Small Molecule Inhibitors of the Bacterial Cell Division Protein FtsZ: Critical Analyses and Cross-Species Comparisons

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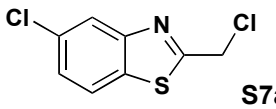
Supporting Information

Materials: Unless otherwise specified, all commercially available reagents were used as received. All reactions using dried solvents were carried out under an atmosphere of argon in flame-dried glassware with magnetic stirring. Dry solvent was dispensed from a solvent purification system that passes solvent through two columns of dry neutral alumina.

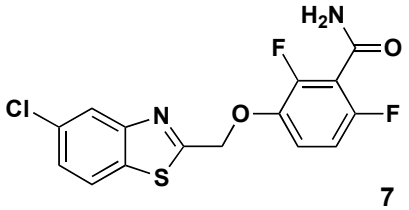
Instrumentation: ^{1}H NMR spectra and proton-decoupled ^{13}C NMR spectra were obtained on a 300 or 400 MHz Varian NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual solvent ($CHCl_3$, s, δ 7.26). Multiplicities are given as: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), m (multiplet), br m (broad multiplet), br s (broad singlet). ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ (t, δ 77.0) unless otherwise noted. High resonance mass spectra were recorded on positive ESI mode in methanol or acetonitrile. Melting points were taken on an EZ-melting apparatus and were uncorrected. Infrared spectra were taken on a Bruker Tensor 27 spectrometer. Gas chromatography-mass spectrometry data was recorded on a GCMS-QP 2010 Shimadzu spectrometer. Silica gel chromatographic purifications were performed by flash chromatography with silica gel (Silicycle, 40–63 μm) packed in glass columns. The eluting solvent for each purification was determined by thin layer chromatography (TLC) on glass plates coated with EMD silica gel 50 F254 and visualized by ultraviolet light. The following abbreviations are used throughout: ethyl acetate (EtOAc), hexanes (Hex), dichloromethane (DCM), triethylamine (TEA), acetonitrile (ACN).

Experimental:
i) Synthesis of 8J (7)



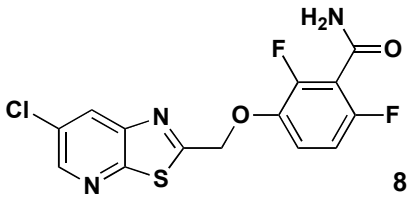
S7a

5-chloro-2-(chloromethyl)-1,3-benzothiazole (S7a). 2-amino-4-chlorobenzenethiol (1.0 g, 6.3 mmol) was dissolved in anhydrous MeOH (20 mL), to this solution 2-chloro-1,1,1-triethoxyethane (1.2 mL, 6.2 mmol) was added and allowed to reflux for 12 h. The solvent was reduced under vacuum and the crude material was purified by flash chromatography (5-50% EtOAc:Hex) yielding a yellow amorphous solid (0.68 g, 50%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, J = 8.6, 1H), 8.12 (d, J = 2.0, 1H), 7.55 (dd, J = 8.6, 2.1, 1H), 5.25 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.2, 153.9, 134.8, 132.0, 126.6, 124.8, 123.1, 42.5; IR (neat) 1585, 1546 cm^{-1}; R_f = 0.60 in 10% EtOAc:Hex.



7

3-[(5-Chloro-1,3-benzothiazol-2-yl)methoxy]-2,6-difluorobenzamide (7). The compound was prepared according to the reported procedure[1] and the NMR spectrum was in accordance with the reported values: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.19 – 8.15 (m, 2H), 8.10 (d, J = 2.1, 1H), 7.87 (br s, 1H), 7.51 (dd, J = 2.1, 8.6 Hz, 1H), 7.37 (m, 1H), 7.09 (t, J = 8.9 Hz, 1H), 5.68 (2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.1, 161.1, 153.4, 153.3 (dd, J = 242.42, 6.5), 148.8 (dd, J = 240.22, 8.7), 141.96-141.86 (dd, J = 3.13, 2.95), 133.2, 131.2, 125.6, 124.0, 122.2, , 116.97-116.67 (dd, J = 20.19, 4.6), 116.3 (d, J = 9.3), 111.2-111.0 (dd, J = 3.13, 3.83), 68.6; IR (neat) 3369, 3183, 1666 cm^{-1}.



8

3-({6-Chloropyrido[3,2-d][1,3]thiazol-2-yl}methoxy)-2,6-difluorobenzamide (8). The compound was prepared according to the reported procedure[2] and the NMR spectrum was in accordance with the reported values: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.73 (d, J = 2.2 Hz, 1H), 8.68 (d, J = 2.2 Hz, 1H), 8.18 (bs, 1H), 7.90 (br s, 2H), 7.41 (m, 2H), 7.12 (m, 2H), 5.73 (s, 2H).

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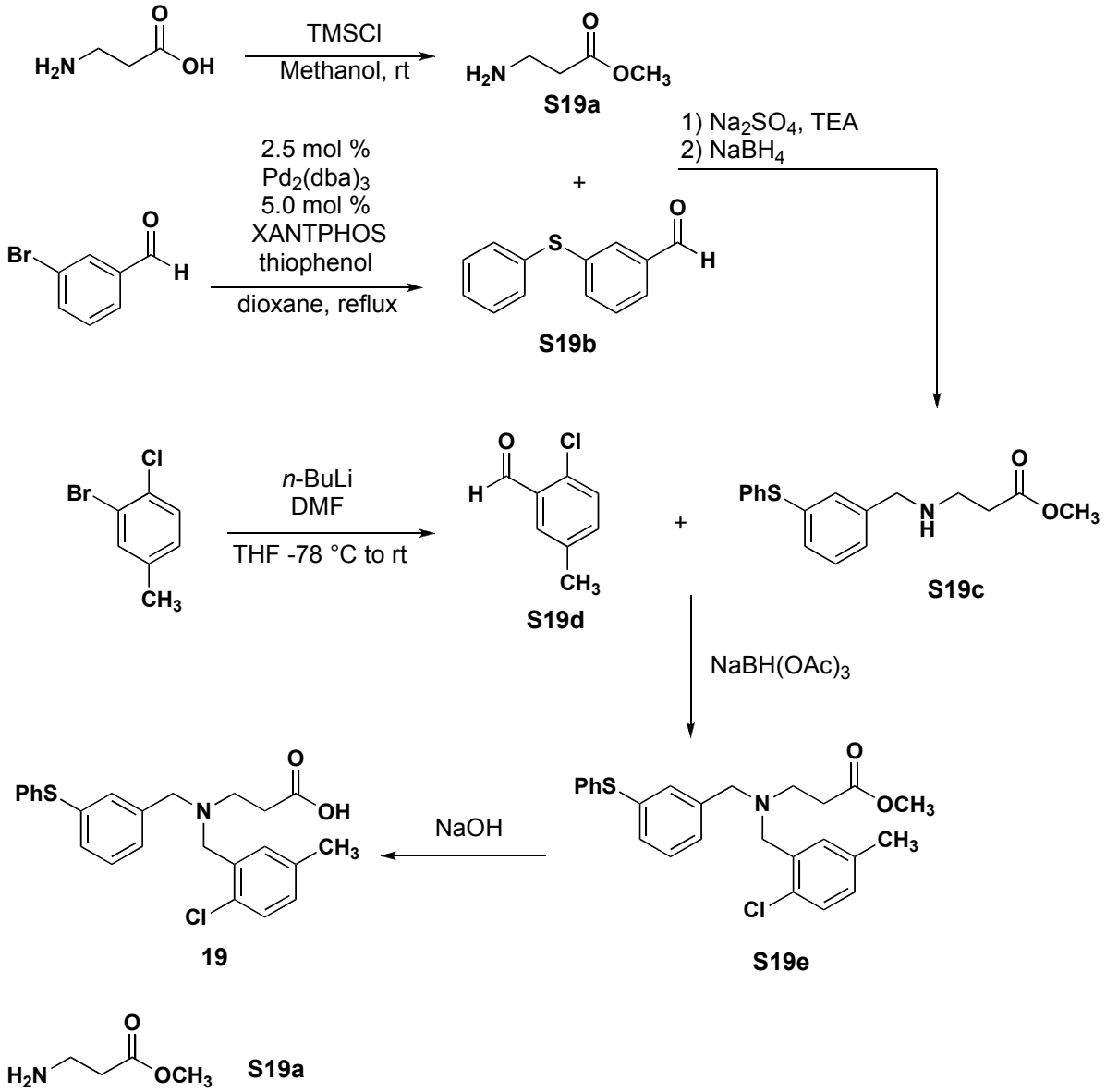
[1] Haydon, D. J.; Bennett, J. M.; Brown, D.; Collins, I.; Galbraith, G.; Lancett, P.; Macdonald, R.; Stokes, N. R.; Chauhan, P. K.; Sutariya, J. K.; Nayal, N.; Srivastava, A.; Beanland, J.; Hall, R.; Henstock, V.; Noula, C.; Rockley, C.; Czaplewski, L. *J. Med. Chem.* **2010**, *53*, 3927-3936.

[2] Sorto, N. A.; Olmstead, M. M.; Shaw, J. T. *J. Org. Chem.* **2010**, *75*, 7946-7949.

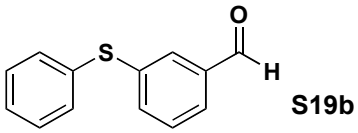
ii) Synthesis of PC170423 (19)



S19a

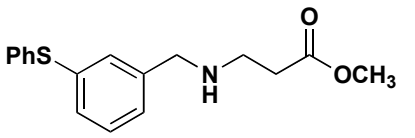
Methyl 3-aminopropanoate (S19a). The compound was prepared according to the reported procedure[3] and the NMR spectrum was in accordance with the reported values: ^{1}H NMR matched the published data: ^{1}H NMR (300 MHz, D_2O) δ 3.73 (s, 3H), 3.28 (t, J = 6.5 Hz, 2H), 2.81 (t, J = 6.5 Hz, 2H).

[3] Li, J. B.; Sha, Y. W. *Molecules* **2008**, *13*, 1111-1119.



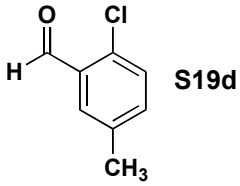
S19b

3-(Phenylthio)benzaldehyde (S19b). The compound was prepared according to the reported procedure[4] and the NMR spectrum was in accordance with the reported values: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.93 (s, 1H), 7.76 – 7.74 (m, 1H), 7.69-7.71 (m, 1H), 7.50-7.52 (m, 1H), 7.42-7.45 (m, 3H), 7.26-7.38 (m, 3H).



S19c

Methyl 3-(3-(phenylthio)benzylamino)propanoate (S19c). To a flame dried flask was added aldehyde **S19b** (0.11 g, 0.50 mmol) in CH_2Cl_2 (1 mL). To the reaction mixture was added protected amino acid **S19a** (77 mg, 0.55 mmol), triethylamine (77 µL, 0.51 mmol) and enough sodium sulfate to cover the stir bar. The reaction mixture was stirred at room temperature for 2h. The reaction mixture was filtered over a pad of cotton and the solvent was removed *in vacuo.* The residue was dissolved in anhydrous methanol (2 mL) and sodium borohydride (9.5 mg, 0.25 mmol) was added to the reaction mixture. The reaction mixture was allowed to stir overnight. The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 and washed with water (2 x 10 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo.* The crude product was purified by flash chromatography (5:10:85 TEA:EtOAc:Hex) to afford the product as a clear oil (0.10 g, 68%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.17 (m, 9H), 3.76 (s, 2H), 3.68 (s, 3H), 2.87 (dd, J = 3.4, 9.5 Hz, 2H), 2.52 (t, J = 6.5 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.1, 141.4, 135.9, 135.7, 130.9, 130.6, 129.7, 129.3, 129.2, 127.0, 126.9, 53.4, 51.6, 44.4, 34.6; IR (neat) 3327, 2949, 2840, 1732 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{17}H_{20}NO_2S$ $(M + H)^+$ 302.1209, found 302.1215.

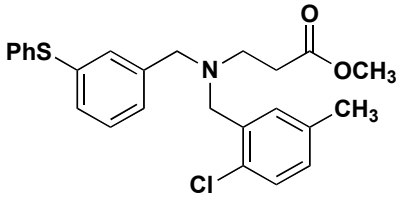


S19d

2-Chloro-5-methylbenzaldehyde (S19d). To a flame dried flask was added 3-bromo-4-chlorotoluene (1.0 g, 5.0 mmol) in anhydrous THF (12 mL). The solution was cooled (-78 °C) and *n*-butyllithium (2.5 M in hexanes, 2.0 mL, 5.0 mmol) was added over 15 min. The solution continued stirring for 45 min at -78 °C. DMF (0.56 mL, 7.25 mmol) in THF (6 mL) was added over a period of 15 min. The solution was allowed to warm to room temperature and allowed to stir overnight. The reaction mixture was poured over ice and the aqueous mixture was extracted ether (3 x 50 mL). The organic layer was washed with 5%HCl followed by sat. $NaHCO_3$ and dried over Na_2SO_4. The mixture was filtered and the organic layer was concentrated *in vacuo.* The crude yellow oil was purified by flash chromatography (30% DCM:Hex) to afford the

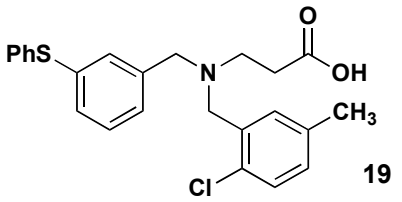
[4] Itoh, T.; Mase, T. *Org. Lett.* **2004**, *6*, 4587-4590.

product as an oil (0.45 g, 58%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.45 (s, 1H), 7.72 (s, 1H), 7.33 (s, 2H), 2.37 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.2, 137.5, 136.1, 135.2, 132.1, 130.4, 129.7, 20.8; IR (neat) 2925, 2854, 2754, 1697 cm^{-1}; HRMS (ESI) *m / z* calcd for C_8H_8ClO $(M + H)^+$ 155.0258, found 155.0464.



S19e

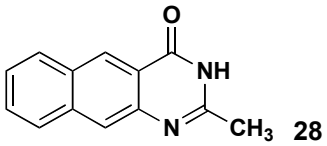
Methyl 3-((2-chloro-5-methylbenzyl)(3-(phenylthio)benzyl)amino)propanoate (S19e). To a solution of **S19c** (33 mg, 0.11 mmol) in CH_2Cl_2 (0.3 mL) was added aldehyde **S19d** (19 mg, 0.12 mmol) and sodium triacetoxyborohydride (35 mg, 0.17 mmol). The mixture was allowed to stir overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with water. The aqueous layer was extracted CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude oil was purified by flash chromatography (10% EtOAc:Hex) to provide the product as a clear oil (31 mg, 63%): ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.38 (s, 1H), 7.35 – 7.18 (m, 7H), 7.00 (dd, *J* = 1.9, 8.0 Hz, 1H), 3.65 (2, 1H), 3.61 (s, 3H), 3.58 (s, 2H), 2.79 (t, *J* = 7.2 Hz, 2H), 2.49 (t, *J* = 7.2 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.5, 141.1, 137.0, 136.7, 136.5, 136.0, 132.0, 131.8, 131.5, 131.4, 130.4, 129.7, 129.6, 129.6, 129.4, 128.2, 127.5, 58.6, 55.5, 52.1, 50.0, 33.0, 21.6; IR (neat) 3057, 2948, 2922, 2849, 1735 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{25}H_{27}ClNO_2S$ $(M + H)^+$ 440.1446, found 440.1443.



19

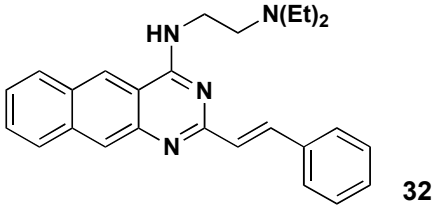
(PC170423) 3-((2-Chloro-5-methylbenzyl)(3-(phenylthio)benzyl)amino)propanoic acid (19). To a solution of ester **S19d** (19 mg, 0.043 mmol) dissolved in THF (1.25 mL) was added 0.4 mL of sodium hydroxide (1.15 M) and the mixture was refluxed overnight. After cooling to room temperature the mixture was diluted with water. The pH of the solution was adjusted to 2 with small aliquots of 10% HCl solution. The mixture was extracted with $CHCl_3$ (5 x 10 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (4% MeOH:DCM) to provide the product as an oil (13.9 mg, 76%): ^{1}H NMR (600 MHz, CD_3CN) δ 7.53 (s, 1H), 7.51 (s, 1H), 7.47 (d, *J* = 7.5 Hz, 1H), 7.38 – 7.26 (m, 7H), 7.16 (d, *J* = 7.8 Hz, 1H), 4.08 (s, 4H), 3.12 (t, *J* = 7.0 Hz, 2H), 2.78 (t, *J* = 7.0 Hz, 2H), 2.28 (s, 3H); ^{13}C NMR (150 MHz, CD_3CN) δ 173.7, 139.7, 138.0, 136.5, 136.3, 135.4, 133.5, 132.9, 132.1, 131.8, 131.0, 130.8, 130.4, 130.3, 130.2, 129.6, 128.3, 58.1, 55.9, 49.5, 31.5, 20.8; IR (neat) 3056, 2924, 2853, 1711 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{24}H_{25}ClNO_2S$ $(M + H)^+$ 426.1289, found 426.1290.

iii) Synthesis of zantrin Z3 and related analogs (9, 28-37)



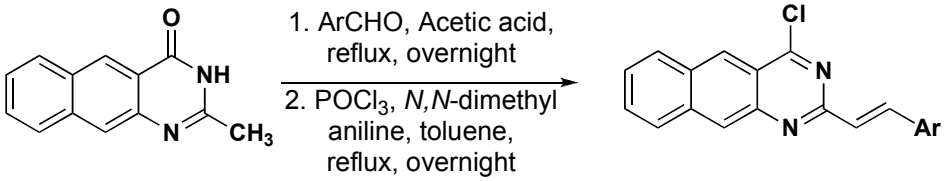
28

2-Methylbenzo[g]quinazolin-4(3H)-one (28). Acetic anhydride (3 mL) was added to 3-amino-2-naphthoic acid (0.14 g, 0.76 mmol) and heated to reflux for 3 h. The solvent was removed *in vacuo* and 28% NH_4OH (3 mL) was added and the solution was heated at 100 °C for 12 h. The precipitate was filtered and washed with H_2O (2 x 15 mL) and with MeOH (1 x10 mL) yielding a solid (0.12 g, 75%): ^{1}H NMR; (400 MHz, DMSO): δ 11.99 (s, 1H), 8.78 (s, 1H), 8.17 (d, J = 8.3, 1H), 8.11 (s, 1H), 8.05 (d, *J* = 8.4 Hz, 1H), 7.68 – 7.59 (m, 1H), 7.59 – 7.49 (m, 1H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, DMSO) 162.1, 153.3, 144.4, 136.2, 130.5, 129.2, 128.4, 127.7, 127.1, 125.9, 123.7, 120.0, 21.7 IR (neat) 3172, 3041, 2911, 1678, 1613 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{13}H_{11}N_2O$ $(M + H)^+$ 211.0866, found 211.0864; Melting point. 299 – 303 °C.

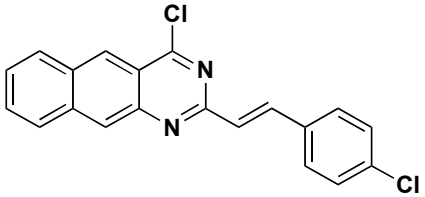


32

(E)-*N*-(2-(Diethylamino)ethyl)-2-styrylbenzo[g]quinazolin-4-amine (32). Acetic acid was added (2 mL) to a mixture of **28** (69 mg, 0.33 mmol) and benzaldehyde (33 µL, 0.32 mmol) and heated to 120 °C for 12 h. The mixture was allowed to cool to room temperature and the yellow precipitate was filtered and washed with cold MeOH (2 x 15 mL). The product was collected and used in the next step without further purification. This product was dissolved in a 10:90 solution of $POCl_3$ and toluene (5 mL total volume) in the presence of *N,N*-dimethylaniline (42 µL, 0.33 mmol). The mixture was allowed to reflux for 4 h then the solvent removed under vacuum and re-dissolved in $CHCl_3$ and then washed H_2O (2 x 10 mL). The organic layers were combined and dried over Na_2SO_4 and then passed through a plug of silica using $CHCl_3$. The solvent was removed under vacuum yielding an oil (53 mg, 0.17 mmol). The crude product was dissolved in toluene (6 mL) and DIPEA (43 µL, 0.25 mmol) and *N,N*-diethylethylenediamine (30 µL, 0.21 mmol) was added and refluxed for 12 h. The solvent was removed *in vacuo* and the crude product was purified in a gradient of 100-95% $CHCl_3$:MeOH yielding an amorphous yellow solid (0.045 g, 35%): ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1H), 8.32 (s, 1H), 8.05 (d, *J* = 15.8 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 8.5 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 2H), 7.54 – 7.49 (m, 1H), 7.46 – 7.37 (m, 4H), 7.32 (d, *J* = 6.9 Hz, 1H), 7.30 (s, 1H), 3.94 (t, *J* = 5.8 Hz, 2H), 2.98 (t, *J* = 5.8 Hz, 2H), 2.80 (q, *J* = 7.1 Hz, 4H), 1.18 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.3, 159.4, 145.7, 137.3, 136.8, 136.1, 131.2, 129.5, 129.0, 128.8, 128.7, 128.0, 127.7, 127.6, 125.7, 125.2, 121.5, 114.3, 51.5, 47.3, 38.1, 11.5; IR (neat) 3257, 2964, 1542, 1431, 1374 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{26}H_{29}N_4$ $(M + H)^+$ 397.2387, found 397.2378; R_f = 0.20 in 5% MeOH:DCM.

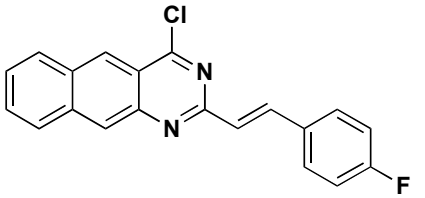


General procedure A: To a solution of lactam **28** (1 equiv.) in acetic acid (0.10 M) was added an aryl aldehyde (1.1 equiv.). The solution was refluxed overnight and cooled to room temperature. The product precipitated out of solution and was collected by vacuum filtration. The unpurified product was dried under vacuum for 3 h. This product was dissolved in toluene (0.020 M) and dimethylaniline (2 equiv.) and phosphoryl chloride (2 equiv.) were added to the solution. This mixture was heated to reflux overnight. The solution turned purple and was cooled to room temperature. The solvent was removed *in vacuo* and the product was purified by flash chromatography to yield products **29a-29e**.



29a

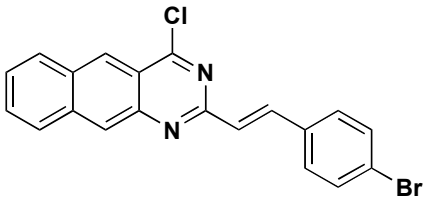
(*E*)-2-(4-Chlorostyryl)-4-chlorobenzo[*g*]quinazoline (29a). This compound was prepared according to general procedure A using **28** (0.80 g, 3.8 mmol) and 4-chlorobenzaldehyde (0.59 mL, 4.2 mmol). The product of this reaction was collected by vacuum filtration (0.68 g). A portion of this intermediate (0.67 g, 2.0 mmol) was subjected to the second step of general procedure A using dimethylaniline (0.51 mL, 4.0 mmol) and phosphoryl chloride (0.37 mL, 4.0 mmol). The solvent was removed *in vacuo* and the crude product was purified by flash chromatography (gradient 40% DCM:Hex to 3% MeOH:DCM) to afford the product as a yellow amorphous solid (0.51 g, 38% over two steps): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.85 (s, 1H), 8.54 (s, 1H), 8.13 (d, J = 15.8 Hz, 1H), 8.13 (d, J = 8.5 Hz, 1H), 8.09 (d, J = 8.5 Hz, 1H), 7.70 – 7.65 (m, 1H), 7.64 – 7.59 (m, 3H), 7.40 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 15.8 Hz, 1H); ^{13}C NMR (150 MHz, DMSO) δ 162.2, 151.4, 143.9, 138.1, 136.9, 134.9, 134.7, 131.5, 129.7, 129.6, 129.5, 129.0, 128.2, 127.8, 126.7, 124.2, 122.3, 120.7; IR (neat) 3052, 1623 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{20}H_{12}Cl_2N_2$, $(M + H)^+$ 351.0450 found 351.0422.



29b

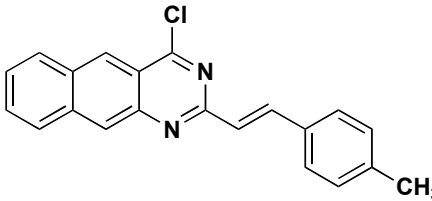
(*E*)-2-(4-Chlorostyryl)-4-fluorobenzo[*g*]quinazoline (29b). This compound was prepared according to general procedure A using **28** (0.16 g, 0.76 mmol) and 4-fluorobenzaldehyde (90 μL, 0.84 mmol). The product of this reaction was collected by vacuum filtration (79 mg). A portion of this intermediate (60 mg, 0.19 mmol) was subjected to the second step of general procedure A using dimethyl aniline (48 μL, 0.38 mmol) and phosphoryl chloride (35 μL, 0.038 mmol). The solvent was removed *in vacuo* and the crude product was purified by flash chromatography (gradient 40% DCM:Hex to 10% MeOH:DCM) to afford the product as a

yellow amorphous solid (48.6 mg, 19%, two steps): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.85 (s, 1H), 8.53 (s, 1H), 8.14 (m, 2H), 8.09 (d, J = 8.5 Hz, 1H), 7.67 (m, 3H), 7.61 (ddd, J = 7.9, 6.6, 1.1 Hz, 1H), 7.28 (d, J = 14.5 Hz, 1H), 7.12 (m, 2H).; ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.5, 163.4 (d, J = 250.1 Hz), 158.7, 146.0, 138.7, 137.1, 132.3, 132.2 (d, J = 3.2 Hz), 129.5 (d, J = 8.3 Hz), 129.3, 128.9, 128.3, 127.2, 126.9, 126.5 (d, J = 2.4 Hz), 126.4, 120.4, 116.0 (d, J = 21.8 Hz); IR (neat) 3052, 1598, 1553, 1505 cm^{-1}; HRMS (ESI) m / z calcd for $C_{20}H_{13}ClFN_2$ $(M + H)^+$ 335.0746, found 335.0750.



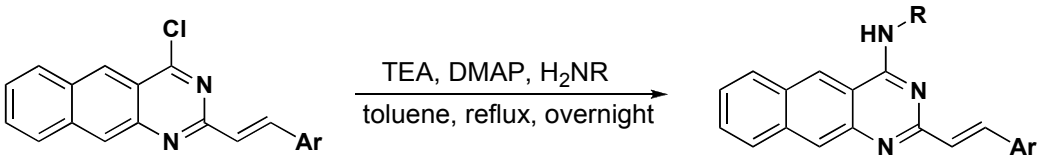
29c

(***E*)-2-(4-Bromostyryl)-4-chlorobenzo[g]quinazoline (29c)**. This compound was prepared according to general procedure A using **28** (69 mg, 0.33 mmol) and 4-bromobenzaldehyde (60 mg, 0.33 mmol). The product of this reaction was collected by vacuum filtration. The intermediate was subjected to the second step of general procedure A using dimethylaniline (83 µL, 0.65 mmol) and phosphoryl chloride (60 µL, 0.65 mmol). The solvent was removed in vacuo and the resulting solid was then purified by flash column chromatography (100 % dichloromethane) to give a bright yellow amorphous solid (42 mg, 32% over two steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.52 (s, 1H), 8.10 (dd, J = 5.5, 8.8 Hz, 2H), 8.06 (s, 1H), 7.66 (m, 1H), 7.60 (m, 1H), 7.54 (s, 4H), 7.32 (d, J = 15.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.7, 158.7, 146.1, 138.7, 137.3, 135.1, 132.6, 132.3, 129.5, 129.4, 129.2, 128.5, 127.7, 127.4, 127.1, 126.7, 123.7, 120.5; IR (neat) 2923, 1599, 1578, 1523 cm^{-1}; HRMS (ESI) m / z calcd for $C_{20}H_{13}BrClN_2$ $(M + H)^+$ 394.9951, found 394.9951.

