Europium(III) DOTA-derivatives having ketone donor pendant arms display dramatically slower water exchange

Supporting Information

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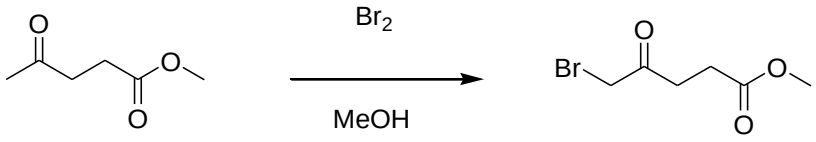
Synthetic procedures for the ligands

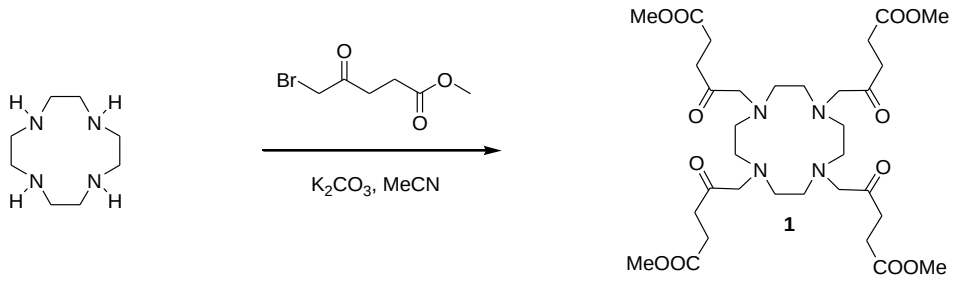
Methyl 5-bromo-4-oxopentanoate (or methyl 5-bromolevulinate) (1a).



1a

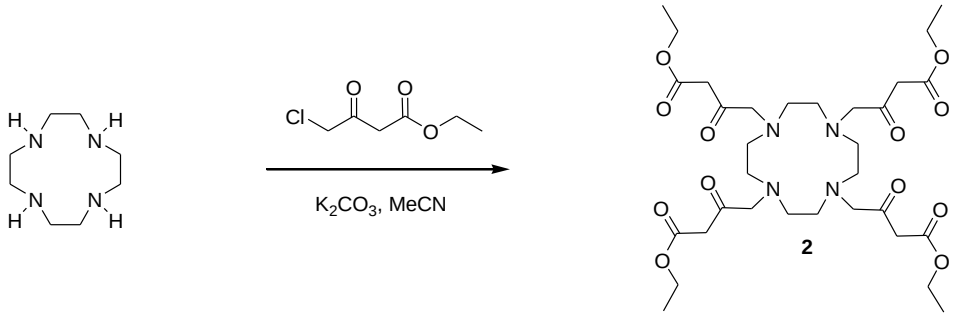
A methanol solution (200 mL) of methyl levulinate (28 mL) was prepared in a 1000 mL round bottom flask. While stirring vigorously at room temperature, bromine (40 mL) was added drop wise over the course of 1 hour. The resulting light orange solution was allowed to stir overnight. A nitrogen stream was introduced to the solution for 1 hour to remove any excess bromine. The methanol solvent was carefully removed to yield a yellow oil which was then dissolved in ether and washed with a solution of sodium bicarbonate. The ether layer was collected and the solvent removed to yield a clear oil. Further purification followed one of the following two routes. (1) The oil was dissolved in a mixture of diethyl ether and cyclohexane (1:1) and cooled to -30° C. White crystals formed, which were quickly filtered from the remaining solution and collected. The melting point of the crystals was approximately 3° C. This was repeated 3-4 times with the remaining solution to give the desired product in about 55% yield. (2) A gradient silica column (MeOH:CH_2Cl_2) yielded the product in 41% yield as the last of three clear bands. **CAUTION!!** *This compound is volatile and causes severe irritation to the skin. Wear appropriate protective clothing including goggles and rubber gloves. Wash immediately upon exposure.* ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.595 (-C*H*$_2$-, t), 2.9 (-C*H*$_2$-, t), 3.6(-OC*H*$_3$, s), 3.9 (Br-C*H*$_2$-C(O), 1); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.2 (O*C*H$_3$), 34.6 (Br*C*H$_2$-), 34.7 (BrCH$_2$C(=O)*C*H$_2$-), 52.1 (-*C*H$_2$C(=O)OCH$_3$), 172.9 (BrCH$_2$C(=O)(CH$_2$)$_2$*C*(=O)OCH$_3$), 200.8 (BrCH$_2$*C*(=O)(CH$_2$)$_2$C(=O)OCH$_3$).

1,4,7,10-Tetraazacyclododecane-1,4,710-tetrakis(methyl-oxopentanoate) (1).



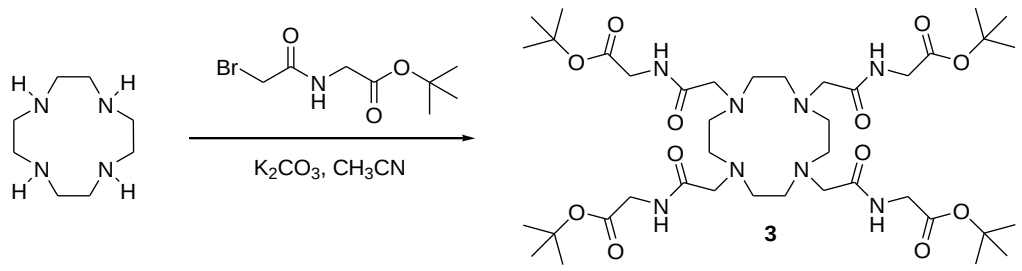
1,4,7,10-Tetraazacyclododecane (cyclen) (309 mg, 1.8 mmol) and K_2CO_3 (1.119 g, 38.5 mmol) were combined with methyl 5-bromolevulinate **1a** (1.5 g, 7.3 mmol) in CH_3CN (300 mL) and stirred for 5 days at 55 °C. The solution was cooled, filtered, and evaporated to give a dark red, thick oil. Purification by column chromatography (silica, MeOH, CH_2Cl_2) afforded the product as a red solid in 37 % yield. ^{13}C NMR (100 MHz, D_2O) δ =212.9, 175.9, 62.1, 52.4, 48.4, 35.0, 27.8. ^{1}H NMR (400 MHz, D_2O) δ = 4.6(s), 3.6 (s), 3.2 (s), 2.0-2.8 (br. mult.). MALDI^{+}: *m/z* 684.14 (100%) $[M+H]^{+}$, 708 (60%) $[M+Na]^{+}$. HPLC: (95% H_2O → 95% CH_3CN) Retention time; 15.7 min.

1,4,7,10-Tetraazacyclododecane-1,4,710-tetrakis(ethyl-oxobutanoate) (2).



