pH Modulation of the luminescence emission of a new europium cryptate complex

Carla Bazzicalupi,[a] Andrea Bencini,*[a] Antonio Bianchi,*[a] Claudia Giorgi,[a] Vieri Fusi,[b] Andrea Masotti,[a] Barbara Valtancoli,[a] Ana Roque[c] and Fernando Pina*[c]

[a] Department of Chemistry, University of Florence, Via Maragliano 75/77, 50144 Florence, Italy.
  E-mail: benc@chim1.unifi.it

[b] Institute of Chemical Sciences, University of Urbino, Italy. E-mail: vieri@chim.uniurb.it

[c] Departamento de Química, Centro de Química-Fina e Biotecnologia, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Quinta da Torre 2825, Monte de Caparica, Portugal. E-mail: fjp@dq.fct.unl.pt

The luminescence emission of a new dipyridyl-containing Eu(III) cryptate complex is modulated by pH, due to protonation of the polyamine chain strategically inserted within the ligand backbone.

The use of europium and terbium complexes as luminescent labels in fluoroimmunoassay is limited by the fact that they have extremely low absorption coefficients.[1] This drawback can be overcome through encapsulation of the metal inside the cavity of phenanthroline or dipyridyl-containing cryptands.[2] These moieties contain two nitrogen donors which act as binding sites for the metal cation and can efficiently protect the metal from the solvent, especially in aqueous solution where metal-bound water molecules may act as quencher of the emission. In addition, these efficient chromophore units allow the collection and transfer of electronic energy to the metal.[1] A further development of this study is the design of water-soluble cryptate complexes whose fluorescence emission can be modulated by pH. To this purpose we have synthesized the new cryptand **L**.
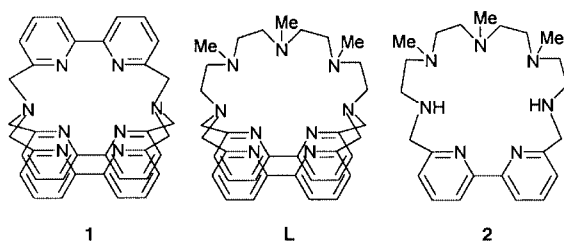
The major difference from previously reported dipyridyl-containing cryptands, such as **1**,[1,2] is the strategic insertion in **L** of a polyamine chain within the ligand backbone, which connects the two dipyridyl chromophore units. We hoped that this ligand could be able to lead to a stable Eu(III) cryptate complex in aqueous solution. At the same time, the aliphatic amine groups, which are more basic than heteroaromatic nitrogens,[3] may be protonated, leading to protonated cryptate complexes.

Ligand **L** was obtained, as the sodium complex, [Na⊂L]ClO$_4$, by reaction of 2,2′-bis(bromomethyl)dipyridyl[4] with the macrocyclic precursor **2** (MeCN, Na$_2$CO$_3$, reflux). The latter was obtained by condensation of 2,2′-bis(bromomethyl)dipyridyl with 1,13-ditosyl-4,7,10-trimethyl-1,4,7,10,13-pentaazatridecane,[5] by using the procedure of Richman and Atkins.[6] Reaction of [Na⊂L]ClO$_4$ with EuCl$_3$ (1:1 molar ratio) in aqueous solution (pH 3, 100 °C, 24 h) affords the protonated complex [EuCl$_3$⊂LH$_2$](ClO$_4$)$_2$·4H$_2$O in 64% yield.†

The crystal structure of [EuCl$_3$⊂LH$_2$]$^{2+}$ **3** (Fig. 1)‡ shows the metal enclosed within the cryptand cavity, coordinated by dipyridyl nitrogens N(2), N(3), N(5) and N(6) and by the two bridgehead amine groups N(1) and N(4). The polyamine chain N(7)–N(8)–N(9) is not coordinated, due to binding of the two acidic protons within this triamine subunit. The Eu(III) cation completes its coordination environment by binding three chloride anions. The coordination geometry is best described as a distorted tricapped trigonal prism, the two basal planes being defined by N(5), N(6) and Cl(1) and N(2), N(3) and Cl(2), respectively. The capped positions are occupied by the N(1), N(4) and Cl(3) donors. It is also of interest that, although the metal is lodged inside the cryptand cavity, the protonated ligand leaves free binding sites at the metal, available for the binding of exogenous species, such as chloride anions in the present structure. Most likely, water molecules replace these anions in aqueous solutions in the absence of chloride.

