Supplementary Information for

Fluorous-Soluble Metal Chelate for Sensitive Fluorine-19 Magnetic Resonance Imaging Nanoemulsion Probes

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1. Synthesis and Characterization

General method for perfluoroalkylation of salicylaldehydes. The overall synthetic scheme is displayed in Figure 1 of the manuscript. The salicylaldehyde (10 mM), perfluoroalkyl iodide (20 mM) and anhydrous cesium carbonate (40 mM) were heated in dry DMF (50 mL) at 100 °C overnight with vigorous stirring under a dry N_2 atmosphere. The dark reaction mixture was cooled and cautiously poured into ~4M HCl (100 mL), and the crude product was extracted with EtOAc (3× 50 mL), dried over Na_2SO_4 and evaporated to dryness. The product was purified by silica gel chromatography eluted with EtOAc-hexane (1:50 to 1:10 ratio).

3-Bromo-5-perfluorohexyl-salicylaldehyde, 2a. Prepared from 3-bromosalicylaldehyde (**1a**) and perfluorohexyl iodide. Purified by silica gel chromatography eluted with 5 % EtOAc-hexane to give a white solid. Yield, 63%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.98 (s, 1H), 9.96 (s, 1H), 8.00 (s, 1H), 7.82 (s, 1H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -80.94, -110.22 – -110.71 (m), -121.60 (dt, *J* = 50.9, 15.6 Hz), -122.95, -126.31 (d, *J* = 15.1 Hz). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 195.51, 160.98, 137.77, 131.77, 121.77 (t, *J* = 26.1 Hz), 120.67, 112.49. MS (m/z, ESI-TOF) for $[M-H]^+$ calculated 516.9114, found 516.9110.

5-Bromo-3-perfluorohexyl-salicylaldehyde, 2b. Prepared from 5-bromosalicylaldehyde (**1b)** and perfluorohexyl iodide. Purified by silica gel chromatography eluted with 5 % EtOAc-hexane to give a white solid. Yield, 75%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.74 (s, 1H), 9.90 (s, 1H), 7.90 (s, 1H), 7.85 (s, 1H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -80.95 (d, *J* = 25.4 Hz), -108.48 – -110.07 (m), -121.46 (d, *J* = 113.9 Hz), -121.94, -122.91, -126.32. ^{13}C NMR (101 MHz, Chloroform-*d*) δ 195.33, 159.75, 140.25, 139.00, 122.65, 119.25 (t, *J* = 24.1 Hz), 111.05. MS (m/z, ESI-TOF) for $[M-H]^+$ calculated 516.9114, found 516.9112.

5-Perfluorohexyl-salicylaldehyde, 3a *(route 2).* **2a**, (3.2 g, 6.3 mmol) was dissolved in MeOH (100 ml), sodium acetate (0.57 g, 7 mmol) and Pd-C (5%, 50 mg), and the mixture was hydrogenated at room temperature and pressure until uptake was complete (30 min). The reaction mixture was filtered through Celite and evaporated to dryness, dissolved in EtOAc (50 ml) and washed with water (3× 25 ml). The organic layer was dried (Na_2SO_4) and evaporated to give the product as a white solid. Yield, 2.71 g (98%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.32 (s, 1H), 9.95 (s, 1H), 7.80 (s, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.12 (d, *J* = 8.8 Hz, 1H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -80.75 (t, *J* = 10.2 Hz), -110.24 (t, *J* = 14.0 Hz), -121.64 (d, *J* = 145.9 Hz), -122.83, -126.14. ^{13}C NMR (101 MHz, Chloroform-*d*) δ 196.12, 164.36, 134.97, 133.01, 120.34, 118.90, 122-108 (m, weak). MS (m/z, ESI-TOF) for $[M-H]^+$ calculated 439.0009, found 439.0010.

3-Perfluorohexyl-salicylaldehyde, 3b *(route 2).* Prepared from **2b** using the procedure used for **3a** to give **3b** as a white solid, yield 100%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.83 (s, 1H), 9.94 (s, 1H), 7.76 (dd, *J* = 10.7, 8.0 Hz, 2H), 7.14 (t, *J* = 7.8 Hz, 1H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -80.81 (d, *J* = 8.2 Hz), -109.01 (t, *J* = 14.7 Hz), -121.65

(d, *J* = 144.0 Hz), -122.80, -126.19. ^{13}C NMR (101 MHz, Chloroform-*d*) δ 196.55, 160.96, 138.27, 136.58, 121.55, 119.58, 122-108 (m, weak). MS (m/z, ESI-TOF) for $[M-H]^+$ calculated 439.0009, found 439.0008.

3,5-Bis-perfluorohexyl-salicylaldehyde, 3c *(route 1).* Prepared from salicylaldehyde, **1c** and perfluorohexyl iodide (3 equivalent). Purified by silica gel chromatography eluted with 15% EtOAc-hexane producing a white solid. Yield, 68%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.21 (s, 1H), 10.02 (s, 1H), 8.03 (s, 1H), 7.93 (s, 1H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -80.96 (d, *J* = 7.7 Hz), -109.49 (t, *J* = 15.5 Hz), -110.56 – -111.15 (m), -121.54 (q, *J* = 17.7, 16.6 Hz), -121.94 (t, *J* = 16.8 Hz), -122.93, -126.31 (d, *J* = 14.3 Hz). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 195.76, 163.30, 136.71 (t, J = 6.9 Hz), 134.61 (t, J = 8.4 Hz), 121.31, 120.64 (t, J = 24.2 Hz), 118.52 (t, J = 22.4 Hz), 120-108 (m, weak). MS (m/z, ESI-TOF) for $[M-H]^+$ calculated 756.9723, found 756.9719.

5-Methyl-3-perfluorohexyl-salicylaldehyde, 3d *(route 1).* Prepared from 5-methylsalicylaldehyde (1d) and perfluorohexyl iodide. Purified by short pathlength distillation 90-120 °C at 100 mTorr to give a yellow-brown oil. Yield, 75%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.62 (s, 1H), 9.90 (s, 1H), 7.48-7.62 (m, 2H), 2.40 (s, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -80.82 (t, *J* = 10.7 Hz), -108.92 (t, *J* = 15.6 Hz), -121.44 (d, *J* = 16.4 Hz), -121.87 (t, *J* = 14.7 Hz), -122.81 (d, *J* = 15.9 Hz), -126.18 (d, *J* = 16.9 Hz). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 196.47, 158.75 (t, J = 2.7 Hz), 138.22, 137.07 (t, J = 7.9 Hz), 133.56, 129.12, 121.31, 20.32. MS (m/z, ESI-TOF) for $[M-H]^+$ calculated 453.0166, found 453.0164.

3-Methyl-5-perfluorooctyl-salicylaldehyde, 3f *(route 1).* Prepared from 3-methylsalicylaldehyde (1f) and perfluorooctyl iodide. Purified by short pathlength distillation 135 °C at 100 mTorr to give a yellow-brown oil that slowly solidified. Yield, 78%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.59 (s, 1H), 9.94 (s, 1H), 7.66 (s, 1H), 7.57 (s, 1H), 2.34 (s, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -80.72 (t, *J* = 9.5 Hz), -110.15 (t, *J* = 14.5 Hz), -121.08 – -121.42 (m), -121.54 – -122.10 (m), -122.70, -126.11 (t, *J* = 14.3 Hz). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 196.27, 162.72, 135.16 (t, J = 6.2 Hz), 130.50 (t, J = 6.8 Hz), 128.57, 120.11(t, J =25.2 Hz), 119.52, 15.40. MS (m/z, ESI-TOF) for $[M-H]^+$ calculated 553.0102, found 553.0099.

1,1,1-(Tris(3'(5')-perfluorosalicylidene)methyl)-ethane, 4a. 2-(Aminomethyl)-2-methyl-1,3-propanediamine trihydrochloride (226 mg, 1 mmol) was added to a solution of **3a** (0.88 g, 2 mmol), **3b** (0.44 g, 1 mmol) and triethylamine (0.46 ml, 3.33 mmol) in absolute EtOH (10 ml). The mixture was refluxed for 4 h until a yellow solution was formed, then cooled and evaporated to dryness. The residue was dissolved in EtOAc/water (1:1 v/v, 100 ml), separated and the EtOAc layer washed with water (2× 25 ml). The combined organic layers were dried (Na_2SO_4) to give the crude product as a mixture of isomers that was used without further purification. For analysis, a small amount these intermediates was separated by HPLC on a C18 reverse-phase column (Luna-2, Phenomenex, Torrance, CA) using isocratic elution with acetonitrile.

1,1,1-(Tris(5'-perfluorohexylsalicylidene)methyl)-ethane, 4a *PPP* (R_2, R_4, R_6 = H; R_1, R_3, R_5 = perfluorohexyl). ^{1}H NMR (400 MHz, Methylene Chloride-d_2) δ 13.87 (s, 3H), 8.46 (s, 3H), 7.53 (d, *J* = 7.5 Hz, 6H), 7.07 (d, *J* = 9.3 Hz, 3H), 3.71 (s, 6H), 1.20 (s, 3H). ^{19}F NMR (376 MHz, Methylene Chloride-d_2) δ -81.16 (d, *J* = 8.6 Hz), -110.04 (t, *J* = 13.7 Hz), -121.74, -122.15, -123.09, -126.43.

1,1,1-(Bis(5'-perfluorohexylsalicylidene)(3'-perfluorohexylsalicylidene) methyl)-ethane, 4a *POP* (R_2, R_3, R_6 = H; R_1, R_4, R_5 = perfluorohexyl). ^{1}H NMR (400 MHz, Methylene Chloride-d_2) δ 14.52 (s, 1H, OH of ortho), 13.86 (s, 2H, OH of para), 8.46 (d, *J* = 8.3 Hz, 3H), 7.53 (q, *J* = 8.8, 7.9 Hz, 6H), 7.18 – 6.92 (m, 3H), 3.71 (d, *J* = 13.7 Hz, 6H), 1.20 (s, 3H). ^{19}F NMR (376 MHz, Methylene Chloride-d_2) δ -81.18 (t, *J* = 9.8 Hz), -108.82 (t, *J* = 14.5 Hz), -110.06 (t, *J* = 13.4 Hz), -120.24 – -125.45 (m), -126.45.

1,1,1-(Bis(3'-perfluorohexylsalicylidene)(5'-perfluorohexylsalicylidene) methyl)-ethane, 4a *OOP* (R_2, R_3, R_5 = H; R_1, R_4, R_6 = perfluorohexyl). ^{1}H NMR (400 MHz, Methylene Chloride-d_2) δ 14.55 (s, 2H, OH of ortho), 13.89 (s, 1H, OH of para), 8.52 (d, *J* = 4.7 Hz, 3H), 7.64 – 7.52 (m, 6H), 7.14 – 6.99 (m, 3H), 3.75 (s, 6H), 1.23 (s, 3H). ^{19}F NMR (376 MHz, Methylene Chloride-d_2) δ -81.18, -108.81 (t, *J* = 14.3 Hz), -110.07 (t, *J* = 13.1 Hz), -120.31 – -125.21 (m), -126.43.

1,1,1-(Tris(3'-perfluorohexylsalicylidene)methyl)-ethane, 4a *OOO* (R_1, R_3, R_5 = H; R_2, R_4, R_6 = perfluorohexyl). ^{1}H NMR (400 MHz, Methylene Chloride-d_2) δ 14.49 (s, 3H, OH), 8.49 (s, 3H, CH=N), 7.53 (dd, *J* = 19.4, 7.7 Hz, 6H), 7.00 (t, *J* = 7.7 Hz, 3H), 3.72 (s,

6H), 1.19 (s, 3H). ^{19}F NMR (376 MHz, Methylene Chloride-d_2) δ -81.11 (dt, J = 20.6, 10.0 Hz), -108.80 (t, J = 14.0 Hz), -120.23 – -124.17 (m), -126.40.

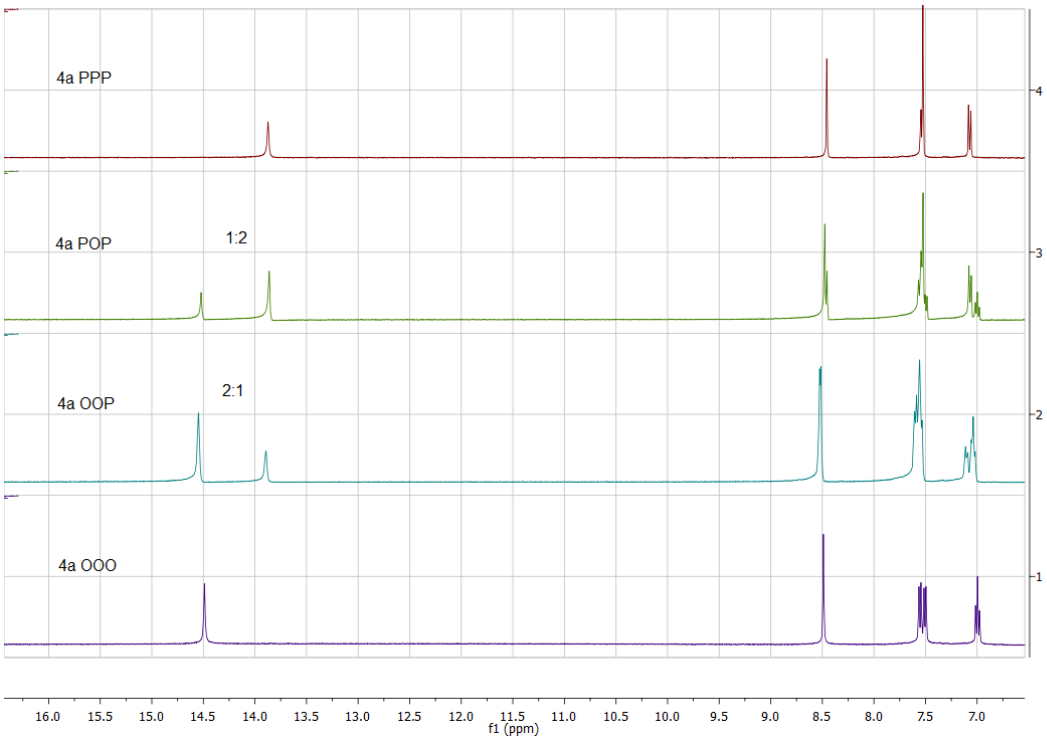


Figure S1. A comparison of ^{1}H NMR spectra of 4a isomers in the aromatic region.

Preparation of Fe SALTAME isomers (5a *PPP*, *POP*, *OOP*, and *OOO*). A solution of **3a** (0.78 g, 1.77 mmol) in absolute EtOH (5 ml) was added dropwise to a stirred suspension of 2-(aminomethyl)-2-methyl-1,3-propanediamine, trihydrochloride (210 mg, 0.88 mmol) and triethylamine (0.49 ml, 3.52 mmol) in absolute EtOH (10 ml) at 80 °C. **3b** (387 mg, 0.88 mmol) dissolved in 2.5 ml EtOH was then added and further heated for 30 min, followed by the addition of a solution of anhydrous ferric chloride (162 mg, 1 mmol), then anhydrous sodium acetate (0.24 g, 3 mmol) in absolute EtOH (5 ml). The resulting deep red reaction mixture was cooled, evaporated to dryness, dissolved in EtOAc:water (1:1 v/v, 100 ml) and separated. The aqueous layer was extracted (2× 50 ml) with EtOAc; the combined organic layers dried over Na_2SO_4 and evaporated to dryness. The products were separated by SiO_2 column chromatography eluted with 0-55% EtOAc-hexane. Three distinct, red products were collected:

i. **5a Fe *PPP*** eluted with 20% EtOAc-hexane. Yield, 228 mg (18%). ^{19}F NMR (376 MHz, Methanol-d_4) δ -82.31, -108.47, -117.87, -123.11, -127.14. MS (m/z, ESI-TOF) for $[M+H]^+$ calculated 1437.0383, found 1437.0405.

ii. **5a Fe *POP*** eluted with 40% EtOAc-hexane. Yield, 346 mg (27%). ^{19}F NMR (376 MHz, Methanol-d_4) δ -82.31, -108.92, -114.90 – -130.03 (m). MS (m/z, ESI-TOF) for $[M+H]^+$ calculated 1437.0383, found 1437.0400.

iii. **5a Fe *OOP*** eluted with 50% EtOAc-hexane. Yield, 183 mg (14%). ^{19}F NMR (376 MHz, Methanol-d_4) δ -82.22 (d, J = 98.2 Hz), -117.04 – -124.61 (m), -125.89, -127.17. MS (m/z, ESI-TOF) for $[M+H]^+$ calculated 1437.0383, found 1437.0397.

Only trace amount of **5a *OOO*** was observed on TLC. **5a *OOO*** was also prepared by using **3b** (1.16 g, 2.65 mmol) without adding **3a**.

5a Fe *OOO*: ^{19}F NMR (376 MHz, Methanol-d_4) δ -82.04, -121.06, -125.89. MS (m/z, ESI-TOF) for $[M+H]^+$ calculated 1437.0383, found 1437.0407.

Compound **5a Fe *PPP*** can also be obtained using **3a** (1.16 g, 2.65 mmol) only. The independent preparation of **5a Fe *OOO*** and **5a Fe *PPP*** helps assign the four isomers on TLC.

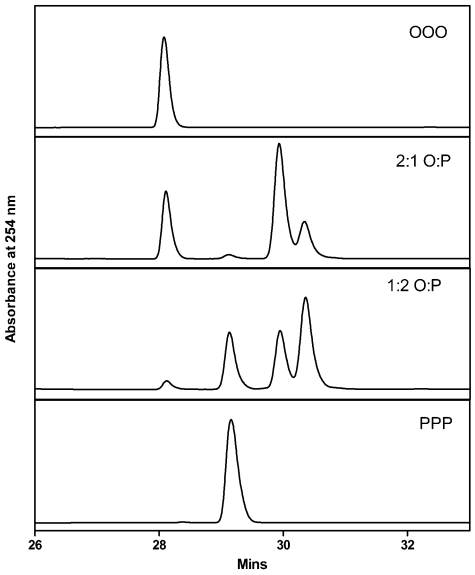


Figure S2. Identification of **5a Fe *OP*** isomers from HPLC product profiles of reactions using either **3a** (O), 2:1 **3a:3b**, 1:2 **3a:3b**, or **3b** (P).

