Synthesis and Dynamic Properties of Kinetically Inert Lanthanide Compounds: Lanthanum(III) and Europium(III) Complexes of 1,4,7,10-Tetrakis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane

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The synthesis and dynamic properties of La(THED)$(CF_3SO_3)_3$ and Eu(THED)$(CF_3SO_3)_3$ are described. (THED is 1,4,7,10-tetrakis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane.) Variable-temperature ^{1}H and ^{13}C NMR studies indicate that the ethylenic groups of the 12-membered tetraaza macrocycle are rigid on the NMR time scale at low temperatures (–40 °C), and all adopt the same conformation. Macrocycle rigidity is lost at elevated temperatures. A dynamic process involving conformational changes of the ethylenic groups in the tetraazamacrocycle is consistent with NMR data. An energy barrier of 52 (±0.7) kJ mol^{-1} is calculated for this process from line-shape analysis of ^{13}C NMR spectra of La(THED)$(CF_3SO_3)_3$ at four temperatures. Both complexes are stable in water at near-neutral pH. The dissociation of La^{3+} or Eu^{3+} from their respective complexes is investigated in the presence of excess Cu^{2+} by monitoring an absorbance at 312 nm attributed to the Cu(THED)$^{2+}$ complex. For La(THED)$(CF_3SO_3)_3$, dissociation is first-order in lanthanum complex and independent of Cu^{2+} with a first-order rate constant of 9.2 (±0.5) × 10^{-6} s^{-1} at 37 °C, pH 6.0. Eu(THED)$(CF_3SO_3)_3$ is substantially more inert to metal ion release than is the lanthanum complex. The first-order rate constant for dissociation of the europium complex at pH 6.0 and 37 °C is 7.1 (±0.4) × 10^{-7} s^{-1}; there is a slight dependence of the rate constant on pH in the pH range 3.0–6.8.

Introduction

Many medicinal applications of metal complexes require that the complex be inert to metal ion release in water. Much effort has been devoted recently to the synthesis of complexes of the lanthanides(III) and yttrium(III) that are inert to dissociation under physiological conditions. Most of these complexes have been developed for magnetic resonance imaging[1] or radiotherapeutic[2] applications. Our interests lie in the design of new lanthanide complexes that are inert to metal ion dissociation and are active in catalyzing RNA cleavage.[3] Many good ligands for the lanthanides such as DTPA (diethylenetriaminepentaacetic acid) and DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) form anionic complexes with trivalent lanthanides. However, polyamino carboxylate complexes of lanthanide(III) ions that are anionic are not good catalysts for RNA cleavage;[3] thus, we set out to synthesize new ligands for chelation of lanthanide(III) ions. Our goal was to find neutral ligands that would strongly bind trivalent lanthanides and form cationic complexes.

DOTA is a highly sterically efficient ligand for the coordination of lanthanides.[4] The formation constant for Gd(DOTA)$^-$ is high with reported values[5] ranging from 10^{22} to 10^{25}, and the complex is kinetically inert to metal ion release. Other tetraazacyclododecane derivatives with four pendant groups such as phosphinic acids[6] appear to be good ligands for Gd^{3+} as well. These studies suggest that the 12-membered cyclen macrocycle (1,4,7,10-tetraazacyclododecane) is a good framework to build ligands for the lanthanide(III) ions. We reasoned that addition of four neutral pendent ligating groups to cyclen might produce a neutral octadentate ligand that would coordinate strongly to trivalent lanthanide ions. For this task, we chose to use neutral pendent groups that would conserve the five-membered chelate rings found in DOTA complexes. Here we report the synthesis and solution properties of the lanthanum(III) and europium(III) complexes of 1,4,7,10-tetrakis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (THED). The THED complexes are remarkably resistant to lanthanide ion release in water at 37 °C. The solution properties of the complexes have been studied by NMR and are compared with those of lanthanide(III) DOTA complexes.

Experimental Section

The free base form of cyclen (1,4,7,10-tetraazacyclododecane) was generated by passing the tetrahydrochloride salt (Parish Chemicals or Strem Chemicals) through a Dowex 1X8-200 anion-exchange column (30 cm × 2.5 cm, hydroxide form). La$(CF_3SO_3)_3$ and Eu$(CF_3SO_3)_3$ were obtained by treating the respective lanthanide oxides with concentrated trifluoromethanesulfonic acid as reported previously.[7] 1,4,7,10-Tetrakis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (THED) was prepared as reported previously[8,9] by treating cyclen with a 50% excess of ethylene oxide in either absolute ethanol or water at 0 °C for 3–4 h.