The Eu(III) cryptate complex can be obtained in aqueous solution by addition of Eu(ClO$_4$)$_3$ to [Na⊂L]ClO$_4$ (1:1 molar ratio, 60 °C, 12 h at pH 7); the luminescence spectrum of the europium cryptate in aqueous solution shows the characteristic visible emission of the complexed metal (λ_{max} = 617 nm) which mainly arises from intramolecular transfer to the metal ion from the highest energy triplet state of the cryptand.§ The
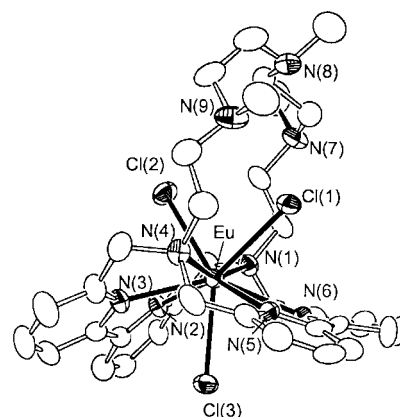

Fig. 1 ORTEP[11] drawing of [EuCl$_3$⊂H$_2$L](ClO$_4$)$_2$·4H$_2$O. Selected bond lenghts (Å) and angles (°): Eu–N(3) 2.606(10), Eu–N(5) 2.607(10), Eu–N(6) 2.610(9), Eu–N(2) 2.632(10), Eu–N(4) 2.712(9), Eu–Cl(2) 2.713(4), Eu–N(1) 2.771(9), Eu–Cl(3) 2.800(4), Eu–Cl(1) 2.820(4), N(3)–Eu–N(5) 106.6(3), N(3)–Eu–N(6) 144.4(3), N(5)–Eu–N(6) 62.6(3), N(3)–Eu–N(2) 61.1(3), N(5)–Eu–N(2) 144.1(3), N(6)–Eu–N(2) 106.4(3), N(3)–Eu–N(4) 64.1(3), N(5)–Eu–N(4) 62.7(3), N(6)–Eu–N(4) 124.2(3), N(2)–Eu–N(4) 124.2(3), N(3)–Eu–Cl(2) 76.9(2), N(5)–Eu–Cl(2) 136.3(2), N(6)–Eu–Cl(2) 135.7(2), N(2)–Eu–Cl(2) 76.8(2), N(4)–Eu–Cl(2) 82.0(2), N(3)–Eu–N(1) 122.7(3), N(5)–Eu–N(1) 125.6(3), N(6)–Eu–N(1) 64.0(3), N(2)–Eu–N(1) 62.8(3), N(4)–Eu–N(1) 158.6(3), Cl(2)–Eu–N(1) 80.4(2), N(3)–Eu–Cl(3) 71.8(2), N(5)–Eu–Cl(3) 71.4(2), N(6)–Eu–Cl(3) 72.6(2), N(2)–Eu–Cl(3) 72.7(2), N(4)–Eu–Cl(3) 99.7(3), Cl(2)–Eu–Cl(3) 143.91(12), N(1)–Eu–Cl(3) 101.7(2), N(3)–Eu–Cl(1) 141.6(2), N(5)–Eu–Cl(1) 72.2(2), N(6)–Eu–Cl(1) 70.7(2), N(2)–Eu–Cl(1) 139.6(2), N(4)–Eu–Cl(1) 83.2(3), Cl(2)–Eu–Cl(1) 79.15(12), N(1)–Eu–Cl(1) 81.6(2), Cl(3)–Eu–Cl(1) 136.94(11).
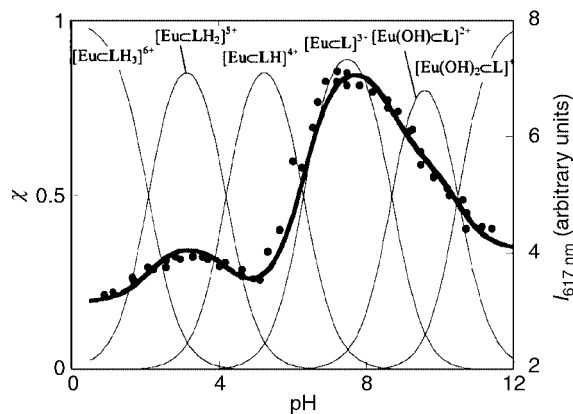
Fig. 2 Luminescence emission (●) of the Eu(III) complex with **L** (λ_{exc} = 260 nm; λ_{em} = 617 nm, [**L**] = [Eu³⁺] = 5 × 10⁻⁵ M, T = 300 K) and calculated molar fractions of the Eu(III) complexes (——) as a function of pH.

