Electronic Supplementary Information for:

^{19}F NMR indicator displacement assay using synthetic receptor with appended relaxation agent

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1. Supplementary Figures

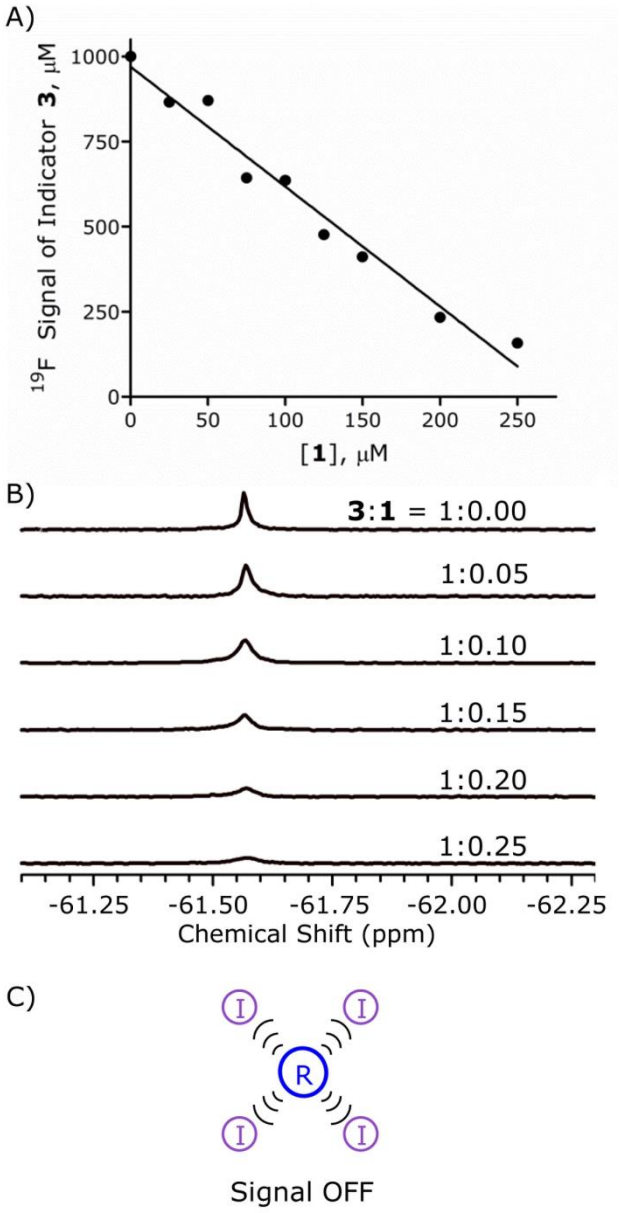


Figure S1. (A) Plot of ^{19}F NMR peak height of indicator **3** (1000 μM) upon addition of receptor **1**. (B) Representative ^{19}F NMR spectra (376 MHz) used to create the plot. N = 8 scans, 10 mM HEPES, pH 7.4, 25 °C, external trifluoroethanol (TFE) as reference. (C) Schematic rationalization of data showing receptor **R** quenching an average of four copies of indicator **I** due to rapid exchange.

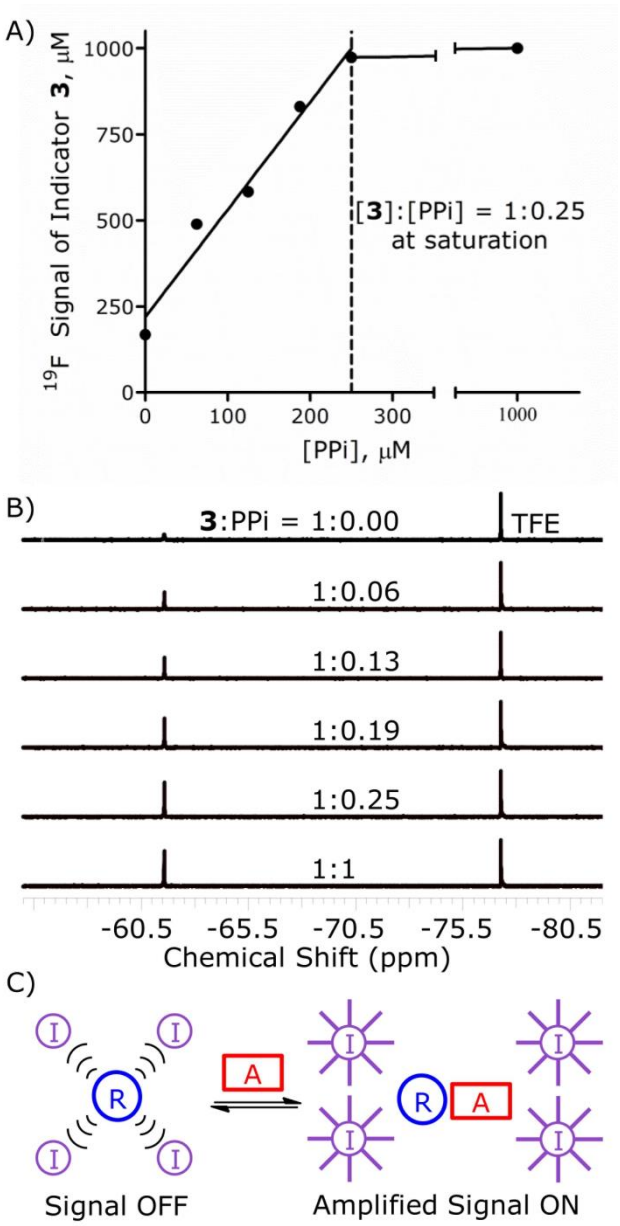


Figure S2. (A) Plot of ^{19}F NMR peak height of indicator **3** (1000 μM) upon addition of PPi (0 – 1.0 mM) to the admixture of **1**:**3** (250 μM:1000 μM). (B) Representative ^{19}F NMR spectra (376 MHz) used to create the plot. N = 8 scans, 10 mM HEPES, pH 7.4, 25 ° C, external trifluoroethanol (TFE) as reference. (C) Schematic rationalization of the data.

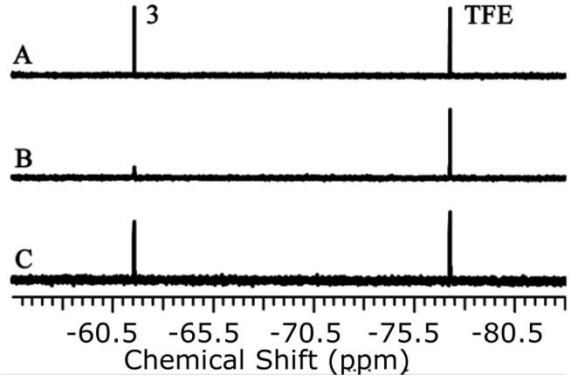


Figure S3: ^{19}F NMR spectra of indicator **3** alone (A, 1.0 mM **3**), after addition of receptor **1** (B, 0.25 mM **1**), and after further addition of PPi (C, 5.0 mM PPi). N = 8 scans, 10 mM HEPES, pH 7.4, 25 ° C, external trifluoroethanol (TFE) as reference.

