Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Halogenated Quinolines Discovered Through Reductive Amination with Potent Eradication Activities against MRSA, MRSE and VRE Biofilms

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Supporting Information

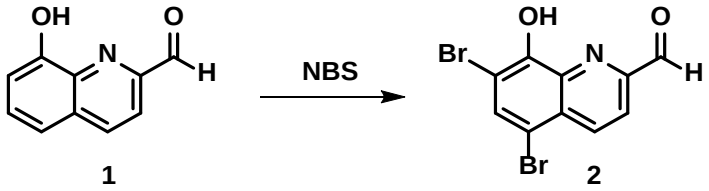
1.) General Information:

All reactions were carried out under an atmosphere of argon using anhydrous solvents unless otherwise specified. All chemical reagents for synthesis were used without further purification. 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.

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 (Mnova) software and ^{1}H NMR are typically displayed from 10.7 to -0.7 ppm 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), 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: br. = broad, s = singlet, d = doublet, t = triplet, p = pentet, m = multiplet. High-resolution mass spectra were obtained for all new compounds from the Chemistry Department at the University of Florida.

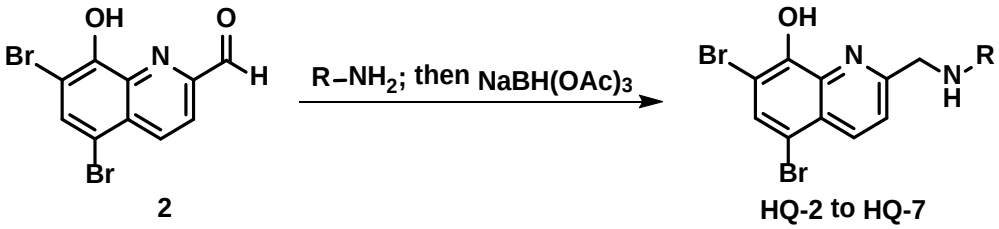
DMSO stocks of quinoline compounds tested were stored at room temperature in the absence of light for several months at a time without observing a loss in biological activity. To ensure compound integrity of our DMSO stock solutions, we did not subject DMSO stocks of test compounds to freeze-thaw cycles.

2.) Chemical Synthesis Supporting Information:

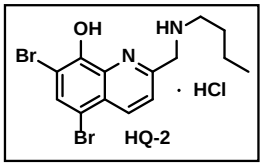


5,7-dibromo-8-hydroxyquinoline-2-carbaldehyde (2): To a stirring solution of 8-hydroxyquinoline-2-carbaldehyde (100 mg, 0.58 mmol) in 15 mL toluene at room temperature, *N*-bromosuccinimide (226 mg, 1.27 mmol) was added and the reaction mixture was stirred for 8 hours. The completion of the reaction was confirmed *via* TLC before being concentrated *in vacuo*. The crude material was then adsorbed onto silica using dichloromethane and concentrated *in vacuo* before being applied to a column. The crude product was then purified via flash column chromatography using hexanes:methylene chloride (2:1 to 1:1) to elute pure quinoline derivative **2** as a yellow solid (140 mg, 73%).

^{1}H NMR (400 MHz, d_6-DMSO): 11.34 (br. s, 1H), 10.20 (s, 1H), 8.64 (d, *J* = 8.7 Hz, 1H), 8.26 (s, 1H), 8.15 (d, *J* = 8.7 Hz, 1H).
^{13}C NMR (100 MHz, d_6-DMSO): 192.5, 151.8, 151.3, 138.7, 137.4, 136.0, 128.2, 119.2, 109.0, 106.6.
HRMS (DART): calcd for $C_{10}H_6Br_2NO_2$ $[M+H]^+$: 329.8760, found: 329.8764.
MP: 154-156 °C.

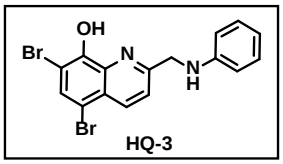


General reductive amination procedure (HQ-2 to HQ-7): To a stirring solution of **2** (100 mg, 0.3 mmol) in 1,2-dichloroethane (10 mL) at room temperature, aliphatic amine or aniline (0.45 mmol) was added and the resulting reaction mixture was continued to stir (for 15 minutes with aliphatic amines and 1 hour with anilines) until the **2** was consumed *via* TLC. Then sodium triacetoxyborohydride (77 mg, 0.45 mmol) was added to the reaction mixture which was allowed to stir at room temperature for an additional 12 hours. After that time, the reaction was concentrated *in vacuo* and then stirred in 10 mL saturated sodium bicarbonate solution for 30 minutes. The crude product was extracted with dichloromethane (2x30 mL) and the combined organic layer was washed with brine, dried with anhydrous sodium sulfate, filtered and absorbed in silica before being applied to a column. The crude products were then purified via flash column chromatography using hexanes:ethyl acetate (5:1 to 3:1) to elute pure quinolines **HQ-2** to **HQ-7**.

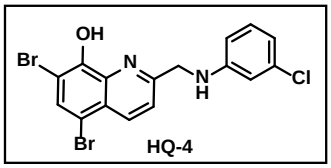


Yield: 68% yield; 120 mg of **HQ-2** was isolated as a yellow solid.
^{1}H NMR (400 MHz, d_6-DMSO): 9.59 (br. s, 2H), 8.40 (d, *J* = 8.7 Hz, 1H), 7.96 (s, 1H), 7.72 (d, *J* = 8.7 Hz, 1H), 4.55 (s, 2H), 3.05 – 2.90 (m, 2H), 1.72 (p, *J* = 7.7 Hz, 2H), 1.30 (sextet, *J* = 7.6 Hz, 2H), 0.82 (t, *J* = 7.5 Hz, 3H).
^{13}C NMR (100 MHz, d_6-DMSO): 153.0, 151.0, 137.8, 137.0, 133.8, 126.2, 122.8, 109.3, 105.6, 50.5, 47.4, 27.6, 19.7, 13.8.
HRMS (ESI): calcd for $C_{14}H_{16}Br_2N_2ONa$ $[M+Na]^+$: 408.9522, found: 408.9518.

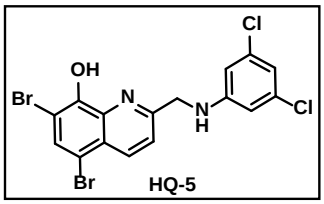
MP: 184-186 °C.
Note: The free base of **HQ-2** was used in biological experiments; however, the HCl salt of **HQ-2** was made for characterization purposes.



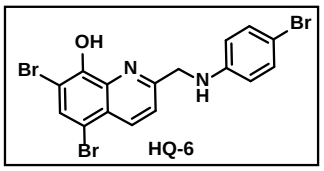
Yield: 44% yield; 35 mg of **HQ-3** was isolated as a white solid.
^{1}H NMR (400 MHz, $CDCl_3$): 8.38 (d, *J* = 8.7 Hz, 1H), 7.87 (s, 1H), 7.64 (d, *J* = 8.7 Hz, 1H), 7.18 (dd, *J* = 8.5, 7.3 Hz, 2H), 6.75 (t, *J* = 7.3 Hz, 1H), 6.67 (dd, *J* = 8.6, 1.2 Hz, 2H), 4.70 (s, 2H).
^{13}C NMR (100 MHz, $CDCl_3$): 160.0, 149.4, 147.6, 138.1, 137.1, 133.5, 129.6, 126.0, 121.7, 118.5, 113.3, 110.4, 104.4, 49.8.
HRMS (ESI): calcd for $C_{16}H_{12}Br_2N_2ONa$ $[M+Na]^+$: 428.9209, found: 428.9202.
MP: 181-183 °C.



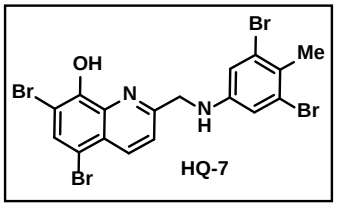
Yield: 65% yield; 60 mg of **HQ-4** was isolated as a light yellow solid.
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (d, *J* = 8.7 Hz, 1H), 7.88 (s, 1H), 7.61 (d, *J* = 8.7 Hz, 1H), 7.08 (t, *J* = 8.0 Hz, 1H), 6.71 (dd, *J* = 7.8, 1.8 Hz, 1H), 6.66 (t, *J* = 2.2 Hz, 1H), 6.53 (dd, *J* = 8.2, 2.3 Hz, 1H), 4.73 (s, 1H), 4.68 (s, 2H).
^{13}C NMR (100 MHz, $CDCl_3$): 159.1, 149.4, 148.7, 138.1, 137.3, 135.4, 133.7, 130.6, 126.0, 121.6, 118.4, 113.0, 111.5, 110.4, 104.6, 49.4.
HRMS (ESI): calcd for $C_{16}H_{11}Br_2ClN_2ONa$ $[M+Na]^+$: 462.8798, found: 462.8782.
MP: 175-177 °C.



Yield: 60% yield; 45 mg of **HQ-5** was isolated as a white solid.
^{1}H NMR (400 MHz, $CDCl_3$): 8.41 (d, *J* = 8.7 Hz, 1H), 7.88 (s, 1H), 7.55 (d, *J* = 8.7 Hz, 1H), 6.71 (m, 1H), 6.56 (d, *J* = 1.8 Hz, 2H), 4.95 (br. s, 1H), 4.64 (s, 2H).
^{13}C NMR (100 MHz, $CDCl_3$): 158.1, 149.4, 149.2, 138.0, 137.4, 135.9, 133.8, 126.1, 121.5, 118.1, 111.4, 110.4, 104.9, 49.1.
HRMS (ESI): calcd for $C_{16}H_{10}Br_2Cl_2N_2ONa$ $[M+Na]^+$: 496.8429, found: 496.8399.
MP: 164-166 °C.



Yield: 60% yield; 42 mg of **HQ-6** was isolated as a light yellow solid.
^{1}H NMR (400 MHz, $CDCl_3$): 8.38 (d, *J* = 8.6 Hz, 1H), 7.87 (s, 1H), 7.58 (d, *J* = 8.6, 1H), 7.25 (d, *J* = 8.1 Hz, 2H), 6.54 (d, *J* = 8.2 Hz, 2H), 4.65 (s, 2H).
^{13}C NMR (100 MHz, $CDCl_3$): 159.3, 149.3, 146.5, 138.1, 137.2, 133.6, 132.3, 126.0, 121.6, 114.8, 110.4, 110.1, 104.5, 49.6.
HRMS (DART): calcd for $C_{16}H_{12}Br_3N_2O$ $[M+H]^+$: 484.8494, found: 484.8492.
MP: 161-163 °C.



Yield: 45% yield; 55 mg of **HQ-7** was isolated as a light yellow solid.
^{1}H NMR (400 MHz, $CDCl_3$): 8.42 (d, *J* = 8.7 Hz, 1H), 7.89 (s, 1H), 7.57 (d, *J* = 8.7 Hz, 1H), 6.87 (s, 2H), 4.63 (s, 2H), 2.43 (s, 3H).
^{1}H NMR (400 MHz, d_6-DMSO): 10.76 (s, 1H), 8.41 (d, *J* = 8.7 Hz, 1H), 8.04 (s, 1H), 7.70 (d, *J* = 8.7 Hz, 1H), 7.06 (s, 2H), 7.01 (t, *J* = 4.9 Hz, 1H), 4.63 (d, *J* = 4.8 Hz, 2H), 2.35 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): 158.6, 149.5, 146.9, 138.2, 137.4, 133.9, 126.5, 126.1, 125.9, 121.6, 116.6, 110.5, 104.8, 49.4, 22.6.

^{13}C NMR (100 MHz, d_6-DMSO): 157.8, 150.4, 148.1, 137.6, 135.9, 132.8, 125.5, 124.9, 122.3, 122.3, 115.5, 109.0, 105.1, 47.7, 22.0.
HRMS (ESI): calcd for $C_{17}H_{12}Br_4N_2ONa$ $[M+Na]^+$: 598.7575, found: 598.7564.
MP: 144-146 °C.

3.) Biological Supporting Information:

A.) Minimum Inhibitory Concentration (MIC) Susceptibility Assay (in 96-well plate):
The minimum inhibitory concentration (MIC) for each compound was determined by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI). 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 test compound that prevented bacterial growth after incubating 16 to 18 hours at 37 °C. The concentration range tested for each compound 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. Bacterial strains used: methicillin-resistant *Staphylococcus aureus*-2 (MRSA-2; clinical isolate from Shands Hospital in Gainesville, FL), methicillin-resistant *Staphylococcus epidermidis* (MRSE; ATCC 35984), vancomycin-resistant *Enterococcus faecium* (VRE, ATCC 700221).

