

Aqueous Ln(III) Luminescence Agents Derived from a Tasty Precursor

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The synthesis, aqueous stability and photophysical properties are reported for a novel tetradentate ligand derived from maltol, a commonly used flavor enhancer. In aqueous solution, this chelate forms stable complexes with Ln(III) cations, and sensitized emission was observed from Eu(III), Yb(III), and Nd(III). A comparison with recently reported and structurally analogous ligands reveals a slightly higher basicity but lower complex stability with Eu(III) [$pK_a = 14.7$ (1)]. A very poor metal centered quantum yield with Eu(III) was observed ($\Phi_{tot} = 0.04\%$), which can be rationalized by the similar energy of the ligand triplet state and the Eu(III) 5D_0 emissive level. Instead, sensitized emission from the Yb(III) and Nd(III) cations was observed, which emit in the Near Infra-Red (NIR).

Optical fluorescent assays are ubiquitous in biotechnology and medicinal chemistry. The advantages of visible emitting Eu(III) or Tb(III) complexes (which emit characteristic red or green luminescence) have long been recognized for the detection of numerous analytes in biological samples, and consequently, a large number of these compounds have been developed. However, Near Infra-Red (NIR) emitting lanthanides such as Yb(III) and Nd(III) have only recently begun to receive more attention for this application.¹ For the further development of NIR assays, the knowledge gained from masking lanthanide toxicity *in vivo* can be transferred from our experience in the related field of MRI contrast agent development utilizing Gd(III),^{2,3} and from our prior experience with Tb(III) and Eu(III) based luminescence agents.^{4,5}

The recent discovery^{5,6} of highly efficient 1,2-HOPO sensitized emission from Eu(III) prompted our investigation of similar oxoligands available in our group as sensitizers for lanthanide luminescence. Of these, the hexadentate TREN-MAM ligand is one of the chelates we have studied recently, which forms a Gd(III) complex that achieves high chelate stability and good efficiency as a contrast agent for Magnetic Resonance Imaging (MRI), primarily due to the presence of two inner sphere

coordinated water molecules.⁷ While coordinated water molecules are desirable for improved MRI contrast agents, such nearby OH oscillators diminish the quantum yield of lanthanide emission. Hence, for the development of highly luminescent materials, the ligand should shield the metal ion from water coordination, while retaining sufficient stability in aqueous solution.

The 5LIO-1,2-HOPO ligand (**2**) shown in Chart 1 is known to form stable ternary complexes with Ln(III) cations, and can support eight coordinate geometries (CN = 8) at the metal center, as demonstrated by several X-ray crystal structures which have been recently reported.^{5,6} Hence, using these observations as a guide, we have utilized the identical 5LIO- backbone to prepare the novel ligand **1**, to investigate the chelating properties of the maltol group (MAM). Herein, we report the synthesis, solution stability and photophysical properties of 5LIO-MAM **1** and several of its Ln(III) complexes.

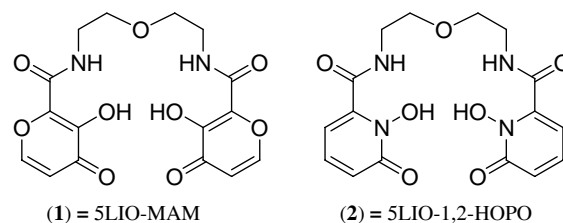


Chart 1. Chemical structures of tetradentate ligands forming stable ML_2 complexes with Ln(III), and featuring exclusively oxygen donors.

The ligand **1** is readily prepared using the inexpensive flavor enhancer, maltol, as the starting material, in a five step synthesis (see Schemes S1 and S2, Supp Info). The crucial step in this preparation is the oxidation of benzyl protected maltol to the corresponding aldehyde, where care must be taken to prevent the entry of moisture into the reaction mixture to obtain a reasonable yield.⁸ DCC and NHS are used for coupling of the benzyl protected maltol acid with 2-(2-aminoethoxy)ethylamine (5LIO-). The protected ligand is readily purified by column chromatography and deprotection in 1:1 (v/v) concentrated hydrochloric acid and glacial acetic acid affords the desired product in 15% overall yield from maltol.