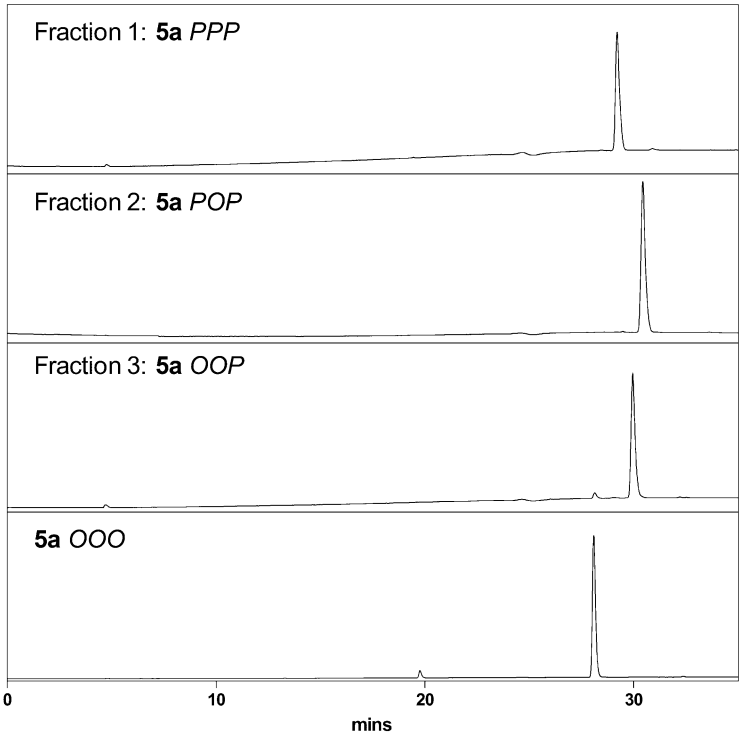


Figure S3. Purity of column fractions (1-3) in separation of **5a Fe** isomers by HPLC analysis and comparison with authentic **5a Fe** OOO.

Preparation of Fe SALTAMEs (5c-f). Perfluoroalkyl-substituted salicylaldehyde **3c-f** (3.3 mmol), 2-(aminomethyl)-2-methyl-1,3-propanediamine trihydrochloride (226 mg, 1 mmol) and triethylamine (0.46 ml, 3.3 mmol) in absolute EtOH (10 ml) were heated at 80 °C for 3 h. A solution of anhydrous ferric chloride (243 mg, 1.5 mmol) in absolute EtOH (5 ml) was added, followed by anhydrous sodium acetate (287 mg, 3.5 mmol) to give a red colored solution. The reaction mixture was cooled, evaporated to dryness, dissolved in EtOAc:water (20 ml / 20 ml) and separated. The aqueous layer was extracted with EtOAc (3× 20 ml); the combined organic layers were dried over anhydrous Na_2SO_4 and evaporated to dryness. The products were separated by SiO_2 column chromatography eluted with EtOAc-hexane.

5c (R_1-R_6 = perfluorohexyl) was obtained by starting with **3c** in a 70% yield. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -80.77, -81.04, -109.32, -117.57, -121.42, -122.48, -126.13. MS (m/z, ESI-TOF) for $[M+H]^+$ calculated 2412.9346, found 2412.9307.

5d (R_1, R_3, R_5 = CH_3; R_2, R_4, R_6 = perfluorohexyl) was obtained by starting with **3d** at 69% yield. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -80.82, -120.49, -122.28, -126.36. MS (m/z, ESI-TOF) for $[M+H]^+$ calculated 1479.0853, found 1479.0843.

5e (R_1, R_3, R_5 = CF_3; R_2, R_4, R_6 = H) was obtained by starting with **1e** (or **3e**) at yield of 65%. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -61.86. MS (m/z, ESI-TOF) for $[M+H]^+$ calculated 687.0862, found 687.0865.

5f (R_1, R_3, R_5 = perfluorooctyl; R_2, R_4, R_6 = CH_3) was obtained by starting with **3f** at yield of 49%. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -80.72, -105.82, -116.21, -121.24, -121.82, -122.69, -126.11. MS (m/z, ESI-TOF) for $[M+H]^+$ calculated 1779.0661, found 1779.0644.

Preparation of 5a Mn, Co and Ga SALTAME. Prepared as described above for the Fe complex, except replacing $FeCl_3$ with $MnCl_2$, $CoCl_2$ and $GaCl_3$, to form brown, green and colorless reaction mixtures, respectively, that were isolated as isomer mixtures (predominately ***POP*** and ***OOP*** in approximately equal amounts for Co and Ga but one major isomer for Mn) by silica gel chromatography. **Ga *POP*** and ***OOP*** complexes were separated by preparative HPLC. Complexes formed with other Period 4 metals were detected by LC-MS or, if unstable to the acidic LC conditions, by MS only, if not isolatable by silica gel chromatography.

5a Ga *OOP* ^{1}H NMR (400 MHz, Methylene Chloride-d_2) δ 11.92 (s, 1H), 11.39 (s, 1H), 10.00 (s, 1H), 8.32 – 8.16 (m, 2H), 7.88 (d, *J* = 8.6 Hz, 1H), 7.79 (dd, *J* = 16.6, 8.4 Hz, 1H), 7.52 – 7.41 (m, 2H), 7.26 – 7.16 (m, 1H), 6.92 – 6.66 (m, 2H), 4.26 (dt, *J* = 27.8, 14.2 Hz, 3H), 3.65 – 3.43 (m, 3H), 1.38 – 1.08 (m, 3H). ^{19}F NMR (376 MHz, Methylene Chloride-d_2) δ -76.34, -79.87 – -82.57 (m), -107.68 – -111.95 (m), -120.11 – -124.32 (m), -126.41. MS (m/z, ESI-TOF) for $[M+H]^+$ calculated 1450.0289, found 1450.0281.

5a Ga *POP* ^{1}H NMR (400 MHz, Methylene Chloride-d_2) δ 8.29 – 8.06 (m, 3H), 7.56 – 7.21 (m, 6H), 6.80 – 6.55 (m, 3H), 4.26 – 4.07 (m, 3H), 3.48 (t, *J* = 16.5 Hz, 3H), 1.19 (s, 3H). ^{19}F NMR (376 MHz, Methylene Chloride-d_2) δ -81.08 – -81.66 (m), -107.56 – -111.35 (m), -121.14 – -123.63 (m), -126.60 (d, *J* = 61.7 Hz). ^{13}C NMR (101 MHz, Methylene Chloride-d_2) δ 172.02, 169.75, 169.68, 169.56, 168.03, 139.06, 138.10, 135.57, 135.48, 135.21, 133.05, 132.45, 123.88, 120.99, 120.57, 120.32, 118.95, 115.12, 114.60, 114.36, 114.31,122.55 – 105.27 (m, weak), 67.27, 66.09, 65.88, 35.47, 23.43. MS (m/z, ESI-TOF) for $[M+H]^+$ calculated 1450.0289, found 1450.0282.

5a Mn^{4+} SALTAME MS (m/z, ESI) for $[M]^+$ calculated 1435.0, found 1435.0.

5a Co^{3+} SALTAME MS (m/z, ESI) for $[M+H]^+$ calculated 1440.0, found 1440.2.

2. X-ray Crystallography

Crystals of the subject compound were grown by dissolving approximately 20 mg of sample in 1 mL of perfluorooctyl bromide, which was then vapor diffused with pentane over several days. A 0.299 x 0.283 x 0.13 mm piece of a dark red crystal was mounted on a Cryoloop with Parabar oil. The single crystal X-ray diffraction data were collected on a Bruker D8 Venture kappa diffractometer equipped with a Photon 100 CMOS detector. An Iμs microfocus source provided the Mo Kα radiation (0.71073 Å) that was monochromated with multilayer mirrors. Data were collected in a nitrogen gas stream at 100(2) K. Crystal-to-detector distance was 34 mm and exposure time was 60 seconds per frame using a scan width of 0.50°. Data collection was 99.9% complete to 25.242° in θ. A total of 147,277 reflections were collected covering the indices -22 ≤ h ≤ 22, -25 ≤ k ≤ 25, and -27 ≤ l ≤ 27. A total of 27,918 reflections were found to be symmetry independent, with R_{int} = 0.0522. Indexing and unit cell refinement indicated a triclinic lattice. The space group was found to be *P-1*. The data were integrated using the Bruker SAINT software program and scaled using the SADABS software program.[1] Solution by direct methods (SHELXT)[2, 3] produced a complete phasing model consistent with the proposed structure.

All nonhydrogen atoms were refined anisotropically by full-matrix, least-squares using SHELXL-2014 software. All hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-2014. Please see additional information in the cif for explanation of disorder and the restraints and constraints that were imposed on the structure model. Crystallographic parameters are summarized in Tables S1-S3.

Table S1. Crystal data and structure refinement for 5a PPP.

Identification code	dd40g1s_sq
Empirical formula	C44 H21 F39 Fe N3 O3
Formula weight	1436.49
Temperature	100(2) K

Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/n$
Unit cell dimensions	a = 18.7587(13) Å = 90°. b = 14.9103(10) Å = 100.778(2)°. c = 41.473(3) Å = 90°.
Volume	11395.3(13) $Å^3$
Z	8
Density (calculated)	1.675 Mg/m^3
Absorption coefficient	0.433 mm^{-1}
F(000)	5,656
Crystal size	0.476 x 0.186 x 0.066 mm^3
Theta range for data collection	2.121 to 25.403°.
Index ranges	-22 ≤ h ≤ 18, -17 ≤ k ≤ 17, -49 ≤ l ≤ 49
Reflections collected	146,200
Independent reflections	20,891 [R(int) = 0.0439]
Completeness to theta = 25.242°	99.8%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7452 and 0.6602
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	20891 / 3332 / 2048
Goodness-of-fit on F^2	1.128
Final R indices [I>2sigma(I)]	R1 = 0.0699, wR2 = 0.1600
R indices (all data)	R1 = 0.0844, wR2 = 0.1677
Extinction coefficient	n/a
Largest diff. peak and hole	1.441 and -0.912 $e.Å^{-3}$

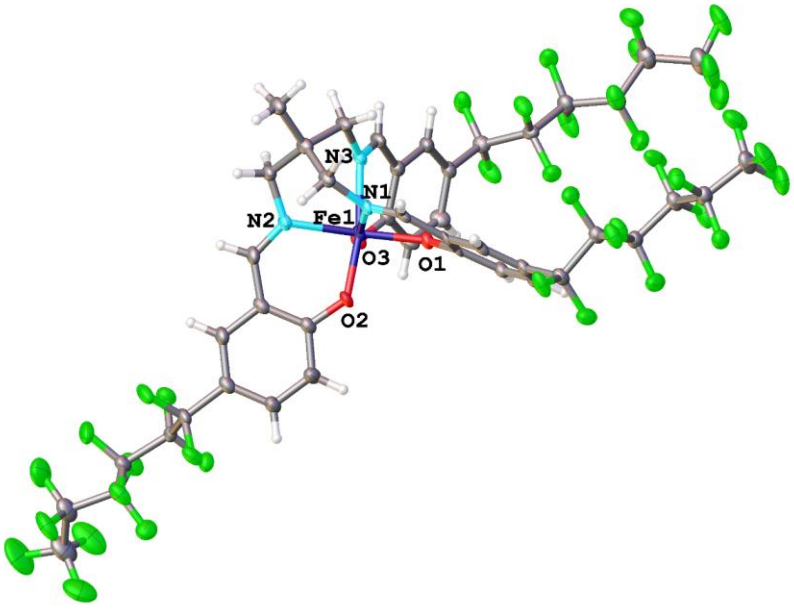


Figure S4. Structure of 5a *PPP*.

Table S2. Crystal data and structure refinement for 5a POP.

Identification code	dd39g1s	
Empirical formula	C44 H21 F39 Fe N3 O3	
Formula weight	1436.49	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 18.9287(12) Å	= 69.132(2)°.
	b = 20.9323(13) Å	= 67.049(2)°.
	c = 22.6097(13) Å	= 72.740(2)°.
Volume	7,577.4(8) $Å^3$	
Z	6	
Density (calculated)	1.889 Mg/m^3	
Absorption coefficient	0.489 mm^{-1}	
F(000)	4,242	
Crystal size	0.299 x 0.283 x 0.13 mm^3	
Theta range for data collection	2.268 to 25.443°.	
Index ranges	-22 ≤ h ≤ 22, -25 ≤ k ≤ 25, -27 ≤ l ≤ 27	
Reflections collected	147,277	
Independent reflections	27,918 [R(int) = 0.0522]	
Completeness to theta = 25.242°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7452 and 0.6127	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	27,918 / 882 / 2,590	
Goodness-of-fit on F^2	1.123	
Final R indices [I>2sigma(I)]	R1 = 0.0703, wR2 = 0.1882	
R indices (all data)	R1 = 0.0860, wR2 = 0.2052	
Extinction coefficient	0.00211(15)	
Largest diff. peak and hole	1.637 and -0.788 $e.Å^{-3}$	

Table S3. Crystal data and structure refinement for 5a OOO.

Identification code	ooo	
Empirical formula	C44.33 H21.67 Cl0.67 F39 Fe N3 O3	
Formula weight	1,464.80	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Trigonal	
Space group	R-3	
Unit cell dimensions	a = 21.9360(10) Å	= 90°.
	b = 21.9360(10) Å	= 90°.
	c = 55.501(3) Å	= 120°.

Volume	23128(2) $Å^3$
Z	18
Density (calculated)	1.893 Mg/m^3
Absorption coefficient	0.516 mm^{-1}
F(000)	12978
Crystal size	0.317 x 0.255 x 0.176 mm^3
Theta range for data collection	1.299 to 26.382°.
Index ranges	-27 ≤ h ≤ 27, -27 ≤ k ≤ 17, -69 ≤ l ≤ 68
Reflections collected	61,788
Independent reflections	10,520 [R(int) = 0.0442]
Completeness to theta = 25.000°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.1495 and 0.1220
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	10,520 / 465 / 1,068
Goodness-of-fit on F^2	1.041
Final R indices [I>2sigma(I)]	R1 = 0.0395, wR2 = 0.0887
R indices (all data)	R1 = 0.0629, wR2 = 0.1010
Extinction coefficient	n/a
Largest diff. peak and hole	0.669 and -0.536 $e.Å^{-3}$

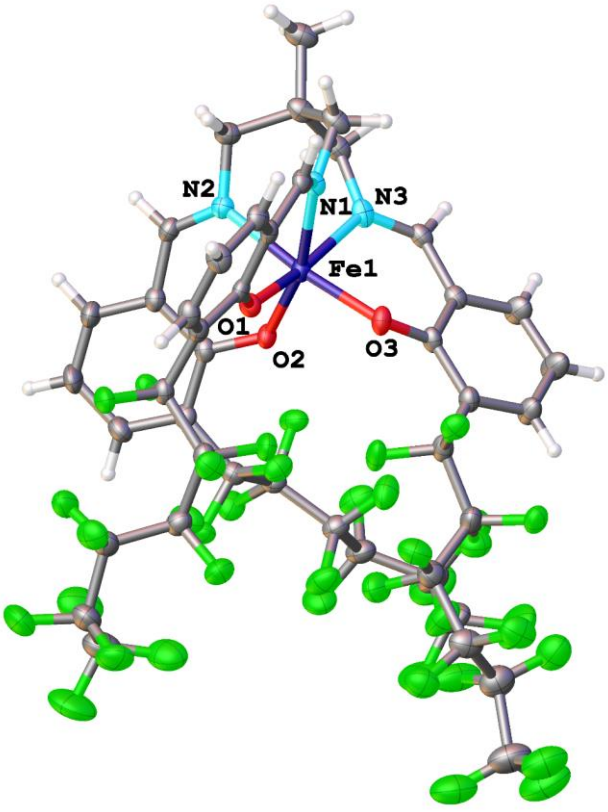


Figure S5. Structure of 5a *OOO*.

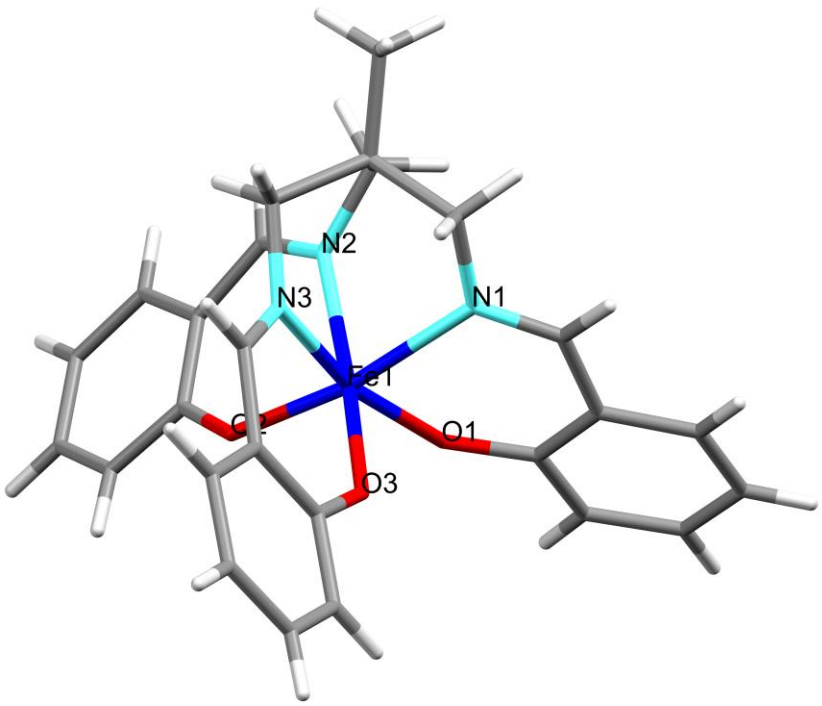


Figure S6. Structure of unsubstituted Fe SALTAME ((5, R_1-R_6=H). Data taken from Reference 5.

3. Sensitivity Modeling

Numerical simulation was used to predict the approximate ^{19}F MRI sensitivity gain using P-PFOB NE agents. With repetitive signal averaging (N_{av} is the number of acquisitions) signal is additive, whereas noise tends to diminish, thereby increasing overall image signal-to-noise ratio (SNR) by $SNR = SNR_1\sqrt{N_{av}}$, where SNR_1 is the SNR for $N_{av} = 1$. We assume a conventional spoiled gradient-echo (GRE) imaging sequence,[4] where the signal S_1 acquired per acquisition is given by

$$S_1 = \frac{(1-e^{-n})e^{-TE/T_2^*}\sin\alpha}{1-e^{-n}\cos\alpha} \quad (1)$$

where $n = TR/T_1$, TR is the repetition time, TE is the echo time, T_1 and T_2 are longitudinal and transverse relaxation times, and a is the flip angle set at the optimal Ernst angle value

given by $a = cos^{-1}(e^{-n})$. The total imaging time $t \approx N_{av}TR$. We assume $t = 1$, $T_2^* \sim T_2$, and $TR < T_1$ with a value fixed such that $TR \propto T_1$. For two different materials designated a and b with differing T_1 and T_2 values (*e.g.*, a = P-PFOB NE and b = PFOB) and using (Eq. 1), the model defines sensitivity gain G as

$$G = \frac{SNR_a}{SNR_b} = \sqrt{\frac{T_{1b}}{T_{1a}}} \frac{e^{-TE/T_{2a}}}{e^{-TE/T_{2b}}} \quad (2)$$

Simulated results are displayed in Figure 3a using empirical, magnetic field dependent relaxivities measured at 3 T and 9.4 T.

