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Crown ether lanthanide complexes as building blocks for luminescent ternary complexes

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Abstract

Lanthanide complexes of the macrocyclic ligands 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane-*N*,*N*′-diacetic acid (H_2(dacda)) and 1,4,7,10-tetraoxa-13-azacyclopentadecane-13-acetic acid (H(macma)) have been isolated and characterised. The photophysical properties of the red and green emissive, Eu(III) and Tb(III), respectively, complexes have been elucidated. Upon addition of mono- or bi-dentate aromatic carboxylates or dibenzoylmethide in solutions of the Eu(III) macrocycles triggering of the lanthanide emission is observed. The formation of the ternary complex between the lanthanide macrocycle and the sensitiser is controlled by the available coordinaton sites of the lanthanide macrocycle prior to the binding event.

Keywords: Luminescence; Lanthanide; Macrocycles; Ternary complexes

1. Introduction

Lanthanide ions, particularly Eu^{3+} and Tb^{3+}, are popular luminescent probes for the development of fluoroimmunoassays [1–6]. Their photophysical properties, narrow line luminescence with long lifetimes, are attractive in comparison with the broad luminescence of the organic fluorescent dyes. The disadvantage of low absorption coefficients of the lanthanides can be overcome by the introduction of ligands that act as light-harvesting centres (LHCs) to sensitise the lanthanide emission [7–13]. In the homogeneous immunoassay area the design of labels for direct signal response upon interaction of an antigen/antibody remains a challenge. Most of the methods employed so far are based on heterogeneous fluoroimmunoassays involving indirect detection of the lanthanide label content, by dissociation of the lanthanide label, a method which gives increased errors and tedious procedures to avoid contamination [14]. An approach employed for direct signal response is based on fluorescence resonance energy transfer mechanism (FRET) [15] where a europium cryptate plays the role of both the light harvester and the energy donor to an energy acceptor such as allophycocyanin [16]. We are interested in triggering fluorescence based on intramolecular energy transfer mechanism in order to avoid the limited choice of applications imposed by donor/acceptor concentration or energy levels difference in FRET. Our design is based on using two building blocks for lanthanide coordination, one based on a ligand that is non-absorbing (NAL), and the other based on a LHC (Scheme 1) [17]. Addition of the LHC to a complex of NAL with the lanthanide will then sensitise lanthanide luminescence by filling in the coordination sphere of the lanthanide centre. Triggering of the lanthanide emission by a sensitiser external to the lanthanide complex has been successfully used for the development of chemosensors [18] using non-covalent interactions to bring the participating units in one supramolecular structure; although lanthanide sensitisation has been reported in ternary lanthanide complexes

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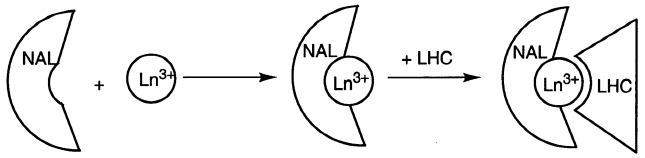
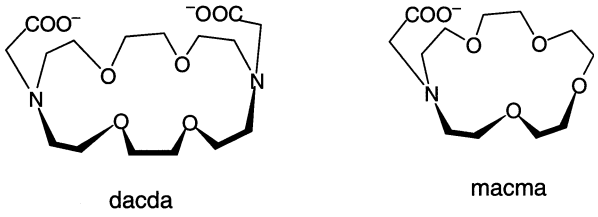


Scheme 1.



Scheme 2.

[19,20], there is little known of their controlled formation in solutions [21–24] and in most cases is based on either a synergistic coordination of the different ligands or addition of excess of one of the ligands [25,26].

We have chosen to use macrocycles with ionisable pendant donor groups as NALs. The macrocyclic ligands 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane-*N*,*N*′-diacetic acid (H_2(dacda)) and 1,4,7,10-tetraoxa-13-azacyclopentadecane-13-acetic acid (H(macma)) were chosen for the role of the NAL (Scheme 2). The pendant ionic groups greatly increase the thermodynamic and kinetic stability of their complexes compared with the non-functionalised analogues. This results from the greater preorganisation of the ligand for metal binding (i.e. it is held in a conformation close to that required for complex formation) and the electrostatic metal–ligand interactions introduced by the ionisable pendant arms [27]. There have been a number of studies involving Ln^{3+} ions and dacda [28–30]. These have been concerned with the solution formation of the complexes and measurement of the stability constants with Ln^{3+} ions (log $K = 10.8$–12.2) [31]. As the ligand macma has only one pendant arm, its lanthanide complexes will possess a 2+ charge. It also provides two fewer donor atoms than dacda; four oxygen atoms and one nitrogen atom in the ring, and the carboxylate group.

We report the isolation and characterisation of lanthanide complexes of dacda and macma, their luminescent properties and the triggering of luminescence by the formation of ternary complexes with LHCs.

2. Experimental

^{1}H, ^{13}C{^{1}H} and ^{31}P{^{1}H} NMR spectra were recorded on Bruker AC-200, 250 and 360 MHz spectrometers. Positive ion FAB mass spectra were recorded on a Kratos MS-50 mass spectrometer. Elemental analyses were performed on a Perkin–Elmer 2400 CHN elemental analyser. UV–Vis absorption spectra were recorded on a Perkin–Elmer Lambda 9 spectrometer. All measurements were made at room temperature (≈ 20 °C) in aerated solutions. Luminescence emission and excitation spectra were recorded on a Photon Technology International QM-1 emission spectrometer, previously described [32]. The luminescence studies of the lanthanide complexes were performed in Tris [tris(hydroxymethyl)-aminomethane] buffer, 0.05 mol dm^{-3}, at pH 7.5 (25 °C), unless otherwise stated.

2.1. [Eu(dacda)]Cl

H_2(dacda) [33] (0.097 g, 0.26 mmol) and NaOMe (0.028 g, 0.53 mmol) were dissolved in methanol (3 cm^3) and $EuCl_3$ (0.110 g, 0.30 mmol) in H_2O (2 cm^3) was added dropwise to this solution. After stirring for 30 min, the solution was filtered and acetone (50 cm^3) added. The white solid that crystallised overnight was filtered, washed with a 5% H_2O/acetone mixture (1 cm^3) and dried under vacuum (0.11 g, 73%). Single crystals of [Eu(dacda)]Cl suitable for an X-ray diffraction analysis were grown by slow evaporation from an acetonitrile solution. FAB MS *m*/*z* 527 $[M-Cl]^+$. (Found: C, 32.38; H, 5.33; N, 4.73%. $C_{16}H_{28}ClEuN_2O_8 \cdot 2H_2O$ requires C, 32.04; H, 5.38; N, 4.67%).

