

Supporting Information

Rational Design of a Red Fluorescent Sensor for ALDH1A1 Displaying Enhanced Cellular Uptake and Reactivity

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Materials

Thin-layer chromatography (TLC) was performed on 0.25 mm silica-coated TLC plates containing an UV254 fluorescent indicator from Machery-Nagel. Compounds were visualized using a UVP UVGL-25 Compact UV Lamp. Flash chromatography was performed with 230-400 mesh silica gel P60 from SiliCycle Inc. Triethylamine was purchased from Acros Organics. Hydrogen gas was purchased from Airgas. 2-iodobenzoic acid, 4-bromo-3,5-dimethoxybenzaldehyde, and methylboronic acid were purchased from AK Scientific. 2,4-dinitrobenzenesulfonyl chloride and anhydrous pyridine were purchased from Alfa Aesar. 4-bromo-3,5-difluorobenzoic acid was purchased from Arctom. 3-methoxybenzyl alcohol was purchased from ArkPharm. All deuterated solvents were purchased from Cambridge Isotope Laboratories. 1,4-dibromo-2-fluoro-5-methylbenzene was purchased from Santa Cruz Biochemicals. 4-bromo-2,3-difluorobenzoic acid, 4-bromo-3-(trifluoromethyl)benzoic acid, 4-bromo-3-fluorotoluene, and methyl 4-bromo-3-methylbenzoate were purchased from Combi-Blocks. CH₂Cl₂, THF, Et₂O, EtOAc, hexanes, HNO₃, iPrOH, NaHCO₃, toluene, and the Pierce™ BCA Protein Assay Kit were purchased from Thermo Fisher Scientific. Cyclohexane, DMSO, H₂SO₄, MeOH, NaOH, and THF were purchased from Macron. CaCO₃ was purchased from Mallinckrodt Pharmaceuticals. 1,4-dioxane, 2,4,5-trifluorobenzoic acid, 3-bromophenol, Celite®, CuBr₂, dimethylcarbonyl chloride, imidazole, K₃PO₄, methyl iodide, N-bromosuccinimide, N-iodosuccinimide, Na₂S₂O₃, NaBH₄, Oxone®, Pd(dppf)Cl₂, *tert*-butyldimethylsilyl chloride, triethanolamine, and trifluoroacetic acid were purchased from Oakwood Chemical. 1.0 M BH₃-THF in THF, 1.0 M DIBAL-H in CH₂Cl₂, 1.0 M DIBAL-H in hexanes, 1.0 M LiHMDS in THF, 1.4 M *sec*-butyllithium in cyclohexane, 1.7 M *tert*-butyllithium in pentane, 10% w/w Pd/C, 2.5 M *n*-butyllithium in hexanes, 3.0 M methylmagnesium bromide in Et₂O, 4-bromobenzoic acid, AIBN, anhydrous MeCN, dichlorodimethylsilane, DMF, LiAlH₄, *tert*-butyl nitrite, and trypan blue were purchased from MilliporeSigma. 1,4-bis(trifluoromethyl)benzene was purchased from SynQuest Laboratories. Benzylamine was purchased from TCI America. HCl was purchased from VWR. THF used for anhydrous reactions was dried over activated 4 Å molecular sieves for a minimum of 24 hours. All other chemicals were used as received.

pKa Determination

Buffers used for pH titrations were glycine (pH = 2.3-3.7), NaOAc (pH = 4.0-5.6), MES (pH = 5.7-6.7), HEPES (pH = 6.9-8.1), and CHES (pH = 8.6-9.5). All buffers were prepared to a concentration of 50 mM in H₂O using aqueous NaOH and HCl to adjust pH. For each condition, 1 μM red-AIDeSense was excited at 594 nm, and an emission spectrum was collected from 600-650 nm. Each condition was performed in triplicate. Data was analyzed using Microsoft Excel.

Expression and Purification of ALDH Isoforms

Plasmids for ALDH1A1, ALDH1A2, ALDH1A3, ALDH2, ALDH3A1, ALDH4A1, and ALDH4A1 were generously provided by Professor Daria Mochly-Rosen (Stanford). Expression and purification of each isoform was performed as previously described.¹

ALDH Isoform Selectivity

Each ALDH isoform was added to a 50 mM triethanolamine solution (pH 7.4) containing 2.0 mM NAD⁺ and 100 μM substrate at room temperature. Benzaldehyde was the substrate for ALDH3A1. Propanal was the substrate for all other isoforms. ALDH isoform activity was determined by monitoring the rate of NADH production through the increase in absorbance at 340 nm ($\epsilon = 6220 \text{ M}^{-1}\text{cm}^{-1}$). For these assays, 1 unit is

defined as the amount of enzyme that catalyzes the conversion of 1 μM substrate per minute. For every combination of dye and enzyme, 2 μM dye was preincubated with 2.0 mM NAD^+ in 50 mM triethanolamine solution (pH 7.4). The reaction was initiated by addition of 1 unit of ALDH. Dyes were excited at 590 nm, and fluorescence was measured continuously at 610 nm.

ALDH1A1 Kinetics

Solutions were prepared of 2.0 mM NAD^+ and 0.1-2.5 μM AIDeSense or red-AIDeSense in 50 mM triethanolamine solution (pH 7.4). For every concentration, 1 unit of enzyme (as defined and determined in ALDH Isoform Selectivity) was added. Fluorescence was monitored for 30 seconds to determine V_0 . AIDeSense was excited at 490 nm and monitored at 510 nm. Red-AIDeSense was excited at 590 nm and monitored at 610 nm. Each condition was performed in triplicate. Protein concentration was determined using the Pierce™ BCA Protein Assay Kit. Data was analyzed using Microsoft Excel.

Trypan Blue Viability Assay

K562 cells were plated in a 96-well plate with 50,000 cells per well. Each well was filled to a final volume of 100 μL IMDM media with 0.25% DMSO. Wells were tested using red-AIDeSense or Ctrl- red-AIDeSense (1 μM and 5 μM each) or using a DMSO control. After 6-, 12-, and 24-hour incubation periods at 37 °C and 5% CO_2 , a 10 μL sample was removed from each condition and combined with 10 μL 0.4% w/v trypan blue in PBS. Samples were incubated for 3 minutes at room temperature before being loaded on a hemocytometer where live and dead cells were counted. Conditions were tested in triplicate for each time point. Samples were analyzed on an EVOS digital inverted microscope using transmitted white light for brightfield imaging and the Cy5 filter cube for trypan blue visualization.

Cell Culture

K562 cells were obtained from Prof. Paul Hergenrother (UIUC). K562 cells were cultured using Iscove's Modified Dulbecco's Medium (IMDM, ATCC) supplemented with 10% fetal bovine serum (FBS, Sigma) and 1% penicillin/streptomycin (pen-strep, Corning). A549 cells were purchased from the American Type Culture Collection (ATCC) and cultured in Dulbecco's Modified Eagle Medium (DMEM, ATCC) supplemented with 10% FBS, 1% pen-strep, and non-essential amino acids. All cells were grown at 37 °C in a humidified incubator with 5% CO_2 . For each cell line, media was changed or cells were passaged every 3 days. Passage numbers were kept below 20 for all experiments.

Flow Cytometry

For all cell lines, 200,000 cells in a single-cell suspension were transferred into 1.5 mL Eppendorf tubes. Cells were pelleted by centrifugation at 400g for 5 minutes at room temperature. After removing the supernatant by aspiration, cells were resuspended in 300 μL premixed PBS supplemented with verapamil, probenecid, and novobiocin. For the indicated samples, DEAB (final concentration 20 μM) was added. Samples were immediately stored on ice. Cells were stained with red-AIDeSense or Ctrl- red-AIDeSense (100 μL of 4 μM dye solution; dyes were normalized by fluorescence spectroscopy). Samples were analyzed on a BD LSR II Flow Cytometry Analyzer using a 488 nm laser and a 550 LP and 610/30 filter set. Samples were analyzed every 5 minutes for 15 minutes, with the samples being placed back on ice between data collection. Data were analyzed on FCS Express 6.04.

Confocal Imaging

Nunc™ Lab-Tek™ 8-well Chamber Coverglass (Thermo Fisher) were coated with poly-L-lysine (Trevigen). For cells that were also stained with an antibody, 2,000,000 cells were transferred to an Eppendorf tube. The cells were resuspended in 200 μ L of antibody buffer (5% BSA and 1 mg/mL NaN_3 in PBS) and treated with 2 μ L FITC-labeled anti-CD44 antibody (10 mg/mL, Sino Biological) and left on ice for 20 minutes. In all cases, cells were added at 150,000 cells/well to the coated slides 1 hour prior to imaging. Before imaging, the media was aspirated and each chamber was refilled with 300 μ L premixed imaging buffer (100 μ M verapamil, 2.5 mM probenecid, and 200 μ M novobiocin in PBS plus 5% BSA and 1 mg/mL NaN_3 for antibody-stained cells) at room temperature. Cells were treated with 100 μ L red-ALDeSense or Ctrl-red-ALDeSense solution (4 μ M dye in imaging buffer; dyes were normalized by fluorescence spectroscopy) and 0.8 μ L Hoescht 33342 (5 mg/mL in PBS) for 10 minutes. Live cell imaging was performed on a Multiphoton Confocal Microscope Zeiss 710. Hoescht 33342 was excited with a 405 nm laser and detected with a 415-502 filter set. FITC was excited with a 488 nm laser and detected with a 502-561 filter set. Hoescht 33342 was excited with a 594 nm laser and detected with a 600-735 filter set. For each condition, three images were taken for each of four different wells using the 40x objective. The same optical settings were used for images within each set of matched experiments. Images were analyzed using ZEN 3.0 (blue edition) software. Mean fluorescence for the FITC-labeled anti-CD44 antibody was multiplied by the cell diameter to account for its 1-dimensional cell membrane staining, as opposed to 2-dimensional intracellular staining of (Ctrl-)red-ALDeSense. Cells were considered to have high ALDH activity when they had a mean red-ALDeSense fluorescence signal greater than 99% of Ctrl-red-ALDeSense-stained cells.

Supporting References

(1) Anorma, C.; Hedhli, J.; Bearrood, T. E.; Pino, N. W.; Gardner, S. H.; Inaba, H.; Zhang, P.; Li, Y.; Feng, D.; Dibrell, S. E.; et al. (2018) Surveillance of Cancer Stem Cell Plasticity Using an Isoform-Selective Fluorescent Probe for Aldehyde Dehydrogenase 1A1. *ACS Cent. Sci.* 4, 1045–1055.

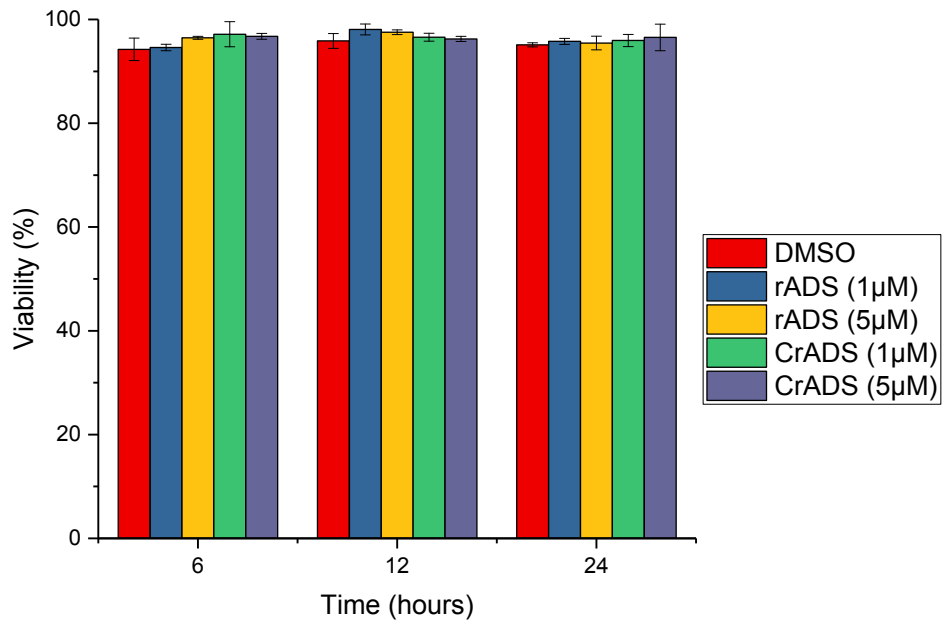


Figure S1. Trypan blue cytotoxicity assay using K562 cells for red-AIDeSense (rADS) and Ctrl-red-AIDeSense (CrADS) over 6, 12, and 24 hours.

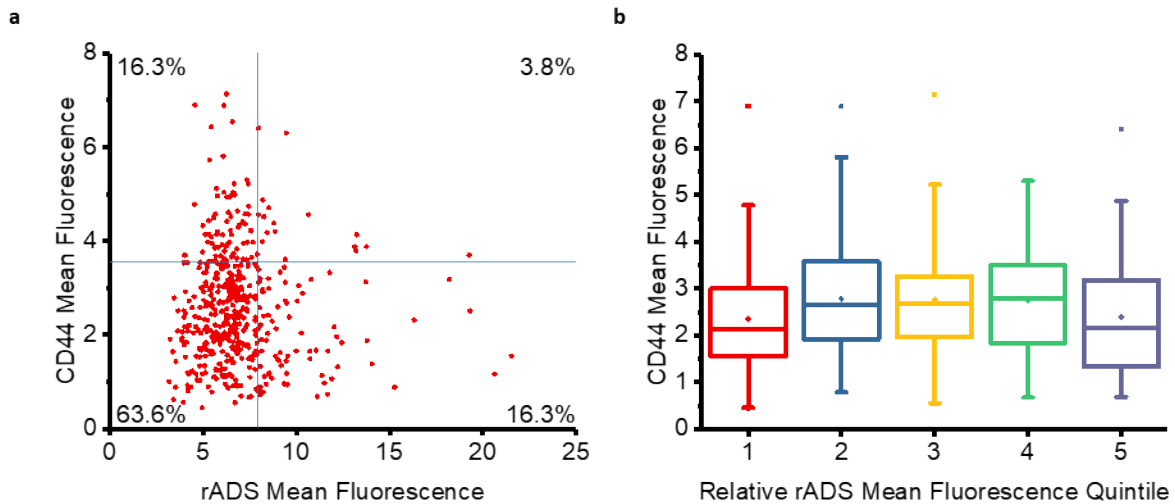


Figure S2. A549 were cells co-stained with red-AIDeSense or Ctrl-red-AIDeSense and a FITC-labeled anti-CD44 antibody (CD44). Mean fluorescence values of both CD44 and red-AIDeSense were quantified for every cell. This was (a) plotted with quadrants indicating the top 20% of each population and (b) sorted into quintiles, wherein the first quintile comprises the lowest red-AIDeSense mean fluorescence. All assays were performed using 1 µM red-AIDeSense or Ctrl-AIDeSense.

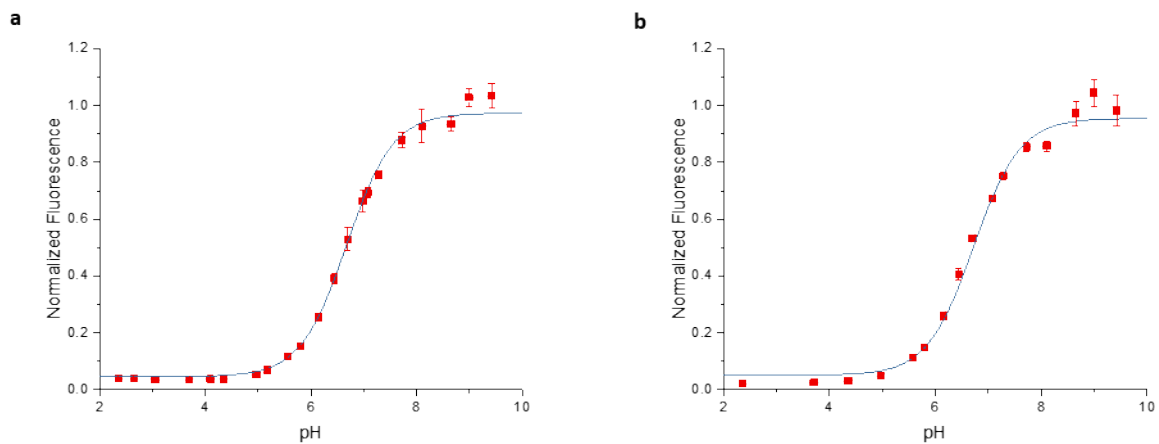
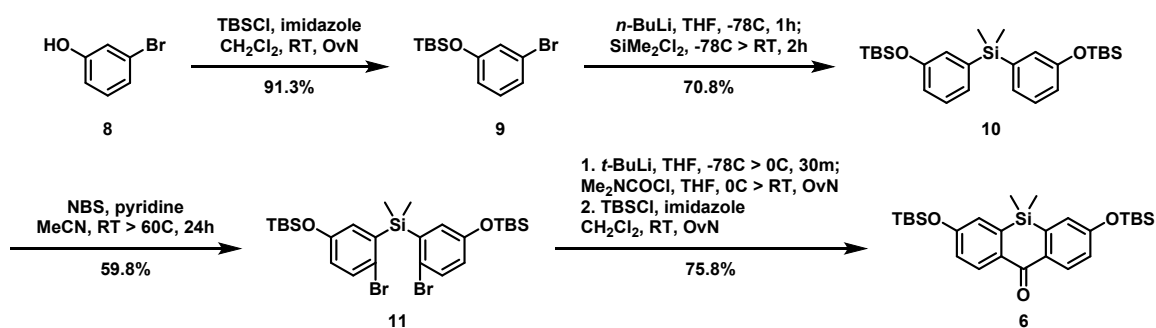


Figure S3. pH profiles of (a) red-AIDeSense and (b) Ctrl-red-AIDeSense.



Scheme S1. Synthesis of Xanthone 6.

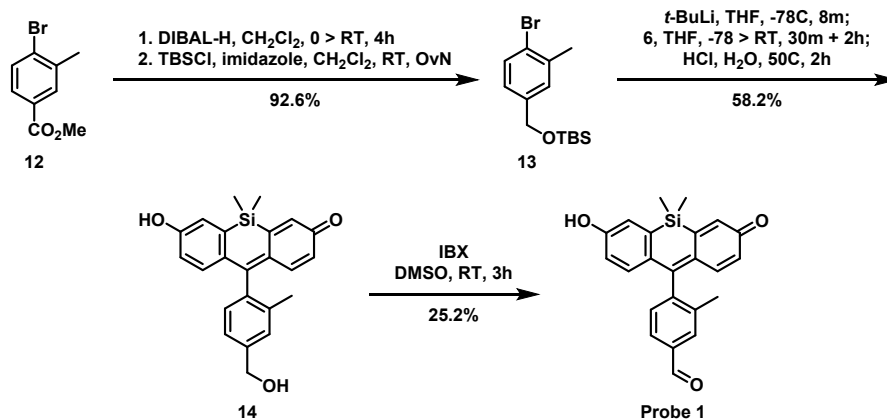
(3-Bromophenoxy)(*tert*-butyl)dimethylsilane (9) A round-bottom flask was charged with 3-bromophenol (8.65 g, 50.0 mmol, 1.0 eq.), *tert*-butyldimethylsilyl chloride (7.9 g, 52 mmol, 1.05 eq.), imidazole (3.6 g, 52 mmol, 1.05 eq.), and CH₂Cl₂ (70 mL). After stirring overnight at room temperature, the reaction was washed sequentially with 1.0 M HCl in H₂O (50 mL) and 1.0 M NaOH in H₂O (50 mL). The organics were collected, dried over Na₂SO₄, and concentrated under reduced pressure to afford the title compound as a colorless oil (13.12 g, 45.7 mmol, 91.3%). ¹H NMR (500 MHz, CDCl₃) δ 7.12 – 7.04 (m, 2H), 7.03 – 6.98 (m, 1H), 6.76 (ddq, *J* = 6.5, 4.2, 2.2 Hz, 1H), 0.98 (s, 9H), 0.20 (s, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 156.68, 130.55, 124.61, 123.67, 122.61, 118.96, 25.76, 18.33, -4.31.

Bis(3-((*tert*-butyldimethylsilyl)oxy)phenyl)dimethylsilane (10) A flame-dried round-bottom flask was charged with **9** (13.12 g, 45.7 mmol, 2.0 eq.) and anhydrous THF (25 mL). The reaction was cooled to -78 °C and then treated dropwise with 2.5 M *n*-butyllithium in hexanes (18 mL, 45.7 mmol, 2.0 eq.) over 25 minutes. After stirring for an additional hour at -78 °C, the reaction was treated dropwise with a solution of dimethyldichlorosilane (2.75 mL, 22.8 mmol, 1.0 eq.) in anhydrous THF (15 mL) over 5 minutes via syringe addition. After addition, the reaction was warmed to room temperature and stirred for 2 hours, then poured over sat. NaHCO₃ (50 mL) and diluted with H₂O (50 mL). The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (0:100 to 2:98 v/v EtOAc:hexanes gradient) to afford the title compound as a colorless oil (7.65 g, 16.2 mmol, 70.8% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.22 (t, *J* = 7.6 Hz, 2H), 7.09 (dt, *J* = 7.3, 1.1 Hz, 2H), 6.97 (dd, *J* = 2.5, 1.1 Hz, 2H), 6.83 (ddd, *J* = 8.1, 2.6, 1.1 Hz, 2H), 0.97 (s, 18H), 0.51 (s, 6H), 0.16 (s, 12H). ¹³C NMR (126 MHz, CDCl₃) δ 155.25, 139.87, 129.09, 127.09, 125.71, 120.92, 25.88, 18.39, -2.32, -4.23.

Bis(2-bromo-5-((*tert*-butyldimethylsilyl)oxy)phenyl)dimethylsilane (11) A round-bottom flask was charged with **10** (1.42 g, 3.0 mmol, 1.0 eq.), anhydrous MeCN (20 mL), and anhydrous pyridine (2.0 mL, 24 mmol, 8.0 eq.). The reaction was treated with *N*-bromosuccinimide (2.35 g, 13 mmol, 4.4 eq.). The reaction was heated to 60 °C for 24 hours. After cooling to room temperature, the reaction was diluted with Et₂O and EtOAc, then washed sequentially with 1.0 M HCl in H₂O, 1.0 M NaOH in H₂O, and brine. The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (2:98 v/v EtOAc:hexanes) to afford the title compound as a white solid (1.13 g, 1.79 mmol, 59.8% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.35 (d, *J* = 8.5 Hz, 2H), 6.91 (d, *J* = 3.0 Hz, 2H), 6.71 (dd, *J* = 8.6, 3.0 Hz, 2H), 0.95 (s, 18H), 0.72 (s, 6H), 0.14 (s, 12H). ¹³C NMR (126 MHz, CDCl₃) δ 154.59, 140.03, 133.90, 128.76, 123.17, 121.41, 25.83, 18.39, -1.07, -4.27.

3,7-Bis((*tert*-butyldimethylsilyl)oxy)-5,5-dimethyldibenzo[*b,e*]silin-10(5H)-one (6) A flame-dried round-bottom flask was charged with **11** (3.60 g, 5.7 mmol, 1.0 eq.) and anhydrous THF (50 mL). The reaction

was cooled to $-78\text{ }^{\circ}\text{C}$ and then treated dropwise with 1.7 M *t*-butyllithium in pentane (14 mL, 23.4 mmol, 4.1 eq.) over 10 minutes. After stirring for 30 minutes, the reaction was warmed to $0\text{ }^{\circ}\text{C}$ and treated dropwise with 1.0 M dimethylcarbonyl chloride in THF (11.5 mL, 11.5 mmol, 2.0 eq.) over 40 minutes. The reaction was slowly warmed to room temperature with the melting ice bath. After stirring overnight, the THF was removed *in vacuo*. The crude residue was suspended in CH_2Cl_2 and treated with imidazole (0.82 g, 12 mmol, 2.1 eq.) and *tert*-butyldimethylsilyl chloride (1.9 g, 13 mmol, 2.2 eq.). After stirring overnight, the reaction was washed with H_2O and concentrated. The crude material was purified via flash chromatography on a silica column (0:100 to 2:98 v/v EtOAc:hexanes gradient) to afford the title compound. This was further dissolved in EtOH and triturated with H_2O to afford a white solid (2.16 g, 1.33 mmol, 75.8% yield). ^1H NMR (500 MHz, CDCl_3) δ 8.37 (d, $J = 8.7$ Hz, 2H), 7.04 (d, $J = 2.5$ Hz, 2H), 6.99 (dd, $J = 8.7, 2.5$ Hz, 2H), 1.01 (s, 18H), 0.46 (s, 6H), 0.26 (s, 12H). ^{13}C NMR (126 MHz, CDCl_3) δ 186.11, 158.87, 141.31, 134.71, 132.42, 123.85, 121.92, 25.82, 18.47, -1.39, -4.13.

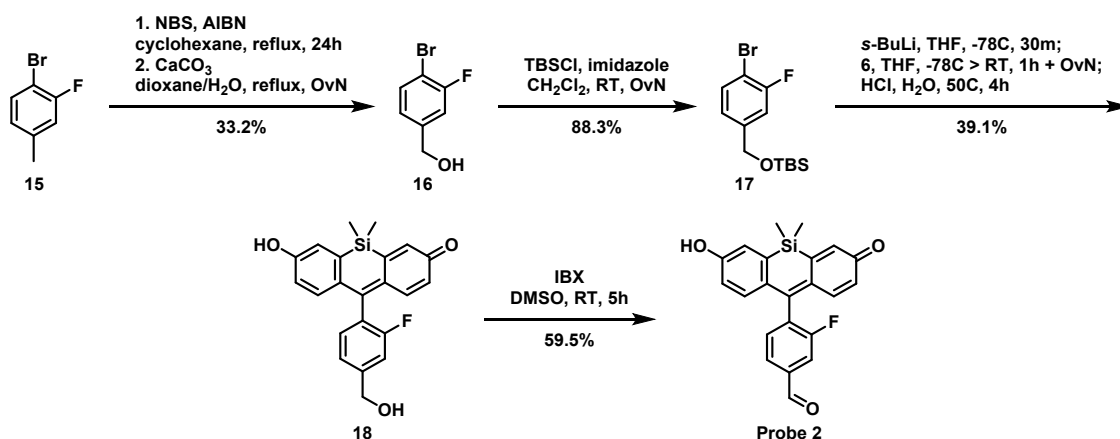


Scheme S2. Synthesis of Probe 1.

((4-Bromo-3-methylbenzyl)oxy)(*tert*-butyl)dimethylsilane (13) A flame-dried round-bottom flask was charged with methyl 4-bromo-3-methylbenzoate (22.9g, 50 mmol, 1.0 eq.) and anhydrous CH_2Cl_2 (100 mL). The reaction was cooled to $0\text{ }^{\circ}\text{C}$ before treating dropwise with 1.0 M DIBAL-H in CH_2Cl_2 (110 mL, 110 mmol, 2.2 eq.) over 23 minutes. After stirring for 30 minutes, the reaction was warmed to room temperature. After 4 hours, the reaction was cooled back to $0\text{ }^{\circ}\text{C}$ before quenching with the sequential addition of H_2O , 1.0 M NaOH in H_2O , and more H_2O . The mixture was filtered. The organics were collected, washed with brine, and concentrated. The crude material was treated with imidazole (6.8 g, 100 mmol, 2.0 eq.) and CH_2Cl_2 (50 mL). Once a solution had formed, *tert*-butyldimethylsilyl chloride (8.2 g, 50 mmol, 1.1 eq.) was added. After stirring overnight at room temperature, the reaction was filtered. The filtrate was washed with aqueous NH_4Cl and concentrated under reduced pressure. The crude material was purified via flash chromatography on a silica column (2:98 v/v EtOAc:hexanes) to afford the title compound as a colorless oil (14.6 g, 46.3 mmol, 92.6% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.47 (d, $J = 8.1$ Hz, 1H), 7.18 (d, $J = 1.4$ Hz, 1H), 7.01 (dd, $J = 8.2, 2.1$ Hz, 1H), 4.66 (s, 2H), 2.39 (s, 3H), 0.94 (s, 9H), 0.10 (s, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 140.83, 137.67, 132.23, 128.64, 125.21, 123.16, 64.49, 26.09, 23.08, 18.57, -5.10.

7-Hydroxy-10-(4-(hydroxymethyl)-2-methylphenyl)-5,5-dimethyldibenzo[b,e]silin-3(5H)-one (14) A flame-dried round-bottom flask was charged with **13** (0.325 g, 1.0 mmol, 10 eq.) and anhydrous THF (5 mL). The reaction was cooled to -78 °C and then treated dropwise with 1.7 M *t*-butyllithium in pentane (0.59 mL, 1.0 mmol, 10 eq.) over 1 minute. After stirring for an additional 8 minutes at -78 °C, the reaction was treated dropwise with a solution of **6** (0.051 g, 0.10 mmol, 1.0 eq.) in anhydrous THF (2 mL) over 2 minutes. 30 minutes after addition, the reaction was warmed to room temperature and stirred for 2 hours. The reaction was treated with 1.0 M HCl in H₂O (3 mL, 3.0 mmol, 30 eq.) and heated to 50 °C. After stirring for 2 hours, the reaction was cooled to room temperature. The reaction was diluted with Et₂O and THF and washed with brine. The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (5:95 v/v MeOH:CH₂Cl₂). The crude material was washed with Et₂O to afford the title compound as a red solid (0.0218 g, 0.0582 mmol, 58.2% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.33 (d, *J* = 1.5 Hz, 1H), 7.29 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.07 (d, *J* = 7.7 Hz, 1H), 7.00 (s, 2H), 6.76 (d, *J* = 9.5 Hz, 2H), 6.43 (d, *J* = 7.8 Hz, 2H), 5.29 (s, 1H), 4.57 (s, 2H), 1.98 (s, 3H), 0.46 (s, 3H), 0.44 (s, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 156.30, 143.67, 142.55, 138.07, 137.45, 134.98, 129.14, 128.84, 128.09, 123.98, 122.02, 62.66, 19.08, -1.28, -1.61. HRMS (ESI⁺): *m/z* calculated for [M+H]⁺ = 375.1411; found = 375.1411.

4-(7-Hydroxy-5,5-dimethyl-3-oxo-3,5-dihydrodibenzo[b,e]silin-10-yl)-3-methylbenzaldehyde (Probe 1) A round-bottom flask was charged with **14** (0.0187 g, 0.050 mmol, 1.0 eq.), 2-iodoxybenzoic acid (0.0169 g, 0.060 mmol, 1.2 eq.), and DMSO (1 mL). After stirring 3 hours at room temperature, the reaction was quenched with brine and filtered. The filtrate was extracted from with 33:67 v/v *i*PrOH:CH₂Cl₂. The organics were combined with the precipitate and purified via flash chromatography on a silica column (0:100 to 5:95 v/v *i*PrOH:CH₂Cl₂ gradient). The resultant solid purified via flash chromatography on a silica column (Et₂O) to afford the title compound as a red solid (0.0047 g, 0.0126 mmol, 25.2% yield). ¹H NMR (500 MHz, CDCl₃) δ 10.09 (s, 1H), 7.86 (s, 1H), 7.85 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.31 (d, *J* = 7.7 Hz, 1H), 7.06 (d, *J* = 2.4 Hz, 2H), 6.81 (d, *J* = 9.5 Hz, 2H), 6.52 (dd, *J* = 9.4, 2.4 Hz, 2H), 2.15 (s, 3H), 0.48 (s, 3H), 0.47 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 191.92, 146.01, 145.13, 138.97, 137.63, 136.51, 131.37, 130.31, 130.15, 129.51, 127.46, 122.56, 19.60, -1.12, -1.34. HRMS (ESI⁺): *m/z* calculated for [M+H]⁺ = 373.1254; found = 373.1259.



Scheme S3. Synthesis of Probe 2.

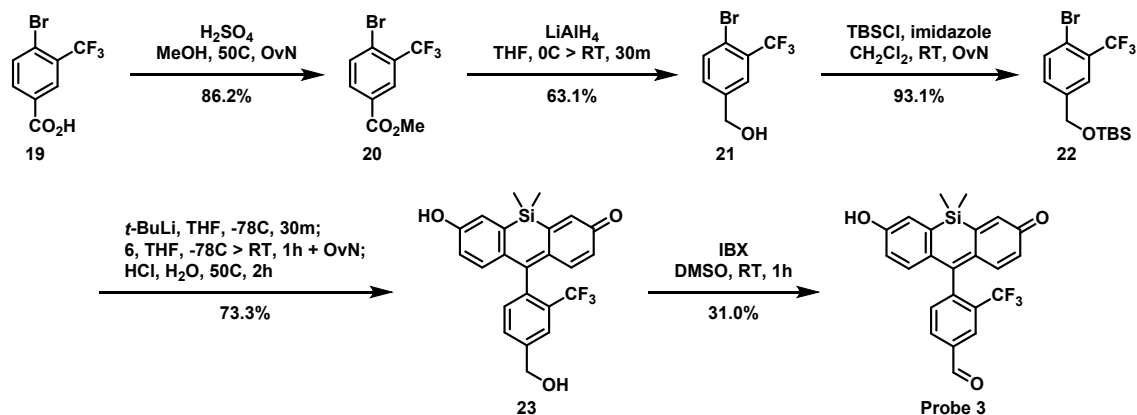
(4-Bromo-3-fluorophenyl)methanol (16) A flame-dried round-bottom flask was charged with 4-bromo-3-fluorotoluene (0.67 mL, 5.3 mmol, 1.0 eq.), *N*-bromosuccinimide (1.04 g, 5.8 mmol, 1.1 eq.), AIBN (0.086 g, 0.53 mmol, 0.1 eq.), and cyclohexane (20 mL). The reaction was heated to reflux. After stirring for 24 hours, the reaction was cooled to RT and concentrated *in vacuo*. The crude material treated with dioxane (10 mL), H₂O (10 mL), and CaCO₃ (1.59 g, 16 mmol, 3.0 eq.). The reaction was heated to reflux. After stirring overnight, the reaction was cooled to room temperature and concentrated *in vacuo*. The crude material was purified via flash chromatography on a silica column (5:95 to 30:70 v/v EtOAc:hexanes gradient) to afford the title compound as a colorless oil (0.360 g, 1.76 mmol, 33.2% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.46 (t, *J* = 7.5 Hz, 1H), 7.08 (d, *J* = 9.1 Hz, 1H), 6.94 (d, *J* = 7.7 Hz, 1H), 4.57 (s, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 159.12 (d, *J* = 247.4 Hz), 142.75 (d, *J* = 6.8 Hz), 133.47, 123.34 (d, *J* = 3.4 Hz), 114.71 (d, *J* = 22.6 Hz), 107.62 (d, *J* = 20.9 Hz), 63.74. ¹⁹F NMR (471 MHz, CDCl₃) δ -107.22 (dd, *J* = 9.2, 7.4 Hz).

((4-Bromo-3-fluorobenzyl)oxy)(tert-butyl)dimethylsilane (17) A round-bottom flask was charged with **16** (0.360 g, 1.76 mmol, 1.0 eq.), imidazole (0.135 g, 1.9 mmol, 1.1 eq.), and CH₂Cl₂ (20 mL). Once a solution had formed, *tert*-butyldimethylsilyl chloride (0.35 g, 2.3 mmol, 1.3 eq.) was added. After stirring overnight at room temperature, the reaction was poured through filter paper. The filtrate was purified via flash chromatography on a silica column (0:100 to 5:95 v/v EtOAc:hexanes gradient) to afford the title compound as a colorless oil (0.490 g, 1.55 mmol, 88.3% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.48 (dd, *J* = 8.2, 7.0 Hz, 1H), 7.12 (d, *J* = 9.6 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 4.68 (s, 2H), 0.94 (s, 9H), 0.10 (s, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 159.25 (d, *J* = 247.1 Hz), 143.72 (d, *J* = 6.3 Hz), 133.27, 122.61 (d, *J* = 3.6 Hz), 114.17 (d, *J* = 22.7 Hz), 106.95 (d, *J* = 21.1 Hz), 63.93 (d, *J* = 1.7 Hz), 26.04, 18.52, -5.16. ¹⁹F NMR (471 MHz, CDCl₃) δ -107.75 (dd, *J* = 9.5, 7.0 Hz).

10-(2-Fluoro-4-(hydroxymethyl)phenyl)-7-hydroxy-5,5-dimethyldibenzo[b,e]silin-3(5H)-one (18) A flame-dried round-bottom flask was charged with **17** (0.095 g, 0.60 mmol, 6.0 eq.) and anhydrous THF (10 mL). The reaction was cooled to -78 °C and then treated dropwise with 1.4 M *s*-butyllithium in cyclohexane (0.46 mL, 0.60 mmol, 6.0 eq.) over 1 minute. After stirring for an additional 30 minutes at -78 °C, the reaction was treated dropwise with a solution of **6** (0.052 g, 0.10 mmol, 1.0 eq.) in anhydrous THF (3 mL) over 3 minutes. 1 hour after addition, the reaction was transferred to an ice bath. The reaction was stirred overnight in the melting ice bath before being treated with 1.0 M HCl in H₂O (4.0 mL, 4.0 mmol, 40 eq.) and heated to 50 °C. After stirring for 4 hours, the reaction was cooled to room temperature and quenched with H₂O. The THF was removed *in vacuo*. The resulting mixture was extracted from with 33:67 v/v *i*PrOH:CH₂Cl₂. The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (0:100 to 5:95 v/v MeOH:CH₂Cl₂ gradient). The resulting solid was washed with CH₂Cl₂ to afford the title compound as a red solid (0.0148 g, 0.0391 mmol, 39.1% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.54 (s, 1H), 7.32 (d, *J* = 9.0 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.03 (s, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.49 (s, 2H), 5.45 (s, 1H), 4.63 (s, 2H), 0.46 (s, 3H), 0.45 (s, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 158.76 (d, *J* = 243.1 Hz), 149.83, 146.30 (d, *J* = 6.8 Hz), 131.99 – 129.79 (m), 124.70 (d, *J* = 17.3 Hz), 122.26 (d, *J* = 2.5 Hz), 113.20 (d, *J* = 21.8 Hz), 62.01, -1.29, -1.55. ¹⁹F NMR (471 MHz, DMSO-*d*₆) δ -115.72 (dd, *J* = 9.4, 7.7 Hz). HRMS (ESI⁺): *m/z* calculated for [M+H]⁺ = 379.1160; found = 379.1162.

3-Fluoro-4-(7-hydroxy-5,5-dimethyl-3-oxo-3,5-dihydrodibenzo[b,e]silin-10-yl)benzaldehyde (Probe 2) A round-bottom flask was charged with **18** (0.0078 g, 0.021 mmol, 1.0 eq.), 2-iodoxybenzoic acid (0.0068 g, 0.025 mmol, 1.2 eq.), and DMSO (0.5 mL). After stirring 5 hours at room temperature, the reaction was quenched with brine. The mixture was chilled and filtered. The filtrate was extracted from with 33:67

iPrOH:CH₂Cl₂. + organics were combined with the precipitate and concentrated. The crude material was purified via flash chromatography on a silica column (0:100 to 2:98 v/v iPrOH:CH₂Cl₂ gradient) to afford the title compound as a red solid (0.0046 g, 0.012 mmol, 59.5% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.62 (s, 1H), 10.12 (s, 1H), 7.95 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.93 (d, *J* = 8.9 Hz, 1H), 7.63 (t, *J* = 7.3 Hz, 1H), 7.27 – 7.19 (m, 1H), 6.93 – 6.79 (m, 2H), 6.79 – 6.68 (m, 2H), 6.25 – 6.07 (m, 1H), 0.47 (s, 3H), 0.46 (s, 3H). ¹⁹F NMR (376 MHz, DMSO-*d*₆) δ -114.38 (t, *J* = 8.2 Hz). HRMS (ESI⁺): *m/z* calculated for [M+H]⁺ = 377.1004; found = 377.1025.



Scheme S4. Synthesis of Probe 3.

Methyl 4-bromo-3-(trifluoromethyl)benzoate (20) A round-bottom flask was charged with 4-bromo-3-(trifluoromethyl)benzoic acid (1.35 g, 5.0 mmol, 1.0 eq.), MeOH (10 mL), and conc. H₂SO₄ (0.5 mL). The reaction was heated to 50 °C. After stirring overnight, the reaction was neutralized with saturated NaHCO₃. The resulting mixture was poured through filter paper. The precipitate was further washed with H₂O and dried to afford the title compound as an off-white solid (1.22 g, 4.3 mmol, 86.2% yield). ¹H NMR (500 MHz, CDCl₃) δ 8.34 (d, *J* = 2.1 Hz, 1H), 8.03 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.80 (d, *J* = 8.3 Hz, 1H), 3.95 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 165.32, 135.47, 133.75, 130.78 (q, *J* = 32.1 Hz), 129.72, 129.06 (q, *J* = 5.4 Hz), 125.58 (q, *J* = 2.0 Hz), 122.64 (q, *J* = 273.7 Hz), 52.83. ¹⁹F NMR (471 MHz, CDCl₃) δ -62.94.

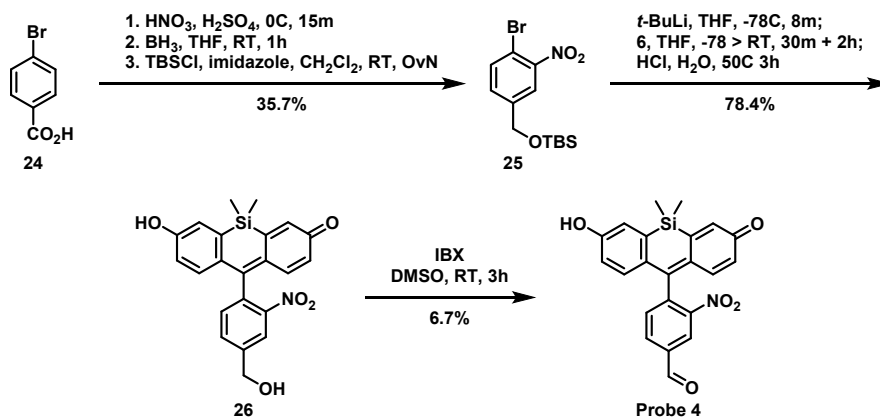
(4-Bromo-3-(trifluoromethyl)phenyl)methanol (21) A flame-dried round-bottom flask was charged with **20** (0.283 g, 1.0 mmol, 1.0 eq.) and anhydrous THF (10 mL). The reaction was cooled to 0 °C before treating with LiAlH₄ (0.038 g, 1.0 mmol, 1.0 eq.) portionwise over 1 minute. After effervescence ceased, the reaction was warmed to room temperature. After 30 minutes, the reaction was cooled to 0 °C and quenched via the sequential addition of H₂O (2 mL), 1.0 M NaOH in H₂O (2 mL), and more H₂O (2 mL). The mixture was diluted with CH₂Cl₂ and filtered. The organics were collected, dried over Na₂SO₄, and concentrated. The crude residue was recrystallized in hexanes to afford the title compound as a white crystalline solid (0.161 g, 0.631 mmol, 63.1% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.69 (s, 1H), 7.68 (d, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 7.4 Hz, 1H), 4.71 (s, 2H), 1.96 (s, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 140.55, 135.16, 131.22, 130.37 (q, *J* = 31.2 Hz), 126.22 (q, *J* = 5.4 Hz), 123.01 (q, *J* = 273.4 Hz), 118.88 (q, *J* = 2.0 Hz), 64.02. ¹⁹F NMR (471 MHz, CDCl₃) δ -62.66.

((4-Bromo-3-(trifluoromethyl)benzyl)oxy)(tert-butyl)dimethylsilane (22) A round-bottom flask was charged with **21** (0.161 g, 0.63 mmol, 1.0 eq.), imidazole (0.055 g, 0.77 mmol, 1.2 eq.), and CH₂Cl₂ (10 mL).

Once a solution had formed, *tert*-butyldimethylsilyl chloride (0.134 g, 0.89 mmol, 1.4 eq.) was added. After stirring overnight at room temperature, the reaction was washed with H₂O. The organics were collected and concentrated under reduced pressure. The crude material was purified via flash chromatography on a silica column (2:98 v/v EtOAc:hexanes) to afford the title compound as a colorless oil (0.217 g, 0.588 mmol, 93.1% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.66 (d, *J* = 7.8 Hz, 1H), 7.66 (s, 1H), 7.34 (d, *J* = 8.3 Hz, 1H), 4.72 (s, 2H), 0.95 (s, 9H), 0.11 (s, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 141.44, 134.86, 130.42, 130.05 (q, *J* = 31.0 Hz), 125.44 (q, *J* = 5.5 Hz), 123.13 (q, *J* = 273.3 Hz), 118.04 (q, *J* = 1.9 Hz), 63.87, 26.00, 18.50, -5.18. ¹⁹F NMR (471 MHz, CDCl₃) δ -62.64.

7-Hydroxy-10-(4-(hydroxymethyl)-2-(trifluoromethyl)phenyl)-5,5-dimethyldibenzo[b,e]silin-3(5H)-one (23) A flame-dried round-bottom flask was charged with **22** (.367 g, 1.0 mmol, 10 eq.) and anhydrous THF (5 mL). The reaction was cooled to -78 °C and then treated dropwise with 1.7 M *t*-butyllithium in pentane (0.6 mL, 1.0 mmol, 10 eq.) over 1 minute. After stirring for an additional 8 minutes at -78 °C, the reaction was treated dropwise with a solution of **6** (.050 g, 0.10 mmol, 1.0 eq.) in anhydrous THF (2 mL) over 30 seconds. After 30 more minutes at -78 °C, the reaction was warmed to room temperature and stirred for 2 hours. The reaction was treated with 1.0 M HCl in H₂O (3 mL, 3 mmol, 30 eq.) and heated to 50 °C. After stirring overnight, the reaction was cooled to room temperature and quenched with brine and diluted with 33:67 v/v *i*PrOH:CH₂Cl₂. The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (10:90 v/v MeOH:CH₂Cl₂). The crude residue was suspended in Et₂O and filtered. The precipitate was dried under vacuum to afford the title compound as a red solid (0.0314 g, 0.0733 mmol, 73.3% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.50 (s, 1H), 7.87 (d, *J* = 1.6 Hz, 1H), 7.74 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.19 (s, 1H), 6.83 (s, 1H), 6.69 (d, *J* = 11.9 Hz, 2H), 6.56 (d, *J* = 8.8 Hz, 1H), 6.13 (d, *J* = 8.8 Hz, 1H), 5.53 (t, *J* = 5.7 Hz, 1H), 4.70 (d, *J* = 5.6 Hz, 2H), 0.50 (s, 3H), 0.39 (s, 3H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 182.83, 159.24, 152.50, 146.66, 143.93, 140.94, 139.98, 136.36, 135.81, 135.54, 131.87, 131.40, 130.15, 127.61, 127.25 (q, *J* = 29.5 Hz), 126.80, 124.01 (q, *J* = 4.6 Hz), 123.99 (q, *J* = 274.6 Hz), 121.81, 116.90, 61.92, -0.84, -2.47. ¹⁹F NMR (471 MHz, DMSO-*d*₆) δ -57.90. HRMS (ESI⁺): *m/z* calculated for [M+H]⁺ = 429.1128; found = 429.1129.

4-(7-Hydroxy-5,5-dimethyl-3-oxo-3,5-dihydrodibenzo[b,e]silin-10-yl)-3-(trifluoromethyl)benzaldehyde (Probe 3) A round-bottom flask was charged with **23** (0.0269 g, 0.063 mmol, 1.0 eq.), 2-iodoxybenzoic acid (0.021 g, 0.075 mmol, 1.2 eq.), and DMSO (3 mL). After stirring 1 hour at room temperature, the reaction was quenched with brine. The resulting mixture was poured through over filter paper. The precipitate was purified via flash chromatography on a silica column (2:98 to 10:90 v/v MeOH:CH₂Cl₂ gradient) to afford the title compound as a red solid (0.0083 g, 0.019 mmol, 31.0% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.55 (s, 1H), 10.21 (s, 1H), 8.46 (d, *J* = 1.4 Hz, 1H), 8.32 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.21 (s, 1H), 6.86 (s, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.67 (d, *J* = 9.5 Hz, 1H), 6.52 (d, *J* = 8.8 Hz, 1H), 6.13 (d, *J* = 10.2 Hz, 1H), 0.51 (s, 3H), 0.40 (s, 3H). ¹⁹F NMR (471 MHz, DMSO-*d*₆) δ -58.37. HRMS (ESI⁺): *m/z* calculated for [M+H]⁺ = 427.0972; found = 427.0977.

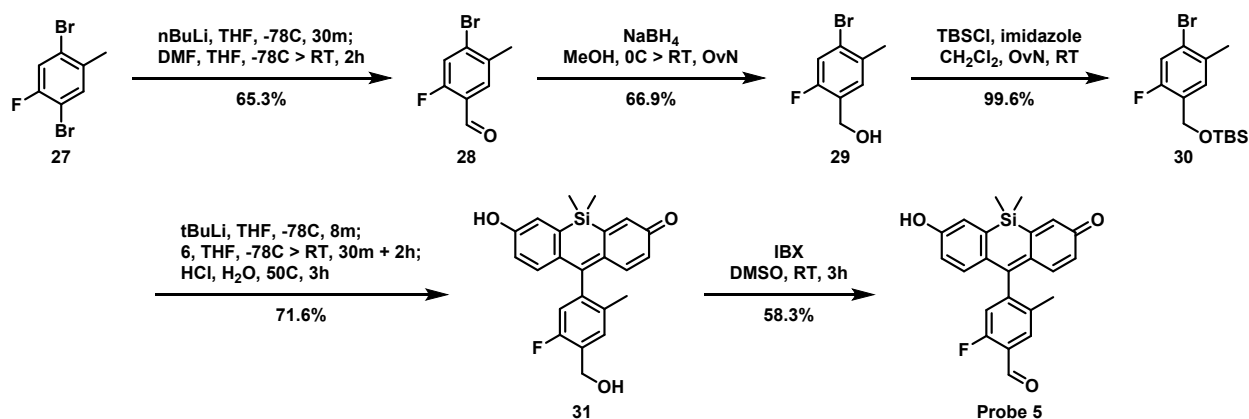


Scheme S5. Synthesis of Probe 4.

((4-Bromo-3-nitrobenzyl)oxy)(tert-butyl)dimethylsilane (25) A round-bottom flask was charged with 4-bromobenzoic acid (1.04 g, 5.62 mmol, 1.0 eq.) and H_2SO_4 (20 mL). The reaction was cooled to 0°C and the treated dropwise with a solution of HNO_3 (0.25 mL, 5.9 mmol, 1.05 eq.) in H_2SO_4 (3 mL) over 4 minutes. After stirring for 15 minutes at 0°C , the reaction poured over ice. Product was extracted with EtOAc, dried over Na_2SO_4 , and concentrated. The white solid was dissolved in anhydrous THF. While stirring at room temperature, the reaction was treated dropwise with 1.0 M $\text{BH}_3\cdot\text{THF}$ in THF (17 mL, 17 mmol, 3.3 eq.) over 30 seconds. After stirring for 1 hour, the reaction was quenched by slow addition of 1.0 M NaOH in H_2O . The mixture was diluted with Et_2O . The organics were collected, dried over Na_2SO_4 , and concentrated. The crude residue was treated with imidazole (0.36 g, 5.4 mmol, 1.0 eq.), and CH_2Cl_2 (10 mL). Once a solution had formed, *tert*-butyldimethylsilyl chloride (0.82 g, 5.4 mmol, 1.0 eq.) was added. After stirring overnight at room temperature, the reaction was filtered. The crude filtrate was purified via flash chromatography on a silica column (2:98 to 5:95 v/v EtOAc:hexanes gradient) to afford the title compound as a yellow oil (0.695 g, 2.01 mmol, 35.7% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.81 (dd, $J = 2.0, 0.9$ Hz, 1H), 7.68 (d, $J = 8.2$ Hz, 1H), 7.37 (ddd, $J = 8.2, 2.0, 0.9$ Hz, 1H), 4.74 (s, 2H), 0.94 (s, 9H), 0.12 (s, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 149.91, 143.04, 134.88, 130.51, 123.02, 112.39, 63.44, 26.00, 18.49, -5.19.

7-Hydroxy-10-(4-(hydroxymethyl)-2-nitrophenyl)-5,5-dimethyldibenzo[b,e]silin-3(5H)-one (26) A flame-dried round-bottom flask was charged with **25** (0.345 g, 1.0 mmol, 10 eq.) and anhydrous THF (5 mL). The reaction was cooled to -78°C and then treated dropwise with 1.7 M *t*-butyllithium in pentane (0.60 mL, 1.0 mmol, 10 eq.) over 1 minute. After stirring for an additional 8 minutes at -78°C , the reaction was treated dropwise with a solution of **6** (0.050 g, 0.10 mmol, 1.0 eq.) in anhydrous THF (2 mL) over 30 seconds. 30 minutes after addition, the reaction was warmed to room temperature and stirred for 2 hours. The reaction was treated with 1.0 M HCl in H_2O (3 mL, 3.0 mmol, 30 eq.) and heated to 50°C . After stirring for 3 hours, the reaction was cooled to room temperature. The reaction was diluted with CH_2Cl_2 and THF and washed with brine. The organics were collected, dried over Na_2SO_4 , and concentrated. The crude material was purified via flash chromatography on a silica column (5:95 to 10:90 v/v *i*PrOH: CH_2Cl_2 gradient). The crude material was washed with CH_2Cl_2 to afford the title compound as a red solid (0.0318 g, 0.0784 mmol, 78.4% yield). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 10.51 (s, 1H), 8.28 (s, 1H), 7.85 (d, $J = 8.5$ Hz, 1H), 7.48 (d, $J = 7.8$ Hz, 1H), 7.02 (s, 2H), 6.69 (d, $J = 9.3$ Hz, 2H), 6.43 (s, 2H), 5.65 (s, 1H), 4.74 (s, 2H), 0.50 (s, 3H), 0.44 (s, 3H). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 152.56, 147.71, 145.49, 132.72, 131.93, 131.80, 122.20, 61.58, -1.05, -2.19.

4-(7-Hydroxy-5,5-dimethyl-3-oxo-3,5-dihydrodibenzo[b,e]silin-10-yl)-3-nitrobenzaldehyde (Probe 4) A round-bottom flask was charged with **26** (0.0164 g, 0.040 mmol, 1.0 eq.), 2-iodoxybenzoic acid (0.0138 g, 0.049 mmol, 1.2 eq.), and DMSO (1 mL). After stirring 3 hours at room temperature, the reaction was concentrated under reduced pressure. The crude material was purified via flash chromatography on a silica column (5:95 v/v iPrOH:CH₂Cl₂). The resultant solid was washed with CH₂Cl₂ to afford the title compound as a red solid (0.0011 g, 0.0027 mmol, 6.7% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.60 (s, 1H), 8.72 (s, 1H), 8.37 (d, *J* = 7.9 Hz, 1H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.23 (s, 1H), 6.71 (s, 4H), 6.14 (s, 1H), 0.49 (s, 3H), 0.44 (s, 3H). HRMS (ESI⁺): *m/z* calculated for [M+H]⁺ = 404.0949; found = 404.0963.



Scheme S6. Synthesis of Probe 5.

4-Bromo-2-fluoro-5-methylbenzaldehyde (28) A flame-dried round-bottom flask was charged with 1,4-dibromo-2-fluoro-5-methylbenzene (3.2 g, 12 mmol, 1.0 eq.) and anhydrous THF (30 mL). The reaction was cooled to -78 °C and then treated dropwise with 2.5 M *n*-butyllithium in hexanes (5.03 mL, 12.6 mmol, 1.05 eq.) over 10 minutes. After stirring an additional 30 minutes at room -78 °C, the reaction was treated dropwise with a solution of DMF (1.67 mL, 21.6 mmol, 1.8 eq.) in anhydrous THF (3 mL) over 5 minutes. The reaction was warmed to room temperature. After 2 hours, the reaction was quenched with brine and diluted with EtOAc. The organics were collected, dried over Na₂SO₄, and concentrated under reduced pressure. The crude material was purified via flash chromatography on a silica column (10:90 v/v CH₂Cl₂:hexanes) to afford the title compound as a white solid (1.70 g, 7.83 mmol, 65.3% yield). ¹H NMR (500 MHz, CDCl₃) δ 10.29 (s, 1H), 7.71 (d, *J* = 7.3 Hz, 1H), 7.42 (d, *J* = 9.6 Hz, 1H), 2.41 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 186.58 (d, *J* = 5.8 Hz), 162.27 (d, *J* = 260.3 Hz), 135.12 (d, *J* = 3.9 Hz), 132.53 (d, *J* = 9.7 Hz), 129.56 (d, *J* = 2.4 Hz), 123.09 (d, *J* = 8.2 Hz), 120.78 (d, *J* = 23.2 Hz), 22.20. ¹⁹F NMR (471 MHz, CDCl₃) δ -125.06 (t, *J* = 8.5 Hz).

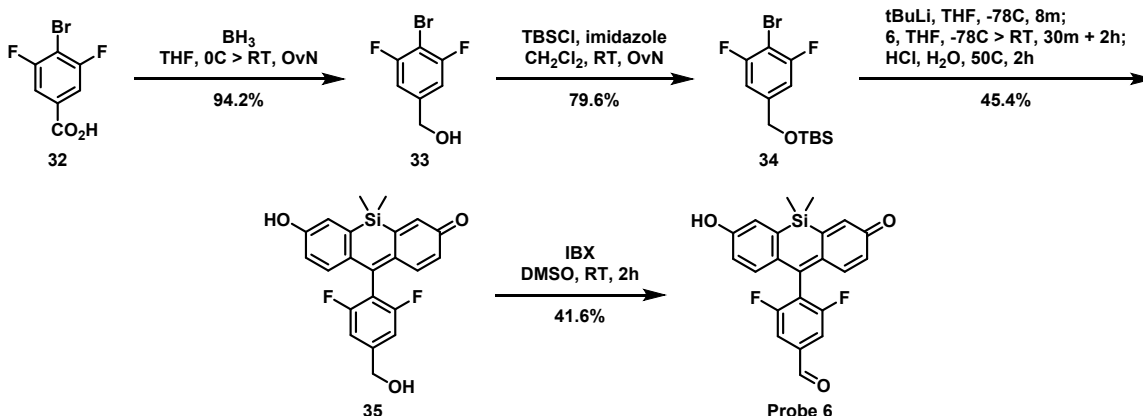
(4-Bromo-2-fluoro-5-methylphenyl)methanol (29) A round-bottom flask was charged with **38** (0.508 g, 2.34 mmol, 1.0 eq.) were treated with MeOH (10 mL). After cooling to 0 °C, the solution was treated with NaBH₄ (0.090 g, 2.34 mmol, 1.0 eq.) The reaction was warmed to room temperature. After stirring overnight, the reaction was quenched with sequential addition of H₂O (5 mL), 1.0 M NaOH in H₂O (5 mL), and more H₂O (5 mL). MeOH was removed under reduced pressure. Product was extracted with CH₂Cl₂. The organics were collected, dried over Na₂SO₄, and concentrated to afford the title compound as a colorless oil (0.343 g, 1.57 mmol, 66.9% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.29 (d, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 9.3 Hz, 1H), 4.69 (s, 2H), 2.36 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 158.42 (d, *J* = 248.1 Hz), 133.98 (d, *J*

= 3.7 Hz), 130.81 (d, $J = 4.8$ Hz), 126.92 (d, $J = 14.7$ Hz), 123.71 (d, $J = 9.6$ Hz), 119.40 (d, $J = 24.5$ Hz), 59.08 (d, $J = 3.8$ Hz), 22.18. ^{19}F NMR (471 MHz, CDCl_3) δ -122.30 (t, $J = 8.7$ Hz).

