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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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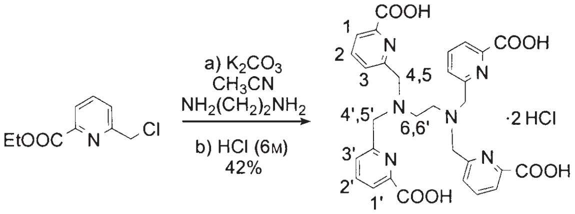
The unique electronic properties of lanthanide ions (long-lived luminescence and sharp emission spectra) make them particularly suitable for the development of diagnostic tools in medical analysis.[1,2] Lanthanide complexes are increasingly used for sensor development[3] and as luminescent probes in time-resolved high-throughput assays and fluorescence imaging because of their ability to discriminate between background fluorescence and the target signal.[4–8] Numerous studies have been directed to design efficient luminescence probes containing the long-lived visible emitters Eu^{III} and Tb^{III}[9–14] and more recently near-infrared (NIR) emitters (Pr^{III}, Er^{III}, Yb^{III}, Nd^{III}).[15–18]

As the Laporte-forbidden 4f–4f transition prevents direct excitation of lanthanide luminescence, lanthanide ions require sensitization by suitable organic chromophores. Furthermore, for practical applications under physiological conditions, the lanthanide ion should be incorporated into highly stable complexes. The efficiency of ligand-to-lanthanide energy transfer, which requires compatibility between the energy levels of the ligand excited states and the accepting levels of lanthanide ions, is crucial in the design of high-performance probes.[19] Moreover, high quantum yield cannot be obtained without the prevention of nonradiative deactivation of the lanthanide excited states by O–H oscillators of coordinated or closely diffusing water molecules. The incorporation of suitable chromophores in carefully designed polydentate ligands leads to increased stability of the lanthanide chelate in solution and allows for the metal center to be well protected from water molecules. However, the tendency of lanthanide ions to adopt high coordination numbers and their lack of stereochemical preferences make the design of such ligands very challenging.[20] A successful strategy adopted by several investigators relies on tripodal architectures for the organization of three tridentate binding units in nine-coordinate Ln^{III} complexes.[21–25] The design of polydentate ligands that allow the arrangement of four bidentate moieties around a lanthanide ion has received less attention in spite of the excellent luminescent properties observed for tetrakis complexes of bidentate chomophores, such as quinolinates[26] or tropolonates.[27] Recently, octadentate ligands incorporating four bidentate chromophores have been shown to yield lanthanide complexes with very efficient emissions in the visible or NIR regions.[15,28] The structures of these complexes have not been elucidated, but the highly flexible structure of the backbone that connects the bidentate units suggests nonoptimal protection of the metal center in such systems.

Herein, we describe a new and particularly efficient way of assembling four picolinate chromophores around a lanthanide center with a multidentate ligand that yields highly luminescent and water-stable lanthanide complexes. The decadentate ligand *N*,*N*,*N*′*N*′-tetrakis[(6-carboxypyridin-2-yl)methyl]ethylenediamine (H_4tpaen) is readily obtained in five steps from commercially available pyridine-2,6-dicarboxylic acid and ethylenediamine in a yield of 26% (Scheme 1).



Scheme 1. Synthesis of the ligand $[H_6tpaen]^{2+}$, and its numbering scheme for NMR spectral assignment.