2.1.1. Crystallographic data for $\{[Eudacda(H_2O)]Cl\}_n \cdot 6nH_2O$

$C_{16}H_{42}ClEuN_2O_{15}$, $M = 689.93$, monoclinic, space group $P2_1/n$, $a = 13.207(4)$, $b = 12.110(4)$, $c = 16.681(5)$ Å, $\alpha = \gamma = 90°$, $\beta = 96.28(3)°$, $V = 2651.8(13)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.728$ g cm^{-3}, $F(000) = 1408$, Mo Kα radiation ($\lambda = 0.71073$ Å), $T = 220$ K, $\mu = 2.536$ mm^{-1}. The structure was solved by Patterson methods (DIRDIF) [34] and refined by full-matrix least squares against F^2 (SHELXL); [35] H-atoms of the water molecules were located in a Fourier map and refined subject to restraints applied to OH and HH distances. $R_1 = 0.0317$ [$\theta_{max} = 25°$, 3889 data $F > 4\sigma(F)$], $wR = 0.0701$ for 4602 independent reflections, GOF = 1.060.

2.2. [La(dacda)]Cl

A mixture of $LaCl_3 \cdot 7H_2O$ (0.20 g, 0.53 mmol), H_2(dacda) (0.21 g, 0.53 mmol) and $NaHCO_3$ (0.10 g, 1.2 mmol) was dissolved in H_2O (100 cm^3). This, initially effervescent, solution was stirred for 1 h, and the initial white precipitate that formed was filtered off and discarded. The volume was reduced to 10 cm^3 and acetone (120 cm^3) was added, resulting in the precipitation of a white solid, which was isolated and identified as the desired product (0.17 g, 56%). δ_H (360 MHz, D_2O) 4.11–4.18 (4H, m, OCH_2CH_2N), 3.94–4.03 (8H,

m, OCH_2CH_2O), 3.72–3.77 (4H, m, OCH_2CH_2N), 3.49 (4H, s, NCH_2COO), 3.19–3.27 (4H, m, OCH_2CH_2N), 2.79–2.83 (4H, m, OCH_2CH_2N); $\delta_C\{^1H\}$ (91 MHz, $CDCl_3$) 180.6 (CO), 69.9 (OCH_2CH_2O), 69.1 (OCH_2CH_2N), 59.9 (NCH_2COO), 59.1 (OCH_2CH_2N); FAB MS *m/z* 515 $[M-Cl]^+$. (Found: C, 33.69; H, 5.96; N, 4.23%. $C_{16}H_{28}ClLaN_2O_8\cdot 2H_2O\cdot 1/2CH_3COCH_3$ requires C, 34.13; H, 5.73; N, 4.55%).

2.3. [Tb(dacda)]Cl

H_2(dacda) (0.077 g, 0.20 mmol) and KOH (0.024 g, 0.43 mmol) were dissolved in H_2O (10 cm^3) and $TbCl_3$ (0.075 g, 0.20 mmol) in H_2O (2 cm^3) was added dropwise to this solution. The volume was reduced to 3 cm^3 under reduced pressure and acetone (40 cm^3) added. The white solid that crystallised was filtered, washed with a 5% H_2O/acetone mixture (1 cm^3) and dried under vacuum (0.10 g, 85%). Single crystals of [Tb(dacda)]Cl suitable for an X-ray diffraction analysis were grown by slow evaporation from an acetonitrile solution. FAB MS *m/z* 535 $[M-Cl]^+$(thioglycerol matrix). (Found: C, 24.55; H, 4.91; N, 3.53%. $C_{16}H_{28}ClTbN_2O_8\cdot 6H_2O\cdot KCl$ requires C, 24.55; H, 4.91; N, 3.53%).

2.3.1. Crystallographic data for $[Tbdacda]_n\cdot Cl_n\cdot 4nH_2O$

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$C_{16}H_{36}ClN_2O_{12}Tb$, $M = 642.84$, orthorhombic, space group $Pna2_1$, $a = 12.634(3)$, $b = 14.828(2)$, $c = 12.620(6)$ Å, $\alpha = \beta = \gamma = 90°$, $V = 2364.3(12)$ $Å^3$, $Z = 4$, $\rho_{calcd} = 1.806$ g cm^{-3}, $F(000) = 1296$, Mo Kα radiation ($\lambda = 0.71073$ Å), $T = 220$ K, $\mu = 3.166$ mm^{-1}. The structure was solved by Patterson methods (DIRDIF) and refined by full-matrix least squares against F^2 (SHELXL). H-atoms of the water molecules were not evident in Fourier maps, and have not been placed. This is likely owed to the lower crystal quality of this sample compared to that of the Eu complex. Low crystal quality is probably also to be the cause of the difficulties experienced in applying an absorption correction; the program SHELXA was used as a last resort, empirical procedures having failed to yield acceptable refinement statistics (for example difference peaks of over 3 $eÅ^{-3}$ in the region of the Tb atom). $R_1 = 0.0412$ [$\theta_{max} = 25°$, 2284 data $F > 4\sigma$ (F)], $wR = 0.0975$ for 2976 independent reflections, GOF = 1.019.

2.4. H(macma)·HCl·nH₂O (1 < n < 2) [36]

Ethyl 1,4,7,10-tetraoxa-13-azacyclopentadecane-13-acetate [37] (1.6 g, 5.2 mmol) was stirred at reflux with concentrated hydrochloric acid (3 cm^3) in H_2O (300 cm^3) for 67 h. The clear solution was cooled, the solvent removed under reduced pressure and the compound dried under vacuum, yielding a viscous, pale yellow oil that turned to a glass on standing (quantitative yield based on ^1H NMR spectrum). δ_H (250 MHz, $CDCl_3$) 8.18 (1H, br s, CO_2H), 4.47 (2H, s, NCH_2COOH), 4.17–3.65 (20H, m, CH_2); $\delta_C\{^1H\}$ (62.9 MHz, $CDCl_3$) 167.1 (CO_2H), 70.3 (OCH_2CH_2O), 69.8 (OCH_2CH_2O), 69.6 (OCH_2CH_2O), 65.0 (OCH_2CH_2N), 55.1 (OCH_2CH_2N), 54.6 (NCH_2CO_2H).

