Microwave-Enhanced Friedländer Synthesis for the Rapid Assembly of Halogenated Quinolines with Antibacterial and Biofilm Eradication Activities against Drug Resistant and Tolerant Bacteria

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Supporting Information

1.) General Information:

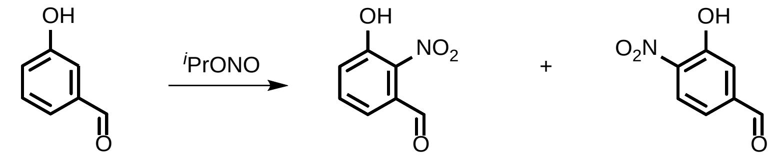
All synthetic reactions were carried out under an inert atmosphere of argon unless otherwise specified. Reagents and commercially available biological controls (i.e., antibiotics) were purchased at ≥ 95% purity and used without further purification. All microwave reactions were carried out in sealed tubes in an Anton Paar Monowave 300 Microwave Synthesis Reactor. A constant power was applied to ensure reproducibility. Temperature control was automated via IR sensor and all indicated temperatures correspond to the maximal temperature reached during each experiment. Analytical thin layer chromatography (TLC) was performed using 250 μm Silica Gel 60 F254 pre-coated plates (EMD Chemicals Inc.). Flash column chromatography was performed using 230-400 Mesh 60Å Silica Gel from Sorbent Technologies. All melting points were obtained, uncorrected, using a Mel-Temp capillary melting point apparatus from Laboratory Services, Inc.

NMR experiments were recorded using broadband probes on a Varian Mercury-Plus-400 spectrometer via VNMR-J software (400 MHz for ^{1}H and 100 MHz for ^{13}C). All spectra are presented using MestReNova 8.1 (Mnova) software and are displayed without the use of the signal suppression function. Spectra were obtained in the following solvents (reference peaks also included for ^{1}H and ^{13}C NMRs): $CDCl_3$ (^{1}H NMR: 7.26 ppm; ^{13}C NMR: 77.23 ppm), CD_3OD (^{1}H NMR: 3.31 ppm; ^{13}C NMR: 49.00 ppm), and d_6-DMSO (^{1}H NMR: 2.50 ppm; ^{13}C NMR: 39.52 ppm). All NMR experiments were performed at room temperature. Chemical shift values (δ) are reported in parts per million (ppm) for all ^{1}H NMR and ^{13}C NMR spectra. ^{1}H NMR multiplicities are reported as: s = singlet, br. s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High-resolution mass spectra were obtained for new compounds from the Mass Spectrometry Facility in the Chemistry Department at the University of Florida.

Bacterial strains used during these investigations include: methicillin-resistant Staphylococcus aureus (Clinical Isolate from Shands Hospital in Gainesville, FL: MRSA-2; ATCC strains: BAA 1707, BAA 44) methicillin-resistant Staphylococcus epidermidis (MRSE strain ATCC 35984), and vancomycin-resistant Enterococcus faecium (VRE strain ATCC 700221). All compounds were stored as DMSO stocks at room temperature in the absence of light for several months at a time without observing any loss in biological activity. To ensure compound integrity of our DMSO stock solutions, we did not subject DMSO stocks of our test compounds to freeze-thaw cycles.

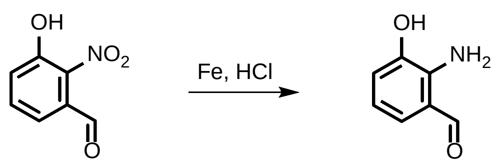
This work was supported by the Emerging Pathogens Institute and the College of Pharmacy at the University of Florida.

2.) Synthetic Procedures with Tabulated Characterization Data:



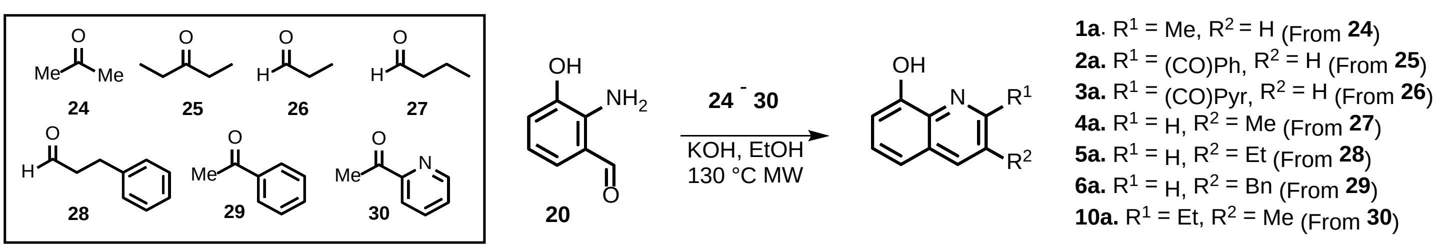
21 22 23

3-Hydroxy-2-nitrobenzaldehyde (22). To a stirring solution of 3-hydroxybenzaldehyde **21** (618 mg, 5.0 mmol) in dichloromethane (10 mL) was added tetrabutylammoniumhydrogen sulfate (85.0 mg, 0.25 mmol) and isopropyl nitrate (1.27 mL, 12.5 mmol). Concentrated sulfuric acid (610 µL) was added dropwise and the resulting reaction mixture was allowed to stir at room temperature for 15 minutes. The reaction contents were then transferred to a separatory funnel containing 50 mL of an aqueous saturated sodium bicarbonate solution. Dichloromethane was then used to extract the crude product. The combined organic layers were dried with anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The resulting solid was adsorbed onto silica gel and purified via flash column chromatography eluting with 99:1 to 4:1 hexanes:ethyl acetate to give isomer **23** (R_f = 0.44 in 3:1 hexanes:ethyl acetate) as a yellow solid (201 mg, 24% yield) followed by the desired product **22** (R_f = 0.19 in 3:1 hexanes:ethyl acetate) as a pale yellow solid (411 mg, 47% yield). **Note:** We have previously reported this procedure.[1] We were able to obtain **22** (CAS number: 42123-33-1) in 64% yield (2.18 g) during the course of these investigations.

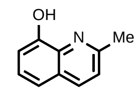


22 20

Synthesis of 2-amino-3-hydroxybenzaldehyde (20). Iron powder (895 mg, 16 mmol) was added to a stirring solution of 3-hydroxy-2-nitrobenzaldehyde **22** (268 mg, 1.60 mmol) dissolved in 4:1 mixture of ethanol:water (6 mL). Concentrated hydrochloric acid (14 µL, 0.16 mmol) was added to the reaction mixture which was heated to reflux for 5 hours until complete (determined by TLC analysis). After the completion of the reaction, the reaction was cooled and the resulting suspension was passed through a short plug of celite eluting with ethanol. The resulting solution was concentrated *in vacuo* to afford pure **20** (1.49 g, 84% yield). **Note:** We have previously reported this procedure.[1]



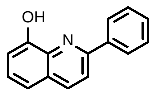
Method A Friedländer synthesis of 8-hydroxyquinolines (1a – 6a, 10a). To a sealed microwave vial was added 2-amino-3-hydroxybenzaldehyde **20** (47.6 mg, 0.35 mmol), potassium hydroxide (43.0 mg, 0.76 mmol) and 3-pentanone **25** (56 µL, 0.52 mmol). The resulting mixture was then heated to 130 °C in the microwave reactor for 40 minutes. After that time, the reaction was then allowed to cool and the solvent was removed *in vacuo*. The crude residue was taken up in dichloromethane, transferred to a separatory funnel and then neutralized with 2N hydrochloric acid. The solution was then extracted with dichloromethane three times. The organic layers were combined, dried with anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The crude solid was purified via flash column chromatography eluting with 95:5 to 4:1 hexanes:ethyl acetate to afford quinoline **10a** as a white solid (61.4 mg, 95%).



1a

Yield: 84% yield; 27.1 mg of **1a** was isolated as a white solid.

Note: We have previously reported a synthesis of this compound, without microwave assistance.[1]



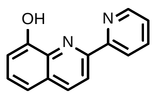
2a

Yield: 76% yield; 63.9 mg of **2a** was isolated as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 – 8.33 (br. s, 1H), 8.22 (d, *J* = 8.6 Hz, 1H), 8.19 – 8.14 (m, 2H), 7.92 (d, *J* = 8.6 Hz, 1H), 7.59 – 7.40 (m, 3H), 7.35 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.21 (dd, *J* = 7.5, 1.3 Hz, 1H).

MP: 45 - 47 °C, lit. 55 °C.

Note: ^{1}H NMR spectrum and melting point match previously reported values.[3]



3a

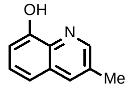
Yield: 83% yield; 57.1 mg of **3a** was isolated as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 – 8.68 (m, 1H), 8.59 (d, *J* = 8.6 Hz, 1H), 8.56 (dt, *J* = 8.0, 1.1 Hz, 1H), 8.35 (br. s, 1H), 8.26 (d, *J* = 8.6 Hz, 1H), 7.85 (td, *J* = 7.8, 1.8 Hz, 1H), 7.46 (dd, *J* = 7.9, 7.9 Hz, 1H), 7.39 – 7.32 (m, 2H), 7.20 (dd, *J* = 7.6, 1.3 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.5, 153.8, 152.3, 149.2, 137.6, 137.0, 136.9, 128.5, 128.0, 124.2, 121.5, 119.7, 117.9, 110.2.

MP: 121 - 123 °C, lit. 119 - 121 °C.

Note: NMR spectra and melting point match previously reported values.[4]



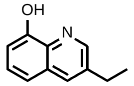
4a

Yield: 52% yield; 40.0 mg of **4a** was isolated as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (d, *J* = 2.0 Hz, 1H), 7.90 (d, *J* = 2.0 Hz, 1H), 7.42 (dd, J = 8.3, 7.6 Hz, 1H), 7.25 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.12 (dd, *J* = 7.6, 1.2 Hz, 1H), 2.51 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.4, 150.0, 136.9, 135.0, 131.5, 128.6, 128.0, 117.5, 109.5, 19.0.

Note: NMR spectra match previously reported values.[5]



5a

Yield: 48% yield; 48.6 mg of **5a** was isolated as a white solid.

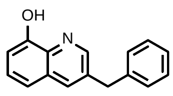
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (d, *J* = 2.1 Hz, 1H), 7.92 (dt, J = 2.1, 0.8 Hz 1H), 7.43 (dd, *J* = 8.3, 7.6 Hz, 1H), 7.28 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.13 (dd, *J* = 7.6, 1.2 Hz, 1H), 2.84 (qd, *J* = 7.6, 0.8 Hz, 2H), 1.35 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.4, 149.5, 137.7, 137.1, 133.8, 128.7, 127.9, 117.7, 109.4, 26.5, 15.5.

HRMS (DART): calc. for $C_{11}H_{12}NO$ $[M+H]^+$: 174.0913, found: 174.0914.

MP: 66 - 68 °C.

Note: Compound has an assigned CAS number (11470-98-5), but no published spectra or melting point were found.



6a

Yield: 52% yield; 75.7 mg of **6a** was isolated as a white solid.

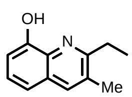
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (d, *J* = 2.0 Hz, 1H), 7.91 (d, *J* = 2.0 Hz, 1H), 7.51 – 7.03 (m, 8H), 4.19 (s, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.4, 149.7, 139.8, 137.2, 135.2, 134.9, 129.1, 129.0, 128.6, 128.1, 126.8, 117.8, 109.8, 39.5.

HRMS (DART): calc. for $C_{16}H_{14}NO$ $[M+H]^+$: 236.1070, found: 236.1075.

MP: 97 - 99 °C.

Note: Compound has an assigned CAS number (457948-80-0), but no published spectra were found for comparison.



10a

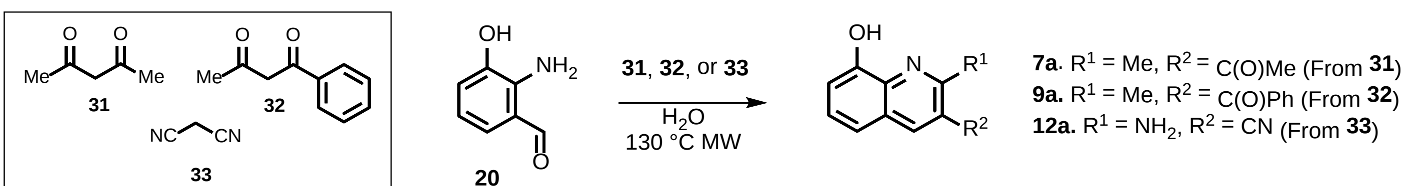
Yield: 95% yield; 61.4 mg of **10a** was isolated as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (s, 1H), 7.35 (dd, *J* = 8.2, 7.7 Hz, 1H), 7.20 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.09 (dd, *J* = 7.7, 1.2 Hz, 1H), 2.94 (q, *J* = 7.4 Hz, 2H), 2.44 (s, 3H), 1.41 (t, *J* = 7.4 Hz, 3H).

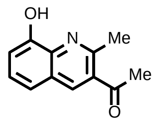
^{13}C NMR (100 MHz, $CDCl_3$): δ 160.8, 151.9, 136.5, 135.6, 130.7, 127.4, 126.8, 117.0, 108.8, 28.8, 19.2, 12.0.

MP: 44 - 46 °C, lit. 46 - 47 °C.[6]

Note: Compound has an assigned CAS number (88611-52-3), but no published spectra were found for comparison.



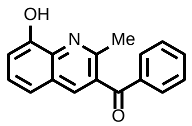
Method B Friedländer synthesis of 8-hydroxyquinolines (7a, 9a, 12a). To an 8 mL sealed microwave vial was added 2-amino-3-hydroxybenzaldehyde **20** (40.8 mg, 0.30 mmol) in water (4 mL) and malononitrile **33** (29 μL, 0.45 mmol). The resulting mixture was then heated at 130 °C in the microwave reactor for 15 minutes at 120 °C. After that time, the reaction was then allowed to cool and the contents were transferred to a separatory funnel containing a saturated aqueous solution of sodium bicarbonate. The solution was then extracted with ethyl acetate three times. The organic layers were combined, dried with anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The crude solid was purified via flash column chromatography eluting with 95:5 to 4:1 hexanes:ethyl acetate to afford quinoline **12a** as a white solid (50.5 mg, 92%). **Note:** Reaction times for complete conversion of starting material ranged from 10 to 45 minutes.



7a

Yield: 90% yield; 31.0 mg of **7a** was isolated as a white solid.

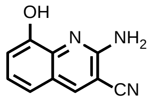
Note: We have previously reported a synthesis of this compound, without microwave assistance.[1]



9a

Yield: 77% yield; 35.8 mg of **9a** was isolated as a clear oil.

Note: We have previously reported a synthesis of this compound, without microwave assistance.[1]



12a

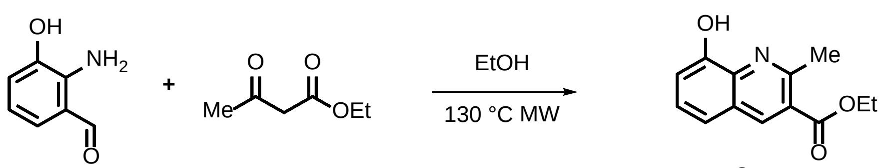
Yield: 92% yield; 50.5 mg of **12a** was isolated as a white solid.

^{1}H NMR (400 MHz, Methanol-*d*$_4$): δ 8.44 (s, 1H), 7.20 (dd, J = 8.1, 1.5 Hz, 1H), 7.14 (dd, J = 8.1, 7.4 Hz, 1H), 7.05 (dd, J = 7.4, 1.5 Hz, 1H).

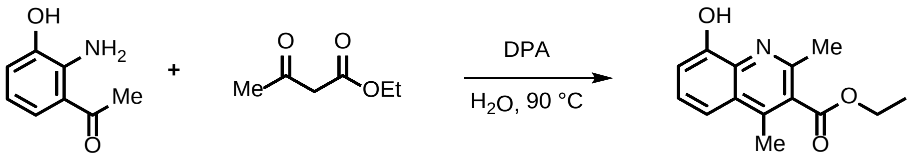
^{13}C NMR (100 MHz, Methanol-*d*$_4$): δ 156.3, 151.8, 146.1, 140.5, 124.8, 123.3, 119.7, 117.2, 115.1, 96.7.

MP: 206 - 208 °C, lit. 202 - 203 °C.[2]

Note: Compound has an assigned CAS number (90800-72-9), but no published spectra were found for comparison.



Method C Friedländer synthesis of ethyl 8-hydroxy-2-methylquinoline-3-carboxylate (8a). To an 8 mL sealed microwave vial was added 2-amino-3-hydroxybenzaldehyde **20** (28.5 mg, 0.21 mmol) and ethyl acetoacetate **34** (53 µL, 0.42 mmol) in ethanol (3 mL). The resulting mixture was then heated at 130 °C in the microwave reactor for 65 minutes. After that time, the reaction was then allowed to cool and the contents were then transferred to a separatory funnel containing a saturated aqueous solution of sodium bicarbonate. The solution was then extracted with ethyl acetate three times. The organic layers were combined, dried with anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The crude solid was purified via flash column chromatography eluting with 95:5 to 9:1 hexanes:ethyl acetate to give **8a** as a white solid (36.4 mg, 78%). **Note:** We have previously reported the synthesis of this compound, without microwave assistance.[1]



35 **34** **11a**

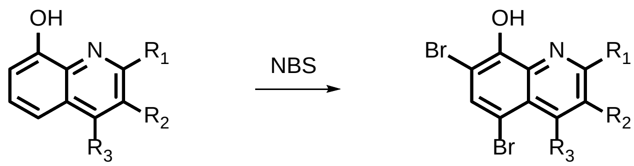
Synthesis of ethyl 8-hydroxy-2,4-dimethylquinoline-3-carboxylate (11a). To 0.3 mL of water was added 1-(2-amino-3-hydroxyphenyl)ethan-1-one (136 mg 0.90 mmol), ethyl acetoacetate **34** (170 µl, 1.35 mmol) and dodecylphosphonic acid (22.5 mg, 0.1 mmol). The resulting mixture was then heated at 90 °C for 6 hours. After that time, the reaction was then allowed to cool and the contents were transferred to a separatory funnel containing a saturated aqueous solution of sodium bicarbonate. The solution was then extracted with dichloromethane three times. The organic layers were combined, dried with anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The crude solid was purified via flash column chromatography eluting with 95:5 to 4:1 hexanes:ethyl acetate to give **11a** as an off-white solid (206 mg, 93%). **Note:** This protocol was adopted from a previously reported procedure.[7] The microwave-mediated synthesis of this compound was carried out via Method C (8.4 mg, 38%).

^{1}H NMR (400 MHz, CDCl$_3$): δ 7.40 – 7.35 (m, 2H), 7.14 (m, 1H), 4.47 (q, *J* = 7.2, Hz, 2H), 2.66 (s, 3H), 2.58 (s, 3H), 1.43 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (100 MHz, CDCl$_3$): δ 169.1, 152.4, 152.1, 142.2, 136.9, 128.7, 127.2, 126.1, 114.4, 110.6, 61.8, 23.6, 15.9, 14.4.

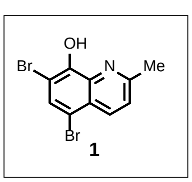
HRMS (DART): calc. for $C_{14}H_{16}NO_3$ $[M+H]^+$: 246.1125, found: 246.1125.

MP: 31 - 33 °C.

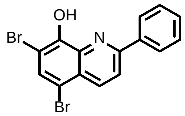


1a - 11a **1 - 11**

General procedure for 5,7-dibromo-8-hydroxyquinolines (1-11). 8-Hydroxyquinoline **7a** (53.2 mg, 0.24 mmol) was dissolved in dichloromethane (8 mL) before *N*-bromosuccinimide (89.5 mg, 0.50 mmol) was added and allowed to stir at room temperature for two hours until reaction was complete (monitored by TLC). At this time, the reaction was concentrated and adsorbed onto silica gel (via dissolving the crude reaction contents and silica gel in dichloromethane, then concentrating via rotavap) and purified via column chromatography using 80:20 hexanes:ethyl acetate to elute pure halogenated quinoline **7** (120.3 mg , 94%).



Note: We have previously reported a synthesis of this compound.[1]



2

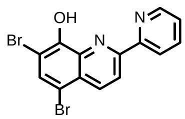
Yield: 22% yield; 10.4 mg of **2** was isolated as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 (br. s, 1H), 8.49 (d, *J* = 8.8 Hz, 1H), 8.16 – 8.11 (m, 2H), 8.02 (d, *J* = 8.8 Hz, 1H), 7.87 (s, 1H), 7.60 – 7.49 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 156.7, 149.8, 138.5, 137.8, 137.2, 133.5, 130.5, 129.3, 127.8, 125.7, 121.0, 110.3, 104.3.

HRMS (DART): calc. for $C_{15}H_{10}Br_2NO$ $[M+H]^+$: 379.9104, found: 379.9091.

MP: 148 - 150 °C.



3

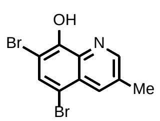
Yield: 78% yield; 70.9 mg of **3** was isolated as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (ddd, *J* = 4.8, 1.8, 1.1 Hz, 1H), 8.73 (d, *J* = 8.8 Hz, 1H), 8.66 (br. s, 1H), 8.54 (d, *J* = 8.8 Hz, 1H), 8.53 (ddd, *J* = 8.0, 1.1, 1.1 Hz, 1H), 7.91 (ddd, *J* = 8.0, 7.7, 1.8 Hz, 1H), 7.90 (s, 1H), 7.42 (ddd, *J* = 7.7, 4.8, 1.1 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.6, 154.6, 149.8, 149.7, 138.2, 137.3, 137.3, 134.1, 126.8, 125.0, 122.0, 121.2, 110.6, 104.3.

HRMS (DART): calc. for $C_{14}H_9Br_2N_2O$ $[M+H]^+$: 380.9056, found: 380.9066.

MP: 225 - 227 °C.



4

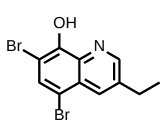
Yield: 62% yield; 27.2 mg of **4** was isolated as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.62 (d, *J* = 1.7 Hz, 1H), 8.16 (d, *J* = 1.7 Hz, 1H), 7.84 (s, 1H), 2.56 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 151.2, 149.8, 137.1, 134.9, 133.9, 133.3, 126.6, 109.7, 102.9, 19.2.

HRMS (DART): calc. for $C_{10}H_8Br_2NO$ $[M+H]^+$: 317.8947, found: 317.8943.

MP: 153 - 155 °C.



5

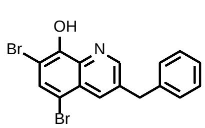
Yield: 31% yield; 12.9 mg of **5** was isolated as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (d, *J* = 1.7 Hz, 1H), 8.19 (d, *J* = 1.7 Hz, 1H), 7.87 (s, 1H), 2.90 (q, *J* = 7.6 Hz, 2H), 1.39 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 150.8, 149.8, 139.4, 137.4, 133.9, 133.8, 126.8, 110.0, 102.9, 26.7, 15.4.

HRMS (DART): calc. for $C_{11}H_{10}Br_2NO$ $[M+H]^+$: 331.9104, found: 331.9096.

MP: 117 - 119 °C.



6

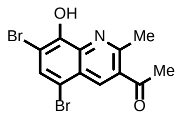
Yield: 28% yield; 15.4 mg of **6** was isolated as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (d, *J* = 2.0 Hz, 1H), 8.21 (d, *J* = 2.0 Hz, 1H), 7.88 (s, 1H), 7.34 (dd, *J* = 8.0, 6.6 Hz, 2H), 7.29 – 7.19 (m, 3H), 4.21 (s, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 151.1, 149.8, 139.1, 137.4, 136.6, 135.1, 134.2, 129.1, 129.1, 127.1, 126.7, 110.0, 103.3, 39.6.

HRMS (DART): calc. for $C_{16}H_{12}Br_2NO$ $[M+H]^+$: 393.9260, found: 393.9270.

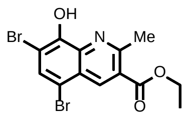
MP: 176 - 178 °C.



7

Yield: 94% yield; 120.3 mg of **7** was isolated as a white solid.

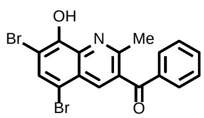
Note: We have previously reported the synthesis of this compound.[1]



8

Yield: 90% yield; 232.7 mg of **8** was isolated as a white solid.

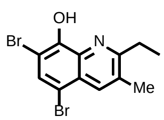
Note: We have previously reported the synthesis of this compound.[1]



9

Yield: 62% yield; 32.8 mg of **9** was isolated as an off-white solid.

Note: We have previously reported the synthesis of this compound.[1]



10

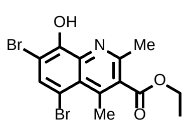
Yield: 90% yield; 69.4 mg of **10** was isolated as an off-white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (d, *J* = 1.0 Hz, 1H), 7.74 (s, 1H), 2.96 (q, *J* = 7.4 Hz, 2H), 2.48 (d, *J* = 1.0 Hz, 3H), 1.39 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 162.7, 149.3, 136.6, 135.3, 132.6, 132.4, 125.4, 109.3, 102.3, 28.6, 19.3, 11.9.

MP: 144 - 146 °C, lit. 150 °C.[6]

Note: Compound has an assigned CAS number (857758-48-6), but no published spectra were found.



11

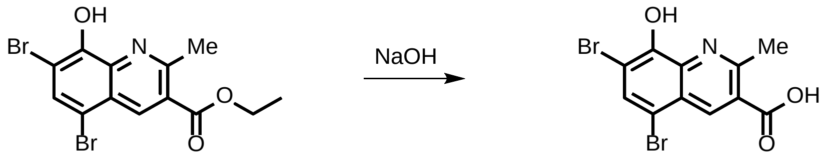
Yield: 79% yield; 53.0 mg of **11** was isolated as an off-white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H), 4.48 (q, *J* = 7.1 Hz, 2H), 2.97 (s, 3H), 2.65 (s, 3H), 1.44 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 168.9, 153.9, 149.6, 143.7, 138.2, 136.6, 131.7, 124.0, 107.4, 104.2, 62.3, 23.3, 21.3, 14.4.

