Kinetics of the Formation of Macrocyclic Polyaminocarboxylate Ligand Complexes: A Laser-Excited Luminescence Study of the Eu^{3+}−dtpa-dien System

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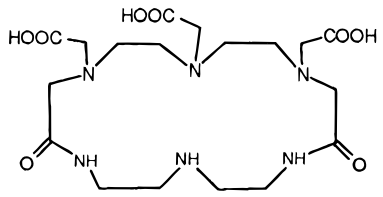
Excitation spectroscopy of the $^7F_0 \rightarrow {}^5D_0$ transition of Eu^{3+} is used to detect and characterize a kinetic intermediate in the formation of a complex between Eu^{3+} and the macrocyclic ligand dtpa-dien (1,4,7-tris(carboxymethyl)-9,17-dioxo-1,4,7,10,13,16-hexaazacyclooctadecane). Both the long-lived intermediate and the final product, $[Eu(Hdtpa\text{-}dien)(H_2O)]^+$, are formed immediately upon mixing the components, as evidenced by separate peaks in the excitation spectrum. The transformation of the intermediate to the final product, monitored by excitation spectroscopy, occurs by both proton-assisted and non-proton-assisted pathways. It is proposed that the intermediate represents a "blind alley" in the pathway to a final nine-coordinate tricapped trigonal prismatic product. The intermediate with an "up, down, up" configuration of carboxylate arms must undergo some decoordination to form the final complex. Molecular mechanics calculations and excited state lifetimes suggest that the intermediate is eight-coordinate with one coordinated water molecule. The stability constant of the final complex is found to be $\log K = 14.11 \pm 0.05$.

Introduction

Complexation reactions between lanthanide ions, Ln^{3+}, and flexible acyclic polyaminocarboxylate ligands, e.g., edta, dtpa, and heda, are generally extremely rapid and therefore difficult to study.[1] Although such rates can sometimes be estimated from the outer-sphere association constant (K_{os}) and the characteristic water exchange constant ($k_{Ln\text{-}H_2O}$) or from the measured dissociation rate and the equilibrium constant for formation,[1−5] the mechanistic details of such reactions remain poorly understood. For macrocyclic ligands in this class, on the other hand, complexation reactions can be relatively slow, as exemplified by recent studies on dota and related species.[6−13] Owing to the application of Gd^{3+} complexes of this type as actual or potential magnetic resonance imaging (MRI) contrast agents, it is important to achieve an understanding of the mechanisms of formation and the factors, such as cavity size, degree of protonation, conformational flexibility, and steric effects, which affect the rates of formation.

Trinegative ligands such as diethylenetriaminepentaacetic acid bis(methylamide), dtpa-bma, are attractive as MRI ligands since they form neutral complexes with Gd^{3+}.[14,15] Ln^{3+} complexes of dtpa-bma have been shown to conform to a tricapped trigonal prismatic coordination polyhedron about the metal ion both in the solid state and in solution.[16−19] Studies on complexes of the related cyclic amide, dtpa-dien, Structure **1**, have demonstrated that linking the amide moieties reduces the number of possible isomers present in solution from eight to four and that the pathways available for their interconversion are limited.[15,18,19] Cyclization is also expected to limit the conformational mobility of the free ligand. Coordination by dtpa-dien can be categorized according to whether each of the pendant carboxylate coordinating groups is "up" or "down" with respect to the macrocyclic ring. The presence of the ring itself limits the number of energetically accessible isomers and impedes the interconversion of certain isomers into others. The present research provides evidence for the rapid formation of the final product if the initially formed collisional complex is of the correct conforma-



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tion; however, a fairly long-lived intermediate is detected for conformational situations where direct interconversion to the final complex is blocked by the macrocyclic ring structure. A detailed mechanism for this interconversion is proposed. Laser excitation spectroscopy of the $^7F_0 \rightarrow {}^5D_0$ transition of Eu^{3+} is used to detect the transient intermediate and to follow its transformation into the final product. The number of water molecules coordinated to the Eu^{3+} ion and the thermodynamic stability constant are also determined using these methods.

Experimental Section

Materials. Hydrated $EuCl_3$, HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid), D_2O (99.8%) (Aldrich Chemical Co.), MES (2-(*N*-morpholino)ethanesulfuric acid), piperazine hexahydrate (Sigma Chemical Co.), and HOMOPIPES (homopiperazine-*N*,*N*′-bis-2-(ethanesulfuric acid)) (Research Organic Inc.) were purchased from the sources indicated. dtpa-dien (1,4,7,-tris(carboxymethyl)-9,17-dioxo-1,4,7,10,-13,16-hexaazacyclooctadecane) was synthesized by a method reported in the literature.[18] The water used was deionized and doubly distilled, and all remaining reagents were the purest commercially available. Europium chloride solutions were prepared at ~10 mM and standardized by means of an edta titration using arsenazo III as indicator. The concentration of the dtpa-dien stock solution was determined via a titration of a ~2 μM solution with standardized Eu^{3+} buffered at pH 6 on equilibrated samples using laser-excited Eu^{3+} luminescence to monitor complex formation.

