Synthesis of luminescent heterometallic bis-lanthanide complexes *via* selective, sequential metallation†

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A modular synthetic method for the differential incorporation of two lanthanide ions into a single molecular scaffold is reported; the mixed bimetallic Tb/Eu complex displays an interesting solvent polarity-dependent ratiometric luminescence.

The utility of lanthanide chelates as luminescent probes and magnetic resonance (MR) contrast agents in biological systems is well established.[1] The sharp emission bands of Tb^{3+} and Eu^{3+} occur in a useful wavelength domain (500–700 nm) and often have lifetimes in the order of a few milliseconds. Although direct excitation of the metal center leads to forbidden transitions, sensitization *via* energy transfer from an appended "antenna" occurs readily with a variety of organic chromophores, including the aromatic amino acids tyrosine and tryptophan.[2] The highly paramagnetic Gd^{3+} ion is used frequently in MR imaging applications owing to its ability to provide image contrast by decreasing the T_1 relaxation time of nearby water molecules.[3] Responsive lanthanide probes capable of sensing analyte binding or enzymatic activity have been developed for both the luminescent[4] and magnetic modes.[5]

The toxicity of lanthanide aqua ions makes their residence in a soluble chelate obligatory for biological applications. In addition, inner sphere water molecules can be detrimental to Tb^{3+} and Eu^{3+} luminescence quantum efficiency due to the strong coupling of O–H vibrations. Fulfilment of the high coordination requirements (7–9) of the lanthanides is typically accomplished using the water-soluble polyaminocarboxylates 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) and 1,1,4,7,7-diethylenetriamine pentaacetic acid (DTPA),[6] although several other motifs have been studied.[7] As a result of their popularity, several methods for the preparation of DOTA and DTPA bioconjugates are available.[8]

The incorporation of two different lanthanide ions into a single probe molecule may afford interesting properties, in either a dual-emissive (Tb^{3+} and Eu^{3+}) or bimodal (Eu^{3+}/Tb^{3+} and Gd^{3+}) context.[7b] Although several lanthanide-containing heterometallic complexes have been prepared by exploiting differences in ligand preference between lanthanides and other transition metals,[7b,9] the minute differences in coordination behavior[10] across the lanthanide period—particularly the immediate neighbors Eu^{3+}, Gd^{3+} and Tb^{3+}—severely limit the synthesis of heterometallic bis-lanthanide complexes. To the best of our knowledge, there is only one example of a discretely synthesized heterometallic complex with two different lanthanide ions,[11] though other complexes containing two different lanthanide ions have been prepared and studied as components of statistical mixtures.[7b,12]

As part of a broad program aimed at sensing chemical and enzymatic events with fluorescent[13] and luminescent[14] probes, we wanted to develop a modular synthetic strategy for the differential incorporation of two lanthanide ions into a single molecular scaffold, preferably using the stable, soluble, and readily bioconjugatable DOTA and DTPA chelates. Although the two chelates are almost indistinguishable thermodynamically,[10,15] only the acyclic DTPA chelate is prone to kinetic dissociation.[7a,10,16] We postulated that the sequential, differential complexation of a molecule containing both DOTA and DTPA chelates could be accomplished by capitalizing on this difference in decomplexation rates (Fig. 1). Specifically, treatment of DOTA/DTPA bis-chelate **1** with an excess of $Ln_A{}^{3+}$ should result in homobimetallic complex **2**, which could be prompted to undergo selective kinetic dissociation (*e.g.* acid-promoted demetallation) to give the corresponding monometallic species **3**. Further complexation of this complex with $Ln_B{}^{3+}$ should yield the pure heterobimetallic complex **4**. A conceptually similar approach has been used elegantly by Horrocks, Jr. *et al.* to study the distances between calcium binding sites in proteins.[17] The proposed sequential, differential metallation may represent a more general approach than a previously reported method,[11] since it obviates the need to couple metallated fragments.

The branched tetrapeptide-based DOTA/DTPA bis-chelate **5** was prepared *via* standard solid phase peptide synthesis on Rink Amide AM resin using the orthogonally-protected diamino acid Fmoc-Dpr(Mtt)-OH for the selective introduction of the DOTA(^{t}Bu)$_3$ and DTPA(^{t}Bu)$_4$ units (Scheme 1). After cleavage and global deprotection, the crude peptide **5** was purified by reverse phase HPLC (RP-HPLC) and complexed with excess Tb^{3+} in pH 5 triethylammonium acetate buffer. When analyzed by RP-HPLC using a H_2O/MeCN eluent system buffered with 0.1% TFA, the crude mixture contained a single major peak, which was isolated by preparative RP-HPLC and identified by mass spectrometry as the mono-Tb^{3+} species **6**, containing only a trace amount of the corresponding bis-Tb^{3+} species. The TFA-promoted dissociation of Tb:DTPA complexes during preparative HPLC has been noted by others[4b] and found by us to be nearly quantitative. Following the isolation of **6**, complexation with Eu^{3+} or Gd^{3+} took place quantitatively in unbuffered aqueous media; the resulting heterobimetallic complexes **7** and **8** could be used directly for photophysical measurements.[6] This simple protocol permits the rational design and synthesis of heterobimetallic

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† Electronic supplementary information (ESI) available: Synthetic protocols and characterization; experimental details and additional data for photophysical experiments.