((4-Bromo-2-fluoro-5-methylbenzyl)oxy)(tert-butyl)dimethylsilane (30) A round-bottom flask was charged with **39** (0.219 g, 1.0 mmol, 1.0 eq.), imidazole (0.136 g, 2.0 mmol, 2.0 eq.), and CH_2Cl_2 (10 mL). Once a solution had formed, *tert*-butyldimethylsilyl chloride (0.301 g, 2.0 mmol, 2.0 eq.) was added. After stirring overnight at room temperature, the reaction was washed with 1.0 M HCl in H_2O . The organics were collected, dried over Na_2SO_4 , and concentrated under reduced pressure to afford the title compound as a colorless oil (0.332 g, 0.996 mmol, 99.6% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.32 (d, $J = 7.8$ Hz, 1H), 7.20 (d, $J = 9.3$ Hz, 1H), 4.70 (s, 2H), 2.36 (s, 3H), 0.94 (s, 9H), 0.11 (s, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 157.66 (d, $J = 247.3$ Hz), 133.59 (d, $J = 3.4$ Hz), 129.98 (d, $J = 5.2$ Hz), 127.66 (d, $J = 14.4$ Hz), 122.65 (d, $J = 9.2$ Hz), 118.88 (d, $J = 24.4$ Hz), 58.76 (d, $J = 4.3$ Hz), 26.06, 22.31, 18.57, -5.21. ^{19}F NMR (471 MHz, CDCl_3) δ -122.56 (t, $J = 8.6$ Hz).

10-(5-Fluoro-4-(hydroxymethyl)-2-methylphenyl)-7-hydroxy-5,5-dimethyldibenzo[b,e]silin-3(5H)-one (31) A flame-dried round-bottom flask was charged with **40** (0.334 g, 1.0 mmol, 10 eq.) and anhydrous THF (5 mL). The reaction was cooled to -78 °C and then treated dropwise with 1.7 M *t*-butyllithium in pentane (0.59 mL, 1.0 mmol, 10 eq.) over 1 minute. After stirring for an additional 8 minutes at -78 °C, the reaction was treated dropwise with a solution of **6** (0.051 g, 0.10 mmol, 1.0 eq.) in anhydrous THF (2 mL) over 1 minute. After addition, the reaction was warmed to room temperature and stirred for 2 hours. The reaction was treated with 1.0 M HCl in H_2O (3 mL, 3 mmol, 30 eq.) and heated to 50 °C. After stirring for 3 hours, the reaction was cooled to room temperature and quenched with brine. The organics were collected, dried over Na_2SO_4 , and concentrated. The crude material was purified via flash chromatography on a silica column (2:98 v/v MeOH: CH_2Cl_2). The crude residue was dissolved in CH_2Cl_2 , triturated with hexanes, and collected by filtration to afford the title compound as a red solid (0.0281 g, 0.0716 mmol, 71.6% yield). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 10.52 (s, 1H), 7.47 (d, $J = 7.4$ Hz, 1H), 7.18 (s, 1H), 7.03 (d, $J = 10.1$ Hz, 1H), 6.77 (s, 4H), 6.19 (s, 1H), 5.35 (t, $J = 5.7$ Hz, 1H), 4.62 (d, $J = 5.3$ Hz, 2H), 1.96 (s, 3H), 0.47 (s, 3H), 0.44 (s, 3H). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 157.66 (d, $J = 244.5$ Hz), 139.18 (d, $J = 7.6$ Hz), 131.14 (d, $J = 3.3$ Hz), 130.59 (d, $J = 4.8$ Hz), 128.81 (d, $J = 15.0$ Hz), 115.60 (d, $J = 22.2$ Hz), 56.62 (d, $J = 3.6$ Hz), 18.25, -1.33, -1.65. ^{19}F NMR (471 MHz, $\text{DMSO}-d_6$) δ -124.04 (t, $J = 8.8$ Hz). HRMS (ESI⁺): m/z calculated for $[\text{M}+\text{H}]^+$ = 393.1317; found = 393.1321.

2-Fluoro-4-(7-hydroxy-5,5-dimethyl-3-oxo-3,5-dihydrodibenzo[b,e]silin-10-yl)-5-methylbenzaldehyde (Probe 5) A round-bottom flask was charged with **41** (0.0069 g, 0.018 mmol, 1.0 eq.), 2-iodoxybenzoic acid (0.0060 g, 0.021 mmol, 1.2 eq.), and DMSO (1 mL). After stirring 3 hours at room temperature, the reaction was concentrated. The crude material was purified via flash chromatography on a silica column (5:95 v/v *i*PrOH: CH_2Cl_2) to afford the title compound as a red solid (0.0040 g, 0.0102 mmol, 58.3% yield). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 10.57 (s, 1H), 10.29 (s, 1H), 7.87 (d, $J = 6.8$ Hz, 1H), 7.38 (d, $J = 10.5$ Hz, 1H), 7.22 (d, $J = 2.7$ Hz, 1H), 6.85 (s, 1H), 6.80 (d, $J = 10.4$ Hz, 1H), 6.75 (d, $J = 7.8$ Hz, 1H), 6.67 (d, $J = 8.6$ Hz, 1H), 6.17 (d, $J = 10.1$ Hz, 1H), 2.03 (s, 3H), 0.48 (s, 3H), 0.45 (s, 3H). ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 187.58, 182.82, 161.48 (d, $J = 257.5$ Hz), 159.62 (d, $J = 1.8$ Hz), 152.52, 147.70 (d, $J = 8.8$ Hz), 146.58, 140.54, 140.16, 136.66, 134.78, 132.71 (d, $J = 3.2$ Hz), 130.57, 130.01, 127.51, 126.48, 123.29 (d, $J = 8.0$ Hz), 122.30, 117.66 (d, $J = 20.7$ Hz), 117.29, 18.00, -1.36, -1.64. ^{19}F NMR (471 MHz, $\text{DMSO}-d_6$) δ -124.88 (dd, $J = 10.5, 7.1$ Hz). HRMS (ESI⁺): m/z calculated for $[\text{M}+\text{H}]^+$ = 391.1160; found = 391.1163.



Scheme S7. Synthesis of Probe 6.

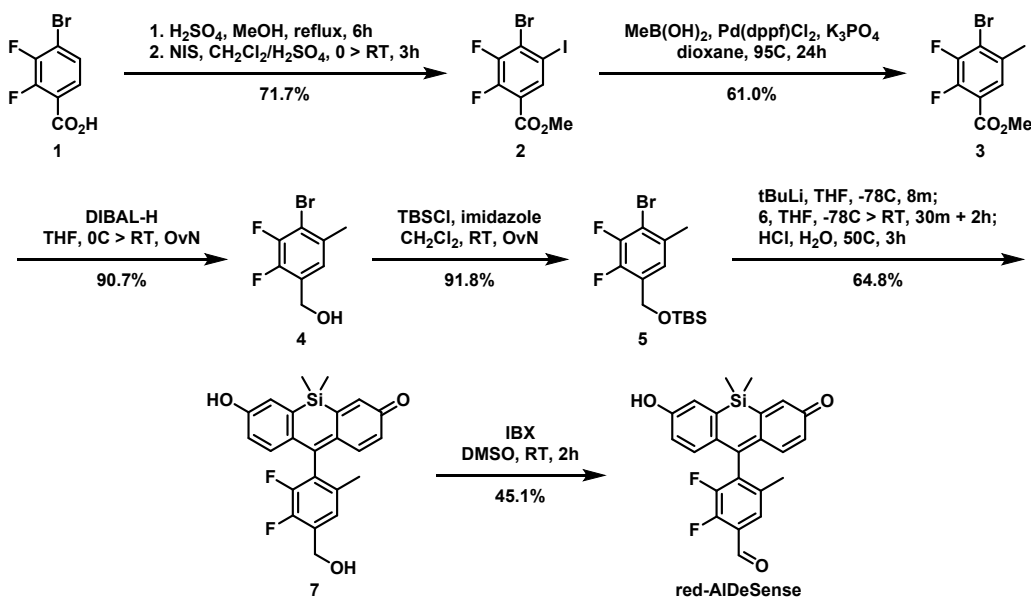
(4-Bromo-3,5-difluorophenyl)methanol (33) A round-bottom flask was charged with 4-bromo-3,5-difluorobenzoic acid (0.234 g, 1.0 mmol, 1.0 eq.) and anhydrous THF (10 mL). The solution was cooled to 0 °C and then treated dropwise with 1.0 M BH₃-THF in THF (3.0 mL, 3.0 mmol, 3.0 eq.) over 3 minutes. The reaction was removed from the cold bath. After stirring at room temperature overnight, the reaction was cooled to 0 °C and quenched with H₂O. The THF was removed under reduced pressure. Product was extracted with EtOAc. The organics were collected, washed with brine, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (25:75 to 50:50 v/v EtOAc:hexanes gradient) to afford the title compound as a white solid (0.210 g, 0.942 mmol, 94.2% yield). ¹H NMR (500 MHz, CDCl₃) δ 6.97 (d, *J* = 7.0 Hz, 2H), 4.67 (s, 2H), 1.91 (s, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 160.13 (dd, *J* = 249.2, 4.5 Hz), 143.27 (t, *J* = 8.1 Hz), 111.98 – 108.81 (m), 96.48 (t, *J* = 24.5 Hz), 63.78 (t, *J* = 2.0 Hz). ¹⁹F NMR (471 MHz, CDCl₃) δ -105.06 (d, *J* = 7.1 Hz).

((4-Bromo-3,5-difluorobenzyl)oxy)(tert-butyl)dimethylsilane (34) A round-bottom flask was charged with **33** (0.492 g, 2.21 mmol, 1.0 eq.) imidazole (0.165 g, 2.4 mmol, 1.1 eq.) and CH₂Cl₂ (10 mL). Once a solution had formed, *tert*-butyldimethylsilyl chloride (0.365 g, 2.4 mmol, 1.1 eq.) was added. After stirring overnight at room temperature, the reaction was filtered. The filtrate was washed with 1.0 M HCl in H₂O, dried over Na₂SO₄, and concentrated under reduced pressure to afford the title compound as a colorless oil (0.592 g, 1.76 mmol, 79.6% yield). ¹H NMR (500 MHz, CDCl₃) δ 6.93 (d, *J* = 7.8 Hz, 2H), 4.67 (s, 2H), 0.94 (s, 9H), 0.11 (s, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 160.01 (dd, *J* = 248.4, 4.5 Hz), 144.23 (t, *J* = 8.1 Hz), 109.40 – 108.88 (m), 95.65 (t, *J* = 24.7 Hz), 63.70 (t, *J* = 2.1 Hz), 26.00, 18.50, -5.22. ¹⁹F NMR (471 MHz, CDCl₃) δ -105.65 (d, *J* = 7.1 Hz).

10-(2,6-Difluoro-4-(hydroxymethyl)phenyl)-7-hydroxy-5,5-dimethyldibenzo[b,e]silin-3(5H)-one (35) A flame-dried round-bottom flask was charged with **35** (0.340 g, 1.0 mmol, 10 eq.) and anhydrous THF (5 mL). The reaction was cooled to -78 °C and then treated dropwise with 1.7 M *t*-butyllithium in pentane (0.60 mL, 1.0 mmol, 1.0 eq.) over 5 minutes. After stirring for an additional 8 minutes at -78 °C, the reaction was treated dropwise with a solution of **6** (0.050 g, 0.10 mmol, 1.0 eq.) in anhydrous THF (3 mL) over 4 minutes. After addition, the reaction was warmed to room temperature and stirred for 2 hours. The reaction was treated with 3.0 M HCl (1.0 mL, 3.0 mmol, 30 eq.) and heated to 50 °C. After stirring for 2 hours, the reaction was cooled to room temperature and quenched with brine. The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (5:95 v/v MeOH:CH₂Cl₂ gradient) to afford the title compound as a red solid (0.018 g,

0.0454 mmol, 45.4% yield). ^1H NMR (500 MHz, Methanol- d_4) δ 7.19 (d, J = 8.1 Hz, 2H), 7.05 (s, 2H), 7.03 (d, J = 5.2 Hz, 2H), 6.50 (d, J = 9.4 Hz, 2H), 4.74 (s, 2H), 0.50 (s, 6H). ^{13}C NMR (126 MHz, Methanol- d_4) δ 161.04 (dd, J = 246.8, 7.4 Hz), 148.71 (d, J = 8.6 Hz), 147.83, 131.39, 115.64 (t, J = 22.1 Hz), 111.97 – 106.78 (m), 63.77, -1.50. ^{19}F NMR (471 MHz, Methanol- d_4) δ -114.31 (d, J = 8.1 Hz). HRMS (ESI $^+$): m/z calculated for $[\text{M}+\text{H}]^+$ = 397.1066; found = 397.1070.

3,5-Difluoro-4-(7-hydroxy-5,5-dimethyl-3-oxo-3,5-dihydrodibenzo[b,e]silin-10-yl)benzaldehyde (Probe 6) A round-bottom flask was charged with **36** (0.0157 g, 0.040 mmol, 1.0 eq.), 2-iodoxybenzoic acid (0.014 g, 0.048 mmol, 1.2 eq.), and DMSO (1 mL). After stirring 2 hours at room temperature, the reaction was concentrated. The crude material was purified via flash chromatography on a silica column (5:95 v/v iPrOH:CH $_2$ Cl $_2$) to afford the title compound as a red solid (0.0065 g, 0.0165 mmol, 41.6% yield). ^1H NMR (500 MHz, CDCl $_3$) δ 10.05 (t, J = 1.6 Hz, 1H), 7.61 (d, J = 6.0 Hz, 2H), 7.03 (d, J = 2.5 Hz, 2H), 6.89 (dt, J = 9.4, 1.0 Hz, 2H), 6.55 (dd, J = 9.5, 2.4 Hz, 2H), 0.46 (s, 6H). ^{13}C NMR (126 MHz, CDCl $_3$) δ 188.90, 160.41 (dd, J = 252.7, 6.3 Hz), 144.37, 142.98, 138.84 (t, J = 7.3 Hz), 137.48, 130.61, 129.90, 123.05, 122.75 (t, J = 22.1 Hz), 112.98 – 112.28 (m), -1.30. ^{19}F NMR (471 MHz, CDCl $_3$) δ -107.90 (d, J = 6.1 Hz). HRMS (ESI $^+$): m/z calculated for $[\text{M}+\text{H}]^+$ = 395.0910; found = 395.0916.



Scheme S8. Synthesis of red-AIDeSense.

Methyl 4-bromo-2,3-difluoro-5-iodobenzoate (2) A round-bottom flask was charged with 4-bromo-2,3-difluorobenzoic acid (1.00 g, 4.22 mmol, 1.0 eq.) and MeOH (5 mL). The reaction was cooled to 0 °C and then treated dropwise with conc. H $_2$ SO $_4$ (1 mL) over 30 seconds. The reaction was heated to reflux. After 6 hours, the reaction was poured into H $_2$ O and diluted with EtOAc. The organics were collected, washed with 1.0 M NaOH in H $_2$ O, dried over Na $_2$ SO $_4$, and concentrated under reduced pressure. The material was dissolved in CH $_2$ Cl $_2$ (4 mL) and cooled to 0 °C. The reaction was treated dropwise with conc. H $_2$ SO $_4$ (4 mL) over 2 minutes. While still cold, the reaction was treated portionwise with *N*-iodosuccinimide (1.41 g, 6.3

mmol, 1.5 eq.) over 2 minutes. The reaction was warmed to room temperature. After stirring for 3 hours, the reaction was quenched via addition of ice. The organics were collected, washed with saturated NaHCO₃, washed with aqueous Na₂S₂O₃, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (10:90 v/v CH₂Cl₂:hexanes) to afford the title compound as a white solid (1.14 g, 3.02 mmol, 71.7% yield). ¹H NMR (500 MHz, CDCl₃) δ 8.21 (dd, *J* = 6.4, 1.3 Hz, 1H), 3.94 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 162.38 (t, *J* = 3.4 Hz), 150.25 (dd, *J* = 267.8, 15.4 Hz), 148.70 (dd, *J* = 253.4, 15.2 Hz), 136.20 (d, *J* = 4.0 Hz), 124.04 (d, *J* = 18.7 Hz), 120.69 (d, *J* = 8.0 Hz), 94.51 (dd, *J* = 5.1, 2.5 Hz), 53.12. ¹⁹F NMR (471 MHz, CDCl₃) δ -116.92 (d, *J* = 21.2 Hz), -130.87 (dd, *J* = 21.1, 6.6 Hz).

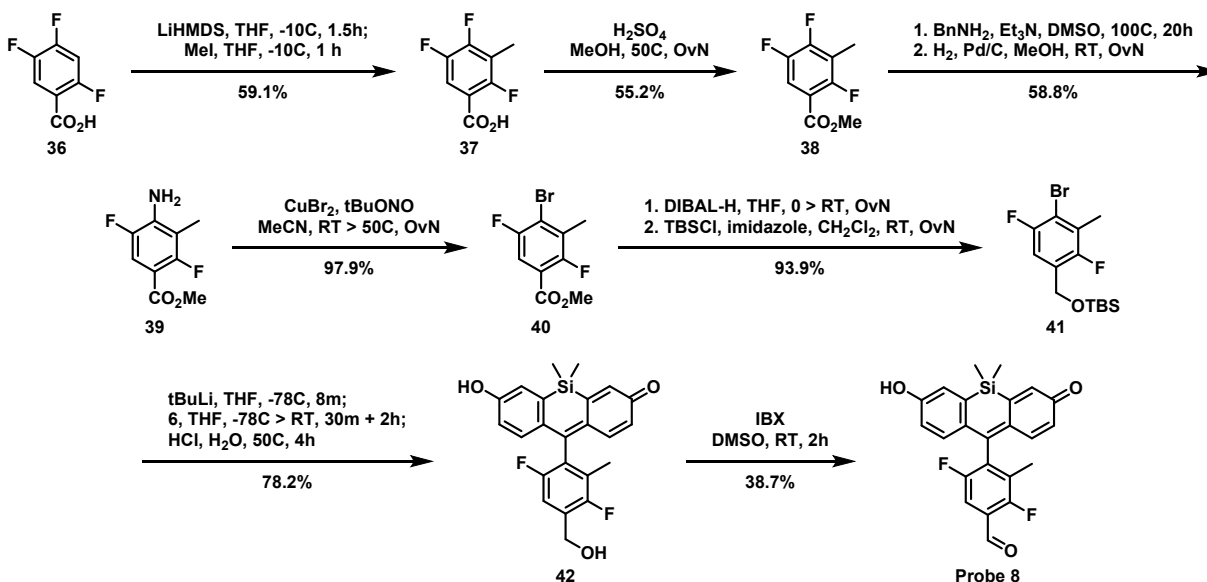
Methyl 4-bromo-2,3-difluoro-5-methylbenzoate (3) A pressure flask was charged with **2** (0.900 g, 2.39 mmol, 1.0 eq.), methylboronic acid (0.286 g, 4.78 mmol, 2.0 eq.), K₃PO₄ (1.01 g, 4.78 mmol, 2.0 eq.), and anhydrous 1,4-dioxane (10 mL). The mixture was degassed for 15 minutes via concurrent sparging with N₂ and sonication before being treated with Pd(dppf)Cl₂ (0.053 g, 0.072 mmol, 0.03 eq.). The reaction was sealed and heated to 95 °C. After stirring for 24 hours, the reaction was cooled, poured into H₂O. Product was extracted with Et₂O. The organics were combined, washed with H₂O, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (10:90 v/v CH₂Cl₂:hexanes) to afford the title compound as a white solid (0.386 g, 1.46 mmol, 61.0% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.59 (dd, *J* = 6.7, 2.1 Hz, 1H), 3.93 (s, 3H), 2.40 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 163.80 (t, *J* = 3.6 Hz), 148.63 (dd, *J* = 247.9, 14.8 Hz), 148.51 (dd, *J* = 263.8, 15.4 Hz), 134.65 (d, *J* = 4.5 Hz), 126.62 (d, *J* = 3.4 Hz), 118.52 (d, *J* = 7.3 Hz), 117.99 (d, *J* = 17.4 Hz), 52.77, 22.12 (d, *J* = 2.3 Hz). ¹⁹F NMR (471 MHz, CDCl₃) δ -125.94 (dd, *J* = 21.7, 2.5 Hz), -135.08 (dd, *J* = 21.4, 6.7 Hz).

(4-Bromo-2,3-difluoro-5-methylphenyl)methanol (4) A flame-dried round-bottom flask was charged with **3** (0.386 g, 1.46 mmol, 1.0 eq.) and anhydrous THF (10 mL). The reaction was cooled to 0 °C before treating dropwise with 1.0 M DIBAL-H in hexanes (3.2 g, 3.2 mmol, 2.2 eq.) over 3 minutes. The reaction was slowly warmed to room temperature with the melting ice bath. After stirring overnight, the reaction was concentrated and resuspended in 1.0 M NaOH in H₂O. Product was extracted with Et₂O, dried over Na₂SO₄, and concentrated to afford the title compound as a white solid (0.313 g, 1.32 mmol, 90.7% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.12 (dd, *J* = 6.8, 2.0 Hz, 1H), 4.73 (d, *J* = 4.9 Hz, 2H), 2.39 (s, 3H), 1.84 (t, *J* = 6.1 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 147.74 (dd, *J* = 247.0, 14.3 Hz), 146.70 (dd, *J* = 249.0, 14.4 Hz), 134.76 (d, *J* = 4.2 Hz), 128.30 (d, *J* = 11.6 Hz), 124.23 (t, *J* = 3.4 Hz), 111.74 (d, *J* = 17.2 Hz), 58.78 (dd, *J* = 3.7, 2.7 Hz), 22.26 (d, *J* = 2.4 Hz). ¹⁹F NMR (471 MHz, CDCl₃) δ -128.37 (dd, *J* = 21.7, 2.5 Hz), -144.51 (dd, *J* = 21.9, 6.7 Hz).

((4-Bromo-2,3-difluoro-5-methylbenzyl)oxy)(tert-butyl)dimethylsilane (5) A round-bottom flask was charged with **4** (0.313 g, 1.32 mmol, 1.0 eq.), imidazole (0.111 g, 1.6 mmol, 1.2 eq.), and CH₂Cl₂ (10 mL). Once a solution had formed, *tert*-butyldimethylsilyl chloride (0.235 g, 1.6 mmol, 1.2 eq.) was added. After stirring overnight at room temperature, the reaction was washed with 1.0 M HCl in H₂O. The organics were collected, dried over Na₂SO₄, and concentrated under reduced pressure to afford the title compound as a colorless oil (0.426 g, 1.21 mmol, 91.8% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.14 (dd, *J* = 7.1, 2.0 Hz, 1H), 4.74 (s, 2H), 2.39 (s, 3H), 0.95 (s, 9H), 0.13 (s, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 147.48 (dd, *J* = 246.3, 14.2 Hz), 146.02 (dd, *J* = 248.2, 14.4 Hz), 134.29 (d, *J* = 4.1 Hz), 129.08 (d, *J* = 11.4 Hz), 123.51 (t, *J* = 3.5 Hz), 110.73 (d, *J* = 17.1 Hz), 58.64 (dd, *J* = 4.0, 2.9 Hz), 26.01, 22.35 (d, *J* = 2.3 Hz), 18.53, -5.26. ¹⁹F NMR (471 MHz, CDCl₃) δ -129.36 (dd, *J* = 22.0, 2.2 Hz), -144.86 (dd, *J* = 21.8, 6.9 Hz).

10-(3,6-Difluoro-4-(hydroxymethyl)-2-methylphenyl)-7-hydroxy-5,5-dimethyldibenzo[b,e]silin-3(5H)-one (7) A flame-dried round-bottom flask was charged with **5** (0.352 g, 1.0 mmol, 10 eq.) and anhydrous THF (5 mL). The reaction was cooled to -78 °C and then treated dropwise with 1.7 M *t*-butyllithium in pentane (0.59 mL, 1.0 mmol, 10 eq.) over 1 minute. After stirring for an additional 8 minutes at -78 °C, the reaction was treated dropwise with a solution of **6** (0.052 g, 0.10 mmol, 1.0 eq.) in anhydrous THF (2 mL) over 30 seconds. After addition, the reaction was warmed to room temperature and stirred for 2 hours. The reaction was treated with 1.0 M HCl in H₂O (3 mL, 3 mmol, 30 eq.) and heated to 50 °C. After stirring for 3 hours, the reaction was cooled to room temperature and quenched with brine. The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (5:95 to 10:95 v/v MeOH:CH₂Cl₂ gradient). The crude material was further purified via flash chromatography on a silica column (Et₂O) to afford the title compound as a red solid (0.0266 g, 0.0648 mmol, 64.8% yield). ¹H NMR (500 MHz, Methanol-*d*₄) δ 7.33 (d, *J* = 6.1 Hz, 1H), 7.06 (d, *J* = 2.5 Hz, 2H), 6.97 (d, *J* = 9.4 Hz, 2H), 6.51 (d, *J* = 8.7 Hz, 2H), 4.79 (s, 2H), 2.05 (s, 3H), 0.54 (s, 3H), 0.51 (s, 3H). ¹⁹F NMR (471 MHz, Methanol-*d*₄) δ -142.01 (d, *J* = 21.7 Hz), -149.82 (dd, *J* = 21.7, 6.4 Hz). HRMS (ESI⁺): *m/z* calculated for [M+H]⁺ = 411.1223; found = 411.1226.

2,5-Difluoro-4-(7-hydroxy-5,5-dimethyl-3-oxo-3,5-dihydrodibenzo[b,e]silin-10-yl)-3-methylbenzaldehyde (red-ALDeSense aka Probe 7) A round-bottom flask was charged with **7** (0.0259 g, 0.063 mmol, 1.0 eq.), 2-iodoxybenzoic acid (0.0214 g, 0.076 mmol, 1.2 eq.), and DMSO (1 mL). After stirring 2 hours at room temperature, the reaction was concentrated. The crude material was purified via flash chromatography on a silica column (5:95 v/v *i*PrOH:CH₂Cl₂) to afford the title compound as a red solid (0.0116 g, 0.0284 mmol, 45.1% yield). ¹H NMR (500 MHz, CDCl₃) δ 10.41 (s, 1H), 7.63 (d, *J* = 5.4 Hz, 1H), 7.05 (d, *J* = 2.5 Hz, 2H), 6.83 (d, *J* = 9.4 Hz, 2H), 6.56 (dd, *J* = 9.5, 2.4 Hz, 2H), 2.09 (s, 3H), 0.47 (s, 3H), 0.45 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 185.76, 171.89, 150.99 (dd, *J* = 261.5, 14.4 Hz), 148.04, 147.71 (dd, *J* = 249.6, 12.5 Hz), 144.72, 137.45, 135.42 (d, *J* = 14.1 Hz), 134.27 (d, *J* = 3.9 Hz), 129.92, 129.68, 125.21 (d, *J* = 5.3 Hz), 124.02 (d, *J* = 3.6 Hz), 123.14, 18.98 (d, *J* = 1.8 Hz), -1.24, -1.44. ¹⁹F NMR (471 MHz, CDCl₃) δ -136.14 (d, *J* = 22.2 Hz), -149.06 (dd, *J* = 22.4, 5.9 Hz). HRMS (ESI⁺): *m/z* calculated for [M+H]⁺ = 409.1066; found = 409.1071.



Scheme S9. Synthesis of Probe 8.

2,4,5-Trifluoro-3-methylbenzoic acid (37) A round-bottom flask was charged with anhydrous THF (6 mL) and 1.0 M LiHMDS in THF (6.5 mL, 6.5 mmol, 2.3 eq.). The solution was cooled to -10 °C and then treated dropwise with a solution of 2,4,5-trifluorobenzoic acid (0.503 g, 2.8 mmol, 1.0 eq.) in anhydrous THF (3 mL) over 4 minutes. After stirring for 1.5 hours, the reaction was treated dropwise with methyl iodide (0.193 mL, 3.1 mmol, 1.1 eq.). After stirring for 1 hour, the reaction was quenched with cold 3 M HCl (2 mL) followed by cold 6 M HCl (2.5 mL) and warmed to room temperature. The THF was removed under reduced pressure. Product was extracted with EtOAc. The organics were collected, dried over Na₂SO₄, and concentrated. The crude residue was recrystallized in H₂O to afford the title compound as a white solid (0.321 g, 1.69 mmol, 59.1% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.70 (td, *J* = 9.5, 6.6 Hz, 1H), 2.29 (t, *J* = 2.3 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 167.64, 157.44 (ddd, *J* = 259.7, 7.2, 2.4 Hz), 152.89 (ddd, *J* = 256.8, 14.1, 8.6 Hz), 146.54 (ddd, *J* = 246.4, 13.6, 3.5 Hz), 117.38 (dd, *J* = 23.4, 17.5 Hz), 117.00 (dt, *J* = 20.5, 1.9 Hz), 113.05 (ddd, *J* = 11.7, 5.5, 4.0 Hz), 7.77 (dt, *J* = 3.8, 1.9 Hz). ¹⁹F NMR (471 MHz, CDCl₃) δ -112.01 – -112.19 (m), -126.87 – -127.01 (m), -141.35 (ddd, *J* = 21.8, 16.3, 10.3 Hz).

Methyl 2,4,5-trifluoro-3-methylbenzoate (38) A round-bottom flask was charged with **43** (3.58 g, 18.8 mmol, 1.0 eq.), MeOH (40 mL), and conc. H₂SO₄ (2 mL). The reaction was heated to 50 °C. After stirring overnight, the reaction was neutralized with saturated NaHCO₃ and diluted with EtOAc. The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (5:95 v/v EtOAc:hexanes) to afford the title compound as a colorless oil (2.12 g, 10.4 mmol, 55.2% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.61 (td, *J* = 9.3, 6.6 Hz, 1H), 3.92 (s, 3H), 2.26 (t, *J* = 2.2 Hz, 3H). ¹⁹F NMR (376 MHz, CDCl₃) δ -114.06 – -114.71 (m), -129.48 (dt, *J* = 19.4, 9.3 Hz), -142.34 (ddd, *J* = 22.0, 16.0, 10.1 Hz).

Methyl 4-amino-2,5-difluoro-3-methylbenzoate (39) A pressure flask was charged with **44** (0.97 g, 5.0 mmol, 1.0 eq.), benzylamine (0.65 mL, 6.0 mmol, 1.2 eq.), triethylamine (1.03 mL, 7.4 mmol, 1.5 eq.), and DMSO (2 mL). The reaction was heated to 100 °C for 20 hours. After cooling to room temperature, the reaction was poured into brine and diluted with EtOAc. The organics were washed with more brine, dried over Na₂SO₄, and concentrated under reduced pressure. To the crude residue was added 10% w/w Pd/C

(0.069 g) and MeOH (20 mL). The mixture was stirred vigorously under hydrogen (1 atm) overnight at room temperature. The reaction filtered through Celite®. The crude material was purified via flash chromatography on a silica column (10:90 v/v EtOAc:hexanes) to afford the title compound as a white solid (0.579 g, 2.88 mmol, 58.8% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.41 (dd, *J* = 11.4, 6.4 Hz, 1H), 4.17 (s, 2H), 3.84 (s, 3H), 2.06 (d, *J* = 2.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 164.63 (dd, *J* = 4.4, 2.4 Hz), 157.46 (dd, *J* = 252.7, 1.2 Hz), 146.09 (dd, *J* = 234.7, 2.1 Hz), 139.30 (dd, *J* = 14.1, 8.3 Hz), 114.57 (dd, *J* = 22.3, 3.1 Hz), 110.90 (dd, *J* = 22.4, 3.4 Hz), 106.06 (dd, *J* = 13.2, 7.0 Hz), 51.97, 8.67 (dd, *J* = 6.8, 2.4 Hz). ¹⁹F NMR (471 MHz, CDCl₃) δ -116.80 (ddd, *J* = 15.5, 6.1, 2.1 Hz), -140.73 (dd, *J* = 16.0, 11.4 Hz).

Methyl 4-bromo-2,5-difluoro-3-methylbenzoate (40) A flame-dried round-bottom flask was charged with **45** (0.310 g, 1.54 mmol, 1.0 eq.), CuBr₂ (1.72 g, 7.7 mmol, 5.0 eq.), and anhydrous MeCN (10 mL). The reaction was treated with *tert*-butyl nitrite (0.28 mL, 2.3 mmol, 1.5 eq.) and heated to 50 °C. After stirring overnight, the reaction was cooled and diluted with saturated NaHCO₃. Product was extracted with EtOAc. The organics were combined, dried over Na₂SO₄, and concentrated to afford the title compound as a yellow solid (0.40 g, 1.51 mmol, 97.9% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.54 (dd, *J* = 8.3, 6.0 Hz, 1H), 3.93 (s, 3H), 2.41 (d, *J* = 2.9 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 163.62 (dd, *J* = 4.3, 2.3 Hz), 156.27 (dd, *J* = 123.6, 3.1 Hz), 154.28 (dd, *J* = 109.3, 3.1 Hz), 129.33 (d, *J* = 21.2 Hz), 118.01 (dd, *J* = 22.2, 5.2 Hz), 117.90 (dd, *J* = 13.6, 7.1 Hz), 115.17 (dd, *J* = 26.8, 1.9 Hz), 52.70, 15.08 (dd, *J* = 4.9, 2.2 Hz). ¹⁹F NMR (471 MHz, CDCl₃) δ -109.15 (dd, *J* = 16.9, 8.2 Hz), -113.13 (ddq, *J* = 16.6, 5.9, 2.7 Hz).

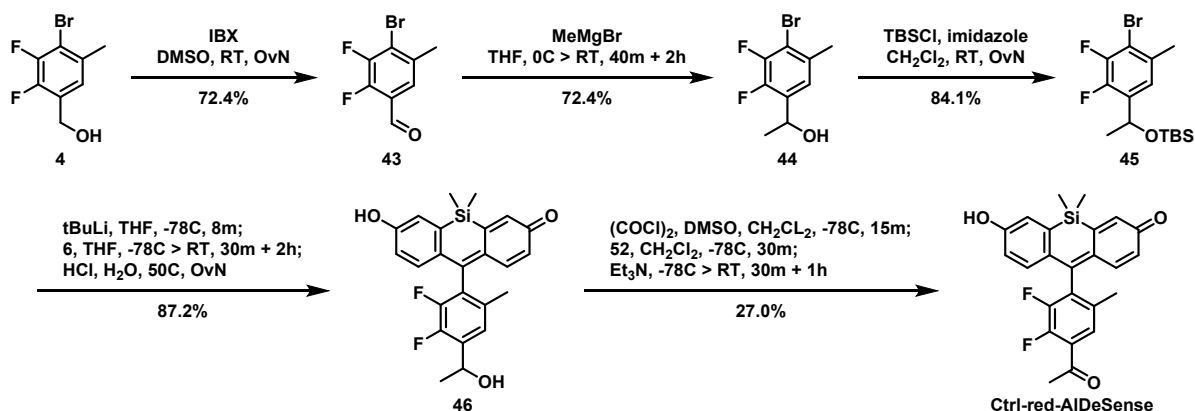
((4-Bromo-2,5-difluoro-3-methylbenzyl)oxy)(*tert*-butyl)dimethylsilane (41) A flame-dried round-bottom flask was charged with **46** (0.525 g, 2.0 mmol, 1.0 eq.) and anhydrous THF (10 mL). The reaction was cooled to 0 °C before treating dropwise with 1.0 M DIBAL-H in CH₂Cl₂ (5.0 mL, 5.0 mmol, 2.5 eq.) over 2 minutes. The reaction was slowly warmed to room temperature with the melting ice bath. After stirring overnight minutes, the reaction was quenched via the sequential addition of H₂O (5 mL), 1.0 M NaOH in H₂O (5 mL), and more H₂O (5 mL). The reaction was diluted with Et₂O. The organics were collected, dried over Na₂SO₄, and concentrated. The crude residue was treated imidazole (0.21 g, 3.0 mmol, 1.5 eq.) and CH₂Cl₂ (20 mL). Once a solution had formed, *tert*-butyldimethylsilyl chloride (0.47 g, 3.0 mmol, 1.5 eq.) was added. After stirring overnight at room temperature, the reaction was purified via flash chromatography on a silica column (2:98 v/v EtOAc:hexanes) to afford the title compound as a colorless oil (0.660 g, 1.88 mmol, 93.9% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.13 (dd, *J* = 8.7, 6.2 Hz, 1H), 4.72 (s, 2H), 2.34 (d, *J* = 2.6 Hz, 3H), 0.95 (s, 9H), 0.12 (s, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 155.71 (dd, *J* = 241.8, 2.9 Hz), 153.43 (dd, *J* = 242.4, 2.7 Hz), 129.15 (dd, *J* = 18.2, 7.2 Hz), 126.55 (d, *J* = 20.5 Hz), 112.00 (dd, *J* = 26.3, 5.6 Hz), 110.20 (dd, *J* = 22.8, 5.5 Hz), 58.68 (d, *J* = 5.3 Hz), 26.03, 18.53, 14.81 (dd, *J* = 4.1, 2.2 Hz), -5.26. ¹⁹F NMR (471 MHz, CDCl₃) δ -110.43 (dd, *J* = 16.3, 8.8 Hz), -124.14 (ddd, *J* = 15.9, 6.1, 2.7 Hz).

10-(3,6-Difluoro-4-(hydroxymethyl)-2-methylphenyl)-7-hydroxy-5,5-dimethyldibenzo[b,e]silin-3(5H)-one (42) A flame-dried round-bottom flask was charged with **47** (0.349 g, 1.0 mmol, 10 eq.) and anhydrous THF (5 mL). The reaction was cooled to -78 °C and then treated dropwise with 1.7 M *t*-butyllithium in pentane (0.59 mL, 1.0 mmol, 10 eq.) over 1 minute. After stirring for an additional 8 minutes at -78 °C, the reaction was treated dropwise with a solution of **6** (0.050 g, 0.10 mmol, 1.0 eq.) in anhydrous THF (2 mL) over 1 minute. After addition, the reaction was warmed to room temperature and stirred for 2 hours. The reaction was treated with 1.0 M HCl in H₂O (3 mL, 3 mmol, 30 eq.) and heated to 50 °C. After stirring for 4 hours, the reaction was cooled to room temperature and quenched with brine. The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (5:95 to 10:90 v/v iPrOH:CH₂Cl₂ gradient). The crude residue was suspended in CH₂Cl₂

and collected by filtration to afford the title compound as a red solid (0.0321 g, 0.0782 mmol, 78.2% yield). ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 10.58 (s, 1H), 7.29 (dd, $J = 9.2, 5.6$ Hz, 1H), 7.04 (s, 2H), 6.81 (d, $J = 9.4$ Hz, 2H), 6.49 (s, 2H), 5.51 (s, 1H), 4.65 (s, 2H), 1.91 (d, $J = 2.2$ Hz, 3H), 0.47 (s, 3H), 0.45 (s, 3H). ^{13}C NMR (126 MHz, $\text{DMSO-}d_6$) δ 154.67 (d, $J = 237.9$ Hz), 153.80 (d, $J = 241.6$ Hz), 147.20, 131.25 (dd, $J = 18.4, 7.7$ Hz), 128.97, 125.98 (dd, $J = 20.0, 4.4$ Hz), 124.40 (dd, $J = 19.5, 3.1$ Hz), 112.38 (dd, $J = 25.6, 5.4$ Hz), 56.59 (d, $J = 4.1$ Hz), 11.54 (d, $J = 2.2$ Hz), -1.26, -1.84. ^{19}F NMR (471 MHz, $\text{DMSO-}d_6$) δ -119.71 (dd, $J = 18.1, 9.3$ Hz), -126.85 (dd, $J = 18.1, 5.3$ Hz). HRMS (ESI⁺): m/z calculated for $[\text{M}+\text{H}]^+ = 411.1223$; found = 411.1216.

2,5-Difluoro-4-(7-hydroxy-5,5-dimethyl-3-oxo-3,5-dihydrodibenzo[b,e]silin-10-yl)-3-methylbenzaldehyde (Probe 8)

Probe 8 A round-bottom flask was charged with **48** (0.0174 g, 0.042 mmol, 1.0 eq.), 2-iodoxybenzoic acid (0.016 g, 0.051 mmol, 1.2 eq.), and DMSO (1 mL). After stirring 4 hours at room temperature, the reaction was concentrated. The crude material was purified via flash chromatography on a silica column (5:95 v/v $i\text{PrOH}:\text{CH}_2\text{Cl}_2$) to afford the title compound as a red solid (0.0067 g, 0.0164 mmol, 38.7% yield). ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 10.62 (s, 1H), 10.29 (d, $J = 2.4$ Hz, 1H), 7.66 (dd, $J = 8.2, 5.1$ Hz, 1H), 7.24 (s, 1H), 6.88 (s, 2H), 6.77 (s, 2H), 6.20 (s, 1H), 2.00 (d, $J = 2.0$ Hz, 3H), 0.48 (s, 3H), 0.46 (s, 3H). ^{19}F NMR (471 MHz, $\text{DMSO-}d_6$) δ -118.07 (dd, $J = 18.3, 8.5$ Hz), -127.14 (dd, $J = 18.5, 4.8$ Hz). HRMS (ESI⁺): m/z calculated for $[\text{M}+\text{H}]^+ = 409.1066$; found = 409.1077.



Scheme S10. Synthesis of Ctrl-red-AIDeSense.

4-Bromo-2,3-difluoro-5-methylbenzaldehyde (43) A round-bottom flask was charged with **4** (0.460 g, 1.94 mmol, 1.0 eq.), 2-iodoxybenzoic acid (0.81 g, 2.9 mmol, 1.5 eq.), and DMSO (10 mL). After stirring overnight at room temperature, the reaction was poured into brine and diluted with Et_2O . The organics were collected, washed with H_2O , dried over Na_2SO_4 , and concentrated under reduced pressure to afford the title compound as a light yellow solid (0.330 g, 1.40 mmol, 72.4% yield). ^1H NMR (500 MHz, CDCl_3) δ 10.27 (s, 1H), 7.52 (d, $J = 6.3$ Hz, 1H), 2.43 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 185.50 (dd, $J = 6.0, 3.1$ Hz), 150.71 (dd, $J = 261.8, 14.7$ Hz), 148.15 (dd, $J = 249.7, 13.5$ Hz), 135.79 (d, $J = 4.3$ Hz), 123.82 (d, $J = 5.8$ Hz), 123.17 (d, $J = 3.7$ Hz), 120.31 (d, $J = 17.1$ Hz), 22.27 (d, $J = 2.3$ Hz). ^{19}F NMR (471 MHz, CDCl_3) δ -126.06 (d, $J = 21.8$ Hz), -147.66 (dd, $J = 21.6, 6.2$ Hz).

1-(4-Bromo-2,3-difluoro-5-methylphenyl)ethan-1-ol (44) A flame-dried round-bottom flask was charged with **49** (0.388 g, 1.65 mmol, 1.0 eq.) and anhydrous THF (5 mL). The reaction was cooled to 0°C and then

treated dropwise with 3.0 M methylmagnesium bromide in Et₂O (0.66 mL, 1.98 mmol, 1.2 eq.) over 1 minute. After stirring for an additional 40 minutes at 0 °C, the reaction was warmed to room temperature and stirred for 2 hours. The reaction was quenched with brine and diluted with Et₂O and CH₂Cl₂. The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was dissolved in CH₂Cl₂ and treated with imidazole (0.011 g, 0.165 mmol, 0.1 eq.) and *tert*-butyldimethylsilyl chloride (0.024 g, 0.165 mmol, 0.1 eq.). After stirring for 10 minutes, the reaction was quenched with MeOH and concentrated. The crude residue purified via flash chromatography on a silica column (10:90 v/v EtOAc:hexanes) to afford the title compound as a colorless oil (0.300 g, 1.19 mmol, 72.4% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.17 (dd, *J* = 6.8, 2.0 Hz, 1H), 5.16 (q, *J* = 6.5 Hz, 1H), 2.39 (s, 3H), 1.95 (s, 1H), 1.50 (d, *J* = 6.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 147.66 (dd, *J* = 246.7, 14.6 Hz), 145.91 (dd, *J* = 248.2, 14.4 Hz), 134.74 (d, *J* = 4.2 Hz), 133.27 (d, *J* = 10.4 Hz), 121.75 (t, *J* = 3.4 Hz), 111.07 (d, *J* = 17.2 Hz), 64.25 (d, *J* = 2.4 Hz), 24.28, 22.35 (d, *J* = 2.3 Hz). ¹⁹F NMR (471 MHz, CDCl₃) δ -128.62 (dd, *J* = 21.9, 2.2 Hz), -144.99 (dd, *J* = 22.3, 6.8 Hz).

(1-(4-Bromo-2,3-difluoro-5-methylphenyl)ethoxy)(*tert*-butyl)dimethylsilane (45) A round-bottom flask was charged with **50** (0.300 g, 1.19 mmol, 1.0 eq.), imidazole (0.163 g, 2.4 mmol, 2.0 eq.), and CH₂Cl₂ (10 mL). Once a solution had formed, *tert*-butyldimethylsilyl chloride (0.358 g, 2.4 mmol, 2.0 eq.) was added. After stirring overnight at room temperature, the reaction was concentrated under reduced pressure. The crude material was purified via flash chromatography on a silica column (hexanes) to afford the title compound as a colorless oil (0.367 g, 1.00 mmol, 84.1% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.19 (ddd, *J* = 6.7, 2.1, 0.9 Hz, 1H), 5.11 (q, *J* = 6.3 Hz, 1H), 2.38 (s, 3H), 1.39 (d, *J* = 6.3 Hz, 3H), 0.90 (s, 9H), 0.07 (s, 3H), -0.01 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 147.40 (dd, *J* = 246.2, 14.8 Hz), 145.23 (dd, *J* = 247.1, 14.3 Hz), 134.52 (d, *J* = 11.0 Hz), 134.34 (d, *J* = 4.1 Hz), 122.13 (t, *J* = 3.4 Hz), 110.33 (d, *J* = 17.3 Hz), 64.36 (t, *J* = 2.5 Hz), 25.93, 22.42 (d, *J* = 2.3 Hz), 18.35, -4.81, -4.87. ¹⁹F NMR (471 MHz, CDCl₃) δ -129.38 (d, *J* = 22.2 Hz), -145.34 (dd, *J* = 22.4, 6.9 Hz).

10-(3,6-Difluoro-4-(1-hydroxyethyl)-2-methylphenyl)-7-hydroxy-5,5-dimethyldibenzo[*b,e*]silin-3(5H)-one (46) A flame-dried round-bottom flask was charged with **51** (0.357 g, 1.0 mmol, 5 eq.) and anhydrous THF (10 mL). The reaction was cooled to -78 °C and then treated dropwise with 1.7 M *t*-butyllithium in pentane (0.59 mL, 1.0 mmol, 5 eq.) over 1 minutes. After stirring for an additional 8 minutes at -78 °C, the reaction was treated dropwise with a solution of **6** (0.100 g, 0.20 mmol, 1.0 eq.) in anhydrous THF (5 mL) over 3 minutes. After stirring for 30 minutes, the reaction was warmed to room temperature and stirred for 2 hours. The reaction was treated with 1.0 M HCl in H₂O (6 mL, 6 mmol, 60 eq.) and heated to 50 °C. After stirring overnight, the reaction was cooled to room temperature, quenched with brine, and diluted with Et₂O. The organics were collected, dried over Na₂SO₄, and concentrated. The crude material was purified via flash chromatography on a silica column (5:95 v/v MeOH:CH₂Cl₂). The crude material was further purified via flash chromatography on a silica column (0:100 to 10:90 v/v MeOH:Et₂O gradient) to afford the title compound as a red solid (0.074 g, 0.174 mmol, 87.2% yield). ¹H NMR (500 MHz, Methanol-*d*₄) δ 7.44 – 7.30 (m, 1H), 7.04 (d, *J* = 2.5 Hz, 2H), 6.95 (dd, *J* = 9.5, 0.9 Hz, 1H), 6.93 (dd, *J* = 9.4, 0.9 Hz, 1H), 6.51 (dd, *J* = 9.4, 2.4 Hz, 1H), 6.48 (dd, *J* = 9.4, 2.4 Hz, 1H), 5.21 (q, *J* = 6.5 Hz, 1H), 2.04 (s, 3H), 1.55 (d, *J* = 6.5 Hz, 3H), 0.52 (s, 3H), 0.49 (s, 3H). ¹³C NMR (126 MHz, Methanol-*d*₄) δ 152.40, 146.43, 139.50, 136.99 (d, *J* = 10.6 Hz), 134.10, 130.63, 130.27, 128.30 (d, *J* = 14.2 Hz), 126.12, 123.77, 123.39, 64.42, 24.45, 18.93 (d, *J* = 2.0 Hz), -1.39, -1.67. ¹⁹F NMR (471 MHz, Methanol-*d*₄) δ -142.00 (d, *J* = 21.8 Hz), -150.09 (dd, *J* = 21.7, 6.3 Hz). HRMS (ESI⁺): *m/z* calculated for [M+H]⁺ = 425.1379; found = 425.1376.

10-(4-Acetyl-3,6-difluoro-2-methylphenyl)-7-hydroxy-5,5-dimethyldibenzo[b,e]silin-3(5H)-one (Ctrl-red-ALDeSense) A flame-dried round-bottom flask was charged with anhydrous CH_2Cl_2 (1 mL) and oxalyl chloride (0.10 mL, 1.2 mmol, 20 eq.). The reaction was cooled to $-78\text{ }^\circ\text{C}$ and then treated dropwise with a solution of DMSO (0.17 mL, 2.4 mmol, 40 eq.) in anhydrous CH_2Cl_2 (1 mL) over 2 minutes. After stirring for an additional 15 minutes at $-78\text{ }^\circ\text{C}$, the reaction was treated dropwise with a solution of **121** (0.026 g, 0.061 mmol, 1.0 eq.) in anhydrous CH_2Cl_2 (2.5 mL) over 5 minutes. After stirring for 30 minutes, the reaction was treated dropwise with Et_3N (0.34 mL, 2.4 mmol, 40 eq.) over 1 minute. After stirring for 30 minutes, the reaction was warmed to room temperature and stirred for 1 hour. The reaction poured into a dilute HCl solution and diluted with CH_2Cl_2 . The organics were collected, dried over Na_2SO_4 , and concentrated. The crude material was purified via flash chromatography on a silica column (5:95 v/v iPrOH: CH_2Cl_2). The crude material was further purified via flash chromatography on a silica column (0:100 to 100:0 v/v Et_2O : CH_2Cl_2 gradient) to afford the title compound as a red solid (0.0070 g, 0.0166 mmol, 27.0% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.63 (d, $J = 6.0$ Hz, 1H), 7.03 (d, $J = 2.5$ Hz, 2H), 6.83 (d, $J = 9.5$ Hz, 2H), 6.53 (dd, $J = 9.5, 2.5$ Hz, 2H), 2.73 (d, $J = 4.5$ Hz, 3H), 2.07 (s, 3H), 0.48 (s, 3H), 0.46 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 194.56, 151.65, 147.13, 144.22, 137.17, 135.91, 133.69 (dd, $J = 8.9, 5.4$ Hz), 130.07, 128.39, 126.80 (d, $J = 9.9$ Hz), 125.66, 123.12, 31.65 (d, $J = 6.8$ Hz), 18.98, -1.22, -1.40. ^{19}F NMR (471 MHz, CDCl_3) δ -136.39 (d, $J = 23.0$ Hz), -137.81 (dq, $J = 21.6, 5.2$ Hz). HRMS (ESI⁺): m/z calculated for $[\text{M}+\text{H}]^+ = 423.1223$; found = 423.1229.