luminescence emission, however, is strongly affected by pH, displaying a maximum at neutral pH and significant decreases both at acidic and alkaline pH, as shown in Fig. 2. pK_a values of 2.1, 4.2, 6.3, 8.7 and 10.5 for the Eu(III) complex can be derived from the titration curve in Fig. 2 by least-squares analysis using the bracketing technique.[7][¶] Most likely, the first three constants correspond to protonation of the complex to give [Eu⊂LH$_n$]$^{(n+3)+}$ (n = 1–3) species at acidic pH. This process involves the aliphatic polyamine chain, as strongly supported by the crystal structure of the [EuCl₃⊂LH₂]²⁺ cation. Protonation does not allow the amine groups to bind to the Eu(III) cation, and, therefore, the ligand donors do not fulfill the coordination sphere of the metal. The resulting free binding sites at the metal are occupied by water molecules, which can quench the luminescence of the complex. This hypothesis is confirmed by the analysis of the lifetimes (τ) of the Eu(III) cryptate at different pH values in H₂O and D₂O at 300 K. For instance, at pH 2, τ = 0.39 ms in H₂O and 1.4 ms in D₂O, while, at pH 6.8, τ = 0.52 and 1.34 ms in H₂O and D₂O, respectively. These data account for the coordination of *ca.* two water molecules at pH 2 and just one at pH 6.8.[8] Binding of amine groups in the unprotonated [Eu⊂L]³⁺ complex gives a more 'protected' metal cation from the solvent molecules, thus enhancing the luminescence emission, with a maximum at pH 7.2. Finally, the observed decrease of the emission at alkaline pH is due to the formation of the hydroxylated species [Eu(OH)⊂L]²⁺ and [Eu(OH)₂⊂L]⁺, with pK_a values of 8.7 and 10.5, respectively. Binding of hydroxide anions to Eu(III) gives rise to a quenching of the emission.

The spectral features of the present complex are similar to those reported for other dipyridyl-containing Eu(III) cryptate complexes.[1,2,9] At pH 6.8, the quantum yield (Φ = 0.014 at 300 K), the radiative rate constant (k_r = 746 s⁻¹), the non-radiative temperature independent rate constant due to the coupling with the high-energy O–H oscillators [k_{nr}(OH) = 1177 s⁻¹], and the temperature dependent decay rate constant [$k_{nr}(T) \cong 0$], are somewhat lower than those found for [Eu⊂**1**]³⁺, as expected considering the replacement of a dipyridyl chromophore unit of **1** by an aliphatic polyamine chain in **L**. The present complex, however, still remains an efficient luminescent system. At the same time, the pH dependence of the emission intensity is a novel characteristic of our cryptate complex, which can be defined a 'pH modulated antenna device'.