4. Nanoemulsion and Biological Studies

Nanoemulsion preparation. The fluorous phase consisted of a solution of 36 mg of Fe **5a** *POP* and 102 mg of 1-(perfluoro-n-hexyl)decane (Fluoryx) in 2.4 g PFOB (Acros, Geel, Belgium). The aqueous phase consisted of lipids, mannitol and water. A lipid solution of 139 mg egg yolk phospholipids (EYP, Sigma-Aldrich), 28 mg cholesterol (Avanti Polar Lipids, Alabaster, AL) and 3 mg 1,2-dihexadecanoyl-sn-glycero-3-phospho-L-serine, sodium salt (DPPS, Avanti) was prepared in chloroform, from which a lipid film was made and dried under high vacuum overnight. The lipid film was hydrated with 3.07 g H_2O and 90 mg mannitol was added. The aqueous phase was vortexed for 1 min and sonicated for 2 mins (Omni Ruptor 250 W, 30% power, 2 min, Omni International, Kennesaw, GA). The fluorous phase and 132 mg Cremophor (Sigma-Aldrich) were added subsequently, followed by ultrasonication for 2 mins. The crude emulsion was passed 5 times through a LV1 microfluidizer (Microfluidics, Newton, MA) operating at 20,000 psi and then filtered through a 0.2 µm Supor membrane (no. 4187, Pall, Port Washington, NY) into sterile glass vials. Nanoemulsion size characterizations (Figs. S8-S9) were performed using a dynamic light scattering (DLS) instrument (Malvern Zetasizer ZS, Malvern, PA).

***In vitro* cell labeling.** The murine macrophage cell line RAW 267.4 (TIB-71, ATCC, Manassas, VA) were maintained in Dulbecco's modified eagle media containing 10% fetal bovine serum (FBS), 100 µg/mL streptomycin and 100 U/mL penicillin at 37 ºC in 5% CO_2 atmosphere. Cells were plated in 10 cm dishes in media supplemented with 10% (v/v) FBS, and PFOB-**5a** *POP* NE, [F] = 5 mg/mL, was added. After 24 h incubation at 37 °C, cells were washed three times in phosphate-buffered saline (PBS) and resuspension in

1 mL of PBS. A portion of the cell suspension was used for cell number estimates using Cell Titer Glo assay (Promega, Madison, WI) using vendor instructions, as well as ^{19}F uptake measurements in cell pellets (Fig. S11).

Phenotype assay. Phycoerythrin conjugated Rat anti-mouse CD86 (CD86-PE) antibody was purchased from BD Bioscience (#553692, San Jose, CA). The NE-labeled and control cells were incubated with anti-CD86 antibody for 30 minutes, followed by three washes. Samples were analyzed using flow cytometry (BD ™ LSR Fortessa) and 10,000 events were recorded. As a pro-inflammatory positive control, bacterial lipopolysaccharide (LPS, Salmonella enterica, #L7770, Sigma-Aldrich) was added to RAW cells (200 ng/mL for 16 h).

Viability assay. 10-N-nonyl acridine orange (NAO) was obtained from Invitrogen (A1372, Carlsbad, CA). NE labeled and control RAW cells were stained with 100 ng/ml of NAO for 15 minutes followed by washes according to manufacturer's instructions. As a positive control, cells were stressed by incubation with 10% ethanol at 37 °C containing 5% CO_2 for 90 minutes before NAO staining. Green fluorescence from NAO dye (emission max at 520 nm) was detected using flow cytometry (LSR Fortessa, BD Biosciences).

Inflammation mouse model. Animal experiments were performed in accordance with the guidelines provided by the UCSD Institutional Animal Care and Use Committee (IACUC) and the National Institute of Health Guide for the Care and Use of Laboratory Animals. C57BL/6 mice (N=3, female, 6-8 weeks, Jackson Laboratory, Bar Harbor, ME) were anesthetized with 1.5% isoflurane and the neck was shaved. Local inflammation was induced with a 0.3 ml injection of a LPS and Matrigel (Corning, Oneonta, NY) mixture containing 800 µg LPS subcutaneously into the posterior neck area. Two hours after Matrigel implantation, a single bolus of P-PFOB NE (200 µl of 0.6% w/w emulsion, [F] = 271 mg/ml, [SALTAME] = 5.5 mM, 54.2 mg total F), was injected intravenously. $^{1}H/^{19}F$ MRI scans were performed 24 h after injection.

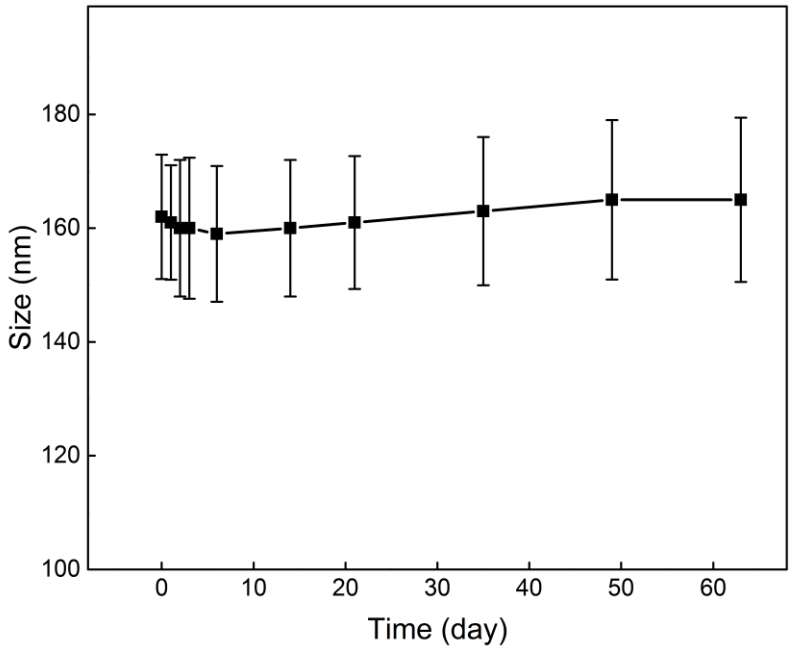


Figure S7. Dynamic light scattering (DLS) characterization of P-PFOB NE ([Fe^{3+}-SALTAME **5**a POP] = 20 mM in PFOB) at 4 °C. Full height of error bars represents polydispersity index (PDI).

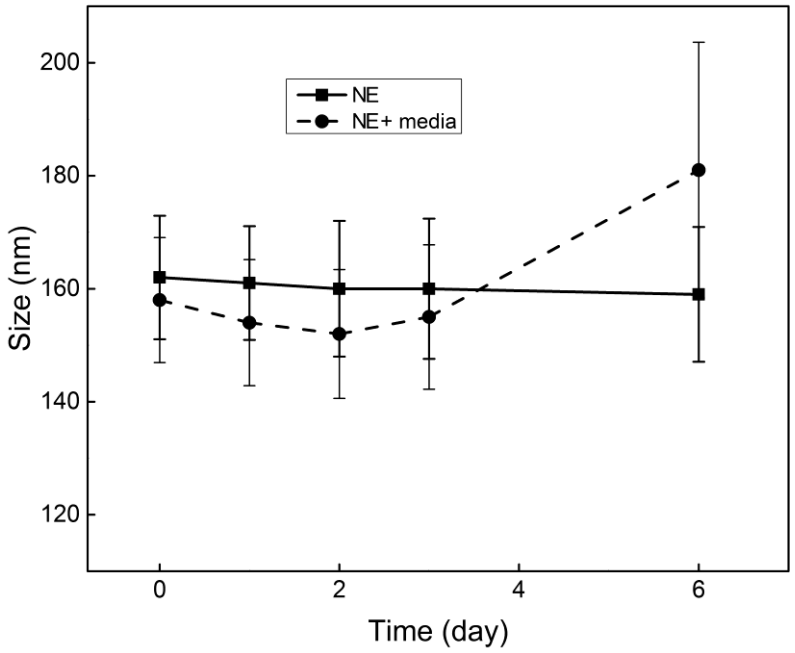


Figure S8. NE stability in the presence of proteinaceous media. Here, DLS measurements were made with P-PFOB NE ([Fe^{3+}-SALTAME **5**a POP] = 20 mM in PFOB) in PBS solution with or without 10% fetal bovine serum (NE+media and NE,

respectively) and stored at 37 °C. Full height of error bars represents polydispersity index (PDI).

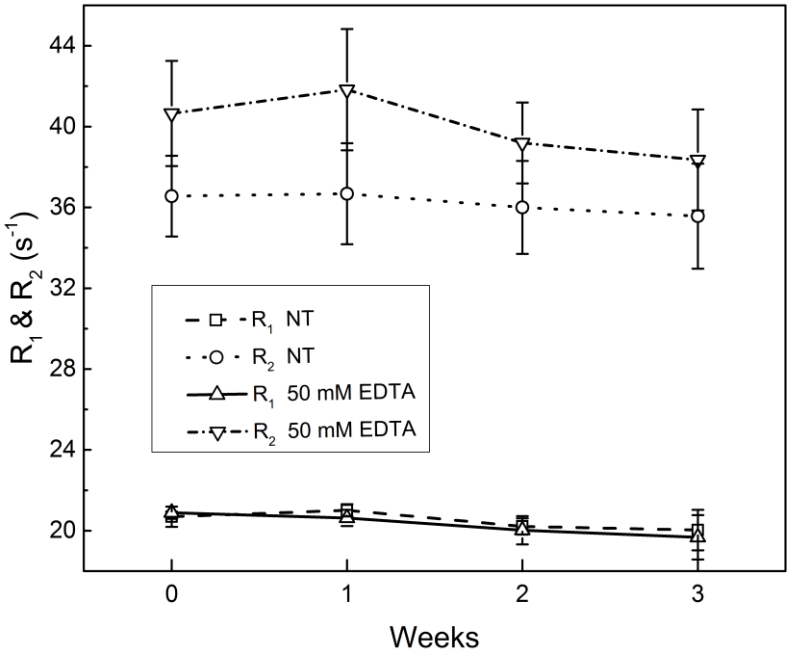


Figure S9. Stability of P-PFOB NE ([Fe^{3+}-SALTAME **5**a POP] = 20 mM in PFOB) over time in the presence of EDTA, a competing iron chelate in the aqueous phase. The P-PFOB NE was treated with 50 mM EDTA or with no treatment (NT). Shown are ^{19}F $R_1 = 1/T_1$ and $R_2 = 1/T_2$ values of 6 middle CF2 units of PFOB over a period of 3 weeks. Error bars are standard deviations from three independent replicates. The changes in R_1 and R_2 values upon addition of EDTA are not statistically significant ($p>0.05$).

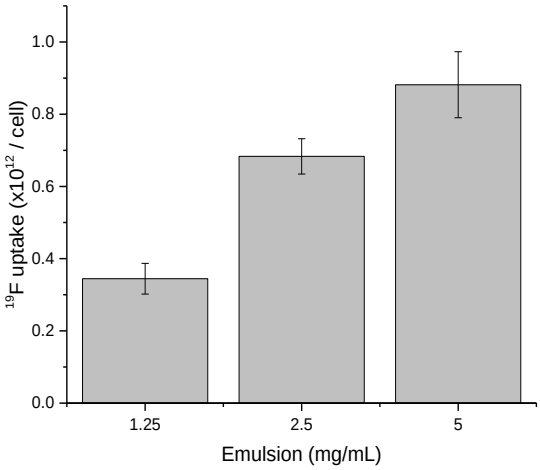
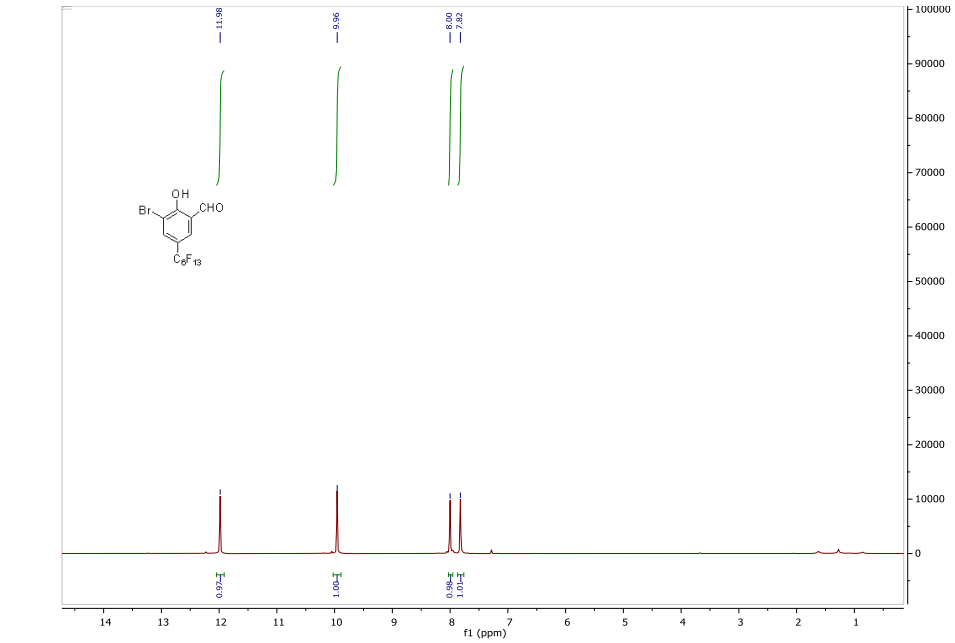
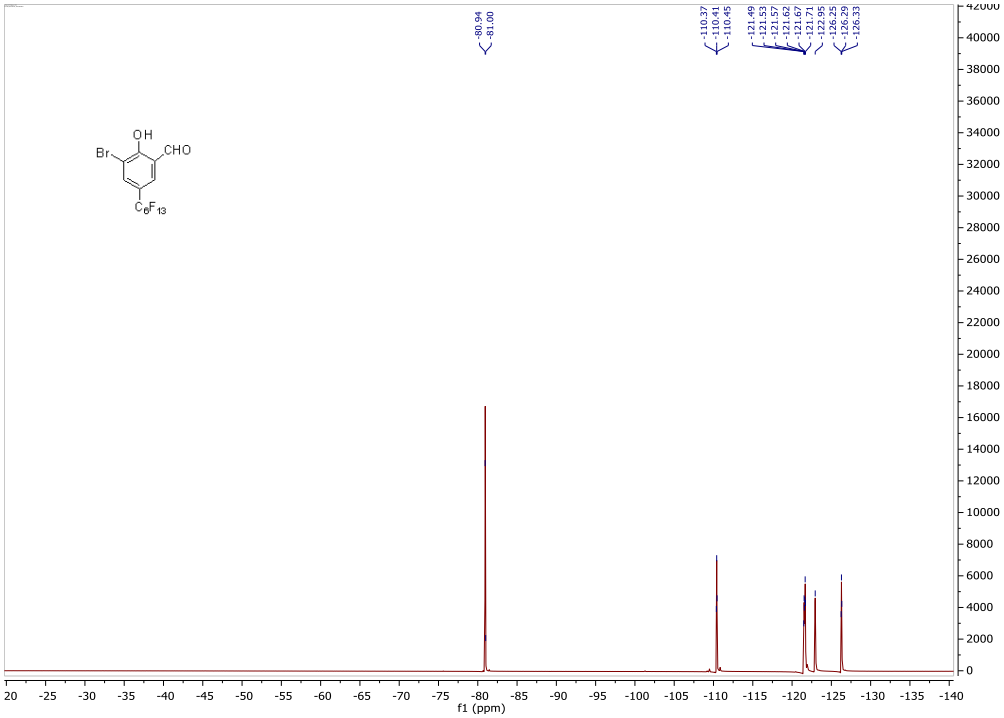


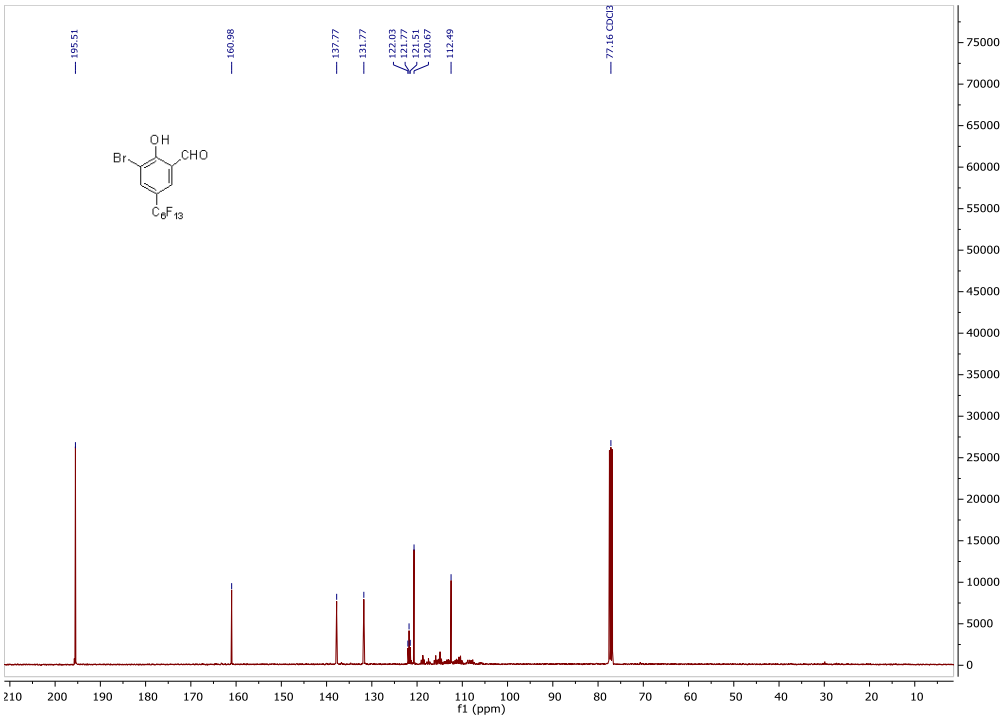
Figure S10. Cellular uptake of NE, as measured by ^{19}F NMR. RAW cells were labelled in culture for 16 h, using P-PFOB NE ([Fe^{3+}-SALTAME **5**a POP] = 20 mM in PFOB). Error bars represent standard error of mean from three independent experiments.