Mechanistic Investigations with Metal(II)-Cations:
Mechanistic investigations with metal(II)-cation were performed similar to standard MIC assays, with the addition of 200 μM of the metal salt (copper (II) sulfate, magnesium (II) sulfate, ammonium iron (II) sulfate hexahydrate and zinc (II) chloride). See Supporting Table 1 for "loss of antibacterial activity" results with MRSA-2.

Supporting Table 1. Mechanistic investigations of select HQ compounds against MRSA-2. All concentrations are in μM.

	MRSA-2								
Compound	MIC	MIC w/ Fe^{2+}	Fold Δ	MIC w/ Cu^{2+}	Fold Δ	MIC w/ Mg^{2+}	Fold Δ	MIC w/ Zn^{2+}	Fold Δ
HQ-1	1.13[a]	>100	+ 88	25	+ 22	1.56	n.a.	0.15[a]	- 8
HQ-3	1.13[a]	6.2[a]	+ 6	0.78	n.a.	1.56	n.a.	0.39	- 3
HQ-6	4.69[a]	9.38[a]	+ 2	1.13[a]	+ 4	3.13	n.a.	0.78	- 6

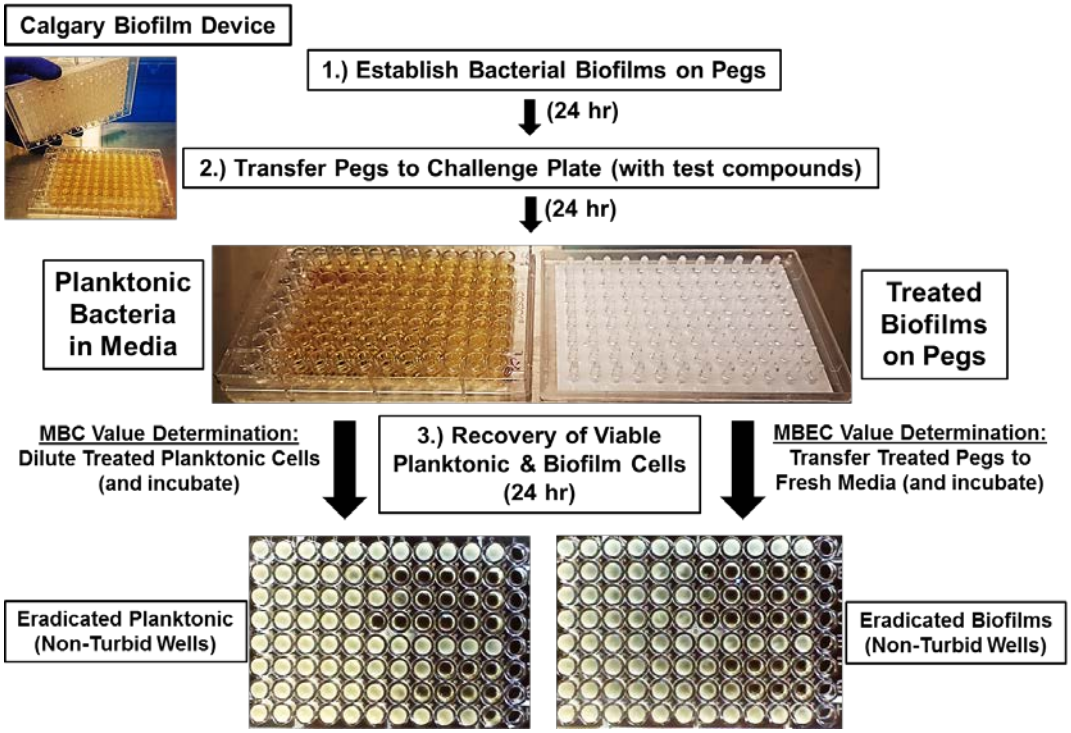
Notes: [a] corresponds to the midpoint value of a 2-fold range (in MIC) of a two independent experiments. "Fold Δ" denotes the change in antibacterial activity according to MIC values; (+) for increase in MIC value/loss of antibacterial activity; (-) for decrease in MIC value/increase of antibacterial activity; (n.a.) means insignificant changes in MIC values.

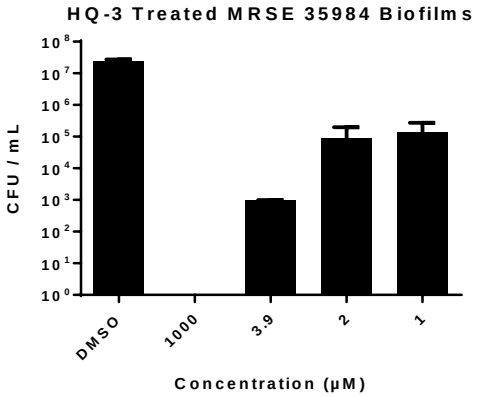
B.) Calgary Biofilm Device (CBD) Experiments:

Determination of Minimum Bactericidal Concentrations (MBC) and Minimum Biofilm Eradication Concentrations (MBEC) using the Calgary Biofilm Device

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 96 total pegs to establish biofilms; 1 peg/well) 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 was 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 period). In select experiments, treated pegs from the Calgary device were removed from lead biofilm eradicators after final incubation, sonicated for 30 minutes in PBS and plated out to determine biofilm cell killing (i.e., colony forming unit per milliliter, CFU/mL).

Note: MRSA-2, *S. epidermidis* (ATCC 35984) and VRE (ATCC 700221) were tested using these assay parameters.



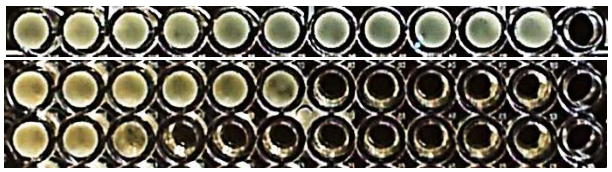


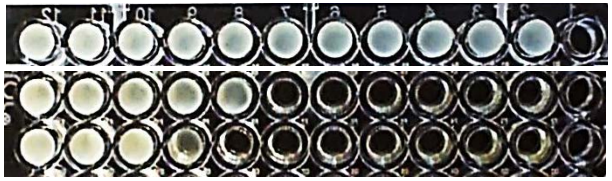
Viable biofilm cells determined from CBD assay (from treated and untreated pegs).

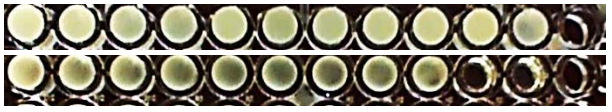
D.) Hemolysis assay:

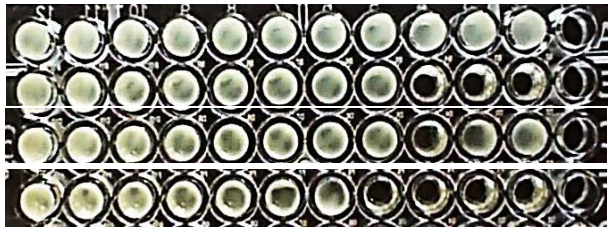
Freshly drawn human red blood cells (hRBC with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant) were washed with Tris-buffered saline (0.01M Tris-base, 0.155 M sodium chloride (NaCl), pH 7.2) and centrifuged for 5 minutes at 3500 rpm. The washing was repeated three times with the buffer. In 96-well plate, the test compounds were added to the buffer. Then 50 µL of 2% of hRBCs in the buffer were added to the test plate to make the final concentration to be 200 µM of each compound. The plate was then incubated for 1 hour at 37 °C. After incubation, the plate is centrifuged for 5 minutes at 3500 rpm. Then 80 µL of the supernatant were transferred to another 96-well plate and the Optical Density (OD) was read at 405 nm. DMSO served as our negative control (0% hemolysis) and Triton X served as our positive control (100% hemolysis). The percent of hemolysis was calculated as (OD_{405} of the compound- OD_{405} DMSO) / (OD_{405} Triton X- OD_{405} buffer).

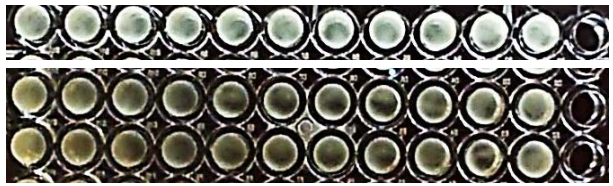
MRSA-2 Biofilm Eradication (CBD Assay)

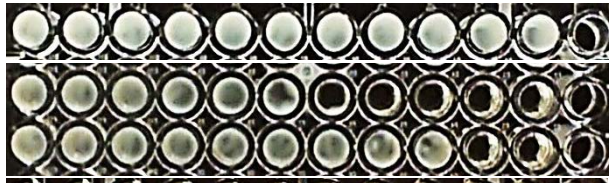


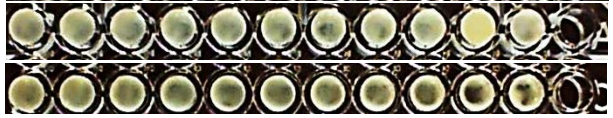












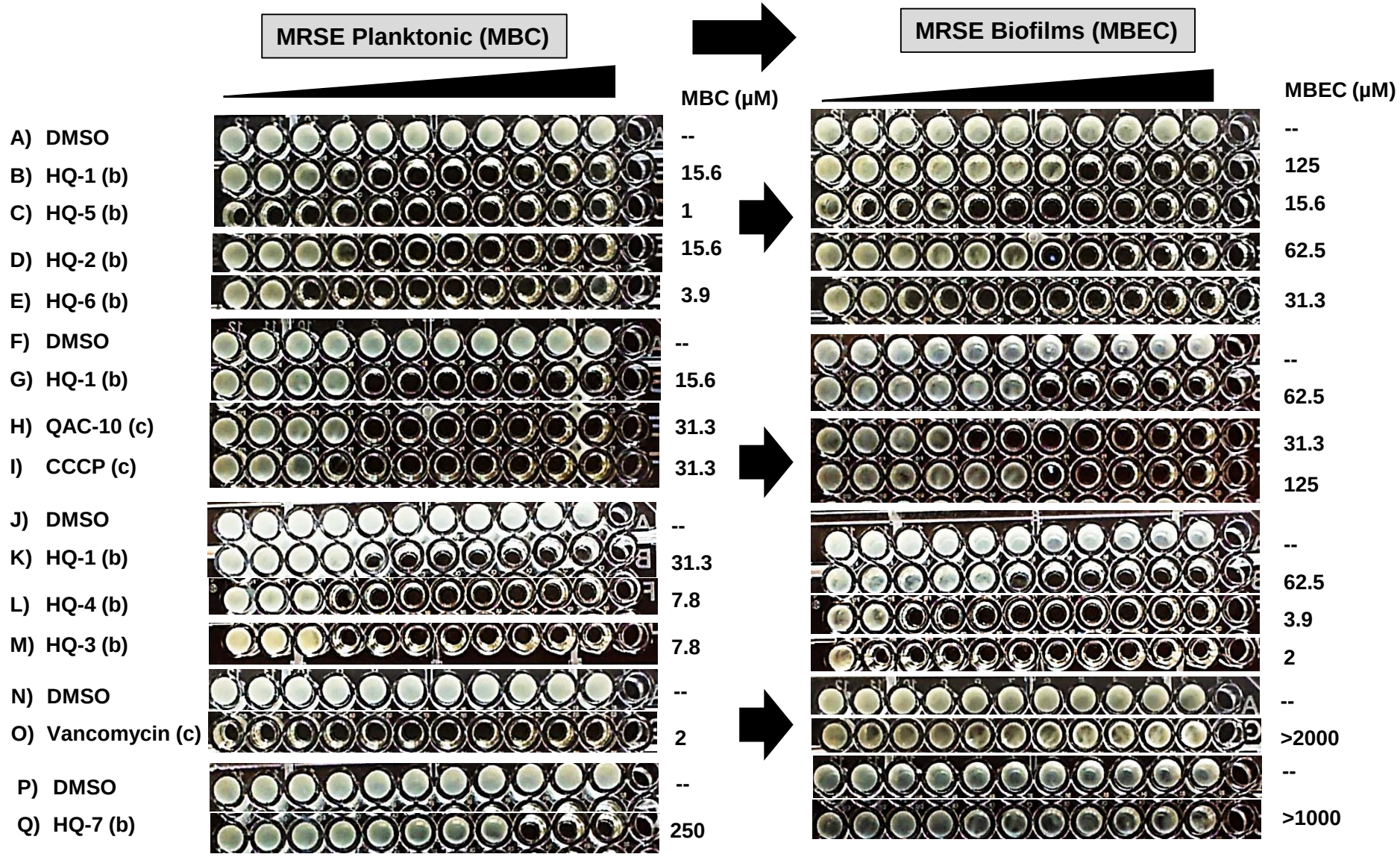
MRSA-2 Planktonic (MBC)

MRSA-2 Biofilms (MBEC)

