Supporting Information for

Silicon Substitution in Oxazine Dyes Yields Near-Infrared Azasiline Fluorophores that Absorb and Emit Beyond 700 nm

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Materials and Methods

Figure S1a: Excitation and Emission of Azasilines in Ethanol

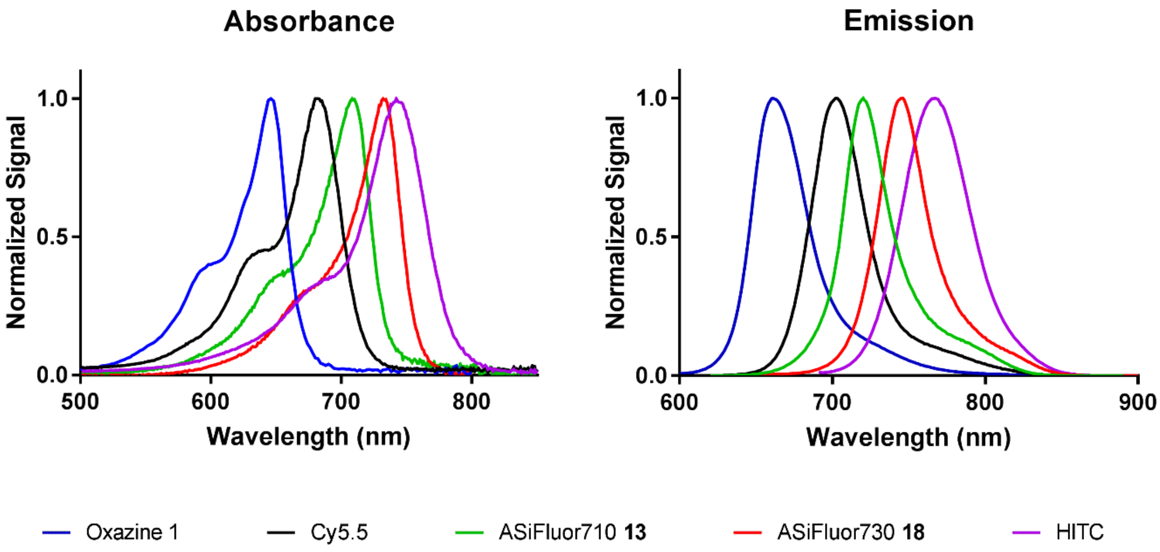


Figure S1b: Excitation and Emission of ASiFluor730 in Various Solvents

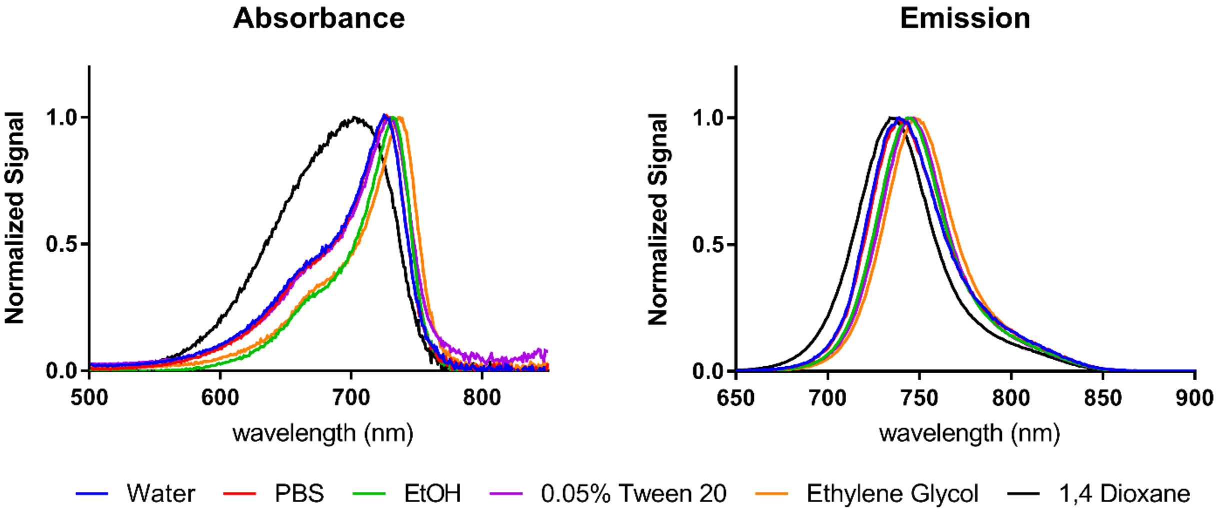
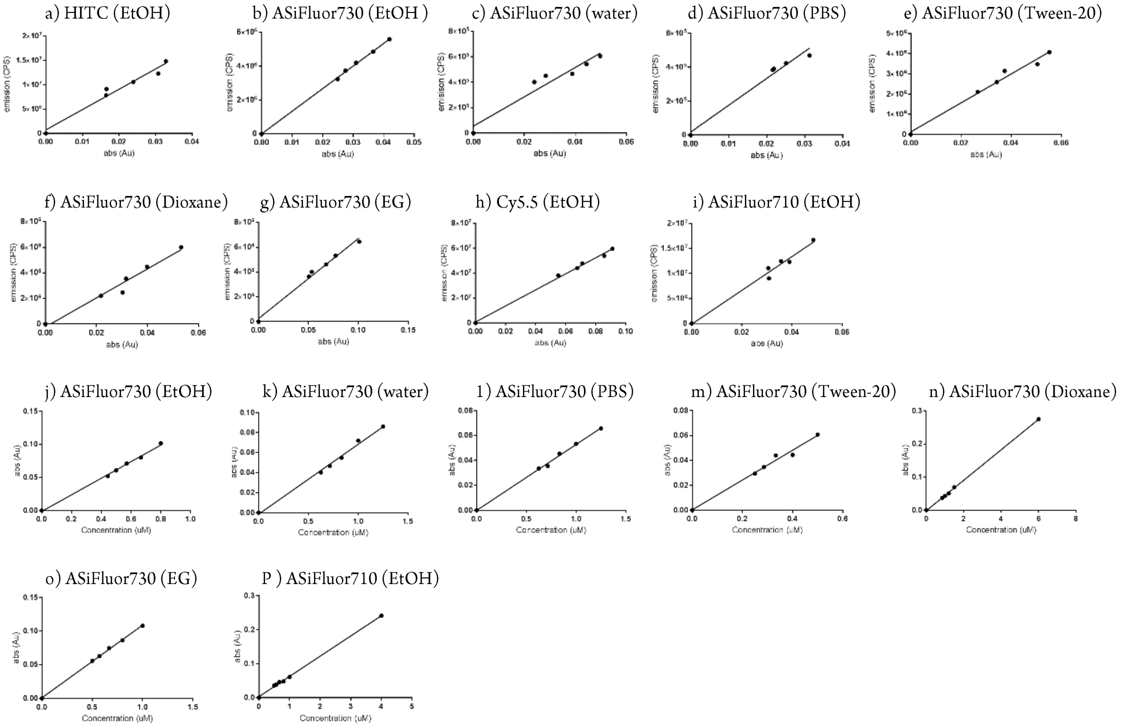


Figure S2: Measurement of Quantum Yields and Molar Extinction Coefficients of ASiFluors



a) HITC (EtOH) b) ASiFluor730 (EtOH) c) ASiFluor730 (water) d) ASiFluor730 (PBS) e) ASiFluor730 (Tween-20)

f) ASiFluor730 (Dioxane) g) ASiFluor730 (EG) h) Cy5.5 (EtOH) i) ASiFluor710 (EtOH)

j) ASiFluor730 (EtOH) k) ASiFluor730 (water) l) ASiFluor730 (PBS) m) ASiFluor730 (Tween-20) n) ASiFluor730 (Dioxane)

o) ASiFluor730 (EG) P) ASiFluor710 (EtOH)

Compound	Solvent	Slope	r^2	ϕ	ε (M−1 cm−1)	r^2	Brightness
ASiFluor730	EtOH	133,500,434	0.9991	0.11	120,601	0.9998	13,817
	water	11,515,019	0.9337	0.01	69,980	0.9949	631
	PBS	15,807,667	0.9750	0.01	52,943	0.9941	549
	0.05% Tween-20	71,799,640	0.9802	0.06	95,030	0.9949	5,592
	Ethylene Glycol	132,590,701	0.9759	0.13	108,600	0.9999	13,661
	Dioxane	120,154,108	0.9980	0.13	46,270	0.9990	5,942
ASiFluor710	EtOH	338,139,559	0.9788	0.11	59,570	0.9989	6,285
HITC	EtOH	349,572,566	0.9784	0.30			
Cy5.5	EtOH	641,024,853	0.9964	0.20			

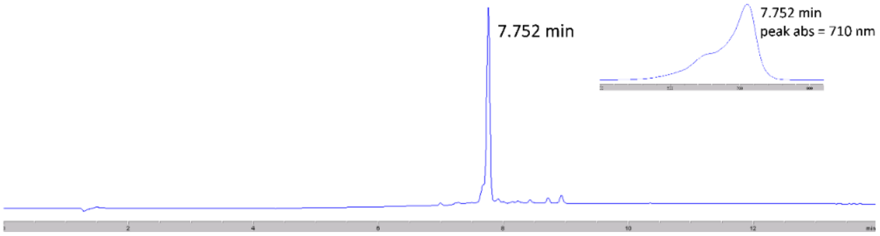
Quantum yields (a-i) and molar extinction coefficients (l-p) of ASiFluors.
Quantum yields were calculated using equation:

$$\phi_x = \phi_{ST}\left[\frac{(slope)_x}{(slope)_{ST}}\right]\left[\frac{n_x^2}{n_{ST}^2}\right]$$

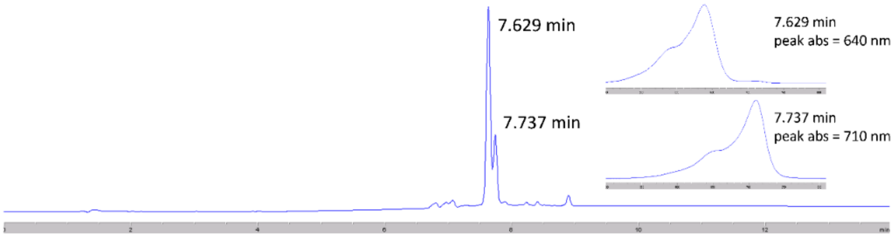
where slope refers to the slope of absorbance vs. integrated emission and *n* was the refractive index of solvents if different solvents were used between the standard and sample x. HITC was used for ASiFluor730 and Cy5.5 was used for ASiFluor710 as references. Results are given in the table below the graphs.

Figure S3a: HPLC of ASiFluors after PBS treatment

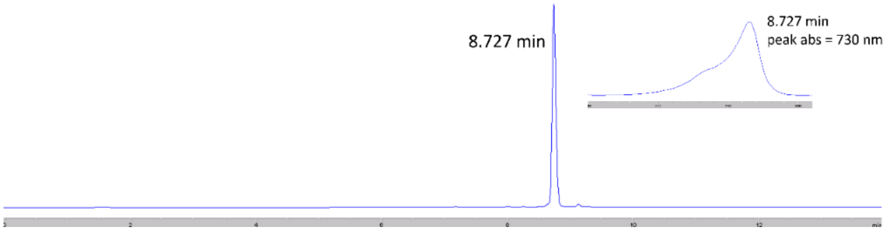
a) ASiFluor710 (No treatment)



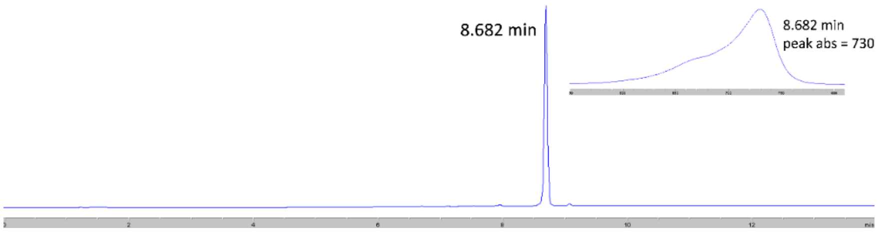
b) ASiFluor710 (48 hours in PBS)



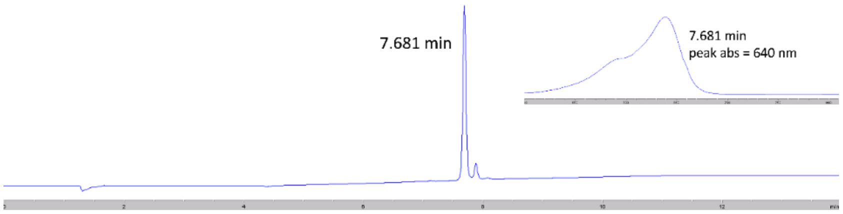
c) ASiFluor730 (No treatment)



d) AsiFluor730 (48 hours in PBS)



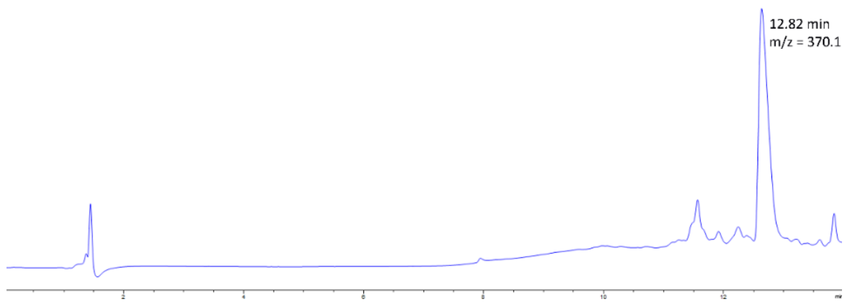
i) Isolated ASiFluor710 degradation product



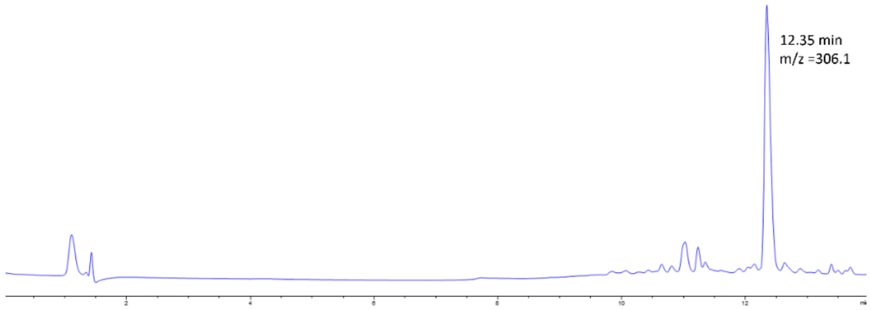
HPLC spectra of ASiFluor710 (a-b) and ASiFluor730 (c-d), before (a and c), after 48 hour incubation in PBS (b and d), and isolated degradation product of ASiFluor710 (e). Each peak's corresponding diode array detector (DAD) spectrum is displayed on the right of each trace. HPLC detection wavelength was set to 650 nm.

Figure S3b: LCMS of ASiFluors after PBS treatment

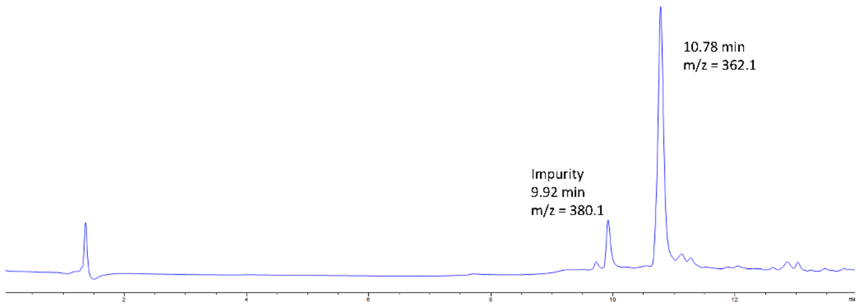
a) ASiFluor710 (No treatment)



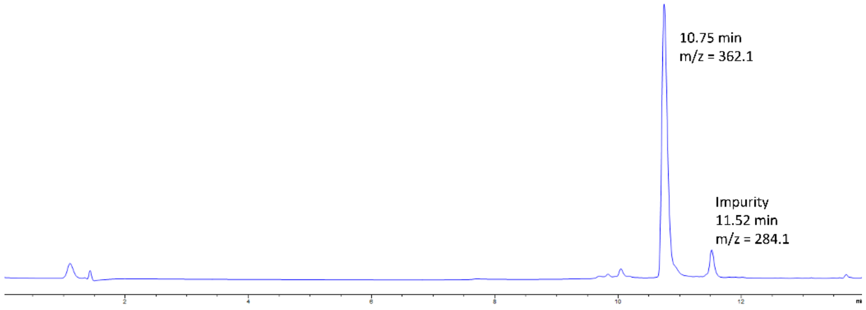
b) ASiFluor710 (48 hours in PBS)



c) ASiFluor730 (No treatment)



d) ASiFluor730 (48 hours in PBS)



LCMS spectra of ASiFluor730 (a-b) and ASiFluor730 (c-d), before (a and c) and after 48 hour incubation in PBS (b and d). Detected mass is reported below the retention time. LCMS detection wavelength was set to 280 nm.

Table S1: Size Comparison of Fluorophores

Fluorophore	Ex (nm)	Em (nm)	MW	Length (Å)	Ex : Length	Ex : MW	Distance Between	Ref.
ASiFluor710	710	720	370	13.08	54.28	1.92	C(22)-C(25)	this work
AsiFluor730	730	740	362	13.08	55.81	2.02	C(24)-C(21)	this work
SiR700	691	712	423	11.93	57.92	1.63	C(8)-C(19)	1
SiR720	721	740	532	14.52	49.66	1.36	C(37)-C(33)	1
ICG	775	831	752	17.20	45.06	1.03	C(2)-C(30)	2
Cy5.5	679	696	483	14.89	45.60	1.41	C(2)-C(25)	2
Oxazine 1	646	662	324	14.39	44.89	1.99	C(19)-C(24)	3
HITC (Cy7)	743	772	409	18.03	41.21	1.82	C(2)-C(26)	3
TTAB	733	757	521	11.51	63.68	1.41	C(29)-C(22)	4
Sq2	729	739	521	14.27	51.09	1.40	C(35)-C(37)	5

The length was calculated as the distance between the two outer most atoms using Chem3D. The excitation wavelength to molecular weight ratio estimates the compactness of the molecule. ICG = Indocyanine Green. TTAB and Sq2 are examples of NIR aza-BODIPY and squarine fluorophores, respectively.

Materials and Methods

General: Commercially available products were used without purification and purchased form Combi-Block, Chem-Impex, Sigma-Aldrich, Acros or Oakwood Chemicals. Palladium catalysts and ligands were purchased from Combi-Blocks and Chem-Impex. Cy5.5 free carboxylic acid was purchased from Lumiprobe. Anhydrous solvents were purchased from Acros. Compounds were purified with the CombiFlash Rf+ system and HPLC-grade ChromoSolv solvents were purchased from Sigma-Aldrich.

All NMR spectra (^{1}H, ^{19}F, and ^{13}C) were obtained on a Bruker Ascend 500. Analytical HPLC was performed on an Agilent 1100 equipped with a Zorbex C8 column and a PDA detector (G1315A DAD). Preparatory HPLC was performed on a Varian ProStar equipped with Aglient 10-Prep C18 21.2 x 250 mm Column. LCMS was performed on an Agilent 1260 Infinity equipped with a Zorbex SB-C18 column and a 6130 Quadrupole detector. High resolution mass-spec data were obtained on an Agilent 6520 Q-TOF. Absorption and emission data were obtained on Cary-50 and Fluoromax-4 instruments, respectively. Data were graphed and fitted using Graphpad Prism 7. NMR spectra were analyzed with MestReNova.

Photostability

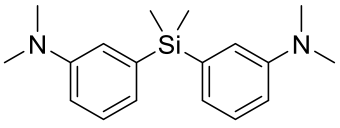
Each fluorophore was diluted from a 10 mM DMSO stock to 3.3 μM in ethanol or 10 μM in PBS. The excitation wavelength was set to match each fluorophore's absorption maxima with a 20 nm slit width. The emission detection wavelength was set to 40 nm away from the absorption maxima at the indicated slit width. The fluorescence signal was collected every 0.1 seconds for 3700 seconds and normalized to the initial signal. The average μAs are listed as an estimate of the relative irradiation intensity at each wavelength.

Compound	[μM]	Solvent	Excitation	Slit	Emission	Slit	Avg. μA
Oxazine 1	10	PBS	646	20	686	1	116
	3.3	EtOH				1	
ASiFluor730 (**18**)	10	PBS	730		770	10	95
	3.3	EtOH				2	
Cy5.5	10	PBS	684		724	2	141
	3.3	EtOH				1	
ASiFluor710 (**13**)	3.3	EtOH	710		750	2	105

Quantum Yield and Extinction Coefficient

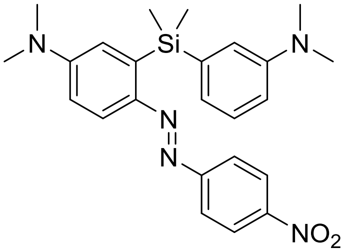
Compounds were first diluted from a 10 mM DMSO stock into the desired solvent (e.g. PBS, water, EtOH, 1,4-Dioxane, 0.05% Tween-20 or ethylene glycol). The first dilution solution was allowed to equilibrate in the dark at room temperature for 15 minutes before a second dilution was made. The second dilution was allowed to equilibrate in the dark at room temperature for another 15 minutes before the absorption or fluorescence signal was read. Absorbance readings were kept below 0.1 for quantum yield samples. Slit widths were set to 2 nm (ex) and 5 nm (em) with 0.1s integration time for fluorescence measurements.

Synthetic Methods



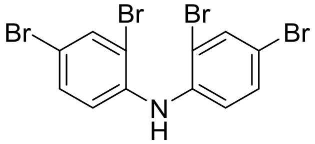
3,3'-(Dimethylsilanediyl)bis(N,N-dimethylaniline) (**1**)

In a 50 mL oven dried round bottom flask, 3-bromo-N,N-dimethylaniline (200 mg, 1 mmol) was dissolved in anhydrous diethyl ether (10 mL) and anhydrous THF (0.5 mL) at room temperature and then cooled to 0 °C. *n*-BuLi (0.49 ml of 2.5 M, 1.2 mmol) was added drop-wise to the reaction. After 1 hour, dichlorodimethylsilane (77 mg, 0.6 mmol) was added and the reaction was warmed to room temperature. After 16 hours, the reaction was poured into water (20 mL), and the product was extracted with diethyl ether (25 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), then dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by silica gel chromatography (0-30% EtOAc/hexanes) to afford the product as a yellow oil (37 mg, 0.12 mmol, 24%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 – 7.27 (m, 2H), 6.98 (d, *J* = 2.7 Hz, 2H), 6.95 (m, 2H), 6.80 (ddd, *J* = 8.2, 2.7, 0.6 Hz, 2H), 2.96 (s, 12H), 0.57 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.1, 139.1, 128.6, 122.9, 118.5, 113.7, 40.8, -2.0. HR-EIMS m/z calculated for C18H27N2Si $[M + H]^+$:299.1938, found: 299.1917.



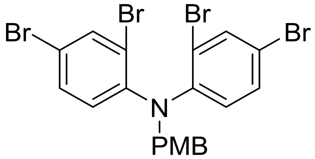
(E)-3-((3-(Dimethylamino)phenyl)dimethylsilyl)-N,N-dimethyl-4-((4-nitrophenyl)diazenyl) aniline (**2**)

In a 100 mL round bottom flask, compound **1** (149 mg, 0.5 mmol) was dissolved in aq 2 M HCl (25 mL) and MeOH (10 mL) at 0 °C. 4-Nitrobenzenediazonium tetrafluoroborate (119 mg, 0.5 mmol) was separately dissolved in aq 2 M HCl (1 mL) and MeOH (5 mL) and added dropwise with an addition funnel, then stirred at 4 °C. After 16 hours, the reaction was poured into sat'd $NaHCO_3$ (100 mL), and the product was extracted with ethyl acetate (3 X 20 mL). The combined organic layers were washed with $NaHCO_3$ (50 mL) and brine (50 mL), then dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by silica gel chromatography (0-20% EtOAc/hexanes) to afford the product as a purple solid (105 mg, 0.23 mmol, 47%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (d, *J* = 8.9 Hz, 2H), 7.91 (d, *J* = 9.1 Hz, 1H), 7.60 (d, *J* = 8.9 Hz, 2H), 7.22 (t, *J* = 7.7 Hz, 1H), 6.95 (d, *J* = 2.2 Hz, 1H), 6.92 (d, *J* = 7.2 Hz, 1H), 6.87 (d, *J* = 2.8 Hz, 1H), 6.78 – 6.71 (m, 2H), 3.08 (s, 6H), 2.88 (s, 6H), 0.61 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.0, 152.5, 150.2, 148.3, 147.2, 145.3, 140.0, 128.5, 124.7, 123.0, 122.9, 118.6, 118.2, 117.4, 113.6, 113.1, 40.9, 40.3, -0.2. HR-EIMS m/z calculated for C24H30N5O2Si [M + H]$^+$: 488.2163, found: 488.2143.



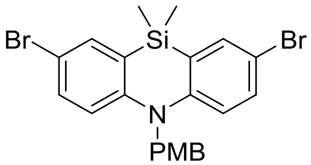
Bis(2,4-dibromophenyl)amine (3)

In a 1 L round bottom flask, diphenylamine (5 g, 29.9 mmol) was dissolved in acetone (225 mL) at room temperature. The temperature was lowered to 0 °C, then NBS (23.2 g) was added in small portions. After 15 minutes, H_2O (225 mL) was added to the reaction and the white solid was filtered and washed with 50/50 acetone/H_2O (400 mL). Finally, the white solid was recrystallized in toluene (300 mL) giving **3** as a white solid (9.75 g, 20.1 mmol, 69%). ^{1}H NMR (500 MHz, C_6D_6) δ 7.45 (d, *J* = 2.2 Hz, 2H), 6.88 (dd, *J* = 8.7, 2.2 Hz, 2H), 6.39 (d, *J* = 8.7 Hz, 2H), 6.15 (s, 1H). ^{13}C NMR (126 MHz, C_6D_6) δ 139.0, 135.6, 131.3, 119.2, 115.3, 114.2. HR-EIMS m/z calculated for C12H8Br4N [M + H]$^+$: 485.7344, found: 485.7339.