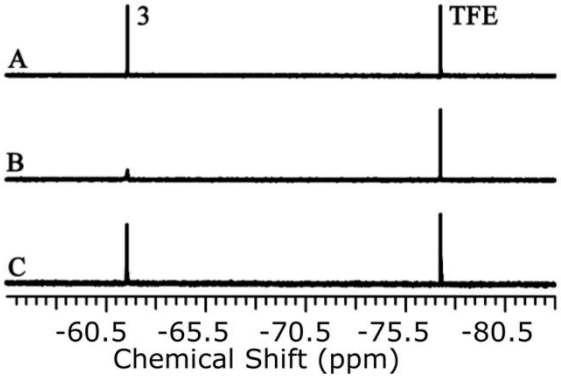


Figure S4: ^{19}F NMR spectra of indicator **3** (A, 1.0 mM **3**) after addition of receptor **2** (B, 0.25 mM **1**), and after further addition of PPi (C, 5.0 mM PPi). N = 8 scans, 10 mM HEPES, pH 7.4, 25 ° C, external trifluoroethanol (TFE) as reference.

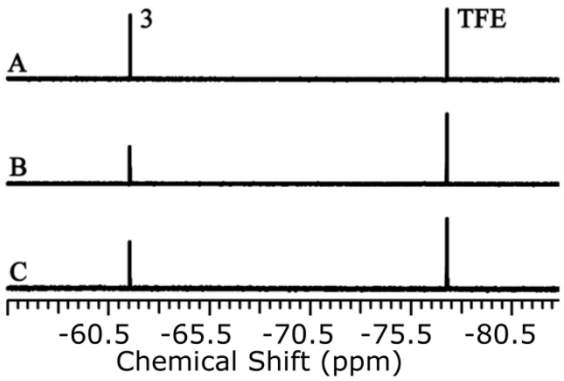


Figure S5: ^{19}F NMR spectra of indicator **3** alone (A, 1.0 mM **3**), after addition of RA **apo-2** (B, 0.25 mM **1**), and after further addition of PPi (C, 5.0 mM PPi). N = 8 scans, 10 mM HEPES, pH 7.4, 25 ° C, external trifluoroethanol (TFE) as reference.

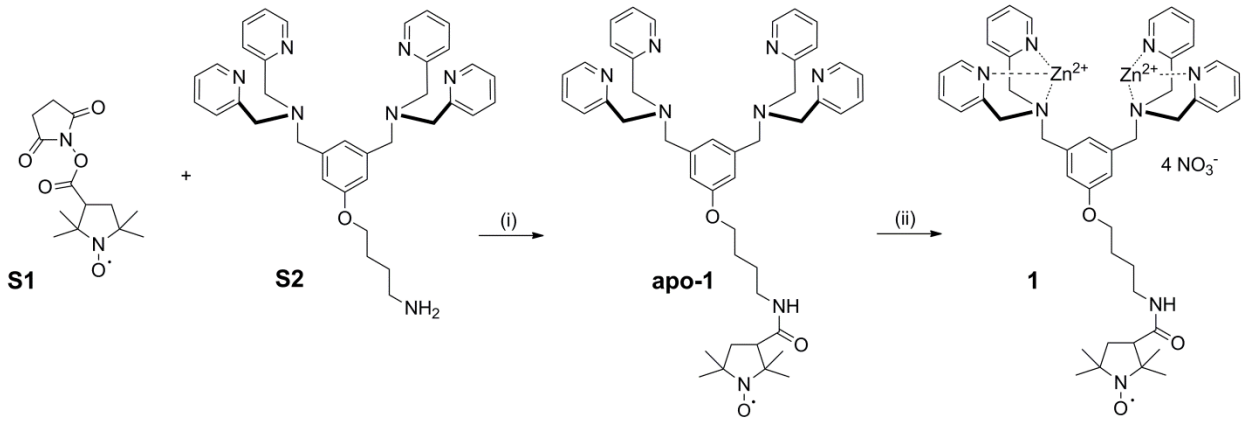
2. ^{19}F NMR Acquisition:

^{19}F NMR spectra were acquired using a Bruker instrument (376 MHz) with proton-decoupling and external trifluoroethanol as a reference (5.0 mM trifluoroethanol in D_2O). T_1 relaxation times were measured using an inversion recovery pulse sequence, and a spin-echo pulse sequence was used to measure T_2 relaxation times.

3. Chemical Synthesis:

General Schemes

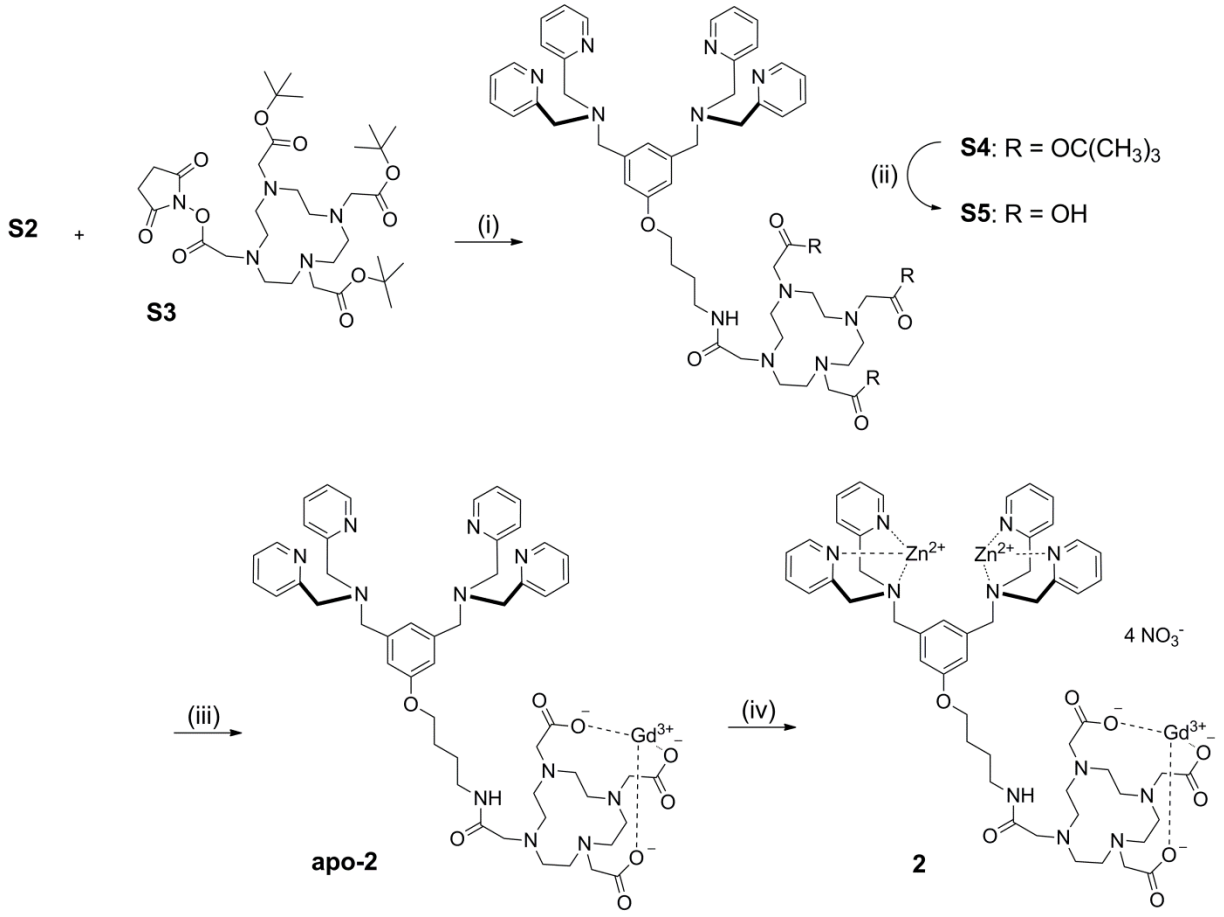
The synthetic route to prepare ZnBDPA receptor **1** is shown in Scheme S1. Compound **S1** was prepared from 3-Carboxy-proxyl (Sigma Aldrich, St. Louise, MO, USA) following literature precedent.[1] Treatment of activated NHS ester **S1** with the known amine **S2**[2] resulted in amide bond formation and **apo-1**. Subsequent treatment with $Zn(NO_3)_2$ in methanol produced ZnBDPA receptor **1**.



