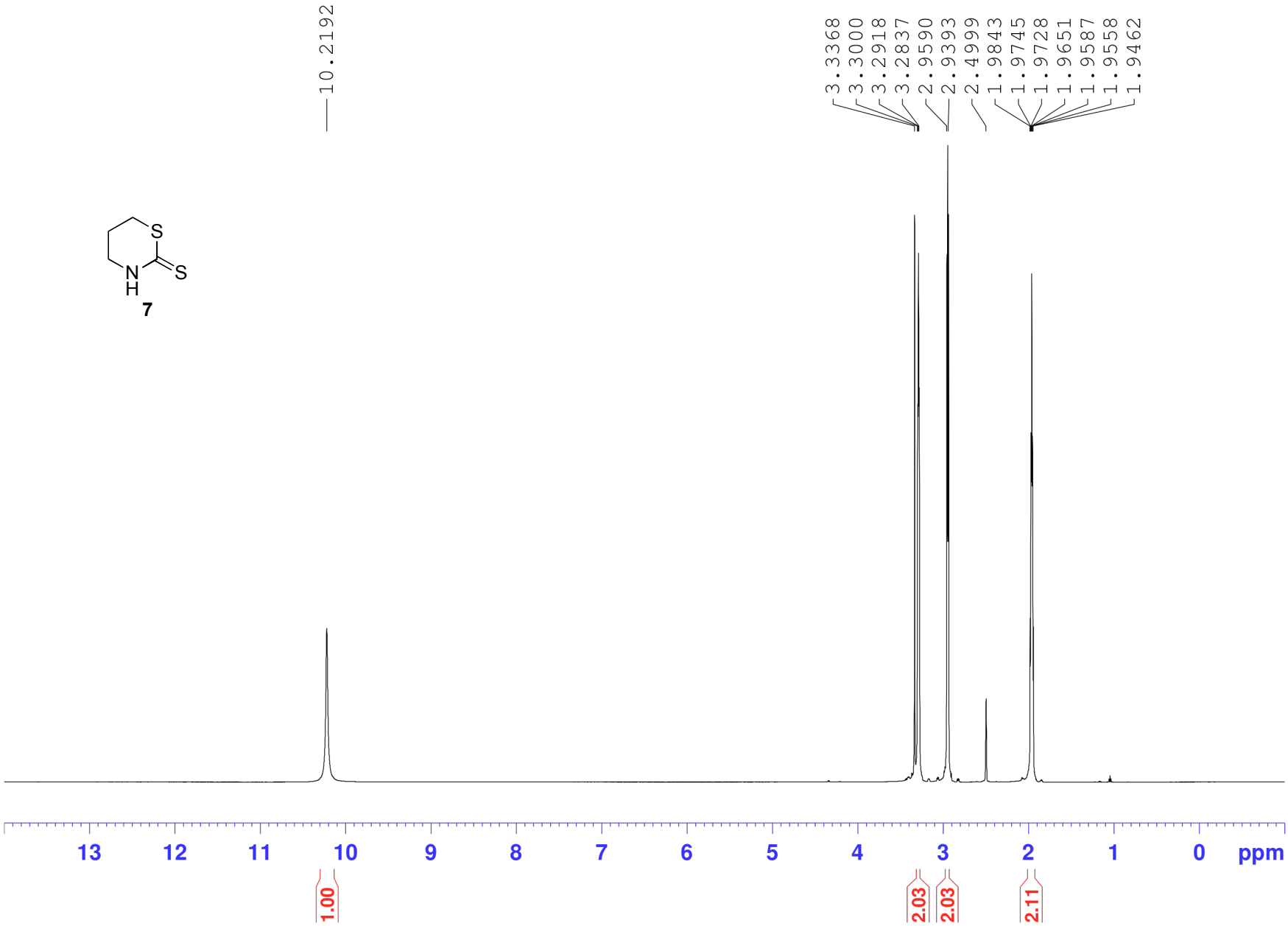
130.94
127.69
123.78
117.51
104.36
55.40
42.37
39.92
39.78
39.64
39.50
39.36
39.22
39.08
33.43
S
NH
O
S
O
kb-NB165-09
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


7.6524
3.8250
3.8148
3.8046
3.3776
3.1604
2.8825
2.8727
2.8612
2.8506
2.8391
2.8291
2.5026
2.5001
2.4975
1.8224
1.8117
1.7998
1.7884
1.7776
H2N
OSO3H
3-aminopropyl hydrogen sulfate
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
2.95
2.00
2.05
1.97



H_2N OSO_3H

3-aminopropyl hydrogen sulfate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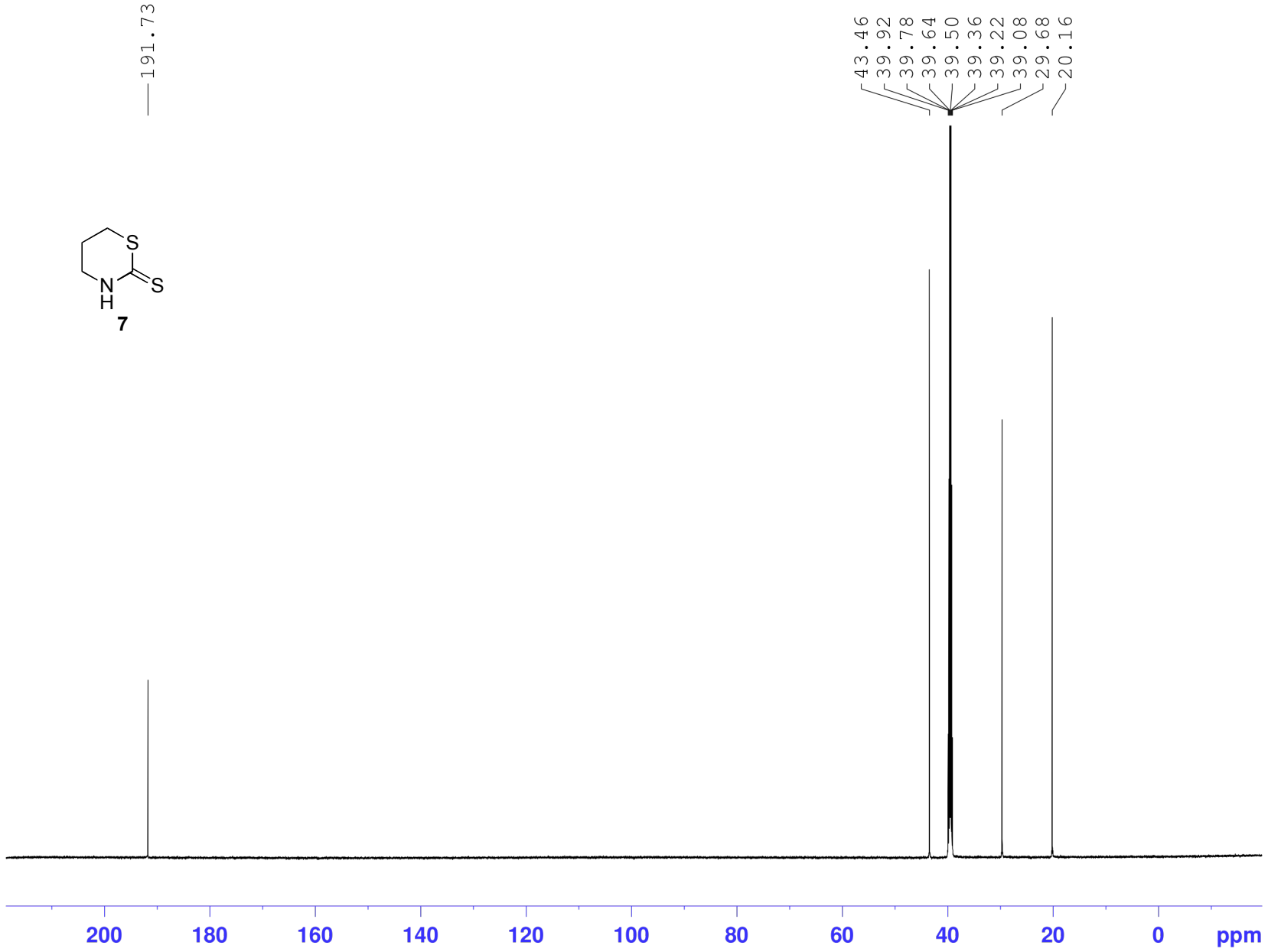
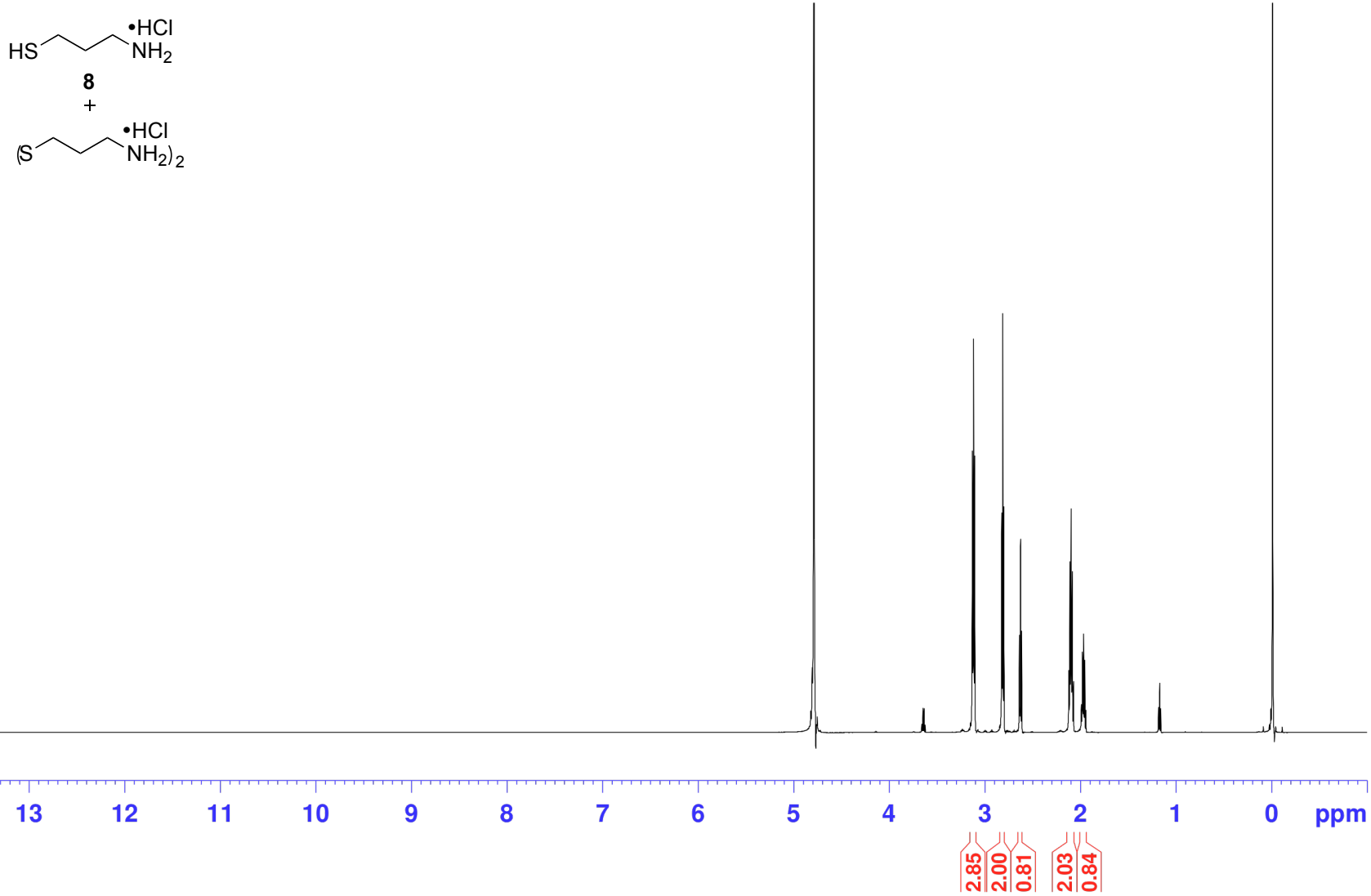
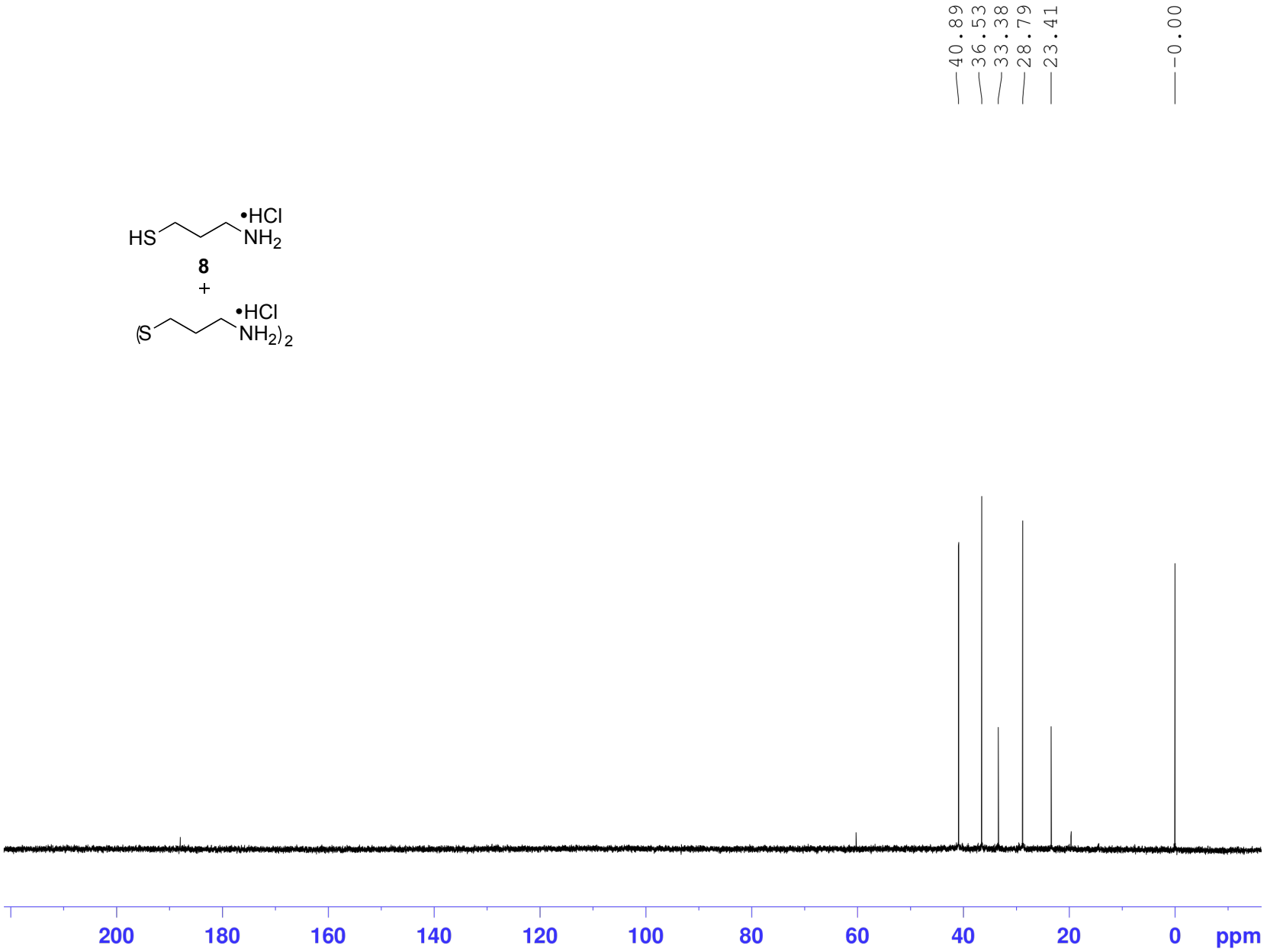
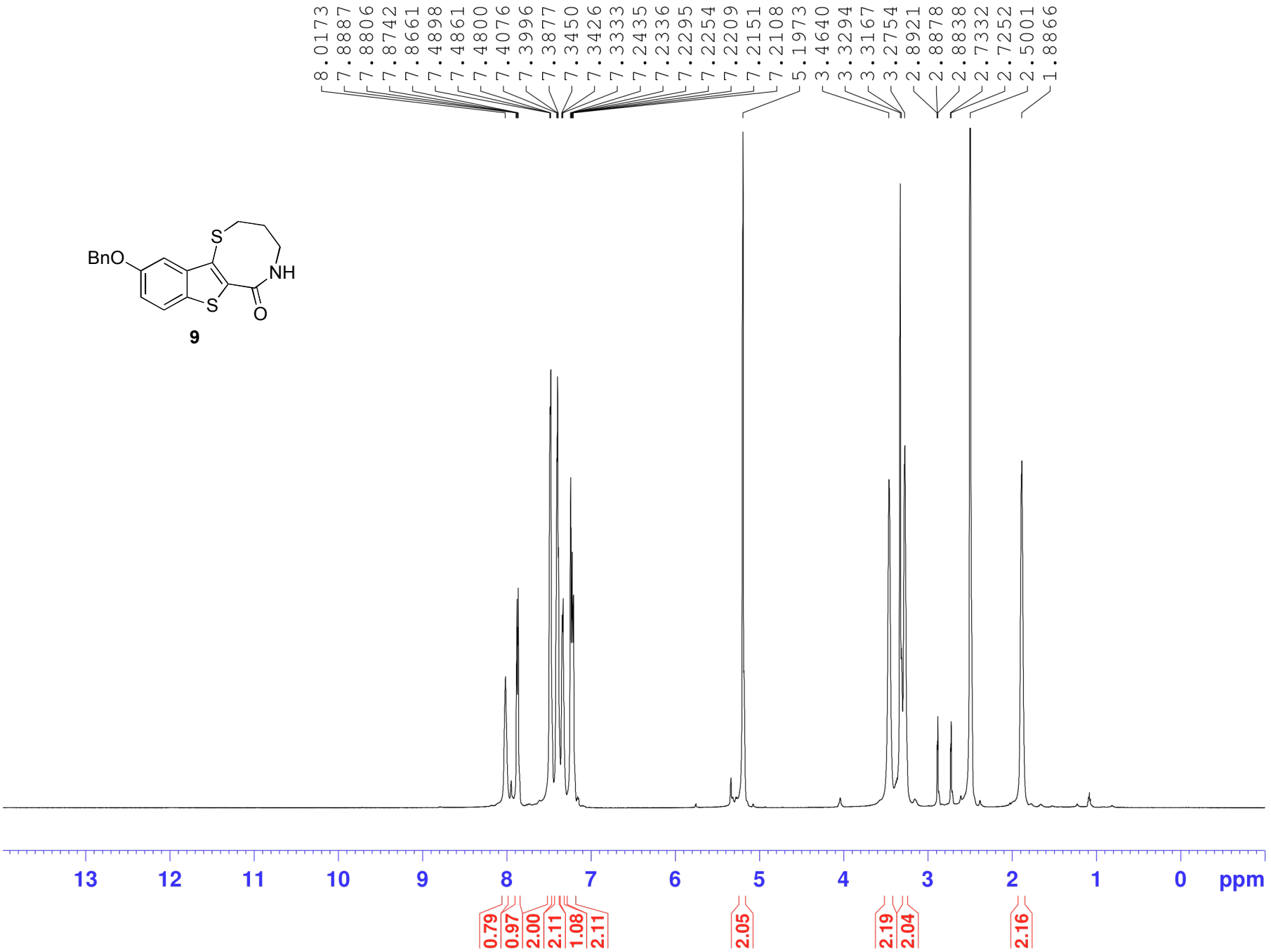
3.3368
3.3000
3.2918
3.2837
2.9590
2.9393
2.4999
1.9843
1.9745
1.9728
1.9651
1.9587
1.9558
1.9462
S
N
H
S
7
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
2.03
2.03
2.11


