Crystal Structure and Spectroscopic Characterization of a Luminescent Europium Chelate

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We report the crystal structure at 1 Å resolution of europium bound to diethylenetriaminepentaacetate covalently coupled to an organic chromophore, carbostyril 124 (DTPA-cs124; carbostyril 124 = 7-amino-4-methyl-2(1*H*)-quinolinone). The complex is highly luminescent in solution and in the crystal form. The Eu-DTPA-cs124 crystallizes in the space group *C*2/*c* with two molecules in the asymmetric unit. The two molecules form a dimer which is stabilized by π-stacking of two carbostyrils and by the ligation of the carbonyl oxygen of one cs124 to the europium of the second half of the dimer. This ligation removes the last water bound to the primary coordination sphere of europium, resulting in no waters bound to the lanthanide. The crystal structure also shows the amide oxygen between the cs124 and the DTPA is ligated to the metal. Spectroscopic measurements of the europium luminescence in the crystal show that 66% of the luminescence is centered around 617 nm with a full width at half-maximum of 2.5 nm. In solution, 48% of the europium luminescence intensity is in the 617 nm peak, with a fwhm of 3.5 nm. The europium lifetime in the crystal structure is 0.90 ms; the lifetime of the monomeric Eu-DTPA-cs124 is 0.62 ms in H_2O solution and 2.4 ms in D_2O solution. The differing lifetimes can be explained by the well-known effects of H_2O on nonradiative europium emission rates and by postulating an electric (crystal) field surrounding the Eu which is more anisotropic in the crystal than in the solution, creating a faster radiative rate of Eu excited state deactivation. This greater anisotropy is expected on the basis of the crystal structure. The crystal structure also suggests improvements in the linkage of cs124 to DTPA which may improve the energy transfer between the organic chromophore and the europium and sharpen the Eu emission spectrum in solution.

Introduction

Luminescent lanthanide (terbium and europium) chelates are alternatives to radioactive and fluorescent probes in imaging and detection applications.[1−10] They are also excellent donors in resonance energy transfer experiments.[6,11−13] Their utility arises because of the lanthanide's unusual spectroscopic emission characteristics, which include millisecond lifetime, spiked emission peaks (<10 nm full width at half-maximum), large Stokes shifts (>150 nm), potentially high quantum yields (∼1), and excellent solubility. These characteristics enable discrimination against background fluorescence, which tends to have nanosecond lifetime and be broadly spread in emission. The result is high signal to noise, even when background fluorescence (such as autofluorescence from cells, or from solid supports such as nylon or nitrocellulose) is large. Detection limits of 2×10^{-15} M in an ethanolic solution[14] and $\leq 10^{-12}$ M in aqueous solutions[9,15−17] have been reported. Detection limits of biological macromolecules can be considerably lower because several hundred chelates per macromolecule can be attached with negligible self-quenching and minimal loss of biological specificity.[16,18,19]

A number of different luminescent lanthanide complexes have been synthesized, including those based on β-diketones,[20] cryptates,[21] and a number of polyamino carboxylates.[15,17,19,22−26] A critical element in the design of highly luminescent lanthanide chelates is the attachment of an organic chromophore to the chelate, such that the chromophore absorbs excitation light and transfers energy to the lanthanide with high efficiency. The chromophore is necessary because the intrinsic absorbance of terbium and europium is very low (≤ 1 M^{-1} cm^{-1}).[27] The exact

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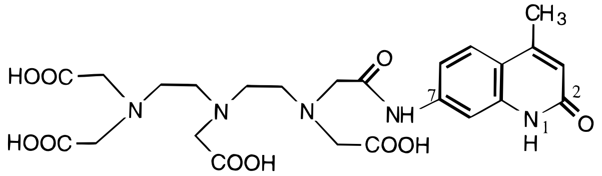

Figure 1. Structural formula of diethylenetriaminepentaacetic acid covalently coupled to carbostyril 124 (DTPA-cs124). The bis complex, DTPA-(cs124)$_2$, contains two carbostyrils, one at each end of the DTPA.

mechanism of energy transfer is not well understood, although a through-bond electron exchange via the chromophore triplet state and a through-space dipole−dipole interaction appear to be two mechanisms.[22,28] For the dipole−dipole mechanism, the distance between the chromophore and lanthanide is critical, and for electron exchange, the number of bonds between the chromophore and lanthanide is critical, with direct ligation being optimal. A greater understanding of these mechanisms, as well as the ability to systematically improve luminescent lanthanide complexes, has been hindered by the limited number of atomic-resolution structures.[20,21,28]

