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Supporting information

Macrocyclic complexes of Fe(III) with mixed hydroxypropyl and phenolate or amide pendants as T_1 MRI probes

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Experimental – Instrumentation and Materials

Instrumentation. ^{1}H NMR spectra, ^{13}C NMR spectra, and ^{31}P NMR spectra were acquired using a Bruker Neo-500 NMR spectrometer (broadband, autosampler) operating at 500 MHz, 125 MHz, and 202.5 MHz respectively. A Varian Inova 400 MHz spectrometer equipped with a 5 mm broad-band probe operating at a resonance frequency of 54.24 MHz was used for ^{17}O NMR experiments. All pH measurements were obtained using a Thermo Scientific 9110DJWP double junction, glass, semi-micro pH electrode connected to a 702 SM Titrino pH meter. A Thermo Fisher Linear Ion Trap (LTQ) Mass Spectrometer was used to collect all mass spectral data. Absorbance spectra were collected using a Beckman-Coulter DU 800 UV–Vis Spectrophotometer equipped with a Peltier temperature controller. Cyclic voltammograms were collected using a WavenowXV potentiostat from Pine Research using a glassy carbon working electrode, an Ag/AgCl reference electrode, and a Pt counter electrode, all purchased from Pine Research. T_1 relaxivity values were obtained using a Varian Inova 400 MHz spectrometer as well as a Nanalysis NMReady-60 Benchtop 60 MHz spectrometer. Iron concentration of complexes was determined by using Thermo X-Series 2 inductively coupled plasma mass spectrometer (ICP-MS).

Materials. 1,4,7-triazacyclononane (TACN) and o-cresol were purchased from TCI America. (S)−(−)−propylene oxide was purchased from either TCI America or Thermo Fisher Scientific. 4-nitrobenzyl bromide, meglumine, dibenzylamide, dibromomethane, hydrobromic acid (ACS, 47% - 49%), triethyl phosphite, N-bromosuccinimide, N,N-dimethylformamide dimethyl acetal, celite (standard super cell), and 65–70% nitric acid with greater than 99.999% purity (trace metals basis) were purchased from BeanTown Chemical. Triethylamine, and acetic acid were purchased from EMD Millipore Corporation. Human serum albumin, apo-transferrin,

10% palladium on carbon, azobisisobutyronitrile, and HEPES were purchased from Sigma-Aldrich. Methanesulfonyl chloride, ferrous bromide anhydrous, and hydrochloric acid were purchased from Thermo Fisher Scientific. 2-chloroacetyl chloride was purchased from Acros Organics and ferrous chloride tetrachloride was purchased Alfa Aesar. Basic alumina (50-200 μm) and silica gel (63 – 200 μm, 60 Å) were purchased from Sorbtech. 10 ppm Fe and 10 ppm Co standard solutions were purchased from Inorganic Ventures. The TOAB ligand and Fe(TOAB) were synthesized as reported previously.[1]

Experimental – Methods

Magnetic susceptibility. The effective magnetic moment (μ_{eff}) for each compound was calculated using Evans Method (Equations S1 and S2).[2] Samples containing 5 – 10 mM iron complex in a solution of 5% t-butanol in D_2O were placed in a coaxial NMR insert. The coaxial insert was placed inside of a 5 mm NMR tube containing a solution of 5% t-butanol in D_2O. All samples were ran on a 500 MHz NMR spectrometer at 298 K.

$$\chi_g = \frac{3\Delta f}{4\pi f m} + \chi_0 \qquad \text{(Equation S1)}$$

$$\mu_{eff} = 2.84(\chi_m T)^{1/2} \qquad \text{(Equation S2)}$$

The mass susceptibility (χ_g) was calculated using Equation S1, where Δf is the shift in frequency (Hz), f is the operating frequency of NMR spectrometer (Hz), m is the concentration of the substance (g/mL), and χ_0 is the mass susceptibility of the solvent ($\chi_0 = -0.6466 \times 10^{-6}$ cm^3/g).[3] The molar susceptibility (χ_m) is obtained by multiplying the mass susceptibility (χ_g) by the molar mass. This result was used to calculate the effective magnetic moment μ_{eff} (Equation S2).

Inductively coupled plasma mass spectrometry (ICP-MS) measurement. Iron concentration of each of the complexes was determined using a Thermo X-Series 2 ICP-MS. Samples were dissolved in 65–70% metal free nitric acid for 3 days to undergo digestion. After the digestion was complete, a cobalt internal standard was added, and the samples were diluted with Milli-Q water to contain 2% nitric acid and 50 ppb cobalt. A linear calibration curve ranging from 0.1 ppb to 100 ppb iron was prepared as well and used for quantification of the samples. Quantification and data analysis were performed using Thermo Fisher PlasmaLab.

UV–Vis spectroscopy. Kinetic inertness was tested over the course of 24 hours by measuring the absorbance of the complexes from 200 to 800 nm. For all conditions tested a temperature of 37 °C was maintained using a Peltier temperature controller. Solutions contained 100 μM or 150 μM iron for all samples. For testing the kinetic inertness of the iron complexes at pH 7, either 20 mM HEPES (pH 7.4) or meglumine (pH 7) was used to maintain the pH. Kinetic inertness in the presence of biologically relevant anions was tested with solutions containing 25 mM $NaHCO_3$, 0.5 mM Na_2HPO_4. The pH of the anion containing solutions was brought to 7.4 using either 20 mM HEPES buffer or meglumine (specified for each complex within the results). For kinetic inertness studies done in acidic condition, solutions containing 0.10 M HCl. Transmetallation studies were performed in the presence of a molar equivalent of zinc chloride along with 20 mM HEPES (pH 7.4) or meglumine to bring the pH to 7. Kinetic inertness to transferrin binding of iron was also tested. Solutions for this study contained two molar equivalents of apo-transferrin, 20 mM HEPES (pH 7.4) or meglumine (pH 7), and 100 mM NaCl.

Cyclic voltammetry (CV). Aqueous solutions containing 5 mM iron complex and 1 M KCl as a supporting electrolyte were used to perform cyclic voltammetry measurements. Fe(TOAB)

solutions did not contain buffer and pH was adjusted using meglumine. A standard three electrode cell consisting of a glassy carbon working electrode, a Ag/AgCl reference electrode, and a Pt wire counter electrode were used. Solutions were purged with N_2 gas before each measurement to remove any dissolved oxygen in the system. Current was measured from − 1.5 V to + 1.5 V with a 10 second pre-scan delay at a sweep rate of 100 mV/s as well as at variable sweep rates. A standard of 5 mM ferrocyanide was used. Alternatively, experiments were conducted in acetonitrile with 0.10 M $TBAPF_6$ as an electrolyte. In these experiments, a standard three electrode cell consisting of a platinum working electrode, a Ag/AgCl reference electrode, and a Pt wire counter electrode were used with ferrocene as a reference. Redox potential of Fe(II)/Fe(III) was calculated from the average of the anodic (E_{pa}) and the cathodic (E_{pc}) potentials in reference to Ag/AgCl and then converted to the normal hydrogen electrode (NHE).

T_1/T_2 proton relaxation measurements. T_1 and T_2 values were measured at 1.4 T (60 MHz NMR) and experiments were performed at 33 °C. T_1 and T_2 experiment solutions contained 20 mM HEPES buffer (pH 7.4) and 100 mM NaCl for Fe(PTOB). Solutions for Fe(TOAB) contained 100 mM NaCl and were brought to pH 6.8 – 7.2 with meglumine. Concentrations of 0.050 mM to 0.40 mM were used and studies where HSA was present contained 35 mg/mL of HSA.

An inversion recovery True FISP acquisition was used to measure T_1 relaxation rate constants. T_2 relaxation rates were measured by using multi-echo, Carr-Purcell-Meiboom-Gill spin-echo sequence with a fixed TR of 3000 ms and TE times ranging from 20 to 1200 ms. The T_1 and T_2 relaxivity values were calculated by using linear regression fitting of $1/T_1$ (s^{-1}) and $1/T_2$ (s^{-1}) versus concentration (mM) in GraphPad Prism 9.0.2.

The pH dependence of T_1 water proton relaxivity values were measured at 1.4 T at 33 °C with solutions containing 0.20 mM iron complex, 50 mM HEPES buffer (pH 7.4), and 100 mM NaCl. pH values ranged from 1 to 10 and were adjusted with 1 M hydrochloric acid or 1 M sodium hydroxide. T_1 relaxivity values were determined as mentioned above.

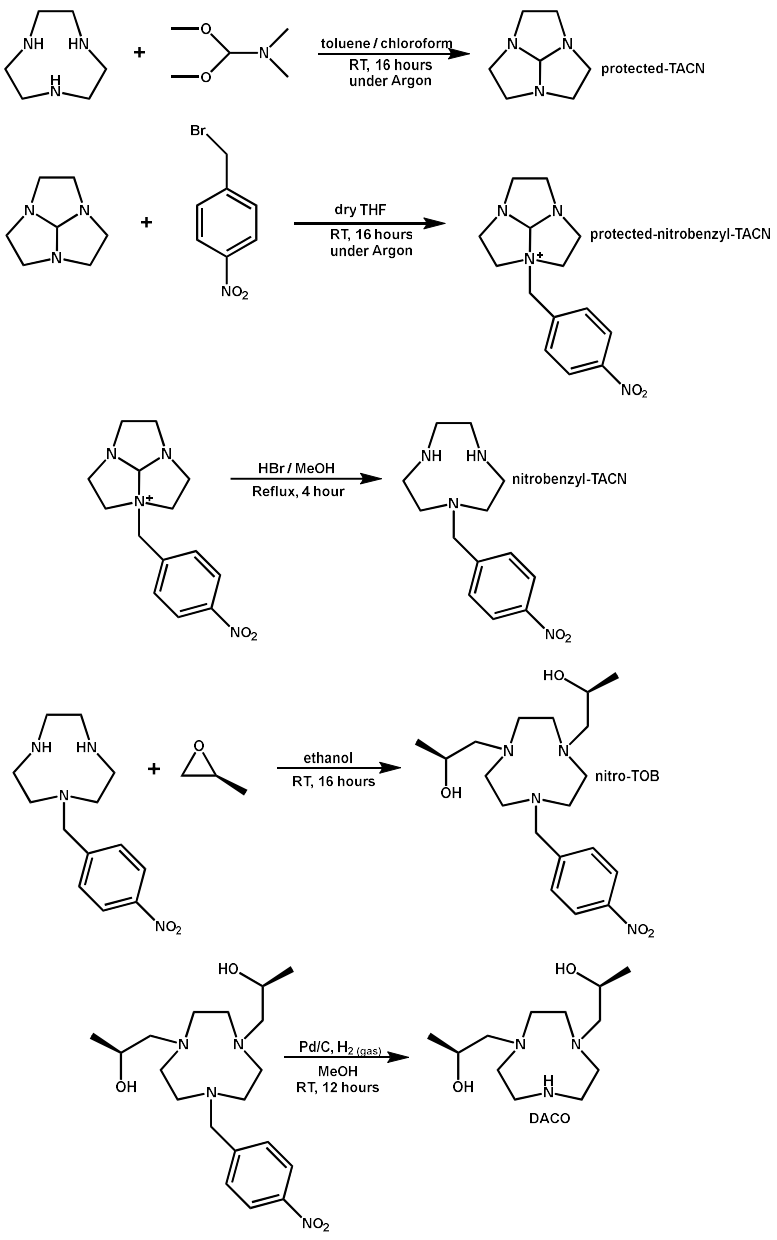
Variable temperature ^{17}O NMR spectroscopy. Solutions containing 10 mM iron complex, 20 mM HEPES buffer (pH 7.4), and 100 mM NaCl were prepared in a 1% $^{17}OH_2$ water and studied from 20 °C to 80 °C at intervals of 5 °C. The transverse relaxation rate constant ($1/T_2$) was calculated by determining the full width at half maximum (FWHM) of the ^{17}O resonance with iron complex ($\Delta V_{complex}$) and subtracting that of the ^{17}O resonance without iron complex ($\Delta V_{solvent}$) as show in equation S3.[4] Plotting of r_2^o (equation S4, where C is concentration of iron complex) as a function of temperature allows for the determination of the number of inner-sphere waters (q) the complex contains.

$$\frac{1}{T_2} = \pi * (\Delta V_{complex} - \Delta V_{solvent}) \quad \text{(Equation S3)}$$

$$r_2^o = \frac{1/T_2}{C} \quad \text{(Equation S4)}$$

Experimental – Synthesis of Ligands and Complexes

Synthesis of (2S,2'S)-1,1'-(1,4,7-triazonane-1,4-diyl)bis(propan-2-ol) (DACO). This ligand precursor has been synthesized previously,[5] however the procedure used for this series of ligands was updated and modified substantially from the originally published protocol. See Scheme S1 for the overall procedure. The TOAB ligand and $[Fe(TOAB)^{2+}]Br_2$ were synthesized as published previously.[1]



Scheme S1: *Synthesis of (2S,2'S)-1,1'-(1,4,7-triazonane-1,4-diyl)bis(propan-2-ol) (DACO).*

Synthesis of 1,4,7-triazatricyclo[5.2.1.04,10]-decane (protected-TACN). The first step in this synthesis has been reported in multiple publications,[6] but the ratio of solvents was modified in this preparation. TACN (17 mmol) was dissolved in chloroform (15 mL) and once the TACN was completely dissolved toluene (60 mL) was added. The solution was placed under argon and N,N-dimethylformamide dimethyl acetal (2.55 mL, 1.1 equivalent) was added. The reaction was stirred at room temperature for 16 hours. Solvent and excess N,N-dimethylformamide dimethyl acetal were removed under reduced pressure yielding protected TACN as a pale yellow oil. Yield: 99%. ESI-MS (positive) m/z, calculated: 139.20 (M), found: 140.24 ($M + H^+$) (100%) where M equals the ligand as drawn in scheme S1. ^{1}H NMR ($CDCl_3$): 2.66 – 2.80 (6H, TACN CH_2, multiplet), 2.96 – 3.09 (6H, TACN CH_2, multiplet), 4.96 (1H, CH, singlet). ^{13}C NMR ($CDCl_3$): 51.74, 103.90.

Synthesis of N-4-nitrobenzyl-1,4,7-triazatricyclo[5.2.1.04,10]-decane (protected-nitrobenzyl-TACN). Protected TACN (3.4 mmol) was dissolved in dry THF (24.5 mL) and the solution was placed under argon. 4-nitrobenzyl bromide (3.75 mmol, 3 equivalent) was added under argon and stirred at room temperature for 16 hours. The protected-nitrobenzyl-TACN precipitated out upon formation, and was collected by vacuum filtration. The product was washed with dry THF resulting in a pale-yellow solid. Yield: 94.1%. ESI-MS (positive) m/z, calculated: 275.33 (M^+), found: 275.34 (M^+) (100%) where M equals the ligand as drawn in scheme S1. ^{1}H NMR (CD_3CN/ $CDCl_3$): 3.17 – 3.45 (6H, TACN CH_2, multiplet), 3.58 – 3.87 (6H, TACN CH_2, multiplet), 4.76 (2H, benzyl CH_2, singlet), 5.97 (1H, CH, singlet), 7.94 (2H, benzyl CH, doublet, J = 10.50 Hz), 8.29 (2H, benzyl CH, doublet, J = 10.50 Hz). ^{13}C NMR (CD_3CN/ $CDCl_3$): 51.87, 56.30, 57.18, 58.62, 124.07, 129.05, 133.19, 136.53, 148.73.