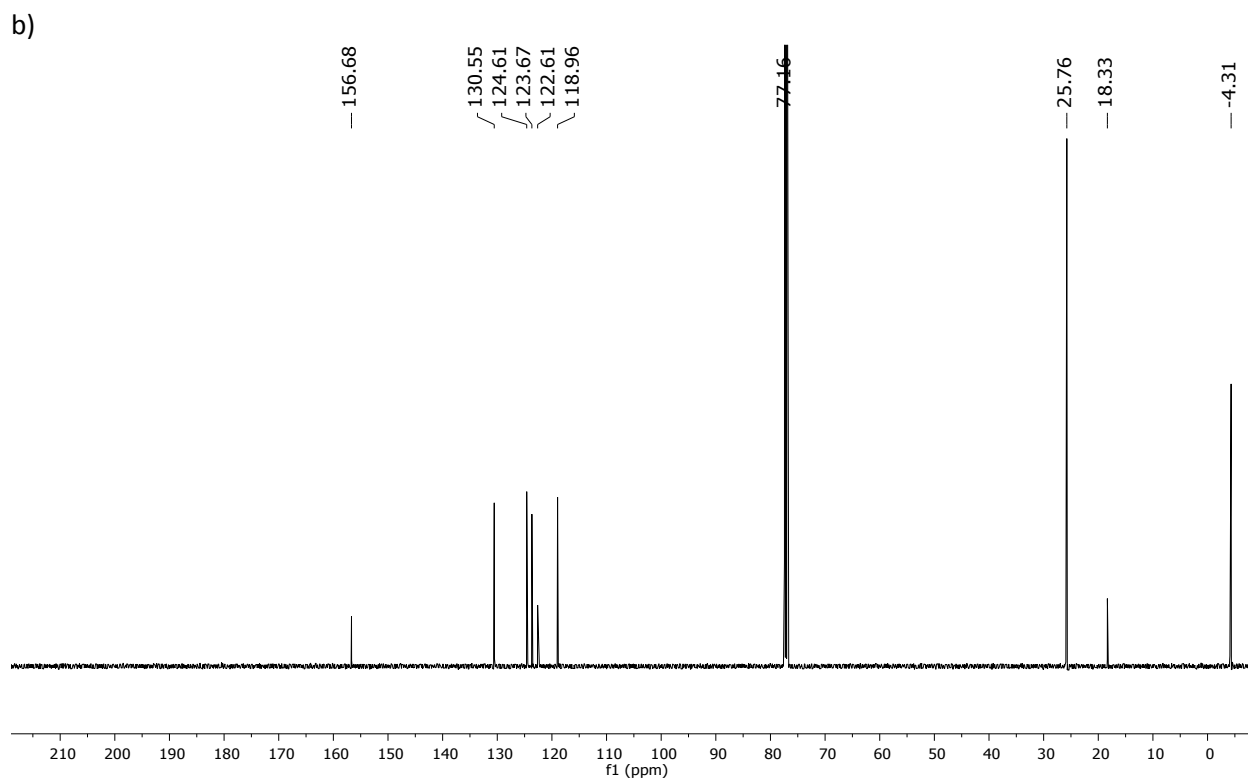
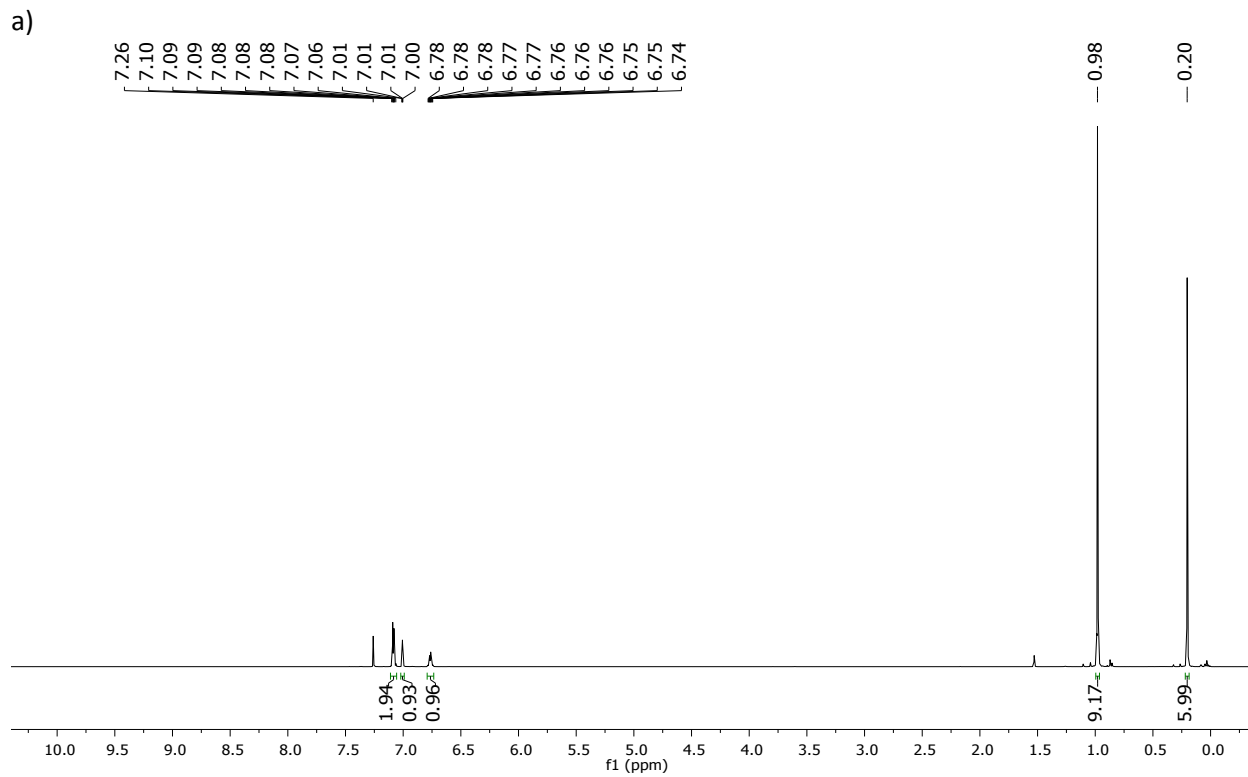


Figure S4. a) ^1H NMR and b) ^{13}C NMR spectra of **9**

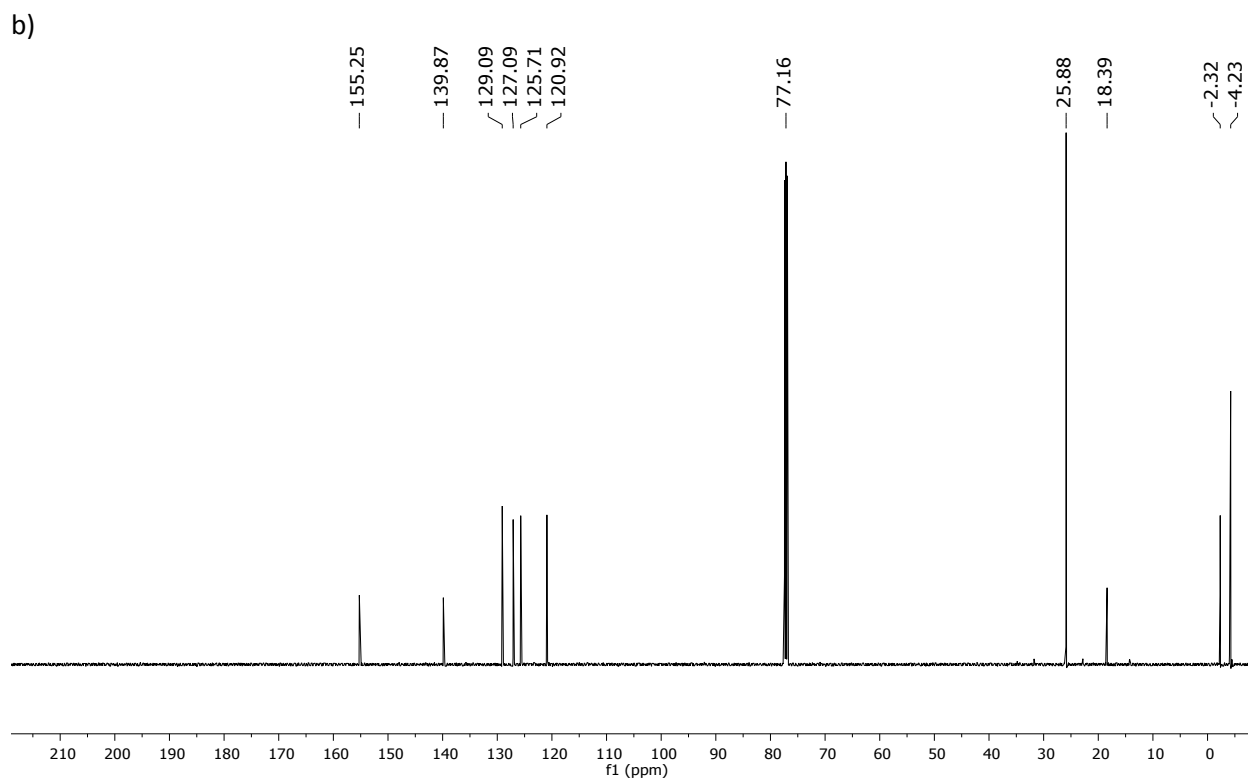
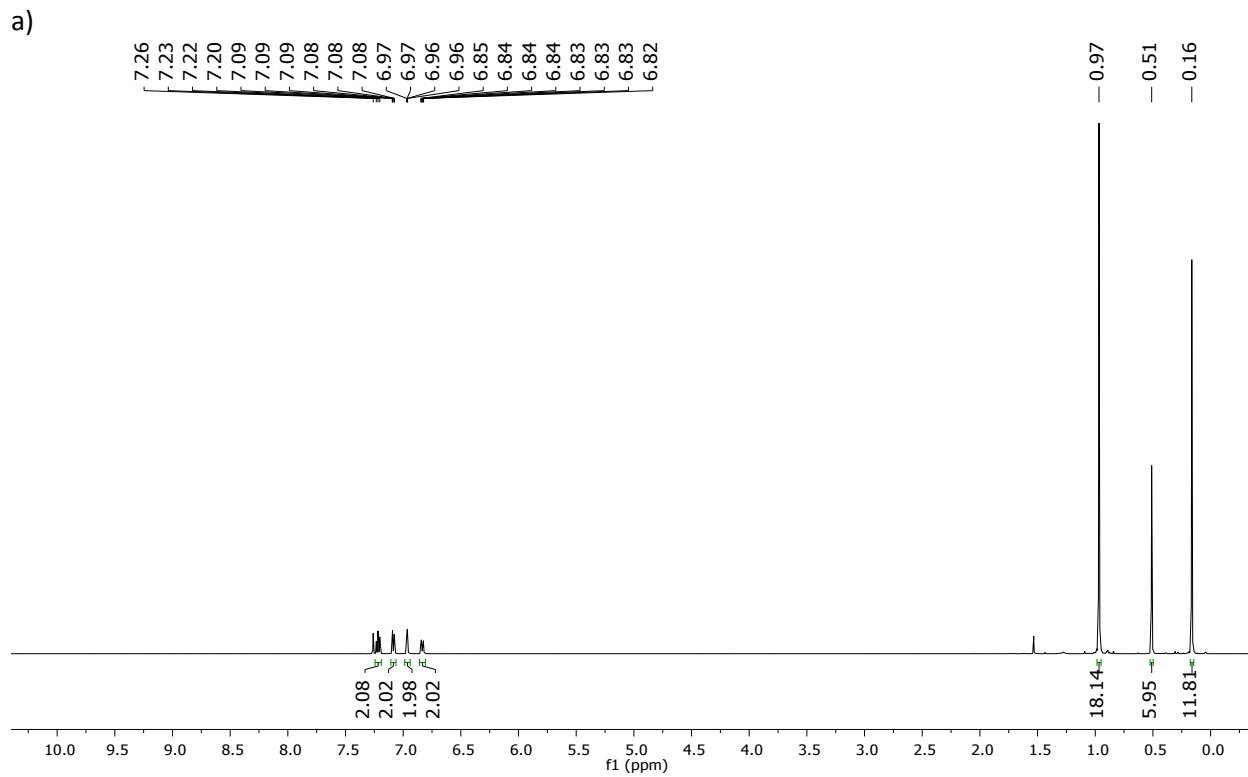


Figure S5. a) ^1H NMR and b) ^{13}C NMR spectra of **10**

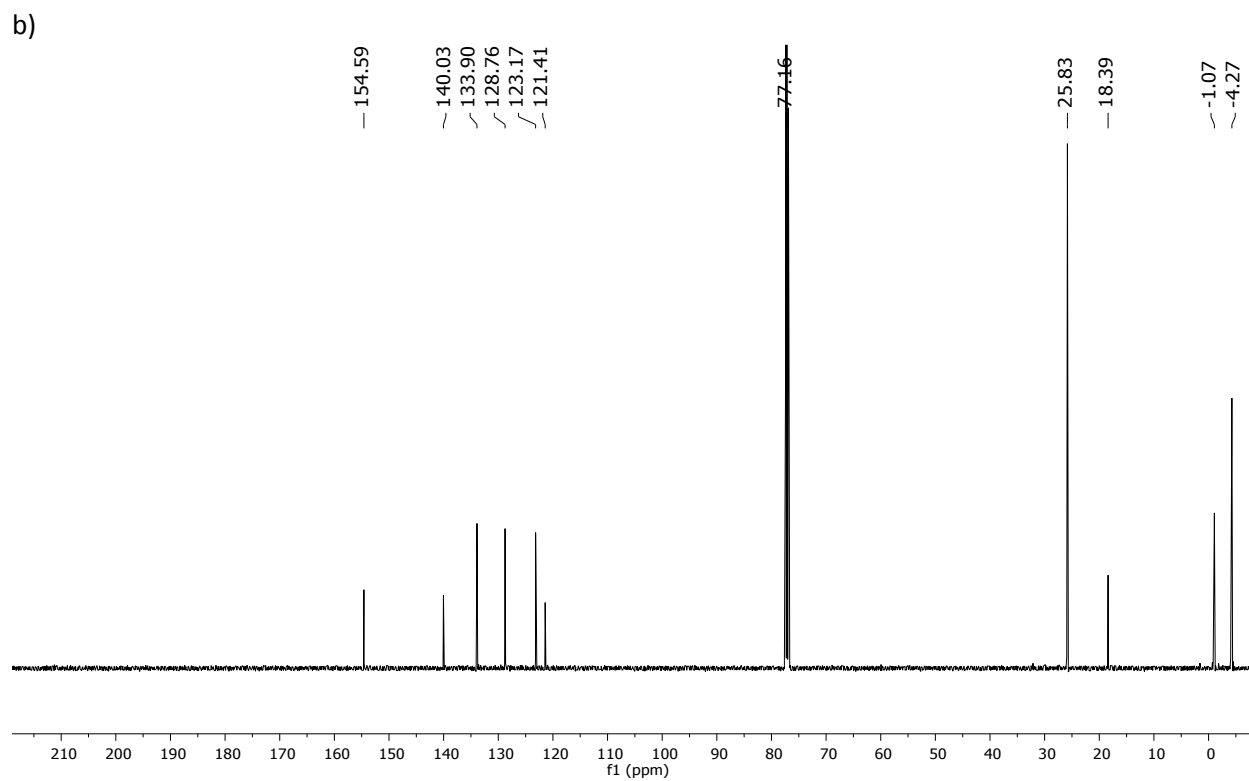
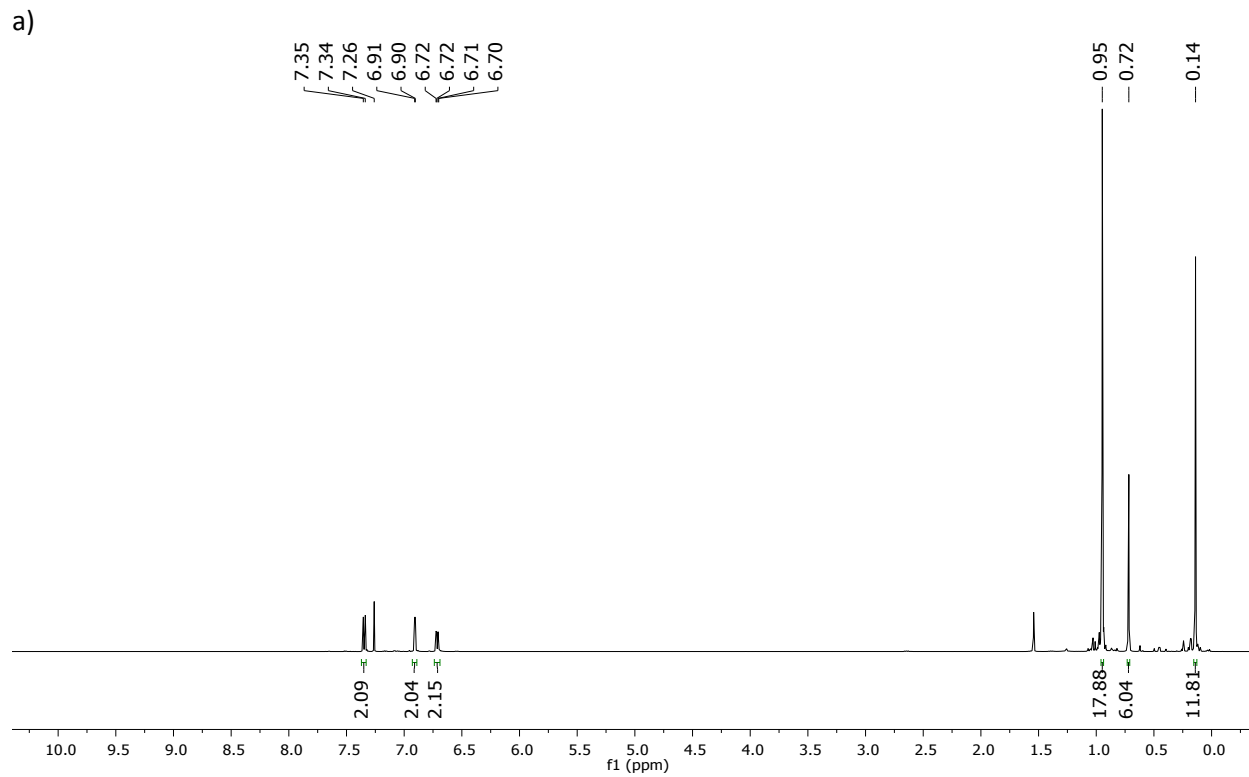


Figure S6. a) ^1H NMR and b) ^{13}C NMR spectra of **11**

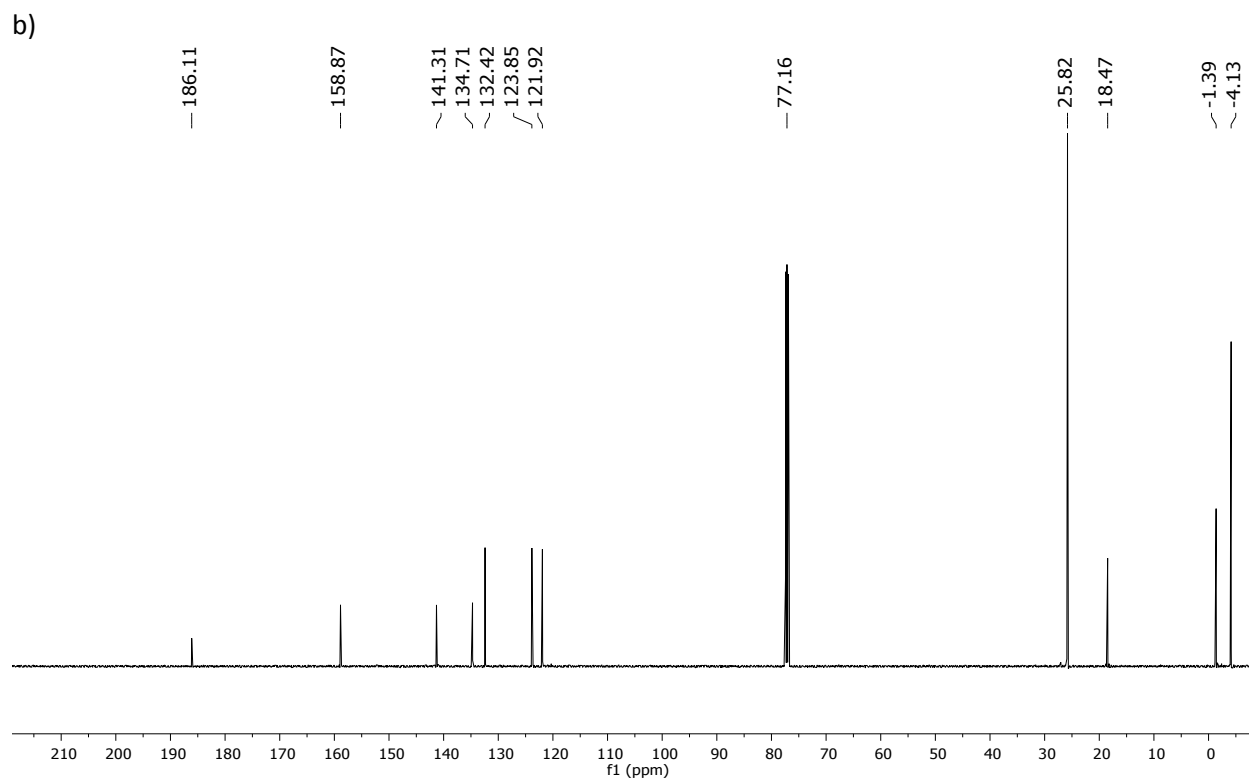
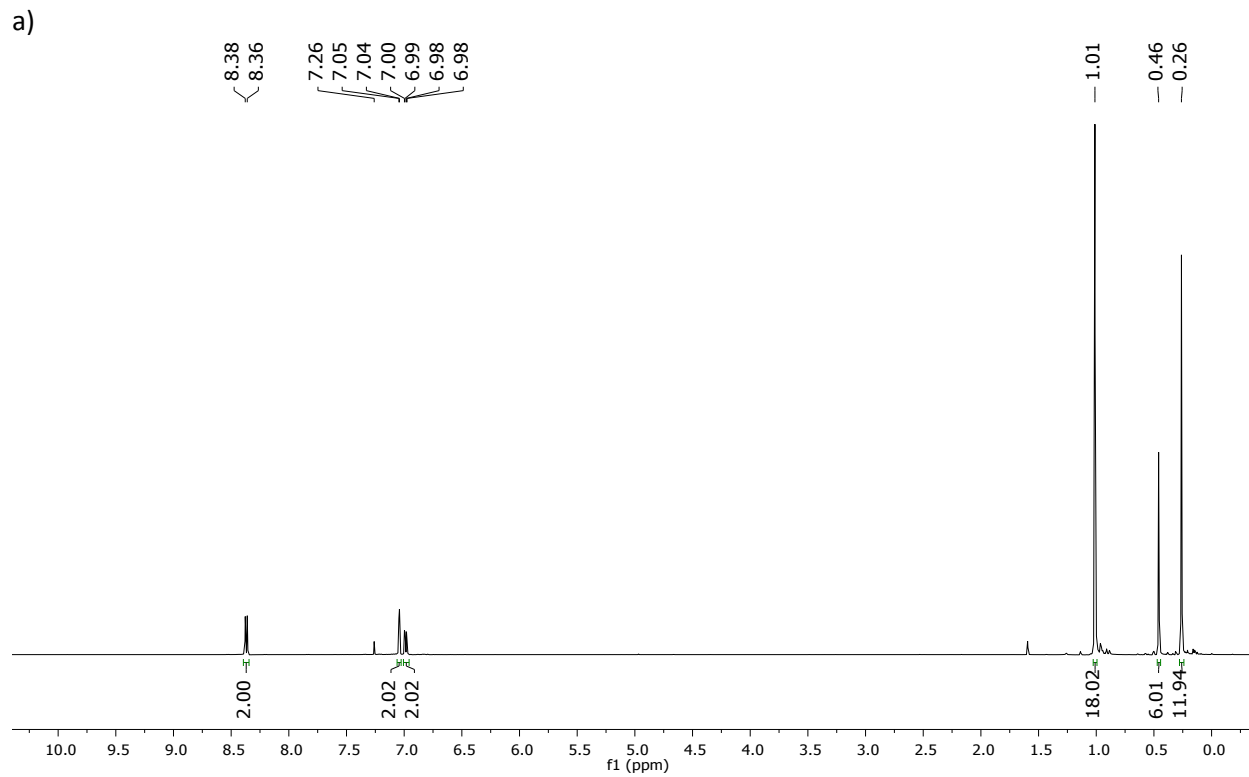


Figure S7. a) ^1H NMR and b) ^{13}C NMR spectra of **6**

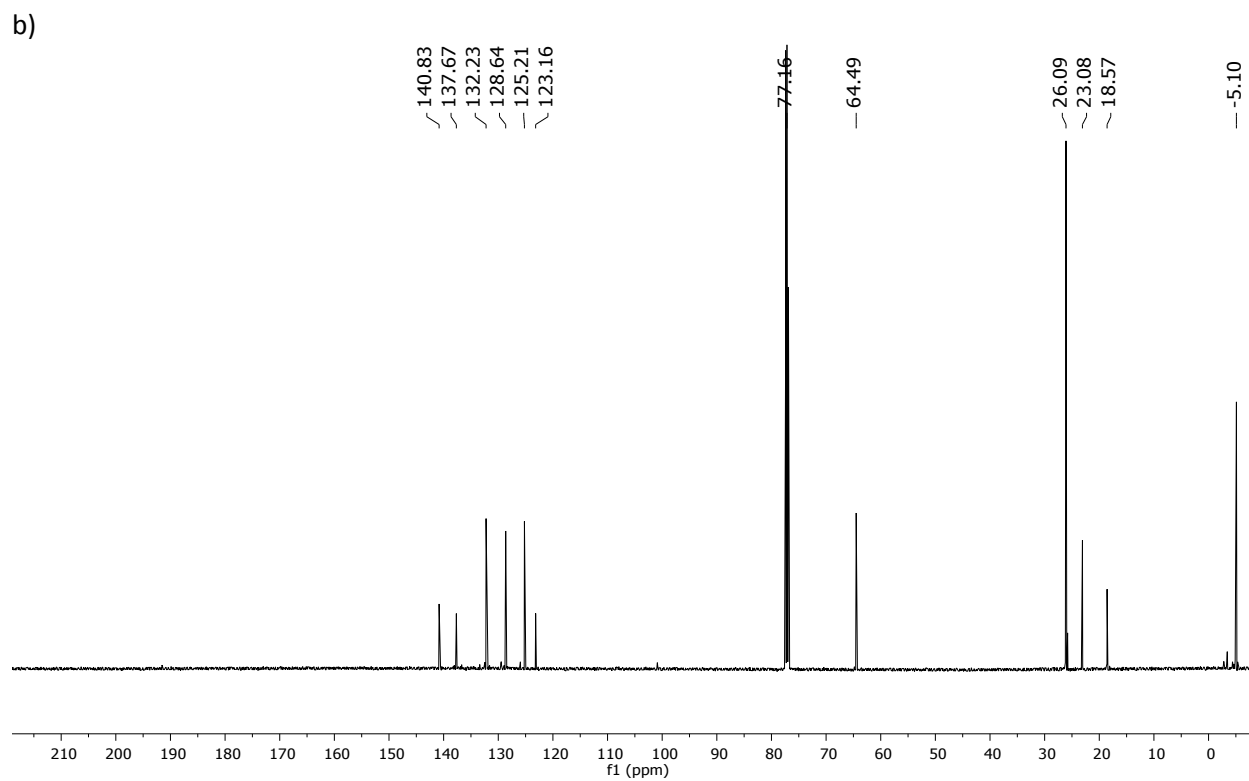
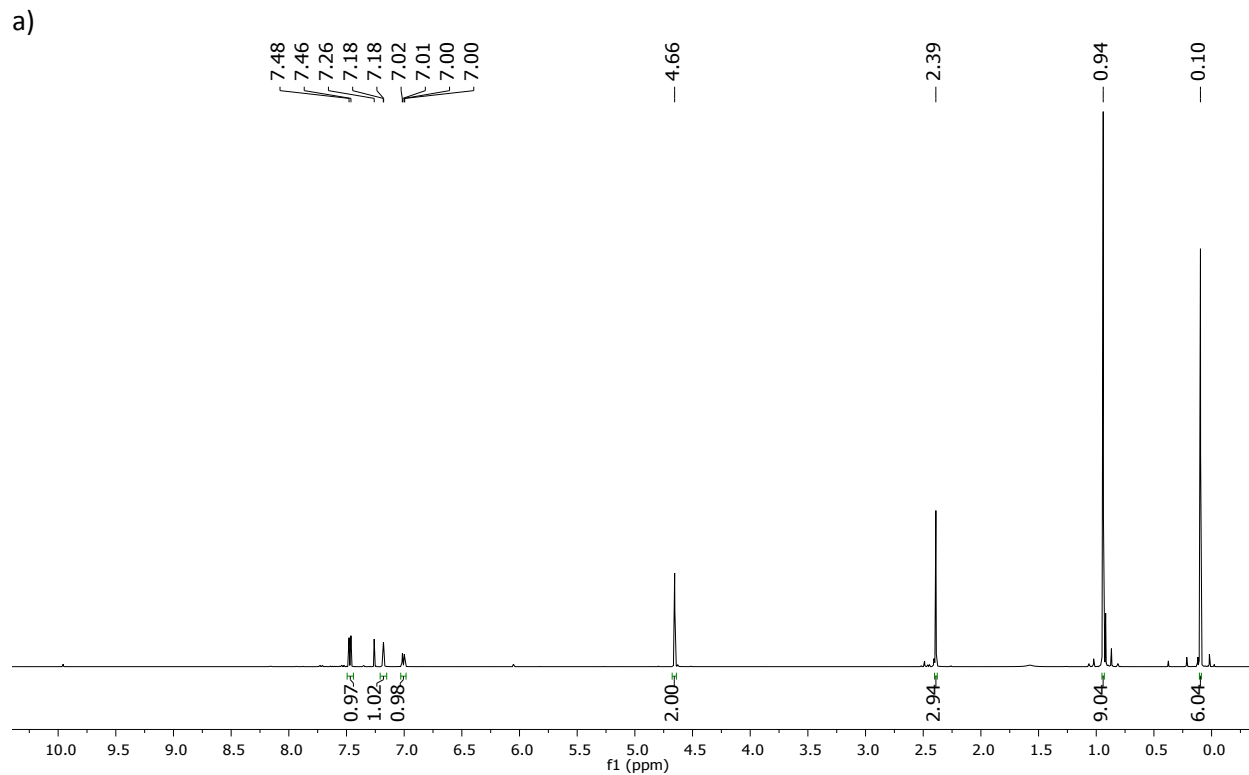


Figure S8. a) ^1H NMR and b) ^{13}C NMR spectra of **13**

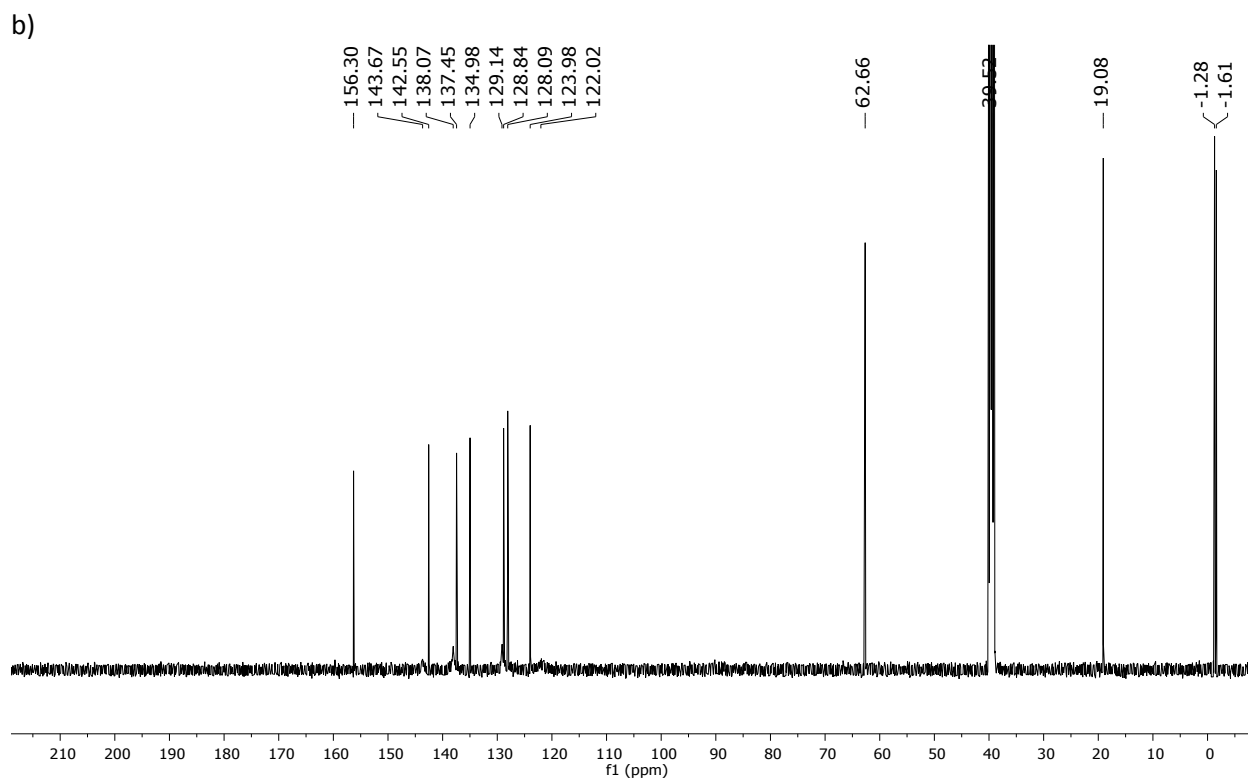
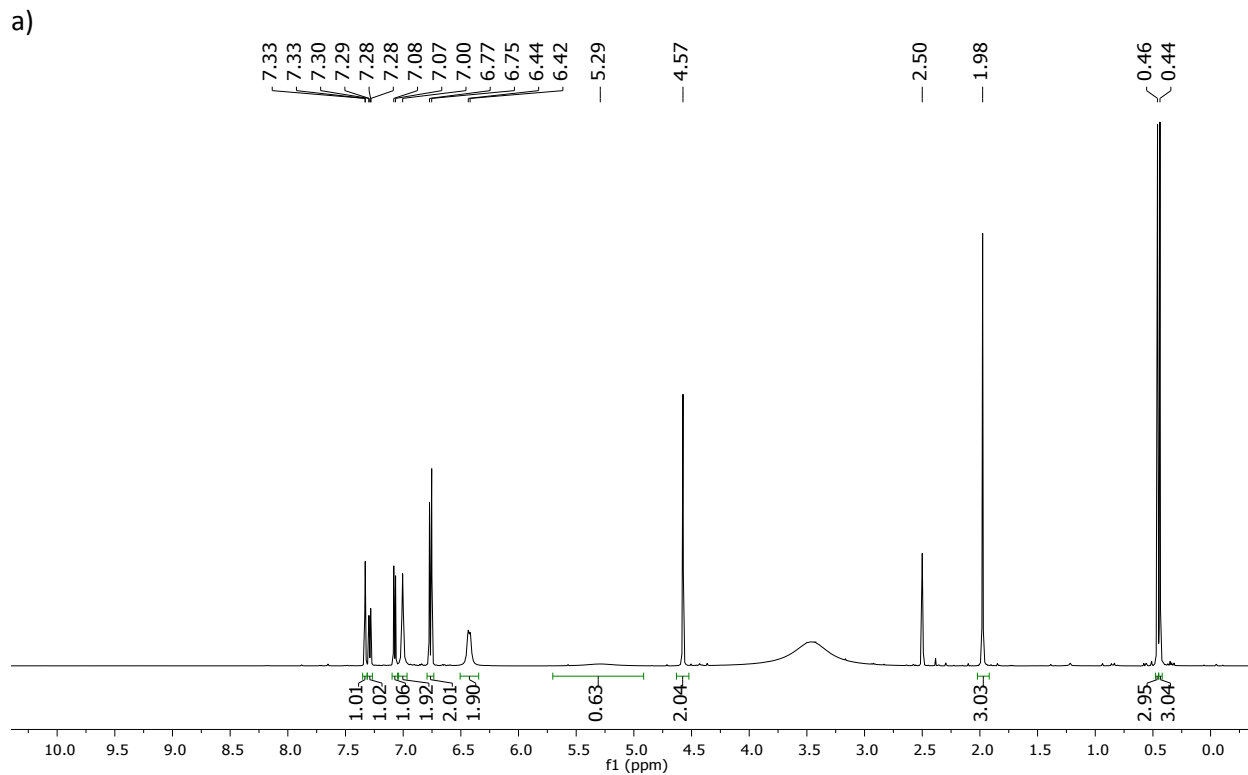


Figure S9. a) ^1H NMR and b) ^{13}C NMR spectra of **14**

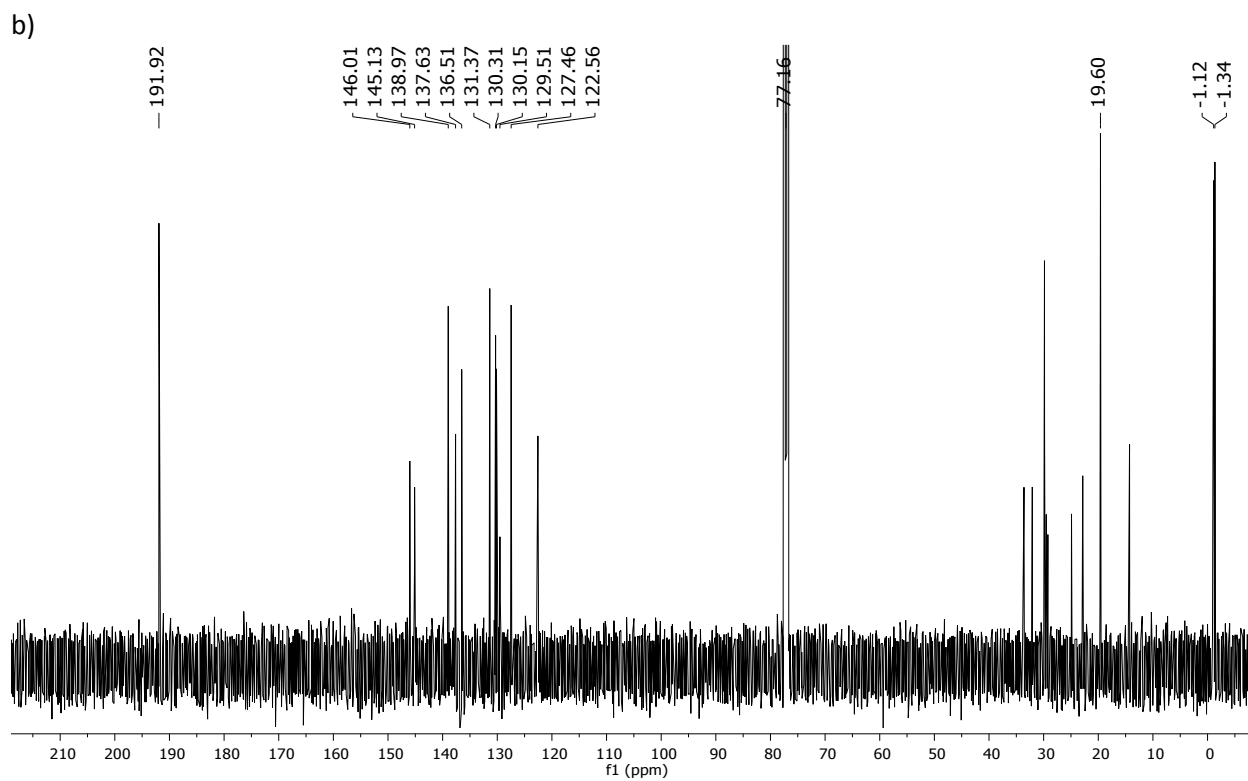
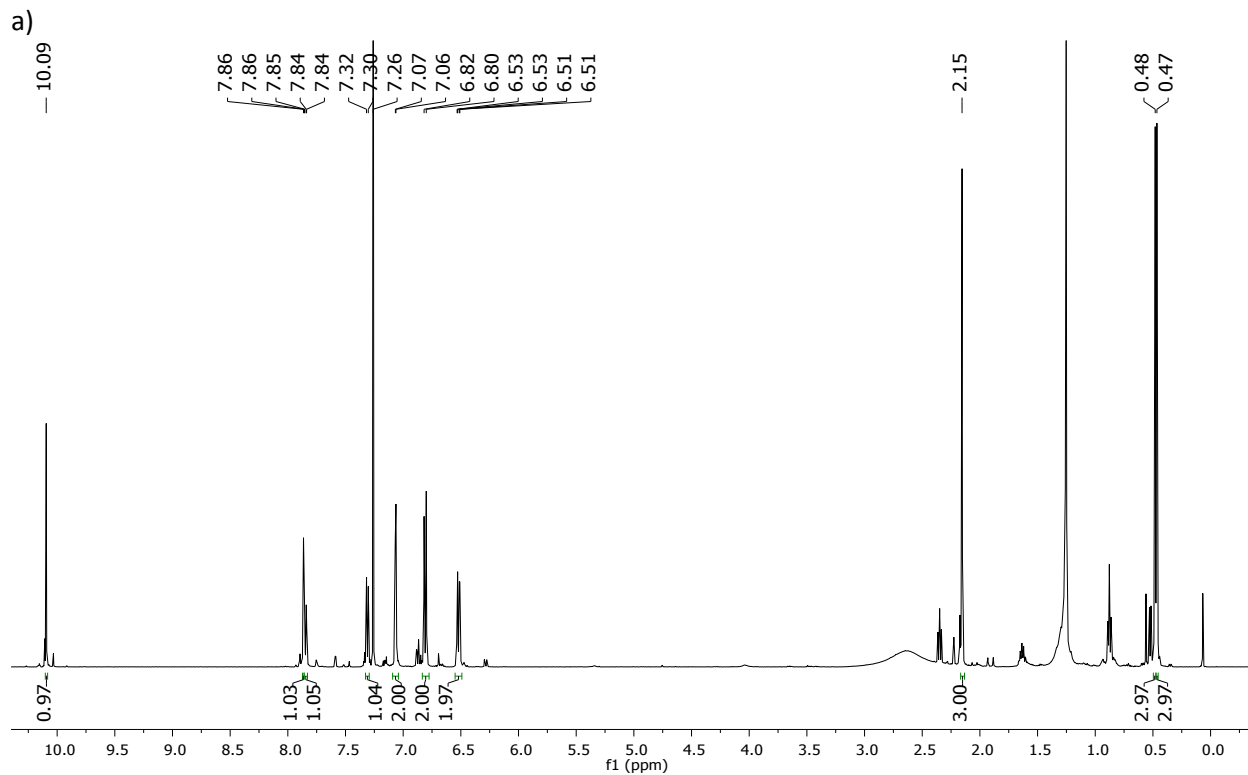


Figure S10. a) ^1H NMR and b) ^{13}C NMR spectra of **Probe 1**

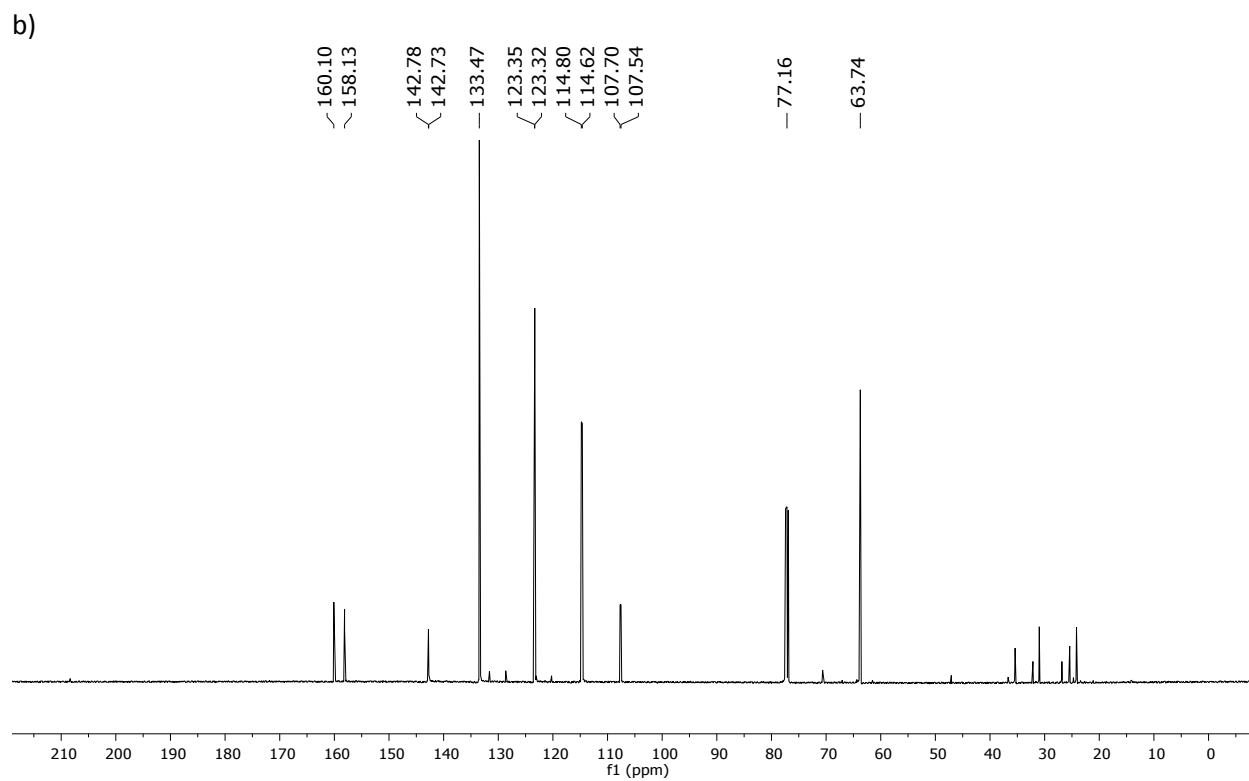
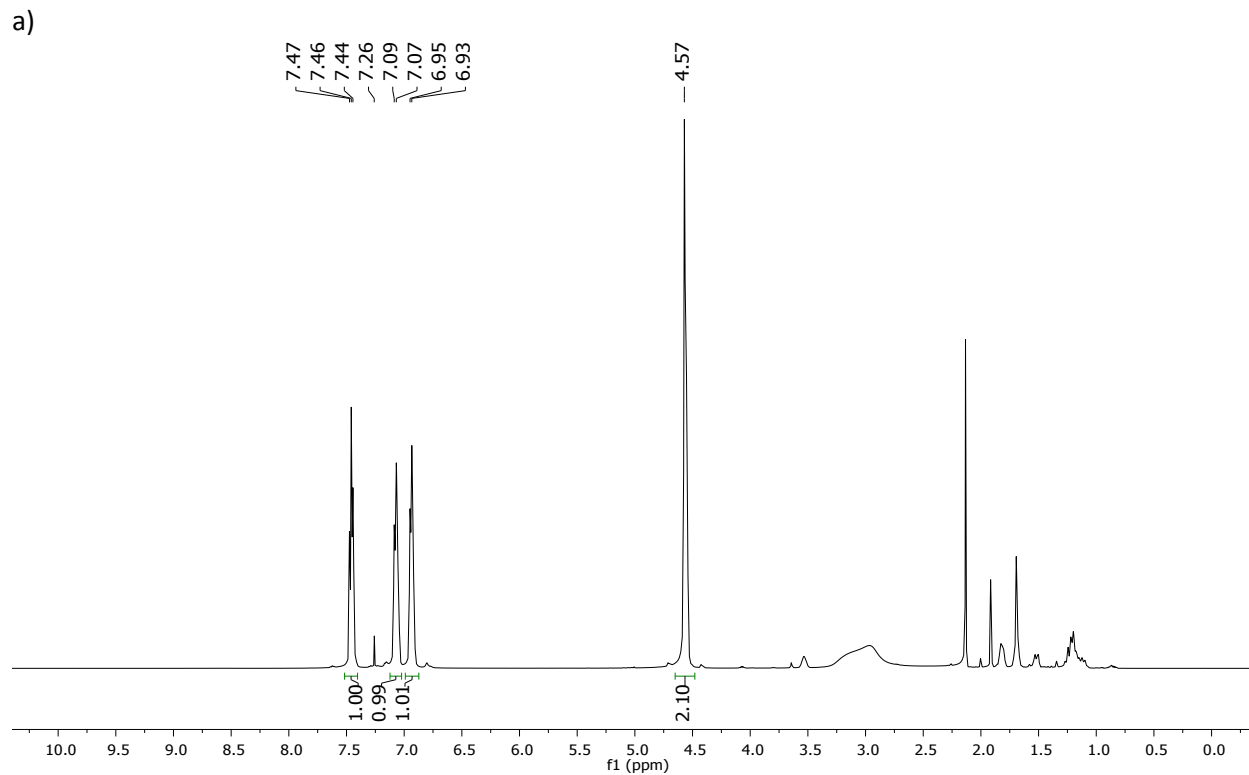


Figure S11. a) ^1H NMR and b) ^{13}C NMR spectra of **16**

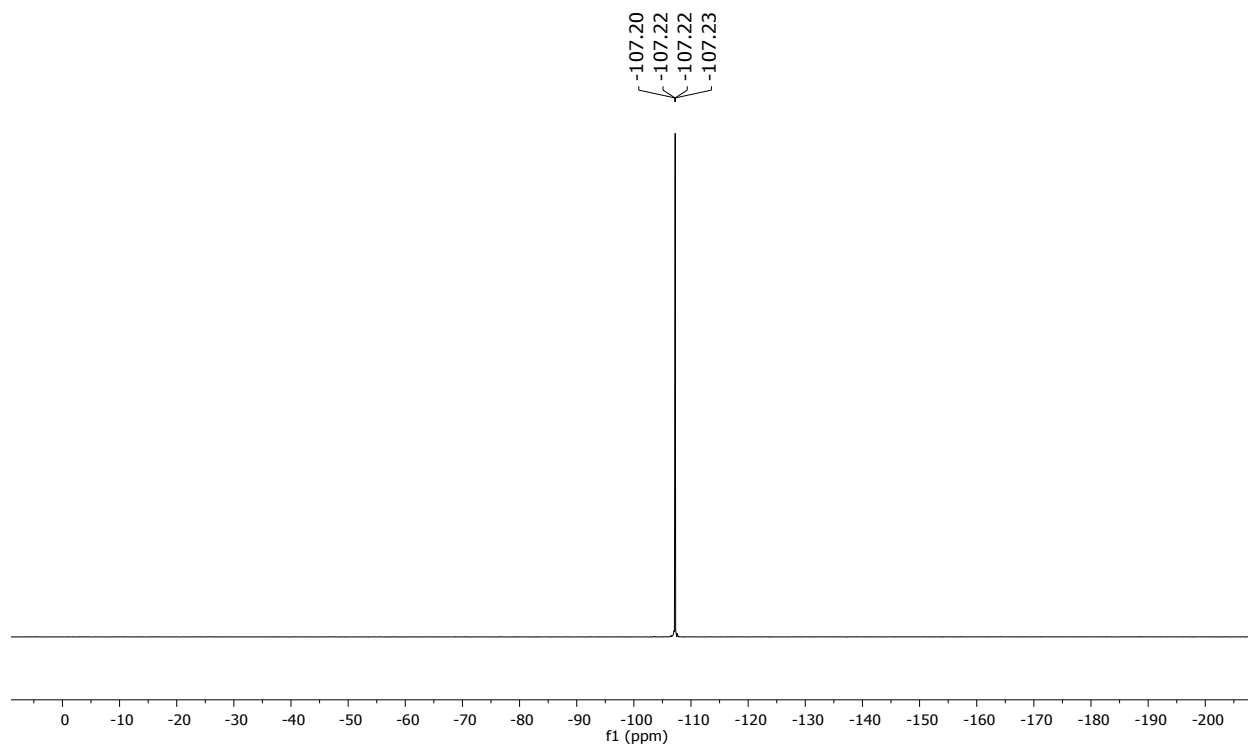


Figure S12. ^{19}F NMR spectrum of **16**

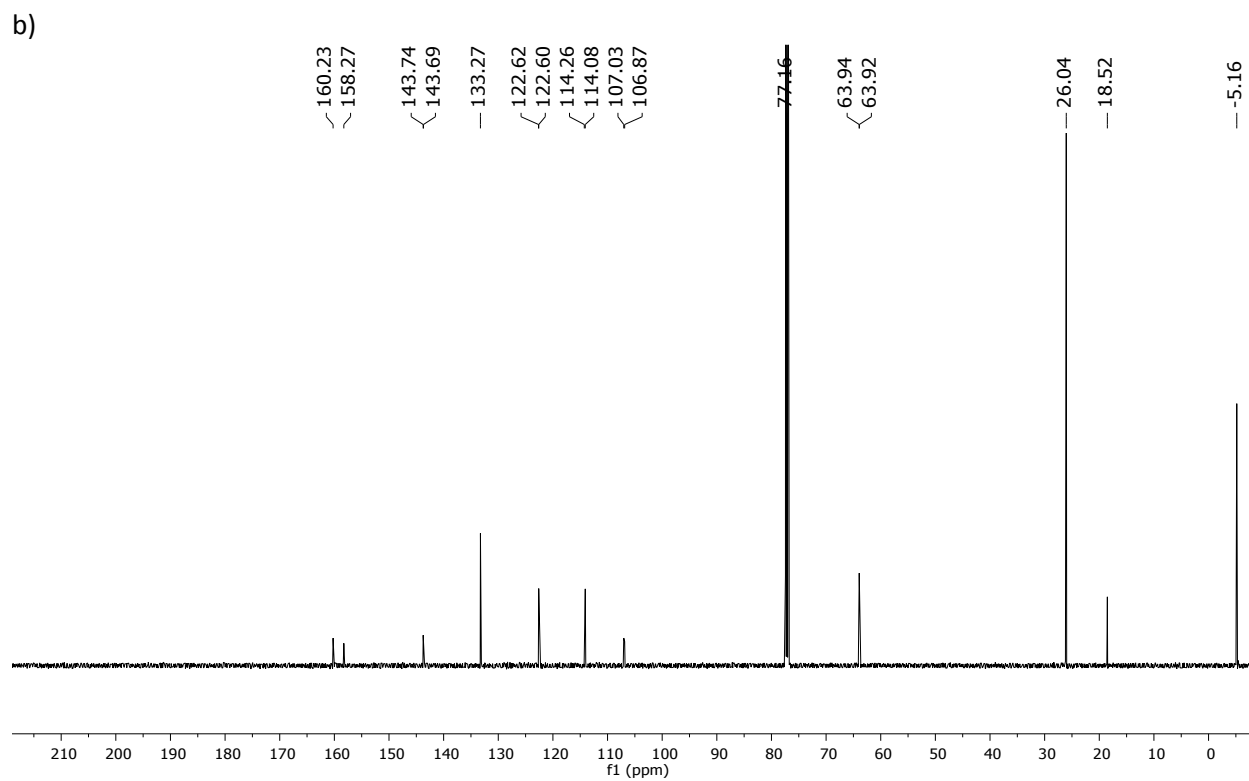
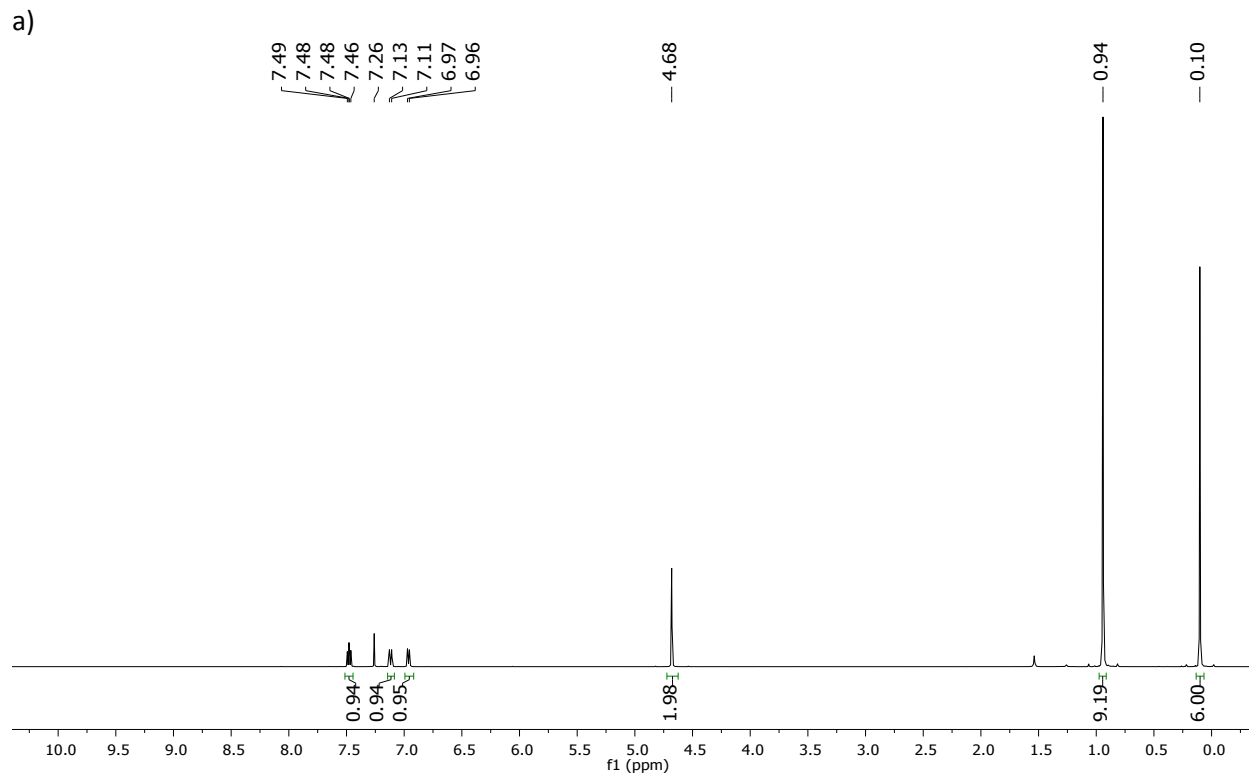


Figure S13. a) ^1H NMR and b) ^{13}C NMR spectra of **17**

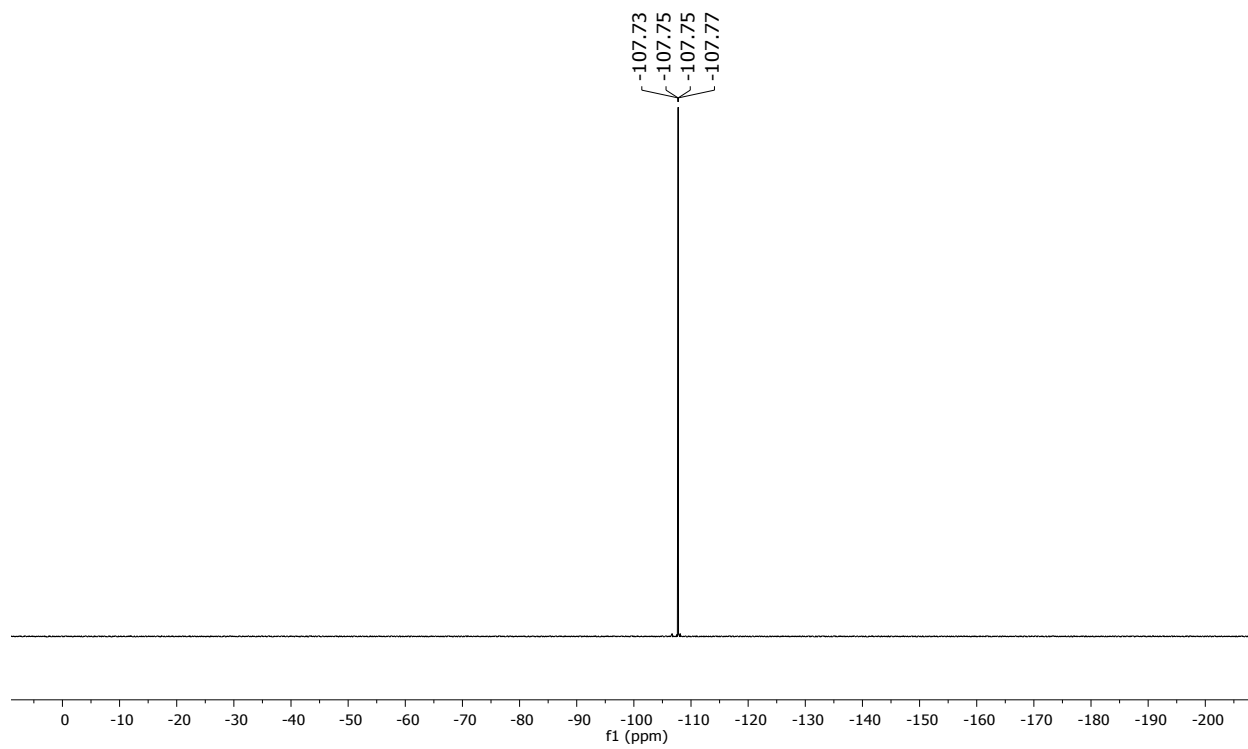


Figure S14. ^{19}F NMR spectrum of **17**

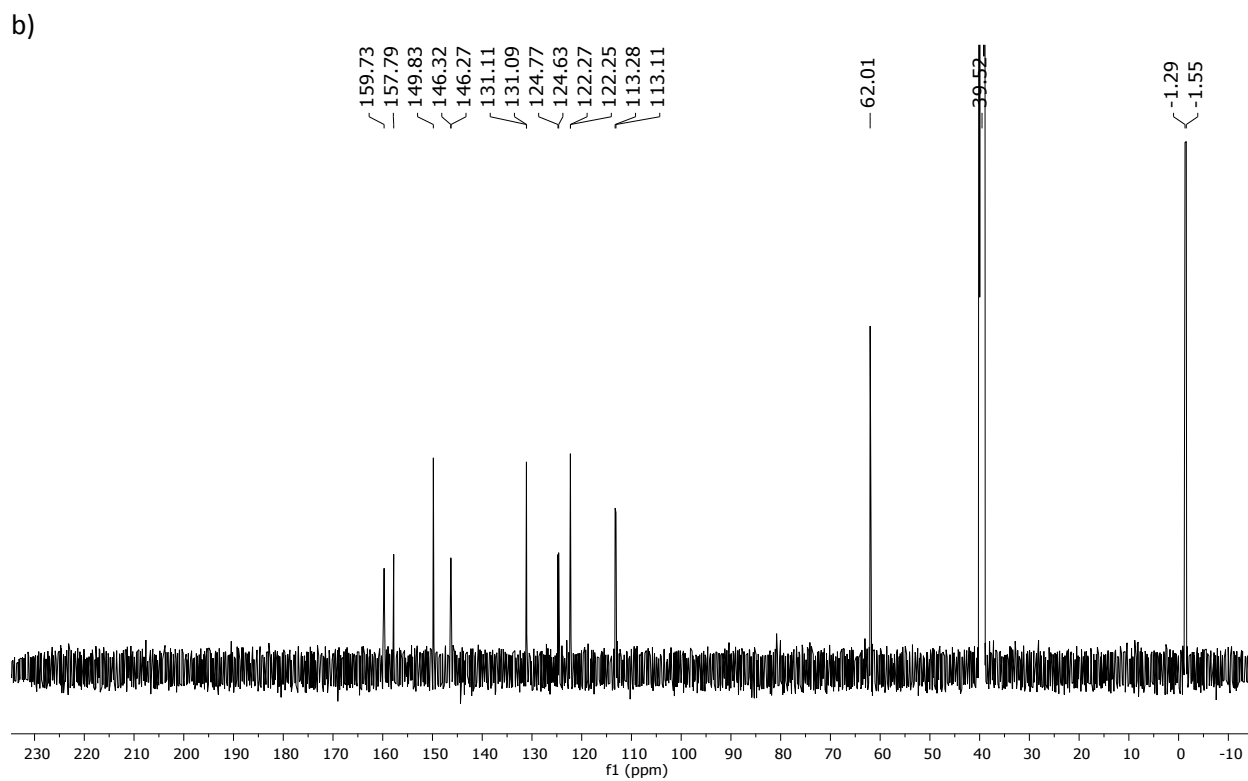
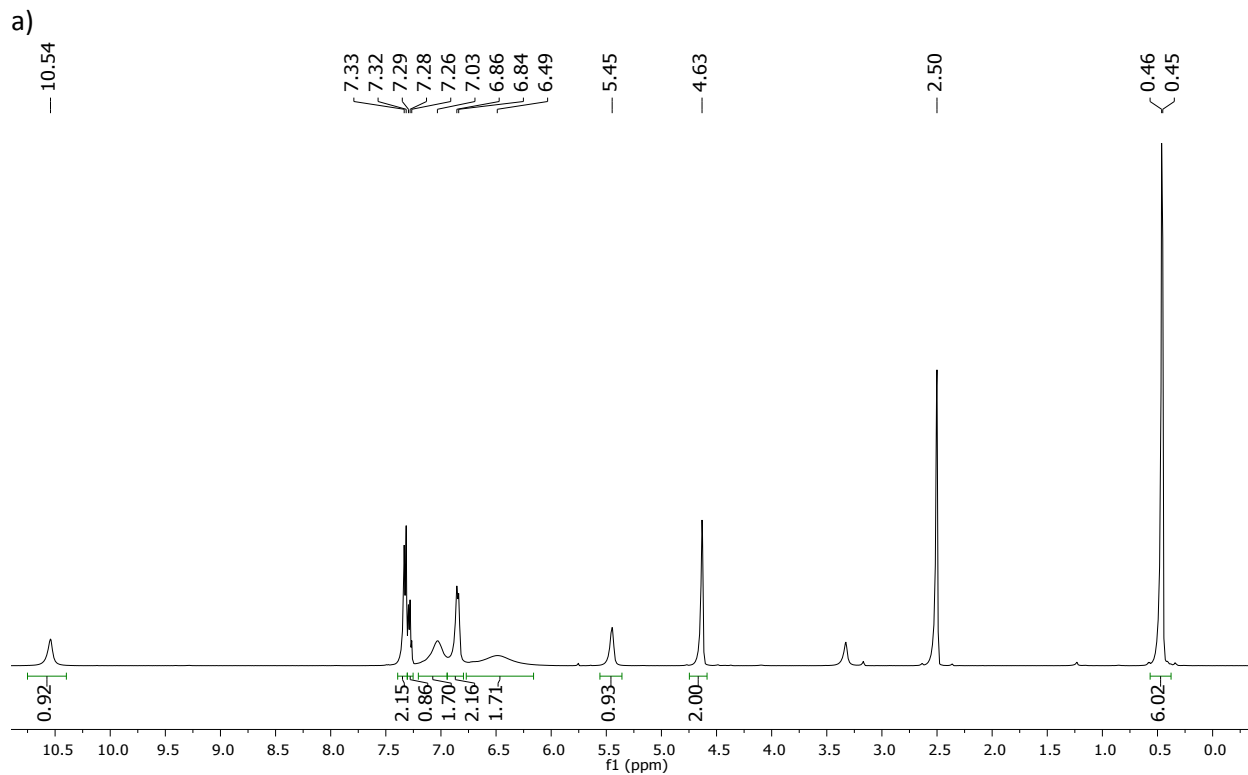


