Lanthanide Homobimetallic Triple-Stranded Helicates: Insight into the Self-Assembly Mechanism

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The self-assembly mechanism leading to the exclusive formation of a triple-stranded bimetallic helicate upon reaction of EuIII with a ditopic hexadentate ligand L bearing two carboxylate moieties has been fully elucidated in water for a wide range of [Eu]tot/[L]tot ratios. Using a fruitful combination of electrospray mass spectrometry, potentiometry, UV/Vis spectrophotometry, luminescence, and 1H NMR spectroscopy, the final product Eu3L3 and the intermediate species Eu2L2 and Eu2L3 have been characterised. The presence of terminal carboxylates in L significantly reduces the electrostatic repulsions of the coordination sites in Eu2L2 and Eu2L3 compared with the corresponding complexes formed with analogous neutral ligands and thus increases the stability of the L-europium(III) complexes. Kinetic investigations carried out with an excess of L and with an excess of EuIII, show that the self-assembly proceeds through either EuL2 or EuL3 intermediates depending on the experimental conditions and leads to a pre-organized Eu3L3 complex by either a “braiding” or a “keystone” mechanism. In the last step, a fast and efficient wrapping of the third ligand strand leads to the target Eu3L3 helicate. The overall process is mainly governed by electrostatic interactions and proceeds via a key double stranded intermediate helicate Eu2L2. To the best of our knowledge, as a result of the fine-tuning of the coordination properties of L, we present one of the most efficient and cooperative metal/ligand systems for the spontaneous organization of a bimetallic triple-stranded structure.

Introduction

The self-assembly of supramolecular edifices, which was inspired from one of the basic principles in biology,[1] is an appealing approach to the design of novel nanomaterials with predetermined and specific properties.[2] In particular, combining the toolbox of non-covalent interactions with the intriguing chemical and physicochemical properties of transition metal ions allows one to build functional supramolecular architectures via metal-ion assisted self-assembly.[3] This strategy has successfully generated fascinating two and three-dimensional edifices from polytopic ligands, which include rods,[4] grids,[5] cages,[6] helices,[7] ladders,[8] and rings.[9] Initial studies of metallo-supramolecular helicates have mainly involved double- or triple-stranded structures containing cations such as CuII, Ag+, CuII, NiII, GaIII, or FeIII and ligands derived from bipyridine, terpyridine, or catechol frameworks.[10,11] The specific magnetic and luminescent[12–14] properties of the trivalent lanthanide cations[15] are increasingly being taken advantage of in medical diagnoses[16,17] and therapy[18] as well as in the catalytic cleavage of DNA and RNA.[19] In this context, the synthesis of elaborate polymetallic lanthanide helicates, which are potentially chiral, may contribute to the development of such applications. However, the poor stereochemical preferences and the variable coordination numbers adopted by LnIII ions render difficult a reliable molecular programming in solution. In spite of this difficulty, we have demonstrated that a careful ligand design based on the induced fit principle[20] leads to the strict self-assembly of lanthanide-containing triple-stranded helicates in organic media.[21,22] Ditopic pentadentate, hexadentate or nonadentate ligands derived from 2-pyridin-2-yl-1H-benzimidazole are indeed versatile building blocks and can be used to prepare bimetallic 3d–4f and 4f–4f assemblies[23] as well as trimetallic 4f–4f–4f edifices.[24] Recently, we have also shown that grafting different substituents at the end of the hexadentate ligands results in ditopic hosts able to specifically recognize a pair of lanthanide ions in solution.[25] Bioanalytical and medical applications require water-soluble probes, so that the ditopic ligand L with two terminal carboxylate moieties was synthesized (Figure 1) and was found to yield highly stable bimetallic helicates.

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Supporting information for this article is available on the WWW under http://www.eurjic.org or from the author.
Ln₂L₃ in water, despite the large hydration enthalpy of the metal ions.[26]

The numerous helicates reported so far[10] have been essentially characterised by their molecular structures and, sometimes, by their thermodynamic parameters. Kinetic data, which are essential for deciphering the self-assembly mechanism and, therefore for understanding the recognition process, are extremely scarce.[27–30] As part of our ongoing kinetic investigations of supramolecular edifices with polytopic ligands, dealing with either copper(II) trimetallic double-stranded[29] or lanthanide(III) bimetallic triple-stranded[30] helicates in acetonitrile, we present here a detailed physicochemical study of the Eu₂L₃ helicate in aqueous solution. The work is focused on getting a better understanding of the key steps of the self-assembly process. An original combination of potentiometry, absorption and emission spectrophotometry, ES mass spectrometry, and stopped-flow techniques, allowed us to determine both the thermodynamic and kinetic parameters characterising the formation of the bimetallic europium(III) triple-stranded helicate in water.

Results and Discussion

Ligand Properties

Detailed interpretation of thermodynamic and kinetic data requires knowledge of the ligand pKₐ values, at least in the pH range in which the data are collected. Indeed, pKₐ values of the carboxylic acid functions are difficult to determine in view of the extremely poor solubility of the ligand below pH 6 and its precipitation at pH 4. We have therefore investigated the acid-base properties of the ligand in the sole pH range 10.5–5.8 by various techniques. Potentiometric titrations of ca. 10⁻³ M solutions (Figure S1 in the Supporting Information; see also the footnote on the first page of this article) followed by a statistical treatment of the data[31] yielded the three protonation constants listed in Table 1. The corresponding distribution diagram is presented in Figure S2 (see Supporting Information).

Table 1. Successive protonation constants of ligand L; solvent: water; T = 25.0 ± 0.2 °C; I = 0.1 M (NEt₄ClO₄); for the sake of simplicity charges have been omitted

<table>
<thead>
<tr>
<th>Equilibrium</th>
<th>log K (± 3σ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L + H ⇌ LH</td>
<td>10.1 ± 0.1</td>
</tr>
<tr>
<td>LH + H ⇌ LH₂</td>
<td>9.5 ± 0.2</td>
</tr>
<tr>
<td>LH₂ + H ⇌ LH₃</td>
<td>6.0 ± 0.2</td>
</tr>
</tbody>
</table>

In order to confirm the first pKₐ, UV/Visible absorption spectra of the free ligand (see Supporting Information, Figure S3) were recorded in the range 7.38 < p[H] < 11.59 and a single cumulative protonation constant logβ₁LH₂ = 19.8 ± 0.6 could be extracted using statistical methods[31] compared with 19.6 obtained by potentiometry.