We recently presented work on the design and application of lanthanide complexes which are luminescent with both terbium and europium.[11,12,17,29] In order to further improve these chelates, we have crystallized one of the complexes, Eu-DTPA-cs124 (Figure 1). Among the questions of interest are the following: (a) Is the amide oxygen connecting the cs124 to the DTPA ligated to the metal? (b) Is the carbonyl oxygen at the 2-position of the carbostyril ligated to the metal? (c) Is the 7-position on the carbostyril the optimal position for linkage to the DTPA? (d) What is the number of water molecules ligated to the lanthanide's primary coordination sphere? (e) What is the overall size of the complex? (f) How do the spectra and lifetime of the europium emission in the crystal compare to those in solution, and what can be learned about the relative anisotropy of the crystal (electric) field surrounding the europium?

Experimental Section

Synthesis and Crystallization of Eu-DTPA-cs124. Synthesis of DTPA-cs124. A 73 mg sample of cs124 (0.42 mmol) in 1.5 mL of DMF was added dropwise to a solution of dianhydride DTPA (150 mg, 0.42 mmol) in 11.25 mL of DMF and 0.75 mL of triethylamine. The reaction was quenched after 2 h by addition of 15 mL of 1 M triethylammonium acetate, pH 6.5. The mixture was then purified by reversed-phase HPLC using a 21.4 mm × 250 mm C18 column (Dynamax 60 Å) with a flow rate of 12.5 mL/min. A 30 min linear gradient, from 15% to 60% solvent B (solvent A, 0.1 M aqueous triethylammonium acetate, pH 6.5; solvent B, acetonitrile) was used. The peak at retention time 12 min was detected at 328 nm, collected, and dried to give DTPA-cs124 (MW 549) as a light yellow solid: yield 120 mg, 52%. FAB-MS $m/e = 550$ ($M + H^+$). ^{1}H NMR: δ (D_2O) 2.70 (3H, s), 3.50 (2H, t), 3.57 (2H, t), 3.63 (2H, t), 3.75 (2H, t), 3.92 (2H, s), 4.02 (2H, s), 4.11 (4H, s), 4.30 (2H, s), 6.70 (1H, s), 7.52 (1H, d), 7.92 (1H, s), 8.01 (1H, d). Anal. Calcd (found) for $C_{24}H_{31}N_5O_{10}\cdot 3H_2O\cdot 1.5(CH_3CH_2)_3N$: C, 52.47 (52.36); H, 7.94 (8.12); N, 12.05 (12.11).

Lanthanide and Counterion Labeling. An 86 μL portion of a 0.1 M $EuCl_3$ solution was added to 4 mg of solid DTPA-cs124 dissolved in 3 mL of 100 mM sodium bicarbonate, pH 7.0. After ≥1 h, the solution was divided in half and 0.5 mL of 200 mM NaCl was added to each aliquot. Each resulting aliquot was then passed over a C-18 column (Waters, Sep-Pak No. 23635) and washed with ≥5 mL of H_2O, followed by elution with 50% methanol/50% H_2O. The eluant was evaporated to dryness in a speed-vac. Absorbance and luminescence measurements of the europium complex displayed the expected characteristics.[17]

Crystallization. Initial screening for crystallization conditions was carried out by sparse matrix sampling using a set of 50 different conditions.[30] The procedure yielded small needlelike crystals in a solution containing 30% PEG 4000 (poly(ethylene glycol)), 100 mM cacodylate buffer, pH 6.5, and 200 mM magnesium chloride. Further improvement was made by the vapor-diffusion sitting drop method.[31] Drops containing 7 μL of Eu-DTPA-cs124 (19 mg/mL) were mixed with 7 μL of reservoir solution containing 15% PEG 4000, 100 mM cacodylate buffer, pH 6.5, and 200 mM $MgCl_2$ in nine depression spot plates (Corning No. 7220). The samples were then sealed inside a plastic sandwich box which held 20 mL of the above reservoir solution. Crystallization was complete in approximately 1 week.

X-ray Diffraction. Data Collection. A colorless needlelike crystal of $EuN_5O_{18.5}C_{24}H_{43}$ having approximate dimensions of 0.01 mm × 0.03 mm × 0.45 mm was mounted on a glass fiber. All measurements were made on a Siemens SMART CCD diffractometer with graphite-monochromated Mo Kα radiation ($\lambda = 0.710\,69$ Å).

Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the measured positions of 3972 reflections with $I > 10\sigma(I)$ corresponded to a primitive monoclinic cell with dimensions $a = 34.547(2)$ Å, $b = 18.8540(9)$ Å, $c = 12.8900(6)$ Å, $V = 8148.8(6)$ Å^3, and $\beta = 103.936(1)°$.

For $Z = 8$ and fw = 849.59, the calculated density is 1.38 g/cm^3. On the basis of the systematic absences of hkl ($h + k \neq 2n$) and $h0l$ ($l \neq 2n$), a statistical analysis of intensity distribution, and the successful solution and refinement of the structure, the space group was determined to be $C2/c$ (No. 15).

The data were collected at −105 ± 2 °C. Area detector frames were collected using ω scans of 0.30° collected for a total of 30 s per scan using graphite-monochromated Mo Kα radiation, with a $2\theta_{\max}$ of 46.5°.

Data Reduction. The frame data were integrated using the program SAINT and a 1.6° × 1.6° × 0.6° box. The data were corrected for Lorentz and polarization effects. No decay correction was necessary. The linear absorption coefficient, μ, for Mo Kα radiation is 16.1 cm^{-1}. An empirical absorption correction based on multiply redundant data was performed using the Siemens version of XPREP (ellipsoid function, $\mu R = 0.04$, $T_{\max} = 0.98$, $T_{\min} = 0.78$). The 15 266 integrated reflections were averaged to yield 5907 unique reflections ($R_{\text{internal}} = 0.131$).

Structure Solution and Refinement. The structure was solved by heavy-atom Patterson methods[32] and expanded using Fourier techniques.[33] Due to limitations of the data, only the Eu and full-occupancy oxygen atoms were refined anisotropically, while the rest were refined isotropically. Hydrogen atoms on the ligand were included but not refined. The final cycle of full-matrix least-squares refinement[34] was based on 2134 observed reflections ($I > 4.00\sigma(I)$) and 296 variable parameters and converged (largest parameter shift was 0.08 times its esd) with unweighted and weighted agreement factors of:

$$R = \sum||F_o| - |F_c||/\sum|F_o| = 0.072$$

$$R_w = [\sum w(|F_o| - |F_c|)^2/\sum wF_o^{\,2})]^{1/2} = 0.081$$

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The standard deviation of an observation of unit weight[35] was 3.04. The weighting scheme was based on counting statistics and included a factor (p = 0.033) to downweight the intense reflections. Plots of $\sum w(|F_o| - |F_c|)^2$ versus $|F_o|$, reflection order in data collection, $(\sin\theta)/\lambda$, and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 1.00 and -0.77 e$^-$/Å^3, respectively.

Neutral-atom scattering factors were taken from Cromer and Waber.[36] Anomalous dispersion effects were included in F_c;[37] the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley.[38] The values for the mass attenuation coefficients are those of Creagh and Hubbel.[39] All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corp.[40]

Luminescence Spectroscopy. All lifetime and wavelength emission spectra were recorded on a laboratory-built spectrometer. A pulsed nitrogen laser (337 nm, 5 ns pulse-width, 40 Hz repetition rate; Laser Photonics) excited the Eu-DTPA-cs124 sample, and emission was passed through a double-monochromator (SPEX 1680B, blaze = 500 nm, *f*/4) and detected by a gallium arsenide photomultiplier tube operating in photon-counting mode (Hamamatsu R943-02) with associated electronics (MITEQ 500 MHz preamplifier; Ortec 854 gated discriminator; Canberra FMS multichannel scalar with 2 μs time resolution). The Eu-DTPA-cs124 crystals were thoroughly washed with H_2O so no mother liquor remained and then placed in a quartz capillary tube (Vitro Dynamics, Rockaway, NJ). For solution measurements, Eu-DTPA-cs124 (or Eu-DTPA-$(cs124)_2$) was dissolved in either a D_2O- or H_2O-based buffer in 10 mM Tris, pH 8.0, and placed in a 3 mm × 3 mm quartz cuvette. Details of the solution measurement have been reported.[17]

Results and Discussion

Crystal Structure. The structural formula of DTPA-cs124 is shown in Figure 1. Figure 2a shows a top view of the crystal structure of the dimer $(Eu\text{-}DTPA\text{-}cs124)_2$ which makes up the unit cell. Figure 2b is a side view of the same structure, and Figure 2c is the structure of half the dimer, with the europium of the second half of the dimer shown as well. A list of bond lengths appears in Table 1.