2.5. Preparation of [Eu(macma)](NO₃)₂

$Eu(NO_3)_3\cdot 5H_2O$ (0.219 g, 0.511 mmol) was dissolved in dry acetonitrile (10 cm^3) and heated under reflux with triethylorthoformate (1 cm^3) for 4 h, followed by the dropwise addition of a solution of H(macma)·HCl (0.157 g, 0.494 mmol) in dry acetonitrile (4 cm^3). The solution was heated under reflux for 24 h. Diethyl ether (20 cm^3) was added and the resultant solid was washed with diethyl ether (2 × 10 cm^3) and dried under vacuum (0.12 g, 20%). δ_H (200 MHz, CD_3NO_2) 3.37–3.70 (6H, m, CH_2), 2.89 (16H, br s, CH_2); FAB MS *m/z* 489 $[M-NO_3]^+$. (Found: C, 23.75; H, 4.21; N, 7.16%. $C_{12}H_{22}EuN_3O_{12}\cdot 3H_2O$ requires C, 23.77; H, 4.65; N, 6.93%).

2.6. Preparation of [Y(macma)](NO₃)₂

$Y(NO_3)_3\cdot 6H_2O$ (0.201 g, 0.525 mmol) was dissolved in dry acetonitrile (10 cm^3) and heated under reflux with triethylorthoformate (1 cm^3) for 4 h, followed by the dropwise addition of a solution of H(macma)·HCl (0.168 g, 0.529 mmol) in dry acetonitrile (3 cm^3). The solution was heated under reflux for 17 h. Diethyl ether (20 cm^3) was added and the resultant solid was washed with diethyl ether (2 × 10 cm^3) and dried under vacuum (0.191 g, 67%). δ_H (360 MHz, D_2O) 3.68–3.97 (18H, m, CH_2), 3.42 (4H, br s, CH_2); FAB MS *m/z* 427 $[M-NO_3]^+$.

3. Results and discussion

3.1. Characterisation of lanthanide dacda complexes

The crystal structure of [Eu(dacda)]Cl is shown in Fig. 1. Each Eu^{3+} ion is ten-coordinate, and is bound to four oxygen and two nitrogen atoms of the dacda ring, one oxygen atom from each of the ligand's carboxylate arms and a water molecule. One of the carboxylate arms is monodentate, whilst the other is 1,3-binucleating, linking adjacent Eu^{3+} centres to form a polymeric chain. The bridging carboxylate arm from a neighbouring ligand completes the coordination sphere of the metal. The Eu^{3+} ion is situated in the middle of the dacda ring, the two carboxylate arms from the same ligand are on one side of the ring, and the other carboxylate and water molecule are on the other side of the ring. While it is

