# Lawrence Berkeley National Laboratory

Lawrence Berkeley National Laboratory

### Title

Using the Antenna Effect as a Spectroscopic Tool; Photophysics and Solution Thermodynamics of the Model Luminescent Hydroxypyridonate Complex [EuIII(3,4,3-LI(1,2-HOPO))]-

**Permalink** https://escholarship.org/uc/item/2sq8n7kp

Author Abergel, Rebecca J.

### **Publication Date**

2010-11-30

Peer reviewed

## Using the Antenna Effect as a Spectroscopic Tool; Photophysics and Solution Thermodynamics of the Model Luminescent Hydroxypyridonate Complex [Eu<sup>III</sup>(3,4,3-LI(1,2-HOPO))]<sup>-</sup>

# Rebecca J. Abergel, Anthony D'Aléo, Clara Ng Pak Leung, David K. Shuh and Kenneth N. Raymond\*

Chemical Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, and Department of Chemistry, University of California, Berkeley, California 94720-1460.

RECEIVED DATE (will be automatically inserted after manuscript is accepted)

While widely used in bioassays, the spectrofluorimetric method described here uses the antenna effect as a tool to probe the thermodynamic parameters of ligands that sensitize lanthanide luminescence. The Eu<sup>3+</sup> coordination chemistry, solution thermodynamic stability and photophysical properties of the spermine-based hydroxypyridonate octadentate chelator 3,4,3-LI(1,2-HOPO) are reported. The complex [Eu<sup>III</sup>(3,4,3-LI(1,2-HOPO))]<sup>-</sup> luminesces with a long lifetime (805  $\mu$ s) and a guantum yield of 7.0% in agueous solution, at pH 7.4. These remarkable optical properties were exploited to determine the high (and proton-independent) stability of the complex (log  $\beta_{110} = 20.2(2)$ ) and to define the influence of the ligand scaffold on the stability and photophysical properties.

The high-affinity bidentate hydroxypyridonate (HOPO) metal-chelating groups are related to microbial siderophores: they combine the structural features of hydroxamic acids with the electronic properties of catechols. The 6-amide derivative of 1-hydroxy-pyridin-2one (1,2-HOPO) has been linked to multiple polyamine scaffolds through amide coupling, to form multidentate ligand structures used for a variety of applications such as actinide (An) and iron chelation,<sup>1,2</sup> MRI contrast enhancement<sup>3</sup> lanthanide and (Ln) luminescence sensitization.<sup>4</sup> The coordination chemistry properties of these ligands can be fine-tuned by systematic modifications of the denticity, geometry and acidity of the backbone. Octadentate ligands, each incorporating four 1,2-HOPO functionalities, are known to strongly bind Ln(III), An(III), and An(IV) and to act as antennae that sensitize the emission of Eu(III).<sup>1,5</sup> The backbone geometry of the ligand must affect the thermodynamic stability and photophysical properties of the corresponding Ln- and An-complexes, but this has not been investigated in a systematic way. While the octadentate ligand 3,4,3-LI(1,2-HOPO) (1, Fig. 1) is composed of 1,2-HOPO units linked to a central linear spermine scaffold and has shown potential as a therapeutic Pu(IV) and Am(III) chelator,<sup>1</sup> the branched tetrapodal ligand H(2,2)-1,2-HOPO (2, Fig. 1) has been studied for its ability to form a highly stable luminescent Eu(III) complex that contains a molecule of water in the inner coordination sphere at physiological pH.5 The work presented herein probes the coordination chemistry and photophysical properties of the Gd(III) and Eu(III) complexes of 1, showing that the geometry of the ligand scaffold strongly affects the inner coordination sphere of the metal ion and consequently its emissive properties. In addition, the Eu luminescence sensitization properties of the antenna ligand 1 are used as a spectroscopic tool to determine the solution thermodynamic stability of the corresponding metalcomplex, which provides a new method for the accurate determination of these thermodynamic parameters.



**Figure 1.** Structures of the octadentate hydroxypyridonate ligands 3,4,3-LI(1,2-HOPO) (1, left) and H(2,2)-1,2-HOPO (2, right); the metal-coordinating oxygen atoms are indicated in red.