Methods. Eu^{3+} excitation spectra, excited state lifetimes, and excitation intensities were measured using a Continuum YG-581 C pulsed (10 Hz) Nd:Y laser-pumped tunable TDL-50 dye laser described previously.[20] The $^7F_0 \rightarrow {}^5D_0$ transition of the Eu^{3+} ion (578−581 nm) was excited by using a mixture of Rhodamine 590 (Excition Co.) and 610 (Kodak Chemical Co.). The $^5D_0 \rightarrow {}^7F_0$ emission band at 614 nm was monitored in each case. An Orion pH meter (Model 720A) with an Orion glass electrode was used for all pH measurements. The commercially available Peakfit program (Jandel Scientific), which applies a nonlinear regression method, was employed in the data analyses. All spectroscopic measurements were carried out at 25.0 ± 0.1 °C or at another temperature indicated in the text, by using a Haake water bath temperature controller.

The stability constant, *K*, of $[Eu(Hdtpa\text{-}dien)H_2O]^+$ was determined as follows.[21] A series of solutions each containing 1.8 μM Eu^{3+} and 1.8 μM dtpa-dien are prepared in separate sealed containers at various pH values ranging from pH 2 to pH 8. Following equilibration at room temperature for 24 h, the excited state lifetime and associated excitation amplitude were recorded for each sample at the peak maximum, 579.89 nm, for 5 min at 25 °C. These amplitudes, which are proportional to the concentration of the complex in solution, are corrected for fluctuations in the laser power over the course of the series of measurements. *K* was obtained from a nonlinear regression analysis fit of the intensity data, *I*, to eq 3, which relates this quantity to *K*, the ligand protonation constants, and the initial concentrations of Eu^{3+} and ligand.

The formation kinetics of the Eu^{3+}−dtpa-dien reaction was studied by recording the $^7F_0 \rightarrow {}^5D_0$ excitation spectra over the 578.5−580.5 nm range as a function of time or by monitoring the intensity increase of the signal of the final complex or the intensity decrease of the intermediate, after mixing 1 mL samples of Eu^{3+} and dtpa-dien, usually buffered at the same pH value. At pH values greater than 8, to avoid forming a precipitate of $Eu(OH)_3(s)$, individual Eu^{3+} solutions were prepared in doubly distilled H_2O and diluted with appropriate concentrations of buffer solution at the time of mixing. In addition, excess ligand was used in runs at high pH.

The buffers used were 0.02 M potassium formate (pH < 4), 0.02 M HOMOPIPES (pH 4−5), 0.02 M MES (pH 5−7), and 0.02 M HEPES (pH 7−9), with ionic strength adjusted to 0.10 with KCl. The protonation constants of dtpa-dien were determined by potentiometry as described elsewhere:[22,23] $\log K_1 = 10.02(4)$, $\log K_2 = 8.87(2)$, $\log K_3 = 4.10(5)$, $\log K_4 = 2.62(8)$, and $\log K_5 = 1.8(2)$. The value of $K_w = [H^+][OH^-]$ was taken as $10^{-13.78}$. The pD for the D_2O solutions was calculated as pH + 0.41.

Results

Characterization of the Complex $[Eu(Hdtpa\text{-}dien)(H_2O)]^+$ and the Determination of Its Stability Constant. The $^7F_0 \rightarrow {}^5D_0$ excitation spectrum of the final complex formed between dtpa-dien and Eu^{3+} consists of a single symmetric peak centered at 579.9 nm (not shown). A titration carried out by monitoring the intensity of this peak as a function of Eu^{3+} added to 4 μM dtpa-dien (not shown) disclosed a 1:1 stoichiometry, as expected. Excited state lifetimes, τ, measured separately in H_2O and D_2O are useful in determining the number of water molecules, *q*, coordinated to the Eu^{3+} ion via eq 1, as shown by Horrocks and Sudnick.[24]

$$q = 1.05(\tau_{H_2O}{}^{-1} - \tau_{D_2O}{}^{-1}) \qquad (1)$$

The values of τ_{H_2O} and τ_{D_2O} obtained, 0.592 and 2.41 ms, respectively, lead to a *q* value of 1.3. This is in agreement with X-ray determinations on Ln^{3+} complexes of ligands of this type, which show that the ligand generally supplies eight coordinating atoms, and there is one water molecule for a total coordination number of nine.[15−17,25]

