

Supporting Information

For

A general approach to biocompatible branched fluororous tags for
increased solubility in perfluorocarbon solvents

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Supporting Tables, Schemes, and Figures.

Scheme S1.

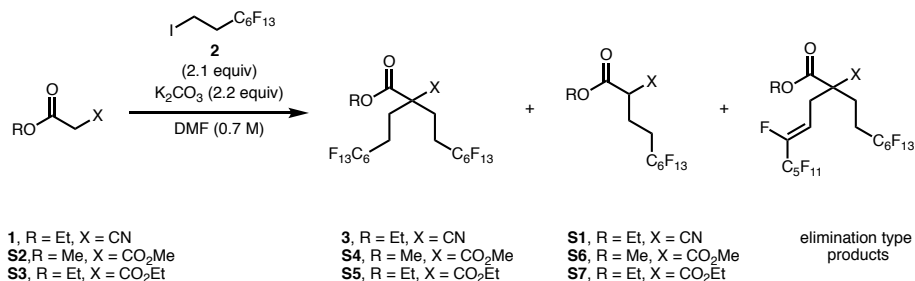


Table S1. Dialkylation and monoalkylation of different nucleophiles: ethyl cyanoacetate **1**, dimethylmalonate **S2**, and diethylmalonate **S3**. Percent conversion to alkylated products and elimination side products were determined by crude NMR using 1,3,5-trimethoxybenzene as an external standard for ¹H NMR. ^aDetermined by ¹⁹F NMR.

SM	time (h)	temp	di-alkylation	mono-alkylation	elimination
1	24	rt	3 , 97%	S1 , <2%	<1%
1	48	rt	3 , 97%	S1 , <2%	<1%
S2	72	rt	S4 , 26%	S6 , 74%	--
S3	72	rt	S5 , 30-40% ^a	S7 , 60-70% ^a	--

Scheme S2. Perfluorocarbon nanoemulsion formation using Pluronic F-68 as a surfactant.

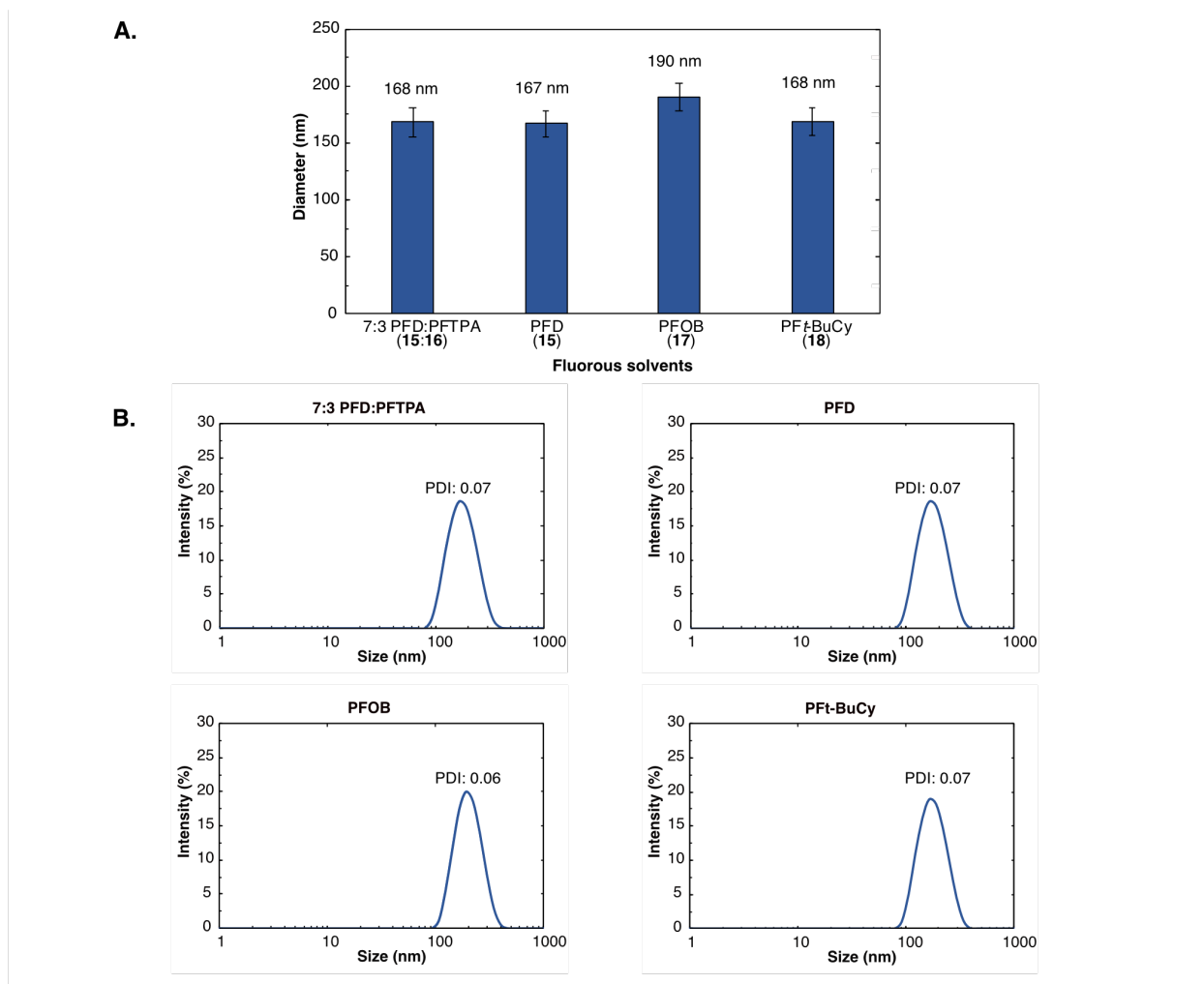
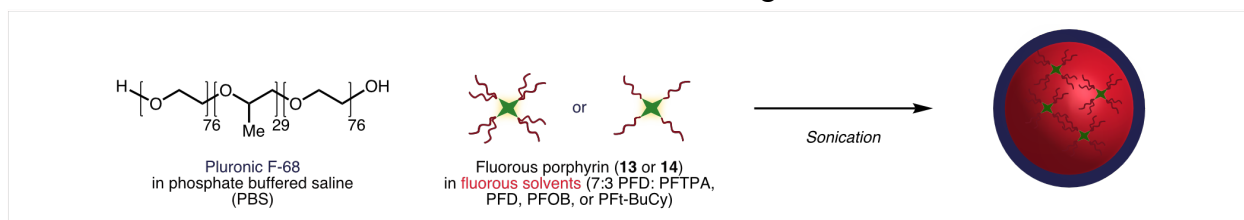


Figure S1. (A) Initial size distribution of PFC nanoemulsions composed of different fluoruous solvents. Emulsions were prepared by sonicating a solution of 2.8 wt% Pluronic F-68 in phosphate buffered saline (PBS) with 10 vol% fluoruous solvent (7:3 PFD **15**:PFTPA **16**, PFD **15**, PFOB **17**, or PFT-BuCy **18**). (B) Dynamic light scattering data for the PFC nanoemulsions stabilized by Pluronic F-68. Data is an average of three replicate measurements.

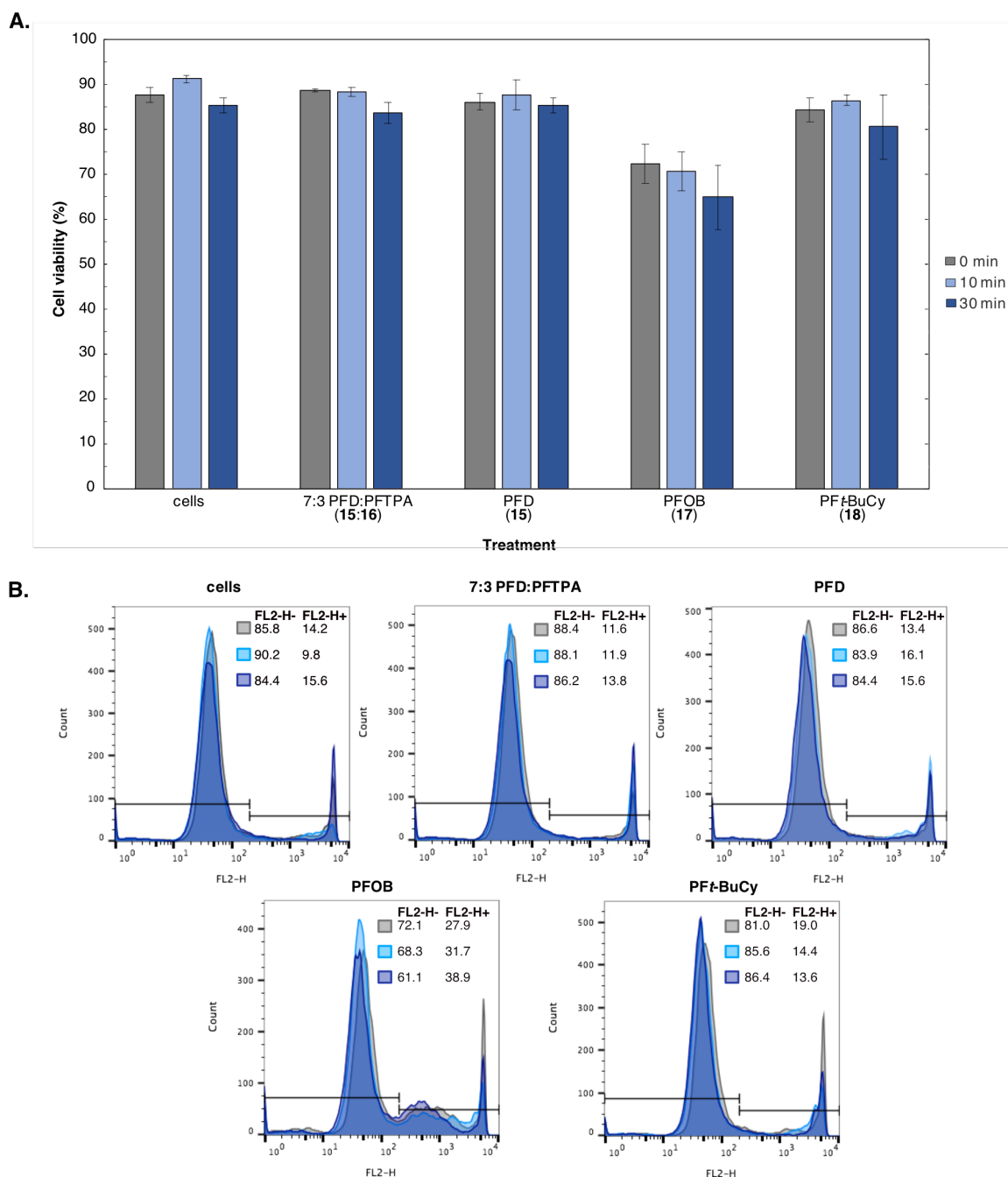


Figure S2. Photodynamic therapy with empty perfluorocarbon nanoemulsions composed of different fluoros solvents without photosensitizer **13**. Light treatment (420 nm LED, 8.5 mW/cm²) is indicated as follows: grey = 0 min, light blue = 10 min, dark blue = 30 min. (A) Cell viability with emulsions composed of different fluoros solvents (7:3 PFD **15**: PFTPA **16**, PFD **15**, PFOB **17**, PFt-BuCy **18**) and no photosensitizer. Error bars represent the standard deviation of 3 replicate samples. (B) Histograms for A375 cell viability flow cytometry data.

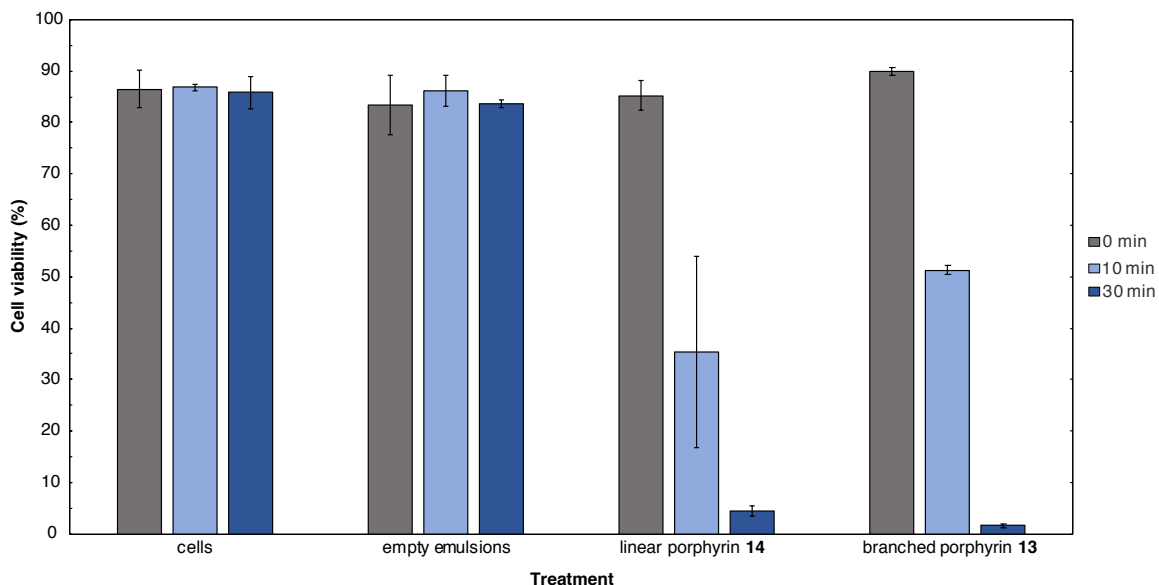


Figure S3. Cell viability comparison of photodynamic therapy efficiency of linear porphyrin **14** and branched porphyrin **13**. For comparison, emulsions composed of 7:3 PFD **15**: PFTPA **16** were employed as linear porphyrin **14** was not readily soluble in the other fluorosolvents. We found that linear porphyrin **14** and branched porphyrin **13** were similarly effective at inducing cell death when irradiated, suggesting that the branched tags do not affect the ability of porphyrin **13** to sensitize oxygen. Light treatment (420 nm LED, 8.5 mW/cm²) is indicated as follows: grey = 0 min, light blue = 10 min, dark blue = 30 min. Error bars represent the standard deviation of 2 replicate samples.

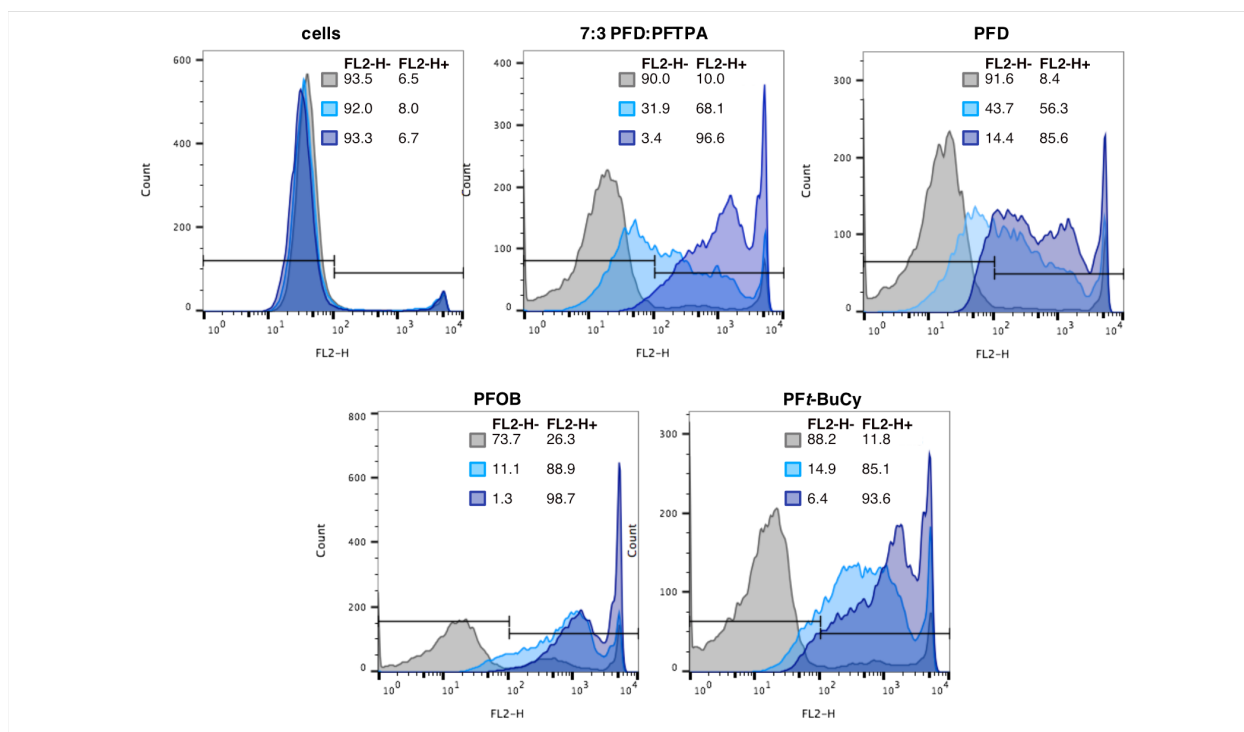


Figure S4. Histograms for flow cytometry data in Figure 3B. Light treatment (420 nm LED, 8.5 mW/cm²) is indicated as follows: grey = 0 min, light blue = 10 min, dark blue = 30 min.

