Supporting Information

A Modular Synthetic Route Involving *N*-Aryl-2-Nitrosoaniline Intermediates Leads to a New Series of 3-Substituted Halogenated Phenazine Antibacterial Agents

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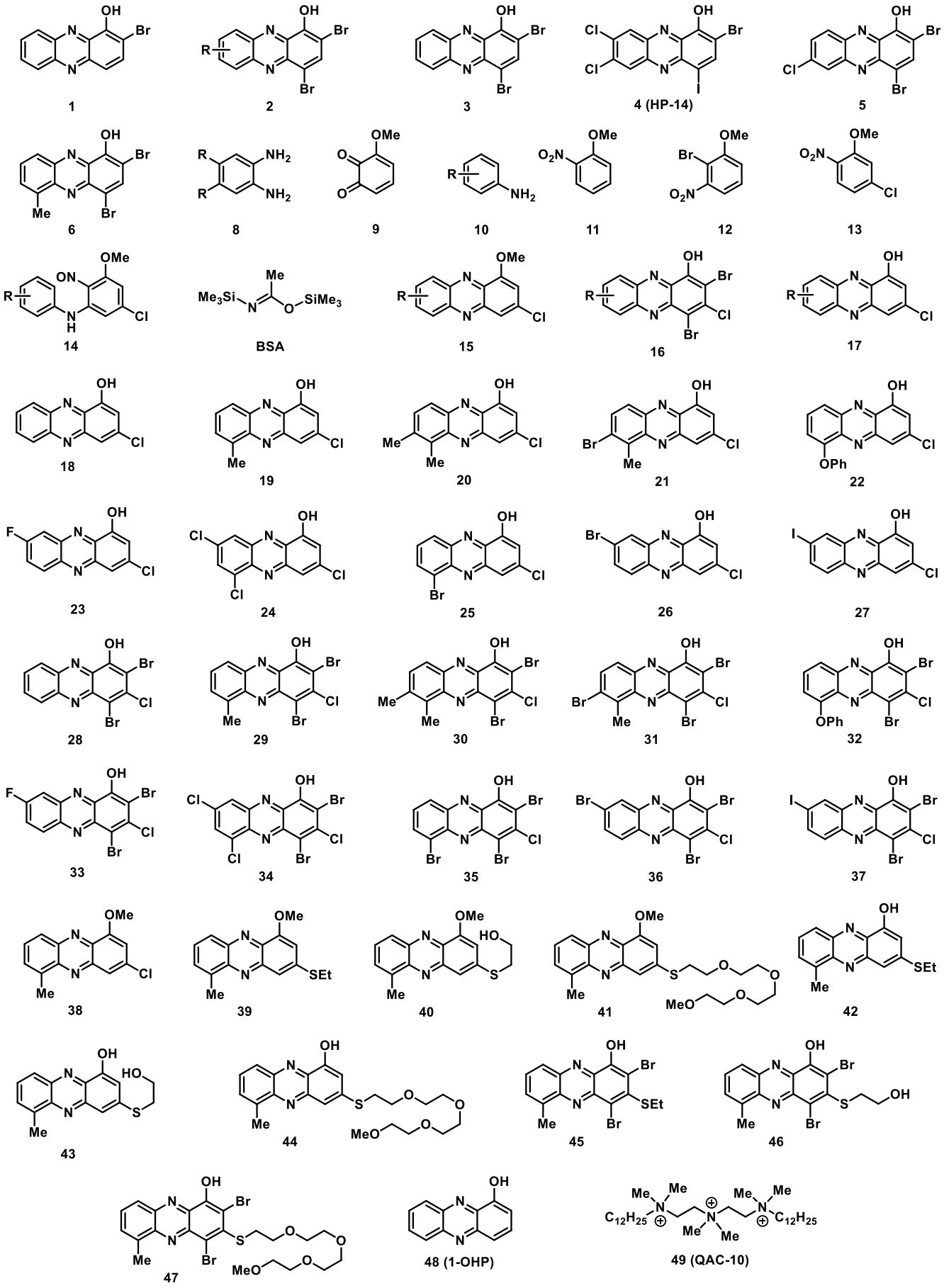
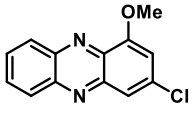
1.) <u>Figure S1.</u> Compounds reported in the manuscript.



2.) <u>Figure S2.</u> Compounds reported in the supporting information.



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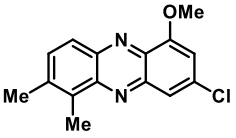

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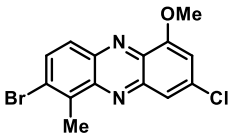

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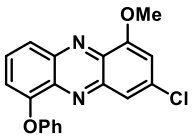

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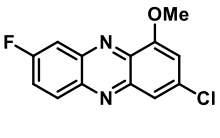

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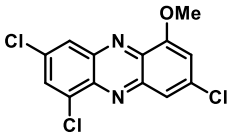

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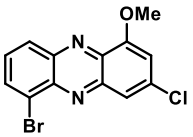

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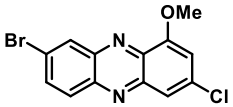

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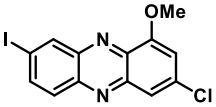

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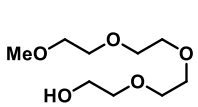

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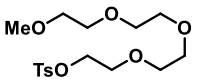

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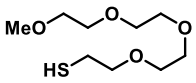

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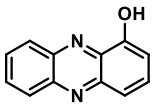
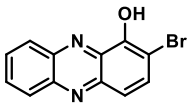
3.) Figure S3. Activity profiles with p*K*a and CLogP values for select HP analogues.



48 (1-OHP)

CLogP: 3.17
p*K*a: 9.37

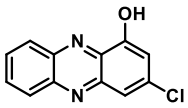
	MIC	/	MBEC (μM)
MRSA:	50	/	--
MRSE:	37.5	/	--
VRE:	> 50	/	--



1

CLogP: 3.80
p*K*a: 7.61

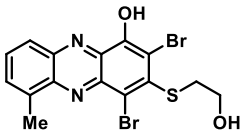
	MIC	/	MBEC (μM)
MRSA:	3.13	/	--
MRSE:	3.13	/	--
VRE:	6.25	/	--



18

CLogP: 3.93
p*K*a: 8.04

	MIC	/	MBEC (μM)
MRSA:	25	/	--
MRSE:	18.8	/	--
VRE:	> 50	/	--



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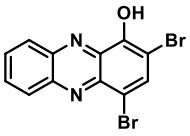
CLogP: 4.61
p*K*a: 8.07

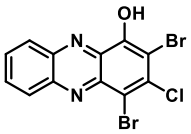
	MIC	/	MBEC (μM)
MRSA:	6.25	/	--
MRSE:	4.69	/	--
VRE:	12.5	/	--



3

CLogP: 4.68
p*K*a: 7.12

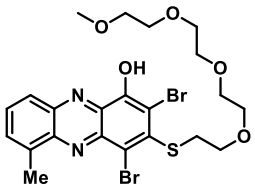
	MIC	/	MBEC (μM)
MRSA:	1.56	/	100
MRSE:	2.35	/	50
VRE:	4.69	/	12.5



28

CLogP: 5.12
p*K*a: 5.93

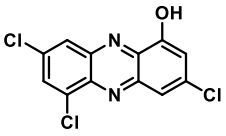
	MIC	/	MBEC (μM)
MRSA:	0.15	/	50
MRSE:	0.59	/	25
VRE:	1.17	/	2.35



47

CLogP: 5.12
p*K*a: 7.93

	MIC	/	MBEC (μM)
MRSA:	2.35	/	--
MRSE:	2.35	/	--
VRE:	3.13	/	--



24

CLogP: 5.37
p*K*a: 6.89

	MIC	/	MBEC (μM)
MRSA:	0.30	/	75
MRSE:	1.17	/	--
VRE:	2.35	/	--

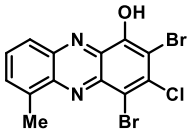
KEY:

Increased Potency

MIC (μM)	MBEC (μM)
< 0.10	< 1
0.10 - 0.59	1 - 4.69
1.17 - 3.13	6.25 - 25
4.69 - 6.25	37.5 - 75
12.5 - 50	100 - 200
> 50	> 200

Most Potent Antibacterial Activities
CLogP > 5.6

p*K*a does not dramaticaly impact activities



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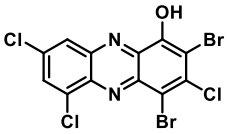
CLogP: 5.62
p*K*a: 6.79

	MIC	/	MBEC (μM)
MRSA:	0.075	/	2.35
MRSE:	0.075	/	0.59
VRE:	0.10	/	0.59



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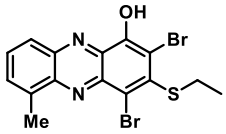
CLogP: 6.55
p*K*a: 6.07

	MIC	/	MBEC (μM)
MRSA:	0.59	/	3.13
MRSE:	0.05	/	0.30
VRE:	0.075	/	0.20



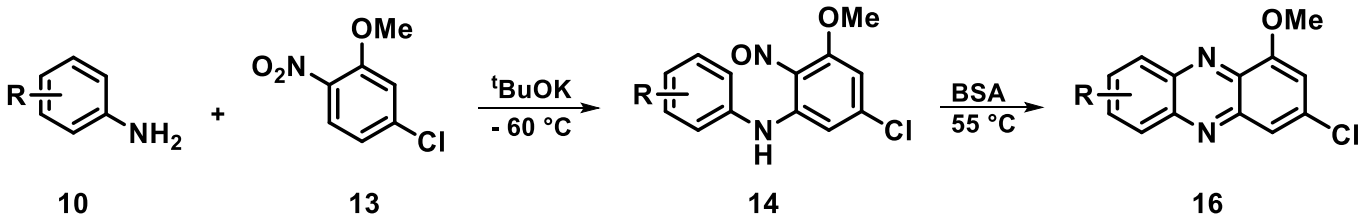
45

CLogP: 6.45
p*K*a: 7.88

	MIC	/	MBEC (μM)
MRSA:	0.30	/	200
MRSE:	0.30	/	4.69
VRE:	0.39	/	3.13

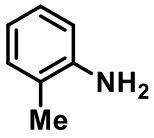
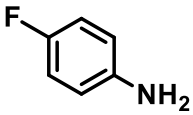
4.) <u>Table S1.</u> Select nitroso intermediates characterized.

Select nitroso intermediates en route to 1-methoxyphenazines were identified using low resolution MS. Comparative yields of 1-methoxyphenazine from cyclization with purified nitroso intermediates and cyclization with crude intermediates are included below.



10 13 14 16

Nitroso Intermediate Confirmed by Low Resolution MS

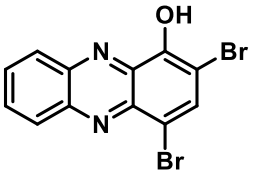
Aniline	Nitroso 14 %Yield	1-OMe HPs (16) Yield Over 2 Steps	
		Purified Nitroso	Crude Nitroso
	84%	73%	80%
	77%	70%	82%

Note: Crude nitroso intermediates were synthesized and divided into two equal portions. One portion was purified by flash chromatography to get the pure nitroso intermediate (confirmed by low resolution MS) before cyclization to the corresponding 1-methoxyphenazine. The other nitroso portion was directly subjected to cyclization crude, similar to the synthetic approach detailed in the manuscript. Comparison of the final yield of 1-methoxyphenazines showed that cyclization of the crude nitroso gave slightly higher yields than with pure nitroso intermediates in our hands.

5.) <u>Table S2.</u> Summary of MIC assays against MRSA clinical isolates. All MIC values are reported in micromolar (μM) concentrations.

Compound	MRSA-1707	MRSA 1	MRSA 2	*S. aureus* 129	*S. aureus* 147	*S. aureus* 138	*S. aureus* 156
3	1.56[b]	1.25	1.88[a]	1.88[a]	1.88[a]	2.5	1.25
29	0.08[a]	0.06[a]	0.12[a]	0.12[a]	0.12[a]	0.08	0.08
34	0.59[a]	1.25	0.94	0.63	0.47[a]	0.94[a]	0.47[a]
45	0.30[a]	0.31	0.63	0.47[a]	0.47[a]	0.47[a]	0.47[a]
Vancomycin	0.39	0.39	0.39	0.39	0.59[a]	0.39	0.39
Methicillin	18.8[a]	37.5[a]	>100	37.5[a]	6.25	37.5[a]	37.5[a]
Ciprofloxacin	0.59[a]	0.78	>100	>100	1.17[a]	>100	0.78[b]

Notes: All MIC values were recorded from a minimum of three independent biological assays. [a] = midpoint of a 2-fold range in observed MIC values. [b] = midpoint of a 4-fold range in observed MIC values. For these MIC assays, HPs were tested at a range of 0.01 - 10 μM, while antibiotics were tested at a range of 0.1 - 100 μM.



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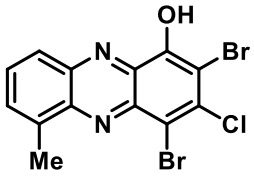

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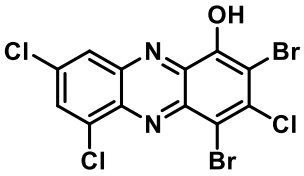

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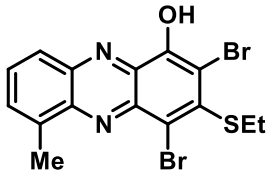

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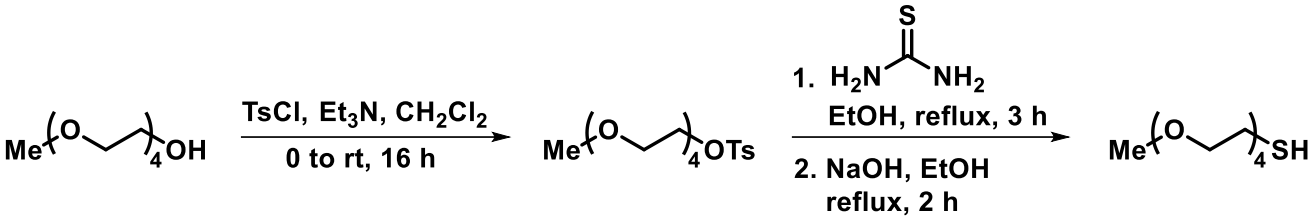
Clinical isolates MRSA 1, MRSA 2, *S. aureus* 129, *S. aureus* 147, *S. aureus* 138 and *S. aureus* 156 were obtained from the Emerging Pathogens Institute (EPI) at the University of Florida and isolated from patients treated at UF Health Shands Hospital (Gainesville, FL). Methicillin has an MIC = 0.78 μM against *S. aureus* strain ATCC 29213, which is susceptible to this antibiotic. MRSA-1707 was obtained from ATCC (MRSA strain BAA-1707) and included in this table as a comparator.

6.) Synthetic Procedures.



59 **60** **61**

Procedures. The title compound was synthesized according to modified literature procedures.[1,2]

First step:

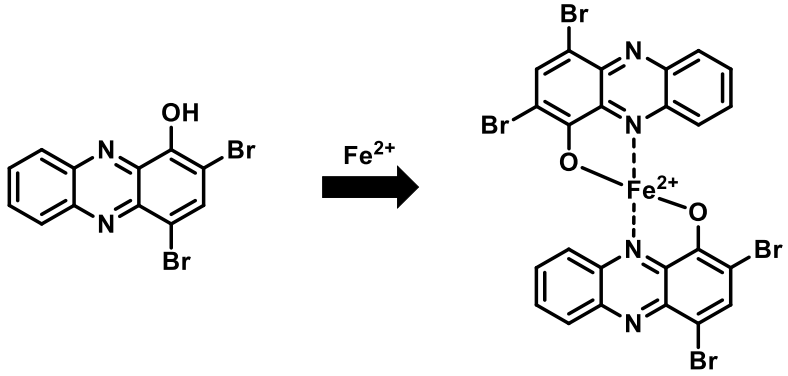
Tetraethylene glycol monomethyl ether (2.18 g, 13.4 mmol) was dissolved in dichloromethane (12 mL) before triethylamine (2.25 mL, 16.02 mmol) was added. Then, a solution of 4-toluenesulfonyl chloride (2.55 g, 13.4 mmol) in dichloromethane (2 mL) was added at 0 °C and the resulting reaction mixture was allowed to stir at room temperature for 3 days before being quenched with brine. The mixture was then transferred to a separatory funnel and extracted with dichloromethane (3 x 30 mL). The organic layers were collected and dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to afford **60** as a colorless oil (100%, 5.5 g). Note: Our ^{1}H NMR align with the previously reported spectra for **60** (CAS: 62921-76-0).[1]

Second step:

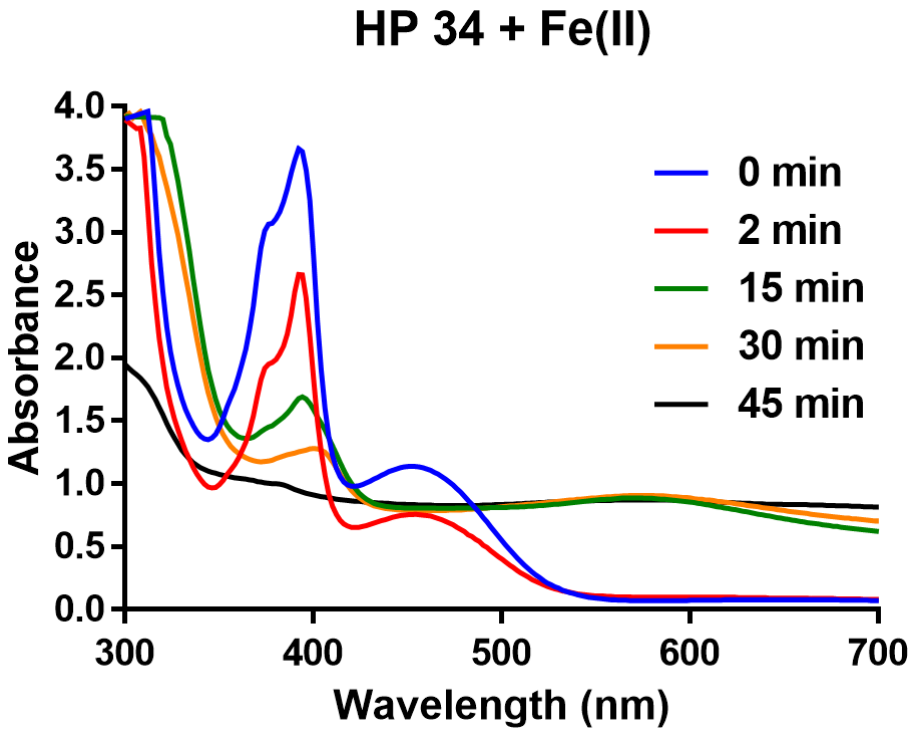
Intermediate **60** (2.90 g, 8.00 mmol) was dissolved in ethanol (5 mL). Then, thiourea (609 mg, 8.00 mmol) was added to the solution along with deionized water (3.5 mL). The resulting reaction mixture was then heated to reflux and allowed to stir for 3 hours. After that time, a solution of sodium hydroxide (384 mg, 9.60 mmol) in water (2.5 mL) was added to the mixture, which was heated to reflux and allowed to stir for an additional 2 hours. When the reaction was complete, the mixture was cooled to room temperature and concentrated to 6 mL *in vacuo*, neutralized with 1 M hydrochloric acid until pH 7, and extracted with dichloromethane. The organic layers were combined and dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The resulting crude oil was purified using flash column chromatography with 4:1 to 1:1 hexanes:ethyl acetate to yield **61** as a colorless oil (82%, 1.38 g). Note: Our ^{1}H NMR align with the previously reported spectra for **61** (CAS: 52190-55-3).[2]

7.) UV-Vis Spectroscopy.

We use UV-Vis to show iron(II) binding, which form a 2:1 HP:iron(II) complex as shown below.[3-5]

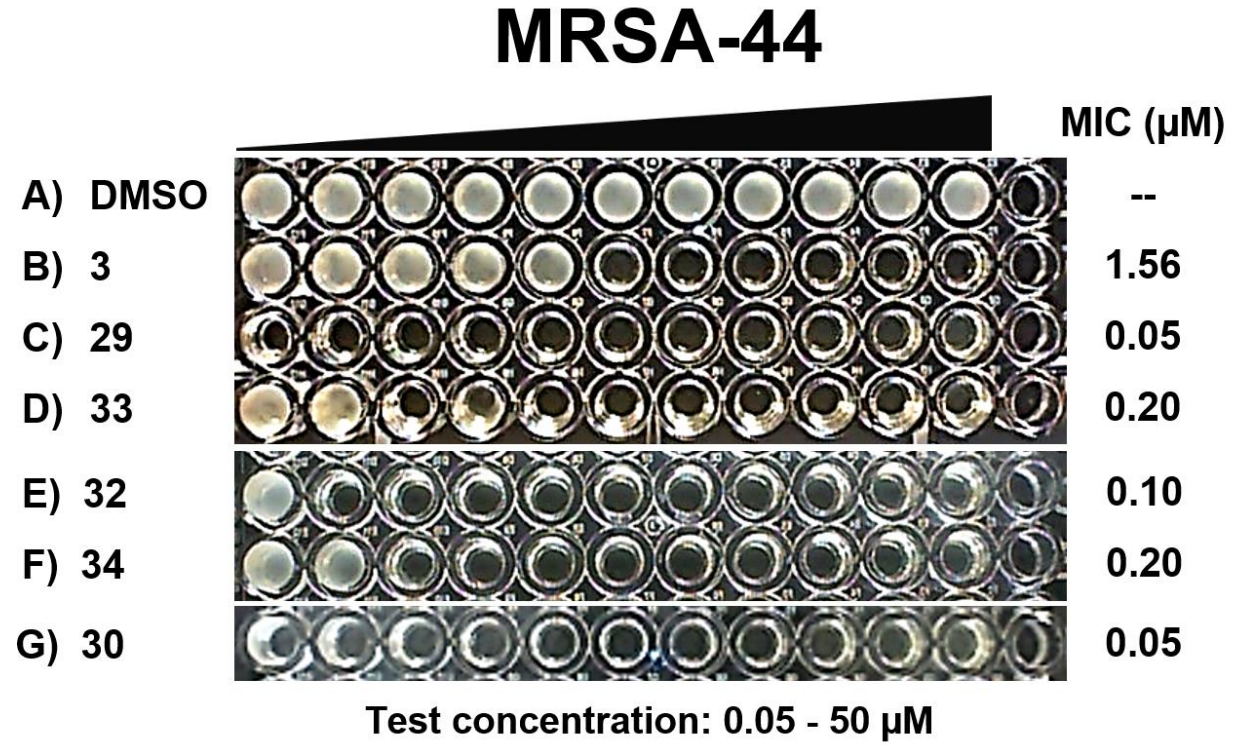


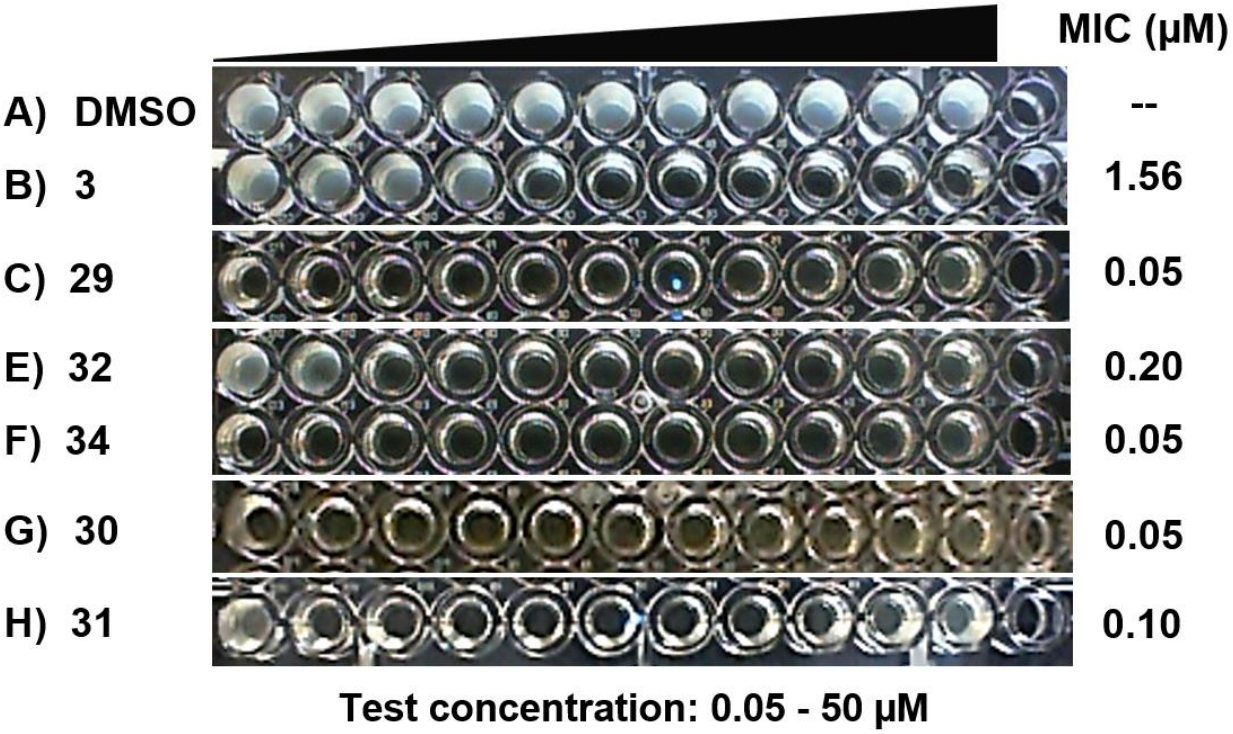
UV-Vis analysis of iron(II) binding with halogenated phenazine **34** (other HPs are presented in the manuscript).



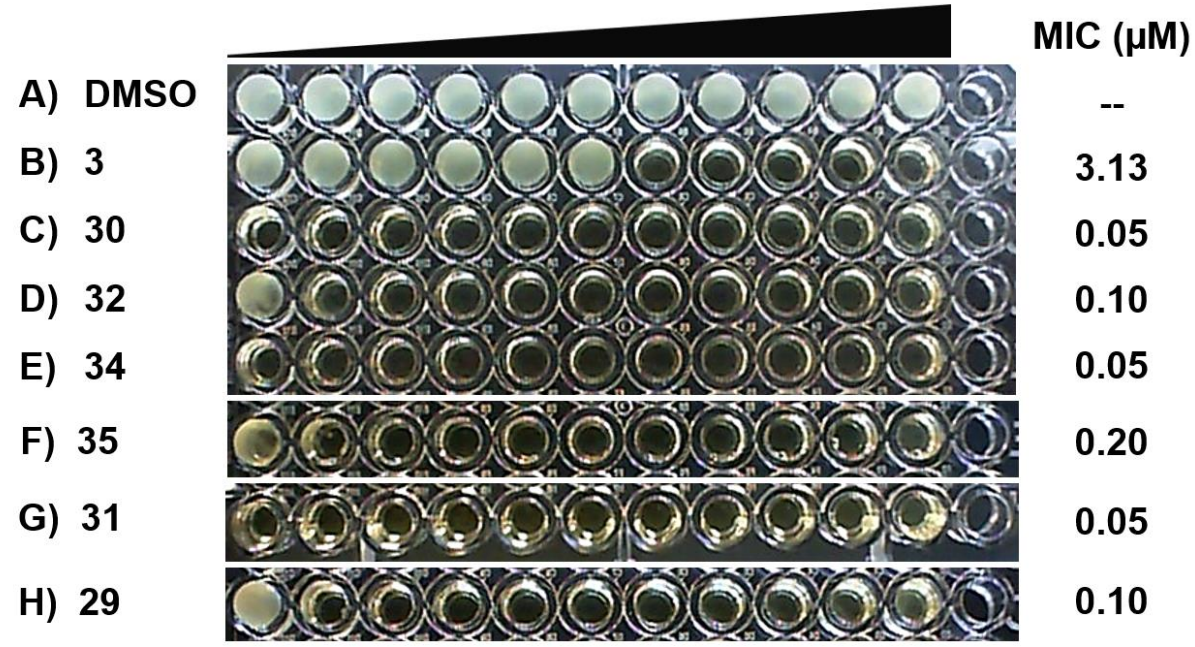
8.) <u>MIC Assays.</u>

Images of representative MIC assays performed during these studies (2-fold serial dilution concentrations).



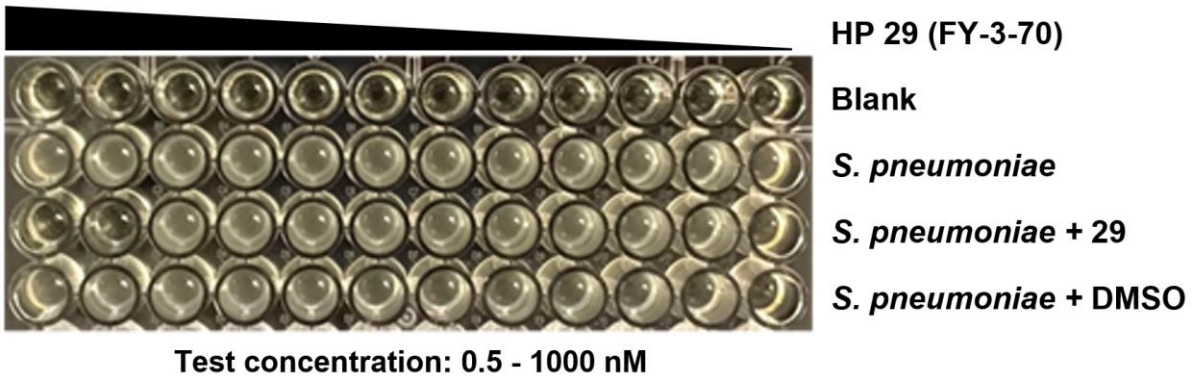


VRE 700221



Test concentration: 0.05 - 50 μM

HP 29 (FY-3-70) against S. pneumoniae 6303

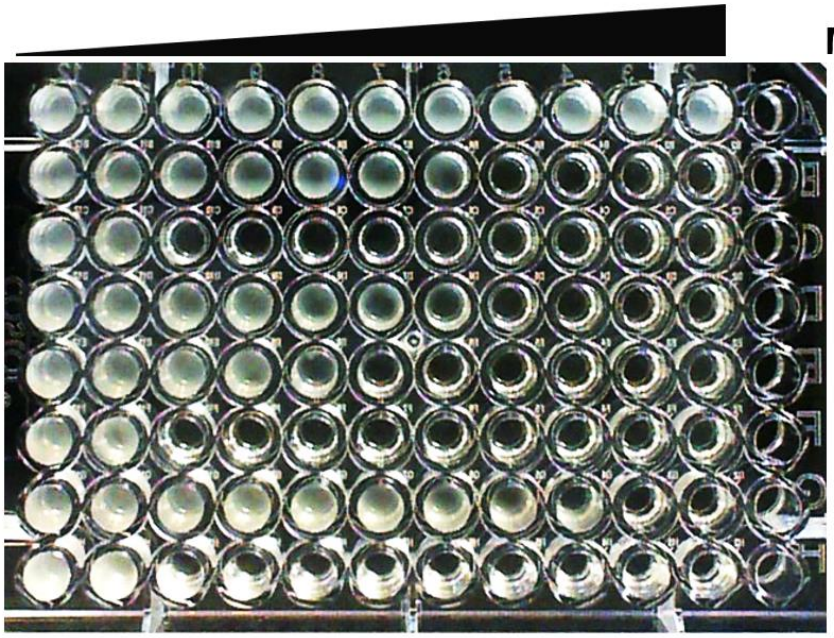


Test concentration: 0.5 - 1000 nM

Evaluation against MRSA clinical isolates from patients at UF Health Shands Hospital (Gainesville, FL)

Vanco. = vancomycin, Cipro. = ciprofloxacin

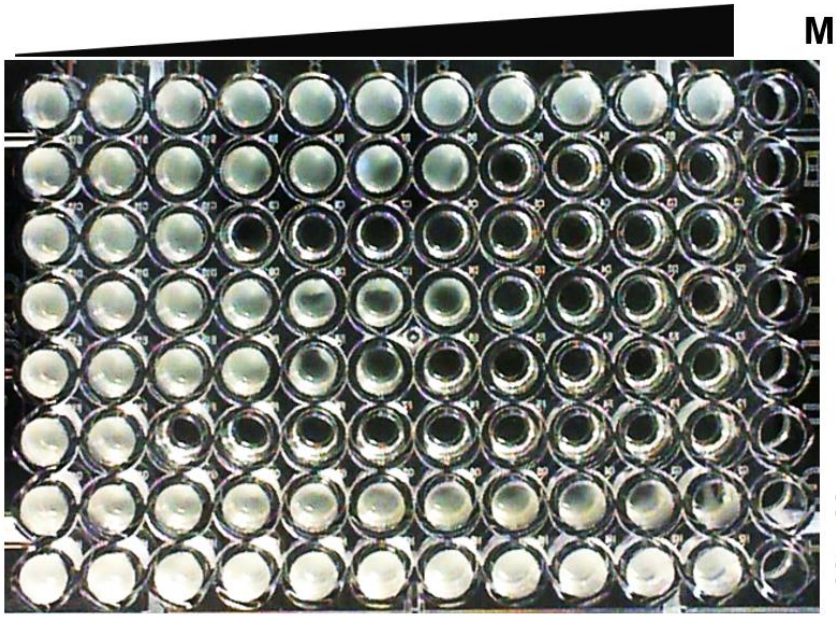
MRSA 1



	MIC (μM)
A) DMSO	--
B) 3	1.25
C) 29	0.04
D) 34	1.25
E) 45	0.31
F) Vanco.*	0.39
G) Methicillin*	25
H) Cipro.*	0.78

Test concentration: 0.01 - 10 μM; 0.1 - 100 μM*

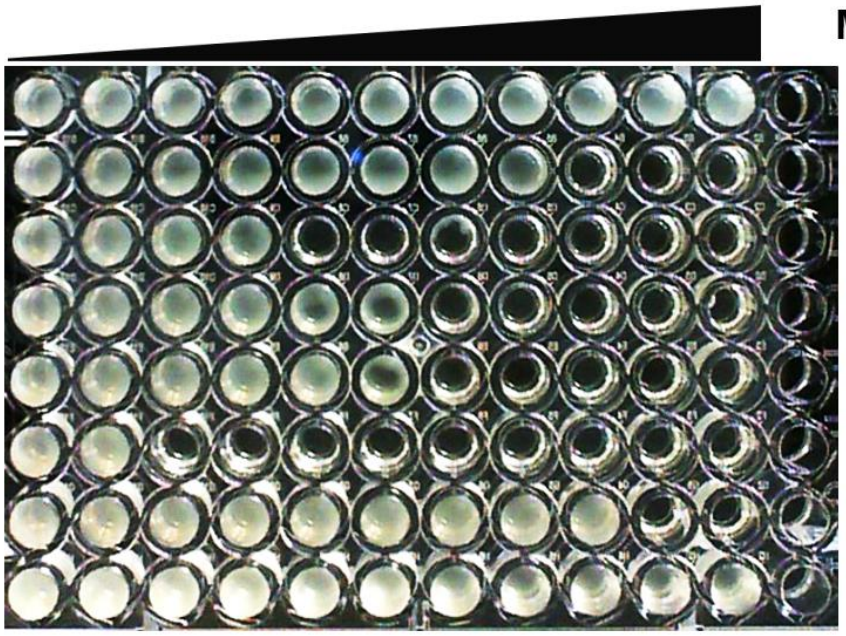
MRSA 2



	MIC (μM)
A) DMSO	--
B) 3	1.25
C) 29	0.08
D) 34	1.25
E) 45	0.63
F) Vanco.*	0.39
G) Methicillin*	> 100
H) Cipro.*	> 100

Test concentration: 0.01 - 10 μM; 0.1 - 100 μM*

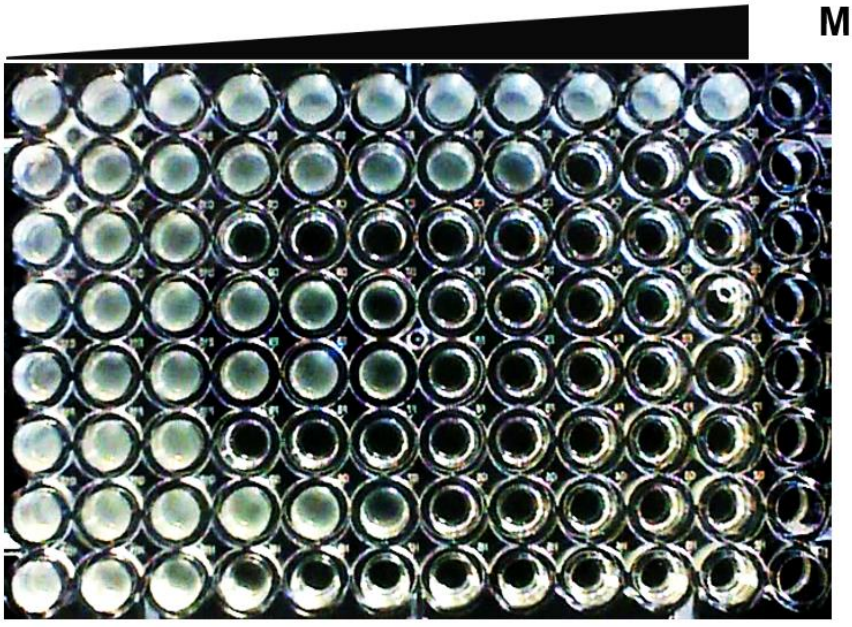
S. aureus 129



	MIC (μM)
A) DMSO	--
B) 3	2.5
C) 29	0.16
D) 34	0.63
E) 45	0.63
F) Vanco.*	0.39
G) Methicillin*	50
H) Cipro.*	> 100

Test concentration: 0.01 - 10 μM; 0.1 - 100 μM*

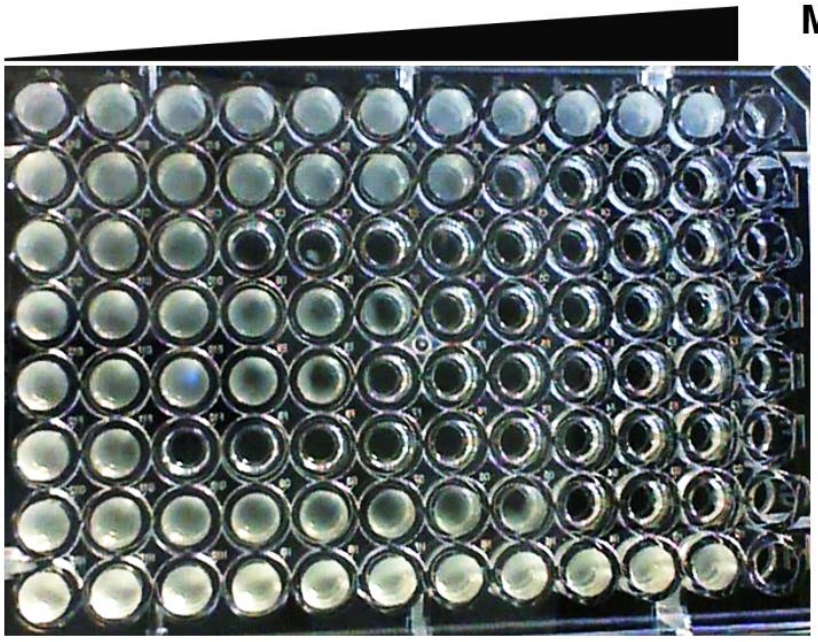
S. aureus 147



	MIC (μM)
A) DMSO	--
B) 3	2.5
C) 29	0.08
D) 34	0.63
E) 45	0.63
F) Vanco.*	0.78
G) Methicillin*	6.25
H) Cipro.*	0.78

Test concentration: 0.01 - 10 μM; 0.1 - 100 μM*

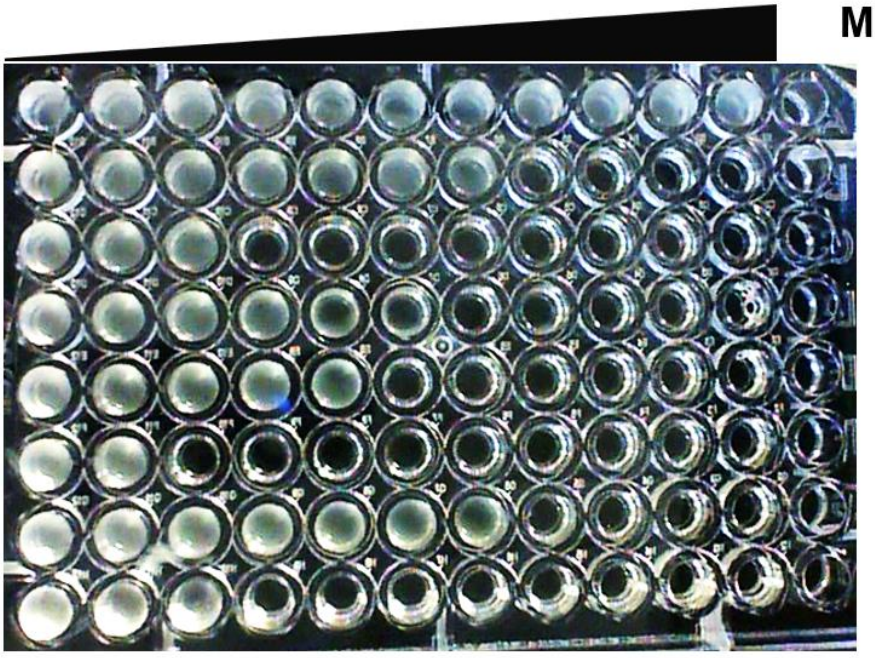
S. aureus 138



	MIC (μM)
A) DMSO	--
B) 3	2.5
C) 29	0.08
D) 34	0.63
E) 45	0.31
F) Vanco.*	0.39
G) Methicillin*	25
H) Cipro.*	> 100

Test concentration: 0.01 - 10 μM; 0.1 - 100 μM*

S. aureus 156

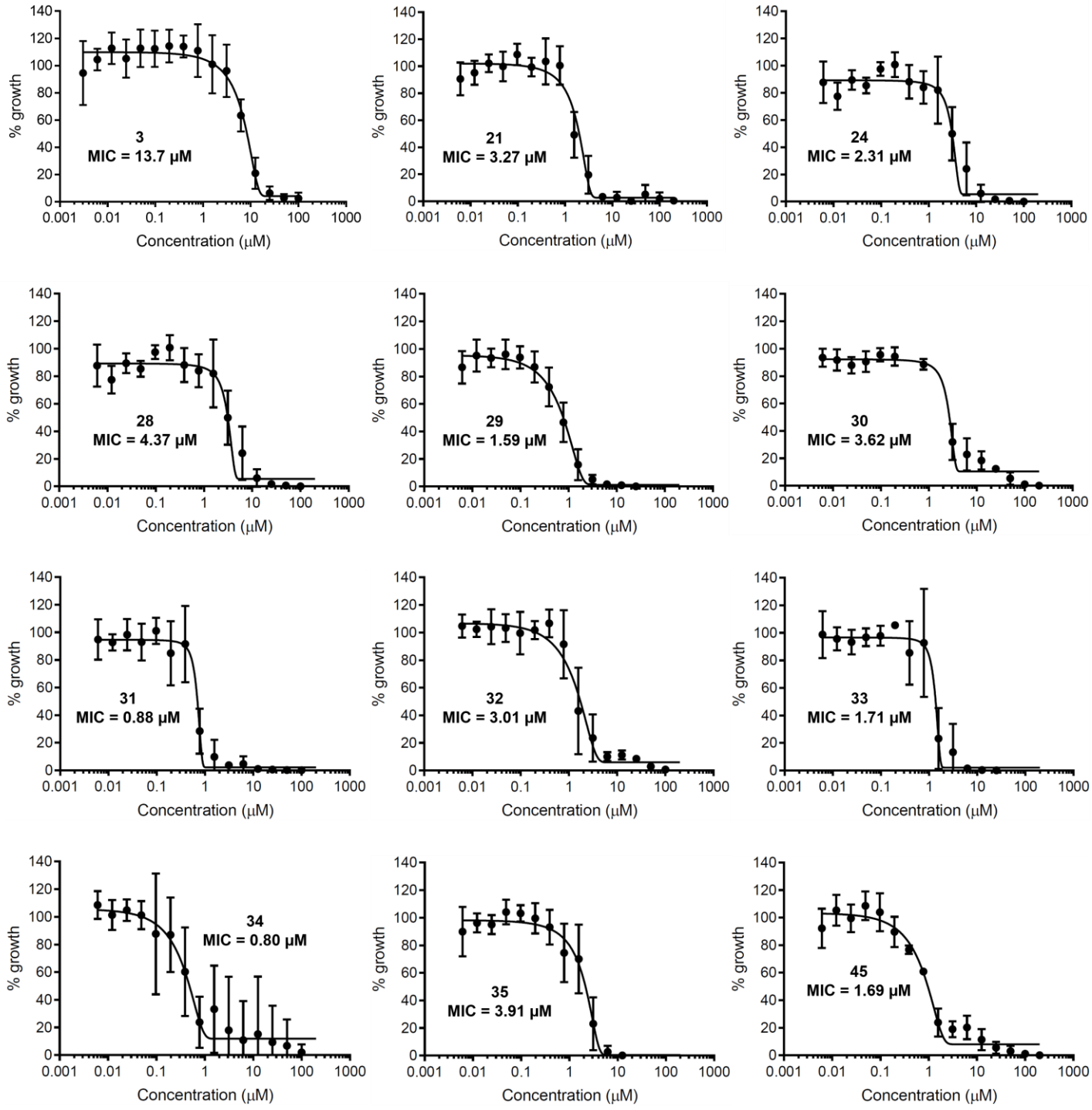


	MIC (μM)
A) DMSO	--
B) 3	1.25
C) 29	0.08
D) 34	0.63
E) 45	0.31
F) Vanco.*	0.39
G) Methicillin*	25
H) Cipro.*	0.78

Test concentration: 0.01 - 10 μM; 0.1 - 100 μM*

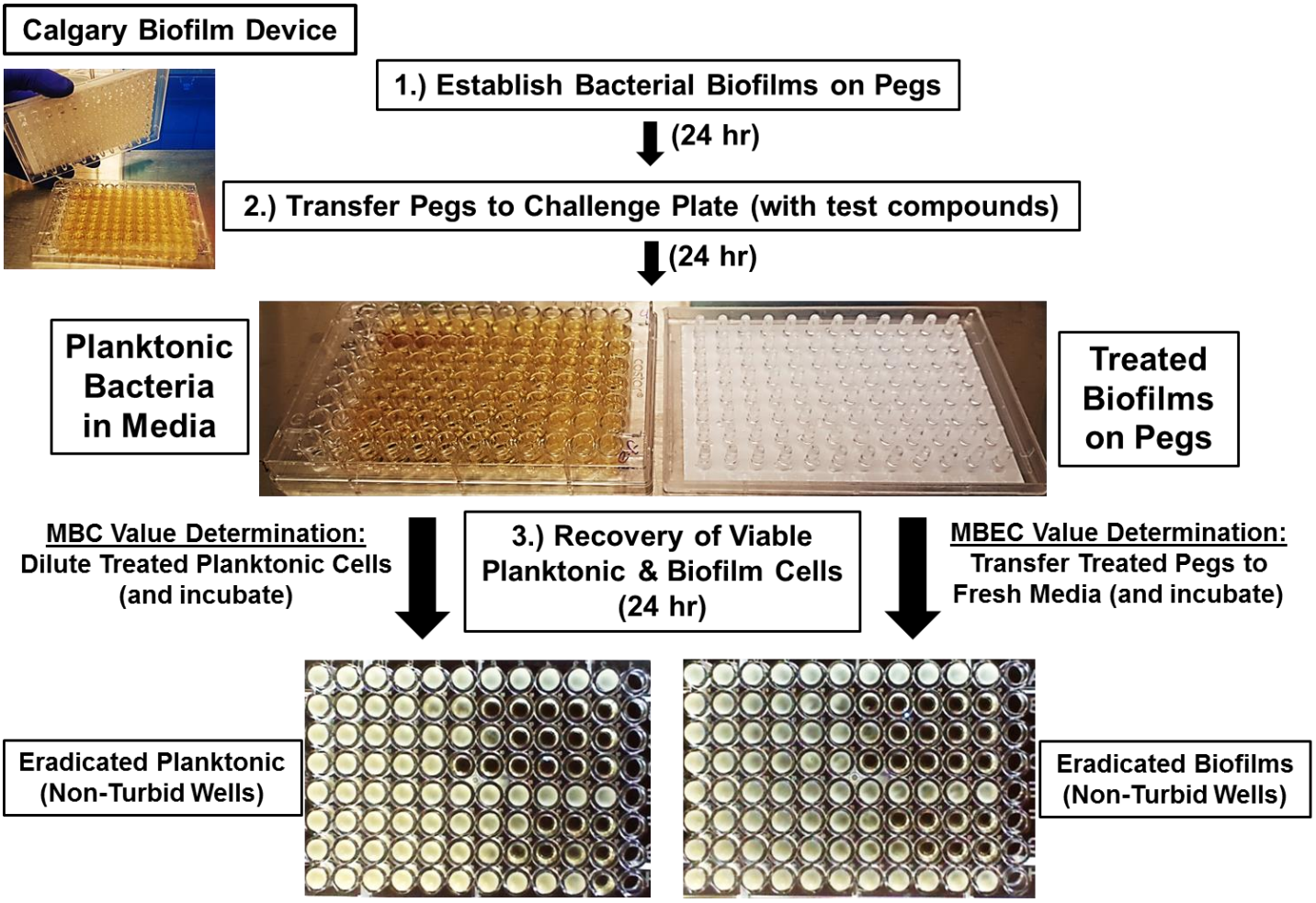
9.) Dose-Response Curves of HPs against *Mycobacterium tuberculosis* CDC1551.

Dose-response curves determined from OD readings of MIC assays performed in 96-well plates.



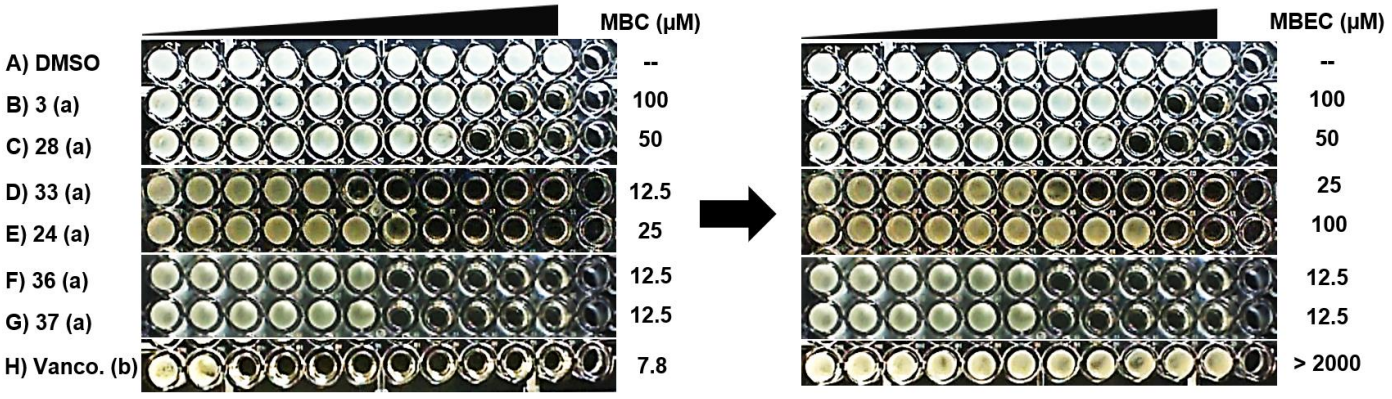
10.) <u>Biofilm Eradication Assays with Calgary Biofilm Devices.</u>

Assay workflow to determine planktonic and biofilm eradication activities.



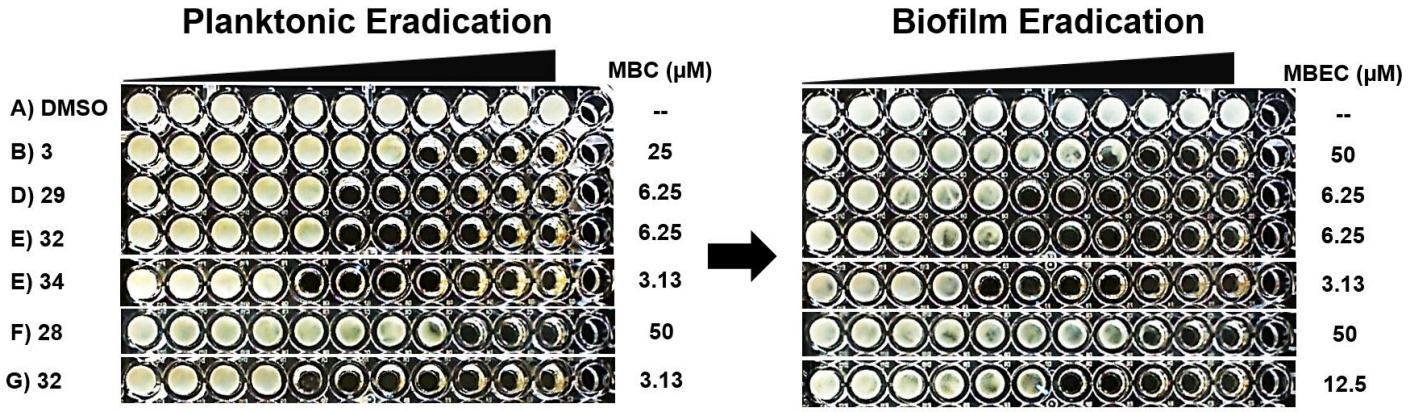
Images of representative CBD assays performed during these studies.

MRSA-1707



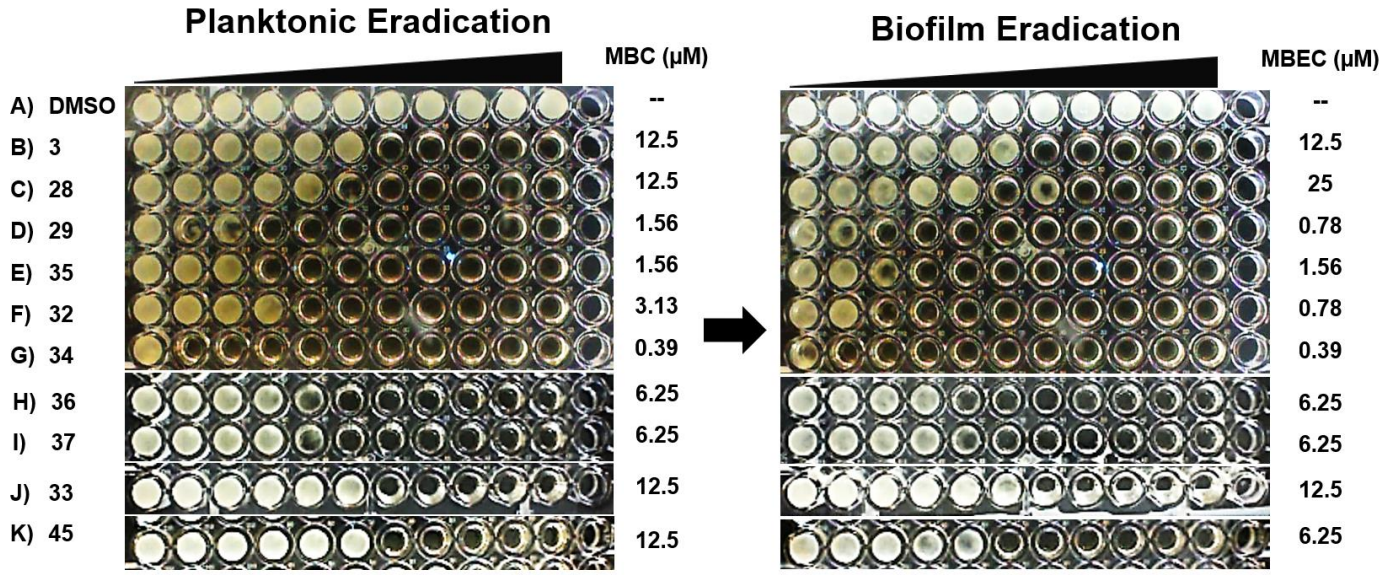
(a) Test concentration: 0.2 – 200 μM; (b) Test concentration: 2-2000 μM; Vanco. (Vancomycin).