Synthesis of 1-(4-nitrobenzyl)-1,4,7-triazacyclononane (nitrobenzyl-TACN). Protected nitrobenzyl-TACN (16 mmol) was added to a mixture of HBr (80 mL) and methanol (80 mL). The mixture refluxed for 4 to 5 hours after which it was cooled to room temperature. Once the reaction mixture reached room temperature, another 80 mL of HBr was added, and the flask was placed in the freezer overnight. Nitrobenzyl-TACN precipitated as an off-white solid which was collected via vacuum filtration. Yield: 95%. ESI-MS (positive) m/z, calculated: 264.33 (M), found: 265.42 ($M + H^+$) (100%) where M equals the ligand as drawn in scheme S1. ^{1}H NMR (D_2O): 2.92 (4H, TACN CH_2, triplet, J = 11.45 Hz), 3.20 (4H, TACN CH_2, triplet, J = 11.45 Hz), 3.58 (4H, TACN CH_2, singlet), 3.87 (2H, benzyl CH_2, singlet), 7.45 (2H, benzyl CH, doublet, J = 8.55 Hz), 8.01 (2H, benzyl CH, doublet, J = 8.55 Hz). ^{13}C NMR (D_2O): 42.26, 43.80, 47.52, 57.86, 123.76, 131.00, 143.29, 147.09.

Synthesis of (2S,2'S)-1,1'-(7-(4-nitrobenzyl)-1,4,7-triazonane-1,4-diyl)bis(propan-2-ol) (nitro-TOB). Nitrobenzyl-TACN (6.8 mmol) was dissolved in ethanol (25 mL) and minimal water (< 1 mL) was added to fully dissolve the starting material. S-(–) propylene oxide (2.26 mL, 4.8 equivalents) was added and the mixture stirred at room temperature for 2 days. Excess solvent and propylene oxide was removed under reduced pressure resulting in a yellow solid. Yield: 61%. ESI-MS (positive) m/z, calculated: 380.24 (M), found: 381.42 ($M + H^+$) (100%) where M equals the ligand as drawn in scheme S1. ^{1}H NMR (D_2O, pD 1.5): 1.12 (6H, hydroxypropyl CH_3, doublet, J = 6.22 Hz), 2.95 – 3.07 (2H, H1 hydroxypropyl CH_2, multiplet), 3.13 – 3.29 (6H, H2 hydroxypropyl CH_2/ TACN CH_2, multiplet), 3.30 – 3.44 (4H, TACN CH_2, multiplet), 3.50 – 3.71 (4H, TACN CH_2, multiplet), 4.02 – 4.21 (4H, hydroxypropyl CH/ benzyl CH_2, multiplet), 7.55 (2H, benzyl CH,

doublet, J = 8.62 Hz), 8.11 (2H, benzyl CH, doublet, J = 8.62 Hz). ^{13}C NMR (D_2O, pD 1.5): 20.08, 50.48, 58.32, 62.35, 63.38, 66.53, 67.87, 124.03, 131.62, 140.30, 147.77.

Synthesis of (2S,2'S)-1,1'-(1,4,7-triazonane-1,4-diyl)bis(propan-2-ol) (DACO). Nitro-TOB (0.96 mmol) was dissolved in 10-15 mL of methanol and placed under argon. Under argon atmosphere, 10% palladium on carbon (Pd/C) catalyst (0.56 mmol, 0.6 equivalents) was added, along with 1-2 mL of acetic acid. The reaction mixture was shaken under a hydrogen atmosphere for 16 hours at room temperature. The mixture was filtered through Celite to remove the catalyst. The crude product was purified via basic alumina column with a DCM/ methanol gradient. DACO was collected in fractions containing 4% – 8% methanol as a yellow oil. Yield: 59%. ESI-MS (positive) m/z, calculated: 245.21 (M), found: 246.50 (M + H^+) (100%) where M equals the ligand as drawn in scheme S1. ^{1}H NMR and ^{13}C NMR spectra are shown in Figure S1, and Figure S2. ^{1}H NMR (D_2O): 1.06 (6H, hydroxypropyl CH_3, doublet, J = 6.24 Hz), 2.43 – 2.54 (2H, H1 hydroxypropyl CH_2, multiplet), 2.54 – 2.61 (2H, TACN CH_2, multiplet), 2.62 – 2.75 (6H, TACN CH_2, multiplet), 2.78 – 2.86 (2H, H2 hydroxypropyl CH_2, multiplet), 2.87 – 2.96 (4H, TACN CH_2, multiplet), 3.82 – 3.94 (2H, hydroxypropyl CH, multiplet). ^{13}C NMR (D_2O): 19.99, 43.73, 48.02, 50.80, 63.38, 65.02.

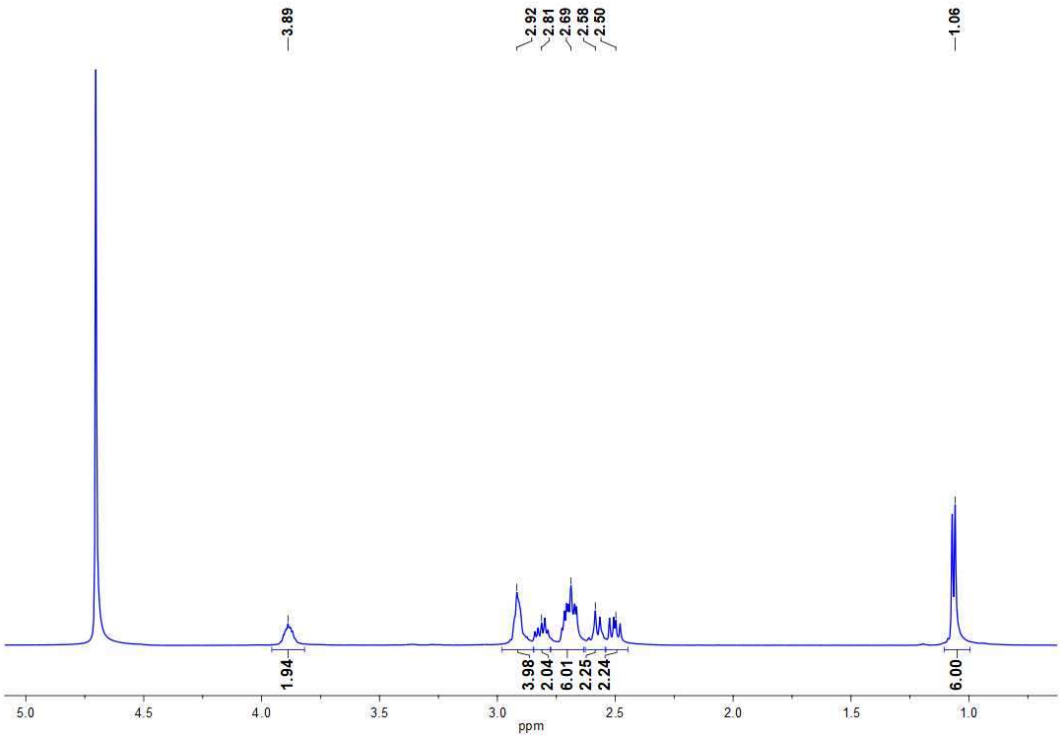


Figure S1: ^{1}H NMR spectrum of DACO ligand. (500 MHz, D_2O, 298 K).

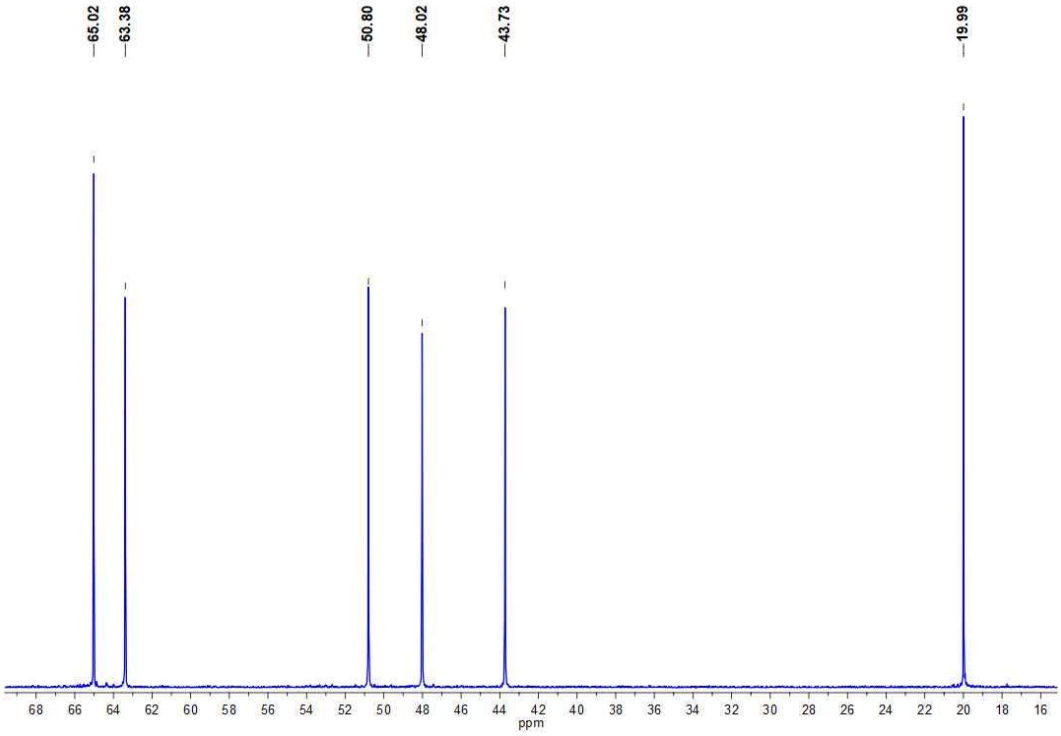
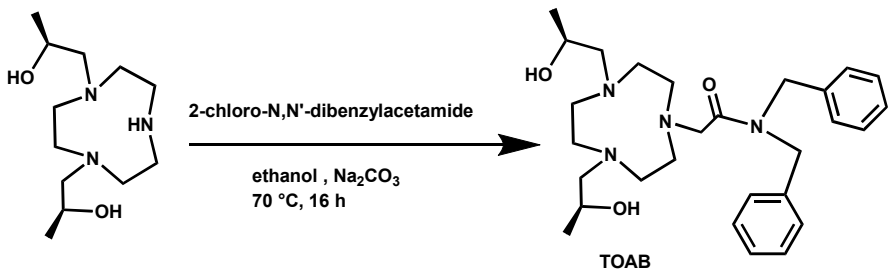
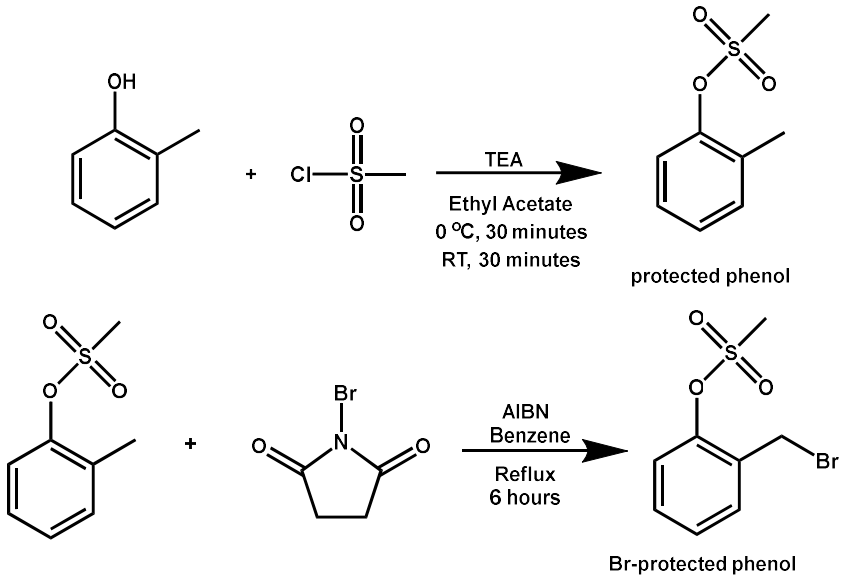


Figure S2: ^{13}C NMR spectrum of DACO ligand. (500 MHz, D_2O, 298 K).



Scheme S2. *Synthesis of TOAB ligand (from reference 1).*

Synthesis of 2-(bromomethyl)phenyl methanesulfonate (Br-protected phenol). Br-protected phenol is a precursor used in the synthesis of PTOB. The procedure used in step one to form protected phenol was previously reported.[7]



Scheme S3: *Synthesis of 2-(bromomethyl)phenyl methanesulfonate (Br-protected phenol).*

Synthesis of 2-Methylphenyl mesylate (protected phenol). Following the reported procedure,[7] O-cresol (0.05 mol, 1 equivalent) was dissolved in 150 mL of ethyl acetate and placed in an ice bath. Triethylamine (0.1 mol, 2 equivalents) was added to the stirred solution. The reaction solution was stirred for 15 minutes. Methanesulfonyl chloride (0.065 mmol, 1.3

equivalents) was added dropwise to the stirring mixture at 0 °C. Once all of the methanesulfonyl chloride was added, the reaction was stirred at 0 °C for 10 minutes and then removed from the ice bath and stirred for 30 minutes at room temperature. To quench the reaction, 150 mL of water was added. The organic layer was washed with water (3 x 150 mL) and then dried over sodium sulfate. The organic layer was evaporated under reduced pressure to yield the product as a colorless oil. Yield 88%. ^{1}H NMR: (500 MHz, $CDCl_3$): 2.39 (3H, CH_3, singlet), 3.20 (3H, protecting group CH_3, singlet), 7.20 – 7.27 (2H, benzyl CH, multiplet), 7.27 – 7.34 (2H, benzyl CH, multiplet). ^{13}C NMR: (500 MHz, $CDCl_3$): 16.61, 38.08, 122.05, 127.29, 131.30, 131.91, 147.89.

Synthesis of 2-(bromomethyl)phenyl methanesulfonate (Br-protected phenol). Protected phenol (0.036 mol) was dissolved in 180 mL of benzene. N-bromosuccinamide (NBS) (0.054 mol, 1.5 equivalents) and Azobisisobutyronitrile (AIBN) (0.018 mol, 0.5 equivalents) were added to the solution. The reaction was refluxed for 6 hours and then cooled to room temperature. As the reaction cooled, a solid by-product precipitated out. The solid was removed by vacuum filtration and the filtrate was dried on a rotary evaporator. The crude was purified via silica gel column with a petroleum ether / ethyl acetate gradient. Br-protected phenol eluted in fractions containing 16% and 18% ethyl acetate as a colorless oil. Yield: 52%. ^{1}H NMR and ^{13}C NMR spectra are shown in Figures S3 and S4. ^{1}H NMR: (500 MHz, $CDCl_3$): 3.21 (3H, CH_3, singlet), 4.49 (2H, CH_2, singlet), 7.22 (1H, benzyl CH, triplet, J = 6.81 Hz), 7.29 (1H, benzyl CH, triplet, J = 6.91 Hz), 7.34 (1H, benzyl CH, doublet, J = 9.34 Hz), 7.41 (1H, benzyl CH, doublet, J = 9.18 Hz). ^{13}C NMR: (500 MHz, $CDCl_3$): 27.15, 38.56, 122.37, 127.62, 130.31, 130.76, 131.90, 146.99. 130.76, 131.90, 146.99. 131.90, 146.99. 131.90, 146.99.