X-ray structures of the La^{3+} and Eu^{3+} complexes of dtpa-dien[15] determined on crystals grown at pH 7.2 and 6.8, respectively, reveal the presence of the protonated complexes $Ln(Hdtpa\text{-}dien)^+$, with the uncoordinated central nitrogen of the bis(amide) linker being the locus of the proton. Potentiometric titrations and other luminescence studies yield a pK_a value in the 7−8 range for the dissociation of a proton from $Eu(Hdtpa\text{-}dien)^+$. There is no evidence for a diprotonated species, which in any case would be unexpected since it would carry two positive charges. There is no change in the shape of the excitation peak or in the excited state lifetime of the final complex in the pH range 2.0−8.2, suggesting that the central nitrogen of the amide linker remains uncoordinated throughout this range. As shown in Figure 1, at low pH protons effectively compete with Eu^{3+} for the ligand, which leads to the observed decrease in the intensity of the excitation peak at low pH values. In addition to the Eu^{3+} complex formation equilibrium (eq 2), the five ligand protonation equilibria for the formation of species from $Hdtpa\text{-}dien^{2-}$ (K_1) through $H_5dtpa\text{-}dien^{2+}$ (K_5) were included in the analysis. Equation 2 does not imply anything about the mechanism of formation of the complex in the low-pH region, where the ligand species $H_4dtpa\text{-}dien^+$ and $H_3dtpa\text{-}dien$ predominate. These highly protonated ligand forms would, of course, release most of their protons upon complexation to the Eu^{3+} ion.

$$Eu^{3+} + Hdtpa\text{-}dien^{2-} = Eu(Hdtpa\text{-}dien)^+, K \qquad (2)$$

The intensity, *I*, corresponding to the complex $[Eu(Hdtpa\text{-}dien)(H_2O)]^+$ may be expressed as eq 3.

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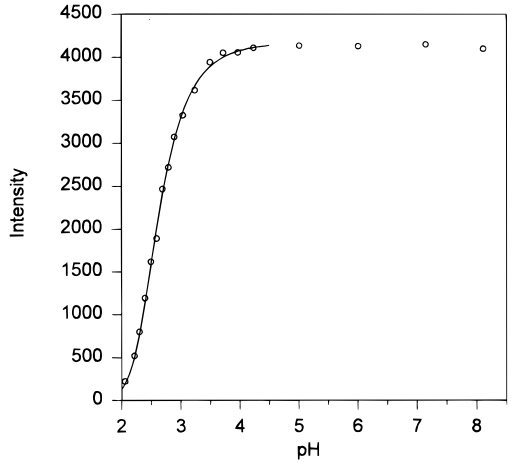

Figure 1. Plot of the intensity at 579.9 nm vs pH, for solutions where $[Eu^{3+}] = [dtpa\text{-}dien] = 1.8\ \mu M$, 25 °C, and $\mu = 0.1$. The solid line is the theoretical curve (see text).

$$I = k'\{[Eu]_i + [L]_i + (K\alpha)^{-1} - \{([Eu]_i + [L]_i + (K\alpha)^{-1})^2 - 4[Eu]_i[L]_i\}^{1/2}\} \quad (3)$$

where $[Eu^{3+}]_i$ and $[L]_i$ stand for the initial total concentration of Eu^{3+} and ligand, respectively, k' is the proportionality constant between intensity and concentration of complex, and α is defined by eq 4.

$$\alpha = K_1[H^+]/(1 + K_1[H^+] + K_1K_2[H^+]^2 + K_1K_2K_3[H^+]^3 + K_1K_2K_3K_4[H^+]^4 + K_1K_2K_3K_4K_5[H^+]^5) \quad (4)$$

Fitting the intensity data in the pH range 2−4 to eq 3 by nonlinear regression analysis (Figure 1, solid curve) leads to a log K value of 14.11 ± 0.05 for $[(Eu(Hdtpa\text{-}dien)(H_2O)]^+$.