MRSA-44



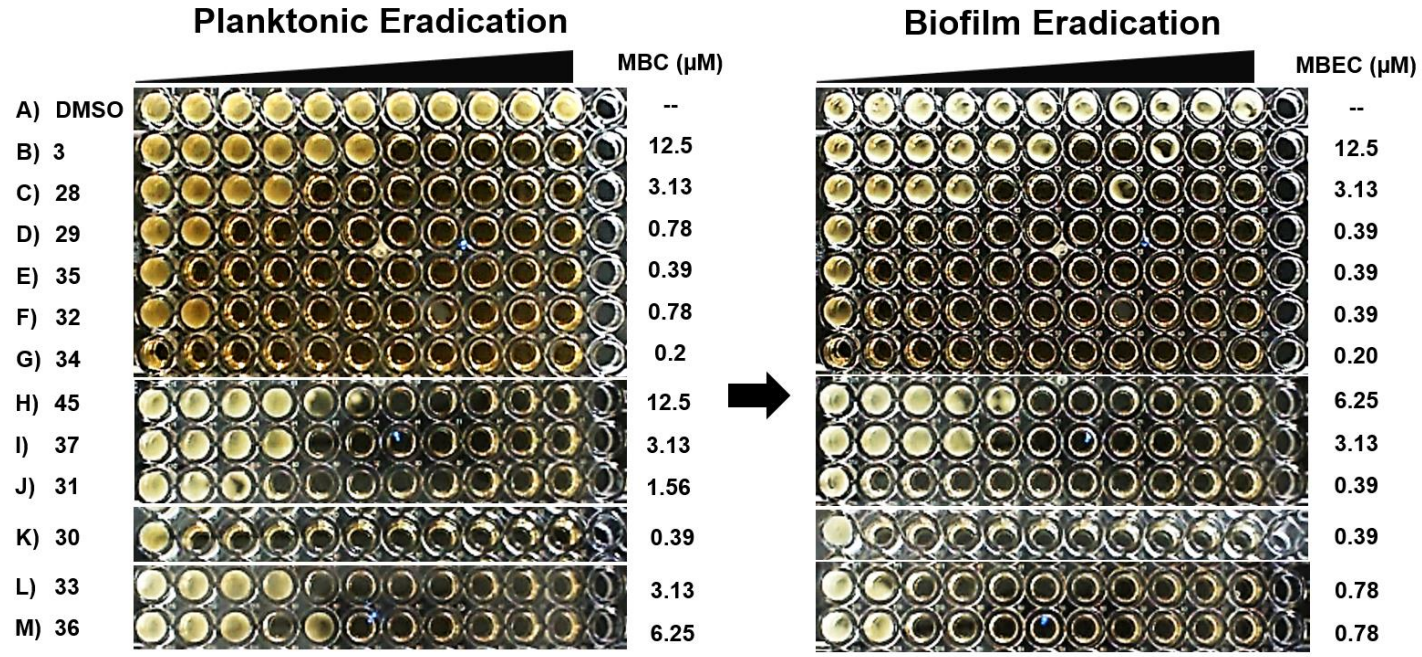
Test concentration: 0.2 – 200 μM

MRSE 35984



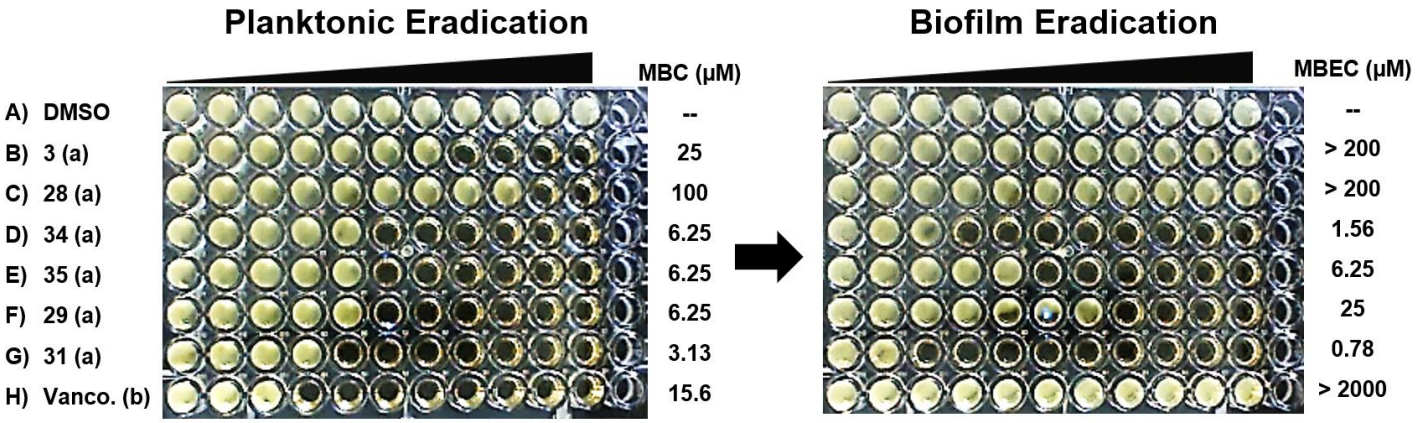
Test concentration: 0.2 – 200 μM

VRE 700221



Test concentration: 0.2 – 200 μM

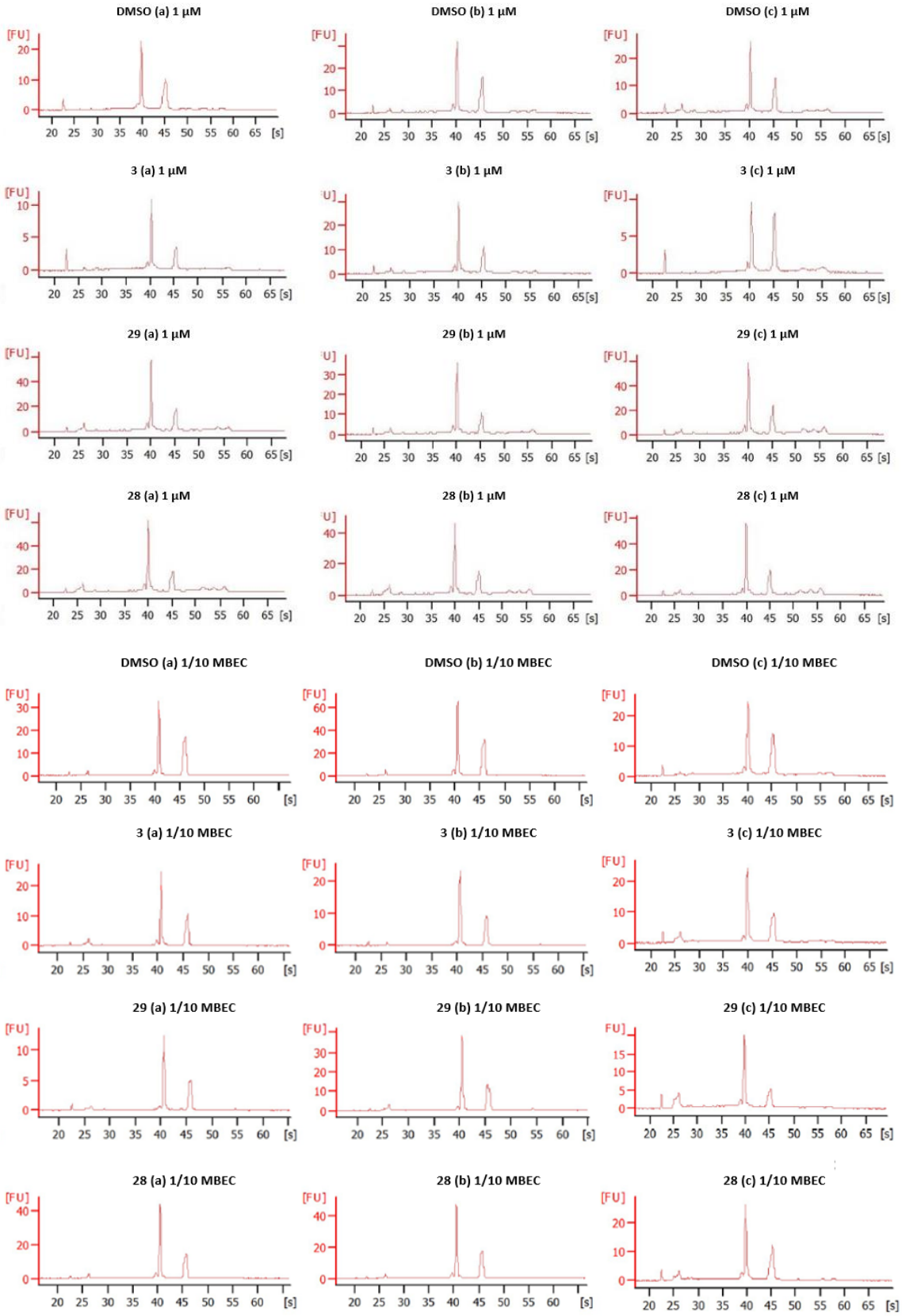
E. faecalis OG1RF



(a) Test concentration: 0.2 – 200 μM; (b) Test concentration: 2-2000 μM; Vanco. (Vancomycin).

11.) Iron Starvation in MRSA Biofilms via RT-qPCR.

Time course quality control data for biofilm experiments obtained from the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida.



Time course RT-qPCR validation of MRSA-1707 biofilm upon treatment with **3**, **28**, and **29** at (A) 1 µM, (B) 1/10 MBEC for 4 hours.

(A) MRSA-1707 biofilms treated with HPs at 1 µM.

Gene	DMSO	3	28	29	Gene Information / Function
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
isdB	1.0 ± 0.02	5.3 ± 4.00	7.6 ± 2.13	7.0 ± 3.31	hemoglobin receptor required for heme iron utilization
sbnC	1.0 ± 0.02	5.4 ± 1.85	10.8 ± 2.16	12.7 ± 2.98	staphyloferrin B (siderophore) biosynthesis; iron acquisition
MW0695	1.0 ± 0.05	4.4 ± 3.10	6.1 ± 4.08	10.2 ± 4.99	hypo. protein, similar to ferrichrome ABC transporters
sfaA	1.0 ± 0.02	3.4 ± 0.50	4.4 ± 0.48	3.9 ± 1.47	staphyloferrin A (siderophore); iron acquisition

(B) MRSA-1707 biofilms treated with HPs at 1/10 MBEC.

Gene	DMSO	3	28	29	Gene Information / Function
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
isdB	1.0 ± 0.01	7.3 ± 1.70	14.4 ± 5.73	8.6 ± 2.04	hemoglobin receptor required for heme iron utilization
sbnC	1.1 ± 0.08	15.5 ± 4.35	14.1 ± 5.86	15.3 ± 4.82	staphyloferrin B (siderophore) biosynthesis; iron acquisition
MW0695	1.0 ± 0.04	1.9 ± 0.66	2.4 ± 1.84	1.9 ± 0.87	hypo. protein, similar to ferrichrome ABC transporters
sfaA	1.0 ± 0.02	3.2 ± 1.06	2.9 ± 1.45	3.5 ± 2.66	staphyloferrin A (siderophore); iron acquisition

Primers used for qPCR validation experiments.

Gene	Symbol	Forward primer	Reverse primer
MW0091	*sbnC*	TCCGGTACATCCTTGGCAAT	GACATGGTTATACGGTGCGC
MW1011	*isdB*	CCAGCAGCAAAAGCCACTAA	CGAGAGTTTGGTGCGCTATG
MW0695	n.a.	CTGGAACGATGGAATGGGCT	CGTGCGCAATGAGATGAAGG
MW2106	*sfaA*	GACGATGTGTGCCATTGGTG	TTGTCGTACGTGCCCATTGA
MW1668	*ptaA*	TCCTAGCGAGTTCAGTTGCA	CCAATGGAATGTAGCTGCGA

Notes: Primers were designed using OligoPerfect Primer Designer (Thermofisher). During these studies, *ptaA* was used as the housekeeping gene for MRSA-1707 (*S. aureus* MW2) biofilms.

Materials used added to a 1.5 mL Eppendorf tube on ice.

Material	Amount / Reaction (20 µL)	Triplicate + Excess (88 µL)
SYBR	10 µL	44 µL
Primer (5 nM)_forward	1.5 µL	6.5 µL
Primer (5 nM)_reverse	1.5 µL	6.5 µL
Rt enzyme	0.16 µL	0.704 µL
RNA	30 ng	132 ng
Water	20 µL	88 µL

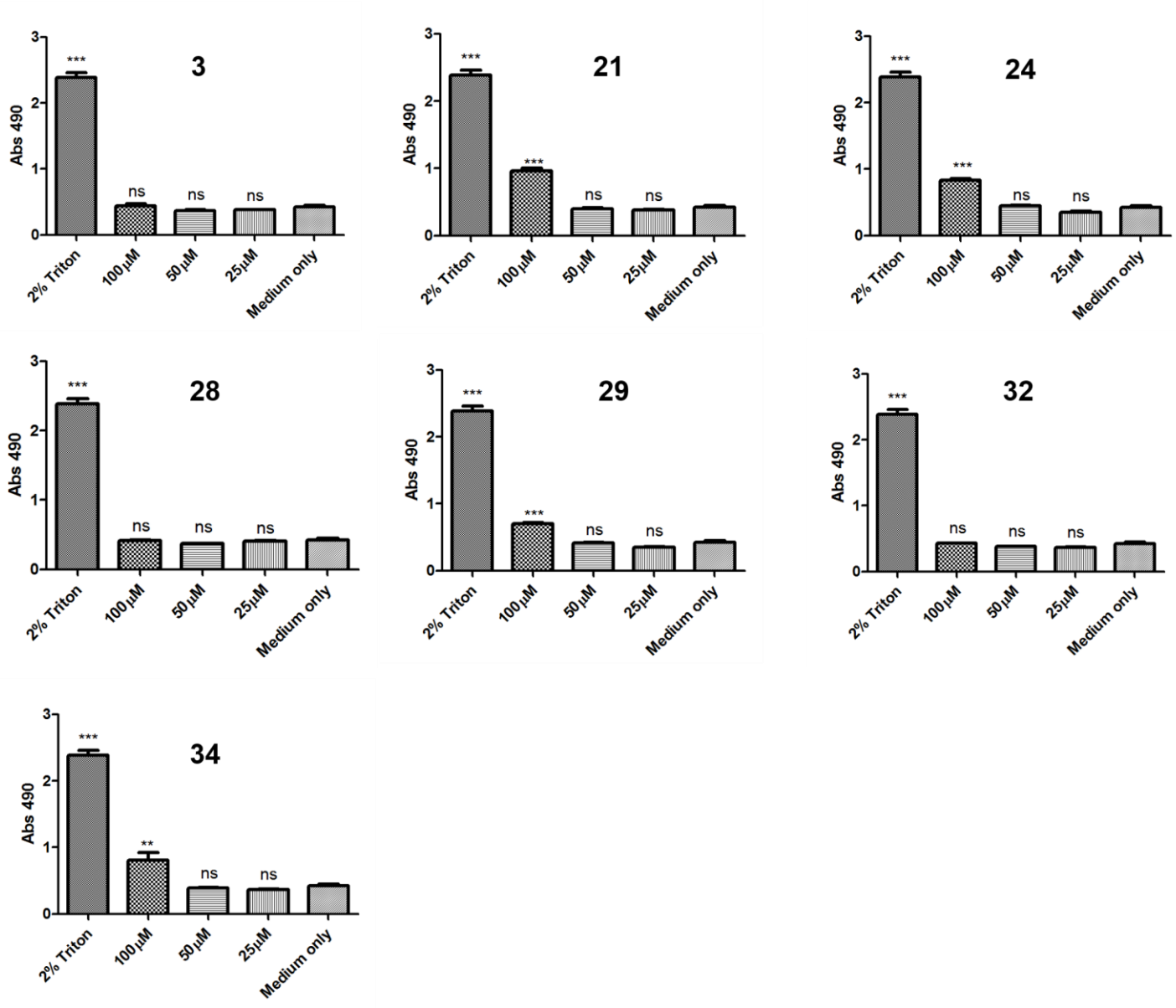
Summary of the RNA concentrations and the RIN (RNA Integrity Number) numbers obtained from the RNA samples (independent experiments) isolated from MRSA-1707 biofilms.

Sample	1 μM		1/10 MBEC	
	[ng / μL]	RIN	[ng / μL]	RIN
DMSO (a)	133.8	8.6	141.467	9.0
DMSO (b)	135.64	8.3	193.387	10.0
DMSO (c)	97.64	8.3	135	8.2
3 (a)	52.84	7.7	112.507	9.0
3 (b)	186.52	7.9	93.467	9.0
3 (c)	78.2	8.8	116.68	8.1
29 (a)	231.24	8.3	40.587	9.0
29 (b)	214.68	8.0	121.707	10.0
29 (c)	319.08	8.5	102.76	7.4
28 (a)	324.92	8.3	146.667	9.0
28 (b)	260.52	8.2	149.467	9.0
28 (c)	236.2	8.7	111.32	8.2

Data obtained from the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida.

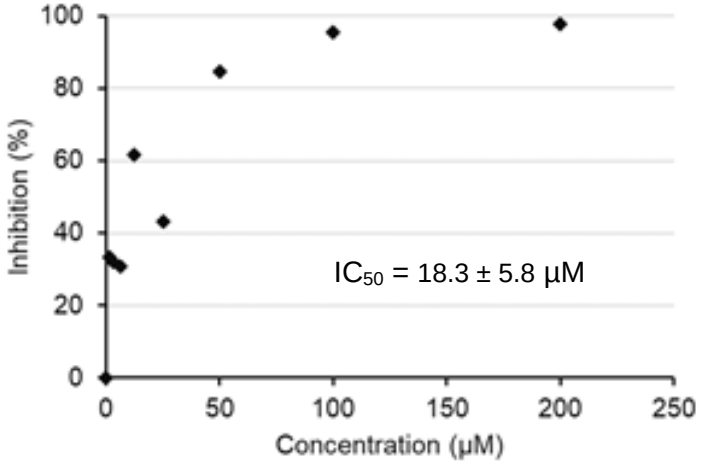
12.) <u>HeLa Cytotoxicity: LDH Release Results.</u>

HP analogues demonstrate low levels of cytotoxicity against HeLa cells at 100 μM.



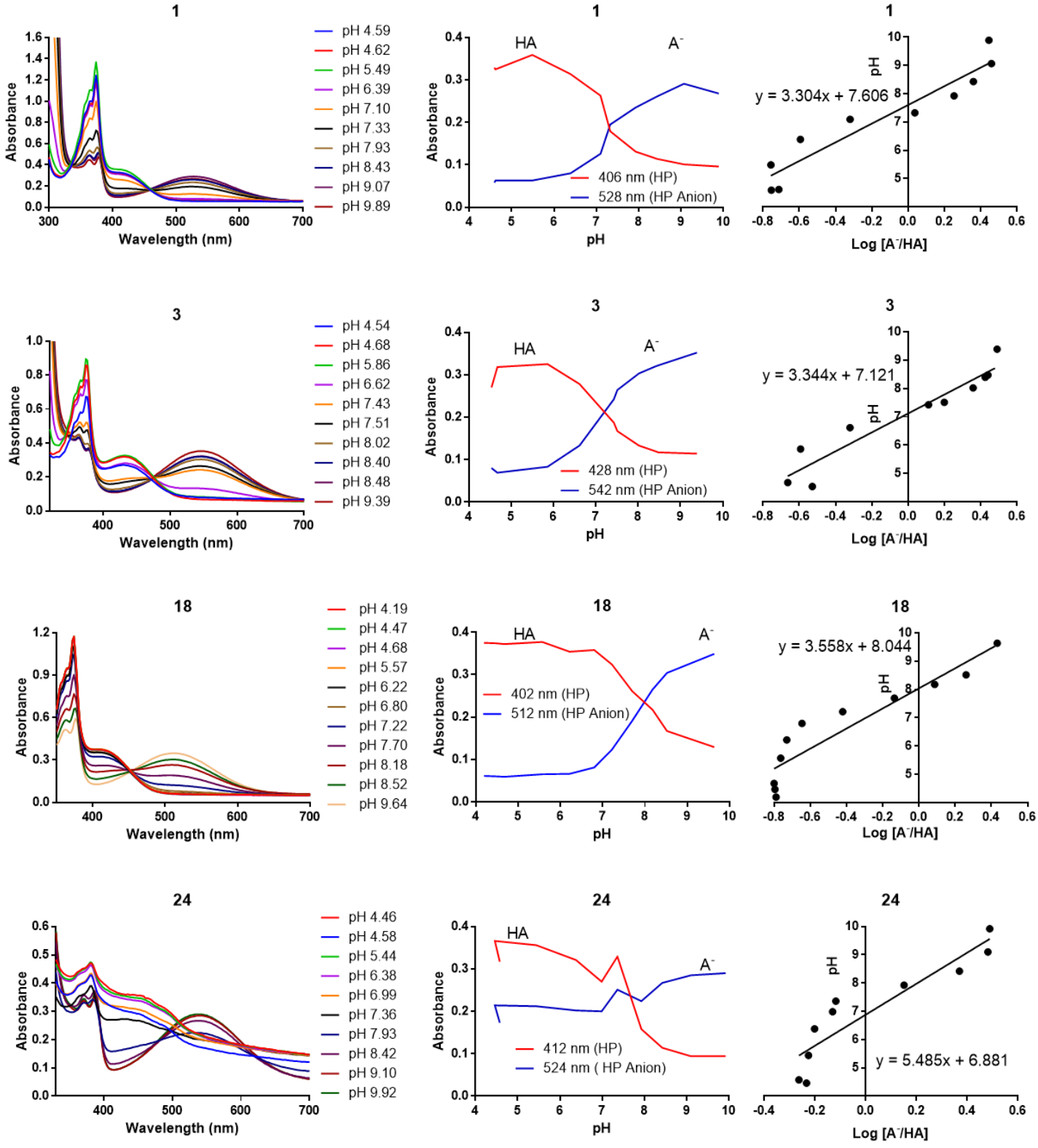
Key: Triton-X = 100% cell death; Medium Only = 0% cell death.

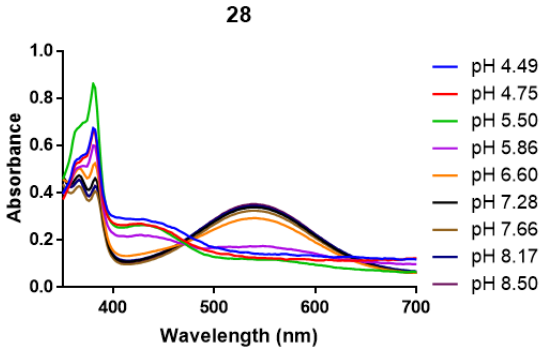
13.) <u>HEK-293 Cytotoxicity of HP 29.</u>

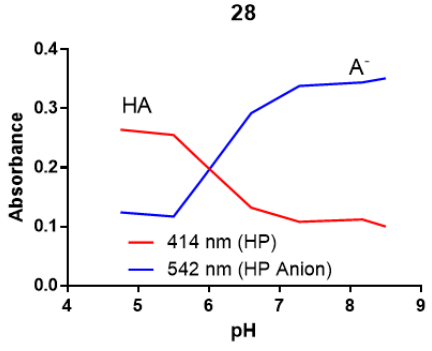


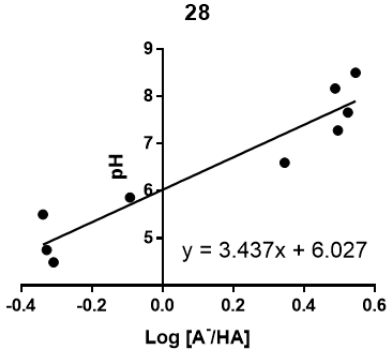
14.) pKa Value Determination for Select HPs.

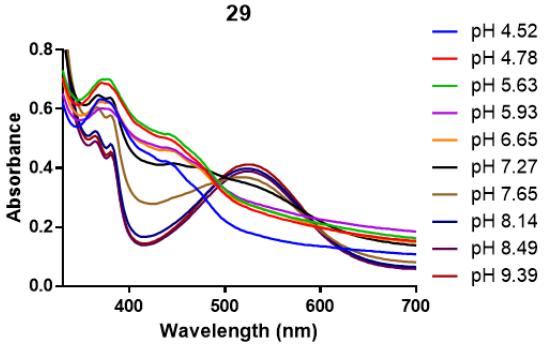
pH-Dependent spectral scan of HPs in buffer; normalized plot of absorbance vs. pH for protonated (HA) and deprotonated (A^-) HPs and plot of pH versus log[A^-/HA] for HPs. A linear regression line is shown with a Y-intercept corresponding to a calculated pKa value.

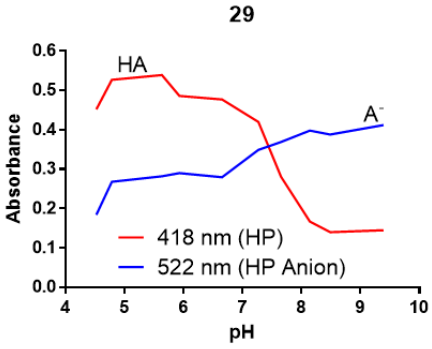


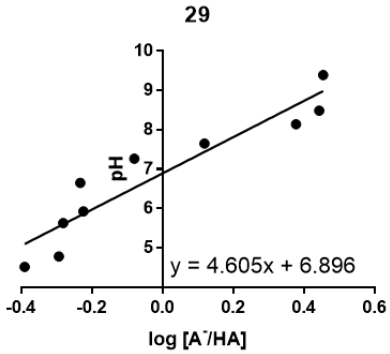

28
Absorbance
Wavelength (nm)
pH 4.49
pH 4.75
pH 5.50
pH 5.86
pH 6.60
pH 7.28
pH 7.66
pH 8.17
pH 8.50

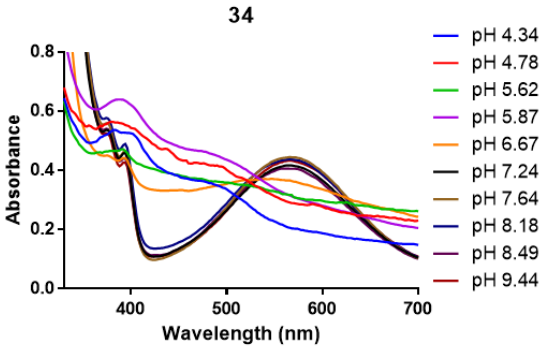

28
HA
A-
414 nm (HP)
542 nm (HP Anion)
Absorbance
pH

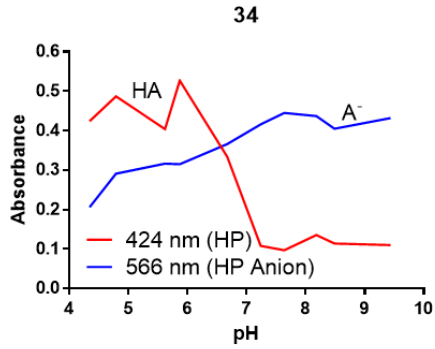

28
y = 3.437x + 6.027
pH
Log [A-/HA]

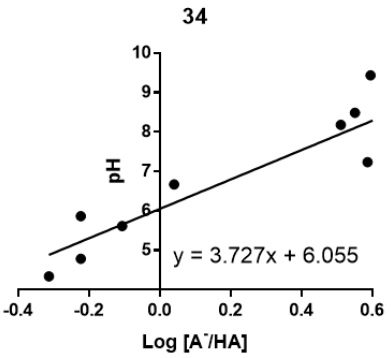

29
Absorbance
Wavelength (nm)
pH 4.52
pH 4.78
pH 5.63
pH 5.93
pH 6.65
pH 7.27
pH 7.65
pH 8.14
pH 8.49
pH 9.39

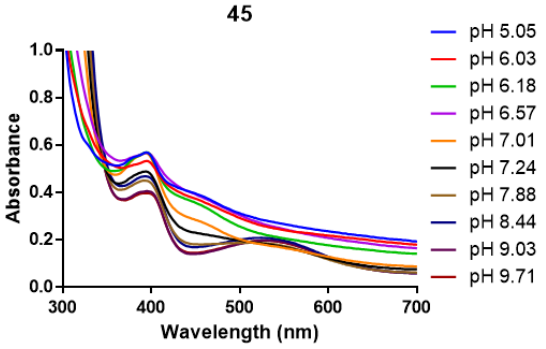

29
HA
A-
418 nm (HP)
522 nm (HP Anion)
Absorbance
pH

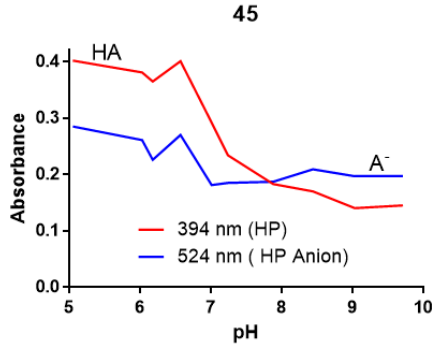

29
y = 4.605x + 6.896
pH
log [A-/HA]

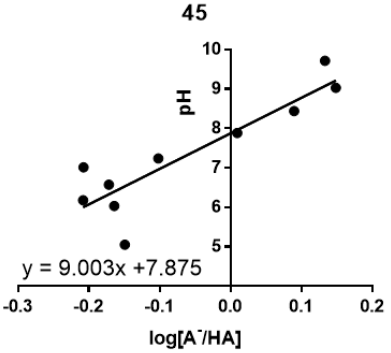

34
Absorbance
Wavelength (nm)
pH 4.34
pH 4.78
pH 5.62
pH 5.87
pH 6.67
pH 7.24
pH 7.64
pH 8.18
pH 8.49
pH 9.44

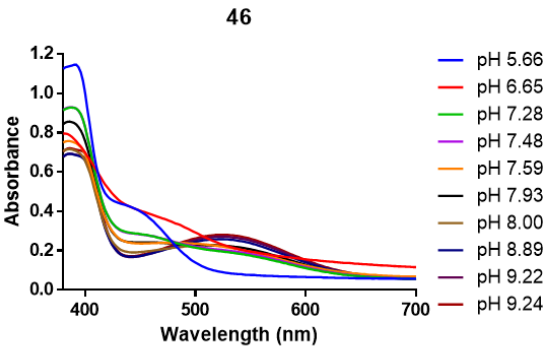

34
HA
A-
424 nm (HP)
566 nm (HP Anion)
Absorbance
pH

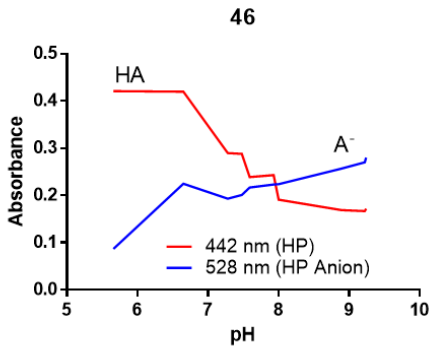

34
y = 3.727x + 6.055
pH
Log [A-/HA]

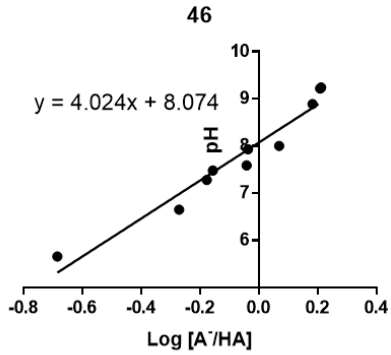

45
Absorbance
Wavelength (nm)
pH 5.05
pH 6.03
pH 6.18
pH 6.57
pH 7.01
pH 7.24
pH 7.88
pH 8.44
pH 9.03
pH 9.71

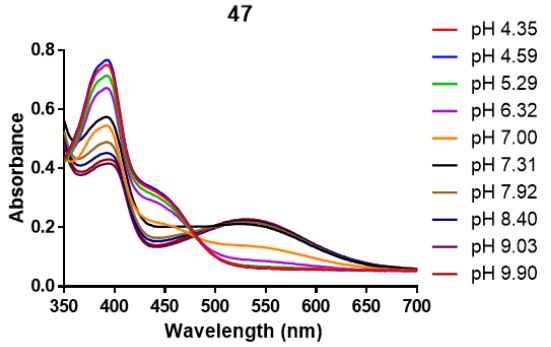

45
HA
A-
394 nm (HP)
524 nm (HP Anion)
Absorbance
pH

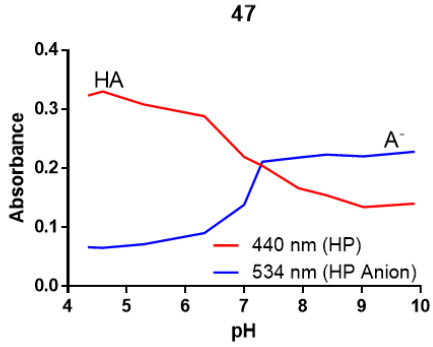

45
y = 9.003x +7.875
pH
log[A-/HA]

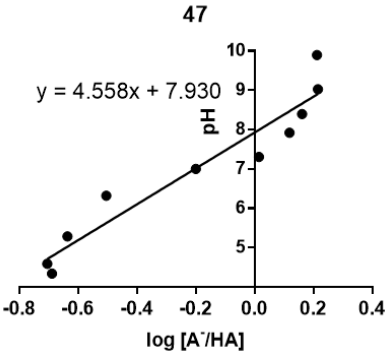

46
Absorbance
Wavelength (nm)
pH 5.66
pH 6.65
pH 7.28
pH 7.48
pH 7.59
pH 7.93
pH 8.00
pH 8.89
pH 9.22
pH 9.24

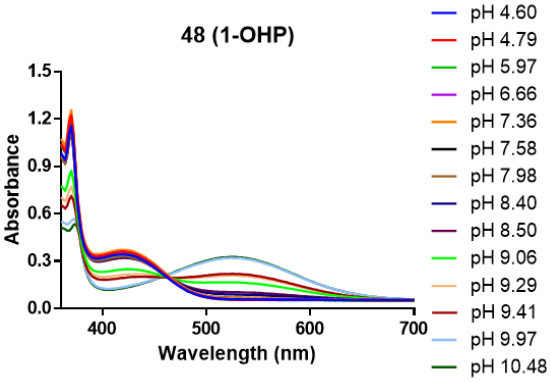

46
HA
A⁻
Absorbance
pH
442 nm (HP)
528 nm (HP Anion)

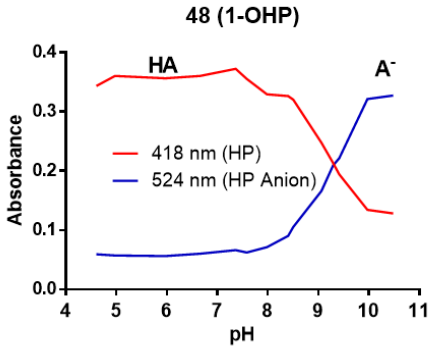

46
y = 4.024x + 8.074
pH
Log [A⁻/HA]

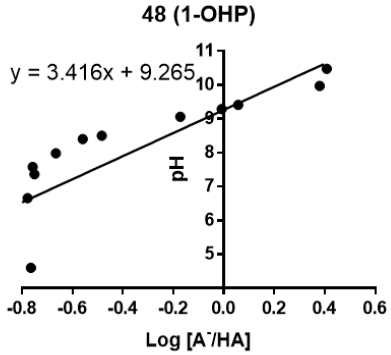

47
Absorbance
Wavelength (nm)
pH 4.35
pH 4.59
pH 5.29
pH 6.32
pH 7.00
pH 7.31
pH 7.92
pH 8.40
pH 9.03
pH 9.90


47
HA
A⁻
Absorbance
pH
440 nm (HP)
534 nm (HP Anion)


47
y = 4.558x + 7.930
pH
log [A⁻/HA]

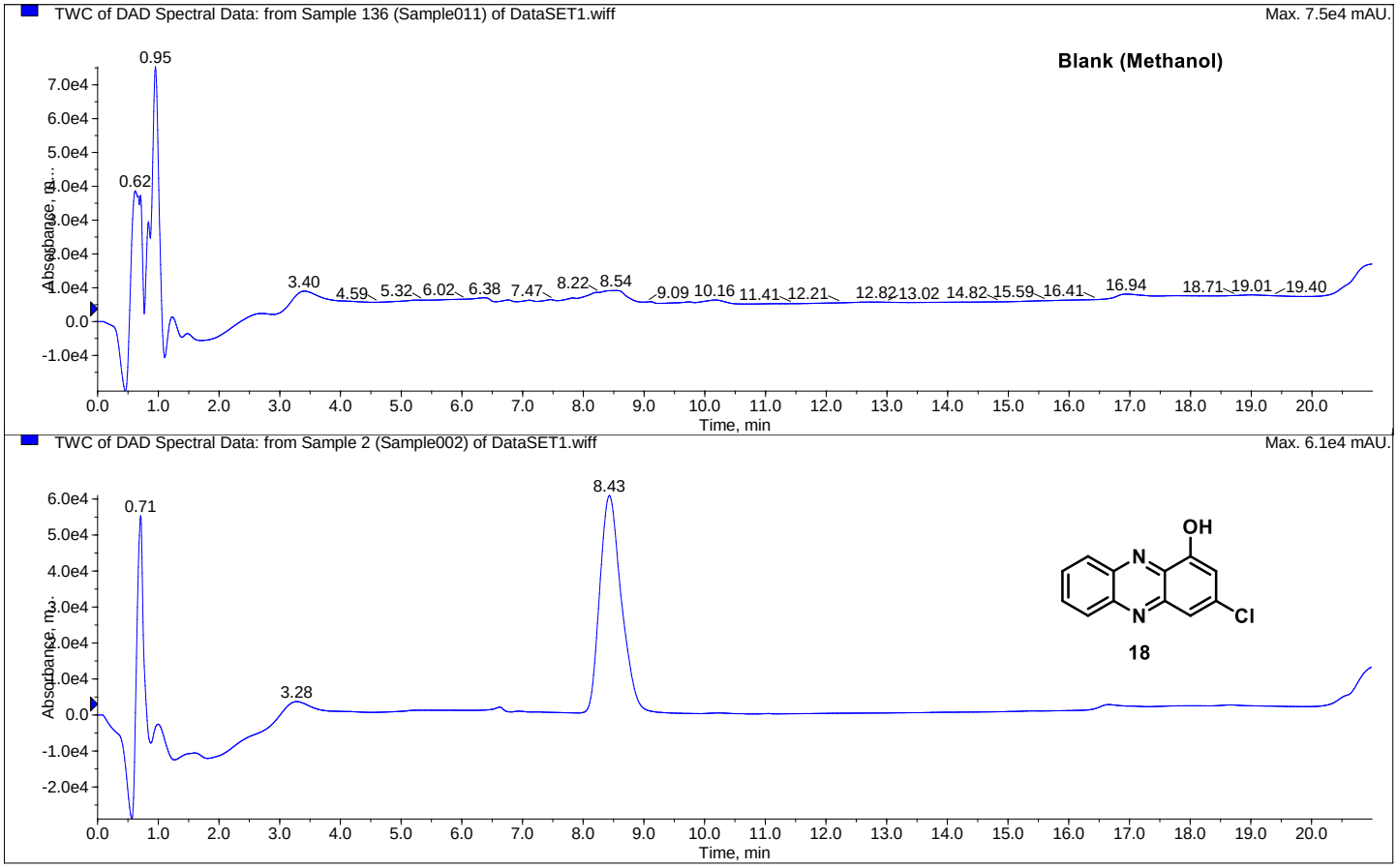

48 (1-OHP)
Absorbance
Wavelength (nm)
pH 4.60
pH 4.79
pH 5.97
pH 6.66
pH 7.36
pH 7.58
pH 7.98
pH 8.40
pH 8.50
pH 9.06
pH 9.29
pH 9.41
pH 9.97
pH 10.48


48 (1-OHP)
HA
A⁻
Absorbance
pH
418 nm (HP)
524 nm (HP Anion)

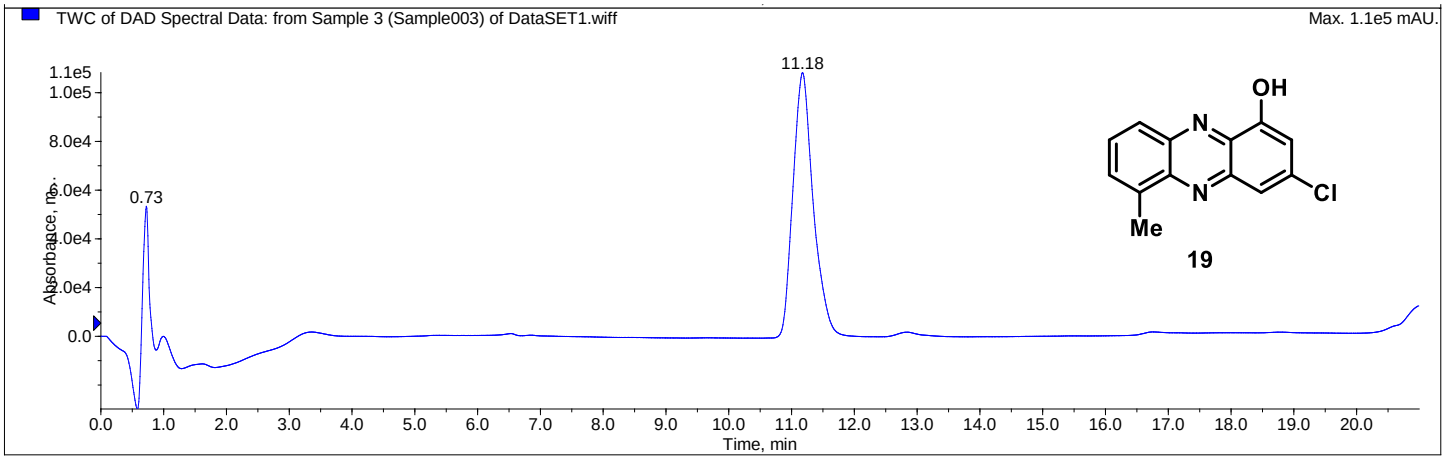

48 (1-OHP)
y = 3.416x + 9.265
pH
Log [A⁻/HA]

15.) <u>Compound Purity Analysis.</u>

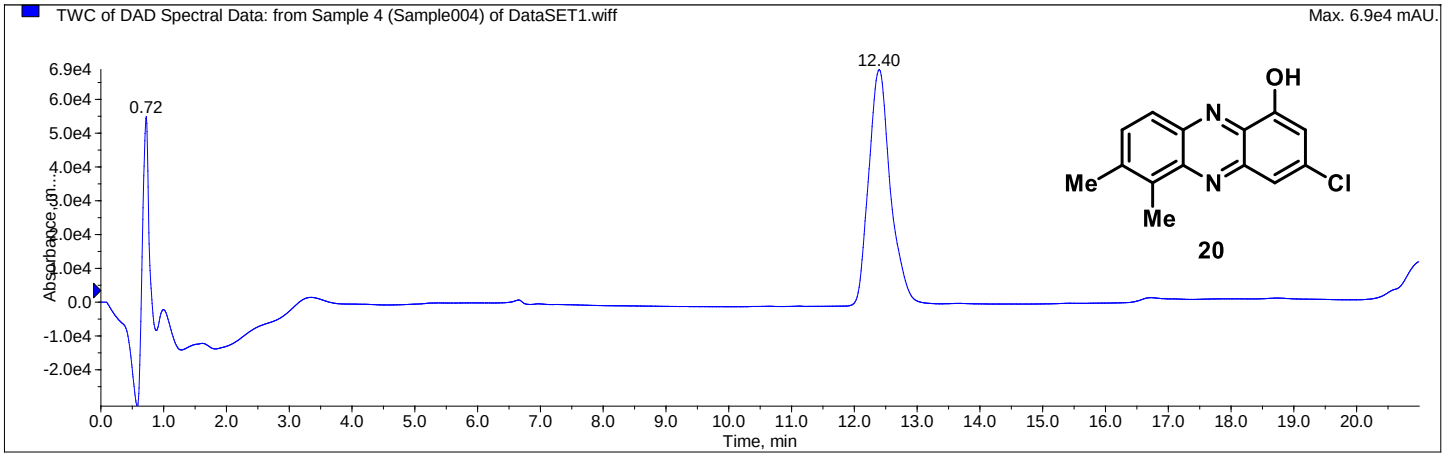
Each HPLC experiment to determine the purity of halogenated phenazine analogues was carried out over 20 minutes. The purity of each compound was determined following the characterization via $^{1}H/^{13}C$ NMR, HRMS and melting point analysis. A blank methanol run is shown below and used to remove background noise from the purity assessment of each compound. Overall, the purity range from these experiments was found to be 95.5 to >99.9% pure based on LC traces.



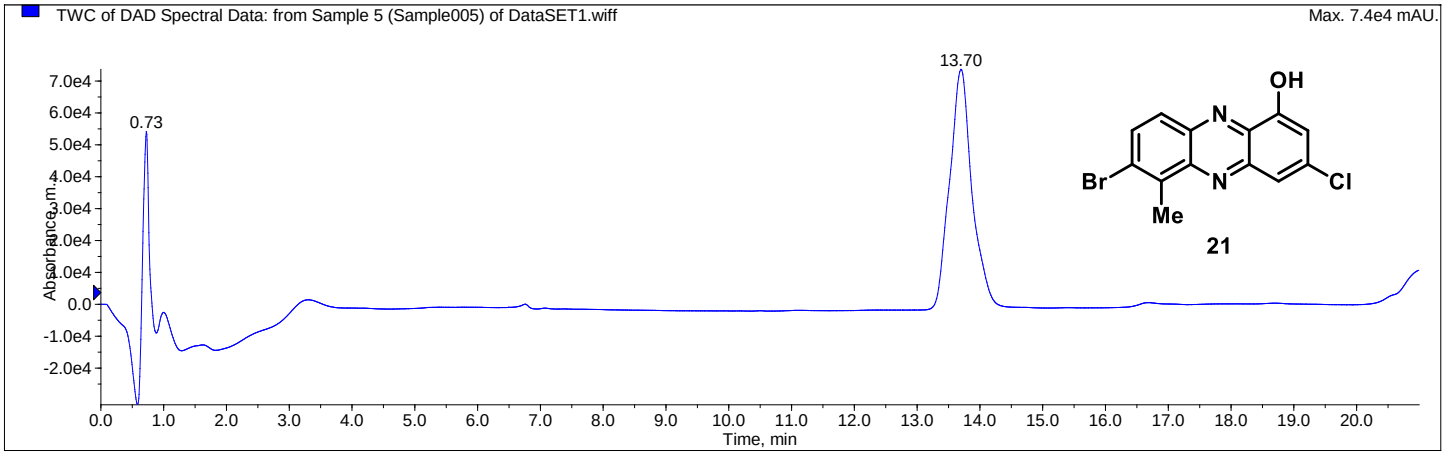
Retention Time (Min)	Peak Area
6.63	17500
6.95	2034
8.43 (**18**)	1480000
Sum of Area	1499534
% Purity	**98.7%**



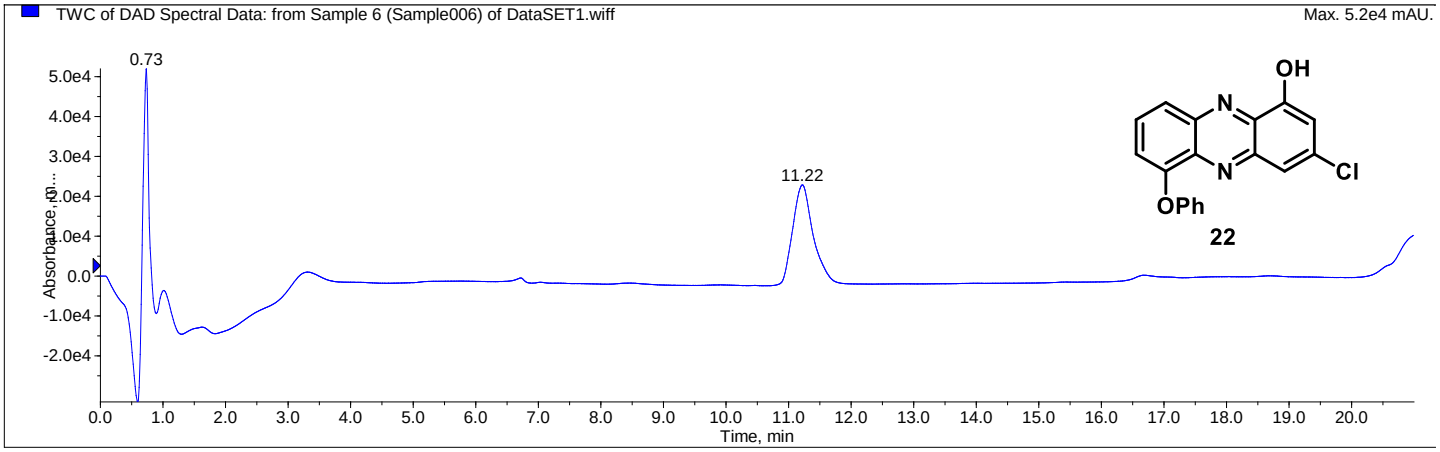
Retention Time (Min)	Peak Area
6.53	11800
11.18 (**19**)	2440000
12.84	44300
Sum of Area	2496100
% Purity	**97.8%**



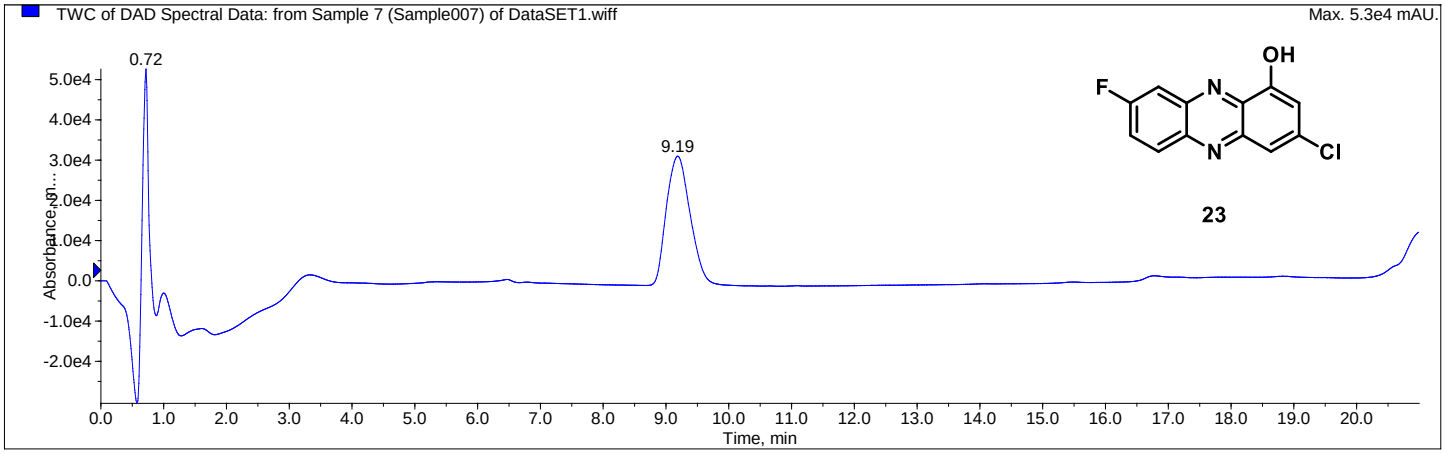
Retention Time (Min)	Peak Area
6.65	15800
12.40 (**20**)	1670000
Sum of Area	1685800
% Purity	**99.1%**



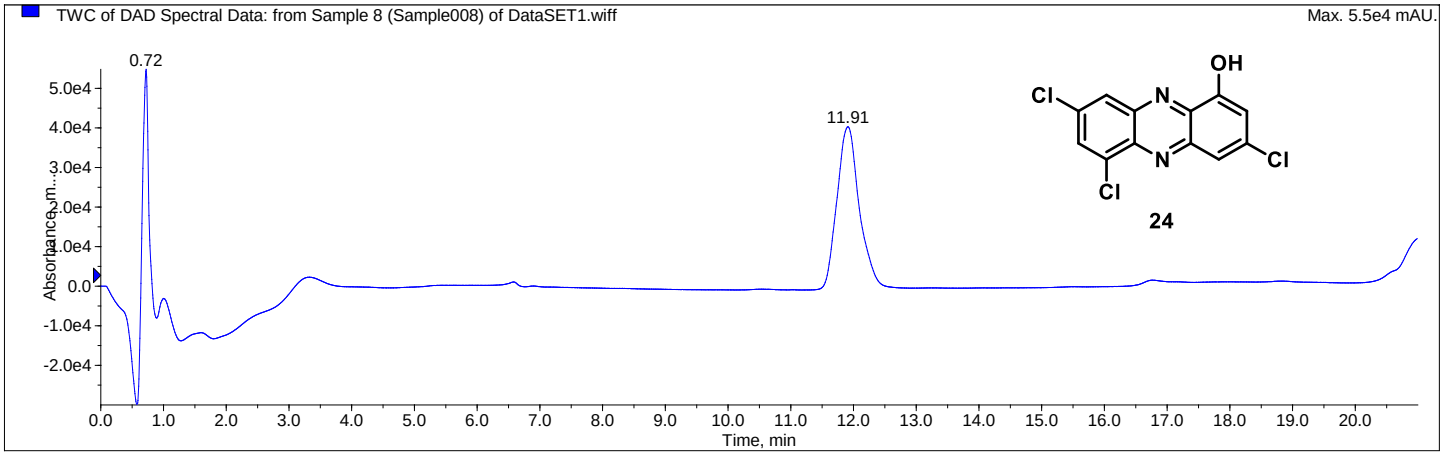
Retention Time (Min)	Peak Area
6.76	13800
13.70 (**21**)	1850000
Sum of Area	1863800
% Purity	**99.3%**



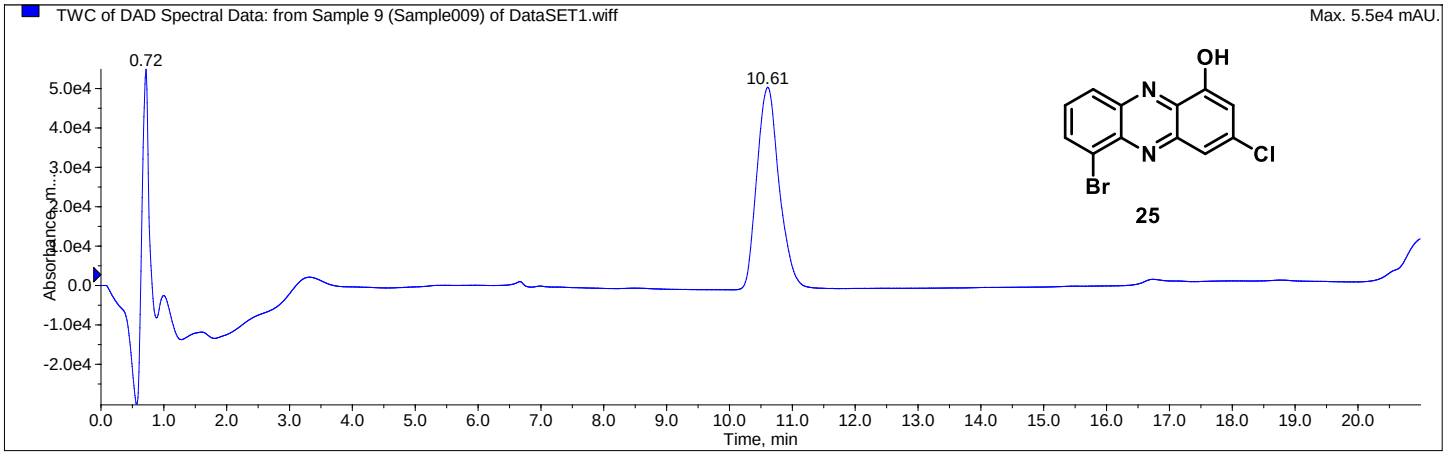
Retention Time (Min)	Peak Area
6.71	13200
7.03	1806
11.22 (**22**)	571000
Sum of Area	586006
% Purity	**97.4%**



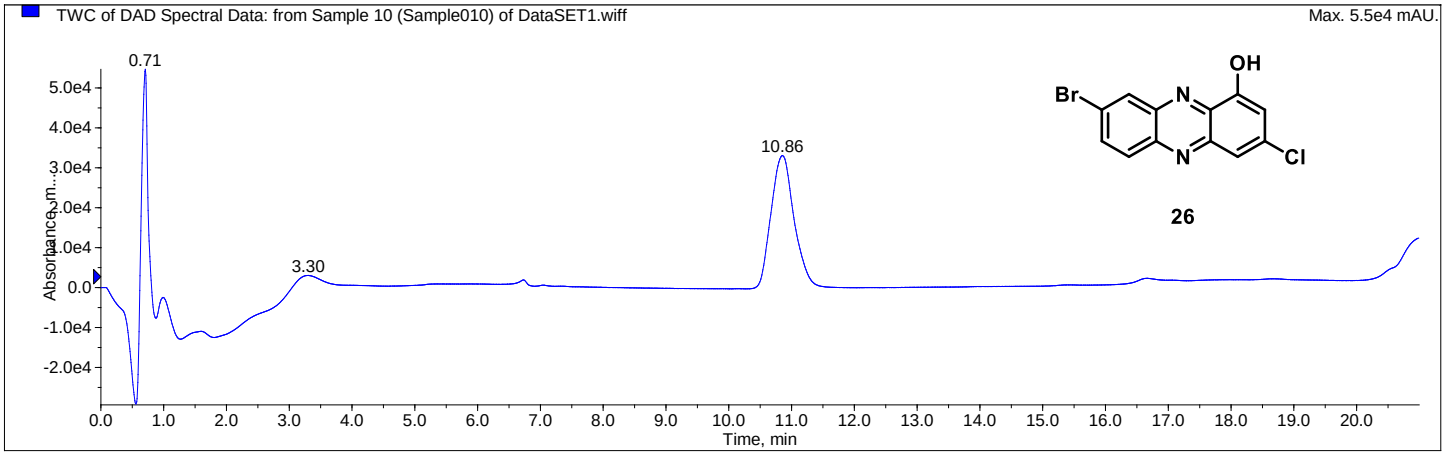
Retention Time (Min)	Peak Area
9.19 (**23**)	842000
10.31	147
Sum of Area	842147
% Purity	**>99.9%**



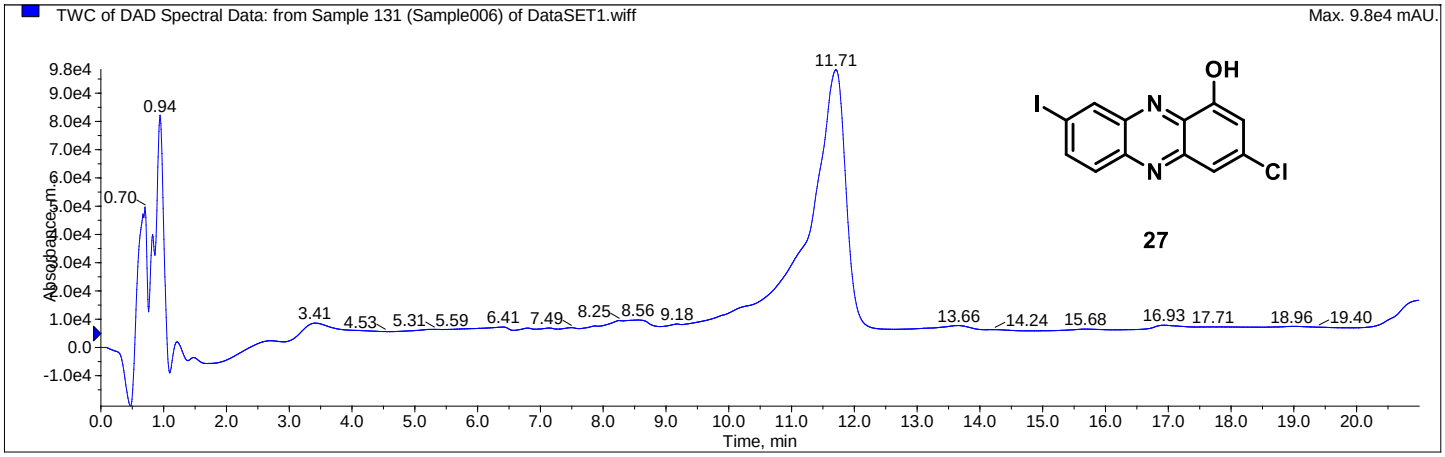
Retention Time (Min)	Peak Area
6.58	17000
6.90	1888
10.62	5083
11.91 (**24**)	1010000
15.52	367
Sum of Area	1034338
% Purity	**97.6%**



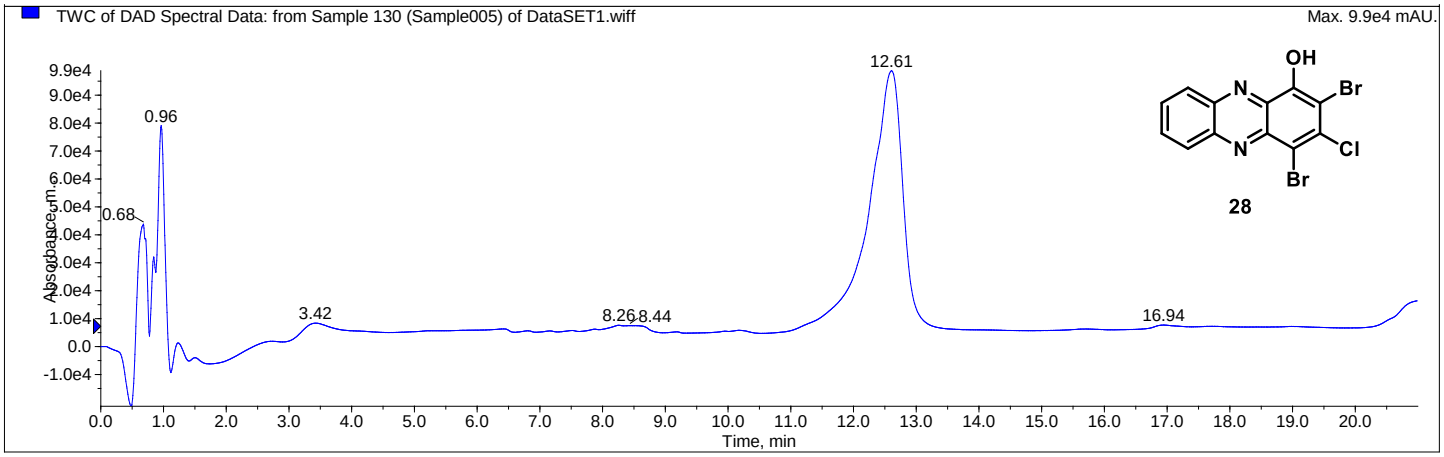
Retention Time (Min)	Peak Area
6.67	20400
6.99	2430
10.61 (**25**)	1250000
17.98	256
Sum of Area	1273086
% Purity	**98.2%**



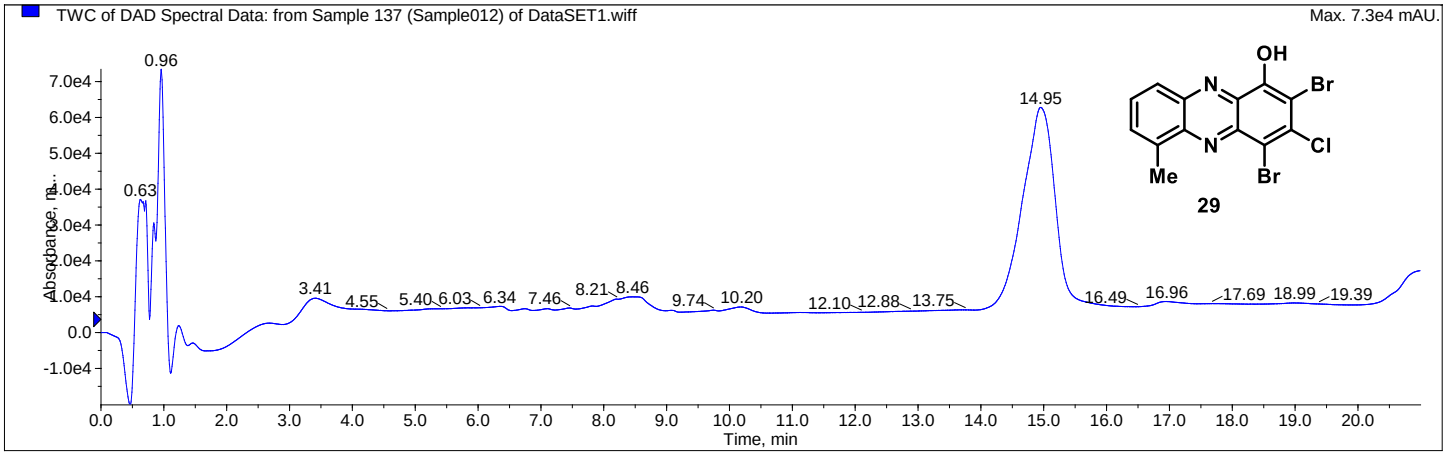
Retention Time (Min)	Peak Area
6.73	14000
7.04	2179
10.86 (**26**)	847000
Sum of Area	863179
% Purity	**98.1%**



