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Synthesis of Novel Macrocyclic Lanthanide Chelates Derived from Bis-pyrazolylpyridine

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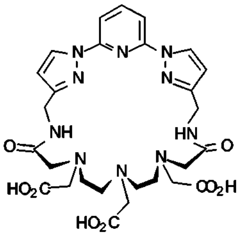
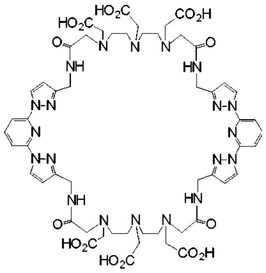
ABSTRACT





New macrocyclic chelates based on bis-pyrazolylpyridine and diethylenetriaminepentaacetic acid are synthesized, and the remarkable luminescence properties of their lanthanide chelates are reported.

The metal-centered emission of lanthanide chelates has been successfully applied in various analytical methods based on time-resolved fluorescence (TRF)[1] and fluoroinmmunoassay.[2] The design of the organic part of the chelate is paramount in attaining the required conditions for these chelates to be practical. A useful ligand should bear a good yield for intersystem crossing, a reasonable matching between the chromophore first triplet state and the resonance level of the metal, and the closest possible metal-to-chromophore distance. Last but not least, the ligand should be reasonably soluble in aqueous media but must effectively shield the metal from the water OH oscillators that easily quench lanthanide emission. The fulfillment of all these requirements is not an easy task, and research in this area is very active.[3]

Heteroaromatic rings such as pyridine, pyrazine, or pyrazole have revealed excellent building blocks for designing photoactive probes, and we have already prepared a number of useful chelates based on these motifs.[4] On the other hand, polycarboxylate units are widely used in lanthanide chelates because of their high binding constants for lanthanides.[5]

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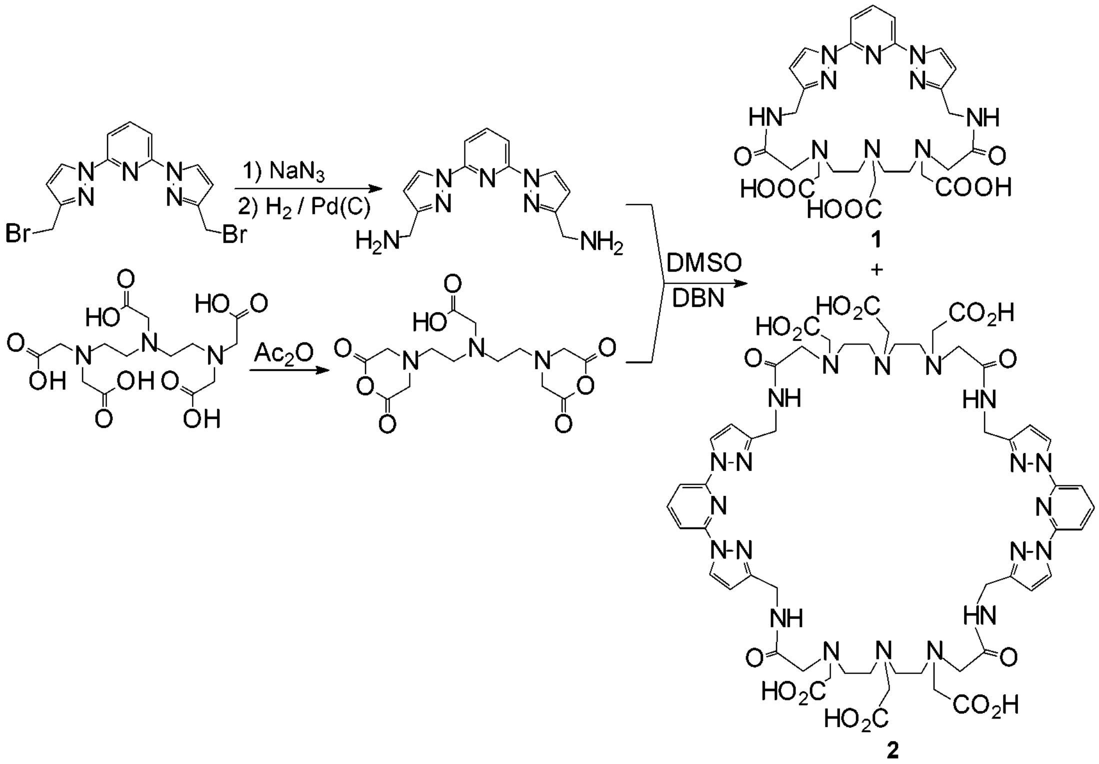
Scheme 1