All photophysical properties were measured in buffered aqueous solutions at pH 7.4 and relevant parameters are summarized in Table 1. The electronic absorption spectrum of  $[\text{Eu}^{\text{III}}(1)]^{-}$  (Fig. 2) shows an absorption maximum due to  $\pi - \pi^{*}$  transitions at  $\lambda_{\text{max}} = 315$  nm ( $\varepsilon = 17,700 \text{ M}^{-1}\text{cm}^{-1}$ ). This spectrum is blue-shifted and has a lower molar absorption coefficient than the spectrum of  $[\text{Eu}^{\text{III}}(2)]^{0}$  ( $\lambda_{\text{max}}$ 

<sup>\*</sup> To whom correspondence should be addressed. Email: raymond@socrates.berkeley.edu. Fax: +1 (510) 486 5283

= 343 nm,  $\varepsilon$  = 18,200 M<sup>-1</sup>cm<sup>-1,5</sup> Fig. 2). The shift towards higher energies is attributed to the different ligand scaffolds: the four protonated amide nitrogen atoms in the molecular structure of **2** can form hydrogen bonds with the hydroxyl groups from the pyridinone rings, thereby stabilizing the first singlet excited state of the corresponding europium complex, whereas the backbone of **1** only contains two protonated amide nitrogen atoms yielding a complex with a singlet excited state slightly higher in energy, and a shoulder at lower energy on the main absorption peak.



**Figure 2.** Electronic absorption (solid, left) and normalized steady-state emission spectra (solid, right,  $\lambda_{exc} = 325$  nm) of  $[Eu^{III}(1)]^-$ , and electronic absorption spectrum (dash) of  $[Eu^{III}(2)]^0$ , in 0.1 M TRIS buffer (pH 7.4).

The Gd(III) complex of 1 was prepared in situ, to determine the ligand centered triplet excited state energy. Because the Gd<sup>3+</sup> ion exhibits a size and atomic weight similar to Eu<sup>3+</sup> but lacks an appropriately positioned electronic acceptor level, the phosphorescence of the ligand can be observed by luminescence measurements in a solid matrix (1:3 (v/v) MeOH:EtOH) at 77 K. Upon cooling to 77 K, the spectrum of  $[Gd^{III}(1)]^{-}$  reveals an intense unstructured emission band from 400 to 570 nm (Fig. S1), assigned to phosphorescence from the ligand  $T_1$  excited state. The lowest T<sub>1</sub> state energy was estimated by spectral deconvolution of the 77 K luminescence signal into several overlapping Gaussian functions.<sup>6</sup> The resulting  $T_1$  energy was calculated at 24,390 cm<sup>-1</sup>, which is higher than the values found for other 1,2-HOPO derivatives ( $T_1 \sim 19,500$ -21,500 cm<sup>-1</sup>),<sup>4</sup> and the energy gap between T<sub>1</sub> and the  ${}^{5}D_{1}$ accepting state was determined at 5,360 cm<sup>-1</sup>, a value larger than that found for 2, implying that the energy transfer efficiency of  $[Eu^{III}(1)]^{-}$  should be less efficient. This increase of the triplet excited state energy is in line with the increase of the singlet excited state energy determined by UV-Visible absorption spectroscopy and can also be attributed to the absence of two protonated amide nitrogen (destabilizing the triplet excited state as compared to that of  $[Eu^{III}(2)]^0$ ).

The luminescence spectrum of  $[Eu^{III}(1)]^{-}$  displays the characteristic features of  $Eu^{III}(1,2\text{-HOPO})$  complexes; the very intense  ${}^{5}D_{0} \rightarrow {}^{7}F_{2}$  hypersensitive transition results in almost pure red luminescence ( $\lambda_{em} = 612 \text{ nm}$ , Fig. 2). The luminescence quantum yield of  $[Eu^{III}(1)]^{-}$  in aqueous

solution (pH = 7.4),  $\Phi_{tot} = 7.0\%$ , is two-fold higher than that of  $[Eu^{III}(2)]^0$  (3.6%),<sup>5</sup> thus  $[Eu^{III}(1)]^-$  is much brighter than  $[Eu^{III}(2)]^0$  (the brightness is defined as the product of molar absorption coefficient and luminescence quantum yield). The optical properties of the 3,4,3-LI backbone are superior to those of the H(2,2) backbone at high energy, up to 337 nm, and are slightly inferior at lower energies (Fig. S2).

Table 1. Summary of Photophysical Parameters for [Eu<sup>III</sup>(1)]<sup>- a</sup>

| $\lambda_{\rm max}, \epsilon_{\rm max}  ({\rm nm}, {\rm M}^{-1} {\rm cm}^{-1})$ | 315, 17700        | $\tau_{\rm rad} (\mu { m s})$    | 1860  |
|---|-------------------|----------------------------------|-------|
| $\tau_{\rm obs}$ {H <sub>2</sub> O} ( $\mu$ s)                                  | $805 \pm 81$      | $k_{\rm rad}~({\rm s}^{-1})$     | 540   |
| $\tau_{obs} \{D_2 O\} (\mu s)$  | $1120 \pm 112$    | $k_{\rm nonrad}  ({\rm s}^{-1})$ | 705   |
| $\Phi_{\rm tot}$ (H <sub>2</sub> O)   | $0.070 \pm 0.007$ | $\eta^{\text{Eu}}$               | 0.432 |
| 9   | $0.1 \pm 0.1$     | $\dot{\eta}_{ m sens}$           | 0.162 |

<sup>&</sup>lt;sup>a</sup>The uncertainties were determined from the standard deviation between three independent experiments performed in aqueous buffered solutions (TRIS, pH 7.4).