HRMS (DART): calc. for $C_{14}H_{14}Br_2NO_3$ $[M+H]^+$: 403.9315, found: 403.9302.

MP: 97 - 99 °C.



8 **13**

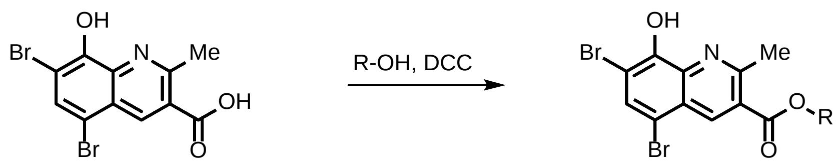
Synthesis of 5,7-dibromo-8-hydroxy-2-methylquinoline-3-carboxylic acid (13). To a round-bottom flask containing quinoline **8** (61.8 mg, 0.16 mmol) was added 1N sodium hydroxide (20 mL). The reaction was left to stir for 17 hours at room temperature. Upon completion, the reaction mixture was transferred to a separatory funnel, neutralized with 1N hydrochloric acid, and then extracted with dichloromethane. The organic layers were combined, dried with anhydrous sodium sulfate, and then filtered. The solvent was removed *in vacuo* to afford **13** as a pure white solid (56.3 mg, >99%), which was used without further purification.

^{1}H NMR (400 MHz, *d_6*-DMSO): δ 8.79 (s, 1H), 8.05 (s, 1H), 2.96 (s, 3H).

^{13}C NMR (100 MHz, *d_6*-DMSO): δ 166.9, 158.1, 150.3, 138.7, 138.3, 133.2, 126.2, 124.3, 109.4, 107.3, 25.0.

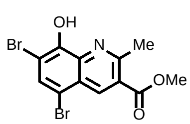
HRMS (DART): calc. for $C_{11}H_8Br_2NO_3$ $[M+H]^+$: 361.8845, found: 361.8831.

MP: 247 - 249 °C.



13 **14 - 16**

General procedure for the synthesis of 5,7-dibromo-8-hydroxy-2-methylquinoline-3-esters (14 - 16). To a stirring solution of 5,7-dibromo-8-hydroxy-2-methylquinoline-3-carboxylic acid **13** (65.0 mg, 0.18 mmol) and dicyclohexyl carbodiimide (44.6 mg, 0.22 mmol) in dichloromethane (4 mL) was added methanol (1 mL, 24.7 mmol). The reaction was then brought to reflux and allowed to react for 6 hours. Upon completion by TLC, the reaction contents were washed with water and partitioned between ethyl acetate and water in a separatory funnel. The organic contents were collected and dried with anhydrous sodium sulfate. The solvent was filtered and removed *in vacuo*. The resulting crude solid was purified via column chromatography using 99:1 to 1:1 hexanes:ethyl acetate to afford **14** as a white solid (15.5 mg, 23%).



14

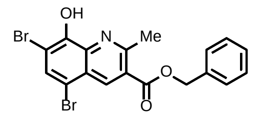
Yield: 23% yield; 15.5 mg of **14** was isolated as a white solid.

^{1}H NMR (400 MHz, CDCl$_3$): δ 8.92 (s, 1H), 7.86 (s, 1H), 4.01 (s, 3H), 3.00 (s, 3H).

^{13}C NMR (100 MHz, CDCl$_3$): δ 166.3, 159.0, 149.2, 139.9, 138.6, 133.9, 125.7, 124.5, 110.8, 106.3, 53.0, 25.4.

HRMS (DART): calc. for $C_{12}H_{10}Br_2NO_3$ $[M+H]^+$: 375.9002, found: 375.9005.

MP: 158 - 160 °C.



15

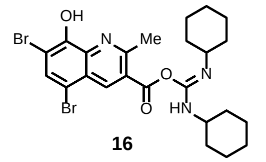
Yield: 16% yield; 14.9 mg of **15** was isolated as a white solid.

^{1}H NMR (400 MHz, CDCl$_3$): δ 8.98 (s, 1H), 7.87 (s, 1H), 7.55 – 7.47 (m, 2H), 7.47 – 7.34 (m, 3H), 5.45 (s, 2H), 2.99 (s, 3H).

^{13}C NMR (100 MHz, CDCl$_3$): δ 165.8, 159.0, 149.2, 140.0, 138.6, 135.6, 134.0, 129.0, 128.8, 128.6, 125.8, 124.5, 110.9, 106.4, 67.8, 25.5.

HRMS (DART): calc. for $C_{18}H_{14}Br_2NO_3$ $[M+H]^+$: 451.9316, found: 451.9326.

MP: 105 - 107 °C.



16

Yield: 37% yield; 38.2 mg of **16** was isolated as a white solid.

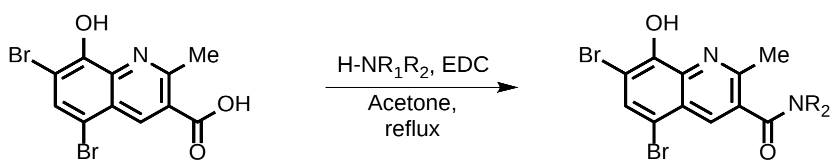
^{1}H NMR (400 MHz, CDCl$_3$): δ 8.22 (s, 1H), 7.86 (s, 1H), 3.89 (m, 1H), 3.49 (dt, *J* = 10.7, 8.3 Hz, 1H), 2.78 (s, 3H), 2.07 – 1.94 (m, 2H), 1.93 – 1.85 (m, 2H), 1.84 – 1.76 (m, 2H), 1.75 – 1.66 (m, 2H), 1.64 – 1.49 (m, 4H), 1.31 – 1.19 (m, 2H), 1.19 – 0.97 (m, 6H).

^{13}C NMR (100 MHz, CDCl$_3$): δ 169.7, 155.7, 153.1, 149.3, 137.7, 134.0, 132.8, 132.6, 124.1, 110.1, 105.2, 58.2, 50.0, 32.7, 31.1, 26.3, 25.5, 25.3, 24.7, 23.1.

HRMS (DART): calc. for $C_{24}H_{30}Br_2N_3O_3$ $[M+H]^+$: 568.0630, found: 568.0632.

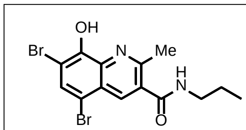
MP: 96 - 98 °C.

Note: This compound was isolated as a side product obtained during the synthesis of halogenated quinoline **14**.



13 **17 - 19**

General procedure for the synthesis of 5,7-dibromo-8-hydroxy-2-methylquinoline-3-amides (17 - 19). To a solution of 5,7-dibromo-8-hydroxy-2-methylquinoline-3-carboxylic acid **13** (59.1 mg, 0.16 mmol) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (37.7 mg, 0.20 mmol) in acetone (6 mL) was added benzylamine (18 µL, 0.16 mmol). The reaction was then brought to reflux and allowed to react for 5 hours. Upon completion by TLC, the reaction contents were washed with water and partitioned between ethyl acetate and water in a separatory funnel. The organic contents were collected and dried with anhydrous sodium sulfate. The solvent was filtered and removed *in vacuo*. The resulting crude solid was purified via column chromatography using 95:5 to 1:1 hexanes:ethyl acetate to afford the product, **18**, as a white solid (21.1 mg, 29%).



17

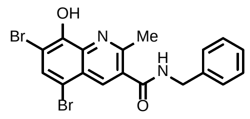
Yield: 26% yield; 14.8 mg of **17** was isolated as a white solid.

^{1}H NMR (400 MHz, CDCl$_3$): δ 8.20 (s, 1H), 7.78 (s, 1H), 6.35 (br. s, 1H), 3.48 (q, *J* = 6.7 Hz, 2H), 2.79 (s, 3H), 1.72 (qt, *J* = 7.3, 7.3 Hz, 2H), 1.05 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, CDCl$_3$): δ 167.9, 156.9, 148.9, 137.5, 134.1, 133.7, 132.6, 124.1, 110.2, 105.0, 42.2, 23.4, 23.1, 11.7.

HRMS (DART): calc. for $C_{14}H_{15}Br_2N_2O_2$ $[M+H]^+$: 402.9475, found: 402.9479.

MP: 184 - 186 °C.



18

Yield: 29% yield; 21.1 mg of **18** was isolated as a white solid.

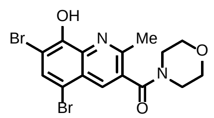
[1]H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, 1H), 7.82 (s, 1H), 7.43 – 7.37 (m, 4H), 7.34 (m, 1H), 6.44 (m, 1H), 4.69 (s, 2H), 2.83 (s, 3H).

[13]C NMR (100 MHz, $CDCl_3$): δ 167.6, 156.9, 149.1, 137.8, 137.7, 134.3, 133.8, 132.1, 132.1, 129.2, 128.2, 128.2, 124.2, 110.2, 105.3, 44.6, 44.4, 23.6.

HRMS (DART): calc. for $C_{18}H_{15}Br_2N_2O_3$ $[M+H]^+$:450.9475, found: 450.9463.

MP: 208 - 210 °C.

Note: Observed additional [13]C NMR peaks which likely correspond to amide rotamers. This was also observed in d_6-DMSO. No impurities were observed via [1]H NMR or HRMS.



19

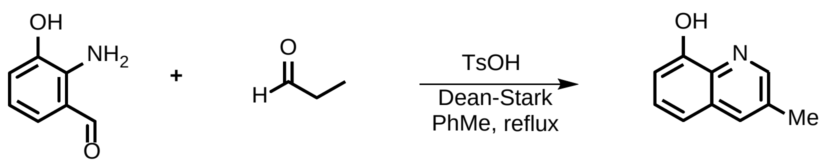
Yield: 15% yield; 18.4 mg of **19** was isolated as a white solid.

[1]H NMR (400 MHz, $CDCl_3$): δ 8.22 (s, 1H), 7.87 (s, 1H), 3.90 (m, 2H), 3.84 (m, 2H), 3.64 (m, 2H), 3.29 (m, 2H), 2.75 (s, 3H).

[13]C NMR (100 MHz, $CDCl_3$): δ 167.5, 155.4, 149.3, 137.8, 134.0, 133.9, 131.5, 124.6, 110.2, 105.1, 67.0, 67.0, 47.7, 42.5, 22.9.

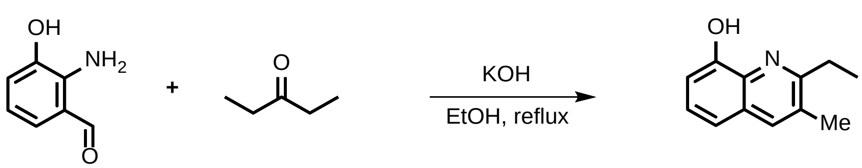
HRMS (DART): calc. for $C_{15}H_{15}Br_2N_2O_3$ $[M+H]^+$: 430.9424, found: 430.9407.

MP: 186 - 188 °C.



20 **26** **4a**

General procedure for the synthesis of 8-hydroxyquinolines under acidic conditions and traditional oil bath heating. To a stirring solution of 2-amino-3-hydroxybenzaldehyde **20** (74.0 mg, 0.54 mmol) in toluene (27 mL) was added propionaldehyde **29** (46 µL, 0.65 mmol) and *p*-toluenesulfonic acid (5.4 mg, 0.03 mmol). The resulting mixture was then heated to reflux for 24 hours using a Dean-Stark trap. After that time, the reaction was then allowed to cool and the mixture was washing with saturated sodium bicarbonate. The solution was then extracted with ethyl acetate, which was then dried with anhydrous sodium sulfate, filtered, and removed in vacuo. The crude solid was purified via flash column chromatography eluting with 95:5 to 4:1 hexanes:ethyl acetate to afford quinoline **4a** as a white solid (9.2 mg, 11%). **Note:** This protocol was modified from a previously published procedure.[8]



20 **25** **10a**

General procedure for the synthesis of 8-hydroxyquinolines under basic conditions and traditional oil bath heating. To a stirring solution of 2-amino-3-hydroxybenzaldehyde **20** (128.0 mg, 0.93 mmol) in ethanol (10 mL) was added potassium hydroxide (141 mg, 2.52 mmol) and 3-pentanone **25** (148 µL, 1.40 mmol). The resulting mixture was then heated to reflux for 24 hours. After that time, the reaction was then allowed to cool and the solvent was removed *in vacuo*. The crude residue was taken up in dichloromethane, transferred to a separatory funnel and then neutralized with 2N hydrochloric acid. The solution was then extracted with dichloromethane three times. The organic layers were combined, dried with anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The crude solid was purified via flash column chromatography eluting with 95:5 to 4:1 hexanes:ethyl acetate to afford quinoline **10a** as a white solid (42.0 mg, 24%). **Note:** All reaction yields for traditional oil bath conversion are listed in the manuscript.

3.) Biological Methods:

A.) Minimum Inhibitory Concentration (MIC) Susceptibility Assay (in 96-well plate):

The minimum inhibitory concentration (MIC) for each quinoline was determined by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI).[9] In a 96-well plate, eleven two-fold serial dilutions of each compound were made in a final volume of 100 µL Luria Broth. Each well was inoculated with ~10^5 bacterial cells at the initial time of incubation, prepared from a fresh log phase culture (OD_{600} of 0.5 to 1.0 depending on bacterial strain). The MIC was defined as the lowest concentration of compound that prevented bacterial growth after incubating 16 to 18 hours at 37 °C (MIC values were supported by spectrophotometric readings at OD_{600}). The concentration range tested for each quinoline/antibacterial during this study was 0.10 to 100 µM. DMSO served as our vehicle and negative control in each microdilution MIC assay. DMSO was serially diluted with a top concentration of 1% v/v. Each experiment was conducted in three replicates from separate, individual bacterial colonies.

B.) Calgary Biofilm Device (CBD) Experiments

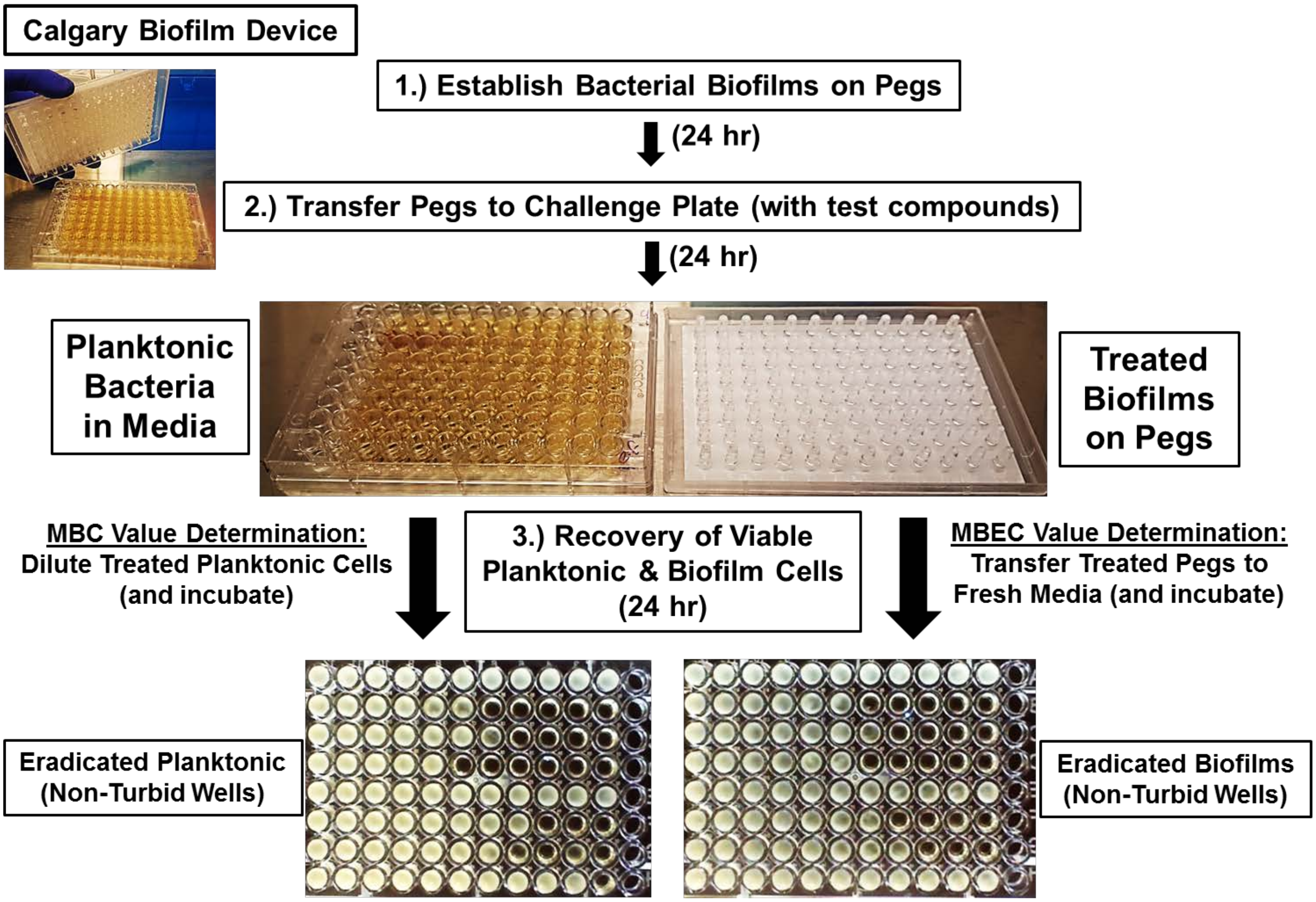
Minimum Bactericidal Concentrations (MBC) and Minimum Biofilm Eradication Concentrations (MBEC)

Biofilm eradication experiments were performed using the Calgary Biofilm Device to determine MBC/MBEC values for various compounds of interest (Innovotech, product code: 19111). The Calgary device (96-well plate with lid containing pegs to establish biofilms on) was inoculated with 125 µL of a mid-log phase culture diluted 1,000-fold in tryptic soy broth with 0.5% glucose (TSBG) to establish bacterial biofilms after incubation at 37 °C for 24 hours. The lid of the Calgary device was then removed, washed and transferred to another 96-well plate containing 2-fold serial dilutions of the test compounds (the "challenge plate"). The total volume of media with compound in each well in the challenge plate is 150 µL. The Calgary device was then incubated at 37 °C for 24 hours. The lid was then removed from the challenge plate and MBC/MBEC values were determined using different final assays. To determine **MBC values**, 20 µL of the challenge plate was transferred into a fresh 96-well plate containing 180 µL TSBG and incubated overnight at 37 °C. The MBC values were determined as the concentration giving a lack of visible bacterial growth (i.e., turbidity). For determination of **MBEC values**, the Calgary device lid (with attached pegs/treated biofilms) was transferred to a new 96-well plate containing 150 µL of fresh TSBG media in each well and incubated for 24 hours at 37 °C to allow viable biofilms to grow and disperse resulting in turbidity after the incubation period. MBEC values were determined as the lowest test concentration that resulted in eradicated biofilm (i.e., wells that had no turbidity after final incubation). Each experiment was conducted in three replicates from separate, individual bacterial colonies.

Supporting Table 1 MBC/MBEC for select HQs against MRSA BAA 1707 and BAA 44.

Compound	MRSA BAA 1707 MBC / MBEC	MRSA BAA 44 MBC / MBEC
1	31.3 [a] / 31.3 [a]	46.9 [a] / 93.8 [a]
4	46.9 [a] / 1000 [a]	93.8 [a] / > 2000
5	62.5 / 250	125 / > 1000
7	> 1000 / > 1000	> 1000 / > 1000
8	500 [a] / > 1000	> 1000 / > 1000
9	7.8 / 3.9	23.5 [a] / 15.6
Vancomycin	5.9 [a] / > 2000	3.0 [a] / > 2000

Note: [a]Midpoint value of a two-fold range. All concentrations have been recorded in µM.



F.) Haemolysis Assay:

We performed haemolysis assays based on a previous procedure.[5] Freshly drawn human red blood cells (hRBC with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant) were washed with Tris-buffered saline (0.01M Tris-base, 0.155 M sodium chloride (NaCl), pH 7.2) and centrifuged for 5 minutes at 3,500 rpm. The washing was repeated three times with the buffer. In 96-well plate, test compounds were added to the buffer from DMSO stocks. Then 2% hRBCs (50 µL) in buffer were added to test compounds to give a final concentration of 200 µM. The plate was then incubated for 1 hour at 37 °C. After incubation, the plate was centrifuged for 5 minutes at 3,500 rpm. Then 80 µL of the supernatant was transferred to another 96-well plate and the optical density (OD) was read at 405 nm. DMSO served as our negative control (0% haemolysis) while Triton X served as our positive control (100% haemolysis). The percent of haemolysis was calculated as (OD_{405} of the compound- OD_{405} DMSO) / (OD_{405} Triton X- OD_{405} buffer). Each experiment was conducted in three replicates.

4.) Literature References:

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8.) J. M. Muchowski, M. L. Maddox, *Can. J. Chem*., 2004, **82**, 461 – 478.

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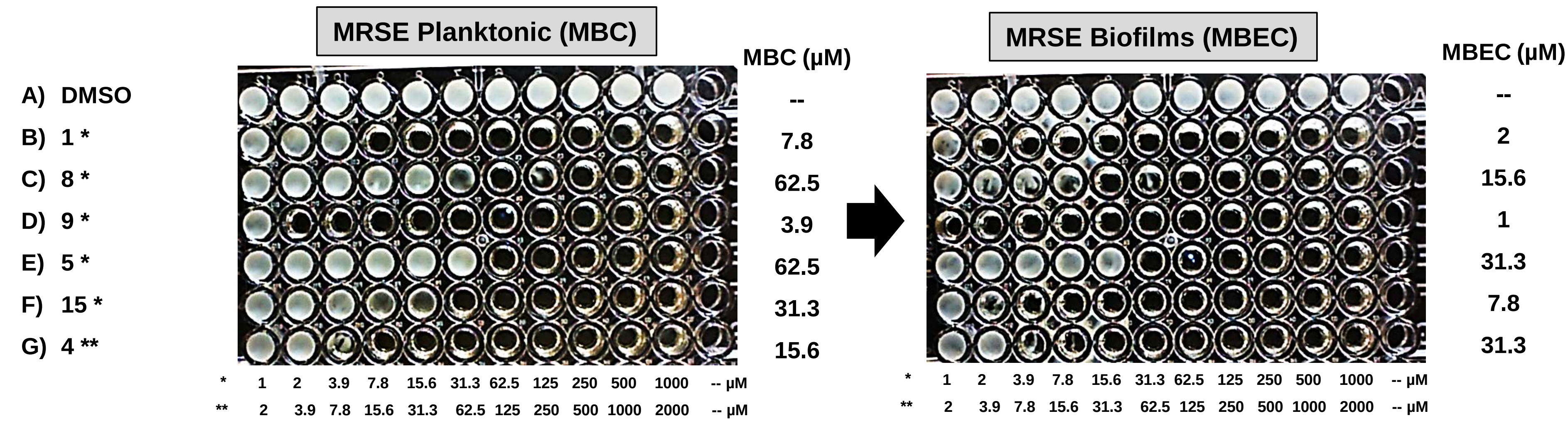
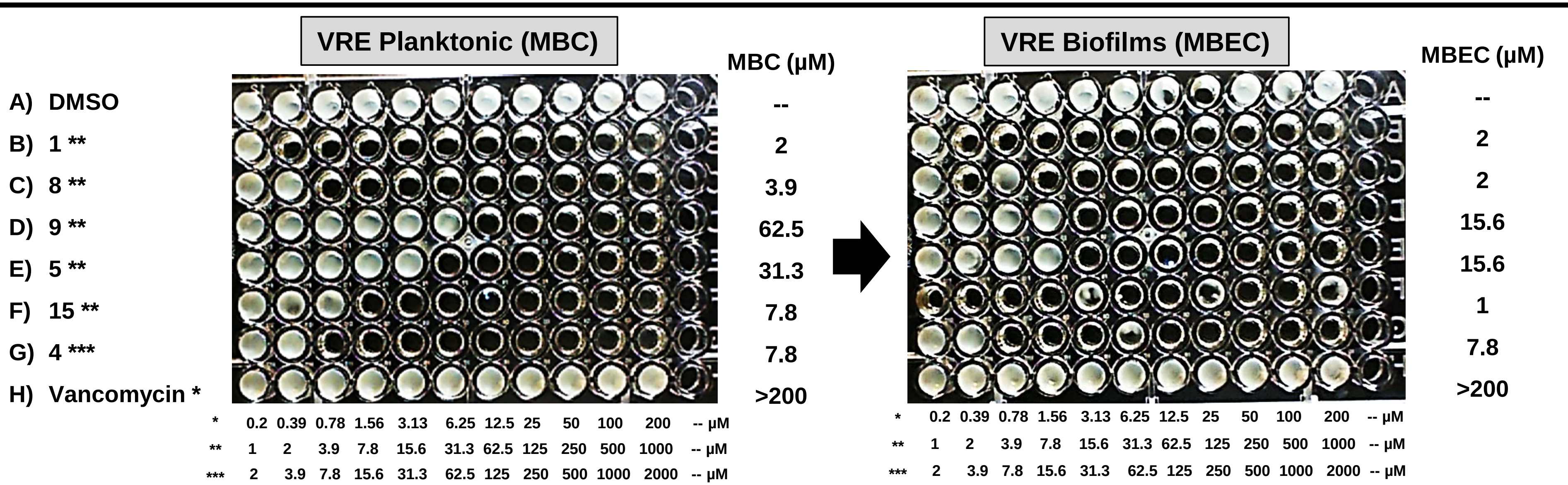
S. epidermidis (MRSE 35984) Biofilm Eradication (CBD Assay)

