PERKIN 2 COMMUNICATION

Small singlet–triplet energy gap of acridone enables longer wavelength sensitisation of europium(III) luminescence

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Incorporation of 9(10*H*)-acridone as a chromophore into a seven-coordinate chelating ligand allows observation of sensitised luminescence from the europium(III) complex, even with excitation wavelengths greater than 410 nm.

Traditional fluorescent markers in biological assays tend to suffer from problems of interference arising from various other fluorescent species also present in the sample matrix.[1] Recent trends have tended to move away from so-called 'conventional' fluorophores towards the use of luminescent lanthanide complexes, which with millisecond order lifetimes allow the use of time-gating techniques to improve signal–noise ratios.[2] Particular interest has centred on Tb^{3+} and Eu^{3+} complexes with ligands containing antenna chromophores. Such sensitising chromophores effectively increase the inherently low molar absorptivities of lanthanide ions in aqueous media.[3] A number of mechanisms for sensitisation of the lanthanide have been suggested: in the most commonly accepted, light energy absorbed by the chromophore is transferred to a lanthanide ion *via* a chromophore-centred triplet state.[4] Research has centred on incorporating chromophores with high molar extinction coefficients and high triplet yields.[5] The ideal chromophore would also permit long wavelength excitation of the chelate, avoiding the unwanted excitation of biomolecules in the sample and allowing the use of standard glass optics rather than quartz.

Phenanthridine has recently been incorporated into an octadentate ligand allowing selective excitation at 370 nm on *N*-protonation.[6,7] Red emission in the *intra*molecular phenanthridinium europium(III) complex is 'switched on' following *N*-protonation. However, in the analogous terbium(III) complex the green luminescence is found to 'switch off' in the phenanthridinium species, due to rapid back energy transfer to the low-lying triplet state of phenanthridinium. Recently, benzophenone-conjugated europium and terbium complexes have been reported to show strong absorption bands under neutral conditions extending to 370 nm, with quantum yields of 0.095 and 0.27 respectively.[8] Elsewhere, *inter*molecular energy transfer between Michler's ketone and $Eu(fod)_3$ (fod = 6,6,7,7,8,8,8-heptafluoro-2,2-dimethyloctane-3,5-dione) in benzene has led to a red glow emerging from the solution under daylight illumination (λ_{max} 414 nm), with excitation extending beyond 450 nm.[9]

In our search for longer-wavelength sensitisers of Eu^{3+}, as potential markers in biological applications, we have been investigating the use of acridone chromophores as long wavelength sensitisers, in complexes such as **EuL**^{1} and **EuL**^{2} (Fig. 1). The ligands were prepared from acridone and 2,7-dibromoacridone **1a** and **1b** respectively, alkylating at the amine position to provide a C_5 spacer in **2a** and **2b** (Scheme 1). These were then coupled to 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, yielding the DO3A-derived acids **H$_3$L^1** and **H$_3$L^2** after deprotection.† The europium complexes were then prepared in aqueous HEPES-buffered solution at pH 7.4.

The absorption spectrum of the free ligand **H$_3$L^1** in aqueous solution is little changed by complexation with europium in **EuL**^{1} (λ_{max} 257, 394, 410 nm). Excitation at 406 nm gave rise to the characteristic red emission of europium at 615 nm. Similar studies were carried out on **EuL**^{2}; the UV-absorption and fluorescence spectra of the ligand **H$_3$L^2** showed an additional band at 427 nm, which tailed off at 450 nm. Upon complexation with Eu^{3+}, excitation of the sample at both 406 and 428 nm (Fig. 2) led to europium emission.