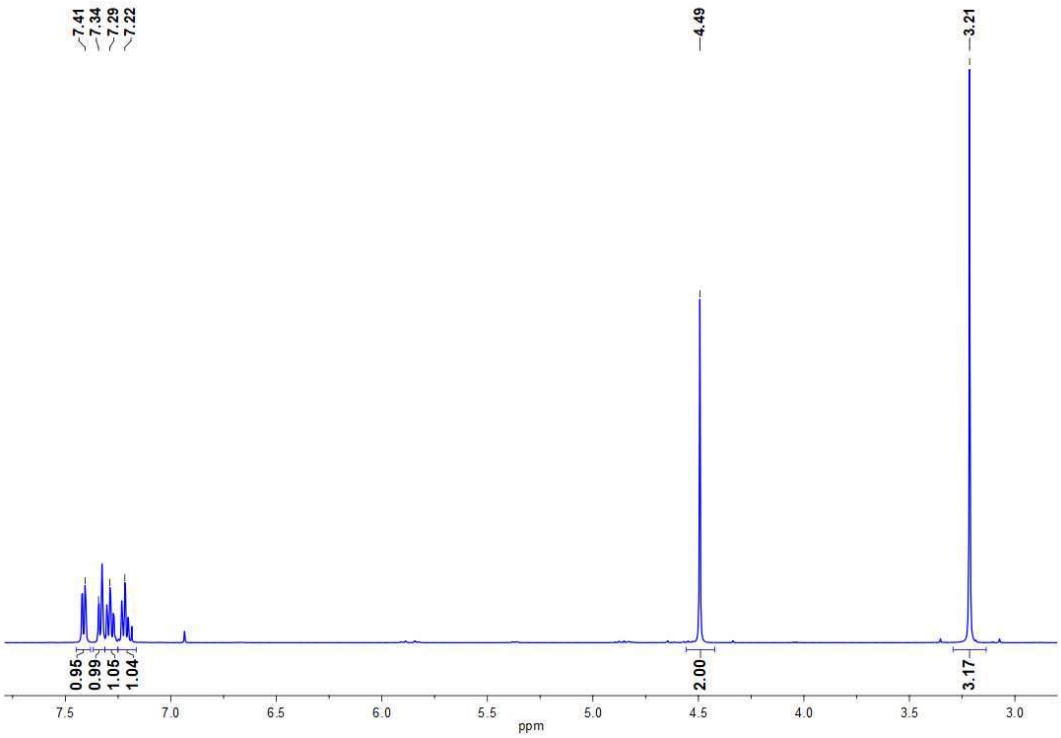


Figure S3: ^{1}H NMR spectrum of Br-protected phenol ligand. (500 MHz, $CDCl_3$, 298 K).

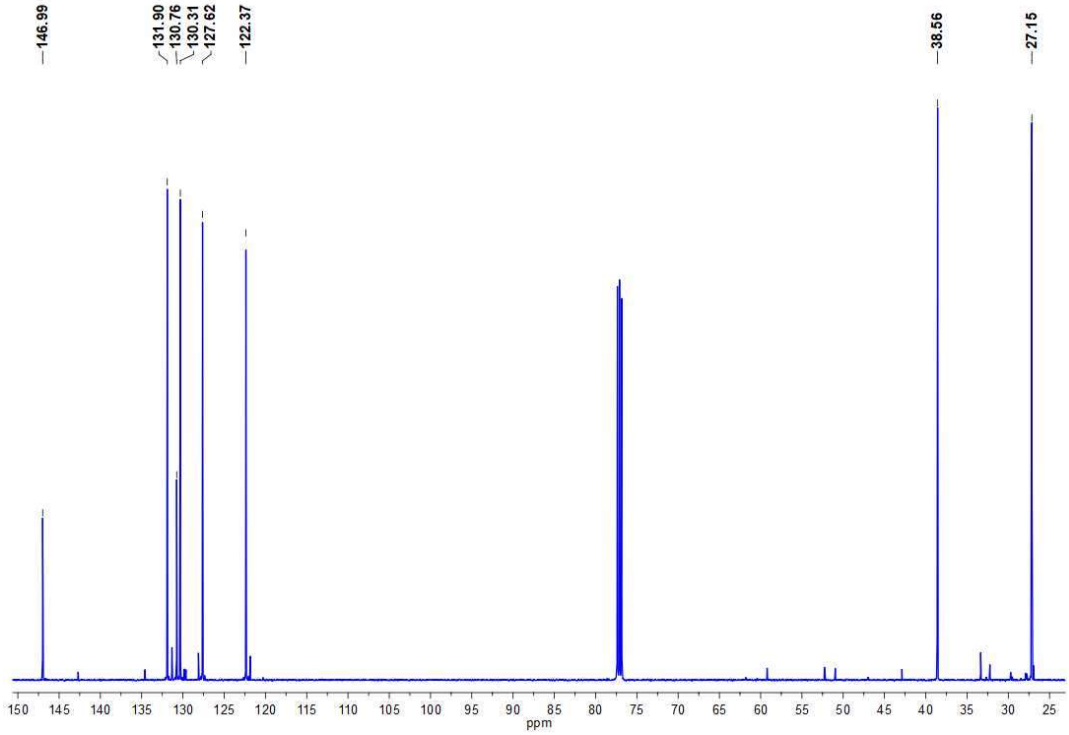
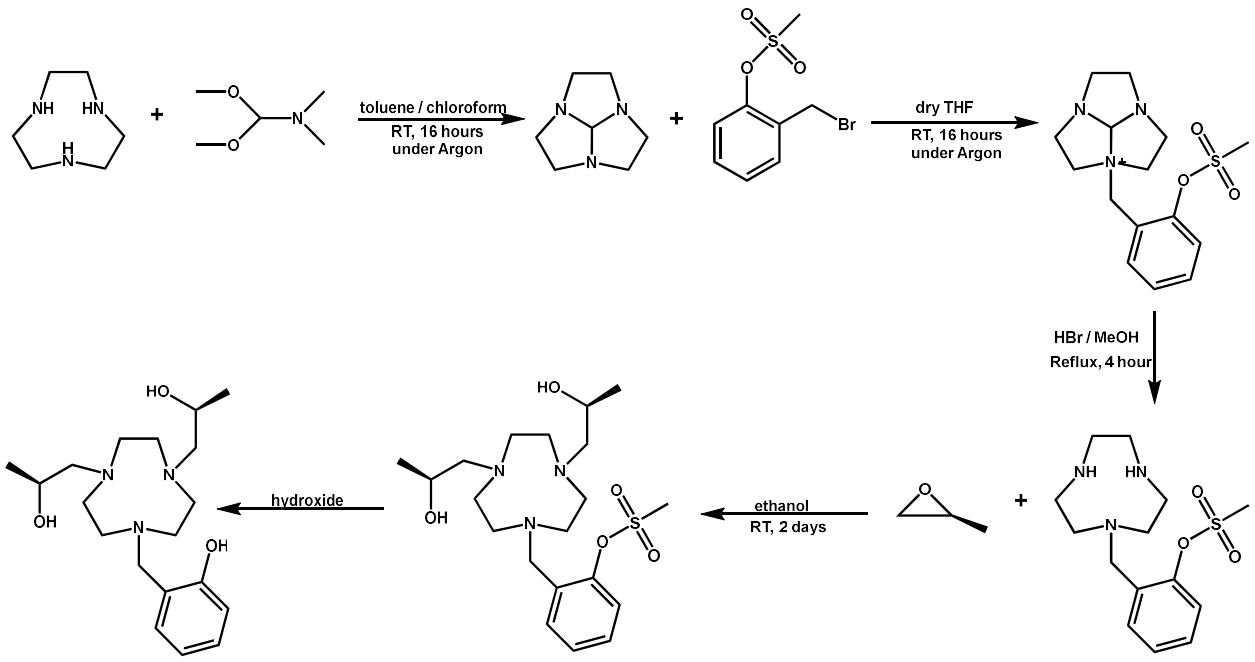


Figure S4: ^{13}C NMR spectrum of Br-protected phenol ligand. (500 MHz, $CDCl_3$, 298 K).



Scheme S4. *Synthesis of PTOB ligand.*

Synthesis of N-(2-((methylsulfonyl)oxy)benzyl)-(1,4,7-triazatricyclo[5.2.1.04,10]-decane) (protected phenol protected TACN). Protected TACN (8.2 mmol) was dissolved in 48 mL of dry THF. The solution was placed under inert atmosphere and Br-protected phenol (9.0 mmol, 1.1 equivalents) was added. The reaction was stirred at room temperature under inert atmosphere overnight and the product precipitated out as a cream-colored solid. The product was collected by vacuum filtration and washed with dry THF. Yield: 98%. ESI-MS (positive) m/z, calculated: 324.14 (M^+), found: 324.15 (M^+), where M equals the ligand as drawn in scheme S4. ^{1}H NMR: (500 MHz, $CD_3CN/CDCl_3$): 3.16 – 3.38 (6H, TACN CH_2, multiplet), 3.44 (3H, CH_3, singlet), 3.73 – 3.89 (6H, TACN CH_2, multiplet), 4.62 (2H, benzyl CH_2, singlet), 5.98 (1H, CH, singlet), 7.51 (1H, benzyl CH, triplet, J = 7.53 Hz), 7.57 (1H, benzyl CH, doublet, J = 8.19 Hz), 7.65 (1H, benzyl CH, triplet, J = 7.79 Hz), 7.86 (1H, benzyl CH, doublet, J = 7.64 Hz). ^{13}C NMR: (500 MHz,

$CD_3CN/CDCl_3$): 38.84, 52.09, 54.49, 56.48, 57.42, 123.22, 123.60, 128.39, 129.43, 132.73, 134.57, 148.97.

Synthesis of 2-((1,4,7-triazonan-1-yl)methyl)phenyl methanesulfonate (protected phenol TACN). Protected phenol protected TACN (9 mmol) was dissolved in 45 mL HBr and 45 mL MeOH. The reaction was refluxed for 4 – 5 hours, after which it was cooled to room temperature. An additional 45 mL of HBr was added to the solution and it was placed into the freezer for about 3 days. The HBr salt of the ligand precipitated out as a pale pink-white solid that was collected by vacuum filtration. Yield: 92%. ESI-MS (positive) m/z, calculated: 313.15 (M), found: 314.17 ($M + H^+$), where M equals the ligand as drawn in scheme S4. ^{1}H NMR: (500 MHz, D_2O): 2.81 (4H, TACN CH_2, triplet, J = 5.69 Hz), 3.15 (4H, TACN CH_2, triplet, J = 5.79 Hz), 3.31 (3H, CH_3, singlet), 3.53 (4H, TACN CH_2, singlet), 3.83 (2H, benzyl CH_2, singlet), 7.22 – 7.34 (3H, benzyl CH, multiplet), 7.38 (1H, benzyl CH, doublet, J = 7.28 Hz). ^{13}C NMR: (500 MHz, D_2O): 38.19, 42.20, 43.66, 47.24, 52.57, 122.33, 128.09, 128.49, 130.29, 132.88, 147.78.

Synthesis of 2-((4,7-bis((S)-2-hydroxypropyl)-1,4,7-triazonan-1-yl)methyl)phenyl methanesulfonate (protected PTOB). Protected phenol TACN (5 mmol) was dissolved in 20 mL ethanol and S–(–) propylene oxide (20 mmol, 4 equivalents) was added to it. The reaction stirred for 2 days at room temperature and the solvent was removed using a rotary evaporator. The crude was purified via silica gel column with a DCM / MeOH gradient. Protected PTOB eluted in fractions containing 5 – 10% methanol. The fractions were dried under reduced pressure, yielding protected PTOB as an off-white solid. Yield: 27%. ESI-MS (positive) m/z, calculated: 429.23 (M), found: 430.33 ($M + H^+$), where M equals the ligand as drawn in scheme S4. ^{1}H NMR: (500 MHz, D_2O): 1.10 (6H, hydroxypropyl CH_3, doublet, J = 6.20 Hz), 2.91 – 3.05 (2H, H1 hydroxypropyl CH_2,

multiplet), 3.06 – 3.27 (6H, TACN CH_2, H2 hydroxypropyl CH_2, multiplet), 3.28 – 3.48 (7H, protecting group CH_3, TACN CH_2, multiplet), 3.52 – 3.747 (4H, TACN CH_2, multiplet), 3.97 – 4.19 (4H, benzyl CH_2, hydroxypropyl CH, multiplet), 7.37 (1H, benzyl CH, triplet, J = 7.40 Hz), 7.41 (1H, benzyl CH, doublet, J = 7.42 Hz), 7.45 (1H, benzyl CH, triplet, J = 8.37 Hz), 7.51 (1H, benzyl CH, doublet, J = 7.44 Hz). ^{13}C NMR: (500 MHz, D_2O): 20.09, 37.96, 49.71, 53.67, 62.30, 63.09, 122.59, 126.57, 128.41, 131.32, 133.21, 147.82.

Synthesis of (2S,2'S)-1,1'-(7-(2-hydroxybenzyl)-1,4,7-triazonane-1,4-diyl)bis(propan-2-ol) (PTOB). Protected PTOB (0.9 mmol) was dissolved in 10 mL methanol and heated to 55 °C. Potassium hydroxide (4.3 mmol, 5 equivalents) in 1 mL water was added to the solution and the reaction stirred at 55 °C for 18 hours. The solvent was removed under reduced pressure and dissolved into 25 mL chloroform. The chloroform layer was washed with 1 M NaOH (5 x 25 mL), dried with sodium sulfate, and the solvent was removed yielding PTOB as an off-white solid. Yield: 57%. ESI-MS (positive) m/z, calculated: 350.48 (M), found: 352.33 ($M + 2H^+$), where M equals the ligand as drawn in scheme S4. ^{1}H NMR and ^{13}C NMR spectra are shown in Figure S5, and Figure S6. ^{1}H NMR: (500 MHz, D_2O): 1.01 (6H, hydroxypropyl CH_3, doublet, J = 6.32 Hz), 2.31 – 2.42 (2H, H1 hydroxypropyl CH_2, multiplet), 2.45 – 2.51 (2H, H2 hydroxypropyl CH_2, multiplet), 2.51 – 2.74 (8H, TACN CH_2, multiplet), 3.03 – 3.23 (4H, TACN CH_2, multiplet), 3.72 (2H, benzyl CH_2, singlet), 4.13 (2H, hydroxypropyl CH, doublet of doublet, J = 12.49 Hz, 32.12 Hz), 6.53 (1H, benzyl CH, triplet, J = 7.70 Hz), 6.64 (1H, benzyl CH, doublet, J = 7.78 Hz), 7.12 – 7.21 (2H, benzyl CH, multiplet). ^{13}C NMR: (500 MHz, D_2O): 19.69, 48.28, 51.02, 58.24, 64.70, 64.80, 114.20, 119.58, 119.89, 131.37, 131.68, 166.18.

