pH-Dependent Modulation of Relaxivity and Luminescence in Macrocyclic Gadolinium and Europium Complexes Based on Reversible Intramolecular Sulfonamide Ligation

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Abstract: A series of macrocyclic Eu, Gd, and Tb complexes has been prepared in which the intramolecular ligation of a β-arylsulfonamide nitrogen is rendered pH-dependent, giving rise to changes in the hydration state, q, at the lanthanide center. In complexes based on DO3A, variation of the *p*-substituent in the arylsulfonamide moiety determines the apparent protonation constant log K_{MLH} with values of 5.7, 6.4, and 6.7 for the -CF_3, -Me, and -OMe substituents, respectively. Introduction of three β-carboxyalkyl substituents, α to three ring nitrogens, inhibits displacement of the bound water by added protein and also suppresses intermolecular binding by endogenous anions (lactate, HCO_3^-). Measurements of the pH dependence of the form and intensity of the Eu complexes revealed that intramolecular carboxylate coordination occurred competitively. This was reduced either by enhancing the electron density at the sulfonamide nitrogen or by enlarging the chelate ring from 7−8. Amplification of the relaxivity changes in the pH range 8−5 occurred on protein binding, and over the pH range 7.4−6.8 a 48% change in relaxivity was defined for [Gd·**3a**] (298 K, 65.6 MHz) in 50% human serum solution.

Magnetic resonance imaging (MRI) is an established clinical modality for defining changes in tissue anatomy that may arise from disease or injury. In the late 1980s, a series of extracellular gadolinium contrast agents emerged which allowed contrast to be enhanced between tissues containing differing water concentrations.[1,2] A second generation of paramagnetic contrast agents is now beginning to emerge, in which the relaxivity of the contrast agent is a defined function of a particular biochemical variable. Thus, Ca^{2+}-dependent gadolinium complexes have been devised for application in vitro to problems in developmental biology.[3] A series of Eu^{II} (f^7) complexes has also been reported which may be expected to exhibit pO_2 or time-dependent relaxivity[4]—although this has yet to be proven in an in vivo model. Attention has also turned to pH-responsive agents. Such work has been stimulated by the observation that the extracellular pH of tumors (ca. 6.8−6.9) is more acidic than both tumor intracellular pH (7.2) and normal extracellular tissue (ca. 7.4). Although this acidity has been ascribed to overexpression of lactate[5] it may be related to a change in the equilibrium position of the Na^+/H^+ exchanger.[6] Such acidity has important consequences: it may stimulate metastasis[7] and it may enhance the uptake of weakly acidic chemotherapeutic drugs.

The implementation of a pH-dependent contrast agent for use in vivo requires that several criteria are satisfied. In addition to being nontoxic (hence, kinetically stable with regard to the biological half-life) and relatively cheap, the agent should exhibit pH-dependent relaxivity modulation in the applied field range of 20−80 MHz—corresponding to the field strengths of most commercial MRI instruments. However, as the intensity of the image will be directly proportional to the local contrast agent concentration, either the distribution of the complex in the tissue must be determined or a difference image must be obtained, for example following sequential administration of two contrast agents with different relaxivity/pH profiles but identical (or very similar) tissue biodistributions.[7] Evidently, the practicalities associated with the application of pH-dependent contrast agents require that a closely related series of well-tolerated complexes be examined in detail.

The different ways in which the relaxivity of a given contrast agent may be modulated can be appreciated by consideration

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(1) *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Merbach, A. E., Toth, E., Eds.; Wiley: New York, 2001.

(2) (a) Caravan, P.; Ellison, J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293. (b) Aime, S.; Botta, M.; Fasano, M.; Terreno, M. *Chem. Soc.* Rev., **1998**, *27*, 19. (c) Lauffer, R. B. *Chem. Rev.*, **1987**, *87*, 901.

(3) Li, W. H.; Fraser, S. E.; Meade, T. J. *J. Am. Chem. Soc.*, **1999**, *121*, 1413.

(4) (a) Burai, L.; Toth, E.; Seibig, S.; Scopellitti, R.; Merbach, A. E. *Chem. Eur. J.* **2000**, *6*, 3761 (b) For related work on pO_2-dependent $Mn^{II/III}$ complexes see: Aime, S.; Botta, M.; Gianolio, E.; Terreno, E. *Angew. Chem., Int. Ed.* **2000**, *39*, 747.

(5) Newell, K.; Franchi, A.; Ponyssegur, J.; Tannock, I. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1127.

(6) Mclean, J. A.; Roscoe, J.; Jorgensen, N. K.; Gorin, F. A.; Cala, P. M. *Am. J. Physiol.* **2000**, *278*, C676.

(7) Beauregard, D. A.; Parker, D.; Brindle, K. M. *Proc. Int. Soc. Magn. Reson. Med.* **1998**, *6*, 53.

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of the equations defining the dependence of the water proton relaxation rate, R_1. The paramagnetic contribution (eq 1) is made up of outer-sphere, second-sphere and inner-sphere terms, of which the inner-sphere is usually the most significant.[1,2]

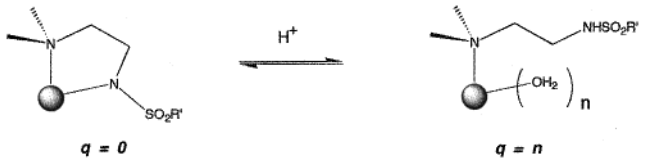
$$R_{1p} = R_{1p}^{OS} + R_{1p}^{IS} + R_{1p}^{SS} \quad (1)$$

$$R_{1p}^{IS} = \frac{cq}{55.6}\left[\frac{1}{T_{1m} + \tau_m}\right] \quad (2)$$

The inner sphere contribution is a function of four variables (eq 2): the concentration of the complex, c; the number of directly bound water molecules, q; the exchange lifetime of the coordinated water molecule, τ_m; the longitudinal water proton relaxation time, T_{1m}. Gadolinium complexes in which the bound water proton exchange rate is a function of pH have been defined recently,[8] in which deprotonation of the coordinated water proton is assisted by a high charge density at the lanthanide center.[9] Labilization of the water molecule itself may be rendered pH-dependent in cases where water exchange is a function of the nature of proximate anionic groups which, in turn, define the structure and dynamics of the second sphere of hydration.[8a,b,10] Within the complex series of terms that control the value of T_{1m}, the pH dependence of the rotational correlation, time τ_r, may be picked out as an example. Conjugates of gadolinium complexes have been devised in which a large pH-dependent change in the conformation and molecular volume of a macromolecule occurs, thereby perturbing τ_r and hence T_{1m}.[11] Finally, if a change in the hydration state of a gadolinium complex is rendered pH-dependent, then R_{1p} may be varied significantly. Such a variation may be amplified if the complex is noncovalently associated with a macromolecule, provided that τ_m remains relatively fast. These PRE effects are usually largest for field strengths in the range 10 → 100 MHz. Therefore, if a pH-dependent agent is sought for application at higher fields (e.g., at 300 or 400 MHz and hence higher spatial resolution), then it is more appropriate to seek a system which does not bind strongly to an endogenous macromolecule. Preliminary examples of lanthanide complexes possessing pH dependence of the hydration state have begun to appear,[12] including cases where reversible binding of hydrogen carbonate allows switching between differently hydrated complexes over the pH range 5−8.[9,13,14]

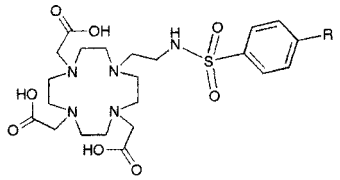
We report herein examples of gadolinium complexes exhibiting pH-dependent relaxivity by virtue of a switch in hydration state, associated with the on/off ligation of a sulfonamide nitrogen donor.[12] Early work by Kimura established that the protonation constants associated with the binding of a sulfonamide nitrogen to a zinc center fall in the pH range 5−7.5.[15] With this in mind, we sought to append a sulfonamide nitrogen β to a ligand nitrogen donor and hence allow a variation in the coordination environment of the Ln center as a function of pH (Scheme 1). Control over the facility of complex protonation may be exerted by variation of the N electron density which, in turn, is determined by the nature of the substituent R′. Because europium complexes exhibit a metal-based emission which is particularly sensitive to coordination environment, both Eu and Gd complexes of a series of sulfonamido-ligands have been prepared and the pH dependence of their emission spectra and relaxivity behavior has been defined, in the presence and absence of serum or added protein.