Observation and Characterization of an Intermediate in the Formation Reaction. The $^7F_0 \rightarrow {}^7D_0$ excitation spectra taken at various elapsed times after mixing Eu^{3+} and dtpa-dien at pH 6.01 are shown in Figure 2. Initially the signal is resolvable into peaks at 579.5 and 579.9 nm, which appear simultaneously. The higher wavelength peak is characteristic of the final complex, while the band at 579.5 nm represents an intermediate which slowly transforms into the final product. Measurement of the excited state lifetime at this wavelength immediately after mixing ($\tau_{H_2O} = 579\ \mu s$ and $\tau_{D_2O} = 2.45$ ms) yields (eq 1) 1.4 ± 0.5 inner-sphere water molecules. A model for the intermediate which accommodates these findings involves coordination of the Eu^{3+} to the three carboxylates, three amino nitrogen atoms, a single amide carboxyl, and a water molecule for a total coordination number of eight. Support for this model is provided by a recent correlation of $^7F_0 \rightarrow {}^5D_0$ excitation frequencies with the sum of nephelauxetic parameters of the coordinated liganding atoms.[26,27] This correlation predicts an excitation peak maximum at 579.6 nm for the intermediate and one at 579.9 nm for the nine-coordinate final product, in reasonable agreement with the results.

Owing to the slowness of the conversion of the intermediate into the final product, it is possible to monitor, as a function of pH, the relative concentrations of the intermediate and the final complex immediately after mixing by measuring the emission

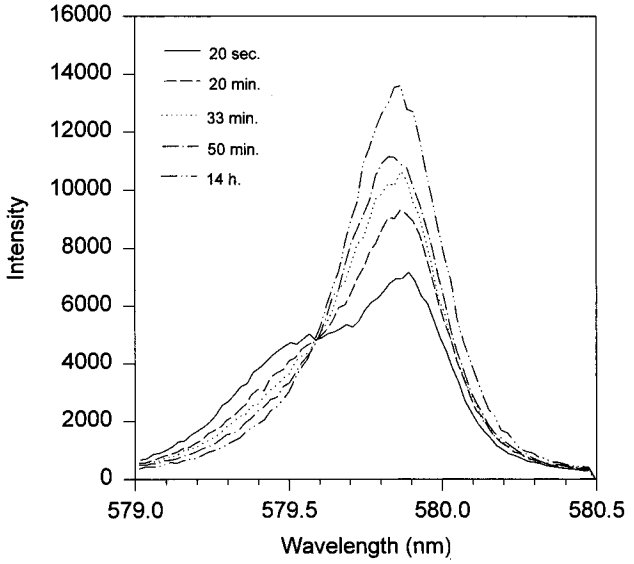

Figure 2. $^7F_0 \rightarrow {}^5D_0$ excitation spectra of the reacting mixture recorded (0.01 nm/s) at various elapsed times after the reaction was started. $[Eu^{3+}]_i = 2\ \mu M$, $[dtpa\text{-}dien]_i = 10\ \mu M$, pD = 6.01, and $\mu = 0.1$, 25 °C. The times refer to the instant at which the spectral scans were started at 579.0 nm.

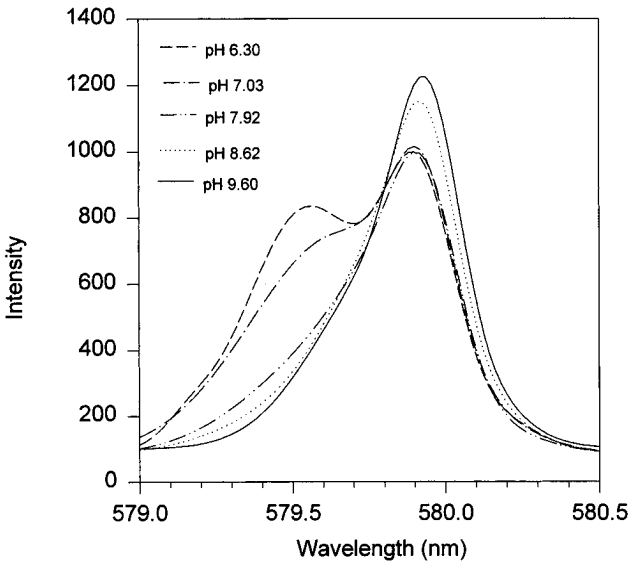

Figure 3. Plots of $^7F_0 \rightarrow {}^5D_0$ excitation spectra recorded (0.01 nm/s) immediately after mixing of Eu^{3+} and dtpa-dien at various pH values. $[Eu^{3+}]_i = 5\ \mu M$, $[dtpa\text{-}dien]_i = 20\ \mu M$, 25 °C, and $\mu = 0.1$.

intensities, $I_{579.5}$ and $I_{579.9}$ for excitation at 579.5 and 579.9 nm, respectively. The ratio of the intensities of the two bands ($I_{579.5}/I_{579.9}$) remains constant at about 0.87 for pH values less than 6.5. However, as the pH is raised from 7 to 9.6, this ratio decreases until the 579.5 nm band is undetectable in the presence of the band due to the final product. Representative $^7F_0 \rightarrow {}^5D_0$ excitation spectra measured immediately after mixing at several pH values (Figure 3) illustrate this effect. The decrease in the concentration of the intermediate as the pH is raised coincides with the marked decrease in the concentration of the H_2L^- and rapid increase in the concentration of HL^{2-} free ligand species in the pH range 7−9.6. It is possible that the HL^{2-} species are preorganized differently from the H_2L^- monoanion, which predominates in the pH 4−8.5 region, and this influences the pathway of the reaction. No influence on the $I_{579.5}/I_{579.9}$ ratio at pH 5.98 of either temperature (in the range 5−90 °C) or the initial ratio ($[Eu^{3+}]/[dtpa\text{-}dien]$ in the range 2 μM/100 μM to 100 μM/2 μM) was observed.

