Supplementary Material for:

A PARACEST agent responsive to inner- and outersphere phosphate ester interactions for MRI applications

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Materials and methods.

[Eu(**S-THP**)](CF_3SO_3)$_3$ was prepared as reported previously in reference 18. MES buffer was used at pH 5.5 to 6.5 and HEPES buffer was used at pH 6.5 to 7.5. CEST experiments were acquired on an Inova-500 Spectrometer at room temperature, B_1 = 40-44 db with an irradiation time of 3 seconds. Eu(III) excitation spectra and excited state lifetimes were obtained using a Spectra-Physics Quanta Ray PRO-270–10 Q-switched Nd:YAG pump laser (10 Hz, 60–70 mJ pulse^{-1}) and a MOPO SL for all luminescence measurements. Time resolved luminescence measurements were collected by using a digital Tektronix TDS 3034B oscilloscope. Data were fit to single exponential decays by using GraphPad Prism4. CEST spectra were fit to modified Bloch equations with three exchangeable sites to obtain rate constants for exchange by using Matlab.[S1]

References

S1. Woessner, D. E.; Zhnag, S.; Merritt, M. E.; Sherry, A. D. *Magn. Reson. Med.* **2005**, *53*, 790.

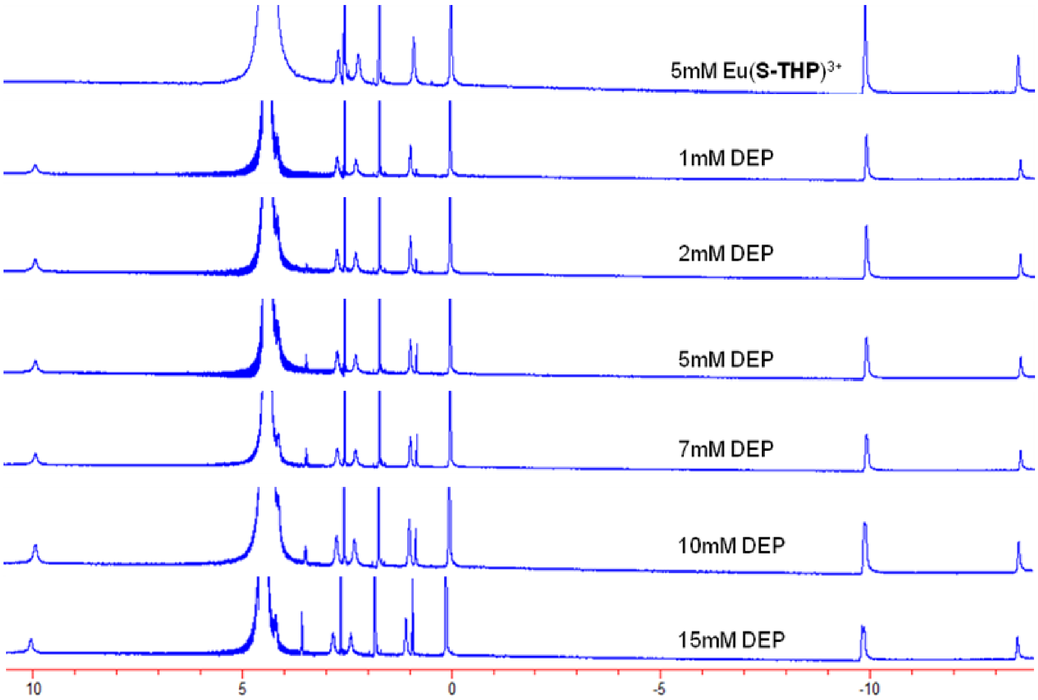

Figure S1. ^{1}H NMR spectra of 5.00 mM Eu(**S-THP**)$^{3+}$ titrated with diethyl phosphate at pH 5.6. The addition of **DEP** slows the exchange rate of the alcohol protons with water, resulting in a sharpening of the alcohol proton resonance. The resonance at 10.0 ppm is assigned as an alcohol proton based on NMR spectra of the complex in D_2O and from previous work in acetonitrile/water solutions.[17] The integration of this peak is consistent with a contribution from the alcohol protons only. The bound water peak is observed at 3 ppm in wet acetonitrile.[17]

