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

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The synthesis, characterization, and photophysical properties are reported for several Ln(III) complexes of a tetradentate chelate, 5LIO-MAM, derived from the common flavor enhancer "maltol". Eu(III), Yb(III), and Nd(III) form stable ML_2 complexes in aqueous solution that emit in the red or near-infrared (NIR) upon excitation at ca. 330 nm. 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) [pEu = 14.7(1)]. A very poor metal-centered quantum yield with Eu(III) was observed (Φ_{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 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-infrared (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 magnetic resonance imaging (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]

Chart 1. Chemical Structures of Tetradentate Ligands Forming Stable ML_2 Complexes with Ln(III) and Featuring Exclusively Oxygen Donors

(1) = 5LIO-MAM (2) = 5LIO-1,2-HOPO
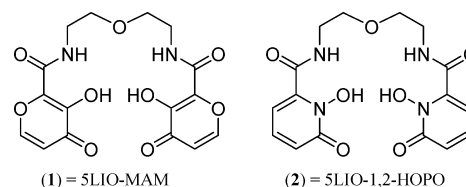
The recent discovery[5,6] of highly efficient 1,2-HOPO-sensitized emission from Eu(III) prompted our investigation of similar oxo ligands available in our group as sensitizers for lanthanide luminescence. Of these, the hexadentate TREN-MAM ligand is one of the chelates we have studied recently, which forms a Gd(III) complex that achieves high chelate stability and good efficiency as a contrast agent for MRI, primarily because of 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 that have recently been 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.

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 in the Supporting

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Table 1. Solution Thermodynamic Data Obtained for **1** and **2** Performed at 298 K with a 0.1 M KCl Supporting Electrolyte

constant	**1**	**2**[a]
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)

[a] Data taken from ref 5.

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

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

Information). 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] Dicyclohexyl-carbodiimide and *N*-hydroxysuccinimide 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 spectrophotometry in 0.1 M KCl (see Figure S2 in the Supporting Information), yielding pK_a's of 4.91(6) and 6.09(2), which are slightly more basic than the corresponding values for **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-HOPO[9] and TREN-MAM[7] ($\log \beta_{014} = 20.7$ 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 diethylenetriaminepentaacetic acid (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 a competitor determined the formation constants as summarized in Table 1. The observed decrease in the complex stability for the EuL and EuL₂ complexes results in a significant drop in the calculated pEu to 14.7 for **1**. Also, as a result of these low stability constant values and given the second-order dependence of speciation on the ligand concentration for a ternary complex, we calculate that the $[Eu(\mathbf{1})_2]^-$ complex will begin to partially hydrolyze at pH 7.4, forming the ML complex below ca. 0.1 mM in aqueous solution (see Figure 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 lanthanide hydroxy species (which convert to insoluble oxides) becomes apparent.

Having established the aqueous stability of the $[Eu(\mathbf{1})_2]^-$ complex, we sought to evaluate the photophysical properties. As shown in Figure 2, the UV−visible spectrum of the complex is typical for that expected from the aromatic chromophore, with a broad electronic envelope centered at
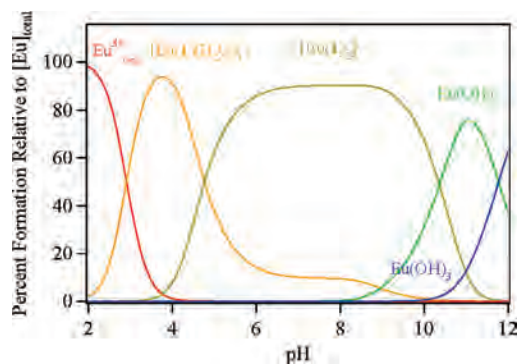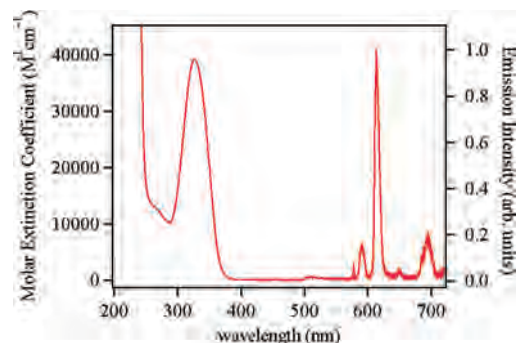
ca. 326 nm. As verified by time-dependent density functional theory techniques (see Figure S3 in the Supporting Information), we assign this peak to an essentially $\pi-\pi^*$ transition. Also shown in Figure 2 is the observed luminescence spectrum upon ligand-centered excitation at 326 nm. Several characteristic 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, respectively. To quantify the observed luminescence, we determined the overall quantum yield using standard techniques (see Figure S1 in the Supporting Information), resulting in a very low value of $\Phi_{tot} = 0.04\%$.

In order to rationalize the observed poor Eu(III) luminescence behavior, we determined the position of the lowest excited-state triplet energy, using the $[Gd(\mathbf{1})_2]^-$ 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 previously[10] that matching of the triplet energy and Ln(III) emission levels is a key factor for efficient metal emission.

