The Selectivity of Reversible Oxy-Anion Binding in Aqueous Solution at a Chiral Europium and Terbium Center: Signaling of Carbonate Chelation by Changes in the Form and Circular Polarization of Luminescence Emission

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Abstract: Reversible anion binding in aqueous media at chiral Eu^{III} and Tb^{III} centers has been characterized by ^1H NMR and by changes in the emission intensity and circular polarization following direct or sensitized (365 nm) excitation via an alkylphenanthridinium chromophore. Using a series of heptadentate tri-amide or polycarboxylate ligands, the affinity for CO_3^{2-}/HCO_3^-, phosphate, lactate, citrate, acetate, and malonate at pH 7.4 was found to decrease as a function of the overall negative charge on the complex: citrate and malonate bound most strongly, and lactate and hydrogen carbonate also formed chelated ternary complexes in which displacement of both of the metal-bound water molecules occurred, which was confirmed by VT 17-O NMR measurements of the corresponding Gd complexes. The binding of carbonate was studied in particular, and ^1H NMR and CPL data were obtained that were consistent with the formation of a complex with a reduced helical twist about the metal center. Monohydrogen phosphate was bound in a monodentate manner, giving a mono-aqua adduct. The binding of carbonate to cationic Eu complexes in the presence of a simulated extra-cellular anionic background at pH 7.4 was monitored by variation in the emission intensity, ratio of intensities (615/594 nm), and dissymmetry factors as a function of added total carbonate.

Introduction

The selective recognition of anions in aqueous media by natural and synthetic receptors constitutes an important element of supramolecular chemistry.[1] Despite the importance of anion recognition in biological chemistry,[2,3] the majority of published work with synthetic receptors has concentrated on their behavior in nonaqueous solvents. This is not surprising, because anions generally have large free energies of hydration, ΔG_{hyd} (HCO_3^-, -335 kJ mol^{-1}; $H_2PO_4^-$ and F^-, -465 kJ mol^{-1}; SO_4^{2-}, -1080 kJ mol^{-1}; CO_3^{2-}, -1315 kJ mol^{-1}),[4] possess different ionic geometries (trigonal planar, tetrahedral, octahedral), and often exhibit pH-dependent speciation. These features necessarily make it difficult to design a selective synthetic receptor for a given anion. Obvious exceptions to this generalization include protonated polyamines which are used to bind, compact, and transport nucleic acids and the large number of natural receptors and the smaller cohort of synthetic systems possessing guanidinium groups.[1]

An obvious approach to the development of anion binding in aqueous media is to study the anion affinity of well-defined, positively charged mono- and dinuclear metal complexes bound to one or two labile water molecules. Such features are also of interest to those seeking to model the structure and function of metalloenzymes. Indeed, there are numerous reports of anion ligation to zinc and copper complexes, and their behavior in the presence of various anions[5−10] has been studied. With this in mind, we set out to explore the interaction of the common bioactive anions with an aquated metal center. Complexes of the lanthanide ions with macrocyclic heptadentate ligands

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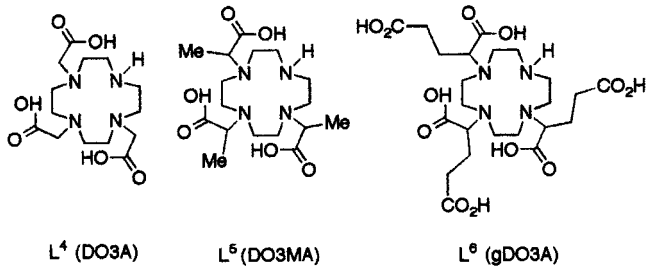
are particularly appropriate for this purpose for several reasons. First, the 1:1 ML complexes are well-defined and relatively stable in aqueous solution and may be studied by NMR and luminescence methods. Second, the lanthanide ion is likely to be bound to one or two water molecules, and the solution hydration state of the aquated and anion-bound complex may be estimated for Eu and Tb using established methodology.[11] Third, stepwise displacement of water molecules by a coordinating anion is signaled by an increase in the lifetime of the lanthanide excited state and the emission intensity: coordinated and closely diffusing OH (and NH) oscillators have been shown to quench the $^5D_0$ and $^5D_4$ excited states of Eu and Tb in a defined manner.[11] Finally, the $\Delta J = 2$ and $\Delta J = 4$ transitions in the Eu emission spectrum are hypersensitive[12a] and are perturbed significantly by variations in the Eu coordination environment. Moreover, the splitting and intensity of the $\Delta J = 1$ manifold is sensitive to the local symmetry at the metal center and to the magnetic anisotropy factor, D, which in turn is a function of the nature of the ligand and axial donor atoms and their polarizability.[12b]

Accordingly, we set out to study the anion binding behavior of the lanthanide complexes of the chiral ligands L^{1a} (the *p*-carboxymethyl derivative L^2), its N-methylated analogue L^{1b}, and a further derivative, L^{1c}, which bears an N-alkylphenanthridinium group to allow near-UV excitation.[13] In each case, the (*RRR*) and (*SSS*) complexes may be prepared to allow the polarization of the metal-based emission to be monitored.[14] Changes in the circularly polarized luminescence (CPL) from the Eu (or Tb) excited state are sensitive to the nature (e.g., Δ vs Λ) and degree of local helicity, because this mixes the electric and magnetic dipole transition moments, enhancing the "allowedness" (intensity) of a given transition.[15] Thus, anion binding at the lanthanide center may, in principle, be signaled by changes in the form and polarization of the CPL.[16] For purposes of comparison, we also prepared the lanthanide complexes of the alanine derivative L^{3a}[17] and the complexes of ligands L^4, L^5, and L^6 that possess zero or net overall negative charge at ambient pH. In this series, the reduction of residual charge density at the lanthanide center and the variation in the peripheral electrostatic gradient was expected to modulate the affinity of the complex for a given anion.



Ligand and Complex Synthesis. Reaction of three equivalents of (*R*)-N-(2-chloroethanoyl)-2-phenyl-ethylamine[13] with 1-(*p*-methoxyphenylsulfonyl)-1,4,7,10-tetraazacyclododecane (Cs_2CO_3, DMF, 70 °C), followed by reductive deprotection (Na, liq. NH_3, THF-EtOH) afforded the ligand L^{1a}. Alternatively, direct reaction of 1,4,7,10-tetraazacyclododecane (cyclen) with 3 equiv of the same α-haloamide in EtOH, using Et_3N as the base, afforded a mixture of the di-, tri-, and tetra-amides from which L^{1a} was separated by chromatography on neutral alumina. Using similar methods (Scheme 1), the *p*-CO_2Me derivative L^2 was prepared,[18] and the N-methylated ligand L^{1b} was isolated following reductive amination using the Eschweiler-Clarke procedure. A related procedure using (*S*)-ethyl-*N*-2-chloroethanoyl-alanate allowed the isolation of L^{3b} following chromatographic purification on silica gel (eluent CH_2Cl_2; 0−3% $^iPr_2NH$). In this case, the lanthanide complex was prepared in dry MeCN using the appropriate trifluoromethanesulfonate, and basic hydrolysis followed by cation exchange chromatography yielded the complex, for example, $[EuL^{3a}]^{3+}$.

For the synthesis of L^{1c}, it was not found possible to isolate significant quantities of the desired product by N-alkylation of L^{1a} with 2-bromomethylphenanthridine nor via reductive alkylation of L^{1a} using 2-formylphenanthridine in the presence of $NaB(OAc)_3H$. The successful route (Scheme 2) involved monoalkylation of cyclen (by using a 5-fold or excess of the tetraamine) in MeCN, followed by alkylation with the appropriate α-haloamide. Complexation with $Eu(CF_3SO_3)_3$ in dry MeCN followed by N-methylation of the phenanthridine moiety (the Eu serving as a protecting group for the ligand heteroatoms) yielded the target tetracationic complex $[EuL^{1c}]^{4+}$, which has an extinction coefficient of 5×10^3 M^{-1} cm^{-1} at 365 nm in water or aqueous methanol solution.

Variation of Emission Spectra with Anion. The CPL spectra for (*SSS*)-$[EuL^{1a}]^{3+}$ and (*RRR*)-$[EuL^{1a}]^{3+}$, as their triflate salts at pD 6, revealed the expected mirror image behavior (Figure 1a). Examination of the emission spectrum revealed three components for the $\Delta J = 1$ transition, which is consistent with the absence of axial symmetry. A relatively strong $\Delta J = 0$ transition was observed which gave no CPL, because this transition is not magnetic-dipole allowed. This is explained by the fact that the axial crystal field term, which accounts for the electric dipole intensity of the transition, is of odd parity. It cannot, therefore, mix the $^7F_1$ and $^7F_0$ states, both of which arise from the same f^6 configuration and which would be required

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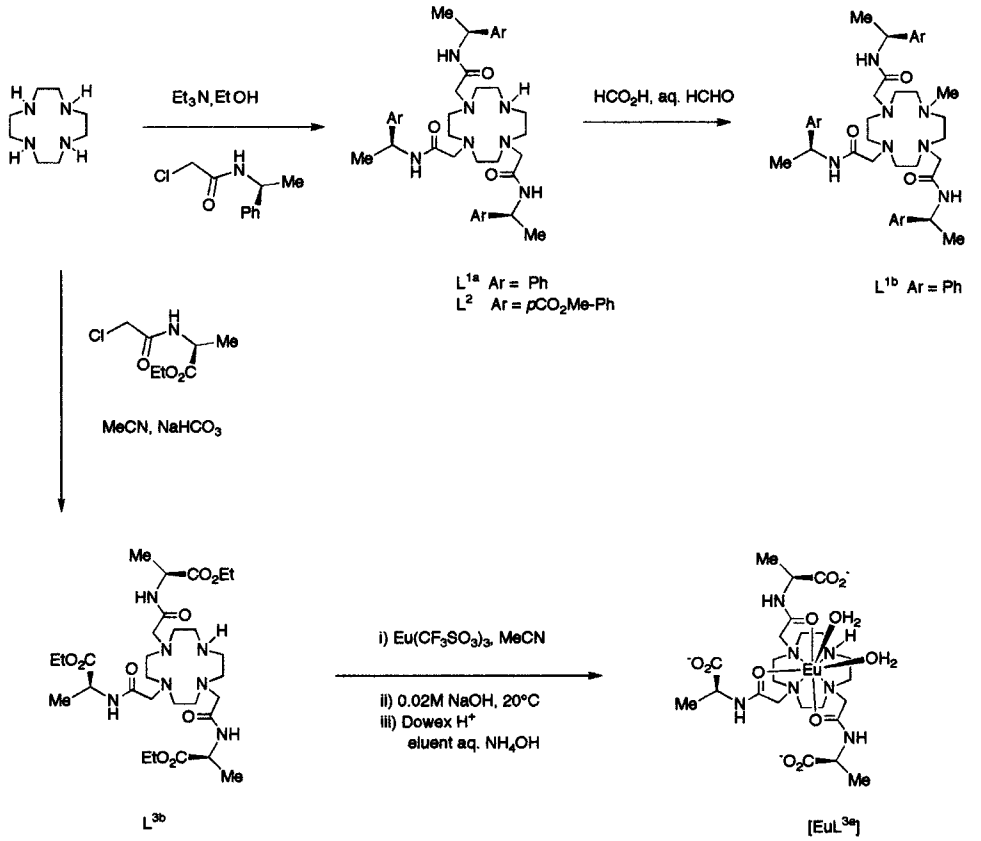
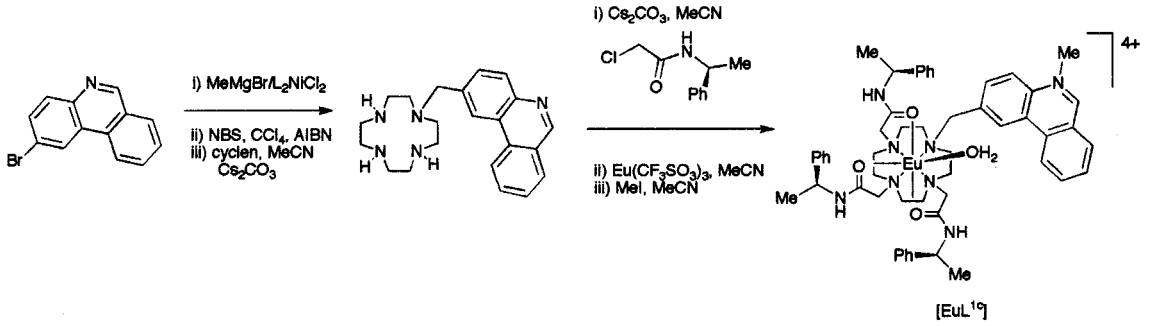
Scheme 1