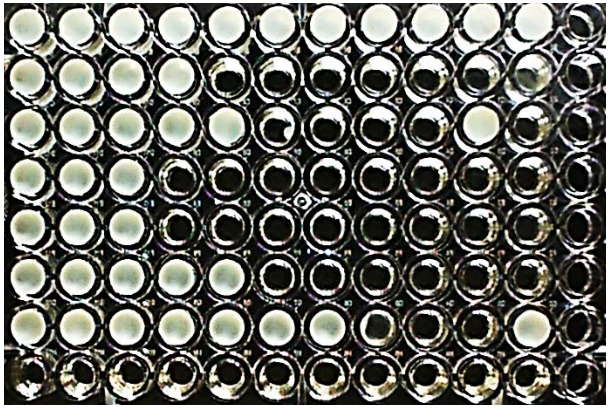


E. faecium (VRE 700221) Biofilm Eradication (CBD Assay)

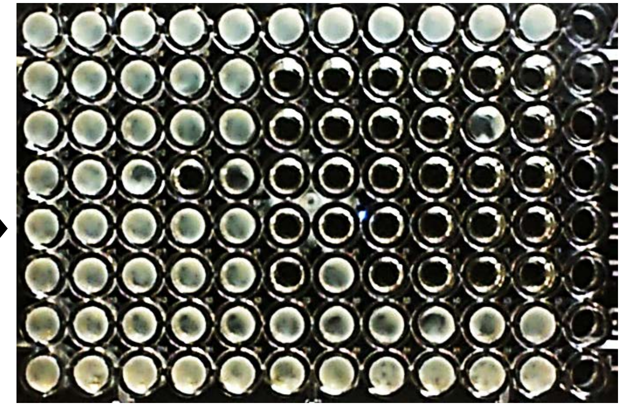


MRSA-2 Biofilm Eradication (CBD Assay)

MRSA Planktonic (MBC)



MRSA Biofilms (MBEC)

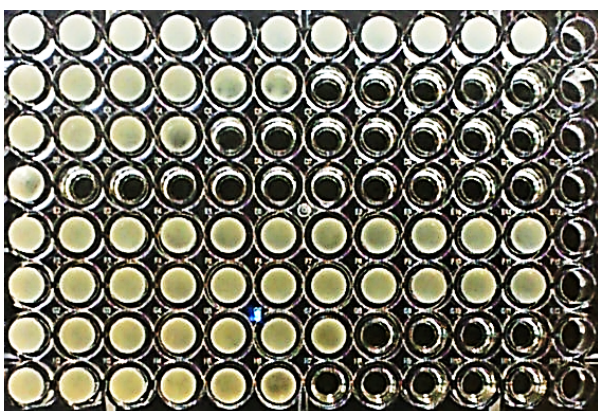


	MBC (µM)	MBEC (µM)
A) DMSO	--	--
B) 1 *	15.6	31.3
C) 8 *	31.3	31.3
D) 9 *	7.8	31.3
E) 4 **	15.6	62.5
F) 5 *	31.3	125
G) 15 *	250	>1000
H) Vancomycin **	2	>2000

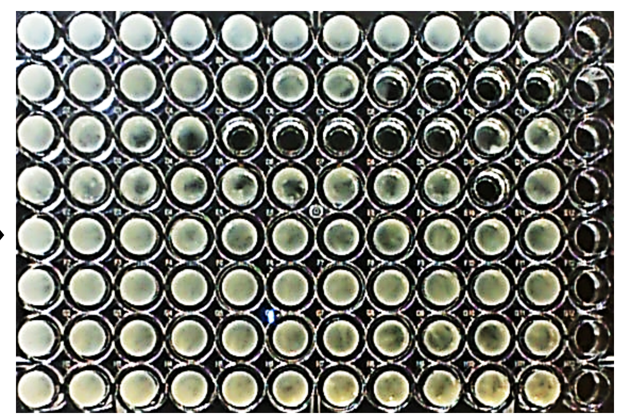
*	1	2	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	-- µM
**	2	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	2000	-- µM

MRSA BAA 44 Biofilm Eradication (CBD Assay)

MRSA BAA 44 Planktonic (MBC)



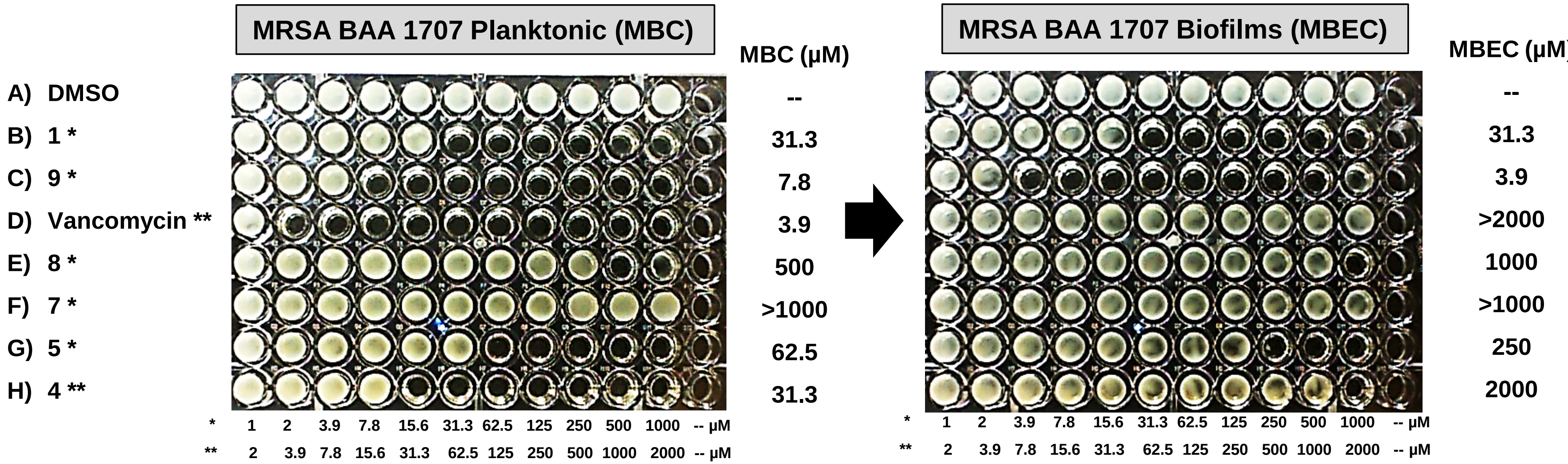
MRSA BAA 44 Biofilms (MBEC)



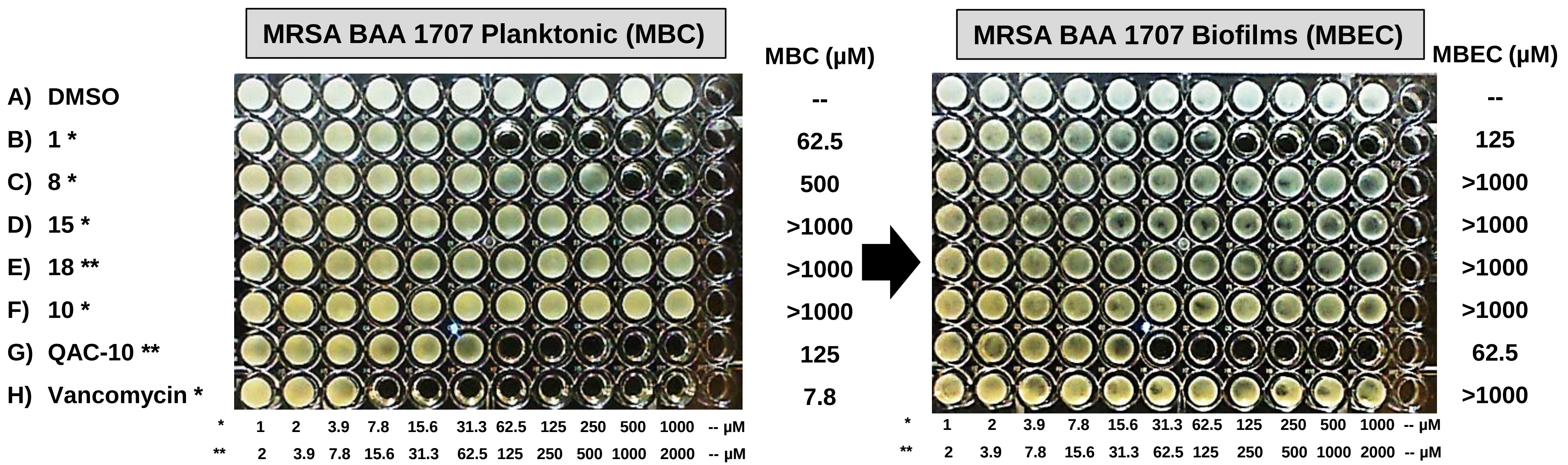
	MBC (µM)	MBEC (µM)
A) DMSO	--	--
B) 1 *	62.5	125
C) 9 *	15.6	15.6
D) Vancomycin **	3.9	>2000
E) 8 *	>1000	>1000
F) 7 *	>1000	>1000
G) 5 *	125	>1000
H) 4 **	125	>2000

*	1	2	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	-- µM
**	2	3.9	7.8	15.6	31.3	62.5	125	250	500	1000	2000	-- µM

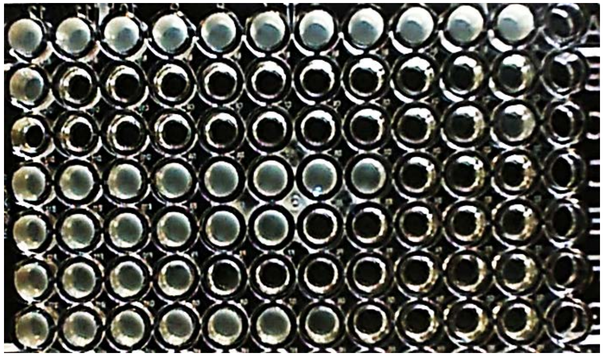
MRSA BAA 1707 Biofilm Eradication (CBD Assay)



MRSA BAA 1707 Biofilm Eradication (CBD Assay)

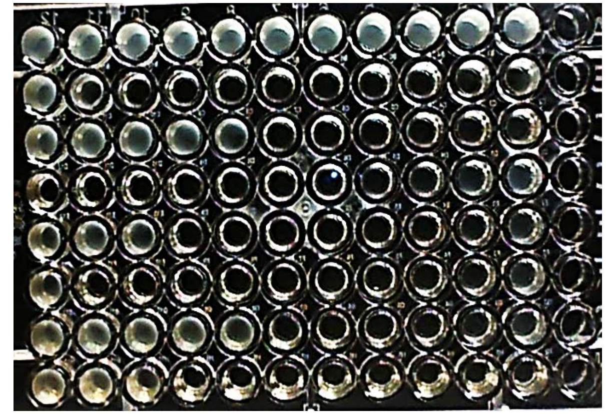


S. epidermidis (MRSE 35984) MIC



Row	Compound	MIC (µM)
A)	DMSO	--
B)	1	0.2
C)	8	0.1
D)	13	25
E)	7	6.25
F)	6	3.13
G)	17	12.5

Columns (µM): 0.1, 0.2, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, --



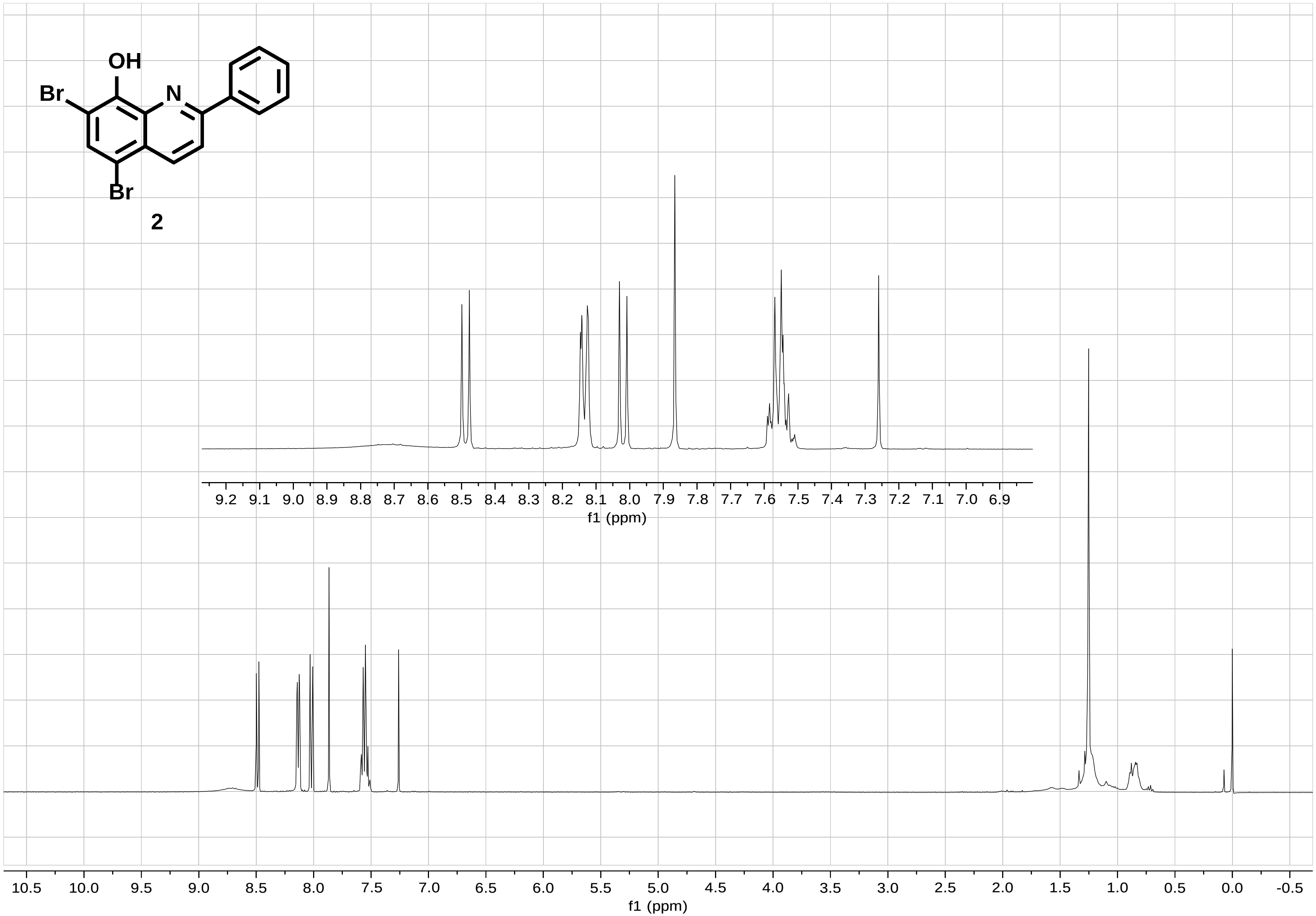
Row	Compound	MIC (µM)
A)	DMSO	--
B)	2	0.39
C)	3	3.13
D)	10	0.1
E)	4	0.78
F)	14	0.2
G)	18	6.25
H)	11	0.78

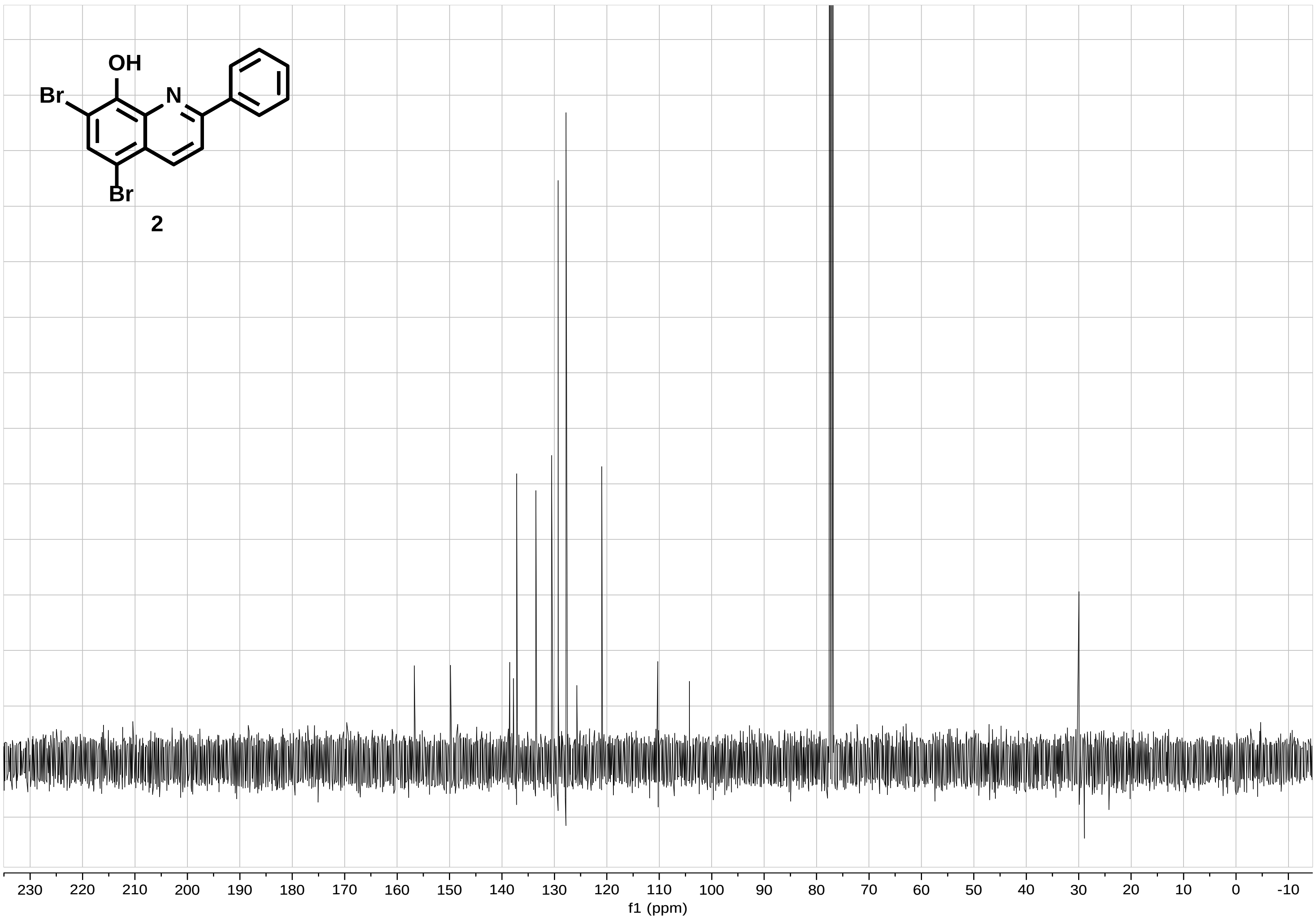
Columns (µM): 0.1, 0.2, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, --

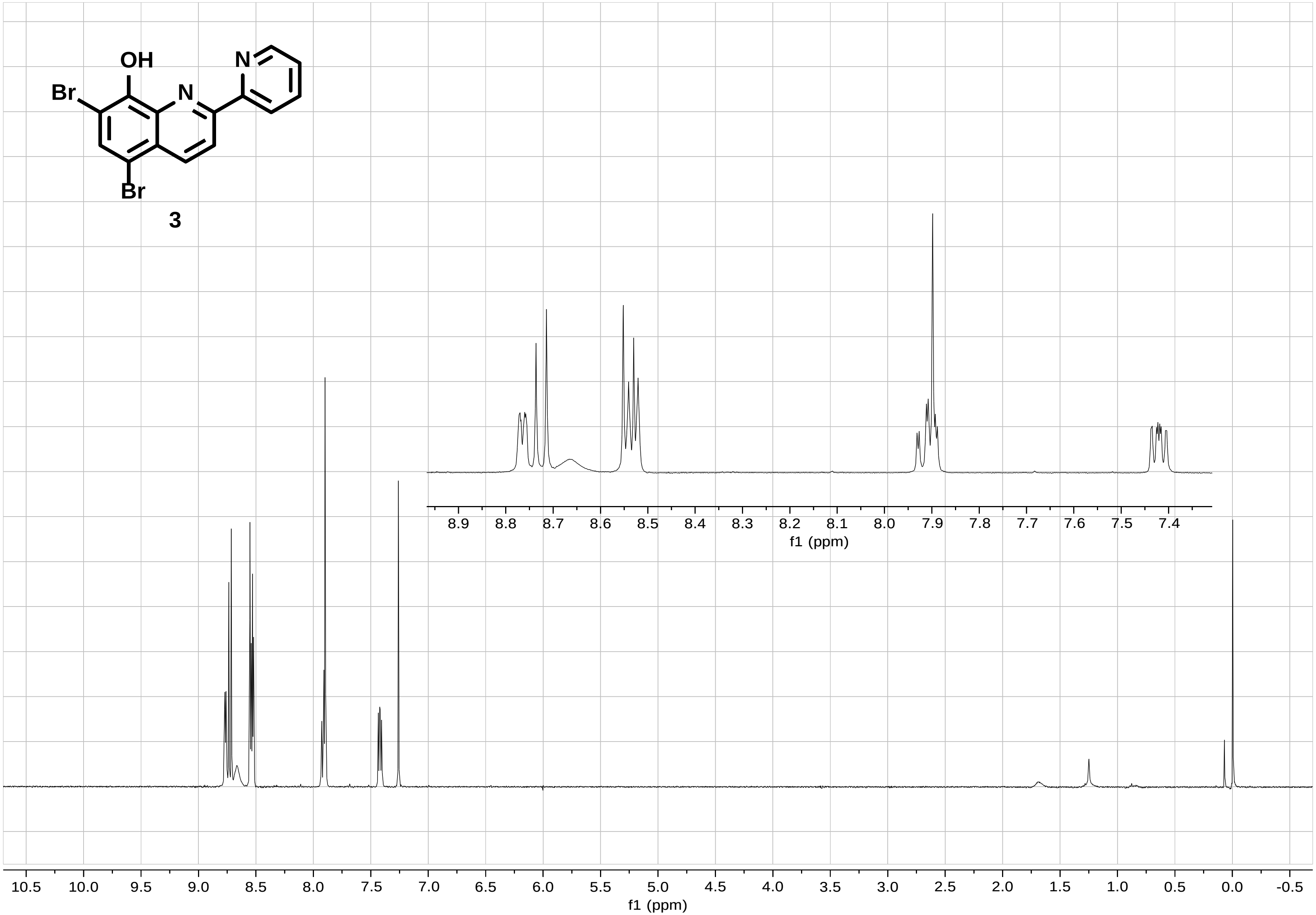
E. faecium (VRE 700221) MIC

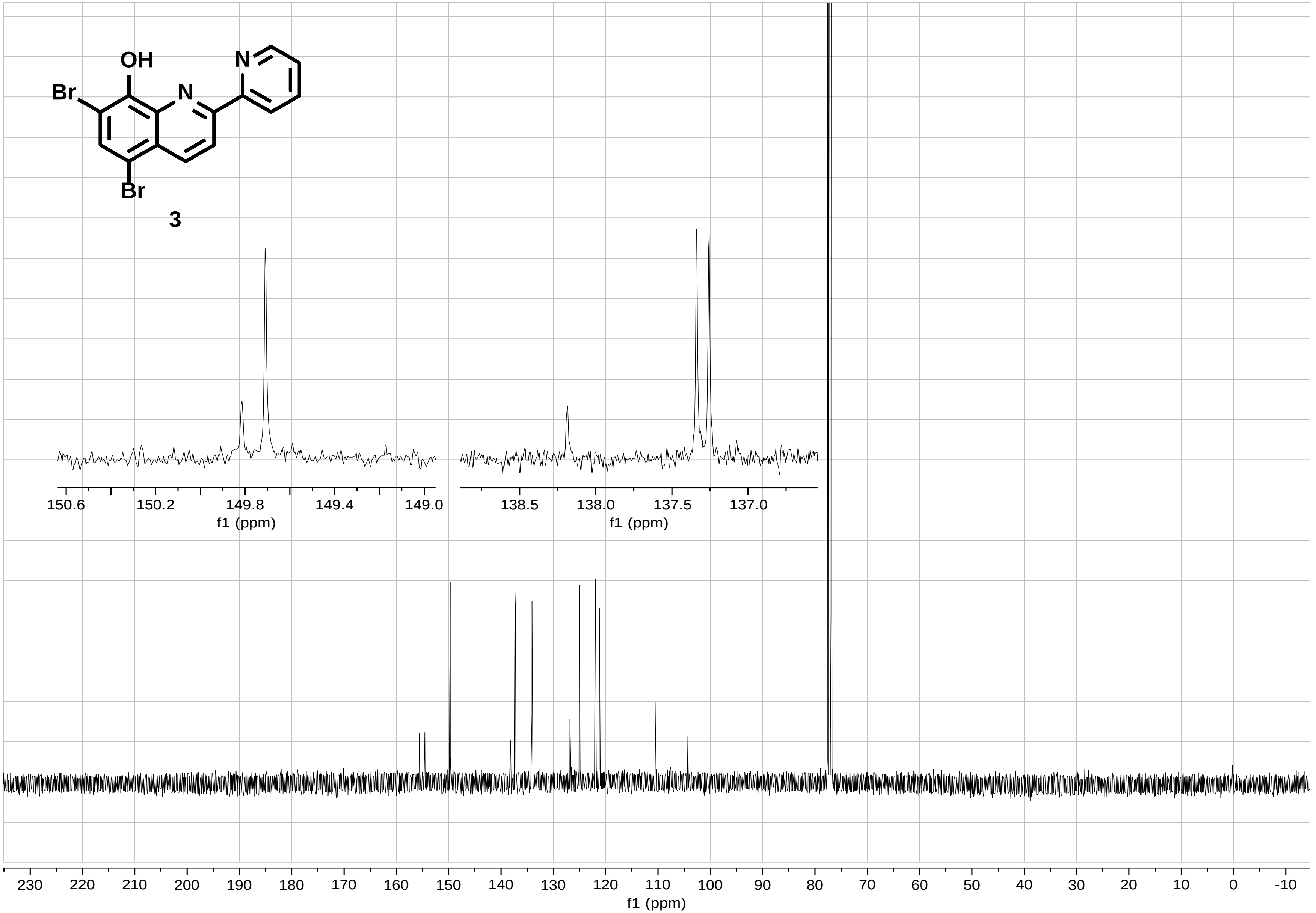
Row	Compound	MIC (µM)
A)	DMSO	--
B)	1	1.56
C)	8	0.78
D)	17	50
E)	10	0.78
F)	4	3.13
G)	14	3.13
H)	18	25