General Experimental Procedures.

Chemical reagents were purchased from Sigma-Aldrich, Alfa Aesar, Fisher Scientific, Acros Organics, Synquest, or TCI and used without purification unless noted otherwise. Anhydrous DMSO was obtained from a Sure-Seal™ bottle (Aldrich). Anhydrous and deoxygenated solvents DCM, MeOH, THF, and DMF were dispensed from a Grubb's-type Phoenix Solvent Drying System. Anhydrous, but oxygenated Et₂O was prepared by drying over 4 Å molecular sieves for at least 3 days. Thin layer chromatography was performed using Silica Gel 60 F₂₅₄ (EMD Millipore) plates. Flash chromatography was executed with technical grade silica gel with 60 Å pores and 40 – 63 µm mesh particle size (Sorbtech Technologies). Solvent was removed under reduced pressure with a Büchi Rotovapor with a Welch self-cleaning dry vacuum pump and further dried with a Welch DuoSeal pump. Bath sonication was performed using a Branson 3800 ultrasonic cleaner. Emulsions were prepared using a QSonica (Q125) sonicator. Masses for analytical measurements were taken on a Sartorius MSE6.6S-000-DM Cubis Micro Balance. Size and surface charge were measured with Malvern Zetasizer Nano instrument. All ¹H, ¹³C, and ¹⁹F NMR spectra are reported in ppm and relative to residual solvent signals (¹H and ¹³C). Spectra were obtained on Bruker AV-300, AV-400, DRX-500, or AV-500 instruments and processed with MestReNova software. Mass spectra (electron impact (EI), electrospray ionization (ESI), and matrix assisted laser desorption ionization (MALDI)) were collected on either an Agilent 7890B-7250 Quadrupole Time-of-Flight GC/MS, Thermo Scientific Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ with Dionex UltiMate 3000 RSLCnano System, or Bruker Ultraflex MALDI TOF-TOF.

General photophysics procedures. Absorbance spectra were collected on a JASCO V-770 UV-Visible/NIR spectrophotometer with a 4000 nm/min scan rate after blanking with the appropriate solvent. Photoluminescence spectra were obtained on a Horiba Instruments PTI QuantaMaster Series fluorometer. Quartz cuvettes (1 cm) were used for absorbance and photoluminescence measurements unless otherwise noted.

All irradiation was performed with THORLabs M420L3 (420 nm) LED with SM1P25-A lens. Power was supplied with a KORAD KD3005D Digital-control DC Power Supply: 0-30V, 0-5A. Samples were placed 18 cm from the lens and kept in the center of the light. Power densities were measured with a FieldMate Laser Power Meter + Iris.

General nanoemulsions formation procedures.

Emulsions were prepared by predissolving **13** or **14** (for amounts see individual figure procedures) in fluorosolvent (10 vol%, 20 µL) in an S33 Eppendorf tube. Pluronic F-68 was predissolved in PBS pH 7.4 (28 mg/mL), and this solution (200 µL, 2.8 wt%) was added to the fluorosolvent. The mixture was sonicated at 35% amplitude for 90 seconds at 0 °C on a QSonica (Q125) sonicator. Sonication was performed by lowering the probe directly at the liquid-liquid interface of the two immiscible solvents, resulting in emulsions of sizes shown in Figure S1A without filtration.

General cell culture procedures. A375 cells were purchased from ATCC. A375 cells were cultured in Dulbecco's Modified Eagle Media (Life Technologies, cat# 11995073) supplemented with 10% fetal bovine serum (Corning, lot# 35016109) and 1% penicillin-streptomycin (Life

Technologies, cat# 15070063). Cells were washed with PBS, or PBS supplemented with 1% fetal bovine serum (FACS buffer). Cells were incubated at 37 °C, 5% CO₂, during treatments and throughout culturing, in HERACell 150i CO₂ incubators. Cells were pelleted through use of Sorvall ST 40R centrifuge. All cell work was performed in 1300 Series A2 biosafety cabinets.

For cell viability experiments: following incubation, cells were washed three times by centrifugation (526xg, 3 min, 4 °C). Propidium iodide solution (1 µL of 0.25 mg/mL in DI H₂O) was added to each well. Cells were transferred to FACS tubes with a final volume of 300 µL FACS buffer (PBS + 1% FBS). Cells were incubated on ice for 15 minutes prior to flow cytometry measurement. Propidium iodide fluorescence was measured on FL2 channel. Data was analyzed by splitting the population at 10² as a live/dead line. Flow cytometry was performed on a BDBiosciences FACSCalibur equipped with 488 nm and 635 nm lasers.

Abbreviations:

DCM = dichloromethane; DMF = dimethylformamide; DMSO = dimethylsulfoxide; EtOH = ethanol; EtOAc = ethyl acetate; Et₂O = diethyl ether; FACS = phosphate buffered saline supplemented with 1% fetal bovine serum; MeOH = methanol; PBS = phosphate buffered saline; PFD = perfluorodecalin; PFTPA = perfluorotripropylamine; PFOB = perfluorooctylbromide; PF*t*-BuCy = perfluoro(*tert*-butylcyclohexane); TEA = triethylamine.

Synthetic experimental procedures

2,2-bis(perfluorohexyl)ethyl ethyl cyanoacetate (3). To perfluorohexyl ethyl iodide (0.65 mL, 2.6 mmol, 2.1 equiv), was added ethyl cyanoacetate (135 μ L, 1.27 mmol, 1.0 equiv), freshly powdered and flame dried K_2CO_3 (388 mg, 2.80 mmol, 2.2 equiv), and anhydrous DMF (1.8 mL, 0.70 M). The vial was sealed under nitrogen and stirred at rt. Note: if the reactants begin to solidify, the mixture can be warmed to 30 °C without formation of significant byproducts. After 72 h, the reaction mixture was evaporated to dryness. The resulting solids were dissolved in acetone and absorbed onto SiO_2 . The crude product was purified by column chromatography (silica gel, 98% \rightarrow 95% \rightarrow 90% pentane/ Et_2O) to afford **3** as a white solid (922 mg, 1.14 mmol, 90%). R_f = 0.8 in 20% EtOAc/hexanes. 1H NMR (400 MHz, $CDCl_3$): δ 4.36 (q, J = 7.1 Hz, 2H), 2.49 – 2.08 (m, 8H), 1.36 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.0, 117.1, 64.1, 47.7, 28.3, 27.6 (t, J_{CF} = 22.1 Hz), 14.1. ^{19}F NMR (376 MHz, $CDCl_3$): δ -80.7 (t, J = 9.8 Hz, 6F), -114.2 (m, 4F), -122.0 (m, 4F), -122.7 (m, 4F), -123.2 (m, 4F), -126.1 (m, 4F). HRMS (ESI) calcd. for $C_{21}H_{13}F_{26}NO_2Cl^-$ [$M + Cl$] $^-$: 840.0225; found 840.0221.

2,2-bis(perfluorohexyl)ethyl nitrile (4). To **3** (307 mg, 0.382 mmol, 1.0 equiv), was added lithium chloride (56 mg, 1.3 mmol, 3.5 equiv), DMSO (3.0 mL, 0.13 M), and H_2O (1.0 mL). The reaction mixture was heated to 160 °C and stirred for 12 h. The reaction mixture was allowed to cool to room temperature, and product crashes out of solution. The crude product was rinsed with water, dissolved in EtOAc, and evaporated to dryness. The crude product was dissolved in EtOAc, absorbed onto SiO_2 , and purified by column chromatography (silica gel, 97% \rightarrow 95% hexanes/EtOAc) to afford **4** as a white solid (201 mg, 0.274 mmol, 72%). R_f = 0.3 in 10% DCM/hexanes. 1H NMR (500 MHz, $CDCl_3$): δ 2.78 (sep, J = 5.0 Hz, 1H), 2.52 – 2.18 (m, 4H), 2.07 – 1.91 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 119.8–108.0 (m, CF_n peaks), 119.3 (CN), 30.8, 28.7 (t, J_{CF} = 22.6 Hz), 23.6. ^{19}F NMR (376 MHz, $CDCl_3$): δ -80.8 (t, J = 9.9 Hz, 6F), -112.7 – -115.9 (m, 4F), -121.8 (bs, 4F), -122.8 (bs, 4F), -123.3 (bs, 4F), -126.1 (bs, 4F). HRMS (EI) calcd. for $C_{18}H_8F_{25}N^+$ [$M - HF$] $^+$: 713.0252; found 713.0256.

2,2-bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)ethylamine (5). To nitrile **4** (96 mg, 0.13 mmol, 1.0 equiv) was added dry Et_2O (5.5 mL, 0.02 M) under nitrogen. Nitrile **4** was stirred until it was fully dissolved, and then cooled to 0 °C. Lithium aluminum hydride (1.0 M in THF, 0.26 mmol, 2.0 equiv) was added. After 1 h, the reaction was let warm to room temperature and stirred for 5 h. The reaction was quenched by dropwise addition of H_2O with stirring, and then extracted with EtOAc, dried ($MgSO_4$), filtered, and evaporated to dryness. The crude mixture was evaporated onto SiO_2 from EtOAc and purified by column chromatography (silica gel, 100% DCM \rightarrow 1% MeOH/DCM \rightarrow 5% MeOH/DCM (all with 1% TEA)) to provide the product **5** as a yellow oil (29 mg, 0.04 mmol, 30%). R_f = 0.5 in 10% MeOH/DCM. 1H NMR (500 MHz, MeOD- d_4): δ 2.65 (d, J = 5.5 Hz, 2H), 2.28 – 2.15 (m, 4H), 1.79 – 1.48 (m, 5H). ^{13}C NMR (126 MHz, MeOD- d_4): δ 122.2 – 109.8 (CF_n), 44.6, 40.3, 29.02 (t, J_{CF} = 22.1 Hz), 22.42. ^{19}F NMR (282 MHz, MeOD- d_4): δ -83.9 – -84.1 (m, 6F), -116.7 – -117.4 (m, 4F), -124.5 (bs, 4F), -125.4 (bs, 4F), -126.0 (bs, 4F), -127.9 – -130.4 (m, 4F). HRMS (ESI) calcd. for $C_{18}H_{14}F_{26}N^+$ [$M + H$] $^+$: 738.0706; found 738.0684.

2,2-bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-1-ethanal (6). To nitrile **4** (30 mg, 0.04 mmol, 1.0 equiv) dissolved in dry Et₂O (2.0 mL, 0.02 M) at -78 °C, DIBAL-H (1.0 M in Hexanes, 0.09 mmol, 2.3 equiv) was added dropwise. After 2 h, additional DIBAL-H (45 μL, 1.2 equiv) was added, and the reaction was stirred for 2 h more at -78 °C. EtOAc (2 mL) was added slowly, then a saturated aq Rochelle salt solution (2 mL) was added, and the mixture was stirred at room temperature until there were two clear layers (~ 1 h). The aqueous layer was extracted with additional EtOAc (2 x 3 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated. The crude mixture was dissolved in EtOAc (2 mL) and stirred with 1 M HCl (2 mL) to hydrolyze any remaining imine. The organic layer was separated, evaporated onto SiO₂, and purified by column chromatography (silica gel, 3% DCM/hexanes → 5% DCM/hexanes → 10% DCM/hexanes). This procedure yielded the aldehyde **6** as a white solid (14 mg, 0.02 mmol, 47% yield). R_f = 0.5 in 20% DCM/hexanes. ¹H NMR (500 MHz, CDCl₃): δ 9.68 (d, *J* = 1.6 Hz, 1H), 2.56 – 2.42 (m, 1H), 2.24 – 1.99 (m, 6H), 1.86 – 1.73 (m, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 201.6, 49.6, 28.2 (t, *J*_{CF} = 22.4 Hz), 19.2. ¹⁹F NMR (376 MHz, CDCl₃): δ -80.8 (t, *J* = 10.0 Hz, 6F), -114.6 (bs, 4F), -121.9 (bs, 4F), -122.8 (bs, 4F), -123.4 (bs, 4F), -126.0 – -126.3 (m, 4F). HRMS (ESI) calcd. for C₁₈H₉F₂₆O⁻ (M – H)⁻: 735.0244; found 735.0239.

2,2-bis((perfluorohexyl)ethyl)-1-carboxylic acid (7). To ethyl cyanoacetate **3** (1.39 g, 1.73 mmol, 1.00 equiv.) and powdered KOH (297 mg, 5.29 mmol, 3.1 equiv), absolute ethanol (21 mL, 0.08 M) and H₂O (21 mL, 0.08 M) were added. The reaction was heated to 80 °C. After 3 h, 1 M HCl (~20 mL) was added, and the mixture was extracted with Et₂O (3 x 60 mL). The organics were washed with brine, dried (MgSO₄), and evaporated to dryness to yield branched fluoruous cyanoacid as a white solid (1.26 g, 1.61 mmol, 93% crude yield). Branched fluoruous cyanoacid (938 mg, 1.20 mmol) suspended in conc. HCl (27 mL, 0.04 M) was stirred and heated to 100 °C for 2 h, then cooled to 0 °C, and conc. H₂SO₄ (27 mL, 0.04 M) was added slowly. The reaction mixture was heated to 160 °C. Note: reaction is connected to a base trap before venting into a fume hood. After 14 h, the reaction was stopped, diluted with H₂O, and extracted with Et₂O (4 x 50 mL). The organics were dried (MgSO₄), filtered, and concentrated to yield **7** as an off-white solid (832 mg, 1.11 mmol, 92%). From **3**, the two step yield is 89%. R_f = 0.05 in 30% Et₂O/pentane (streaks). ¹H NMR (500 MHz, MeOD-*d*₄): δ 2.55 (sep, *J* = 5.0 Hz, 1H), 2.32 – 2.17 (m, 4H), 1.99 – 1.79 (m, 4H). ¹³C NMR (101 MHz, MeOD-*d*₄): δ 177.2, 44.5, 29.6 (t, *J*_{CF} = 22.6 Hz), 23.7. ¹⁹F NMR (376 MHz, MeOD-*d*₄): δ -82.5 (tt, *J* = 10.2, 2.7 Hz, 6F), -114.9 – -116.4 (m, 4F), -123.0 (bs, 4F), -123.9 (bs, 4F), -124.6 (bs, 4F), -127.3 – -127.4 (m, 4F). HRMS (ESI) calcd. for C₁₈H₉F₂₆O₂⁻ [M – H]⁻: 751.0193; found 751.0183.

2,2-bis((perfluorohexyl)ethyl)-1-ethanol (8). To lithium aluminum hydride (1.0 M in THF, 3.6 mmol, 2.4 equiv) in Et₂O (15 mL), was added branched fluoruous carboxylic acid **7** (1.11 g, 1.47 mmol, 1.00 equiv) in Et₂O (15 mL) slowly under nitrogen. After 3.5 h, the reaction was quenched by slow addition of H₂O and saturated NaHCO₃, and then extracted with Et₂O (4 x 20 mL). The organics were dried (MgSO₄), filtered, and concentrated. Crude reaction mixture was absorbed onto SiO₂ and purified by flash column chromatography (silica gel, 15% Et₂O/pentane) to provide the product **8** as a colorless oil (981 mg, 1.33 mmol, 90%). R_f = 0.4 in 30% Et₂O/pentane. ¹H NMR (500 MHz, CDCl₃): δ 3.64 (apparent t, *J* = 4.3 Hz, 2H), 2.29 – 1.99 (m, 4H), 1.78 – 1.62 (m, 5H), 1.44 (t, *J* = 4.6 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃): δ 120.8 – 106.3

(CF_n), 64.4, 39.1, 28.6 (t, $J_{CF} = 22.4$ Hz), 21.6. ¹⁹F NMR (376 MHz, CDCl₃): δ -80.9 (tt, $J = 10.0, 5.2$ Hz, 6F), -114.6 – -114.7 (m, 4F), -122.0 (bs, 4F), -122.9 (bs, 4F), -123.5 (bs, 4F), -126.2 – -126.3 (m, 4F). HRMS (ESI) calcd. for C₁₈H₁₂F₂₆OCl [M + Cl]⁻: 773.0167; found 773.0165.

2,2-bis((perfluorohexyl)ethyl)-1-ethyl tosylate (9). Tosylate **9** was synthesized using a procedure adapted from Yoshida, et. al.¹ To alcohol **8** (453 mg, 0.614 mmol, 1.00 equiv), was added DCM (3 mL, 0.2 M), triethylamine (0.21 mL, 1.53 mmol, 2.49 equiv), and trimethylamine hydrochloride (60 mg, 0.61 mmol, 1.0 equiv). The reaction mixture was stirred vigorously, cooled to 0 °C, and tosyl chloride (175 mg, 0.921 mmol, 1.50 equiv) was added in DCM (1.9 mL). The reaction was let warm to room temperature and stirred for 12 h. The organics were washed with H₂O (2x) and brine (2x), dried (MgSO₄), filtered, and evaporated to dryness. The crude product was evaporated onto silica from DCM and purified by column chromatography (silica gel, 50% toluene/hexanes) to afford **9** as a white solid (481 mg, 0.539 mmol, 88%). $R_f = 0.2$ in 10% EtOAc/hexanes. ¹H NMR (500 MHz, CDCl₃): δ 7.79 (d, $J = 8.0$ Hz, 2H), 7.37 (d, $J = 8.0$ Hz, 2H), 3.98 (d, $J = 4.5$ Hz, 2H), 2.45 (s, 3H), 2.04 – 1.95 (m, 4H), 1.83 – 1.76 (m, 1H), 1.66 – 1.62 (m, 4H). ¹³C NMR (101 MHz, CDCl₃): δ 145.5, 132.5, 130.2, 128.0, 70.3, 36.7, 28.1 (t, $J_{CF} = 22.4$ Hz), 21.7, 21.5. ¹⁹F NMR (376 MHz, CDCl₃) δ -80.8 (t, $J = 10.0$ Hz, 6F), -114.5 – -114.6 (m, 4F), -121.9 (bs, 4F), -122.8 (bs, 4F), -123.4 (bs, 4F), -126.0 – -126.2 (m, 4F). HRMS (EI) calcd. for C₂₅H₁₈F₂₆O₃S⁺ [M]⁺: 892.0561; found 892.0560.

