Europium(III) DOTA-tetraamide complexes as redox active MRI sensors

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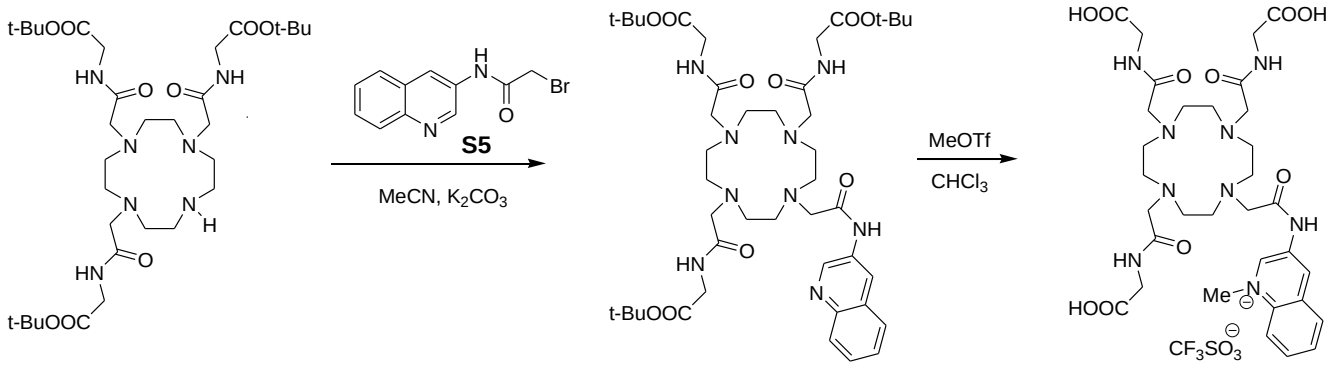
Experimental

General Remarks. All solvents and reagents were purchased from commercial sources and used as received unless otherwise stated. ^{1}H CEST spectra and ^{13}C NMR and ^{13}C DEPT analysis were recorded on a Varian VNMRS direct drive Varian console spectrometer operating at 400 and 100 MHz respectively. MALDI mass spectra were acquired on an Applied Biosystems Voyager-6115 Voyager -DE-' Pro Biospectrometry workstation mass spectrometer. Analytical HPLC was performed on a Dionex Summit P580 system equipped with a Phenomenex Gemini C18, 5 μ, 250×4.6 mm analytical column or a 250×4.6 mm, Phenomenex silica (2) normal phase, 5 μ analytical column. The elemental analyses were done at the Galbraith Laboratories, Inc (Knoxville, TN). ESI mass spectra were recorded at HT Laboratories (San Diego, CA). Cyclic voltammetry was performed on a BAS Epsilon Electrochemical Workstation with glassy carbon as working electrode, Pt wire as counter electrode, Ag^{+}/AgCl as reference electrode, and potassium chloride as supporting electrolyte. The voltage sweep rate for each scans were 100 mV. CEST images were acquired with a 20 mm Varian probe at 400 MHz using a modified fast spin echo sequence with an 8 echo train length for each 10 second repetition period, resulting in an effective echo time of 8.5 msec. An in plane resolution of approximately 0.1 mm^2 was achieved using a 40 x 40 field of view and 128 x 128 data matrix. A 10 μT saturation pulse was administered for 3 seconds on resonance at the exchange pool (50 ppm), on the bulk water, and off resonance (-50 ppm). The final CEST image of the phantom was derived by subtracting the off resonance image with the on resonance image.