Scheme 2



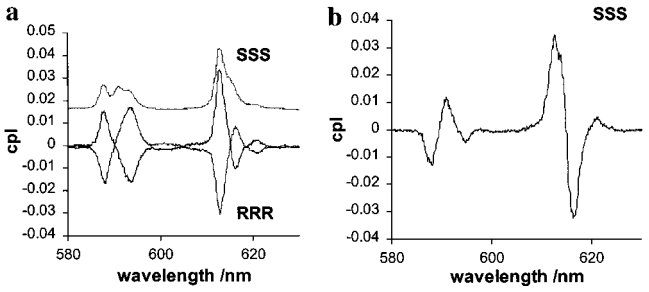


Figure 1. (a) Circularly polarized emission spectrum of (*RRR*)-$[EuL^{1a}](CF_3SO_3)_3$ and the (*SSS*) enantiomer showing the $\Delta J = 1$ and $\Delta J = 2$ transitions (295 K, D_2O), 1 mM complex, pD 6, $\lambda_{exc} = 265$ nm. (b) CPL spectrum for (*SSS*)-$[EuL^{1b}]^{3+}$ under the same conditions; the spectrum for (*SSS*)-$[EuL^{1c}]^{4+}$ was identical.

to give magnetic dipole intensity to the $^5D_0 \rightarrow {}^7F_0$ transition. Thus, the $^5D_0 \rightarrow {}^7F_0$ transition is electric, but not magnetic, dipole-allowed and so shows no CPL.[19] The magnitude of the observed emission dissymmetry factors [(*SSS*)-$EuL^{1a}]^{3+}$ (g_{em}^{593}, -0.035; g_{em}^{612}, $+0.06$) was very similar to those observed with the related (*SSSS*)-Δ C_4-symmetric tetraamide complex[18,19] in D_2O (e.g., g_{em}^{593}, -0.03), which suggests a similar degree of helical twist about the principal axis. The sign of the CPL for a given transition also confirmed that the configuration of the (*SSS*) complex was Δ, as found in the C_4-symmetric series.[19]

The CPL spectra for the triflate salts of the N-alkylated complexes $[EuL^{1b}]^{3+}$ and $[EuL^{1c}]^{3+}$ revealed significant differences in the $\Delta J = 2$ and, more particularly, the $\Delta J = 1$ transitions as compared to $[EuL^{1a}]^{3+}$. Thus, the sign of the three observable transitions for (*SSS*)-$[EuL^{1c}]^{3+}$ followed the sequence $-$, $+$, $-$ for the transitions at 587, 590, and 593 nm, whereas for (*SSS*)-$[EuL^{1a}]^{3+}$, the order was $+$, $-$, with the transition at 591 nm apparently carrying little CPL. In the $\Delta J = 2$ manifold, the (*SSS*)-N-alkylated complexes possessed a value for g_{em} at 616 nm that was 2 times smaller than that of (*SSS*)-$[EuL^{1a}]^{3+}$ (Figure 1b). Evidently, there may be a change in the coordination environment at the metal between $[EuL^{1a}]^{3+}$ and its N-alkylated analogues. CPL and total emission spectra were also recorded

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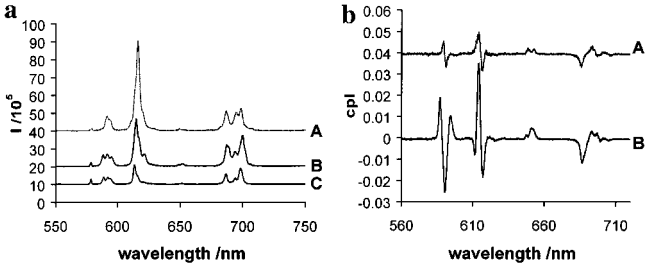


Figure 2. (a) Metal-based emission spectrum of (*SSS*)-[EuL^{1a}](CF$_3$SO$_3$)$_3$ in the presence of (A) $NaHCO_3$, (B) Na_2HPO_4, and (C) at pD 6 in the absence of added anions (295 K, H_2O, 1 mM complex, 10 mM salt). (b) Circularly polarized emission spectra ($\lambda_{exc} = 265$ nm) in the presence of phosphate (B) and hydrogen carbonate (A) under the same conditions.

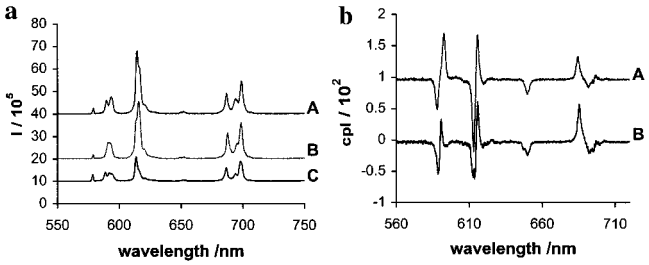


Figure 3. (a) Metal-based emission spectrum of (*RRR*)-[EuL^{1a}](CF$_3$SO$_3$)$_3$ in the presence of (A) sodium lactate, (B) disodium malonate, and (C) at pD 6 (295 K, 1 mM complex, 10 mM salt). (b) CPL spectra ($\lambda_{exc} = 265$ nm) in the presence of lactate (B) and malonate (A) under the same conditions.

for [EuL^{1a}]$^{3+}$ in the presence of 10 equivalents of added anion. Addition of a 100-fold excess gave rise to no further change for the following examples. A comparison of spectra in the presence of $NaHCO_3$ and Na_2HPO_4 (Figure 2) revealed significant differences in each case when compared to the triflate salt. With added hydrogen carbonate, the $\Delta J = 0$ emission band reduced in intensity by a factor of 4, and each observable circularly polarized transition was less intense by a factor of 2 or more. Only two components were resolved in the $\Delta J = 1$ manifold, and the $\Delta J = 2/\Delta J = 1$ intensity ratio doubled. With added $H_2PO_4^-$ (at pH 9, addition of HPO_4^{2-} gave a similar spectrum), another significant change was apparent: the overall emission and CPL intensity was similar to that observed in the presence of triflate alone, but the $\Delta J = 1$ manifold was characterized by a $-, +, -$ sign sequence for the three observed transitions. With [EuL^{1b}]$^{3+}$ and [EuL^{1c}]$^{4+}$, addition of $H_2PO_4^-$ caused relatively little change to the $\Delta J = 1$ and $\Delta J = 2$ manifold, whereas with added HCO_3^-, the $\Delta J = 2$ transition quintupled in intensity and the circular polarization of the 593-nm component ($\Delta J = 1$) was 4 times as large as in the presence of triflate.

A similar series of experiments was carried out to examine the spectral behavior following the addition of sodium lactate, citrate, and malonate to [EuL^{1a}]$^{3+}$ and [EuL^{1b}]$^{3+}$. The behavior of added lactate and citrate was very similar in each case, and with [EuL^{1c}]$^{4+}$, the observed emission and CPL spectra were also almost identical. More marked differences were observed with [EuL^{1a}]$^{3+}$ (Figure 3). The ratio of the $\Delta J = 2/\Delta J = 1$ band intensities remained constant. The splitting of the components of the $\Delta J = 1$ and $\Delta J = 4$ manifold was lower for malonate, and changes in the $\Delta J = 2$ transitions were also apparent, for example, the relative intensity of the 612- and 616-nm bands.

Apparent Affinity Constants for Anion Binding. Given the spectral variations induced by addition of different anions, binding constants were able to be measured that characterized the ternary complex formation. It was found expedient to use 0.1 M collidine/HCl buffers to maintain a pH of 7.4,[20] and titrations were carried out using 1 mM concentrations of complex, with direct excitation of the Eu^{3+} or Tb^{3+} ion ($\lambda_{exc} =$ 395 and 355 nm, respectively). Changes in the intensity of a $\Delta J = 2$ (Eu, e.g., 618 nm) or $\Delta J = 1$ transition (Tb, e.g., 545 nm) were followed as a function of added anion concentration at 295 K. The resultant binding isotherms were fitted to a 1:1 binding model by least-squares iterative analysis, and, for the binding of hydrogen carbonate and hydrogen phosphate, complex formation was assumed to be characterized by an apparent binding constant, *K*, for example, where $K = [LnLHCO_3^{2+}]/[LnL^{3+}][HCO_3^-]$. Representative binding curves (Figures 4 and 5) revealed that particularly large changes in emission intensity occurred following addition of HCO_3^- to [EuL^{1a}]$^{3+}$ or [EuL^{1b}]$^{3+}$. Similar data, giving binding constants within 10%, were obtained by examining the intensity ratio at two emission wavelengths (e.g., 612/591 nm).

