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Synthesis and Luminescence of Lanthanide Complexes of a Branched Macrocyclic Ligand Containing 2,2′-Bipyridine and 9-Methyl-1,10-phenanthroline Subunits

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The synthesis of the branched-macrocyclic ligand **1** incorporating two 2,2′-bipyridine units in the macrocycle and two 9-methyl-1,10-phenanthroline units in the branches is described as well as the synthesis and the photophysical properties of its Eu^{3+}, Tb^{3+}, and Gd^{3+} complexes. These complexes do not decompose in water in contrast to those of the related ligand containing 2,2′-bipyridine instead of 1,10-phenanthroline. They show intense absorption bands in the UV region due to absorption in the ligand. The emission spectra of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ and $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$ complexes obtained upon ligand excitation show the usual Eu^{3+} and Tb^{3+} transitions. The pattern of the emission spectrum of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex allows us to assess a low (presumably C_2) symmetry as the probable site symmetry of the metal ion in the complex. For $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ and $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$, the metal luminescence excitation spectra in water match the ligand absorption spectra while in methanol the absorption due to the phenanthroline is missing. This suggests that in water the efficiency of the ligand-to-metal energy transfer is similar for the two chromophores while in methanol phenanthroline transfers energy to the metal ion less efficiently than bipyridine. The luminescence quantum yield values in water and methanol confirm this interpretation. The lifetimes of the Eu^{3+} and Tb^{3+} emitting states indicate that the shielding of the metal ion from solvent molecules is rather inefficient. For the $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$ complex the lifetimes are temperature dependent which is attributed to the presence of an equilibrium between the metal emitting state and triplet excited states of the ligand; this process is most likely responsible for the low luminescence quantum yields and the oxygen effect on the Tb^{3+} luminescence. A detailed comparison between the photophysical properties in water and methanol allows us to conclude that the ligand **1** coordinates better to the metal ion in water than in methanol because of a stronger interaction in water between the phenanthroline branches and the metal ion. As to the application in fluoroimmunoassay, the Eu^{3+} and Tb^{3+} complexes of the ligand **1** present the highest molar extinction coefficients among the water-stable lanthanide complexes studied up to now. The value of the incident light-emitted light conversion efficiency, obtained from the absorption and emission efficiencies, makes the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex interesting as a luminescent label.

Introduction

The Eu^{3+} and Tb^{3+} ions have revealed to be efficient emitters when encapsulated by cage-type ligands incorporating chromophoric groups.[1] In these complexes the luminescence of the metal is obtained by the "antenna effect", which is defined as a light conversion process via an absorption–energy transfer–emission sequence involving distinct absorbing (ligand) and emitting (metal ion) components.[2,3] In such a process, the quantities that contribute to the luminescence intensity are (i) the intensity of the ligand absorption, (ii) the efficiency of the ligand-to-metal energy transfer, and (iii) the efficiency of the metal luminescence.

Among the species investigated up to now,[1] complexes of branched-macrocyclic ligands containing four chromophoric units play a special role because they absorb incident light very efficiently and shield, to a great extent, the metal ion from interaction with solvent molecules which would cause nonradiative decay of the luminescent level.[4] However, some of these complexes decompose in water,[5,6] which represents a disadvantage in view of the use of lanthanide complexes as luminescent labels in fluorimmunoassay (FIA).[7–9]

In this paper we report on the synthesis and the luminescence of Eu^{3+}, Tb^{3+}, and Gd^{3+} complexes of the branched-macrocyclic ligand **1**. Interestingly, the lanthanide complexes of the ligand **1** do not decompose in water in contrast to those of the analogous ligand containing 2,2′-bipyridine (bpy) instead of 1,10-phenanthroline (phen).[5] The study of their photophysical properties in water and methanol revealed that the ligand **1** coordinates better to the ion in water than in methanol, which is in contrast with the results of other cage-type, bpy-based ligands.[1,5] The behavior of the complexes of the ligand **1** shows that water as solvent can favor the complexation more than methanol, at least for certain types of ligands.

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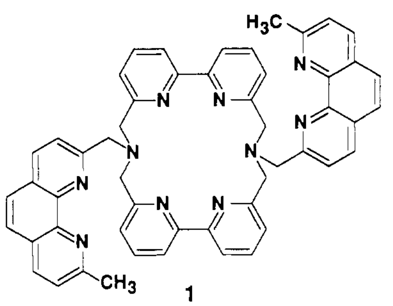

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A detailed analysis of the excited-state properties of the metal ion as well as of the ligand is performed, in order to elucidate the role played by the various factors that determine the metal luminescence properties.