2,2-bis((perfluorohexyl)ethyl)-1-ethyl azide (10). To tosylate **9** (60 mg, 0.07 mmol, 1.0 equiv.) was added sodium azide (11 mg, 0.17 mmol, 2.5 equiv) and DMF (3 mL, 0.02 M). The mixture was heated to 80 °C. After 12 h, the reaction mixture was evaporated to dryness. The crude product was taken up in DCM and filtered, to provide the product **10** as a colorless oil (47 mg, 0.060 mmol, 92%). $R_f = 0.6$ in 10% EtOAc/hexanes. ¹H NMR (500 MHz, CDCl₃): δ 3.38 (d, $J = 4.2$ Hz, 2H), 2.19 – 2.05 (m, 4H), 1.74 – 1.64 (m, 5H). ¹³C NMR (101 MHz, CDCl₃): δ 54.1, 37.1, 28.3 (t, $J_{CF} = 22.6$ Hz), 22.3. ¹⁹F NMR (282 MHz, CDCl₃): δ -80.7 – -80.8 (m, 6F), -114.4 – -114.6 (m, 4F), -121.9 (bs, 4F), -122.9 (bs, 4F), -123.4 (bs, 4F), -126.1 – -126.2 (m, 4F). HRMS (EI) calcd. for C₁₈H₁₁F₂₆N⁺ [M – N₂]⁺: 735.0471; found 735.0491.

2,2-bis((perfluorohexyl)ethyl)-1-ethanethiol (11). To tosylate **9** (169 mg, 0.190 mmol, 1.00 equiv), was added DMF (5.6 mL, 0.03 M) and potassium thioacetate (38 mg, 0.33 mmol, 1.7 equiv). The reaction was heated to 80 °C and stirred for 4 h. The reaction mixture was evaporated to dryness, taken up in DCM, filtered, and concentrated to yield an off-white solid (135 mg, 0.169 mmol, 90% crude yield). Branched fluororous thioacetate (122 mg, 0.150 mmol) was dissolved in MeOH (1.0 mL, 0.2 M) and under nitrogen, acetyl chloride (80 μ L, 1.0 mmol, 7.5 equiv) was added dropwise. The reaction mixture was stirred at 65 °C for 5.5 h, and then evaporated to dryness. The crude product was absorbed onto silica and purified by column chromatography (silica gel, 1% Et₂O/pentane → 5% Et₂O/pentane) to afford the product **11** as a colorless oil (105 mg, 0.139 mmol, 91%). The two step yield from **9** is 82%. $R_f = 0.6$ in 10% EtOAc/hexanes. ¹H NMR (500 MHz, CDCl₃): δ 2.60 (dd, $J = 8.2, 4.7$ Hz, 2H), 2.17 – 1.99 (m, 4H), 1.82 – 1.65 (m, 5H), 1.26 (t, $J = 8.2$ Hz, 1H). ¹³C NMR (101 MHz, CDCl₃): δ 39.0, 28.3 (t, $J_{CF} = 22.3$ Hz), 27.5, 22.7. ¹⁹F NMR (376 MHz, CDCl₃): δ -80.8 (t, $J = 10.0$ Hz, 6F), -114.4 – -114.5 (m, 4F), -121.9 (bs, 4F), -122.9 (bs, 4F), -123.4 (bs, 4F), -126.1 – -126.2 (m, 4F). HRMS

(EI) calcd. for $C_{18}H_{12}F_{26}S^+$ [M]⁺: 754.0245; found 754.0262.

5,10,15,20-tetrakis(4-(2,2-bis(perfluorohexylethyl)ethyl-thio)-2,3,5,6-tetrafluorophenyl)porphyrin (13). Procedure was adapted from Tüxen, et. al.² Branched thiol **11** (104 mg, 0.138 mmol, 10.0 equiv) was dissolved in DMF (2.0 mL), EtOAc (0.5 mL), and diethylamine (41 μ L, 0.40 mmol, 28.5 equiv). To this mixture, 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin **12** (13.4 mg, 0.0140 mmol, 1.0 equiv) dissolved in DMF (1.4 mL) was added. The reaction mixture was stirred for 17 h at room temperature, at which point the product had precipitated from solution. The mixture was filtered, and the precipitate was dissolved in chloroform. The crude product was evaporated onto silica from acetone and purified by column chromatography (silica gel, 3% \rightarrow 4% \rightarrow 5% \rightarrow 10% acetone/pentane) to afford the product **13** as a dark purple solid (46 mg, 0.12 mmol, 85%). R_f = 0.4 in 20% in acetone/hexanes. ¹H NMR (500 MHz, CDCl₃): δ 8.87 (s, 8H), 3.29 (d, J = 4.7 Hz, 8H), 2.27 – 2.17 (m, 16H), 2.07 – 1.89 (m, 20H), -2.89 (s, 2H). ¹⁹F NMR (282 MHz, CDCl₃): δ -80.9 (t, J = 9.8 Hz, 24F), -114.2 – -114.4 (m, 16F), -121.8 (bs, 16F), -122.9 (bs, 16F), -123.3 (bs, 16F), -126.1 – -126.3 (m, 16F), -134.0 (dd, J = 24.9, 12.3 Hz, 8F), -136.5 (dd, J = 24.8, 12.2 Hz, 8F). HRMS (MALDI): calcd for $C_{116}H_{54}F_{120}N_4S_4^+$ (M + H)⁺: 3911.1388; found 3911.2753. Absorbance (7:3 PFD **15**:PFTPA **16**): 408, 502, 584 nm. Emission (7:3 PFD **15**:PFTPA **16**, Ex 410 nm): 651, 709 nm.

Figure experimental procedures.

Figure 2B. Porphyrins **13** and **14** were added to individual dram vials from stock solutions (1.0 mg/mL) in acetone, solvent was removed, and porphyrins were dried on high vacuum (<100 mTorr). Fluorous porphyrins **13** (0.02 mg, 0.07 mmol) and **14** (0.03 mg, 0.07 mmol) were dissolved in perfluoromethylcyclohexane (1.5 mL) and then sonicated for 1 min. Toluene (1.5 mL) was added. The dram vials containing each porphyrin were allowed to roll freely on a KJ-201BD Orbital shaker for 22 h. Aliquots (200 μ L) from each layer (toluene and perfluoromethylcyclohexane) were dried on high vacuum to remove residual solvent. Samples were diluted in DMF (5.0 mL). Absorbance values were obtained on a JASCO V-770 UV-Visible/NIR spectrophotometer with a 2000 nm/min scan rate. The fluorous partition coefficient was determined by taking the ratio of the absorbance in perfluoromethylcyclohexane and toluene. Photographs were taken under long-wave UV light (UVGL-25, 365 nm).

Figure 2D. Emulsions were prepared as described in general nanoemulsion formation procedure with **13** (0.14 mg, 0.04 μ mol) or **14** (0.13 mg, 0.04 μ mol) in 7:3 PFD/PFTPA (10 vol%, 20 μ L). The emulsions (200 μ L) were diluted to 5 mL in PBS. A portion of these solutions (1 mL) was taken and placed in the presence of 1-octanol (0.5 mL) in an Eppendorf tube. The biphasic samples were then rocked for 2 weeks. The Eppendorf tubes containing the samples were allowed to roll freely on a KJ-201BD Orbital shaker at 20 rpm. The fluorescence of the 1-octanol layer was monitored over time to determine the amount of **13** and **14** that diffused out of the emulsions. Emission values were obtained by taking an aliquot (200 μ L) of the 1-octanol in a 0.3 mL quartz cuvette and measuring the fluorescence on a Horiba Instruments PTI QuantaMaster Series fluorometer with the following settings: slits 5 nm, step size 1 nm, integration time 0.1 s (Ex 410 nm, collection 450-800 nm). The 1-octanol was returned to the sample after the measurement.

Figure 2E. Porphyrins **13** and **14** (0.36 mmol) were dissolved in the indicated fluororous solvents (1 mL) through inversion or minimal sonication (<10 min, bath sonicator). Photographs were taken under visible light.

Figure 3B/Figure S4. Emulsions with fluororous solvents (7:3 PFD **15** :PFTPA **16**, PFD **15**, PFOB **17**, or PF*t*-BuCy **18**) were prepared as described in the general nanoemulsion formation procedure with porphyrin **13** (0.04 mg, 0.01 μ mol). A375 cells were placed in a 96-well plate (200,000 cells per 150 μ L/well). The emulsions were diluted in FACS buffer (200 μ L emulsions in 1 mL, PBS buffer + 1% FBS). Cells suspended in 150 μ L media were treated with 50 μ L of the diluted emulsions. Cells were incubated in the presence of emulsions (37 $^{\circ}$ C, 5% CO₂, 3 h). Cells were washed by centrifugation (3x, 526xg, 3 min) and resuspended in FACS buffer (PBS with 1% FBS) to a final volume of 200 μ L. Cells were irradiated (30 min or 10 min, 420 nm LED, 8.5 mW/cm²). After irradiation, the cells were diluted to 300 μ L with FACS buffer and incubated (0 $^{\circ}$ C, 15 min) with propidium iodide (1 μ L, 0.25 mg/ml solution). Cell death was analyzed by FL2 channel on FACSCalibur. 15,000 cells were collected per sample.

Figure S1. Empty emulsions were prepared as described in the general nanoemulsion formation procedure by sonicating fluororous solvent (10 vol%, 20 μ L, 7:3 PFD **15**:PFTPA **16**, PFD **15**, PFOB **17**, or PF*t*-BuCy **18**) with Pluronic F-68 predissolved in PBS pH 7.4 (28 mg/mL, 2.8 wt%, 200 μ L). Emulsions were diluted in MilliQ H₂O (20 μ L emulsions in 2 mL MilliQ H₂O, Ω = -18 mV) in a plastic cuvette. The size was monitored by dynamic light scattering (Malvern Zetasizer Nano) and data is representative of three replicate measurements. Error bars represent the standard deviation of the product of the Z_{average} and the polydispersity index of three measurements.

Figure S2. Cell viability with empty perfluorocarbon nanoemulsions was determined using the same procedure described for Figure 3B.

Figure S3. Cell viability comparison with **14** (7 nmol) and **13** (7 nmol) in perfluorocarbon nanoemulsions composed of 7:3 PFD **15**:PFTPA **16** was determined using the same procedure described for Figure 3B. Less porphyrin was used due to the lower solubility of **14** in 7:3 PFD:PFTPA.

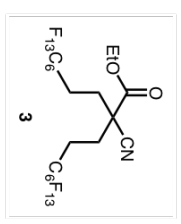
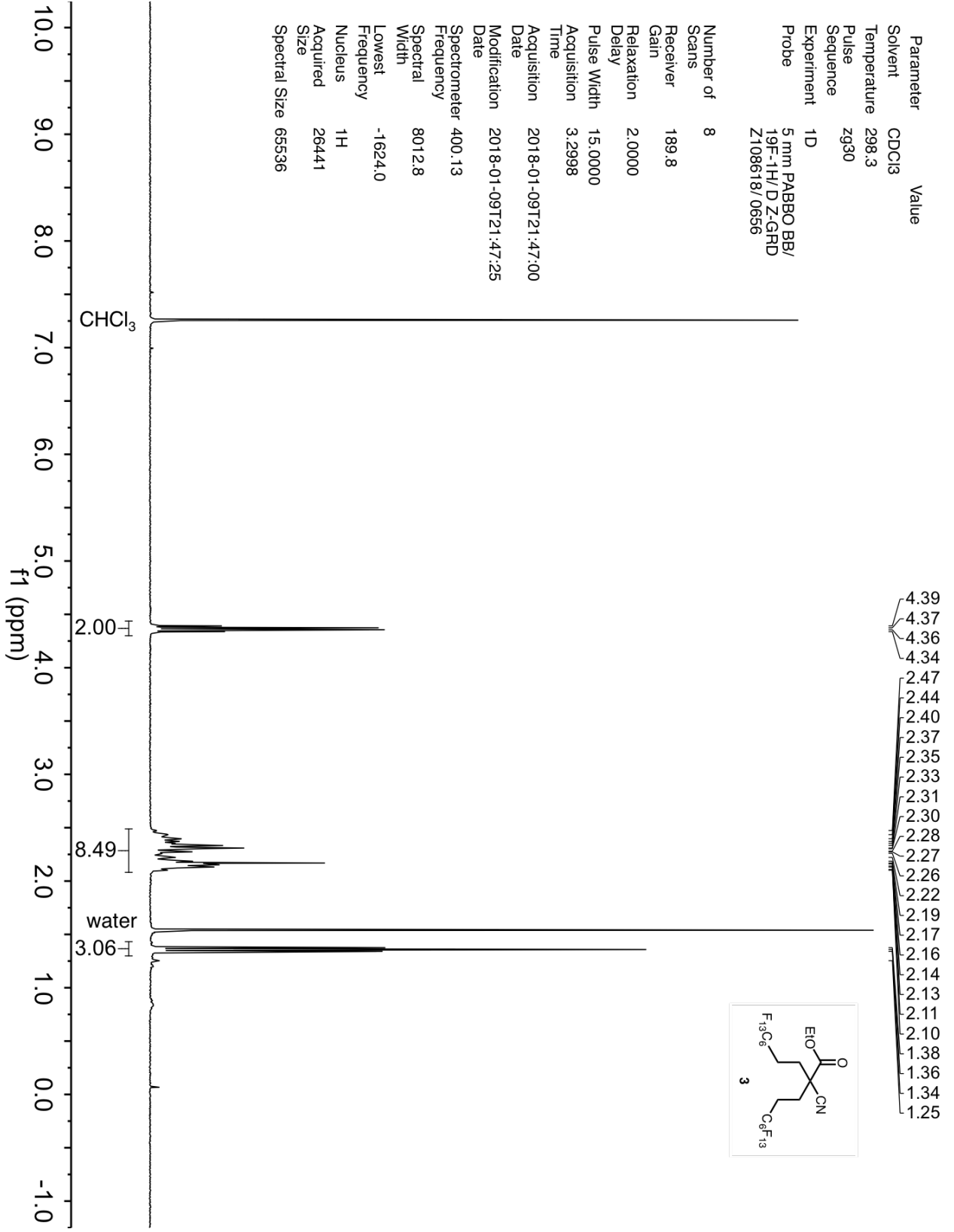
Supplemental references.

- (1) Yoshida, Y.; Sakakura, Y.; Aso, N.; Okada, S.; Tanabe, Y. *Tetrahedron* **1999**, *55*, 2183-2192.
- (2) Tüxen, J.; Eibenberger, S.; Gerlich, S.; Arndt, M.; Mayor, M. *Eur. J. Org. Chem.* **2011**, 4823-4833.