Corresponding time resolved analysis of  $[\text{Eu}^{\text{III}}(1)]^{-1}$ luminescence, measured at 612 nm in H<sub>2</sub>O and D<sub>2</sub>O, revealed monoexponential decays with decay times of *ca*. 805 µs and *ca*. 1120 µs, respectively, which are slightly longer than the typical lifetimes determined for bistetradentate Eu<sup>III</sup>(1,2-HOPO) complexes.<sup>6,7</sup> Using the improved Horrocks equation,<sup>8</sup> the number of inner sphere water molecules on the  $[\text{Eu}^{\text{III}}(1)]^{-1}$  complex was determined as  $q = 0.1 \pm 0.1$ , essentially zero. In contrast to complexes formed with H(2,2) ligand derivatives,<sup>5</sup> the 3,4,3-LI linear backbone promotes the formation of a Eu(III) complex with no inner sphere water molecule.

In order to investigate in detail further particularities of the sensitization process by the linear ligand 1, the kinetic parameters were determined.<sup>9,10</sup> The sensitization efficiency,  $\eta_{sens}$ , defined as the product of the efficiency of the energy transfer,  $\eta_{\rm ET}$ , and the quantum yield of the antenna,  $\Phi_{antenna}$ , was determined using the equation:  $\Phi_{tot}$  $= \Phi_{\text{antenna}} \times \eta_{\text{ET}} \times \Phi_{\text{Eu}}$ . The radiative decay rate,  $k_{\text{rad}}$ , of  $[\operatorname{Eu}^{III}(1)]^{-1}$  is higher than that of  $[\operatorname{Eu}^{III}(2)]^{0}$  (538 s<sup>-1</sup> vs. 333 s<sup>-1</sup> <sup>1</sup>), and the non-radiative decay rate,  $k_{\text{nonrad}}$ , is much lower for the linear complex than for the branched complex with values of 705 s<sup>-1</sup> and 1750 s<sup>-1</sup>, respectively. This result reflects the absence of water molecule in the inner sphere of  $[Eu^{III}(1)]^{-}$ , inducing a decrease of the quenching by OH vibrations. Consequently, the metal centered luminescence efficiency  $(\eta^{\text{Eu}})$ , is higher for  $[\text{Eu}^{\text{III}}(1)]^{-}$  than for  $[\text{Eu}^{\text{III}}(2)]^{0}$ (43.2% vs. 16.0%), while the sensitization efficiency,  $\eta_{sens}$ , is lower (16.2% vs. ca. 22.5%). The intersystem crossing and the energy transfer are therefore affected when using the 3,4,3-LI backbone, which limits the corresponding luminescence quantum yield to 7.0%. These values emphasize the important trade off existing between the sensitization and metal efficiency of the Eu<sup>III</sup> complexes.

The protonation constants of **1** were determined by potentiometric titrations, and four protonation equilibria (Table 2) were assigned to sequential removal of one proton from each of the four 1,2-HOPO units. The overall basicity of **1** (quantified from the sum of the log  $K_a$  values associated to only those protonation steps that result in the neutral ligand species,<sup>11</sup>  $\Sigma \log K_a = 21.2$ ) is increased when compared to the branched ligand **2** ( $\Sigma \log K_a = 18.9$ ).<sup>5</sup> Initial attempts were made to determine the Eu(III) affinity

of 1 by direct spectrophotometric titration (Fig. S3). However, few changes occur in the UV-Visible absorption of the complex over the pH range 3.0-8.0, limiting accurate spectral deconvolution and data refinement. The Eu<sup>3+</sup> complexation of **1** was thus studied by spectrofluorimetric titration: a solution containing an equimolar ratio of Eu and 1 was divided into separate aliquots, and base was added to each sample to give a pH range from 2.2 to 9.5. After a 24 h equilibration time, each emission spectrum ( $\lambda_{exc} = 325$ nm) and pH was recorded and analyzed to ascertain the proton-independent stability constant ( $K_f = \beta_{110} = 10^{20.2(2)}$ ) and protonation constant ( $K_a = 10^{4.6(1)}$ ) of the corresponding europium complex (Table 2). In contrast to previous studies,<sup>12</sup> the present method relies on the sensitization of the Eu luminescence by the excited ligand rather than on the emission resulting from direct excitation of the metal ion. Upon acidification, the luminescence of the solution decreases (Fig. 3), corresponding to the disappearance of the emissive species  $[Eu^{III}(1)]^{-1}$  and the formation of the protonated complex [Eu<sup>III</sup>(1H)]<sup>0</sup>. In addition, only a single mono-exponential decay lifetime (805  $\mu$ s +/- 10%), corresponding to the parent complex  $[Eu^{III}(1)]^{-}$ , was detected through time-resolved measurements of the titration samples.