Preliminary results of this work have been previously published.[10]

Results and Discussion

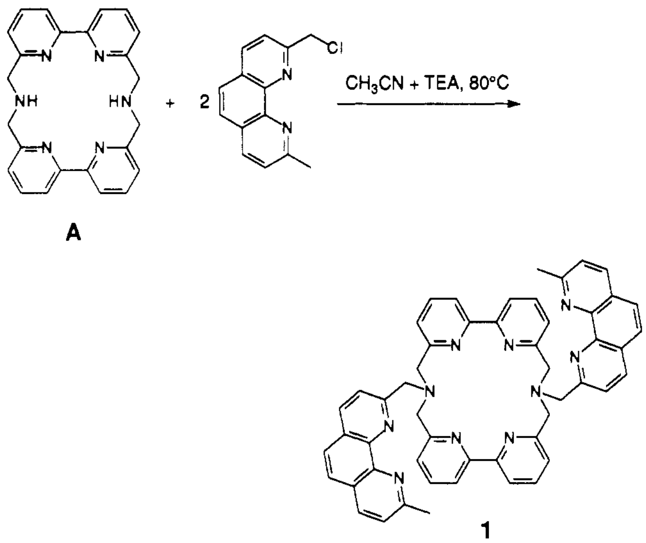
The synthesis of the branched-macrocyclic ligand **1** is depicted in Scheme 1. This ligand has been obtained by a procedure adapted from the method described in the literature[11] for the analogous ligand containing only bpy units. As a matter of fact, the synthetic procedure previously described could not be used because of the high reactivity of the phenanthroline derivatives with water in the presence of Na_2CO_3. Hence the following method was developed. The macrocycle incorporating 2,2′-bipyridine was mixed with water-free 2-(chloromethyl)-9-methyl-1,10-phenanthroline in anhydrous acetonitrile and heated at reflux in the presence of triethylamine as base. After recrystallization, the branched macrocycle **1** was obtained in good yield (75%).

The Eu^{3+}, Tb^{3+}, and Gd^{3+} complexes of the ligand **1** were readily prepared by reacting equimolar quantities of the ligand **1** and the lanthanide salt ($MCl_3 \cdot 6H_2O$; M = Eu, Tb, or Gd) in a methanol/dichloromethane solution (77–85% yield).

All the photophysical properties of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$, $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$, and $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complexes resulted to be constant for several days in water or methanol solutions, indicating that these complexes are kinetically inert in both solvents. This is of particular interest because the complexes of the analogous ligand incorporating two bpy's instead of two phen's decompose in water.[1,5] The more efficient complexation of the metal ion by ligand **1** may be related to the rigidity of the phenanthroline.

The absorption spectrum of the ligand **1** in methanol (Figure 1), where a quantitative spectrum could be obtained, is the sum of the absorption spectra of the separate chromophores. The maximum at 272 nm corresponds to the absorption maximum of the phen moiety while the maximum of the bpy absorption, which lies at 288 nm for the free 6,6′-dimethyl-2,2′-bipyridine, cannot be distinguished because it is overlapped by the phen absorption. Comparison of the absorption spectra of the ligand **1** and the Eu^{3+}, Tb^{3+}, and Gd^{3+} complexes in methanol (Figure 1) reveals that in going from the free ligand to the complex the absorption due to the phen subunits shows no appreciable shift and that a new band with a maximum at about 305 nm appears. This band is attributed to the bpy absorption, which would undergo some red shift in the complex. Such a behavior suggests a stronger complexation of the bpy's than of the phen's. This is not surprising if one considers that, most likely, the macrocycle encloses the metal ion,[5] and therefore the bpy's in the macrocycle may well approach the ion more than the phen's in the branches. In water a quantitative absorption spectrum of the ligand **1** could not be obtained because of the low solubility, and consequently a comparison analogous to that in methanol cannot be made. Anyway, it is interesting to notice that the absorption spectra of the complexes in water and methanol show important differences (Figure 1). In line with the interpretation given below for the different luminescence properties shown by the complexes in the two solvents, the changes of the absorption spectra are attributed to different complexation of the ligand to the metal ion rather than to a solvent effect.

Scheme 1



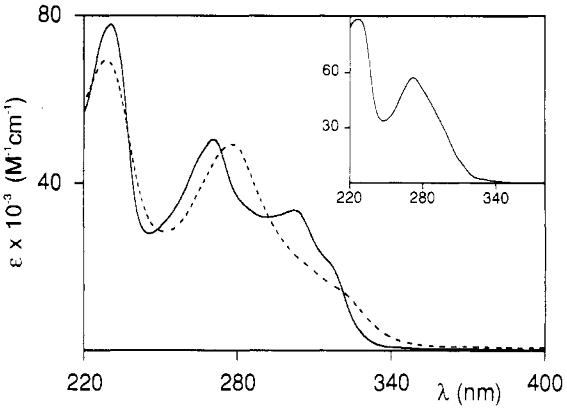


Figure 1. Absorption spectra of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex in water (- -) and methanol (—) solutions (the $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$ and $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complexes show similar spectra). Inset: absorption spectrum of the ligand **1** in methanol solution.