5. NMR Spectra

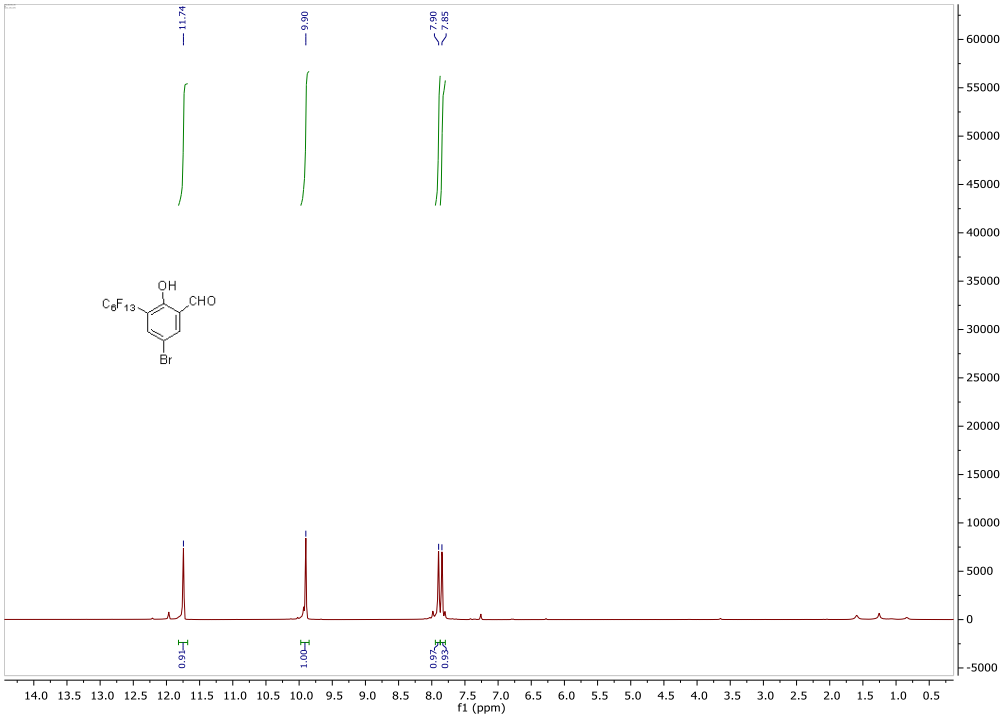
3-Bromo-5-perfluorohexyl-salicylaldehyde (2a)

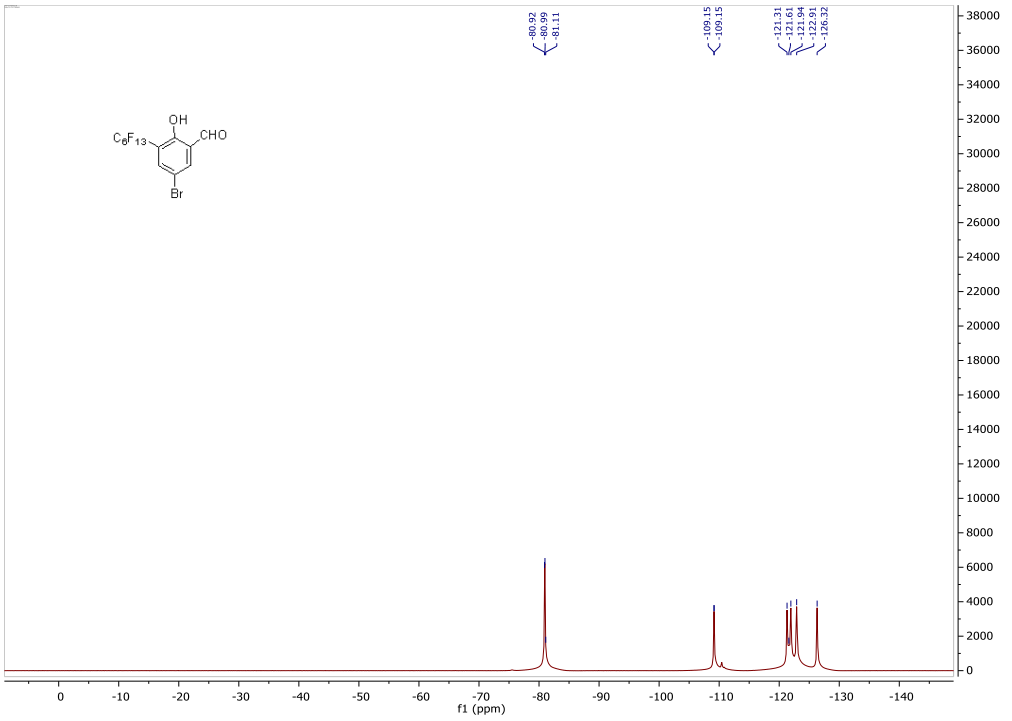






5-Bromo-3-perfluorohexyl-salicylaldehyde (2b)

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80.99
81.11
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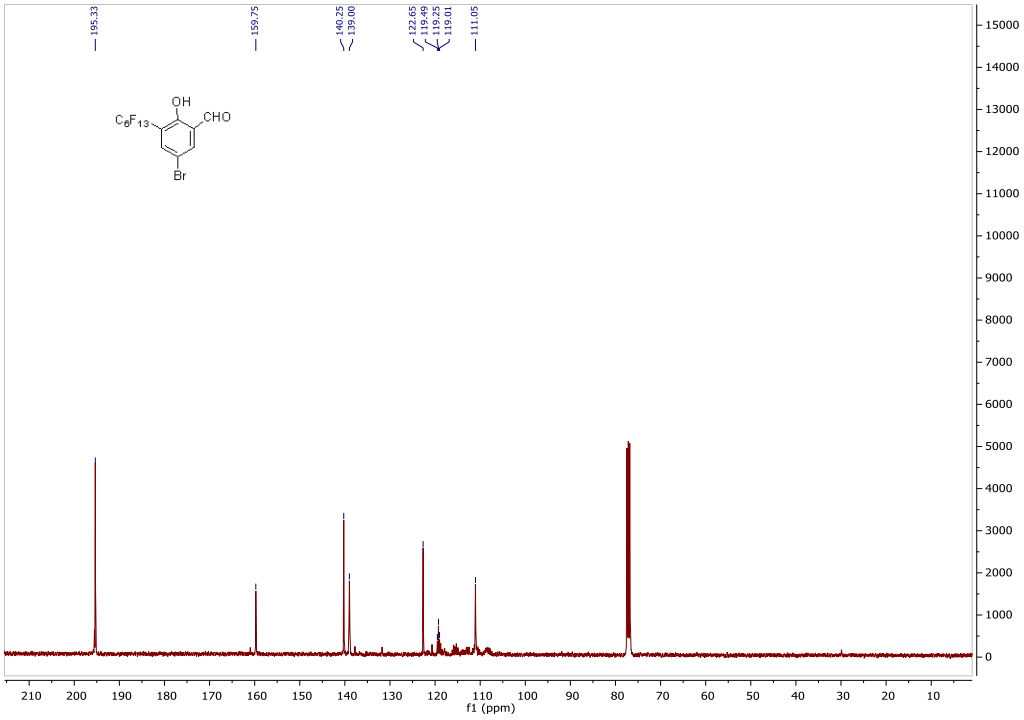
109.15
121.31
121.64
121.94
122.91
126.32
OH
CHO
C_6F_{13}
Br
f1 (ppm)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139.00
122.65
119.49
119.25
119.01
111.05
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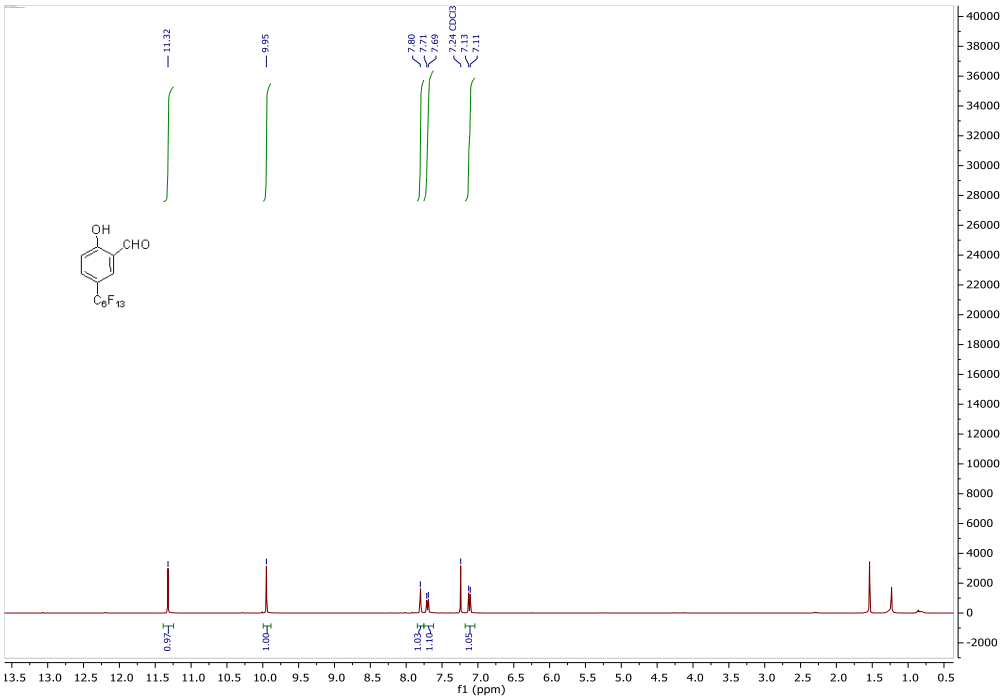
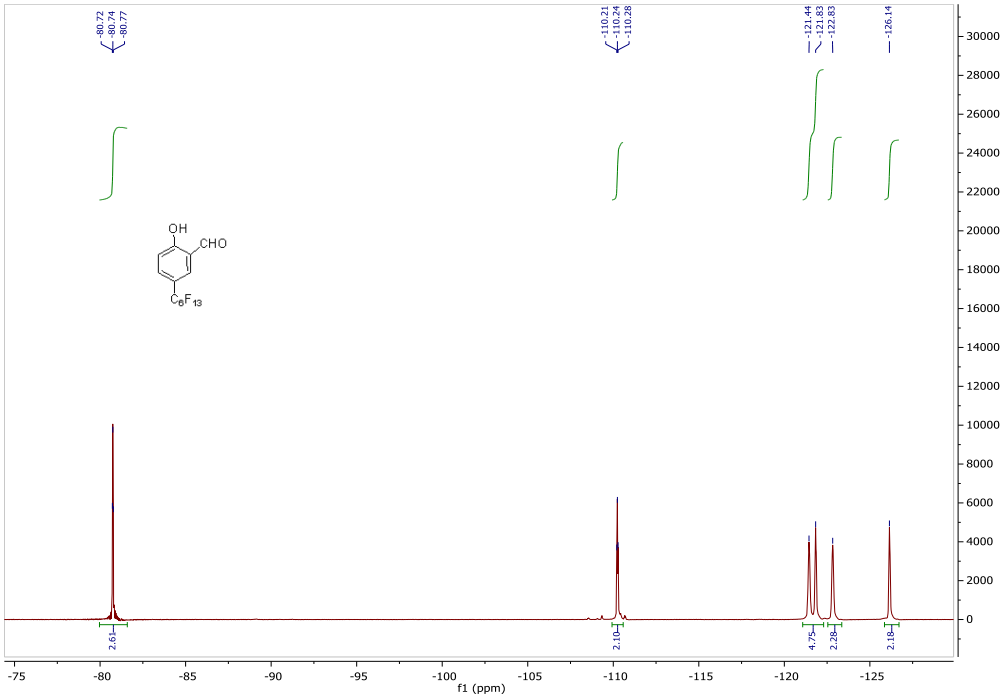
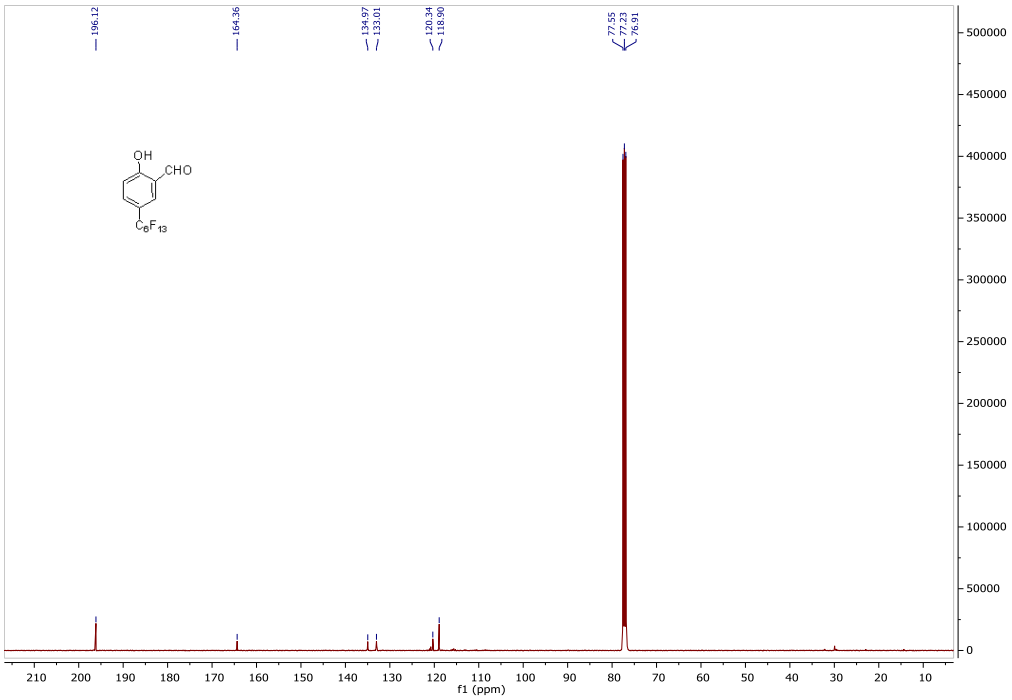
CHO
C_6F_{13}
Br
f1 (ppm)

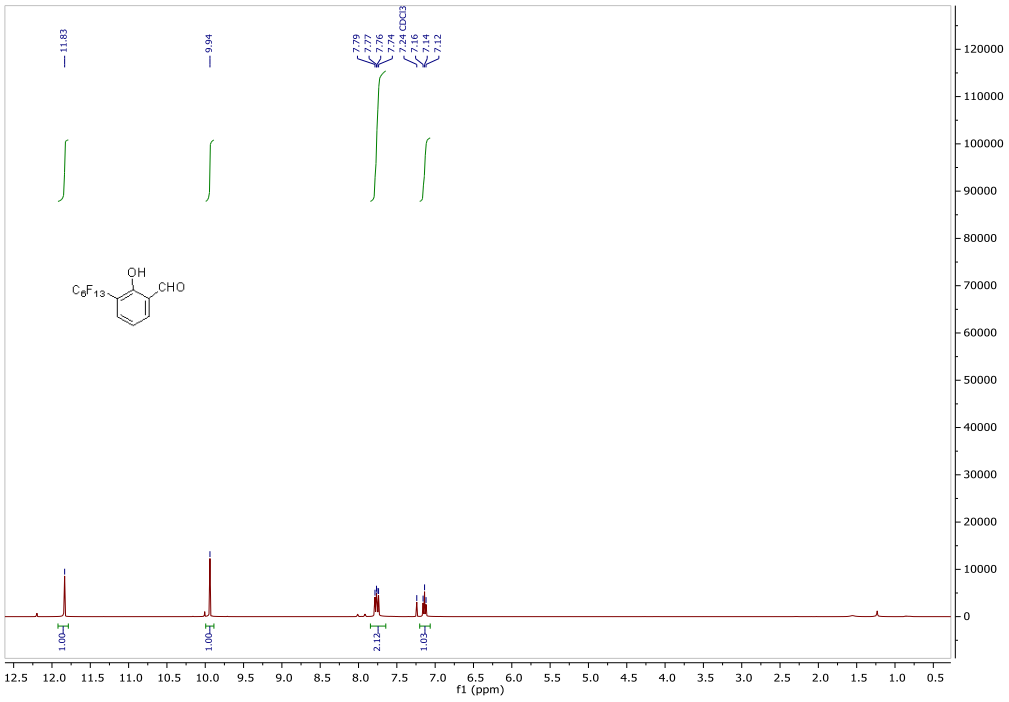
5-Perfluorohexyl-salicylaldehyde (3a)

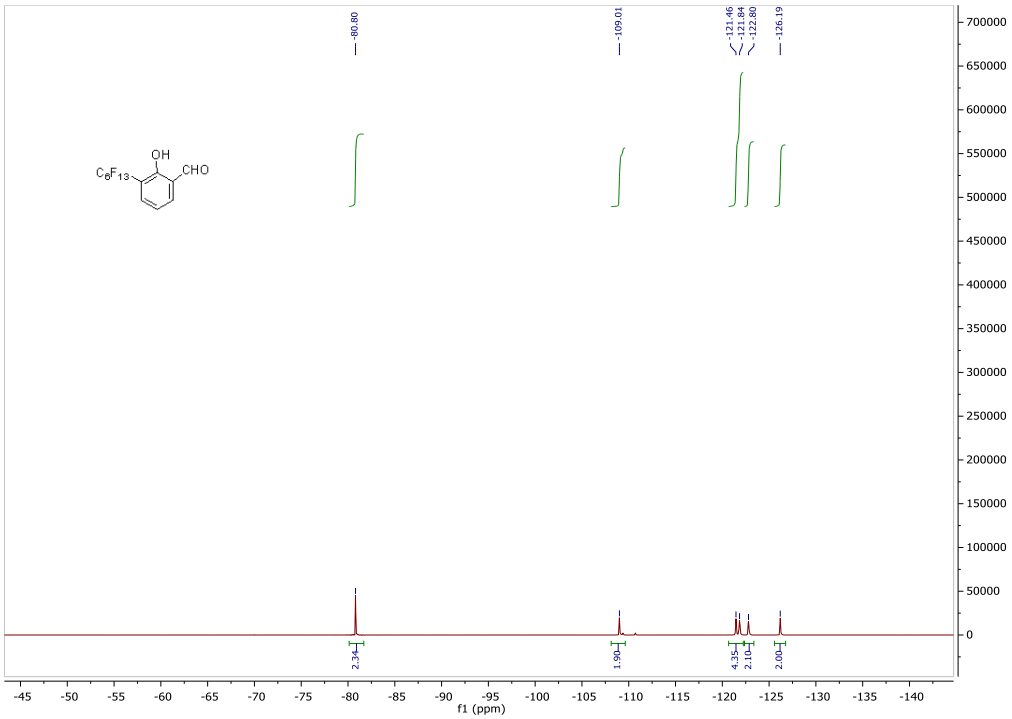






3-Perfluorohexyl-salicylaldehyde (3b)