**Figure 3.** Spectrofluorimetric titration of  $[Eu^{III}(1)]^{-}$  ( $[Eu^{3+}] = [1] = 0.01$  mM, 0.1 M KCl, 0.2 mM Hepes, 0.2 mM Mes, 25.0 °C,  $\lambda_{exc} = 325$  nm). *Insert:* Normalized integration of the emission spectra (data points) and calculated speciation diagram corresponding to the titration conditions (solid lines).

Table 2. Protonation and Eu Complex Formation Constants for Ligand 1<sup>a</sup>

| species                  | m. l, h | $\log \beta_{mlh}$ |  |
|--------------------------|---------|--------------------|--|
| LH                       | 0, 1, 1 | 6.64(1)            |  |
| $LH_2$                   | 0, 1, 2 | 12.32(1)           |  |
| $LH_3$                   | 0, 1, 3 | 17.33(1)           |  |
| $LH_4$                   | 0, 1, 4 | 21.20(1)           |  |
| EuL                      | 1, 1, 0 | 20.2(2)            |  |
| EuLH                     | 1, 1, 1 | 24.8(1)            |  |
| pEu <sup>III b</sup>     |         | 21.1(1)            |  |
| $\overline{pEu}^{III c}$ |         | 21.3(2)            |  |

<sup>a</sup>The figures in parentheses give the uncertainties determined from the standard deviation between three independent titrations, pEu<sup>III</sup> values indicate the negative log value of the free Eu(III) concentration at pH 7.4, 1  $\mu$ M [Eu], and 10  $\mu$ M [1]. <sup>b</sup>Value calculated from protonation and complex formation constants. <sup>c</sup>Value determined by direct competition against DTPA at pH 7.4.

The conditional stability constant pEu<sup>III</sup> could then be calculated as a function of pH for a standard set of

conditions (Fig. S4, initial concentrations:  $[Eu] = 10^{-6}$  M,  $[L] = 10^{-5}$  M), to allow comparisons between **1** and other ligands of varying denticity and/or acidity. Both **1** (pEu<sup>III</sup><sub>7,4</sub>) = 21.1(1)) and **2** (pEu<sup>III</sup><sub>7,4</sub> = 21.2) exhibit similar affinities for Eu(III), not only at pH = 7.4, but over the pH range 2.0–10, despite the differences in ligand acidity and water coordination of the corresponding complexes. To validate the use of this spectrofluorimetric method, the affinity of **1** for Eu(III) was verified *via* direct competition against diethylenetriaminepentaacetic acid (DTPA) at pH = 7.4, following a previously described method (Fig. S5-S6, Table 2).<sup>5</sup>

While the linear spermine-based 1,2-HOPO ligand 1 and the tetrapodal ligand 2 both exhibit similar affinities for Eu(III), the photophysical properties of the resulting complexes differ significantly. In contrast to  $[Eu^{III}(2)]^0$ , the high quantum yield of the bright complex  $[Eu^{III}(1)]^-$  comes mainly from its high metal-centered luminescence efficiency and from the lack of an inner-sphere water molecule. The remarkable luminescence properties of  $[Eu^{III}(1)]^-$  were used to design a direct method of stability constant determination. This method will be applied to other ligand systems that sensitize lanthanide and actinide luminescence.

Acknowledgment. This work was supported by the National Institutes of Health (Grant AI074065-01), and the Director, Office of Science, Office of Basic Energy Sciences, and the Division of Chemical Sciences, Geosciences, and Biosciences of the U.S. Department of Energy at LBNL under Contract No. DE-AC02-05CH11231. The authors thank Dr. Jide Xu for providing the ligands and Prof. Gilles Muller (San Jose State University) for the use of a low temperature time-resolved luminescence spectrometer. This technology is licensed to Lumiphore, Inc. in which some of the authors have a financial interest.

**Supporting Information Available:** Detailed experimental procedures and spectral data. This material is available free of charge via the Internet at <u>http://pubs.acs.org</u>.