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(1) (a) Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901–927 and references within. (b) Konings, M. S.; Dow, W. C.; Love, D. B.; Raymond, K. N.; Quay, S. C.; Rocklage, S. M. *Inorg. Chem.* **1990**, *29*, 1488–1491. (c) Smith, P. H.; Brainard, J. R.; Morris, D. E.; Jarvinen, G. D.; Ryan, R. R. *J. Am. Chem. Soc.* **1989**, *111*, 7437–7443. (d) Dischino, D. D.; Delaney, E. J.; Emswiler, J. E.; Gaughan, G. T.; Prasad, J. S.; Srivastava, S. K.; Tweedle, M. F. *Inorg. Chem.* **1991**, *30*, 1265–1269.

(2) (a) Moi, M. K.; Meares, C. F.; DeNardo, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 6266–6267. (b) Cox, J. P. L.; Jankowski, K. J.; Kataky, R.; Parker, D.; Beeley, N. R. A.; Boyce, B. A.; Eaton, M. A. W.; Millar, K.; Millican, A. T.; Harrison, A.; Walker, C. *J. Chem. Soc., Chem. Commun.* **1989**, 797–800.

(3) Morrow, J. R.; Buttrey, L. A.; Shelton, V. M.; Berback, K. A. *J. Am. Chem. Soc.* **1992**, *114*, 1903–1905.

(4) Desreux, J. F. *Inorg. Chem.* **1980**, *19*, 1319–1324.

(5) (a) Wang, X.; Jin, T.; Comblin, V.; Lopez-Mut, A.; Merciny, E.; Desreux, J. F. *Inorg. Chem.* **1992**, *31*, 1095–1099. (b) Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1991**, *190*, 37–46. (c) Cacheris, W. P.; Nickle, S. K.; Sherry, A. D. *Inorg. Chem.* **1987**, *26*, 958–960. (d) Chang, C. A. *Eur. J. Solid State Chem.* **1991**, *28*, 237–241.

(6) Broan, C. J.; Jankowski, K. J.; Kataky, R.; Parker, D.; Randall, A. *J. Chem. Soc., Chem. Commun.* **1990**, 1739–1740.

(7) Smith, P. H.; Raymond, K. N. *Inorg. Chem.* **1985**, *24*, 3469–3477.

(8) Buoen, S.; Dale, J.; Groth, P.; Krane, J. *J. Chem. Soc., Chem. Commun.* **1982**, 1172–1174.

(9) Madeyski, C. M.; Michael, J. P.; Hancock, R. D. *Inorg. Chem.* **1984**, *23*, 1487–1489.

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The ligand was recrystallized from 2-propanol and isolated in 50% yield. All other chemicals used were of analytical grade. Acetonitrile was dried over CaH_2. Milli-Q purified water was used for kinetic experiments.

An Orion Research Model 510 digital ion analyzer equipped with a temperature compensation probe was used for all pH measurements. The pD was calculated as pH + 0.4.[10] A Hewlett-Packard diode array 8452A spectrophotometer with a thermostated cell compartment was employed for UV–vis spectra and for kinetic measurements. All ^1H, ^{13}C, and ^{139}La NMR spectra were recorded by use of a Varian 400 XL spectrophotometer. ^1H and ^{13}C chemical shifts are reported as parts per million downfield of $SiMe_4$ and ^{139}La chemical shifts are reported as parts per million downfield of $La(NO_3)_3$. NMR sample temperatures were measured by use of a thermocouple located near the probe. Elemental analyses were performed by E and R Microanalytical Laboratories. A VG 70-SE mass spectrometer with fast atom bombardment was utilized.

