Tuning the Kinetic Inertness of Bi^{3+} Complexes: The Impact of Donor Atoms on Diaza-18-crown-6 Ligands as Chelators for ^{213}Bi Targeted Alpha Therapy

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1. EXPERIMENTAL PROCEDURES

General. All solvents and reagents, unless otherwise noted, were of ACS grade of higher and were purchased from sommercial sources. Solvents noted as dry were obtained following storage over 3 Å molecular sieves. Deionized water (≥18 MΩ·cm) was obtained from an Elga Purelab Flex 2 water purification system and was used in all experiments. Macropa,[1,2] macrophosphi,[3] macroquin-SO_3,[4] CHX-macropa,[5] macropaquin,[4] 2-(chloromethyl)pyridine-1-oxide (**2**),[6] and diethyl (6-chloromethyl)pyridin-2-yl)phosphonate (**3**)[7] were prepared according to published literature procedures.

High-performance liquid chromatography (HPLC) consisted of a CBM-20A communications bus module, an LC-20AP (preparative) or LC-20AT (analytical) pump, and an SPD-20AV UV/vis detector monitoring at 270 nm (Shimadzu, Japan). Analytical chromatography was carried out at a flow rate of 1.0 mL/min using an Ultra Aqueous C18 column, 100 Å, 5 µm, 250 mm × 4.6 mm (Restek, Bellefonte, PA). Semi-preparative purification was performed using an Epic Polar preparative column, 120 Å, 10 µm, 25 cm × 20 mm (ES Industries, West Berlin, NJ) at a flow rate of 14 mL/min. The solvents used in the analyses were as follows:

Solvent A: 0.1% trifluoroacetic acid (TFA) in H_2O

Solvent B: 0.1% TFA in MeOH

The following linear-gradient HPLC methods were employed:

Method 1 (solvents A/B): 10% B (0–5 min), 10–100% B (5–25 min)

Method 2 (solvents A/B): 10% B (0–10 min), 10–100% B (10–40 min)

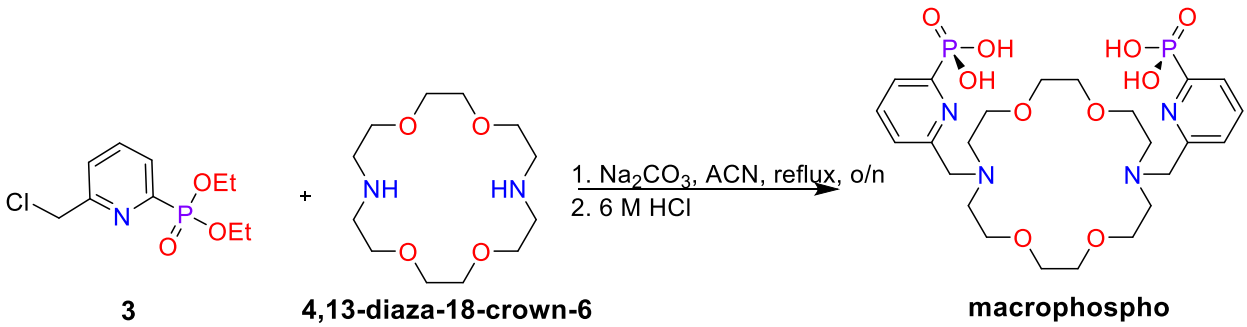
NMR spectra were recorded at 298 K, unless otherwise noted, on a Bruker AV III HD 500 MHz spectrometer equipped with a broadband Prodigy cryoprobe. Chemical shifts are reported in parts

per million (ppm). ^{1}H NMR and ^{13}C{^{1}H} NMR spectra were acquired in D_2O or DMSO-d_6 and referenced to the tetramethylsilane (TMS) internal standard (0 ppm) for ^{1}H spectra taken in DMSO-d_6 and DMSO-d_6 (39.52 ppm) for ^{13}C spectra taken in DMSO-d_6, or an internal standard of CH_3CN (2.06 ppm, ^{1}H NMR; 1.47 ppm, ^{13}C NMR) for spectra taken in D_2O. The splitting of proton resonances in the reported ^{1}H NMR spectra is defined as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. ^{19}F NMR spectra were referenced to an internal standard of fluorobenzene (–113.15 ppm). Quantitative ^{1}H and ^{19}F NMR spectra were acquired using a 30 s relaxation delay. ^{31}P{^{1}H} NMR spectra were aquired in D_2O or DMSO-d_6 and referenced to internal standards of potassium phosphate monobasic (0.08 ppm) contained within capillary tubes.

High-resolution mass spectra (HRMS) were obtained on an Exactive Orbitrap mass spectrometer in positive electrospray ionization (ESI) mode (ThermoFisher Scientific, Waltham, MA). UV/visible spectra were recorded on a Cary 8454 UV-Vis (Agilent Technologies, Santa Clara, CA) using 1-cm quartz cuvettes. Elemental analysis (EA) was performed by Atlantic Microlab, Inc. (Norcross, GA).

1.1 Ligand and Complex Syntheses

Synthesis of macrophospho

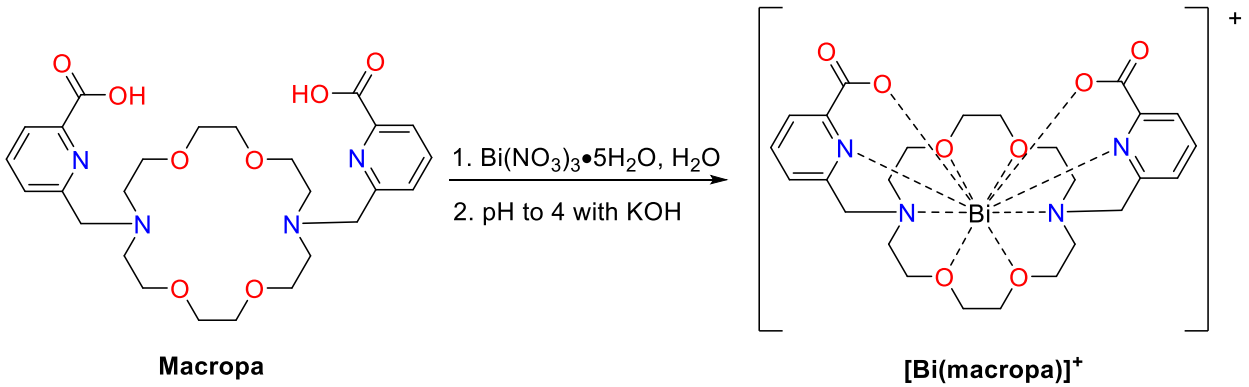


3 **4,13-diaza-18-crown-6** **macrophospho**

To a 100 mL round-bottom flask was added 4,13-diaza-18-crown-6 (0.824 g, 3.14 mmol) and dry acetonitrile (50 mL), giving a clear, colorless solution. To this solution was added anhydrous Na_2CO_3 (2.001 g, 18.88 mmol) and **3** (1.65 g, 6.27 mmol), and the resulting mixture was refluxed at 90 °C for 19.5 h. After allowing to cool to RT, the solvent was evaporated under vacuum, giving a yellow oil. This oil was then dissolved in dichloromethane (DCM) (170 mL), to which H_2O (10 mL) was added. The organic layer was then separated and dried over Na_2SO_4, and the solvent was removed under vacuum to obtain the ethyl ester, which was used in the following step without further charcterization.. The ethyl ester was then hydrolyzed by heating a solution of the compound at 90 °C in 6 M HCl (15 mL) for a total period of 48 h. The solvent was then removed under vaccum, and the title compound was afforded after semi-preparative reverse-phase HPLC (method 2, with impure fractions undergoing an additional purification using method 2). The solvent was removed under vacuum before the obtained TFA salt of the compound was changed to an HCl salt through three consecutive washings with 6 M HCl, with the solvent removed under vacuum between each washing. H_2O was then added and the title compound was obtained after lyophilization (0.823 g, 34.0%). A residual amount of TFA was obseved by NMR, but quantitative ^1H and ^{19}F NMR with an internal fluorobenzene standard found the average number of TFA per ligand to be insignificant (<0.1). ^1H NMR (500 MHz, 298 K, D_2O) δ = 8.02 (m, 2H), 7.89 (t, *J* = 6.81 Hz, 2H), 7.60 (d, *J* = 7.81 Hz, 2H), 4.73 (s, 4H), 3.94 (br t, *J* = 4.66 Hz, 8H), 3.66 (m, 16 H). $^{13}C\{^1H\}$ NMR (126 MHz, D_2O) δ = 155.5 (d, *J* = 211.6 Hz), 149.7 (d, *J* = 19.2 Hz), 140.7 (d, *J* = 11.1 Hz), 128.0, 127.8, 127.8, 127.7, 70.4, 64.7, 57.7, 54.7. Due to overlap, while the four peaks from δ 128 – 127 belong to two ^{13}C doublets, the four peaks can not be assigned unambiguously, nor can *J* values be determined. ^{19}F NMR (470 MHz, 298 K, D_2O) δ = –75.0 (TFA). $^{31}P\{^1H\}$ NMR (202 MHz, 298 K, D_2O) δ 6.3. Elem. anal. Found: C, 37.27; H, 5.74;

N, 7.02. Calcd for $[C_{24}H_{38}N_4O_{10}P_2]\cdot 4.2HCl\cdot H_2O$: C, 37.16; H, 5.74; N, 7.22. ESI-MS: Found: *m/z* 303.11142. Calcd for $[C_{24}H_{40}N_4O_{10}P_2]^{2+}$: *m/z* 303.11043. Found: *m/z* 605.21561. Calcd for $[C_{24}H_{39}N_4O_{10}P_2]^{+}$: *m/z* 605.21359. Found: *m/z* 627.19747. Calcd for $[C_{24}H_{38}N_4NaO_{10}P_2]^{+}$: *m/z* 627.19554. HPLC: t_R = 14.105 (Method 1).

Synthesis of [Bi(macropa)]$^+$

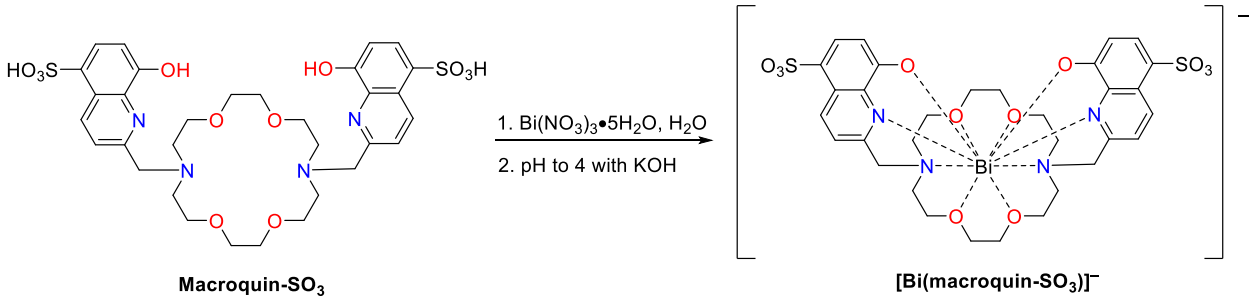


Macropa **[Bi(macropa)]$^+$**

The syntheseses of all Bi^{3+} complexes followed a procedure that was previously reported in the literature for related ligands.[8] To a solution of H_2macropa·2HCl·4H_2O (0.407 g, 0.600 mmol) in H_2O (30 mL) was added $Bi(NO_3)_3\cdot 5H_2O$ (0.295 g, 0.608 mmol). The mixture, which became cloudy immediately, was stirred at RT for 30 minutes, and then the pH was adjusted to 4 via aliquots of 2 M KOH. The resulting white suspension was heated at reflux at 100 °C for 19 h and then allowed to cool to RT before methanol (30 mL) was added. The white suspension was filtered through a 0.20 µm nylon membrane, and the clear, colorless filtrate was concentrated under vacuum before an aliquot of H_2O was added and the solution lyophilized to give a white, fluffy solid. This solid was purified by preparative HPLC with method 1. Fractions containing pure material were concentrated under vacuum before an aliquot of H_2O was added for lyophilization to give the compound as a TFA salt (0.428 g, 70.0%). The average number of TFA molecules per complex was determined to be 2.3 by quantitative ^{1}H and ^{19}F spectroscopy using fluorobenzene as

an internal standard. ^{1}H NMR (500 MHz, 298 K, D_2O) δ = 8.47 (t, *J* = 7.87 Hz, 2H), 8.20 (d, *J* = 7.70 Hz, 2 H), 8.14 (d, *J* = 7.91 Hz, 2H), 4.63 (d, 16.39 Hz, 2H), 4.49 (d, *J* = 16.44 Hz, 2H), 3.98–3.83 (m, 4H), 3.81–3.62 (m, 4H), 3.57–3.45 (m, 4H), 3.44–3.33 (m, 2H), 3.25–3.08 (m, 4H), 2.92–2.81 (m, 2H) 2.81–2.71 (m, 2H), 2.57–2.46 (m, 2H). ^{13}C{^{1}H} NMR (126 MHz, 298 K, D_2O) δ = δ 170.9, 163.7 (TFA), 163.4 (TFA), 159.0, 148.6, 143.8, 128.5, 126.5, 118.1 (TFA), 115.8 (TFA), 69.3, 68.9, 68.1, 65.4, 61.9, 56.3, 55.6. ^{19}F NMR (470 MHz, 298 K, D_2O) δ = –74.9 (TFA). Elem anal. Found: C, 36.27; H, 3.61; N, 5.51. Calcd for $[C_{26}H_{34}BiN_4O_8][C_2F_3O_2]\cdot 1.3C_2HF_3O_2\cdot H_2O$: C, 36.07; H, 3.69; N, 5.50. ESI-MS: Found: *m/z* 739.21601. Calcd for $[C_{26}H_{34}BiN_4O_8]^+$: *m/z* 739.21750. HPLC: t_R = 19.092 min (Method 1).

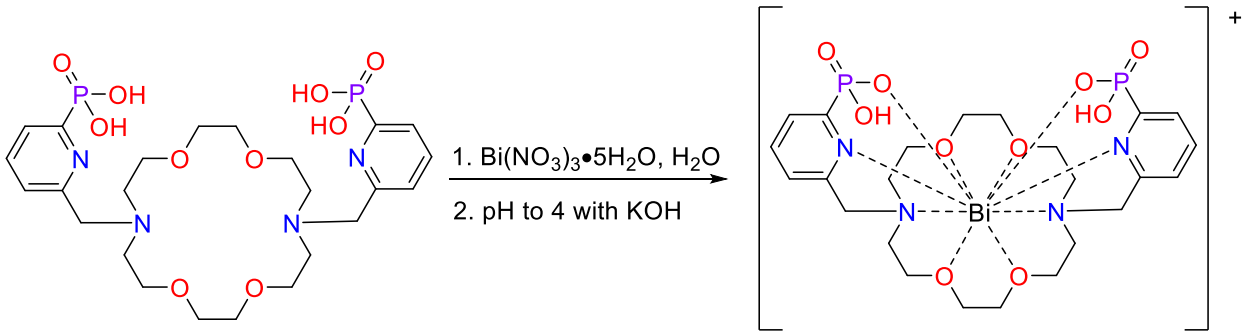
Synthesis of [Bi(macroquin-SO$_3$)]$^-$



To an opaque, off-white suspension of H_4(macroquin-SO_3)·$6H_2O$ (0.150 g, 0.178 mmol) in H_2O (4 mL) was addded $Bi(NO_3)_3\cdot 5H_2O$ (0.120 g, 0.247 mmol). The mixture, which became an intensely yellow immediately, was stirred at RT for 30 minutes, and then , the pH was adjusted to 4 with aliquots of 2 M KOH. The resulting opaque, yellow suspension was heated at reflux at 100 °C for 21 h. The suspension was then allowed to cool to RT before being transfered to a centrifuge tube and centrifuged at 3000 rpm for 5 minutes. The resulting clear, yellow supernatant was then purified through preparative HPLC (Method 1) to give the title coumpound following concentration under vacuum of fractions containing pure material and subsequent addition of H_2O

for lyophilization (0.141 g, 75.1%). The average number of TFA molecules per complex was determined to be < 0.1 by quantitative ^{1}H and ^{19}F NMR spectroscopy using flurobenzene as an internal standard. ^{1}H NMR (500 MHz, 298 K, D_2O) δ = 9.30 (d, *J* = 8.71 Hz, 2H), 8.23 (d, *J* = 8.23 Hz, 2H), 8.14 (d, *J* = 8.83 Hz, 2H), 6.86 (d, *J* = 8.29 Hz, 2H), 4.33 (d, *J* = 16.97 Hz), 3.76–3.63 (m, 4H), 3.63–3.54 (m, 4H), 3.40 (t, *J* = 10.51 Hz), 3.28–3.22 (2H), 3.21–3.13 (m, 4H), 2.95 (d, *J* = 12.91 Hz, 2H), 2.83–2.76 (m, 2H), 2.57–2.50 (m, 2H), 2.01–1.95 (m, 2H). A peak overlaps with the solvent peak, which is most likely the remaining 2H from the methylene arm linker. $^{13}C\{^{1}H\}$ NMR (126 MHz, 298 K, D_2O) δ 160.6, 160.0, 143.4, 139.8, 130.8, 126.9, 126.6, 122.9, 115.8, 68.8, 68.5, 68.0, 65.9, 61.9, 56.7, 55.0. ^{19}F NMR (470 MHz, 298 K, D_2O) δ = –74.9 (TFA). Elem anal. Found: C, 36.59; H, 4.69; N, 5.11. Calcd for $[C_{32}H_{37}BiN_4O_{12}S_2]\cdot 6H_2O$: C, 36.51; H, 4.88; N, 5.32. ESI: Found *m/z*: 943.16595. Calcd for $[C_{32}H_{38}BiN_4O_{12}S_2]^+$: 943.17260. Found *m/z*: 965.14764. Calcd for $[C_{32}H_{37}BiN_4NaO_{12}S_2]^+$: *m/z* 965.15454. Found *m/z*: 987.12954. Calcd for $[C_{32}H_{36}BiN_4Na_2O_{12}S_2]^+$: 987.13649. HPLC: t_R = 15.663 minutes (Method 1).

Synthesis of [Bi(macrophospho)]$^+$

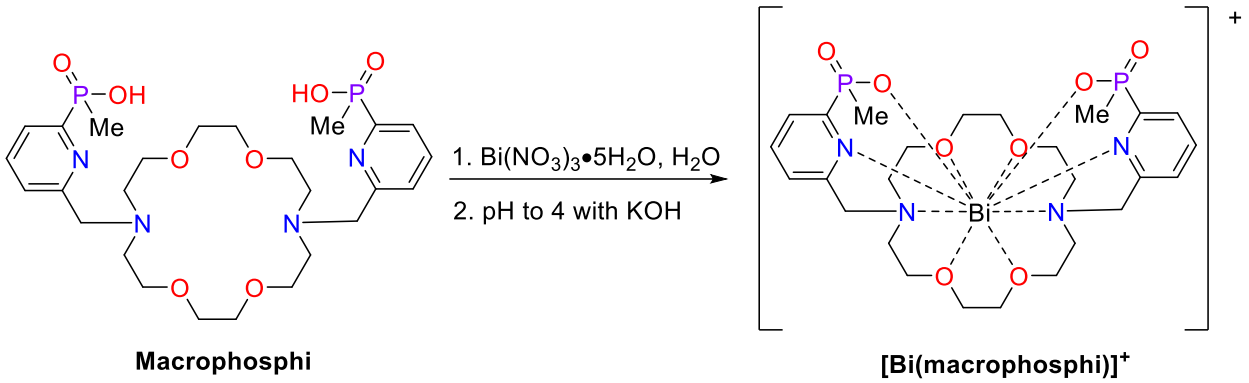


Macrophospho **[Bi(macrophospho)]$^+$**

To a clear, colorless aqeuous stock solution of macrophospho (18.0 mL of 22.5 mM ligand, 0.404 mmol) was added $Bi(NO_3)_3\cdot 5H_2O$ (0.259 g, 0.534 mmol). The mixture, which became a white, cloudy suspension immediately, was stirred at RT for 30 minutes, and then the pH was adjusted to

4 using aliquots of 2 M KOH. The resulting white suspension was then heated at reflux at 100 °C for 21 h before being allowed to cool to RT followed by an addition of methanol (5 mL) and subsequent filtration through a 0.22 μm nylon membrane. The clear, colorless filrate was then concentrated down over vacuum before being purified to give the title compound through preparative HPLC with method 1. Fractions containing pure material were concentrated under vacuum before an aliquot of H_2O was added for lyophilization to give the compound as a TFA salt (0.321 g, 70.2% yield). The average number of TFA molecules per complex was determined to be 2.5 by quantitative ^{1}H and ^{19}F NMR spectroscopy using flurobenzene as an internal standard. ^{1}H NMR (500 MHz, 298 K, D_2O) δ = 8.37 (br s, 2H), 8.06 – 7.92 (m, 4H), 4.54 (br d, *J* = 15.42 Hz, 2H), 4.41 (br d, *J* = 15.51 Hz, 2H), 4.03 (br s, 2H), 3.84 (br s, 2H), 3.72 (br s, 4H), 3.58 – 3.34 (m, 6H), 3.24 – 3.14 (m, 2H), 3.09 (br s, 2H), 2.91 (br s, 2H), 2.55 (br s, 2H). $^{13}C\{^{1}H\}$ (126 MHz, 298 K, D_2O) δ = 163.5 (q, *J* = 36.1 Hz, TFA), 159.3 (d, *J* = 21.1 Hz), 155.8, 154.2, 142.3, 128.3 (d, *J* = 18.4 Hz) , 127.3, 119.7 (CH_3CN), 116.9 (q, *J* = 294.60 Hz, TFA), 68.9, 68.6, 67.6, 65.3, 62.7, 56.5, 55.3. ^{19}F NMR (470 MHz, 298 K, D_2O) δ = –74.9 (TFA). $^{31}P\{^{1}H\}$ NMR (202 MHz, 298 K, D_2O) δ = 13.46. Elem anal. Found: C, 30.93; H, 3.65; N, 4.83. Calcd for $[C_{24}H_{36}BiN_4O_{10}P_2][C_2F_3O_2]\cdot 1.5C_2HF_3O_2\cdot 2H_2O$: C, 30.78; H, 3.70; N, 4.95. ESI-MS: Found *m/z* 406.08818. Calcd for $[C_{24}H_{37}BiN_4O_{10}P_2]^{2+}$: *m/z* 406.08889. Found *m/z* 811.16912. Calcd for $[C_{24}H_{36}BiN_4O_{10}P_2]^{+}$: *m/z* 811.17050. Found *m/z*: 833.15062. Calcd for $[C_{24}H_{35}O_{10}N_4BiNaP_2]^{+}$: *m/z* 833.15245. HPLC: t_R = 18.041 min (Method 1).

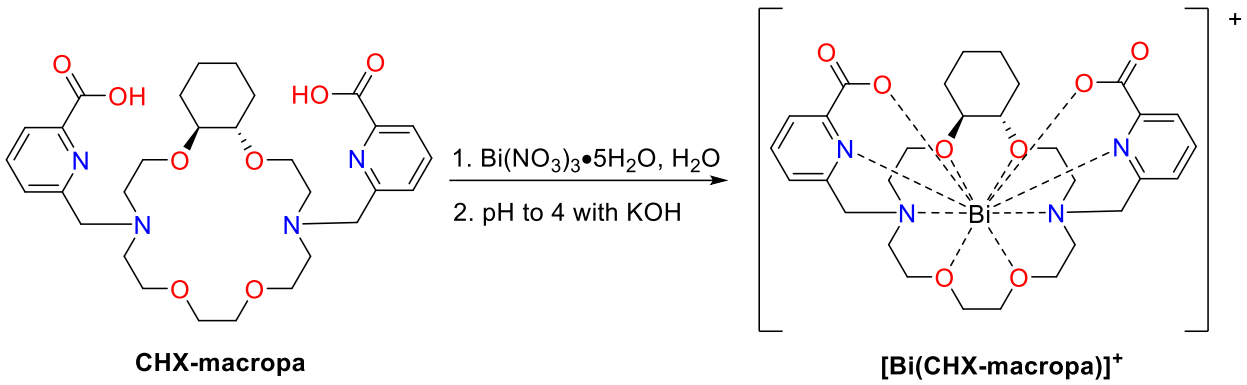
Synthesis of [Bi(macrophosphi)]$^{+}$



To a clear, colorless aqueous solution of macrophosphi (12 mL of 21.8 mM ligand, 0.262 mmol) was added $Bi(NO_3)_3 \cdot 5H_2O$ (0.167 g, 0.344 mmol). The mixture, which became a cloudy, white suspension immediately, was stirred at RT for 30 minutes before being adjusted to pH 4 with aliquots of 1 M KOH. The cloudy, white suspension was then heated at reflux at 100 °C for 14 h before the suspension was allowed to cool to RT and methanol (5 mL) was added, followed by filtration through a 0.20 µm nylon membrane. The clear, colorless filtrate was then concentrated down under vacuum to a white, fluffy solid. The title compound was then obtained through preparative HPLC (method 1). Fractions containing pure material were concentrated under vacuum, after which an aliquot of H_2O was added for lyophilization, giving the title compound as a white solid as a TFA salt. (0.213 g, 73.3%). The average number of TFA molecules per complex was determined to be 2.5 by quantitative ^{1}H and ^{19}F NMR spectroscopy using flurobenzene as an internal standard. ^{1}H NMR (600 MHz, 393.2 K, DMSO-d_6) δ = 8.33 (td, J = 7.61, 3.36 Hz, 2H), 7.93 (d, J = 7.70 Hz, 2H) 7.88 (t, J = 6.35 Hz, 2H), 3.87 (br s, 3H), 3.73 – 3.44 (m, 10H), 3.44 – 3.28 (m, 5H), 3.06 (m, 2H), 2.96 – 2.85 (m, 2H), 2.85 – 2.73 (m, 2H), 2.73 – 2.64 (m, 2H), 2.63 – 2.51 (m, 2H), 1.27 (d, J = 14.74 Hz, 6H). A large, broad peak is present around 4.69 – 3.96. Because the solid complex contains some waters of hydration, these peaks are likely the effect of heating the sample over the boiling point of water. $^{13}C\{^{1}H\}$ NMR: (126 MHz, 393.2 K, DMSO-d_6) δ 160.1 (d, J = 129.2 Hz), 158.4 (d, J = 12.9 Hz), 140.1 (d, J = 8.9 Hz), 126.9 (d, J = 18.4 Hz),

125.2, 67.7, 67.0, 66.5, 64.9, 61.0, 56.1, 53.8, 20.4 (d, J = 99.2 Hz). ^{19}F NMR (470 MHz, 298 K, D_2O) δ = –74.5 (TFA). $^{31}P\{^1H\}$ NMR: (202 MHz, 393.2 K, DMSO-d_6) δ = 26.4. Elem anal. Found: C, 33.58; H, 4.17; N, 5.17. Calcd for $[C_{26}H_{40}BiN_4O_8P_2][C_2F_3O_2]\cdot 1.5C_2HF_3O_2\cdot H_2O$: C, 33.56; H, 3.95; N, 5.05. ESI-MS: Found: *m/z* 404.10820. Calcd for $[C_{26}H_{41}BiN_4O_8P_2]^{2+}$: *m/z* 404.10962. Found: *m/z* 807.20897. Calc for $[C_{26}H_{40}BiN_4O_8P_2]^{+}$: *m/z* 807.21197. HPLC: t_R = 21.253 min (Method 1).