Table 1. Estimated Apparent Anion Binding[a] Affinities (log K, ±0.2) for 1:1 Complex Formation (295 K, 0.1 M collidine/HCl buffer, pH 7.4)[b]

	log K			
complex	HCO_3^- [c]	(*S*)-lactate[d]	$CH_3CO_2^-$	HPO_4^{2-} [e]
[EuL^{1a}]$^{3+}$	2.6 (23)	3.7 (64)	<1.0 (<1)	4.15 (77)
[EuL^{1b}]$^{3+}$	3.75 (65)	>4.7 (>87)	2.4 (17)	>4.7 (>87)
[TbL^{1a}]$^{3+}$	3.8 (67)	4.4 (82)	2.3 (15)	≥4.7 (>87)
[TbL^{1b}]$^{3+}$	≥4.7 (>87)	>4.7 (>87)	3.5 (57)	>4.7 (>87)

[a] Values for K for binding malonate and citrate were $= 4 \times 10^4$ M^{-1} in each case; quoted values represent the mean of three determinations. [b] Values in parentheses show the apparent percent of anion bound for a 1:1 ratio of complex/anion (1 mM). [c] The affinity of [EuL^{1a}]$^{3+}$, [EuL^{3a}], and [EuL^{6}]$^{3-}$ for HCO_3^- was also examined at pH 8.9 in a 0.1 M CHES buffer. Apparent affinity constants were 25 (±5) M^{-1} for [EuL^{6}]$^{3-}$, 500 (±50) M^{-1} for [EuL^{3a}] and $=5 \times 10^4$ for [EuL^{1a}]$^{3+}$, in accord with the variation of complex charge.[22] The bound species is believed to be CO_3^{2-}, but the apparent affinity constants measured refer to the putative equilibrium in which $K = [LnLHCO_3^{2+}]/[LnL^{3+}][HCO_3^-]$. The concentration of carbonate at a given pH is readily calculated from the reported species distribution diagrams,[23] so that the affinity constants for carbonate binding will be much higher than presented. [d] Values of K for (*R*)-lactate were within the stated experimental error (mean of at least 2 independent measurements). [e] The pK_a of $H_2PO_4^-$ is 7.23, and the most likely species to bind the tripositive metal complex is the doubly charged anion, monohydrogenphosphate (see text for a discussion). The affinity for HPO_4^{2-} may also be slightly higher than presented in the table.

Values of apparent binding constants are collated in Table 1 and revealed some notable trends. First, the affinity for HCO_3^- under these conditions was apparently lower than that for phosphate, citrate, lactate, and malonate.[21] However, given that it is CO_3^{2-} (and HPO_4^{2-}) rather than HCO_3^- (or $H_2PO_4^-$) which binds, vide infra, then the values given in Table 1 will be much lower than the true affinity constant for carbonate and only slightly lower for hydrogen phosphate binding, respectively. This apparent binding constant was found to increase by an order of magnitude, either following N-alkylation or by replacement of Eu by Tb. Second, the singly charged anion lactate also bound strongly, and again the terbium complex gave the higher apparent binding affinity; N-alkylated complexes again bound

(20) HEPES or related zwitterionic buffers may also be used, but the high affinity of the complexes for HCO_3^-/CO_3^{2-}, which was evident by monitoring the emission spectrum prior to addition of an additional anion, meant that the pH needed to be adjusted up to the required value by addition of base. No evidence for catalysis of CO_2 aquation was found in control experiments at pH 7.4.

(21) Control experiments with added NaX (X = Cl, Br, I, NO_3^-) revealed that none of these anions bound to the triflate salt of [EuL^{1a}] with an affinity constant of greater than ca. 5 M^{-1} at pH 7.4.

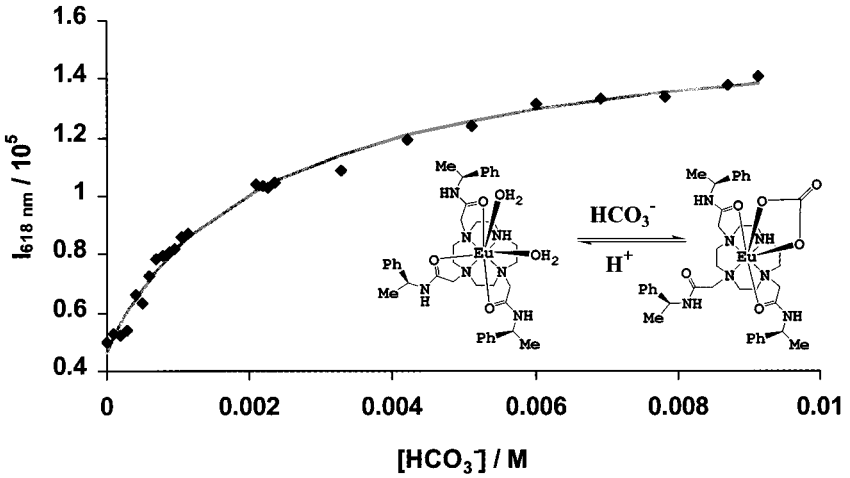


Figure 4. Variation of europium emission intensity (618 nm) for $[EuL^{1a}](CF_3SO_3)_3$ as a function of added $NaHCO_3$ (1 mM complex, pH 7.4, 0.1 M collidine/HCl) showing the fit to the experimental data.

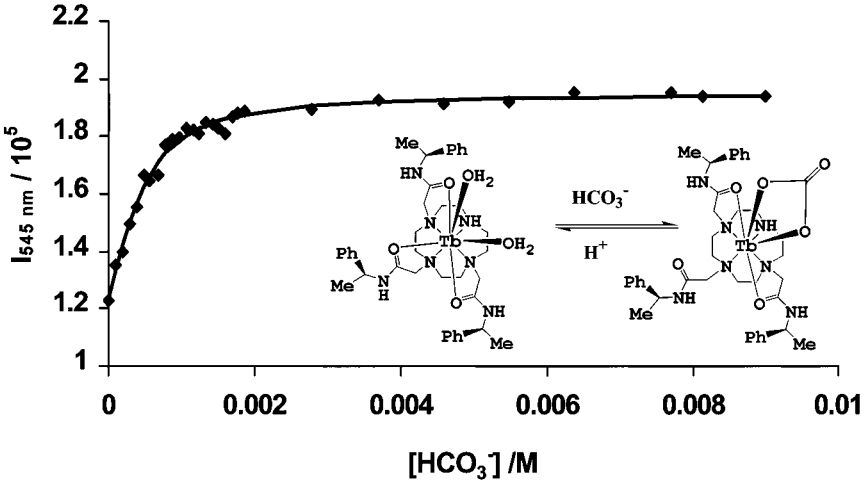


Figure 5. Variation of terbium emission intensity (545 nm) for $[TbL^{1a}](CF_3SO_3)_3$ as a function of added $NaHCO_3$ (1 mM complex, pH 7.4, 0.1M collidine/HCl) showing the fit to the experimental data points.

most strongly. Citrate and malonate also bound to the Ln ion *very* strongly ($K \geq 40\,000\ M^{-1}$ in all cases), whereas acetate bound very weakly to $[EuL^{1a}]^{3+}$ ($K < 10\ M^{-1}$). The affinity for acetate increased by an order of magnitude either by N-methylation or by replacing Eu by Tb, ($[EuL^{1b}]^{3+}$ and $[TbL^{1b}]^{3+}$, $K = 200$ and $3000\ M^{-1}$, respectively). The fact that acetate is bound by the Eu and Tb complexes an order of magnitude more weakly than is CO_3^{2-}/HCO_3^- for each of the 4 complexes examined suggests that the nature of the bound species is quite different.

Finally, in the case of added HCO_3^-, measurements of the apparent affinity constants were also undertaken at pH 8.9 in a CHES buffer. At this pH, the speciation profile of the aqueous carbonate system revealed that the concentration of HCO_3^- was a maximum.[23] However, the positively charged complexes bound HCO_3^- (or, more likely, CO_3^{2-}) very strongly ($K > 5 \times 10^4\ M^{-1}$). Apparent affinity constants[22] were measured for the neutral complex $[EuL^{3b}]$ ($500\ M^{-1}$) and for the anionic complex $[EuL^6]^{3-}$ ($K \sim 25\ M^{-1}$); the reduced affinity followed the order dictated by the degree of electrostatic repulsion.

Degree of Hydration of Ternary Complexes. Having established that for a 1 mM concentration of each of the cationic lanthanide complexes, a 10-fold excess of added citrate/lactate/HPO_4^{2-}/malonate would give rise to a ternary complex that was > 95% associated at pH 7.4, measurements of the mean hydration state for each of these complexes were undertaken. Such information is readily available following analysis of the rate constants for radiative decay of the Eu $^5D_0$ or Tb $^5D_4$ excited state in H_2O and D_2O.[11] Corrections were applied, as appropriate (Table 2), to account for the quenching contribution of closely diffusing (second-sphere) water molecules and, in the case of Eu complexes, the quenching effect of the amide NH oscillators.[11]

The europium and terbium triflate complexes of L^{1a} possess two bound water molecules, and a hydration state of $q = 2$ was also observed in the presence of 0.1 M added Cl^-, Br^-, I^-, and NO_3^-. Addition of fluoride gave a species with $q = 1$ for $[EuL^{1a}]^{3+}$ and $[TbL^{1a}]^{3+}$, whereas malonate gave a $q = 0$ complex in each case. Acetate did not bind very strongly itself to this complex ($K < 10\ M^{-1}$ in Table 1); in the presence of 10 mM acetate, $q \approx 1.5$ with $[EuL^{1a}]^{3+}$, and even when 100 mM acetate was added, $q = 0.85$, which is consistent with either partial hydration or incomplete complex formation. The zero-

(22) Examples of other binding isotherms are given in the Supporting Information.

(23) (a) Covington, A. K. *Chem. Soc. Rev.* **1985**, *14*, 265. Flear, C. T. G.; Covington, A. K.; Stoddart, J. C. *Ann. Intern. Med.* **1984**, *144*, 2285: at pH 8.9, HCO_3^- makes up 90% of all carbonated species; at pH 7.4, the value is ca. 45%, but CO_3^{2-} is <0.2%. (b) For the concentration of anions in extracellular media, see ref *1* and Parker, D. In *Crown Compounds: Towards Future Applications*; Cooper, S. R., Ed.; VCH: New York, 1992; Chapter 4, p 53.