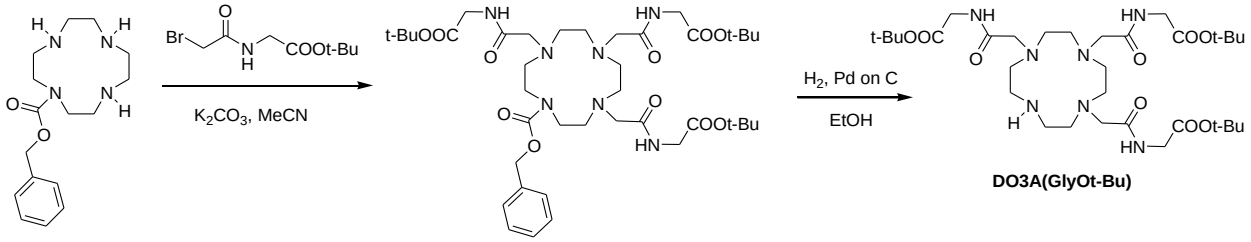
1,4,7,10-Tetraazacyclododecane (cyclen) (1.26 g, 7.0 mmol) and K_2CO_3 (4.2 g, 38.5 mmol) were combined with ethyl-4-chloro-3-oxobutanoate (5 g, 3.04 mol) in CH_3CN (500 mL) and stirred for 3 days at 55° C. The solution was cooled, filtered, and evaporated by rotary evaporation to give an orange oil. Purification by column chromatography (silica, MeOH, CH_2Cl_2) afforded the product as a yellow solid in 72% yield. ^{13}C NMR (100 MHz, $CDCl_3$) δ =169.0, 152.8, 117.6, 62.0, 60.2, 43.3, 38.0, 14.0.; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.41 (q), 2-4 (br. mult.) 1.40 (t) .MALDI^{+}: *m/z* 685.47 (100%) $[M+H]^{+}$.

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrakis[acetic acid glycine amide] (3).



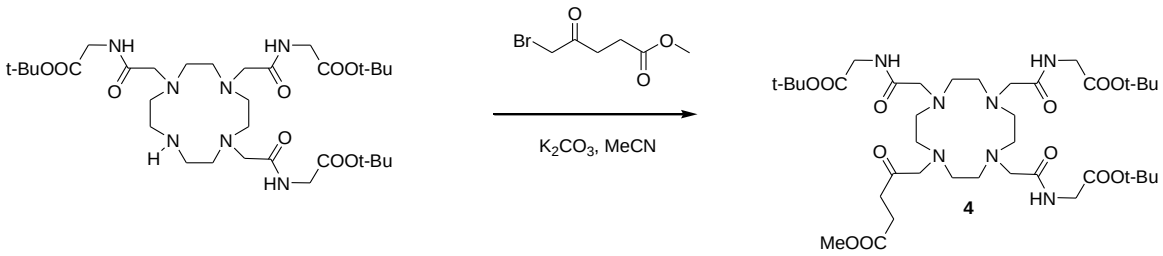
Cyclen (56.8 mg, 0.32 mmol) and K_2CO_3 (265.4 mg, 1.92 mmol) were combined with 200 mL of CH_3CN. An CH_3CN solution of N-bromoacetyl glycine t-Bu ester **(**349.0 mg, 1.38 mmol) was then added. The reaction mixture was stirred vigorously for 4 days at 60 °C and then cooled to room temperature. The solution was filtered and solvents removed under reduced pressure to afford the product as a white solid in 75 % yield. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.4, 168.9, 81.7, 59.2, 53.2, 41.6, 27.9; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.58, 3.75, 3.04, 2.62, 1.36. MALDI^{+}: *m/z* 858.1 (100%) $[M+H]^{+}$.

1,4,7,10-Tetraazacyclododecane-1,4,7-tris[acetic acid-(glycine t-Bu ester) amide] (DO3A(GlyOt-Bu))



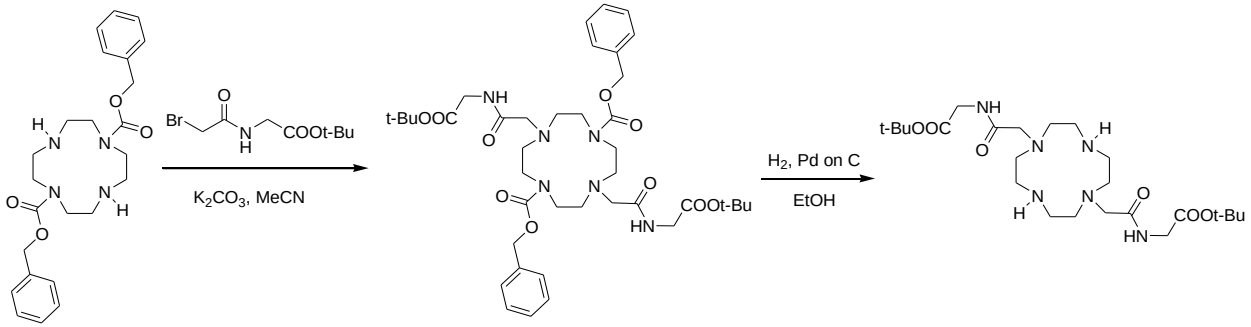
A solution of 1-benzyloxycarbonyl-1,4,7,10-tetraazacyclododecane[1] and potassium carbonate were combined with 3.2 equivalents of N-bromoacetylglycine t-Bu ester. The reaction mixture was heated at 60 °C for 4 days while stirring and then cooled, filtered and evaporated by rotary evaporation. The crude product was purified by column chromatography (silica, MeOH, CH_2Cl_2) before the catalytic hydrogenation. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 215.0, 192.3, 127-128, 84.0, 81.5, 66.9, 60.24, 52.2, 48.9, 41.2, 28.0; ^{1}H NMR (400 MHz, CDC_3l) δ = 8.46, 8.1, 7.77 7.61, 5.14, 3.92, 3.89, 3.44, 3.43, 3.25, 3.13, 2.64-2.9 (br. mult.), 1.45. Removal of the benzyloxycarbonyl protecting group was accomplished by catalytic hydrogenation in the presence of Pd/C catalyst (dry, 10%) in ethanol under hydrogen pressure (40 psi) for 3 days. ^{13}C NMR (100 MHz, $CDCl_3$) δ =174.98, 173.98, 172.2, 172.1, 84.55, 84.49, 63.03, 60.89, 58.38, 55.94, 53.70, 47.77, 44.11, 30.55; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.87, 3.83, 3.32, 3.30, 3.01, 2.90, 2.74, 2.66, 1.89, 1.39.

1,4,7,10-Tetraazacyclododecane-1,4,7-tris[acetic acid-(glycine t-Bu ester) amide] -10-methyl-oxopentanoate (4).



A slight excess of methyl 5-bromo-levulinate **1a** (139.87 mg, 0.669 mmol) was added to an CH_3CN solution of **DO3A(GlyOt-Bu)** (383 mg, 0.558 mmol) and K_2CO_3 (154.1mg, 1.12 mmol). The mixture was stirred at 55 °C for 5 days. It was allowed to cool and then filtered. The solvent was removed under reduced pressure to give a yellow oil. Purification by column chromatography (silica, MeOH, CH_2Cl_2) afforded the product as a light yellow solid in 41 % yield. ^{13}C NMR (100 MHz, $CDCl_3$) δ =205.12, 172.98, 172.67, 171.53, 81.92, 67.95, 58.76, 53.692, 51.86, 41.79, 33.34, 28.58, 27.27; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.5-8.1 (br. mult.) 4.7, 2.2-3.8 (mult.), 2.1, 1.5; MALDI$^+$: *m/z* 814.48 (100%) $[M+H]^+$; HPLC: (C18 reversed phase column, 95% H_2O → 95% CH_3CN) Retention time; 16.7 min.