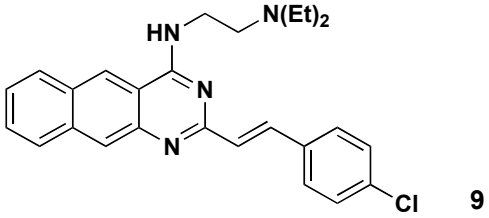


29e

(*E*)-2-(4-Chlorostyryl)-4-methylbenzo[*g*]quinazoline (29e). This compound was prepared according to general procedure A using **28** (0.18 g, 0.83 mmol) and 4-methylbenzaldehyde (0.11 mL, 0.91 mmol). The product of this reaction was collected by vacuum filtration (93 mg). A portion of this intermediate (83 mg, 0.27 mmol) was subjected to the second step of general procedure A using dimethyl aniline (68 µL, 0.54 mmol) and phosphoryl chloride (50 µL, 0.054 mmol). The solvent was removed *in vacuo* and the crude product was purified by flash chromatography (gradient 40% DCM:Hex to 3% MeOH:DCM) to afford the product as a yellow amorphous solid (38.5 mg, 43%, two steps): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.51 (s, 1H), 8.16 (d, J = 15.8 Hz, 1H), 8.10 (d, J = 8.5 Hz, 1H), 8.07 (d, J = 8.6 Hz, 1H), 7.67 – 7.63 (m, 1H), 7.61 – 7.57 (m, 3H), 7.31 (d, J = 15.8 Hz, 1H), 7.23 (d, J = 7.9 Hz, 2H), 2.40 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.9, 159.6, 140.7, 140.3, 137.7, 133.8, 132.8, 130.2, 129.9, 129.4, 128.8, 128.4, 127.7, 127.5, 126.8, 126.4, 120.9, 22.1; IR (neat) 2845, 2834 cm^{-1}; HRMS (ESI) m / z calcd for $C_{21}H_{16}ClN_2$, $(M + H)^+$ 331.0997, found 331.0998.

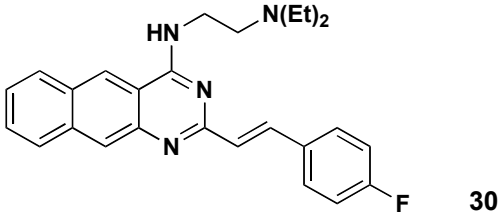


General procedure B: To a solution of intermediate (**29a-29e**) in toluene (0.025 M) was added triethylamine (2 equiv.), a primary or secondary amine (1.1 equiv.), and 4-dimethylaminopyridine (0.1 equiv). The solution was refluxed overnight. Upon cooling to room temperature, the solvent was removed *in vacuo* and the crude product was purified by flash chromatography.



9

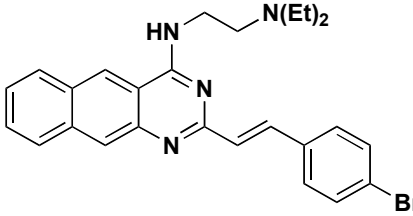
Z3 (9). This compound was prepared according to general procedure B using **29a** (200 mg, 0.57 mmol), triethyl amine (160 µL, 1.14 mmol), 4-dimethylaminopyridine (6.4 mg, 0.06 mmol), and *N,N*-diethylethylenediamine (89 µL, 0.63 mmol). The solvent was removed *in vacuo* and the product was purified by flash chromatography (5% MeOH/CH_2Cl_2 to 8% MeOH/CH_2Cl_2) to afford the product as a yellow amorphous solid (238 mg, 97%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.28 (s, 1H), 7.94 (dd, *J* = 16.2, 8.6 Hz, 3H), 7.50 (m, 3H), 7.40 (m, 1H), 7.33 (m, 2H), 7.20 (d, *J* = 15.7 Hz, 1H), 3.93 (t, *J* = 5.9 Hz, 2H), 3.01 (t, *J* = 5.9 Hz, 2H), 2.82 (q, *J* = 7.1 Hz, 4H), 1.18 (t, *J* = 7.1 Hz, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ 160.0, 159.5, 145.6, 136.2, 135.9, 135.3, 134.5, 131.3, 130.1, 129.2,129.1, 128.9, 128.1, 127.8, 125.9, 125.2, 121.9, 114.3, 51.7, 47.5, 38.0, 11.3; IR (thin film) 2972, 1629, 1563, 1539 cm^{-1}; HRMS (FTMS + P ESI) *m / z* calcd for $C_{26}H_{28}ClN_4$ $(M + H)^+$ 431.2002, found 432.2017.



30

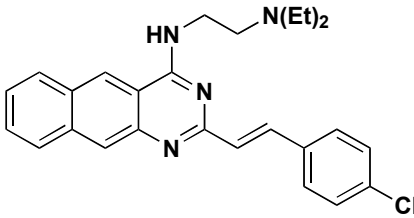
(*E*)-2-(4-Fluorostyryl)-*N*-(2-(diethylamino)ethyl)benzo[*g*]quinazolin-4-amine (30). This compound was prepared according to general procedure B using **29b** (34 mg, 0.10 mmol), triethyl amine (28 µL, 0.20 mmol), 4-dimethylaminopyridine (1.2 mg, 0.01 mmol), and *N,N*-diethylethylenediamine (16 µL, 0.11 mmol). The solvent was removed *in vacuo* and the product was purified by flash chromatography (5% MeOH/CH_2Cl_2) to afford the product as a yellow-orange amorphous solid (48 mg, 98%): ^{1}H NMR (600 MHz, CD_3OD) δ 8.56 (s, 1H), 8.04 (s, 1H), 7.87 (m, 3H), 7.59 (m, 2H), 7.48 (ddd, *J* = 8.2, 6.6, 1.1 Hz, 1H), 7.40 (ddd, *J* = 8.0, 6.6, 1.0 Hz, 1H), 7.08 (t, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 15.7 Hz, 1H), 4.07 (t, *J* = 6.7 Hz, 2H), 3.40 (t, *J* = 6.7 Hz, 2H), 3.22 (q, *J* = 7.2 Hz, 4H), 1.27 (t, *J* = 7.3 Hz, 6H); ^{13}C NMR (150 MHz, CD_3OD) δ 164.09 , 162.44 , 160.05 , 159.52 , 143.55 , 137.17 , 135.99 , 132.33 (d, *J* = 3.3 Hz), 131.17 , 129.22 , 129.17 , 127.95 (d, *J* = 203.5 Hz), 127.91 , 126.38 , 125.78 , 122.67 (d, *J* = 4.8 Hz),

115.39 (d, J = 22.0 Hz), 113.27 , 50.30 , 47.55 , 36.13 , 8.32; IR (neat) 3246, 2966, 1628, 1561 cm^{-1}; HRMS (ESI) m / z calcd for $C_{26}H_{28}FN_4$, $(M + H)^+$ 415.2293 found 415.2294.



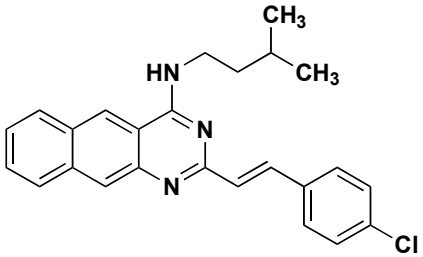
31

(*E*)-2-(4-Bromostyryl)-*N*-(2-(diethylamino)ethyl)benzo[*g*]quinazolin-4-amine (31). This compound was prepared according to general procedure B using **29c** (16 mg, 0.040 mmol) was dissolved in toluene (5 mL) and to this TEA (0.11 mL), *N,N*-dimethylaminopyridine (0.35 mg, 0.003 mmol) and *N,N*-diethylethylenediamine (0.06 mL, 0.42 mmol) were added and heated to reflux overnight. The solvent was removed *in vacuo* and the crude material was purified by flash column chromatography (5% MeOH:DCM) to give an amorphous yellow solid (12.5 mg, 66%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.29 (s, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 15.8 Hz, 1H), 7.50 (m, 5H), 7.44 (m, 1H), 7.23 (d, J = 15.8 Hz, 1H), 4.11 (t, J = 5.4 Hz, 2H), 3.23 (t, J = 5.4 Hz, 2H), 3.02 (q, J = 7.1 Hz, 4H), 1.30 (t, J = 7.1 Hz, 6H).); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.5, 159.5, 145.0, 136.1, 135.9, 135.4, 131.9, 131.3, 129.7, 129.3, 128.9, 127.8, 125.8, 124.8, 122.7, 122.5, 113.9, 52.0, 47.9, 37.3, 10.1; IR (neat) 2941, 1628, 1604, 1568, 1537 cm^{-1}; HRMS (ESI) m / z calcd for $C_{26}H_{27}BrN_4$ $(M + H)^+$ 475.1492, found 475.1487.



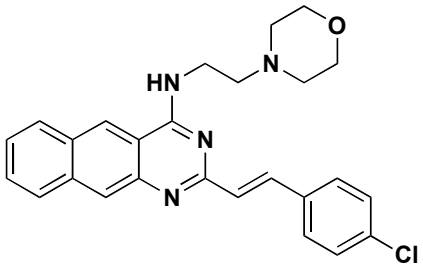
33

(*E*)-2-(4-Methylstyryl)-*N*-(2-(diethylamino)ethyl)benzo[*g*]quinazolin-4-amine (33). This compound was prepared according to general procedure B using **29e** (30 mg, 0.09 mmol), triethylamine (25 µL, 0.18 mmol), 4-dimethylaminopyridine (1.1 mg, 0.009 mmol), and *N,N*-diethylethylenediamine (14 µL, 0.10 mmol). The solvent was removed *in vacuo* and the product was purified by flash chromatography (10% MeOH:DCM) to afford the product as a yellow-orange amorphous solid (33 mg, 89%): ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.74 (s, 1H), 8.25 (s, 1H), 8.05 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 15.8 Hz, 1H), 7.97 (d, J = 9.2 Hz, 1H), 7.59 – 7.52 (m, 3H), 7.47 (t, J = 7.2 Hz, 1H), 7.23 (d, J = 7.9 Hz, 2H), 7.19 (d, J = 15.8 Hz, 1H), 4.13 – 3.99 (m, 2H), 3.23 – 3.11 (m, 2H), 2.96 (q, J = 7.1 Hz, 4H), 2.38 (s, 3H), 1.26 (t, J = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, CD_2Cl_2) δ 160.5, 159.8, 146.2, 139.3, 137.2, 136.3, 134.2, 131.5, 129.9, 129.4, 128.9, 128.0, 127.9, 127.8, 125.9, 125.1, 122.7, 114.7, 52.4, 48.0, 37.7, 21.5, 10.5; IR (neat) 3227, 2965, 2926, 1628 cm^{-1}; HRMS (ESI) m / z calcd for $C_{27}H_{31}N_4$, $(M + H)^+$ 411.2543 found 411.2554.



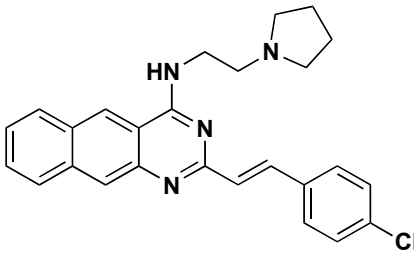
34

(*E*)-2-(4-Chlorostyryl)-N-isopentylbenzo[g]quinazolin-4-amine (34). The compound was prepared according to general procedure B using **29a** (24 mg, 0.068 mmol), triethylamine (16 μL, 0.14 mmol), 4-dimethylaminopyridine (1.2 mg, 0.01 mmol), and isoamylamine (8.0 μL, 0.07 mmol). The solvent was removed in vacuo and the crude product was purified by flash chromatography (5% MeOH:DCM) to afford the product as a yellow-orange amorphous solid (0.014 g, 51%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.23 (s, 1H), 8.00 (d, *J* = 15.7 Hz, 1H), 7.94 (dd, *J* = 8.4, 16.6 Hz, 2H), 7.55 (d, *J* = 7.3 Hz, 2H), 7.52 (m, 1H), 7.44 (m, 1H), 7.35 (m, 2H), 7.24 (m, 1H), 6.12 (s, 1H), 3.84 (q, *J* = 6.8 Hz, 2H), 1.82 (m, 1H), 1.72 (q, *J* = 6.8 Hz, 2H), 1.05 (d, *J* = 1.5 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.0, 159.2, 136.2, 136.1, 135.9, 135.1, 134.3, 131.0, 129.0, 128.9, 128.8, 128.6, 128.6, 128.5, 128.0, 128.0, 125.3, 120.5, 114.0, 39.7, 38.3, 26.1, 22.7, 22.6; IR (neat) 2924, 1628, 1604, 1561, 1537 cm^{-1}; HRMS (ESI) m / z calcd for $C_{25}H_{24}ClN_3$ $(M + H)^+$ 402.1732, found 402.1346.



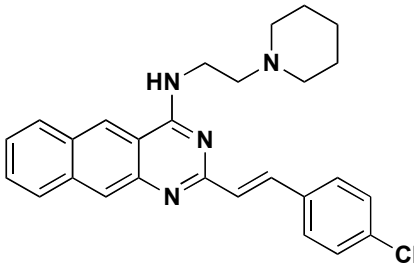
35

(*E*)-2-(4-Chlorostyryl)-*N*-(2-morpholinoethyl)benzo[*g*]quinazolin-4-amine (35). The compound was prepared according to general procedure B using **29a** (24 mg, 0.068 mmol), triethylamine (16 μL, 0.14 mmol), 4-dimethylaminopyridine (1.2 mg, 0.01 mmol), and *N,N*-diethylethylenediamine (16 μL, 0.11 mmol). The solvent was removed *in vacuo* and the crude product was purified by flash chromatography (5% MeOH:DCM) to afford the product as a yellow-orange amorphous solid (0.014 g, 51%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.26 (s, 1H), 7.99 (m, 3H), 7.57 (t, *J* = 7.8 Hz, 3H), 7.49 (m, 1H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 15.8 Hz, 1H), 6.90 (s, 1H), 3.91 (m, 2H), 3.82 (t, *J* = 4.5 Hz, 4H), 2.83 (t, *J* = 5.9 Hz, 2H), 2.63 (s, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.0, 159.3, 136.0, 135.1, 134.3, 131.0, 129.8, 129.0, 128.7, 128.6, 128.1, 127.7, 125.9, 125.4, 120.7, 114.1, 67.1, 56.6, 53.4, 37.2; IR (neat) 2926, 1628, 1603, 1559, 1536 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{26}H_{25}ClN_4O$ $(M + H)^+$ 445.1790, found 445.1791.



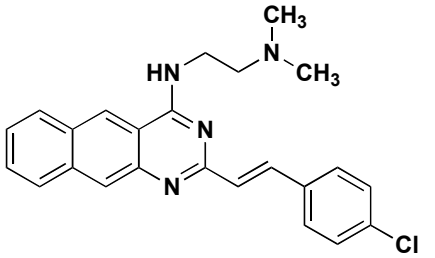
36

(*E*)-2-(4-Chlorostyryl)-*N*-(2-(pyrrolidin-1-yl)ethyl)benzo[*g*]quinazolin-4-amine (36). This compound was prepared according to general procedure B using **29a** (0.10 g, 0.28 mmol), triethylamine (80 µL, 0.57 mmol), 4-Dimethylaminopyridine (3.4 mg, 0.028 mmol), and 1-(2-aminoethyl)pyrrolidine (39 µL, 0.31 mmol). The solvent was removed *in vacuo* and the crude product was purified by flash chromatography (10% MeOH:DCM) to afford the product as a yellow-orange amorphous solid (0.12 g, 97%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.53 (s, 1H), 8.02 (s, 1H), 7.86 (d, J = 9.7 Hz, 2H), 7.83 (d, J = 16.4 Hz, 1H), 7.52 (d, J = 8.5 Hz, 2H), 7.51 – 7.45 (m, 1H), 7.43 – 7.37 (m, 1H), 7.32 (d, J = 8.5 Hz, 2H), 6.96 (d, J = 15.8 Hz, 1H), 4.08 (t, J = 6.3 Hz, 2H), 3.49 (t, J = 6.3 Hz, 2H), 3.37 (t, J = 6.5 Hz, 4H), 2.10 – 2.01 (m, 4H); ^{13}C NMR (150 MHz, CD_3OD) δ 161.7, 161.1, 145.5, 138.3, 137.5, 136.2, 135.9, 132.7, 130.1, 130.0, 130.0, 129.4, 129.1, 128.8, 127.3, 124.5, 124.1, 115.0, 55.6, 55.5, 39.1, 24.1; IR (neat) 2473, 2359 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{26}H_{26}ClN_4$, $(M + H)^+$ 429.1841 found 429.1848.



37

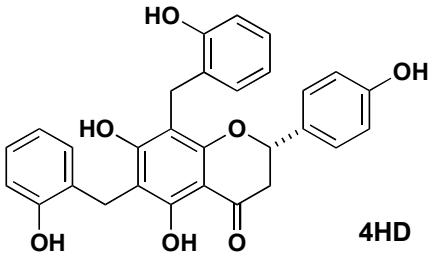
(*E*)-2-(4-Chlorostyryl)-*N*-(2-(piperidin-1-yl)ethyl)benzo[*g*]quinazolin-4-amine (37). This compound was prepared according to general procedure B using **29a** (0.10 g, 0.28 mmol), triethyl amine (0.08 mL, 0.57 mmol), 4-dimethylaminopyridine (3.4 mg, 0.028 mmol), and 1-(2-aminoethylpiperidine (0.044 mL, 0.31 mmol). The solvent was removed *in vacuo* and the product was purified by flash chromatography (gradient 5% MeOH:DCM to 8% MeOH:DCM) to afford the product as a yellow-orange amorphous solid (0.12 g, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.30 (s, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.95 (d, J = 9.6, 1H), 7.94 (d, J = 15.8 Hz, 1H), 7.55 (d, J = 8.5 Hz, 2H), 7.54 – 7.51 (m, 1H), 7.47 – 7.41 (m, 1H), 7.34 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 15.8 Hz, 1H), 4.00 (t, J = 5.5 Hz, 2H), 2.98 (t, J = 5.1 Hz, 2H), 2.77 (s, 4H), 1.89 – 1.74 (m, 4H), 1.57 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.3, 160.0, 145.9, 136.5, 136.3, 135.7, 134.8, 131.7, 130.5, 129.6, 129.5, 129.2, 128.4, 128.2, 126.3, 125.6, 122.5, 114.7, 57.8, 54.9, 37.4, 25.6, 24.1; IR (neat) 2937, 2182, cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{27}H_{28}ClN_4$, $(M + H)^+$ 443.1997 found 443.1997.



38

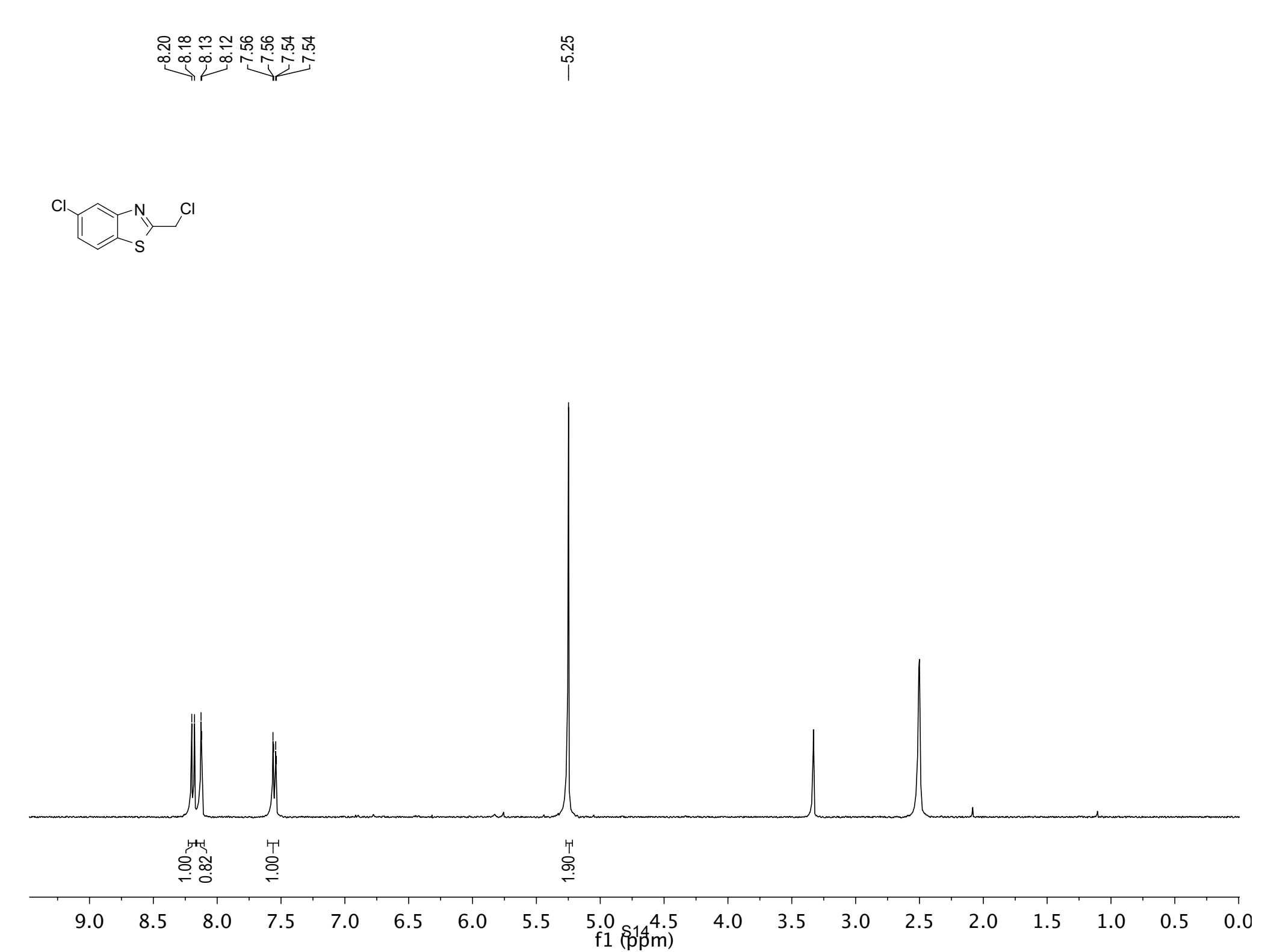
(*E*)-2-(4-Chlorostyryl)-*N*-(2-(dimethylamino)ethyl)benzo[*g*]quinazolin-4-amine (38). This compound was prepared according to general procedure B using **29a** (0.030 g, 0.085 mmol), triethyl amine (0.024 mL, 0.17 mmol), 4-dimethylaminopyridine (1 mg, 0.009 mmol), and *N,N*-dimethylethylenediamine (0.010 mL, 0.094 mmol). The solvent was removed *in vacuo* and the product was purified by flash chromatography (gradient 7% MeOH:DCM to 10% MeOH:DCM) to afford the product as a yellow-orange amorphous solid (0.033 g, 96%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.32 (s, 1H), 8.05 – 7.95 (m, 3H), 7.57 (d, *J* = 8.5, 2H), 7.54 (t, *J* = 7.1, 1H), 7.47 (t, *J* = 7.1, 1H), 7.36 (d, *J* = 8.4, 2H), 7.24 (d, *J* = 15.8, 1H), 3.94 (t, *J* = 5.6, 2H), 2.84 (t, J = 5.8, 2H), 2.47 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.0, 159.5, 145.3, 136.1, 136.1, 135.2, 134.4, 131.2, 129.8, 129.1, 128.8, 128.8, 128.1, 127.7, 125.9, 125.1, 121.4, 114.2, 57.7, 45.3, 38.3; IR (neat) 2973, 1630, 1562 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{24}H_{24}ClN_4$, $(M + H)^+$ 403.1689 found 403.1674.