Protonation constants of **1** were determined by potentiometry (added acid vs pH) and verified by

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spectrophotometry in 0.1 M KCl (see Fig. S2, Supp Info), yielding pK_a 's of 4.91(6) and 6.09(2), which are slightly more basic than the corresponding values with **2**. The higher acidity of the 1,2-HOPO group versus the maltol is reflected by an average decrease of *ca.* 0.5 pK_a units, and this agrees with the trend observed for the analogous hexadentate ligands TREN-1,2-HOPO⁹ and TREN-MAM⁷ ($\log\beta_{014} = 20.7$ and 21.9 respectively). The hexadentate ligands have very similar stability constants for Gd(III) binding resulting in identical conditional stability constants $pGd = 19.3$. However, in the case of our tetradentate ligands this situation is not maintained. Our initial spectrophotometric titrations with Eu(III) established that 5LIO-MAM cannot compete with DTPA for Ln(III). Under the chosen experimental conditions this indicates a formation constant of $\log\beta_{120} < 20$, and further analysis in the absence of competitor determined the formation constants as summarized in Table 1. The observed decrease in complex stability for the EuL and EuL₂ complexes results in a significant drop in the calculated pEu to 14.7 for 5LIO-MAM (**1**). Also, as a result of these low stability constant values, and given the second order dependence of speciation on ligand concentration for a ternary complex, we calculate that the $[\text{Eu}(\mathbf{1})_2]^-$ complex will begin to partially hydrolyze at pH 7.4, forming the ML complex below *ca.* 0.1 mM in aqueous solution (see Fig. 1). Nonetheless, ternary complex formation is still favored (especially at higher concentrations) and the ML₂ complex will predominate in solution at pH 7.4. Above *ca.* pH 8, the competitive formation of Ln hydroxy species (which convert to insoluble oxides) becomes apparent.

Table 1. Solution thermodynamic data obtained for **1** and **2** performed at 298 K with 0.1 M KCl supporting electrolyte.

Constant	1	2 *
pK_{a1}	4.91 (6)	4.19 (3)
pK_{a2}	6.09 (2)	5.79 (1)
$\log\beta_{110}$	11.0 (1)	12.46 (2)
$\log\beta_{120}$	19.0 (1)	22.85 (10)
pEu	14.7 (1)	18.6 (1)

* Data taken from ref. 5.

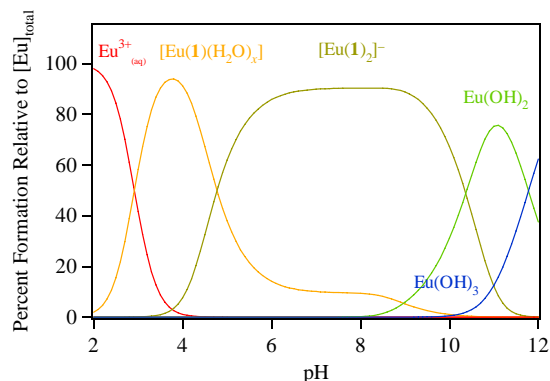


Figure 1. Calculated speciation diagram from solution thermodynamic data for $[\text{Eu}(\mathbf{1})_2]^-$ in aqueous solution at *ca.* 10^{-6} M.

Having established the aqueous stability of the $[\text{Eu}(\mathbf{1})_2]^-$ complex, we sought to evaluate the photophysical properties. As shown in Fig. 2, the UV-Visible spectrum of the complex is typical for that expected from the aromatic chromophore, with a broad electronic envelope centered at

ca. 326 nm. As verified by TD-DFT techniques (see Fig S3, Supp Info), we assign this peak to an essentially π - π^* transition. Also shown in Fig. 2 is the observed luminescence spectrum upon ligand centered excitation at 326 nm. Several characteristic Eu(III) bands were observed, corresponding to the $^5D_0 \rightarrow ^7F_J$ transitions, with peak maxima at *ca.* 578, 590, 613, 650 and 694 nm for the $J = 0, 1, 2, 3$ and 4 levels. To quantify the observed luminescence, we determined the overall quantum yield using standard techniques (see Fig S1, Supp Info), which resulting in a very low value of $\Phi_{\text{tot}} = 0.04$ %.

