Supporting Information
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An Efficient Design for the Rigid Assembly of Four Bidentate Chromophores in Water Stable Highly Luminescent Lanthanide Complexes

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Experimental Details

General Information – $^1$H and $^{13}$C NMR spectra were recorded on a Varian Mercury 400 spectrometer. Chemical shifts are reported in ppm with solvent or 3-(trimethylsilyl)-1-propane-sulfonic acid, sodium salt as internal reference. Elemental analyses were performed by the Service Central d'Analyses (Vernaison, France).

Solvents and starting materials were obtained from Aldrich, Fluka, Acros and Alfa and used without further purification. 6-chloromethylpyridine-2-carboxylic acid ethyl ester was obtained from the commercially available 2,6-dipicolinic acid according to a published procedure. \cite{1}

Synthesis of the ligand H$_4$tpaen

N,N,N’N’-tetrakis[(6-carboxypyridin-2-yl)methyl]-ethylenediamine (H$_4$tpaen).

To a solution of 6-chloromethylpyridine-2-carboxylic acid ethyl ester (2.95 g, 14.8 mmol) in anhydrous acetonitrile (50 mL), freshly distilled ethylenediamine (250 $\mu$L, 3.6 mmol) and anhydrous K$_2$CO$_3$ (2.04 g, 14.8 mmol) were successively added under argon atmosphere. The reaction mixture was refluxed for 20 hours. After filtration and evaporation of the solvent a yellow oil was obtained which was dissolved in dichloromethane. The resulting solution was washed twice with water (100 mL) and then dried over anhydrous Na$_2$SO$_4$. After evaporation of the solvent the resulting yellow oil was used without further purification. The crude product (2.56g) was refluxed overnight in a 6M HCl aqueous solution (40 mL). After evaporation of the solvent to ~5 mL the solution was stored at 5°C overnight, whereupon a solid had precipitated which was collected by filtration, washed with 6 M HCl and dried under vacuum to yield H$_4$tpaen.6HCl.5H$_2$O (1.37g, 42%).

H$_6$tpaen. $^1$H NMR (D$_2$O, 400 MHz, 298 K, pH = 5): $\delta$ 3.57 (s, 4H, NCH$_2$CH$_2$N), 4.36 (s, 8H, NCH$_2$py), 7.48 (d, 4H, CH), 7.77 (d, 4H, CH), 7.84 (t, 4H, CH).

$^{13}$C NMR (D$_2$O, 100 MHz): $\delta = 51.6$ (CH$_2$); 56.4 (CH$_2$); 57.8 (CH$_2$); 125.8 (CHpy); 128.4 (CHpy); 142.9 (CHpy); 147.4 (Cpy); 152.0 (Cpy); 166.4 (COOH); 172.1 (COOH).

**Potentiometric Titrations.** Ligand protonation constants and metal ion stability constants with H$_6$tpaen were determined by potentiometric titrations. Eu(III) solutions were prepared by dissolving the appropriate amounts of EuCl$_3$.6H$_2$O (Aldrich) in water. The deprotonation constants of [H$_6$tpaen]$^{2+}$ defined as $K_{a_i} = [H_6-L]/[H_5-L]^{1+i}[H]^+$ were determined to be $pK_{a_1} = 2.8(1)$, $pK_{a_2} = 3.2(1)$, $pK_{a_3} = 3.9(2)$, $pK_{a_4} = 5.1(1)$, and $pK_{a_5} = 7.8(1)$. The exact Eu$^{3+}$ ion concentration was determined by colorimetric titration in acetate buffer (pH=4.5) using standardized H$_2$Na$_2$edta solution (Aldrich) and xylenol orange as the indicator. Ca(II) solutions were prepared by dissolving CaCl$_2$.2H$_2$O (Aldrich) in water. The exact Ca$^{2+}$ ion concentration was determined by colorimetric titration at pH ~ 12.5 using standardized H$_2$Na$_2$edta solution (Aldrich) and calgonite as the indicator. 20 mL solution of H$_4$tpaen alone (3.10$^{-4}$ M), acidified (pH~2.5) 1:1 Ln:ligand mixtures ([L] 3.10$^{-4}$ M), acidified (pH~2.5) 1:1 Ca:ligand mixtures ([L] 7.10$^{-5}$M) were titrated in a thermostated cell (25.0°C +/- 0.1°C) under a stream of argon with a 0.1 M KOH solution added by means of a 5 mL piston burette (Metrohm). The ionic strength was fixed with KCl ($\mu$=0.1 M). Titrations were carried out with a Metrohm 751 GPD Titrino potentiometer equipped with a combined pH glass electrode (Metrohm). Calibration of the electrode system was performed prior to each measurement. The electromotrive force is given by $E=E^o+xp[H^+]$ and both $E^o$ and $s$ were determined by titrating a known amount of HCl by 0.1 M KOH at $\mu=0.1$ M (KCl), using the acid range of the titration. The value used for the ion product of water is $pK_w = 13.77$. More than 50 data points were collected for each experiment. The data were mathematically treated by the software HYPERQUAD2000. All values and errors represent the average of at least three independent experiments.
Spectroscopic Measurements

Absorption spectra were recorded on a Cary 50 Probe UV-visible spectrometer with Perkin Elmer Luminescence Cells with a pathlength of 1cm. Luminescence lifetime measurements were recorded on a Perkin-Elmer LS-50B spectrometer at 293 K. The phosphorescence lifetime ($\tau_L$) was measured by recording the decay at the maximum of the emission spectra. The instrument settings were as follows: a gate time of 10 ms, a cycle time of 200 ms, a flash count of 5, an integration time of 1s, with excitation and emission slit widths of 2.5 nm, and a varied delay time. Phosphorescence excitation and emission spectra were recorded on the same instrument with a delay of 0 seconds, a gate time of 20 ms, a cycle time of 200 ms and a flash count of 5. For quantum yield measurements, $10^{-6}$ M solutions of [Tb(tpaen)]$^+$, [Eu(tpaen)]$^+$, [Tb(dpa)$_3$]$^{3-}$, [Eu(dpa)$_3$]$^{3-}$ were prepared in situ by mixing appropriate volumes of Ln(III) in mQ water (concentration having been determined by titration with EDTA in acetate buffer, with xylenol orange indicator) and tpaen (in 0.1 M tris buffer, pH =7.4) or dpa (in 0.1 M tris buffer, pH = 7.4). Quantum yields $Q$ have been calculated using the equation

$$Q/Q_r = A_x(\tilde{\nu}).n_x^2.D_x / A_r(\tilde{\nu}).n_r^2.D_r,$$

where $x$ refers to the sample, and $r$, to the reference; $A$ to the absorbance, $\tilde{\nu}$, to the excitation wavenumber used, $n$, to the refractive index, and $D$, to the integrated emitted intensity.
Figure S1. Titration curves (pH versus \( a = \text{mol of OH} / \text{mol of tpaen} \)) for: a) \( \text{H}_4\text{tpaen} \) (0.38 mM) b) \( \text{H}_4\text{tpaen} \) and \( \text{Eu}^{3+} \) (0.3 mM) c) \( \text{H}_4\text{tpaen} \) and \( \text{Ca}^{2+} \) (0.07 mM).
Figure S2. Molecular structure of \{[\text{Ce(tpaen)}]\text{K(H}_2\text{O)}_3]\_216\text{H}_2\text{O} (2) 30\% probability level)
Figure S3. $^1$H NMR at 333 K of [Ce(tpaen)]$^{+}$ in D$_2$O at pD=7.7. (# denotes an Hydrolite impurity and * denotes DSS added as an external reference).