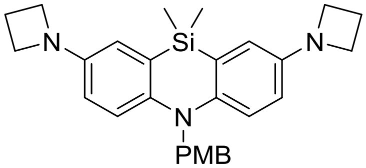
2,4-Dibromo-N-(2,4-dibromophenyl)-N-(4-methoxybenzyl)aniline (4)

In a 500 mL round bottom flask, compound **3** (9.75 g, 20.1 mmol) was dissolved in THF (250 mL) at 0 °C. Sodium hydride (2.4 g, 100 mmol) was added to the reaction in small portions. After one hour, *p*-methoxybenzyl chloride (PMB-Cl) (4.1 mL, 30.1 mmol) was added, then the reaction was warmed to room temperature and stirred for 72 hours. The solvent was then removed under reduced pressure. The resulting solid was triturated in MeOH (10 mL) and the product was collected by filtration (10.8 g, 17.9 mmol, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, *J* = 2.0 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.36 – 7.21 (m, 2H), 6.83 (d, *J* = 8.5 Hz, 4H), 4.72 (s, 2H), 3.78 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.8, 145.8, 136.7, 131.1, 128.8, 128.7, 126.5, 122.0, 117.2, 114.0, 56.1, 55.2. HR-EIMS m/z calculated for C20H14Br4NO [M - H]$^+$: 603.7762, found: 603.7756. The NMR is consistent with previously reported values.[6]



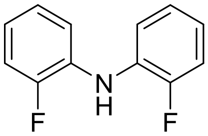
2,4-Dibromo-N-(2,4-dibromophenyl)-N-(4-methoxybenzyl)aniline (**5**).

In a 100 mL oven-dried round bottom flask, compound **4** (1.2 g, 1.99 mmol) was dissolved in anhydrous diethyl ether (25 mL) at room temperature and then cooled to 0 °C. *n*-BuLi (2.6 mL of 1.6 M) was added drop-wise to the reaction. After 1 hour, dichlorodimethylsilane (460 mg, 3.6 mmol) was added and the reaction mixture was warmed to room temperature. After 16 hours, the product was extracted with ether (50 mL), washed with water (50 mL) and brine (50 mL), and finally purified with silica gel chromatography (0-50% DCM/hexanes) to afford a white solid (515 mg, 1 mmol, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 2.5 Hz, 2H), 7.30 (dd, *J* = 9.0, 2.5 Hz, 2H), 7.11 (d, *J* = 8.7 Hz, 2H), 6.90 – 6.85 (m, 2H), 6.78 (d, *J* = 9.0 Hz, 2H), 5.09 (s, 2H), 3.81 (s, 3H), 0.47 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.9, 148.4, 135.8, 133.2, 128.7, 127.4, 124.5, 118.3, 114.5, 113.9, 55.6, 55.5, -0.8. HR-EIMS m/z calculated for C22H22Br2NOSi $[M + H]^{+}$: 503.9811, found: 503.9805.



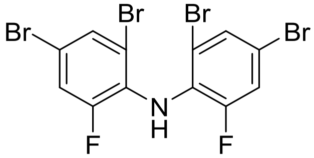
2,8-Di(azetidin-1-yl)-5-(4-methoxybenzyl)-10,10-dimethyl-5,10 dihydrodibenzo[b,e][1,4] azasiline (**6**)

In a 15 mL sealed tube, compound **5** (200 mg, 0.4 mmol), $Pd_2(dba)_3$ (18.3 mg, 0.02 mmol), BINAP (25 mg 0.04 mmol), sodium *tert*-butoxide (92 mg, 0.96 mmol) and azetidine (50 mg, 0.87 mmol) were dissolved in anhydrous toluene (10 mL). The reaction tube was flushed with argon, then heated to 80 °C for 24 hours. The reaction was filtered through Celite and the product was purified with silica gel chromatography (0-30% EtOAC/hexanes) to afford a yellow-tinted solid (129 mg, 0.28 mmol, 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (d, *J* = 8.6 Hz, 2H), 6.88 – 6.84 (m, 2H), 6.78 (d, *J* = 8.9 Hz, 2H), 6.60 (d, *J* = 2.9 Hz, 2H), 6.41 (dd, *J* = 8.9, 2.9 Hz, 2H), 5.05 (s, 2H), 3.83 (t, *J* = 7.1 Hz, 8H), 3.80 (s, 3H), 2.33 (p, *J* = 7.1 Hz, 4H), 0.45 (d, *J* = 3.2 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.5, 146.0, 142.8, 131.1, 127.6, 122.1, 116.5, 115.8, 114.4, 114.2, 55.6, 55.4, 53.2, 17.3, -0.7. HR-EIMS m/z calculated for C28H34N3OSi $[M + H]^{+}$: 456.2466, found: 456.2436.



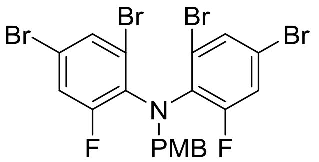
Bis(2-fluorophenyl)amine (8)

In a 100 mL round bottom flask equipped with a reflux condenser, 2-fluoroaniline (2 g, 18 mmol), 1-bromo-2-fluorobenzene (3.5 g, 19.8 mmol), $Pd_2(dba)_3$ (833 mg, 0.9 mmol), Xantphos (1 g, 1.8 mmol), and sodium *tert*-butoxide (2.1 g, 21.8 mmol) were dissolved in anhydrous toluene (15 ml). The reaction was refluxed at 110 °C. After 16 hours, the reaction was filtered through Celite and the solvent was removed under reduced pressure. The product was purified with silica gel chromatography (0-5% DCM/hexanes) to afford a clear oil (3.3 g, 16 mmol, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (dt, *J* = 8.3, 1.4 Hz, 2H), 7.19 – 7.12 (m, 2H), 7.12 – 7.06 (m, 2H), 6.98 – 6.91 (m, 2H), 5.90 (s, 1H). ^{19}F NMR (471 MHz, $CDCl_3$) δ -131.24 (ddd, *J* = 11.6, 8.6, 4.9 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.7 (d, J_{CF} = 242.2 Hz), 130.7 (d, J_{CF} = 11.1 Hz), 124.4 (d, J_{CF} = 3.8 Hz), 121.7 (d, J_{CF} = 7.3 Hz), 118.3 (d, J_{CF} = 1.8 Hz), 115.8 (d, J_{CF} = 19.2 Hz). HR-EIMS m/z calculated for C12H10F2N [M + H]$^+$: 206.0776, found: 206.0776.



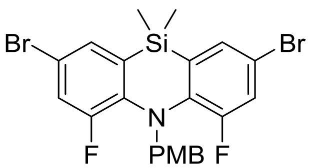
Bis(2,4-dibromo-6-fluorophenyl)amine (9)

In a 50 mL round bottom flask, compound **8** (2 g, 9.74 mmol) was dissolved in acetic acid (20 mL). The reaction temperature was raised to 110 °C, followed by the addition of bromine (2.1 mL, 58.4 mmol). After 10 minutes, the reaction was cooled to room temperature to allow for crystallization of the product. The product was filtered and washed with acetic acid (60 ml) and H_2O (200 mL) to afford a white solid (4.21 g, 8.1 mmol, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 – 7.50 (m, 2H), 7.18 (dd, *J* = 10.6, 1.9 Hz, 2H), 5.64 (s, 1H). ^{19}F NMR (471 MHz, $CDCl_3$) δ -119.23 (d, *J* = 10.4 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.4 (dd, J_{CF} = 254.0, 2.3 Hz), 130.6 (d, J_{CF} = 2.8 Hz), 128.8 (dd, J_{CF} = 11.7, 2.0 Hz), 119.0 (d, J_{CF} = 23.5 Hz), 116.9 – 116.4 (m), 114.4 – 114.1 (m). HR-EIMS m/z calculated for C12H6Br4F2N [M + H]$^+$: 521.7155, found: 521.7135.



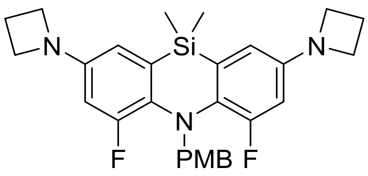
2,4-Dibromo-N-(2,4-dibromo-6-fluorophenyl)-6-fluoro-N-(4-methoxybenzyl)aniline (10)

In a 100 mL round bottom flask, compound **9** (2 g, 3.9 mmol) and 15-Crown 5 (1.6 mL, 7.8 mmol) were dissolved in THF (40 mL). Sodium hydride (120 mg, 5 mmol) was added to the reaction in small portions. After 1 hour, *p*-methoxybenzyl chloride (529 ml, 5.85 mmol) was added to the solution and refluxed at 75 °C. After 24 hours, the reaction was poured into brine (100 mL) and extracted with EtOAc (3 X 50 mL). The combined organic layers were washed with brine (100 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the product was purified by silica gel chromatography (0-100% DCM/hexanes) to afford a white solid (2.07 g, 3.22 mmol, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 – 7.44 (m, 2H), 7.41 (d, *J* = 8.6 Hz, 2H), 7.11 (dd, *J* = 10.9, 2.2 Hz, 2H), 6.80 – 6.72 (m, 2H), 4.82 (s, 2H), 3.75 (s, 3H). ^{19}F NMR (471 MHz, $CDCl_3$) δ -111.59. ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.1 (d, J_{CF} = 256.5 Hz), 159.6, 133.1 (d, J_{CF} = 11.9 Hz), 132.2 (d, J_{CF} = 3.4 Hz), 129.7, 128.4, 123.4 (d, J_{CF} = 3.9 Hz), 119.6 (d, J_{CF} = 25.3 Hz), 117.8 (d, J_{CF} = 11.0 Hz), 113.7 , 56.7 (t, J_{CF} = 5.67 Hz), 55.3. HR-EIMS m/z calculated for C20H12Br4F2NO [M - H]$^+$: 639.7574, found: 639.7564.



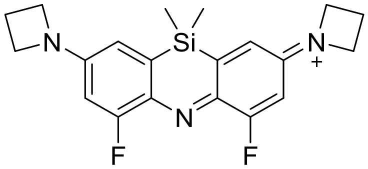
2,8-Dibromo-4,6-difluoro-5-(4-methoxybenzyl)-10,10-dimethyl-5,10-dihydrodibenzo[b,e][1,4] azasiline (11)

In a 50 mL oven-dried round bottom flask, compound **10** (642 mg, 1 mmol) was dissolved in anhydrous diethyl ether (25 mL) at room temperature and then cooled to -78 °C. *n*-BuLi (0.96 ml of 1.6 M, 2.4 mmol) was added drop-wise to the reaction. After 1 hour, dichlorodimethylsilane (645 mg, 5 mmol) was added and the reaction mixture was warmed to room temperature. After 4 hours, the product was extracted with ether (25 mL) and then washed with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by silica gel chromatography (0-50% DCM/hexanes). The resulting white solid was washed with MeOH (2 mL) and filtered to afford the product (114 mg, 0.21 mmol, 21%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (dd, *J* = 11.7, 2.2 Hz, 2H), 7.21 (d, *J* = 2.1 Hz, 2H), 6.80 – 6.73 (m, 2H), 6.71 – 6.62 (m, 2H), 4.86 (s, 2H), 3.70 (s, 3H), 0.46 (s, 3H), -0.26 (s, 3H). ^{19}F NMR (471 MHz, $CDCl_3$) δ -113.89 (d, *J* = 11.6 Hz).^{13}C NMR (126 MHz, $CDCl_3$) δ 159.3, 156.2, 154.2, 137.9 (d, J_{CF} = 6.4 Hz), 134.0, 131.1 (d, J_{CF} = 4.0 Hz), 130.6, 129.4, 121.0 (d, J_{CF} = 24.8 Hz), 116.0 (d, J_{CF} = 7.6 Hz), 113.8, 58.0 (t, J_{CF} = 9.2 Hz), 55.4. HR-EIMS m/zcalculated for C22H20Br2F2NOSiNa [M + Na]$^+$: 561.9442, found: 561.9432.



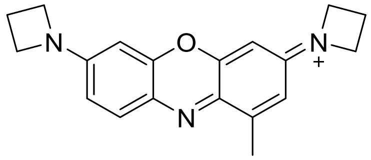
2,8-Di(azetidin-1-yl)-4,6-difluoro-5-(4-methoxybenzyl)-10,10-dimethyl-5,10 dihydrodibenzo [b,e][1,4]azasiline (12)

In a 15 mL sealed tube, compound **11** (50 mg, 0.093 mmol), $Pd_2(dba)_3$ (4.2 mg, 0.0046 mmol), BINAP (5.8 mg, 0.009 mmol), sodium *tert*-butoxide (21 mg, 0.22 mmol) and azetidine (21 mg, 0.37 mmol) were dissolved in anhydrous toluene (2 mL). The reaction tube was flushed with argon, then heated to 110 °C. After 24 hours, the reaction mixture was filtered through Celite. The solvent was removed under reduced pressure and the product was purified by silica gel chromatography (0-30% EtOAC/hexanes) to afford a light yellow solid (46 mg, 0.093 mmol, quantitative). ^1H NMR (500 MHz, $CDCl_3$) δ 6.71 – 6.53 (m, 4H), 6.29 (dd, *J* = 13.9, 2.5 Hz, 2H), 6.13 (d, *J* = 2.5 Hz, 2H), 4.71 (s, 2H), 3.85 (t, *J* = 7.2 Hz, 8H), 3.69 (s, 3H), 2.34 (p, *J* = 7.5 Hz, 4H), 0.36 (s, 3H), -0.47 (s, 3H). ^{19}F NMR (471 MHz, $CDCl_3$) δ -117.72 (d, *J* = 13.9 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.9, 156.3 (d, J_{CF} = 249.5 Hz), 149.2 (d, J_{CF} = 8.6 Hz), 133.2, 130.9, 130.5 (t, J_{CF} = 3.6 Hz), 113.4, 110.1 (d, J_{CF} = 3.1 Hz), 101.6 (d, J_{CF} = 24.9 Hz), 58.5 (t, J_{CF} = 7.4 Hz), 55.3, 52.9, 17.0, -1.0, -2.6. HR-EIMS m/z= calculated for C28H32F2N3OSi $[M + H]^+$: 492.2277, found: 492.2257.



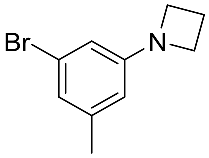
2,8-Di(azetidin-1-yl)-4,6-difluoro-5-(4-methoxybenzyl)-10,10-dimethyl-5,10-dihydrodibenzo[b,e][1,4]azasiline (13), ASiFluor710

In a 25 mL round bottom flask, compound **12** (20 mg, 0.04 mmol) was dissolved in MeOH (10 mL) at 0 °C. Iodine (21 mg, 0.08 mmol) was separately dissolved in MeOH (5 mL) and added to the reaction. After 20 minutes, the resulting green solid was collected by filtration and washed with hexane (10 mL). The product can used without further purification (13 mg, 0.028 mmol, 70%). To increase solubility 10 mg (0.021 mmol) was eluted through a reverse phase C18 column (20-85% ACN/H_2O, 0.1% TFA) to afford the TFA salt (4 mg, 0.008 mmol, 40%). ^1H NMR (500 MHz, CD_3CN) δ 6.83 (d, *J* = 2.3 Hz, 2H), 6.39 (dd, *J* = 12.3, 2.3 Hz, 2H), 4.62 – 4.20 (m, 8H), 2.53 (p, *J* = 8.0 Hz, 4H), 0.48 (s, 6H). ^{19}F NMR (471 MHz, CD_3CN) δ -105.67 (d, *J* = 12.4 Hz). ^{13}C NMR (126 MHz, CD_3CN) δ 166.5 (d, J_{CF} = 272.2 Hz), 157.3 (d, J_{CF} = 14.2 Hz), 140.1 (d, J_{CF} = 2.2 Hz), 134.9 (d, J_{CF}= 7.1 Hz), 120.5, 100.2 (d, J_{CF} = 25.2 Hz), 55 – 54 (m), 16.4, -1.0. HR-EIMS m/z $[M]^+$ calculated for C20H22F2N3Si $[M + H]^+$: 370.1546, found: 370.1545.



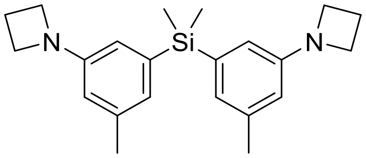
1-(7-(Azetidin-1-yl)-1-methyl-3H-phenoxazin-3-ylidene)azetidin-1-ium (13′)

In a 50 mL round bottom flask, compound **13** (6 mg, 0.01 mmol) was dissolved in acetonitrile (10 mL) and PBS (10 mL) and stirred in the dark at room temperature. After 48 hours, the reaction was poured into brine (50 mL) and extracted with acetonitrile (3 X 20 mL). The combined organic layers were washed with brine (50 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the product was purified by preparatory HPLC (0-65% 0.1% TFA ACN/H_2O) to afford a blue solid (>1 mg). ^{1}H NMR (500 MHz, MeOD) δ 7.75 (d, *J* = 9.2 Hz, 1H), 6.87 (dd, *J* = 9.2, 2.4 Hz, 1H), 6.83 – 6.79 (m, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 6.40 (d, *J* = 2.4 Hz, 1H), 4.52 – 4.32 (m, 8H), 2.64 – 2.49 (m, 7H). HR-EIMS m/z calculated for C19H20N3O M^+: 306.1601, found: 306.1596.



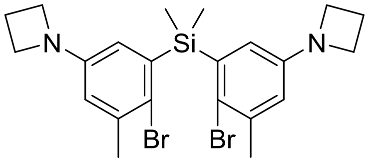
1-(3-Bromo-5-methylphenyl)azetidine (14)

In a 75 mL sealed pressure tube, 2,5-dibromotoluene (4 g, 16 mmol), azetidine (457 mg , 8 mmol), $Pd_2(dba)_3$ (366 mg, 0.4 mmol), BINAP (498 mg, 0.8 mmol), and sodium *tert*-butoxide (921 mg, 9.6 mmol) were dissolved in anhydrous toluene (62 mL). The reaction was heated to 110 °C for 24 hours. The reaction was then filtered through Celite and the solvent was removed under reduced pressure. The product was purified with silica gel chromatography (0-30% EtOAc/hexanes) to afford a white solid (1.2 g, 5.3 mmol, 66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.68 (s, 1H), 6.39 – 6.35 (m, 1H), 6.14 (s, 1H), 3.85 (t, *J* = 7.2 Hz, 4H), 2.35 (p, *J* = 7.5 Hz , 2H), 2.25 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.1, 140.5, 122.8, 120.9, 111.3, 110.6, 52.3, 21.4, 16.9. HR-EIMS m/z calculated for C10H13BrN $[M + H]^+$: 226.0226, found: 226.0197.



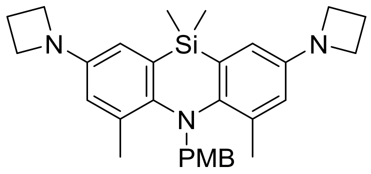
Bis(3-(azetidin-1-yl)-5-methylphenyl)dimethylsilane (15)

In a 100 mL oven-dried round bottom flask, compound **14** (500 mg, 2.21 mmol) was dissolved in anhydrous diethyl ether (50 mL) and anhydrous THF (1 mL) at room temperature and then cooled to 0 °C. *n*-BuLi (1.76 ml of 2.5 M, 4.4 mmol) was added drop-wise to the reaction. After 1 hour, dichlorodimethylsilane (171 mg, 1.3 mmol) was added and the reaction was warmed to room temperature. After 16 hours, the reaction was poured into water (20 mL), and the product was extracted with diethyl ether (25 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), then dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by silica gel chromatography (0-50% DCM/hexanes) to afford a yellow solid (170 mg, 0.49 mmol, 44%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.72 (s, 2H), 6.44 (d, *J* = 2.2 Hz, 2H), 6.38 – 6.25 (m, 2H), 3.85 (t, *J* = 7.2 Hz, 8H), 2.33 (p, *J* = 7.5 Hz, 4H), 2.28 (s, 6H), 0.48 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.8, 138.9, 137.9, 124.4, 114.2, 113.1, 52.6, 21.8, 17.2, -2.0. HR-EIMS m/z calculated for C22H31N2Si $[M + H]^+$: 351.2251, found: 351.2261.



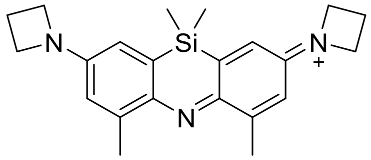
Bis(5-(azetidin-1-yl)-2-bromo-3-methylphenyl)dimethylsilane (16)

In a 50 ml round bottom flask, **15** (170 mg, 0.48 mmol) was dissolved in dichloromethane (19 mL) at 0 °C. NBS (172 mg, 0.97 mmol) was separately dissolved in dichloromethane (1 mL) and added dropwise to the reaction. After 15 minutes, the solvent was removed under reduced pressure and the product was purified by silica gel chromatography (0-20% EtOAc/hexanes) to afford a white solid (170 mg, 0.35 mmol, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.39 (d, *J* =2.8 Hz, 2H), 6.33 (d, *J*= 2.7, 2 H), 3.80 (t, *J* = 7.2 Hz, 8H), 2.37 – 2.27 (m, 10H), 0.71 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.8, 140.2, 138.2, 120.5, 118.4, 115.0, 52.6, 24.3, 17.1, -0.2. HR-EIMS m/z calculated for C22H29Br2N2Si $[M + H]^+$: 509.0441, found: 509.0457.



2,8-Di(azetidin-1-yl)-5-(4-methoxybenzyl)-4,6,10,10-tetramethyl-5,10-dihydrodibenzo[b,e][1,4]azasiline (17)

In a 15 mL sealed tube, compound **16** (150 mg, 0.3 mmol), $Pd_2(dba)_3$ (13.7 mg, 0.015 mmol), BINAP (18.4 mg 0.03 mmol), sodium *tert*-butoxide (68 mg, 0.71 mmol) and 4-methoxybenzylamine (49 mg, 0.35 mmol) were dissolved in anhydrous toluene (3 mL). The reaction tube was flushed with argon, then heated to 110 °C. After 48 hours the reaction mixture was filtered through Celite. The solvent was removed under reduced pressure and the product was purified by silica gel chromatography (0-20% EtOAc/hexanes) to afford a yellow solid (50 mg, 0.1 mmol, 33%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.61 (d, *J* = 8.6 Hz, 2H), 6.51 (d, *J* = 8.6 Hz, 2H), 6.37 (d, *J* = 2.6 Hz, 2H), 6.34 (d, *J* = 2.7 Hz, 2H), 4.15 (s, 2H), 3.85 (t, *J* = 7.1 Hz, 8H), 3.72 (s, 3H), 2.40 (s, 6H), 2.33 (p, *J* = 7.0 Hz, 4H), 0.31 (s, 3H), -0.24 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.8, 148.6, 144.9, 134.5, 134.1, 132.0, 130.2, 115.8, 113.8, 113.2, 61.8, 55.4, 52.9, 19.6, 17.2, -0.4, -1.5. HR-EIMS m/z calculated for C30H38N3OSi [M + H]$^+$: 484.2779, found: 484.2762.



1-(8-(Azetidin-1-yl)-4,6,10,10-tetramethyldibenzo[b,e][1,4]azasilin-2(10H)-ylidene)azetidin-1-ium (18), ASiFluor730

In a 25 mL round bottom flask, compound **17** (8.5 mg, 0.018 mmol) was dissolved in MeOH (1 mL) at 0 °C. Iodine (4.4 mg, 0.176 mmol) was separately dissolved in MeOH (5 mL) and then added to the reaction. After 15 minutes, the solvent was removed under reduced pressure and the product was purified by silica gel chromatography (0-20% MeOH/DCM). The product was further purified with preparatory HPLC (20-85% 0.1% TFA ACN/H_2O) to afford a green solid (3 mg, 0.006 mmol, 36%). ^{1}H NMR (500 MHz, MeOD) δ 6.88 (d, *J* = 2.6 Hz, 2H), 6.59 – 6.55 (m, 2H), 4.47 (t, *J* = 7.7 Hz, 8H), 2.57 (p, *J* = 7.5 Hz, 4H), 2.50 (s, 6H), 0.44 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 155.2, 154.2, 144.4, 140.5, 121.3, 115.0, 53.7, 19.8, 16.7, -1.4. HR-EIMS m/z calculated for C22H28N3Si M$^+$: 362.2047, found: 362.2043.

Supplementary References

(1) Koide, Y.; Urano, Y.; Hanaoka, K.; Piao, W.; Kusakabe, M.; Saito, N.; Terai, T.; Okabe, T.; Nagano, T. Development of NIR Fluorescent Dyes Based on Si–rhodamine for in Vivo Imaging. *J. Am. Chem. Soc.* **2012**, *134*, 5029–5031.

(2) Pansare, V. J.; Hejazi, S.; Faenza, W. J.; Prud'homme, R. K. Review of Long-Wavelength Optical and NIR Imaging Materials: Contrast Agents, Fluorophores, and Multifunctional Nano Carriers. *Chem. Mater.* **2012**, *24*, 812–827.

(3) Rurack, K.; Spieles, M. Fluorescence Quantum Yields of a Series of Red and Near-Infrared Dyes Emitting at 600−1000 Nm. *Anal. Chem.* **2011**, *83*, 1232–1242.