		MBC (μM)	MBEC (μM)
A)	DMSO	--	--
B)	HQ-1 (b)	31.3	250
C)	HQ-2 (b)	31.3	>1000
D)	HQ-6 (b)	125	125
E)	DMSO	--	--
F)	Daptomycin (c)	125	>2000
G)	Linezolid (c)	15.6	>2000
H)	DMSO	--	--
I)	HQ-1 (b)	15.6	250
J)	HQ-5 (b)	62.5	500
K)	HQ-4 (b)	31.3	125
L)	HQ-3 (b)	125	125
M)	DMSO	--	--
N)	QAC-10 (c)	62.5	125
O)	CCCP (c)	31.3	1000
P)	DMSO	--	--
Q)	HQ-7 (b)	500	>1000

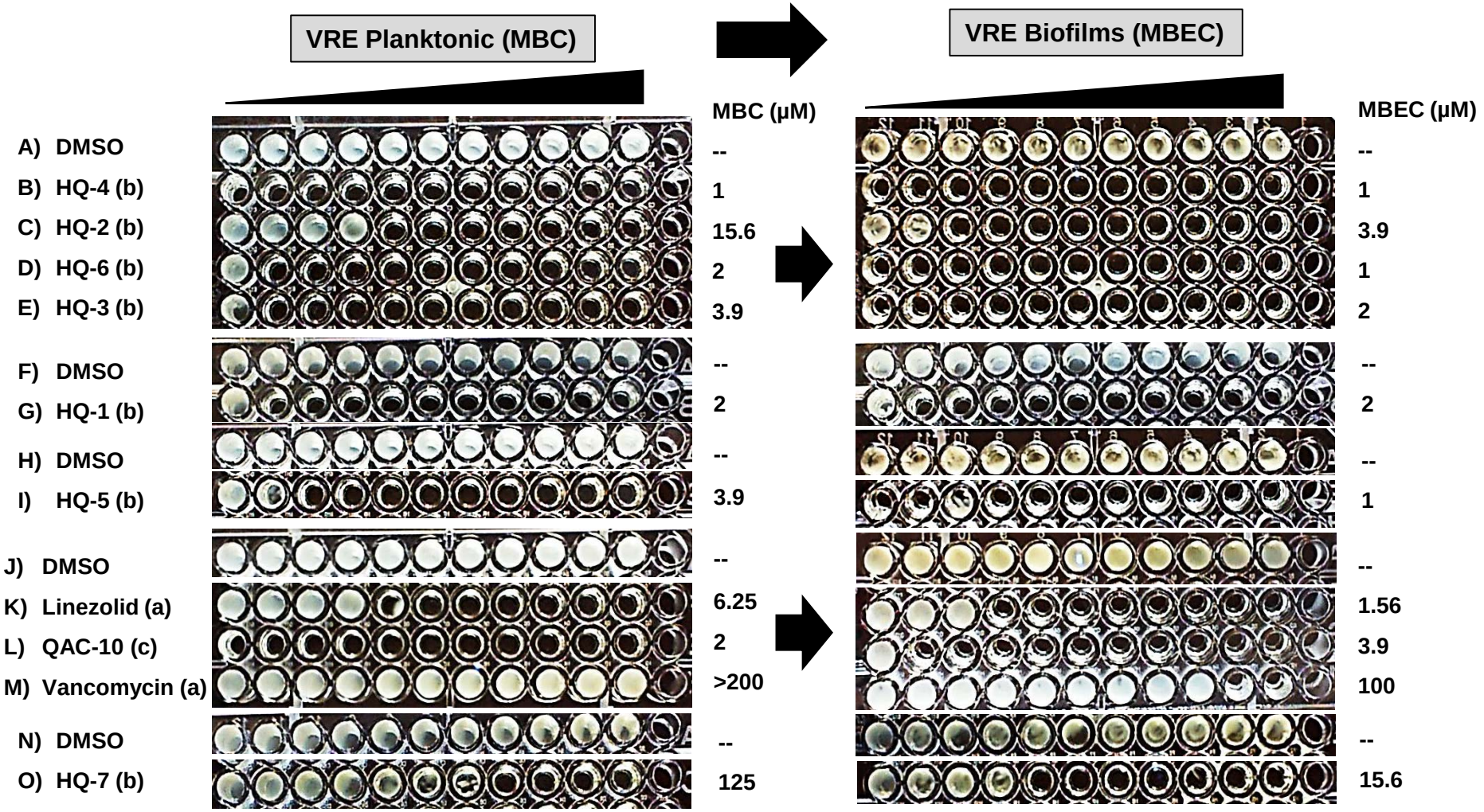
DMSO stock (with test range): (a) 10 mM (0.2 - 200 μM), (b) 50 mM (1 - 1,000 μM), (c) 100 mM (2 - 2,000 μM).

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



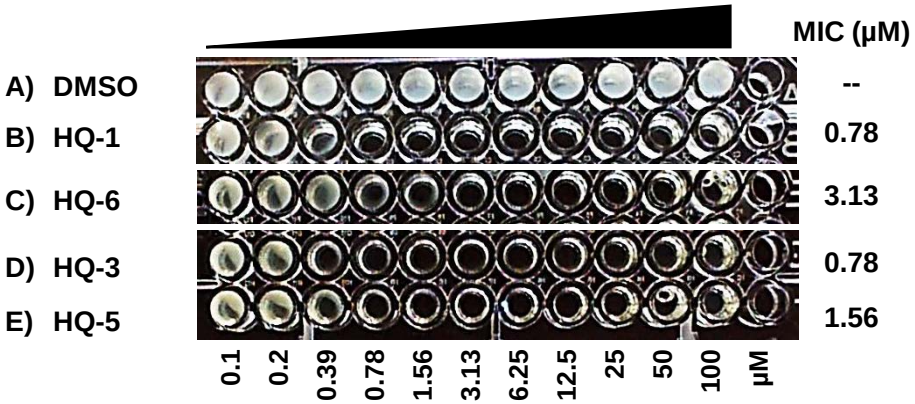
DMSO stock (with test range): (a) 10 mM (0.2 - 200 µM), (b) 50 mM (1 - 1,000 µM), (c) 100 mM (2 - 2,000 µM).

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

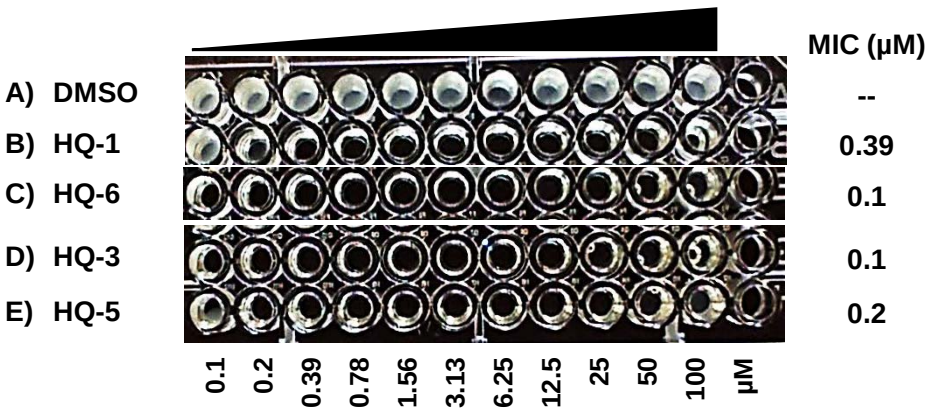


DMSO stock (with test range): (a) 10 mM (0.2 - 200 μM), (b) 50 mM (1 - 1,000 μM), (c) 100 mM (2 - 2,000 μM).

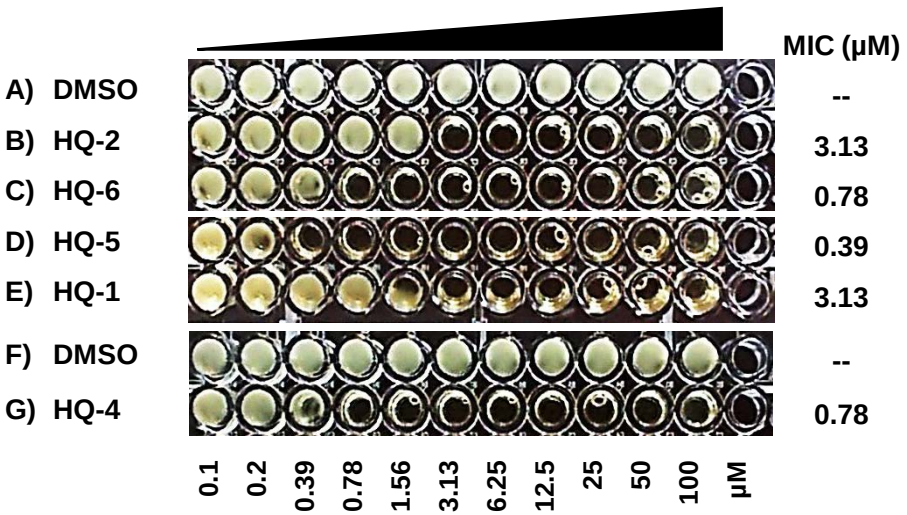
MIC Assay against MRSA-2



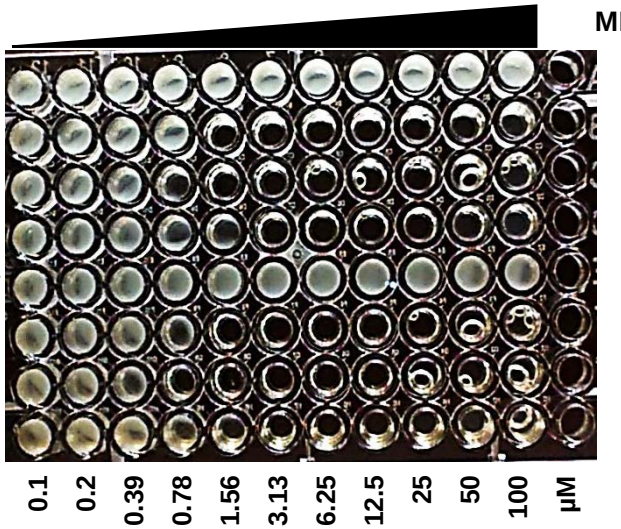
MIC Assay against *S. epidermidis* (ATCC 35984)

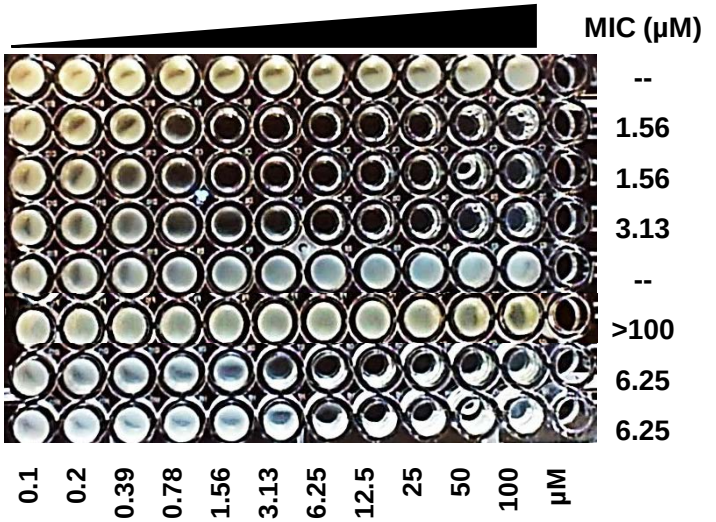


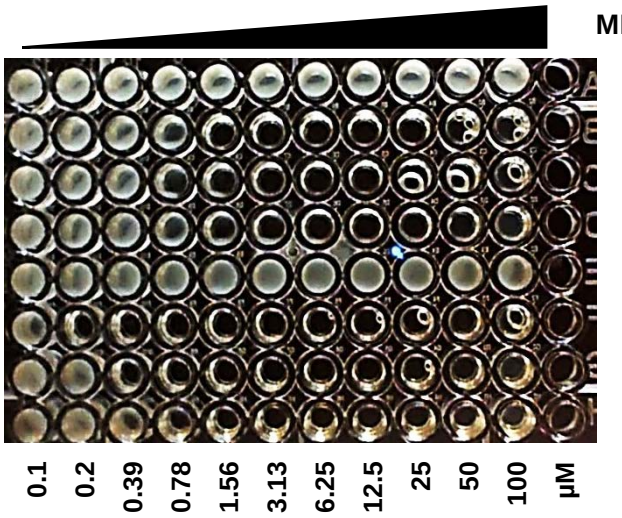
MIC Assay against *E. faecium* (ATCC 700221)