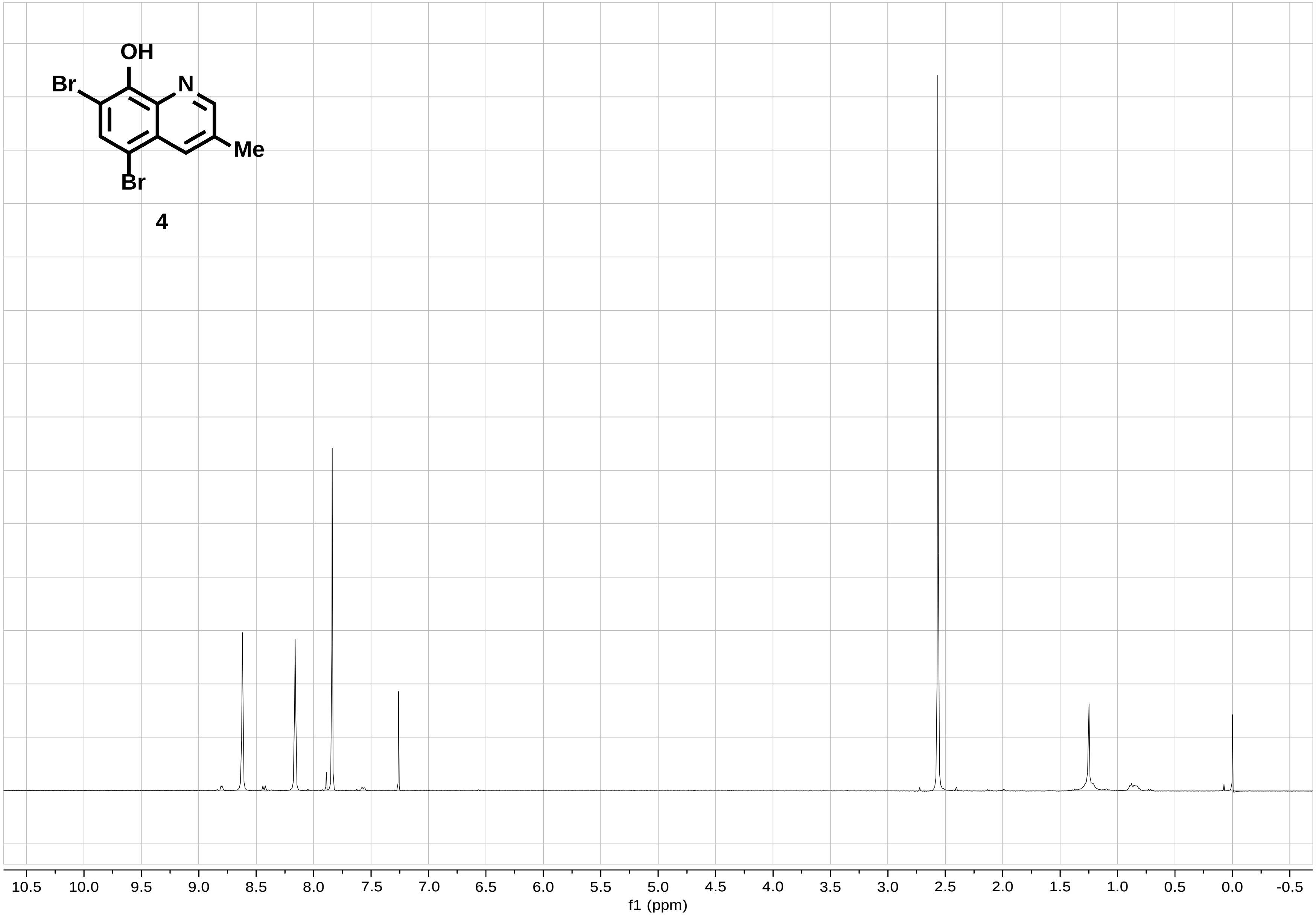
Columns (µM): 0.1, 0.2, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, --

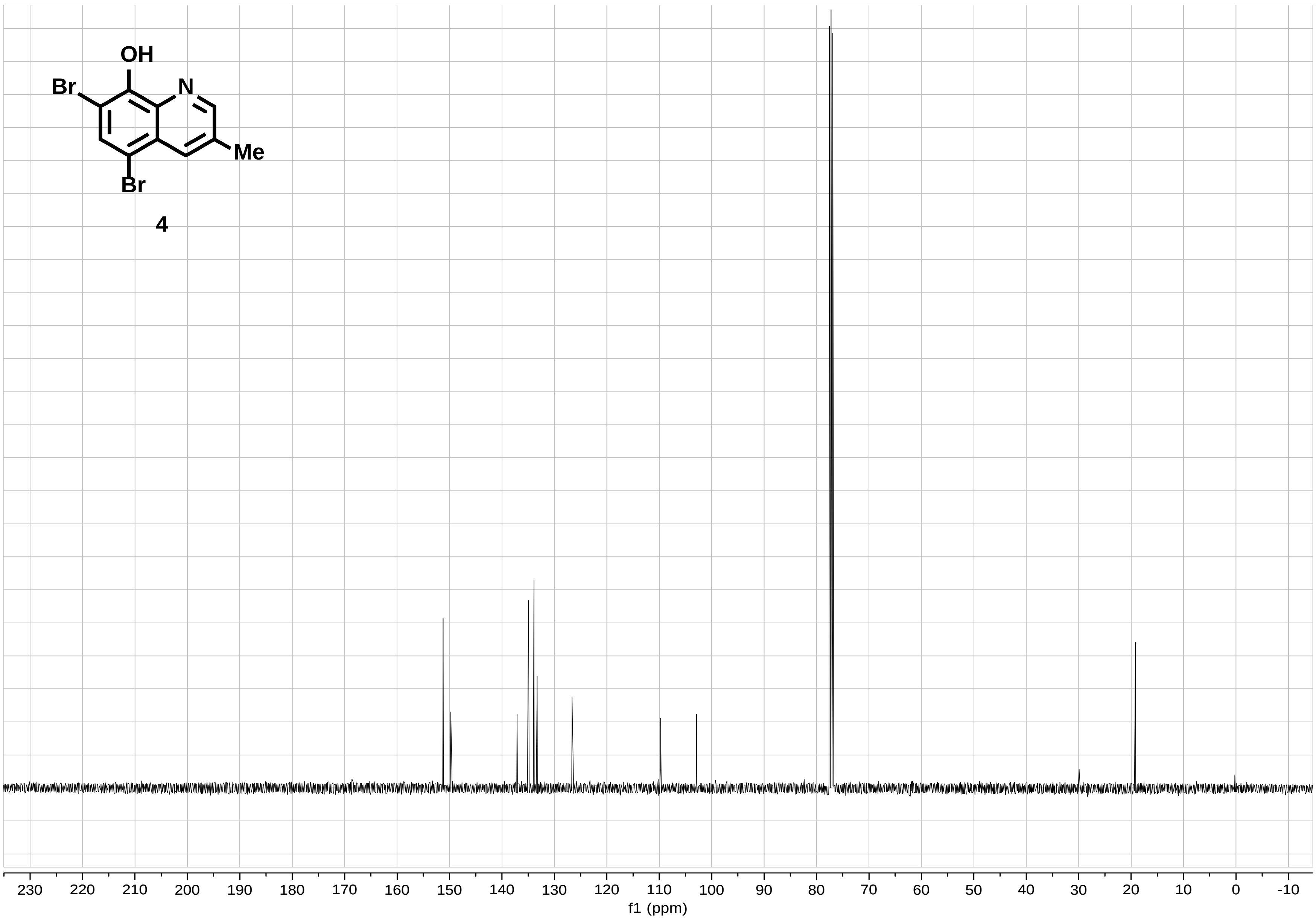

OH
Br
N
Br
2
9.2 9.1 9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9
f1 (ppm)
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

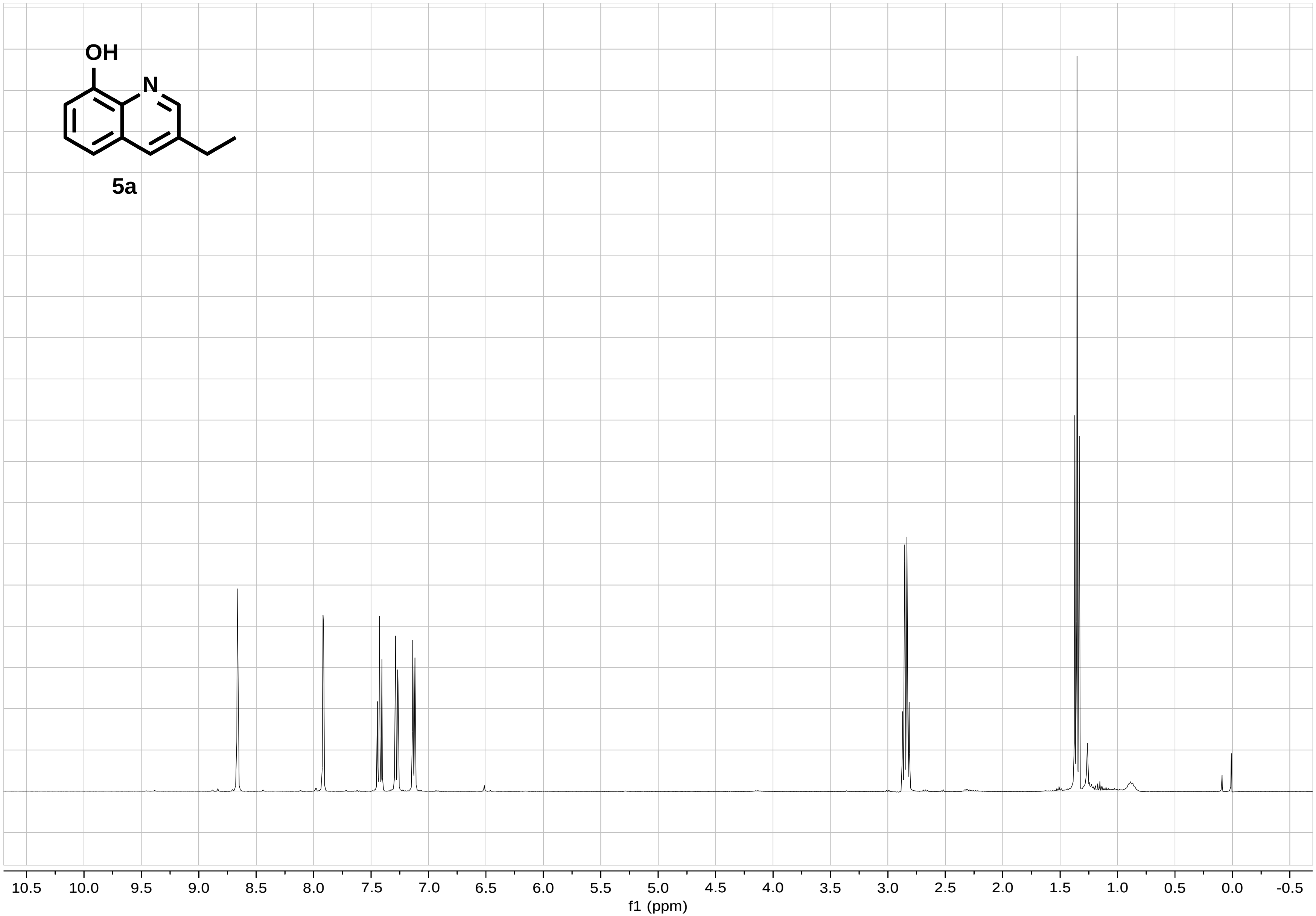

OH
Br
N
Br
2
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

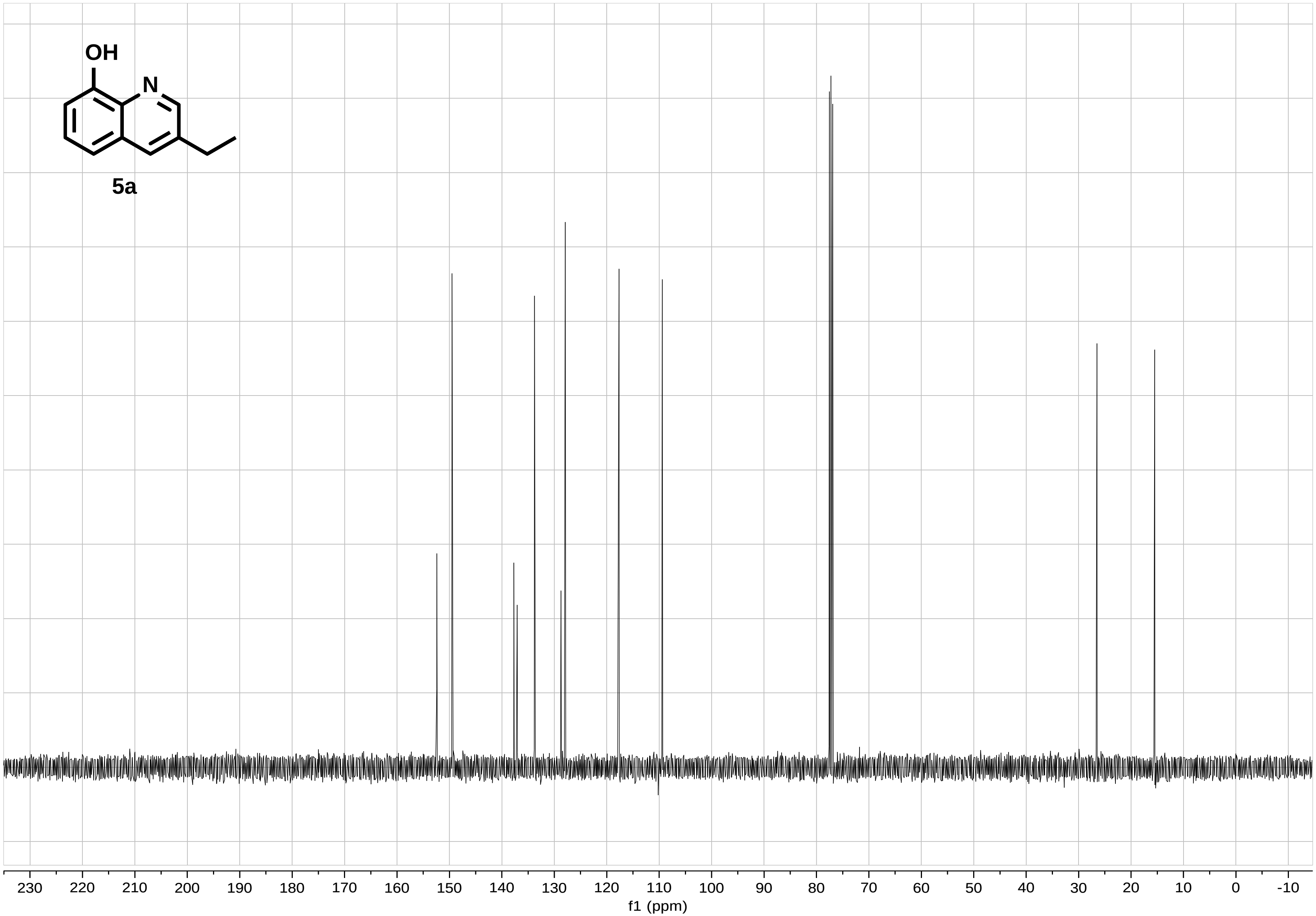

OH
Br
N
N
Br
3
8.9
8.8
8.7
8.6
8.5
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

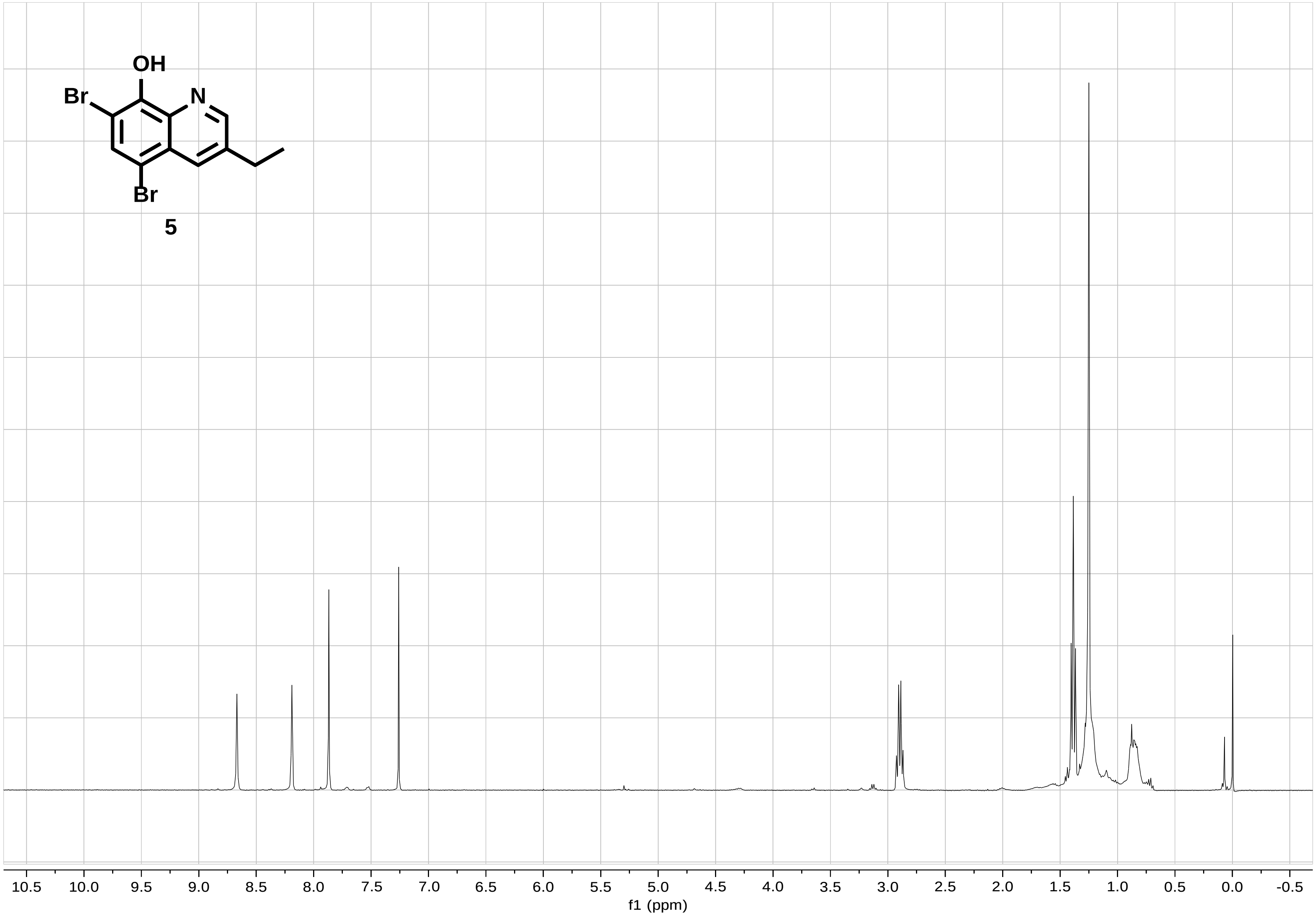

3
150.6
150.2
149.8
149.4
149.0
f1 (ppm)
138.5
138.0
137.5
137.0
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