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-121.46
-121.84
-122.80
-126.19
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CHO
C_6F_{13}
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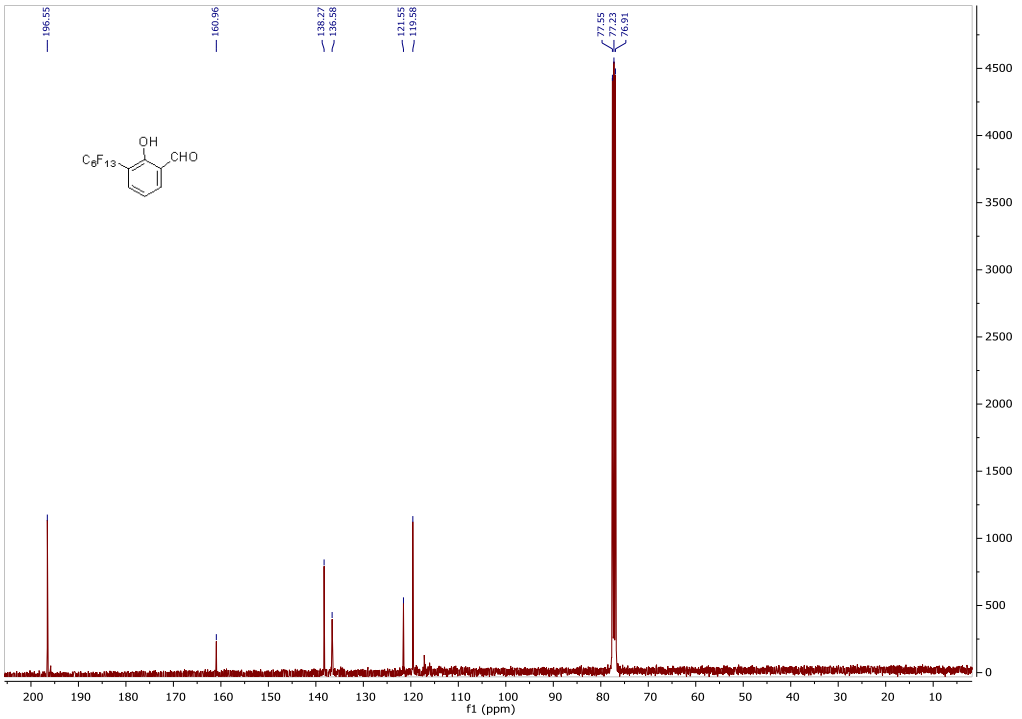
1.90
4.35
2.10
2.00
f1 (ppm)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160.96
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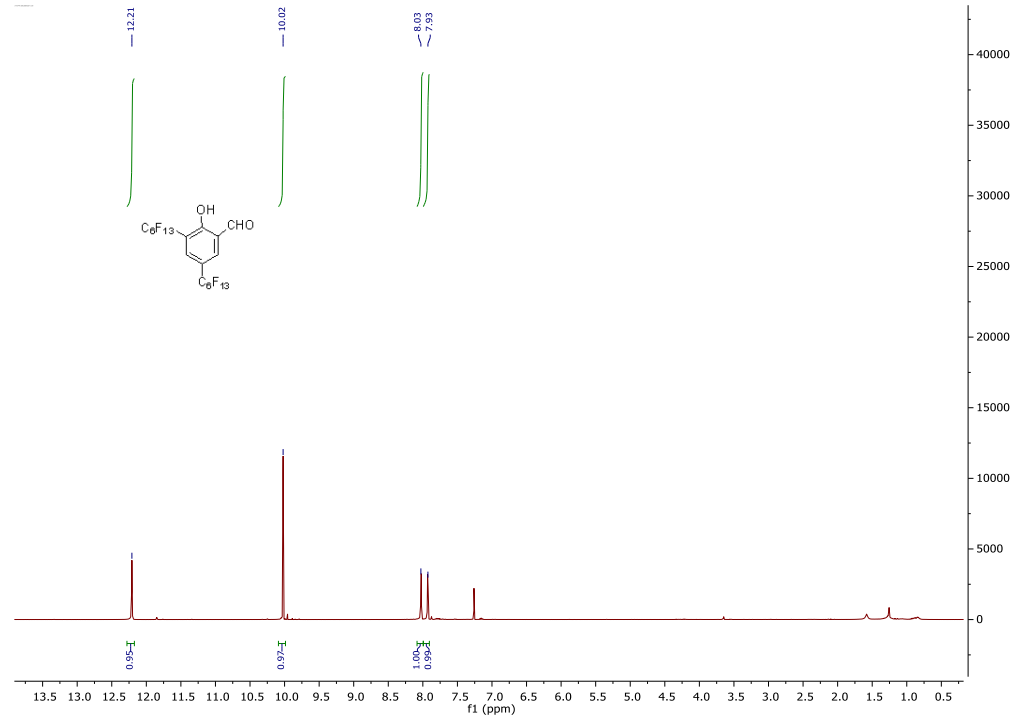
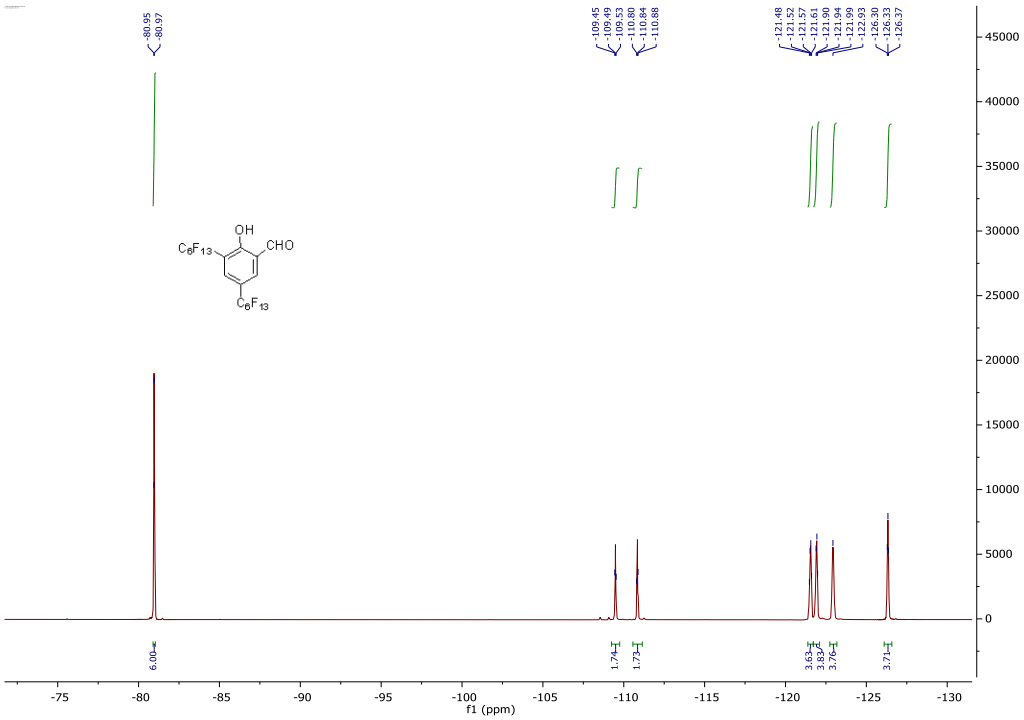
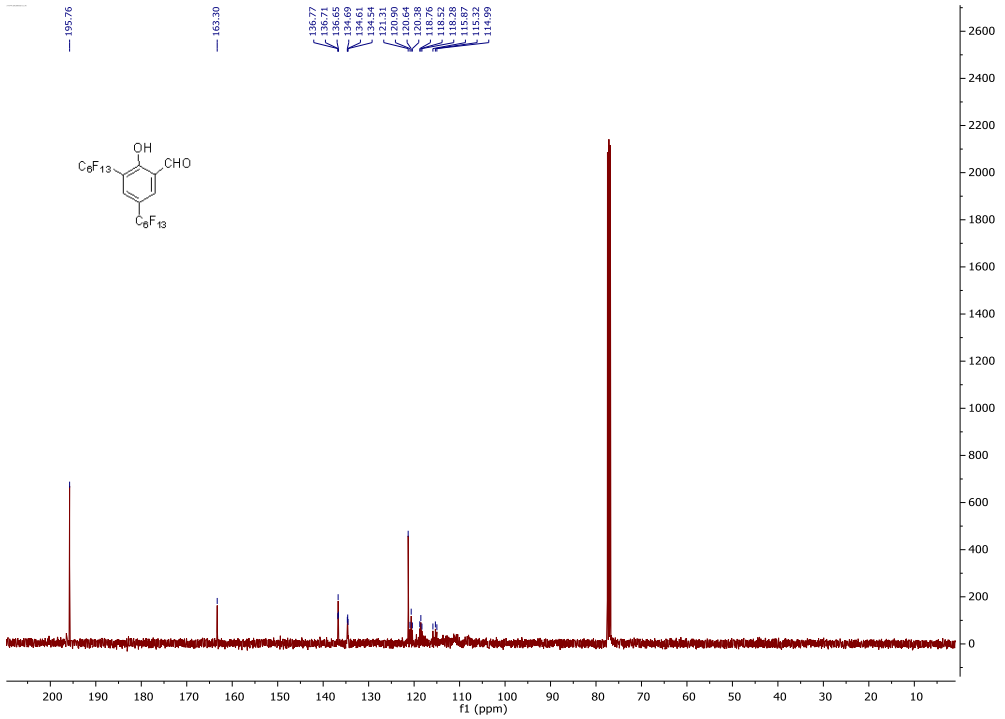
121.55
119.58
77.55
77.23
76.91
OH
CHO
C_6F_{13}
f1 (ppm)

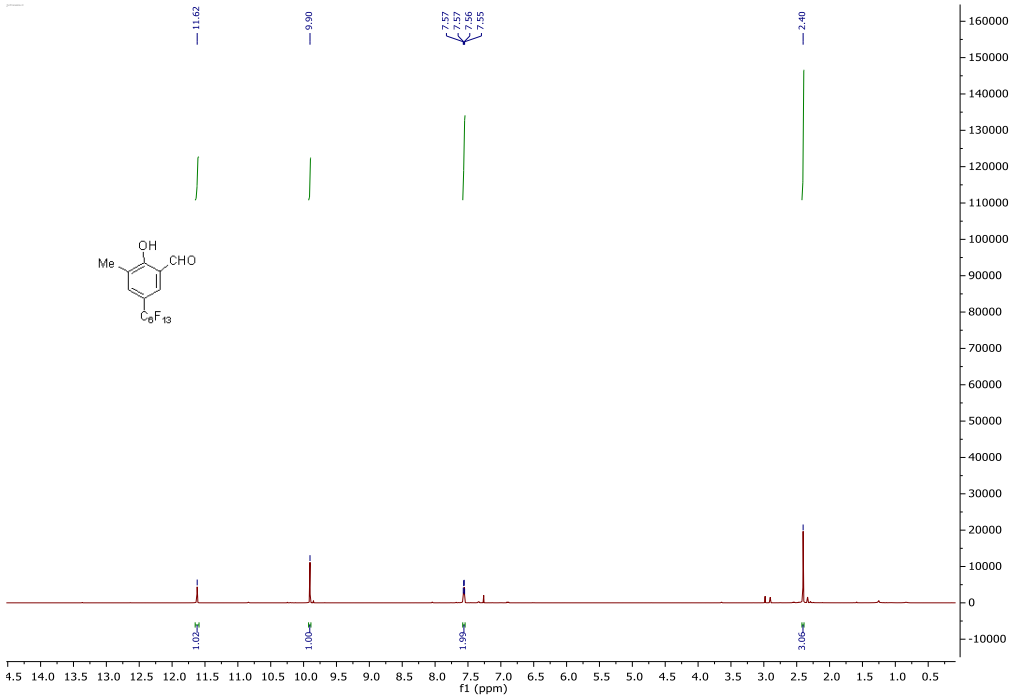
3, 5-di-perfluorohexyl-salicylaldehyde (3c)

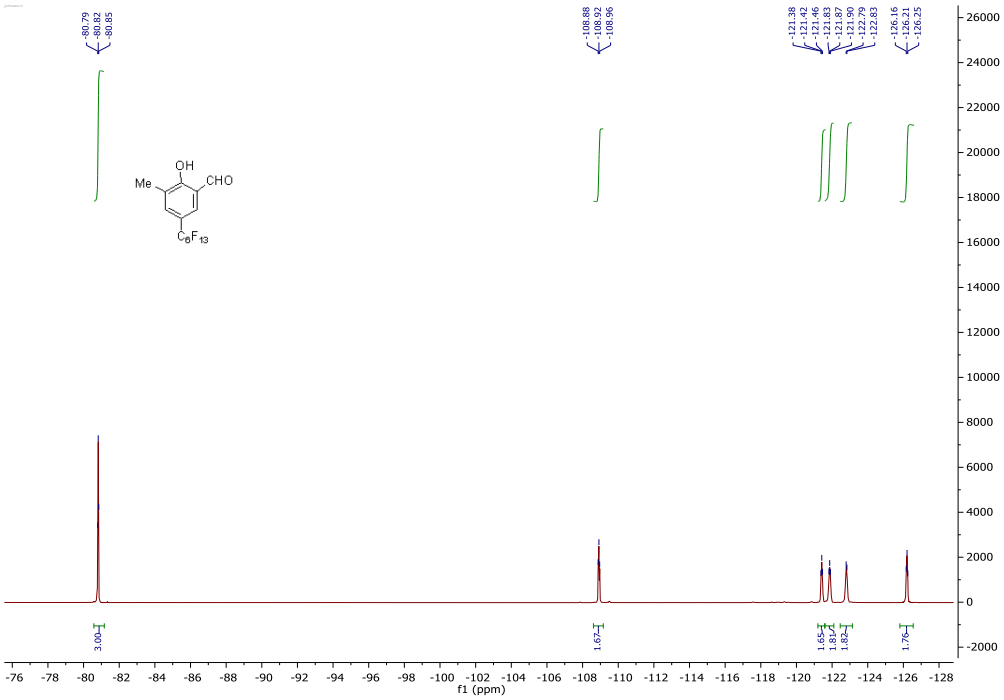






5-methyl-3-perfluohexyl-salicylaldehyde (3d)

