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Eu(III) Complex of a Macrocyclic Ligand derived from 2,2'-Bipyridine and Iminodiacetic Acid : Synthesis, Luminescence and Phosphate Diester Transesterification Properties.

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Abstract: A very convenient synthesis of a macrocyclic ligand bearing two endocyclic amide and two exocyclic carboxylate groups (lanthanide binding sites) and one sensitizer (2,2'-bipyridine unit) is described. It is shown that the corresponding Eu(III) complex bearing a single positive charge and incorporating two water molecules in the first coordination sphere of the metal ion is strongly fluorescent on the basis of energy-transfer luminescence ($\Phi = 0.36$ in D_2O solution at 300 K) and cleaves a RNA model phosphate diester in aqueous solution. © 1998 Elsevier Science Ltd. All rights reserved.

The luminescence and Lewis acid properties of lanthanidic species have been of much recent concern because of their potential use for a variety of chemical and biological applications.[1,2] The latter ones include the use of luminescent [Eu(III), Tb(III)] ions as labels in fluoroimmunoassays, DNA/RNA hybridization assays, enzyme analyses, cellular applications or receptor-ligand interactions[3] and the use of lanthanide [Ln(III)] ions as artificial ribonucleases for the sequence-specific cleavage of RNA.[4] Although simple salts of some trivalent lanthanide ions have excellent luminescence properties[2] or promote rapid phosphate ester transesterification under mild conditions,[5] the development of organic Ln(III) complexes is crucial for a specific labeling of biomolecules or a specific cleavage of a target nucleic acid.

Luminescence or catalytic properties of lanthanide complexes are dependent on the type of ligand donor groups, the number of vacant coordination sites on the metal ion likely to bind small molecules such as water, the overall charge of the complex. On the other hand the ligand may enhance the properties of the free metal ion as shown by the use of ligands bearing chromophoric groups which balance the inherently weak metal centered absorption band (antenna effect[6]) thus yielding highly luminescent Eu^{3+}, Tb^{3+} species. The organic ligand should also form kinetically stable complex (with respect to dissociation) in aqueous solution. It should have reasonable water solubility if the complex is used under physiological conditions. At last, it should be obviously readily synthesized.

In recent years a considerable research effort has been directed toward two main classes of lanthanide receptors, i.e. macrocyclic ligands[7] and aminopolycarboxylate chelates.[8] Our interest in the properties of europium macrocyclic complexes[9] led us to investigate the preparation of ligands presenting into the same structure these two features. In the present paper we report the synthesis, the characterization, the luminescence properties and reactivity studies of an europium complex derived from a new macrocyclic ligand (**5**) which is built from diamide complexing moiety associated to an intracyclic 2,2'-bipyridine chromophore and two exocyclic carboxylate groups. We have noticed only few reports about lanthanides binding by macrocyclic compounds incorporating both an intracyclic chromophoric unit and pendant carboxylate groups.[10]

