

Polymetallic Lanthanide Complexes with PAMAM-Naphthalimide Dendritic Ligands: Luminescent Lanthanide Complexes Formed in Solution

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Luminescent lanthanide reporters emitting in the visible and near-infrared domains are in increasing demand due to unique electronic properties such as long luminescence lifetimes, photostability, and sharp emission bands. These properties make them superior reporters for applications such as drug discovery, genomic and proteomic screening, medical diagnostics, and biological imaging.^{1,2} To take advantage of these properties, it is crucial to have lanthanide complexes that are strongly emissive in solution, the result of strong absorption, efficient sensitization and protection of the metal ion.^{3,4} A promising strategy to maximize the number of absorbed and emitted photons is the development of reporters that possess more than one luminescent metal ion by synthesizing polymetallic lanthanide complexes. This can be achieved by using large, flexible ligands such as dendrimers. Attaching sensitizing groups to the end branches of the dendrimer allows for high absorption of the complex, and the presence of multiple coordinating groups within the dendrimer core enables the binding of several luminescent lanthanide ions. Reports of metal complexes formed with dendrimer ligands have recently appeared in the literature.^{5–9} When polynuclear complexes are formed, characterization of the species in solution is an important aspect of rationalizing the luminescent properties of the complex. For this purpose, a particularly useful family of dendrimers are PAMAM (polyamidoamine) dendrimers,^{10a,b} whose branches contain internal amide groups capable of binding hard Lewis acid lanthanide ions through their oxygen atoms, and which have terminal primary amine end branches for functionalization with suitable chromophores. PAMAM dendrimers are also shown to have low toxicity and have been employed in a large variety of biomedical applications.¹¹ We report the synthesis of a generation 3 (32 end-branches) PAMAM dendrimer **1** (Figure 1) that contains a total number of 60 internal amide groups suitable for the coordination of lanthanide ions and functionalized with 32 external 2,3-naphthalimide groups. 2,3-Naphthalimide was chosen as a sensitizer for Eu^{3+} , as it displays high molar absorptivity and efficient intersystem crossing, which leads to high population of the triplet state.¹² In addition, absorption centered at $27\,780\text{ cm}^{-1}$ allows for sensitization of the Eu^{3+} ion at relatively low energy, which is desirable for the use of compounds in biological applications since shorter excitation wavelengths can damage biological substrates. The triplet-state energy is located at $20\,280\text{ cm}^{-1}$,¹² a suitable energy for the sensitization of Eu^{3+} due to a good energy match between the donating level of the ligand and the accepting level of Eu^{3+} ($17\,200\text{ cm}^{-1}$). For the first time, polymetallic dendrimer complexes formed in solution have been characterized, and their stoichiometry determined through luminescence titrations. The results indicate that this is a powerful method for characterizing such complexes. Experimental data shows that a metal complex is formed and the lanthanide cations have a coordination number ranging from 7 to 9 for the most luminescent species. These coordination numbers are typical for complete coordination of Eu^{3+} in solution.¹ To our knowledge, this is also the first reported use

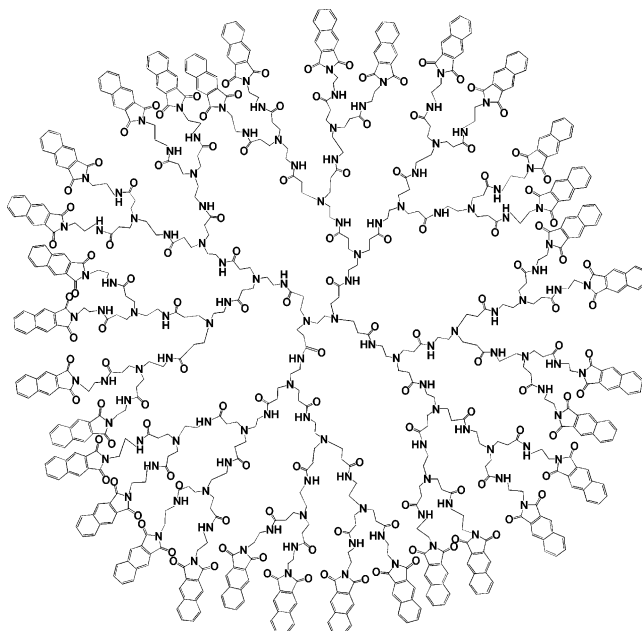


Figure 1. Synthesized dendrimer ligand **1**.