The observation of the Eu^{3+} and Tb^{3+} luminescence upon excitation in the ligand absorption indicates that an energy transfer from the ligand to the metal ion takes place. Comparison of the absorption and excitation spectra in water (Figure 2a) shows that the energy transfer involves both the bpy's and the phen's. More detailed, the shoulder above 300 nm is more pronounced in the excitation than in the absorption spectrum. This may be explained by a slightly more efficient energy transfer for the bpy's than for the phen's, considering that the bpy absorption becomes more important going to the red part of the spectrum. In methanol (Figure 2b) the excitation spectrum does not show the phen band with maximum at 272 nm expected on basis of the absorption spectrum, and a band with a maximum at 308 nm appears. This indicates that the difference in energy transfer efficiency for the bpy's and the phen's is much greater in methanol than in water, as also shown by the values of the luminescence quantum yields upon excitation in the bpy and phen absorption (Table 1).

As concerns the metal emission spectra of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ and $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$ complexes, we discuss in detail only the Eu^{3+} spectrum because the Tb^{3+} emission results in a spectrum difficult to be analyzed. The emission spectrum of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex in water (Figure 3) shows the bands corresponding to the $^5D_0 \rightarrow {}^7F_J$

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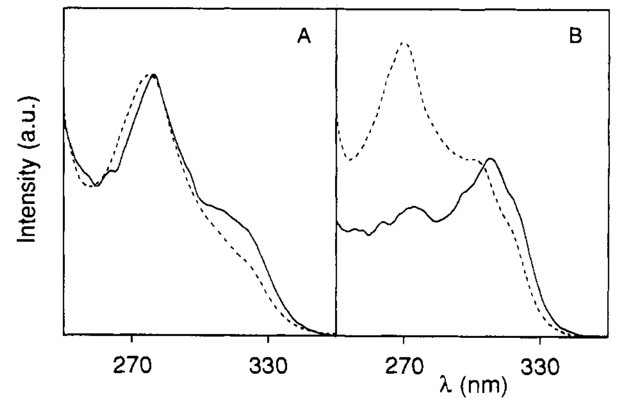

Figure 2. Luminescence excitation spectra (—) for the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex (λ_{em} = 618 nm) in water (A) and methanol (B) solutions (the $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$ complex shows similar spectra). For the sake of comparison also the absorption spectra of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex (- -) are shown.

(J = 0, 1, 2, 3, 4) transitions. The observation of only one component in the $^5D_0 \rightarrow {}^7F_0$ region suggests that only one Eu^{3+} emitting species is present, even though one cannot exclude the presence of species in which the $^5D_0 \rightarrow {}^7F_0$ transition is symmetry-forbidden.[4b,12] The pattern of the Eu^{3+} emission spectrum can be related to the site symmetry of the metal ion.[4b,12] By inspection of the CPK model of the molecule, D_{4h} symmetry may be likely attributed, in first approximation, to the eight coordinating nitrogens of the bpy and phen moieties. In such a symmetry, two components are expected for the $^5D_0 \rightarrow {}^7F_1$ transition, and the symmetry-forbidden $^5D_0 \rightarrow {}^7F_0$, $^5D_0 \rightarrow {}^7F_2$, and $^5D_0 \rightarrow {}^7F_4$ transitions must be very weak. Although this is certainly not the case, the $^5D_0 \rightarrow {}^7F_1$ transition is split in first approximation into two components and has a relatively high intensity. However, we see that one component of the $^5D_0 \rightarrow {}^7F_1$ transition splits and the $^5D_0 \rightarrow {}^7F_0$, $^5D_0 \rightarrow {}^7F_2$, and $^5D_0 \rightarrow {}^7F_4$ transitions are considerably intense. These observations point to a site symmetry of the Eu^{3+} ion lower than D_{4h}. As a matter of fact, C_2 or lower symmetries are compatible with the pattern of the emission spectrum. Such low symmetries may originate from different coordination of the metal ion by the phen's of the branches and the bpy's of the macrocycle. Moreover, the site symmetry of the metal ion may be affected by the presence of coordinated solvent molecules. The emission spectrum of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex in methanol is different from that in water. The presence of three regularly spaced components for the $^5D_0 \rightarrow {}^7F_1$ transition points to a lower site symmetry in methanol than in water.