191.73
43.46
39.92
39.78
39.64
39.50
39.36
39.22
39.08
29.68
20.16
S
N
H
S
7
200
180
160
140
120
100
80
60
40
20
0
ppm


4.7900
3.6616
3.6497
3.6379
3.6261
3.1326
3.1204
3.1074
2.8260
2.8142
2.8025
2.6402
2.6285
2.6169
2.1231
2.1112
2.0988
2.0865
2.0744
1.9923
1.9806
1.9683
1.9559
1.9441
1.1845
1.1726
HS
•HCl
NH2
8
+
•HCl
NH2)2
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
2.85
2.00
0.81
2.03
0.84


40.89
36.53
33.38
28.79
23.41
0.00
•HCl
HS
NH2
8
+
•HCl
S
NH2)2
200
180
160
140
120
100
80
60
40
20
0
ppm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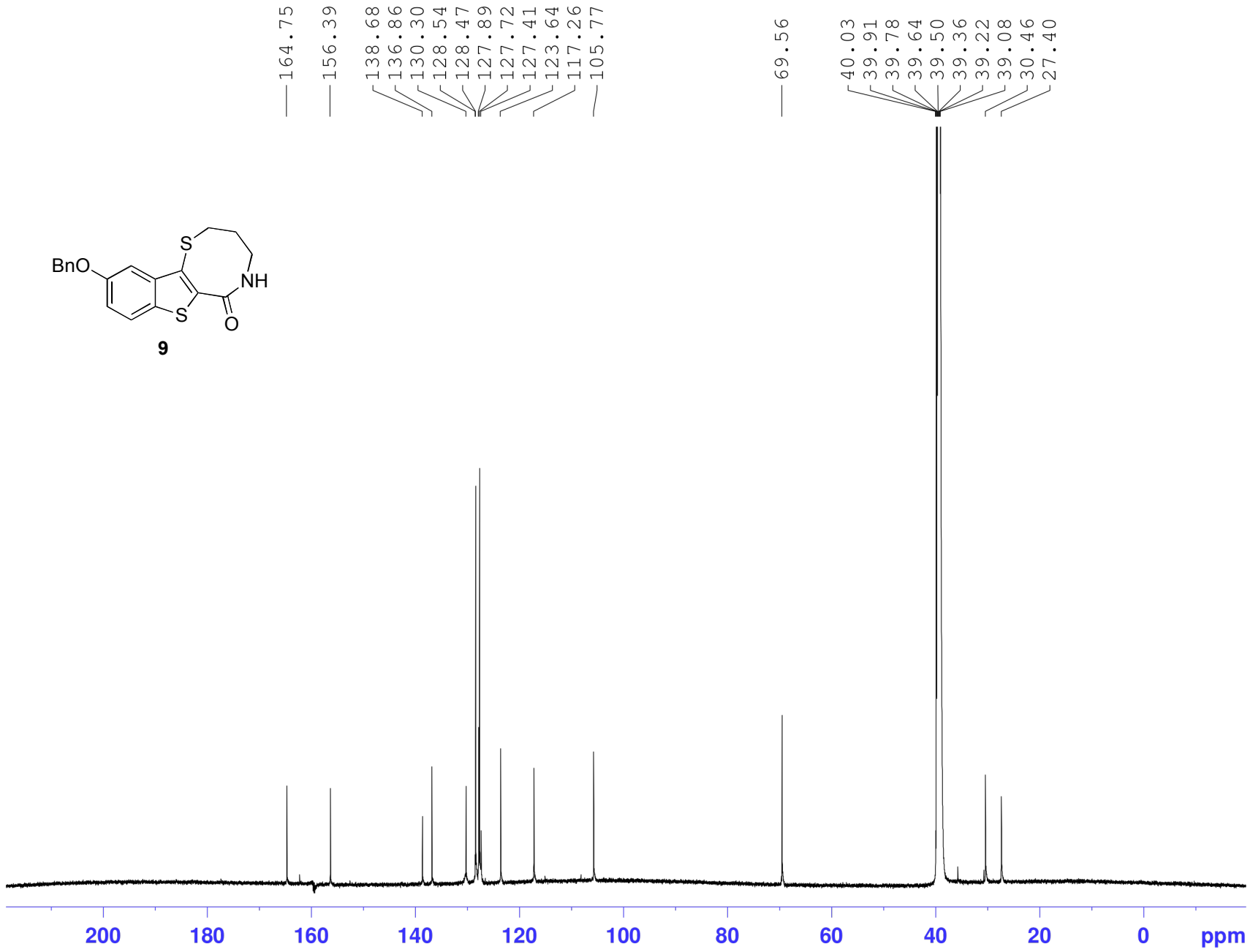
7.8887
7.8806
7.8742
7.8661
7.4898
7.4861
7.4800
7.4076
7.3996
7.3877
7.3450
7.3426
7.3333
7.2435
7.2336
7.2295
7.2254
7.2209
7.2151
7.2108
5.1973
3.4640
3.3294
3.3167
3.2754
2.8921
2.8878
2.8838
2.7332
2.7252
2.5001
1.8866
BnO
S
NH
S
O
9
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.79
0.97
2.00
2.11
1.08
2.11
2.05
2.19
2.04
2.16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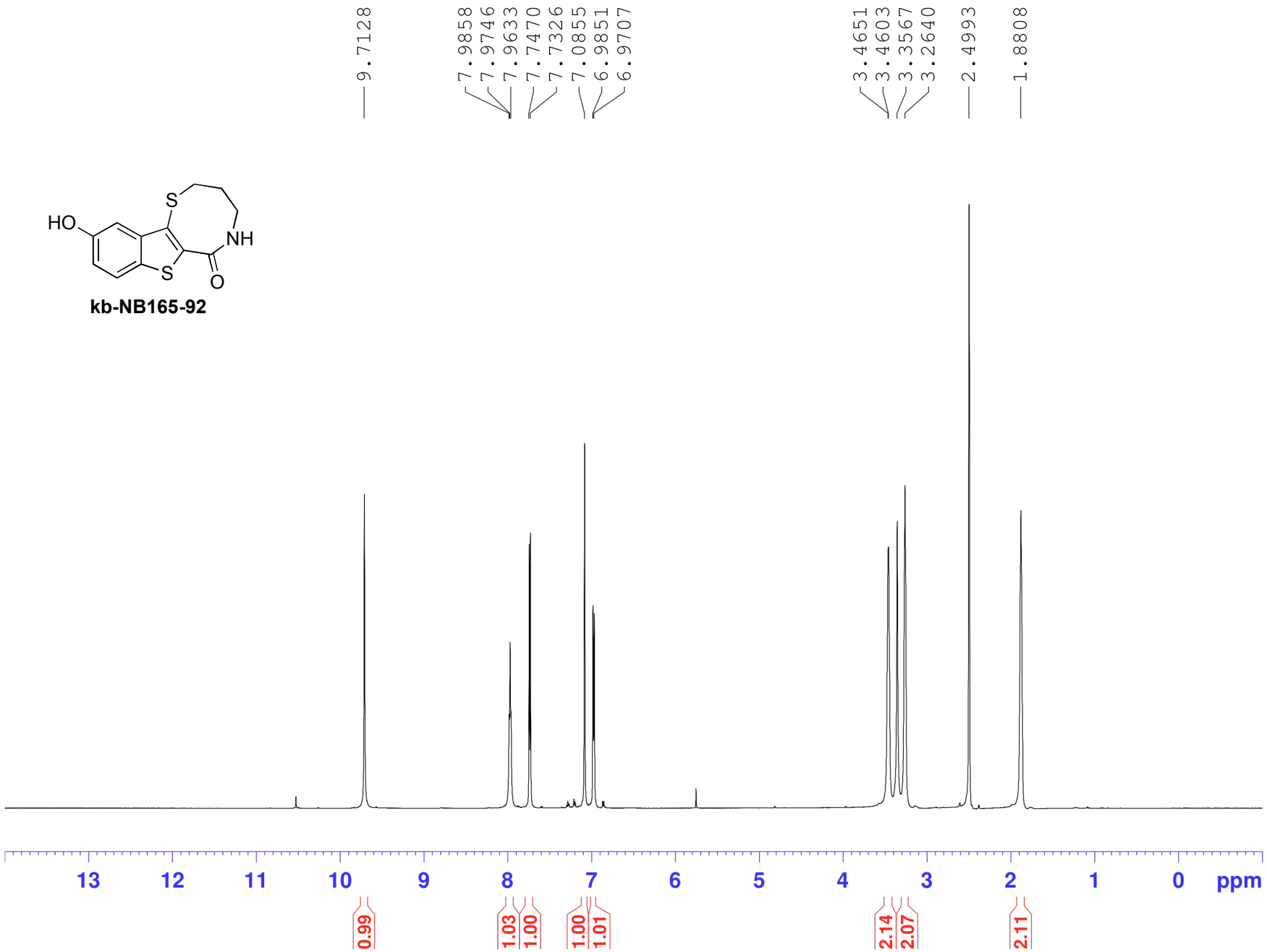
156.39
138.68
136.86
130.30
128.54
128.47
127.89
127.72
127.41
123.64
117.26
105.77
69.56
40.03
39.91
39.78
39.64
39.50