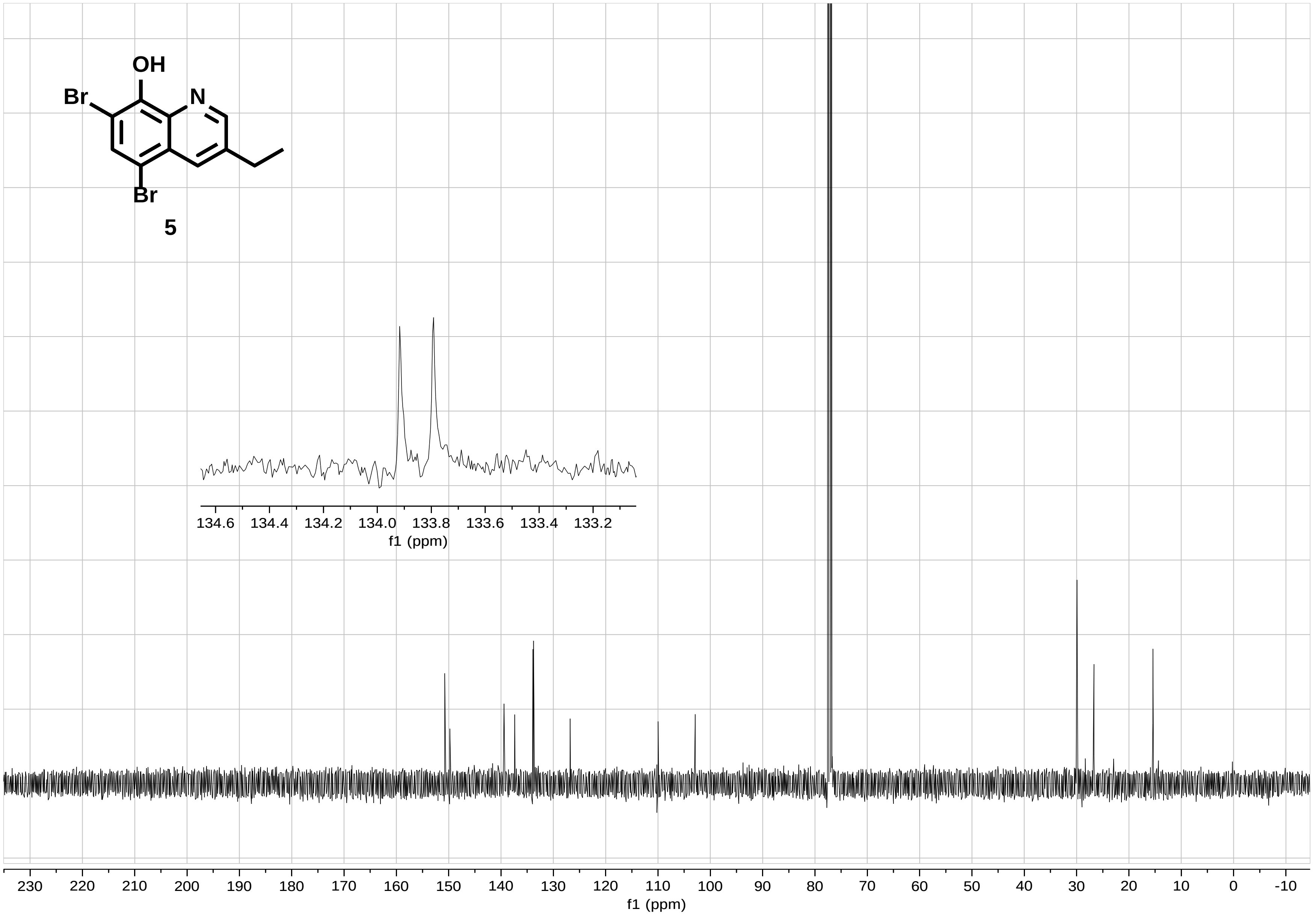

4
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

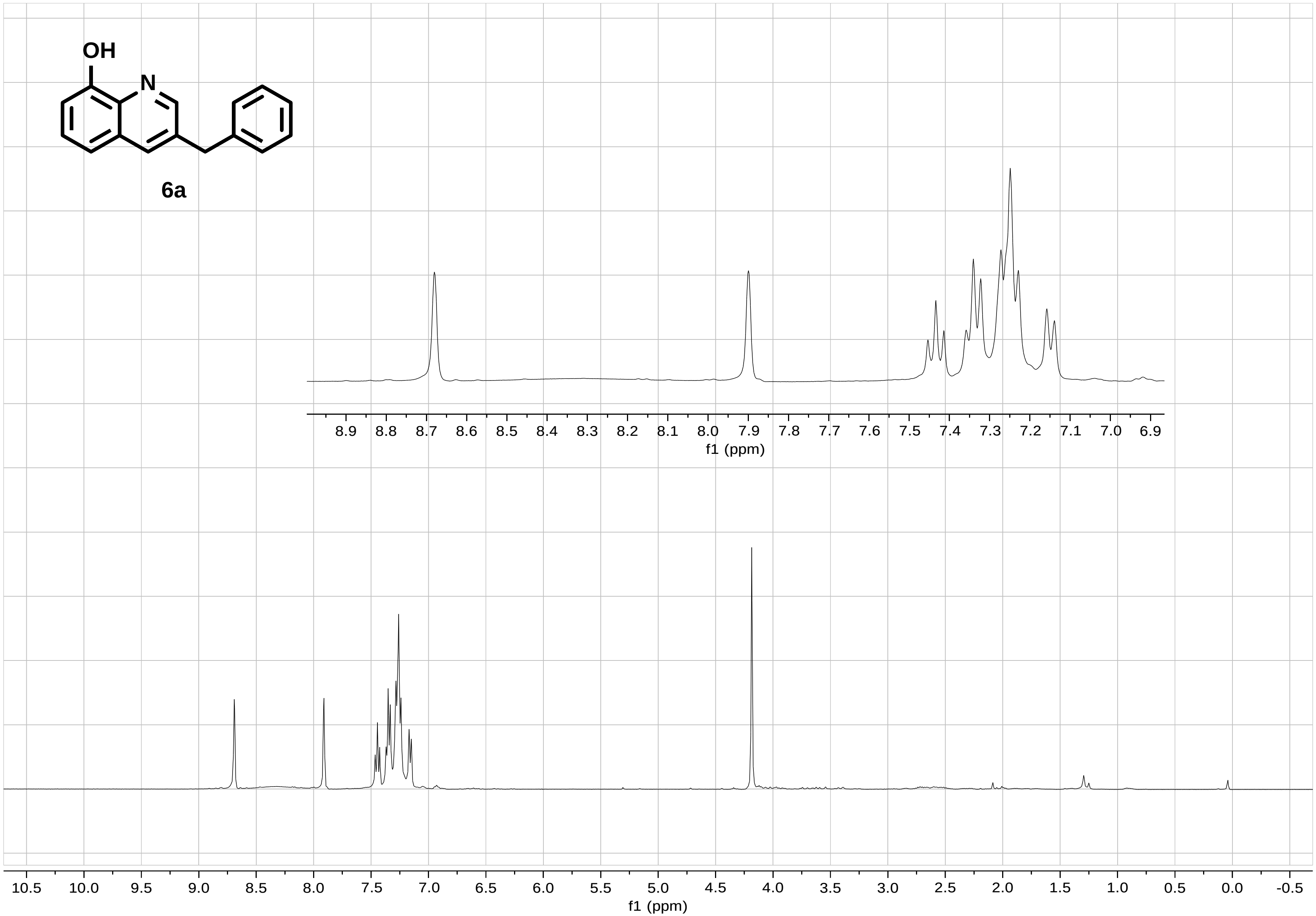

OH
Br
N
Me
Br
4
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

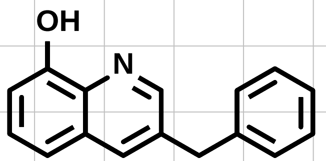

OH
N
5a
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


OH
N
5a
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

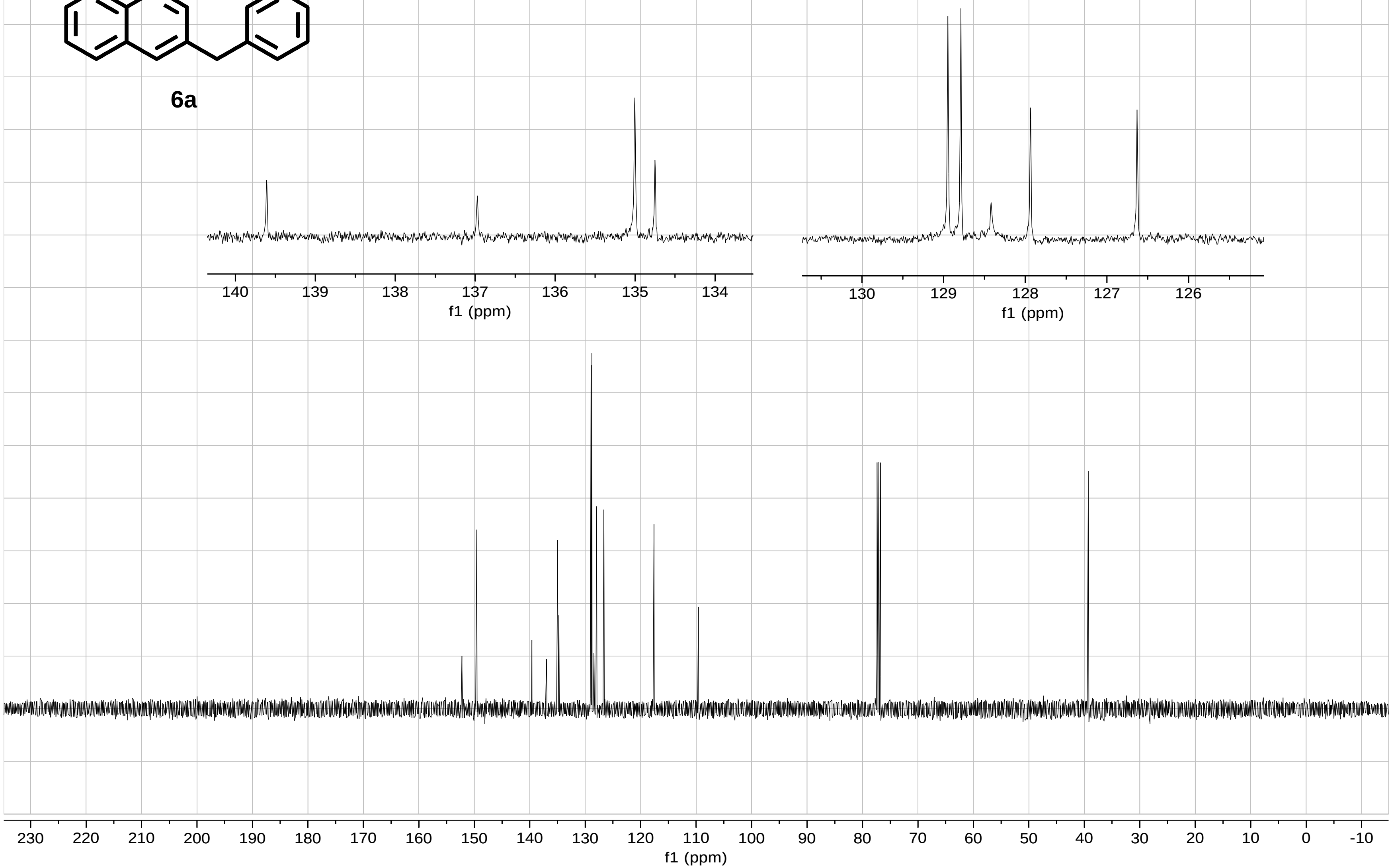

OH
Br
N
Br
5
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


OH
Br
N
Br
5
134.6
134.4
134.2
134.0
133.8
133.6
133.4
133.2
f1 (ppm)
230
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)

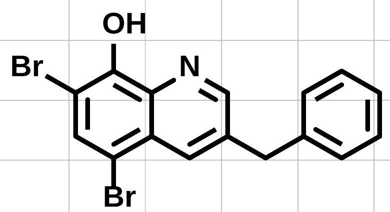

OH
N
6a
8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9
f1 (ppm)
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)



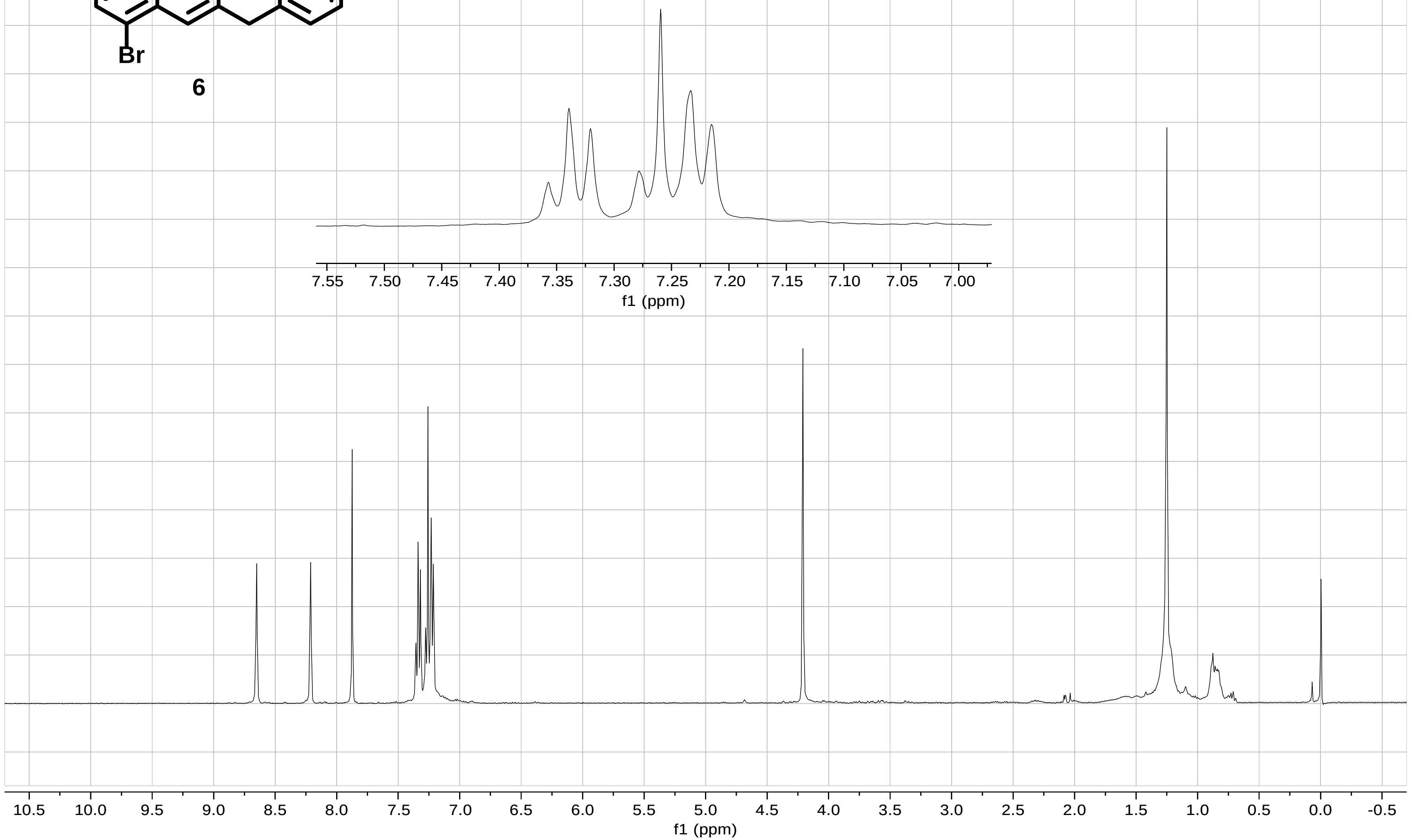
6a

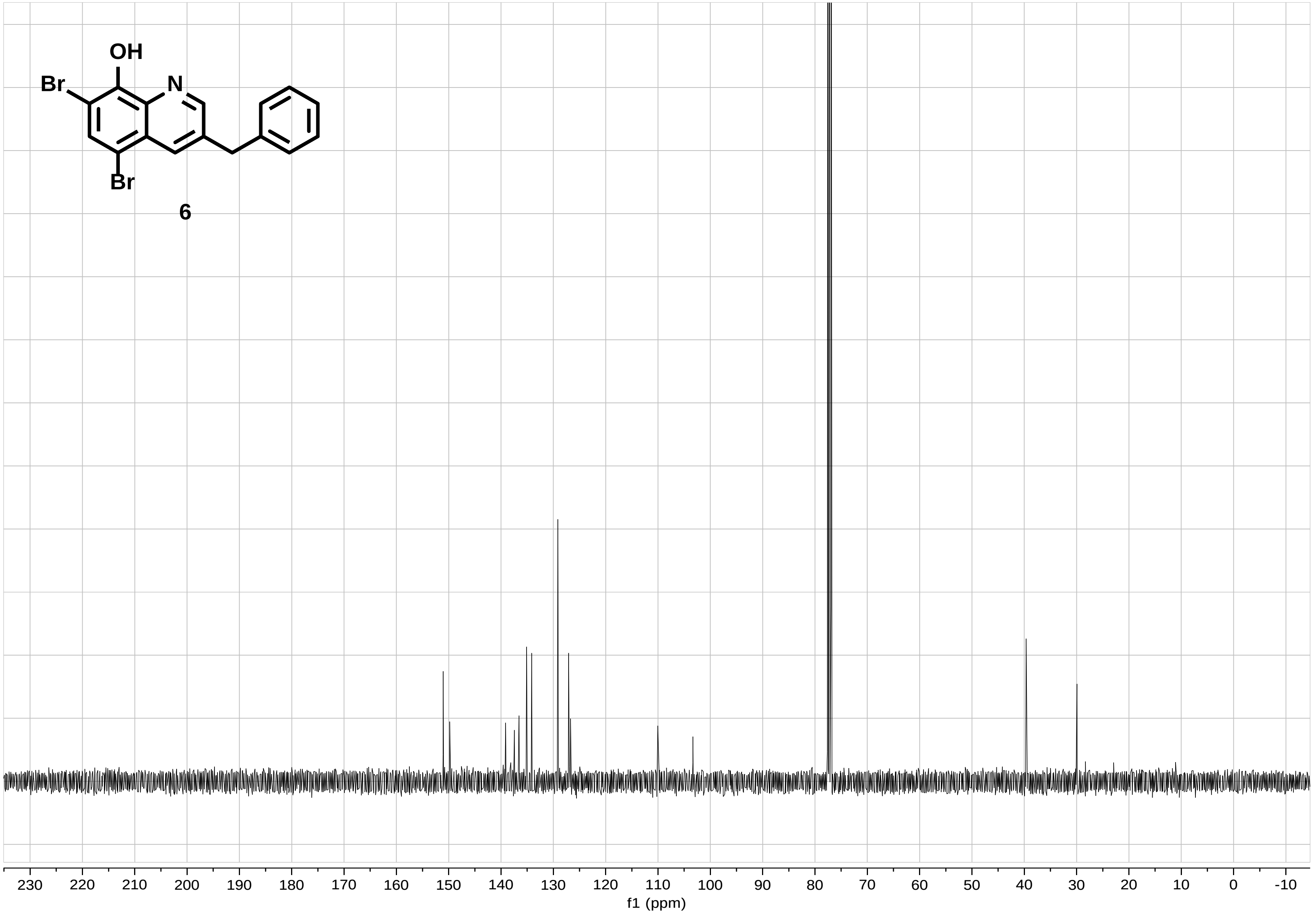


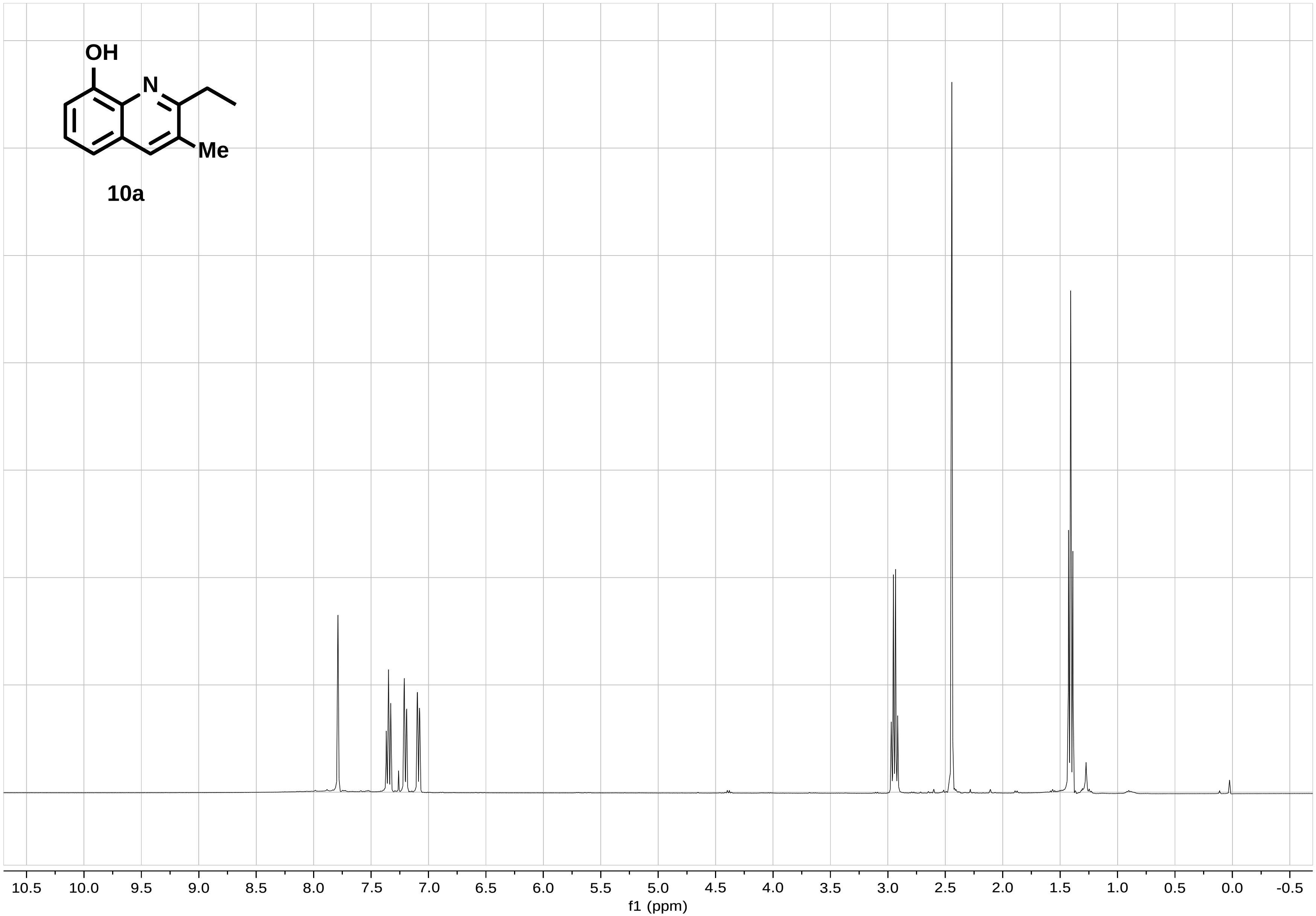
140
139
138
137
136
135
134
f1 (ppm)
130
129
128
127
126
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

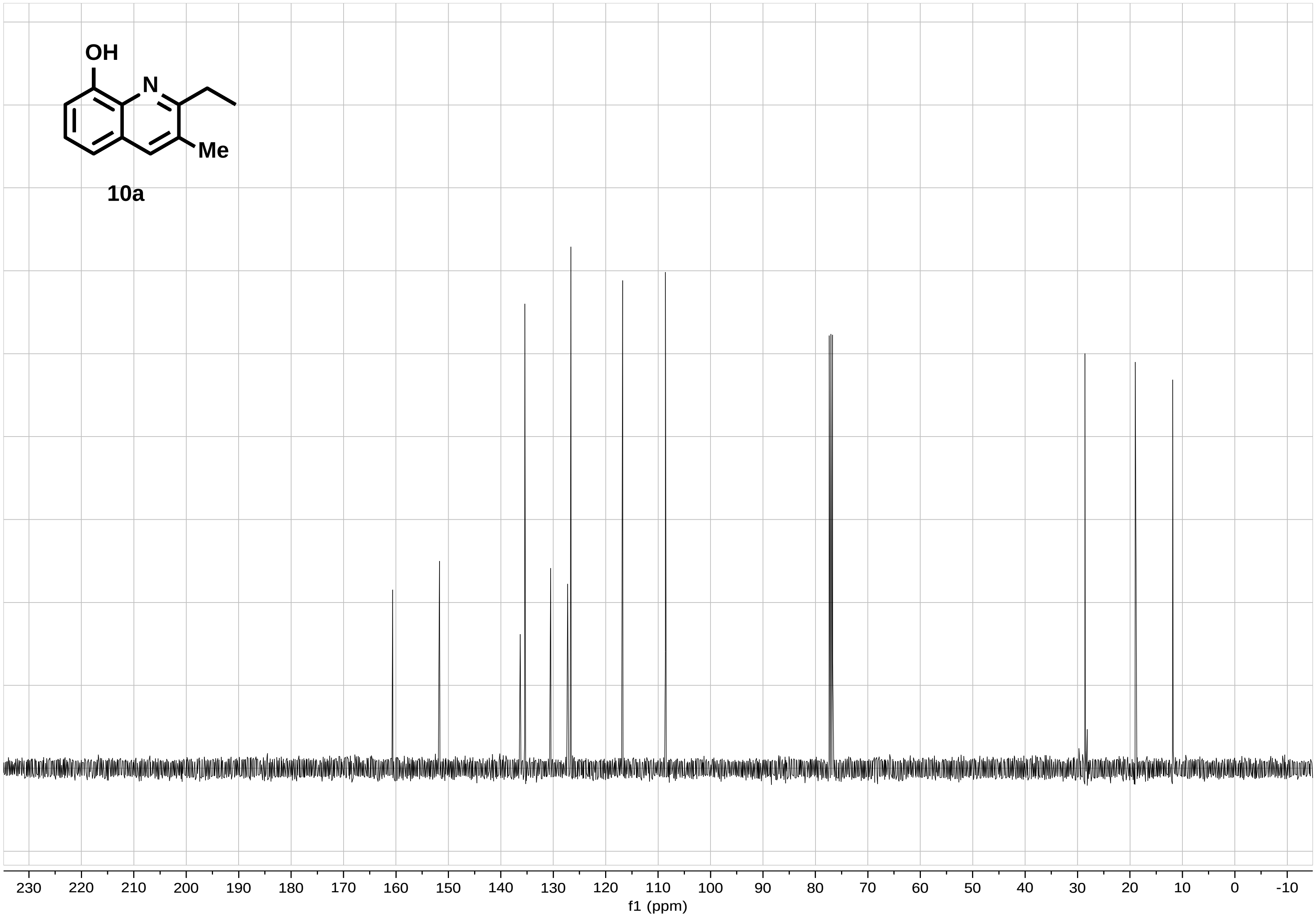

OH
Br
N
Br

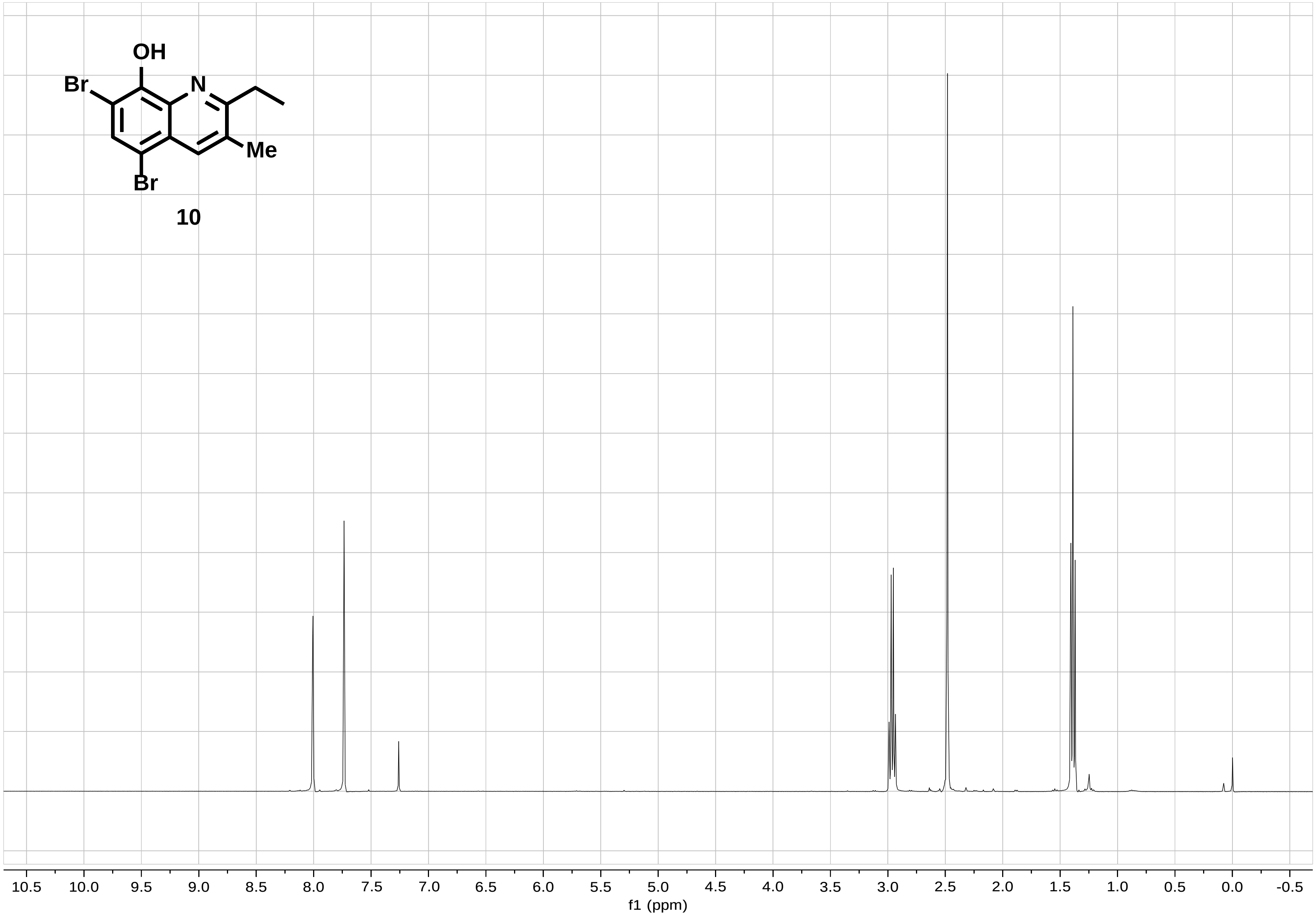
6


7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20 7.15 7.10 7.05 7.00
f1 (ppm)
10.5 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)

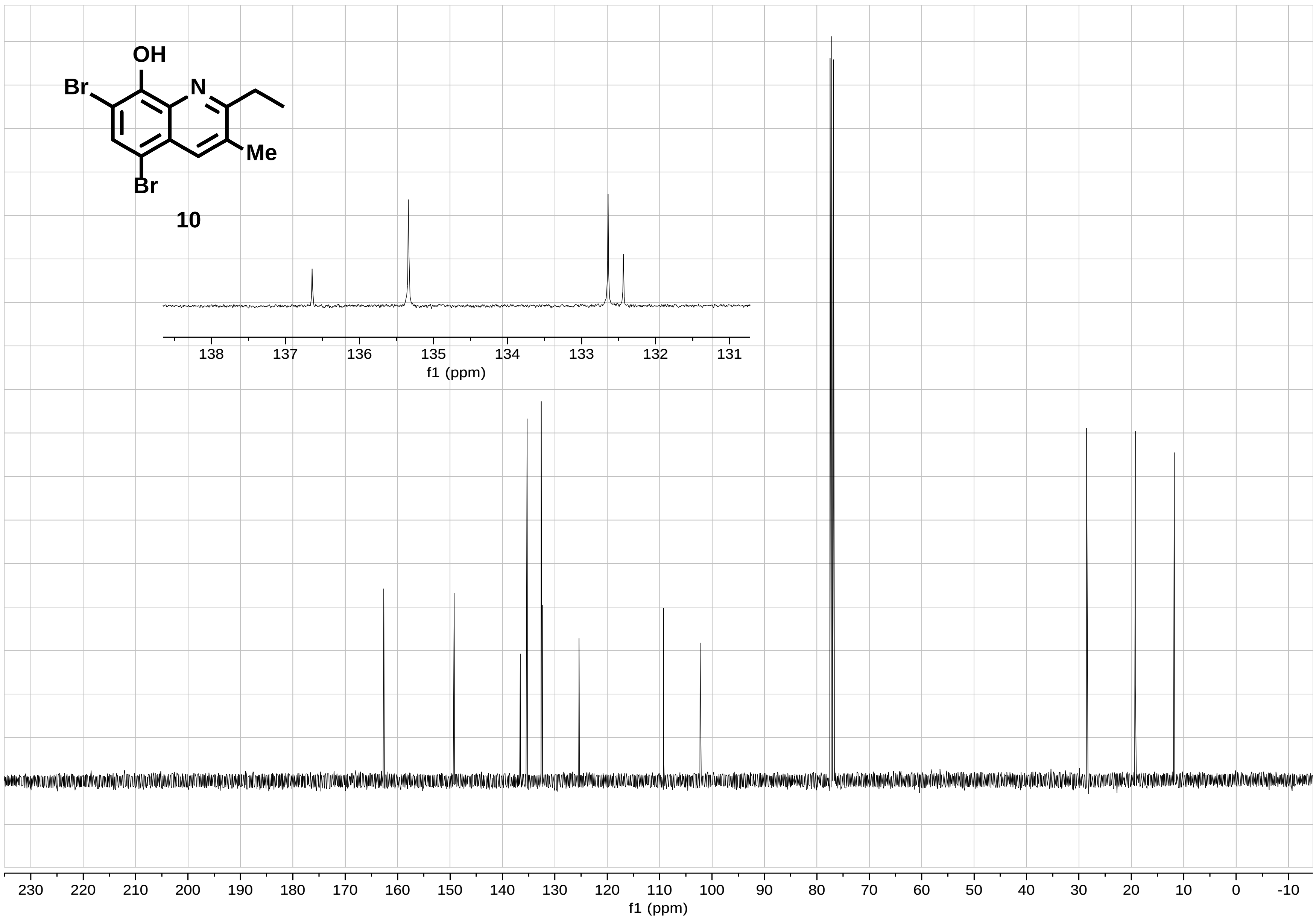

OH
Br
N
Br
6
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


OH
N
Me
10a
10.5 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)