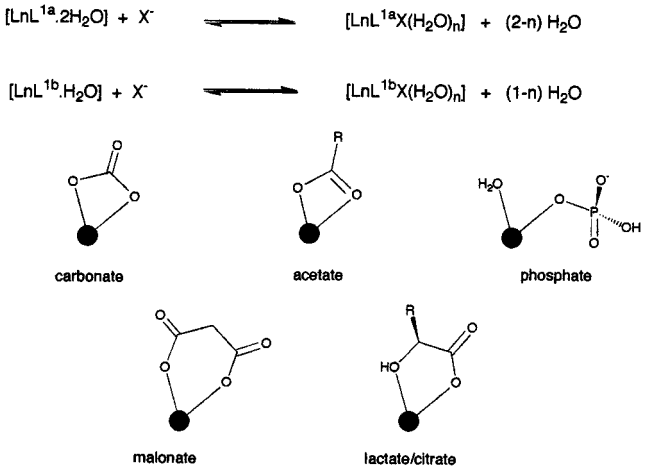
Table 2. Effect of Added Anions on the Rate Constants (±10%) for Depopulation of the Excited States of [LnL^{1a}], [LnL^{1b}], and [EuL^{1c}] (295 K, 1 mM complex, 10 mM anion unless otherwise stated) and Derived Hydration Numbers, q (±20%)[a,b,c]

complex/anion	k_{H_2O}/ms^{-1}	k_{D_2O}/ms^{-1}	Δk_{corr}/ms^{-1}	q
EuL^{1a}/CF_3SO_3	3.85[d]	1.54	1.82	2.2
CO_3^{2-}/HCO_3^-	2.37	1.80	0.08	0.1
F^-	2.70	1.39	0.82	1.0
Cl^-/Br^-/I^-	3.80	1.55	1.76	2.1
HPO_4^{2-} [b]	2.50	1.46	0.55	0.7
lactate	2.17	1.40	0.28	0.3
citrate	2.20	1.45	0.26	0.3
malonate	1.65	1.16	0	0
acetate[e]	3.45	1.69	1.27	1.52
TbL^{1a}/CF_3SO_3	0.84	0.39	0.39	2.0
CO_3^{2-}/HCO_3^-	0.53	0.45	0.02	0.1
F^-	0.61	0.36	0.19	1.0
Cl^-/Br^-/I^-	0.87	0.39	0.42	2.1
HPO_4^{2-}	0.57	0.37	0.14	0.7
lactate	0.56	0.40	0.10	0.5
citrate	0.55	0.41	0.08	0.4
malonate	0.49	0.43	0.0	0
acetate[f]	0.58	0.45	0.07	0.3
EuL^{1b}/CF_3SO_3	2.33[h]	0.53	1.33	1.6
CO_3^{2-}/HCO_3^-	1.39	0.72	0.18	0.2
HPO_4^{2-}	1.41	0.54	0.38	0.5
lactate	1.38	0.68	0.21	0.3
citrate	1.54	0.71	0.34	0.4
malonate	0.91	0.51	0	0
acetate[e]	1.28	0.68	0.11	0.1
TbL^{1b}/CF_3SO_3	0.76[g]	0.40	0.30	1.5
CO_3^{2-}/HCO_3^-	0.50	0.40	0.04	0.2
HPO_4^{2-}	0.51	0.31	0.14	0.7
lactate	0.58	0.46	0.06	0.3
citrate	0.59	0.45	0.08	0.4
malonate	0.49	0.40	0.03	0.1
acetate[f]	0.55	0.47	0.02	0.1
EuL^{1c}/CF_3SO_3	1.60[h]	0.28	0.83	1.0
CO_3^{2-}/HCO_3^-	1.18	0.66	0.03	0
HPO_4^{2-}	1.37	0.33	0.55	0.7
lactate	1.43	0.58	0.36	0.4
citrate	1.35	0.51	0.35	0.4
malonate	0.95	0.50	0	0
acetate[e]	1.25	0.56	0.20	0.2

[a] Apparent values of q were derived using $q^{Eu} = 1.2$ (Δk_{corr}) and $q^{Tb} = 5$ (Δk_{corr}) after correcting for the estimated effect of unbound water molecules (Eu, −0.25 ms^{-1}; Tb, −0.06 ms^{-1}) and for amide NH oscillators (0.08 ms^{-1} each).[11] [b] Addition of SO_4^{2-} behaved very similarly to HPO_4^{2-}, giving apparent q values of 1. [c] Reported values of k represent the mean value of three independent sets of measurements, for each of which the k value quoted was the mean of at least 3 separate determinations. [d] An amine NH oscillator at a distance of 3.1 Å from a Eu ion (i.e., as in [EuL^{1a}]$^{3+}$) has been shown to be twice as efficient at quenching the Eu $^5D_0$ excited state as a bound OH oscillator which is ca. 3 Å distant.[11] The secondary amide NH oscillators are at a distance of ca. 4.5 Å from the Eu ion[18] and quench with a reduced effciency as a result.[11] [e] For [EuL^{1a}] in the presence of 100 mM acetate, $q = 0.85$ ($k_{H_2O} = 2.97$ and $k_{D_2O} = 1.76$ ms^{-1}), consistent with partial complex formation ($K < 10$ M^{-1}, Table 1). For [EuL^{1b}], titration data at pH 7.4 (Table 1) revealed an affinity constant of 250 M^{-1}; data were recorded, therefore, in the presence of 25 mM and 100 mM acetate and gave identical values (±7%). [f] For [TbL^{1a}] and [TbL^{1b}], data are given for addition for 100 mM added acetate. For [TbL^{1a}] in the presence of 0.3 M NaOAc, a q value of 0.1 was found. In contrast, q values of 1 have been observed in the presence of a large excess of acetate with the neutral Tb complex, [TbL^4] in which there is a reduced charge demand at the Ln center. [g] The slower rate of decay for the NH vs NMe Tb complexes reflects the combined quenching effect of the presence of the additional water molecule and the NH oscillator, in the former case.[11,31] [h] Bi-exponential decay was observed with both direct and indirect excitation of the Eu ion, suggesting that $q = 1$ and $q = 2$ species may be present and in slow exchange.

hydration state of the malonate-bound complex strongly suggests the formation of a complex involving an 0,0′-bound six-membered chelate ring (Scheme 3). With [TbL^{1a}]$^{3+}$, acetate was bound more strongly ($K = 200$ M^{-1}, Table 1) and a q value of 0.1 in the presence of 300 mM acetate suggested that *both* waters had been displaced in the ternary complex.

Scheme 3. Postulated Structures of Ternary Anion Complexes



Lactate and citrate again behaved in a parallel manner and gave a residual q value varying between 0.3 and 0.4 (Table 2). In each case with [EuL^{1a}]$^{3+}$, q was 0.3 to 0.4. Given the similarity of their emission spectral behavior and the lower binding affinity of lactate (1−) as compared to malonate (2−), the data is consistent with a ternary complex structure in which a 5-ring chelate is formed. This complex possesses one (exchangeable) OH oscillator close to the Ln ion, which may independently quench the excited state with approximately half the efficiency of a coordinated water molecule. Such an interpretation is consistent with the partial q values obtained.

Independent support for such a $q = 0$ chelated structure has been obtained by VT ^{17}O NMR measurements on the rate of exchange of water in [GdL^{1a}]$^{3+}$ [24] using a 3.4 mM solution of [GdL^{1a}]$^{3+}$ in the presence of 80 mM sodium lactate in 2.6% ^{17}O enriched H_2O. The measured transverse relaxation rate, R_2, at 9.4 T was 40 (±10) s^{-1} and was *independent* of temperature over the range 275−360 K (see Supporting Information for details). Related complexes for which $q = 1$ and $q = 2$ have been shown to produce bell-shaped rate vs T profiles over this T range.[24,25] Indeed, such behavior was exhibited by [GdL^{1a}]$^{3+}$ itself as the triflate salt. In a separate study with a 12 mM solution of [GdL^{1a}](CF_3SO_3)$_3$, the measured R_2 vs T profile was consistent with that expected for a $q = 2$ complex, with $\tau_m = 1.5$ μs at 298 K.[24] Following addition of a 10-fold excess of Na_2HPO_4 (pH 7.45), the water exchange lifetime decreased to 175 ns and the profile fitted very well for a species with $q = 1$, (Figure 6). Similar behavior was exhibited when a 30-fold excess of NaF (pH 5.6) was added to [GdL^{1a}]$^{3+}$: a bell-shaped profile was obtained that fits well to a $q = 1$ species with $\tau_m = 130$ ns.

(24) Further and more detailed aspects of this work will be described in a forthcoming publication. Measurements of the variation of the relaxivity of [GdL^{1a}]$^{3+}$ as a function of added lactate and malonate gave binding constants (298 K) of 4000 M^{-1} and >20 000 M^{-1}, respectively (an example is provided in the Supporting Information), in agreement with those derived from emission spectral changes (Table 2). In the VT-17-O NMR study, the observed profiles (Figure 6 and supporting data) were fitted to the Swift−Connick equations using established procedures (Swift, T. J.; Connick, R. E. *J. Chem. Phys.* **1962**, *37*, 307; **1964**, *41*, 2553. Aime, S.; Botta, M.; Fasano, M.; Paoletti, S.; Terreno, E. *Chem. Eur. J.* **1997**, *3*, 1499). The pronounced dependence of the water exchange rate on the nature of the counteranion has been observed in related (monoaqua) cationic tetraamide complexes.[28]

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

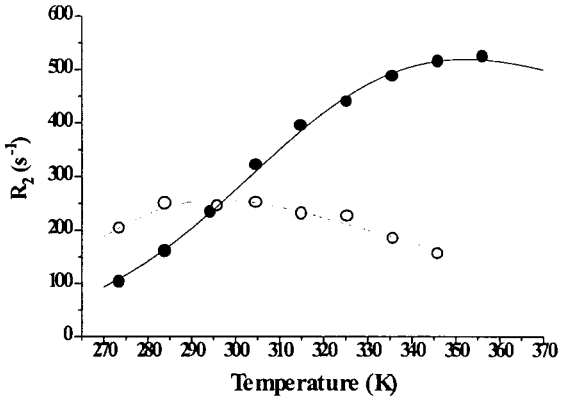


Figure 6. Variation of the ^{17}O transverse relaxation rate with temperature (9.4 *T*) for $[GdL^{1a}](CF_3SO_3)_3$ (12 mM, filled circles) and in the presence of 120 mM Na_2HPO_4 (open circles) showing the fit to the experimental data. The least-squares fitting procedure[24] gave (values in the presence of phosphate in brackets) $\Delta^2 = 2.0(4.1) \times 10^{19}$ s^{-2}, $\tau_v = 3.4(4)$ ps, $k_{ex}298 = 0.6(57) \times 10^6$ s^{-1}, $\Delta H_M = 24.3(12)$ kJ mol^{-1}, $q = 2(1)$. The hyperfine coupling constant was taken to be $A/h = -3.8 \times 10^6$ rad s^{-1} in each case. The faster rate of dissociative water exchange for the phosphate-bound complex reflects the lower net charge at the metal center, labilizing the bound water molecule.

The N-alkylated lanthanide complexes of L^{1b} and L^{1c}, as their triflate salts, seem to be bound to either one or two water molecules on the millisecond time-scale (Table 2). With these complexes, addition of malonate again gave a $q = 0$ adduct; citrate and lactate behaved very similarly, and adding HCO_3^- also displaced the inner-sphere water to give a $q = 0$ species. For the Eu and Tb complexes of these N-alkylated ligands, higher binding affinities for acetate had been revealed ($K =$ 200 and 3000 M^{-1} respectively, Table 1), and the measured q values indicated that no residual inner sphere water was bound. Thus acetate, like HCO_3^-, must also chelate, but with a reduced affinity. The higher affinity constant, measured following the addition of HCO_3^-, is consistent with preferential binding of the symmetrical carbonate dianion (Scheme 3). Support for this hypothesis comes from a comparison of the "fully bound" (acetate vs carbonate) $[EuL^{1b}]^{3+}$ emission spectra; in each complex, the $\Delta J = 2$ band was about five times as intense as the $\Delta J = 1$ manifold, but the form of the hypersensitive $\Delta J = 4$ manifold was distinctly different (see Supporting Information for comparative spectra).