The main goal of this work was thus to build compound **1** derived from bis-pyrazolylpyridine[6] and diethylenetriaminepentaacetic acid (DTPA)[7] (Figure 1). Its molecular modeling (HyperChem, AM1 and MM+) based on published data for DTPA−lanthanide complexes[8] (assuming nine-coordination of the metals by means of the three carboxylates and aliphatic amines) showed that the macro ring of **1** would adequately embrace the metal, therefore suggesting that the Eu^{3+} and Tb^{3+} complexes of **1** would very likely comply with the stringent structural features imposed by an efficient lanthanide luminescence.

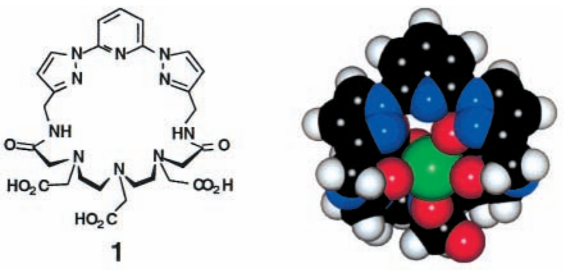


Figure 1. Structure and molecular model of the target macrocycle.

Scheme 1 shows the synthetic approach. The starting 2,6-bis(3-bromomethylpyrazol-1-yl)pyridine was prepared as previously described from 2,6-dibromopyridine and 3-ethoxycarbonyl pyrazol.[4] The treatment of the dibromo derivative in standard conditions with sodium azide and further catalytic hydrogenation of the resultant diazide led to 2,6-bis(3-aminomethylpyrazol-1-yl)pyridine. Addition of freshly prepared diethylenetriaminepentaacetic dianhydride (DTPA) over the diamine derivative dissolved in DMSO (concentration of reactants, 37 mM) in the presence of DBN (1,5-diazabicyclo[3.4.0]non-5-ene) afforded a mixture of the two macrocycles.

Although the assembly of very large macrocycles with DTPA has rarely been reported,[9] the MS spectrum (MALDI-TOF) of the crude reaction hinted its production. It showed two main groups of peaks at m/z 627.3 ($\mathbf{1}H^+$), 649.2 ($\mathbf{1}Na^+$), 665.2 ($\mathbf{1}K^+$) and 1253.5, 1275.5, 1291.5, the latter group being attributable either to a noncovalent dimer of **1** or to $\mathbf{2}H^+$, $\mathbf{2}Na^+$, and $\mathbf{2}K^+$, respectively. Isolation and fragmentation in the mass spectrometer of the species responsible for the peak at m/z 1253.5 led to new fragments of higher and equal m/z ratio than that of **1**, i.e., 879, 727, 653, and 627, strongly suggesting the presence of **2** since a noncovalent dimer of **1** is very unlikely to render fragments of m/z ratio larger than that of **1**. After several unsuccessful attempts of separation, **2** precipitated (15−20% yield) treating the crude product with an 1:1 water−methanol mixture containing 0.5% TFA. The residue was eluted with water over an ion-

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exchange AG1-X8 resin (acetate form), the key fractions were lyophilized, and the residue was washed with methanol, yielding **1** in 10% yield. The two macrocycles exhibited very different ^{1}H NMR behavior in that **1**, in contrast to **2**, displayed very broad signals over the observable range of temperatures in D_2O.[10]

The Eu^{3+} and Tb^{3+} complexes of the macrocycles were prepared by the addition of the corresponding aqueous solution of lanthanide chloride to the buffered solution (pH 8.6) of ligand at a concentration of 3×10^{-5} M in water. Ligand-to-metal stoichiometry of the complexes resulted 1:1 and 1:2 for **1** and **2**, respectively, as determined by luminescence titrations and Job's plots (Figure 2).[11]

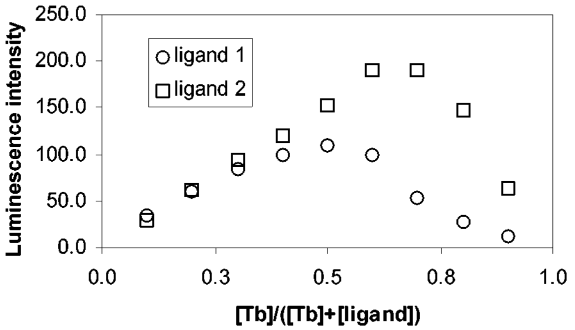

Figure 2. Job's plots of the complexation of ligands **1** and **2** with Tb^{3+}.