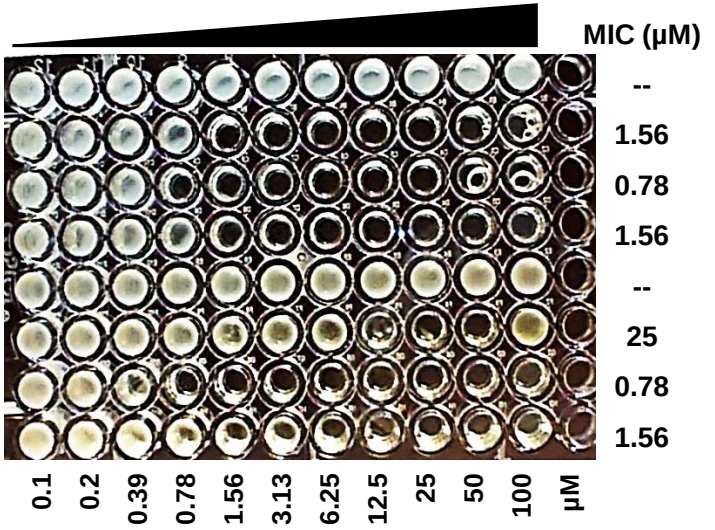


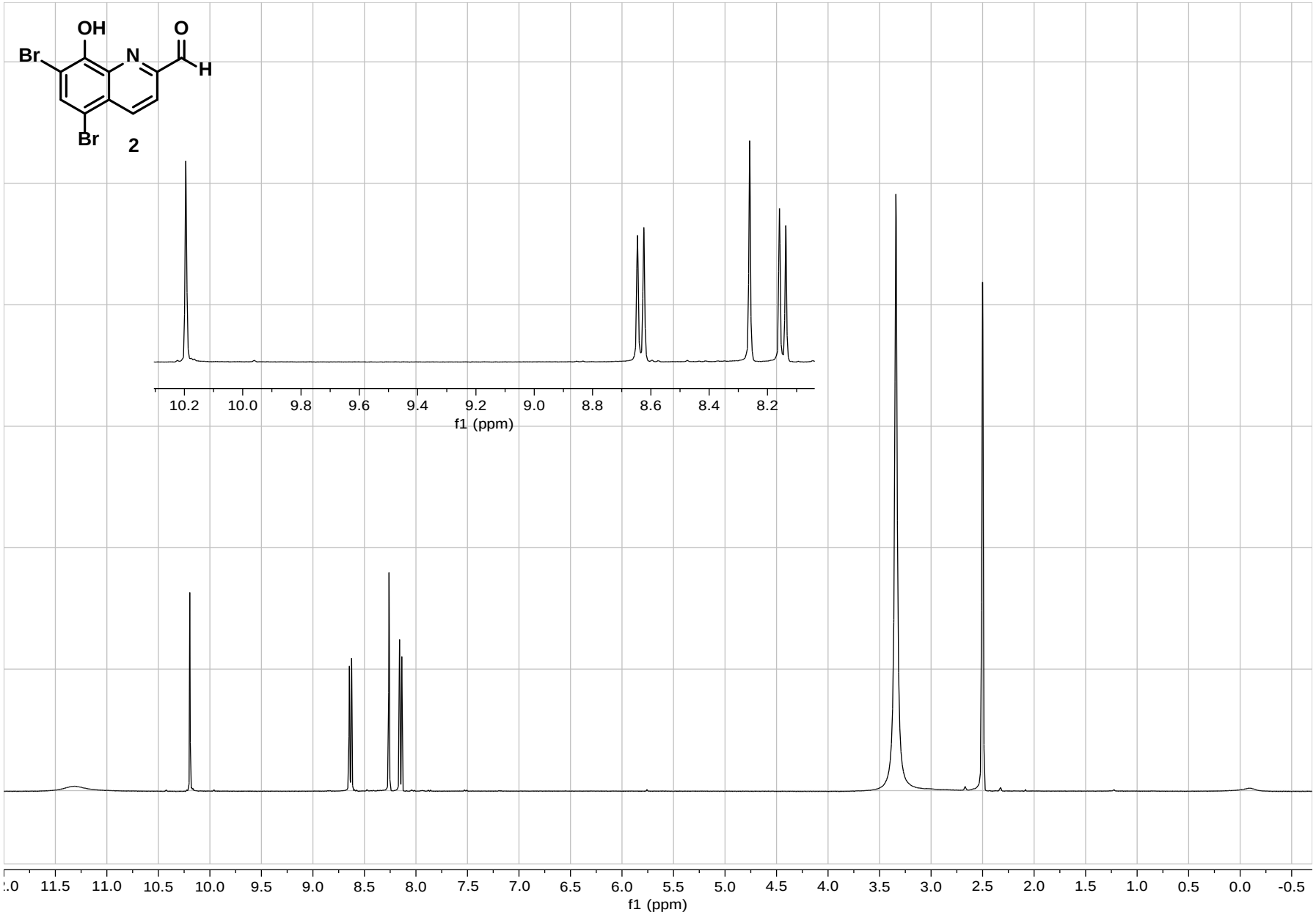
MRSA-2 Mechanistic Studies (Metal(II)-Cation Co-treatment)