iv) Synthesis of 4'-hydroxydichamanetin



4HD

4'-Hydroxydichamanetin (4HD). In a microwave vial, (±)-narningen (0.24 g, 0.92 mmol), o-hydroxybenzyl alchol (0.24 g, 1.9 mmol) and $ZnCl_2$ (0.26 g, 1.9 mmol) were dissolved in dioxane (8 mL) and then purged with nitrogen. This was heated at 100 °C using microwave irradition. The reaction was rotovapped to remove solvent and then purified using flash column chromatography in 40% EtOAc:Hex to yield a white solid (0.20 g, 45%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.18 (t, *J* = 8.1 Hz, 3H), 6.99 (d, *J* = 6.6 Hz, 1H), 6.89 (q, *J* = 4.2, 11.1 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 2H), 6.72 (q, *J* = 8.1, 10.8, 2H), 6.61 (m, 2H), 5.07 (dd, *J* = 2.4, 13.2 Hz, 1H), 3.82 (q, *J* = 5.4, 15 Hz, 2H), 3.75 (t, *J* = 15.9 Hz, 2H), 2.90 (dd, *J* = 4.7, 17.4 Hz, 1H), 2.50 (dd, J= 2.7, 17.1 Hz, 1H), -OH protons were not observable; ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.1, 161.9, , 159.6, 159.4, 158.8, 157.4, 153.5, 153.4, 130.2, 129.8, 127.7, 126.8, 126.8, 126.7, 126.7, 119.9 ,119.8115.0, 114.5, 114.4, 107.7, 106.9, 102.3, 78.8, 48.6, 42.4, 22.4, 21.6; IR (neat) 3238, 2451, 2072, 1603, 1490 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{29}H_{25}O_7$, $(M + H)^+$ 485.1600 found 485.1590.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8.13
8.12
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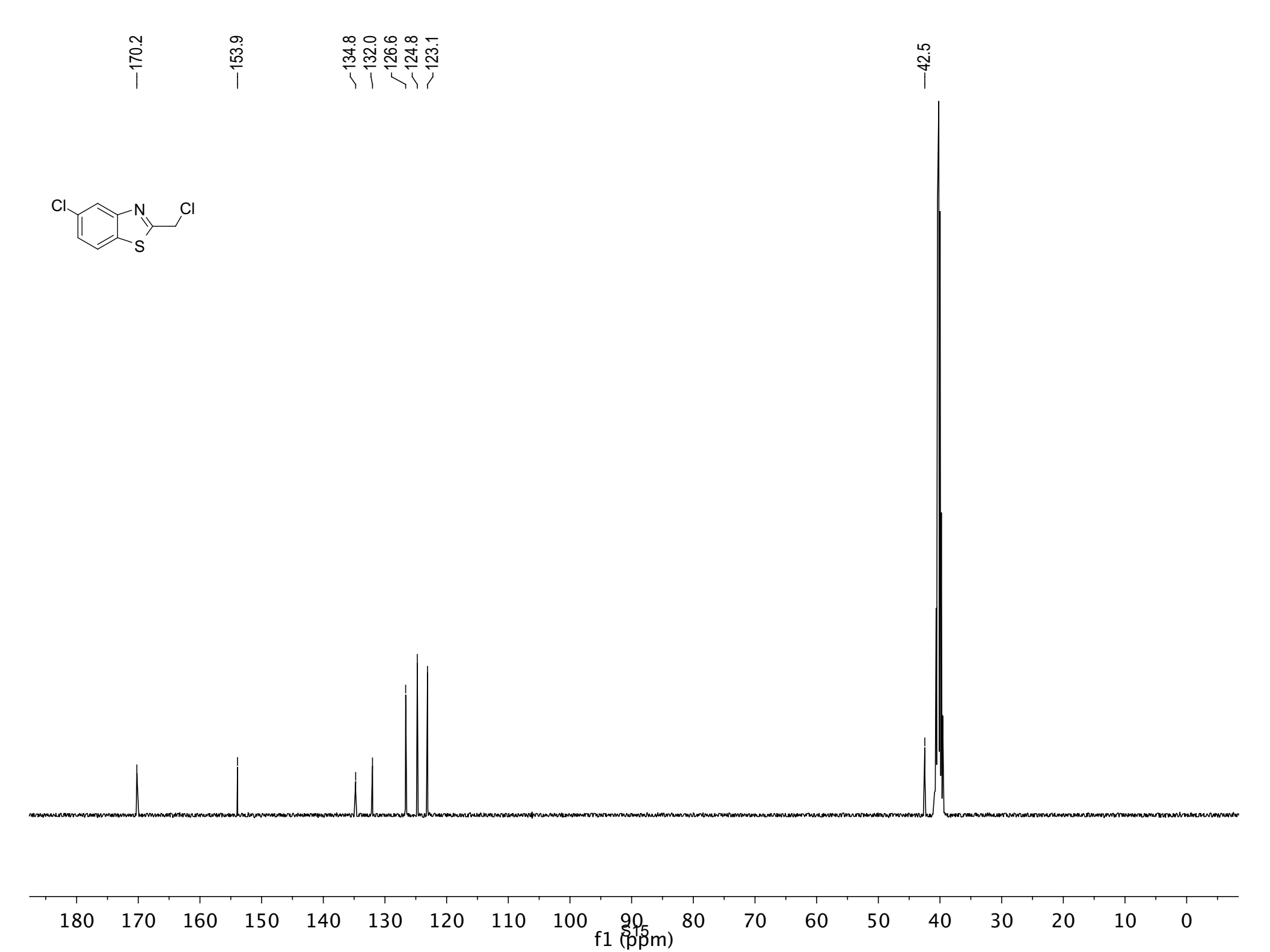
7.56
7.54
7.54
5.25
Cl
N
Cl
S
1.00
0.82
1.00
1.90
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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153.9
134.8
132.0
126.6
124.8
123.1
42.5
Cl
N
Cl
S
180
170
160
150
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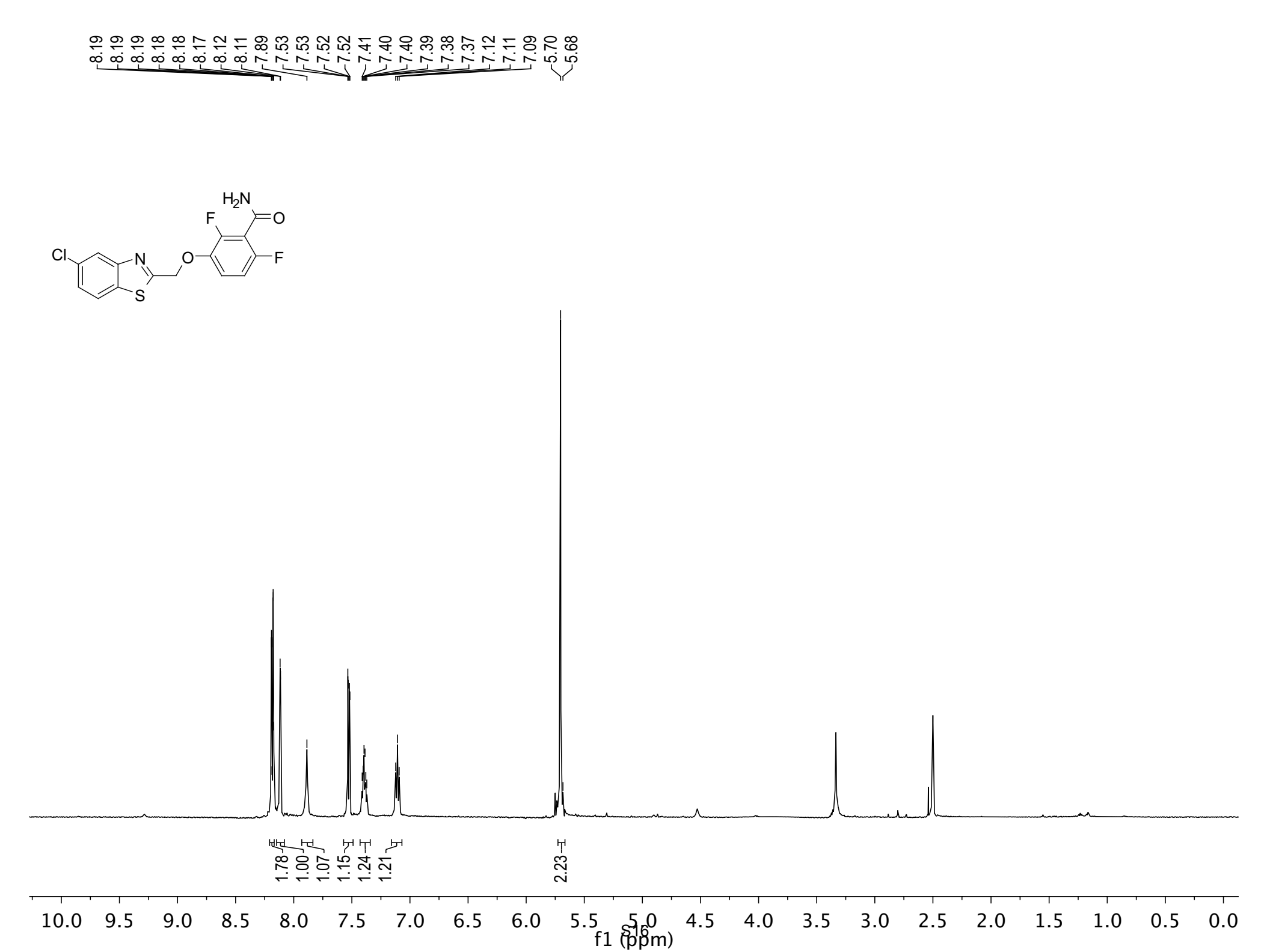
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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8.19
8.19
8.18
8.18
8.17
8.12
8.11
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7.53
7.53
7.52
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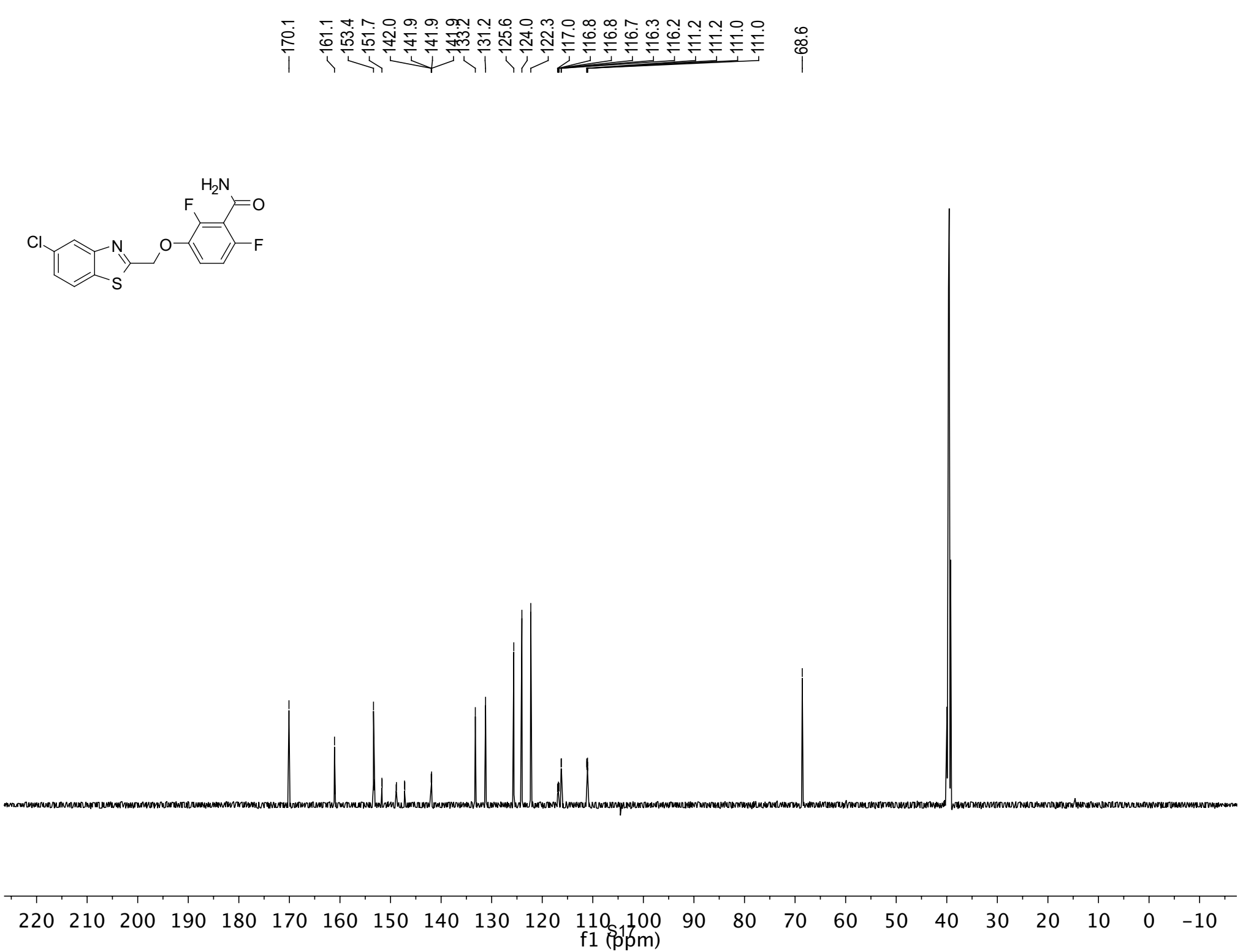
7.41
7.40
7.40
7.39
7.38
7.37
7.12
7.11
7.09
5.70
5.68
H2N
O
F
F
Cl
N
O
S
1.78
1.00
1.07
1.15
1.24
1.21
2.23
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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161.1
153.4
151.7
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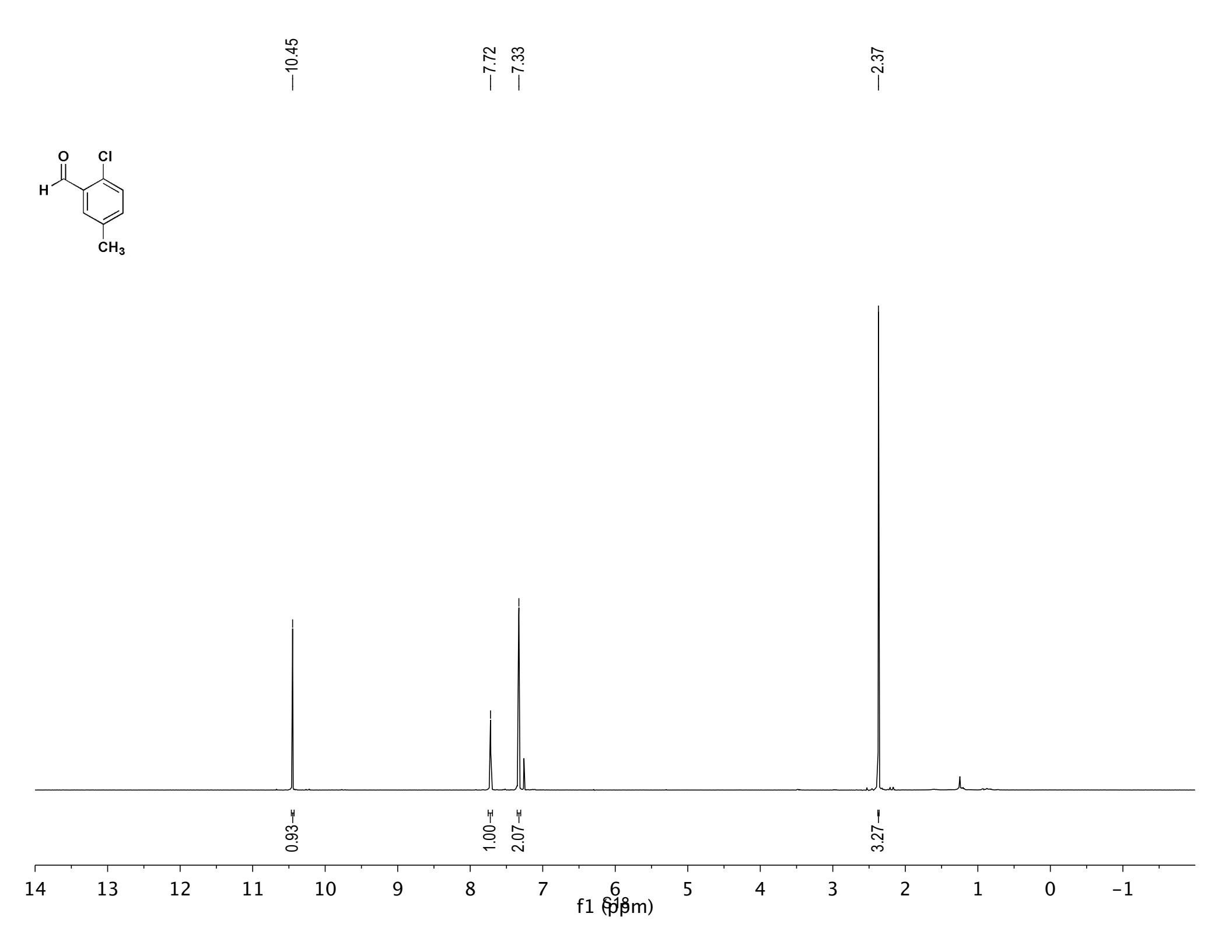
141.9
141.9
141.9
133.2
131.2
125.6
124.0
122.3
117.0
116.8
116.8
116.7
116.3
116.2
111.2
111.2
111.0
111.0
68.6
H2N
O
F
F
Cl
N
O
S
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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7.72
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Cl
H
CH3
0.93
1.00
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14
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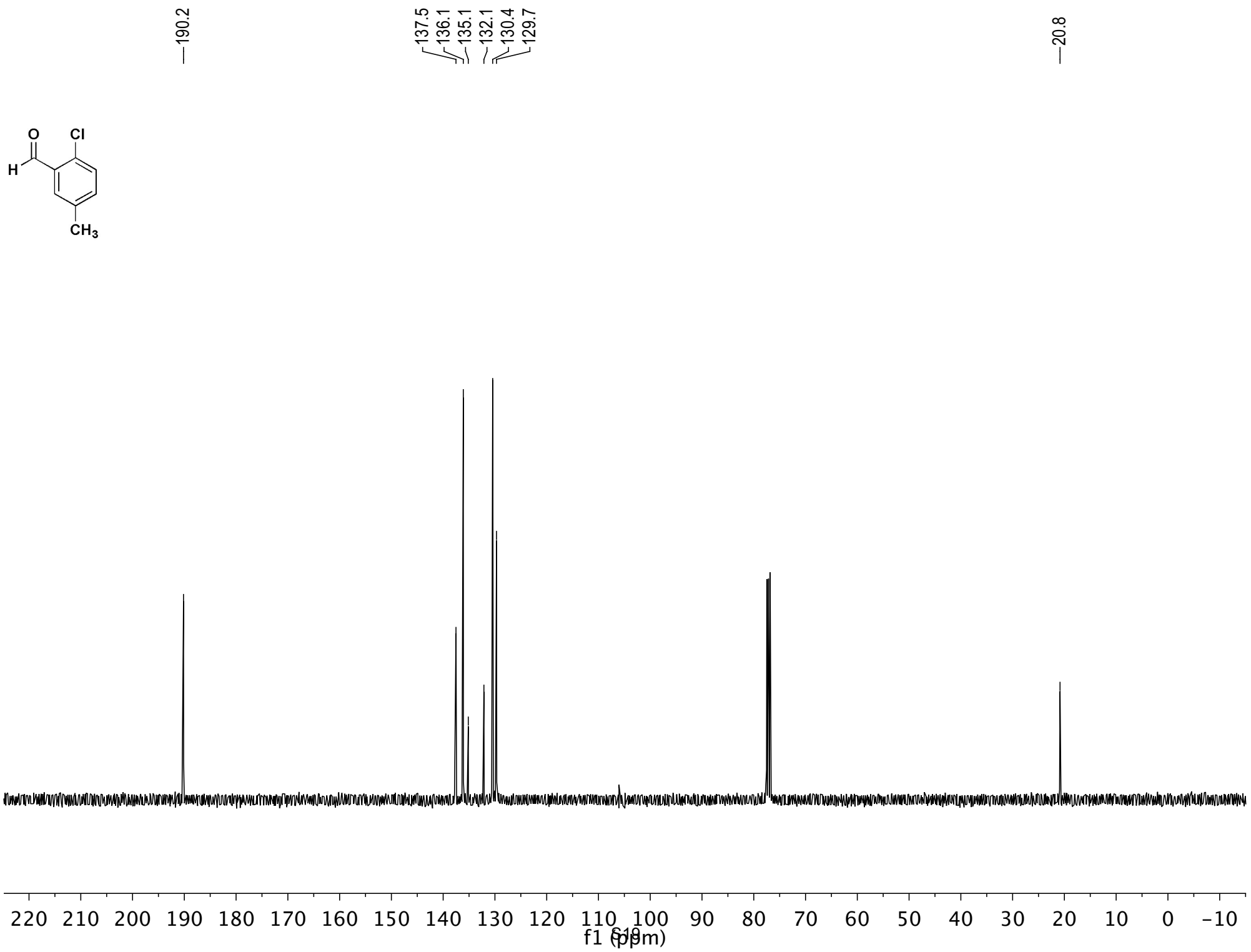
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)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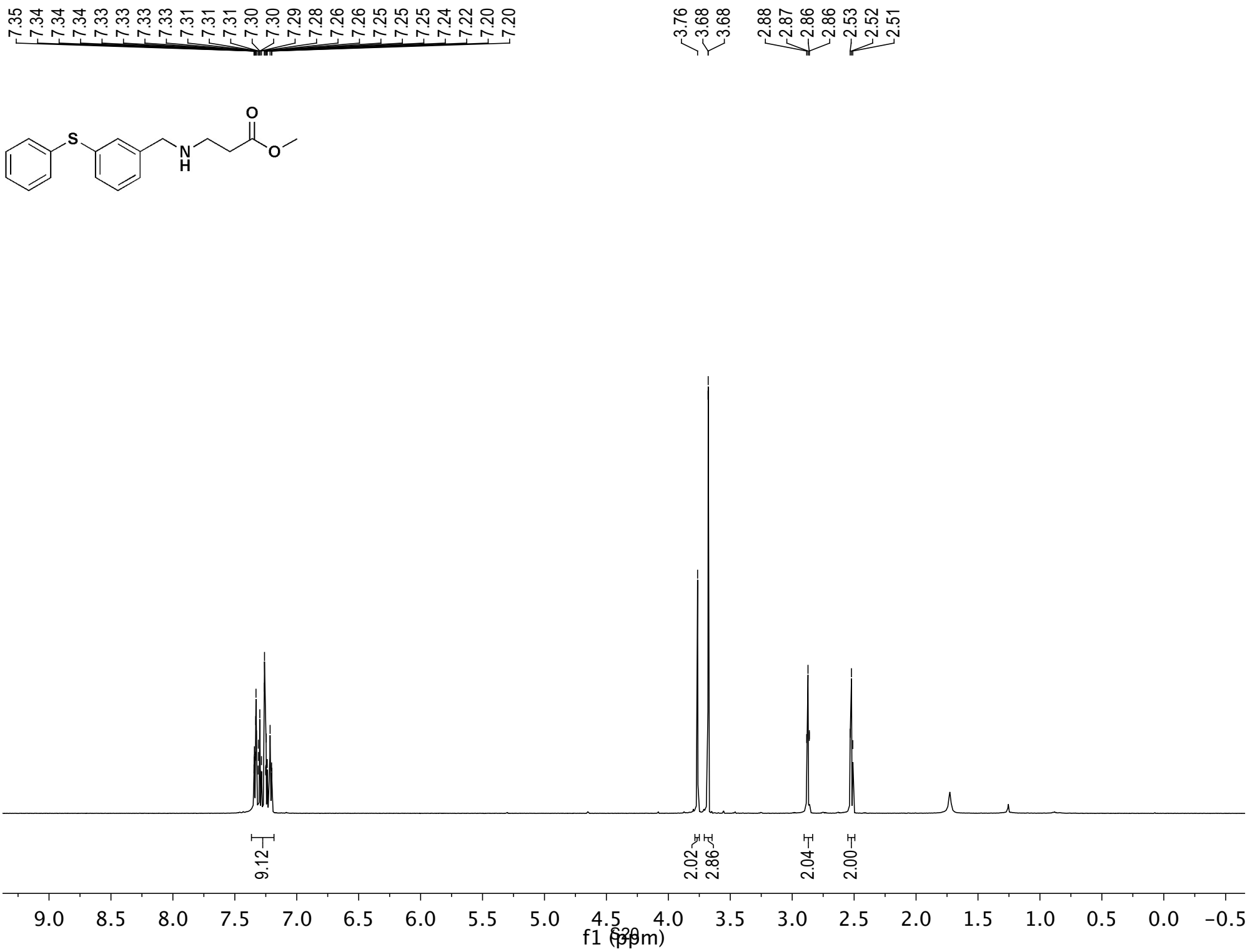
137.5
136.1
135.1
132.1
130.4
129.7
20.8
O
Cl
H
CH3
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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7.30
7.29
7.28
7.26
7.26
7.25
7.25
7.25
7.24
7.22
7.20
7.20
3.76
3.68
3.68
2.88
2.87
2.86
2.86
2.53
2.52
2.51
S
N
H
O
O
9.12
2.02
2.86
2.04
2.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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3.0
2.5
2.0
1.5
1.0
0.5
0.0
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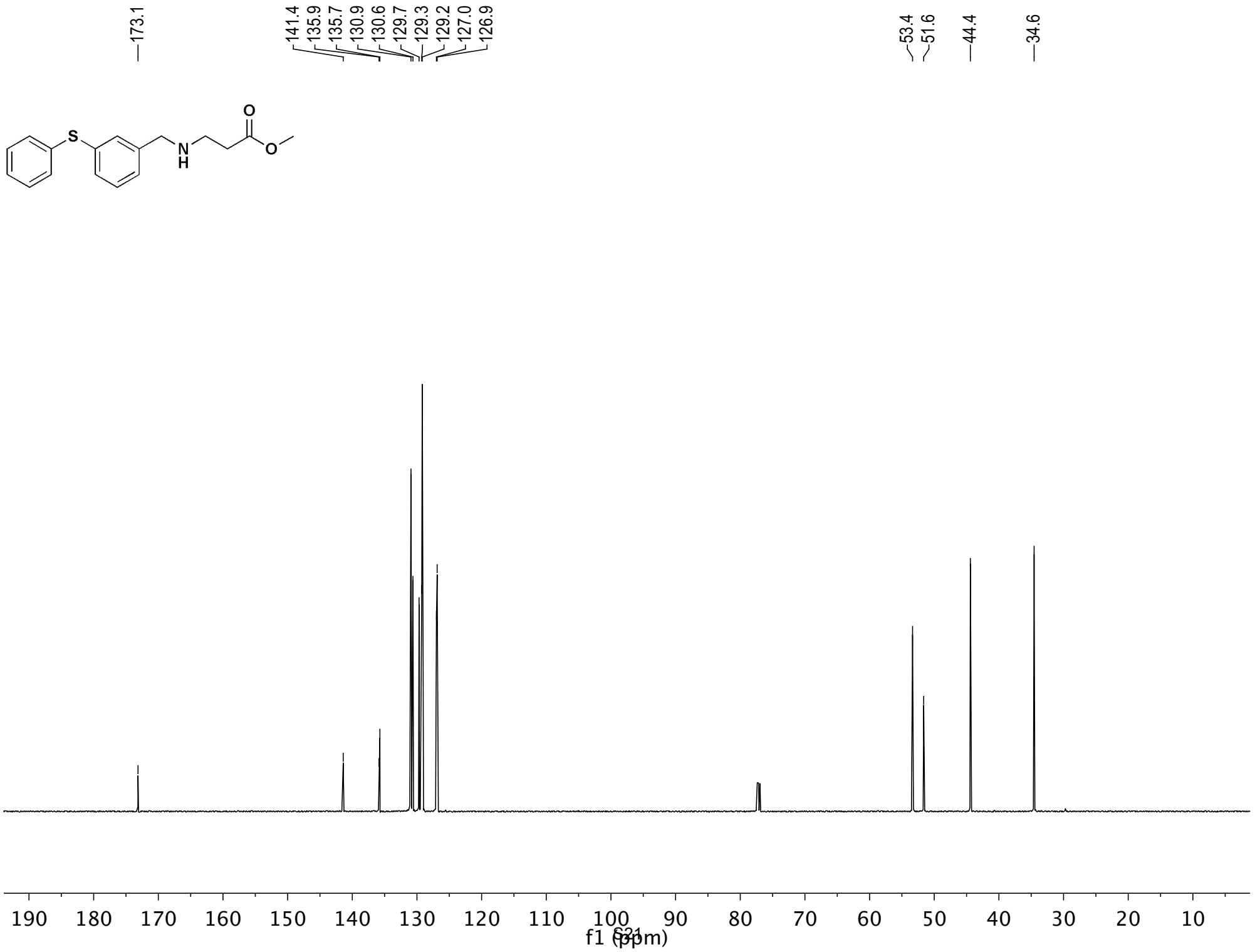
f1 (ppm)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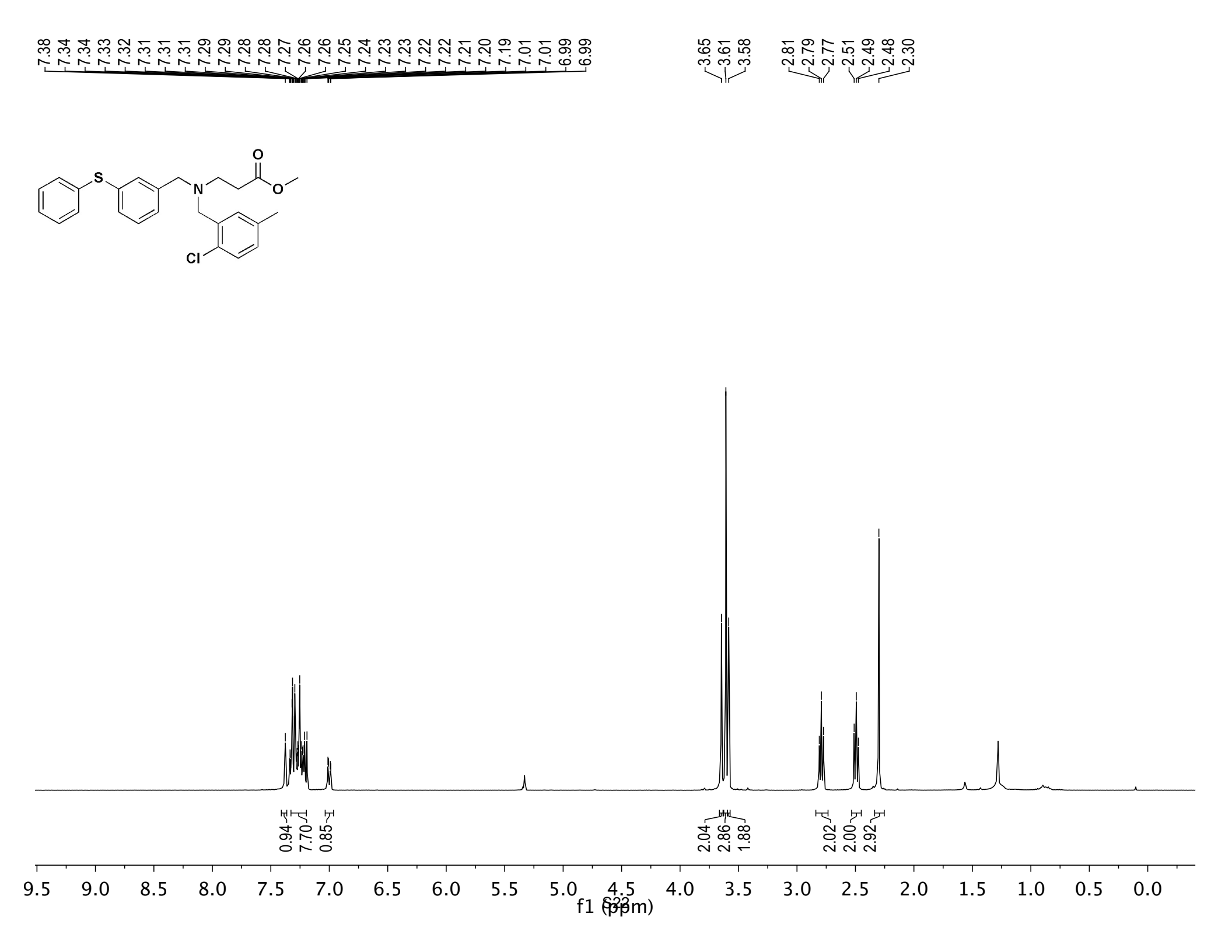
141.4
135.9
135.7
130.9
130.6
129.7
129.3
129.2
127.0
126.9
53.4
51.6
44.4
34.6
S
N
H
O
O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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7.31
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7.29
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7.28
7.27
7.26
7.26
7.25
7.24
7.23
7.23
7.22
7.22
7.21
7.20
7.19
7.01
7.01
6.99
6.99
3.65
3.61
3.58
2.81
2.79
2.77
2.51
2.49
2.48
2.30
O
S
N
O
Cl
0.94
7.70
0.85
2.04
2.86
1.88
2.02
2.00
2.92
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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2.5
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1.0
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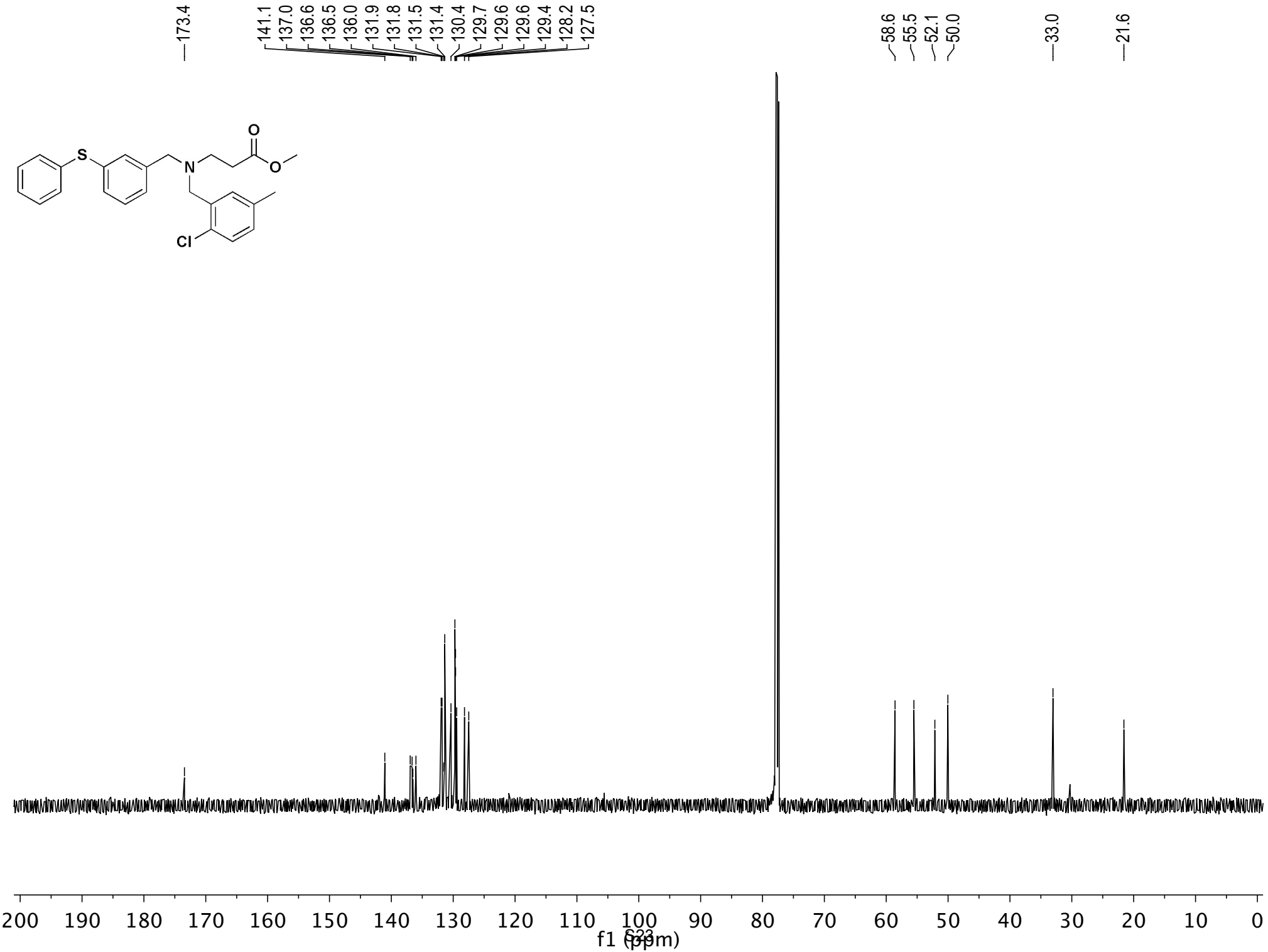
0.0
f1 (ppm)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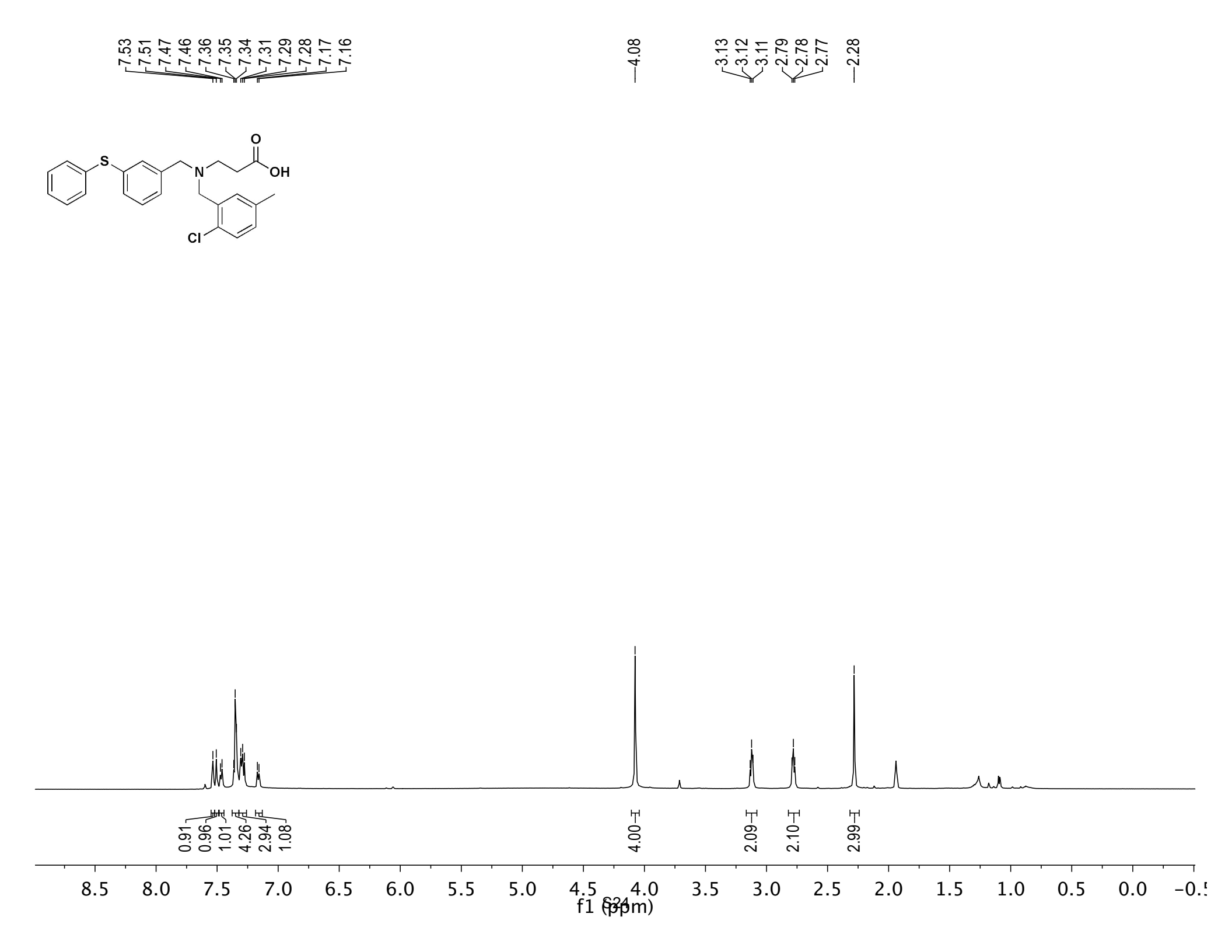
136.0
131.9
131.8
131.5
131.4
130.4
129.7
129.6
129.6
129.4
128.2
127.5
58.6
55.5
52.1
50.0
33.0
21.6
S
N
O
O
Cl
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.35
7.34
7.31
7.29
7.28
7.17
7.16
4.08
3.13
3.12
3.11
2.79
2.78
2.77
2.28
O
OH
S