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-121.90
-122.79
-122.83
-126.16
-126.21
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Me
CHO
C6F13
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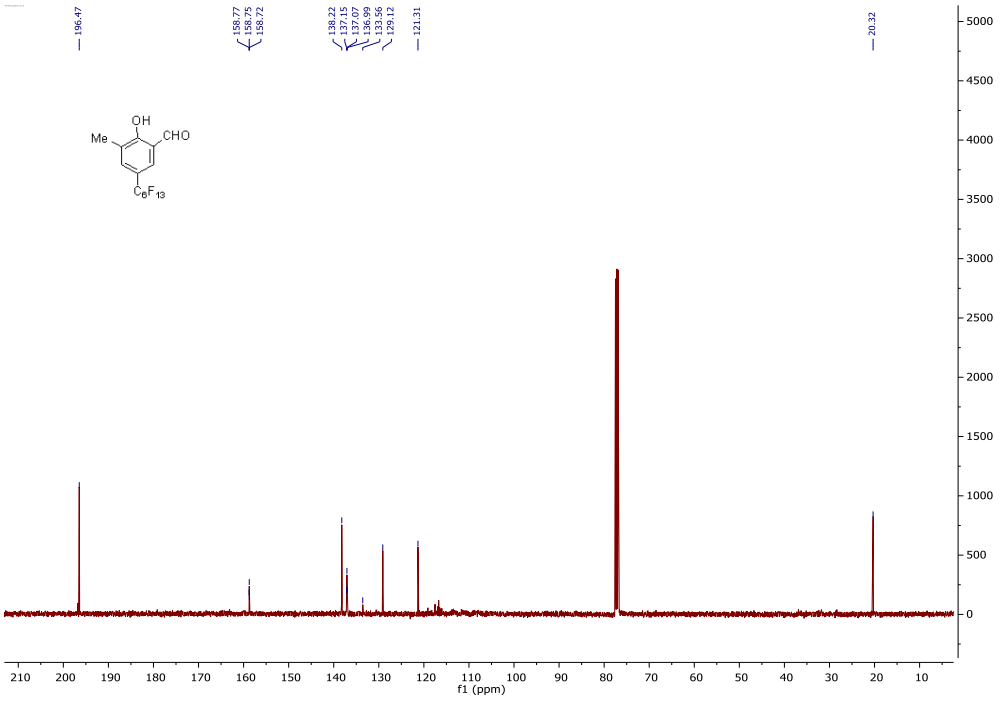
1.67
1.65
1.81
1.82
1.76
f1 (ppm)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137.15
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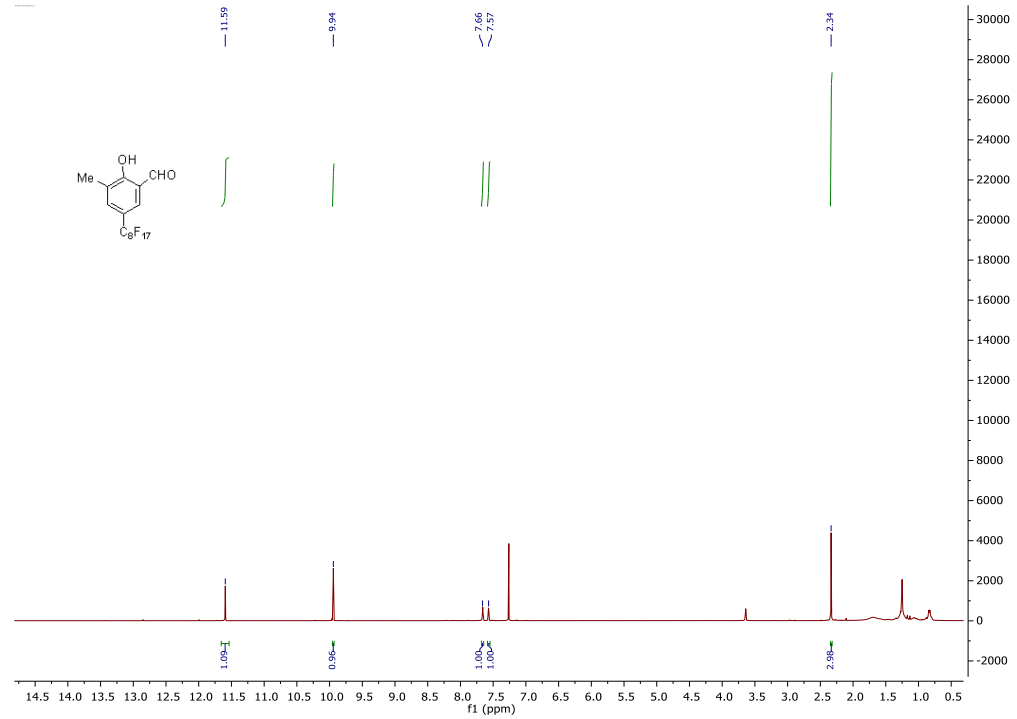
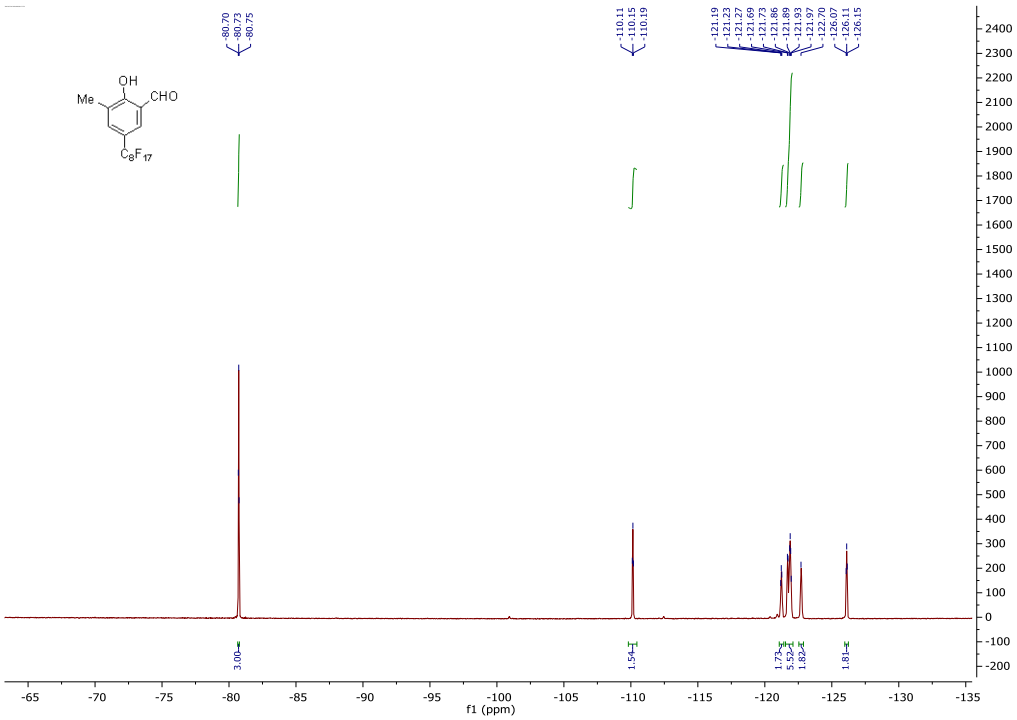
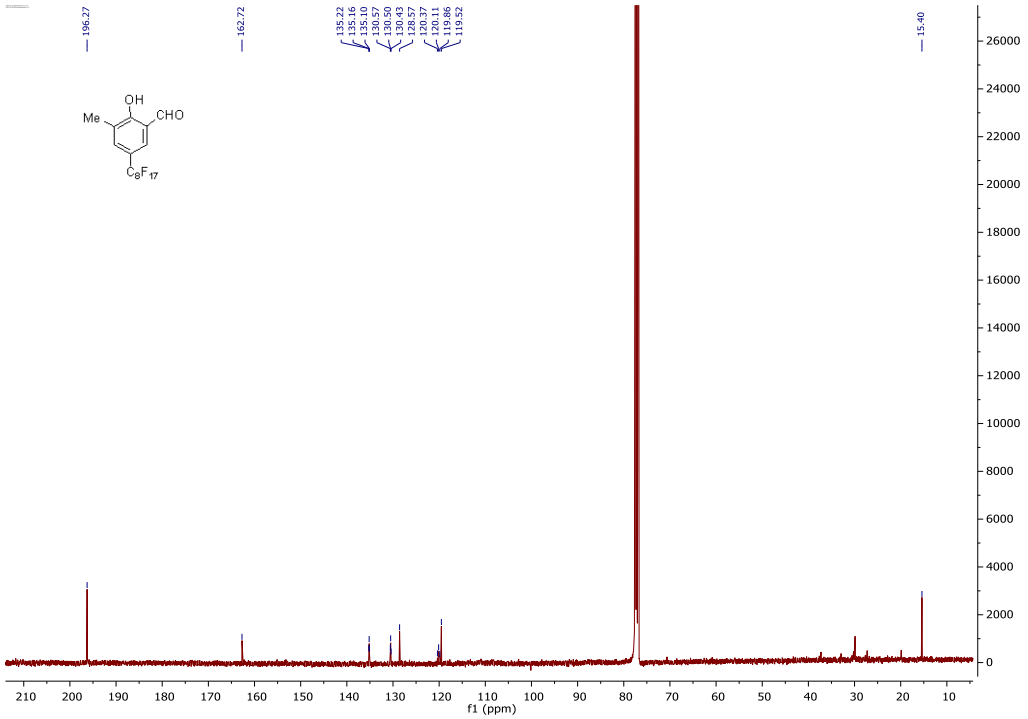
133.56
129.12
121.31
20.32
OH
Me
CHO
C6F13
f1 (ppm)

3-methyl-5-perfluorooctyl-salicylaldehyde (3f)



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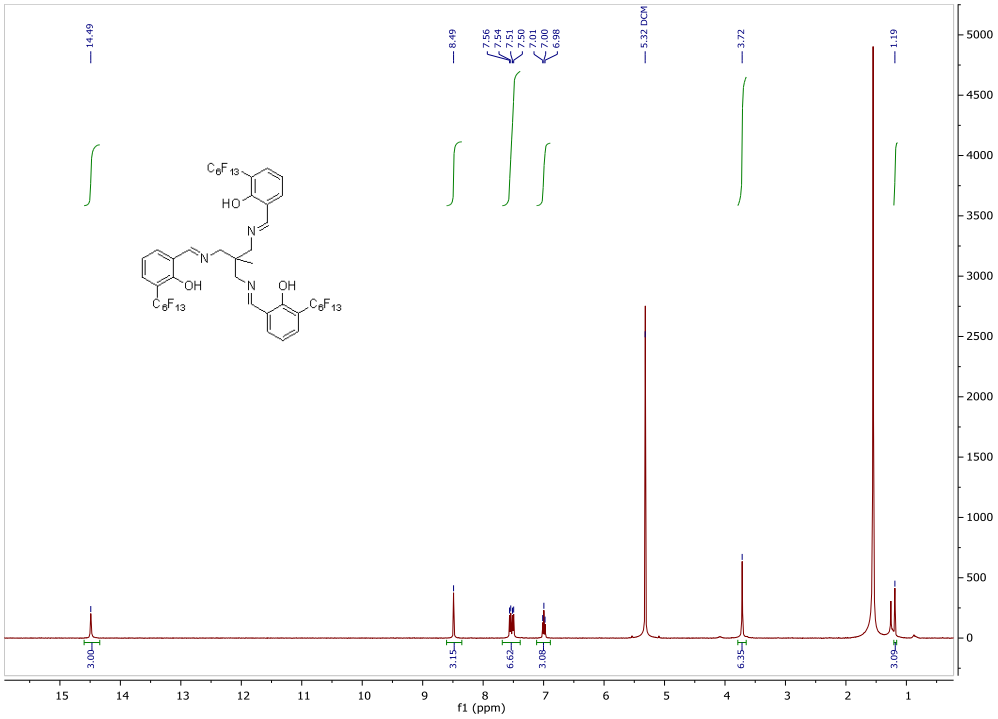
135.16
135.10
130.57
130.50
130.43
128.57
120.37
120.11
119.86
119.52
15.40
OH
Me
CHO
C8F17
f1 (ppm)

4a OOO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7.51
7.50
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7.00
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C6F13
HO
N
OH
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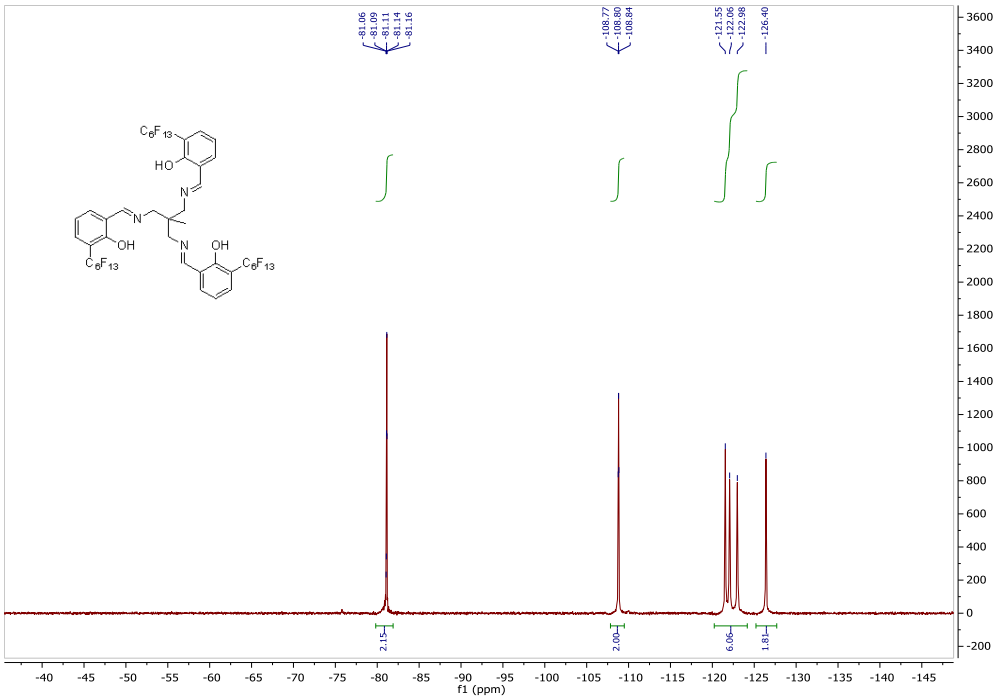
3.15
6.62
3.08
6.35
3.09
f1 (ppm)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-108.80
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-122.98
-126.40
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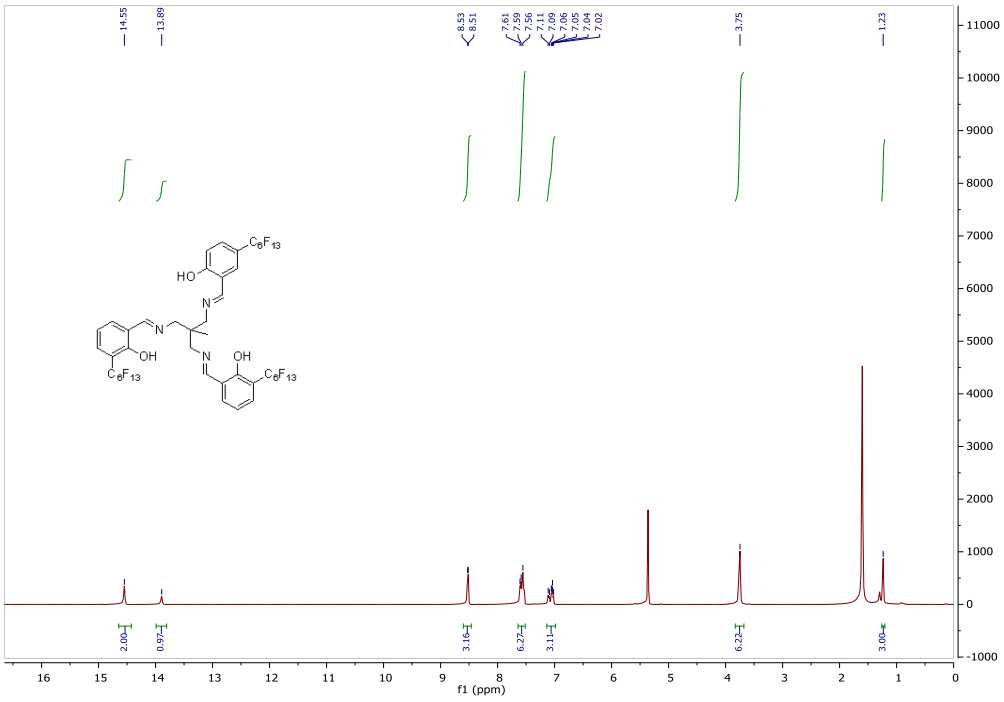
2.00
6.06
1.81
f1 (ppm)

4a OOP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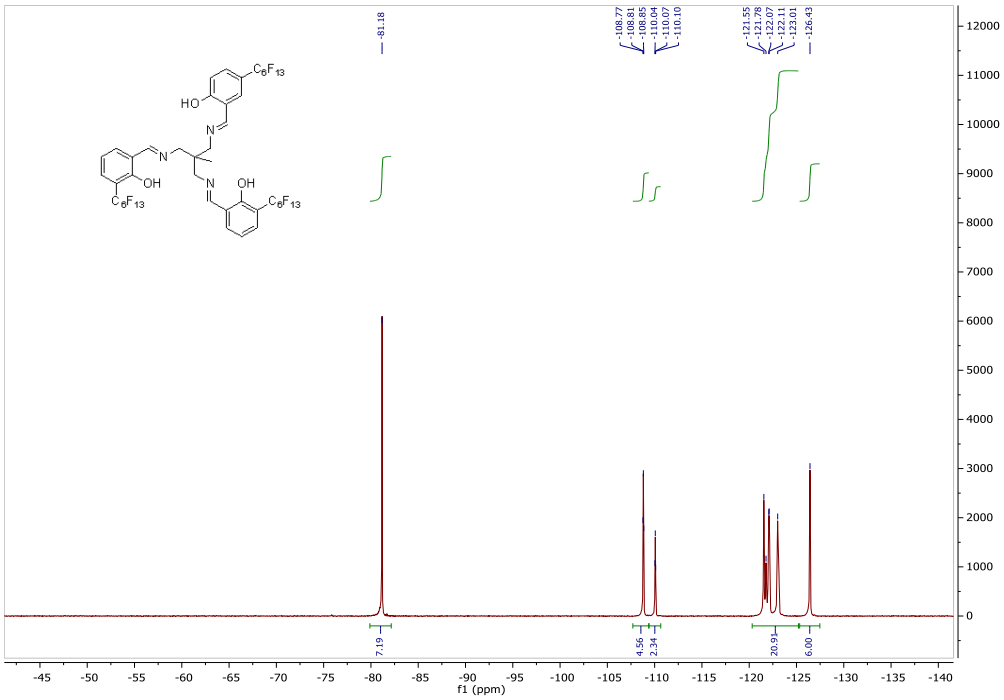
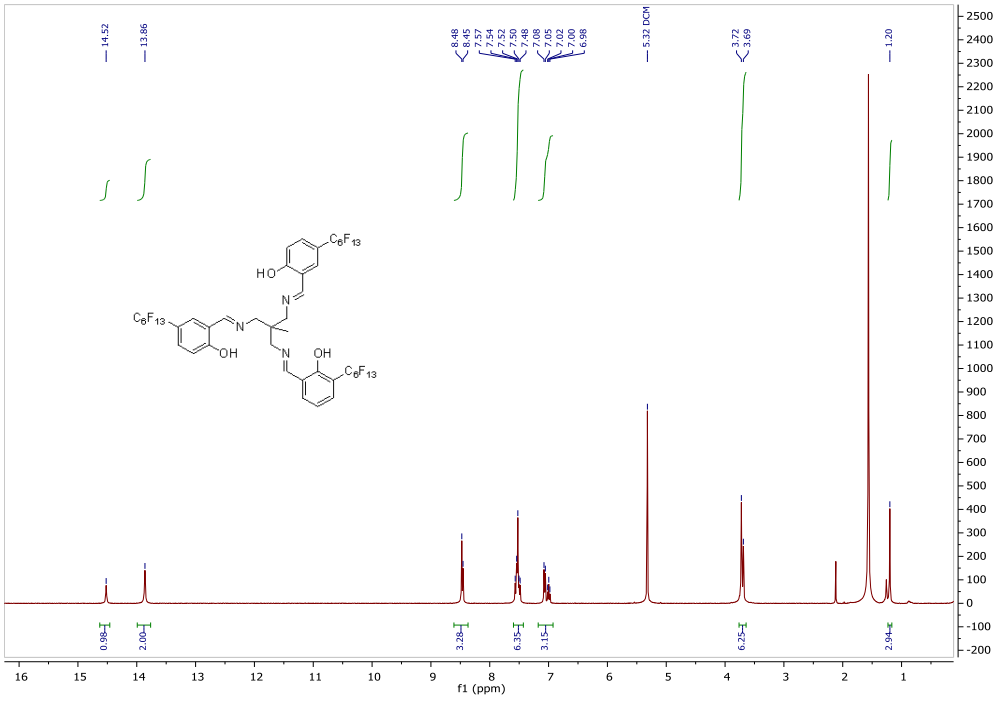
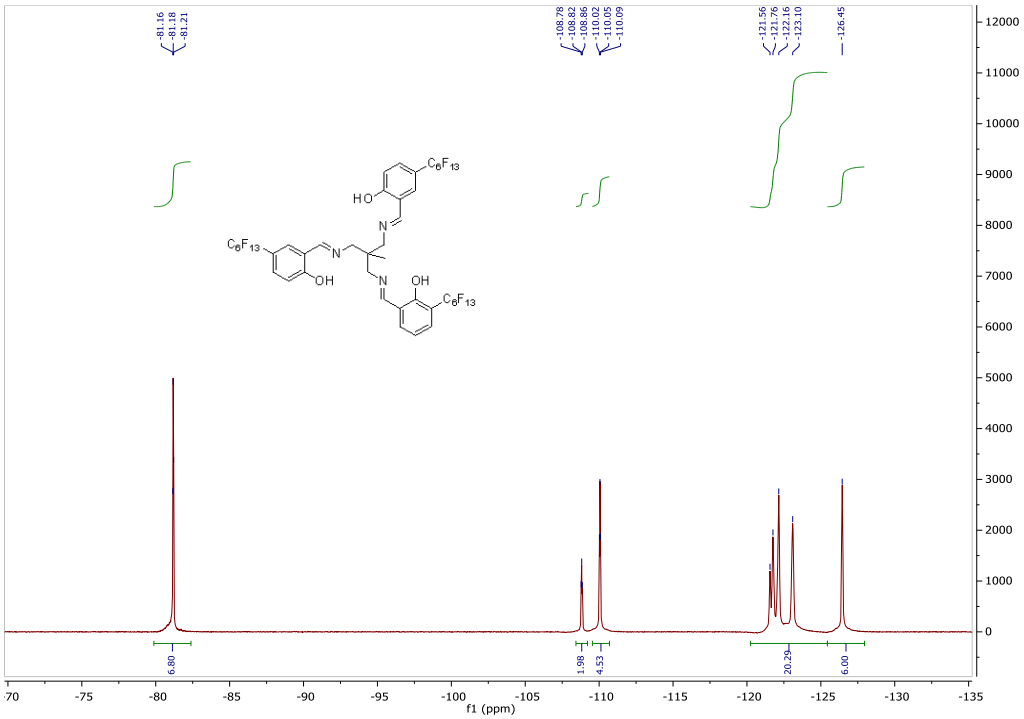
8.53
8.51
7.61
7.59
7.56
7.11
7.09
7.06
7.05
7.04
7.02
3.75
1.23
2.00
0.97
3.16
6.27
3.11
6.22
3.00
f1 (ppm)