[a]Reagents and conditions: (i) DMF, $CHCl_3$ 87%; (ii) $Zn(NO_3)_2 \cdot 6H_2O$, MeOH, quantitative.

Scheme S1: Synthesis of receptor **1**[a]

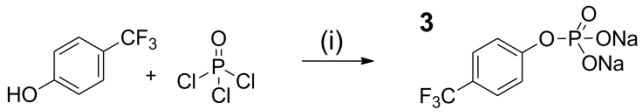
The synthetic route to prepare ZnBDPA receptor **2** is shown in Scheme S2. Treatment of **S2** with DOTA-mono-NHS-tris(^{t}Bu-Ester) **S3** produced **S4** in 60% yield. The ^{t}Bu-ester groups were removed using standard deprotection conditions to yield unprotected DOTA derivative **S5**. Standard lanthanide chelation protocols were used to yield **apo-2**.[3] Subsequent treatment with $Zn(NO_3)_2$ in methanol produced ZnBDPA receptor **2**.



[a]Reagents and conditions: (i) TEA, $CHCl_3$, 18 h, 60%; (ii) TFA, CH_2Cl_2, rt, 12 h, 94%; (iii) $GdCl_3$, 0.1 M NH_4OAc pH 8.0, 18 h; (iv) $Zn(NO_3)_2 \cdot 6H_2O$, MeOH, quantitative.

Scheme S2: Synthesis of receptor **2**[a]

A literature route was used to prepare indicator **3** (Scheme S3).[4]



[a]Reagents and conditions: (i) Pyridine, 0 °C, 45 m; H_2O, cyclohexylamine, pH 9; Ion exchange chromatography.

Scheme S3: Synthesis of ^{19}F NMR indicator **3**[a]

Synthetic Procedures

Apo-1: NHS-3-carboxy-proxyl **S1** was prepared using the method of Liu[1]. Butylamine DPA **S2** (52 mg, 88 μmol) and **S1** (52 mg, 180 μmol) were combined in a 1:1 solution of DMF:$CHCl_3$ (4 mL) and allowed to stir at room temperature for 36 h. The solvent was evaporated and the residue purified by silica gel column chromatography with 2-10% MeOH in $CHCl_3$ as the eluent to yield the desired product (58.6 mg, 87 %) as a sticky yellow oil. LCMS analysis was performed to verify compound purity (see Figure S6). ^{1}H NMR: (500 MHz, $CDCl_3$) δ 1.68-1.95 (m, 6H), 3.32-3.55 (m, 2H), 3.67 (br s, 4H), 3.82 (br s, 8H), 3.96-4.06 (m, 2H), 6.85 (br s, 2H), 7.08 (br s, 1H), 7.16 (br s, 4H), 7.61 (br s, 8H), 8.52 (br s, 4H); Mass Spectroscopy: MS (ESI+) calculated for $C_{45}H_{56}N_8O_3$ ($[M+H]^+$) 756.4470, found 756.4498.

S4: To a solution of 72 mg (120 μmol) butylamine DPA **S2** in 250 μL anhydrous $CHCl_3$ and 10 μL triethylamine was added 25 mg (31 μmol) DOTA-mono-NHS-tris(^{t}Bu-Ester) **S3**. The reaction stirred for 18 h after which the solvent was removed *in vacuo*. The crude material was purified using silica gel column chromatography with 0-10% MeOH in $CHCl_3$ as the eluent to yield 24.2 mg (60 % yield) of the desired product as a colorless oil. ^{1}H NMR: (600 MHz, $CDCl_3$) δ 1.41 (s, 18H), 1.44 (s, 9H), 1.68 (p, J = 7 Hz, 2H), 1.78 (p, J = 7 Hz, 2H), 2.10-3.00 (br m, 24 H), 3.26 (q, J = 6 Hz, 2H), 3.63 (s, 4H), 3.79 (s, 8H), 3.92 (t, J = 6 Hz, 2H), 6.64 (t, J = 5 Hz, 1H), 6.82 (s, 2H), 7.04 (s, 1H), 7.13 (qd, J = 7 Hz, J = 1 Hz, 4H), 7.60 (d, J = 7 Hz, 4H), 7.66 (td, J = 7 Hz, J = 2 Hz, 4H), 7.48 (dq, J = 5 Hz, J = 1 Hz, 4H) ppm; ^{13}C NMR: (600 MHz, $CDCl_3$) δ 14.2, 21.0, 25.9, 26.7, 27.9, 39.1, 55.7, 55.9, 58.6, 60.0, 60.4, 67.4, 81.7, 81.8, 113.5, 121.3, 122.0, 122.8, 136.7, 140.4, 148.8, 159.2, 159.5, 171.2, 171.6, 172.4 ppm; Mass Spectroscopy: MS (ESI+) calculated for $C_{64}H_{91}N_{11}NaO_8$ ($[M+Na]^+$) 1164.6944, found 1164.6964.

