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A Water-Stable and Strongly Luminescent Terbium(III) Macrocyclic Complex Incorporating an Intracyclic Pyrazolylpyridine Chromophore

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A 15-membered macrocyclic ligand $\mathbf{L^1}H_3$ was designed with the aim of sensitizing the visible luminescence of the Tb^{III} ion in water: this octadentate chelating ligand is based on an intracyclic *N*,*C*-pyrazolylpyridine chromophore and a diethylenetriaminetriacetic acid core. This ligand reacts with equimolar amounts of $LnCl_3$ salts to yield water-soluble and stable complexes of the general formula $[\mathbf{L^1}\cdot Ln(H_2O)]^0$. Detailed photophysical studies were performed in aerated water on the free ligand and its Gd^{III} complex and complexes formed with Ln^{III} ions commonly used in fluoroimmunoassays (Ln = Sm, Eu, Tb, and Dy). The results show a wide range in the emission properties of the complexes, which can be rationalized in terms of the chromophore triplet-state energy. In particular, the Tb^{III} complex has a remarkable overall quantum yield of ca. 50 % in H_2O (80 % in D_2O) and an energy-transfer efficiency from sensitizer to the terbium ion of ca. 85 %. According to these results and in view of its kinetic inertness in aqueous solutions, the $\mathbf{L^1}\cdot Tb$ complex may be considered as a very promising candidate as long-lived luminescent label for bioaffinity assays.

Introduction

The design of ligands to form stable and highly luminescent Eu^{III} and Tb^{III} materials in aqueous solutions is of great interest owing to their potential applications as luminescent labels in the biochemical, biological, and biomedical sciences. The use of these luminescent labels in fluoroimmunoassays, protein and nucleic acid detection, bioactive ion detection, luminescence resonance energy transfer (LRET) experiments, and optical microscopy is nowadays well established.[1] In comparison with conventional dyes, the power of these lanthanide labels is that they display a large Stokes shift, a long-emission lifetime in the microsecond/millisecond range, which can serve as the basis of the time-resolved spectroscopy technique, and a relative insensitivity to photobleaching. For long-lifetime measurements, the use of temporal resolution is not technically demanding and can permit easier distinction from the shorter-lived (nanosecond range) endogenous fluorescence present in most biological matrices, and it also obviates problems associated with light scattering in the apparatus. In this method, only the signals of the desired labels are detected, as short-lived background emission is allowed to decay before data recording begins.[2]

A general strategy in the design of luminescent lanthanide complexes of practical use in bioanalytical applications is to synthesize a ligand that comprises an efficient chelating system for Ln^{3+} ions in aqueous solutions and an organic chromophore in close proximity to the bound lanthanide ion. The ligand is expected to form water-soluble lanthanide complexes of high kinetic stability with respect to metal decomplexation and to protect the metal from surrounding water molecules, as they cause vibronic deactivation by the OH oscillators. The role of the chromophore is to capture the excitation light and to transfer it to the luminescent f–f excited states of the lanthanide ions that are not readily accessible by direct excitation (antenna effect).[3,4] In this photochemical pathway, the most probable channel corresponds to (i) the absorption of UV light by a ground-singlet–excited-singlet transition, (ii) an energy migration by nonradiative intersystem crossing to a ligand triplet state, (iii) an intramolecular energy transfer from the triplet state to the closest emitting level of the metal, and (iv) the deactivation of the metal-centered level by radiative and nonradiative paths. This phenomenon was reviewed by Crosby et al.[5] and energy-transfer mechanisms have been discussed in detail.[6] Consequently, to work efficiently (thereby producing large quantum yields), the relative energies of the excited states of the ligand (singlet $^1S_{00}$ and triplet $^3S_{00}$ excited states) and the emissive states of the lanthanide ions are of great importance and particularly the energy difference between the ligand triplet state and the metal excited state.

In attempts to optimize the stability and the luminescence properties of Eu^{III} and Tb^{III} complexes in aqueous

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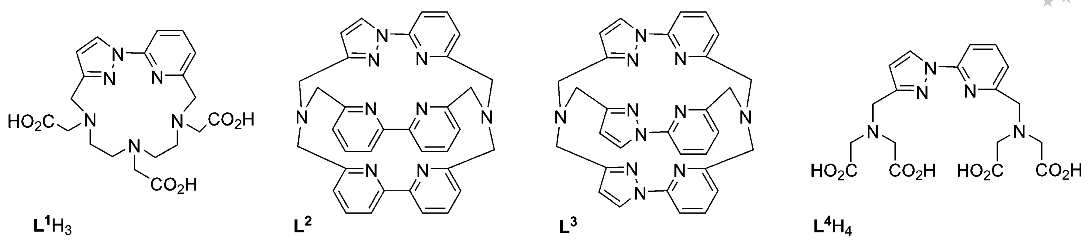


Scheme 1. Ligands based on the *N*,*C*-pyrazolylpyridine chromophore.

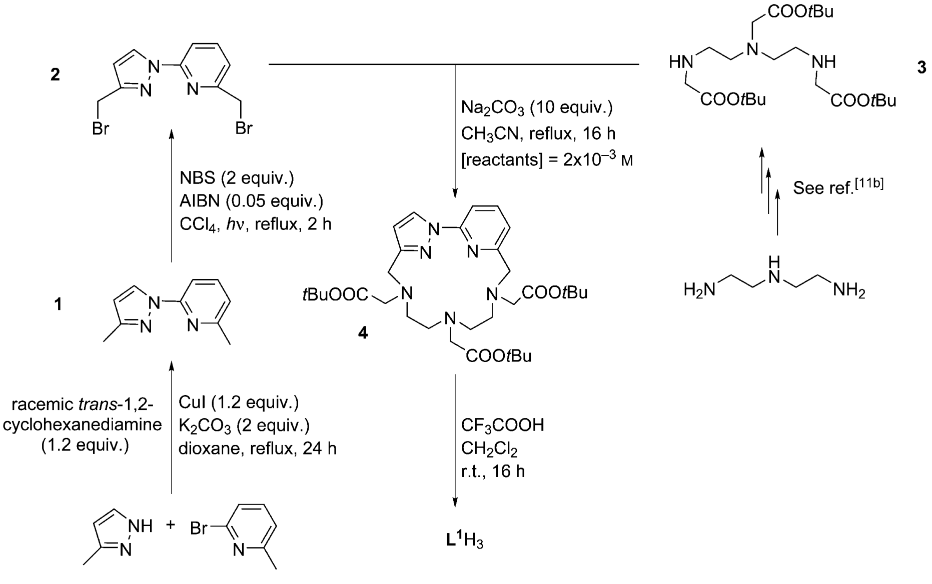
solutions, many ligands (cryptands, branched macrocycles, podands, acyclic ligands)[4,7] have been synthesized and numerous chromophores have been investigated for the sensitization of luminescence of these ions.[8] However, as a consequence of these requirements of optimal kinetic inertness and luminescence, only a few viable lanthanide labels (essentially based on Eu^{III} complexes) have been developed and tested up to now.[1a,9]