39.36
39.22
39.08
30.46
27.40
BnO
S
NH
S
O
9
200
180
160
140
120
100
80
60
40
20
0
ppm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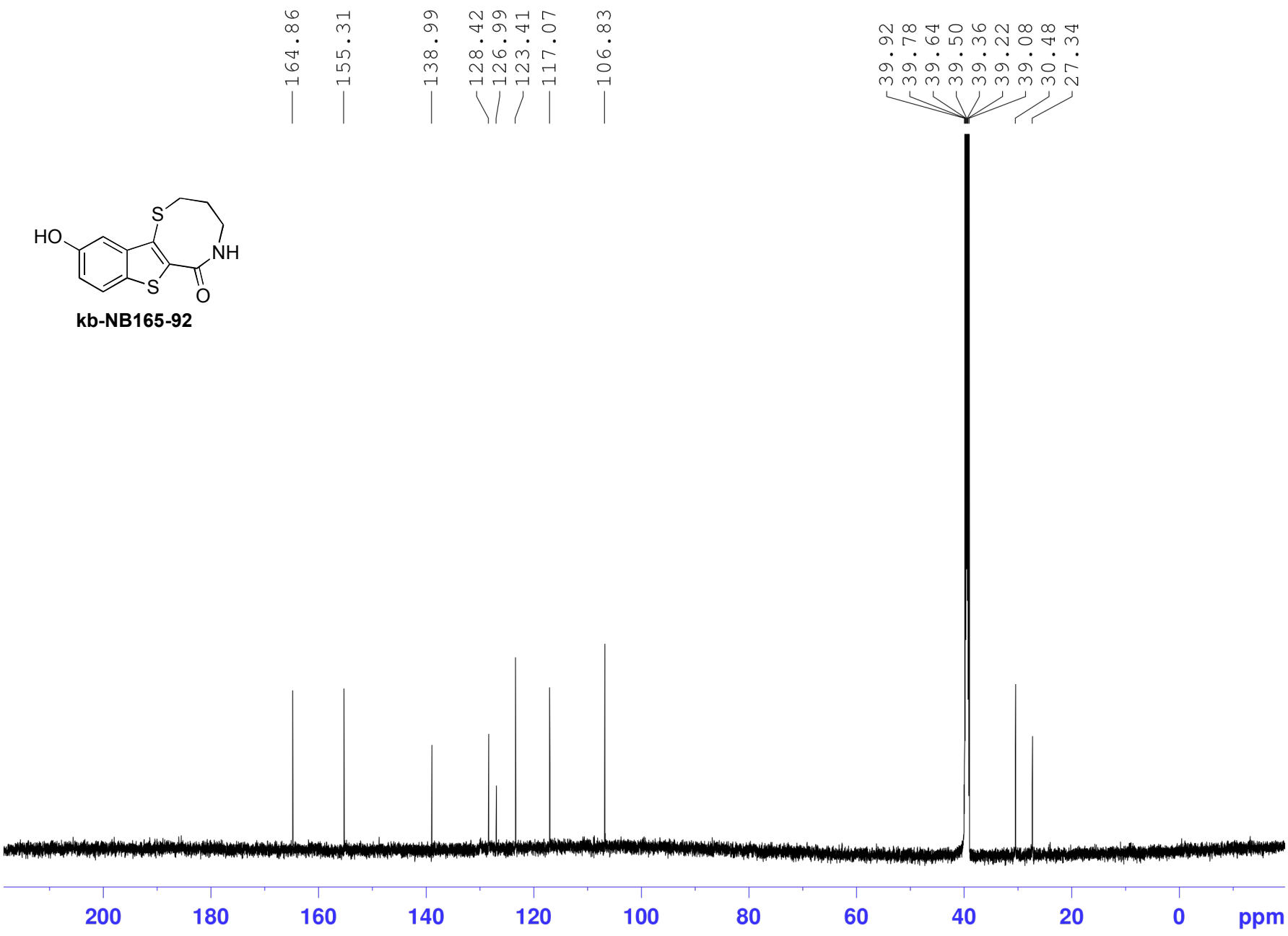
7.9858
7.9746
7.9633
7.7470
7.7326
7.0855
6.9851
6.9707
3.4651
3.4603
3.3567
3.2640
2.4993
1.8808
HO
S
NH
S
O
kb-NB165-92
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.99
1.03
1.00
1.00
1.01
2.14
2.07
2.11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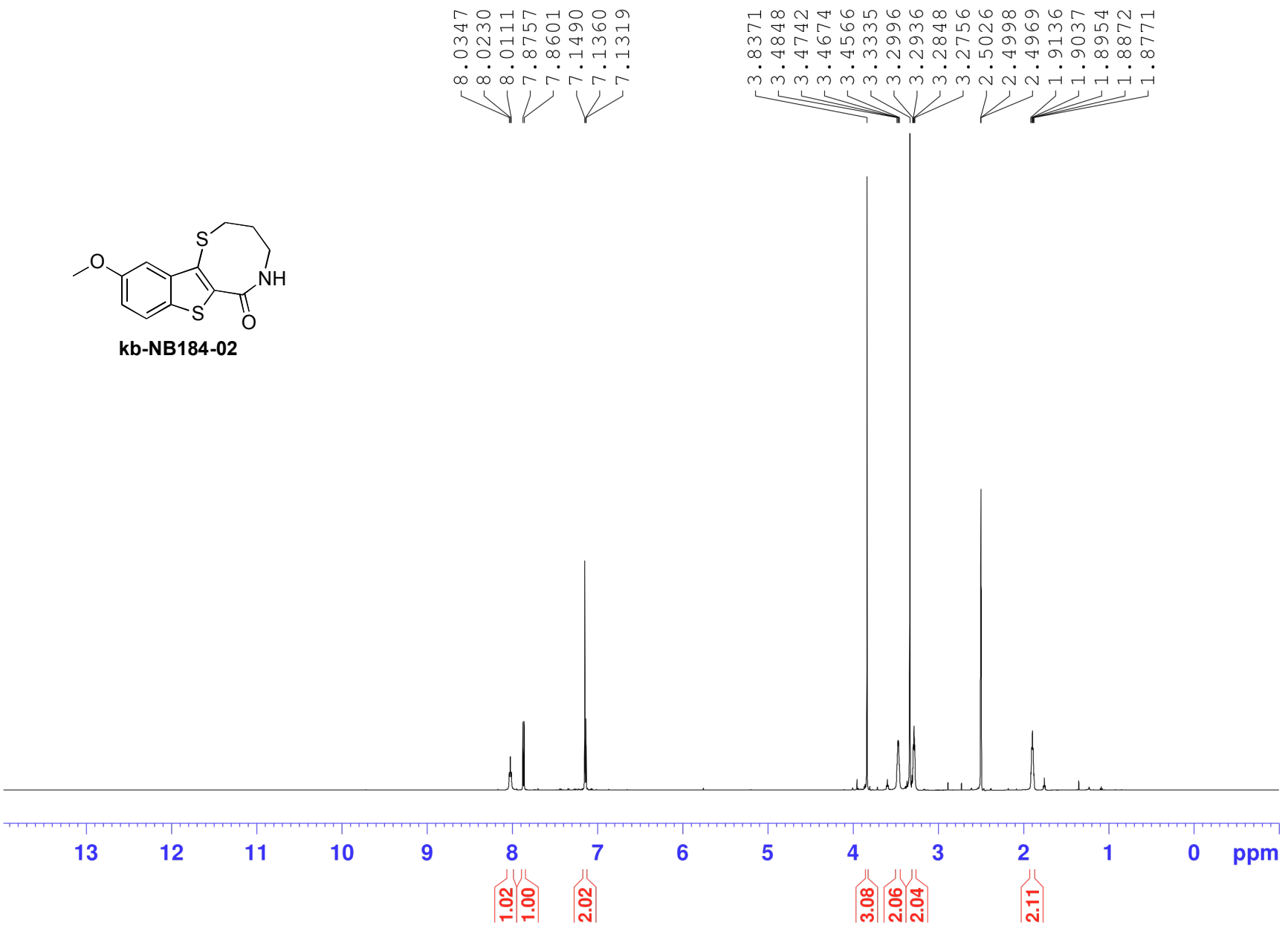
155.31
138.99
128.42
126.99
123.41
117.07
106.83
39.92
39.78
39.64
39.50
39.36
39.22
39.08
30.48
27.34
HO
S
NH
S
O
kb-NB165-92
200
180
160
140
120
100
80
60
40
20
0
ppm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8.0230
8.0111
7.8757
7.8601
7.1490
7.1360
7.1319
3.8371
3.4848
3.4742
3.4674
3.4566
3.3335
3.2996
3.2936
3.2848
3.2756
2.5026
2.4998
2.4969
1.9136
1.9037
1.8954
1.8872
1.8771
O
S
NH
S
O
kb-NB184-02
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
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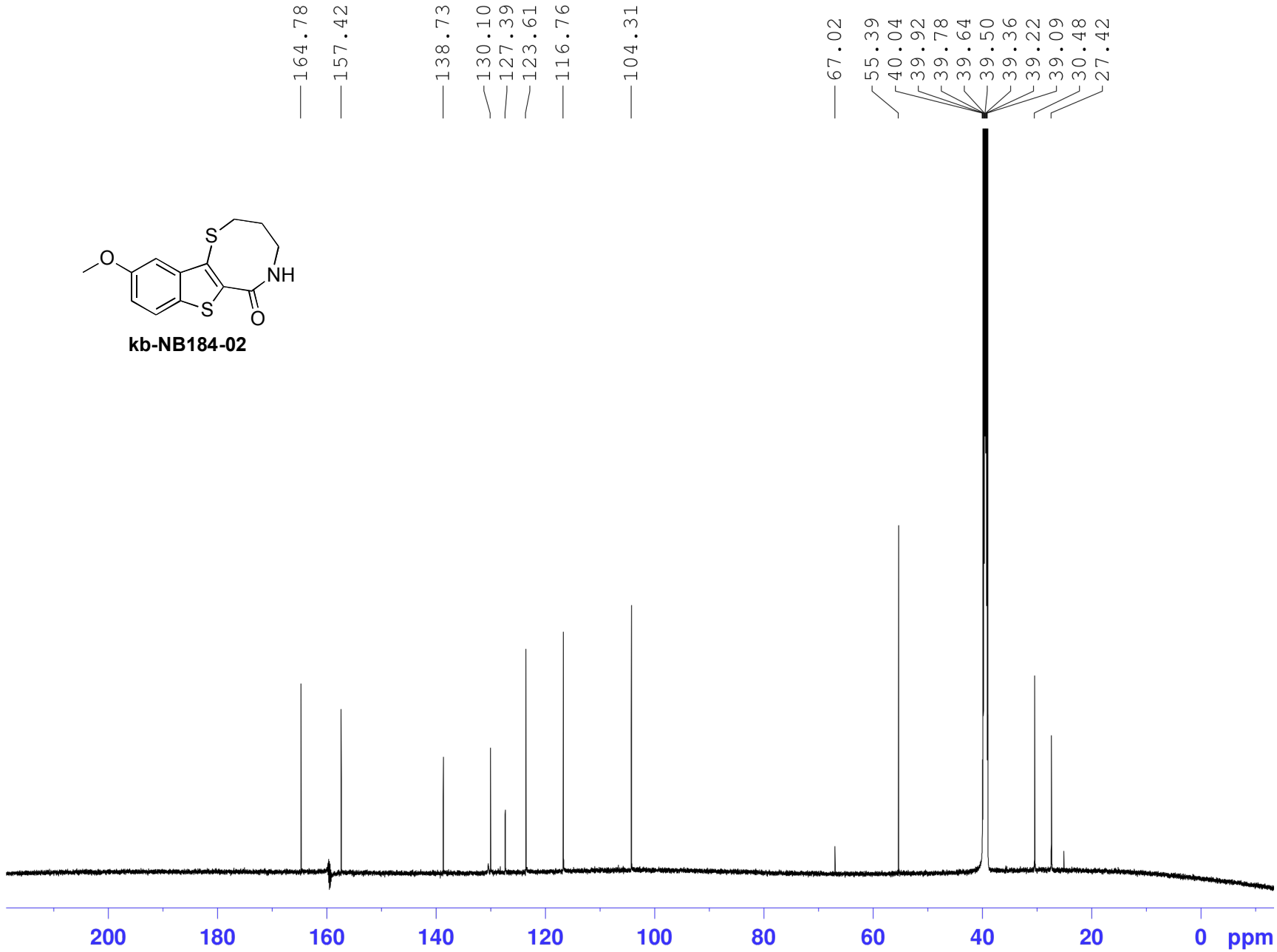
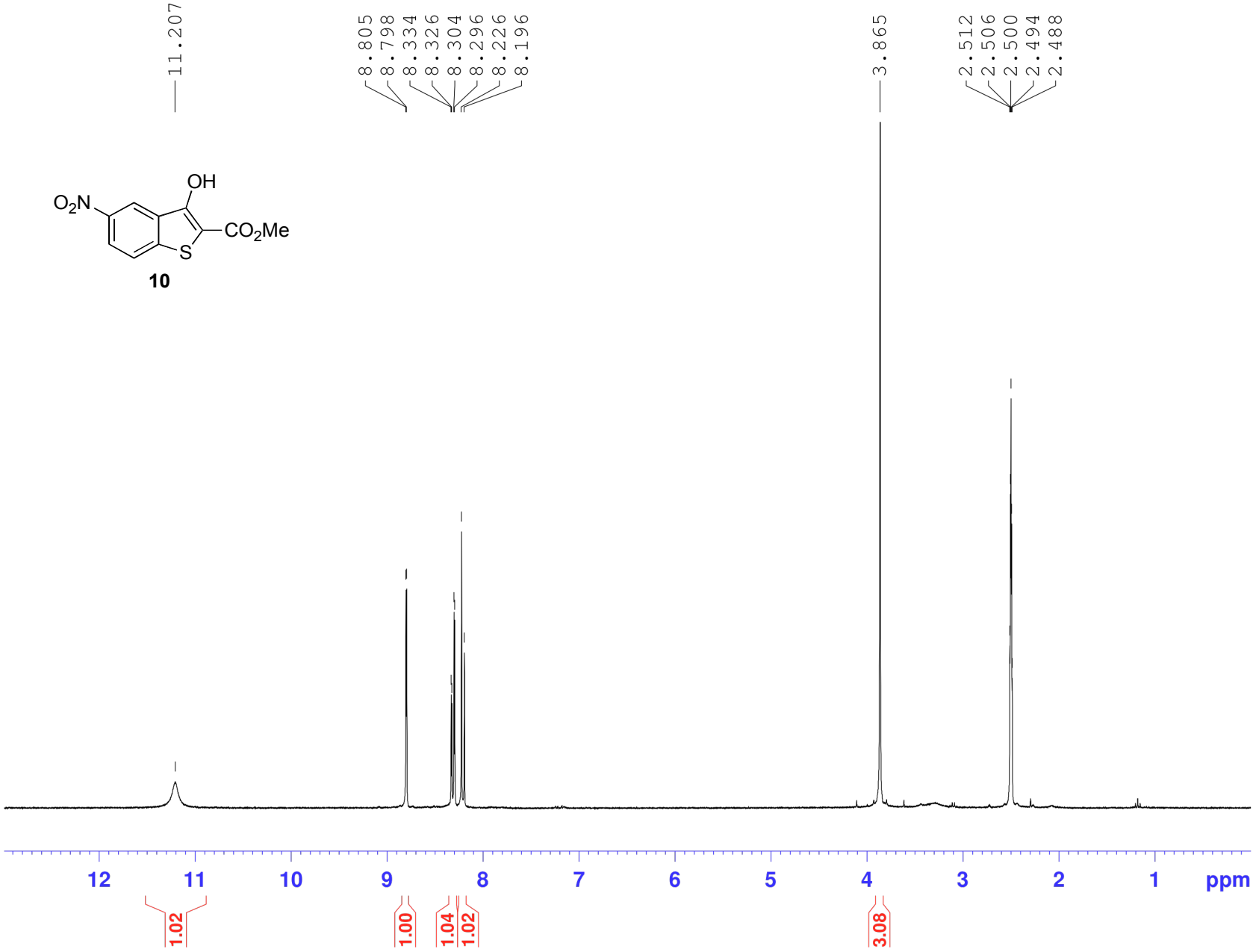
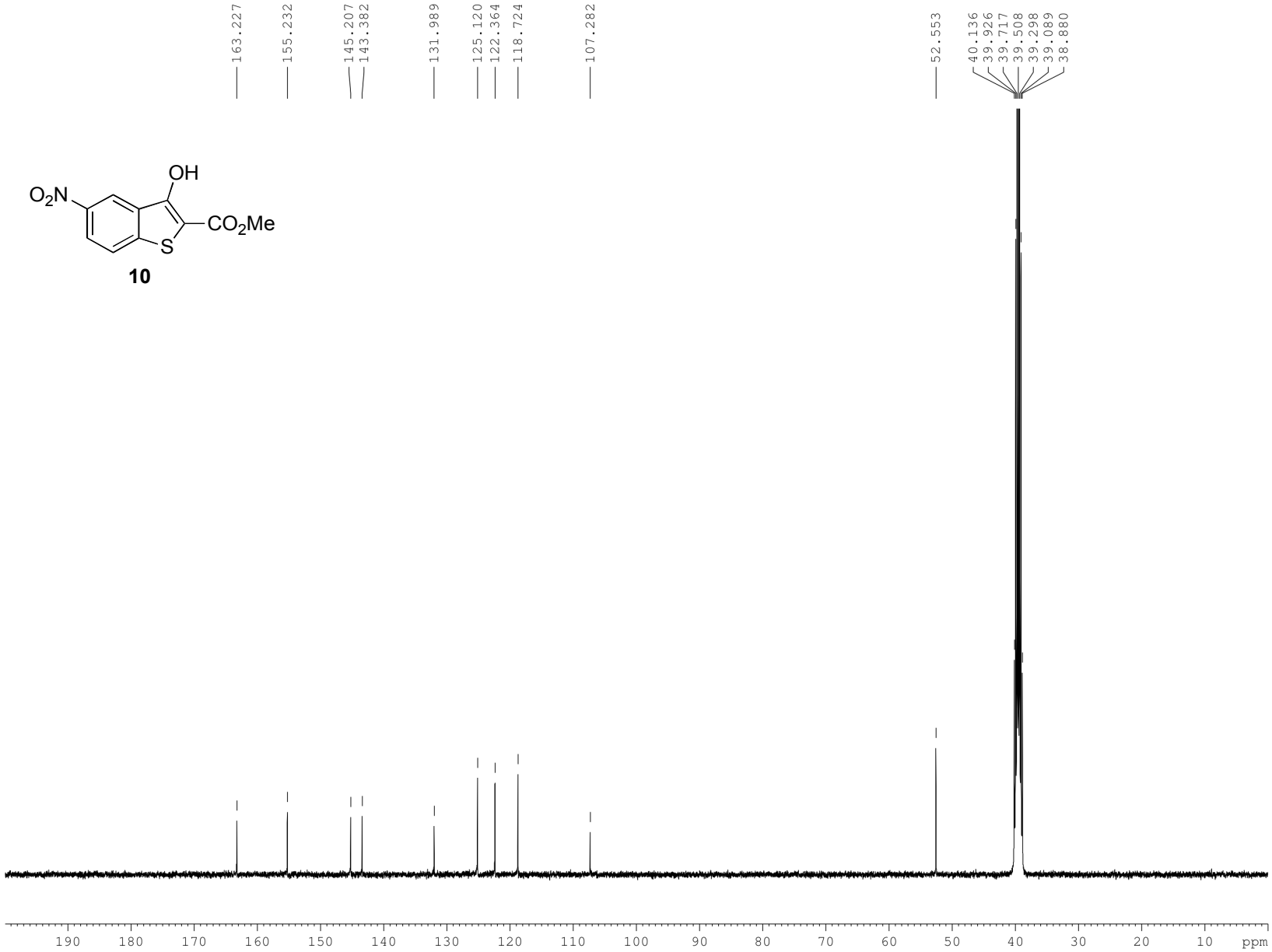
1.00
2.02
3.08
2.06
2.04
2.11


