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Luminescent Sensing of Dicarboxylates in Water by a Bismacrocyclic Dinuclear Eu(III) Conjugate

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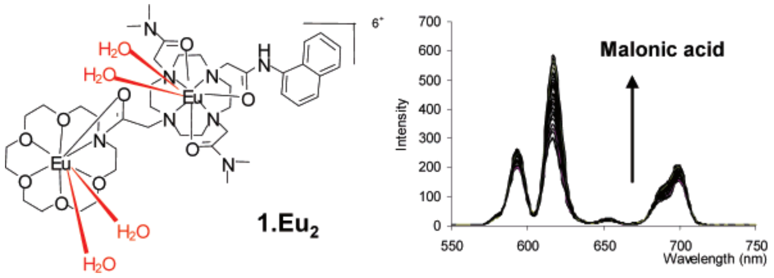
ABSTRACT



The design, synthesis, and characterization of a novel dinuclear Eu(III) bismacrocyclic conjugate 1·Eu$_2$ as a delayed luminescent lanthanide sensor for dicarboxylates is discussed. The sensor was shown to bind small dicarboxylic acids such as aspartic, malonic, succinic, or glutaric acid in pH 6.5 solutions. However, only malonic acid gave rise to selective Eu(III) luminescent enhancements, as the emission was reduced for all of the other acids.

Luminescence from lanthanide ions is a particularly attractive tool for use in optical imaging and sensing of biological systems.[1−3] This is due to their unique photophysical properties, such as long-lived excited states, long wavelength emission (occurring in the visible or in the NIR), which overcomes autofluorescence and light scattering from biological backgrounds, and sharp line-like emission bands, which are absent in traditional organic fluorophores.[4−7] As the lanthanides have forbidden f−f electronic transitions, their excited states are often produced through sensitization via ligand antennae,[4] or by the displacement of metal bound water molecules (themselves being efficient quenchers of the lanthanide excited state), by coordination ligands, such as antennae.[8,9] Both methods have been employed for developing delayed luminescent sensing assays,[4,6] with the latter

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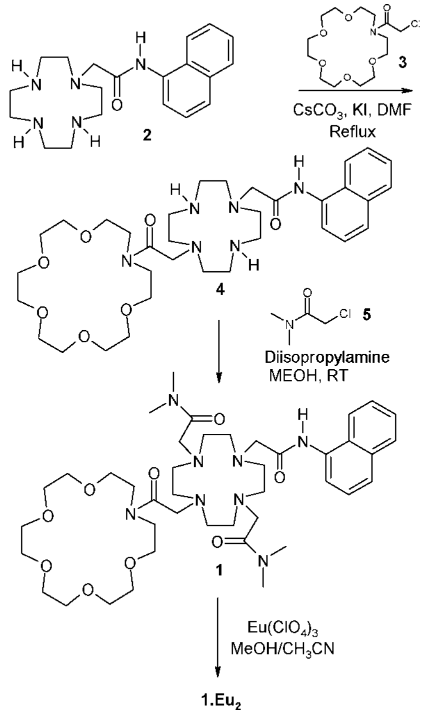
being particularly employed in the sensing of anions.[9,10] We have recently developed luminescent sensors for anions,[11] such as for medium size aliphatic dicarboxylates, e.g., pimelic and tartaric acids, or aromatic acids, e.g., salicylic or terephthalic acid with Tb(III)-based complexes.[12] While the Tb(III) emission of this complex was modulated upon anion recognition, the sensor lacked an appropriate antenna, the emission occurred at relatively short wavelengths, and the emission enhancement was minor for aliphatic acids. Consequently, we set out to improve our design, and developed **1·Eu₂**, a bismacrocyclic dinuclear Eu(III) complex[13] designed to achieve the luminescent sensing of small biologically important dicarboxylates such as malonic, succinic, and glutaric acids. Herein, the results from this investigation are presented, which demonstrate that *only* for malonic acid was the Eu(III) emission significantly modulated upon sensing by **1·Eu₂**.