We have also taken advantage of the fluorescent properties of the benzimidazole derivatives[32,33] Decreasing the p[H] from 11.43 to 7.59 resulted in a drastic quenching of the ligand-centered fluorescence (Figure 2) and statistical treatment[31] of the data leads to the following protonation constants: logK₁LH = 10.9 ± 0.7 and logK₁LH₂ = 8.7 ± 0.5. The re-calculated spectra for L, LH and LH₂ are presented in Figure S4 (see Supporting Information).
Finally, the assignment of the pKₐ values was made on the basis of structural information extracted from ¹H and ¹³C NMR spectra recorded at various p[D] values. These data clearly point to both monoprotonated LH and diprotonated LH₂ species retaining C₂ symmetry in the p[D] range 13.00–7.10, indicating that no intramolecular interaction occurs between the two identical subunits of the ditopic strand (Figure 1). This lack of interaction can be further confirmed by the difference between logK₁LH and logK₁LH₂ (ΔlogK = 0.6, Table 1), which is in excellent agreement with a statistical evaluation.[34] NOE effects are consistent with a transoid conformation for the ligand at p[D] = 13.[36] When the p[D] was decreased from 13.00 to 7.10, small but significant variations in the chemical shifts of the aromatic protons of the benzimidazole moiety (H₄, H₆, and H₇) were observed whereas the ¹³C chemical shifts related to the terminal carboxylates did not vary (Table 2). These NMR spectroscopic data strongly suggest that the first two protonation processes occur at the imidazolyl groups, an assumption confirmed by the absence of a red shift in the absorption and emission spectra in the p[H] range 11.4–7.4 (Figure 2 and Figure S3 in the Supporting Information). Such a shift would be expected if the N-pyridyl groups were protonated.[35,36]

In spite of the converging of the information provided by our measurements, one has to be aware that intra- and intermolecular hydrogen bonding may occur which could modify the above reasoning. For instance, in the case of 2-(2’-pyridyl)benzimidazole in hydrochloric acid, ¹⁵N NMR spectroscopic measurements result in only two resonances and are consistent with the presence of fast exchanging resonance structures, one of which having a proton linked to both nitrogen atoms.[37] A similar situation is met for 1,10-phenanthroline-2,9-dicarboxylic acid (logK₂ = 9.04 ± 0.14).[41] Solubility problems below p[H] 5.89 prevented the determination of K₁LH₄, K₂LH₄, and K₃LH₄. In fact, K₁LH₄ is related to K₁LH₂ in terms of symmetry (L adopting C₂ symmetry) and statistically should be smaller than the latter by approximately 0.6.[13] Much lower values of K₁LH₄ and K₂LH₄ are expected according to data available in the literature for picolinic (logK₂ = 1)[43,44] and 1,10-phenanthroline-2,9-dicarboxylic acids (logK₁ = 2.37 ± 0.21).[41,45] In 1.5 M HClO₄, slow and irreversible

<table>
<thead>
<tr>
<th>p[H]</th>
<th>H⁴</th>
<th>H⁶</th>
<th>δ(¹H)</th>
<th>H³</th>
<th>H⁴</th>
<th>H⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.6</td>
<td>7.56</td>
<td>7.12</td>
<td>7.30</td>
<td>7.87</td>
<td>7.92</td>
<td>7.85</td>
</tr>
<tr>
<td>10.0</td>
<td>7.54</td>
<td>7.08</td>
<td>7.26</td>
<td>7.86</td>
<td>7.91</td>
<td>7.82</td>
</tr>
<tr>
<td>9.0</td>
<td>7.53</td>
<td>7.07</td>
<td>7.24</td>
<td>7.86</td>
<td>7.90</td>
<td>7.81</td>
</tr>
<tr>
<td>6.7</td>
<td>7.49</td>
<td>7.03</td>
<td>7.20</td>
<td>7.86</td>
<td>7.88</td>
<td>7.79</td>
</tr>
</tbody>
</table>

Scheme 1. Potential hydrogen bonding in two protonated subunits of L

The protonation constants logK₁LH and logK₁LH₂ are at least 4–5 orders of magnitude larger than those reported for benzimidazole (logK = 5.58)[39,40] or 2-(2’-pyridyl)benzimidazole (logK = 4.41)[36] but less than two orders of magnitude smaller than the first protonation constant determined for 1,10-phenanthroline-2,9-dicarboxylic acid in water (logK₁ = 11.77 ± 0.08).[41] Carboxylic functions in positions 2 and 9 of the phenanthroline indeed induce a drastic increase in the basicity of the N-pyridyl atoms compared with phenanthroline (logK₁ = 4.77).[42] A similar trend can be observed for the ditopic ligand L, and we note that intramolecular hydrogen bonds between N(3’) and N(1) would also contribute to increasing logK₁LH and logK₁LH₂ (Scheme 1). If the third protonation were to take place at N(1), a large decrease in K₁LH could be expected, due to both statistical effects and hydrogen bonding. The value of K₁LH₂ is about four orders of magnitude lower than K₁LH₂ and could therefore reflect these contributions and be satisfactorily compared with the constant relative to the second protonation of 1,10-phenanthroline-2,9-dicarboxylic acid (logK₂ = 9.04 ± 0.14).[41]

Table 2. ¹H- and ¹³C-chemical shifts (ppm vs. TMS) as a function of p[H]; p[H] values were determined by using p[H] = p[D] - 0.40[38]; solvent: D₂O; [L]tot = 10⁻² M; T = 25 ± 1 °C.