N
Cl
0.91
0.96
1.01
4.26
2.94
1.08
4.00
2.09
2.10
2.99
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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2.5
2.0
1.5
1.0
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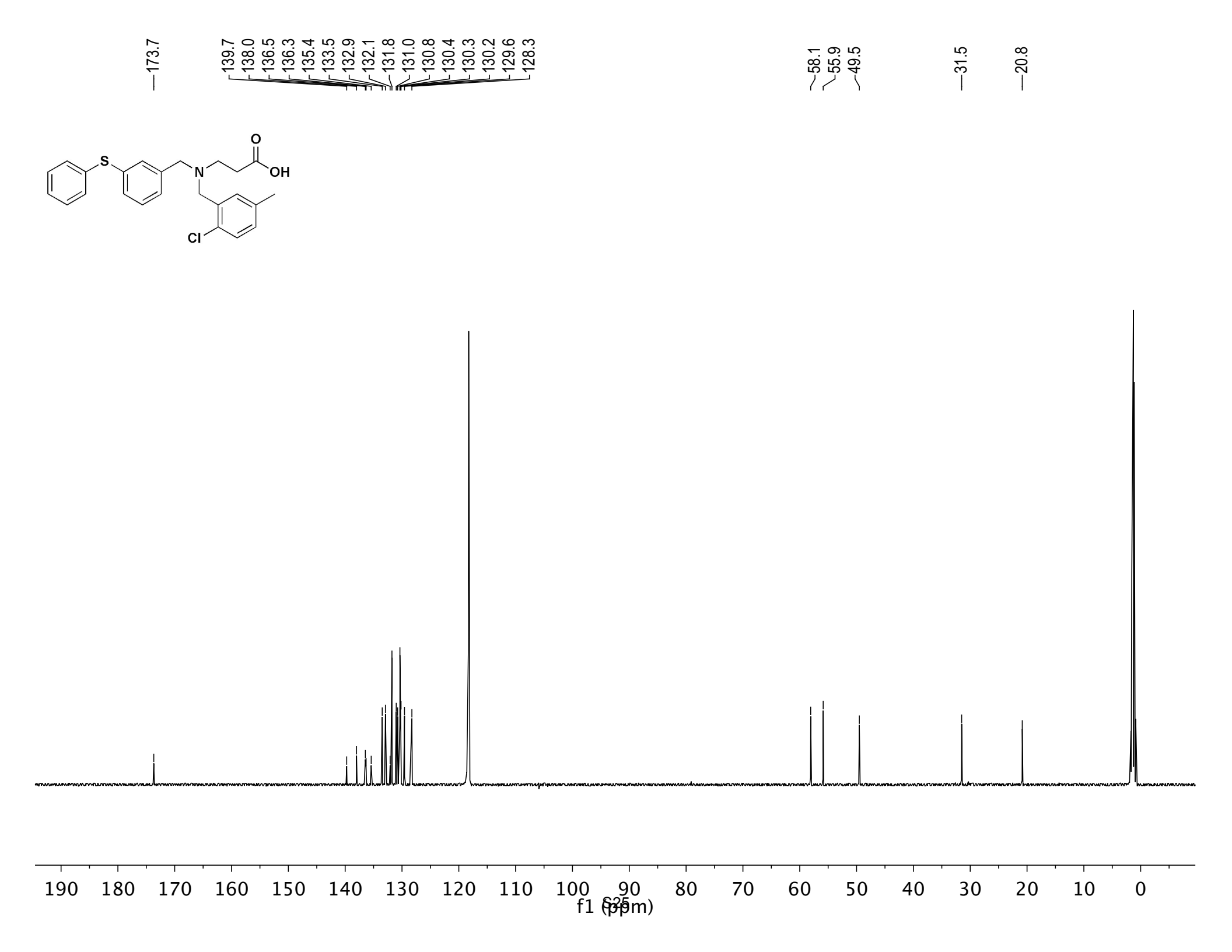
0.0
f1 (ppm)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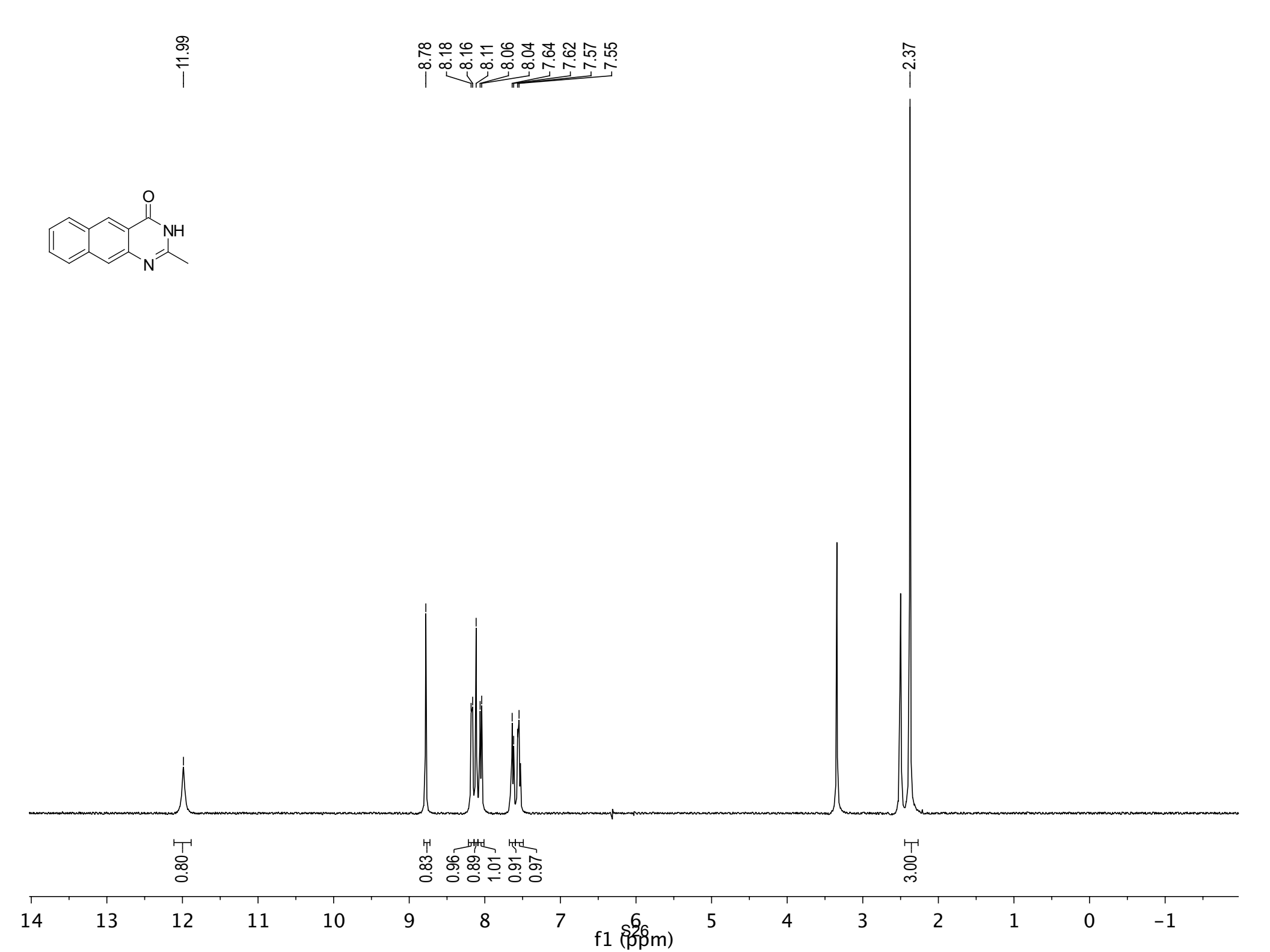
131.0
130.8
130.4
130.3
130.2
129.6
128.3
58.1
55.9
49.5
31.5
20.8
O
OH
N
S
Cl
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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8.16
8.11
8.06
8.04
7.64
7.62
7.57
7.55
2.37
O
NH
N
0.80
0.83
0.96
0.89
1.01
0.91
0.97
3.00
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10
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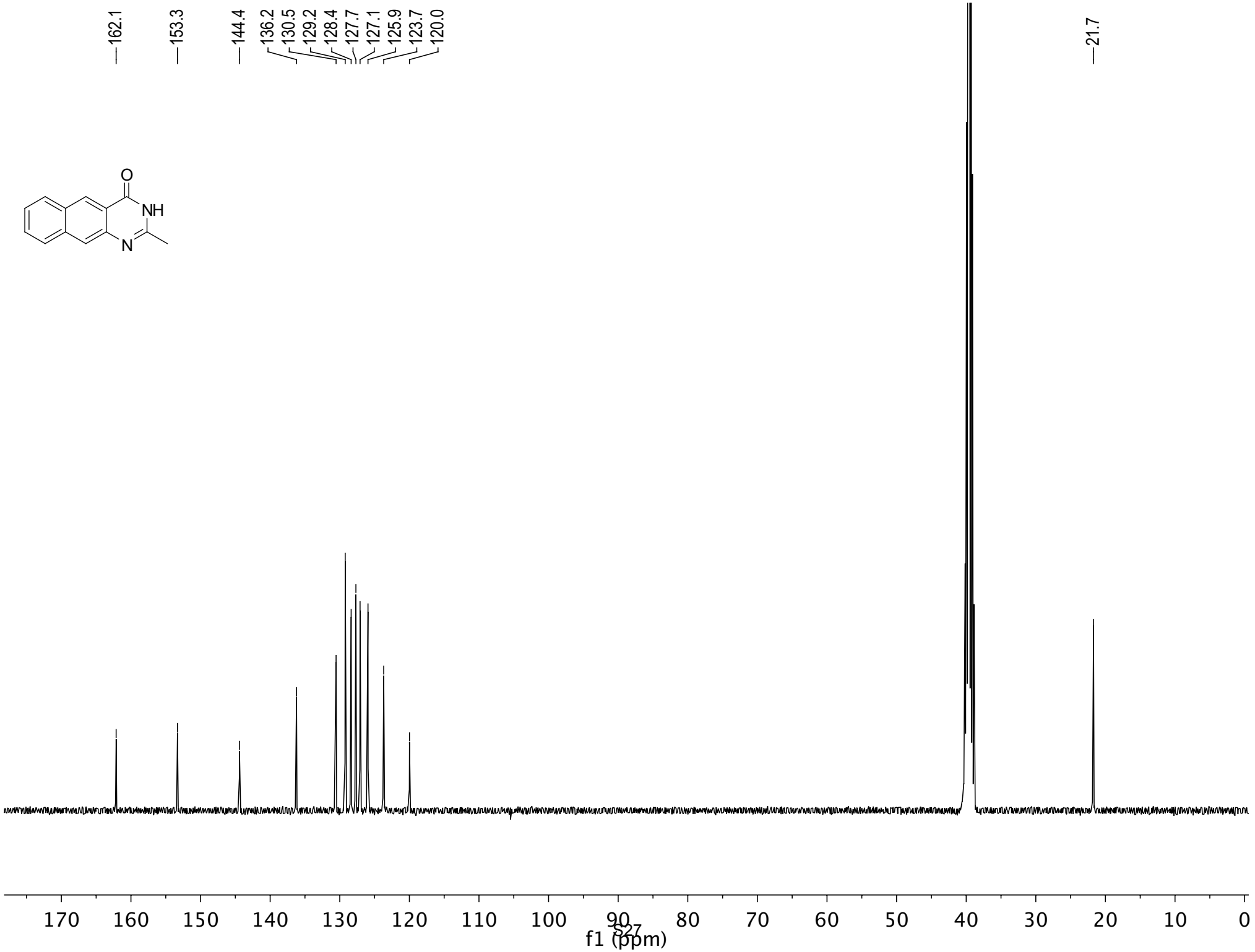
8
7
6
5
4
3
2
1
0
−1
f1 (ppm)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153.3
144.4
136.2
130.5
129.2
128.4
127.7
127.1
125.9
123.7
120.0
21.7
O
NH
N
170
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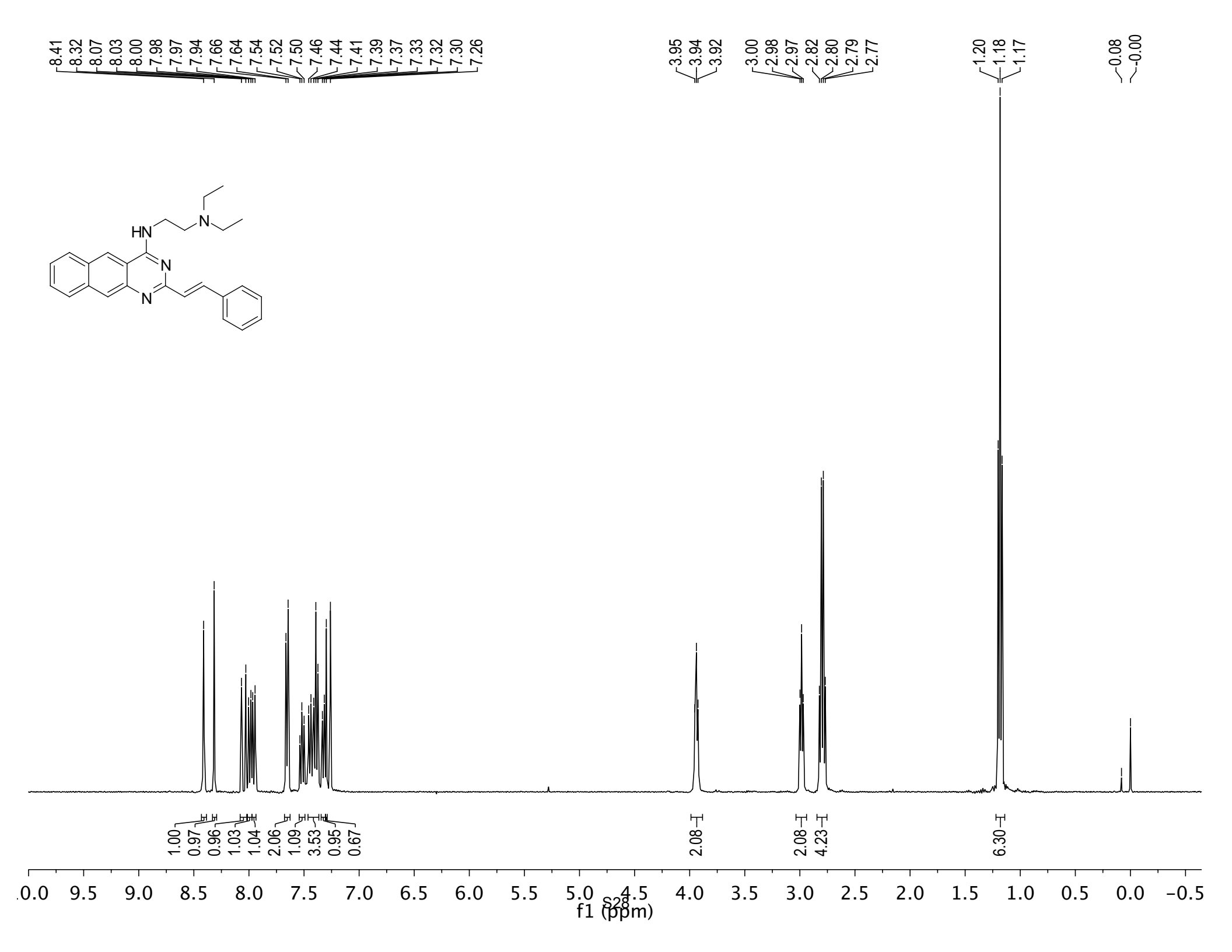
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.50
7.46
7.44
7.41
7.39
7.37
7.33
7.32
7.30
7.26
3.95
3.94
3.92
3.00
2.98
2.97
2.82
2.80
2.79
2.77
1.20
1.18
1.17
0.08
-0.00
HN
N
N
N
1.00
0.97
0.96
1.03
1.04
2.06
1.09
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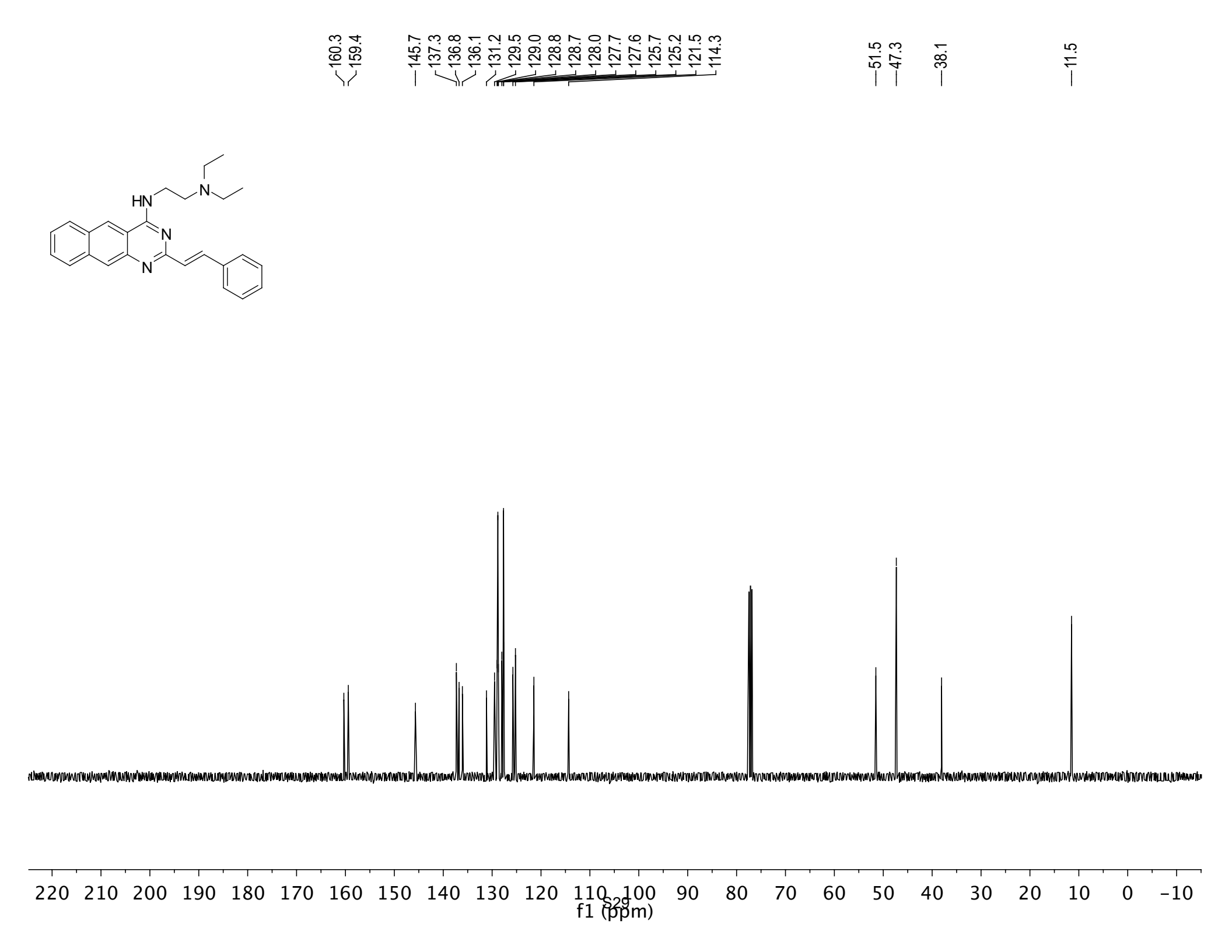
0.95
0.67
2.08
2.08
4.23
6.30
f1 (ppm)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128.0
127.7
127.6
125.7
125.2
121.5
114.3
51.5
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N
N
N
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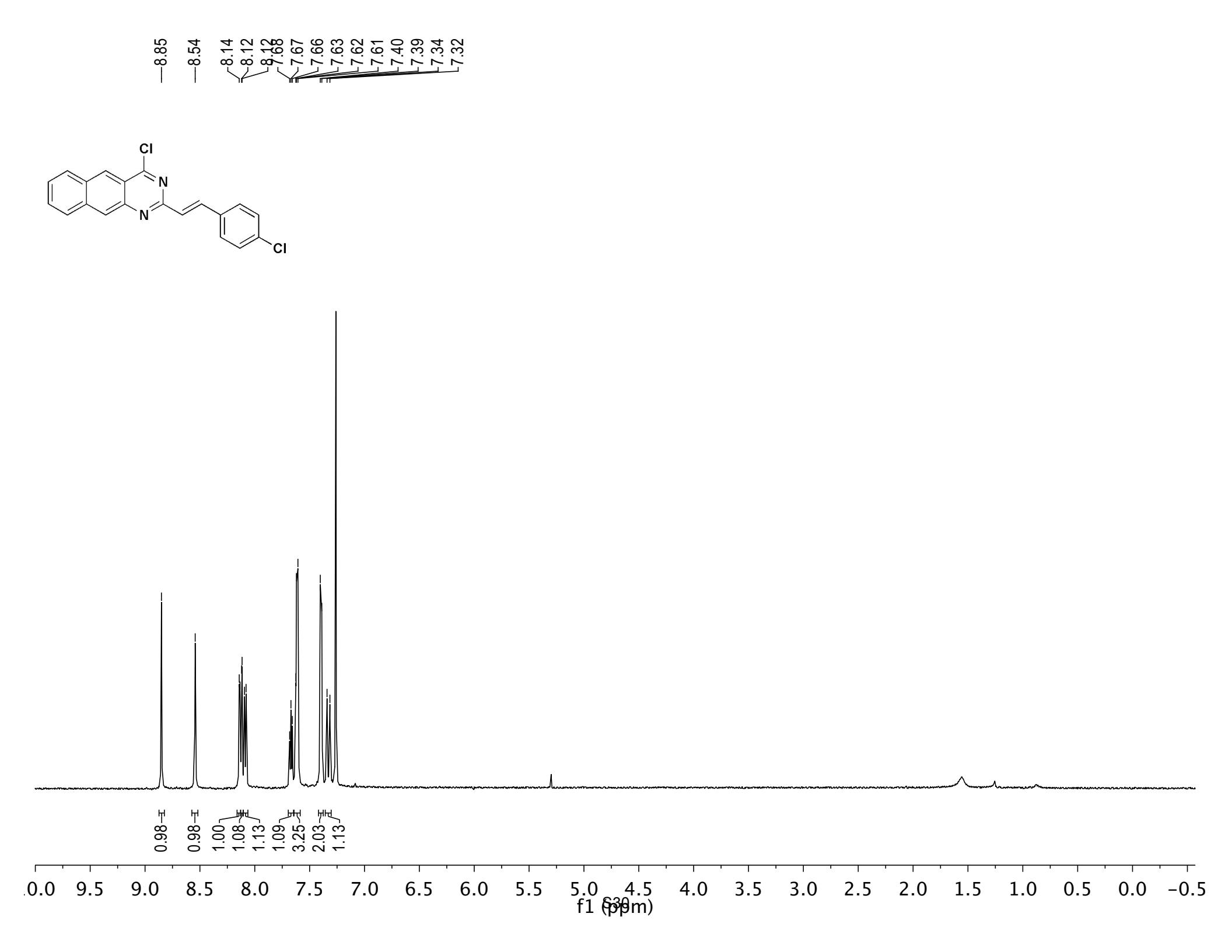
f1 (ppm)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7.63
7.62
7.61
7.40
7.39
7.34
7.32
Cl
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Cl
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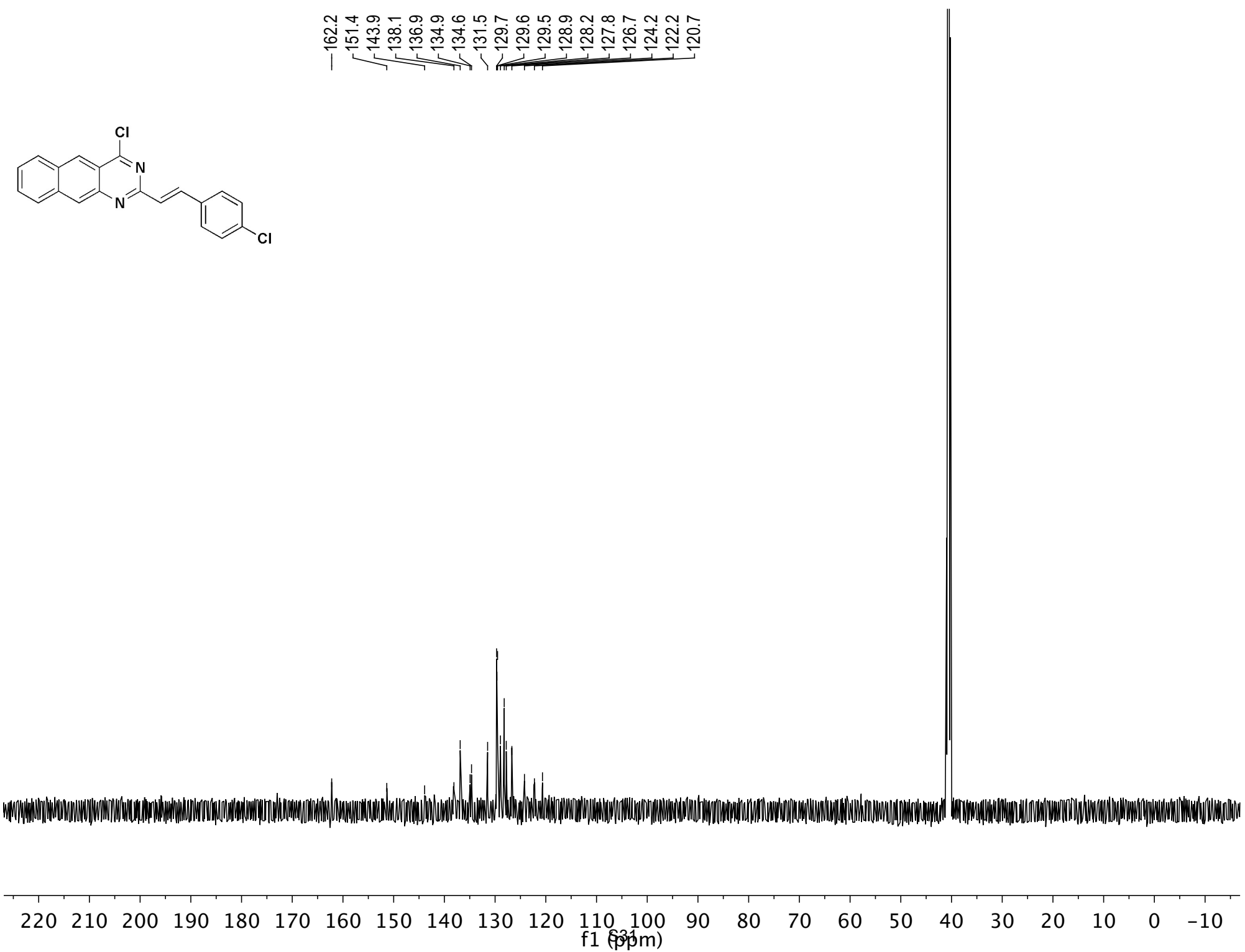
1.08
1.13
1.09
3.25
2.03
1.13
f1 (ppm)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134.9
134.6
131.5
129.7
129.6
129.5
128.9
128.2
127.8
126.7
124.2
122.2
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Cl
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Cl
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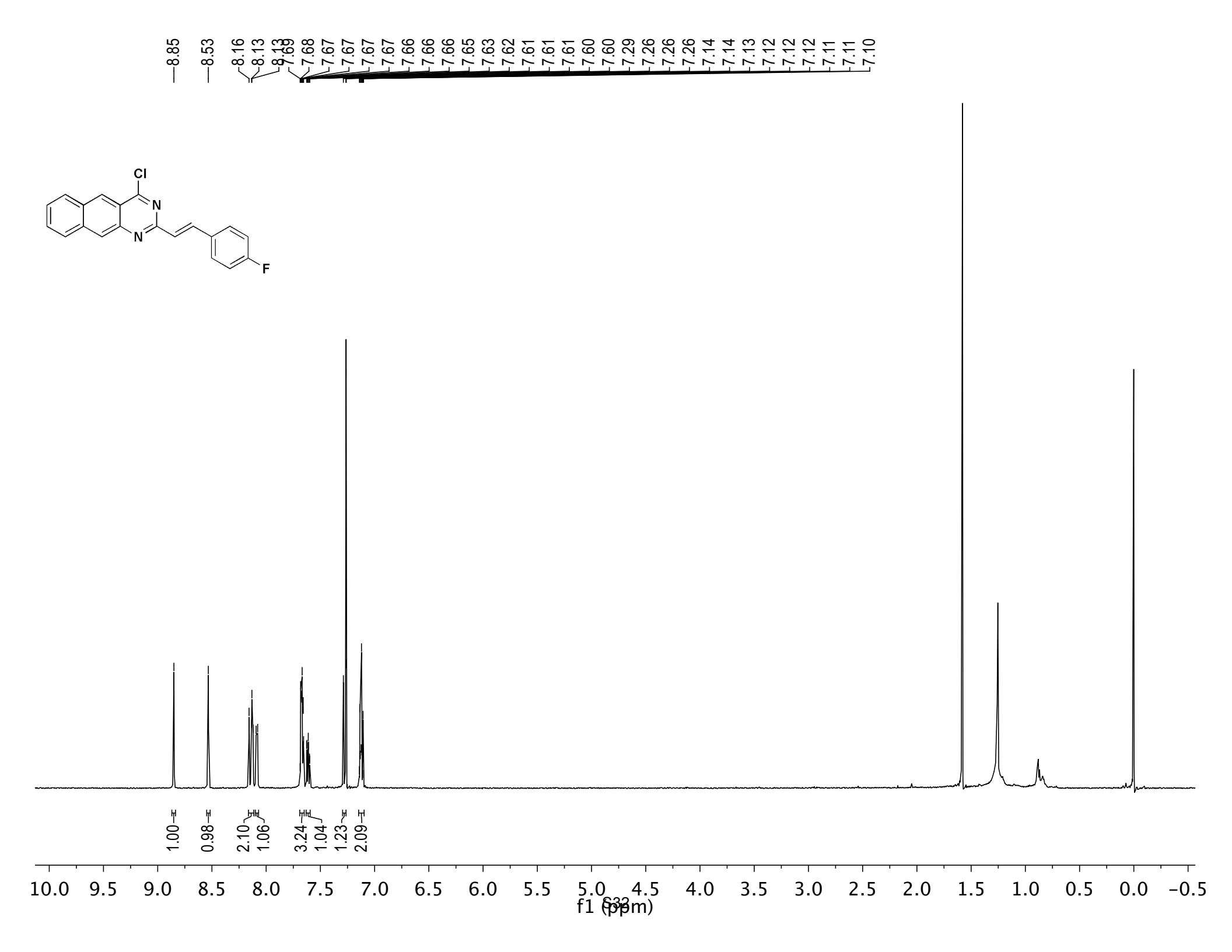
f1 (ppm)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7.67
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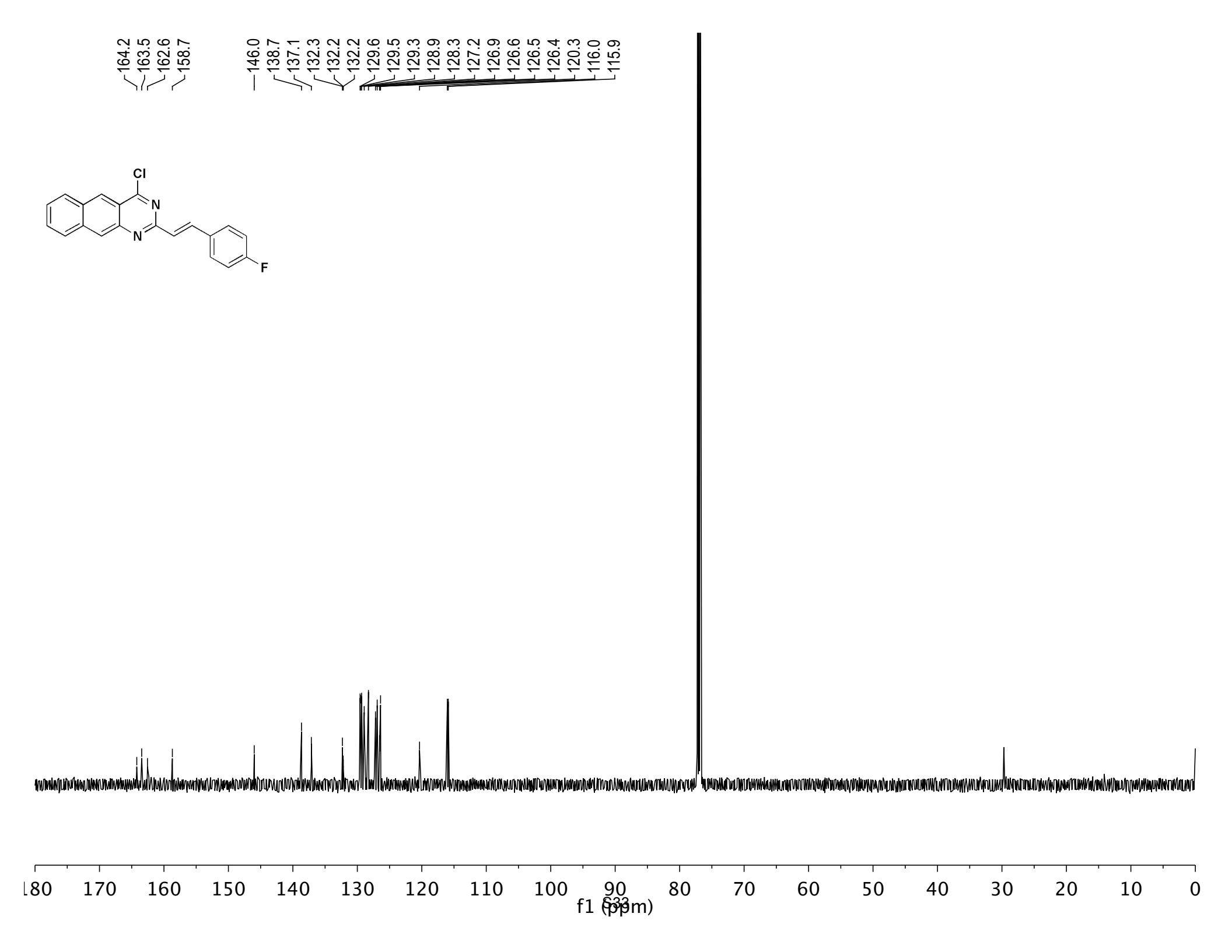
7.14
7.14
7.13
7.12
7.12
7.12
7.11
7.11
7.10
Cl
N
N
F
1.00
0.98
2.10
1.06
3.24
1.04
1.23
2.09
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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158.7
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138.7
137.1
132.3
132.2
132.2
129.6
129.5
129.3
128.9
128.3
127.2
126.9
126.6
126.5
126.4
120.3
116.0
115.9
Cl
N
N
F
180
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120
110
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90
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50
40
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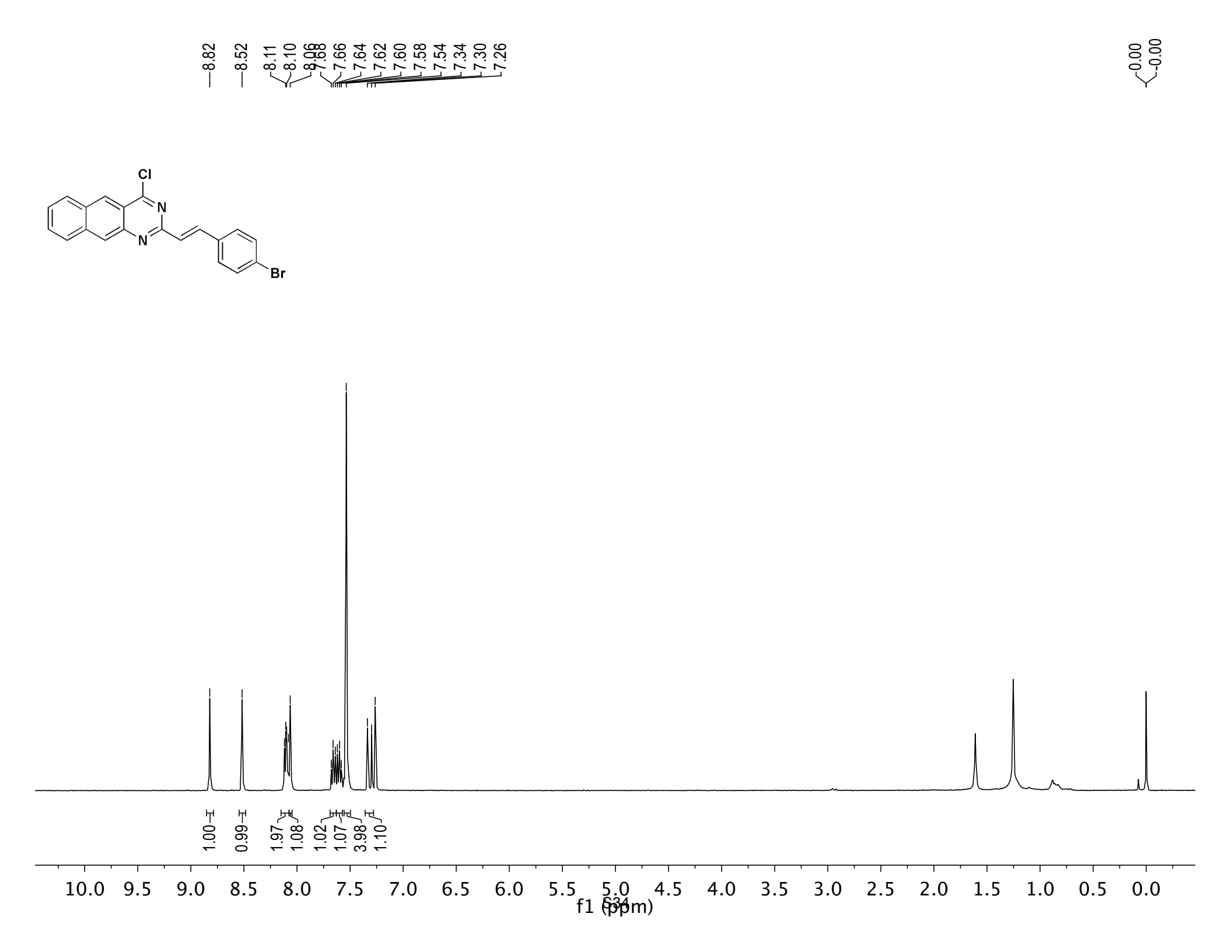
f1 (ppm)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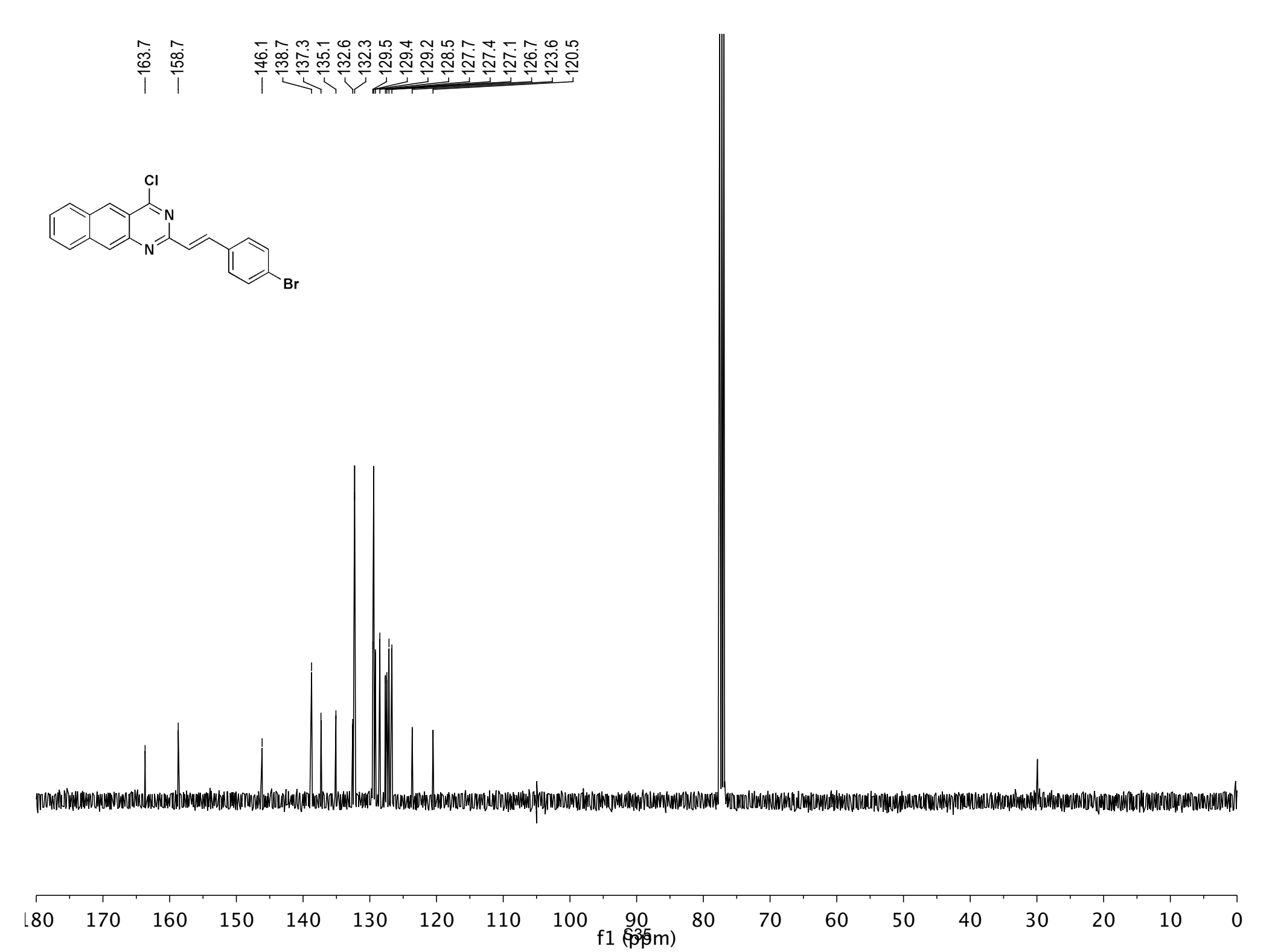
7.62
7.60
7.58
7.54
7.34
7.30
7.26
0.00
-0.00
Cl
N
N
Br
1.00
0.99
1.97
1.08
1.02
1.07
3.98
1.10
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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132.3
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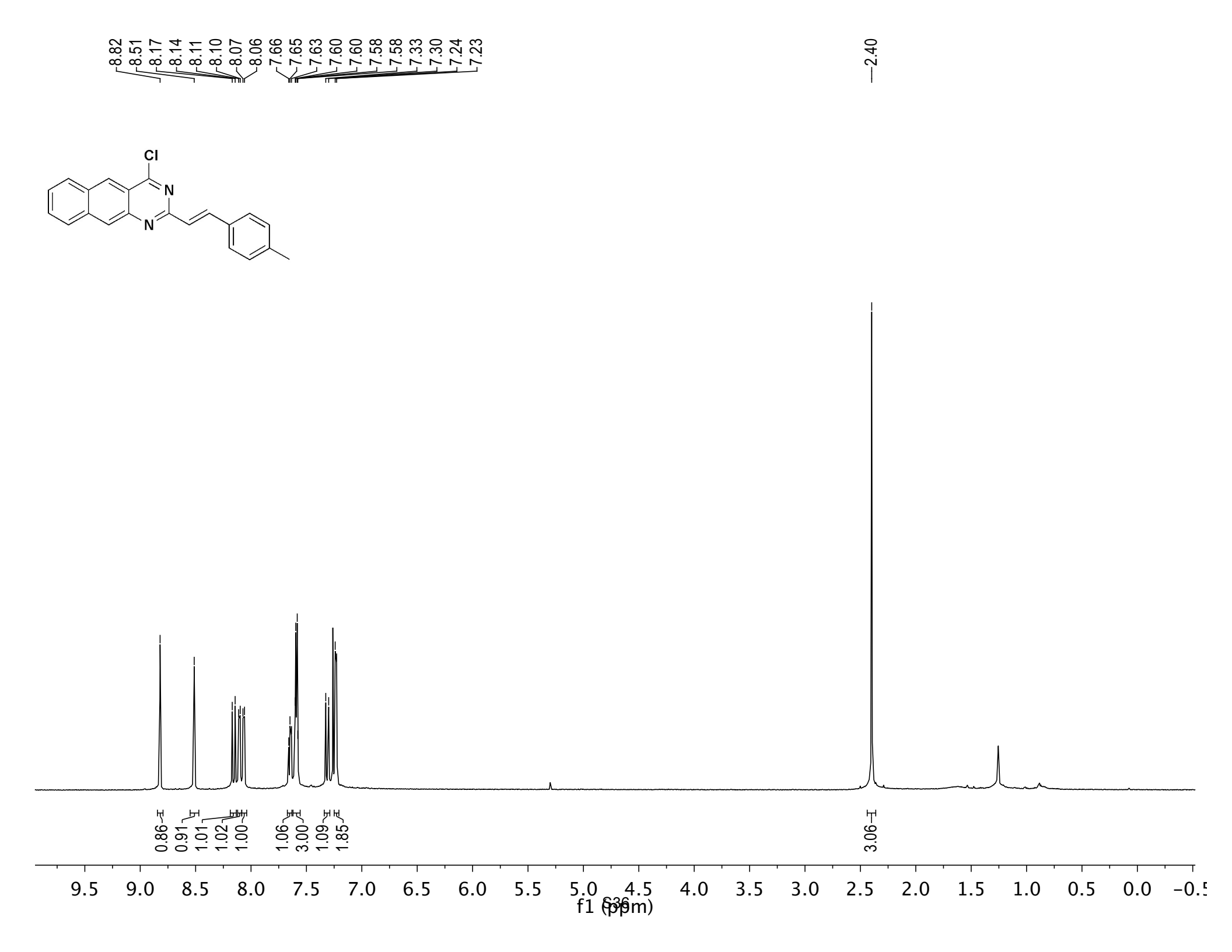
129.4
129.2
128.5
127.7
127.4
127.1
126.7
123.6
120.5
Cl
N
N
Br
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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8.06
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7.63
7.60
7.60
7.58
7.58
7.33
7.30
7.24
7.23
2.40
Cl
N
N
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0.91
1.01
1.02
1.00
1.06
3.00
1.09
1.85
3.06
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9.0
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8.0
7.5
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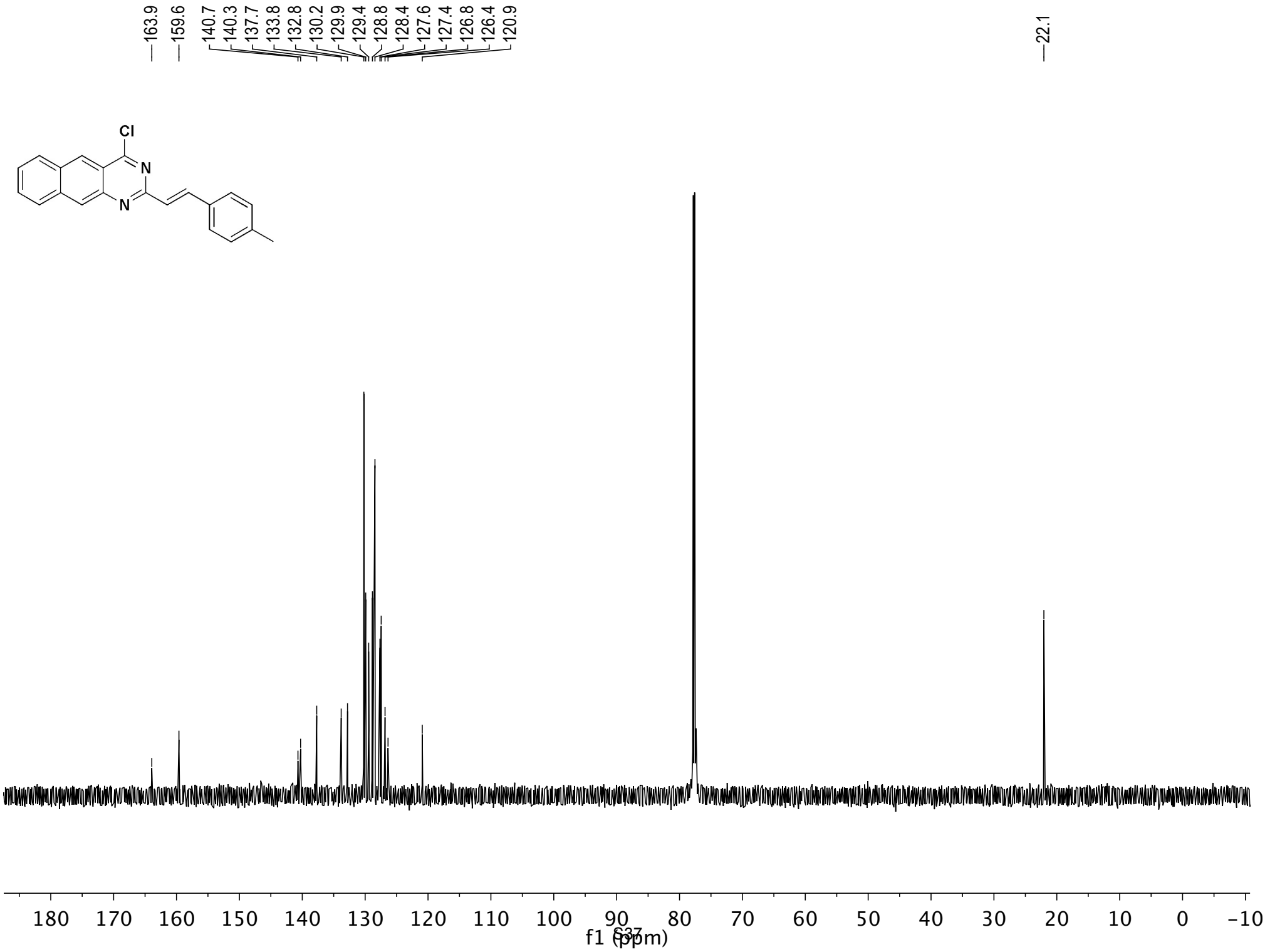
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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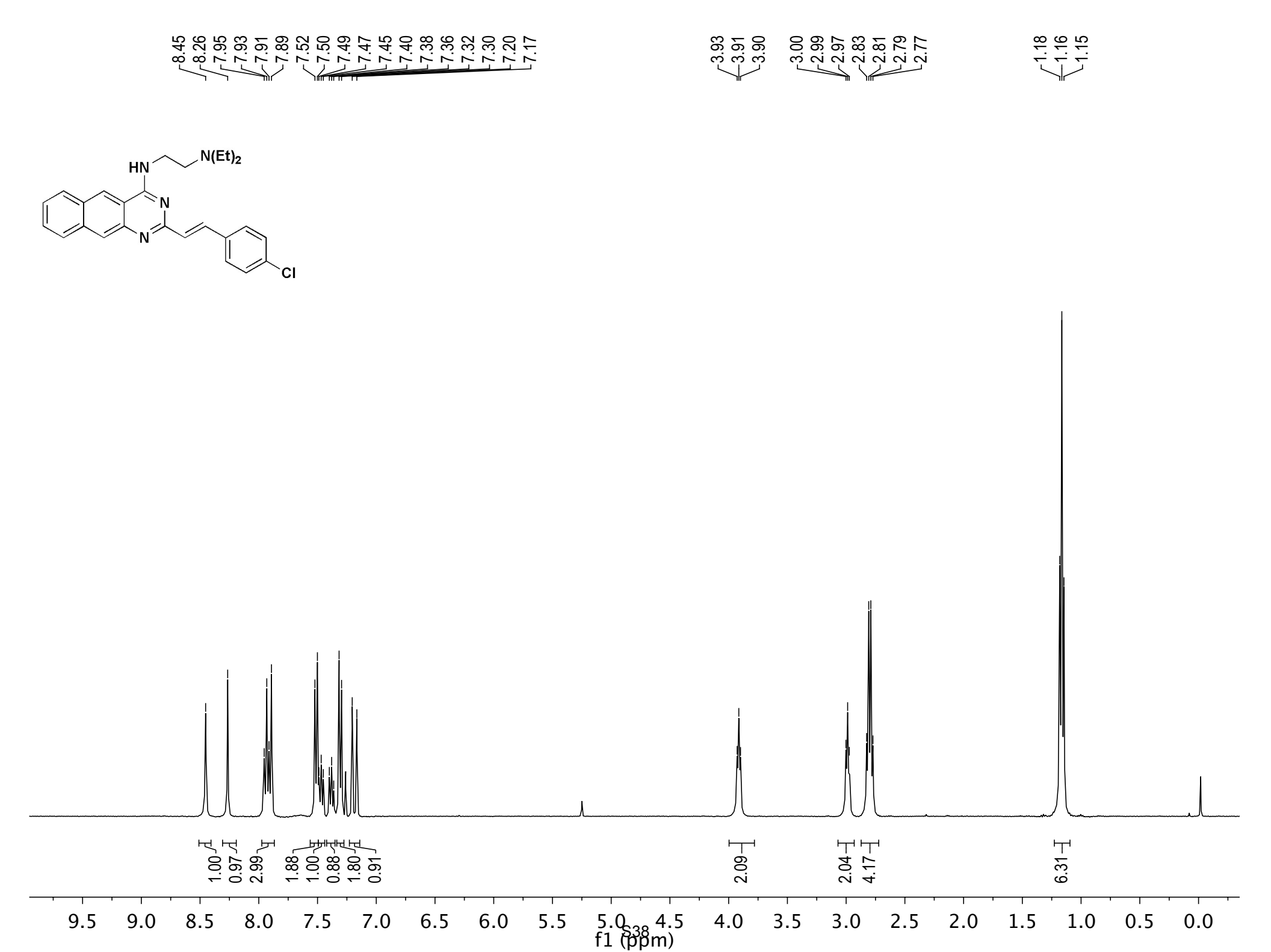
140.7
140.3
137.7
133.8
132.8
130.2
129.9
129.4
128.8
128.4
127.6
127.4
126.8
126.4
120.9
22.1
Cl
N
N
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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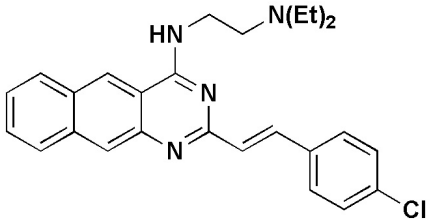
7.89
7.52
7.50
7.49
7.47
7.45
7.40
7.38
7.36
7.32
7.30
7.20
7.17
3.93
3.91
3.90
3.00
2.99
2.97
2.83
2.81
2.79
2.77
1.18
1.16
1.15
HN
N(Et)2
N
N
Cl
1.00
0.97
2.99
1.88
1.00
0.88
1.80
0.91
2.09
2.04
4.17
6.31
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