In designing **1·Eu₂**, several important objectives were kept in mind: (i) a suitable antenna was needed for the population of the lowest Eu(III) excited state (17,200 cm^{-1}); (ii) a second binding site for the Eu(III) ion was needed to enable the binding of small biscarboxylates, and hence (iii) the coordination environment of each lanthanide ion should include two metal bound water molecules (e.g., $q = 2$ at each), at the same time as; and (iv) the resulting dinuclear complex should be stable toward metal dissociation. The outcome of this design is the bismacrocyclic ligand **1**, the synthesis of which is shown in Scheme 1. Here, a naphthalene fluorophore was chosen as an antenna, which is known to successfully populate the lanthanide excited state.[14] Secondly, hepta- or octadentate cyclen (1,4,7,10-tetraazacyclododecane) ligands form stable complexes with lanthanide ions.[15] Moreover, cyclen is easily functionalized. Therefore, we decided to use cyclen as our central macrocyclic unit.[16] However, a second coordination moiety was needed, and we decided on using monoaza-18-crown-6. Such crown ethers are known to bind lanthanide ions, at the same time as enabling the ions to coordinate the necessary water molecules.[17]

Scheme 1. Synthesis of **1** and the Corresponding Eu(III) Complex



The synthesis of the mixed cyclen−crown ether conjugate ligand **1** involved the initial formation of the monoalkylated cyclen derivative **2** in 69% yield, which was achieved by reacting an excess of cyclen with the α-naphthalene chloroamide, in DMF in the presence of Cs_2CO_3. The second alkylation of this product, using the 18-crown-6 based α-chloroamide **3**, Cs_2CO_3, and KI in DMF, gave the 1,7-disubstituted cyclen−crown ether conjugate **4**, in 67% yield after heating at 80 °C for 3 days, following purification with alumina column chromatography (CH_2Cl_2:MeOH 10:0.5). Finally, the remaining two amines of the cyclen moiety were alkylated, giving **1**, in 80% yield, using the *N*,*N*-dimethylacetamide **5** in the presence of diisopropylamine in MeOH at room temperature for 12 h. The bis-Eu(III) complex of **1**, **1·Eu₂**, was formed by adding $Eu(ClO_4)_3$ to a stirring solution of **1** (20 mg) in MeOH (2 mL). This led to the formation of an off-white precipitate that was redissolved in 2 mL of CH_3CN. The solution was then left to evaporate at room temperature overnight. This gave a solid that was washed with MeOH and collected with suction filtration to give the desired complex in 62% yield. Analysis of this complex clearly showed that two metal ions were present; the ^1H NMR (400 MHz, $CDCl_3$) showed the characteristic shifting

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of the macrocyclic resonances, indicating the presence of the Eu(III) ions.[6] Elemental analysis indicated the presence of four water molecules, suggesting that each Eu(III) had two metal bound waters.[18]

We had foreseen that while the cyclen moiety would be expected to form a strong complex with Eu(III), the columbic repulsion might effect the introduction and the stability constant of the second Eu(III) at the crown ether moiety. Because of this, the pK_a values of **1** (Figure S1, Supporting Information) and the stability constants (log K for the formation of the mono- and dinuclear complexes **ML** and **M$_2$L**) were determined potentiometricially in 50:50 MeOH:H_2O solution. Three pK_a values were determined for **1**, by fitting the titration data using the nonlinear least-squares regression program HYPERQUAD, as 9.98(±0.02), 6.90(±0.02), and 2.89(±0.01), assigned to the protonation of the nitrogen moieties of the two macrocycles. These have five protonation sites, but we were unable to determine more than three of these, and their exact order. The stability constants for the possible **1·Eu** (**ML**) and **1·Eu$_2$** (**M$_2$L**) complex formations were determined in the presence of both 1.5 and 3 equiv (Figures S2 and S3, respectively, Supporting Information) of $Eu(ClO_4)_3$. For the former, the 1:1 complex **1·Eu** was the major species in solution [$\log K_1 = 21.83(\pm 0.03)$]. With 3 equiv of $Eu(ClO_4)_3$, the **1·Eu$_2$** complex (Figure S3, Supporting Information) was the dominant species [$\log K_1 = 21.68(\pm 0.03)$, $\log K_2 = 14.70(\pm 0.02)$]. From these titrations, two pK_a values were obtained as 6.39(±0.02) and 7.39(±0.01), assigned to the deprotonation of metal-bound water molecules.[19]