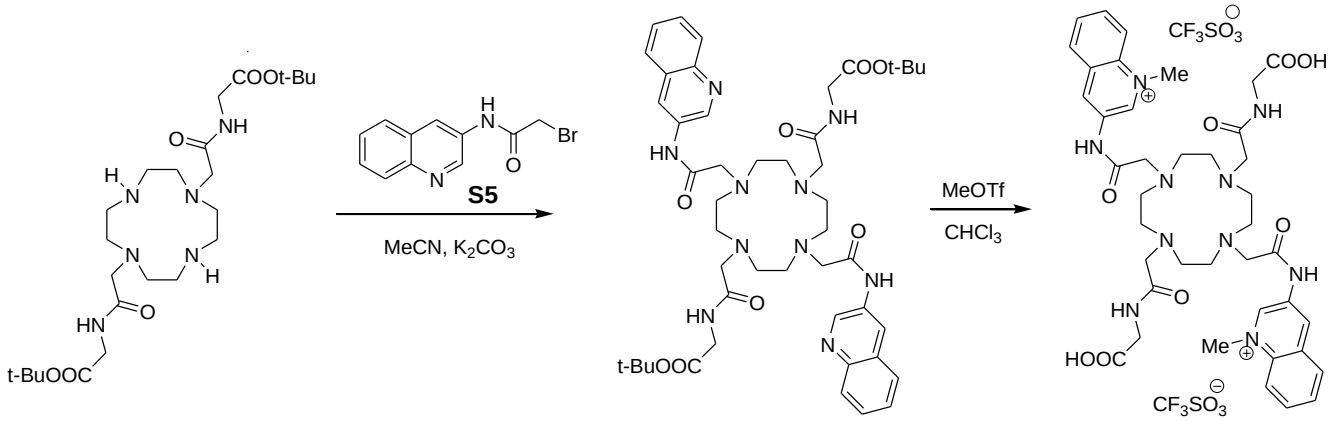
Synthetic procedures

The synthesis of ligand **1** and **2** is outlined in Scheme **S1** and **S2**, respectively. The macrocyclic precursors DO3A(GlyOt-Bu)$_3$ (**S3**) and DO2A(GlyOt-Bu)$_2$ (**S4**) have been prepared following previously published procedures.[1-3]



S3 **S6** **1**

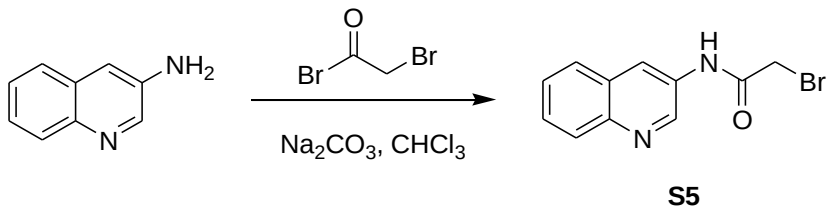
Scheme S1. Synthesis of ligand **1**.



S4 **S7** **2**

Scheme S2. Synthesis of ligand **2**.

3-(2-Bromoacetamido)quinoline (S5).



S5

Scheme S3. Synthesis of 3-(2-bromoacetamido)quinoline (**S5**)

To a solution of 3-aminoquinoline (1.4g, 10 mmol) in chloroform (100 mL) was added sodium carbonate (1.05g, 10 mmol). The reaction mixture was stirred vigorously and cooled to 0 °C in an ice bath. Bromoacetyl bromide (4.04 g, 20 mmol) in chloroform (25 mL) was added dropwise over a period of 1 hr. The reaction mixture was then allowed to warm to room temperature and stirred for a further 8 h. The yellow solid that separated was filtered and washed with water (3 x 25 mL) and air-dried to give a pale yellow powder. Yield: 3.2 g, 95 %. ^{1}H NMR (400 MHz, CD_3OD), δ = 9.40 (1H, s br, NH), 8.92 (1H, s, Ar), 8.69 (1H, s, Ar), 7.98 (1H, Ar), 7.72 (1H, Ar), 7.59 (1H, Ar), 7.49 (1H, Ar), 4.13 (2H, s); ^{13}C NMR (100 MHz, CD_3OD) δ = 165.2 (C=O), 145.12 (1H, Ar), 143.93 (1H, Ar), 131.4 (1H, Ar), 129.0 (1H, Ar), 128.7 (1H, Ar), 128.0 (1H, Ar), 127.7 (1H, Ar), 125.9 (1H, Ar), 29.6 (2H, C*H*$_2$Br); Anal. found: C 37.9 %, H 2.85 %, N 7.70 %; calculated for $C_{11}H_9N_2O\times HBr$: C 38.18 %, H 2.91%, N 8.10 %.

1,4,7,10-tetraazacyclododecane-1-[acetic acid 3-aminoquinoline amide]-4,7,10-tris[acetic acid (glycine tert-butyl ester) amide] (S6) DO3A(GlyOt-Bu)$_3$ (**S3**) (1.0 g, 1.46 mmol) was dissolved in acetonitrile (50 mL) and potassium carbonate (0.207 g, 1.46 mmol) was added. 3-Bromoacetylamino