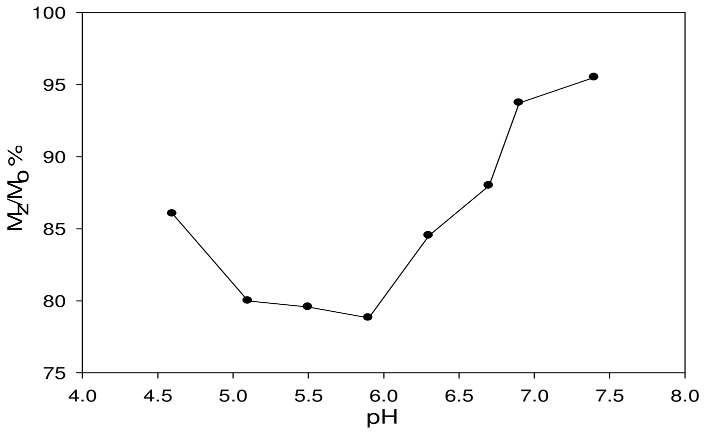

Figure S2. pH dependence of CEST effect of 5.00 mM Eu(**S-THP**)$^{3+}$, 20.0 mM buffer and 100 mM NaCl with an offset frequency of 6 ppm and *B1* = 40 db. (solid line is for guidance only.)

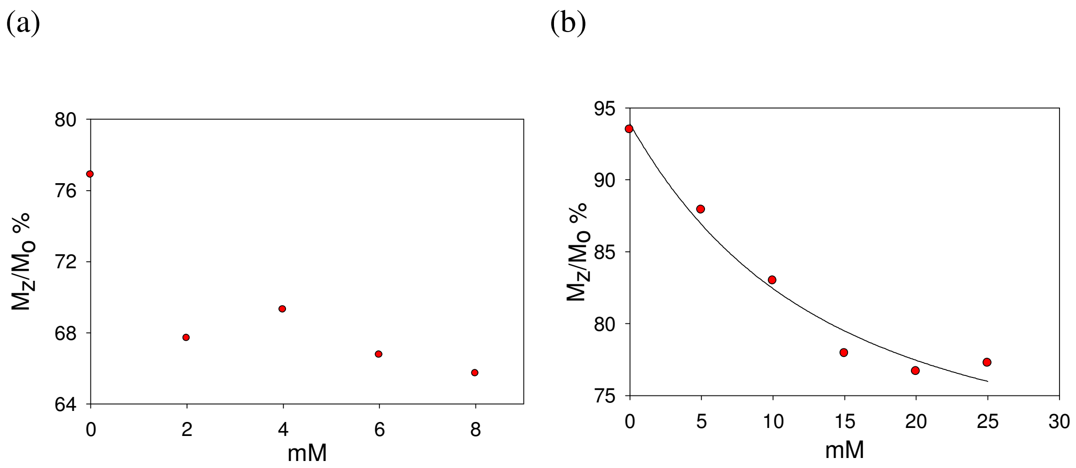

Figure S3. (a) CEST response of 5.00 mM Eu(**S-THP**)$^{3+}$, 20.0 mM buffer and 100 mM NaCl at an offset of 6 ppm as a function of **DEP** with *B1* = 44 db. (b) CEST response of 5.00 mM Eu(**S-THP**)$^{3+}$, 20.0 mM buffer and 100 mM NaCl at an offset of 9 ppm as a function of **MP** with *B1* = 44 db. The data in (b) is fit to a 1:1 binding isotherm (eq. S1) with K_d = 10.2 mM, r^2= 0.97.

$$F = [\mathbf{MP}]_{tot} + [Eu(\mathbf{S} - \mathbf{THP})^{3+}]_{tot} + K_d \qquad \text{eq. S1-a}$$

$$[Eu(\boldsymbol{S} - \boldsymbol{THP})(\boldsymbol{MP})^{+}] = \frac{F-\sqrt{F^2-4[Eu(S-THP)^{3+}]_{tot}[MP]_{tot}}}{2} \qquad \text{eq. S1-b}$$

$$X_{Eu(S-THP)(MP)^+} = [Eu(\boldsymbol{S} - \boldsymbol{THP})(\boldsymbol{MP})^{+}]/[Eu(\boldsymbol{S} - \boldsymbol{THP})^{3+}]_{tot} \qquad \text{eq. S1-c}$$

$$X_{Eu(\mathrm{S-THP})^{3+}} = 1 - X_{Eu(S-THP)(MP)^+} \qquad \text{eq. S1-d}$$

$$y = X_{Eu(\mathrm{S-THP})^{3+}} k_{Eu(\mathrm{S-THP})^{3+}} + X_{Eu(S-THP)(MP)^+} k_{Eu(S-THP)(MP)^+} \qquad \text{eq. S1-e}$$