S5: To a solution of 25 mg (22 μmol) **S4** in 400 μL CH_2Cl_2 was added 600 μL of TFA. The reaction mixture was allowed to proceed at ambient temperature for 16 h. The solvent was removed and the residue was washed 3 times with Et_2O after which the residue was subjected to high vacuum. 20.0 mg (94 % yield) of the desired product was obtained as a yellow oil. ^{1}H NMR: (600 MHz, DMSO-d_6) δ 1.57 (p, J = 8 Hz, 2H), 1.72 (p, J = 8 Hz, 2H), 3.00-3.80 (br m, 24 H), 3.92 (t, J = 7.5 Hz, 2H), 4.03 (s, 4H), 4.08 (br s, 2H), 4.15 (s, 8H), 6.98 (s, 2H), 7.14 (s, 2H), 7.40-7.54 (m, 4H), 7.53 (d, J = 8 Hz, 4H), 7.86 (dt, J = 8 Hz, J = 2 Hz, 4H), 8.58-8.59 (m, 4H) ppm; Mass Spectroscopy: MS (ESI+) calculated for $C_{52}H_{68}N_{11}O_8$ ($[M+H]^+$) 974.5225, found 974.5247.

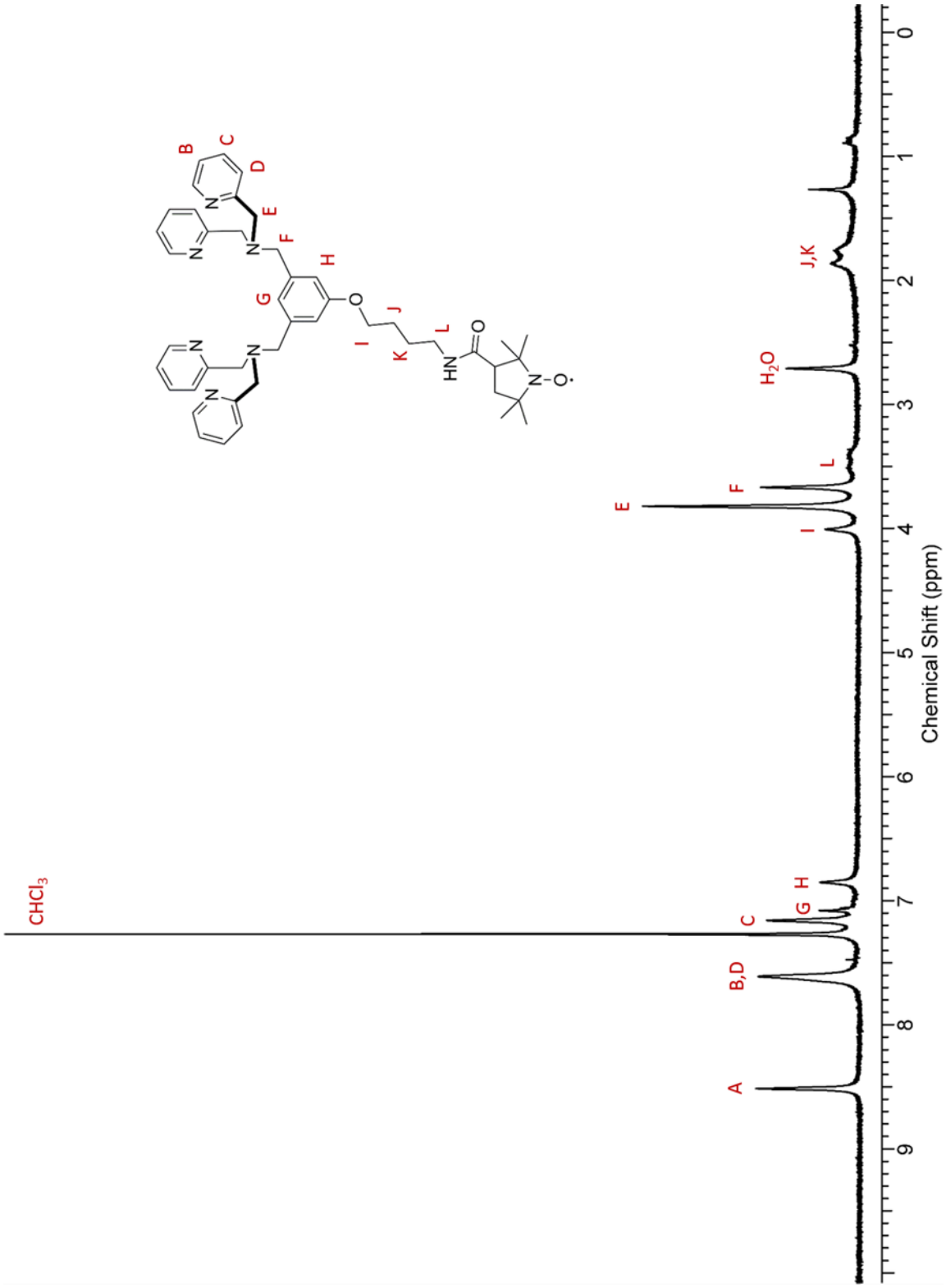
Apo-2: Standard lanthanide chelation protocols were used to yield **apo-2**.[3] Chelator **S5** (9.5 mg, 8.4 nmol) was dissolved in 0.1 M ammonium acetate (1.0 mL, pH adjusted to 8 with aqueous NH_4OH) and treated with 3 molar equivalents of $GdCl_3$ (10.9 mg, 29.0 nmol). The reaction mixture was stirred at room temperature overnight. Excess $GdCl_3$ and ammonium salts were removed using a SEP-PAK C_{18} reverse-phase column with repetitive washing (10 mL DI H_2O). The final product was eluted using 50% aqueous acetonitrile (2 mL) and lyophilized. Previous work by Meade & co-workers has shown that lanthanide metals preferentially coordinate to DOTA chelators in the presence of dipicolylamine moieties.[3a] LCMS analysis was performed to verify chelate purity (see Figure S7). Mass Spectroscopy: MS (ESI+) calculated for $C_{52}H_{66}GdN_{11}O_8$ ($[M+2H]^{2+}$) 567.7177, found 565.2169.

Receptors 1 or **2**: Stock solutions of $Zn(NO_3)_2 \cdot 6H_2O$ and BDPA scaffold (**apo-1** or **apo-2**) were prepared in MeOH. Separate samples were mixed at a $[Zn^{2+}]$:[DPA] molar ratio of 1:1 and allowed to shake for 1 h before the solvent was removed by rotary evaporation followed by sitting under vacuum for 1 h. The resulting samples of ZnBDPA receptors **1** or **2** were used without further purification.

