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Characterization of Lanthanide Complexes with a Series of Amide-Based Macrocycles, Potential MRI Contrast Agents, Using Eu^{3+} Luminescence Spectroscopy and Molecular Mechanics

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Eu^{3+} luminescence spectroscopy was used to characterize the solution complexes of the following ligands: 4,10,13-tris(carboxymethyl)-8,15-dioxo-1,4,7,10,13-pentaazacyclopentadecane (edta-dam); 1,4,7-tris(carboxymethyl)-9,14-dioxo-1,4,7,10,13-pentaazacyclopentadecane (edta-dam); 1,4,7-tris(carboxymethyl)-9,14-dioxo-1,4,7,10,13-pentaazacyclopentadecane (dtpa-eam); 1,4,7-tris(carboxymethyl)-9,20-dioxo-13,16-dioxa-1,4,7,10,19-pentaazacycloheneicosane (dtpa-oam); 1,4,7,16,19,22-hexa(carboxymethyl)-9,14,24,29-dioxo-1,4,7,10,13,16,19,22,25,28-decaazacyclotriacontane (bis(dtpa-eam)), 1,4-bis((4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetyl)piperazine (piperazine do3a-dimer). A single isomeric form of the Eu^{3+} complex with each ligand is observed. Edta-dam, dtpa-eam, and dtpa-oam form 1:1 stoichiometric metal–ligand complexes while bis(dtpa-eam) and the do3a-dimer form 2:1 stoichiometric metal–ligand complexes. Formation constants were measured for the Eu^{3+} complexes of dtpa-eam, dtpa-oam, and edta-dam. Relative formation constants for the other members of the lanthanide series were measured by competition experiments for dtpa-eam, dtpa-oam, and edta-dam. The number of coordinated water molecules were determined for the Eu^{3+} complex of each ligand. Inter-metal ion energy transfer between the Eu^{3+} ion and various other coordinated lanthanide ions is observed in dinuclear complexes of bis(dtpa-eam) and the piperazine do3a-dimer. Deductions regarding the composition of the first coordination sphere of the Eu^{3+} ion were made on the basis of molecular mechanics calculations and the number of coordinated water molecules determined from excited-state lifetime measurements in H_2O and D_2O.

Introduction

The utility of Gd^{3+} and Dy^{3+} chelate complexes to act as contrast agents for magnetic resonance imaging (MRI)[1] has generated an interest in techniques which can monitor the solution-state characteristics of lanthanide ion complexes. The important characteristics of a successful lanthanide ion-based contrast agent have recently been discussed in detail.[2] Particularly important requirements include a high thermodynamic stability constant for the complex and a selectivity toward Ln^{3+} ion coordination that minimizes replacement by such ions as Mg^{2+}, Ca^{2+}, and Zn^{2+}, which are present in relatively high concentrations in human plasma. Ideally, the chelate complexes themselves should be kinetically inert toward dissociation of the Ln^{3+} ion. Gd^{3+} MRI contrast agent complexes must, however, involve the coordination of at least one water molecule to allow the ^{1}H nuclear relaxation of the Ln^{3+}-bound water to be transferred to the bulk aqueous solution. Furthermore, it is advantageous that the complex be of neutral charge in order to minimize osmolality.[2–4]

Laser-excited Eu^{3+} luminescence spectroscopic techniques developed over the past decade in our laboratory allow us to obtain a more detailed characterization of solution-state Eu^{3+} complexes than is possible by any other means.[5,6] The information obtained includes the determination of the number of isomeric complexes present, metal–ligand stoichiometries, values for the formation constants, the number of water molecules bound to the Eu^{3+} ion in each complex, formation constants for the other members of the lanthanide series including Gd^{3+} and Dy^{3+} (from competition experiments), and the measurement of the inter-metal ion distances in the case of dinuclear complexes (from Förster-type energy transfer experiments).

Laser-induced Eu^{3+} excitation spectroscopy has been used extensively in our laboratory to characterize Eu^{3+} interaction with proteins,[5–8] nucleic acids,[9,10] and small chelating ligands.[11–14] In general, the transition between the ground ($^7F_0$) and excited ($^5D_0$) states of Eu^{3+} is accessed by a tunable dye laser in the range 577–581 nm, while the "hypersensitive" $^5D_0 \rightarrow {}^7F_2$ emission band is monitored at 614 nm. Since both ground and excited states are nondegenerate, a single excitation band is observed for each unique Eu^{3+} environment with an intensity proportional to the complex concentration. Complex stoichiometry and stability is therefore revealed by changes in the Eu^{3+} excitation intensity as a function of ligand or metal concentration. The excited-state lifetime, measured in both H_2O and D_2O is diagnostic of the number of coordinated water molecules, owing to an isotope effect.[15]

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(1) Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901–927.

(2) Cacheris, W. P.; Quay, S. C.; Rocklage, S. M. *Magn. Reson. Imaging* **1990**, *8*, 467–481.

(3) Konings, M. S.; Dow, W. C.; Love, D. B.; Raymond, K. N.; Quay, S. C.; Rocklage, S. M. *Inorg. Chem.* **1990**, *29*, 1488–1491.

(4) Chang, C. A.; Sieving, P. F.; Watson, A. D.; Dewey, T. M.; Karpishin, T. B.; Raymond, K. N. *J. Magn. Reson. Imaging* **1992**, *2*, 95–98.

(5) Horrocks, W. DeW., Jr.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384–392.

(6) Horrocks, W. DeW., Jr. In *Methods of Enzymology*; Riordan, J. F., Vallee, B. L., Eds.; Academic Press: New York, 1993; Vol. 226, 495–538.

(7) Horrocks, W. DeW., Jr.; Tingey, J. M. *Biochemistry* **1988**, *27*, 413–419.

(8) Bruno, J.; Horrocks, W. DeW., Jr.; Zauhar, R. J. *Biochemistry* **1992**, *31*, 7016–7026.

(9) Klakamp, S. L.; Horrocks, W. DeW., Jr. *J. Inorg. Biochem.* **1992**, *46*, 175–192.

(10) Klakamp, S. L.; Horrocks, W. DeW., Jr. *J. Inorg. Biochem.* **1992**, *46*, 193–205.

(11) Albin, M.; Horrocks, W. DeW., Jr.; Liotta, F. J. *Chem. Phys. Lett.* **1982**, *85*, 61–64.

(12) Holz, R. C.; Chang, C. A.; Horrocks, W. DeW., Jr. *Inorg. Chem.* **1991**, *30*, 3270–3275.

(13) Holz, R. C.; Klakamp, S. L.; Chang, C. A.; Horrocks, W. DeW., Jr. *Inorg. Chem.* **1990**, *29*, 2651–2658.

(14) Bruno, J.; Herr, B. R.; Horrocks, W. DeW., Jr. *Inorg. Chem.* **1993**, *32*, 756–762.

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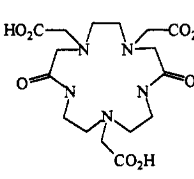
Chart 1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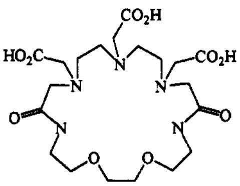
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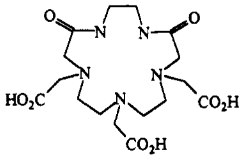
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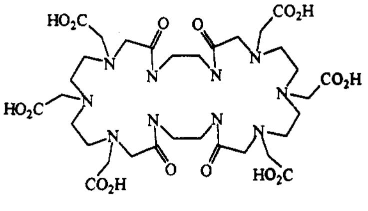
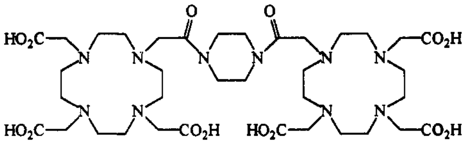

bis(dtpa-eam)


piperazine do3a-dimer

We have used these techniques to carry out a fairly complete characterization of the Eu^{3+} complexes of a series of amide-based macrocyclic ligands (Chart 1) which were designed as potential MRI contrast agent ligands.[16] Since Gd^{3+} is the periodic table neighbor of Eu^{3+}, with a nearly identical ionic radius (Eu^{3+}, 1.260 Å, CN = 9; Gd^{3+}, 1.247 Å, CN = 9), their chemistries are virtually identical. Thus any findings made concerning Eu^{3+} complexes of MRI contrast ligands can generally be applied to Gd^{3+} and, with some caution, to Dy^{3+} (ionic radius: 1.223 Å, CN = 9), three elements away on the periodic table.