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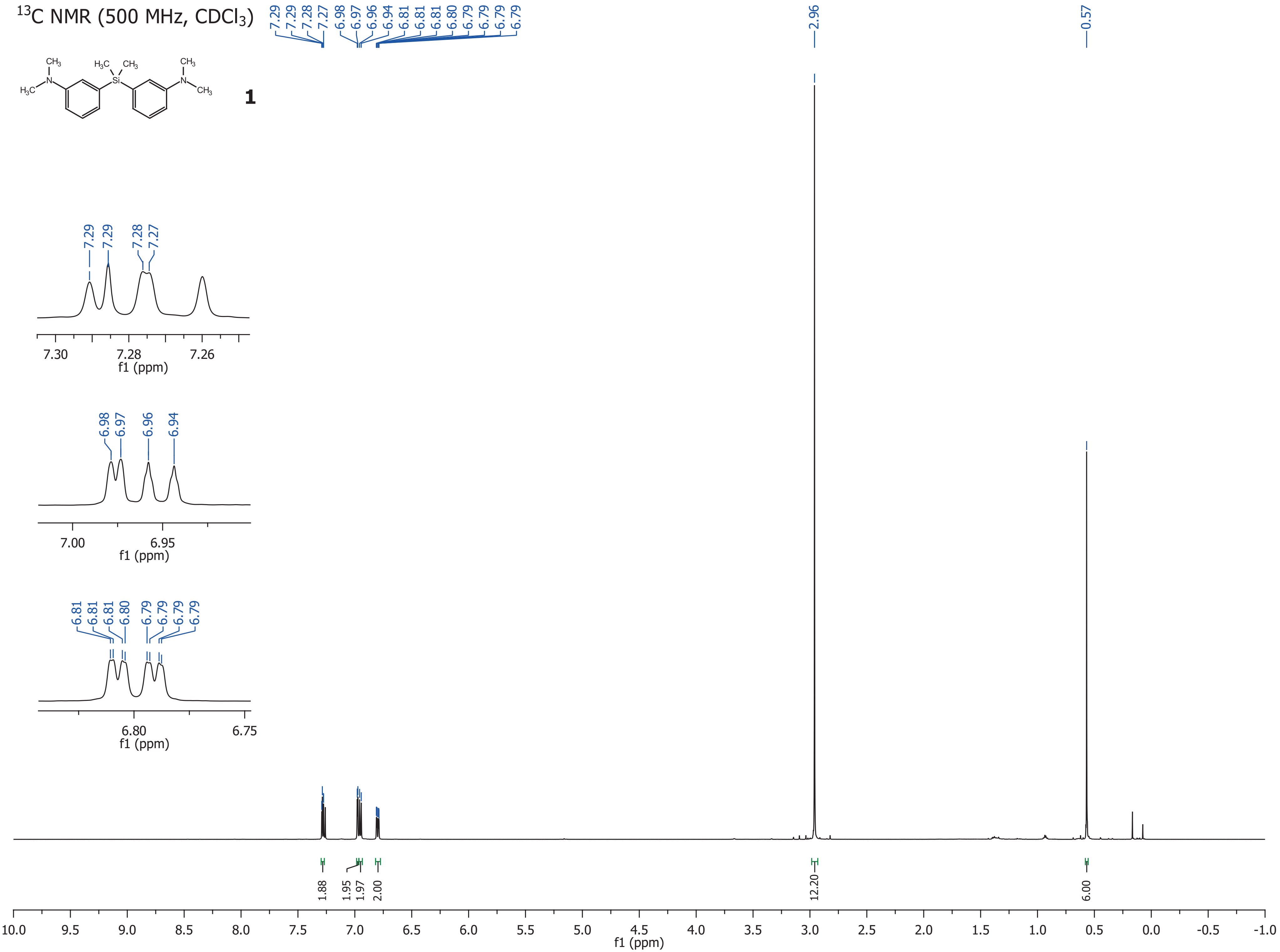
(6) Suzuki, R.; Tada, R.; Hosoda, T.; Miura, Y.; Yoshioka, N. Synthesis of Ester-Substituted Dihydroacridine Derivatives and Their Spectroscopic Properties. *New J. Chem.* **2016**, *40*, 2920–2926.

^{13}C NMR (500 MHz, $CDCl_3$)



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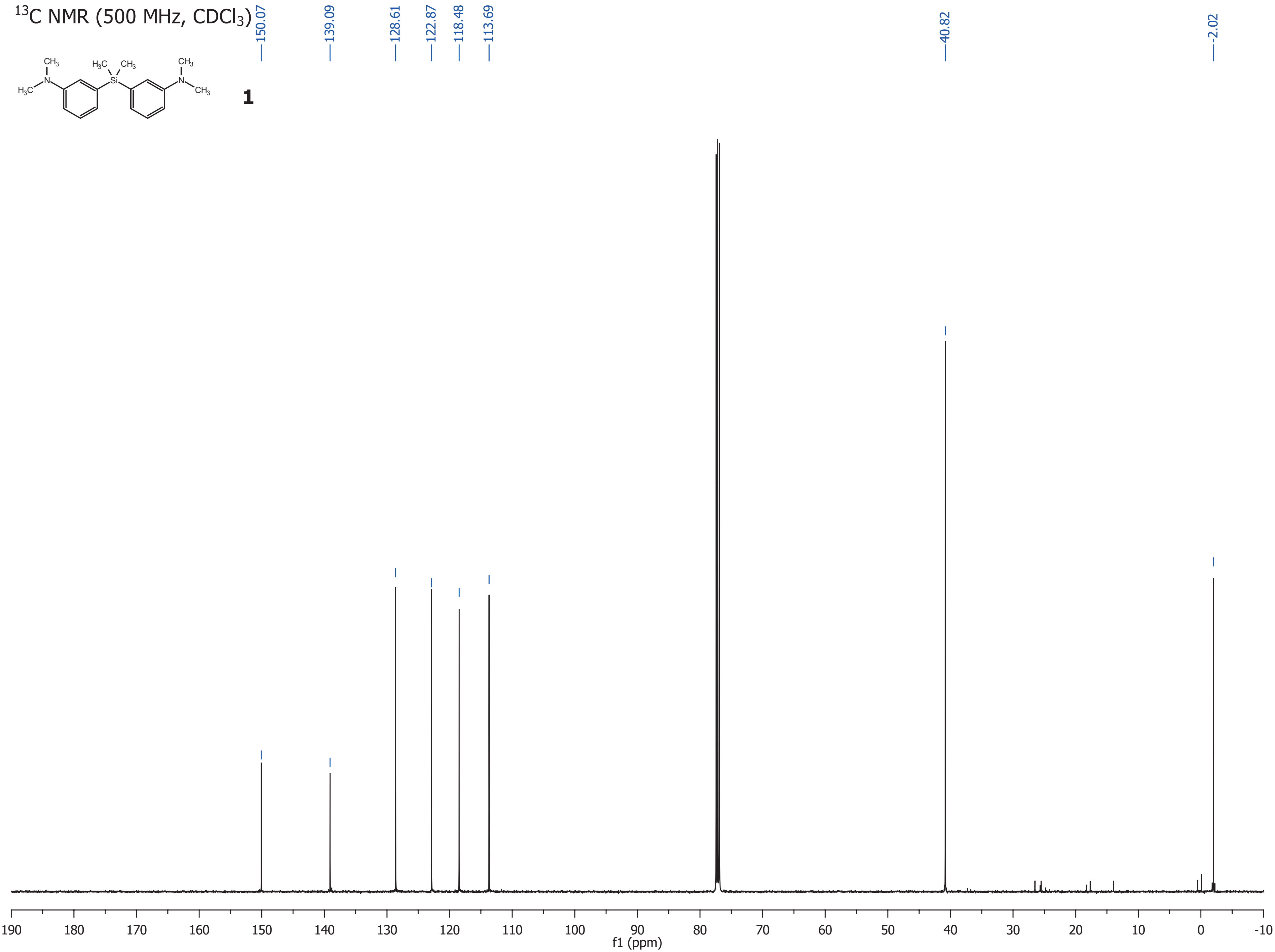
^{13}C NMR (500 MHz, CDCl$_3$)



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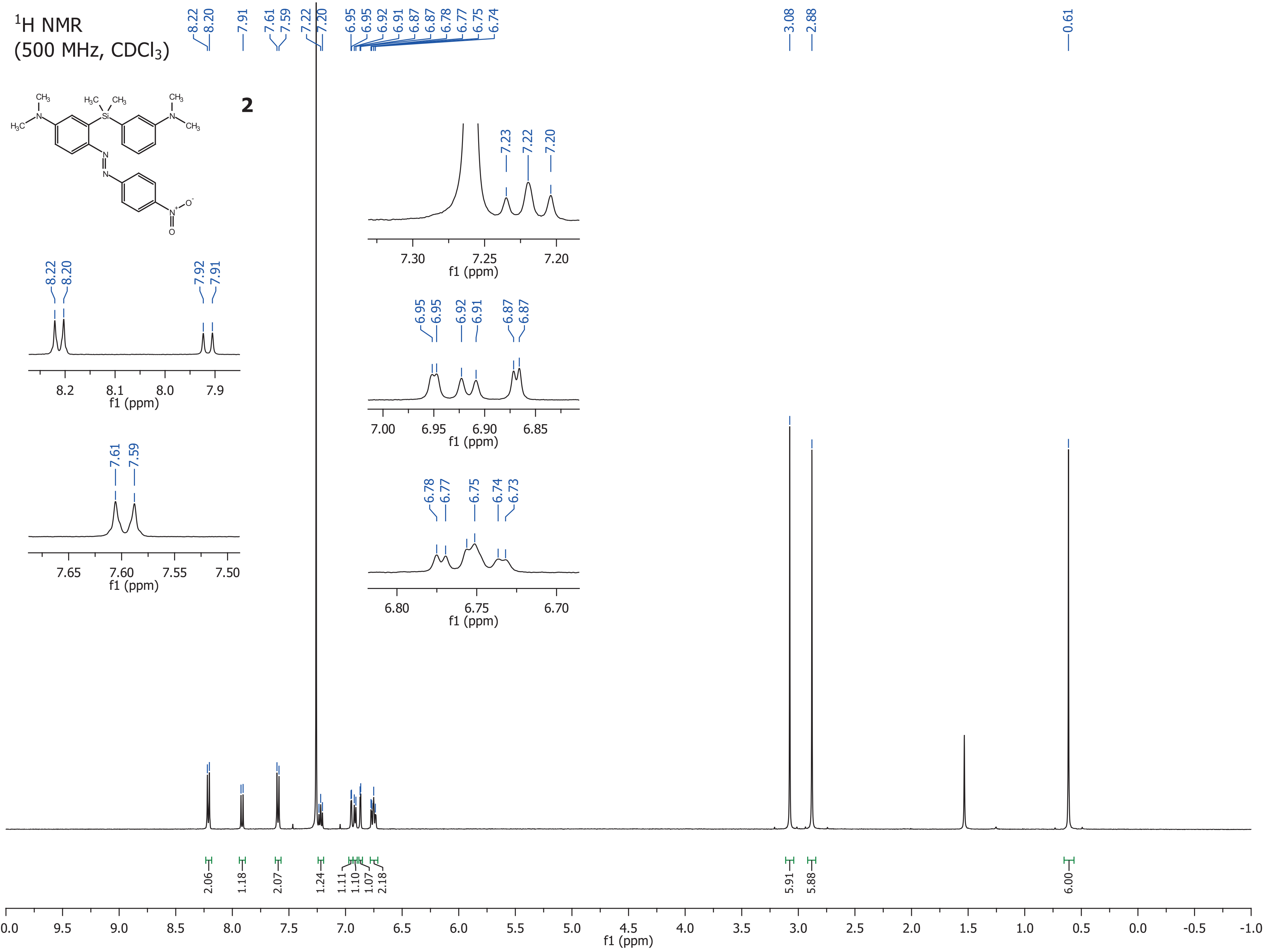
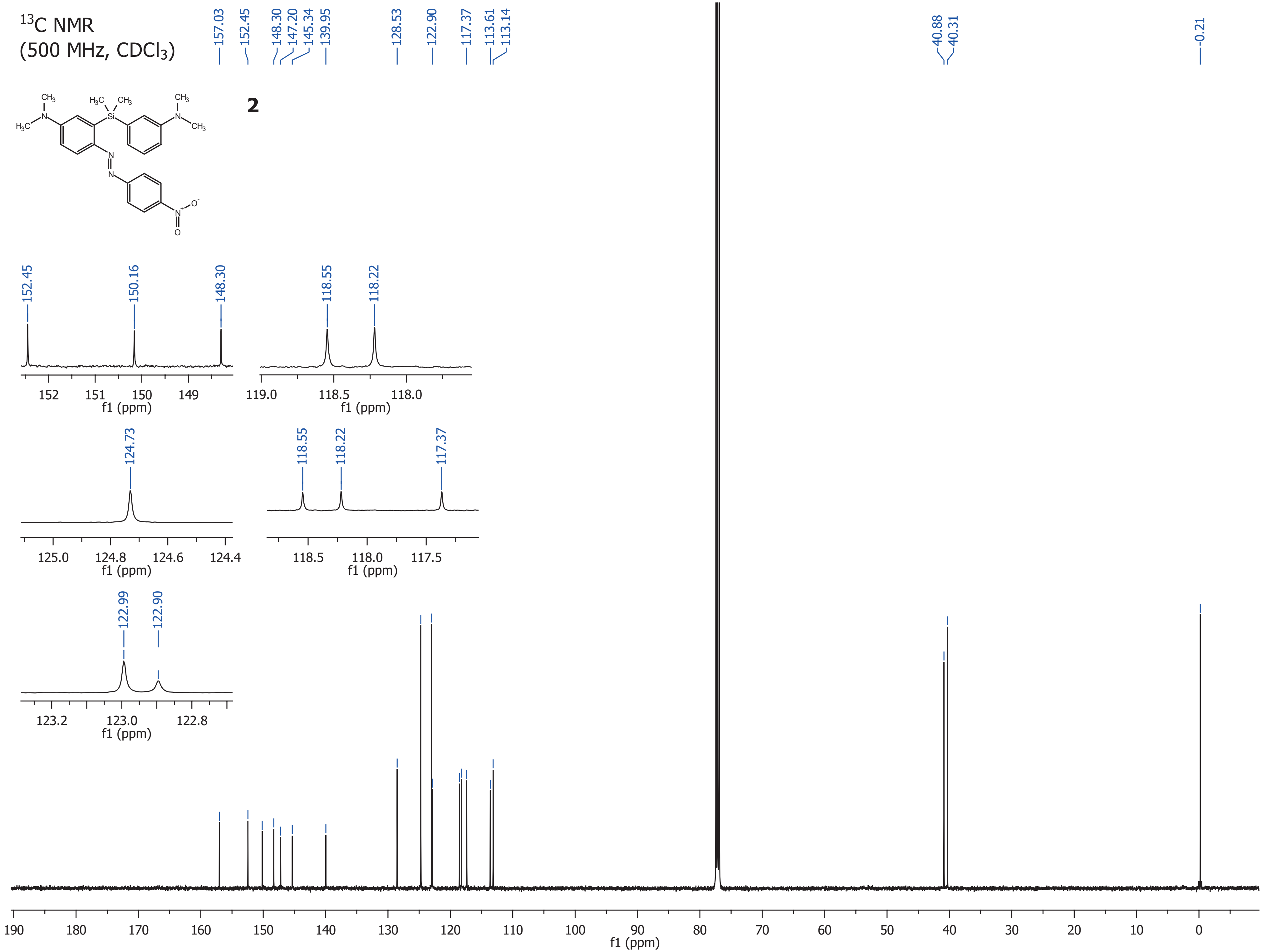
^{1}H NMR
(500 MHz, $CDCl_3$)

2



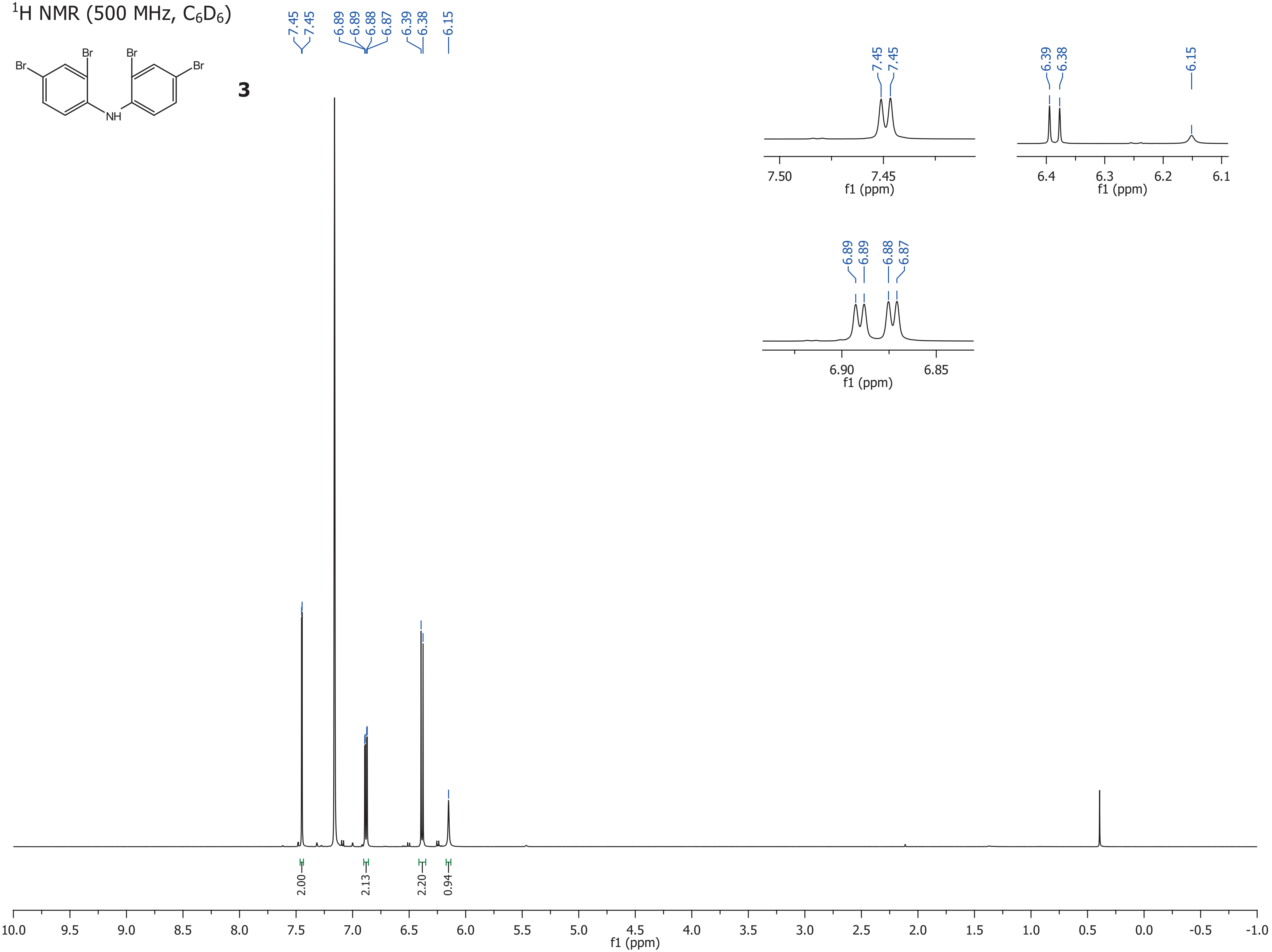


^{13}C NMR
(500 MHz, $CDCl_3$)

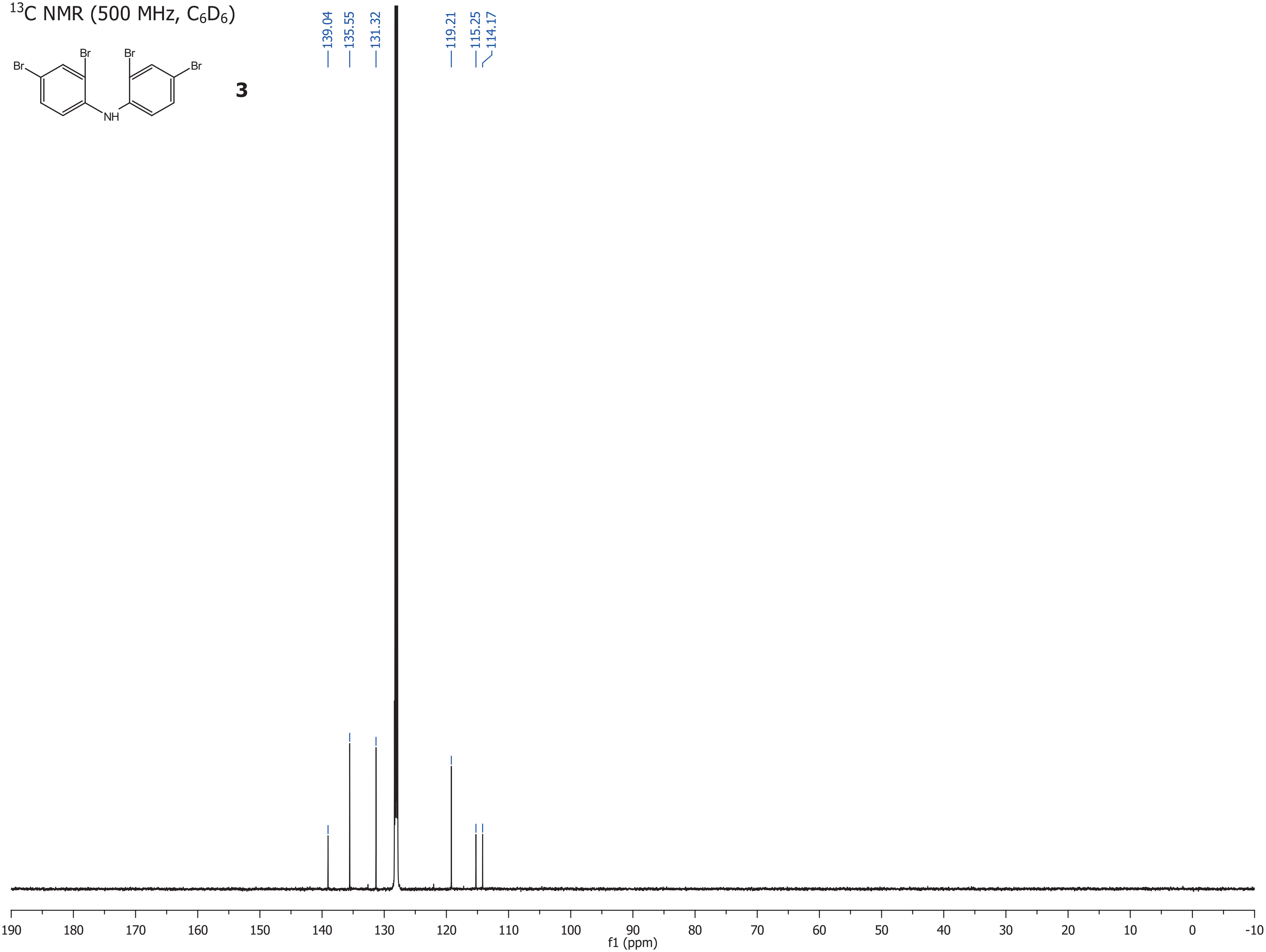
2

^{1}H NMR (500 MHz, C_6D_6)

3



^{13}C NMR (500 MHz, C_6D_6)



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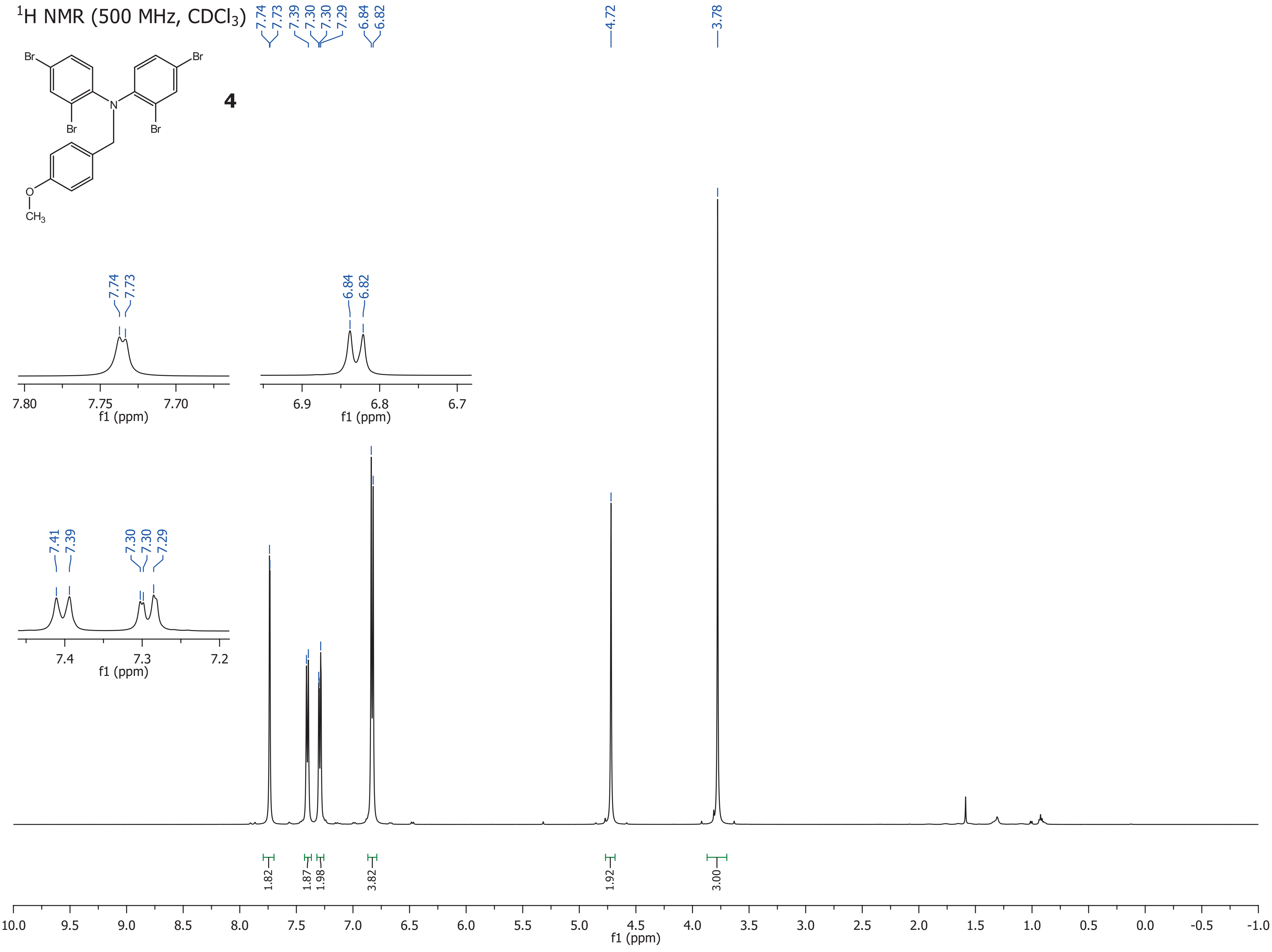
^{1}H NMR (500 MHz, CDCl$_3$)