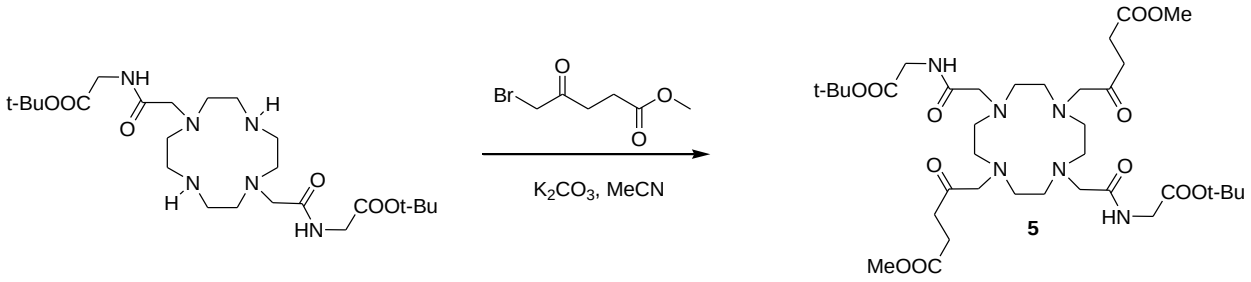
1,4,7,10-Tetraazacyclododecane-1, 7-bis[acetic acid-(glycine t-Bu ester) amide]



1,7-Bis (benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane[2] (4.13 g, 9.38 mmol) and K_2CO_3 (6.483 g, 46.9 mmol) was combined in CH_3CN (~200 mL). A CH_3CN solution of N-bromoacetylglycine t-Bu ester (5.913 g, 23.5 mmol) was added. The reaction mixture was stirred for 6 d at 60 ^oC, cooled to room temperature and filtered to remove the inorganic salts. The solvent was removed under reduced pressure to yield a hygroscopic yellow solid, which was purified by column chromatography (silica, CH_2Cl_2: MeOH, 95:5) to give the product in 65% yield. ^{13}C NMR (100 MHz, $CDCl_3$) δ =172.5, 171.3, 156.8, 136.5, 128.5-128.1, 82.5, 67.3, 58.0, 45-57 (br.), 41.4, 28.03. Removal of the benzyloxycarbonyl protecting group was accomplished

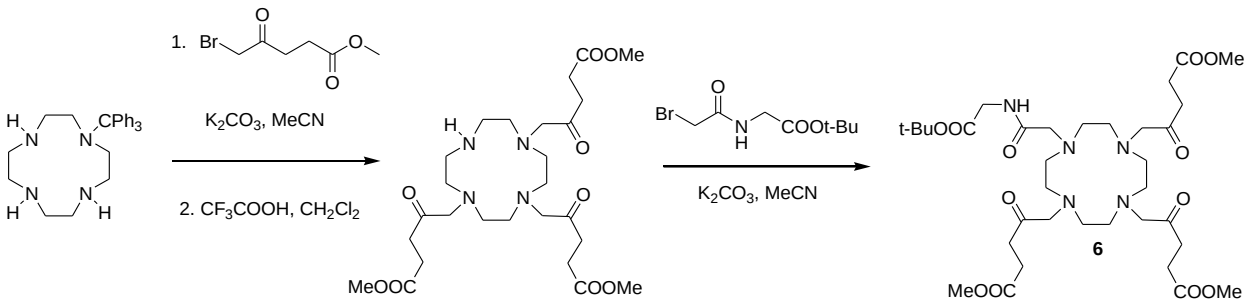
by catalytic hydrogenation in the presence of Pd/C catalyst (dry, 10% Pd on C) in ethanol under hydrogen pressure (40 psi) for 3 days in quantitative yield. ^{13}C NMR (100 MHz, $CDCl_3$) δ =171.56, 169.45, 81.96, 60.3, 53.04, 46.43, 41.61, 28.00.

1,4,7,10-Tetraazacyclododecane-1,7-bis[acetic acid-(glycine t-Bu ester) amide]-4,10-bis(methyl-oxopentanoate) (5).



A slight excess of methyl 5-bromo-levulinate **1a** (268.1 mg, 1.283 mmol) was added to an mixture of 1,4,7,10-tetraazacyclododecane-1,7-bis[acetic acid-(glycine t-Bu ester) amide] (300 mg, 0.583 mmol) and K_2CO_3 (242 mg, 1.75 mmol) in acetonitrile and stirred vigorously at 55 °C for 5 days. The resulting orange oil was purified by column chromatography (silica, CH_2Cl_2, MeOH) to give the desired product as an orange solid. Yield: 51.2 %. ^{13}C NMR (100 MHz, $CDCl_3$) δ =205.6, 173.2, 172.2, 169.0, 130.87, 81.6, 71.7, 62.5, 56.7, 51.8, 41.6, 34.6, 28.2, 27.9.; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.4, 4.0, 3.8 3.6, 3.28 (br. mult.) 2.6 (br. mult.) 1.4, 0.93; MALDI^{+}: *m/z* 771.526 (100%) $[M+H]^{+}$, 793.504 (40 %) $[M+Na]^{+}$; HPLC: (C18 reversed phase column, 95% H_2O → 95% CH_3CN) Retention time; 15.9 min.

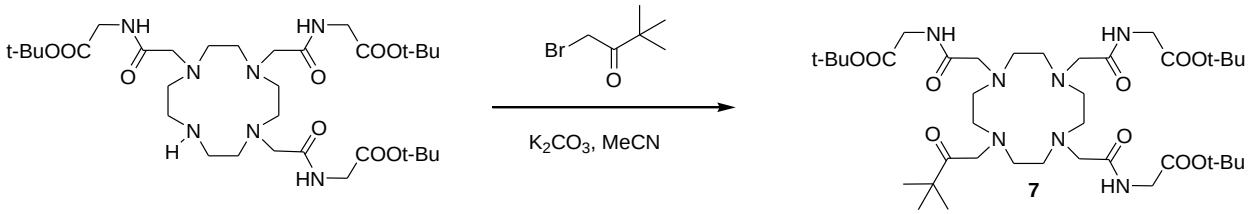
1,4,7,10-Tetraazacyclododecane-1-[acetic acid-(glycine t-Bu ester) amide]-4,7,10-tris(methyl-oxopentanoate) (6)



Monotrityl cyclen[3] (0.518 g, 1.25 mmol) was dissolved in CH_3CN and K_2CO_3 (1.03 g, 7.5 mmol) was added. The mixture was heated to 60 °C and **1a** was added, and the reaction mixture was stirred for 3 days. The solution was filtered and solvent removed to yield the trityl protected intermediate as a dark colored solid. The trityl protection was removed by dissolving the product in a 5% TFA solution in CH_2Cl_2 (20 mL). The solution was stirred for 15 min and then

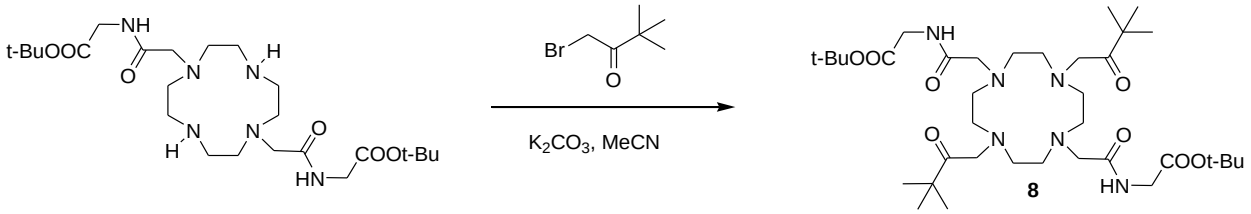
concentrated to about 5 mL by rotary evaporation. Ether was added to produce an oily red solid upon filtration. This process was repeated 2x with the remaining filtrate. The fractions were combined to afford 1,4,7,10-tetraazacyclododecane-1,4,7-tris(methyl-oxopentanoate) trifluoroacetic acid salt as a dark red solid (410 mg). This compound (189 mg) was alkylated with excess N-bromoacetylglycine tert-butyl ester (106 mg, 0.423 mmol) in the presence of K_2CO_3 (233 mg, 1.692 mmol) in CH_3CN at 60° C for 4 days. The mixture was then cooled, filtered and evaporated to yield a red oil which was purified by column chromatography (silica gel, CH_2Cl_2: MeOH) to give the final product as a red solid (51%). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 205.4, 160-170, 131.2, 81.2, 82.4, 68.25, 62.1, 53.4, 41.4, 41.1, 28.3, 27.9; ^{1}H NMR (100 MHz, $CDCl_3$) δ = 6.8-7.2 (br. mult.), 4.8, 4.6, 3.1-4.0 (Br. mult.), 2.3, 1.6; MALDI^{+}: *m/z* 727.95 (100%) $[M]^{+}$. HPLC: (C18 reversed phase column, 95% H_2O → 95% CH_3CN) Retention time; 16.3 min.