The water-soluble complexes of tpaen were isolated in 50–60% yield by treating the ligand with $LnCl_3\cdot6H_2O$ (Ln = Ce, Eu) and adjusting the pH value to approximately 6. Five deprotonation constants ($pK_{a,1} = 2.8(1)$, $pK_{a,2} = 3.2(1)$, $pK_{a,3} = 3.9(2)$, $pK_{a,4} = 5.1(1)$, and $pK_{a,5} = 7.8(1)$) were determined for the $[H_6tpaen]^{2+}$ ions by potentiometric titration as well as the stability constants of the resulting Eu^{III} and Ca^{II} complexes ($\log\beta_{EuL} = 15.3(3)$) and Ca^{II} ($\log\beta_{CaL} = 8.5\ (5)$; see the Supporting Information). The values of pEu = 15.7, pGd = 15.0, and pCa = 8.5 ($-\log[M]_{free}$ at pH 7.4, $[M]_{total} = 1\ \mu M$, and $[tpaa]_{total} = 10\ \mu M$) relative to the value of pEu = 14.0 for ethylenediaminetetraacetate (edta) indicate that the tpaen ligand forms lanthanide complexes of sufficient stability for potential biomedical applications and shows a good selectivity for europium with respect to calcium.

The crystal structure of the complexes [{Eu(tpaen)}K-$(H_2O)_3$]$\cdot4H_2O$ (**1**; Figure 1) and [{Ce(tpaen)}K-

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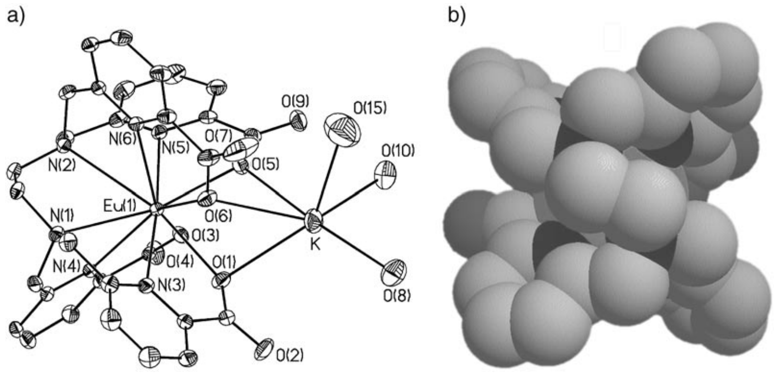

Figure 1. a) Molecular structure of $[\{Eu(tpaen)\}K(H_2O)_3]\cdot 4H_2O$ (**1**) (30% probability level). b) CPK (or space-filling) model of $[Eu(tpaen)]^+$ in **1**.

$(H_2O)_3]_2\cdot 16H_2O$ (**2**; see the Supporting Information) were analyzed by X-ray diffraction. In both complexes, the Ln^{III} ion is ten-coordinate with the four oxygen atoms (mean values of the M–O distances: 2.42(1) Å for Eu and 2.50(4) Å for Ce) and the six nitrogen atoms (mean values of the $M-N_{pyridine}$ distances: 2.65(4) Å for Eu and 2.72(1) Å for Ce, and mean values of the $M-N_{amine}$ distances: 2.91(1) Å for Eu and 2.91(4) Å for Ce) of the ligand. The bridging mode of the six-coordinate K^+ counterion differs in the two structures, thus leading to a monomeric structure for the Eu complex and to a dimeric structure for the Ce complex. The picolinate arms of the tpaen ligand wrap around the metal ion in a pseudo-C_2-symmetric helical arrangement that results in a chiral complex. Both complexes crystallize as a racemic mixture of Λ and Δ enantiomers in a centrosymmetric space group.

The ^1H NMR spectra of the Eu (Figure 2) and Ce complexes of tpaen in a solution of D_2O at room temperature and at pH 7 is consistent with the presence of rigid C_2-symmetric species in solution, in which the four chelating arms remain coordinated to the metal center on the NMR timescale. The observed symmetry is in agreement with a chiral helical solution structure, as found in the solid state. The complexes maintain their rigid structure in the temperature range 298–363 K. The presence of similar rigid C_2-symmetric species was also observed in the range 298–363 K for solutions of the tpaen complexes of La and Tb in D_2O prepared in situ. The high rigidity of these complexes in solution, rarely observed for lanthanide complexes with high denticity,[29] indicated that the arrangement of the ten donor atoms provided by the simple ethylenediamine backbone is very well adapted to lanthanide complexation, thus providing highly effective protection of the metal center from interaction with solvent molecules.