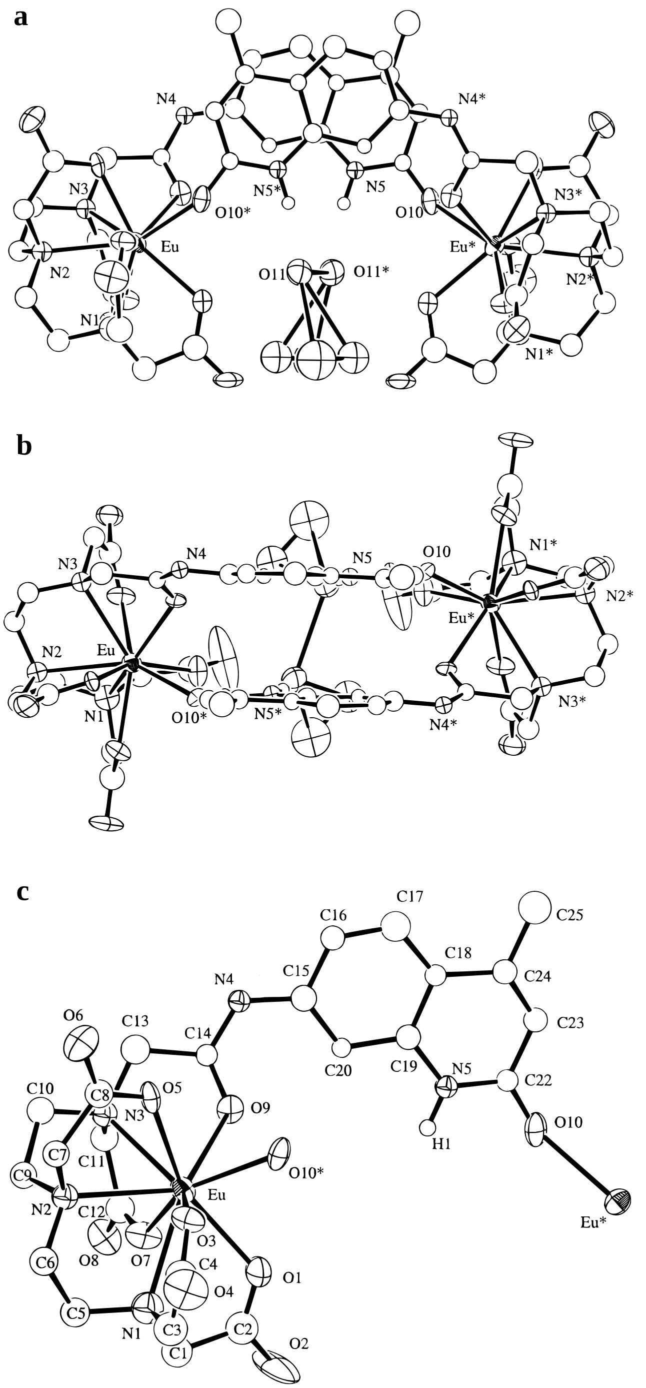

Figure 2. Crystal structure of Eu-DTPA-cs124. The unit cell consists of a dimer of Eu-DTPA-cs124. Two out of the 8−9 water molecules in the unit cell are also shown. Note the stacking of the carbostyril chromophores and the ligation of the carbostyril carbonyl (O10 and O10*). Hydrogen atoms are not shown except for those bonded to N5 and N5* and to the water oxygens, O11 and O11*.

The europium ion is coordinated by nine ligands, consisting of three amines N1, N2, N3, four carboxylate oxygens O1, O3, O5, O7, one amide oxygen O9 from the linkage between DTPA and cs124, and one amide oxygen O10* from the carbostyril of the second half of the dimer. The asymmetric unit consists of two molecules of Eu-DTPA-cs124 which are joined (1) by the ligation of the amide oxygen at position 2 in the carbostyril ring to the europium of the other half of the dimer and (2) by strong π-stacking of the two carbostyrils—each carbostyril is planar and separated by 3.38 Å from the other. The Eu−Eu distance is 9.9 Å. There are an additional 8−9 water molecules in the asymmetric unit, two of which are shown in Figure 2a. The unit cell displays *C*2/*c* symmetry. However, the environment around each europium is highly unsymmetrical. Eu-DTPA (no carbostyril and no extra ligation due to dimerization) is fairly asymmetric,[41] and the symmetry is further reduced by the replacement of one carboxylate group by an amide group (forming the linkage to cs124) and by the replacement of one coordinated water by the amide oxygen of the second carbostyril.