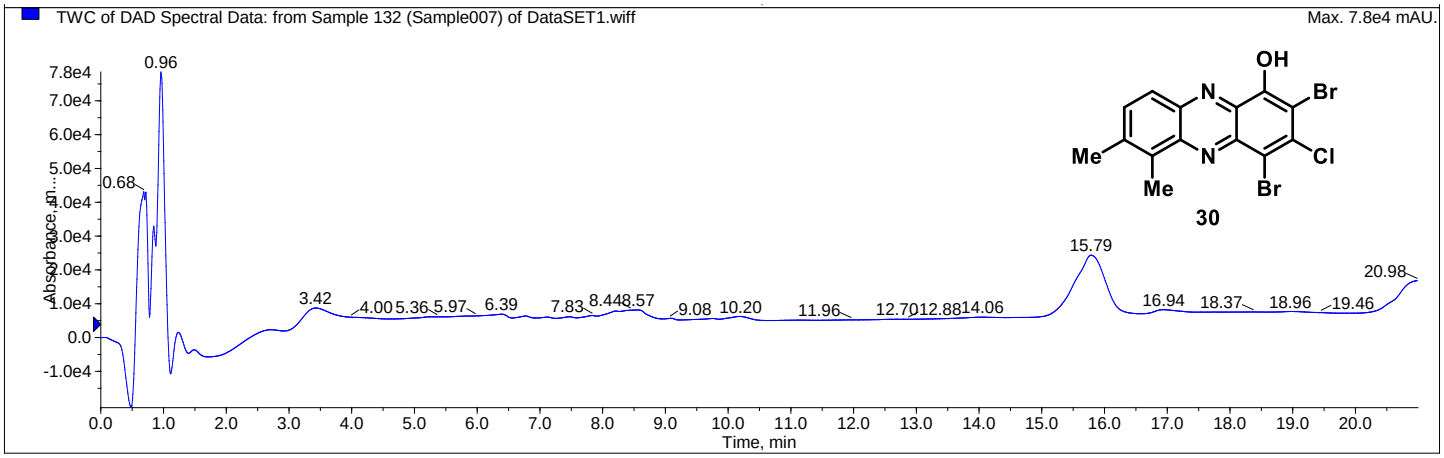
Retention Time (Min)	Peak Area
6.41	32600
6.80	4841
9.18	3346
11.71 (**27**)	3800000
13.67	40300
Sum of Area	3881087
% Purity	**97.9%**



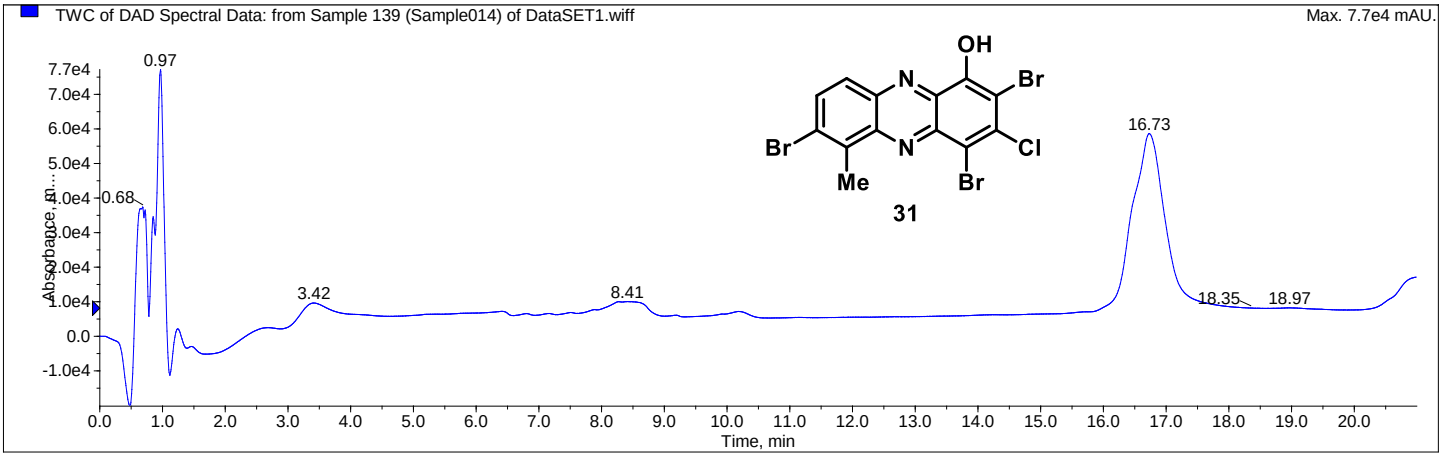
Retention Time (Min)	Peak Area
12.61(**28**)	3530000
15.67	6526
17.72	10003
Sum of Area	3546529
% Purity	**99.5%**



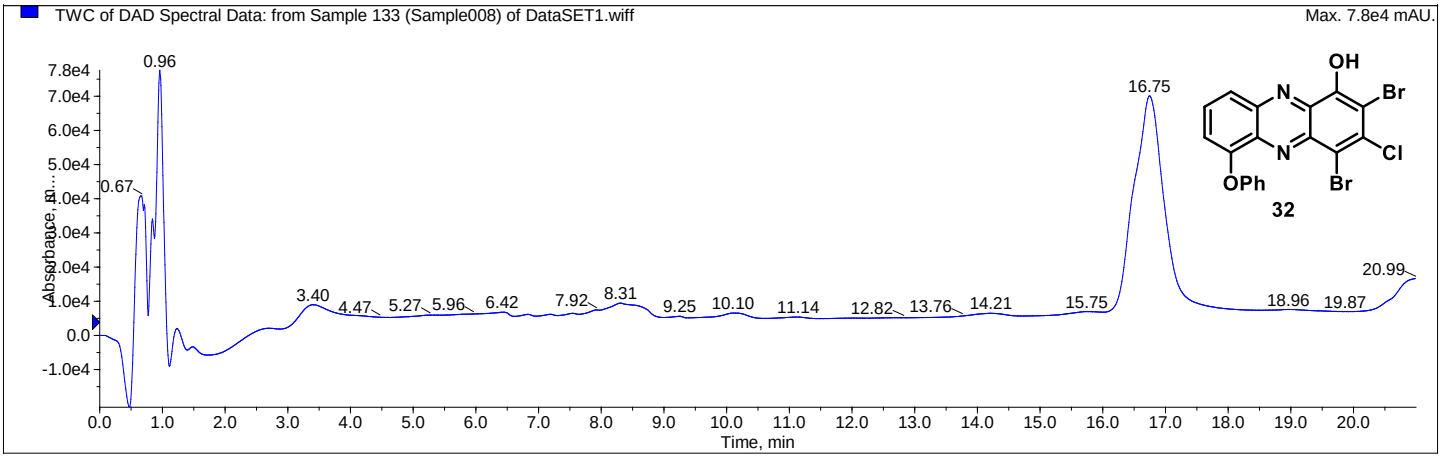
Retention Time (Min)	Peak Area
6.28	24300
11.19	337
14.95 (**29**)	1960000
Sum of Area	1984637
% Purity	**98.8%**



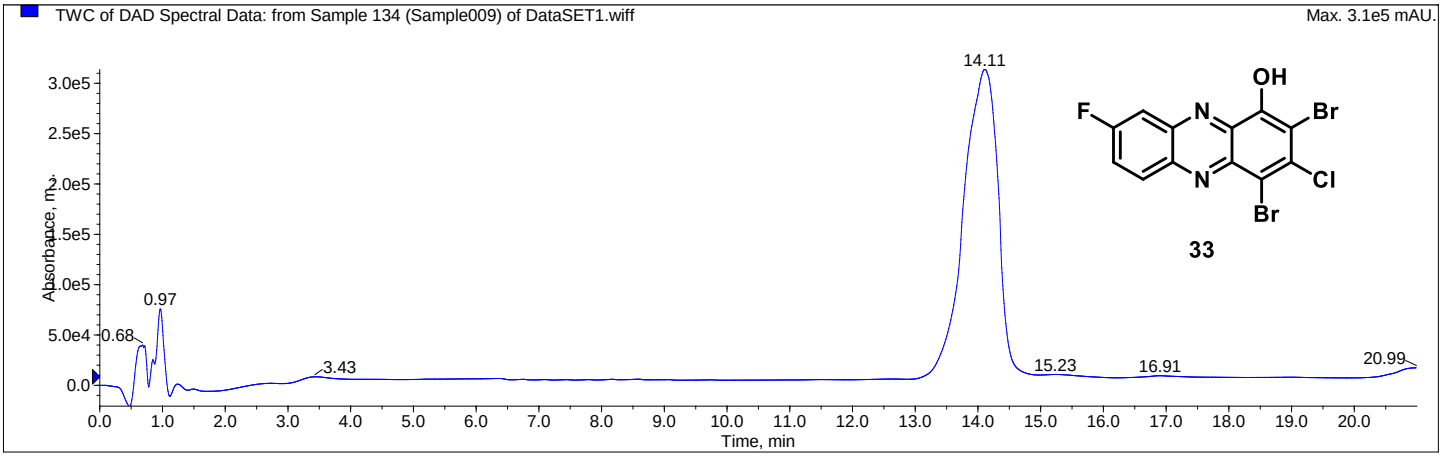
Retention Time (Min)	Peak Area
6.39	22700
6.76	5874
15.79 (**30**)	601000
Sum of Area	629574
% Purity	**95.5%**



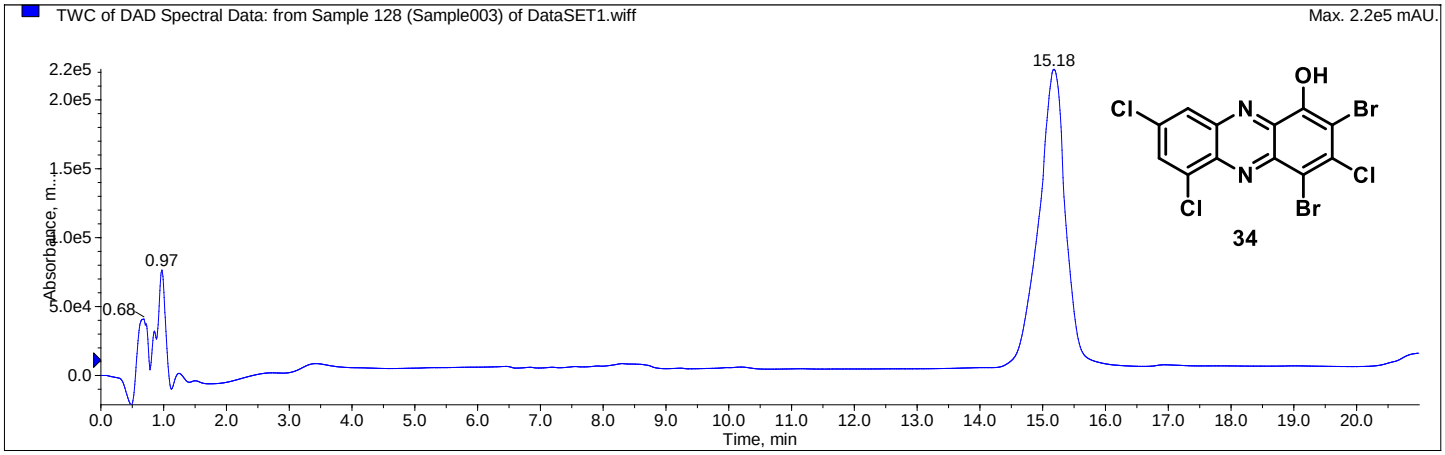
Retention Time (Min)	Peak Area
12.24	727
14.25	488
16.73 (**31**)	1810000
18.35	6
Sum of Area	1811221
% Purity	**>99.9%**



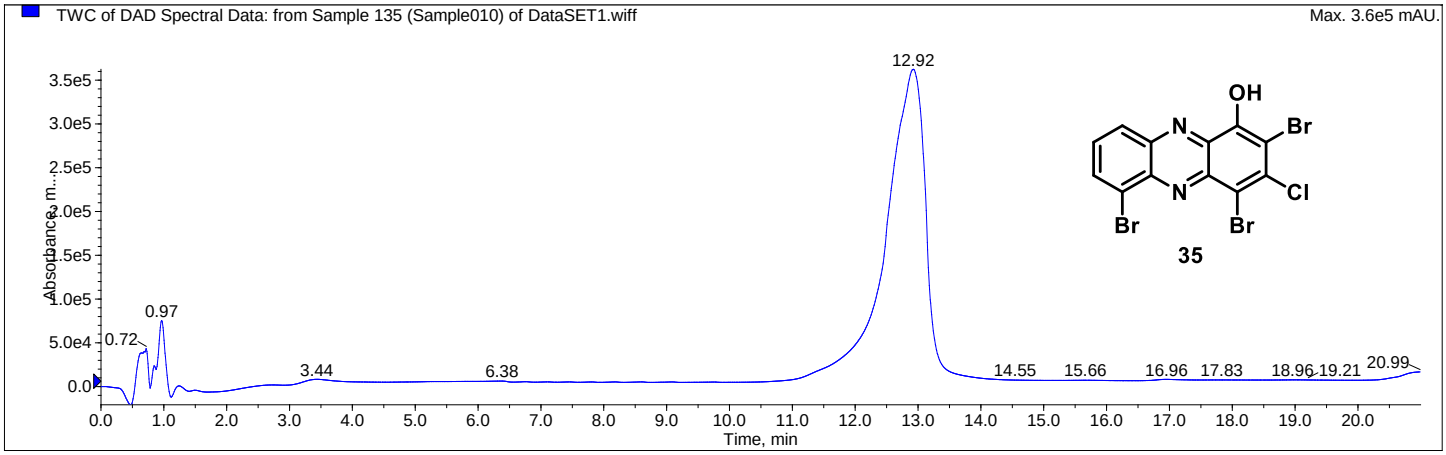
Retention Time (Min)	Peak Area
11.14	8699
13.76	499
14.21	27200
15.75	2104
16.75 (**32**)	2030000
Sum of Area	2068502
% Purity	**98.1%**



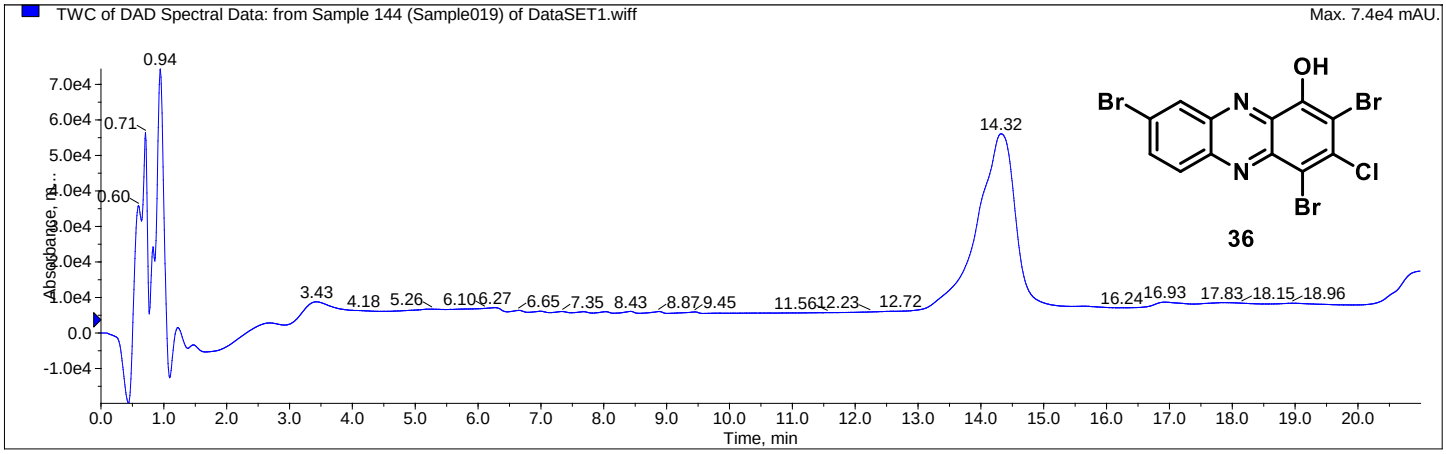
Retention Time (Min)	Peak Area
11.51	4725
12.64	4539
14.11 (**33**)	11600000
15.23	32900
Sum of Area	11642164
% Purity	**99.6%**



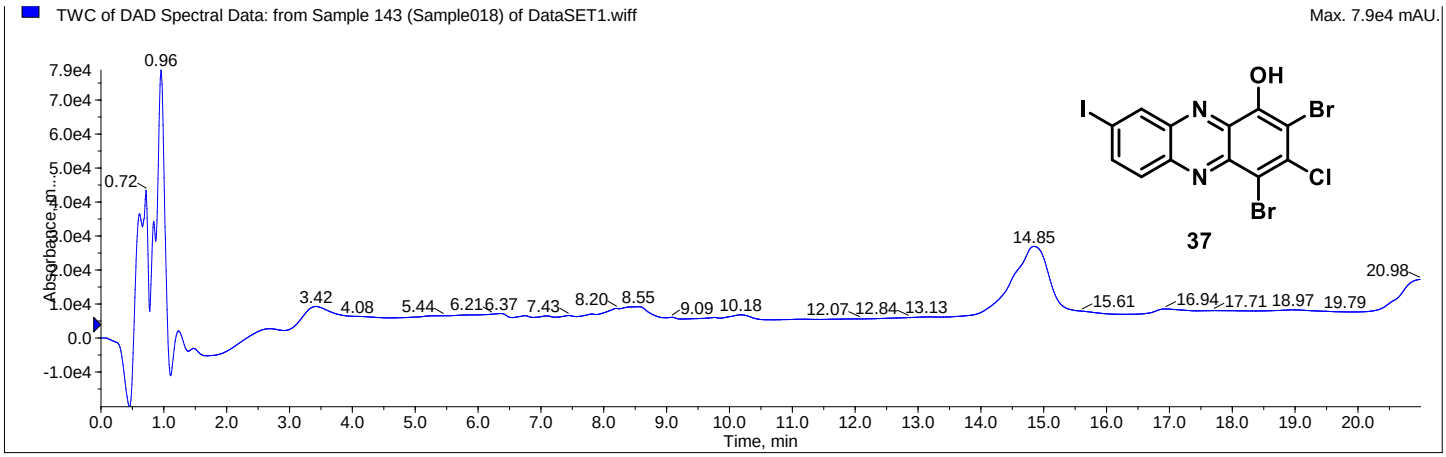
Retention Time (Min)	Peak Area
11.27	5207
15.18 (**34**)	6400000
17.73	2988
Sum of Area	6408195
% Purity	**99.9%**



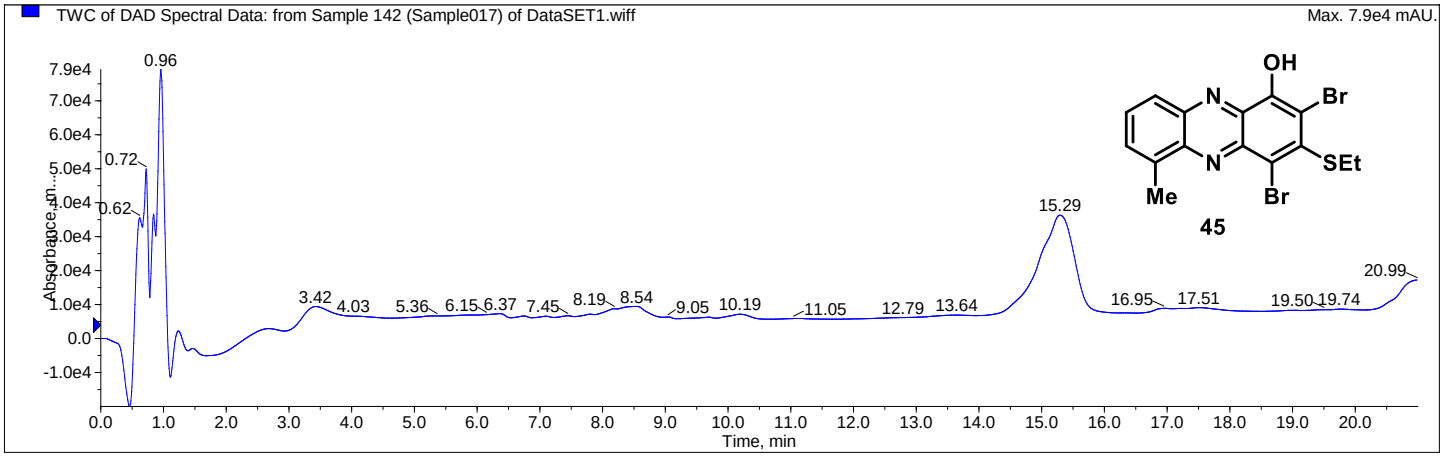
Retention Time (Min)	Peak Area
12.92 (**35**)	15700000
15.66	3507
17.66	5228
Sum of Area	15708735
% Purity	**>99.9%**



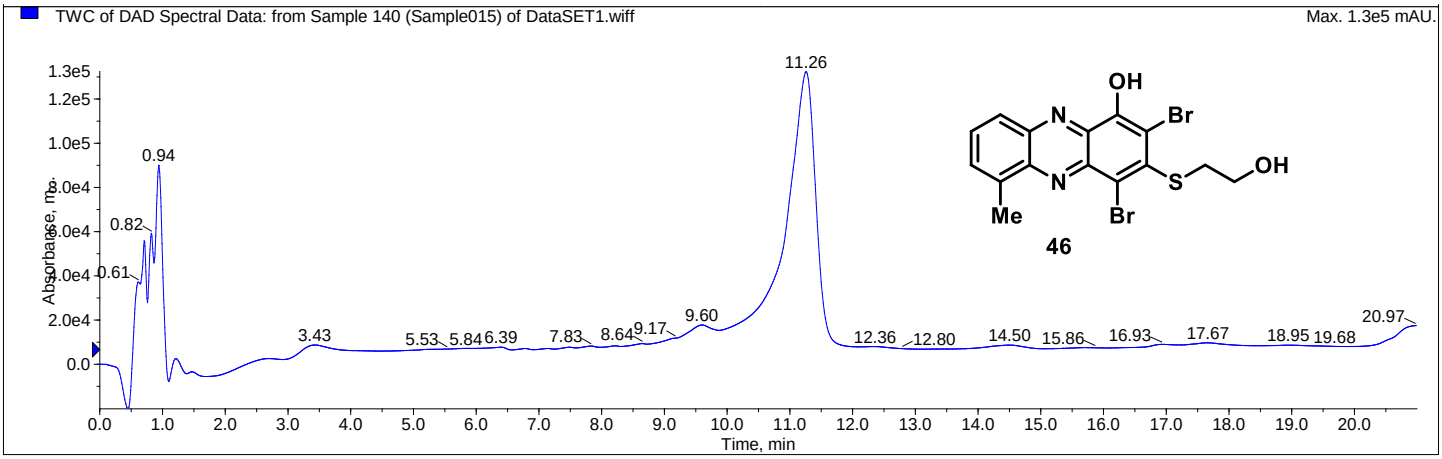
Retention Time (Min)	Peak Area
8.87	5904
9.45	5161
14.32 (**36**)	1990000
17.87	11086
Sum of Area	2012151
% Purity	**98.9%**



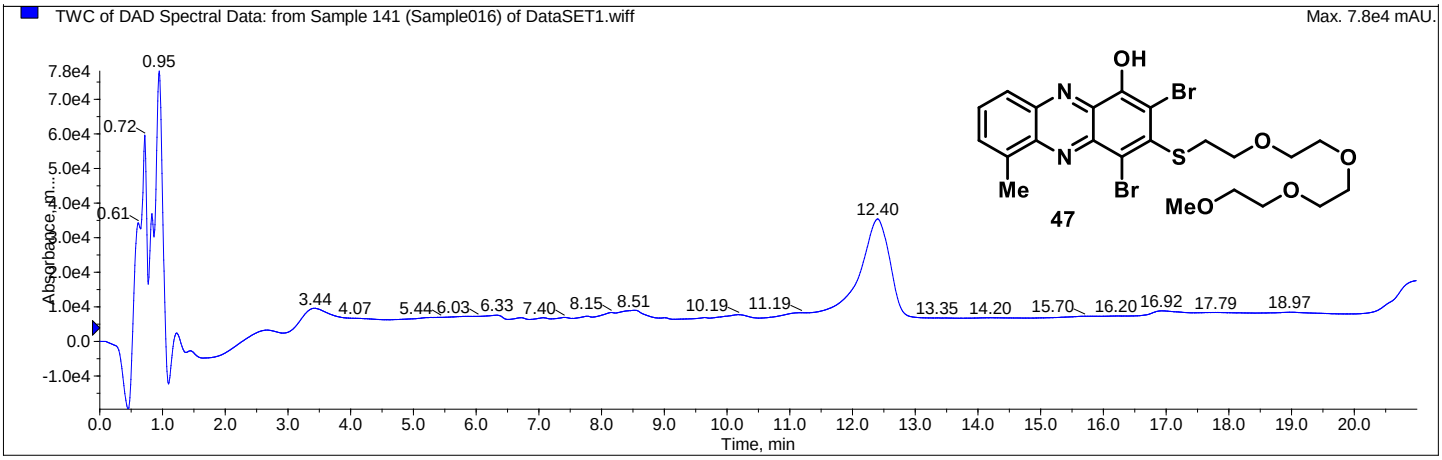
Retention Time (Min)	Peak Area
11.18	1420
13.13	825
14.85 (**37**)	760000
Sum of Area	762245
% Purity	**99.7%**



Retention Time (Min)	Peak Area
11.15	4579
13.64	3454
15.29 (**45**)	1140000
17.51	17500
Sum of Area	1165533
% Purity	**97.8%**



Retention Time (Min)	Peak Area
9.17	1184
9.60	76600
11.26 (**46**)	3860000
14.50	51900
17.67	45300
Sum of Area	4034984
% Purity	**95.7%**

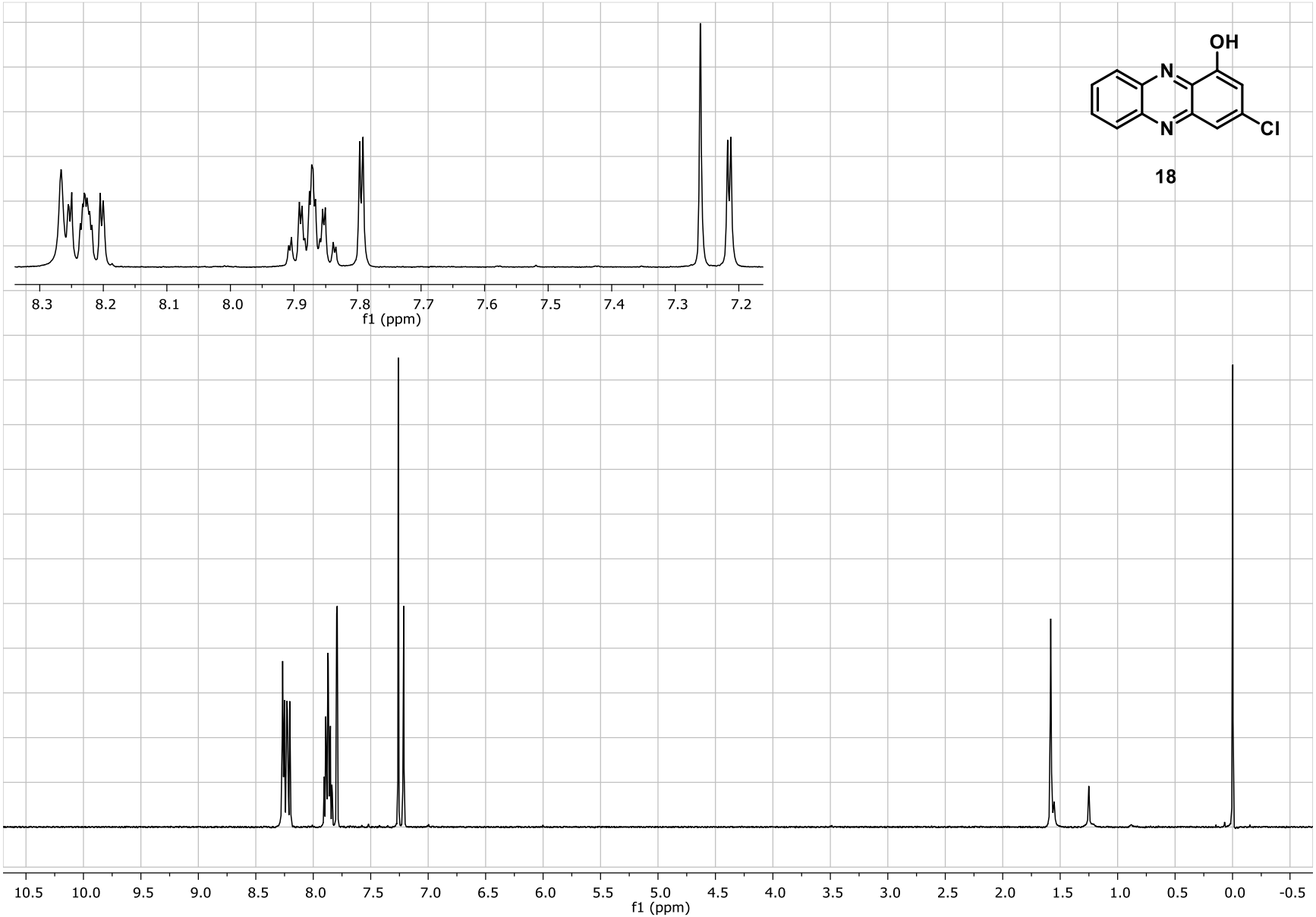


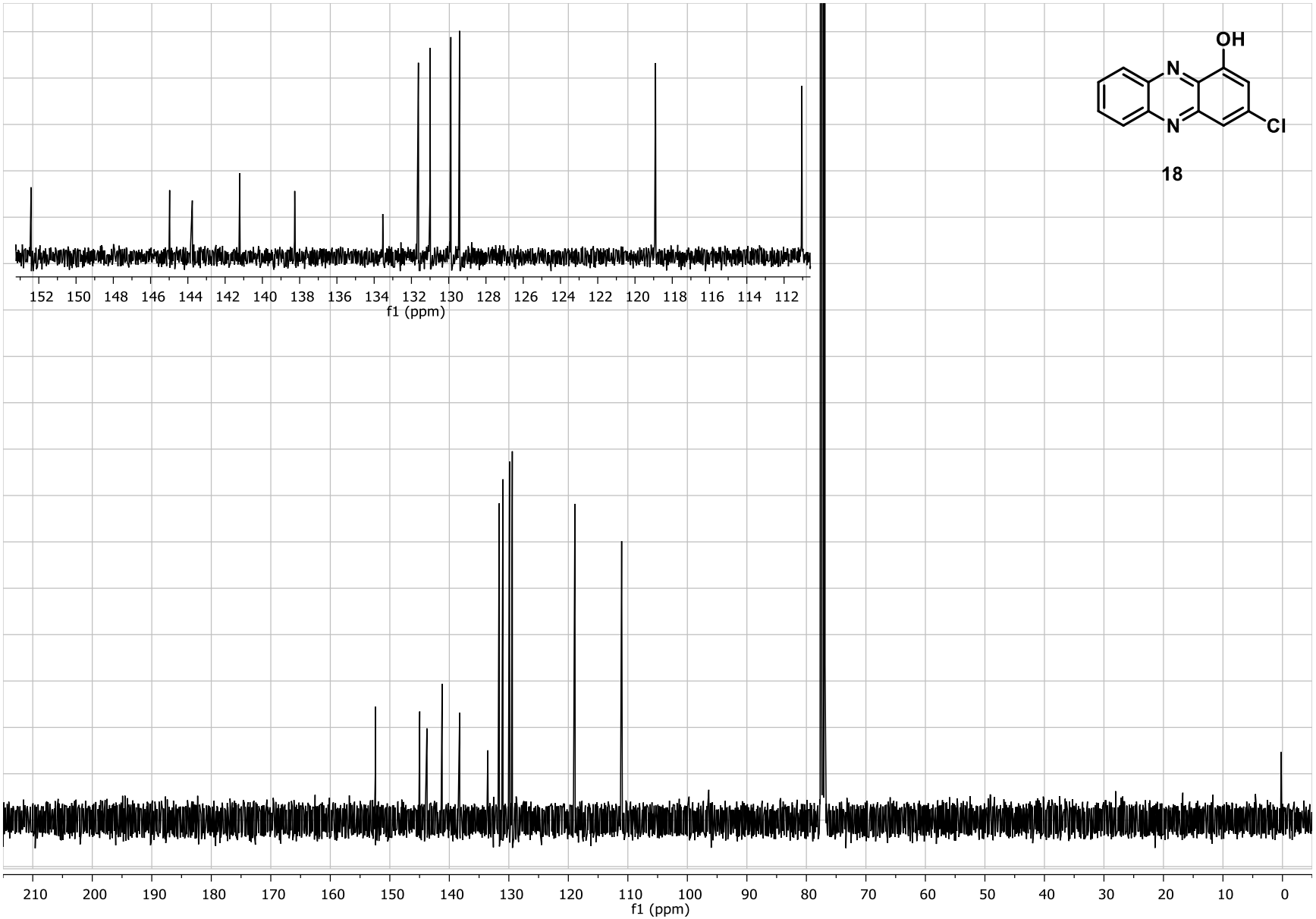
Retention Time (Min)	Peak Area
11.19	176
12.40 (**47**)	887000
13.35	67
Sum of Area	887243
% Purity	**>99.9%**

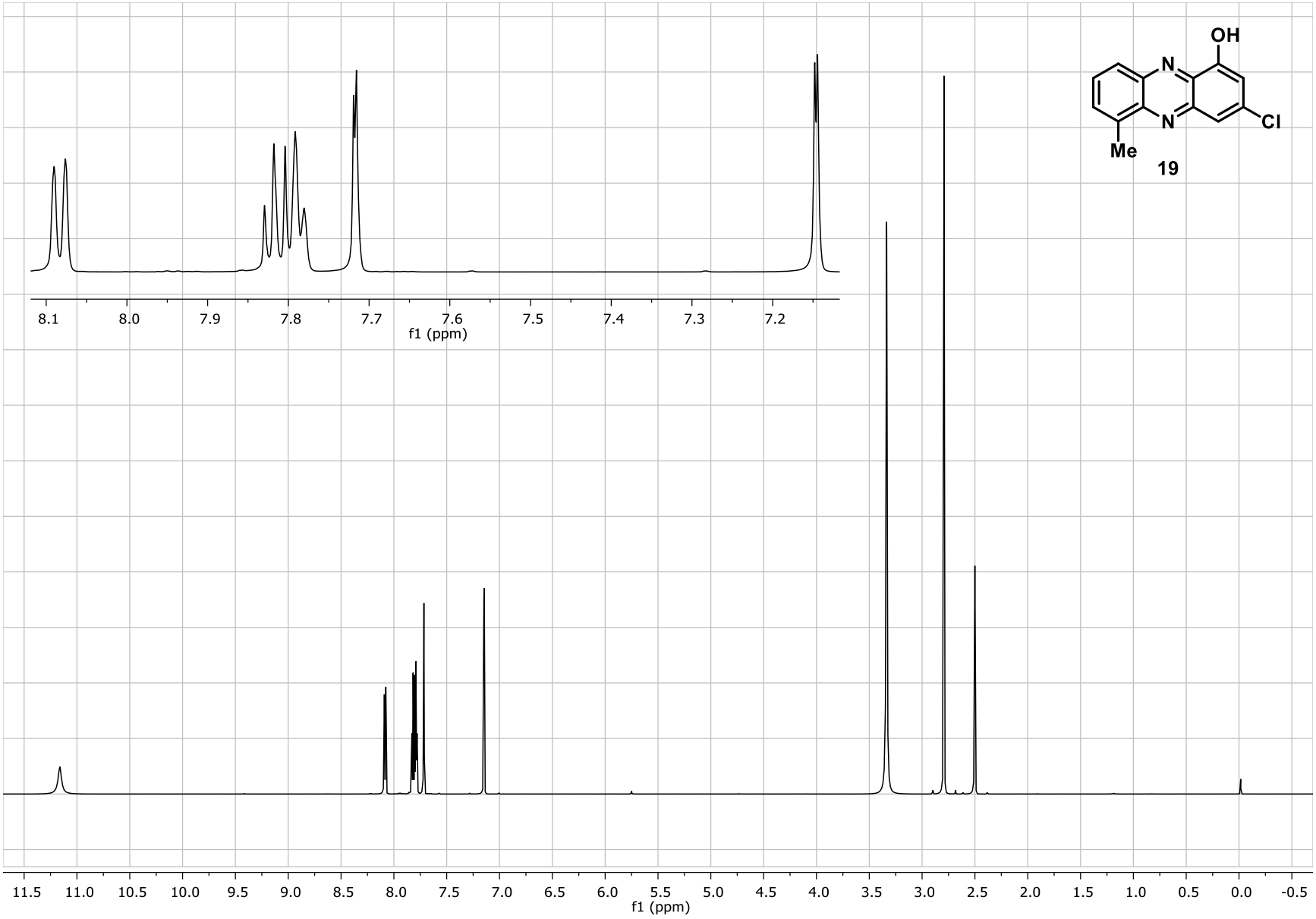
16.) Literature References.

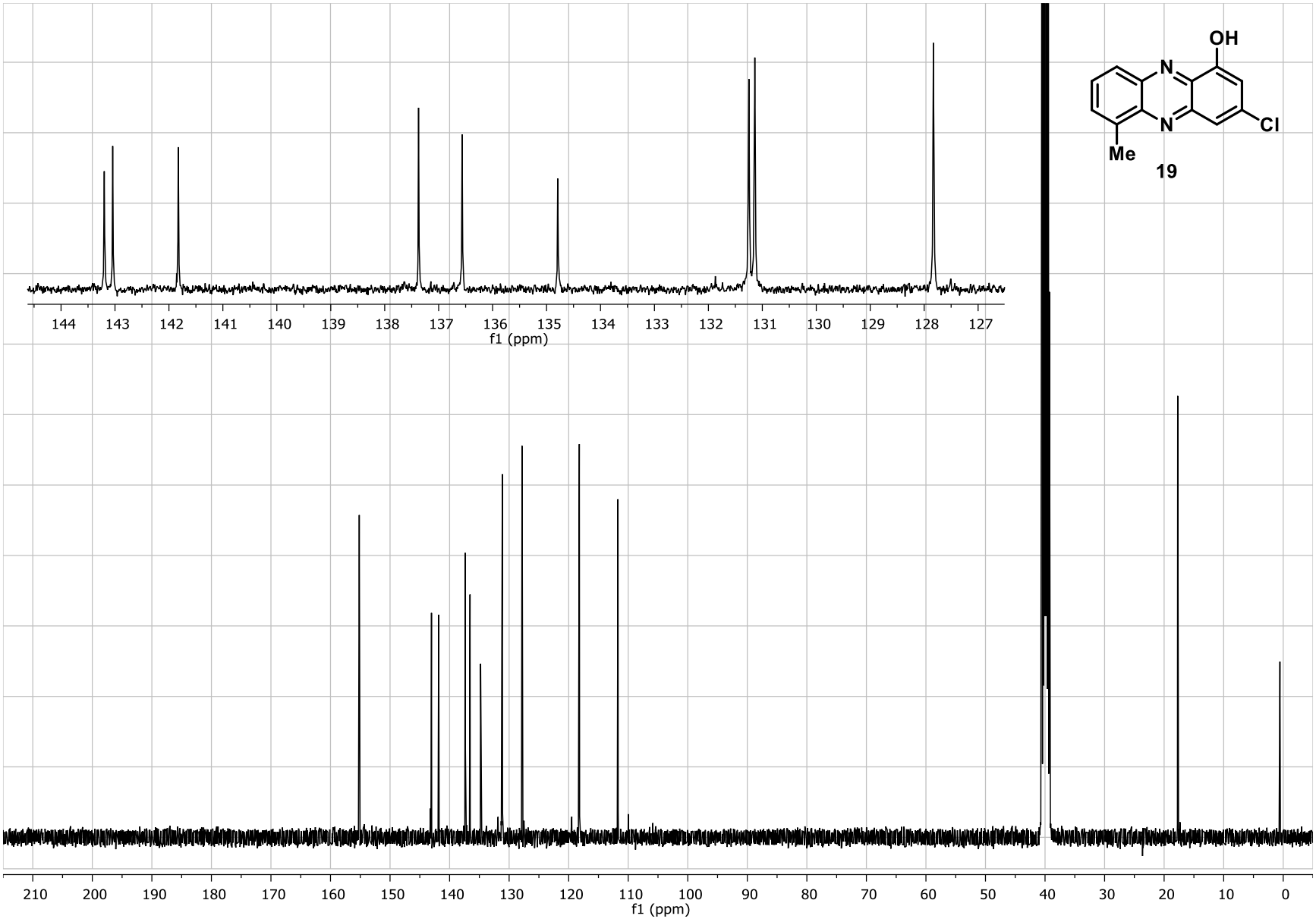
1. Sachin, K.; Jadhav, V.H.; Kim, E.M.; Kim, H.L.; Lee, S.B.; Jeong, H.J.; Lim, S.T.; Sohn, M.H.; Kim, D.W. *Bioconjug. Chem.* **2012**, *23*, 1680-1686.
2. Snow, A.W.; Foos, E.E. *Synthesis* **2003**, *14*, 0509-0512.
3. Garrison, A.T.; Abouelhassan, Y.; Norwood IV, V.M.; Kallifidas, D.; Bai, F.; Nguyen, M.; Rolfe, M.; Burch, G.M.; Jin, S.; Luesch, H.; Huigens III, R.W. *J. Med. Chem.* **2016***, 59*, 3808-3825.
4. Yang, H.; Abouelhassan, Y.; Burch, G.M.; Kallifidas, D.; Huang, G.; Yousaf, H.; Jin, S.; Luesch, H.; Huigens III, R.W. *Sci. Rep.* **2017**, *7*, 2003.
5. Garrison, A.T.; Abouelhassan, Y.; Kallifidas, D.; Tan, H.; Kim, Y.S.; Jin, S.; Luesch, H.; Huigens III, R.W. *J. Med. Chem.* **2018**, *61*, 3962-3983.

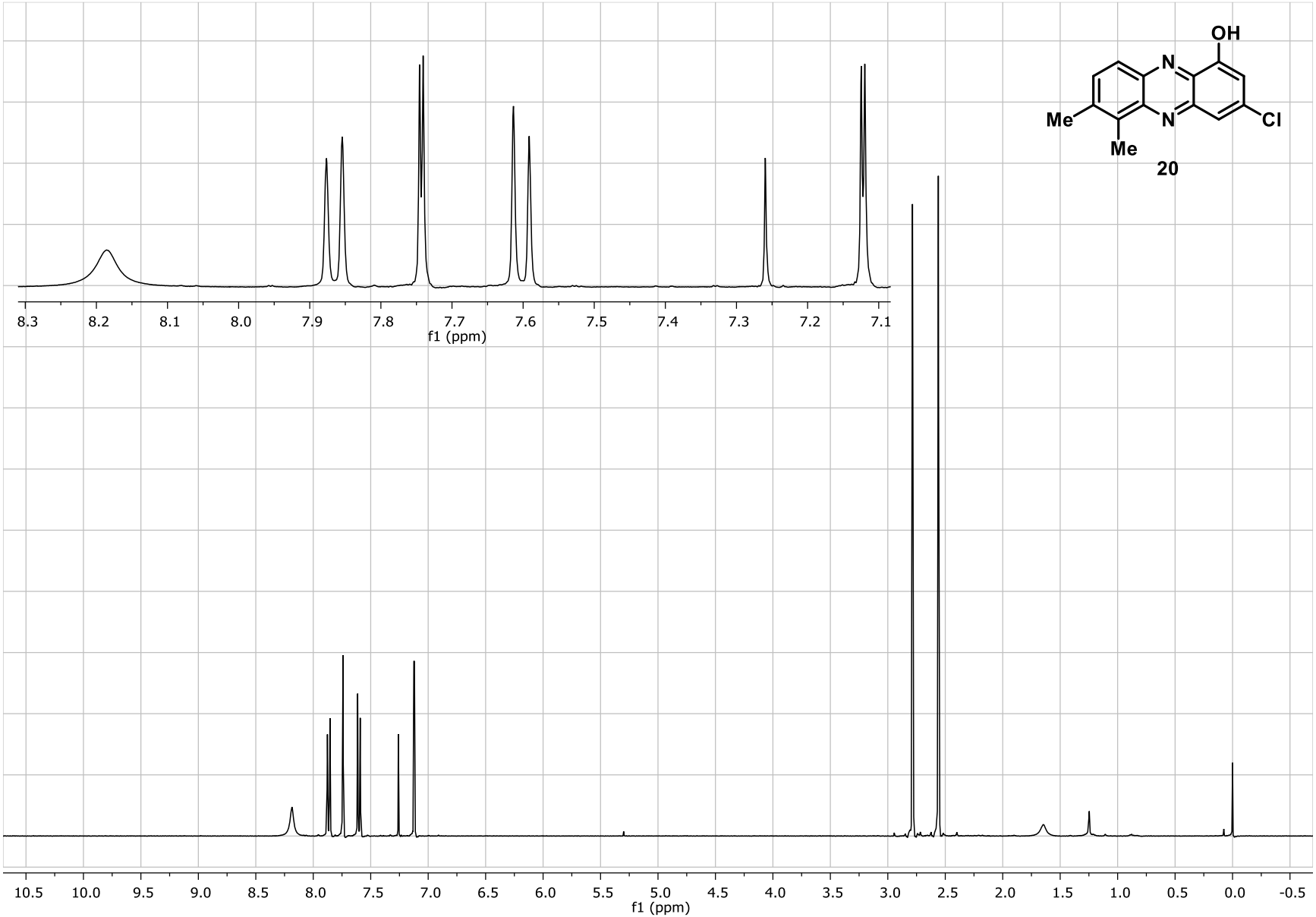
17. NMR Spectra.

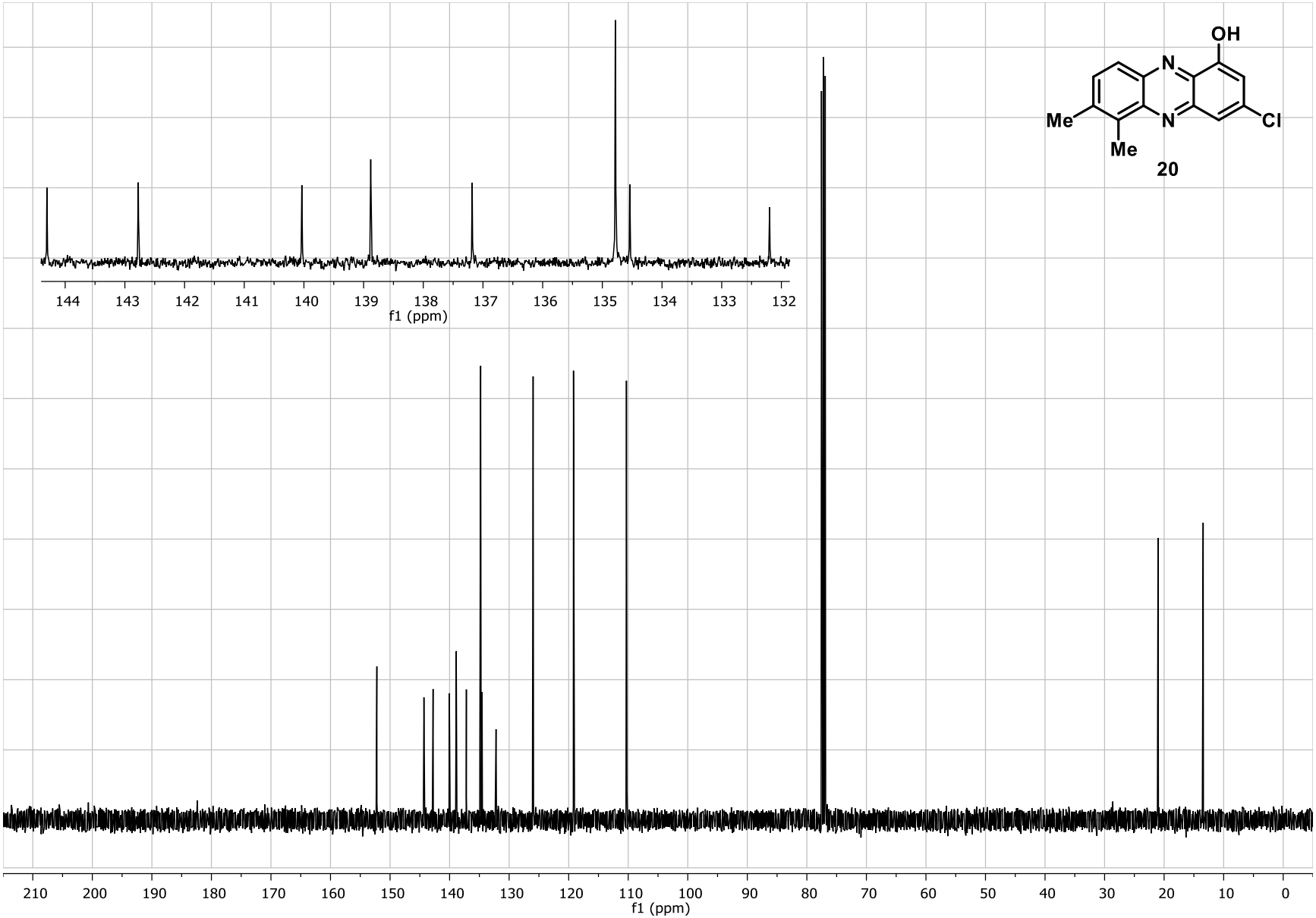

OH
N
N
Cl
18
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

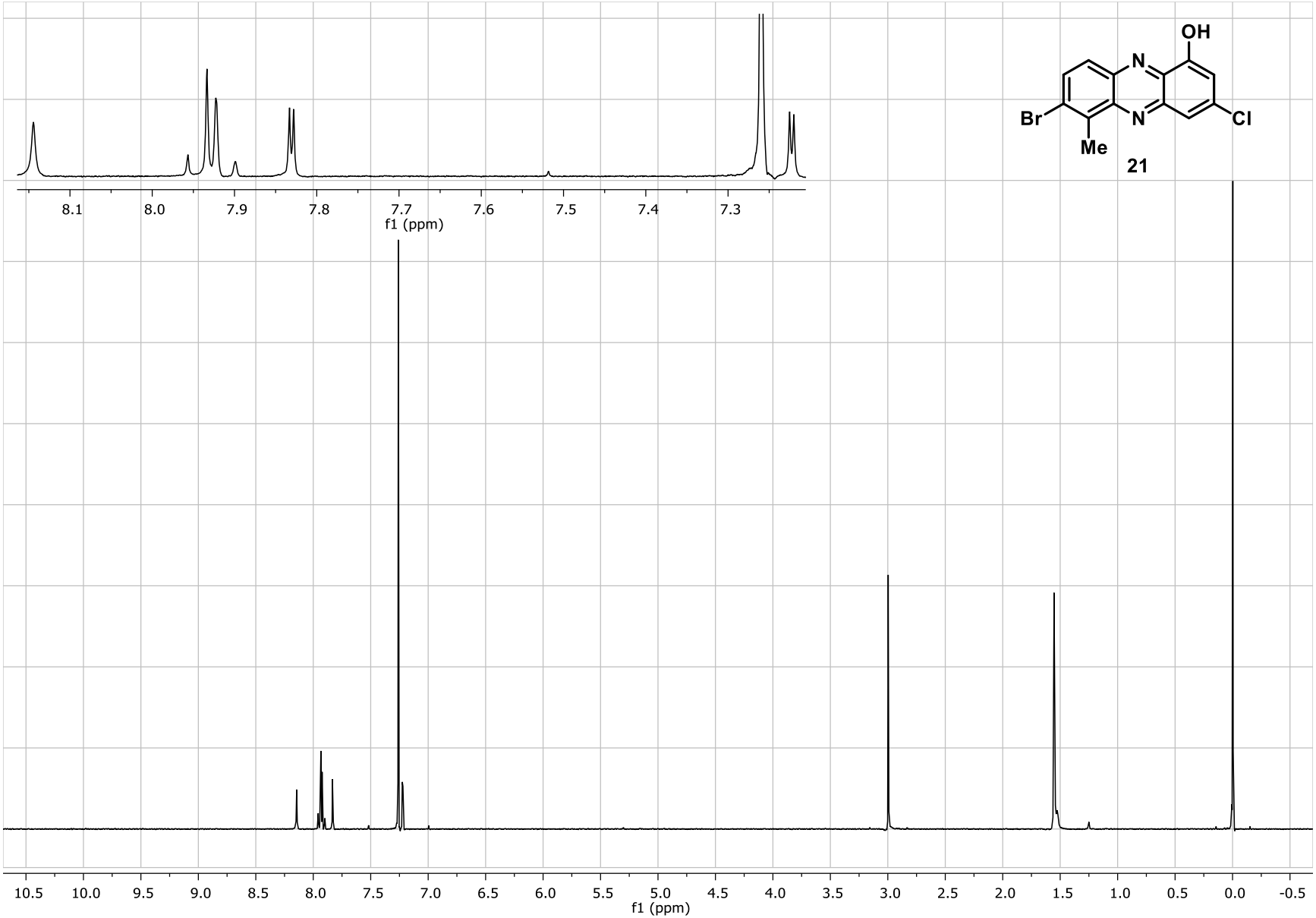

OH
N
N
Cl
18
152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

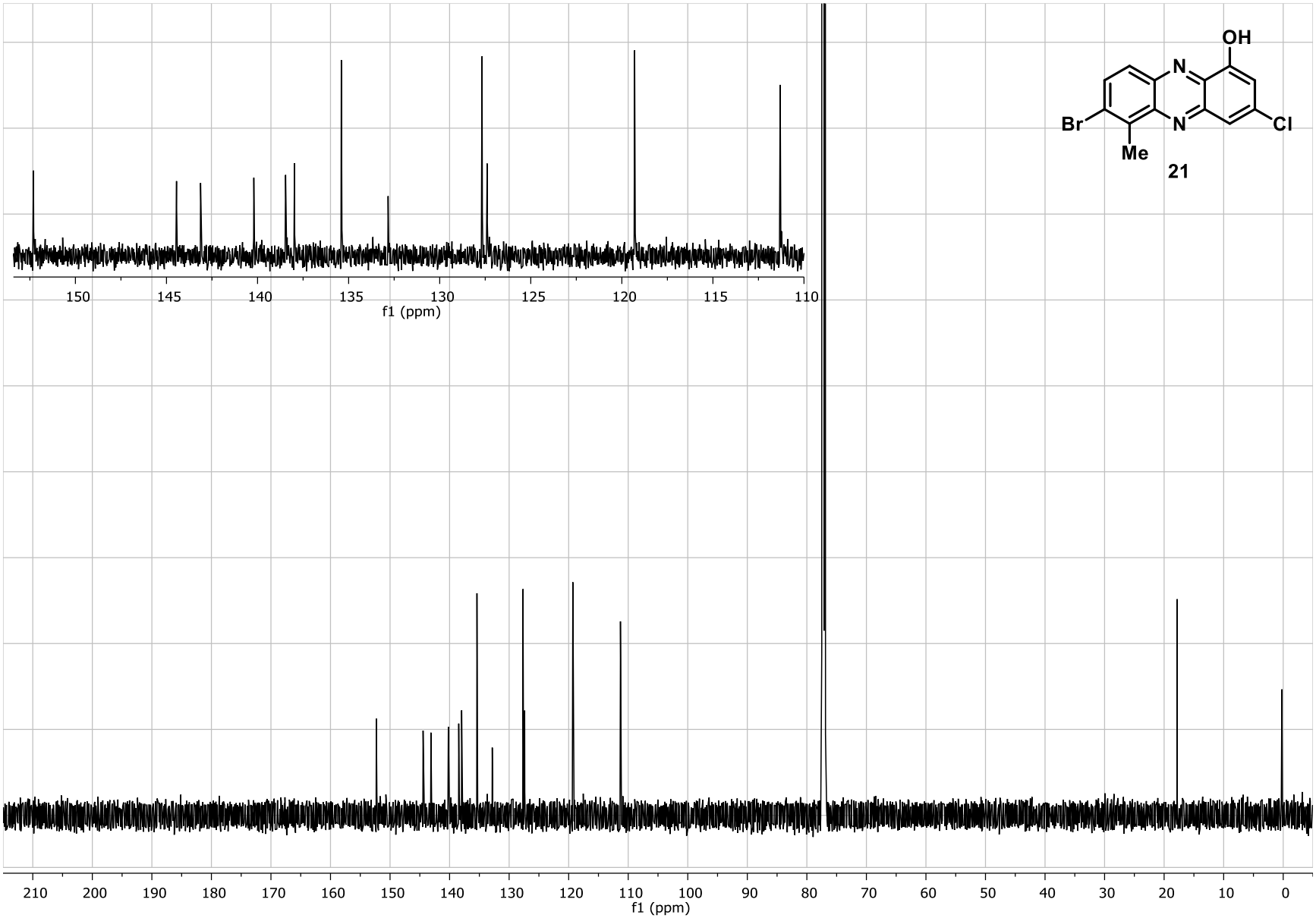

OH
N
N
Cl
Me
19
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
f1 (ppm)
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

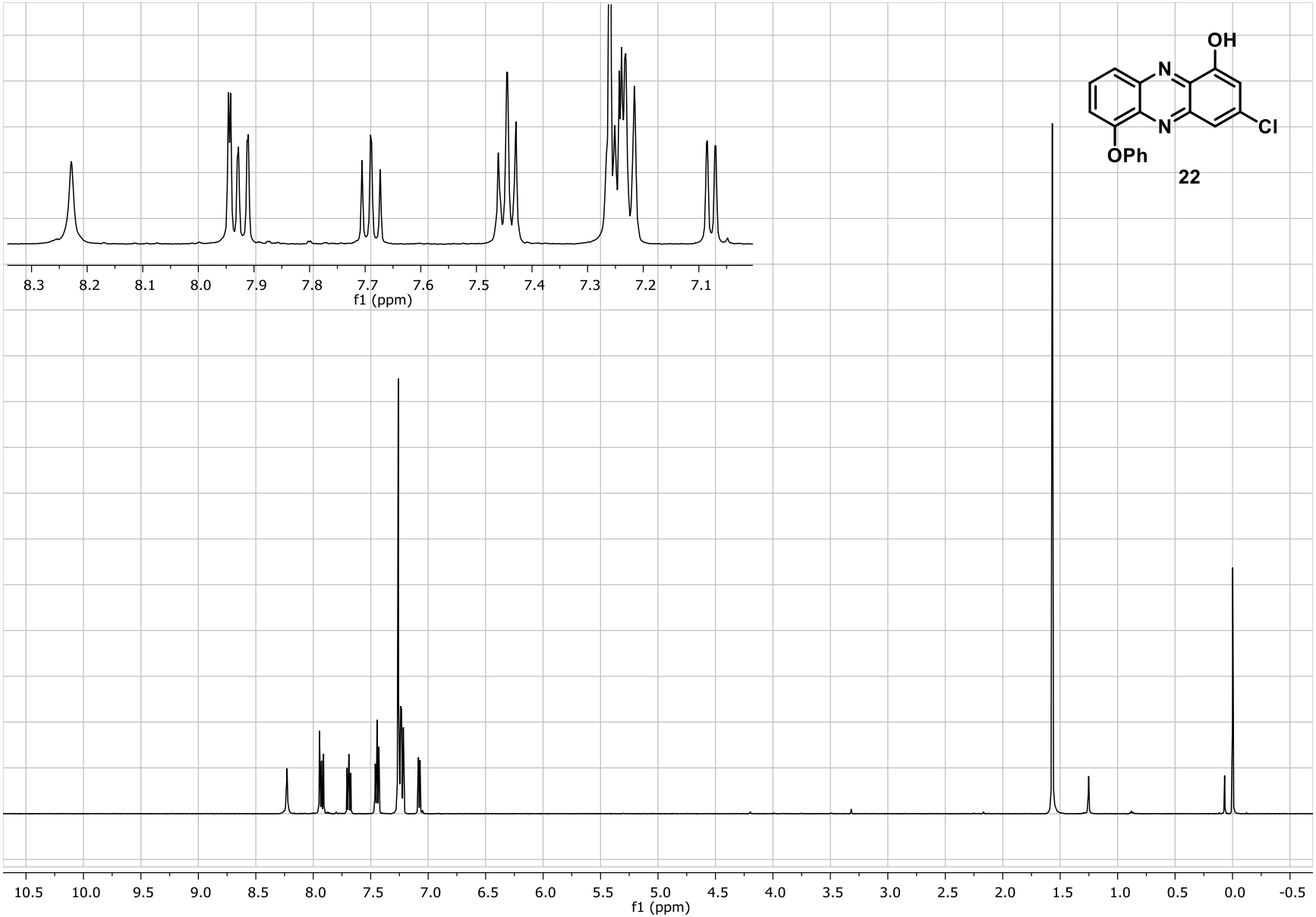

OH
N
N
Cl
Me
19
144 143 142 141 140 139 138 137 136 135 134 133 132 131 130 129 128 127
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