164.78
157.42
138.73
130.10
127.39
123.61
116.76
104.31
67.02
55.39
40.04
39.92
39.78
39.64
39.50
39.36
39.22
39.09
30.48
27.42
S
O
NH
S
O
kb-NB184-02
200
180
160
140
120
100
80
60
40
20
0
ppm


11.207
8.805
8.798
8.334
8.326
8.304
8.296
8.226
8.196
3.865
2.512
2.506
2.500
2.494
2.488
OH
O2N
CO2Me
S
10
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.02
1.00
1.04
1.02
3.08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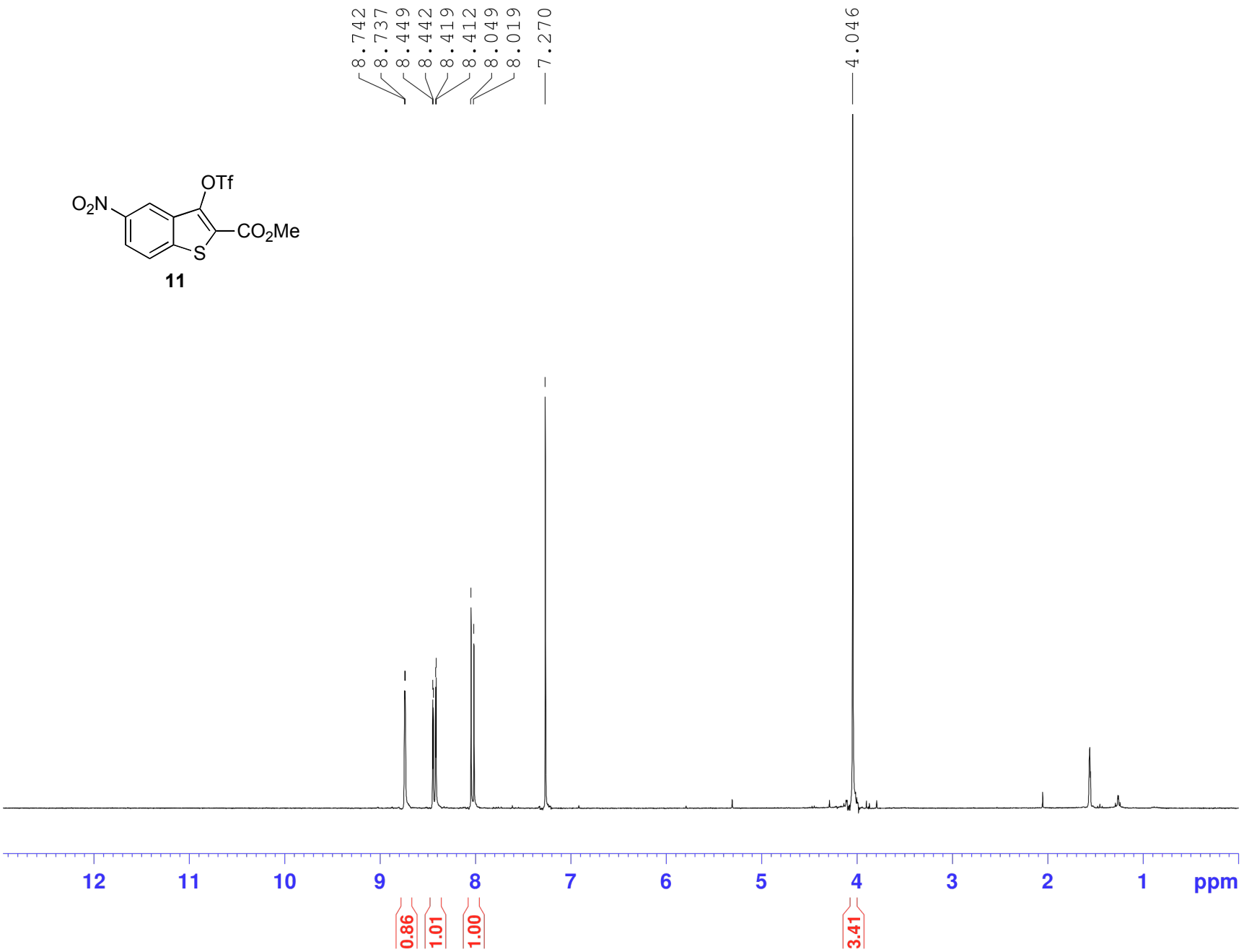
145.207
143.382
131.989
125.120
122.364
118.724
107.282
52.553
40.136
39.926
39.717
39.508
39.298
39.089
38.880
OH
O2N
CO2Me
S
10
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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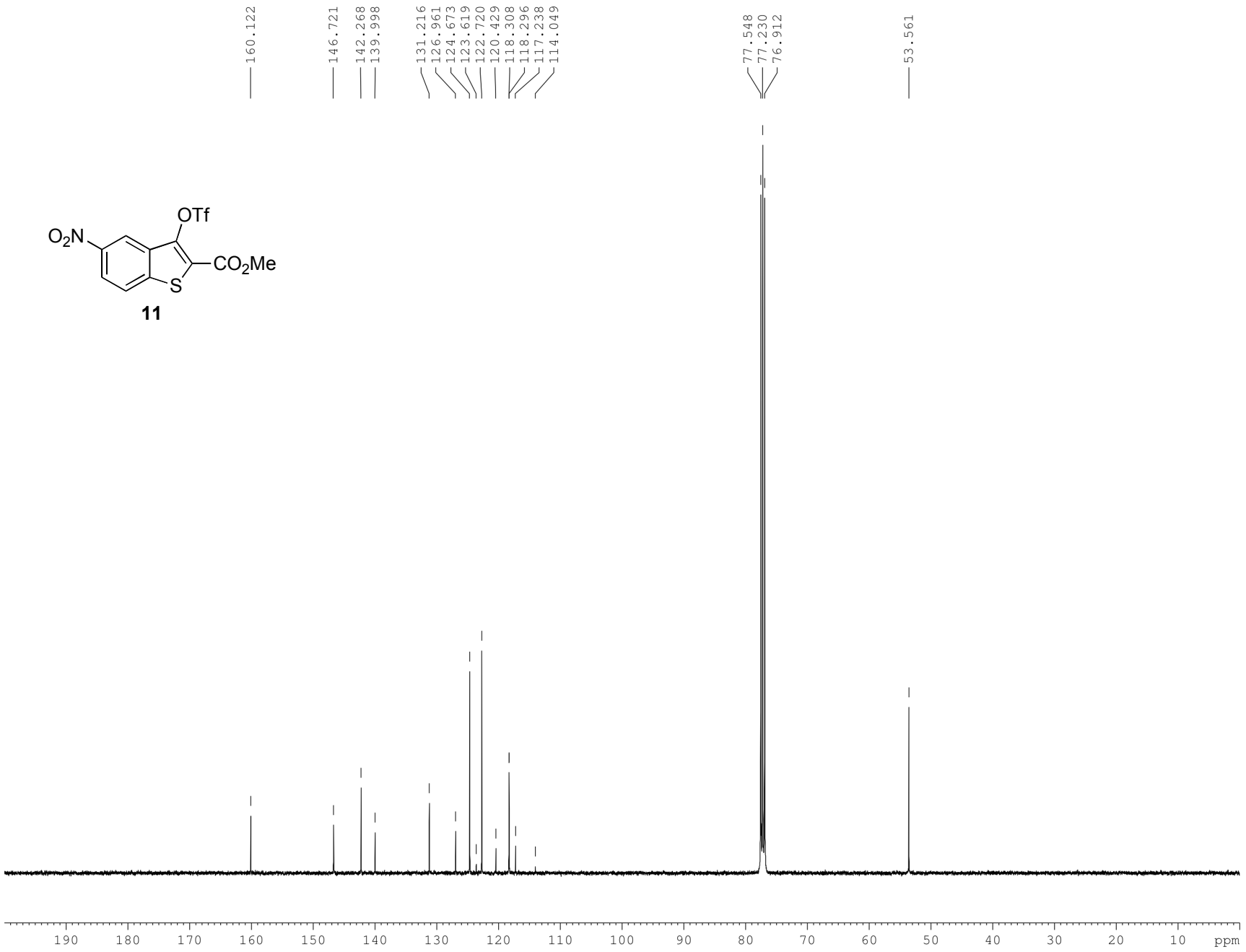
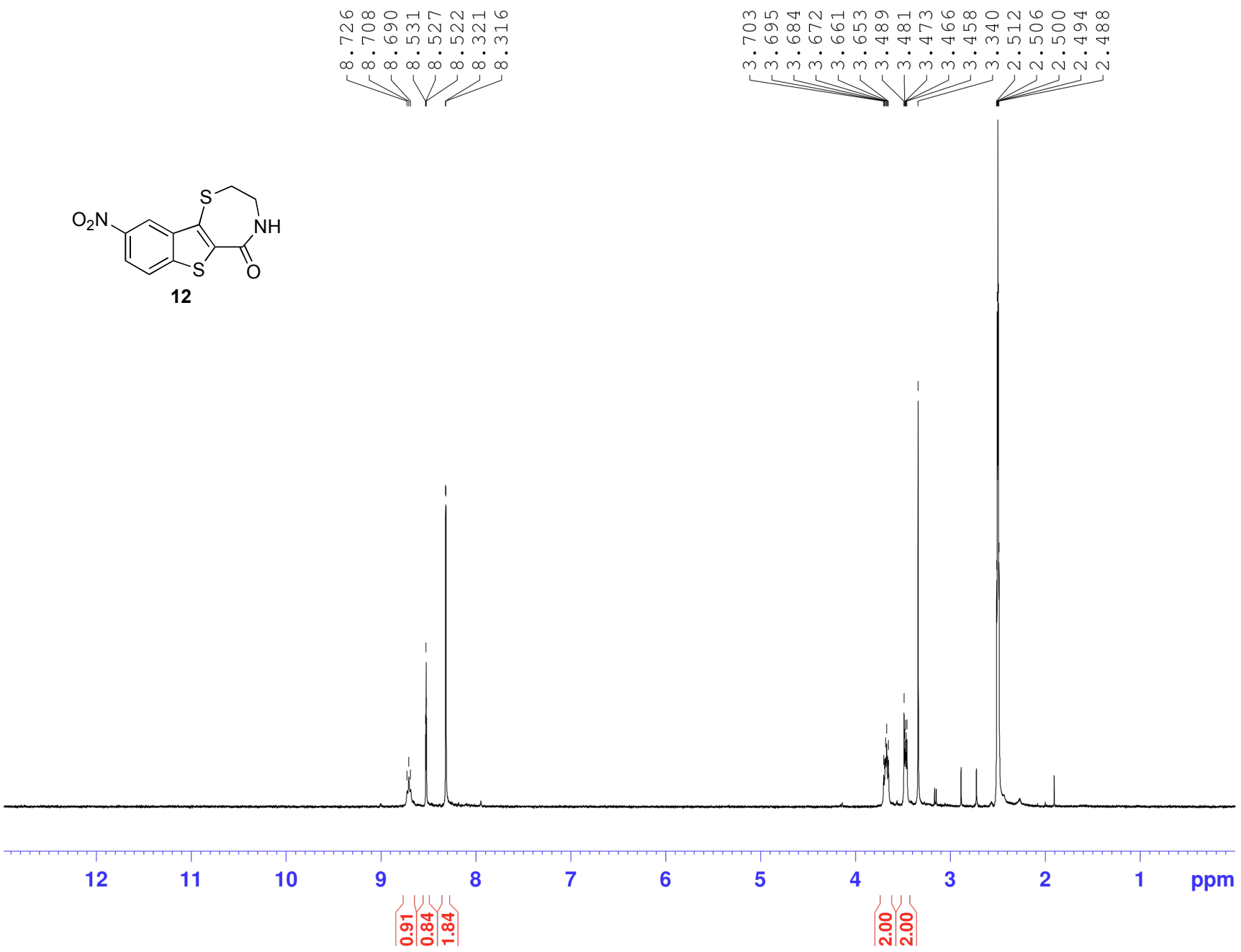
8.737
8.449
8.442
8.419
8.412
8.049
8.019
7.270
4.046
OTf
O_2N
CO_2Me
S
11
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.86
1.01
1.00
3.41


160.122
146.721
142.268
139.998
131.216
126.961
124.673
123.619
122.720
120.429
118.308
118.296
117.238
114.049
77.548
77.230
76.912
53.561
OTf
O2N
CO2Me
S
11
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