Synthesis of [Bi(CHX-macropa)]$^+$

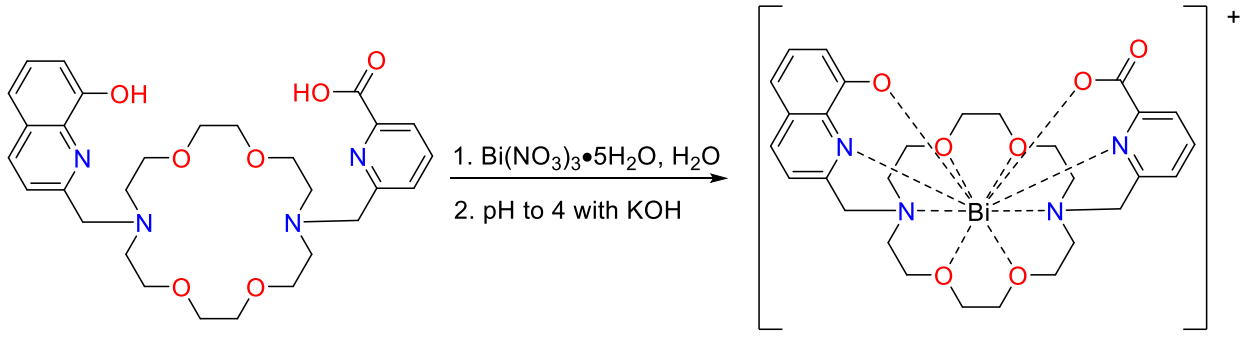


CHX-macropa **[Bi(CHX-macropa)]$^+$**

To a solution of H_2CHX-macropa·3.4HCl·1.4H_2O (0.0684 g, 0.0930 mmol) in H_2O (4 mL) was added $Bi(NO_3)_3\cdot 5H_2O$ (0.0589 g, 0.121 mmol). The mixture, which became cloudy immediately, was stirred at RT for 30 minutes, after which the pH was adjusted to 4 with aliquots of 2 M KOH. The resulting white, cloudy suspension was heated at reflux at 100 °C for 19 h before being allowed to cool to RT followed by an addition of methanol (4 mL). The suspension was then filtered through a 0.22 µm nylon membrane, giving a clear, colorless filtrate that was then concentrated down under vacuum to a white residue before being purified through preparative HPLC with method 1. Fractions containing pure material were concentration under vacuum, after which an aliquot of H_2O was added for subsequent lyophilization to give the title compound as a

TFA salt (0.074 g, 74.9%). The average number of TFA molecules per complex was determined to be 2.0 by quantitative ^{1}H and ^{19}F NMR spectroscopy using flurobenzene as an internal standard. ^{1}H NMR (500 MHz, 298 K, D_2O) δ = 8.46 (q, *J* = 8.02 Hz, 2H), 8.22 (t, *J* = 6.93 Hz, 2H), 8.15 (t, *J* = 9.20 Hz, 2H), 4.61 (d, *J* = 15.95 Hz, 2H), 4.51 (d, *J* = 15.95 Hz, 1H), 4.39 (*J* = 15.98 Hz, 1H), 4.21 – 4.10 (m, 1H), 4.05 – 3.91 (m, 2H), 3.90 – 3.80 (m, 1H), 3.77 – 3.62 (m, 3H), 3.62 – 3.54 (m, 2H), 3.53 – 3.44 (m, 1H), 3.44 – 3.31 (s, 1H), 3.31 – 3.18 (m, 2H), 3.18 – 3.04 (m, 3H), 3.04 – 2.87 (m, 3H), 2.77 – 2.67 (s, 2H), 1.94 (d, *J* = 10.94 Hz, 1H), 1.53 (d, 10.87 Hz, 2H), 1.37 (s, 1H), 1.08 – 0.97 (m, 1H), 0.97 – 0.84 (m, 1H), 0.75 – 0.61 (m, 1H), 0.58 – 0.45 (m, 1H). $^{13}C\{^{1}H\}$ NMR (126 MHz, 298 K, D_2O) δ 170.8, 170.3, 163.6 (q, *J* = 34.0 Hz, TFA), 159.1, 158.7, 149.1, 148.9, 143.8, 143.6, 129.1, 128.6, 126.7, 126.5, 119.8 (CH_3CN), 116.9 (q, *J* = 291.3 Hz), 80.9, 78.3, 69.5, 68.5, 67.4, 64.7, 63.4, 62.6, 61.3, 60.5, 57.2, 55.9, 55.6, 54.1, 28.8, 28.1, 23.8, 23.7. ^{19}F NMR (470 MHz, 298 K, D_2O) δ = –74.9 (TFA). Elem anal. Found: C, 38.52; H, 4.12; N, 5.25; Calcd for $[C_{30}H_{40}BiN_4O_8][C_2F_3O_2]\cdot C_2HF_3O_2\cdot 2H_2O$: C, 38.65; H. 4.29; N, 5.30. ESI: Found *m/z*: 793.26753. Calcd for $[C_{30}H_{40}BiN_4O_8]^+$: *m/z* 793.26445. HPLC: t_R = 22.159 min (Method 1).

Synthesis of [Bi(macropaquin)]$^+$



Macropaquin **[Bi(macropaquin)]$^+$**

To a clear, slightly greenish solution of H_2macropaquin·4HCl·4.5H_2O (0.101 g, 0.130 mmol) in H_2O (5 mL) was added $Bi(NO_3)_3$·5H_2O (0.097 g, 0.199 mmol). The mixture, which became a cloudy green suspension immediately, was stirred for 30 minutes at RT before the pH was adjusted to 4 with aliquots of 2 M KOH. The resulting cloudy greenish-yellow suspension was heatedat 90 °C for 18 h and then allowed to cool to RT. The suspension was transferred to a centrifuge tube and centrifuged at 3000 rpm for 5 minutes, giving a clear, yellow supernatant that was separated from the yellow pellets. The supernatant was then concentated down under vacuum until most of the solvent was removed, followed by purification through preparative HPLC (method 1). Fractions containing pure material were concentrated down under vacuum, followed by addition of an aliquot of H_2O for subsequent lyophilization to give the title compound as a yellow, fluffy solid (0.104 g, 80.8%). The average number of TFA molecules per complex was determined to be 1.7 by quantitative ^{1}H and ^{19}F NMR spectroscopy using flurobenzene as an internal standard. ^{1}H NMR (500 MHz, 298 K, D_2O) δ = 8.60 (m, 1H), 8.40 (m, 1H), 8.17 (m, 1H), 8.10 (m, 1H), 7.88 (m, 1H), 7.63 (m, 1H), 7.18 (m, 1H), 6.86 (m, 1H). 4.74 – 4.67 (m, 1H), 4.65 – 4.58 (m, 1H), 4.43 – 4.32 (m, 1H), 4.28 – 4.17 (m, 1H), 3.86 – 3.63 (m, 5H), 3.61 – 3.30 (m, 8H), 3.25 – 3.14 (m, 1H), 3.14 – 2.90 (m, 4H), 2.86 – 2.65 (m, 3H), 2.45 (br s, 1H), 2.37 – 2.26 (m, 1H), 2.23 – 2.10 (m, 1H). $^{13}C\{^{1}H\}$ NMR (126 MHz, D2O) δ = 170.7, 163.5 (q, *J* = 34.78 Hz, TFA), 160.5, 159.0, 156.3, 148.9, 143.5, 142.9, 142.2, 130.8, 130.6, 128.1, 126.1, 121.7, 119.7 (CH_3CN), 118.5, 116.9 (q, *J* = 290.0 Hz, TFA), 117.1, 68.9, 68.7, 68.7, 68.1, 67.8, 65.7, 65.7, 62.1, 61.7, 56.4, 56.3, 55.3. ^{19}F NMR: –74.9 (TFA). Elem. anal. Found: C, 39.27; H, 4.05; N, 5.71. Calcd for $[C_{29}H_{36}BiN_4O_7][C_2F_3O_2]$·0.7$C_2HF_3O_2$·$H_2O$: C, 39.29; H, 4.14; N, 5.66. ESI: Found *m/z*: 761.23338. Calcd for $[C_{29}H_{36}BiN_4O_7]^+$: *m/z* 761.23823. HPLC: t_R = 22.300 min (Method 1).

1.2 X-Ray Diffraction Studies

Single crystals of [Bi(macropa)](NO_3)·dioxane were obtained by the slow vapor diffusion of dioxane into an aqueous solution containing a 1:1 mixture of the ligand and $Bi(NO_3)_3 \cdot 5H_2O$. Single crystals of [Bi(macrophospho)](TFA)·H_2O were grown from the slow vapor diffusion of acetone into a DMF solution containing a TFA salt of the purified complex. Single crystals of [Bi(macrophosphi)](TFA)·H_2O were grown from slow by the slow vapor diffusion of diethyl ether into a DMF solution containing the TFA salt of the purified complex.

Low-temperature X-ray diffraction data for the crystals were collected on a Rigaku XtaLAB Synergy diffractometer coupled to a Rigaku Hypix detector with Cu Kα radiation (λ = 1.54184 Å), from a PhotonJet micro-focus X-ray source at 100 K ([Bi(macropa)](NO_3)·dioxane and [Bi(macrophosphi)](TFA)·H_2O) or 200 K ([Bi(macrophospho)](TFA)·H_2O). The diffraction images were processed and scaled using the CrysAlisPro software.[9] The structures were solved through intrinsic phasing using SHELXT[10] and refined against F^2 on all data by full-matrix least squares with SHELXL[11] following established refinement strategies.[12] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms bound to carbon were included in the model at geometrically calculated positions and refined using a riding model. Hydrogen atoms bound to oxygen were located in the difference Fourier map and subsequently refined semi-freely with the help of distance restraints. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the Ueq value of the atoms they are linked to (1.5 times for methyl groups). [Bi(macrophospho)](TFA)·H_2O contains disordered solvent molecules of DMF that were included in the unit cell but could not be satisfactorily modeled. Therefore, those solvents were treated as diffuse contributions to the overall scattering without specific atom positions using the solvent mask routine in Olex2.[13] Details of the data quality and a summary of the residual values of the refinements are listed in Table S1–S15.

1.3 Thermodynamic Solution Studies

Protonation constants of macrophospho were obtained by potentiometric titration using a Metrohm Titrando 888 titrator equipped with a Ross Orion combination electrode (8103BN, ThermoFisher Scientific), a Metrohm 806 exchange unit with an automatic buret (10 mL), and *Tiamo 2.5* software. Detailed descriptions of the titration setup and procedures have been reported in previous publications.[4,5] Briefly, all titration solutions were maintained at a constant ionic strength of 0.1 M using KCl and equilibrated for 25 min prior to the addition of titrant. Before every ligand titration, the electrode was calibrated in terms of the hydrogen-ion concentration by titrating a solution of standardized HCl (10 mM) containing supporting electrolyte (H/KCl = 0.1 M) with standardized KOH. Data within the pH ranges of 2.2−3.2 and 10.8−11.3 were analyzed using the program *Glee* (version 3.0.21)[14] to obtain the standard electrode potential (E_0) and slope factor. The H_2O ion product (pK_w = 13.78) was taken from the literature.[15] A stock solution of macrophosphi was prepared in H_2O.

The ligand protonation constants were measured by adding standardized KOH to an aqueous solution (20 mL) of ligand (~0.02 mmol), HCl (0.1 mmol, and KCl (1.9 mmol). The titration method employed a 0.1 mV min^{-1} drift limit and a maximum wait time of 180 s between additions of aliquots of base.

The protonation constants were refined using the program *Hyperquad2013*.[16] The proton and ligand concentrations were admitted as a refineable parameter. The protonation constants, defined in Eq. 1 below, were calculated from the averge of three independent titrations. The errors provided correspond to 1 standard deviation.

$$K_{ai} = \frac{[H_iL]}{[H_{i-1}L][H^+]} \quad (1)$$

1.4 DTPA Challenges

The pH of a 100 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer was adjusted to 7.4 using aqueous NMe_4OH. The ionic strength was set at 100 mM using NMe_4Cl. A stock solution of diethylenetriaminepentaacetic acid (DTPA, 125 mM) was made in this MOPS buffer by adjusting the pH of the initial suspension to 7.4 using aqueous NMe_4OH. The preformed Bi^{3+} complexes were challenged with DTPA. Challenges were initiated by adding an aliquot of complex solution to a solution containing DTPA in MOPS buffer so that the initial concentrations of macrocyclic Bi^{3+} complex were 100 μM ($[Bi(macropa)]^+$, $[Bi(CHX\text{-}macropa)]^+$, $[Bi(macrophospho)]^+$, and $[Bi(macrophosphi)]^+$) or 50 μM ($[Bi(macroquin\text{-}SO_3)]^-$ and $[Bi(macropaquin)]^+$) and the initial concentrations of DTPA were 100 mM ($[Bi(macropa)]^+$, $[Bi(CHX\text{-}macropa)]^+$, $[Bi(macrophospho)]^+$, and $[Bi(macrophosphi)]^+$) or 50 mM ($[Bi(macroquin\text{-}SO_3)]^-$ and $[Bi(macropaquin)]^+$). The solutions were analyzed by UV spectroscopy until no further spectral changes were observed. For $[Bi(macroquin\text{-}SO_3)]^-$, the solution was tracked repeatedly over the course of 21 days for any spectral changes. The final solution was then analyzed with HPLC to ensure complete transchelation had occured for labile complexes or no transchelation had occured for $[Bi(macroquin\text{-}SO_3)]^-$. The half-lives reported are the average of three replicates.

1.5 Computational Details

DFT calculations were performed using the Gaussian16 software.[17] The geometries of the complexes were optimized in the gas phase using the crystal structure of the complexes as starting geometries when available. The optimizations were performed using the TPSSh functional[18] and the TZVP[19] basis set for all atoms except Bi, which was treated with the large-core relativistic effective core potential (ECP60MDF) and related basis set.[20] Frequency calculations were

performed to ensure that the optimized geometry was at a local minimum on the potential energy surface. Natural bond order analysis was performed using the NBO software as implemented in Gaussian.[21] Quantum theory of atoms in molecules (QTAIM) analysis was completed using the program Multiwfn (version 3.7).[22] The (3,-1) critical points in ρ(r) were located between the donor atoms and the Bi center and the values of ρ(r), the energy density parameters V(r), G(r), and H(r), the ratio of potential and kinetic energies |V|/G, and the normalized total energy density H/ρ were calculated at the bond critical point. The values of ρ(r) and $\nabla^2\rho$(r) were also evaluated along the length of the bond path connecting the donor atoms and Bi.

1.6 ^{213}Bi Radiolabeling Experiments

Caution! Work with radioactive isotopes such as ^{213}Bi should only be carried out by trained personnel at facilities equipped to safely handle and store these isotopes.

An ^{225}Ac/^{213}Bi generator system was used as a source of ^{213}Bi. Isolation of ^{213}Bi from ^{225}Ac was performed using an analogous approach to previously reported methods for clinically proven generator systems.[23,24] AGMP-50 cation exchange resin (50 mg) was packed into a 1 mL reservoir, equipped with polyethylene frits and pre-equilibrated with 4 M HNO_3 (2 mL). Approximately 6.5 MBq (620 mL) of first pass $^{225/227}$Ac in 4 M HNO_3 (originating from the spallation of solid thorium targets[25]) was loaded onto the column. After washing the column with 4 M HNO_3 (2 mL), ^{213}Bi was eluted with freshly prepared 0.2 M NaI/0.1 M HCl solution (300 – 600 µL), wherein the bulk of the activity was eluted in the first 150 µL. Subsequent elutions (with or without 2 mL of 1 mM HCl prewash of the generator) proceeded no earlier than 3 hours after the last elution, therefore optimizing the ratio of ^{213}Bi to the amount of other radionuclide impurities such as ^{209}Pb and ^{209}Bi.[23] The generator was sealed between each elution to minimise evaporation from the resin. ^{213}Bi activity and radionuclidic purity was determined using a High Purity Germanium (HPGe)

detector (Mirion Technologies (Canberra) Inc.) with Genie 2000 software by measurement of gamma emission line of ^{213}Bi ($t_{1/2}$ = 45.6 min, 440 keV, 25.9% abundance).

Stock solutions (1 mM) of macropa, macropaquin, macroquin-SO_3, and 1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid (DOTA) were made with ultra-pure deionised water. Serial dilutions were used to prepare initial ligand solutions of 10^{-4} M, 10^{-5} M, 10^{-6} M, and 10^{-7} M with ultra-pure water. Concentration-dependent radiolabeling studies were performed by the addition of $[^{213}Bi]BiI_4^-$/$[^{213}Bi]BiI_5^{2-}$ (30 kBq – 210 kBq) to a solution containing ligand sock (10 µL; or deionized water for negative controls) in MES buffer (80 µL; 0.5 M, pH 5.5 – 6). All radiolabeling studies were carried out within 5 minutes post-elution of the $^{225}Ac/^{213}Bi$ generator. The reaction mixtures were gently agitated using a vortex mixer and the pH confirmed to be between 5 – 5.5 by spotting a portion (1 – 2 µL) of reaction mixture onto pH paper. Macropa, macropaquin, and macroquin-SO_3 were incubated at room temperature, while DOTA was incubated at 95 °C. The radiochemical yield (RCY) was analyzed after 8 minutes at room temperature/elevated temperatures by spotting an aliquot (5 – 7 µL) on the bottom of instant thin layer chromatography plates (iTLC's) or aluminum backed silica TLC plates. TLC imaging was performed using an AR-2000 imaging scanner equipped with PD-10 gas, and analysis of radiochemical conversion yields (RCYs) was carried out using WinScan V3_14 software. TLC/iTLC plate systems were optimized for each ligand. For macropa and DOTA, the system consisted of paper backed iTLC-silicic acid (iTLC-SA, Agilent Technologies), with ethylenediaminetetraacetic acid (EDTA) (50 mM, pH 5.5) as the mobile phase. For macropaquin, the system consisted of aluminum backed silica TLC plates (TLC-SG, MERCK), with citrate buffer (0.4 M, pH 4.0) as the mobile phase. For macroquin-SO3, the system consisted of aluminum backed silica TLC plates (TLC-SG, MERCK), with EDTA (50 mM, pH 5.5) as the mobile phase.

For all these systems, free ^{213}Bi migrates with the solvent front (R_f = 1) while ^{213}Bi complexes will remain at the baseline (R_f = 0). The resulting iTLC data are present in detailed below.

2 SUPPORTING FIGURES, TABLES, AND SCHEMES

2.1 Ligand Characterization

2.1.1 Macrophospho

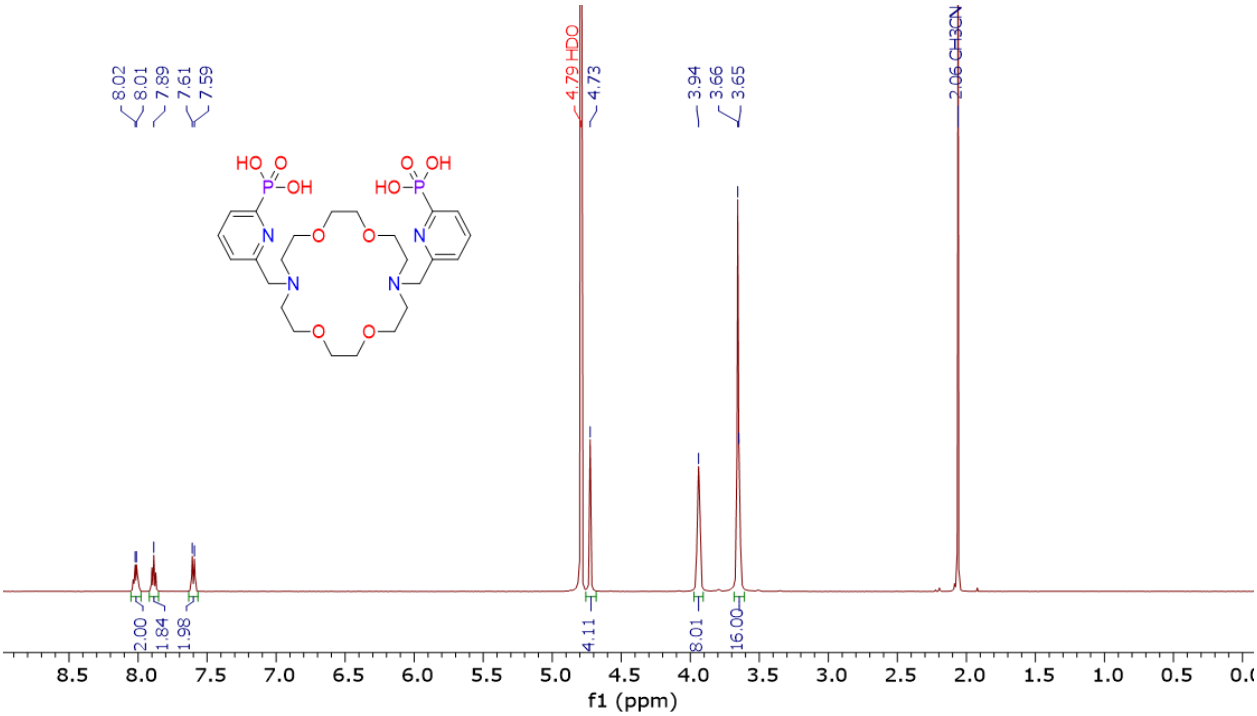


Figure S1. ^{1}H NMR spectrum of macrophospho. 500 MHz, 298 K, D_2O.

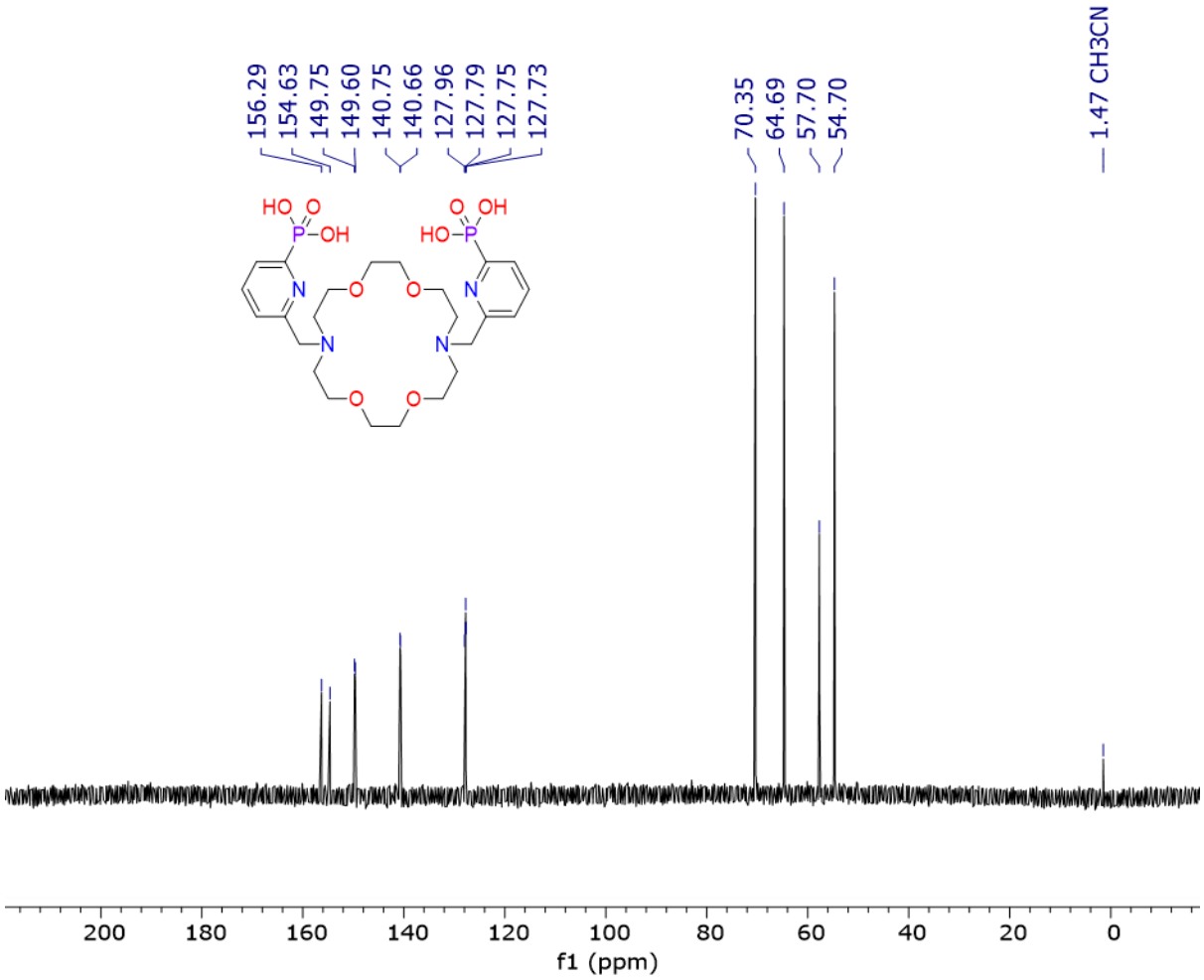


Figure S2. $^{13}C\{^{1}H\}$ NMR spectrum of macrophospho. 126 MHz, 298 K, D_2O.

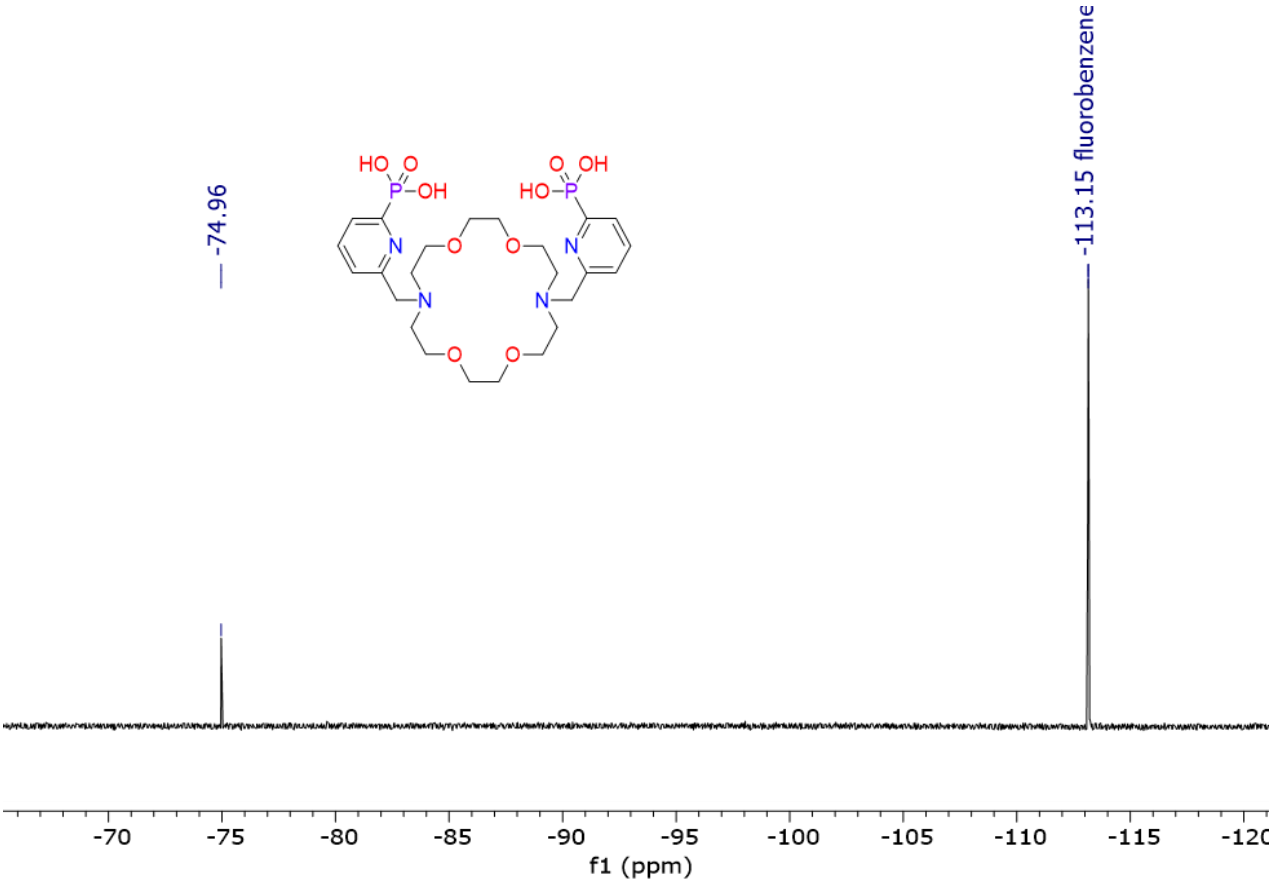


Figure S3. ^{19}F NMR spectrum of macrophospho. 470 MHz, 298 K, D_2O.

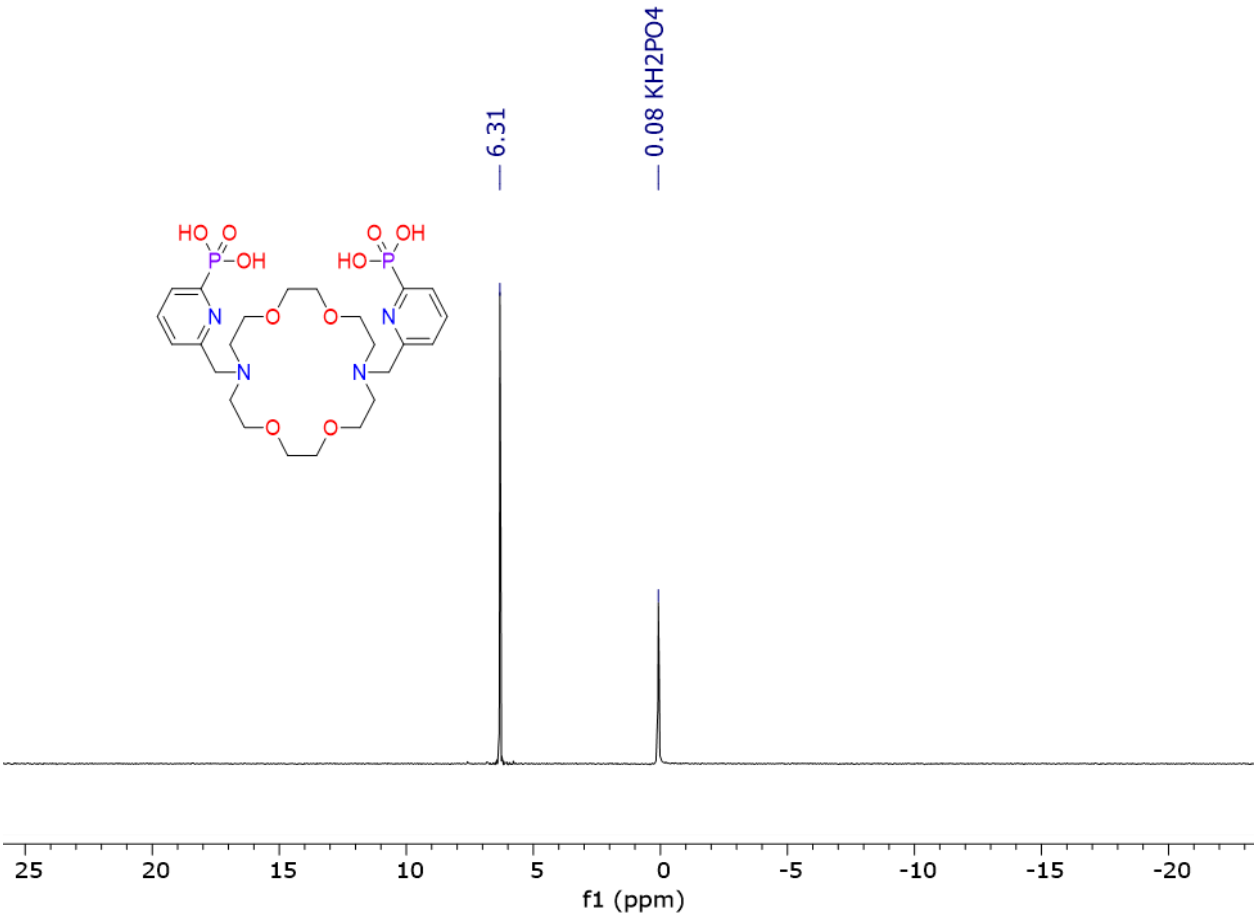


Figure S4. $^{31}P\{^{1}H\}$ NMR spectrum of macrophospho. 202 MHz, 298 K, D_2O.

