Lanthanide(III) Complexes with a Reinforced Cyclam Ligand Show Unprecedented Kinetic Inertness

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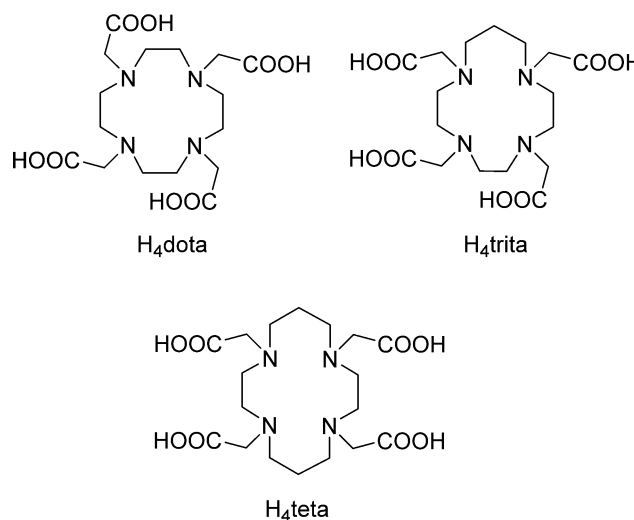
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S *Supporting Information*

ABSTRACT: Lanthanide(III) complexes of a cross-bridged cyclam derivative containing two picolinate pendant arms are kinetically inert in very harsh conditions such as 2 M HCl, with no dissociation being observed for at least 5 months. Importantly, the $[Ln(dota)]^-$ complexes, which are recognized to be extremely inert, dissociate under these conditions with lifetimes in the range ca. 1 min to 12 h depending upon the Ln^{3+} ion. X-ray diffraction studies reveal octadentate binding of the ligand to the metal ion in the $[Eu(cb\text{-}tedpa)]^+$ complex, while ^1H and ^{13}C NMR experiments in D_2O point to the presence of a single diastereoisomer in solution with a very rigid structure. The structure of the complexes in the solid state is retained in solution, as demonstrated by the analysis of the Yb^{3+}-induced paramagnetic shifts.

Chart 1. Ligands Discussed in the Present Work

Lanthanide(III) coordination chemistry in aqueous solutions is currently receiving a great deal of attention due to the important biomedical and bioanalytical applications of the complexes of these metal ions. Indeed, stable Gd^{3+} complexes with polyaminocarboxylate ligands are commonly used as contrast agents in magnetic resonance imaging (MRI),[1] while luminescent complexes of Eu^{3+} and Tb^{3+} have found applications in fluoroimmunoassays and as luminescent probes for optical imaging.[2] These applications require a very robust complexation of the metal ion in highly competitive biological media. For instance, macrobicyclic structures such as the famous cryptates reported by Lehn have found application in homogeneous fluoroimmunoassays thanks to their very low dissociation rates in biological media.[3] However, stable Ln^{3+} complexation is normally achieved with the use of macrocyclic ligands based on 1,4,7,10-tetraazacyclododecane (cyclen) such as dota (H_4dota = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, Chart 1), which forms lanthanide complexes of exceptionally high thermodynamic stability and kinetic inertness.[4] In spite of the intense research efforts carried out in the past 20 years to develop stable Ln^{3+} chelates for medical or biochemical applications, none of the systems reported to date can rival with dota derivatives in terms of combined thermodynamic stability and kinetic inertness. Some Ln^{3+}

complexes of dota-tetraamides and cyclen-based polyazaphosphinic acids were shown to be somewhat more inert than the parent dota complexes, but their thermodynamic stability is considerably lower.[5]

Kinetic inertness of Ln^{3+} complexes with functionalized tetraazamacrocyclic ligands decreases by several orders of magnitude from the 12-membered dota to the 13-membered trita and the 14-membered teta, which is based on a cyclam platform (Chart 1).[6] An interesting approach to develop kinetically inert complexes of transition metal complexes is the use of cross-bridged cyclam derivatives, which commonly contain a 1,8-ethylene unit bridging two nitrogen atoms of the tetraazamacrocycle in *trans* positions.[7] However, to the best of our knowledge cross-bridged cyclam derivatives have never been used for Ln^{3+} complexation. In this contribution we report the cross-bridged cyclam derivative cb-tedpa (Scheme 1), which forms Ln^{3+} complexes endowed with a very high kinetic inertness.