<table>
<thead>
<tr>
<th>C¹</th>
<th>C⁶</th>
<th>δ(¹³C)</th>
<th>C³</th>
<th>C⁴</th>
<th>C⁵</th>
<th>C=O</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.1</td>
<td>110.8</td>
<td>124.0</td>
<td>117.9</td>
<td>125.6</td>
<td>138.4</td>
<td>124.7</td>
</tr>
<tr>
<td>9.1</td>
<td>110.1</td>
<td>124.3</td>
<td>117.4</td>
<td>123.8</td>
<td>137.5</td>
<td>125.1</td>
</tr>
<tr>
<td>7.1</td>
<td>110.1</td>
<td>124.4</td>
<td>117.4</td>
<td>125.1</td>
<td>137.9</td>
<td>123.9</td>
</tr>
</tbody>
</table>
degradation of ligand L via a potential de-carboxylation reactions was observed by UV/Visible spectrophotometry (see Supporting Information, Figure S5).

**Stoichiometry and Stability of Europium(III) Complexes with L**

The use of electrospray mass spectrometry (ES-MS) to characterize the stoichiometry of metallic complexes in solution is well documented,[10,46] and has been employed by us for this purpose on previous occasions.[21,27-30,47-49] ES-MS measurements were carried out at two different [Eu]_{tot}/[L]_{tot} ratios (Figure 3) and the corresponding spectra clearly indicate the formation of three europium(III) complexes, Eu_{2}L_3, Eu_{2}L_2, and EuL_2 (see Supporting Information, Table S1). The peaks of the triple-stranded Eu_{2}L_3 helicate dominate the spectra, even with an excess of Eu(III). The inner coordination spheres of the minor species Eu_{2}L_2 and EuL_2 are completed with water molecules. Since lanthanide cations possess large solvation energies,[47,48] the ES-MS responses[50] will be drastically decreased for the latter complexes compared with that of the Eu_{2}L_3 helicate. Loss of neutral CO_2 was also observed, as previously reported for amino acid- or peptide-metal complexes in positive[51,52] and in negative modes.[53]

To quantify the interaction between L and Eu(III), we titrated the ligand in water at p[H] 6.15±0.05 with europium(III) and monitored the changes occurring in solution using both absorption (Figure 4a, and Figure S6 in the Supporting Information) and gated luminescence spectroscopy (Figure 4b).

The formation of the helicate induces a significant red shift to 332 nm in the ligand π→π* transition band centered at 308 nm (Figure 4a). Moreover, L was reported to be a fair luminescence sensitizer for europium(III),[26] the absolute quantum yield of the metal-centered luminescence reaching 1.3% at p[H] = 7.0 with a lifetime of 2.13(2) ms. Data were satisfactorily fitted to the simple model represented by Equation (2) and both experiments yielded very similar values (Table 3), in agreement with the previously mentioned quantum yield and lifetime.

Table 3. Cumulative stability constants for the Eu_{2}L_3 helicate; solvent: water; T = 25.0 ± 0.2°C

<table>
<thead>
<tr>
<th>Titration method</th>
<th>log/β_{Eu_{2}L_{3}} ± 3σ[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV/Vis absorption, direct</td>
<td>50.6 ± 2.0</td>
</tr>
<tr>
<td>Gated luminescence, direct</td>
<td>49.9 ± 1.3</td>
</tr>
<tr>
<td>1H NMR, competition with DOTA</td>
<td>52.3 ± 2.3</td>
</tr>
</tbody>
</table>

[a] The stability constants were calculated[89] from conditional values with the protonation constants given for L in Table 1.
reported one obtained via \(^1\text{H} \) NMR competitive titrations with various concentrations of DOTA (1,4,7,10-tetraazacyclododecane-\(N,N',N'',N'''\)-tetraacetic acid) in D\( _2\text{O}\).[26]

\[
3\text{L} + 2\text{Eu} \rightarrow \text{Eu}_2\text{L}_3
\]  

(2)

Spectrophotometric (absorption and luminescence) and NMR spectroscopic data clearly demonstrate that the major species in aqueous solution at pH 6.15 and in a large range of [Eu\(_{\text{tot}}\)]/[L\(_{\text{tot}}\)] ratios is the triple-stranded helicate Eu\(_2\)L\(_3\). In addition to this main species ES-MS, which is by far a more sensitive method, indicated the presence of small amounts of Eu\(_2\)L\(_2\) with an excess of Eu\(_{\text{III}}\), and of EuL\(_2\) with an excess of L. The triple stranded helicate is with no doubt a thermodynamic product and its structure in aqueous solution is the same as that determined by single-crystal X-ray diffraction.[26] Although the Eu\(_2\)L\(_2\) species is not seen in the spectrophotometric titrations, 2:2 species have been observed with similar ligands.[21,22] In particular, [Eu\(_2\)(LB)\(_2\)]\(^{6+}\) (LB: \(\text{R}^1 = \text{C(O)NEt}_2\) and \(\text{R}^2 = \text{CH}_3\), see Figure 1) adopts a box-like structure in the solid state, in which the ligand strands are arranged “side-by-side” and the two metal are ions connected via hydrogen bonds produced by four water molecules.[22] Therefore, the 2:2 species may also be considered to be a thermodynamic product. The presence of the terminal carboxylate functions of L could be expected to significantly reduce the electrostatic repulsions of the coordination sites in Eu\(_2\)L\(_2\) and Eu\(_2\)L\(_3\) by comparison with the corresponding complexes with L\(_A\) (R\(_1 = 1-(3,5\text{-dimehoxybenzyl}-1\text{-H}-\text{benzimidazole and R}^2 = \text{CH}_3\), see Figure 1) and LB and thus to increase the stability of the L-europium(III) complexes.[54]

### Formation Kinetics in Excess of Ligand

Two series of experiments carried out with two different metal concentrations, [Eu\(_{\text{tot}}\)] = 1.06 \times 10^{-2} \text{ M} and 2.73 \times 10^{-6} \text{ M}, allowed us to observe two rate-limiting steps in the second and minute time spans, respectively (see Supporting Information, Figure S7). The corresponding spectrophotometric signals recorded at 350 nm undergo an increase in absorbance followed by a decrease. The variation of the two corresponding pseudo-first-order rate constants \(k_{2,\text{obs}}\) and \(k_{3,\text{obs}}\) (s\(^{-1}\)) versus [L\(_{\text{tot}}\)] (see Supporting Information, Tables S2 and S3) is presented in Figure 5.