$La(THED)(CF_3SO_3)_3$. The lanthanum complex of THED was prepared in acetonitrile by the following method. Typically, $La(SO_3CF_3)_3$ (5.75×10^{-4} mol) was refluxed under nitrogen in a mixture of 50 mL of dry acetonitrile and 5.6 mL of trimethyl orthoformate for several hours. THED (5.75×10^{-4} mol) dissolved in acetonitrile was introduced by cannula into the solution containing lanthanum. The mixture was refluxed for 1 h. The acetonitrile solution was concentrated in vacuo, and methylene chloride was added. The crystalline product was obtained in 60% yield. Anal. Calcd for $C_{19}H_{38}N_4O_{14}F_9S_3La$: C, 24.00; H, 3.99; N, 5.88; La, 14.59. Found: C, 24.20; H, 4.02; N, 5.74; La, 14.45. FABMS (m/z): 785 ($La(THED)(CF_3SO_3)_3 - CF_3SO_3$). ^1H NMR (d^4-methanol, −40 °C): 4.33 (t, 4H, $HOCH_2CH_2$), 3.99 (m, 4H, $HOCH_2CH_2$), 3.80 (t, 4H, axial ring protons), 3.60 (t, 4H, axial ring protons), 3.49 (m, 4H, $HOCH_2CH_2$), 2.44 (d, 8H, $HOCH_2CH_2$ and equatorial ring protons), 2.28 (d, 4H, equatorial ring protons). ^{13}C NMR (d^4-methanol, 25 °C): 50.80 (broad, NCH_2CH_2N), 53.80 ($HOCH_2CH_2$), 60.75 ($HOCH_2CH_2$). ^{139}La NMR (H_2O/D_2O): 230.

$Eu(THED)(CF_3SO_3)_3$. The europium complex of THED was prepared in a procedure similar to that for the lanthanum complex. A white powder was isolated in 60% yield. Anal. Calcd for $C_{19}H_{36}N_4O_{13}F_9S_3Eu$: C, 24.10; H, 3.80; N, 5.91; Eu, 16.05. Found: C, 23.82; H, 3.82; N, 5.79; Eu, 16.29. FABMS (m/z): 798 ($Eu(THED)(CF_3SO_3)_3 - CF_3SO_3$). ^1H NMR (d^4-methanol, 17 °C) (all are broad, featureless resonances): 6.63 (4H), 2.40 (4H), 1.64 (4H), −0.93 (4H), −3.30 (4H), −9.08 (4H), −9.62 (4H), −13.21 (4H). ^{13}C NMR (d^4-methanol, 17 °C): 76.4, 84.2 (broad), 88.6.

^1H NMR was used to monitor formation and dissociation of the $La(THED)(CF_3SO_3)_3$ complex. Samples for experiments to study complex formation contained 0.043 M $La(CF_3SO_3)_3$ and 0.01 M THED at pD 8.0. Solutions of $La(THED)(CF_3SO_3)_3$ in D_2O were monitored to determine qualitatively the extent of dissociation of La^{3+} from the macrocycle. Solutions were approximately 5 mM in complex, pD 8.0, and some experiments had a 2-fold excess of DTPA (diethylenetriaminepentaacetic acid).

A few experiments were done using the dye Arsenazo III to monitor coordination of Eu^{3+} or La^{3+} to THED. Typical reactions had 2.5×10^{-5} M $La(CF_3SO_3)_3$, 2.5×10^{-4} M dye, 25 mM Hepes, 0.1 M $NaClO_4$, and 1 mM THED at pH 7.4, 37 °C. An absorbance peak of the dye–lanthanide complex at 660 nm was monitored.[11]

Kinetics. The rate of dissociation of La^{3+} or Eu^{3+} from the THED complex at 37 °C in the presence of Cu^{2+} was monitored by following the increase in absorbance at 312 nm. Beer's law plots with varying concentrations of the $Cu(THED)^{2+}$ complex (0.100–1.00 mM) gave an extinction coefficient at 312 nm of 5000, 7320, 6830, and 6530 M^{-1} cm^{-1} for pH = 3.0, 5.0, 6.0, and 6.8, respectively. Solutions for reactions at pH 5.00 or 6.00 were self-buffered because of the presence of excess $Cu(Cl)_2$. Alternately, pH was maintained constant by addition of 0.01 M MES buffer (pH = 6.0) or 0.01 M HEPES buffer (pH = 6.8). Although reactions at pH 3.0 contained no buffer, the pH changed during the reaction by less than 0.1 pH unit. All reaction solutions were tested at the end of kinetic runs to insure that pH had remained constant. Kinetics experiments with the lanthanum complex showed good pseudo-first-order kinetics for more than 4 half-lives. First-order rate constants were obtained by a linear least-squares fitting of $\log(A_\infty - A_t)$ vs time data. The first-order rate constant for dissociation of the lanthanum complex was independent of Cu^{2+} for copper concentrations in the range from 0.1–0.5 mM at pH 6.0. Because of the slow rate of dissociation of lanthanide ions, some experiments used the method of initial rates. First-order rate constants, determined by dividing the rate by the concentration of lanthanide complex, were independent of the concentration of complex. The rate of dissociation of the europium complex was measured at pH 3.0, 5.0, 6.0, and 6.8.