$y = \frac{M_z}{M_o}\%$, saturation transfer effect from CEST spectra

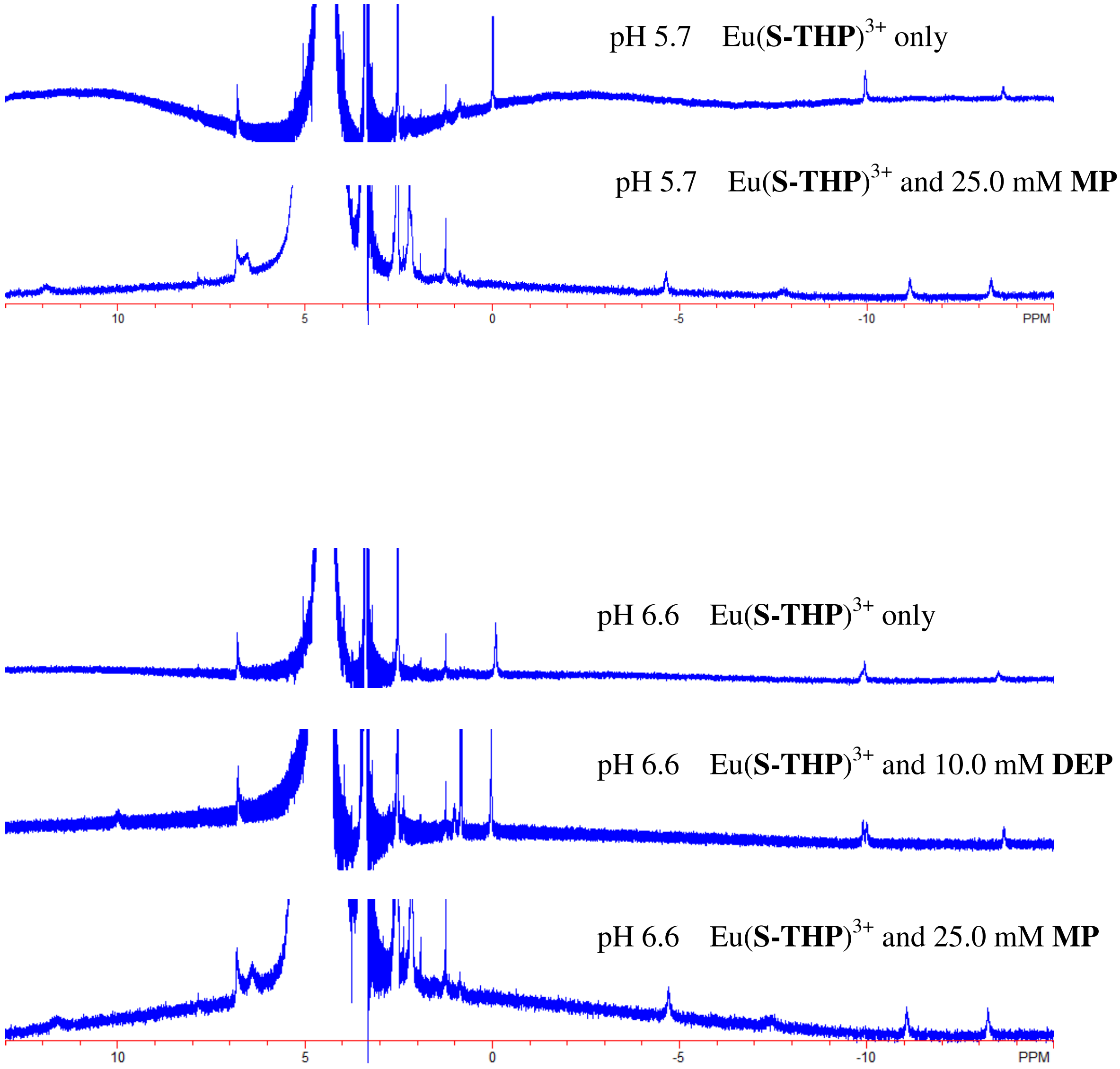

Figure S4. ^{1}H NMR spectra of Eu(**S-THP**)$^{3+}$ in H_2O at pH shown with complex only, or upon addition of **DEP** or **MP.** Resonances at approximately 10 ppm and 12 ppm are observed, respectively, for exchangeable hydroxyl protons of the **DEP** or **MP** complexes with Eu(**S-THP**)$^{3+}$.