The variation of \(k_{2,\text{obs}}\) is in good agreement with a fast pre-equilibrium for the formation of EuL, followed by a slower binding of L to EuL:

\[
\text{Eu} + \text{L} \rightarrow \text{EuL} \quad \text{fast}
\]

(3)

\[
\text{EuL} + \text{L} \rightarrow \text{EuL}_2 \quad \text{slow}
\]

(4)

The rate law corresponding to the equilibrium according to Equation (4) is expressed by the following Equation (5).

\[
d[\text{EuL}_2]/dt = k_1 \times [\text{EuL}] \times [\text{L}]_{\text{tot}} - k_2 \times [\text{EuL}]
\]

(5)

and leads to Equation (6).[55]

\[
k_{2,\text{obs}} = \frac{k_1 \times K_{\text{EuL}} \times [\text{L}]_{\text{tot}}^2}{1 + K_{\text{EuL}} \times [\text{L}]_{\text{tot}} + k_{-2}}
\]

(6)

The rate constant \(k_2\) and the stability constant \(K_{\text{EuL}}\) were determined by a non-linear least-squares method[56] (Table 4) but \(k_{-2}\) could not be obtained under our experimental conditions.

On the other hand, the variation of \(k_{3,\text{obs}}\) versus [L\(_{\text{tot}}\)] suggests the coordination of a second Eu\(_{\text{III}}\) cation to EuL\(_2\):

\[
\text{EuL}_2 + \text{EuL} \rightarrow \text{EuL}_3
\]

(7)

The corresponding rate law can be written:

\[
d[\text{EuL}_3]/dt = k_3 \times [\text{EuL}_2] \times [\text{Eu}] - k_3 \times [\text{EuL}_2]
\]

(8)

Resolution of the differential Equation (8)[55] leads to the following reduced expression, under our experimental conditions:

\[
k_{3,\text{obs}} = \frac{4 \times k_1 \times \beta_{\text{EuL}} \times [\text{L}]_{\text{tot}} \times [\text{EuL}]_2}{(1 + K_{\text{EuL}} \times [\text{L}]_{\text{tot}} + \beta_{\text{EuL}} \times [\text{L}]_{\text{tot}}^2)} + k_{-3}
\]

(9)
Table 4. Formation mechanism of Eu₂L₃ with an excess of L; solvent: water; T = 25.0 ± 0.2 °C; p[H] = 6.15±0.05 (MES buffer 0.1 M); the errors are given as standard errors[56]

<table>
<thead>
<tr>
<th>Steps</th>
<th>Experimental values</th>
<th>Calculated values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu + L $\xrightarrow{k_{1}}$ EuL</td>
<td>$K_{int} = (1.9 \pm 0.8) \times 10^4$ M⁻¹</td>
<td></td>
</tr>
<tr>
<td>EuL + L $\xrightarrow{k_{2}}$ EuL₂</td>
<td>$k_{3} = (4.4 \pm 0.9) \times 10^6$ M⁻¹ s⁻¹</td>
<td>$k_{3} = \frac{k_{2} \times K_{int}}{[L]}$</td>
</tr>
<tr>
<td>EuL₂ + Eu $\xrightarrow{k_{3}}$ Eu₂L₃</td>
<td>$k_{3} = (7.0 \pm 0.6) \times 10^6$ M⁻¹ s⁻¹</td>
<td>$K_{int,3} = \frac{k_{3}}{k_{4}}$</td>
</tr>
<tr>
<td>Eu₂L₃ + L $\xrightarrow{k_{4}}$ Eu₃L₄</td>
<td>not determined</td>
<td></td>
</tr>
</tbody>
</table>

The corresponding rate constants $k_{3}$, $k_{-3}$ are given in Table 4.

The absorbance at 350 nm at the end of the third step (Figure S7 in the Supporting Information) is in good agreement, within experimental error, with the sum of the absorbance of the excess of free ligand and of the bimetallic triple-stranded helicate ([Eu₂L₃]ₜot = 1/2[Eu₄tot]). This result constitutes a good indication that the last step is faster than the formation of the Eu₂L₂ precursor and that the final step in the formation of Eu₂L₃ involves the addition of a third ligand to Eu₂L₂, in a “braiding” mechanism, as opposed to a “keystone” mechanism in which the second metal ion is added to a pre-organized EuL₃ complex.[57]

Formation Kinetics with an Excess of Europium(III)

Kinetic measurements were carried out under pseudofirst order conditions. Two exponential signals were recorded at 332 nm in the second time range using a stopped-flow technique, whereas an exponential signal was measured in the hour time range on a classical spectrophotometer (Figure 6).

The pseudo-first-order rate constant $k'_{2,obs}$ increased slightly, but significantly as [Eu]tot was increased. A large increase of the spectrophotometric absorption occurred during the dead-time ($\sim 3$ ms) of the stopped-flow device and could be attributed to the initial and fast formation of EuL [Equation (3)]. The following equilibria are in satisfactory agreement with these experimental data:

\[
\text{EuL} + \text{Eu} \xrightarrow{k'_{2}} \text{Eu}_{2}\text{L} \quad \text{(10)}
\]

The rate law corresponding to equilibrium (10) is expressed by:

\[
d[\text{EuL}] / dt = k'_{2} \times [\text{EuL}] \times [\text{Eu}]_{tot} - k'_{2} \times [\text{EuL}] \quad \text{(11)}
\]

and leads to Equation (12).[55]

\[
k'_{2,obs} = k'_{2} \times [\text{Eu}]_{tot} \times [\text{EuL}]_{tot}^{2} / (1 + K_{int} \times [\text{Eu}]_{tot} + k'_{2}) \quad \text{(12)}
\]