The addition of HPO_4^{2-} at pH 8 gave a similar q value of 0.7 to that obtained at pH 5.5 with added $H_2PO_4^-$. Given the similarity of the emission spectra in each case, such behavior suggests that the phosphate adduct (as with $[EuL^{1a}]^{3+}$) involves monodentate ligation of a doubly charged phosphorus−oxygen donor, with a water molecule still bound to the Ln center (Scheme 3). Such an interpretation is consistent with the established tendency of phosphate to act as a mono-dentate ligand to a single metal center[26a] and is inconsistent with phosphate chelation. A recent database analysis of X-ray structures involving the binding of phosphate to Na^+, Mg^{2+}, K^+, Ca^{2+}, and Zn^{2+} revealed no examples of phosphate chelation.[26a] This may be contrasted with the situation for carboxylate ligation. For metal−oxygen distances of 2.35−2.50 Å, the preferred and most commonly observed mode of binding to a cationic Ln center involves four-membered chelate ring formation. Such M−O distances are typical of those found in lanthanide-carboxylate complexes, when the carboxylate itself is unable to form other chelated structures, such as the N−O chelates found in many polyaminocarboxylate ligands.[26b] There have been some reports of carbonate binding to related macrocyclic lanthanide complexes: the Gd complex of DO3A was crystallized as a novel dimeric, hydrated complex in which each Gd was 9-coordinate and bound to the ligand heteroatoms and to two oxygens of a bridging carbonate anion. No water molecule was directly bound to Gd.[26c] In addition, some preliminary relaxivity measurements on [GdDO3A] have suggested that competitive binding to carbonate may limit the overall relaxivity by partial displacement of the bound waters.[26d]

The possibility of different hydration states for $[Ln^{1a}]^{3+}$ vs $[LnL^{1b}]^{3+}$ and $[LnL^{1c}]^{3+}$ offers an explanation for the higher apparent affinity constants observed with the N-alkylated complexes following addition of HCO_3^- (Table 1). The enthalpic cost of displacing two water molecules in the former case (although compensated partially by the likely small entropic gain: Scheme 3), compared to one in the latter, may make up some of the observed free energy difference ($\Delta\Delta G \sim 6.5$ kJ mol^{-1}, 295 K), as well as differential complex hydration energies. The differences in anion affinity observed at pH 7.4 between Tb and Eu complexes remain intriguing, because they apparently are not related to any change in the initial or final hydration state. A possible explanation is that the pK_a of the Eu aqua complex may be lower than that of the Tb analogue. Such behavior has been observed with related tetraamide macrocyclic complexes in which the Eu and Gd monoaqua complexes had pK_a values of 7.3 and 7.9.[28] Thus, the higher apparent affinity in the Tb series simply may be related to the greater electrostatic attraction between the tripositive Tb species and the anion in comparison to the Eu analogues, where more of the dipositive [EuLOH] species may be present.

Solution ^{1}H NMR Studies. Further information about the nature of the complex solution structure was provided by some preliminary ^{1}H NMR investigations. The ^{1}H NMR spectrum of $[EuL^{1a}]^{3+}(CF_3SO_3)_3$ (5 mM, 295 K) in D_2O at pD 6 revealed the presence of a single major species having a shifted NH resonance at +27.8 ppm and a singlet for each of the most shifted set of 4 nonequivalent axial ring protons (Figure 7). Addition of an excess of $NaHCO_3$ (40 mM) caused a slight sharpening of the observed resonances, with a dramatic shift to lower frequency of the ring axial protons and a general decrease in the magnitude of the paramagnetic shift for all paramagnetically shifted resonances. With $[EuL^{1b}]^{3+}$, a similar pattern of behavior was observed: in the monoaqua species, the 4 H_{ax} resonances appeared at +25, +19.5, and +19.0 ppm (1:2:1) and following addition of $NaHCO_3$, in the limiting spectrum, each resonated at least 13 ppm to lower frequency.[27] A partial assignment of the carbonate-bound complex was achieved with the aid of a ^{1}H−^{1}H COSY spectrum (500 MHz) and by examination of the spectrum at different field strengths. The overall spectral pattern for the aquated and carbonate-bound complexes was very different, with the former resembling the shifts observed for related monocapped square-antiprismatic tetraamide cyclen Eu complexes, and the latter corresponding more closely to spectra characteristic of a twisted square-

(26) (a)Schneider, B.; Kabelac, M. *J. Am. Chem. Soc.* **1998**, *120*, 161. Alexander, R. S.; Kanyo, Y. F.; Chirlian, L. E.; Christianson, D. W. *J. Am. Chem. Soc.* **1990**, *112*, 933. (b) Carrell, C. J.; Carrell, H. L.; Erlebacher, J.; Glusker, J. P. *J. Am. Chem. Soc.* **1988**, *110*, 8651. (c) Chang, C. A.; Francesconi, L. C.; Malley, M. F.; Kumar, K.; Gougoutas, J. Z.; Tweedle, M. F.; Lee, D. W.; Wilson, L. J. *Inorg. Chem.* **1993**, *32*, 3501. (d) Burai, L. PhD thesis, University of Debrecen, 1997. See also: Burai, L.; Hietopelto, V.; Kiraly, R.; Toth, E.; Brucher, E. *Magn. Reson. Imaging* **1997**, *38*, 146.

(27) Similar behavior was also apparent with $[EuL^{3a}]$ following addition of $NaHCO_3$ to the diaqua complex.

(28) 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*, 5762.

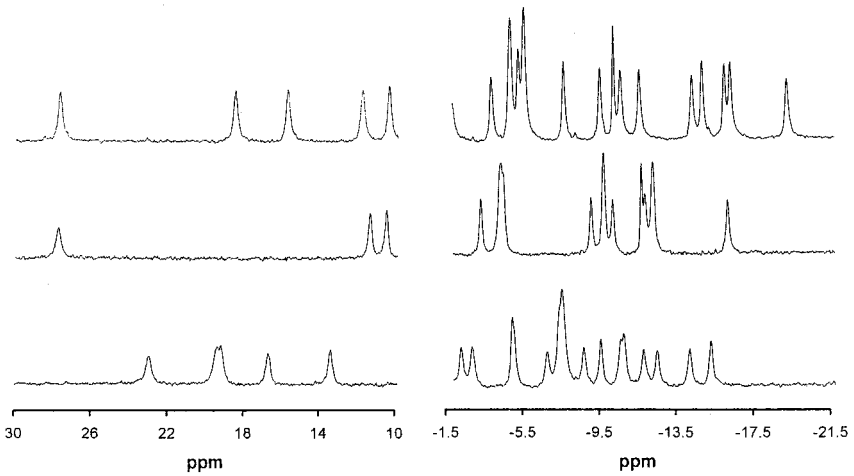


Figure 7. Partial ^{1}H NMR spectrum of $[EuL^{1a}]^{3+}$ at pH 5.5 (upper) in the presence of 10 equiv of $NaHCO_3$ (center) and sodium lactate (lower) (295 K, D_2O, 500 MHz). The resonance to highest frequency is the ring NH proton, followed by one set of axial ring protons.

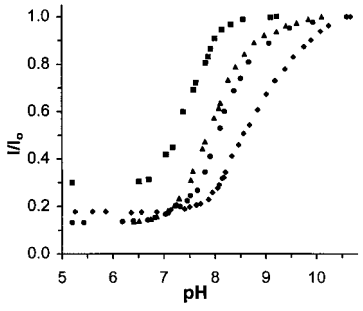


Figure 8. Relative emission intensity (I/I_o) of europium complexes as a function of pH showing the change in affinity for HCO_3^-/CO_3^{2-} as the residual charge at, or near, the Eu(III) ion is varied ($\lambda_{exc} = 397$ nm, $\lambda_{em} = 618$ nm, 295 K, H_2O, 30 mM $NaHCO_3$, 100 mM NaCl, 0.9 mM Na_2HPO_4, 0.13 mM sodium citrate, 2.3 mM sodium lactate). Filled squares, $[EuL^{1a}]^{3+}$; triangles, $[EuL^{3a}]$; circles, $[EuL^5]$; diamonds, $[EuL^6]^{3-}$.

antiprismatic structure, with a reduced helicity about the lanthanide center.[28]

Changes in the ^{1}H NMR spectrum following addition of (*S*)-lactate to [(*SSS*)-$EuL^{1a}]^{3+}$ were also significant but less dramatic than for added hydrogen carbonate (Figure 7). A reduction in the magnitude of the paramagnetic shift was apparent, but the relative shift of the axial, equatorial, and CH_2CO protons apparently remained constant. With excess added (*R*)-lactate, the ^{1}H NMR spectrum of the diastereoisomeric complex was only slightly different from that observed with (*S*)-lactate, with $\Delta\delta_H$ values of up to 0.8 ppm for the shifted ring protons.

Affinity for Carbonate in a Competitive Anion Background and the Effect of pH. . The speciation of the $CO_3^{2-}/HCO_3^-/aqCO_2$ system in aqueous media is complex but has been fully defined.[23a] The concentration of HCO_3^- falls from ca. 90% at pH 8.9 to <2% at pH 4.9. Accordingly, the solution pH determines the effective concentration of HCO_3^- in solution and, of course, above pH 9 significant quantities of CO_3^{2-} are present ($pK_a = 10.2$ at 298 K, $I = 0.1$). By examining the emission spectra of $[EuL^{1a}]^{3+}$, $[EuL^{3a}]$, $[EuL^5]$, and $[EuL^6]^{3-}$ as a function of pH in a solution containing initially (pH 10) 30 mM total carbonate, 100 mM NaCl, 0.9 mM $NaHPO_4$, 2.3 mM lactate, and 0.13 mM citrate (i.e., simulating an extracellular anionic environment[23b]), a considerable amount of useful information may be gleaned (Figure 8). The form and relative intensity of the Eu emission spectra for each complex of the individual amides was characterized earlier. The behavior of $[EuL^4]$ and $[EuL^5]$ was identical, which indicates that neither α-substitution nor the presence of the various stereoisomers of $[EuL^5]$ has any influence on the observed pH response.

For each complex in the stated anion mixture at pH 10, the emission spectrum resembled that of the carbonate adduct, with a very intense $\Delta J = 2$ transition at ca. 618 nm, that was five times as intense as the $\Delta J = 1$ transition at 591 nm. Lowering of pH sequentially reduced the effective concentration of first carbonate and then hydrogen carbonate, and the emission intensity (and $\Delta J = 2/\Delta J = 1$ ratio) fell, reaching a limiting value below pH 6.5 in all cases. Complexes which bound HCO_3^-/CO_3^{2-} relatively weakly (e.g., the anionic complex $[EuL^6]^{3-}$) were more sensitive to this pH change than the complexes which bound more strongly, for example, $[EuL^{1a}]^{3+}$. Moreover, the residual emission intensity at pH 5.5 for the cationic complexes, for example, $[EuL^{1a}]^{3+}$, was higher than that for any other complex (Figure 8), presumably reflecting the fact that it still bound lactate/citrate relatively strongly at lower pH (and, hence, had a smaller proportion of any water bound species), an assumption that was corroborated by the observed spectral emission profile in the pH range 5−6.5. This resembled that observed in the presence of lactate alone and differed from the behavior of $[EuL^6]^{3-}$, in which the emission spectrum over the pH range 4−6 was the same as that of the aqua complex.