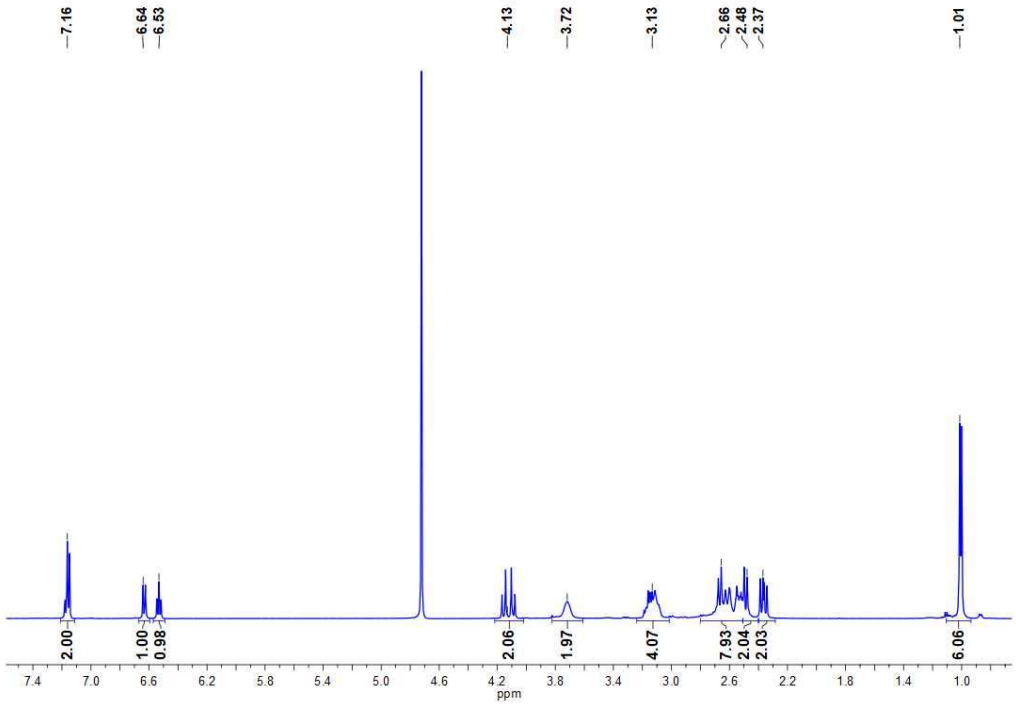


Figure S5: ^{1}H NMR spectrum of PTOB. (500 MHz, D_2O, 298 K).

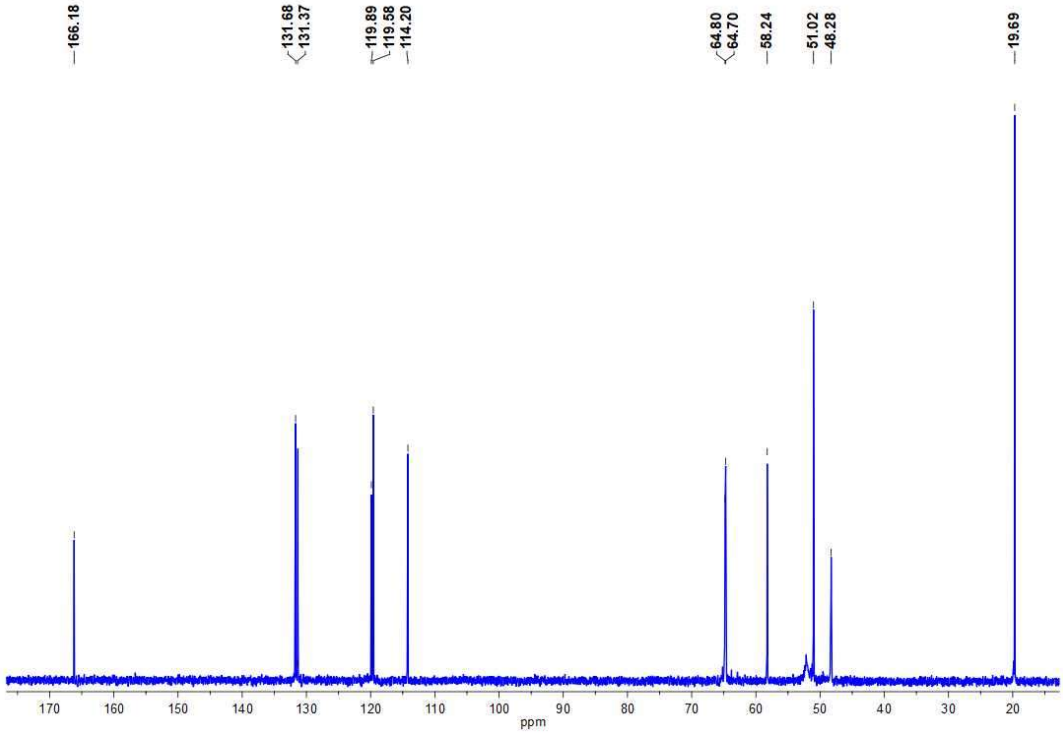
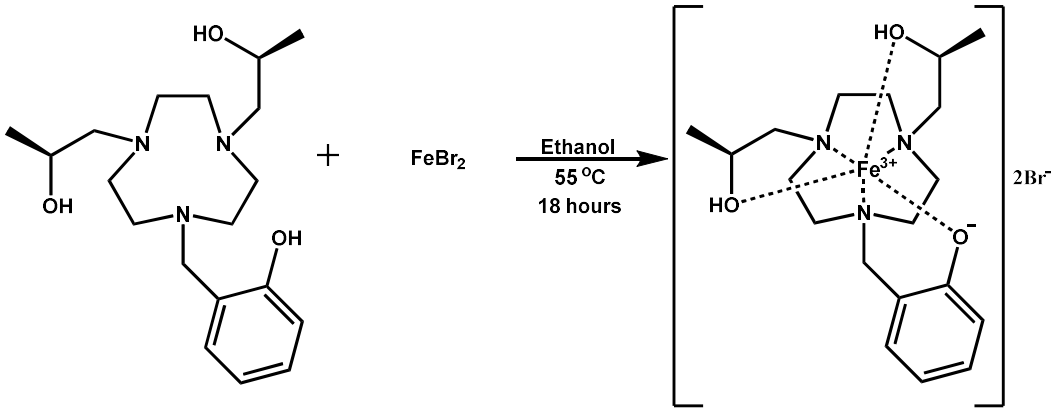


Figure S6: ^{13}C NMR spectrum of PTOB. (500 MHz, D_2O, 298 K).

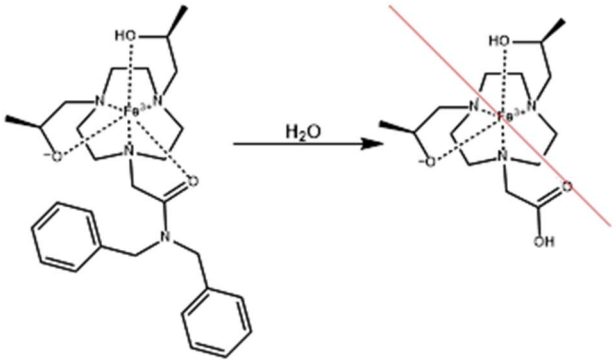


Scheme S5: Synthesis of $[Fe(PTOB)^{2+}]Br_2^-$.

PTOB ligand (0.010 mmol) was dissolved in water and the pH was adjusted to 7. The ligand was then dried under vacuum and, subsequently, dissolved in 3 mL of ethanol. This solution was heated to 55 °C. Iron(II) bromide (0.010 mmol, 1 equivalent) was dissolved in 3 to 5 mL of ethanol and added dropwise to the ligand solution. The solution was stirred for 18 hours and then the solvent was reduced to 2 mL using a rotary evaporator. To the dark purple ethanolic solution, 15 mL of anhydrous ethyl ether was added, to produce a solid. The solid was collected via centrifugation and washed 3 times with ethyl ether to produce a purple solid ESI-MS (positive) *m/z*, calculated: 406.33 (M), found: 405.25 ($M - H^+$, 100%) where M equals $Fe(PTOB)^{2+}$ as drawn in scheme S5. Fe content of the solid was determined using ICP-MS calculated for $[Fe(PTOB)]Br_2$: 9.89%, found: 9.49%.

Hydrolysis studies of Fe(TOAB). A potential challenge in using amide pendants, such as in Fe(TOAB), is the hydrolysis of the amide to a carboxylate group (Scheme S6). This is often acid catalyzed, and while most of the intended studies will be done at neutral pH, iron(III) is a Lewis acid and may catalyze the reaction. Since there is a 178 mass unit different between these two species, mass spectrometry was used to determine if hydrolysis would occur over

time when Fe(TOAB) was dissolved in water. Fe(TOAB) was studied at pH 4, pH 7, and pH 9 over the course of two weeks. For all pH conditions, no evidence of the hydrolysis product was observed after two weeks at room temperature (~ 25 °C). Mass spectroscopy spectrum of the study conducted at pH 7 is shown in Figure S7.



Scheme S6: *Fe(TOAB) did not hydrolyze to form the carboxylate analog of the complex.*

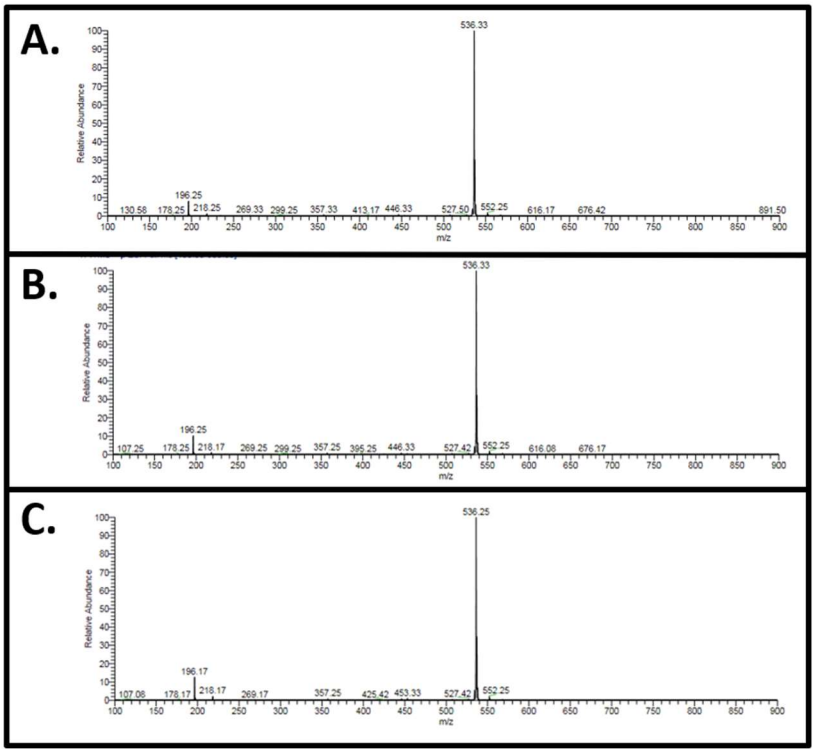


Figure S7: LTQ-MS (positive) spectrum monitoring aqueous solutions of 2 mM Fe(TOAB) at pH 7.2 was obtained using meglumine to adjust pH. Fe(TOAB) m/z = 536.33, meglumine m/z = 196.25, hydrolysis product m/z = 358.15. A) initial scan, B) 24 hours, C) 2 weeks.

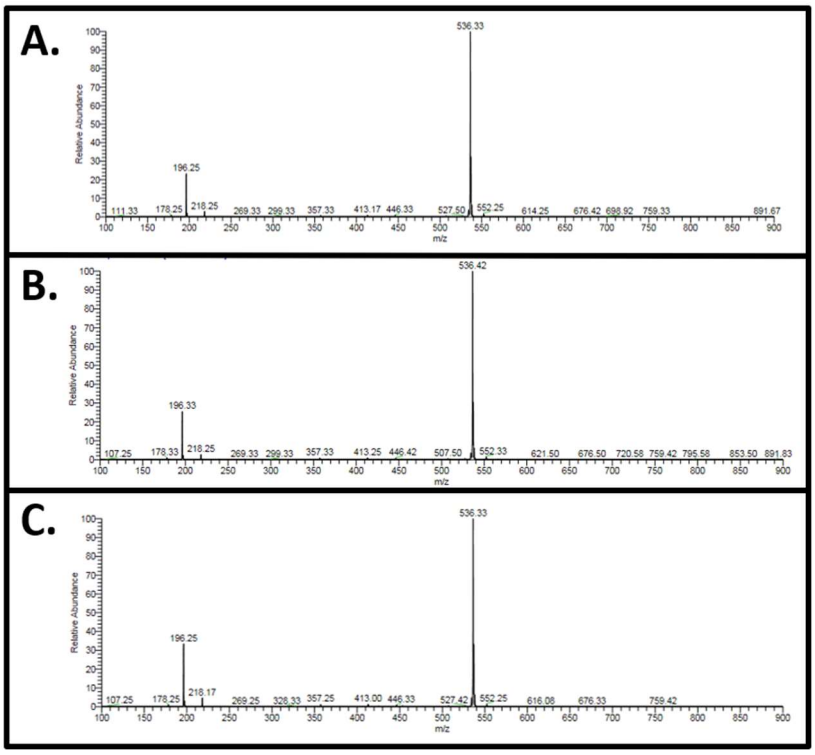


Figure S8: LTQ-MS (positive) spectrum monitoring aqueous solutions of 2 mM Fe(TOAB) at pH 9.7 was obtained using meglumine to adjust pH. Fe(TOAB) m/z = 536.33, meglumine m/z = 196.25, hydrolysis product m/z = 358.15. A) initial scan, B) 24 hours, C) 96 hours.

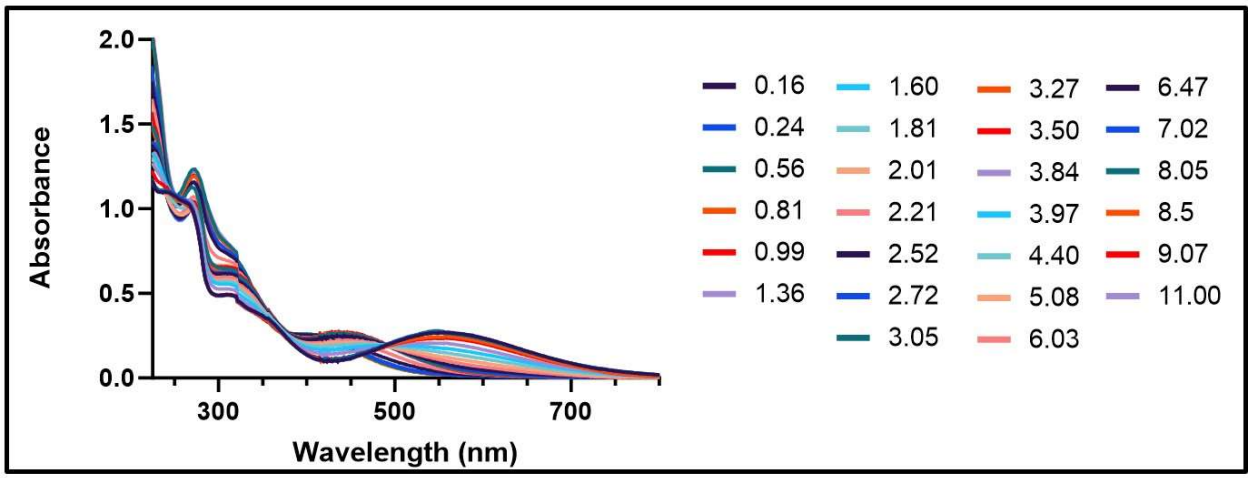


Figure S9: Spectrophotometric titration of Fe(PTOB) as a function of pH. All solutions contained 20 mM HEPES buffer, 100 mM NaCl, 200 µM Fe(PTOB) and were adjusted to the indicated pH value using HCl or NaOH.