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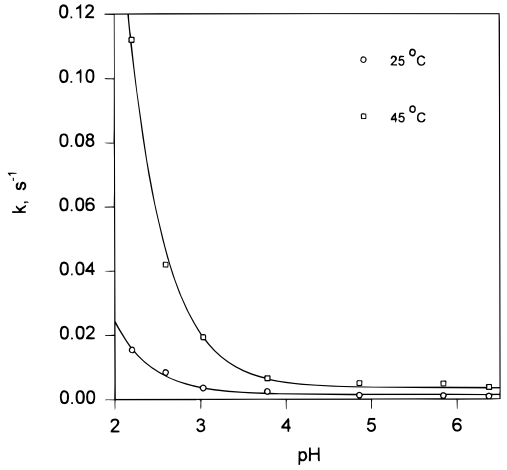

Figure 4. Plots of k_{obs} vs pH at 25 and 45 °C. $[Eu^{3+}]_i = 100$ μM, $[dtpa\text{-}dien]_i = 2$ μM, and $\mu = 0.1$. The solid curves represent the theoretical fit according to eqs 5 and 6, as described in the text.

Kinetics of the Conversion of the Intermediate to the Final Product. Although the intermediate and some of the final product form instantly upon mixing Eu^{3+} and dtpa-dien, the conversion of the intermediate to the final product is slow and this can be followed easily by monitoring the increase in the excitation peak at 579.9 nm as a function of time. The rate constants (k, s^{-1}) were obtained by fitting the intensity as a function of time, $I(t)$, to a first-order model:

$$I(t) = I_0 + (I_\infty - I_0)\{1 - \exp(-kt)\} \quad (5)$$

where I_0 is the intensity measured immediately upon mixing and I_∞ is its value at $t = \infty$. The k values, so obtained, at various pH values and at 25 or 45 °C are presented in Figure 4. Since the rate constants are nonzero at higher pH values, the results suggest both proton-assisted and proton-independent pathways, with the former becoming dominant at pH values less than 4, where the k values increase greatly. The data are consistent with the rate law:

$$k = k_0 + k_H[H^+] \quad (6)$$

Fitting the data to eqs 5 and 6 (Figure 4, solid curves) yields at 25 °C $k_0 = (1.4 \pm 0.3) \times 10^{-3}$ s^{-1}, $k_H = 2.3 \pm 0.1$ M^{-1} s^{-1}; and at 45 °C $k_0 = (3.6 \pm 0.1) \times 10^{-3}$ s^{-1}, $k_H = 16.9 \pm 0.4$ M^{-1} s^{-1}.

Discussion

The structures of the final complex in solution are key in the elucidation of the mechanism of the formation reaction. The solid state structures of Ln^{3+} complexes of acyclic dtpa-bis(amide) ligands such as Gd(dtpa-bea)[16] and Dy(dtpa-bma)[17] reveal that the Ln^{3+} ion is coordinated to the three amine nitrogens, three carboxylate oxygens, two amide carboxyl oxygens, and the oxygen of a water molecule. The coordination geometry may be described as a tricapped trigonal prism (TTP) with the two outer amine nitrogens and the water molecule occupying the capping positions. Recent NMR studies[18,19] suggest that Ln^{3+} complexes of this type have the same structures in solution. The TTP model generates a maximum of eight possible isomers (Figure 5, bottom). Indeed, all eight isomers have been shown to be present in solution by NMR studies on Ln^{3+} complexes of the acyclic ligands, dtpa-bpa.[19]