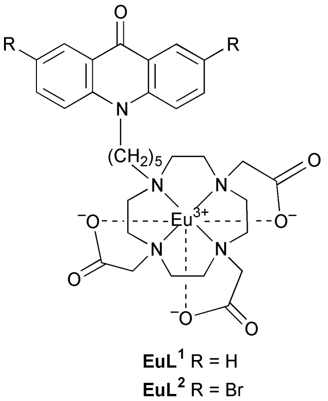
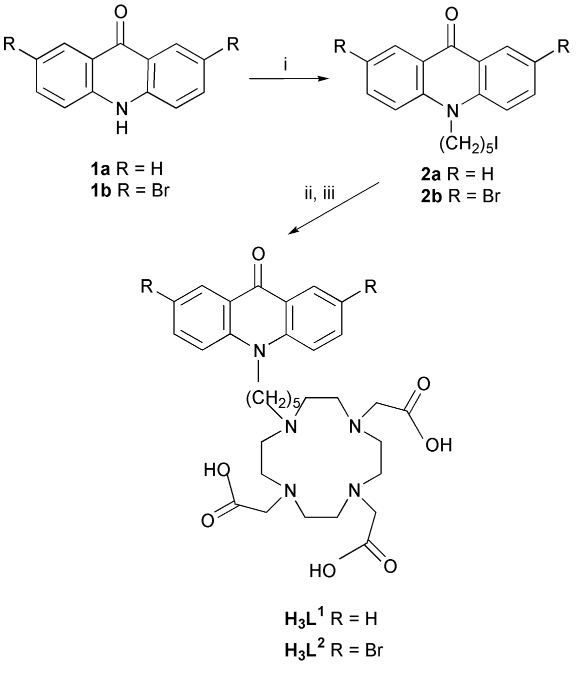


Fig. 1 The europium(III) complexes **EuL**^{1} and **EuL**^{2}.



Scheme 1 (i) NaH, DMF, $I(CH_2)_5I$, 60 °C, (ii) 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, K_2CO_3, MeCN, reflux; (iii) CF_3COOH, DCM, rt.

The luminescence lifetimes of the complexes in H_2O and D_2O (Table 1) were used to determine the inner sphere hydration number, q_{corr}. Parker and co-workers have pioneered the use of the eqn. (1),[10] where k_{H_2O} and k_{D_2O} are the rate constants

$$q_{corr} = A'_{Eu}\,[(k_{H_2O} - k_{D_2O}) + corr_{Eu}] \qquad (1)$$

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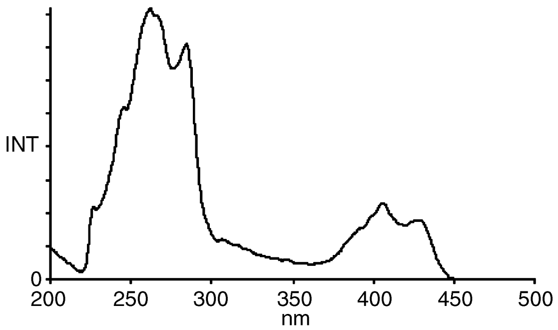


Fig. 2 Time-resolved excitation spectrum of the europium(III) complex **EuL**^{2} (monitoring the $^5D_0 \rightarrow {}^7F_2$ emission at 615 nm), with a long wavelength band at 428 nm.

Table 1 Photophysical properties of the europium(III) complexes, **EuL**^{1} and **EuL**^{2}

	τ_{H_2O}/ms	τ_{D_2O}/ms	q_{corr}	$\phi_{H_2O}{}^a$	ϕ_{D_2O}	$E_T{}^b$/cm^{-1}
EuL^1	0.37	1.67	2.2	0.014	0.035	21 500
EuL^2	0.38	1.52	2.1	0.010	0.025	19 800

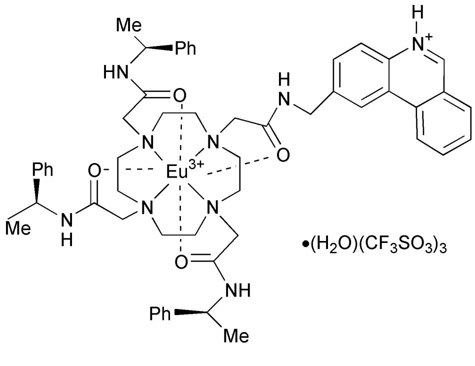
^{a} Measured in aerated solution (λ_{exc} = 380 nm), using protonated [EuL^3(H$_2$O)](CF$_3$SO$_3$)$_3$ (Fig. 3) as the reference compound.[7] ^{b} Measured using the Gd^{3+} complex at 77 K in aqueous glass. **EuL**^{1} shows an identical profile for a short-lived triplet state.