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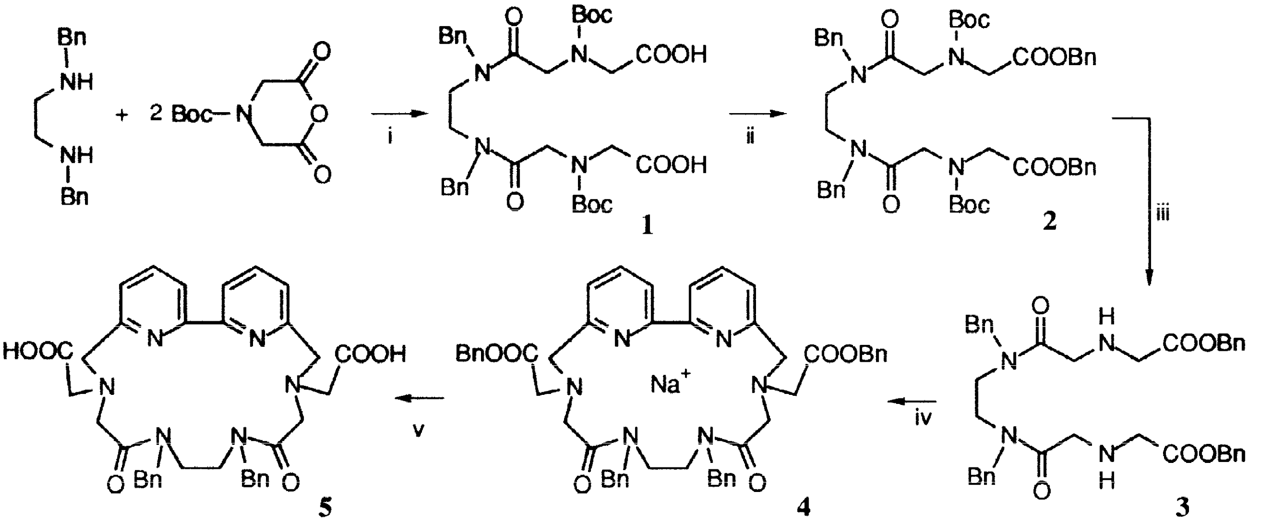
The procedure developed for the synthesis of ligand **5** utilized a simple intermediate, the diamide diacid compound **1**, which can be readily obtained by reacting N,N'-dibenzylethylenediamine with the cyclic anhydride of N-Boc iminodiacetic acid prepared *in situ*[11] (Scheme 1). Esterification of **1** with benzyl chloroformate according to the procedure reported by S. Kim *et al*,[12] followed by acidic cleavage of the Boc protecting group gave the building block **3** in good yield. The condensation of 6,6'-bis(bromomethyl)-2,2'-bipyridine[13] with the secondary diamine **3** in MeCN at reflux in the presence of Na_2CO_3 afforded the NaBr complex of the macrocycle **4** in **93% yield.** The very high yield of macrocycle formation obtained without using high dilution techniques is noteworthy. It may result from both a templating effect of the sodium cation (very likely chelated by amide and ester groups) and from a rigid group effect of the bridging unit introduced. Finally the free ligand **5**[14] was obtained by saponification of the ester groups of **4**.



Reagents and conditions: i) THF, 50 °C, 24 h, 87% yield from N-Boc iminodiacetic acid; ii) ClCOOBn, Et_3N, DMAP, CH_2Cl_2, 69%; iii) CF_3COOH/CH_2Cl_2, 85%; iv) 6,6'-bis(bromomethyl)-2,2'-bipyridine, Na_2CO_3, CH_3CN, 80 °C, 24 h, reagent concentrations 0.002 M, 93%: v) a) KOH, EtOH, b) HCl 1N, 80%.

Scheme 1

The europium complex of **5** was prepared, using standard methods,[15] by stirring equimolar amounts of ligand **5** and $EuCl_3.H_2O$ in slightly acidic aqueous solution (pH = 6.5). The complex[16] was precipitated by the addition of acetone (77% yield).

The mass spectrometric data and microanalytical results were consistent with a 1:1 stoichiometry for the complex. The ES^+ mass spectrum showed a major ion at m/z 801 (based on ^{153}Eu) which presented the characteristic isotopic abundance for europium and which was assigned to the $[(5-2H)Eu]^+$ species. No peak ascribed to the free ligand was observed in the ES^+ mass spectrum and no loss of metal was detected when Vc (extraction cone voltage) was increased. This indicated a high affinity of ligand **5** for europium ion.

In the complex the participation in binding of carboxylate, amide oxygen atoms and pyridinic nitrogen atoms was provided by recording IR and UV spectra of the ligand and the complex.[14, 16] The participation of the nitrogen pyridinic atoms in the complexation was confirmed by the luminescence spectrum (*vide infra*). Moreover luminescence data[17] indicated that two water molecules were present in the first coordination sphere of the metal ion in the complex. Thus in $[Eu \subset 5-2H]^+$ complex the europium ion is in an eight-coordinate ligand environment[18] or in a nine-coordinate ligand environment if the chloride counter ion remains bound to the metal in the complex. These two coordinates are the most favourable ones for the europium ion.