1,4,7,10-Tetraazacyclododecane-1,4,7-tris[acetic acid-(glycine t-Bu ester) amide]-10-pinacolone (7).



A slight excess of bromo-pinacolone (47 mg, 0.26 mmol) was added to an CH_3CN solution of 1,4,7,10-tetraazacyclododecane-1,4,7-tris[acetic acid-(glycine t-Bu ester) amide] (150 mg, 0.218 mmol) and K_2CO_3 (61 mg, 0.44 mmol). The reaction mixture was vigorously stirred at 55 °C for 5 days, allowed to cool and then filtered. Solvent was removed under reduced pressure to yield the product as a yellow solid. Yield: 67.5 %. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 217.4, 217.1, 171.8, 171.6, 169.2, 168.9, 81.8, 81.6, 58.9, 58.4, 57.8, 54.1, 53.6, 43.1, 41.8, 28.0, 26.5, 26.1; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.15, 7.91, 7.77, 3.81, 3.81-2.64 (br. mult.), 1.39, 1.04. MALDI^{+}: *m/z* 784.327 (100%) $[M+H]^{+}$, 806.282 (30%) $[M+Na]^{+}$, 822.231 (35%) $[M+K]^{+}$; HPLC: (C18 reversed phase column, 95% H_2O → 95% CH_3CN) Retention time; 33.054 min.

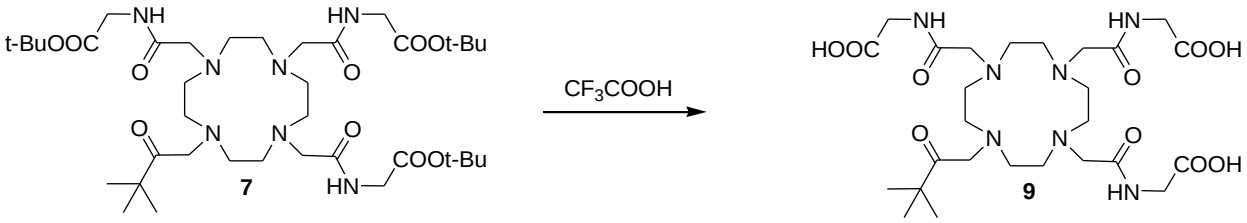
1,4,7,10-Tetraazacyclododecane-1,7-bis[acetic acid-(glycine t-Bu ester) amide]-4,10-bis(pinacolone) (8).



A slight excess of bromo-pinacolone (365.2 mg, 2.04 mmol) was added to an CH_3CN solution of 1,4,7,10-tetraazacyclododecane-1,7-bis[acetic acid-(glycine t-Bu ester) amide] (500 mg, 0.9715

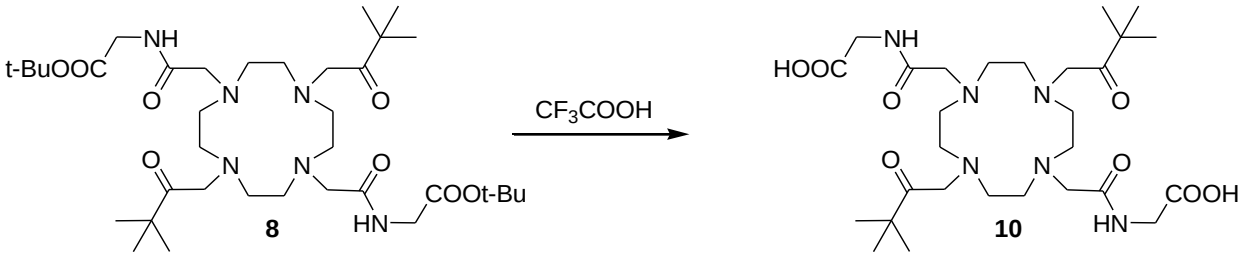
mmol) and K_2CO_3 (671 mg, 4.85 mmol) and stirred vigorously at 55 °C for 4 days. The product was obtained as an orange solid following the removal of solvent under reduced pressure. Yield: 74.8 %. ^{13}C NMR (100 MHz, $CDCl_3$) δ =214.34, 171.1, 168.3, 80.6, 58.5, 57.5, 42.9, 41.1, 37.5, 34.5, 27.9, 26.0; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.31, 4.1-2 (br. mult.), 1.21, 0.895; MALDI^{+}: *m/z* 712.497 (100%) $[M+H]^{+}$, 749.396 (80%) $[M+K]^{+}$. HPLC: (C18 reversed phase column, 95% H_2O → 95% CH_3CN) Retention time; 33.11 min.

1,4,7,10-Tetraazacyclododecane-1,4,7-tris[acetic acid glycine amide]-10-pinacolone (9).



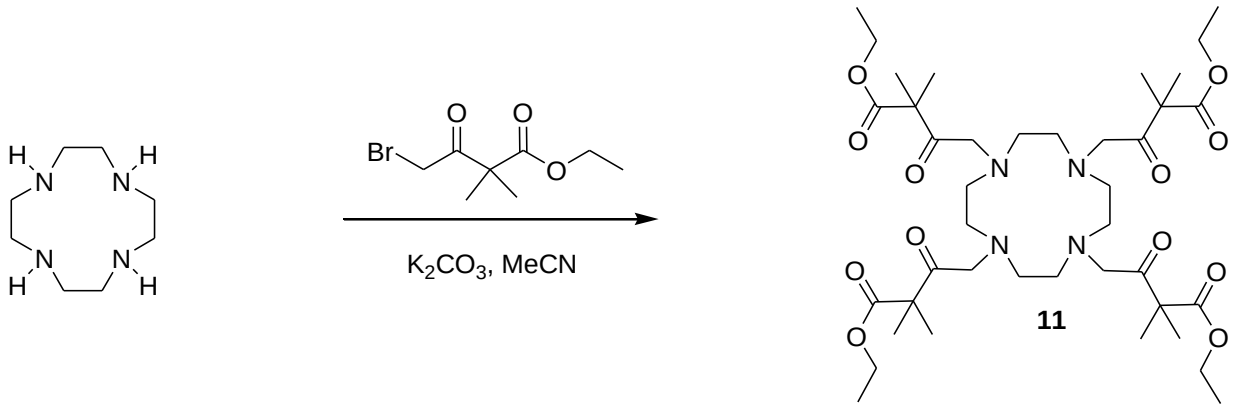
Ligand **7** was dissolved in neat trifluoroacetic acid and allowed to stir for 18 h. Excess TFA was removed under reduced pressure. The residue was dissolved in water and the solution was lyophilized to afford the product (TFA salt) as white solid. MALDI^{+}: *m/z* 616.641 (100%) $[M+H]^{+}$. HPLC: (C18 reversed phase column, 100% H_2O → 100% CH_3CN) Retention time; 14.74 min.