Excitation of aqueous solutions of Eu^{3+} and Tb^{3+} complexes of **1** and **2** from the lowest energy absorption band gave rise to the usual, well-structured emission of the lanthanide cations (Figure 3). In all cases, excitation and absorption spectra matched very well, indicating that the ligand did transfer the absorbed photons to the metal. However, the UV spectra of the complexes were very similar to the those of the ligand, suggesting that the bis-pyrazolylpyridine moiety did not suffer major conformational changes upon complexation.The intense Eu^{3+} and Tb^{3+} luminescence observed at the relatively low concentrations used (3×10^{-5} M) suggests that the ligand-to-metal energy transfer should be effective. The long decay times (τ) in the ms range (Table 1) make these complexes very serious candidates as luminescent probes for time-resolved techniques.

Despite the difference in flexibility between **1** and **2** as shown by their different behavior in ^{1}H NMR (see above), the number of water molecules in the coordination sphere of the metals, calculated by three different methods (Horrocks,[12] Kimura,[13] and Parker[14]), was the same for the complexes of the two ligands and only 1.1 ± 0.5. This suggests that coordination should be very similar in the two cases, probably not involving the aromatic heterocycles, in agreement with the small changes observed in the UV spectra upon complexation (vide supra).

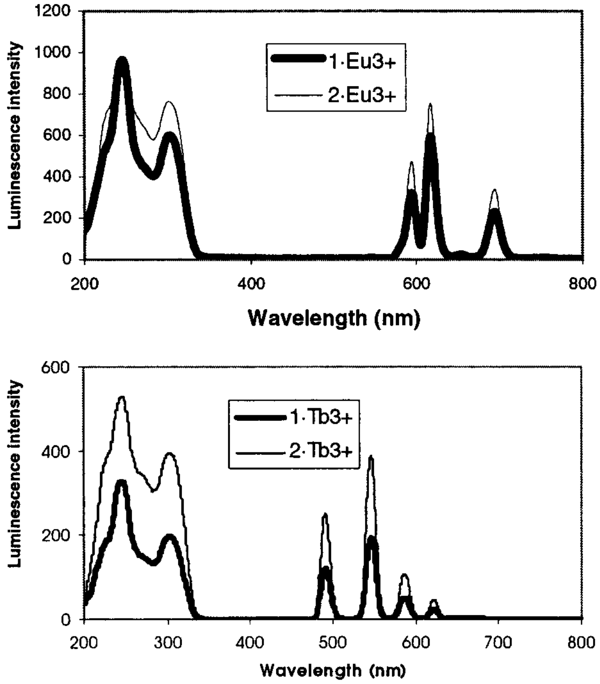

Figure 3. Luminescence excitation and emission spectra of Eu^{3+} and Tb^{3+} complexes of **1** and **2**.

The larger lifetimes measured in D_2O (factors of ca. 3.5 and 1.7 for Eu^{3+} and Tb^{3+}, respectively; cf. Table 1) indicate that the complexes were sensitive to vibronic deactivation via O−H oscillators.

Reconsideration of lifetimes and quantum yields in the light of the equations reported by Sabbatini et al.[15] facilitates the discussion of the ligand-to-metal energy transfer process.

Table 1. Emission (Eu^{3+}, 616 nm; Tb^{3+}, 546 nm) Lifetimes (ms) and Quantum Yields (ϕ) after Excitation of Metal Complexes of Ligands **1** and **2** at 304 nm in Borate Buffered (pH 8.6) Solutions

complex	ϕ	$\tau(H_2O)$	$\tau(D_2O)^a$
1·Eu^{3+}	0.02	0.7	2.2 (2.3)
1·Tb^{3+}	0.29	1.7	2.9 (2.9)
2·Eu^{3+}	0.01	0.6	2.3 (2.3)
2·Tb^{3+}	0.23	1.7	3.0 (3.0)

[a] Data at 77 K in parentheses.