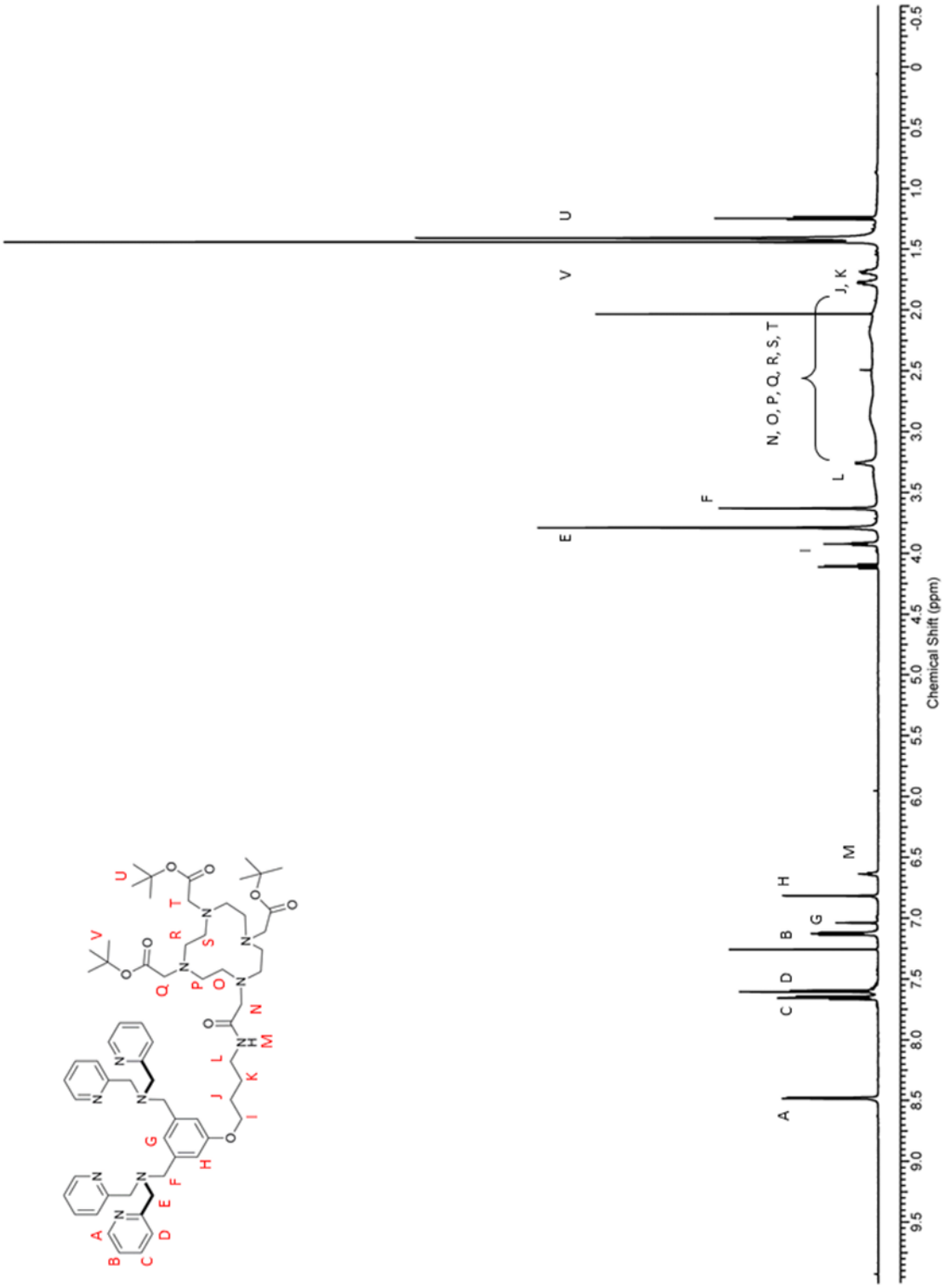
Indicator 3: A solution of 4-(trifluoromethyl)phenol (1.0 g, 6.2 mmol) in anhydrous pyridine (6.0 mL) was added dropwise to a stirred, cooled solution of $POCl_3$ (920 μL, 9.9 mmol) dissolved in anhydrous pyridine (6.0 mL). The reaction mixture was allowed to stir for 45 m and was then poured onto ice. The pH of the solution was adjusted to 9 upon addition of cyclohexylamine. The crude product was isolated by filtration to yield a white solid that was recrystallized from hot ethanol to obtain **3** as a cyclohexylammonium salt. Ion exchange chromatography with Amberlite 200c resin (Na form) produced **3** as the sodium salt (1.1 g, 41% Yield). ^{1}H NMR (400 MHz, D_2O) δ 7.23 (d, J = 12 Hz, 2H), 7.57 (d, J = 12 Hz, 2H) ppm; ^{19}F NMR (376 MHz, D_2O) δ 88.93 ppm (relative to an internal KBF_4 standard); ^{31}P NMR (160 MHz, D_2O) δ 147.81 ppm (relative to an internal $NaPF_6$ standard); Mass Spectroscopy: MS (negative ESI) calculated for $C_7H_5F_3O_4P$ ($[M-H]^-$) 240.9883, found 240.9906.