Scheme 1



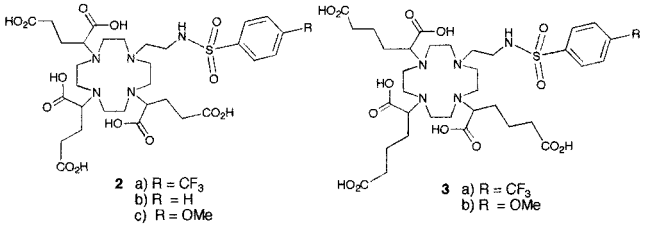
Results and Discussion

The initial target complexes, prepared in order to prove the concept, were the Eu and Gd complexes of the DO3A-derived[16] ligands **1a**−**1c**. It was appreciated at the outset that, when the



1 a) R = CF_3
b) R = Me
c) R = OMe

sulfonamide nitrogen was not bound to the Gd ion, then binding of either endogenous anions[12,14] or of protein (e.g. via chelation of amide carbonyl or carboxylate groups) might displace the bound water and hence suppress the change in relaxivity.[17] Therefore, the related series of ligands **2a**−**2c** and **3a**,**3b** were also prepared. In each case, the nature of the *p*-substituent in



2 a) R = CF_3
b) R = H
c) R = OMe

3 a) R = CF_3
b) R = OMe

the arylsulfonamide was considered likely to determine the sensitivity of the derived lanthanide complex to protonation. The presence of the carboxyalkyl substituents had earlier been demonstrated to inhibit intermolecular anion binding at a coordinatively unsaturated lanthanide center.[14]

(8) (a)Aime, S.; Barge, A.; Botta, M.; Parker, D.; de Sousa, A. S. *J. Am. Chem. Soc.* **1997**, *119*, 4767. (b) Aime, S.; Barge, A.; Bruce, J. I.; Botta, M.; Howard, J. A. K.; Moloney, J. M.; Parker, D.; de Sousa, A. S.; Woods, M. *J. Am. Chem. Soc.* **1999**, *121*, 5672. (c) Hall, J.; Haner, R.; Aime, S.; Botta, M.; Faulkner, S.; Parker, D.; de Sousa, A. S. *New J. Chem.* **1998**, *22*, 627.

(9) Note that the pH-dependent labilization of exchangeable protons that are close to the paramagnetic center may constitute a large contribution to the second-sphere term, R_{1p}^{SS}; for examples see ref 8b and Aime, S.; Barge, A.; Botta, M.; Howard, J. A. K.; Kataky, R.; Lowe, M. P.; Moloney, J. M.; Parker, D.; de Sousa, A. S.; *Chem. Commun.* **1999**, 1047.

(10) Zhang, S. R.; Wu, K. C.; Sherry, A. D. *Angew. Chem., Int. Ed.* **1999**, *38*, 3192.

(11) Aime, S.; Botta, M.; Crich, S. G.; Giovenzana, G.; Palmisano, G.; Sisti, M. *Chem. Commun.* **1999**, 1577.

(12) Parker, D.; Lowe, M. P. *Chem. Commun.* **2000**, 707.

(13) Aime, S.; Batsanov, A. S.; Botta, M.; Howard, J. A. K.; Lowe, M. P.; Parker, D. *New J. Chem.* **1999**, *23*, 669.

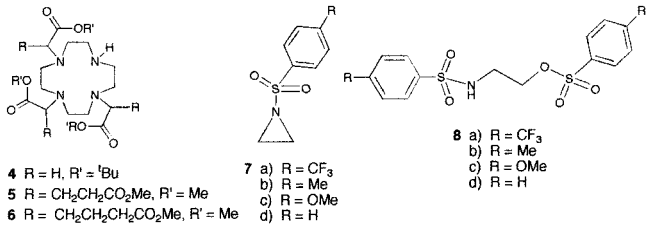
(14) Bruce, J. I.; Dickins, R. S.; Govenlock, L. J.; Gunnlaugsson, T.; Lopinski, S.; Lowe, M. P.; Parker, D.; Peacock, R. D.; Perry, J. J. B.; Aime, S.; Botta, M. *J. Am. Chem. Soc.* **2000**, *122*, 9674.

(15) (a) Koike, T.; Kimura, E.; Nakamura, I.; Hashimoto, Y.; Shiro, M. *J. Am. Chem. Soc.* **1992**, *114*, 7338. (b) Koike, T.; Kimura, E. *Chem. Soc. Rev.* **1998**, *27*, 179.

(16) DO3A is 1,4,7-tris(carboxyethyl)-1,4,7,10-tetraazacyclododecane; the tris[α-carboxyethyl]substituted ligand **2** is given the acronym gDO3A, and the homologue **3**, is aDO3A.

(17) Protein binding to Gd DO3A derivatives produces $q = 0$ species: Aime, S.; Gianolio, E.; Terreno, E.; Pagliarin, R.; Giovenzana, G. B.; Lowe, M. P.; Sisti, M.; Palmisano, G.; Parker, D.; Botta, M. *J. Biol. Inorg. Chem.* **2000**, *5*, 488.

The synthesis of the desired sulfonamide-functionalized ligands relied upon the established ring-opening reaction of *N*-sulfonylaziridines with secondary amines.[18] For example,



reaction of equimolar quantities of *N*-*p*-methoxyphenylsulfonylaziridine **7c** (prepared by treatment of the corresponding sulfonate ester, **8c** with base) with 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, **4**, in hot acetonitrile followed by deprotection with CF_3CO_2H afforded the desired ligand **1c**. In the case of the ligands **2a**−**2c** and **3a**,**3b**, the products were isolated as a mixture of the RRR/SSS, RSR/SRS, and RRS/SSR stereoisomers. Lanthanide complexes were prepared in aqueous solution following reaction of the ligand with hydrated $LnCl_3$ at pH 6 (18 h, 90 °C). The purification of [Ln·**1**], [Ln·**2**], and [Ln·**3**] involved extraction of the lyophilized complex into 5−40% MeOH/CH_2Cl_2 at pH 5, The purified complexes were rather hygroscopic, but each gave a satisfactory high-resolution electrospray mass spectrum (negative ion mode).

Analysis of the ^{1}H NMR spectrum of [Eu·**1c**] in basic media (277 K, pD 10, 300 MHz) revealed resonances for the most shifted ring axial protons at 37.5, 30.7, 27.9, and 18.9 ppm. Such values—and the shift pattern of the remaining shifted ligand protons—are similar to those observed for monoamide tricarboxylate[19] complexes of europium and to similar neutral monopyridyltriacetate complexes reported more recently.[13] They are consistent with a mono-capped square-antiprismatic coordination environment about the lanthanide center. The proton NMR spectra for [Eu·**1**] recorded in more acidic media were much less well-defined: exchange broadened spectra were observed (300 MHz, pD 4) over the temperature range 30° to −5 °C and the form of the spectra closely resembled those defined for N-alkylated complexes of DO3A[17] in which cooperative arm rotation occurs at a relatively fast rate on the NMR time-scale. The ^{1}H NMR spectra of the Eu complexes of **2** and **3** were more complex, owing to the presence of the three stereoisomeric species (RRS, RRR, RSR and their enantiomers). In the case of [Eu·**2c**], a sample enriched in the RRR/RRS species (ca. 70/30) was prepared following partial chromatographic separation of the precursor esters. In this case, the NMR spectra recorded at pD 4 and 10 were similar in general form to those obtained with [Eu·**1**], except that at lower pD the spectrum exhibited reduced line-broadening consistent with some degree of inhibition of arm rotation caused by substitution α to the ring nitrogen. Such effects have been defined in detail recently in each of the stereoisomeric tetra {α-(carboxyalkyl)} series of complexes.[20]

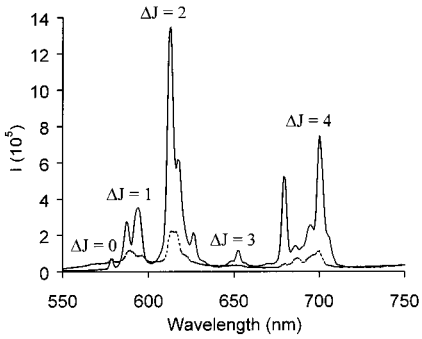


Figure 1. Europium emission spectra for [Eu·**1b**] at pH 10 (more intense) and pH 4, revealing the changes in the hypersensitive $\Delta J = 2$ (ca. 616 nm) and $\Delta J = 4$ (e.g., 682 nm) transitions (λ_{exc} 270 nm; 295 K, H_2O, $I = 0.1$ M NaCl).