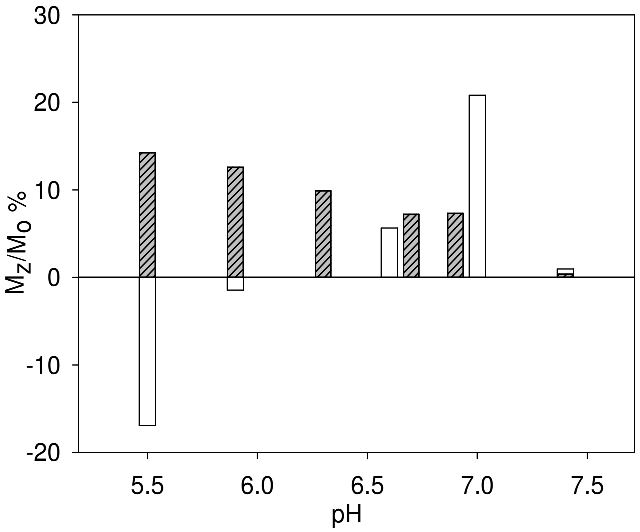

Figure S5. The pH dependence of the Eu(**S-THP**)$^{3+}$ CEST effect in the presence of phosphate esters. Data shown is the difference between the CEST effect of Eu(**S-THP**)$^{3+}$ alone and that of a solution containing Eu(**S-THP**)$^{3+}$ and one equivalent of **DEP** (□) or three equivalents of **MP** (▨). The CEST effect was monitored at 6.0 ppm for **DEP** addition and at 8.0 ppm for **MP** addition. All solutions contained 100 mM NaCl and 20.0 mM buffer.

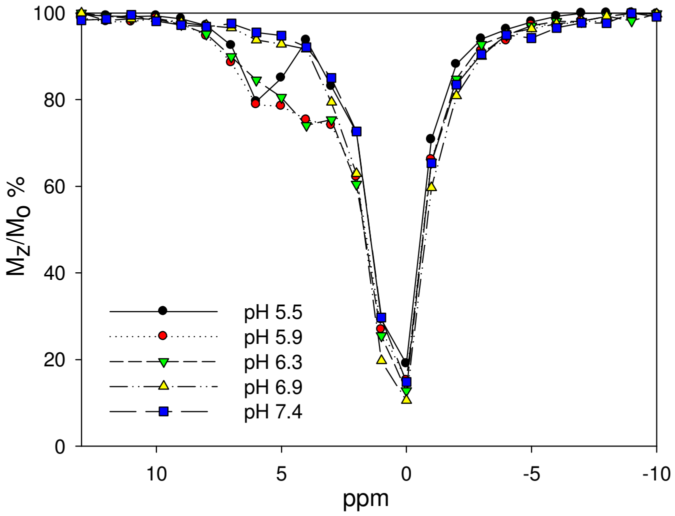

Figure S6. The pH dependence of CEST for Eu(**S-THP**)$^{3+}$. Solutions contained 5.00 mM Eu(**S-THP**)$^{3+}$, 20.0 mM buffer and 100 mM NaCl. Fitting of the spectrum at pH 6.6 with modified Bloch equations according to reference S1 gives a rate constant for proton exchange of ~8,000 s^{-1}.

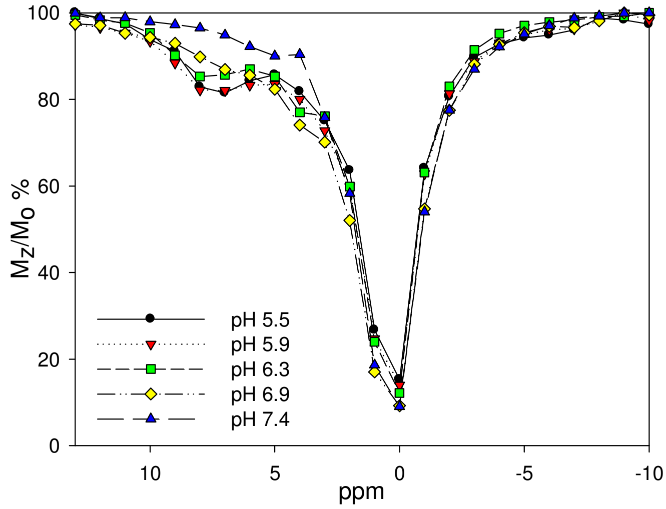

Figure S7. The pH dependence of CEST for the Eu(**S-THP**)$^{3+}$ complex with **MP**. Solutions contained 5.00 mM Eu(**S-THP**)$^{3+}$, 15.0 mM **MP**, 20.0 mM buffer and 100 mM NaCl.