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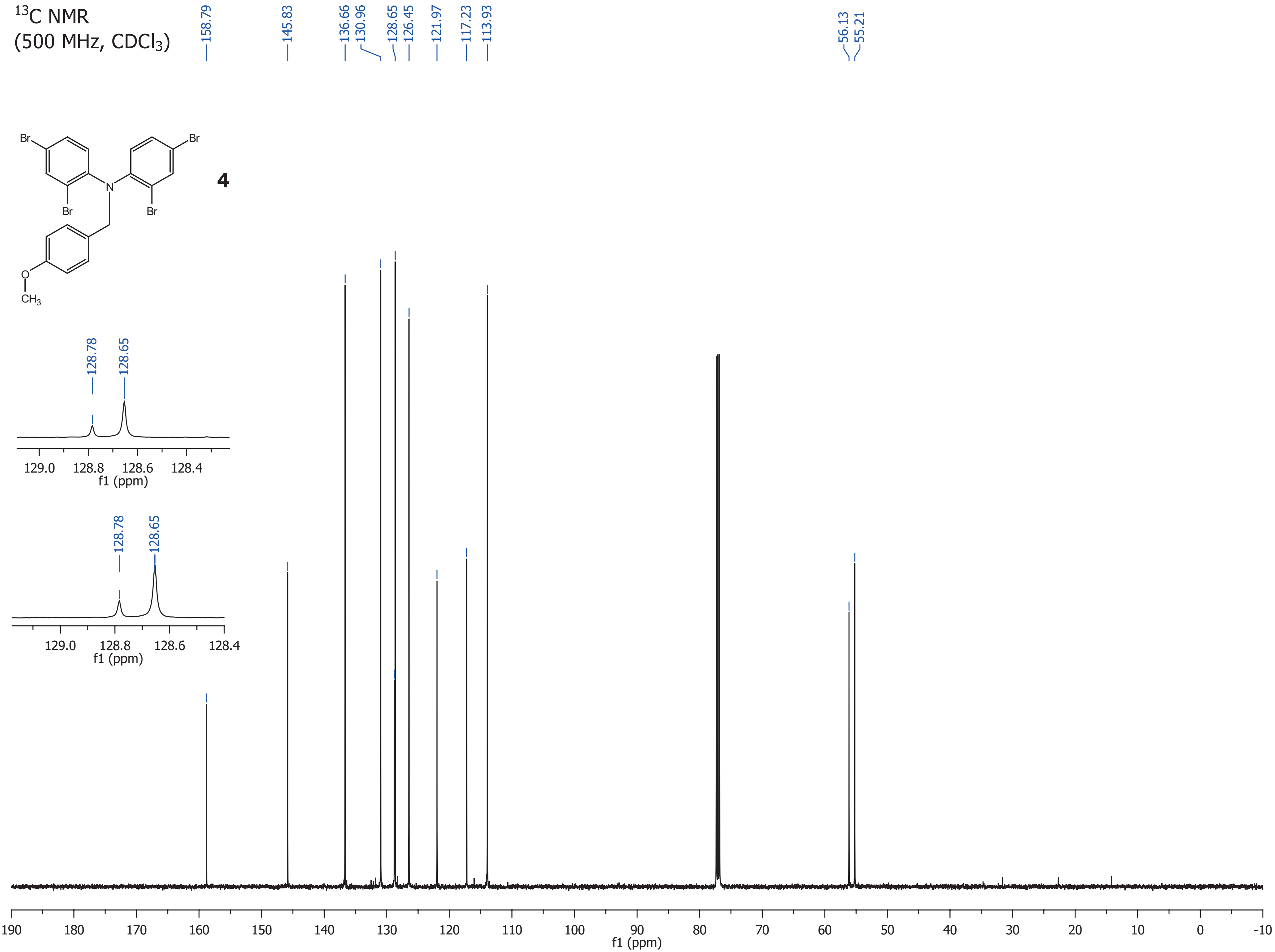
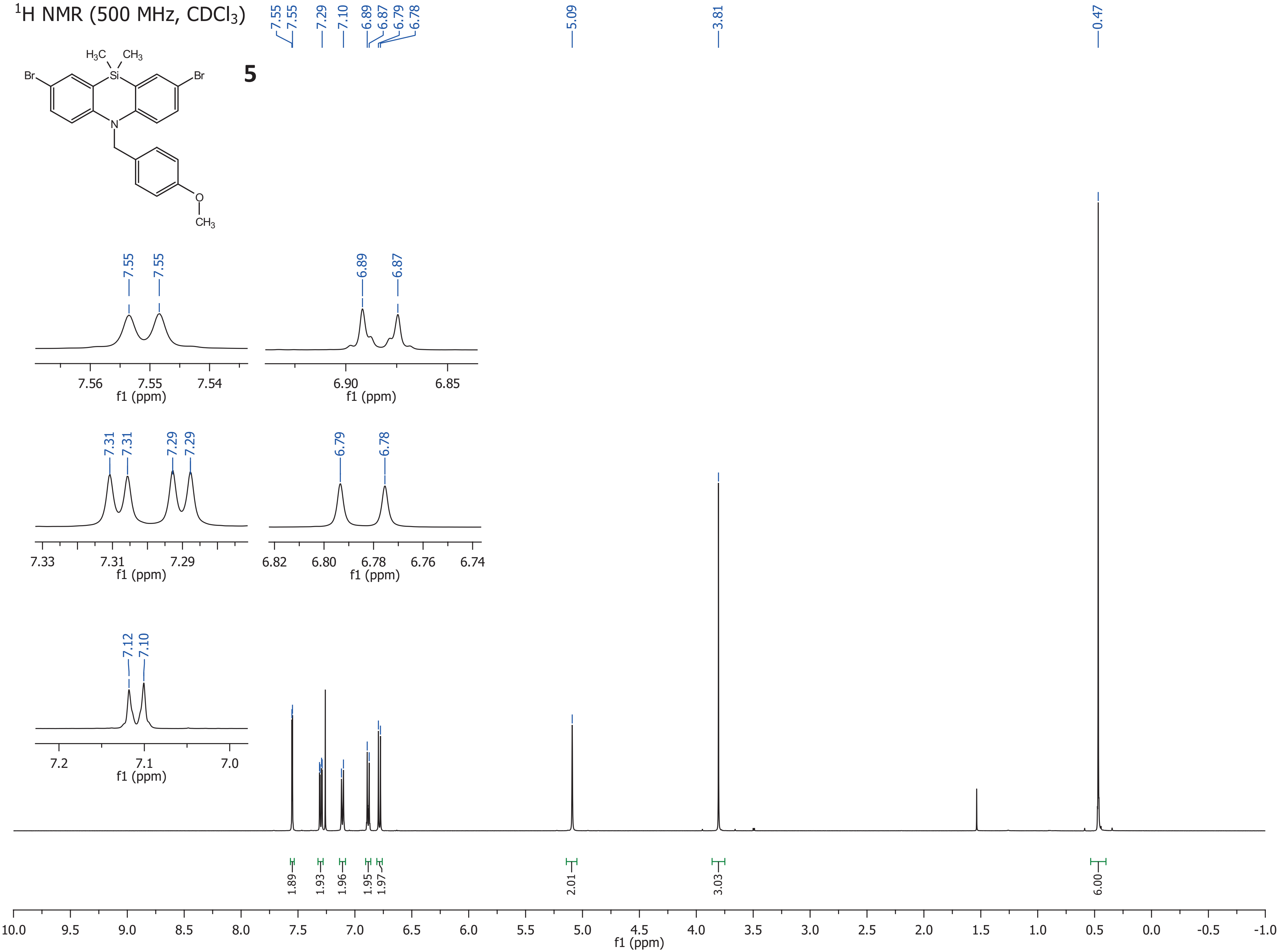
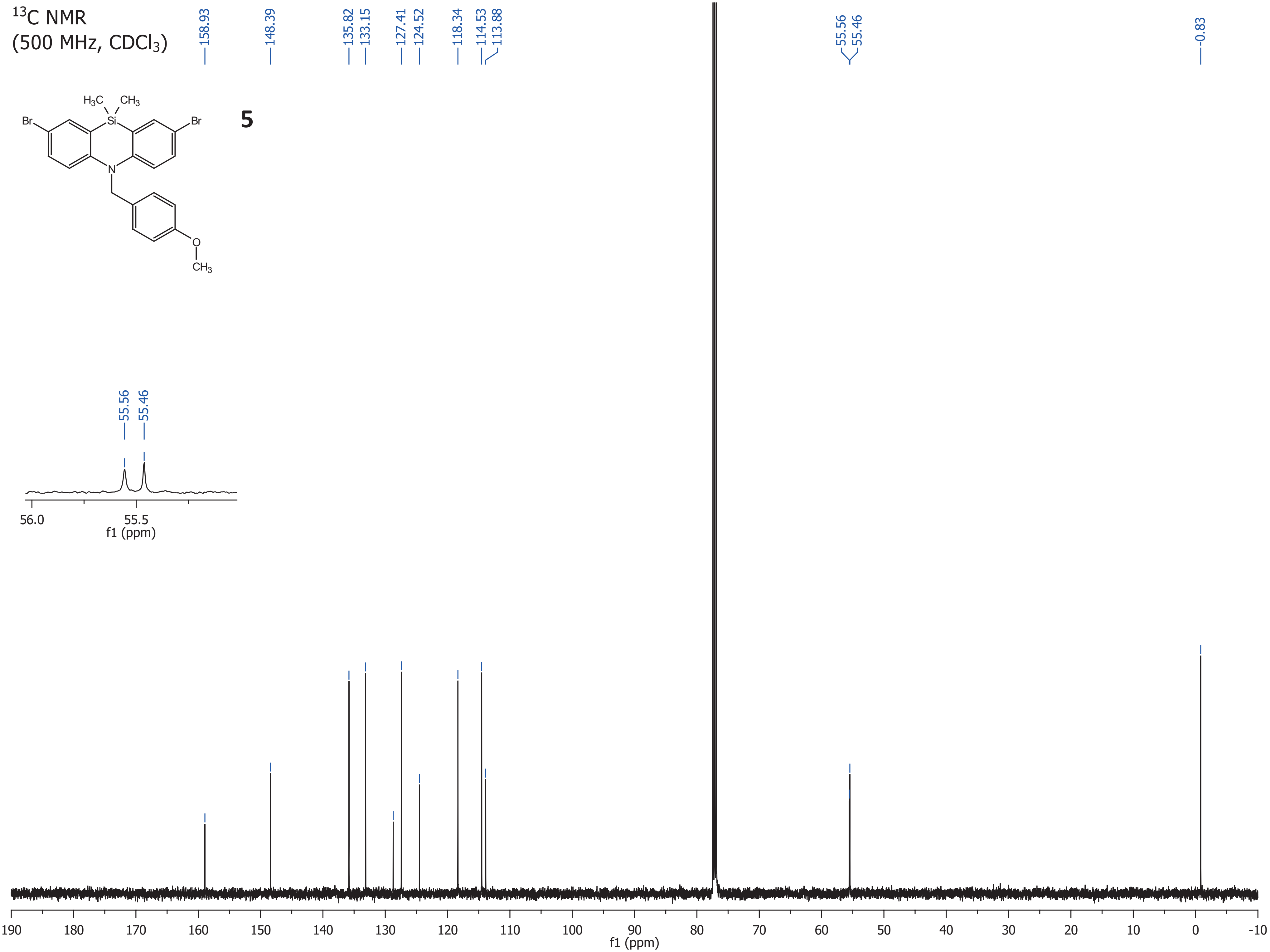
^{13}C NMR
(500 MHz, $CDCl_3$)



^{1}H NMR (500 MHz, CDCl$_3$)

5

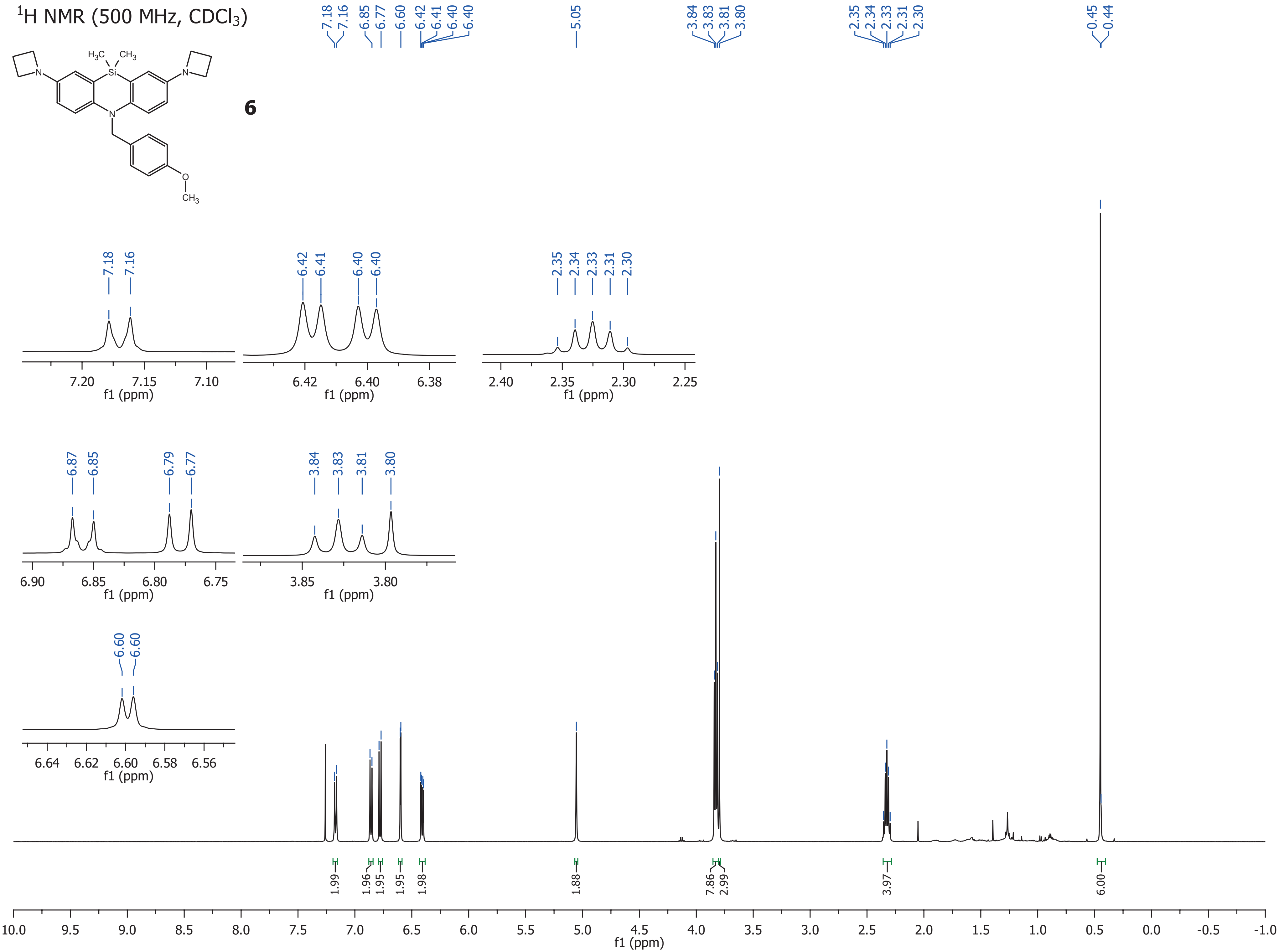




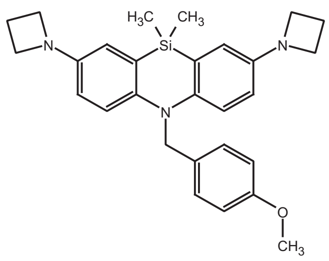
5

^{13}C NMR
(500 MHz, CDCl$_3$)
158.93
148.39
135.82
133.15
127.41
124.52
118.34
114.53
113.88
55.56
55.46
-0.83
H$_3$C
CH$_3$
Si
Br
Br
N
O
CH$_3$
55.56
55.46
56.0
55.5
f1 (ppm)
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR
(500 MHz, $CDCl_3$)



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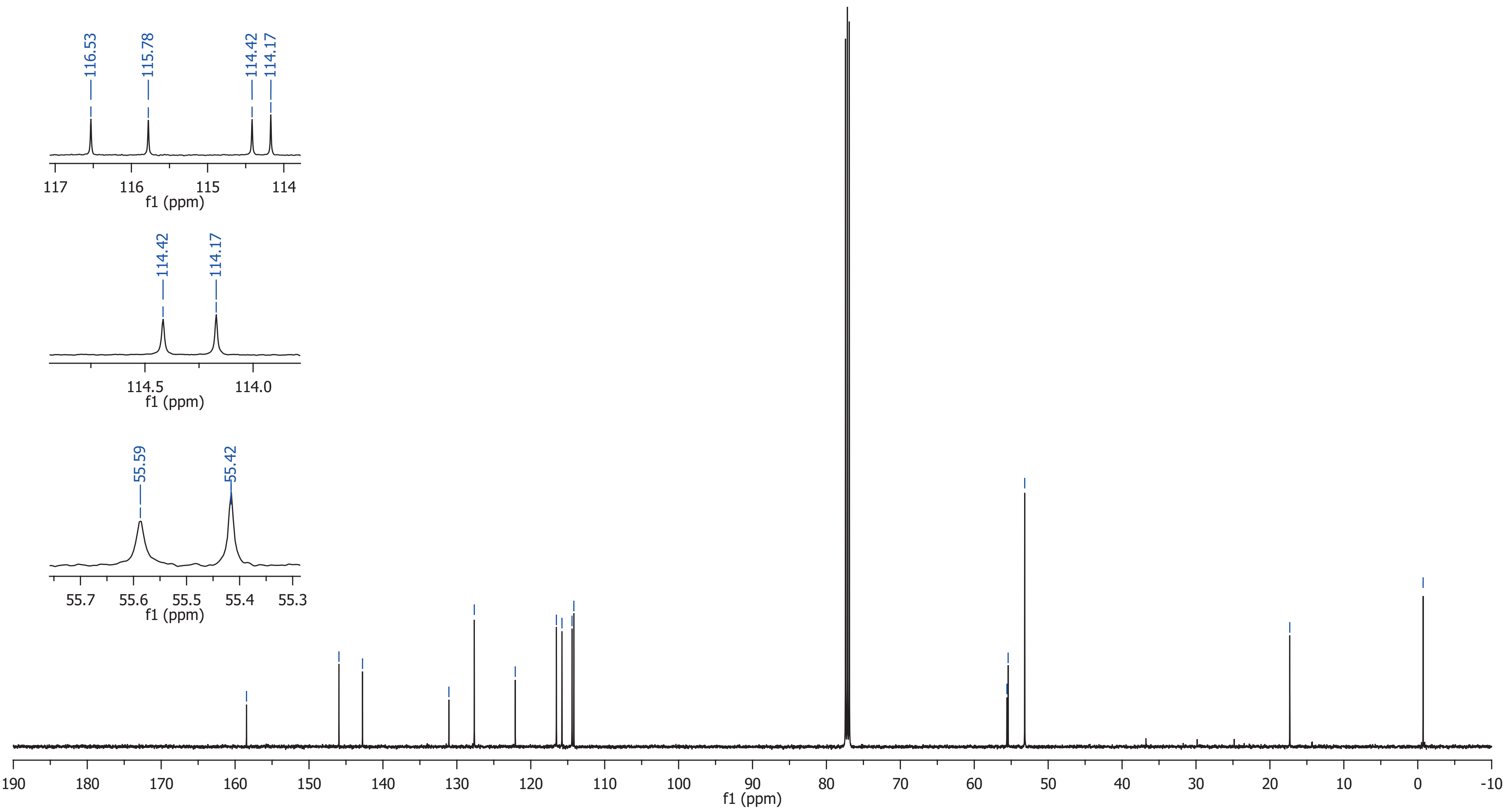
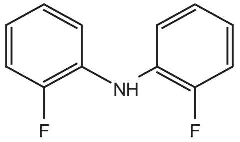
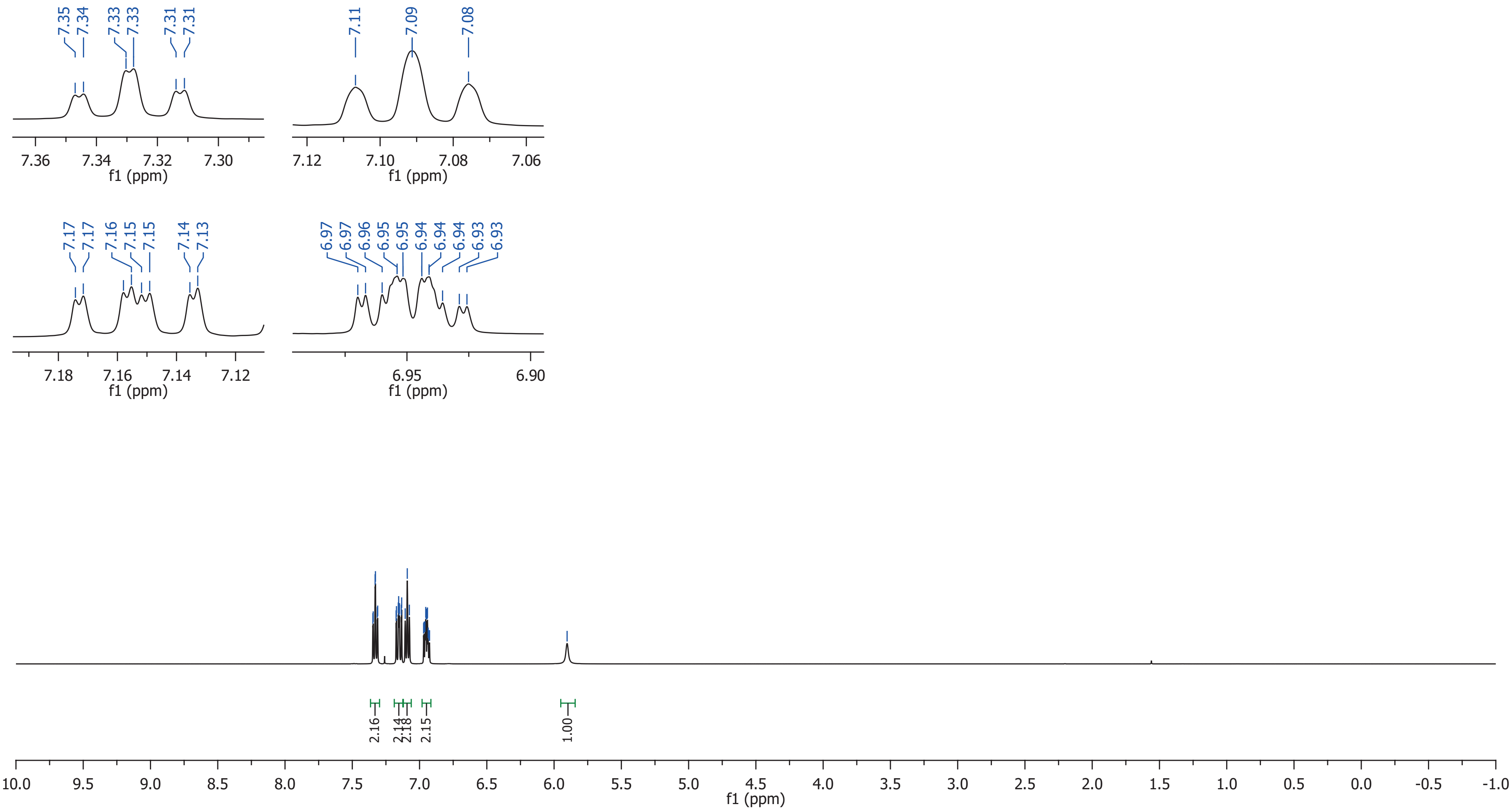


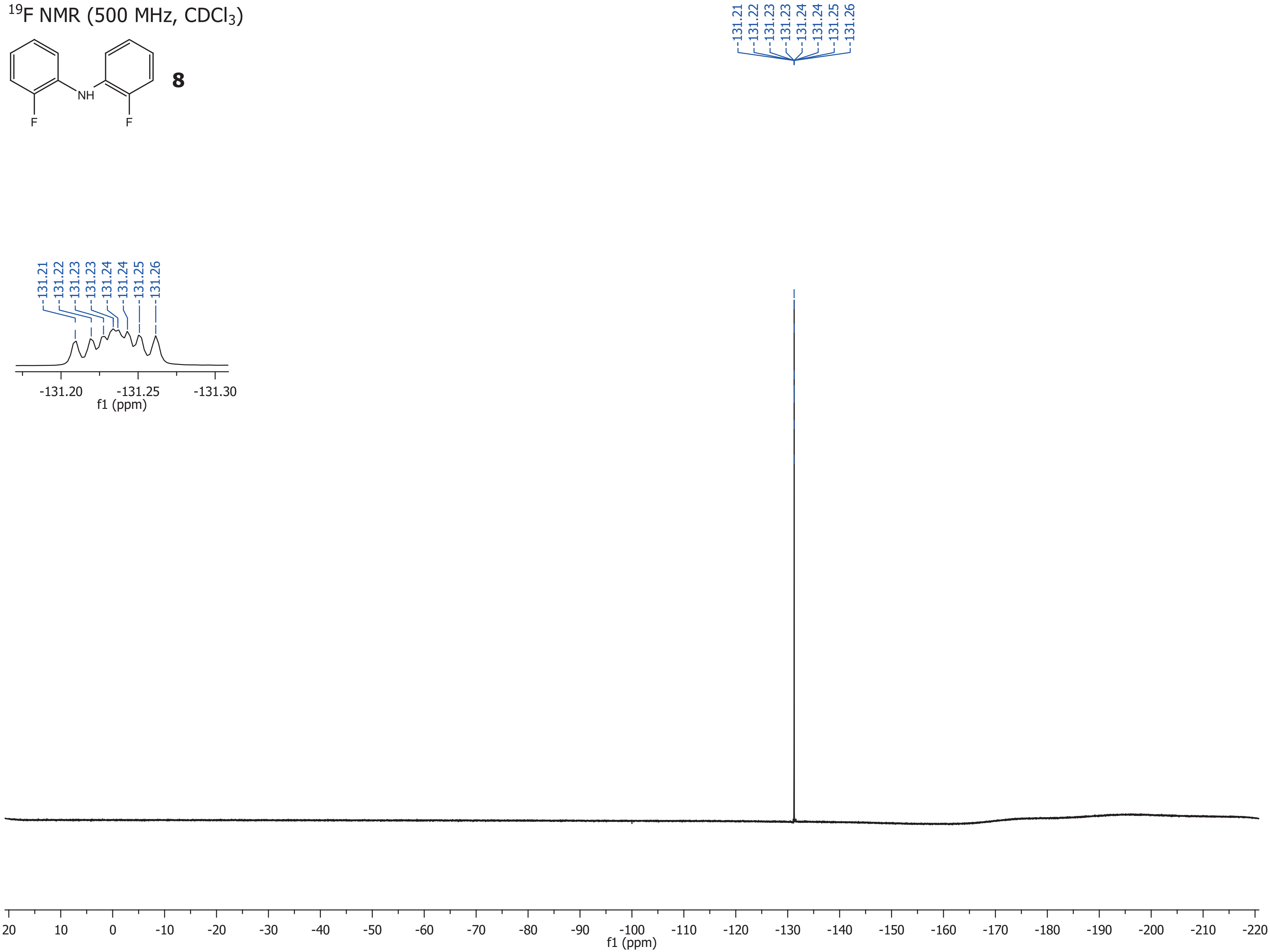
^{1}H NMR
(500 MHz, $CDCl_3$)