Now we will discuss the processes involved in the deactivation of the Eu^{3+} and Tb^{3+} luminescent states. The role played by the vibronic deactivation mechanism involving the O–H oscillators is evaluated by comparison of the metal luminescence lifetimes and quantum yields in hydrogenated and deuterated solvents.[3,4] The importance of the thermally activated nonradiative decays is studied by analyzing the lifetime measurements at 300 and 77 K.[3,13]

Let us now discuss the results obtained for the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex (Table 1). As far as the measurements performed in water solutions are concerned, the lifetimes and quantum yields at 300 K are much lower in H_2O than in D_2O, which indicates that the vibronic deactivation mechanism is quite efficient. By using Horrock's equation[4] it is possible to calculate that ~3.3 water molecules (estimated error ± 0.5) are coordinated to the metal ion at room temperature. The lack of any temperature dependence of the lifetimes in D_2O suggests that thermally activated decay processes do not play any important role in the deactivation of the metal emitting state. In water the decay of the Eu^{3+} metal luminescence at 77 K is nonexponential and can be analyzed to give two lifetimes, which are attributed to the presence of different coordination spheres of the metal ion at low temperature. The fact that at 77 K a single-exponential decay is obtained in D_2O suggests that the coordination spheres may involve a different number of coordinated water molecules. As a matter of fact, changes in the number of coordinated water molecules in going from room temperature to low temperature has previously been observed for lanthanide complexes of other cage-type ligands exhibiting some flexibility.[1] As to the luminescence quantum yields, their values slightly depend on the nature of the excited chromophore. This indicates a different efficiency of the ligand-to-metal energy transfer for the two chromophores, as also suggested while discussing the excitation spectrum. In methanol solutions the photophysical behavior of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex shows some differences compared to water solutions. The increase of the lifetimes and the quantum yields observed in going from MeOH to MeOD is smaller than that found in going from H_2O to D_2O which is in line with the lower efficiency of MeOH in inducing nonradiative deactivation processes via O–H groups. The dependence of the luminescence quantum yields on the nature of the excited chromophores is much more evident than in water, suggesting that in methanol the conformation of the complex may be different (see below).

Now the results of the $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$ complex will be discussed (Table 1). The strong increase of the lifetime values in water solution upon lowering the temperature indicates that thermally activated decay processes of the Tb^{3+} emitting state via short-lived, upper-lying excited states are very efficient. The participation of f–d states or charge-transfer states involving the ligand and the metal ion[14] to these decay processes is unlikely because these states lie at energy levels much higher than the $^5D_4$ emitting state of the Tb^{3+} ion. Therefore, the thermally activated decay processes most likely involve triplet excited states of the ligand, as previously found for $[\mathrm{Tb}\subset\mathrm{bpy{\cdot}bpy{\cdot}bpy}]^{3+}$.[3] In order to localize these states, the phosphorescence spectrum of the ligand in the $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complex is measured[3] because in the $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$ complex the ligand phosphorescence is completely quenched by the energy transfer to the metal ion. In this spectrum (Figure 4) the band at highest energy lies at 480 nm, indicating a triplet excited state of the ligand at ~20 800 cm^{-1}. This triplet can be attributed to the phenanthroline moiety on basis of its energy location (the bpy triplet was found to lie at about 21 600 cm^{-1} in several lanthanide complexes[1]) and of the pattern of the phosphorescence spectrum (see below). Therefore, thermally activated decay via a back-energy-transfer from the $^5D_4$ Tb^{3+} emitting state, which lies at 20 400 cm^{-1},[15] to the phen triplet excited state is possible. Moreover, the increase of the lifetime and quantum yield values upon deaeration proves that the $^5D_4$ Tb^{3+} level is in equilibrium with a triplet excited state of the ligand. In fact, the quenching of the Tb^{3+} luminescence (k_q = 3.5×10^7 $\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ in H_2O or D_2O solutions) should reflect the interaction of O_2 with the triplet excited state of the ligand because, as is known,[3] the luminescent states of the lanthanide ions are not quenched by oxygen. As a matter of fact, in the $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complex the ligand triplet excited state is quenched by oxygen, the lifetimes in water at 300 K being 3 and 40 μs in aerated and deaerated solutions, respectively. From these values the rate constant 7.7×10^8 $\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ is obtained for the quenching by oxygen. As to the vibronic deactivation mechanism, at 300 K this mechanism is detectable only in deaerated solutions. From the lifetime values a number of ~2.7 coordinated water molecules can be calculated (note that in the presence of this number of coordinated water molecules the lifetimes in aerated H_2O and D_2O solutions should differ only by 3%, which lies within the experimental error). At low temperature the effect of the vibronic deactivation mechanism is more evident because the bimolecular quenching by O_2 is absent and the back-energy-transfer is much

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Table 1. Metal Luminescence Lifetimes (in ms) and Quantum Yields[a]