quinoline (**S5**) (387 mg, 1.12 mmol) was added and the reaction mixture was heated at 60°C for 3 days. The inorganic salts were removed by filtration and the solvent was removed under reduced pressure by rotary evaporation. The oily residue was taken up in dichloromethane, washed with brine and water several times, dried over sodium sulfate and the solvent was evaporated by rotary evaporation. The residue was purified over neutral alumina using CH_3OH/CH_2Cl_2 (5%/95%), to give the product as an oil (0.83 g). Analytical HPLC, R_f = 15.7 min, (95 %) (GEMINI C18 reversed phase analytical column, 250mm x 4.6mm, 5μ, 100 A°, water (0.1% TFA)/acetonitrile (0.1% TFA), 90/10 %, flow rate 1 mL/min, 30 min ramp); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.90 (4H, s br, NH), 8.81 (1H, s, Ar), 8.71 (1H, Ar), 7.99 (1H, Ar), 7.60 (1H, Ar), 7.51 (1H, Ar), 7.45 (1H, Ar), 3.92 (6H, $NHCH_2CO$), 3.80 (6H, CH_2CO), 3.19 (2H, CH_2O), 2.82, 2.48 (16H, m, CH_2 ring), ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.2 (C=O), 171.3 (2 × C=O), 169.0 (C=O), 145.02 (2-Ar), 144.5 (9-Ar), 132.8 (3-Ar), 132.0 (10-Ar), 129.1 (4-Ar) 128.2 (8-Ar), 127.9, (7-Ar) 127.4 (5-Ar) 123.7 (6-Ar), 82.3 ($C(CH_3)_3$ 57.3 (2 × OCH_2), 59.2 (br, NCH_2CO), 53.2, (br, ring CH_2N),41.7 ($NHCH_2CO_2Et$), 28.2($C(CH_3)_3$. MS, MALDI-TOF, positive mode, found *m/z*, 870.2 $[M]^+$; calculated for $[C_{43}H_{67}N_9O_{10}]^+$ *m/z*, 870.05. Anal. found: C 54.9 %, H 7.71%, N 13.1 %; calculated for $C_{43}H_{68}N_9O_{10}$·HBr: C 54.31%, H 7.21 %, N 13.26 %.

1,4,7,10-tetraazacyclododecane-1-[acetic acid (3-amino-1-methylquinolinium) amide]-4,7,10-tris[acetic acid glycine amide] (1). To a solution of **S6** (0.150 g, 0.17 mmol) in chloroform (20 mL) was added methyl triflate (0.150 g, 0.93 mmol). The reaction was allowed to stir at room temperature for 4 h when a yellow oil started to form, which was then separated from the chloroform layer and washed 3 times with chloroform and dried for 8 hr in high vacuum. It was dissolved in water and lyophilized to yield an off-white solid in 60% yield. The methyl triflate treatment also resulted in the cleavage of the *tert*-butyl ester groups. Analytical HPLC, R_f = 6.5 min, (95 %), (GEMINI C18 reversed phase analytical column, 250mm x 4.6mm, 5μ, 100 A°, water (0.1% TFA)/acetonitrile (0.1% TFA), 90/10 %, flow rate 1 mL/min, 30 min ramp);^{1}H NMR (400 MHz, D_2O) δ = 9.3 (1H, Ar), 9.0 (1H, Ar),

8.2 (1H, Ar), 8.1 (1H, Ar), 8.0 (1H, Ar), 7.8 (1H, Ar), 4.5 (3H, NCH_3), 3.8 (6H, CH_2CO), 3.3 (26H, m, CH_2 ring); ^{13}C NMR (100 MHz, D_2O) δ = 172.2 (C=O), 142.02 (2-Ar), 136.1 (9-Ar), 134.8 (3-Ar), 130.6 (10-Ar), 129.1 (4-Ar), 124.3 (8-Ar), 121.2, (7-Ar) 118.4 (5-Ar) 114.6 (6-Ar), 55.1 (s, OCH_2), 54.7 (br, $N\underline{C}H_2CO$), 50.4 (br, ring CH_2N), 45.2 (s, NCH_3), 41.0 ($NH\underline{C}H_2CO_2Et$). MS, MALDI-TOF, positive mode, found: *m/z*, 715.9 $[M]^+$; calculated for $[C_{32}H_{46}N_9O_{10}]^+$: *m/z*, 716.3; Anal. found: C 30.68 %, H 4.18 %, N 8.48 %; calculated for $[C_{32}H_{46}N_4O_{10}]^+[CF3SO3]^-\times 3CF_3SO_3H\times 6H_2O$: C 30.36%, H 4.32%, N 8.85%.

Eu^{3+}-(1) complex. The free ligand (**S1**) (0.05 g, 0.05 mmol) was dissolved in water (10 mL) and the pH was adjusted to 7 with NaOH (0.1 M). To this solution was added excess $EuCl_3.6H_2O$ and the pH was again adjusted to 6.5 and allowed to stir at room temperature overnight. The pH was raised above 8 using 1 M aqueous NaOH, which caused the excess Eu^{3+} to precipitate as $Eu(OH)_3$. The solution was filtered and the pH was readjusted to 7 using 1 M HCl and the solution was lyophilized to give the desired complex. An aqueous solution of the **Eu^{3+}-(1)** complex was checked for the absence of free Eu^{3+} ion with the xylenol orange indicator test. HPLC, R_f = 20 min (95 %) (40% H_2O)/ 5% 10 mM ammonia/ammonium chloride buffer (pH 9.5)/55% acetonitrile) on a normal phase Silica (2), 5µ 150 x 4.6 mm analytical column with a flow rate of 1/min. ^1H NMR (400 MHz, D_2O) δ = 25.7 (4H, br, ring ax^S), 11.0 (4H, br, ring eq^S), 9.0 – 6.5 (6H, Ar), 4.0 – 3.0 (8H, m, NCH_2), 5.3 (3H, s, $ArCH_3$), -2.8 (4H, br, ring ax^C), -5.1 (4H, br, ring eq^C), -8.6, -12.8 (6H, br, *ac*). MS, MALDI-TOF, positive mode, found: *m/z*, 867.2 $[EuL-3H]^+$; calculated for $[C_{32}H_{43}N_9O_{10}Eu]^+$: *m/z*, 866.2.