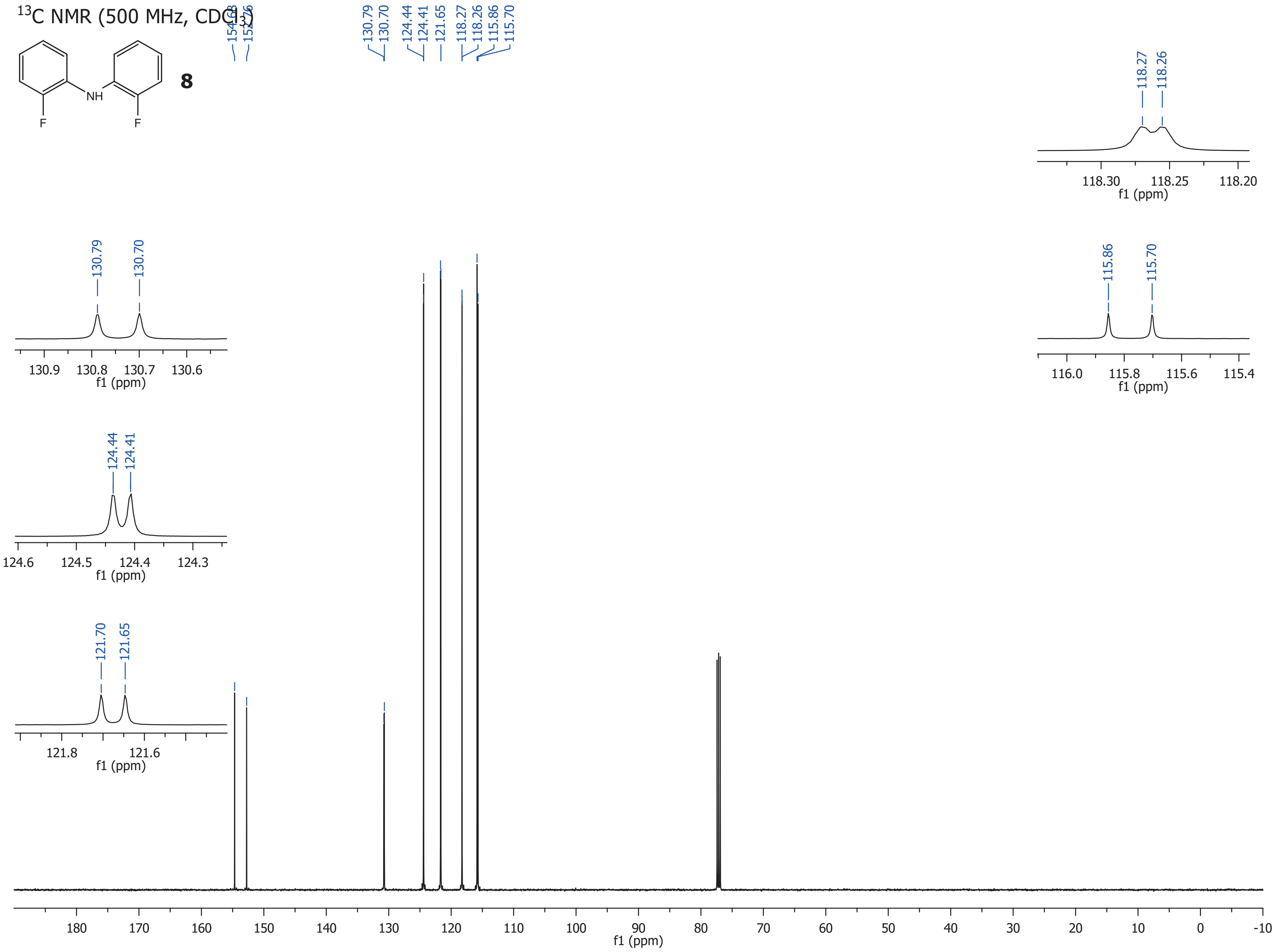
8



^{19}F NMR (500 MHz, CDCl$_3$)


8

^{13}C NMR (500 MHz, $CDCl_3$)



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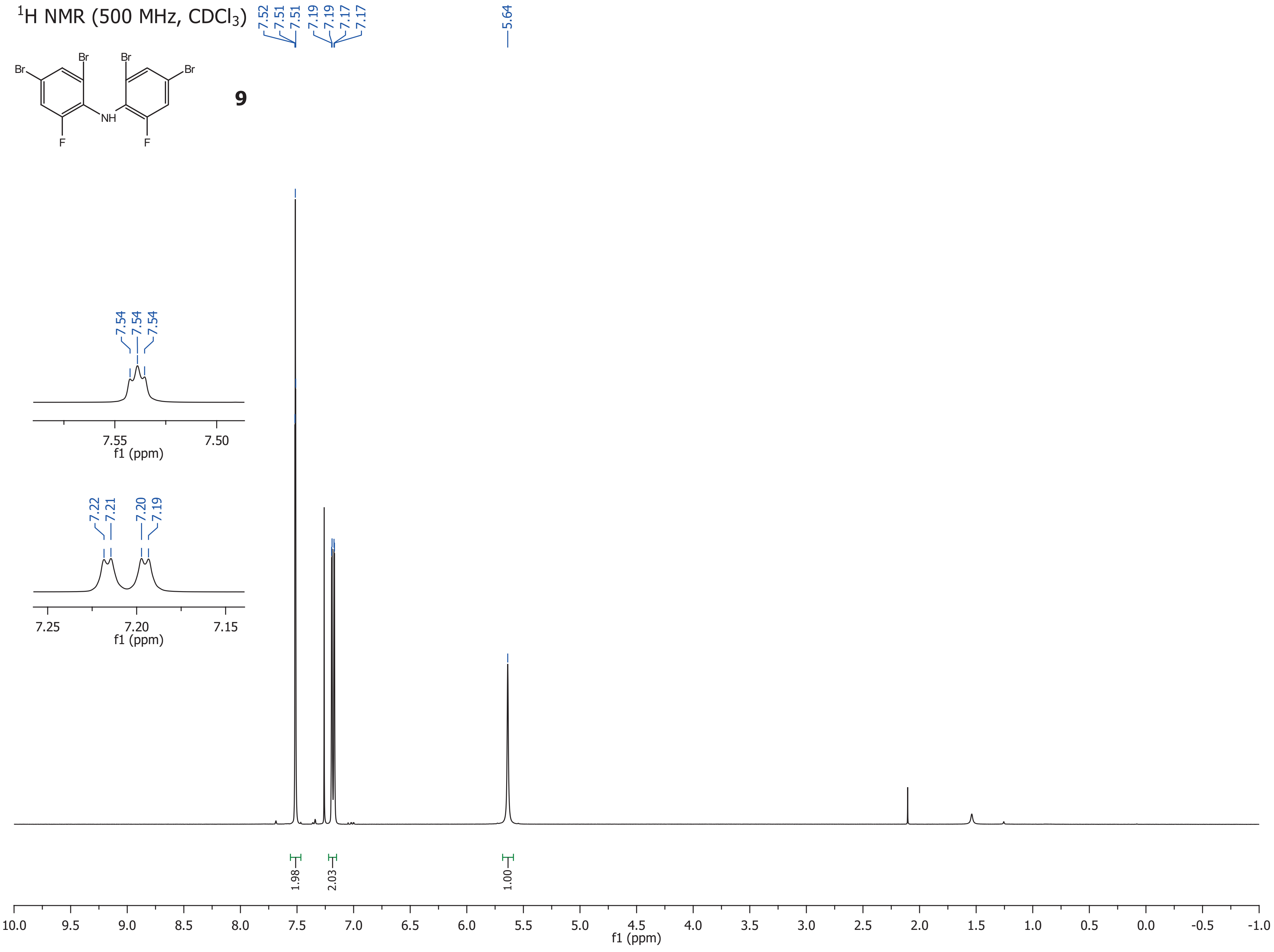
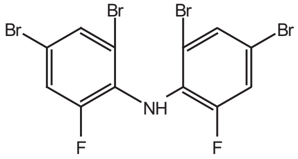

^{1}H NMR (500 MHz, $CDCl_3$)

9

^{19}F NMR (500 MHz, $CDCl_3$)



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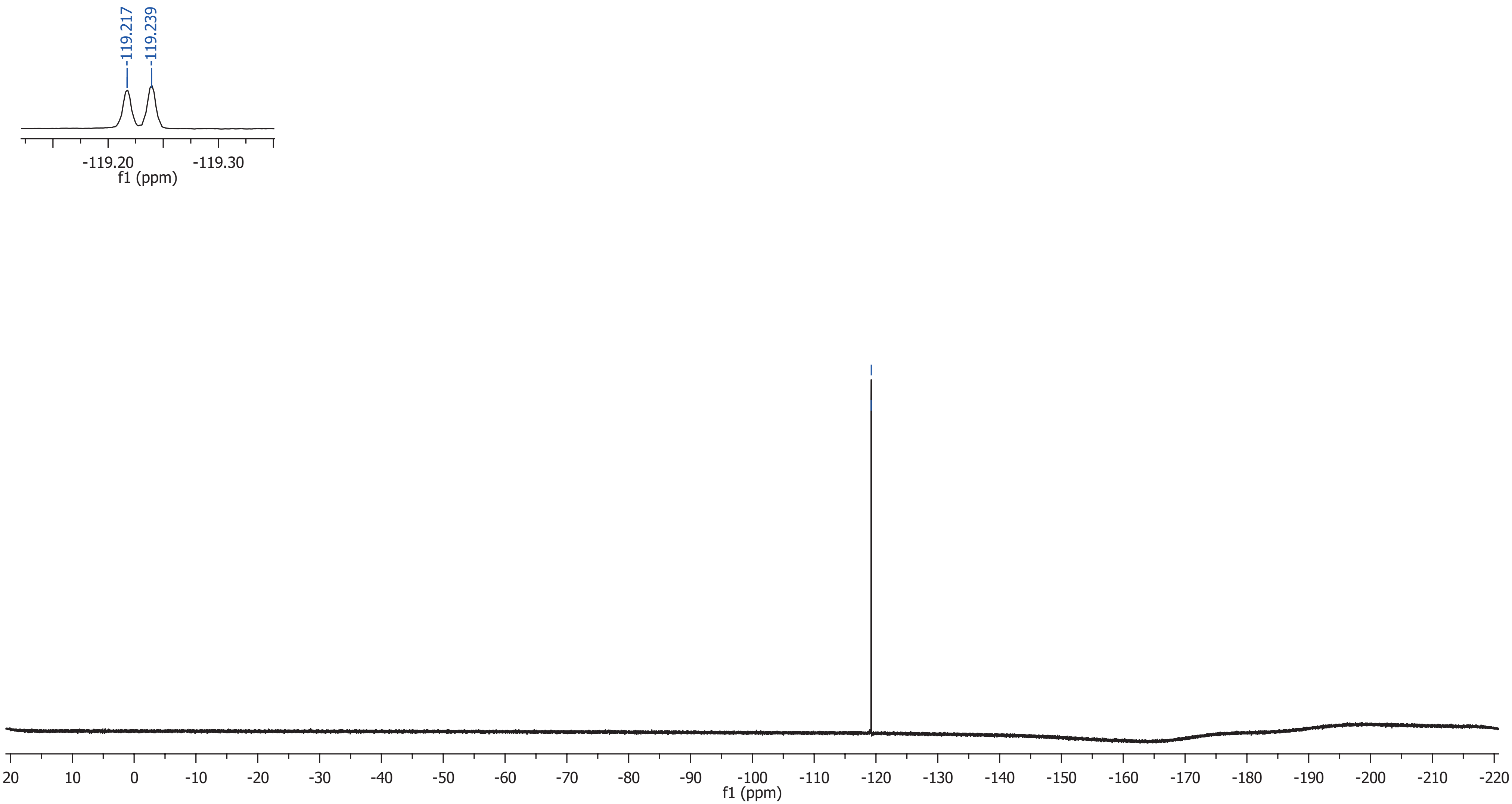
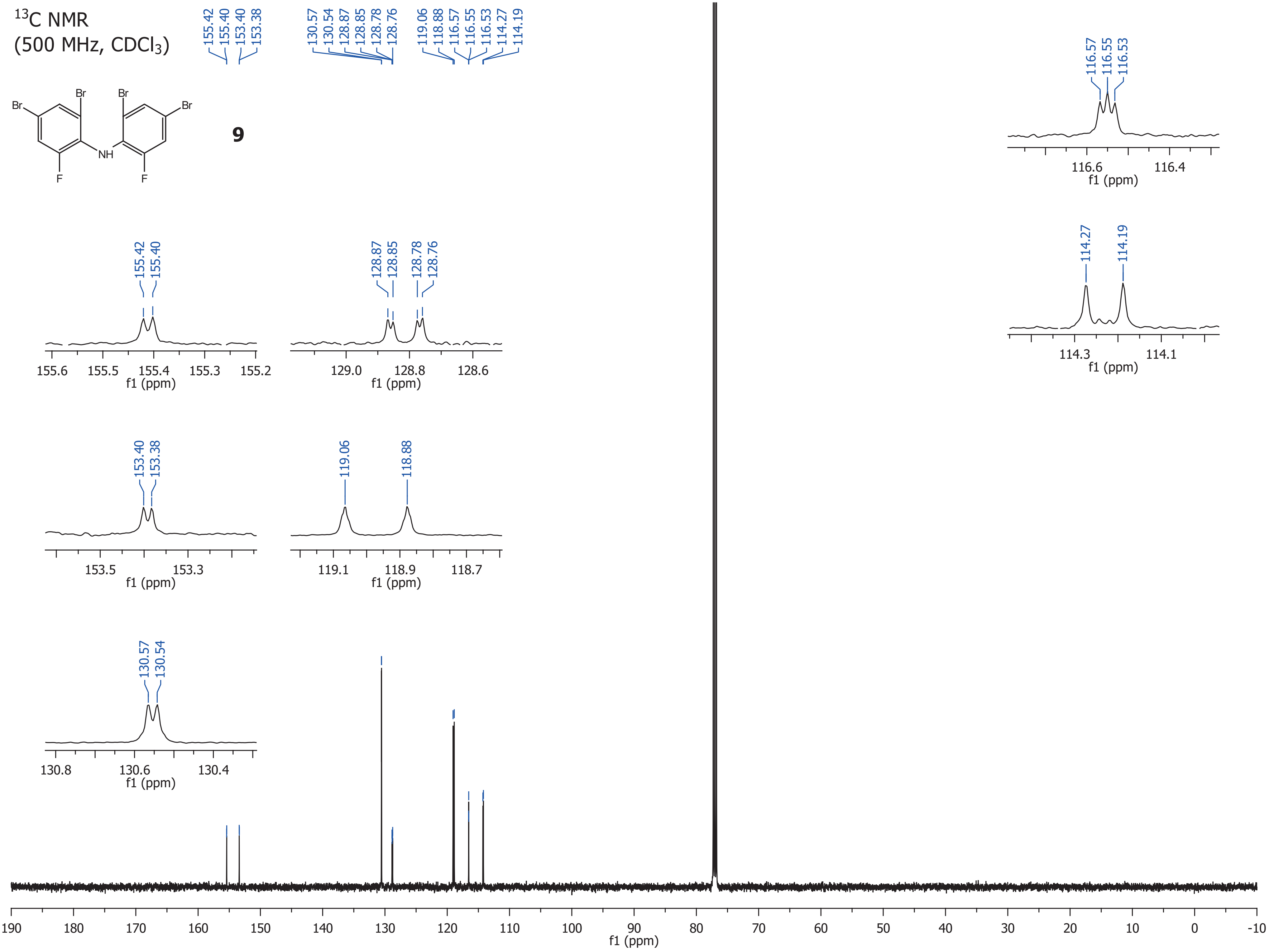


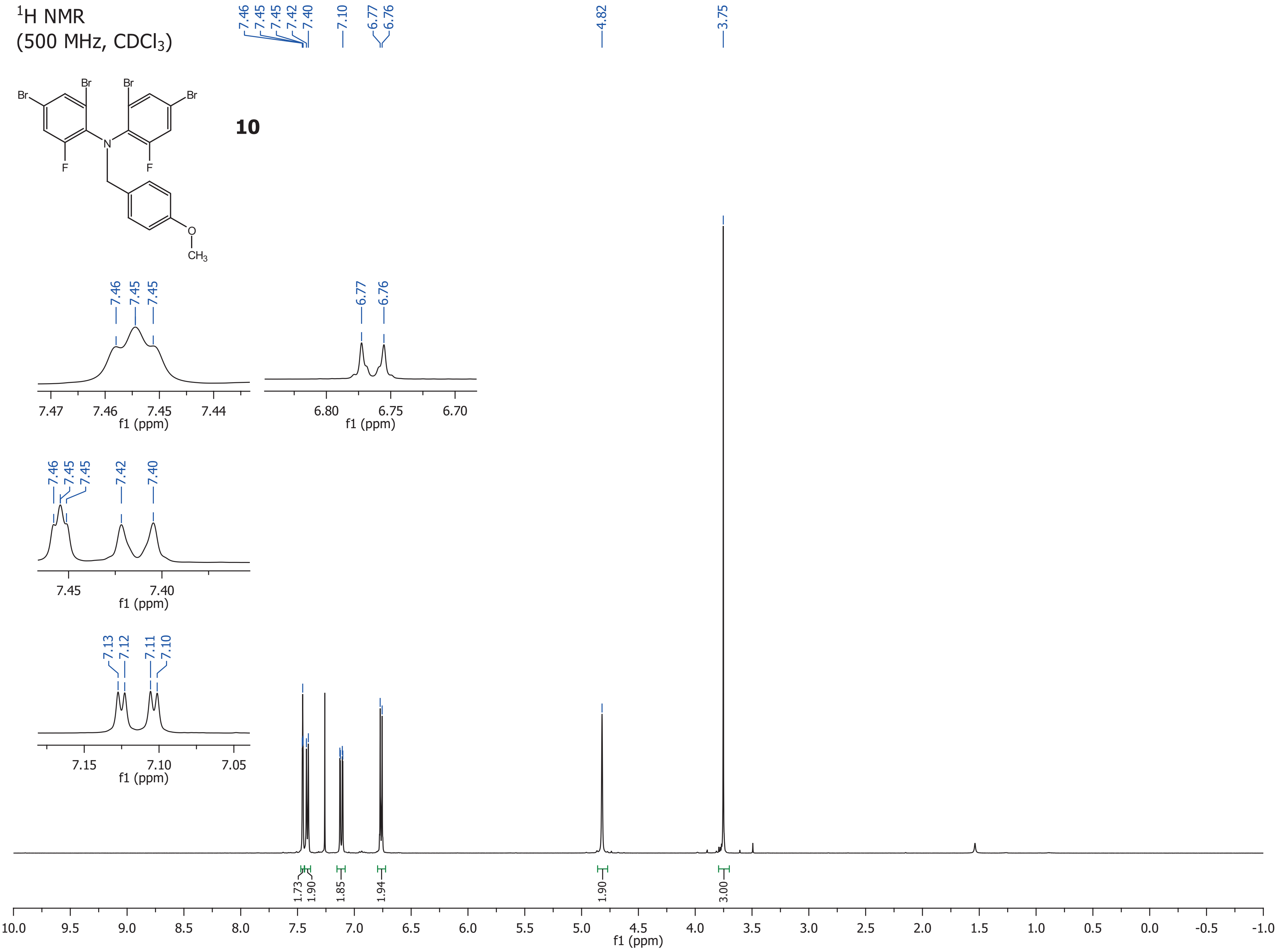
^{13}C NMR
(500 MHz, $CDCl_3$)



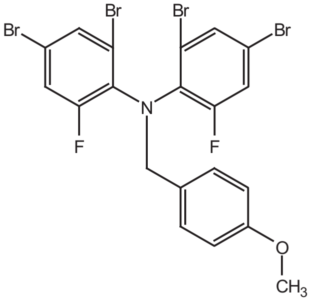
9

^{1}H NMR
(500 MHz, $CDCl_3$)

10



^{19}F NMR (500 MHz, $CDCl_3$)



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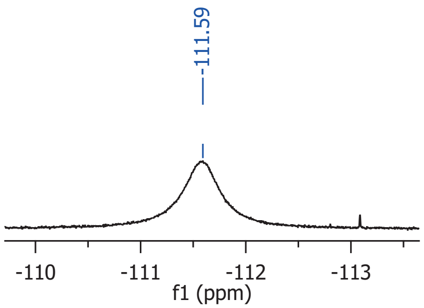
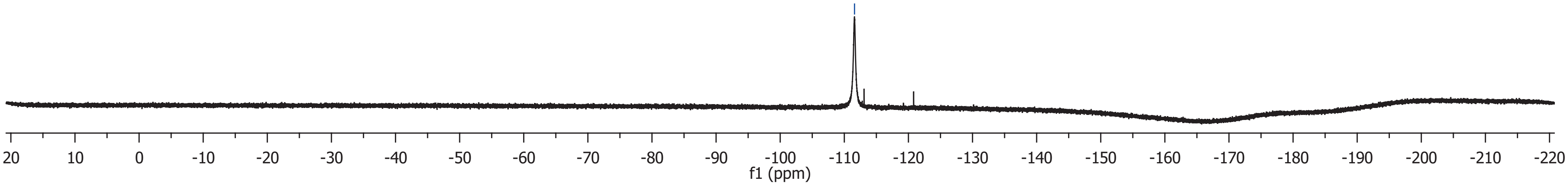
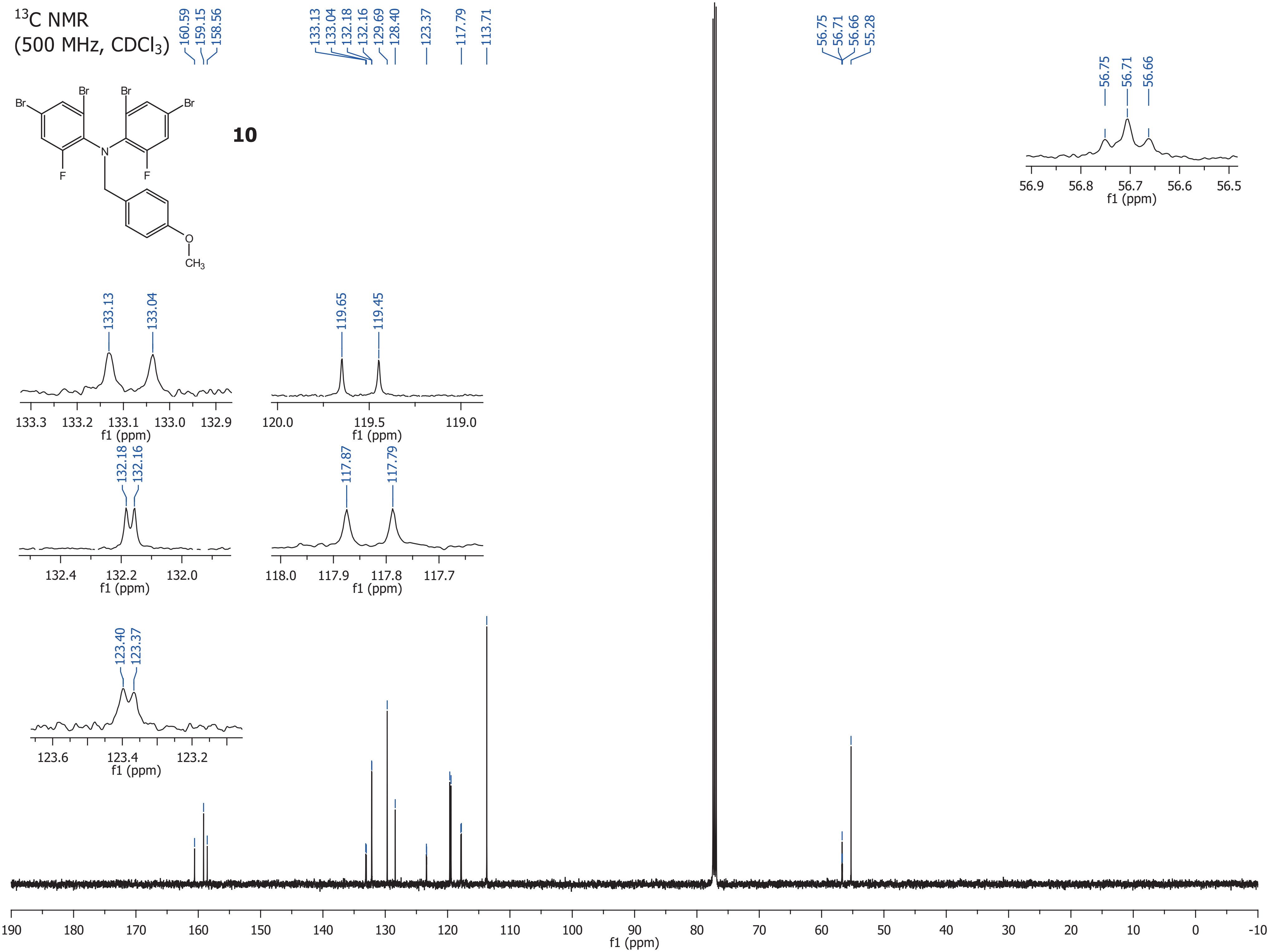


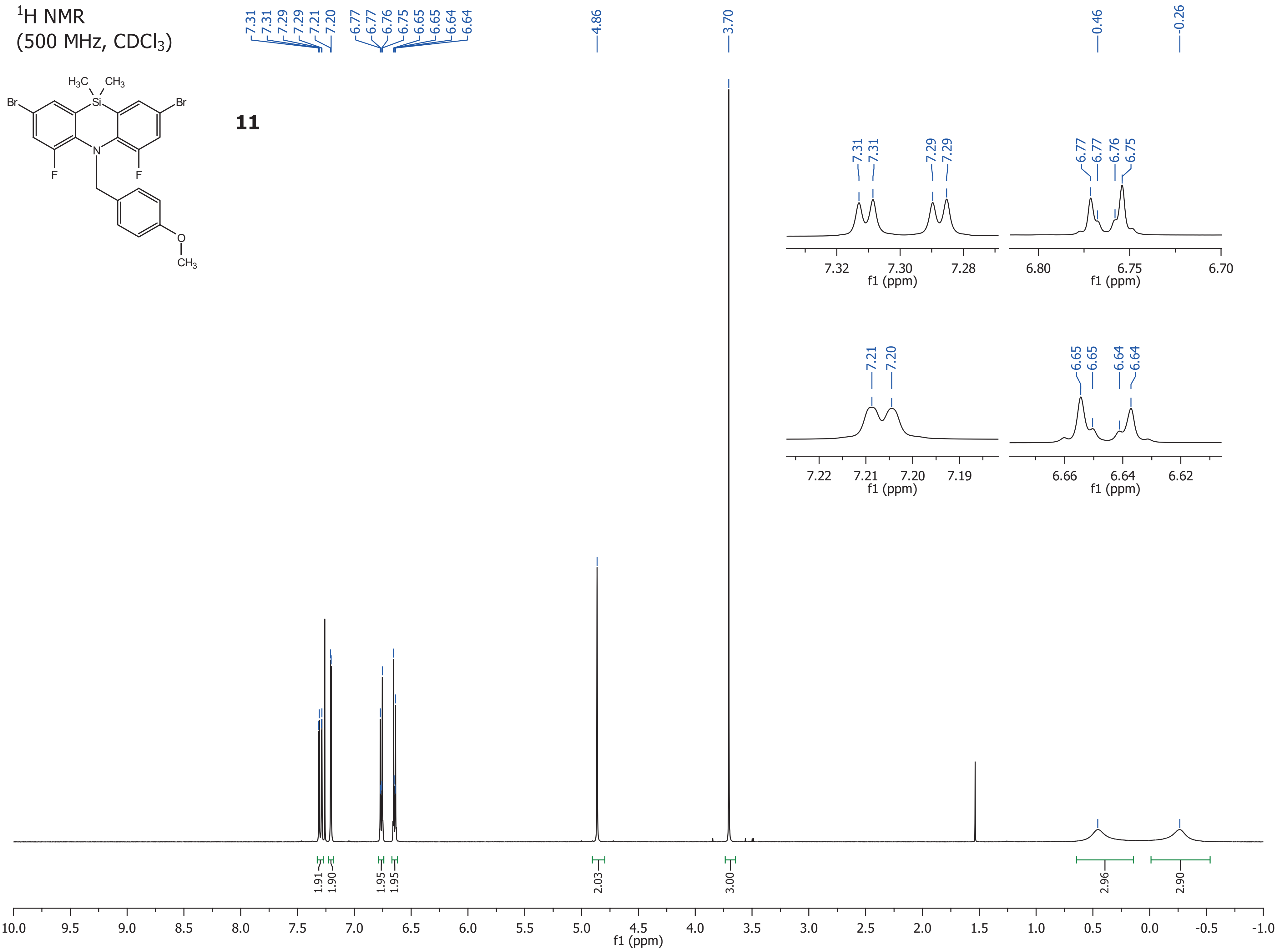


^{13}C NMR (500 MHz, CDCl$_3$)

