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Novel terpyridine macrocyclic complexing agent and luminescence of its neutral Ln(III) complexes (Ln = Eu, Tb, Sm, Dy) in aqueous solution

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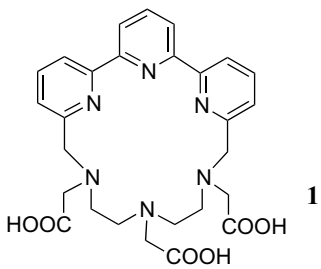
Abstract—A very convenient synthesis of an 18-membered triaza-macrocyclic ligand bearing one endocyclic terpyridine unit and three acetate pendant groups is reported. This nonadentate ligand forms very stable neutral lanthanide complexes in aqueous solution and efficiently shields the metal ion from the water environment. In this medium the Eu(III) and Tb(III) complexes are strongly fluorescent through an energy-transfer luminescence pathway ($\tau \sim 1$ ms and $\Phi \sim 20\%$). © 2001 Elsevier Science Ltd. All rights reserved.

The development of lanthanide luminescence spectroscopy for in vitro and in vivo biomedical diagnostics[1–5] has prompted the search for new photoactive ligands for Eu(III), Tb(III) and to a lesser extent Sm(III) and Dy(III)) ions. An efficient luminescent lanthanide complex should be obviously characterized by a high luminescence lifetime and a high quantum yield. However, other features such as solubility and kinetic stability of the complexes in aqueous solution may be critical and must also be controlled where biological applications are concerned. As a consequence of these requirements of optimal kinetic inertness and luminescence, only a few viable lanthanide labels have been developed and tested.[6]

For complexes of practical use the ligand must satisfy important conditions. It must have a suitable excitation spectrum to sensitize by ligand-to-metal energy transfer the luminescent f–f excited states of the lanthanide ion. Furthermore, it must display a high dendicity to prevent coordination of water molecules which cause non-radiative vibronic deactivation via the O–H oscillators[7] and which are often involved in the first step of decomplexation reactions. The net charge of the complex is also an important factor to take into account; since positively or negatively charged complexes may lead to non specific binding in biological systems.[8–10]

With these points in mind and in the course of our studies of luminescent lanthanide complexes,[11–13] we planned the preparation of the triaza-triacetate ligand **1** containing a terpyridine moiety as part of its macrocyclic ring.



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The presence of three carboxylic acids allows on one hand the formation of strong neutral complexes owing to the establishment of electrostatic interactions and on the other hand an increased solubility in water solution of the ligand and its lanthanide complexes. The terpyridine group is a very suitable energy-absorbing and energy-transferring moiety. It exhibits an intense absorption band in the near UV region and has its lowest excited state ($^3\pi\pi^*$) sufficiently high in energy to be able to transfer excitation to the luminescence lanthanide levels. In addition, the nine coordination sites reported for these lanthanide ions[14] in aqueous solutions may be occupied by the nonadentate ligand **1**. The

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preparation of this ligand and some relevant photophysical data of the corresponding Ln(III) (Ln = Eu, Tb, Sm, Dy) complexes are reported in this paper.

As evidenced in numerous reports,[15] the most crucial problem in the synthesis of macrocyclic compounds consists of the macrocyclization process. Initial route to obtain the 18-membered macrocyclic ligand **1** involved the treatment of 1,4,7-tritosyl-1,4,7-triazaheptane **2** with 6,6″-bis-bromomethyl-[2,2′:6′,2″] terpyridine **3** in the presence of potassium carbonate as a base and acetonitrile as a solvent (Scheme 1). This modified Richman and Atkins method[16] was used recently for obtaining analogous 12- or 15-membered macrocycles embodying pyridine or phenanthroline moieties, respectively.[17,18] Following this procedure, the above reaction did not give the 1:1 cyclization product, but it mainly afforded the dimeric 36-membered macrocycle **4** in 20% yield. In the second strategy (Scheme 2), it was decided to use the triamine **6** in order to benefit from the presence of coordinating groups in this building block and for controlling the formation of the 18-membered ring by a metal 'template' ion effect. The target compound **6** was readily obtained (72% overall yield) in a three-step sequence: (i) reductive amination of diethylenetriamine with benzaldehyde, (ii) alkylation of the resulting secondary triamine with *tert*-butylbromoacetate, (iii) conventional catalytic hydrogenolysis (Pd/C) under hydrogen pressure and at room temperature for the debenzylation reaction. Condensation of the building block **6** with dibromide **3** was carried out in CH_3CN in the presence of M_2CO_3 as a base (M = Li, Na, K). The selectivity of the macrocyclization reaction is strongly dependent on the nature of the alkaline carbonate used. The procedure using Na_2CO_3 mainly generated the 18-membered macrocycle **7** which was isolated, after column chromatography, as its NaBr complex in 36% yield. When a lithium ion was used, the 1:1 macrocyclization process was also favored, but this reaction was accompanied by polymerization to a higher extent. In contrast, the same reaction conditions using K_2CO_3 yielded the dimer structure **8** (31% isolated yield). Finally, treatment of **7** with trifluoroacetic acid gave the target ligand **1** in 77% yield.