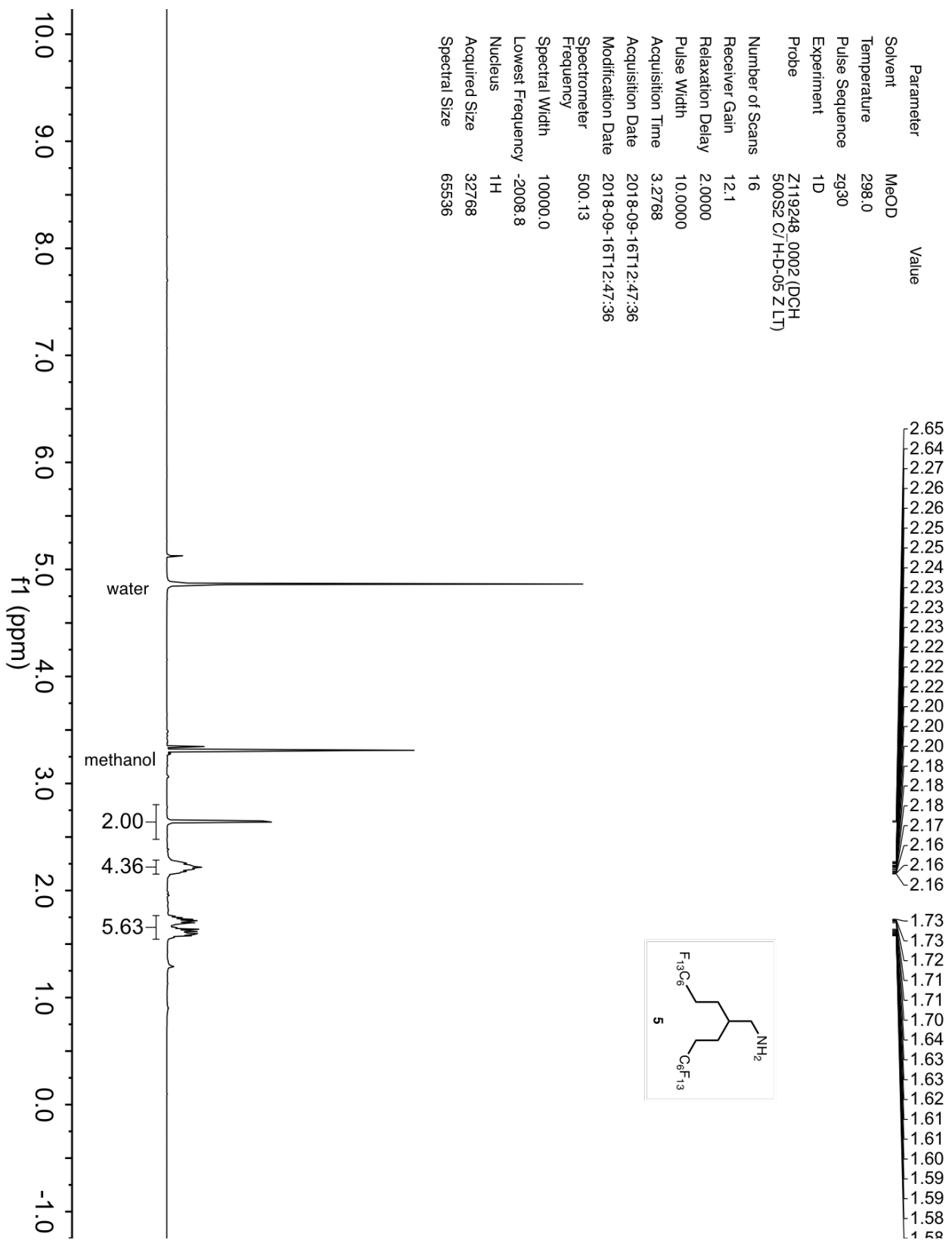
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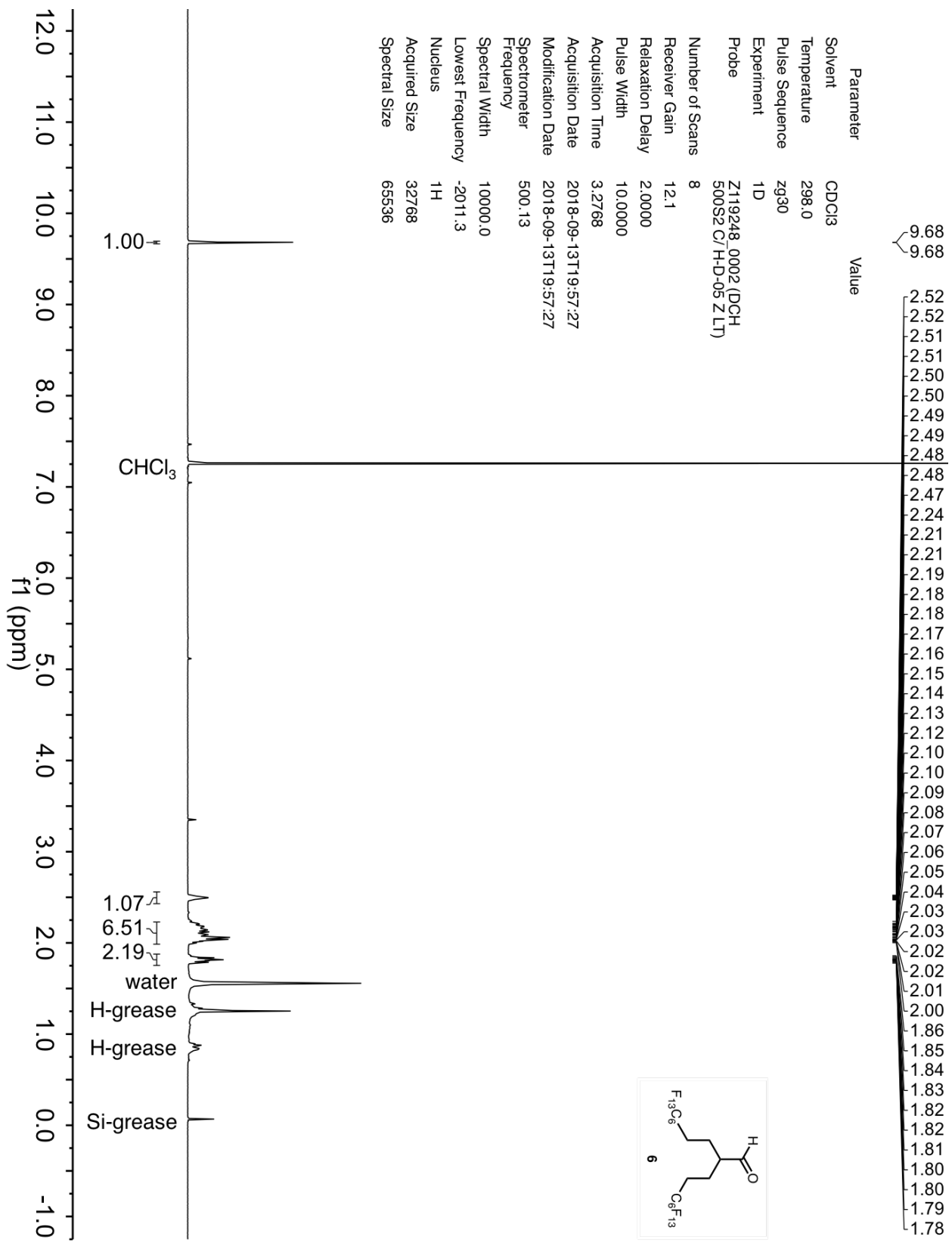
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 Spectral Size 65536

¹H NMR

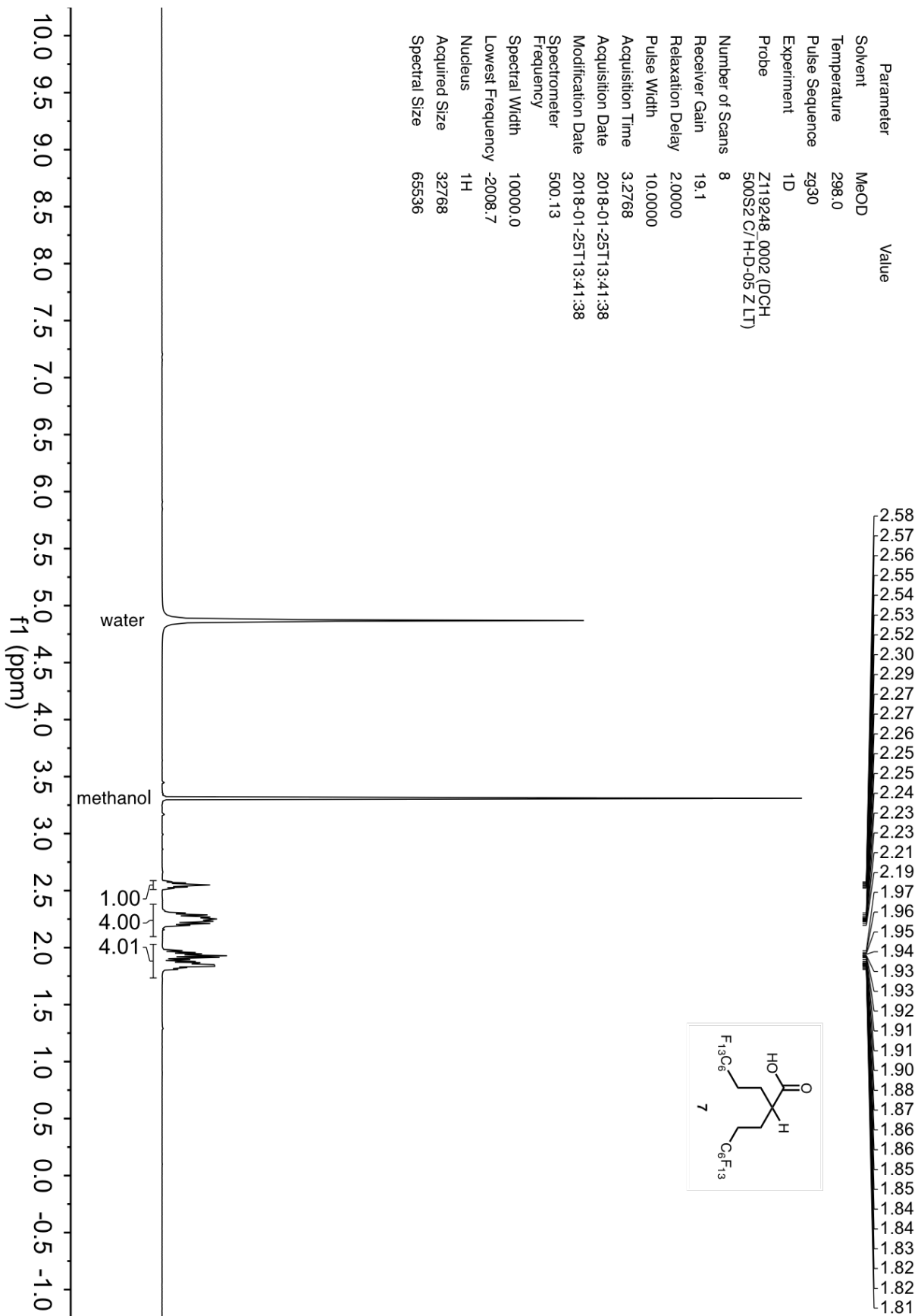


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Nucleus	¹ H
Acquired Size	32768
Spectral Size	65536



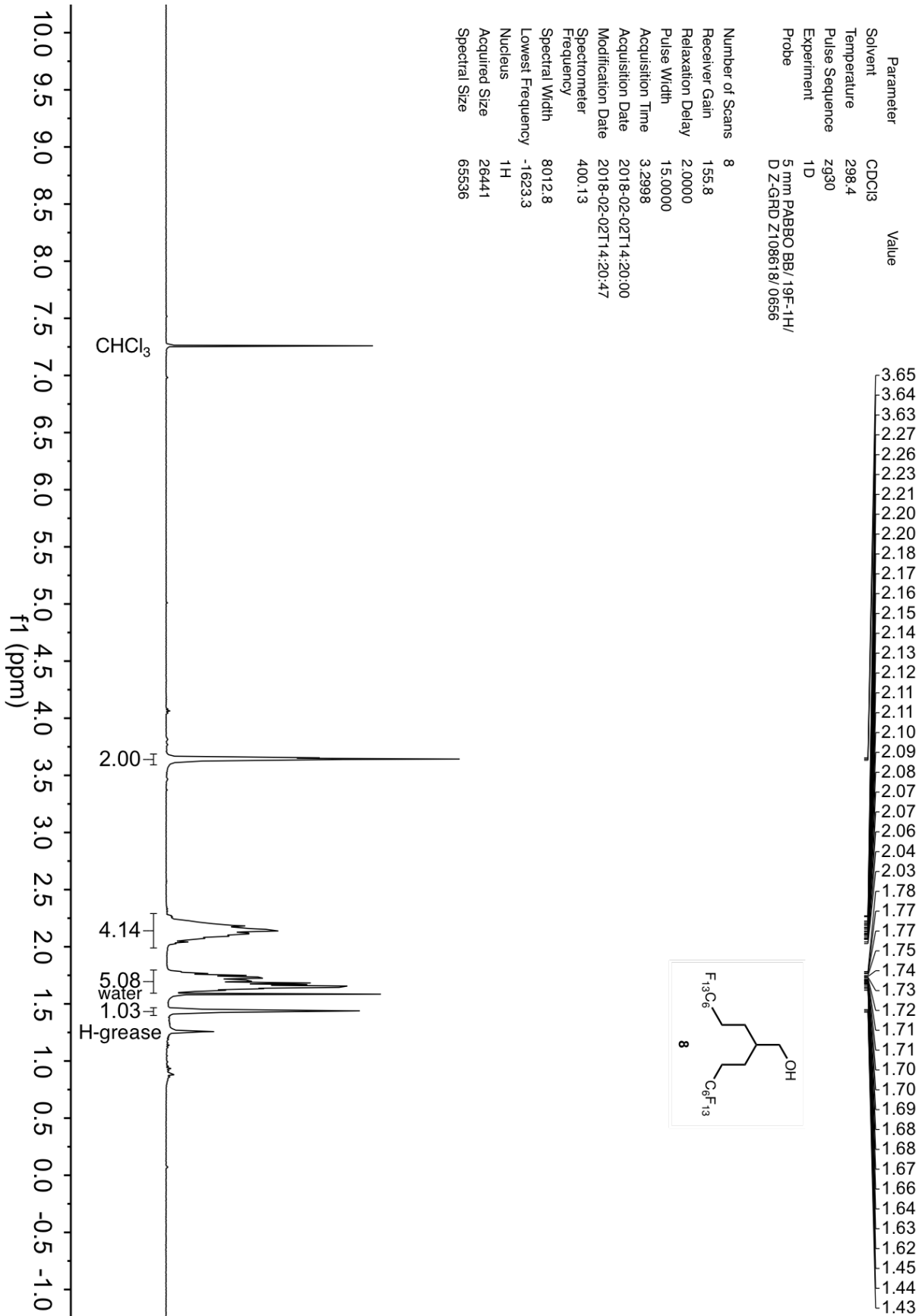


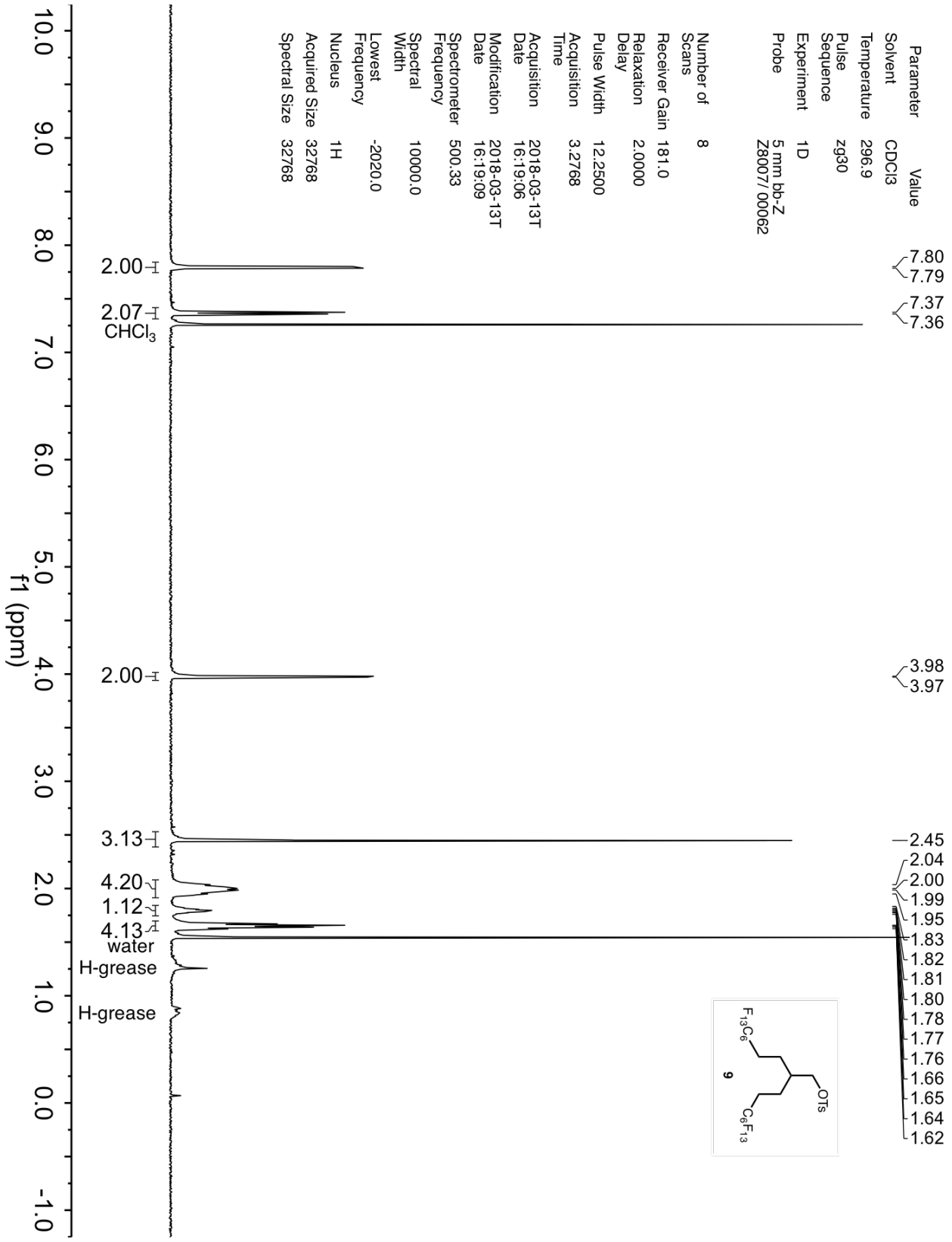
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Temperature	298.0
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Pulse Width	10.0000
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Nucleus	¹ H
Acquired Size	32768
Spectral Size	65536