Excitation of the aqueous solution of the [Eu $\subset$ 5–2H]$^+$ complex at 310 nm gave the usually ligand-to-metal energy transfer process with $^5D_0 \rightarrow ^7F_j$ emissions (J = 0-4); 50% of the total emission was centered in the 616 nm peak. The excitation and absorption spectra match very well (Fig. 1).

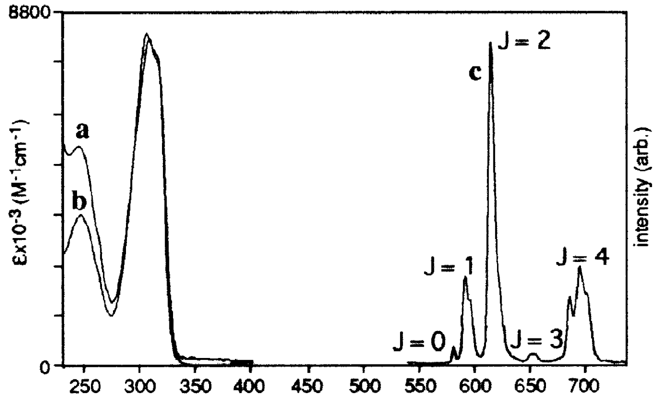


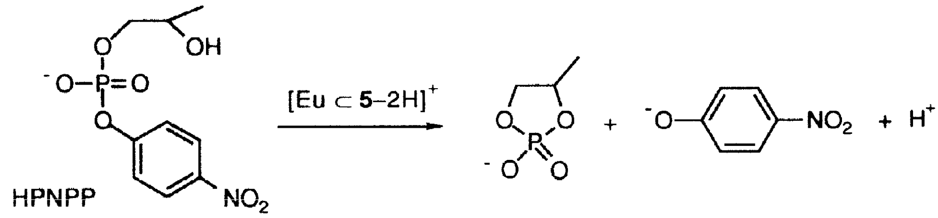
Figure 1: Absorption **a** (—), luminescence excitation **b** (----), luminescence **c** (—) spectra of [Eu $\subset$ **5**–2H]$^+$ in H_2O solution at 300 K.

The luminescence spectrum of the complex did not change in H_2O solution over a period of several days and in spite of the presence of two metal-bound water molecules, the complex exhibited at 300K a 0.42 ms lifetime in H_2O solution. It is noteworthy that this lifetime compares well with those reported for Eu^{3+} complexes derived from ligands bearing one bipyridine unit and where the complexing moiety is based on a macrocyclic backbone (τ = 0.28 ms for bipyridine-[18]-N_2O_4)[19] or a polyaminocarboxylate chelate (τ = 0.59 ms for bipyridine-bis(methylamine) tetraacetic acid).[20] For [Eu $\subset$ 5–2H]$^+$ complex in D_2O solution, we noticed a relatively high quantum yield (Φ = 0.36) and no temperature dependence of the lifetime, indicating that thermally activated decay process was absent.

On the other hand the water quenching of the complex luminescence can be minimized by suitable ion-pair perturbation.[21] In the presence of 4×10^{-1} molar concentration of F^- anions we observed a lifetime of 0.92 ms and a hydration state of 0.6. Similar trend occurred in phosphate buffer (100 mM, pH = 7.0, τ = 0.75 ms).

To investigate also the ability of [Eu $\subset$ 5–2H]$^+$ complex to promote intramolecular RNA transesterification, we undertook a kinetic study of transesterification of 2-hydroxypropyl-4-nitrophenyl phosphate ester (HPNPP) which was widely used as an RNA model compound[22] (Scheme 2).