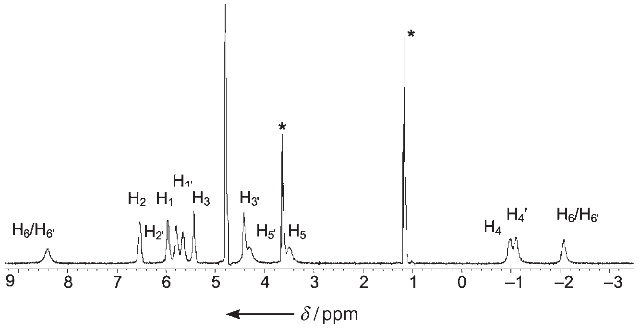

Figure 2. ^1H NMR spectrum at 298 K of [Eu(tpaen)]K in D_2O at pD = 6.9 (* denotes EtOH).

As a result, the Eu^{III} and Tb^{III} complexes of tpaen showed a bright and long-lived luminescence in solutions of both H_2O and D_2O. The observed lifetimes of the $Eu(^5D_0)$ and $Tb(^5D_4)$ levels for the $[Tb(tpaen)]^-$ and $[Eu(tpaen)]^-$ ions are in agreement with the presence of 0.04 ± 0.2 and 0.03 ± 0.2 coordinated water molecules in the Eu and Tb complexes, respectively. The luminescent properties of the visible-light emitting lanthanide ions Eu and Tb are efficiently sensitized by the ligand tpaen (Figure 3). An efficient ligand-to-metal energy transfer is suggested by close matching of the excitation and absorption spectra of the Eu and Tb chelates (Figure 4). The value of the quantum yield of the $[Tb(tpaen)]^-$ complex ($\Phi = 45\%$) measured relative to $[Tb(dpa)_3]^{3-}$ in aerated 0.1 M tris buffer with an experimental error of 15% is one the highest reported to date.[28,30–32] The tpaen chromophore also efficiently sensitize the Eu ion to lead to a quantum yield value $\Phi = 7\%$, which is lower than that of the Tb^{III} complex but still

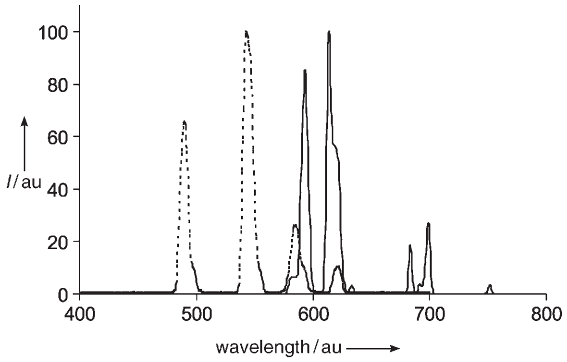

Figure 3. Normalized emission spectrum of $[Tb(tpaen)]^-$ (-----) and $[Eu(tpaen)]^-$ (——) upon ligand excitation at 274 nm.

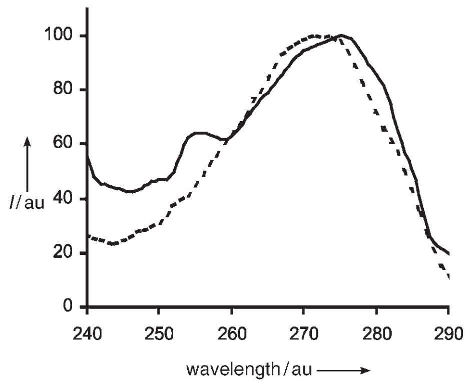

Figure 4. Absorption (•••••) and excitation spectrum (——) of $[Tb(tpaen)]^-$ in tris Buffer.