The observed pH profile for $[EuL^4]$ (EuDO3A) was identical to that of $[EuL^5]$ (EuDO3MA)[29] and the more complex pH dependence in these cases, and that of $[EuL^6]^{3-}$ may reflect the combined effect of competitive HCO_3^- and CO_3^{2-} binding, superimposed on the Eu complex and HCO_3^- protonation.

The europium complex, bearing an N-methylphenanthridinium group, allows sensitized emission from the Eu ion to be observed following excitation at 365 or 370 nm. The variation in the ratio of the $\Delta J = 2/\Delta J = 1$ bands at 615/594 nm was monitored as a function of added $NaHCO_3$ at pH 7.4 in the presence of a simulated extra-cellular anionic background [23b] (Figure 9). The intensity ratio increased with added $NaHCO_3$, reaching a limiting value after addition of ca. 15 mM $NaHCO_3$. Examination of the corresponding europium emission spectra highlighted the characteristic increase in the $\Delta J = 2$ band intensity (Figure 10) and also revealed the concomitant changes in the $\Delta J = 1$ manifold and the complex, hypersensitive $\Delta J = 4$ region (675−705 nm). The limiting emission spectra observed

(29) Limiting emission spectra for $[EuL^5]$ in the absence and presence of HCO_3^- (i.e., at pH 9 and 4) in an initial background of 0.1 M NaCl, 0.9 mM Na_2HPO_4, 30 mM $NaHCO_3$, 0.13 mM sodium citrate and 2.3 mM sodium lactate are given in the Supporting Information.

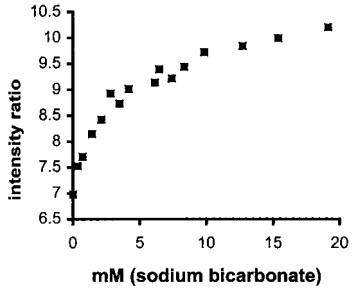


Figure 9. Variation in the ratio of the $\Delta J = 2/\Delta J = 1$ bands for $[EuL^{1c}]^{4+}$ ($\lambda_{exc} = 365$ nm, $\lambda_{em} = 615/594$ nm) as a function of added $NaHCO_3$ (1.5 mM complex, 0.1 M collidine/HCl buffer, pH 7.4, 100 mM NaCl, 0.9 mM Na_2HPO_4, 0.13 mM sodium citrate, 2.3 mM sodium lactate).

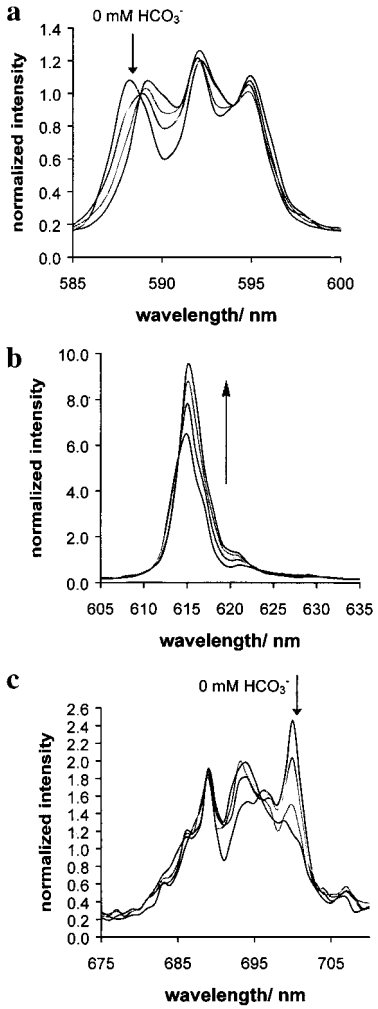


Figure 10. Selected europium emission spectra for $[EuL^{1c}]^{4+}$ (1 mM, 295 K, H_2O, $\lambda_{exc} = 365$ nm) in the presence of increasing concentrations of $NaHCO_3$ (0, 2.1, 4.2, 8.3, 19 mM), highlighting the large increase in intensity of the $\Delta J = 2$ manifold (b) and the change in the form of emission of the $\Delta J = 1$ and $= 4$ bands (a and c, respectively).

under these conditions resembled those found for the carbonate-bound species and for a lactate-bound complex. As discussed above, significant changes in the circular polarization of the $\Delta J = 1$ and $\Delta J = 2$ transitions accompany reversible carbonate binding. This may be interpreted in terms of a change in the local helicity at the metal center. An enhanced intensity is likely to be found as the twist angle increases, because it is the mixing of the electric and magnetic dipole transition moments which governs the allowedness of the observed CPL transitions.

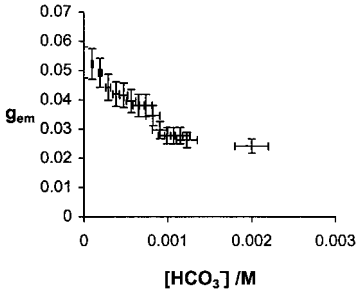


Figure 11. Variation in emission polarization (g_{em}) for the $\Delta J = 1$ transition at 614 nm with (*SSS*)-$[EuL^{1c}]$ (0.4 mM complex, $\lambda_{exc} = 365$ nm, 1 mM complex) as a function of added $NaHCO_3$ (pH 7.4, 100 mM NaCl, 0.9 mM Na_2HPO_4, 0.13 mM sodium citrate, 2.3 mM sodium lactate).

Accordingly, a titration was undertaken to measure the change in the emission circular intensity differential ($\Delta\Delta I$, $\lambda_{em} = 614$ nm, $\lambda_{exc} = 365$ nm) as a function of added anion (Figure 11). The form of the resultant binding curve closely resembled that obtained by observation of the emission intensity (618 nm) or intensity ratio ($\Delta J = 2/\Delta J = 1$) changes. Thus, reversible anion binding may be signaled by changes in the circular polarization of luminescent emission. The affinity for the target anion (HCO_3^-) may be tuned by modification of the structure of the complex, for example, by modification of the overall complex charge. Such behavior augurs well for the application of these and related luminescent complexes as new sensory systems for anion binding in competitive aqueous media.

Experimental Section

Details of the general methods and instrumentation used for NMR, relaxometry and luminescence are given in refs 11, 28 and 30.

Representative Luminescence Titration. Typically, to a 1mM solution of the lanthanide complex (absorbance < 0.1 at 265 nm) in 3 mL of the appropriate buffer solution (collidine/HCl for pH 7.4 and 0.1 M cyclohexylamine-ethanesulfonate (CHES) for pH 8.9) was added a freshly prepared solution of $NaHCO_3$, varying in molarity from 5 mM to 500 mM, such that the total volume change was less than 7%. The pH of the solution was checked at the beginning and end of the titration and did not vary by more than 0.05 pH units. In the case of titrations carried out in a background of competing ions (with pH variation following addition of HCl), a freshly prepared solution containing 100 mM NaCl, 0.9 mM Na_2HPO_4, 2.3 mM sodium lactate, 0.13 mM sodium citrate, and 30 mM sodium carbonate was used, and the starting pH was adjusted to ca. 10.5 by the addition of KOH solution. In the single example of the titration of $[EuL^{1c}]^{4+}$ with added $NaHCO_3$ solution, the same background was used (without the hydrogen carbonate), and the pH varied from 7.4 at the outset to 7.58 after the addition of 30 mM $NaHCO_3$. In those cases in which the pH of the solution was varied deliberately, a time delay of up to 20 min was used until a stable pH reading was attained.

Corrected emission spectra were recorded using a Fluorolog-3 instrument (Instruments s.a.) having a 420-nm cutoff filter and an excitation wavelength of 397 nm (Eu) or 355 nm (Tb) for direct excitation, or using sensitized emission at 265 or 365 nm with $[EuL^{1c}]$. Data analysis was performed using an iterative least-squares fitting procedure, assuming a 1:1 binding stoichiometry, operating in Microsoft Excel. For the binding of a given anion A^- to the lanthanide complex, [LnL], the following equilibrium was assumed:

$$[LnL]^{n+} + [A]^{m-} \overset{K}{\rightleftharpoons} [[LnLA]^{(n-m)+}$$

The following equation was minimized in terms of K, where A_i is the

(30) 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*, accepted (JA994492V).

(31) Salama, S.; Richardson, F. S. *J. Phys. Chem.* **1980**, *84*, 512.

total anion concentration and Ln_i is the complex concentration.

$$2[Ln_i]\frac{(I_{obs}-I_{init})}{(I_{final}-I_{init})} = [Ln_i] + [A_i] + 1/K - (\{[Ln_i] + [A_i] + 1/K\}^2 - 4[Ln_i][A_i])^{1/2}$$

Statistical errors were estimated be <10%, and experimental errors (the mean of three values is given in Table 1) were within 20%.

Ligand and Complex Synthesis. (*SSS*)-1,4,7-Tris[1-(1-phenyl)-ethylcarbamoylmethyl]-1,4,7,10-tetraazacyclododecane, L^{1a}. (*S*)-*N*-(2-Chloroethanoyl)-2-phenylethylamine (1.17 g, 5.9 mmol) in dry ethanol (10 mL) was added dropwise over 8 h to a stirred solution of 1,4,7,10-tetraazacyclododecane (0.34 g, 2.0 mmol) and triethylamine (0.83 mL, 5.9 mmol) in dry ethanol (30 mL) at 60 °C. The mixture was boiled under reflux for 18 h, concentrated to small volume, poured into aqueous hydrochloric acid (0.1 M, 40 mL), and washed with diethyl ether (3 × 30 mL). The aqueous phase was neutralized by careful addition of sodium hydroxide (1 M), the pH was adjusted to 13, and the solution extracted with dichloromethane (3 × 30 mL). Extracts were dried (K_2CO_3) and the solvent was removed to yield an oily residue which was purified by chromatography on neutral alumina (100% CH_2Cl_2 to 2% MeOH/CH_2Cl_2) to yield a pale yellow solid (380 mg, 29%): mp 63−65 °C; R_f (Al_2O_3; 10% MeOH/CH_2Cl_2) 0.5. HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{38}H_{54}N_7O_3$, 656.4288; found, 656.4287. ^{1}H NMR ($CDCl_3$, δ): 7.30 (3H, s, NHCO), 7.29−7.21 (15H, mult, ArH), 5.12 (3H, dq + dq, CH), 2.97 (4H, s, CH_2CO), 2.93 (2H, s, $CH_2C'O$), 2.58 (16H, br mult, CH_2N), 1.80 (1H, br s, NH), 1.48 (6H, d, $J = 7.2$, CH_3), 1.45 (3H, d, CH_3). ^{13}C NMR ($CDCl_3$, δ): 170.8, 170.6, 143.7 (quaternary), 129.1 (meta ArC), 127.8 (para ArC), 127.0, 59.9 (CH_2-CO), 58.0 (CH_2CO), 54.5 (CH_2N), 53.6 (CH_2N), 48.9 (CHN), 48.6 (CHN), 22.1, 21.9. Anal. Calcd for $C_{38}H_{53}N_7O_3 \cdot 2H_2O$: C, 66.0; H, 8.30; N, 14.2. Found: C, 65.7; H, 8.54; N, 14.0.