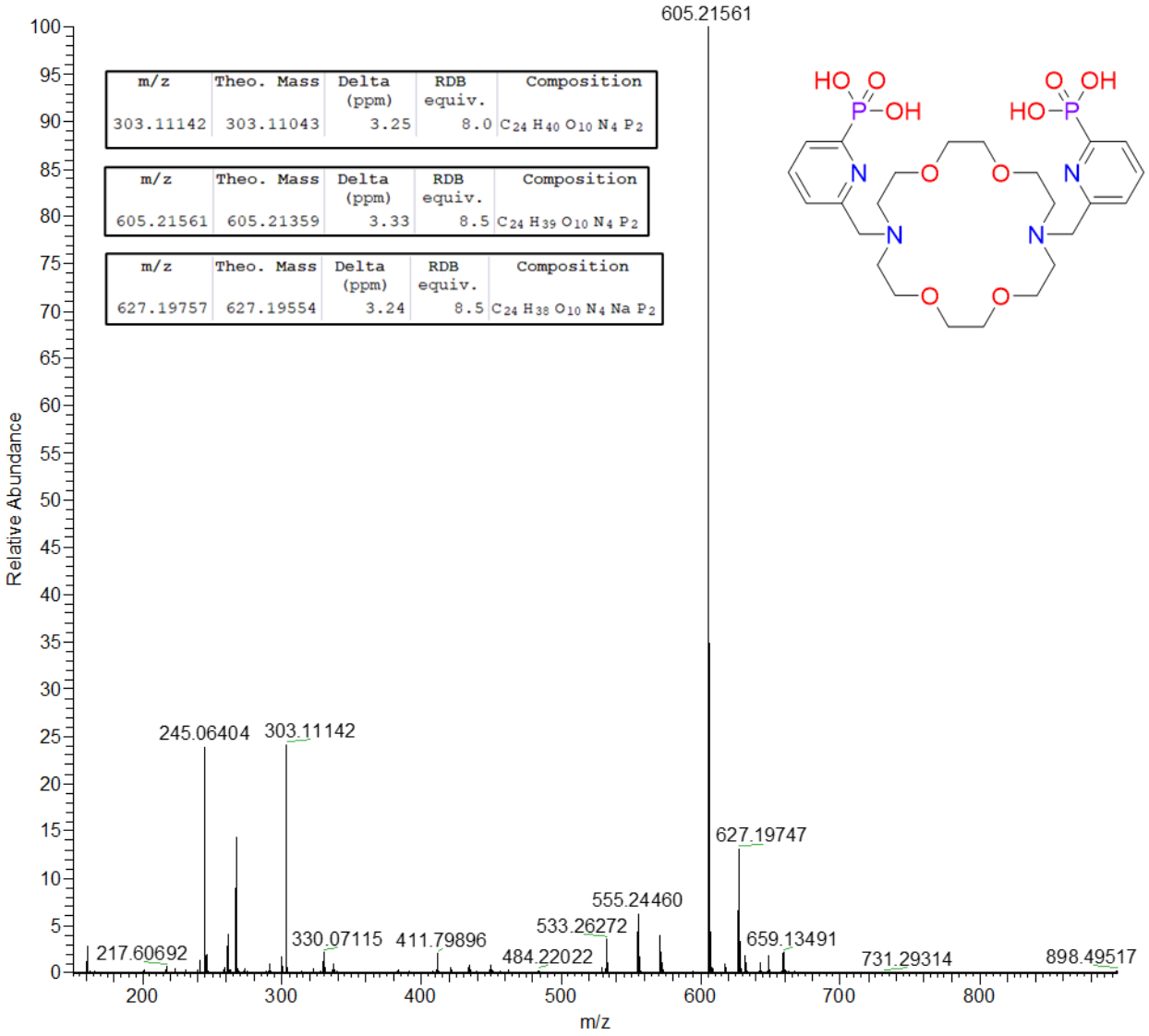


Figure S5. ESI-HRMS of macrophospho. The sample was diluted using a solution of CH_3CN:H_2O (7:3) containing 1% formic acid. The sample was analyzed using a mobile phase of CH_3CN:H_2O (7:3). Three ion peaks were observed, corresponding to $[M+2H]^{2+}$ (*m/z* 303.11142), $[M+H]^{+}$ (*m/z* 605.21561), and $[M+Na]^{+}$ (*m/z*) 627.19747.

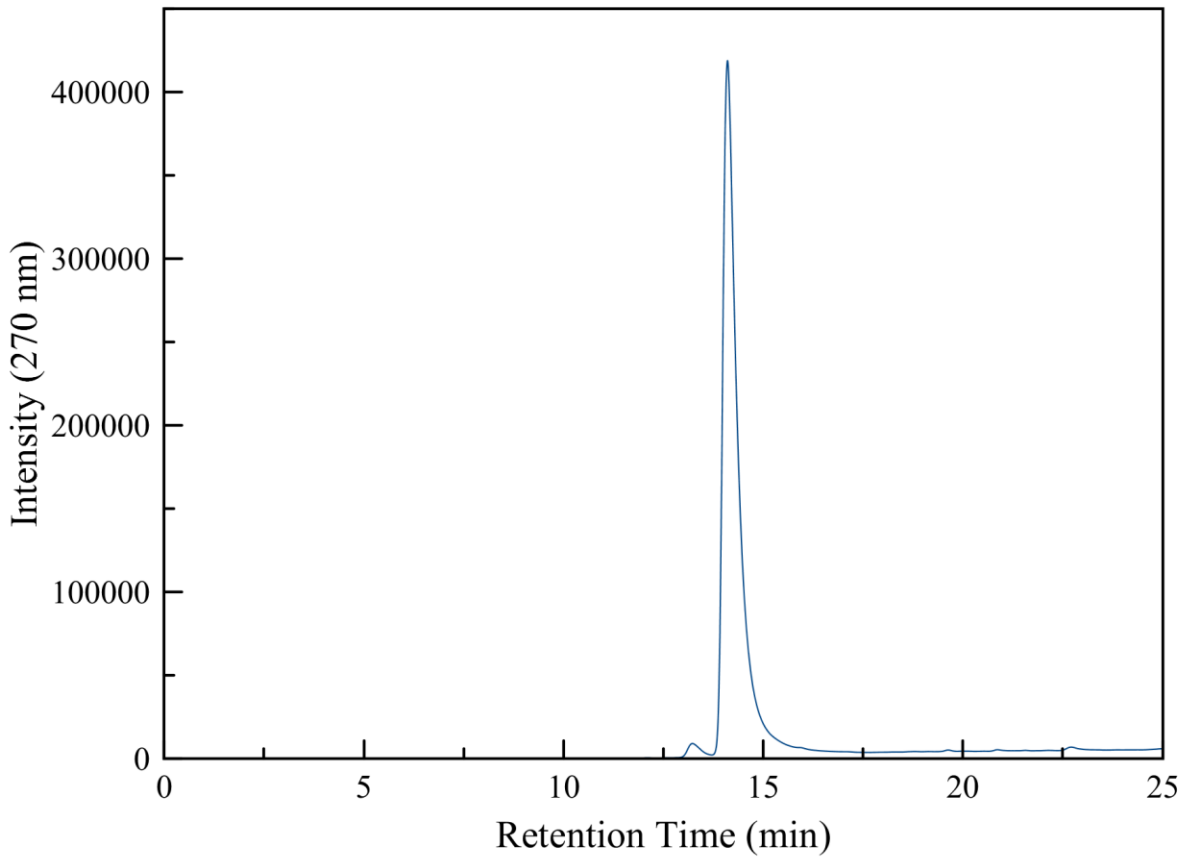


Figure S6. HPLC chromatogram of macrophospho. Retention time (t_R) = 14.105 min using a binary MeOH/H_2O mobile phase containing 0.1% TFA (program: 10% MeOH for 5 min, followed by a linear gradient to 100% MeOH over 20 min).

2.2 Bi^{3+} Complex Characterization

2.2.1 $Bi(macropa)]^{+}$

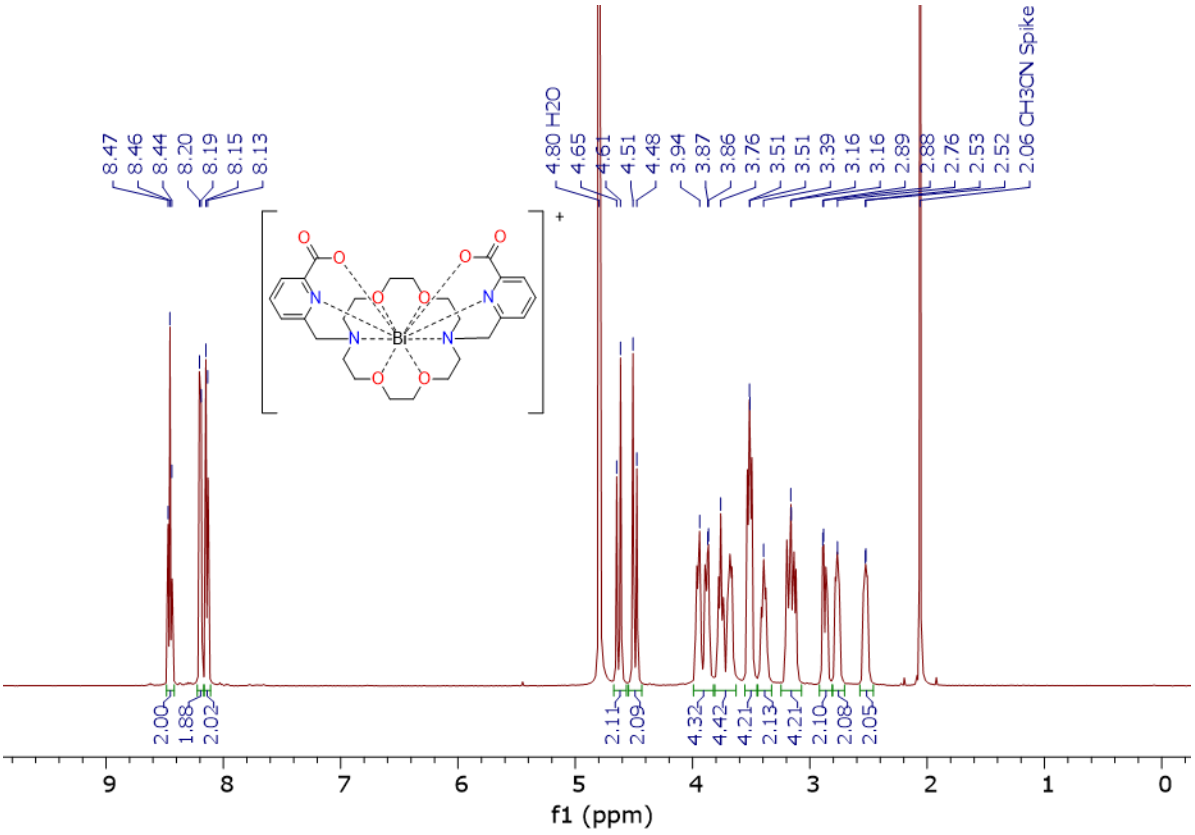


Figure S7. ^{1}H NMR spectrum of $[Bi(macropa)]^{+}$. 500 MHz, 298 K, D_2O.

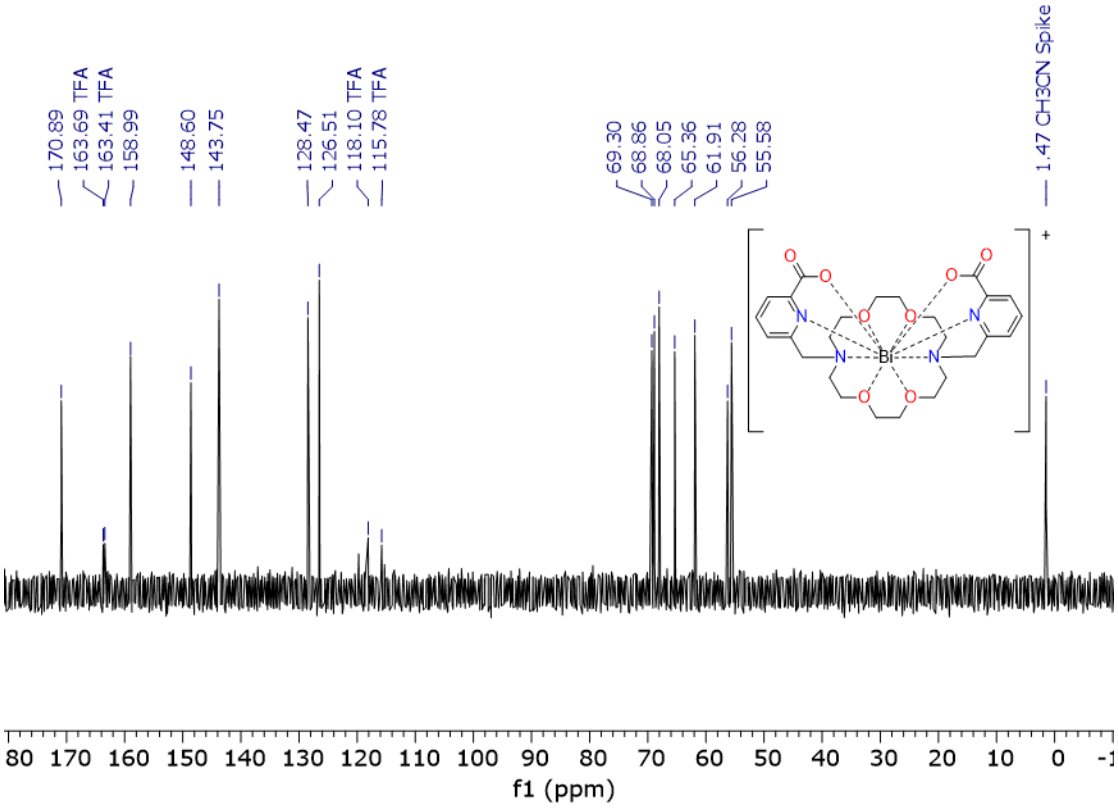


Figure S8. $^{13}C\{^{1}H\}$ NMR spectrum of [Bi(macropa)]$^{+}$. 126 MHz, 298 K, D_2O.

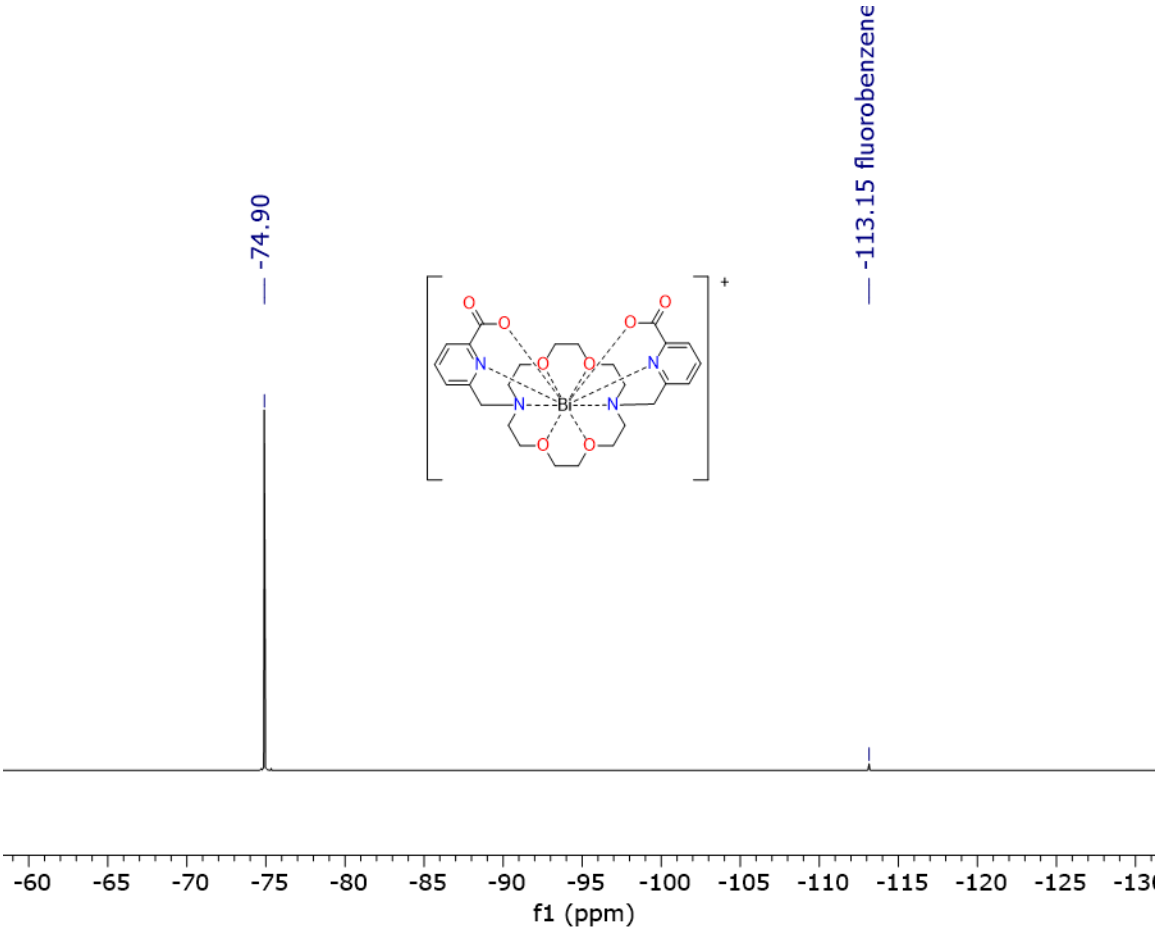


Figure S9. ^{19}F NMR spectrum of [Bi(macropa)]$^{+}$. 470 MHz, 298 K, D_2O.

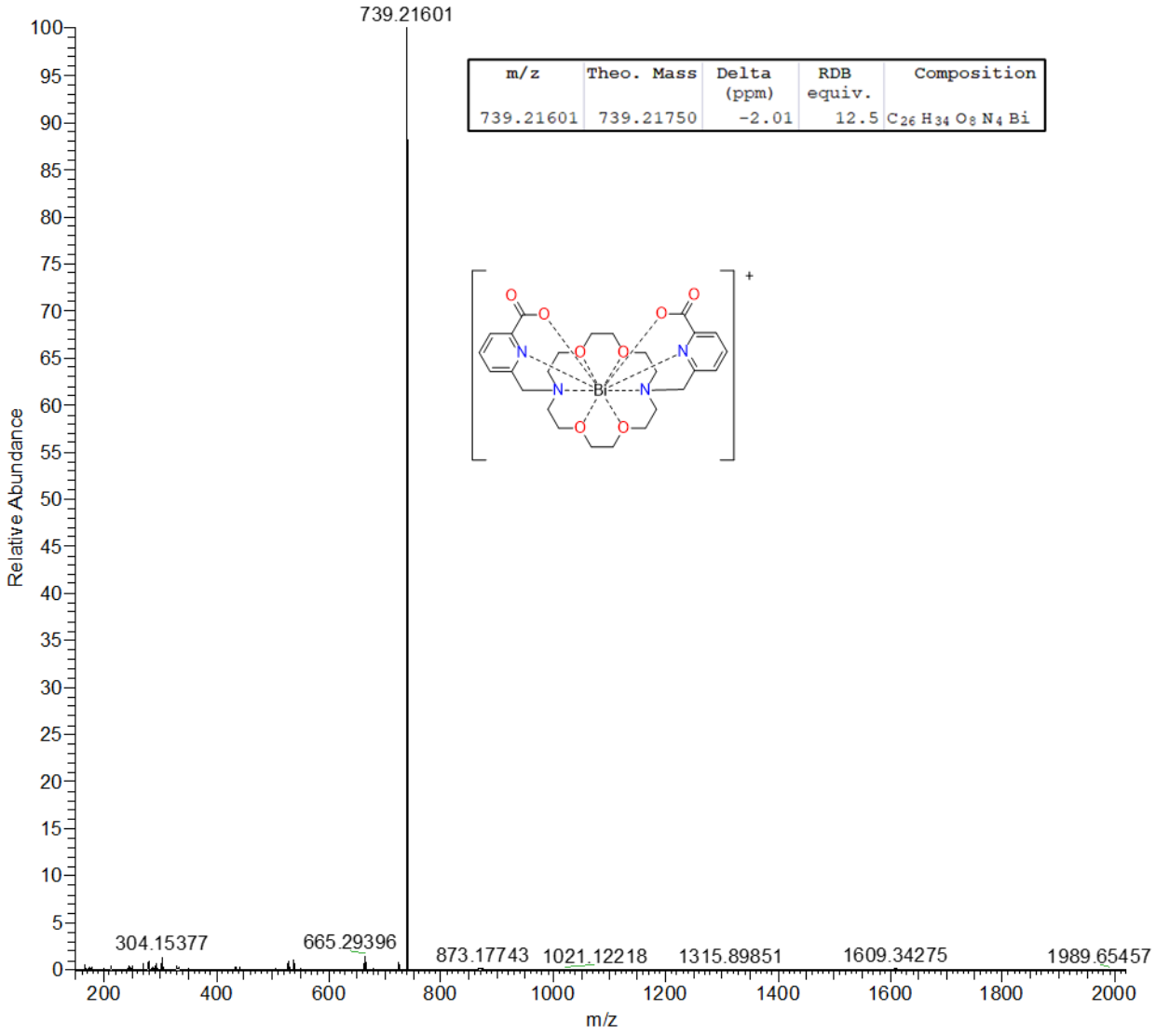


Figure S10. ESI-HRMS of [Bi(macropa)]$^+$. The sample was diluted using a solution of CH_3CN containing 1% formic acid. The sample was analyzed using a mobile phase of CH_3CN. One ion peak was observed, corresponding to $[M]^+$ (*m/z* 739.21601).

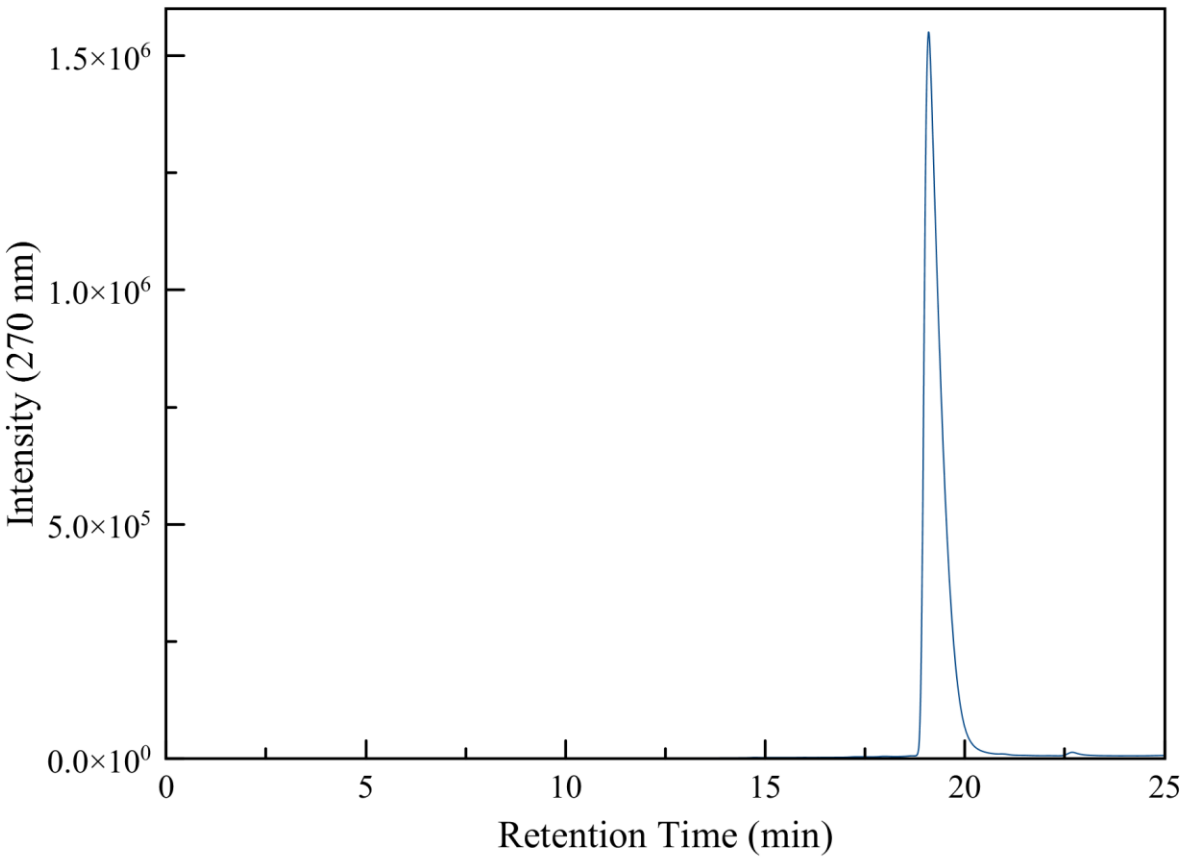


Figure S11. HPLC chromatogram of [Bi(macropa)]$^+$. Retention time (t_R) = 19.092 min using a binary MeOH/H_2O mobile phase containing 0.1% TFA (program: 10% MeOH for 5 min, followed by a linear gradient to 100% MeOH over 20 min).

2.2.2 [Bi(macroquin-SO_3]$^-$

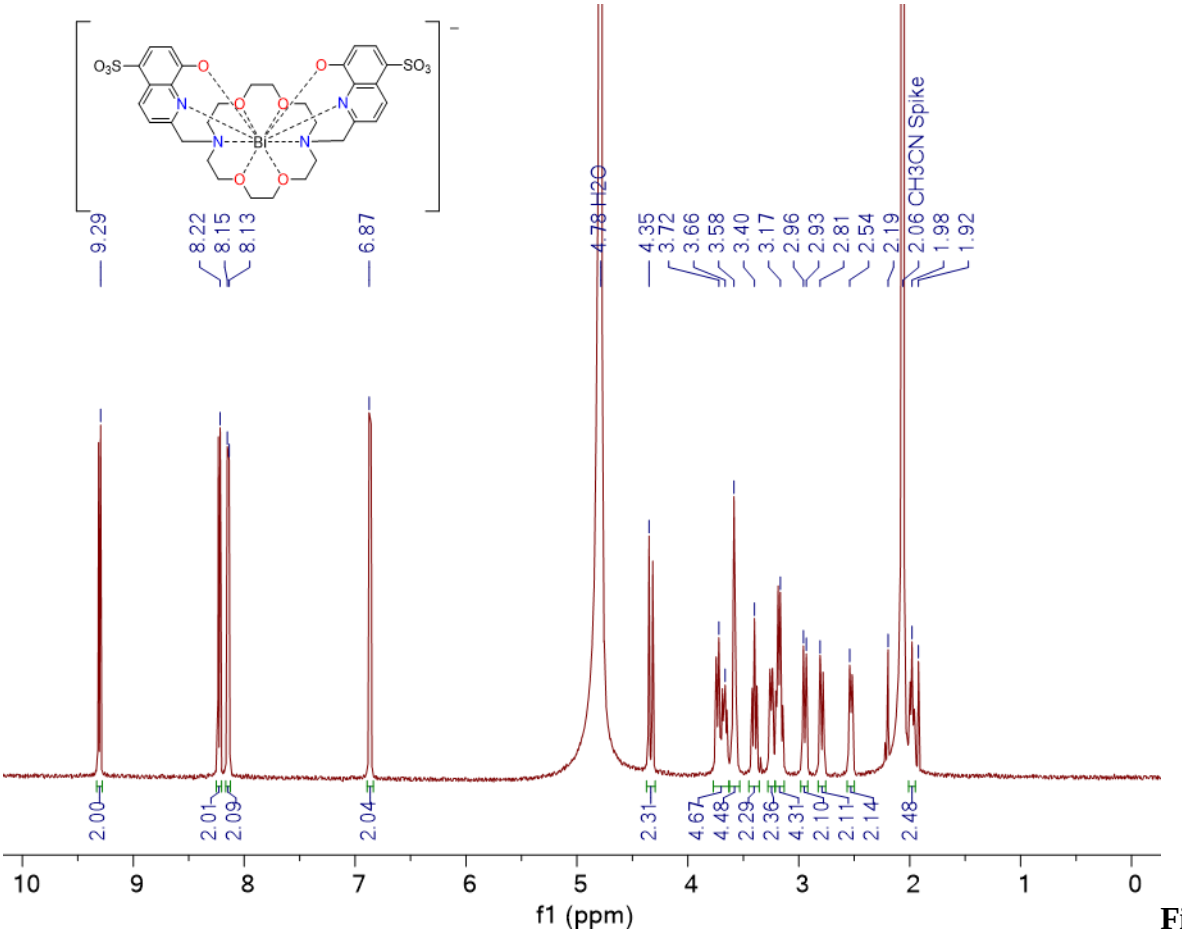
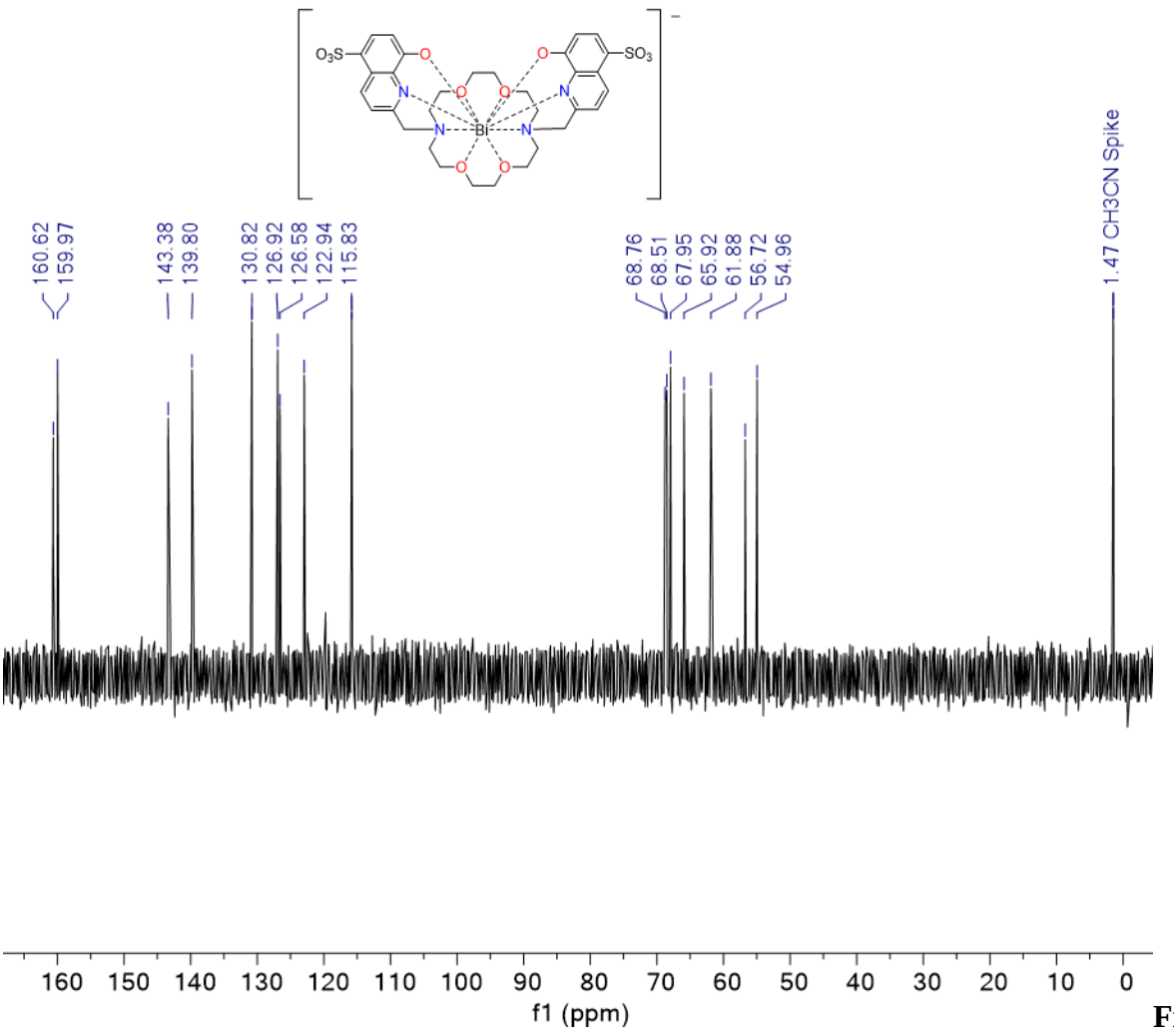


Figure S12. ^{1}H NMR spectrum of [Bi(macroquin-SO_3)]$^-$. 500 MHz, 298 K, D_2O.