Table 1. Radiative Rate Constants k (ms^{-1}, ±10%) and Derived Hydration States, q, for Decay of Europium Luminescence at Limiting pH/pD Values (295 K, $I = 0.1$ M NaCl)

	pH/pD 4			pH/pD 10		
complex	k_{H_2O}	k_{D_2O}	q^{Eu}	k_{H_2O}	k_{D_2O}	q^{Eu}
[Eu·**1a**]	2.35	0.76	1.6	1.09	0.74	0.1
[Eu·**1b**]	2.27	0.70	1.6	1.20	0.80	0.2
[Eu·**1c**]	2.32	0.66	1.7	1.43	0.93	0.3
[Eu·**2c**]	2.21	0.65	1.6	1.05	0.72	0.1
[Eu·**3a**]	2.44	0.75	1.7	0.94	0.65	0.05

[a] Values of q were estimated using the equation: $q^{Eu} = 1.2[(k_{H_2O} - k_{D_2O}) - 0.25]$ which allows for the contribution of unbound water molecules.[22]

Infrared spectra of lyophilized samples (pH 4 and 10) were recorded in methanol solution and, in the solid state, in a KBr matrix and compared to values for the free ligand. Under both sets of conditions, strong sulfur−oxygen stretching bonds were observed at 1329 (asymmetric) and 1159 (symmetric) cm^{-1}. The lack of variation of these stretching frequencies (ligand versus complex, solid-state versus solution) suggests that the sulfonyl oxygen does not participate in metal binding. In contrast, in a recent report of the intramolecular ligation of a dansyl sulfonamide group at a cationic yttrium center, N−O chelation was revealed with well-defined shifts in the sulfonyl-stretching frequencies.[21] Further information about the nature of the lanthanide coordination environment as a function of pH was provided by examination of the Eu emission spectrum following direct (λ_{exc} 397 nm) or indirect (λ_{exc} 270 nm) excitation. Profound changes in the form and relative intensity of the hypersensitive $\Delta J = 2$ and $\Delta J = 4$ transitions were observed (Figure 1) as a function of pH for [Eu·**1**]; similar spectra were recorded at the acidic (pH 3) and basic (pH 10) limits with [Eu·**2**] and [Eu·**3**]. Spectra recorded at pH 7 → 10 were much more intense than those measured in more acidic media. Independent measurements of the radiative rate constants for decay of the Eu excited state were undertaken in H_2O and D_2O at pH/pD 4 and 10 (Table 1). This allowed an estimation of the hydration state at Eu.[22] In each case q was near zero in more alkaline media and was greater than 1 (1.6−1.7) in the acidic limit. The more intense emission observed at higher pH was

(18) Martin, A. E.; Ford, T. M.; Bulkowski, J. E. *J. Org. Chem.* **1982**, *47*, 412; Chandrasekhar, S.; McAuley, A. *J. Chem. Soc., Dalton Trans.* **1992**, 2967; Howard, C. C.; Marckwald, W. *Chem. Ber.* **1899**, *32*, 2036.

(19) Monoamide-triacetate DOTA−Ln complexes typically reveal >90% of the square-antiprismatic isomer in solution; this isomer undergoes water exchange more slowly than the twisted square-antiprismatic isomer in which the faster exchange is ascribed to a later transition state for water interchange (independent of complex charge or structure)[20]: Aime, S.; Anelli, P. L.; Botta, M.; Fedeli, F.; Grandi, M. P.; Padi, P.; Uggeri, F. *Inorg. Chem.* **1992**, *31*, 2422.

(20) Woods, M.; Aime, S.; Botta, M.; Howard, J. A. K.; Moloney, J. M.; Navet, M.; Parker, D.; Port, M.; Rousseaux, O. *J. Am. Chem. Soc.* **2000**, *122*, 9781.

(21) The chelation of a sulfonamide dansyl group has been defined at a tripositive Y center: Aoki, S., Kawatani, H., Goto, T., Kimura, E., Shiro, M. *J. Am. Chem. Soc.* **2001**, *123*, 1123; in analogous anionic Eu and Tb complexes with a lower charge demand at the metal, the sulfonamide acts as a monodentate N donor: Lowe, M. P., Parker, D. *Inorg. Chim. Acta* **2001**, *317*, 163.

(22) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, 493.

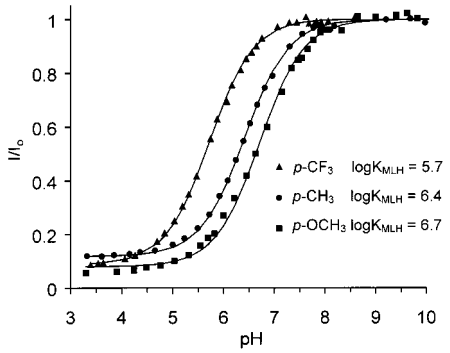


Figure 2. pH Dependence of the europium luminescence at 612 nm for [Eu·**1**](I/I_o, $\Delta J = 2$; 295 K; $I = 0.1$M NaCl); the line shows the fit to the experimental data points for the estimated protonation constants.

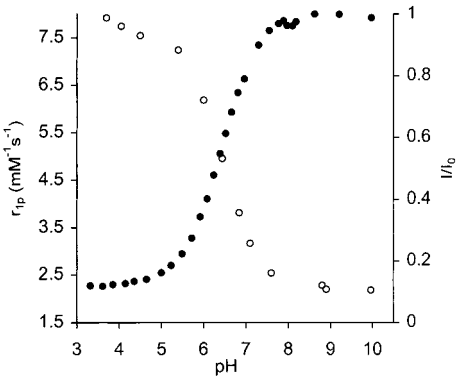


Figure 3. Correlation of the pH-dependent relaxivity of [Gd·**1b**] [open circles] (20 MHz, 298 K) and the luminescence behavior of [Eu·**1b**] [filled circles] (298 K, I/I_o at 612 nm).

quantified in terms of the overall quantum yield for sensitized emission with [Eu·**2c**] (λ_{exc} 270 nm): at pH 10, $\varphi_{em} = 0.4\%$, and at pH 4, $\varphi_{em} = 0.1\%$.[23] The relatively low values obtained here reflect the competitive deactivation of the intermediate aryl singlet excited state caused by charge transfer to the Eu^{III} center. With [Eu·**2a**] at pH 10, a higher emission quantum yield of 0.8% was measured, reflecting the partial suppression of this deactivating process as the *p*-CF_3 substituent renders the aryl singlet less readily oxidized.

The pH variation of the change in the intensity of the $\Delta J = 2$ transition at 612 nm for [Eu·**1**] revealed a sigmoidal curve (Figure 2) characteristic of a simple two-state equilibrium process. Analysis by iterative least-squares fitting afforded apparent protonation constants (log K_{MLH}) of 5.7, 6.4, and 6.7 for the *p*-CF_3-, Me-, and OMe-substituted sulfonamide Eu complexes. This variation is in accord with the increase in electron density at the nitrogen.

Similar results (± 0.05 log K_{MLH}) were obtained by analyzing the intensity changes at 682 nm which are also likely to be associated with sulfonamide nitrogen coordination to the europium center. Taken together with the observed pH-dependent NMR and IR spectra, the behavior of the simple DO3A derivatives is consistent with a binding equilibrium between a $q = 0$, nitrogen-bound species, and a $q > 1$ species which predominates in acidic media (Figure 2 and Scheme 1).[24]

The relaxivity of [Gd·**1**] was recorded as a function of pH in the range 4−10 (20 MHz, 298 K). At low pH, the limiting relaxivity was of the order of 7.5 mM^{-1} s^{-1} and fell to a value of 2.0 mM^{-1} s^{-1} at pH 10. Such behavior (Figure 3) mirrors the pH dependence of luminescence emission found with the analogous Eu complexes. Such a comparison highlights the utility of examining the Eu luminescence behavior in assessing the likely relaxivity profile. Examination of the properties of these lanthanide DO3A complexes served to vindicate the appropriateness of the pH-dependence of the on/off ligation mechanism involving the sulfonamide nitrogen. This determines the local hydration at the lanthanide. However, prior work had established that N-alkylated Gd−DO3A complexes are not good candidates for use in vivo. They are not sufficiently stable with respect to metal dissociation[1,2] to allow their safe use. Furthermore, noncovalent protein binding—facilitated by interaction of the aryl moiety to serum albumin—has been shown to lead to displacement of the metal-bound waters, reducing any relaxivity change.[17] Therefore the [Ln·**2**]$^{n-}$ and [Ln·**3**]$^{n-}$ complexes were examined, as such α-N-alkylated complexes exhibit greater kinetic and thermodynamic stability with respect to metal ion dissociation.[25] In addition, the peripheral carboxyalkyl substituents have been shown to inhibit binding of endogenous anions, present in serum, such as lactate (2.3 mM), HCO_3^- (ca. 27 mM), and HPO_4^{2-} (ca. 0.9 mM).[14]

pH Dependence of Luminescence and Relaxivity for [Ln·2]$^{n-}$ and [Ln·3]$^{n-}$. The variation of luminescence emission intensity with pH for europium and terbium complexes was examined in 0.1 M NaCl solution and in a simulated extracellular ionic background, that is a solution containing additionally 2.3 mM lactate, 0.13 mM citrate, 30 mM HCO_3^-, and 0.9 mM phosphate. For the terbium complexes, although some slight changes in spectral form were evident at the two limiting pH values (i.e., pH 3 and 10), differences were masked by the spectral complexity inherent in terbium emission spectra, deriving from the multiplicity of allowed transitions from the $^5D_4$ excited-state manifold. Such complexity is absent in the corresponding Eu emission spectra. In addition the use of direct excitation (λ_{exc} 397 nm) averts any complications that arise from differing efficiencies of sensitization for chemically distinct lanthanide species.