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(10) ^{1}H NMR of **1** (D_2O, 80 °C, broad signals) δ (ppm): 8.4 (2H, Pz-H5), 7.7 (3H, Py), 6.4 (2H, Pz-H4), 4.4 (4H, PzC*H*$_2$N), 3.3−2.7 (10H, NC*H*$_2$CO) and 2.6−2.3 (8H, NC*H*$_2$C*H*$_2$N). ^{13}C NMR (D_2O, 80 °C) δ (ppm): 179.9 and 179.0 (CO_2H), 174.0 ($CONH_2$), 152.5 (Pz-C3), 147.8 (Py-C4), 141.4 (Py-C2,6), 128.6 (Pz-C5), 107.8 (Py-C3,5), 106.7 (Pz-C4), 58.8, 58.3 [(NCH_2CH_2N, NCH_2CO_2H)], 52.1 (NCH_2CONH) and 36.5 ($PzCH_2N$). Spectra of **2** were almost identical but displaying sharp lines at room temperature.

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Table 2. Reinterpretation of Parameters of Table 1 in Terms of Sabbatini's Equations (see text)

ion	ϕ_M	η	k_{nr}(T), s^{-1}	k_{nr}(OH), s^{-1}
1·Eu^{3+}	0.30	0.07	20	994
1·Tb^{3+}	0.59	0.49	≈0	243
2·Eu^{3+}	0.26	0.04	≈0	1232
2·Tb^{3+}	0.59	0.39	≈0	255

Thus, Table 2 shows values obtained for $\eta = \phi/\phi_M$, which measures the energy transfer efficiency from the S_1 state of the ligand to the emitting state of the metal via previous intersystem crossing ($S_1 \Longrightarrow T_1$) in the ligand, where ϕ and ϕ_M are quantum yields of emission upon ligand and metal excitation, respectively.

Assuming that the decay process in D_2O at 77 K is purely radiative, $\phi_M \approx \tau^{298}_{H_2O}/\tau^{77}_{D_2O}$, where $\tau^{298}_{H_2O}$ and $\tau^{77}_{D_2O}$ are the lifetimes in H_2O at rt and D_2O at 77 K, respectively (Table 1).

It can be seen that η is much lower for Eu^{3+} than for Tb^{3+}. The study of a relatively large body of compounds[16] related to **1** revealed that, to achieve efficient energy transfer to Eu^{3+}, it is necessary for the ligand T_1 level to lie well above the $^5D_2$ state of the metal (21 400 cm^{-1}). Since T_1 of both ligands fulfilled this condition (ca. 24 300 cm^{-1}, measured as usual in the Gd^{3+} complexes), the reason for the energy transfer inefficiency to Eu^{3+} must be found in the effective population of nonemitting LMCT states, especially favored by the presence of the donor amine nitrogens in the DTPA moiety. LMCT states for Tb^{3+} are much less accessible, and the ca. 4000 cm^{-1} difference between the emitting $^5D_4$ level of Tb^{3+} (20 490 cm^{-1}) and the T_1 level of the ligand minimizes the problem of metal-to ligand back energy transfer.[17]

Apart from the sought radiative route, the metal emitting level may be deactivated by other processes. Table 2 contains calculated rate constants for nonradiative processes, namely thermal [$k_{nr}(T) = (1/\tau^{298}_{D_2O}) - (1/\tau^{77}_{D_2O})$] and O−H vibronic deactivation [$k_{nr}(OH) = (1/\tau^{298}_{H_2O}) - (1/\tau^{77}_{D_2O})$]. The former, k_{nr}(T), displays negligible values showing that the studied macrocycles do not introduce excited states which can be thermally populated from the emitting state. The second, k_{nr}(OH), reveals the usual,[18] higher efficacy of the O−H oscillators in deactivating Eu^{3+}, even though the number of water molecules is similar in all cases. Complexes of **2** resulted somewhat more sensitive to O−H vibronic deactivation.

We thus conclude that the described macrocyclic chelates possess enough water solubility and remarkable luminescence properties with Eu^{3+} and Tb^{3+} ions that strongly posit its application in TRF analytical methods. Research is in progress to provide the complexes with additional functionality to allow their covalent conjugation to biomolecules.

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