1,4,7,10-tetraazacyclododecane-1,7-bis[acetic acid 3-aminoquinoline amide]-4,10-bis[acetic acid (glycine tert-butylester) amide] (S7). 1,4,7,10-Tetraazacyclododecane-1,7-bis[acetic acid (glycine tert-butyl ester) amide] (**S4**) (0.49 g, 1 mmol) was alkylated with 3-bromoacetylamino quinoline as described for compound **S6** to afford the product as an oil (0.65 g) after purification by column chromatography on neutral alumina using CH_3OH/CH_2Cl_2 (5%/95%).Analytical HPLC, R_f = 12.0 min,

95 % (GEMINI C18 reversed phase analytical column, 250mm x 4.6mm, 5μ, 100 A°, water (0.1% TFA)/acetonitrile (0.1% TFA), 90/10 %, flow rate 1 mL/min, 30 min ramp). ^{1}H NMR (400 MHz, CD_3Cl) δ = 10.4 (2H, s br, NH), 10.0 (2H, br, NH), 9.08 (1H, Ar), 8.89 (1H, Ar), 7.91 (1H, Ar), 7.69 (1H, Ar), 7.52 (1H, Ar), 7.40 (1H, Ar), 3.68 (4H, $NHCH_2CO$), 3.40 (4H, CH_2CO), 3.09 (4H, CH_2O), 2.76 (8H, m, CH_2 ring), 2.33 (8H, m, CH_2 ring) 1.08 (18 H, CH_3); ^{13}C NMR (100 MHz, CD_3Cl) δ = 171.0 (C=O), 170.3 (C=O), 169.0 (C=O), 168.6 (C=O), 145.0 (2-Ar), 144.2 (9-Ar), 132.5 (3-Ar), 131.6 (10-Ar), 128.8 (4-Ar) 128.2 (8-Ar), 127.7, (7-Ar) 127.1 (5-Ar) 123.7 (6-Ar), 82.0 $C(CH_3)_3$ 59.5 (2 × OCH_2), 58.8 ($N\underline{C}H_2CO$), 53.4, (br, ring CH_2N),41.5 ($NH\underline{C}H_2CO_2Et$), 27.8 ($C(\underline{C}H_3)$)$_3$. MS, MALDI-TOF, positive mode, found: *m/z*, 883.5 $[M+H]^+$; calculated for $[C_{46}H_{63}N_{10}O_8]^+$: *m/z*, 883.48. Anal. found: C, 58.50%, H, 6.62 %, N, 14.88 %.; Calculated for $C_{46}H_{62}N_{10}O_8$×0.75HBr: C, 58.54 %; H, 6.70 %; N, 14.84 %.

1,4,7,10-tetraazacyclododecane-1,7-bis[acetic acid (3-amino-1-methylquinolinium amide)]-4,10-bis[acetic acid (glycine amide)] (2). 1,4,7,10-tetraazacyclododecane-1,7-bis[acetic acid 3-aminoquinoline amide]-4,10-bis[acetic acid (glycine *tert*-butylester) amide] (**S7**) was quaternerized with 10-fold excess methyl triflate as described for ligand **1** to afford ligand **2** as an off white , extremelty hygroscopic solid after lyophilization. HPLC R_f = 22.0 min (95 %) (39% H_2O)/1% 10mM ammonia/ammonium chloride buffer (pH 9.5)/60% acetonitrile) on a normal phase silica (2), 5μ 150 x 4.6 mm analytical column with a flow rate of 1/min; ^{1}H NMR (400 MHz, D_2O) δ = 9.45 (2H, s br, NH), 9.32 (2H, br, NH), 8.96, 8,84, 7.77, 7.64, 7.50, 7.36 (6H, Ar), 4.10 (6H, s, CH_3), 3.91 (4H, $NHCH_2CO$), 3.83 (4H, CH_2CO), 3.49 (4H, CH_2O), 3.29 (8H, m, CH_2 ring), 3.09 (8H, m, CH_2 ring) 1.08 (18 H, CH_3); ^{13}C NMR (100 MHz, D_2O) δ = 172.2 (C=O), 171.1 (C=O), 165.8 (C=O), 143.2 (2-Ar), 141.2 (9-Ar), 134.2 (3-Ar), 132.4 (10-Ar), 130.4 (4-Ar), 128.2 (8-Ar) 121.3 (7-Ar), 117.9 (5-Ar), 117.5 (6-Ar), 62.1 (CH_3), 59.5 (OCH_2), 55.01 ($N\underline{C}H_2CO$), 52.2 (ring CH_2N) , 51.8 (ring CH_2N), 40.5

(NHCH$_2$CO$_2$Et).MS, MALDI-TOF, positive mode, found: *m/z*, 799.6 $[M\text{-}H]^+$; calculated for $[C_{40}H_{52}N_{10}O_8]^+$: *m/z*, 799.4.