With each of the [Eu·**2**] complexes, three distinctively different europium emission spectra were observed (Figures 4 and 5). In the pH range 6−9, sulfonamide N ligation was characterized by large changes in the form and intensity of the hypersensitive $\Delta J = 2$ (e.g., 614 nm) and $\Delta J = 4$ (e.g., 682 nm) manifolds, with an apparent log K_{MLH} around 6.7 for [Eu·**2c**] and 5.7 for [Eu·**2a**]. Further changes were observed in the pH range 6−3, associated with an increase in emission intensity at 617 and 594 nm. For the $\Delta J = 2$ band, a maximum in the emission intensity was observed around pH 5 for [Eu·**2c**], [Eu·**2b**], and [Eu·**2a**] (Figure 5). The spectrum recorded at intermediate pH (inset to Figure 4), represents a chemically distinct species, as it is not just a superposition of the spectra recorded at pH 10 (sulfonamide N-bound) and at pH 3. In more acidic media, (pH < 4), the spectra resembled those observed for the complex [Eu·**1**], where the carboxyalkyl side chain is not present. The observed pH/intensity changes were also found to be independent of the composition of the salt background.

Taken together, such behavior may be rationalized in terms of the formation of an intermediate carboxylate-bound species, involving intramolecular ligation of one β-carboxyethyl sub-

(23) For [Tb·**2c**], at pH 10 $\varphi_{em} = 3.5\%$ (λ_{exc} 270 nm) and at pH $\varphi_{em} = 0.17\%$; in this case the combined effect of the proximity of the chromophore (enhancing the efficiency of intramolecular energy transfer) and the absence of quenching of the Tb $^5D_4$ excited state by OH oscillators serve to enhance the emission quantum yield for the N-bound complex.

(24) Kang, S. I.; Ranganathan, R. S.; Emswiler, J. E.; Kumar, K.; Gougoutas, J. Z.; Malley, M. F.; Tweedle, M. F. *Inorg. Chem.* **1993**, *32*, 2912.

(25) Details of emission spectral changes and individual wavelength variations for selected Eu complexes are given in the Supporting Information.

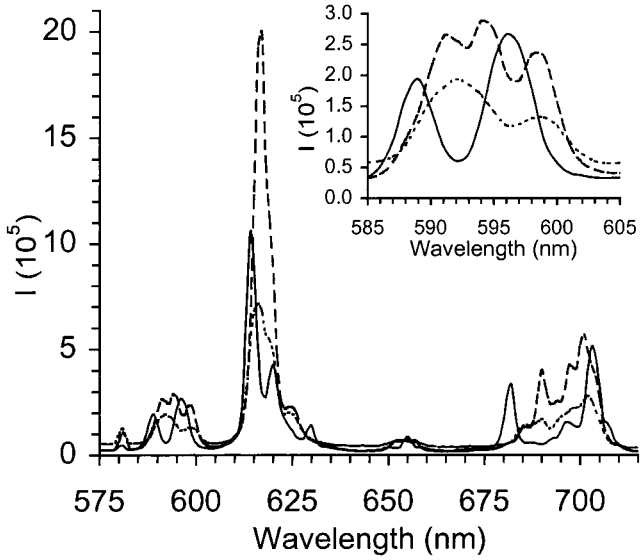


Figure 4. Changes in the europium emission spectrum for [Eu·**2c**] at pH 9.93, (line) 5.49 (— — —) and 3.00 (- - -) following direct excitation of the Eu^{3+} ion at 397 nm, revealing the presence of three chemically distinct species.

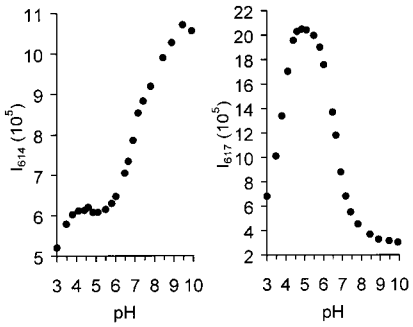


Figure 5. Spectral emission intensity changes with pH for [Eu·**2c**] characterizing sulfonamide nitrogen binding (614 nm; left) and intramolecular carboxylate participation (617 nm; right) (295 K, $I = 0.1$M NaCl); very similar profiles were observed in a simulated extracellular ionic background.

stituent, generating a seven-ring chelate.[26] Protonation of the carboxylate oxygen in the complex occurs significantly below pH 4, leading to the same nine-coordinate "diaqua"-europium species observed for [Eu·**1**].

Support for this hypothesis was provided by examining the pH-dependence of the relaxivity for the corresponding gadolinium complexes and comparing with the pH-dependent changes found in the Eu series. Correlation of the pH/relaxivity changes for [Gd·**2c**] with the pH dependence of the emission intensity profile for [Eu·**2c**] reveal a near mirror-image relationship (Figure 6). Such behavior is consistent with the idea that *increases* in r_{1p} are directly proportional to the hydration state *q*, while the Eu emission intensity *decreases* in direct proportion to it. Moreover, the behavior in the range 9−5 supports the idea that carboxylate ligation and sulfonamide N binding are in competition. Therefore, by decreasing the donor ability of either the sulfonamide N or the carboxylate O, the position of the equilibrium distribution of complexes should be perturbed. A simple example is provided by the *p*-CF_3 complex [Gd·**2a**]: the lesser nucleophilicity of the sulfonamide N leads to enhanced competition from the carboxylate oxygen at higher pH. Thus,

(26) The pH dependence of the emission spectral profile for [Eu·**2c**] was unchanged when using a stereoisomeric mixture containing a 87/13 mixture of RRR/RRS isomers (compared to the 70/30 ratio used elsewhere).

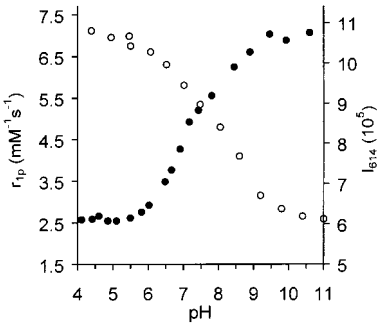


Figure 6. Correlation of the pH-dependence of relaxivity for [Gd·**2c**] [open circles] (298 K, 20 MHz) with emission intensity changes for [Eu·**2c**] [filled circles] (295 K, $I = 0.1$ M NaCl, λ_{exc} 397 nm, I_{em} 614 nm).

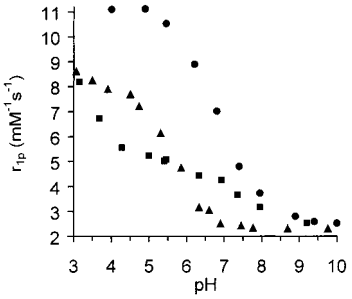


Figure 7. Variation of relaxivity (298 K, 20 MHz, $I = 0.1$ MNaCl) with pH for [Gd·**1a**] [triangles], [Gd·**2a**] [squares], and [Gd·**3a**] [circles], highlighting the competition between sulfonamide N and side-chain carboxylate binding.

carboxylate ligation may occur even at pH > 7, leading to a flatter relaxivity/pH profile (Figure 7). In comparison with [Gd·**1a**], the anionic complex [Gd·**2a**] reveals a steadily increasing relaxivity value from pH 9 to 5 as the mean hydration state increases. In more acidic media protonation of the ligating carboxylate occurs leading to a preponderance of a $q = 2$ species, associated with the higher relaxivity value of ca. 8.5 observed below pH 3.

Intramolecular carboxylate participation may be suppressed either by reducing the nucleophilicity of the carboxylate oxygen or by enlarging the chelate ring by adding spacer groups to the pendant arm. An example of the latter class is provided by the complexes of ligand **3**, with an additional methylene group in the substituents α to the ring N. The variation of Eu emission intensity with pH for [Eu·**3a**] and [Eu·**3b**] revealed significant differences compared to the "gDO3A" analogues. The pH change in emission intensity at 614 and 682 nm (N-binding) for the *p*-CF_3 substituted complex revealed an apparent protonation constant, log K_{MLH}, of 6.1, and the corresponding value for the *p*-OMe example was 7.0. Little change in the form of these pH profiles was observed in a competitive anion background. Examination of the intensity variation for [Eu·**3b**] at 617 nm—characterizing carboxylate ligation—revealed no defined intensity maximum at pH 5. The change in the emission intensity change at 617 nm, between pH 7.4 and pH 5, differed by 60 and 250% compared to [Eu·**2a**] and [Eu·**2c**] respectively.

The pH dependence of the relaxivity of [Gd·**3a**] (20 MHz, 298 K) was simpler in form than for [Gd·**2a**]. An increase from $r_{1p} = 2.7$ mM^{-1} s^{-1} at pH 9 ($q = 0$) to 10.9 mM^{-1} s^{-1} at pH 5. Such a relaxivity value suggests that a $q = 2$ complex predominates in solution below pH 5. This variation was also examined at 65.6 MHz in a competitive anion background, and the observed change (Figure 9) was similar with limiting values of 2.5 mM^{-1} s^{-1} (pH 8.5) and 8.7 mM^{-1} s^{-1} (pH 5).