(*SSS*)-1,4,7-Tris[1-(1-phenyl)ethylcarbamoylmethyl]-10-methyl-1,4,7,10-tetraazacyclododecane, L^{1b}. Compound L^{1a} (0.1 g) was added to a solution of formic acid (2 mL) and aqueous formaldehyde (38%, 2 mL) and the mixture was boiled under reflux for 20 h. After removal of the solvent, the residue was treated with aqueous sodium hydroxide solution (2 M, 10 mL) and extracted with chloroform (3 × 10 mL). The combined extracts were dried (K_2CO_3) and the solvent was removed under reduced pressure to yield a colorless solid: mp 54−56 °C. HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{39}H_{56}N_7O_3$, 670.4444; found, 670.4446. ^{1}H NMR ($CDCl_3$, δ): 7.20 (15H, m, ArH), 5.05 (3H, dq + dq, CHN), 2.90 (3H, s, N−CH_3), 2.45−2.01 (22H, br m, CH_2N), 1.41 (6H, d, $J = 7.2$, CH_3), 1.38 (3H, d, CH_3). ^{13}C NMR ($CDCl_3$, δ): 171.0, 170.3, 143.8, 129.4, 129.3, 128.2, 128.1, 127.2, 126.9, 60.5, 59.8 (CH_2-CO), 56.8, 54.8, 54.7, 53.4 (CH_2N), 48.9, 48.8 (CH), 44.8 (CH_3N), 28.1, 21.9. λ_{max}(H_2O), 255 (510 dm^3 mol^{-1} cm^{-1}).

(*SSS*)-1,4,7-Tris[1-(1-*p*-carboxymethylphenyl)ethylcarbamoylmethyl]-1,4,7,10-tetraazacyclododecane, L^{2}. This was prepared as described for L^{1a} using (*S*)-*N*-(2-chloroethanoyl)-2-(*p*-carboxymethylphenyl)ethylamine and was purified by chromatography on neutral alumina by gradient elution (CH_2Cl_2, 1−5% MeOH (CH_2Cl_2) to yield a pale yellow solid: mp 71−72 °C. HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{44}H_{60}N_7O_9$, 830.4452; found, 830.4451. ^{1}H NMR ($CDCl_3$, δ): 7.86 (6H, d, ArH), 7.26 (6H, d, ArH), 5.05 (3H, dq + dq, CHN), 3.84 (9H, s, OCH_3), 2.89−2.50 (22H, br mult, CH_2N), 1.65 (6H, d, CH_3), 1.38 (3H, d, CH_3). λ_{max}(H_2O), 256 (710 dm^{3}mol^{-1}cm^{-1}).

[EuL^{1a}](CF$_3$SO$_3$)$_3$(H$_2$O)$_2$. Europium triflate (0.09 g, 0.15 mmol) and L^{1a} (0.10 g, 0.15 mmol) were dissolved in dry MeCN (2 mL) and the solution was boiled under reflux for 18 h. The volume was reduced to ca. 0.5 mL, and the solution was added with stirring to cold, dry diethyl ether (50 mL). A colorless solid precipitated which was filtered, dried under high vacuum, and reprecipitated by following the same procedure to yield a solid (0.12 g, 65%): mp 178−79 °C. HR−ESMS (*m*/*z*): [M + $(CF_3SO_3)]^{2+}$ calcd for $C_{39}H_{53}EuF_3N_7O_6S$, 957.2959; found, 957.2969. IR (KBr): 3288 (br, NH), 1620 (CO) cm^{-1}. ^{1}H NMR (D_2O, pD 6, 300 MHz, δ): (partial data and assignment) 27.3 (ring NH), 18.2, 15.5, 11.6, 10.1 (ring H_{axial}), 1.2 (3H, s), 0.1 (3H, s), −1.0 (3H, s, CH_3), −3.04 (1H, s), −4.04 (2H, s), −4.45 (1H, s), −4.8 (2H, s), −6.85 (1H, s), −8.74 (1H, s), −9.45 (1H, s), −9.80 (1H, s), −10.8 (1H, s), −13.6 (1H, s), −14.1 (1H, s), −15.2 (1H, s), −15.5 (1H,s), −18.5 (1H,s). In the presence of 20 mM $NaHCO_3$ (pD 8.6): 27.5 (1H, s, NH), 11.5 (1H, s), 10.6 (1H, s), 1.8 (3H, s, CH_3), 1.6 (6H, CH_3), 0.0 (1H, s), −2.5 (1H, s), −3.5 (3H, br s), −8.2 (1H, s), −8.9 (2H, s), −9.4 (1H, s), −10.9 (1H, s), −11.1 (1H, s), −11.5 (1H, s), −15.4 (1H, s).

Complexes of Tb with L^{1a}, L^{1b}, and L^{1c} were prepared analogously and gave identical IR spectra and ESMS data in accord with the theoretical isotope patterns for the complex plus one or two triflate counterions. Complexes of Eu with L^{1b} and L^{1c} also gave confirmatory accurate mass data and the following NMR data in the presence of the given anion: **[EuL^{1b}(H$_2$O)(CF$_3$SO$_3$)$_3$]$^{3+}$** partial ^{1}H NMR (pD 6, 400 MHz, 293K) δ: 25.3 (1H, s H_{ax}), 19.8 (2H, br s, H_{ax}), 19.1 (1H, s, H_{ax}), −0.9 (3H, s, CH_3), −1.1 (6H, s, CH_3), −3.8 to −4.3 (4H, mult), −6.1 (1H, s), −6.8 (1H, s), −7.2 (1H, s), −7.5 (1H, s), −7.9 (1H, s), −8.0 (1H, s), −8.8 (1H, s), −12.2 (1H, s), −12.5 (1H, s), −12.8 (1H, s), −13.9 (1H, s), −18.1 (1H, s). **[EuL^{1b}(CO$_3$)]$^+$** partial ^{1}H NMR (pD 6, 500 MHz, 293 K; primed protons are geminally coupled, e.g.,H_x and H′$_x$) δ: 13.9 (1H, s, H_a), 9.46 (2H, s, ArH), 8.56 (3H, s, ArH + H_b), 8.06 (1H, br s, ArH), 7.80 (3H, br s, ArH + H_c), 7.69 (2H, s, ArH), 7.25 (2H, br m, ArH + H_d), 6.98 (2H, s, ArH), 6.19 (2H, s, ArH), 6.08 (1H, s, C**H**CH_3), 6.07 (1H, s, C**H′**(CH_3), 5.68 (1H, s, CH′CH_3), 5.51 (3H, s, NCH_3), 4.11 (1H, s, H_e), 3.67 (1H, s, H_f), 3.38 (1H, s, Hg), 1.36 (6H, s, CH_3), 1.06 (3H, s, CH_3), 0.03 (1H, s, H′$_a$), −0.93 (1H, s, CH_xCO), −2.59 (1H, s, H′$_e$), −6.00 (1H, s, H′$_d$), −6.41 (2H, s, H′$_b$ + H_h), −7.77 (1H, s, H′$_f$), −8.31 (1H, s, H′$_h$), −8.73 (1H, s, H′$_e$), −10.35 (1H, s, H′$_g$), −10.69 (1H, s, CH_yCO), −11.77 (1H, s, CH′$_x$CO), −13.32 (1H, s, CH_zCO), −13.83 (1H, s, CH′$_y$CO), −18.47 (1H, s, CH′$_z$CO). **[EuL^{1b}(CH$_3$CO$_2$)]$^{2+}$** partial ^{1}H NMR (pD 6, 300 MHz, 295 K) δ: 18.2 (1H, s), 18.0 (1H, s), 16.7 (1H, s), 15.9 (1H, s, H_{ax}); 11.5 (1H, s, ArH); −2.19 (1H, s), −2.46 (5H, br s), −5.5 (1H, s), −6.2 (1H, s), −6.4 (1H, s), −7.4 (1H, s), −7.8 (2H, s), −9.1 (1H, s), −10.1 (1H, s), −10.5 (2H, s), −12.0 (1H, s), −15.3 (1H, s), −17.0 (1H, s).

1-(2′-Methylphenanthridinyl)-1,4,7,10-tetraazacyclododecane. 2-Methylphenanthridine (317 mg, 1.64 mmol) was dissolved in CCl_4 (20 mL) and a small amount of AIBN (4 mg) was added. *N*-Bromosuccinimide (297 mg, 1.66 mmol, 1.0 equiv.) was added and the resultant solution was stirred at 60 °C. The progress of the reaction was monitored by TLC analysis (silica, 5% MeOH/CH_2Cl_2, product R_f 0.3), and after 1.5 h the solution was cooled and filtered and the solvent was removed under reduced pressure to give the 2-bromomethyl compound (ca. 290 mg, 65%, as estimated by ^{1}H NMR analysis) as a cream-colored solid which was used immediately, in the next step. ^{1}H NMR ($CDCl_3$): δ 4.71 (2H, s, CH_2Br), 7.68 (1H, dd, H-9), 7.71 (1H, dd, H-3), 7.82 (1H, dt, $J = 8.4$, H-8), 8.00 (1H, br d, $J = 7.5$, H-4), 8.11 (1H, br d, $J = 8.4$, H-10), 8.57 (1H, d, $J = 8.1$, H-7) 8.52 (1H, d, $J = 1.8$, H-1), 9.23 (1H, s, H-6).

The crude 2-bromomethylphenanthridine was taken up in dry CH_3-CN (15 mL), cyclen (1.9 g, 11.0 mmol) and Cs_2CO_3 (540 mg, 1.65 mmol) were added, and the resultant mixture was stirred at 80 °C. TLC analysis (5% MeOH/CH_2Cl_2) after 45 min indicated that all of the 2-bromomethylphenanthridine had been consumed. The reaction mixure was filtered, and the solvent was removed under vacuum to leave a yellow hygroscopic oil. The residue was taken into CH_2Cl_2(30 mL) and washed extensively with Purite water (5 × 100 mL) to remove residual cyclen. The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness to afford the title compound (285 mg, 71%) as a hygroscopic yellow solid, which was used without further purification. ^{1}H NMR ($CDCl_3$): δ 2.46−2.78 (20H, m, ring CH_2N + NH), 3.78 (2H, s, CH_2), 7.54 (1H, t, H-9), 7.60 (1H, br d, H-3), 7.72 (1H, t, $J =$ 8.0, H-8), 7.91 (1H, d, $J = 8.0$, H-4), 8.04 (1H, d, $J = 8.4$, H-10), 8.47 (1H, br s, H-1), 8.55 (1H, d, $J = 8.0$, H-7) 9.14 (1H, s, H-6). HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{22}H_{30}N_5$, 364.2501; found, 364.2500.