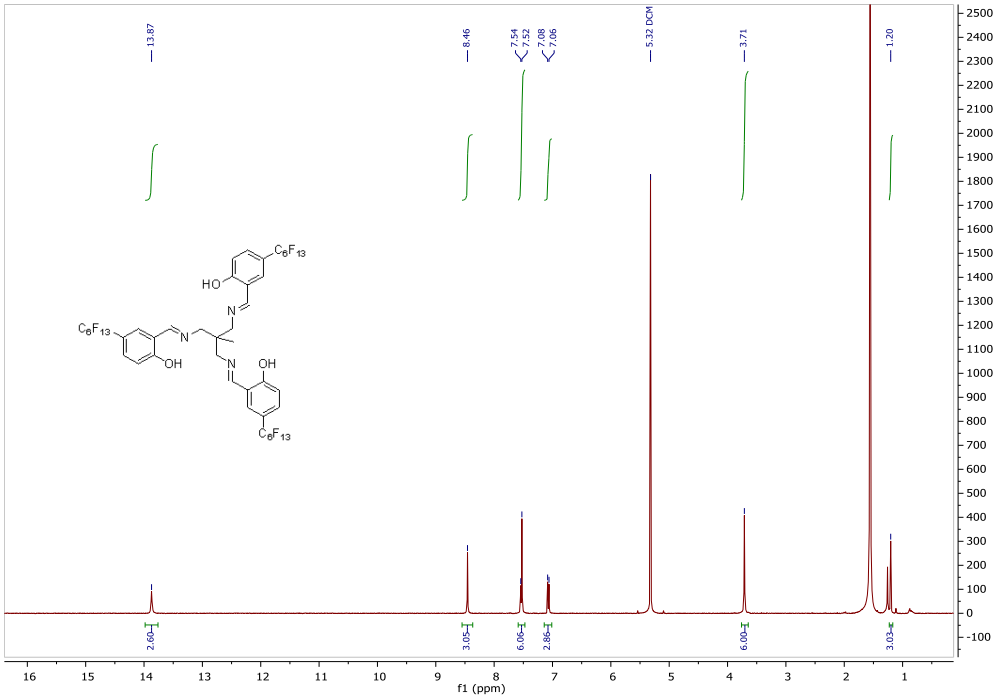

-81.18
-108.77
-108.81
-108.85
-110.04
-110.07
-110.10
-121.55
-121.78
-122.07
-122.11
-123.01
-126.43
7.19
4.56
2.34
20.91
6.00
f1 (ppm)

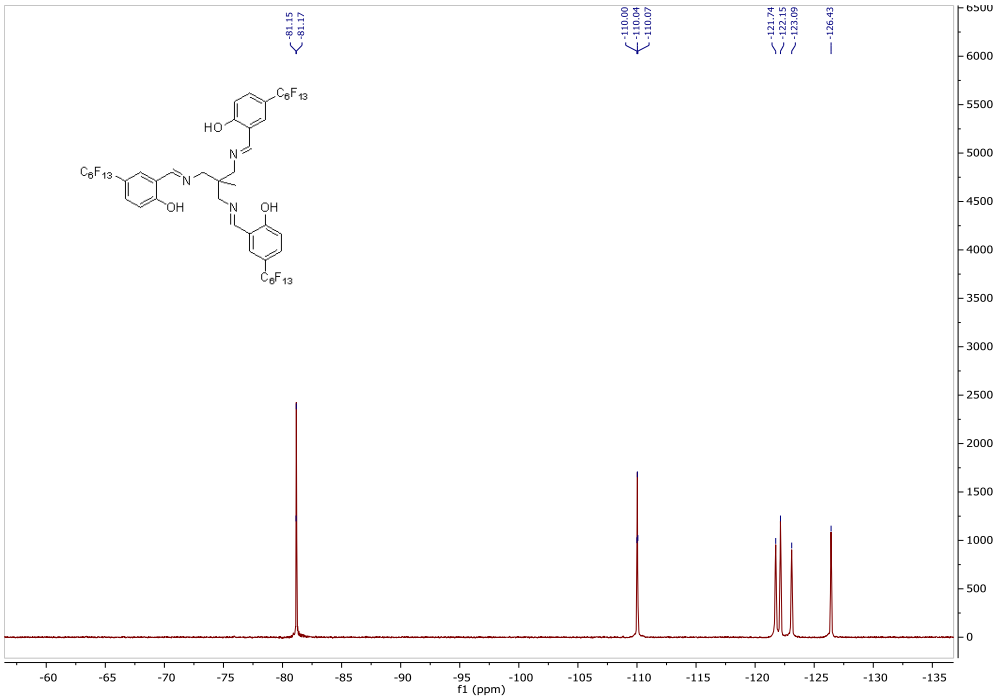
4a POP


14.52
13.86
8.48
8.45
7.57
7.54
7.52
7.50
7.48
7.08
7.05
7.02
7.00
6.98
5.32 DCM
3.72
3.69
1.20
0.98
2.00
3.28
6.35
3.15
6.25
2.94
f1 (ppm)

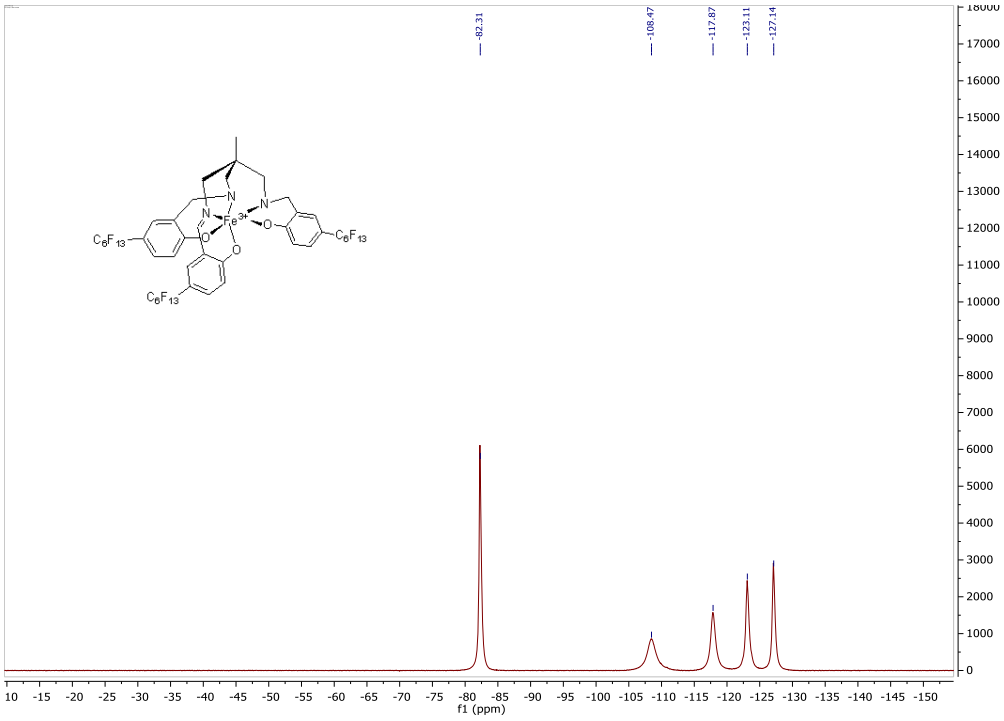

-81.16
-81.18
-81.21
-108.78
-108.82
-108.86
-110.02
-110.05
-110.09
-121.56
-121.76
-122.16
-123.10
-126.45
HO
OH
OH
f1 (ppm)

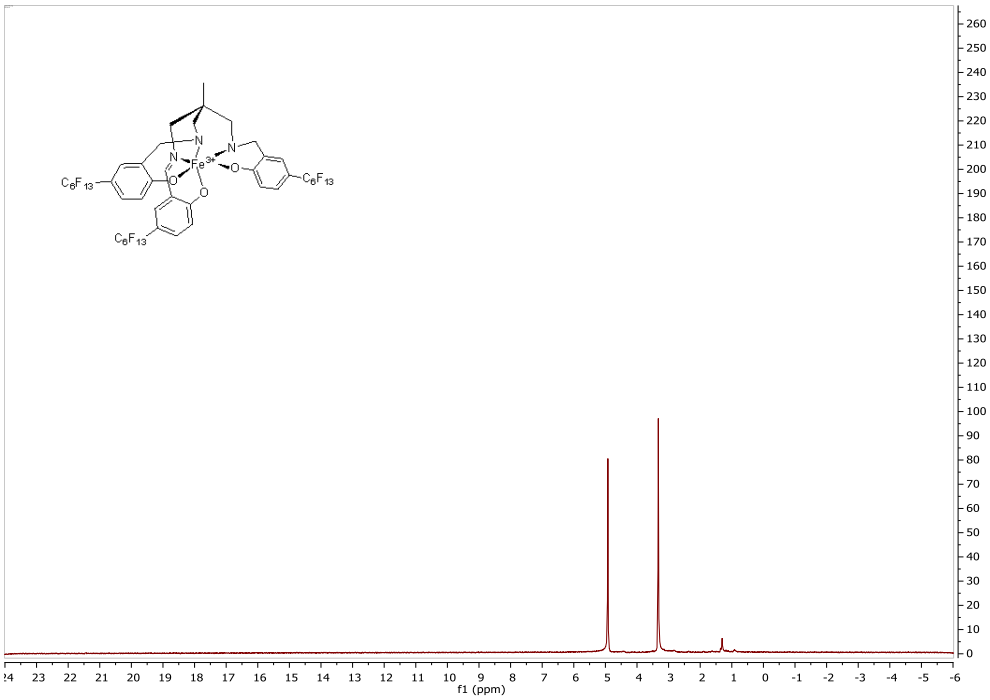
4a PPP

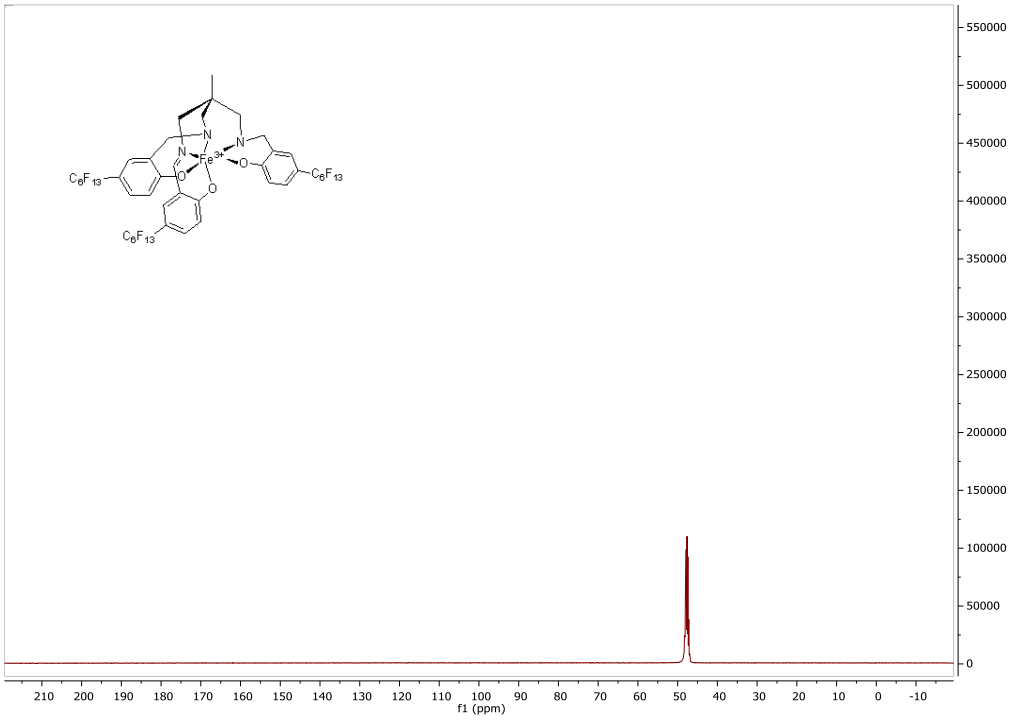

13.87
8.46
7.54
7.52
7.08
7.06
5.32 DCM
3.71
1.20
HO
OH
OH
f1 (ppm)



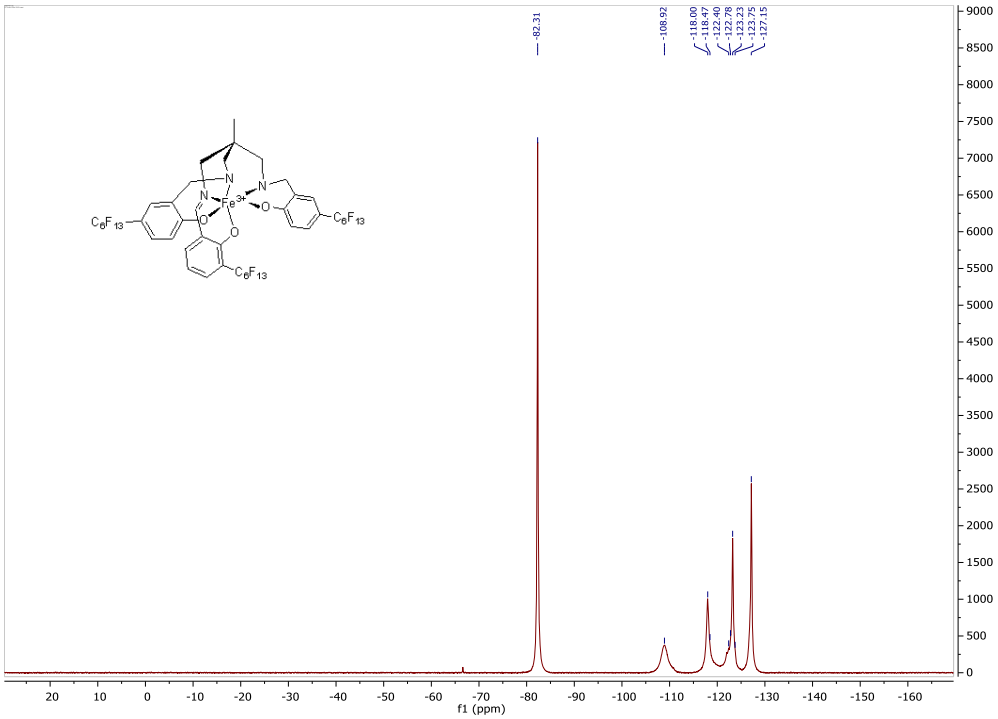
Fe SALTAME (5a PPP)




f1 (ppm)


f1 (ppm)

Fe SALTAME (5a POP)



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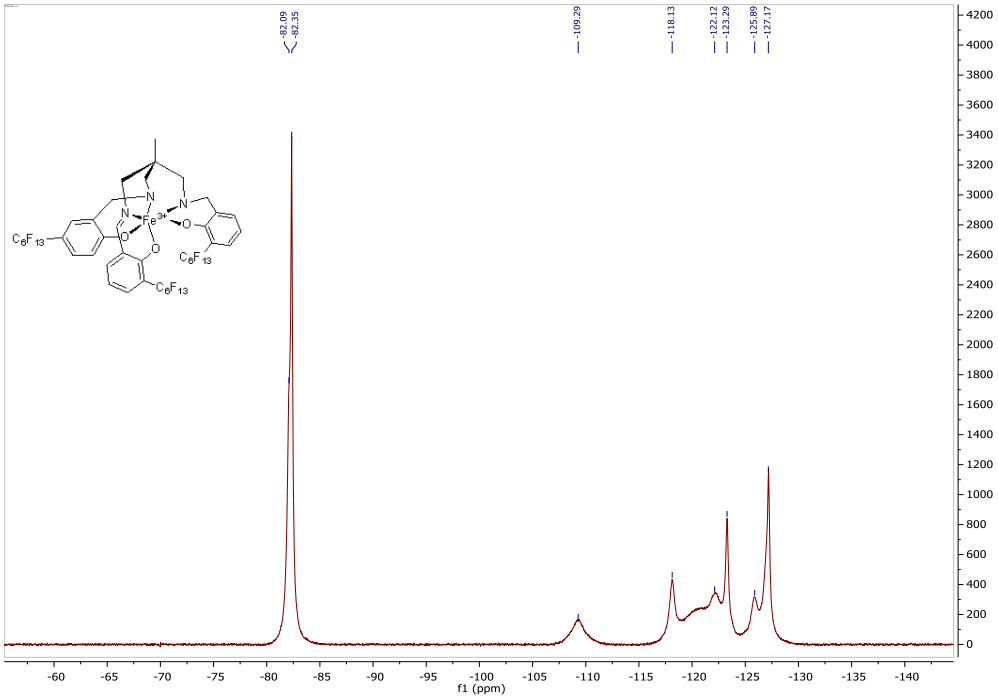
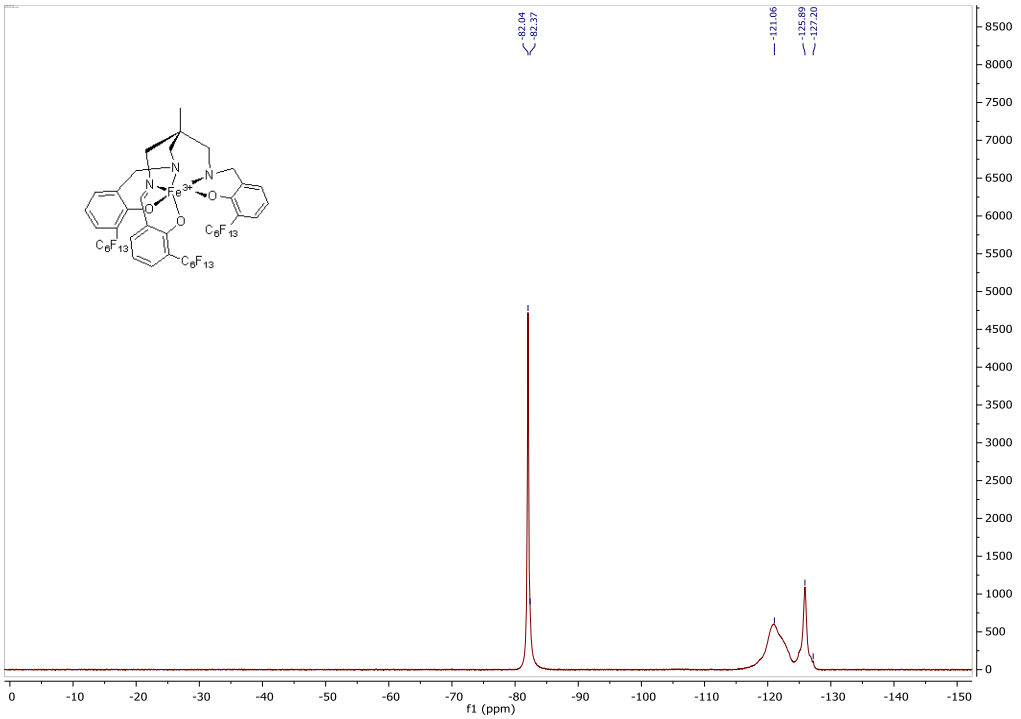