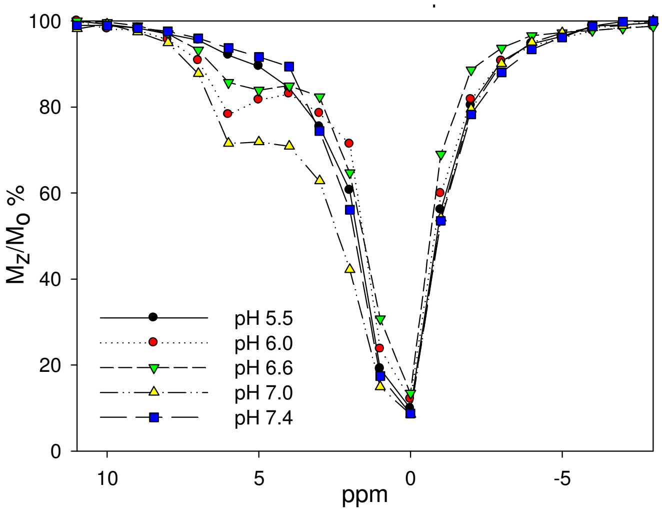

Figure S8. The pH dependence of CEST for the Eu(**S-THP**)$^{3+}$ complex with **DEP**. Solutions contained 5.00 mM Eu(**S-THP**)$^{3+}$, 5.00 mM **DEP**, 20.0 mM buffer and 100 mM NaCl.