#### References

- (1) Durbin, P. W. Health Phys. 2008, 95, 465-492.
- (2) Scarrow, R. C.; Riley, P. E.; Abu-Dari, K.; White, D. L.; Raymond, K. N. *Inorg. Chem.* 1985, 24, 954-967.
- (3) Werner, E. J.; Datta, A.; Jocher, C. J.; Raymond, K. N. Angew. Chem. Int. Ed. 2008, 47, 8568-8580.
- (4) Moore, E. G.; Samuel, A. P. S.; Raymond, K. N. Acc. Chem. Res. 2009, 42, 542-552.
- (5) Moore, E. G.; Jocher, C. J.; Xu, J.; Werner, E. J.; Raymond, K. N. *Inorg. Chem.* 2007, *46*, 5468-5470.
- (6) Moore, E. G.; Xu, J.; Jocher, C. J.; Castro-Rodriguez, I.; Raymond, K. N. *Inorg. Chem.* 2008, *47*, 3105-3118.
- (7) D'Aléo, A.; Xu, J.; Moore, E. G.; Jocher, C. J.; Raymond, K. N. Inorg. Chem. 2008, 47, 6109-6111.
- (8) Supkowski, R. M.; Horrocks, W. D. Inorg. Chimi. Acta 2002, 340, 44-48.
- (9) Beeby, A.; Bushby, L. M.; Maffeo, D.; Williams, J. A. G. J. Chem. Soc. Dalton Trans. 2002, 48-54.
- (10) Werts, M. H. V.; Jukes, R. T. F.; Verhoeven, J. W. Phys. Chem. Chem. Phys. 2002, 2002, 1542-1548.
- (11) Kumar, K.; Tweedle, M. F.; Malley, M. F.; Gougoutas, J. Z. Inorg. Chem. 1995, 34, 6472-6480.
- (12) Wu, S. L.; Horrocks, W. D. J. Chem. Soc. Dalton Trans. 1997, 1497-1502.



The complex  $[Eu^{III}(3,4,3-LI(1,2-HOPO))]^{-1}$  luminesces with a long lifetime (805  $\mu$ s) and quantum yield of 7.0% in aqueous solution, at pH 7.4. These remarkable optical properties are used to determine the stability of the complex (log  $\beta_{110} = 20.2(2)$ ) and to define the influence of the ligand scaffold on the stability and photophysical properties. The described spectrofluorimetric method utilizes the antenna effect as a tool to probe the thermodynamic parameters of a lanthanide luminescence sensitizing ligand.

## Using the Antenna Effect as a Spectroscopic Tool; Photophysics and Solution Thermodynamics of the Model Luminescent Hydroxypyridonate Complex [Eu<sup>III</sup>(3,4,3-LI(1,2-HOPO))]<sup>-</sup>

Rebecca J. Abergel, Anthony D'Aléo, Clara Ng Pak Leung, David K. Shuh and Kenneth N. Raymond\*

Chemical Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, and Department of Chemistry, University of California, Berkeley, California 94720-1460

**Supporting Information** 



**Figure S1.** Steady-state emission spectra ( $\lambda_{exc} = 325 \text{ nm}$ ) of [Gd<sup>III</sup>(1)]<sup>-</sup> (5  $\mu$ M in 1:3 (v/v) MeOH:EtOH, 77 K).



**Figure S2.** Brightness of  $[Eu^{III}(1)]^{-}$  (solid) and  $[Eu^{III}(2)]^{0}$  (dash) in 0.1 M TRIS buffer (pH 7.4).



**Figure S3.** Spectrophotometric titration of  $[Eu^{III}(1)]^{-}$  by KOH in water. I = 0.1 (KCl), T = 25.0 °C, l = 1 cm.



**Figure S4.** Change of pEu with pH for ligands **1** (solid) and **2** (dash), between pH 2 and 10. Calculated for total concentrations:  $[Eu] = 10^{-6} \text{ M}, [L] = 10^{-5} \text{ M}.$ 



**Figure S5.** Spectrofluorimetric competition titration of  $[Eu^{III}(1)]^-$  against DTPA ( $[Eu^{3+}] = [1] = 0.005 \text{ mM}$ , [DTPA] from 0 to 5 mM, 0.1 M KCl, 0.1 M Hepes, pH = 7.4, 25.0 °C,  $\lambda_{exc} = 325 \text{ nm}$ ).



**Figure S6.** Spectrofluorimetric competition titration of  $[Eu^{III}(1)]^{-}$  against DTPA, The x intercepts indicate the difference in pEu between the ligand and the competing poly(amino-carboxylate).



**Figure S7.** Decay profile observed for  $[Eu^{III}(1)]^-$  at pH 7.4 (0.1 M TRIS buffer) in H<sub>2</sub>O (black) and D<sub>2</sub>O (blue). The raw data points were fit (red and light blue lines) to a monoexponential decay ( $I_t = A_0 + A_1 \times exp(-\tau/t)$ , to determine the associated lifetimes.



**Figure S8.** Quantum yield determination for  $[Eu^{III}(1)]^-$  at pH 7.4 (0.1 M TRIS buffer). The blue line is a linear fit to the data for the reference quinine sulphate, the pink line is a linear fit to the data for the sample.

