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# Expanding the Versatility of Dipicolinate-Based Luminescent Lanthanide Complexes: A Fast Method for Antenna Testing

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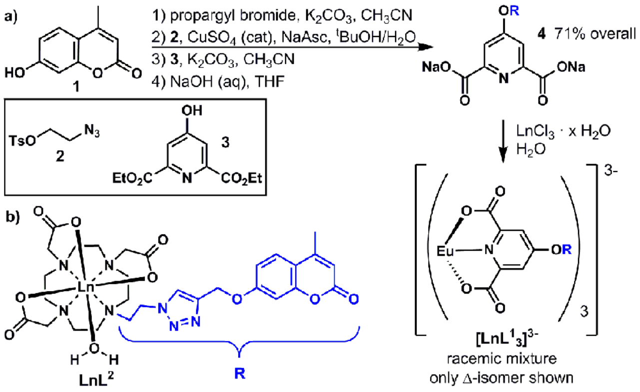
Supporting Information

**ABSTRACT:** A dipicolinate (dpa)-based platform for the rapid testing of potential lanthanide-sensitizing antennae was developed; 4-methyl-7-*O*-alkylcoumarin-appended dpa could sensitize four lanthanides. The platform could be used to estimate the photophysical properties of a more difficult-to-prepare 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid based structure carrying the same antenna.

More and more applications take advantage of lanthanide (Ln) luminescence.[1] Investigating new Ln architectures and increasing their sophistication affords versatile systems for solving a wide scope of physicochemical and biological problems.[2] Ln ions are unique emitters but rely on photosensitizers (antennae) for significant excitation in coordination complexes.[1a,b] Despite the abundance of antennae, there is still a craving for new Ln sensitizers, not least to enable a refined understanding of Ln photophysics.[3]

Here, we address the problem of antenna-dependent ligand synthesis by taking advantage of a dipicolinate (dpa) framework (Scheme 1). Dpa complexes are well described in the literature and provide stable complexes with three ligands coordinated to a single Ln ion in a tricapped trigonal-prismatic geometry.[2c,4−8] Their inner coordination sphere is devoid of $H_2O$ molecules, affording good luminescent properties. The dpa chromophore is also a good standard sensitizer for multiple Ln ions.[4] The diversity of dpa-based structures alone justifies expanding its adaptability and functionality.[2a,c,5−7,9] As a model antenna, we chose 4-methyl-7-hydroxycoumarin, which has been shown to sensitize europium (Eu) and terbium (Tb).[6] Coumarin-sensitized Ln complexes are increasingly used as chemical or biochemical probes.[10] The utility of our design lies in its ability to rapidly identify promising antennae. Thus, we have prioritized complex accessibility over brightness. Ln sensitization has $1/r^6$ distance dependence. Our linker with a considerable Ln-antenna distance facilitates complex assembly, but results in low quantum yields. Importantly, modestly emissive systems can be successfully used for in vivo imaging with a sufficiently low background.[11]

**Scheme 1. (a) Synthesis of the dpa Ligand $Na_2L^1$ and Its Ln Complexes $[LnL^1_3]^{3-}$ and (b) the DO3A Analogue of $LnL^1_3$**



Dpa-based ligand $L^1$ is prepared in 4 steps from readily available building blocks (Scheme 1). Ln complexation by $L^1$ is virtually instantaneous. A 1:3 Ln-to-ligand stoichiometry gives $LnL^1_3$ in $H_2O$ at neutral pH,[4,5,6a,8] the formation of which was evidenced by measuring the emission of the $EuL^1_3$ complex upon titration of $L^1$ with a solution of $EuCl_3$ (Figure S1). The procedure also provides a means of complexometric titration for $L^1$. Eu emission of $EuL^1_3$ is quite stable in the pH 7−10 range and drops below pH 6 (Figure S2).

The photophysical properties of a series of $LnL^1_3$ (Ln = Eu, Tb, Gd, Sm, and Dy) were then evaluated in $H_2O$ (0.01 M Tris, pH 7.4; Figures 1 and S3−S5). The absorbance spectra showed that all of the complexes have the same extinction coefficient (Figure S3). Under coumarin excitation (i.e., at 320 nm), the molar extinction coefficient is $\varepsilon_{320}$ = 26900 (±200) $M^{-1}$ $cm^{-1}$. This excitation wavelength was used in most of the subsequent measurements.