10

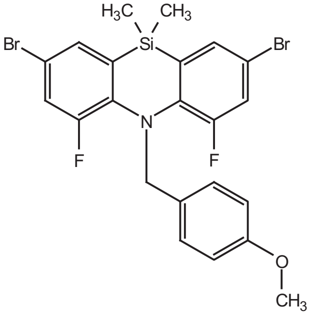


^{1}H NMR
(500 MHz, $CDCl_3$)



11

^{19}F NMR (500 MHz, $CDCl_3$)



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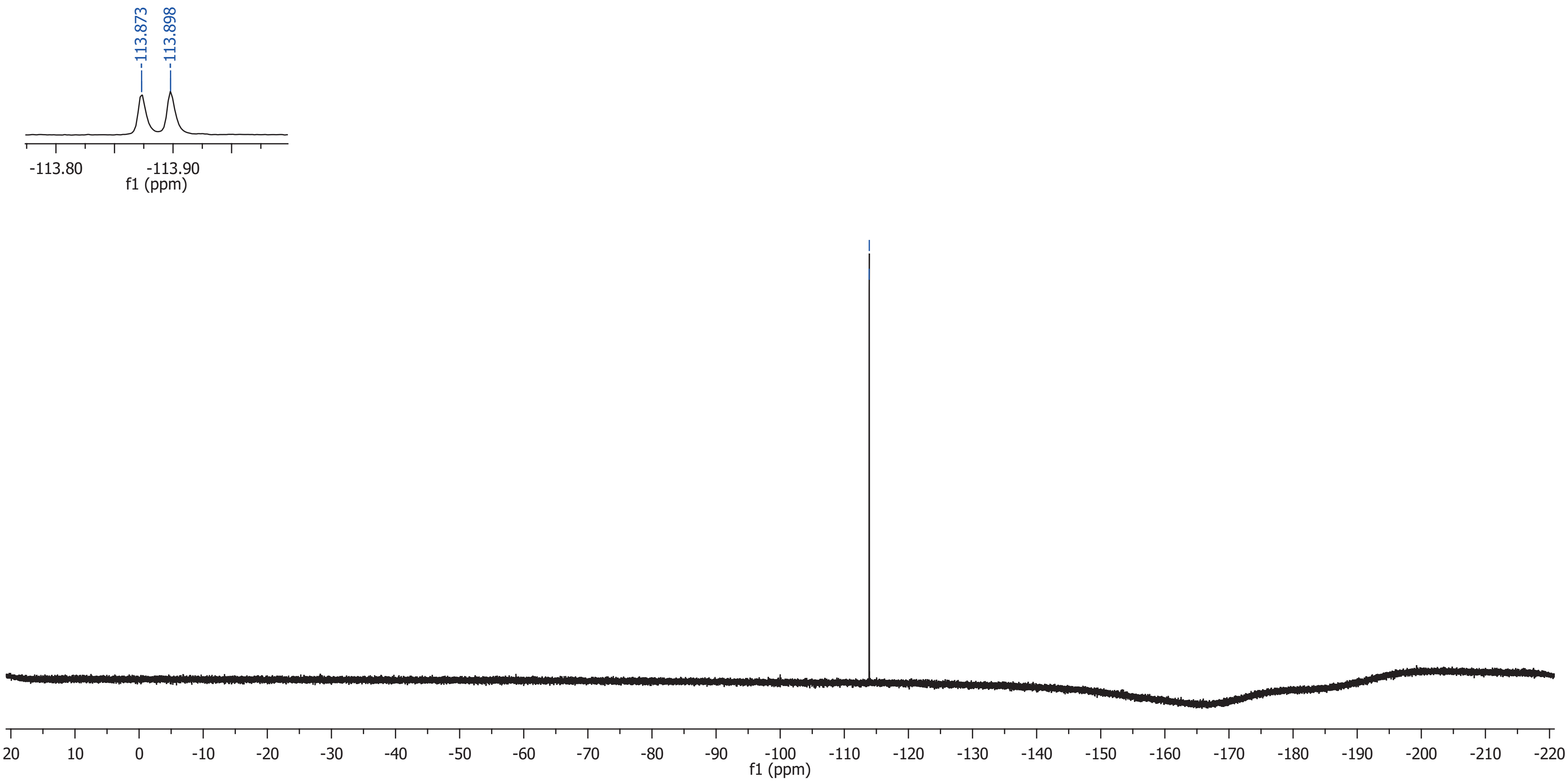
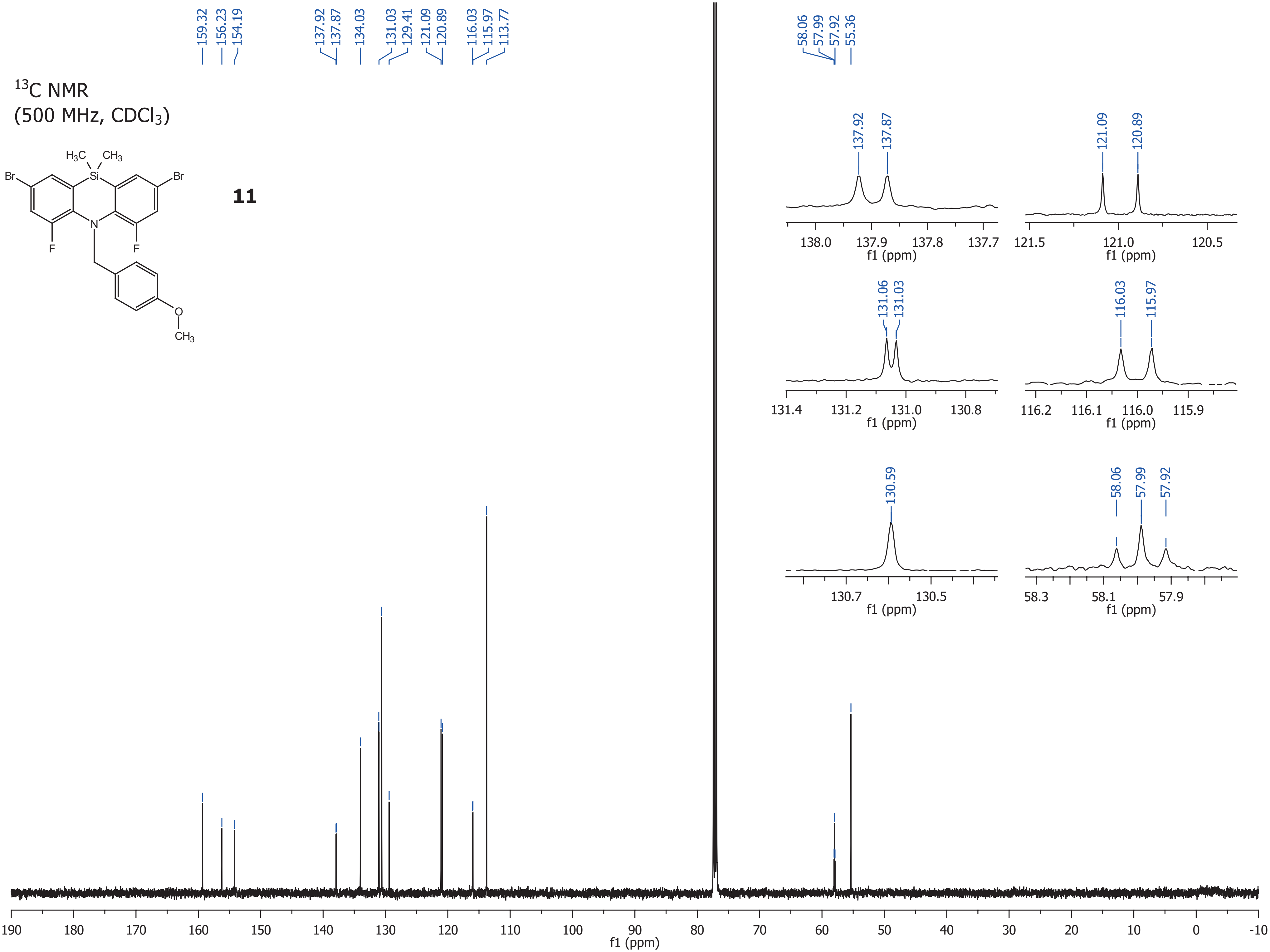
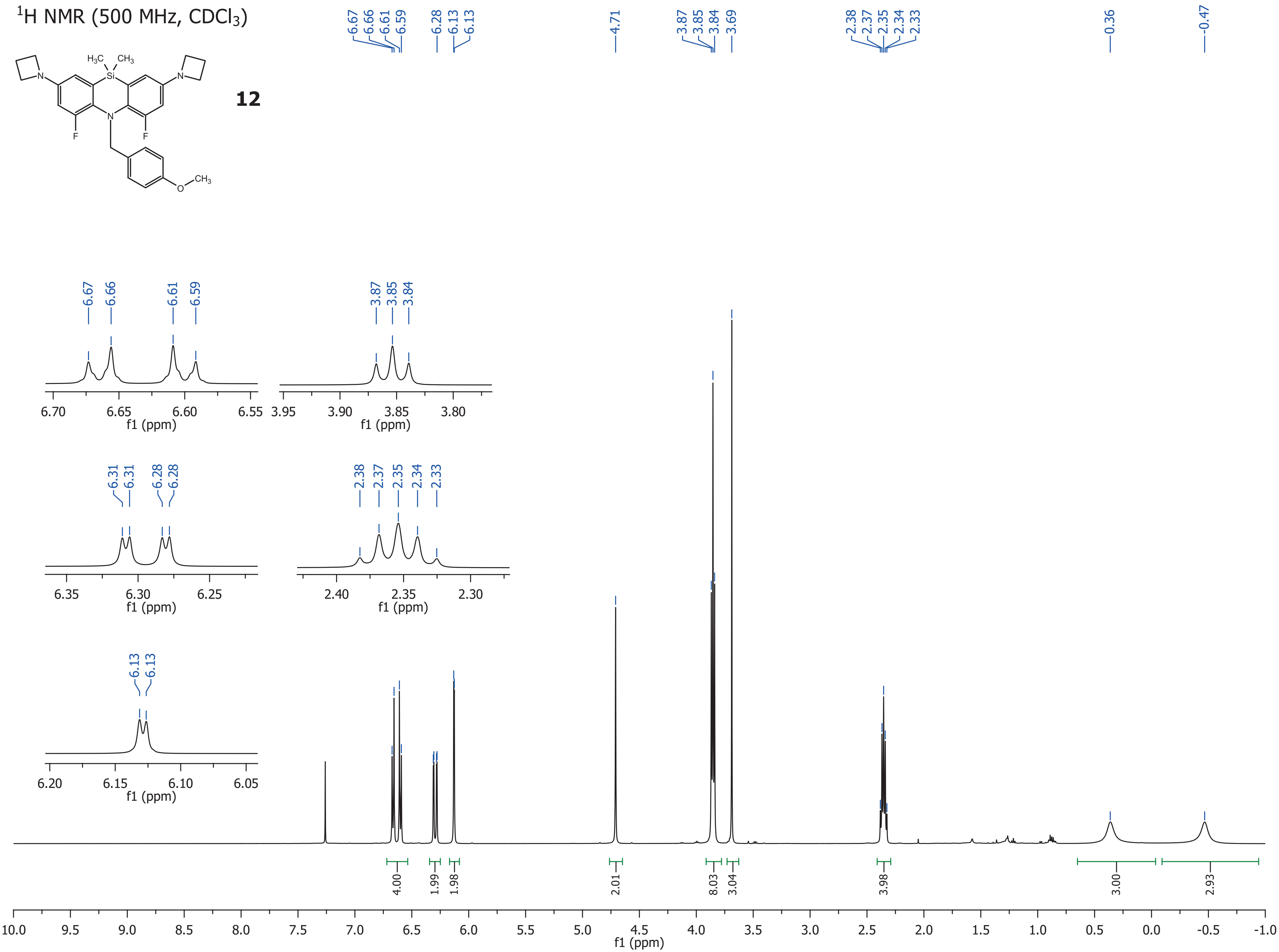


^{13}C NMR
(500 MHz, $CDCl_3$)

11

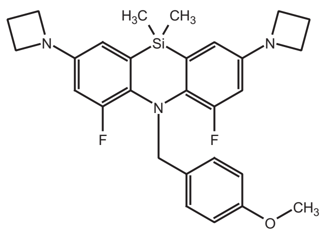


^{1}H NMR (500 MHz, CDCl$_3$)

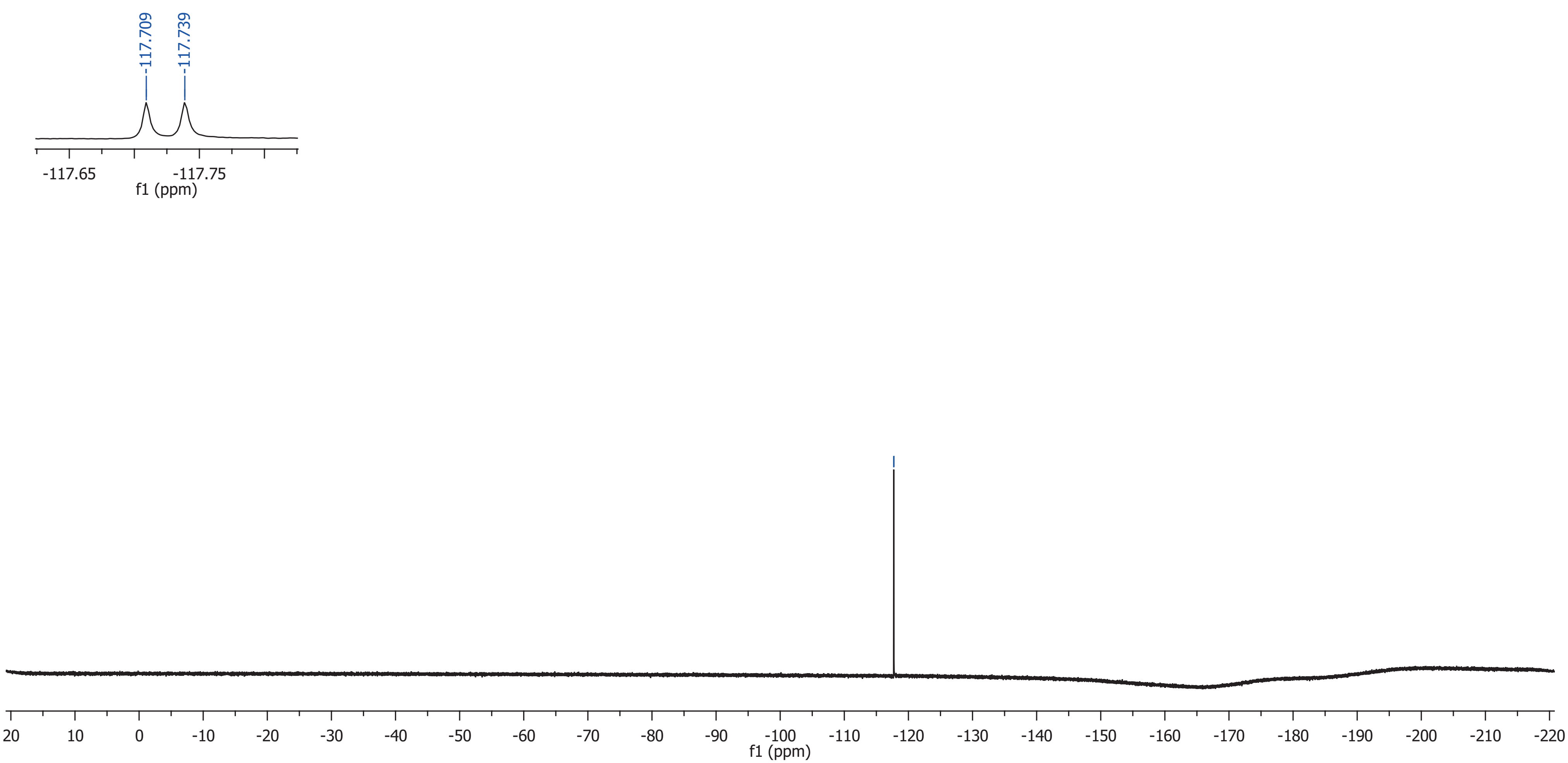


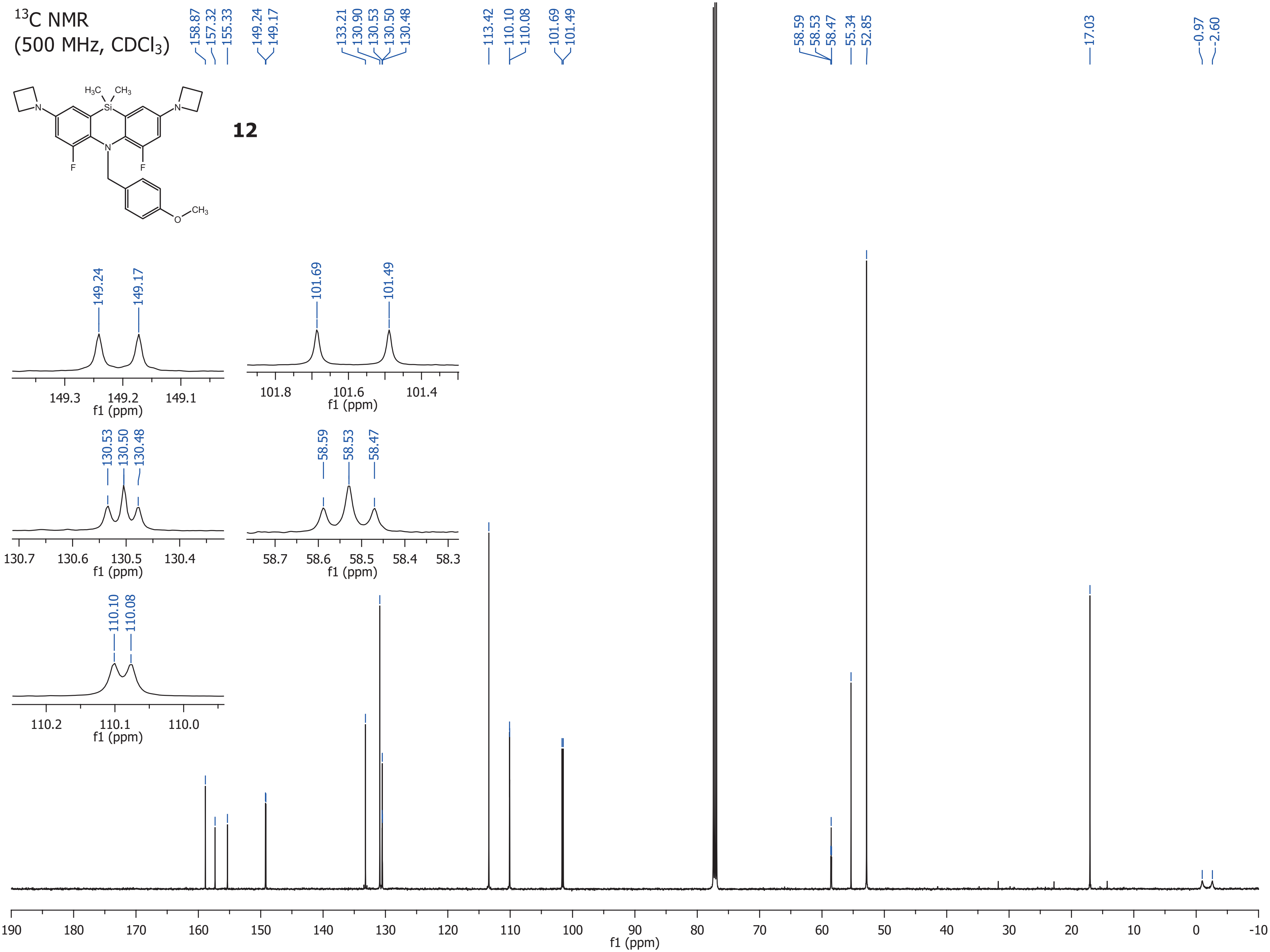
12

^{19}F NMR (500 MHz, CDCl$_3$)



12

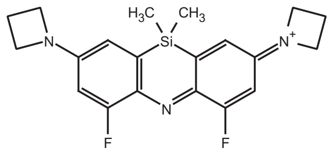




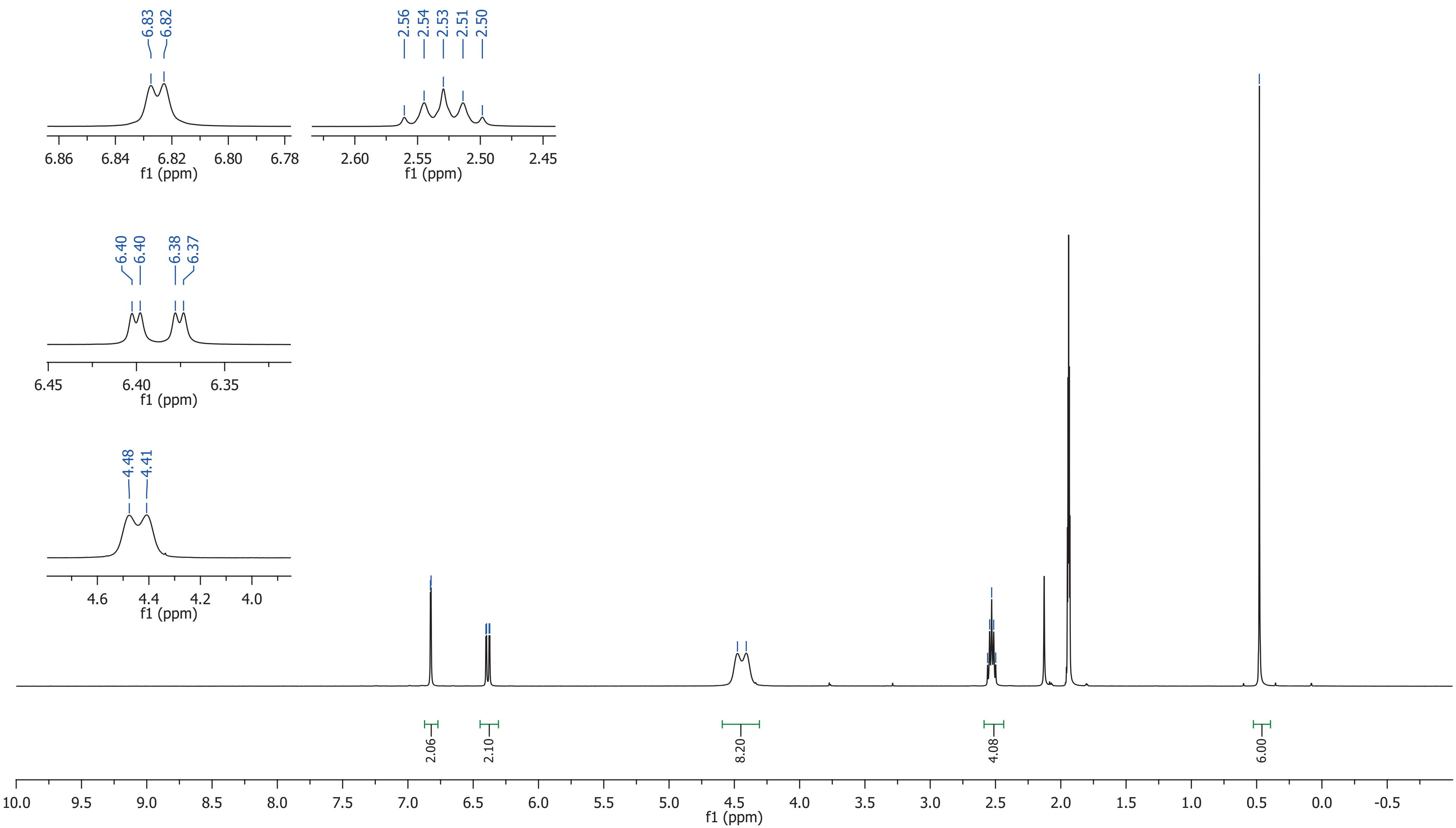
12

^{13}C NMR
(500 MHz, CDCl$_3$)
158.87
157.32
155.33
149.24
149.17
133.21
130.90
130.53
130.50
130.48
113.42
110.10
110.08
101.69
101.49
58.59
58.53
58.47
55.34
52.85
17.03
-0.97
-2.60
H$_3$C
CH$_3$
Si
N
F
O
CH$_3$
149.3
149.2
149.1
101.8
101.6
101.4
130.7
130.6
130.5
130.4
58.7
58.6
58.5
58.4
58.3
110.2
110.1
110.0
f1 (ppm)
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

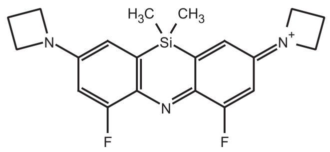
^{1}H NMR (500 MHz, CD_3CN)



13



^{19}F NMR (500 MHz, CD_3CN)