To augment our luminescence work, we have carried out a molecular mechanics study of the Eu^{3+} complexes with each ligand and the appropriate number of water molecules. These modeling studies reveal which of the potentially coordinating atoms on a ligand are likely to be coordinated to the metal. These results, coupled with the luminescence data, provide a detailed assessment of the solution state structure and characteristics of the Eu^{3+} complexes of each macrocycle employed in this study.

Experimental Section

Materials. Bis(dtpa-eam),[17] dtpa-eam,[16] dtpa-oam,[16] edta-dam,[16] and piperazine do3a-dimer were a gift from Nycomed Salutar, Inc., Sunnyvale, CA. These compounds were estimated to be greater than 98% pure. D_2O (99.8%), hydrated $LaCl_3$, $EuCl_3$, $GdCl_3$, and $TbCl_3$ were purchased from the Aldrich Chemical Co. Hydrated $CeCl_3$, $NdCl_3$, $Sm(NO_3)_3$, and $ErCl_3$ were purchased from the Research Organic/Inorganic Chemical Corp. Hydrated $HoCl_3$, $YbCl_3$, and $Lu(NO_3)_3$ were purchased from Alfa Products. Hydrated $Pr(NO_3)_3$ and $Dy(NO_3)_3$ were purchased from the Apache Chemical Co. Hydrated $TmCl_3$ was purchased from the Strem Chemical Co. All metal salts were greater than 99% pure. *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (HEPES) was purchased from the Sigma Chemical Co. Sodium formate and potassium chloride were purchased from the Baker Chemical Co. Rhodamine 590 and 610 dyes were purchased from Exciton and Kodak Chemical companies respectively. The water used was deionized and doubly distilled. All chemicals were used as obtained without further purification.

Methods. Stock solutions, 10 mM in $LaCl_3$, $CeCl_3$, $Pr(NO_3)_3$, $NdCl_3$, $Sm(NO_3)_3$, $EuCl_3$, $GdCl_3$, $TbCl_3$, $Dy(NO_3)_3$, $HoCl_3$, $ErCl_3$, $TmCl_3$, $YbCl_3$, and $Lu(NO_3)_3$, were prepared and standardized by an edta arsenazo titration.[18] Stock solutions, 10 mM in the individuals ligands, were prepared at approximately pH 7.

Luminescence Measurements. Eu^{3+} excitation spectra, excited-state lifetimes, and excitation intensities were recorded with a Continuum YG-581C pulsed (10 Hz) Nd:YAG laser pumped tunable TDL-50 dye laser, as an excitation source. A mixture of rhodamine 590 and 610 dyes was used to access the $^7F_0 \rightarrow {}^5D_0$ transition of Eu^{3+} (577–581 nm) while monitoring the $^7F_0 \rightarrow {}^5D_2$ emission at 614 nm. The wavelength of the dye laser was calibrated by recording a spectrum of solid $EuCl_3(H_2O)_6$ which has a $^7F_0 \rightarrow {}^5D_0$ transition peak maximum at 579.273 nm. The laser operates at 10 Hz producing between 60 and 90 mJ/pulse with this dye mixture. The luminescence, sampled at 90° to the incident laser beam, is focused onto a 0.2-m double monochrometer (Instruments SA, DH-20-V-IR) and subsequently detected by a Hammamatsu type R928 HA photomultiplier tube. The signal is collected by a LeCroy data acquisition system interfaced to a Swan 386 SX computer.

Spectral peak fitting and lifetime analysis was achieved using the program PeakFit (Jandel Scientific). This program employs a nonlinear regression based on the Marquardt algorithm. Excitation spectra were fit to individual peaks describable by a Lorentzian–Gaussian product function discussed elsewhere.[19]

Determination of the Number of Isomers and the Metal–Ligand Stoichiometry. Solutions prepared in 50 mM HEPES containing 10 μM Eu^{3+}, pH 7, were titrated with each ligand while the excitation spectrum was recorded from 577 to 581 nm. An excited-state lifetime was also recorded for each point in the titration at the peak maximum with transients collected over a period of 3 min and averaged.

Formation Constant Measurements. For each of the ligands which form mononuclear complexes, 10 samples were prepared: 10 μM Eu^{3+}, 15 mM formate, and 85 mM KCl (pH 2.97 for edta-dam, pH 3.98 for dtpa-eam, and pH 2.12 for dtpa-oam), 0–50 μM in ligand. Following incubation at room temperature for at least 24 h, the luminescence intensity of each sample was recorded at the excitation peak maximum for a period of time (usually 5 min), at 25 °C. The intensities were then corrected for background counts.

Determination of Relative Formation Constants. For each of the ligands which form mononuclear complexes, samples were made up in 50 mM HEPES at pH 7, 10 μM each in Eu^{3+}, Ln^{3+}, and ligand. Three samples were made up with each Ln^{3+} ion for each ligand. Following equilibration for at least 24 h, the excited state lifetime and associated amplitude was recorded for each sample at the peak maximum for a period of 3 minutes at 25° C.

Determination of the Number of Coordinated Water Molecules. Samples were made up in H_2O (pH = 7) and D_2O (pD = 7) containing 50 mM HEPES and 10 μM Eu^{3+} with 10 μM ligand in the case of the mononuclear complexes, and 5 μM ligand in the case of the dinuclear complexes. Excited state lifetimes were then recorded at the peak maximum for each sample.

Inter-Metal Ion Distances with Dinuclear Ligands. Samples were made up in H_2O, pH 7, with 50 mM HEPES, containing 10 μM Eu^{3+}, as well as 10 μM Nd^{3+}, Pr^{3+}, Ho^{3+}, or Er^{3+}, and 10 μM ligand. The samples were allowed to equilibrate for at least 24 h, following which their excited-state lifetimes were recorded at the peak maximum. Absorption spectra of 5 mM samples of each ligand, unbuffered, with 5 mM Nd^{3+}, Pr^{3+}, Ho^{3+}, or Er^{3+}, pH 7, were recorded on a Varian/Cary 210 spectrophotometer from 500 to 720 nm. Fluorescence emission spectra of unbuffered 5 mM samples of each ligand with 5 mM Eu^{3+}, pH 7, were recorded on a Perkin-Elmer MPF 44A fluorescence spectrophotometer from 500 to 720 nm. It was necessary to record the emission of piperazine do3a-dimer in the absence of Eu^{3+} and to subtract this emission from that which was recorded for the complex. Spectral integration and the

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(15) Horrocks, W. DeW., Jr.; Sudnick, D. R. *J. Am. Chem. Soc.* **1979**, *101*, 334–340.

(16) Carvalho, J. F.; Kim, S. H.; Chang, C. A. *Inorg. Chem.* **1992**, *31*, 4065–4068.

(17) Varadarajan, J.; Carvalho, J.; Kim, S-H.; Fellman, J. Presented at the 12th Annual Scientific Meeting of the SMRM, New York, 1993; Book of Abstracts, No. 752.

(18) Fritz, J. S.; Oliver, R. T.; Pietrzyk, D. J. *Anal. Chem.* **1958**, *30*, 1111–1114.

(19) McNemar, C. W.; Horrocks, W. DeW., Jr. *Appl. Spectrosc.* **1989**, *43*, 816–821.

calculation of spectral overlap integrals were carried out with computer programs based on Simpson's rule, written in this laboratory.