Eu, Tb, samarium (Sm), and dysprosium (Dy) are the main visible-emitting Ln ions. Because of its inaccessible high-energy excited state, gadolinium (Gd) provides a reference complex where energy transfer onto the Ln ion is absent while retaining the possible heavy-atom effect of the metal ion. The complexes of all four luminescent Ln ions exhibited sensitization upon coumarin excitation, as shown by the characteristic spectral fingerprints of the Ln ions besides the coumarin fluorescence (Figure 1). Eu displayed the strongest luminescence, followed by Tb, Dy, and finally the barely detectable Sm emission.

Because of their long lifetimes ($\tau_{Ln}$; Table 1), the Ln peaks can be discriminated by time-resolved measurements. Under these conditions, the short-lived coumarin fluorescence disappears, leaving only the long-lived Ln peaks. Dy and Sm lifetimes are, as expected, much shorter than those of Eu and Tb (21 and 24 $\mu$s vs 1.4 and 1.1 ms, respectively). The resolution of Sm emission required dpa excitation. Because dpa is closer to the Ln ion, it is a more efficient sensitizer.[6]

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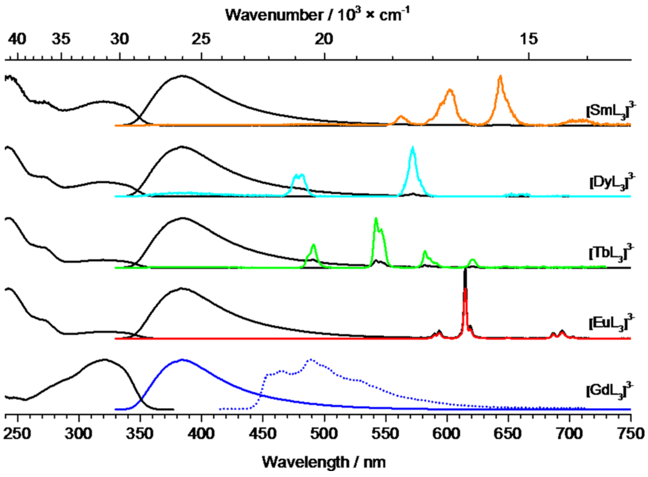

**Figure 1.** Excitation spectra of Sm, Dy, Tb, Eu, and coumarin ($[GdL_3]^{3-}$) at 298 K (solid lines, left). Emission spectra of $LnL^1_3$ at 298 K (solid lines, right, black and blue) and time-resolved emission spectra showing Sm, Dy, Tb, and Eu luminescence (298 K, colored lines) and coumarin phosphorescence ($[GdL_3]^{3-}$, 77 K, blue dotted line). Excitation at $\lambda_{ex}$ = 320 nm, except the orange line ($\lambda_{ex}$ = 256 nm). $[LnL^1_3]$ = 1.8 × $10^{-5}$ M, 0.01 M Tris in $H_2O$, pH 7.4.

**Table 1. Photophysical Properties of $LnL^1_3$ and $LnL^2$ in Tris, pH 7.4, and HEPES, pH 7.0, Buffered Aqueous Solutions, Respectively, with $\lambda_{ex}$ = 322 nm and at Room Temperature**

| | $\Phi_L$/% | $\Phi_{Ln}$/% | $\tau_{Ln}$/ms |
|---|---|---|---|
| $[GdL^1_3]^{3-}$ | 13.7(2) | | |
| $[EuL^1_3]^{3-}$ | 12.3(2) | 1.08(2) | 1.4(1) |
| $[TbL^1_3]^{3-}$ | 12.6(2) | 0.26(3) | 1.1(1)[a] |
| $[DyL^1_3]^{3-}$ | 12.5(2) | 0.13(1) | 0.021(2) |
| $[SmL^1_3]^{3-}$ | 12.3(2) | 0.05(1) | 0.024(2) |
| $[GdL^2]$ | 25.3(4) | | |
| $[EuL^2]$ | 18.7(3) | 0.57(1) | 0.47(5) |
| $[TbL^2]$ | 23.4(4) | 0.64(1) | 1.3(1) |
| $[DyL^2]$ | 24.4(4) | 0.10(1) | 0.010(5) |
| $[SmL^2]$ | 23.3(4) | <0.01 | <0.005 |

[a] Double exponential decay gives a better fit: 0.5(1) ms (25% of the amplitude); 1.3(1) ms (75% of the amplitude). See the Supporting Information for details.