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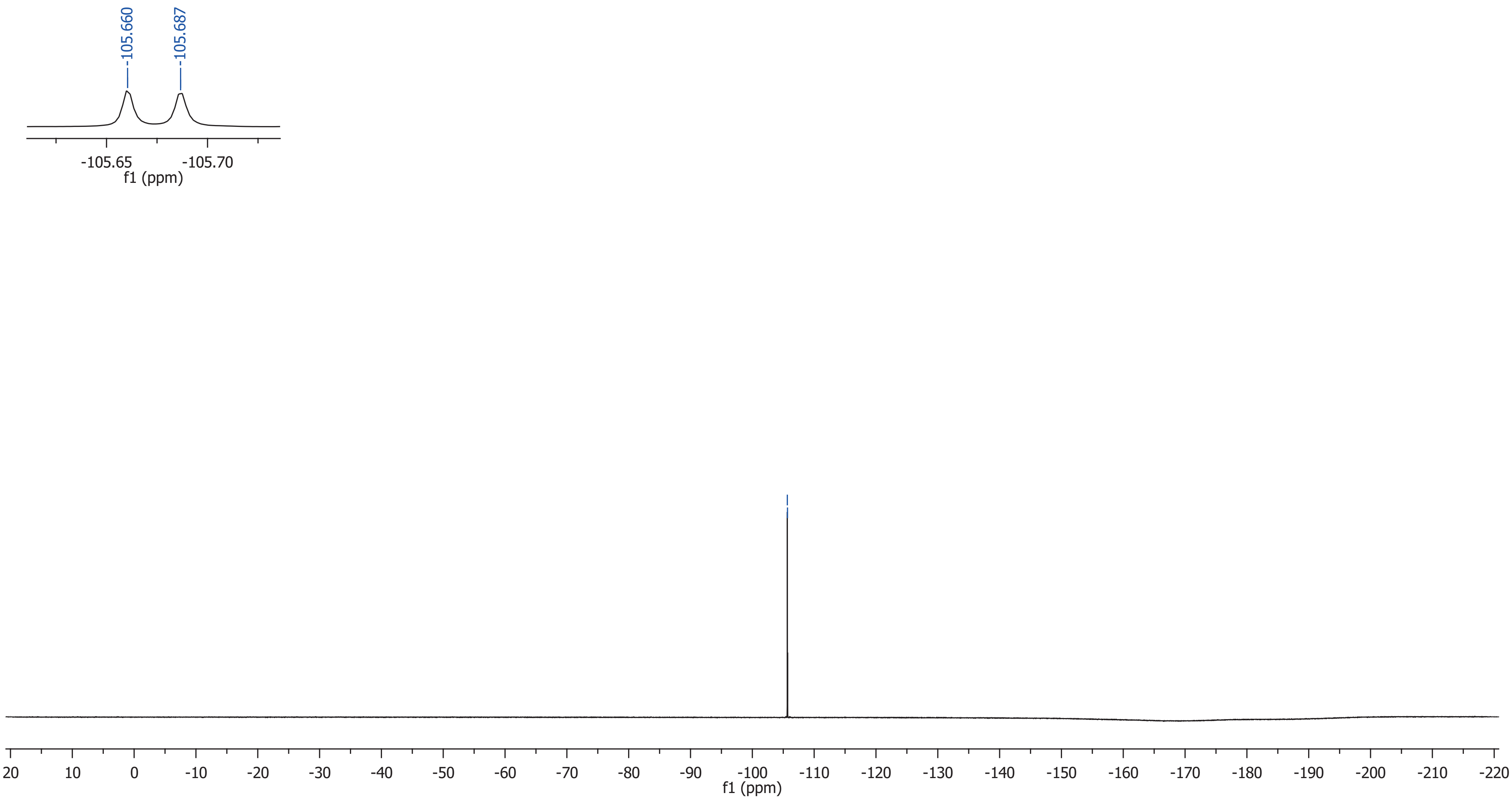
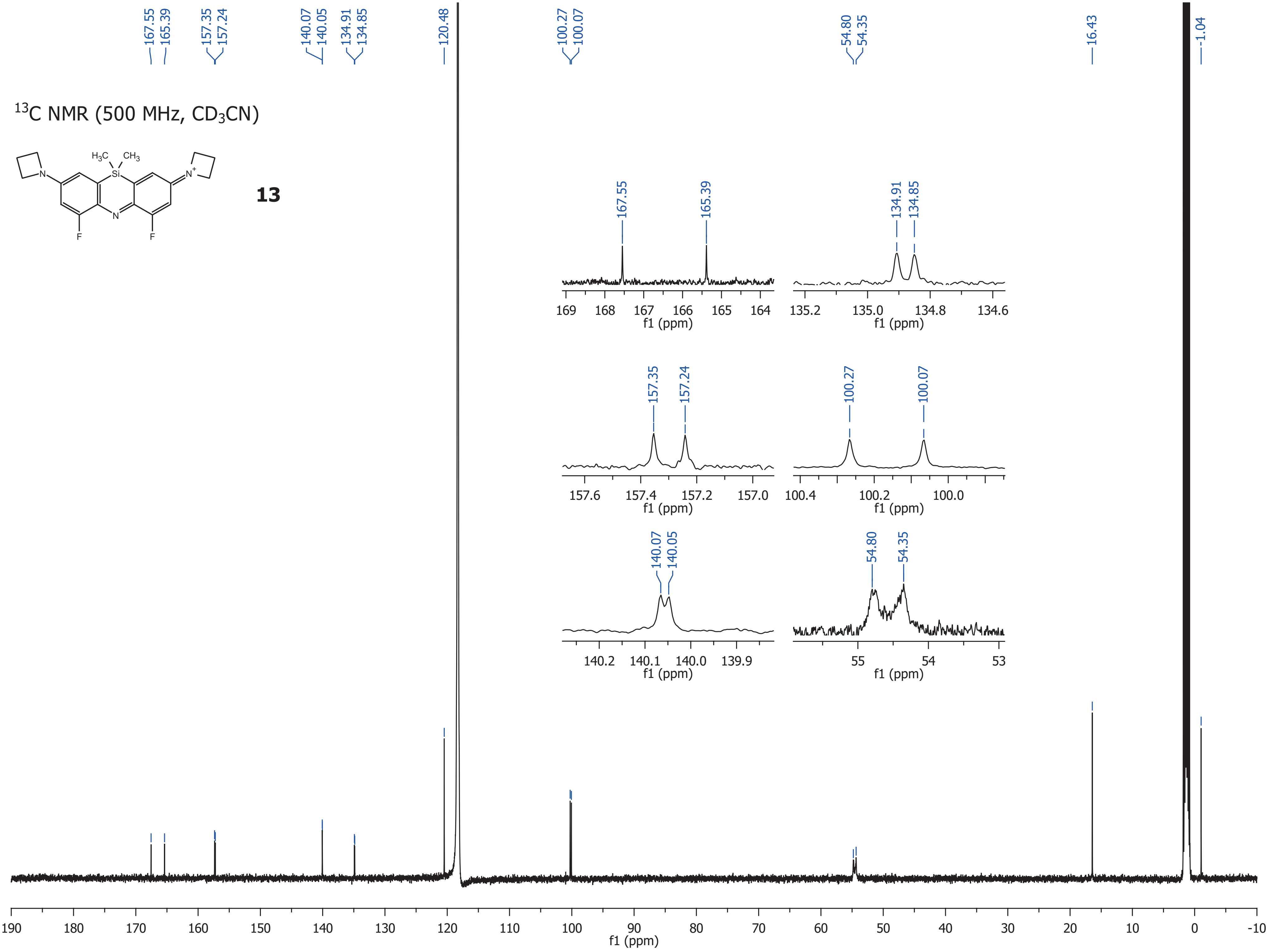
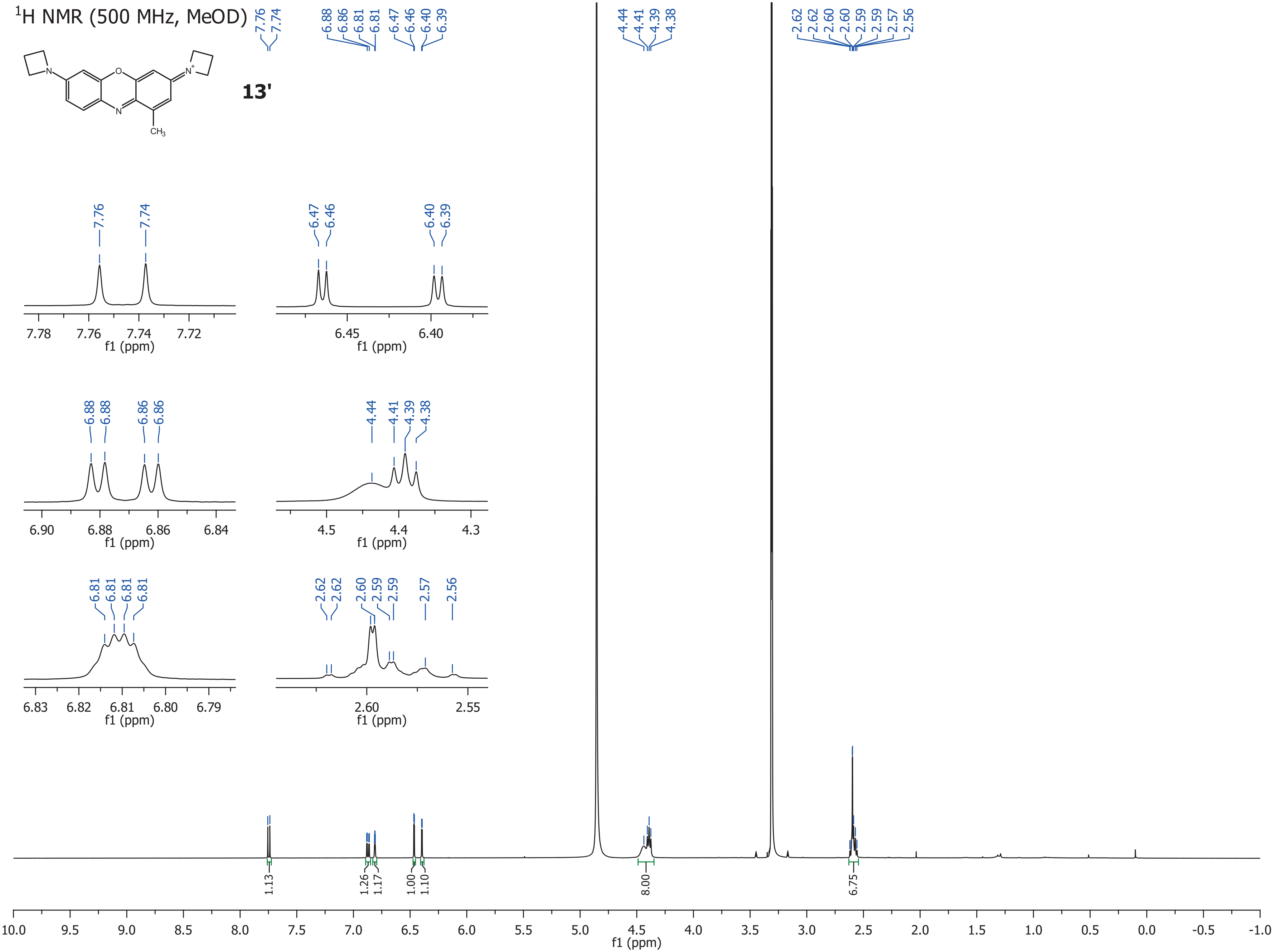




^{13}C NMR (500 MHz, CD_3CN)

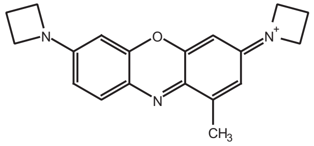
13

^{1}H NMR (500 MHz, MeOD)

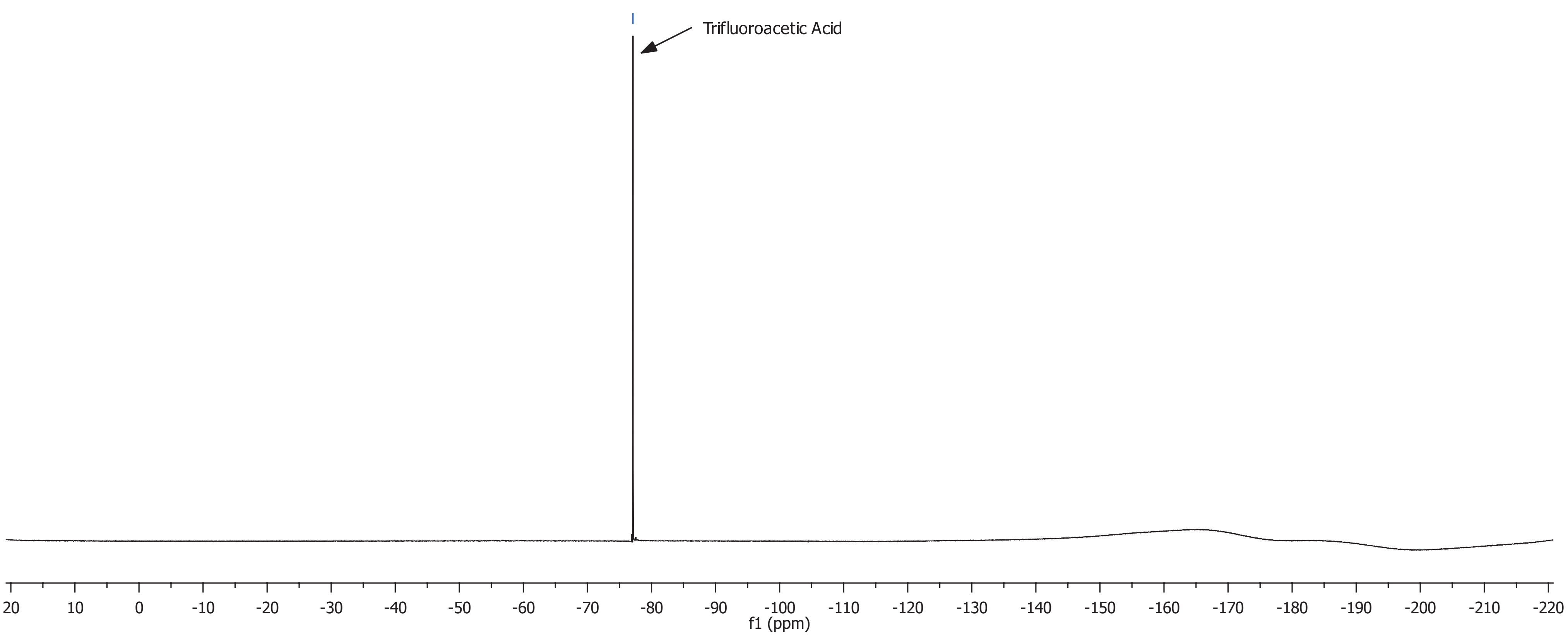


13'

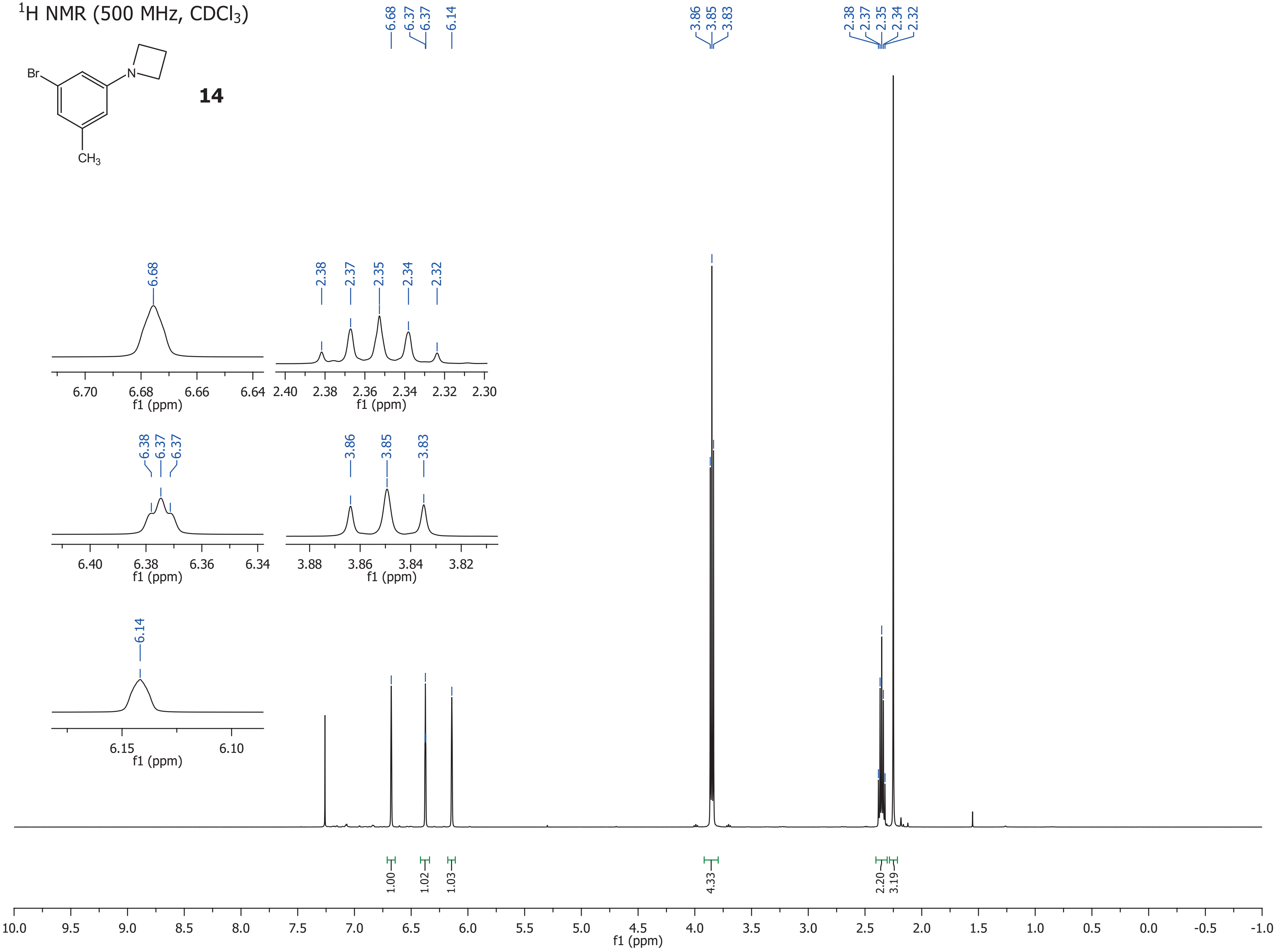
^{19}F NMR (500 MHz, MeOD)



13'

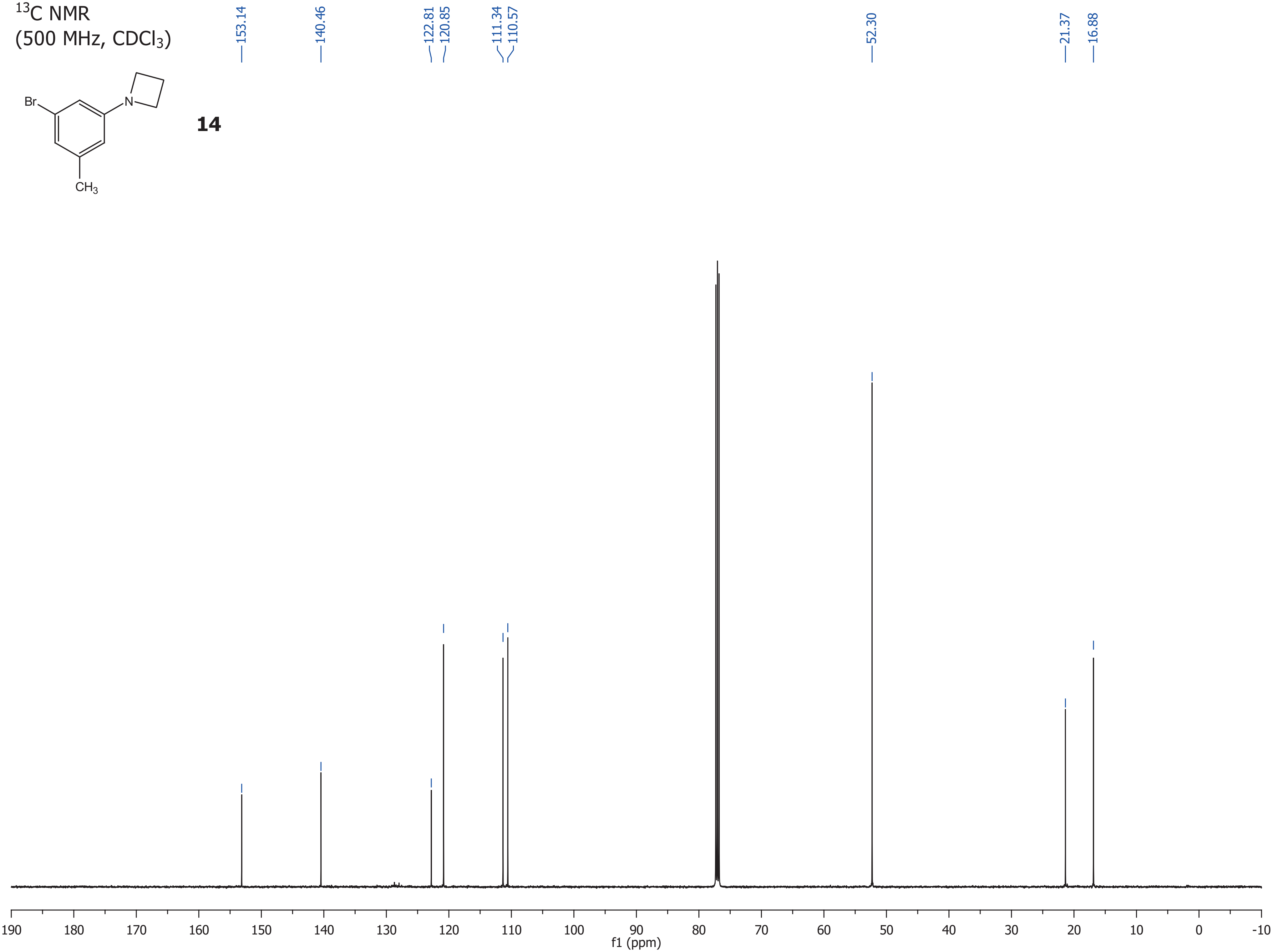


^{1}H NMR (500 MHz, CDCl$_3$)



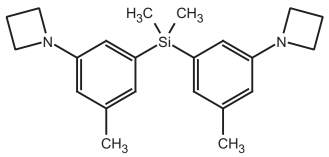
14

^{13}C NMR
(500 MHz, CDCl$_3$)



14

^{13}H NMR (500 MHz, CDCl$_3$)



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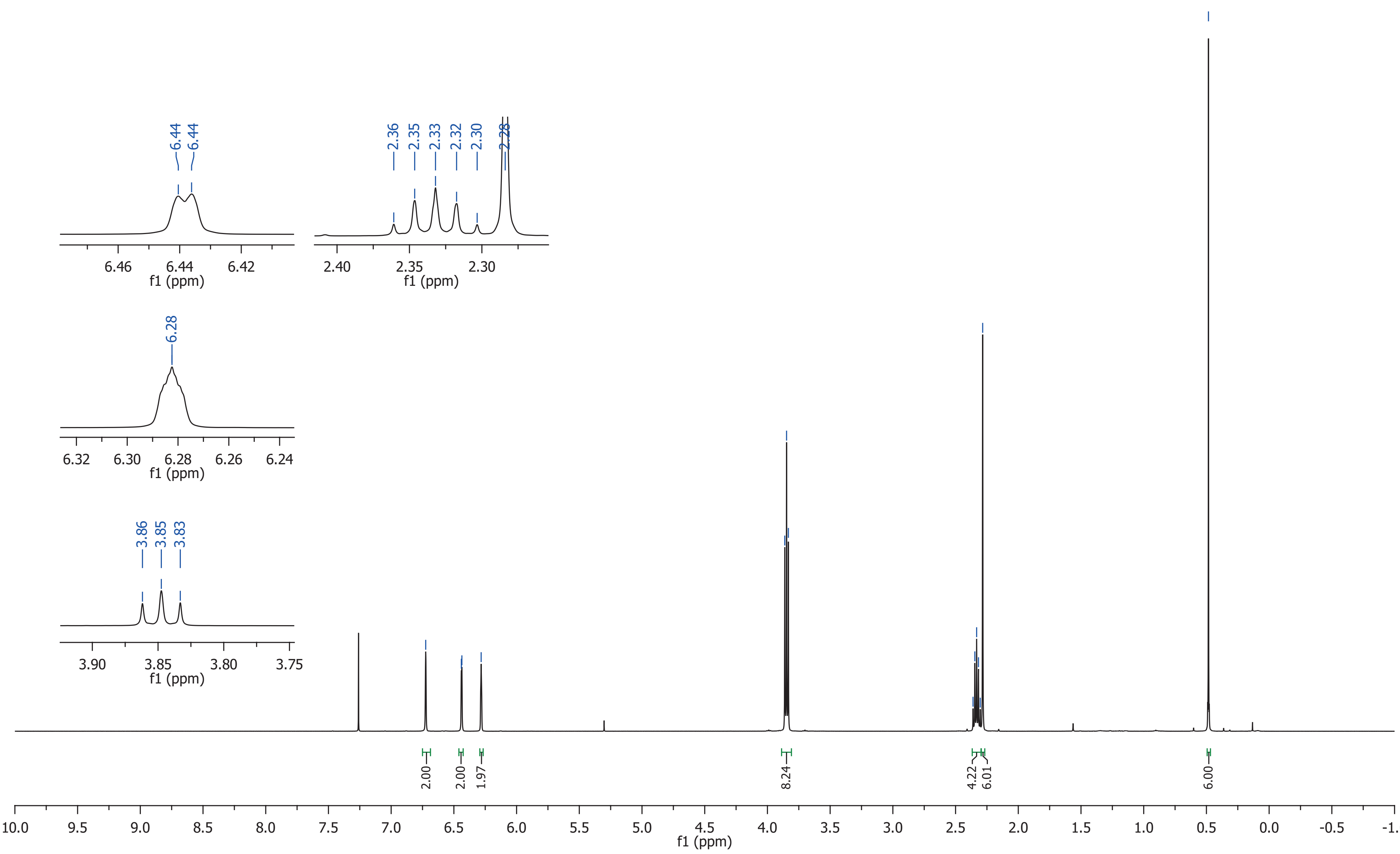
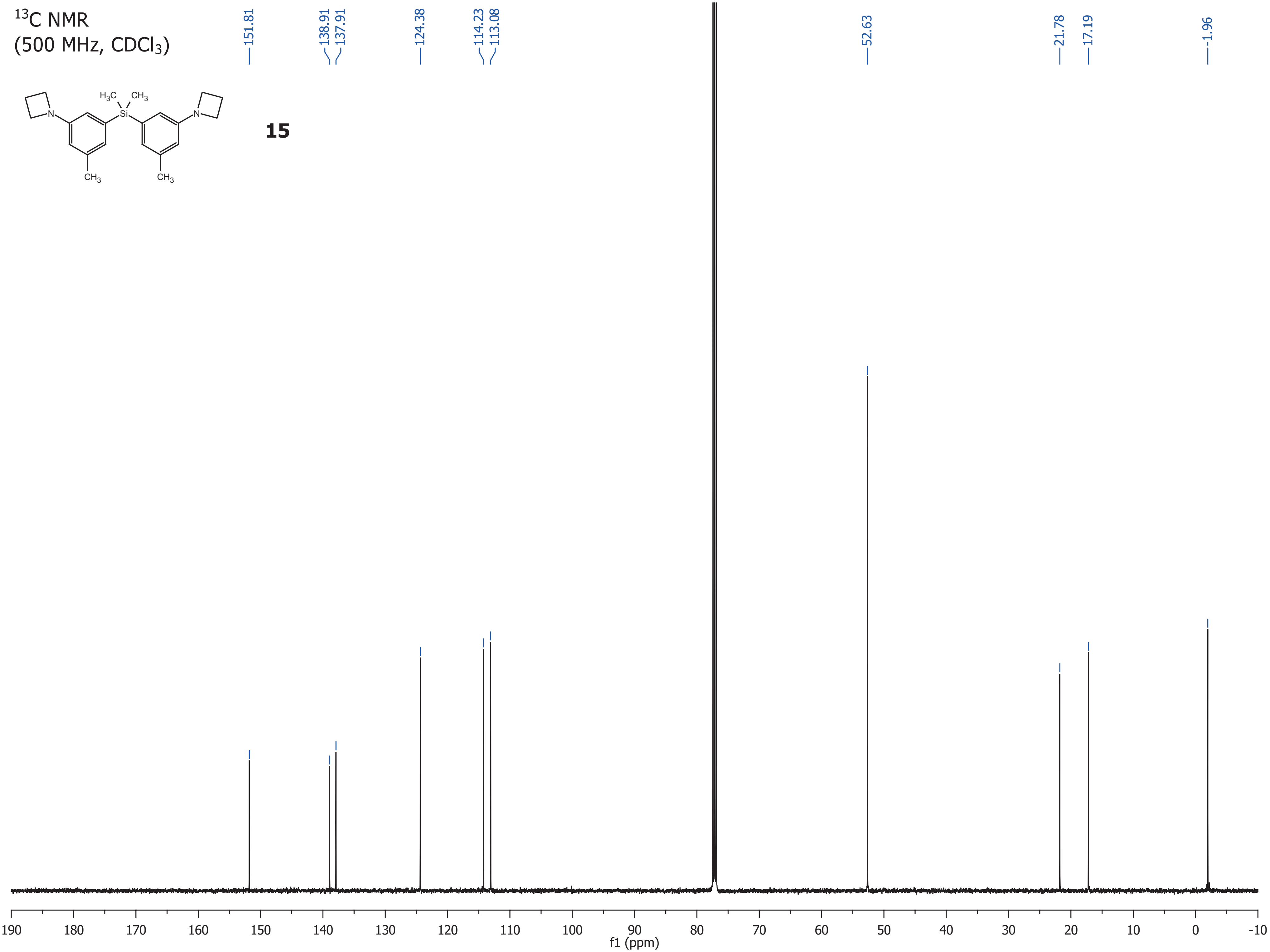
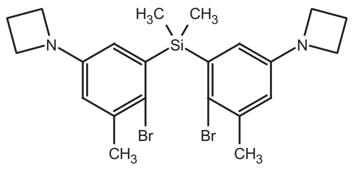