Three corresponding pseudo-first-order rate constants $k'_{2,obs}$, $k'_{3,obs}$ and $k'_{4,obs}$ (s⁻¹) extracted from these data are reported in Table 5 (see also Figure S8 in the Supporting Information).
Table 6. Formation mechanism of the Eu$_2$L$_3$ helicate with an excess of Eu$^{III}$; solvent: water; $T = 25.0 \pm 0.2$ °C; $p$[H] = 6.15 $\pm$ 0.05 (MES buffer 0.1 M); the errors are given as standard errors$^{[56]}$

<table>
<thead>
<tr>
<th>Steps</th>
<th>Experimental values</th>
<th>Calculated values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu + L</td>
<td>$\frac{k_1}{k_2}$</td>
<td>EuL</td>
</tr>
<tr>
<td>EuL + Eu</td>
<td>$\frac{k_3}{k_4}$</td>
<td>EuL$_3$</td>
</tr>
<tr>
<td>Eu$_2$L + L</td>
<td>$\frac{k_5}{k_6}$</td>
<td>Eu$_2$L$_2$</td>
</tr>
<tr>
<td>Eu$_2$L$_2$ + L</td>
<td>$\frac{k_7}{k_8}$</td>
<td>Eu$_2$L$_3$</td>
</tr>
</tbody>
</table>

If we consider that under our experimental conditions $K_{EuL} \times [Eu]_{tot} \gg 1$:

$$k'_{3,obs} = k'_{3} \times [Eu]_{tot} + k'_{-3} \quad (13)$$

The values of the rate constants $k'_3$ and $k'_{-3}$ were calculated by a least-squares method$^{[56]}$ and are reported in Table 6.

When $[Eu]_{tot}$ is increased, $k'_{3,obs}$ is decreases (Table 5 and Figure S8b in the Supporting Information), which suggests that the coordination of a second ligand strand to Eu$_2$L takes place and leads to the bimetallic double-stranded species Eu$_2$L$_2$:

$$EuL + L \xrightarrow{k'_3} EuL_2 \quad (14)$$

The rate law is then expressed by:

$$d[EuL_2]/dt = k'_3 \times [EuL] \times [L] - k'_{-3} \times [EuL_2] \quad (15)$$

If we assume that equilibrium is reached at the end of the previous steps, we can use the corresponding stability constants to solve the mass balance equation and evaluate $[L]$:

$$[L] = \frac{[L]_{tot} - 2 \times [EuL_2]}{1 + K_{EuL} \times [Eu]_{tot} + \beta_{EuL} \times [Eu]_{tot}^2} \quad (16)$$

The new expression of the rate law becomes:

$$\frac{d[EuL_2]}{dt} = k'_3 \times \beta_{EuL} \times [Eu]_{tot} \times [L]_{tot} - k'_{-3} \times [EuL_2]$$

$$= \left(1 + K_{EuL} \times [Eu]_{tot} + \beta_{EuL} \times [Eu]_{tot}^2\right)^{-1} \times k'_{3,obs} \times [Eu]_{tot} \times [L]_{tot} \quad (17)$$

The resolution of this differential equation$^{[55]}$ taking our experimental conditions into account, gives the reduced equation:

$$k'_{3,obs} = \frac{4 \times k'_3 \times \beta_{EuL} \times [L]_{tot} \times [Eu]_{tot}^2}{(1 + K_{EuL} \times [Eu]_{tot} + \beta_{EuL} \times [Eu]_{tot}^2)^2} + k'_{-3} \quad (18)$$

Statistical processing$^{[56]}$ of the kinetic data allows estimation of $k'_{3,obs}$, $K_{EuL}$ and $\beta_{EuL}$ (Table 6), but not of $k'_{-3,obs}$. It is noteworthy that the absorbance at the end of this step (Figure 6) corresponds to the absorption at 332 nm of the bimetallic triple-stranded helicate Eu$_2$L$_3$ (see Supporting Information, Figure S6). This is a sound indication that the formation of Eu$_2$L$_2$ is followed by the fast addition of a third ligand strand leading to the final thermodynamically stable helicate Eu$_2$L$_3$:

$$EuL_2 + L \xrightarrow{k'_{4,obs}} EuL_3 \quad (19)$$

A decrease of more than four orders of magnitude was observed for $k'_{4,obs}$ compared with $k'_{3,obs}$ (Table 5), but $k'_{4,obs}$ did not vary significantly with $[Eu]_{tot}$ (see Supporting Information, Figure S8c). These data strongly suggest a slow release of L from Eu$_2$L$_3$ which was rapidly formed in the previous step:

$$EuL_3 \xrightarrow{k'_{-4}} EuL_2 + L \quad (20)$$

The corresponding rate law can now be expressed by the following relationship:

$$-d[EuL_3]/dt = k'_{-4} \times [EuL_3]$$

with:

$$k'_{4,obs} = k'_{-4} \quad (22)$$

We will now discuss the overall self-assembly mechanism of the bimetallic triple-stranded helicate Eu$_2$L$_3$ presented in
coordination of Eu III limited by the exchange rate of the

cogenerated 

costant.

The stability constant

to a slower binding rate constant and to an increased inert-

ated an outer-sphere stability constant

be estimated

as

slow complex formation is related to the ligand

stoichiometric ratio \([\text{Eu}]_{\text{tot}}/\text{[L]}_{\text{tot}}\). In contrast, the intermedi-

Figure 7. Schematic representation of the self-assembly mechanism

of EuL3

Figure 7. The initial and final steps are independent of the stoichiometric ratio \([\text{Eu}]_{\text{tot}}/\text{[L]}_{\text{tot}}\). In contrast, the intermedi-

dates depend on whether the kinetics were measured in

the presence of an excess of ligand or metal.