#### **Experimental Procedures**

**General.** All chemicals were obtained from commercial suppliers and were used as received. The ligands 3,4,3-LI(1,2-HOPO) (1) and H(2,2)-1,2-HOPO (2) were prepared by Dr. Jide Xu, as described previously.<sup>1,2</sup>

Photophysical Characterization. UV-Visible absorption spectra were recorded on a Varian Cary 300 double beam absorption spectrometer, using quartz cells of 1.00 cm path length. Emission spectra were acquired on a HORIBA Jobin Yvon IBH FluoroLog-3 spectrofluorimeter, equipped with 3 slit double grating excitation & emission monochromators (2.1 nm/mm dispersion, 1200 grooves/mm). Spectra were reference corrected for both the excitation light source variation (lamp and grating) and the emission spectral response (detector and grating). Luminescence lifetimes were determined on a HORIBA Jobin Yvon IBH FluoroLog-3 spectrofluorimeter, adapted for time-correlated single photon counting (TCSPC) and multichannel scaling (MCS) measurements. A sub-microsecond Xenon flashlamp (Jobin Yvon, 5000XeF) was used as the lightsource, with an input pulse energy (100 nF discharge capacitance) of ca. 50 mJ, yielding an optical pulse duration of less than 300 ns at FWHM. Spectral selection was achieved by passage through a double grating excitation monochromator (2.1 nm/mm dispersion, 1200 grooves/mm). Emission was monitored perpendicular to the excitation pulse, again with spectral selection achieved by passage through a double grating excitation monochromator (2.1 nm/mm dispersion, 1200 grooves/mm). A thermoelectrically cooled single photon detection module (HORIBA Jobin Yvon IBH, TBX-04-D) incorporating fast rise time PMT, wide bandwidth pre- amplifier and picosecond constant fraction discriminator was used as the detector. Signals were acquired using an IBH DataStation Hub photon counting module and data analysis was performed using the commercially available DAS 6 decay analysis software package from HORIBA Jobin Yvon IBH. Goodness of fit was assessed by minimizing the reduced chi squared function,  $\chi^2$ , and a visual inspection of the weighted residuals. Each trace contained at least 10,000 points and the reported lifetime values result from at least three independent measurements (Figure S7).

Quantum Yield Determination. Quantum yields were determined by the optically dilute method using eq. S1, where A is the absorbance at the excitation wavelength, I is the intensity of the excitation light at the same wavelength, n is the refractive index and D is the integrated luminescence intensity. The subscripts 'x' and 'r' refer to the sample and reference respectively.

$$\frac{\Phi_x}{\Phi_r} = \frac{A_r(\lambda_r)}{A_x(\lambda_x)} \frac{I(\lambda_r)}{I(\lambda_x)} \frac{n_x^2}{n_r^2} \frac{D_x}{D_r}$$
(S1)

For quantum yield calculations, an excitation wavelength of 325 nm was utilized for both the reference and sample, hence the  $I(\lambda r)/I(\lambda x)$  term is removed. Similarly, the refractive indices term,  $n_x^2/n_r^2$ , was taken to be

identical for the aqueous reference and sample solutions. Hence, a plot of integrated emission intensity (i.e.  $D_r$ ) versus absorbance at 325 nm (i.e.  $A_r(\lambda_r)$ ) yields a linear plot with a slope which can be equated to the reference quantum yield  $\Phi r$ . Quinine sulfate in 0.5 M (1.0 N) sulfuric acid was used as the reference ( $\Phi_r = 0.546$ ). By analogy, for the sample, a plot of integrated emission intensity (i.e.  $D_x$ ) versus absorbance at 330 nm (i.e.  $A_x(\lambda_x)$ ) yields a linear plot and  $\Phi x$  can then be evaluated. The value reported in the manuscript is the average of three independent measurements (Figure **S8**).

**Kinetic Parameters Determination.** The efficiency of the sensitization was estimated using eq. **S2** that defines the overall quantum yield of luminescence ( $\phi_{Eu}$ ) as the product of the efficiency of the intersystem crossing ( $\eta_{ISC}$ ), the efficiency of the energy transfer ( $\eta_{ET}$ ) and the efficiency of metal centered luminescence ( $\eta_{Eu}$ ):

$$\phi_{\mathsf{Eu}} = \eta_{\mathsf{ISC}} \,\eta_{\mathsf{ET}} \,\eta_{\mathsf{Eu}} = \eta_{\mathsf{sens}} \,\eta_{\mathsf{Eu}} \tag{S2}$$

The overall quantum yield of luminescence,  $\phi_{Eu}$ , is determined experimentally while  $\eta_{Eu}$  is determined using eq. **S3**, where  $\tau_{Eu}$  is the measured Eu lifetime and  $\tau_{R}$  is the pure radiative lifetime that can be estimated from the emission spectra (eq. **S4**):

$$\eta_{\rm Eu} = \tau_{\rm Eu}/\tau_{\rm R} \tag{S3}$$

$$k_{\rm B} = 1/\tau_{\rm B} = A(0,1)[I_{\rm tot}/I(0,1)]$$
 (S4)