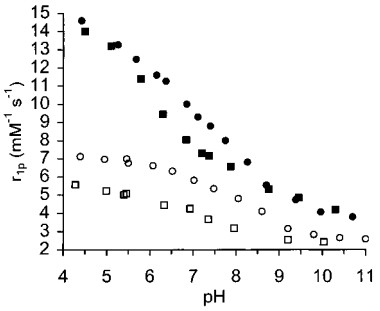


Figure 8. Dependence of the relaxivity of [Gd·**2a**] (circles) and [Gd·**2c**] (squares) in the absence (open) and presence (filled) of 1.5 mM serum albumin (20 MHz, 298 K).

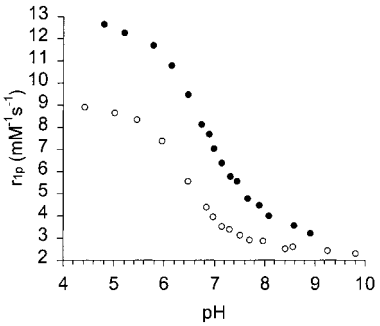


Figure 9. Variation of the relaxivity of [Gd·**3a**] (65.6 MHz, 298 K,) with pH in a simulated extracellular anion background (open circles, 0.5 mM complex) and in the presence of human serum solution, diluted by a half (filled circles, 0.7 mM complex). Very similar behavior was observed with an 0.2 mM solution of complex in 100% serum, with an increase of 37% in relaxivity between pH 7.4 and pH 6.8.

Independent measurement of q on the analogous Eu complexes using luminescence methods (Table 1) gave values of 0.1 for [Eu·**3a**] in the N-bound form at pH 8.5, and $q = 1.7$ at pH 4. Similar values (± 0.2) were recorded for [Eu·**3b**].

The NMRD profile for [Gd·**3a**] was recorded at pH 5 (298 K) and was parametrized in terms of $\tau_m = 35$ ns (a value measured independently by standard VT ^{17}O NMR methods), $\tau_r = 1.86 \times 10^{-10}$ s, $\tau_v = 2.1 \times 10^{-11}$ s, $q = 1.7$, and $\Delta^2 = 4.5 \times 10^{-19}$ s^{-2}. Taken together, these NMRD, luminescence and relaxivity measurements are consistent with a significantly reduced degree of intramolecular carboxylate ligation in [Ln·**3**] which is apparently not compromised by intermolecular anion binding.

Behavior in the Presence of Protein and Serum. In the "field" range 20−100 MHz, the noncovalent interaction of a gadolinium complex undergoing relatively fast water exchange, for example, $k_{ex} = 2 \times 10^7$ s^{-1}, with a more slowly tumbling macromolecule may lead to pronounced relaxivity enhancements, provided that the bound water is not displaced. Such an effect has been established[1,2] for several anionic gadolinium complexes bearing an aryl residue. Typically such anionic complexes form a complex with serum albumin with an apparent affinity constant of the order of 10^4 M^{-1}.[1,2]

Relaxivity measurements at 20 MHz, probing the interaction of [Gd·**2a**] and [Gd·**2c**] with human serum albumin (1.5 mM), revealed modest but significant enhancements (Figure 8). An increase in the slope of the relaxivity/pH plot was observed over the range 7.5−6.5. This change was more marked for the *p*-OMe derivative [Gd·**2c**]. Such behavior may be related to an enhanced binding affinity of the complex with the unbound arylsulfonamide. At lower pH, the sulfonamide N is not coordinated, and the aryl moiety is more sterically accessible to interact with the protein. When the sulfonamide N is bound to Gd, the structure of the complex is much more compact, and the aryl group possesses much less conformational freedom to interact with a protein binding pocket. The enhancement of relaxivity in the presence of albumin was less pronounced around pH 7 for the *p*-CF_3 analogue, [Gd·**2a**] (Figure 8). In this case, the greater extent of intramolecular carboxylate binding in this pH range reduces slightly the PRE effect. With [Gd·**3a**], the relaxivity enhancement in the presence of serum albumin was larger still. An r_{1p} value of 19.4 mM^{-1} s^{-1} was measured in the presence of 1.5 mM serum albumin at pH 5 compared to 13 mM^{-1} s^{-1} for [Gd·**2a**].

Measurements of relaxivity for [Gd·**3a**] and [Gd·**3b**] were also undertaken at 65.6 MHz, examining the pH range 10−4 in a clinical anion background and in 50% human serum solution.[27] In a simulated extracellular "anion" background, the percentage change in relaxivity for [Gd·**2c**] between pH 7.4 and 6.8 was only 7%, whereas in the human serum solution the enhancement of relaxivity was 15%. The corresponding percentage enhancements for [Gd·**3a**], measured under identical conditions, were 35 and 48% respectively (Figure 9). With [Gd·**3b**], the relaxivity value in the anionic background changed by 19% and in serum by 20% between pH 7.4 and 6.8. Such behavior—with a significant percentage change over the physiologically important pH change at a clinically relevant magnetic field strength—augurs well for the development of such pH-responsive contrast agents for their application in devising pH maps in MR images. The different slopes of relaxivity/pH for structurally similar complexes—tuned by the nature of the sulfonamide group or the ring N "sidearm" substituent—suggests that it may be feasible to produce ratios of intensity maps to eliminate the concentration dependence of the primary MR "intensity" images.

Experimental Section

Reagents and Solvents. Acetonitrile was dried over calcium hydride before use. Water and H_2O refer to high purity water with conductivity ≤ 0.04 μS cm^{-1}, obtained from the PURITE purification system. 1,4,7,10-Tetraazacyclododecane is commercially available (Strem) and was used as received.

Chromatography. Column chromatography was carried out using "gravity" silica (Merck). Cation exchange chromatography was performed using Dowex 50W 50 × 4-200 strong ion-exchange resin, which had been pretreated with 3 M HCl.

Spectroscopy. ^1H NMR spectra were recorded at 65.6 MHz on a 1.53 T magnet connected to a Varian VXR400 console, at 199.99 MHz on Varian Mercury-200 and at 299.91 MHz on Varian Unity-300. ^{13}C NMR were recorded on the Varian Mercury-200 at 50.2 MHz and a Varian Unity-300 at 75.4 MHz. Mass spectra were recorded using a VG Platform II electrospray mass spectrometer with methanol or water as a carrier solvent. Accurate masses were determined at the EPSRC National MS Service at Swansea; isotope calculations for Gd and Eu complexes refer to ^{153}Eu and ^{158}Gd. Luminescence measurements for europium and terbium complexes were recorded using an Instrument SA Fluorolog 3-11 using DataMax for Windows v2.1. Second-order diffraction effects were obviated by using a 375 nm cutoff filter.

pH measurements were made using a Jenway 3320 pH meter (fitted with a BDH Glass + combination electrode−microsample) calibrated with pH 4, 7, and 10 buffer solutions.

(27) Measurements of the pH dependence (pH 4−9) of the Eu emission spectrum for [Eu·**3b**] were undertaken in 0.1 M NaCl, in a simulated clinical anion background and in 50% aqueous human serum solution. A very similar spectral form and relative emission intensity was observed at a given pH value, in each case ($\lambda_{exc} = 397$ nm). Such behavior strongly suggests that the Eu coordination environment is very similar in each of these situations, supporting the conclusions drawn from the pH dependence of Gd relaxivity.

The longitudinal water proton relaxation rates were measured either using a Stelar Spinmaster (Mede, Pavia, Italy) spectrometer, operating at 0.47 T or a modified Varian VXR instrument tuned to 65.6 MHz (^{1}H), by means of the standard inversion−recovery technique (16 experiments, 2 scans). A typical 90° pulse width was 3.5 μs, and the reproducibility of the T_1 data was ±0.5%. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper−constantan thermocouple (uncertainty ±0.1 °C). The concentration of the complex was typically 0.1−0.4 mM in deionized water.

The proton $1/T_1$ NMRD profiles were measured for 1−2 mM solutions over a continuum of magnetic field strength from 0.00024−0.28 T (corresponding to 0.01−12 MHz proton Larmor Frequency) on a Stelar field-cycling relaxometer. The relaxometer works under complete computer control with an absolute uncertainty in $1/T_1$ of ±1%. Data points at 0.47 T (20 MHz) and 2.1 T (90 MHz) were added to the experimental NMRD profiles and were recorded on the Stelar Spinmaster and on a JEOL EX-90 spectrometer, respectively.

pH Titrations. Luminescence pH titrations were carried out in a background of constant ionic strength (I = 0.1 NaCl, 295 K) on solutions with absorbances of <0.3 at wavelengths ≥λ_{ex} to avoid any errors due to the inner filter effect. Solutions were made basic by addition of 1 M NaOH and titrated to acidic pH using small aliquots (typically 0.5 μL) of 1 M or 0.1 M HCl. The luminescence spectra were recorded at each point (30−40 points per titration). Excitation wavelengths of 270 nm (sensitization via phenyl chromophore) or 397/355 nm (direct excitation at Eu/Tb) were used to obtain the luminescence spectra. Excitation and emission slits were 2−5 and 1 nm bandpass, respectively. Points were recorded at 1 nm intervals with a 0.25 s integration time.