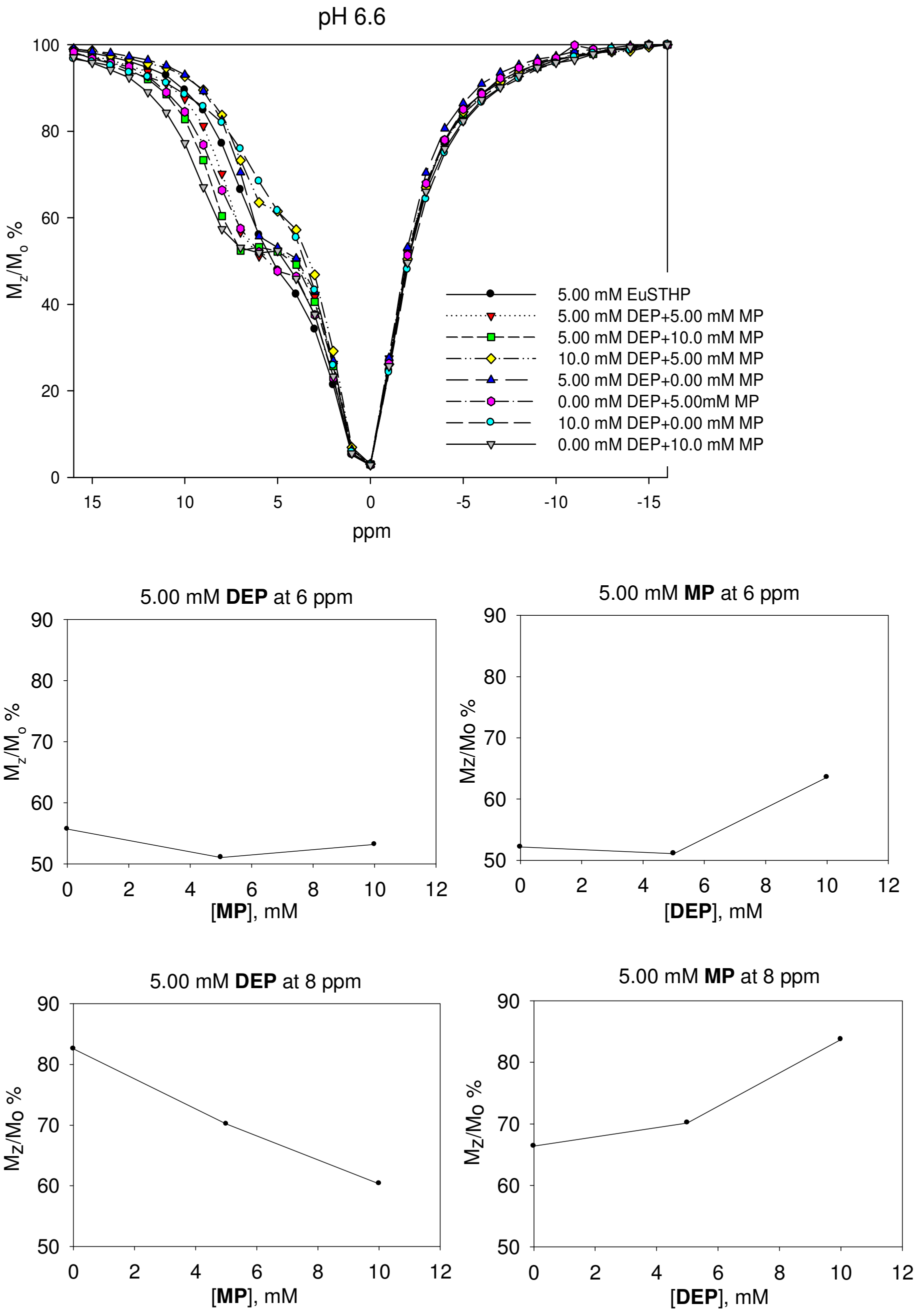

Figure S9. Shown above is a CEST experiment with solutions containing 5.00 mM Eu(**S-THP**)$^{3+}$, 20.0 mM buffer and 100 mM NaCl and variable concentrations of **MP** and **DEP**. In this experiment, the saturating pulse power was increased to better observe the CEST effect. The data is plotted at left to show the CEST effect of Eu(**S-THP**)$^{3+}$, at 8.0 and 6.0 ppm with the **DEP** concentration fixed at 5.00 mM and increasing **MP** concentration. At right is a plot of the CEST effect at 8.0 and 6.0ppm with **MP** concentration fixed at 5.00 mM and increasing **DEP**. This data shows that the CEST effect response of Eu(**S-THP**)$^{3+}$ is dependent on the concentration of **MP** and **DEP** together (compare to Figure 1 and Figure S3) to produce CEST spectra that are characteristic of each combination of phosphate ester.

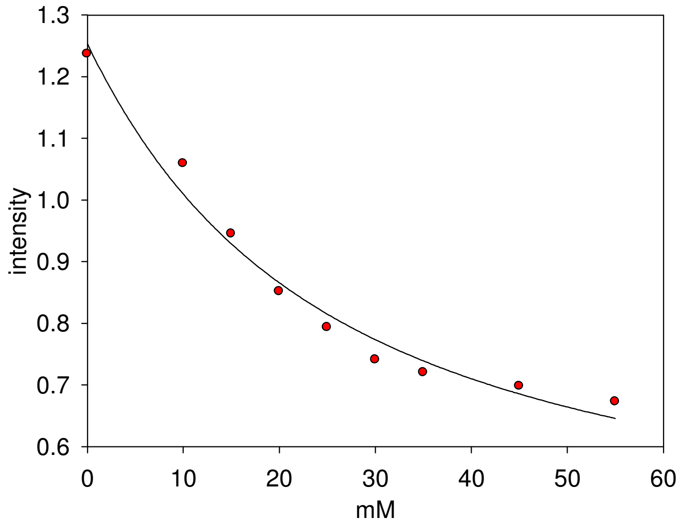

Figure S10. Binding curve for 5.00 mM Eu(**S-THP**)$^{3+}$, 20.0 mM buffer and 100 mM NaCl with **MP** at pH 6.6 by using excitation peak intensity at 579.32 nm. Data was fit to a 1:1 binding model (eq. S1) to give a K_d of 21.9 mM for **MP** (r^2 = 0.98) with $y = intensity\ (au)$ of excitation peak.

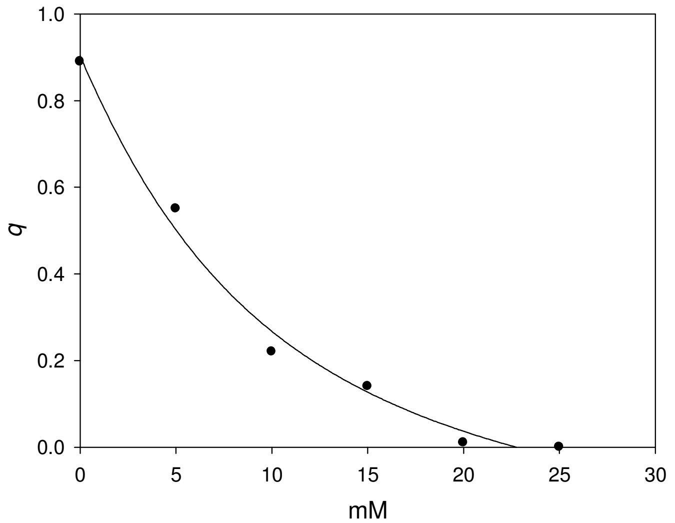

Figure S11. Binding curves for 5.00 mM Eu(**S-THP**)$^{3+}$, 20.0 mM buffer and 100 mM NaCl with **MP** at pH 6.6 by using numbers of bound water at 579.32 nm. Data was fit to a 1:1 binding model (eq. S1) to give a K_d of 7.0 mM (r^2 = 0.99) with $y = q$, the number of metal complex bound waters.