The superior Ln sensitization efficiency of the dpa unit is clear from the excitation spectra. The excitation at 250 nm is stronger than that at 320 nm, whereas the extinction coefficient at 250 nm is lower than that at 320 nm (Figure S3). The good efficiency of the dpa sensitizer also creates a glitch in the excitation spectra, which appears as an unusual excitation band at ~500 nm with significant intensity. This band is not observed in blank solutions or in solutions of complexes without dpa-coordinating groups. Filtering the excitation source by a UV cutoff filter removes the abnormal band, leaving only the sharp Ln direct absorption spikes (Figure S4). The absorption range of the individual dpa and coumarin moieties can be found in Figure S5. The quantum yields were measured (Table 1) and are valid above 300 nm, where the dpa moiety does not absorb. Below 300 nm, the excitation of both the dpa and coumarin moieties varies with the wavelength, and $\Phi_{Ln}$ is continuously changing, as illustrated in Figure S5 for $\Phi_{Eu}$.[6a] The quantum yields of Ln emission ($\Phi_{Ln}$) of $EuL^1_3$ (1.1%) and $TbL^1_3$ (0.3%) are comparable to those of similar systems.[4−6] The intrinsic quantum yield (41%) and radiative rate constant (290 $s^{-1}$) of the Eu ion were calculated from the ratio of the $^5D_0 \rightarrow {}^7F_1$ magnetic dipole transition.[1a,3] These values are also close to those reported for dpa derivatives. Thus, this triazole-containing linker does not seem to have a dramatic impact on the sensitization efficiency, which is around 3% for $EuL^1_3$. This is beneficial, as CuAAC is increasingly used for Ln complex assembly.[7a,b,10b,c,12] A clear quenching of the coumarin fluorescence is observed when the antenna quantum yields $\Phi_L$ of the Eu, Tb, Sm, and Dy complexes are compared with that of $GdL^1_3$. This quenching represents an 8−11% relative decrease of the $\Phi_L$ value of $GdL^1_3$, which is higher than the sensitization efficiency calculated for $EuL^1_3$ but could include processes other than energy transfer. No significant quenching difference between the emissive Ln complexes was seen (within experimental error; see the Supporting Information for a discussion on $\Phi_{Ln}$ errors).

At low temperature (77 K), the coumarin triplet excited-state phosphorescence was observed (Figure 1). This phosphorescence is very long-lived, with triplet lifetimes around 1.5(2) s. The Ln excited-state lifetimes are much shorter than this, suggesting that this phosphorescent state is not transferring energy to the Ln ions.

1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic acid (DO3A)-based emissive Ln complexes are commonly used in vitro and in vivo. However, their syntheses can be lengthy making the testing of new antennae time-consuming. A noncovalent system has been reported for the identification of potential antennae, which relies on chromophore incorporation into the core of $Eu^{3+}$-loaded micelles.[13] $LnL^1_3$ was envisioned as an accessible screening platform that captures key features of the DO3A complexes, such as antenna and linker type.