Scheme 1. Synthesis of cb-tedpa and Numbering Scheme Used for NMR Spectral Assignment
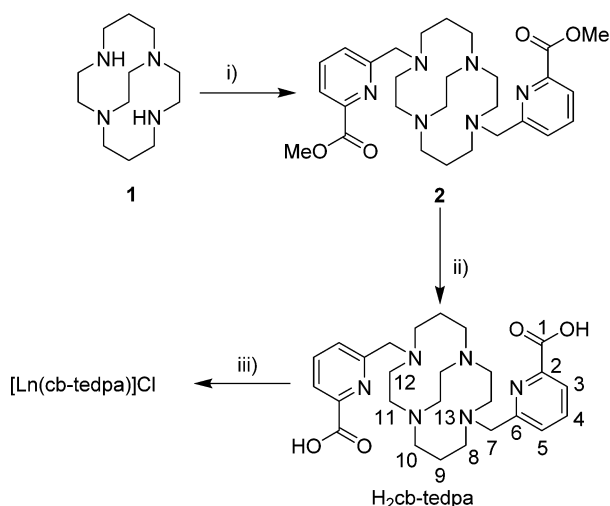
[a] Reagents and conditions: (i) methyl 6-(chloromethyl)picolinate, Na_2CO_3, CH_3CN, 95%; (ii) 6 M HCl, reflux, 97%; (iii) $LnCl_3 \cdot xH_2O$, [n]BuOH, Δ, DIPEA.

Compound **1** was obtained from cyclam following the previously reported synthesis in 70% yield.[8] Alkylation of **1** with methyl 6-(chloromethyl)picolinate[9] followed by deprotection of the methyl ester groups with HCl 6 M gave the desired ligand in 92% yield over the two steps. Attempts to prepare Ln^{3+} complexes of cb-tedpa in 2-propanol, EtOH, and H_2O by reaction of equimolar amounts of the ligand and lanthanide triflates or chlorides were unsuccessful. However, reaction of H_2cb-tedpa with lanthanide chlorides in refluxing [n]-butanol in the presence of diisopropylethylamine (DIPEA) resulted in the formation of compounds of formula [Ln(cb-tedpa)]Cl (Ln = La, Eu, Gd, Tb, or Yb). Even so, the complexes were purified using reverse-phase MPLC to remove unreacted ligand and salts. Thus, the formation of the Ln^{3+} complexes of cb-tedpa appears to be rather slow and requires the use of a solvent with a high boiling point and long reaction times (ca. 4 days). The high-resolution mass spectra of compounds [Ln(cb-tedpa)]Cl (ESI^+-MS) show peaks due to the [Ln(cb-tedpa)]$^+$ entities, thereby confirming the formation of the desired compounds. However, the yields obtained were rather low (ca. 24%). To overcome this, the synthesis of the Eu^{3+} complex was carried out under microwave radiation (150 °C, 250 psi, maximum power 300 W) in [n]-butanol using DIPEA as a base and a 100% excess of $EuCl_3 \cdot 6H_2O$. The formation of the complex was quantitative after 8 h, as demonstrated by NMR spectroscopy (see Supporting Information for details).