The constant A(0,1) is the spontaneous emission probability of the  ${}^{5}D_{0} \rightarrow {}^{7}F_{1}$  transition (32.3 s<sup>-1</sup> in water) and  $I_{tot}/I(0,1)$  is the ratio of the total integrated emission intensity to the intensity of the  ${}^{5}D_{0} \rightarrow {}^{7}F_{1}$ transition. The result of the  $k_{R}$  can be correlated to the variation of the sum of the non-radiative decay constant (eq. **S5**):

$$\Sigma k_{\rm nr} = \left[ (1/\tau_{\rm Eu}) - k_{\rm R} \right] \tag{S5}$$

**Solution Thermodynamics.** Ligands protonation and complex formation constants were determined using procedures and equipment following previous descriptions.<sup>3-5</sup>

**Titration solutions and equipment.** Corning high performance combination glass electrodes (response to [H<sup>+</sup>] was calibrated before each titration)<sup>6</sup> were used together with either an Accumet pH meter or a Metrohm Titrino to measure the pH of the experimental solutions. Metrohm autoburets (Dosimat or Titrino) were used for incremental addition of acid or base standard solutions to the titration cell. The titration instruments were fully automated and controlled using LabView software.<sup>7</sup> Titrations were performed in 0.1 M KCl supporting electrolyte under positive Ar gas pressure. The temperature of the experimental solution was maintained at 25 °C by an external circulating water bath. UV-Visible spectra for spectrophotometric titrations were recorded on a Hewlett-Packard 8452a spectrophotometer (diode array). Solid reagents

were weighed on a Metrohm analytical balance accurate to 0.05 mg. All titrant solutions were prepared using distilled water that was further purified by passing through a Millipore Milli-Q reverse osmosis cartridge system. Titrants were degassed by boiling for 1 h while being purged under Ar. Carbonate-free 0.1 M KOH was prepared from Baker Dilut-It concentrate and was standardized by titrating against potassium hydrogen phthalate using phenolphthalein as an indicator. Solutions of 0.1 M HCl were similarly prepared and were standardized by titrating against sodium tetraborate to Methyl Red endpoint. Stock solutions of DTPA were obtained by dissolving DTPA (Fischer) in Milli-Q water. Stock solutions of europium ion were obtained by dissolving solid EuCl<sub>3</sub> in standardized 1 M HCl.

**Protonation Constants: Incremental Potentiometric Titrations.** The protonation constants of 3,4,3-LI(1,2-HOPO) were determined by potentiometric titration. Solutions were assembled from a weighed portion of ligand and the supporting electrolyte solution, with resulting ligand concentrations between 0.2 and 0.5 mM, and were incrementally perturbed by the addition of either acid (HCl) or base (KOH) titrant, followed by a time delay for equilibration (180 seconds). All titrations were conducted in pairs: first a forward titration from low to high pH, then a reverse titration back to low pH. An average of 60 - 90 data points were collected in each pair of titrations (forward and back), each data point consisting of a volume increment and a pH reading over the pH range 2.5 to 11. Refinement of the protonation constants was accomplished using the program Hyperquad,<sup>8</sup> which allows simultaneous nonlinear least squares refinement of the data from multiple titration curves.

**Formation Constants: Incremental Spectrophotometric Titrations.** Solutions were assembled from a weighed portion of ligand, a measured aliquot of the europium stock solution and the supporting electrolyte solution, with resulting ligand and europium concentrations of ~ 50  $\mu$ M, and were incrementally perturbed by the addition of either acid (HCl) or base (KOH) titrant, followed by a time delay for equilibration (600 seconds). Buffering of the solution was assured by the addition of Hepes and Mes buffers (500  $\mu$ M). An average of 30 – 40 data points were collected in each pair of ligand titrations (forward and back), each data point consisting of a pH measurement and an absorbance spectra over the pH range 2.5 to 10.5. Tentative nonlinear least squares refinement of the complex formation constants was performed using the program pHab.<sup>9</sup> However, few changes occurred in the absorption spectra over this range of pH, preventing accurate refinement and constant determination.

**Formation Constants: Batch Spectrofluorimetric Titrations.** Solutions were assembled from a weighed portion of ligand, a measured aliquot of the europium stock solution and the supporting electrolyte solution, with resulting ligand and europium concentrations of ~ 10  $\mu$ M. Buffering of the solution was assured by the addition of Hepes and Mes buffers (200  $\mu$ M). The pH was adjusted manually (over a range of 2.2 to 9.5), and 3 ml aliquots were sampled at each pH (10-15 data points per titration) and equilibrated in a thermostatic shaker at 25 °C until equilibrium was reached and measurements were stable (24 h). Each data point consisted of a pH measurement and an emission spectrum between 570 and 720 nm. The data was corrected for dilution, imported into the refinement program pHab<sup>9</sup> and analyzed by non-linear least-squares refinement.