Similar solid state and solution structures have also been found for Ln^{3+} complexes of dtpa-based macrocyclic ligands.[15,25] The X-ray structure of Eu(Hdtpa-dien)$^{+}$[15] shows it to have a TTP geometry. The ligand is octadentate, and nine-coordination is achieved by means of η^1 bridging of a carboxylate moiety in the solid state. In aqueous solution this complex has been shown to be monomeric by vapor pressure osmometry,[15] the bridging carboxylate having been replaced by a water molecule. NMR experiments show that the presence of the macrocyclic ring in this ligand reduces the number of isomers based on a TTP model from eight in analogous acyclic ligands to four in the case of complexes of dtpa-dien. As indicated in Figure 5, the connected amide oxygens are required to lie at the apexes of the same TTP triangular face, otherwise the linking group ($-CH_2CH_2$-$NH_2CH_2CH_2-$) would be forced to pass over one of the three capping positions in a sterically unacceptable manner. Thus, the four geometries 1, 2, 3, and 4 shown on the left-hand side of Figure 5 are prohibited for the Eu(Hdtpa-dien)$^{+}$ complex.[15] The notation indicating the chirality at the various nitrogen centers that was used in the earlier NMR study[15] is shown in Figure 5.

The simultaneous appearance of both an intermediate and the final complex immediately after mixing (Figure 2) requires that there be more than one route to the final product in the complexation reaction. The intermediate represents a "blind alley" in the complexation process from which the final product can only be reached by a slow, largely proton-assisted pathway. This implies that the intermediate must undergo a "decoordination" event, which requires activation energy, on the pathway to the final complex.

The free ligand form of dtpa-dien can take up three principal conformations classifiable by whether the carboxylate arms are "up" or "down" with respect to the ring of the macrocycle. Figure 5 (top) shows the three conformational forms (I-III), each of which represents a population of different conformers, all in rapid equilibrium. Nothing is implied about the state of protonation of the reactive ligand species, and protons have been omitted in Figure 5.

Upon the initial encounter of the ligand with Eu^{3+}, an outer-sphere complex will be formed.[1] Then a series of precursor species will form as water molecules are successively replaced by coordinating atoms on the ligand. These steps are indicated below

$$Eu(H_2O)_9^{3+} + L \leftrightarrows Eu(H_2O)_9L$$

$$Eu(H_2O)_9L \rightarrow Eu(H_2O)_8L^1 + H_2O$$

$$Eu(H_2O)_8L^1 \rightarrow Eu(H_2O)_7L^2 + H_2O$$

where the superscripts indicate the number of ligand atoms coordinating to Eu^{3+}. Since it is expected that $Eu^{3+}-$carboxylate bonds will form first,[1] one can expect three different precursors to form on the basis of rapid reactions with free ligand forms I, II, and III. Structures for these precursors (I′, II′, III′) are shown schematically in Figure 5. There are no particular steric restraints to the formation of any of these structures, each of which should exhibit a high degree of flexibility since only three carboxylate groups and an unspecified number of water molecules are coordinated to the Eu^{3+} ion. Once the three $Eu^{3+}-$carboxylate bonds are formed, however, their positions (up or down) with respect to the macrocyclic ring will remain fixed unless decoordination occurs. As discussed below, the three different precursors must follow very different pathways in the formation of final product complexes.

The relative positions of the carboxylate groups with respect to the macrocyclic ring are evident for the energetically accessible isomers (5−8) from Figure 5. Structures 5 and 6