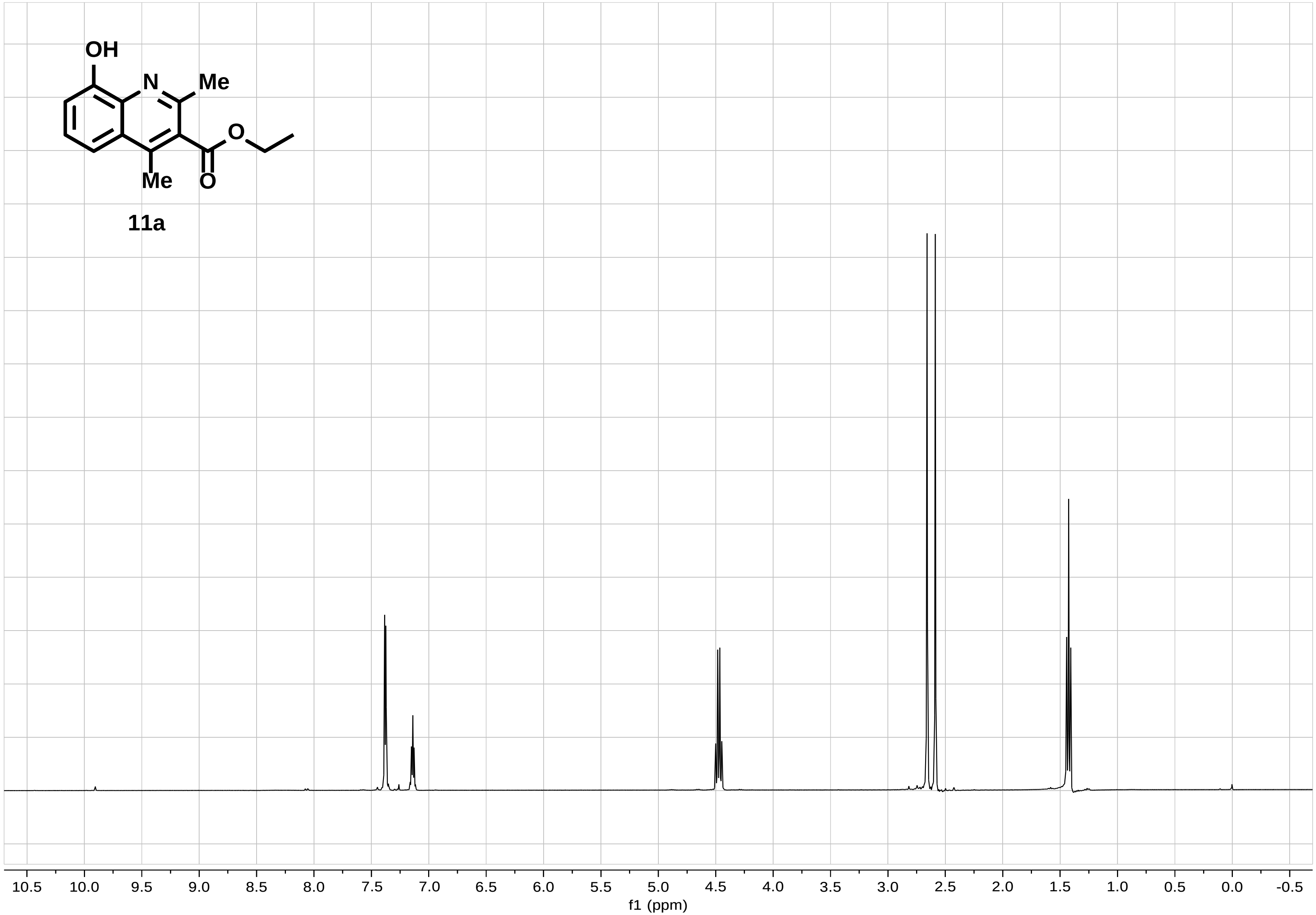

OH
N
Me
10a
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

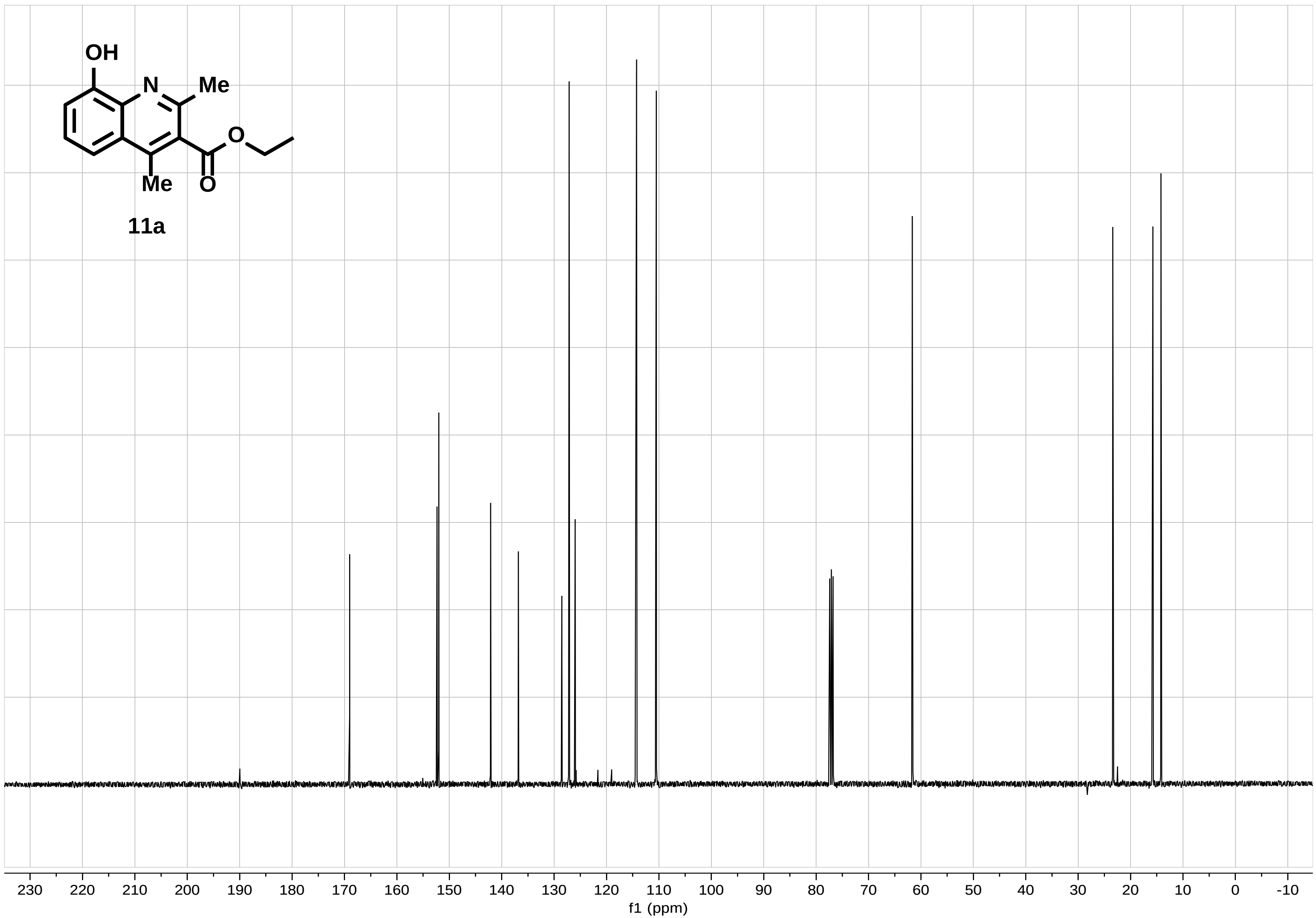


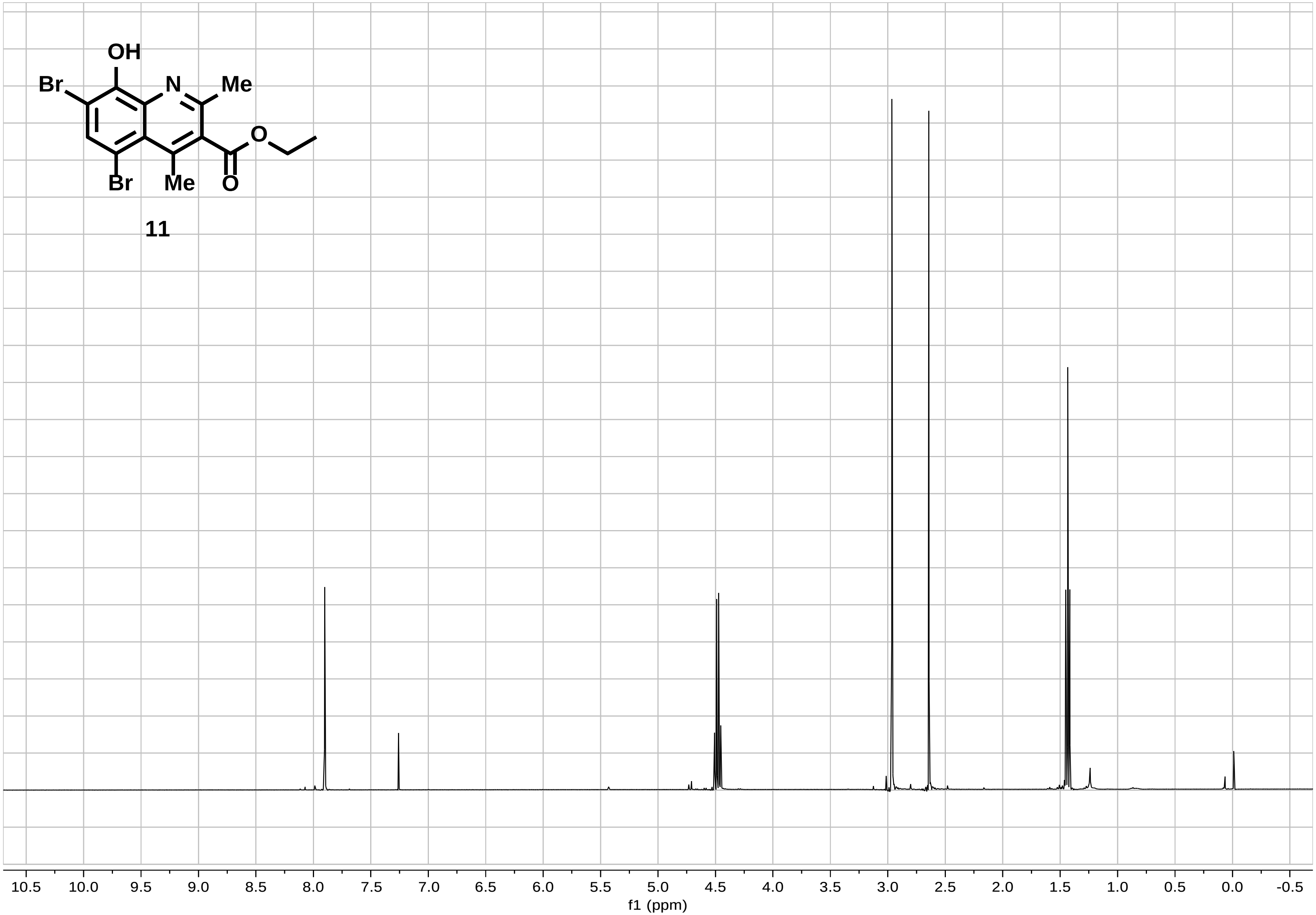
OH
Br
N
Me
Br
10
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

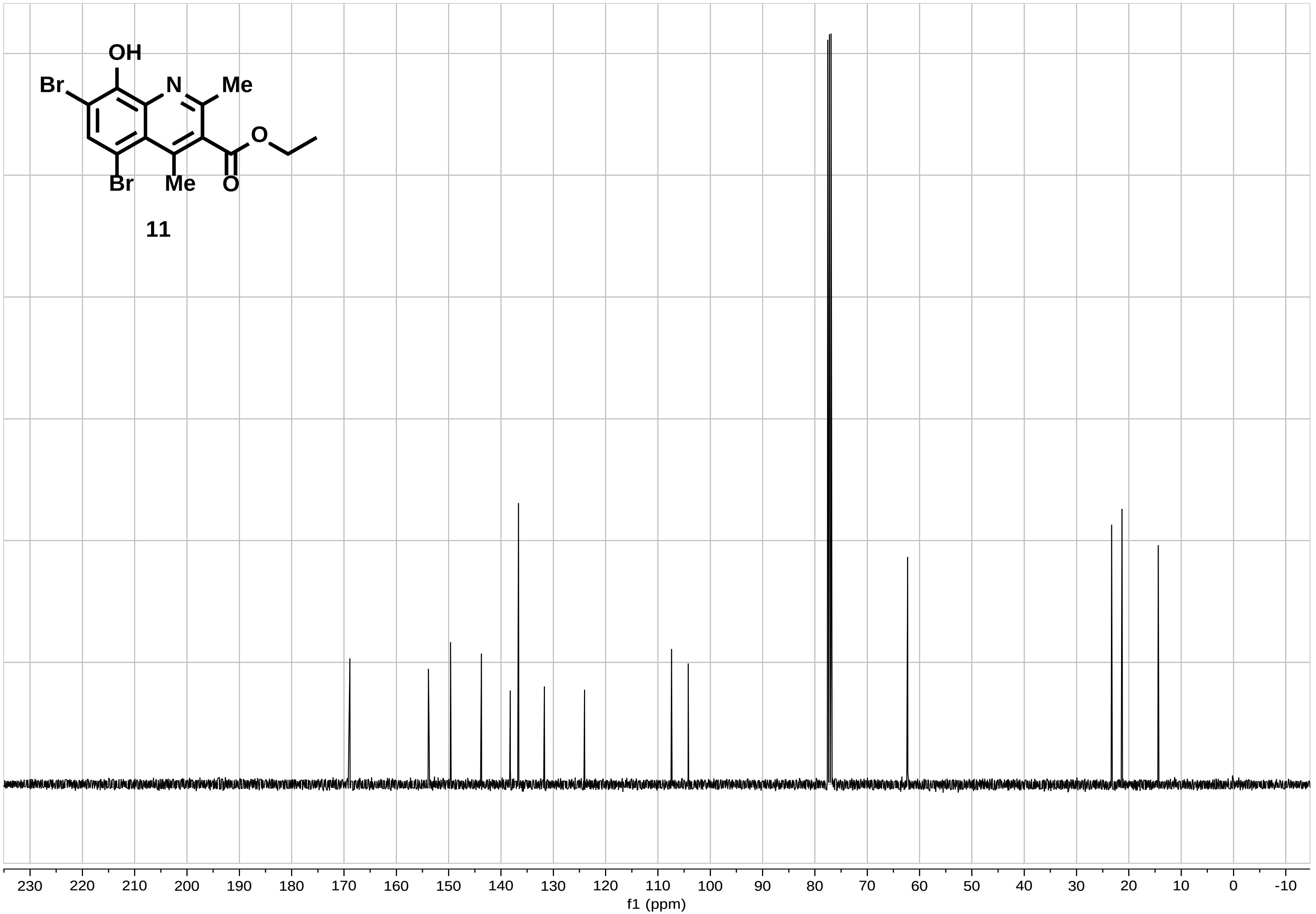


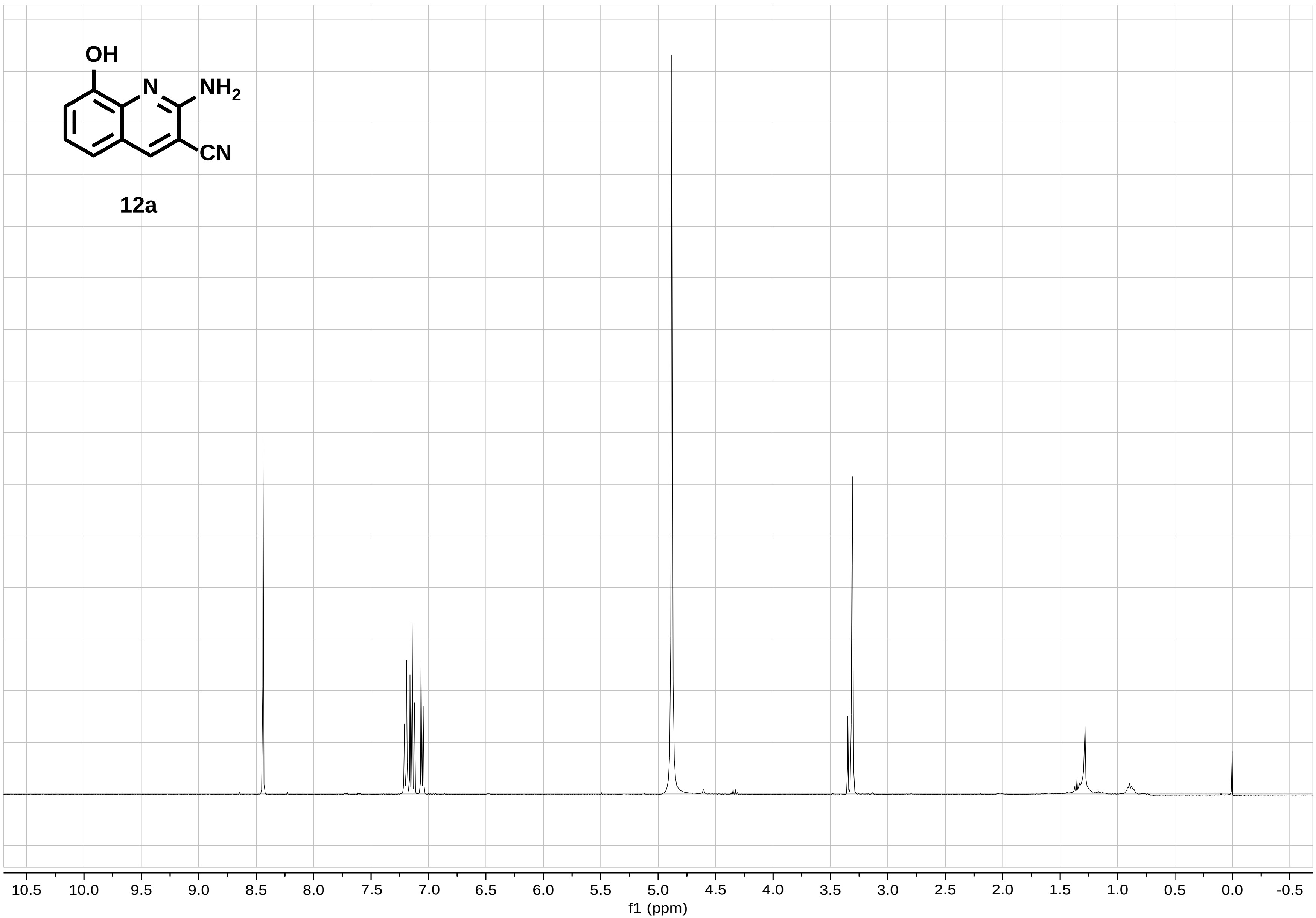
OH
Br
N
Me
Br
10
138
137
136
135
134
133
132
131
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

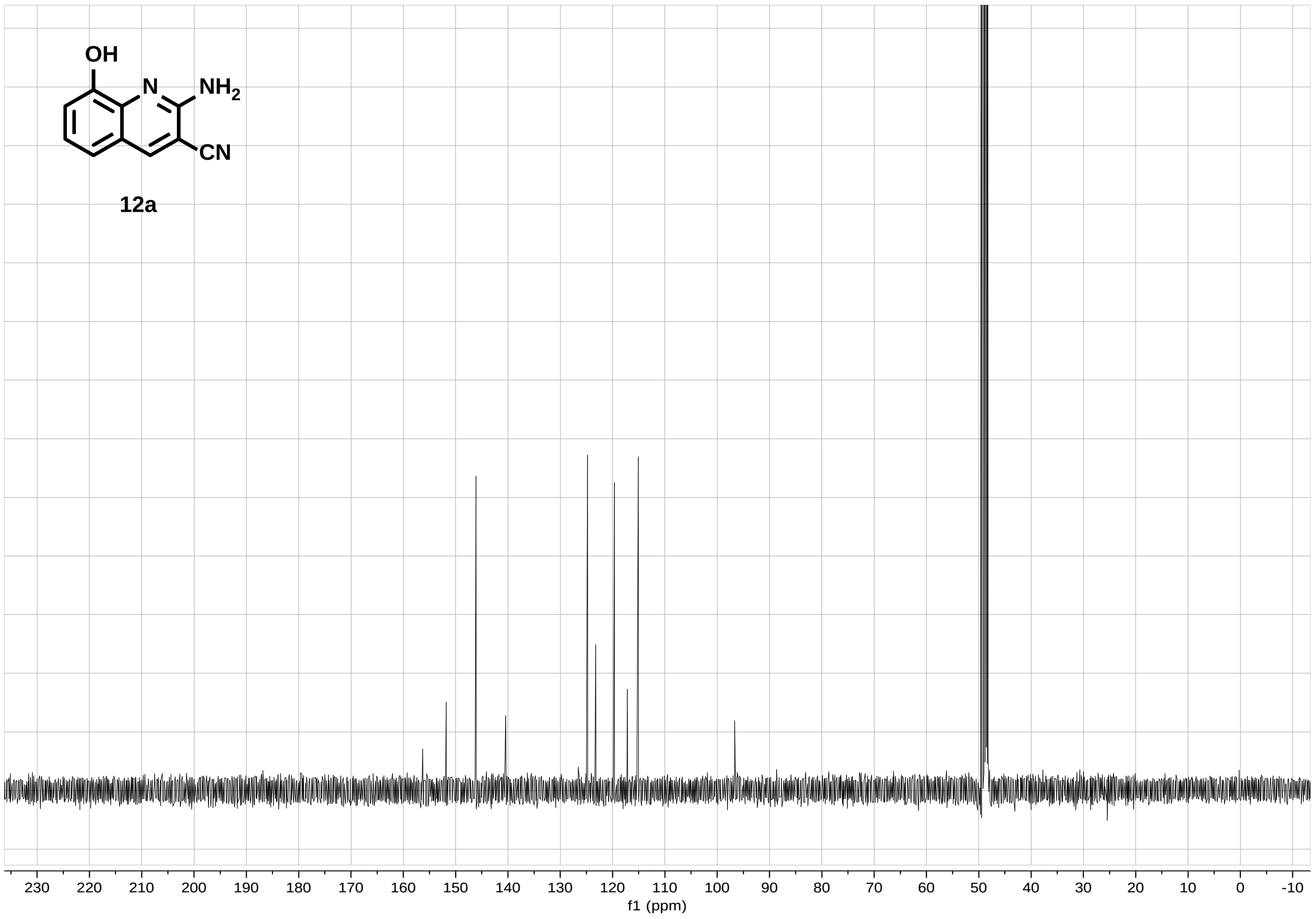

OH
N
Me
O
Me
O
11a
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

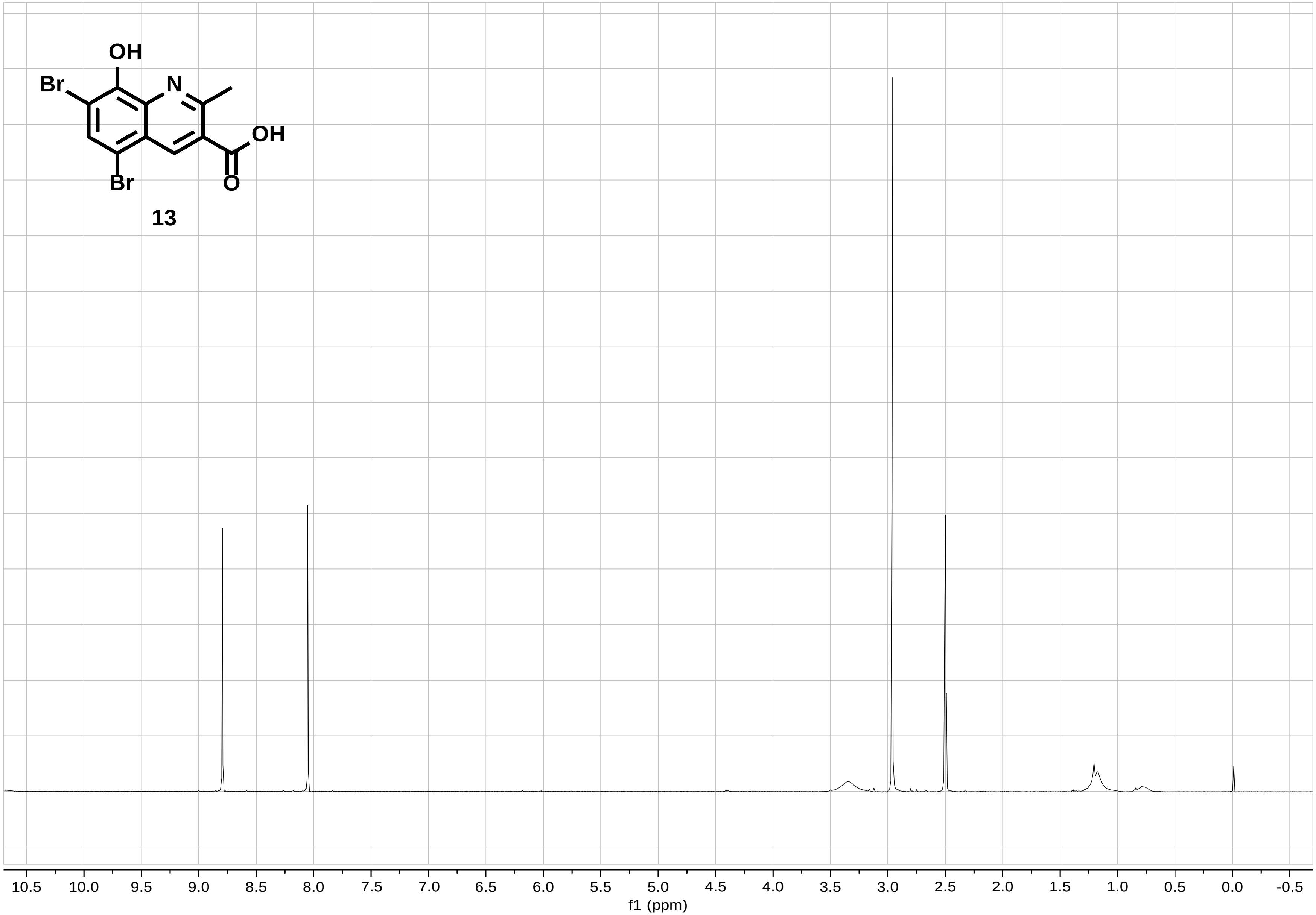

OH
N
Me
O
Me
O
11a
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


OH
Br
N
Me
O
Br
Me
O
11
10.5
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)