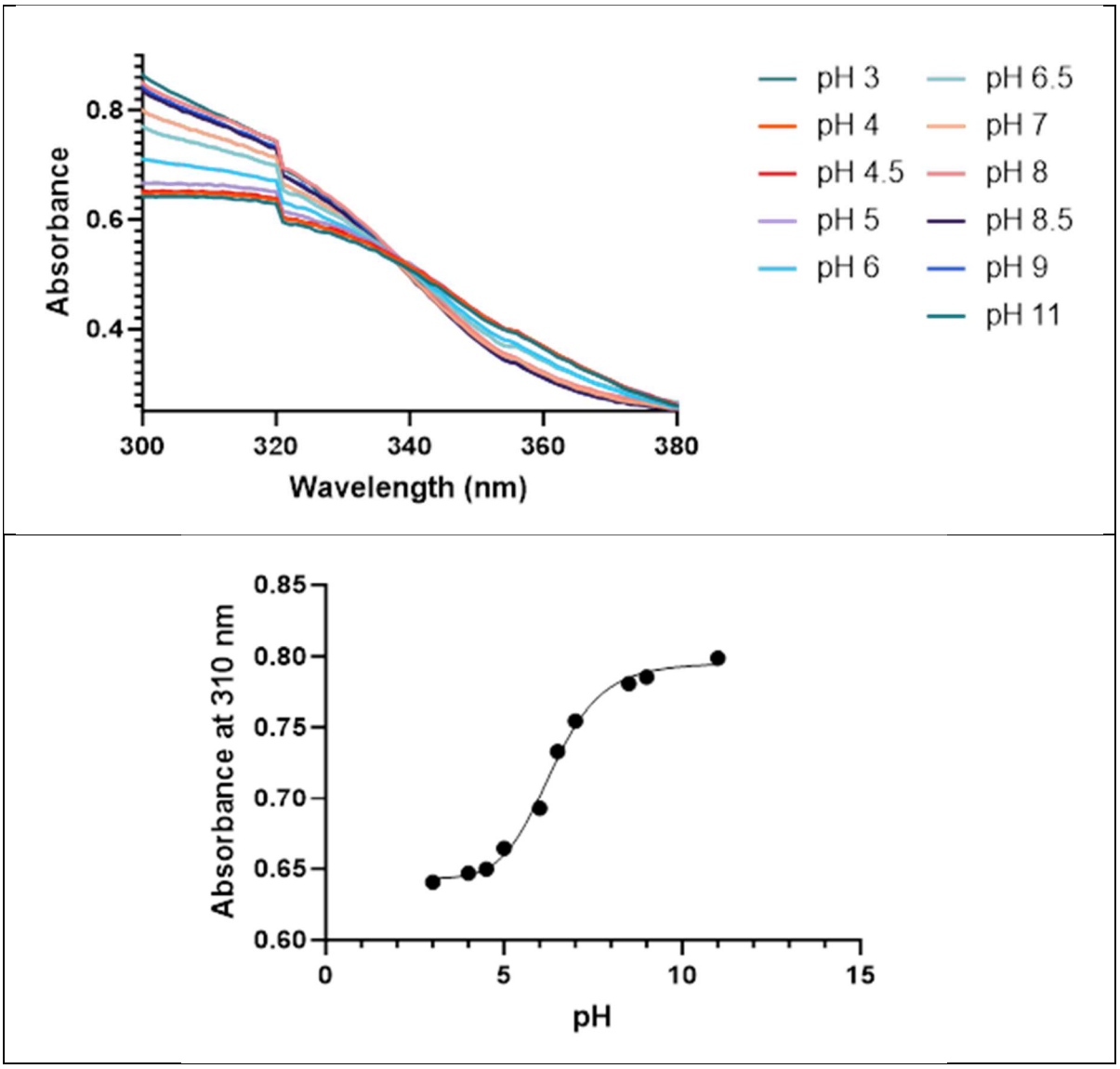


Figure S10. Spectrophotometric titration of Fe(PTOB) as a function of pH (top). A plot of the change in absorbance and fit to a single pK_a value of 6.3 (bottom). All solutions contained 20 mM HEPES buffer, 100 mM NaCl, 200 µM Fe(PTOB) and were adjusted to the indicated pH value using HCl or NaOH.

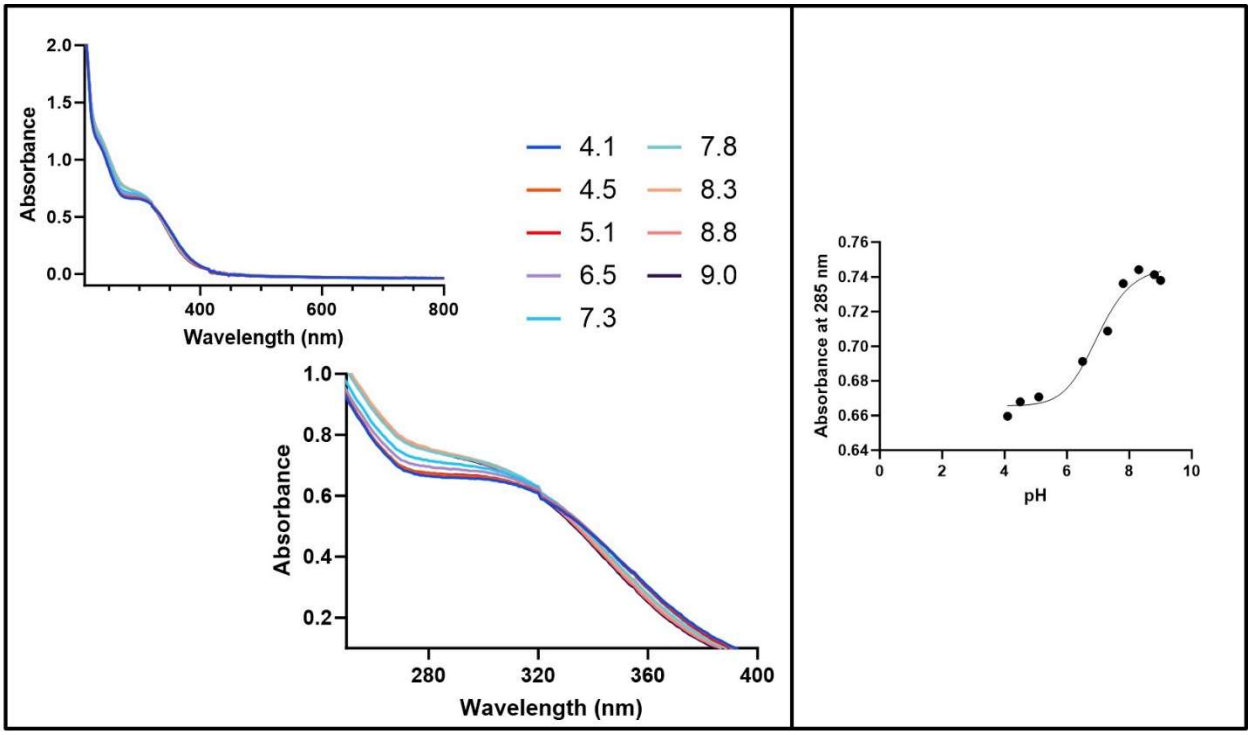


Figure S11. Spectrophotometric titration of Fe(TOAB) as a function of pH (left). At right is shown a plot of the change in absorbance and fit to a single pK_a value of 7.0. All solutions contained 100 mM NaCl, 200 µM Fe(TOAB) and were adjusted to the indicated pH value using HCl or NaOH.

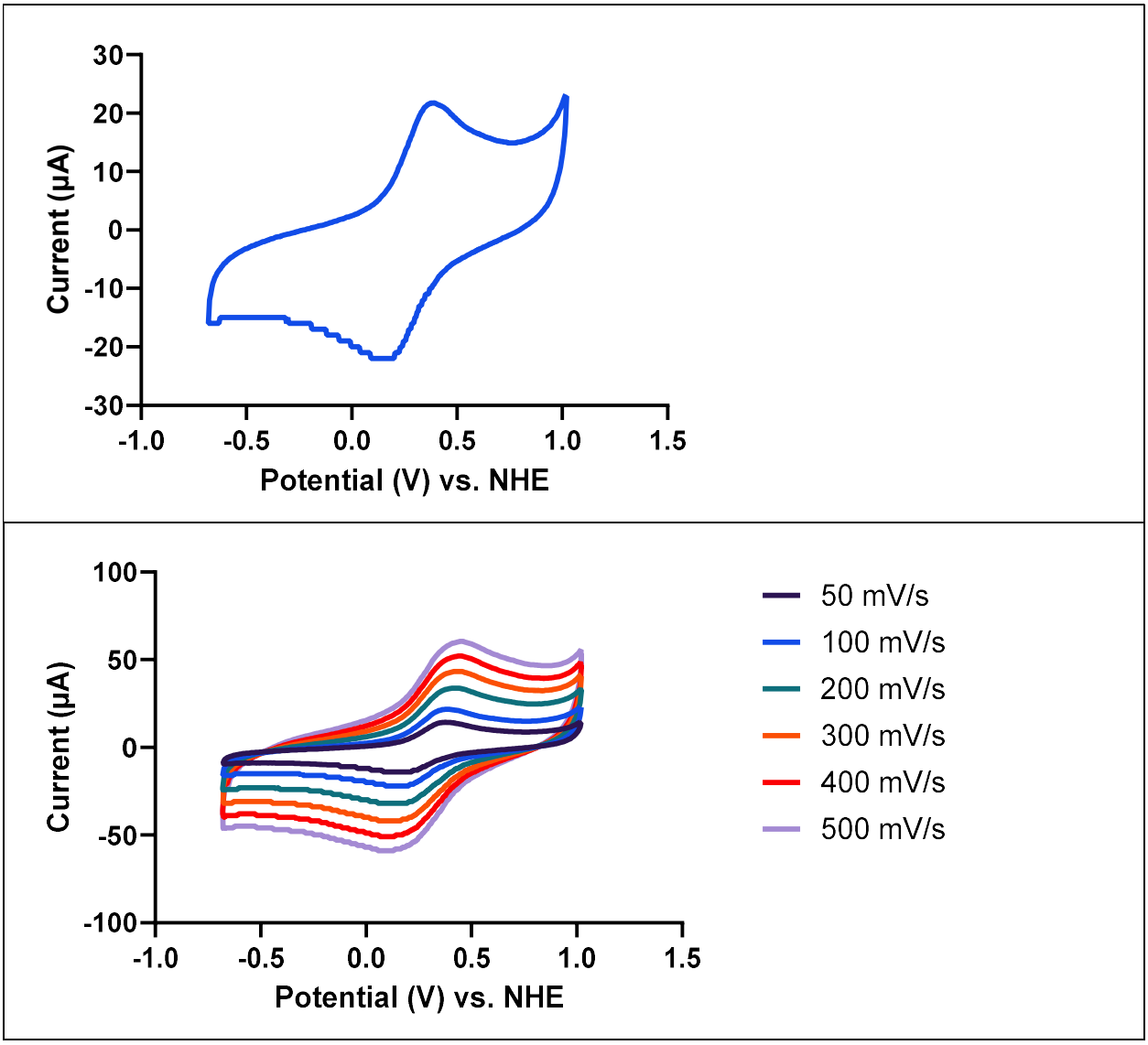
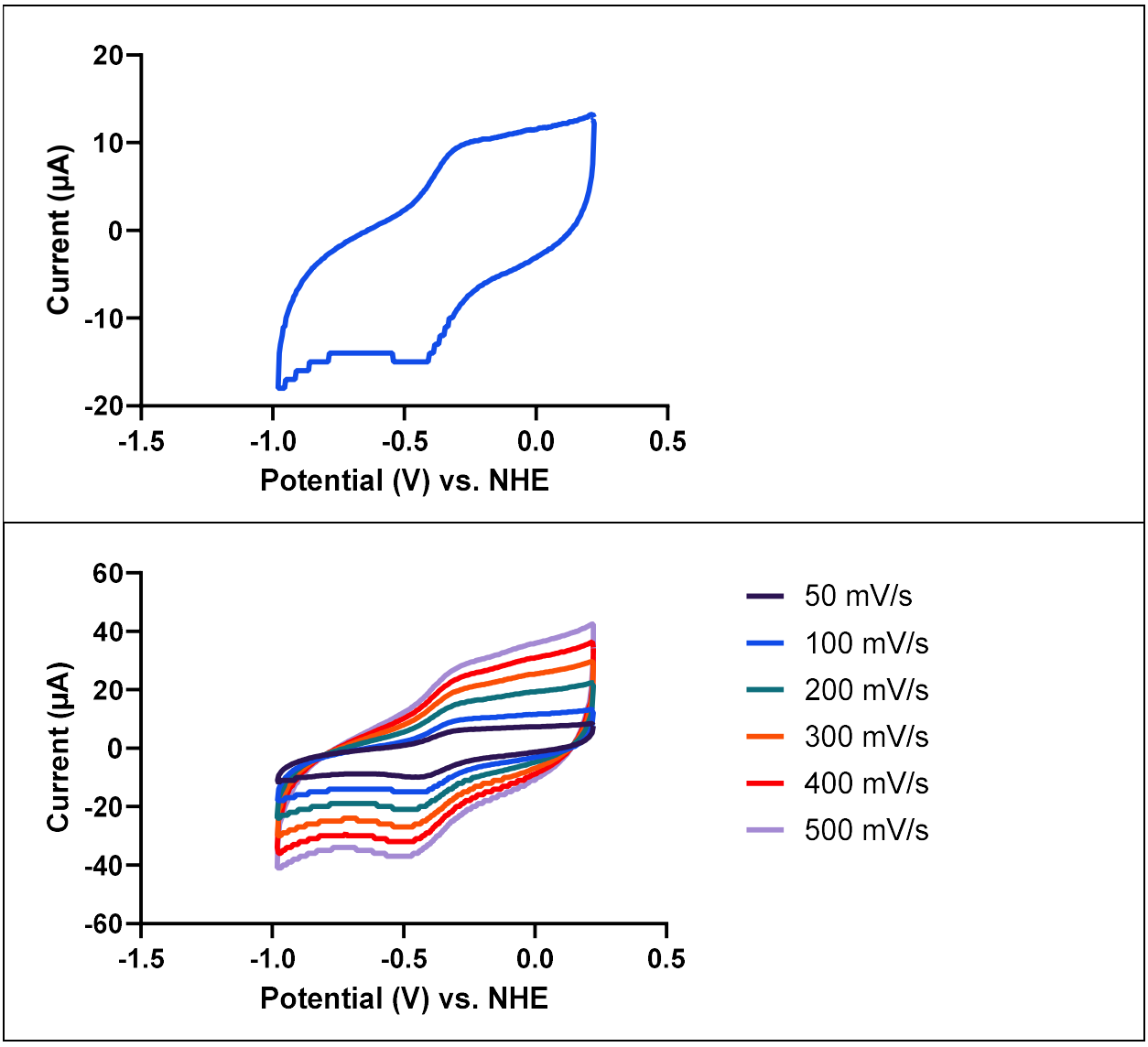


Figure S12. Cyclic voltammograms at pH 3.5 of 5 mM Fe(TOAB) in aqueous solution containing 1 M KCl at 25 °C. Top voltammogram obtained at 100 mV/s.



