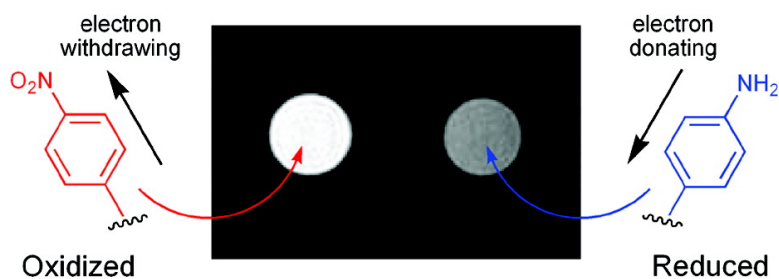Communication

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Modulation of Water Exchange in Europium(III) DOTA−Tetraamide Complexes via Electronic Substituent Effects

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Magnetic resonance image (MRI) contrast originates from differences in tissue proton densities in water and fat and from inherent differences in water relaxation rates. Image contrast can be altered further by administration of a paramagnetic contrast agent, typically a chelated form of Gd^{3+}, that shortens the longitudinal relaxation time (T_1) of bulk water protons.[1] An alternative type of MRI contrast agent has recently been proposed[2] that alters image contrast by transfer of selectively saturated spins from one chemical pool to another, a process called chemical exchange saturation transfer (CEST).[2,3] In order to observe CEST, the rate of exchange between the two pools (k_{ex}) must be slow relative to the frequency difference between the two pools ($\Delta\omega$). Thus, larger shift differences between the two pools permit larger values of k_{ex}. Large $\Delta\omega$ values have the added benefit of eliminating the problem of direct off-resonance saturation of the solvent water. These distinct advantages naturally led to the use of paramagnetic lanthanide ions to induce large hyperfine shifts in the proximate exchangeable protons of ligands. The metal-bound water molecule of Ln^{3+} DOTA−tetraamide complexes has been shown to exhibit unusually slow water exchange rate and large hyperfine shifts, such that these complexes meet the $\Delta\omega \geq k_{ex}$ condition. These paramagnetic CEST agents, often referred to as PARACEST agents, have been widely studied as responsive agents that can report changes in tissue pH,[4] temperature,[5] and glucose,[6] lactate,[7] and Zn^{2+} ion[8] concentrations.

Since the magnitude of CEST is critically dependent upon the water exchange rate in such complexes, modulation of the water exchange rate provides a platform upon which responsive PARACEST agents may be based. It has been established that the water exchange process occurs through a dissociative pathway,[9] and the rate of exchange is related to the free energy difference, ΔG^*, between the transition and the ground states. The parameters that affect water exchange rates in lanthanide complexes have been the subject of intense study over the past decade and have been found to depend upon the coordination geometry,[10] steric crowding,[11] size of the central Ln^{3+} ion,[12] and the electronic properties of the ligand.[13] The idea that water exchange rates could be modulated by changes in the electronic properties of the ligand is an attractive prospect since this could provide the basis of a generalized approach to the design of responsive PARACEST agents. To investigate this hypothesis, a series of complexes were prepared that incorporated either electron-donating (Me, OMe) or electron-withdrawing groups ($CO_2{}^tBu$, CN, F) in the *para*-position (Chart 1).

Cyclen was selectively trialkylated with 3 equiv of ethyl-*N*-bromoacetylglycinate, using $NaHCO_3$ as base.[14] The remaining nitrogen was subsequently alkylated with various *p*-substituted bromoacetyl aniline derivatives ($CH_3CN/K_2CO_3/60$ °C). The Eu^{3+}

Chart 1. The EuDOTA−Tetraamide Complexes Prepared for This Work

X = $CO_2{}^tBu$
CN
F
H
OMe
Me
NO_2 / NH_2
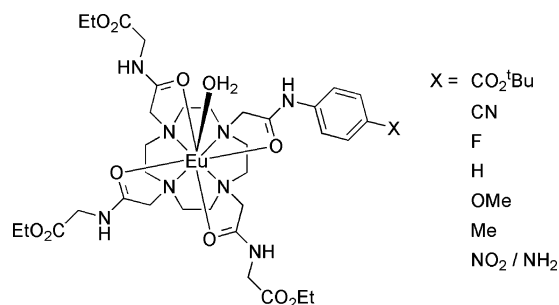
complexes of each ligand were obtained by reaction with $Eu(OTf)_3$ in acetonitrile. The complexes of DOTA−tetraamide ligand systems can adopt two coordination geometries that exhibit different water exchange kinetics.[10b,c] Highly shifted resonances, characteristic of axial protons of the macrocyclic ring from the square antiprismatic isomer,[10b] were observed between +22 and +28 ppm in each ^{1}H NMR spectrum, suggesting that all of these complexes exist as a single isomer in solution.

It was anticipated that electron-donating substituents would push electron density through the coordinating amide and onto the central metal ion, weakening the metal−water interaction and accelerating water exchange. Conversely, electron-withdrawing groups were expected to decelerate water exchange. To examine this hypothesis and establish whether these effects would translate in observable changes, CEST spectra were recorded at 9.4 T. A CEST exchange peak corresponding to the coordinated water molecule was observed in each spectrum near 47 ppm (Figure 1). The magnitude of the CEST peak differed in each complex, demonstrating that CEST can be altered by small differences in electronic properties. To assess the origins of these differences, the CEST spectra were fitted to the Bloch equations (modified to obtain water exchange rates)[15] by use of a nonlinear fitting algorithm written in MATLAB 7 (Mathworks Inc., Natick, MA). The results are summarized in Table 1.