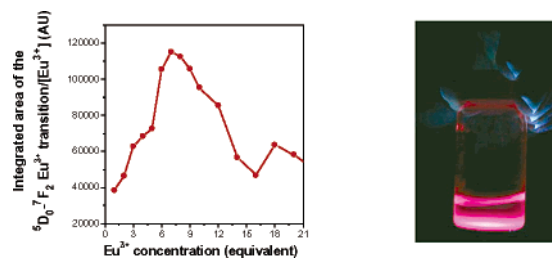


Figure 2. (Left) Plot showing integrated emission of the $\text{Eu}^{3+} {}^5\text{D}_0 \rightarrow {}^7\text{F}_2$ transition at 614 nm with increasing equivalents of Eu^{3+} (298 K, concentration of ligand 10^{-6} M in DMSO, $\lambda_{\text{exc}} = 360\text{ nm}$). (Right) Solution formed by incubating 8 equiv of Eu^{3+} with 1 equiv of dendrimer ligand irradiated with excitation light at 354 nm (298 K, ligand concentration $8.7 \times 10^{-5}\text{ M}$ in DMSO).

of naphthalimides as sensitizers for Eu^{3+} . The synthesis and characterization of **1** are reported in the Supporting Information section.

Addition of Eu^{3+} to a solution of **1** in DMSO leads to the appearance of red-centered lanthanide emission arising from Eu^{3+} which can be easily observed with the naked eye when irradiated with UV light (Figure 2). To identify the species formed in solution, UV/vis absorption and fluorescence/luminescence batch spectrophotometric titrations were performed. A titration experiment was conducted by adding 1–20 molar equiv of Eu^{3+} to a solution of **1** at 10^{-6} M in DMSO. It was anticipated that the coordination of the Eu^{3+} ions would be slow due to the internal location of the amide binding groups. To examine this hypothesis, absorption and

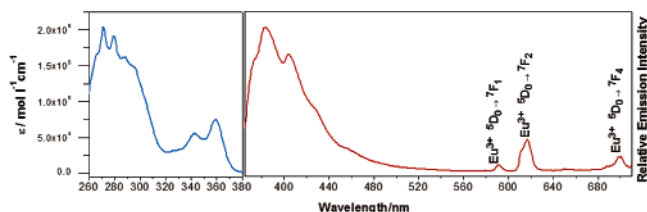


Figure 3. Absorption (left) and emission (right) spectra of 8 equivalents of Eu^{3+} with **1** at 10^{-6} M in DMSO after incubation for 7 days at room temperature; λ_{ex} at 360 nm.

luminescence spectra were recorded on a daily basis for up to 14 days in order to monitor any changes occurring over time. The stability of **1** was assessed by ^1H NMR and ^{13}C NMR, with identical spectra obtained after a period of 14 days. This indicates that there is no fragmentation of the naphthalimide moiety or aliphatic dendrimer core. UV/vis spectra did not reveal significant changes in the absorption of **1**, but it is unlikely that the naphthalimide C=O groups are involved in bonding with the lanthanide ion. A complementary explanation is that the lanthanide ions do not have a strong influence on the electronic properties of the naphthalimide sensitizer due to the probable long distance between them and the Eu^{3+} cations. Luminescence spectra indicated significant changes in emission arising from both the naphthalimide groups and the Eu^{3+} ions. A plot of the area of the emission of the hypersensitive $^5\text{D}_0 \rightarrow ^7\text{F}_2$ Eu^{3+} transition at 614 nm divided by the number of Eu^{3+} molar equivalents against the added molar equivalents of Eu^{3+} (Figure 2) indicates the formation of a luminescent europium complex.