Figure

Figure S13. $^{13}C\{^{1}H\}$ NMR spectrum of [Bi(macroquin-SO_3)]$^-$. 126 MHz, 298 K, D_2O.

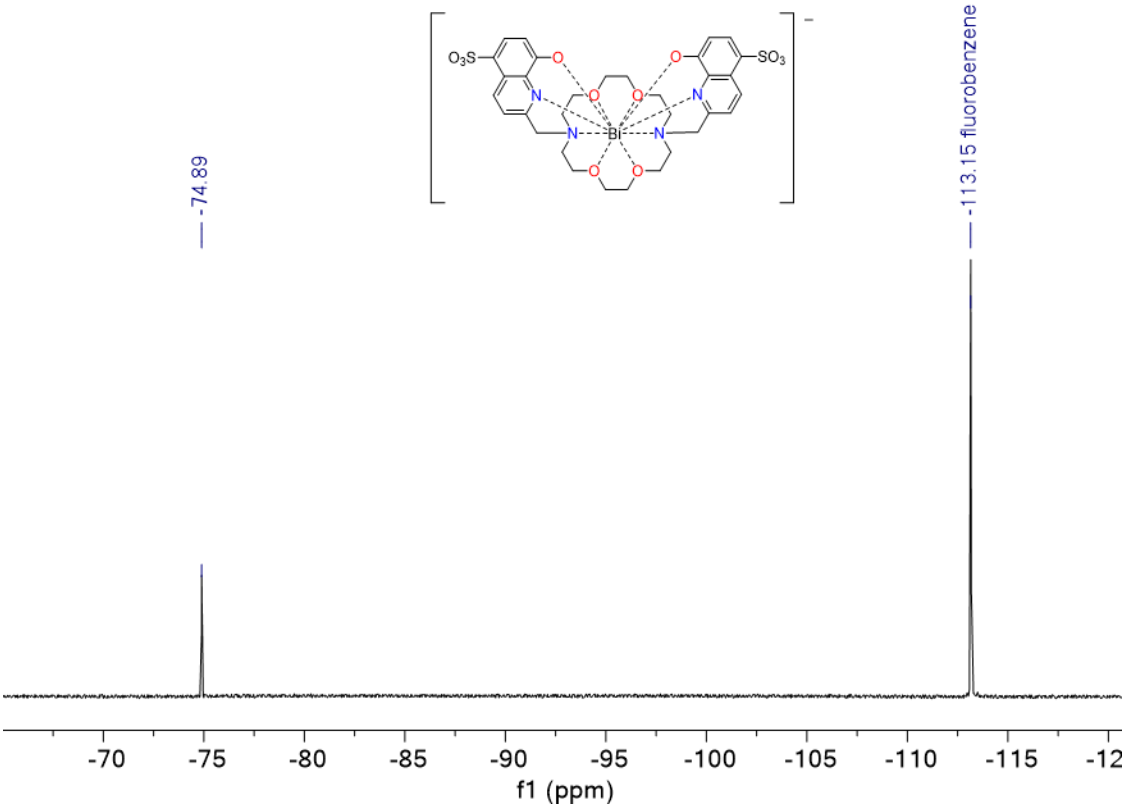


Figure S14. ^{19}F NMR spectrum of [Bi(macroquin-SO$_3$)]$^-$. 470 MHz, 298 K, D_2O.

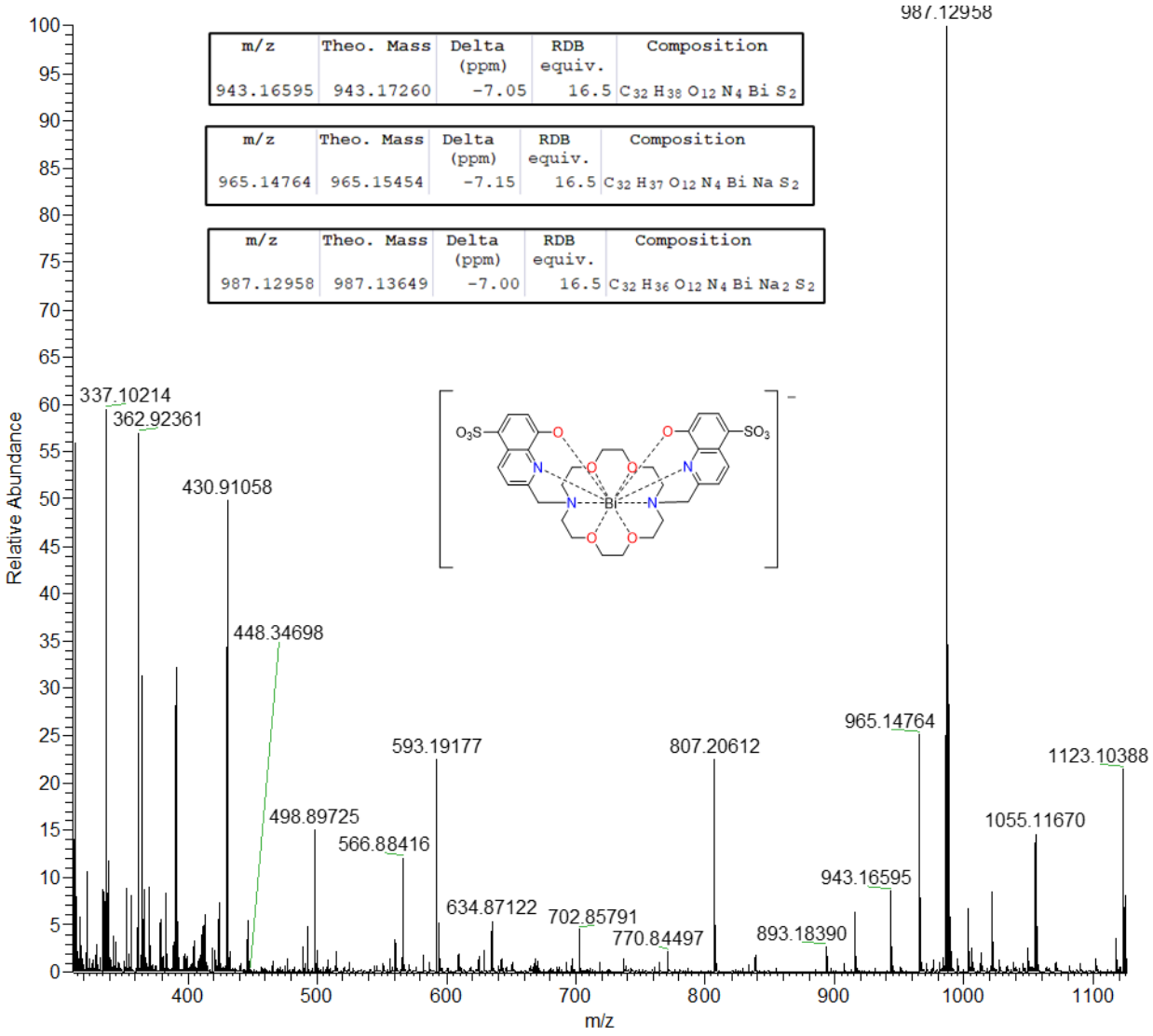


Figure S15. ESI-HRMS of [Bi(macroquin-SO_3)]$^-$. The sample was diluted using a solution of CH_3CN:H_2O (1:1) containing 1% formic acid. The sample was analyzed using a mobile phase of CH_3CN:H_2O (1:1). Three ion peaks were observed, corresponding to $[M+H]^+$ (*m/z* = 943.16595), $[M+Na]^+$ (*m/z* = 965.14764), $[M+2Na-H]^+$ (*m/z* = 987.12958).

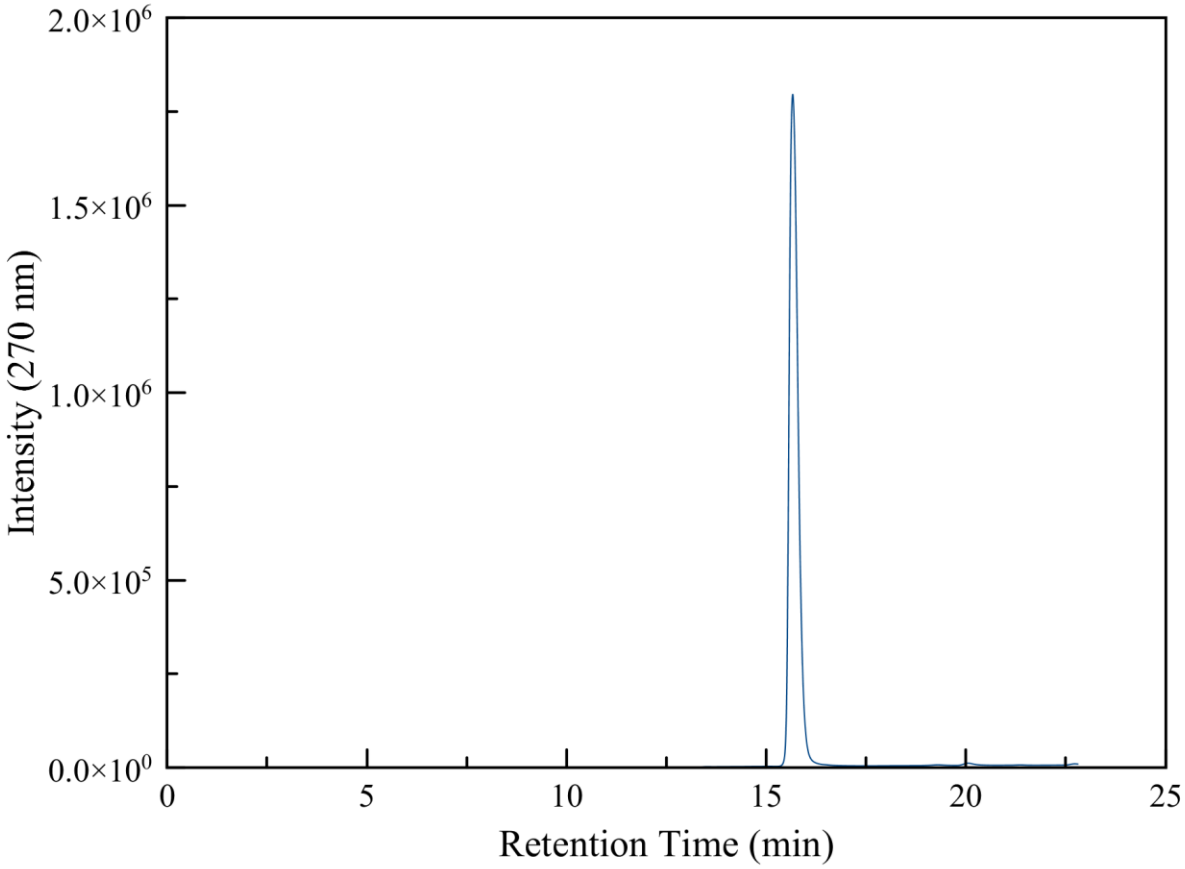


Figure S16. HPLC chromatogram of [Bi(macroquin-SO_3)]$^-$. Retention time (t_R) = 15.663 min using a binary MeOH/H_2O mobile phase containing 0.1% TFA (program: 10% MeOH for 5 min, followed by a linear gradient to 100% MeOH over 20 min).

2.2.3 [Bi(macrophospho)]$^{+}$

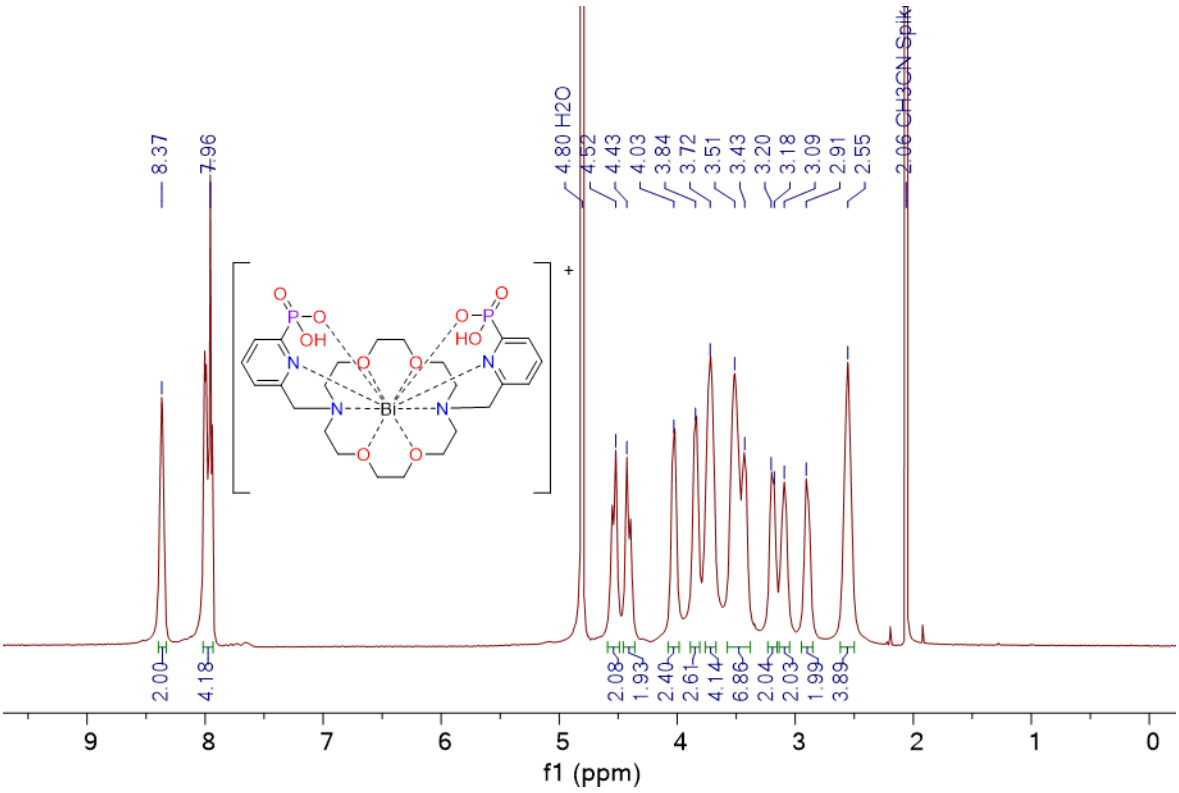


Figure S17. ^{1}H NMR spectrum of [Bi(macrophospho)]$^{+}$. 500 MHz, 298 K, D_2O.

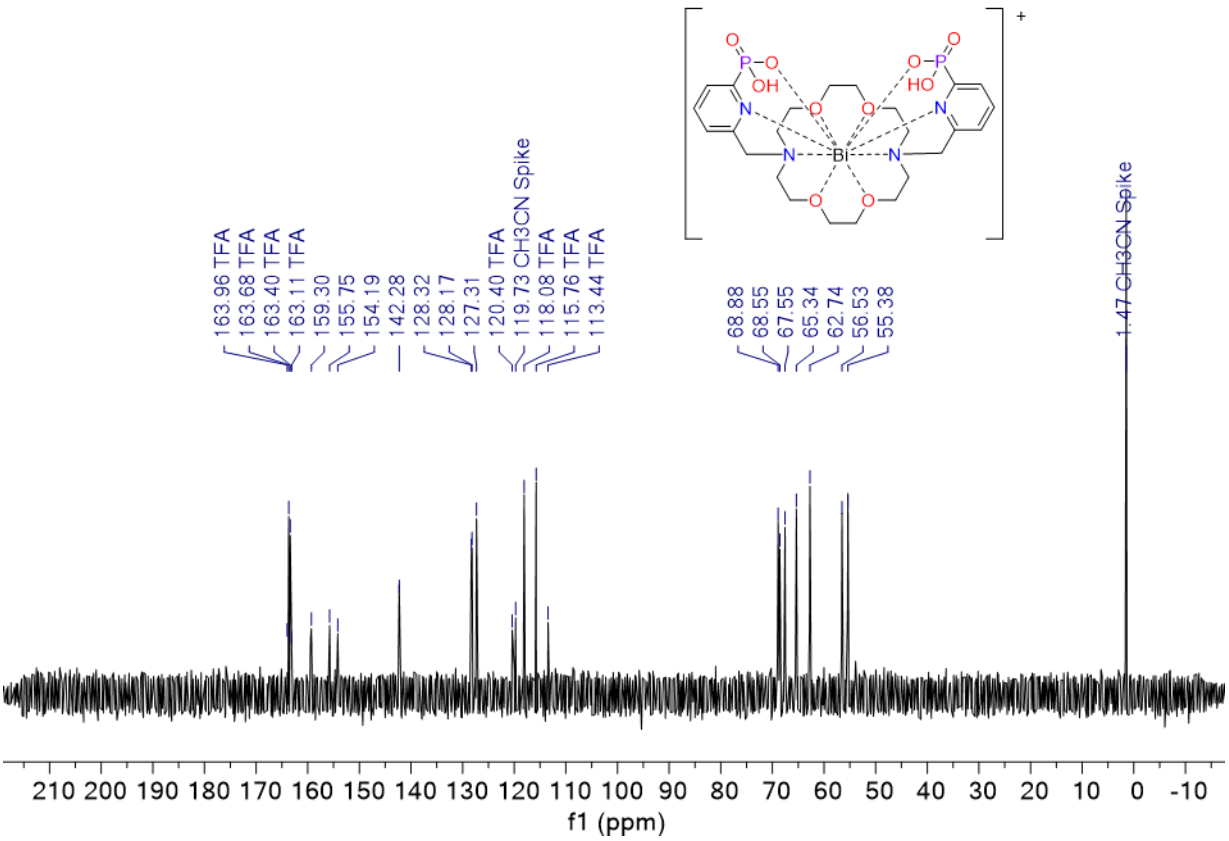


Figure S18. $^{13}C\{^{1}H\}$ NMR spectrum of [Bi(macrophospho)]$^{+}$. 126 MHz, 298 K, D_2O.

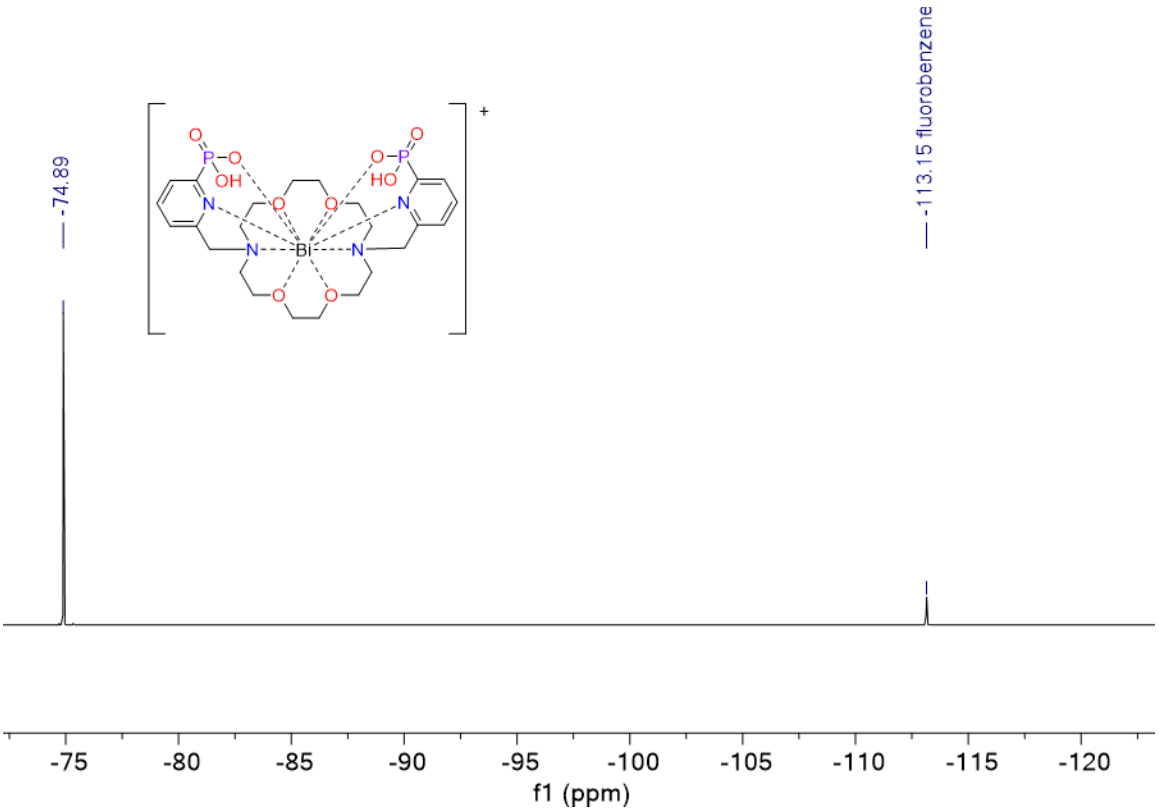


Figure S19. ^{19}F NMR spectrum of [Bi(macrophospho)]$^{+}$. 470 MHz, 298 K, D_2O.

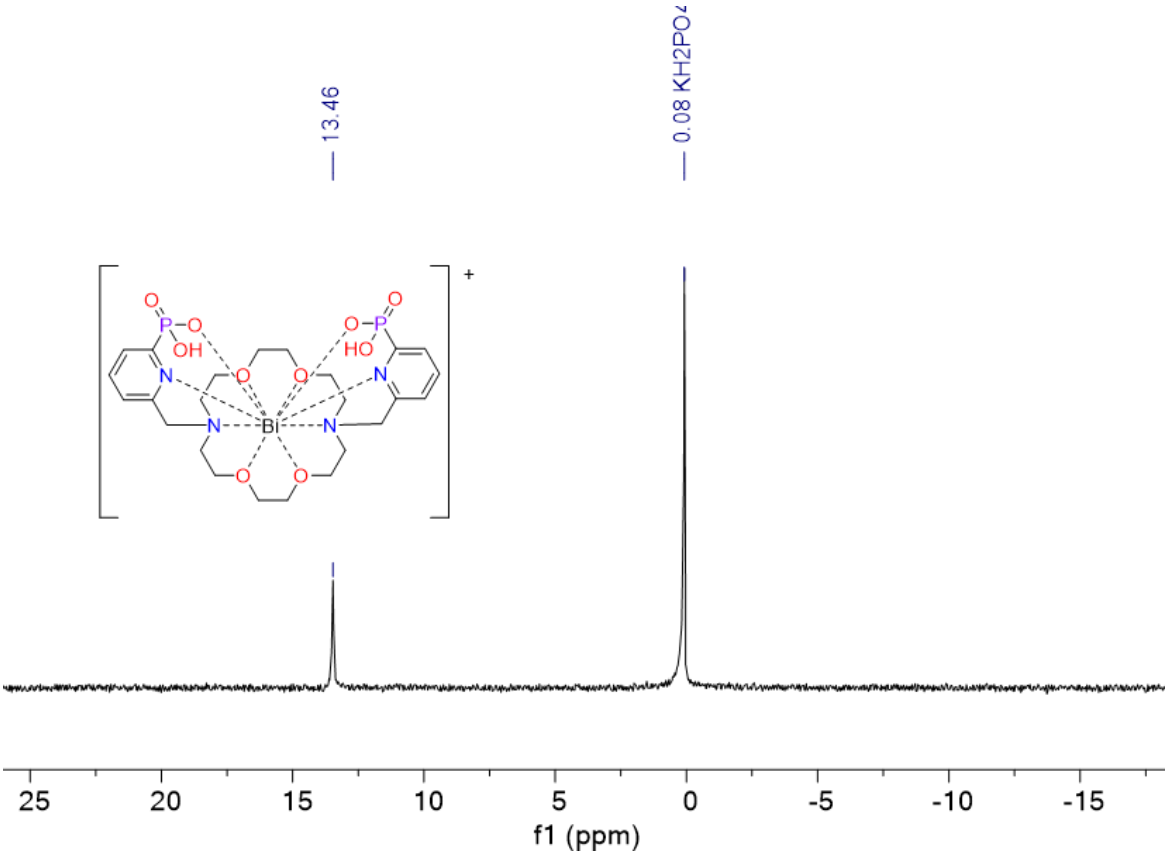


Figure S20. $^{31}P\{^{1}H\}$ NMR spectrum of [Bi(macrophospho)]$^{+}$. 202 MHz, 298 K, D_2O.

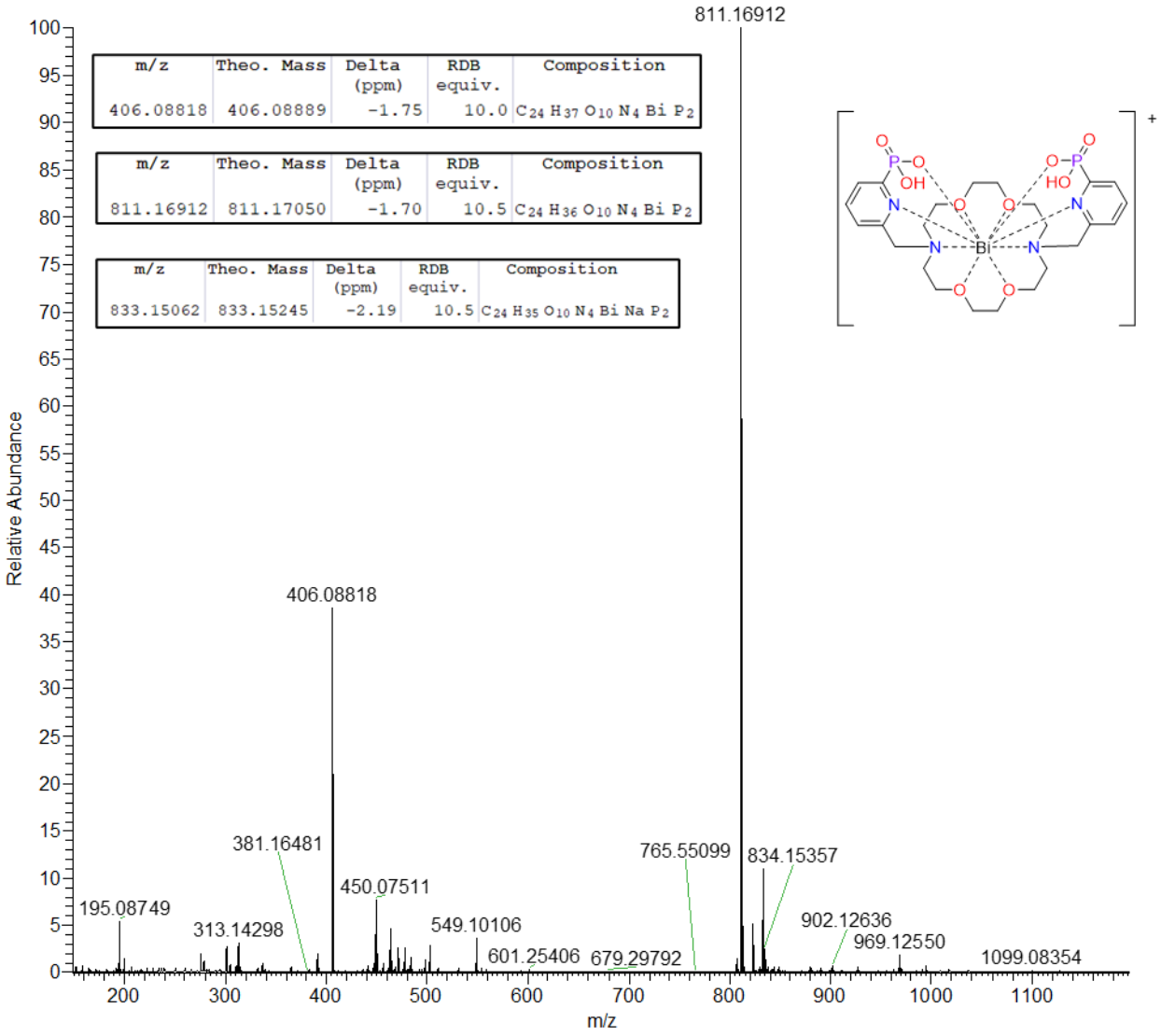


Figure S21. ESI-HRMS of [Bi(macrophospho)]$^{+}$. The sample was diluted using a solution of CH_3CN:H_2O (1:1) containing 1% formic acid. The sample was analyzed using a mobile phase of CH_3CN:H_2O (1:1). Three ion peaks were observed, corresponding to $[M+H]^{2+}$ (*m/z* 406.08818), $[M]^{+}$ (*m/z* 811.16912), and $[M+Na–H]^{+}$ (*m/z* 833.15062).