1,4,7,10-Tetraazacyclododecane-1,7-bis[acetic acid glycine amide]-4,10-bis(pinacolone) (10).



Ligand **10** was obtained as a tan solid in a procedure similar to that described for **9**. MALDI^{+}: *m/z* 599.702 (100%) $[M+H]^{+}$. HPLC: (C18 reversed phase column, 100% H_2O → 100% CH_3CN for 60 min) Retention time; 18.046 min.

1,4,7,10-Tetraazacyclododecane-1,4,710-tetrakis(ethyl-oxobutanoate) (11).



Cyclen (63.7 mg, 0.369 mmol) and K_2CO_3 (254.9 g, 1.84 mmol) were combined with ethyl 4-bromo-2,2-dimethyl-3-oxobutanoate (400 g, 1.59 mmol) in CH_3CN (250 mL) and stirred for 6 days at 55 °C. The reaction mixture was cooled, filtered, and evaporated by rotary evaporation to give an orange oil. A gradient silica column (MeOH, CH_2Cl_2) yielded the expected product as a yellow solid in 62.72% yield. ^{13}C NMR (100 MHz, $CDCl_3$) δ =169.3, 153.2, 118.7, 117.9, 62.3, 51.43, 48.7, 14.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.05 (q), 2-4 (br. mult.) 1.27, 1.16 (t) MALDI$^+$: *m/z* 854.08 (100%) $[M+H]^+$, 876.06 (80%) $[M+Na]^+$.

References

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3 Anelli, P. L.; Calabi, L.; Dapporto, P.; Murru, M.; Paleari, L.; Paoli, P.; Uggeri, F.; Verona, S.; Virtuani, M. *J. Chem. Soc., Perkin Transactions 1: Organic and Bio-Organic Chemistry* **1995**, 2995.

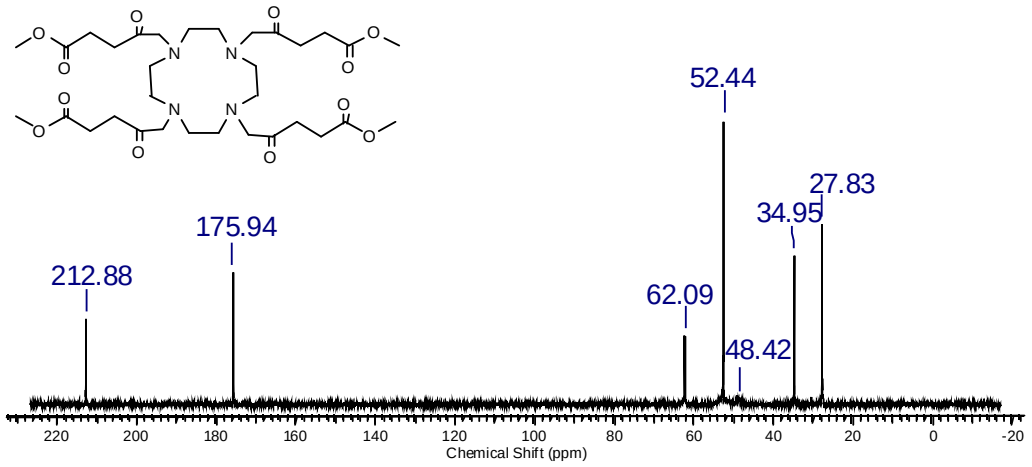


Figure S1. ^{13}C NMR spectrum of **1** in D_2O.

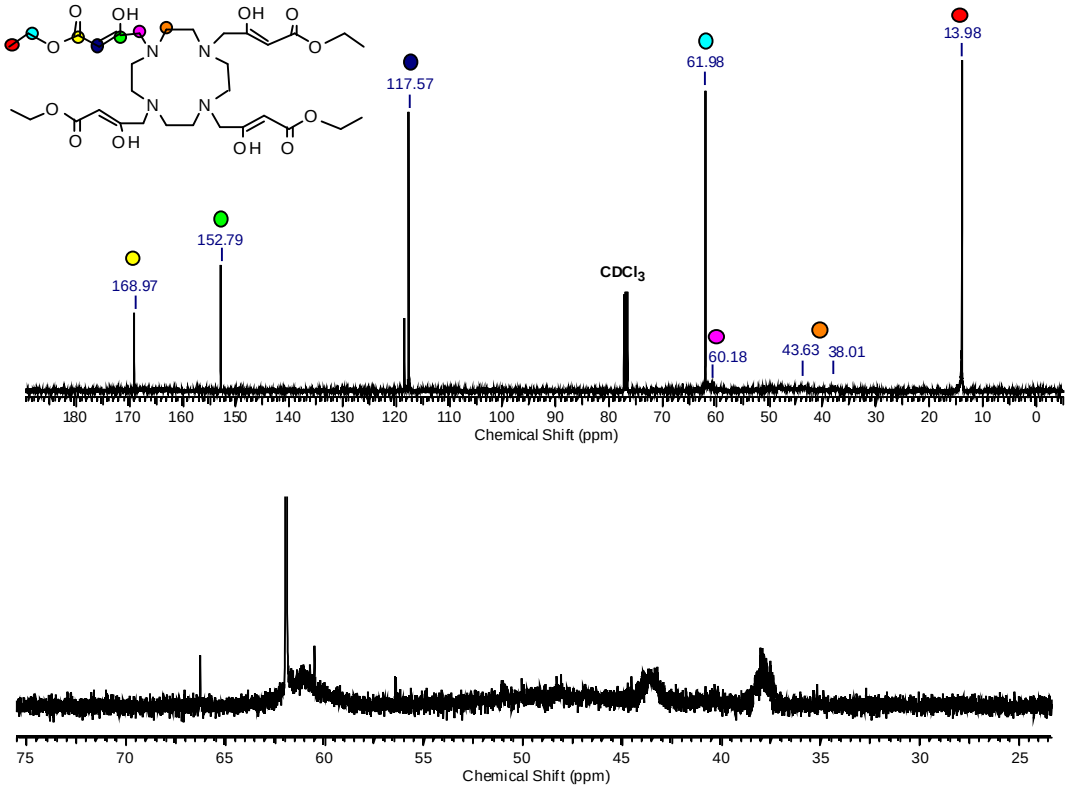


Figure S2. ^{13}C NMR spectrum of **2** in $CDCl_3$.