Figures S13. Cyclic voltammograms at pH 7 of 5 mM Fe(TOAB) in aqueous solution containing 1 M KCl at 25 °C and adjusted to pH 7.4 using meglumine. Top voltammogram obtained at 100 mV/s.

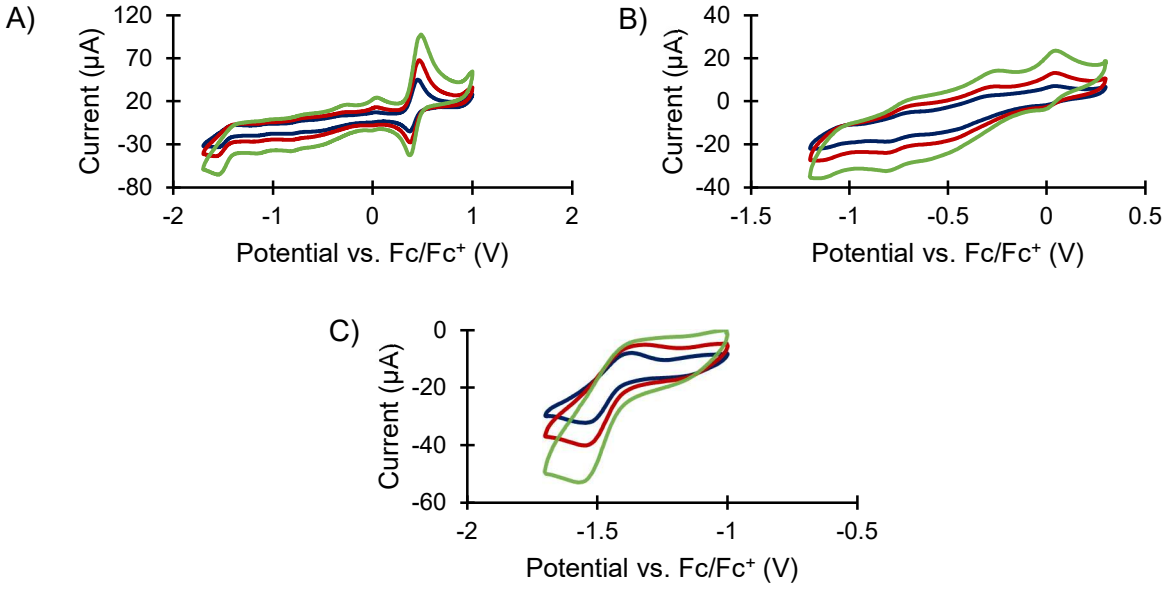
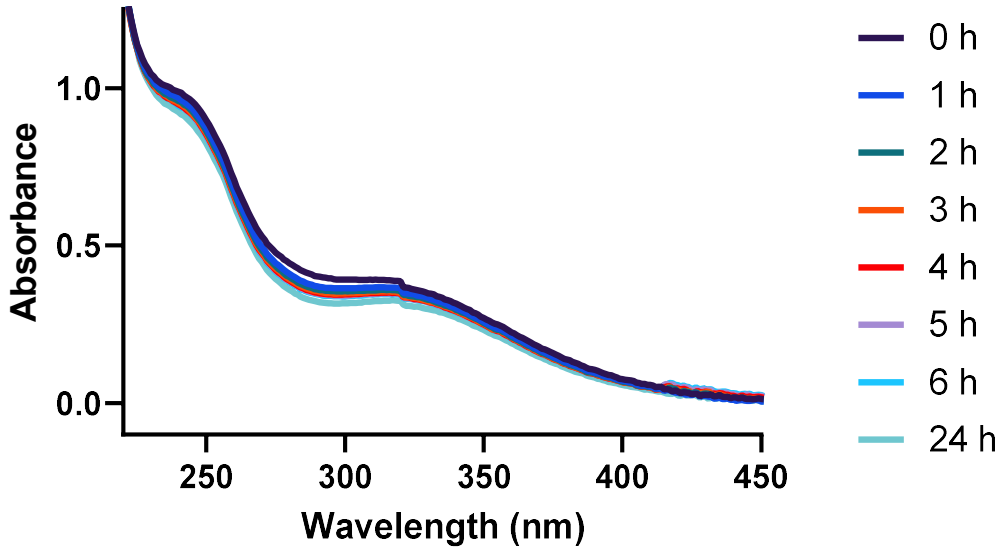


Figure S14. Cyclic Voltammetry of Fe(PTOB). A) Full potential window -1.7 V to 1 V, B) Potential window -1.2 V to 0.3 V, C) Potential window -1.7 V to -1 V. Experimental Conditions: 2.5 mM Fe(PTOB), 1 mM Fc, 0.1 M NBu_4PF_6, and scan rates: 50 mV/s (blue), 100 mV/s (red), 200 mV/s (green).



Figures S15. UV-vis spectra of Fe(TOAB) incubated at 37 ºC in 0.100 M HCl. *Dissociation in the presence of 0.1 M HCl was 17.5% after 24 hours.*

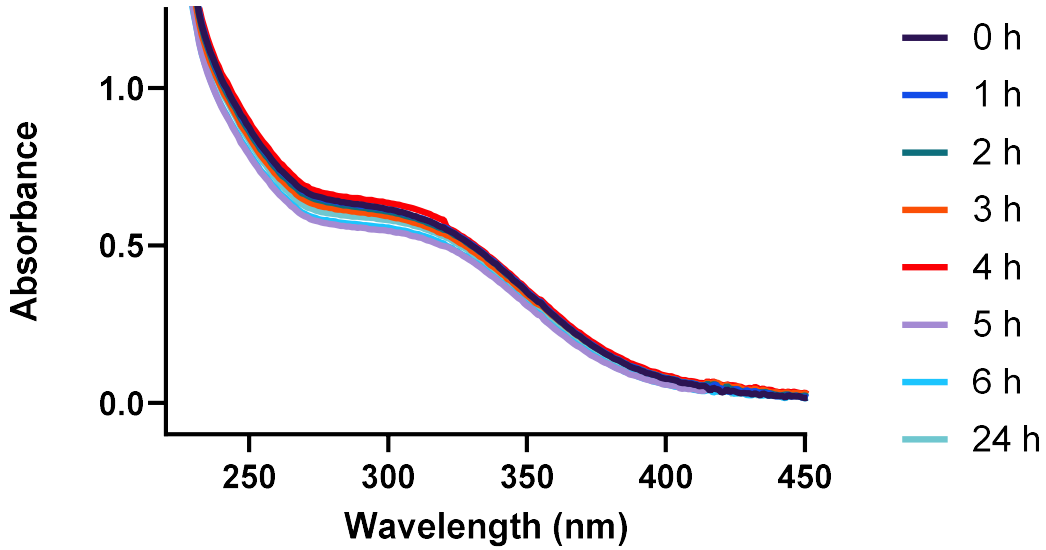


Figure S16. UV-vis spectra of Fe(TOAB) incubated at 37 ºC in 20 mM HEPES and 100 mM NaCl. *Absorbance decrease in the presence of HEPES was 11% after 24 hours due to precipitation of an orange solid.*

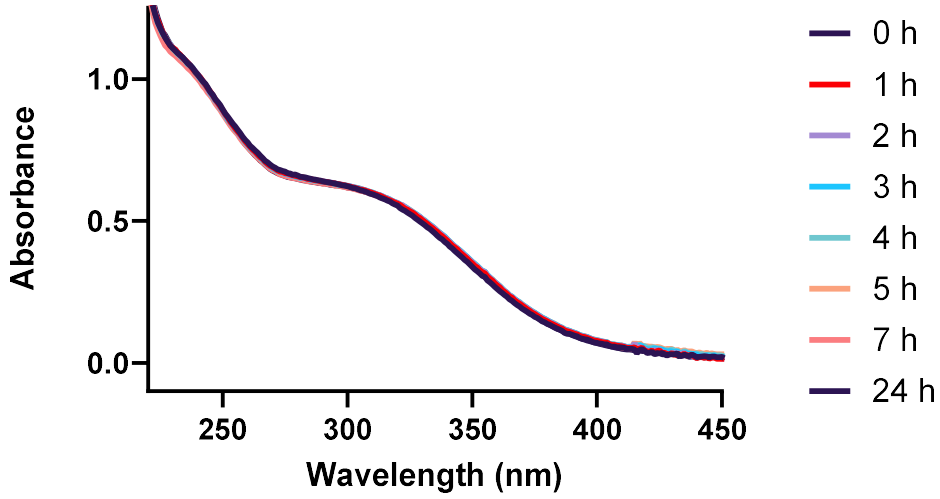


Figure S17. UV-vis spectra of Fe(TOAB) incubated at 37 ºC with pH 7 adjusted by meglumine only.

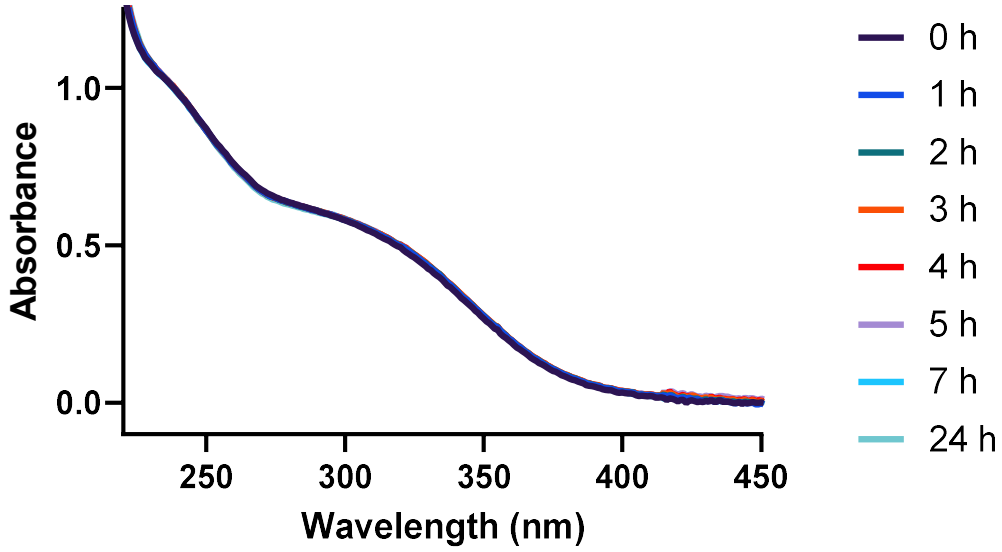


Figure S18. UV-vis spectra of Fe(TOAB) incubated at 37 ºC with 25 mM $NaHCO_3$ and 0.5 mM Na_2PO_4 at pH 7, adjusted with meglumine.

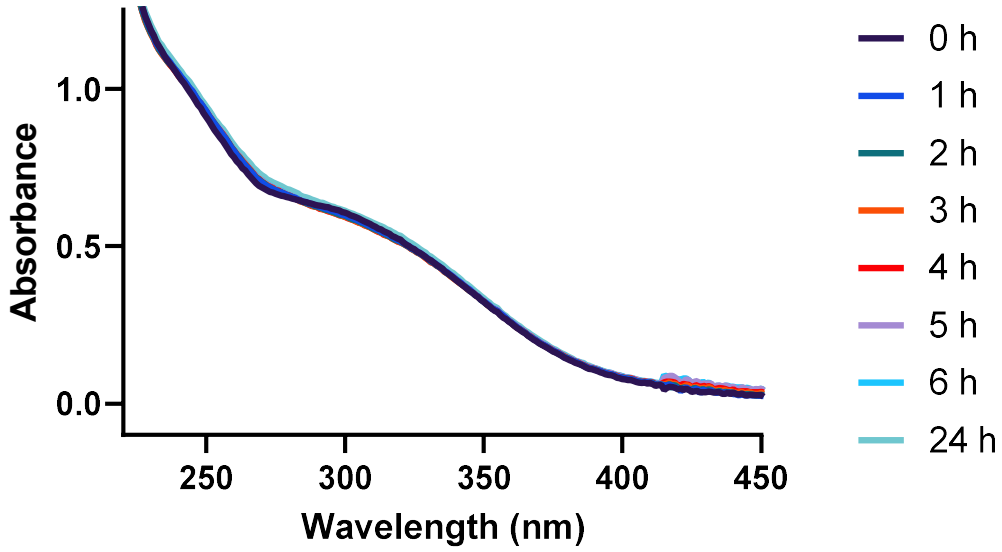
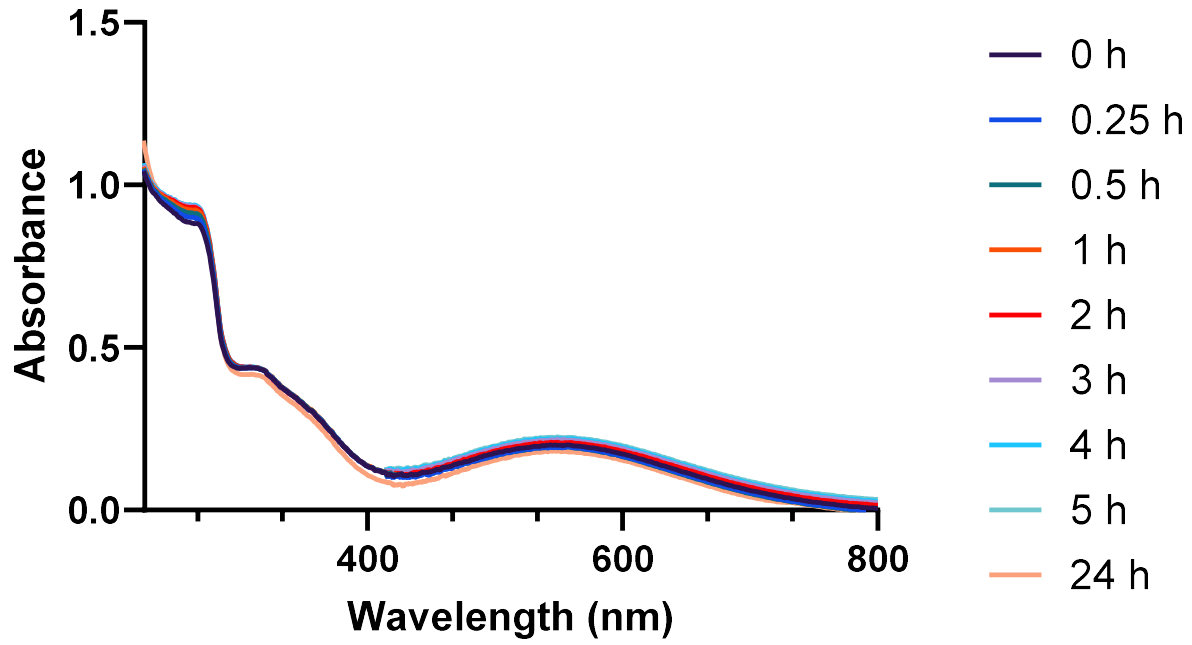


Figure S19. UV-vis spectra of Fe(TOAB) incubated at 37 ºC with one equivalent of $ZnCl_2$ at pH 7.4, adjusted with meglumine.