Notes and references

† **2**: Elemental anal. Calc. for C₂₃H₃₇N₇: C, 67.12; H, 9.06; N, 23.82. Found: C, 66.9; H, 9.1; N, 23.7%. ¹H NMR (D₂O, pH = 11): δ 1.97 (s, 3H), 2.17 (s, 6H), 2.19 (t, 4H), 2.35 (t, 4H), 2.38 (t, 4H), 2.7 (t, 4H), 3.93 (s, 4H), 7.51 (dd, 2H), 7.98 (dd, 2H), 8.02 (dd, 2H).¹³C NMR (D₂O, pH = 11): δ 42.26, 43.74, 45.51, 54.02, 54.66, 54.89, 56.54, 122.56, 125.49, 140.18, 156.54, 160.19.

[Na⊂**L**]ClO₄: Elemental anal. Calc. for C₃₅ClH₄₅NaN₉O₄: C, 58.86; H, 6.35; N, 17.65. Found: C, 58.9; H, 6.5; N, 17.5%. ¹H NMR (CDCl₃): δ 7.94 (dd, 4H), 7.85 (d, 4H), 7.36 (dd, 4H), 3.98 (d, 2H), 3.78 (d, 2H), 3.13 (m, 4H), 2.78 (t, 4H), 2.68 (m, 7H), 2.51 (m, 4H), 2.05 (s, 6H). ¹³C NMR (CDCl₃): δ 42.26, 42.89, 53.84, 54.75, 61.06, 120.49, 124.59, 138.75, 154.61, 158.79.

[EuCl₃⊂LH₂](ClO₄)₂·4H₂O. Elemental anal. Calc. for C₃₅Cl₅EuH₅₅N₉O₁₂: C, 37.43; H, 4.94; N, 11.22. Found: C, 37.4; H, 4.9; N, 11.3%.

‡ *Crystal data* for C₃₅H₅₅Cl₅EuN₉O₁₂: M = 1123.09, triclinic, $P\bar{1}$, a = 13.302(4), b = 13.306(6), c = 15.23(1) Å, α = 83.69(5), β = 64.46(4), γ = 66.14(3)°, V = 2218(2) Å³, T = 298 K, Z = 2, μ(Mo-Kα) = 1.785 mm⁻¹, 5638 reflections collected, 5329 unique (R_{int} = 0.1189) which were used in all calculation, final R indices $R1$ = 0.0769 [$I > 2\sigma(I)$], $wR2$ = 0.2364 (all data).

The structure was solved using direct method (SIR92) and refined by full matrix least squares on F^2 (SHELXL-93).[10] An absorption correction was applied once the structure was solved (DIFABS).[10]

CCDC 182/1554. See http://www.rsc.org/suppdata/cc/a9/a909581k/ for crystallographic files in .cif format.

§ Fluorescence emission spectra at different pH values were recorded on solutions with [**L**] = [Eu³⁺] = 5 × 10⁻⁵ M using a SPEX F111 Fluorolog spectrofluorimeter.

¶ The total emission intensity at each pH value is given by $\Sigma_i c_i \chi_i$, where c_i is a constant proportional to the quantum yield and χ_i the molar fraction of the ith species. The c_i and χ_i values were calculated by least squares fitting of the emission data collected at different pH values. The pK_a values were then derived from the calculated χ_i. The fitting was carried out by using the emission intensity at 617 nm with an excitation wavelength of 260 nm. Absolute quantum yields were calculated by least squares fitting of the integrals of the emission spectra at each pH value, using the fluorescence emission of [Ru(bpy)₃]²⁺ as reference: [Eu⊂**L**H₃]⁶⁺, 0.0022; [Eu⊂**L**H₂]⁵⁺, 0.0098; [Eu⊂**L**H]⁴⁺, 0.00014; [Eu⊂**L**]³⁺, 0.014; [Eu(OH)⊂**L**]²⁺, 0.011; [Eu(OH)₂⊂**L**]⁺, 0.0014.

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