4. NMR Data

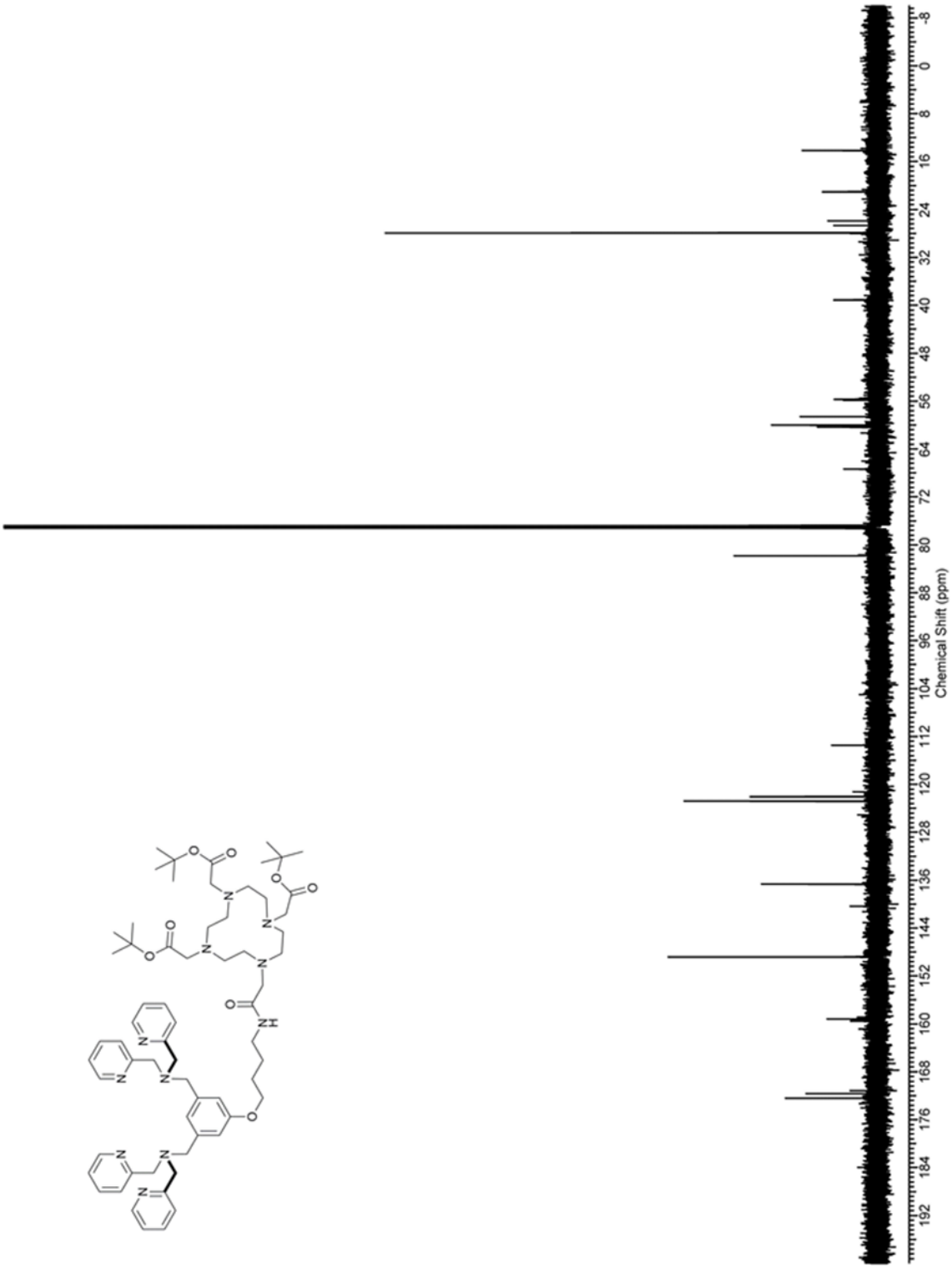
Apo-1 ^{1}H NMR



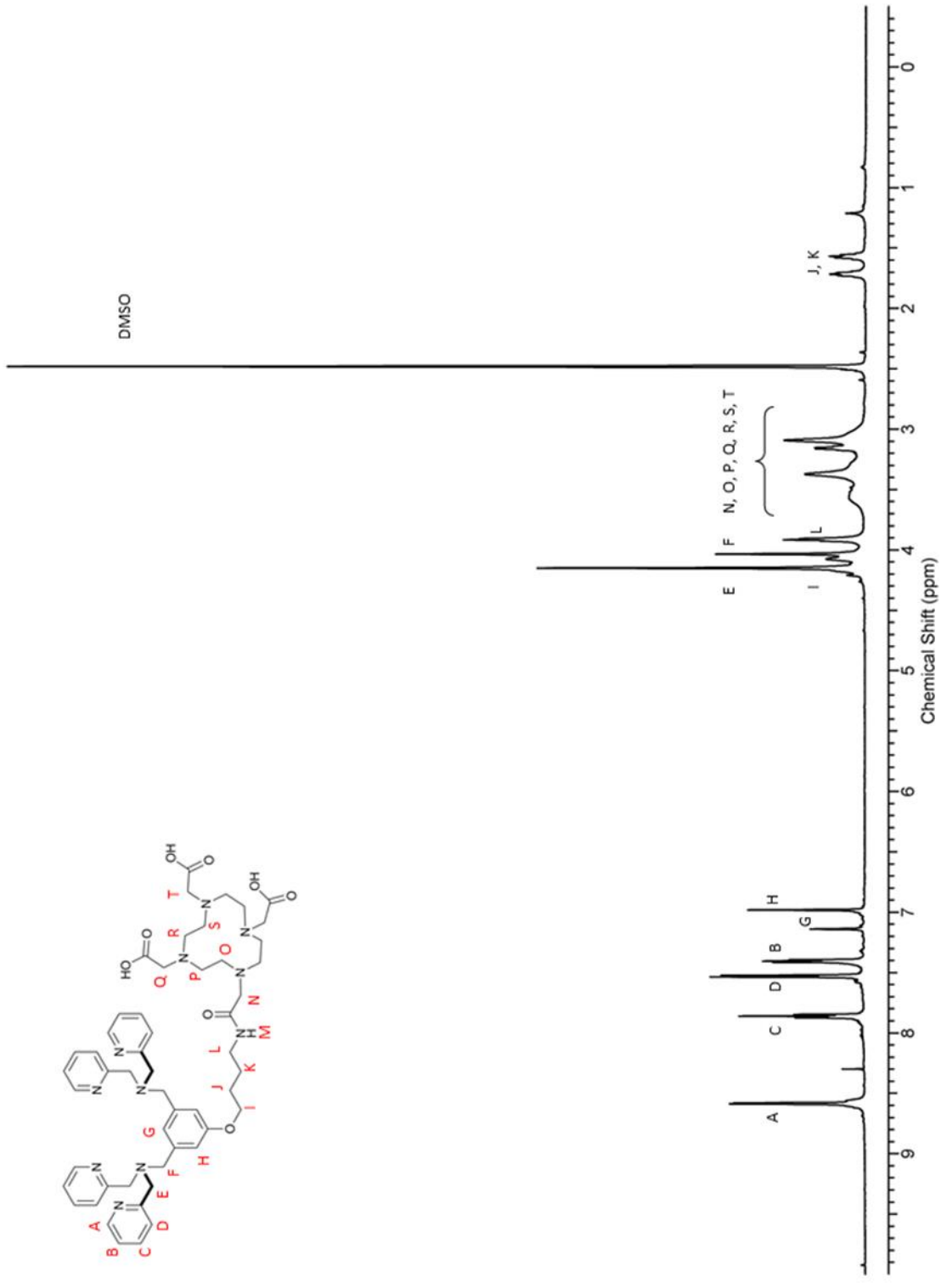
S4 ^{1}H NMR



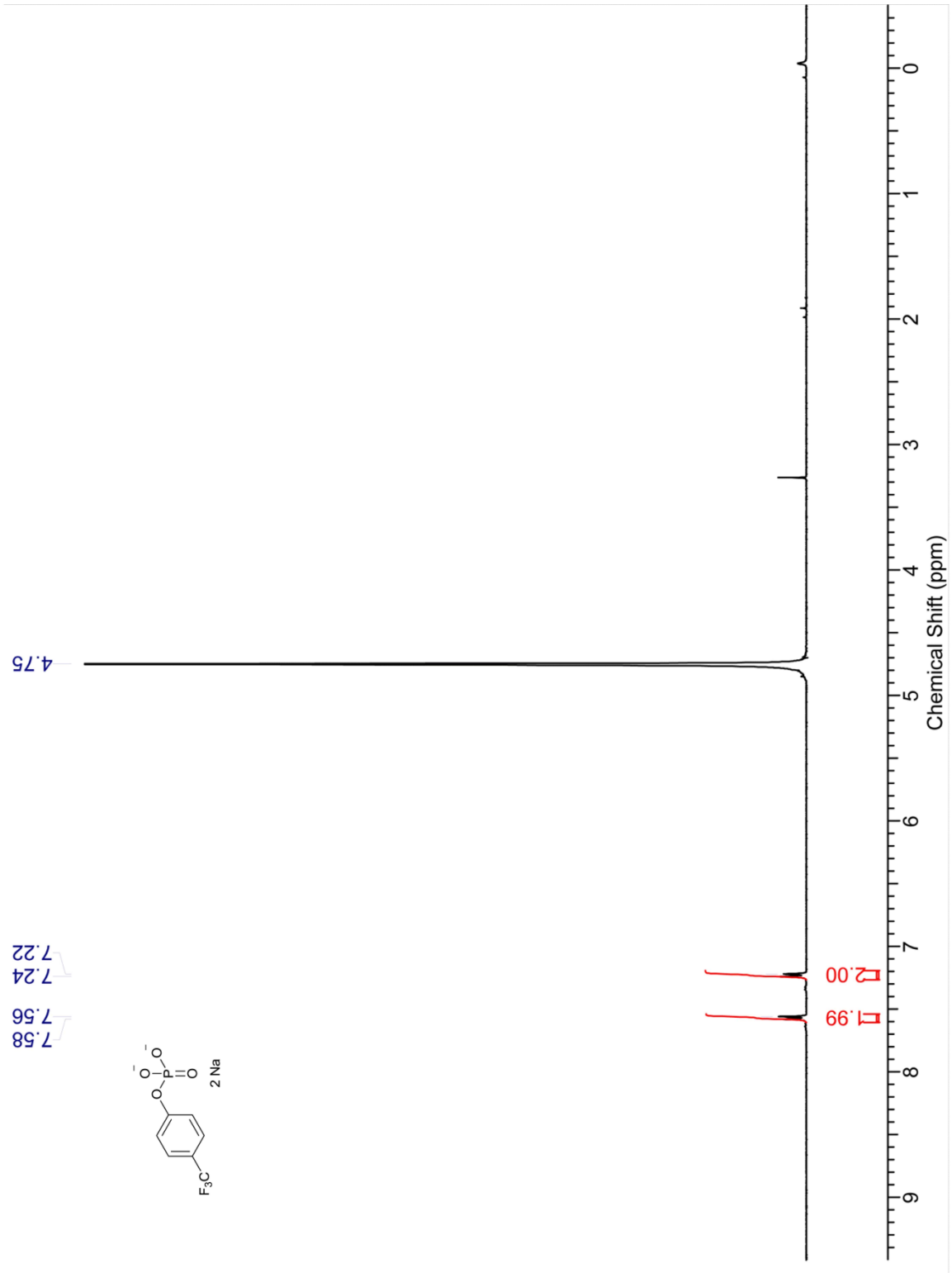
S4 ^{13}C NMR



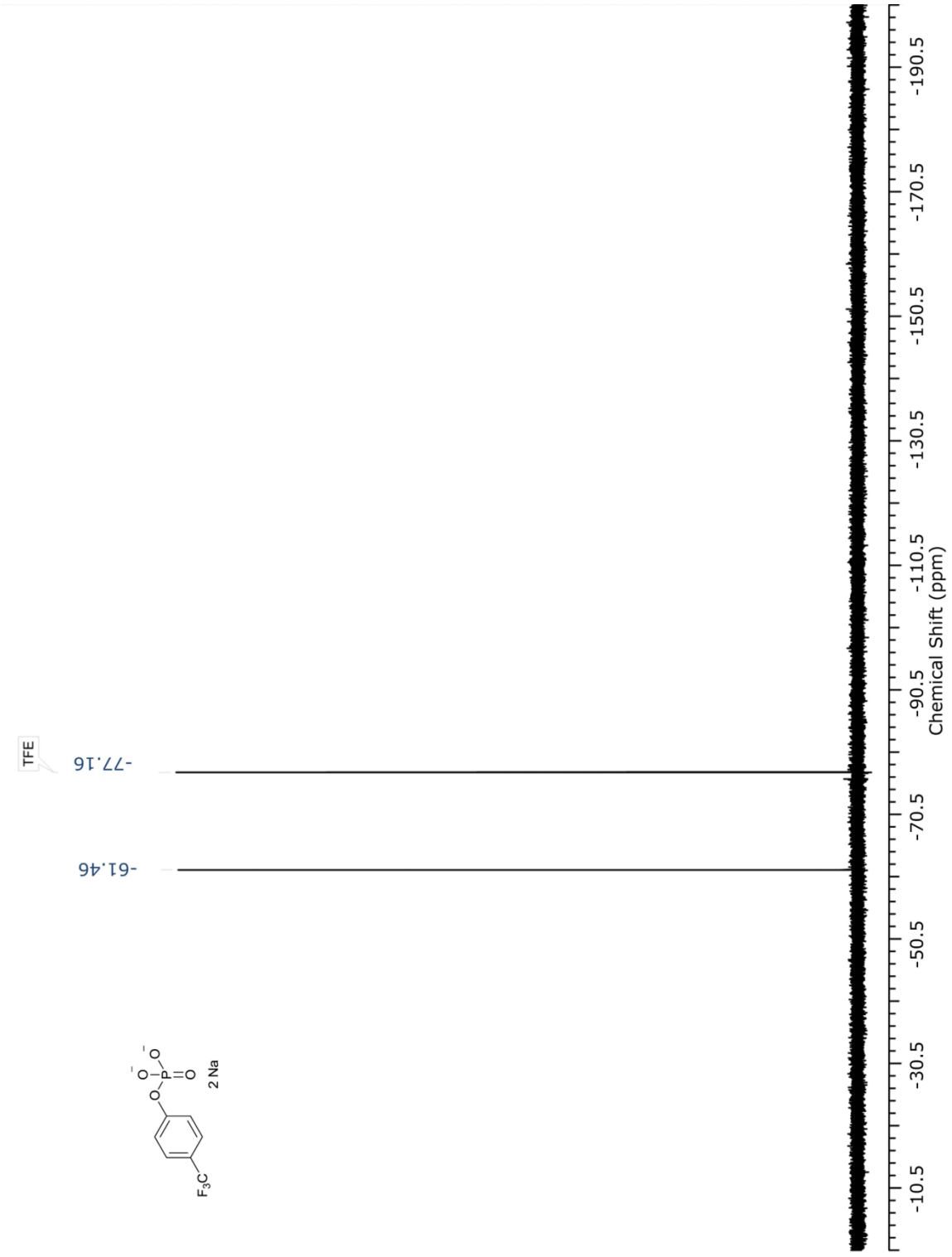
S5 ^{1}H NMR



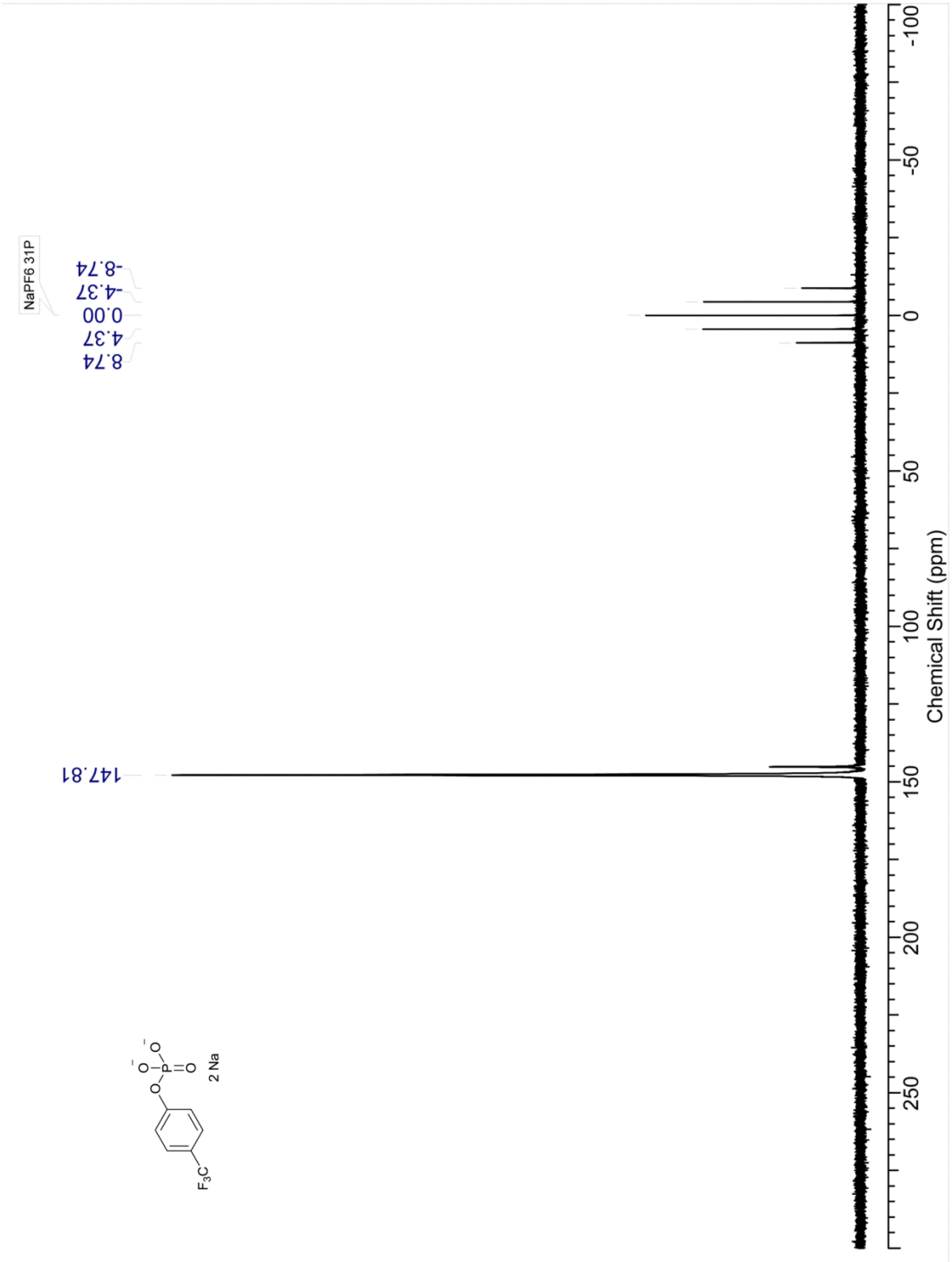
3 ^{1}H NMR



3 ^{19}F NMR (including internal trifluoroethanol (TFE) standard at -150.4 ppm)



3 ^{31}P NMR (including internal $NaPF_6$ standard at 0 ppm)



5. LCMS Data:

Dionex RSLC coupled to a Bruker micrOTOF Q II with a Dionex Acclaim RSLC 120 C18 2.2um 2.1 x 100 mm reversed-phase column. Mobile Phase A = water with 0.1 formic