Eu^{3+}-(2) complex. The free ligand (**2**) (0.05 g, 0.04 mmol) was complexed with excess $EuCl_3.6H_2O$ as described for Eu^{3+}-(**1**). Eu^{3+} content of the product was found to be 3.8 % by ICP-OES. HPLC R_f = 8.4 min (90 %) (39% H_2O)/1% 10mM ammonia/ammonium chloride buffer (pH 9.5)/60% acetonitrile) on a normal phased Silica (2), 5μ 150 x 4.6 mm analytical column with a flow rate of 1/min; ^{1}H NMR (400 MHz, D_2O) δ = 24.1 (4H, br, ring *ax*^{S}), 9.4 (4H, br, ring *eq*^{S}), 8.9 - 7.8 (12H, Ar), 3.8 – 2.7 (8H, m, NCH_2), 5.3 (6H, s, $ArCH_3$), -2.8 (4H, br, ring *ax*^{C}), -5.1 (4H, br, ring *eq*^{C}), -8.9 (2H, br, *ac*), -12.1 (2H, br, *ac*). MS, MALDI-TOF, positive mode, found, *m/z*, 950.4 $[M\text{-}4H]^+$; ESI, positive mode, found, *m/z*, 949.20 $[M\text{-}4H]^+$, 1099.00 $[M\text{-}4H+CF_3SO_3]^+$, 1248.87 $[M\text{-}3H+2CF_3SO_3]^+$; calculated for $[C_{40}H_{48}N_{10}O_8Eu]^+$ *m/z*, 949.3; for $[C_{41}H_{49}EuF_3N_{10}O_{11}S]^+$ *m/z*, 1099.3; for $[C_{42}H_{50}EuF_6N_{10}O_{14}S_2]^+$ *m/z*, 1249.2.

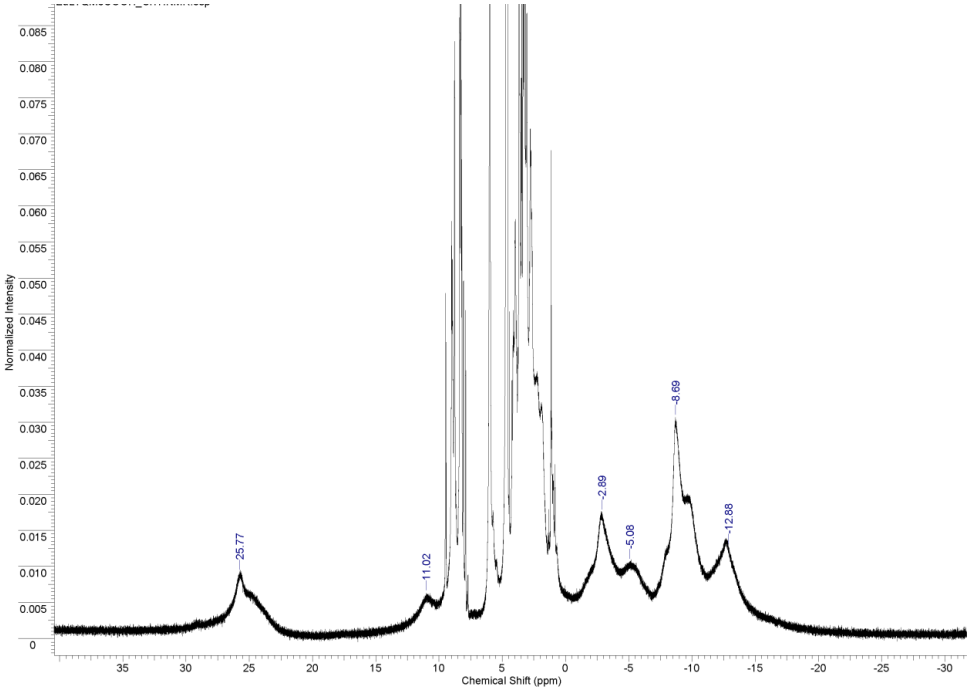


Figure S1. 400 MHz ^{1}H NMR spectrum of Eu^{3+}-(**1**) in D_2O.

