

## An Octadentate Luminescent Eu(III) 1,2-HOPO Chelate with Potent Aqueous Stability

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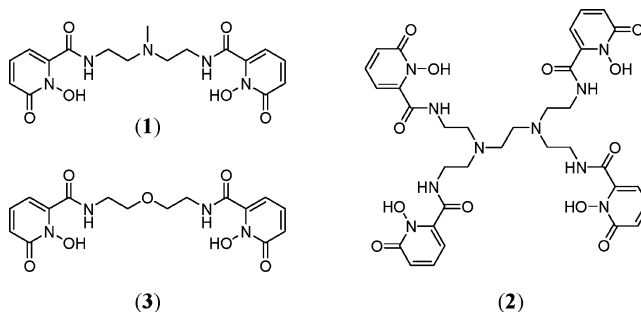
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The synthesis, characterization, and photophysical properties of two novel ligands, 5LIN<sup>Me</sup>-1,2-HOPO (1) and H(2,2)-1,2-HOPO (2), which utilize the 1,2-HOPO chelate as a sensitizer for Eu(III) are reported. In addition, the former ligand was structurally characterized as the Eu(III) complex by X-ray crystallography. The [Eu(1)<sub>2</sub>]<sup>-</sup> complex of the tetradentate ligand (1) is stable in aqueous solution, to a limiting concentration of ca.  $7 \times 10^{-9}$  M, and retains the superior photophysical performance noted for the 1,2-HOPO sensitizer. By contrast, the octadentate ligand (2) has vastly improved stability as the [Eu(2)]<sup>-</sup> complex upon further dilution, to a limiting concentration of ca.  $5 \times 10^{-17}$  M, which is beyond the minimum detectable concentration of most fluorimeters. The presence of a single coordinated water molecule for the latter complex reduces the overall metal-centered luminescence.

Organic complexes of luminescent lanthanides such as Eu(III) have become increasingly useful for biological assays and in high-throughput screening applications,<sup>1,2</sup> where their long-lived luminescence can allow significant improvement in the signal-to-noise ratio. Several Ln(III) chelates are commercially available (e.g., Lance, Perkin-Elmer; LanthaScreen, Invitrogen), and fluorescent assay platforms such as dissociation-enhanced lanthanide fluorescent immuno assay (DELFI) are well developed,<sup>3</sup> offering increased sensitivity compared to colorimetric assay formats such as enzyme-linked immuno sorbent assay (ELISA).<sup>4</sup> Nonetheless, the quantum yield of these commercial luminescence agents are far from optimized (ca. 2–10%), and the improvement of this crucial parameter while maintaining sufficient aqueous solubility and stability for biological compatibility is a challenging area of active current interest.<sup>5</sup>

We recently reported<sup>6</sup> the exceptional photophysical performance of tetradentate 1-hydroxypyridin-2-one deriva-

**Chart 1.** Structures of 5LIO-1,2-HOPO (3) (Top Left), 5LIN<sup>Me</sup>-1,2-HOPO (1) (Bottom Left) and H(2,2)-1,2-HOPO (2) (Right)



tives such as 5LIO-1,2-HOPO (3) that form stable ML<sub>2</sub> complexes with Eu(III). These ligands simultaneously fulfill the criteria of both high thermodynamic stability and excellent photophysical properties. Herein, we extend this family to include an amino-linked version 5LIN<sup>Me</sup>-1,2-HOPO (1) (Chart 1) which was developed as a model for H(2,2)-1,2-HOPO (2), the first example of an octadentate 1,2-HOPO derivative for Eu(III) luminescence. The latter forms an ML complex with improved stability upon dilution in aqueous solution, a feature required for the practical development and application of these complexes in biological assays.