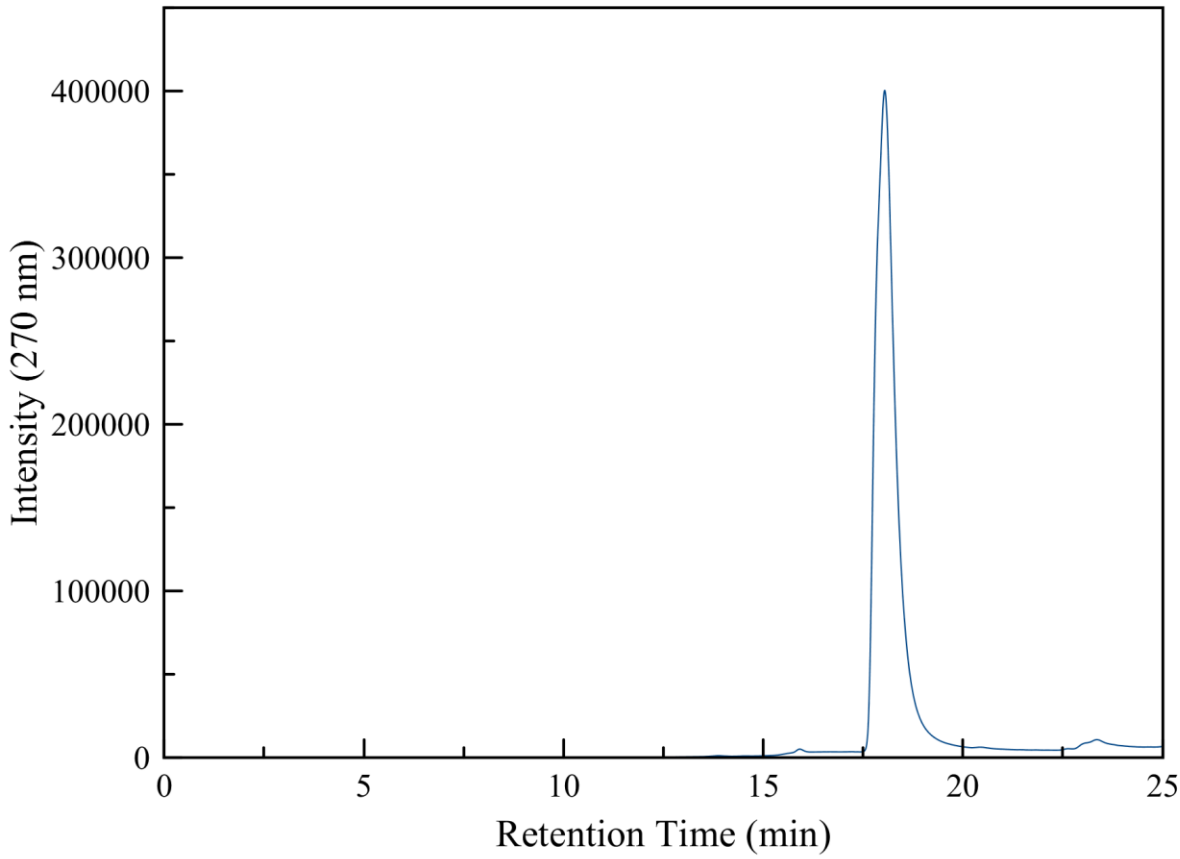


Figure S22. HPLC chromatogram of [Bi(macrophospho)]$^{+}$. Retention time (t_R) = 18.041 min using a binary MeOH/H_2O mobile phase containing 0.1% TFA (program: 10% MeOH for 5 min, followed by a linear gradient to 100% MeOH over 20 min).

2.2.4 [Bi(macrophosphi)]$^{+}$

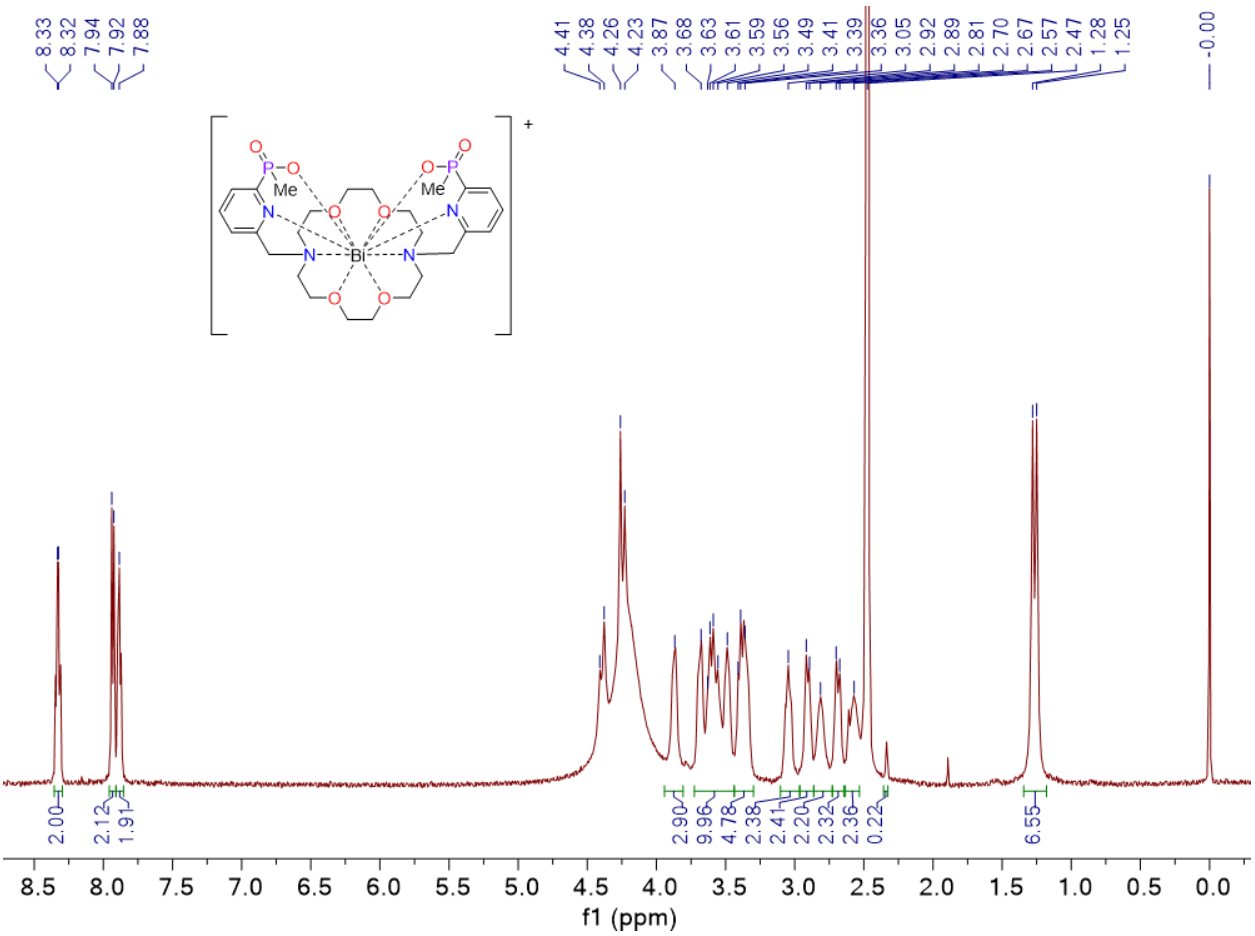


Figure S23. ^{1}H NMR spectrum of [Bi(macrophosphi)]$^{+}$. 500 MHz, 393.2 K, DMSO-d_6.

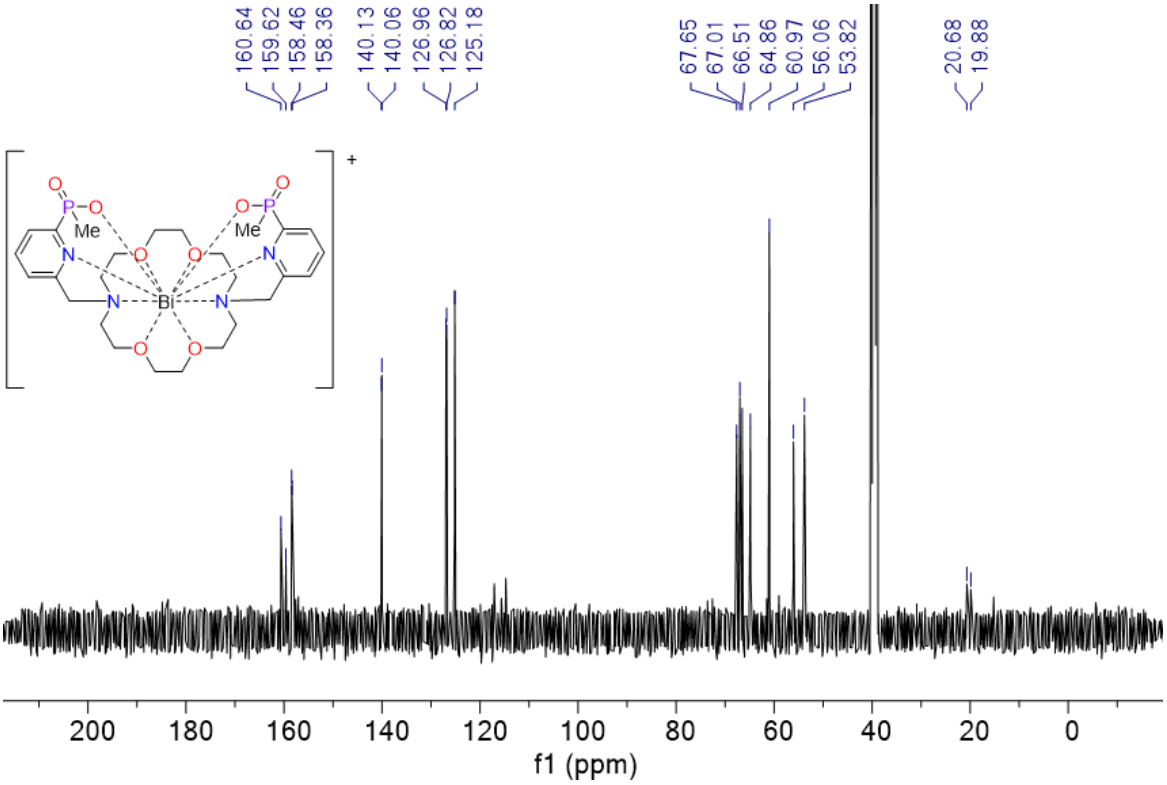


Figure S24. $^{13}C\{^{1}H\}$ NMR spectrum of [Bi(macrophosphi)]$^{+}$. 126 MHz, 393.2 K, DMSO-d_6.

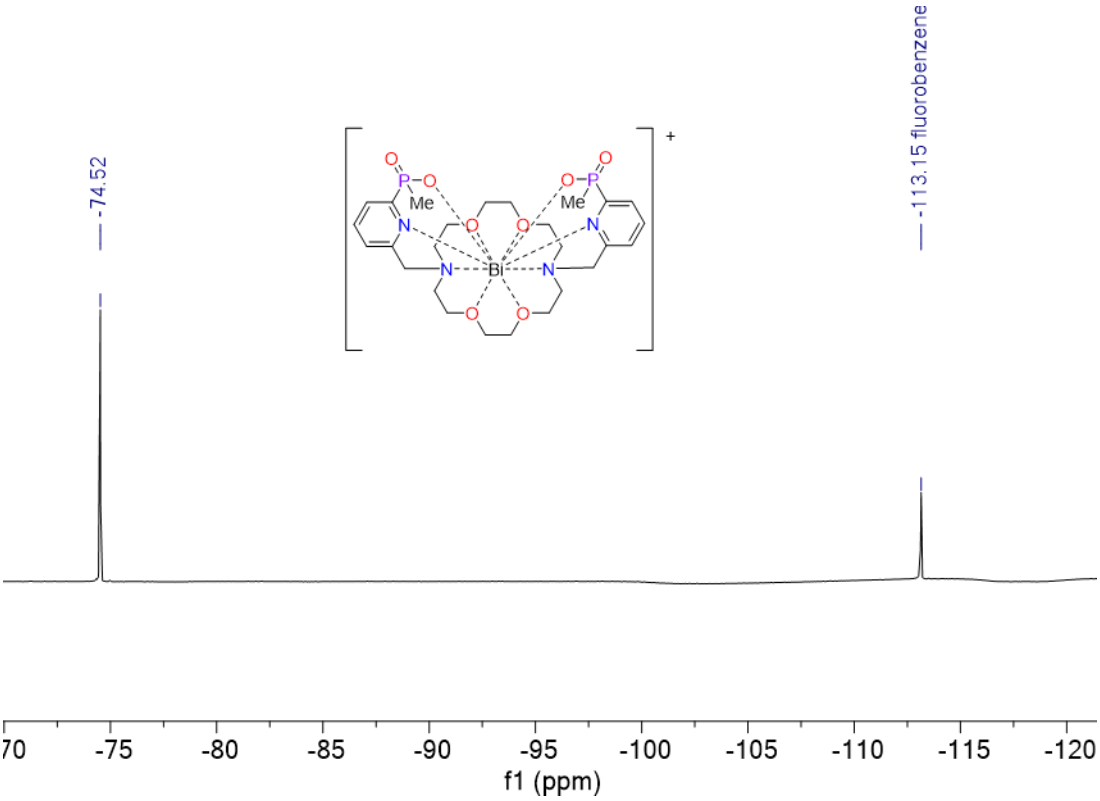


Figure S25. ^{19}F NMR spectrum of [Bi(macrophosphi)]$^{+}$. 470 MHz, 393.2 K, DMSO-d_6.

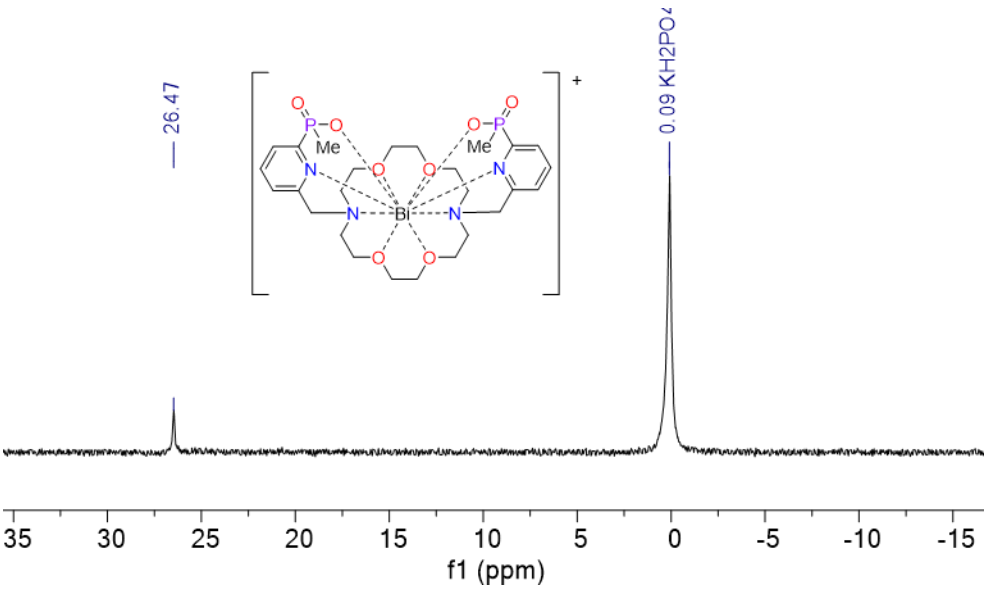


Figure S26. $^{31}P\{^{1}H\}$ NMR spectrum of [Bi(macrophosphi)]$^{+}$. 202 MHz, 393.2 K, DMSO-d_6.

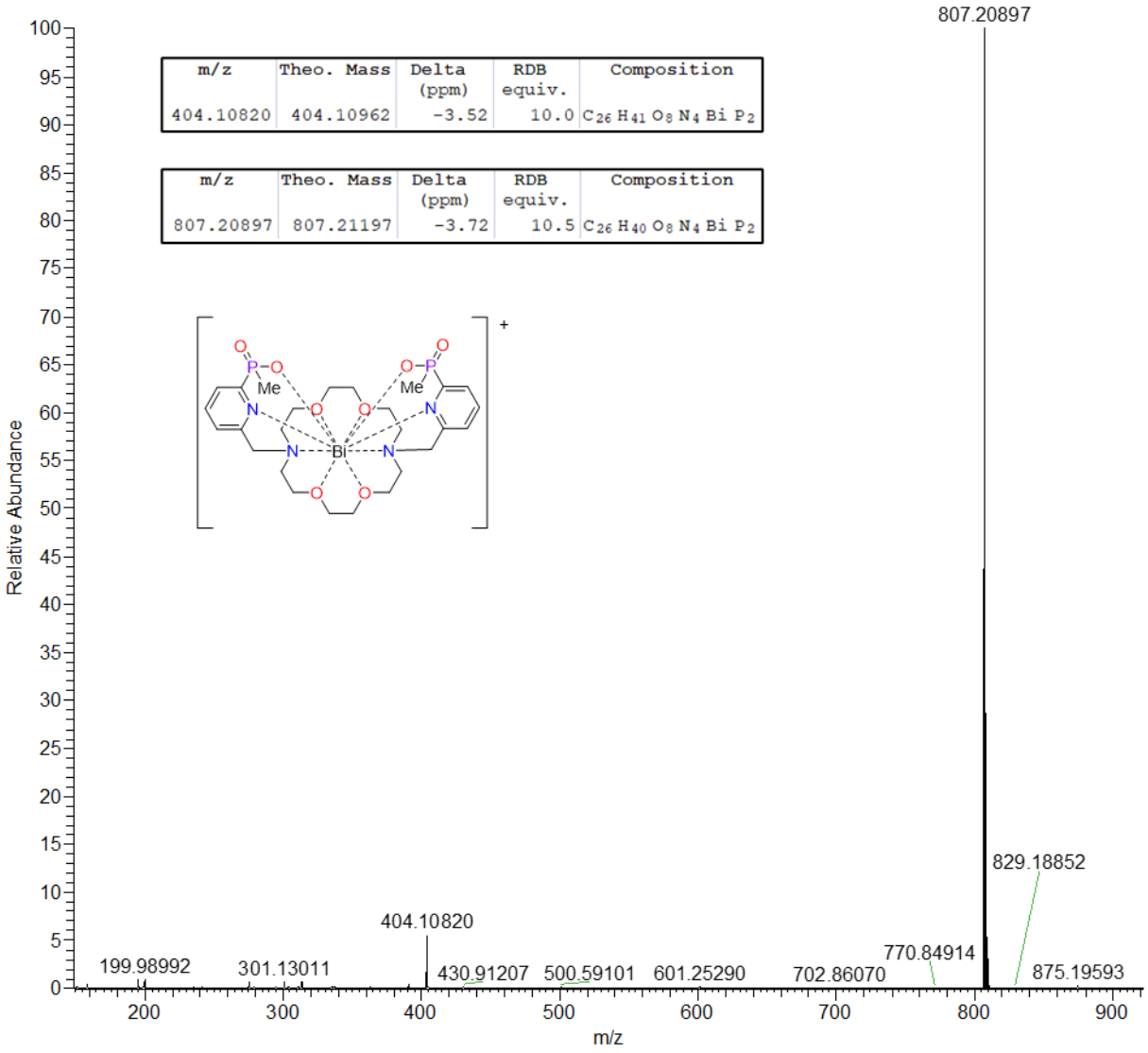


Figure S27. ESI-HRMS of [Bi(macrophosphi)]$^{+}$. The sample was diluted using a solution of CH_3CN:H_2O (1:1) containing 1% formic acid. The sample was analyzed using a mobile phase of CH_3CN:H_2O (1:1). Two ion peaks were observed, corresponding to $[M+H]^{2+}$ (*m/z* 404.10820) and $[M]^{+}$ (*m/z* 807.20897).

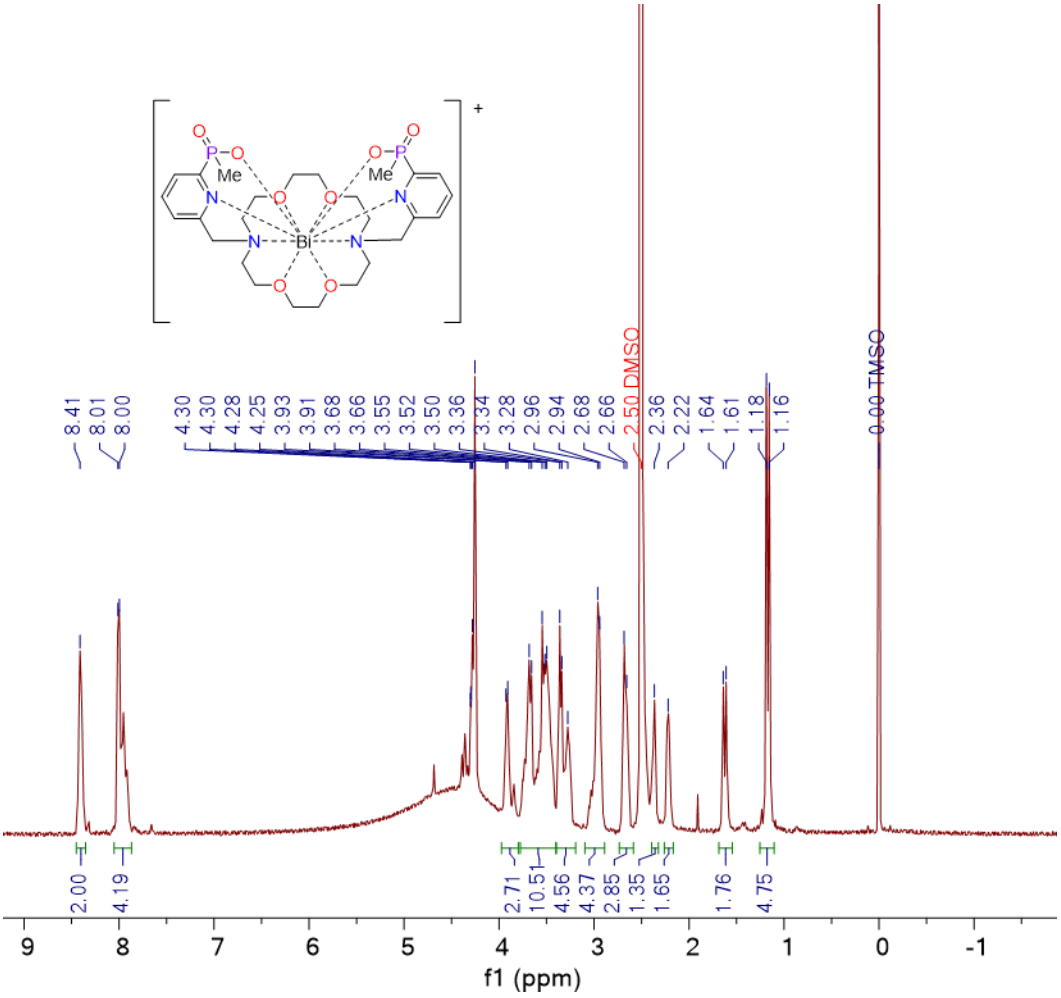


Figure S28. ^{1}H NMR spectrum of [Bi(macrophosphi)]$^{+}$. 500 MHz, 298 K, DMSO-d_6.

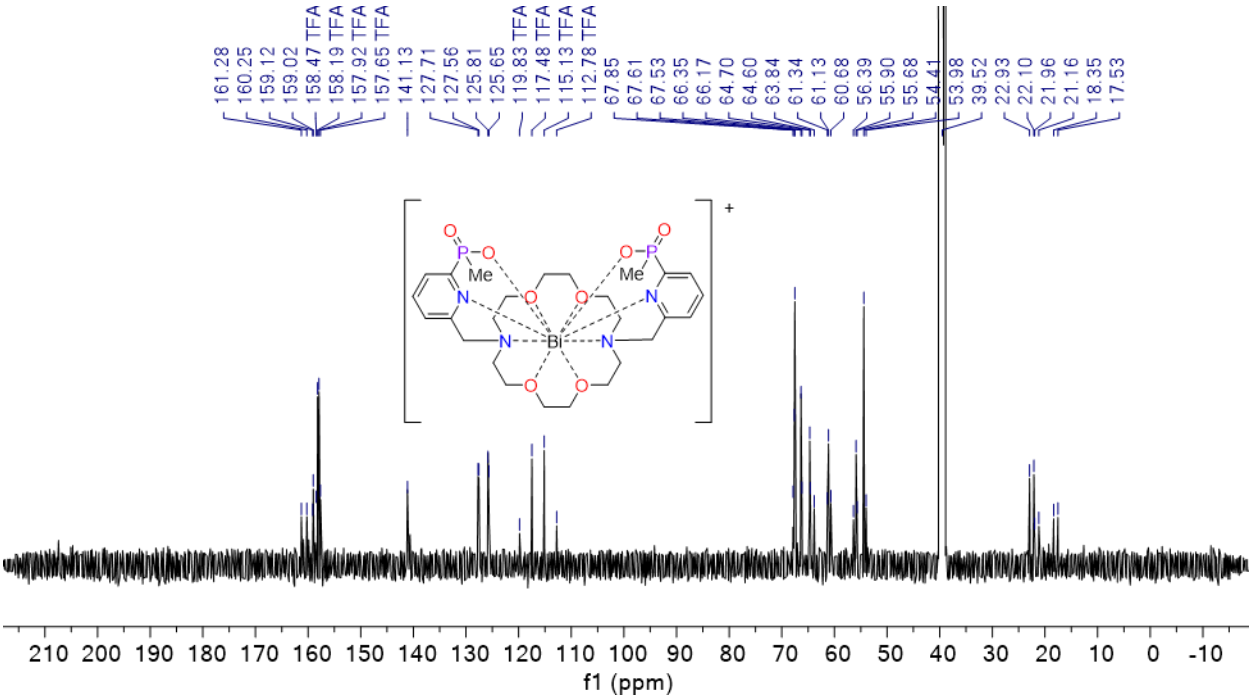


Figure S29. $^{13}C\{^{1}H\}$ NMR spectrum of [Bi(macrophosphi)]$^{+}$. 126 MHz, 298 K, DMSO-d_6.

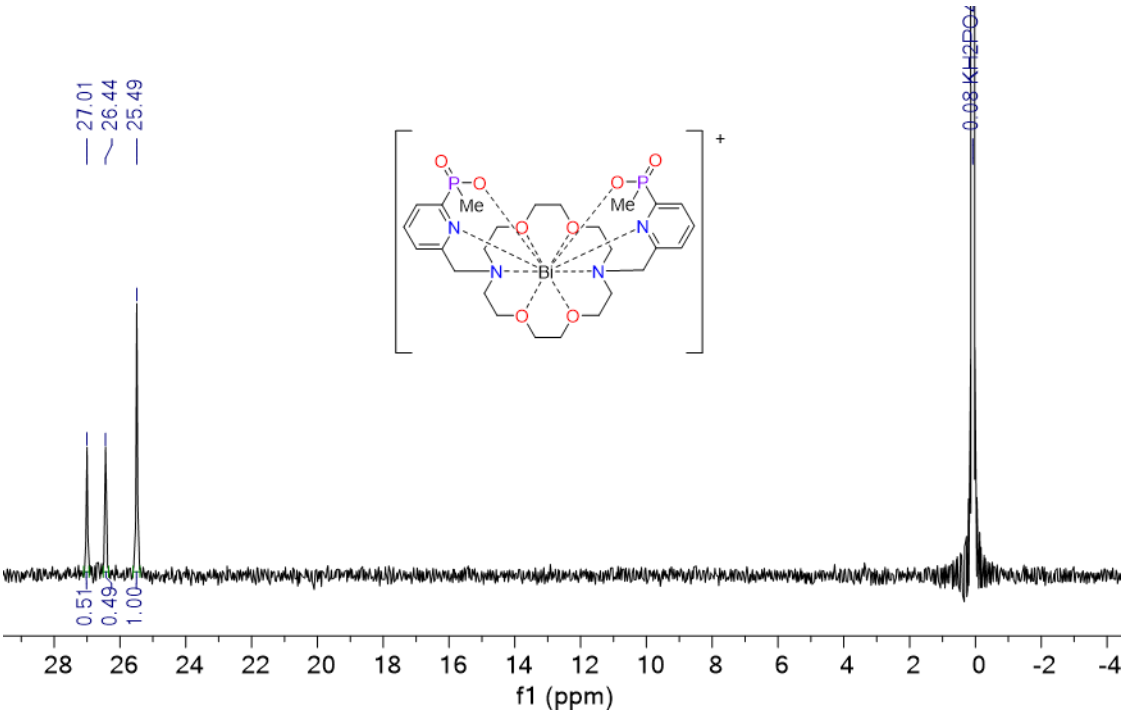


Figure S30. $^{31}P\{^{1}H\}$ NMR spectrum of [Bi(macrophosphi)]$^{+}$. 202 MHz, 298 K, DMSO-d_6.