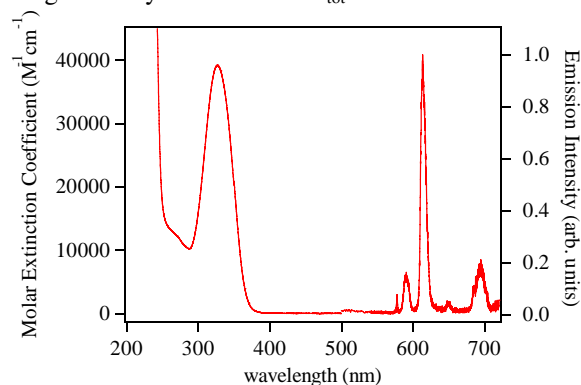


Figure 2. Observed UV-Vis spectra (left) and emission spectra ($\lambda_{\text{ex}} = 326$ nm) of $[\text{Eu}(\mathbf{1})_2]^-$ in 0.1 M TRIS buffered aqueous solution at pH 7.4.

In order to rationalize the observed poor Eu(III) luminescence behavior, we determined the position of the lowest excited state triplet energy, using the $[\text{Gd}(\mathbf{1})_2]^-$ complex. The Gd(III) cation has a similar size and atomic weight compared with Eu(III), yet lacks an appropriately positioned electronic accepting level, so phosphorescence of the ligand can be observed at 77 K. It has been shown previously¹⁰ that matching of triplet energy and Ln(III) emission levels is a key factor for efficient metal emission.

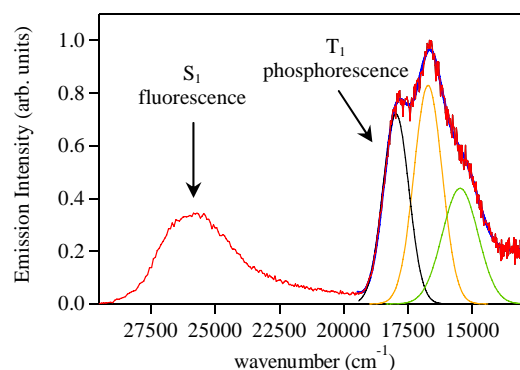


Figure 3. Low temperature (77 K) emission spectra ($\lambda_{\text{ex}} = 326$ nm) of $[\text{Gd}(\mathbf{1})_2]^-$ in 10:10:1 (v/v/v) EtOH:MeOH:H₂O (1.0 M TRIS buffer, pH 7.4), and spectral deconvolution of the phosphorescence into overlapping Gaussian functions to yield the lowest energy triplet level at 17,970 cm^{-1} .

The resulting spectrum is shown in Fig. 3, together with a spectral deconvolution of the observed phosphorescence band from *ca.* 500-700 nm into three overlapping Gaussian functions. From this analysis, we can estimate the position of the zero phonon energy of the lowest triplet state, T₁, to be *ca.* 556 nm (17,970 cm^{-1}). Thus, the triplet energy level of $[\text{Gd}(\mathbf{1})_2]^-$ is barely above the 5D_0 emitting state of Eu(III) (17 300 cm^{-1}) and well below the value previously determined⁶ for $[\text{Gd}(\mathbf{2})_2]^-$ (*ca.* 21,260 cm^{-1}). While this

makes **2** a very efficient sensitizer of Eu(III) emission, **1** performs poorly in this aspect due to highly competitive thermal back energy transfer.

Given that the emissive levels of Yb(III) at 10 200 cm⁻¹ and Nd(III) 12 000 cm⁻¹ are located well below the triplet energy of **1** (17 970 cm⁻¹), a more efficient energy transfer to these metals can be expected. We have also prepared and characterized the corresponding Yb(III) and Nd(III) complexes, and the resulting emission spectra in the NIR region are shown in Fig. 4. Herein, the characteristic ²F_{5/2} → ²F_{7/2} electronic transition of the Yb(III) cation is clearly observed, with a peak at ca. 978 nm, and weakly split due to crystal field effects, while for Nd(III), there are two bands apparent with peaks at ca. 1060 nm and ca. 1330 nm, assigned to transitions from the ⁴F_{3/2} excited state to ⁴I_{11/2} and ⁴I_{13/2} levels respectively.