Molecular Mechanics and Minimization. Calculations were carried out on an IBM RISC 6000 graphics workstation running QUANTA (version 3.3) molecular graphics and CHARMm (version 2.2) molecular mechanics/dynamics software (Molecular Simulations Inc.). The connectivity of the atoms in each ligand, as well as the bond orders, were entered by means of a subprogram of QUANTA called CHEMNOTE. The ligands were entered in their fully deprotonated forms. It was necessary to differentiate between the two carboxylate oxygens to avoid a strong preference towards bidentate coordination during energy minimization. We achieved this by "locking" one oxygen atom as the carboxyl oxygen (CHARMm atom type OAC) and the other oxygen atom as the ionized oxygen (CHARMm atom type OT). The partial charges for each atom were assigned by CHARMm from the most recent parameter list with the exception of amine nitrogens which were assigned a partial charge of –0.40 (CHARMm assignment = –0.30). This was shown to be necessary in previous studies in order to achieve reasonable Eu–N bond distances.[20,21] Eu^{3+} was entered as a separate entity with a charge of +3.0. The parameters which govern nonbonded interactions, minimum van der Waals radius (r_{min}) and well depth potential (e_{min}), were assigned by CHARMm from the most recent parameter list. This list does not include Eu^{3+} which was assigned parameters ($r_{min} = 1.80$, $e_{min} = -0.20$) which were used for Gd^{3+} in previous studies.[20,21] Water molecules used in this study were of the type TIP3P.[22]

The minimization method employed was the adopted-basis set Newton–Raphson (ABNR) technique, with a convergence criteria of 0.01 kcal mol^{-1}. Minimizations were performed *in vacuo*, and a distance-dependent dielectric was used, $\epsilon = r$, where r is the interatomic distance in angstroms. In general, the ligands were energy minimized in the absence of Eu^{3+} and water molecules. Eu^{3+} was then added in close proximity to the ligand, and the system was again energy minimized. It was often necessary to adjust the ligand torsional angles in order to achieve the lowest possible energy upon minimization. Water molecules were then added (based on the experimental determination herein) and the energy of the structure was reminimized. In some cases, especially when more than one water molecule was involved, it was necessary to constrain the water molecules to a distance of 2.4 Å from the Eu^{3+}, minimize the energy of the complex, and then remove the constraints and reminimize. This is because the program has a greater tendency to move the water molecule away than to adjust the ligand to accommodate it.

Results and Discussion

Determination of Number of Isomers. Since both ground and excited states are nondegenerate, the number of peaks in a $^7F_0 \rightarrow {}^5D_0$ excitation spectrum indicates the number of distinct Eu^{3+} ion environments present. In all five cases only a single peak attributable to the Eu^{3+} complex of the ligand of interest was detected (although the spectrum of dtpa-eam contains a small extra peak at 579.9 nm which we attribute to slight contamination (~5%), likely dtpa or bis(dtpa-eam)). A representative excitation spectrum of 10 μM Eu^{3+} with 5 μM piperazine do3a-dimer is shown in Figure 1. Table 1 shows the excitation maximum and full width at half-maximum intensity for each of the complexes. We had expected the excitation maximum for all of the complexes to be centered around 579.4 nm, based on the previously reported correlation between the $^7F_0 \rightarrow {}^5D_0$ transition frequency and formal ligand charge for Eu^{3+} complexes.[23] Curiously, however, the excitation maxima vary between 579.3 and 579.9 nm for the complexes studied. There is an especially intriguing difference between dtpa-eam (579.3 nm) and bis(dtpa-eam) (579.9 nm), suggestive of markedly different coordination environments for Eu^{3+} in the two cases. On the basis of molecular modeling studies (*vide infra*), we postulate that the variation in excitation maxima among the complexes may be reflective of the sum of partial charges of all of the coordinating atoms as opposed to the total formal charge of the unprotonated ligands.

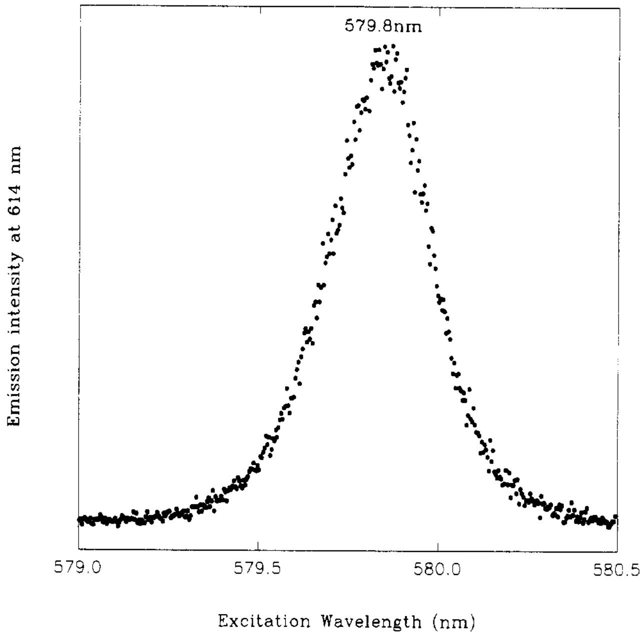

Figure 1. $^7F_0 \rightarrow {}^5D_0$ excitation spectrum of 10 μM Eu^{3+} with 1 equiv of piperazine do3a-dimer in 50 mM HEPES, pH 7.0.

Table 1. Eu^{3+} Excitation Maximum, Full Width at Half-Maximum (Fwhm) Intensity, Metal–Ligand Stoichiometry, Eu^{3+} Lifetimes in H_2O and D_2O, and the Calculated Number of Bound Water Molecules (±0.5 Water Molecules) for the Complex with each Ligand

ligand	$^7F_0 \rightarrow {}^5D_0$ nm	Fwhm, nm	M:L	τ_{H_2O} ms	τ_{D_2O} ms	q^a
edta-dam	579.6	0.35	1:1	0.274	2.25	3.4
dtpa-eam	579.3	0.54	1:1	0.365	1.87	2.3
dtpa-oam	579.9	0.35	1:1	0.605	2.54	1.3
bis(dtpa-eam)	579.9	0.44	2:1	0.639	2.43	1.2
do3a-dimer	579.8	0.44	2:1	0.630	2.10	1.2

[a] q (the number of coordinated water molecules) = $1.05(\tau_{H_2O}{}^{-1} - \tau_{D_2O}{}^{-1})$

Complex Stoichiometries. Each ligand was titrated into a 10 μM solution of Eu^{3+}, while luminescence decays from the $^5D_0$ excited state, $I(t)$, were recorded for a set period of time (usually 3 min)

$$I(t) = I_0 e^{-t/\tau} \qquad (1)$$

where τ is the excited state lifetime, t is the time, and $I_0 = I(t)$ at $t = 0$. Figure 2 shows a typical plot of I_0 vs equivalents of added bis(dtpa-eam). Since the metal ion concentration is well above the dissociation constants for the complexes (2×10^{-12} to 5×10^{-18} M) such curves are expected to break sharply when stoichiometric quantities of each ligand has been added: this is indeed the case. The monomeric ligands dtpa-eam, dtpa-oam, and edta-dam have a 1:1 metal–ligand stoichiometry while the dimers, bis(dtpa-eam), and do3a-dimer exhibit curves that break at ~0.5 equivalents of added ligand, indicative of a 2:1 metal–ligand stoichiometry as is illustrated in Figure 2. These results are listed in Table 1.

Formation Constants. The titration curves used to determine stoichiometries are useless for determining the actual values of the formation constants. However, by working at much lower pH values where H^+ ions compete effectively with Eu^{3+} complex formation, it is possible to obtain binding curves from which conditional stability constants can be determined.[24] Then, with

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[20] Fossheim, R.; Dahl, S. G. *Acta Chem. Scand.* **1990**, *44*, 698–706.