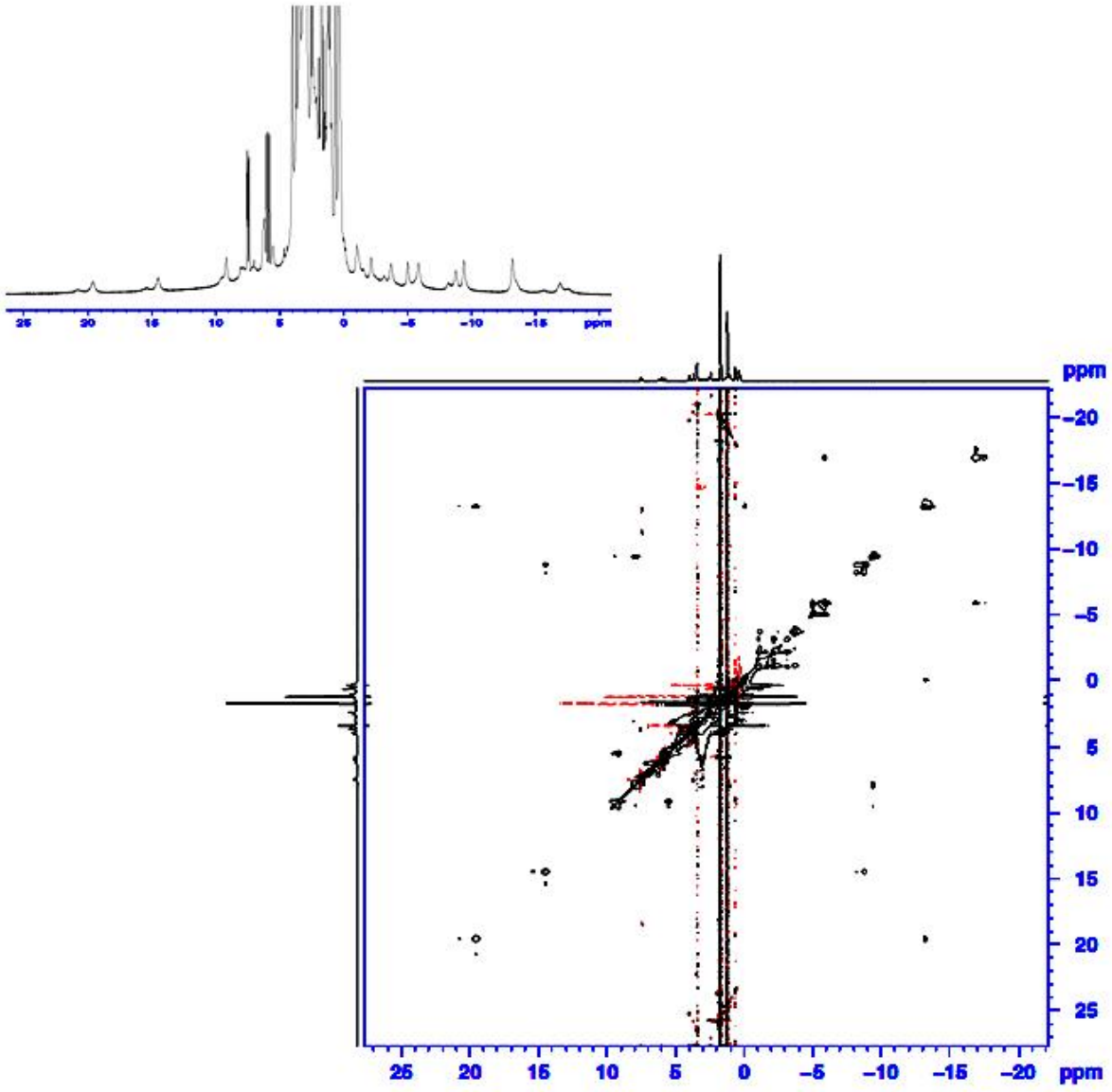


Figure S3. 2D-EXSY spectrum of Eu(**5**) showing eight cross peaks representative of SAP/TSAP isomers. (inset: 1-D ^{1}H NMR).

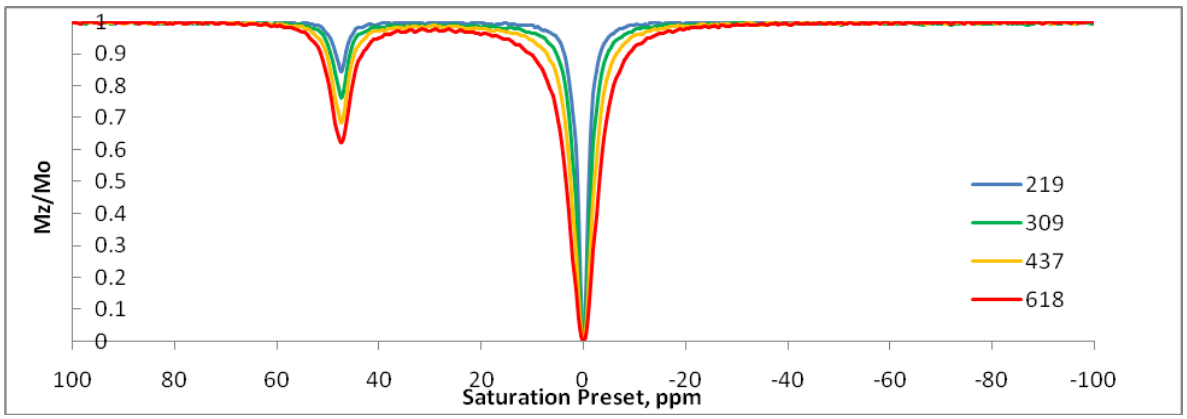


Figure S4. CEST spectrum of a 20 mM solution of Eu(**3**) in H_2O. A 36% CEST effect was observed at 47 ppm and 14.5 μT (618 Hz).

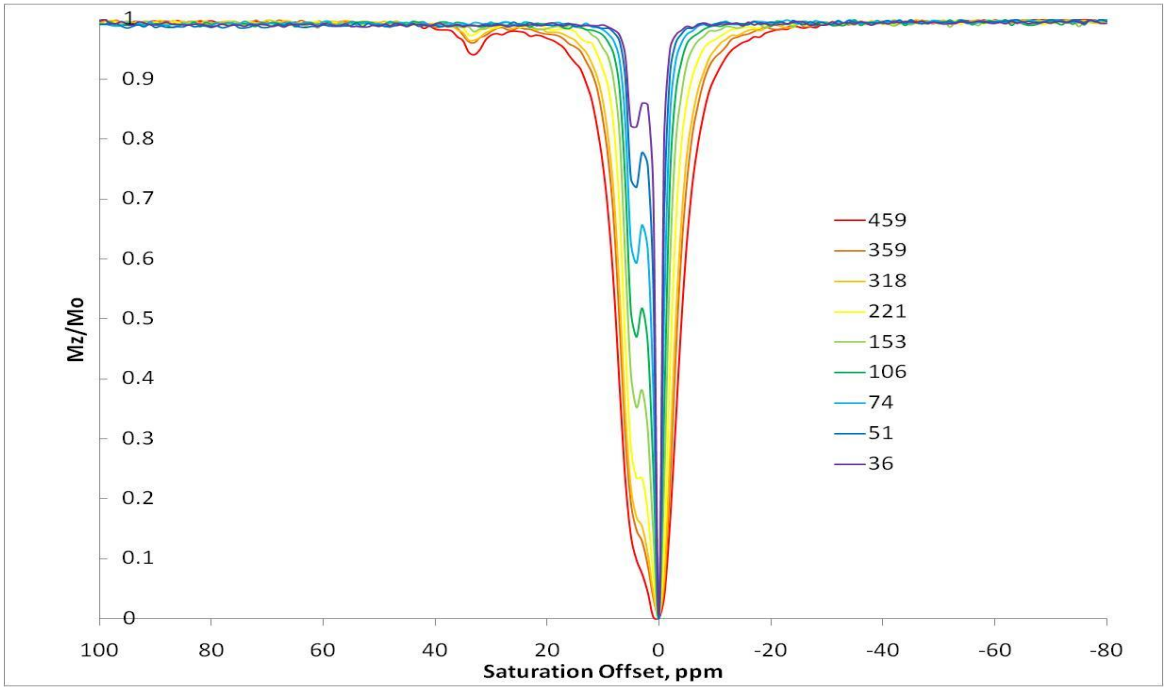


Figure S5. CEST spectrum of a 25 mM solution of Eu**(4)** in wet CH_3CN. A 5 % CEST effect was observed at 34 ppm and 10.7 μT (459 Hz).