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159.5
145.6
136.2
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135.3
134.4
131.3
130.1
129.2
129.1
128.9
128.0
127.8
125.9
125.2
121.9
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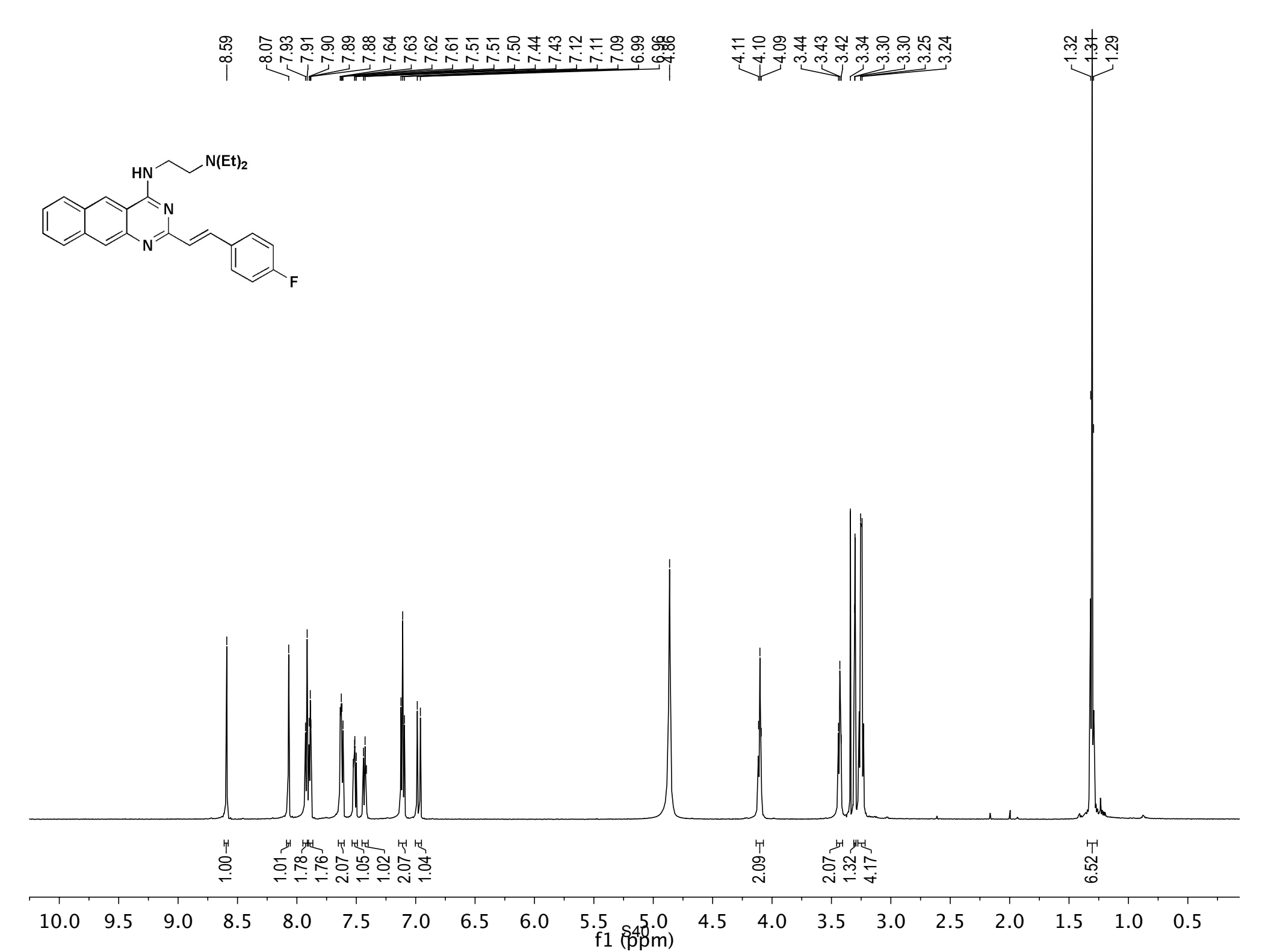
51.7
47.5
38.0
11.3