In order to get a terbium-based probe equipped with the main requirements for a time-resolved luminescence biolabel, we designed macrocyclic ligand $\mathbf{L^1}$H$_3$, which is built from a triamine complexing moiety associated to an intracyclic *N*,*C*-pyrazolylpyridine (pzpy) chromophore and three exocyclic carboxylate groups (Scheme 1). Polyaminocarboxylates have excellent water solubility and a high binding constant for lanthanides,[10] whereas the macrocyclic structure can increase the thermodynamic and (or) kinetic stability of the complexes ("macrocyclic effect"). Moreover, in such system, the antenna-to-cation sensitization step occurs between partners in a rigid conformation that can improve the energy-transfer rates. It is worth noting that only a few reports are based on lanthanide binding by macrocyclic compounds incorporating both an intracyclic chromophoric unit and pendant carboxylate groups,[11] whereas numerous examples of photoactive Ln^{III} complexes based on cyclen-derivatived ligands (cyclen is 1,4,7,10-tetraazacyclododecane) where a chromophoric unit is located on the dangling arms are described in the literature.[12] Although the *N*,*C*-pyrazolylpyridine unit has been the focus of much less attention than the 2,2′-bipyridine moiety, it exhibits promising properties for photon collection and transfer to terbium cations. For example, 6-methyl-2-(3-methylpyrazol-1-yl)pyridine can be excited efficiently (ε = 11200 L mol^{-1} cm^{-1}) at 287 nm in methanol solution, and its lowest excited triplet state is located at 26100 cm^{-1}.[13] The bipyridine analogue (6,6′-dimethyl-2,2′-bipyridine) absorbs light at 290 nm with a comparable efficiency and has a lower-energy triplet state (22850 cm^{-1}). Very recently, the sensitized emission of Tb^{III} complexed in *N*,*C*-pyrazolylpyridine-based ligands has been reported by several groups, including our own. Cryptands $\mathbf{L^2}$,[14] $\mathbf{L^3}$,[15] and acyclic ligand $\mathbf{L^4}$H$_4$[16] are the main representative ligands used in these photophysical studies. In the addition to the preparation of ligand $\mathbf{L^1}$H$_3$, we report here a detailed photophysical study in aqueous solutions of its corresponding Ln^{III} complexes (Ln = Sm, Eu, Gd, Tb, Dy) to help us better understand the role played by the various factors that determine the luminescence properties of these macrocyclic complexes. Attention is also focused on the kinetic stability of these metal probes towards the dissociation and transmetalation processes in aqueous and biological media.

Results and Discussion

Preparation of Ligand $\mathbf{L^1}$H$_3$

Ligand $\mathbf{L^1}$H$_3$ was obtained by a multistep procedure based on key compounds **1** and **3** (Scheme 2). Our first attempt to prepare diheterocyclic compound **1** was based on the traditional method for synthesizing pyrazolylpyridine and involved a direct reaction of potassium 3-methylpyrazolate (by using potassium metal as a base) with 2-bromo-6-methylpyridine in diglyme at 130 °C for several days.[17] Although this coupling method proved to be successful to obtain **1**,[15] it did not provide appreciable results in our hands. An alternative method for the preparation of *N*-arylpyrazoles involves the copper-catalyzed Ullmann-type reactions.[18] In this direction, we were attracted by recent reports claiming that species derived from copper iodide and simple diamines promote efficiently and under mild conditions the N-arylation of nitrogen heterocycles such as pyrazole and indazole.[19] Thus, the reaction between 2-bromo-6-methylpyridine and 3-methylpyrazole was carried out in the presence of K$_2$CO$_3$, CuI (1.2 equiv.), and *trans*-1,2-cyclohexanediamine in refluxing dioxane and afforded compound **1** in a 58% isolated yield. After column chromatography, a second eluting product was obtained in a minor amount (6%) and identified as being an isomeric form of **1**, in which the pyrazolyl ring is linked through the β-nitrogen atom. As expected, the coupling occurred preferentially at the less-hindered atom. These two regioisomers can be easily distinguished through the chemical shifts and coupling constants of the hydrogen atoms of the pyrazolyl ring.[20] Activation of the methyl groups of **1** was achieved by a conventional radical bromination with NBS in CCl$_4$ by using AIBN as an initiator and UV irradiation to initiate the reaction and gave the corresponding bis(bromomethyl) derivative **2**.[14] The second key compound tri-*tert*-butyl 1,4,7-triazaheptane-1,4,7-triacetate (**3**) was prepared in three steps from commercially available diethylenetriamine following our previously reported procedure (overall yield: 74%).[11b] Condensation of acyclic triamine **3** with dibromide **2** was carried out in CH$_3$CN at reflux in the presence of sodium carbonate as a base and without the use of high-



Scheme 2. Synthesis of ligand $\mathbf{L^1}H_3$.

dilution techniques. Subsequent acid hydrolysis of the remaining three *tert*-butyl ester functionalities was conducted on crude macrocyclization product **4** and afforded the targeted ligand $\mathbf{L^1}H_3$ after column chromatography (27% yield, two steps).

Preparation of $\mathbf{L^1}$·Ln Complexes

The Ln^{III} neutral complexes of the ligand $\mathbf{L^1}H_3$ were prepared by the addition of the lanthanide salt ($LnCl_3·6H_2O$) to the aqueous solution of ligand. These solutions were then adjusted in borate buffer (pH 8.6) at a final concentration of 3×10^{-5} M for absorption and 10^{-5} to 10^{-7} M for emission spectroscopy. The complexation was detected by changes in the ligand absorption spectrum upon the addition of the lanthanide salt (vide infra). The stoichiometry of the association between $\mathbf{L^1}H_3$ and the lanthanide ions was 1:1 as evaluated by titration experiments by recording the changes in the lanthanide luminescence intensity upon ligand excitation. Figure 1 illustrates the results obtained in the case of the Tb^{III} complex and shows the change in the luminescence intensity of 2×10^{-6} M of ligand $\mathbf{L^1}H_3$ upon addition of $TbCl_3$. The luminescence intensity increases linearly until a $TbCl_3/\mathbf{L^1}H_3$ concentration ratio of 1, and then a plateau is observed at higher concentration ratios. The luminescence lifetimes were independent of the amount of salt added, and the temporal decays of the metal emission were always rigorously monoexponential, which suggests the presence of one discrete $\mathbf{L^1}$·Ln solution species. This 1:1 stoichiometry was confirmed by ESI mass spectrometry analyses and is consistent with the size of the cavity (15-membered ring) as well the number of donor atoms (octadentate ligand). The photophysical data were collected from the absorption, fluorescence, and luminescence spectra of $\mathbf{L^1}$·Ln complexes measured 24 h after solution preparation to ensure equilibration.

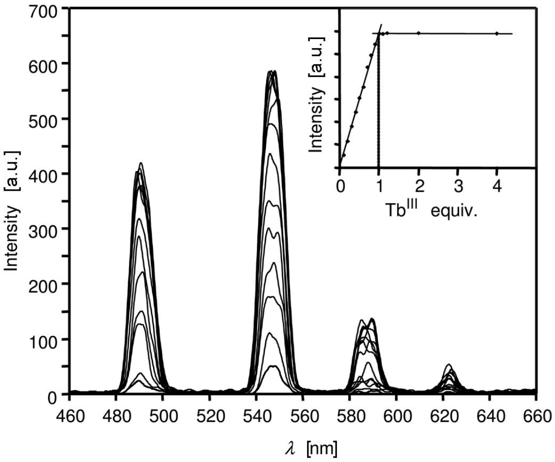


Figure 1. Luminescence spectra of Ligand $\mathbf{L^1}H_3$ (2 μM) in the presence of $TbCl_3$ (0.2–8 μM) in buffered aqueous solution (50 mM borate-buffered medium, pH 8.6). The inset shows the intensities of the $^5D_4\rightarrow^7F_5$ transition peak (545 nm) as the function of $TbCl_3$ added.

Ligand-Centered Transitions

Relevant photophysical data for the free ligand $\mathbf{L^1}H_3$ and $\mathbf{L^1}$·Ln complexes are presented in Table 1. The electronic spectrum of the ligand $\mathbf{L^1}H_3$ in basic aqueous solution (pH 8.6) displays two regions of absorption in the UV domain: a band centered at 255 nm with a shoulder at ca. 259 nm and a band centered at 289 nm. These bands, according to their molar absorption coefficients ($\varepsilon > 10000\ L\,mol^{-1}\,cm^{-1}$), are attributed to $\pi\rightarrow\pi^*$ transitions centered on the pzpy moiety, with a probable contribution from $n\rightarrow\pi^*$ transitions. The ligand–lanthanide interactions lead to weaker absorptions relative to that of the free ligand. We notice also the presence of two resolved maxima at 255 and 261 nm and a slight bathochromic shift (ca. 5 nm) in the low-energy band (Figure 2). Upon excitation through the ligand transitions, **L^1**H$_3$ displays at room temperature a sizeable emission band in the range 300–400 nm with a maximum at 326 nm. The relatively small Stokes shift (ca. 3900 cm^{-1}, estimated from absorption and emission maxima) led us to assign the observed emission band to $^1\pi\pi^*$ transitions. This assignment is corroborated by the fast decay of the emitted light: the intensity of the emission band quickly diminishes when a short delay (10–40 µs) is enforced. The energy of the 0–0 transition of the first excited singlet state is estimated to be 32150 cm^{-1} (intercept of the absorption and fluorescence spectra). At low temperature (77 K), a second emission band is observed with a maximum around 409 nm and a single-exponential time decay with a lifetime of 700 ms. On the bases of its energy level and long-living excited states, this emission is attributed to the $^3\pi\pi^*$ level centered on the pzpy moiety. From the structured phosphorescence profile, an energy level E_T = 26250 cm^{-1} is derived in correspondence to the highest energy band maximum (381 nm).