The syntheses of 5LIN<sup>Me</sup>-1,2-HOPO and H(2,2)-1,2-HOPO are straightforward (see Scheme S1, Supporting Information). Boc amidation of *N*-(2-aminoethyl)-ethane-1,2-diamine (dien) followed by reductive amination with formaldehyde provides the protected backbone which was converted to the 5LIN<sup>Me</sup> amine by reaction with TFA. Alternately, the *N,N,N',N'*-tetrakis-(2-aminoethyl)-ethane-1,2-diamine (H(2,2)) may be prepared using literature methods.<sup>7</sup> Reaction of either backbone with the thiazolide-activated 1,2-HOPO precursor<sup>6,8</sup> yields the benzyl-protected ligands, which were deprotected under acidic conditions with 1:1 (v/v) AcOH/HCl (12 M). Complexation with EuCl<sub>3</sub>·6H<sub>2</sub>O in MeOH at reflux gave the desired complexes on cooling in good yield and analytically pure form. An X-ray-quality crystal of the [Eu(5LIN<sup>Me</sup>-1,2-HOPO)<sub>2</sub>]<sup>-</sup> complex, as the potassium salt,

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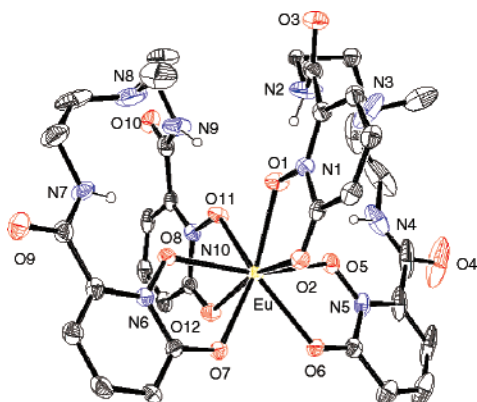
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**Figure 1.** X-ray structure of  $[\text{Eu}(\text{5LIN}^{\text{Me}}\text{-1,2-HOPO})_2]^-$  (30% probability ellipsoids shown, Ortep 3). Solvent molecules, potassium counter cation, and selected H atoms have been omitted for clarity. Only one orientation of the disordered ligand backbone is shown.

**Table 1.** Protonation and Eu(III) Complex Formation Constants of 5LIN<sup>Me</sup>-1,2-HOPO Compared to Previously Reported<sup>6</sup> 5LIO-1,2-HOPO

|                      | 5LIN <sup>Me</sup> -1,2-HOPO | 5LIO-1,2-HOPO |
|----------------------|------------------------------|---------------|
| pK <sub>1</sub>      | 3.76(3)                      | 4.19(3)       |
| pK <sub>2</sub>      | 5.31(1)                      | 5.79(1)       |
| pK <sub>3</sub>      | 8.13(1)                      | N/A           |
| pEu <sup>7.4</sup>   | 17.3(1)                      | 18.6(1)       |
| log β <sub>120</sub> | 23.0(1)                      | 22.9(1)       |
| log β <sub>121</sub> | 30.5(5)                      | 25.2(3)       |
| log β <sub>122</sub> | 36.97(5)                     | N/A           |
| log β <sub>123</sub> | 41.5(1)                      | N/A           |

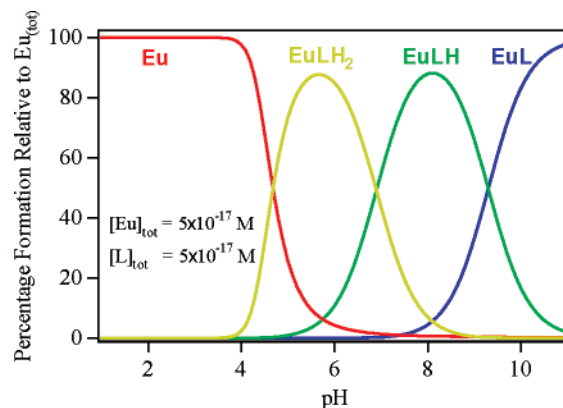
**Table 2.** Protonation and Eu(III) Complex Formation Constants of H(2,2)-1,2-HOPO

| pK <sub>1</sub> | 3.19(4)  | pEu <sub>8.5</sub>   | 21.7(1) |
|-----------------|----------|----------------------|---------|
| pK <sub>2</sub> | 4.27(3)  | pEu <sub>7.4</sub>   | 21.2(1) |
| pK <sub>3</sub> | 5.23(3)  | pEu <sub>6.1</sub>   | 20.0(1) |
| pK <sub>4</sub> | 6.22(2)  | log β <sub>110</sub> | 21.8(5) |
| pK <sub>5</sub> | 7.77(1)  | log β <sub>111</sub> | 31.2(3) |
| pK <sub>6</sub> | 10.43(2) | log β <sub>112</sub> | 38.1(2) |