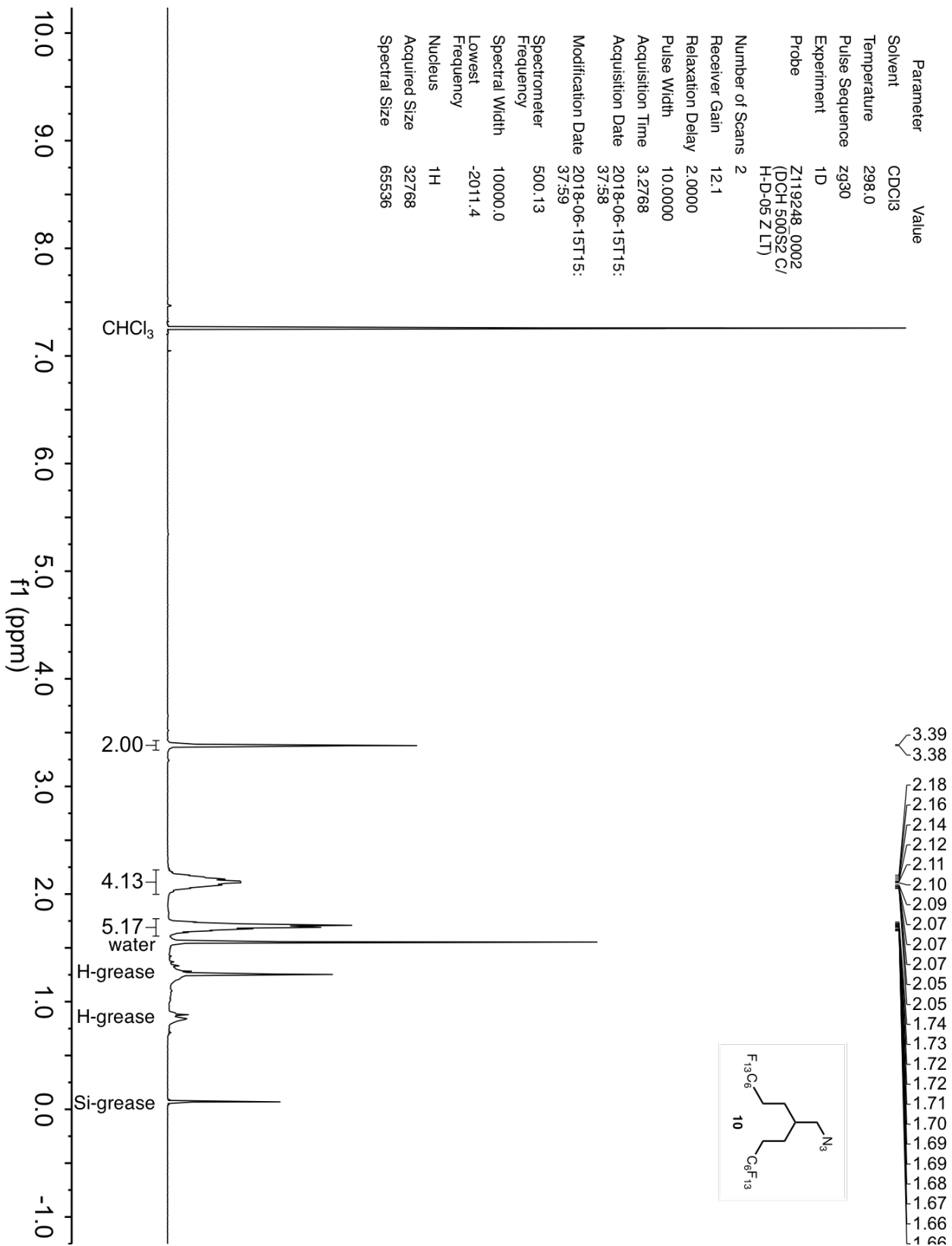
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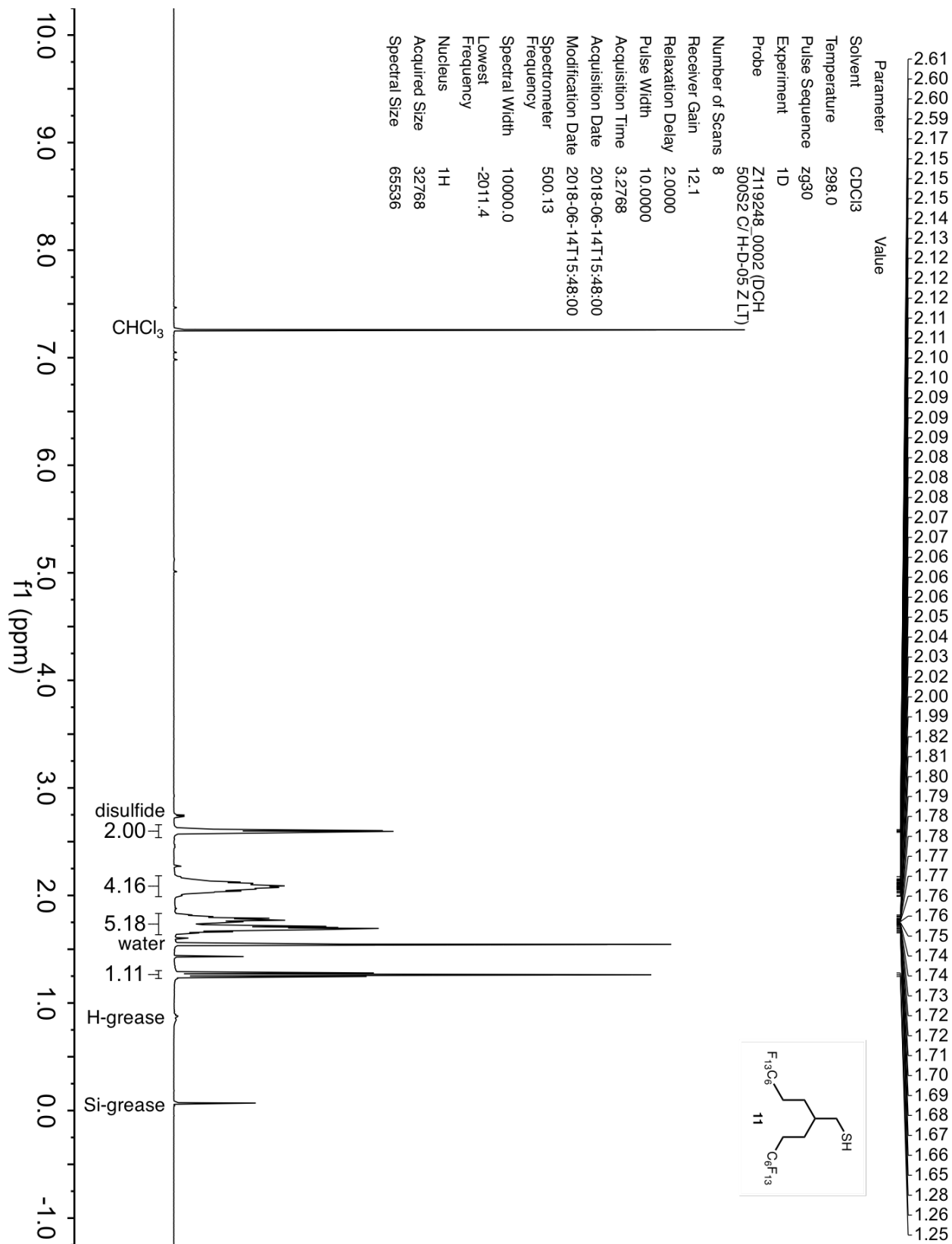
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 Nucleus 26441
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 Spectral Size

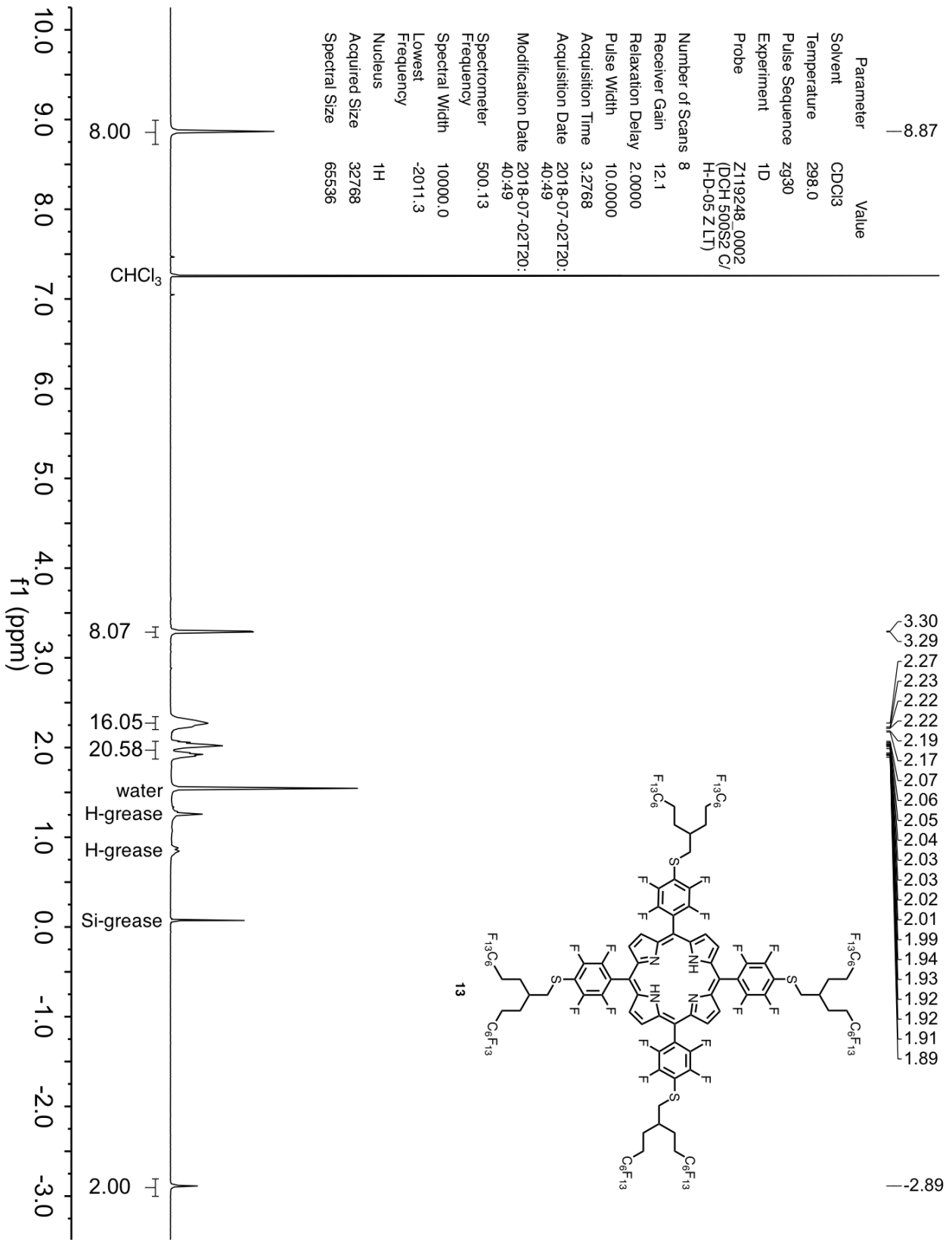




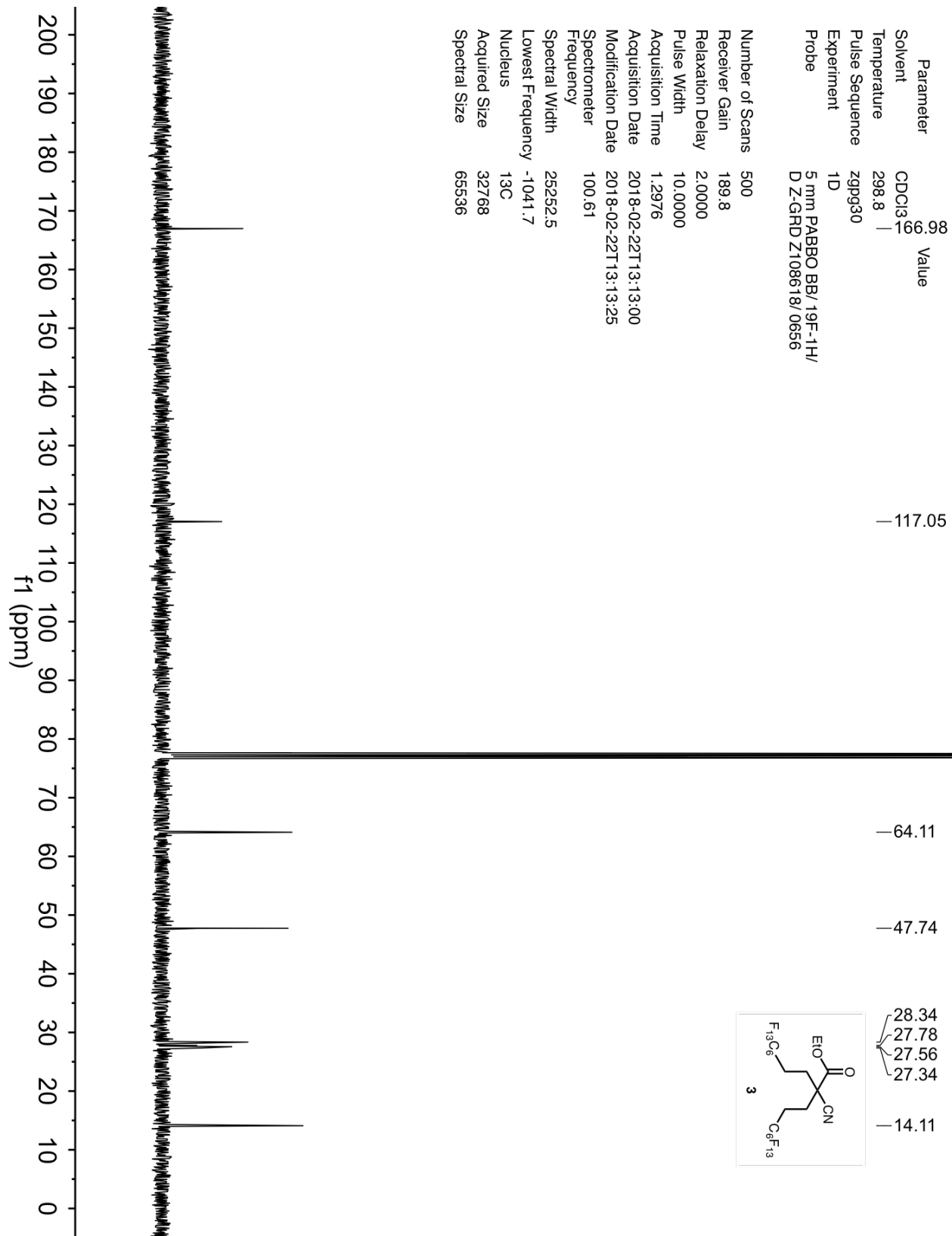
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Relaxation Delay	2.0000
Pulse Width	10.0000
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Nucleus	¹ H
Acquired Size	32768
Spectral Size	65536



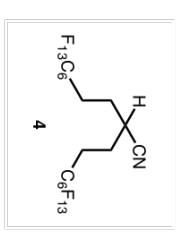
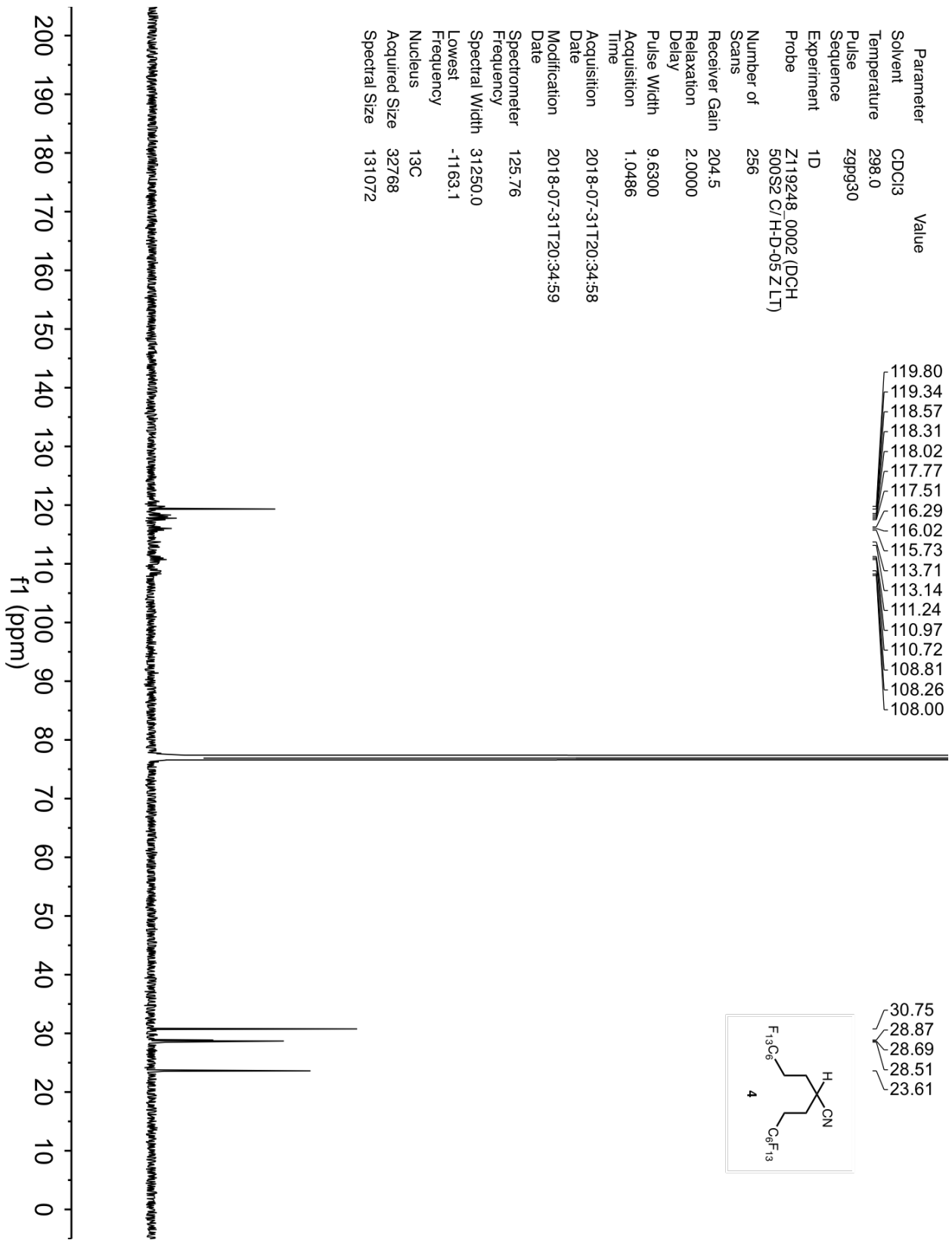