Fe SALTAME (5a OOO)



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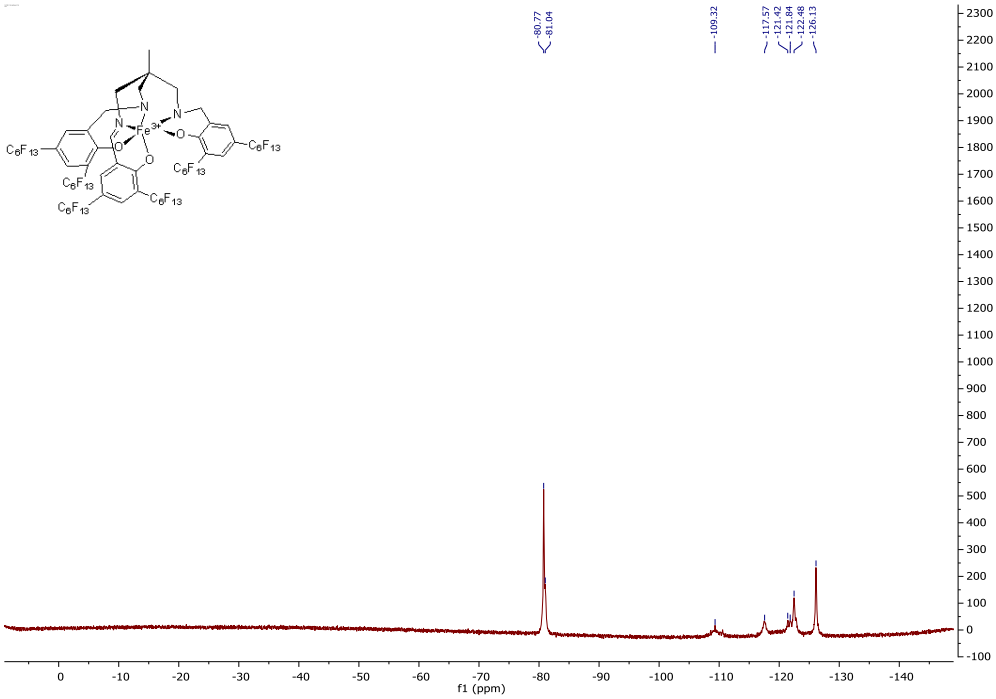
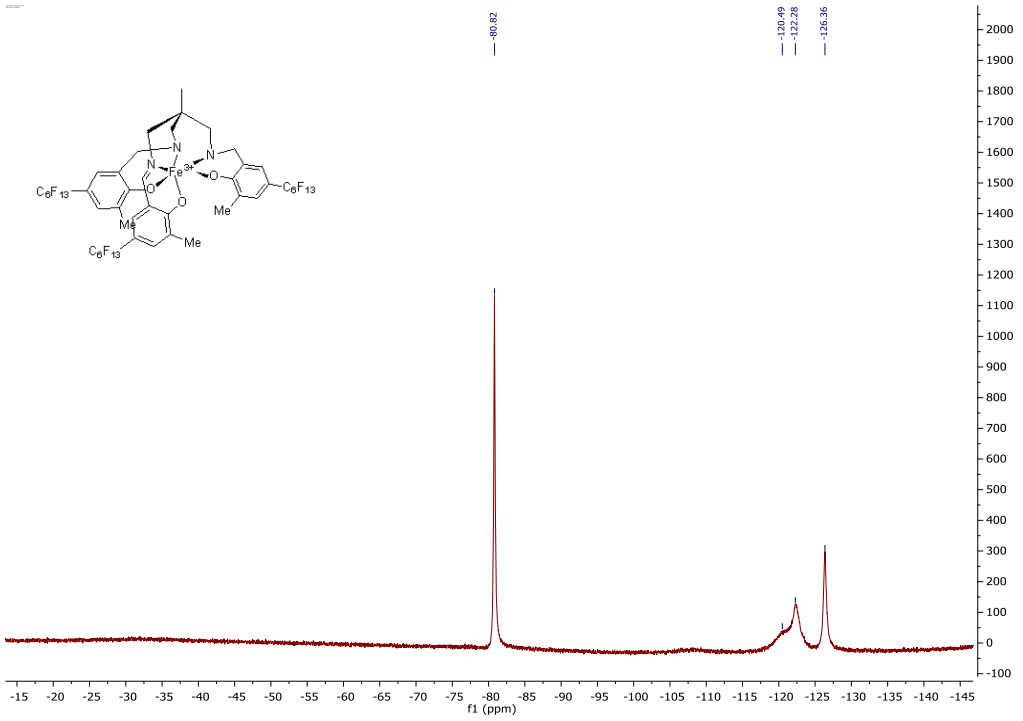
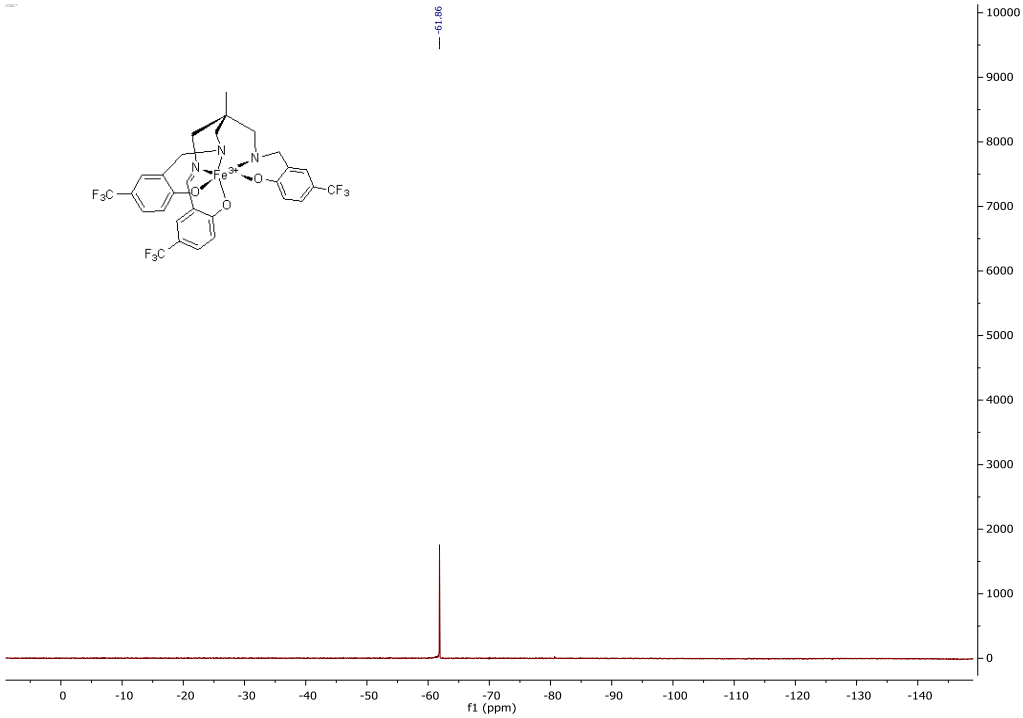


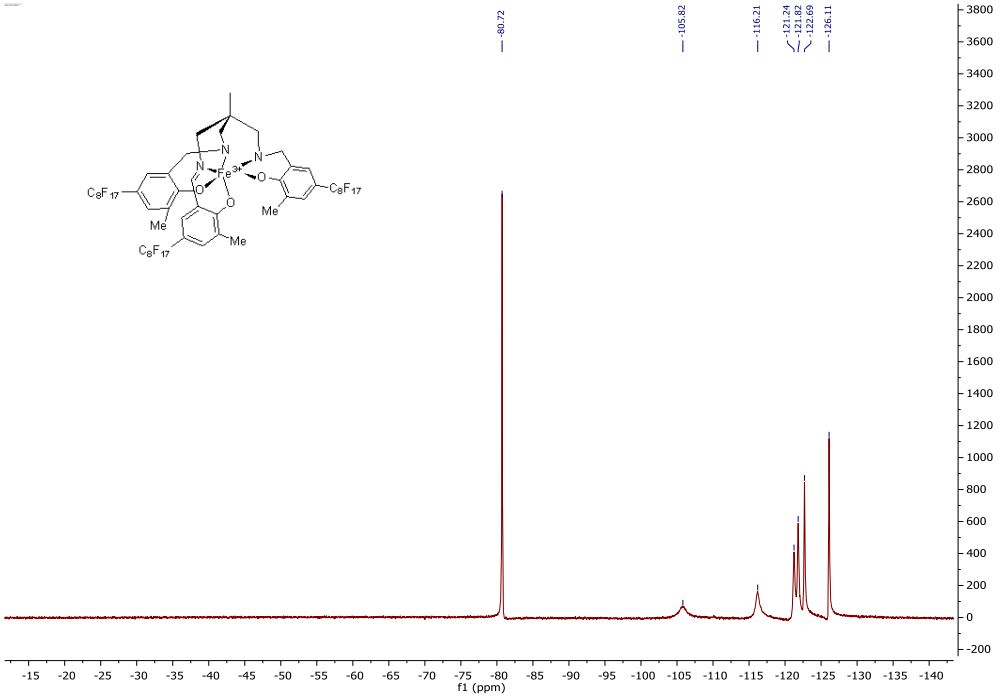
Fe SALTAME (5d)



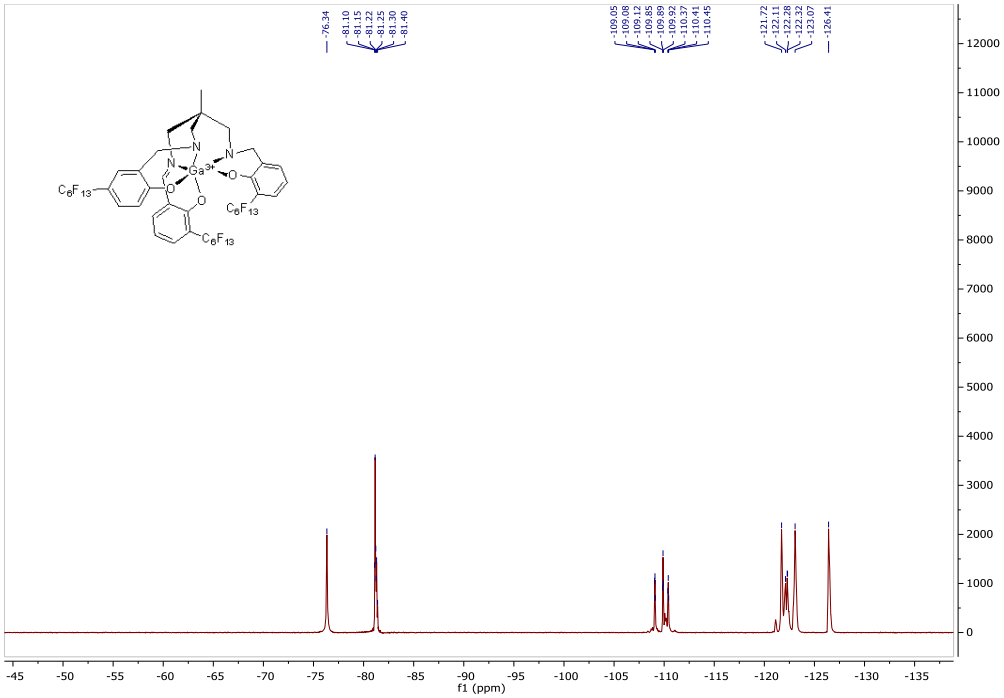
Fe SALTAME (5e)



Fe SALTAME (5f)



5a Ga OOP



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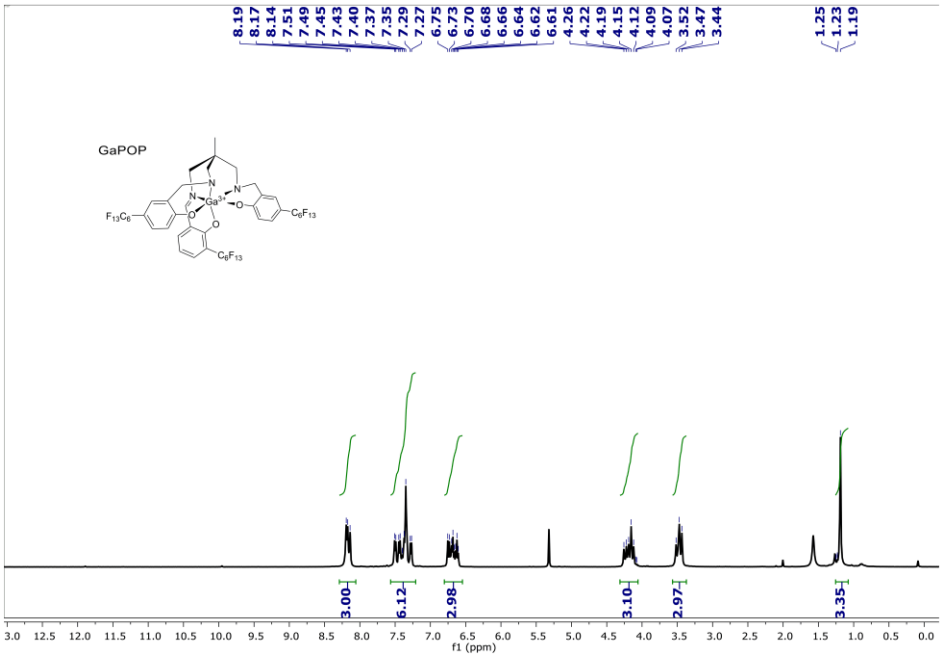
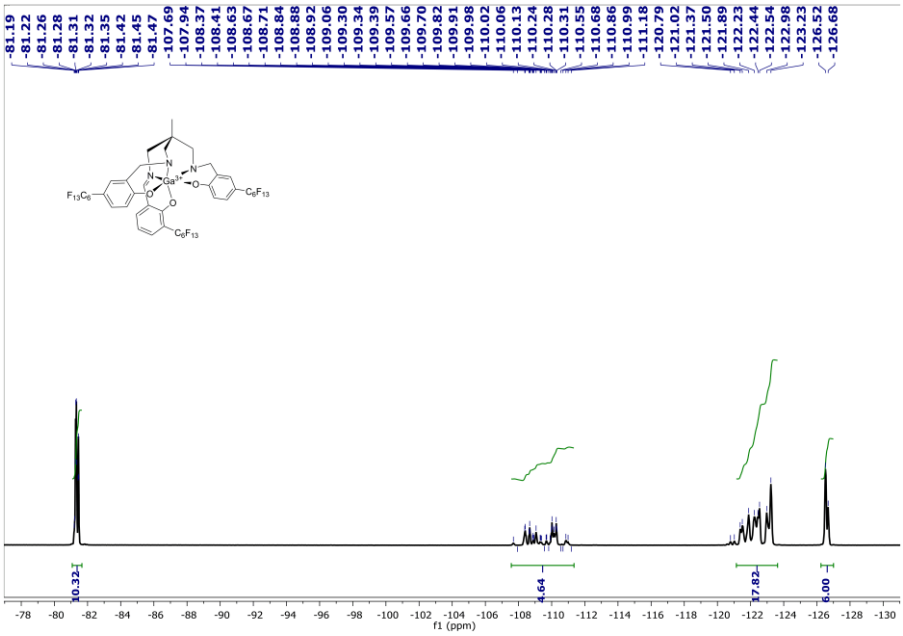
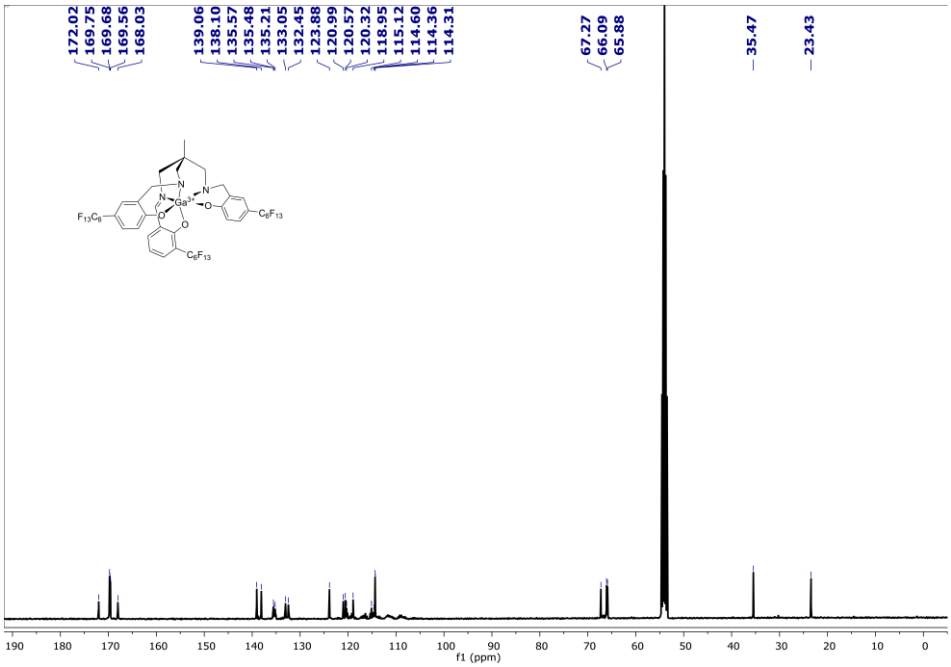


5a Ga POP



5a Ga POP



6. References

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2. Sheldrick, G. M., SHELXT - Integrated Space-Group and Crystal-Structure Determination. *Acta Crystallogr A* 2015, *71*, 3-8.
3. Sheldrick, G. M., Crystal Structure Refinement with SHELXL. *Acta Crystallogr C* 2015, *71*, 3-8.
4. Haase, A.; Frahm, J.; Matthaei, D., FLASH Imaging: Rapid NMR Imaging Using Low Flip-Angle Pulses. *J Magn Reson* 1986, *67*, 258-263.
5. Deeney, F. A.; Harding, C. J.; Morgan, G. G.; McKee, V.; Nelson, J.; Teat, S. J.; Clegg, W., Response to Steric Constraint in Azacryptate and Related Complexes of Iron-(II) and -(III) *. *J Chem Soc Dalton* 1998, 11, 1837-1844.