Table 1. Ligand-centered absorption and emission maxima.

Compd.	$\pi\rightarrow\pi^*$[a] λ_{max} [nm] (log ε)	$^1\pi\pi^*$[a,b] λ_{max} [nm]	$^3\pi\pi^*$[c] λ_{max} [nm][d]	τ [ms][e]
L^{1}H$_3$	289 (4.14)	326 (32600)	409 (26250)	700
L^1·Sm	295 (4.06)	[f]	[f]	–
L^1·Eu	294 (4.06)	[f]	[f]	–
L^1·Gd	294 (4.09)	333 (32000)	407 (25950)	19
L^1·Tb	294 (4.09)	[f]	[f]	–
L^1·Dy	294 (4.07)	[f]	[f]	–

[a] In borate buffer (0.05 M, pH 8.6) solutions at 298 K. [b] From fluorescence data in solutions at 298 K, λ_{max} are given from the maximum of the band envelope, and the energies of the 0–0 transition (within parentheses) are given in cm^{-1}. [c] From phosphorescence data in frozen solutions at 77 K, in borate buffer (pH 8.6)/glycerol (1:1). [d] λ_{max} are given from the maximum of the band envelope, and the energies of the 0–0 transition (within parenthesis) are given in cm^{-1}. [e] Lifetimes of the ligand $^3\pi\pi^*$ states at 77 K. [f] $^1\pi\pi^*$ fluorescence and $^3\pi\pi^*$ phosphorescence quenched by transfer to the lanthanide ion.

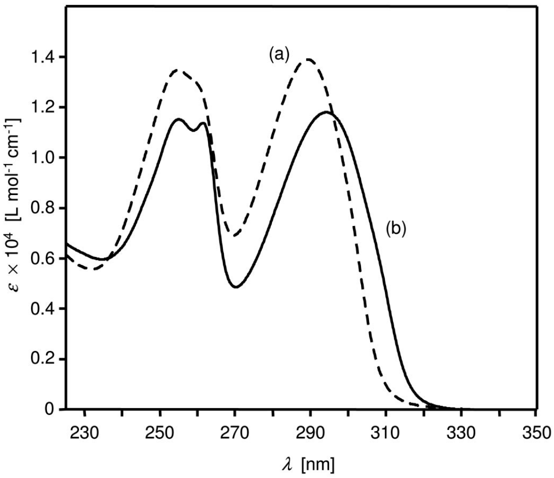


Figure 2. Absorption spectra of (a) the free ligand **L^1**H$_3$ (– – –) and (b) the **L^1**·Dy complex (——) in 50 mM borate buffer (pH 8.6).

As expected, in the gadolinium complex, the ligand-centered (LC) fluorescence and phosphorescence were not quenched, which allowed us to investigate the behavior of the LC excited states in a structure analogous to that of other lanthanide complexes. The LC luminescence of complex **L^1**·Gd displays essentially the same features as those of the free ligand (Table 1 and Figure 3). The energies of both the singlet and triplet LC states are slightly redshifted (600 and 300 cm^{-1}, respectively). The effect from the metal is more apparent on the rate of deactivation of the triplet excited state, whose lifetime at 77 K is decreased by an order of magnitude of about 1.6 relative to that of the free ligand. We can notice that the energy gap between the 0–0 transitions of the lowest singlet and triplet states of **L^1**·Gd amounts to 6050 cm^{-1}, which predicts an efficient singlet-to-triplet intersystem crossing process. Steemers et al. showed that intersystem crossing process becomes effective when ΔE ($^1\pi\pi^*$ – $^3\pi\pi^*$) is at least 5000 cm^{-1}.[21] In **L^1**·Gd, the energy of the $^3\pi\pi^*$ level (25950 cm^{-1}) is higher than that of the emitting excited states of Sm^{III} [$E(^4G_{5/2})$ = 17900 cm^{-1}], Eu^{III} [$E(^5D_0)$ = 17300 cm^{-1}], Tb^{III} [$E(^5D_4)$ = 20500 cm^{-1}], and Dy^{III} [$E(^4F_{9/2})$ = 21100 cm^{-1}], which confirms the suitability of the pzpy antenna as a sensitizer for those lanthanides. In the **L^1**·Ln (Ln = Sm, Eu, Tb, Dy) complexes, the emissions from the LC states are completely quenched and the characteristic emission bands of these lanthanide ions appear in the spectrum, which points out a complete ligand-to-metal energy transfer.

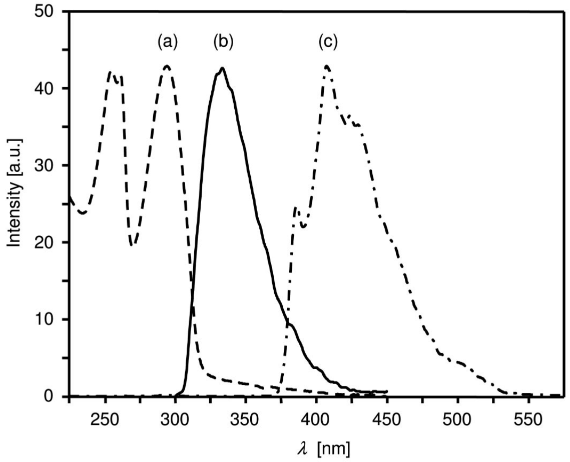


Figure 3. Normalized (a) absorption (– – –), (b) fluorescence (——), and (c) phosphorescence (– · – · –) spectra of the **L^1**·Gd complex. The absorption and fluorescence spectra are measured at 298 K in 50 mM borate buffer (pH 8.6) and the phosphorescence spectrum at 77 K in borate buffer–glycerol (1:1 v/v) glassy matrix.

Eu- and Tb-Centered Luminescence

Figures 4 and 5 show the corrected excitation and emission spectra obtained at room temperature of the **L^1**·Eu and **L^1**·Tb complexes. The excitation spectra are obtained under emission at 616 and 545 nm for **L^1**·Eu and **L^1**·Tb, respectively, and the emission under excitation at the maximum of the ligand-based π–π* transitions (294 nm) for both complexes. The metal luminescence excitation spectra closely resemble the UV absorption profiles and show unequivocally that the excitation of the lanthanide ion in these two complexes is by an indirect process, that is, a ligand-to-metal intersystem energy transfer. In the emission spectrum of **L¹**·Eu, five bands are resolved that correspond to transitions from the $^5D_0$ excited state to the $^7F_0$ and ^7F_J (J = 1–4) levels. The strongest red emission is based on the $^5D_0{\rightarrow}^7F_2$ transition occurring at 616 nm with a shoulder peak. The emission spectrum of the terbium complex is stronger and contains four bands corresponding to transitions from the $^5D_4$ excited state to the ^7F_J (J = 3–6) levels, and the $^5D_4{\rightarrow}^7F_5$ green emission is the most prominent one. All the emission bands have shoulder peaks.

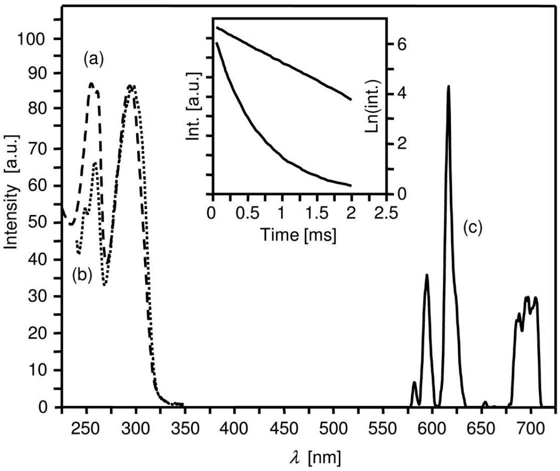


Figure 4. (a) Absorption (– – –), (b) corrected excitation (···, λ_{em} = 616 nm), and (c) emission (——, λ_{exc} = 294 nm) spectra of the **L¹**·Eu complex solution at 298 K (5.0×10^{-6} м in 50 mм borate buffer, pH 8.6). The excitation and emission bandpasses are 5 nm. The insert shows the excited-state lifetime: luminescence decay curve (bottom plot) and ln (intensity) vs. time (upper plot).