[21] Fossheim, R.; Dugstad, H.; Dahl, S. G. *J. Med. Chem.* **1991**, *34*, 819–826.

[22] Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926–935.

[23] Albin, M.; Horrocks, W. DeW., Jr. *Inorg. Chem.* **1985**, *24*, 895–900.

[24] This procedure has been used recently in our laboratory to measure the thermodynamic stability constants for several common Eu^{3+} complexes including Eu(edta)$^-$, Eu(dtpa)$^{2-}$, and Eu(hedta). The stability constants measured for each of these complexes are in excellent agreement with those reported in the literature. Wu, S. L.; Horrocks, W. DeW., Jr. To be submitted for publication.

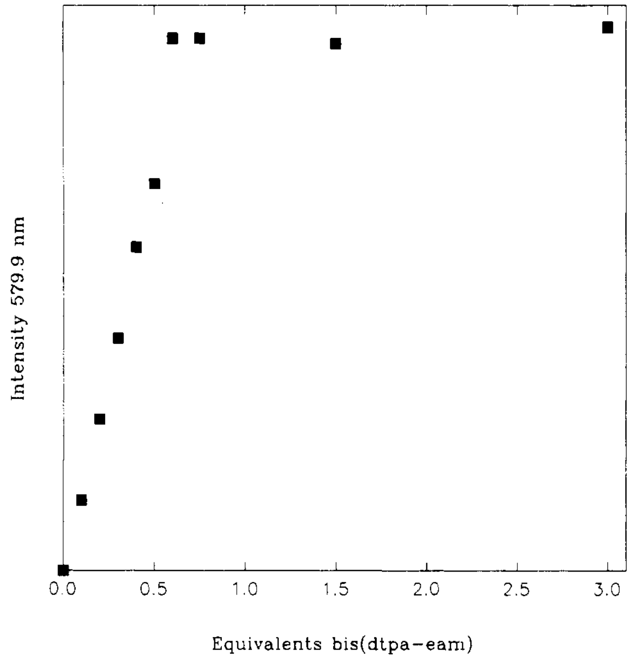

Figure 2. Titration curve of the Eu^{3+} $^5D_0$ luminescence decay intensity (I_0) vs equivalents of bis(dtpa-eam) at pH 7.0, 10 μM Eu^{3+}.

knowledge of the three protonation constants (these studies were not carried out on the dimeric ligands), it is possible to calculate the thermodynamic formation constant for the interaction of the fully deprotonated ligand L^{3-} with Eu^{3+}.

$$K_{therm}(Eu^{3+}) = [EuL]/[Eu^{3+}][L^{3-}] \quad (2)$$

Since our spectroscopic measure (luminescence intensity) is directly proportional to the concentration of EuL, this method is more direct than the traditional potentiometric titration carried out in the presence and absence of metal ion, where only H^+ concentrations are measured. For the present study it is necessary to ascertain that protonated species do not form at the chosen pH value. This is easily accomplished by examining the excitation spectrum, which is sensitive to slight differences in the coordination environment. The low pH spectra of the complexes studied here were identical to their pH 7 counterparts. Thus, we are able to measure the conditional formation constant

$$K_{cond}(Eu^{3+}) = [EuL]/[Eu^{3+}]\{[L^{3-}] + [HL^{2-}] + [H_2L^-] + [H_3L]\} \quad (3)$$

where

$$K_{cond}(Eu^{3+}) = K_{therm}(Eu^{3+})\alpha_H \quad (4)$$

and

$$\alpha^{-1}_H = \{1 + K_{H1}[H^+] + K_{H1}K_{H2}[H^+]^2 + K_{H1}K_{H2}K_{H3}[H^+]^3\} \quad (5)$$

and K_{H1}, K_{H2}, and K_{H3} are the three protonation constants of the ligands in question, which were obtained from potentiometric titrations.[16]

Binding curves were obtained for the three monomeric ligands dtpa-eam, dtpa-oam, and edta-dam, by plotting luminescence intensity against the concentration of ligand. As an example, Figure 3 shows a plot of the emission intensity for excitation of the $^7F_0 \rightarrow {}^5D_0$ transition at 579.9 nm (intensity data for each point was collected over a period of 5 min) vs the concentration

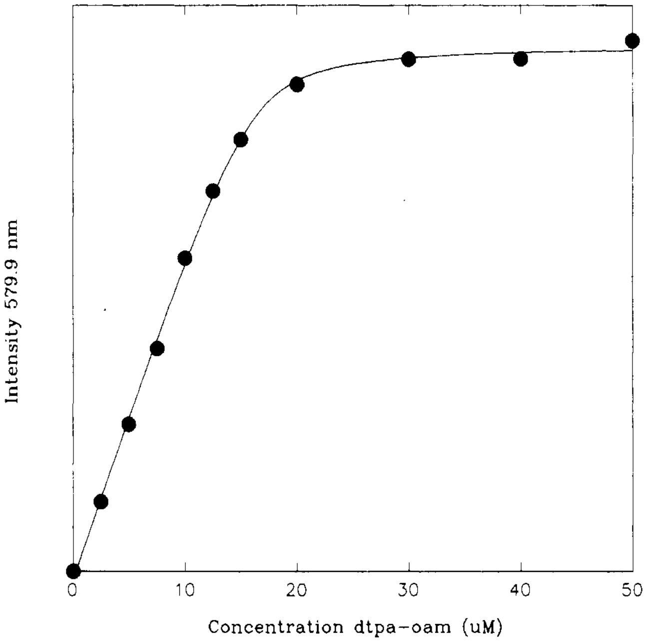

Figure 3. Binding curve of 10 μM Eu^{3+} titrated with varying amounts of dtpa-oam in 15 mM formate, 85 mM KCl, pH 2.12. The filled circles represent actual data points, and the solid line represents the theoretical fit of the data with log $K_{cond}(Eu^{3+})$ = 6.5.

Table 2. α_H^{-1} Values, Conditional Formation Constants, and Thermodynamic Formation Constants for the Three Monomeric Complex-Forming Ligands

ligand	α_H^{-1} (pH)[a]	log $K_{cond}(Eu^{3+})$	log $K_{Eu^{3+}}$	log $K_{Gd^{3+}}$[b]	log $K_{Gd^{3+}}$[a]
edta-dam	3.32×10^9(2.97)	5.8	15.3	15.3	15.14
dtpa-eam	9.25×10^5(3.98)	5.7	11.7	11.4	11.15
dtpa-oam	5.47×10^{10}(2.12)	6.5	17.2	17.5	17.44

[a] Reference 16. [b] Measured from competition experiments.

of added ligand for a titration carried out at pH 2.12 with dtpa-oam. The conditional formation constants for Eu^{3+} with the three monomeric ligands, were thus determined from a nonlinear regression fitting of the binding curves according to eq 3. These were converted to the thermodynamic formation constants using eq 4 and knowledge of α_H^{-1} (eq 5). The values for α_H^{-1}, the conditional formation constants, and the derived thermodynamic formation constants are shown in Table 2, along with the thermodynamic formation constants of the corresponding Gd^{3+} complexes deduced from competition studies (*vide infra*), and log $K_{Gd^{3+}}$ values from the literature.[16] As can be seen, the log $K_{Gd^{3+}}$ values found in our work are in excellent agreement with literature values.[16]

Relative Formation Constants of Other Members of the Ln^{3+} Series. By using the Eu^{3+} luminescence intensity as a measure of the EuL concentration it is possible, with relative ease, to measure the formation constant of complexes of any other metal ion via competition experiments. We have done this for each of the ligands which form mononuclear complexes, with all of the other ions in the Ln^{3+} series. For a competition reaction, equal molar concentrations of ligand, Eu^{3+} ion and a competing Ln^{3+} ion are placed in solution, and the following equilibrium is established.

$$Ln^{3+} + EuL \rightleftarrows Eu^{3+} + LnL$$

It is easily shown that the equilibrium constant for this reaction is the ratio of the formation constant for Ln^{3+} to that of Eu^{3+} as follows:

$$[Eu^{3}][LnL]/[Ln^{3+}][EuL] = K_{Ln^{3+}}/K_{Eu^{3+}} \quad (6)$$

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(25) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th ed.; John Wiley & Sons, Inc.: New York, 1988; pp 959–961.