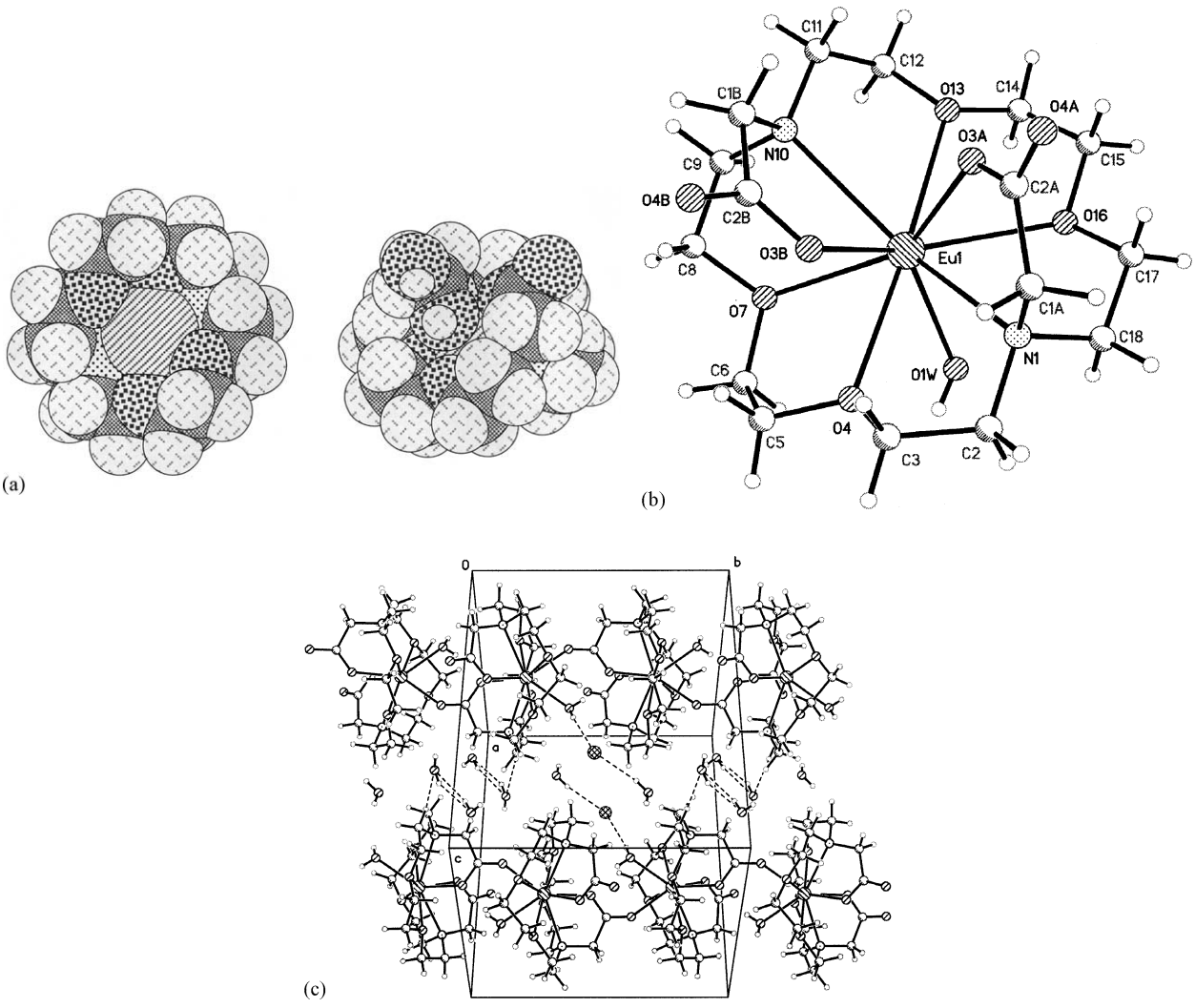


Fig. 1. Crystal structure of $\{Eudacda(H_2O)Cl\}_n \cdot 6nH_2O$ showing (a) the Eu^{3+} coordination sphere with the atomic numbering scheme [selected bond distances (Å): Eu(1)–O(3B) 2.396(3), Eu(1)–O(3A) 2.402(3), Eu(1)–O(4A)#1 2.459(3), Eu(1)–O(1W) 2.461(3), Eu(1)–O(7) 2.528(3), Eu(1)–O(13) 2.612(3), Eu(1)–O(16) 2.613(3), Eu(1)–O(4) and CPK models of top and bottom view and (b) crystal packing diagram, the hydrogens are omitted for clarity.

difficult to directly assess the solution structure from this polymeric crystal structure, it is clear that the ligand is able to wrap around the metal, yet still leave coordination sites free. This is important when considering ternary complex formation. The metal–ligand bond distances depend primarily on the donor atom. The intracomplex carboxylate bonds have an average Ln–O distance of 2.40 Å. These are of a similar length to those found in other complexes (e.g. the average Ln–O bond distance for the coordinated carboxylates in $[EuDOTA]^-$ is 2.39 Å) [38]. Bond distances from the metal to ether oxygens (average 2.60 Å) and nitrogen (average 2.71 Å) are longer, as are the Ln–O distances for the bridging carboxylate ligand and the water molecule (both are 2.46 Å) as expected for lanthanide-aza[15]crown-5 complexes [39]. It is interesting to compare the structure of [Eu(dacda)]Cl with the reported structure of Eu^{3+} complexed with 1,10-diaza-4,7,13,16-tetraoxacyclooctadecane, an analogue of dacda with no carboxylate arms. In this structure, $[Eu(NO_3)_2(C_{12}H_{26}N_2O_4)](NO_3)$, the Eu^{3+} ion is also ten-coordinate, bonding to the six donor atoms of the macrocycle and to the four oxygen atoms of two bidentate nitrates [40]. The average bond distances for Eu–O(ether), Eu–O(NO_3) and Eu–N are 2.60, 2.47 and 2.62 Å, respectively. The addition of the carboxylate arms appears to weaken the Eu–N bonds, possibly due to steric factors. The flexibility of the dacda ligand allows it to optimise bonding interactions with the Ln^{3+} ion by adopting a polymeric structure. In fact, this type of behaviour does not appear to be unusual for complexes of lariat crown ethers. The structure of the cadmium complex of macma is a tetrameric cluster, $[Cd_4(macma)_4][CdCl_4]_2 \cdot 3H_2O$, with bridging acetate

groups [36]. The structure of the cadmium complex with the ligand L = 1,4,7,10-tetraoxa-13-azacyclopentadecane, a macma analogue without the pendant arm, is monomeric $[Cd(L)NO_3)_2]$. Thus, the introduction of pendant carboxylates to crown ethers can result in significant changes to the solid-state structure of the macrocyclic complexes formed. The crystal packing diagram of [Eu(dacda)]Cl shows that the polymeric chains are aligned parallel to the *b* axis and are separated by hydrophilic channels of water molecules and Cl^- ions. There is an intermolecular network of hydrogen bonds, involving coordinated and non-coordinated water molecules, Cl^- ions and ethylenic protons that help to cement the structure. The [Tb(dacda)]Cl complex is isostructural with that of the Eu^{3+} one. The only differences are the shortening of the metal–ligand bond distances and the absence of a coordinated water molecule. Both of these differences can be attributed to the lanthanide contraction.

In an attempt to gain more information about their solution structure, the (dacda) complexes were studied by NMR spectroscopy. The ^1H NMR spectra of both [Eu(dacda)]Cl and [Tb(dacda)]Cl consisted of unresolved, overlapping multiplets. However, NMR spectroscopy of the diamagnetic La^{3+} complex was far more revealing. The ^1H and ^1H–^{13}C COSY NMR spectra of [La(dacda)]Cl in D_2O are shown in Figs. 2 and 3, respectively. There are six intense signals in the ^1H NMR spectrum. The singlet at δ 3.49 is assigned to 4 equiv. acetate protons (e.g. H_{1a} and H_{1b}), indicating that on the NMR timescale the complex has high symmetry. This high symmetry is confirmed by the presence of only five resonances in the ^{13}C NMR spectrum, which must be due to the five unique carbon environments. The signal at δ 181 can be assigned to the carboxylate carbon (carbon 5). From the ^1H–^{13}C COSY NMR spectrum, the ^{13}C resonance at δ 60 is assigned to the acetate carbon (carbon 1). Similarly the multiplet at δ 3.94–4.03 in the ^1H NMR spectrum can be assigned as the eight diether-ethylenic protons (e.g. $H_{4a/b}$), allowing assignment of the carbon resonance at δ 70 to carbon 4. This leaves four ^1H resonances and two carbon resonances still to be assigned. The ^1H–^{13}C COSY shows that each of the remaining carbon resonances correlates with two proton resonances. Based on chemical shift, the carbon resonance at δ 69, which correlates with the proton resonances at δ 4.11–4.18 and 3.72–3.77, is assigned to carbon 3 because it is adjacent to an ether oxygen. Similarly, the carbon resonance at δ 59.12, which correlates with the proton resonances at δ 3.19–3.27 and 2.79–2.83, is assigned to carbon 2 because it is adjacent to an amine nitrogen.

Upon closer inspection of the ^1H NMR spectrum of [La(dacda)]Cl it is evident that there are some smaller peaks which may be due to the presence of a small amount of an isomeric form of the complex. Such solution behaviour is not uncommon for macrocyclic complexes. For example, two isomers are observed in solution in the ^1H NMR spectra of $[LnDOTA]^-$ complexes [41], though only one of these is observed in the solid state. To investigate this possibility, ^1H NMR spectra of [La(dacda)]Cl were recorded at 600 MHz at 5, 25 and 45 °C. The small peaks appear to be quite structured, as would be expected from an isomeric form of [La(dacda)]Cl, although they are too weak to attempt their proper assignment.