Scheme 2



At 30 °C, pH = 7.3, the rate of transesterification of HPNPP mediated by the complex was monitored by UV-Vis spectroscopy by recording the production of the 4-nitrophenolate anion.[23] The observed rate constant (k_{obs} = 0.035 h^{-1}) was *ca.* 2×10^2 greater than in the absence of the complex at pH 7.3. The cleavage activity of [Eu $\subset$ 5–2H]$^+$ complex is consistent with the presence of two available coordination sites for the europium ion, one for binding the substrate P-O$^-$ and the other for furnishing a metal bound hydroxyde ion acting as a general base for the assistance of the attack of the HPNPP hydroxyl group in the cyclization process.[24]

In conclusion, the synthesis of a new photoactivable 18-membered dilactam with pendant carboxylate groups was readily achieved and its coordination ability toward europium ion was established. The introduction of a more absorbing chromophoric unit leading to a more intense luminescence of the europium complex and the phosphate diester transesterification by [Ln $\subset$ 5–2H]$^+$ are in progress.

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References and Notes:

1. Imamoto, T. *Lanthanides in Organic Synthesis*; Academic Press: London, **1994**.
2. Bünzli, J.-C.G.;Choppin, G.R. *Lanthanides Probes in life, Chemical and Earth Sciences*; Elsevier: Amsterdam **1989**.
3. For recent reviews, see: Elbanowski, M.; Makowska, B. *J. Photochem. Photobiol. A: Chem.* **1996**, 99, 85-92. Samme P.G.; Yahioglu, G. *Nat. Prod. Rep.* **1996**, *13*, 1-28. Hemmilä, I.; Webb, S. *Drug Discovery Trends* **1997**, *2*, 373-381.
4. Kalesse, M.; Loos, A. *Bioorg. & Med. Chem. Lett.* **1996**, *6*, 2063-2068 and references therein.
5. Morrow, J.R.; Buttrey, L.A.; Berback, K.A. *Inorg. Chem.* **1992**, *31*, 16-2.
6. Sabbatini, N.; Perathoner, S.; Balzani, V.; Alpha, B.; Lehn, J.-M. *Supramolecular Photochemistry*; Balzani, V. ed., Riedel, D.: Amsterdam **1987**; pp 187-206.
7. See for examples: Sabbatini, N.; Guardigli, M.; Manet, I.; Ungaro, R.; Casnati, A.; Ziessel, R.; Ulrich, G.; Asfari, Z.; Lehn, J.-M. *Pure & Appl. Chem.* **1995**, *67*, 135-140. Steemers, F.J.; Meuris, H.G.; Verboom, W.; Reinhoudt, D.N.; van der Tol, E.B.; Verhoeven, J.W. *J. Org. Chem.* **1997**, *62*, 4229-4235. Parker, D.; Williams, J.A.G. *J. Chem. Soc., Perkin Trans 2* **1996**, 1581-1586.
8. See for examples: Selvin, P.R.; Li, M. *J. Am. Chem. Soc.* **1995**, *117*, 8132-8138. Mukkala, V.-M.; Liitti Hemmilä, I.; Takalo, H.; Matachescu, C.; Kankare *J. Helv. Chim. Acta* **1996**, *79*, 295-306.
9. Galaup, C.; Picard, C.; Cazaux, L.; Tisnès, P.; Aspe, D.; Autiero, H. *New. J. Chem.* **1996**, *20*, 997-999.
10. Sasamoto, K. *European Patent*, EP 0493745A1, **1991**.
11. Cathala, B.; Raouf-Benchekroun, K.; Galaup, C.; Picard, C.; Cazaux, L.; Tisnès, P. *Tetrahedron* **1996**, *52*, 9793-9804.