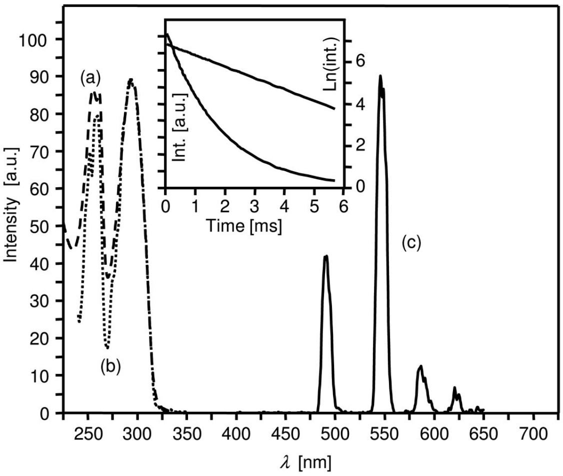


Figure 5. (a) Absorption (– – –), (b) corrected excitation (···, λ_{em} = 545 nm), and (c) emission (——, λ_{exc} = 294 nm) spectra of the **L¹**·Tb complex solution at 298 K (1.0×10^{-6} м in 50 mм borate buffer, pH 8.6). The excitation and emission bandpasses are 5 nm. The insert shows the excited-state lifetime: luminescence decay curve (bottom plot) and ln (intensity) vs. time (upper plot).

The luminescence lifetimes (τ) and quantum yields (Φ) for Eu^{III} and Tb^{III} complexes, obtained under various experimental conditions, are gathered in Table 2. The decay curves of the luminescence from the $^5D_0$ and $^5D_4$ states of the **L¹**·Eu and **L¹**·Tb complexes, respectively, are well described by a single exponential, which indicates that all Eu^{III} or Tb^{III} ions lie in the same average chemical environment, and from which the luminescence lifetime was determined. Both complexes exhibit long excited-state lifetimes in aqueous solutions; in particular, the value of the luminescence lifetime of the Tb^{III} complex at room temperature (1.85 ms) is remarkably high in comparison with those usually observed in monoaquo terbium complexes. In deuterated water at 298 K, the lifetimes are significantly longer, which indicates that there is a degree of metal–solvent (H_2O) interaction in the complexes that favors radiationless deactivation of the Ln^{III} excited state.[22] The lower effect exerted by solvent deuteration on **L¹**·Tb (τ_D/τ_H = 1.7) relative to that observed for **L¹**·Eu (τ_D/τ_H = 2.7) may be rationalized by considering that the energy gap between the lowest luminescent excited state and the highest level of the ground-state multiplet is larger in Tb^{III} than in Eu^{III} (15000 vs. 12000 cm^{-1}),[23] which thus reduces the probability of the Tb^{III} metal to couple with O–H vibronic deactivation. From data obtained in H_2O and D_2O solutions, it is possible to evaluate the number of water molecules directly coordinated to the lanthanide ions, q_{Ln}, owing to a well-established isotope effect[24] and by using the well-known expression developed by Parker et al.[25] [Equation (1)].

$$q_{Ln} = A_{Ln}\,[(1/\tau_H) - (1/\tau_D) - B_{Ln}] \qquad (1)$$

where A_{Eu} = 1.2, B_{Eu} = 0.25 and A_{Tb} = 5, B_{Tb} = 0.06 (with τ values in ms).

Table 2. Metal luminescence data of **L¹**·Ln complexes (Ln = Eu, Tb, Sm, Dy).[a]

Compd.	Lifetime [ms][b]					Quantum yield [×10²][c]		q[d]
	τ_H^{298K}	τ_{H,F^-}^{298K}	τ_D^{298K}	τ_H^{77K}	τ_D^{7K}	Φ_H^{298K}	Φ_D^{298K}	
L¹·Eu	0.68	1.12	1.86	0.81	2.00	5	11	0.82
L¹·Tb	1.85	2.67	3.16	1.89	3.16	49	78	0.82
L¹·Sm	0.011	0.012	0.052	0.011	–	0.3	–	0.90
L¹·Dy	0.015	0.015	–	0.015	–	1.6	–	–

[a] Data obtained in aerated borate buffer (pH 8.6), H_2O (H), or D_2O (D) solutions; in the absence or in the presence of 0.4 м KF (F^-). [b] Determined by excitation into the lowest-energy ligand-centered absorption band and recording the intensity of the $^5D_0{\rightarrow}^7F_2$ (616 nm), $^5D_4{\rightarrow}^7F_5$ (545 nm), $^4G_{5/2}{\rightarrow}^6H_{7/2}$ (602 nm), and $^4F_{9/2}{\rightarrow}^6H_{13/2}$ (578 nm) emissions for Eu^{III}, Tb^{III}, Sm^{III}, and Dy^{III}, respectively. [c] Excitation in ligand-centered band (294 nm). [d] Number of coordinated H_2O molecules at 298 K calculated by using the Parker equation[25] (Eu and Tb complexes) and Hakala equation[38a] (Sm complex).

The results show that both complexes have 0.82 inner-sphere water molecule at room temperature. Owing to the uncertainty of these empirical formulae, it is likely that the true value of the hydration state is q_{Ln} = 1. In fact, Eu^{III} and Tb^{III} ions are known to coordinate nine water molecules [Ln(H_2O)$_9$ species],[24] which strongly suggests that the eight binding sites provided by the ligand **L^1**H$_3$ are coordinated to the lanthanide ion. The presence of directly coordinated water molecules to the central lanthanide ion is also evidenced by the increase in the total emission intensity and in the luminescence lifetime upon the addition of fluoride anions. It has been well established that this small–hard charged anion can displace the residual H_2O molecules from the solvation sphere of Eu^{III} and Tb^{III} ions, which thus limits the radiationless decay of the Ln^{III} excited states through the O–H vibrations.[26] In the presence of a saturating anion concentration (0.4 M, F$^-$), the lifetimes in H_2O are increased by a factor of ca. 1.5 (Table 2) and the emitted light by a factor of 2.2 (Eu) and 1.3 (Tb). The incorporation of the F$^-$ ion in the metal coordination sphere is also reflected in the emission profile of **L^1·**Eu (Figure 6). The major changes are the decrease in the $^5D_0{\rightarrow}^7F_2/^5D_0{\rightarrow}^7F_1$ ratio (≈50 %) and the increase in the $^5D_0{\rightarrow}^7F_4/^5D_0{\rightarrow}^7F_1$ ratio (≈20 %) upon addition of F$^-$. For **L^1·**Tb the relative intensities of the $^5D_4{\rightarrow}^7F_J$ are approximately equal, as the emission spectra of Tb^{III} are not hypersensitive to the detailed nature of the ligand environment.[27]

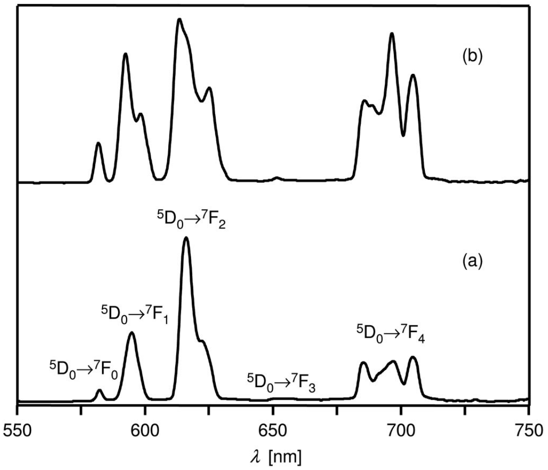


Figure 6. Corrected emission spectra (λ_{exc} = 294 nm) of **L^1·**Eu (5.0×10^{-6} M in 50 mM borate buffer, pH 8.6) in the presence of fluoride ions: (a) no fluoride and (b) 0.4 M fluoride. The excitation and emission passes are 10 and 3 nm, respectively.