	$[\mathrm{Eu}\subset\mathbf{1}]^{3+}$			$[\mathrm{Tb}\subset\mathbf{1}]^{3+}$		
solvent	$\tau_{300\ \mathrm{K}}$[b]	$\tau_{77\ \mathrm{K}}$[b]	$\Phi \times 10^2$[c]	$\tau_{300\ \mathrm{K}}$[b]	$\tau_{77\ \mathrm{K}}$[b]	$\Phi \times 10^2$[c]
H_2O	0.27[d]	(0.3, 1.0)[e]	1.3,[d,f] 1.6[d,g]	0.06 0.35[h]	1.4	0.7,[f] 0.7[g] 2.8,[f,h] 4.0[g,h]
D_2O	1.85	1.9	9,[f] 10[g]	0.06 0.45[h]	2.6	0.7,[f] 0.7[g] 5.0,[f,h] 5.7[g,h]
MeOH	0.70	0.75	2.7,[f] 6.0[g]	(0.03, 0.1)[e] (0.2, 0.8)[e,h]	2.1	0.2,[f] 0.35[g] 3.8,[f,h] 6.0[g,h]
MeOD	1.60	1.65	6.5,[f] 13[g]	(0.03, 0.1)[e] (0.6, 1.4)[e,h]	2.5	0.2,[f] 0.35[g] 4.5,[f,h] 8[g,h]

[a] Measured in aerated solutions, unless otherwise noted. [b] Measured in correspondence with the most intense metal emission bands ($^5D_0 \rightarrow {}^7F_2$ for Eu^{3+} and $^5D_4 \rightarrow {}^7F_6$ for Tb^{3+}). Experimental error < 10%. [c] Measured at 300 K upon excitation in the ligand absorption (see footnotes *f* and *g*). Experimental error ~ 30%. [d] The same results were obtained in deaerated solutions. [e] The decay is nonexponential and has been satisfactory fitted by a biexponential equation. [f] Measured upon excitation of the phen's at 278 nm in water and 270 nm in methanol (see text). [g] Measured upon excitation of the bpy's at 305 nm (see text). [h] Deaerated solution.

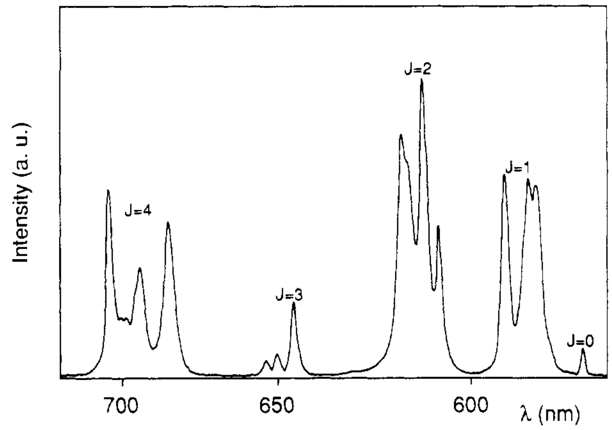

Figure 3. Emission spectrum of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex in water solution (λ_{exc} = 278 nm). The *J* values for the $^5D_0 \rightarrow {}^7F_J$ Eu^{3+} transitions are shown.

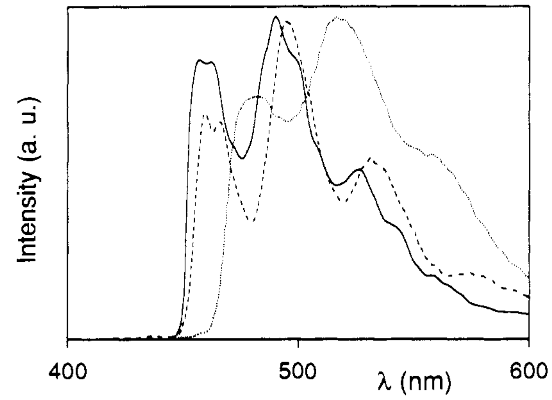

Figure 4. Phosphorescence spectra at 77 K of the ligand **1** in methanol (—) and of the $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complex in methanol (- -) and water (···) solutions, upon excitation with λ = 270 nm for the ligand **1** and the $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complex in methanol and λ = 278 nm for the $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complex in water.

Table 2. Ligand Phosphorescence Data[a]

compound	λ_{max} (nm)[b]	τ (s)[b,c]
6,6′-dimethyl-2,2′-bipyridine	472	1.0
2,9-dimethyl-1,10-phenanthroline	493	2.7
1	491	2.2
$[\mathrm{Gd}\subset\mathbf{1}]^{3+}$	495 517[d]	0.17 0.025[d]

[a] Measured at 77 K in methanol solution, unless otherwise noted. [b] Excitation in correspondence with the absorption maximum. [c] Experimental error < 10%. [d] Measured at 77 K in water solution.