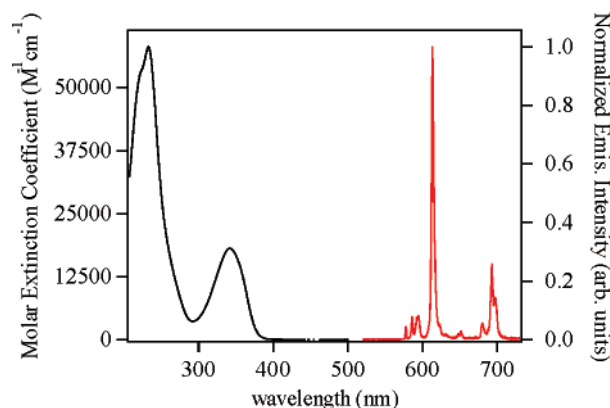
was grown by slow evaporation of a ca. 500 μM solution in 0.1 M KCl at ca. pH 8.

As shown in Figure 1, the anionic Eu(III) complex of **1** forms an ML<sub>2</sub> species, with an overall eight-coordinate central metal ion. Shape analysis, as discussed elsewhere,<sup>9,10</sup> was used to assess the closest idealized coordination polyhedra, yielding the best fit to an approximate C<sub>2v</sub> geometry at the metal (see Table S1). Notably, the observed geometry is very similar to that previously reported<sup>6</sup> for the structurally analogous ligand (**3**) with an ether linkage in the backbone. In the present case, as with the [Eu(**3**)<sub>2</sub>]<sup>-</sup> structure, the presence of four intramolecular hydrogen-bonding interactions is noted between the amide nitrogen protons and proximal phenolic oxygen atoms (av N–H⋯O = 1.97 Å). These interactions have been noted in several previous examples<sup>6,8</sup> and impart additional stability to the ensuing metal complex. A potassium counter cation was also located at the base of each Eu(III) complex, which forms a trifurcated interaction with the O2, O6, and O7 oxygens of the anionic complex, and is bridged by amide carbonyl oxygens from three adjacent molecules (O3, O9, and O10)

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**Figure 2.** Speciation diagram for  $[\text{Eu}(\text{H}(2,2)\text{-1,2-HOPO})]$  in aqueous solution, illustrating the excellent thermodynamic stability of the complex upon very high dilution at ca.  $5 \times 10^{-17}$  M.



**Figure 3.** Electronic absorption spectrum (left) and steady-state emission spectrum (right) ( $\lambda_{\text{ex}} = 340$  nm, 5 nm bandpass) of 5 μM  $[\text{Eu}(\mathbf{2})]^-$  in 0.1 M Tris buffer at pH 7.4

to form an extended coordination polymer network. The coordination sphere of the potassium ion is completed by a solvent water molecule, yielding an overall seven-coordinate cation (see Figure S1).

Ligand pK<sub>a</sub>'s for **1** and **2** were determined by potentiometry, and resulting values are summarized in Tables 1 and 2. For both ligands, the most basic protonation sites were assigned to the amine nitrogen atoms by comparison to previous reports.<sup>6</sup> For the  $[\text{Eu}(\mathbf{1})_2]^-$  complex, a formation constant of log β<sub>120</sub> = 23.0(1) was determined from the conditional stability constant (pEu<sup>7.4</sup> = 17.3(1)), obtained by competition batch titration versus DTPA (see Figure S2).<sup>6</sup> Subsequent potentiometric titrations could be refined with fixed ligand protonation constants and a fixed log β<sub>120</sub> = 23.0, resulting in log β<sub>121</sub> = 30.5(5), log β<sub>122</sub> = 36.97(5), and log β<sub>123</sub> = 41.5(1), with initial protonation sites of the metal complex most likely located at the basic amine functions, and a third (most acidic) protonation at one of the 1,2-HOPO chromophores. Preliminary stability data for the octadentate complex,  $[\text{Eu}(\mathbf{2})]^-$ , were also determined via competition batch titration versus DTPA at pH 7.4, yielding a pEu of 21.2(1). Potentiometric titrations were hampered by a decrease in solubility of the complex below pH 7 (from >0.5 to ~0.1 mM), which thwarted refinement of the titration data. Instead, additional competition batch titration versus DTPA at pH 6.1 and 8.5 were performed (see Figure S3).