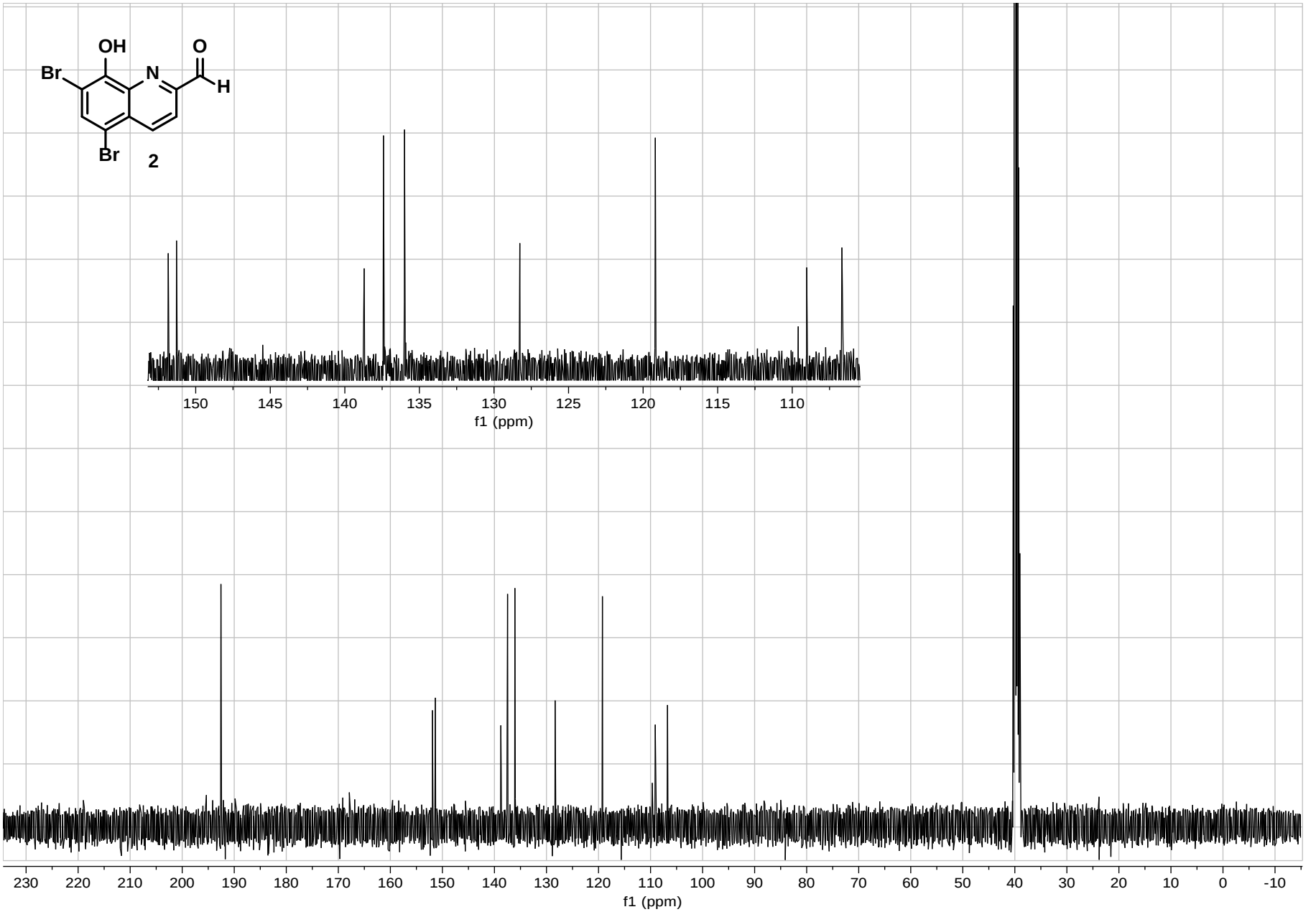


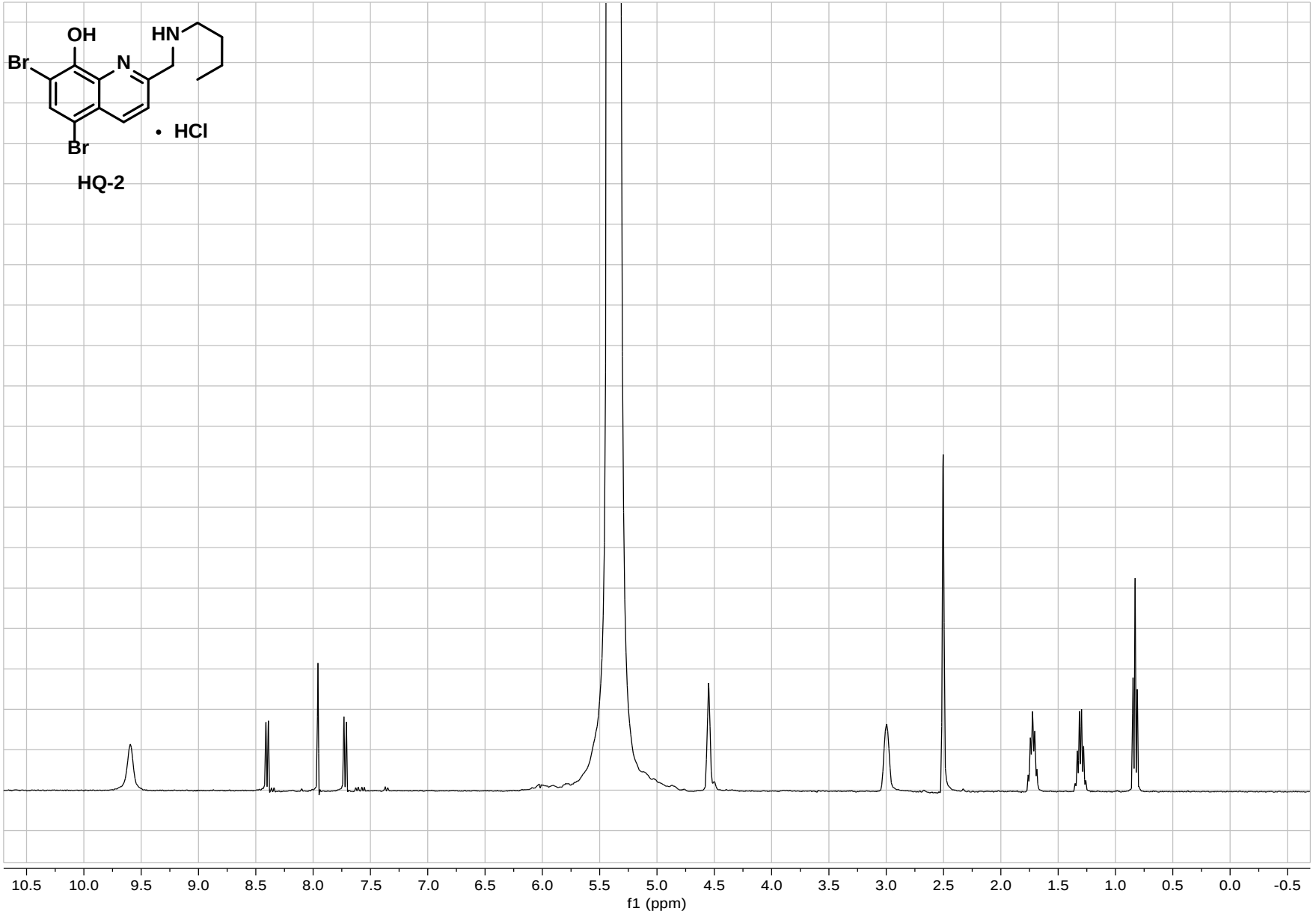


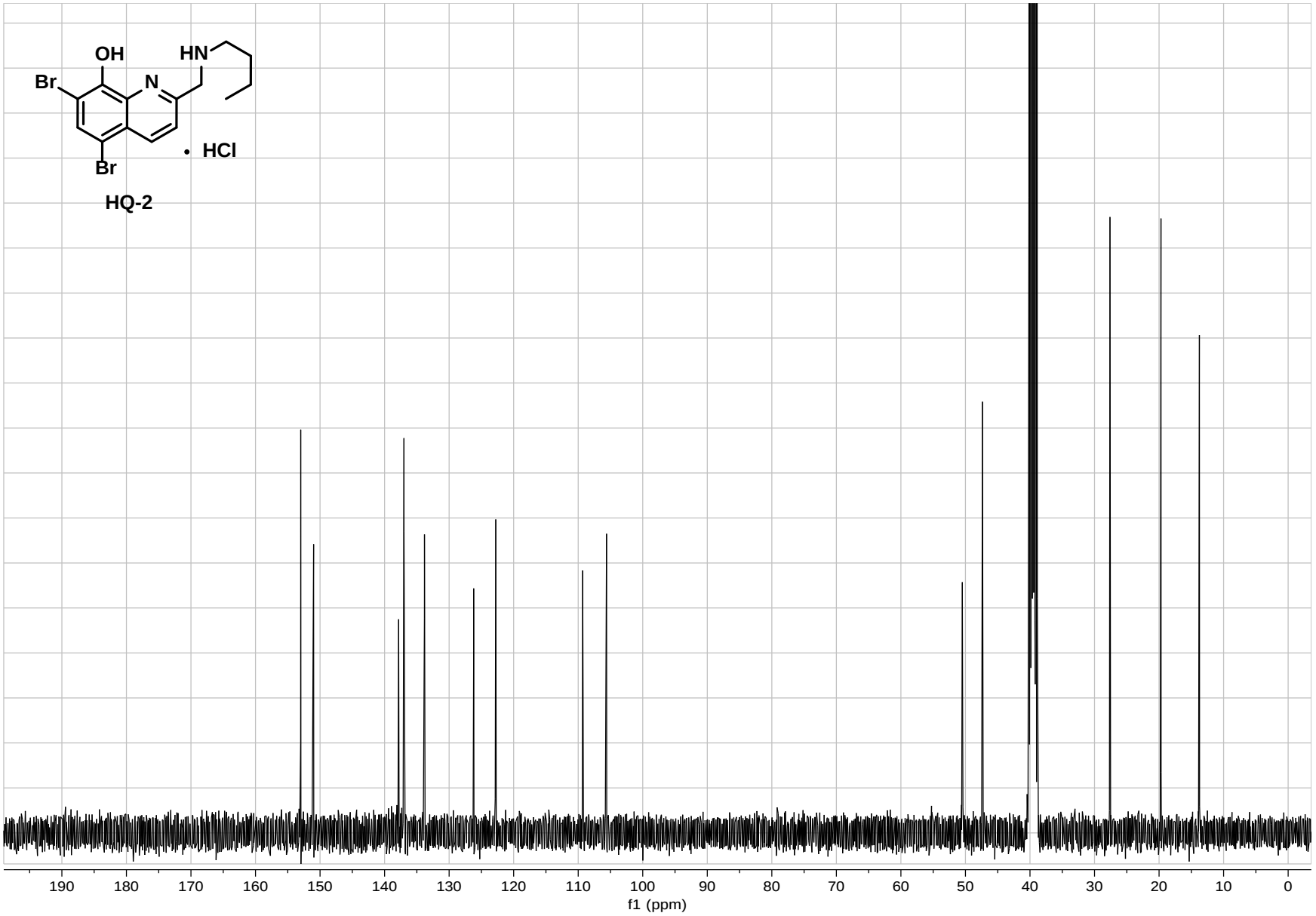


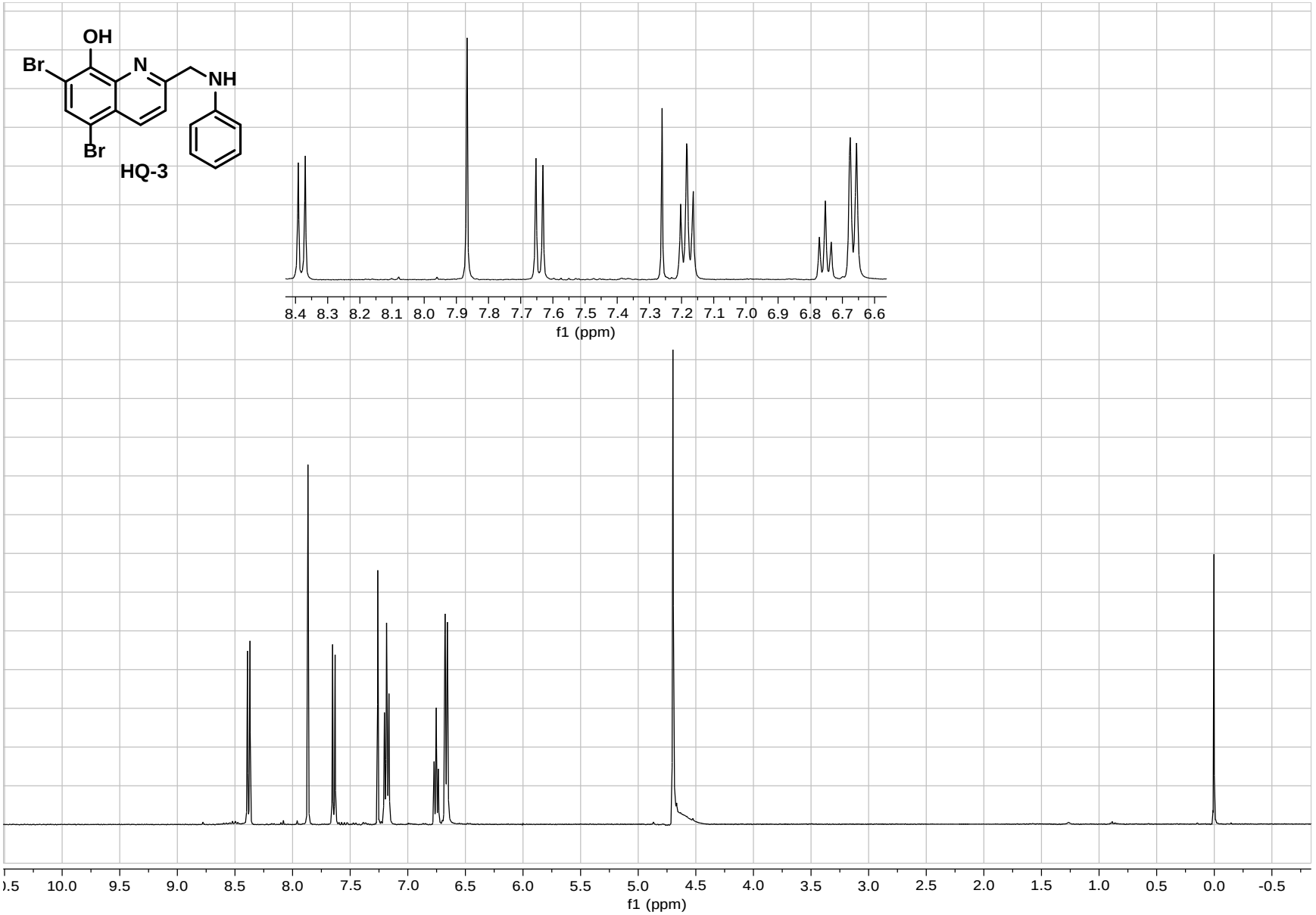


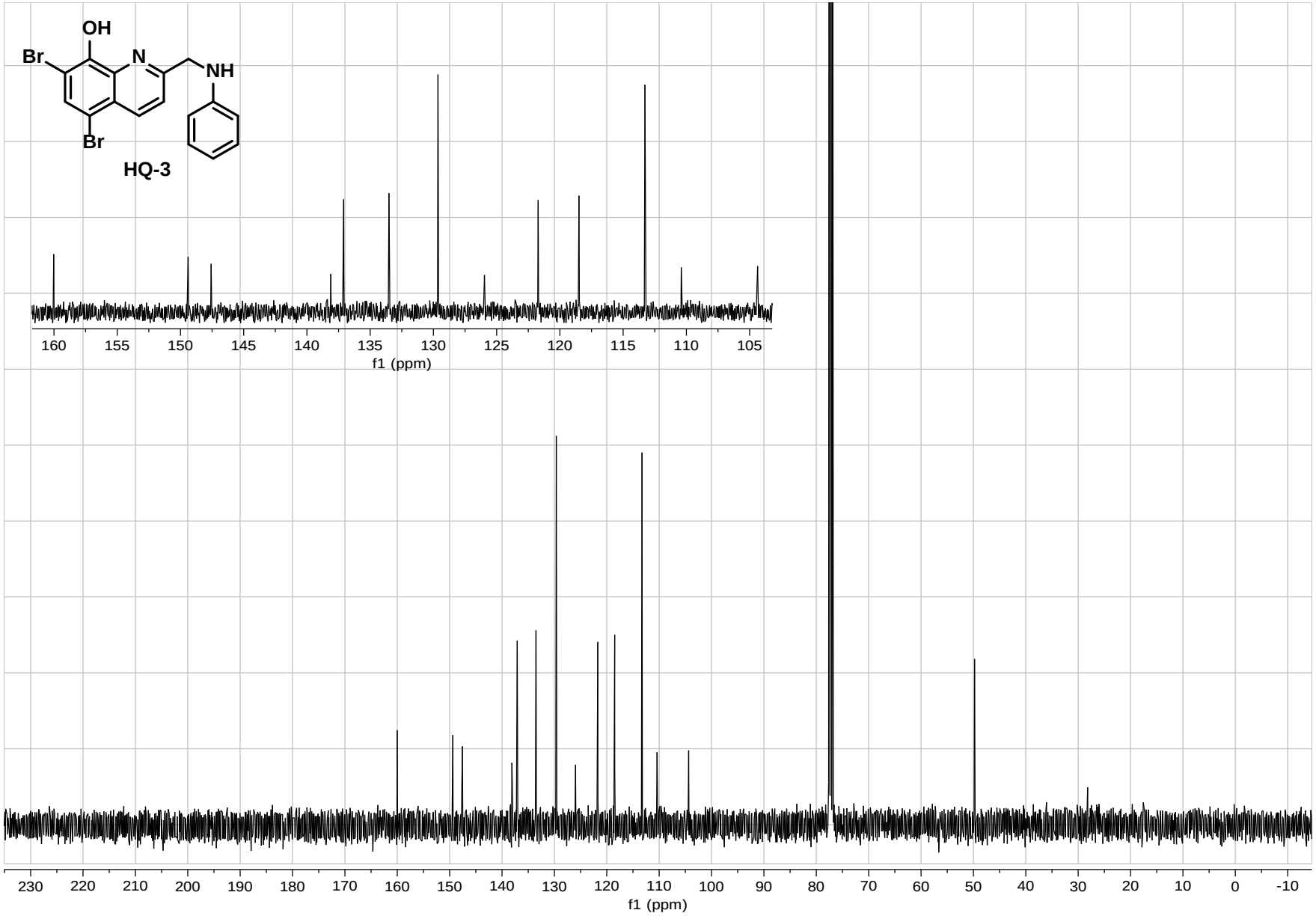

OH
O
Br
N
H
Br
2
10.2
10.0
9.8
9.6
9.4
9.2
9.0
8.8
8.6
8.4
8.2
f1 (ppm)
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