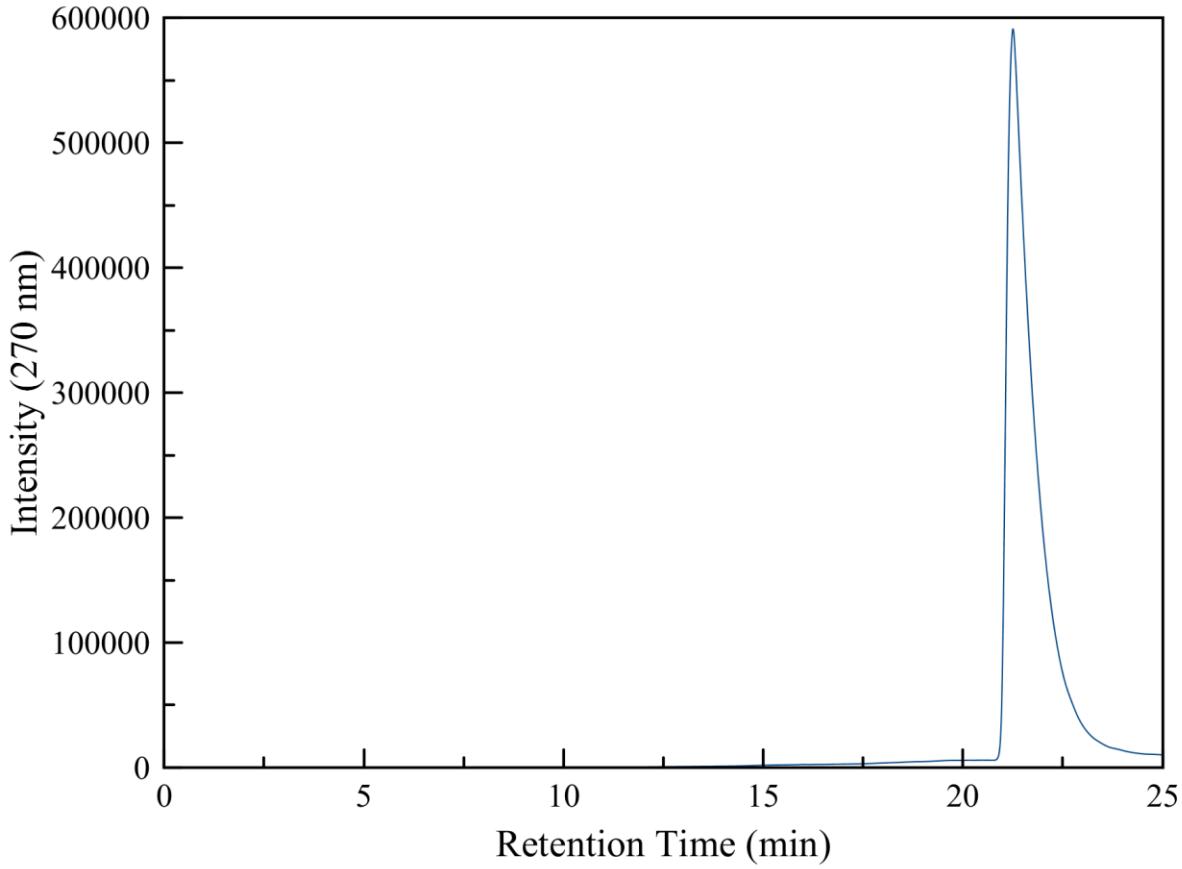


Figure S31. HPLC chromatogram of [Bi(macrophosphi)]$^{+}$. Retention time (t_R) = 21.253 min using a binary MeOH/H_2O mobile phase containing 0.1% TFA (program: 10% MeOH for 5 min, followed by a linear gradient to 100% MeOH over 20 min).

2.2.5 [Bi(CHX-macropa)]$^{+}$

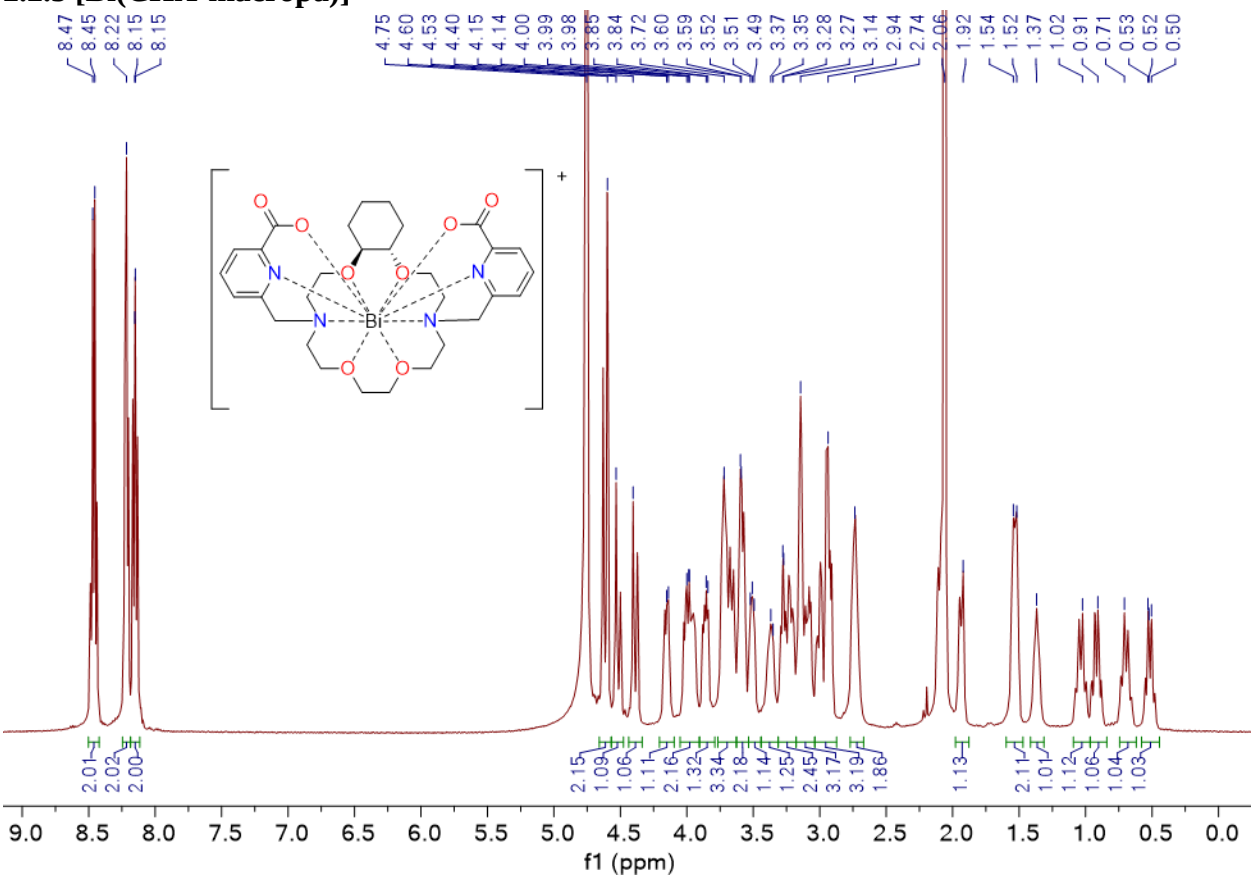


Figure S32. ^{1}H NMR spectrum of [Bi(CHX-macropa)]$^{+}$. 500 MHz, 298 K, D_2O.

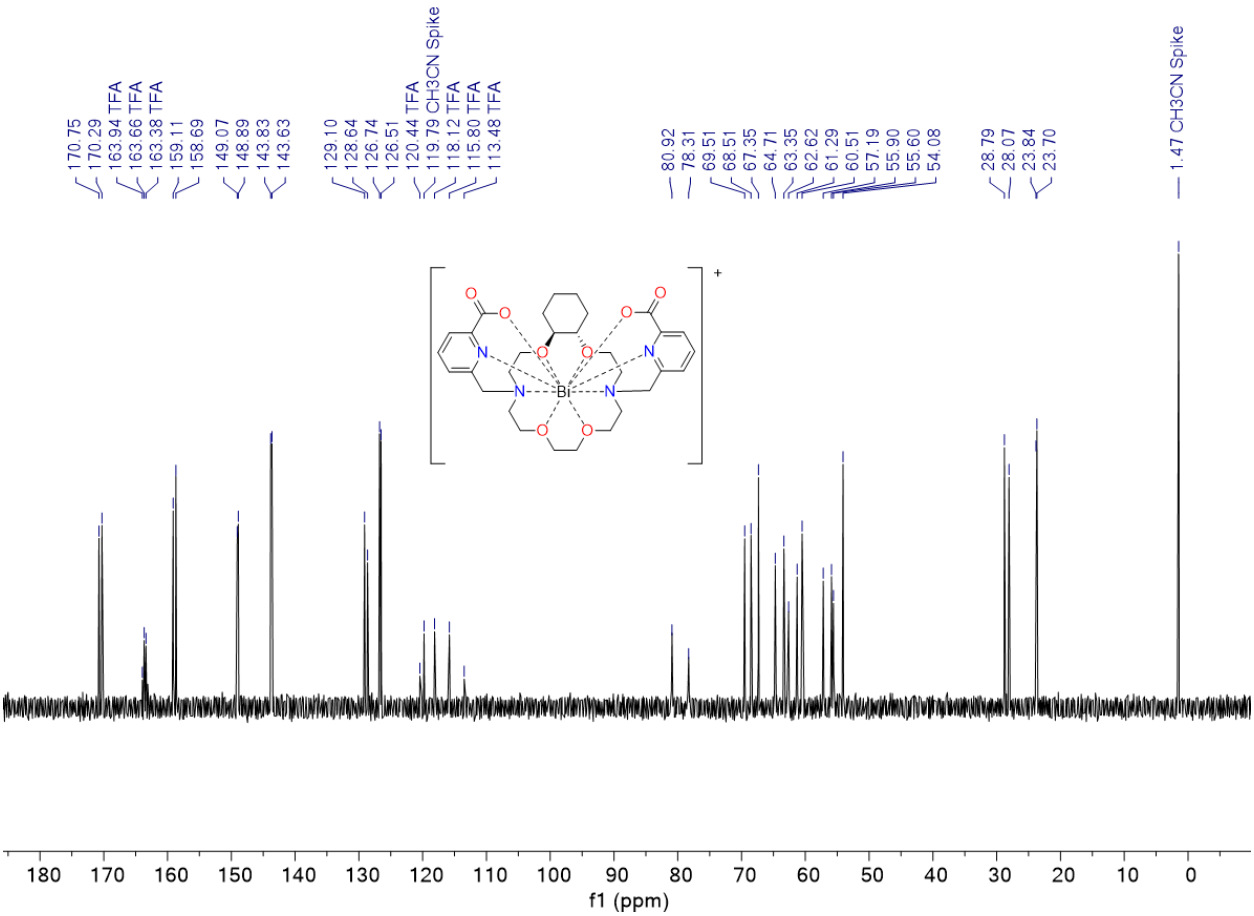


Figure S33. $^{13}C\{^{1}H\}$ NMR spectrum of [Bi(CHX-macropa)]$^{+}$. 126 MHz, 298 K, D_2O.

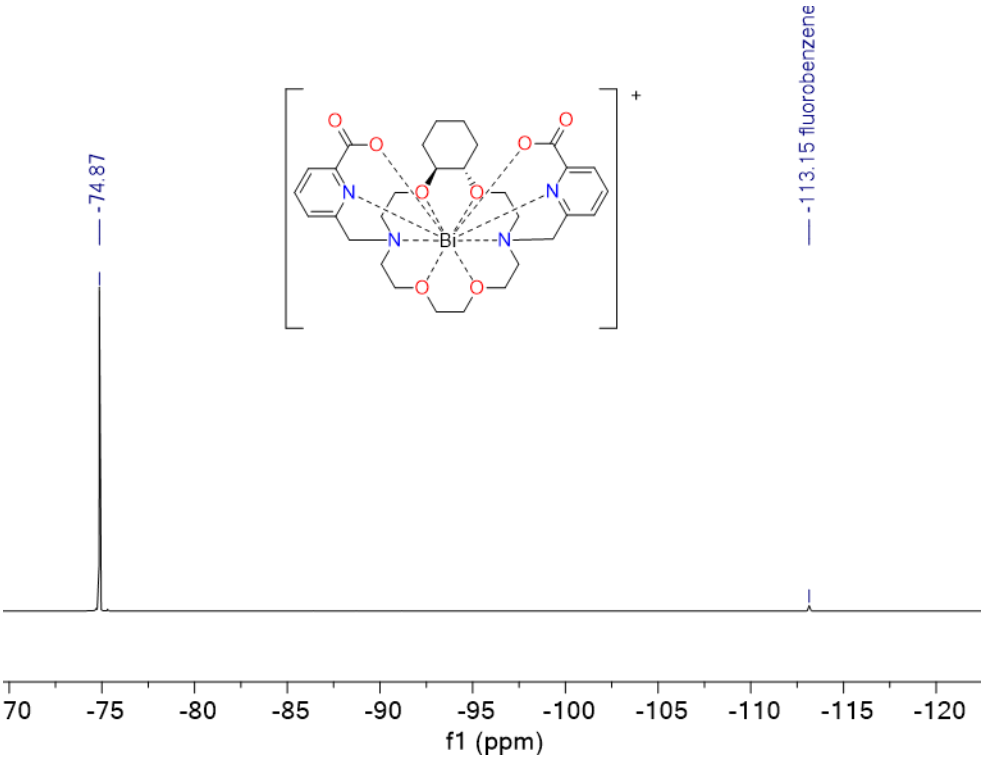


Figure S34. ^{19}F NMR spectrum of [Bi(CHX-macropa)]$^{+}$. 470 MHz, 298 K, D_2O.

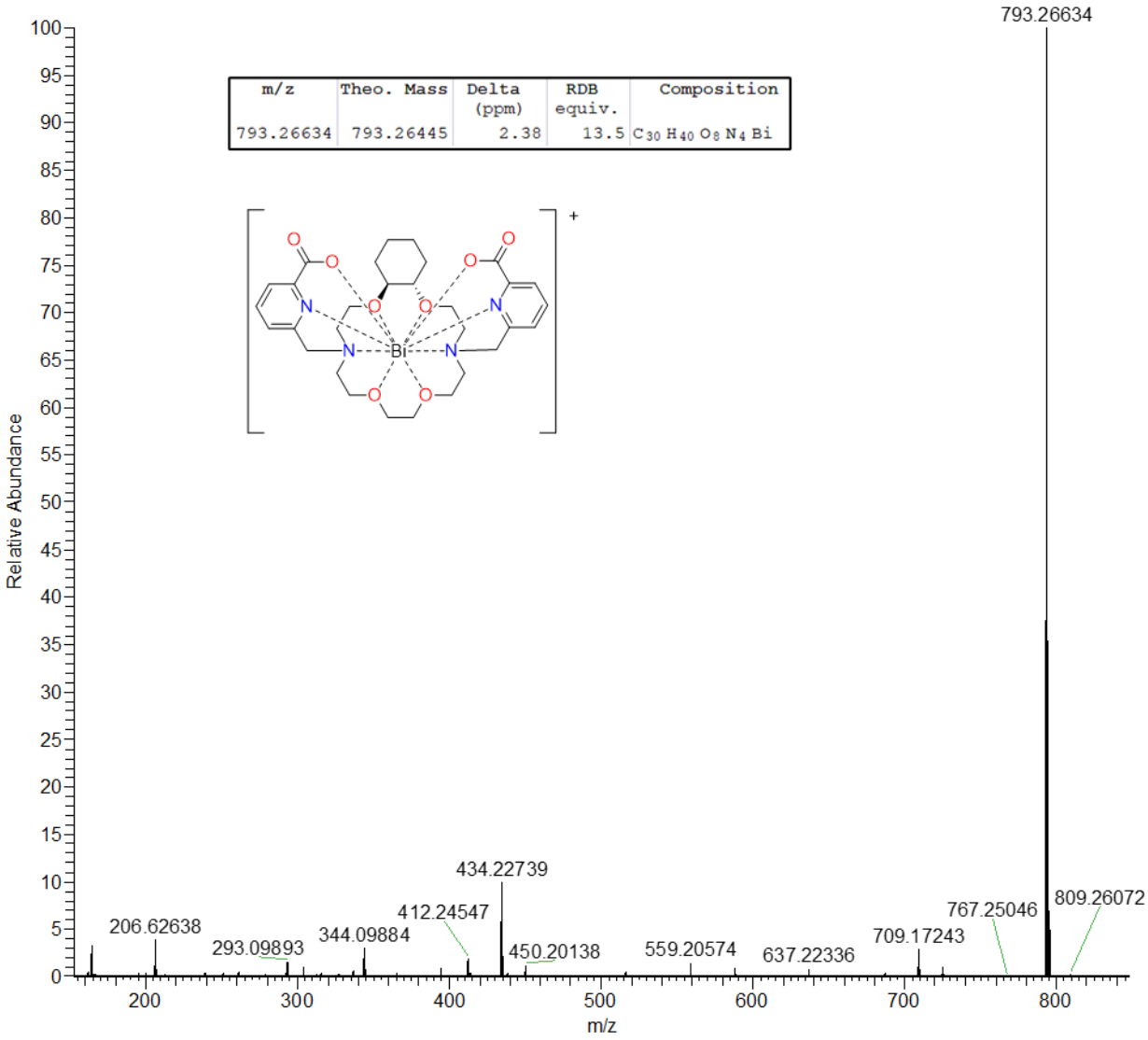


Figure S35. ESI-HRMS of [Bi(CHX-macropa)]$^+$. The sample was diluted using a solution of CH_3CN:H_2O (7:3) containing 1% formic acid. The sample was analyzed using a mobile phase of CH_3CN:H_2O (7:3). One ion peak was observed, corresponding to $[M]^+$ (*m/z* 793.26634).

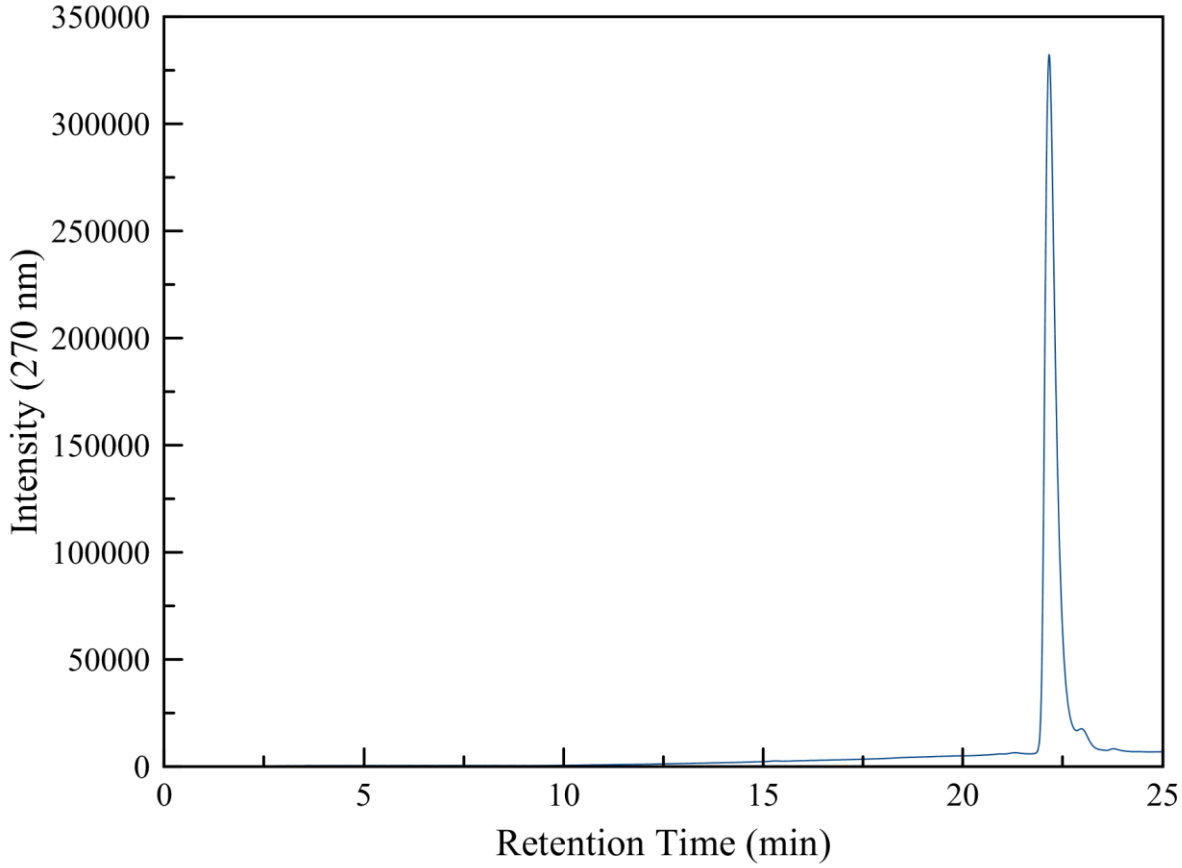


Figure S36. HPLC chromatogram of [Bi(CHX-macropa)]$^+$. Retention time (t_R) = 22.159 min using a binary MeOH/H_2O mobile phase containing 0.1% TFA (program: 10% MeOH for 5 min, followed by a linear gradient to 100% MeOH over 20 min).

2.2.6 [Bi(macropaquin)]$^{+}$

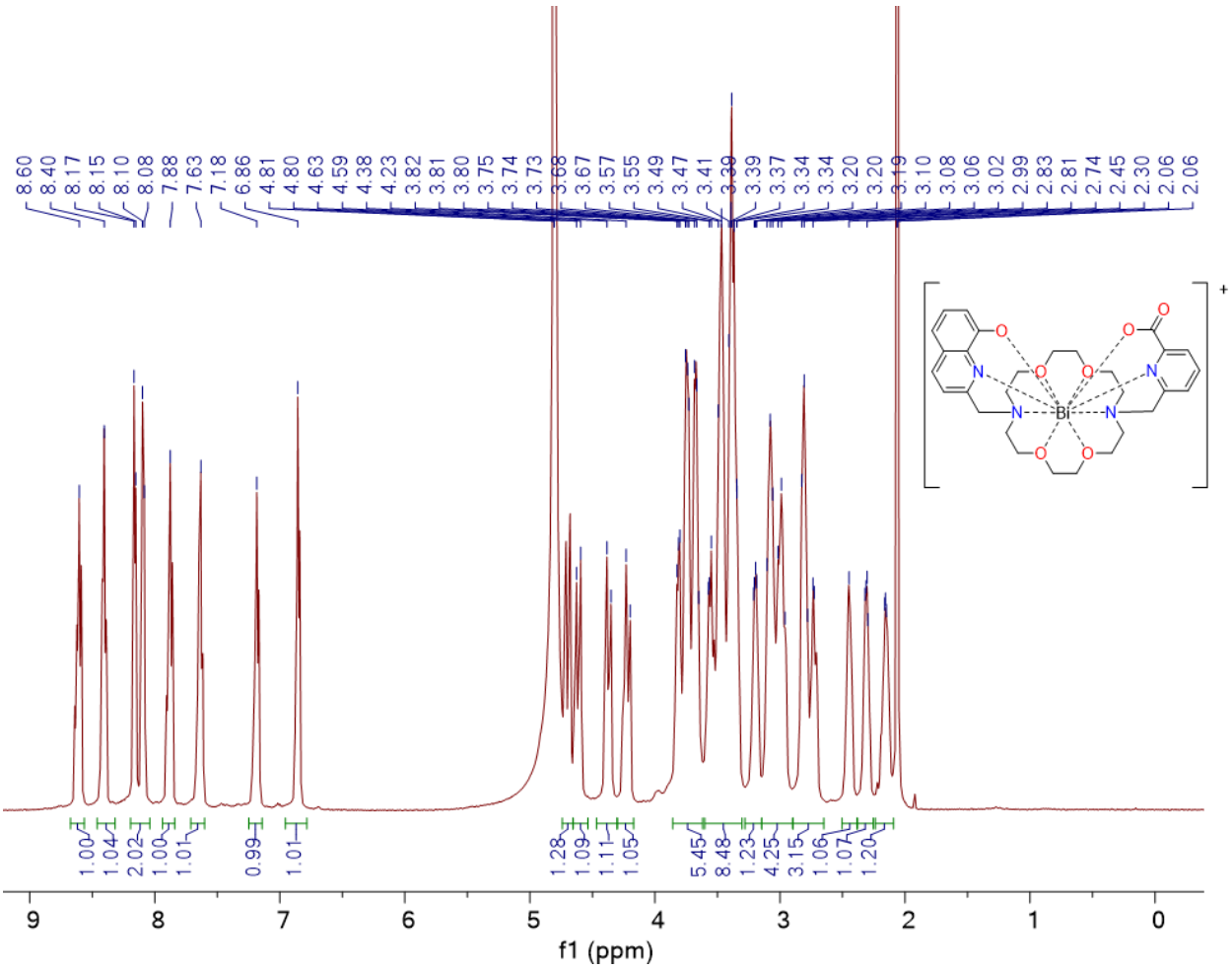


Figure S37. ^{1}H NMR spectrum of [Bi(macropaquin)]$^{+}$. 500 MHz, 298 K, D_2O.

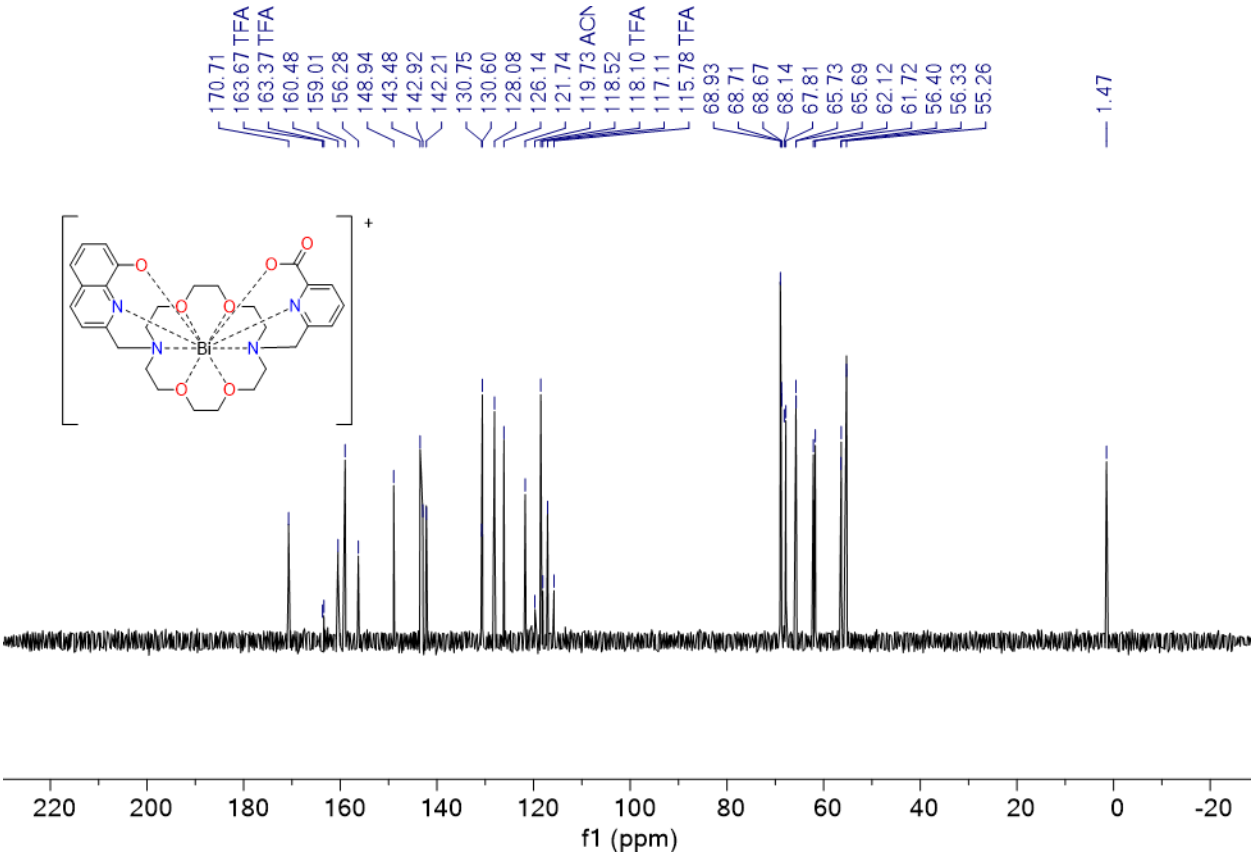


Figure S38. $^{13}C\{^{1}H\}$ NMR spectrum of [Bi(macropaquin)]$^{+}$. 126 MHz, 298 K, D_2O.