**Data Treatment.** All equilibrium constants were defined as cumulative formation constants,  $\beta_{mlh}$  according to eq. **S6**, where the ligand is designated as L. Stepwise protonation constants,  $K_a^n$ , may be derived from these cumulative constants according to eq. **S7** (describes proton association constants).

$$m\operatorname{Eu} + l\operatorname{L} + h\operatorname{H} \Leftrightarrow \left[\operatorname{Eu}_{m}\operatorname{L}_{l}\operatorname{H}_{h}\right]; \ \beta_{mlh} = \frac{\left[\operatorname{Eu}_{m}\operatorname{L}_{l}\operatorname{H}_{h}\right]}{\left[\operatorname{Eu}\right]^{m}\left[\operatorname{L}\right]^{l}\left[\operatorname{H}\right]^{h}}$$
(S6)

$$\mathbf{K}_{a}^{n} = \frac{\left[\mathbf{L}\mathbf{H}_{n}\right]}{\left[\mathbf{H}\right]\left[\mathbf{L}\mathbf{H}_{n-1}\right]} = \frac{\beta_{01n}}{\beta_{01(n-1)}}$$
(S7)

Each pair of titrations (i.e., forward titration against KOH and reverse titration against HCl) was combined for simultaneous refinement. For potentiometric titrations, both the proton and ligand concentrations were refined, only the proton concentration was allowed to vary in the spectrophotmetric and spectrofluorimetric studies, and all other concentrations were held at estimated values determined from the volume of standardized stock or the weight of ligand (measured to 0.01 mg). Refined concentrations were within 5% of the analytical values. The refinements of the overall formation constants  $\beta_{110}$  and  $\beta_{111}$  included in each case the four protonation constants derived from potentiometric titrations and the metal hydrolysis products, whose equilibrium constants were fixed to the literature values<sup>10</sup> (log  $\beta_{10.4} = -7.4$ , log  $\beta_{10.3} = -19.3$ , log  $\beta_{10.4} = -26.2$ , log  $\beta_{20.3} = -15.4$ ) and which do not emit significantly; all species formed with both europium and the ligand were considered to have significant emission to be observed in the emission spectra. The pM(Eu<sup>III</sup>) values at pH varying from 2 to 10 were calculate using the modeling program Hyss.<sup>11,12</sup>

**Spectrofluorimetric Competition Batch Titrations with DTPA.** Varying volumes of a DTPA stock solution were added to solutions of ligand (0.03 mM) and europium (0.03 mM) in 0.1 M KCl buffered at pH 7.4 with 0.1 M HEPES. All solutions were diluted to identical volumes to reach final concentrations of ligand and europium of 0.005 mM. A molar ratio of 1:1 for Eu(III)/ligand, and molar ratios of 1:0 up to 1:1000 for ligand/DTPA were used. All samples were equilibrated in a thermostatic shaker at 25 °C until equilibrium was reached and measurements were stable (7 d). The emission spectrum of each solution was measured using a 1-cm quartz cell. The pM(Eu<sup>III</sup>) value at pH 7.4 for 3,4,3-LI(1,2-HOPO) was determined from three independent titrations as described previously.<sup>1</sup>

### Supporting Information References.

- (1) Moore, E. G.; Jocher, C. J.; Xu, J.; Werner, E. J.; Raymond, K. N. Inorg. Chem. 2007, 46, 5468-5470.
- (2) White, D. L.; Durbin, P. W.; Jeung, N.; Raymond, K. N. J Med Chem 1988, 31, 11-18.
- (3) Cohen, S. M.; O'Sullivan, B.; Raymond, K. N. Inorganic Chemistry 2000, 39, 4339-4346.
- (4) Jurchen, K. M. C.; Raymond, K. N. submitted to Inorganic Chemistry 2005.
- (5) Xu, J.; O'Sullivan, B.; Raymond, K. N. Inorganic Chemistry 2002, 41, 6731-6742.
- (6) Gans, P.; O'Sullivan, B. Talanta 2000, 51, 33-37.
- (7) Xu, J.; Franklin, S. J.; Whisenhunt, D. W.; Raymond, K. N. J Am Chem Soc 1995, 117, 7245-7246.

- (8) Gans, P.; Sabatini, A.; Vacca, A. *HYPERQUAD2000*; Leeds, U. K. and Florence, Italy.
- (9) Gans, P.; Sabatini, A.; Vacca, A. Ann. Chim. (Rome) **1999**, *89*, 45-49.
- (10) Perrin, D. D.; Dempsey, B.; Hall, C. a., Ed. London, 1974.

(11) Alderighi, L.; Gans, P.; Ienco, A.; Peters, D.; Sabatini, A.; Vacca, A. *Coordination Chemistry Reviews* **1999**, *184*, 311-318.

(12) Alderighi, L.; Gans, P.; Ienco, A.; Peters, D.; Sabatini, A.; Vacca, A. *HYSS* Leeds, U.K. and Florence, Italy, 1999.