From the data reported in Table 2, we can notice that, in D_2O solutions, the lifetime of the $^5D_4$ level is temperature independent, whereas the lifetime of the $^5D_0$ level increases marginally (7 %) upon decreasing the temperature. This shows that thermally activated radiationless decay paths play a minor role in the decay of the metal-emitting state. A back energy transfer from the metal-centered level to the ligand-centered triplet level, which is observed particularly for Tb^{III} complexes,[4] does not seem to be operative in **L^1·**Eu and **L^1·**Tb complexes. From the phosphorescence measurements performed on the **L^1·**Gd complex, we can estimate that in **L^1·**Tb and **L^1·**Eu the ligand-centered triplet state is around 5500 cm^{-1} above the $^5D_4$ level of Tb^{III} and 4450 cm^{-1} above the $^5D_2$ level of Eu^{III}. According to the works of Sato et al.,[28] Latva et al.,[29] and Steemers et al.,[21] these energy gaps are so large as to prevent back energy transfer. This is confirmed by the lack of any change in the lifetime and luminescence intensities following deaeration of aqueous solution of these complexes with oxygen-free dinitrogen. Owing to the low value (<40 s^{-1}) of the nonradiative temperature-dependent decay rate constant $k_{nr}(T)$ obtained by taking the difference of the reciprocal lifetimes in D_2O at 298 and 77 K, it can be assumed that luminescence quenching by low-lying ligand-to-metal charge-transfer states does not occur to a significant extent for the **L^1·**Eu complex. As a comparison, Prodi et al. reported a $k_{nr}(T)$ value of 840 s^{-1} for a Eu^{III} complex in which relaxation to the ground state through crossover to Eu^{3+} charge-transfer is efficient.[30]

The overall efficiency of the luminescence of these complexes was assessed by quantum-yield determinations upon ligand excitation. The emission quantum yield of **L^1·**Tb (Φ = 49 %) is one of the highest reported to date in aqueous solutions,[4,29,31] especially for terbium complexes containing one water molecule in the inner coordination sphere of the metal.[11a,12a,32] In contrast, with respect to other *N*,*C*-pyrazolylpyridine-containing **L^n·**Tb complexes (Scheme 1), **L^1·**Tb is 1.2 (**L^4·**Tb)[16] to 4.5 (**L^2·**Tb)[14] times more luminescent. The pzpy chromophore sensitizes the Eu^{III} ion less efficiently with a quantum value of 5 %, which is in the range of the quantum yield of europium-based commercial luminescent probes.[33] The higher susceptibility of Eu^{III} luminescence towards quenching by the hydroxy groups of the solvent cannot account for the reduced emission quantum yield of **L^1·**Eu relative to that of **L^1·**Tb. In D_2O solutions, **L^1·**Eu exhibits a quantum yield seven times lower than that of **L^1·**Tb. This suggests that the relatively high energy of the lowest triplet state of the ligand plays a major role in the luminescent properties of these complexes. Previous studies established that the quantum yield should be highest when the energy is transferred from the triplet-state level of the ligand directly to the lowest excited state of Ln^{III}.[29] Looking at this energy value of 25950 cm^{-1}, it is likely that the excitation energy is primarily transferred to the $^5D_3$ (24500 cm^{-1}), $^5D_2$ (21500 cm^{-1}), or $^5D_1$ (19000 cm^{-1}) levels of the Eu^{III} ion that decay nonradiatively to the lower-lying $^5D_0$ level. During this transfer from upper ^5D_J levels to the emitting $^5D_0$ level, quite significant energy losses can consequently take place. Because the energy of the $^5D_3$ level of the Tb^{III} ion is about 26250 cm^{-1}, the energy is directly transferred to the lowest excited resonance level of Tb^{III} $^5D_4$ in **L^1·**Tb. These results are in agreement with those reported by Latva et al. for a series of 41 Eu^{III} and Tb^{III} chelates derived from acyclic poly(aminocarboxylates) ligands.[29] For this family of photoactive ligands, Tb^{III} chelates exhibit high luminescence quantum yields [$\Phi(D_2O)$ > 40 %] when the $^3\pi\pi^*$ states lie over 23000 cm^{-1}, whereas lower quantum yields [$\Phi(D_2O)$ < 10 %] are observed for Eu^{III} chelates when the $^3\pi\pi^*$ states lie in the range 25000–26000 cm^{-1}.

Several factors contribute to the overall luminescence quantum yield of the complex upon excitation of the chromophore.[1b,12f,34] They are the efficiency of intersystem crossing within the chromophoric group (Φ_{isc}), the efficiency of the ligand-to-metal energy transfer (Φ_{ET}), and the intrinsic luminescence quantum yield of the complexed Ln^{III} ion when it is excited in its own levels (Φ_{Ln}). Equation (2) defines the overall quantum yield of sensitized emission (Φ, as determined experimentally) as a function of these factors.

$$\Phi = \Phi_{\text{isc}} \times \Phi_{\text{ET}} \times \Phi_{\text{Ln}} = \eta_{\text{sens}} \times \Phi_{\text{Ln}} \quad (2)$$

The efficiency of metal-centered luminescence Φ_{Ln} can be estimated through Equation (3) by using the assumption that the decay process at 77 K in deuterated water is purely radiative.[4]

$$\Phi_{\text{Ln}} = \tau_{\text{H}}^{298\text{K}}/\tau_{\text{D}}^{77\text{K}} \quad (3)$$

According to the observed lifetimes given above, the Φ_{Ln} values were determined to be 34.0 (Φ_{Eu}) and 58.5% (Φ_{Tb}), which, in turn, led to η_{sens} values: η(**L^1**·Eu) = 15% and η(**L^1**·Tb) = 84%. With the assumption that Φ_{isc} is similar in the two complexes, the efficiency of the ligand-to-metal energy transfer (Φ_{ET}) is about six times higher for **L^1**·Tb than for **L^1**·Eu, and this may be attributed to a better match between the ligand triplet energy and the acceptor lanthanide state. The values of η_{sens} obtained for the **L^1**·Ln macrocyclic complexes compare well with those noticed for the monoaquo lanthanide complexes derived from the corresponding acyclic ligand **L^4**H$_4$: η(**L^4**·Eu) = 20% and η(**L^4**·Tb) = 80%.[35] This indicates that the ligand structure (macrocyclic or acyclic) does not interfere with the efficiency of the sensitization process of the *N*,*C*-pyrazolylpyridine chromophore.

Sm- and Dy-Centered Luminescence

Sm^{III} and Dy^{III} complexes are generally not as luminescent as their corresponding Tb^{III} and Eu^{III} complexes because of efficient vibrational quenching.[36] However, there is great interest in studying the sensitized luminescence of these ions, because their relatively short emission lifetimes under ambient conditions (microsecond range) allows one to probe shorter time scales. Emission spectra of **L^1**·Sm and **L^1**·Dy complexes show that the same sensitization process discussed for **L^1**·Eu and **L^1**·Tb also occurs in these complexes (Figure 7). Excitation of **L^1**·Sm solutions in ligand-centered band (294 nm) leads to the pink emission of Sm^{III} due to $^4\text{G}_{5/2}\rightarrow{}^6\text{H}_J$ (J = 5/2, 7/2, 9/2, 11/2) transitions; the dominant band in the corrected emission spectrum is the transition $^4\text{G}_{5/2}\rightarrow{}^6\text{H}_{7/2}$ at 602 nm. Similarly, the sensitization process in **L^1**·Dy results in the yellow luminescence of Dy^{III} with the $^4\text{F}_{9/2}\rightarrow{}^6\text{H}_J$ (J = 15/2, 13/2, 11/2, 9/2) transitions observable. The latter spectrum is dominated by the hypersensitive $^4\text{F}_{9/2}\rightarrow{}^6\text{H}_{13/2}$ transition at 578 nm. No ligand emission centered at 333 nm is observed, which indicates the intramolecular ligand-to-lanthanide energy transfer is complete. However, a very small part of the transferred energy is emitted in a radiative way by Sm^{III} and Dy^{III} ions. The quantum yields determined in H_2O solution for **L^1**·Sm and **L^1**·Dy are 0.3 and 1.6%, respectively. The less-efficient quenching observed for **L^1**·Dy can be related to the larger energy gap between the lowest luminescent excited state-$^4\text{F}_{9/2}$ and the highest ground state $^6\text{F}_{3/2}$ of Dy^{III} ($\Delta E_{\text{Dy}} \approx$ 7850 cm^{-1} vs. $\Delta E_{\text{Sm}} \approx$ 7400 cm^{-1}). Moreover, the energy difference between the excited level $^4\text{F}_{9/2}$ of Dy^{III} and the ligand triplet state (4850 cm^{-1}) is more favorable for an efficient energy transfer than that in the Sm^{III} complex (ΔE $\approx$ 8050 cm^{-1} for $^4\text{G}_{5/2}$). The value of 1.6% for **L^1**·Dy is interesting, because the quantum yields for Dy^{III}-containing compounds rarely exceed 2% in aqueous medium.[11a,31b,31c,37]