¹³C NMR



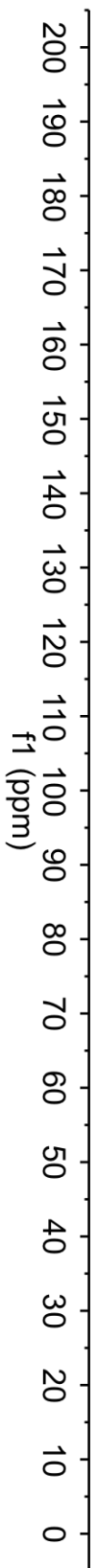
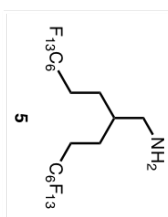
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Lowest Frequency	13C
Nucleus	13C
Acquired Size	32768
Spectral Size	131072



Parameter	Value
Solvent	MeOD
Temperature	298.0
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z119248_0002 (DCH 500S2 C/H-D-05 Z LT)
Number of Scans	256
Receiver Gain	204.5
Relaxation Delay	2.0000
Pulse Width	9.6300
Acquisition Time	1.0486
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Nucleus	¹³ C
Acquired Size	32768
Spectral Size	131072

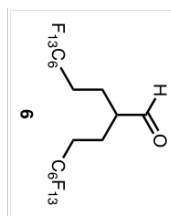
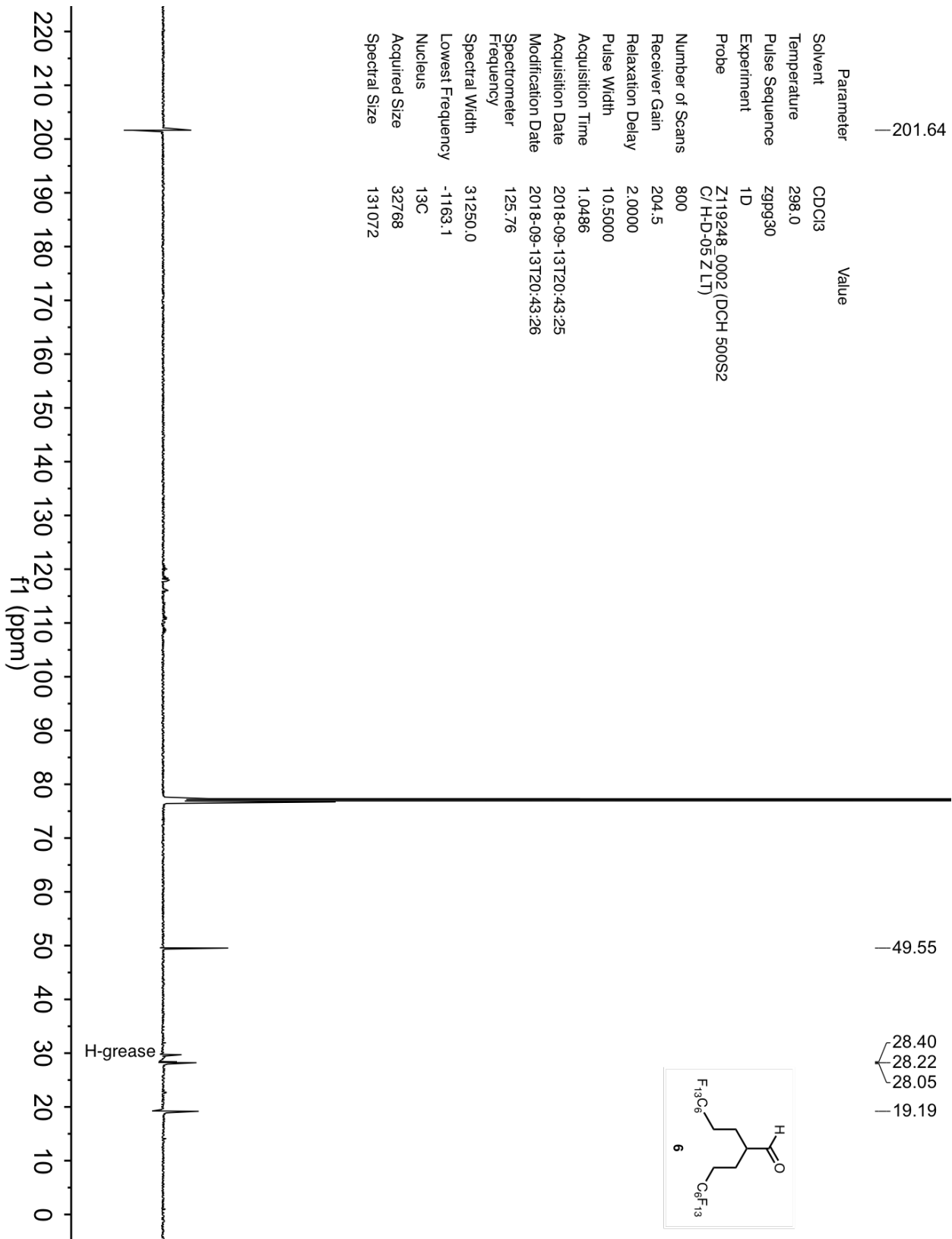
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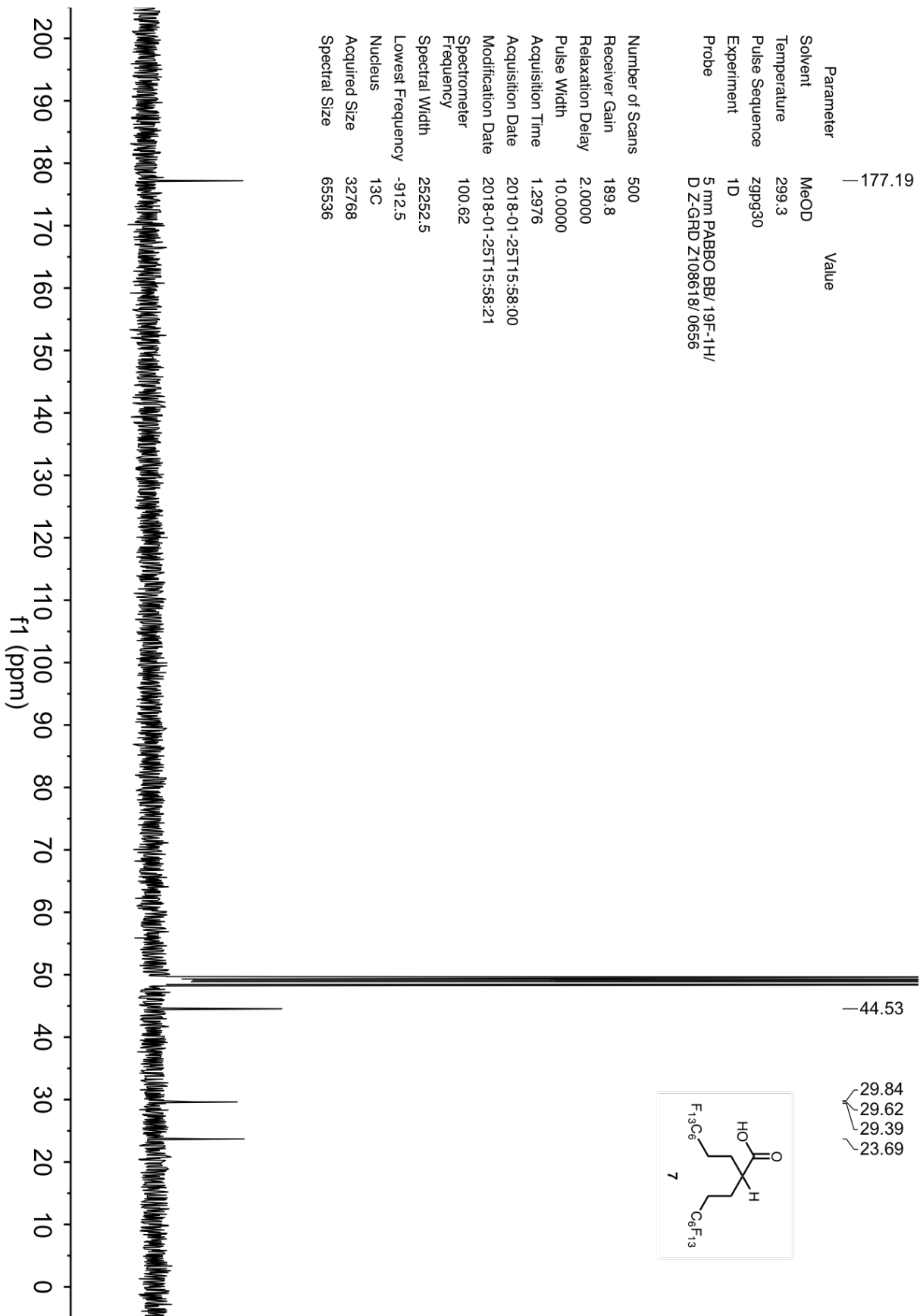
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Temperature	298.0
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Number of Scans	800
Receiver Gain	204.5
Relaxation Delay	2.0000
Pulse Width	10.5000
Acquisition Time	1.0486
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Spectral Size	131072

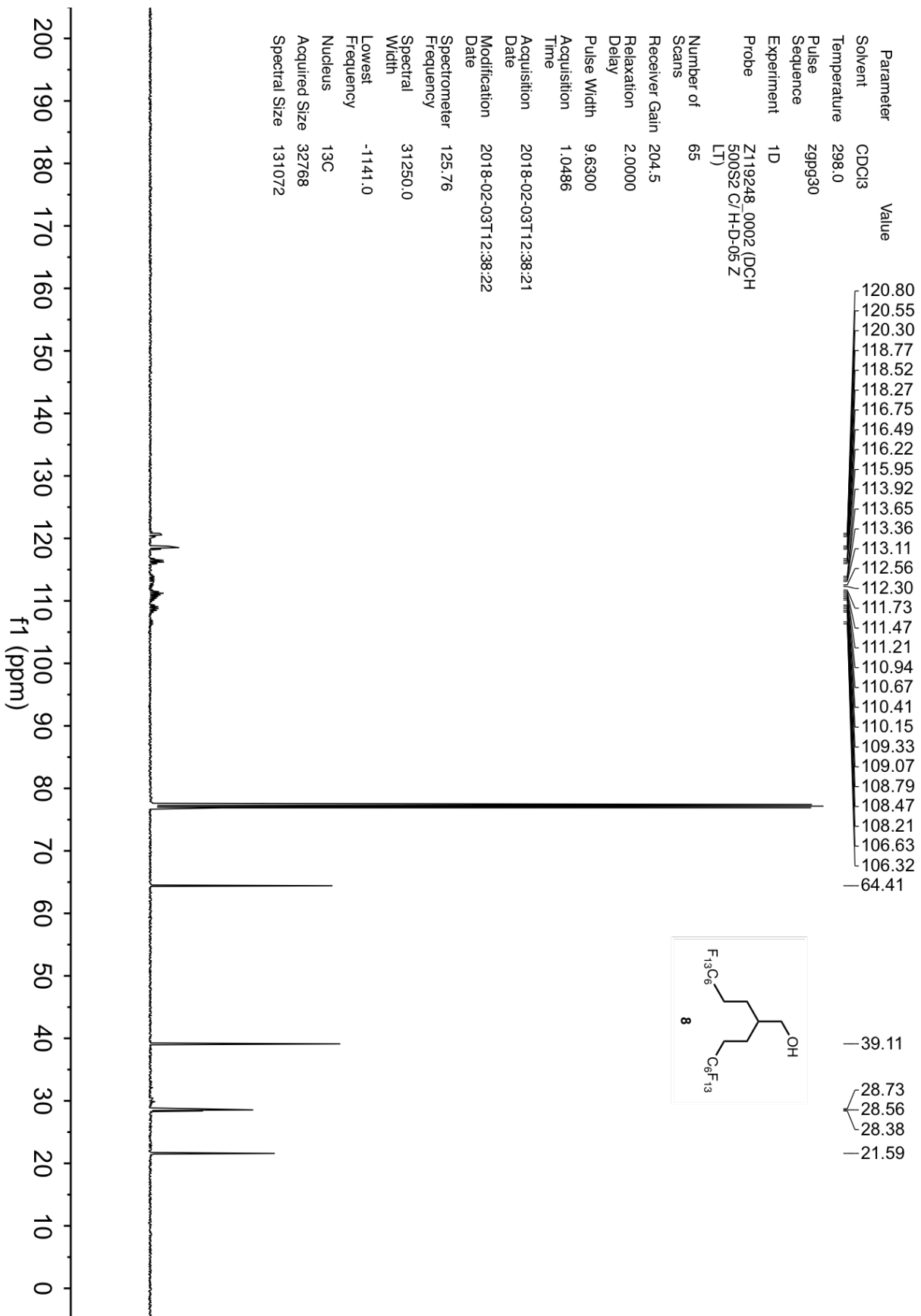


Parameter	Value
Solvent	MeOD
Temperature	299.3
Pulse Sequence	zgpg30
Experiment	1D
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Number of Scans	500
Receiver Gain	189.8
Relaxation Delay	2.0000
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Nucleus	¹³ C
Acquired Size	32768
Spectral Size	65536

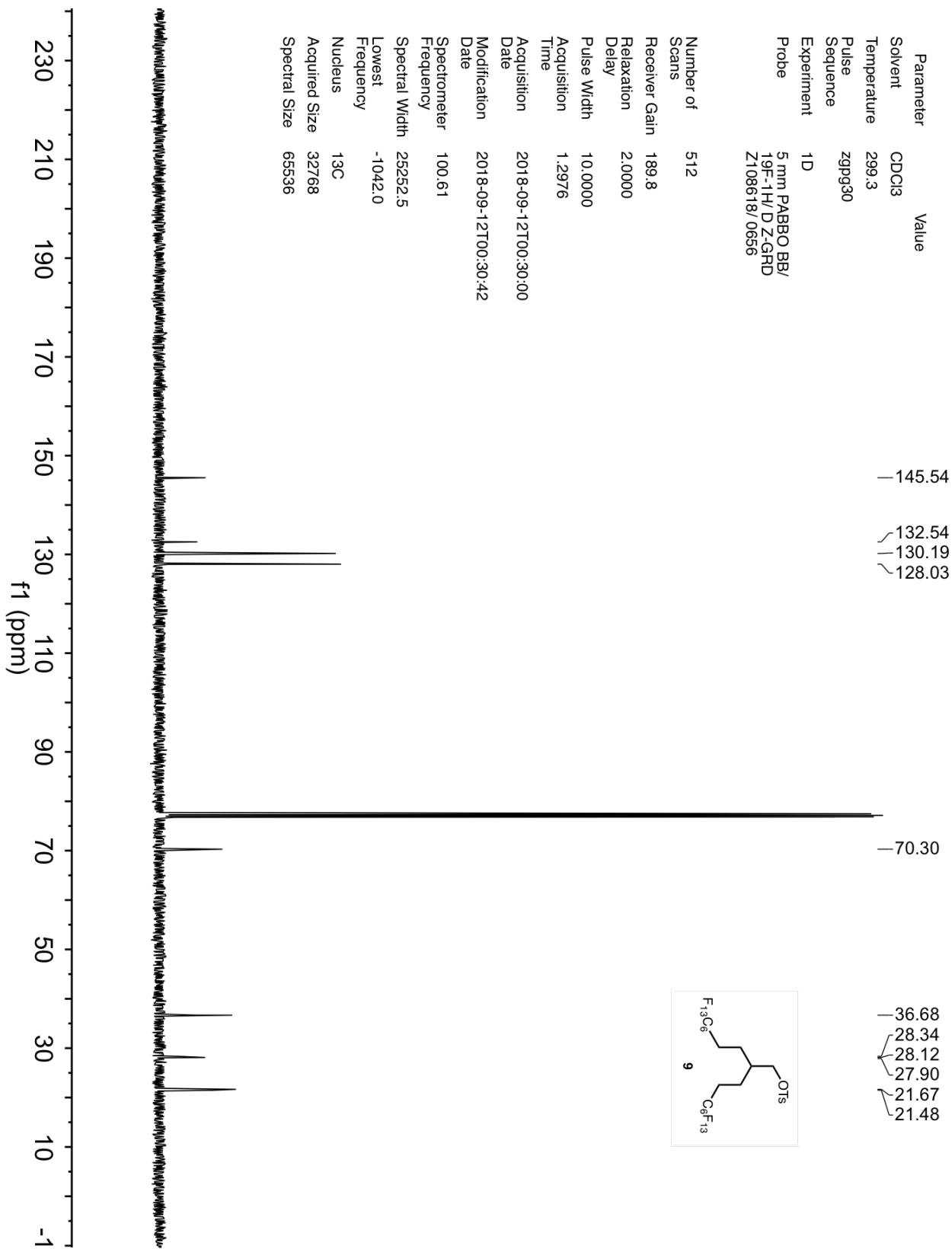


Parameter	Value
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Temperature	298.0
Pulse Sequence	zgpg30
Experiment	1D
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Number of Scans	65
Receiver Gain	204.5
Relaxation Delay	2.0000
Pulse Width	9.6300
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Nucleus	13C
Acquired Size	32768
Spectral Size	131072