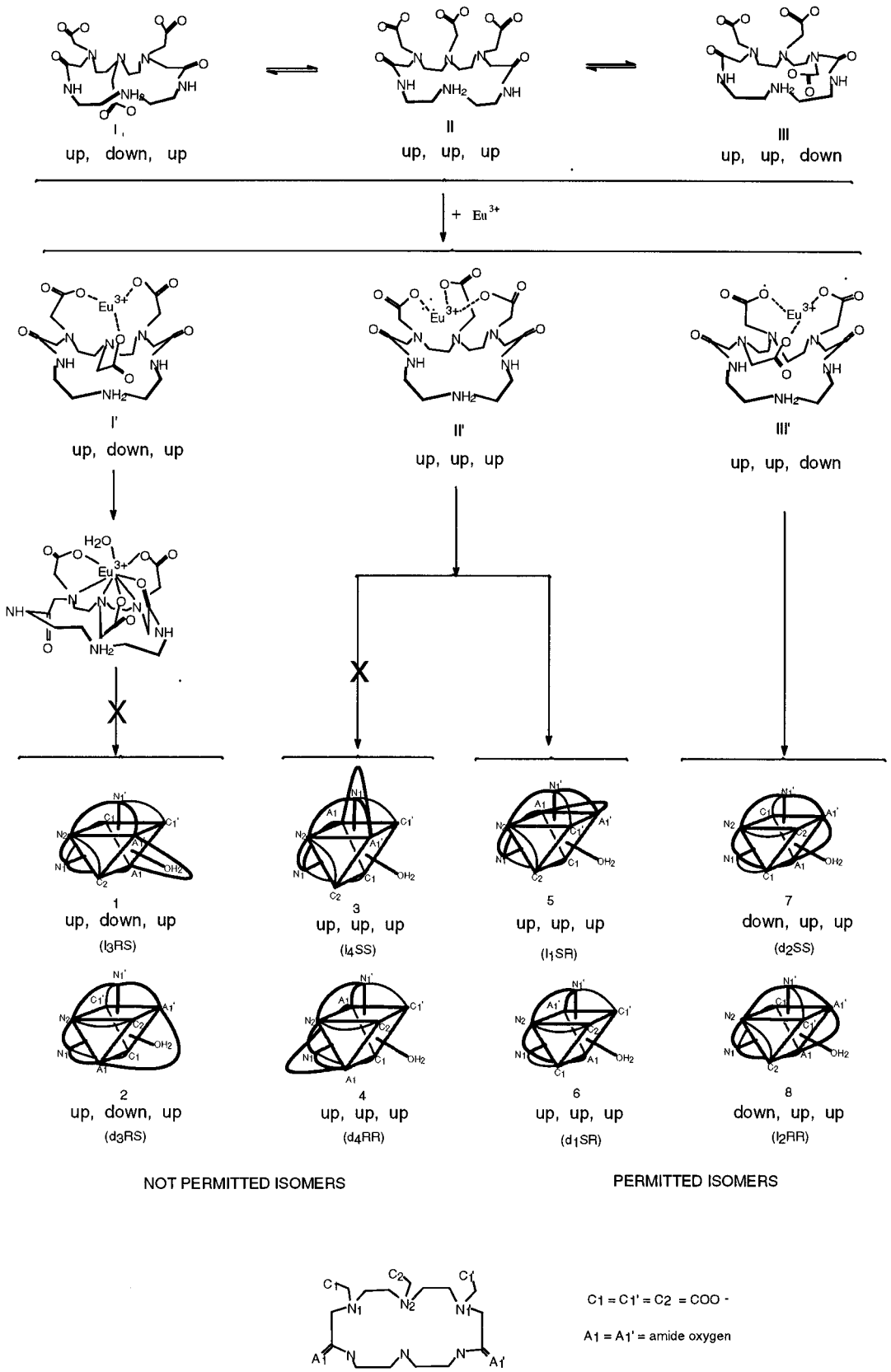

Figure 5. Schematic view of the pathways for formation of the four permitted isomers of the final product, $[Eu(Hdtpa\text{-}dien)(H_2O)]^+$, and of the transient intermediate which does not lead to these without the occurrence of some decoordination in a slow, rate-determining step.

(up, up, up) can only evolve in a straightforward way from precursor II′ (Figure 5), while precursor III′ will lead directly to final structures 7 and 8 (down, up, up). Precursor I′, on the other hand, leads directly only to *prohibited* products 1 and 2 (up, down, up), and coordination of further ligand atoms from this precursor constitutes a "dead-end" stable intermediate. Further progress to a stable final complex is blocked in this pathway, and further reaction can occur only if some degree of decomplexation takes place.

On the basis of our characterization of this intermediate (excitation peak at 579.5 nm), it is proposed that it contains one (or less likely two) coordinated water molecules and seven

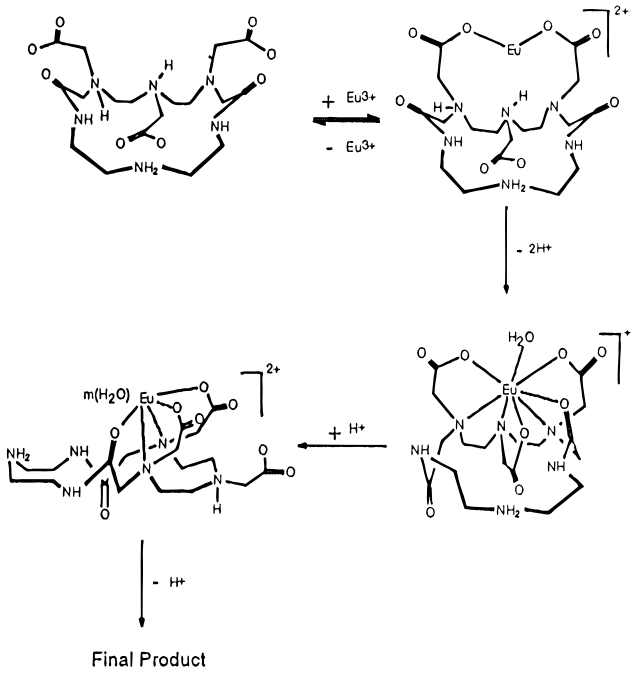

Figure 6. Possible H^+-catalyzed reaction pathway starting with H_2-dtpa-dien$^-$, the predominant ligand species in the pH 4−8.5 range. A parallel acid-independent path also exists, but is not shown.

coordinating atoms provided by the ligand in the first coordination sphere of the metal. Since there is no torsional motion or simple rotation that can carry this intermediate to one of the stable final structures (Figure 5, 5−8), the intermediate must overcome an activation barrier involving decoordination of at least one of the carboxylate groups in order to proceed. Only when one free arm rotates to its opposite position (down to up or up to down) can the intermediate rearrange to the final complex. One of the possibilities for such a reaction route is illustrated in Figure 6, where again only one of the several possible ligand protonation states is considered.