Figure S15. a) ^1H NMR and b) ^{13}C NMR spectra of **18**

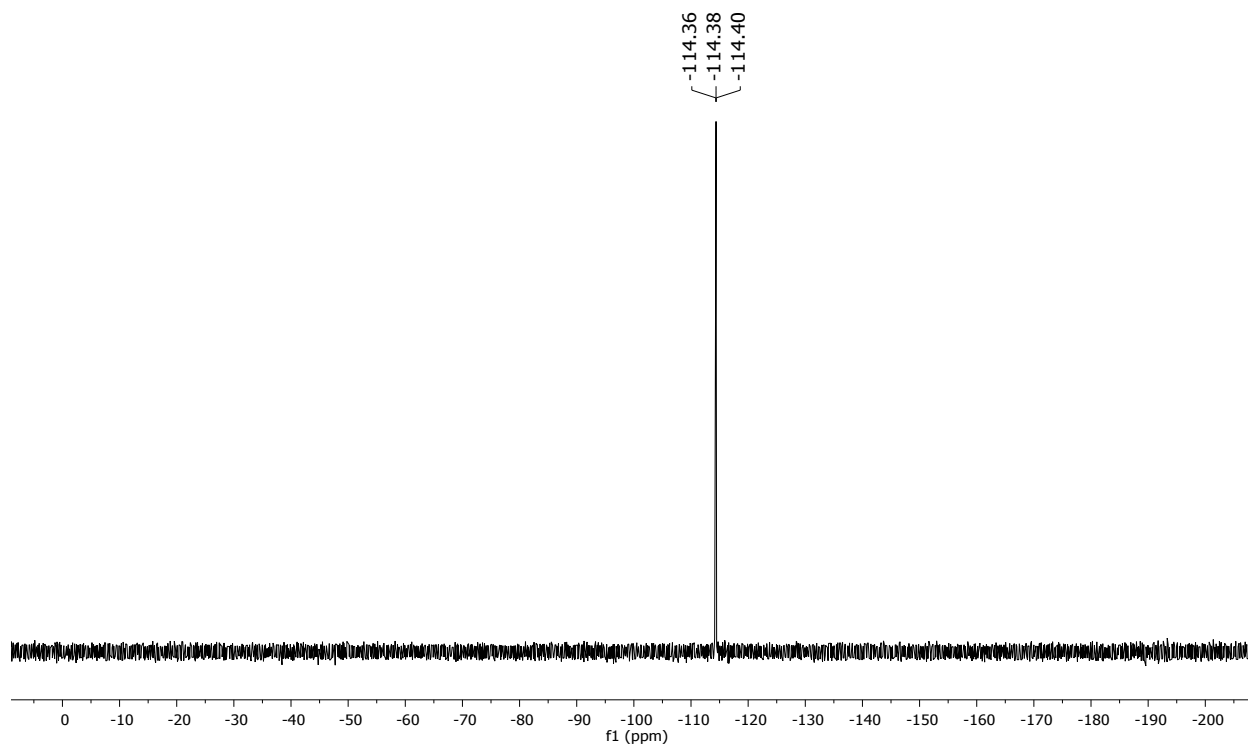


Figure S16. ^{19}F NMR spectrum of **18**

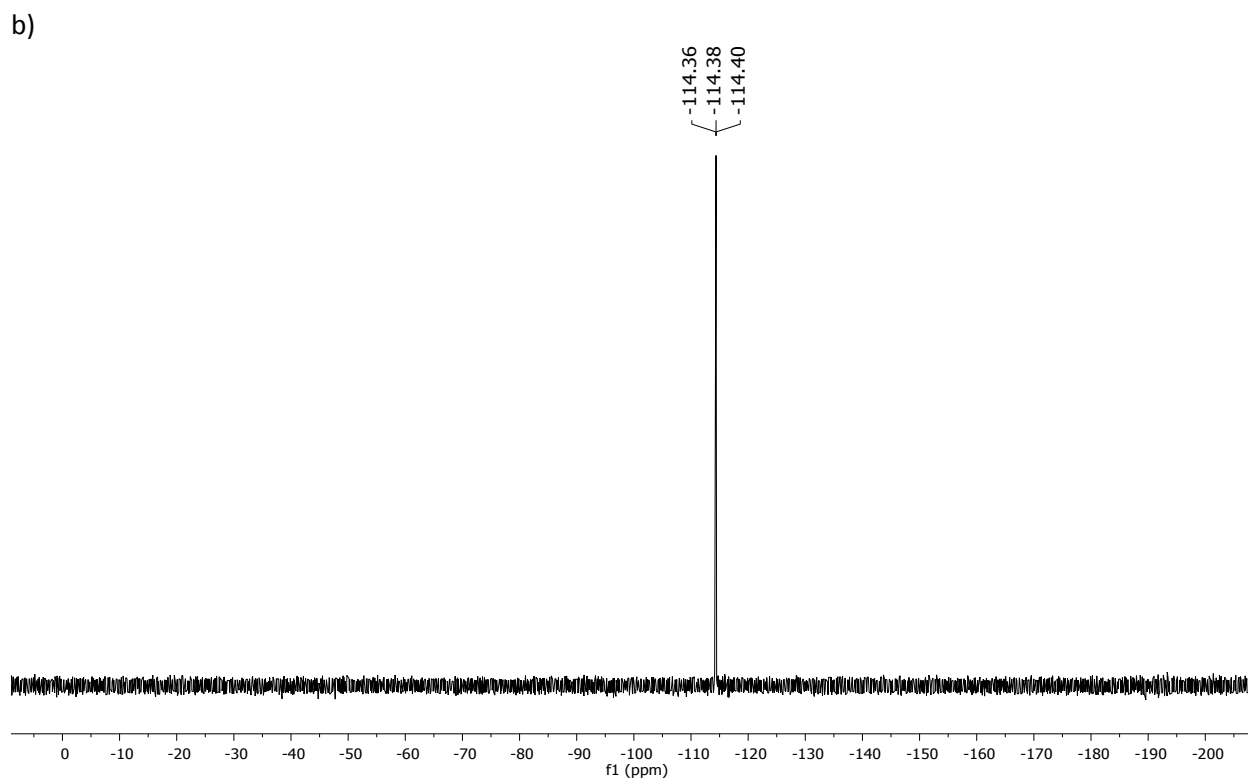
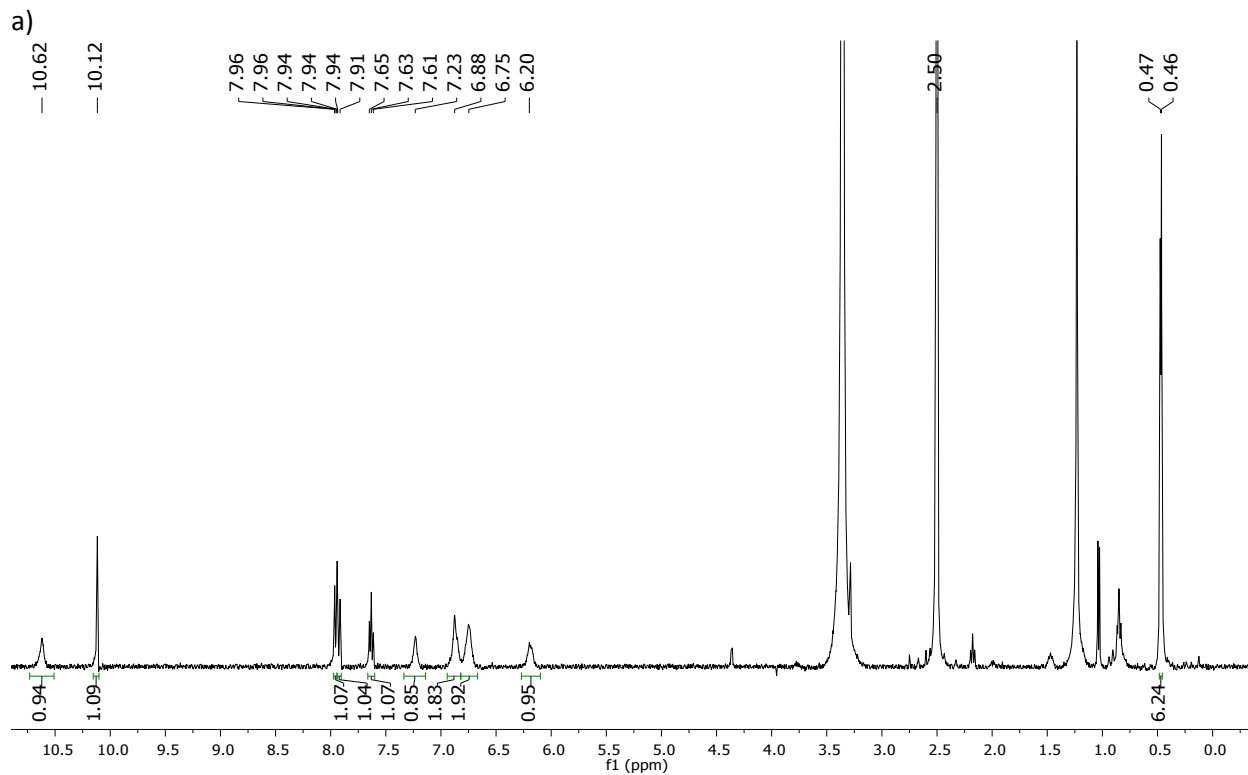


Figure S17. a) ^1H NMR and b) ^{19}F NMR spectra of **Probe 2**

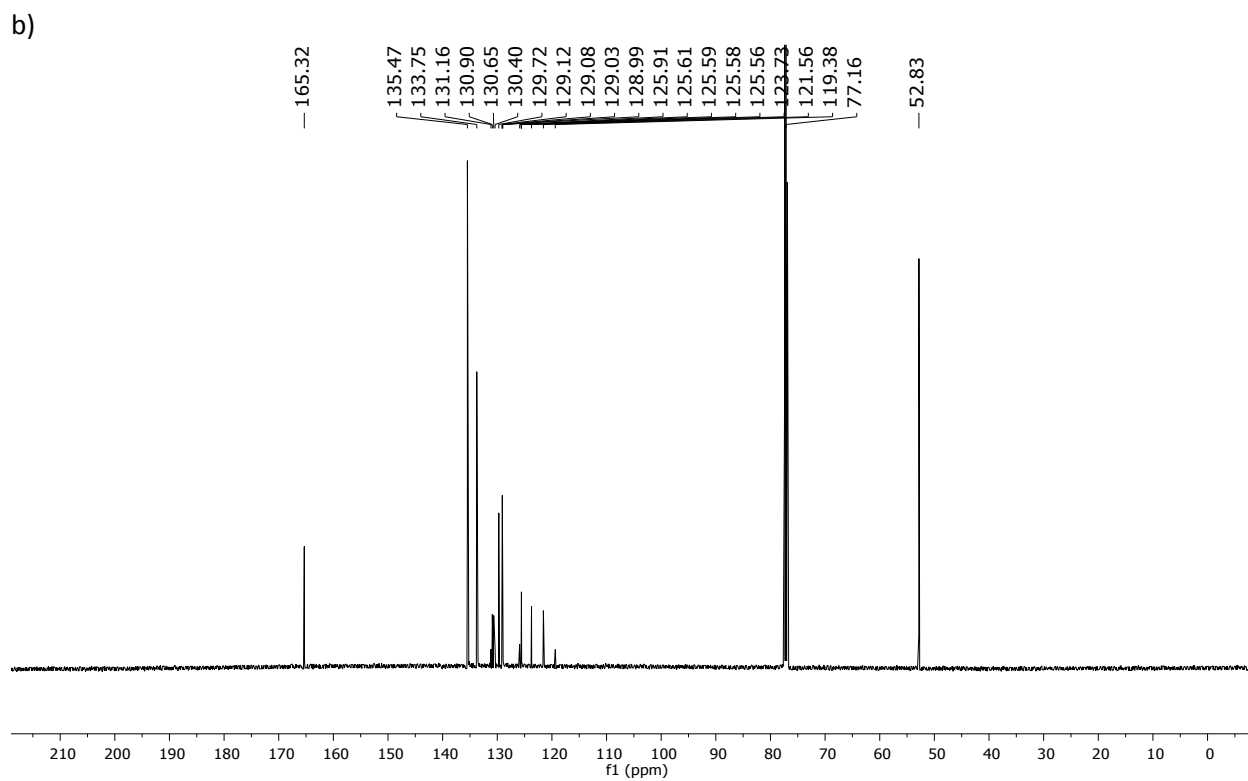
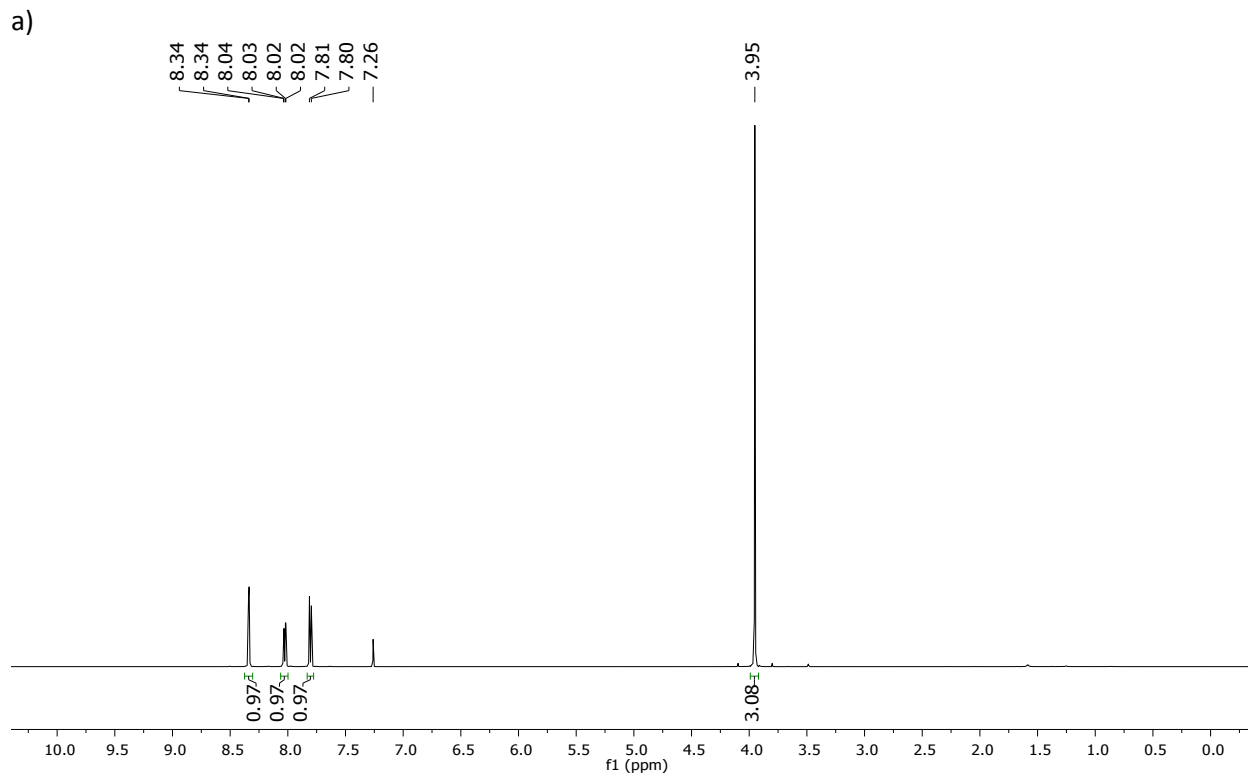


Figure S18. a) ^1H NMR and b) ^{13}C NMR spectra of **20**

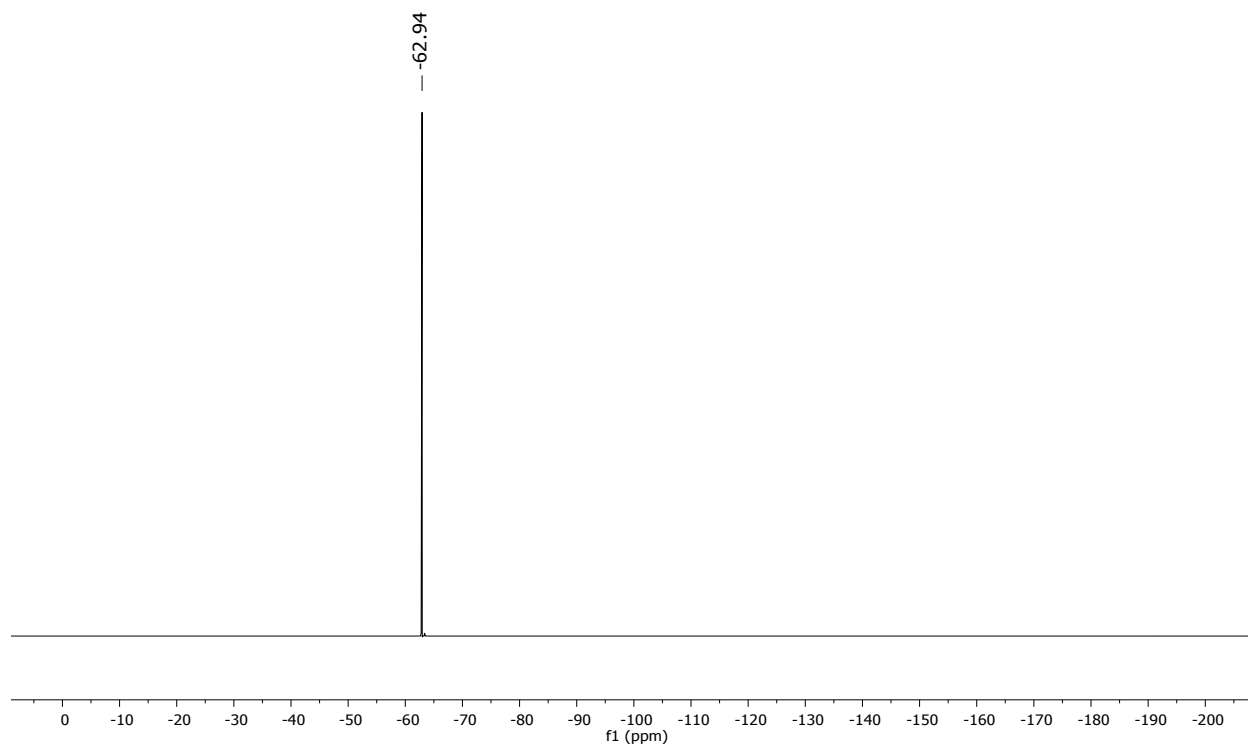


Figure S19. ^{19}F NMR spectrum of **20**

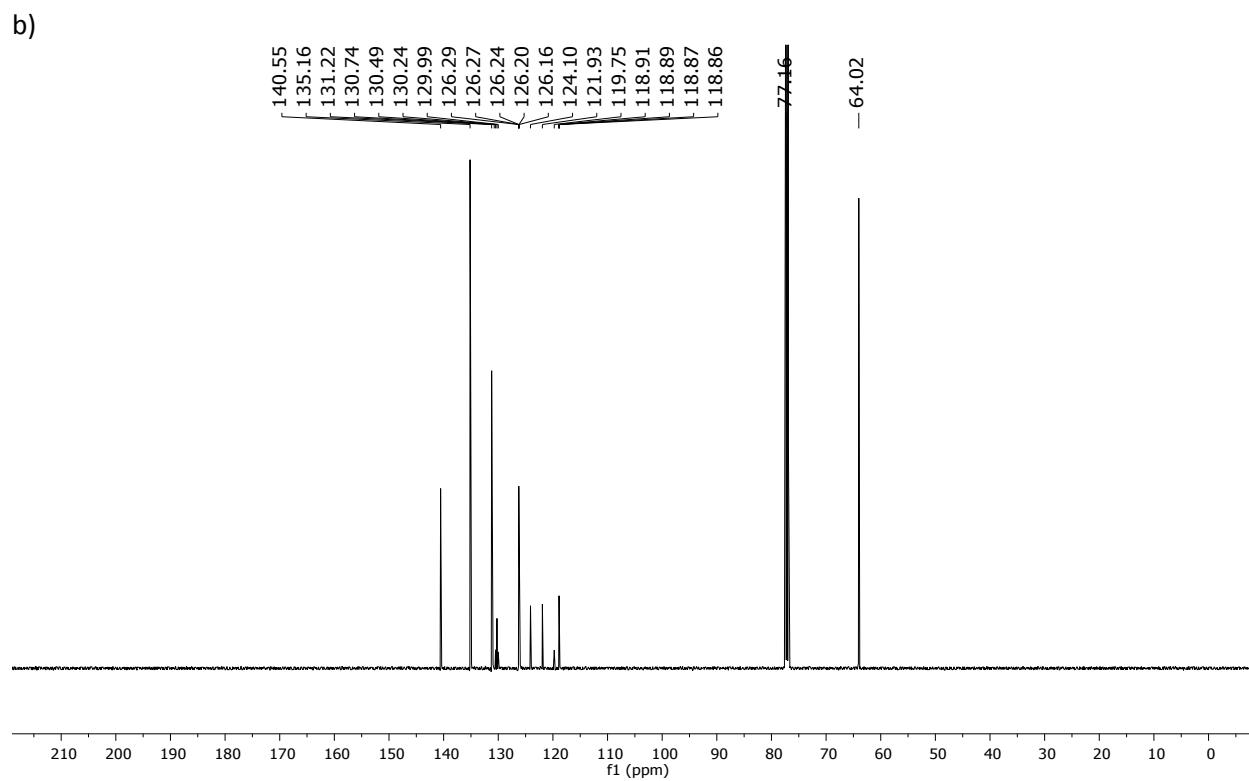
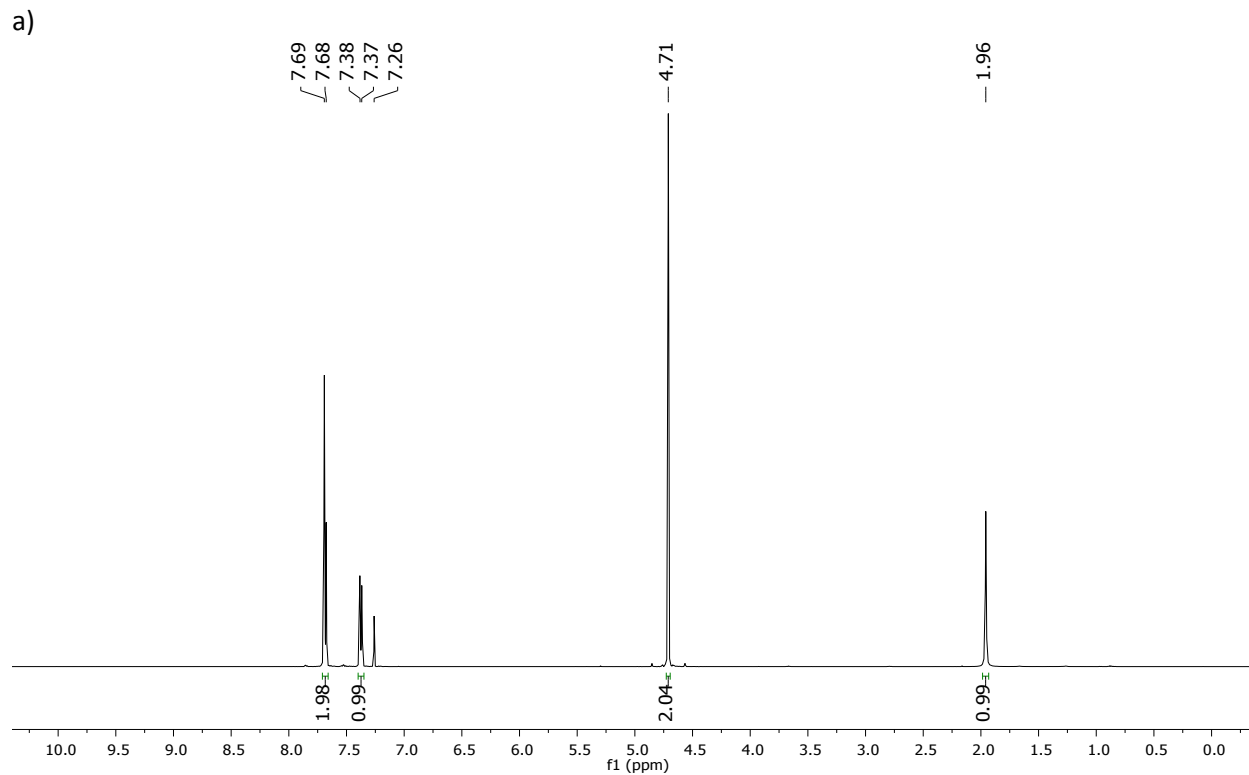


Figure S20. a) ^1H NMR and b) ^{13}C NMR spectra of **21**

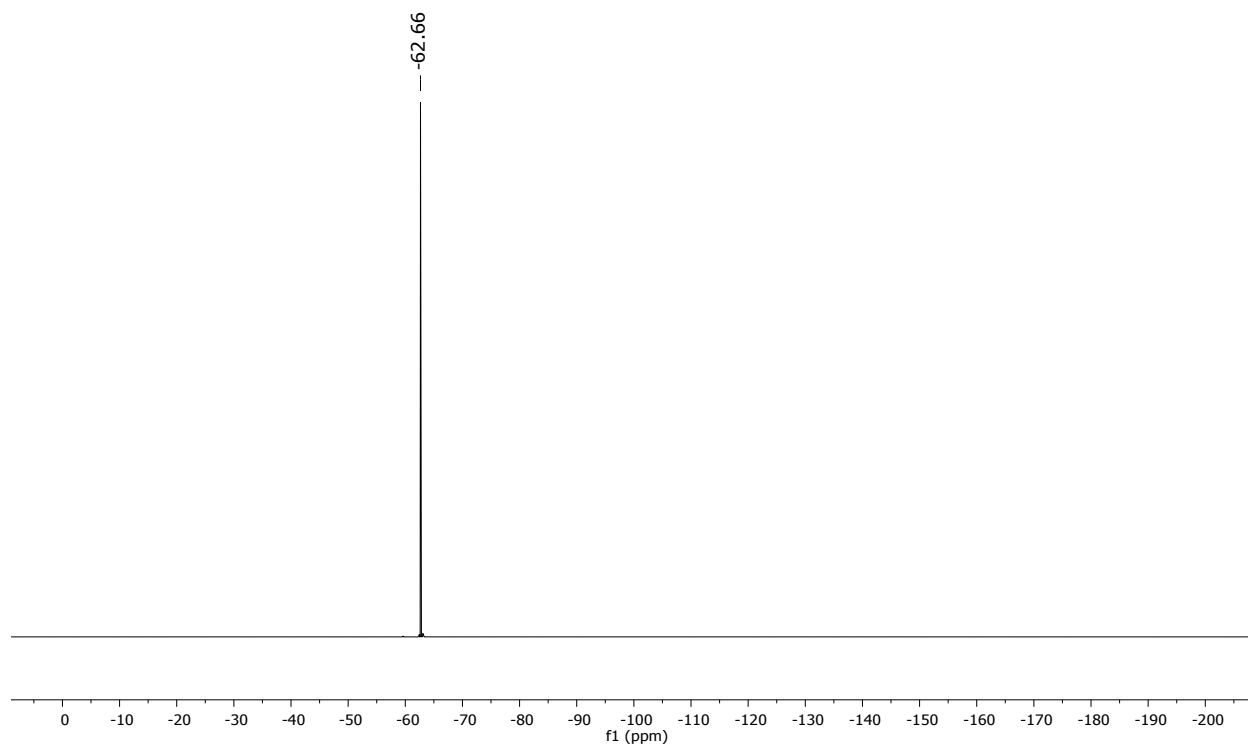


Figure S21. ^{19}F NMR spectra of **21**

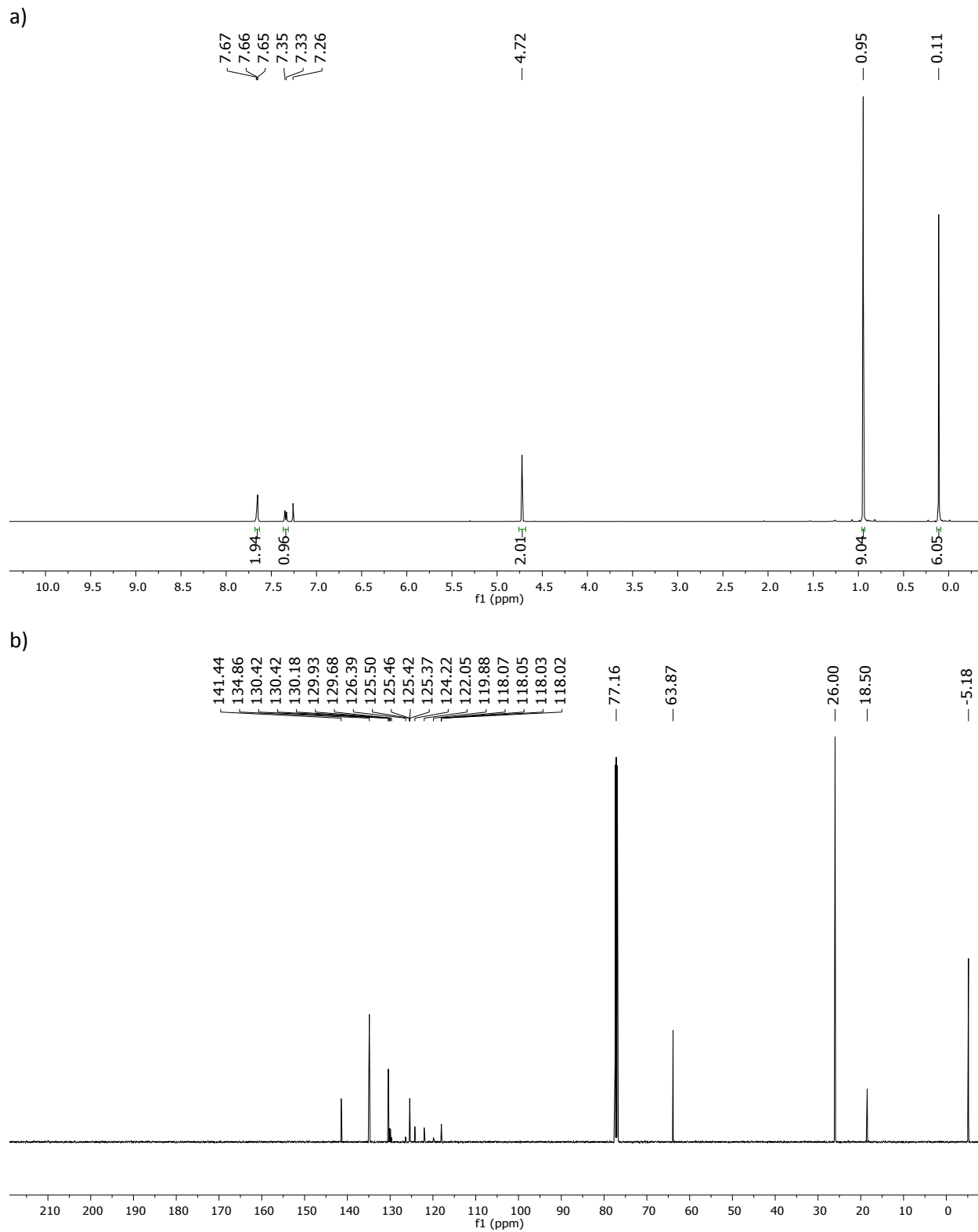


Figure S22. a) ^1H NMR and b) ^{13}C NMR spectra of **22**

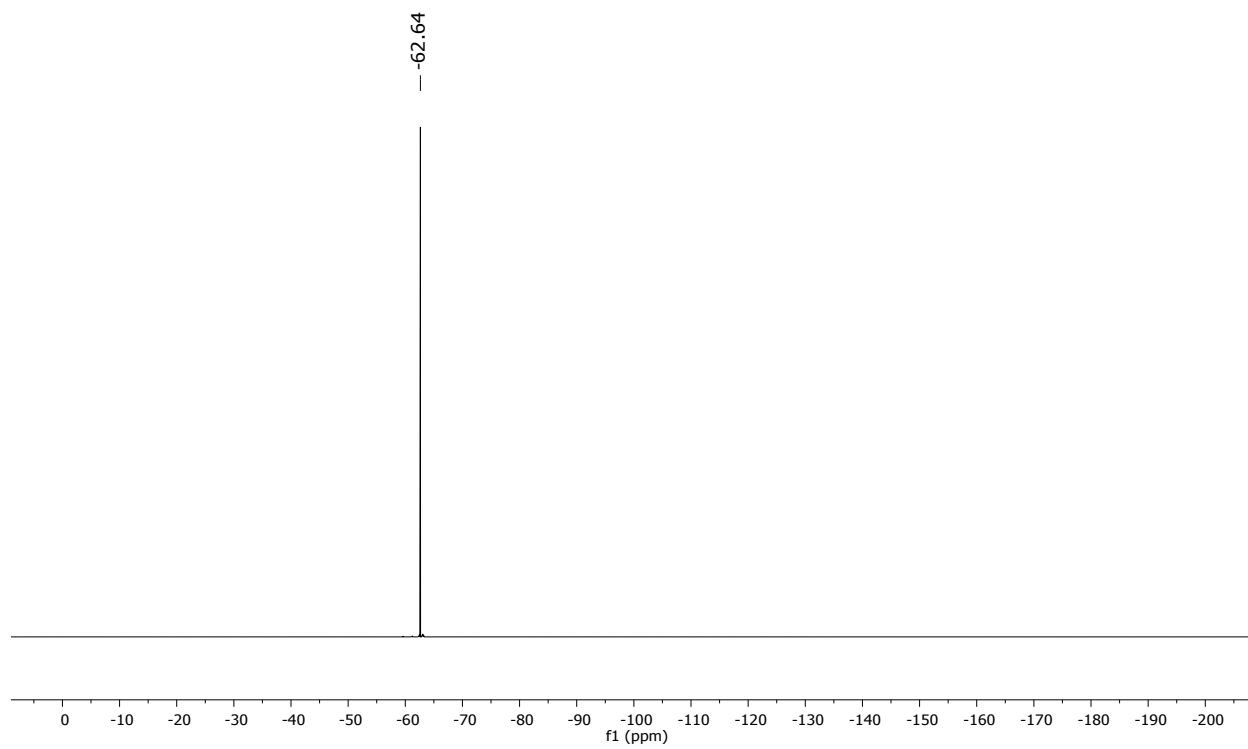


Figure S23. ^{19}F NMR spectrum of **22**

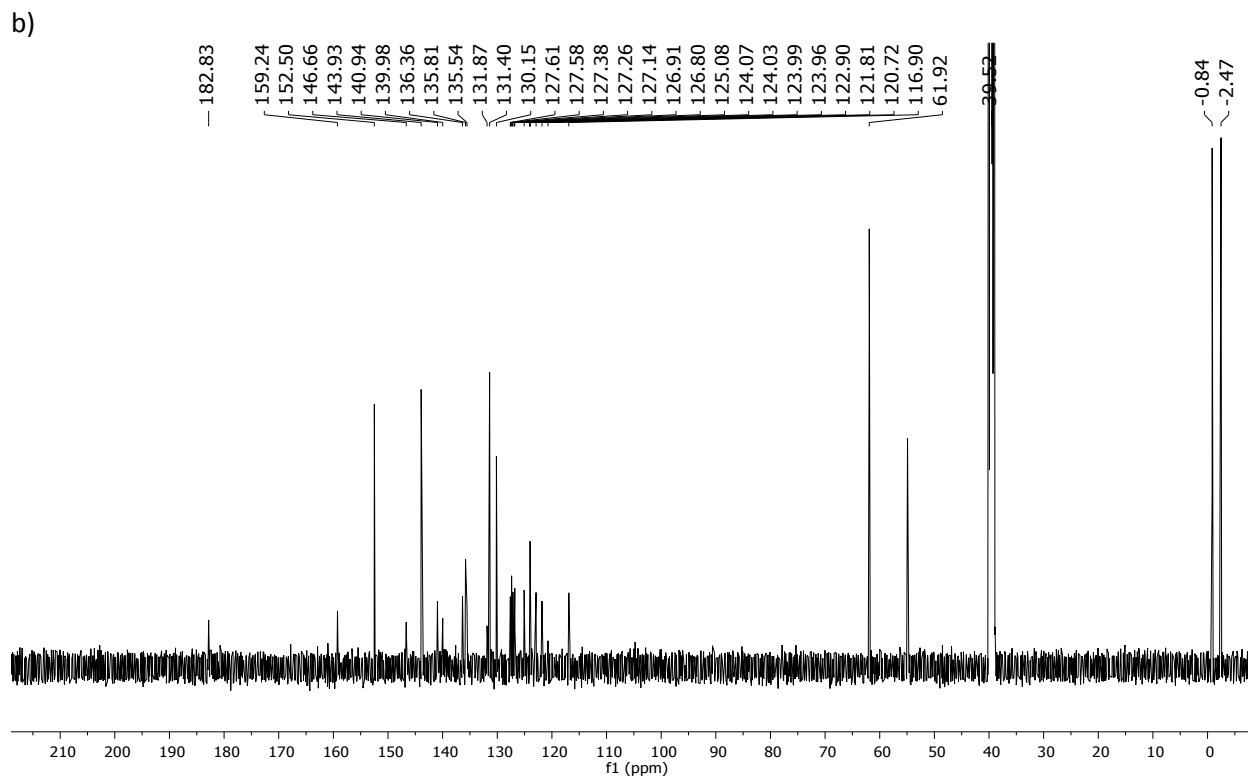
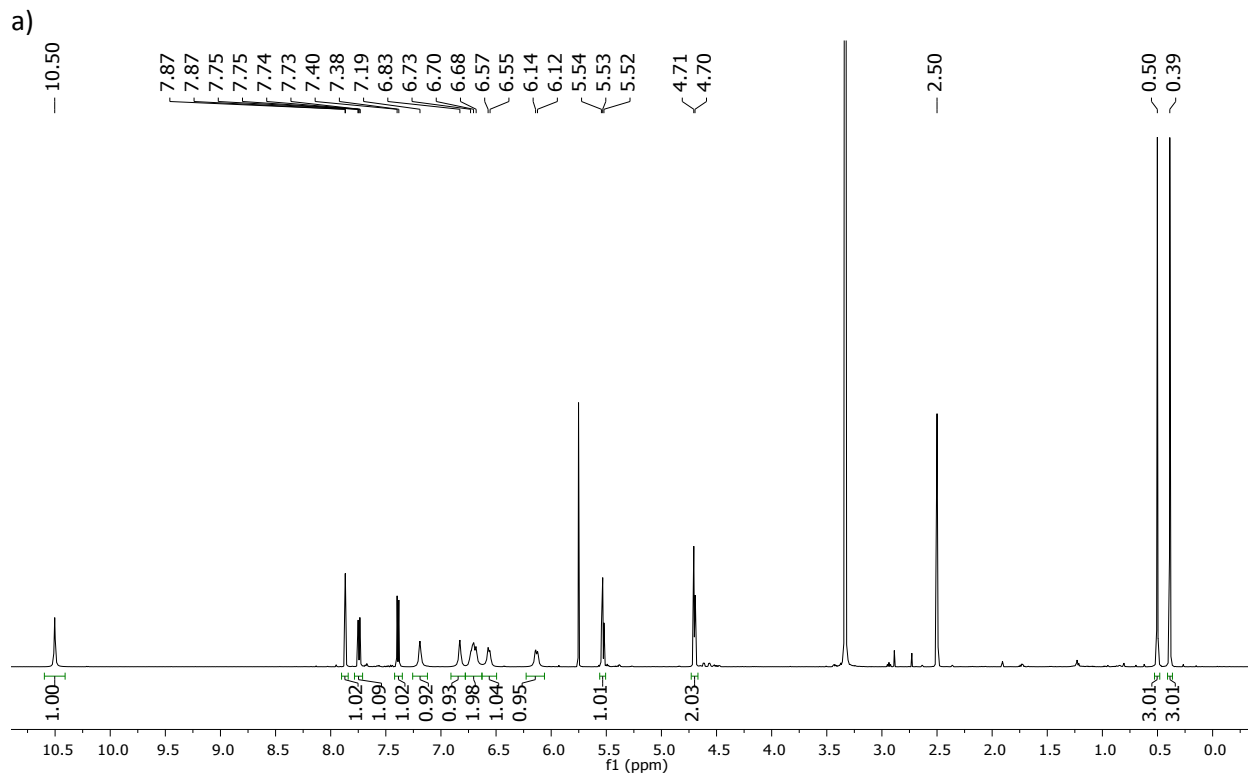


Figure S24. a) ^1H NMR and b) ^{13}C NMR spectra of **23**

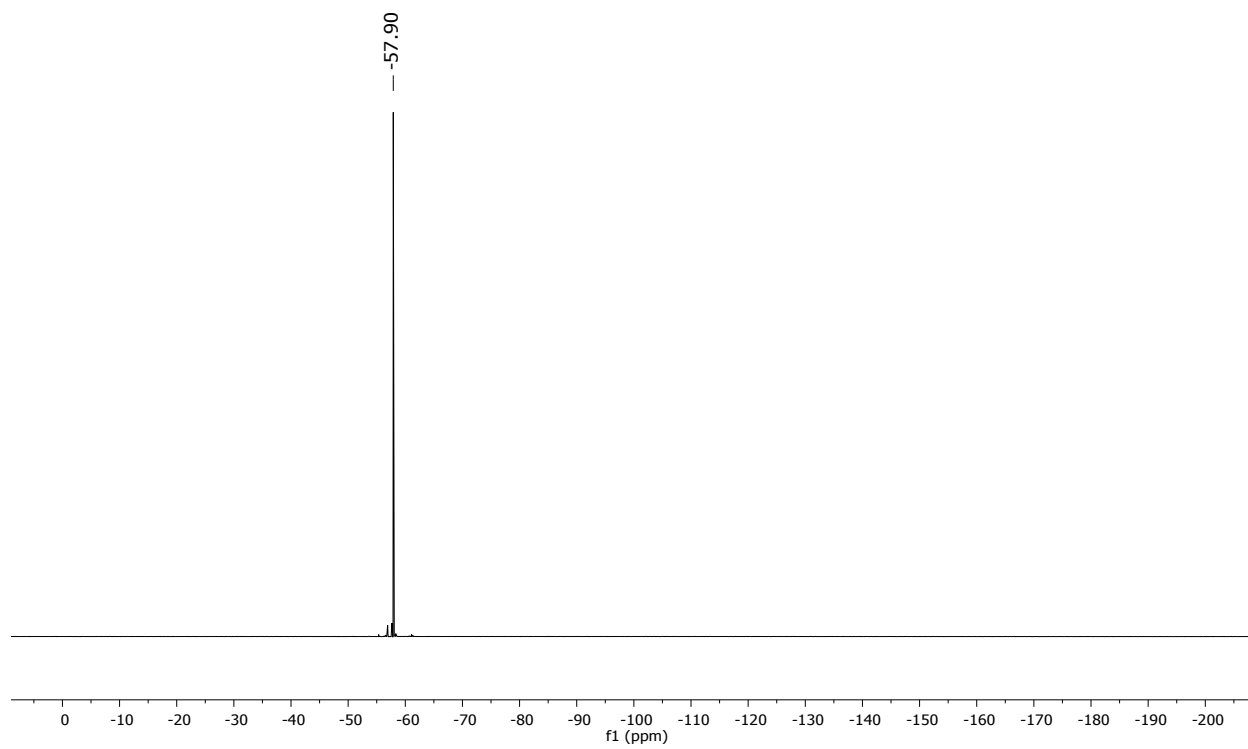


Figure S25. ^{19}F NMR spectra of **23**

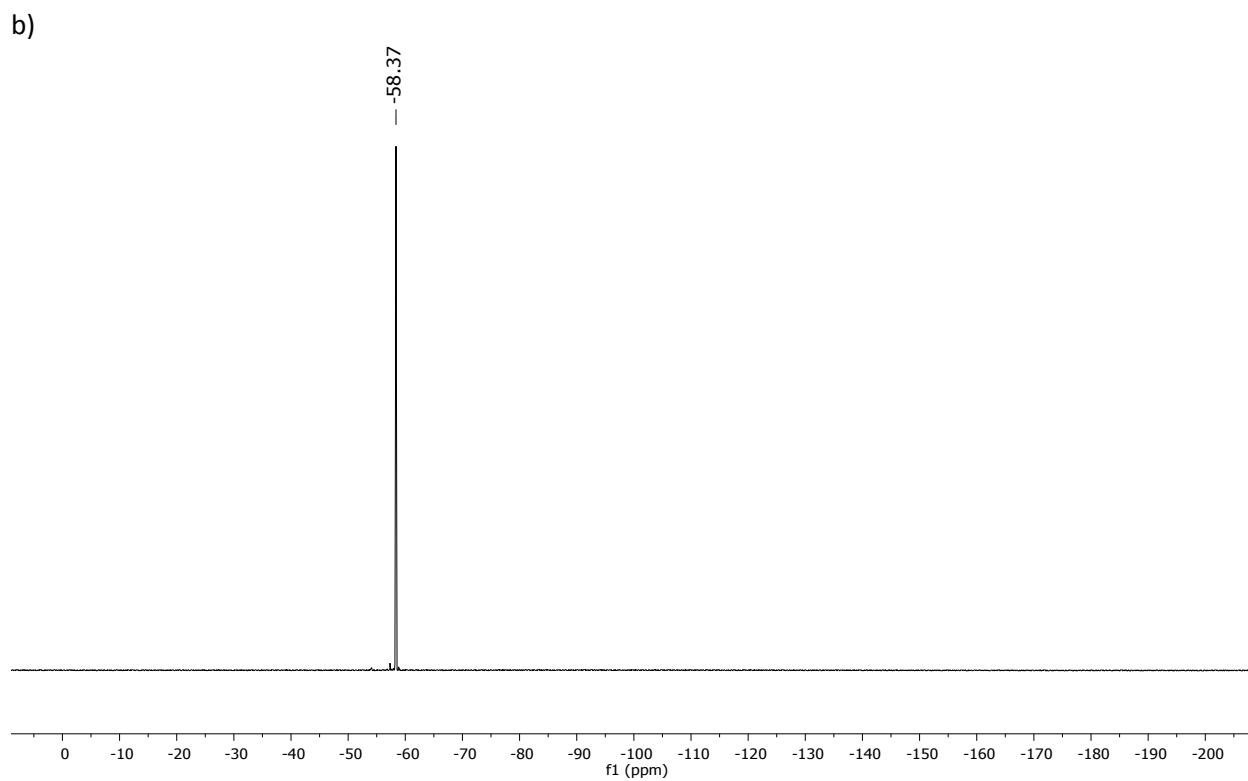
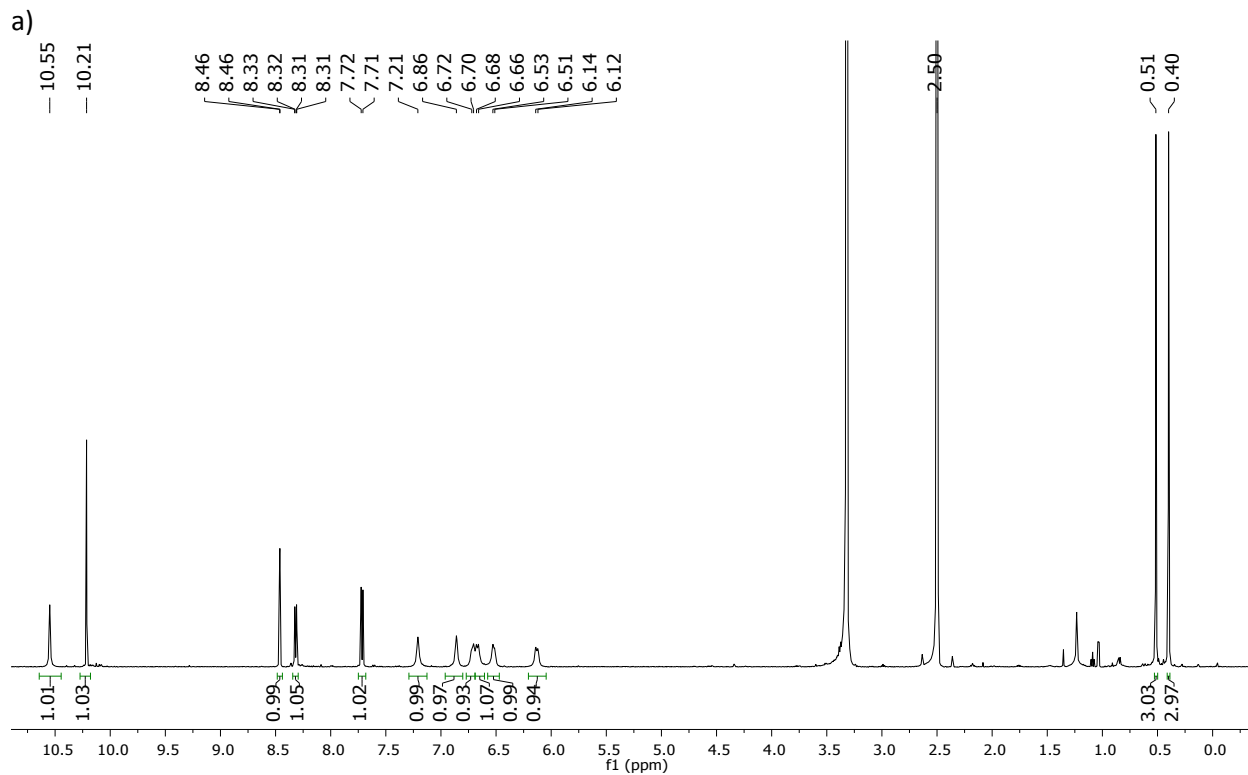


Figure S26. a) ^1H NMR and b) ^{19}F NMR spectra of **Probe 3**

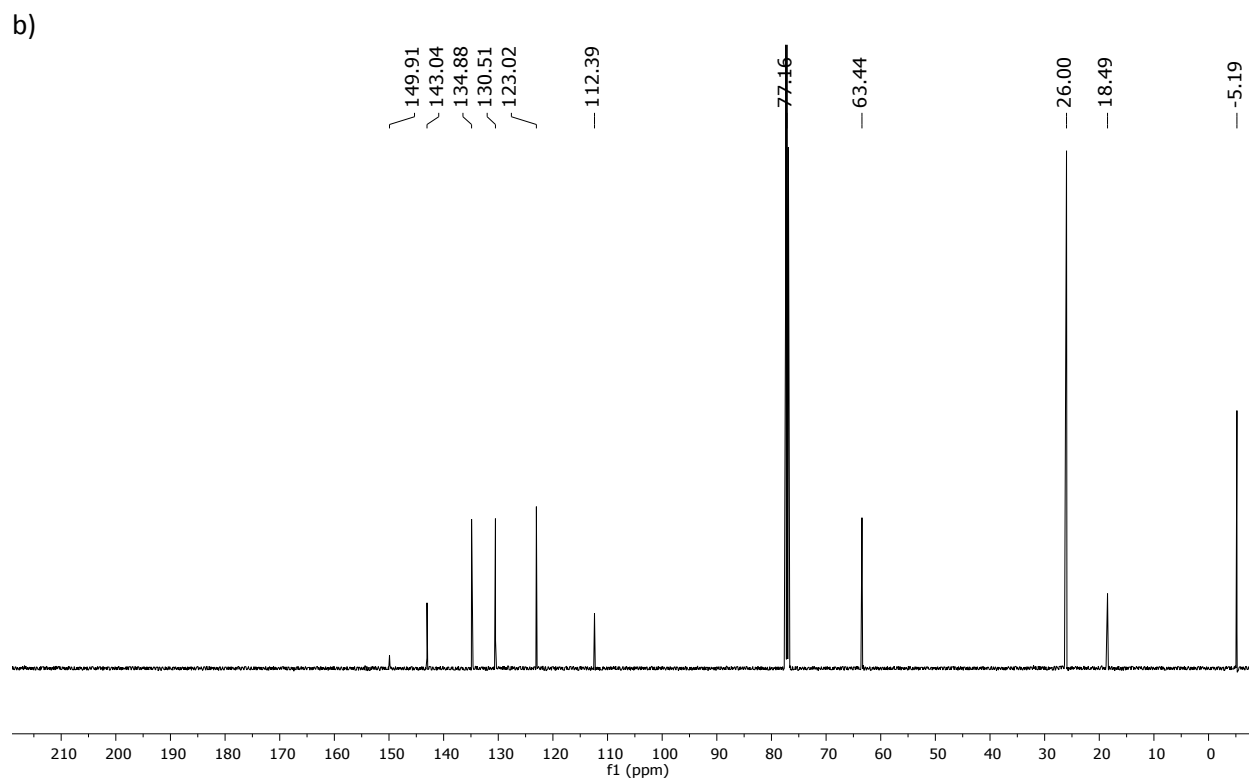
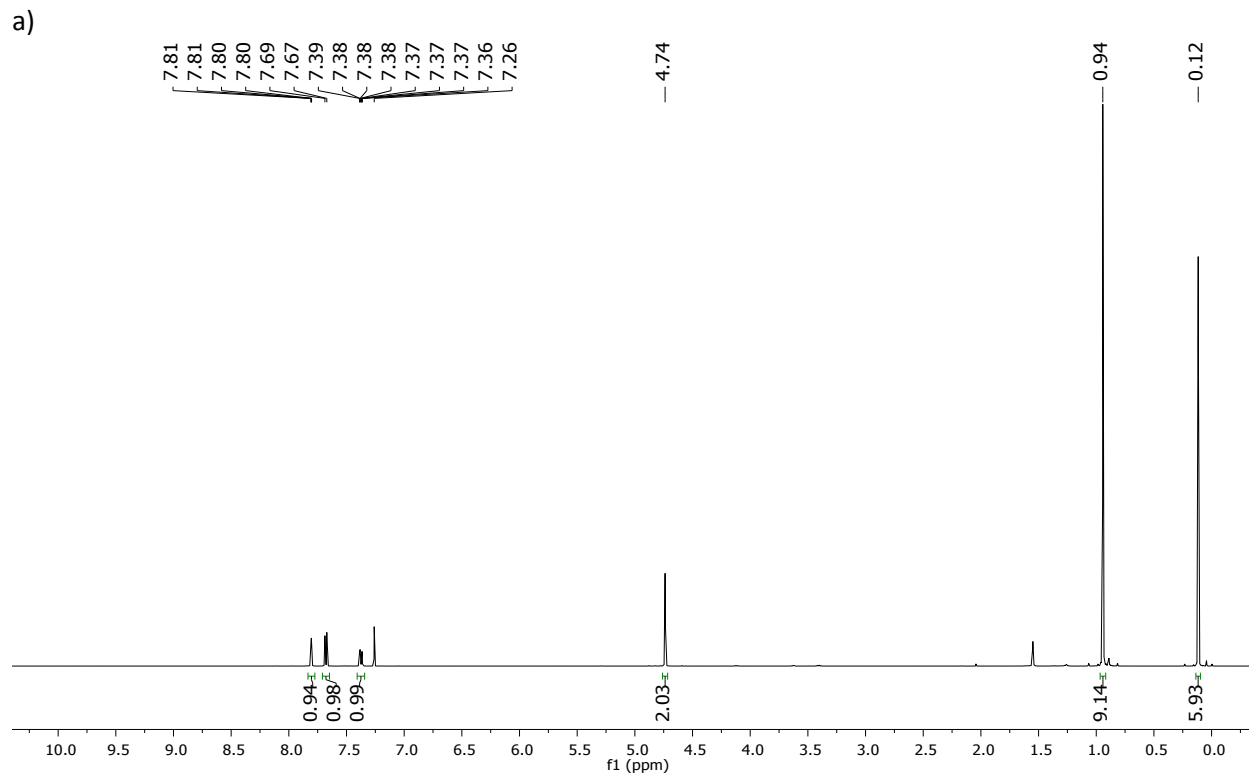