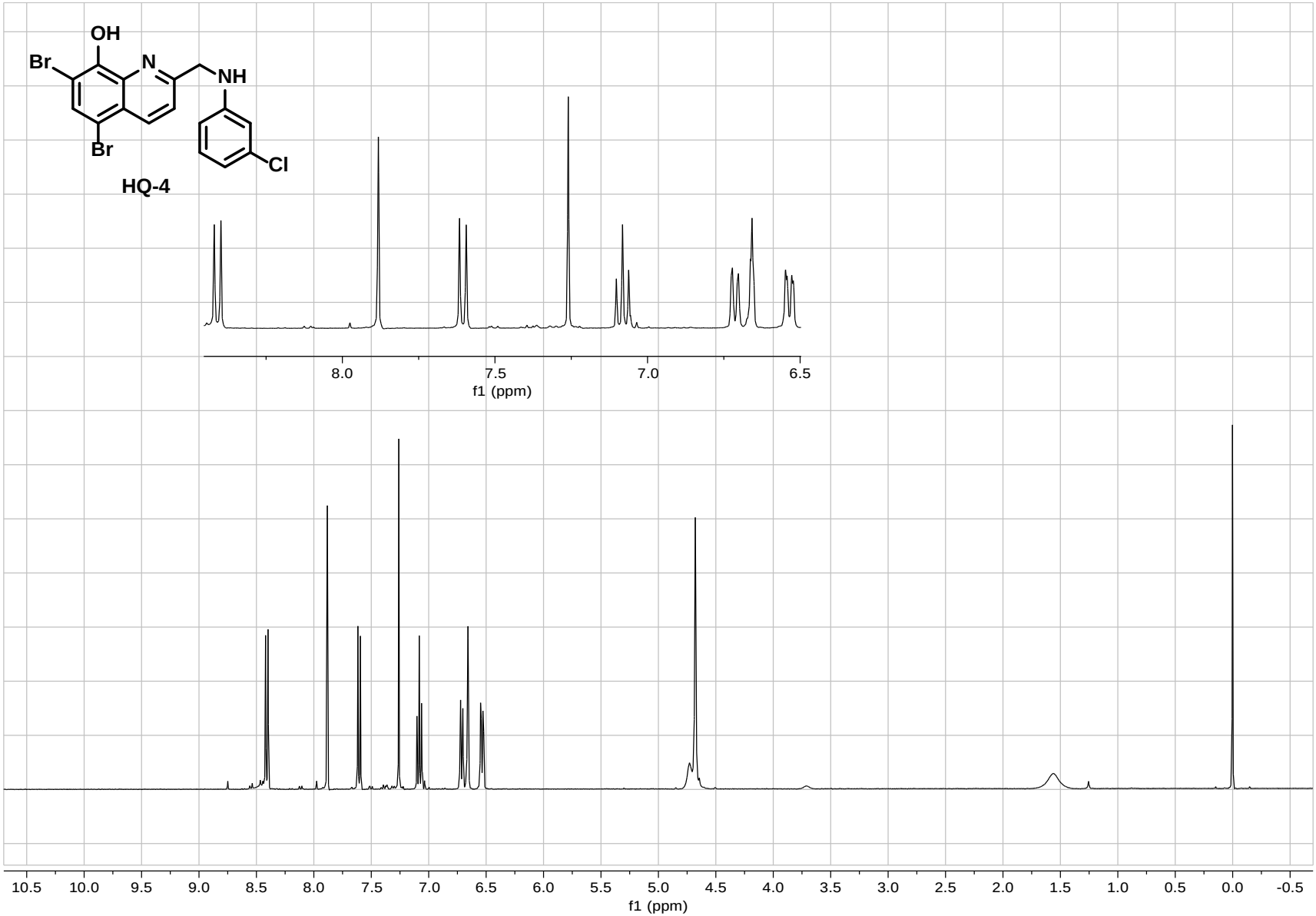

OH
O
Br
N
H
Br
2
150
145
140
135
130
125
120
115
110
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

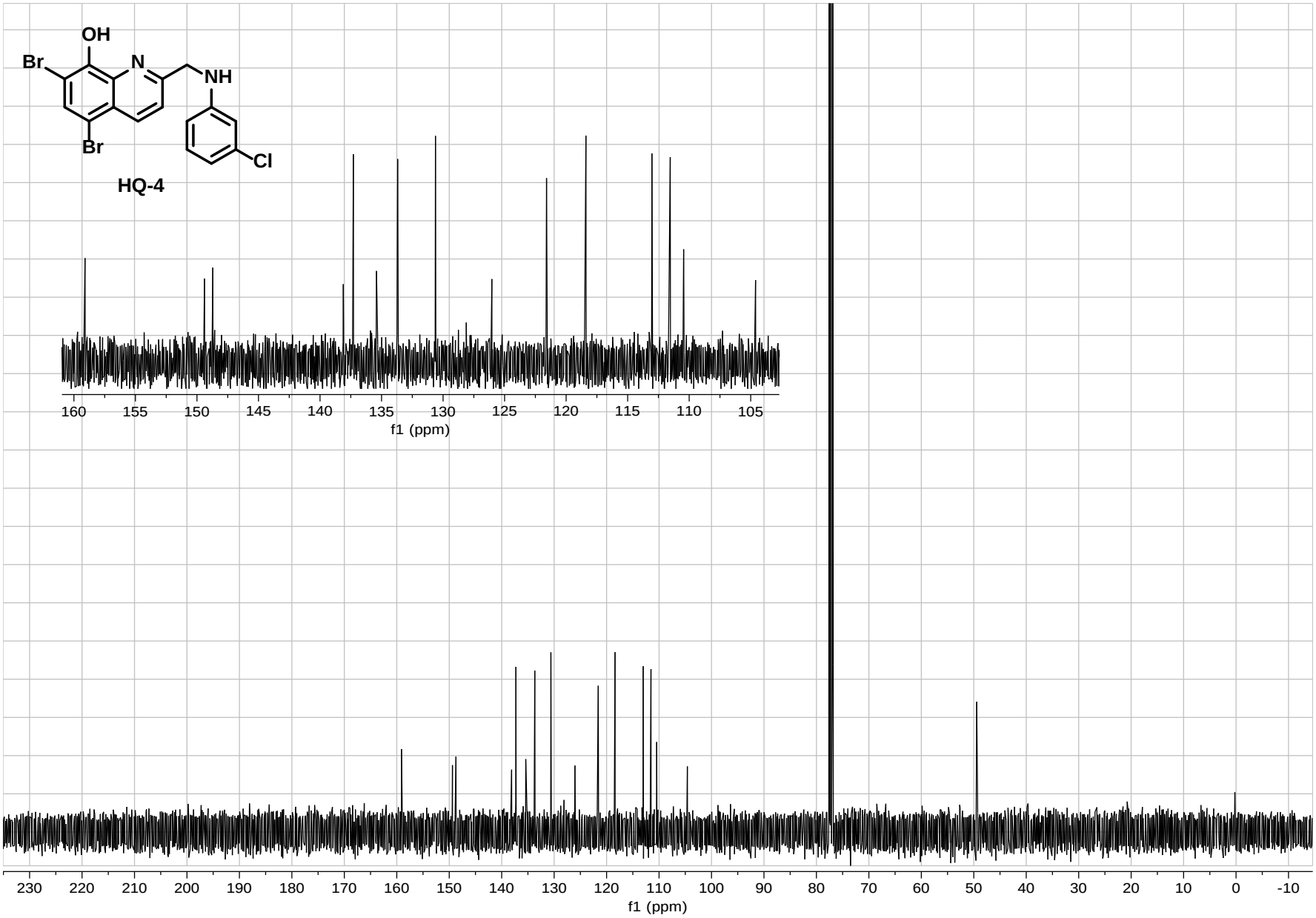

OH
HN
Br
N
• HCl
Br
HQ-2
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

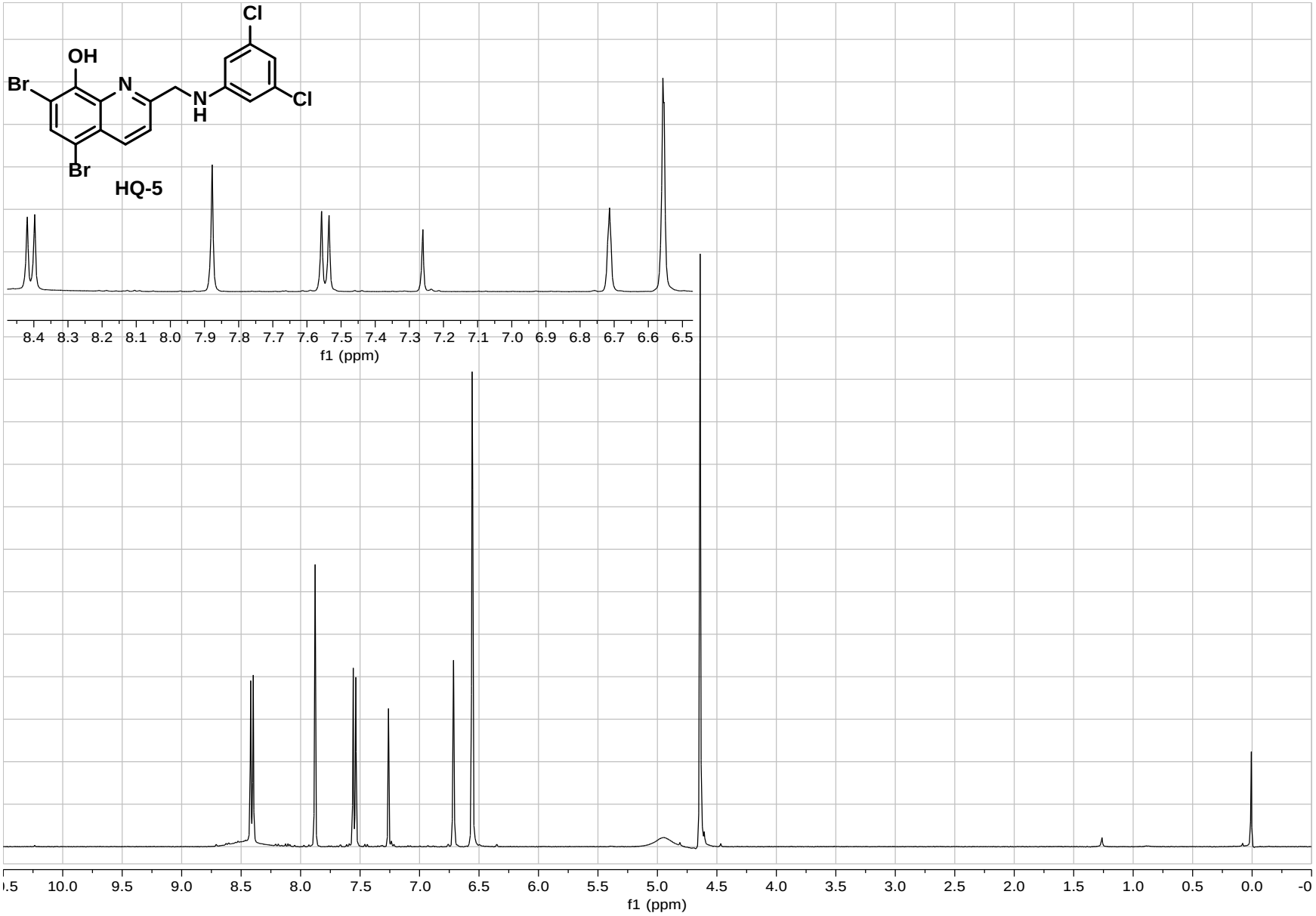

OH
HN
Br
N
• HCl
Br
HQ-2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

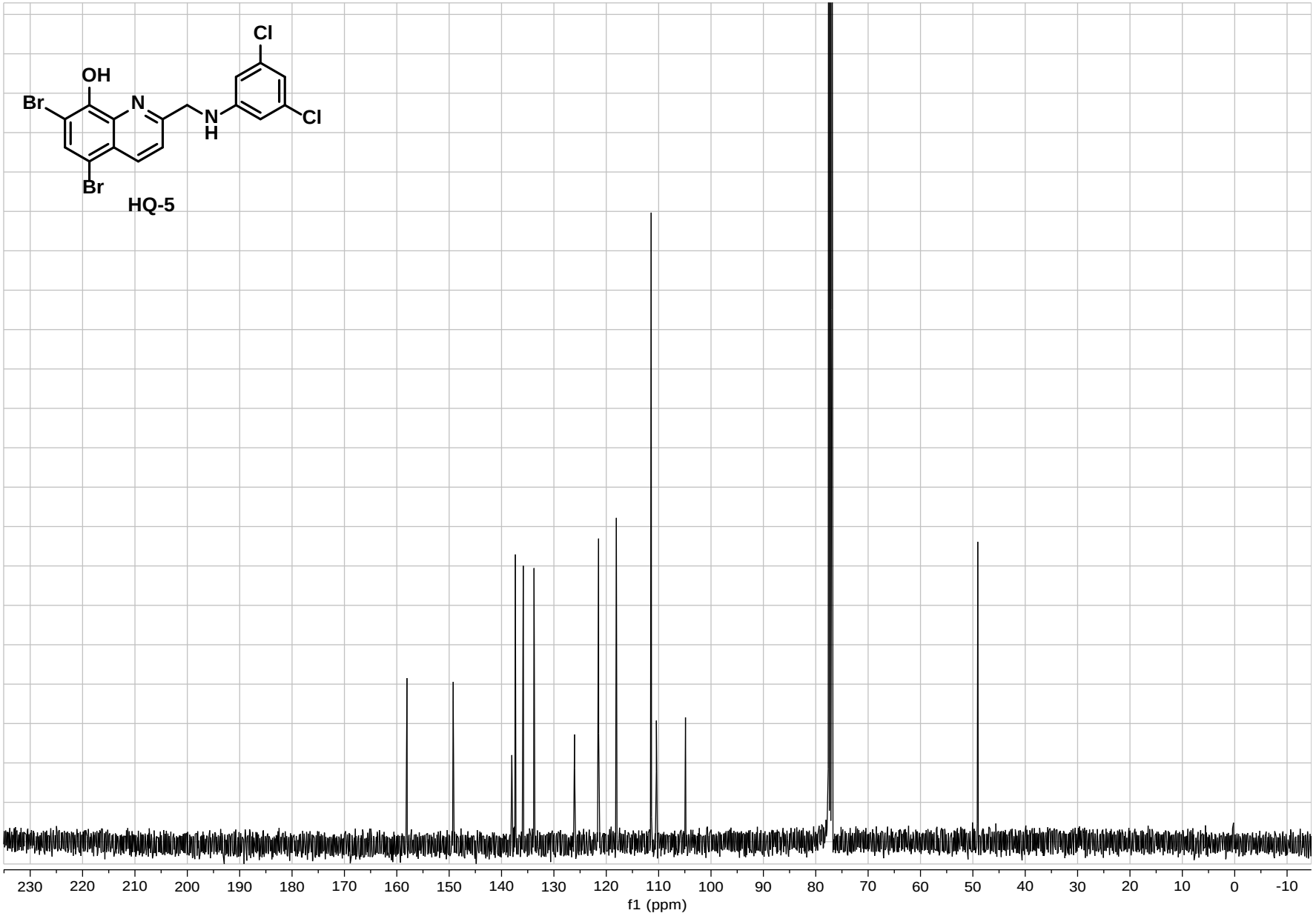

OH
Br
N
NH
Br
HQ-3
8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6
f1 (ppm)
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