Addition of KPF_6 to an aqueous solution of the Eu^{3+} complex gave single crystals of formula [Eu(cb-tedpa)](PF_6)·2.5H_2O suitable for X-ray diffraction analysis. A view of the structure of the [Eu(cb-tedpa)]$^+$ complex and bond distances of the metal coordination environment are given in Figure 1. The metal ion is fully enveloped by the bicyclic ligand, which shows a folded *cis*-V geometry.[10] The Eu^{3+} ion is directly bound to eight donor atoms of the ligand: the four nitrogen atoms of the macrocycle, and the nitrogen and oxygen atoms of each of the two picolinate pendant arms. The distances between the metal ion and the amine nitrogen atoms of the macrocycle fall within the range 2.53−2.63 Å, with an average value of 2.58 Å. A
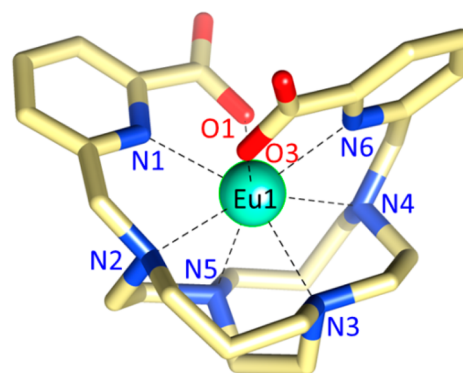

Figure 1. View of the cation complex present in crystals of [Eu(cb-tedpa)](PF_6)·2.5H_2O. Hydrogen atoms are omitted for simplicity. Selected bond distances (Å): Eu1−N1 2.490(6), Eu1−N2 2.602(7), Eu1−N3 2.541(7), Eu1−N4 2.635(6), Eu1−N5 2.544(7), Eu1−N6 2.492(7), Eu1−O1 2.324(5), Eu1−O3 2.309(5).

considerably longer average Eu−N distance was observed for the nine-coordinated [Eu(dota)(H_2O)]$^-$ complex (2.68 Å),[11] while a slightly longer value was found for the eight-coordinated [Eu(teta)]$^-$ analogue (2.62 Å).[12] This result suggests a rather strong interaction between the nitrogen donor atoms of the macrocycle and the metal ion in [Eu(cb-tedpa)]$^+$.

The emission spectra of the Eu^{3+} complex recorded in H_2O and D_2O solutions show the $^5D_0 \rightarrow ^7F_J$ characteristic of this metal ion, with maxima at 580 (J = 0), 592 (J = 1), 617 (J = 2), and 695 nm (J = 4). The emission lifetimes of the $^5D_0$ excited state recorded in H_2O and D_2O are 0.71 and 1.15 ms, respectively. Application of the methodology developed by Horrocks[13] and Beeby[14] allowed to calculate the number of water molecules coordinated to the Eu^{3+} ion in solution, which resulted to be q = 0.25−0.35. This result points to the absence of water molecules in the inner coordination sphere, in line with the X-ray structure of the complex.

The ^1H and ^{13}C NMR spectra of the diamagnetic [La(cb-tedpa)]$^+$ complex have been obtained in D_2O solution at pD ~7.0 and 298 K. The proton spectrum (see Supporting Information) consists of 17 signals corresponding to 17 magnetically non-equivalent proton environments in the ligand (see Scheme 1 for labeling), which points to an effective C_2 symmetry of the complex in solution. This is confirmed by the ^{13}C NMR spectrum, which shows 13 signals for the 26 carbon nuclei of the ligand backbone. The assignments of the proton signals (Table S5, Supporting Information) were based upon HMQC and HMBC 2D heteronuclear experiments as well as standard 2D homonuclear COSY experiments. The sharp and clear diastereotopic nature of the ^1H NMR resonances suggests the presence of a single diastereoisomer in solution with no fluxional behavior being observed at room temperature.