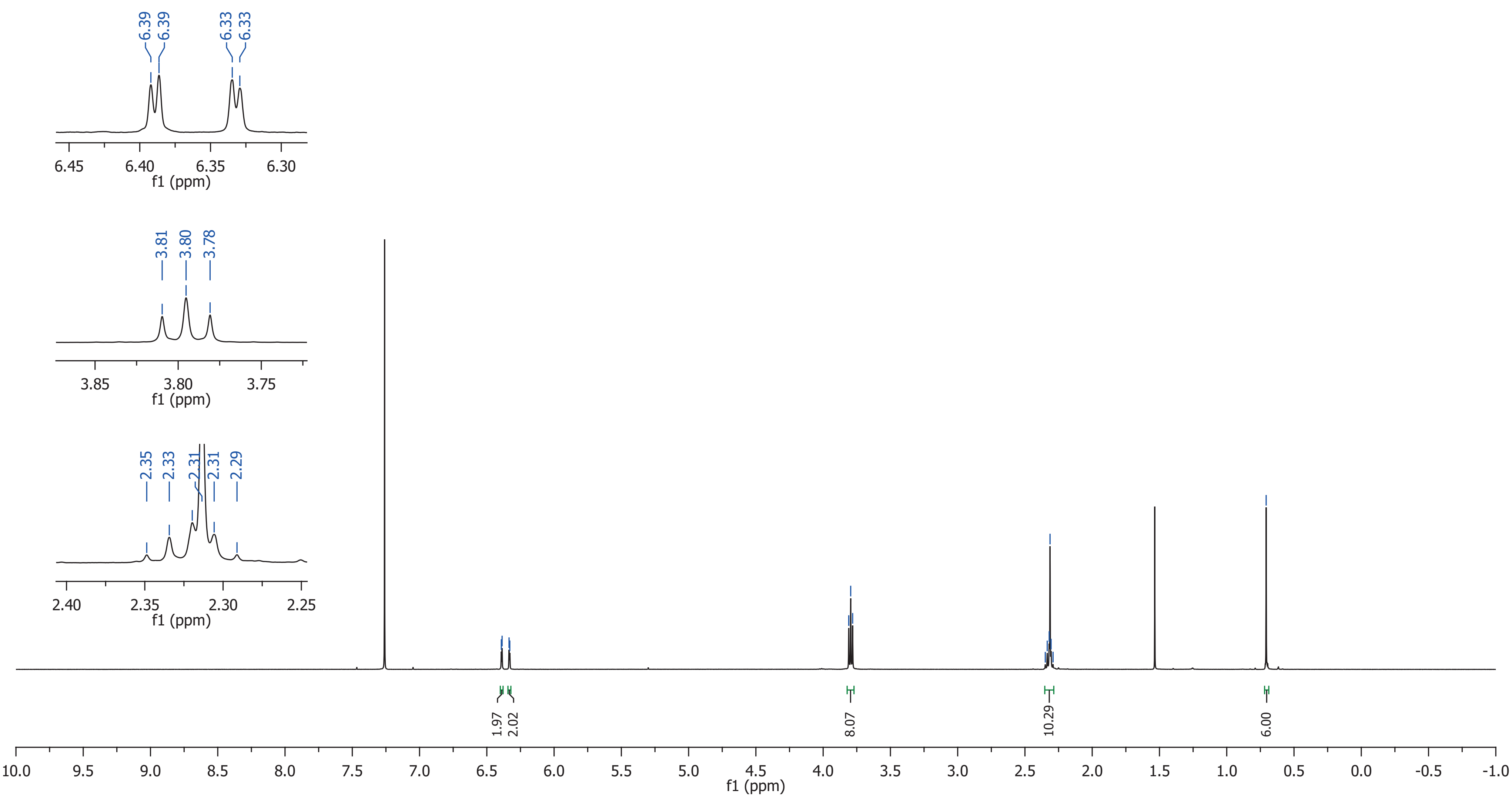

^{13}C NMR
(500 MHz, $CDCl_3$)

15

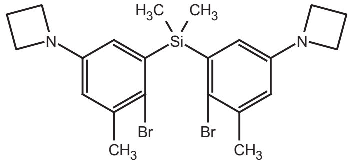
^{1}H NMR (500 MHz, CDCl$_3$)



16



^{13}C NMR (500 MHz, CDCl$_3$)



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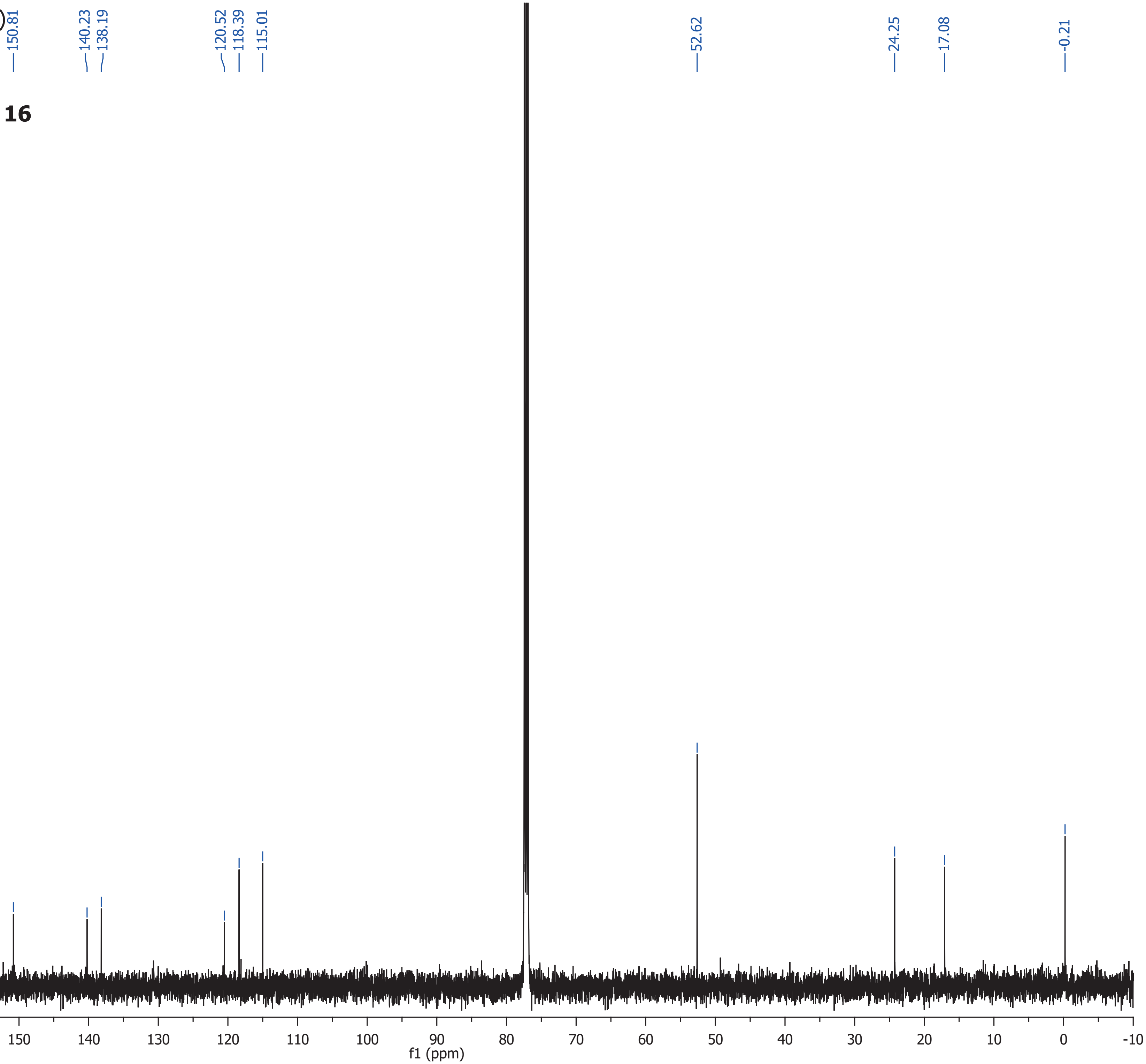
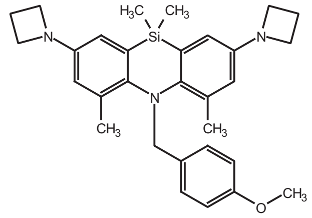


^{1}H NMR (500 MHz, CDCl$_3$)



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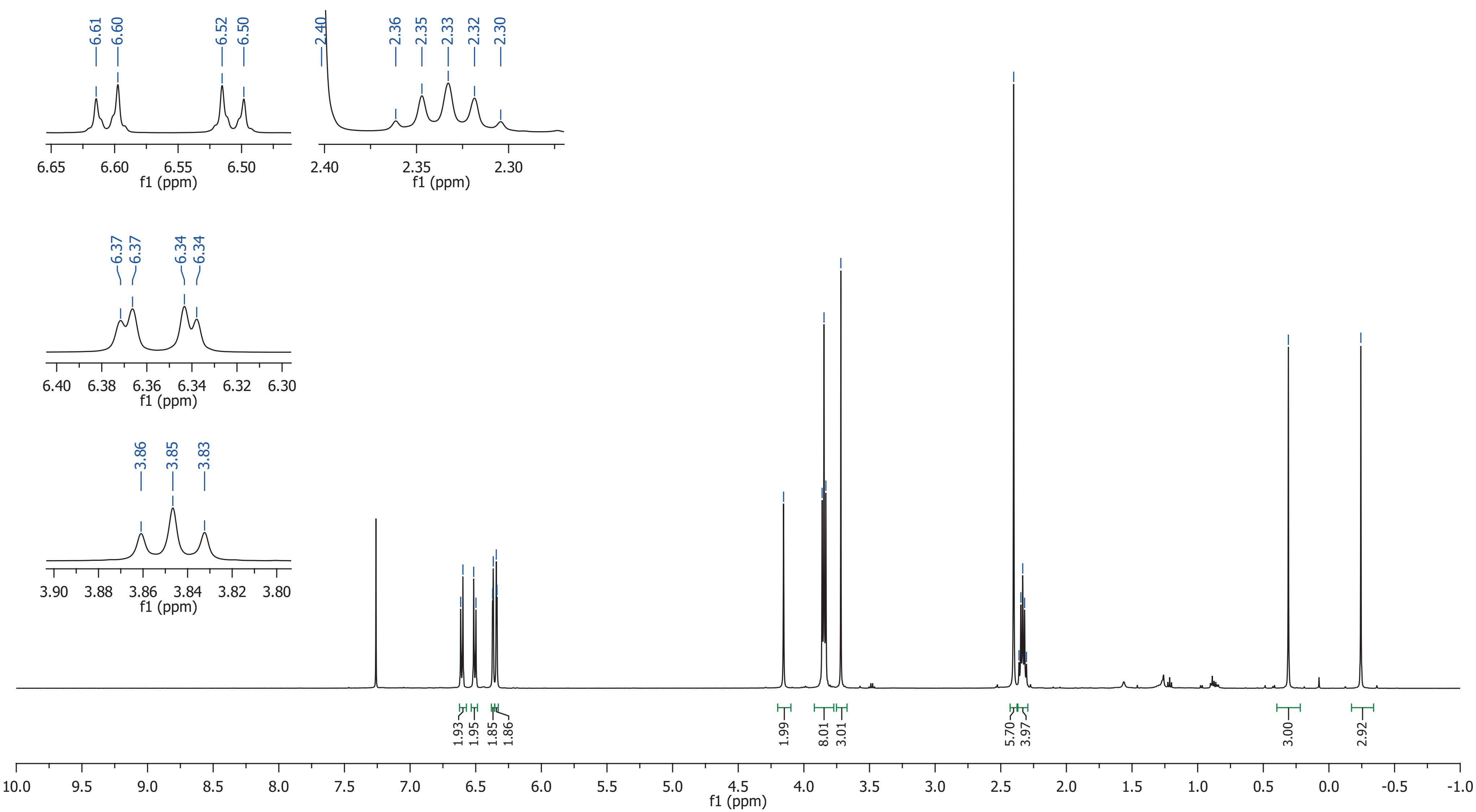
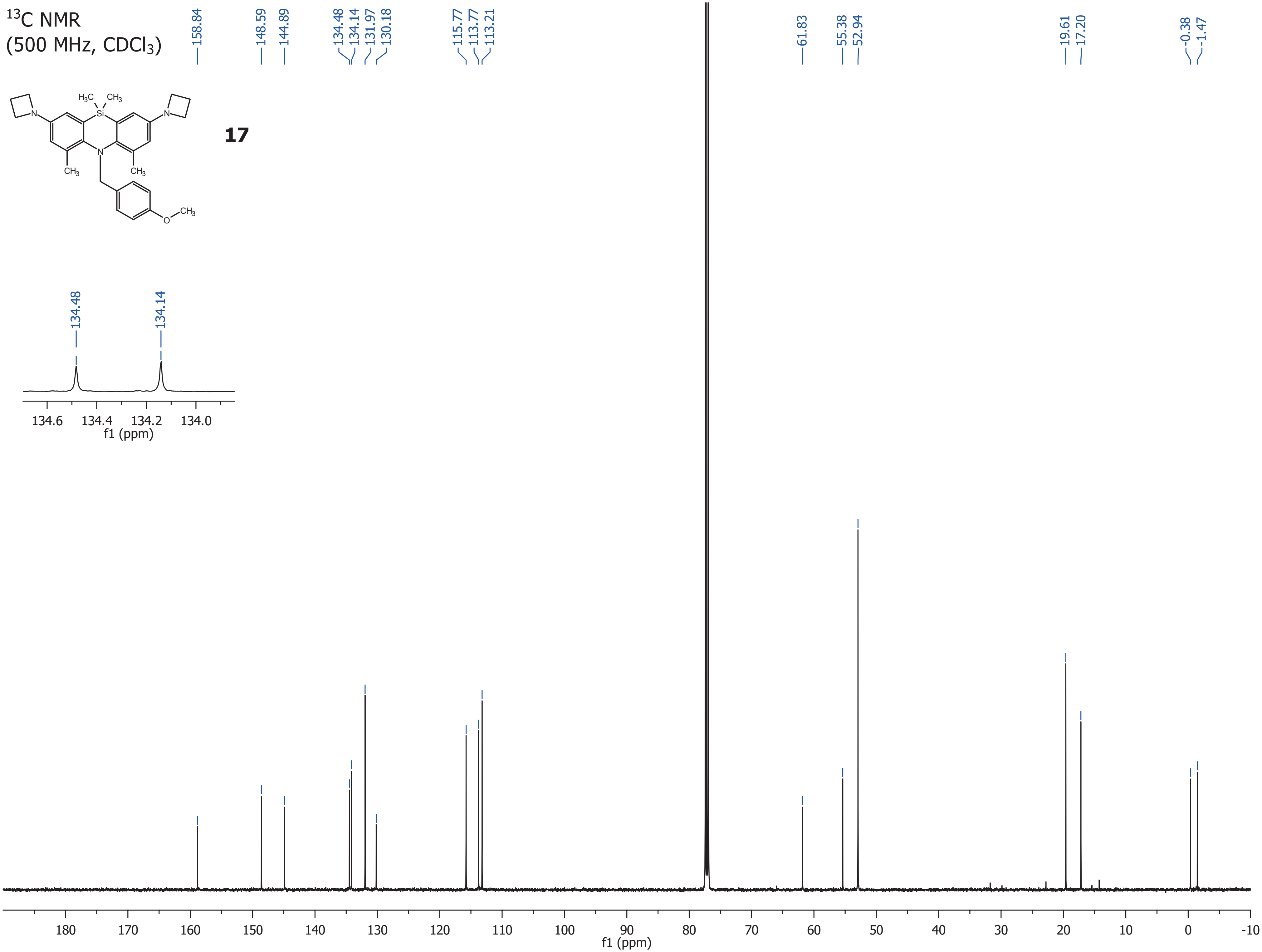
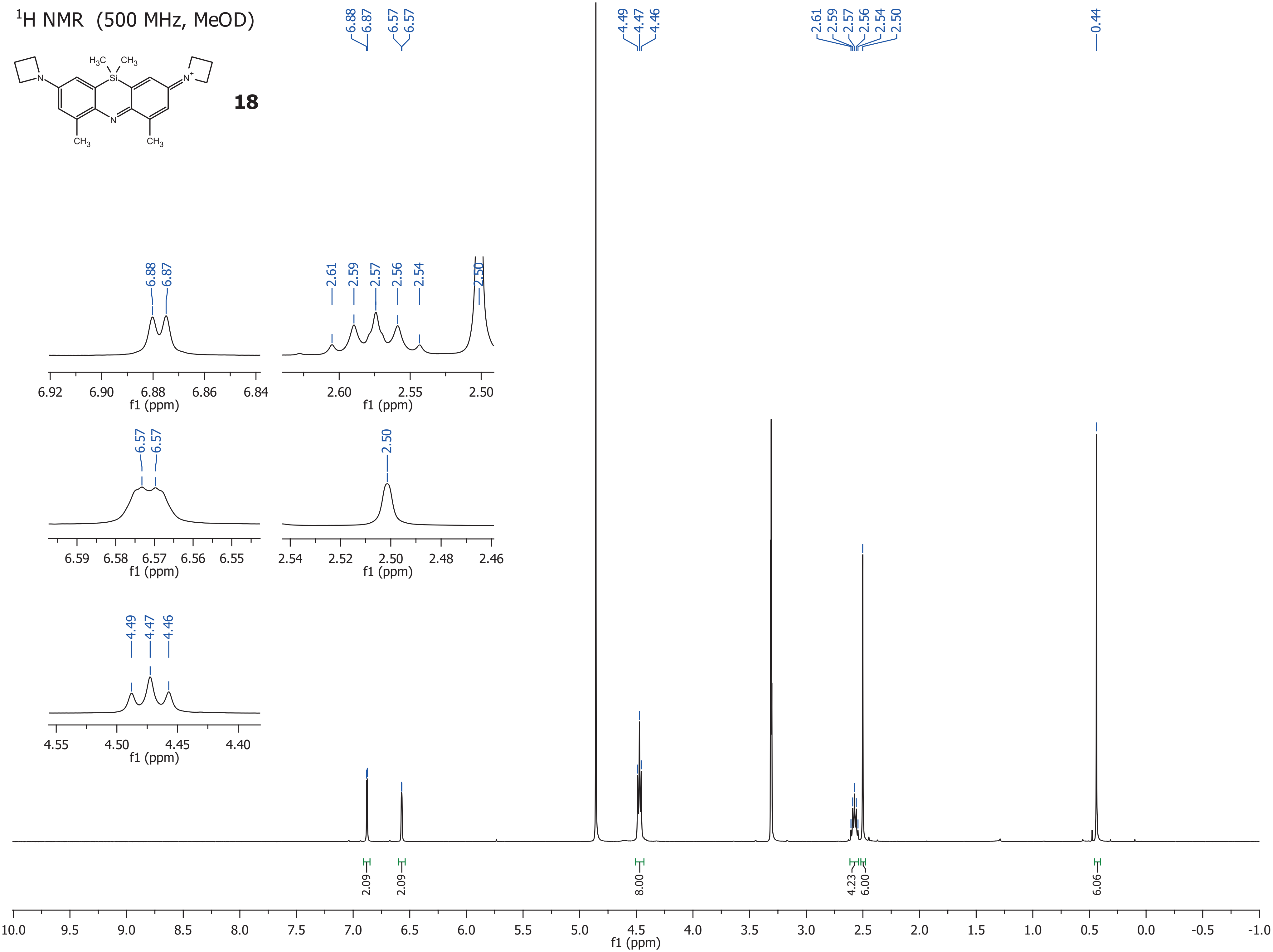


^{13}C NMR
(500 MHz, CDCl$_3$)



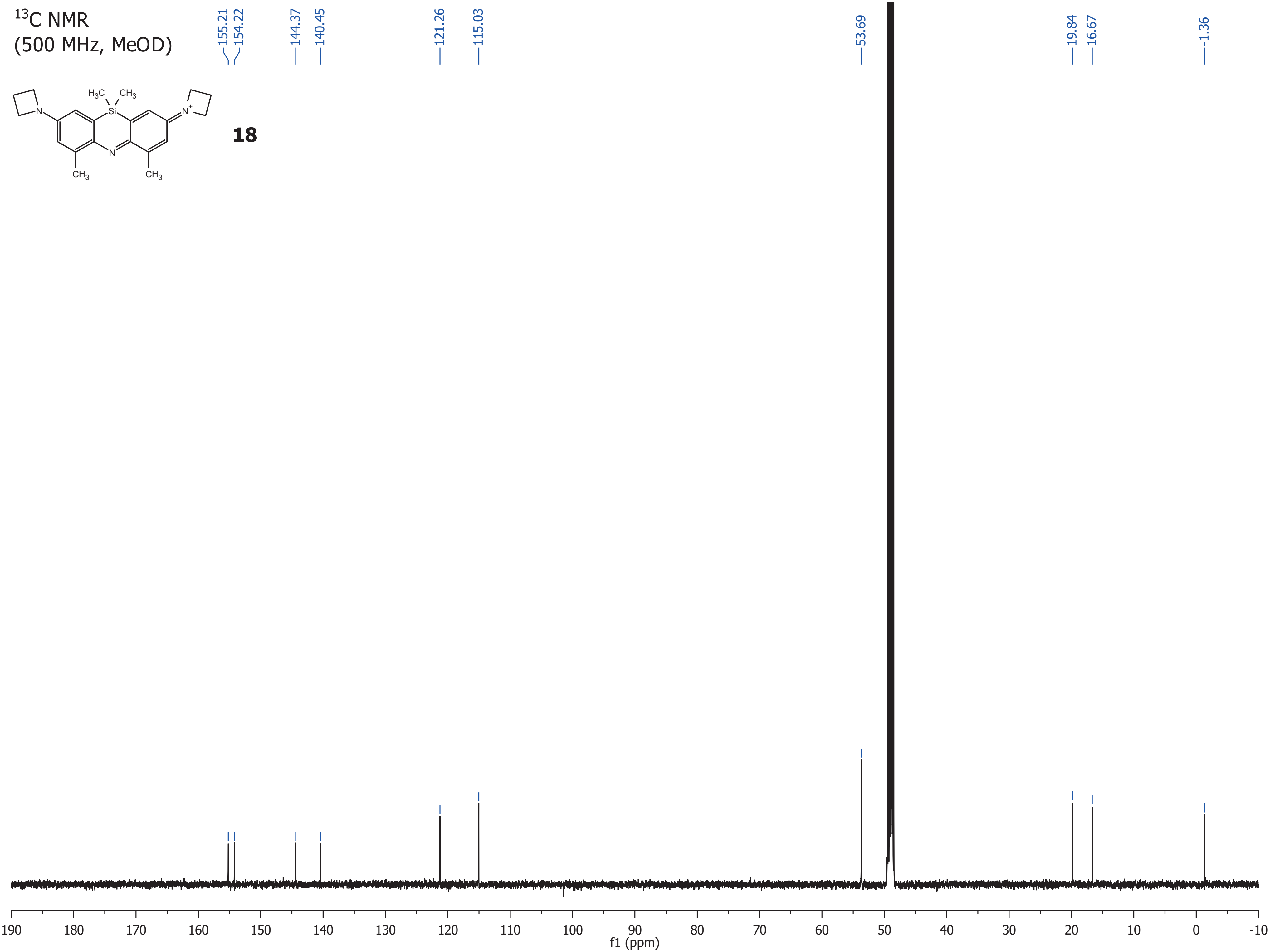
17

^{1}H NMR (500 MHz, MeOD)



18

^{13}C NMR
(500 MHz, MeOD)



18