The macrocyclic ligand **1** formed stable Ln(III) complexes (Ln = Eu, Tb, Sm, Dy) when treated with $LnCl_3$ in aqueous solution. These complexes were characterized in this medium by UV, MS and ligand-sensitized Ln^{3+} luminescence techniques. Upon addition of the Ln^{3+} salts, significant red shifts ($\Delta\lambda \sim 30$ nm) were observed in the ligand absorption spectra (Fig. 1).

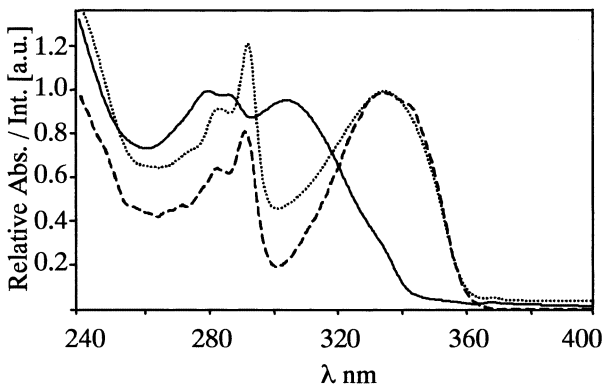
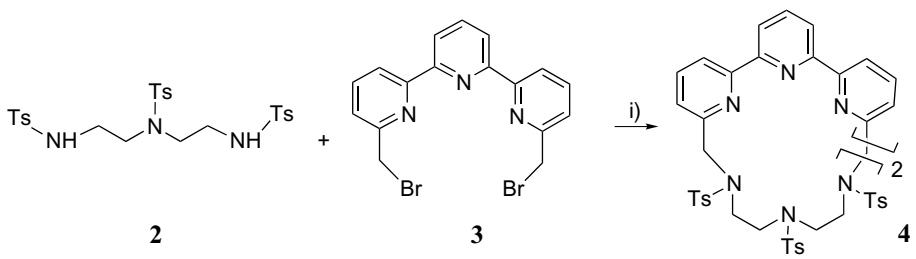
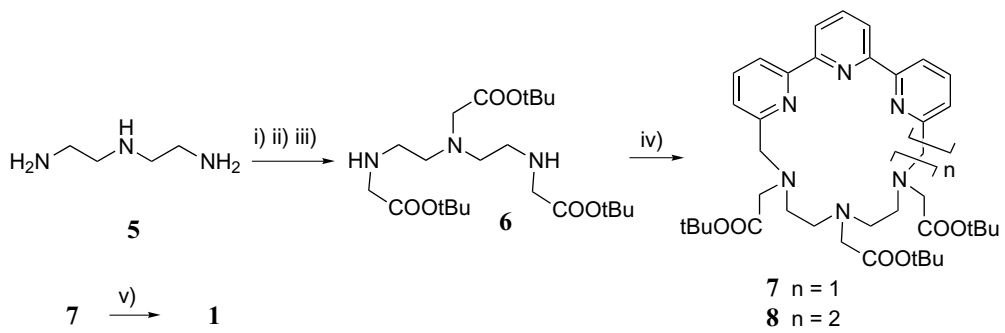


Figure 1. Normalized absorption spectra of **1** (full line) and [(**1**-3H)·Tb] complex (dotted line) in borate buffer solution (pH 8.6). The dashed line represents the excitation spectrum of [(**1**-3H)·Tb] ($\lambda_{em} = 545$ nm).



Scheme 1. (i) K_2CO_3, CH_3CN, reflux, [reactants] = 2.3×10^{-3} M (**4**: 20% yield).