Figure S27. a) ^1H NMR and b) ^{13}C NMR spectra of **25**

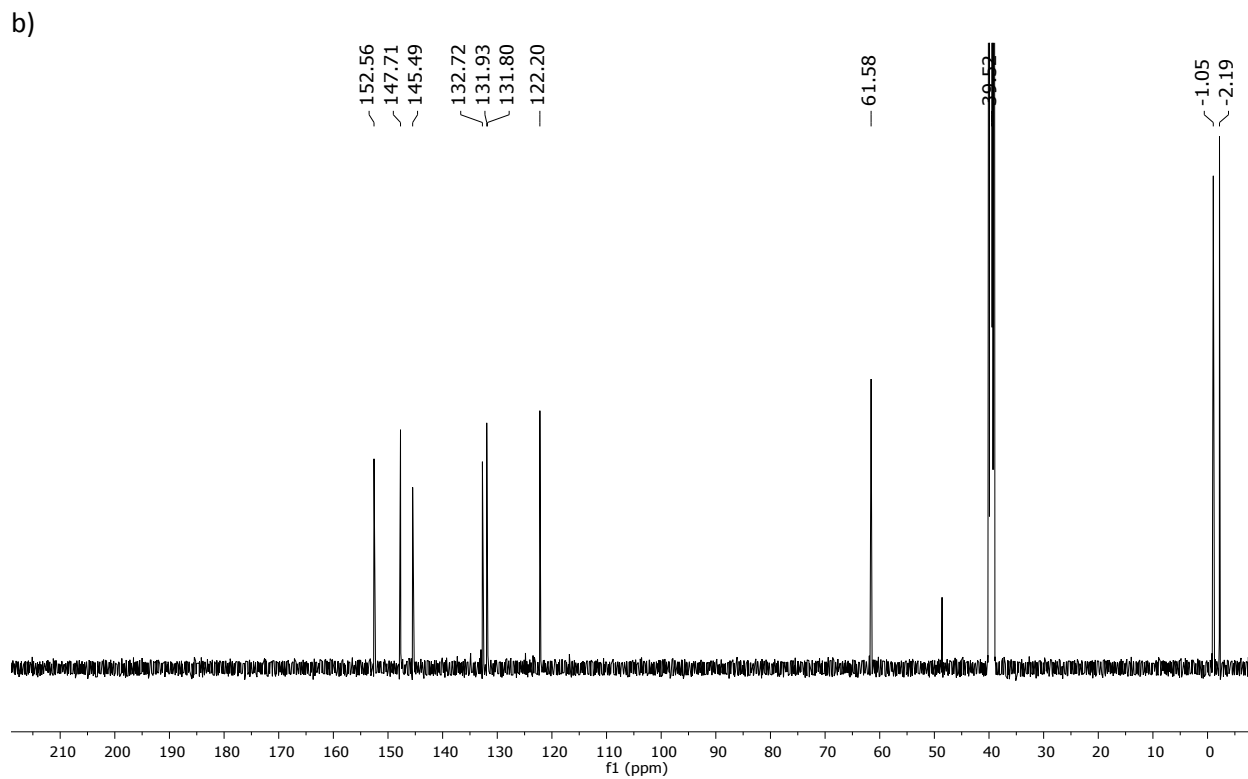
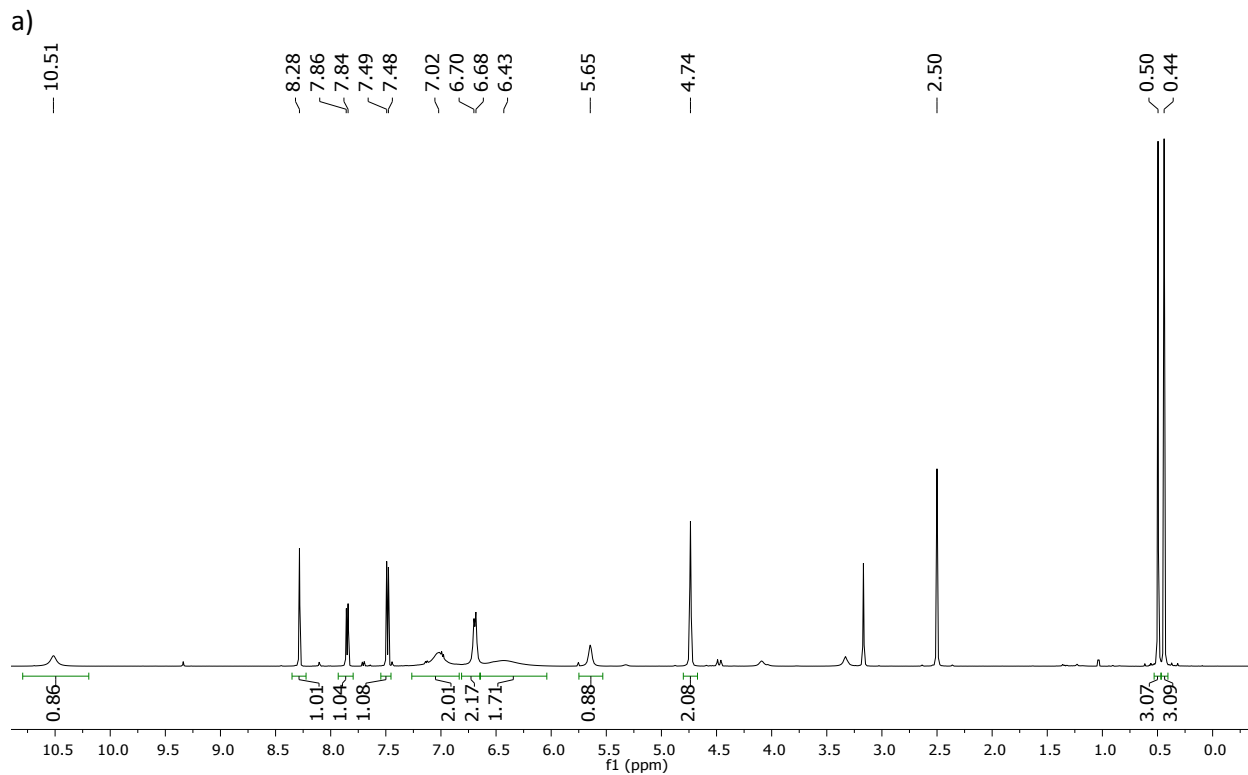


Figure S28. a) ^1H NMR and b) ^{13}C NMR spectra of **26**

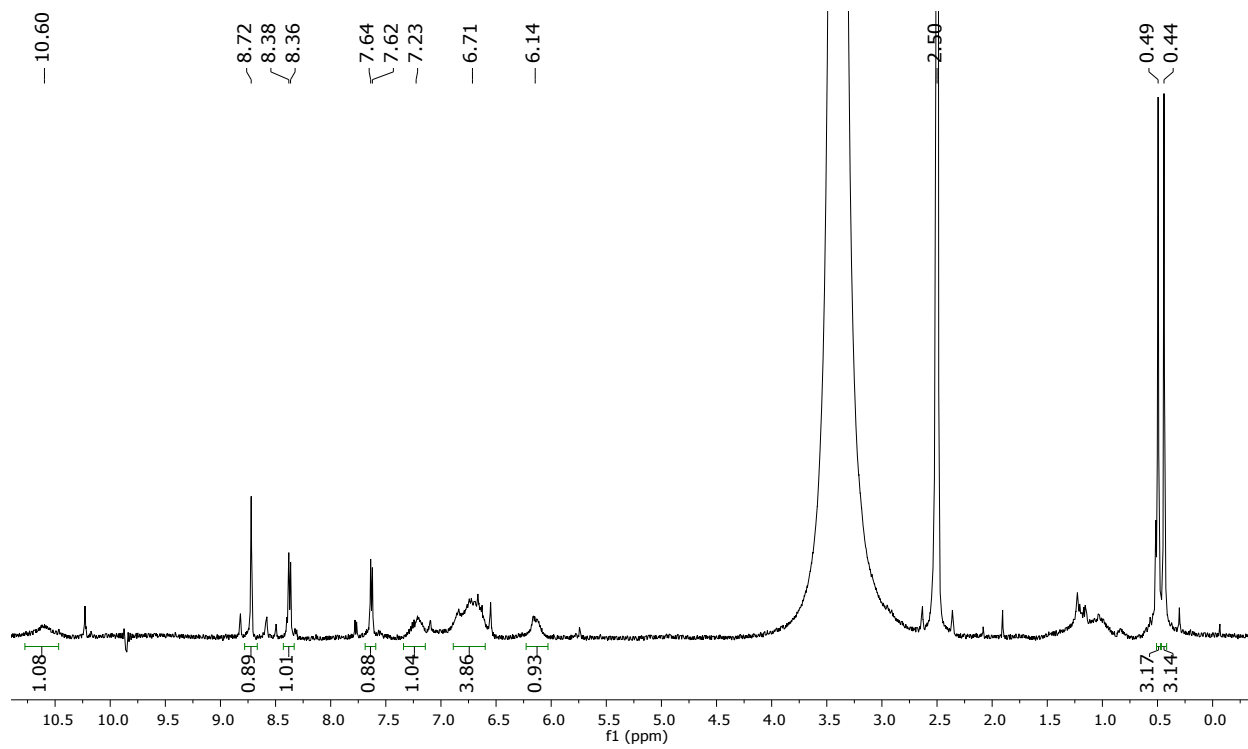


Figure S29. a) ¹H NMR spectrum of **Probe 4**

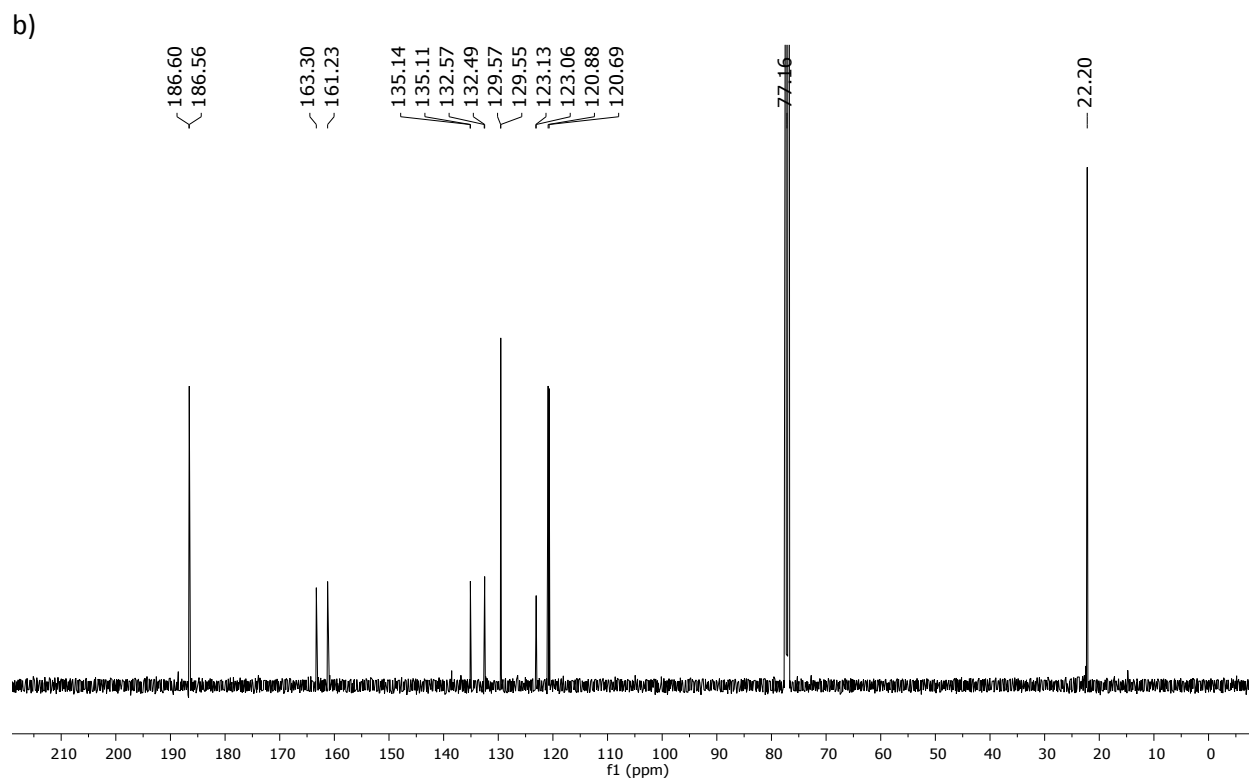
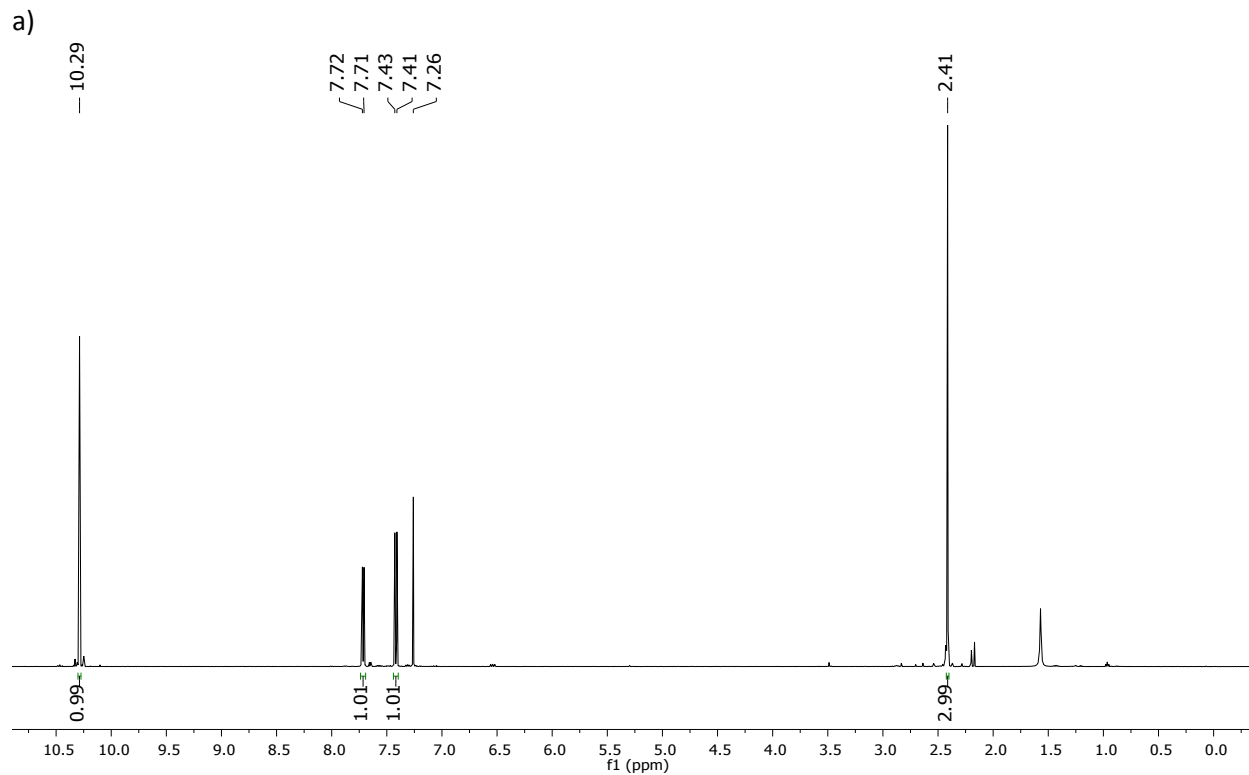


Figure S30. a) ^1H NMR and b) ^{13}C NMR spectra of **28**

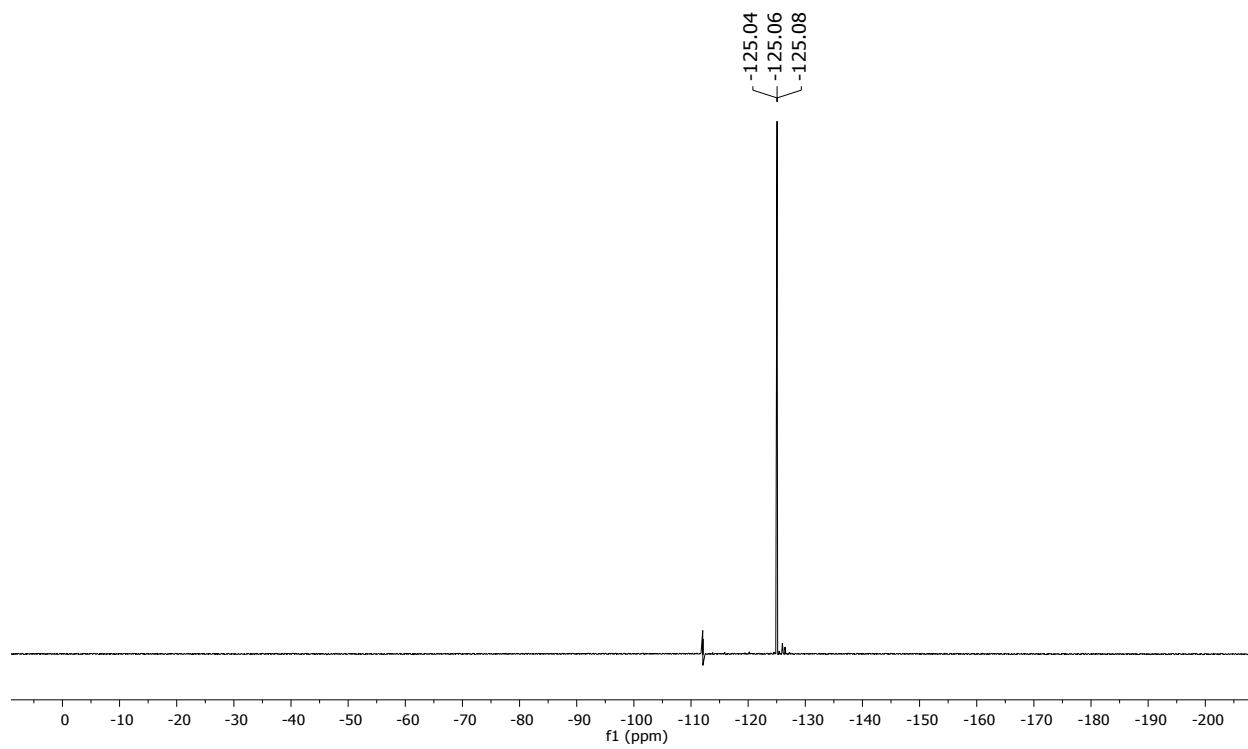
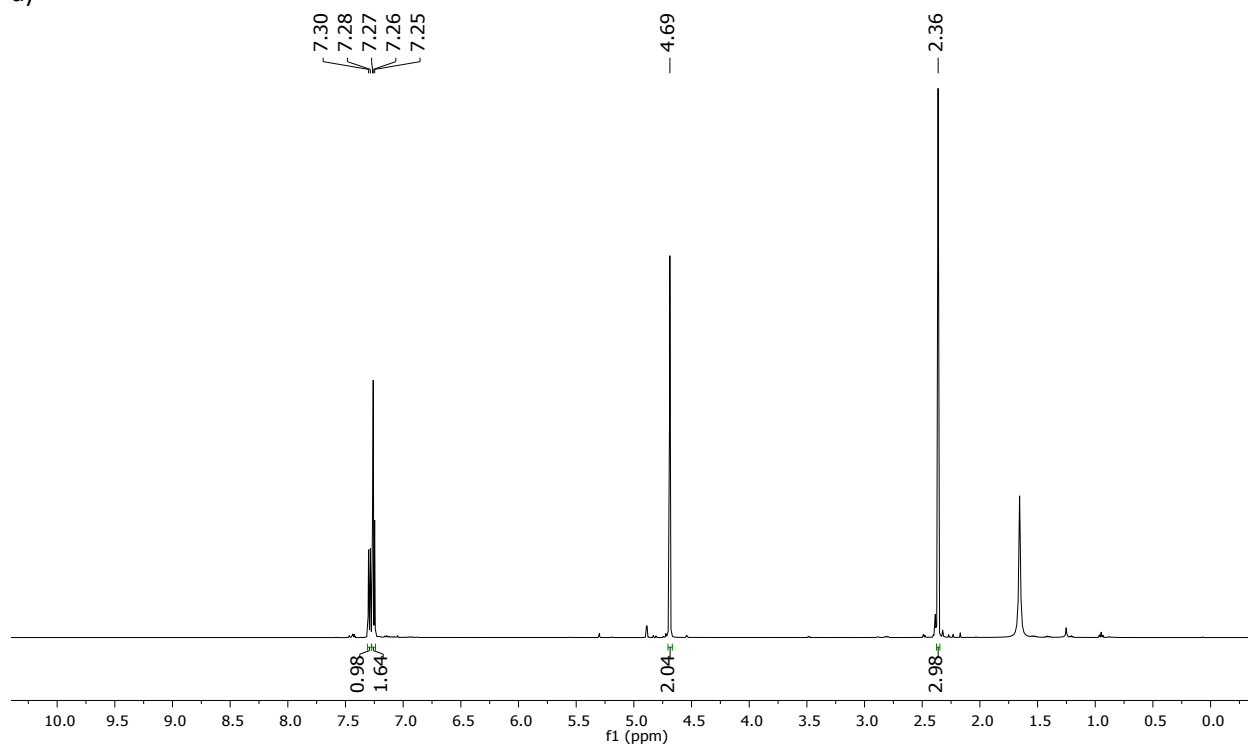


Figure S31. ^{19}F NMR spectrum of **28**

a)



b)

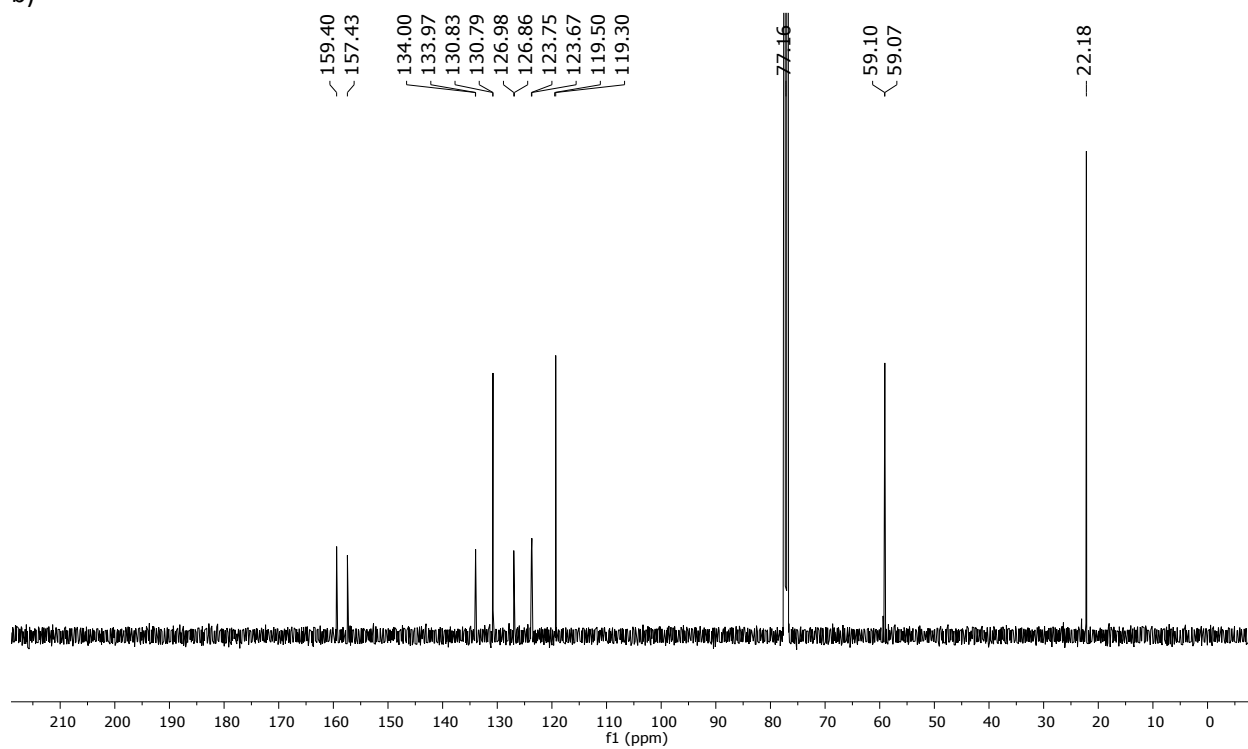


Figure S32. a) ^1H NMR and b) ^{13}C NMR spectra of **29**

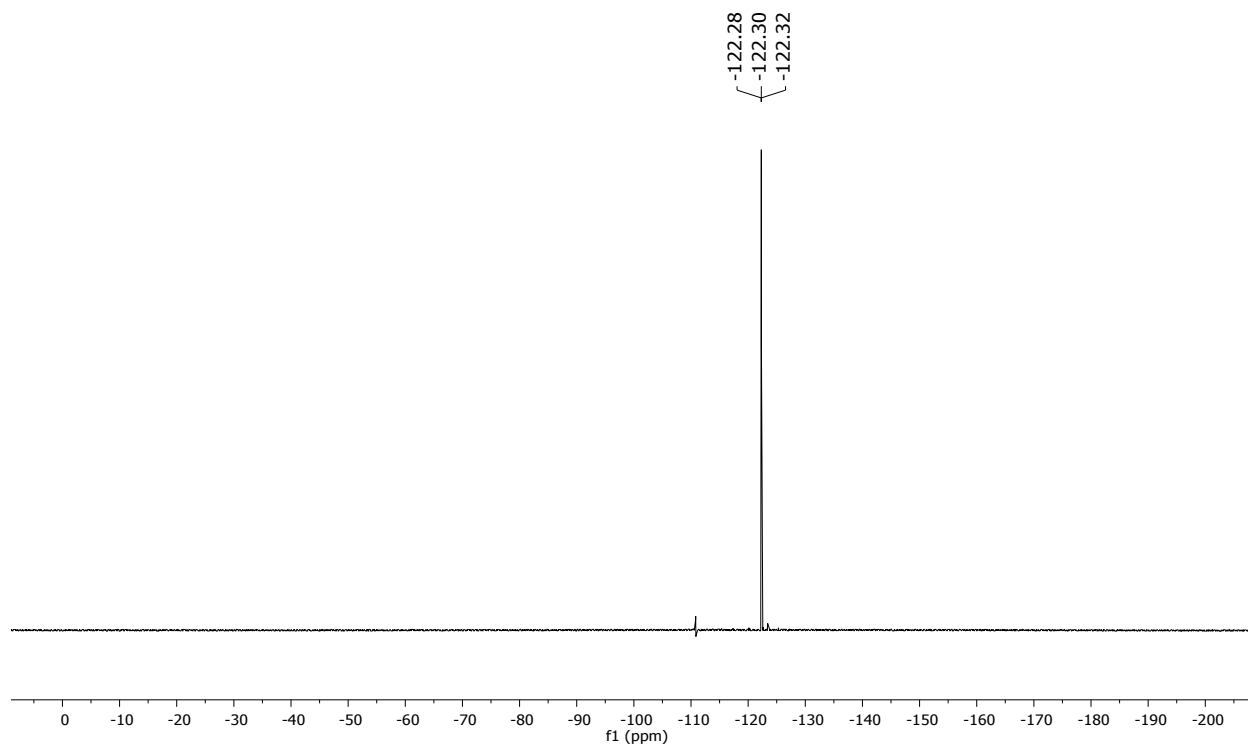


Figure S33. ^{19}F NMR spectrum of **29**

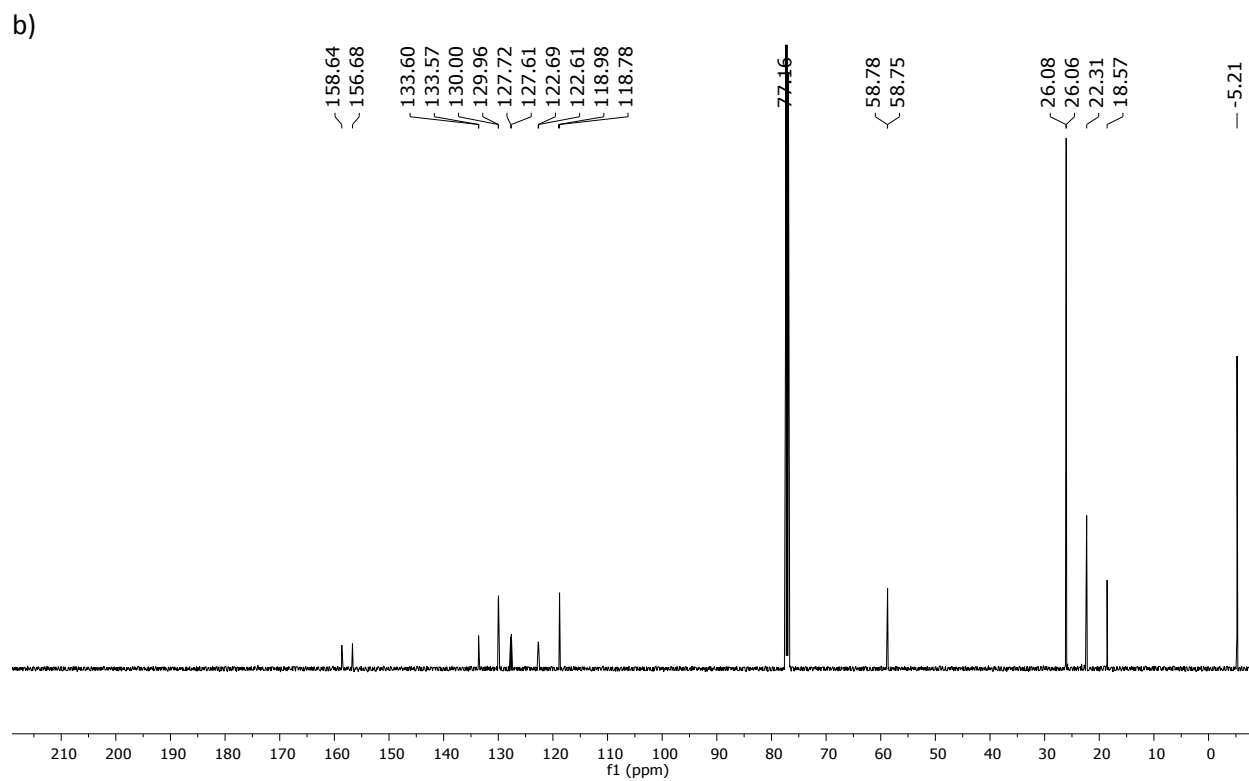
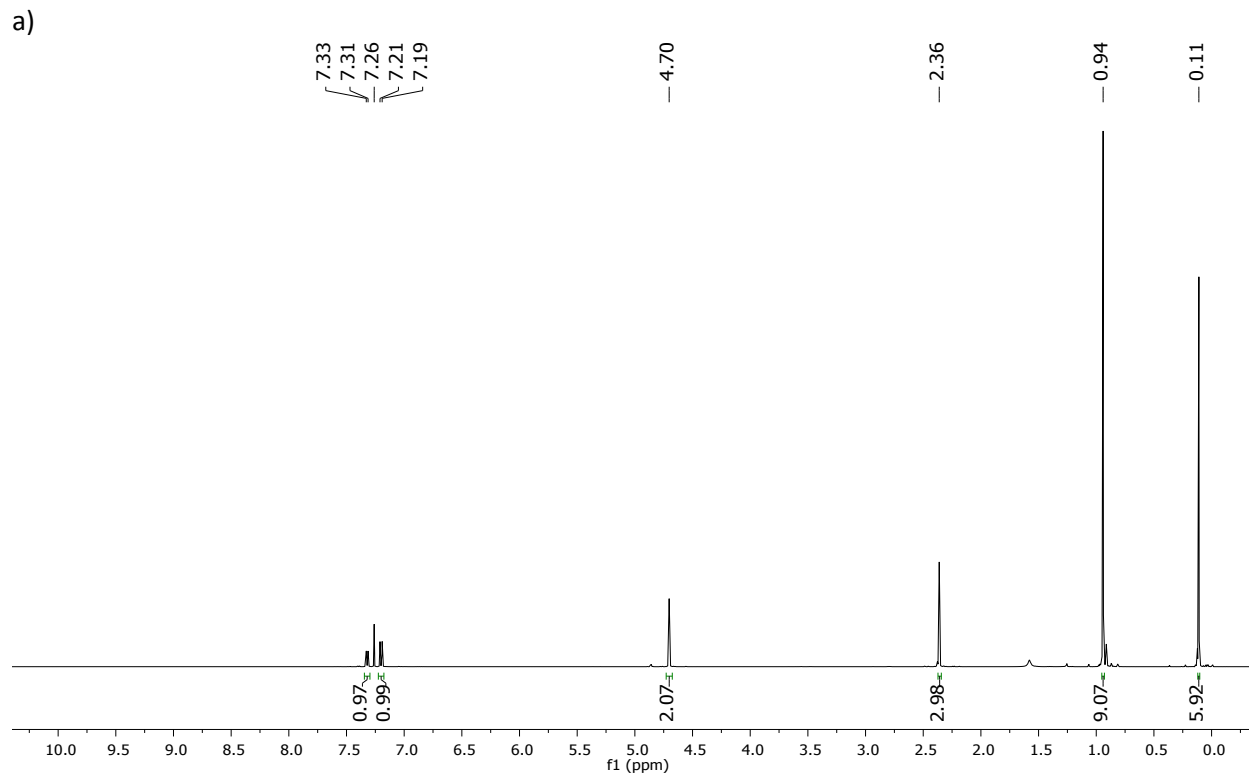


Figure S34. a) ^1H NMR and b) ^{13}C NMR spectra of **30**

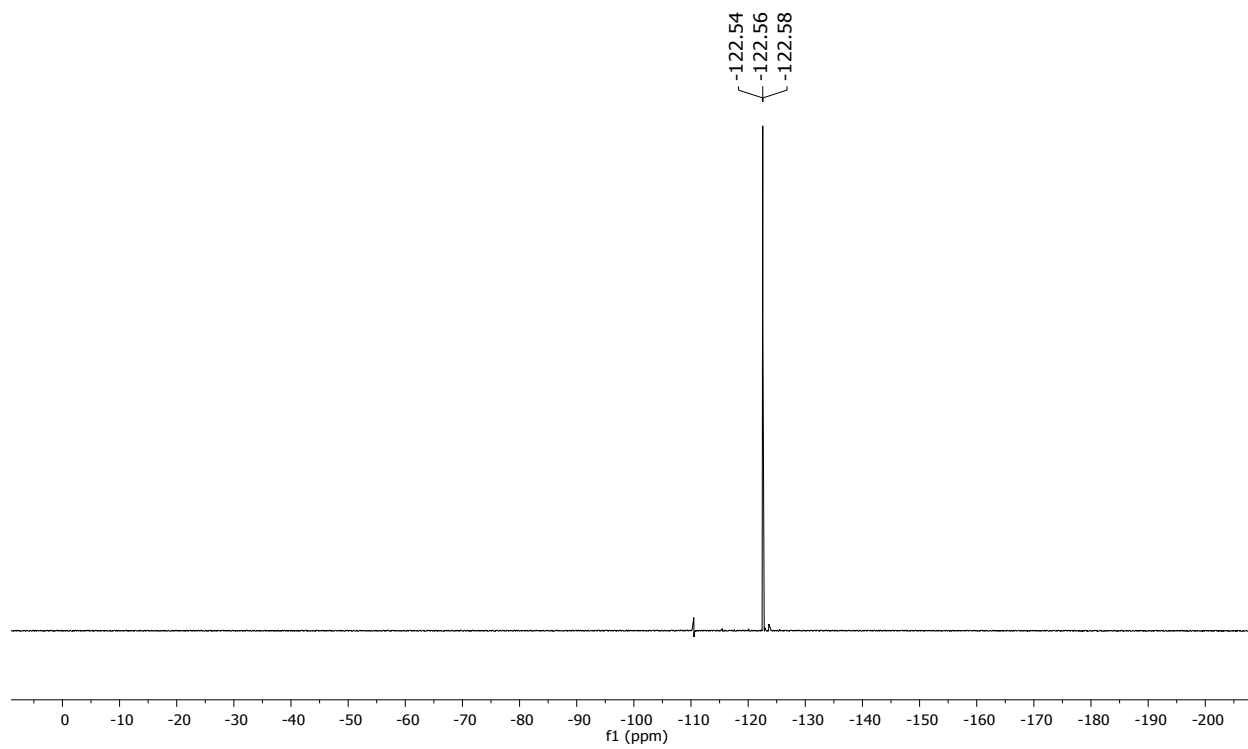


Figure S35. ^{19}F NMR spectrum of **30**

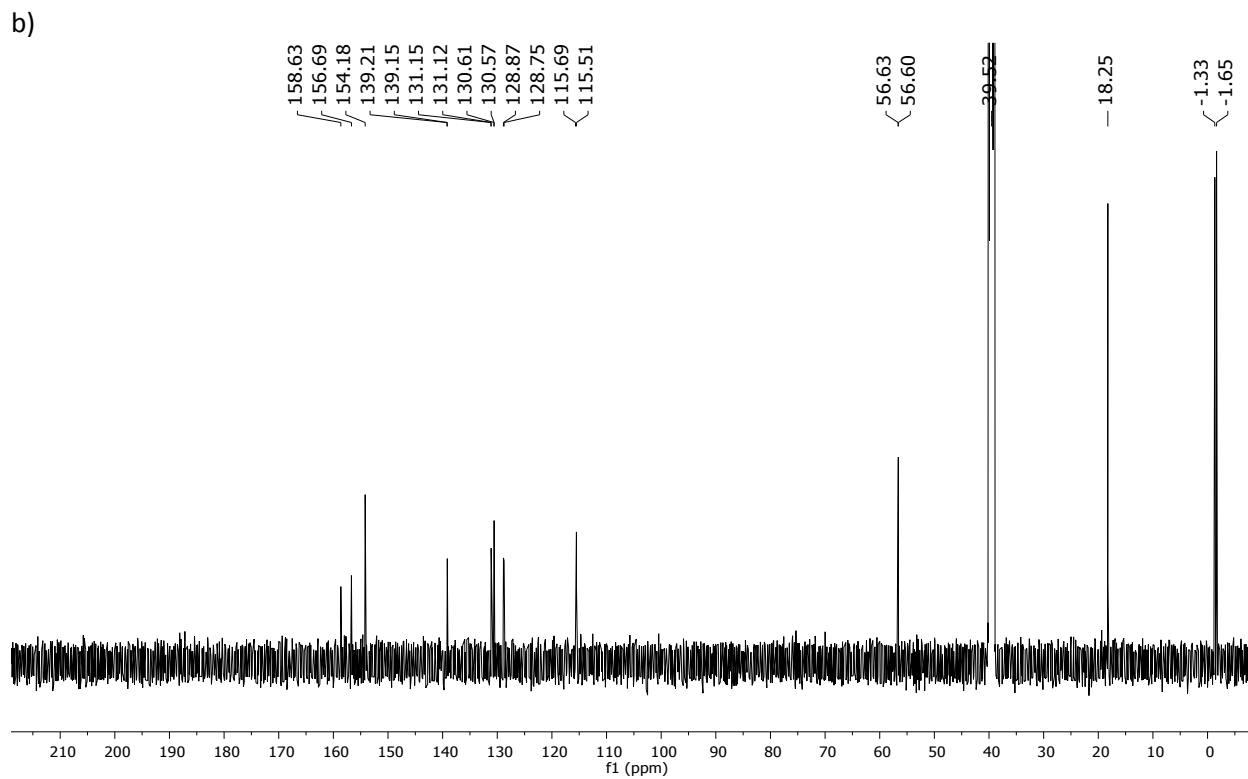
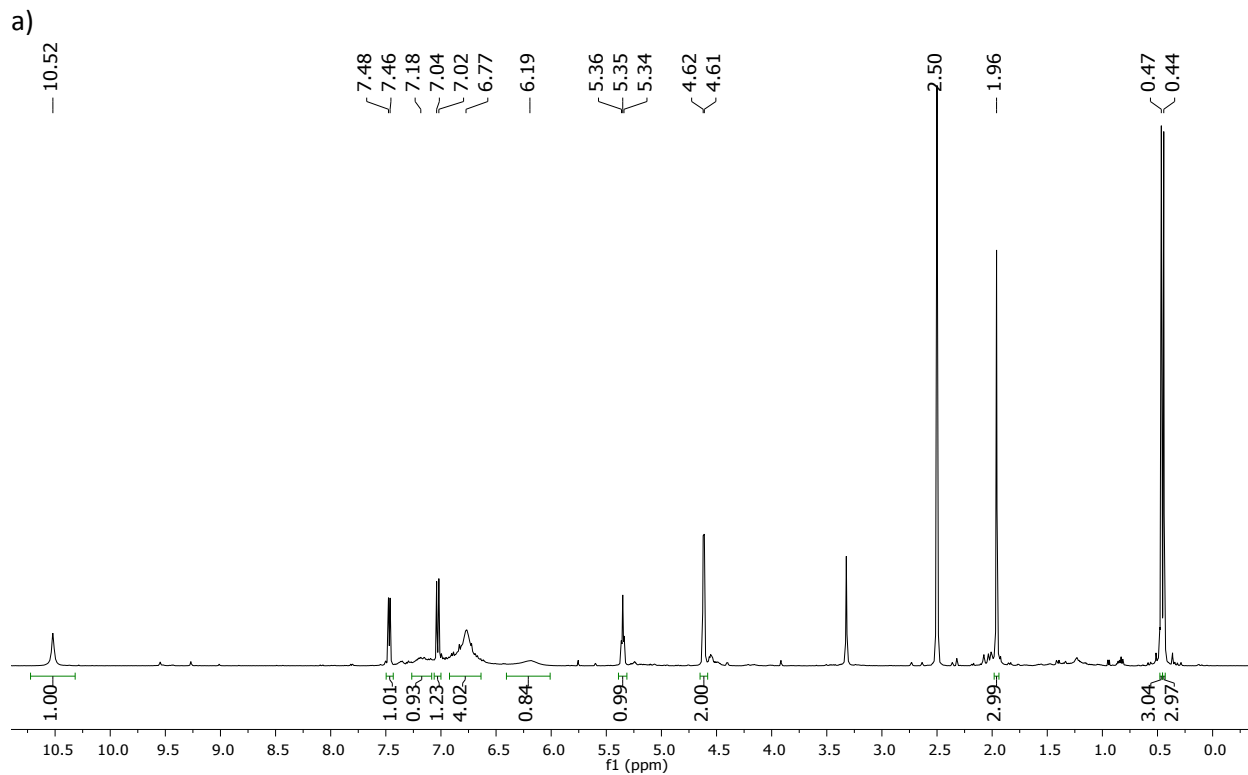


Figure S36. a) ^1H NMR and b) ^{13}C NMR spectra of **31**

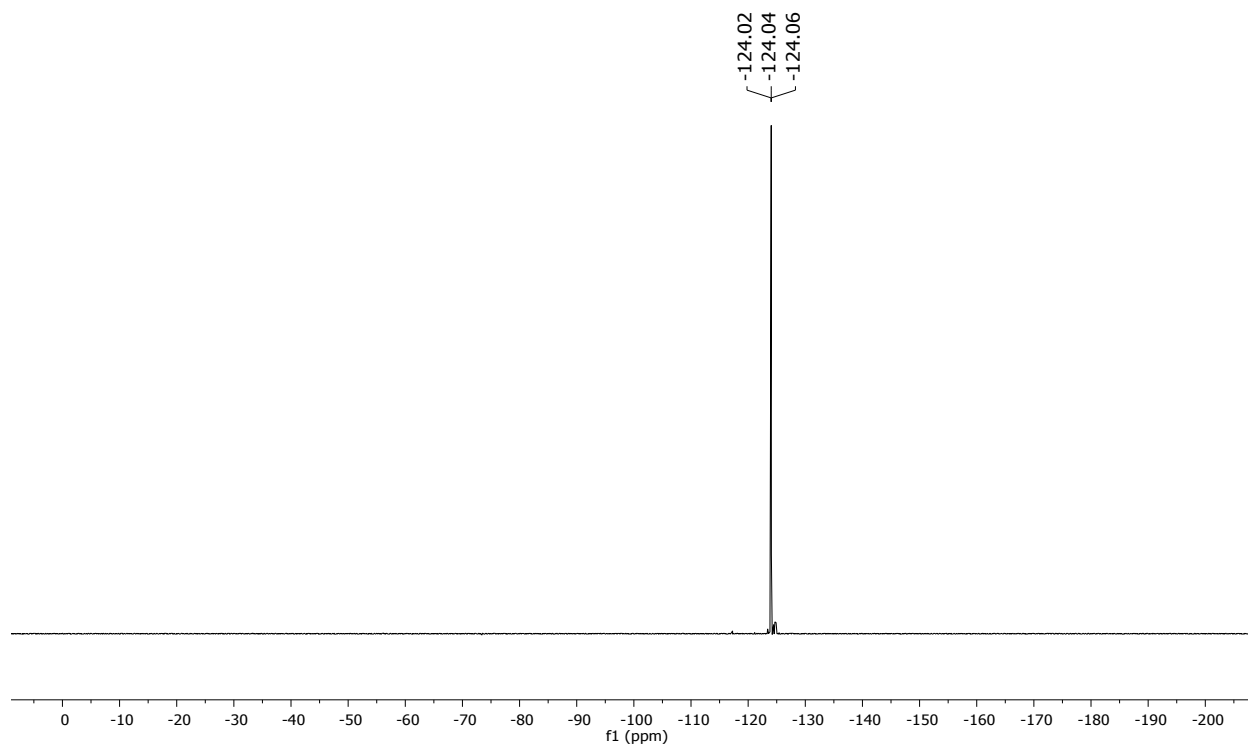


Figure S37. ^{19}F NMR spectrum of **31**

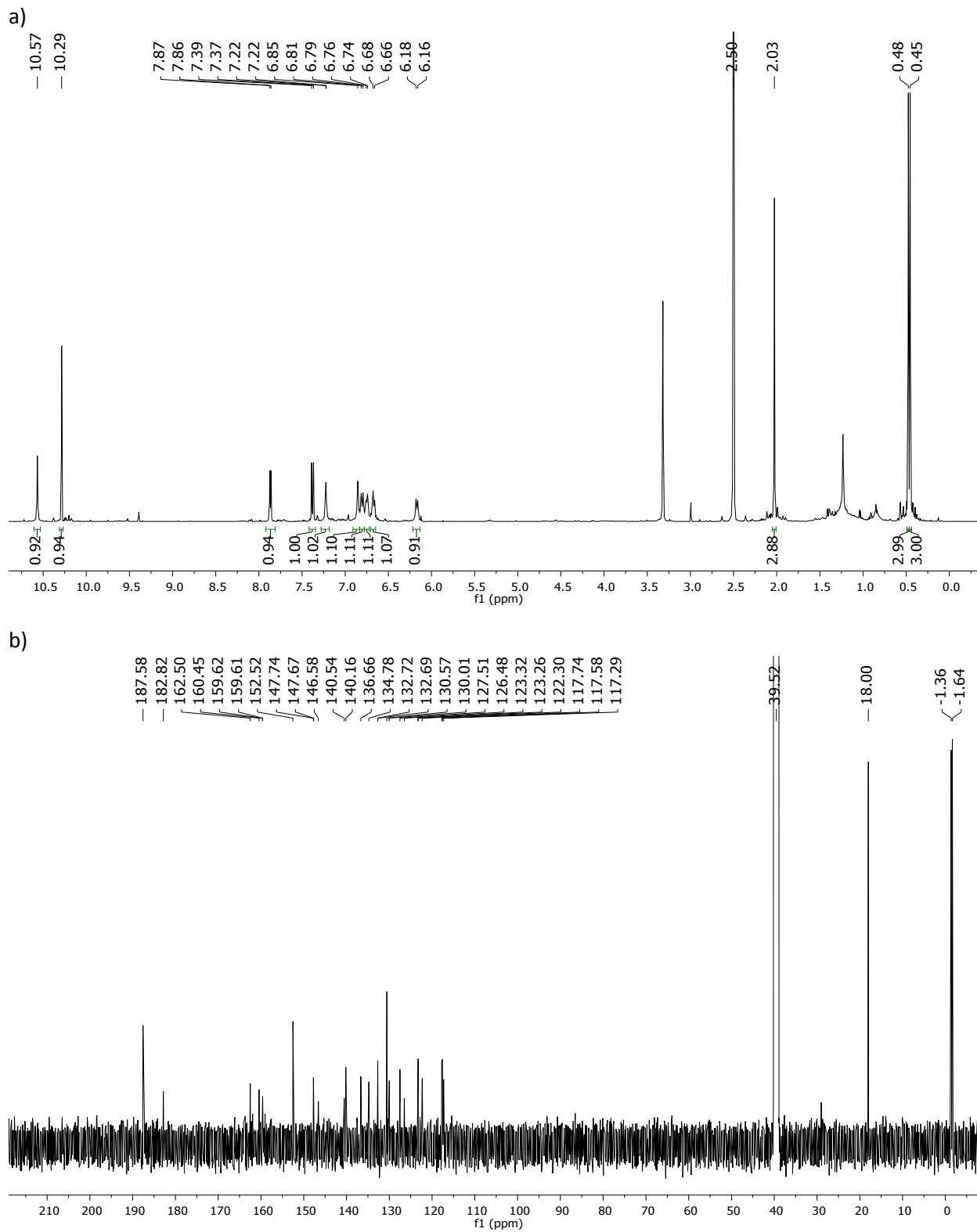


Figure S38. a) ^1H NMR and b) ^{13}C NMR spectra of **Probe 5**

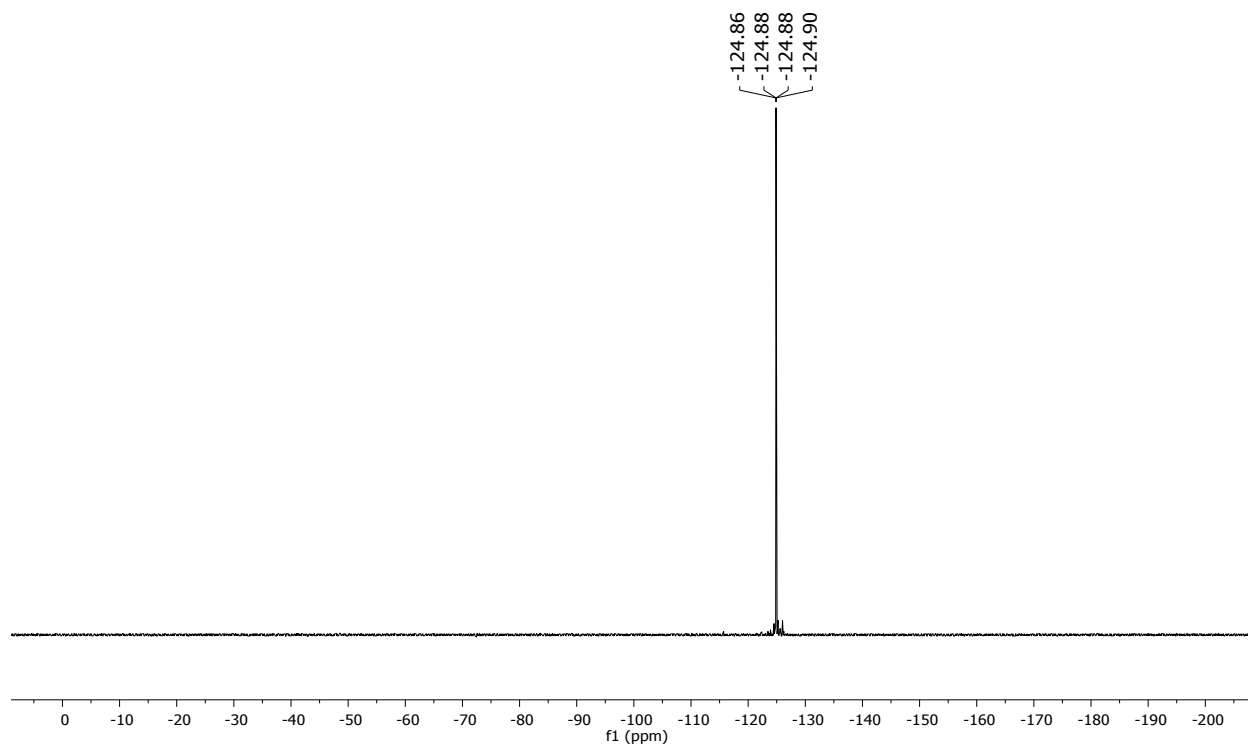


Figure S39. ^{19}F NMR spectrum of **Probe 5**

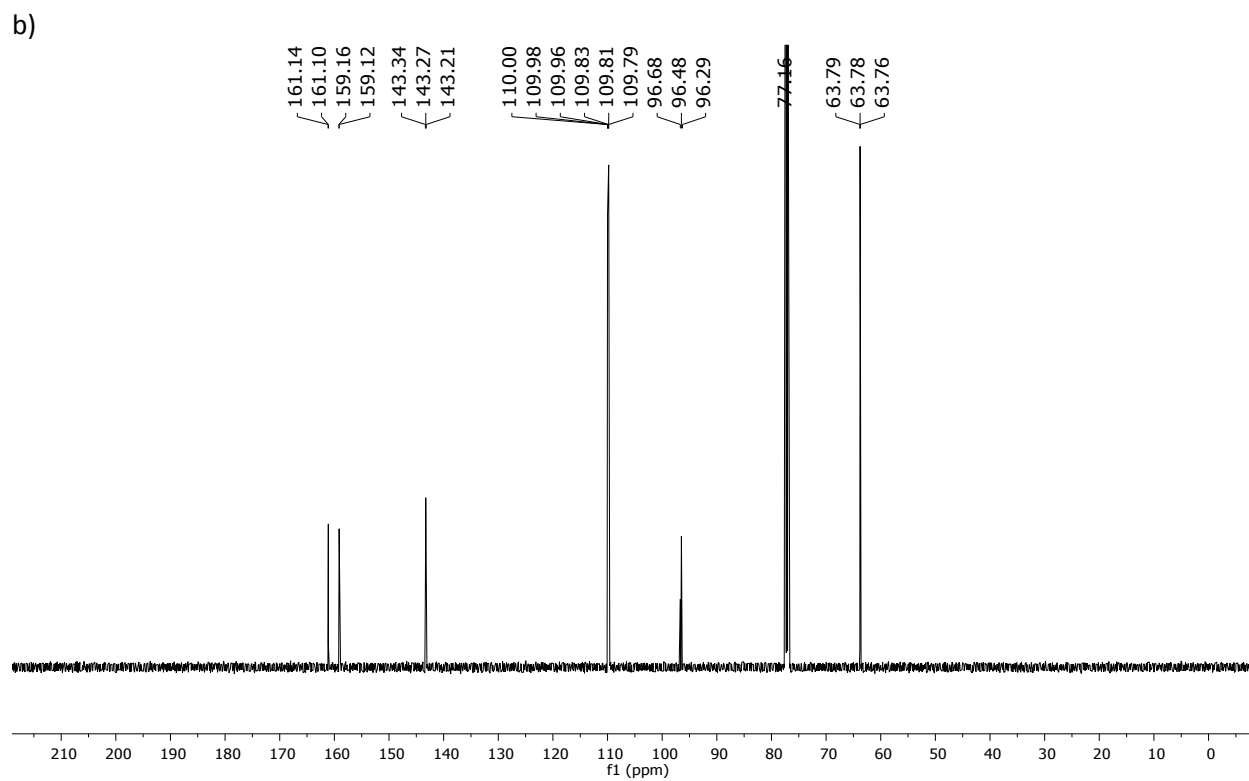
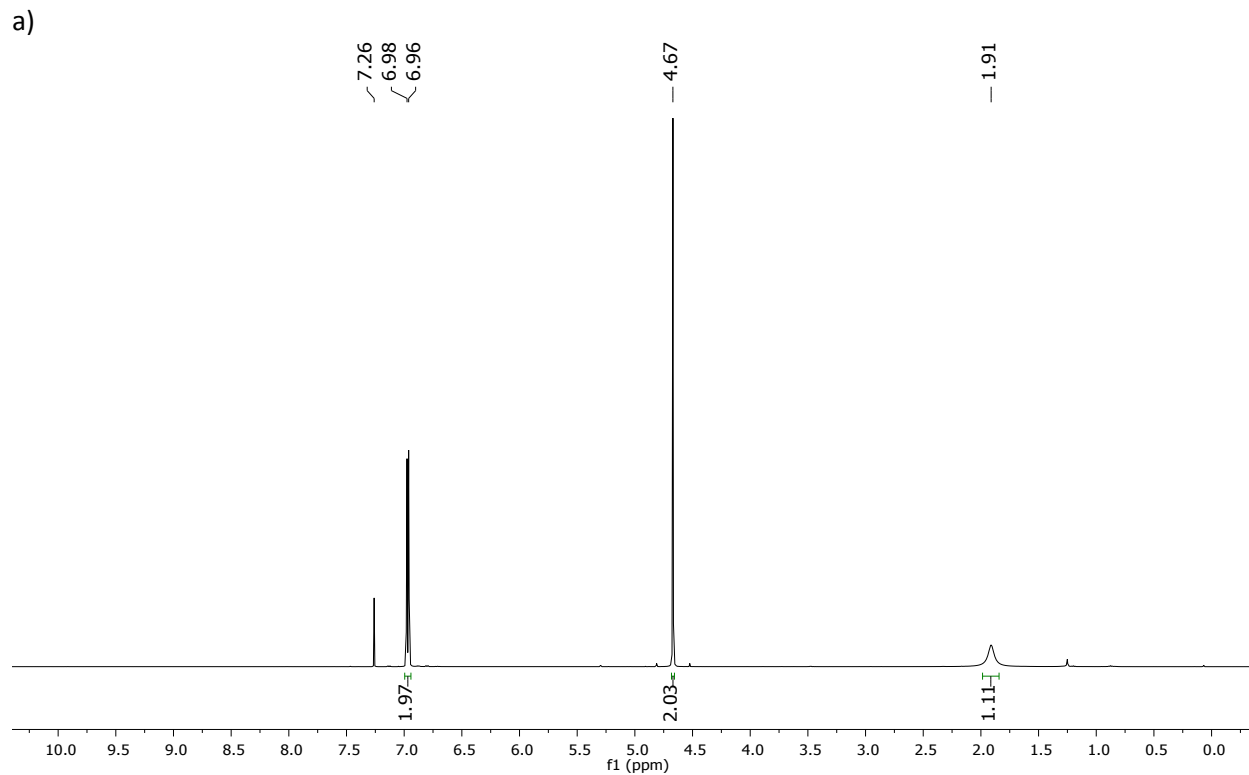


Figure S40. a) ^1H NMR and b) ^{13}C NMR spectra of **33**

c)