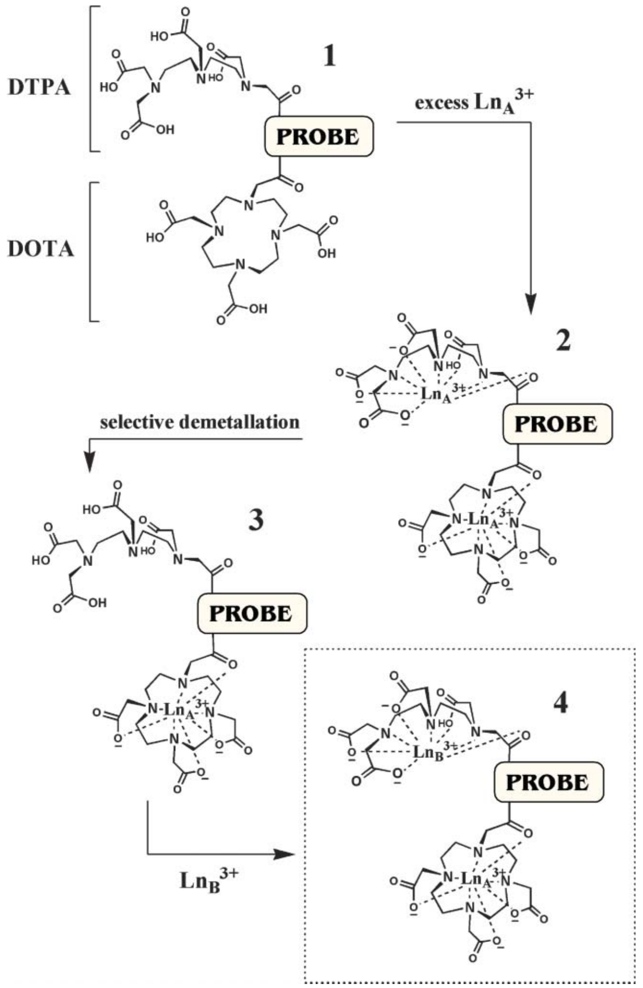

Fig. 1 Schematic representation of the sequential, differential metallation of a DOTA/DPTA bis-chelate with two different lanthanide ions (Ln$_A^{3+}$ and Ln$_B^{3+}$).

complexes containing any pair of lanthanide metals, making available new probes and bioconjugates with potentially interesting properties and applications.

The steady state emission spectra of **6**, **7** and **8** in water are shown in Fig. 2a. As expected, a portion of the energy absorbed by the tryptophan residue is emitted as fluorescence (λ_{max} = 390 nm)‡ and a portion is transferred to the $^5D_4$ state of Tb^{3+} (E = 20 400 cm^{-1}), resulting in sensitized luminescence (λ_{max} = 487, 544, 585 and 620 nm) from the Tb:DOTA moiety. Although the Tb:DOTA and Eu:DTPA chelates are most likely at similar distances from the tryptophan sensitizer in **7**, the triplet energy of tryptophan is too high to efficiently populate the emissive $^5D_0$ state of Eu^{3+} (E = 17 200 cm^{-1}) and no sensitized Eu^{3+} luminescence is expected to be observed.[1,17] However, careful comparison of **6** and **7** reveals a small shoulder at 613 nm in the spectrum of **7** but not in the spectrum of **6**. This emission corresponds to the $^5D_0 \rightarrow {}^7F_2$ transition of Eu^{3+}. This shoulder was more pronounced in less polar solvents, such as *tert*-butanol (Fig. 2b). The luminescence properties of **6** and **7** were compared in a series of alcohol solvents, and the Eu^{3+} emission band increased at the expense of Tb^{3+} emission as the polarity of the solvent was decreased, allowing ratiometric measurement of the relative solvent polarity (Fig. 2c).

This polarity-sensitive emission could be the result of several factors. The first is deactivation of the Tb^{3+} excited state *via* energy transfer to Eu^{3+}, which, although it has limited precedent, is known to be efficient.[7b] The second scenario involves ratiometric proportioning of the tryptophan excited state energy directly to Eu^{3+} in addition to Tb^{3+}; a control compound containing only an Eu^{3+} chelate and tryptophan exhibited similar solvent-dependent sensitized luminescence, albeit significantly weaker.§ A third possibility is direct excitation of the Eu^{3+} chelate, but this has been ruled out by control studies.§ The precise nature of this ratiometric emission and its potential applications to polarity sensing are under investigation. Since both Tb^{3+} and Eu^{3+} have long radiative lifetimes and luminesce at relatively long wavelengths, this is the first system that would provide a ratiometric measurement where both components are outside of the frequency as well as the time domain of cellular autofluorescence.