The structure of the paramagnetic [Yb(cb-tedpa)]$^+$ complex in solution has been established by analyzing the ^1H paramagnetic shifts induced by the Yb^{3+} ion in the ligand nuclei. The ^1H NMR spectrum (D_2O, pH ~7.0, 25 °C) shows 17 paramagnetically shifted resonances in the range +86 to −51 ppm, which confirms the C_2 symmetry of the complex in solution (Figure 2). The ^1H NMR shifts induced by Yb^{3+} are dominated by the pseudocontact contribution, which depends upon the axial $[\chi_{zz} - \frac{1}{3}(\chi_{xx} + \chi_{yy} + \chi_{zz})]$ and rhombic $(\chi_{xx} - \chi_{yy})$ parts of the magnetic susceptibility tensor and the location of the observed nuclei with respect to the magnetic axes.[15] The
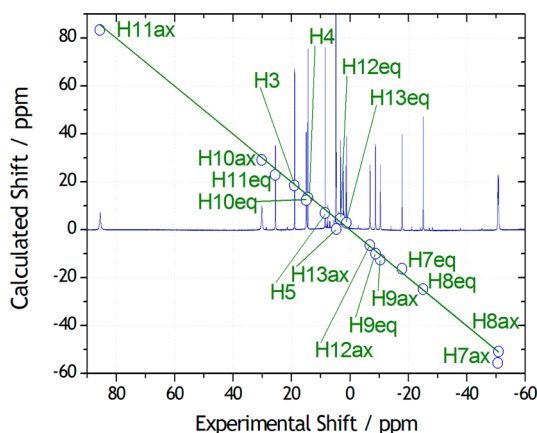
Figure 2. ^{1}H NMR (300 MHz, 298 K) spectrum of [Yb(cb-tedpa)]$^+$ recorded in D$_2$O solution and plot of the experimental shifts versus those calculated with the DFT optimized geometry and pseudocontact contributions. The solid line represents a perfect fit between experimental and calculated values.

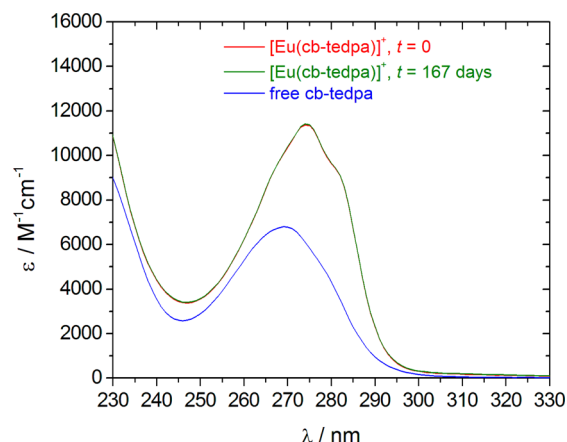**Figure 3.** UV absorption spectra of the cb-tedpa ligand and the [Eu(cb-tedpa)]$^+$ complex recorded in 2 M HCl. The spectra of the complex obtained right after dissolution is identical to those obtained for the same solution after 110 days (samples were thermostated at 25 °C).

analysis of the Yb^{3+}-induced paramagnetic shifts has been carried out following the methodology reported previously,[16] using the ^{1}H NMR shifts observed for the La^{3+} complex to estimate the diamagnetic contribution. A geometrical model of the structure of the Yb^{3+} complex has been obtained using DFT calculations at the TPSSh/LCECP/6-31G(d) level. The geometry of the [Yb(cb-tedpa)]$^+$ complex optimized in aqueous solution is very similar to that observed for the Eu^{3+} analogue in the solid state (Supporting Information). The calculated structure provides an excellent agreement between the observed ^{1}H NMR shift and those calculated using the equation for the pseudocontact shift (Figure 2), with $\chi_{zz} - \frac{1}{3}(\chi_{xx} + \chi_{yy} + \chi_{zz}) = 90 \pm 30$ ppm·Å^3 and $\chi_{xx} - \chi_{yy} = 3018 \pm 39$ ppm·Å^3, and an excellent agreement factor of AF$_j$ = 0.071. This result unambiguously proves that the [Yb(cb-tedpa)]$^+$ complex adopts the same overall structure observed for the Eu^{3+} complex in the solid state (with shorter Ln-donor distances as a consequence of the smaller ionic radius of the Yb^{3+} ion). The Yb^{3+}-induced shifts are dominated by the rhombic contribution, in contrast to the results obtained for [Yb(teta)]$^-$ [$\frac{1}{3}(\chi_{xx} + \chi_{yy} + \chi_{zz}) = 3237$ and $\chi_{xx} - \chi_{yy} = -2526$ ppm·Å^3, with AF$_j$ = 0.089].[17]