Several specific observations can be made:

- The average distance of the $C{=}O_{carboxylate}$ bond is very similar whether the oxygen is bound to the Eu (1.265 ± 0.026 Å) or not bound to the Eu (1.290 ± 0.032 Å). This indicates a resonant delocalization of the electron between the two oxygen molecules of the carboxylate group.
- The amide oxygen of the bond connecting cs124 to DTPA is clearly ligated to the europium. This is consistent with other X-ray data for DTPA−amide complexes[42] and with spectro-

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(35) Standard deviation of an observation of unit weight: $[\sum w(|F_o| - |F_c|)^2/(N_o - N_v)]^{1/2}$ where N_o = number of observations and N_v = number of variables.

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Table 1. Bond Lengths (Å) for Eu-DTPA-cs124[a]

Eu Bond Lengths

Eu−$O_{carboxylate}$		Eu−O_{amide}		Eu−N_{amine}	
Eu−O1	2.41(2)	Eu−O9	2.50(2)	Eu−N1	2.64(2)
Eu−O3	2.40(2)	Eu−O10*	2.30(1)	Eu−N2	2.59(2)
Eu−O5	2.39(2)			Eu−N3	2.76(2)
Eu−O7	2.35(2)			av	2.663 ± 0.087
av	2.388 ± 0.026				

Carbostyril Chromophore Bond Lengths

				chromophore plane 1				
				atoms defining plane	dist*	esd	dist to atoms of plane 2	
C_{sp^2}−C_{sp^2}		C_{sp^2}−N_{amide}		N5	0.0145	0.0175	N5*	3.216
C15−C16	1.43(4)	C14−N4	1.33(3)	C15	−0.0106	0.0237	C15*	3.383
C15−C20	1.37(3)	C22−N5	1.33(3)	C16	−0.0200	0.0228	C16*	3.510
C16−C17	1.37(3)			C17	−0.0312	0.0242	C17*	3.551
C17−C18	1.39(3)	C_{sp^2}−N		C18	0.0088	0.0224	C18*	3.427
C18−C24	1.47(3)	C15−N4	1.38(3)	C19	0.0139	0.0232	C19*	3.303
C19−C20	1.45(3)	C19−N5	1.38(3)	C20	0.0225	0.0213	C20*	3.265
C22−C23	1.42(3)			C22	−0.0593	0.0231	C22*	3.308
C23−C24	1.29(3)	C_{sp^2}=O_{amide}		C23	−0.0216	0.0233	C23*	3.388
C24−C25	1.51(4)	C22−O10	1.28(3)	C24	0.0708	0.0242	C24*	3.383
av	1.411 ± 0.065						av	3.383 ± 0.105
		N−H		mean dev from plane				
		N5−H1	0.95	0.0273 ± 0.0226 Å				
				addnl atoms	dist		addnl atom	dist
				O10	−0.1531		O10*	3.313
				C25	0.0463			

DTPA Chelate Bond Lengths

C−$O_{carboxylate}$		C=$O_{carboxylate}$		N_{amine}−C_{sp^3}		C_{sp^3}−C_{sp^3}		C_{sp^3}−C_{sp^2}	
C2−O1	1.27(4)	C2−O2	1.27(4)	N1−C1	1.51(4)	C5−C6	1.50(4)	C1−C2	1.50(4)
C4−O3	1.30(3)	C4−O4	1.26(4)	N1−C3	1.47(4)	C9−C10	1.51(3)	C3−C4	1.51(4)
C8−O5	1.25(3)	C8−O6	1.30(3)	N1−C5	1.53(3)	av	1.505 ± 0.007	C7−C8	1.55(4)
C12−O7	1.24(3)	C12−O8	1.33(3)	N2−C6	1.49(3)			C11−C12	1.54(4)
av	1.265 ± 0.026	av	1.290 ± 0.032	N2−C7	1.47(3)			C13−C14	1.52(3)
				N2−C9	1.50(3)			av	1.524 ± 0.021
		C=O_{amide}		N3−C10	1.47(3)				
		C14−O9	1.25(3)	N3−C11	1.48(3)				
				N3−C13	1.49(3)				
				av	1.490 ± 0.021				