less efficient. The number of coordinated water molecules for the $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$ complex at 77 K, ~1.5, is lower than that obtained at 300 K. It is noticeable that in H_2O only one lifetime is obtained, in contrast with the observation of two lifetimes for the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex in the same conditions. Most likely the presence at 77 K of coordination spheres containing different numbers of coordinated water molecules is not detectable for the $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$ complex because the Tb^{3+} ion is less sensitive to the vibronic deactivation mechanism.[4] The quantum yields in H_2O and D_2O aerated solutions appear to be independent of the nature of the excited chromophore while in deaerated solutions they are slightly different for excitation in the bpy or in the phen absorption. Considering that the quantum yield values in aerated solutions are much lower, such small differences may not be detectable. In methanol the same nonradiative deactivation processes observed in water, i.e. the vibronic mechanism (only detectable in deaerated solutions), the thermally activated decay process, and the quenching process by oxygen, are present. It is interesting to notice that at 300 K the decays of the luminescence are nonexponential while single-exponential decays are obtained at 77 K. Considering that at 300 K the phen's play an important role in the nonradiative deactivation of the $^5D_4$ Tb^{3+} emitting state while such a mechanism is totally inefficient at 77 K, the presence of species which differ for the coordination of the phen branches to the metal ion may explain this behavior. The observation of exponential decays for the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex at room temperature in methanol is not in contrast with this hypothesis. In fact, the phen's do not play any role in the nonradiative deactivation of the Eu^{3+} excited state. Moreover, the observation that the ratios of the quantum yields upon excitation in the bpy and the phen absorption are higher in methanol than in water both for the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ and $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$ complexes suggests that the phen's are less coordinated to the metal ion in methanol than in water.

The different complexation in methanol and water of the phen's to the metal ion may also explain the ligand phosphorescence properties in the $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complex. Comparison of the phosphorescence spectra (Figure 4) and lifetimes (Table 2) of the ligand **1** and the free chromophores (6,6′-dimethyl-2,2′-bipyridine and 2,9-dimethyl-1,10-phenanthroline) shows that the ligand phosphorescence can be attributed to the phenanthroline moiety. The phosphorescence spectrum of the $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complex in methanol is similar to that of the ligand **1** in methanol (Figure 4), indicating that the phen's are weakly coordinated to the metal ion, since a strong complexation usually causes a significant red shift.[1] Unfortunately, the phosphorescence spectrum of the ligand **1** in water cannot be obtained, so that in this solvent the effect of complexation on the ligand phosphorescence cannot be directly evaluated. However, the pattern of the phosphorescence spectrum of the $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complex in water is similar to that in methanol, but the spectrum is considerably shifted to lower energy. This behavior is in agreement with the stronger complexation of the phen's to the metal ion in water than in methanol suggested while discussing the metal emission properties. As to the phosphorescence lifetimes of the $[\mathrm{Gd}\subset\mathbf{1}]^{3+}$ complex (Table 2), the shorter value measured in water than in methanol is attributed to a stronger heavy atom effect, which still suggests a more efficient complexation.

Conclusions

The lanthanide complexes of the ligand **1** do not decompose in water in contrast to those of the related ligand containing 2,2′-bipyridine instead of the 1,10-phenanthroline. This may be related to the rigidity of the phenanthroline. In methanol, the complexation appears to be worse than in water. This is ascribed to a weaker interaction between the phen branches and the metal ion.

As to the photophysical properties, the Eu^{3+} and Tb^{3+} complexes of the ligand **1** present the highest molar extinction coefficients among the water-stable lanthanide complexes of encapsulating ligands investigated up to now, but conversion of absorbed light into emitted light is rather inefficient compared to other lanthanide complexes.[1] This is ascribed to rather inefficient shielding of the metal ion from interaction with water molecules, to low efficiency of the ligand-to-metal energy transfer, and, in the case of $[\mathrm{Tb}\subset\mathbf{1}]^{3+}$, to deactivation via back-energy-transfer from the metal emitting state to ligand excited triplet states. However, it must be noticed that for the application in FIA the quantity of primary importance is the intensity of the luminescence, which depends not only on the efficiency of the conversion of absorbed light into emitted but also on the efficiency of the absorption. As a matter of fact, in water solution the efficiency of the incident light-emitted light conversion for the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex is close to that for $[\mathrm{Eu}\subset\mathrm{bpy{\cdot}bpy{\cdot}bpy}]^{3+}$, which is considered interesting as luminescent label.[16]

Moreover, if methyl groups are removed in the ligand **1** the intensity of the luminescence could increase significantly. In fact, for Eu^{3+} complexes of other branched macrocyclic ligands containing methyl groups in the branches, removal of these groups gives rise to much more intense luminescence because the number of coordinated water molecules decreases and the efficiency of the ligand-to-metal energy transfer increases.[5]