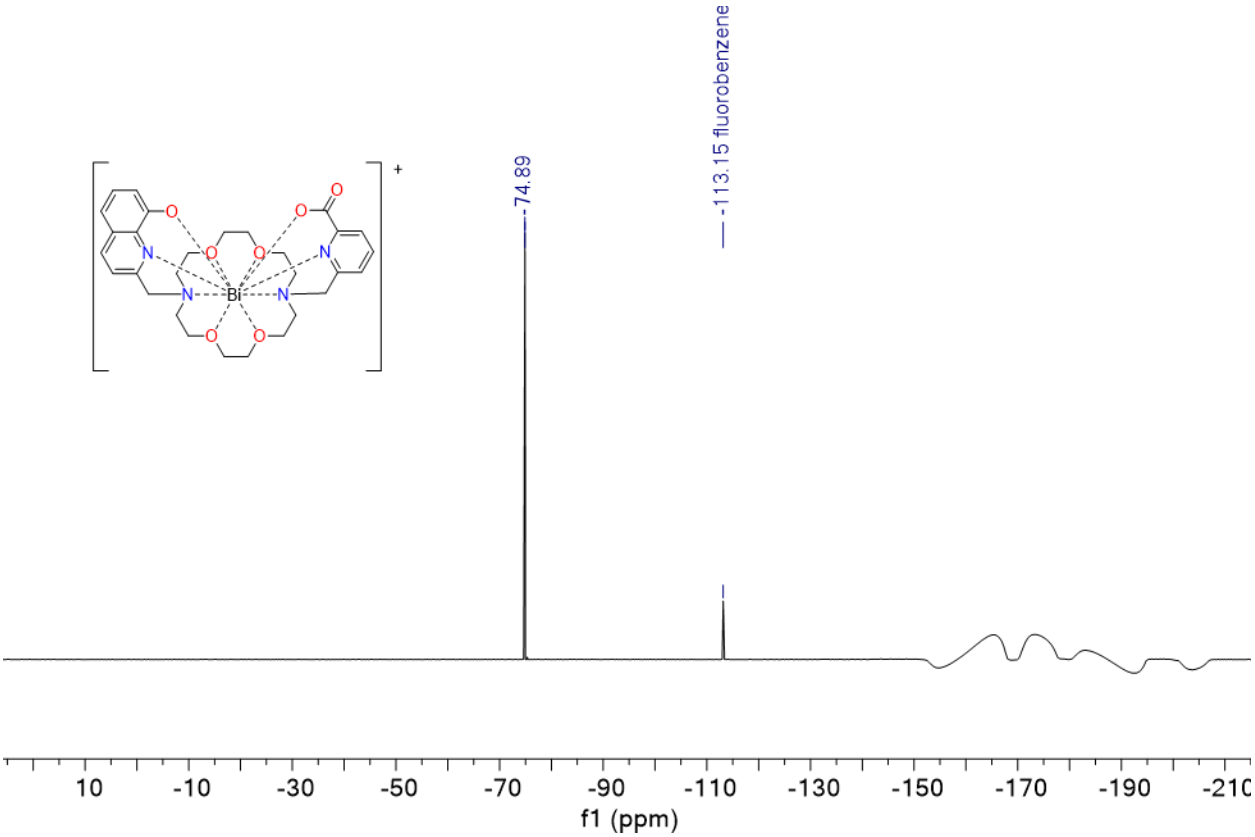


Figure S39. ^{19}F NMR spectrum of [Bi(macropaquin)]$^{+}$. 470 MHz, 298 K, D_2O.

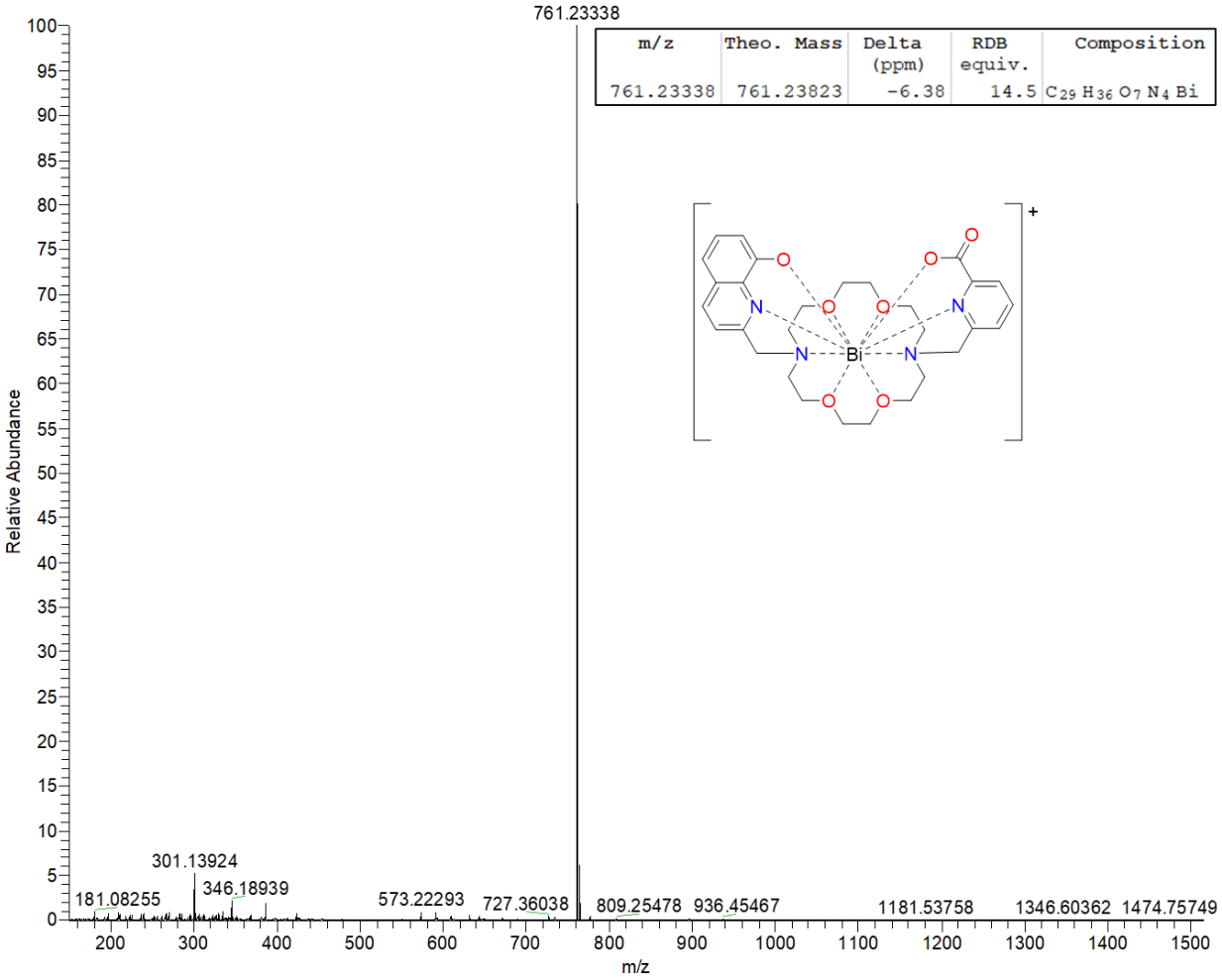


Figure S40. ESI-HRMS of [Bi(macropaquin)]$^{+}$. The sampple was diluted using a solution of CH_3OH. The sample was analyzed using a mobile phase of CH_3OH. One ion peak was observed, corresponding to $[M]^{+}$ (*m/z* 761.23338)

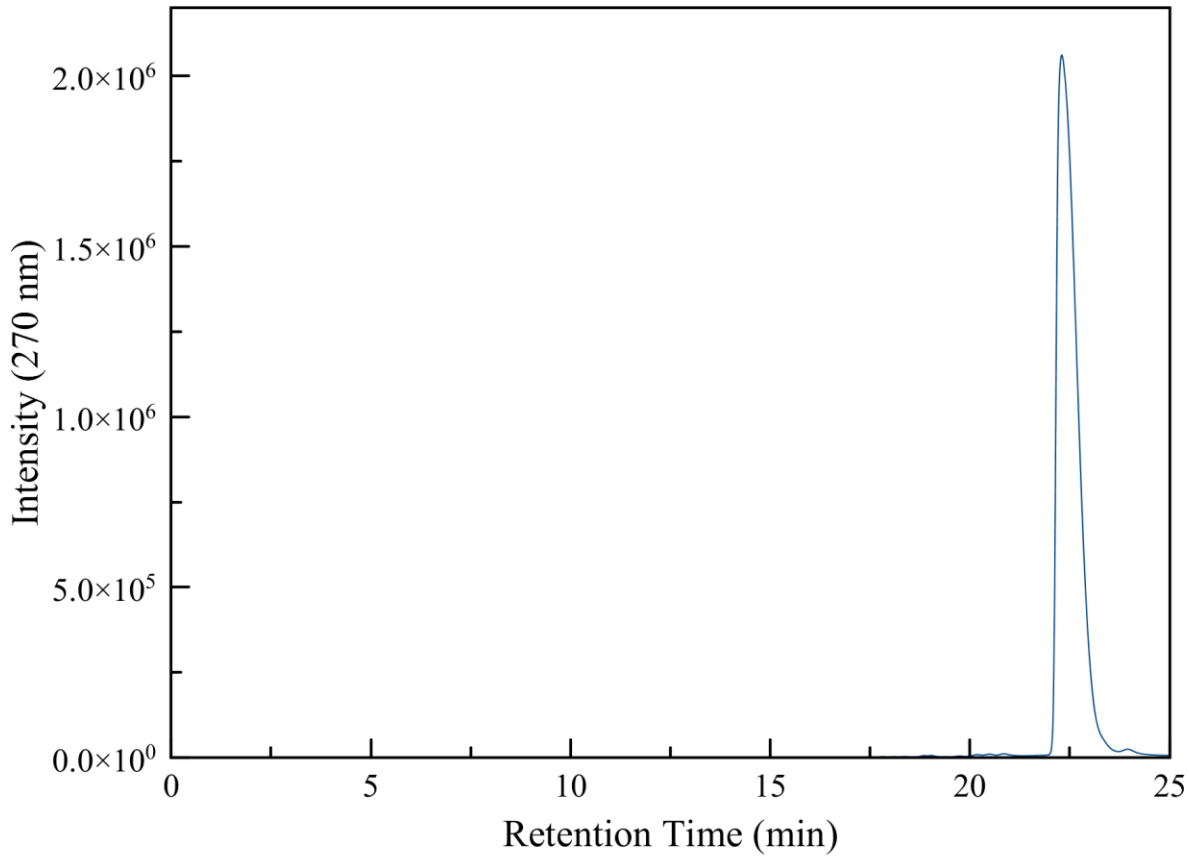


Figure S41. HPLC chromatogram of [Bi(macropaquin)]$^+$. Retention time (t_R) = 22.300 min using a binary MeOH/H_2O mobile phase containing 0.1% TFA (program: 10% MeOH for 5 min, followed by a linear gradient to 100% MeOH over 20 min).

2.3 Crystallography

Table S1. X-ray Crystallographic Data Collection and Refinement Parameters for [Bi(macropa)](NO_3)·dioxane[Bi(macrophospho)](TFA)·H_2O, and [Bi(macrophosphi)](TFA)·H_2O

Compound	[Bi(macropa)](NO_3)·dioxane	[Bi(macrophospho)](TFA)·H_2O	[Bi(macrophosphi)](TFA)·H_2O
Empirical formula	$C_{28}H_{38}BiN_5O_{12}$	$C_{27.50}H_{41.50}BiF_3N_{4.50}O_{13.50}P_2$	$C_{28}H_{42}BiF_3N_4O_{11}P_2$
Formula weight	845.61	979.07	938.57
a (Å)	10.04970(10)	17.76930(10)	10.4640(2)
b (Å)	12.3110(2)	14.48670(10)	12.0924(3)
c (Å)	13.4780(2)	27.9626(2)	13.8540(3)
α (°)	70.3370(10)	90	107.360(2)
β (°)	72.1840(10)	103.3710(10)	91.025(2)
γ (°)	76.3870(10)	90	96.196(2)
V (Å^3)	1478.59(4)	7002.97(8)	1661.07(7)
Z	2	8	2
Crystal system	Triclinic	Monoclinic	Triclinic
Space group	$P\bar{1}$	*I*12/a1	$P\bar{1}$
ρ_{calc} (mg/m^3)	1.899	1.857	1.877
μ (mm^{-1})	12.357	11.531	12.057
T (K)	100.00(10)	200.00(10)	99.9(3)
2*θ* range (°)	3.595 to 70.076	3.249 to 70.069	3.347 to 70.060
Independent reflections	5603	6645	6300
R_{int}	0.0612	0.0690	0.0465
Number of parameters	425	454	479
Max, min peaks (e·Å^{-3})	1.075/–0.862	1.841/–0.705	1.522/–1.110
R1[a]/wR2[b] (all data)	0.0183/0.0442	0.0348/0.0940	0.02633/0.0645
R1[a]/wR2[b] (>2σ)	0.0178/0.0439	0.0337/0.0932	0.0253/0.0638
Goodness of fit[c]	1.037	1.054	1.063

[a] $R_1 = \Sigma||Fo| - |Fc||/\Sigma|Fo|$ for $I > 2\sigma$. [b] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$ for $I > 2\sigma$. [c] $GoF = \{\Sigma[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$, where *n* is the number of data and *p* is the number of refined parameters.

Table S2. SHAPE Output CSMs

Complex	Johnson Pentagonal Pyramid	Trigonal Prism	Octahedron	Pentagonal Pyramid	Hexagon
$[Bi(macropa)]^+$	1.875	18.735	30.965	3.038	23.778
$[Bi(macrophospho)]^+$	1.473	18.688	30.933	2.532	25.685
$[Bi(macrophosphi)]^+$	1.388	19.207	31.580	2.445	25.591

2.4 DTPA Challenges

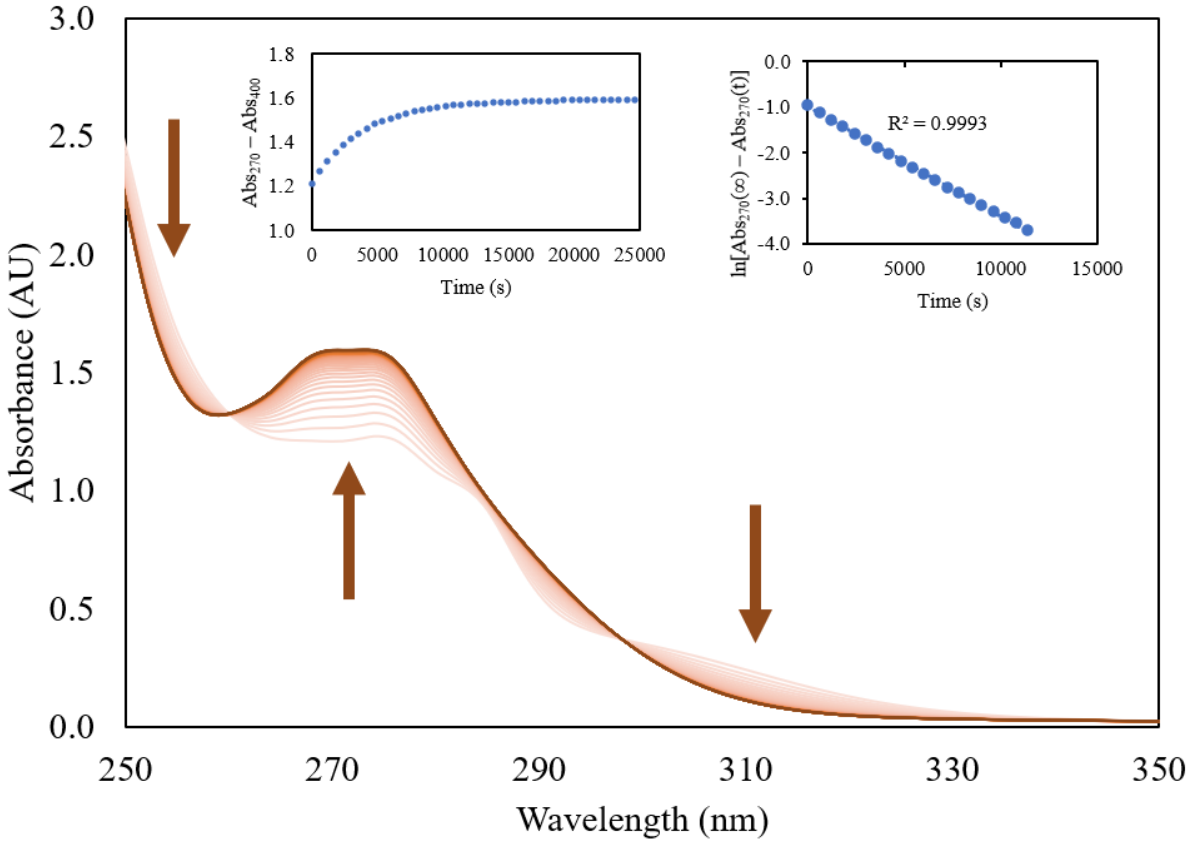


Figure S42. [Bi(macropa)]$^{+}$ DTPA Challenge. Two isosbestic points are observed, demonstrating immediate transchelation. Insets show absorbance at 270 nm with a baseline normalization at 400 nm vs time (left) and the natural log of the difference between the final absorbance and the absorbance at each time (right).

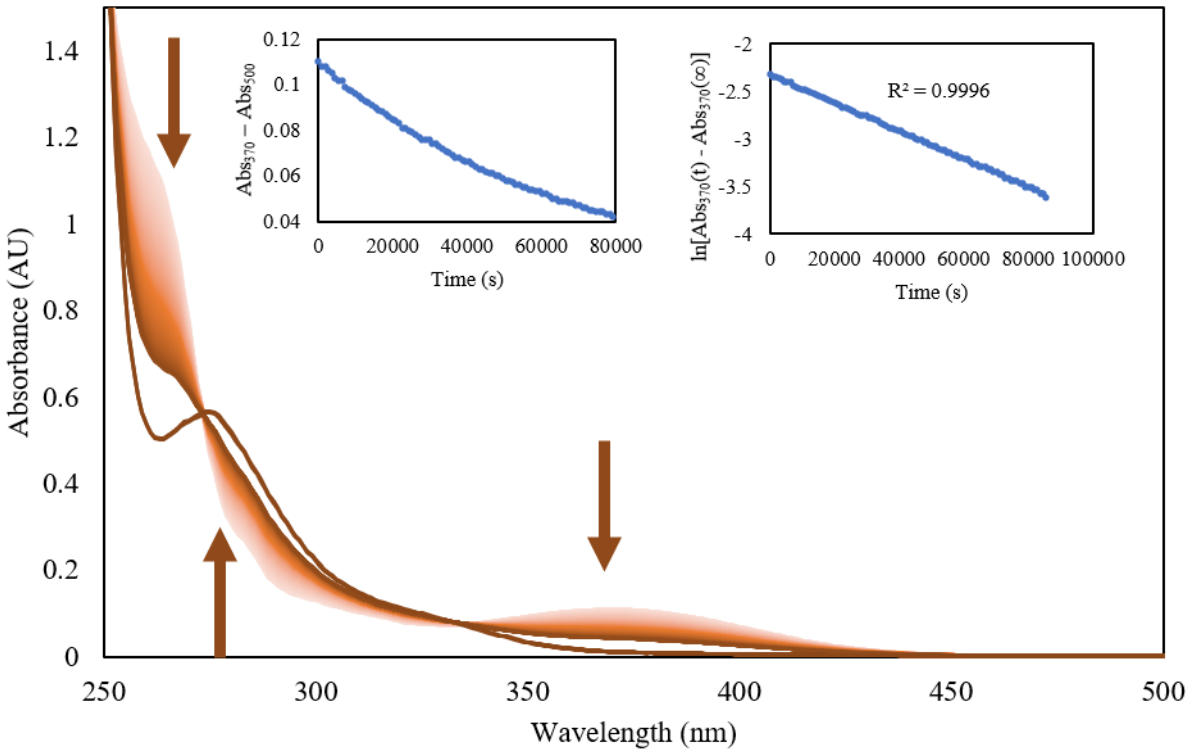


Figure S43. [Bi(macropaquin)]$^{+}$ DTPA Challenge. Spectra were measured regularly throughout the first 24 hours, while the final spectrum was taken 5 days after the start of the challenge. Spectral changes were confirmed to stop by this time point. Two isosbectic points are observed, demonstrating immediate transchelation. Insets show absorbance at 370 nm with a baseline normalization at 500 nm vs time (left) and the natural log of the difference between the final absorbance and the absorbance at each time (right).

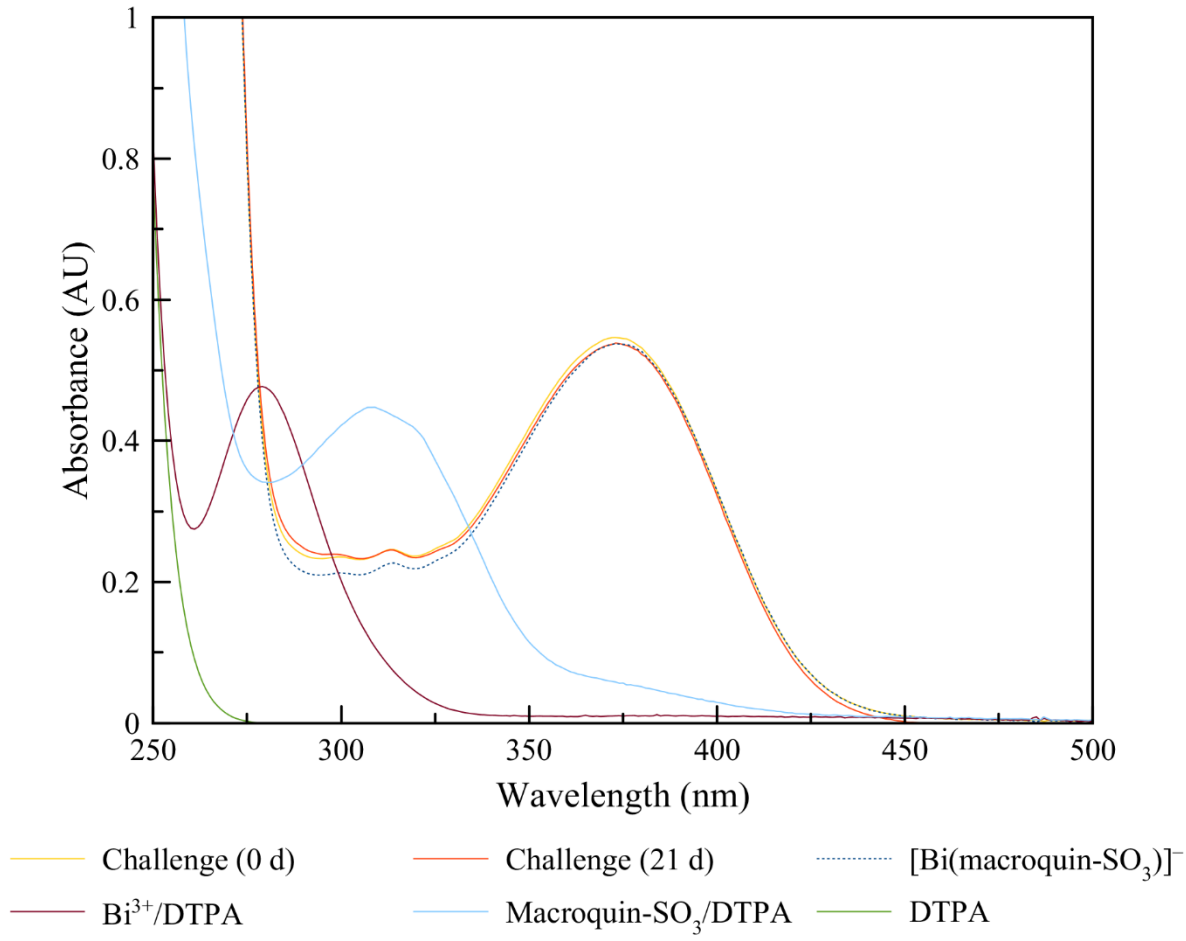


Figure S44. [Bi(macroquin-SO_3)]$^-$ DTPA Challenge. No spectral changes were observed from 0 d (yellow) to 21 d (orange), demonstrating the kinetic inertness of the complex. The absorbance spectra of [Bi(macroquin-SO_3)]$^-$ (dashed blue), free macroquin-SO_3 (light blue), free Bi^{3+} (brown), and DTPA (green) are also shown to demonstrate that transchelation would result in spectral changes.

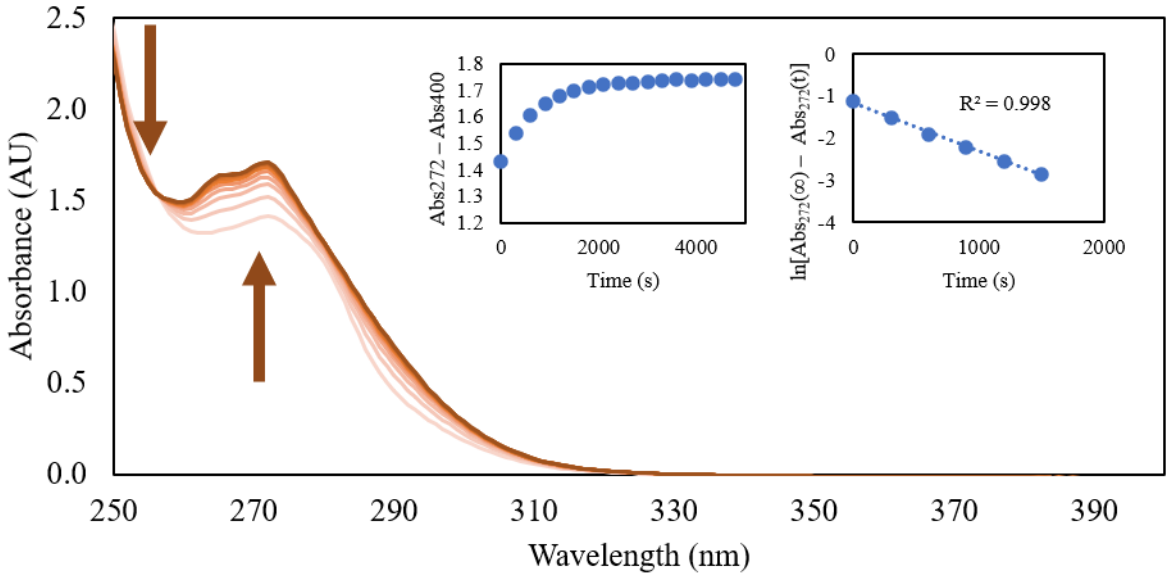


Figure S45. [Bi(macrophospho)]$^{+}$ DTPA Challenge. An isosbectic point is observed, demonstrating immediate transchelation. Insets show absorbance at 272 nm with a baseline normalization at 400 nm vs time (left) and the natural log of the difference between the final absorbance and the absorbance at each time (right).

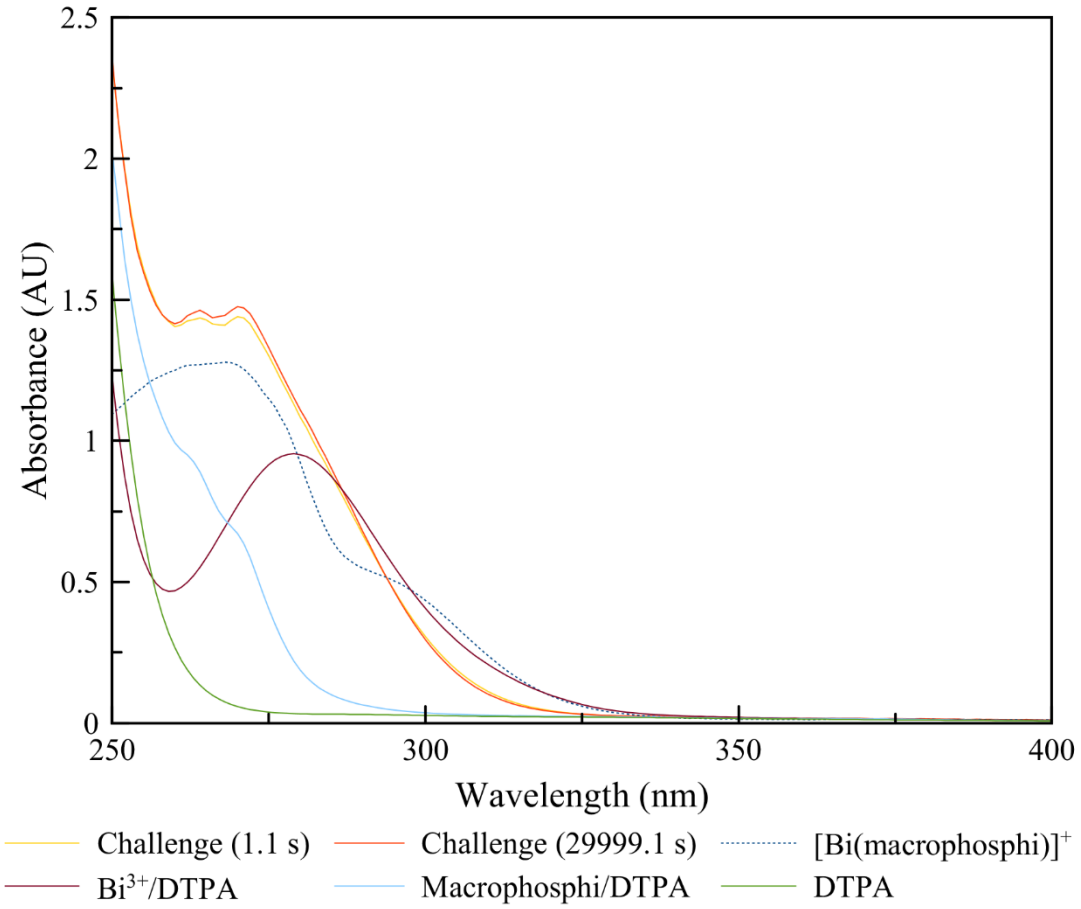


Figure S46. [Bi(macrophosphi)]$^{+}$ DTPA Challenge. No spectral changes were observed from 1.1 s (yellow) to 29999.1 s (orange), while the initial spectrum does not match that of [Bi(macrophosphi)]$^{+}$ (dashed blue). Instead, two observable peaks match the two shoulder peaks present in macrophosphi (light blue). This suggests nearly instantaneous transchelation of the complex. The absorbance spectra of DTPA (green) and free Bi^{3+} (brown) are shown.