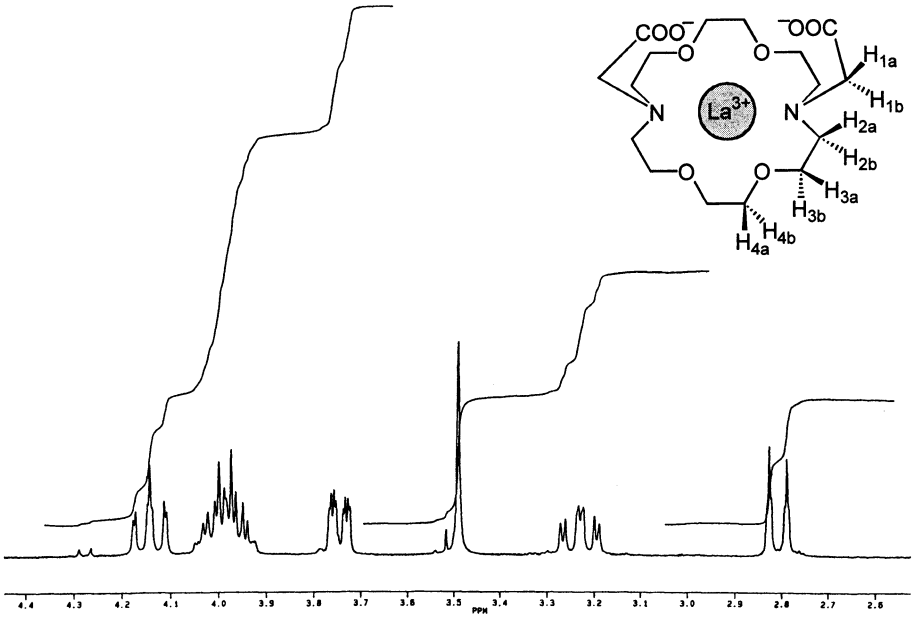


Fig. 2. The 360 MHz ^1H NMR spectrum of [La(dacda)]Cl at room temperature in D_2O; the inset shows a labelled structure (see text for assignments).

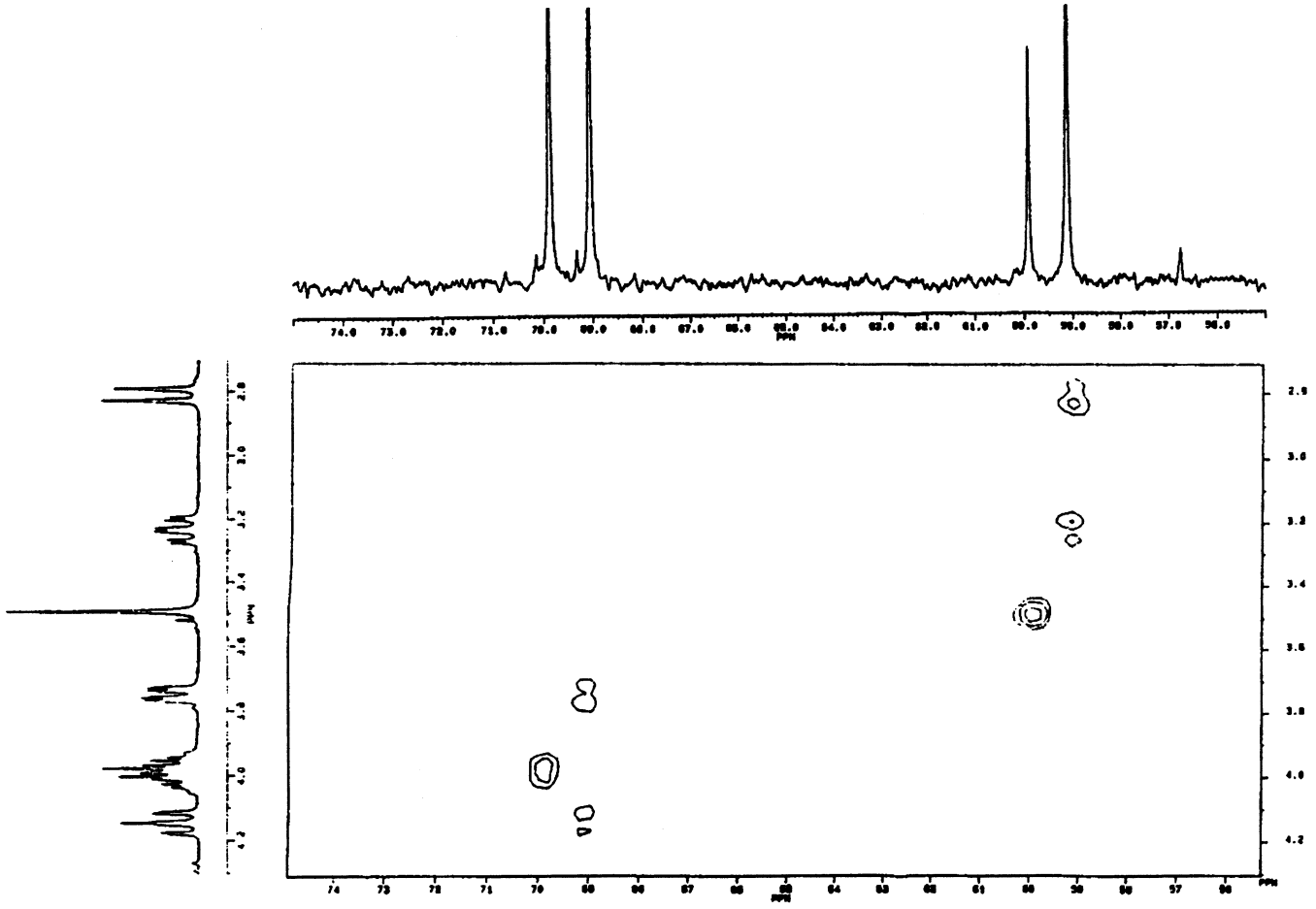


Fig. 3. The 360 MHz $^{1}H-^{13}C$ COSY spectrum of [La(dacda)]Cl at room temperature in D_2O.