**Table 3.** Summary of Experimental and Calculated Photophysical Parameters for  $[\text{Eu}(\text{5LIN}^{\text{Me}}\text{-1,2-HOPO})_2]^-$  and  $[\text{Eu}(\text{H}(2,2)\text{-1,2-HOPO})]^-$  Complexes

| complex  | $\lambda_{\text{max}}, \epsilon_{\text{max}}$<br>(nm, $\text{M}^{-1} \text{cm}^{-1}$ ) | $\Phi_{\text{tot}}$<br>( $\text{H}_2\text{O}$ ) | $\tau_{\text{obs}}$ $\text{H}_2\text{O}, \{\text{D}_2\text{O}\}$<br>( $\mu\text{s}$ ) | $k_{\text{r}}$ , calcd<br>( $\text{ms}^{-1}$ ) | $k_{\text{nr}}$ , calcd<br>( $\text{ms}^{-1}$ ) | $\Phi_{\text{Eu}}$ , calcd | $\eta_{\text{sens}}$ , calcd |
|--|--|---|---|--|---|----------------------------|------------------------------|
| $[\text{Eu}(\text{5LIN}^{\text{Me}}\text{-1,2-HOPO})_2]^-$ | 332, 18 750  | $0.173 \pm 0.026$                               | $728 \pm 2.8 \{1000 \pm 2.5\}$  | 0.61   | 0.77  | 0.442                      | 0.391                        |
| $[\text{Eu}(\text{H}(2,2)\text{-1,2-HOPO})]^-$             | 341, 18 200  | $0.036 \pm 0.005$                               | $480 \pm 1.2 \{1222 \pm 2.5\}$  | 0.38   | 1.75  | 0.178                      | 0.202                        |

Values of  $\log \beta_{110} = 21.8(5)$ ,  $\log \beta_{111} = 31.2(3)$  and  $\log \beta_{112} = 38.1(2)$  resulted from fits to these data, along with  $[\text{Eu}(\mathbf{2})]^-$  complex protonation constants of  $\text{p}K_{\text{a}1} = 9.4(5)$  and  $\text{p}K_{\text{a}2} = 6.9(2)$  which, by analogy to  $[\text{Eu}(\mathbf{1}_2)]^-$ , we assign to protonation of the amine backbone nitrogens. The complete solution thermodynamic model is described by the parameters summarized in Table 2. Assuming a tolerance of 1% free Eu(III), we calculate a theoretical stability limit for the  $[\text{Eu}(\mathbf{2})]^-$  complex of ca.  $5 \times 10^{-17}$  M at pH 7.4 in the absence of competing species (Figure 2), whereupon further dilution leads to hydrolysis of the complex. This limiting concentration is some 8 orders of magnitude improved when compared to the corresponding calculation for  $[\text{Eu}(\mathbf{1}_2)]^-$ , which yields a limiting concentration of ca.  $7 \times 10^{-9}$  M (see Figure S4), demonstrating the dramatic enhancement of stability at high dilution due primarily to the entropic chelate effect when moving from a tetradentate to octadentate ligand topology.

The electronic absorption spectra for the  $[\text{Eu}(\mathbf{2})]^-$  and  $[\text{Eu}(\mathbf{1}_2)]^-$  complexes are shown in Figure 3 and S5, respectively, and are typical of that observed for the 1,2-HOPO chromophore,<sup>6</sup> comprising an  $n-\pi^*$  transition at ca. 330–340 nm and more intense  $\pi-\pi^*$  bands to higher energy. Notably, the spectrum of  $[\text{Eu}(\mathbf{2})]^-$  has a slightly red-shifted  $\lambda_{\text{max}}$  at 341 nm, ca.  $800 \text{ cm}^{-1}$  lower than the  $[\text{Eu}(\mathbf{1}_2)]^-$  complex. The luminescence spectrum of  $[\text{Eu}(\mathbf{2})]^-$  is also shown in Figure 3, and is typical of that for the Eu(III) cation in a low-symmetry environment (vide infra).