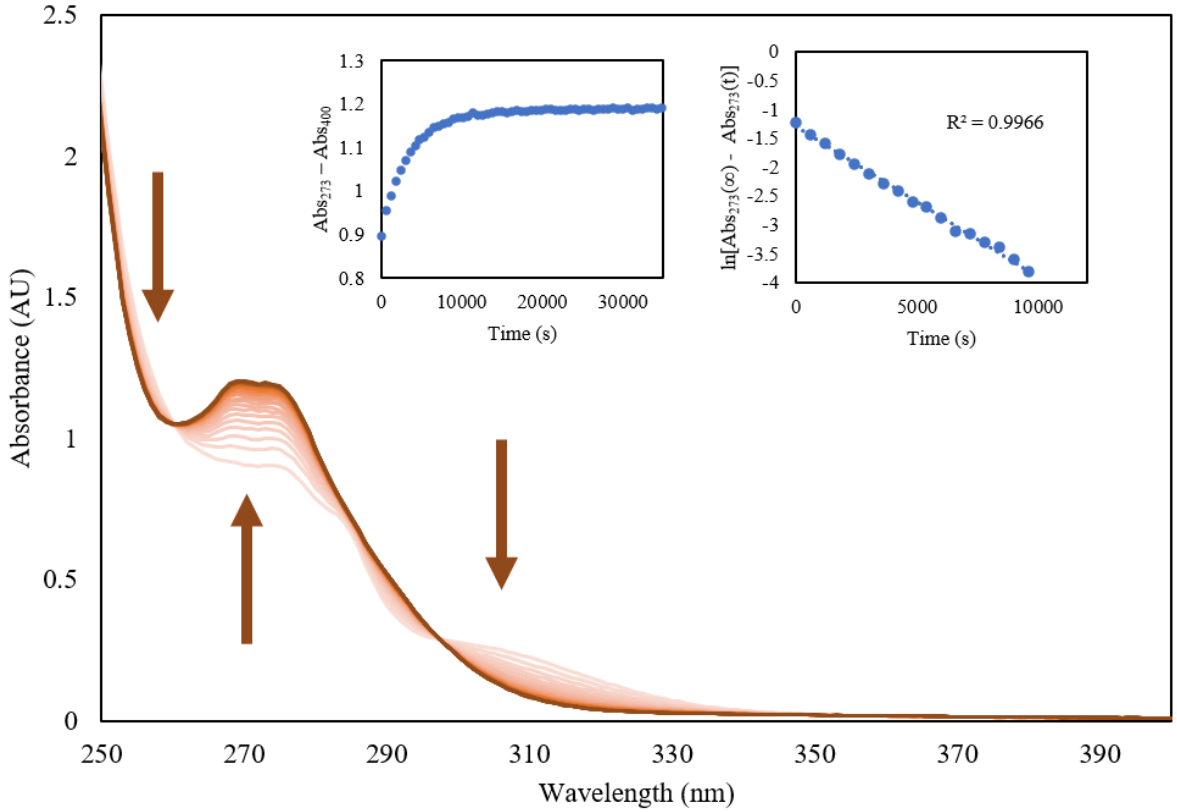


Figure S47. [Bi(CHX-macropa)]$^{+}$ DTPA Challenge. Two isosbectic points are observed, demonstrating immediate transchelation. Insets show absorbance at 273 nm with a baseline normalization at 400 nm vs time (left) and the natural log of the difference between the final absorbance and the absorbance at each time (right).

2.5. Computational Results

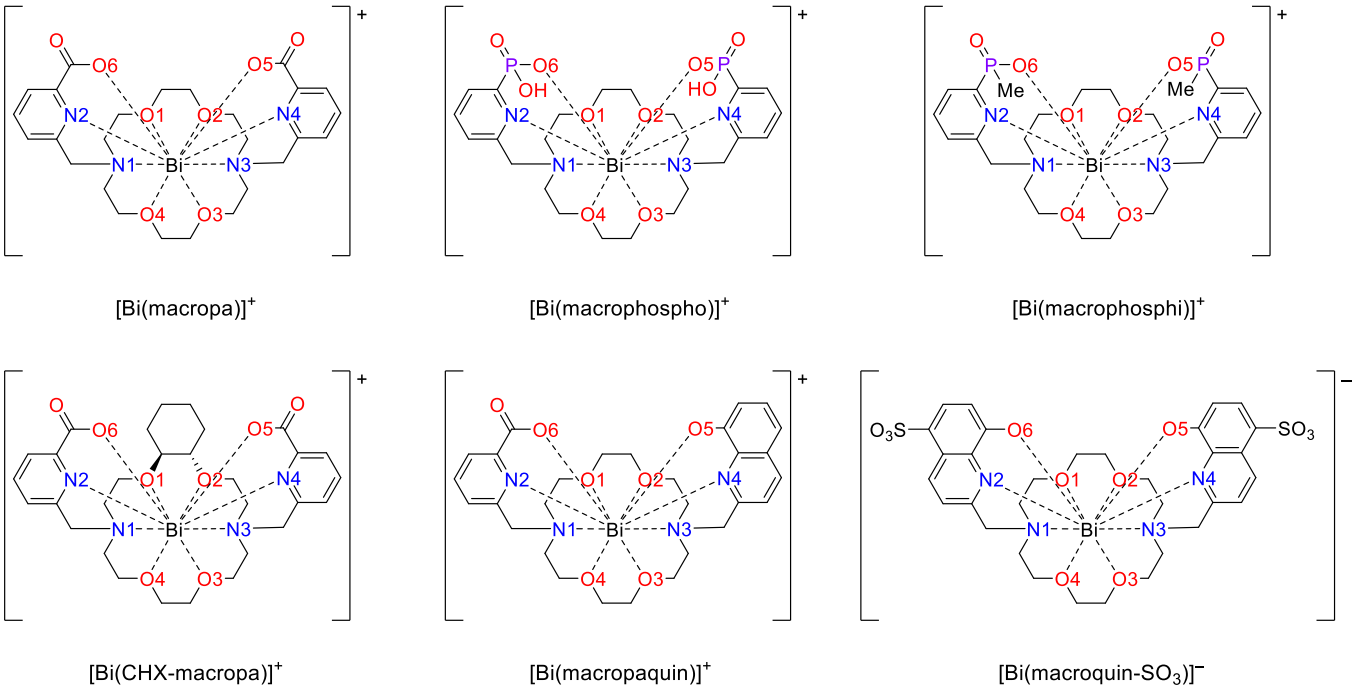


Figure S48. Atom numbering scheme for QTAIM calculations.

Table S3. Compiled QTAIM Results for [Bi(macrophosphi)]$^+$. The Corresponding Units are ρ (e^- Å^{-3}), $\nabla^2\rho(\mathbf{r})$ (unitless), H(**r**) (kJ/mol Å^{-3}), V(**r**) (kJ/mol Å^{-3}), G(**r**) (kJ/mol Å^{-3}), H/ρ (kJ/mol per e^-), |V|/G (unitless), and δ (unitless)

Contact	ρ(**r**)	$\nabla^2\rho(\mathbf{r})$	H(**r**)	V(**r**)	G(**r**)	H/ρ	|V|/G	δ
Bi–O1	0.0183	0.0627	4.0894	–32.9728	37.0622	223.7454	0.8897	0.3274
Bi–O2	0.0151	0.0527	4.7208	–25.1468	29.8676	312.6905	0.8419	0.2917
Bi–O3	0.0246	0.0862	2.7700	–51.0182	53.7882	312.6905	0.8419	0.4188
Bi–O4 [a]	–	–	–	–	–	–	–	0.08694
Bi–O5	0.0816	0.3159	–40.8693	–288.9010	248.0318	–500.7658	1.1648	0.9708
Bi–O6	0.0790	0.3051	–38.0762	–276.2232	238.1469	–481.7647	1.1599	0.9476
Bi–N1	0.0292	0.0691	–3.6798	–52.7400	49.0602	–125.8641	1.0750	0.4697
Bi–N2	0.0507	0.1247	–17.7810	–117.3759	99.5949	–350.5292	1.1785	0.6846
Bi–N3	0.0155	0.0384	1.7174	–21.7582	23.4756	110.5529	0.9268	0.3032
Bi–N4	0.0416	0.1021	–10.8898	–88.7619	77.8721	–261.7623	1.1398	0.6054

a. A bond critical point could not be located between these atoms

Table S4. Compiled QTAIM Results for [Bi(macrophospho)]$^+$. The Corresponding Units are ρ (e^- Å^{-3}), $\nabla^2\rho(\mathbf{r})$ (unitless), H(**r**) (kJ/mol Å^{-3}), V(**r**) (kJ/mol Å^{-3}), G(**r**) (kJ/mol Å^{-3}), H/ρ (kJ/mol per e^-), |V|/G (unitless), and δ (unitless)

Contact	ρ(**r**)	$\nabla^2\rho(\mathbf{r})$	H(**r**)	V(**r**)	G(**r**)	H/ρ	|V|/G	δ
Bi–O1	0.0063	0.0210	3.2236	–7.3687	10.5927	514.9444	0.6956	0.1311

Bi–O2	0.0257	0.0895	2.3668	–54.0253	56.3921	92.1067	0.9580	0.4347
Bi–O3	0.0147	0.0509	4.7168	–23.9546	28.6714	92.1067	0.9580	0.2862
Bi–O4	0.0158	0.0539	4.3781	–26.6463	31.0243	277.8497	0.8589	0.2950
Bi–O5	0.0881	0.3526	–47.5688	–326.3038	278.7350	–540.2123	1.1707	0.9977
Bi–O6	0.0718	0.2697	–31.1072	–239.1356	208.0284	–432.9824	1.1495	0.8771
Bi–N1	0.0306	0.0723	–4.5233	–56.4754	51.9522	–147.6179	1.0871	0.4830
Bi–N2	0.0476	0.1179	–15.3202	–107.9704	92.6502	–321.6876	1.1654	0.6499
Bi–N3	0.0175	0.2484	1.2139	–25.7839	26.9977	69.2168	0.9550	0.3332
Bi–N4	0.0444	0.1093	–12.9238	–97.5587	84.6349	–291.0008	1.1527	0.6323

Table S5. Compiled QTAIM Results for $[Bi(CHX\text{-}macropa)]^+$. The Corresponding Units are ρ (e^- Å^{-3}), $\nabla^2\rho(\mathbf{r})$ (unitless), H(**r**) (kJ/mol Å^{-3}), V(**r**) (kJ/mol Å^{-3}), G(**r**) (kJ/mol Å^{-3}), H/ρ (kJ/mol per e^-), |V|/G (unitless), and δ (unitless)

Contact	ρ(**r**)	$\nabla^2\rho(\mathbf{r})$	H(**r**)	V(**r**)	G(**r**)	H/ρ	|V|/G	δ
Bi–O1	0.0514	0.1292	–18.2421	–121.2608	103.0187	–354.8838	1.1771	0.6843
Bi–O2	0.0571	0.1443	–23.1416	–140.9230	117.7814	–405.3109	1.1965	0.7247
Bi–O3	0.0213	0.0458	–0.7270	–31.4916	30.7646	–405.3109	1.1965	0.3525
Bi–O4	0.0178	0.0550	3.1312	–29.8604	32.9916	175.4914	0.9051	0.2999
Bi–O5	0.0776	0.2831	–38.6701	–262.9884	224.3183	–498.0826	1.1724	0.9523
Bi–O6	0.0786	0.2880	–39.4743	–267.8234	228.3491	–502.2058	1.1729	0.9626
Bi–N1	0.0082	0.0255	3.3099	–10.1021	13.4120	404.5845	0.7532	0.3567
Bi–N2	0.0155	0.0481	3.6336	–24.2898	27.9234	234.3138	0.8699	0.2722
Bi–N3	0.0218	0.0675	2.2519	–39.7922	42.0441	103.1472	0.9464	0.1575
Bi–N4	0.0322	0.0664	–6.6396	–56.8372	50.1976	–206.1312	1.1323	0.4715

Table S6. Compiled QTAIM Results for $[Bi(macropa)]^+$. The Corresponding Units are ρ (e^- Å^{-3}), $\nabla^2\rho(\mathbf{r})$ (unitless), H(**r**) (kJ/mol Å^{-3}), V(**r**) (kJ/mol Å^{-3}), G(**r**) (kJ/mol Å^{-3}), H/ρ (kJ/mol per e^-), |V|/G (unitless), and δ (unitless)

Contact	ρ(**r**)	$\nabla^2\rho(\mathbf{r})$	H(**r**)	V(**r**)	G(**r**)	H/ρ	|V|/G	δ
Bi–O1	0.0155	0.0535	4.5642	–25.9728	30.5370	294.0775	0.8505	0.2954
Bi–O2	0.0182	0.0618	3.9990	–32.5688	36.5678	219.9833	0.8906	0.3273
Bi–O3	0.0070	0.0236	3.4896	–8.4898	11.9793	219.9833	0.8906	0.1517
Bi–O4	0.0206	0.0711	3.7230	–39.2123	42.9353	180.6130	0.9133	0.3681
Bi–O5	0.0792	0.2911	–40.1190	–271.1319	231.0129	–506.3900	1.1737	0.9690
Bi–O6	0.0778	0.2837	–38.7577	–263.5928	224.8352	–498.4372	1.1724	0.9526
Bi–N1	0.0265	0.0636	–2.3712	–46.5008	44.1297	–89.4222	1.0537	0.4341
Bi–N2	0.0566	0.1424	–22.7328	–138.9202	116.1874	–401.4145	1.1957	0.7238
Bi–N3	0.0178	0.0440	1.1498	–26.6088	27.7586	64.6022	0.9586	0.3299
Bi–N4	0.0512	0.1285	–18.0922	–120.5117	102.4195	–353.3262	1.1766	0.6839

Table S7. Compiled QTAIM Results for $[Bi(macropaquin)]^+$. The Corresponding Units are ρ (e^- Å^{-3}), $\nabla^2\rho(\mathbf{r})$ (unitless), H(**r**) (kJ/mol Å^{-3}), V(**r**) (kJ/mol Å^{-3}), G(**r**) (kJ/mol Å^{-3}), H/ρ (kJ/mol per e^-), |V|/G (unitless), and δ (unitless).

Contact	ρ(**r**)	$\nabla^2\rho(\mathbf{r})$	H(**r**)	V(**r**)	G(**r**)	H/ρ	|V|/G	δ

Bi–O1	0.0488	0.1223	–16.1913	–112.6188	96.4275	–331.545	1.1679	0.6674
Bi–O2	0.0600	0.1527	–25.6462	–151.4920	125.8458	–427.1610	1.2038	0.7523
Bi–O3	0.0158	0.0394	1.6805	–22.5243	24.2047	–427.1610	1.2038	0.3010
Bi–O4	0.0182	0.0620	4.0156	–32.6334	36.6489	220.2951	0.8904	0.3309
Bi–O5	0.0797	0.2863	–41.7686	–271.3049	229.5363	–524.1951	1.1820	0.9831
Bi–O6	0.0790	0.2905	–39.7312	–269.9509	230.2197	–503.1691	1.1726	0.9715
Bi–N1	0.0191	0.0015	4.0513	–35.3382	39.3896	211.7137	0.8971	0.3493
Bi–N2	0.0152	0.0522	4.5830	–25.1098	29.6928	301.8964	0.8457	0.2916
Bi–N3	0.0055	0.0181	2.8872	–6.0987	8.9859	529.7280	0.6787	0.1189
Bi–N4	0.0263	0.0631	–2.2831	–45.9558	43.6727	–86.6741	1.0523	0.4341

Table S8. Compiled QTAIM Results for Bi[(macroquin-SO_3)]$^-$. The Corresponding Units are ρ (e^- Å^{-3}), $\nabla^2\rho(\mathbf{r})$ (unitless), H(**r**) (kJ/mol Å^{-3}), V(**r**) (kJ/mol Å^{-3}), G(**r**) (kJ/mol Å^{-3}), H/ρ (kJ/mol per e^-), |V|/G (unitless), and δ (unitless)

Contact	ρ(**r**)	$\nabla^2\rho(\mathbf{r})$	H(**r**)	V(**r**)	G(**r**)	H/ρ	|V|/G	δ
Bi–O1	0.0069	0.0237	3.6309	–8.3062	11.9372	523.5501	0.6958	0.1571
Bi–O2	0.0127	0.0434	4.5507	–19.3726	23.9234	358.8095	0.8098	0.2533
Bi–O3	0.0069	0.0237	3.6316	–8.3095	11.9411	358.8095	0.8098	0.1571
Bi–O4	0.0127	0.0434	4.5510	–19.3822	23.9332	358.7002	0.8098	0.2533
Bi–O5	0.0872	0.3066	–50.5010	–302.0060	251.5050	–579.2698	1.2008	1.0345
Bi–O6	0.0872	0.3066	–50.4962	–301.9797	251.4835	–579.2422	1.2008	1.0345
Bi–N1	0.0152	0.0321	1.9153	–17.2402	19.1555	126.2895	0.9000	0.2921
Bi–N2	0.0581	0.1387	–23.6980	–138.3841	114.6862	–407.7068	1.2066	0.7213
Bi–N3	0.0152	0.0321	1.9156	–17.2384	19.1541	126.3215	0.9000	0.2921
Bi–N4	0.0581	0.1387	–23.6958	–138.3744	114.6786	–407.6882	1.2066	0.7213

2.6 iTLCs

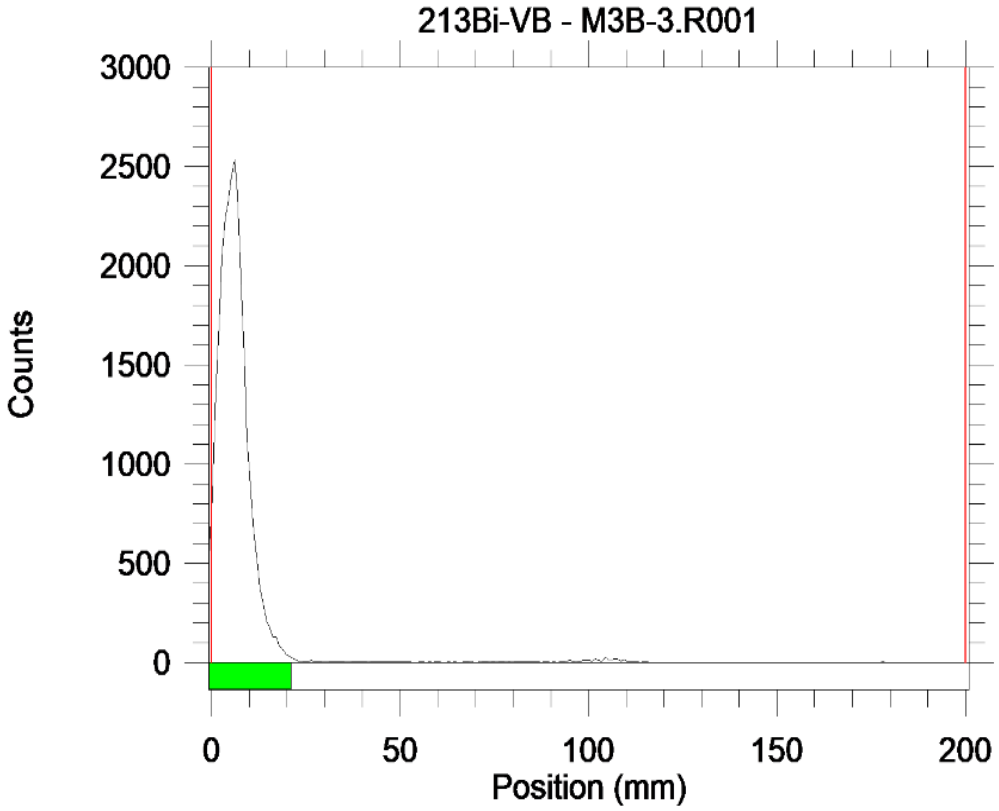


Figure S49. iTLC of macropa at 10^{-4} M, showing >99% RCY.

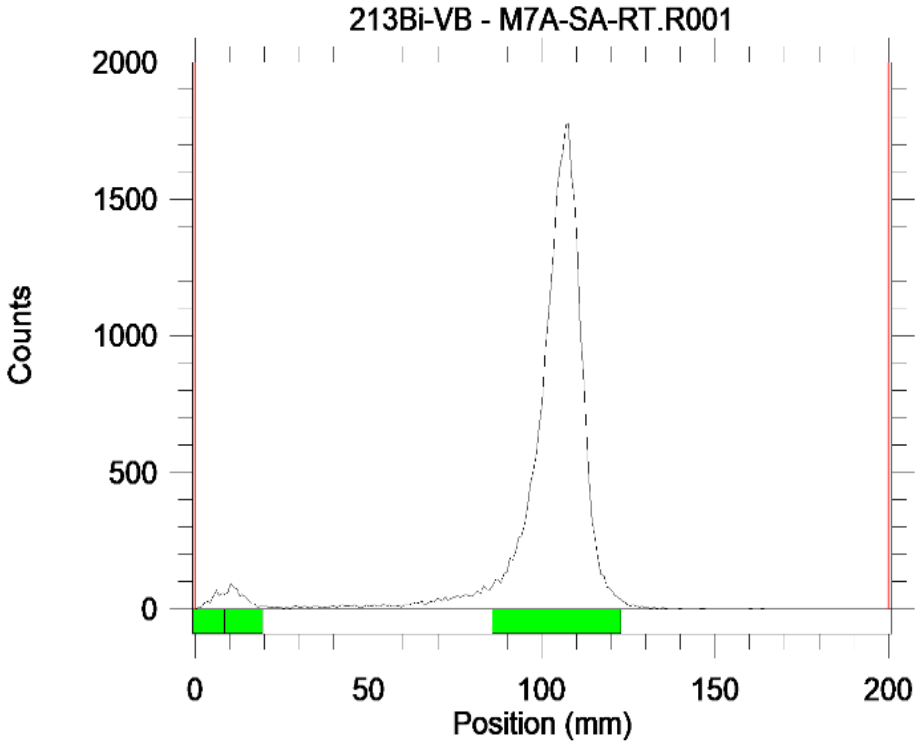


Figure S50. iTLC of macropa at 10^{-8} M, showing 3.21% RCY.

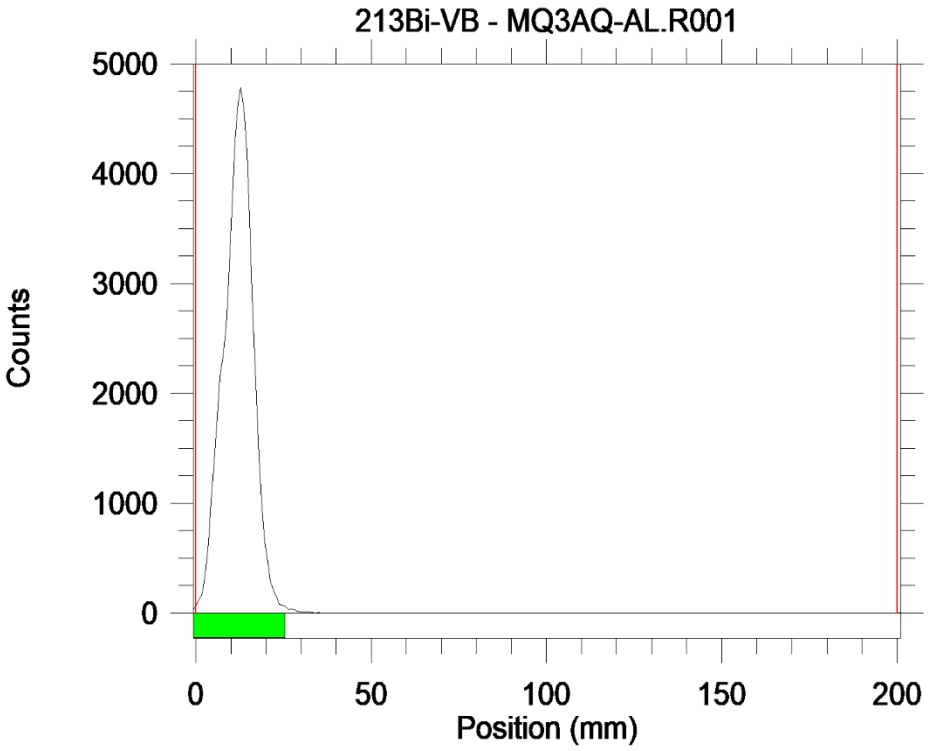


Figure S51. iTLC of macropaquin at 10^{-4} M, showing >99% RCY.

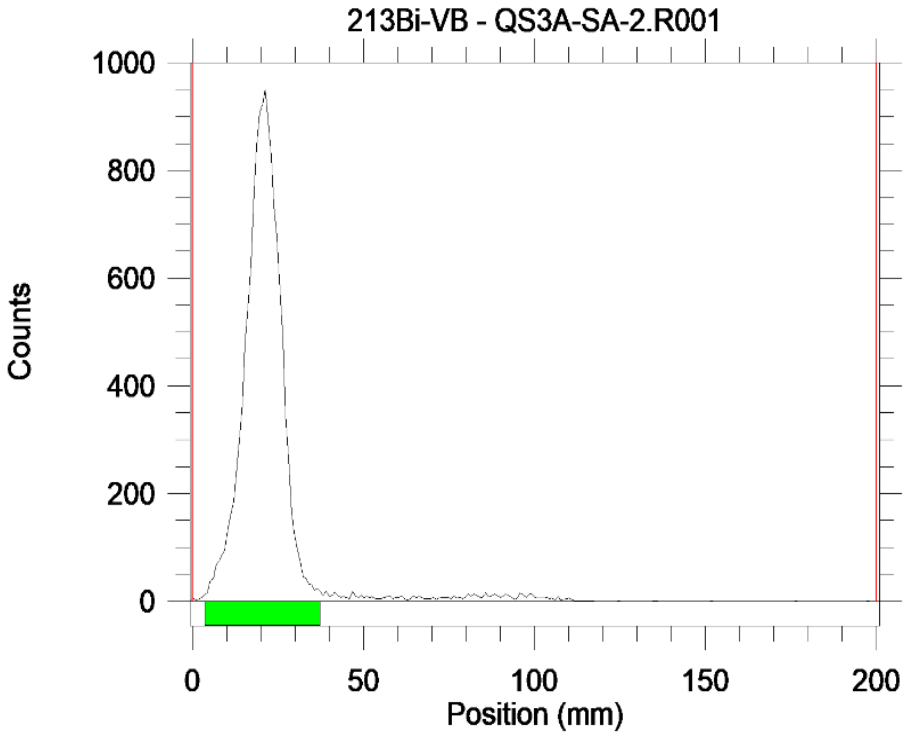


Figure S52. iTLC of macroquin-SO_3 at 10^{-4} M, showing >99% RCY.

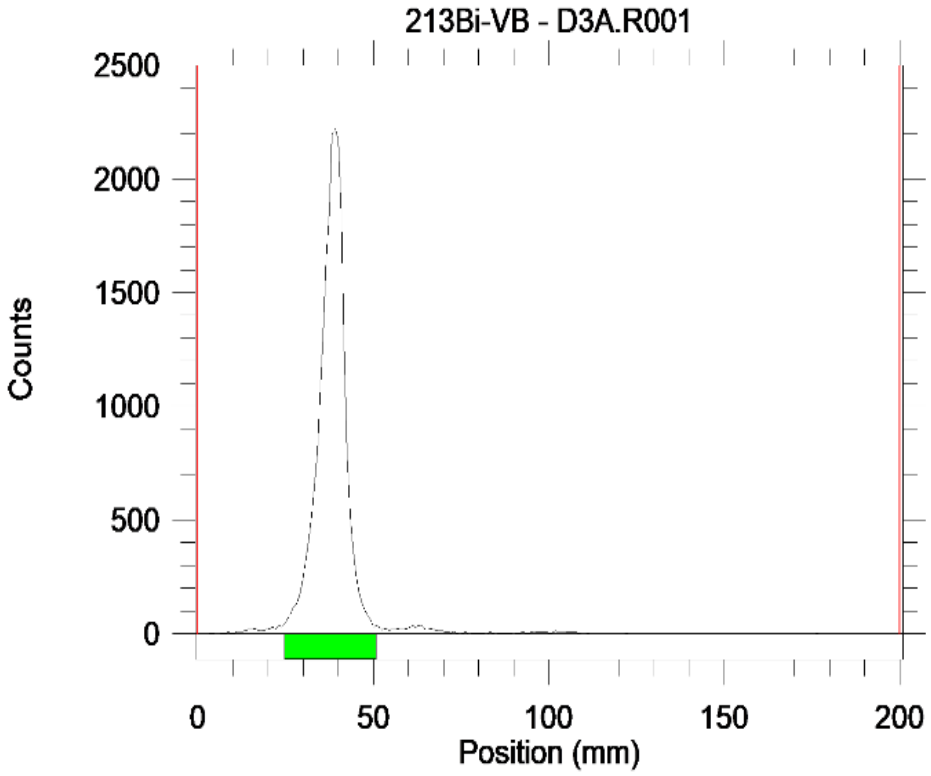


Figure S53. iTLC of DOTA at 10^{-4} M, showing >99% RCY.

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