The other feature of these spectra is the broadening of all the resonances with a lowering of the temperature. This corresponds to the slowing down of fluxional processes and is in agreement with the observed ^{13}C NMR spectra reported for the [Y(dacda)]Cl complex [29]. There were no significant changes in the relative intensities of the weak and strong resonances upon varying the temperature, as might be expected for an equilibrium between isomers. However, these studies were performed over a limited temperature range, and changes may not have been readily discernible due to the small intensity of the peaks. Although the structure of [La(dacda)]Cl is drawn with the carboxylate arms on the same side of the ring, the NMR data do not exclude a structure with the arms on opposite sides of the ring. Indeed, if isomeric forms are present, they may result from changes in the conformation of the acetate arms.

3.2. Luminescence spectroscopy

The steady-state excitation and emission spectra for [Eu(dacda)]Cl are shown in Fig. 4. Both spectra have differences to those reported for the free Eu^{3+} ion in aqueous solution [42], which can be attributed to complexation by dacda. The excitation spectrum consists of sharp f–f transitions, such as the strong $^{7}F_0 \rightarrow {}^{5}L_6$ transition at 394 nm, and broad UV bands centred at 280–290 nm, which are attributed to LMCT transitions associated with oxidation of the amine nitrogens. Similar broad LMCT bands are observed in the excitation spectrum of the Eu^{3+} complex of the 2.2.1 cryptand (**1**) [43].

The emission spectrum of [Eu(dacda)]Cl shows the characteristic $^{5}D_0 \rightarrow {}^{7}F_J$ ($J = 0$–4) Eu^{3+} transitions. The luminescence lifetimes of [Eu(dacda)]Cl (1×10^{-3} mol dm^{-3}) in H_2O and D_2O are 0.43 and 1.50 ms, respectively. The monoexponential decays obtained confirm the existence of the single species in solution. The number of coordinated water molecules can be calculated using equation,

$$q = A(k_{H_2O} - k_{D_2O})$$

where $A = 1.05$ and 4.2 for Eu and Tb, respectively [44] giving a q-value of 1.7.

If the equation which takes into account any outer sphere water molecules [45] is used

$$q_{corr} = A'(k_{H_2O} - k_{D_2O})_{corr}$$

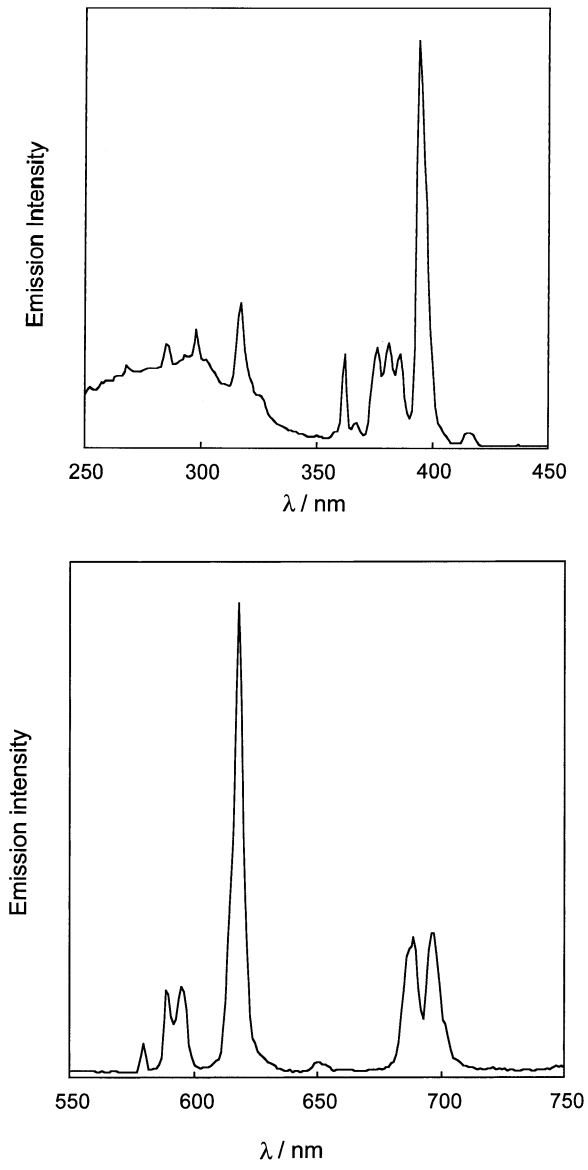


Fig. 4. Excitation spectrum (top) and corrected emission spectrum (bottom) of [Eu(dacda)]Cl in Tris buffer; $\lambda_{em} = 618$ nm, $\lambda_{exc} = 270$ nm.

where $A' = 1.2$ and 5 for Eu and Tb, respectively a q_{corr}-value of 1.7 is also obtained. The high q value may be attributed to the different confirmations that can be adopted from the crown with one or two water molecules coordinated if the carboxylate arms are on the same or opposite sides of the crown, respectively. This is in agreement with the previously reported data when the complexes were formed in solution [29].

3.3. Characterisation of lanthanide macma complexes

The positive-ion electrospray mass spectrum of $[Eu(macma)](NO_3)_2$ in H_2O shows peaks at m/z 489 and 491, corresponding to $[M-NO_3]^+$, as found in the FAB MS spectrum.

In the ^1H NMR spectrum of $[Y(macma)](NO_3)_2$ in D_2O, all of the protons can be accounted for by a poorly resolved multiplet at δ 3.68–3.97 and a broad singlet at δ 3.42, which integrate as 18 and 4 protons, respectively. Similarly, the ^1H NMR spectrum of $[Eu(macma)](NO_3)_2$ in CD_3NO_2 shows an unresolved multiplet at δ 3.37–3.70 and a broad singlet at δ 2.89, which integrate as 6 and 16 protons, respectively. These spectra suggest that fluxional processes, which are slow on the NMR time-scale, are operative for these complexes in solution.