The water exchange data are more easily understood for those substituents operating through mesomeric effects. As expected, the water residence lifetime (τ_M) was found to be longer in complexes bearing an electron-withdrawing group ($CO_2{}^tBu$, CN) and shorter in those with an electron-donating group (OMe). This trend is reversed for groups considered inductive; electron-donating groups (Me) lengthen τ_M, while electron-withdrawing groups (F) shorten τ_M. This suggests that the relationship between water exchange and the electron properties of the amide substituents is more complex than suggested by our initial hypothesis. For example, altering the strength of the metal−water interactions may result in a change in the acidity of the bound water protons such that a stronger metal−

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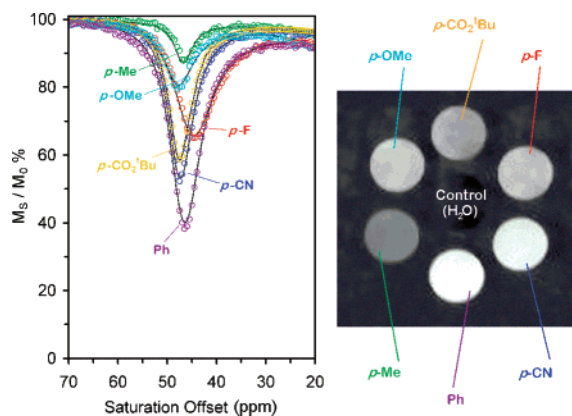‡ Macrocyclics.
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Figure 1. CEST arising from the coordinated water molecules of the Eu^{3+} complexes recorded at 7 T, 296 K, $B_1 = 450$ Hz, concn = 20 mM (50% H_2O in CD_3CN) and irradiation time = 2 s (left). Spin echo CEST difference images of the complexes acquired at 9.4 T and 298 K (right), TR = 10 s, TE = 8.2 ms, irradiation time = 3 s, $B_1 = 24$ μT at −47.5 and +47.5 ppm.

Table 1. Fitting Parameters from the CEST Spectra

X =	effect	τ_M (μs)	$\Delta\omega$ (ppm)	$1- (M_S/M_0)$
		Mesomeric		
$CO_2{}^tBu$	−M	352 ± 17	47.69	0.415
CN	−M	324 ± 33	47.61	0.477
H	0	269 ± 32	46.72	0.611
OMe	+M	198 ± 19	47.25	0.194
		Inductive		
F	−I	144 ± 8	44.98	0.347
H	0	269 ± 32	46.72	0.611
Me	+I	310 ± 55	46.89	0.113

water interaction may increase the acidity of the water protons and thereby increase the rate of proton exchange while a weaker interaction may promote the opposite effect. Proton and whole water exchange are indistinguishable in CEST experiments, and the contribution of proton exchange in this type of complex can be significant.[10b] This may explain the unusual results obtained for the complexes with inductive groups. The magnitude of the CEST effects obtained for the OMe and Me derivatives is smaller than expected based upon their τ_M values, presumably the result of the short T_1 values of these samples.

To demonstrate that the changes in CEST induced by the change in the electronic properties of the aromatic substituent could be detected by MRI, images of a phantom system were acquired simultaneously at 9.4 T. Images were collected following presaturation at +47.5 ppm (sat-on) and −47.5 ppm (sat-off), and CEST images were generated by subtracting the sat-on image from the sat-off image (Figure 1). Each tube in the phantom system exhibited different image intensities, with trends similar to the magnitude of the CEST peak in the spectra.

To prove the concept that altering electronic effects could be used as a platform on which responsive PARACEST agents, and specifically redox-responsive agents, might be designed, a further experiment was performed in which the *p*-nitro derivative was chemically reduced to the *p*-amino derivative by catalytic hydrogenation (40 psi H_2 over Pd/C in EtOH). CEST images acquired of the two samples (Figure 2) clearly show a difference in signal intensity (~30%) between the oxidized (*p*-NO_2) and reduced (*p*-NH_2) forms.
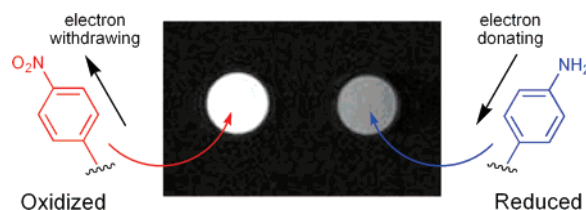

Figure 2. CEST difference images showing that the oxidized and reduced forms of the complexes can be discriminated by MR imaging.

These results clearly demonstrate that the water exchange kinetics, and ultimately the CEST properties, of EuDOTA−tetraamide complexes are acutely sensitive to changes in the electronic properties of the ligand, even at a relatively remote site, providing these sites can communicate electronically with a donor atom coordinated to the metal ion. This sensitivity to changes in electronic effects could provide the basis for a general approach to the design of new responsive PARACEST agents. Our success generating differences in CEST in a chemically reducible system suggests that it should prove possible to design redox-responsive systems applicable to biology.

Supporting Information Available: CEST spectra as a function of applied B_1; CEST fitting; imaging protocols; experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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