significantly larger than the quantum yield of lanthanide-based commercial luminescent probes.[33,34] The very intense luminescence of these ions is a result of efficient (Table 1) ligand-to-metal energy transfer and effective shielding of the metal ion from radiationless deactivation. The long luminescence lifetime observed for the terbium complex in H_2O rules out the presence of a de-excitation pathway that involves back transfer from the excited state of the metal center to the ligand. This leads to a high luminescence quantum yield. The synthesis of the ligand tpaen is simple, can be performed on large scales, and can be readily modified to anchor the complex with functions capable of binding biomolecules so that highly luminescent labels for time-resolved luminescence imaging can be developed.[32]

Table 1: Lifetime and absolute quantum yields in tris buffer (pH 7.4; 298 K) relative to $[Eu(dpa)_3]^{3-}$ and $[Tb(dpa)_3]^{3-}$ ions.

Compound	λ_{exc} [nm]	ε [$M^{-1}cm^{-1}$]	τ_{H_2O} [ms]	τ_{D_2O} [ms]	Φ_{H_2O}
tpaen	270	15 800			
[Eu(tpaen)]	274	21 600	1.70(2)	3.30(1)	0.07
[Tb(tpaen)]	274	21 600	3.0(1)	3.75(1)	0.45

The straightforward approach used to organize four bidentate chromophores in the decadentate ligand tpaen has yielded lanthanide chelates that are highly soluble and stable at physiological pH values. Moreover, the ligand architecture leads to a rigid structure in solution, in which the metal center is effectively protected from interaction with the solvent molecules. This approach opens a wide variety of perspectives on the development of stable visible-light or NIR emitting luminescent probes for biomedical applications from suitable chromophores; investigations towards extending this approach to other chromophores are currently in progress.

Experimental Section

A detailed description of the synthesis of the ligand *N,N,N′N′*-tetrakis[(6-carboxypyridin-2-yl)methyl]ethylenediamine (H_4tpaen) and of the cerium and europium complexes is given in the Supporting Information. The complexes were prepared as follows: A solution of $CeCl_3\cdot7H_2O$ or $EuCl_3\cdot6H_2O$ (0.138 mmol) in water (0.5 mL) was added to a solution of H_4tpaen (0.138 mmol) in water (6 mL) at pH 5, adjusted by the addition of KOH (0.2 M). The resulting solution was then stirred at room temperature for 2 h and its pH value was readjusted to 6 by the addition of further KOH (0.2 M). The water was then evaporated and the resulting solid was suspended in EtOH (5 mL). The insoluble salts were removed by filtration and the filtrate was evaporated. Slow evaporation of a solution of the resulting solid in water (1 mL) yielded the tpaen complexes as yellow ([Ce(tpaen)]K) or white ([Eu(tpaen)]K) crystals after 5 days in 50–60% yield. Elemental analysis (%) calcd for $[Ce(tpaen)]K\cdot4.5H_2O$ ($C_{30}H_{33}N_6O_{12.5}CeK$): C 42.05, H 3.88, N 9.81; found: C 41.85, H 3.67, N 9.77; elemental analysis (%) calcd for$[Eu(tpaen)]K\cdot6H_2O$ ($C_{30}H_{34}N_6O_{13}CeK$): C 40.22, H 4.05, N 9.38; found: C 40.03, H 4.03, N 9.19.

[Ce(tpaen)]: ^{1}H NMR (D_2O, 400 MHz, 298 K, pD = 5.4): $\delta = -2.03$ (s br, 2H, H6/H6′), 0.09 (s br, 2H, H5′), 1.18 (s br, 2H, H6/H6′), 3.25 (d, 2H, H4′), 3.51 (d, 2H, H4), 5.44 (s br, 2H, H5), 7.97 (d, 2H, H3), 8.10 (d, 2H, H3′), 8.45 (d, 2H, H1), 8.91 (t, 2H, H2), 8.94 (d, 2H, H1′), 9.04 ppm (t, 2H, H2′).