(26) Inoue, M. B.; Inoue, M.; Munoz, I. C.; Bruck, M. A.; Fernando, Q. *Inorg. Chim. Acta* **1993**, *209*, 29–34.

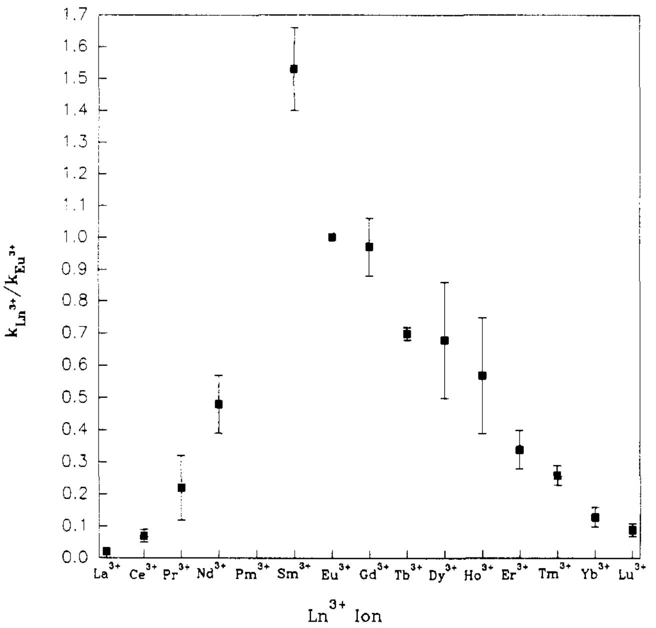


Figure 4. Relative formation constants for all of the trivalent lanthanide ions (with the exception of radioactive Pm^{3+}) with edta-dam as determined from metal ion competition experiments.

Table 3. Relative Formation Constants for all of the Lanthanides with dtpa-eam, dtpa-oam, and edta-dam As Measured from Competition Experiments with Eu^{3+} [a]

	$K_{Ln^{3+}}/K_{Eu^{3+}}$		
Ln^{3+}	dtpa-eam	dtpa-oam	edta-dam
La^{3+}	0.01(1)	0.002(1)	0.02(1)
Ce^{3+}	0.06(1)	0.02(1)	0.07(2)
Pr^{3+}	0.21(8)	0.20(3)	0.2(1)
Nd^{3+}	0.23(5)	0.62(8)	0.48(9)
Sm^{3+}	0.55(4)	1.0(2)	1.5(1)
Gd^{3+}	0.48(2)	1.5(3)	0.97(9)
Tb^{3+}	0.51(5)	1.70(7)	0.70(2)
Dy^{3+}	0.48(2)	1.12(4)	0.7(2)
Ho^{3+}	0.36(2)	1.24(7)	0.6(2)
Er^{3+}	0.34(3)	0.95(8)	0.34(6)
Tm^{3+}	0.31(2)	0.73(3)	0.26(3)
Yb^{3+}	0.08(2)	0.7(1)	0.13(3)
Lu^{3+}	0.23(2)	0.6(1)	0.09(2)

[a] The numbers in parentheses represent the error in the last digit based on three trials.

Equation 6 can be generalized as

$$K_{Ln^{3+}}/K_{Eu^{3+}} = (C_3 - kI)(C_1 - kI)/(kI)(C_2 - C_3 + kI) \quad (7)$$

where C_1 is the total Eu^{3+} concentration, C_2 is the total competing Ln^{3+} concentration, C_3 is the total ligand concentration, I is the intensity in the presence of competing Ln^{3+} ion, and k is a proportionality constant equal to C_1/I_0, where I_0 is the intensity in the absence of competing Ln^{3+} ion. When $C_1 = C_2 = C_3 = C$, eq 7 reduces to

$$K_{Ln^{3+}}/K_{Eu^{3+}} = (C - CI/I_0)^2/(CI/I_0)^2 \quad (8)$$

which, when arranged, yields

$$K_{Ln^{3+}}/K_{Eu^{3+}} = [(I_0 - I)/I]^2 \quad (9)$$

Figure 4 shows a plot of $K_{Ln^{3+}}/K_{Eu^{3+}}$ values as a function of competing Ln^{3+} ion for edta-dam. The results for all three ligands are listed in Table 3. There are considerable changes in affinity for the various Ln^{3+} ions across the series. For each of these ligands, the large La^{3+} ion exhibits the smallest affinity. Both 15-membered macrocycles, dtpa-eam and edta-dam, have similar profiles which rise sharply to a maximum at Eu^{3+} for dtpa-eam and Sm^{3+} for edta-dam and then fall off markedly later in the series. The affinity for Ln^{3+} ions of dtpa-oam, a 21-membered macrocycle, rises more gradually to a maximum at Tb^{3+} and then falls off. While all three ligands exhibit a large change in affinity across the Ln^{3+} series, the sharp profiles of the 15-membered macrocycles indicate that they are more sensitive to slight changes in ionic radius.

Number of Eu^{3+}-Coordinated Water Molecules. Owing to a well established isotope effect[15] whereby O–H oscillators in the first coordination sphere of a Eu^{3+} complex contribute to the quenching of the $^5D_0$ excited state while O–D oscillators do not, the number of coordinated water molecules, q, can be determined by measurement of the excited state lifetime, τ, of Eu^{3+} complexes separately in H_2O and D_2O by application of the following equation.[15]

$$q = 1.05(\tau^{-1}_{H_2O} - \tau^{-1}_{D_2O}). \quad (10)$$

Table 1 lists the excited state lifetimes recorded for the Eu^{3+} complex with each ligand in H_2O and D_2O, as well as the calculated number of coordinated water molecules (with an uncertainty of ±0.5 water molecules). A single lifetime is observed for each Eu^{3+} complex, once again revealing the presence of only a single type of Eu^{3+} environment per complex.

The measurement of the number of coordinated water molecules is suggestive of the number of remaining atoms in the first coordination sphere since Eu^{3+} prefers a coordination number of 8 or 9.[25] For the edta-dam complex, in which the Eu^{3+} ion appears to bind three water molecules, we would expect the ligand to contribute five or six coordinating atoms. Likewise, dtpa-eam would be expected to contribute six or seven coordinating atoms in its complexation, and dtpa-oam, bis(dtpa-eam), and the piperazine do3a-dimer would contribute seven or eight coordinating atoms.

Inter-Metal Ion Distance Measurements in Dinuclear Complexes. If a luminescent Eu^{3+} ion is placed in proximity to another ion whose absorption spectrum overlaps the emission spectrum of Eu^{3+}, Förster-type nonradiative energy transfer can take place. From the efficiency of this transfer it is possible, in principle, to measure the distance, r, between these two ions, provided that no other energy transfer mechanism is operative. We have carried out such measurements for various Eu^{3+}–Ln^{3+} pairs for the dinuclear complex-forming ligands bis(dtpa-eam) and piperazine do3a-dimer. In a typical experiment, samples were prepared containing equal molar quantities of the ligand, Eu^{3+} ion, and the acceptor Ln^{3+} ion in H_2O at pH 7. The excited-state lifetimes were then recorded at the excitation peak maximum. In each case, the decay consists of a double exponential function with τ-values corresponding to the lifetime in the absence and presence of the energy acceptor. This is expected as statistically 50% of the ligand molecules should bind one Eu^{3+} ion and one Ln^{3+} ion, while 25% bind two Eu^{3+} ions and 25% bind two Ln^{3+} ions. The experimental efficiency of energy transfer, E, is related to the lifetime of Eu^{3+} in the absence of a neighboring energy acceptor, τ_0, and the lifetime, τ, in the presence of such an acceptor by

$$E = 1 - (\tau/\tau_0) \quad (11)$$

The distance between the ions, r, is then given by

$$r = R_0[(1 - E)/E]^{1/6} \quad (12)$$

where R_0, the Förster critical distance for 50% energy transfer, is given by

$$R_0 = 8.75 \times 10^{-25}\,\kappa^2\phi\eta^{-4}J \quad (13)$$

where κ^2 is the dipole–dipole orientation factor ($^2/_3$ for metal ions), ϕ is the quantum yield of Eu^{3+} (estimated from τ_{H_2O}/τ_{D_2O}), η is the refractive index of the intervening medium, and