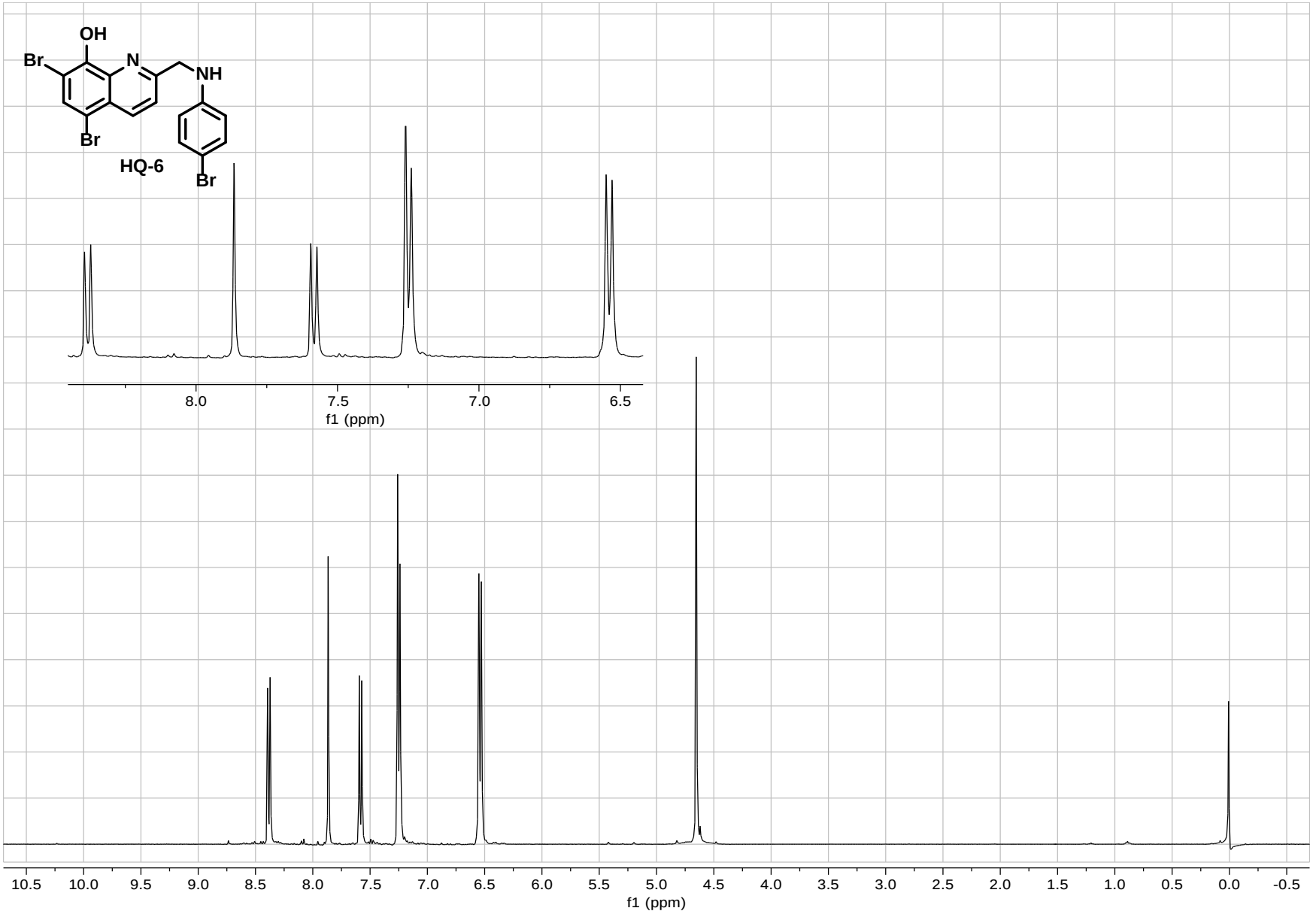

OH
Br
N
NH
Br
HQ-3
160 155 150 145 140 135 130 125 120 115 110 105
f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

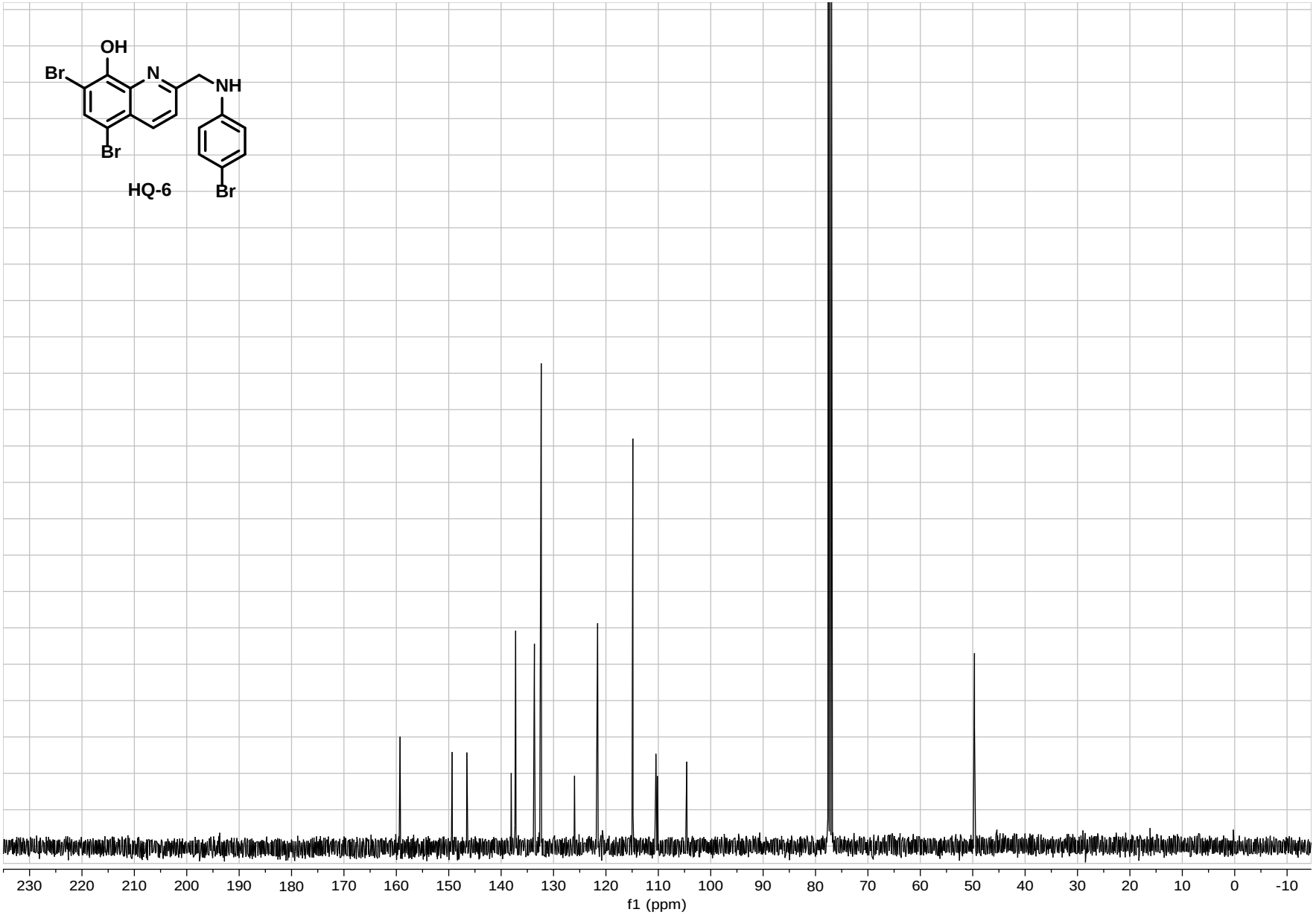

OH
Br
N
NH
Br
Cl
HQ-4
8.0
7.5
7.0
6.5
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

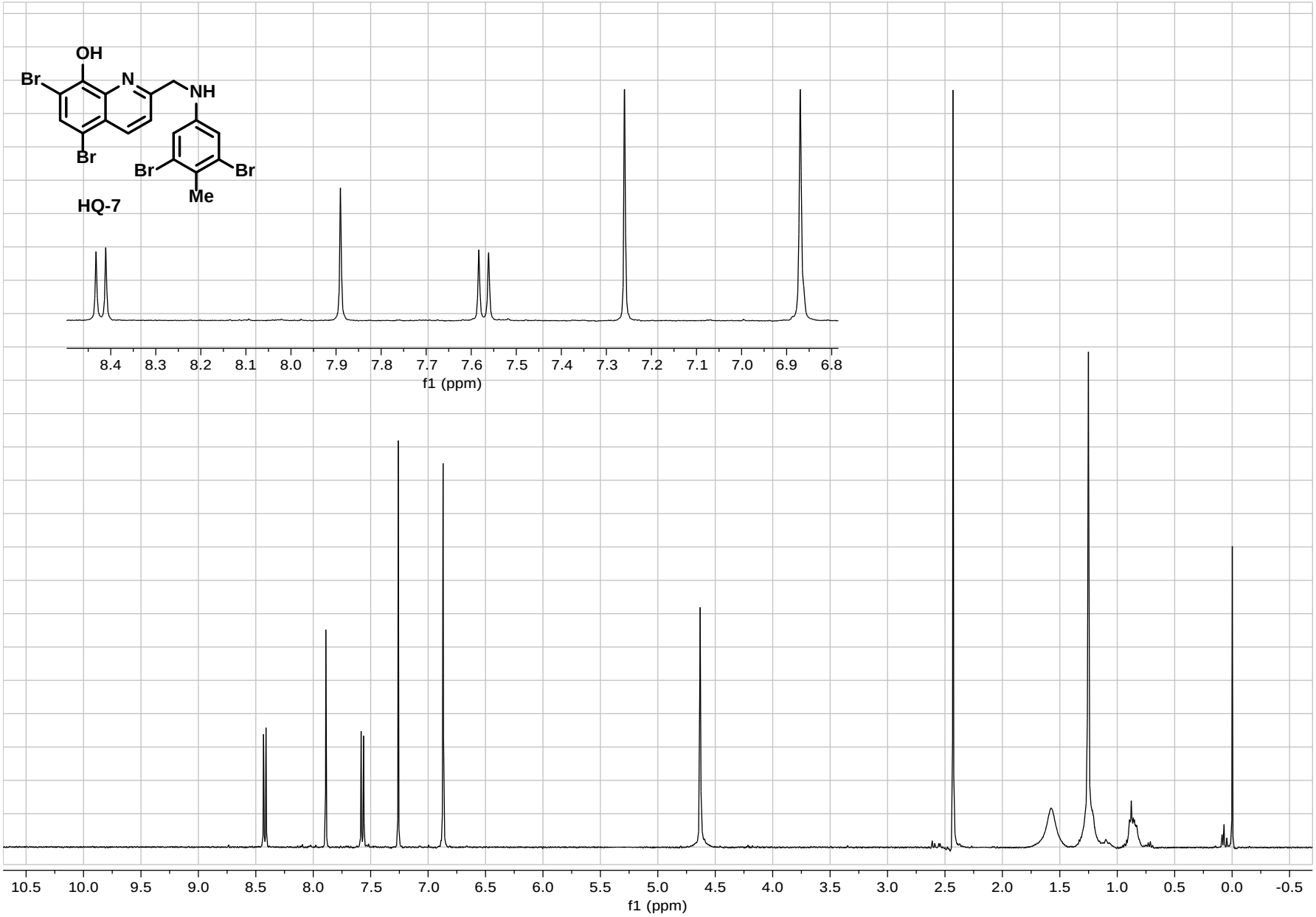

OH
Br
N
NH
Br
Cl
HQ-4
160 155 150 145 140 135 130 125 120 115 110 105
f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