Figures S20. UV-vis spectra of Fe(PTOB) incubated at 37 ºC in 0.100 M HCl.

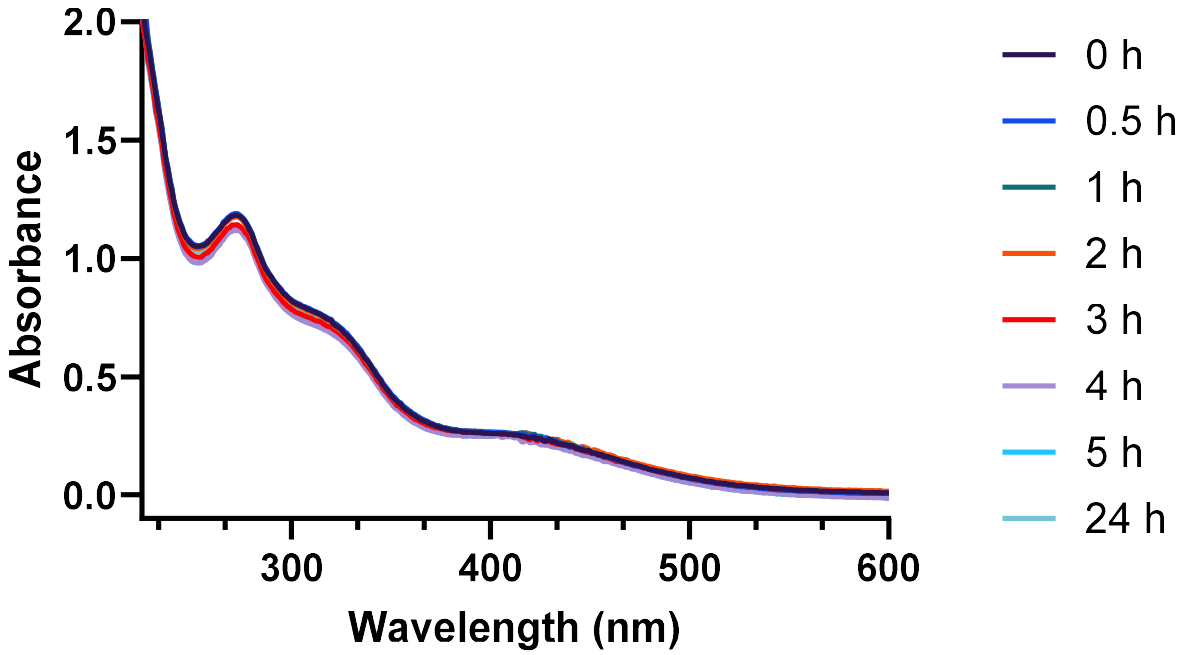


Figure S21. UV-vis spectra of Fe(PTOB) incubated at 37 ºC in 20 mM HEPES (pH 7.4), 100 mM NaCl at 37 ºC.

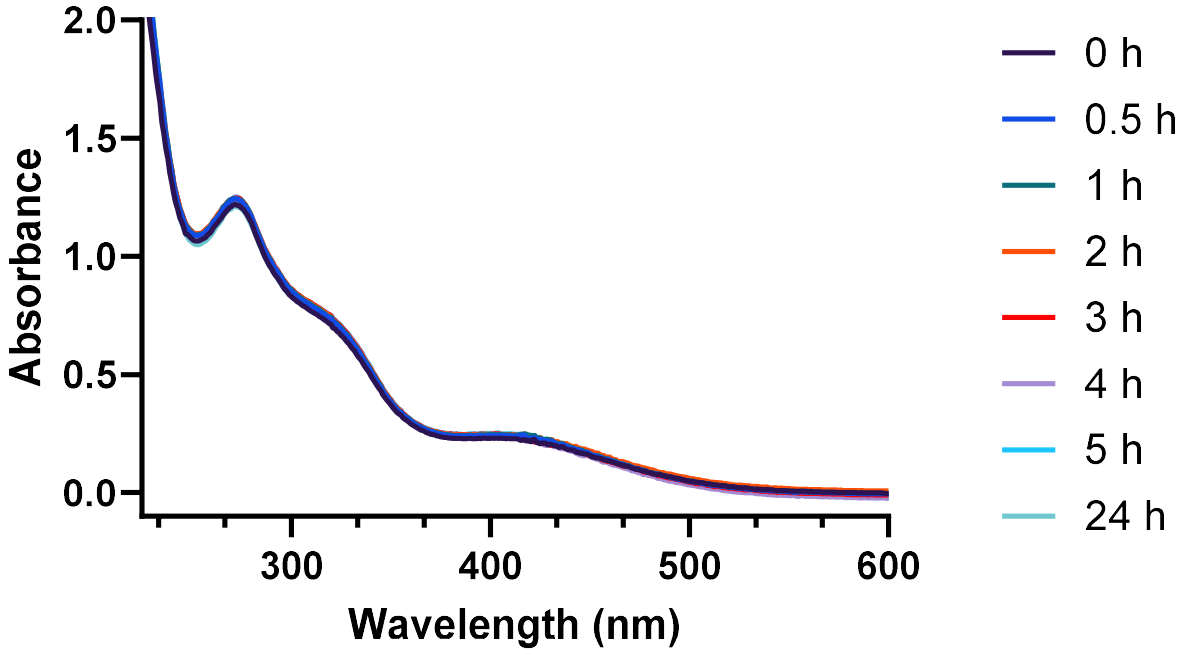


Figure S22. UV-vis spectra of Fe(PTOB) incubated at 37 ºC in 100 mM NaCl, 20 mM HEPES (pH 7.4) with 25 mM $NaHCO_3$ and 0.5 mM Na_2PO_4.

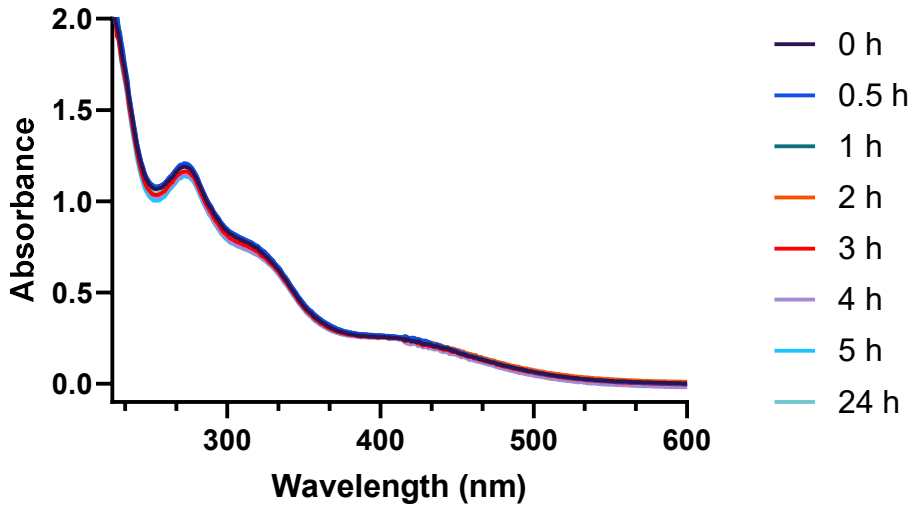


Figure S23. UV-vis spectra of Fe(PTOB) incubated at 37 ºC with one equivalent of $ZnCl_2$ and 20 mM HEPES (pH 7.4).

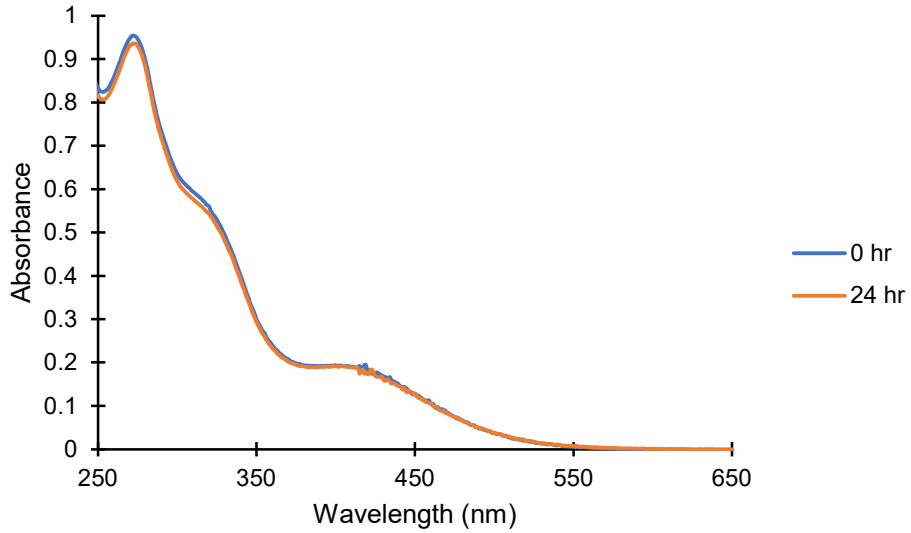


Figure S24. UV-Vis spectra of Fe(PTOB) at pH 9.0. Solutions contained 100 µM Fe(PTOB) with 50 mM HEPES and 100 mM NaCl at pH 7.4 and incubated at 37°C.

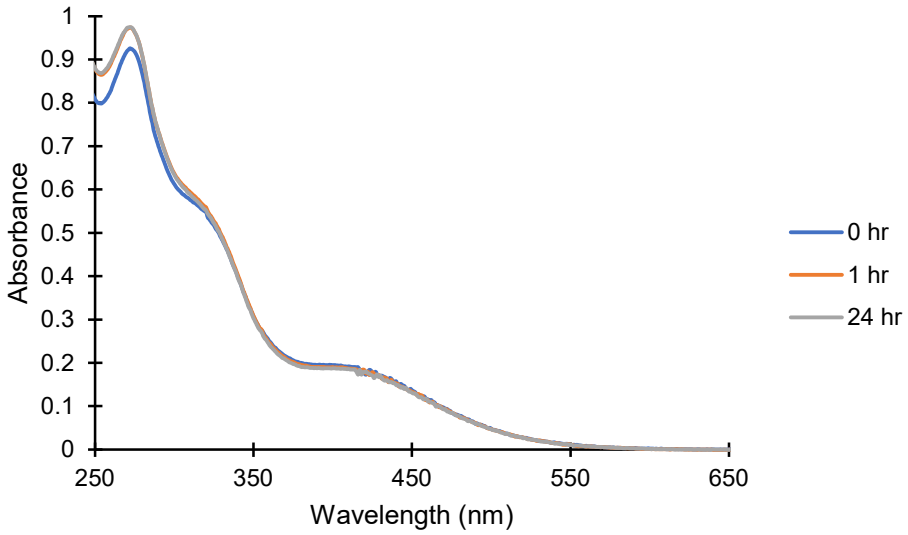


Figure S25. UV-Vis spectra of Fe(PTOB) in the presence of EDTA. Solutions contained 100 µM Fe(PTOB) and 100 µM EDTA with 50 mM HEPES and 100 mM NaCl at pH 7.4 and incubated at 37°C.

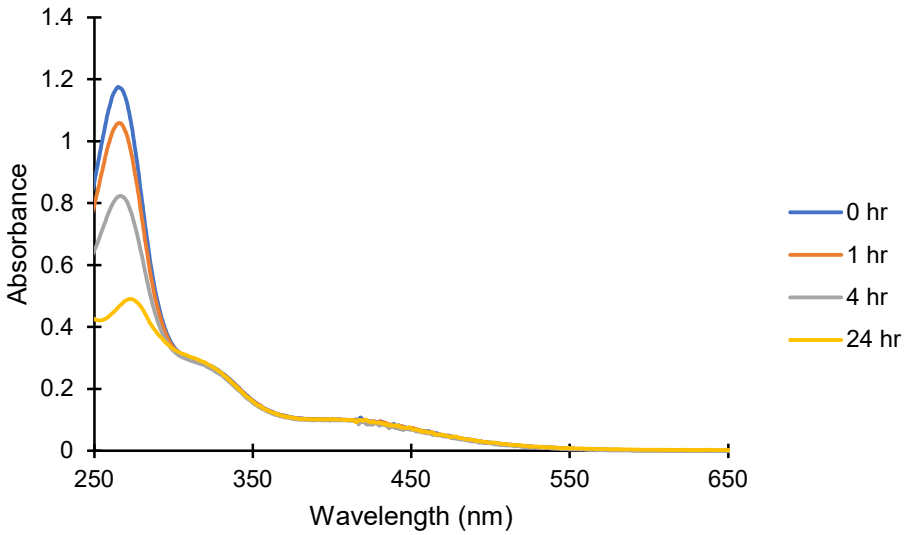


Figure S26. UV-Vis spectra of Fe(PTOB) in the presence of ascorbate. Solutions contained 50 µM Fe(PTOB) and 50 µM ascorbate with 20 mM HEPES and 100 mM NaCl at pH 7.4 and incubated at 37°C.

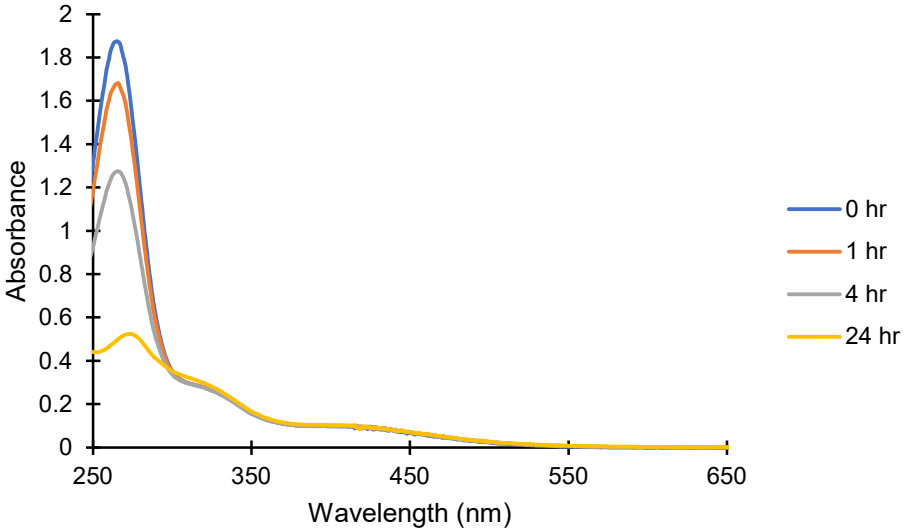


Figure S27. UV-Vis spectra of Fe(PTOB) in the presence of ascorbate. Solutions contained 50 µM Fe(PTOB) and 100 µM ascorbate with 20 mM HEPES and 100 mM NaCl at pH 7.4 and incubated at 37°C.