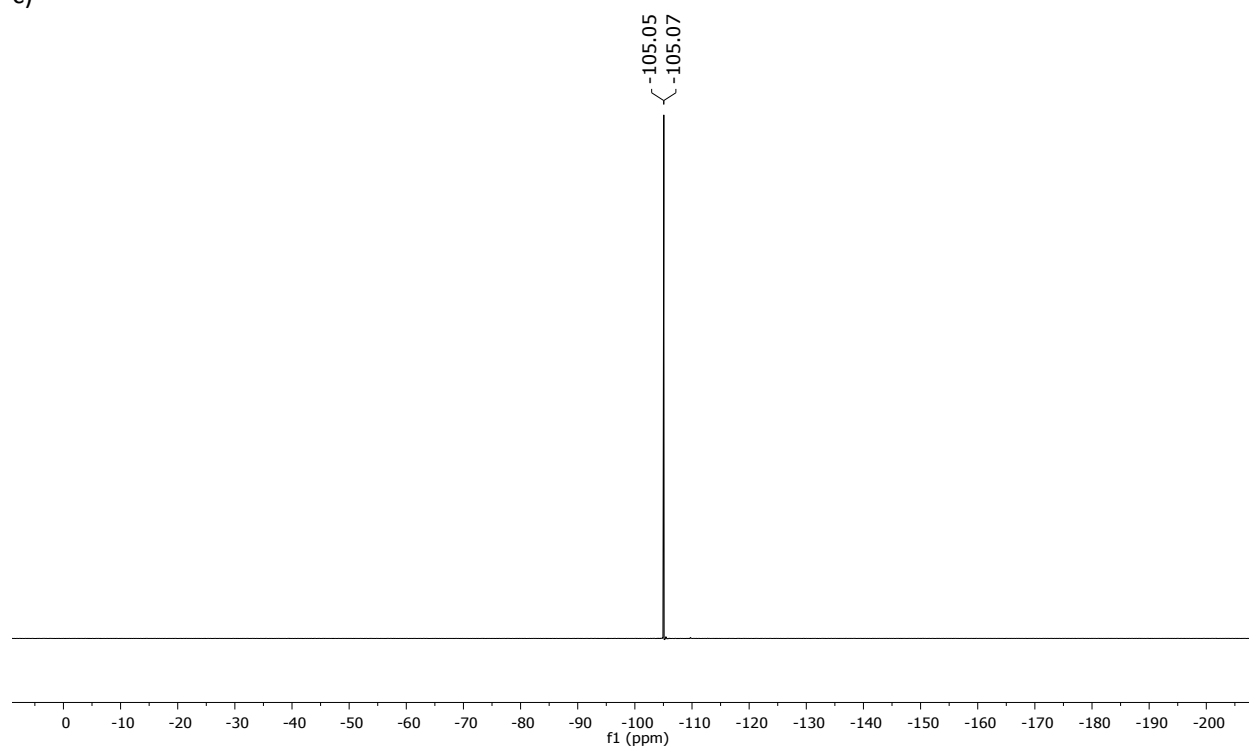


Figure S41. ^{19}F NMR spectrum of **33**

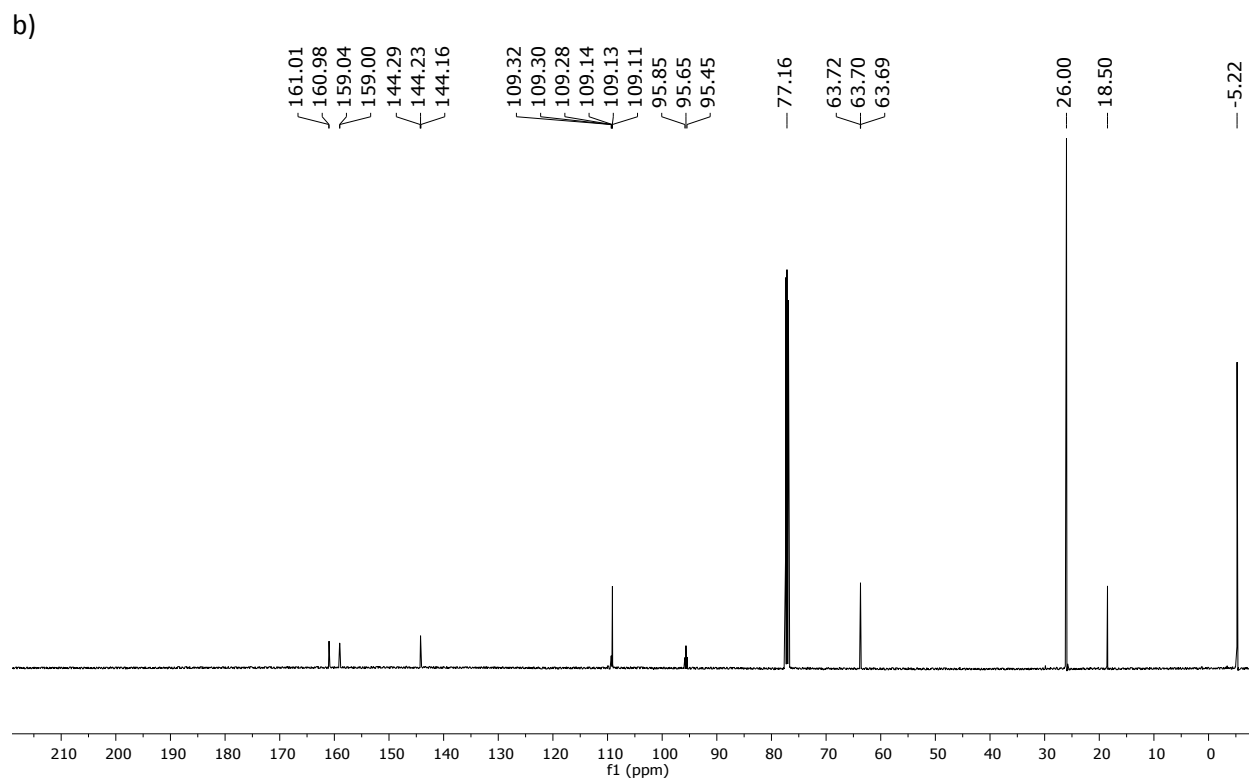
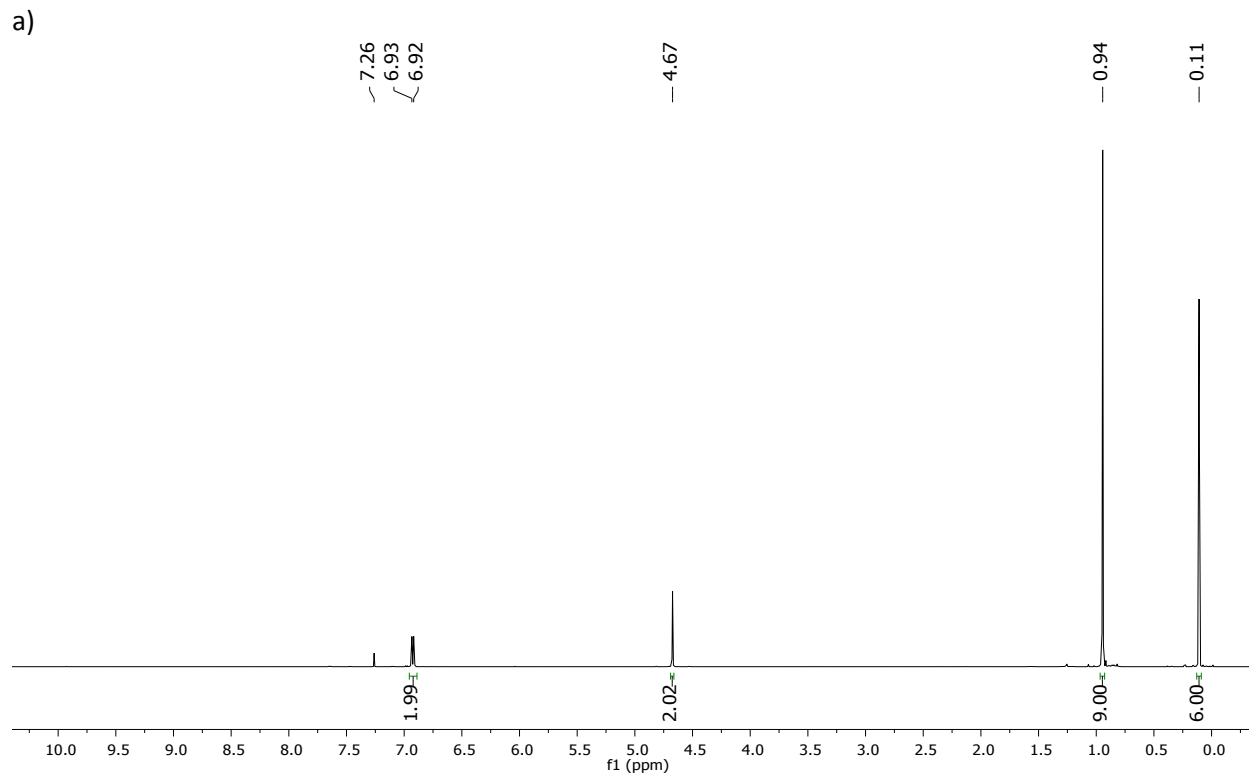


Figure S42. a) ^1H NMR and b) ^{13}C NMR spectra of **34**

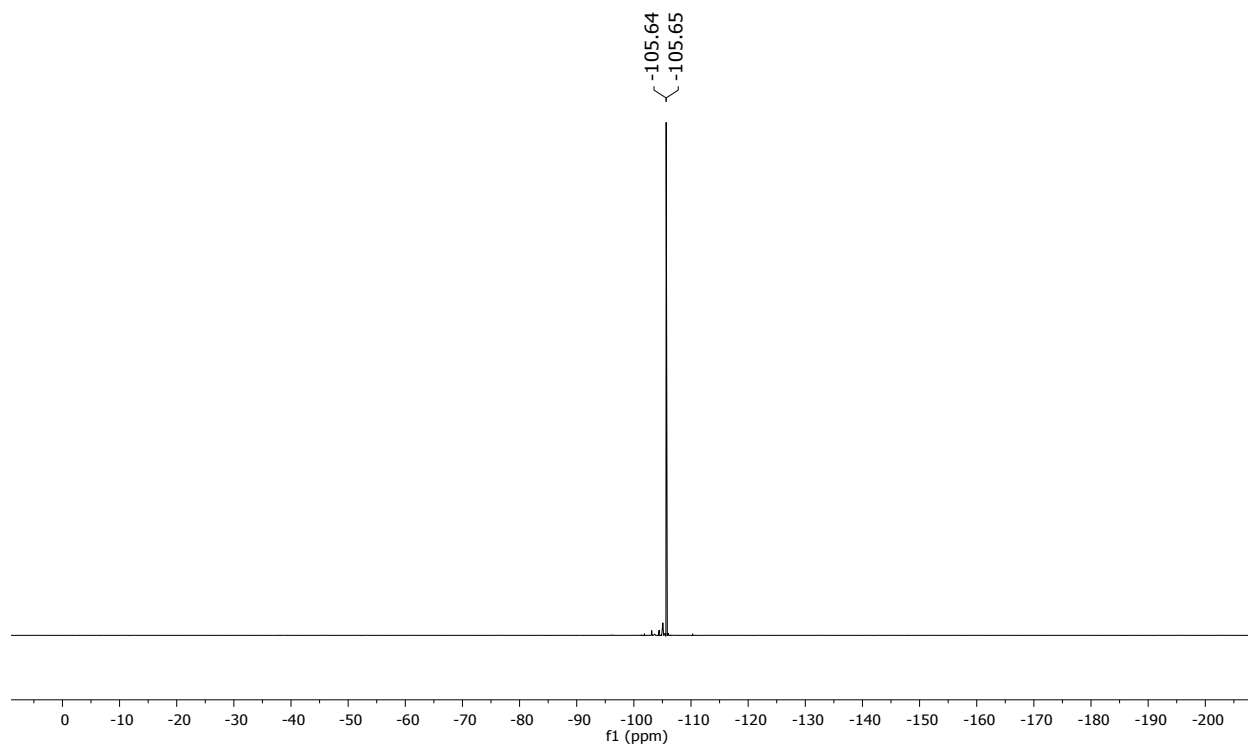
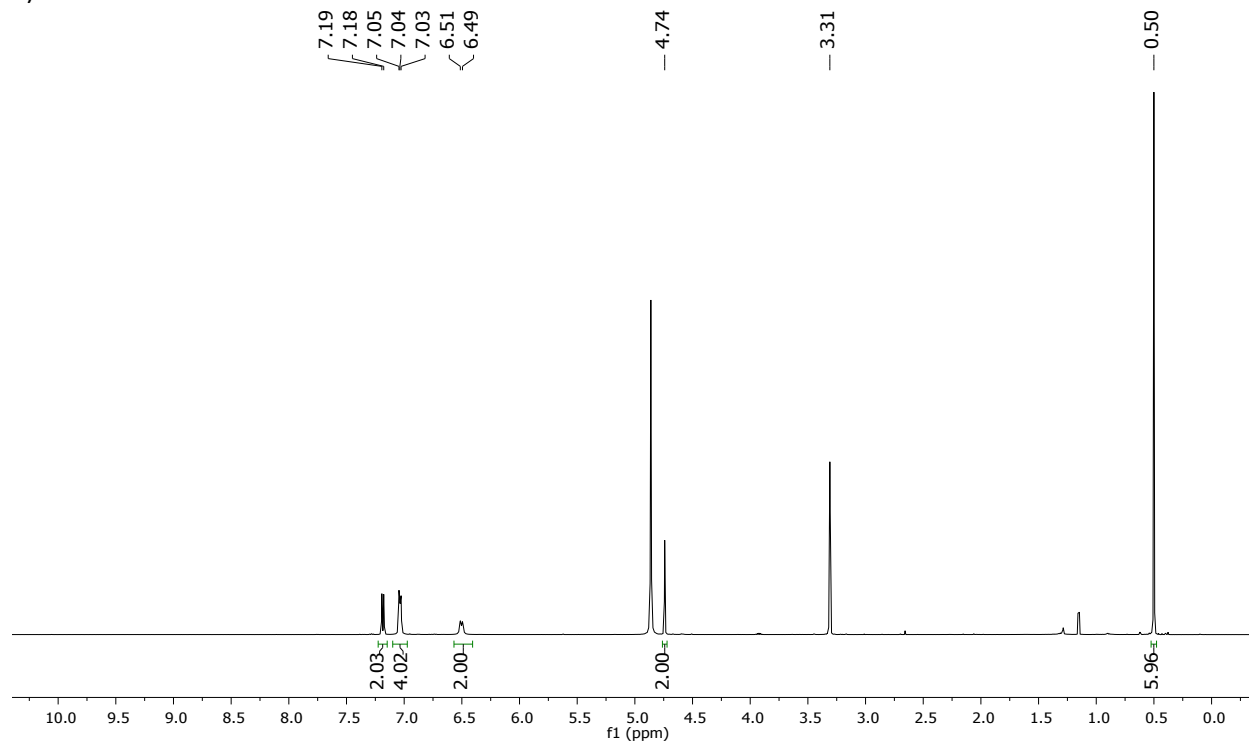


Figure S43. ^{19}F NMR spectrum of **34**

Compound 36

a)



b)

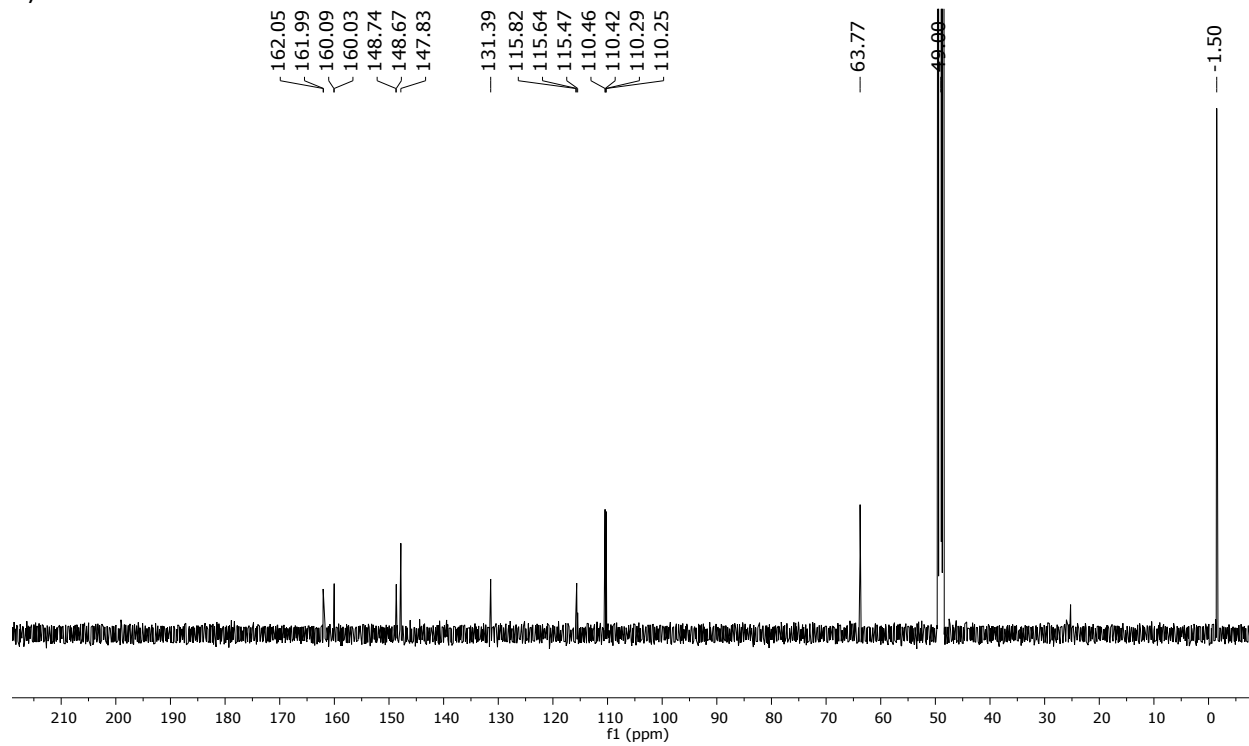


Figure S44. a) ¹H NMR and b) ¹³C NMR spectra of 35

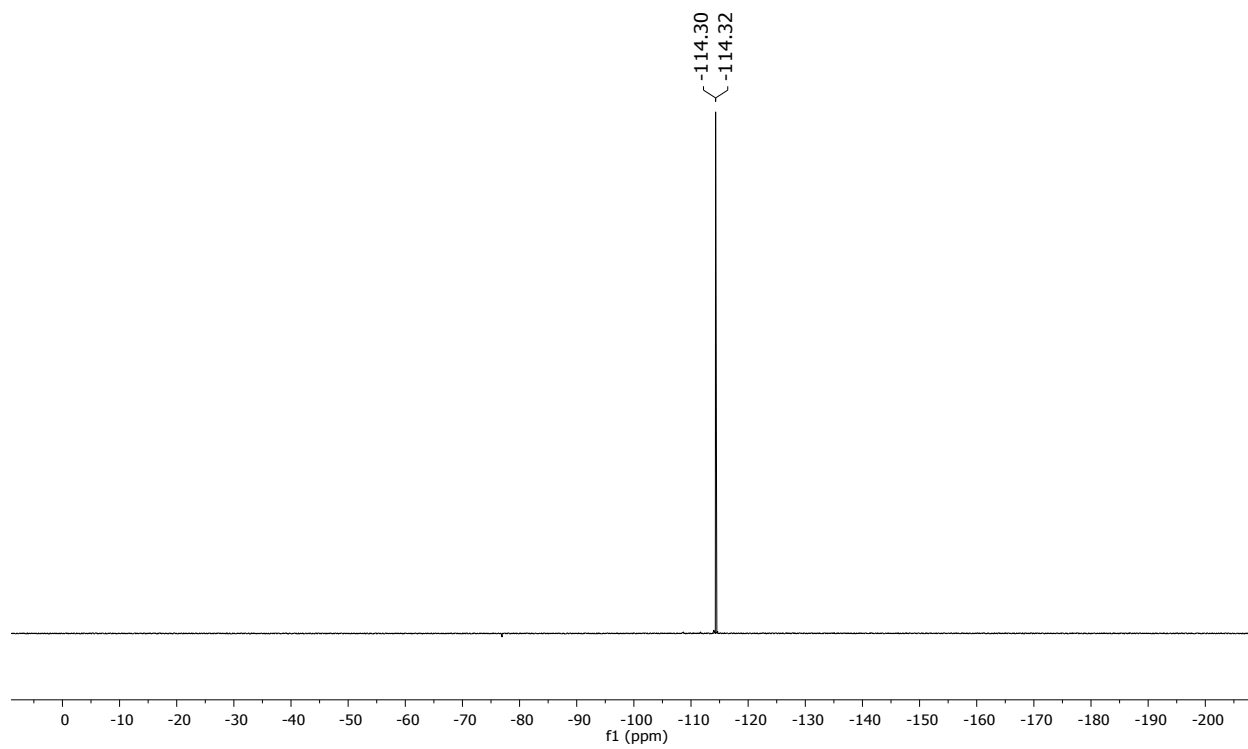


Figure S45. ^{19}F NMR spectrum of **35**

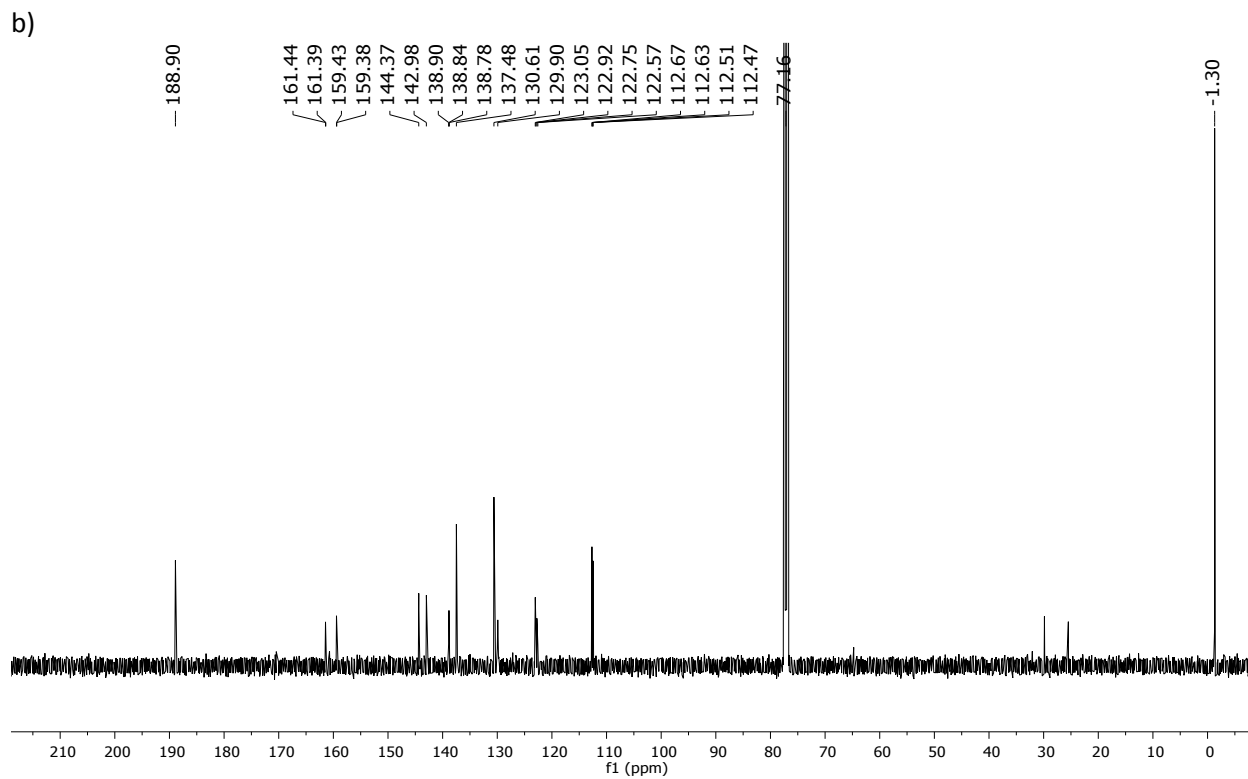
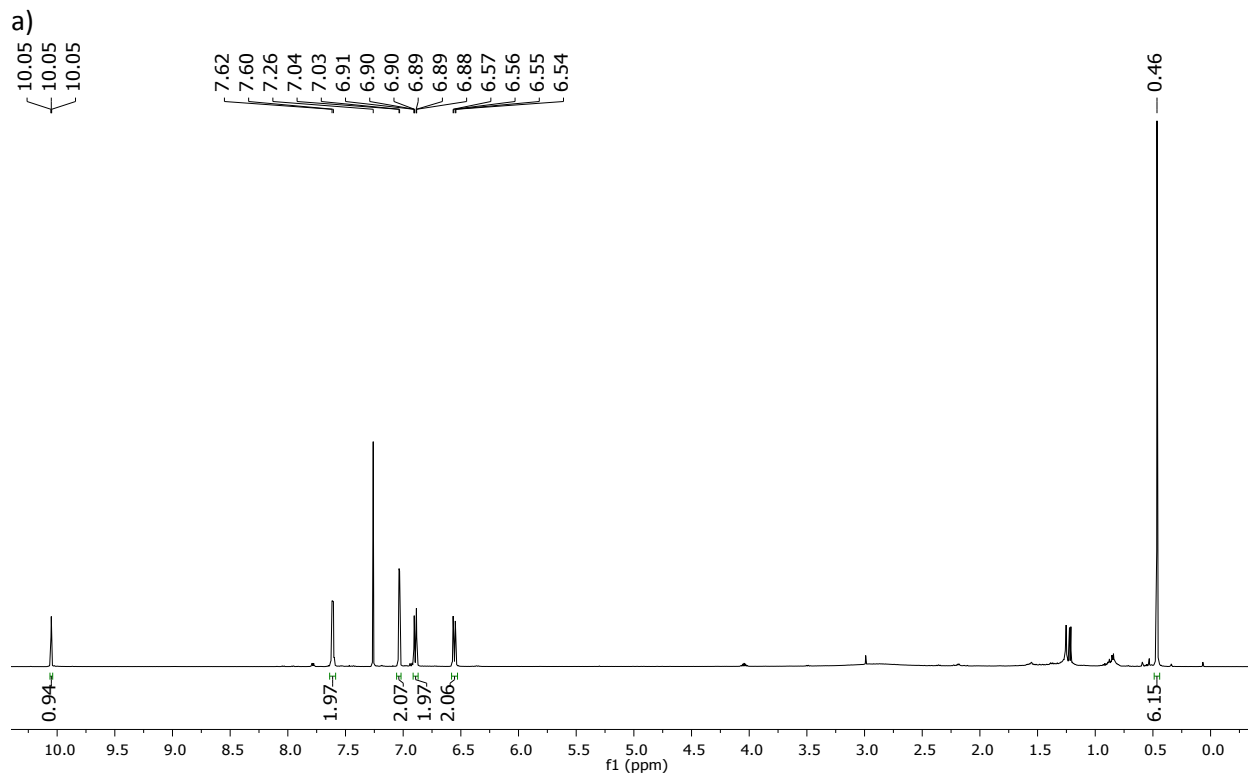


Figure S46. a) ^1H NMR and b) ^{13}C NMR spectra of **Probe 6**

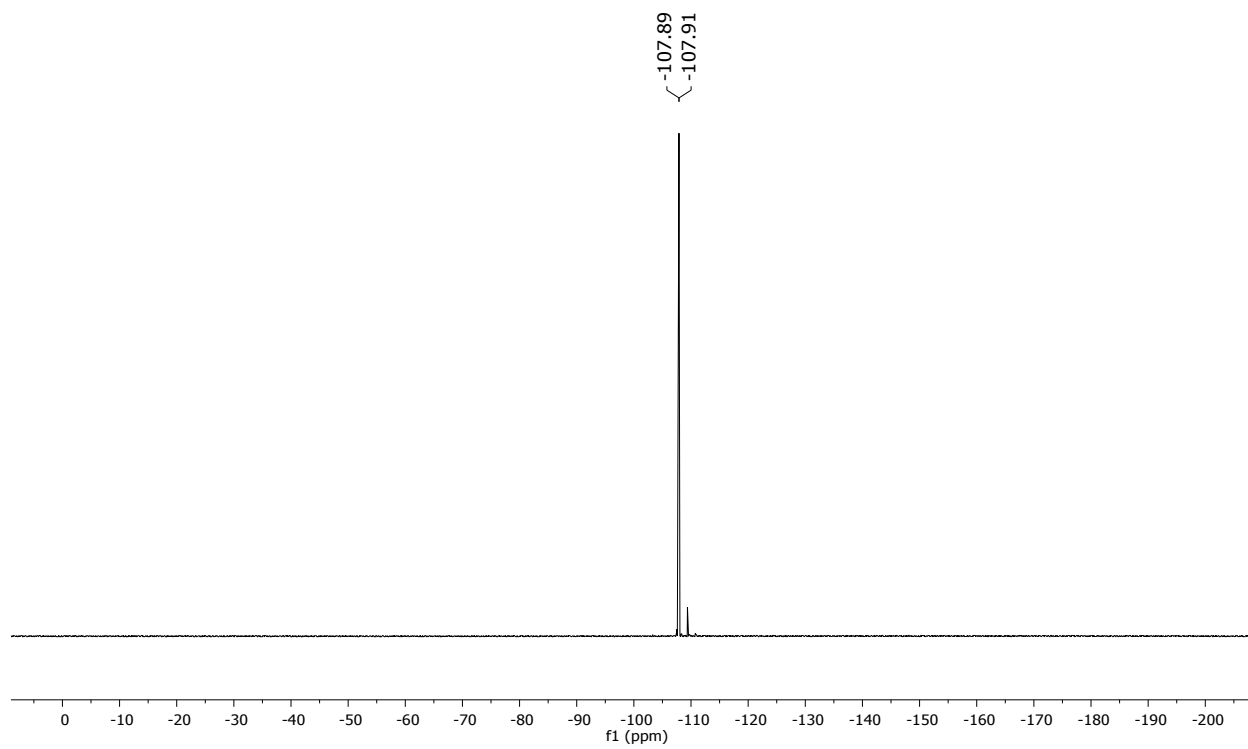


Figure S47. ^{19}F NMR spectrum of **Probe 6**

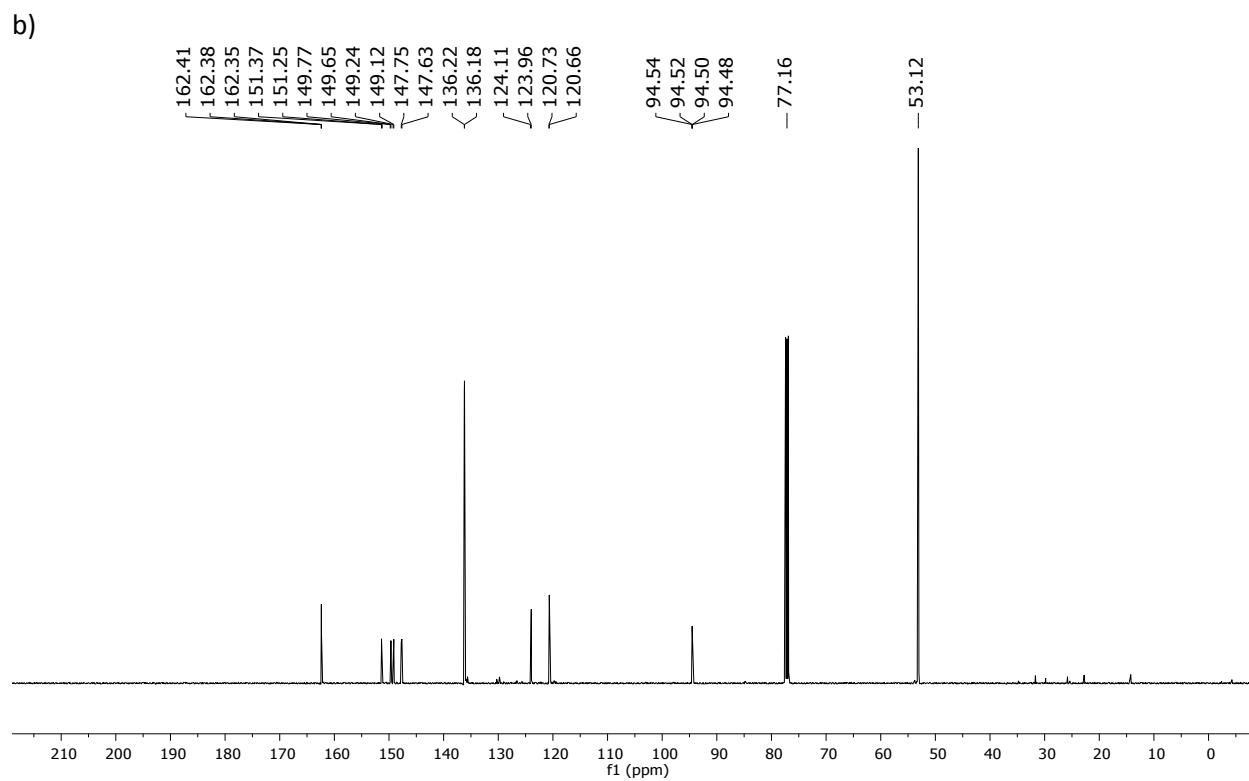
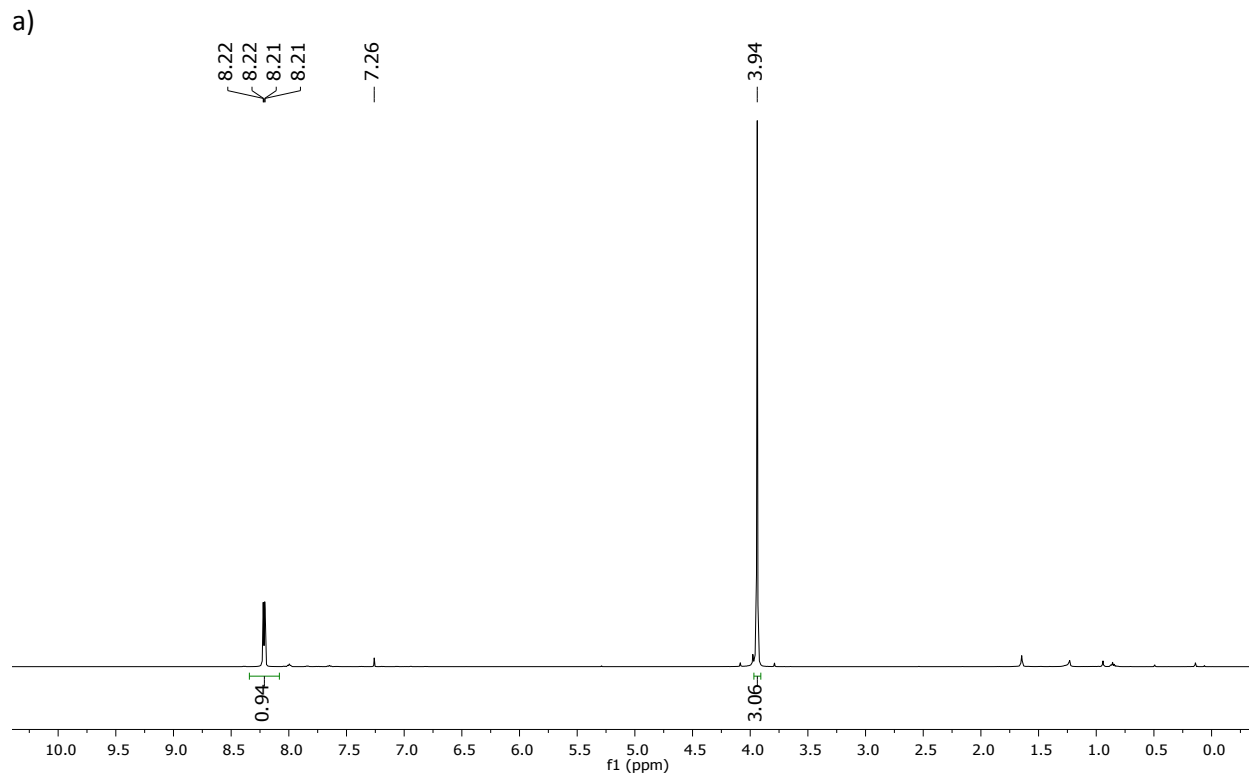


Figure S48. a) ^1H NMR and b) ^{13}C NMR spectra of **2**

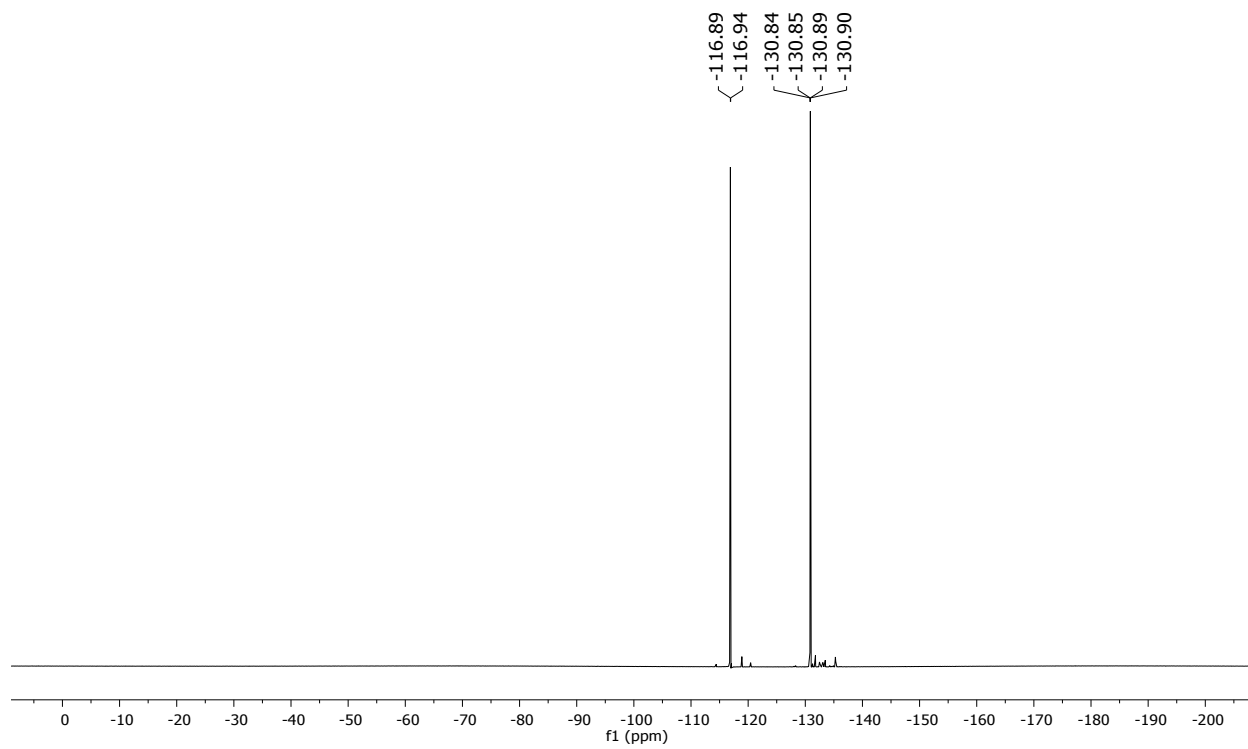


Figure S49. ^{19}F NMR spectrum of **2**

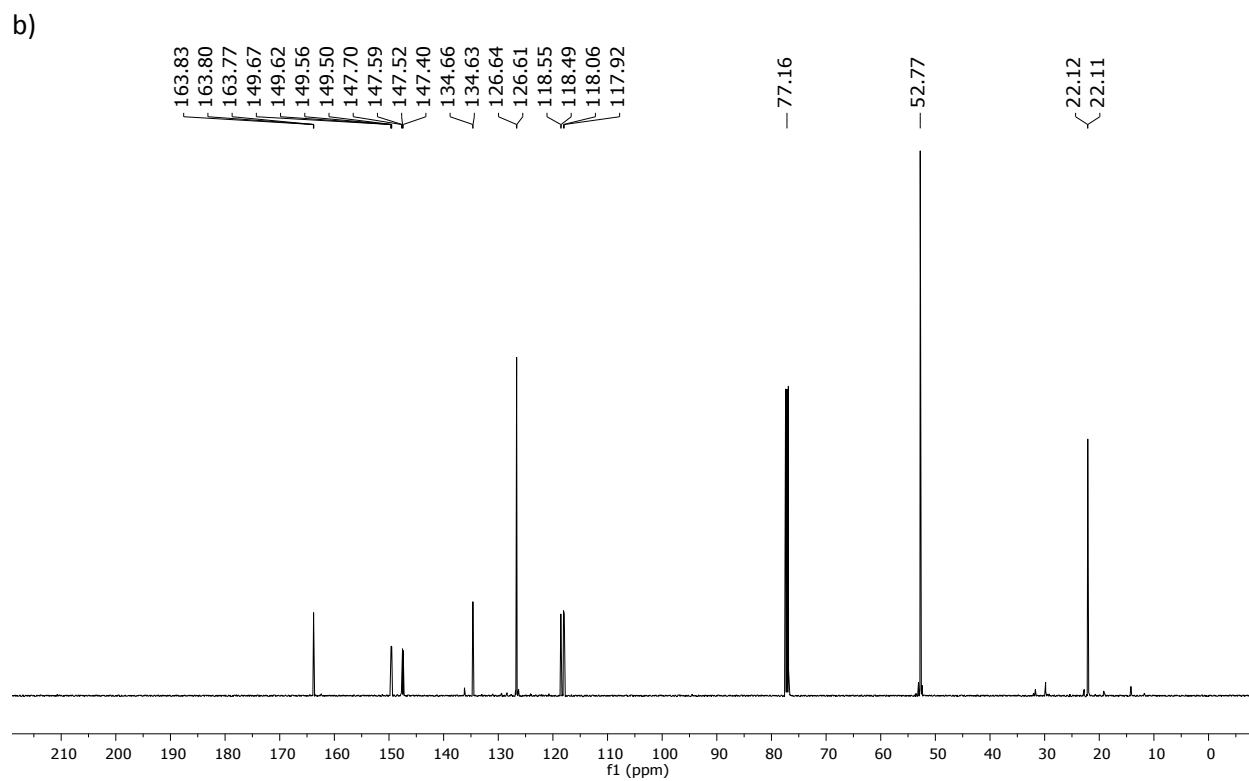
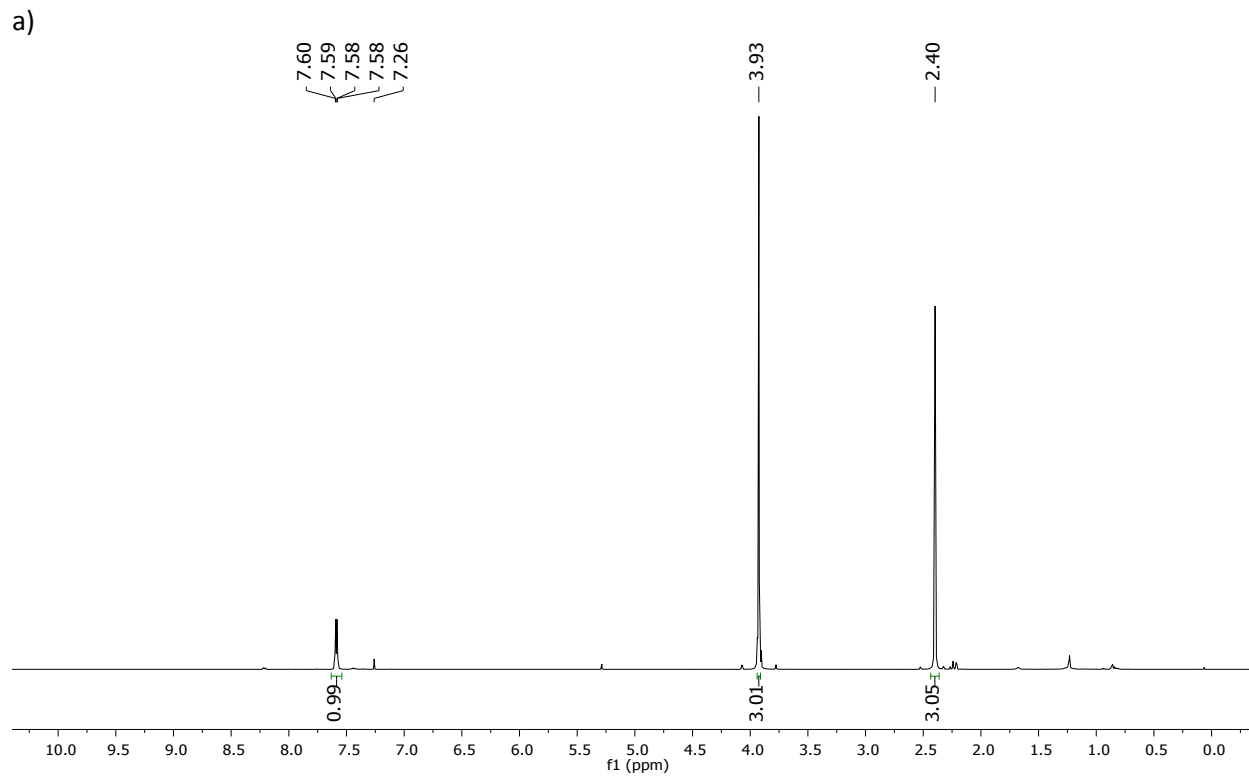


Figure S50. a) ^1H NMR and b) ^{13}C NMR spectra of **3**

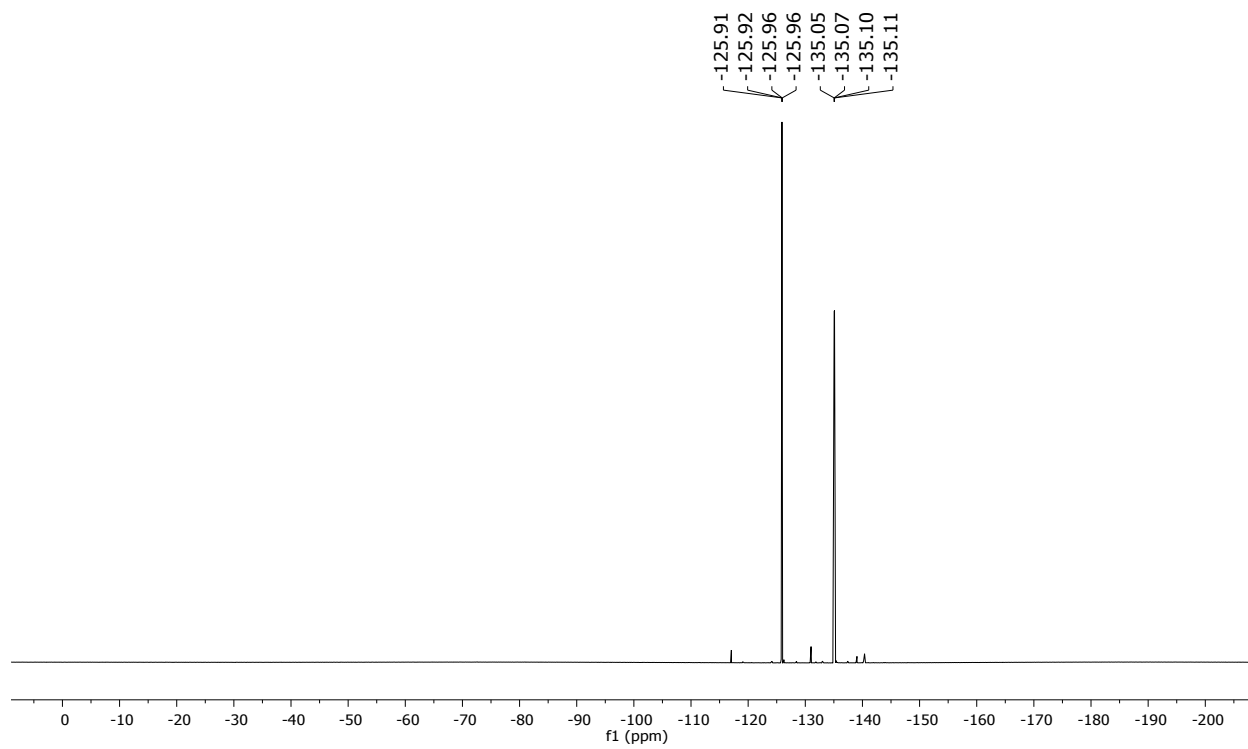


Figure S51. ^{19}F NMR spectrum of **3**

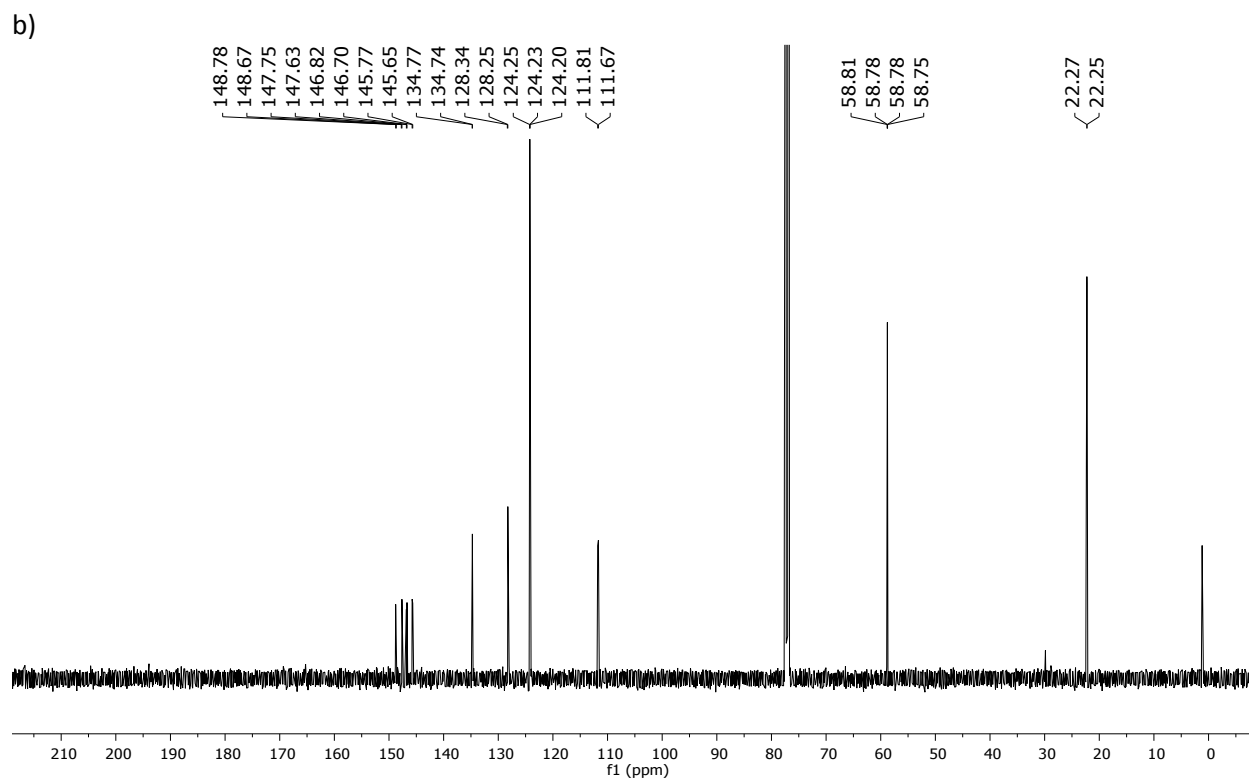
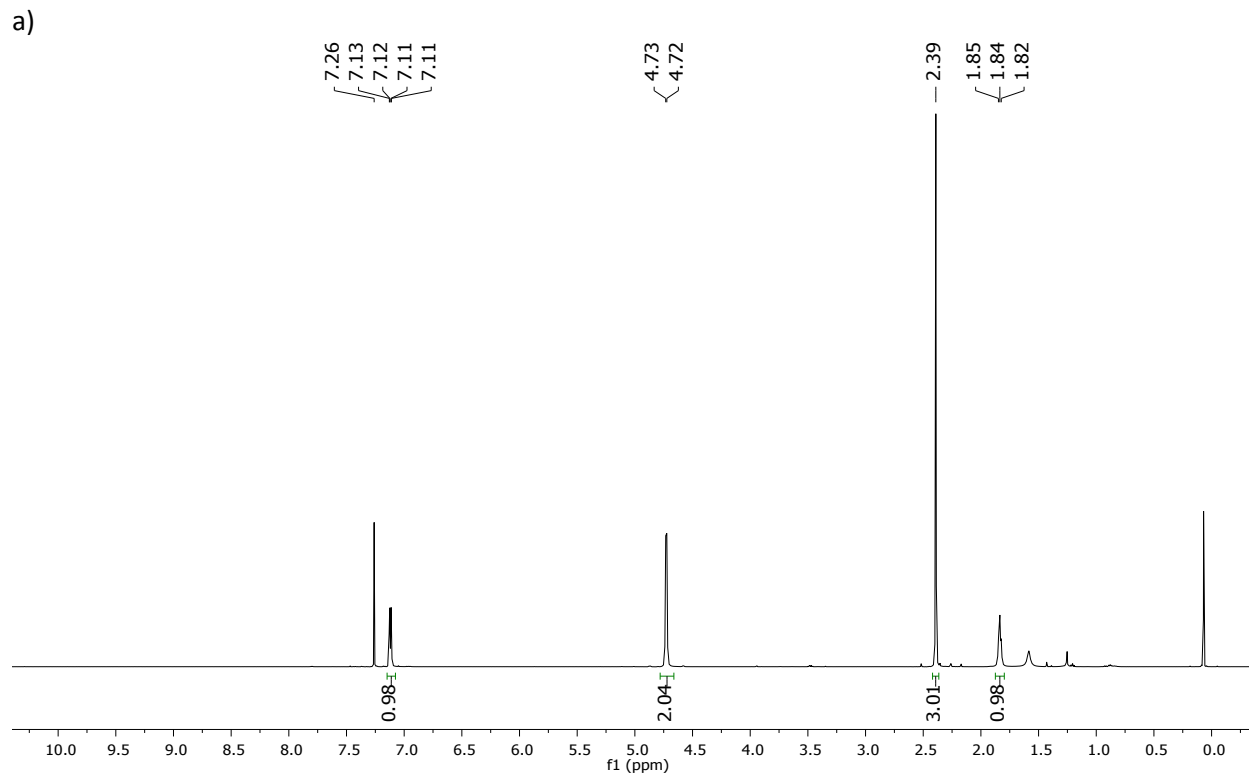


Figure S52. a) ^1H NMR and b) ^{13}C NMR spectra of **4**

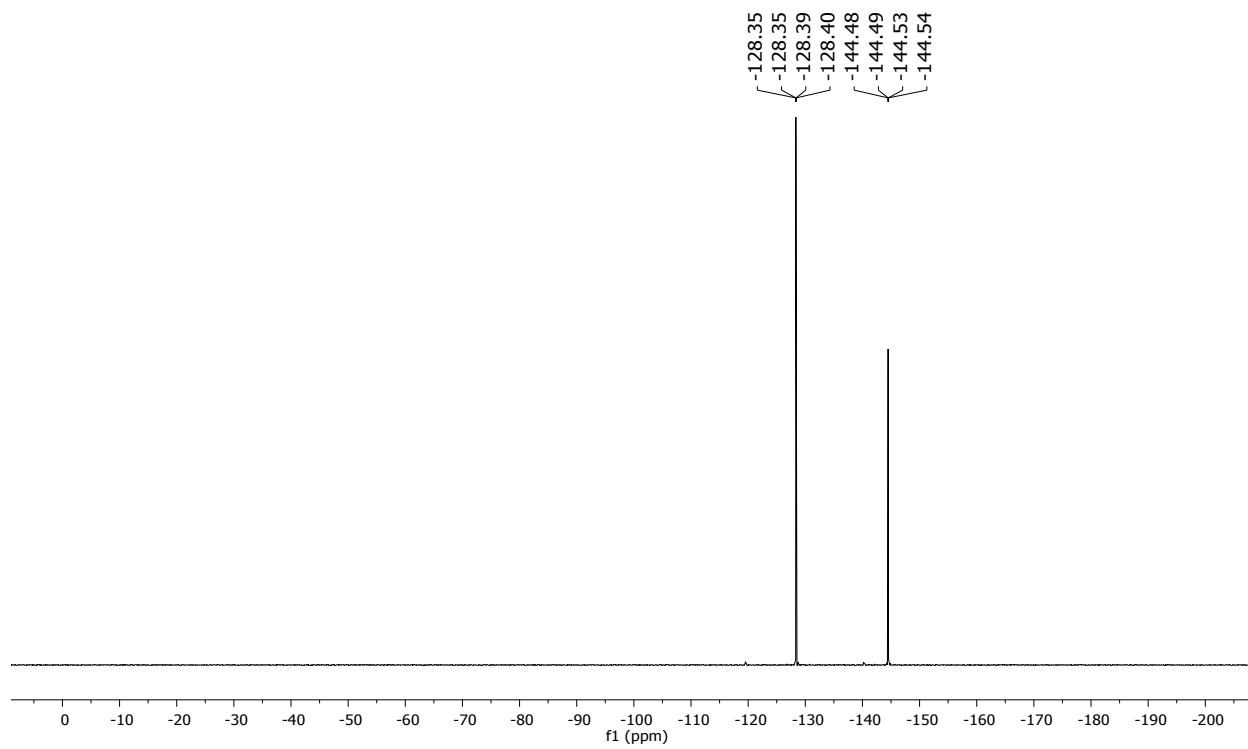


Figure S53. ^{19}F NMR spectrum of **4**

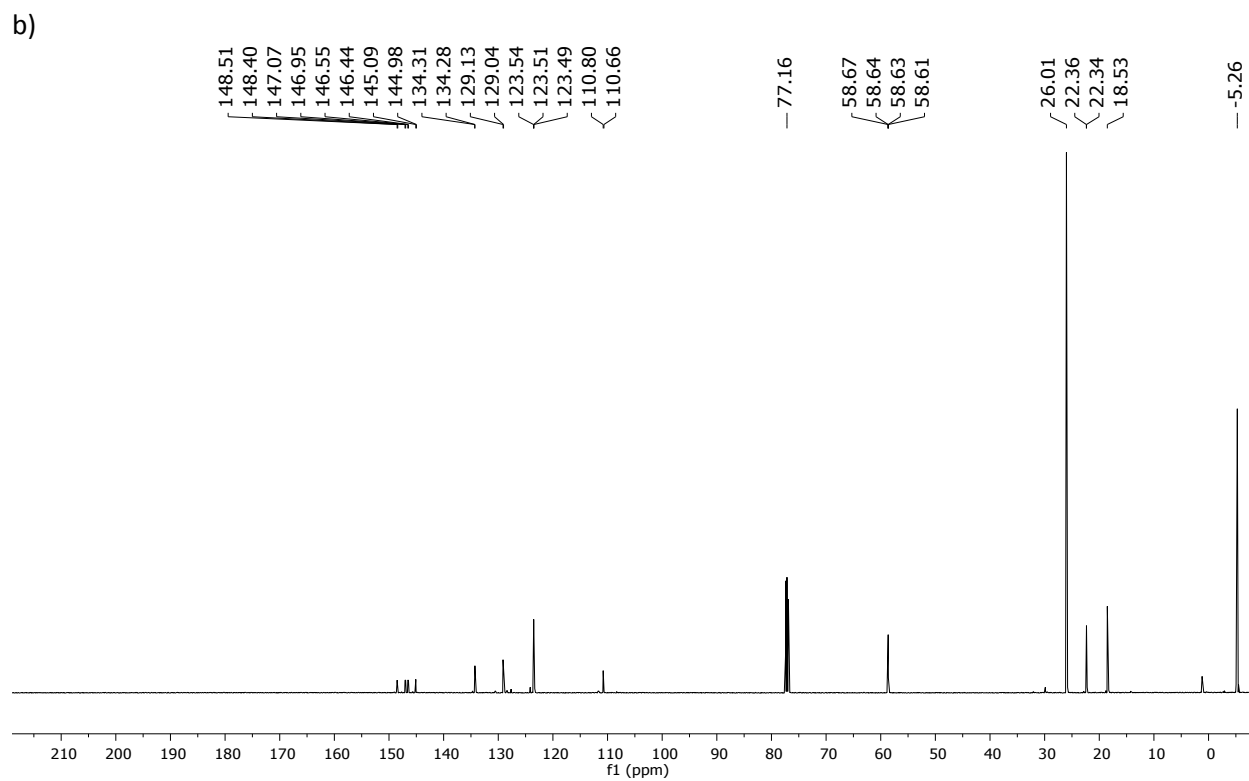
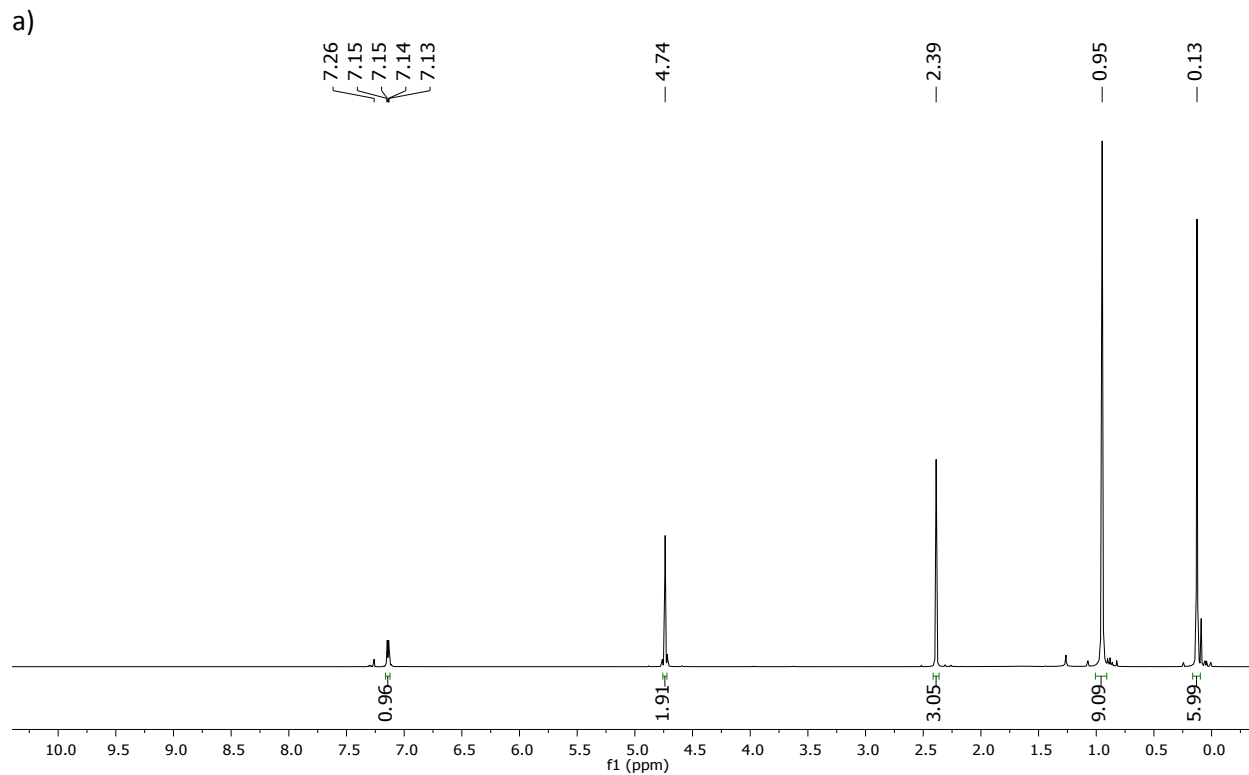