8.726
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8.531
8.527
8.522
8.321
8.316
3.703
3.695
3.684
3.672
3.661
3.653
3.489
3.481
3.473
3.466
3.458
3.340
2.512
2.506
2.500
2.494
2.488
O2N
S
NH
O
S
12
12
11
10
9
8
7
6
5
4
3
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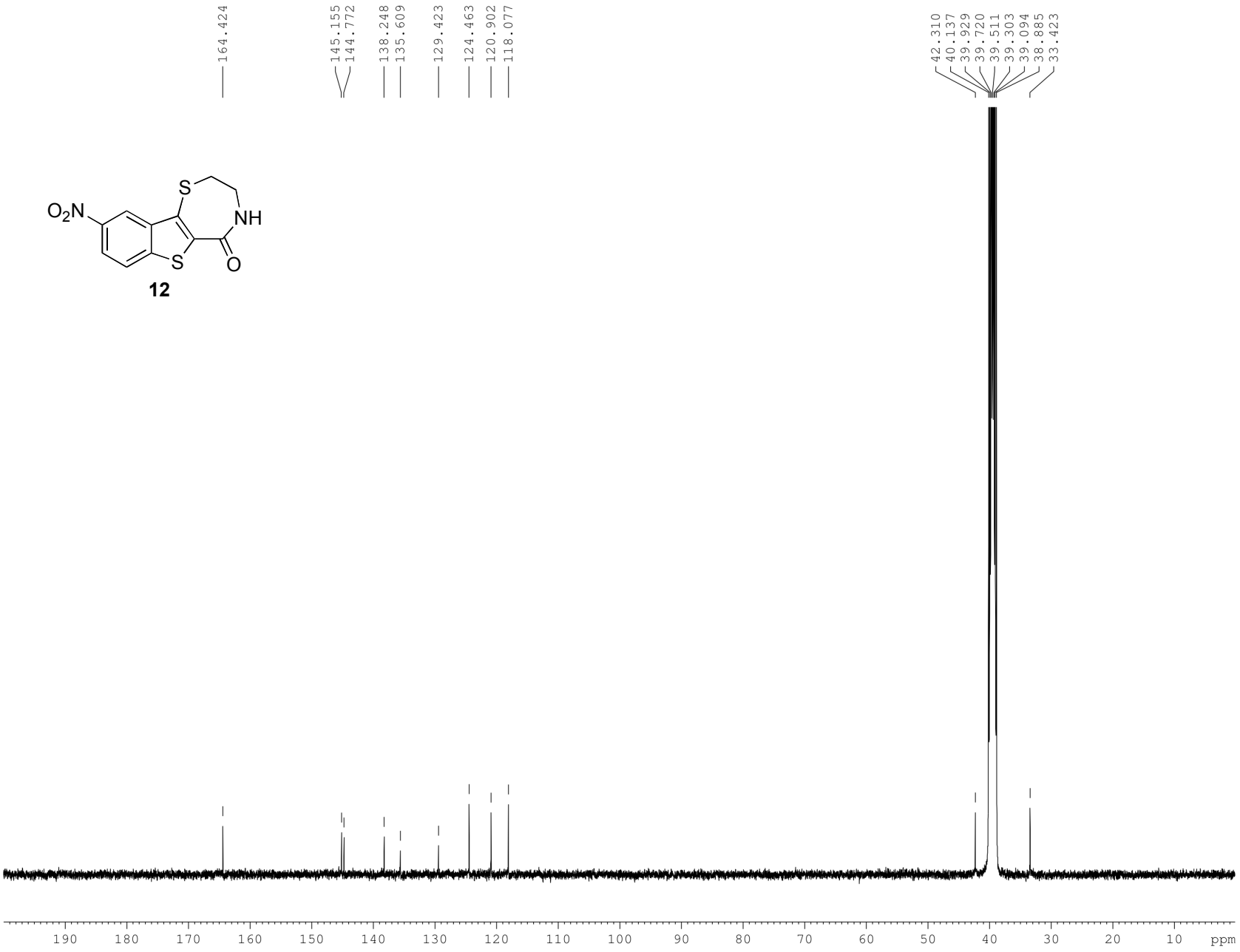
ppm
0.91
0.84
1.84
2.00
2.00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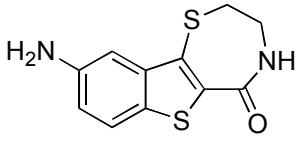
145.155
144.772
138.248
135.609
129.423
124.463
120.902
118.077
42.310
40.137
39.929
39.720
39.511
39.303
39.094
38.885
33.423
O2N
S
NH
O
S
12
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


H2N
S
NH
S
O

13

7.593
7.592
7.571
7.570
7.152
7.151
7.146
7.145
6.997
6.992
6.975
6.970
4.876
3.751
3.744
3.739
3.733
3.727
3.405
3.398
3.393
3.388
3.381
3.318
3.314
3.310
3.306
3.302
12
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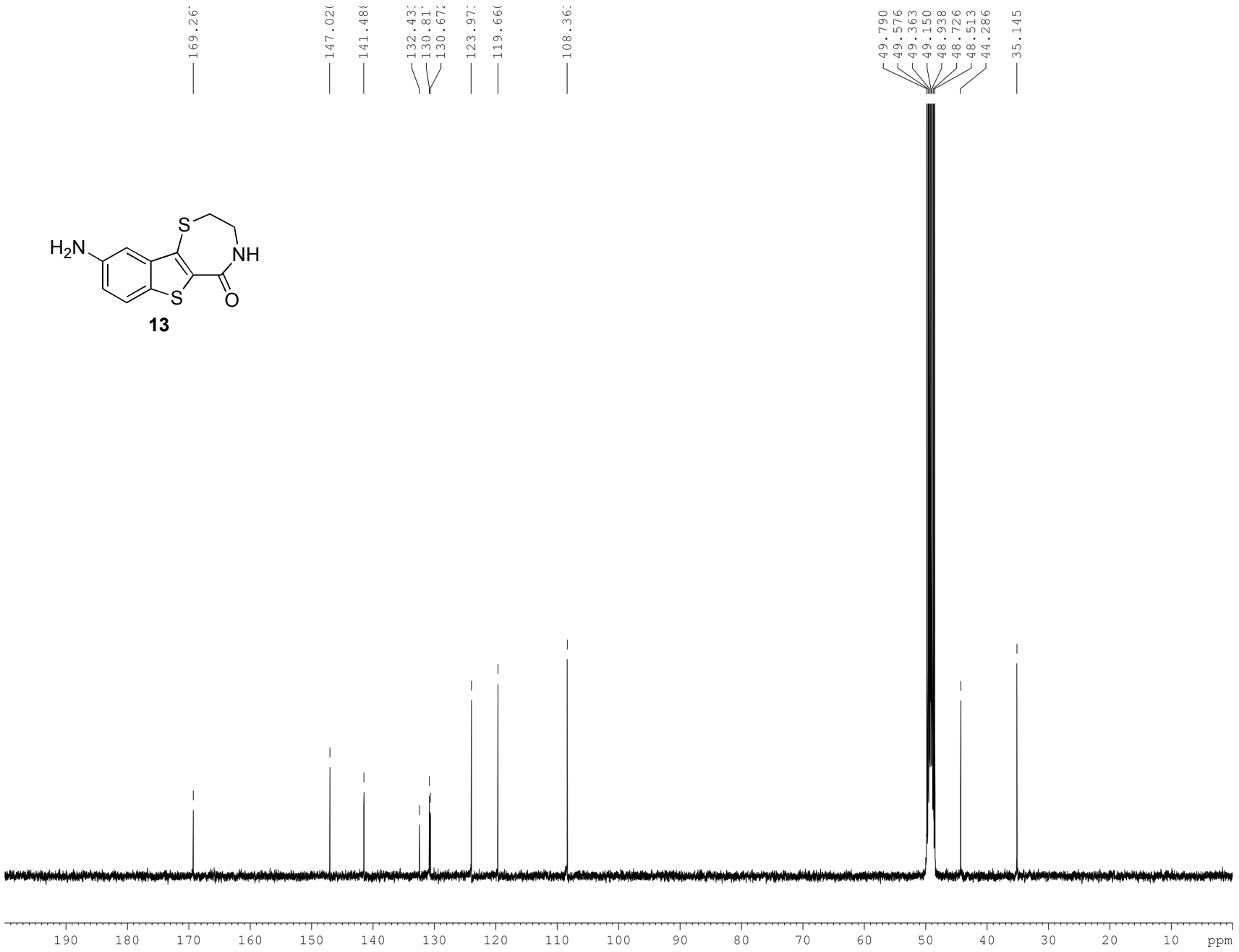
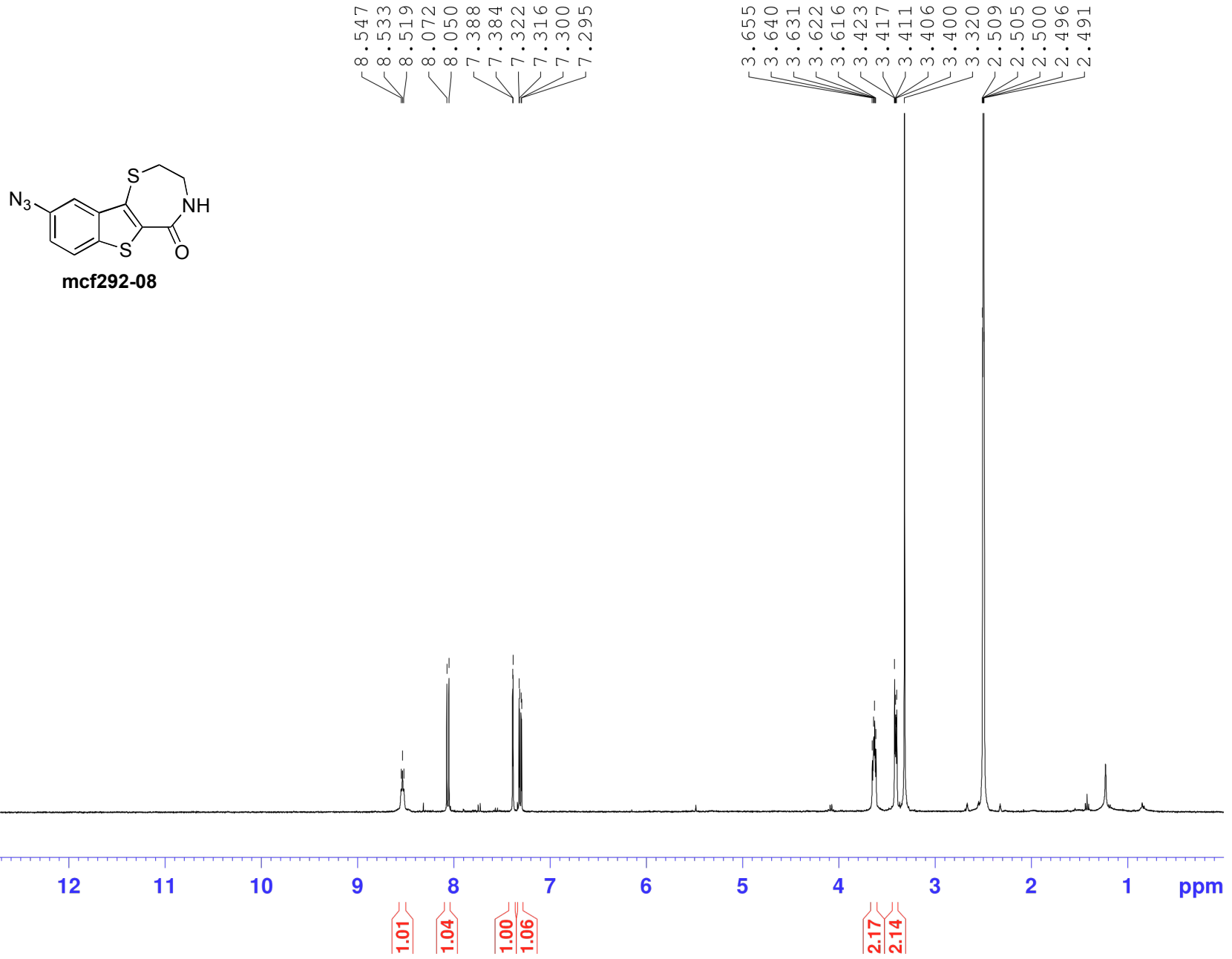
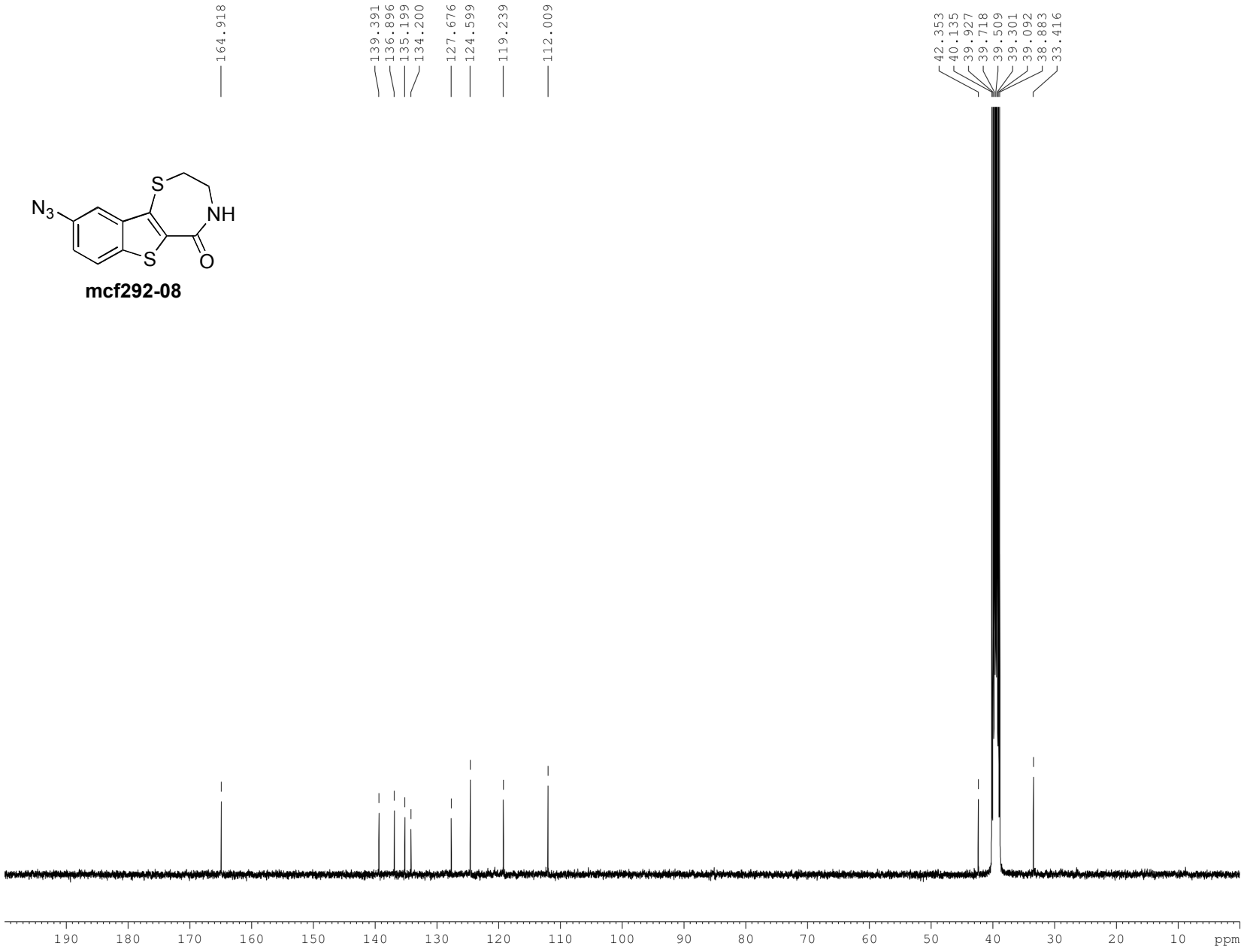
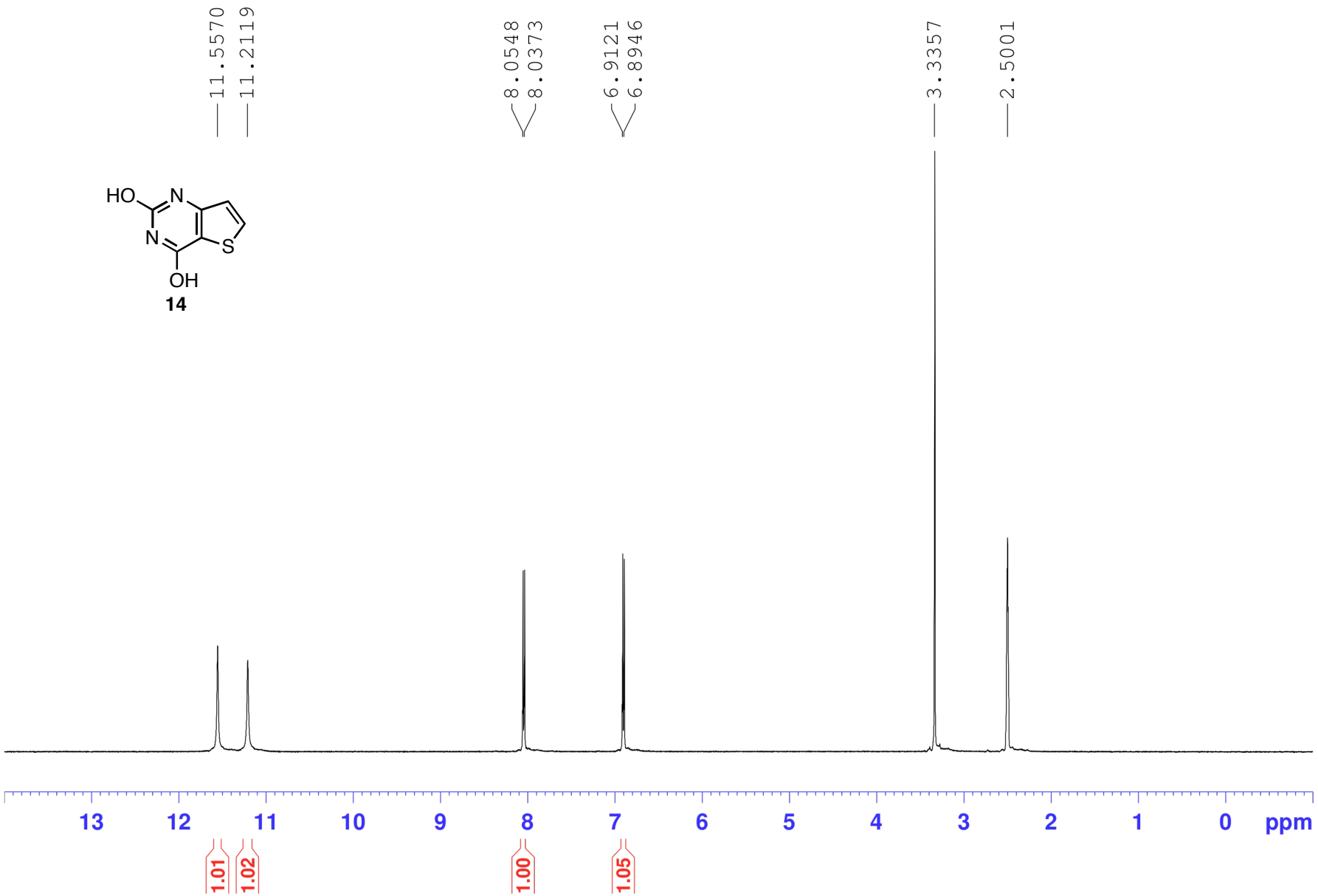
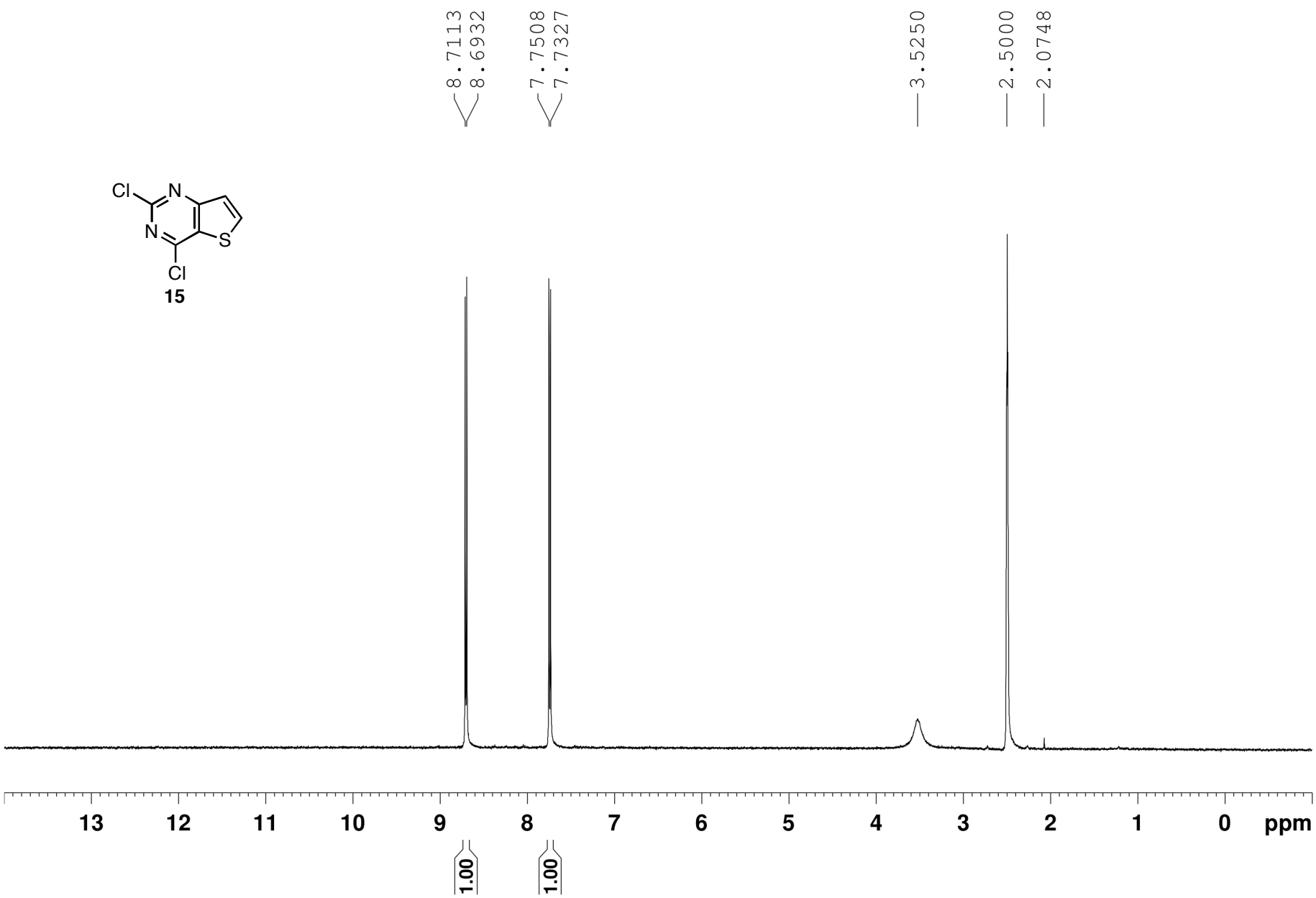
ppm
1.00
0.94
1.00
2.10
2.15