Standard least-squares fitting techniques were used to determine protonation constants from the luminescence intensity data.

Compounds **4**, **5**, **7b**, and **8b** were synthesized by published procedures.[14,18] Compounds **7a**, **8a**, **7c**, **8c**, **7d**, and **8d** were prepared using the published method.[18]

2-(4-Trifluoromethylphenylsulfonylamino)ethyl(4-trifluoromethylphenylsulfonate) (8a): ^{1}H NMR (300 MHz, $CDCl_3$, δ) 8.02 (2H, d, ArH), 7.97 (2H, d, ArH), 7.85 (2H, d, ArH), 7.80 (2H, d, ArH), 5.06 (1H, t, NH), 4.17 (2H, t, CH_2O), 3.34 (2H, q, CH_2N).

2-(4-Methoxyphenylsulfonylamino)ethyl(4-methoxyphenylsulfonate) (8c): ^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.78 (2H, d, ArH), 7.73 (2H, d, ArH), 6.98 (2H, d, ArH), 6.92 (2H, d, ArH), 5.03 (1H, t, NH), 4.04 (2H, t, CH_2O), 3.88 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.20 (2H, q, CH_2N).

2-(Phenylsulfonylamino)ethyl(phenylsulfonate) (8d): ^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.84 (4H, m, ArH), 7.66 (6H, m, ArH), 5.07 (1H, t, NH), 4.07 (2H, t, CH_2O), 3.24 (2H, q, CH_2N).

***N*-(4-Trifluoromethylphenylsulfonyl)aziridine (7a):** ^{1}H NMR (300 MHz, $CDCl_3$, δ) 8.04 (2H, d, ArH), 7.77 (2H, d, ArH), 2.39 (4H, s, CH_2NCH_2).

***N*-(4-Methoxyphenylsulfonyl)aziridine (7c):** ^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.90 (2H, d, ArH), 7.00 (2H, d, ArH), 3.89 (3H, s, CH_3O), 2.36 (4H, s, CH_2NCH_2).

***N*-(Phenylsulfonyl)aziridine (7d):** ^{1}H NMR (300 MHz, $CDCl_3$, δ) 7.96 (2H, m, ArH), 7.60 (3H, m, ArH), 2.40 (4H, s, CH_2NCH_2).

1,4,7-Tris-[(4′-methoxycarbonyl)-1′-methoxycarbonylbutyl]-1,4,7,10-tetraazacyclododecane (6). 1,4,7,10-Tetraazacyclododecane (0.50 g, 2.9 mmol), **6** (2.24 g, 8.9 mmol) and K_2CO_3 (1.0 g, 7.0 mmol) were heated under argon at 60 °C in dry acetonitrile (15 mL) for 48 h. The solids were removed by filtration, and the solvent was removed under reduced pressure. Column chromatography on silica [gradient elution; CH_2Cl_2 to 30% THF−CH_2Cl_2 to 2.5% MeOH−30% THF−67.5% CH_2Cl_2, R_f = 0.30 (70% THF−CH_2Cl_2)] yielded a pale yellow oil (0.75 g, 38%). ESMS + (m/z) = 689 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.65−3.69 (18H, 4 × s, CO_2CH_3), 3.24−2.80 (4H, br, CH), 2.80−2.35 (24H, br, NCH_2), 1.85−1.60 (16H, br, CH_2). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ): 173.5, 170.9 (CO_2), 63.5, 59.0, 51.5, 51.3, 51.0, 46.2, 33.2, 30.0, 21.0 (br, aliphatic C).

1-[2′-(4-Methoxyphenylsulfonylamino)ethyl]-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (1c). A solution containing **4** (0.23 g, 0.45 mmol) and **7c** (0.10 g, 0.47 mmol) in dry acetonitrile (25 mL) was heated at reflux for 18 h. The solvent was removed under reduced pressure and column chromatography on silica [gradient elution; CH_2Cl_2 to 4% MeOH−CH_2Cl_2, R_f = 0.36 (10% MeOH−CH_2Cl_2)] yielded the *tert*-butyl ester of **1c** as a colorless oil (0.14 g, 42%). ESMS + (m/z) = 728 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.70 (2H, d, ArH), 6.91 (2H, d, ArH), 6.09 (1H, t, NH), 3.80 (3H, s, OCH_3), 3.37 (2H, s, CH_2NSO_2), 3.40−2.05 (24H, br, CH_2N), 1.44 (18H, s, $C(CH_3)_3$), 1.41 (9H, s, $C(CH_3)_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ): 173.0, 172.9 (CO_2), 163.1, 130.4, 129.4, 114.5 (Ar), 83.1, 82.6 ($C(CH_3)_3$), 56.6, 56.0, 55.9, 53.7, 52.4, 49.9 (CH_2N), 54.0−47.0 (br, CH_2N), 28.3, 28.1 ($C(CH_3)_3$).

A solution containing the *tert*-butyl ester (0.10 g, 0.14 mmol) in 80% trifluoroacetic acid−CH_2Cl_2 (10 mL) was stirred for 5 h. Solvents were removed under reduced pressure followed by the addition and then evaporation of CH_2Cl_2 (3 × 10 mL) and finally ether (10 mL). The residue was taken up in H_2O, filtered, and lyophilized to give a white solid of the ditrifluoroacetate salt (0.08 g, 77%). ESMS + (m/z) = 560 $[M + H]^+$. ^{1}H NMR (300 MHz, D_2O, δ): 7.62 (2H, d, ArH), 6.95 (2H, d, ArH), 3.88 (2H, br, CH_2NSO_2), 3.70 (3H, s, OCH_3), 3.60−2.80 (24H, br, CH_2N). ^{13}C NMR (75.4 MHz, D_2O, δ): 176.7, 172.8 (CO_2), 163.1, 129.7, 129.3, 115.0 (Ar), 56.9 (OCH_3), 56.2, 55.9, 52.1, 51.3, 50.9, 49.4, 48.7, 38.7 (aliphatic C).

1-[2′-(4-Trifluoromethylphenylsulfonylamino)ethyl]-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (1a). The compound was synthesized by a method similar to that of **1c**.

***tert*-Butyl ester of 1a:** ESMS + (m/z) = 766 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.05 (2H, d, ArH), 7.68 (2H, d, ArH), 7.22 (1H, t, NH), 3.41 (2H, s, CH_2NSO_2), 3.50−2.10 (24H, br, CH_2N), 1.43 (18H, s, $C(CH_3)_3$), 1.40 (9H, s, $C(CH_3)_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ): 177.7, 177.3 (CO_2), 147.7, 132.6, 130.8 (Ar), 87.4, 87.0 ($C(CH_3)_3$), 61.3, 60.2, 58.0, 53.1, 45.7 (br CH_2N), 32.8, 32.6 ($C(CH_3)_3$).

1a: ESMS + (m/z) = 598 $[M + H]^+$. ^{1}H NMR (D_2O, δ): 7.88 (2H, d, ArH), 7.80 (2H, d, ArH), 3.60−2.80 (26H, br, CH_2N). ^{13}C NMR (D_2O, δ): 177.0, 171.2 (CO_2), 141.9, 127.6, 126.9 (Ar), 56.8, 55.9, 52.0, 51.6, 50.8, 48.2, 48.3, 38.9 (aliphatic C).

1-[2′-(4-Methylphenylsulfonylamino)ethyl]-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (1b). The compound was synthesized by a method similar to that of **1c**.

***tert*-Butyl ester of 1b:** ESMS + (m/z) = 712 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.73 (2H, d, ArH), 7.31 (2H, d, ArH), 3.38 (2H, s, CH_2NSO_2), 3.42−2.41 (24H, br, CH_2N), 2.26 (3H, s, CH_3), 1.45 (18H, s, $C(CH_3)_3$), 1.42 (9H, s, $C(CH_3)_3$).

1b: ESMS + (m/z) = 544 $[M + H]^+$. ^{1}H NMR (D_2O, δ): 7.62 (2H, d, ArH), 7.33 (2H, d, ArH), 3.60−2.95 (26H, br, CH_2N), 2.28 (3H, s, CH_3).