The resulting spectrum is shown in Figure 3, together with a spectral deconvolution of the observed phosphorescence band from ca. 500 to 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, T_1, to be ca. 556 nm (17 970 cm^{-1}). Thus, the triplet energy level of $[Gd(\mathbf{1})_2]^-$ is barely above the $^5D_0$ emitting state of Eu(III) (17 300 cm^{-1}) and well below the value previously deter-

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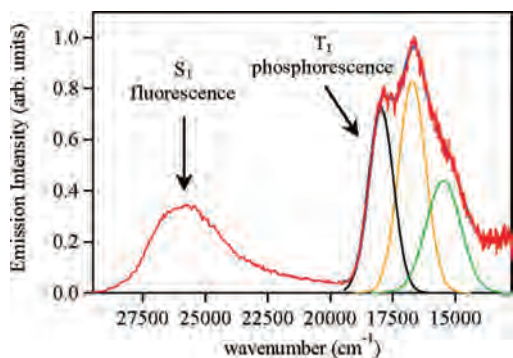
Figure 3. Low-temperature (77 K) emission spectra (λ_{ex} = 326 nm) of [Gd(**1**)$_2$]$^-$ in 10:10:1 (v/v/v) EtOH/MeOH/H$_2$O (1.0 M Tris buffer, pH 7.4), and spectral deconvolution of the phosphorescence into overlapping Gaussian functions to yield the lowest-energy triplet level at 17 970 cm^{-1}.
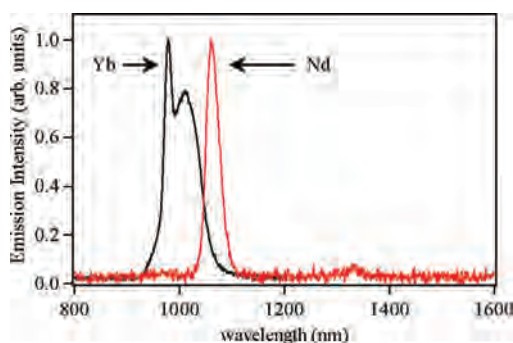

Figure 4. Observed emission spectra (λ_{ex} = 326 nm, 10 nm bandpass) for ca. 10 μM solutions of [Nd(**1**)$_2$]$^-$ and [Yb(**1**)$_2$]$^-$ in a 0.1 M Tris-buffered aqueous solution, pH 7.4.

mined[6] for [Gd(**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 because of highly competitive thermal back energy transfer.

Given that the emissive level of Yb(III) at 10 200 cm^{-1} and that of Nd(III) at 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 Figure 4. Herein, the characteristic $^2F_{5/2} \rightarrow {}^2F_{7/2}$ electronic transition of the Yb(III) cation is clearly observed, with a peak at ca. 978 nm, and weakly split because of crystal-field effects, while for Nd(III), there are two bands apparent with peaks at ca. 1060 and 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, H$_2$O (where possible), and perdeuterated solvent, with the 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 Figure 1), or alternately may be due to the presence of a slowly exchanging equilibrium mixture with a hydrated form of the complex, [Ln(**1**)$_2$(H$_2$O)]$^-$. By contrast, measurements performed in MeOH and MeOD-d_4 were satisfactorily

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**)$_2$]$^-$ (μs)	[Yb(**1**)$_2$]$^-$ (μs)	[Nd(**1**)$_2$]$^-$ (ns)
H$_2$O (0.1 M Tris, pH 7.4)[a]	1.41 (83%)	0.39 (70%)	no data
	19.7 (17%)	1.01 (30%)	(<IRF)
D$_2$O (0.1 M Tris, pD 7.4)[a]	1.07 (95%)	3.30 (71%)	weak
	18.0 (5%)	12.7 (29%)	ca. 482.0
MeOH	1.75	1.76	229.6
MeOD-d_4	1.58	38.4	983.6

[a] The observed decays in H$_2$O and D$_2$O were biexponential (see the text).

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 μs. An examination of the Eu(III) lifetimes in MeOH and MeOD-d_4 revealed an unusual result in that the lifetime is slightly shorter in the deuterated solvent. Apparently, the known differences due to solvent quenching between ν_{OH} and ν_{OD}[11] are overruled by extremely rapid kinetics for the back energy transfer, facilitated by the similarity in the ligand T$_{0-0}$ and Eu(III) $^5D_0$ energy levels. Additional measurements in 1:4 (v/v) MeOH/EtOH at room temperature (RT) and 77 K gave lifetime values of ca. 10 and 865 μs, 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_{H_2O} and τ_{D_2O} 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 overestimation of the anticipated CN = 8 (i.e., q = 0) geometry. For the latter component, the situation is less clear because hydrolysis of the [Yb(**1**)$_2$]$^-$ complex to form [Yb(**1**)(H$_2$O)$_q$] 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**)$_2$(H$_2$O)]$^-$ 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 as a result of 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 (e.g., T$_{0-0}$) and metal acceptor energy levels for efficient sensitization.

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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