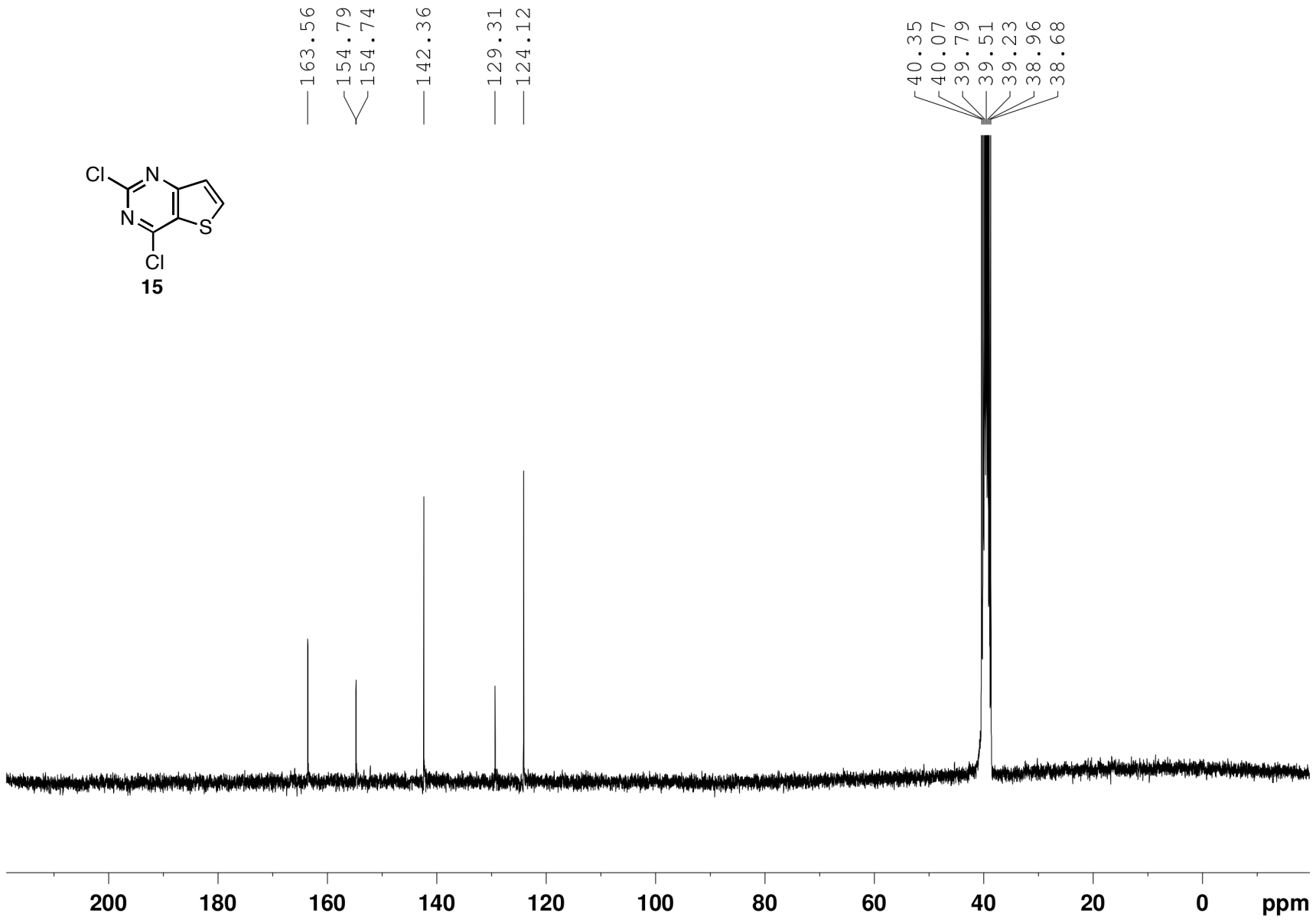

169.26
147.02
141.48
132.43
130.81
130.67
123.97
119.66
108.36
49.790
49.576
49.363
49.150
48.938
48.726
48.513
44.286
35.145
S
NH
O
S
H2N
13
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


8.547
8.533
8.519
8.072
8.050
7.388
7.384
7.322
7.316
7.300
7.295
3.655
3.640
3.631
3.622
3.616
3.423
3.417
3.411
3.406
3.400
3.320
2.509
2.505
2.500
2.496
2.491
N3
S
NH
O
S
mcf292-08
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.01
1.04
1.00
1.06
2.17
2.14

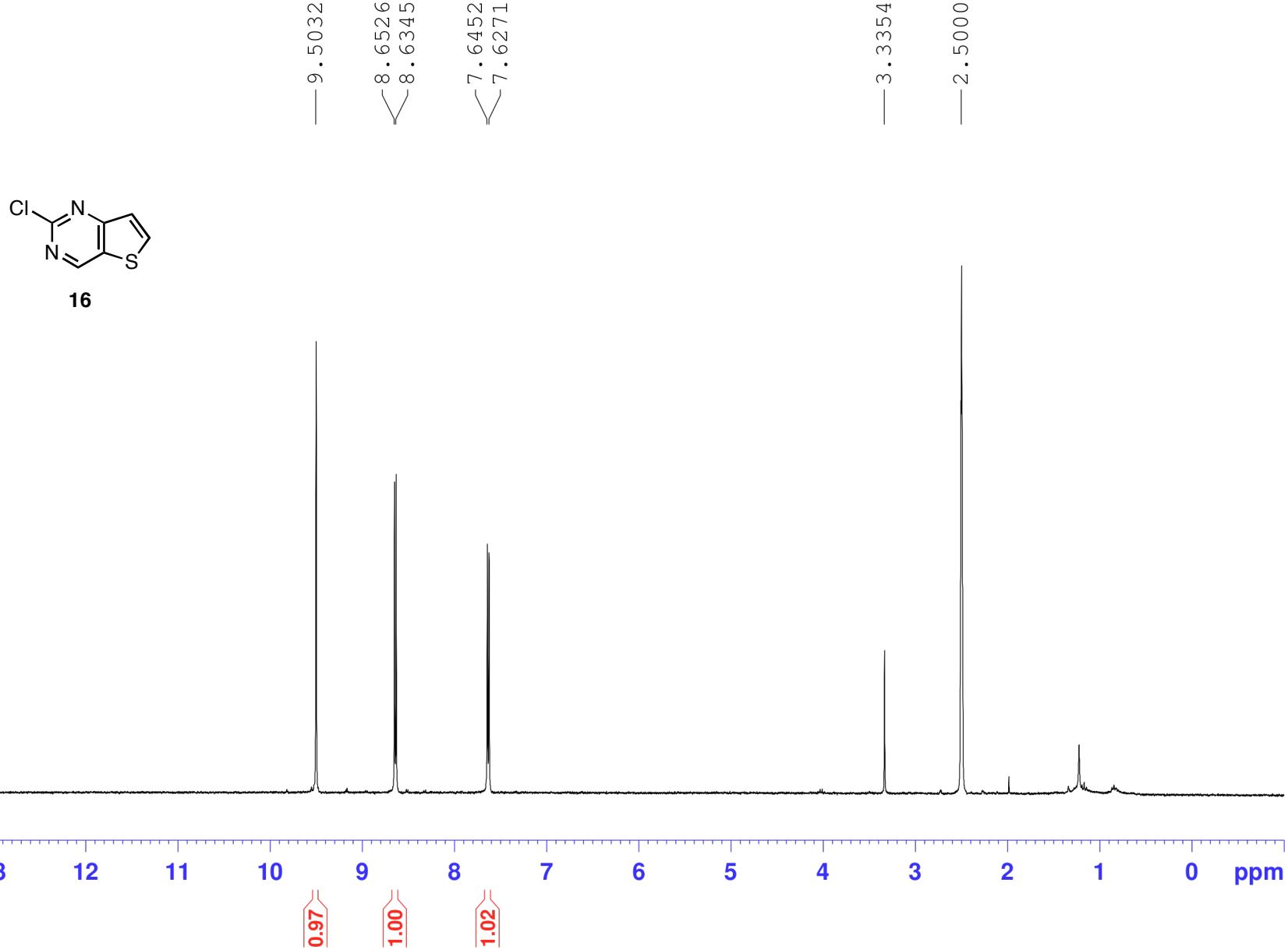

164.918
139.391
136.896
135.199
134.200
127.676
124.599
119.239
112.009
42.353
40.135
39.927
39.718
39.509
39.301
39.092
38.883
33.416
N3
S
NH
O
S
mcf292-08
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