Since the water exchange rates of LnIII ions are large

slow complex formation is related to the ligand

properties.\(^{58-61}\) The ditopic molecule L was designed for

the fine-tuning of the LnIII coordination sphere, with a

short methylene spacer to prevent self-folding. At \(p[H] = 6.15\), LH2, the major species, possesses one proton on each

imidazolyl group. If they are stabilized by hydrogen bonds

as shown in Scheme 1, the tridentate subunits of L adopt

a cisoid conformation (Scheme 1) which is preferable to a

coordination of EuIII limited by the exchange rate of the

first water molecule on the lanthanide cation.\(^{58,62}\) Using

the water exchange rate constant \(k_{\text{ex}}\) determined by \(^{17}\)O

NMR spectroscopy for EuIII \((5 \times 10^8 \text{ s}^{-1})\)^{58,62} we calcu-

lated an outer-sphere stability constant \(K_{\text{ex}} = 0.16 \text{ M}^{-1}\)

for a triple charged cation/neutral ligand system. Under

these conditions, the formation rate constant for EuL can

be estimated\(^{63}\) as \(k_1 = K_{\text{ex}} \times k_{\text{ex}} = 8 \times 10^7 \text{ M}^{-1} \times \text{s}^{-1}\). This value is similar to the literature data reported for the

formation of anthranilate-EuIII \((1.05 \times 10^8 \text{ M}^{-1} \times \text{s}^{-1})\)^{66,67} picolinate-LaIII \((4.9 \times 10^8 \text{ M}^{-1} \times \text{s}^{-1})\) or picolinate-

DyIII \((1.5 \times 10^8 \text{ M}^{-1} \times \text{s}^{-1})\)^{68,69} complexes at various

temperatures and ionic strengths in water. The formation

mechanism of EuL is at odds with the one determined for

the EuL\(^{A}\) analogue, for which conformational changes lead
to a slower binding rate constant and to an increased inert-

ness.\(^{30}\) The stability constant \(K_{\text{EuL}} = (1.8 \pm 0.9) \times 10^4 \text{ M}^{-1}\)

calculated from the variation of the initial absorbance \(A_0\)

versus [Eu]\(_{\text{tot}}\) in the experiments with an excess of metal

(Figure 6 and Figure S9 in the Supporting Information), is

in excellent agreement with the values given in Tables 4 and

6. Comparing this constant with the one obtained for the

complex with the bidentate picolinate ligand \(K_1 = 1.06 \times 10^4 \text{ M}^{-1}\)^{70} gives a good indication of a partial binding to

EuIII by one of the tridentate subunits in L. The disso-

ciation rate constant \(k_{-1} = k_{1}/K_{\text{EuL}}\) can then be estimated
to be \(4 \times 10^3 \text{ s}^{-1}\). EuL is indeed a labile species as was

observed, for instance, for the anthranilate-EuIII complex

\((3.3 \times 10^4 \text{ s}^{-1})\)^{66,67} Step 1 can therefore be considered as

being a fast pre-equilibrium in the formation process of the
target Eu\(_2\)L\(_3\).

With an excess of L, Step 2 implies the binding of a sec-

ond ligand strand to EuL, leading to the more stable com-

plex EuL\(_2\). The bimolecular rate constant \(k_2\) (Table 4) is

about four orders of magnitude lower than \(k_1\). The electro-

static repulsions between the terminal carboxylate groups

of the ligands probably induce conformational rearrange-

ments which drastically slow down the coordination of L
to EuL. The strong negative interactions between the li-

gands will prevent even more efficiently the formation of

EuL\(_3\). The addition of a second EuIII cation to EuL\(_2\) pro-

ceeds in Step 3 and leads to a very inert complex EuL\(_2\) for

which either the release of a ligand \((k_{-3} \approx 10^{-3} \text{ s}^{-1})\) or of a cation \((k'_{-3} \approx 10^{-9} \text{ s}^{-1})\) is dramatically slow. Since EuL\(_2\)
is a minor species, detected by ES-MS but neither by spec-

trophotometry nor by \(^1\)H NMR, its stability constant could

only be calculated from the rate constants (Table 4).

With an excess of EuIII, the binding of a second cation

(Step 2) leads to the formation of EuL. Assuming a disso-

cative Eigen–Wilkins\(^{65}\) mechanism and using the Fuoss

equation,\(^{63}\) a formation rate constant equal to \(5 \times 10^3 \text{ M}^{-1} \times \text{s}^{-1}\) can be calculated, in excellent agreement with the

measured rate constant \(k'_{-2}\) (Table 6). Therefore, the binding

of a second Eu III cation does not involve any structural re-

arrangement, but is mainly governed by the de-solvation of

the entering cation and by intramolecular electrostatic

repulsions. The latter induce a sizeable negative cooperative

effect\(^{27,34}\) as demonstrated by the ratio \(K_{\text{EuL}_3}/K_{\text{EuL}_2} =

10^{-2} - 10^{-3}\). Step 3 is related to the formation of a double-

stranded bimetallic “pre-helicate” from EuL. The ap-

proach of a second ligand is favored because coordination

of the first ligand strand labilises the solvation sphere of the
two cations and reduces the charge on the two binding sites.

The estimation, via \(B_{\text{EuL}_2}\) (Tables 4 and 6), of \(k'_{-3} = (10^{-4}

\text{s}^{-1})\) provides information on the inertness of EuL\(_2\). A

“side-by-side” arrangement, as already reported in the solid

state for a closely related ligand\(^{32}\) with two europium(III)
cations, cannot explain an inert edifice in which the interac-

tions between the two lanthanide ions are drastically re-
duced, perhaps by a “braided” structure (Figure S10, see

Supporting Information).