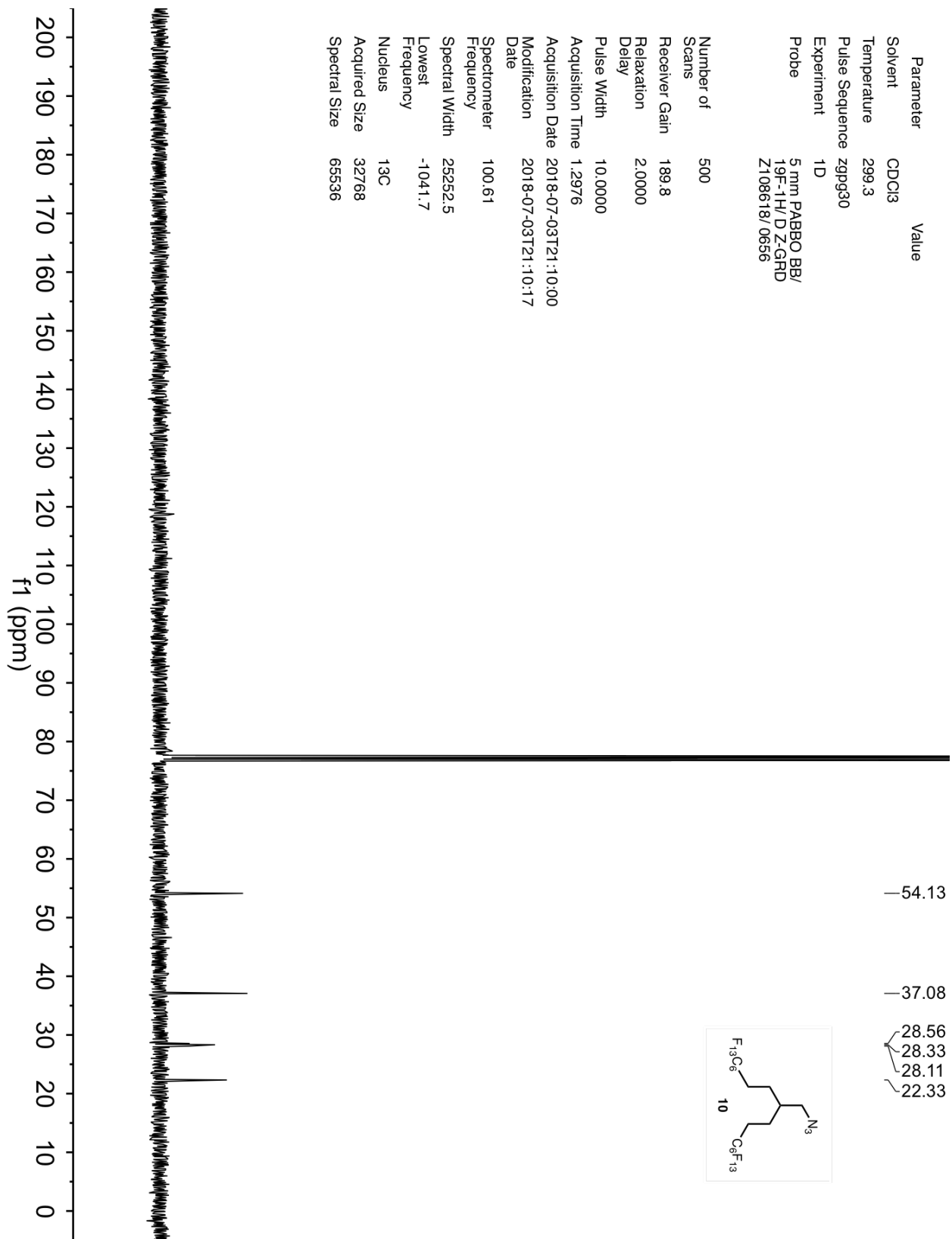
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Nucleus	¹³ C
Acquired Size	32768
Spectral Size	65536



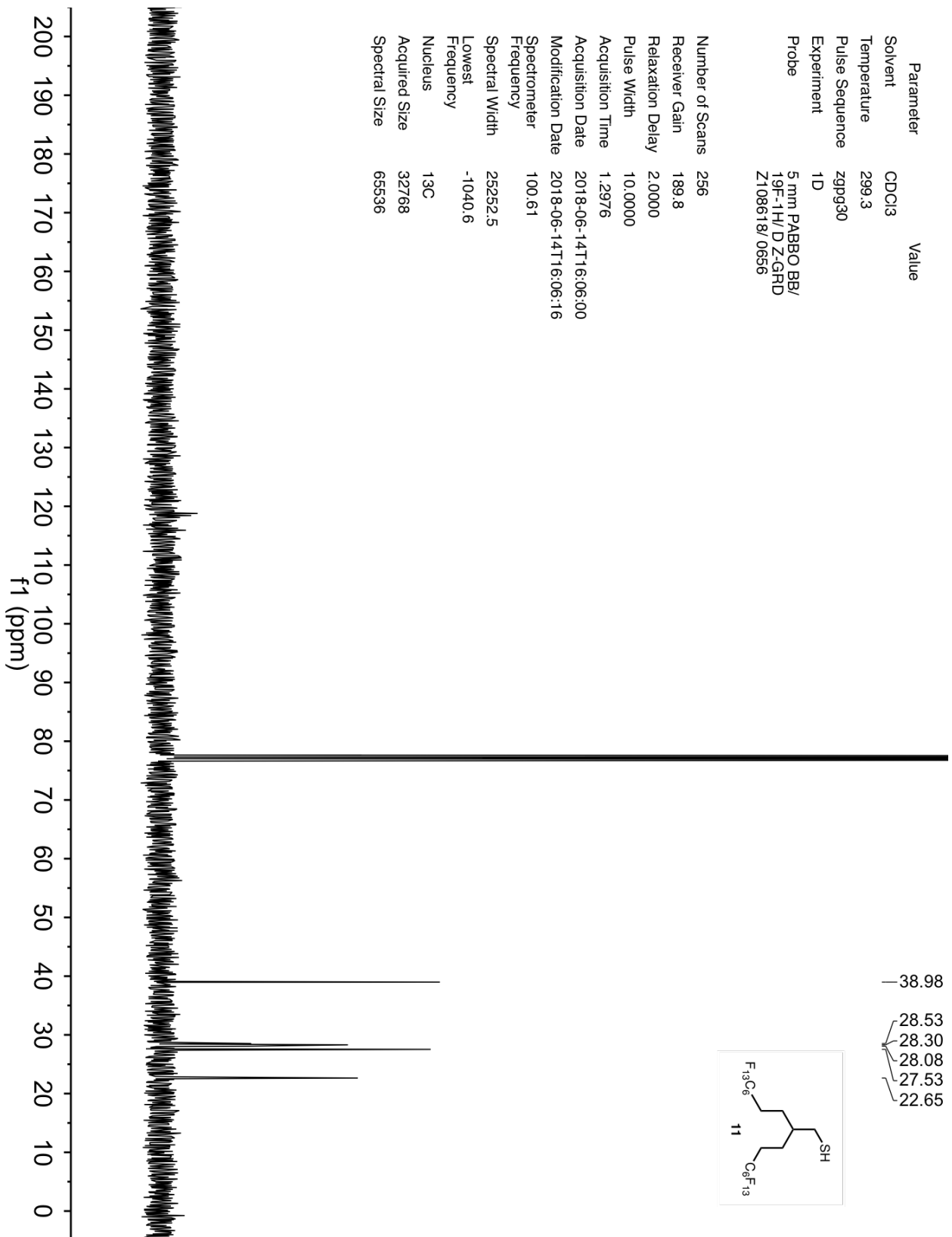
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Temperature	299.3
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Number of Scans	500
Receiver Gain	189.8
Relaxation Delay	2.0000
Pulse Width	10.0000
Acquisition Time	1.2976
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Frequency	25252.5
Spectral Width	25252.5
Lowest Frequency	-1041.7
Nucleus	¹³ C
Acquired Size	32768
Spectral Size	65536



Parameter	Value
Solvent	CDCl ₃
Temperature	299.3
Pulse Sequence	zgpg30
Experiment	1D
Probe	5 mm PABBO BB/ 19F-1H/ D Z-GRD Z108618/ 0656

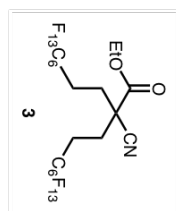
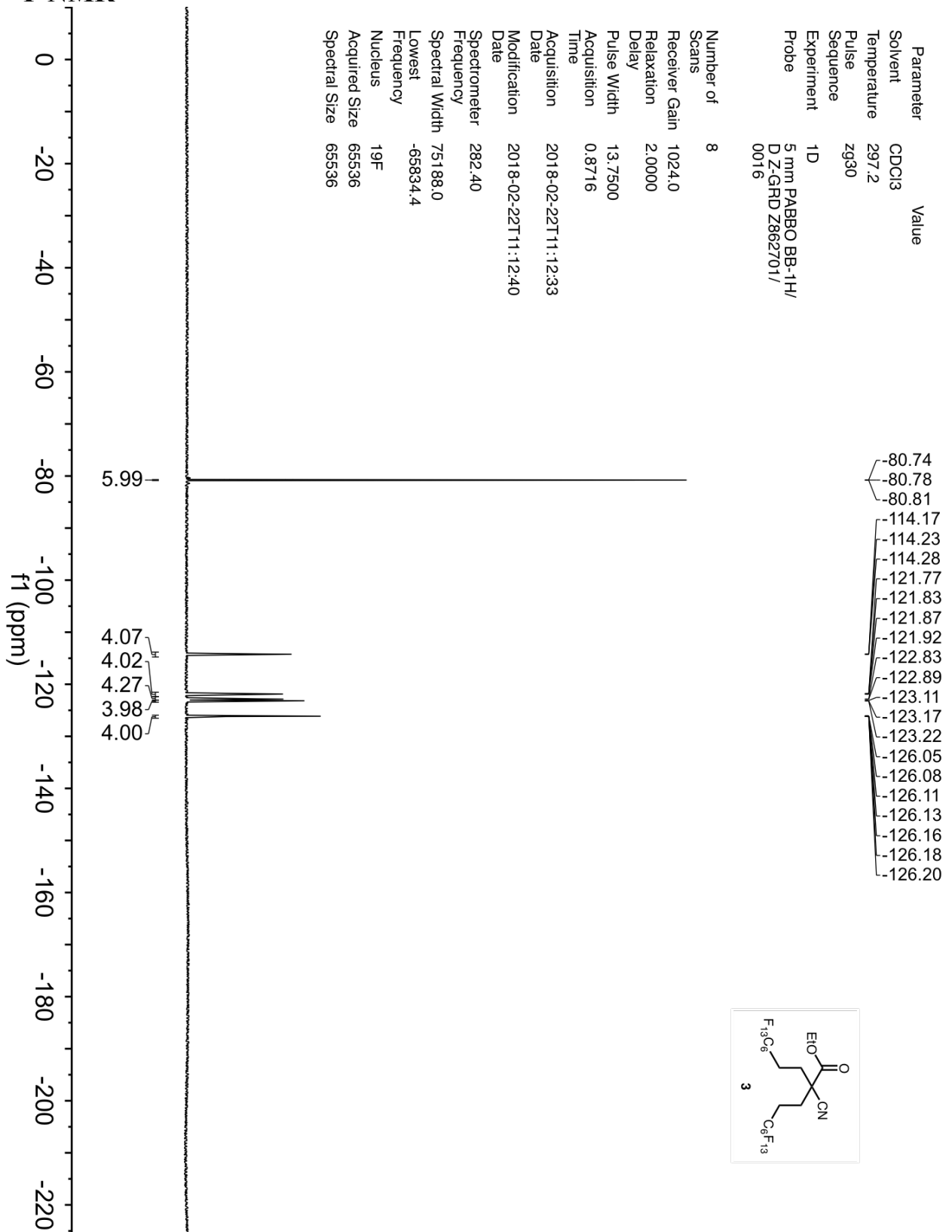
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Spectral Width	-1040.6
Lowest Frequency	13C
Nucleus	32768
Acquired Size	65536
Spectral Size	



Parameter	Value
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Pulse Sequence	zg30
Experiment	1D
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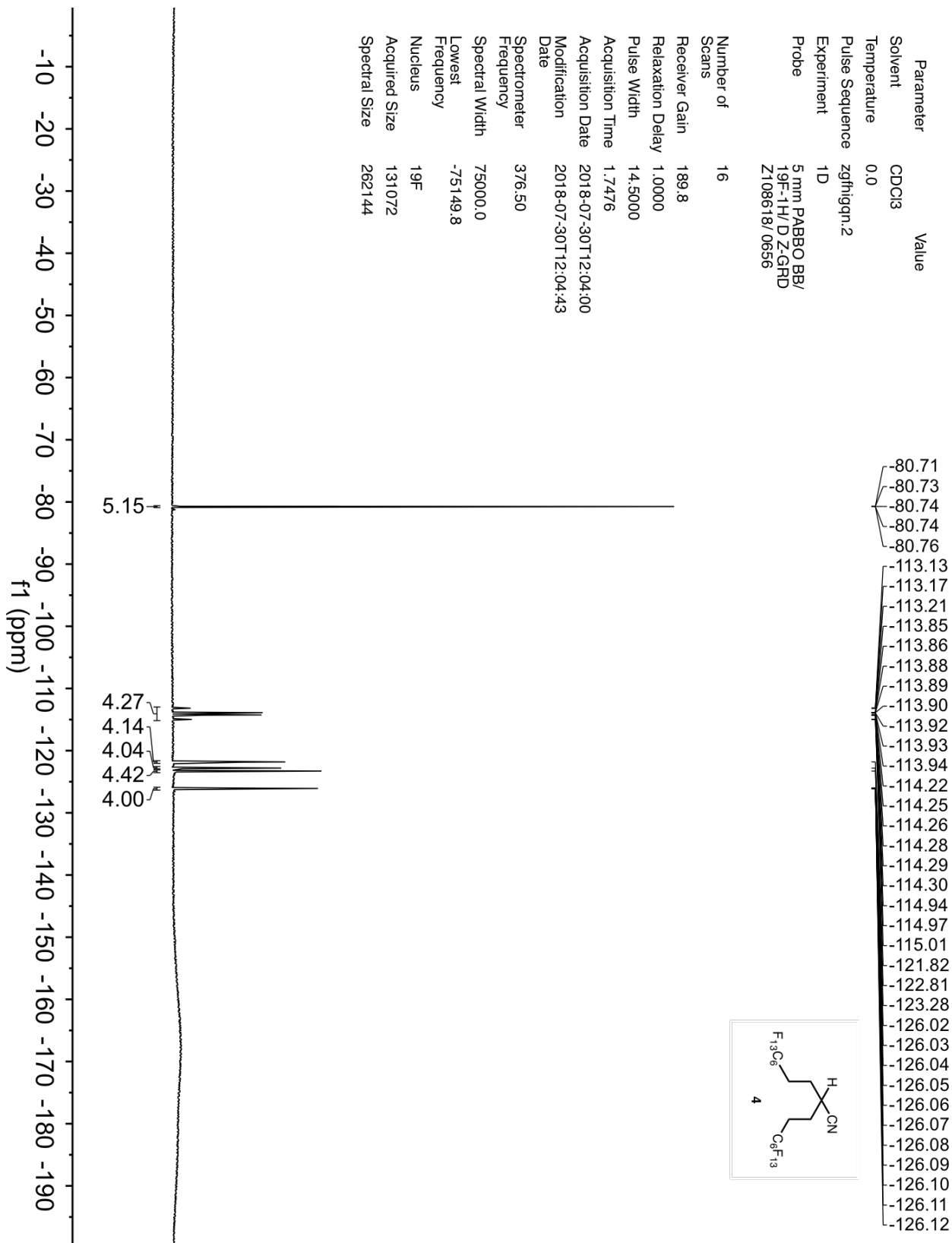
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Acquired Size	65536
Spectral Size	65536