DO3A analogues of $LnL^1_3$ (Scheme 1) were prepared as shown in Scheme S1. Their photophysical properties were evaluated as described for $LnL^1_3$. As is customary for DO3A-based complexes, $LnL^2$ were studied in HEPES (0.1 M) buffered aqueous solutions at pH 7.0 and at $[LnL^2]$ = $10^{-5}$ M. The excitation spectra (Figure S6) are consistent with antenna excitation and, compared to $[LnL_3]^{3-}$, are uncomplicated by the dpa ligand absorbing below 300 nm. The emission spectra (Figure S6) are dominated by coumarin emission, which has a quantum yield twice as high as in $LnL^1_3$ (Table 1). This supports self-quenching in the $dpa_3$ structures, as reported for similar complexes.[6a]

The striking difference between the emission spectra of $LnL^1_3$ and $LnL^2$ is the dramatic decrease of Eu emission in $EuL^2$. $EuL^2$ exhibits nearly half of the quantum yield of $EuL^1_3$. Sm emission was lowered to the point that it could not be quantified anymore, whereas Dy emission is barely affected by the different environment. Unexpectedly, Tb experienced a major increase of 250% of its quantum yield in $TbL^2$.

The lifetimes are significantly shorter in $LnL^2$, except for $TbL^2$. Hydration numbers ($q$) of 1.5 or 1.7 for $EuL^2$ and 1.5 for $TbL^2$ were calculated,[1a] consistent with reported DO3A Ln complexes.[14] The inner-sphere $H_2O$ molecules can account for quenching of the Eu ion, as evidenced by the calculated intrinsic quantum yield in $EuL^1_3$ and $EuL^2$ (41% vs 10%). The sensitization efficiency is significantly different in $EuL^1_3$ and $EuL^2$ [2.6(3)% vs 5.2(6)%] and seems to indicate a more efficient sensitization from the coumarin in $EuL^2$, in line with the enhanced $\Phi_L$ and the shorter sensitizer−Ln distance in the structure lacking the pyridine moiety.

The improved Tb photophysics in $TbL^2$ can be rationalized by the smaller energy gap between the Tb $^5D_4$ spectroscopic level and the ligand excited state, which probably induces more back

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energy transfer than in the Eu complexes. The three dpa units in TbL$^{1}_{3}$ would thus be detrimental to the Tb ion because the higher static concentration of coumarin increases the back-transfer probability (i.e., rate). This explanation is supported by an improved sensitization of Tb by blue-shifted fluorophores and the associated longer lifetimes of the Tb $^{5}D_{4}$ level compared to that of 4-methylumbelliferone.[6b]

A comparison of the emission spectra of LnL$^{1}_{3}$ and LnL$^{2}$ (Figures S6−S10) revealed that coumarin fluorescence of LnL$^{1}_{3}$ is broader, extending farther into the red than that of LnL$^{2}$. This extension of the fluorescence band was also observed during titration of L$^{1}$ by Eu and correlates with the appearance of the 1:3 species. The extra component is probably due to the proximity of three coumarins that may interact with each other (self-quenching supports that the chromophores are within interaction range), or even form exciplexes within LnL$^{1}_{3}$. The bathochromic shift of the fluorescence tail is unfavorable for Tb luminescence because it further shortens the donor−acceptor excited-state energy gap. Therefore, the sensitization efficiency in TbL$^{2}$ could be even better than the 2-fold improvement calculated from EuL$_{3}^{1}$ to EuL$^{2}$.

Overall, complexes LnL$^{1}_{3}$ and LnL$^{2}$ are interesting because of their versatility: sensitization of four Ln ions, concomitant coumarin fluorescence, and time-resolved predisposition. Antennae sensitizing more than two Ln ions is still quite rare.[1a,3,15] For some applications (e.g., multiplex detection[16]), the ability to sensitize multiple Ln ions is an important parameter. For LnL$^{1}_{3}$, the "double sensitization" capability below 300 nm allows a comparison of the new antenna to an internal standard, which is useful for predicting Ln sensitization by the antenna in another setting, i.e., in DO3A-based systems. Deviations from strict analogy between LnL$^{1}_{3}$ and LnL$^{2}$ are moderate and are readily explained. The synthesis of the dpa-based platform is easy and high-yielding and should be adaptable to a wide range of antennae beyond coumarins. The complexes are well-suited to applications at physiological pH and are useful for the screening of antennae for Ln-based biological probes. The dpa platform, hence, greatly facilitates the development of state-of-the-art architectures that are difficult to synthesize and purify.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.5b01579.

Synthesis procedures, titration curves, and additional photophysical characterization (PDF)

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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