CEST spectroscopy

The CEST spectrum or Z-spectrum is representation of the decrease in the signal intensity of the bulk water as it is in slow exchange with the bound water molecule and is visualized as a plot of saturation frequency (ω) relative to the magnitude of the bulk water proton signal (M_z/M_0). The CEST spectrum is obtained by applying a selective radiofrequency pulse for a certain time duration followed immediately a spin-echo observation pulse to attain the net signal intensity of the bulk water and repeating this over a range of frequencies gives CEST spectrum.[4]

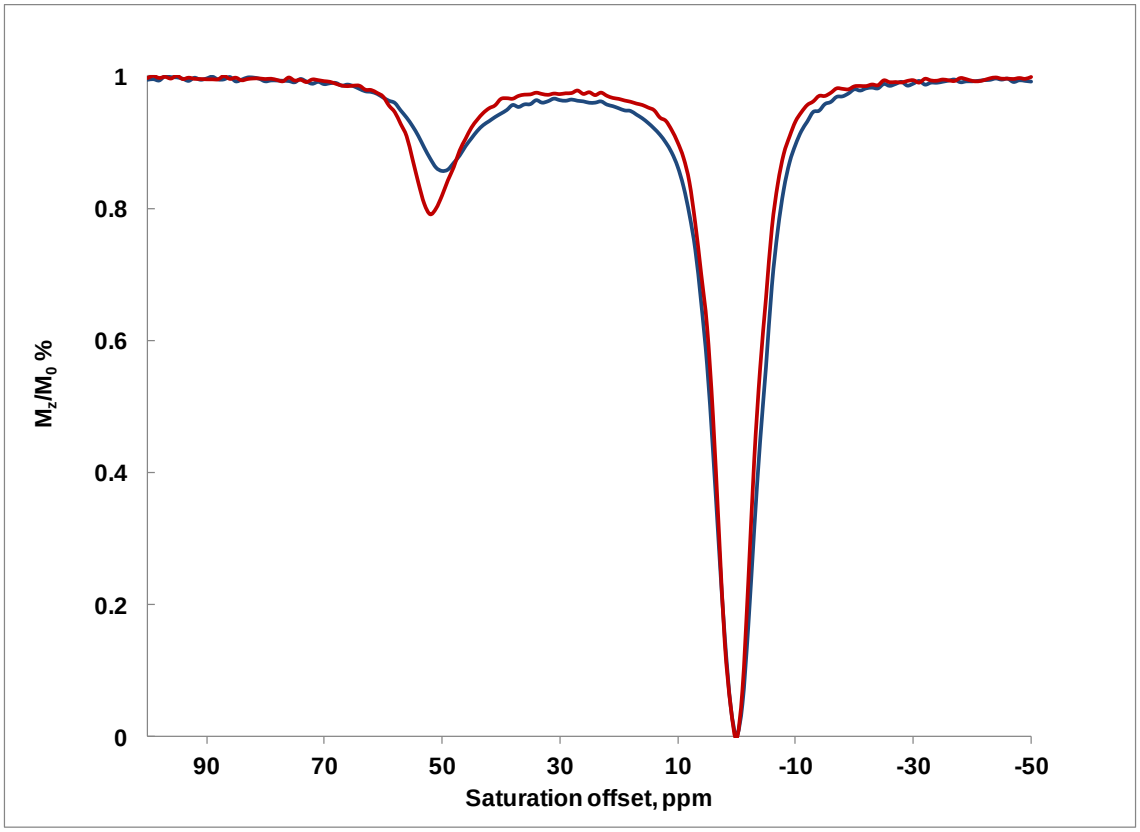


Figure S2. The combined CEST spectra of Eu^{3+}-(**1**) complex at 20 mM concentration before (blue line) and after reduction with β-NADH (red line) recorded at 9.4 T, pH 7, 298 K, with a B_1 = 10 μT and an irradiation time of 5 s.