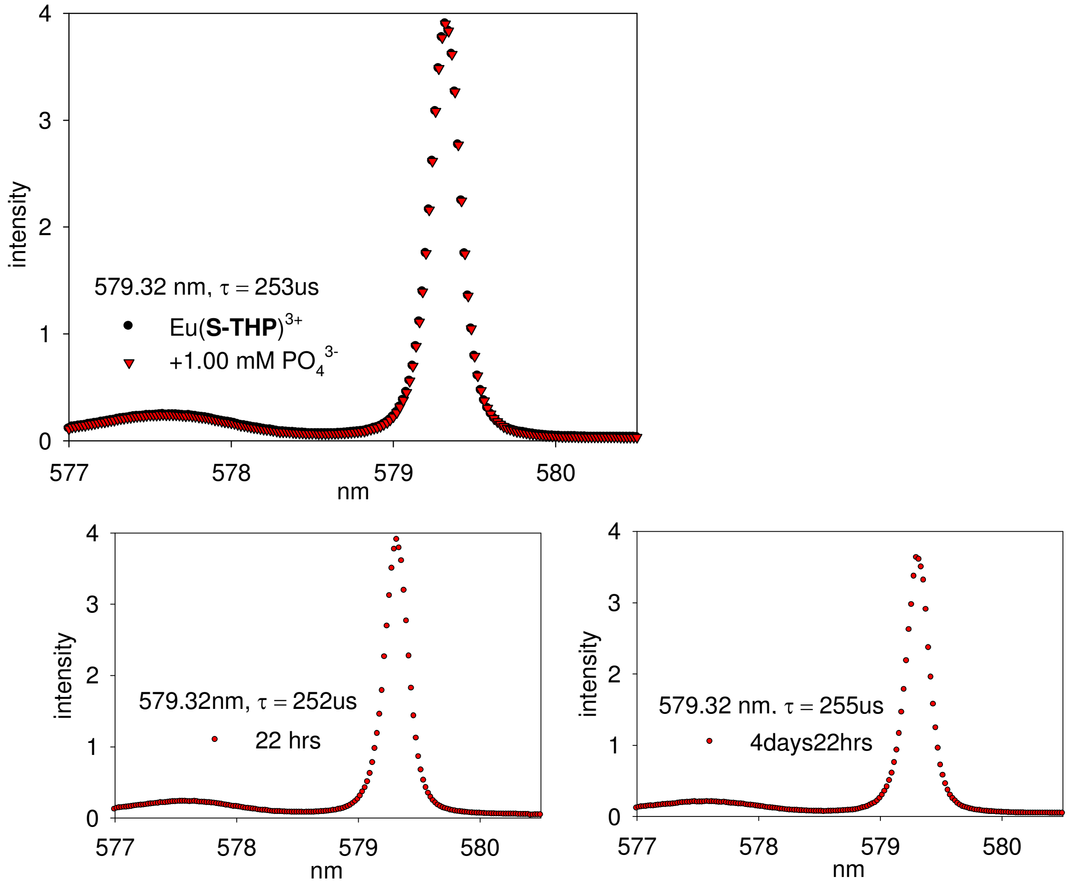

Figure S12. The $^5D_o \rightarrow {}^7F_o$ excitation peak of Eu(**S-THP**)$^{3+}$ at 1.00 mM complex, 1.00 mM PO_4^{3-}, 20.0 mM buffer, 100 mM NaCl at pH 7.4. The red-shifted peak at 577.80 nm is the deprotonated complex as discussed in reference 20. This peak is observed at pH values greater than 7. The excitation spectrum and lifetime of the major species remains the same over 5 days.