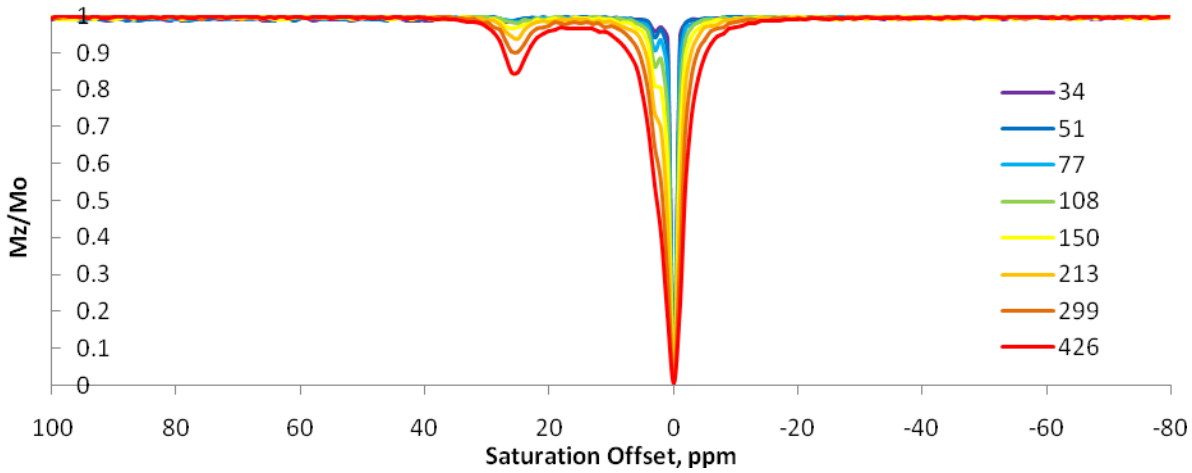


Figure S6. CEST spectrum of a 10 mM solution of Eu(**5**) in H_2O. A 15% CEST effect was observed at 26 ppm and10.0 μT (426 Hz).

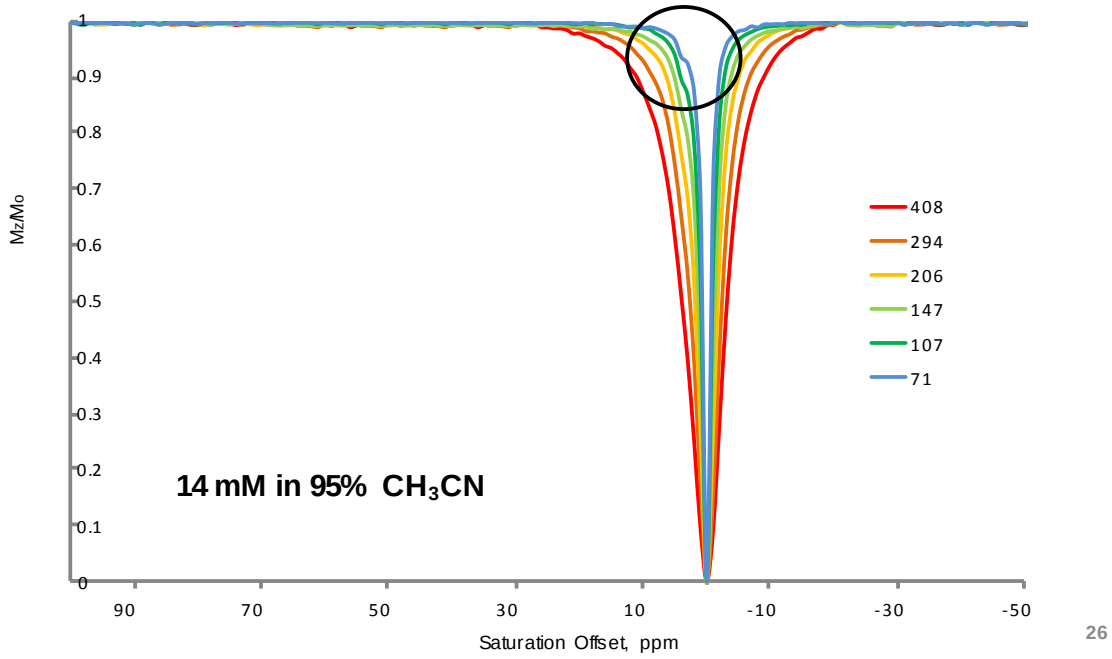


Figure S7. CEST spectrum of **Eu(6)** in CH_3CN.

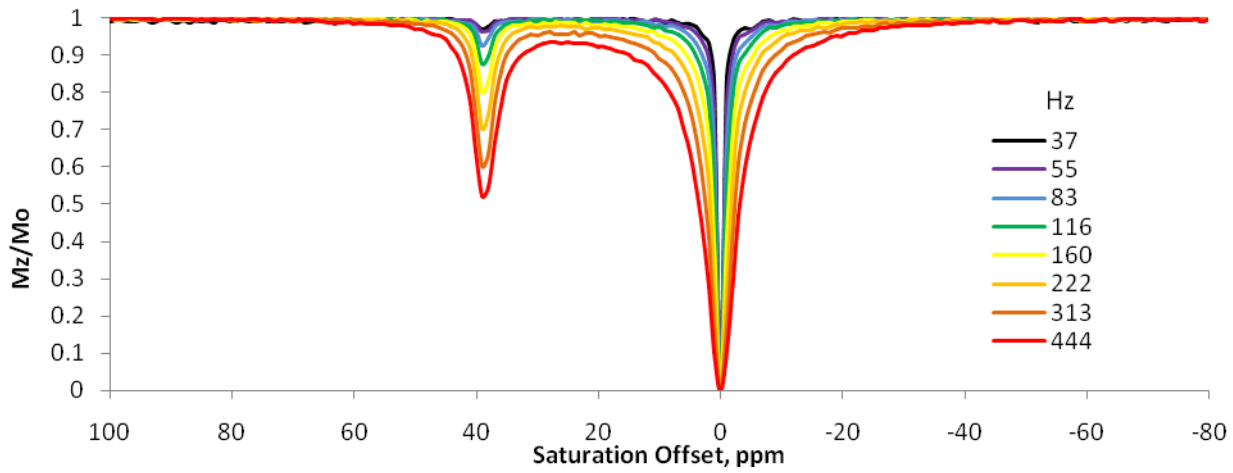


Figure S8. CEST spectrum of a 17 mM solution of Eu(**7**) in H_2O:CH_3CN. A 48% CEST effect was observed at 38 ppm and 10.4 μT (444 Hz).