This plot reveals a bell-shaped curve with a maximum around 8 molar equivalents, indicating the formation of the most luminescent Eu^{3+} complex. This is consistent with the coordination number range of lanthanide ions usually observed in solution. Since there are 60 aliphatic amide donor groups in the dendrimer core, and each dendrimer ligand can coordinate 8 lanthanide ions, an overall average coordination number of 7.5 can be postulated. Values between 8 and 9 are typical for a complete coordination of each lanthanide ion by amide oxygens.^{13,14} The titrations were reproducible on separate occasions under the same experimental conditions. The solution studies were performed at low concentration in a strongly competing solvent (DMSO). Therefore, the observation of Eu^{3+} binding to neutral amide groups is remarkable. This observation indicates that the dendrimeric effect plays a significant role in stabilizing the final complexes.

The absorption spectrum of the lanthanide complex (Figure 3) reveals a high extinction coefficient, enabling excitation of the lanthanide complex at longer wavelength (360 nm). To quantify the ligand–lanthanide energy-transfer efficiency, the absolute quantum yield of the Eu^{3+} -centered emission, obtained by incubating **1** equiv of **1** with 8 equiv of Eu^{3+} , was determined relative to quinine sulfate¹⁵ and found to be 0.06% ($\pm 15\%$) at 298 K.

At room temperature, the luminescent lifetime of the Eu^{3+} $^5\text{D}_0 \rightarrow ^7\text{F}_2$ transition was found to be 1.10 ms ($\pm 5\%$) and was purely monoexponential decay. There was little change in lifetime at 77 K, with the corresponding value being 1.16 ms ($\pm 5\%$). This indicates that there are no significant nonradiative thermally dependent deactivation processes, such as back energy transfer or deactivation due to coordinated solvent interactions. Luminescent lifetimes of Eu^{3+} are strongly dependent on its coordination environment. Different coordination environments for each lanthanide ion would result in multiexponential decay when in solution.⁴ Monoexponential decay is an indication of the presence

of a single luminescent species in solution and that all ions are in an equivalent coordination environment. However, there may be several species in solution that equilibrate at room temperature on a time scale that is shorter than the observed luminescence lifetime.¹⁶ Since the dendritic backbone is a flexible acyclic donor, there may well exist equilibrium between different coordination species where the eight individual Eu^{3+} cations bound to the dendrimer have different coordination environments. This will be addressed in future work by variable-temperature analysis of the Eu^{3+} coordination environment by high-resolution luminescence measurements. Since it has been determined that **1** offers a good protection for the lanthanide ion, the moderate quantum yield may be tentatively attributed to the probable large distance between the chromophore and Eu^{3+} ions, resulting in incomplete ligand-to-lanthanide energy transfer. Significant fluorescence emission arising from the naphthalimide, as shown in Figure 3, confirms that the energy-transfer process is incomplete.

We have shown through luminescence titrations that **1** forms a 1:8 species after incubation over a period of 7 days. Sensitization of europium ions was achieved using a new chromophore, 2,3-naphthalimide, with excitation at low energy. A relatively low quantum yield was obtained, but this was compensated for by the high molar absorptivity of **1** and the large number of lanthanide ions bound in the dendrimer. This work also reveals that luminescence titrations can be a powerful method of characterizing luminescent polymetallic lanthanide complexes formed *in situ*. The results confirm that using dendritic ligands to develop lanthanide complexes emitting in the visible region and suitable for biological applications is a solid strategy.

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Supporting Information Available: Synthetic procedures, characterization, and spectroscopic protocol. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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