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

f1 (ppm)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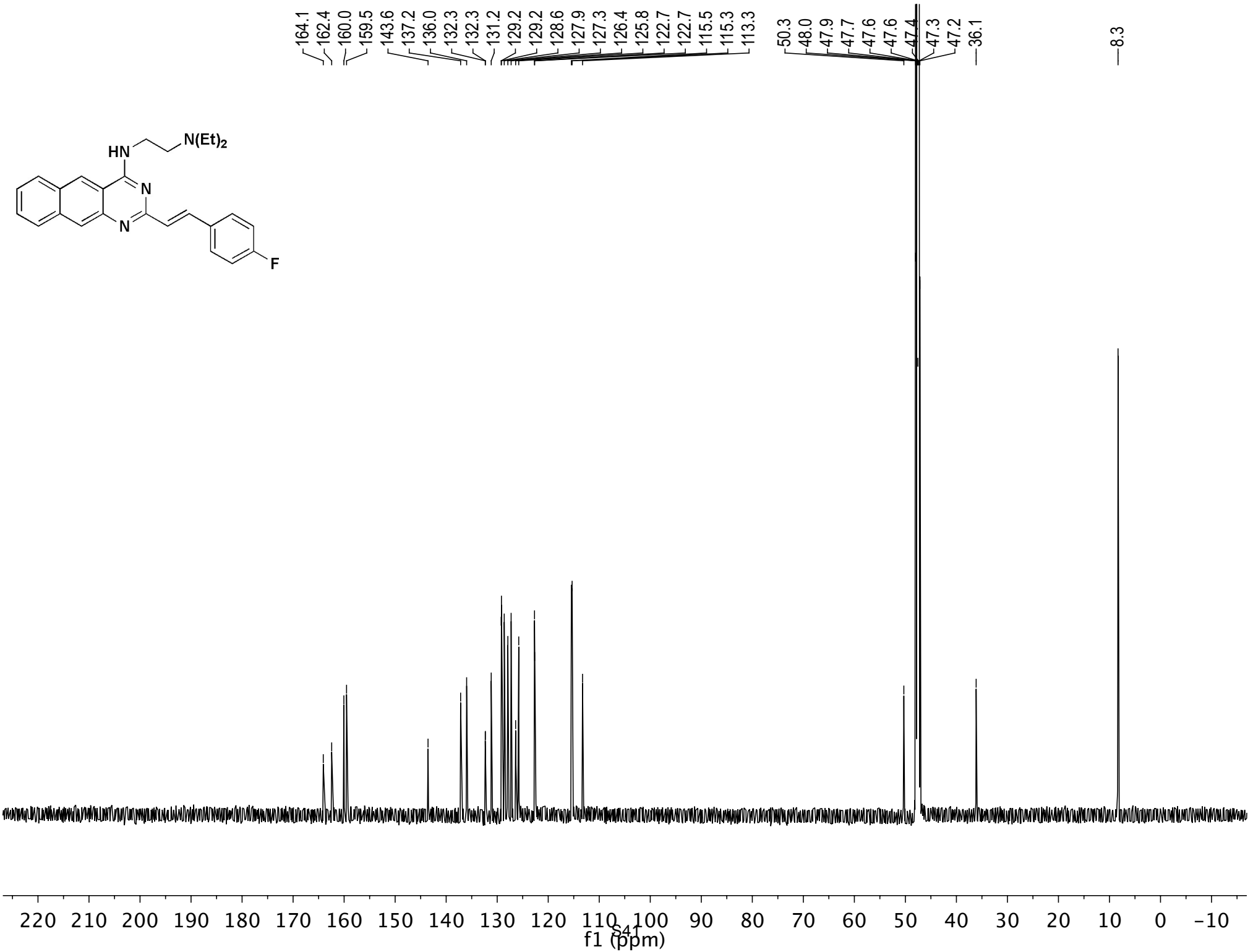
7.62
7.61
7.51
7.51
7.50
7.44
7.43
7.12
7.11
7.09
6.99
6.96
6.86
4.11
4.10
4.09
3.44
3.43
3.42
3.34
3.30
3.30
3.25
3.24
1.32
1.31
1.29
HN
N(Et)2
N
N
F
1.00
1.01
1.78
1.76
2.07
1.05
1.02
2.07
1.04
2.09
2.07
1.32
4.17
6.52
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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162.4
160.0
159.5
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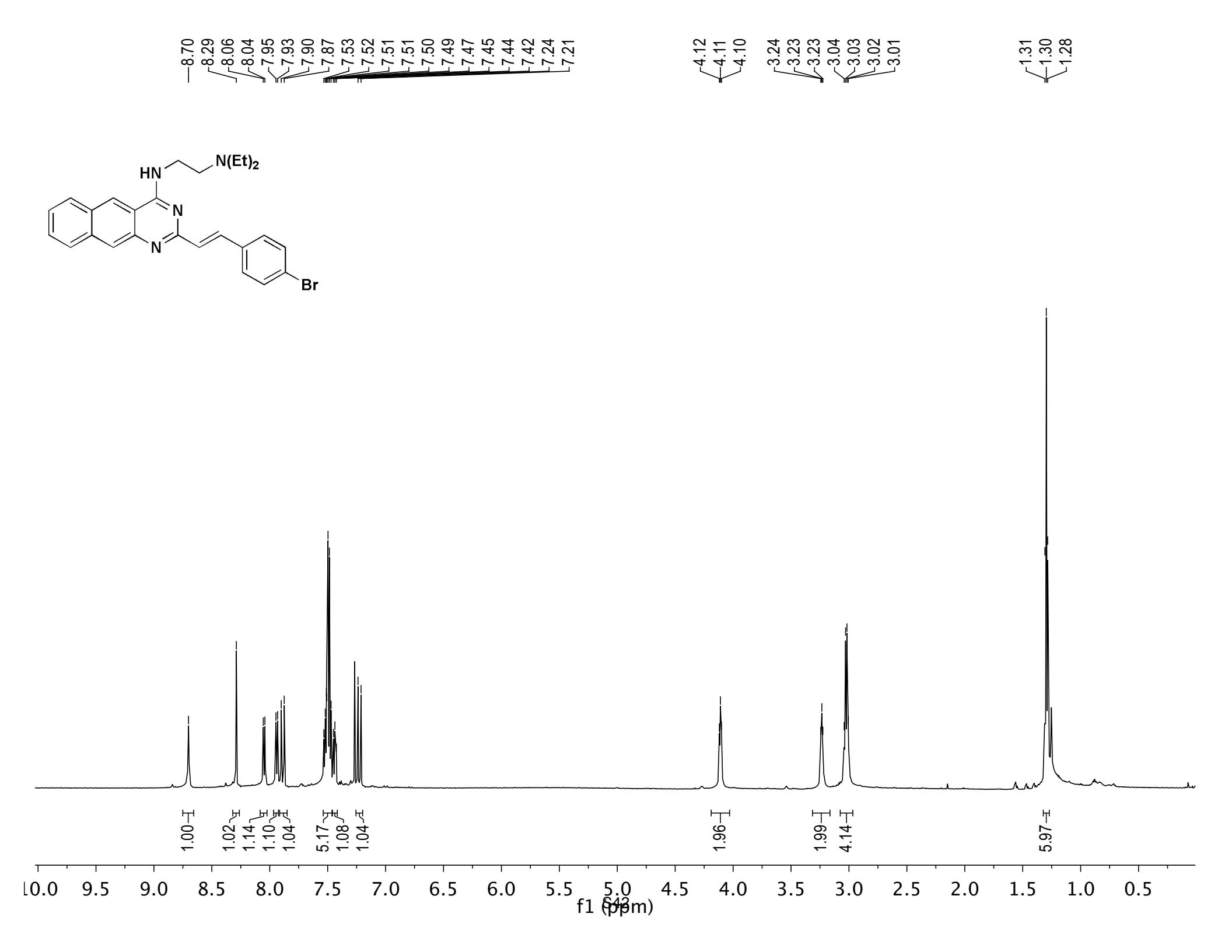
137.2
136.0
132.3
132.3
131.2
129.2
129.2
128.6
127.9
127.3
126.4
125.8
122.7
122.7
115.5
115.3
113.3
50.3
48.0
47.9
47.7
47.6
47.6
47.4
47.3
47.2
36.1
8.3
HN
N(Et)2
N
N
F
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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8.29
8.06
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7.93
7.90
7.87
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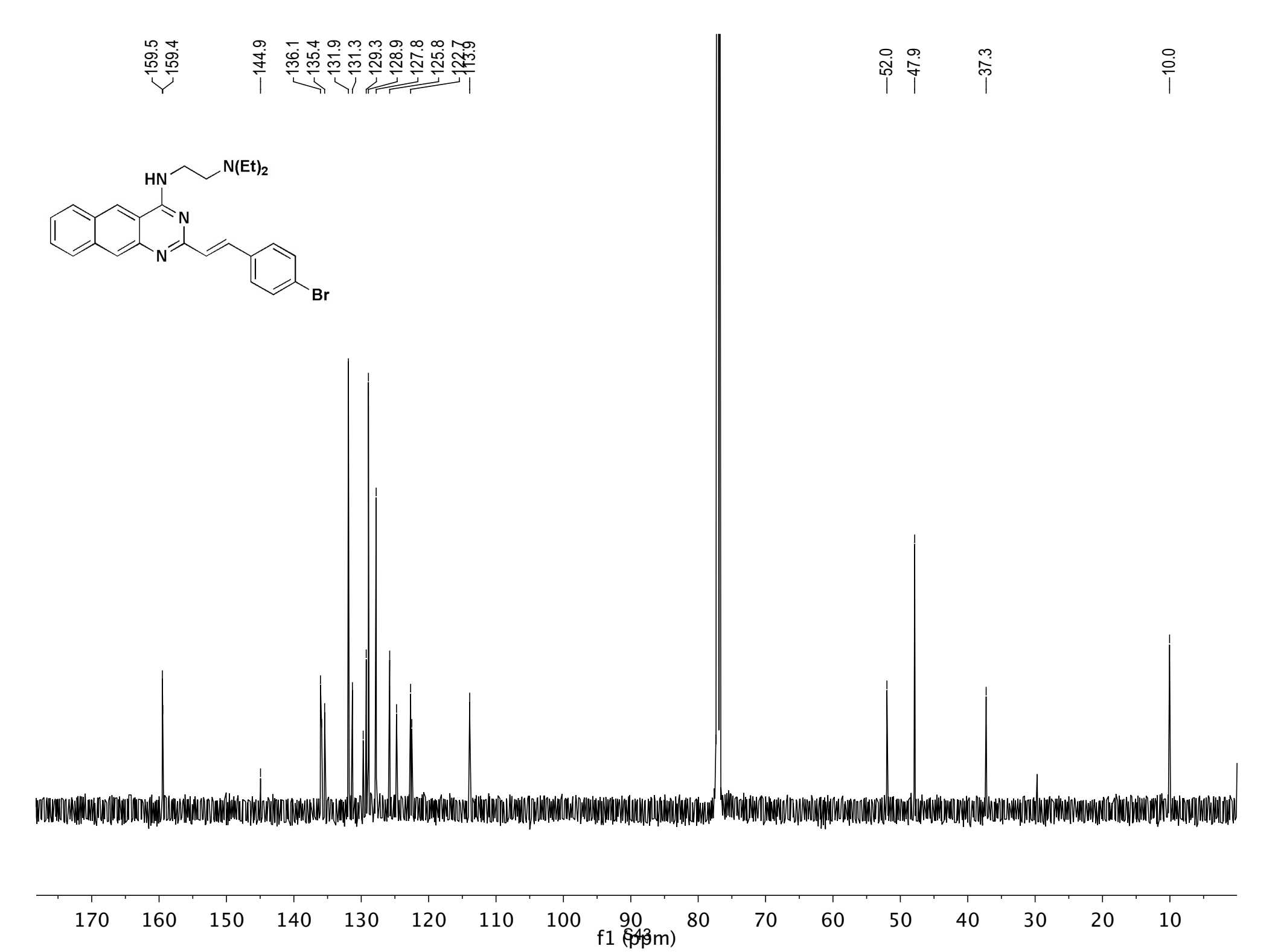
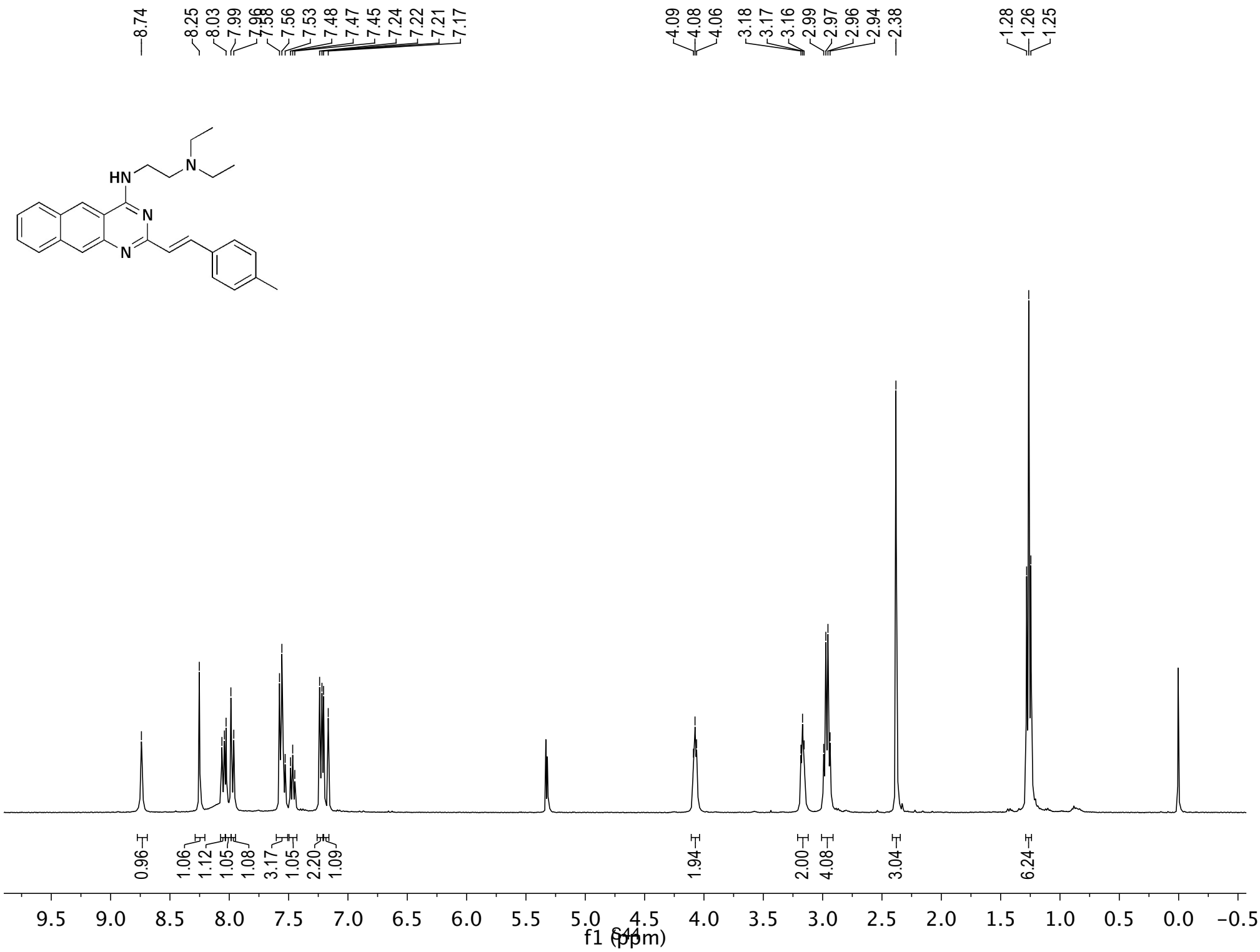
7.52
7.51
7.51
7.50
7.49
7.47
7.45
7.44
7.42
7.24
7.21
4.12
4.11
4.10
3.24
3.23
3.23
3.04
3.03
3.02
3.01
1.31
1.30
1.28
HN
N(Et)2
N
N
Br
1.00
1.02
1.14
1.10
1.04
5.17
1.08
1.04
1.96
1.99
4.14
5.97
f1 (ppm)