Scheme 2. (i) a. C_6H_5CHO, EtOH, 50°C, b. $NaBH_4$, EtOH, 50°C, c. HCl aq. 37%, CH_2Cl_2 (94%); (ii) $BrCH_2COOtBu$, K_2CO_3, CH_3CN, reflux (79%); (iii) Pd/C 10%, H_2/3 bar, MeOH (97%); (iv) 1 equiv. **3**, 10 equiv. M_2CO_3, CH_3CN, reflux, [reactants] = 2.3×10^{-3} M (M = Na, **7** (36%), M = K, **8** (31%); (v) CF_3COOH, CH_2Cl_2 (77%).

This is caused by the polarization of the terpyridine moiety and the variation of the dihedral angle between adjacent aromatic rings during the complexation. The observed absorption maxima at $\lambda \sim 335$ nm were close to those recorded for lanthanide complexes of acyclic ligands derived from terpyridine.[19] The characterization of these complexes by mass spectrometry unambiguously showed complexation of one cation per molecule. In the ESI$^+$-MS spectra of all complexes, the base peaks corresponded to [(**1**-3H)·Ln+H]$^+$ species; the characteristic isotope patterns of Eu^{3+}, Sm^{3+} and Dy^{3+} were clearly observed for these peaks. No peak ascribable to the free ligand was observed in the mass spectra, indicating a high affinity of ligand **1** for lanthanide ion. Upon excitation at 335 nm of the terpyridine chromophore of the [(**1**-3H)·Ln] complexes, the typical lanthanide luminescence bands were observed corresponding to the $^5D_0 \rightarrow {}^7F_j$ (Eu^{3+}), $^5D_4 \rightarrow {}^7F_j$ (Tb^{3+}), $^4G_{5/2} \rightarrow {}^6H_j$ (Sm^{3+}) and $^4F_{9/2} \rightarrow {}^6H_j$ (Dy^{3+}) transitions. Almost the total emission (45–55%) was centered on the 618, 545, 602 and 577 nm peak for these [(**1**-3H)·Ln] complexes (Ln = Eu, Tb, Sm, and Dy, respectively). A representative emission spectrum for the terbium complex is shown in Fig. 2. The close similarity between the excitation spectra of these complexes, which fit their absorption spectra (Fig. 1), strongly suggest that the energy transfer processes involve the same excited state of the ligand.

In aqueous solution the luminescence lifetimes are in the μs range (τSm = 15 μs, τDy <10 μs) or in the ms range (τEu = 1.06 ms, τTb = 1.11 ms). The relative lack of variation (H_2O versus D_2O) of the luminescence lifetimes of the Eu and Tb complexes indicated that no water molecule is coordinated to the metal ion. Use of the conventional analysis of Horrocks[20] reveals the apparent hydration state in [(**1**-3H) Eu] and [(**1**-3H) Tb] to be 0.37 and 0.07, respectively, which is consistent with the presence of water molecules only in the lanthanide secondary coordination sphere. This suggests that the nine binding sites provided by the ligand **1**, which wraps about the metal ion uniformly, coordinate the metal ion. Although the terpyridine chromophore is

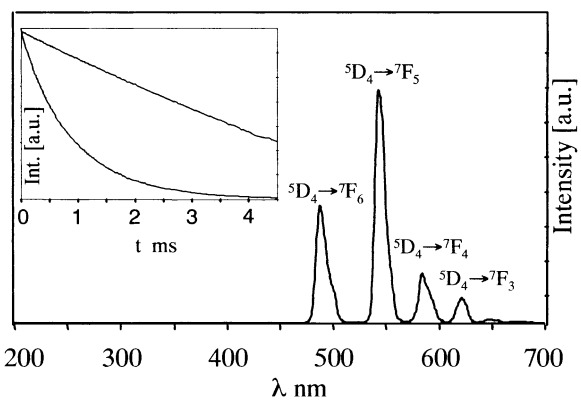


Figure 2. Emission spectrum ($\lambda_{ex} = 335$ nm) of [(**1**-3H)·Tb] in borate buffer solution (pH 8.6) showing $^5D_4 \rightarrow {}^7F_j$ transitions. Insert: excited-state lifetime: luminescence decay curve (bottom plot) and ln(intensity) versus time (upper plot).