Lastly, to further characterize the luminescence of these complexes, we have measured the lifetimes for each of the differing Ln(III) metal centers in MeOH, H₂O (where possible) and in perdeuterated solvent, with results as summarized in Table 2. In aqueous solution, for each Ln(III) cation, the luminescence decay curves were biexponential. This is possibly due to partial hydrolysis of the [Ln(1)₂]⁻ complexes, as expected from the speciation diagram at 10⁻⁶ M (see Fig. 1), or alternately, may be due to the presence of a slowly exchanging equilibrium mixture with a hydrated form of the complex, [Ln(1)₂(H₂O)]⁻. By contrast, measurements performed in MeOH and d₄-MeOD were satisfactorily fit to monoexponential decays. We also note that the lifetimes of the Eu(III) complex are extremely short, with decay times on the order of the 1-20 μsec. An examination of the Eu(III) lifetimes in MeOH and d₄-MeOD revealed an unusual result, in that the lifetime is slightly shorter in the deuterated solvent. Apparently, the known differences due to solvent quenching between νOH vs νOD¹¹ is overruled by extremely rapid kinetics for the back energy transfer, facilitated by the similarity in the ligand T_{0,0} and Eu(III) ⁵D₀ energy levels. Additional measurements in 1:4 (v/v) MeOH:EtOH at room temperature (RT) and 77 K gave lifetime values of ca. 10 μsec and 865 μsec respectively, confirming the effect of this thermal back energy transfer pathway at RT.

Given the complications observed with the Eu(III) lifetime data, the standard *q* determination could not be performed, and instead we utilized the empirical equation given by Beeby *et. al.*¹¹ Substituting the appropriate values of τ_{H2O} and τ_{D2O} for Yb(III) yields an estimated inner sphere hydration of 0.7 and 2.1 for the minor and major components of the biexponential decay. We take the first value to be a slight overestimate of the anticipated CN = 8 (i.e. *q* = 0) geometry. For the latter component, the situation is less clear, since hydrolysis of the [Yb(1)₂]⁻ complex to form [Yb(1)(H₂O)_{*q*}]⁻ should result in a *q* value of 3 or 4. Rather, this supports the proposed identity of the second emitting species as a hydrated [Ln(1)₂(H₂O)]⁻ complex (presumably CN = 9), again with an overestimated *q* value.

To summarize, 5LIO-MAM is a novel ligand for Ln(III) coordination chemistry and forms complexes with a variety

of cations. Of these, Eu(III) is only weakly sensitized due to efficient thermal back energy transfer whereas Yb(III) and Nd(III) are less prone to this effect. This highlights the requirement of a close energy match between the ligand donor (*eg.* T_{0,0}) and metal acceptor energy levels for efficient sensitization.

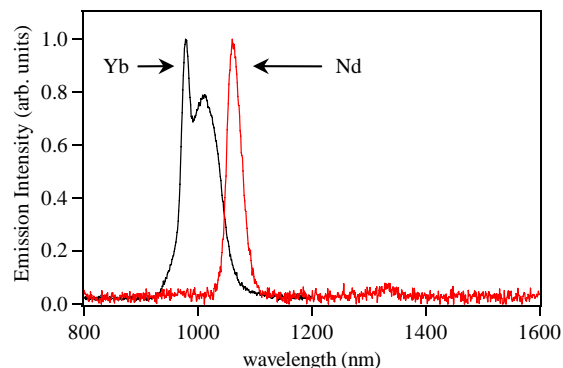


Figure 4. Observed emission spectra ($\lambda_{exc} = 326$ nm, 10 nm bandpass) for ca. 10 μM solutions of [Nd(1)₂]⁻ and [Yb(1)₂]⁻ in 0.1 M TRIS buffered aqueous solution, pH 7.4.

Table 2. Summary of time-resolved luminescence decay data obtained for 1 μM solutions of [Ln(1)₂]⁻ complexes at 298 K in various solvents.

Solvent	[Eu(1) ₂] ⁻ (μsec)	[Yb(1) ₂] ⁻ (μsec)	[Nd(1) ₂] ⁻ (nsec)
H ₂ O (0.1 M Tris, pH 7.4)*	1.41 (83 %) 19.7 (17 %)	0.39 (70 %) 1.01 (30 %)	no data (< IRF)
D ₂ O (0.1 M Tris, pD 7.4)*	1.07 (95 %) 18.0 (5 %)	3.30 (71 %) 12.7 (29 %)	weak ca. 482.0
MeOH	1.75	1.76	229.6
d ₄ -MeOD	1.58	38.4	983.6

*The observed decays in H₂O and D₂O were biexponential (see text).

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Supporting Information Available: Synthetic procedures, experimental data for stability determination and photophysical measurements. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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