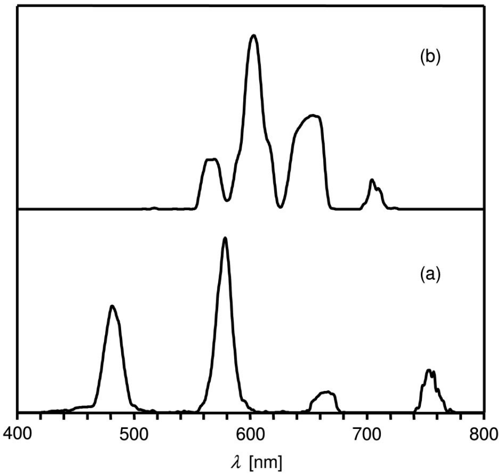


Figure 7. Normalized corrected emission spectra (λ_{exc} = 294 nm) of (a) **L^1**·Dy and (b) **L^1**·Sm at 298 K in 50 mM borate buffer (pH 8.6). The excitation and emission bandpasses are 10 nm.

The observed luminescence lifetimes (Table 2) are longer than the reported values of Sm^{III} and Dy^{III} ions in aqueous solution (≈2.3 µs)[23] and are in the expected range for Sm^{III} and Dy^{III}-containing ligands.[11a,31b,31c,37,38] We may note that the presence of fluoride ions (0.4 M) does not interfere with the luminescence behavior of these complexes (no change in luminescence intensity and lifetime). In contrast, the temperature independence of the lifetimes suggests that we are dealing with multiphonon emissions and excludes thermally activated nonradiative processes. The hydration number q of **L^1**·Sm was determined by using a newly published relationship between q and the reciprocals of the lifetimes in H_2O and D_2O [Equation (4)] proposed on the basis of Sm^{III} chelates containing two iminodiacetic acid moieties.[38a]

$$q_{\text{Sm}} = 0.0225[(1/\tau_{\text{H}}) - (1/\tau_{\text{D}}) - 31.5] \quad (4)$$

where the lifetimes are given in milliseconds. By using this equation, we calculated that essentially one water molecule is bound in the first coordination sphere, which is in line with previous observations for **L^1**·Eu and **L^1**·Tb complexes.

Kinetic Stability of L^1·Tb in Aqueous Media

It is well established that dissociation kinetics plays a significant role for the in vivo stability of a metal chelate and thus, the kinetic stability of a complex may correlate better than thermodynamic stability with the ability of a complex to remain intact in physiological conditions.[39] The kinetic inertness of **L^1·**Tb was studied in various media and in the presence of other ions or strongly binding competing ligands that may be involved in a dissociation pathway. The dissociation of the complex was determined by luminescent experiments, that is, by monitoring the disappearance of the $^5D_4 \rightarrow {}^7F_5$ peak at 545 nm as a function of time. The emission properties of **L^1·**Tb in aerated solution at room temperature remained unchanged for several days in the examined buffers and pH range (borate buffer, pH 8.6; Tris buffer, pH 7.3, phosphate buffer, pH 7.4), which highlights the kinetic inertness of the complex in aqueous media. It is worth noting that **L^1·**Tb is also an efficient emitter at physiological pH where its photophysical characteristics (τ, Φ) are similar to those observed at pH 8.6. One also notes that the presence of phosphate groups, which are ubiquitous in biological systems and also have a strong affinity for lanthanides, does not interfere with the luminescence behavior of this complex at ratios up to 10^4:1; this suggests that the ligand wraps about the metal ion uniformly. No dissociation of **L^1·**Tb was observed when the complex (1 μM) was challenged by an excess amount of the most abundant human serum cations (Ca^{2+}, 126 mM; Mg^{2+}, 0.8 mM; Zn^{2+}, 0.01 mM; Na^+, 140 mM; K^+, 5 mM). In parallel, the sensitivity of **L^1·**Tb to dynamic quenching by urate was assessed. As recently shown by D. Parker et al.,[7b] the insensitivity of luminescent Eu^{III} and Tb^{III} complexes to quenching by this endogenous antioxidant is a favorable factor for in cellulo applications of these probes. The charge neutral **L^1·**Tb complex resists very well to dynamic quenching by urate: the measured lifetime for **L^1·**Tb (10 μM) in the absence and in the presence of urate (50 and 500 μM) is nearly constant (1.85, 1.82, and 1.76 ms, respectively). This complex is also resistant to dissociation in the presence of ethylenediaminetetraacetic acid (EDTA), a competing ligand which binds strongly to Tb^{III} [K_{Tb} (EDTA) = 8.3×10^{17}][40] and is commonly used in biological experiments. As a matter of fact, competitive titration of **L^1·**Tb with EDTA (up to 10-fold excess of EDTA, pH 8.6) showed no evidence of ligand exchange after 2.5 days. This is an improvement over the Tb^{III} complex derived from the corresponding acyclic ligand, **L^4·**Tb, for which 53% dissociation was observed after 2.5 days under the same conditions (Figure 8). This result highlights again the enhanced stability due to the macrocyclic effect, which was attributed to the slow decomposition rates of complexes of metal ions with macrocycles in relation to those of their open-chain analogues.[41] Finally, to evaluate the stability of **L^1·**Tb in biological media, serum stabilities studies were performed. Serum contains a mixture of proteins and small molecules that can potentially compete with **L^1**H_3 for Tb^{III}. Less than a 5% time-dependent change was observed over a 24 h period when **L^1·**Tb (3 μM) was incubated in a mixture of Tris buffer/human serum (2:1) at pH 7.4, which is consistent with high complex kinetic stability. In this medium, the lifetime (1.74 ms, characterized by a monoexponential decay) remained unchanged, which suggests that no interaction occurs between the complex and the serum proteins. This inertness is a promising indication for the use of **L^1·**Tb as a luminescent biolabel.

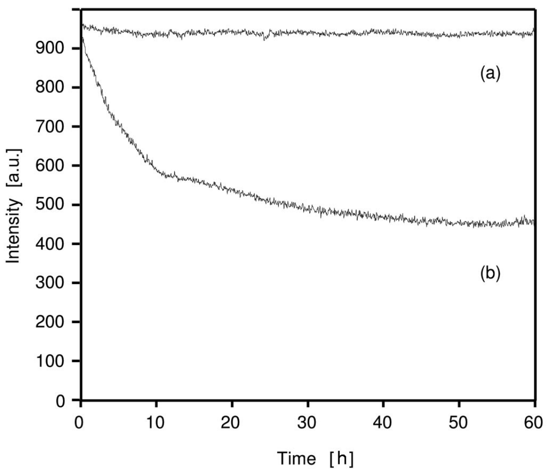


Figure 8. Plot of emission intensity of the $^5D_4 \rightarrow {}^7F_5$ transition (545 nm) of the complexes (a) **L^1·**Tb (5 μM) and (b) **L^4·**Tb (5 μM) in the presence of 10 molar equivalents of EDTA added in borate buffer (pH 8.6).