Step 4 corresponds to the fast wrapping of the third

strand around EuL\(_2\). The kinetic behavior in Step 4 is
dominated by the slow solvolysis of the bimetallic triple-

stranded helicate. The rate constant \(k_{-4}\) listed in Table 6 is

within the range of data reported for the dissociation of

various LnIII-polyaminocarboxylate complexes \(10^{-6} \text{ s}^{-1} <

k < 10^{-3} \text{ s}^{-1}\).\(^{71}\) This could also be in line with the X-ray

structure of EuL\(_3\), in which two slightly different types of

molecules were found, showing that an increase in the sol-

vation of the carboxylates induces a lengthening of EuIII–O
distances.\(^{32}\) It is noteworthy that the self-assembly mecha-
nism of EuL\(_3\) does not exhibit any slow rearrangement

from a precursor species, as previously reported by us for

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a tri-cuprous double-stranded helicate. The situation is summarized in Figure 8 which displays the calculated time-dependences of the concentrations of the various EuIII-containing species formed during the self-assembly process for a stoichiometric [Eu]tot/[L]tot ratio of 0.67. The formation of the unique species Eu₂L₃ clearly occurs via the “braiding” of two ligand strands around the first EuIII cation followed by the “keystone” addition of a second EuIII ion and a fast wrapping of the third ligand around Eu₂L₂, the latter never being significantly accumulated.

![Figure 8](Image)

**Conclusion**

A fruitful combination of potentiometry, absorption and emission spectrophotometry, ¹H and¹³C NMR spectroscopy, and electrospray mass spectrometry allowed us to fully characterize the formation mechanism of a europium-containing triple-stranded Eu₂L₃ helicate with a hexadentate ditopic ligand. The self-assembly process implies a key bimetallic double-stranded species Eu₂L₂. This species is very inert towards the release of either a ligand or a cation, but reacts very rapidly with an additional ligand strand to yield the final triple-stranded helicate. This kinetic behavior points to the Eu₂L₂ intermediate probably adopting a structure different from the “side-by-side” arrangement observed for Eu₂(Lₙ)₂ in the solid state. Indeed the formation of a 2:2 helical structure at this stage will decrease the steric hindrance and, moreover, the presence of negatively charged terminal carboxylate groups obviously contributes to prevent the formation a labile “side-by-side” species. This is in contrast to the formation mechanism of Eu₂(Lₙ)₂ for which we propose the formation of a labile intermediate Eu₂(Lₙ)₂⁻. As far as we can tell, the increased inertness of the key intermediate is the main reason why the rate constant of the last step in the self-assembly process of Eu₂L₃ is 10⁵ times larger than Eu₂(Lₙ)₂. Moreover, the addition of the successive ligand strands leads to a strong diminishing of the electrostatic interactions between the two coordination sites. These two features, which are reflected by the very low equilibrium concentrations of the intermediates, are probably of importance to “cooperatively” drive a fast and efficient self-assembly of the target triple-stranded di-europium(III) helicate.

In conclusion, the hexadentate ditopic ligand L leads to one of the most efficient cation/ligand systems for the self-assembly of a bimetallic triple-stranded helicate in water. This is mainly due to the simultaneous presence of a short spacer and of the one negative charge borne by each extremity.

**Experimental Section**

**Starting Materials and Solvents:** L was synthesized according to a previously published procedure. Eu(ClO₄)₃·6H₂O was prepared from the oxide (Rhône–Poulenc, 99.99%) in the usual way.

**CAUTION!** Perchlorate salts combined with organic ligands are potentially explosive and should be handled in small quantities and with adequate precautions.

Distilled water was further purified by passing through a mixed bed of ion-exchanger (Bioblock Scientific R3-83002, M3-83006) and activated carbon (Bioblock Scientific ORC-83005). Spectrophotometric grade methanol (Merck, p.a.) and water were de-oxygenated using CO₂- and O₂-free argon (Sigma Oxin-clear cartridge). All stock solutions were prepared using an AG 245 Mettler Toledo analytical balance (precision 0.01 mg).

**Electrospray Mass Spectrometric Measurements:** ES mass spectra were recorded on an HP 1100 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA, formerly Hewlett-Packard Company Analytical Products Group). ES-MS was conducted in the positive ion mode. Scanning was performed from m/z 200 to 1500 and the sampling cone voltage (Vₛ) was set at 120 V. For electrospray ionization, the drying gas was heated at 250 °C and its flow set at 3 L/min, the nebuliser pressure was 10 p.s.i. and the capillary voltage 4 kV. Solutions containing ligand L (7.96 × 10⁻⁴, 4.04 × 10⁻⁴, 2.02 × 10⁻³ m) and various equivalents of EuIII were prepared in CH₃OH/H₂O (80:20 by weight). They were injected into the mass spectrometer source with a syringe pump (Harvard type PHD 2000, Harvard Apparatus Inc., South Natick, MA, USA) at a flow rate of 2 µL/min.

**Potentiometric Titrations:** The potentiometric titration of L (1.036 × 10⁻³ m) was performed using an automatic titrator system DMS 716 Titrino (Metrohm) with a combined glass electrode (Metrohm 6.0234.500, Long Life) filled with 0.1 M aqueous NaCl and connected to a microcomputer. The cell was thermostatted at 25.0 ± 0.2 °C by the flow of a Haake FJ thermostat. A stream of argon, pre-saturated with water vapour was passed over the surface of the solution. The potentiometric data (about 160 points collected over the pH range 5.9–11.0) were refined with the Hyperquad 2000[75] program which uses non-linear least-squares methods. Potentiometric data points were weighted by a formula allowing greater pH errors in the region of an endpoint than elsewhere. The weighting factor Wᵢ is defined as the reciprocal of the estimated variance of measurements: 

\[ Wᵢ = \frac{1}{σᵢ^2} = \frac{1}{[σₑᵢ^2 + (DE₅σᵢ^2)]} \]

where σₑᵢ² and σᵢ² are the estimated variances of the potential and volume readings, respectively. The con-
stents were refined by minimizing the error-square sum, \( U \), of the potentials shown below:

\[
U = \sum_i W_i (E_{obs,i} - E_{cal,i})^2
\]

Three titrations were treated either as single sets or as separated entities, for each system, without significant variation in the values of the determined constants. The quality of fit was judged by the values of the sample standard deviation, \( S \), and the goodness of fit, \( \chi^2 \), (Pearson’s test). At \( \sigma_G = 0.1 \text{ mV} (0.0023 \text{ mV}) \) and \( \sigma_V = 0.005 \text{ mL} \), the values of \( S \) in different sets of titrations were between 0.9 and 1.1, and \( \chi^2 \) was below 12.6. The scatter of residuals versus \( pH \) was reasonably random, without any significant systematic trends, thus indicating a good fit of the experimental data. The successive protonation constants were calculated from the cumulative constants determined with the program. The uncertainties in the log \( K \) values correspond to the added standard deviations in the cumulative constants. The distribution curves of the protonated species of \( L \) as a function of \( pH \) (Figure S2, see Supporting Information) were calculated using the Haltafall program.[77]