[a] Estimated standard deviations in the least significant figure are given in parentheses. The C=$O_{carboxylate}$ are defined as the carbon−oxygen bonds containing an oxygen not bound to Eu; the C−$O_{carboxylate}$ are defined as bonds for the oxygen bound to Eu. Eu−O9 is for the amide oxygen in the linkage between cs124 and DTPA, and Eu−O10 is the bond between the carbonyl at the 2-position of the one carbostyrils and the europium of the second half of the dimer (see Figure 2a,b). Chromophore plane 1 is defined as the least-squares planar fit to atoms N5 and C15−24, and the distances and estimated standard deviations of those distances are listed above. Plane 2 is defined as plane 1 except with the other carbostyril, and the distance from each atom in plane 2 to the least-squares plane 1 is shown.

scopic measurements which show that conversion of a DTPA carboxylate to an amide upon attachment of cs124 does not change the ligation state of the europium.[17] The Eu−O_{amide} bond (Eu−O9 = 2.50 Å) formed by the linkage of cs124 to DTPA is, however, significantly longer and hence less strongly ligated to Eu than the carboxylate bonds (average 2.388 ± 0.026 Å). The Eu−O9 amide bond length is 4.3 standard deviations longer than the average bond length of Eu−$O_{carboxylate}$. This is to be expected because the amide oxygen has less negative charge density than a carboxylate oxygen.

- The oxygen−europium bond (O10*−Eu) bridging the halves of the dimer is equal to or shorter than the oxygen−europium bonds within half of the dimer (2.30 Å vs 2.388 Å, a 3.4σ difference). This indicates relatively strong ligation between the dimer in the crystal state. This is consistent with the spectroscopic changes induced by this extra ligation (see below).
- The two carbostyril chromophores are strongly π-stacked and are coplanar, with an average separation of 3.38 Å. The methyl group lies close to the plane of the carbostyril rings. All carbon−carbon lengths in the rings are approximately equal, indicative of the expected resonant structure.
- The structure of the DTPA chelate in the Eu-DTPA-cs124 complex is similar to that of unmodified DTPA bound to heavy metals such as Gd(III) and Nd(III) [42−44] and to a DTPA modified by the conversion of two carboxylate groups to two primary aliphatic amides.[42] This implies that conversion of one (or two) carboxylate groups to amides, whether the amides are formed from primary aliphatic[42] or primary aromatic amines (this work), does not significantly affect the structure of the DTPA chelate. These results are also consistent with solution spectroscopic measurements which show that europium emission, which is a function of the crystal field anisotropy surrounding the lanthanide, is similar in DTPA,[41] in DTPA-cs124,[17] and in DTPA-cs124-ethylenediamine (data not shown). This fact simplifies the synthesis of new luminescent complexes since it implies that attaching a sensitizer does not significantly perturb the chelate, and hence, to first order, the chelate and the sensitizer can be optimized nearly independent of one another. (Of course

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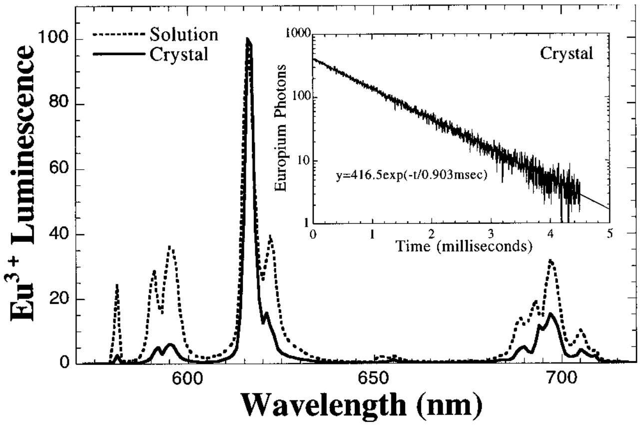

Figure 3. Comparison of Eu-DTPA-cs124 emission line shapes in solution (dotted line) and in the crystal (solid line). Inset is europium lifetime in the crystal. The emission is arbitrarily normalized to the maximum peak height of the $^5D_0 \rightarrow {}^7F_2$ transition at 617 nm. A greater fraction of emission is in the $^5D_0 \rightarrow {}^7F_2$ transition in the crystal than in solution (see text). The absolute intensity of the europium emission under the two conditions was not determined (see text). Concentration of Eu-DTPA-cs124 in solution was approximately 0.5 μM.

the linkage between the two is important, and if the sensitizer is integrated into the structure of the chelate, this independence breaks down.)