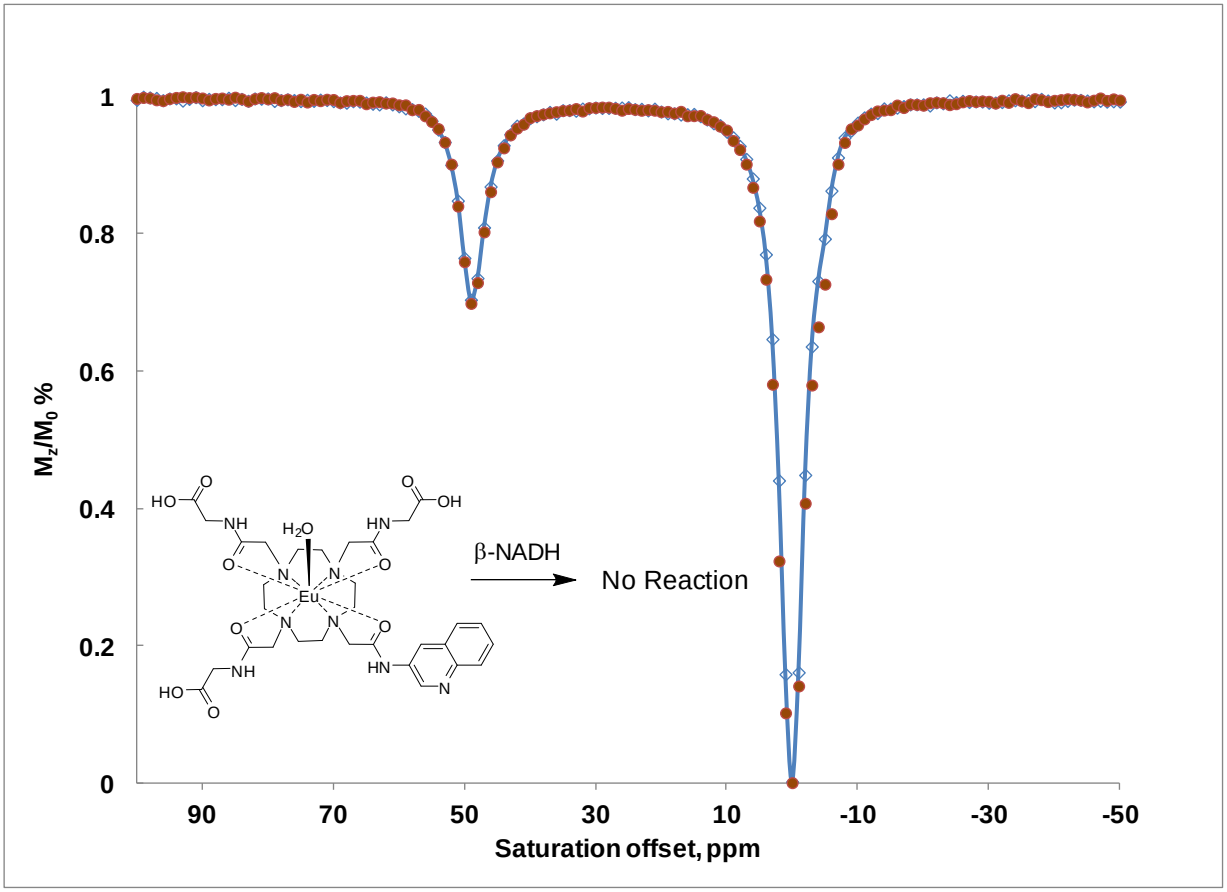
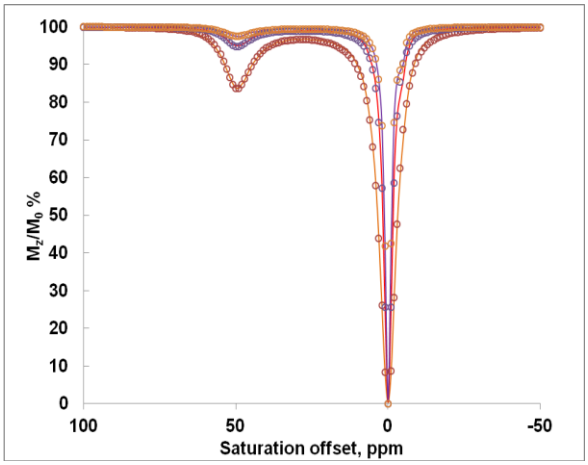
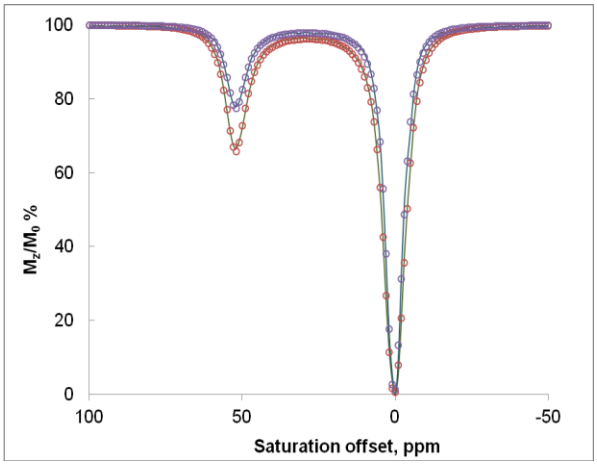


Figure S3. The combined CEST spectra of a 20 mM solution of the non-methylated Eu^{3+}**-(S8)** complex before (red dots) and after reduction (blue) with β-NADH recorded at 9.4 T, pH 7, 298 K, with a B_1 = 10.0 μT, and a irradiation time of 5 s.