The formation of the **1·Eu$_2$** sensor was also observed with a luminescent titration in 50:50 MeOH:H_2O at pH 6.5 upon excitation of the naphthalene antenna at 283 nm. The changes in the Eu(III) emission are shown in Figure 1, which clearly shows the gradual enhancement in the Eu(III) emission, appearing at 592, 615, 624, 654, (686 shoulder), and 702 nm, respectively (for $^5D_0 \rightarrow {}^7F_J$ ($J = 1, 2, 3$ and 4)). This also demonstrates the efficiency of the sensitization of the Eu(III) excited state by the naphthalene antenna. Moreover, plotting the changes in the Eu(III) emission vs Eu(III) equivalents (Figure S4, Supporting Information) clearly showed that the Eu(III) emission increased rapidly from 0 → 1 equiv with further minor changes being observed from 1 → 2 equiv, after which no further changes were observed.[20] Furthermore, fitting these spectral changes, by using the nonlinear least-squares regression program SPECFIT (see Figure 2 insert) gave log K values as 5.92 ± 0.16 for **1·Eu** and 4.78 ± 0.18 for **1·Eu$_2$**. If these stability constants are compared with those of the species which are in predominance at pH 6.5 they are close to those obtained from the potentiometric titration, the log K of **MLH$_{-1}$** is 5.20(±0.01) and that of **M$_2$LH$_{-1}$** is 4.85(±0.02) (Figure S5 and table, Supporting Information).[21]

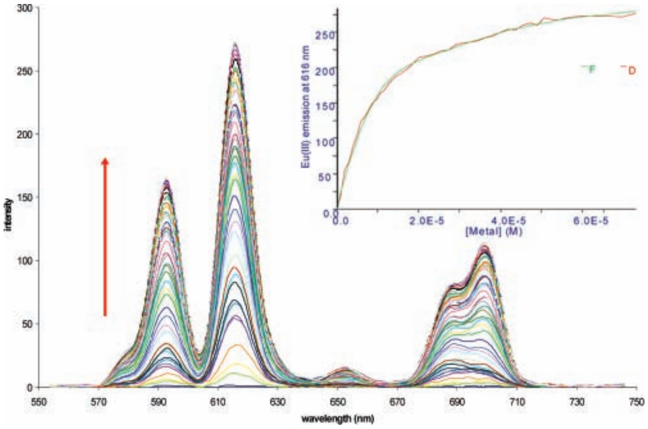

Figure 1. The changes in the Eu(III) emission upon titrating **1** (1×10^{-5} M) with $Eu(ClO_4)_3$ at pH 6.5 (MES buffer) and $I = 0.10$ M Net_4ClO_4 in 50:50 MeOH:H_2O (v/v). Insert: The observed data at 616 nm (red line) and the fitted data (green line).

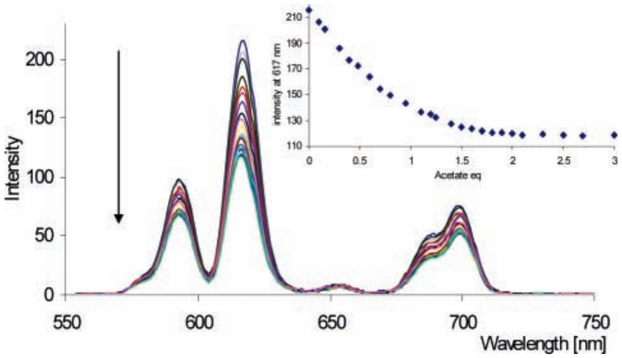

Figure 2. The changes in the Eu(III) emission upon titrating **1·Eu$_2$** (5×10^{-6} M) with acetate at pH 6.5. Insert: The changes in the 617 nm transition vs equivalents of acetate.