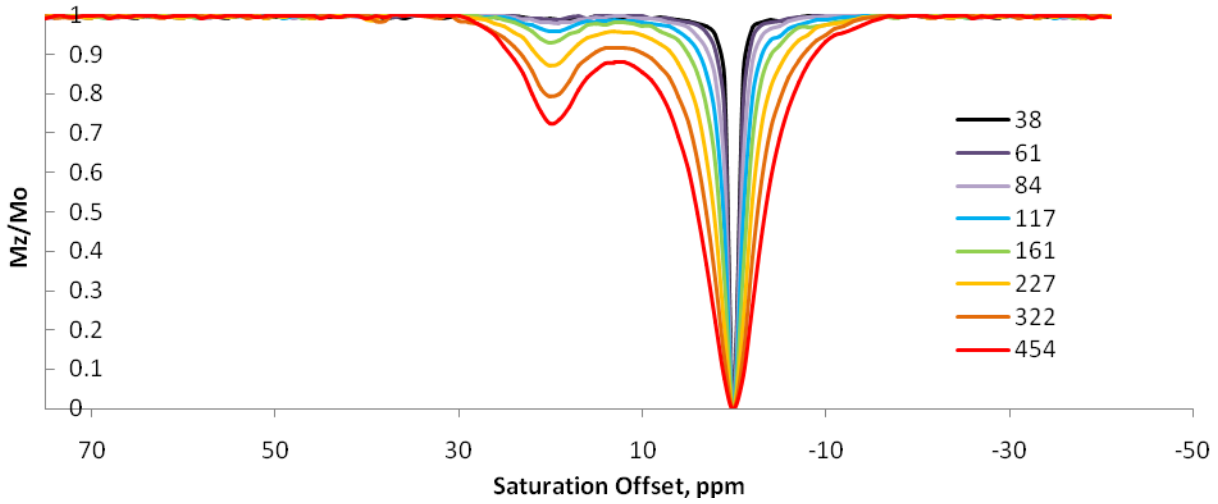


Figure S9. CEST spectrum of a 30 mM solution of Eu(**8**) in H_2O:CH_3CN. A 28% CEST effect was observed at 27 ppm and 10.4 μT (444 Hz).

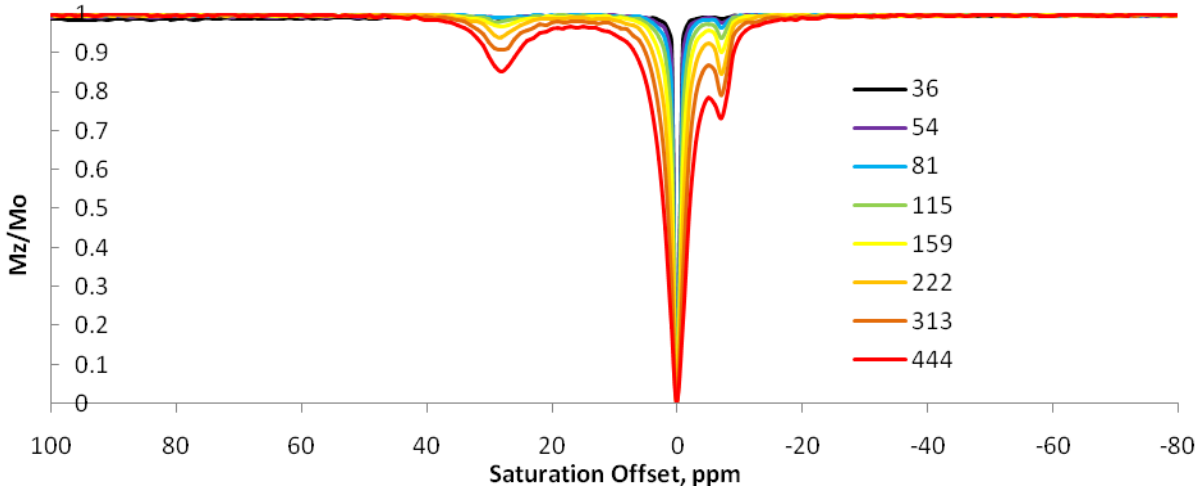


Figure S10. CEST spectrum of a 18 mM solution of Eu(**9**) in H_2O at pH 7. A 15% CEST effect was observed at 28 ppm and 10.4 μT (444 Hz).

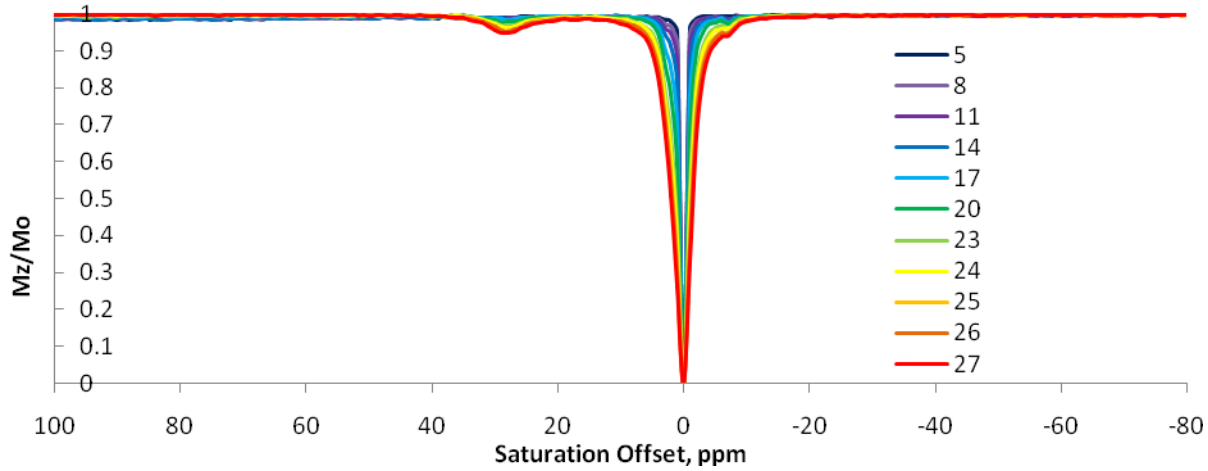


Figure S11. CEST spectrum of a 3.6 mM solution of Eu(**10**) in H_2O at pH 7. A 10% CEST effect was observed at 27 ppm and 10.4 μT (444 Hz).

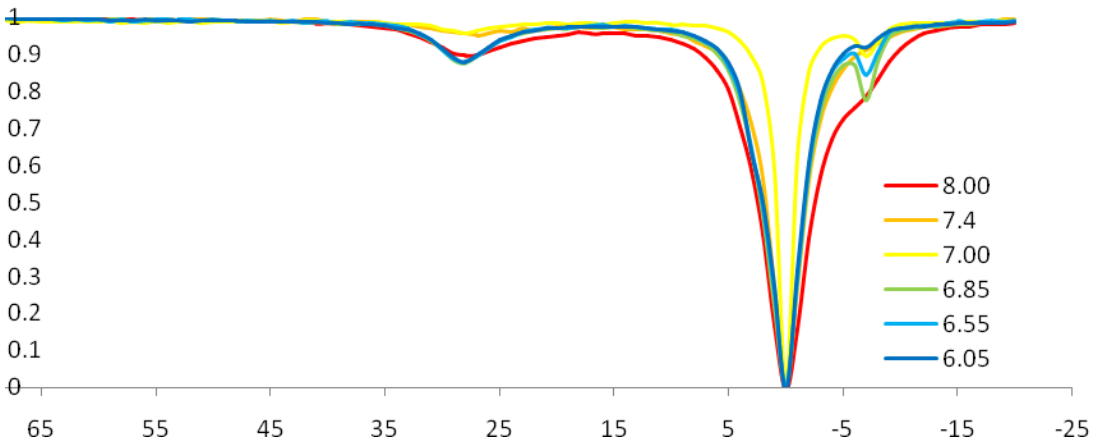
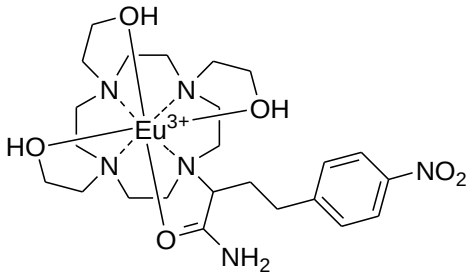


Figure S12. CEST spectrum of a of Eu(**10**) in H_2O at various pH values.



EuCNPHC^{3+}

Chart S1