[Eu(tpaen)]: ^{1}H NMR (D_2O, 400 MHz, 298 K, pD = 6.9): $\delta = -2.07$ (s br, 2H, H6/H6′), −1.11 (s br, 2H, H4′), −0.99 (s br, 2H, H4), 3.48 (s br, 2H, H5), 4.28 (s br, 2H, H5′), 4.41 (d br, 2H, H3′), 5.43 (d br, 2H, H3), 5.65 (s br, 2H, H1′), 5.79 (s br, 2H, H1), 5.96 (br, 2H, H2′), 6.53 (br, 2H, H2), 8.40 ppm (br, 2H, H6/H6′).

Crystal data for $[\{Eu(tpaen)\}K(H_2O)_3]\cdot4H_2O$ (**1**): $C_{30}H_{38}EuKN_6O_{15}$, $M_r = 913.7$, monoclinic, space group $P2(1)/n$, $a = 11.995(2)$, $b = 14.539(3)$, $c = 21.407(5)$ Å, $\beta = 106.186(3)$, $V = 3585.2(12)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.693\ g\,cm^{-3}$, $\mu = 1.944\ mm^{-1}$, $T = 298$ K. Of the 12504 reflections collected, 5133 were unique ($R_{int} = 0.0192$). Refinement on all data converged at $R_1 = 0.0296$, $wR_2 = 0.0667$. Max/min residual density 0.693 and −0.528 e Å^{-3}.

Crystal data for $[\{Ce(tpaen)\}K(H_2O)_3\}_2]\cdot16H_2O$ (**2**): $C_{30}H_{46}CeKN_6O_{19}$, $M_r = 973.95$, monoclinic, space group $P2(1)/c$, $a = 11.7615(10)$, $b = 14.5931(12)$, $c = 22.965(2)$ Å, $\beta = 101.640(1)$, $V = 3860.7(6)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.676\ g\,cm^{-3}$, $\mu = 1.374\ mm^{-1}$, $T = 193$ K. Of the 12929 reflections collected, 7459 were unique ($R_{int} = 0.0310$). Refinement on all data converged at $R_1 = 0.0430$, $wR_2 = 0.1340$. Max/min residual density 1.055 and −2.462 e Å^{-3}.

Data were collected using a Bruker SMART CCD area detector three-circle diffractometer $2\theta_{max} = 59.38°$ (ω scans, $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, graphite monochromator). CCDC-275838 (**1**) and -275839 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

The number of coordinated water molecules present in solution q were determined from lifetime measurements by using the equation of Parker and co-workers ($q = A_{Ln}(1/\tau H_2O - 1/\tau D_2O - \alpha_{Ln})$ with $A_{Tb} = 5$ ms, $A_{Eu} = 1.2$ ms, $\alpha_{Tb} = 0.06\ ms^{-1}$, and $\alpha_{Eu} = 0.25\ ms^{-1}$).[35] Quantum yields Q have been calculated by using the equation $Q_x/Q_r = A_r(\bar{\upsilon})n_x^2D_x/A_x(\bar{\upsilon})n_r^2D_r$, in which x refers to the sample, r to the reference, A to the absorbance, $\bar{\upsilon}$ to the excitation wavenumber used, n to the refractive index, and D to the integrated emitted intensity. The tris(dipicolinate) complexes $[Eu(dpa)_3]^{3-}$ (dpa = pyridine-2,6-dicarboxylate; $\Phi = 13.5\%$, 7.5×10^{-5} M in tris buffer 0.1 M) and $[Tb(dpa)_3]^{3-}$ ($\Phi = 26.5\%$, 6.5×10^{-5} M in tris buffer 0.1 M) were used as references for the determination of the quantum yields of Eu- and Tb-containing samples, respectively.[36] The consistency of data was checked by measuring the quantum yield of the tris(dipicolinate) complexes against rhodamine 101 ($Q_{abs} = 100\%$ in ethanol) and cresyl violet ($Q_{abs} = 54\%$ in methanol).[37]

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