¹⁹F NMR



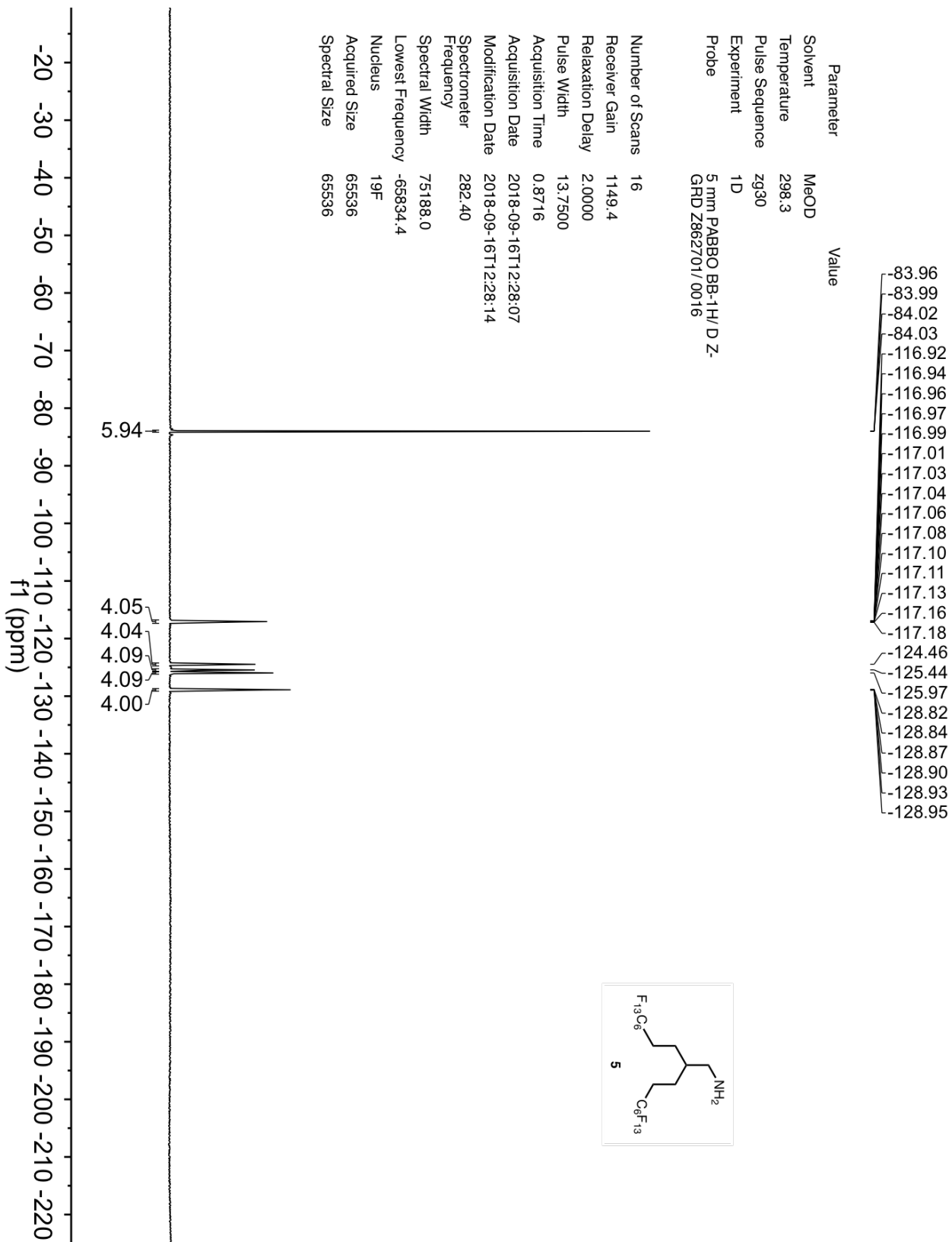
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Acquired Size	131072
Spectral Size	262144



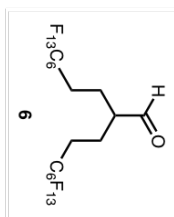
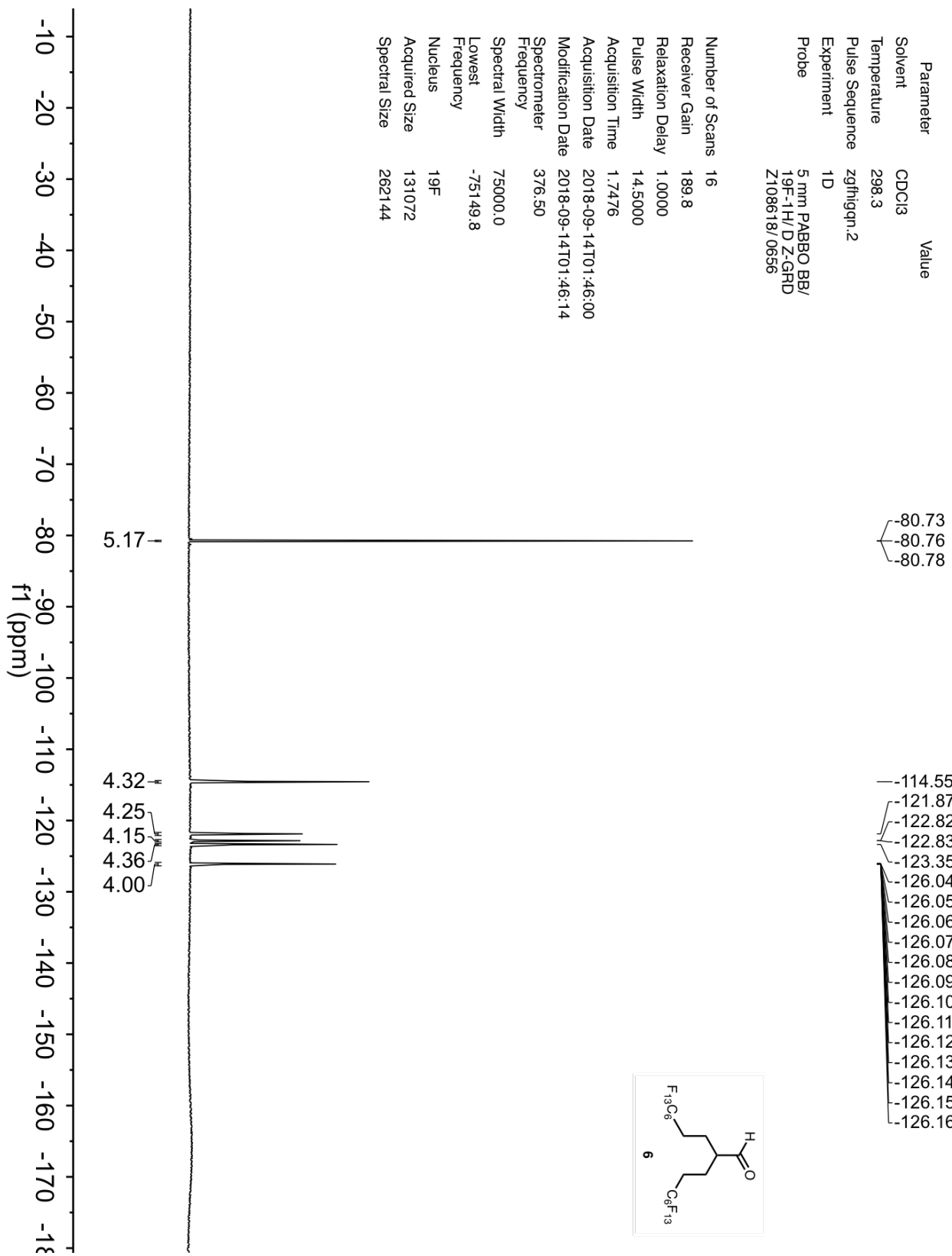
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Receiver Gain	1149.4
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Acquired Size	65536
Spectral Size	65536



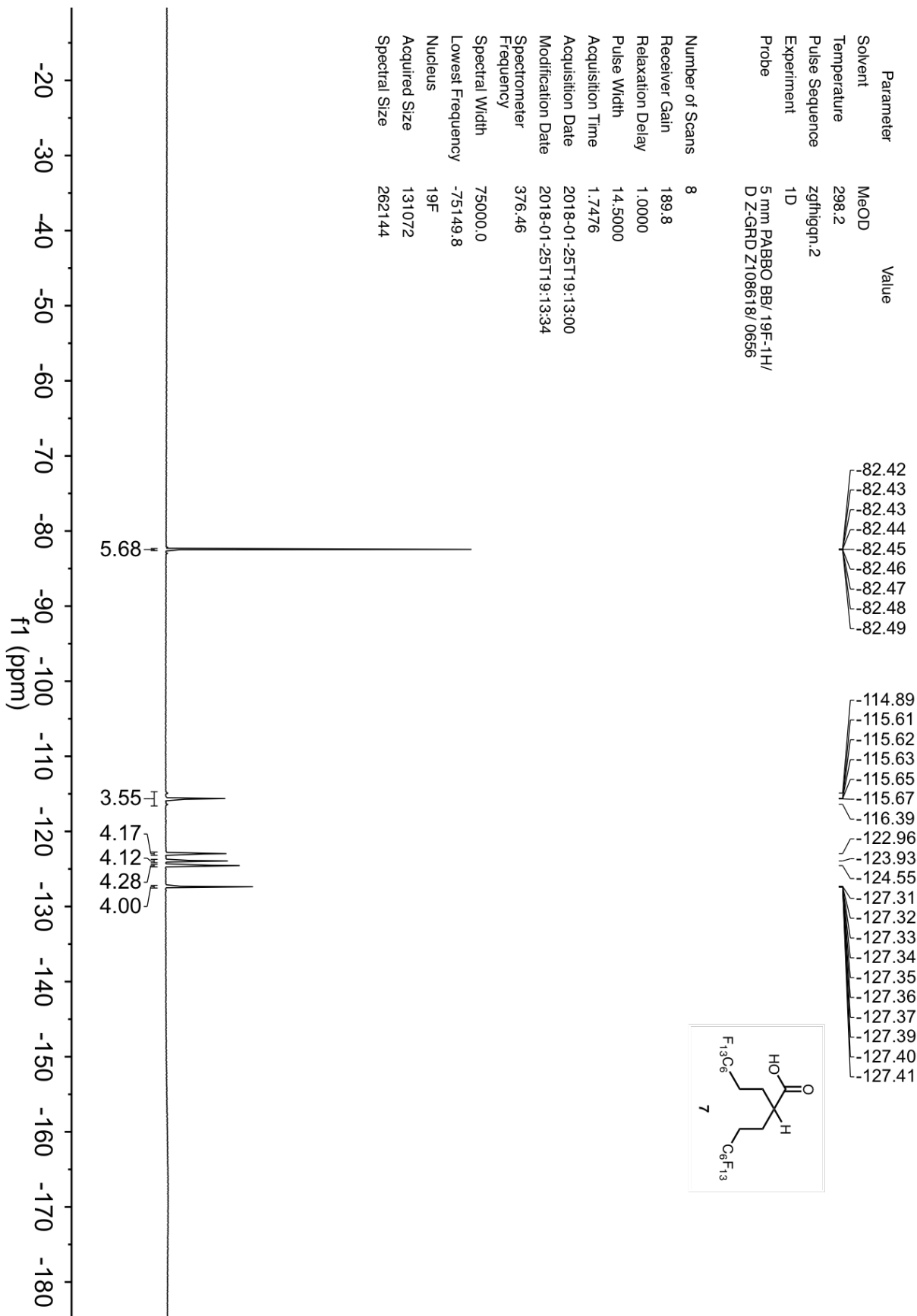
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Spectral Size	



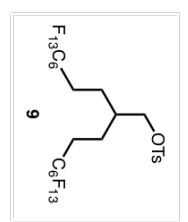
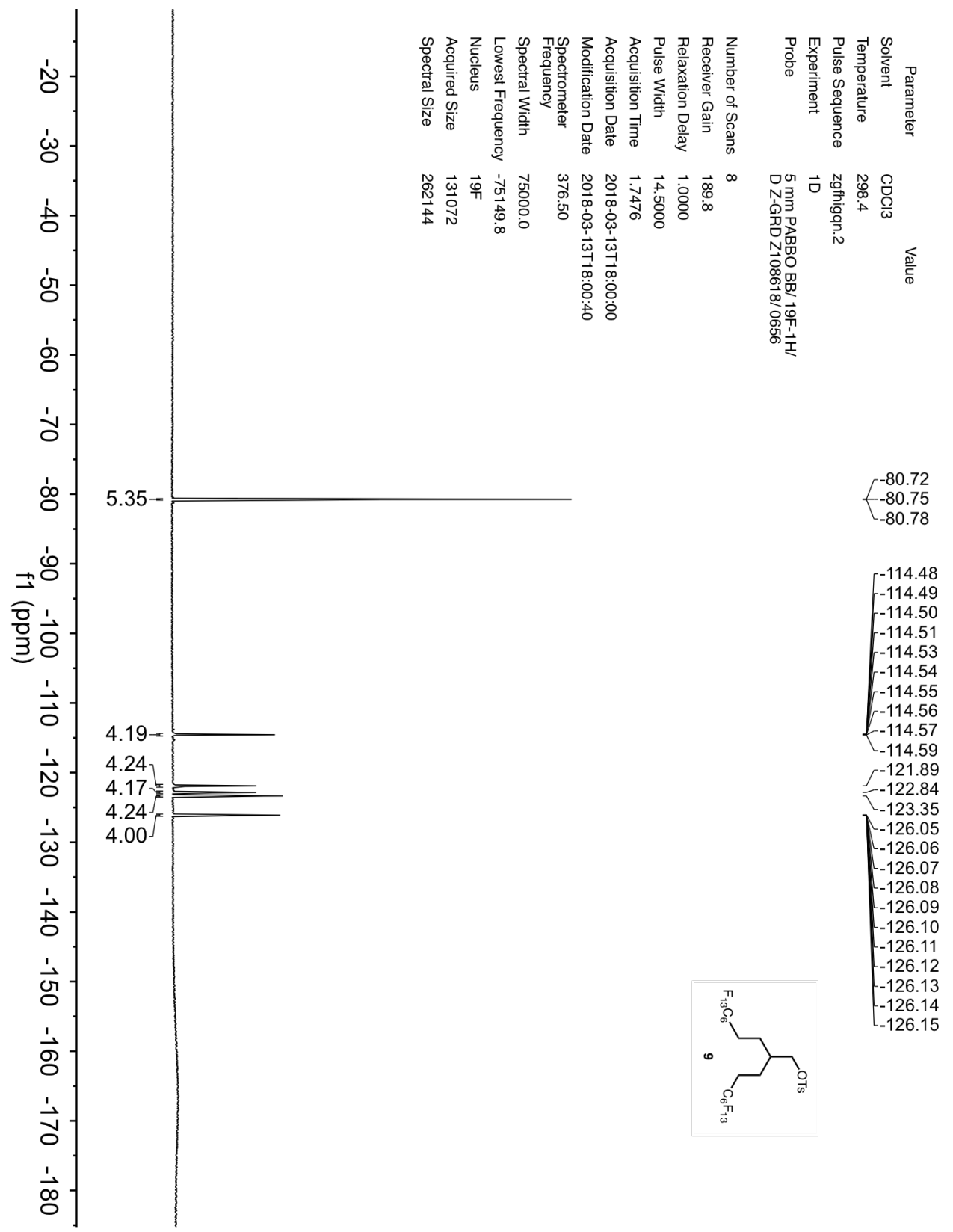
Parameter Value
 Solvent MeOD
 Temperature 298.2
 Pulse Sequence zgfhigqn.2
 Experiment 1D
 Probe 5 mm PABBO BB/ 19F-1H/
 D Z-GRD Z108618/ 0656

Number of Scans 8
 Receiver Gain 189.8
 Relaxation Delay 1.0000
 Pulse Width 14.5000
 Acquisition Time 1.7476
 Acquisition Date 2018-01-25T19:13:00
 Modification Date 2018-01-25T19:13:34
 Spectrometer 376.46
 Frequency 75000.0
 Spectral Width -75149.8
 Lowest Frequency 19F
 Nucleus 131072
 Acquired Size 262144
 Spectral Size



Parameter	Value
Solvent	CDCl3
Temperature	298.4
Pulse Sequence	zgfhigqn.2
Experiment	1D
Probe	5 mm PABBO BB/ 19F-1H/ DZ-GRD Z108618/0656

Number of Scans	8
Receiver Gain	189.8
Relaxation Delay	1.0000
Pulse Width	14.5000
Acquisition Time	1.7476
Acquisition Date	2018-03-13T18:00:00
Modification Date	2018-03-13T18:00:40
Spectrometer	376.50
Frequency	75000.0
Spectral Width	-75149.8
Lowest Frequency	19F
Nucleus	131072
Acquired Size	262144
Spectral Size	



Parameter	Value
Solvent	CDCl ₃
Temperature	298.7
Pulse Sequence	zg30
Experiment	1D
Probe	5 mm PABBO BB-1H/ D Z-GRD Z862701/0016

Number of Scans	8
Receiver Gain	1024.0
Relaxation Delay	2.0000
Pulse Width	13.7500
Acquisition Time	0.8716
Acquisition Date	2018-06-15T14:50:25
Modification Date	2018-06-15T14:50:32
Spectrometer	282.40
Frequency	
Spectral Width	75188.0
Lowest Frequency	-65834.4
Nucleus	¹⁹ F
Acquired Size	65536
Spectral Size	65536