In summary, a novel synthetic route to heterometallic bis-lanthanide complexes *via* sequential, differential metallation has been presented. Complex **7**, containing Tb^{3+} and Eu^{3+}, exhibits ratiometric luminescence as a function of solvent polarity and may provide a useful platform for time-resolved polarity sensing.

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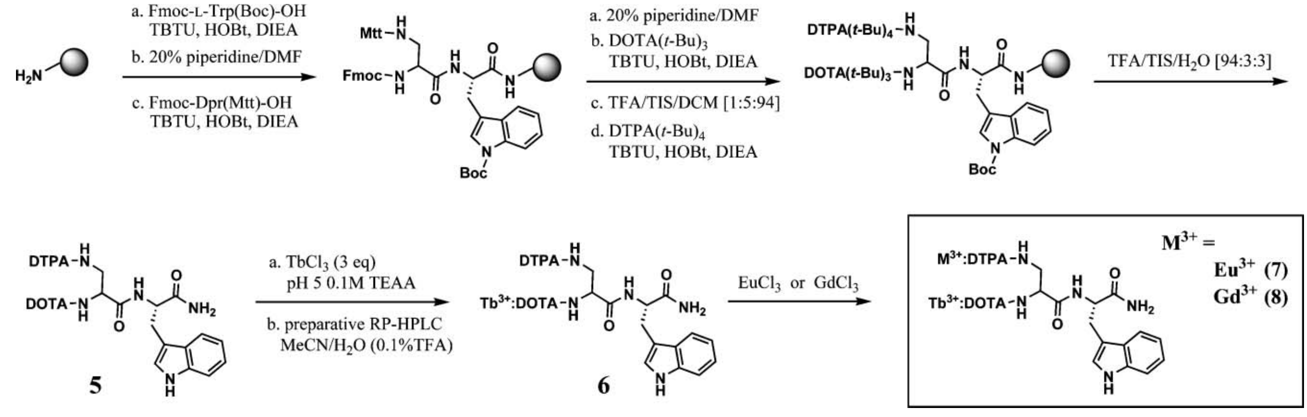

Scheme 1 Synthesis of the branched tetrapeptide ligand DOTA-Dpr(DTPA)-TrpNH$_2$ (**5**) and its sequential, differential metallation, producing the heterometallated bis-lanthanide complexes **7** and **8**.

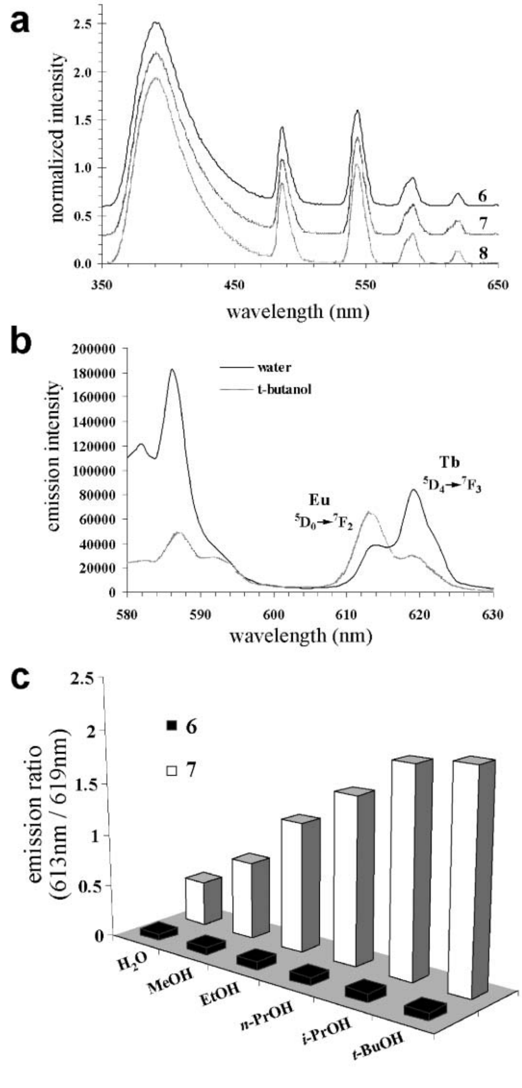

Fig. 2 (a) Emission spectra of compounds **6**, **7** and **8** in H_2O. (b) Emission spectra of **7** in H_2O and in *tert*-butanol. (c) The Eu/Tb emission ratio dependence on solvent polarity of **6** and **7**.

Notes and references

‡ A long pass filter, used to remove diffracted excitation light, truncates the tryptophan emission and shifts its maximum.

§ For additional details, see the ESI.†

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