The hydration state, the q-value, for **1·Eu$_2$** was also evaluated by excitation of the antenna at 283 nm, and by direct excitation of the Eu(III) ion at 395 nm.[22] On both occasions, double exponential decay lifetimes were recorded, making determination of q less "accurate".[23] Nevertheless,

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[18] δ_H (400 MHz; $CDCl_3$) −8.2, -4.5, 0.9, 1.1, 1.2, 2.0, 2.3, 2.9, 3.2, 3.8, 6.1, 7.8, 8.1, 8.5; m/z (ESMS) calcd for $C_{42}H_{68}N_8O_9{\cdot}Eu_2{\cdot}(ClO_4)_3$ m/z 1492.324, found m/z 745.9 [M/2]. Found elemental analysis for $C_{42}H_{68}N_8O_9{\cdot}Eu_2{\cdot}(ClO_4)_3{\cdot}(H_2O)_4$: C 33.37; H 4.87; N 7.36. Calculated: C 33.55; H 5.10; N 7.45.

[19] These titrations showed that the complex formed hydroxide species above pH ~7.5, which caused precipitation, preventing the determination of the pK_a of the deprotonation of the remaining two metal bound waters.

[20] We propose that the first equivalent is due to the complexation of the Eu(III) at the cyclen moiety, which has higher affinity for the Eu(III) then the crown ether, as well as being closer to the antenna, and hence, giving rise to the largest changes in the Eu(III) emission.

[21] Having established that **1·Eu$_2$** was successfully formed, we carried out spectrophotometric pH titrations on **1·Eu$_2$** in water. For these, no spectral changes were seen in either the absorption or the fluorescence emission spectra of the antenna as a function of pH. However, the Eu(III) emission was modulated, between pH 6 and 8 (Figure S6, Supporting Information).

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we determined q as being 4.15 and 1.47($\pm$0.5), from the direct excitation, by using the modified equation of Parker et al.,[22] while for the excitation of the antenna, q was 1.70 and 0.6($\pm$0.5), respectively. Due to the close proximity of the antenna to the cyclen moiety the latter measurements reflected more accurately the q-value of this europium ion at the cyclen moiety. As q was found to be $\sim$2, then it can be postulated that the carboxylic amide, bridging the two macrocyles, is in fact coordinating stronger to the Eu(III) ion in the crown ether moiety (depicted schematically in the graphical abstract), which would mean that each ion had two metal-bound water molecules, which could be displaced upon anion binding.[10]

The ability of **1·Eu$_2$** to sense various carboxylates was evaluated at pH 6.5 buffered (MES) solution in the presence of 0.1 M NEt_4ClO_4, to maintain high constant ionic strength. The changes in the ground and the singlet excited states of the antenna and the Eu(III) emission were all monitored upon titrating **1·Eu$_2$** with various carboxylic acids such as acetate and L-aspartatic, succinic, glutartic, and malonic acids (binding as carboxylates at pH 6.5). However, for all of these, no changes were observed in the absorption and the fluorescence spectra, and only the Eu(III) emission was modulated. Therefore, any changes in the Eu(III) emission are directly related to the binding environment of the Eu(III) ions. At pH 6.5, the **1·Eu$_2$** emission was "switched on", Figure 2. Upon titrating **1·Eu$_2$** with acetate and L-aspartic, succinic, and glutaric acids, the Eu(III) emission was on all occasions quenched, as shown in Figure 2 for the titration of acetate, which was ca. 50% quenched. These changes also demonstrated the formation of 2:1 (ion:complex) stoichiometry (Figure 2 insert).[24] Here, the largest changes were also seen in the hypersensitive $\Delta J = 2$ transition centered at 617 nm, which is highly sensitive to the change in the local coordination environment of Eu(III), indicating that the ion was coordinating directly to the Eu(III) center.[8] The binding constant log K = 5.27($\pm$0.3) was determined from fitting these changes, using the nonlinear least-squares regression program SPECFIT. In a similar manner, L-asparate gave rise to ca. 50% quenching (Figure S7, Supporting Information), and 1:1 binding stoichiometry, from which log K = 4.58($\pm$0.3) was determined.[25] In comparison, the changes in the Eu(III) emission upon titrating with succinic and glutaric acid, gave rise to $\sim$10−15% changes (Figure S7, Supporting Information).