159.5
159.4
144.9
136.1
135.4
131.9
131.3
129.3
128.9
127.8
125.8
122.7
113.9
52.0
47.9
37.3
10.0
HN
N(Et)2
N
N
Br
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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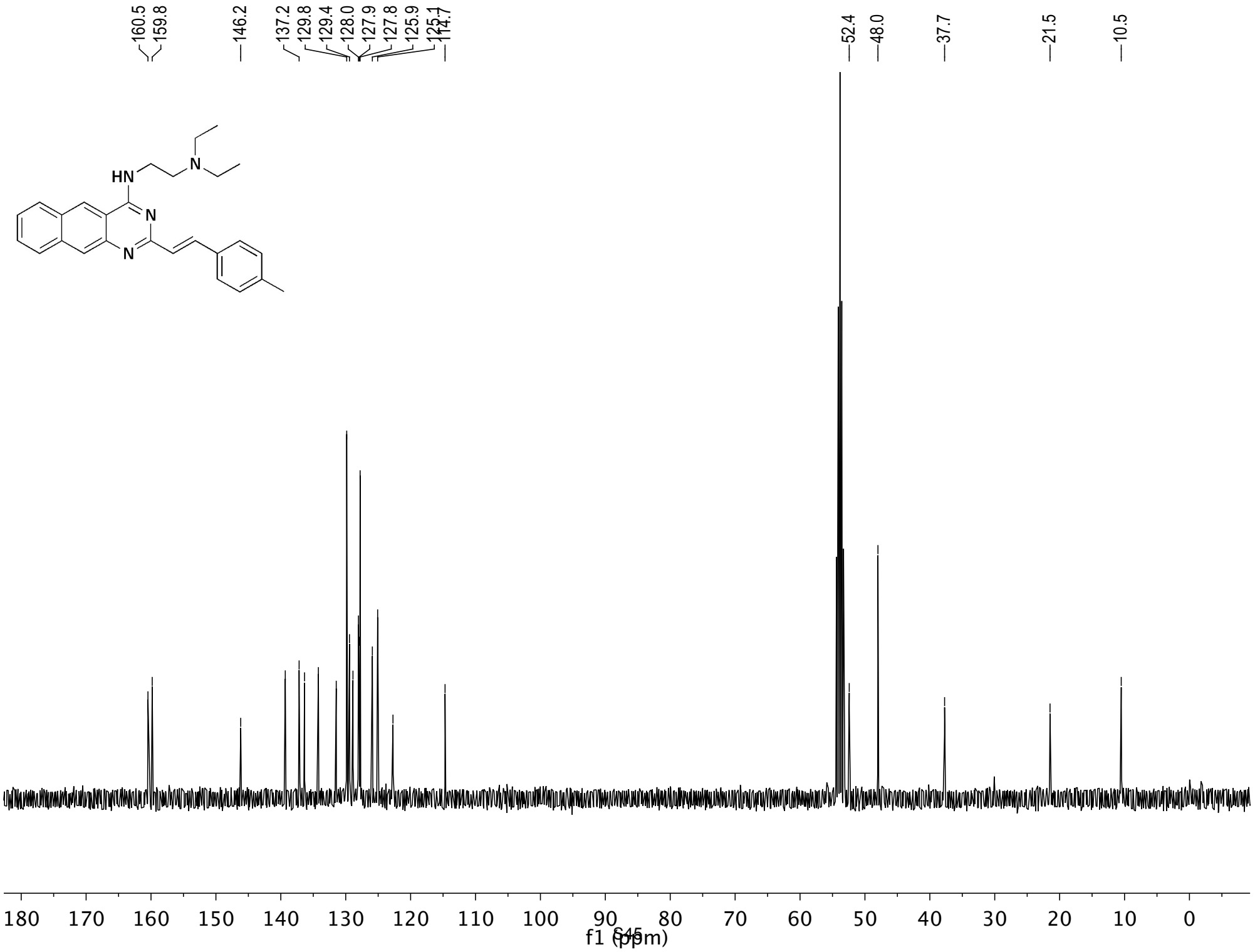
8.03
7.99
7.96
7.58
7.56
7.53
7.48
7.47
7.45
7.24
7.22
7.21
7.17
4.09
4.08
4.06
3.18
3.17
3.16
2.99
2.97
2.96
2.94
2.38
1.28
1.26
1.25
HN
N
N
N
0.96
1.06
1.12
1.05
1.08
3.17
1.05
2.20
1.09
1.94
2.00
4.08
3.04
6.24
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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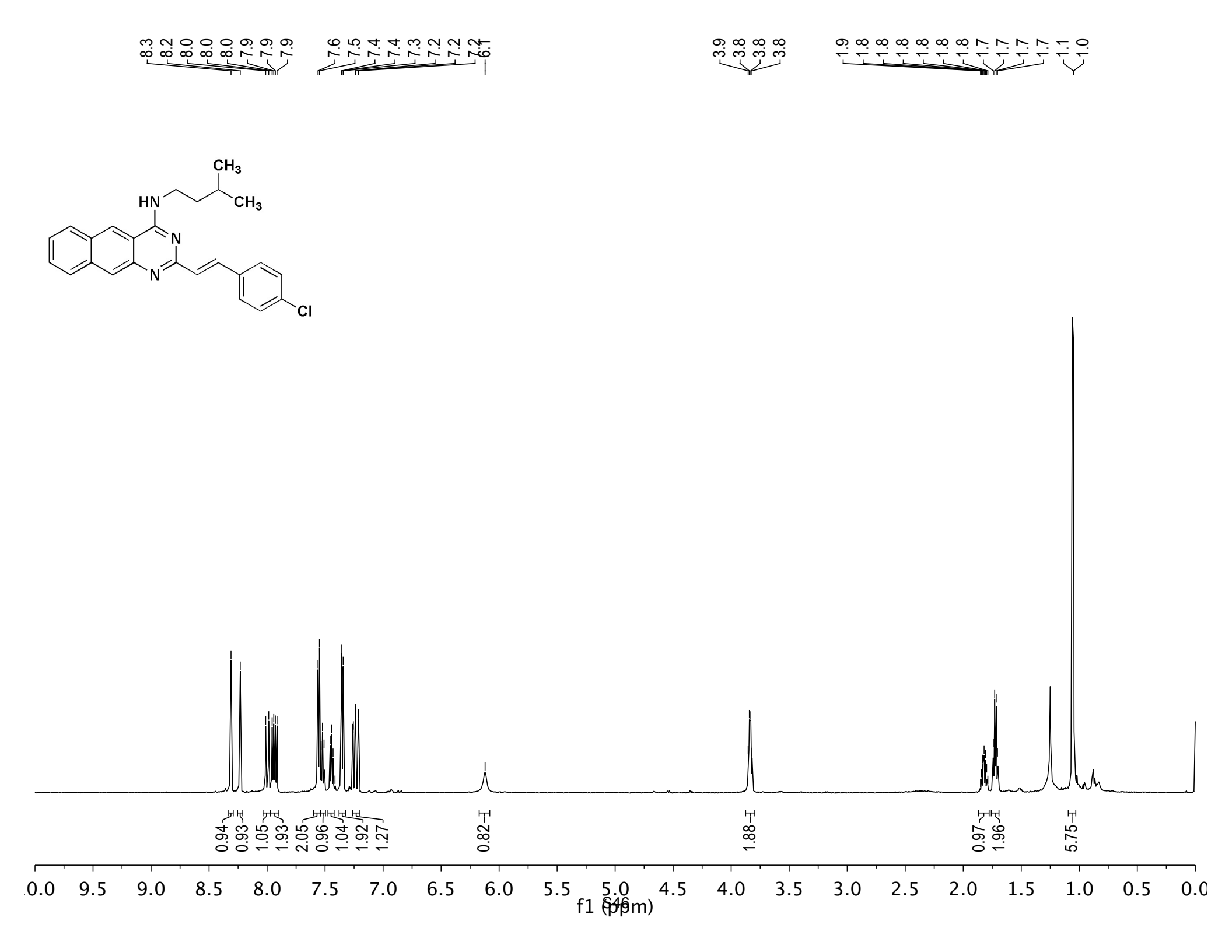
159.8
146.2
137.2
129.8
129.4
128.0
127.9
127.8
125.9
125.1
114.7
52.4
48.0
37.7
21.5
10.5
HN
N
N
N
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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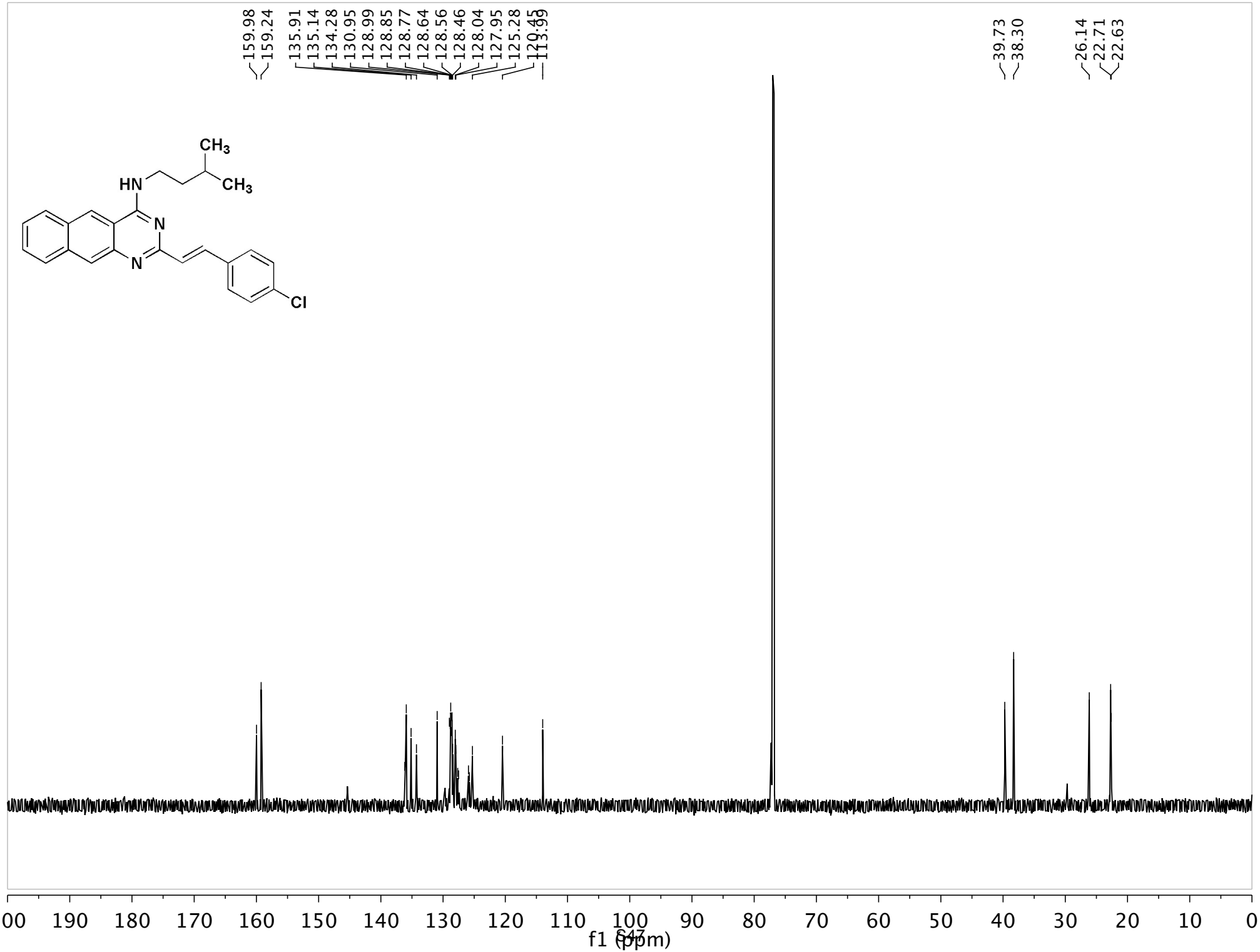
8.2
8.0
8.0
8.0
7.9
7.9
7.9
7.6
7.5
7.4
7.4
7.3
7.2
7.2
7.2
6.1
3.9
3.8
3.8
3.8
1.9
1.8
1.8
1.8
1.8
1.8
1.8
1.7
1.7
1.7
1.7
1.1
1.0
CH3
HN
CH3
N
N
Cl
0.94
0.93
1.05
1.93
2.05
0.96
1.04
1.92
1.27
0.82
1.88
0.97
1.96
5.75
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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159.24
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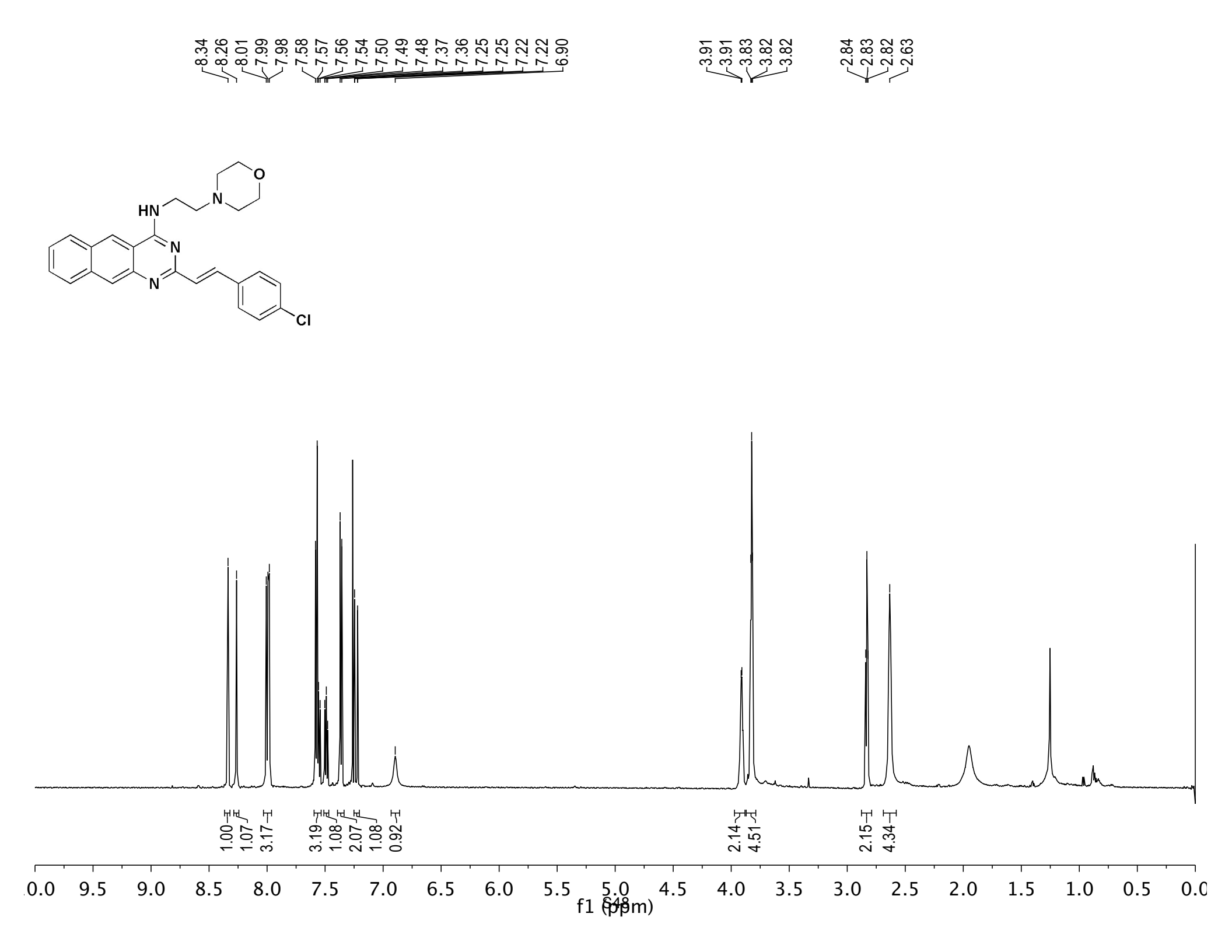
130.95
128.99
128.85
128.77
128.64
128.56
128.46
128.04
127.95
125.28
120.45
113.99
39.73
38.30
26.14
22.71
22.63
CH3
HN
CH3
N
N
Cl
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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8.26
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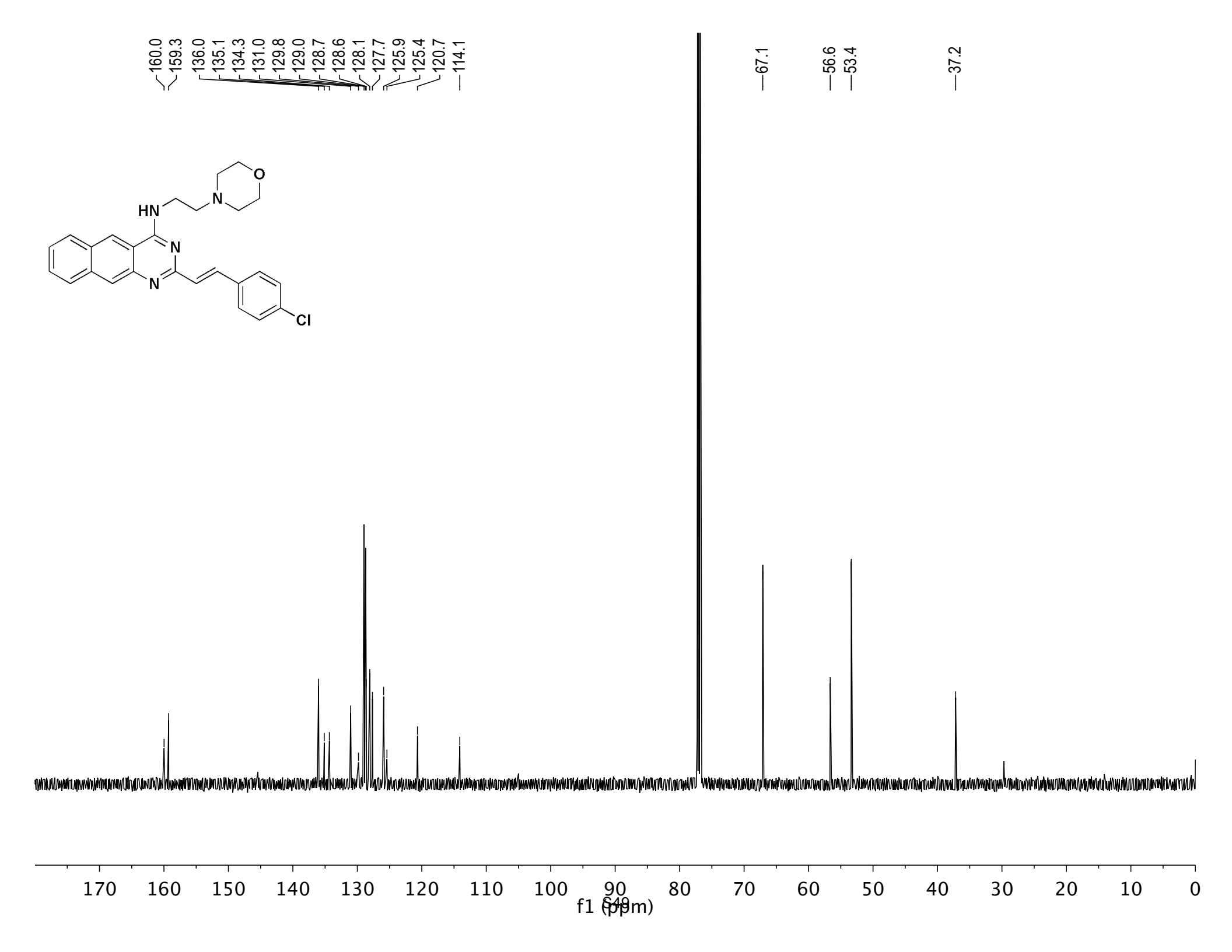
7.98
7.58
7.57
7.56
7.54
7.50
7.49
7.48
7.37
7.36
7.25
7.25
7.22
7.22
6.90
3.91
3.91
3.83
3.82
3.82
2.84
2.83
2.82
2.63
HN
N
O
N
N
Cl
1.00
1.07
3.17
3.19
1.08
2.07
1.08
0.92
2.14
4.51
2.15
4.34
f1 (ppm)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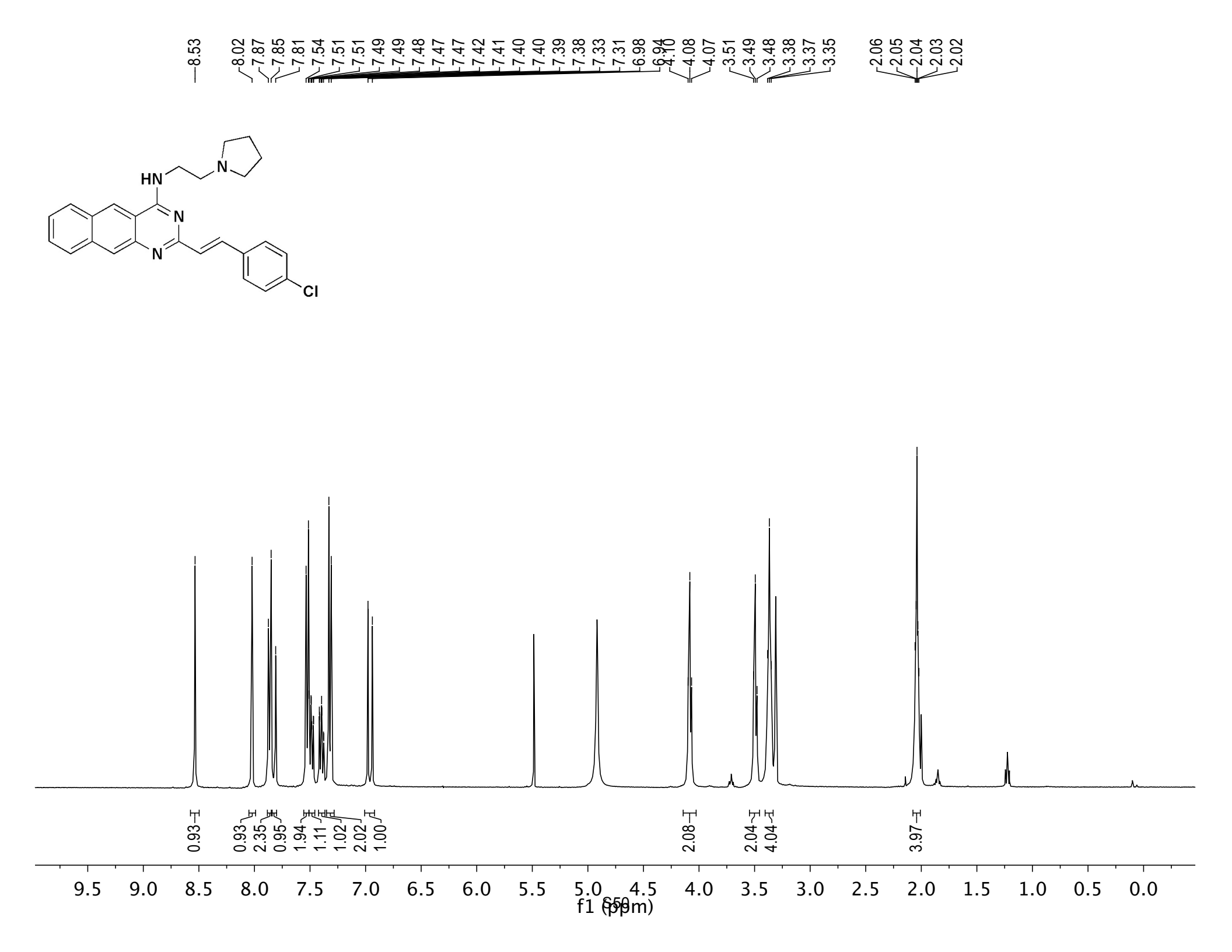
159.3
136.0
135.1
134.3
131.0
129.8
129.0
128.7
128.6
128.1
127.7
125.9
125.4
120.7
114.1
67.1
56.6
53.4
37.2
HN
N
O
N
N
Cl
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.85
7.81
7.54
7.51
7.51
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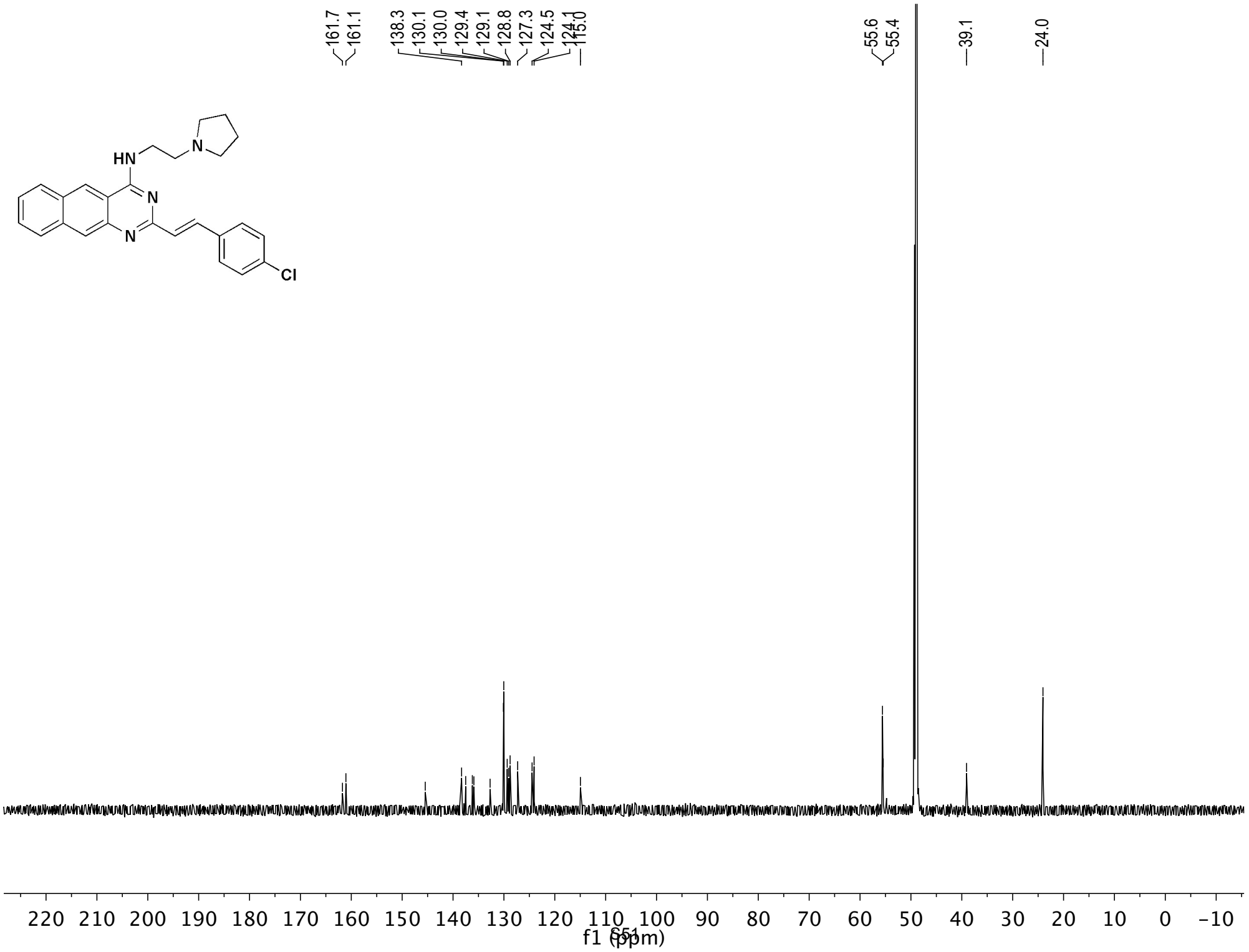
7.49
7.48
7.47
7.47
7.42
7.41
7.40
7.40
7.39
7.38
7.33
7.31
6.98
6.94
4.10
4.08
4.07
3.51
3.49
3.48
3.38
3.37
3.35
2.06
2.05
2.04
2.03
2.02
HN
N
N
N
Cl
0.93
0.93
2.35
0.95
1.94
1.11
1.02
2.02
1.00
2.08
2.04
4.04
3.97
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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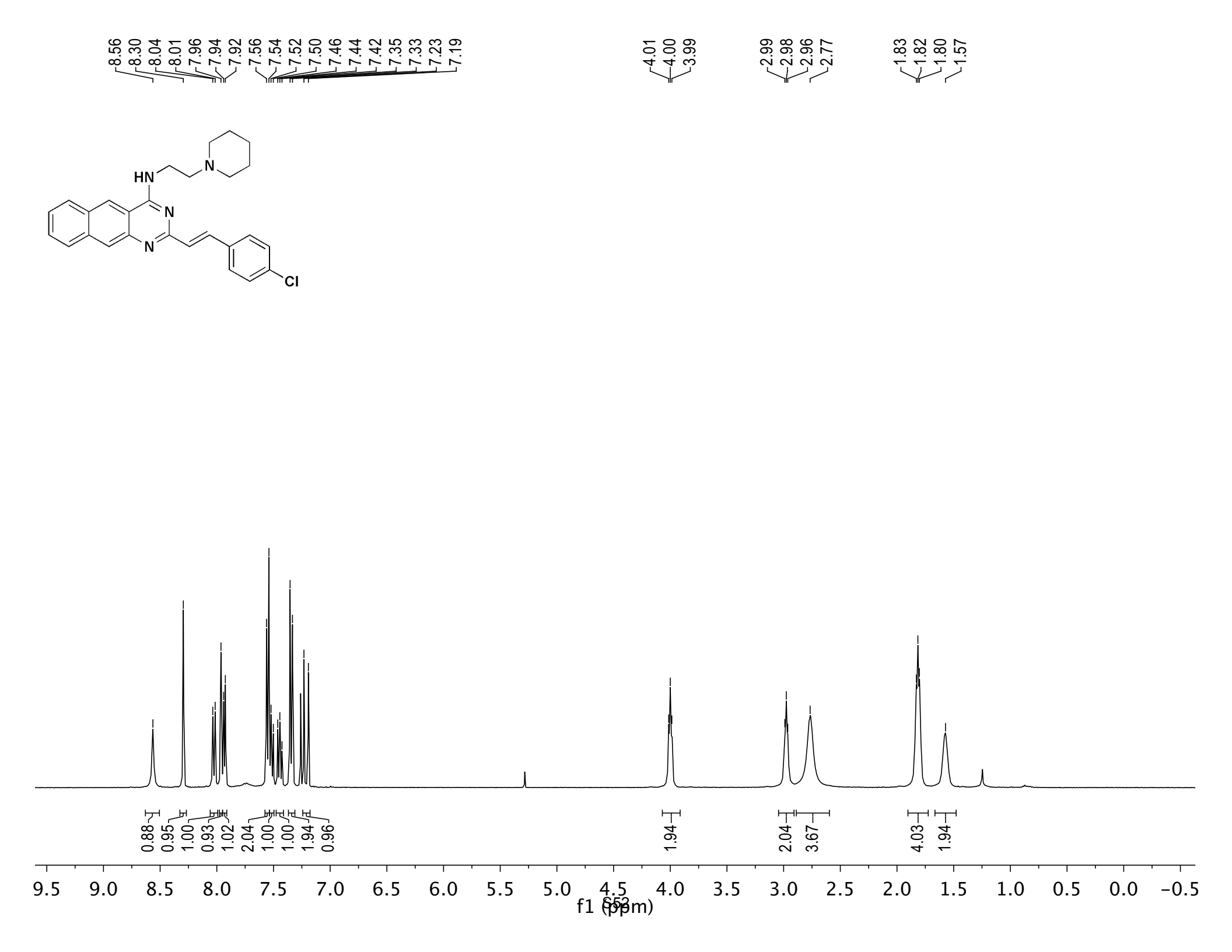
138.3
130.1
130.0
129.4
129.1
128.8
127.3
124.5
124.1
115.0
55.6
55.4
39.1
24.0
HN
N
N
N
Cl
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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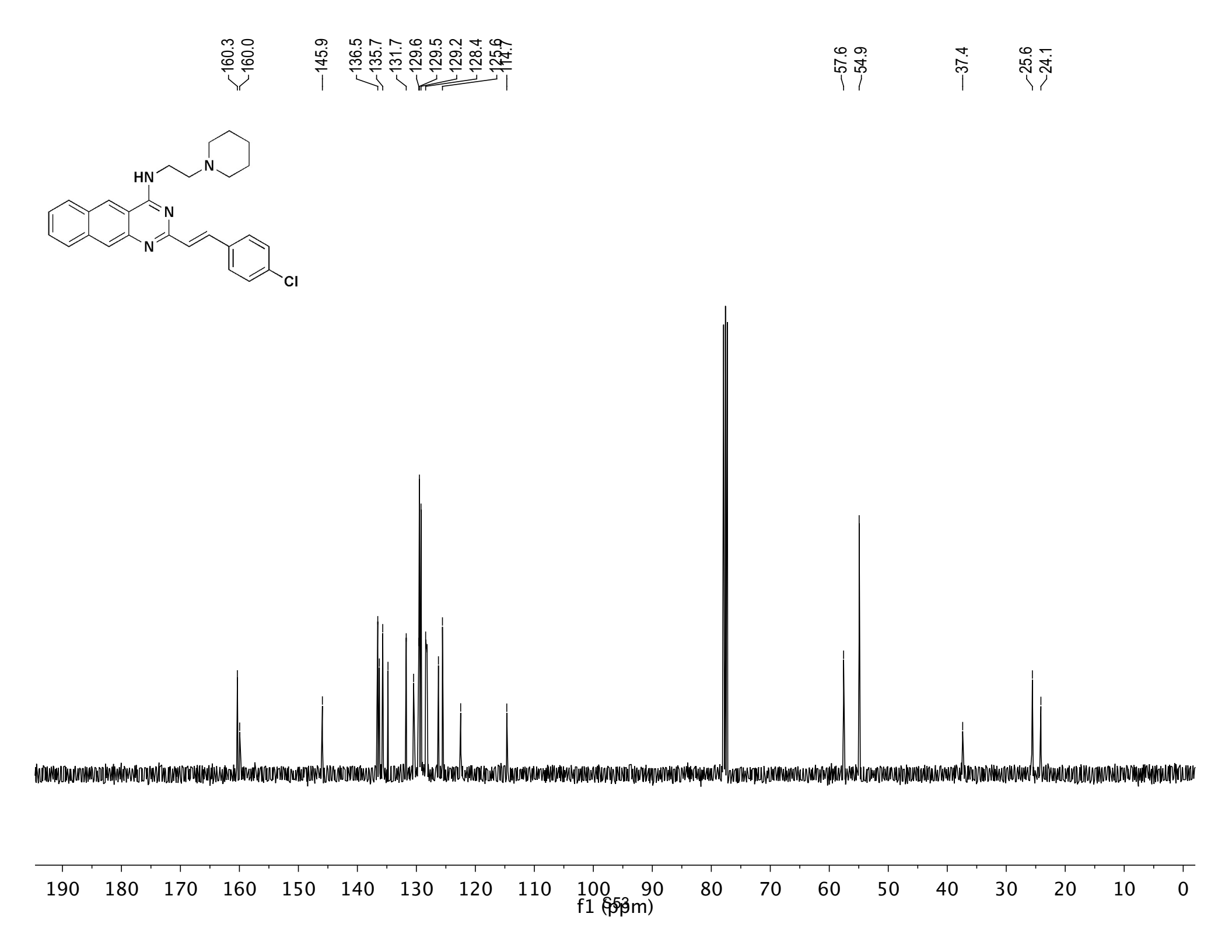
8.01
7.96
7.94
7.92
7.56
7.54
7.52
7.50
7.46
7.44
7.42
7.35
7.33
7.23
7.19
4.01
4.00
3.99
2.99
2.98
2.96
2.77
1.83
1.82
1.80
1.57
HN
N
N
N
Cl
0.88
0.95
1.00
0.93
1.02
2.04
1.00
1.00
1.94
0.96
1.94
2.04
3.67
4.03
1.94
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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136.5
135.7
131.7
129.6
129.5
129.2
128.4
125.6
114.7
57.6
54.9
37.4
25.6
24.1
HN
N
N
N
Cl
190
180
170
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150
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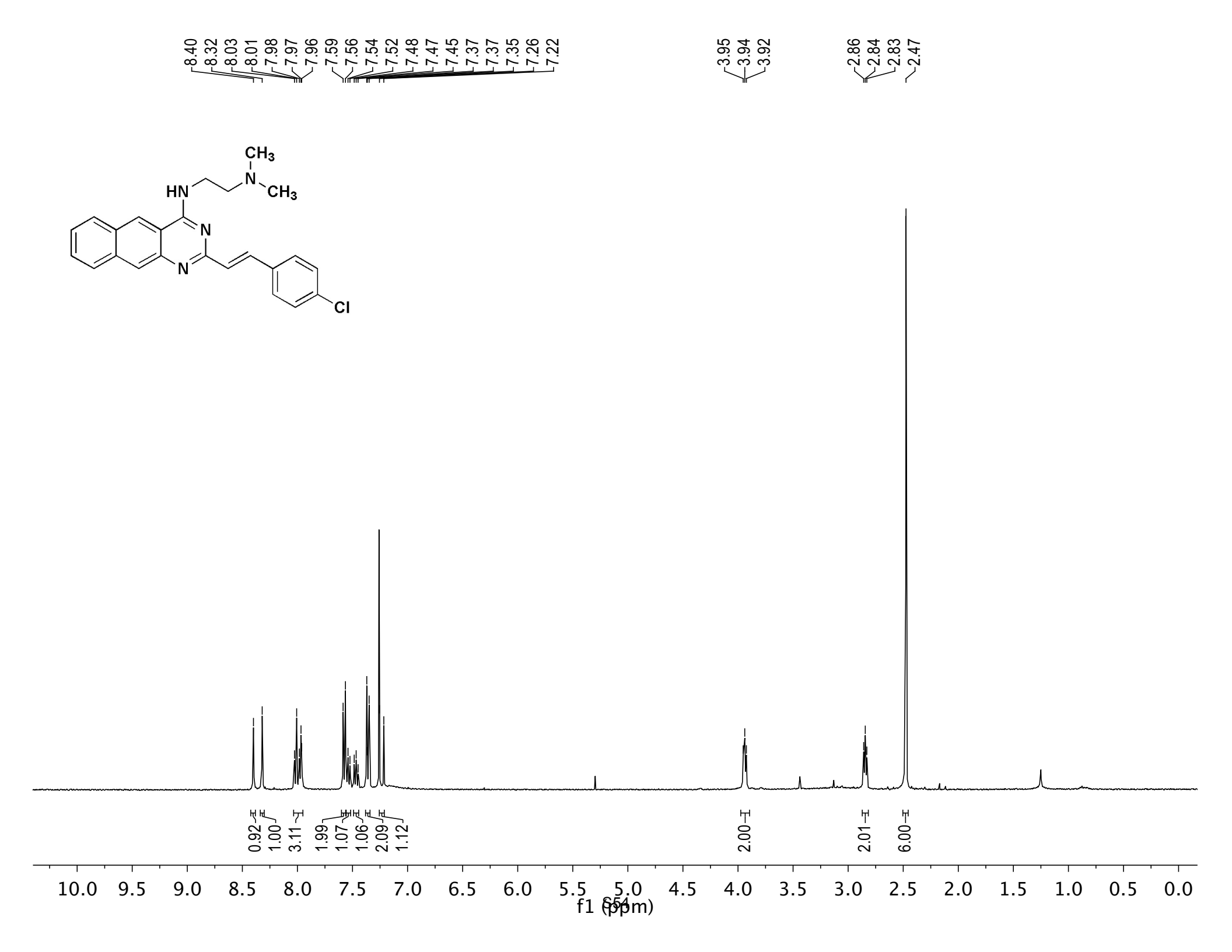
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.97
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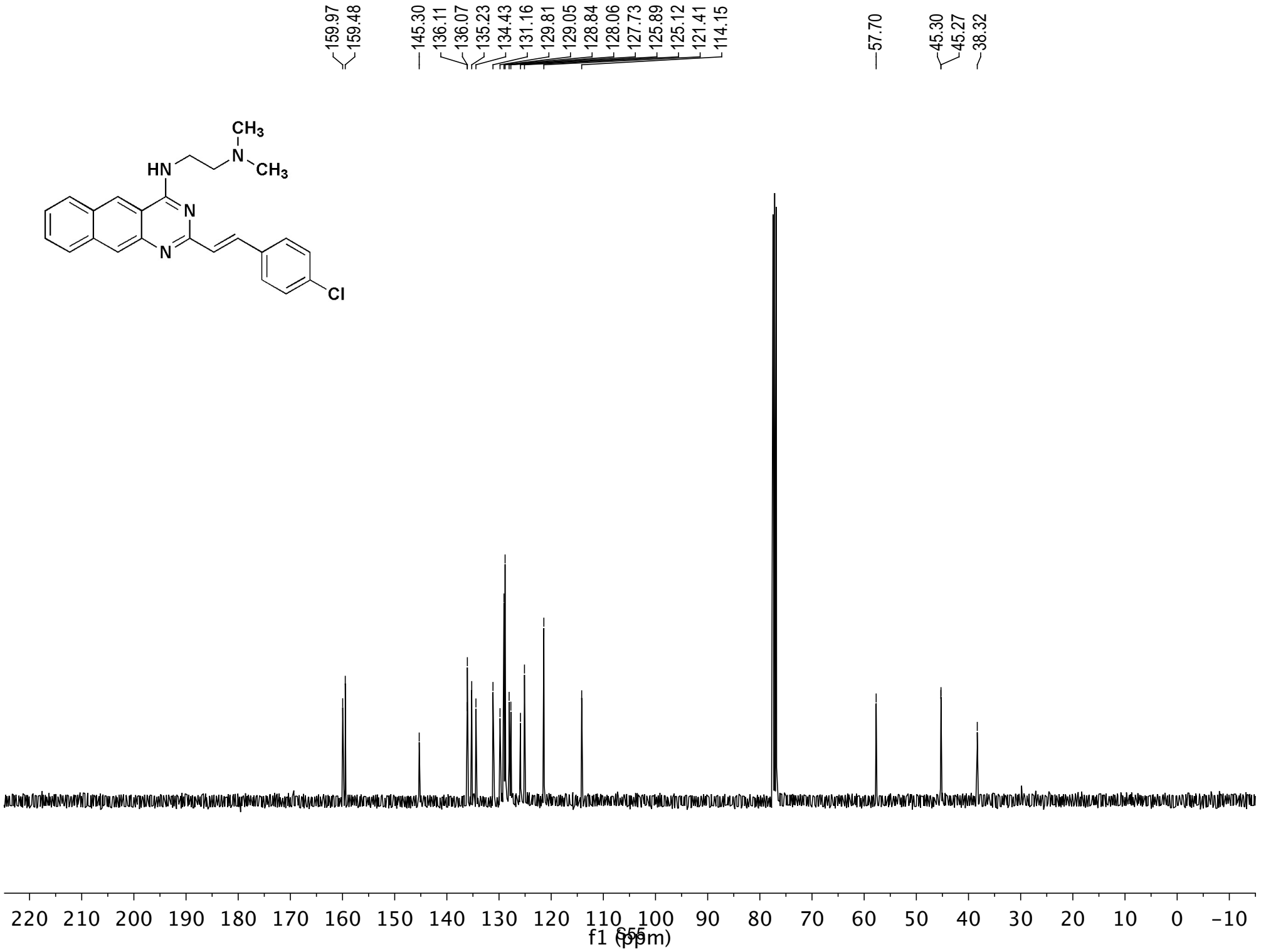
7.59
7.56
7.54
7.52
7.48
7.47
7.45
7.37
7.37
7.35
7.26
7.22
3.95
3.94
3.92
2.86
2.84
2.83
2.47
CH3
N
CH3
HN
N
N
Cl
0.92
1.00
3.11
1.99
1.07
1.06
2.09
1.12
2.00
2.01
6.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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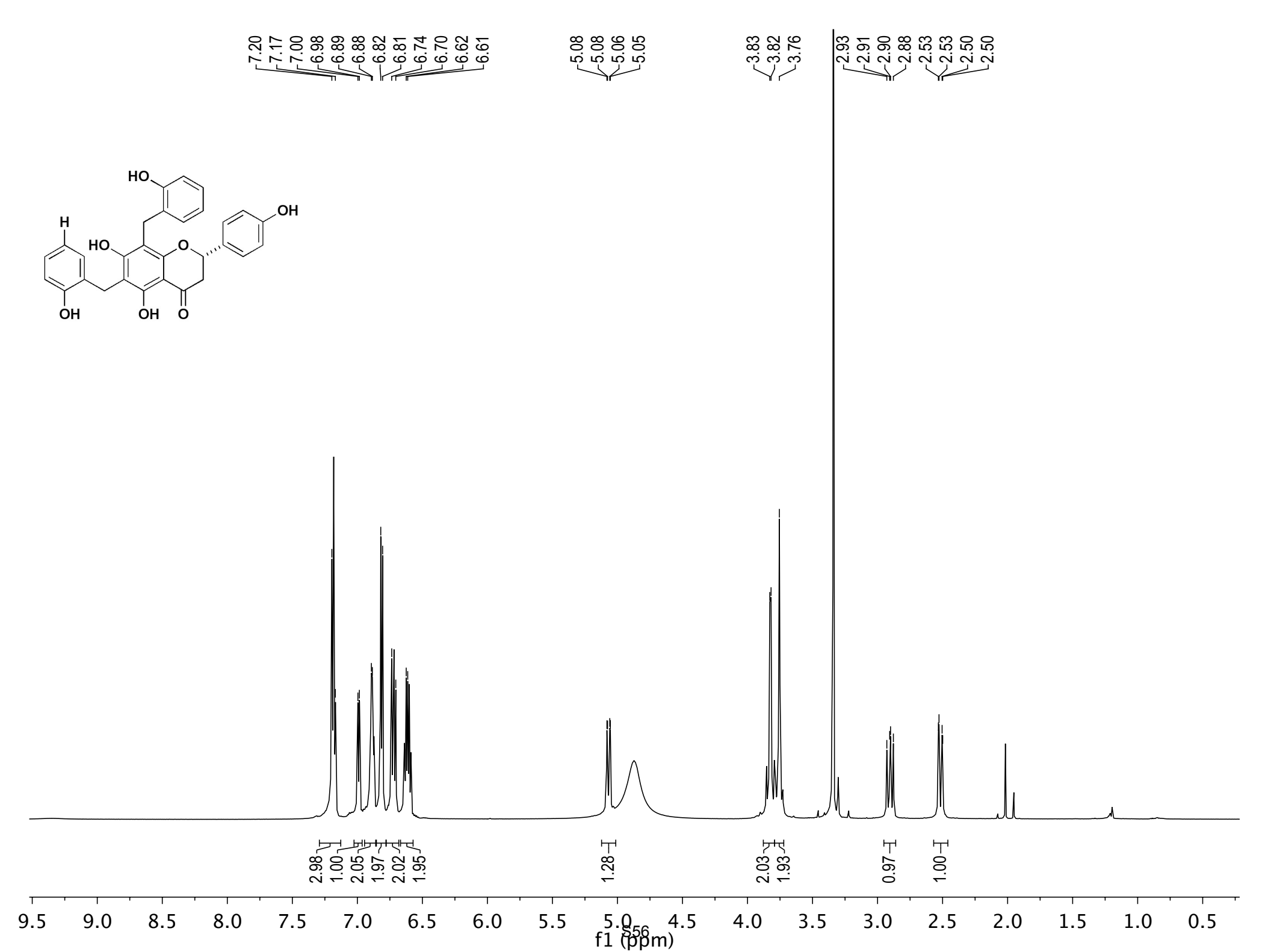
136.07
135.23
134.43
131.16
129.81
129.05
128.84
128.06
127.73
125.89
125.12
121.41
114.15
57.70
45.30
45.27
38.32
CH3
N
CH3
HN
N
N
Cl
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