Conclusions

We synthesized a pentaazamacrocycle containing an intracyclic *N*,*C*-pyrazolylpyridine chromophore and three acetate pendant arms. This ligand efficiently coordinates to trivalent lanthanide cations in aqueous solutions to form neutral 1:1 metal-to-ligand complexes. These complexes display excellent stability versus transmetalation by several bioactive ions and demetalation by blood proteins, which is a prerequisite for their use in biological media. The *N*,*C*-pyrazolylpyridine chromophore provides excellent sensitization of the Tb^{III} luminescence through a particularly efficient ligand-to-lanthanide energy-transfer process that occurrs between the ligand $^3\pi\pi^*$ and Tb($^5D_4$) states. Moreover, it is a good sensitizer of the Eu^{III} luminescence, and it also sensitizes the otherwise poorly luminescent Sm^{III} and Dy^{III} ions. The very low overlap between the emissions of these four lanthanide cations (Figures 4, 5, and 7) opens the way for the use of these lanthanide probes in multiplex assays. Further refinement of the ligand structure may arise from (i) the shift of the absorption maximum to a longer wavelength (introduction of a suitable substitution into the chromophoric unit),[31c] (ii) the elimination of the single water molecule bound to the metal ion (substitution of acetate arms by phosphinates),[42] and (iii) its ability to conjugate the complexes to biomolecules (introduction of an appropriate functionalization into the diethylenetriamine moiety).[43] This work is in progress.

Experimental Section

General Procedures and Starting Materials: Tri-*tert*-butyl 1,4,7-triazaheptane-1,4,7-triacetate (**3**) was prepared according to a literature procedure.[11b] Racemic *trans*-1,2-cyclohexanediamine, copper(I) iodide, and $LnCl_3{\cdot}6H_2O$ salts (99.9%) were purchased from Sigma–Aldrich and used without further purification. HPLC analyses were performed with a Waters Alliance 2690 instrument equipped with a Waters PDA 2996 photodiode array detector. The wavelength used for the analyses was 289 nm. ^{1}H and ^{13}C NMR spectra were recorded with Bruker AC250 and AC400 spectrometers; chemical shifts are given in ppm according to the solvent peak. IR spectra were determined with a Perkin–Elmer FTIR 1725x spectrometer. Electrospray (ES) mass spectra were obtained with a Perkin–Elmer SCIEX API 100 and Waters Q-TOF spectrometers. Absorption measurements were done with a Hewlett Packard 8453 temperature-controlled spectrophotometer.

6-Methyl-2-(3-methylpyrazol-1-yl)pyridine (1): To a mixture of 3-methyl pyrazole (200 mg, 2.44 mmol), CuI (557 mg, 2.92 mmol), and K_2CO_3 (800 mg, 5.8 mmol) in dioxane (10 mL) was added 2-bromo-6-methylpyridine (420 mg, 2.44 mmol) and *trans*-1,2-cyclohexanediamine (333 mg, 2.92 mmol). The mixture was heated at reflux under an atmosphere of argon for 24 h, after which it was cooled to room temperature, diluted with ethyl acetate (100 mL), and filtered through a plug of Celite. The filtrate was washed with saturated EDTA aqueous solution and was then dried with Na_2SO_4. The solvents were evaporated under reduced pressure, and the residue was purified by column chromatography (silica; AcOEt/CH_2Cl_2, 0:100 to 10:90) to give desired product **1** (245 mg, 58%) as a pale-yellow oil. R_f (CH_2Cl_2/AcOEt, 90:10) = 0.35. IR (KBr): $\tilde{\nu}$ = 1599, 1577, 1537, 1472, 1357 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 2.35 (s, 3 H, CH_3), 2.49 (s, 3 H, CH_3), 6.20 (d, $^3J_{H,H}$ = 2.4 Hz, 1 H, pz-H^4), 6.93 (d, $^3J_{H,H}$ = 7.0 Hz, 1 H, py-H^5), 7.55–7.68 (m, 2 H, py-$H^{3,4}$), 8.43 (d, $^3J_{H,H}$ = 2.4 Hz, 1 H, pz-H^5) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 14.1 (1 C, CH_3), 24.4 (1 C, CH_3), 107.8 (1 C, CH), 109.0 (1 C, CH), 120.4 (1 C, CH), 127.8 (1 C, CH), 138.9 (1 C, CH), 151.0 (1 C, C_q), 151.6 (1 C, C_q), 157.5 (1 C, C_q) ppm. MS (DCI, NH_3): *m/z* (%) = 174.0 (100) $[M + H]^+$. UV/Vis (CH_3OH): λ_{max} (ε, $L\,mol^{-1}\,cm^{-1}$) = 255 (10200), 287 (11200) nm. $C_{10}H_{11}N_3$ (173.21): calcd. C 69.34, H 6.40, N 24.26; found C 69.10, H 6.50, N 24.35. 6-Methyl-2-(5-methylpyrazol-1-yl)pyridine was also isolated (25 mg, 6%) as a pale-yellow oil. R_f (CH_2Cl_2/AcOEt, 90:10) = 0.27. IR (KBr): $\tilde{\nu}$ = 1602, 1578, 1531, 1473, 1352 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 2.55 (s, 3 H, CH_3), 2.67 (s, 3 H, CH_3), 6.17 (d, $^3J_{H,H}$ = 1.5 Hz, 1 H, pz-H^4), 7.04 (d, $^3J_{H,H}$ = 7.0 Hz, 1 H, py-H^5), 7.56 (d, $^3J_{H,H}$ = 1.5 Hz, 1 H, pz-H^3), 7.60–7.72 (m, 2 H, py-$H^{3,4}$) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 14.7 (1 C, CH_3), 24.3 (1 C, CH_3), 108.6 (1 C, CH), 113.3 (1 C, CH), 120.8 (1 C, CH), 138.7 (1 C, CH), 140.5 (1 C, CH), 140.8 (1 C, C_q), 153.1 (1 C, C_q), 157.0 (1 C, C_q) ppm. MS (DCI, NH_3): *m/z* (%) = 174.0 (100) $[M + H]^+$. $C_{10}H_{11}N_3$ (173.21): calcd. C 69.34, H 6.40, N 24.26; found C 69.40, H 6.35, N 24.10.

6-Bromomethyl-2-(3-bromomethylpyrazol-1-yl)pyridine (2): To a solution of compound **1** (321 mg, 1.85 mmol) in CCl_4 (20 mL) was added *N*-bromosuccinimide (NBS; 660 mg, 3.71 mmol) and 2,2′-azobis(isobutyronitrile) (AIBN; 16 mg, 0.1 mmol). The solution was irradiated and heated at reflux by using a halogen lamp (150 W) until its fading (2 h). The hot mixture was then filtered and evaporated under reduced pressure, and the residue was purified by column chromatography (silica; CH_2Cl_2/petroleum ether, 40:60) to give dibromo compound **2** (123 mg, 20%) as a white solid. R_f (CH_2Cl_2/petroleum ether, 40:60) = 0.25. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.52 (s, 2 H, CH_2), 4.57 (s, 2 H, CH_2), 6.54 (d, $^3J_{H,H}$ = 2.7 Hz, 1 H, pz-H^4), 7.33 (d, $^3J_{H,H}$ = 7.3 Hz, 1 H, py-H^5), 7.85 (m, 2 H, py-$H^{3,4}$), 8.57 (d, $^3J_{H,H}$ = 2.7 Hz, 1 H, pz-H^5) ppm. M.p. and ^{13}C NMR data are in good agreement with the literature.[15]