(ms^{-1}) for depopulation of the europium excited states in H_2O and D_2O respectively, A'_{Eu} is a proportionality constant equal to 1.2 ms for europium, and $corr_{Eu}$ is a correction factor of -0.25 ms^{-1} for europium, which allows for the effect of closely diffusing OH oscillators. Application of these procedures to **EuL**^{1} and **EuL**^{2} gives inner sphere hydration numbers of 2.2 and 2.1 respectively. These agree within experimental error and correspond to the expected values for DO3A-derived ligands.[11] The addition of oxygen-containing anions such as citrate gives rise to increased lifetimes in water, supporting the observation that coordinated water molecules give rise to the main non-radiative quenching pathway.

Quantum yields for the two complexes were determined relative to a phenanthridinium complex **[EuL**^{3}**H]**$^{4+}$ ($\phi = 0.021$)[7] (Fig. 3). This complex was chosen as it has an absorbance band around 380 nm similar to those of the two complexes to be measured. The total integrated emission intensities were determined for the three solutions, with absorbances ranging from 0.03 to 0.1 at an excitation wavelength of 380 nm. Observed quantum yields of 0.014 and 0.01 were determined for the complexes **EuL**^{1} and **EuL**^{2} in H_2O respectively. Both values increased significantly in D_2O, with the respective quantum yields observed as 0.035 and 0.025.

Degassed solutions of both complexes exhibited slight increases in the relative quantum yields, though no changes in lifetime were observed. This suggests that triplet state quenching can occur, but that back energy transfer is insignificant. Further experiments produced evidence for a triplet-mediated energy transfer mechanism, since luminescence from **EuL**^{1} in an aqueous glass at 77 K, showed a short-lived band corresponding to the aryl triplet.

GdL^1 and **GdL**^{2} were prepared with a view to establishing the triplet state energies more accurately since, having no electronic energy levels below 32 000 cm^{-1}, Gd^{3+} cannot accept any energy from the sensitiser but should induce approximately the same optical shift as Eu^{3+}. Triplet state energies, E_T, were determined from the 0–0 transition, which for **L**^{1} lies at 21 500 cm^{-1} while for **L**^{2} this lies at 19 800 cm^{-1}. The lower lying triplet state of **L**^{2} is still significantly greater than the energy of the $^5D_0$ state in Eu^{3+} (17 200 cm^{-1}), precluding back energy transfer at ambient temperature. **L**^{2} has the advantage of having a small singlet–triplet energy gap in addition to the potential of the 'heavy atom' effect of the bromine atoms, which are known to play a role in catalysing spin-inversions from excited singlet states to excited triplet states through spin-orbit coupling.[12]



[EuL^{3}H]$^{4+}$

Fig. 3 [EuL^3(H$_2$O)](CF$_3$SO$_3$)$_3$, used for quantum yield determinations.

In conclusion, we have succeeded in sensitising europium luminescence at wavelengths over 400 nm, by incorporating acridone into a seven-coordinate ligand. There is strong evidence that energy transfer occurs *via* the triplet state, justifying our belief that long wavelength excitation of Eu^{3+} is best pursued by exploiting ligands with a small singlet–triplet energy gap. The singlet–triplet energy gap in acridone is comparable to that in Michler's ketone,[13] both lying at around 3500 cm^{-1}, but in our systems we have the advantage of the chromophore being incorporated into a stable, charge-neutral, water-soluble luminescent species, ideal for time-resolved fluorescence assay applications. We are currently investigating such potential applications, and endeavouring to optimise the energy transfer process by bringing the antenna into closer proximity with the metal, and by saturating the coordination sphere of the metal to reduce the hydration number.

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Notes and references

† All new compounds were characterised with supporting micro-analytical and/or accurate mass measurements.

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