The observation, at pH values less than 6.5, of a constant initially observed ratio, $I_{579.5}/I_{579.9}$, implies that the probabilities of forming the intermediate or the final complex directly are similar under these conditions. This implies that the initial reaction steps are similar for all pH values in the low range. Ligand species HL^{2-} and L^{3-} are absent under these conditions, while the H_2L^- species is present as 5.2% of the total ligand at pH 3 (where H_3L is dominant) and increases to 99.2% at pH 6.5. Thus, H_2L^- is likely to be the main ligand species to react with Eu^{3+} at pH values less than 6.5. Above pH 6.5 the $I_{579.5}/I_{579.9}$ ratio decreases dramatically, suggesting that the HL^{2-} and L^{3-} species which begin to dominate under these conditions react directly to form the final product.

Thus, the H_2dtpa-dien$^-$ form of the ligand is the one implicated in formation of the intermediate. NMR and X-ray structural investigations[28−31] have shown that in amino polycarboxylate ligands the amino nitrogens take priority over carboxylate groups in being protonated and that the sites of protonation are located so as to minimize electrostatic repulsion between positively charged protonated sites. We propose that the two protons of H_2dpta-dien$^-$ are located on the central nitrogen of the group that links the amide moieties and on one of the remaining three amine nitrogens adjacent to the carboxylate side arms.

Molecular mechanics calculations[26,32,33] for the free ligand species H_2L^-, HL^{2-}, and L^{3-} suggest that the lowest energy configuration is one with the carboxylate arms in the up, down, up configuration, which minimizes electrostatic repulsion among the negatively charged carboxylate groups. It is just this up, down, up structure, I, which leads to the formation of the "dead-end" intermediate. It is proposed that protonation of an amine nitrogen markedly increases the rigidity of the macrocyclic ring by inhibiting inversion at that nitrogen, although the reaction rates for these initial steps are much faster than the rate-determining step in the complexation of Ln^{3+} ions with more rigid macrocyclic ligands such as dota. Protonation of any of the three amine nitrogens (N_1, N_2, N_1') inhibits the flipping (from up to down or vice versa) of the carboxylate side arm attached at that point. Thus, at higher pH values where only the linker nitrogen is protonated, the carboxylate side chains are free to flip between up and down configurations and the up, up, up and up, up, down configurations, which leads to rapid formation of final product complex.

At lower pH values where H_2L^- is dominant, such interconversion are inhibited, and the up, down, up configuration of H_2dtpa-dien$^-$ leads to formation of the intermediate. Nevertheless, there is a proton-assisted pathway for conversion of the intermediate, as depicted in Figure 6. Protonation of one of the coordinated amino nitrogen atoms (N_2 in this example) in the intermediate Eu(Hdtpa-dien)$^+$ to form Eu(H_2dtpa-dien)$^{2+}$ assists the decoordination of the protonated amine nitrogen and its adjacent carboxylate group. This is a slow, proton-assisted process which constitutes the rate-determining step. Subsequent rapid loss of a proton from Eu(H_2dtpa-dien)$^{2+}$ allows the nitrogen to invert and the carboxylate to flip to the up configuration, from which a stable final product complex (Figure 5, isomers 5 or 6) is rapidly formed.

Conclusions. When Eu^{3+} and the ligand dtpa-dien are mixed at pH values less than 6.5, where the diprotonated free ligand species H_2dtpa-dien$^-$ predominates, both the final product complex and a kinetic intermediate are immediately formed. Both forms are observed by $^7F_0 \rightarrow {}^5D_0$ luminescence excitation spectroscopy, and the intermediate is postulated to involve coordination by three carboxylate groups (in a up, down, up configuration with respect to the macrocyclic ring), three amine nitrogens, an amide carboxyl, and likely, one water molecule. This intermediate cannot directly transform into a final stable tricapped trigonal prismatic product without some decomplexation. This slow kinetic process occurs by both proton-assisted and proton-independent processes. At pH values greater than 7 much less intermediate is formed, as complexation with Hdtpa-dien^{2-} and dtpa-dien^{3-} produce the final product directly.

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