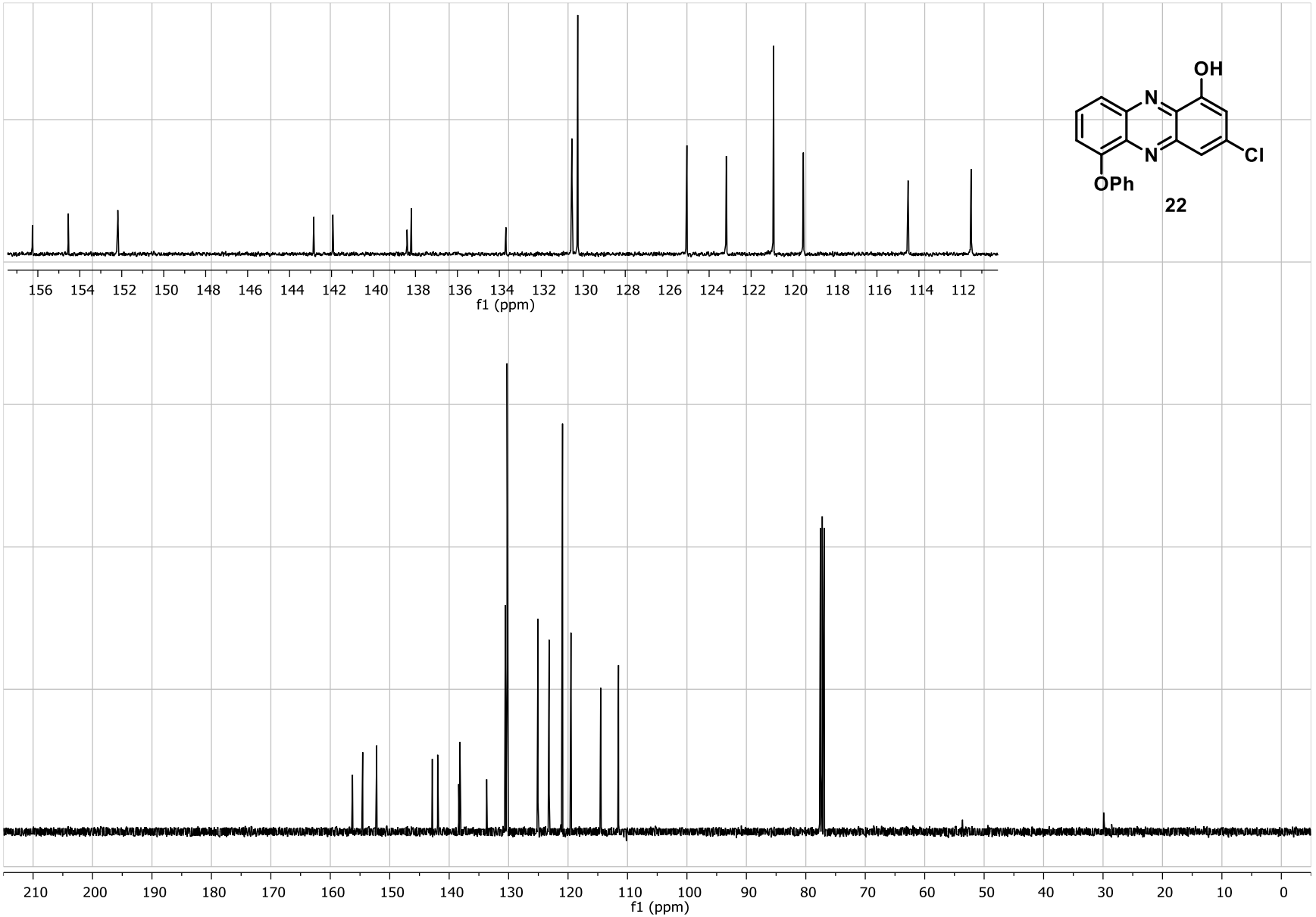

OH
N
N
Me
Me
Cl
20
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

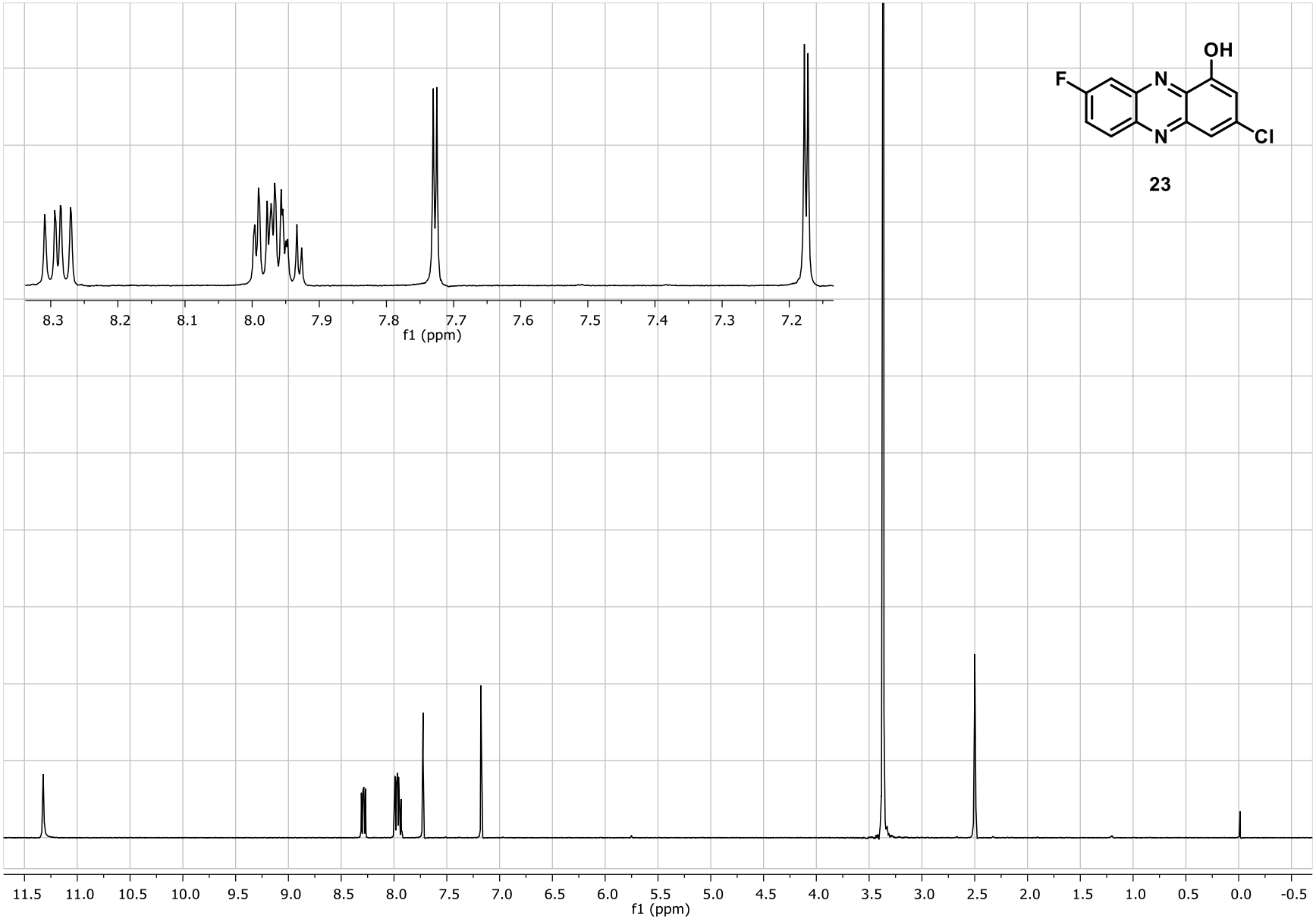

OH
N
N
Me
Me
Cl
20
144 143 142 141 140 139 138 137 136 135 134 133 132
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

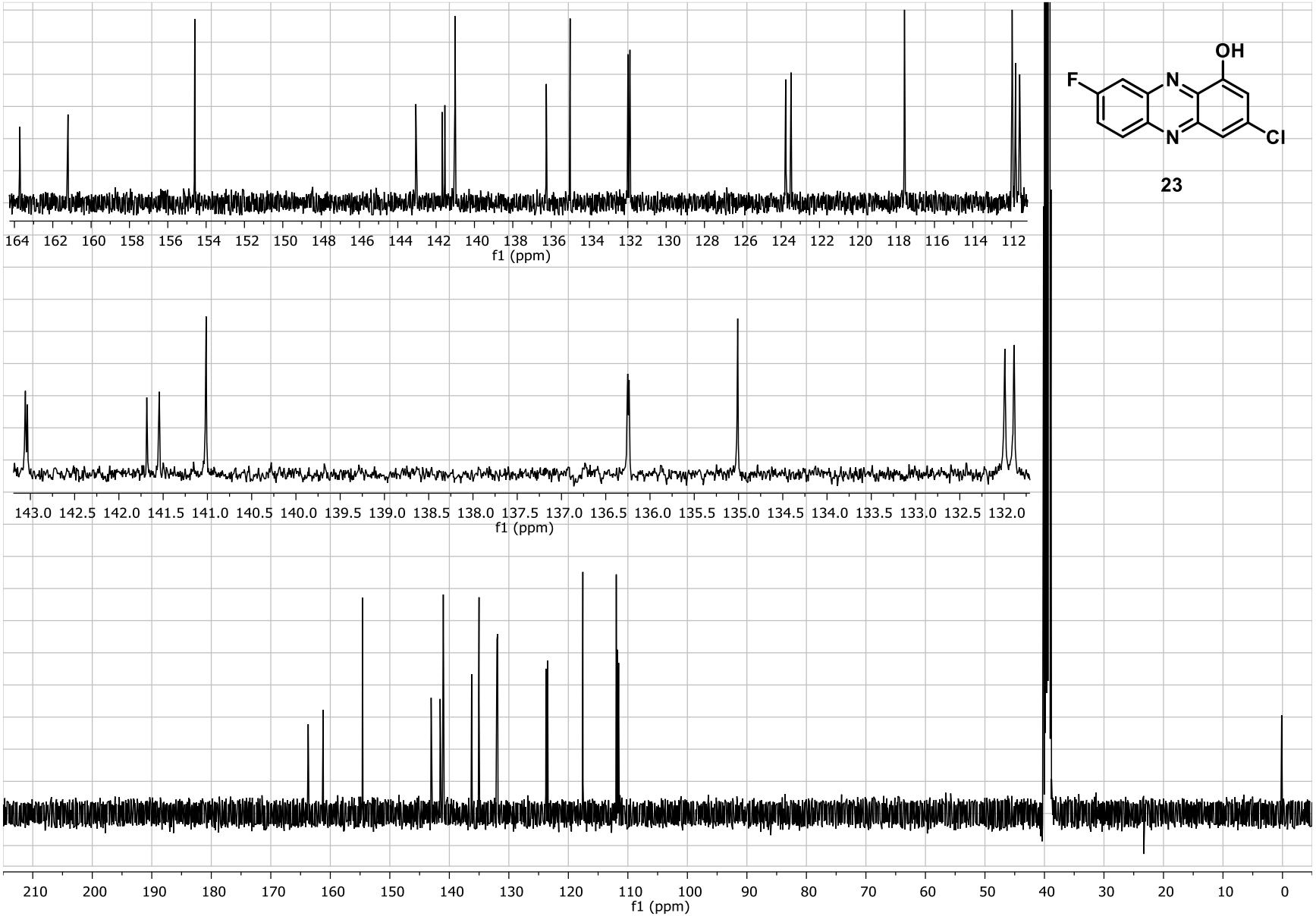

OH
N
N
Br
Cl
Me
21
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

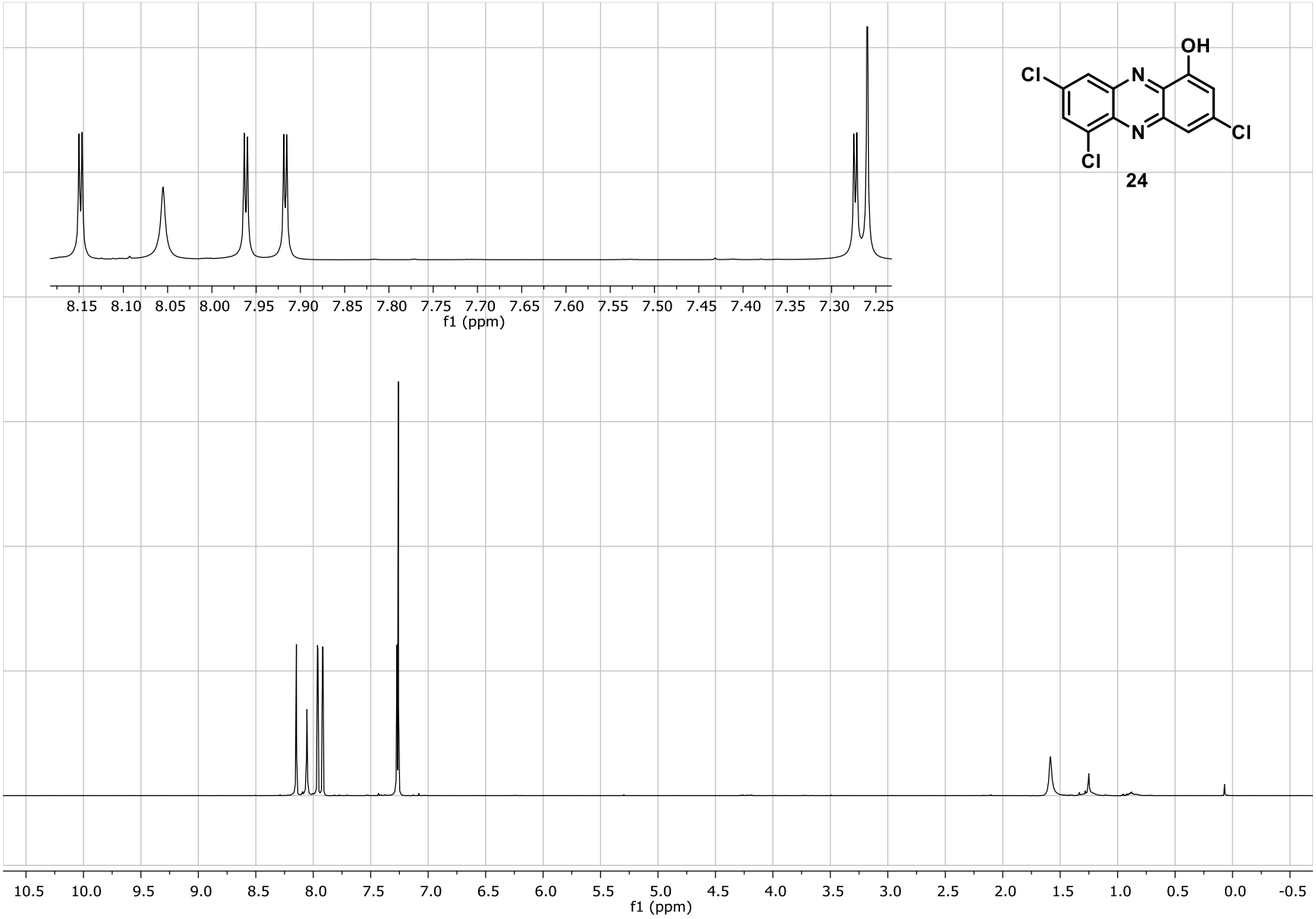

OH
N
N
Br
Cl
Me
21
150
145
140
135
130
125
120
115
110
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

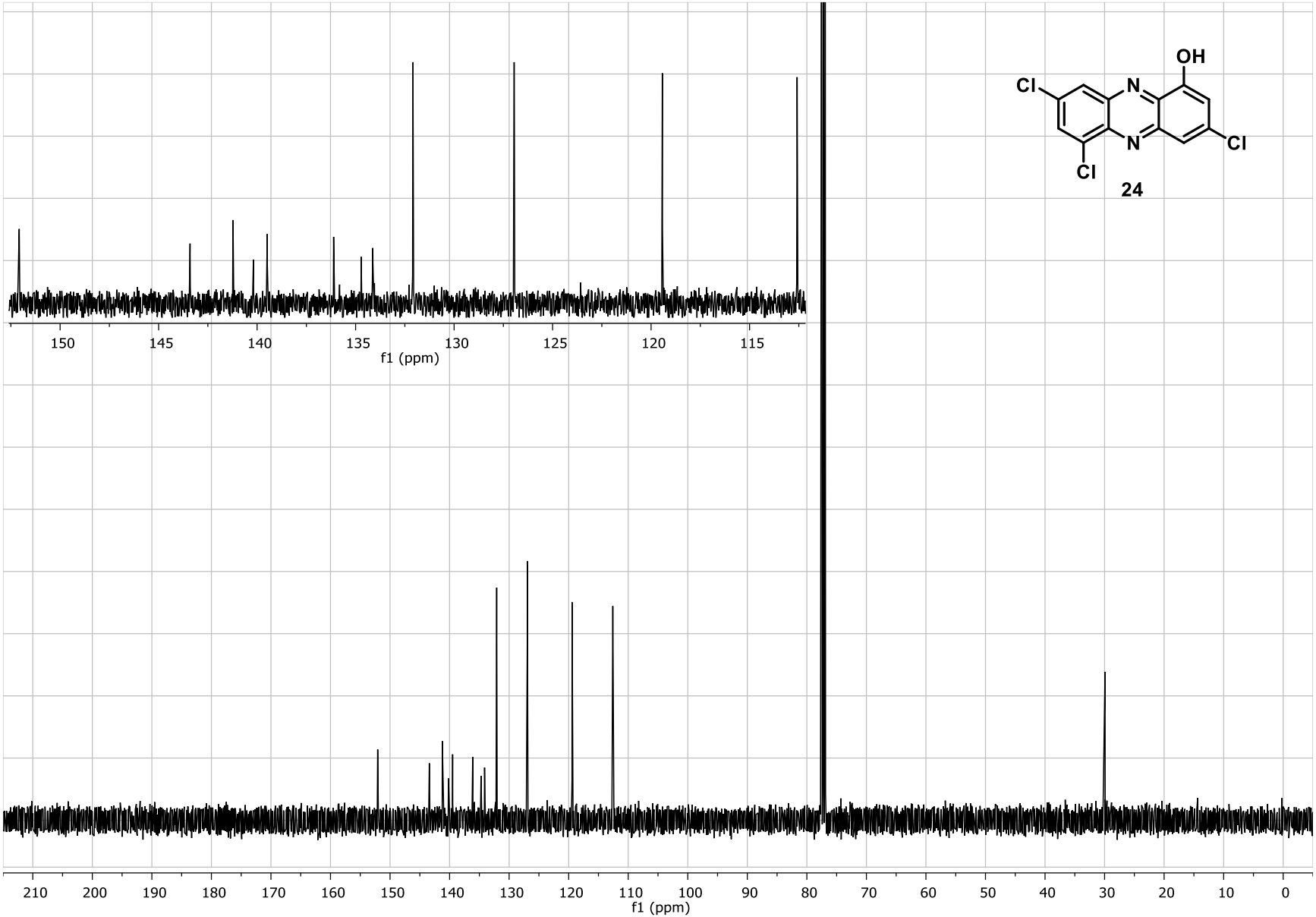

OH
N
N
Cl
OPh
22
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

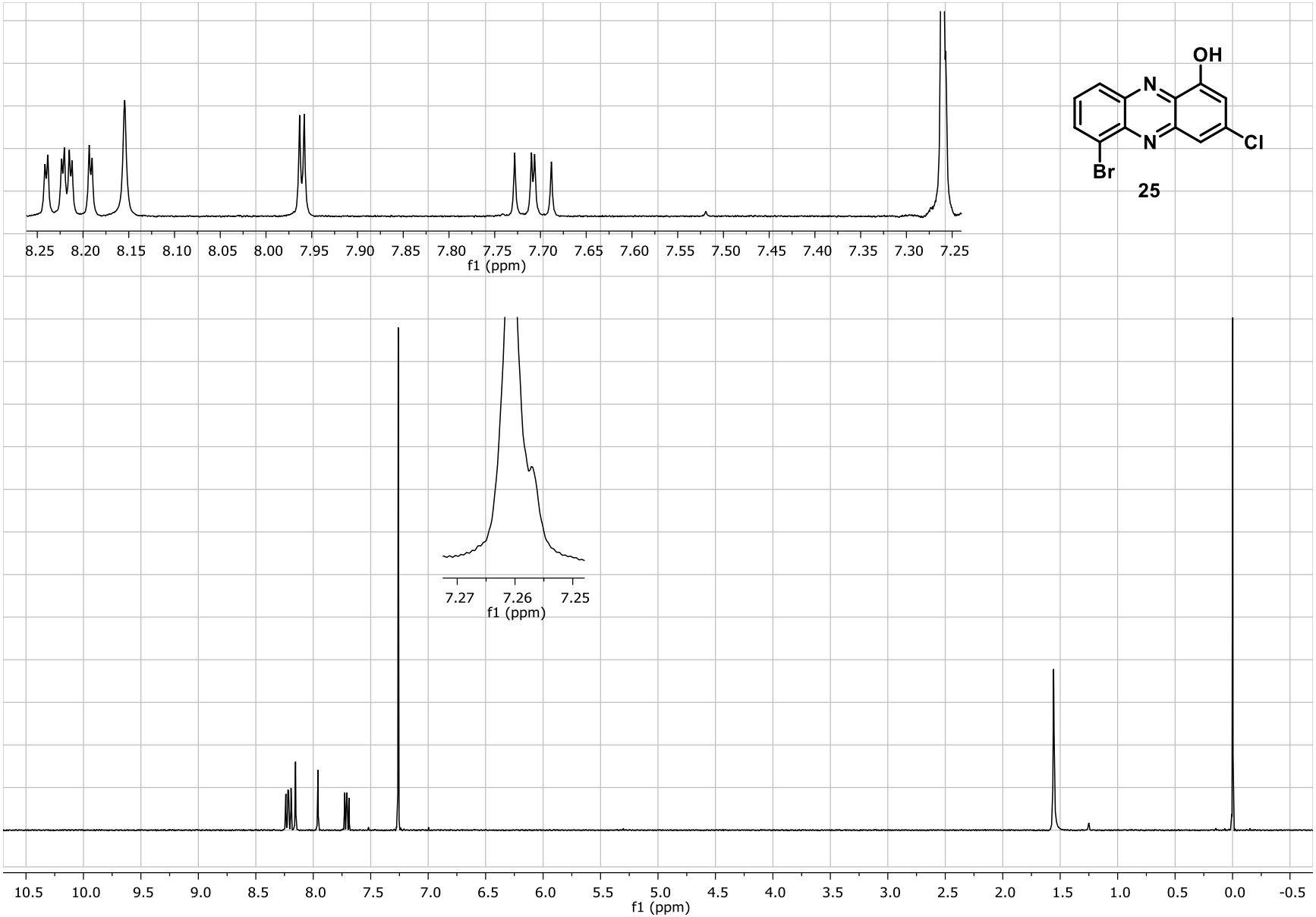

OH
N
N
Cl
OPh
22
156 154 152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

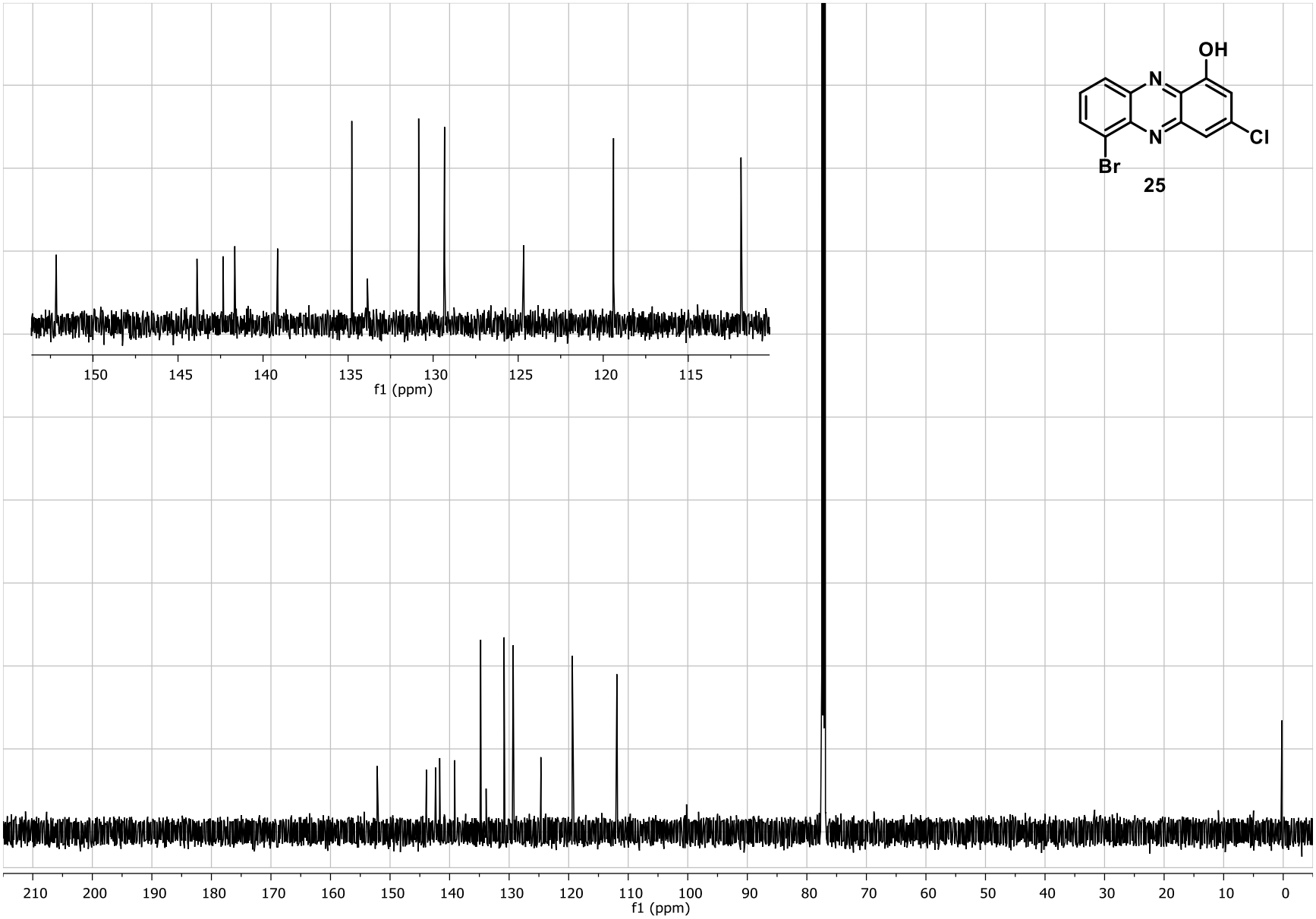

OH
F
N
N
Cl
23
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
f1 (ppm)
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

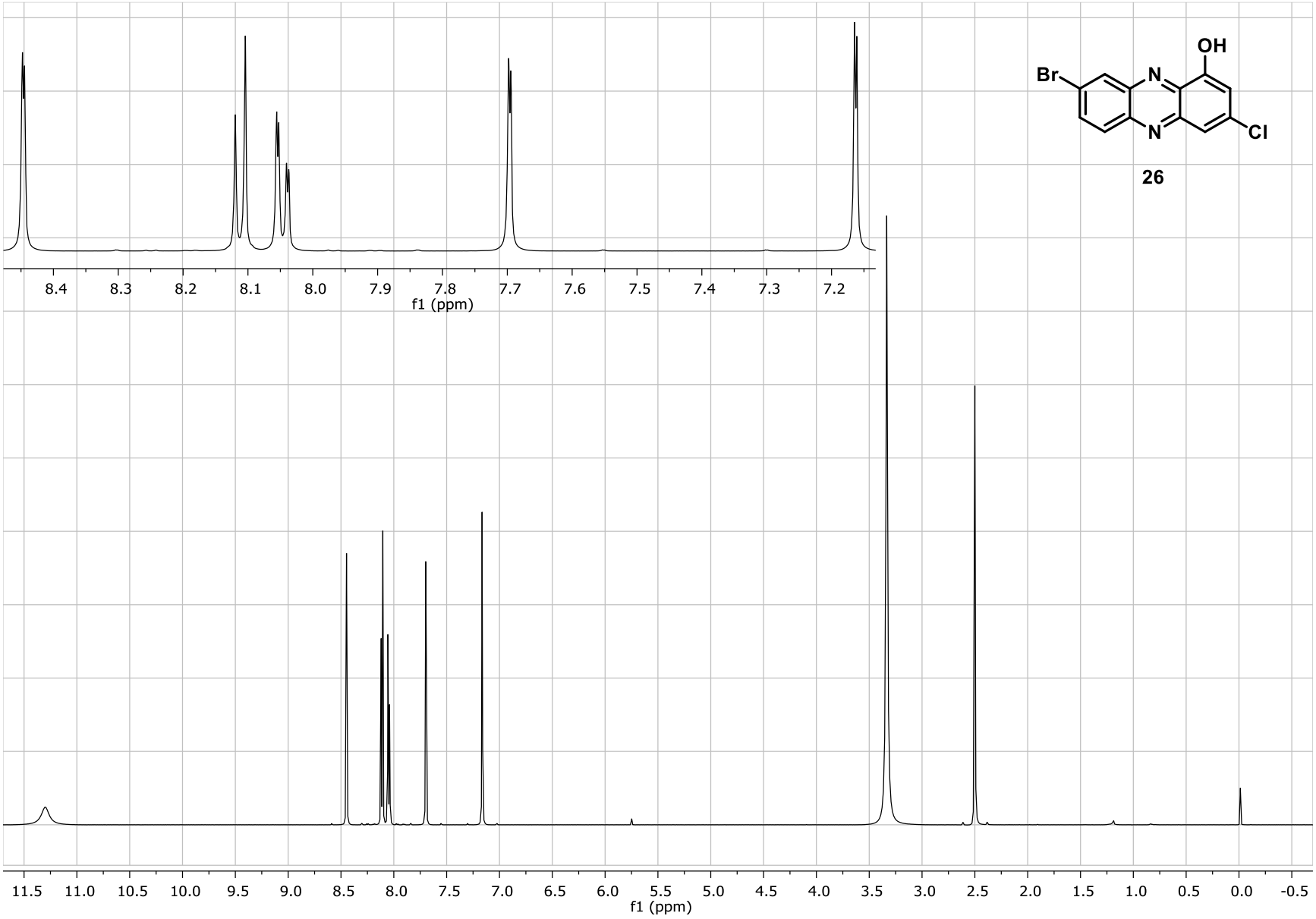

OH
F
N
N
Cl
23
164 162 160 158 156 154 152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112
f1 (ppm)
143.0 142.5 142.0 141.5 141.0 140.5 140.0 139.5 139.0 138.5 138.0 137.5 137.0 136.5 136.0 135.5 135.0 134.5 134.0 133.5 133.0 132.5 132.0
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

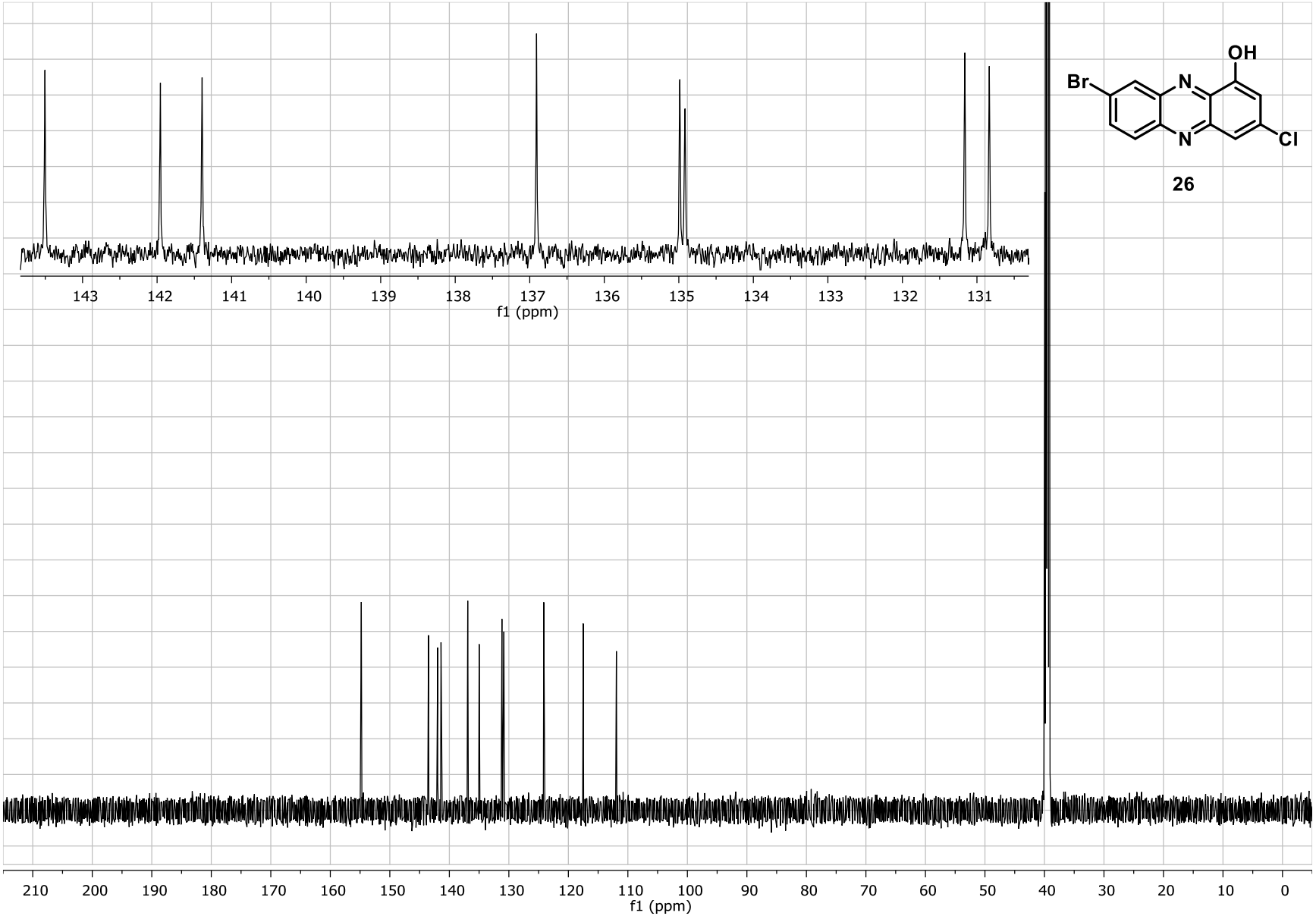

OH
Cl
N
N
Cl
Cl
24
8.15 8.10 8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25
f1 (ppm)
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

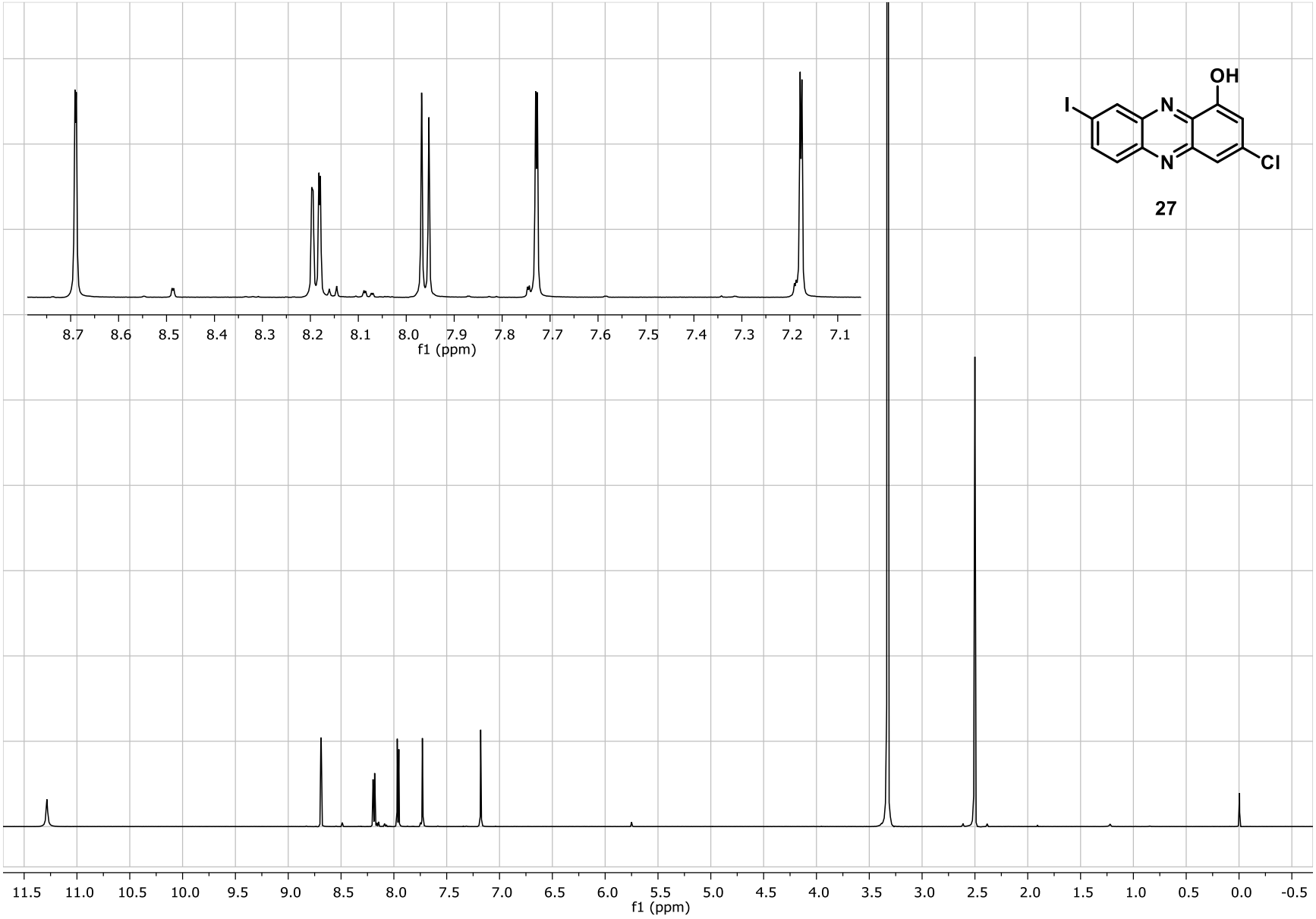

OH
Cl
N
N
Cl
Cl
24
150
145
140
135
130
125
120
115
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

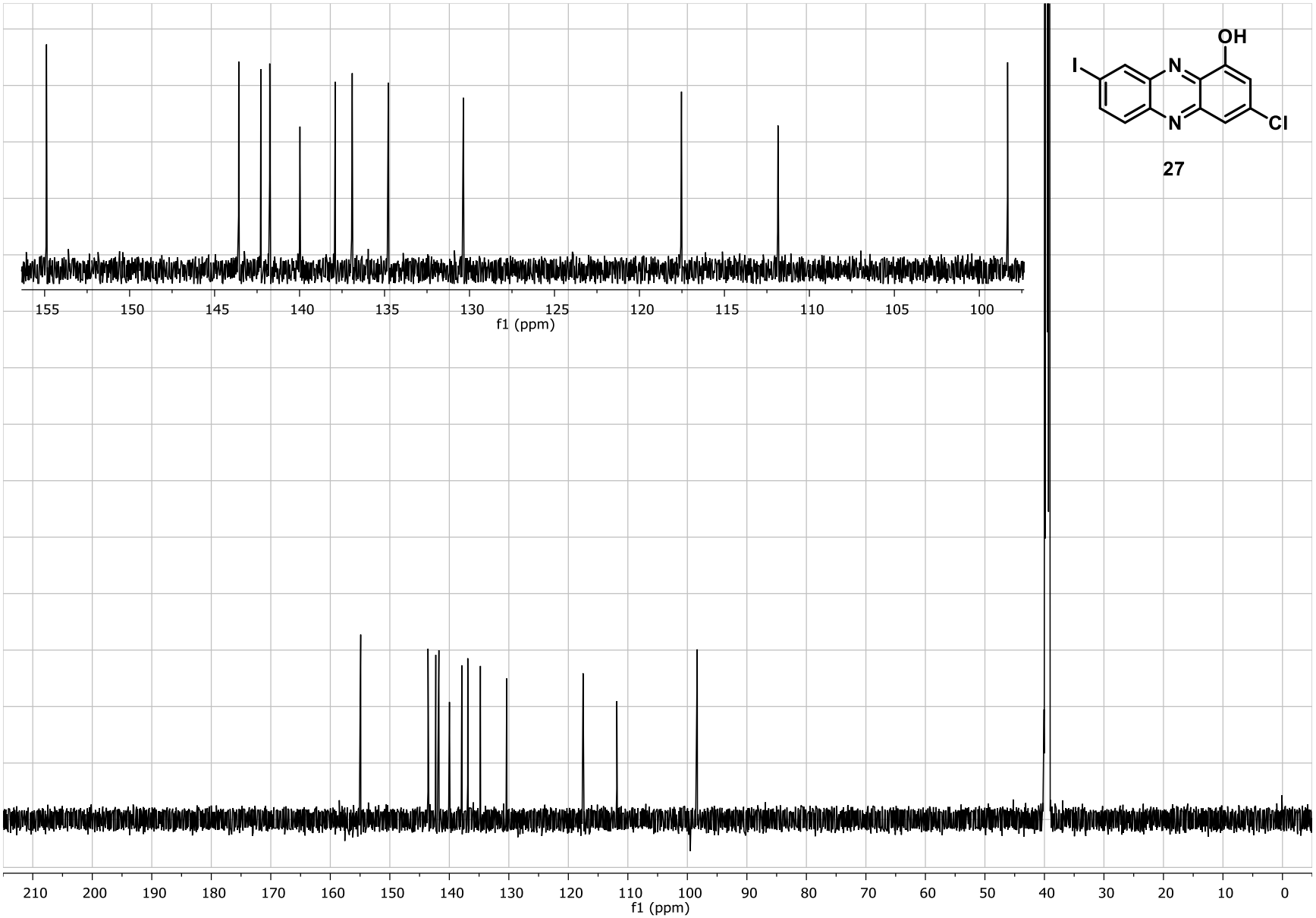

OH
N
N
Cl
Br
25
8.25 8.20 8.15 8.10 8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25
f1 (ppm)
7.27 7.26 7.25
f1 (ppm)
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

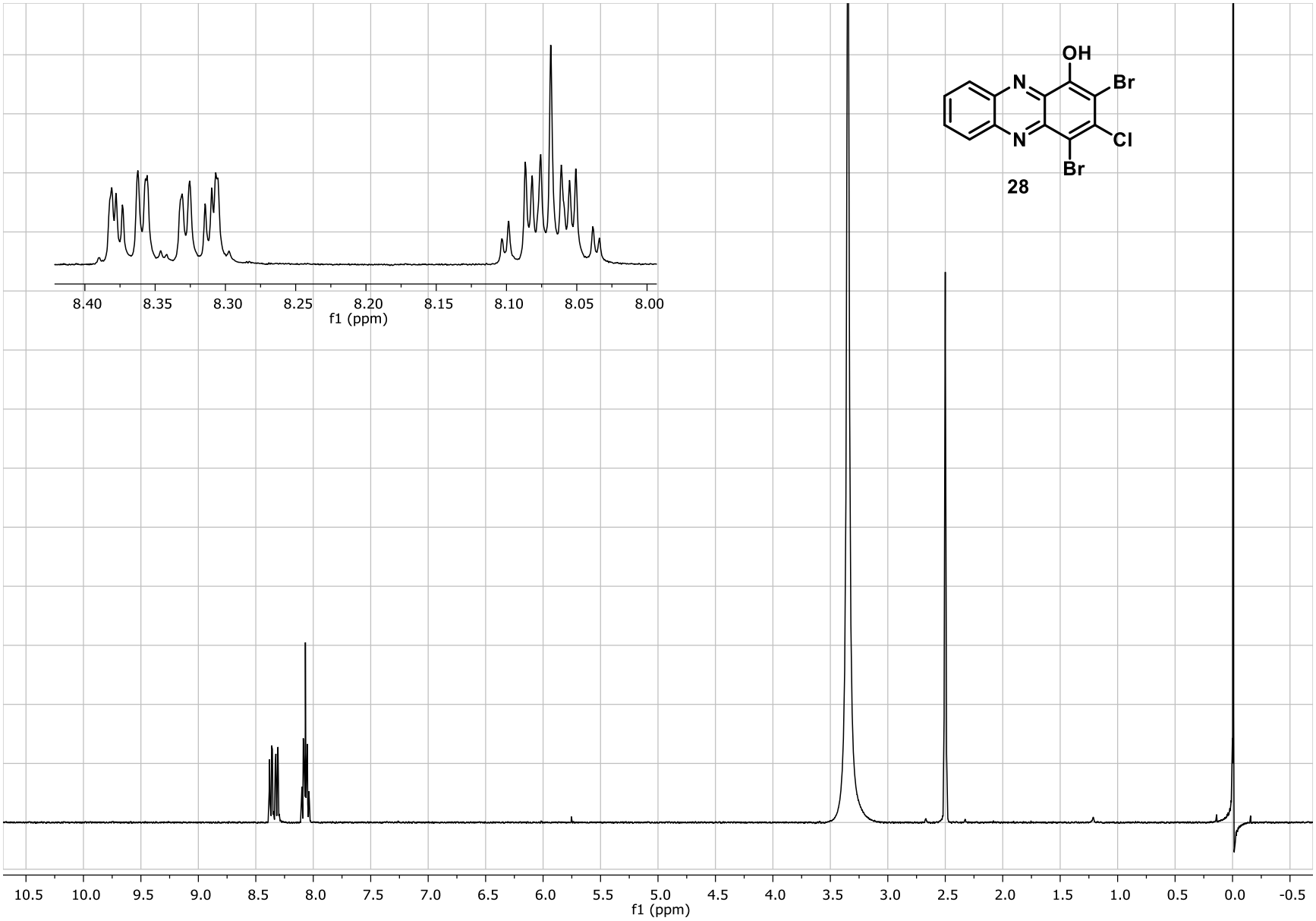

OH
N
N
Cl
Br
25
150
145
140
135
130
125
120
115
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

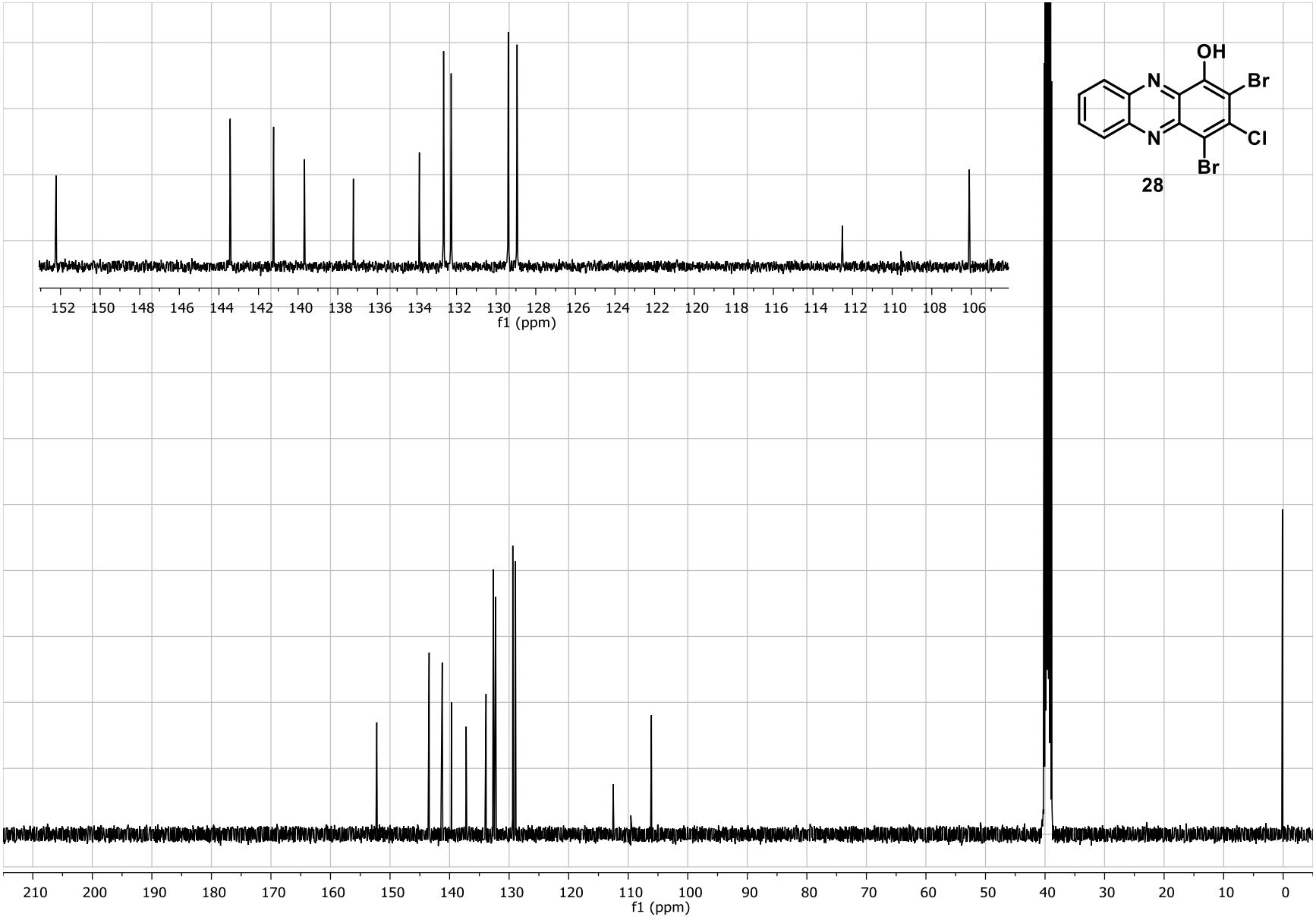

OH
Br
N
N
Cl
26
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
f1 (ppm)
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

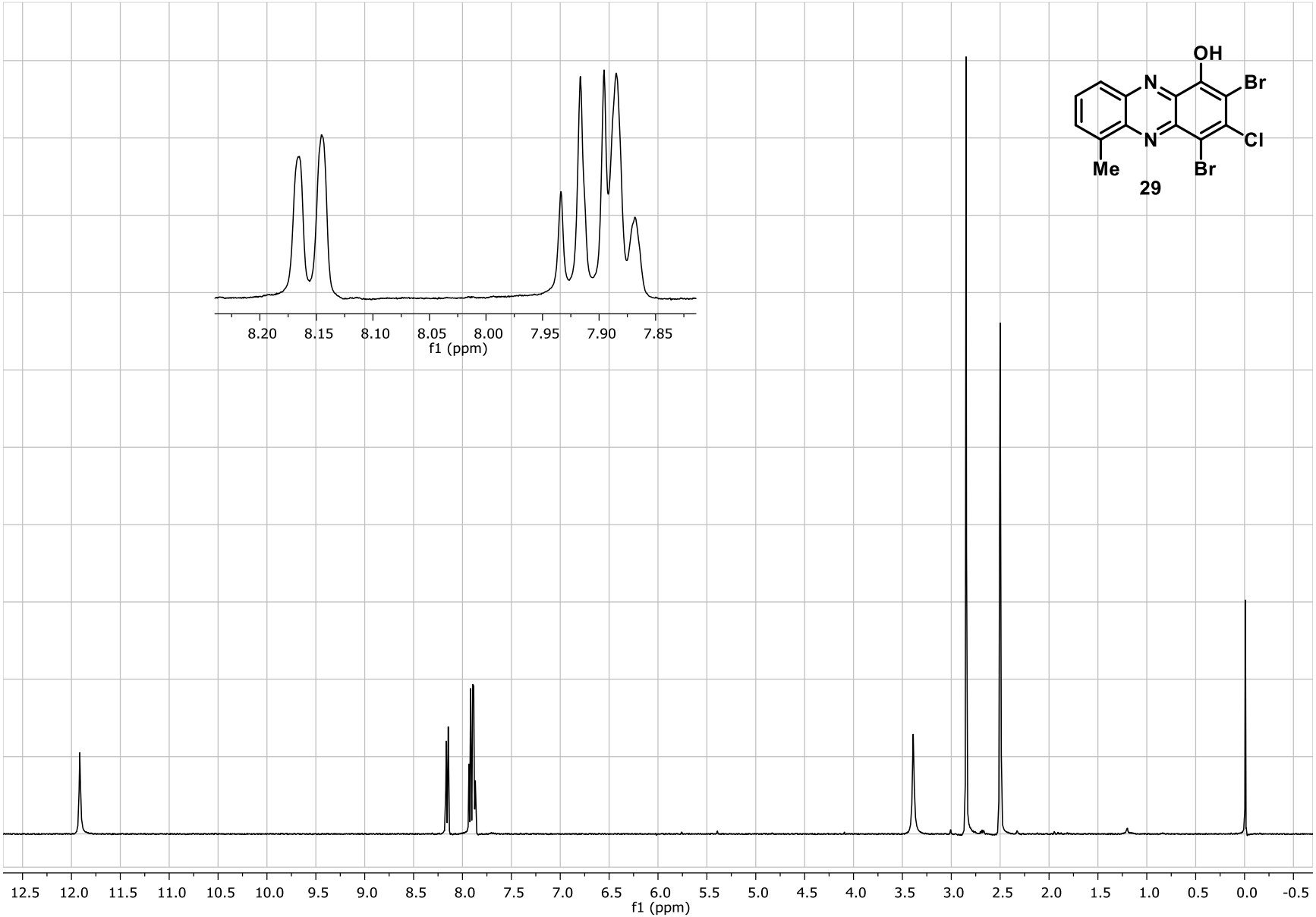

OH
Br
N
N
Cl
26
143
142
141
140
139
138
137
136
135
134
133
132
131
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

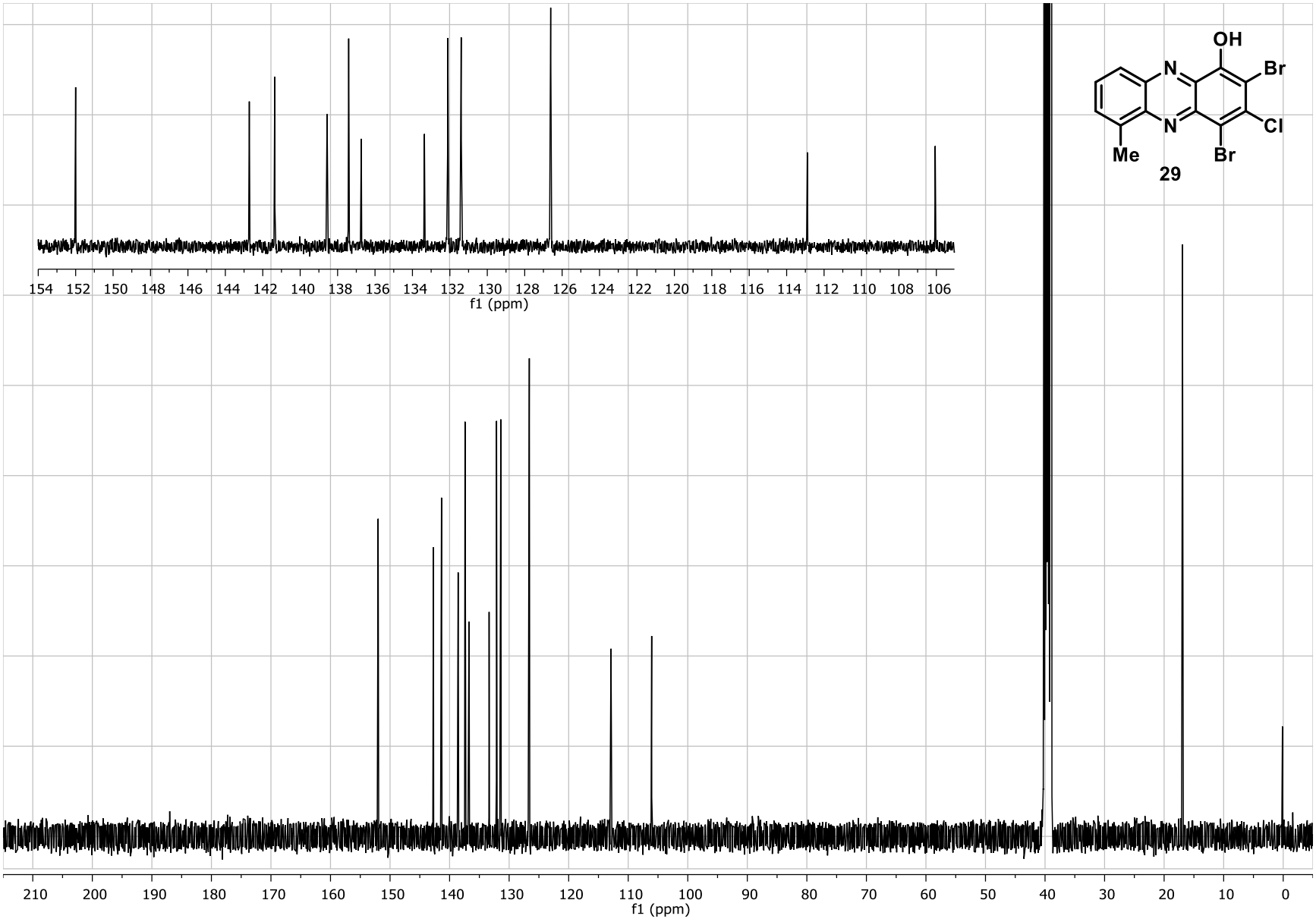

OH
I
N
N
Cl
27
8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1
f1 (ppm)
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