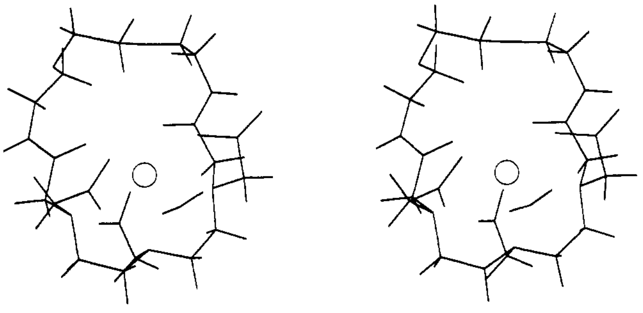
Table 4. Inter-Metal Ion Energy Transfer Data for the Binuclear Complexes of Bis(dtpa-eam) and Piperazine do3a-Dimer[a]

ligand	acceptor	τ_0, ms	τ, ms	E	ϕ	R_0, Å	J, cm^6/mol	r, Å
bis(dtpa-eam)	Nd^{3+}	0.639	0.095	0.85	0.27	9.1	1.22×10^{-17}	6.8
bis(dtpa-eam)	Pr^{3+}	0.644	0.238	0.63	0.27	8.2	6.37×10^{-18}	7.5
bis(dtpa-eam)	Ho^{3+}	0.640	0.322	0.49	0.27	6.8	2.16×10^{-18}	6.8
bis(dtpa-eam)	Er^{3+}	0.640	0.309	0.52	0.27	7.2	2.98×10^{-18}	7.1
do3a-dimer	Nd^{3+}	0.630	0.180	0.71	0.30	9.6	1.49×10^{-17}	8.3
do3a-dimer	Pr^{3+}	0.630	0.233	0.63	0.30	9.1	1.08×10^{-17}	8.3
do3a-dimer	Ho^{3+}	0.630	0.470	0.25	0.30	7.1	2.43×10^{-18}	8.5
do3a-dimer	Er^{3+}	0.630	0.370	0.38	0.30	7.7	3.82×10^{-18}	8.4

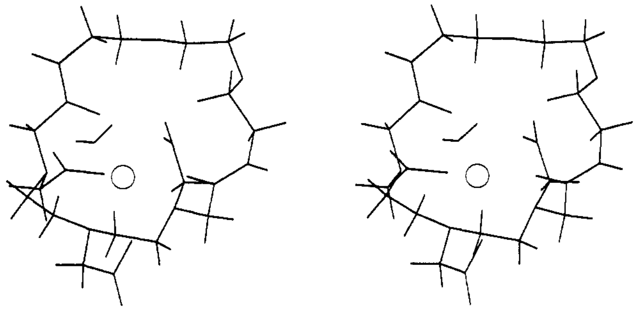
[a] τ_0 = lifetime in the absence of the acceptor. τ = lifetime in the presence of the acceptor. E = efficiency of energy transfer. ϕ = quantum yield. R_0 = critical distance for 50% energy transfer. J = spectral overlap integral. r = inter-metal ion distance.

Table 5. Parameters Used for the Molecular Mechanics Calculations

atom	CHARMm type	partial charge	r_{min}	e_{min}
carboxylate charged O	OT	−0.65	1.550	−0.1521
carboxylate carbonyl O	OAC	−0.55	1.520	−0.1591
amide carbonyl O	O	−0.55	1.550	−0.1591
ether O	OE	−0.37	1.600	−0.1591
amine N	NT	−0.40	1.650	−0.1500
Eu^{3+}	MEU	+3.00	1.800	−0.2000
water O	OW	−0.834	1.768	−0.1521



Eu(dtpa-oam) up, down, up



Eu(dtpa-oam) up, up, down

Figure 5. Stereoscopic drawings of the minimized structures of the low-energy conformations of Eu(dtpa-oam)(H_2O).

J is the spectral overlap integral

$$J = \int \epsilon(\nu)\, F(\nu)\, \nu^{-4}\, d\nu \Big/ \int F(\nu)\, d\nu \qquad (14)$$

where $\epsilon(\nu)$ is the molar absorptivity of the acceptor ion, and $F(\nu)$ is the luminescence emission intensity of the donor ion. The values for these parameters are set out in Table 4. Relatively good agreement is achieved among the various Ln^{3+} ion pairs such that the calculated internuclear distance in bis(dtpa-eam) is about 7.1 Å, while for the piperazine do3a-dimer it is about 8.4Å. It should further be pointed out that the observation of inter-ion energy transfer is absolute proof of the dinuclear nature of these complexes.

Molecular Mechanics. In order to gain a better understanding of the coordination of each of these ligands to Eu^{3+}, we have performed molecular mechanics calculations. We have adopted an approach used by others in a recent study of amino polycarboxylates and their lanthanide ion complexes.[20,21] This approach is to treat "electrostatically bound" ions as "nonbonded" entities. That is, they are bound to the ligand by coulomb and van der Waals forces only, and the metal-ligand bond distances are determined by the balance of coulomb attraction between the negative partial charges on the ligand atom and the positive charge on the metal ion and the van der Waals repulsion between metal and ligand atoms. Table 5 lists the atom types, partial charges, and nonbonded parameters (r_{min} and e_{min}) used in this study.

Figures 5–7 show stereoscopic drawings of the energy-minimized structures of each of the complexes. Table 6 lists the complexes, selected average Eu-atom separation distances, minimization energy, and coordination polyhedra.

Two low-energy conformations exist for the dtpa-oam complex (Figure 5). In both of these conformations, three amine nitrogens, three carboxylate oxygens, two amide carbonyl oxygens, and one water molecule oxygen coordinate the Eu^{3+}. The ether oxygens are not involved in coordinating the Eu^{3+} ion. Lack of coordination by ether oxygens in aqueous solution was noted previously in a study of Eu^{3+}–ether–bis(lactone) macrocyclic complexes.[12] The difference in the two conformations is that in the first conformation the carboxylate groups are up, down, up (with respect to the plane of the macrocycle), and in the second conformation they are up, down, down. The flexibility of the 21-membered macrocycle allows both of the conformations to be very close in energy, differing by only 4 kcal mol^{-1}.