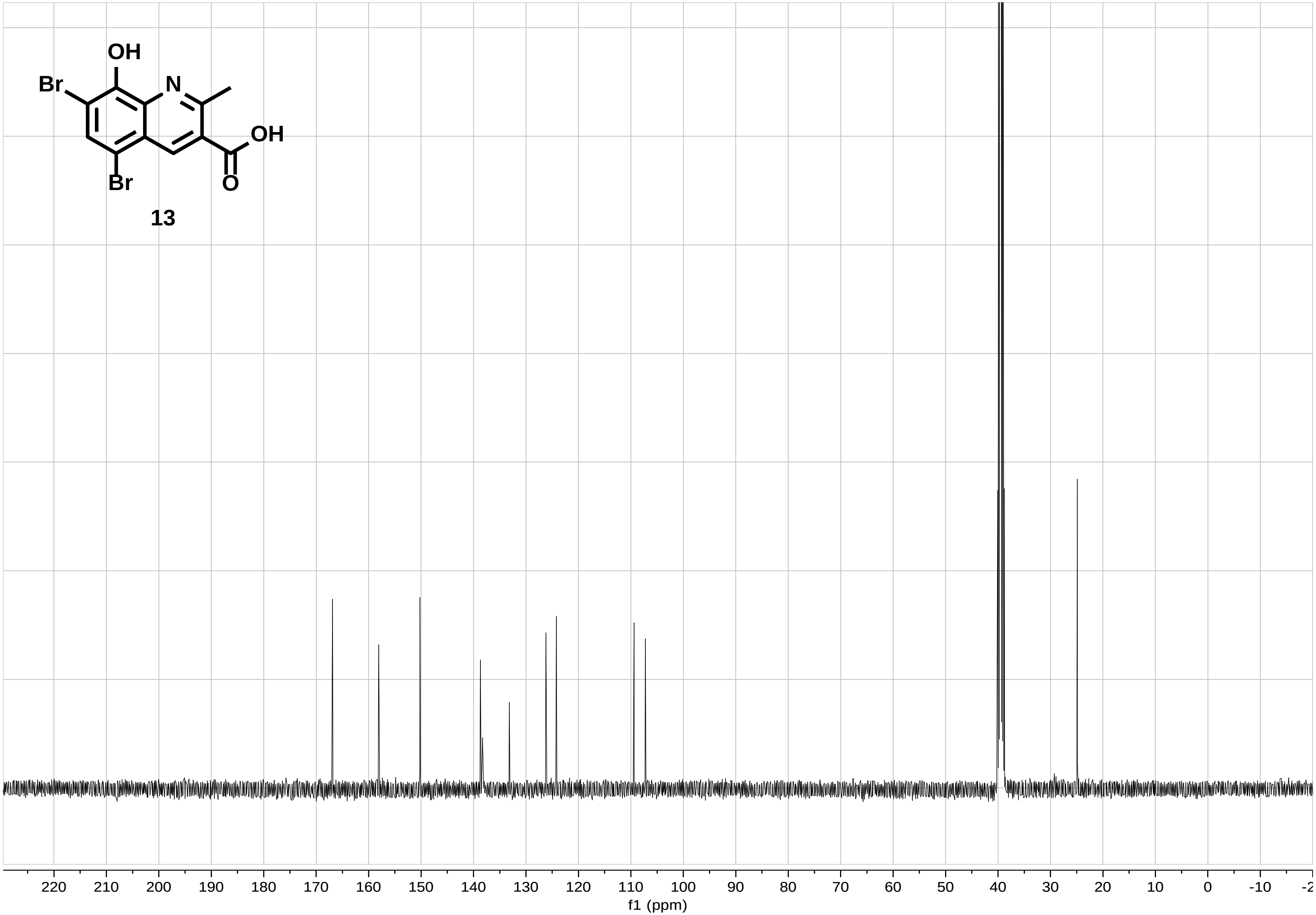

OH
Br
N
Me
O
Br
Me
O
11
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

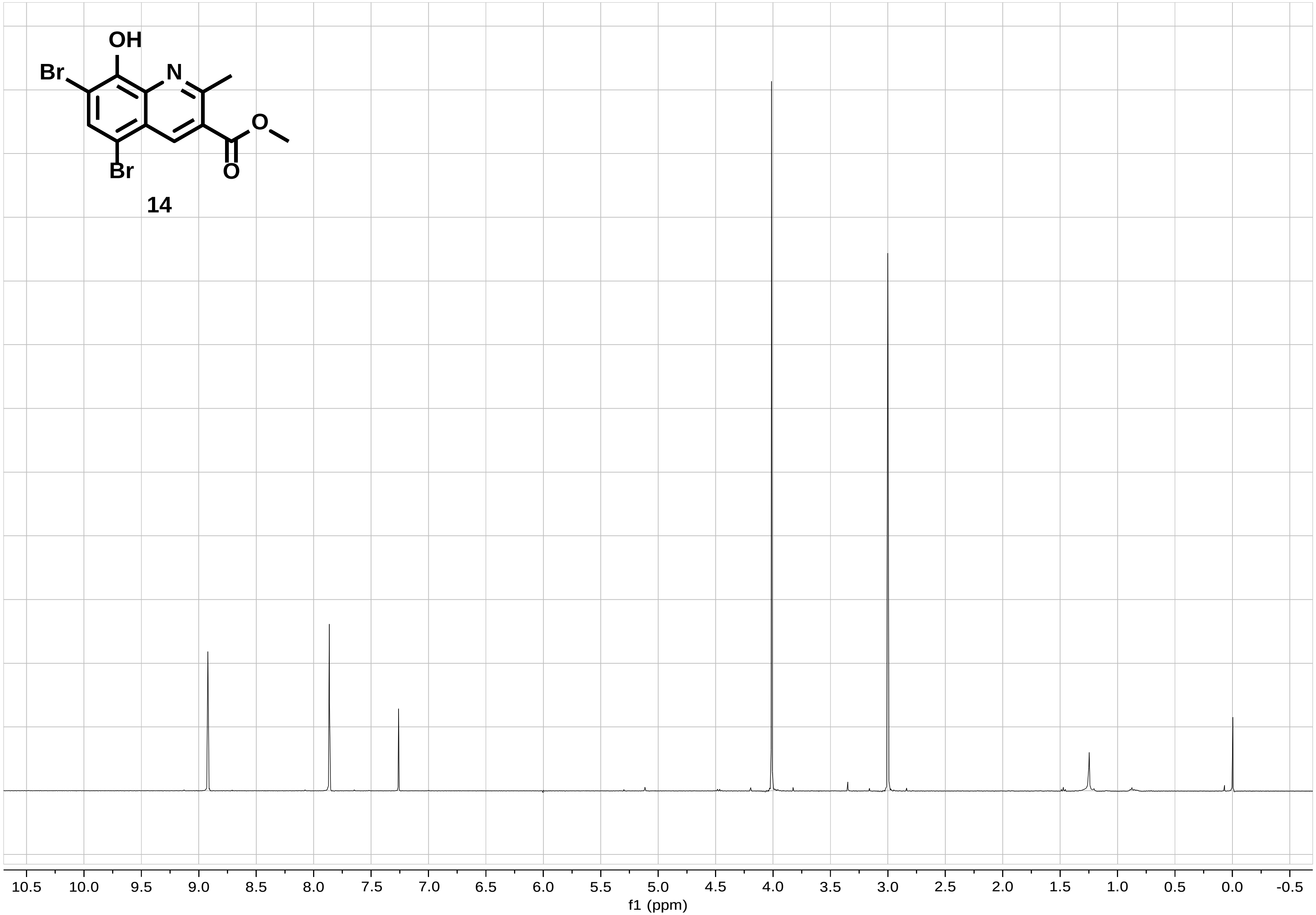

OH
N
NH2
CN
12a
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

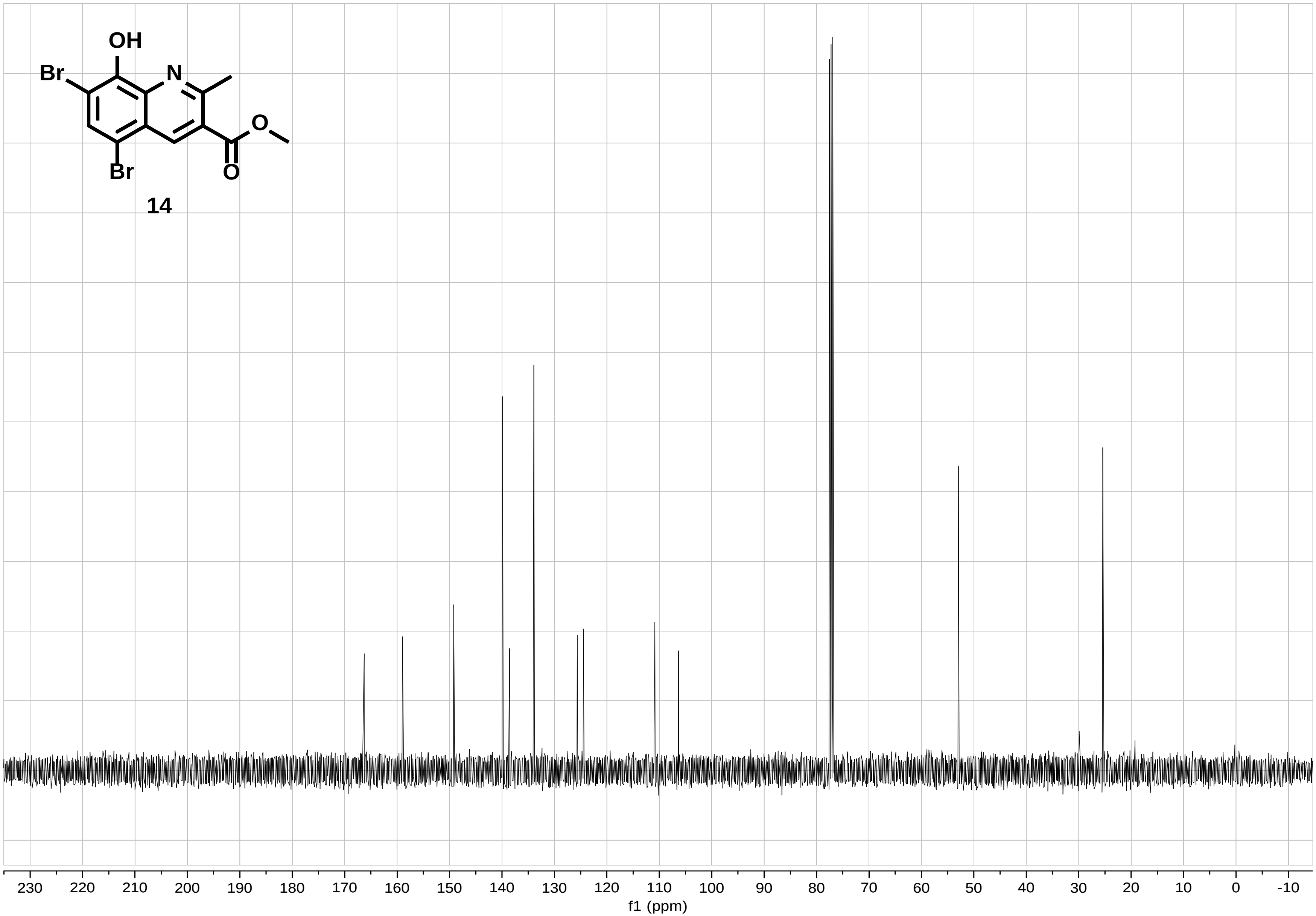

OH
N
NH2
CN
12a
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

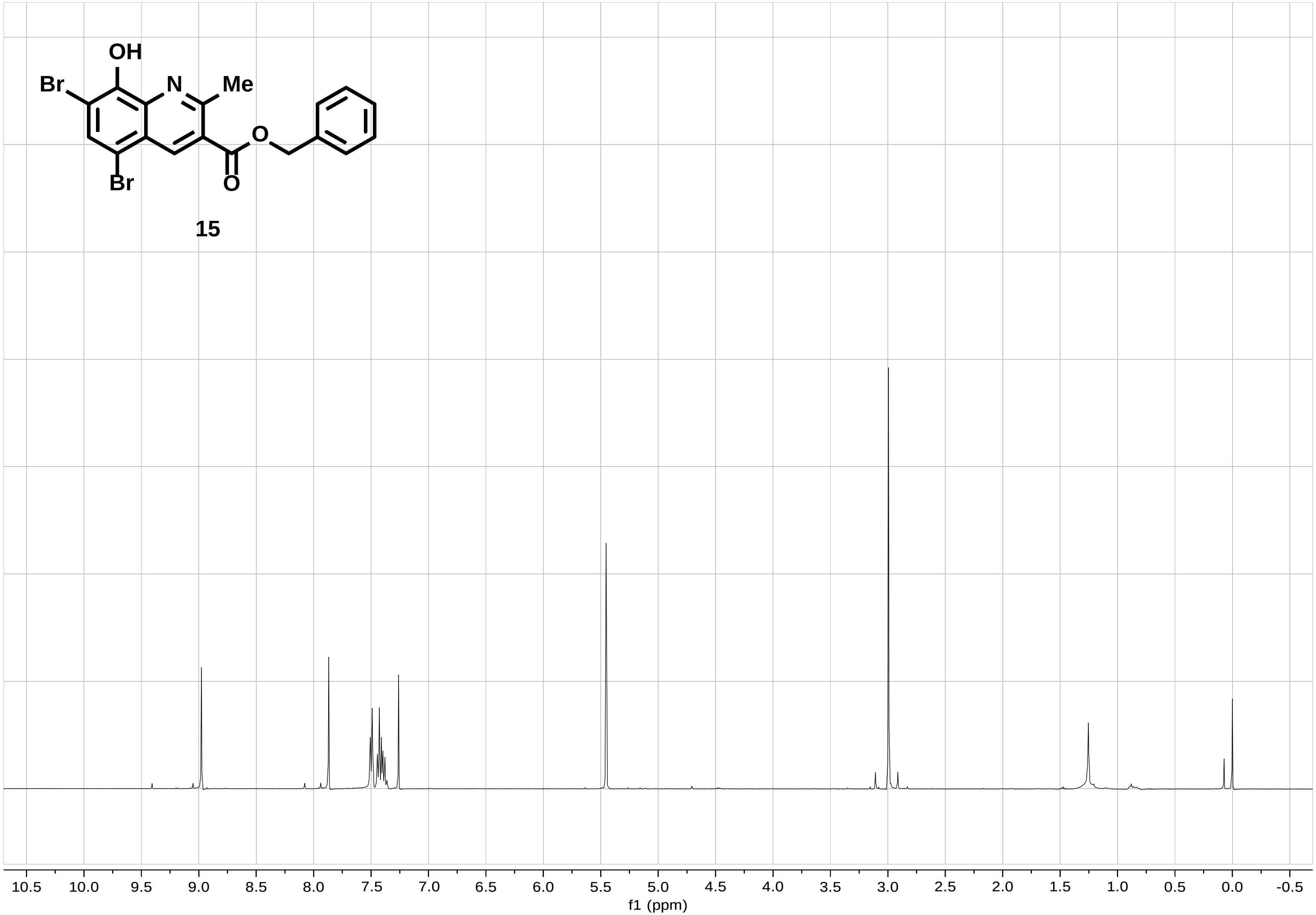


OH
Br
N
OH
Br
O
13
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

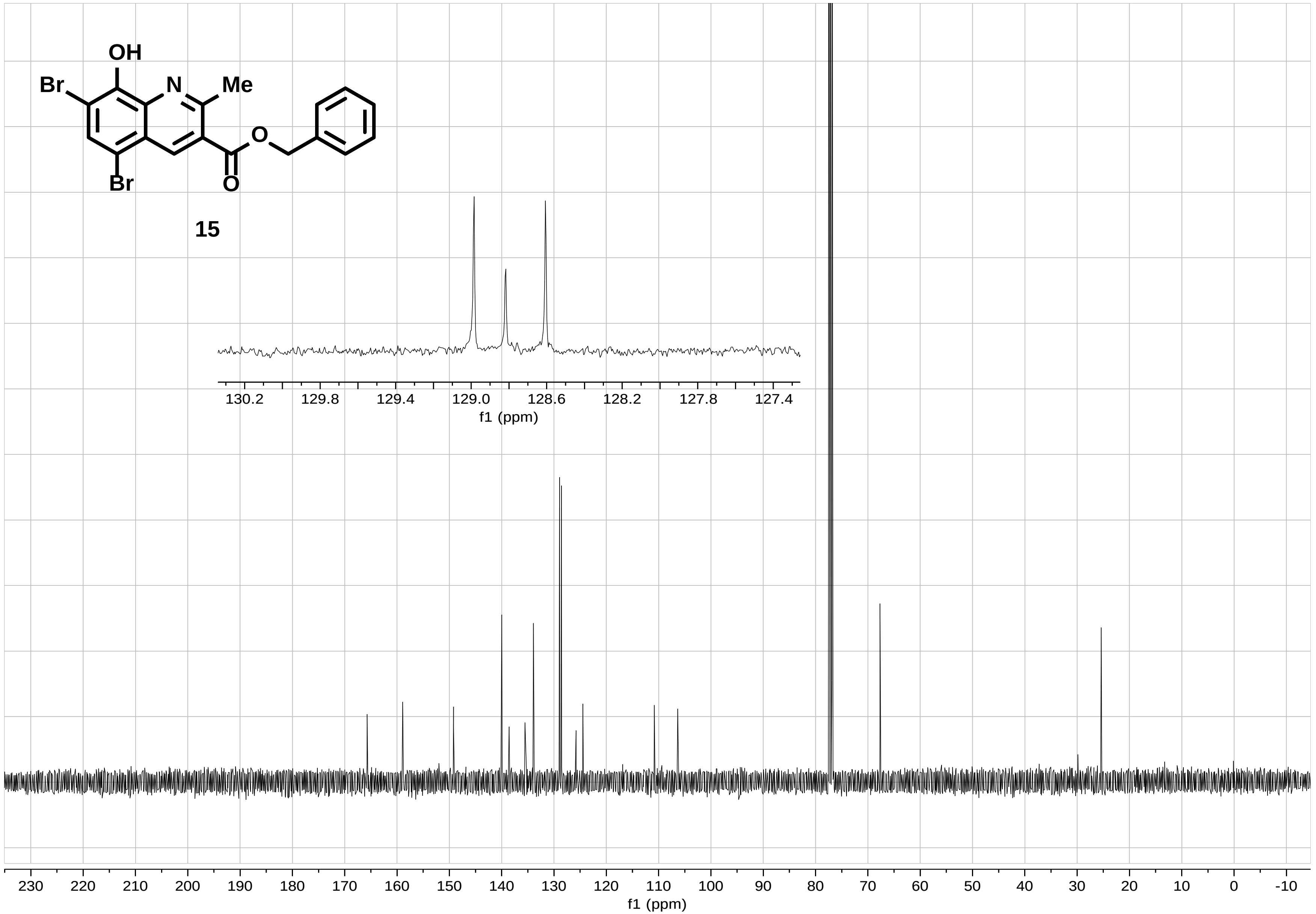

OH
Br
N
OH
Br
O
13
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


14
10.5
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)