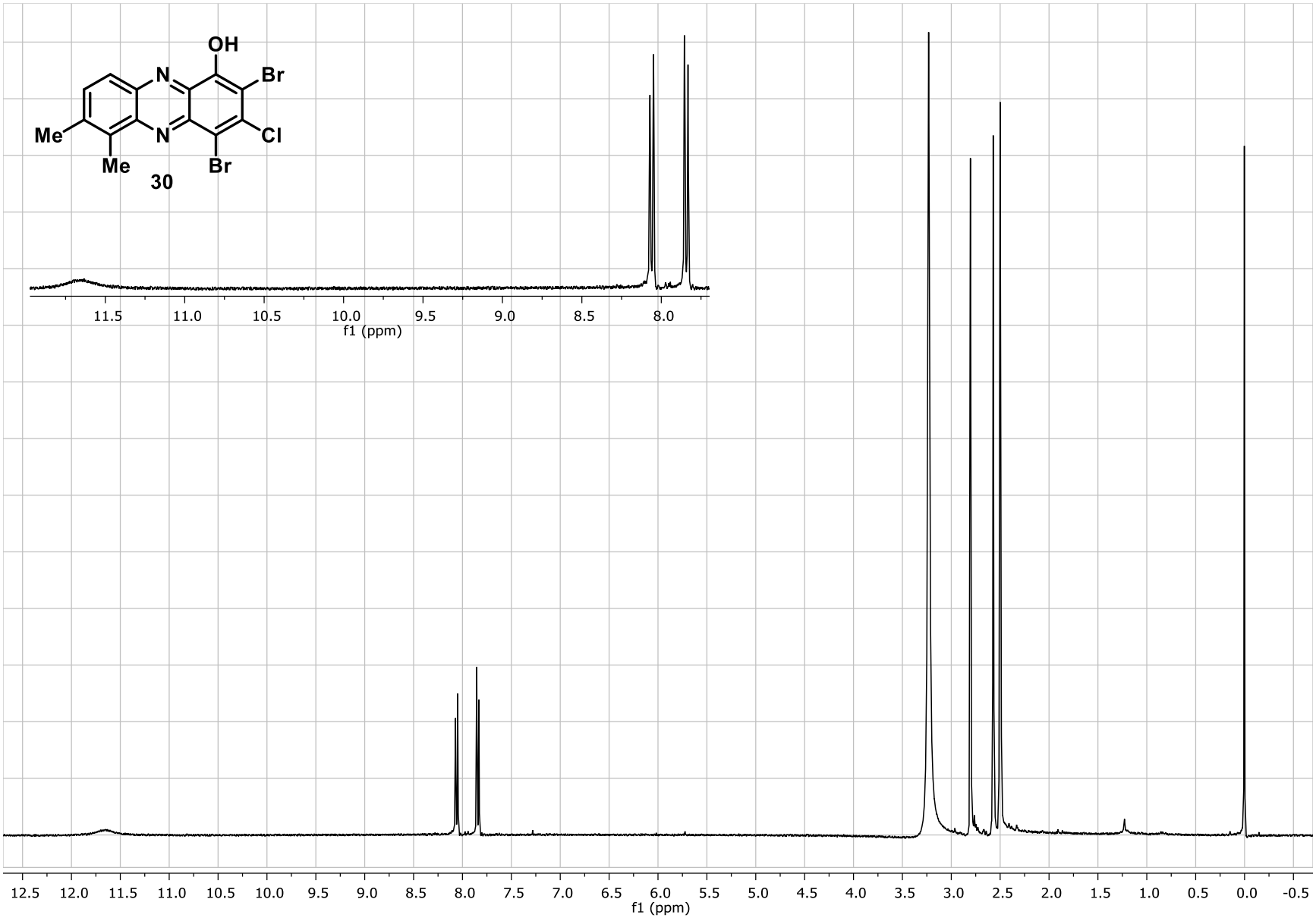

OH
I
N
N
Cl
27
155
150
145
140
135
130
125
120
115
110
105
100
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

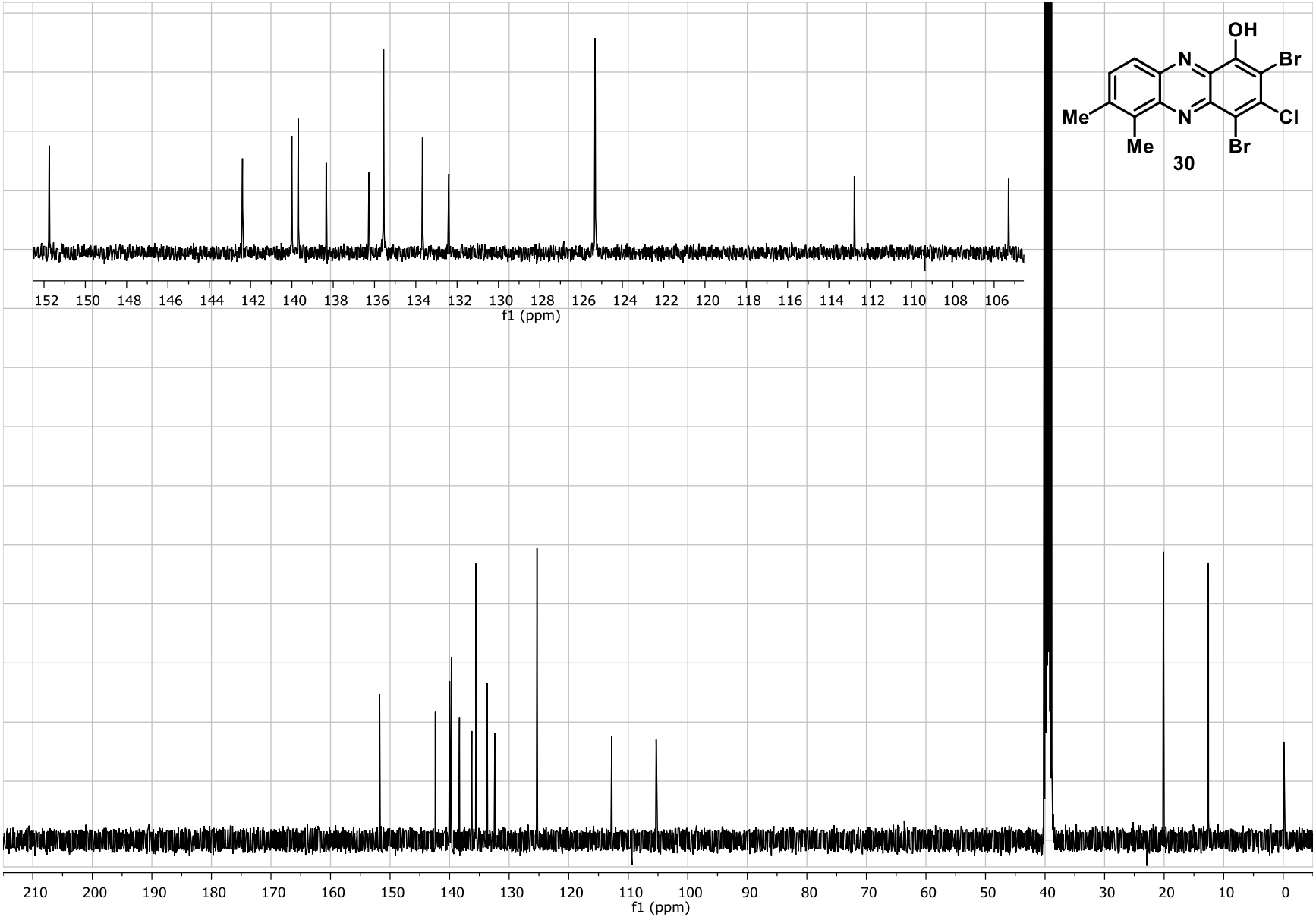

OH
N
Br
N
Cl
Br
28
8.40
8.35
8.30
8.25
8.20
8.15
8.10
8.05
8.00
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

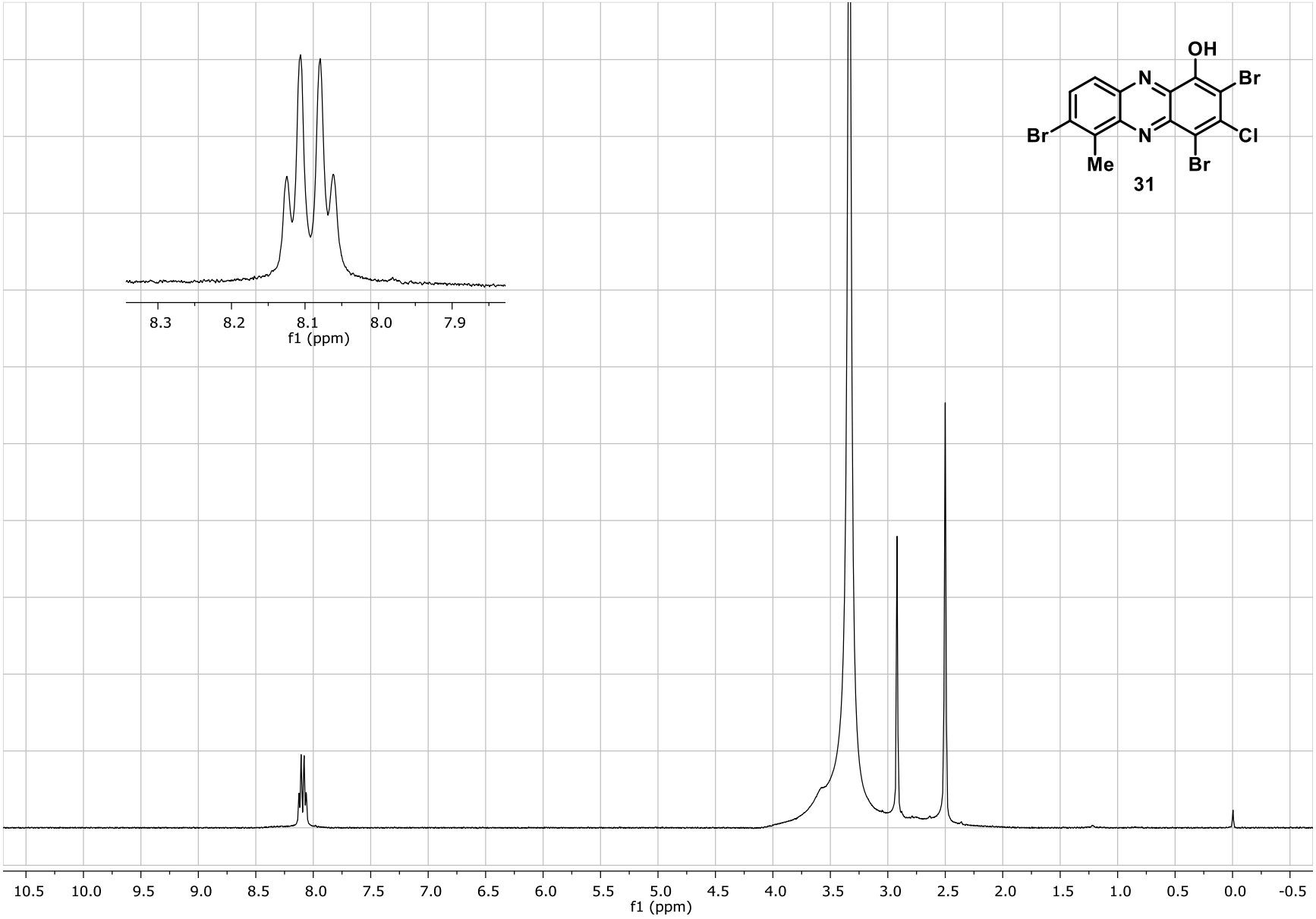

OH
Br
N
N
Cl
Br
28
152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112 110 108 106
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

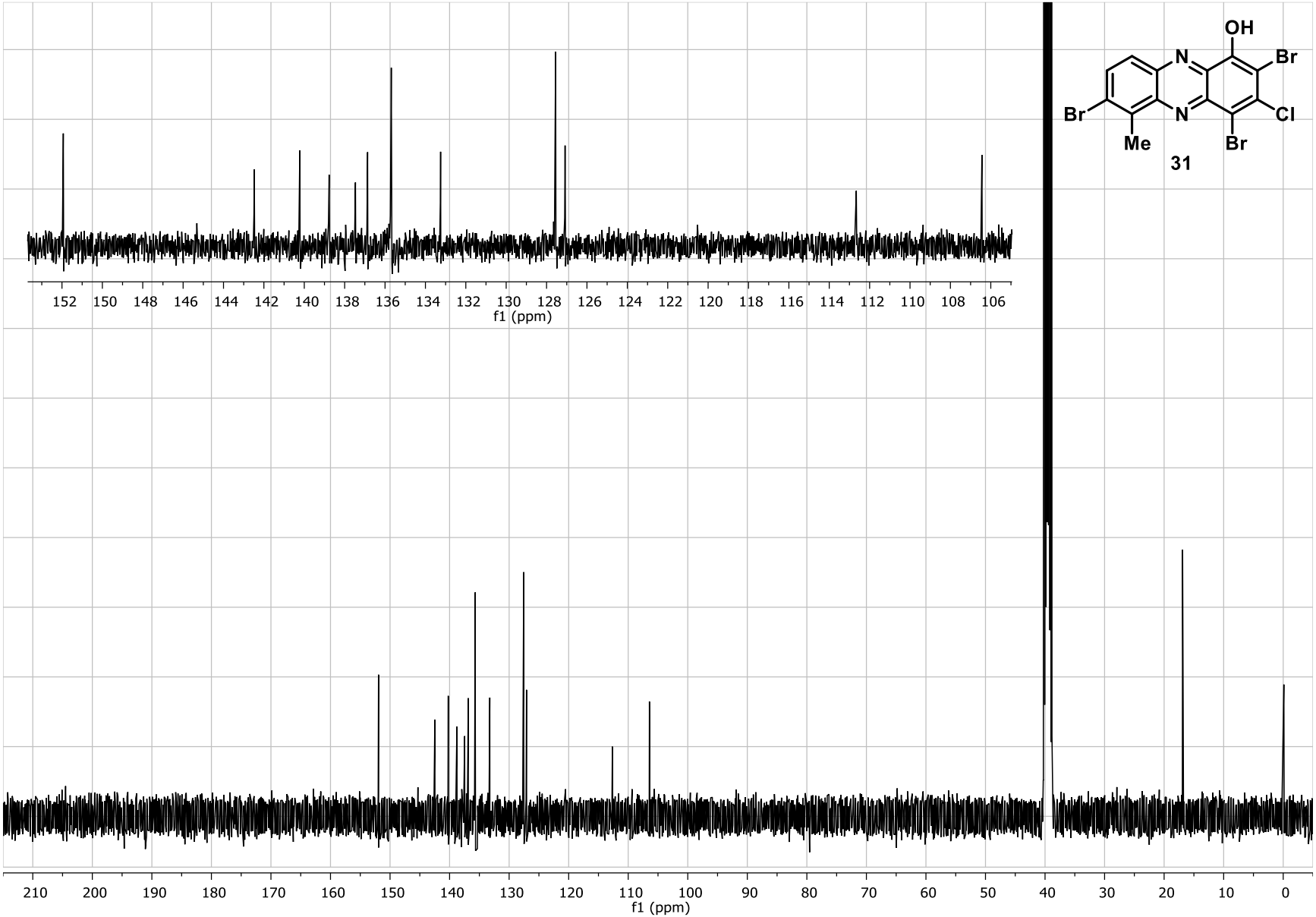

OH
N
Br
N
Cl
Me
Br
29
8.20
8.15
8.10
8.05
8.00
7.95
7.90
7.85
f1 (ppm)
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

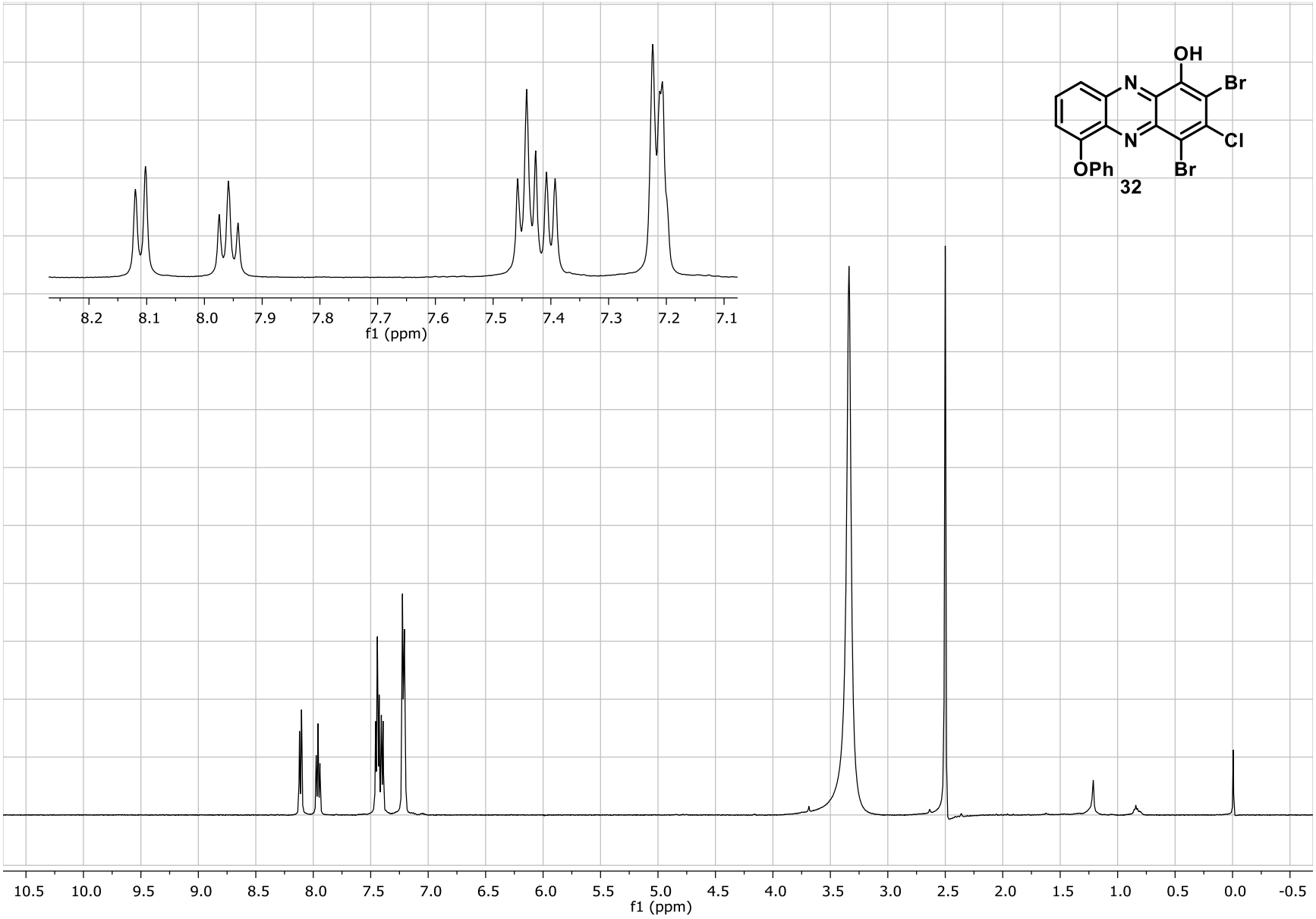

OH
Br
N
N
Cl
Me
Br
29
154 152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112 110 108 106
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

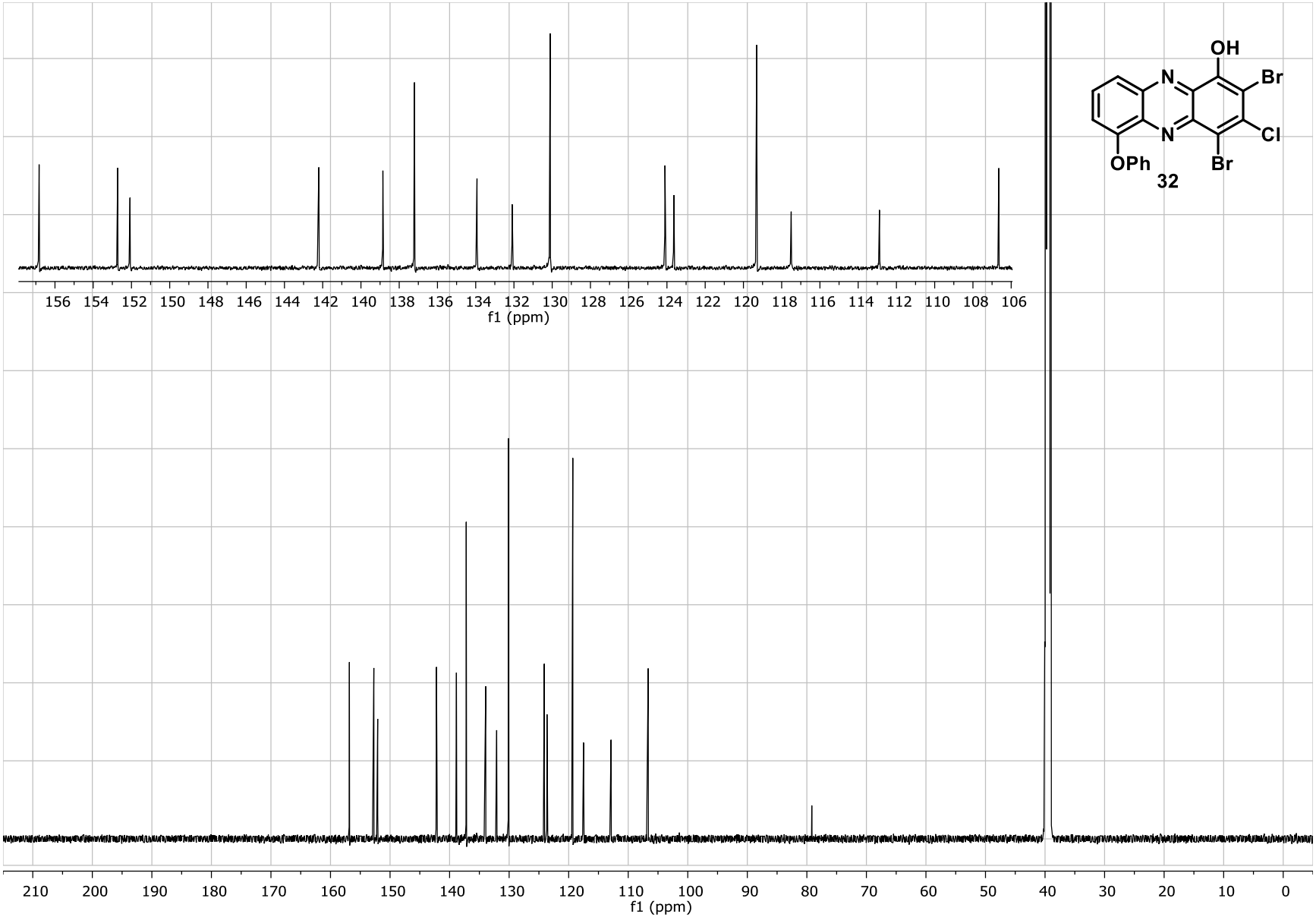

OH
Br
N
N
Me
Cl
Me
Br
30
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
f1 (ppm)
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

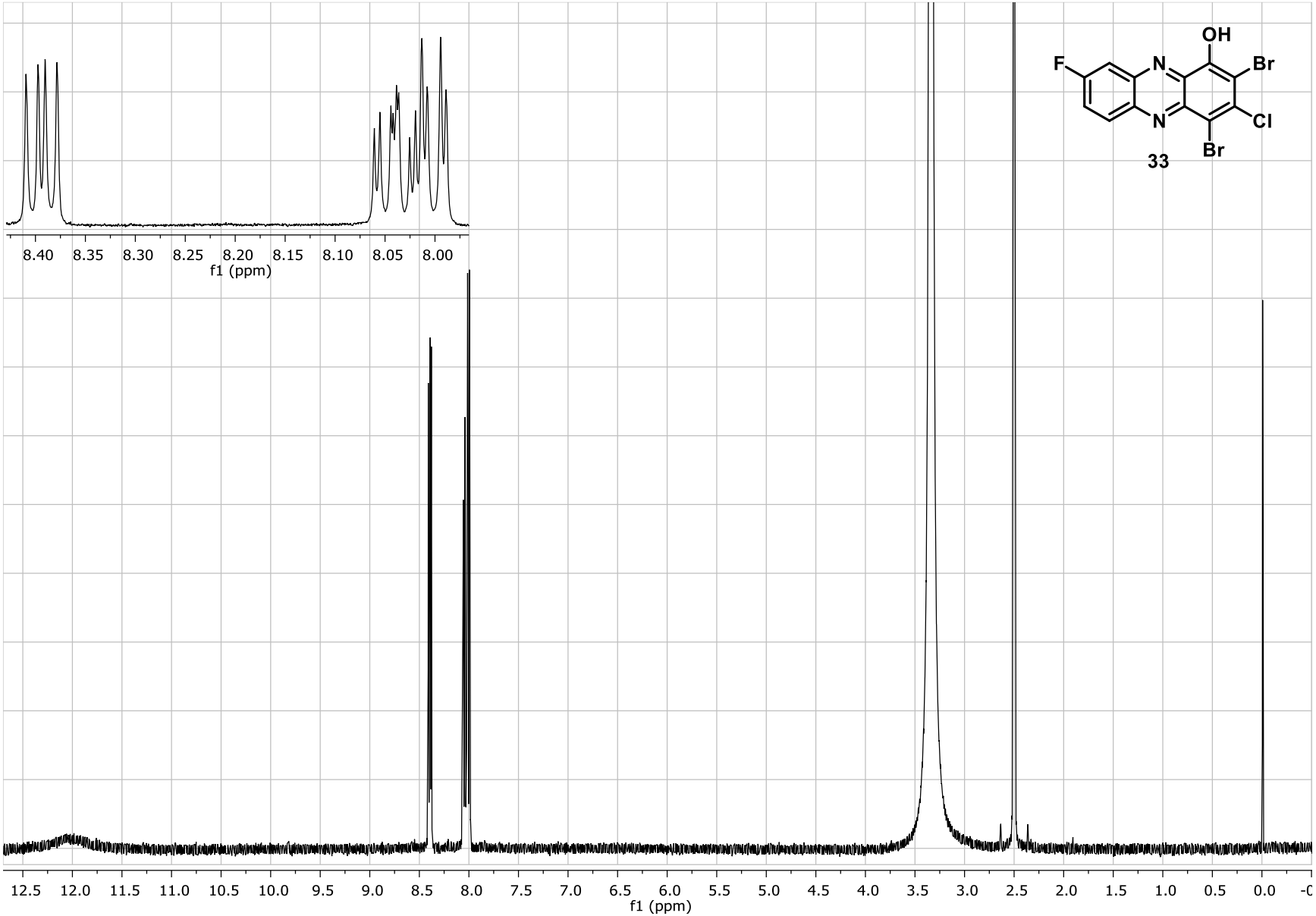

OH
Br
N
Me
N
Cl
Me
Br
30
152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112 110 108 106
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

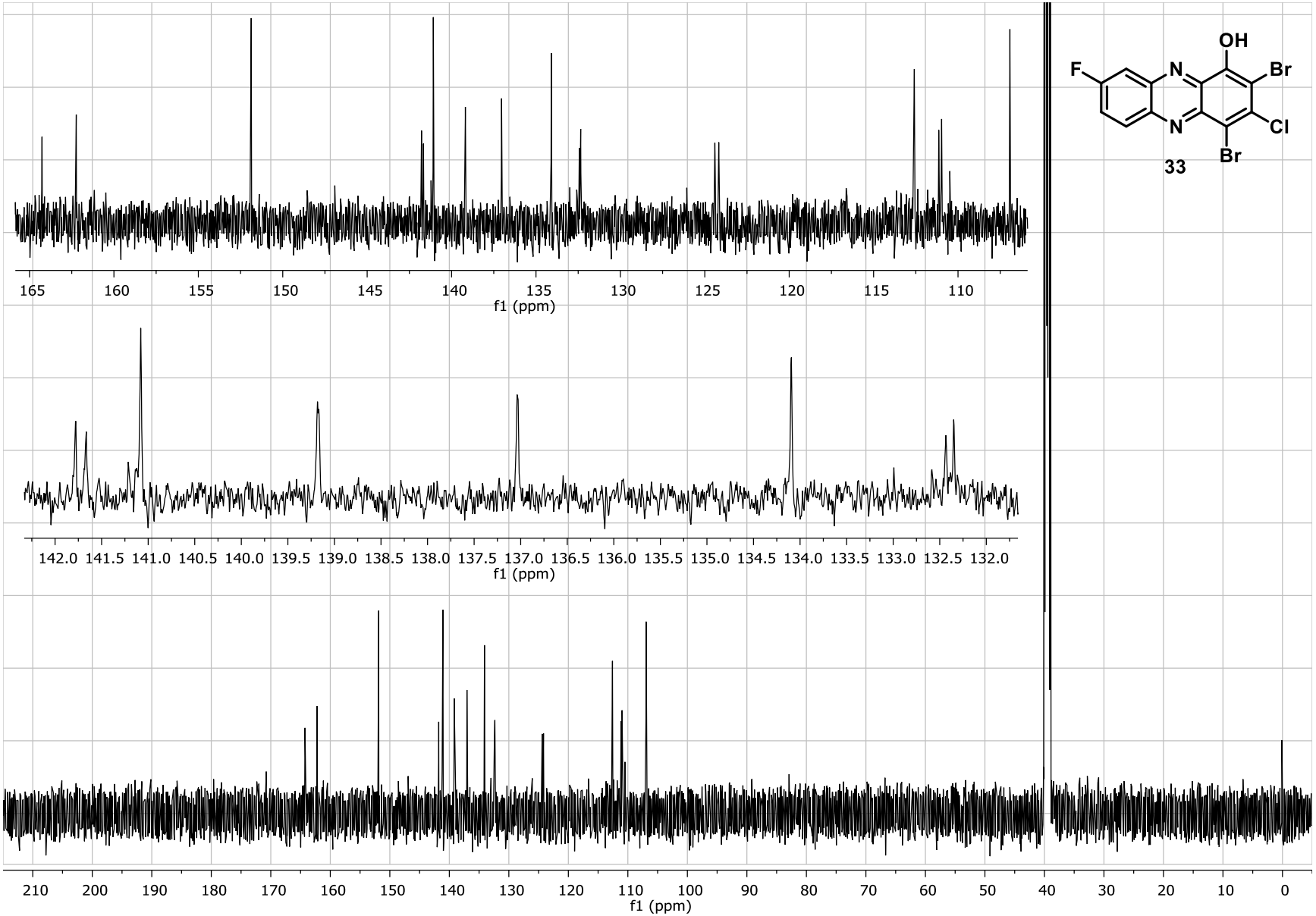

OH
Br
N
Br
N
Cl
Me
Br
31
8.3
8.2
8.1
8.0
7.9
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

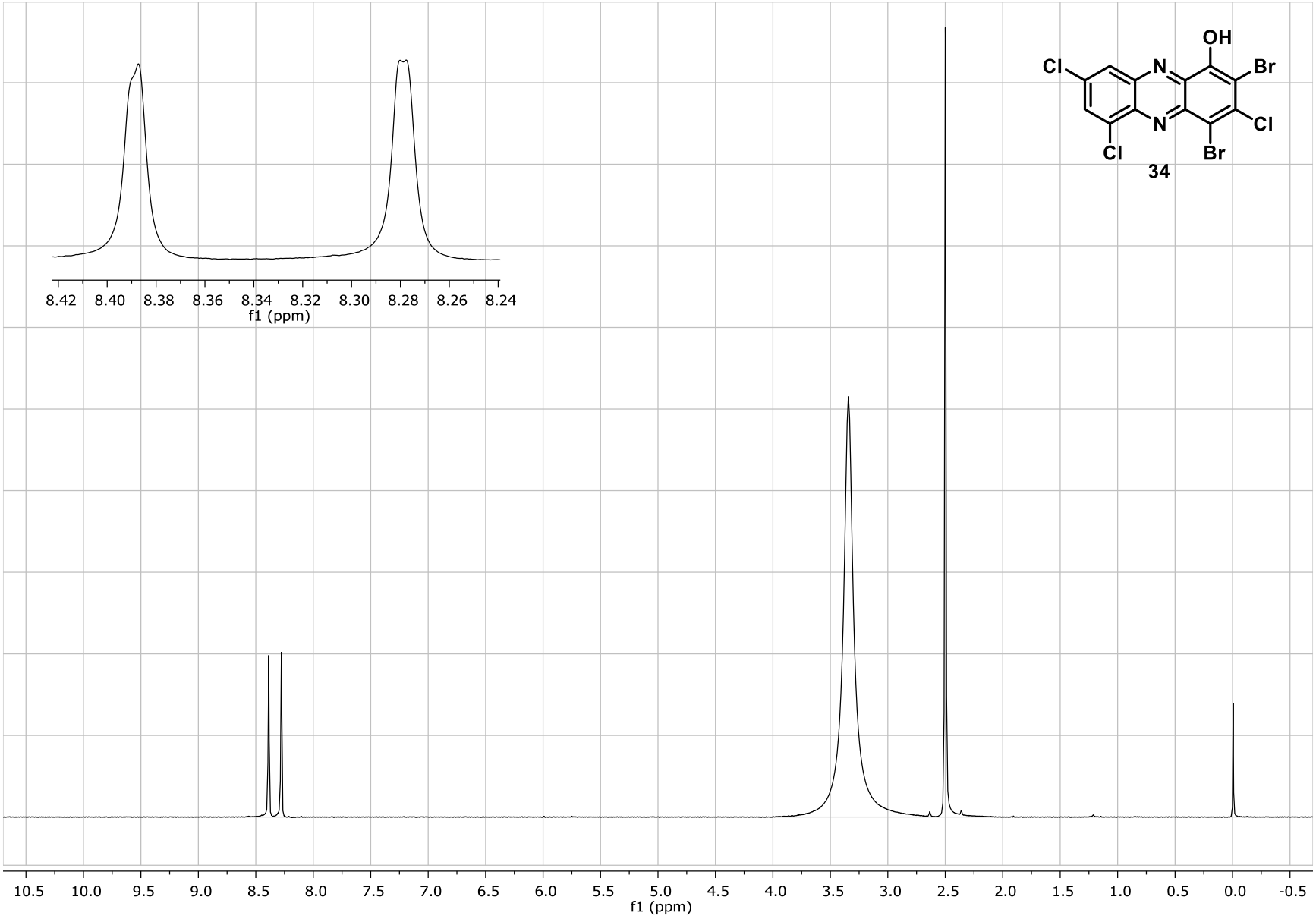

OH
Br
N
Br
N
Cl
Me
Br
31
152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112 110 108 106
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

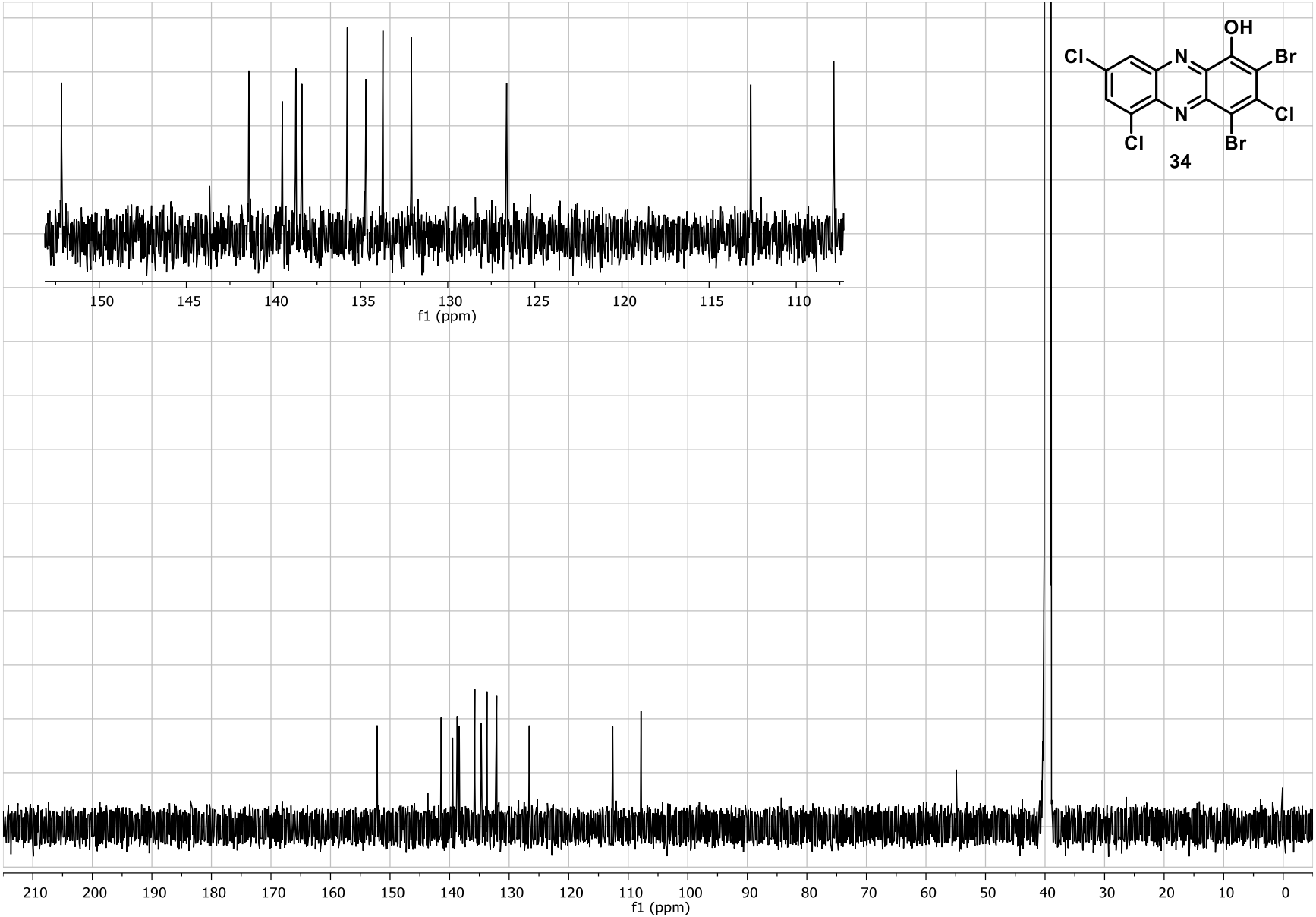

OH
Br
N
N
Cl
OPh
Br
32
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

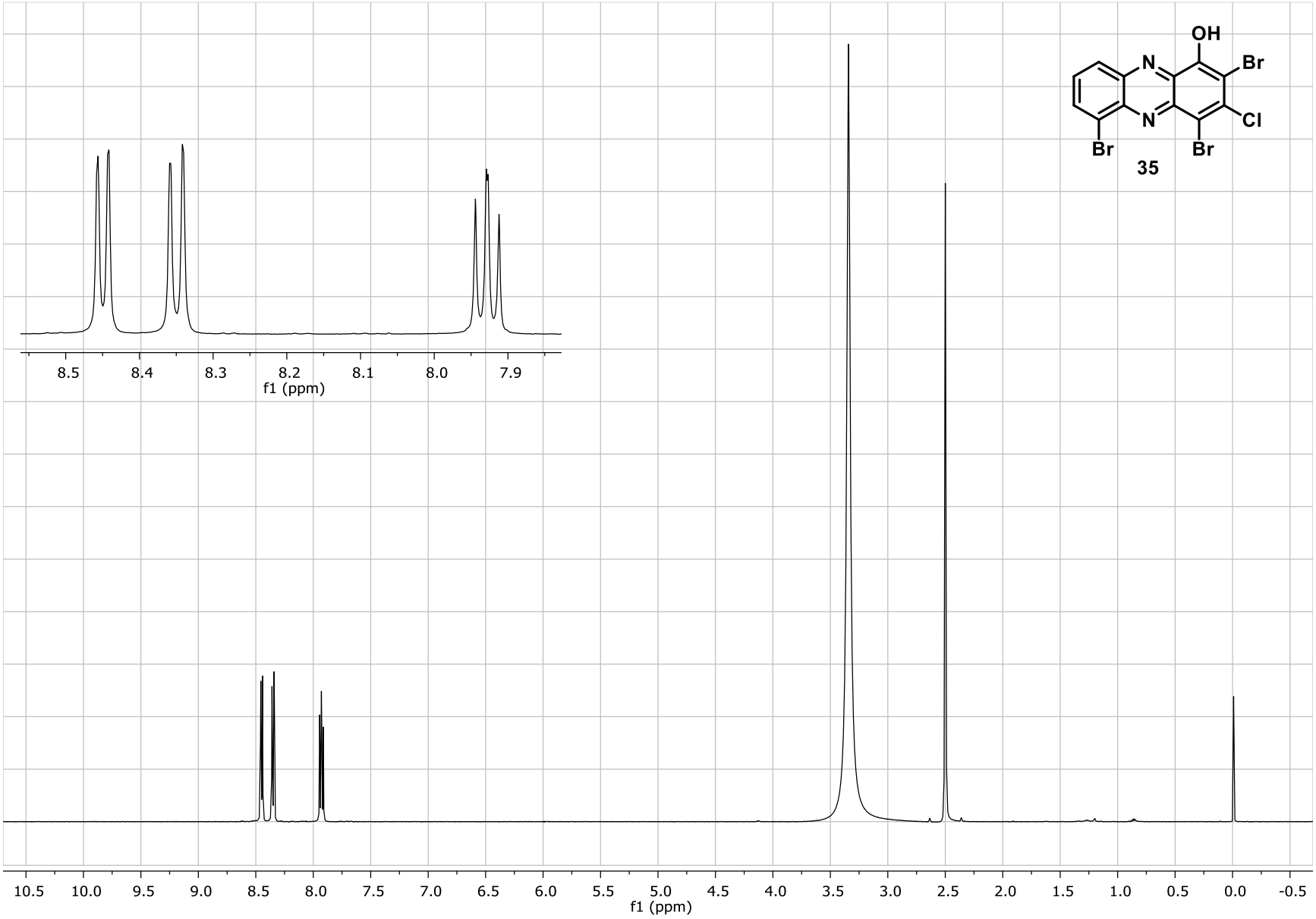

OH
Br
N
N
Cl
OPh
Br
32
156 154 152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112 110 108 106
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

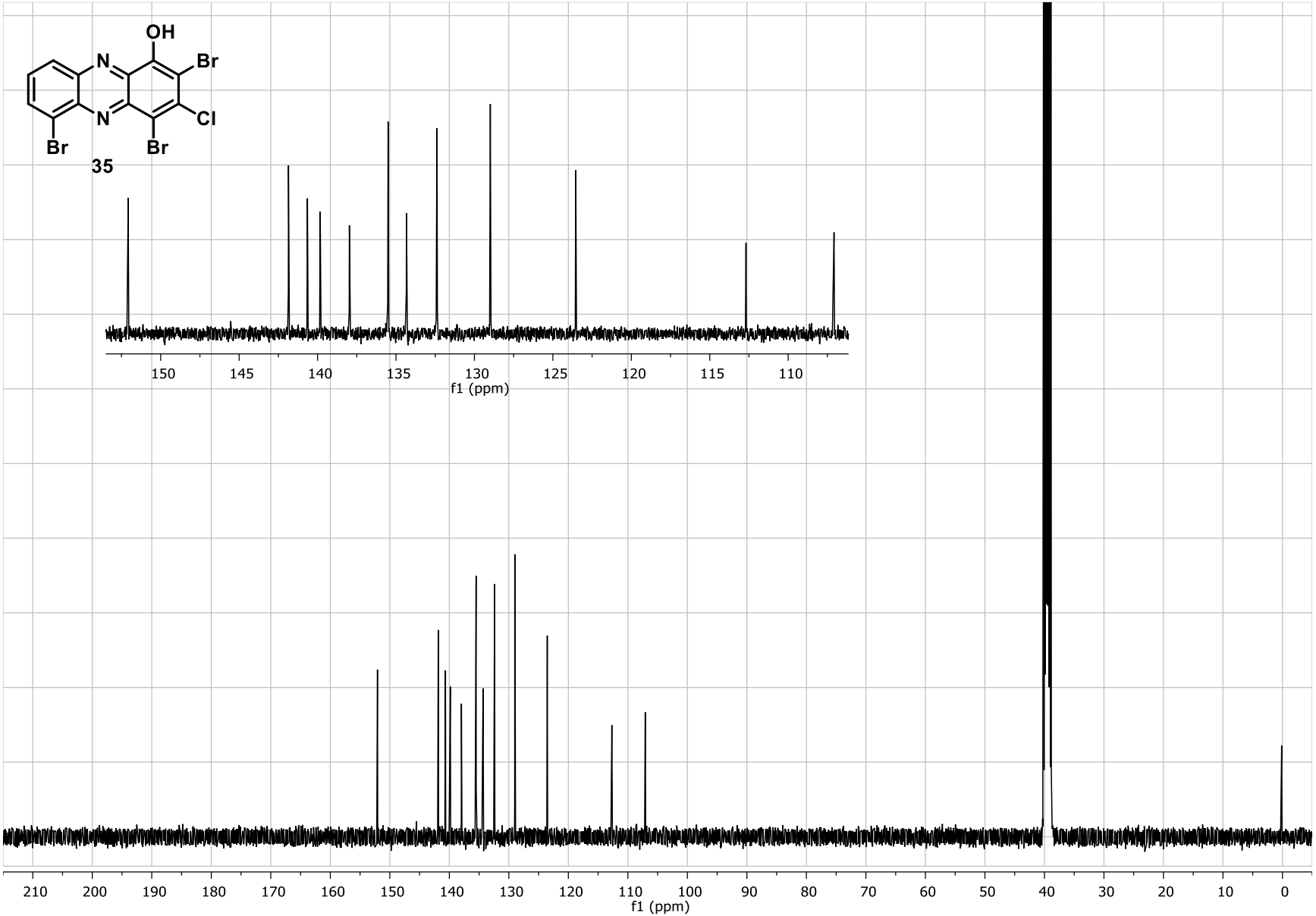

OH
F
N
Br
N
Cl
Br
33
8.40 8.35 8.30 8.25 8.20 8.15 8.10 8.05 8.00
f1 (ppm)
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

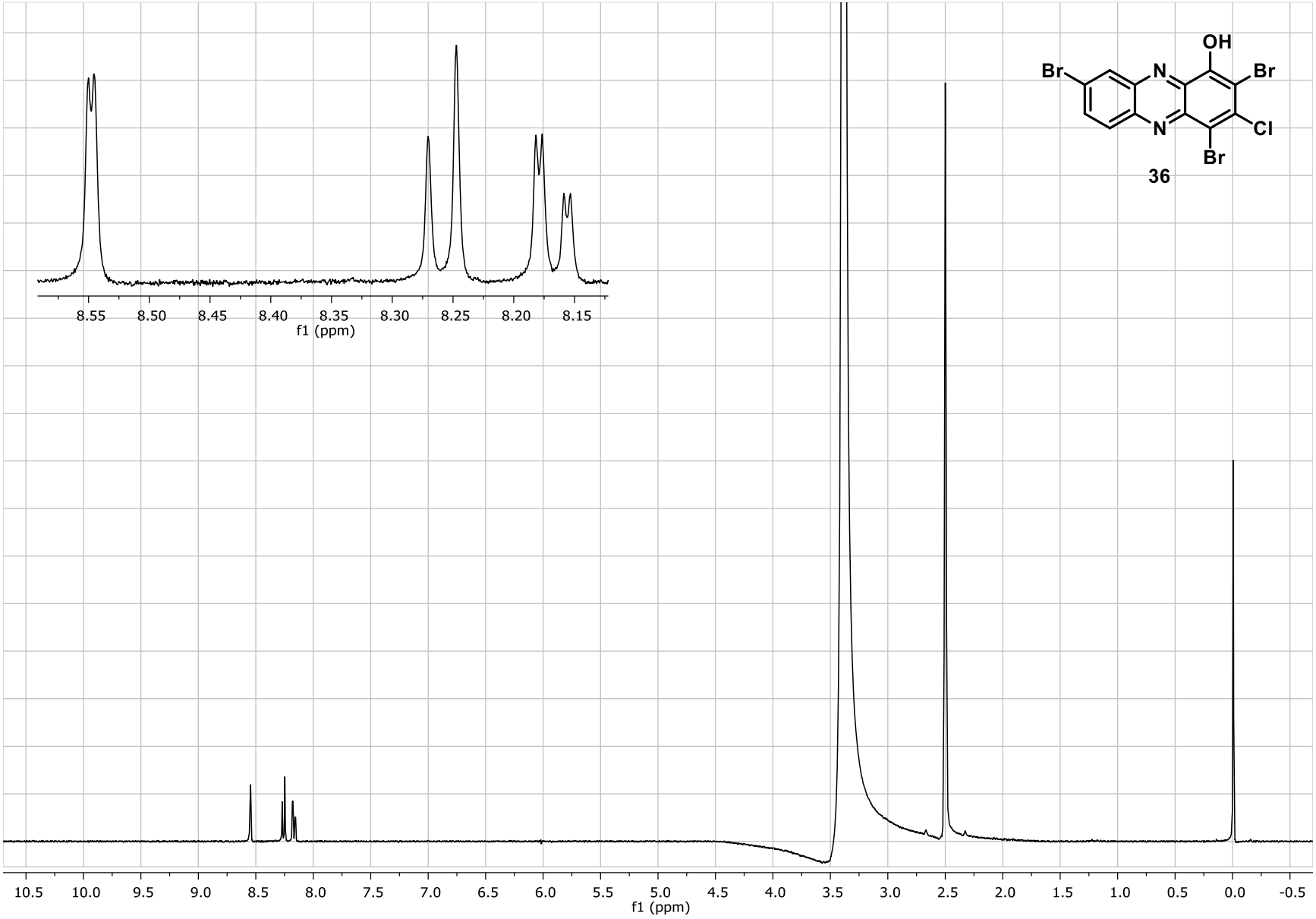

OH
F
N
Br
N
Cl
Br
33
165 160 155 150 145 140 135 130 125 120 115 110
f1 (ppm)
142.0 141.5 141.0 140.5 140.0 139.5 139.0 138.5 138.0 137.5 137.0 136.5 136.0 135.5 135.0 134.5 134.0 133.5 133.0 132.5 132.0
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

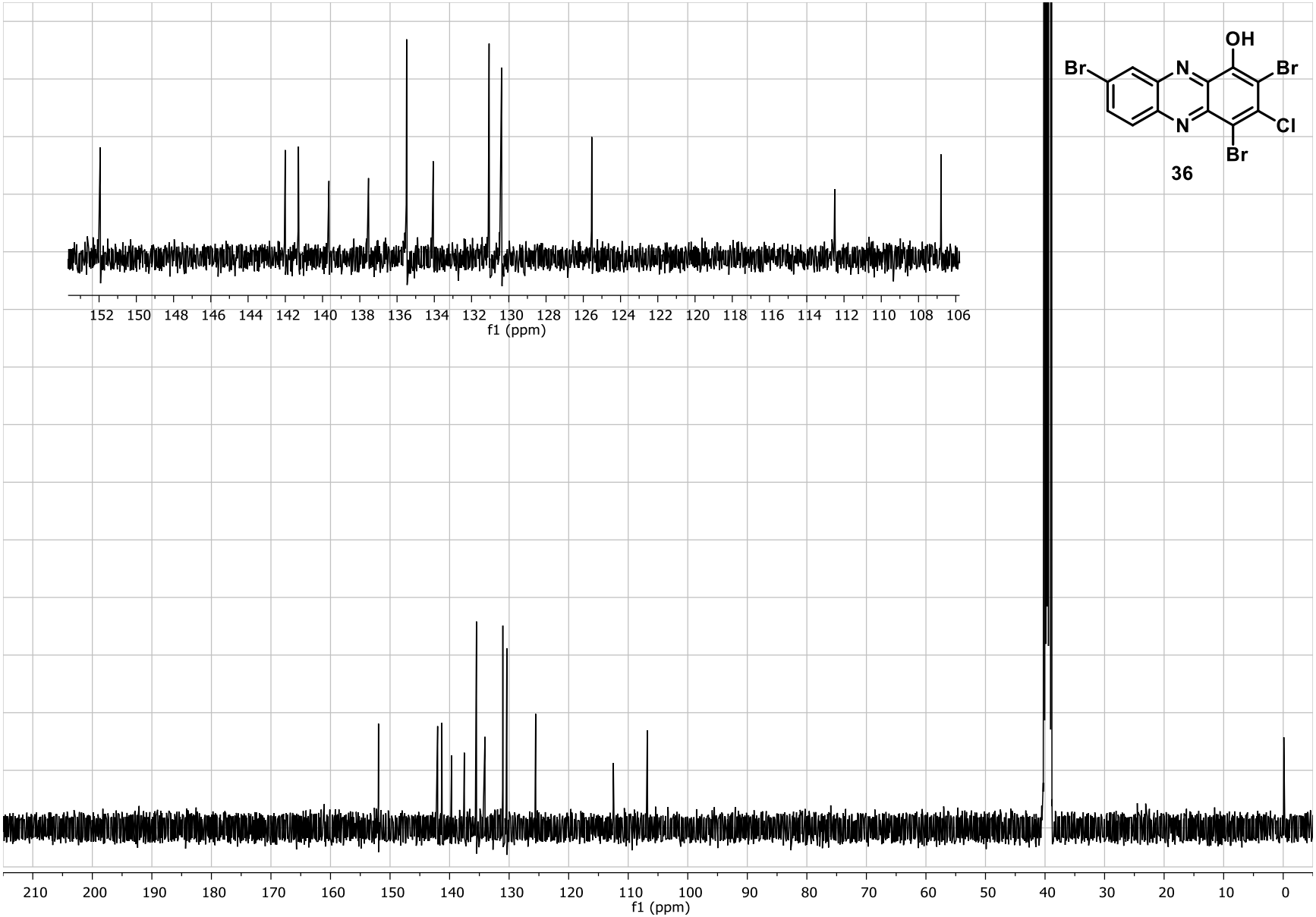

8.42 8.40 8.38 8.36 8.34 8.32 8.30 8.28 8.26 8.24
f1 (ppm)
OH
Cl
N
Br
N
Cl
Cl
Br
34
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

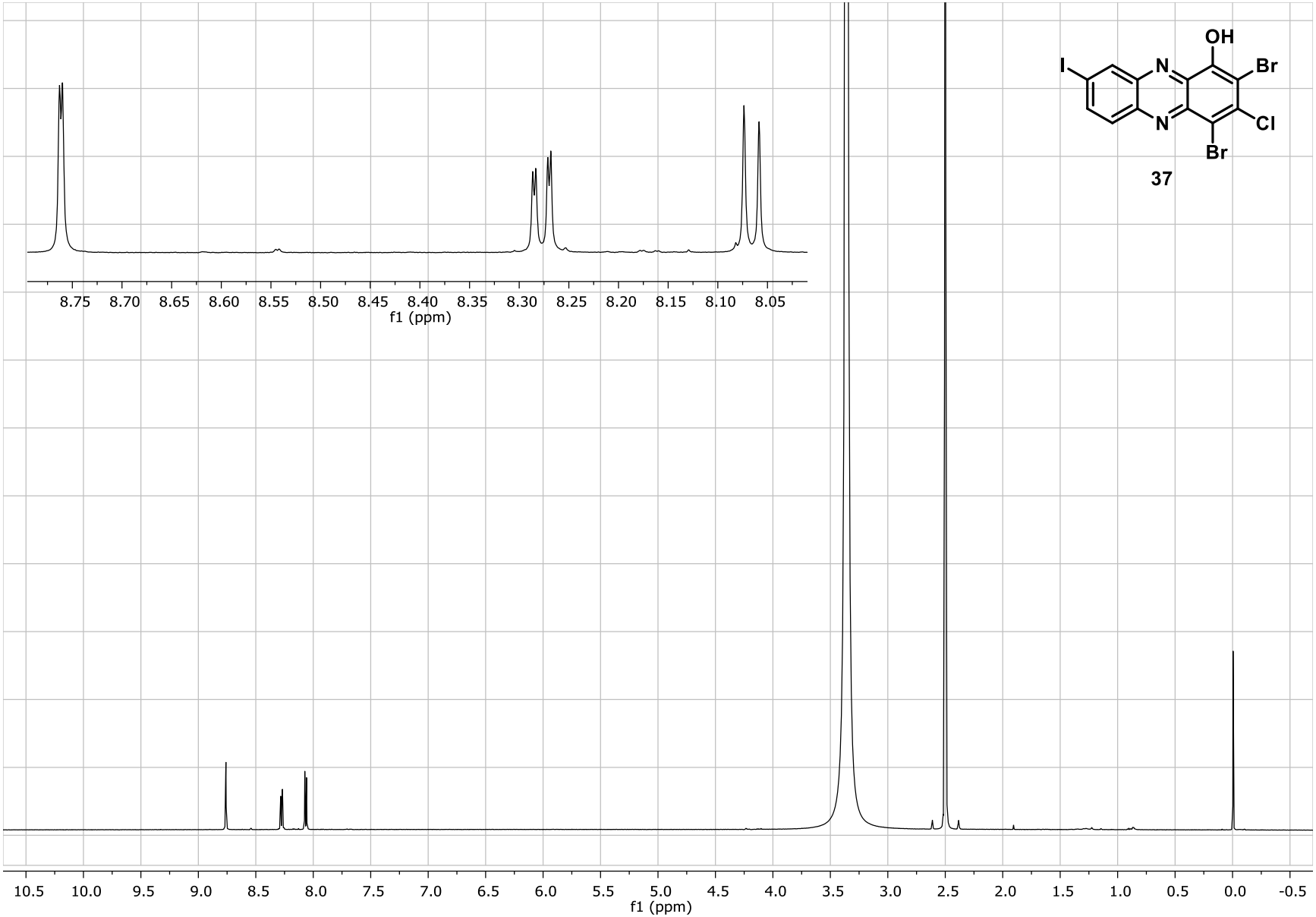

OH
Cl
N
Br
N
Cl
Cl
Br
34
150
145
140
135
130
125
120
115
110
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

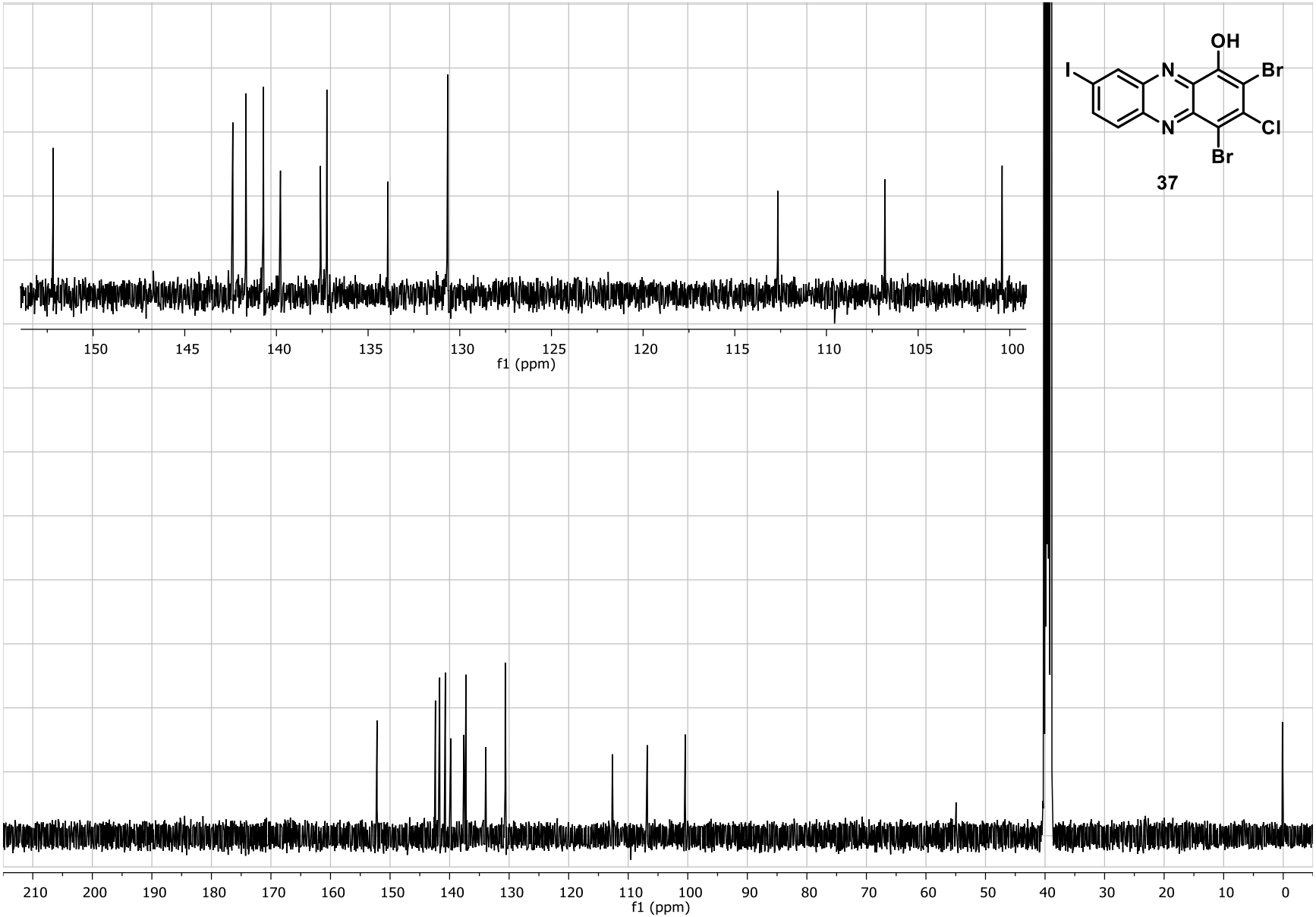

OH
Br
N
N
Cl
Br
Br
35
8.5
8.4
8.3
8.2
8.1
8.0
7.9
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