- The largest distance across Eu-DTPA-cs124 parallel to the cs124 plane is 12.8 Å, from C25 to C5. Perpendicular to this line and still in the cs124 plane, the largest dimension is 8.1 Å, from O2 to O6. The largest distance perpendicular to the cs124 plane is 8.3 Å, from the O4 to O8. Hence, crudely speaking, the monomeric complex can be contained in a rectangular box of dimensions 12.8 Å × 8.1 Å × 8.3 Å.
- The stability of the dimer will be concentration dependent, and at the micromolar levels used in solution-phase spectroscopy, the monomeric form is highly likely to be the predominant form. Indeed, luminescence measurements at these concentrations indicate one water molecule bound to the primary coordination sphere of europium, confirming that ligation by a second carbostyril to the metal is not occurring.[17]
- In solution, cs124 is likely free to rotate about the N4−C15 and N4−C14 bonds, with little steric hindrance or favored orientation. The central two waters (containing atoms O11 and O11*) can have at most one hydrogen bond to the lanthanide complex via the hydrogen atom on N5 and N5*. Consequently, in solution, these waters are likely rapidly exchanging with the solvent and will not significantly hinder the rotation of the cs124. Similarly, the one water bound to the europium in solution (replacing O10*) is capable of forming at most one hydrogen bond to cs124 (possibly to H1) and should therefore not inhibit free rotation of cs124 with respect to Eu-DTPA.

Luminescence Spectroscopy. Figure 3 shows the emission spectrum of europium luminescence in solution and in the crystal structure. No effort has been made to compare the absolute intensities in the crystal and in solution since the relative number of molecules in the excitation beam are unknown. When absolute intensities in europium complexes which do *not* contain a sensitizer are measured, the intensity of the magnetic dipole transition $^5D_0 \rightarrow {}^7F_1$ (around 590 nm) is relatively insensitive to the europium environment, and therefore can be used as an internal standard to account for concentration differences.[27] However, in complexes with a sensitizer, the intensity of this transition is not necessarily the same (in the crystal vs in solution, e.g.) because the efficiency of energy transfer from the sensitizer to the europium may differ under the two conditions.

Table 2. Europium Emission Lifetimes in Different Complexes under Various Conditions

compd	lifetime (ms)
Eu-DTPA-cs124 crystal	0.90
Eu-DTPA-cs124 in soln	0.62 (H_2O) 2.40 (D_2O)
Eu-DTPA-(cs124)$_2$	0.57 (H_2O)

We have measured the europium emission lifetime in the crystal state (this work) and in H_2O- and D_2O-based solutions.[17] In addition, we have measured the lifetime of Eu-DTPA-(cs124)$_2$ ("bis complex") as a model to try to understand the effect of carbostyril stacking on europium emission and lifetime. The europium lifetime in all cases is single exponential, and the values are presented in Table 2. Figure 3 shows that, in the crystal structure, the emission is concentrated in the 617 nm peak which contains 66% of the total emitted photons and has a full width at half-maximum (fwhm) of 2.5 nm. In solution, the europium emission in DTPA-cs124 is somewhat more dispersed, with 48% of the photons emitted in the 617 nm peak, which has a fwhm of 3.5 nm. The shape of the Eu-DTPA-(cs124)$_2$ emission spectrum is very similar to that of the monomeric DTPA-cs124, although the total intensity is 2.7 times less.

The shape of the emission spectrum is a function of the crystal field surrounding the europium. In general, a more anisotropic environment increases the fraction of photons emitted in the 617 nm peak.[17,27,41] In addition, a more anisotropic environment decreases the europium lifetime by increasing the radiative rate. Figure 3 then implies that the crystal symmetry surrounding the europium is more anisotropic in the crystal state than in solution. We believe this is due to the ligation of the amide oxygen of the second carbostyril to the first europium, displacing a water molecule. We would expect the Eu-DTPA-cs124 with one water bound (the solution situation) to have greater symmetry via a rotation about an axis which intersects the water and lanthanide than the same complex where the H_2O (or D_2O) molecule is replaced by an amide oxygen from carbostyril (the crystal situation).