Figure S54. a) ^1H NMR and b) ^{13}C NMR spectra of **5**

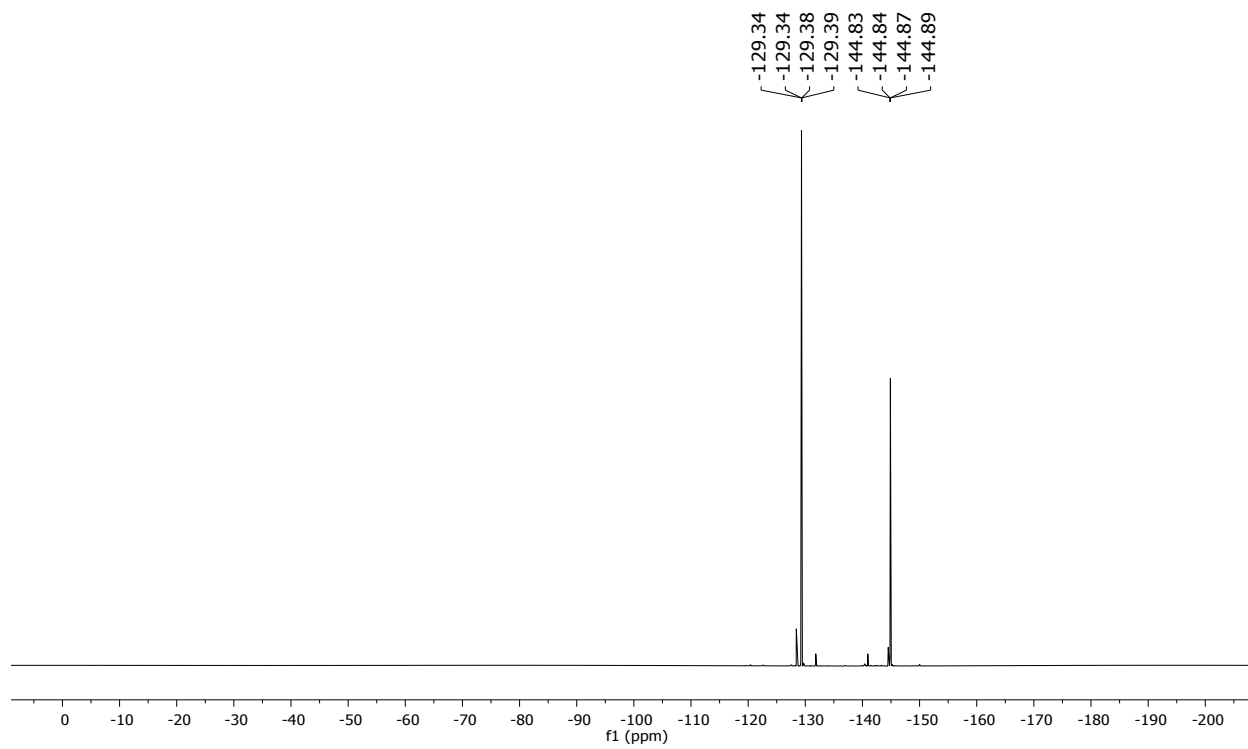


Figure S55. ^{19}F NMR spectrum of **5**

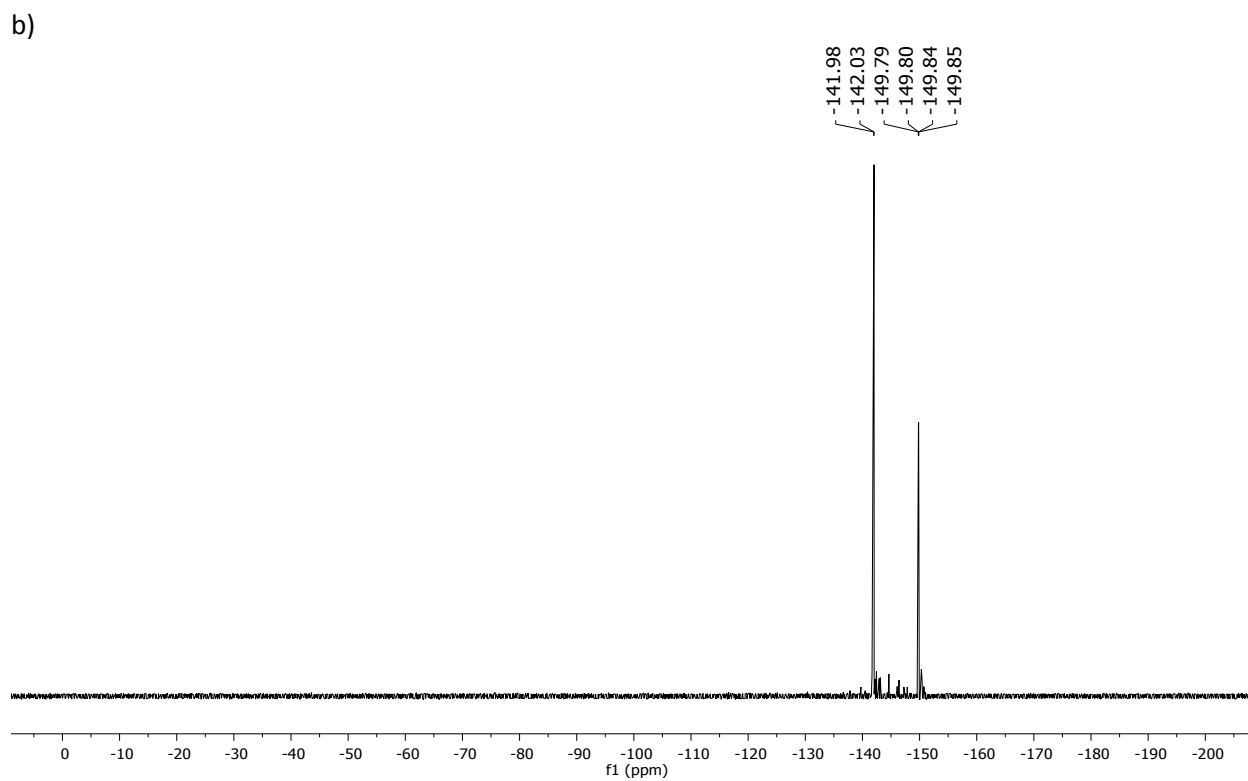
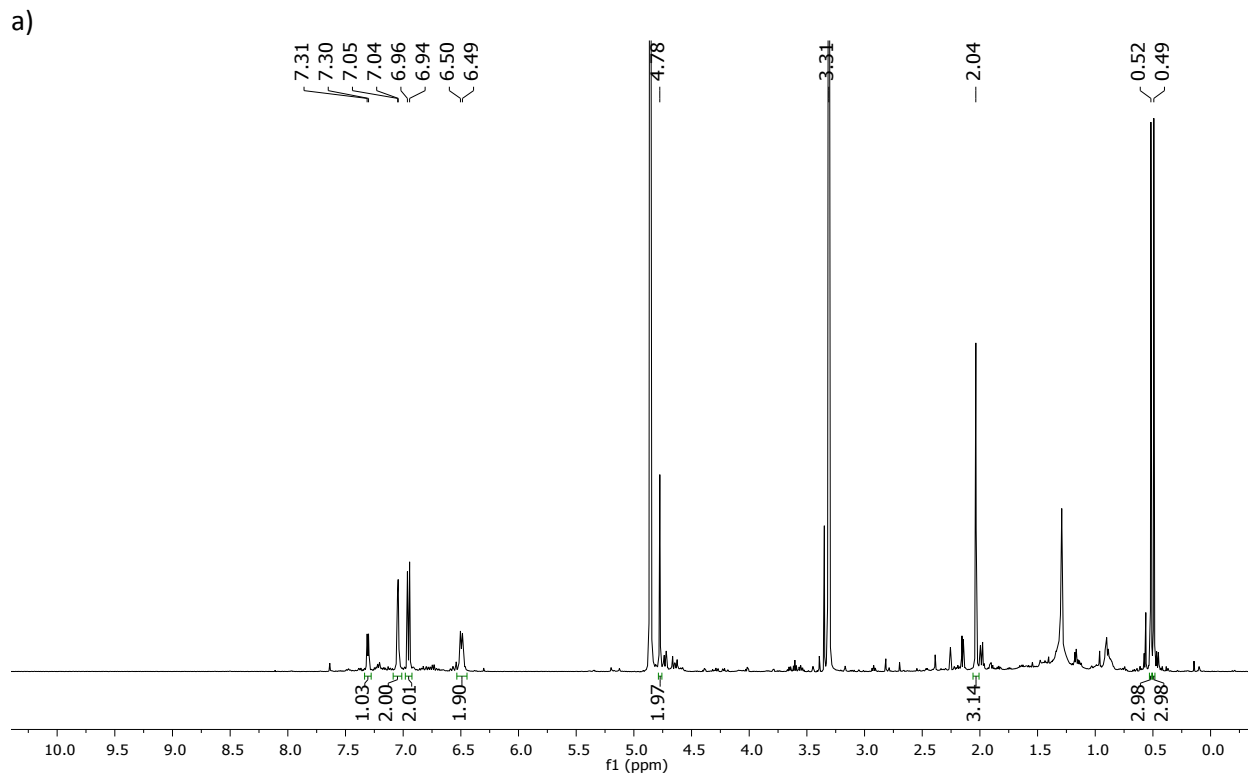


Figure S56. a) ^1H NMR and b) ^{19}F NMR spectra of **7**

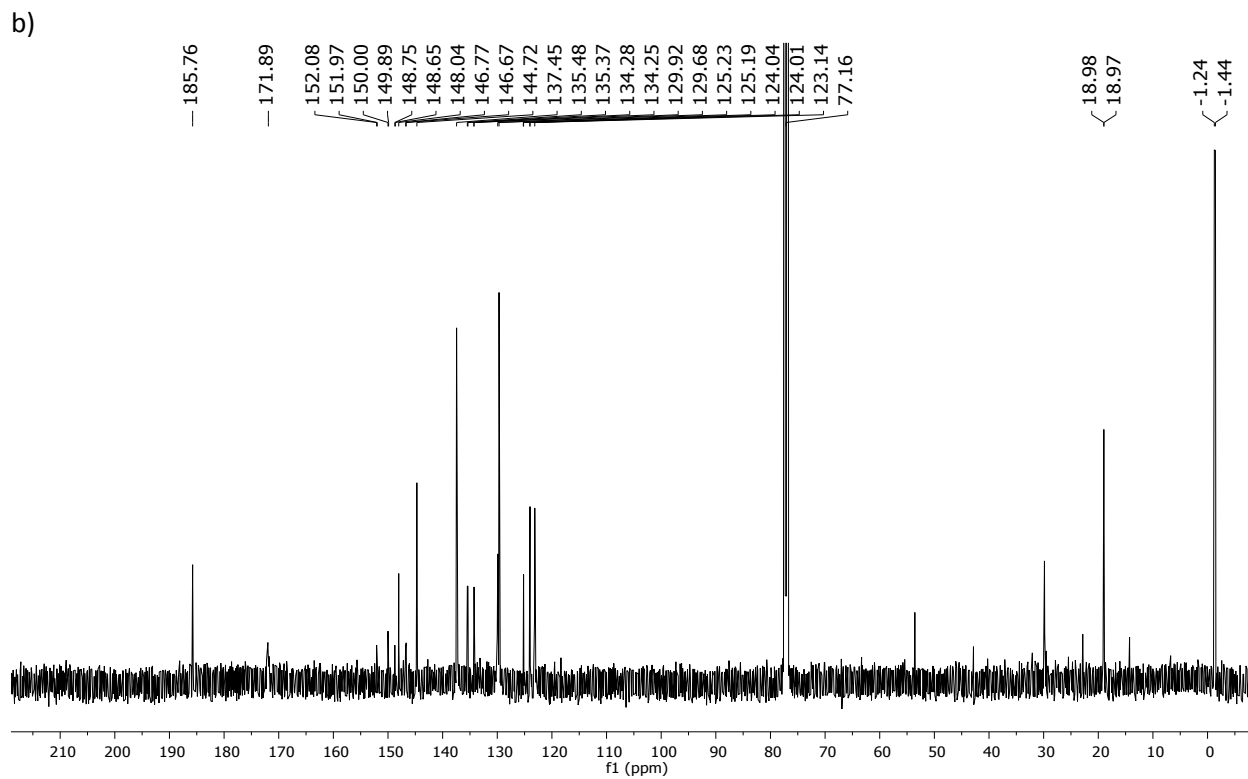
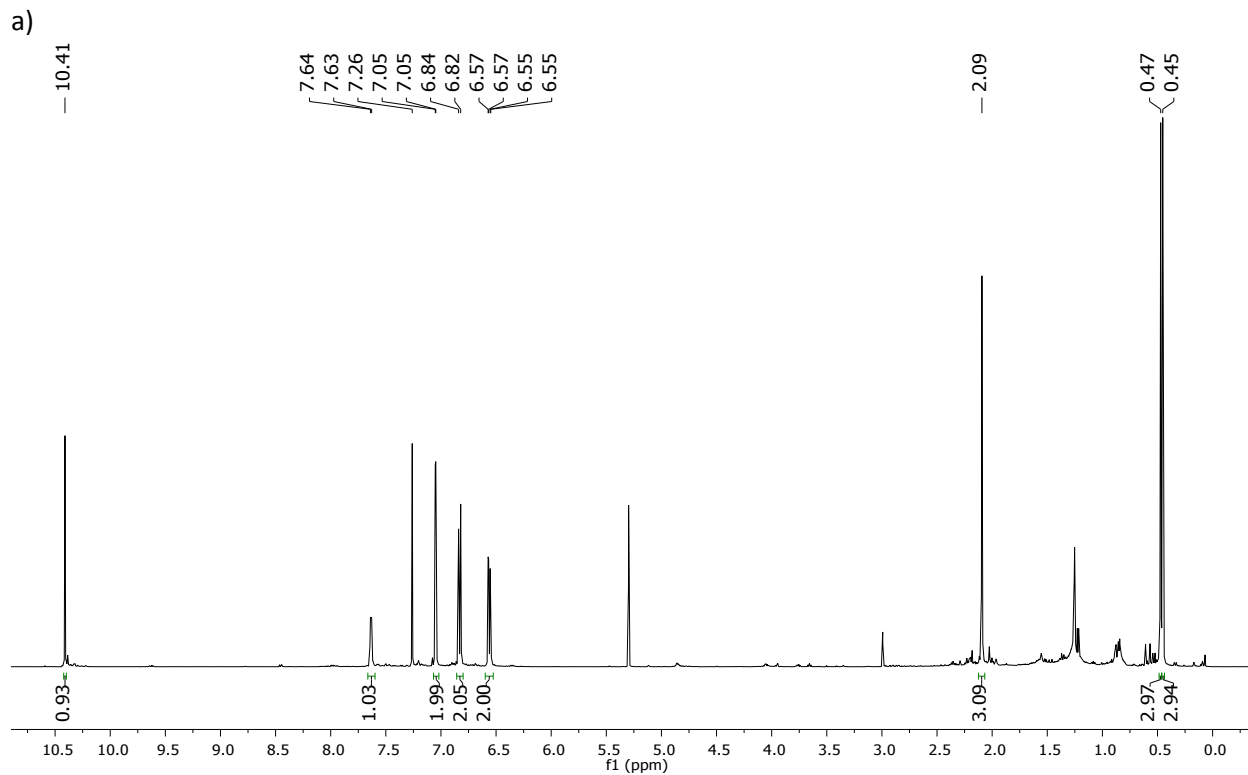


Figure S57. a) ^1H NMR and b) ^{13}C NMR spectra of red-AIDeSense (Probe 7)

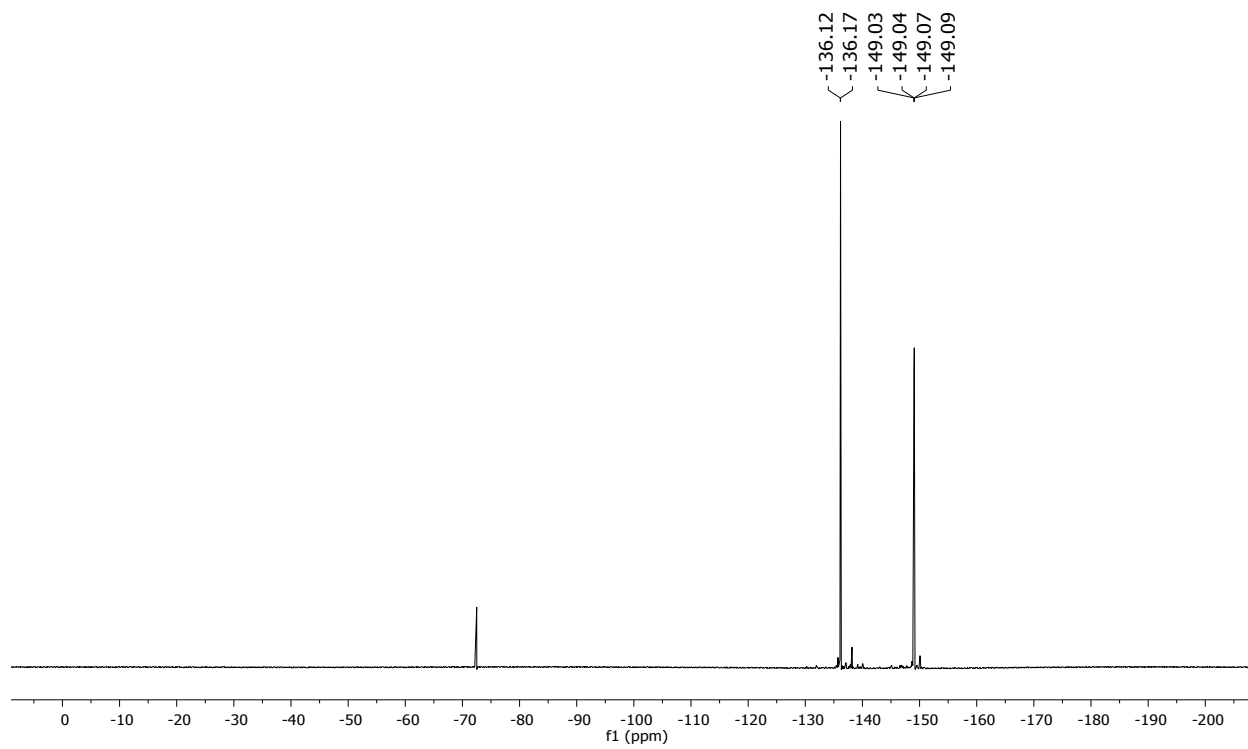


Figure S58. ^{19}F NMR spectrum of red-AIDeSense (Probe 7)

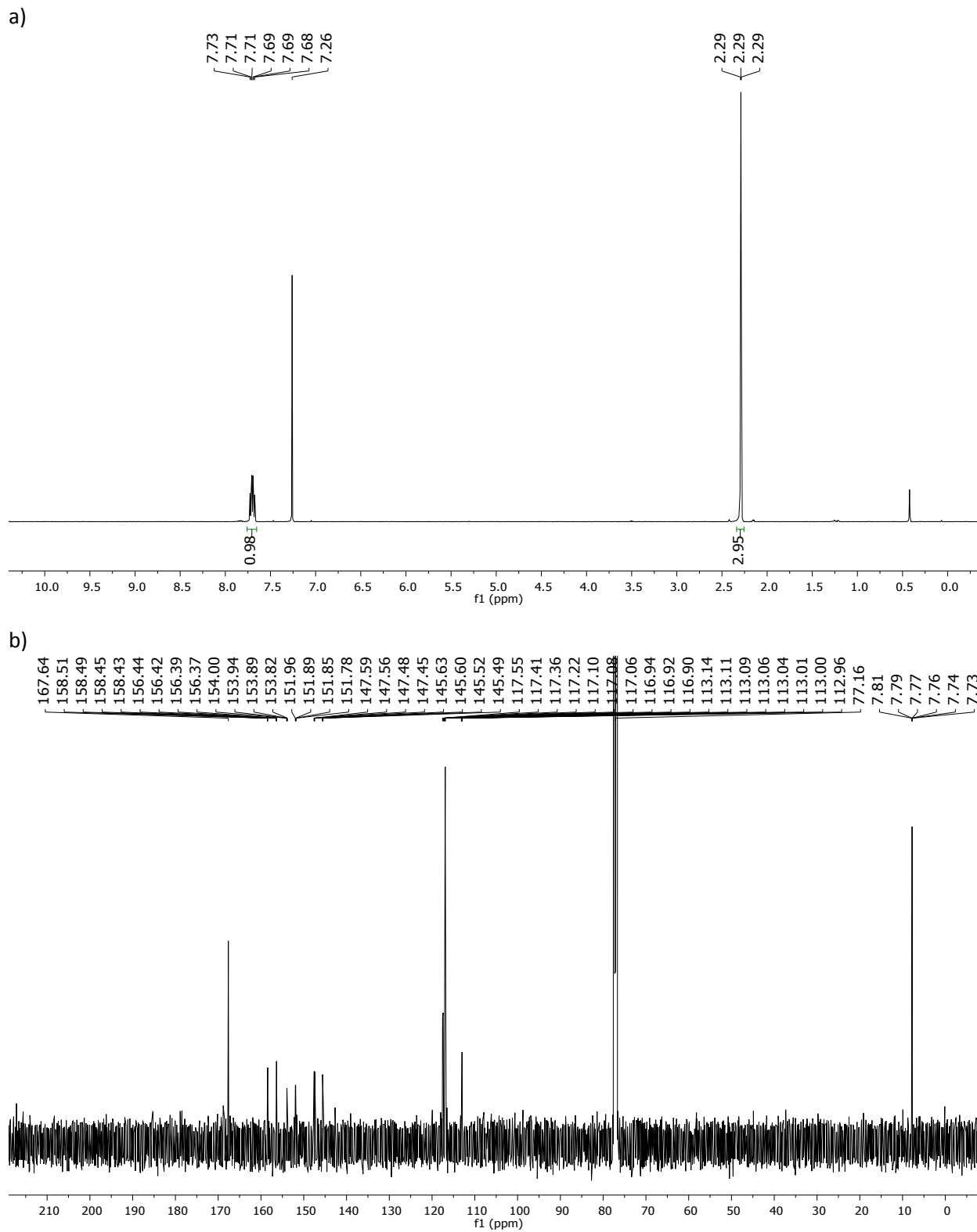


Figure S59. a) ^1H NMR and b) ^{13}C NMR spectra of **37**

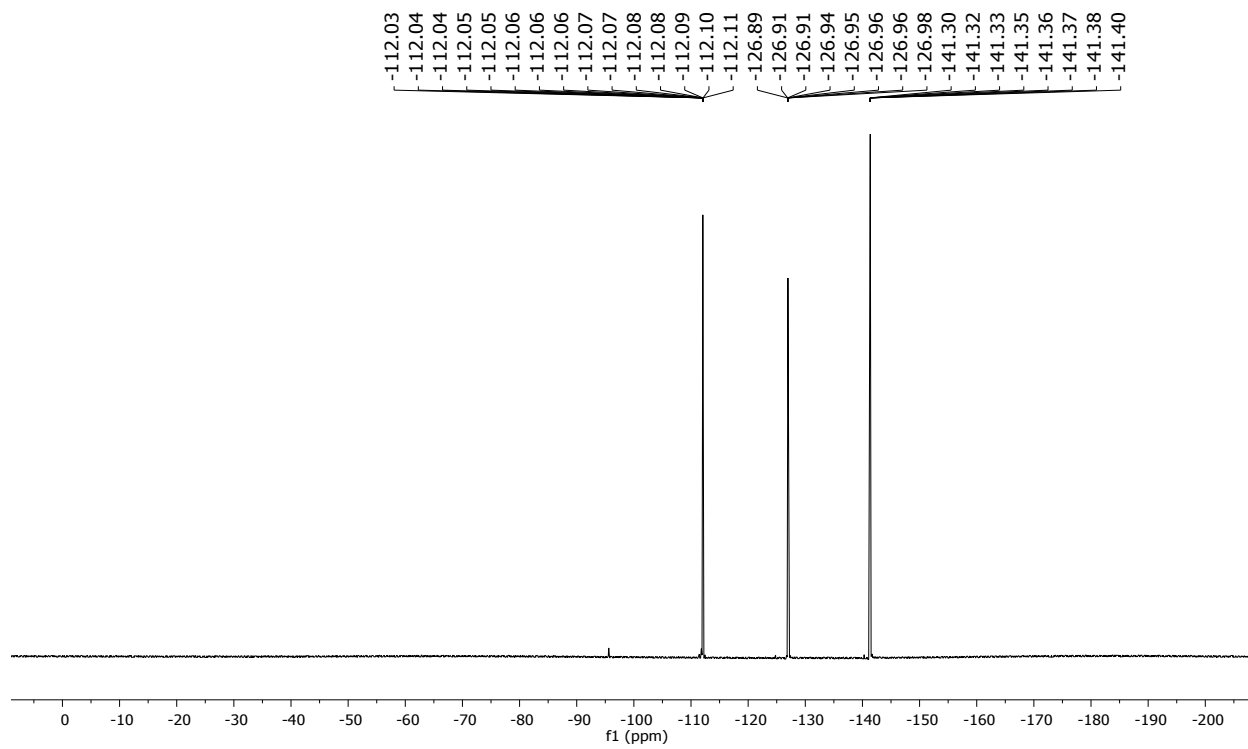


Figure S60. ^{19}F NMR spectrum of **37**

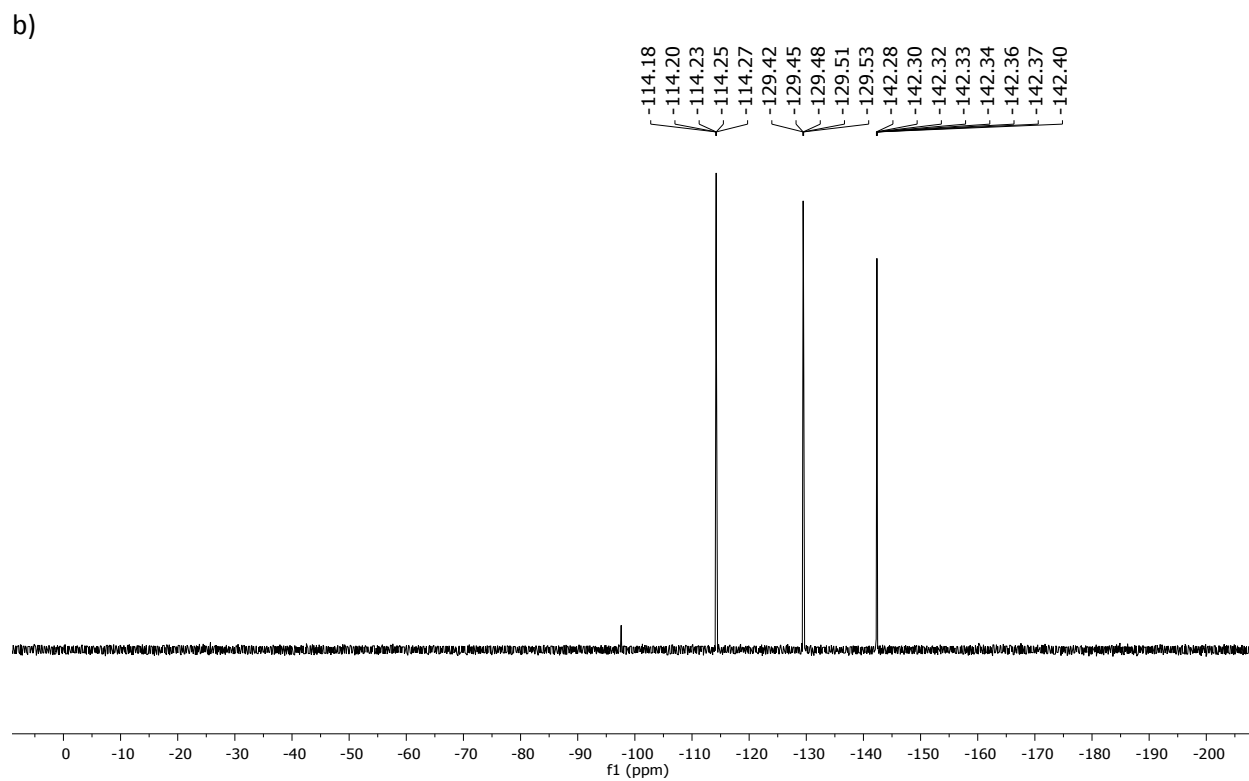
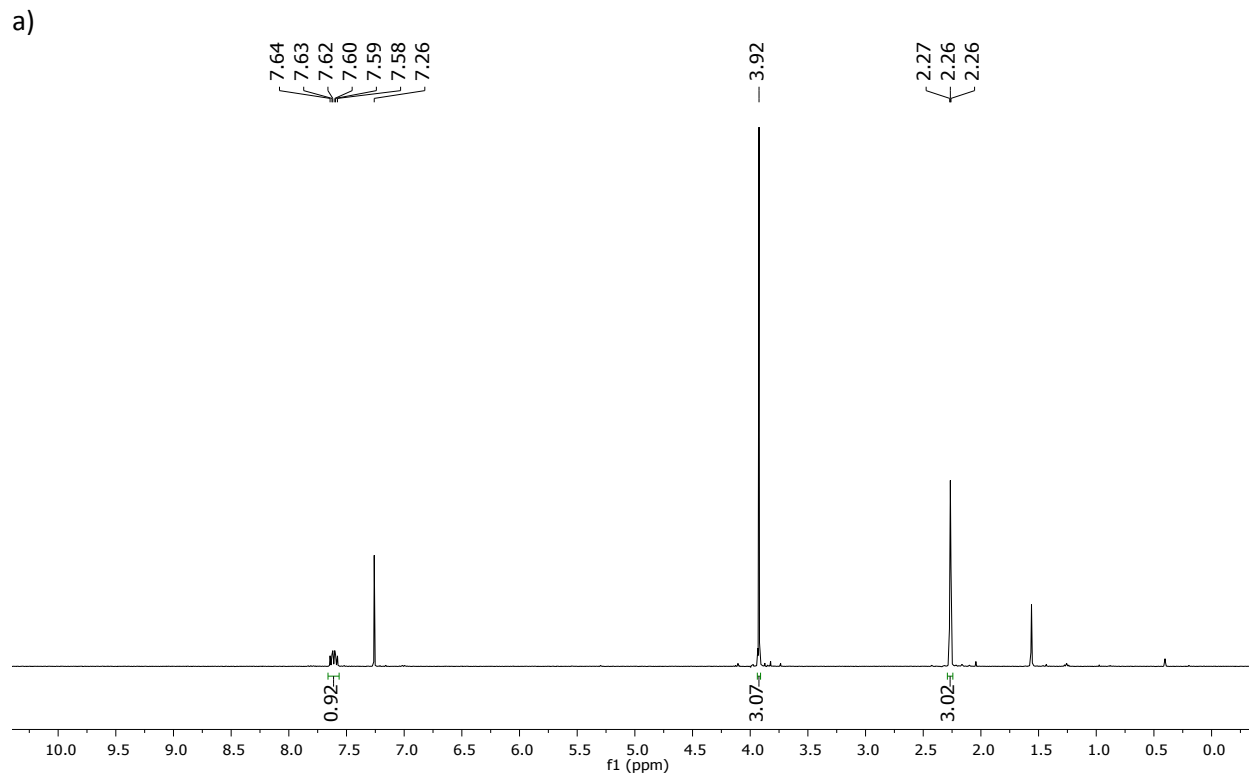


Figure S61. a) ^1H NMR and b) ^{19}F NMR spectra of **38**

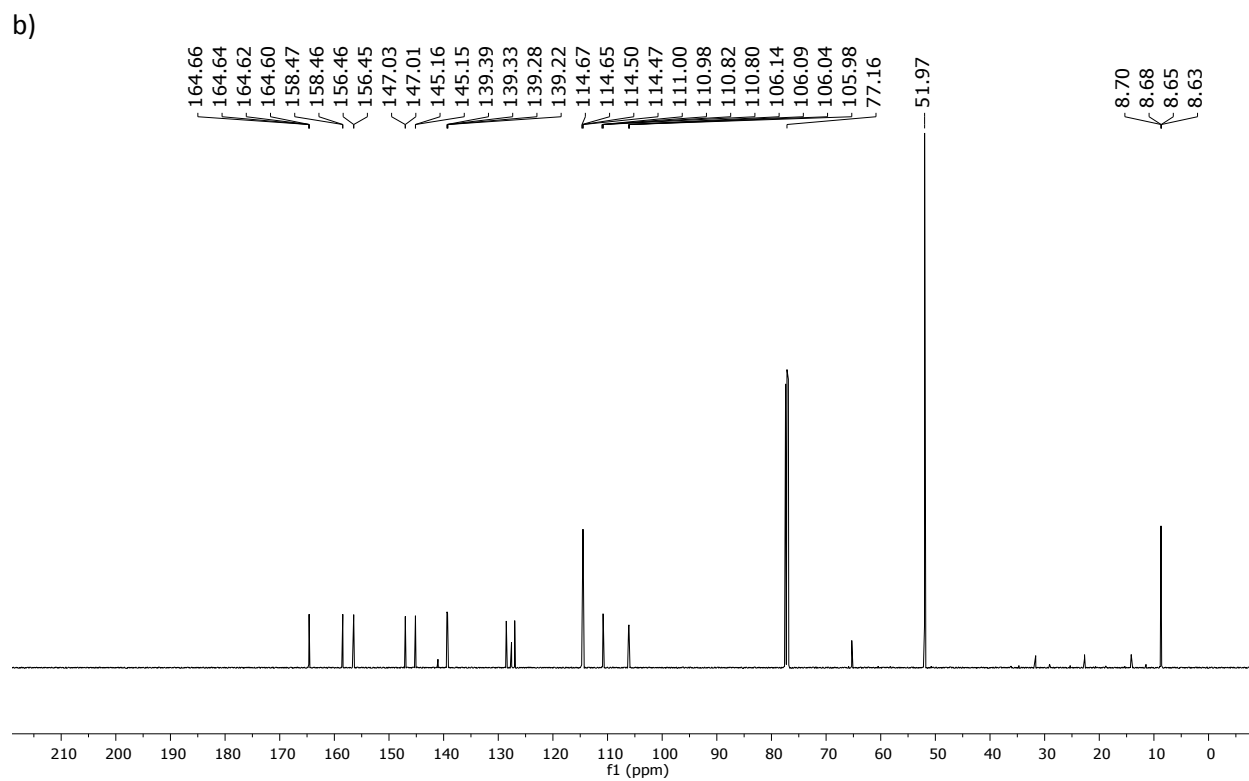
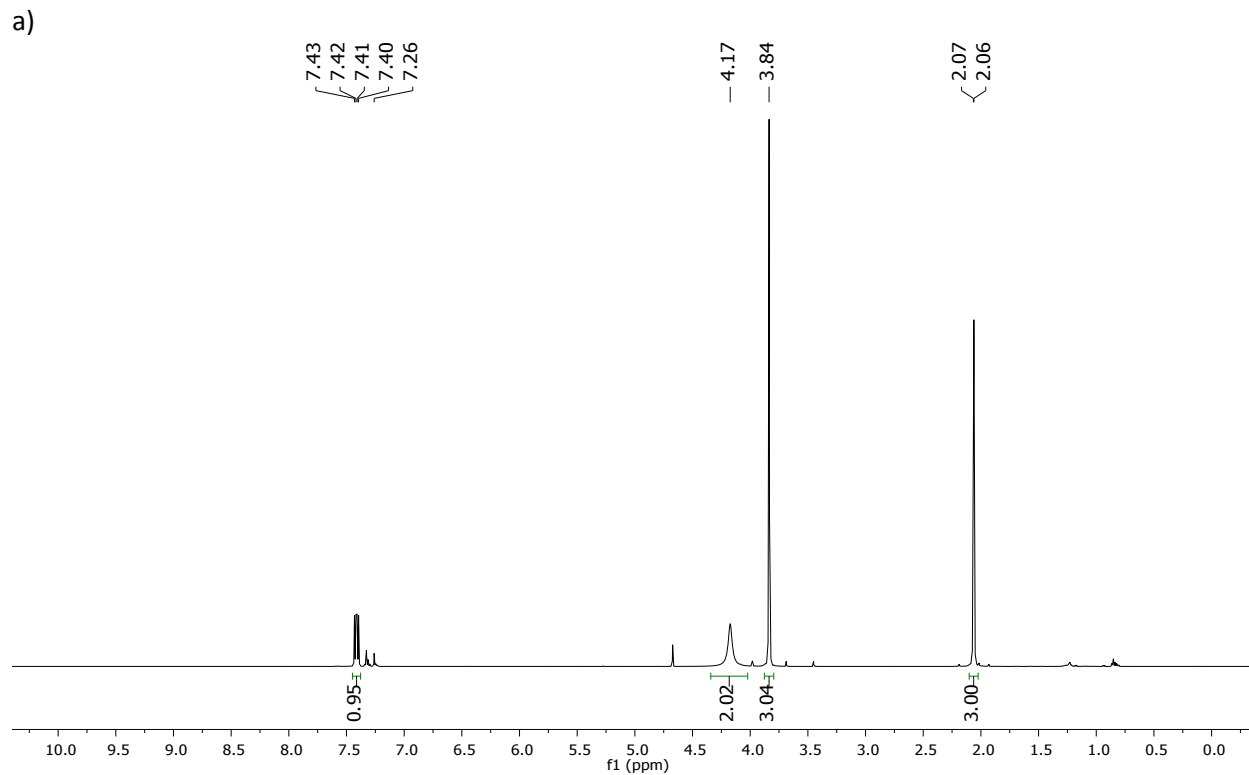


Figure S62. a) ^1H NMR and b) ^{13}C NMR spectra of **39**

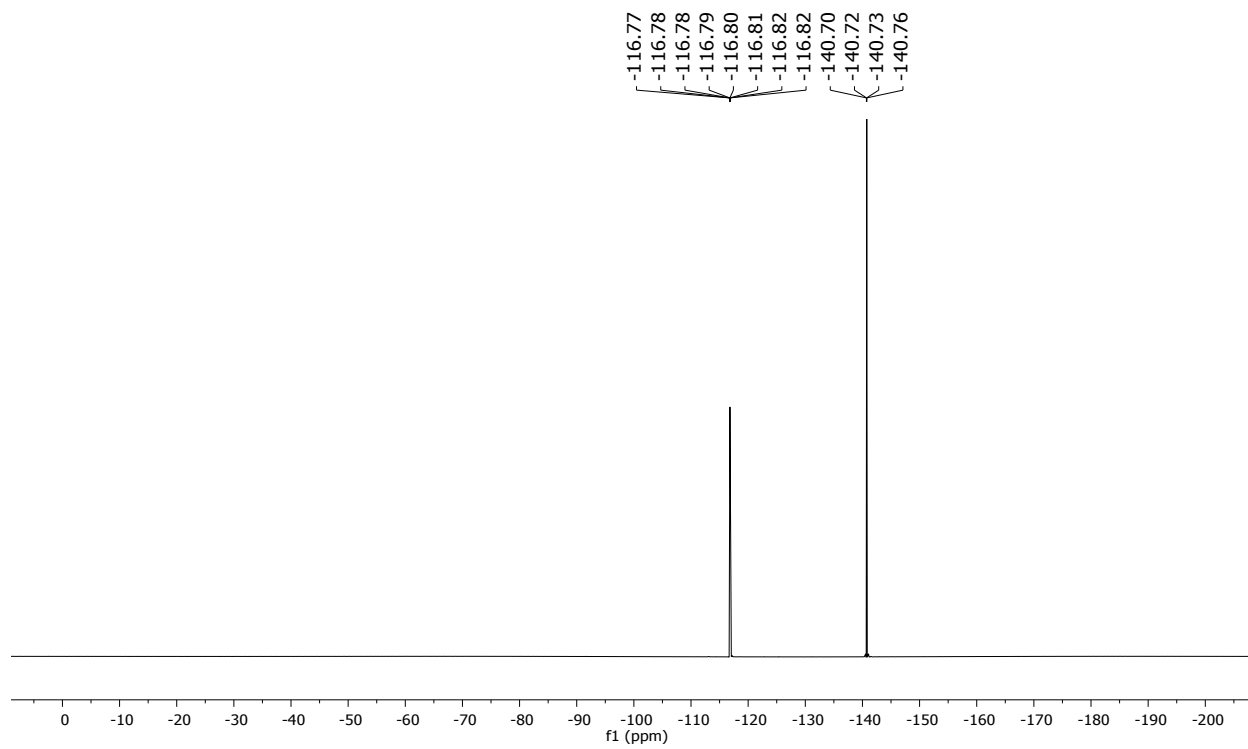


Figure S63. ^{19}F NMR spectrum of **39**

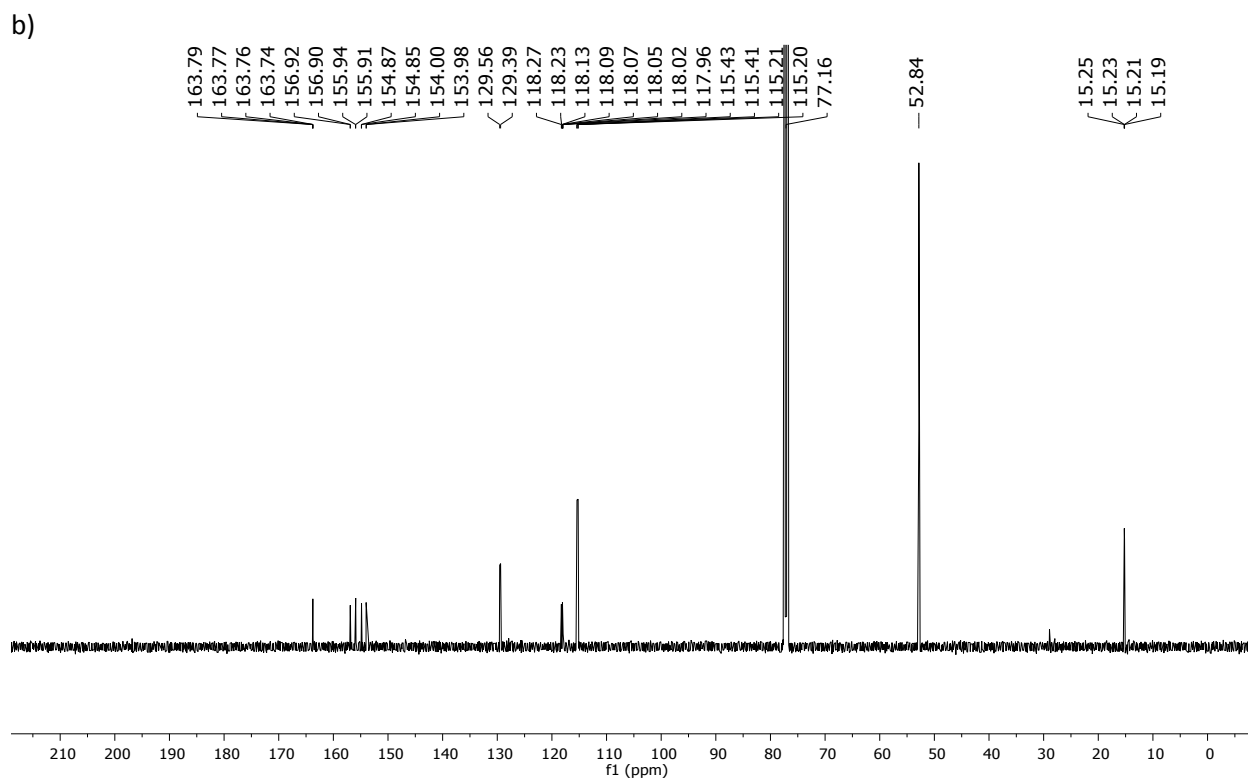
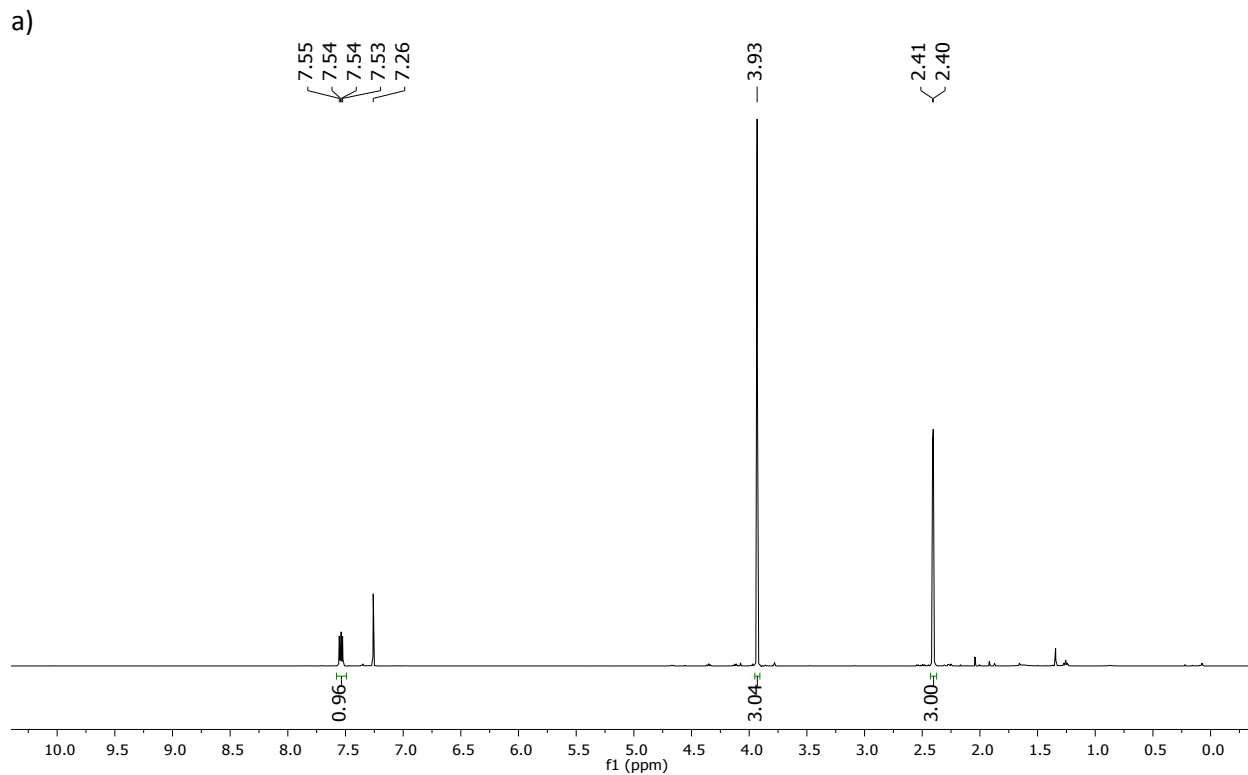


Figure S64. a) ^1H NMR and b) ^{13}C NMR spectra of **40**

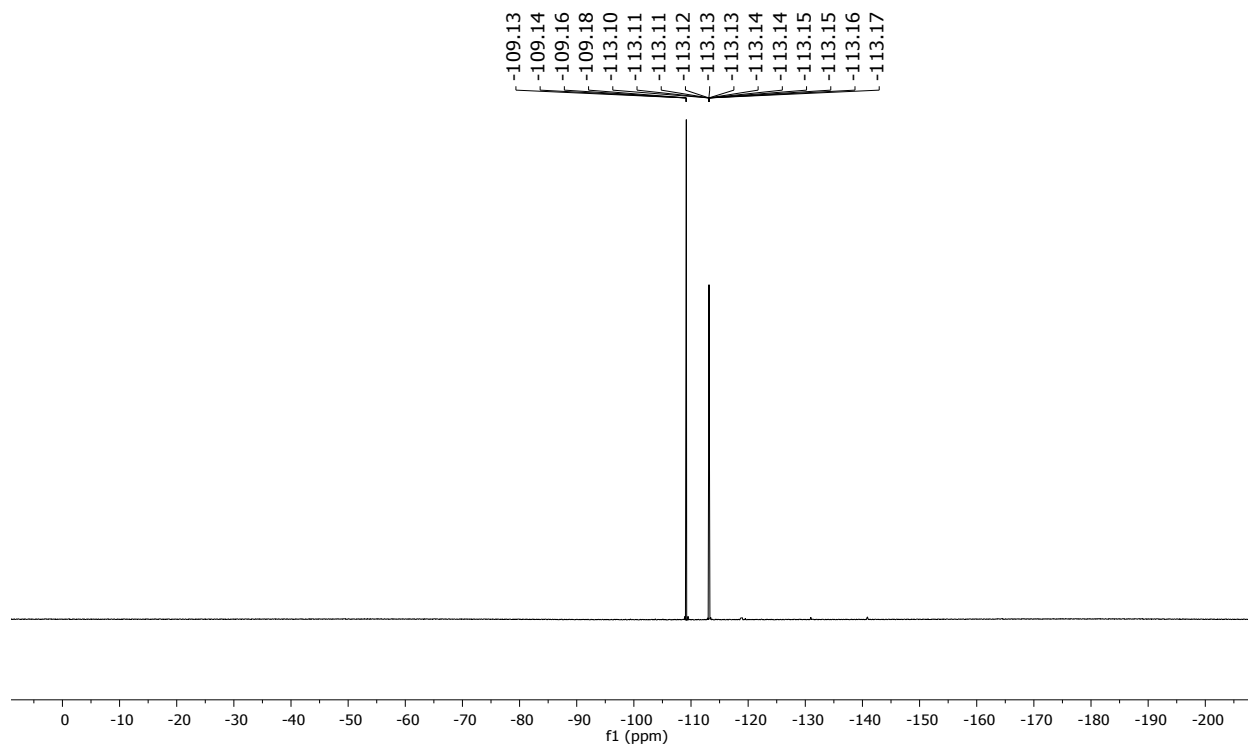


Figure S65. ^{19}F NMR spectrum of **40**

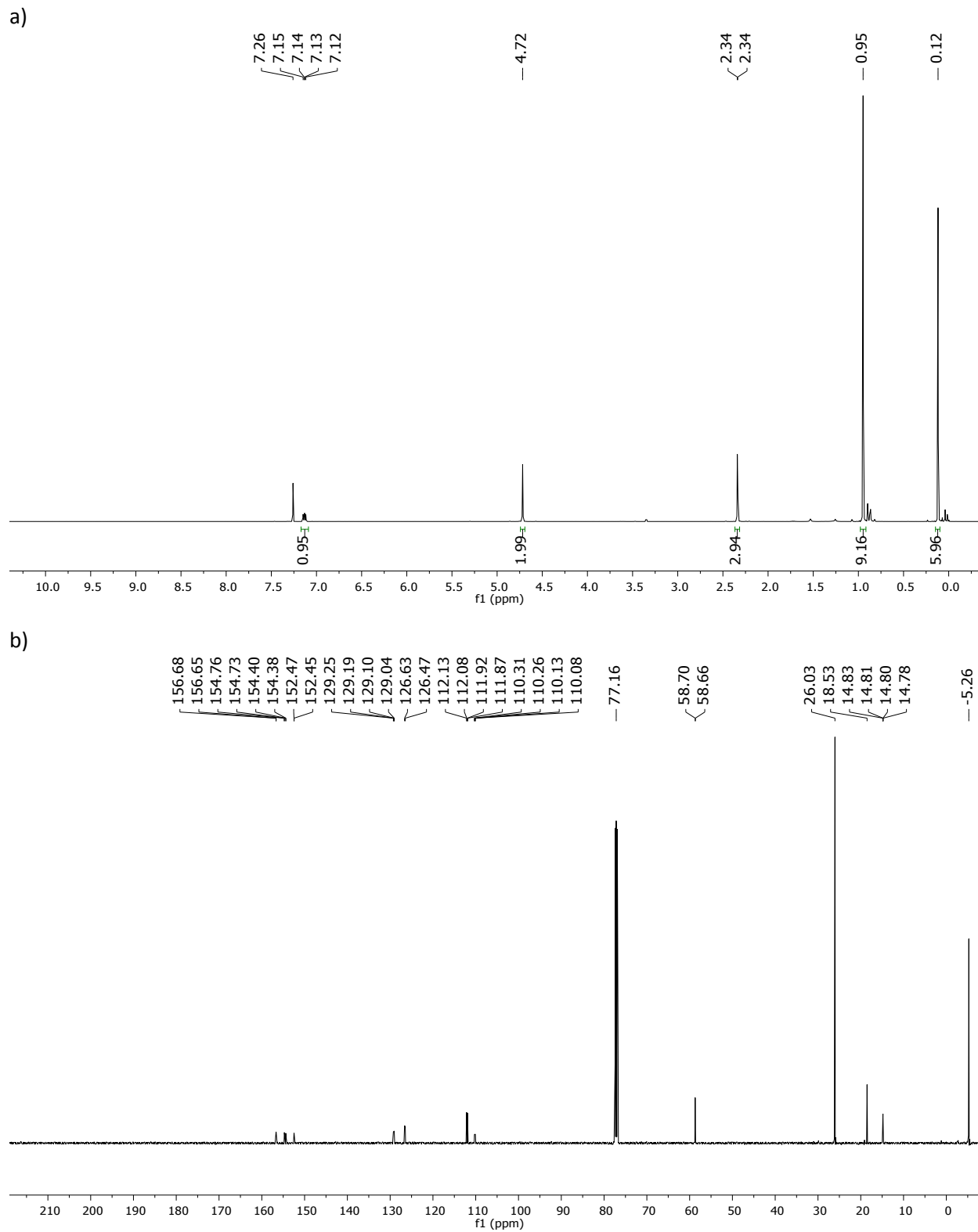


Figure S66. a) ^1H NMR and b) ^{13}C NMR spectra of **41**

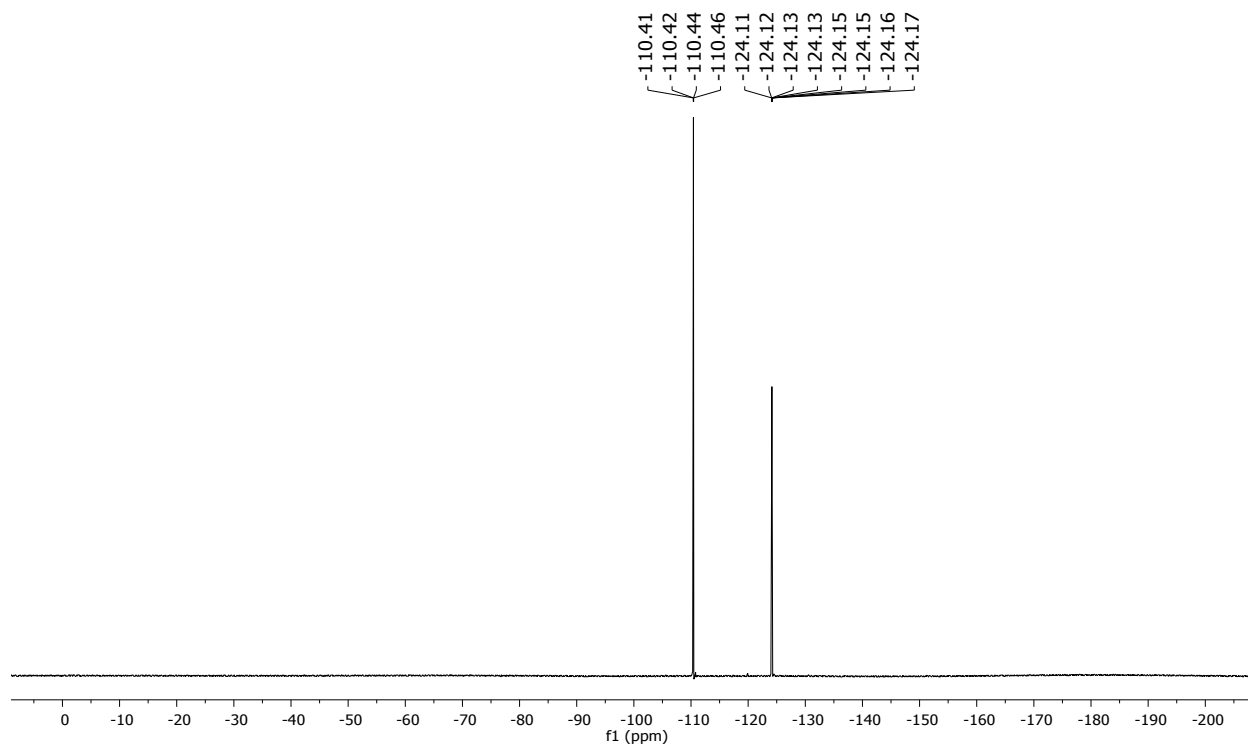


Figure S67. ^{19}F NMR spectrum of **41**

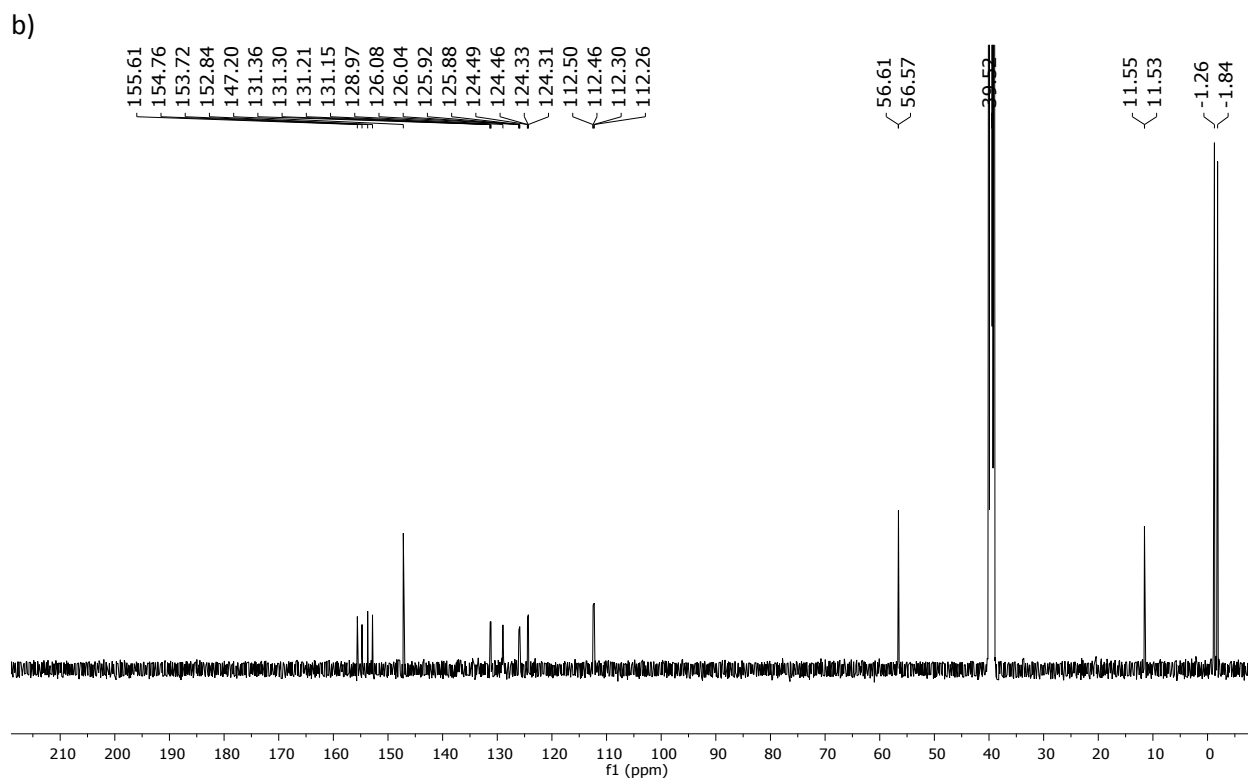
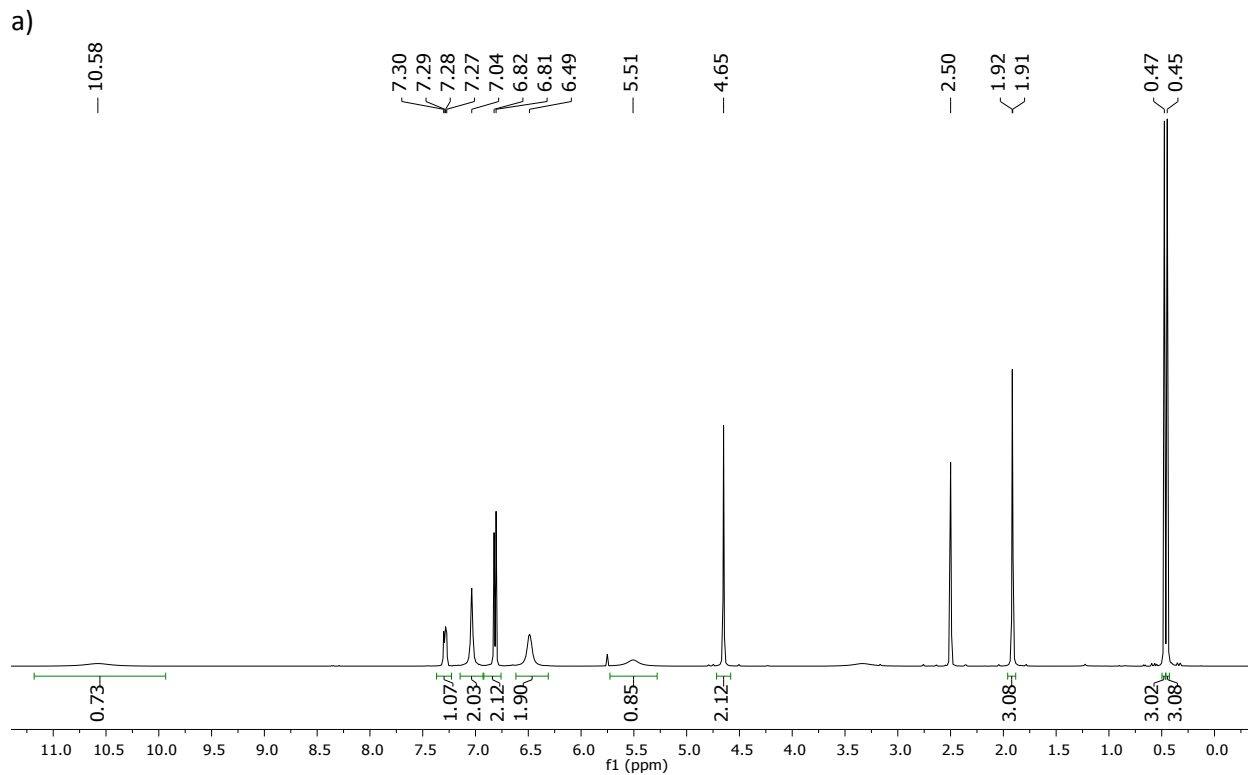