able to sensitize the luminescence of the Sm^{3+} and Dy^{3+} in ligand **1**, the luminescence quantum yield values are very low (<10^{-3}). In contrast [(**1**-3H) Eu] and [(**1**-3H) Tb] complexes display high quantum yields (0.18 and 0.21, respectively) and seem quite competitive with those reported in the literature for either Eu^{3+} or Tb^{3+} complexes in aqueous solution and sensitized in this important excitation wavelength region (around 337 nm).[21,22] The emission properties of these complexes in aerated water solution at room temperature remained unchanged for several days in the examined pH range (6.6–8.6), indicating that they are kinetically inert in this medium. We also studied the resistance of the europium and terbium complexes to dissociation in the presence of other strongly binding competing ligands. No decomposition of these complexes was observed after 1 day in the presence of a 5-fold excess of EDTA and DTPA. These results indicate high stability constants between ligand **1** and lanthanide ions (log β >20).

In conclusion, the synthesis of a new lanthanide photoactive ligand was readily achieved and its coordination ability toward lanthanide ions was established. The described Eu(III) and Tb(III) complexes showed high luminescence properties (decay time, quantum yield, excitation wavelength) and stability in aqueous solutions and appeared to be highly interesting as luminescent biolabels. The introduction in ligand **1** of functional groups for attachment to biological materials is in progress.

All new compounds were characterized spectroscopically, including elemental analysis or (and) mass measurements.[†]

[†] Characterization data for compounds **6**, **1** and complexes [(**1**-3H)·Ln]: triamine **6**: IR (neat): ν 3328 (NH), 1734 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.43 (s, 9H), 1.44 (s, 18H), 2.67 (t, 4H, J = 4.0 Hz), 2.81 (t, 4H, J = 4.0 Hz), 2.91 (s, 2H), 3.32 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 28.1 (CH_3), 28.2 (CH_3), 47.2 (CH_2), 51.2 (CH_2), 53.6 (CH_2), 55.7 (CH_2), 80.9 (C), 81.2 (C), 171.0 (C), 171.2 (C); MS (ES+): m/z 468.3 [M+Na]$^+$ (5%), 446.3 [M+H]$^+$ (100%); Anal. calcd for $C_{22}H_{43}O_6N_3$, 0.5H_2O: C, 58.13; H, 9.76; N, 9.24. Found: C, 58.15; H, 9.89; N, 9.08.

Macrocycle **1**: IR (KBr pellet): ν 3427 (OH), 1680 (CO); ^{1}H NMR (D_2O, 200 MHz): δ 3.0–4.0 (m, 14H), 4.85 (s, 4H), 7.7 (m, 2H), 8.27–8.40 (m, 4H), 8.59 (m, 3H); ^{13}C NMR (D_2O, 100 MHz): δ 51.5 (CH_2), 53.2 (CH_2), 55.0 (CH_2), 55.8 (CH_2), 58.3 (CH_2), 124.2 (CH), 125.4 (CH), 127.9 (CH), 141.5 (CH), 149.0 (C), 151.4 (C), 171.0 (C), 177.8 (C); MS (ES+): m/z 573.0 [M+K]$^+$ (11%), 557.1 [M+Na]$^+$ (65%), 535.1 [M+H]$^+$ (100%); HRMS (FAB+) calcd for [M+H]$^+$ ($C_{27}H_{31}O_6N_6$) 535.23051, found: 535.23189.

Complexes: [(**1**-3H)·Eu]: UV (H_2O): λ_{max} 335; luminescence (H_2O, pH 8.6) λ_{em} 582 (3), 596 (32), 618 (100), 651 (7), 693 (79); MS (ES+): m/z 685.1 [M+H]$^+$ (100%). [(**1**-3H)·Tb]: UV (H_2O): λ_{max} 335; luminescence (H_2O, pH 8.6) λ_{em} 489 (38), 545 (100), 586 (28), 623 (16); MS (ES+): m/z 691.1 [M+H]$^+$ (100%). [(**1**-3H)·Sm]: UV (H_2O): λ_{max} 334; luminescence (H_2O, pH 8.6) λ_{em} 567 (21), 602 (100), 649 (80), 707 (32); MS (ES+): m/z 684.1 [M+H]$^+$ (100%). [(**1**-3H).Dy]: UV (H_2O): λ_{max} 334; luminescence (H_2O, pH 8.6) λ_{em} 480 (56), 577 (100), 664 (7), 756 (10); MS (ES+): m/z 696.2 [M+H]$^+$ (100%).

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