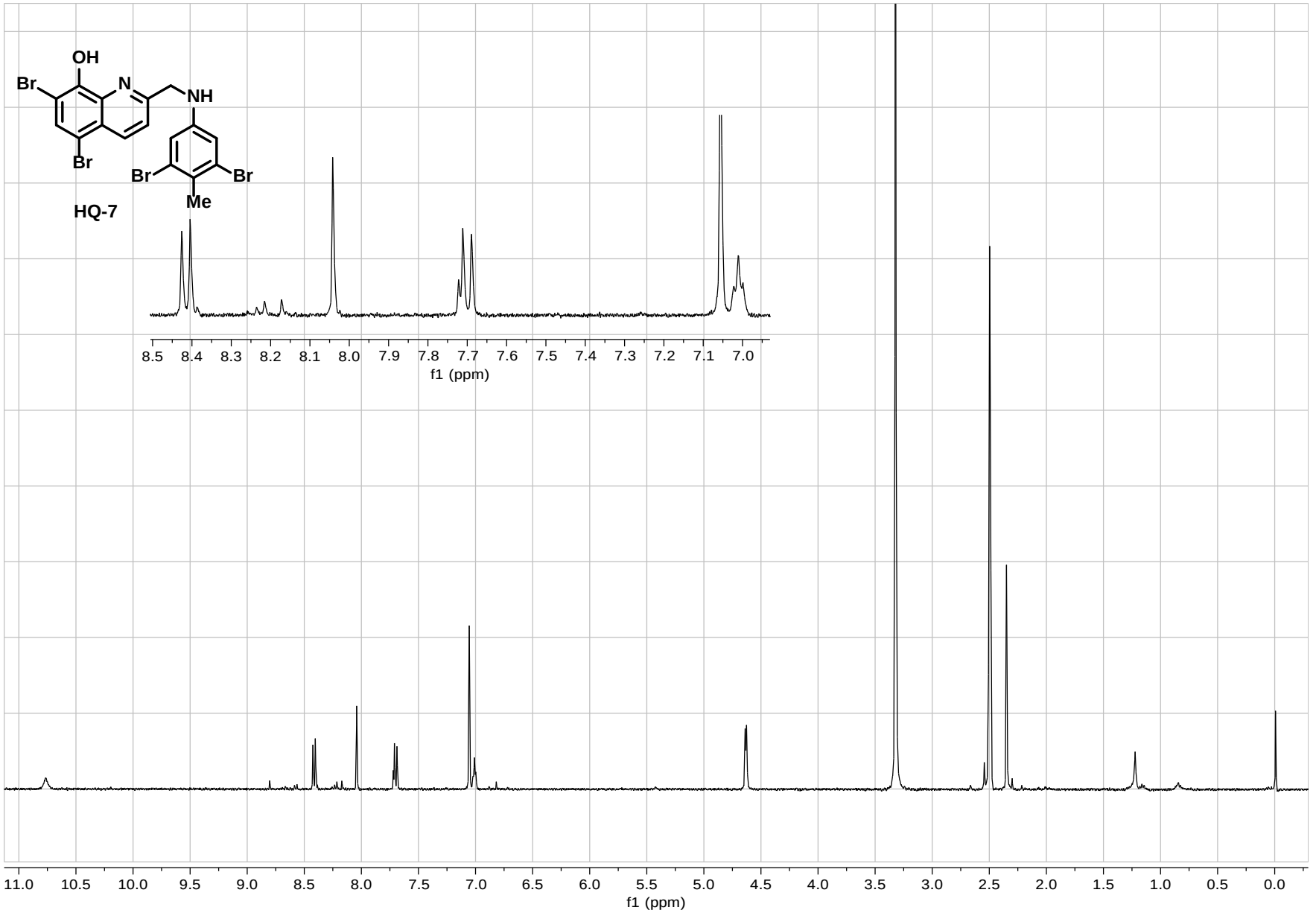

Cl
OH
Br
N
N
H
Cl
Br
HQ-5
8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5
f1 (ppm)
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0
f1 (ppm)

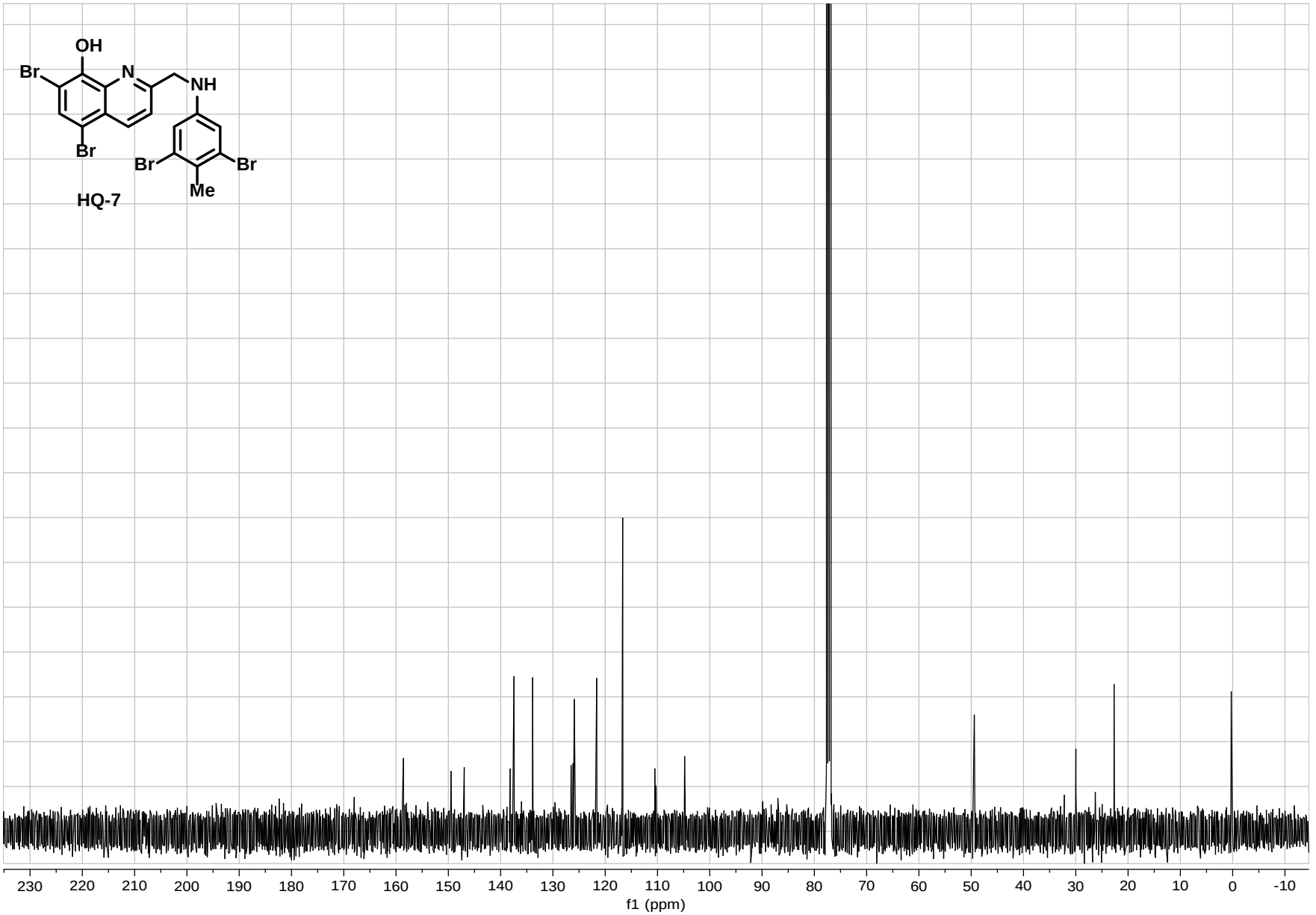

Cl
OH
Br
N
N
H
Cl
Br
HQ-5
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

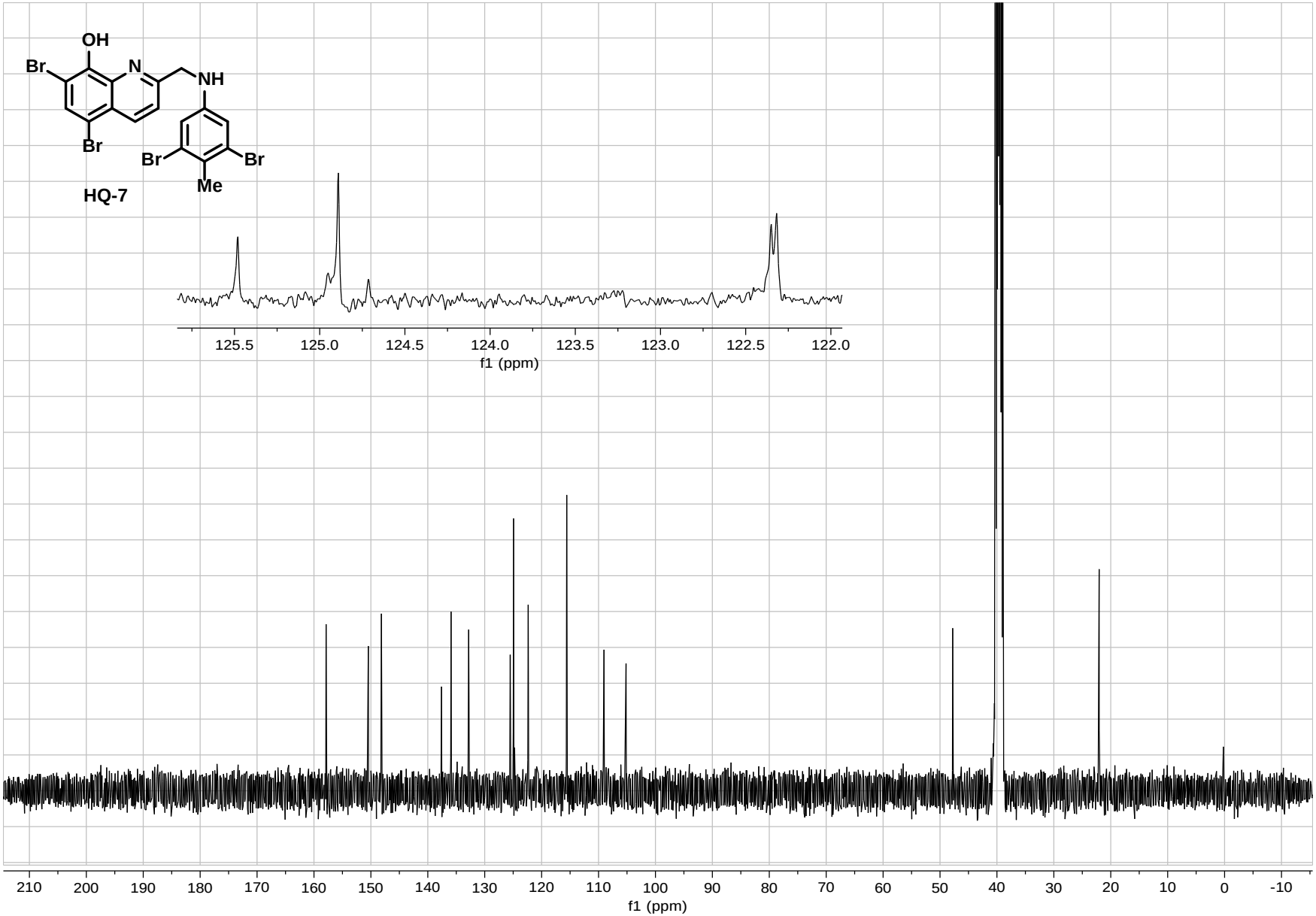

OH
Br
N
NH
Br
HQ-6
Br
8.0
7.5
7.0
6.5
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


OH
Br
N
NH
Br
HQ-6
Br
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
NH
Br
Br
Br
Me
HQ-7
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 6.8
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
NH
Br
Br
Br
Me
HQ-7
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
f1 (ppm)
11.0 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
f1 (ppm)


OH
Br
N
NH
Br
Br
Br
Me
HQ-7
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
NH
Br
Br
Br
Me
HQ-7
125.5
125.0
124.5
124.0
123.5
123.0
122.5
122.0
f1 (ppm)
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)