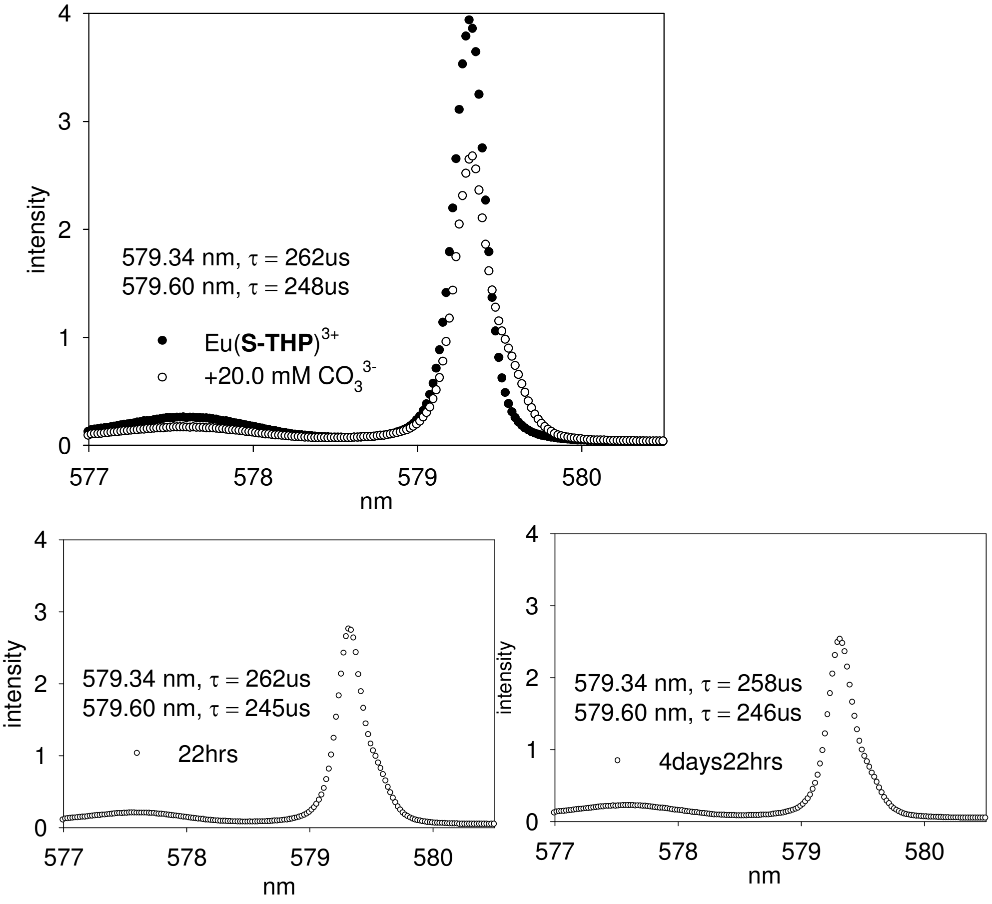

Figure S13. The $^5D_o\rightarrow{}^7F_o$ excitation peak of Eu(**S-THP**)$^{3+}$ at 1.00 mM complex, 20.0 mM CO_3^{2-}, 20.0 mM buffer, 100 mM NaCl at pH 7.4. The excitation spectrum and lifetime of the major species remains the same over 5 days. The red-shifted peak at 577.80 nm is the deprotonated complex as discussed in reference 20. This peak is observed at pH values greater than 7. The new excitation peak at 579.60 nm is characteristic of a carbonate complex.

Table S1. Luminescence lifetime and number of bound waters (*q*) for 5.00 mM Eu(**S-THP**)$^{3+}$, 20.0 mM MES and 100 mM NaCl with addition of **DEP** or **MP**.

Phosphate ester	τ_{H2O}(ms)	k_{H2O}(ms^{-1})	τ_{D2O}(ms)	k_{D2O}(ms^{-1})	*q*
0 eq.	0.239	4.18	0.720	1.39	0.89
1 eq. DEP	0.239	4.18	0.685	1.46	0.80
2 eq. DEP	0.239	4.18	0.737	1.36	0.92
3 eq. DEP	0.239	4.18	0.720	1.39	0.89
5 eq. DEP	0.238	4.20	0.713	1.40	0.90
10 eq. DEP	0.241	4.15	0.690	1.45	0.78
1 eq. MP	0.275	3.64	0.885	1.13	0.55
2 eq. MP	0.305	3.28	0.952	1.05	0.22
3 eq. MP	0.325	3.10	1.075	0.92	0.14
5 eq. MP	0.340	2.94	1.136	0.88	0.01
10 eq. MP	0.354	2.82	1.190	0.84	0.00

$$q = A(k_{H2O}-k_{D2O}-k_{X\text{-}H}) \quad \text{S2-1}$$

$$k_{X\text{-}H}=0.25+0.45*a \quad \text{S2-2}$$

To estimate the number of europium(III) bound waters (*q*), luminescence decays were recorded in H_2O and D_2O, and *q* was calculated by using equation S2-1 and S2-2 with contributions from OH groups as well as second-sphere waters, 0.25 ms^{-1} and the assumption that each hydroxyethyl group (a) will contribute one O-H oscillator at 0.45 ms^{-1}. In the equations, A =1.20 ms^{-1} as the constant for Eu(III), a is the number of alcohol OH bonds, and k=1/τ.