Both steady-state excitation and emission spectra were recorded for solutions of $[Eu(macma)](NO_3)_2$ in Tris buffer (Fig. 5). Complexation by macma is demonstrated by the broad UV bands attributed to LMCT transitions, while the emission spectrum displays a

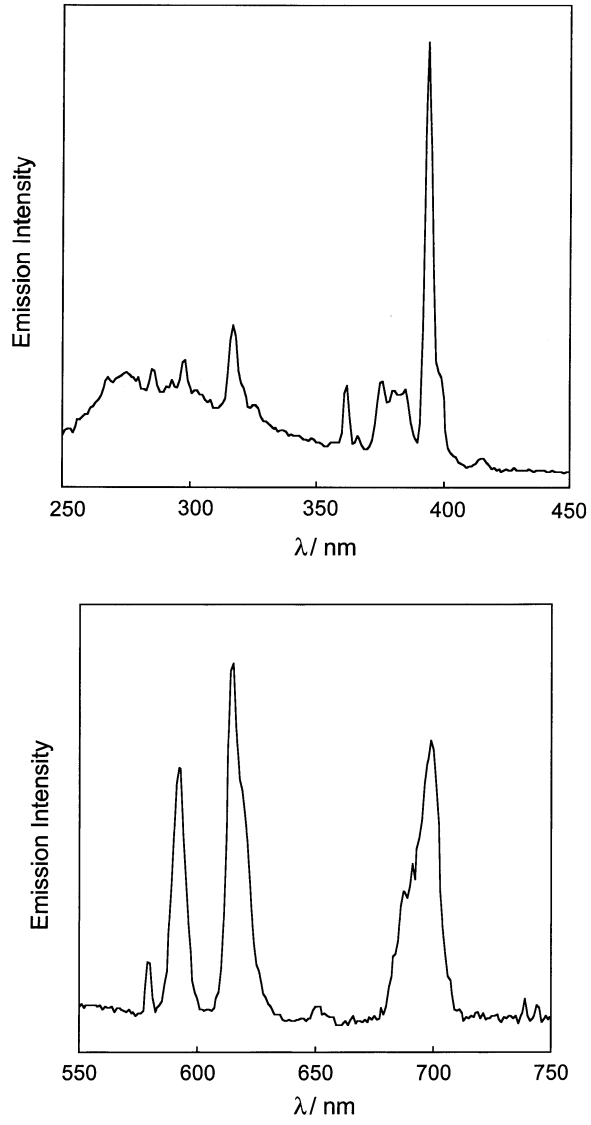


Fig. 5. Excitation spectrum (top) and corrected emission spectrum (bottom) of [Eu(macma)]Cl in Tris buffer; $\lambda_{em} = 618$ nm, $\lambda_{exc} = 270$ nm.

relatively high intensity $^5D_0 \rightarrow {}^7F_4$ transition (≈ 695 nm).

The luminescence lifetimes for $[Eu(macma)](NO_3)_2$ in H_2O and D_2O were found to be 0.18 and 1.0 ms, respectively. Monoexponential decays were obtained which confirms the presence of a single species. The calculated values of q and q_{corr} for this complex are 4.8 and 5.2, indicating five water molecules are coordinated to the Eu^{3+} ion. This high q-value is due to the presence of only five ring donor atoms and one carboxylate group on the ligand.

3.4. Ternary complex formation with LHCs

The formation of ternary complexes of the $[Ln(dacda)]^+$ and $[Eu(macma)]^{2+}$ with different LHCs was monitored by electrospray mass spectrometry and luminescence spectroscopy.

Initially, we chose dibenzoylmethane (DBM) as the LHC. It has good absorption properties and it is known to sensitise lanthanide luminescence. Upon incremental addition of a solution of deprotonated DBM to a solution of $[Eu(dacda)]^+$ in acetonitrile an increase of the lanthanide emission is observed which slows down after 1 equiv. of DBM and results in sensitisation of the europium signal of about fiftyfold (Fig. 6). DBM was deprotonated with triethyl amine prior to addition. The discontinuity in the shape of the titration plot is attributed to the complex equilibrium between the deprotonated and bound DBM, especially due to the possibility of formation of a six-member ring of triethyl amine with deprotonated DBM. The observed plateau confirms the saturation of the lanthanide binding site with the LHC, which is not observed in the absence of the crown ether under the same conditions. The binding of the LHC and the energy transfer process from DBM to lanthanide is also established by excitation spectroscopy where the characteristic profile of coordinated DBM absorption is observed. Electrospray mass spectrometry also confirmed the presence of 1:1 {[Eu(dacda)]:DBM} species (Table 1).

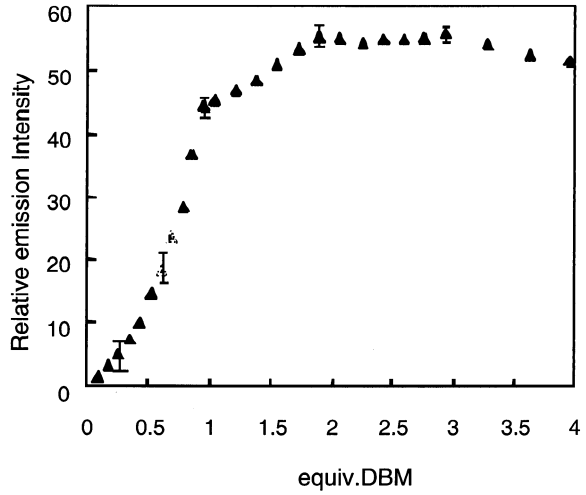


Fig. 6. Relative emission intensity of $[Eu(dacda)]^+$ upon addition of DBM in CH_3CN, $[Eu(dacda)Cl] = 1.1 \times 10^{-5}$ M, $\lambda_{exc} = 350$ nm. Only selected errors bars are shown for clarity.

Picolinic and phthalic acid were also chosen as bidentate LHCs. Addition of those acids to solutions of $[Eu(dacda)]^+$ or $[Tb(dacda)]^+$ was performed in aqueous solutions at pH 7.5. In both cases a plateau of the emission intensity is observed following the addition of the LHC attributed to the formation of a ternary complex between the crown and the LHC. In the case of terbium the signal enhancement with picolinic acid is almost two hundred and fiftyfold whereas in the case of europium is much smaller around one hundred and seventyfold; this is expected due to the better match of the LHC triplet state with terbium as well as the presence of charge transfer deactivating pathways in the case of the europium complex with carboxylates. The titration plots for the terbium complex are presented in Fig. 7. Although the increase of the emission intensity slows down after 1 equiv. of the phthalate or picolinate th saturation point suggests that 1 or 2 equiv. of phthalate or picolinate bind to $[Ln(dacda)]^+$. Although there are only two coordination sites of the lanthanide available for binding the flexibility of the two arms of the crown together with the similarity of the carboxylate binding site leads to a binding competition of the carboxylate arms with the carboxylates of the LHC. Control experiments under the same conditions of the emission titration plots in the absence of the crown moiety do not show saturation plateaus, confirming the role of the crown as a building block for the ternary formation.