**Potentiometric and UV/Visible Titrations:** A stock solution of \( L \) \((8.91 \times 10^{-5} \text{ m})\) was prepared by quantitative dissolution of a solid sample in deionised water and the ionic strength was adjusted to 0.1 m with \( \text{NEt}_4\text{ClO}_4\) (Fluka, puriss). 100 mL of this solution was introduced into a jacketed cell (Metrohm) maintained at 25.0±0.2 °C (Haake FJ thermostat). The free hydrogen ion concentration was measured with a combined glass electrode (Metrohm 6.0234.500, Long Life) and a Tacussel Isis 20,000 millivoltmeter. The \( \text{Ag/AgCl} \) reference electrode was filled with \( \text{NaCl} \) (0.01 m, Fluka, p.a.) and \( \text{NaClO}_4 \) (0.09 m, Fluka, p.a.). Standardization of the millivoltmeter and verification of the linearity \((3.00 < \text{pH} < 9.00)\) of the electrode were performed using commercial buffers (Merck, Tritisol) according to classical methods.[78] The initial \( \text{pH} \) was adjusted to 11.59 with \( \text{NMe}_4\text{OH} \) (Merck, 10 wt.\% solution in water), and the titration of the free ligand \((7.38 < \text{pH} < 11.59)\) was then carried out by addition of known volumes of 1.69 \times 10^{-2} \text{ m} \text{ perchloric acid (Floro, normapur, 70}\% \text{ min})\) and an Eppendorf micro burette. An aliquot (2.0 mL) was taken after each addition of acid, and simultaneous \( \text{pH} \) and UV/Visible emission spectra were recorded \((350-550 \text{ nm})\) with a Hellma quartz optical cells (1 cm) on a Perkin–Elmer LS-50B instrument maintained at 25.0±0.2 °C by the flow of a Haake FJ thermostat. The excitation wavelength was 308±1 nm and the slit width was set at 4 nm for both excitation and emission. The source was a pulsed xenon flash lamp with a pulse width at half peak height < 10 μs and power equivalent to 20 kW.

**Luminescence Titrations:** Luminescence titrations were carried out on solutions with an absorbance less than 0.1 at wavelengths ≥ λ_max in order to avoid any errors due to the inner filter effect. The \( \text{pH} \) was maintained at 6.15±0.05 by the use of 0.1 m MES (Fluka, microselect, 99.5\%) and perchloric acid (Prolabo, normapur, 70% min). The titration of \( L \) \((1.64 \times 10^{-6} \text{ m})\) was carried out in a 1 cm Hellma quartz optical cell by addition of known microvolumes of a buffered solution of \text{Eu}^{III} \((6.55 \times 10^{-5} \text{ m})\) with an Eppendorf multipette\% plus micro burette. The \([\text{Eu}_{tot}/\text{L}_{tot}]\) ratio was varied from 0 to 15.37. Metal-centered luminescence spectra were recorded from 550 nm to 800 nm on a Perkin–Elmer LS-50B instrument at 25.0±0.2 °C. Gated spectra were obtained using phosphorimetry acquisition, with a delay time of 0.05 ms, a 200 ms sample window, 1 ms per flash and 1 flash per point. The slit width was 12.5 nm for both excitation and emission.

**Analysis and Processing of the Spectroscopic Data:** The spectrophotometric data were analyzed with both the Letagrop–Specto[80–82] and Specfit[13] programs which adjust the absorptivities and the stability constants of the species formed at equilibrium. The Letagrop–Specto program uses the Newton–Raphson algorithm to solve mass balance equations and a pit-mapping method to minimize the errors and determine the best parameter values. Specfit uses factor analysis to reduce the absorbance matrix and to extract the eigenvalues prior to the multiwavelength fit of the reduced data set according to the Marquardt algorithm.[83,84]

**Kinetics of Formation:** All kinetic measurements were performed in water at \( \text{pH} = 6.15±0.05 \) (0.1 m MES buffer) using a stopped-flow spectrophotometer (Applied Photophysics SX-18MV) equipped with a diode array device. The reactants were thermostatted at 25.0±0.2 °C (Lauda M12 thermostat) and mixed in a 1 cm optical cell. The data sets, averaged out of at least three replications, were recorded and analyzed with the commercial software Biokine.[85] This program fits up to three exponential functions to the experimental curves with the Simplex algorithm after initialization with the Pade–Laplace method.[86]

The reaction of \( L \) with \text{Eu}^{III} in excess was followed at 332 nm. Ligand \text{L} concentration was equal to 2.55 \times 10^{-5} \text{ m} and \text{Eu}^{III} concentrations were varied from 2.57 \times 10^{-4} \text{ m} to 2.57 \times 10^{-3} \text{ m}. The reaction of \text{Eu}^{III} with ligand \( L \) in excess was followed at 350 nm.
Two sets of experiments were carried out. Eu³⁺ concentration was equal to 1.06 × 10⁻⁶ m or, 2.73 × 10⁻⁶ m and L concentrations were varied from 1.06 × 10⁻⁵ m to 8.15 × 10⁻⁵ m or from 3.08 × 10⁻⁵ m to 1.54 × 10⁻⁴ m.

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In reference 26b, pK_a values are not pK_b values. Recalculating these data in terms of pK_b values gives 9.6 and 6.3, two values in good agreement with those reported in Table 1, given the slightly different experimental conditions.


Values determined at T = 12.5 °C and I = 0.2 M (NaClO_4) using the temperature jump relaxation method.


Values determined at T = 20 °C in aqueous solutions containing 0.5 M tert-butyl alcohol and using the radiation induced pH-jump technique.


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