2,7,10,13,19,20-Hexaazatricyclo[13.3.1.1^{2,5}]icosa-1(19),3,5(20),15,17-pentaene-7,10,13-triacetic Acid (L^1H_3): To a solution of triamine **3** (334 mg, 0.75 mmol) in anhydrous acetonitrile (375 mL) was added Na_2CO_3 (795 mg, 7.5 mmol). The suspension was heated at reflux for 1 h and then dibromide **2** (248 mg, 0.75 mmol) was added in one portion. The mixture was stirred at reflux overnight before filtration. The solvent was removed by rotary evaporation, and the residue was chromatographed (alumina; MeOH/CH_2Cl_2, 1:99 to 20:80). The combined fractions were evaporated to afford 169 mg of the crude macrocycle **4**, which was not further purified {HPLC (column CC125-3 MN C18 HD 5 µm; eluant: 0.1% TFA in H_2O/MeOH, 50:50; flow rate: 0.6 $mL\,min^{-1}$): t_R = 12.68 min. MS (ESI+, CH_3OH): *m/z* (%) = 615.7 (100) $[M + H]^+$, 559.6 (35) $[M - tBu + 2H]^+$, 503.4 (18) $[M - 2tBu + 3H]^+$, 447.6 (5) $[M - 3tBu + 4H]^+$}. A solution of this crude product in a mixture of CH_2Cl_2/CF_3COOH (1:1, 5 mL) was stirred at room temperature overnight. After evaporation, the residue was dissolved in a minimal amount of methanol and diethyl ether was added, which resulted in the formation of a precipitate that was isolated after centrifugation. HPLC purification of the precipitate (C18 silica reverse phase, elution with 0.1% TFA in H_2O/CH_3CN, 95:5) gave the desired product L^1H_3 (90 mg, 27% yield over two steps) as a white solid. M.p. > 250 °C. HPLC (column Xterra MS C18 3.5 µm; eluant: 0.1% TFA in H_2O/CH_3CN, 95:5; flow rate: 0.6 $mL\,min^{-1}$): t_R = 5.83 min. IR (KBr): $\tilde{\nu}$ = 3443, 1686 cm^{-1}. ^{1}H NMR (250 MHz, D_2O): δ = 3.43–3.84 (m, 10 H, CH_2), 4.13 (s, 2 H, CH_2), 4.22 (s, 2 H, CH_2), 4.59 (s, 2 H, CH_2Ar), 4.70 (s, 2 H, CH_2Ar), 6.61 (d, $^3J_{H,H}$ = 2.5 Hz, 1 H, pz-H^4), 7.32 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, py-H^5), 7.63 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, py-H^3), 7.97 (t, $^3J_{H,H}$ = 8.0 Hz, 1 H, py-H^4), 8.32 (d, $^3J_{H,H}$ = 2.5 Hz, 1 H, pz-H^5) ppm. MS (ESI+, H_2O): *m/z* (%) = 485.5 (80) $[M + K]^+$, 469.5 (94) $[M + Na]^+$, 447.6 (100) $[M + H]^+$. HRMS (ESI+, H_2O): calcd. for $C_{20}H_{27}N_6O_6$ $[M + H]^+$ 447.1992; found 447.2002. UV/Vis (Borate buffer, pH 8.6): λ_{max} (ε, $L\,mol^{-1}\,cm^{-1}$) = 255 (13550), 259 sh. (12950), 289 (13900) nm.

In Situ Preparation of $L^1{\cdot}Ln$ Complexes: An equimolar amount of $LnCl_3{\cdot}6H_2O$ in water (2×10^{-3} M) was added to a solution of L^1H_3 (2×10^{-3} M) in water. The solution was stirred for 1 h at room temperature and then adjusted to a final concentration of 3×10^{-5} to 1×10^{-6} M in borate buffer (50 mM, pH 8.6).

$L^1{\cdot}Eu$ Complex: MS (ESI+, H_2O): *m/z* (%) = 635.3 (9) $[M + K]^+$, 619.3 (39) $[M + Na]^+$, 597.3 (43) $[M + H]^+$, 318.2 (100) $[M + H + K]^{2+}$. UV/Vis (Borate buffer pH 8.6): λ_{max} (ε, $L\,mol^{-1}\,cm^{-1}$) = 255 (11500), 261 (11300), 294 (11550) nm. Luminescence (borate buffer pH 8.6, λ_{exc} = 294 nm): λ_{em} (relative intensity, corrected spectrum), 582 (3.7), 595 (34.8), 616 (100.0), 654 (0.5), 695 (81.8) nm

$L^1{\cdot}Tb$ Complex: MS (ESI+, H_2O): *m/z* (%) = 641.2 (8) $[M + K]^+$, 625.3 (33) $[M + Na]^+$, 603.3 (17) $[M + H]^+$, 321.2 (100) $[M + H + K]^{2+}$. UV/Vis (Borate buffer pH 8.6): λ_{max} (ε, $L\,mol^{-1}\,cm^{-1}$) = 255 (11800), 261 (11700), 294 (12175) nm. Luminescence (borate buffer pH 8.6, λ_{exc} = 294 nm): λ_{em} (relative intensity, corrected spectrum), 491 (48.3), 545 (100.0), 586 (34.4), 622 (14.2) nm.

$L^1{\cdot}Gd$ Complex: MS (ESI+, H_2O): *m/z* (%) = 639.8 (3) $[M + K]^+$, 623.9 (8) $[M + Na]^+$, 601.9 (15) $[M + H]^+$, 320.6 (100) $[M + H + K]^{2+}$. UV/Vis (Borate buffer pH 8.6): λ_{max} (ε, $L\,mol^{-1}\,cm^{-1}$) = 255 (11950), 261 (11800), 294 (12175) nm.

$L^1{\cdot}Sm$ Complex: MS (ESI+, H_2O): *m/z* (%) = 634.0 (2) $[M + K]^+$, 617.9 (6) $[M + Na]^+$, 595.9 (15) $[M + H]^+$, 317.5 (100) $[M + H + K]^{2+}$. UV/Vis (Borate buffer pH 8.6): λ_{max} (ε, $L\,mol^{-1}\,cm^{-1}$) = 255 (11200), 261 (10950), 295 (11500) nm. Luminescence (borate buffer pH 8.6, λ_{exc} = 294 nm): λ_{em} (relative intensity, corrected spectrum), 564 (24.5), 602 (100.0), 648 (72.2), 704 (8.7) nm.

$\mathbf{L^1}$·Dy Complex: MS (ESI+, H_2O): *m/z* (%) = 645.9 (13) $[M + K]^+$, 629.9 (48) $[M + Na]^+$, 608.0 (38) $[M + H]^+$, 332.6 (100) $[M + H_2O + H + K]^{2+}$, 323.6 (60) $[M + H + K]^{2+}$. UV/Vis (Borate buffer pH 8.6): λ_{max} (ε, $\mathrm{L\,mol^{-1}\,cm^{-1}}$) = 255 (11500), 261 (11400), 294 (11800) nm. Luminescence (borate buffer pH 8.6, λ_{exc} = 294 nm): λ_{em} (relative intensity, corrected spectrum), 481 (68.6), 578 (100.0), 663 (13.6), 752 (21.1) nm.

Luminescence Measurements: Fluorescence and phosphorescence spectra were obtained with a LS-50B Perkin–Elmer and a Cary Eclipse spectrofluorimeters equipped with a Xenon flash lamp source and a Hamamatsu R928 photomultiplier tube. The measurements were carried out at pH 8.6 in borate buffer (50 mM) and all samples were prepared with an absorbance between 0.01 and 0.05 at the excitation wavelength in order to prevent the inner-filter effect. Phosphorescence spectra of ligand $\mathbf{L^1}H_3$ and complex $\mathbf{L^1}$·Gd were recorded in a borate buffer/glycerol mixture (1:1 v/v). Spectra were corrected for both the excitation light source variation and the emission spectral response. Lifetimes τ (uncertainty $\leq$ 5%) are the average values from at least five separate measurements covering two or more lifetimes made by monitoring the decay at a wavelength corresponding to the maximum intensity of the emission spectrum, following pulsed excitation. The phosphorescence decay curves were fitted by an equation of the form $I(t) = I(0)\exp(-t/\tau)$ by using a curve-fitting program. High correlation coefficients were observed in each case (higher than 0.999). The luminescence quantum yields (uncertainty ±15%) were calculated by using the following equation:

$$\Phi/\Phi_r = [A_r(\lambda_r)/A(\lambda)]\,[I/I_r]\,[n^2/n_r^2]$$

where *A*, *I*, and *n* denote the absorbance at the excitation wavelength (λ), the integrated luminescence intensity, and the refractive index of solvent, respectively. Subscript "r" denotes the reference, and no subscript denotes the unknown sample. Quinine sulfate in 1 N sulfuric acid (Φ = 0.546)[44] and $[\mathrm{Ru(bpy)_3}]^{2+}$ in aerated water (Φ = 0.028)[45] were used as the references for the $\mathrm{Tb^{III}}$ and $\mathrm{Eu^{III}}$ complexes, respectively. The $\mathbf{L^1}$·Tb and $\mathbf{L^1}$·Eu complexes were taken as references for the determination of the quantum yields of the $\mathbf{L^1}$·Dy and $\mathbf{L^1}$·Sm complexes, respectively.

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