The dtpa-eam complex is very similar to the dtpa-oam chelate in that three amine nitrogens, three carboxylate oxygens, and two amide carbonyl oxygens coordinate the Eu^{3+} ion (Figure 6). However, in this complex, the 15-membered macrocycle is not able to encapsulate the metal ion as well as the 21-membered ring of dtpa-oam does, leaving it more exposed. This allows the Eu^{3+} to coordinate two water molecules yielding a 10-coordinate complex. For this complex, only one low-energy conformation is found having the carboxylates in an up, down, up configuration. When the carboxylates are adjusted to an up, down, down configuration, the Eu^{3+} cannot accommodate two water molecules. An X-ray crystal structure determination has been reported for the Gd^{3+} complex of dtpa-eam.[26] However, this complex crystallizes as Gd_2(dtpa-eam)$_2$ where two Gd^{3+} ions are located between two ligands. In the solid-state structure, each Gd^{3+} ion coordinates three amine nitrogens, three carboxylate oxygens, two amide carbonyl oxygens, and one water molecule. Since we have determined that the Eu^{3+} complex of dtpa-eam binds two water molecules in solution, we conclude that dimerization in the case of Gd(dtpa-eam) is a result of crystallization. Interestingly, when a $-CH_2-CH_2-CH_2-$ group is substituted for the $-CH_2-CH_2-$ group in dtpa-eam, the Gd^{3+} complex crystallizes as a monomer with three amine nitrogens, three carboxylate oxygens, two amide carbonyl oxygens, and one water molecule coordinated to the Gd^{3+} ion.[26]

The dinuclear ligand bis(dtpa-eam) binds both Eu^{3+} ions in an identical fashion (Figure 6). Just like the dtpa-eam monomeric complex, each Eu^{3+} ion is coordinated to three amine nitrogens, three carboxylate oxygens, and two amide carbonyl oxygens. However, as in the case of dtpa-oam, the added flexibility of the

Table 6. Average Distances between Eu^{3+} and Selected Atoms, Minimization Energy, and Coordination Polyhedra for the Complex with Each Ligand As Determined from Molecular Mechanics Calculations

complex	average Eu–atom separation, Å	minimization energy, kcal mol^{-1}	coordination polyhedra[e]
Eu(dtpa-oam)[a]	OT, 2.32; O, 2.38; NT, 2.62; OW, 2.63	–406	msa
Eu(dtpa-oam)[b]	OT, 2.31; O, 2.37; NT, 2.62; OW, 2.63	–402	msa
Eu(dtpa-eam)	OT, 2.34; O, 2.46; NT, 2.79; OW 2.60	–410	bsa
Eu(edta-dam)	OT, 2.34; O 2.42; NT, 2.61; OW 2.60	–398	msa
Eu_2(do3a-dimer)[c]	OT, 2.32; O 2.44; NT, 2.61; OW 2.56; Eu, 8.71	–815	msa
Eu_2(do3a-dimer)[d]	OT, 2.31; O 2.45; NT, 2.60; OW 2.57; Eu, 11.83	–804	msa
Eu_2(bis(dtpa-eam))	OT, 2.25; O 2.43; NT, 2.60; OW 2.71; Eu, 7.48	–1073	msa

[a] Up, down, up configuration. [b] Up, up, down configuration. [c] Cupped configuration. [d] Extended configuration. [e] msa = monocapped square antiprism. bsa = bicapped square antiprism.

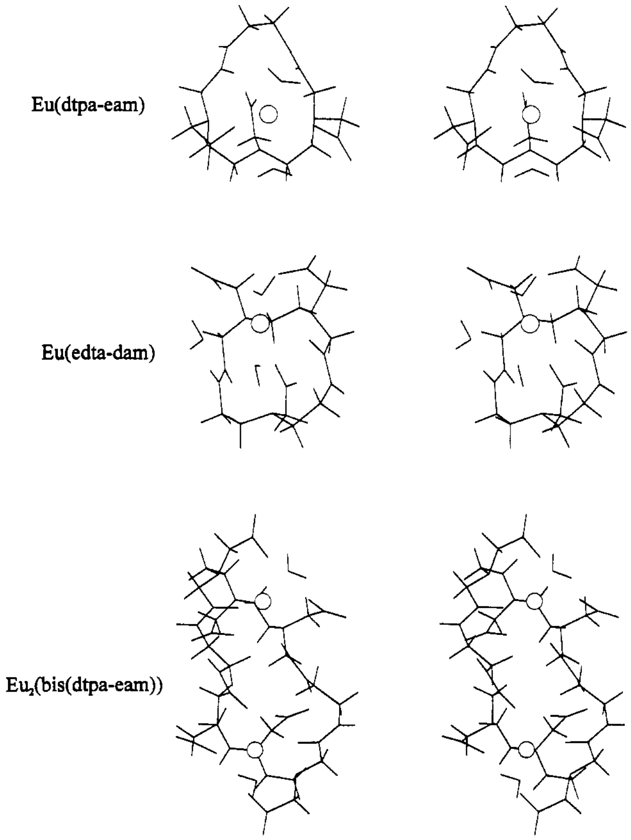


Figure 6. Stereoscopic drawings of the minimized structures of Eu(dtpa-eam)$(H_2O)_2$, Eu(edta-dam)$(H_2O)_3$, and Eu_2(bis(dtpa-eam))$(H_2O)_2$.

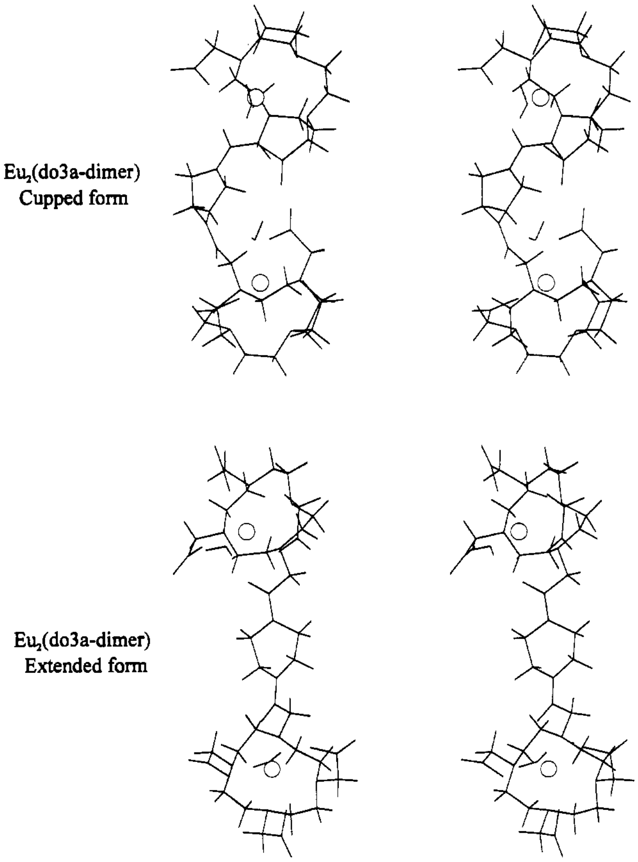


Figure 7. Stereoscopic drawings of the minimized structures of the low-energy minimized structures of Eu_2(do3a-dimer)$(H_2O)_2$.

bis(dtpa-eam) molecule allows the macrocycle to wrap around the Eu^{3+} ions, leaving space for only a single water molecule to coordinate each Eu^{3+} ion. The coordination of a second water molecule in the dtpa-eam complex explains why the Eu^{3+} $^7F_0 \rightarrow {}^5D_0$ transitions of each of these complexes differ so markedly from one another. For the bis(dtpa-eam) complex, the conformation with carboxylate arms in the up, down, up configuration is much lower in energy than any other conformation. This structure gives a Eu^{3+}–Eu^{3+} distance of 7.5 Å which is very close to the distance calculated from energy transfer (7.1 Å).

The edta-dam complex involves the coordination of two amine nitrogens, three carboxylate oxygens, one amide carbonyl oxygen, and three water molecules (Figure 6). Apparently in this case, the strain caused by the small macrocyclic cavity allows only one amide carbonyl oxygen to coordinate and dissuades the third amine nitrogen from coordinating. The Eu^{3+} ion is therefore well exposed and is able to coordinate three water molecules. This conformation has the two adjacent carboxylate arms in the up position, with the opposite arm in the down position. No other configurations allow three water molecules to coordinate the Eu^{3+} ion and are therefore disregarded.

The piperazine do3a-dimer binds two Eu^{3+} ions in an identical fashion. Complexation of Eu^{3+} by the piperazine do3a-dimer is achieved by the coordination of four amine nitrogen atoms, three carboxylate oxygens, one amide carbonyl oxygen, and one water molecule. There are two conformations of this complex that differ by only 11 kcal mol^{-1} (Figure 7). In both conformations, the carboxylate arms are in the up, up, up configuration. Any other configuration results in a significantly higher energy. In one low-energy conformation the piperazine ring and associated linker atoms form a cupped configuration. The Eu^{3+}–Eu^{3+} separation in this conformation is 8.7 Å which is in good agreement with the distance measured from energy transfer (8.4 Å). In the second conformation, the piperazine ring and linking $-COCH_2-$ moieties are in a more extended configuration, and the Eu^{3+}–Eu^{3+} separation is 11.8 Å. We believe, on the basis of the energy transfer results, that in solution the complex exists mainly in the cupped configuration.