The shortened lifetime of europium in the crystal compared to Eu-DTPA-cs124 in D_2O is also consistent with a more anisotropic crystal field. H_2O bound to the primary coordination sphere of europium is the main pathway for nonradiative deexcitation,[27,45] and in D_2O or the crystal, there are no H_2O molecules bound to the europium. Assuming a quantum yield for europium luminescence of unity in the crystal and in D_2O, the faster lifetime in the crystal implies a faster radiative rate. (See also refs 11, 12, and 17 and references therein for a discussion of lanthanide quantum yield. Here we note that the lanthanide quantum yield is defined as the probability that the excited state of the lanthanide will decay to the ground state via emission of a photon. This definition explicitly excludes the efficiency of energy transfer from the sensitizer to the lanthanide, which simply decreases the effective absorption cross section of the complex.) While the assumption of unity quantum yield in D_2O solution for Eu-DTPA-cs124 is probably not in error by too much, it is possible that the dimerization and π-stacking of cs124s in the crystal structure create a low-lying triplet state capable of de-exciting the europium through a nonradiative, back-transfer mechanism. We have found that such a nonradiative pathway exists for Tb-DTPA-(cs124)$_2$ in solution.[17] As a crude model for the stacked carbostyrils, we have examined the europium luminescence and lifetime of Eu-

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DTPA-$(cs124)_2$ in solution. The similarity in the shapes of the emission spectra of the mono and bis forms indicates the crystal fields in the two complexes are quite similar. The europium lifetime in the bis complex is 8% shorter than that in the mono complex, indicating that if a nonradiative pathway exists due to interactions between the cs124s, the pathway is quite inefficient for europium. (It is likely that the bis complex forms a new triplet state which is near or below the terbium excited state energy level and hence is capable of efficiently deactivating the terbium excited state, but significantly above the europium excited state level, and therefore ineffective at deactivating the Eu excited state.) From this, we conclude that the most likely explanation for the shortened lifetime of Eu in the crystal is an increased radiative rate due to an anisotropic crystal field, although an increased nonradiative rate cannot rigorously be excluded.

Conclusion

The crystal structure has answered many of the questions which motivated the crystallization effort: (a) The amide oxygen between carbostyril and DTPA is ligated to the europium. (b) The amide oxygen at the 2-position of carbostyril is not ligated to its own europium but is ligated to the second europium in the unit cell. This ligation is not likely to exist in solution at the (sub)micromolar concentrations used in luminescence spectroscopy. (c) This ligation eliminates all waters potentially bound to the europium primary coordination sphere. (d) The dimerization is aided by the tight stacking of the relatively hydrophobic and planar carbostyrils. (e) The europium lifetime is decreased in the crystal structure as compared to a D_2O solution, likely because of an increased radiative rate, though possibly owing to an increased nonradiative rate as well. (f) The 7-position at carbostyril is not an optimal attachment point for DTPA, and an attachment through the 1-nitrogen might allow the 2-carbonyl of carbostyril to ligate its own europium.

These results are a direct guide to developing better luminescent lanthanide chelates. An anisotropic crystal field around the europium appears to concentrate the luminescence in a single narrow band around 617 nm. The anisotropy can be induced through anisotropic chelates and by ligation of the sensitizer to the lanthanide. Ligation of the sensitizer to the lanthanide in its own chelate would have many advantages. It would likely increase the efficiency of energy transfer from sensitizer to lanthanide: if the dipole−dipole mechanism is operative, then ligation brings the sensitizer physically close to the lanthanide; if electron exchange is operative, then the number of bonds between sensitizer and lanthanide has been reduced to the minimum. Ligation of the sensitizer would also fill the last primary coordination site of the lanthanide, thereby increasing the europium quantum yield to nearly unity, even in a H_2O-based solution. Finally, if ligation increases the efficiency of energy transfer from the sensitizer to the lanthanide to nearly 100%, then the lanthanide intensity would be not only brighter but potentially nearly nonphotobleachable. This is because the lanthanide is indestructible and the chromophore should become so if it quickly and efficiently transfers its excited-state energy to the lanthanide. Such a bright, indestructible luminescent label would have many applications, particularly in energy-transfer experiments and in samples where autofluorescence background prevents imaging and detection using conventional fluorophores.

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Supporting Information Available: Tables of crystal data, positional parameters and B_{eq} for the Eu-dtpa-cs124 dimer, *U* values for the Eu-dtpa-cs124 dimer, intramolecular distances involving the non-hydrogen atoms, intramolecular distances involving the hydrogen atoms, intramolecular bond angles involving the non-hydrogen atoms, intramolecular bond angles involving the hydrogen atoms, torsion or conformation angles, intermolecular distances involving the non-hydrogen atoms, intermolecular distances involving the hydrogen atoms. special contacts involving the non-hydrogen atoms, and least-squares planes (26 pages). Ordering information is given on any current masthead page.

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