1-[2′-(4-Methoxyphenylsulfonylamino)ethyl]-4,7,10-tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane (2c). A solution containing **5** (0.34 g, 0.53 mmol), **7c** (0.12 g, 0.56 mmol) and Na_2CO_3 (0.06 g, 0.57 mmol) in dry acetonitrile (15 mL) was heated at reflux for 18 h. The solution was filtered and the solvent removed under reduced pressure. Column chromatography on silica [gradient elution; CH_2Cl_2 to 4% MeOH−CH_2Cl_2, R_f = 0.40 (10% MeOH−CH_2Cl_2)] yielded the hexamethyl ester of **2c** as a colorless oil (0.25 g, 55%). ESMS + (m/z) = 860 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.51 (2H, d, ArH), 6.91 (2H, d, ArH), 3.97 (3H, s, $ArOCH_3$), 3.66 (2H, s, CH_2NSO_2), 3.61 (18H, s, OCH_3), 3.31 (3H, t, CH), 3.21−1.63 (30H, br, CH_2N). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ): 176.7, 176.5, 176.1, 175.8 (CO_2), 165.7, 133.4, 132.4, 117.5 (Ar), 65.1, 58.7, 56.7, 54.7 (br), 54.3, 53.5 (aliphatic C), 33.8 (CO_2CH_3).

A suspension of the hexamethyl ester (0.21 g, 0.24 mmol) in 1 M LiOH (10 mL) was heated at 80 °C for 18 h. The resulting solution was loaded onto a DOWEX 50W strong acid cation-exchange column (H^+ form), washed with water, and eluted with 12% aqueous ammonia solution. The solvent was removed under reduced pressure, and the residue was taken up in water and then lyophilized to give a white powder of the diaqua-diammonium salt of **2c** (0.17 g, 84%). ESMS + (m/z) = 776 $[M + H]^+$. ^{1}H NMR (D_2O, δ): 7.60 (2H, d, ArH), 6.93 (2H, d, ArH), 3.70 (3H, s, OCH_3), 3.60−1.50 (35H, br, CH_2N, CH). ^{13}C NMR (D_2O, δ): 186.8, 186.1, 185.9, 185.7 (CO_2), 167.7, 133.9, 133.8, 119.5 (Ar), 68.7, (br, aliphatic C), 60.4 (OCH_3), 55.7, 51.5, 39.4 (br, aliphatic C).

1-[2′-(4-Trifluoromethylphenylsulfonylamino)ethyl]-4,7,10-tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane (2a). The compound was synthesized by a method similar to that of **2c**.

Hexamethyl ester of 2a: ESMS + (*m*/*z*) = 898 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.10 (2H, d, ArH), 7.72 (2H, d, ArH), 3.63 (18H, s, OCH_3), 3.60−1.70 (35H, br, CH_2N). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ): 173.3, 172.9 (CO_2), 149.2, 127.7, 126.1, (Ar), 62.1, 53.5, 51.6, (br, aliphatic C), 30.6 (CO_2CH_3).

2a: ESMS + (*m*/*z*) = 814 $[M + H]^+$. ^{1}H NMR (300 MHz, D_2O, δ): 7.92 (2H, d, ArH), 7.85 (2H, d, ArH), 3.80−1.60 (35H, br, CH_2N, CH). ^{13}C NMR (75.4 MHz, D_2O, δ): 181.5, 180.9, (CO_2), 146.5, 130.2, 129.4 (Ar), 66.3, 49.1, 33.4, 26.2 (br, aliphatic C).

1-[2′-(Phenylsulfonylamino)ethyl]-4,7,10-tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane (2b). The compound was synthesized by a method similar to that of **2c**.

Hexamethyl ester of 2b: ESMS + (*m*/*z*) = 830 $[M + H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.90 (2H, m, ArH), 7.48 (3H, m, ArH), 3.62 (18H, s, OCH_3), 3.60−1.75 (35H, br, CH_2N). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ): 173.2, 172.9 (CO_2), 167.4, 132.3, 129.7, 127.1, (Ar), 51.7, (br, aliphatic C), 30.7 (CO_2CH_3).

2b: ESMS + (*m*/*z*) = 746 $[M + H]^+$. ^{1}H NMR (300 MHz, D_2O, δ): 7.74 (2H, m, ArH), 7.55 (3H, m, ArH), 3.70−1.65 (35H, br, CH_2N, CH). ^{13}C NMR (75.4 MHz, D_2O, δ): 183.3, 183.1, (CO_2), 140.3, 136.5, 132.4, 129.5 (Ar), 67.1, 54.2, 49.3, 40.5, 37.1, 26.1 (br, aliphatic C).

1-[(2′-(4-Methoxyphenylsulfonaminoethyl]-4,7,10-tris-[(4′-carboxy)-1′-carboxybutyl]-1,4,7,10-tetraazacyclododecane (3b). A solution of **6** (0.106 g, 0.15 mmol), **7c** (0.035 g, 0.16 mmol) and Na_2CO_3 (0.017 g, 0.16 mmol) in dry acetonitrile (5 mL) was heated at reflux for 48 h. The solution was filtered and the solvent removed under reduced pressure. Following column chromatography on silica [gradient elution; CH_2Cl_2 to 30% THF−CH_2Cl_2, R_f = 0.45 (30% THF−CH_2Cl_2)] the hexamethyl ester of **3b** was obtained as a pale yellow oil (0.118 g, 85%).. HR-ESMS + (*m*/*z*): $[M - H]^+$ calcd for $C_{41}H_{67}N_5O_{15}S_1Na_1$, 924.4243; found, 924.4252.

A suspension of the hexamethyl ester (0.118 g, 0.13 mmol) in 1 M LiOH (10 mL) was heated at 80 °C for 18 h. The resulting solution was loaded onto a DOWEX 50W strong acid cation-exchange column (H^+ form), washed with water, and eluted with 12% aqueous ammonia solution. The solvent was removed under reduced pressure, and the residue taken up in water and then lyophilized to give a white powder of the diaqua-diammonium salt of **3b** (0.067 g, 65%). ESMS (*m*/*z*) = 817 $[M - H]^-$. ^{1}H NMR (200 MHz, D_2O, δ): 7.70 (2H, d, ArH), 7.00 (2H, d, ArH), 3.37 (3H, m, CH), 3.30−2.09 (26H, br, CH_2N), 1.90−1.20 (12H, b, $CH_2CH_2CO_2$). ^{13}C NMR (50.3 MHz, D_2O, δ): 185.4−184.8 (CO_2), 165.7, 131.9, 117.5, (Ar), 66.8−66.0, (br, aliphatic C), 58.4 (OCH_3), 54.0−49.6 (br, aliphatic C), 39.8, 32.0, 26.2 (br, aliphatic C).

1-[(2′-(4-Trifluoromethylphenylsulfonaminoethyl]-4,7,10-tris-[(4′-carboxy)-1′-carboxybutyl]-1,4,7,10-tetraazacyclododecane (3a). Compound **3a** was synthesized in a manner similar to that for **3b**.

ESMS (*m*/*z*) = 854 $[M - H]^-$. ^{1}H NMR (200 MHz, D_2O, δ): 7.99 (4H, m, ArH), 3.46 (3H, m, CH), 3.30−2.09 (26H, br, CH_2N, CH_2CO_2), 1.90−1.20 (12H, b, $CH_2CH_2CO_2$). ^{13}C NMR (50.3 MHz, D_2O, δ): 184.7−180.7 (CO_2), 144.0, 136.6, 130.2, 129.4, 128.8, (Ar), 68.5−66.0, (br, aliphatic C), 58.4 (OCH_3), 51.2−49.7 (br, aliphatic C), 39.4, 33.0−28.6, 25.8 (br, aliphatic C).

Lanthanide Complexes. All of the complexes were synthesized using the method outlined for **Eu[1c]** using the appropriate $LnCl_3 \cdot 6H_2O$ starting material. For **Ln[2a−c]** and **Ln[3a,b]** the complexes were extracted from the lyophilized salt mixture using 20% MeOH−CH_2Cl_2 and 40% MeOH−CH_2Cl_2, respectively. Each complex was rather hygroscopic and was stored in a desiccator under inert gas.

Europium(III) 1-[2′-(4-Methoxyphenylsulfonylamino)ethyl]-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, [Eu·1c]. An aqueous solution (10 mL) containing **1c** (13 mg, 22 μmol) and $EuCl_3 \cdot 6H_2O$ (10 mg, 27 μmol) was adjusted to pH 5.5 using 1 M NaOH and heated at 90 °C for 18 h. After the solution was cooled, the pH was raised to 10.0, and the solution was filtered through a Celite plug to remove the excess europium as $Eu(OH)_3$. The pH was then lowered to 5.0 and the solution lyophilized. The compound was extracted from the salt residues with 5% MeOH−CH_2Cl_2 and filtered, and the solvent was removed under reduced pressure. The residue was taken up in water and lyophilized to give a white powder (14 mg, 90%). HR-ESMS (*m*/*z*): $[M - H]^-$ calcd for $C_{23}H_{33}N_5O_9S_1Eu_1$, 708.1211; found, 708.1210. ^{1}H NMR (300 MHz, D_2O pD = 10, 277 K, δ): partial assignment 37.5 (1H, s, ring CH axial), 30.7 (1H, s, ring CH axial), 27.9 (1H, s, ring CH axial), 18.9 (1H, s, ring CH axial), typical of a square-antiprismatic geometry about Eu, the remaining resonances span 8.8 to −31.1 ppm. IR (KBr and MeOH cm^{-1}) ν_{SO} = 1329 (asymm), 1159 (symm).