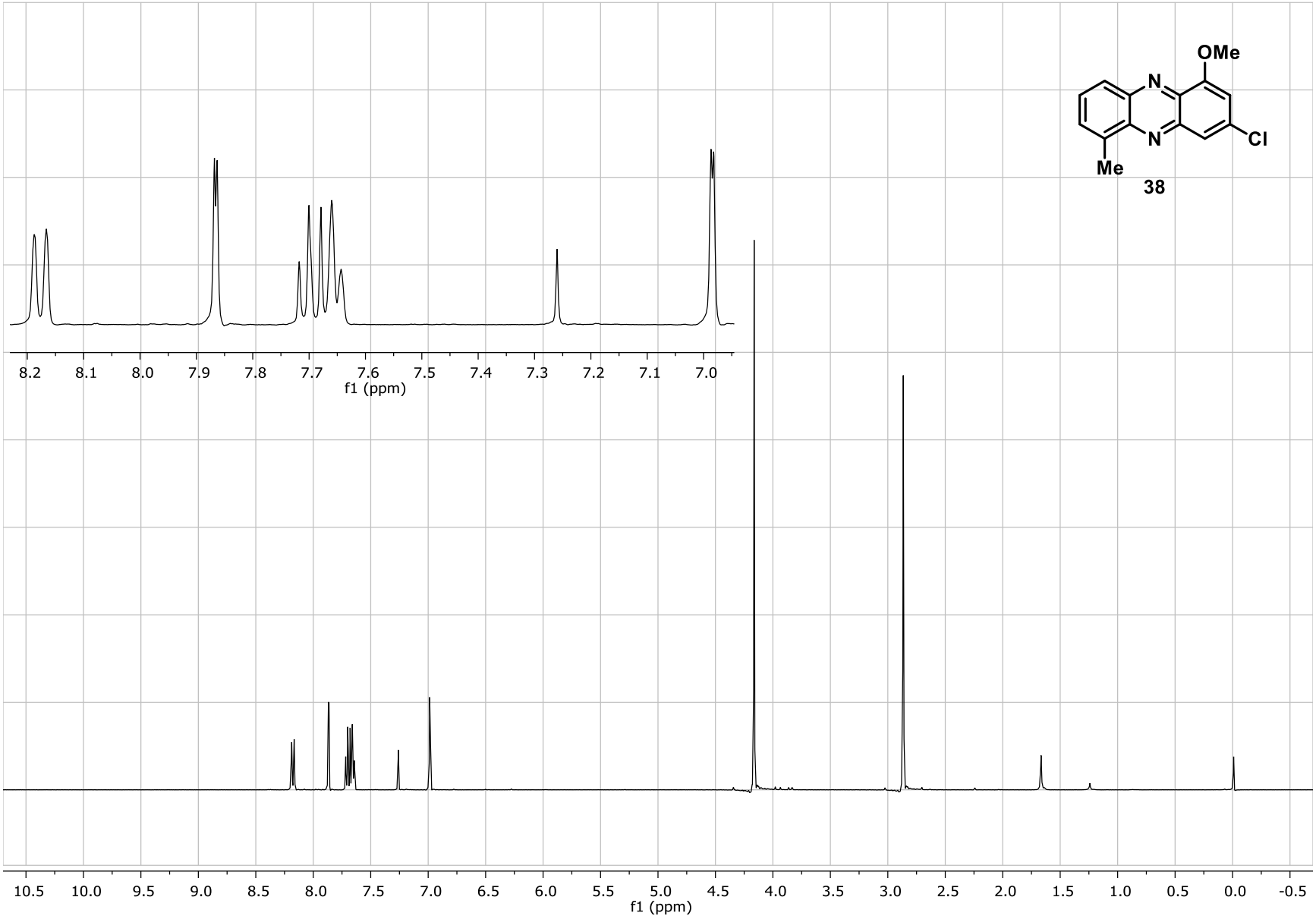

OH
N
Br
N
Cl
Br
Br
35
150
145
140
135
130
125
120
115
110
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

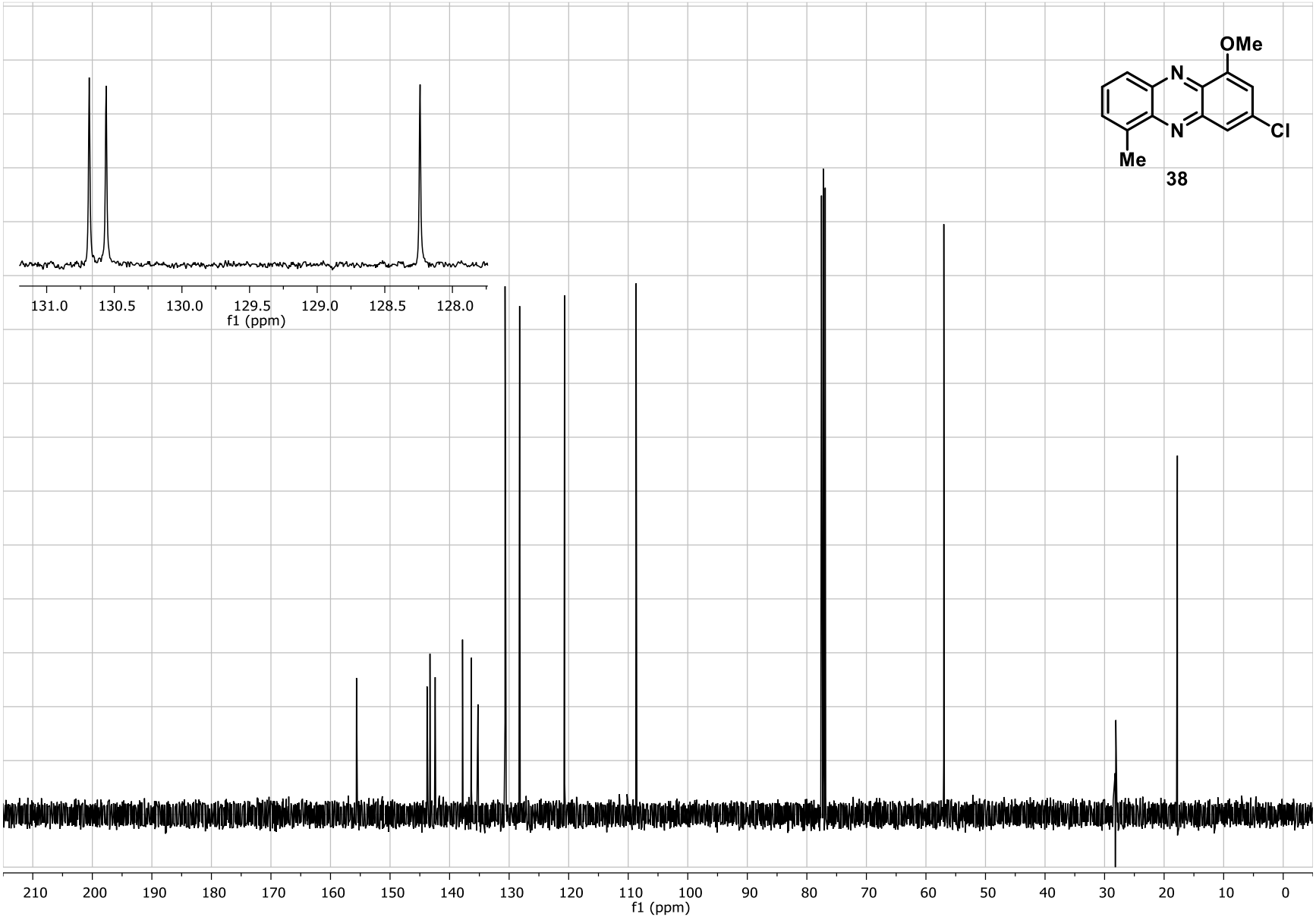

8.55
8.50
8.45
8.40
8.35
8.30
8.25
8.20
8.15
f1 (ppm)
OH
Br
N
Br
N
Cl
Br
36
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

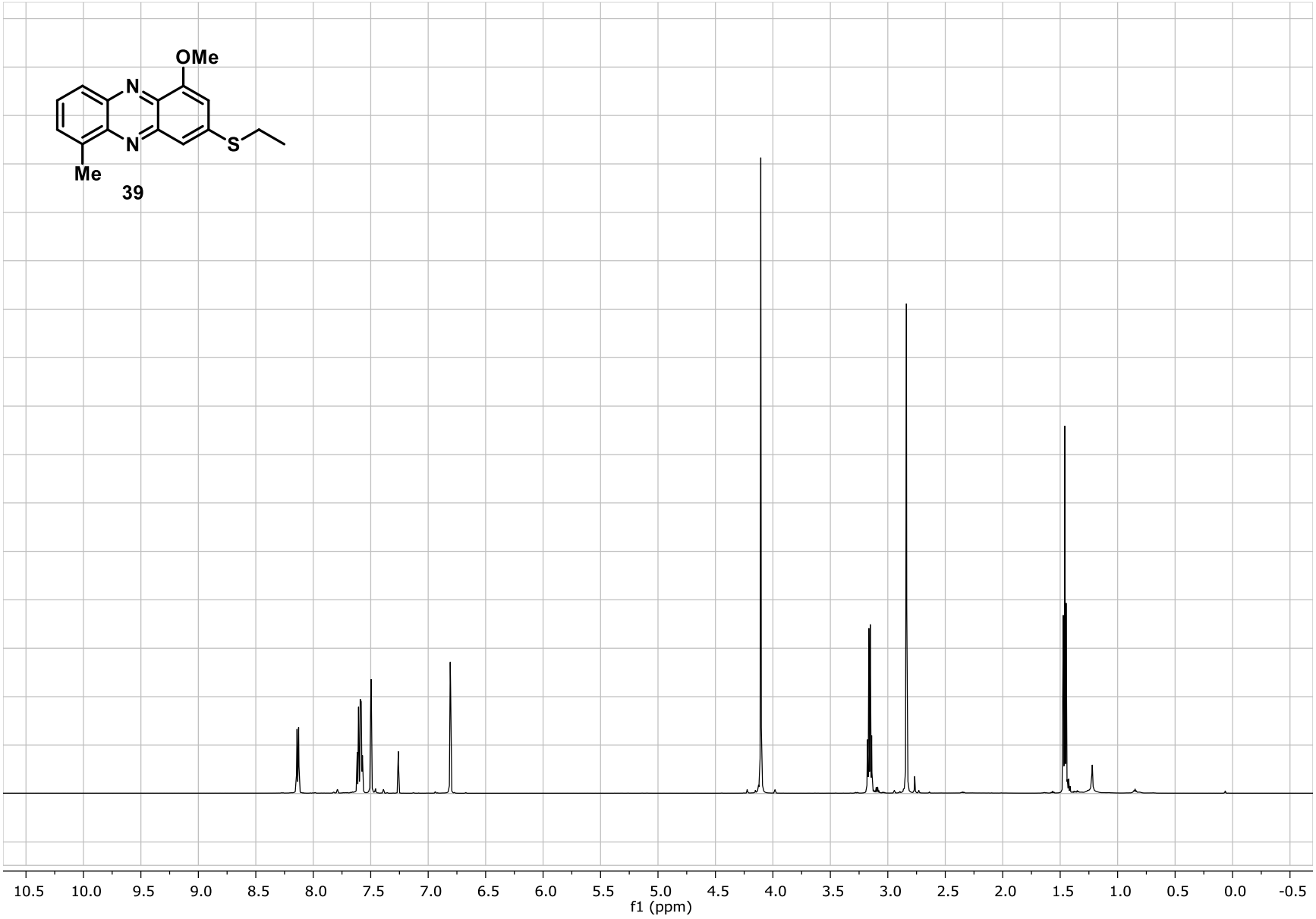

OH
Br
Br
N
N
Cl
Br
36
152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112 110 108 106
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

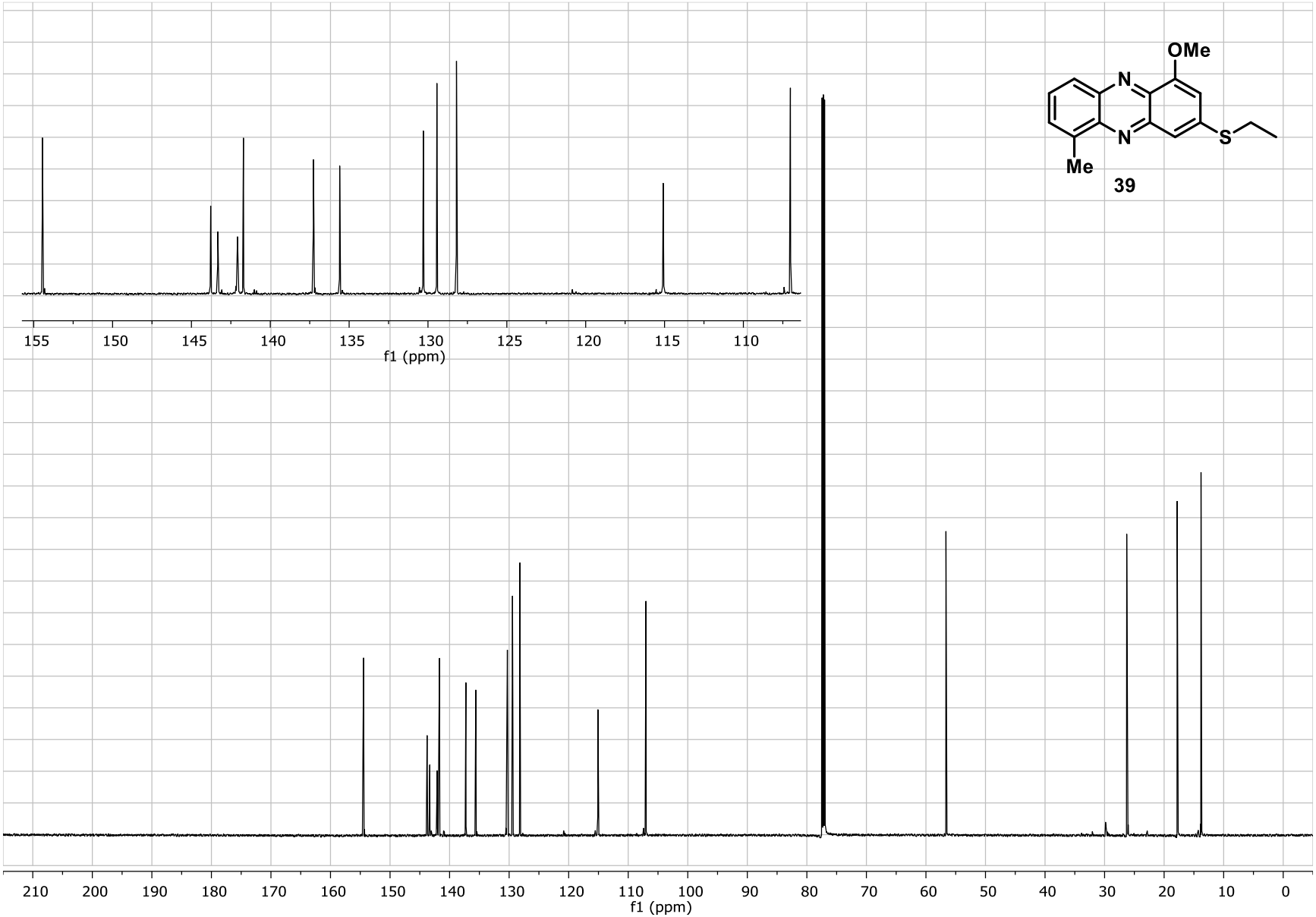

OH
I
N
Br
N
Cl
Br
37
8.75 8.70 8.65 8.60 8.55 8.50 8.45 8.40 8.35 8.30 8.25 8.20 8.15 8.10 8.05
f1 (ppm)
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

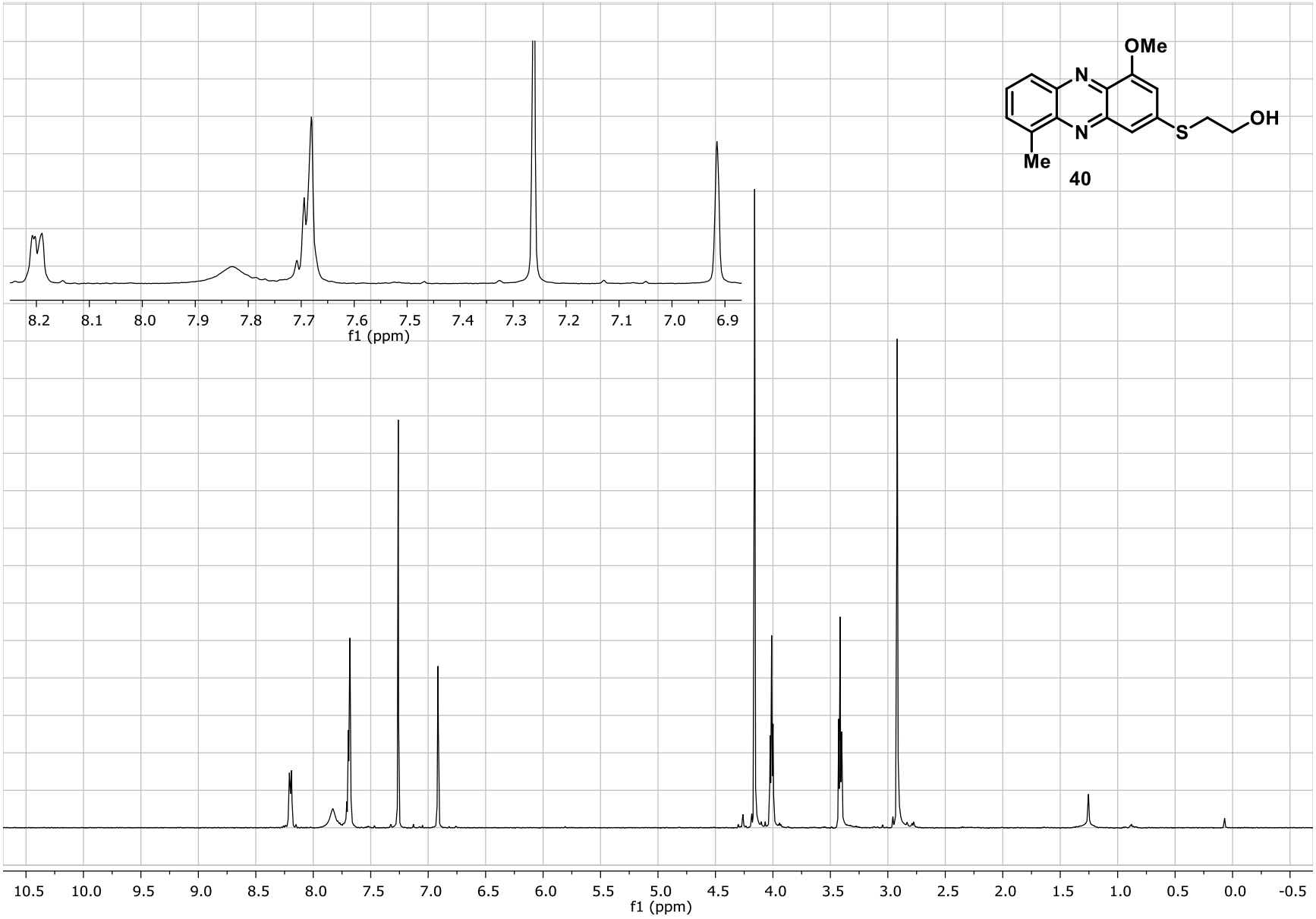

OH
I
N
Br
N
Cl
Br
37
150
145
140
135
130
125
120
115
110
105
100
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

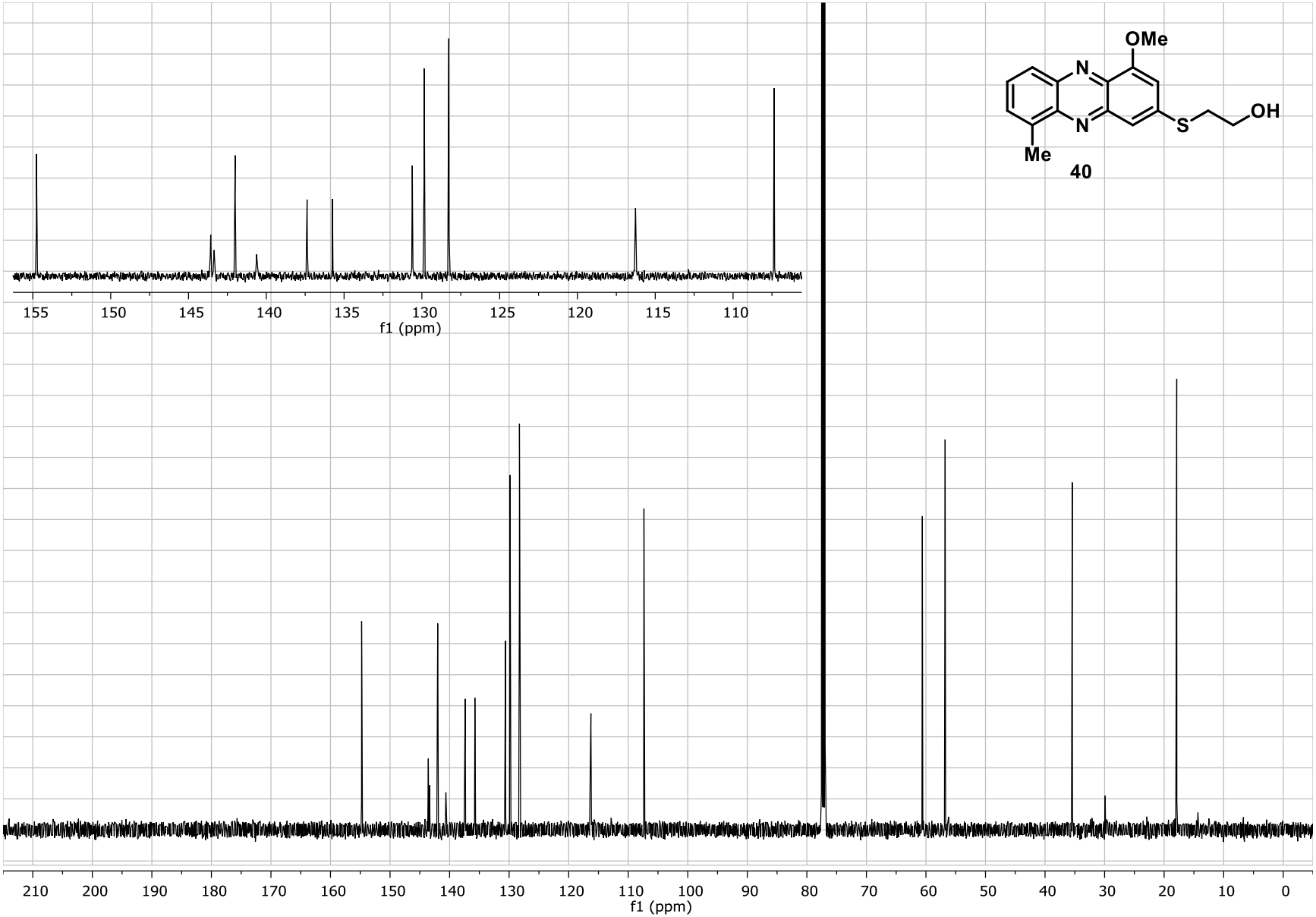

OMe
N
N
Cl
Me
38
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

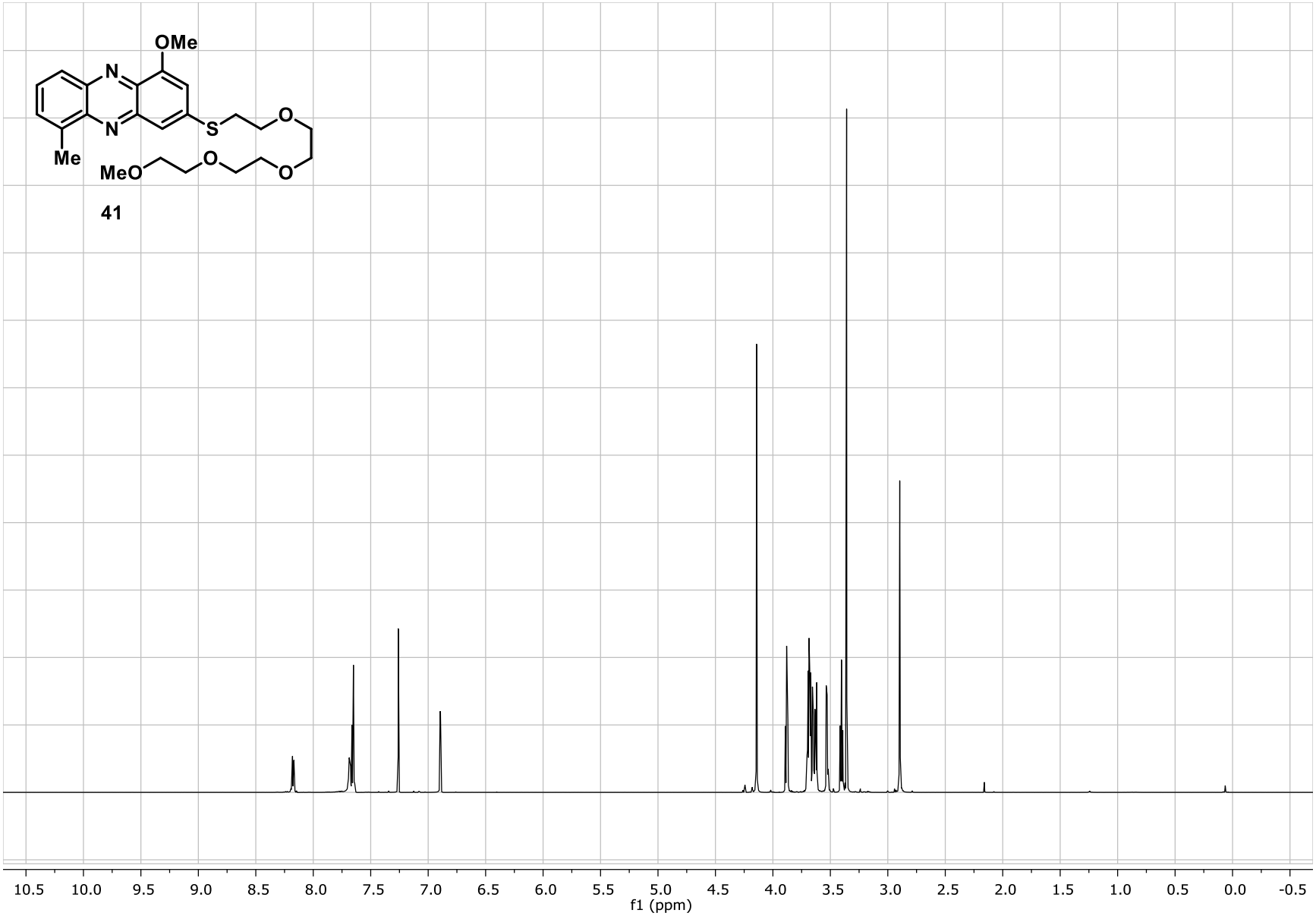

OMe
N
N
Cl
Me
38
131.0
130.5
130.0
129.5
129.0
128.5
128.0
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

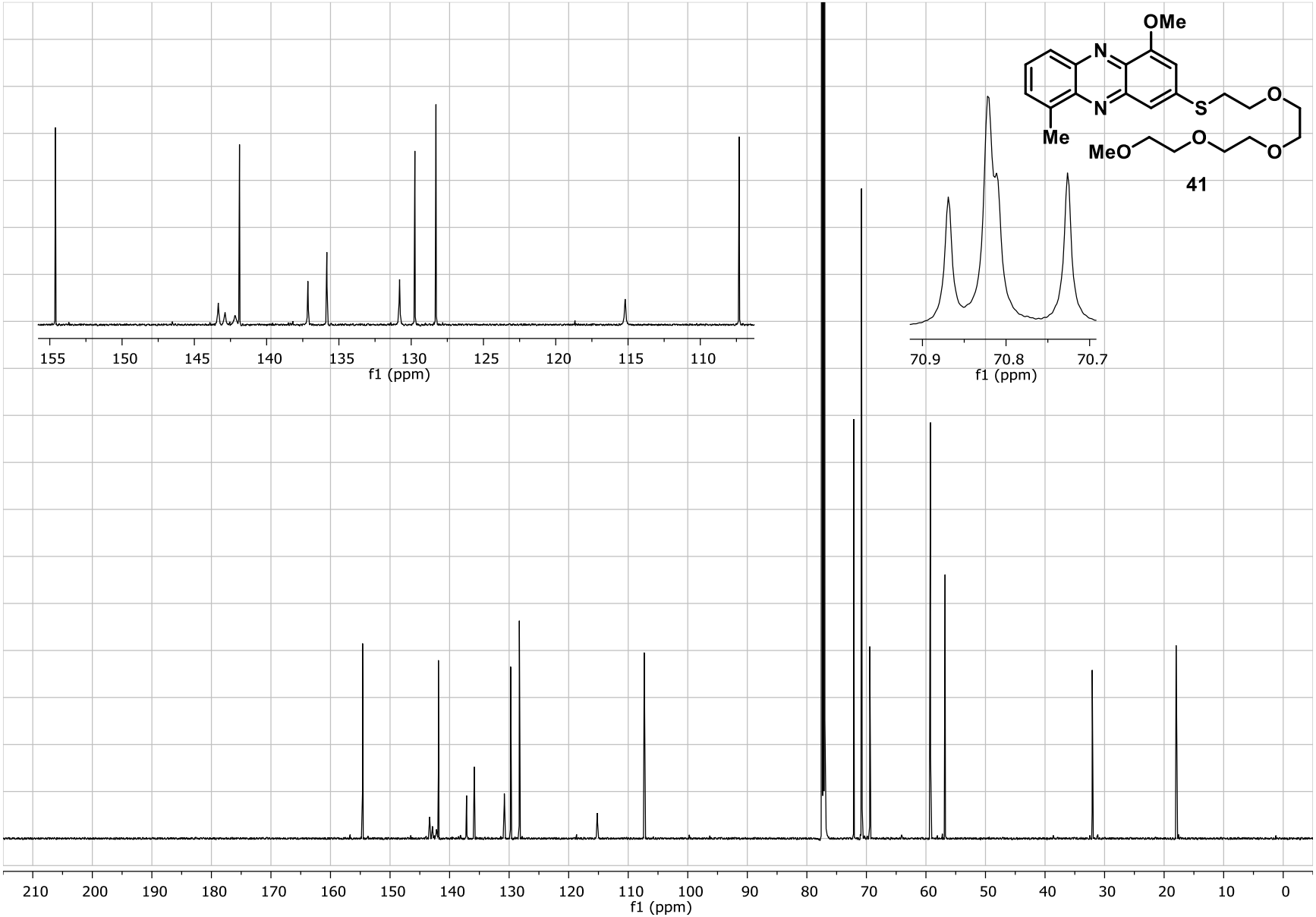

OMe
N
N
S
Me
39
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

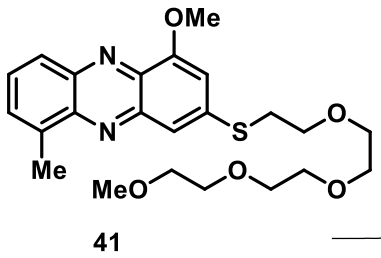

OMe
N
N
S
Me
39
155
150
145
140
135
130
125
120
115
110
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


OMe
N
N
S
OH
Me
40
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)

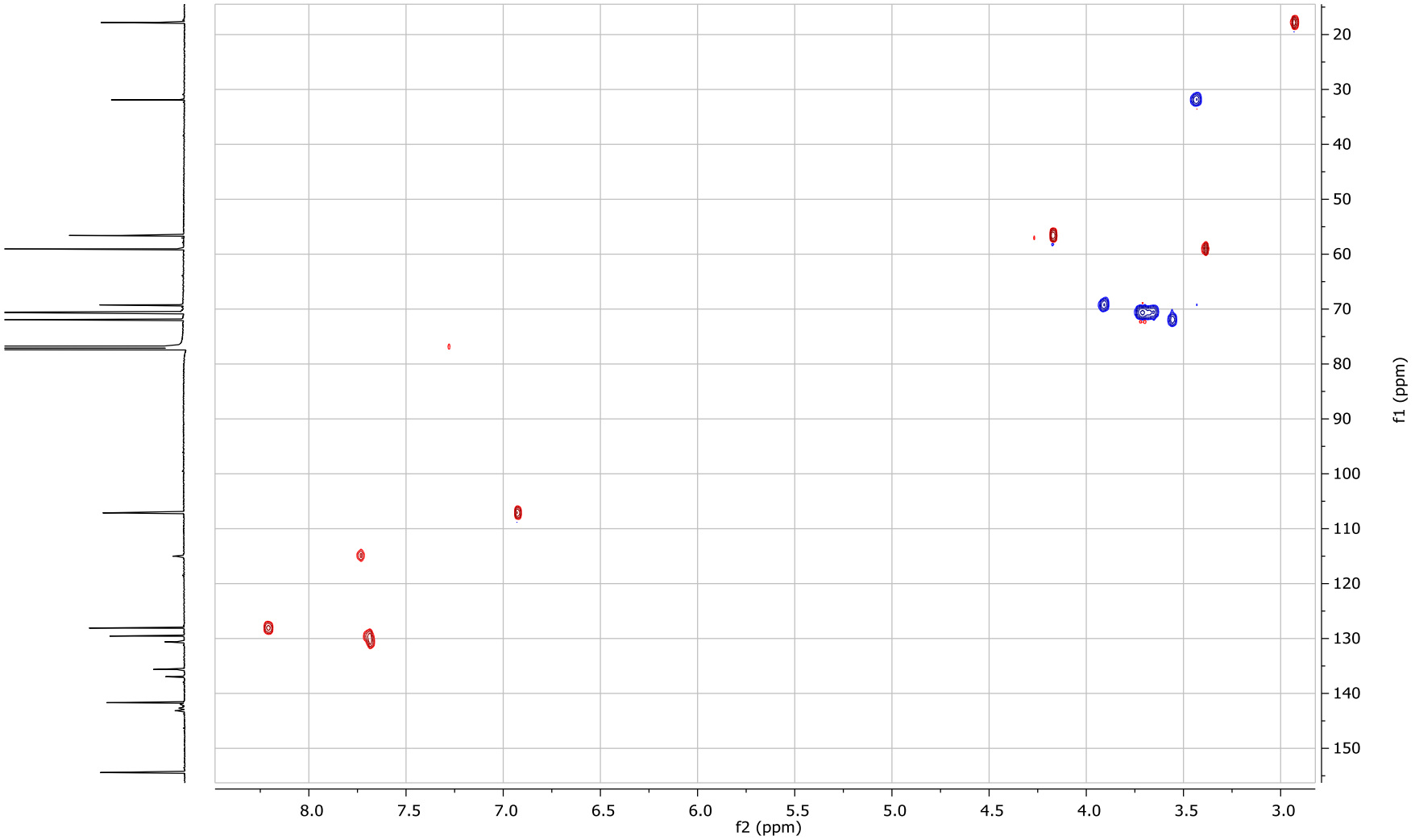

OMe
N
N
S
OH
Me
40
155
150
145
140
135
130
125
120
115
110
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


OMe
N
N
S
O
O
O
O
Me
MeO
41
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)

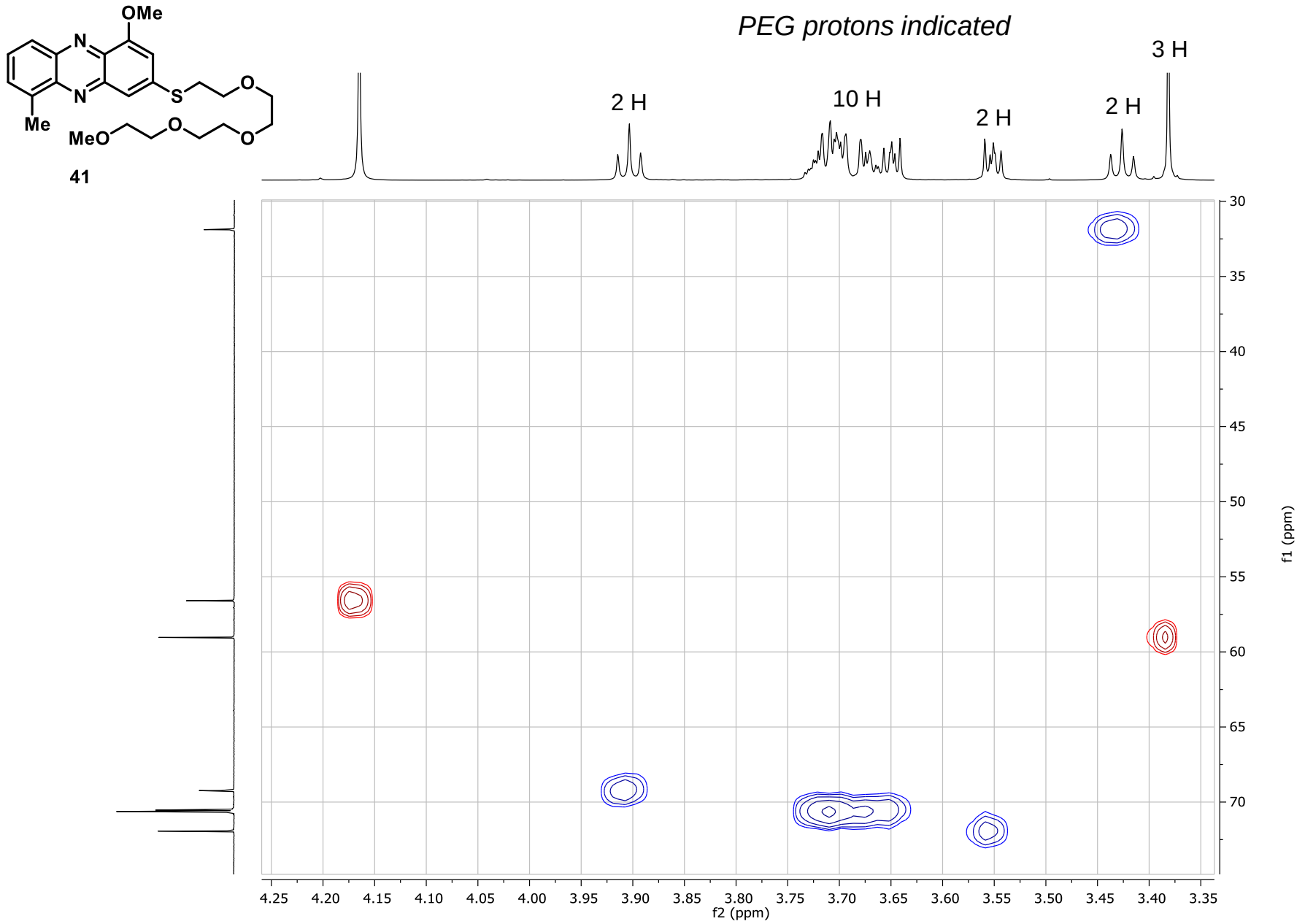

OMe
N
N
S
O
O
O
O
Me
MeO
41
155
150
145
140
135
130
125
120
115
110
f1 (ppm)
70.9
70.8
70.7
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


OMe
N
N
S
O
O
O
O
MeO
Me

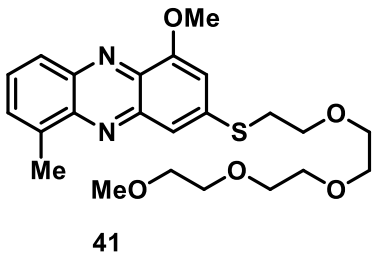
41


8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)

HSQC (full)



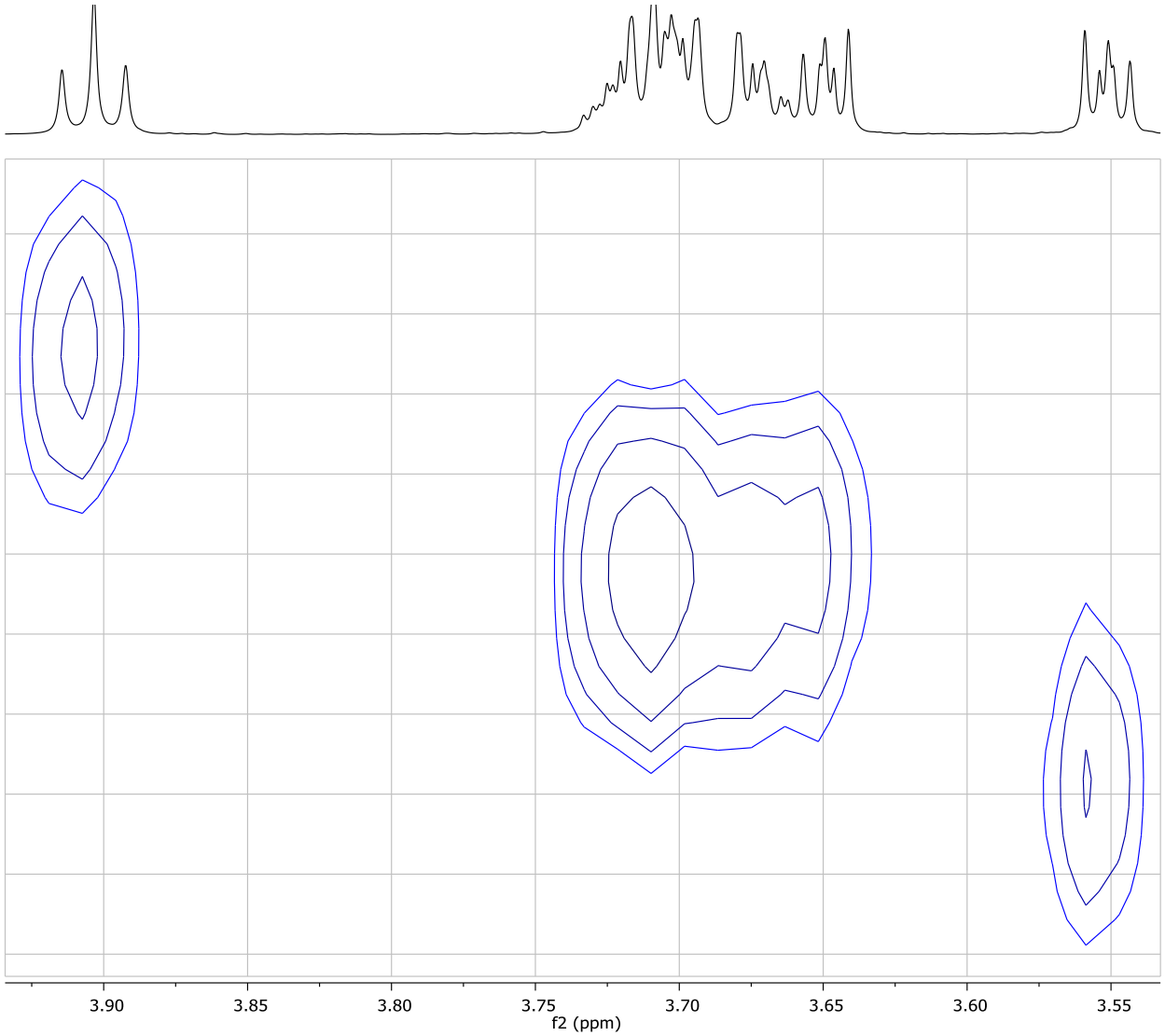
HSQC (zoomed in to relevant region bearing 19 PEG protons)



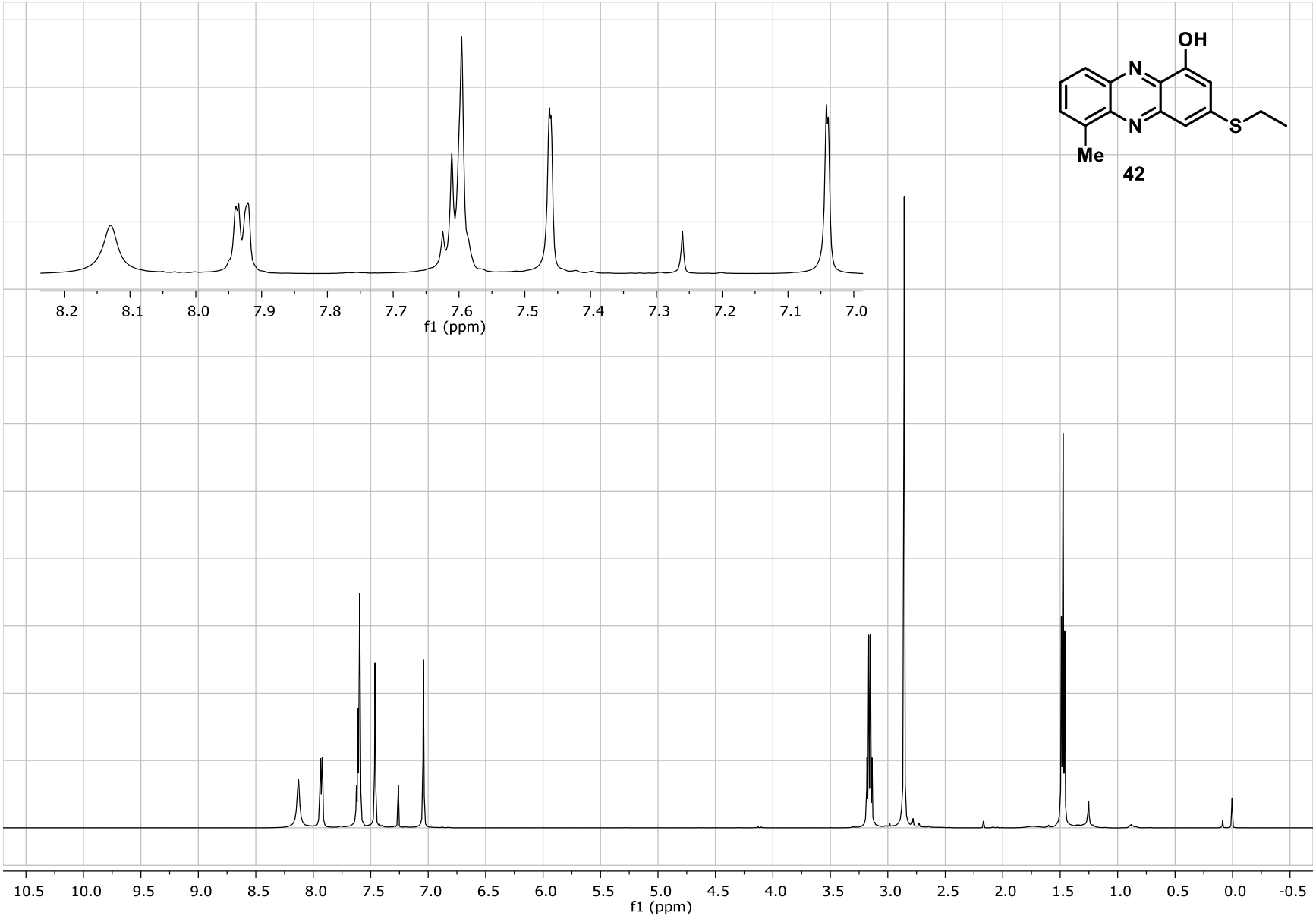
41

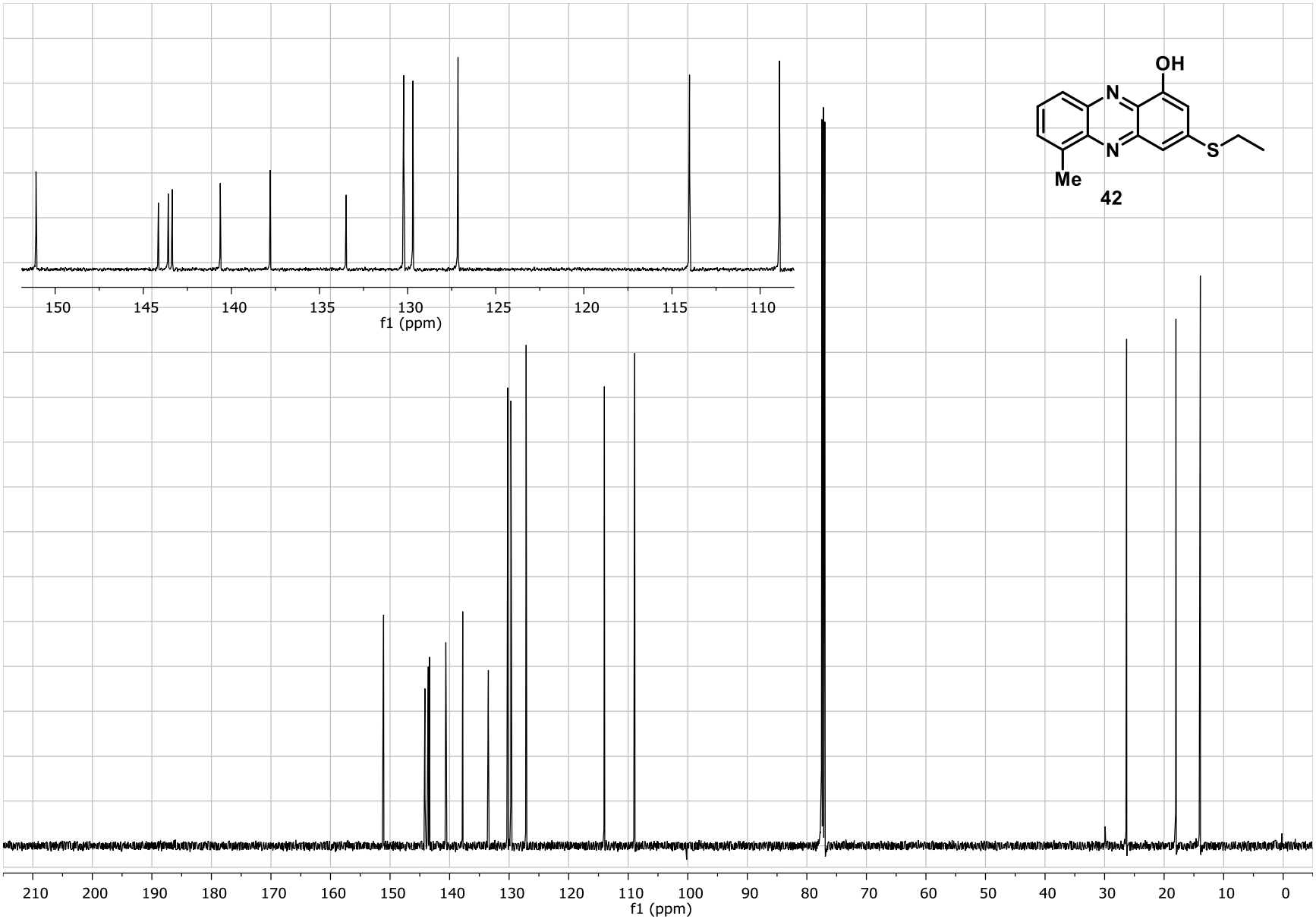
10 protons (3.72 - 3.60 ppm)

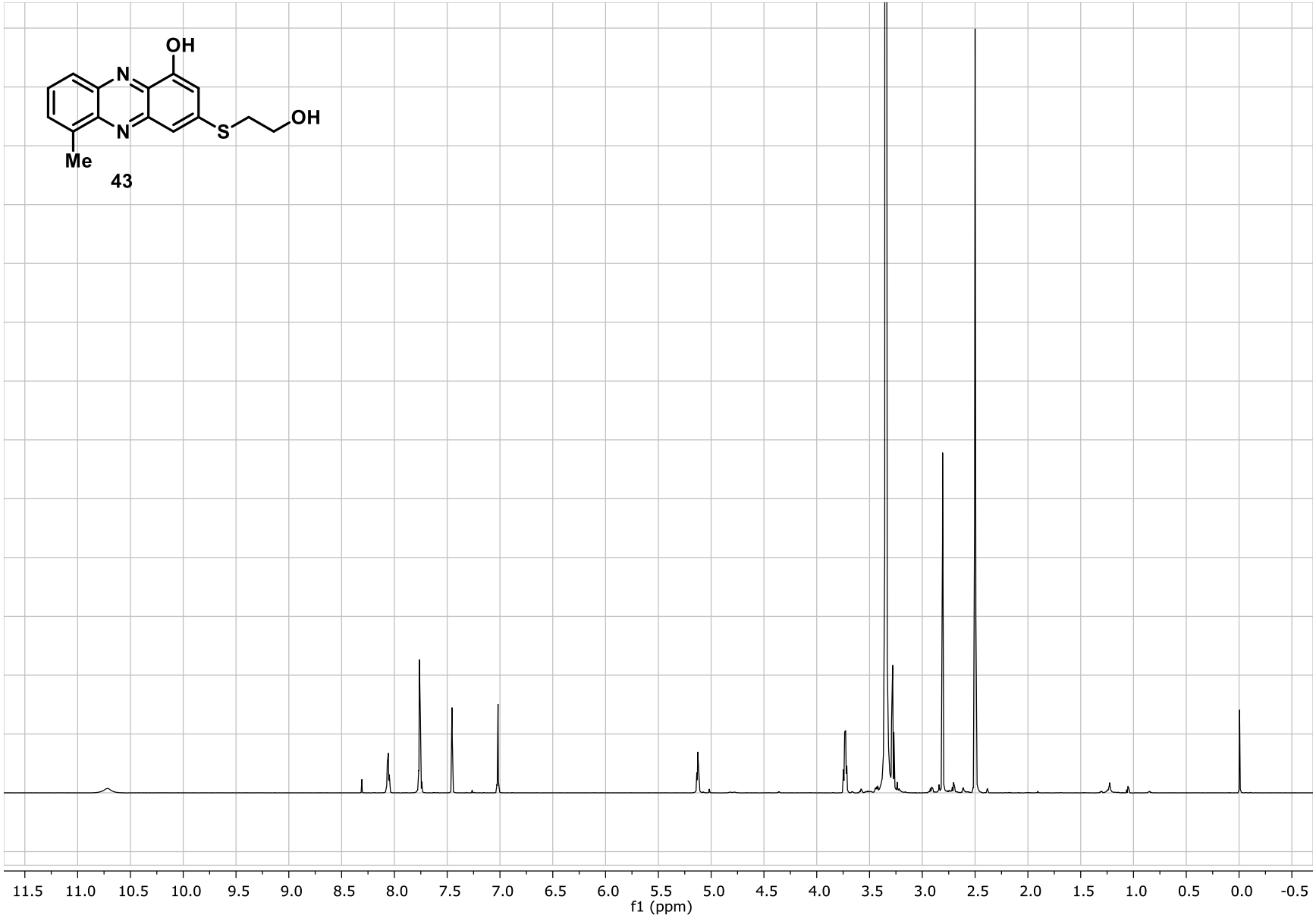
5 carbon atoms
4 clear signals
(1 overlap)

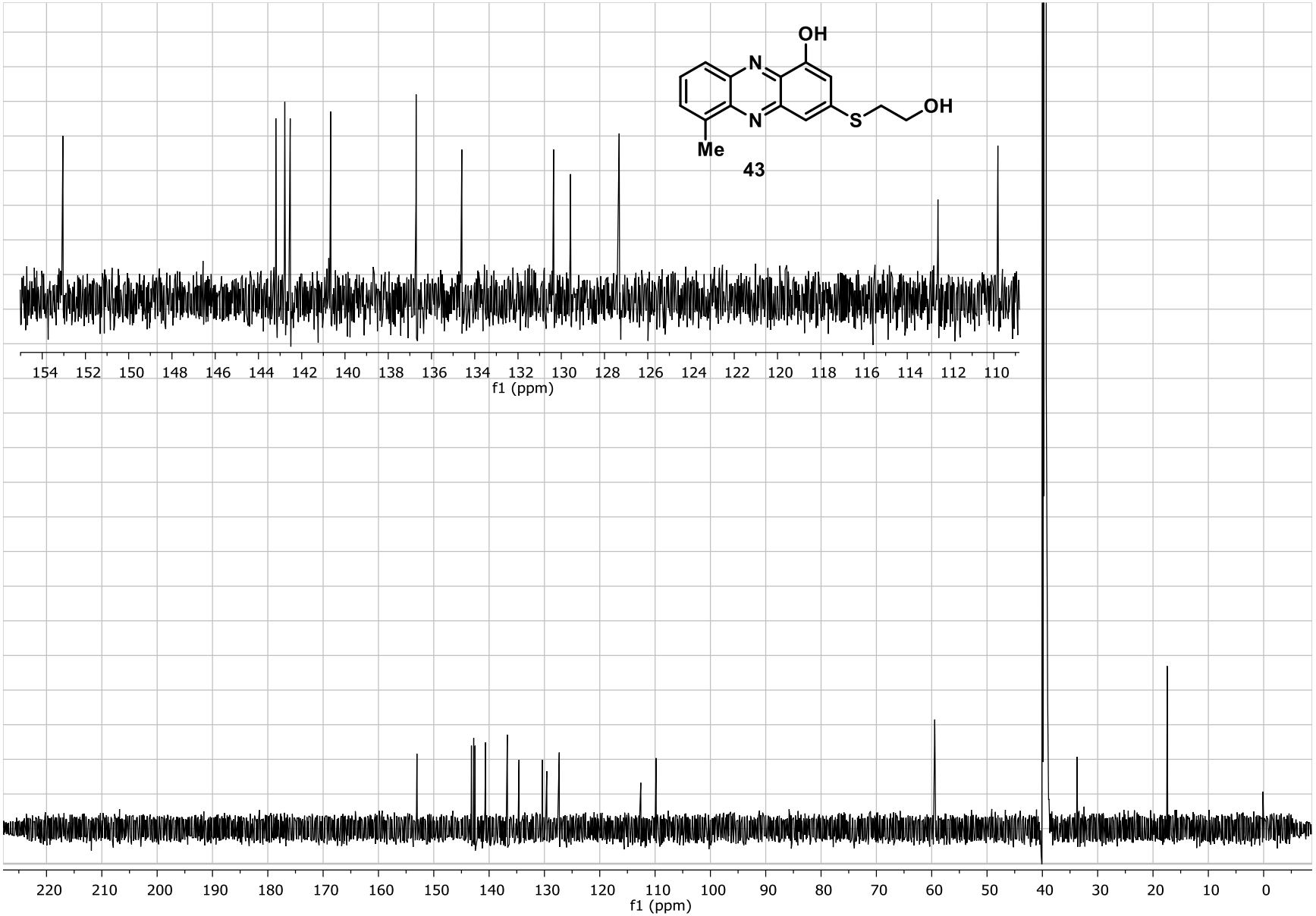


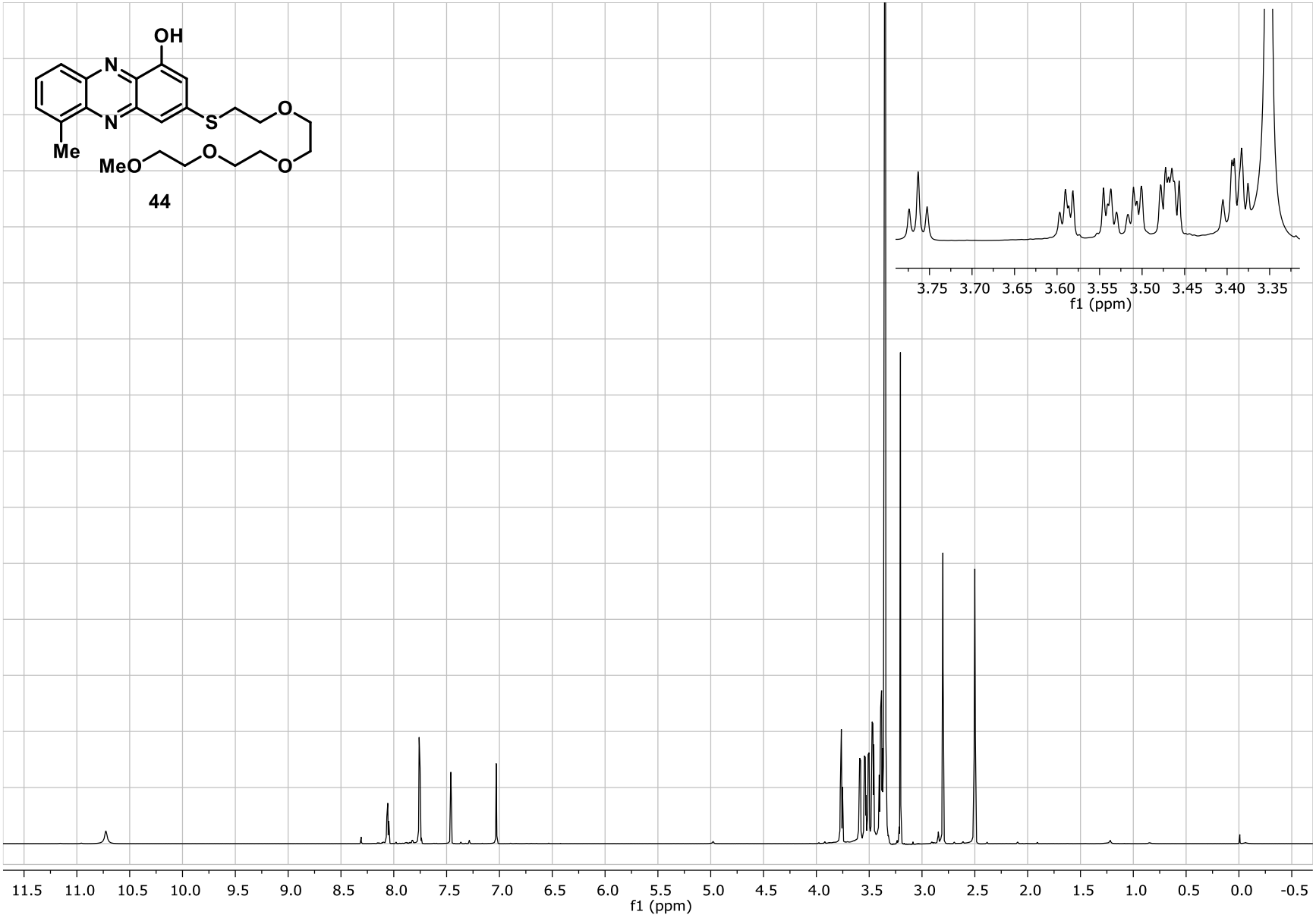
HSQC (zoomed in to show 10 protons on the PEG moiety on 5 carbon atoms in this region)