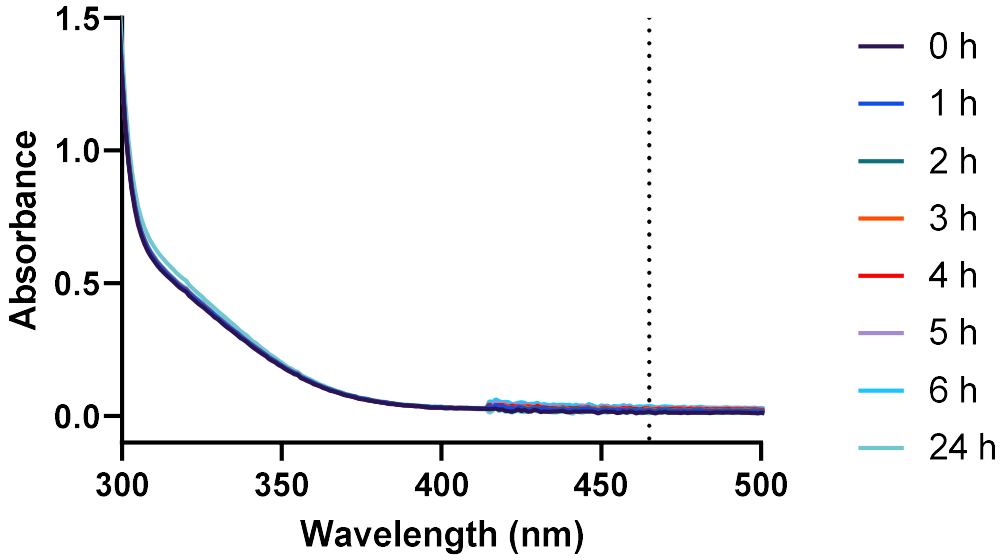


Figure S28: UV-Vis spectra of Fe(NOHP) complex – transferrin competition studies. Solutions contained 100 µM Fe(NOHP) and 200 µM apo-transferrin with 20 mM HEPES (pH 7.4) and 100 mM NaCl. The dotted line indicates 465 nm where Fe-Transferrin Ɛ(465 nm) = 4950 $M^{-1}cm^{-1}$. Over 24 hours, 4.3% transchelation of the Fe was observed.

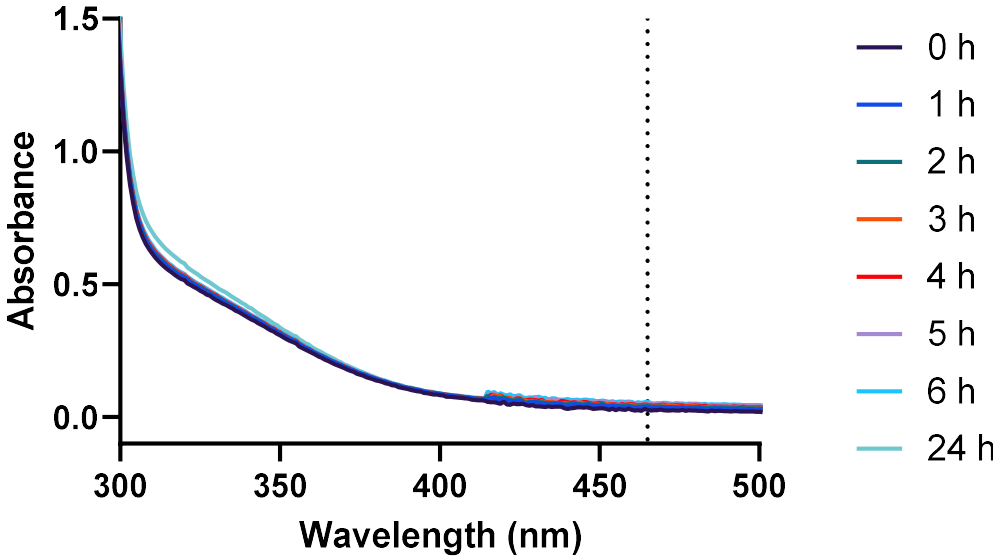


Figure S29: UV-Vis spectra of Fe(TOAB) complex – transferrin competition studies. Solutions contained 100 µM Fe(TOAB) and 200 µM apo-transferrin with 100 mM NaCl at pH 7 (adjusted with meglumine). The dotted line indicates 465 nm where Fe-Transferrin Ɛ(465 nm) = 4950 $M^{-1}cm^{-1}$. Over 24 hours, 5.9% transchelation of the Fe was observed.

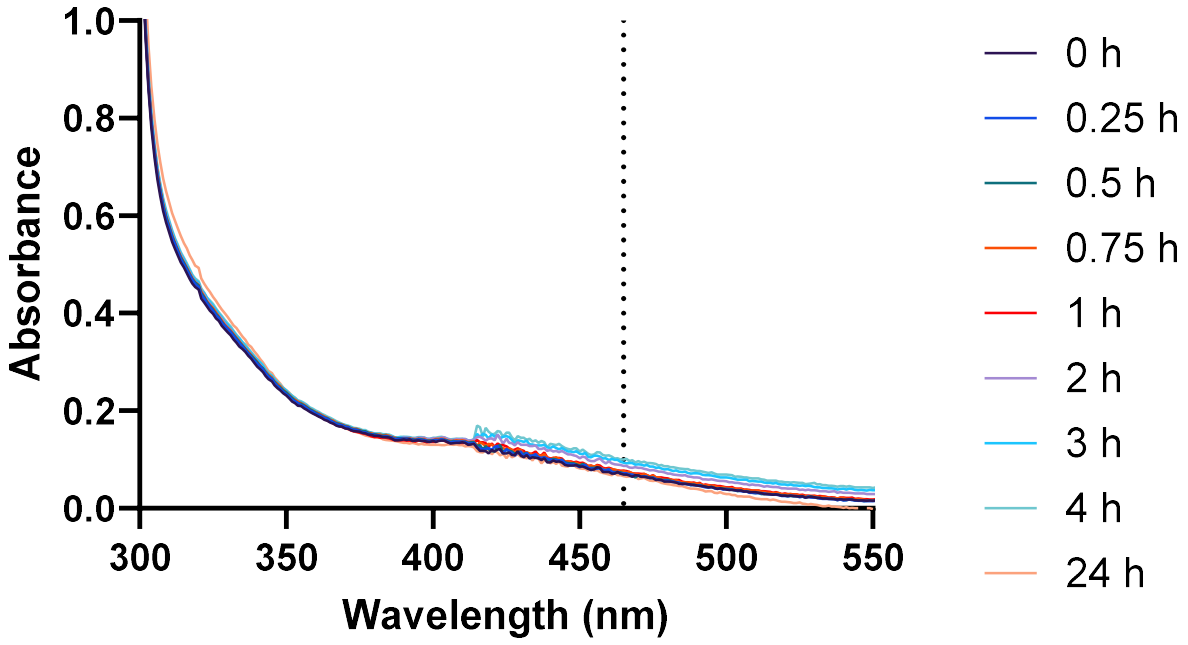


Figure S30: UV-Vis spectra of Fe(PTOB) complex – transferrin competition studies. Solutions contained 100 µM Fe(TOAB) and 200 µM apo-transferrin with 20 mM HEPES (pH 7.4) and 100 mM NaCl. The dotted line indicates 465 nm where Fe-Transferrin Ɛ(465 nm) = 4950 $M^{-1}cm^{-1}$. Over 24 hours, 6.5% transchelation of the Fe was observed.

Table S1. Crystallographic Details.

	Fe_Phenol_TOB
CCDC Number	2309236
Empirical formula	$C_{19}H_{32}Br_{1.5}Cl_{0.5}FeN3O3 \bullet 1.75[H_3CCN]$
Formula weight	615.89
Temperature (K)	100.0(1)
Crystal system	monoclinic
Space group	$P2_1$
a (Å)	12.8951(5)
b (Å)	6.9779(2)
c (Å)	15.6193(6)
α (°)	90
β (°)	97.804(4)
γ (°)	90
Volume (Å^3)	1392.42(9)
Z	2
ρ_{calc} (g cm^{-3})	1.469
μ (mm$^{-1)}$	7.558
F(000)	633.0
Crystal size (mm^3)	0.303 × 0.039 × 0.020
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection (°)	5.712 to 159.536
Index ranges	$-16 \le h \le 16$, $-5 \le k \le 8$, $-19 \le l \le 19$
Reflections collected	12511
Independent reflections	4430 [R_{int} = 0.0550, R_{sigma} = 0.0510]
Data/restraints/parameters	4430/268/259
Goodness-of-fit on F^2	1.046
Final R indexes [I>=2σ (I)]	R_1 = 0.0452, wR2 = 0.1213
Final R indexes [all data]	R_1 = 0.0556, wR2 = 0.1282
Largest diff. peak/hole (e$^-$ Å^{-3})	0.38/–0.58
Flack parameter	–0.009(7)

Table S2: Selected Coordination Sphere Bond Lengths:

Atom	Atom	Length/Å
Fe1	O2	2.022(5)
Fe1	O3	2.059(4)
Fe1	O1	1.858(5)
Fe1	N3	2.162(5)
Fe1	N1	2.185(5)
Fe1	N2	2.184(6)

Table S3: Selected Coordination Sphere Bond Angles:

Atom	Atom	Atom	Angle/˚
O2	Fe1	O3	94.6(2)
O2	Fe1	N3	158.5(2)
O2	Fe1	N1	104.7(2)
O2	Fe1	N2	79.2(2)
O3	Fe1	N3	78.7(2)
O3	Fe1	N1	159.3(2)
O3	Fe1	N2	95.7(2)
O1	Fe1	O2	99.5(2)
O1	Fe1	O3	93.9(2)
O1	Fe1	N3	101.3(2)
O1	Fe1	N1	90.3(2)
O1	Fe1	N2	170.4(2)
N3	Fe1	N1	80.6(2)
N3	Fe1	N2	81.1(3)
N2	Fe1	N1	80.9(3)

Crystallographic Details

Single crystals of **Fe(PTOB)]Br$_2$** were grown from an acetonitrile/diethyl ether mixture. The crystals grew as fine, purple/red needles and were removed from the mother liquor using N-Paratone oil. The sample was mounted on a MiTeGen loop and mounted on a Rigaku XtaLAB Synergy-S diffractometer equipped with PhotonJet-S Cu and Ag microfocus sources and HyPix-6000HE hybrid pixel array detector. The datum crystal (0.303 × 0.039 × 0.020 mm^3) was cooled and maintained at 100 K using an Oxford 1000 cryostream for data collection. Data was collected using Cu Kα (1.54184 Å) radiation. A series of ω scans were used to cover reciprocal space. Data reduction was performed using CrysAlisPro, absorption correction was applied based on the indexed faces of the crystal.[8] Space group assignment was made on the basis of systematic absences, $|E^2-1|$ statistics, and the successful refinement of the structure. **Fe(PTOB)]Br$_2$** was found to crystallize in the $P2_1$ space group. The crystals were small and weakly diffracting and show some evidence of twinning. Attempts to refine the minor twin component (<15%) were fruitless and refinement against data from the primary domain was used. Furthermore, the crystals were found to degrade overtime in the X-ray beam, which may be a result of radiation damage. As a result, a relatively low coverage of Friedel mates were measured; however, this does not detract from the atomic connectivity. Structure solution was performed using ShelXT via Intrinsic Phasing.[9] Structure refinement was by least-squares using ShelXL with the Olex2 software package.[10] Structure refinement was routine and refinement statistics are compiled in Table S1. The Br^- counterions were found to be over modeled at full occupancy and were positionally disordered with Cl^- counterions. Occupancies were freely refined, and displacement parameters and coordinates were constrained with EADP and EXYZ constraints. The final occupancies across both positions refined to 1.5 Br^- and 0.5 Cl^-. During the refinement process channels through the crystal were discovered along the crystallographic *b*-axis (see Figure S31). Considerable electron density was found; however, modeling attempts failed. A solvent mask was applied, and the channels were found to contain 38 e^-/formula unit, which corresponds to 1.75 acetonitrile molecules per formula unit. We cannot rule out the possibility of a mix of acetonitrile and diethyl ether. The assignment of acetonitrile was made on the basis that features in the residual electron density map looked to be linear, rather than the expected “zig-zag” type pattern expected for diethyl ether.

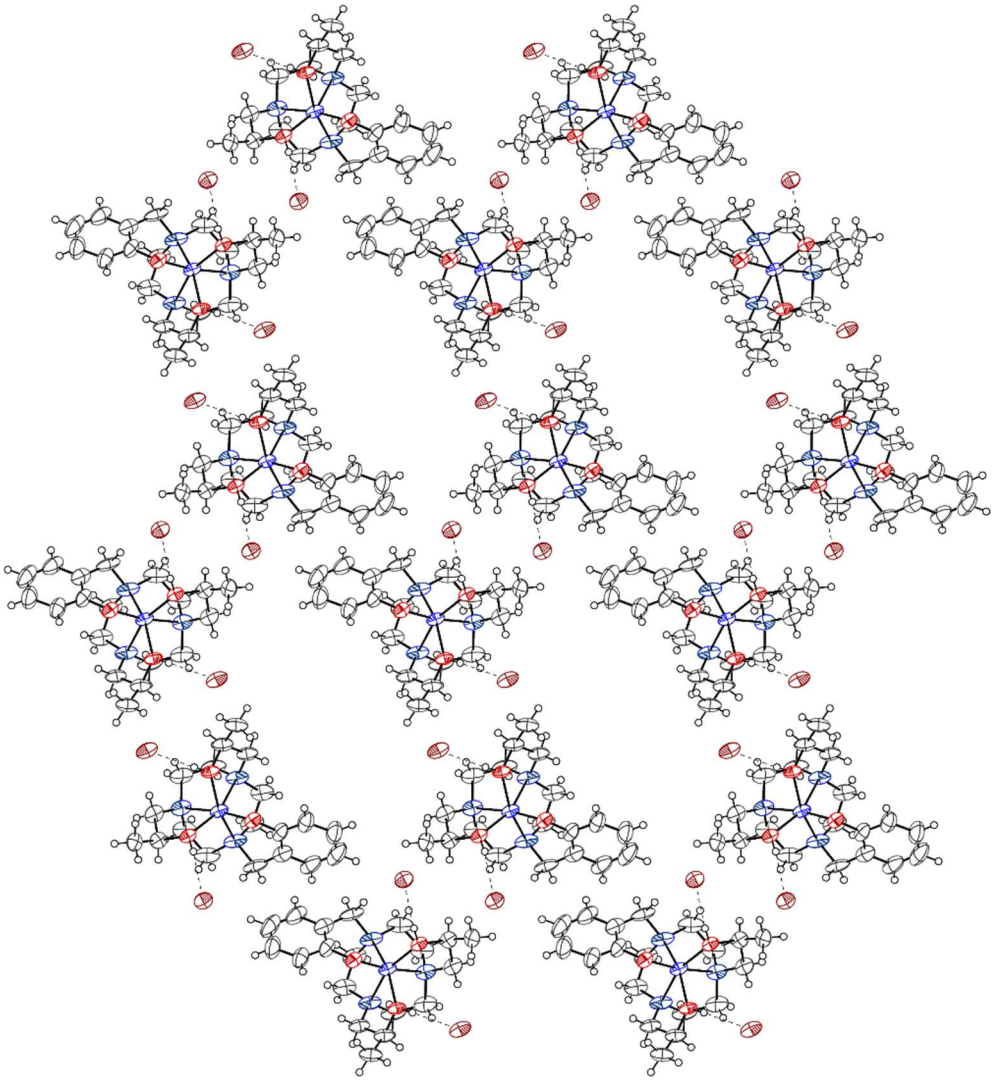


Figure S31. Packed structure of **Fe(PTOB]Br$_2$** viewed down the crystallographic *b*-axis. Thermal ellipsoids are set at the 50% probability level. Hydrogen-bonding interactions between the hydroxy group of the complex and bromide counterions are illustrated by dashed lines. The minor disordered chloride component has been hidden for clarity.

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