A summary of the relevant photophysical parameters determined experimentally (and calculated from the emission spectrum as discussed elsewhere<sup>11</sup>) for  $[\text{Eu}(\mathbf{1}_2)]^-$  and  $[\text{Eu}(\mathbf{2})]^-$  is given in Table 3. Most notably, the quantum yield determined (see Supporting Information) for  $[\text{Eu}(\mathbf{1}_2)]^-$  is comparable to that previously reported<sup>6</sup> for the analogous  $[\text{Eu}(\mathbf{3}_2)]^-$  complex, while the corresponding value of  $\Phi_{\text{tot}} = 3.6\% \pm 0.5\%$  for  $[\text{Eu}(\mathbf{2})]^-$  is almost an order of magnitude less than the tetradentate model compounds.

The corresponding luminescence lifetimes were evaluated for both complexes in aqueous and deuterated solvent, allowing the degree of hydration,  $q$ , to be estimated using

the recently improved Horrocks equation.<sup>12</sup> The resulting  $q$  values for  $[\text{Eu}(\mathbf{1}_2)]^-$  and  $[\text{Eu}(\mathbf{2})]^-$  were 0.07 and  $1.06 \pm 0.1$  respectively. Accordingly, while  $[\text{Eu}(\mathbf{1}_2)]^-$  receives an effective degree of protection from nonradiative deactivation by solvent interactions, it appears that the  $[\text{Eu}(\mathbf{2})]^-$  complex retains a single bound water molecule in the metal's first coordination sphere. Since the non-radiative decay rate of the Eu(III) cation is known<sup>12</sup> to be strongly influenced by the presence of proximal OH oscillators, this explains the poor photophysical performance for  $[\text{Eu}(\mathbf{2})(\text{H}_2\text{O})]^-$ . Furthermore, it appears both the radiative decay rate,  $k_{\text{r}}$ , and the energy transfer efficiency (from ligand to metal),  $\eta_{\text{sens}}$ , are lower for  $[\text{Eu}(\mathbf{2})(\text{H}_2\text{O})]^-$  for reasons which are not yet readily apparent and are currently being investigated.

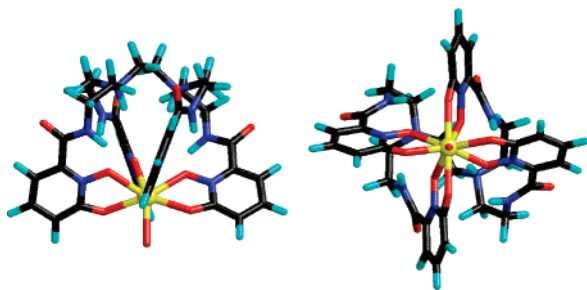
Last, it has been shown<sup>13</sup> that the observed emission spectra can be used to yield an insight into the solution structure of Eu(III) complexes. The corresponding  $q$  measurements determined a nine-coordinate coordination polyhedron for  $[\text{Eu}(\mathbf{2})(\text{H}_2\text{O})]^-$ . However, a consideration of the symmetry-allowed transitions (see Table S3) did not yield a satisfactory match to either the tricapped trigonal prismatic ( $D_{3h}$ ) or monocapped square antiprismatic ( $C_{4v}$ ) geometries. Rather, a closer analysis of the  $J = 1$  band reveals a splitting of the degenerate E state of the latter into two 2B bands (see Figure S6), consistent with a local  $C_2$  site symmetry for the metal ion.<sup>13</sup> This can be rationalized by the  $C_2$  geometry enforced by the ligand. An optimized MM3 model of the  $[\text{Eu}(\mathbf{2})(\text{H}_2\text{O})]^-$  complex is shown in Figure 4 and supports this assignment.

Our efforts are now turning toward eliminating the residual water molecule in order to improve the luminescent characteristics of these octadentate 1,2-HOPO derivatives.

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**Supporting Information Available:** Synthesis of  $5\text{LIN}^{\text{Me}}\text{-1,2-HOPO}$ ,  $\text{H}(2,2)\text{-1,2-HOPO}$ , and Eu(III) complexes. Shape analysis results and crystal data (cif) for  $[\text{K}(\text{H}_2\text{O})\text{Eu}(\mathbf{1}_2)] \cdot \text{H}_2\text{O}$ . Details of solution thermodynamic experiments and photophysical data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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**Figure 4.** Side view (left) and bottom view (right) of an MM3-optimized geometry for the  $[\text{Eu}(\mathbf{2})(\text{H}_2\text{O})]^-$  complex demonstrating local  $C_2$  symmetry about the Eu(III) metal ion.

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