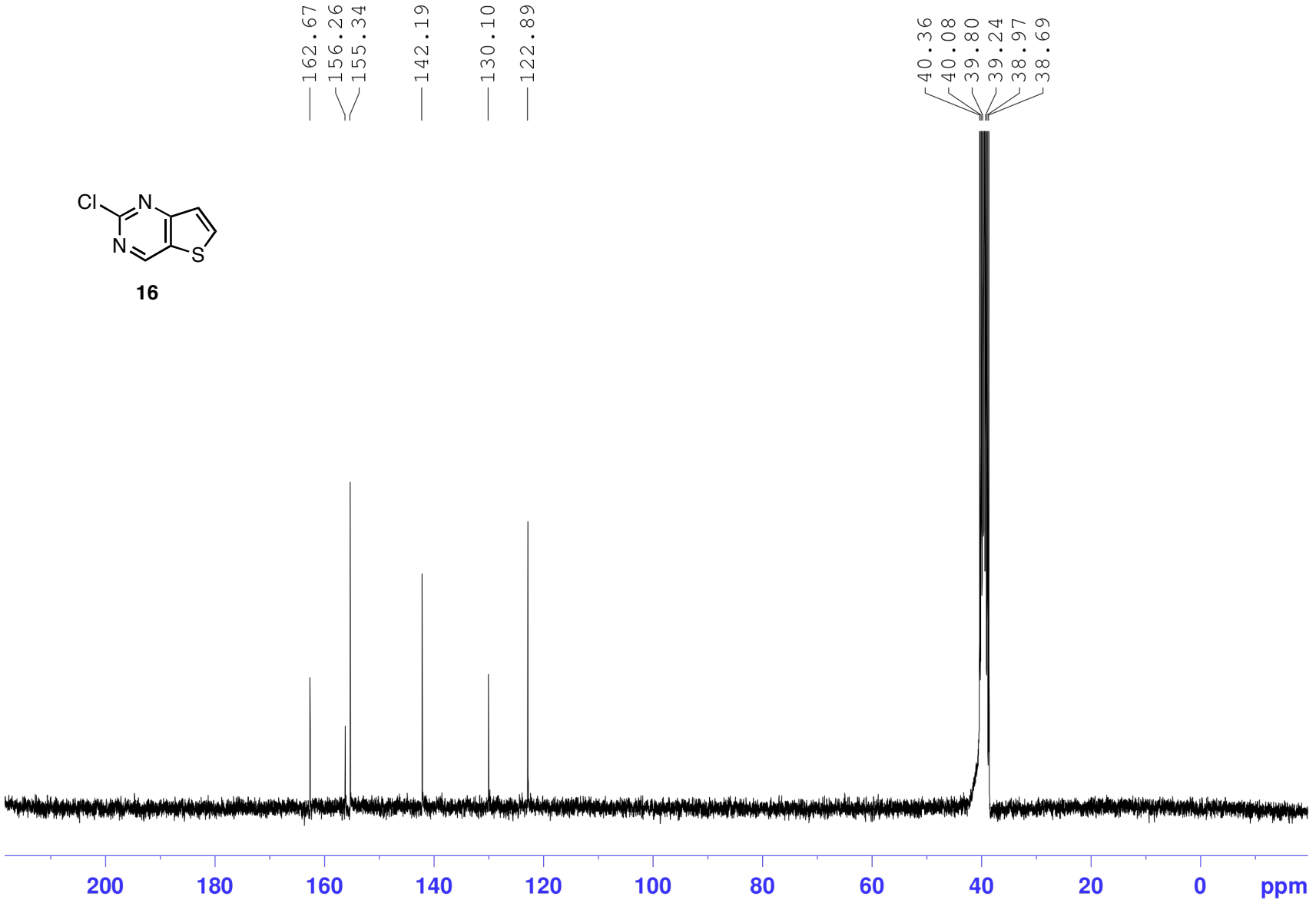

11.5570
11.2119
8.0548
8.0373
6.9121
6.8946
3.3357
2.5001
HO
N
N
S
OH
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.01
1.02
1.00
1.05


8.7113
8.6932
7.7508
7.7327
3.5250
2.5000
2.0748
Cl
N
N
S
Cl
15
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
1.00

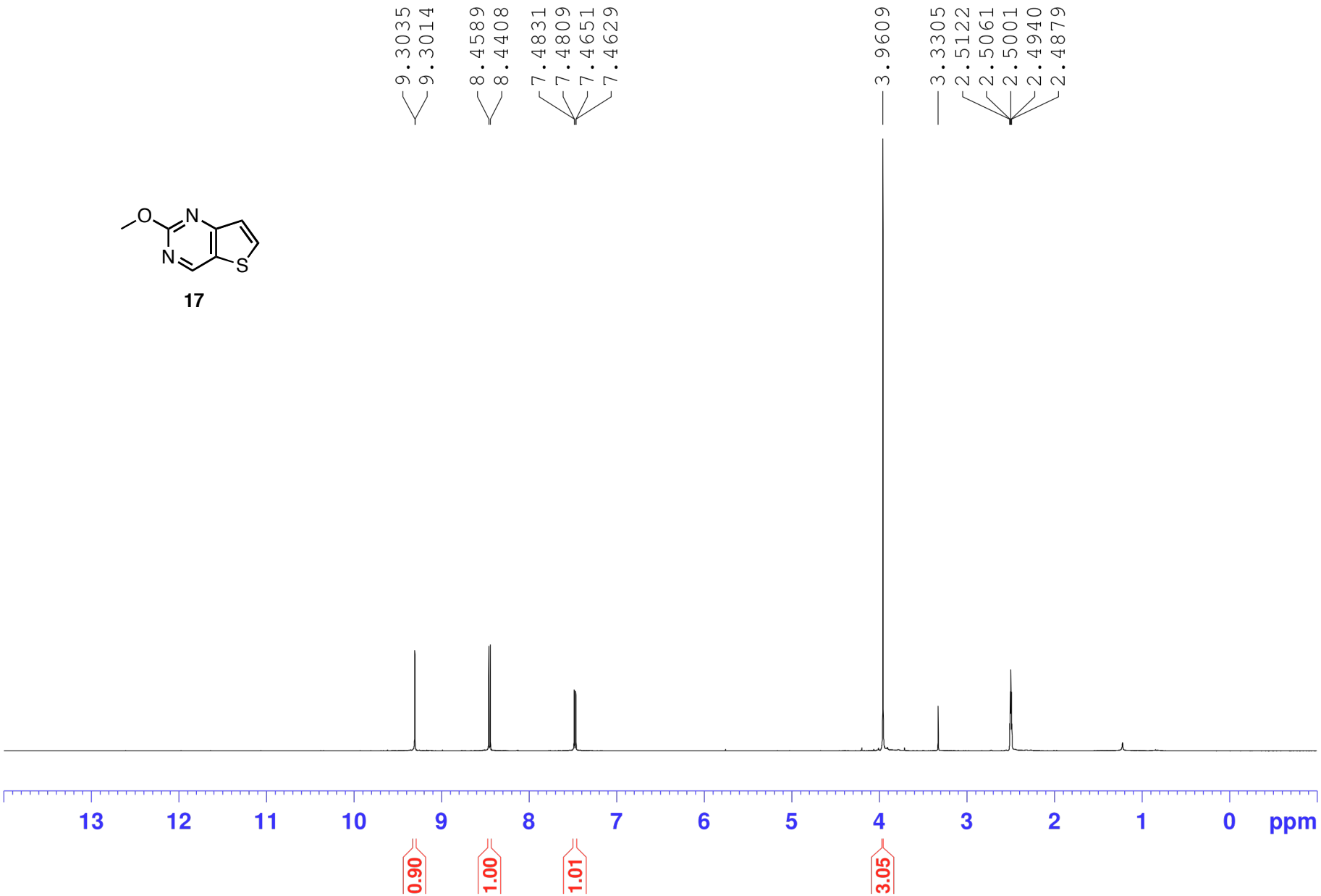

163.56
154.79
154.74
142.36
129.31
124.12
40.35
40.07
39.79
39.51
39.23
38.96
38.68
Cl
N
N
S
Cl
200
180
160
140
120
100
80
60
40
20
0
ppm

15

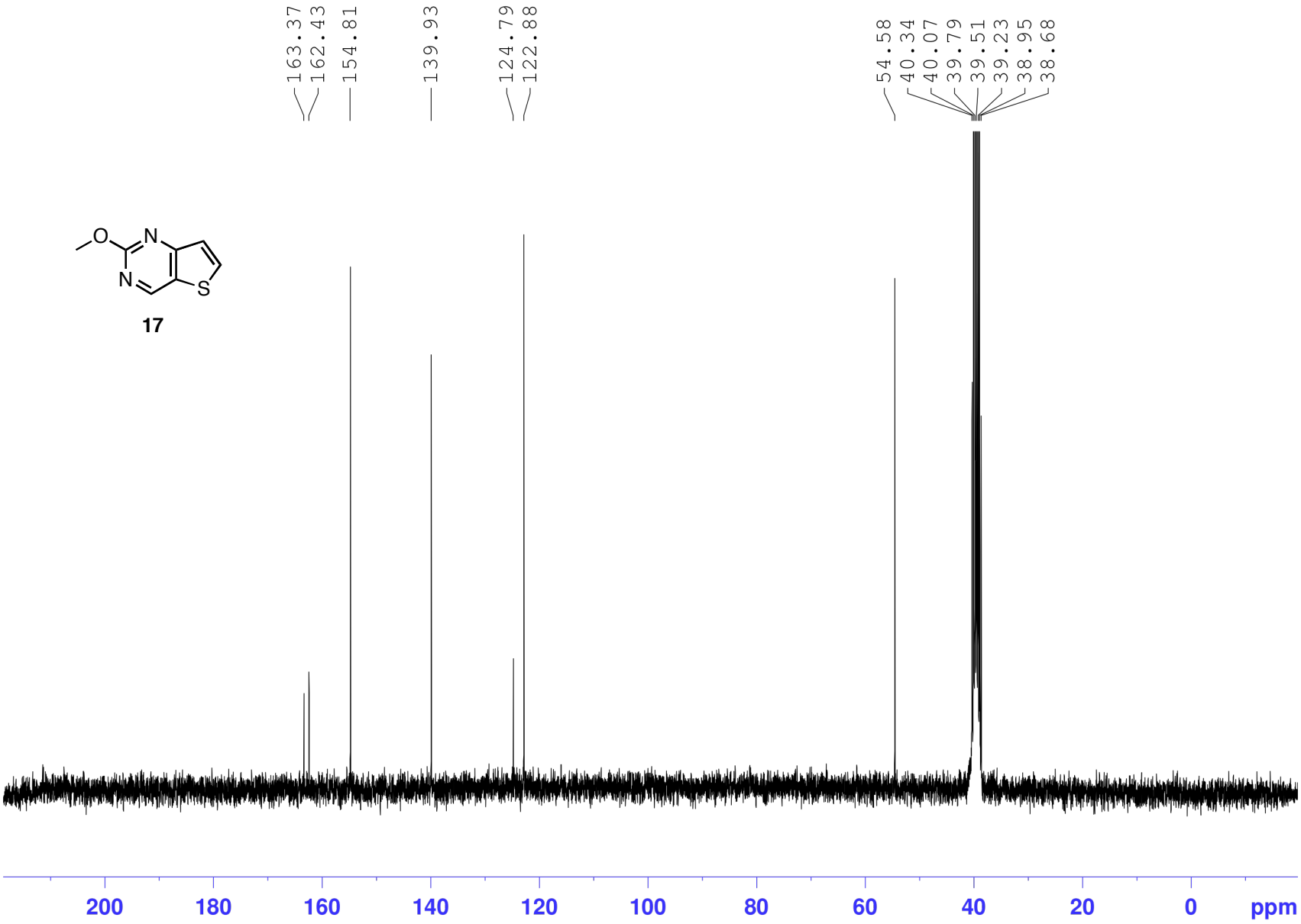

9.5032
8.6526
8.6345
7.6452
7.6271
3.3354
2.5000
Cl
N
N
S
16
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.97
1.00
1.02

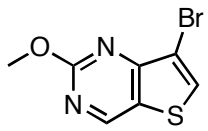

162.67
156.26
155.34
142.19
130.10
122.89
40.36
40.08
39.80
39.24
38.97
38.69
Cl
N
N
S
200
180
160
140
120
100
80
60
40
20
0
ppm

16


9.3035
9.3014
8.4589
8.4408
7.4831
7.4809
7.4651
7.4629
3.9609
3.3305
2.5122
2.5061
2.5001
2.4940
2.4879
O
N
N
S
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.90
1.00
1.01
3.05