7.20
7.17
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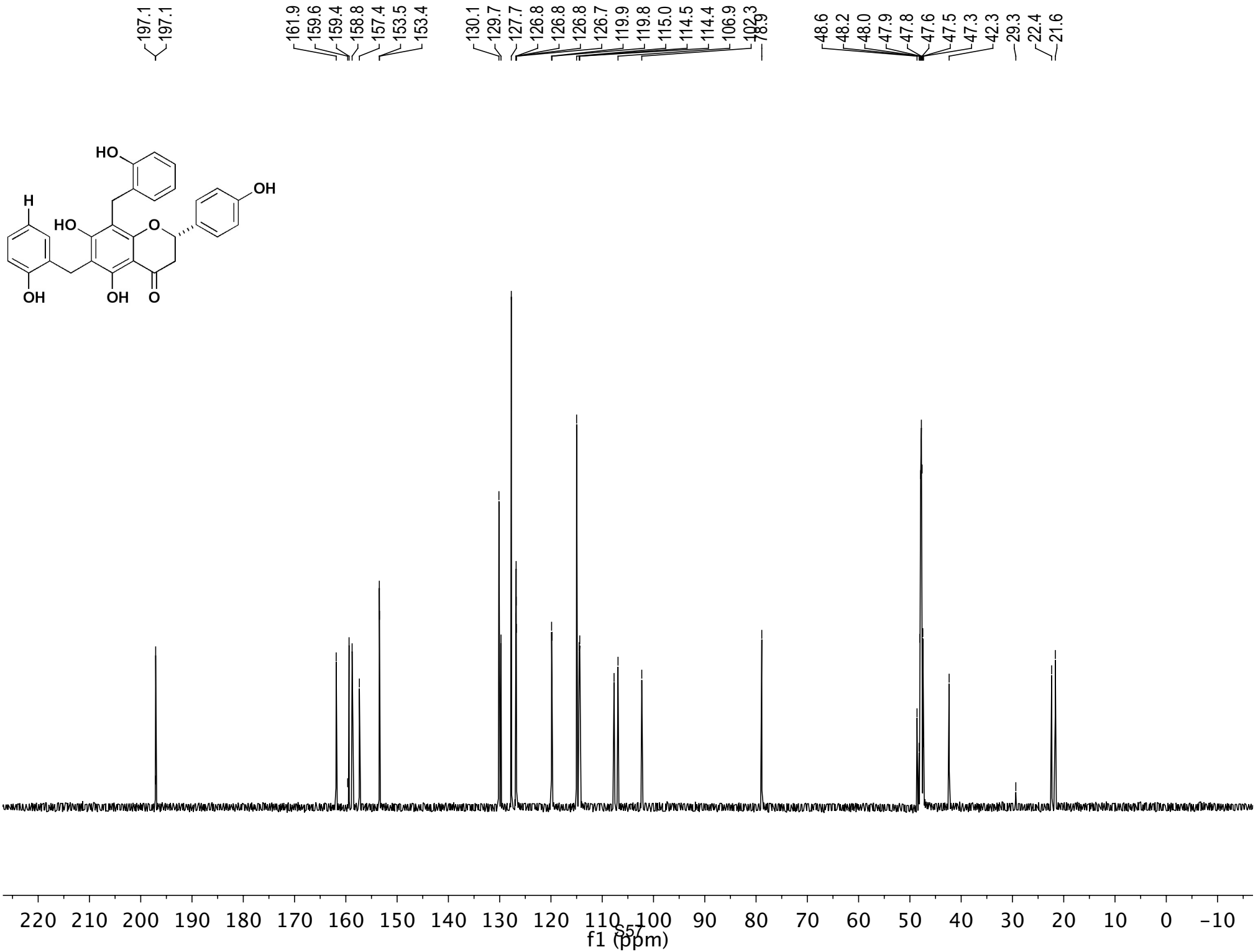
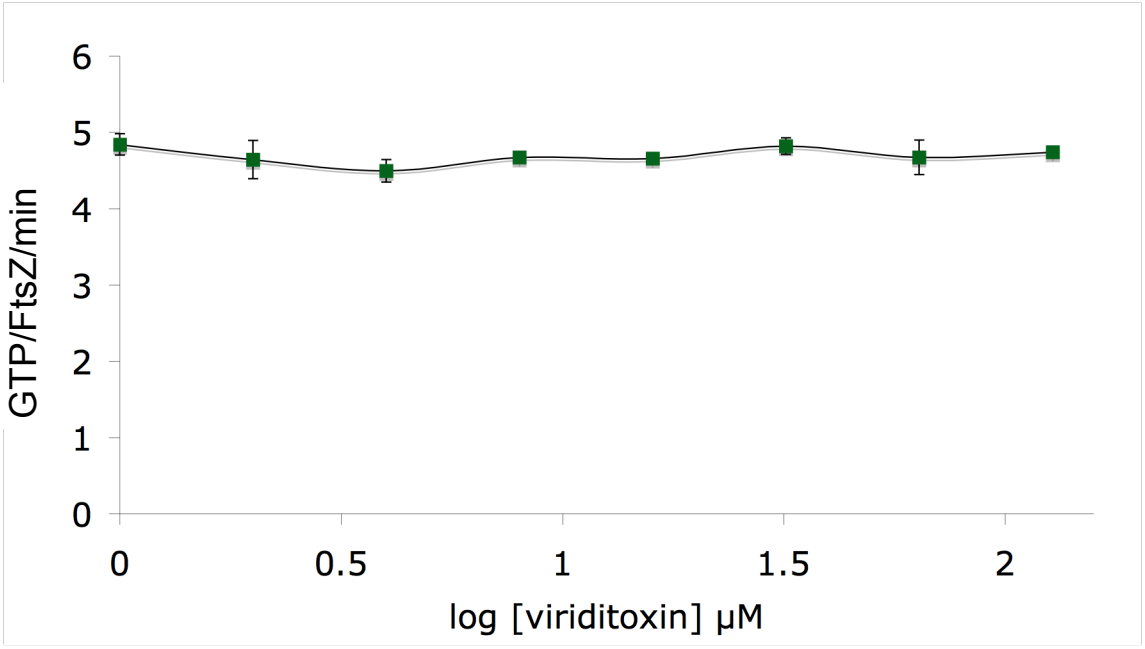
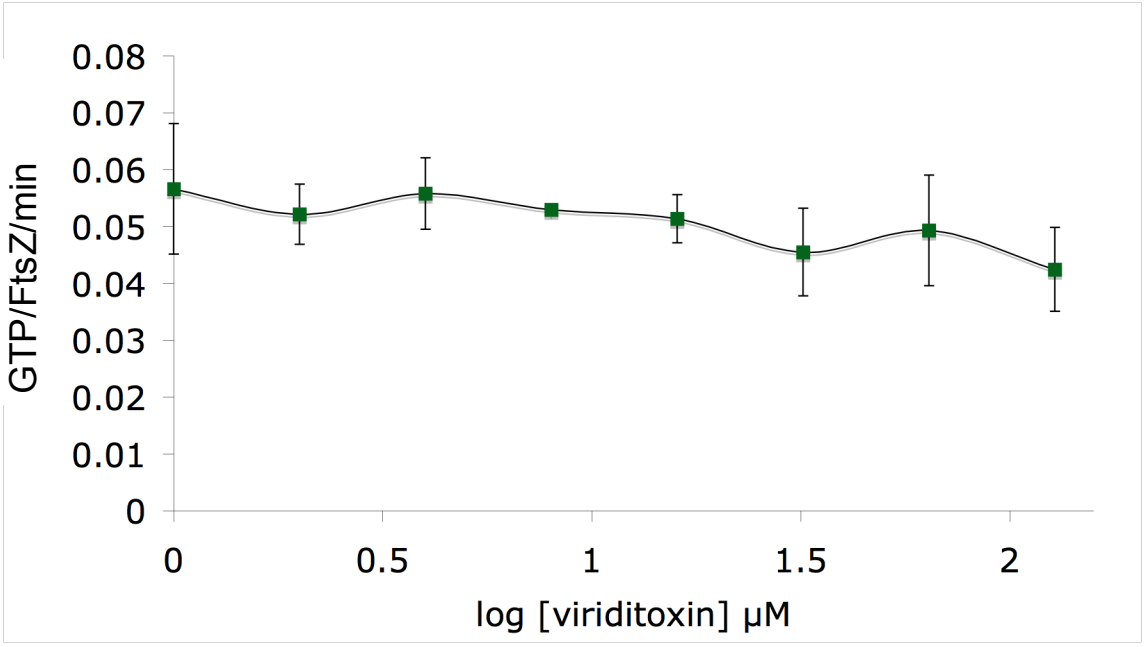
6.89
6.88
6.82
6.81
6.74
6.70
6.62
6.61
5.08
5.08
5.06
5.05
3.83
3.82
3.76
2.93
2.91
2.90
2.88
2.53
2.53
2.50
2.50
HO
OH
H
HO
O
OH
OH
O
2.98
1.00
2.05
1.97
2.02
1.95
1.28
2.03
1.93
0.97
1.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


197.1
197.1
161.9
159.6
159.4
158.8
157.4
153.5
153.4
130.1
129.7
127.7
126.8
126.8
126.8
126.7
119.9
119.8
115.0
114.5
114.4
106.9
102.3
78.9
48.6
48.2
48.0
47.9
47.8
47.6
47.5
47.3
42.3
29.3
22.4
21.6
HO
OH
H
HO
O
OH
OH
OH
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

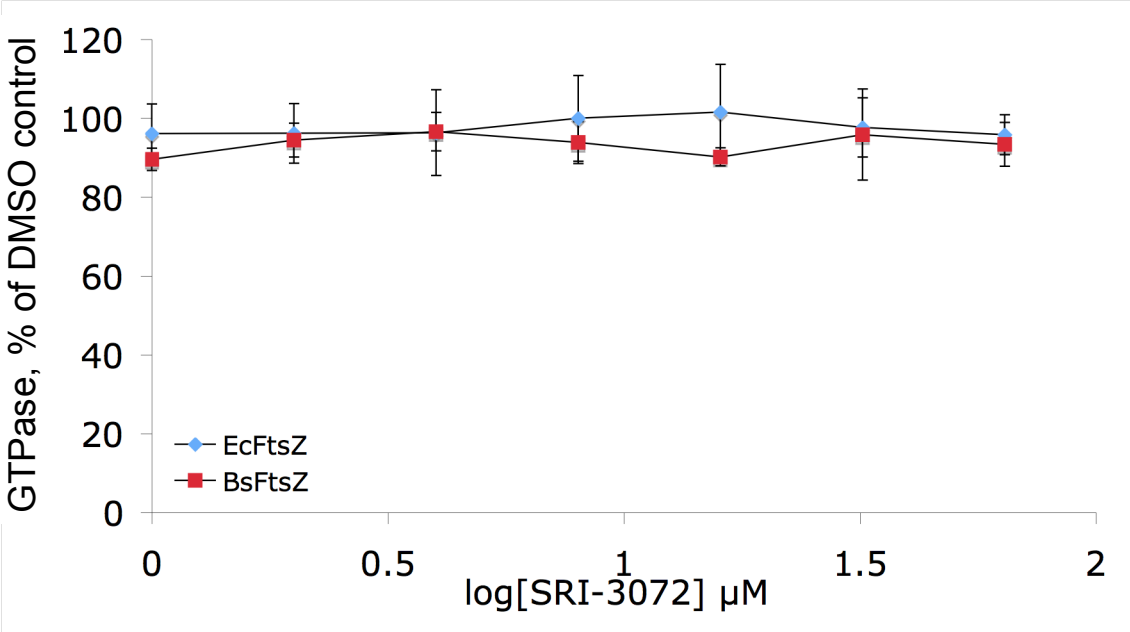
Malachite Green GTPase assay of EcFtsZ with viriditoxin in MMK buffer



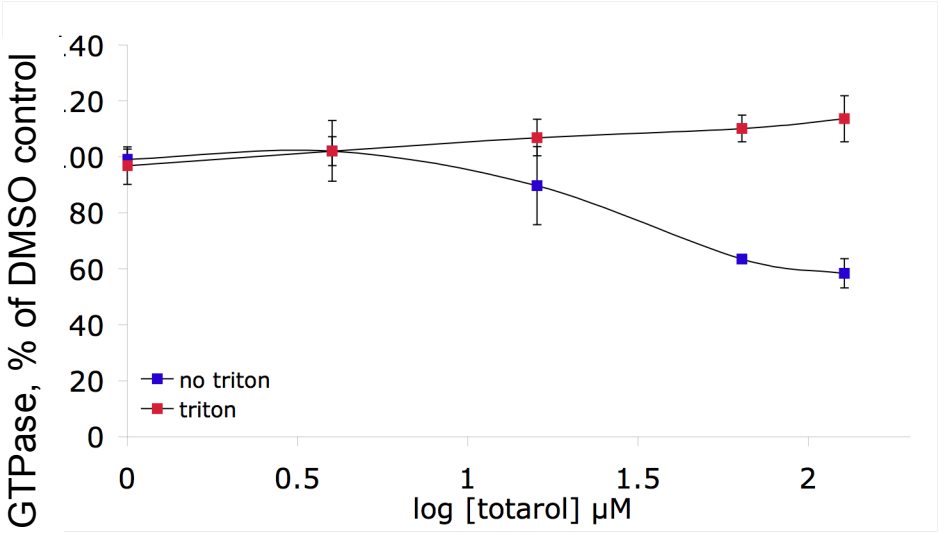
Malachite Green GTPase assay of EcFtsZ with viriditoxin under Merck buffer conditions



Enzyme-coupled GTPase assay of EcFtsZ and BsFtsZ with SRI-3072



Enzyme-coupled GTPase assay of Dnm1 with totarol



His10-Dnm1 was purified from baculovirus infected insect cells as previously described (Ingerman et al., 2005). To measure the GTP hydrolysis activity of Dnm1 in the presence of totarol with and without triton, a continuous regenerative assay was used (Ingerman and Nunnari, 2005). Briefly, the GTPase assays were carried out at 30°C in 25 mM HEPES pH 7.0, 25 mM PIPES pH 7.0, 150 mM NaCl, 7.5 mM KCl, 5 mM MgCl2, 1 mM phospho(enol)pyruvate, 20 U/mL pyruvate kinase/lactate dehydrogenase, 600 µM NADH and 2% DMSO. Totarol concentrations were varied as indicated, and the reactions were carried out in the absence or presence of 0.1% triton as indicated. Dnm1 was present at a final concentration of 0.4 µM. Following a 5 min incubation of the assay components listed above, GTP was added to a final concentration of 1 mM to start the reactions. The GTPase assay reactions were started in a 200 µL volume, of which 150 µL was placed into the well of a 96-well plate. Depletion of NADH, as monitored by reading the A340 of the reaction, was measured every 20 s for a total of 40 min using a SpectraMAX M5 96-well plate reader (Molecular Devices). Spectrophotometric data were transferred to Excel, and the measured steady state depletion of NADH over time was converted to protein activity as previously described (Ingerman and Nunnari, 2005).

Ingerman, E., and Nunnari, J. (2005). A continuous, regenerative coupled GTPase assay for dynamin-related proteins. Methods Enzymol 404, 611-619.

Ingerman, E., Perkins, E.M., Marino, M., Mears, J.A., McCaffery, J.M., Hinshaw, J.E., and Nunnari, J. (2005). Dnm1 forms spirals that are structurally tailored to fit mitochondria. J. Cell Biol. 170, 1021-1027.

Minimum Inhibitory Concentration (MIC) Assay of PC190723 and 8j Antibacterial Activity

An overnight culture of *B. subtilis* strain 168, grown in Luria-Bertani (LB) medium, was diluted into fresh LB medium to a final density of approximately 10^5 cells/mL. Dry powdered stocks of the small molecule inhibitors were dissolved in sterile-filtered DMSO to a concentration of 25 mM. From this, serial dilutions of 50-fold concentrated drug stock were prepared in DMSO and 4 μL of these were loaded into wells of a 96-well microplate in triplicate. 196 μL of diluted cell suspension was then added to each well, giving a total volume of 200 μL, and mixed thoroughly. The final DMSO concentration in each well was therefore 2%. Wells with 2% DMSO only were included as full growth controls and wells containing LB only without cells were included to check for possible contamination. Microplates were incubated for 16 h at 37 °C with shaking. After growth, plates were visually inspected and the concentration of inhibitor that produced no visible growth was designated the MIC. The visual assessment was also confirmed by absorbance measurements at 630 nm. The compound PC190723 gave an MIC of 1 μM and 8j gave an MIC of 0.25 μM. All three replicates showed the same MIC value.