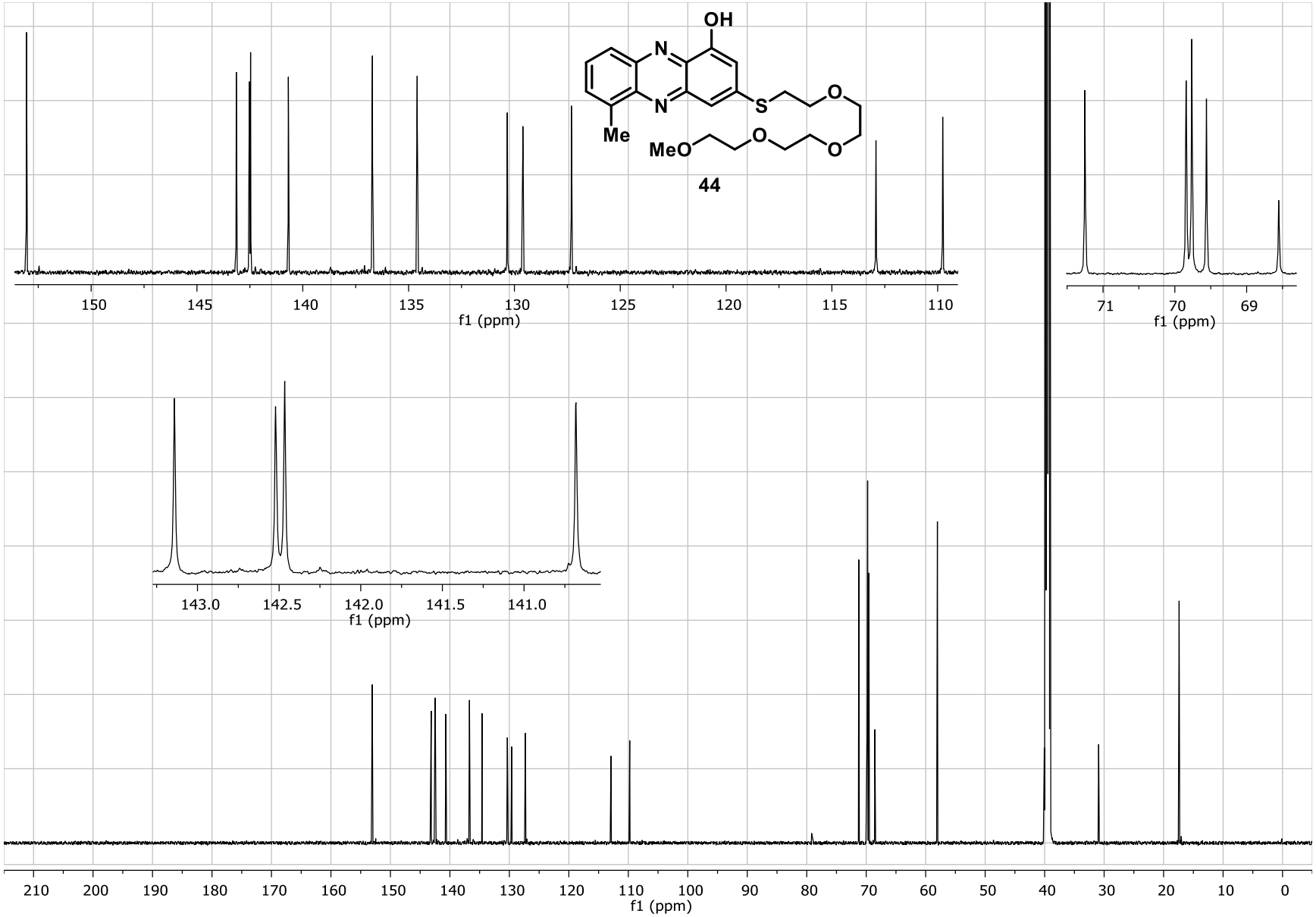

OH
N
N
S
Me
42
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

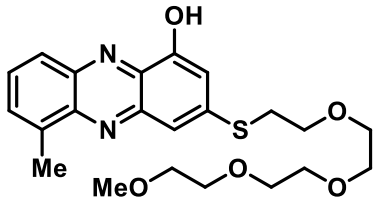

OH
N
N
S
Me
42
150
145
140
135
130
125
120
115
110
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


OH
N
N
S
OH
Me
43
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)

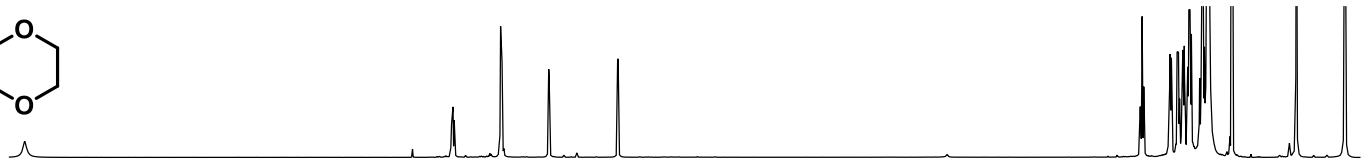

OH
N
N
S
OH
Me
43
154 152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112 110
f1 (ppm)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

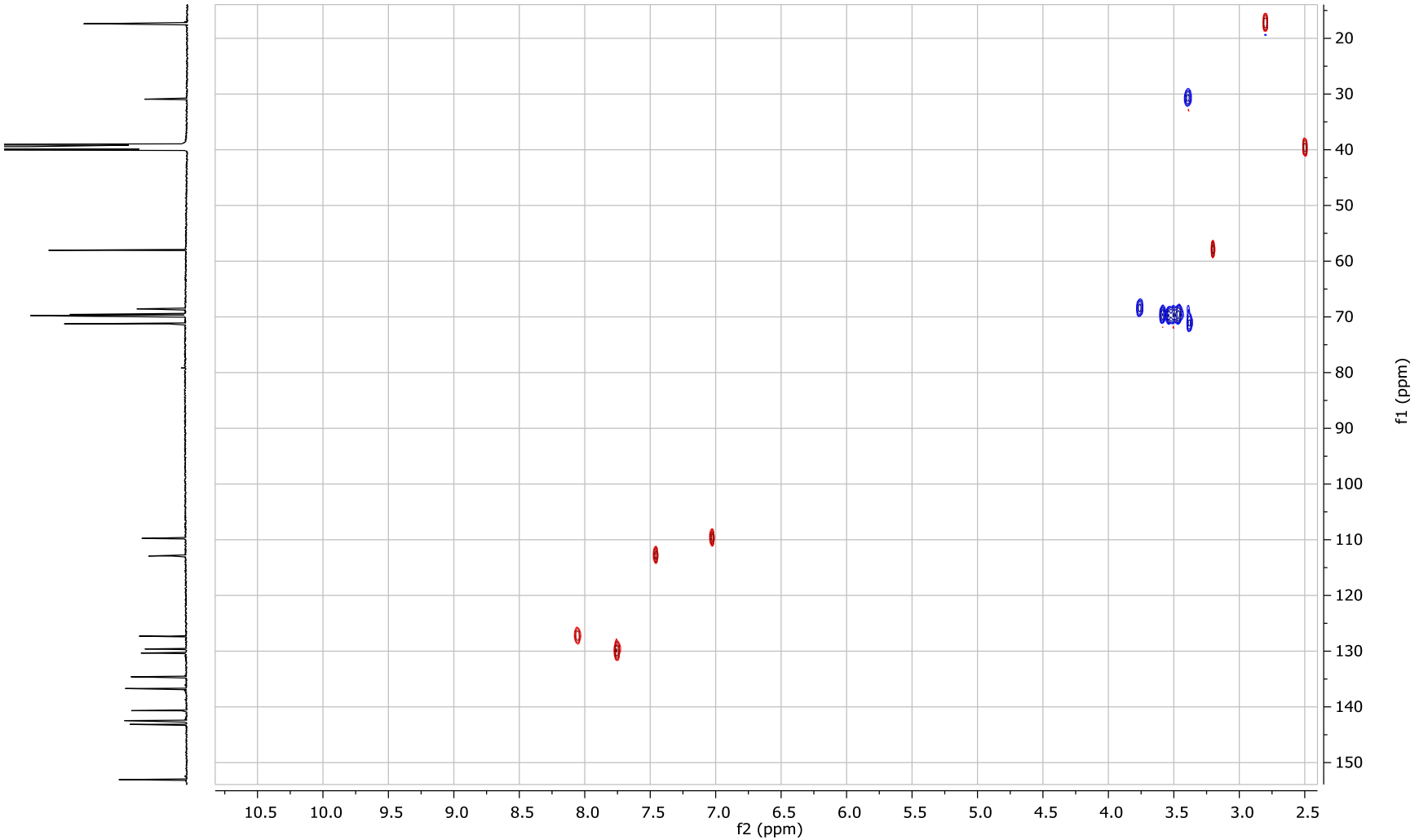

OH
N
N
S
O
O
O
O
Me
MeO
44
3.75 3.70 3.65 3.60 3.55 3.50 3.45 3.40 3.35
f1 (ppm)
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


OH
N
N
S
O
O
O
Me
MeO
44
150
145
140
135
130
125
120
115
110
f1 (ppm)
71
70
69
f1 (ppm)
143.0
142.5
142.0
141.5
141.0
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

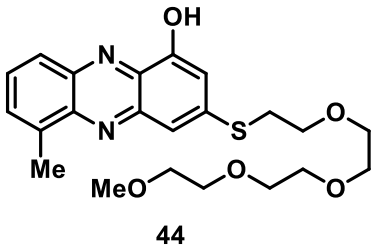

OH
N
N
S
O
O
O
O
Me
MeO

44

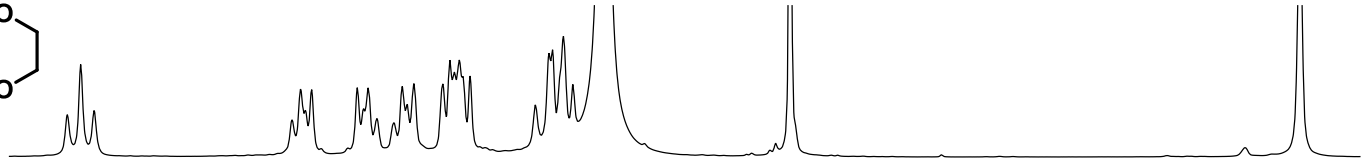


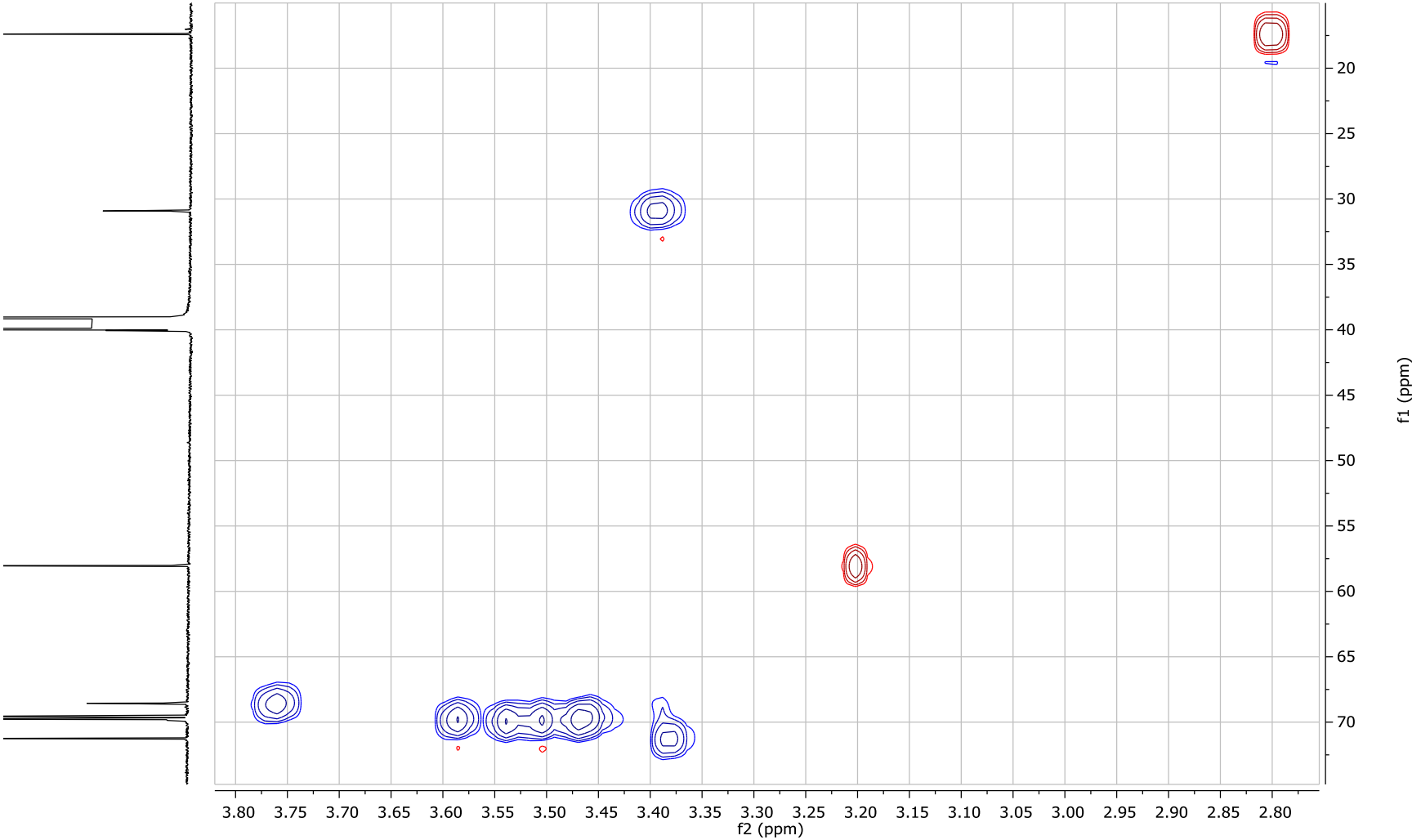

10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)

HSQC (full)

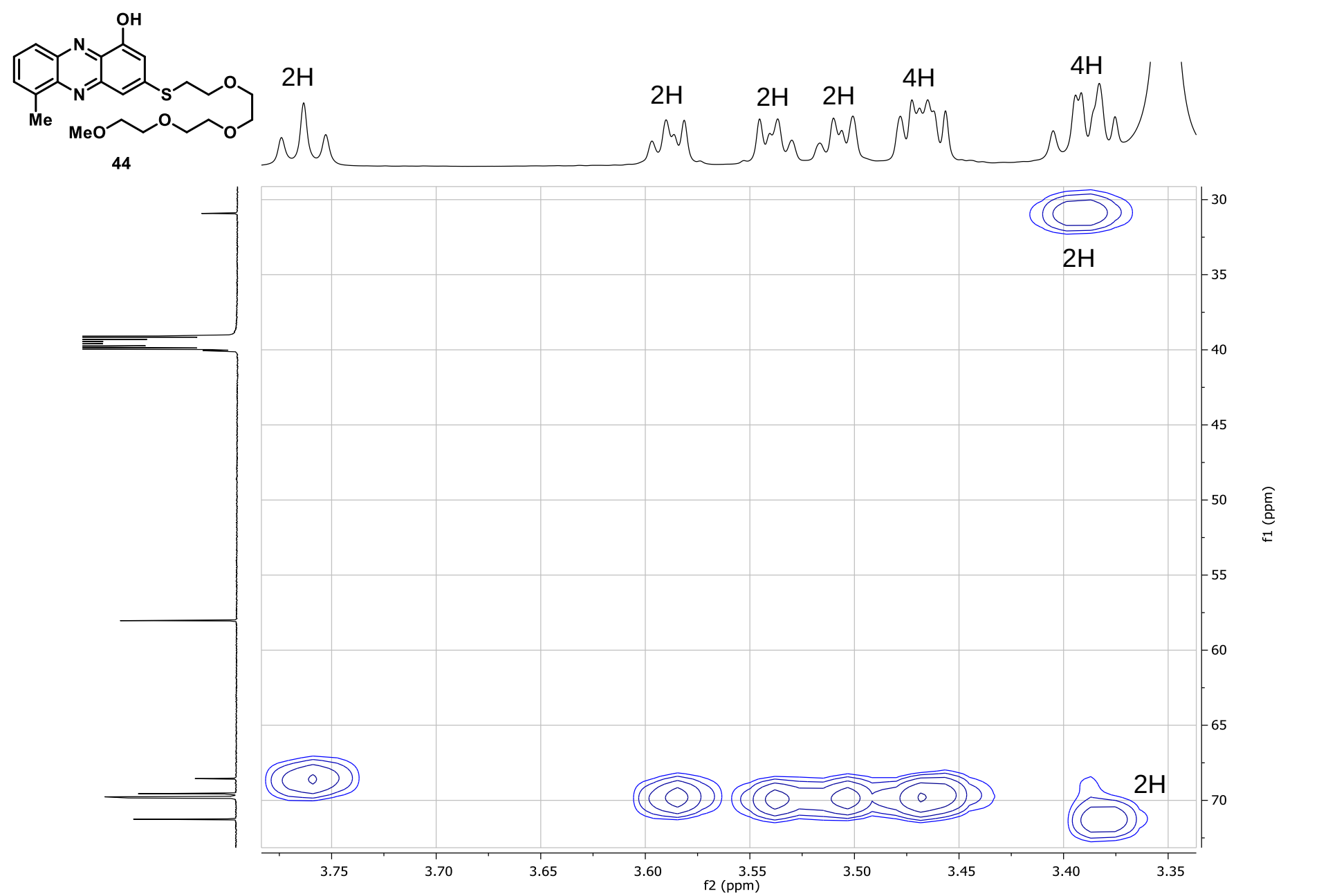


44

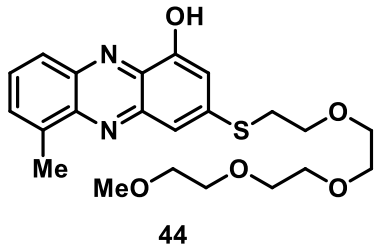




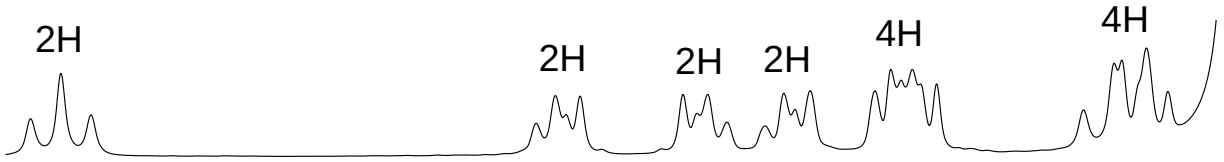
HSQC (zoomed in)

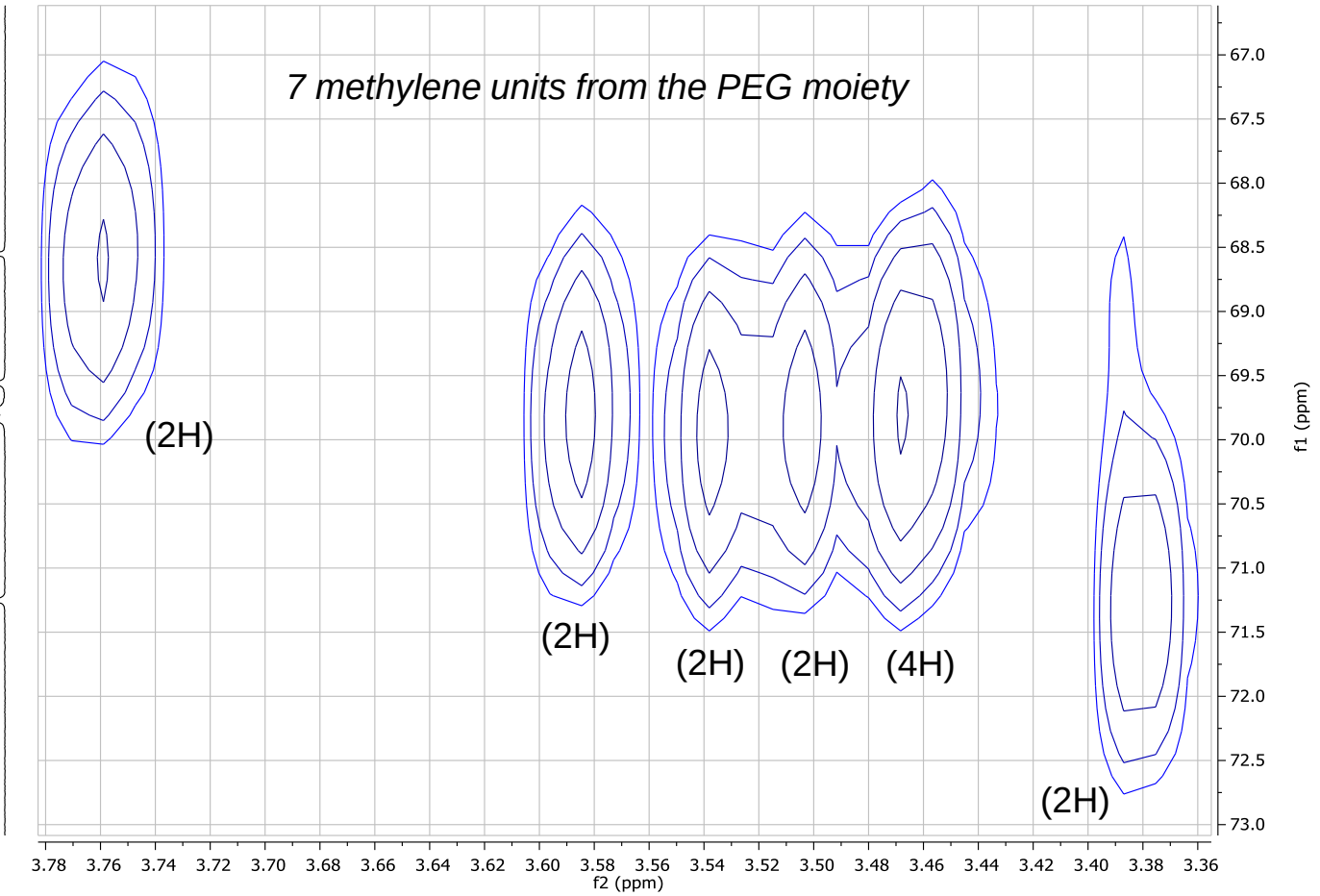


HSQC (zoomed in to relevant PEG protons)



44

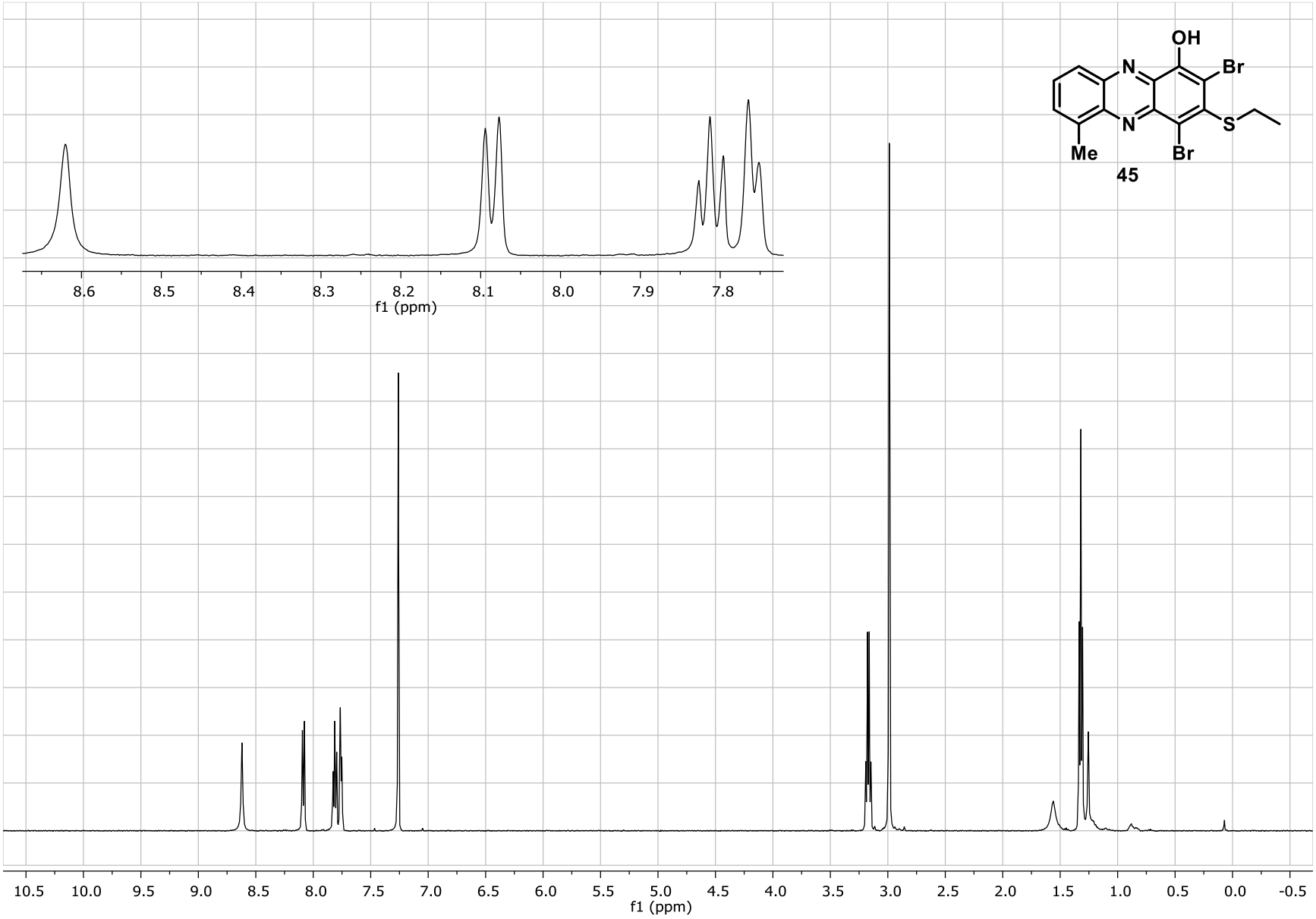


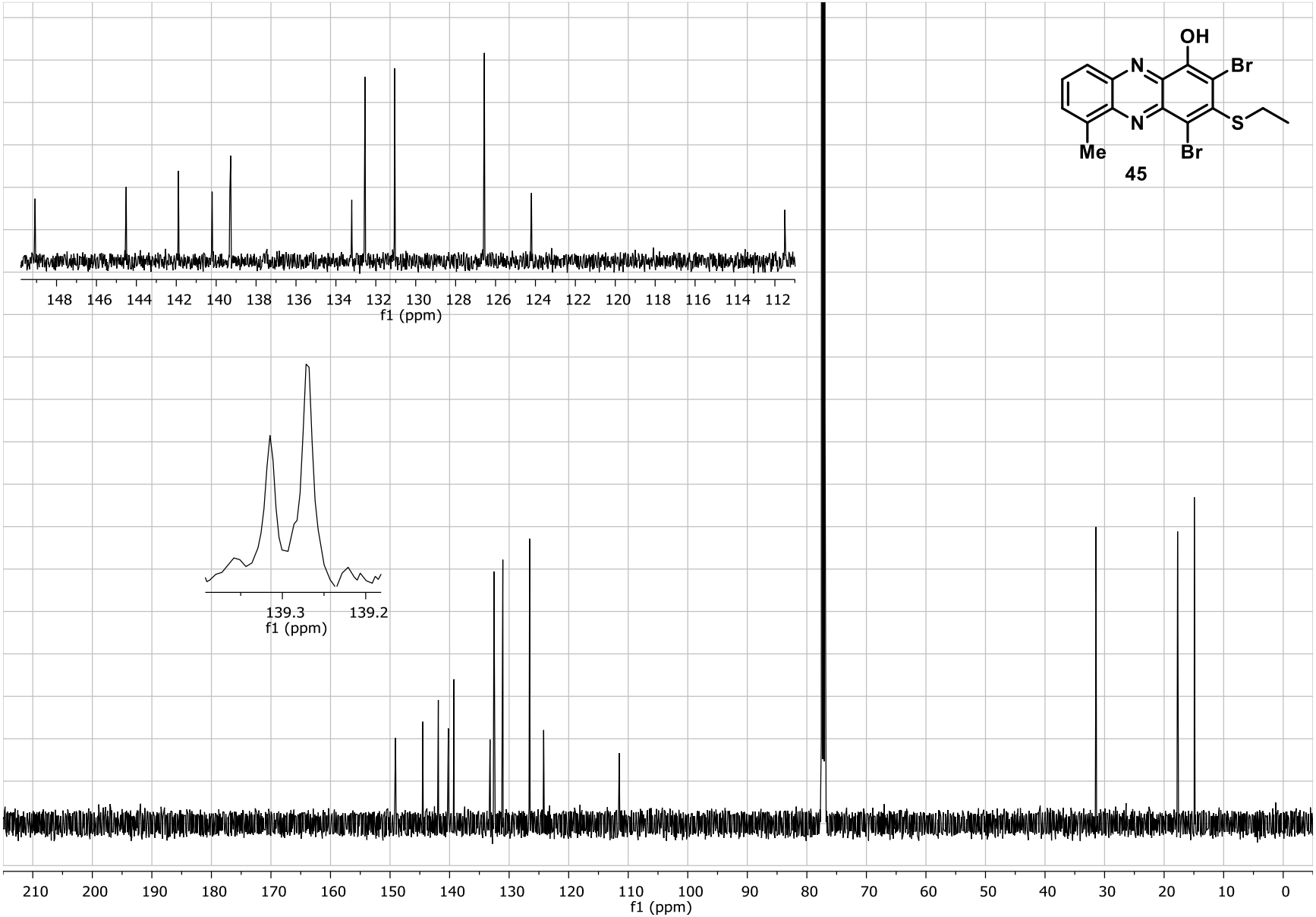


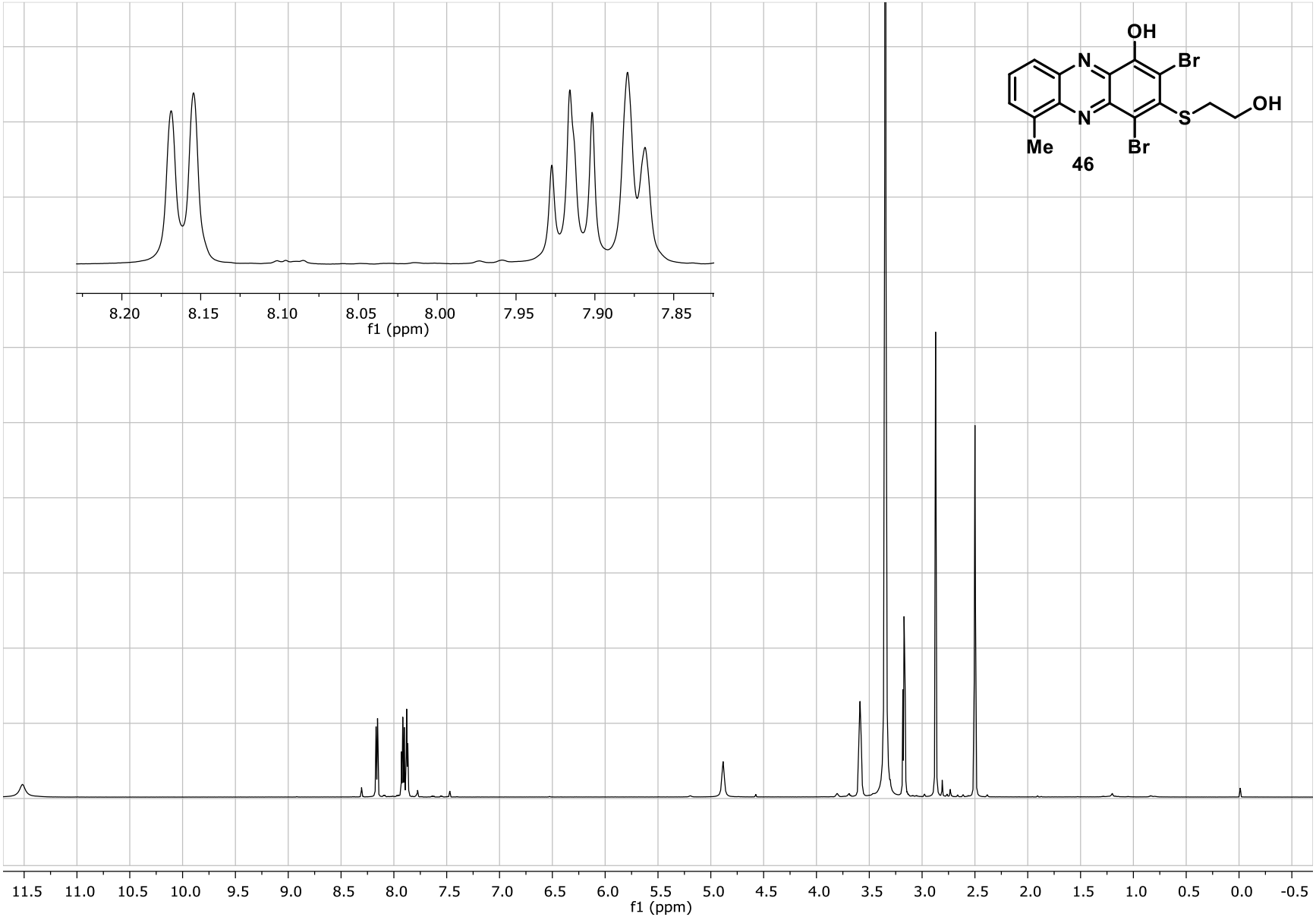
7 carbon atoms
14 protons

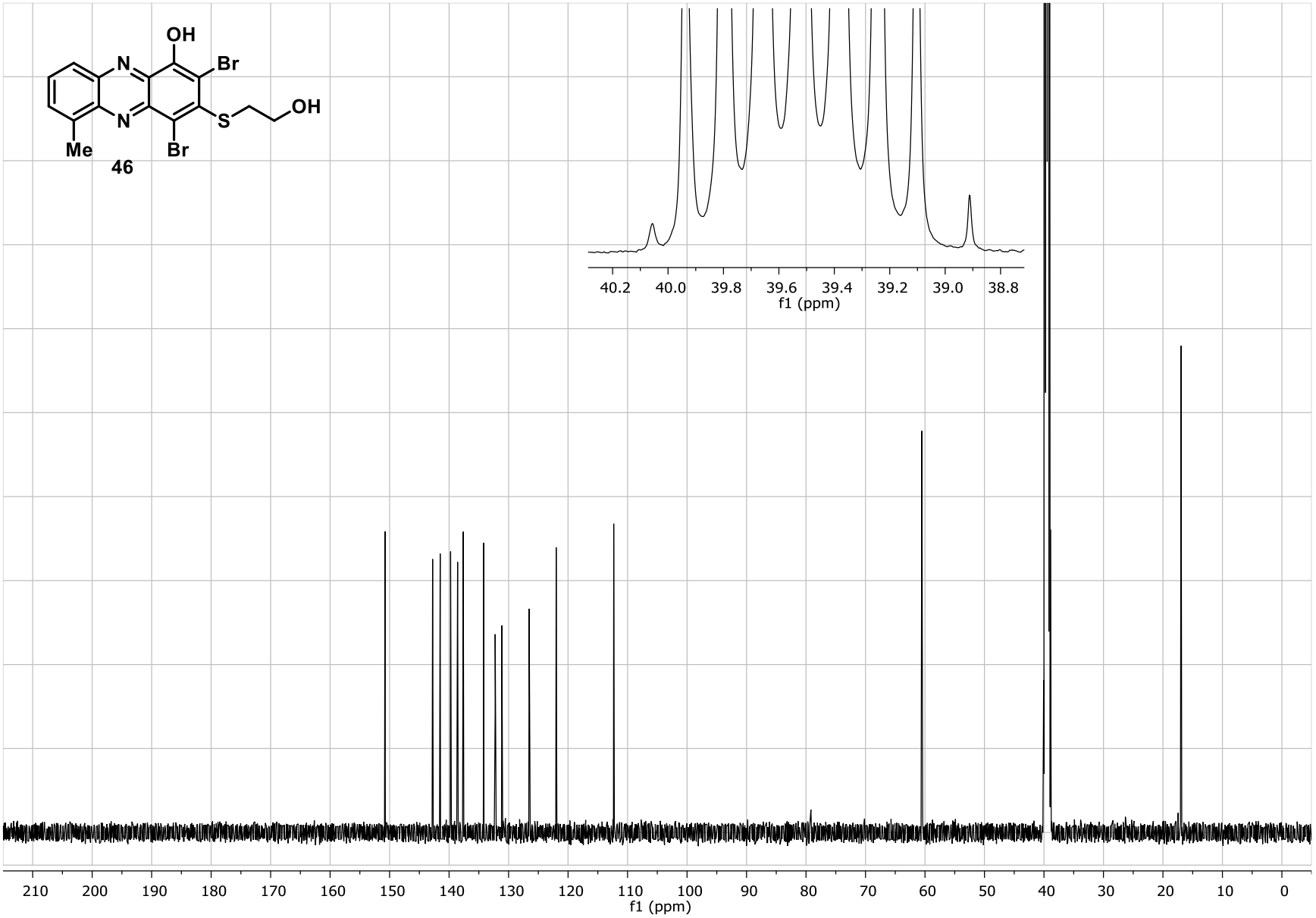
5 carbon signals
(2 missing due to
overlap ~70 ppm)

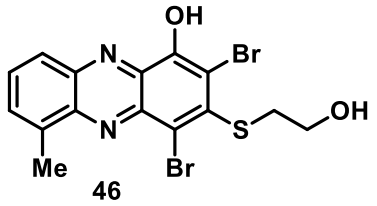
HSQC (zoomed in, relevant PEG methylene units)


OH
Br
N
N
S
Me
Br
45
8.6
8.5
8.4
8.3
8.2
8.1
8.0
7.9
7.8
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)

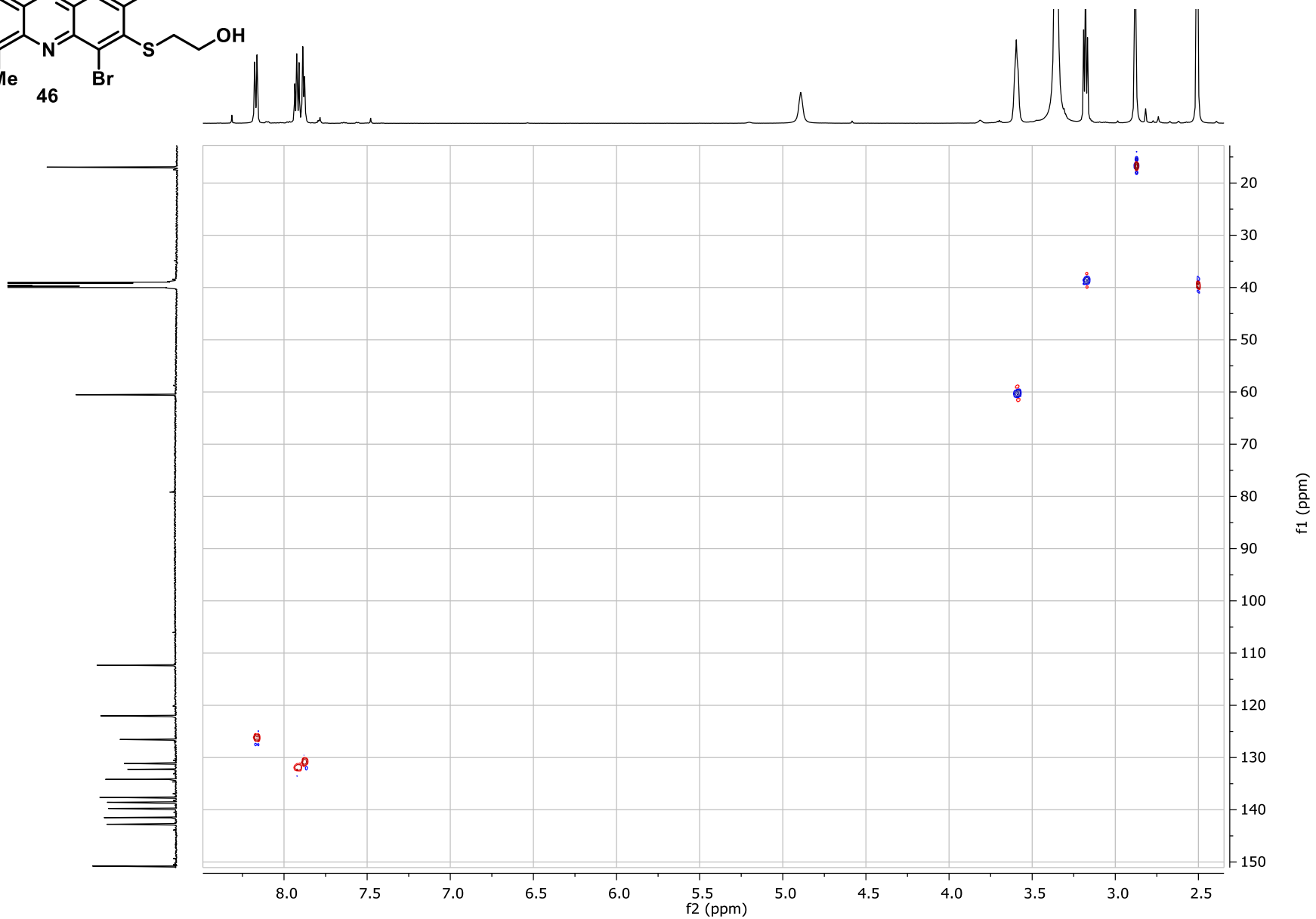

OH
Br
N
N
S
Me
Br
45
148 146 144 142 140 138 136 134 132 130 128 126 124 122 120 118 116 114 112
f1 (ppm)
139.3
139.2
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


OH
Br
N
N
OH
S
Me
Br
46
8.20
8.15
8.10
8.05
8.00
7.95
7.90
7.85
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)

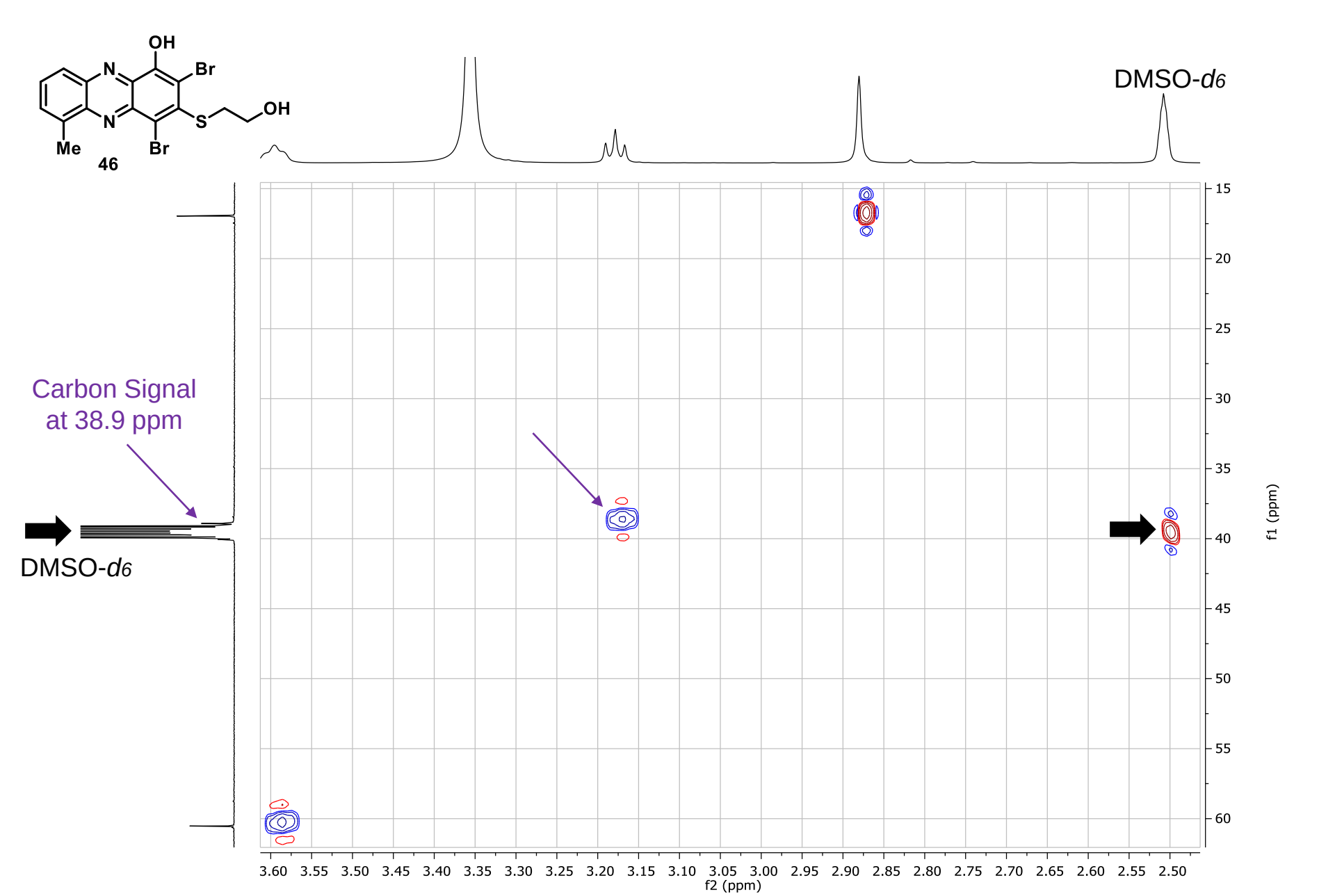

OH
Br
N
N
S
OH
Me
Br
46
40.2
40.0
39.8
39.6
39.4
39.2
39.0
38.8
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



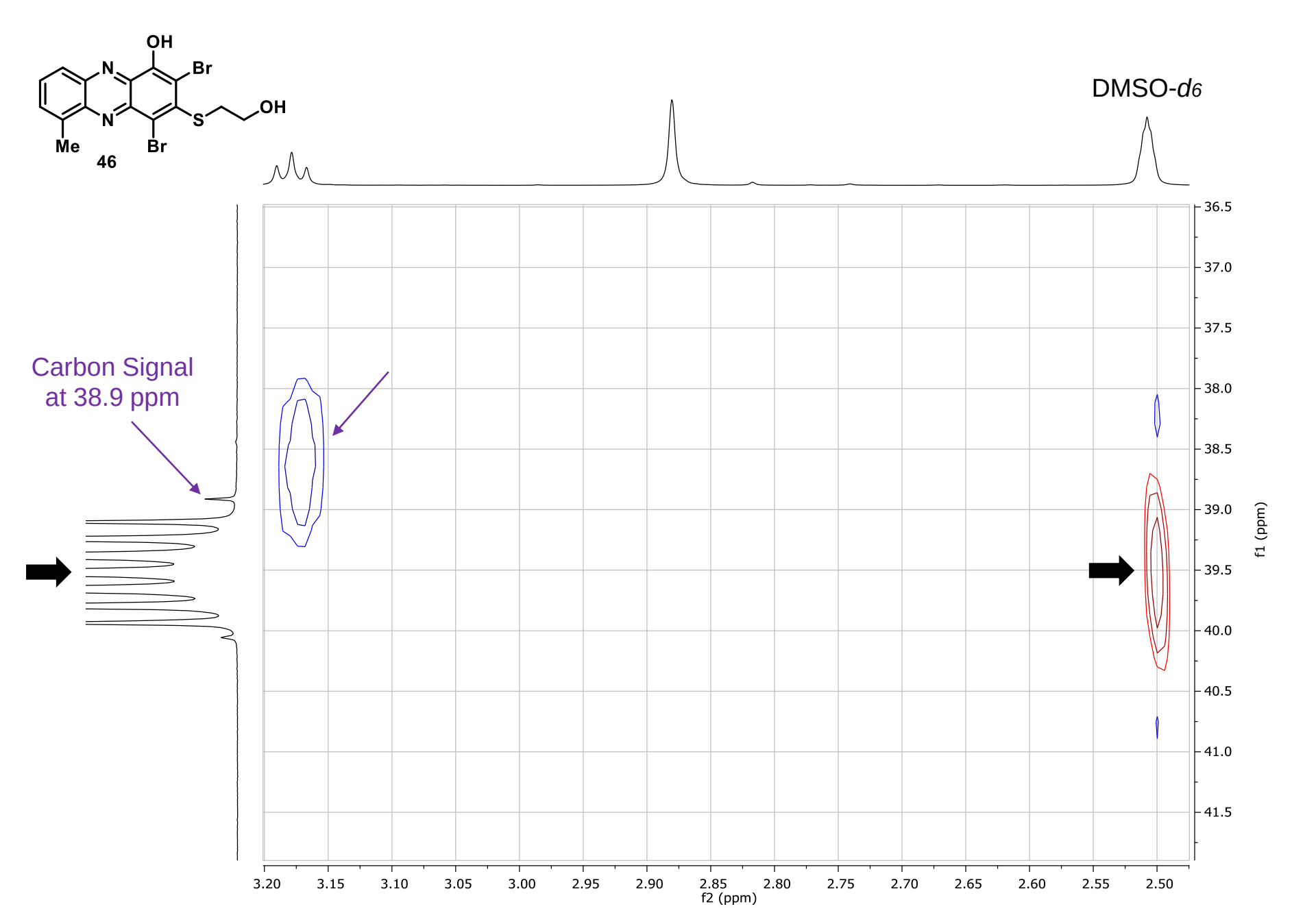
46



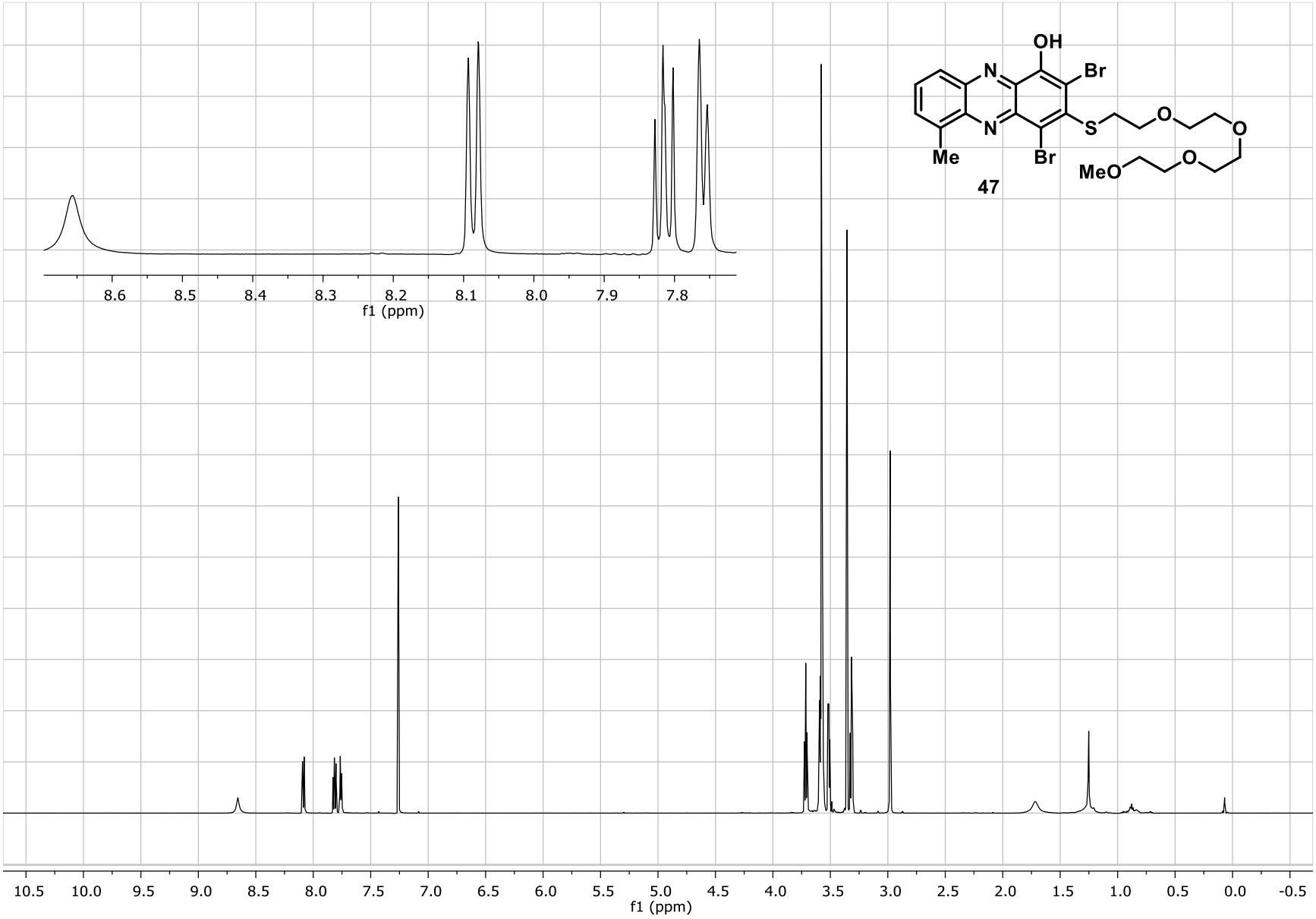
HSQC (full)

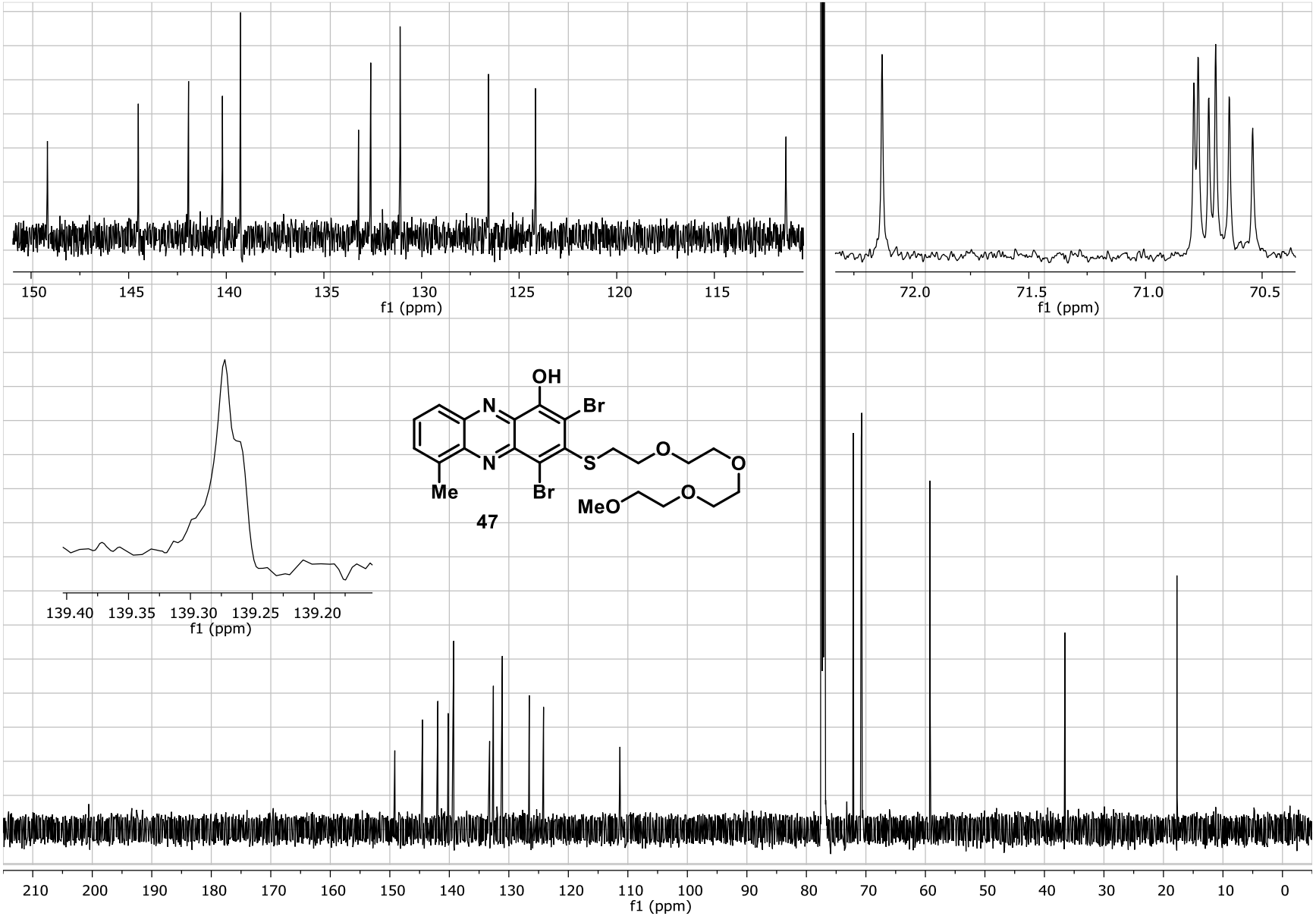


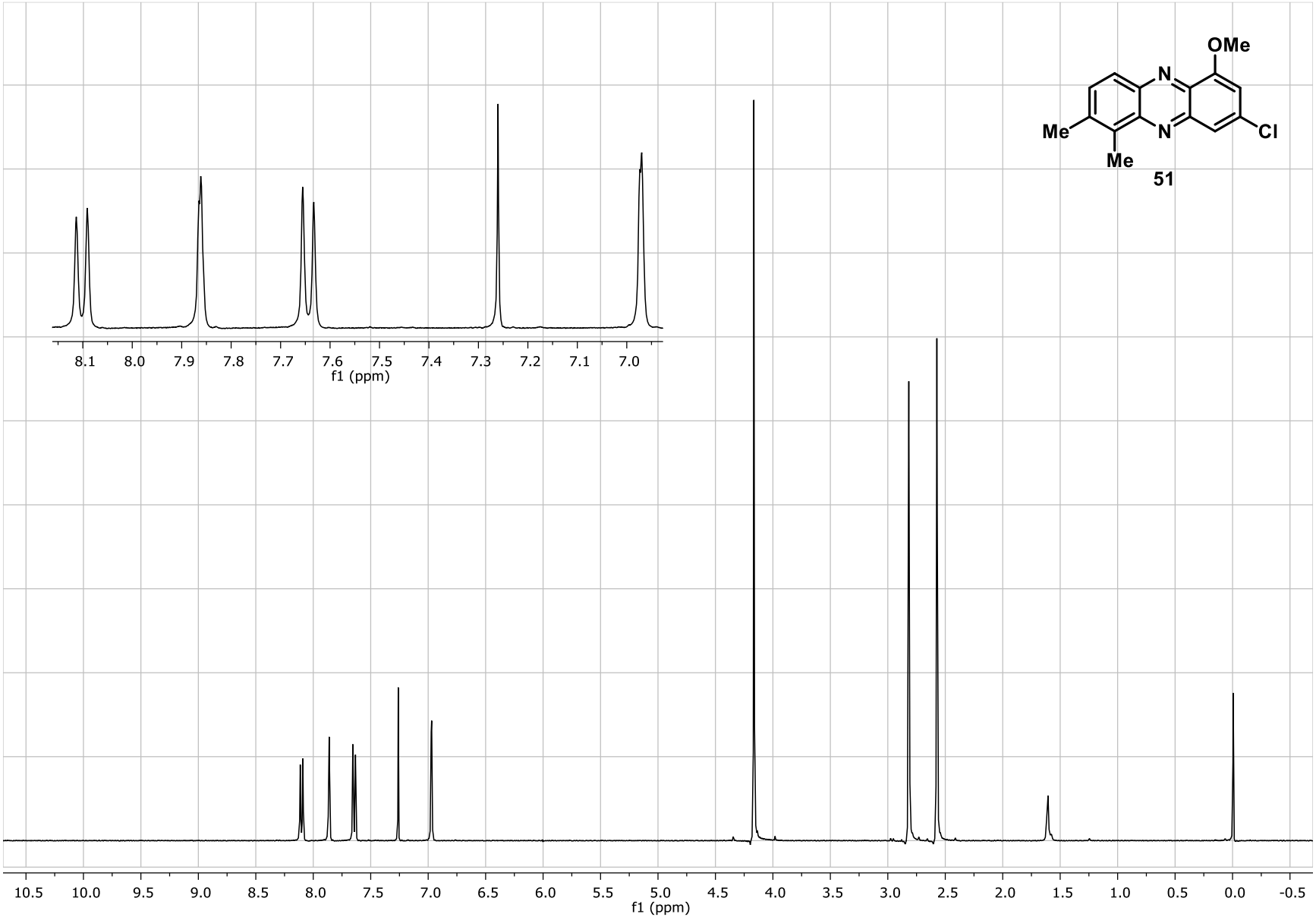
HSQC (zoomed in version)