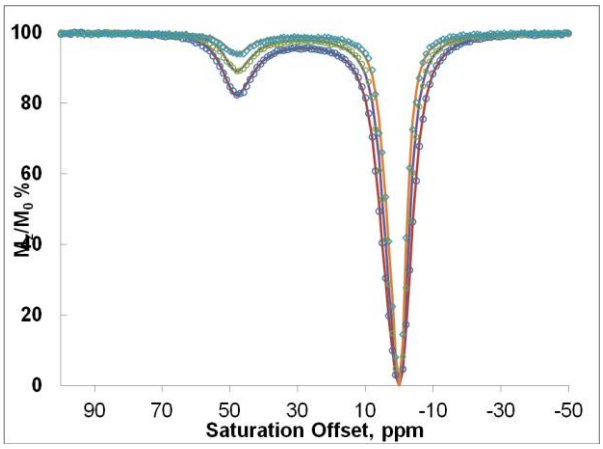


Figure S4 Water exchange rates were calculated by fitting the experimental data to a three pool model using the Bloch equations modified for exchange written in MATLAB.[5] The line represents the experimental data and the points show the data from fitting: Eu^{3+}-(**1**) (oxidized form), top left, Eu^{3+}-(**1**) (reduced form), top right, and Eu^{3+}-(**2**) (reduced form), bottom.

Cyclic voltammetry

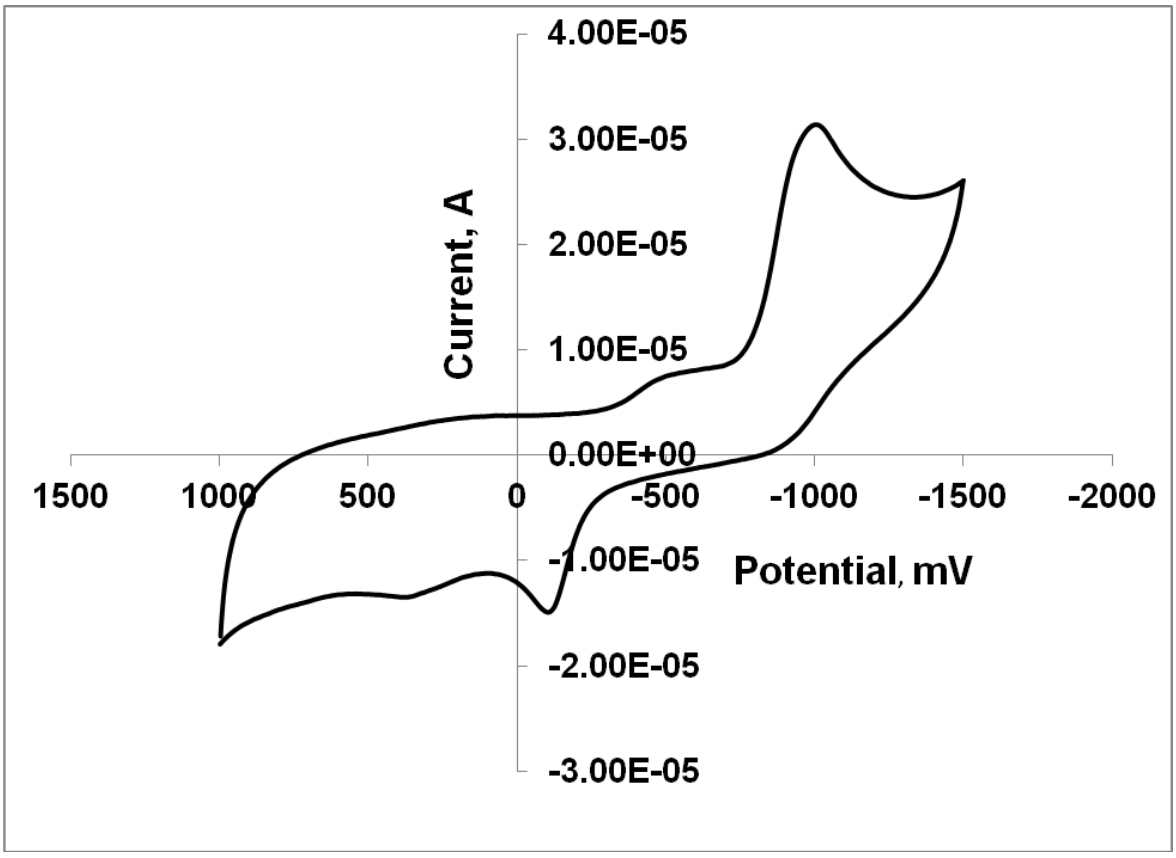


Figure S5 The cyclic voltammogram of Eu^{3+}-**(2)** in water. The cyclic voltammogram was recorded using a three electrode cell setup (glassy carbon as the working electrode, Ag^{+}/AgCl as the reference electrode, and a platinum wire as a counter electrode). The scan rate was 100 mV/min.

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