Experimental Section

The 8,21-bis[(9-methyl-1,10-phenanthroline-2-yl)methyl]-8,21,27,28,29,30-hexaazapentacyclo[21.3.1.$1^{2,6}$.$1^{10,14}$.$1^{15,19}$]triaconta-1(27),2,4,6(30),10,12,14(29),15,17,19(28),23,25-dodecaene ligand **1** was synthesized in a straightforward manner by reacting the bpy·bpy macrocycle **A**[17] with 2-(chloromethyl)-9-methyl-1,10-phenanthroline.[18] The phenanthroline derivative (280 mg, 1.07 mM) was heated at reflux in benzene (60 mL) for 4 h in a Dean–Stark apparatus. After this solution was cooled to room temperature, the solvent was evaporated and the solid was dried under high vacuum. This residue was dissolved in acetonitrile (70 mL, freshly distilled over $\mathrm{P_2O_5}$), and a suspension of macrocycle **A** (200 mg, 0.51 mM) in triethylamine (10 mL, freshly distilled over KOH) was added under argon. The mixture was refluxed for 15 h. After it cooled to room temperature the solvent was evaporated. The residue was dissolved in a minimal amount of dichloromethane (ca. 10 mL). After filtration over Celite, hexane (10 mL) was added as a countersolvent. Slow evaporation of the solvent caused the precipitation of the pure branched macrocycle **1** (380 mg, 75%). The use of hydrated phenanthroline derivatives markedly decreases the yield (~25%). A low yield (<5%) was obtained when 2-(bromomethyl)-9-methyl-1,10-phenanthroline monohydrate and $\mathrm{Na_2CO_3}$ as base were used under the same reaction conditions.

The ligand **1** was characterized by ^{1}H- and ^{13}C-NMR and FAB^{+} spectra as well as by elemental analysis. Mp: >260 °C. ^{1}H-NMR ($\mathrm{CD_2Cl_2}$): δ 8.39 (d, ^{3}J = 8.3 Hz, 2H), 8.30 (d, ^{3}J = 8.3 Hz, 2H), 8.19 (d, ^{3}J = 8.2 Hz, 4H), 7.80 (s, 4H), 7.65 (d, ^{3}J = 7.7 Hz, 2H), 7.61 (d, ^{3}J = 8.2 Hz, 2H), 7.36 (d, ^{3}J = 7.7 Hz, 2H), 7.33 (d, ^{3}J = 7.7 Hz, 2H), 7.18 (d, ^{3}J = 7.6 Hz, 4H), 4.58 (s, 4H), 4.11 (s, 8H), 2.89 (s, 6H). $^{13}\mathrm{C}\{^{1}\mathrm{H}\}$ NMR ($\mathrm{D_2O}$ + DCl 20% + *t*-BuOH): δ 158.33 (CC), 152.07 (CC), 151.97 (CC), 148.75 (CH), 147.57 (CC), 146.72 (CC), 146.50 (CC), 141.06 (CH), 138.66 (CC), 136.55 (CC), 131.55 (CH), 131.25 (CH), 130.62 (CH), 129.63 (CH), 128.58 (CH), 128.31 (CH), 125.71 (CH), 62.15 ($\mathrm{CH_2}$), 60.77 ($\mathrm{CH_2}$), 22.18 ($\mathrm{CH_3}$). FAB-MS (positive mode, *m*-nitrobenzylalcohol (*m*-NBA) as matrix): *m/z* 807.4 $(\mathrm{M + H})^{+}$, 599.3 $(\mathrm{M - phen - H})^{+}$, 391.2 $(\mathrm{M - phen - 2H})^{+}$. Anal. Calcd for $\mathrm{C_{52}H_{42}N_{10}}$: C, 77.39; H, 5.25; N, 17.37. Found: C, 77.32; H, 5.18; N, 17.28.