When $[Eu(macma)]^{2+}$ was used, a clear plateau at the europium emission is observed upon addition of 2 equiv. of picolinate and phthalate (Fig. 8). The 1:2 ternary complex stoichiometry was expected due to the more available coordination sites of the macma complex to

Table 1
Data of electrospray mass spectrometry analysis

Complex	ES-MS mode	m/z; assignment
$[Eu(dacda)]^+$	positive	527/529, $[M]^+$
$[Tb(dacda)]^+$	positive	535, $[M]^+$
$[Eu(macma)]^{2+}$	positive	489/491 $[M+NO_3]^+$
[Eu(dacda):DBM]	positive	751/753, $[M+H]^+$
[Eu(dacda):PCA]	positive	672/674, $[M+Na]^+$
$[Eu(dacda):PTA]^-$	positive	715/717 $[M+Na+H]^+$
$[Eu(dacda):PTA]^-$	positive	691/693 $[M]^-$
[Eu(dacda):BZA]	positive	671/673, $[M+Na]^+$
[Tb(dacda):PCA]	positive	680, $[M+Na]^+$
$[Tb(dacda):PTA]^-$	positive	723, $[M+Na+H]^+$
$[Tb(dacda):PTA]^-$	negative	699, $[M]^-$

PCA, picolinate; PTA, phthalate; BZA, benzoate; DBM, dibenzoylmethide.

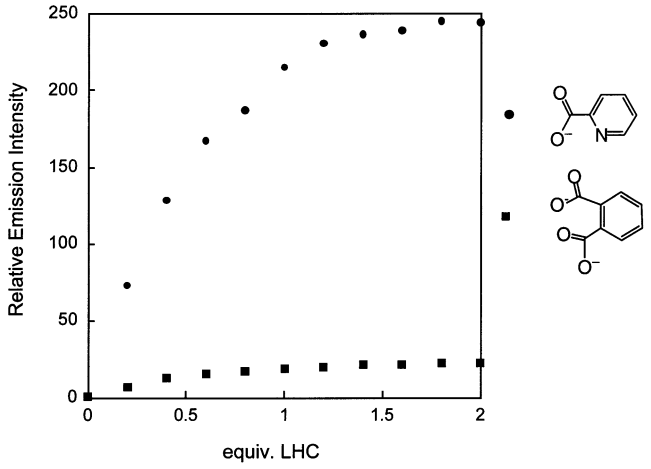


Fig. 7. Relative emission intensity of $[Tb(dacda)]^{+}$ upon addition of picolinate and phthalate in aqueous solution, $[Tb(dacda)Cl] = 1 \times 10^{-4}$ M, $\lambda_{exc} = 280$ nm.

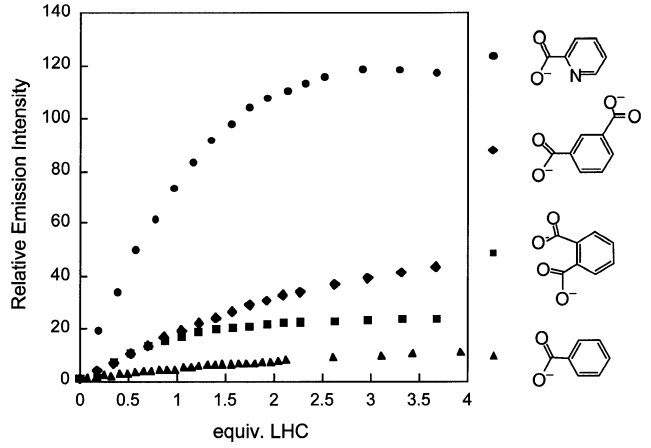
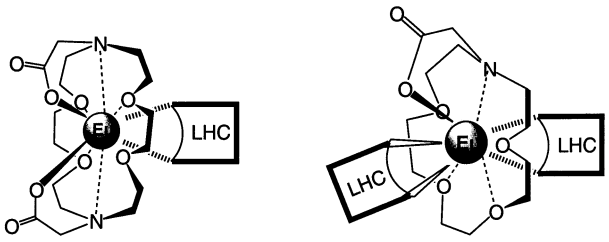


Fig. 8. Relative emission intensity of $[Eu(macma)]^{2+}$ upon addition of benzoate, phthalate isophthalate and picolinate in aqueous solution $[Eu(macma)(NO_3)_2] = 1 \times 10^{-4}$ M, $\lambda_{exc} = 280$ nm.

bind the LHC in comparison with the dacda. Isophthalate was also employed to compare the binding with phthalate. It is shown that there is no saturation plateau due to the weak binding of the monodentate carboxylate and the possibility of forming complexes in both the sites; although the latter is expected to occur in conditions of higher concentrations than the ones recorded in this experiment. Weak binding is also observed for benzoate.

Excitation spectra of the ternary complexes of $[Ln(dacda)]^{+}$ and $[Eu(macma)]^{2+}$ with the relevant aromatic acids also show the characteristic peak corresponding to the absorption of the LHC. Electrospray mass spectra also show the presence of the ternary complexes (Table 1) of the lanthanide crowns with the aromatic acids. Species of 1:2 ternary complexes were only detected for the macma case, probably due to the lack of stability of the higher species under the electrospray conditions.



Scheme 3.

4. Conclusions

We have shown that the luminescent europium(III) and terbium(III) complexes of dacda and macma, have available coordination sites so that the can act as building blocks for the formation of ternary complexes in aqueous solution (Scheme 3). Sensitisation of the luminescence signal up to two hundred and fiftyfold is observed by addition of simple LHCs to the lanthanide crowns. The availability of coordination sites of the lanthanide can be predicted by the number of water molecules around the lanthanide and it is consistent for most of the cases. The flexibility of the carboxylate arms of the crowns can be a problem for the control of the ternary complex formation. The different charge of the crowns examined may also affect the strength of the LHC binding and consequently the sensitisation of the lanthanide luminescence signal. Further studies are underway to extend the control of formation of ternary lanthanide complexes.

5. Supplementary material

The CCDC contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/contc/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk)).

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