In contrast to these results, the changes in the Eu(III) emission were significantly enhanced upon titrating **1·Eu$_2$** with malonate (Figure 3). Again, the main changes can be observed for the hypersensitive $\Delta J = 2$ transition. From these changes, a 1:1 stoichiometry, with log $K = 4.61(\pm.0.29)$, was determined. Analysis of the q-values for this binding again showed double exponential decay lifetimes for the Eu(III) emission. Nevertheless, these were significantly different to that previously observed, with q = 2.3 and 1.0 being determined. While this does not exclusively verify the displacement of both of the sets of metal-bound water molecules, it strongly indicates that the water molecules have been displaced, as the emission is enhanced due to the removal of the O−H vibrational quenching pathway. Importantly, when the above measurements were carried out at pH 8, no changes were observed in the Eu(III) emission. This clearly indicates their importance in the sensing mechanism of these ions, as at this pH the two Eu(III) centers would have coordinated hydroxide species, which would prevent the coordination of the anion. Furthermore, when these measurements were repeated with a mono-Eu(III) based analogue of **1·Eu$_2$**, which lacked the crown ether moiety, *no such* anion sensing was observed at pH 6.5. This clearly demonstrates the importance of the presence of the second Eu(III) metal ion in **1·Eu$_2$**. These results demonstrate that malonic acid can be sensed selectively in competitive aqueous media, as it gives rise to (i) significant luminescent enhancement over other structurally related anions and (ii) strong binding.

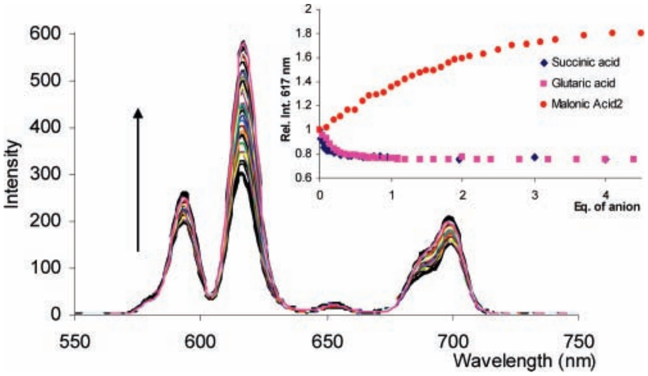

Figure 3. The changes in the Eu(III) emission upon titrating **1·Eu$_2$** (5×10^{-6} M) with malonic acid at pH 6.5. Insert*:* The changes in the 617 nm transition vs equivalents of various anions used.

In summary, we have designed and synthesized a novel stable dinuclear Eu(III) conjugate by tethering a mono-aza-18-crown-6-ether moiety to a cyclen macrocyle. Analysis of this conjugate showed that anions such as acetate and L-aspartate gave rise to quenching of the Eu(III) emission, while malonate gave rise to large luminescent enhancements, where the largest changes were seen in the hypersensitive $\Delta J = 2$ transition. We are currently exploring the use of such dinuclear and trinuclear based lanthanide ion conjugates further for the luminescent sensing of other biologically important structures.

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Supporting Information Available: Synthesis and characterization of **1.Eu$_2$**, as well as titration curves, speciation diagrams, and emission intensity curves (Figures S1−S7). This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) We also carried out titrations using Gly and L-Phe. On both occasion the emission was quenched and the 1:1 stoichiometry was observed.

(25) However, fitting these data also gave good correlation with the 1:2 complex formation, with log $\beta = 6.9(\pm 0.1)$.