Figure S68. a) ^1H NMR and b) ^{13}C NMR spectra of **42**

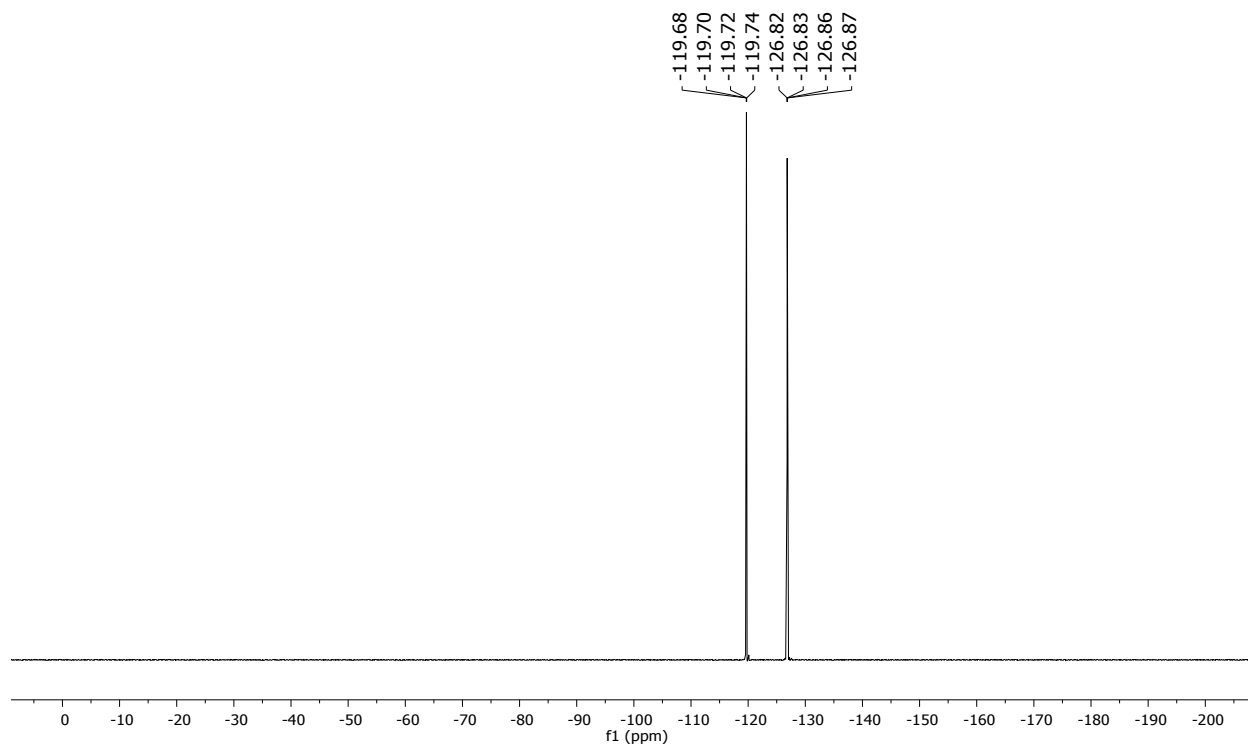


Figure S69. ^{19}F NMR spectrum of **42**

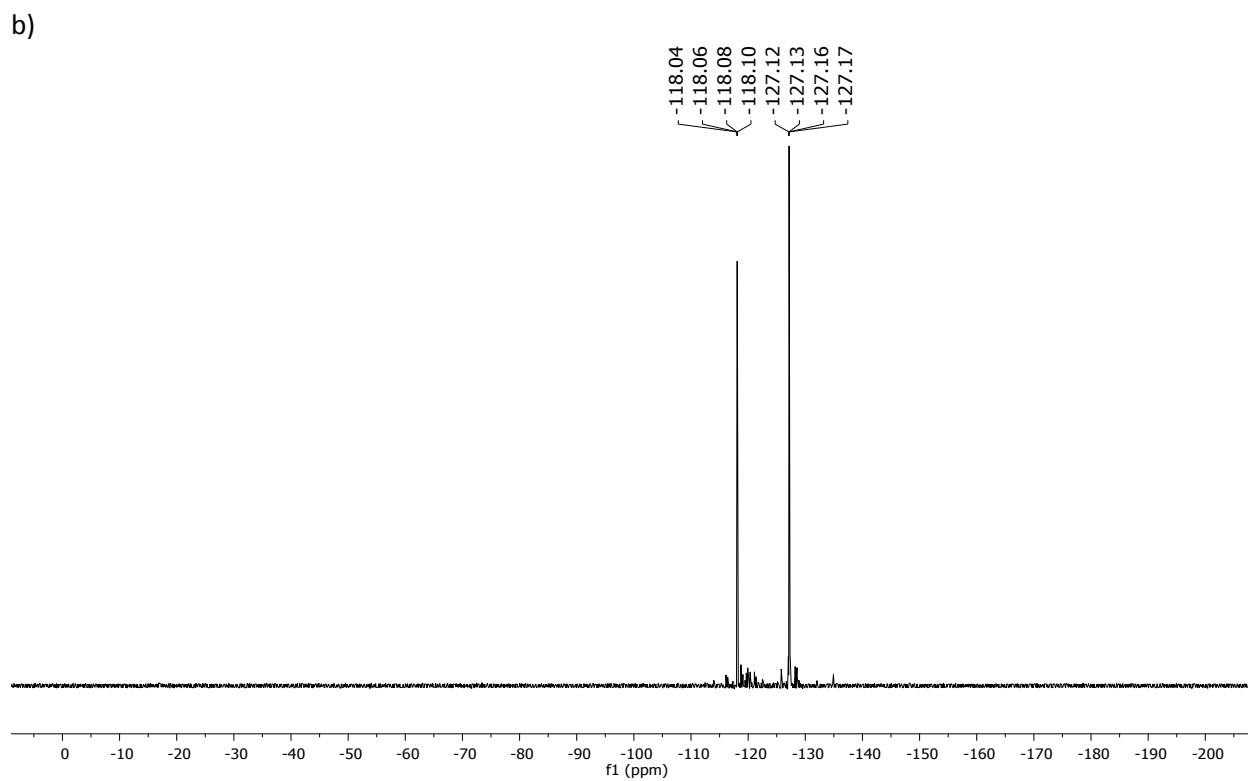
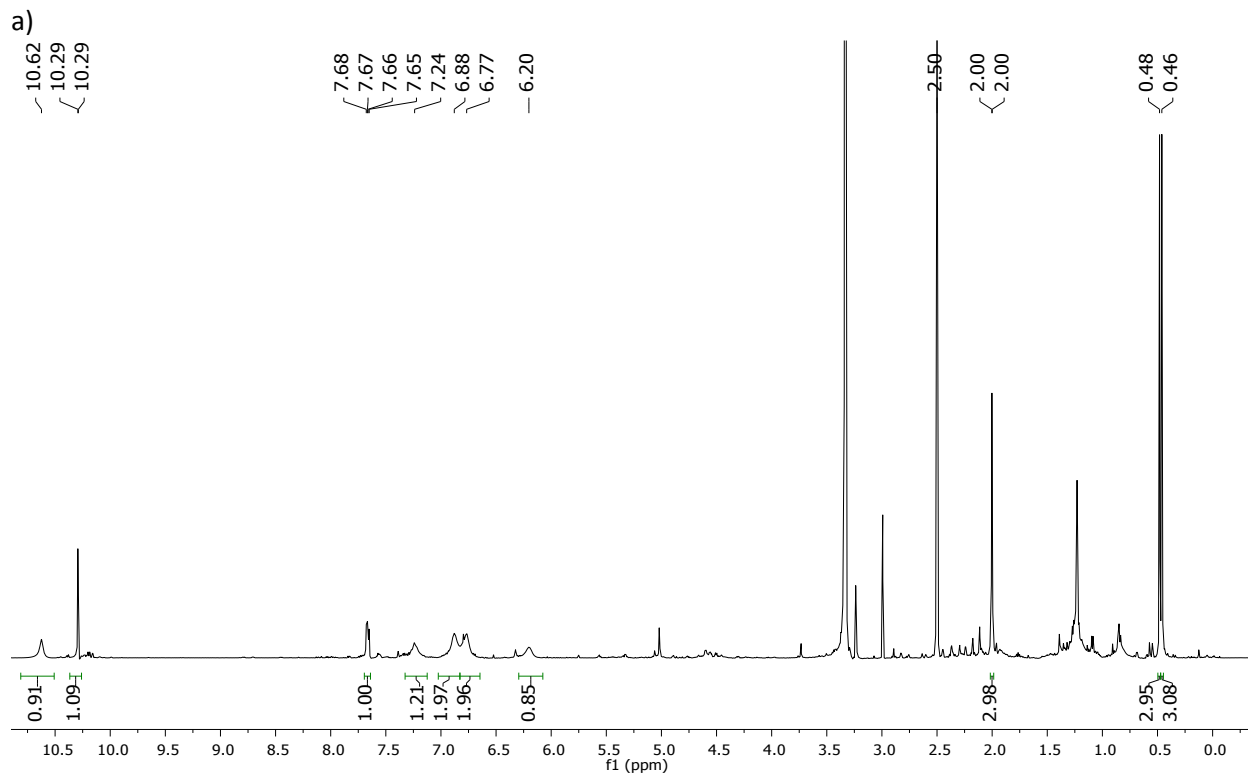


Figure S70. a) ^1H NMR and b) ^{19}F NMR spectra of **Probe 8**

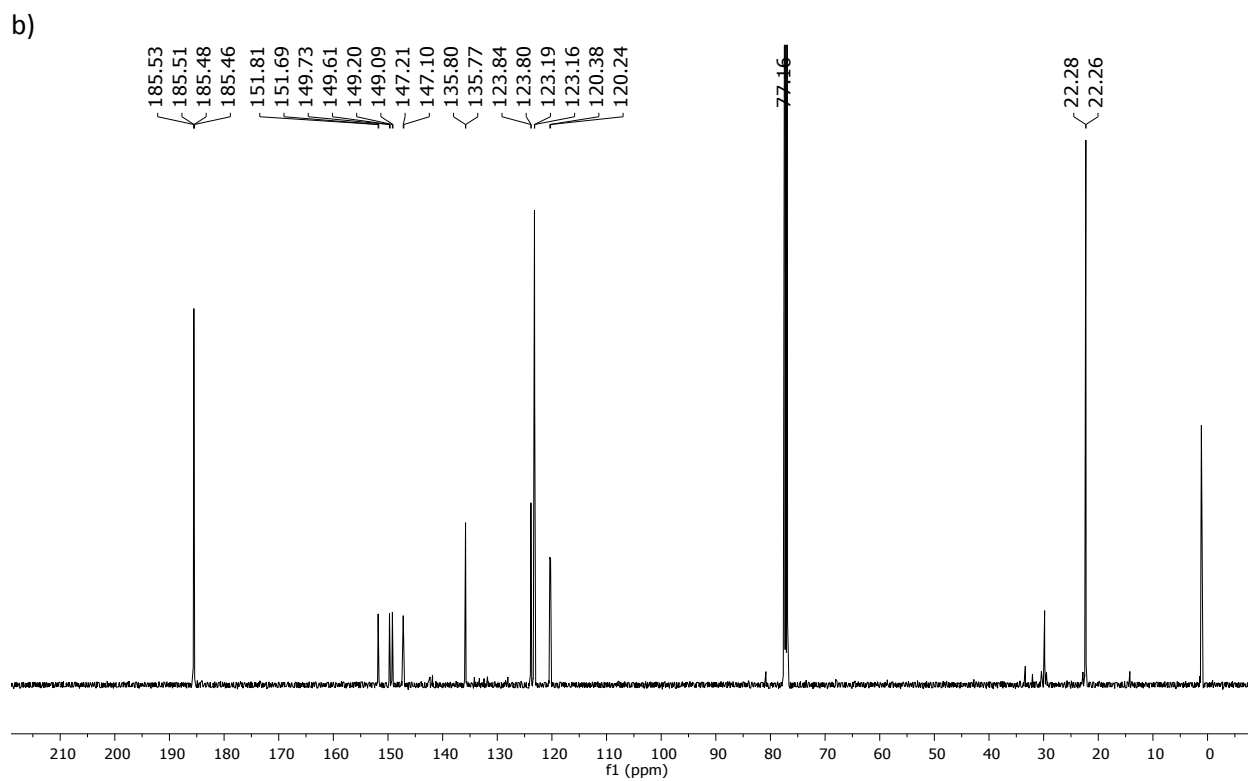
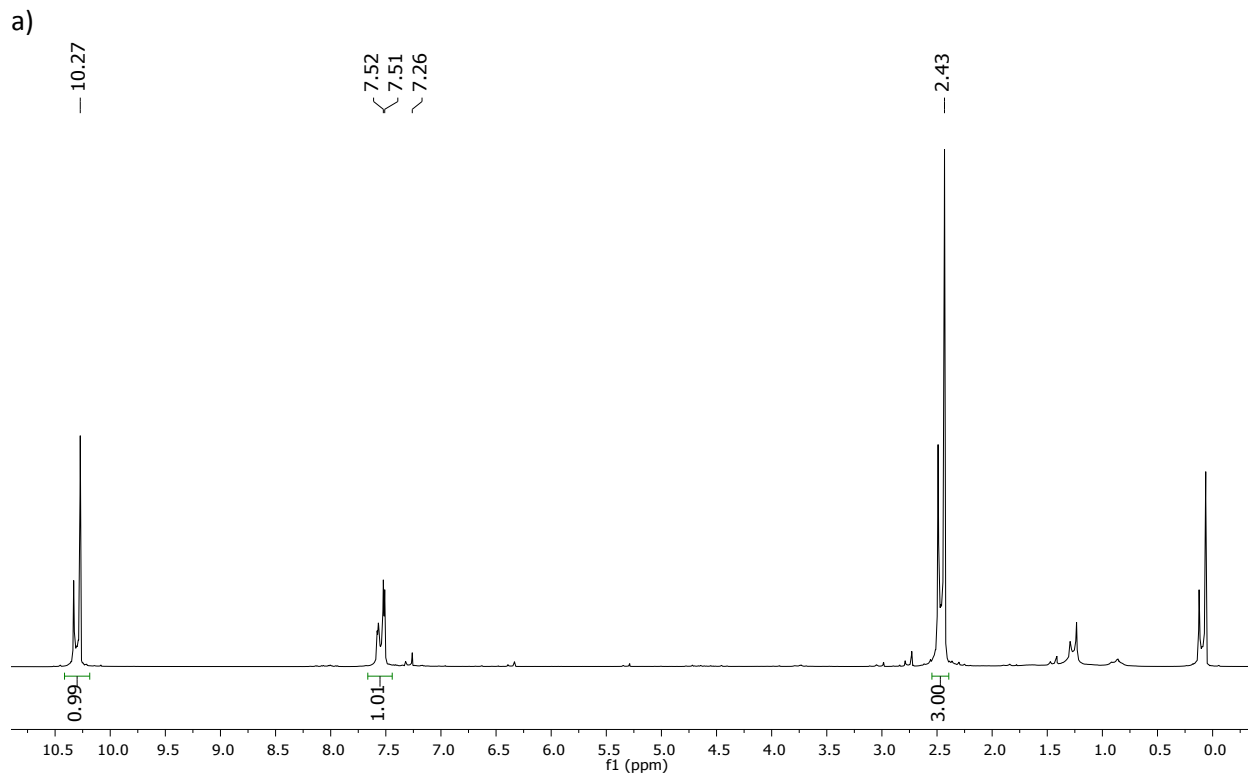


Figure S71. a) ^1H NMR and b) ^{13}C NMR spectra of **43**

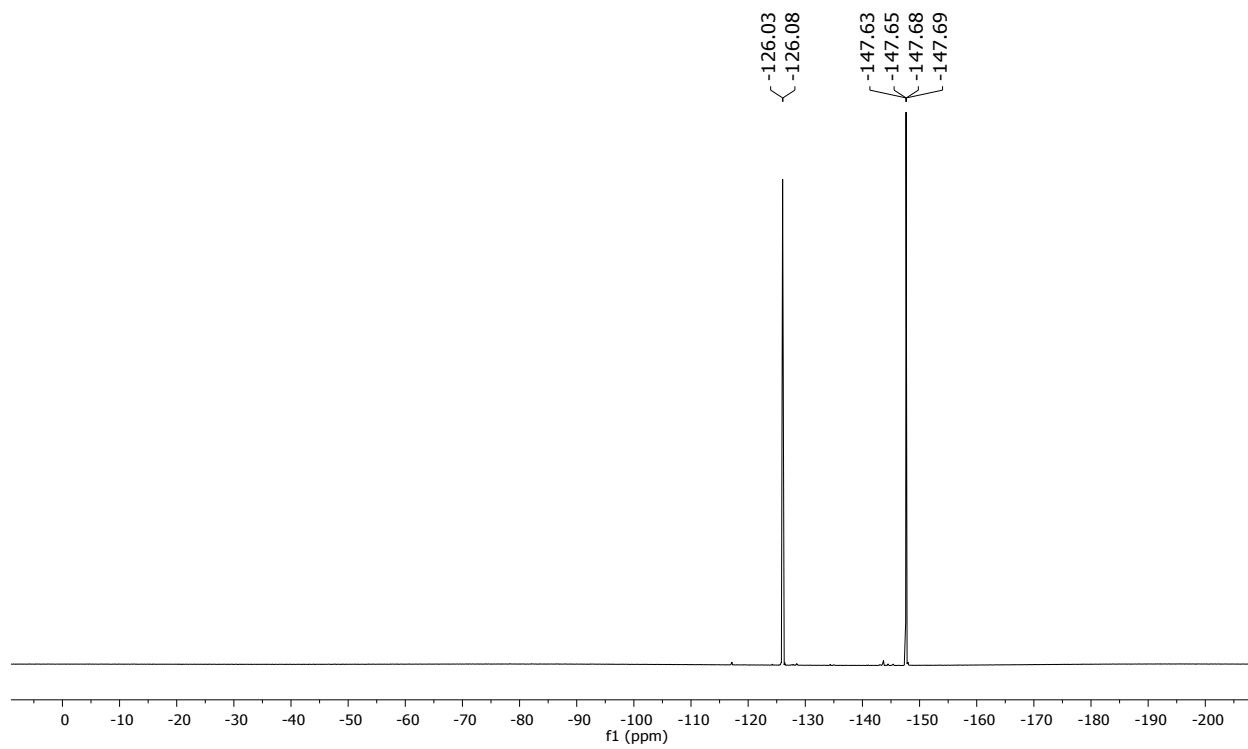


Figure S72. ^{19}F NMR spectrum of **43**

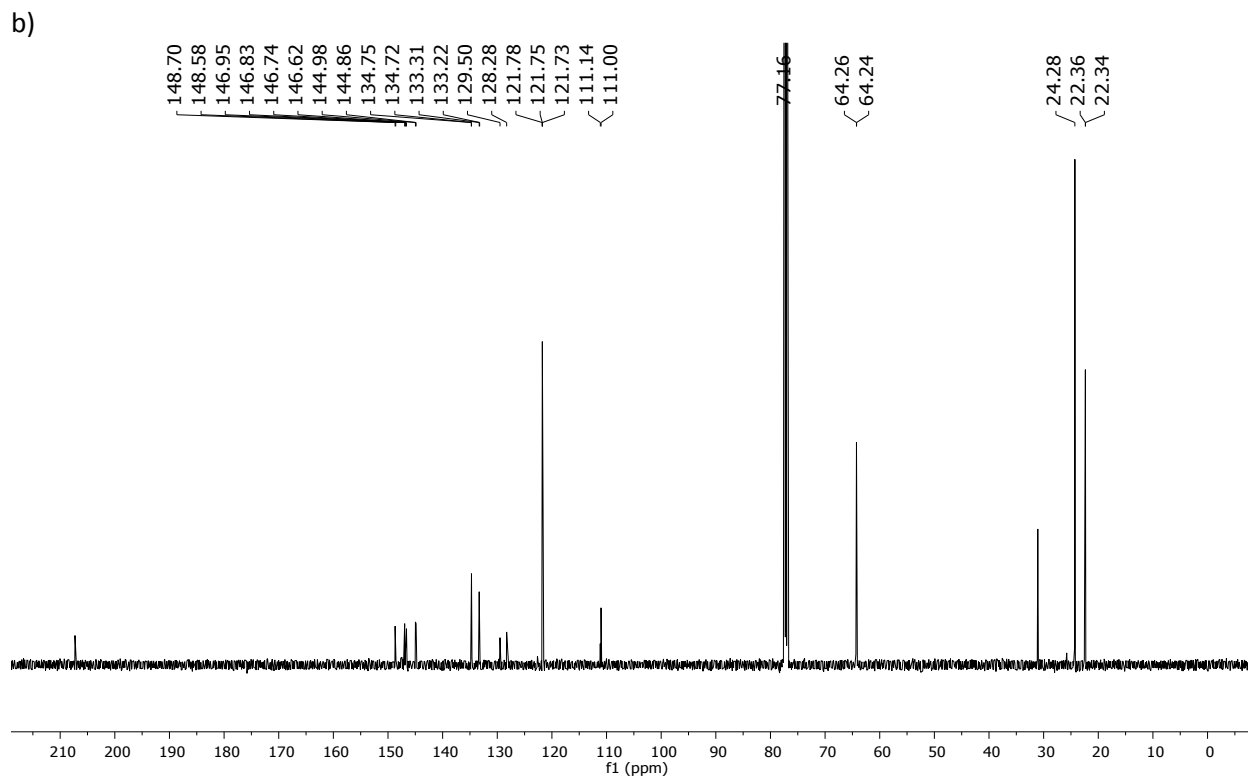
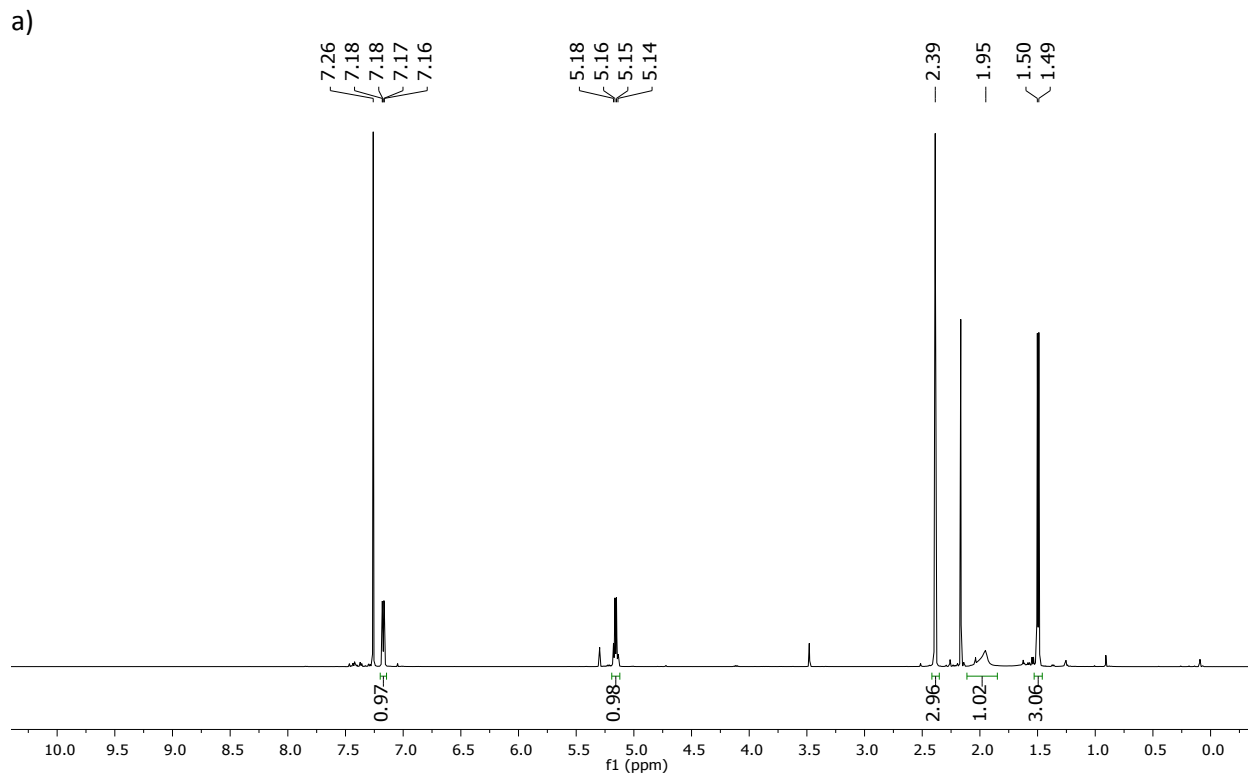


Figure S73. a) ^1H NMR and b) ^{13}C NMR spectra of **44**

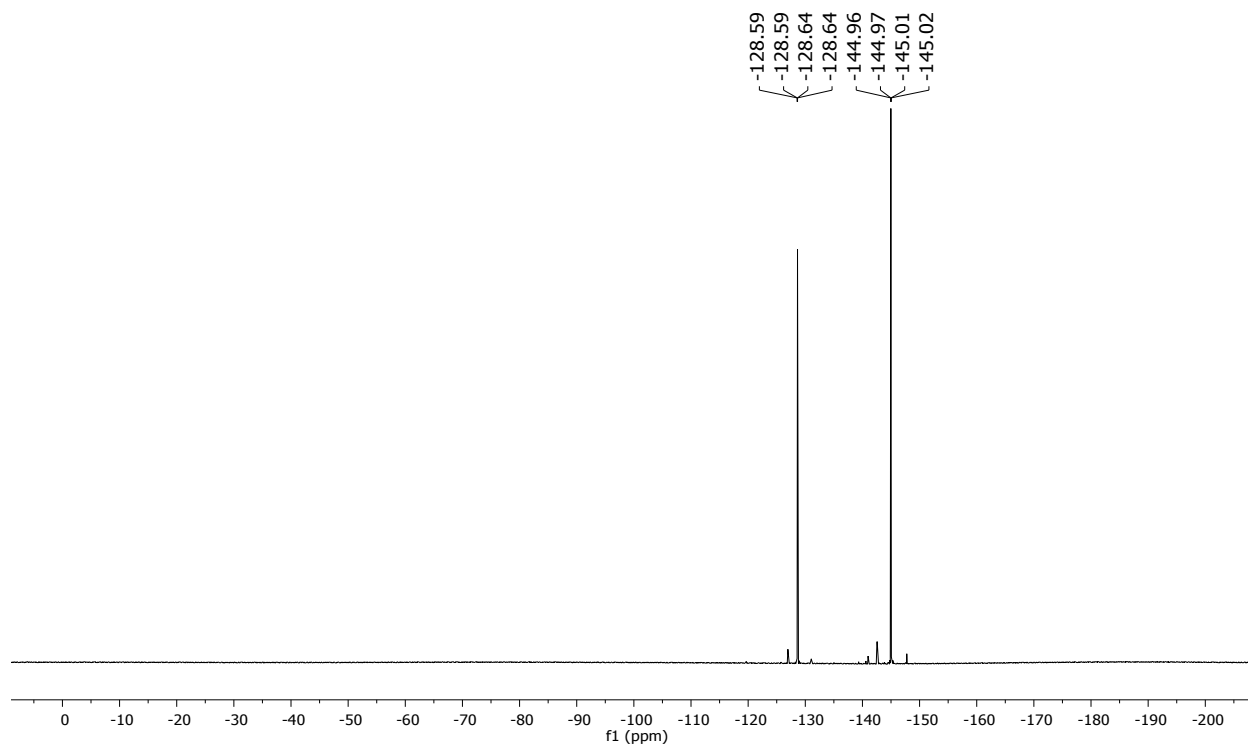


Figure S74. ^{19}F NMR spectrum of **44**

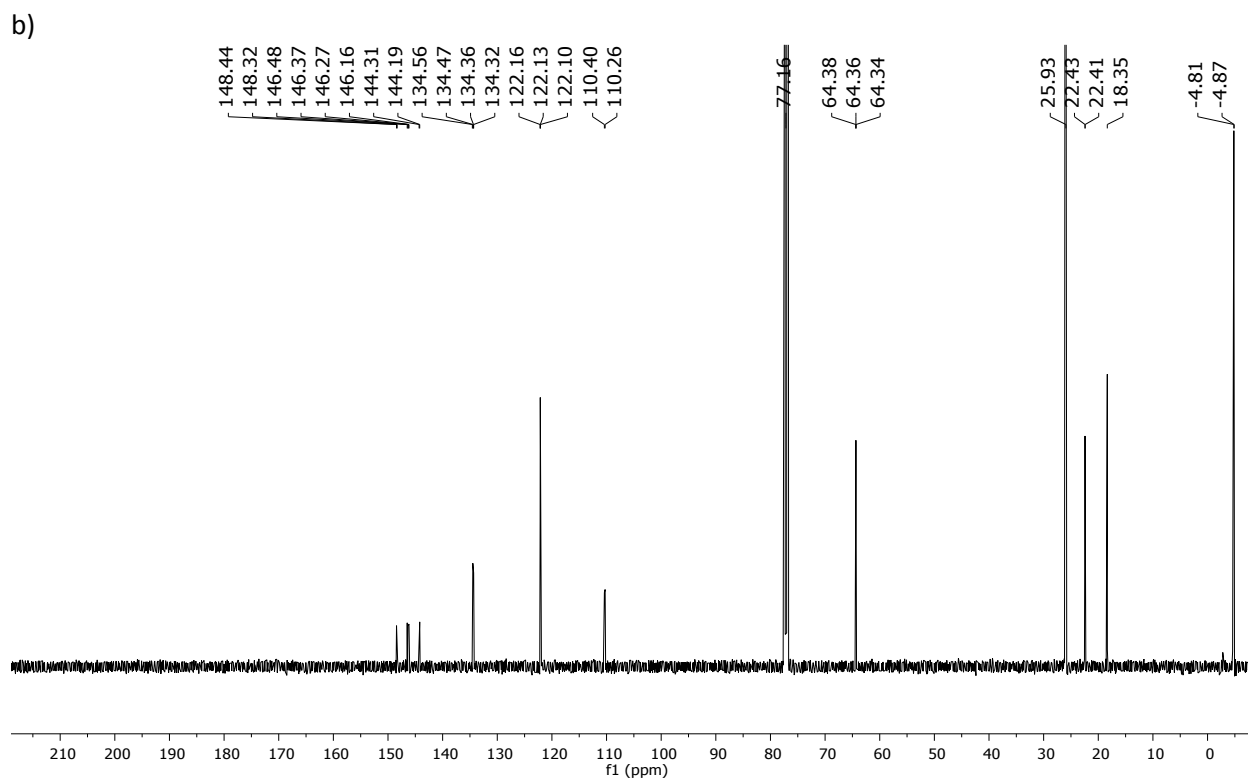
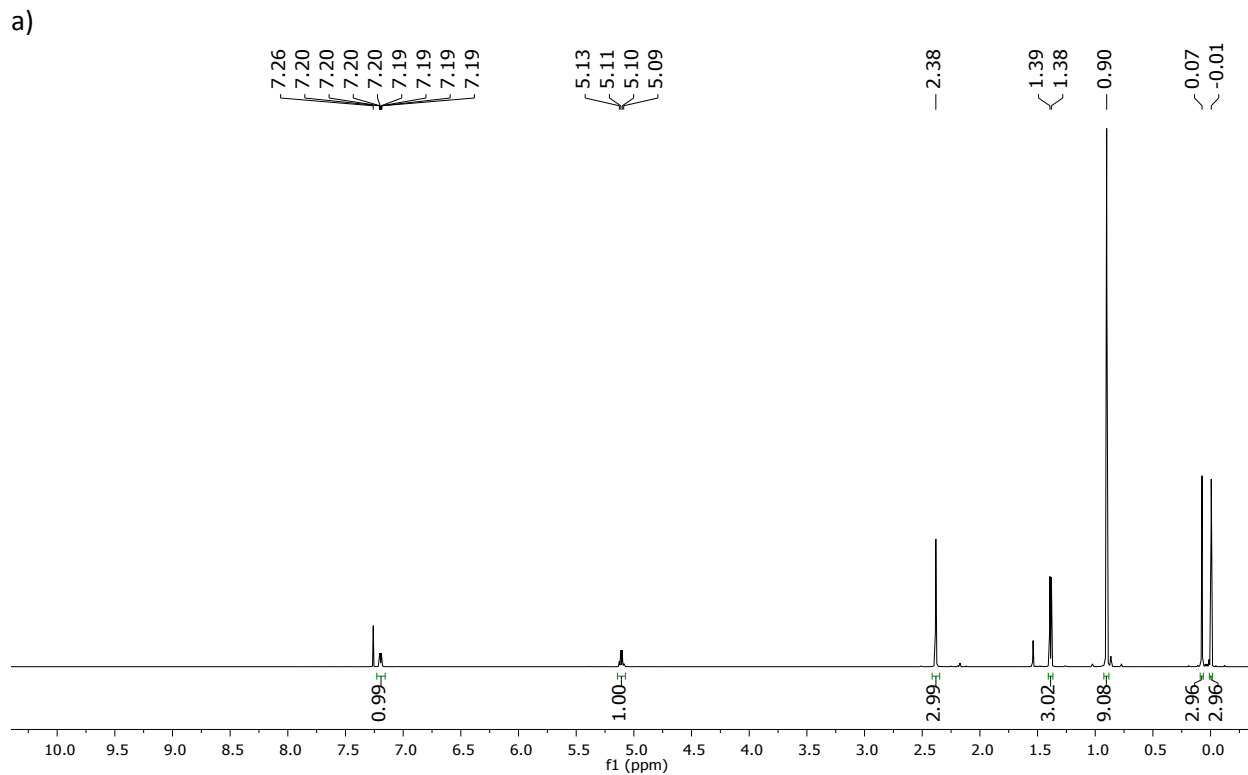


Figure S75. a) ^1H NMR and b) ^{13}C NMR spectra of **45**

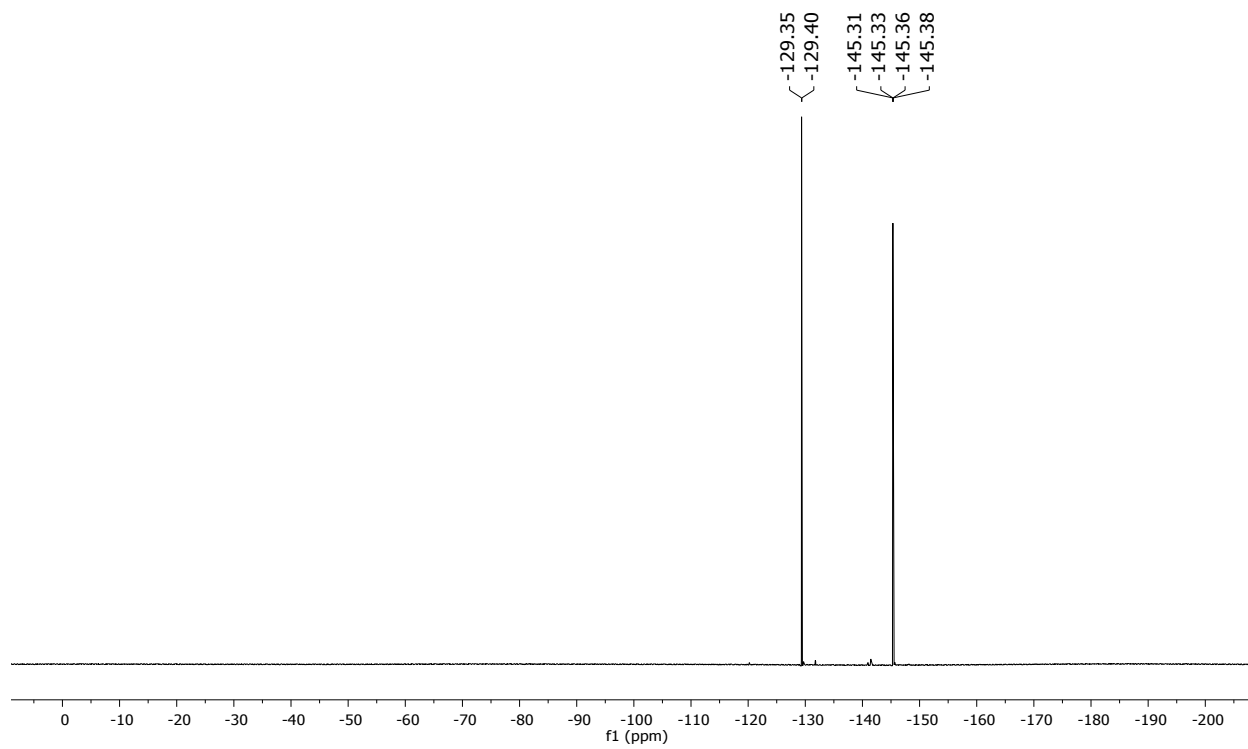


Figure S76. ^{19}F NMR spectrum of **45**

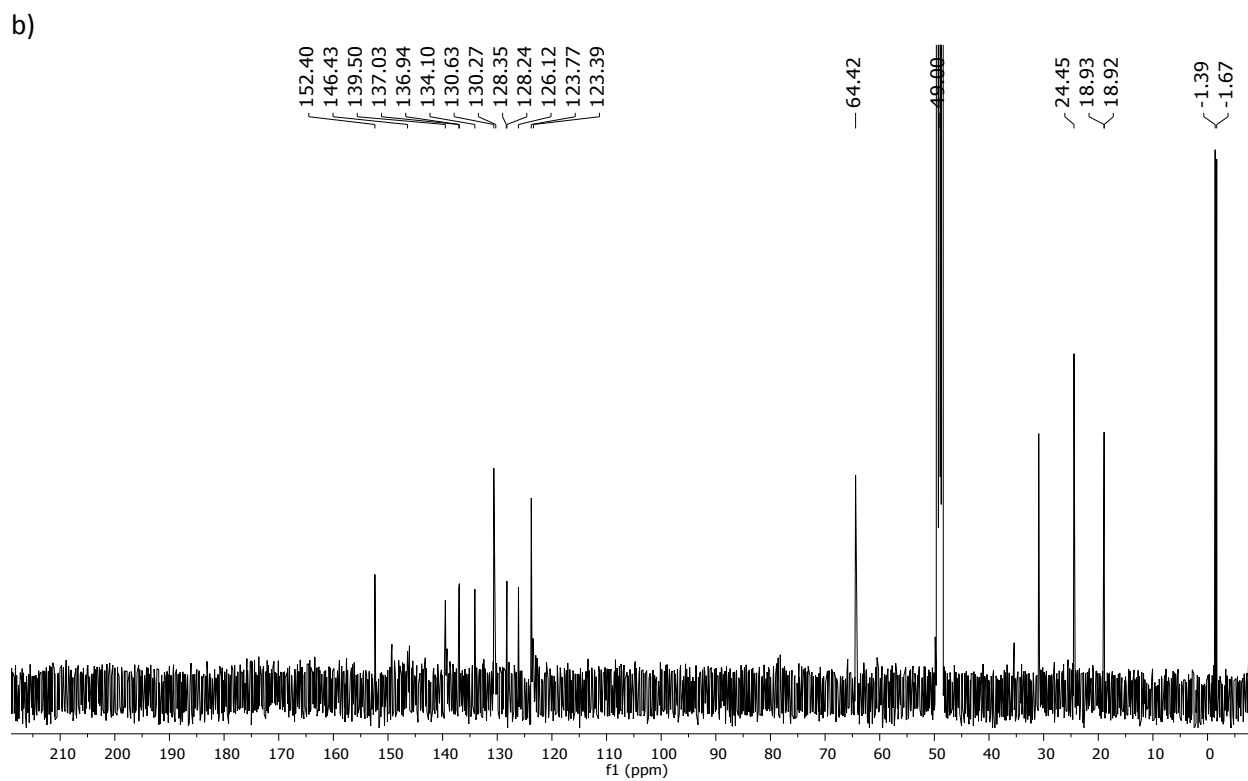
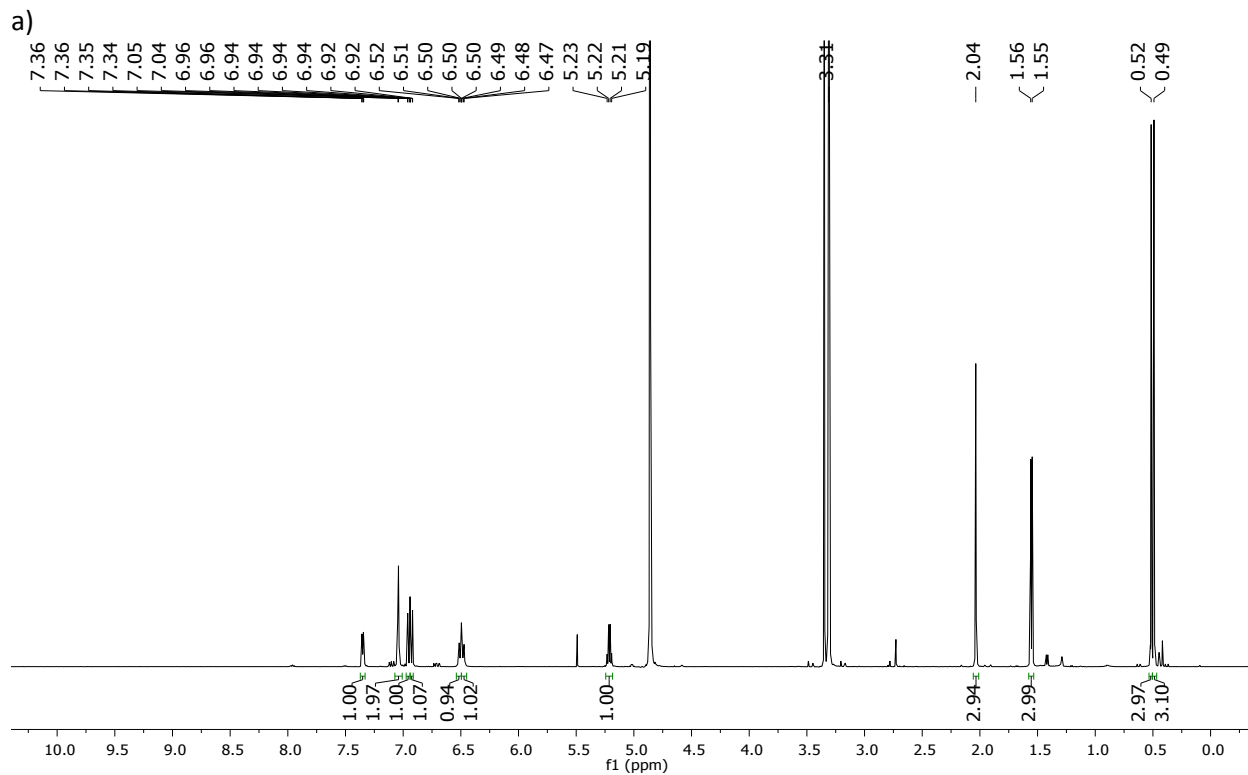


Figure S77. a) ^1H NMR and b) ^{13}C NMR spectra of **46**

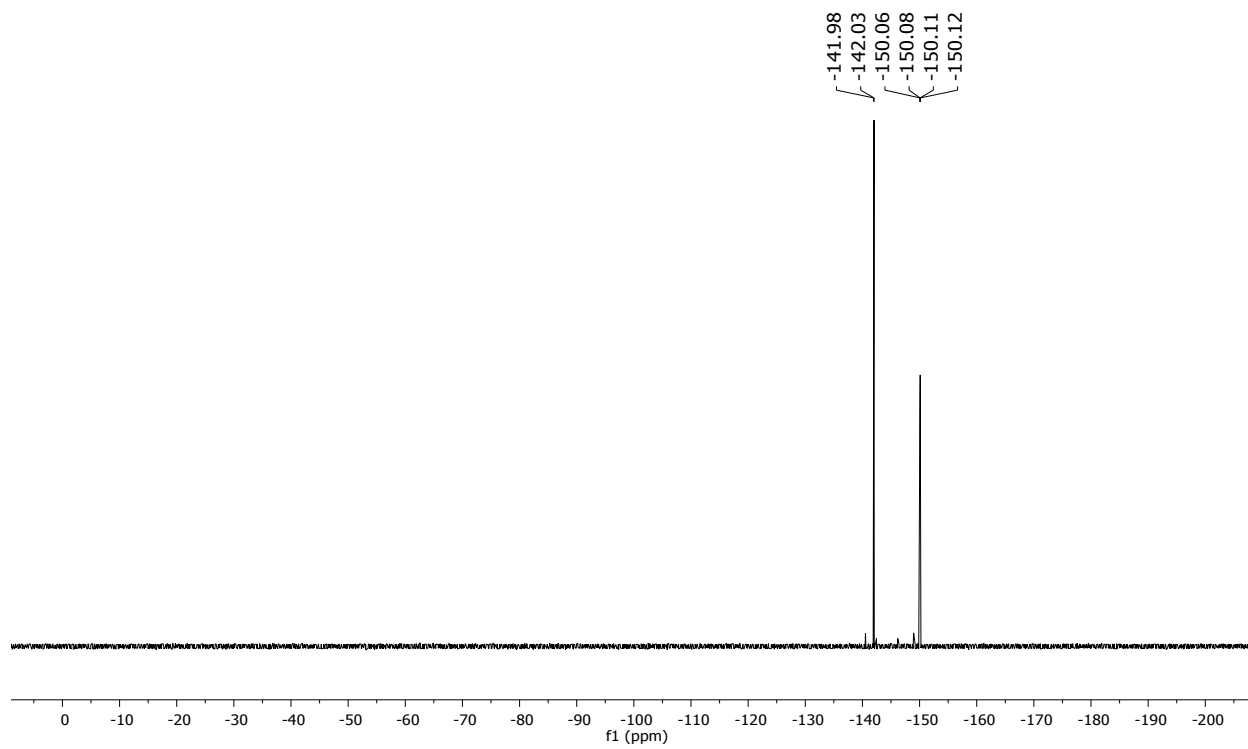


Figure S78. ^{19}F NMR spectrum of **46**

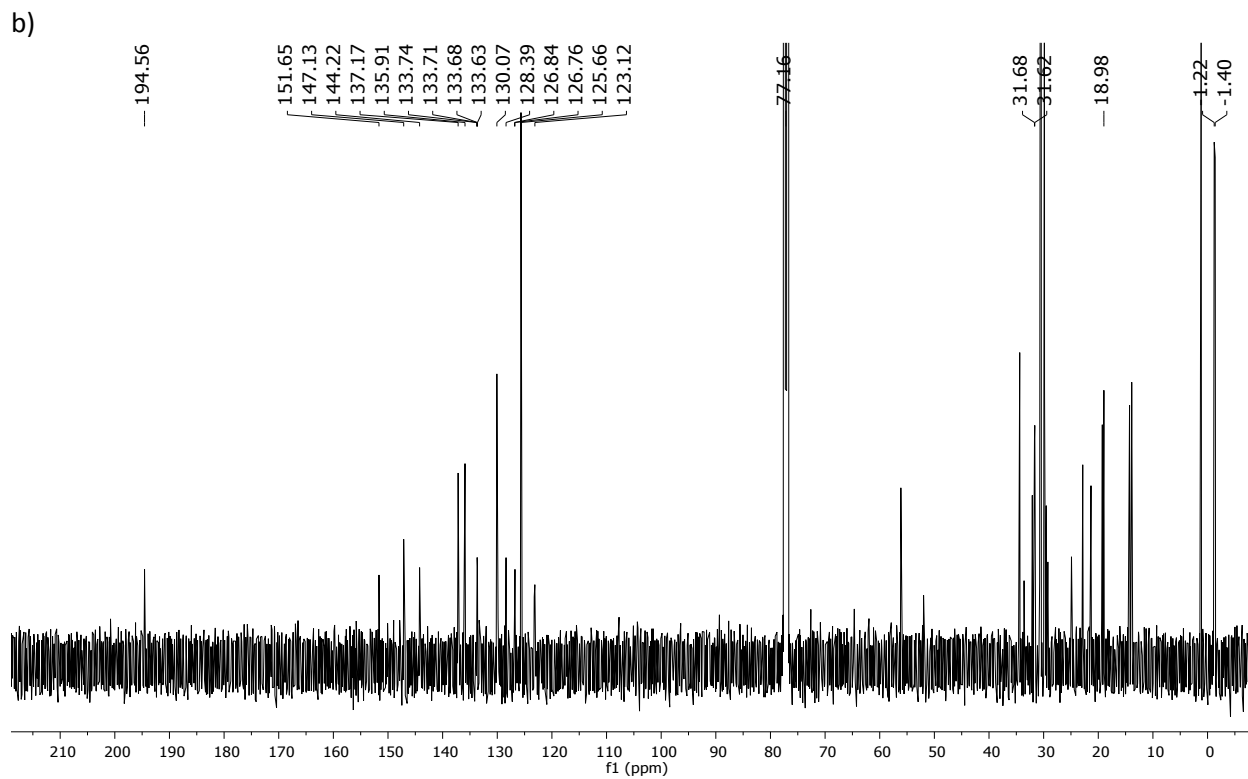
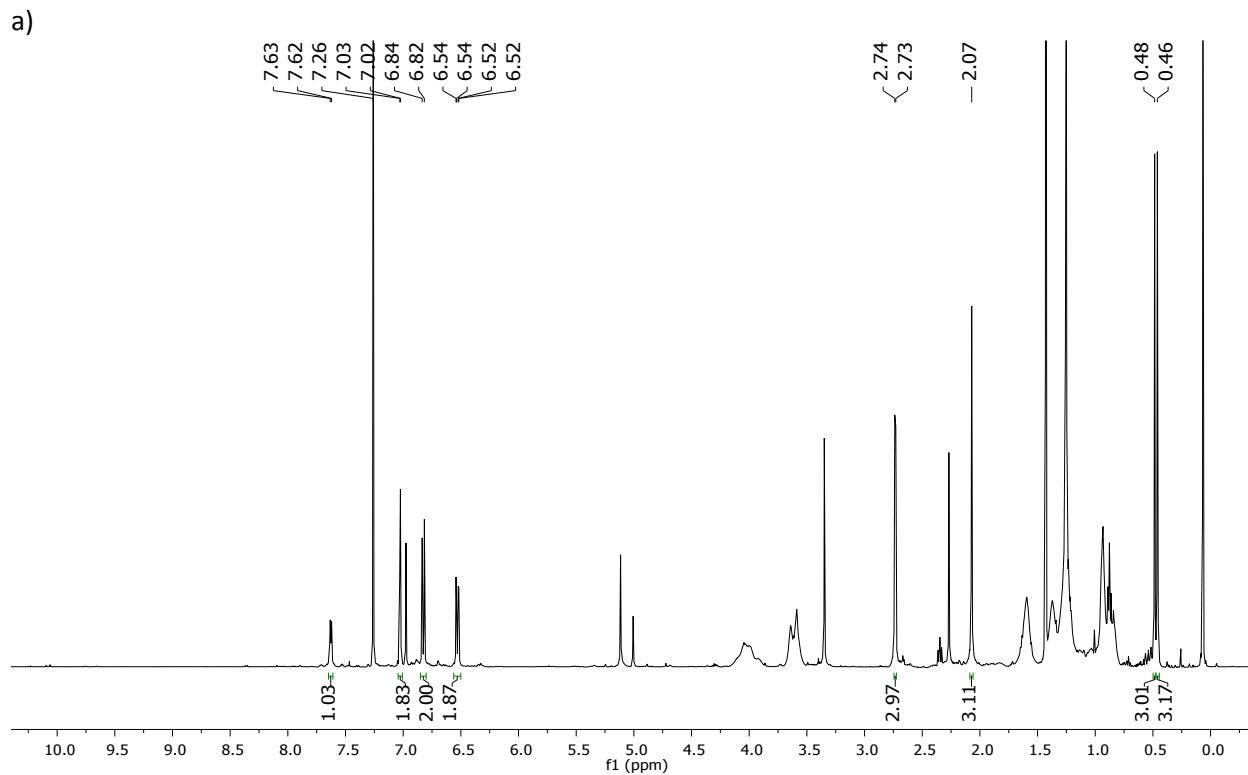


Figure S79. a) ^1H NMR and b) ^{13}C NMR spectra of Ctrl-red-AIDeSense

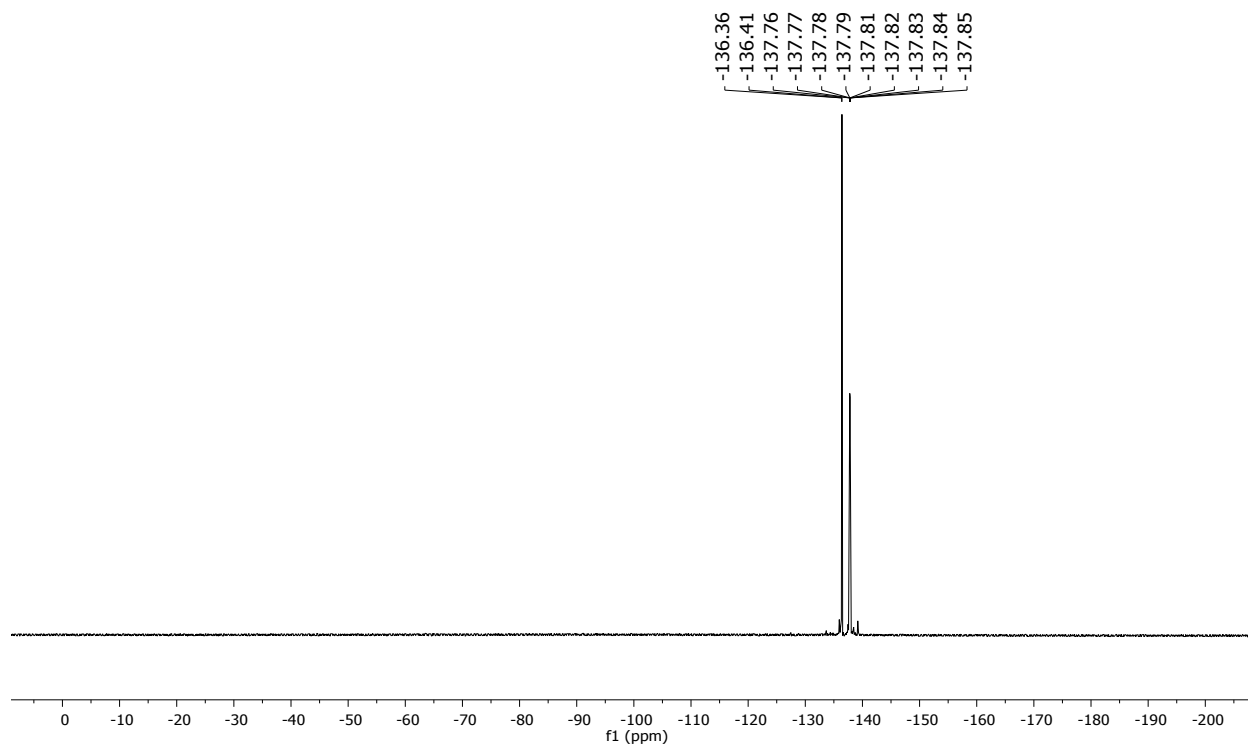


Figure S80. ^{19}F NMR spectrum of Ctrl-red-ALDeSense