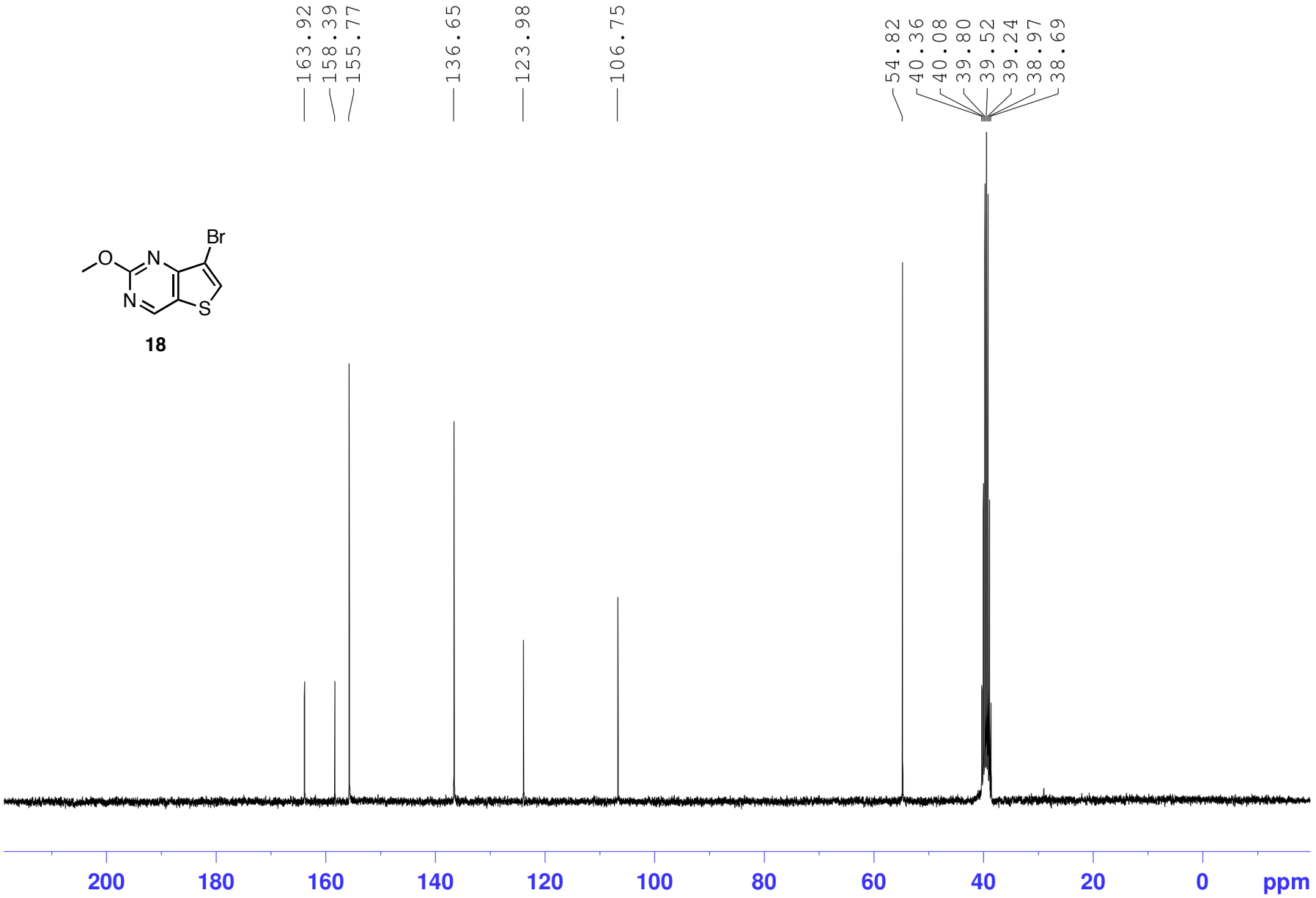
17

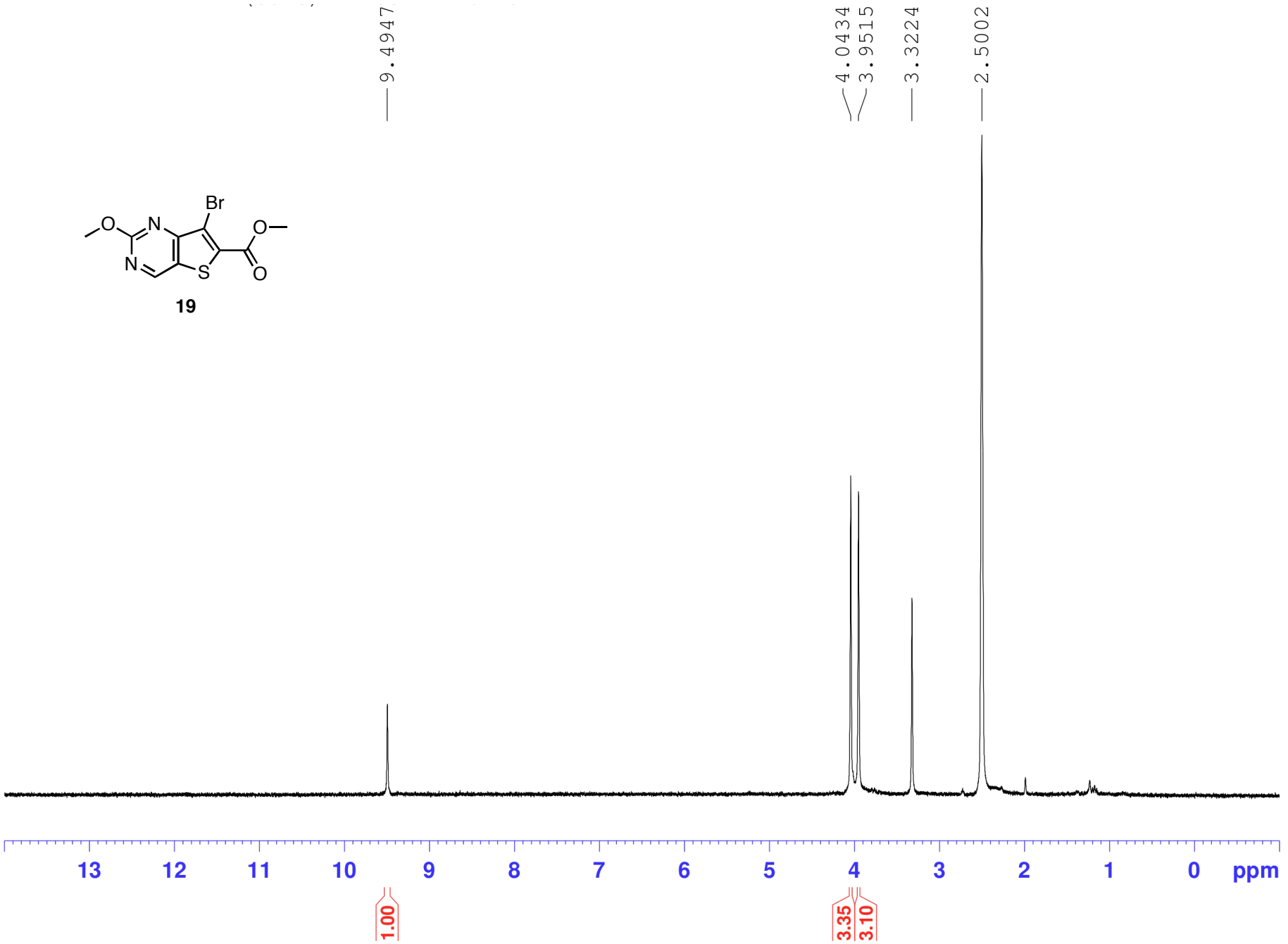

163.37
162.43
154.81
139.93
124.79
122.88
54.58
40.34
40.07
39.79
39.51
39.23
38.95
38.68
O
N
N
S
17
200
180
160
140
120
100
80
60
40
20
0
ppm


Br
O
N
N
S

18

9.3355
8.6097
4.0075
3.3507
2.4999
1.1731
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.98
3.05


163.92
158.39
155.77
136.65
123.98
106.75
54.82
40.36
40.08
39.80
39.52
39.24
38.97
38.69
Br
O
N
N
S
18
200
180
160
140
120
100
80
60
40
20
0
ppm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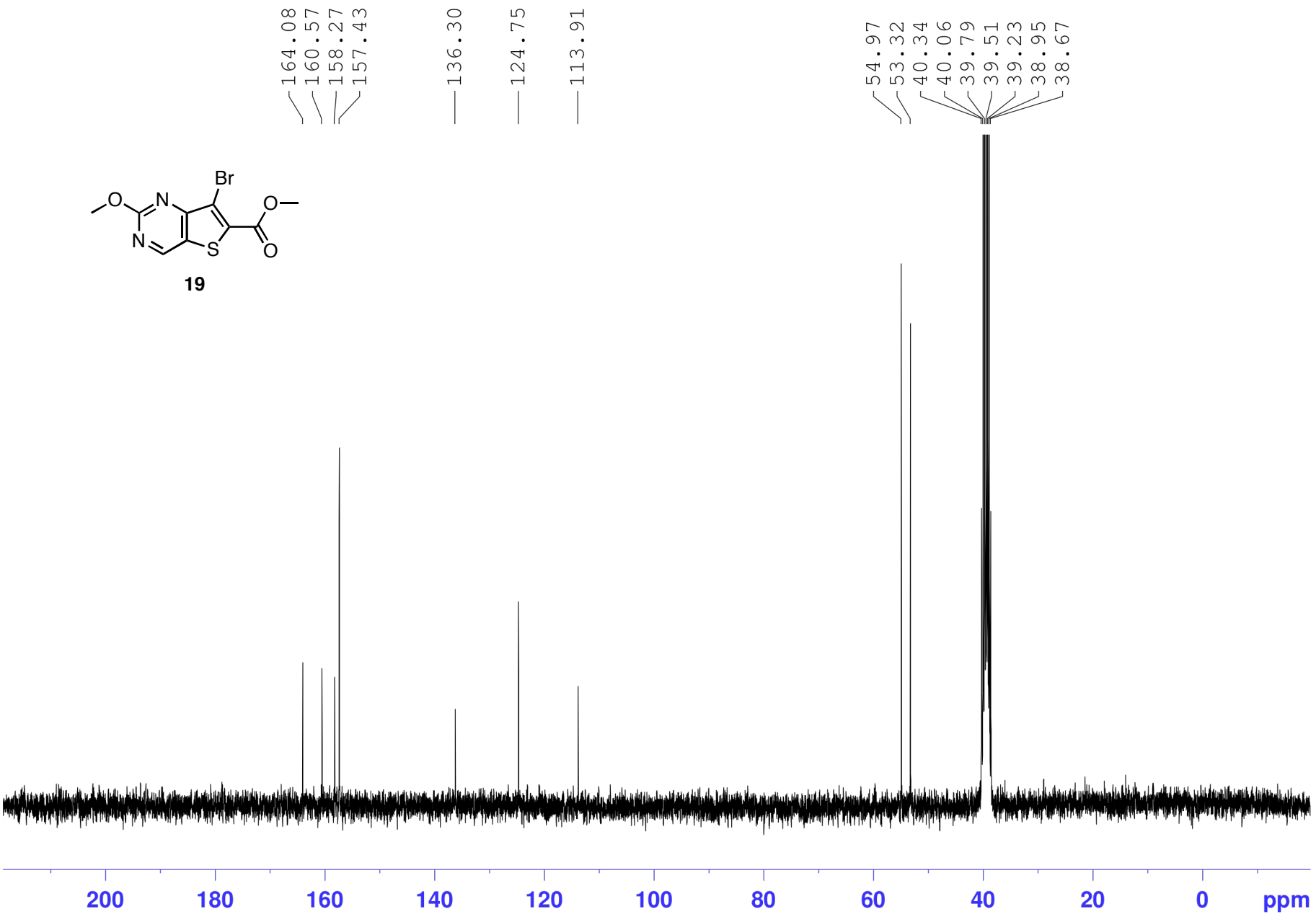
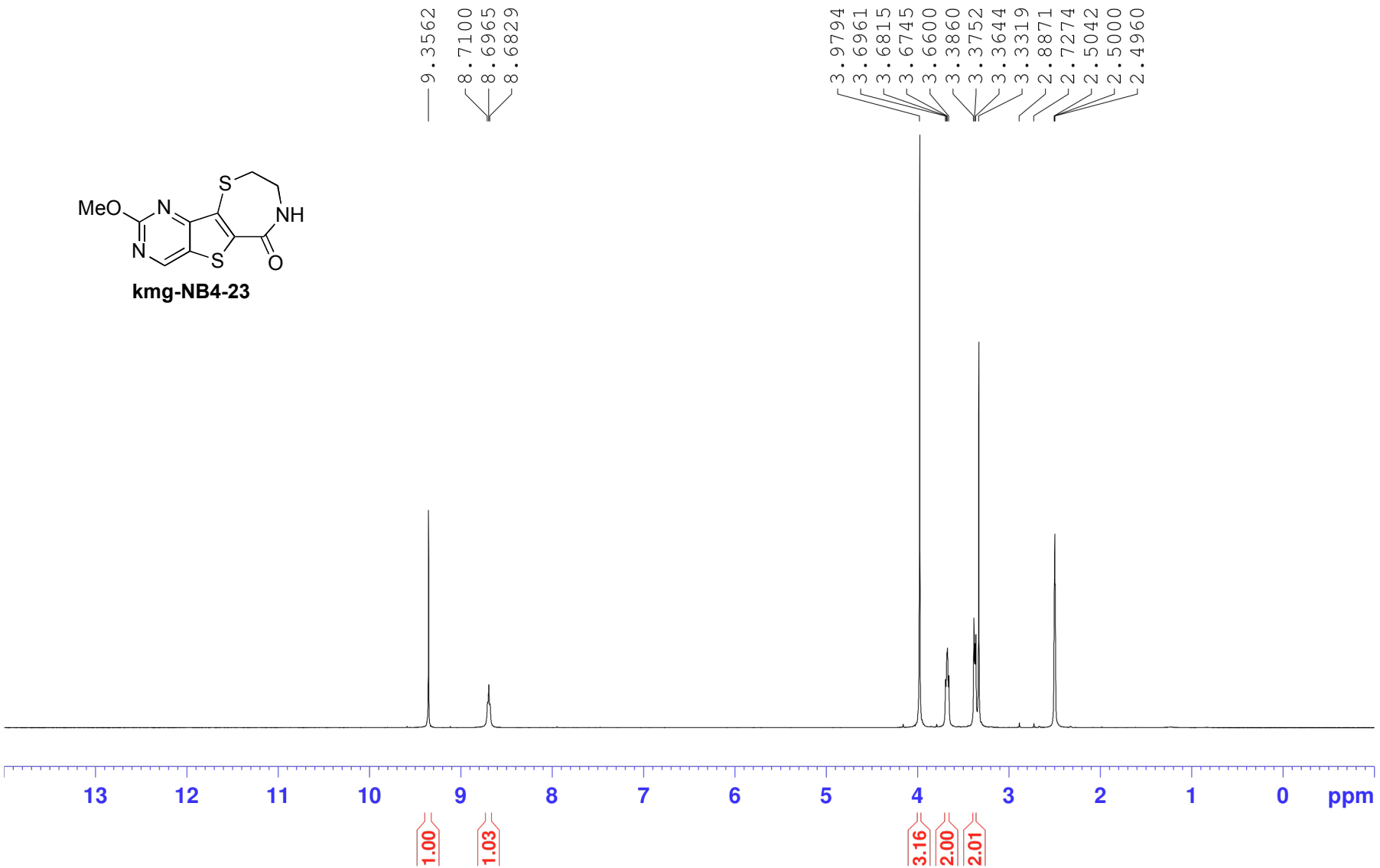
4.0434
3.9515
3.3224
2.5002
Br
O
N
N
S
O
O
19
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
3.35
3.10


164.08
160.57
158.27
157.43
136.30
124.75
113.91
54.97
53.32
40.34
40.06
39.79
39.51
39.23
38.95
38.67
Br
O
N
N
S
O
O
200
180
160
140
120
100
80
60
40
20
0
ppm

19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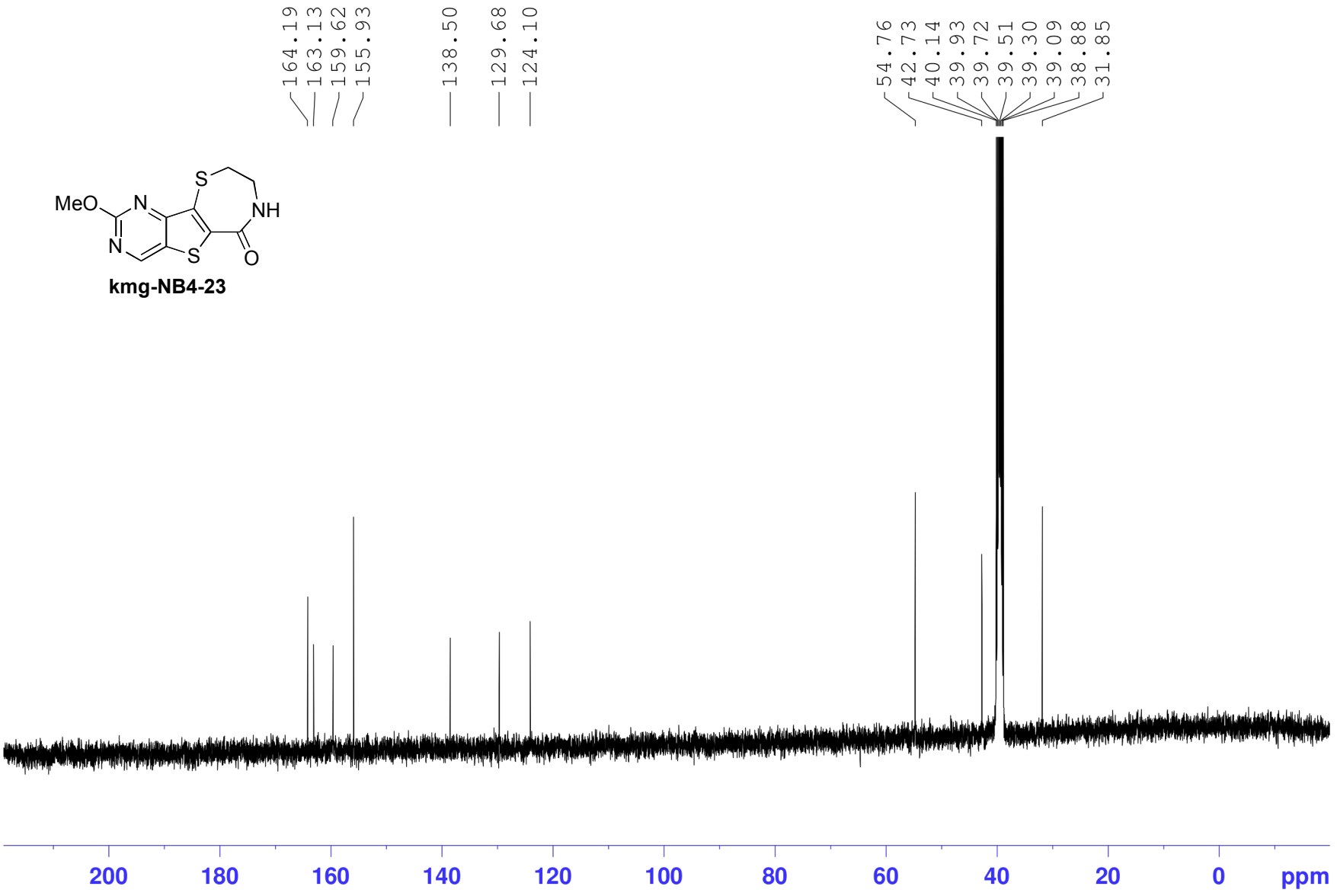
8.7100
8.6965
8.6829
3.9794
3.6961
3.6815
3.6745
3.6600
3.3860
3.3752
3.3644
3.3319
2.8871
2.7274
2.5042
2.5000
2.4960
MeO
N
N
S
S
NH
O
kmg-NB4-23
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
1.03
3.16
2.00
2.01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163.13
159.62
155.93
138.50
129.68
124.10
54.76
42.73
40.14
39.93
39.72
39.51
39.30
39.09
38.88
31.85
MeO
N
N
S
S
NH
O
kmg-NB4-23
200
180
160
140
120
100
80
60
40
20
0
ppm