acid; Mobile Phase B = acetonitrile with 0.1% formic acid. Flow rate = 0.4 mL/min and column temp = 50 degrees. The initial mobile phase was 95% A/5% B which was ramped to 100%B over 6 minutes. 100% B was held for 2 minutes and then the column was re-equilibrated. The LC eluent entered the electrospray source of the micrOTOF Q II and was analyzed by the Q-TOF mass analyzer.

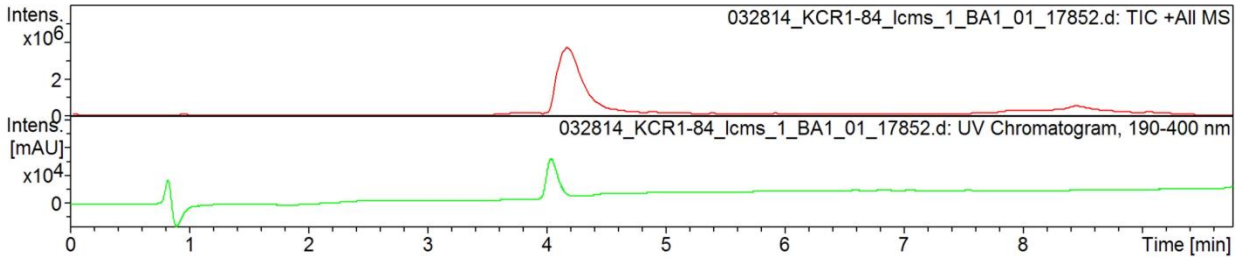


Figure S6: LCMS trace for compound **apo-1**, retention time = 4.2 minutes

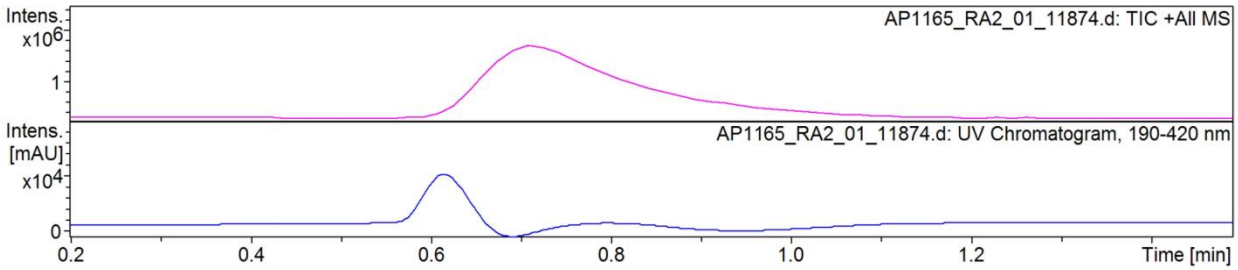


Figure S7: LCMS trace for compound **apo-2**, retention time = 0.6 minutes

6. References:

1. Y. Liu, F. A. Villamena, Y. Song, J. Sun, A. Rockenbauer and J. L. Zweier, *J. Org. Chem.*, 2010, **75**, 7796-7802.
2. C. Lakshmi, R. G. Hanshaw and B. D. Smith, *Tetrahedron*, 2004, **60**, 11307-11315.
3. (a) L. M. Matosziuk, A. S. Harney, K. W. MacRenaris and T. J. Meade, *Eur. J. Inorg. Chem.*, 2012, 2099-2107; (b) C. R. De Silva, J. Vagner, R. Lynch, R. J. Gillies and V. J. Hruby, *Anal. Biochem.*, 2010, **398**, 15-23.
4. (a) T. L. Born, J. K. Myers, T. S. Widlanski and F. Rusnak, *J. Biol. Chem.*, 1995, **270**, 25651-25655; (b) Z. Y. Zhang and R. L. VanEtten, *J. Biol. Chem.*, 1991, **266**, 1516-1525.