Europium(III) 1-[2′-(4-Trifluoromethylphenylsulfonylamino)ethyl]-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, [Eu·1a]: HR-ESMS (*m*/*z*): $[M - H]^-$ calcd for $C_{23}H_{30}F_3N_5O_8S_1Eu_1$, 746.0980; found, 746.0982. ^{1}H NMR (300 MHz, D_2O pD = 10, 277 K, δ): partial assignment 36.8 (1H, s, ring CH axial), 30.6 (1H, s, ring CH axial), 28.8 (1H, s, ring CH axial), 20.4 (1H, s, ring CH axial), typical of a square-antiprismatic geometry about Eu, the remaining resonances span 12.2 to −29.3 ppm.

Europium(III) 1-[2′-(4-Methylphenylsulfonylamino)ethyl]-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, [Eu·1b]: HR-ESMS (*m*/*z*): $[M - H]^-$ calcd for $C_{23}H_{33}N_5O_8S_1Eu_1$, 692.1262; found, 692.1258. ^{1}H NMR (300 MHz, D_2O pD = 10, 277 K, δ): partial assignment 39.0 (1H, s, ring CH axial), 31.9 (1H, s, ring CH axial), 29.2 (1H, s, ring CH axial), 19.8 (1H, s, ring CH axial), typical of a square-antiprismatic geometry about Eu, the remaining resonances span 8.9−31.9 ppm.

Europium(III) 1-[2′-(4-Methoxyphenylsulfonylamino)ethyl]-4,7,10-tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane, [Eu·2c]: HR-ESMS (*m*/*z*): $[M - H]^-$ calcd for $C_{32}H_{45}N_5O_{15}S_1Eu_1$, 924.1845; found, 924.1842. ^{1}H NMR (300 MHz, D_2O pD = 10, 298K, δ): partial assignment 36.7 (1H, s, ring CH axial), 35.7 (1H, s, ring CH axial), 33.7 (1H, s, ring CH axial), 27.4 (1H, s, ring CH axial), typical of a square-antiprismatic geometry about Eu, the remaining resonances span 10.9 to −29.8 ppm.

Europium(III) 1-[2′-(4-Trifluoromethylphenylsulfonylamino)ethyl]-4,7,10-tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane, [Eu·2a]: HR-ESMS (*m*/*z*): $[M - H]^-$ calcd for $C_{32}H_{42}F_3N_5O_{14}S_1Eu_1$, 962.1614; found, 962.1618. ^{1}H NMR (300 MHz, D_2O pD = 10, 298 K, δ): partial assignment 36.3 (1H, s, ring CH axial), 35.8 (1H, s, ring CH axial), 33.4 (1H, s, ring CH axial), 28.3 (1H, s, ring CH axial), typical of a square-antiprismatic geometry about Eu, the remaining resonances span 11.2 to −28.8 ppm.

Europium(III) 1-[2′-(Phenylsulfonylamino)ethyl]-4,7,10-tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane, [Eu·2b]: HR-ESMS (*m*/*z*): $[M - H]^-$ calcd for $C_{31}H_{43}N_5O_{14}S_1Eu_1$, 894.1740; found, 894.1737. ^{1}H NMR (300 MHz, D_2O pD = 10, 298K, δ): partial assignment 36.0 (1H, s, ring CH axial), 35.5 (1H, s, ring CH axial), 33.2 (1H, s, ring CH axial), 27.2 (1H, s, ring CH axial), typical of a square-antiprismatic geometry about Eu, the remaining resonances span 10.8 to −29.8 ppm.

Europium(III) 1-[(2′-(4-Methoxyphenylsulfonaminoethyl]-4,7,10-tris-[(4′-carboxy)-1′-carboxybutyl]-1,4,7,10-tetraazacyclododecane, [Eu·3b]: HR-ESMS (*m*/*z*): $[M - H]^-$ calcd for $C_{35}H_{51}N_5O_{15}S_1Eu_1$, 966.2330; found, 966.2315. ^{1}H NMR (D_2O pD = 10, 298K, δ): partial assignment 40.8 (1H, s, ring CH axial), 32.8 (1H, s, ring CH axial), 31.6 (1H, s, ring CH axial), 28.0 (1H, s, ring CH axial), typical of a square-antiprismatic geometry about Eu, the remaining resonances span 12.7 to −32.2 ppm.

Europium(III) 1-[(2′-(4-Trifluoromethylphenylsulfonaminoethyl]-4,7,10-tris-[(4′-carboxy)-1′-carboxybutyl]-1,4,7,10-tetraazacyclododecane, [Eu·3a]: HR-ESMS (*m*/*z*): $[M - H]^-$ calcd for $C_{35}H_{48}F_3N_5O_{14}S_1Eu_1$, 1004.2083; found, 1004.2081. ^{1}H NMR (D_2O pD = 10, 298 K, δ): partial assignment 40.6 (1H, s, ring CH axial), 33.1 (1H, s, ring CH axial), 28.6 (2H, s, ring CH axial), typical of a square-antiprismatic geometry about Eu, the remaining resonances span 14.5 to −28.6 ppm.

Gadolinium(III) 1-[2′-(4-Methoxyphenylsulfonylamino)ethyl]-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, [Gd·1c]: HR-ESMS (*m*/*z*): $[M - H]^-$ calcd for $C_{23}H_{33}N_5O_9S_1Gd_1$, 713.1240; found, 713.1243.

Gadolinium(III) 1-[2′-(4-Trifluoromethylphenylsulfonylamino)-ethyl]-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, [Gd·1a]:HR-ESMS (*m*/*z*): [M − H]$^-$ calcd for $C_{23}H_{30}F_3N_5O_8S_1Gd_1$, 751.1008; found, 751.1009.

Gadolinium(III) 1-[2′-(4-Methylphenylsulfonylamino)ethyl]-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, [Gd·1b]: HR-ESMS (*m*/*z*): [M − H]$^-$ calcd for $C_{23}H_{33}N_5O_8S_1Gd_1$, 697.1291; found, 697.1295.

Gadolinium(III) 1-[2′-(4-methoxyphenylsulfonylamino)ethyl]-4,7,10-tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane, [Gd·2c]: HR-ESMS (*m*/*z*): [M + Na − 2H]$^-$ calcd for $C_{32}H_{45}N_5O_{15}S_1Na_1Gd_1$, 951.1694; found, 951.1701.

Gadolinium(III) 1-[2′-(4-Trifluoromethylphenylsulfonylamino)-ethyl]-4,7,10-tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane, [Gd·2a]: HR-ESMS (*m*/*z*): [M − H]$^-$ calcd for $C_{32}H_{42}F_3N_5O_{14}S_1Gd_1$, 967.1642; found, 967.1635.

Gadolinium(III) 1-[2′-(Phenylsulfonylamino)ethyl]-4,7,10-tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane, [Gd·2b]: HR-ESMS (*m*/*z*): [M − H]$^-$ calcd for $C_{31}H_{43}N_5O_{14}S_1Gd_1$, 899.1768; found, 899.1761.

Gadolinium(III) 1-[(2′-(4-Methoxyphenylsulfonaminoethyl]-4,7,-10-tris-[(4′-carboxy)-1′-carboxybutyl]-1,4,7,10-tetraazacyclododecane, [Gd·3b]: HR-ESMS (*m*/*z*): [M − H]$^-$ calcd for $C_{35}H_{51}N_5O_{15}S_1Gd_1$, 971.2344; found, 971.2334.

Gadolinium(III) 1-[(2′-(4-Trifluoromethylphenylsulfonaminoethyl]-4,7,10-tris-[(4′-carboxy)-1′-carboxybutyl]-1,4,7,10-tetraazacyclododecane, [Gd·3a]: HR-ESMS (*m*/*z*): [M − H]$^-$ calcd for $C_{35}H_{48}F_3N_5O_{14}S_1Gd_1$, 1009.2112; found, 1009.2109.

Terbium(III) 1-[2′-(4-Methoxyphenylsulfonylamino)ethyl]-4,7,10-tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane, [Tb·2c]: HR-ESMS (*m*/*z*): [M − H]$^-$ calcd for $C_{32}H_{45}N_5O_{15}S_1Tb_1$, 930.1887; found, 930.1883. ^{1}H NMR (65 MHz, D_2O pD = 10, 298 K, δ): partial assignment −442 (1H, s, ring CH axial), −505 (1H, s, ring CH axial), −540 (1H, s, ring CH axial), −561 (1H, s, ring CH axial), typical of a square-antiprismatic geometry about Eu, the remaining resonances span 630 to −180 ppm.

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Supporting Information Available: Several Figures showing the pH dependence of Eu spectral emission and the relaxivity of [Gd·**3a**], in differing salt backgrounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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