$[\mathrm{Gd}\subset\mathbf{1}]\mathrm{Cl_3}\cdot7\mathrm{H_2O}$, $[\mathrm{Eu}\subset\mathbf{1}]\mathrm{Cl_3}\cdot7\mathrm{H_2O}$, and $[\mathrm{Tb}\subset\mathbf{1}]\mathrm{Cl_3}\cdot6\mathrm{H_2O}$ were prepared by the following procedure. To a solution of the ligand **1** (50 mg, 0.06 mM in 10 mL of $\mathrm{CH_2Cl_2}$) was added an equimolar quantity of the lanthanide salt ($\mathrm{MCl_3}\cdot6\mathrm{H_2O}$; M = Gd, Tb, or Eu) dissolved in 3 mL of methanol. When this was heated at 60 °C, the solution became highly fluorescent when irradiated with 365-nm light. After 1 h the solution was cooled to room temperature, and the solvent was removed under vacuum. The white residue was dissolved in ethanol and filtered over Celite, and the complex was crystallized twice by slow diffusion of diethyl ether into the solution. The complexes were characterized by FAB^{+} and by elemental analysis. $[\mathrm{Gd}\subset\mathbf{1}]\mathrm{Cl_3}\cdot7\mathrm{H_2O}$ (yield 77%): FAB^{+} (*m*-NBA) *m/z* 1033.8 $(\mathrm{M - Cl})^{+}$, 827.8 (M – Cl – phen), 619.8 (M – Cl – 2phen – H). Anal. Calcd for $\mathrm{C_{52}H_{42}N_{10}GdCl_3}\cdot7\mathrm{H_2O}$: C, 52.19; H, 4.71; N, 11.70. Found: C, 52.15; H, 4.67; N, 11.73. $[\mathrm{Tb}\subset\mathbf{1}]\mathrm{Cl_3}\cdot6\mathrm{H_2O}$ (yield 82%): FAB^{+} (*m*-NBA) *m/z* 1035.3 $(\mathrm{M - Cl})^{+}$, 1000.3 (M – 2Cl), 828.3 (M – Cl – phen), 621.1 (M – Cl – 2phen). Anal. Calcd for $\mathrm{C_{52}H_{42}N_{10}TbCl_3}\cdot6\mathrm{H_2O}$: C, 52.91; H, 4.61; N, 11.87. Found: C, 52.95; H, 4.52; N, 11.78. $[\mathrm{Eu}\subset\mathbf{1}]\mathrm{Cl_3}\cdot7\mathrm{H_2O}$ (yield 89%): FAB^{+} (*m*-NBA) *m/z* 1028.9 $(\mathrm{M - Cl})^{+}$, 994.0 (M – 2Cl), 958.0 (M – 3Cl – H), 821.9 (M – Cl – phen), 786.9 (M – 2Cl – phen), 751.9 (M – 3Cl – phen). Anal. Calcd for $\mathrm{C_{52}H_{42}N_{10}EuCl_3}\cdot7\mathrm{H_2O}$: C, 52.42; H, 4.74; N, 11.76. Found: C, 52.44; H, 4.57; N, 11.72.

From the absorption spectra in water solution the following numbers were obtained for $\lambda_{\max}$ (nm) and $\epsilon_{\max}$ ($\mathrm{M^{-1}\,cm^{-1}}$), respectively: $[\mathrm{Eu}\subset\mathbf{1}]\mathrm{Cl_3}$: 229, 278; 67 000, 46 000. $[\mathrm{Tb}\subset\mathbf{1}]\mathrm{Cl_3}$: 229, 278; 66 000, 43 000. $[\mathrm{Gd}\subset\mathbf{1}]\mathrm{Cl_3}$: 229, 278; 66 000, 45 000.

The 200.1-MHz ^{1}H- and 50.3-MHz ^{13}C-NMR spectra were obtained at room temperature on a Bruker-AC-200 spectrometer. The spectra were referenced internally to the residual solvent resonances ($\mathrm{CD_2Cl_2}$, ^{1}H, δ 5.32 ppm; *t*-BuOH, ^{13}C, δ 31.6 and 68.7 ppm). Fast-atom bombardment spectra were recorded with a ZAB-HF-VG analytical apparatus. Elemental analyses were performed by the analytical service of the Centre de Recherche Chimie, Strasbourg, France.

The solvents used for the photophysical measurements were triply distilled water, $\mathrm{D_2O}$ (99.8%D, Carlo Erba), MeOH (fluorescence grade, Aldrich), an MeOD (99.8%D, Carlo Erba). Degassed solutions were obtained by repeated freeze–pump–thaw cycles.

The luminescence spectra were obtained with a Perkin-Elmer LS 50 spectrofluorimeter and a Perkin-Elmer 650-40 spectrofluorimeter. The high-resolution emission spectrum of the $[\mathrm{Eu}\subset\mathbf{1}]^{3+}$ complex was registered on a Jasco R300 Raman spectrophotometer, using a filtered (260–350-nm band-pass filter) xenon lamp as an exciting source. The UV–vis absorption spectra were measured with a Perkin-Elmer Lambda 6 spectrophotometer.

The luminescence decays were acquired by a Perkin-Elmer LS 50 spectrofluorimeter and analyzed with a least-squares fitting program.

The lifetimes of the triplet excited states at 300 K have been obtained from triplet-triplet absorption measurements performed using a JK System 2000 neodymium YAG DLPY4 laser (λ_{exc} = 255 nm; pulse half-width = 25 ns) as exciting source and a Varian 300-W xenon lamp as analyzing light. The decays were acquired at 430 nm by using a Tektronix 466 storage oscilloscope and analyzed with a least-squares fitting program.

The luminescence quantum yields were obtained by the method described by Haas and Stein[19] using as standards $\mathrm{Ru(bpy)_3}^{2+}$ (Φ = 0.028 in aerated water[20]) for the Eu^{3+} complex and quinine sulfate (Φ = 0.546 in 1 N $\mathrm{H_2SO_4}$[21]) for the Tb^{3+} complex. The values measured in methanol solutions were corrected for the refraction index.[22]

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