Acid-catalyzed dissociation represents the main dissociation pathway of Ln^{3+} complexes with macrocyclic ligands.[18] Thus, the kinetic inertness of the [Eu(cb-tedpa)]$^+$ complex has been evaluated by following the changes of the UV−vis absorption spectrum in acidic solution (2 M HCl). The spectrum of the free ligand in 2 M HCl shows an absorption band with a maximum at 269 nm ($\varepsilon \approx 6800$ M^{-1} cm^{-1}), while in the spectrum of the complex this band points at 274 nm and is considerably more intense ($\varepsilon \approx 11400$ M^{-1} cm^{-1}) (Figure 3). This absorption band is assigned to a combination of $\pi \rightarrow \pi^*$ and n$\rightarrow \pi^*$ transitions centered on the picolinate pendant arms, and is very similar to those reported for different complexes with macrocyclic ligands containing picolinate groups.[19] Thus, the spectra of the ligand and the complex recorded in 2 M HCl solution are substantially different, which allows the dissociation of the complex to be monitored. Importantly, the spectrum of the complex does not show any variation during a period of more than 5 months (167 days). Furthermore, the integrity of the complex has been confirmed by NMR spectroscopy. The ^{1}H NMR spectrum of the [Eu(cb-tedpa)]$^+$ recorded in D$_2$O

solution at neutral pH presents paramagnetically shifted signals in the range ~+15 to −15 ppm. The spectrum recorded in 2 M DCl is very similar, although some signals are slightly shifted presumably due to the protonation of the complex. The spectrum recorded in 2 M HCl does not show noticeable changes during 90 days (Figure S3, Supporting Information), which confirms an astonishing kinetic inertness of the complex in solution. Indeed, the most inert Ln^{3+} complexes reported to date, the [Ln(dota)]$^-$ complexes, do undergo dissociation under the same conditions: The lifetime of the [Eu(dota)]$^-$ complex in 2 M HCl can be estimated from the rate constants characterizing to acid-catalyzed dissociation to be 6.9 h, while the corresponding values for the Ce^{3+} and Gd^{3+} complexes under these conditions are 1.2 min and 11.5 h.[4,20] It has been shown that Ln^{3+} complexes of dota-tetraamides and cyclen-based polyazaphosphinic acids are even more inert than the parent dota complexes.[5] However, the lifetimes of these complexes in acidic solutions (0.1−2 M acid concentrations) are still in the range of a few days, highlighting once more the exceptional kinetic inertness of [Ln(cb-tedpa)]$^+$ complexes.

An additional set of experiments was carried out to test whether the [Eu(cb-tedpa)]$^+$ complex undergoes dissociation in the presence of competing metal ions (Cu^{2+}, 10-fold excess at pH 4.5), bioligands (250-fold excess of H$_2$PO$_4^{3-}$/HPO$_4^{2-}$ at pH 7.2), or chelating ligands (250-fold excess of the ttha ligand at pH 5.8). The absorption spectra of the samples remained unchanged throughout the examined time period (3 weeks), confirming that the complex does not suffer dissociation under these conditions (Figures S6−S8, Supporting Information). The Gd^{3+} complex with a macrocyclic ligand based on a diaza-12-crown-4 platform containing two picolinate pendant arms was shown to dissociate rather quickly following both the proton-assisted and metal-assisted pathways.[21] Thus, it is clear that the cross-bridged cyclam unit of the ligand plays a critical role in the kinetic inertness of [Ln(cb-tedpa)]$^+$ complexes.

In conclusion, we have reported a new class of very rigid Ln^{3+} complexes with extremely high kinetic inertness, which overcomes by far that of the well-known [Ln(dota)]$^-$ complexes. This property is of crucial importance for the design of Ln^{3+} complexes for medical and bioanalytical applications with reduced toxicities, as the release of the toxic free Ln^{3+} ion can be avoided. For instance, Gd^{3+} release of the

metal ion after the administration of certain Gd^{3+}-based CAs to patients suffering from severe renal failure has been proved to trigger a potentially fatal disease called nephrogenic systemic fibrosis (NSF).[22] Another important property of the cross-bridged ligand reported here is that it can be functionalized by introducing different groups in the β-N position, following our straightforward methodology reported recently.[23]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full details on the synthesis of the ligand and Ln^{3+} complexes, ^1H and ^{13}C NMR data, excitation and emission spectra, and computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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