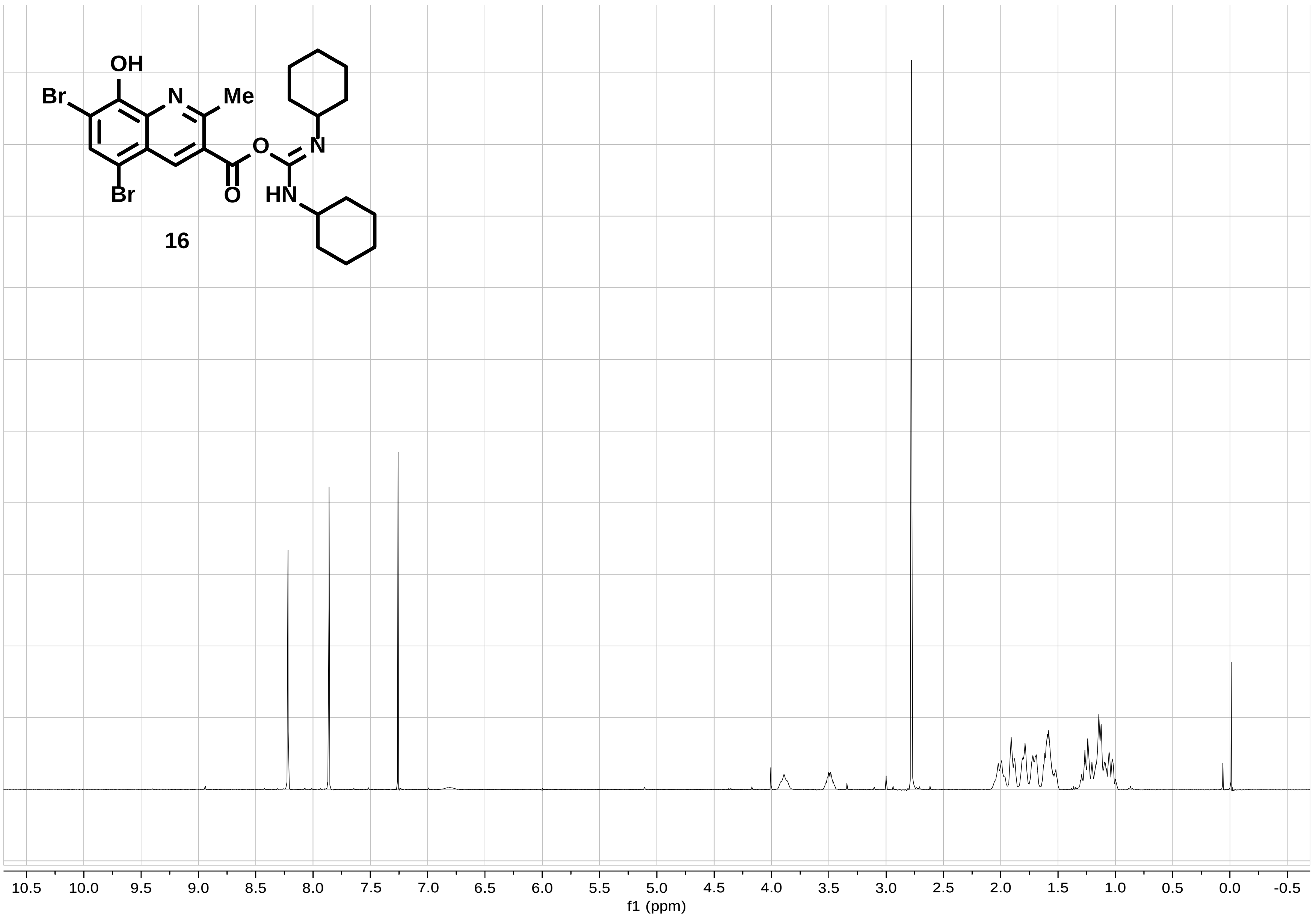

OH
Br
N
O
Br
O
14
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

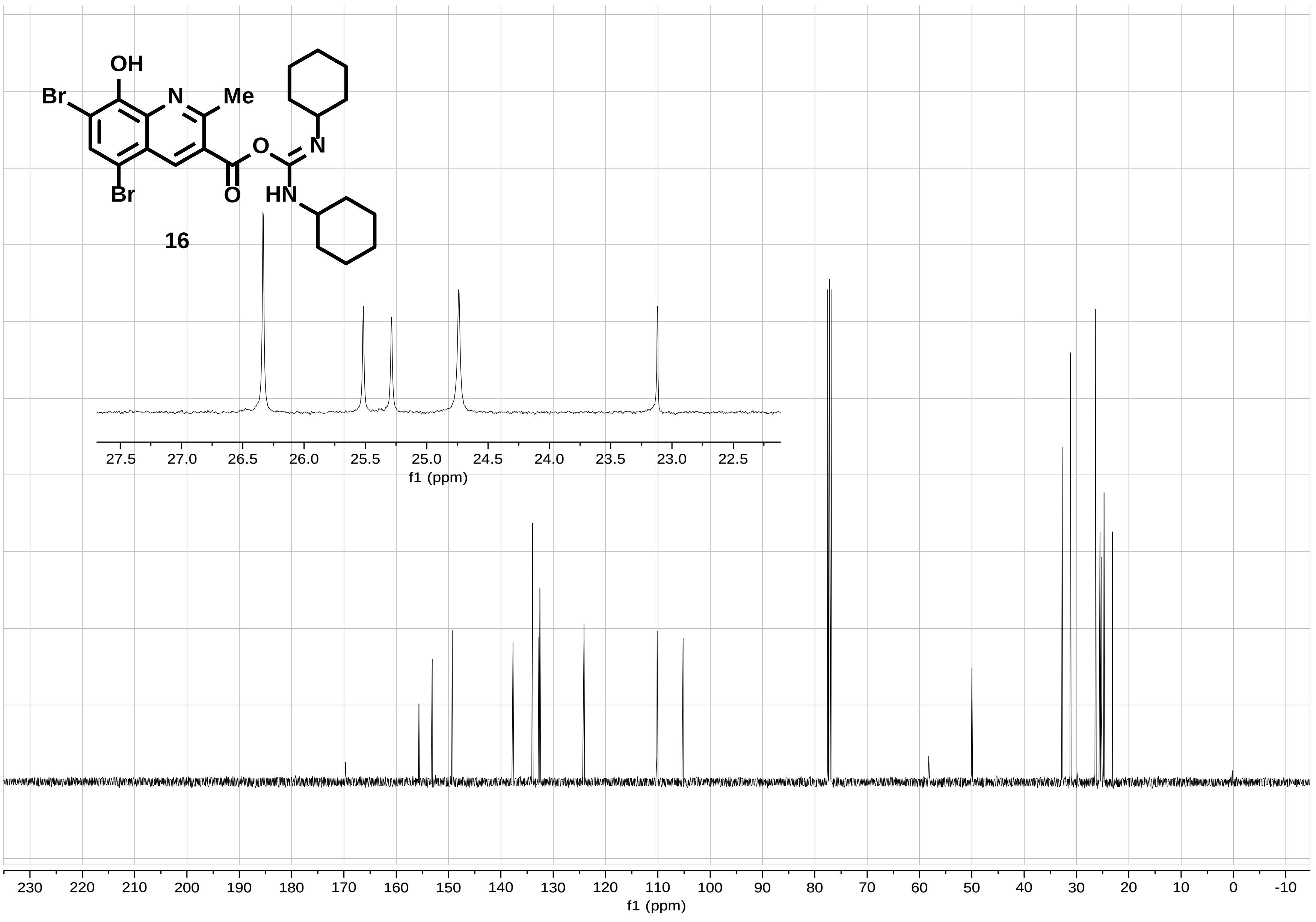


OH
Br
N
Me
O
Br
O
15
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

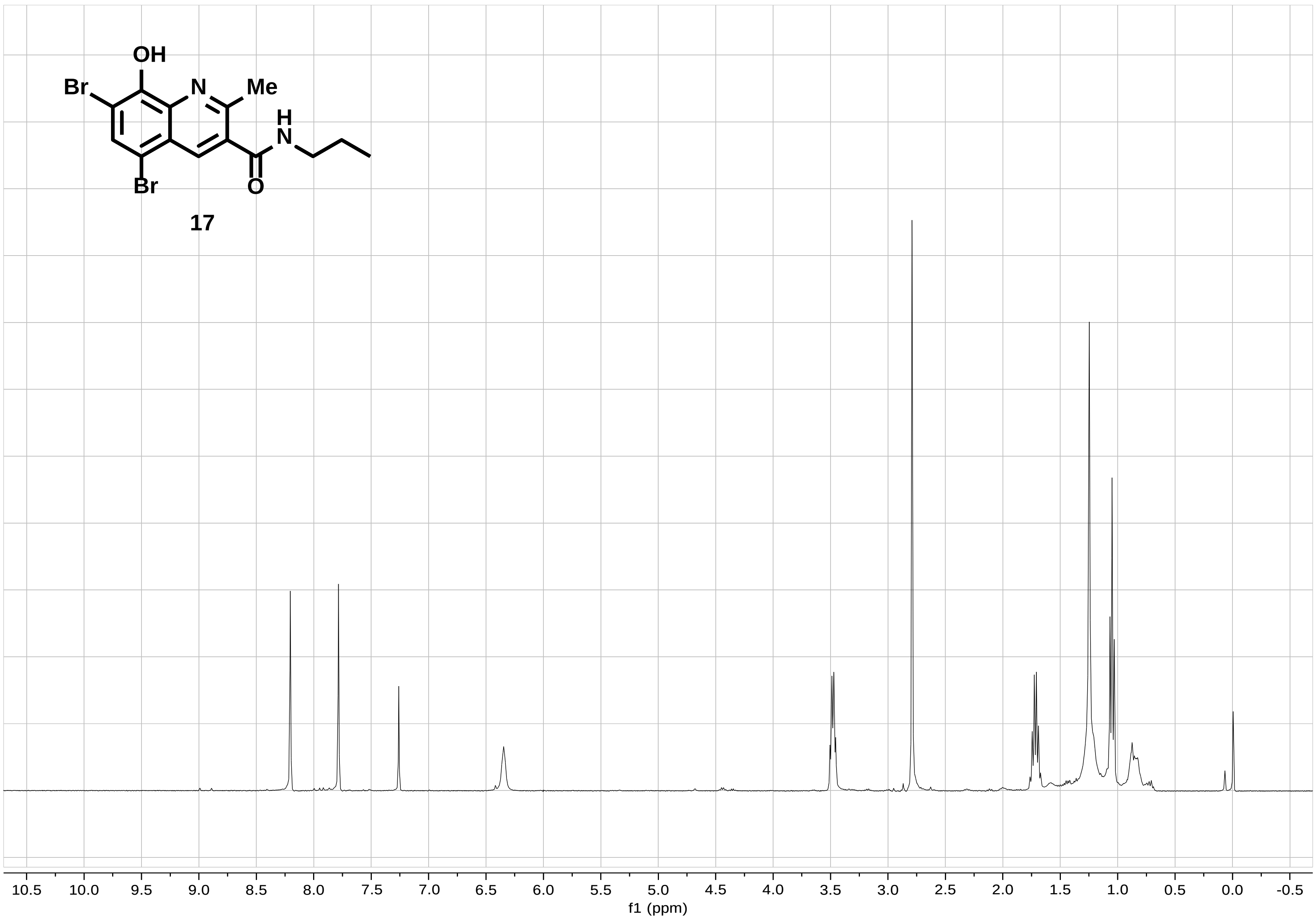


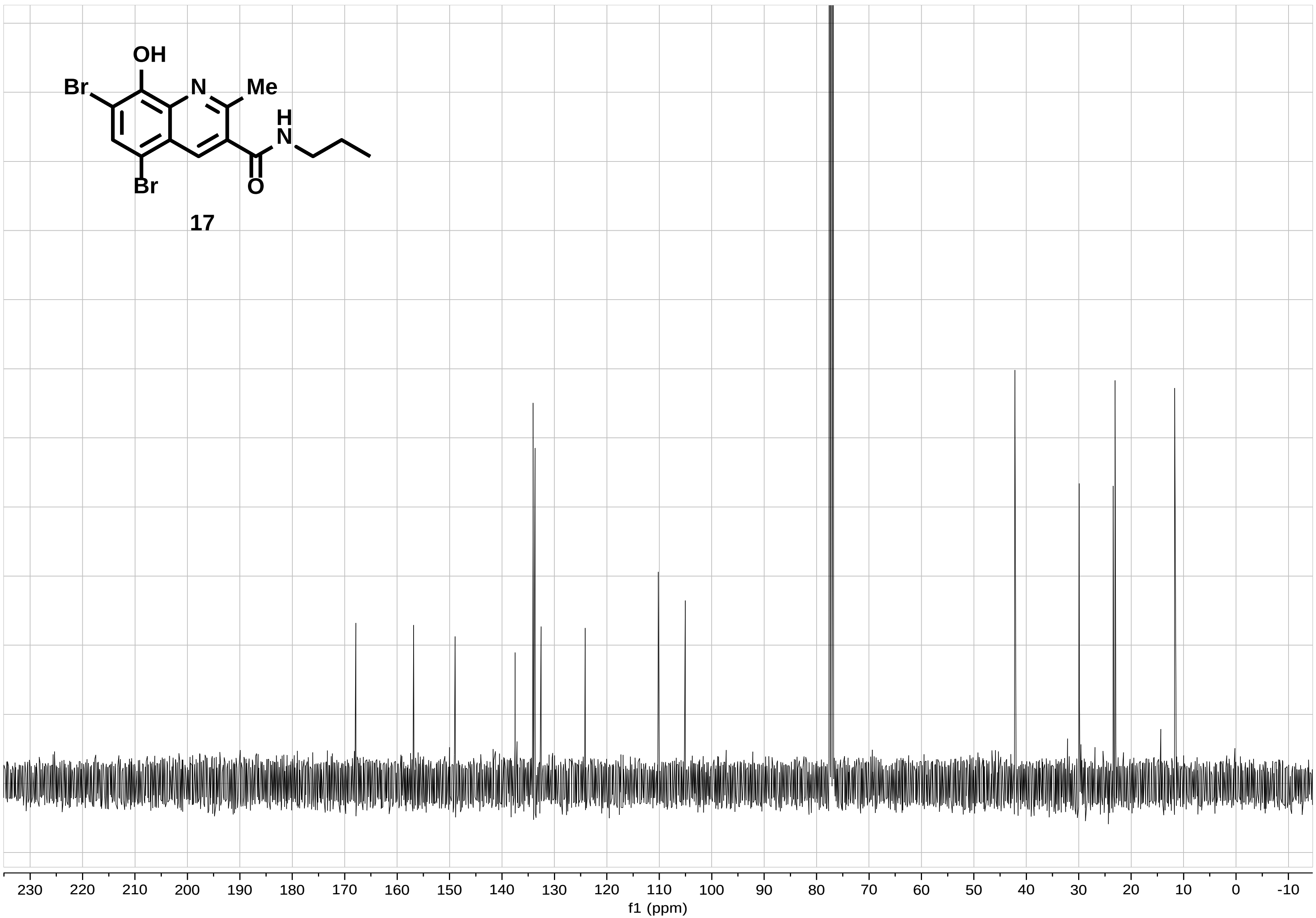
OH
Br
N
Me
O
Br
O
15
130.2
129.8
129.4
129.0
128.6
128.2
127.8
127.4
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


OH
Br
N
Me
O
N
HN
O
Br
16
10.5 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)

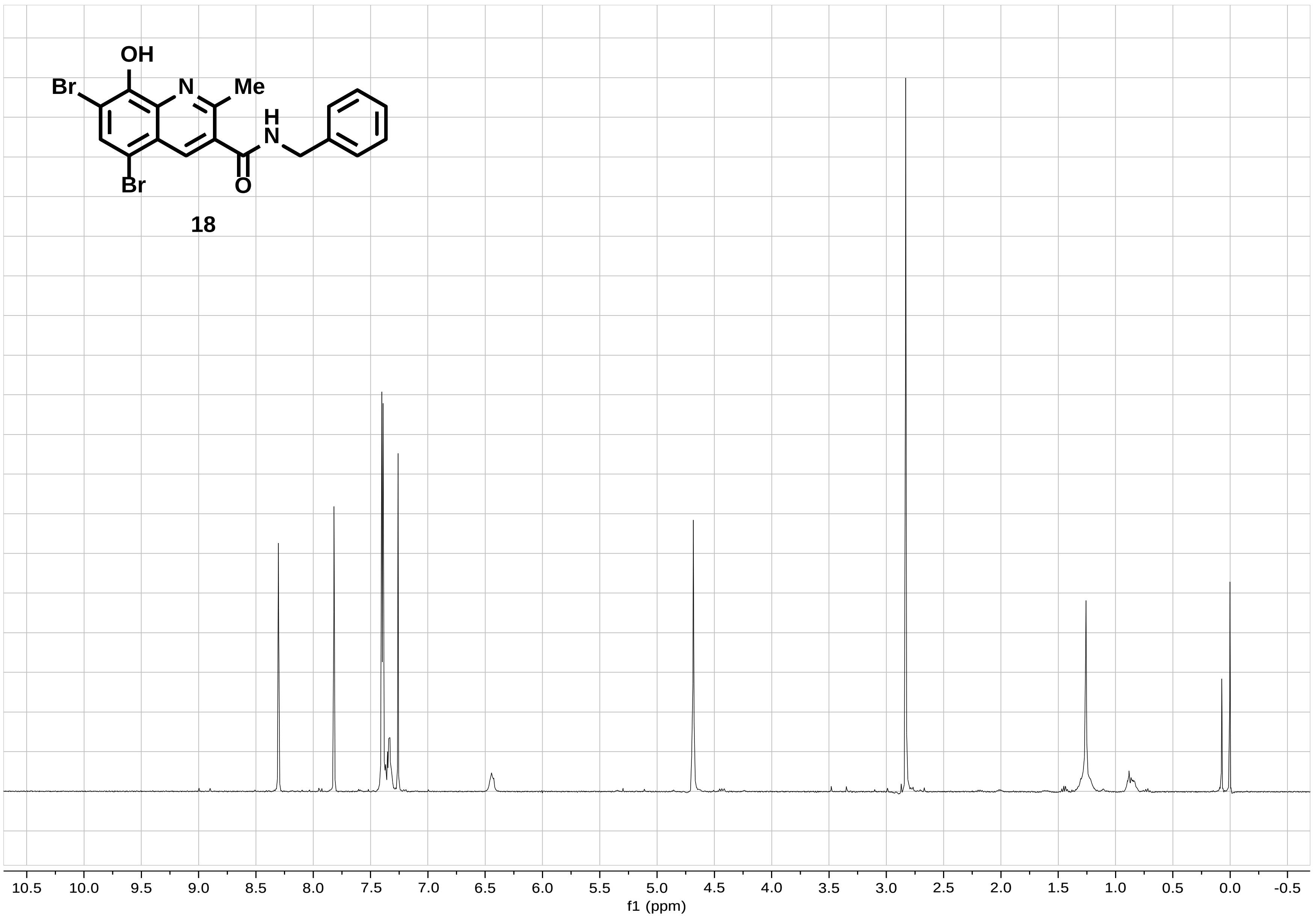


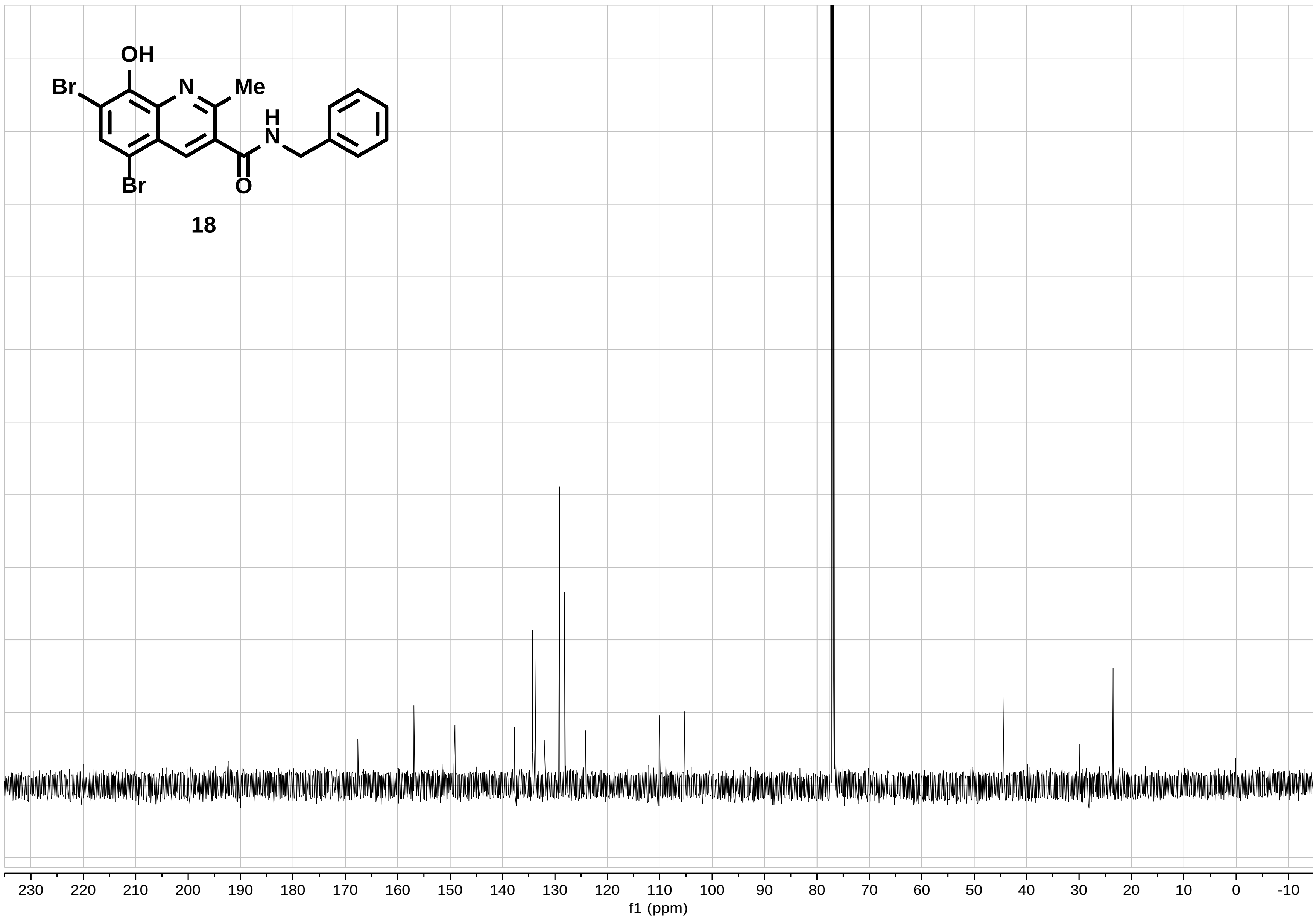
OH
Br
N
Me
O
N
HN
O
Br
16
27.5
27.0
26.5
26.0
25.5
25.0
24.5
24.0
23.5
23.0
22.5
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

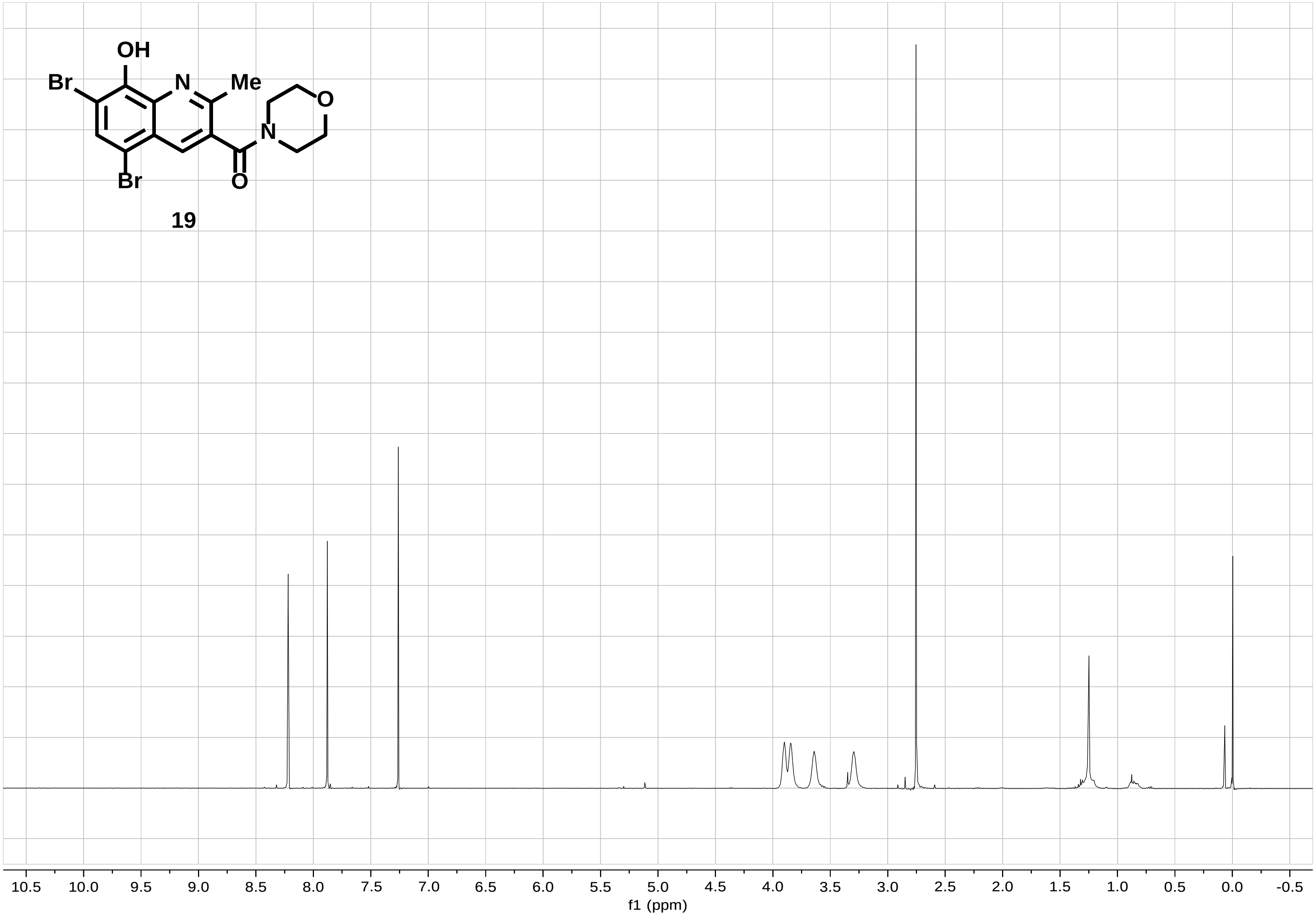

OH
Br
N
Me
H
N
Br
O
17
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

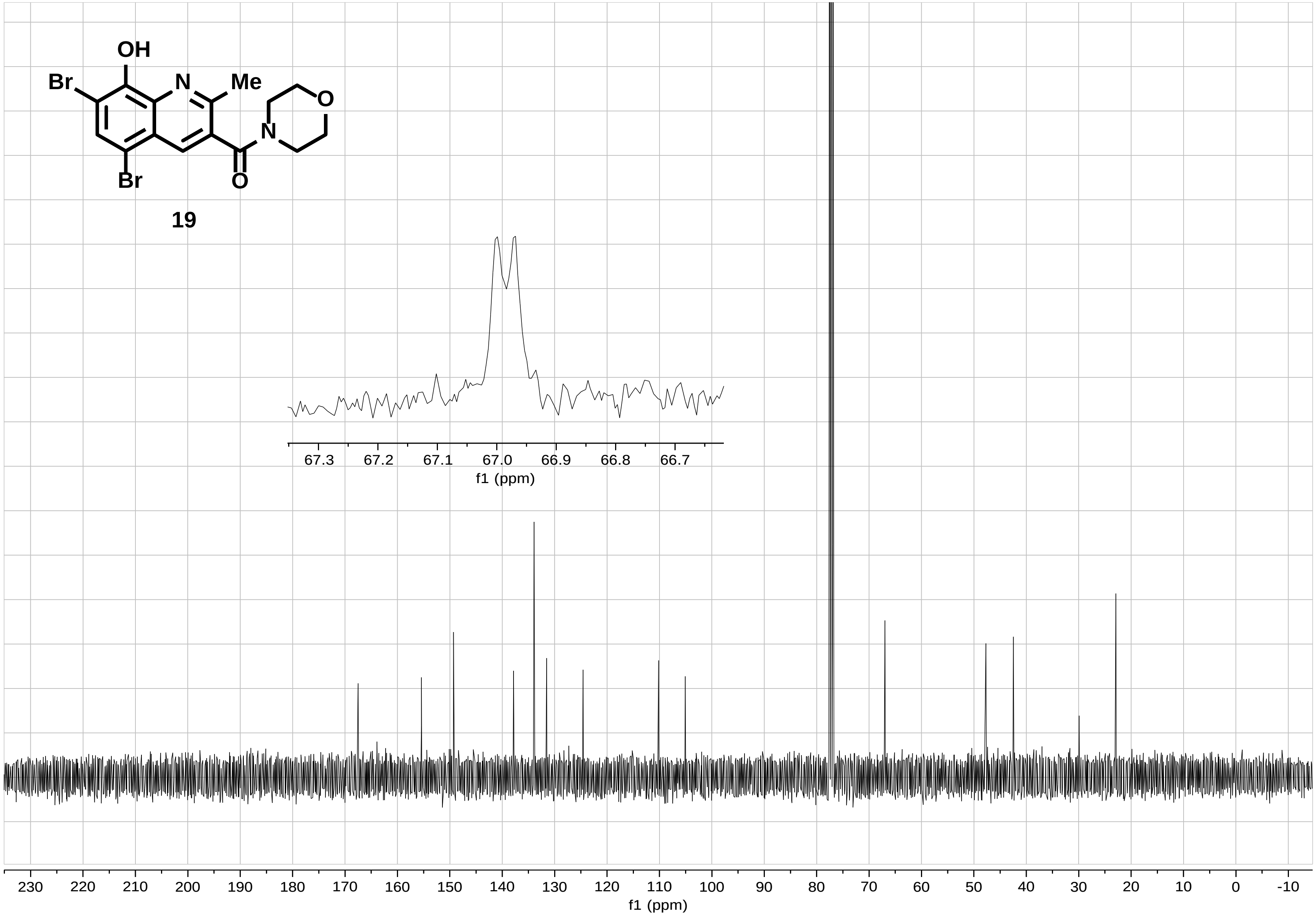


OH
Br
N
Me
H
N
Br
O
17
f1 (ppm)


OH
Br
N
Me
H
N
Br
O
18
f1 (ppm)


OH
Br
N
Me
H
N
Br
O
18
230
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)


OH
Br
N
Me
O
N
Br
O
19
10.5 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)


OH
Br
N
Me
O
N
Br
O
19
67.3 67.2 67.1 67.0 66.9 66.8 66.7
f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)