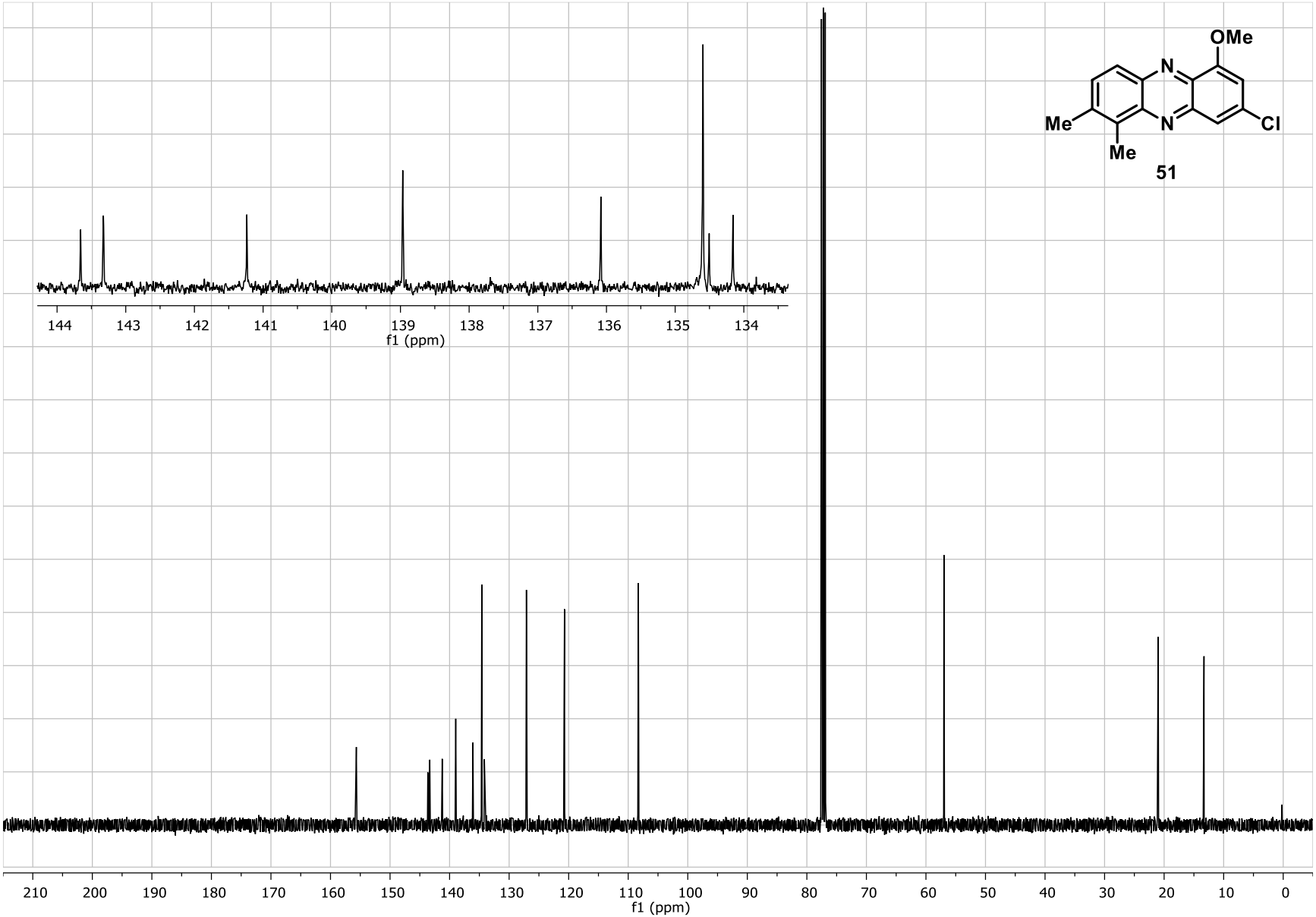


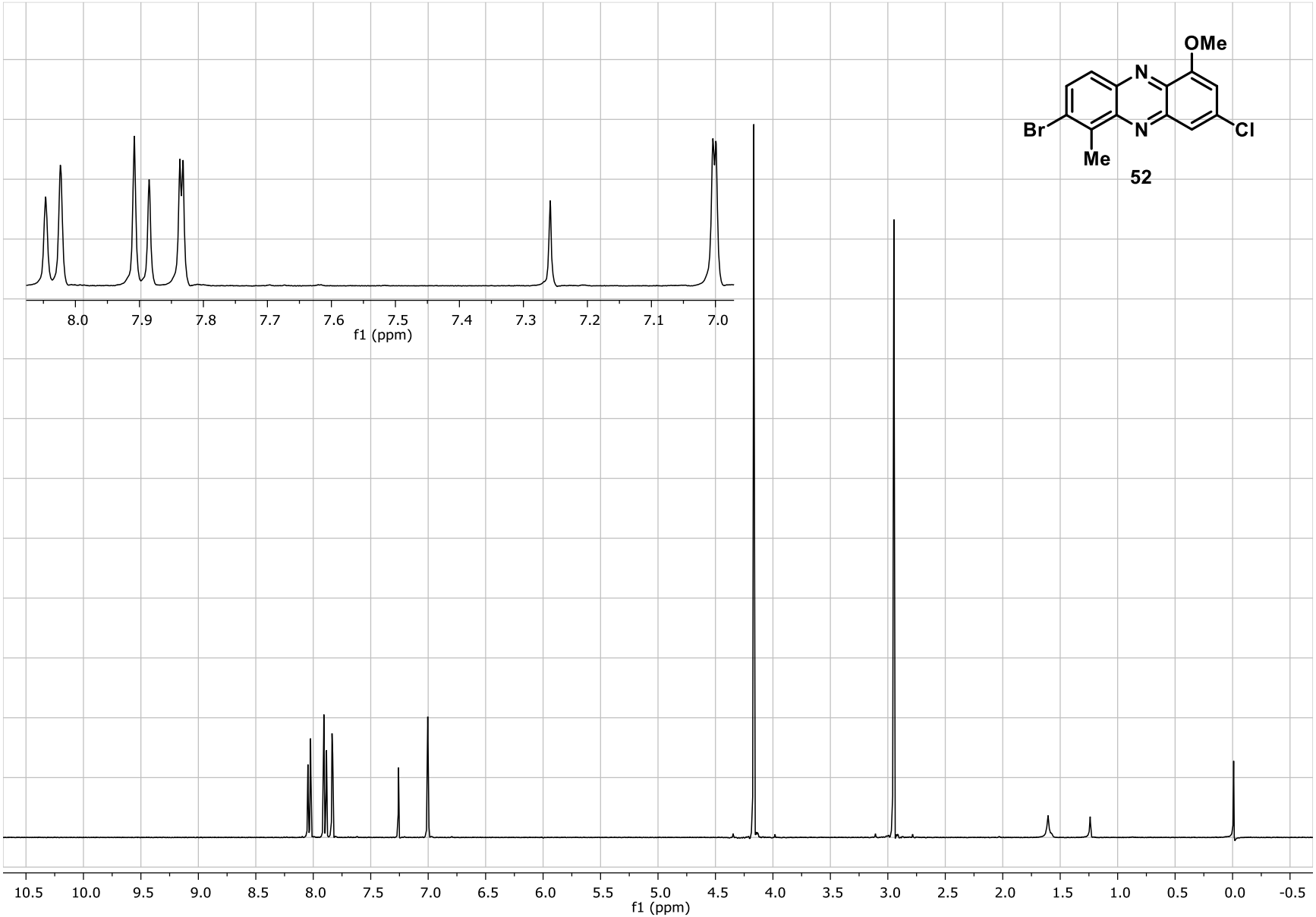
HSQC (zoomed in to assign carbon signal)

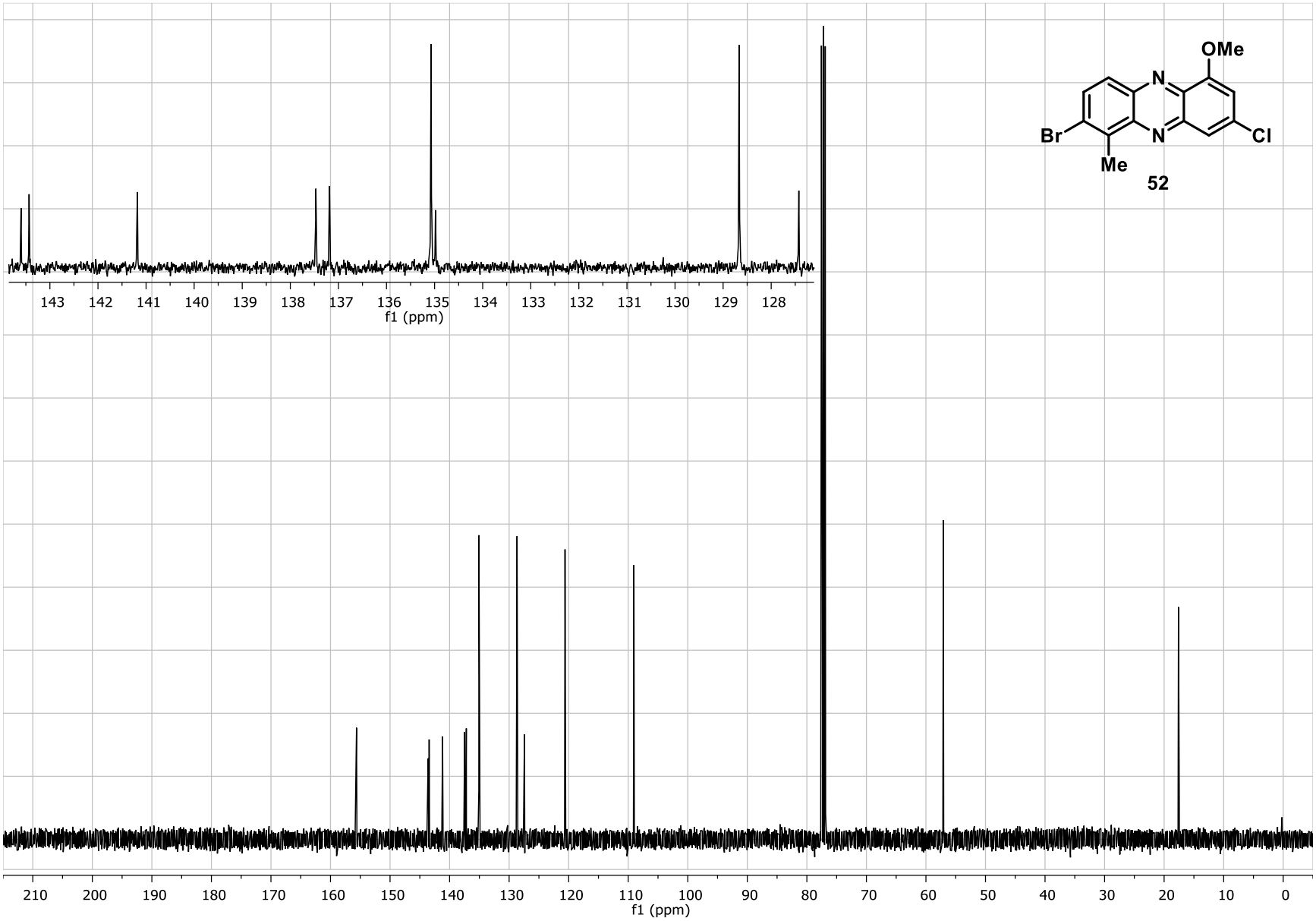

OH
Br
N
N
S
O
O
O
Me
Br
MeO
47
8.6
8.5
8.4
8.3
8.2
8.1
8.0
7.9
7.8
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

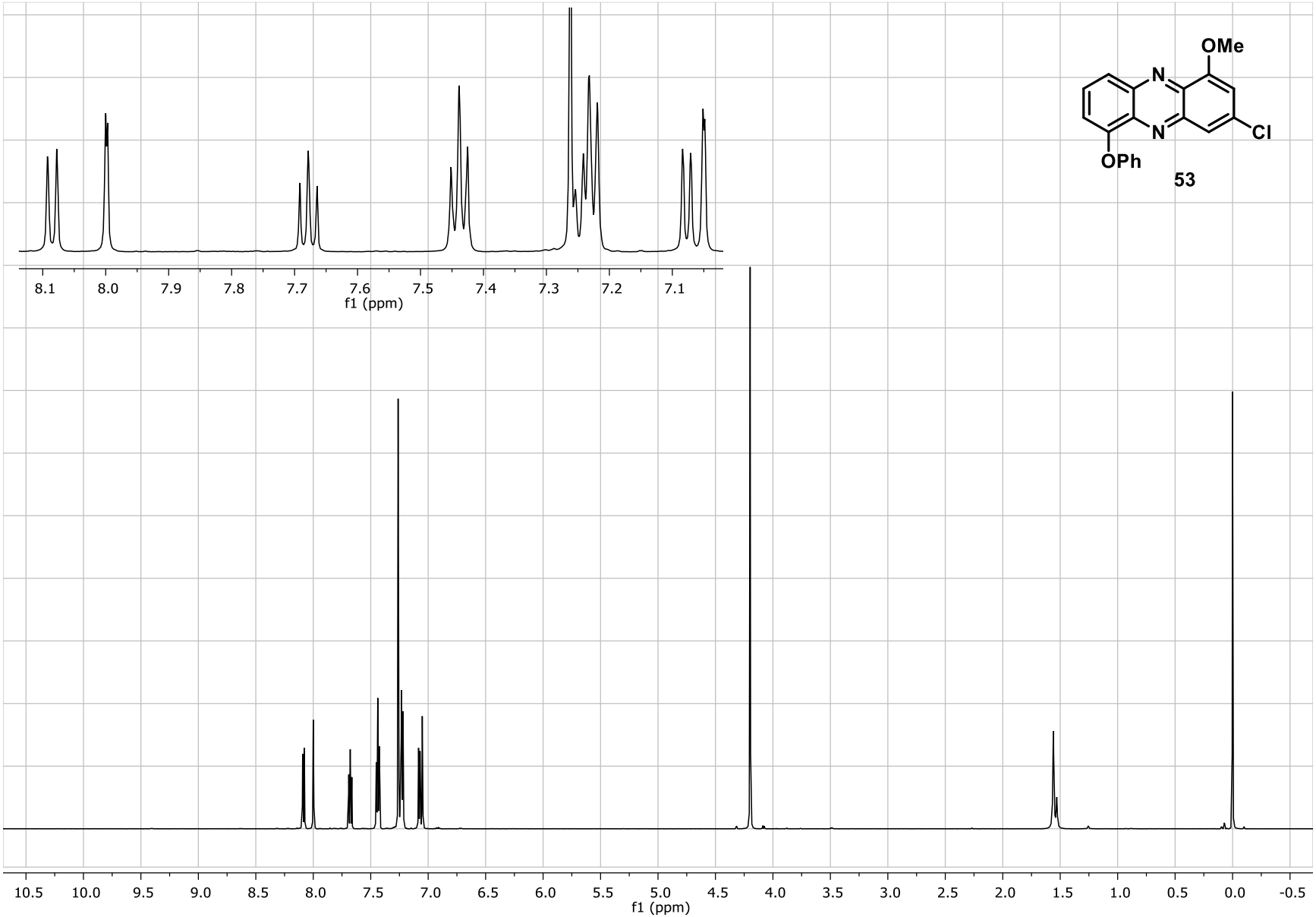

150 145 140 135 130 125 120 115
f1 (ppm)
72.0 71.5 71.0 70.5
f1 (ppm)
OH
Br
N
N
S
O
O
O
Me
Br
MeO
47
139.40 139.35 139.30 139.25 139.20
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

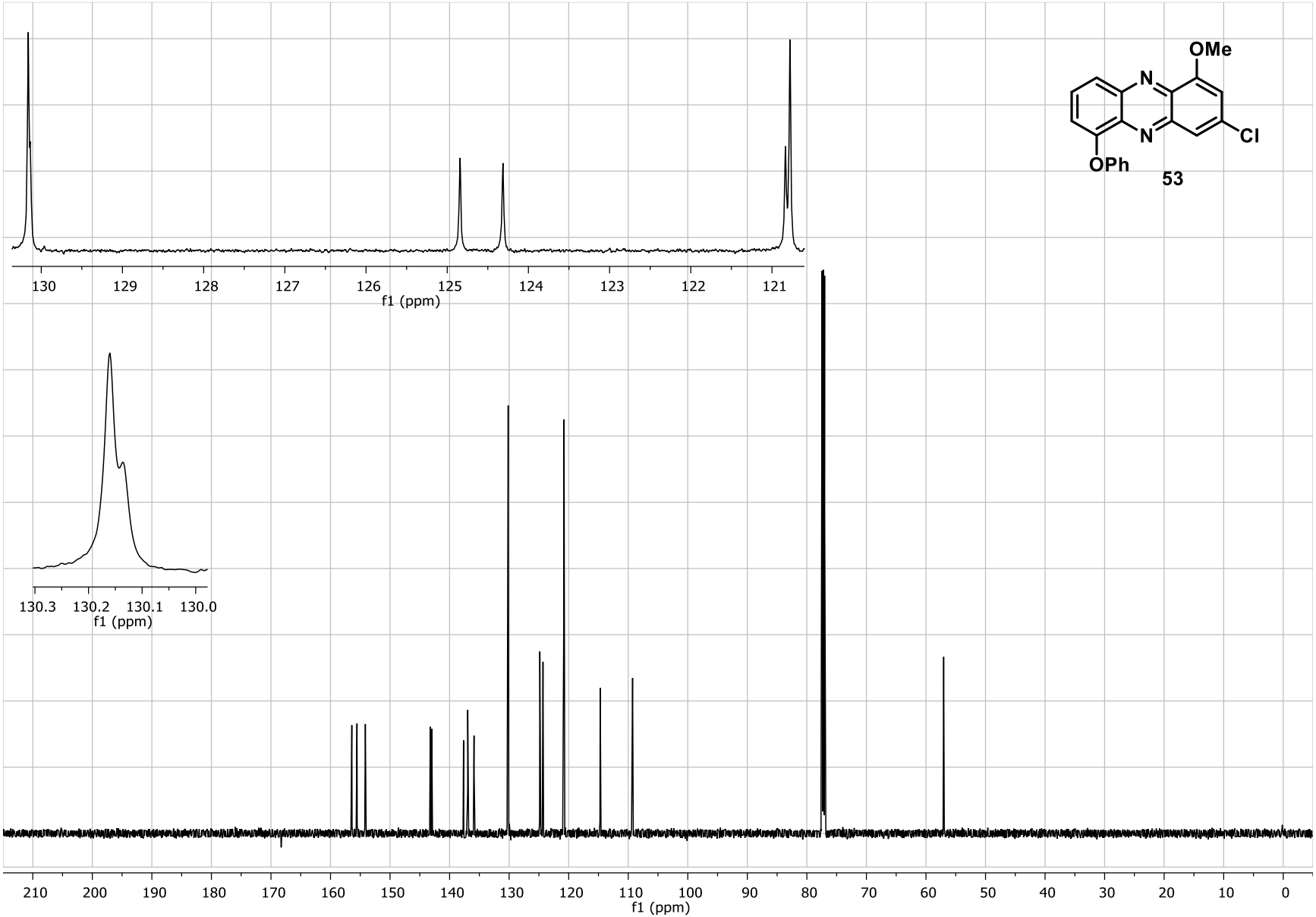

OMe
N
Me
N
Cl
Me
51
8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0
f1 (ppm)
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

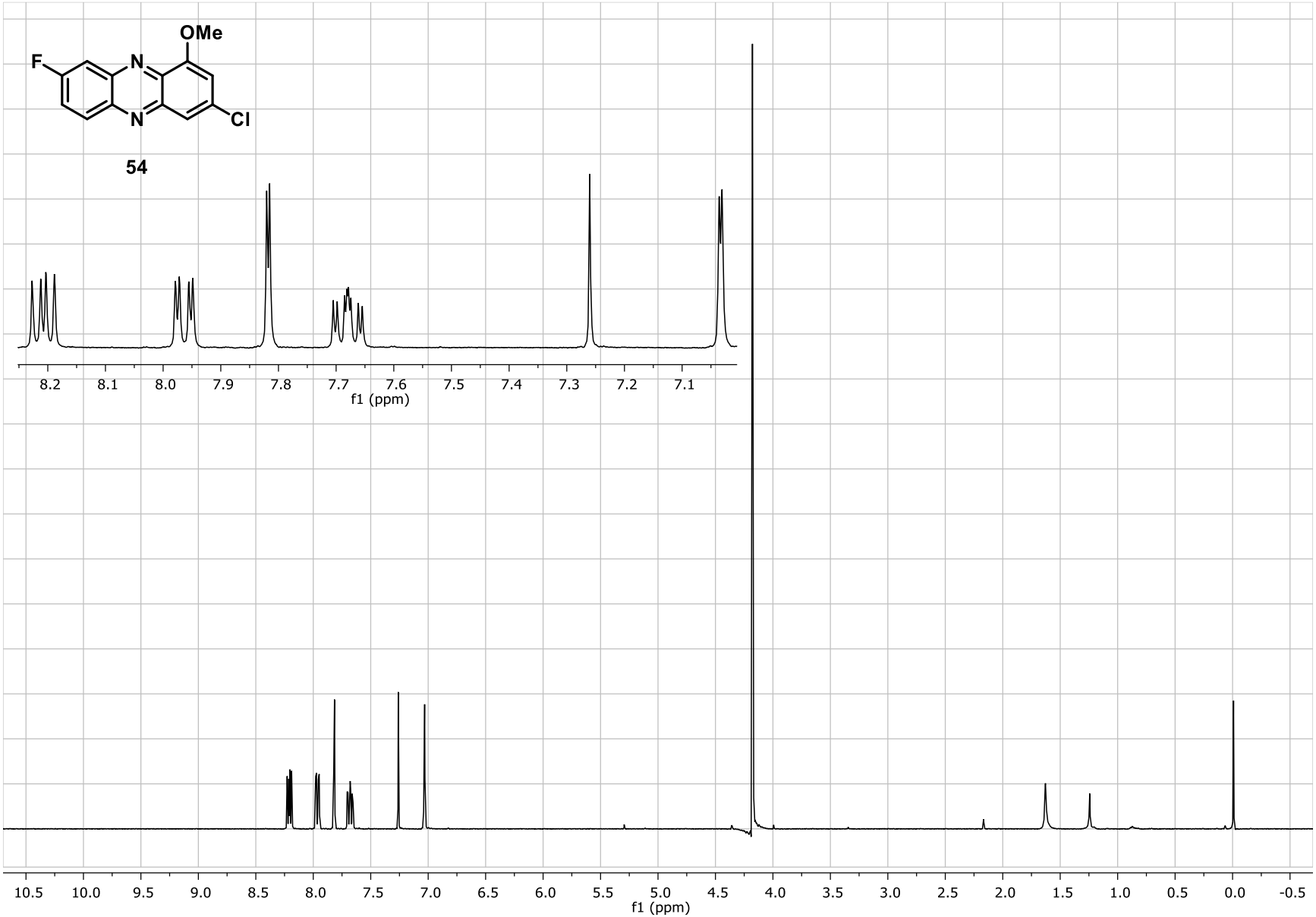

OMe
N
Me
N
Cl
Me
51
144
143
142
141
140
139
138
137
136
135
134
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

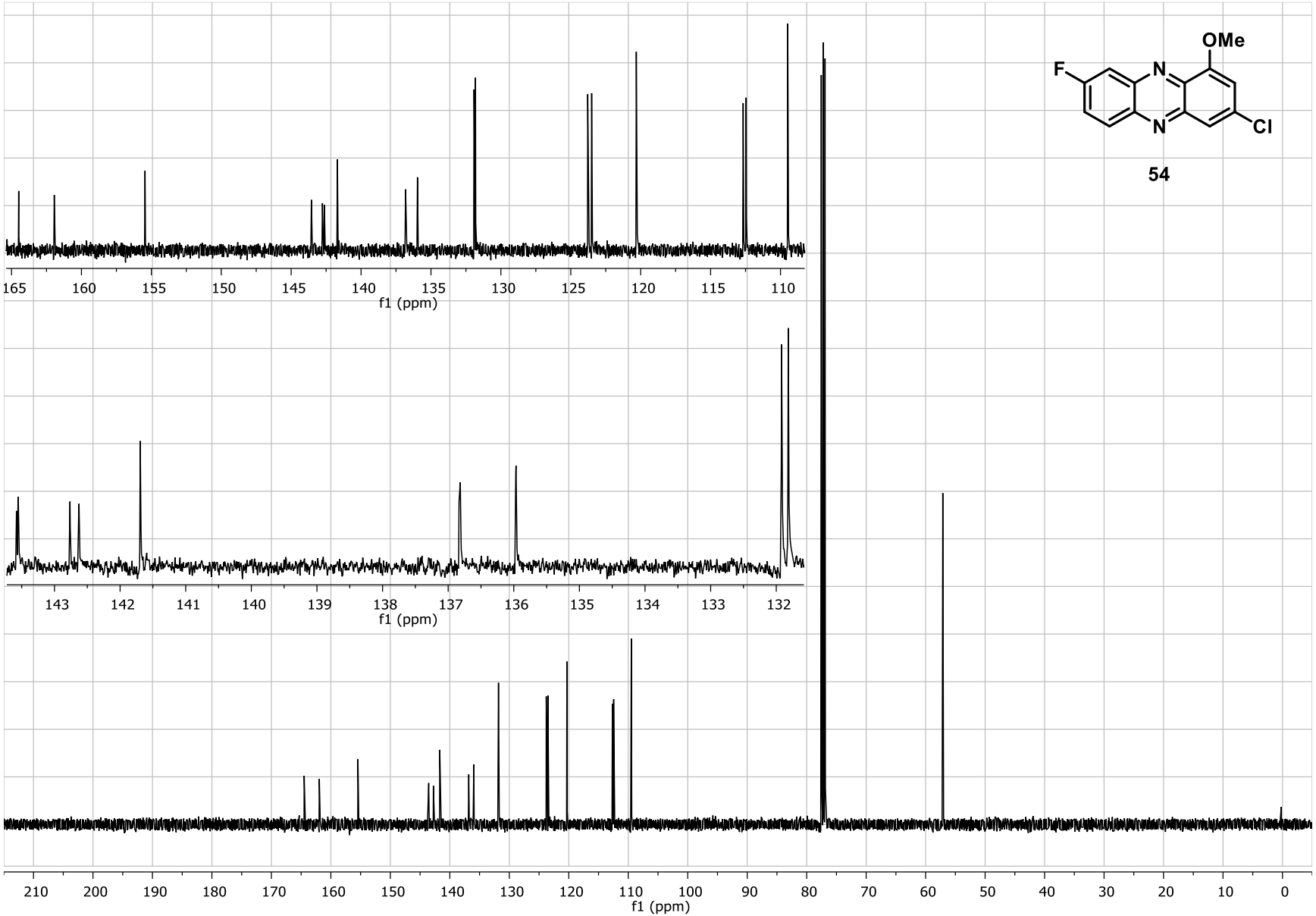

OMe
N
Br
N
Cl
Me
52
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

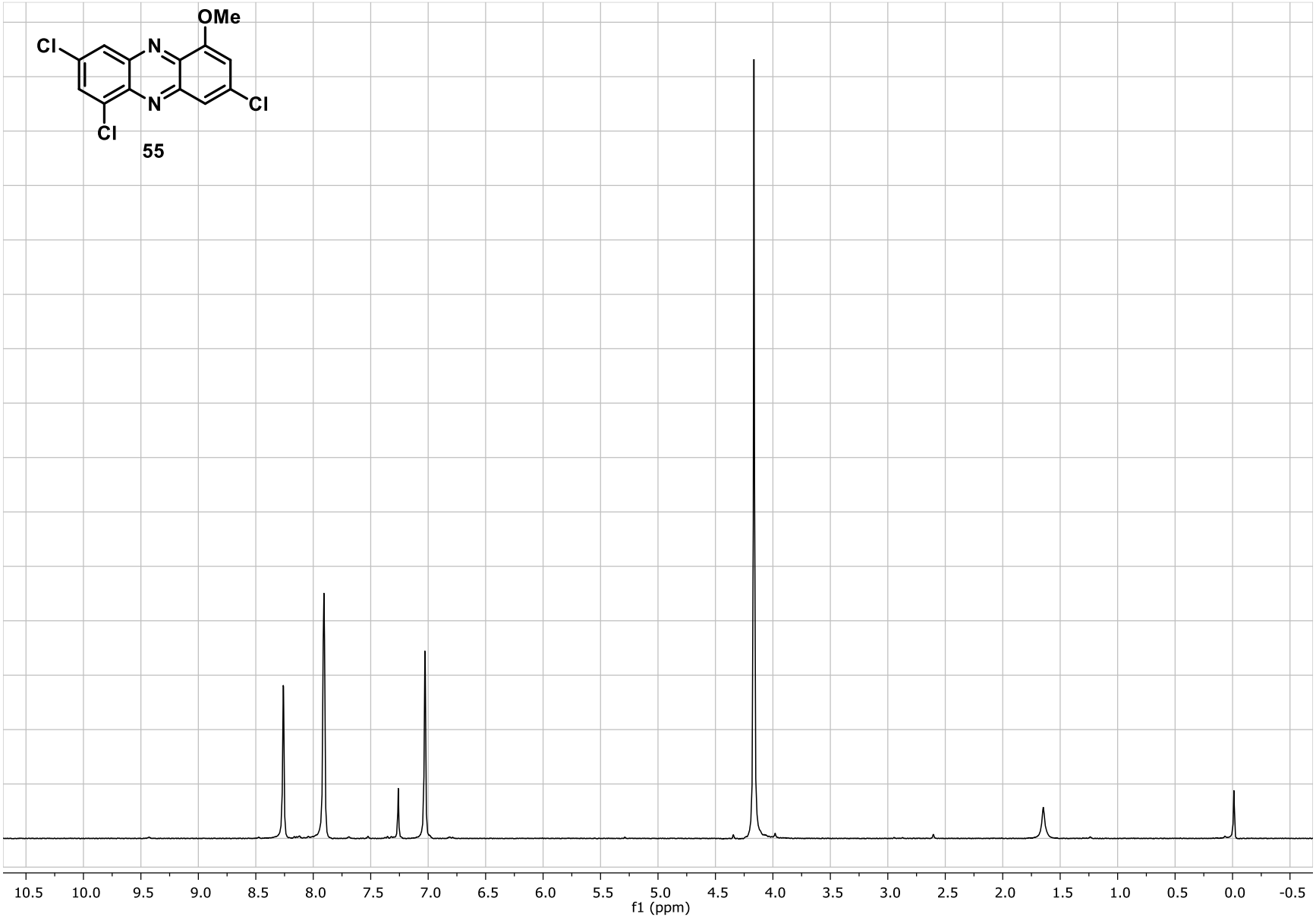

OMe
N
Br
N
Cl
Me
52
143 142 141 140 139 138 137 136 135 134 133 132 131 130 129 128
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

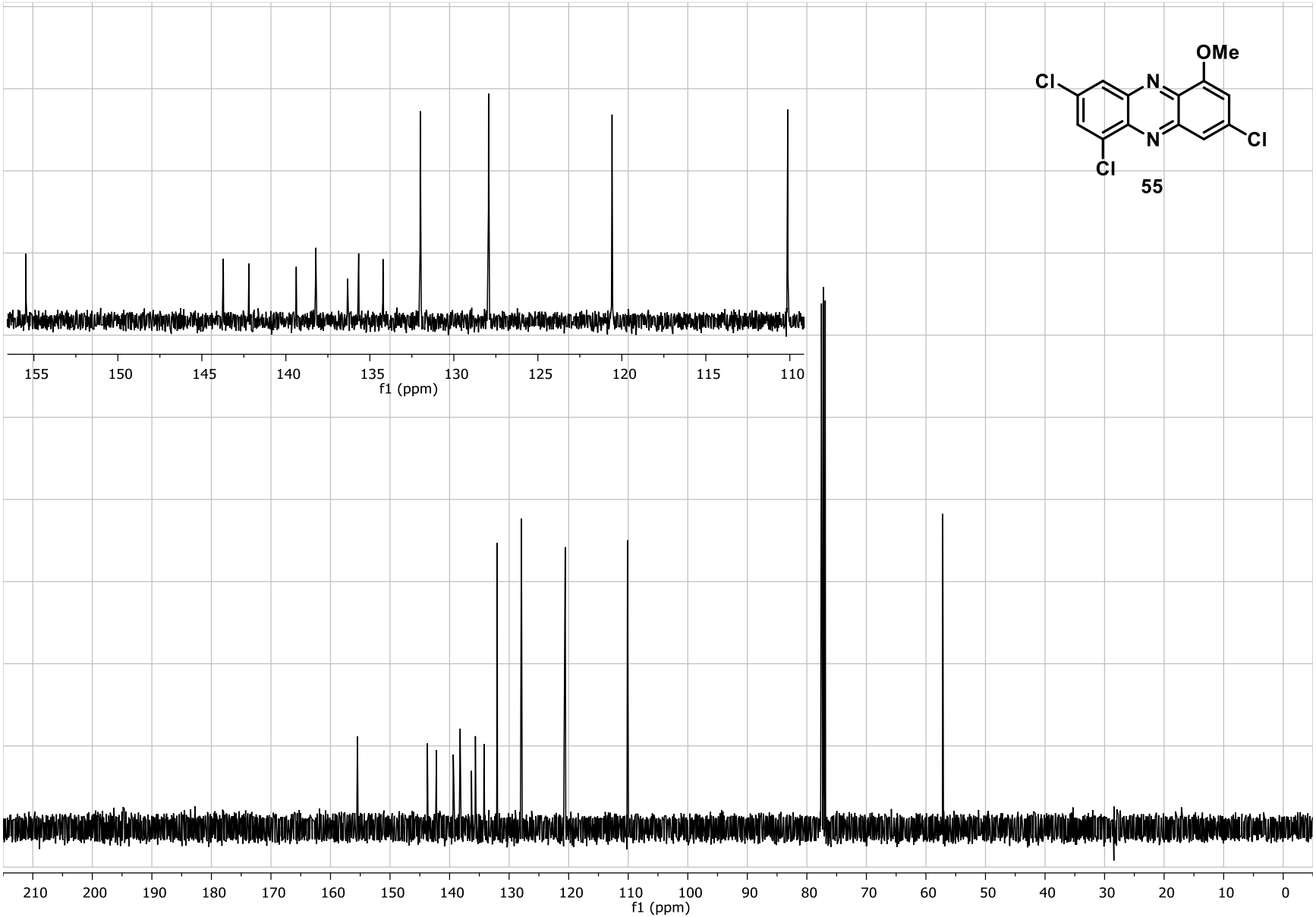

OMe
N
N
Cl
OPh
53
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

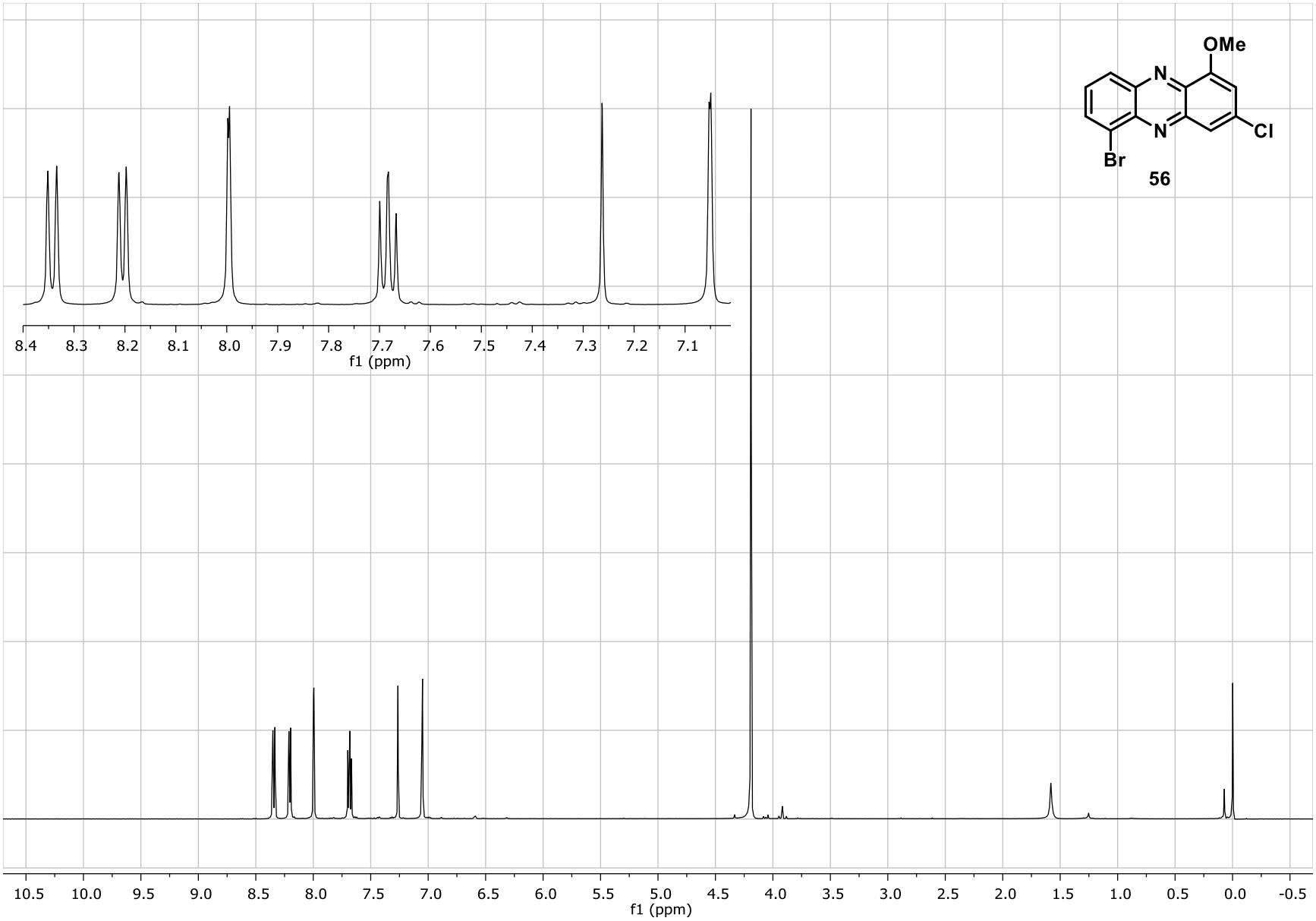

OMe
N
N
Cl
OPh
53
130
129
128
127
126
125
124
123
122
121
f1 (ppm)
130.3
130.2
130.1
130.0
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

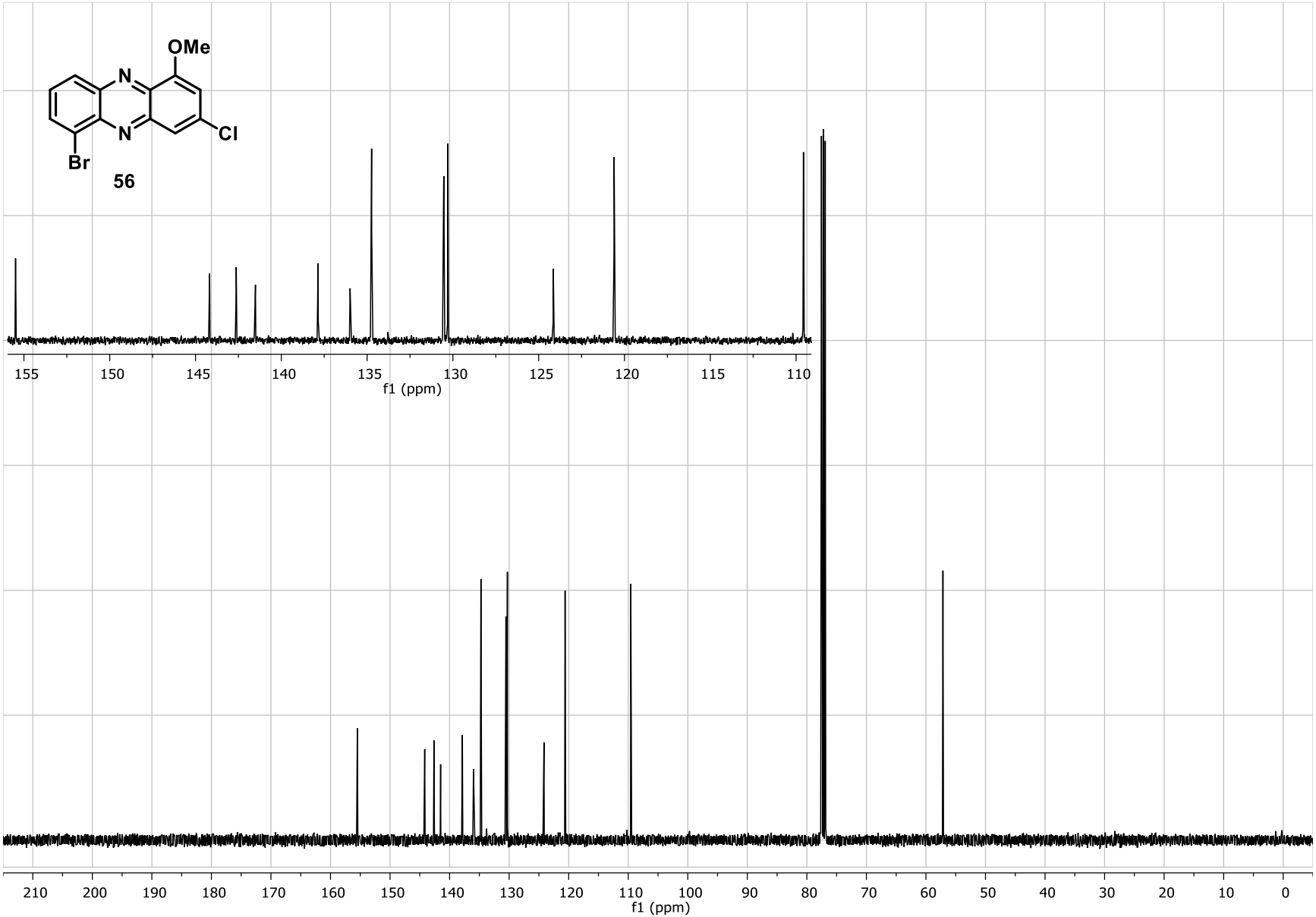

OMe
F
N
N
Cl
54
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
f1 (ppm)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

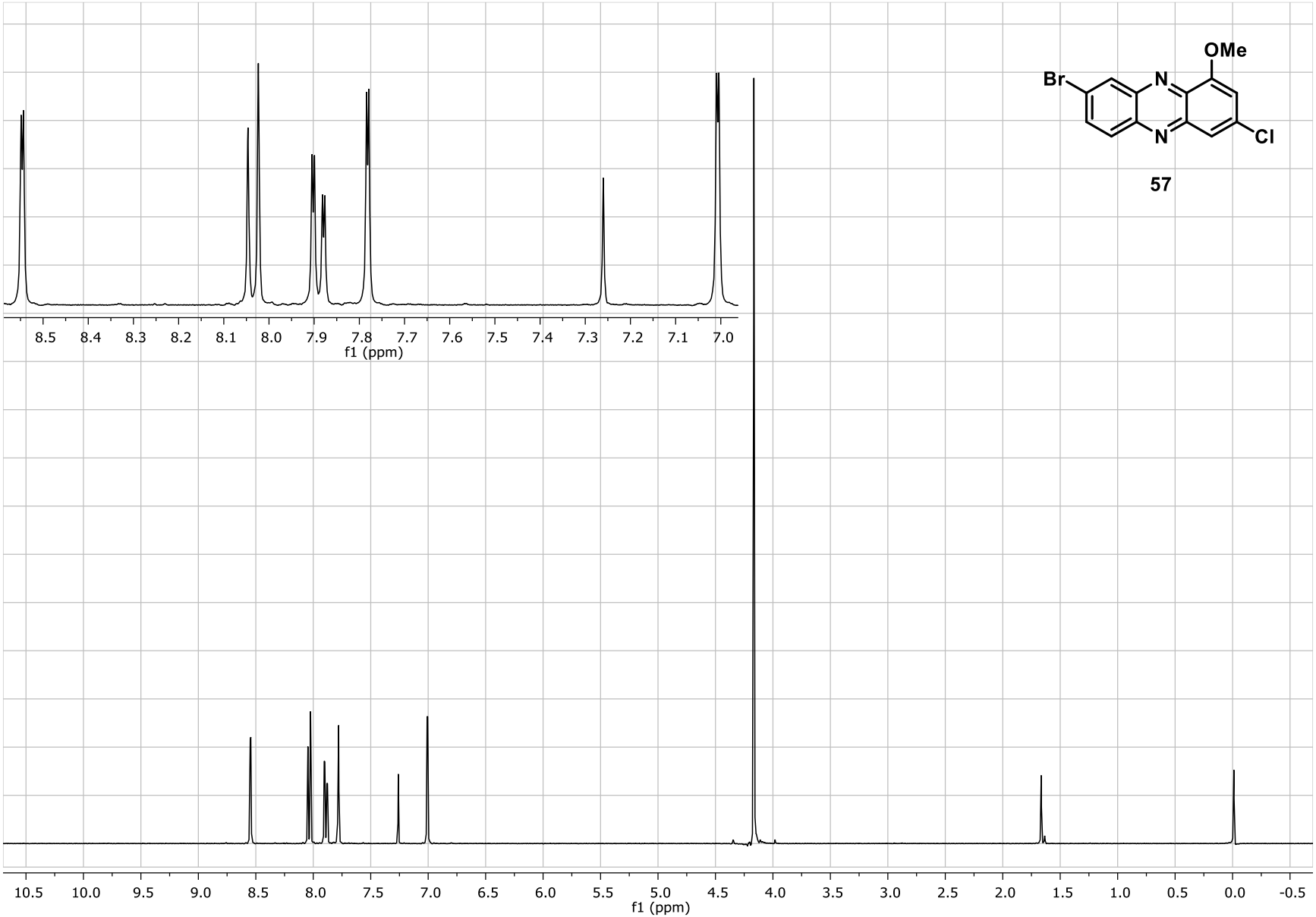

OMe
F
N
N
Cl
54
165 160 155 150 145 140 135 130 125 120 115 110
f1 (ppm)
143 142 141 140 139 138 137 136 135 134 133 132
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

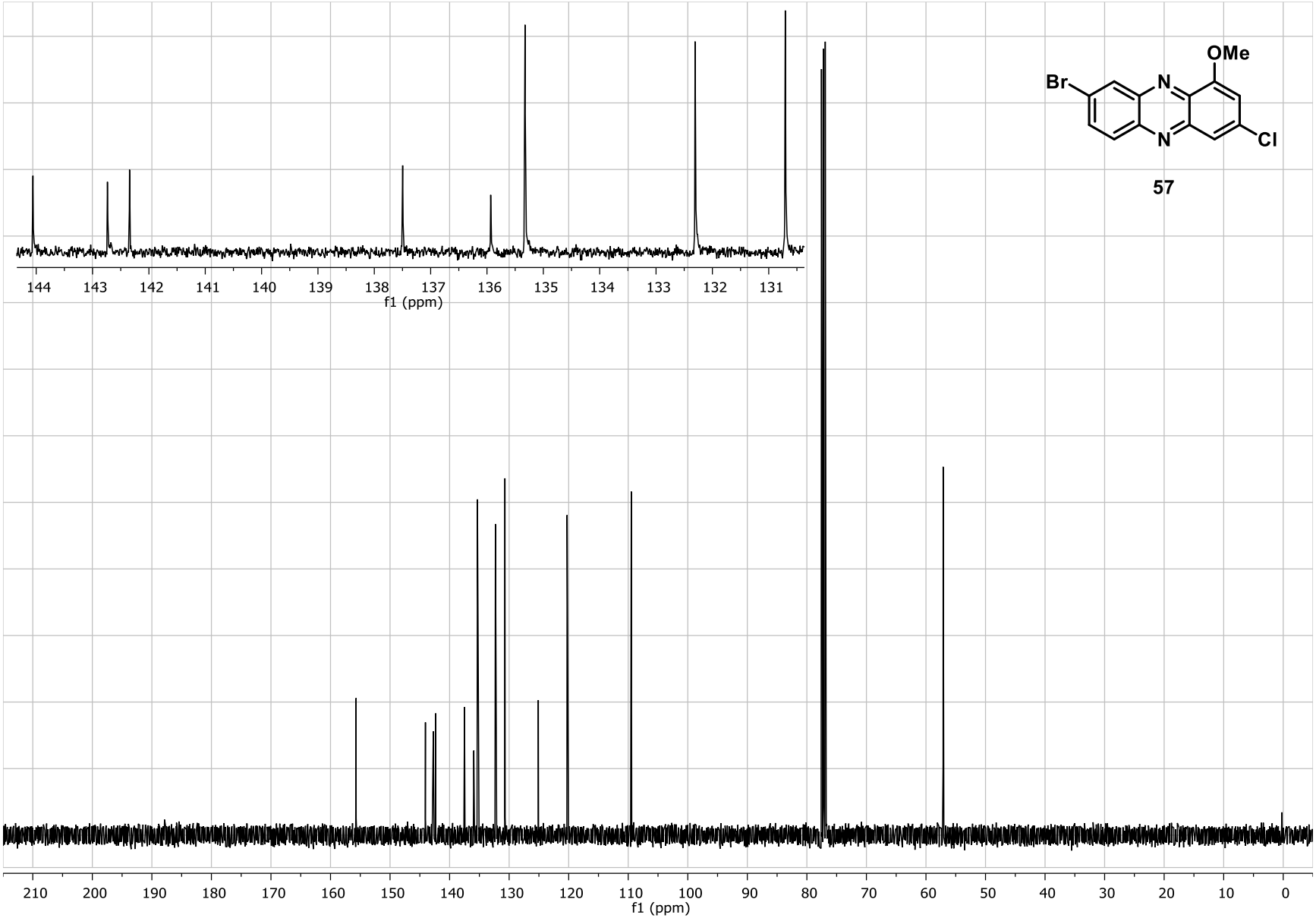

OMe
Cl
N
N
Cl
Cl
55
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

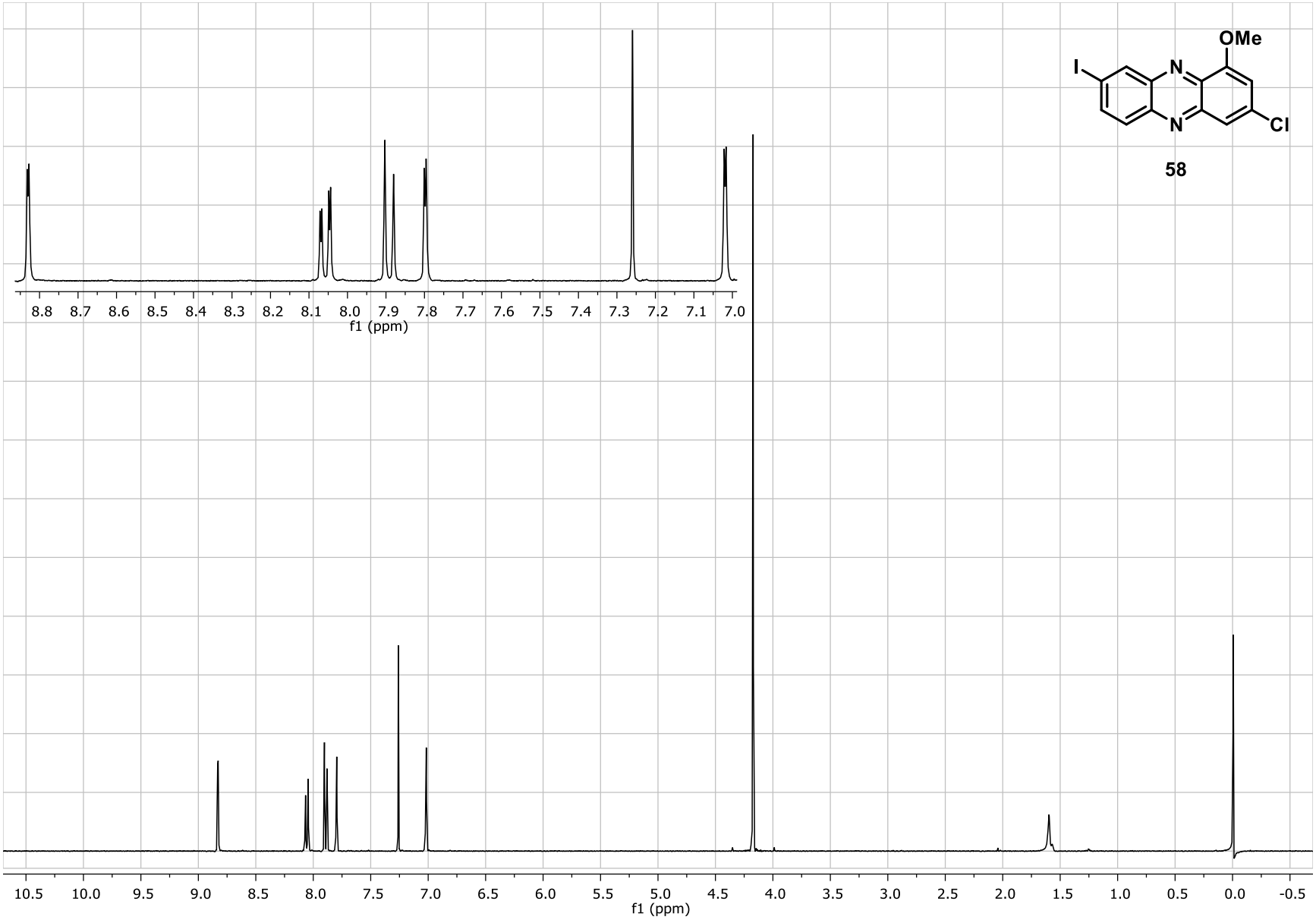

OMe
Cl
N
N
Cl
Cl
55
155 150 145 140 135 130 125 120 115 110
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

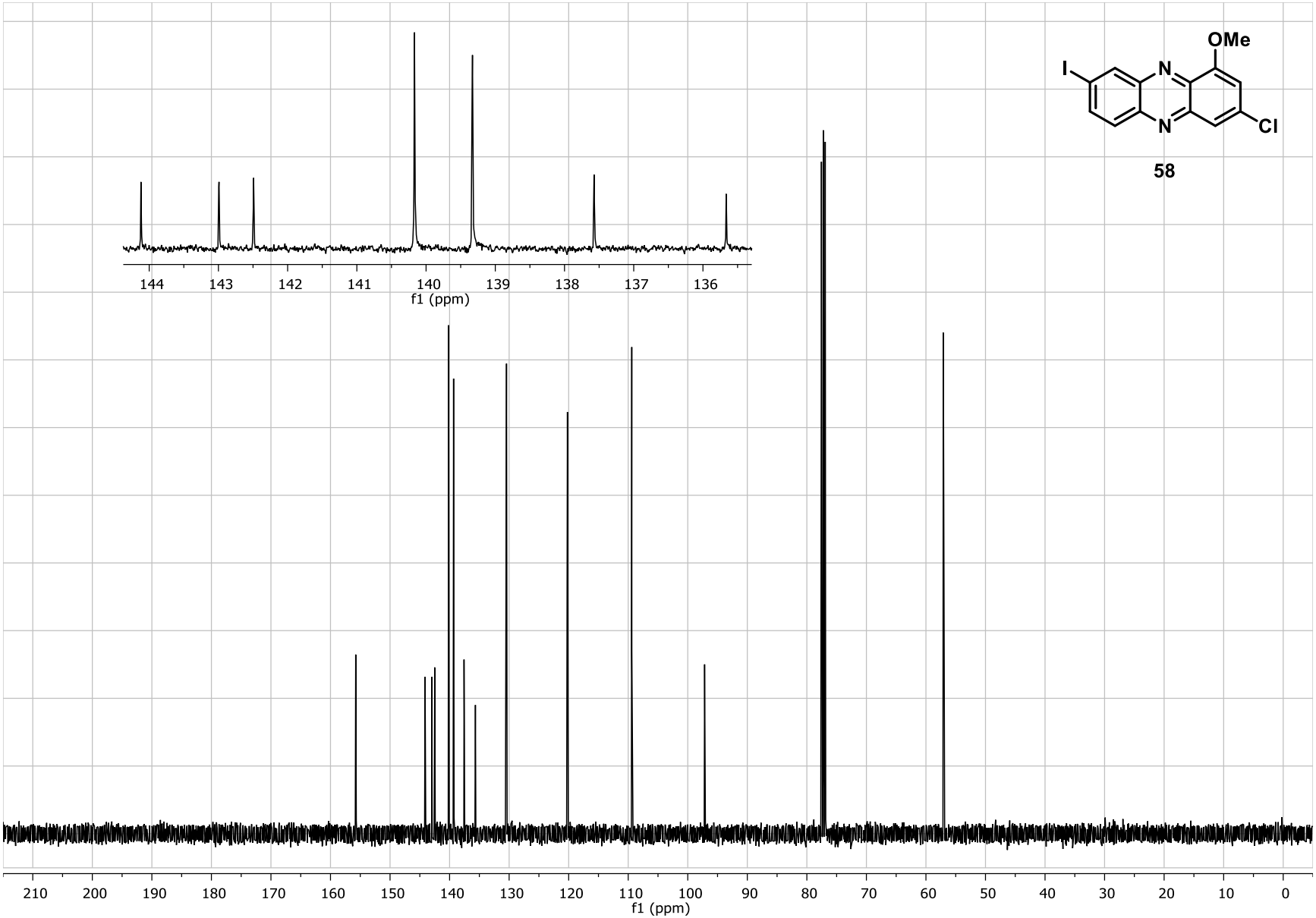

OMe
N
N
Cl
Br
56
8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1
f1 (ppm)
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


OMe
N
N
Cl
Br
56
155
150
145
140
135
130
125
120
115
110
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


OMe
Br
N
N
Cl
57
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)
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)


OMe
Br
N
N
Cl
57
144 143 142 141 140 139 138 137 136 135 134 133 132 131
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


OMe
I
N
N
Cl
58
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
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)


OMe
I
N
N
Cl
58
144
143
142
141
140
139
138
137
136
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)