The most interesting feature of the coordination chemistry of the ligands used in this study is persistence of amide carbonyl coordination to the metal ion even when there is a degree of strain involved. In the case of edta-dam and dtpa-eam the lowest energy conformation of the free ligands has the amide carbonyls pointing away from the center of the macrocyclic ring. In coordinating Eu^{3+}, the dtpa-eam ligand swings both carbonyl oxygens inward, balancing strain with coordination energetics. In the edta-dam complex, where the strain is even greater, only a single amide carbonyl oxygen can swing in and coordinate. In the case of dtpa-oam and bis(dtpa-eam) the strain energy is low enough so that the amide carbonyls can easily be directed inward toward the metal ion. For the piperazine do3a-dimer complex, the amide carbonyl oxygens from the linking piperazine moiety draw the Eu^{3+} ion in to coordinate causing the macrocycles to fold in toward each other.

On the basis of the strain involved with the coordination of the amide carbonyl oxygens to Eu^{3+} in these macrocyclic complexes, its not surprising that a considerable variation in the relative formation constants as a function of the lanthanide ion size is observed. Interestingly the smaller 15-membered macrocycles, dtpa-eam and edta-dam, have the greatest affinity for Eu^{3+} and Sm^{3+} respectively, while the 21-membered macrocycle dtpa-oam prefers the slightly smaller Tb^{3+} ion. From the modeling study, we believe this is because the dtpa-oam macrocycle wraps around the metal ion in coordination, whereas the metal ion sits above the macrocyclic plane in the dtpa-eam and edta-dam complexes. It is easy to understand why dtpa-oam has the highest formation constant for lanthanide ions of these three ligands (Table 2) since it sequesters the ions with little strain. It is less obvious why edta-dam has a stronger affinity for lanthanide ions than dtpa-eam. One would expect, on the basis of the fact that dtpa-eam provides eight coordinating atoms and edta-dam provides six coordinating atoms, that dtpa-eam would be a much stronger ligand. Perhaps the ligand dtpa-eam is held so rigidly in the strain of coordinating both amide carbonyl oxygens that it suffers a great deal entropically.

Frequency of the $^7F_0 \rightarrow {}^5D_0$ Transition. On the basis of these modeling studies, we are now in a better position to understand why the $^7F_0 \rightarrow {}^5D_0$ excitation maxima occur at different wavelengths for each complex. Previously in our laboratory, a correlation was discovered between the overall formal charge on a ligand and the frequency of the $^7F_0 \rightarrow {}^5D_0$ peak maximum of coordinated Eu^{3+} in a variety of complexes.[27] Using what has come to be known as the "charge line," one can predict, using eq 15, the energy of the $^7F_0 \rightarrow {}^5D_0$ transition of a Eu^{3+} complex knowing the overall charge on the ligands, p

$$p = -0.76\nu^2 + 2.29\nu + 17273 \qquad (15)$$

where ν is the energy of the transition (cm^{-1}).[23] This expression works well for many complexes, but many exceptions have been noted, including the complexes examined in the present study. We therefore sought alternative factors in the Eu^{3+} ion environment which might affect the frequency of the $^7F_0 \rightarrow {}^5D_0$ transition. One possible description of the electronic environment in the vicinity of Eu^{3+} is the sum of partial (negative) charges on the ligating atoms. In order to explore this possibility we summed the partial charges of the coordinated atoms for each complex in this study as well as for a selection of other complexes from previous studies, and plotted the frequencies of their $^7F_0 \rightarrow {}^5D_0$ transitions versus the sum of partial charges (Figure 8). The complexes considered, their coordinating atoms, $^7F_0 \rightarrow {}^5D_0$ transition energies, and partial charge sums are listed in Table 7. The partial charges are those used in the molecular mechanics calculations. The resulting plot fits well to a straight line with a correlation coefficient of 0.95 and clearly demonstrates the trend. The present correlation, it should be noted, runs counter to the earlier Albin–Horrocks line in that greater negative charge results in higher, rather than lower, frequency. It may be that physically this means that ligands with greater charge have more ionic character and hence less covalent character, causing the separation between ground and excited states to be larger. We are presently investigating a variety of Eu^{3+} complexes in an attempt to understand and verify our new correlation.

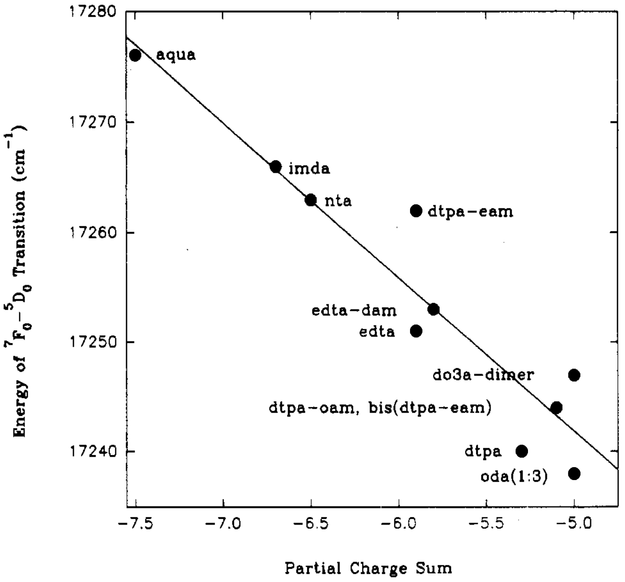


Figure 8. Plot of the sum of partial charges on all of the coordinated atoms vs. the wavelength of the Eu^{3+} $^7F_0 \rightarrow {}^5D_0$ excitation peak maximum for each complex in the present study as well as several other complexes from previous studies.[23,27]

Table 7. Coordinating Atoms, $^7F_0 \rightarrow {}^5D_0$ Transition Energies, and Partial Charge Sums for the Complexes Plotted in Figure 8

complex[c]	coordinating atoms	$^7F_0 \rightarrow {}^5D_0$ transition energy, cm^{-1}	partial charge sum
$Eu(H_2O)_9^{3+}$ [a]	9 OW	17 276	−7.5
$Eu(imda)^{+}$ [a]	1 NT, 2 OT, 6 OW	17 266	−6.7
$Eu(nta)$[a]	1 NT, 3 OT, 5 OW	17 263	−6.5
$Eu(edta)^{-}$ [a]	2 NT, 4 OT, 3 OW	17 251	−5.9
$Eu(dtpa)^{2-}$ [b]	3 NT, 5 OT, 1 OW	17 240	−5.3
$Eu(oda)_3^{3-}$ [a]	3 OE, 6 OT	17 238	−5.0
Eu(dtpa-eam)	3 NT, 3 OT, 1 O, 2 OW	17 262	−5.9
Eu(edta-dam)	4 OT, 2 O, 3 OW	17 253	−5.8
Eu(dtpa-oam)	3 NT, 3 OT, 2 O, 1 OW	17 244	−5.1
Eu_2[bis(dtpa-eam)]	3 NT, 3 OT, 2 O, 1 OW	17 244	−5.1
Eu_2(do3a-dimer)	4 NT, 4 OT, 1 OW	17 247	−5.0

[a] Reference 27. [b] Reference 23. [c] Abbreviations: imda = iminodiacetate; nta = nitrilotriacetate; edta = ethylenediamine-*N*,*N*,*N'*,*N'*-tetraacetate; dtpa = diethylenetriaminepentaacetate; oda = oxydiacetate.

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(27) Sudnick, D. R., Ph.D. Thesis, The Pennsylvania State University, 1979.