(*SSS*)-1-(2′-Methylphenanthridinyl)-4,7,10-tris-[1-(1-phenyl)ethylcarbamoylmethyl]-1,4,7,10-tetraazacyclododecane, L^{1c}. 1-(2′-Methylphenanthridinyl)-1,4,7,10-tetraazacyclododecane (285 mg, 0.785 mmol), (*S*)-*N*-2-chloroethanoyl-1-phenylethylamine (488 mg, 2.47 mmol, 3.1 eq) and cesium carbonate (850 mg, 2.61 mmol) were stirred overnight in a mixture of CH_3CN (4 mL) and CH_2Cl_2 at 80 °C. The solution was filtered, and the solvent was removed under reduced

pressure. The crude residue was purified by chromatography over alumina (gradient elution: CH_2Cl_2, increasing from 0 to 5% MeOH/CH_2Cl_2). The major polar fraction was collected, and the solvent was removed to afford a pale yellow oil (287 mg, 43%): ^{1}H NMR ($CDCl_3$): δ 1.36 (6H, d, $J = 7.1$, CH_3), 1.45 (3H, d, $J = 7.1$, CH_3), 2.60−2.82 (16H, m, ring CH_2N), 3.10 (2H, s, CH_2CO), 3.25 (2H, d, $J = 16.5$, CHCO), 3.29 (2H, d, CH′CO), 3.75 (2H, s, CH_2−Ar), 4.98−5.10 (3H, dq-dq, NHCH), 6.90 (1H, br d, $J = 8.0$, NHCO), 7.12−7.27 (15H, m, phenyl H), 7.55 (1H, br d, NHCO), 7.60 (1H, br d, H-3), 7.75 (2H, br t + br d, H-9 + NHCO), 7.89 (1H, t, $J = 8.0$, H-8), 8.07 (1H, br d, H-10), 8.125 (1H, d, $J = 8.1$, H-4) 8.38 (1H, s, H-1), 8.60 (1H, d, $J = 8.0$, H-7), 9.28 (1H, s, H-6), LR-ESMS (*m*/*z*): $[M + Ca]^{2+}$ 443 (48%), $[M + H]^+$ 847 (100%), $[M + Na]^+$ 869 (90%). HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{52}H_{63}N_8O_3$, 847.5023; found, 847.5021.

Lanthanide Complexes of L^{1c}. The europium complex of L^{1c} was prepared as described for [EuL^{1a}]$(CF_3SO_3)_3$. LR-ESMS (*m*/*z*): $[M + (CF_3SO_3)_2]^+$ 1295, $[M + CF_3SO_3]$ 1148. A solution of the europium complex (65.8 mg, 0.045 mmol) in CH_3CN (1 mL) was stirred with excess methyl iodide (1 mL) at room temperature for 48 h. The solvent and excess reagent were removed under reduced pressure to give the *N*-methylated complex (71 mg, 98%) as a yellow hygroscopic solid. **[EuL^{1c}(D$_2$O)]$^{4+}$** partial ^{1}H NMR (pD 6, 25% CD_3OD, 300 MHz, 293K) δ: 24.8 (1H, br s), 22.8 (1H, br s), 21.5 (1H, br s), 20.5 (1H, br s, H_{ax}), −11.5 (1H, s, ArH), −1.28 (1H, br s), −3.0 (2H, br s), −5.3 (1H, br s), −6.3 (1H, br s), −8.4 (4H, br s), −9.5 (1H, br s), −10.2 (1H, br s), −12.2 (1H, br s), −14.2 (1H, br s), −17.9 (3H, br). LR-ESMS (*m*/*z*): $[MLOH]^{2+}$ 515. UV−vis (H_2O) 368 (5×10^3), 320 (9×10^3). **[EuL^{1c}(CO$_3$)]$^+$** partial ^{1}H NMR (pD 8.6, 25% CD_3OD, 300 MHz, 293K, 3-fold excess of $NaHCO_3$ added) δ: 18.7 (1H, s), 15.3 (1H, s), 14.6 (1H, s), 11.7 (2H, s, ArH + ring CH) −0.05 (1H, s), −2.7 (1H, s), −3.5 (1H, s), −7.0 (1H, s), −7.7 (1H, s), −9.1 (2H, s), −10.25 (1H, s), −12.65 (1H, s), −13.15 (1H, s), −13.9 (1H, s), −14.8 (1H, s), −16.1 (1H, s), −20.0 (1H, s).

(*SSS*)-1,4,7-Tris[1-(1-ethoxycarbonyl)ethylcarbamoylmethyl]-1,4,7,10-tetraazacyclododecane, L^{3b}. To a solution of cyclen (0.283 g, 1.64 mmol) in dry acetonitrile (20 mL) was added (*S*)-*N*-(2-chloroethanoyl)-ethyl alanate (0.965 g, 4.98 mmol) and sodium hydrogen carbonate (0.43 g, 5.1 mmol). The mixture was stirred at room temperature for 18 h and filtered and the solvent was removed to yield a residue which was purified by chromatography on silica gel (eluant: CH_2Cl_2 to 4% $^iPr_2NH/CH_2Cl_2$) to yield a colorless solid: mp 91−92 °C. ^{1}H NMR ($CDCl_3$) δ: 7.91 (2H, d, NHCO), 7.39 (1H, d, NHCO), 4.50 (3H, dq + dq, CHN), 4.13 (6H, mult, OCHH′), 3.18 (6H, d + d + s, CH_2CO), 2.91−2.54 (16H, br m, CH_2N), 1.39 (6H, d, CH_3), 1.33 (3H, d, $CHCH_3$), 1.24 (9H, t + t, CH_3). ^{13}C NMR ($CDCl_3$) δ: 173.6, 173.1, 171.5, 171.4 (CO), 61.6 (CH_2O), 59.6, 53.7 (CH_2-CO), 52.8, 52.6 (CHN), 48.1, 48.0, 47.3 (CH_2N), 18.2, 17.7 (CH_3), 14.4 (CH_3). HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{29}H_{54}N_7O_9$, 644.3983; found, 644.3985. **[EuL^{3a}]**: To a solution of L^{3b} (50 mg, 0.078 mmol) in dry MeCN (6 mL) was added europium triflate (0.051 g, 0.085 mmol) and the mixture was stirred for 18 h at 80 °C. Solvent was removed and the residue was treated with aqueous sodium hydroxide solution (0.02 M, 40 mL) for 24 h at 20 °C. The solution was brought to pH 6, reduced to small volume, and loaded onto a cation exchange column (Dowex 50W H^+), eluting with water then aqueous ammonia solution. The lyophilised solid (30 mg, 54%) was off-white: HR-ESMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{23}H_{38}N_7O_9EuNa$, 732.1841; found, 732.1843. Partial ^{1}H NMR (pD 3.8) δ: 24.9, 17.3, 14.8, 12.0 (H_{ax} ring). In the presence of a 40-fold excess of $NaHCO_3$ (pD 8.6; ca. 150 mM, HCO_3^-), these axial protons shifted to lower frequency: 11.0, 9.2, 7.7, 6.2.

1,4,7-Tris[(3′-carboxyl)-1′-carboxypropyl]-1,4,7,10-tetraazacyclododecane, L^6. To a mixture of cyclen (0.8 g, 4.6 mmol) and sodium hydrogen carbonate (1.2 g, 14 mmol) was added a solution of dimethyl α-bromoglutarate (3.35 g, 14 mmol) in dry acetonitrile (10 mL). The reaction was heated at 50 °C and progress was monitored by ESMS analysis. After 72 h, the mixture was filtered, the solvent was removed under reduced pressure, and the residue was taken up in dichloromethane (20 mL). The solution was washed with water (2×10 mL) and dried over K_2CO_3, and the solvents were removed to yield a residue which was purified by chromatography on silica gel, eluting with CH_2Cl_2, then 30% THF (CH_2Cl_2, and finally to 5% aqueous ammonia, 5% MeOH, 30% CH_2Cl_2, and 60% THF. A pale yellow oil was obtained (0.48 g, 33%; $R_f = 0.55$ [5% ammonia, 5% MeOH, 30% CH_2Cl_2, 60% THF]) as a mixture of (*RRR/SSS*), (*RRS/SSR*) and (*RSR/SRS*) stereoisomers. HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{29}H_{51}N_4O_{12}$, 647.3503; found, 647.3504. IR (film)/cm^{-1} 3443 (br, NH), 2953, 2847, 1745, 1730 (CO), 1453, 1253, 1209, 1169, 919, 732. ^{1}H NMR ($CDCl_3$) δ: 4.31 (1H, dd, CH), 3.89 (2H, dd, $J = 6.0$, CH), 3.70 (3H, s, OCH_3), 3.64 (6H, s, OCH_3), 3.62 (9H, s, OCH_3), 3.54−1.72 (28H, mult br, CH_2N + CH_2C). The hexaester (63 mg, 97 mmol) was treated with aqueous LiOH solution (1 M, 1 mL), the solution heated at 80 °C for 18 h and then passed down a cation exchange column (Dowex 50W H^+), eluting with 12% aqueous ammonia solution. The solvent was removed under reduced pressure to yield L^6 as its tetraammonium salt (50 mg): mp >250 °C. HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{23}H_{39}N_4O_{12}$, 563.2564; found, 563.2566. IR (KBr)/cm^{-1} 3500 (br, OH, NH), 2868, 2812 (CH), 1628 (CO), 1579, 1403, 1226, 1183, 1127, 1036, 1008, 666. ^{1}H NMR (pD 6, D_2O) 3.14 (2H, mult, CH), 3.08 (1H, mult, CH), 3.02−1.42 (28H, br mult, CH_2N + CH_2C). **[EuL^6]** The complex was prepared by heating L^6 (56 mg, 0.1 mmol) and Eu_2O_3 (18 mg, 0.05 mmol) in dilute HCl (pH 3, 2 mL) at 80 °C for 10 h, when the pH was adjusted to 6 and heating continued for 3 h. The pH was raised to 10 (aq. KOH), and the solution was filtered through a 5-μ membrane filter. The solution was readjusted to pH 5 and lyophilized, and the residue was taken up into absolute ethanol (5 mL) and filtered through a short pad of Celite, and the solvent was removed to yield the neutral complex as a colorless solid (60 mg, 85%): HR-ESMS (*m*/*z*): $[M - H]^-$ calcd for $C_{23}H_{34}N_4O_{12}Eu$, 710.1173: found, 710.1170. Partial ^{1}H NMR (pD 6, 300 MHz, D_2O) 39.9 (1H, br s, H_{ax}), 35.9 (1H, br s, H_{ax}), 28.4 (1H, br s, H_{ax}), 26.0 (1H, br s, H_{ax}), −4.6 (3H, br), −5.8 (1H, s), −6.2 (1H, s), −9.8 (1H, s), −10.1 (1H, s), −10.9 (1H, br s), −11.4 (1H, s), −11.9 (1H, br s), −13.9 (1H, br s), −17.9 (1H, s), −22.1 (1H, s).

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Supporting Information Available: NMR and emission spectra data, as detailed in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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