12. Kim, S.; Inlee, J.; Kim, Y.C. *J. Org. Chem.* **1985**, *50*, 560-565.
13. Rodriguez-Ubis, J.-C.; Alpha, B.; Plancherel, D.; Lehn, J.-M. *Helv. Chim. Acta* **1984**, *67*, 2264-2269.
14. Ligand **5**: IR (KBr): $\nu_{C=O}$ = 1729, 1706, 1645 cm^{-1}; UV (H_2O): λ_{max} = 290 nm; ^1H-NMR (200 MHz, $CDCl_3$): δ ppm = 2.90-3.29 (m, 4H), 3.60-3.74 (m, 8H), 3.93-4.09 (m, 4H), 4.21-4.37 (m, 4H), 6.75-7.37 (m, 10H), 7.60 (d, 1H, J = 7.8 Hz), 7.63 (d, 1H, J = 7.8 Hz), 7.80 (t, 1H, J = 7.8 Hz), 7.85 (d, 1H, J = 7.8 Hz), 7.92 (t, 1H, J = 7.8 Hz), 8.13 (d, 1H, J = 7.8 Hz); MS (FAB^+, mNBA): m/z = 673 (100%, $[M+Na]^+$); Anal. calc. For $C_{36}H_{38}N_6O_6$, 1 AcOEt: C, 65.03; H, 6.28; N, 11.37; found C, 65.10; H, 6.14; N, 10.75.
15. Mukkala, V.-M.; Helenius, M.; Hemmilä, I.; Kankare, J.; Takalo, H. *Helv. Chim. Acta* **1993**, *76*, 1361-1378.
16. $[Eu \subset \mathbf{5}{-}2H]^+$ complex: IR(KBr): $\nu_{C=O}$ = 1602 cm^{-1}; UV (H_2O): λ_{max} = 305 nm; MS (ES^+,CH_3OH): m/z = 801 (100%, $[(L-2H)Eu]^+$); Anal. calc. For $C_{36}H_{36}N_6O_6EuCl$, NaCl, 6 H_2O: C, 43.12; H, 4.83; N, 8.38; found C, 43.51; H, 4.94; N, 7.95.
17. Luminescence data of $[Eu \subset \mathbf{5}{-}2H]^+$ complex in aqueous solution (pH = 7): $\tau(H_2O)$ = 0.42 ms; $\tau(D_2O)$ = 2.2 ms; $\Phi(H_2O)$ = 0.073; $\Phi(D_2O)$ = 0.36. Using the empirical equation proposed by Horrocks and Sudnick (See Horrocks, W. de W.; Sudnick, D.R. *Acc. Chem. Res.* **1981**, *14*, 384-392) and the lifetimes in H_2O and D_2O solutions at room temperature, the average number of coordinated H_2O molecules was calculated to be 2.0 (±0.5).
18. The participation of these atoms in the complex was confirmed by a molecular modeling study (PC model program) which in addition excluded the two nitrogen atoms of the amine type as coordination sites.
19. Sabbatini, N.; Perathoner, S.; Balzani, V.; Alpha, B.; Lehn, J.-M. presented at the 13th International Symposium on Macrocyclic Chemistry, Hamburg, September **1988**, *Abstracts Book*, 182-183.
20. Mukkala, V.-M.; Sund, C.; Kwiatkowski, M.; Pasanen, P.; Högberg, M.; Kankare, J.; Takalo, H. *Helv. Chim. Acta* **1992**, *75*, 1621-1632.
21. Sabbatini, N.; Guardigli, M.; Lehn, J.-M.; Mathis, G. *J. Alloys Compounds* **1992**, *180*, 363-367. Sabbatini, N.; Perathoner, S.; Lattanzi, G.; Dellonte, S.; Balzani, V. *J. Phys. Chem.* **1987**, *91*, 6136-6139.
22. Breslow, R.; Huang, D.-L. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 4080-4083.
23. In a typical run, solutions contained 0.1 mM phosphate diester, 0.01 M Hepes buffer (pH = 7.3), 0.1 M NaCl, and 0.5 mM europium complex at 30 °C. Kinetics was monitored at 400 nm, following released p-nitrophenolate ions and rate constants were calculated by using an iterative curve fit (Hewlett-Packard kinetics software package).
24. Chin, K.O.A.; Morrow, J.R. *Inorg. Chem.* **1994**, *33*, 5036-5041.