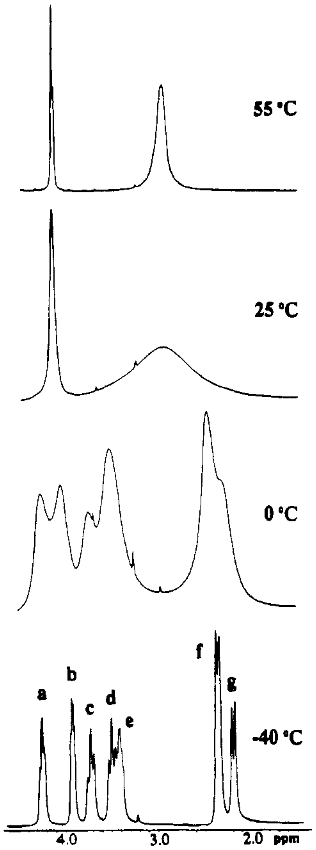

Figure 1. Variable-temperature ^1H NMR spectra of $La(THED)(CF_3SO_3)_3$ from −40 to 55 °C in d^4-methanol.

Results

The THED complexes of La^{3+} and Eu^{3+} are prepared under anhydrous conditions by treatment of the lanthanide(III) trifluoromethanesulfonate salt with the free base form of THED. The use of dry acetonitrile and the addition of the drying agent trimethyl orthoformate is necessary to eliminate the production of lanthanide hydroxide precipitates. Single crystals of diffraction quality are readily formed for the lanthanum complex. Unfortunately, all attempts to obtain a crystal structure were fruitless. After several hours the crystal disintegrated even when the crystal was in contact with mother liquor.

Attempts to form the lanthanum complex in water were not successful. ^1H NMR spectra of D_2O solutions at pD 8.0 containing 43 mM $La(CF_3SO_3)_3$ and 10 mM THED indicated no formation of $La(THED)(CF_3SO_3)_3$ even after incubation of the sample for several days. The extent formation of complex in water was also studied by monitoring the absorbance spectrum of a complex[11] between Arsenazo III and La^{3+} or Eu^{3+}. In this method, the THED ligand competes with the dye for binding the lanthanide ion. None of the $La(CF_3SO_3)_3$ was bound by THED after 8 h at 37 °C, pH 7.4. However, 27% of the $Eu(CF_3SO_3)_3$ was bound to THED after 30 min at 37 °C. The amount of Eu^{3+} bound by THED was unchanged after 8 h.

^1H NMR spectra of $La(THED)(CF_3SO_3)_3$ taken as a function of temperature indicate a dynamic process (Figure 1). At room temperature in d^4-methanol, one broad resonance and one narrower resonance are observed. (A very similar spectrum is observed for the complex at room temperature in D_2O.) In d^4-

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[10] Mikkelsen, K.; Nielson, S. O. *J. Phys. Chem.* **1960**, *64*, 632–637.

[11] Kodama, M.; Koike, T.; Mahatma, A.; Kimura, E. *Inorg. Chem.* **1991**, *30*, 1270–1273.

methanol at 55 °C, one of the peaks resolves into a triplet but the resonance at 2.8 ppm remains fairly broad. Cooling the sample to −40 °C gives a spectrum with seven resonances. Assignments were made by use of 2D-COSY experiments at −40 °C. Comparison was made to the ^{1}H NMR spectrum of the lanthanum(III) DOTA complex[4,12] to distinguish between the ethylenic protons of the hydroxyethyl groups and ethylenic cyclen ring protons. ^{1}H resonances of the hydroxyethyl groups appear as a triplet (a), two multiplets (b and e), and one resonance that overlaps with a cyclen ring proton resonance (f). (Resonance f begins to resolve into two resonances in other solvents.) Axial and equatorial protons of the ring are readily assigned; small gauche coupling constants and large geminal and trans axial coupling constants are observed in similar complexes.[4,12] Thus, cyclen ring protons in axial positions appear as triplets (c and d), and equatorial protons appear as doublets (f and g). The geminal coupling constant for cyclen ring protons is 13.7 Hz. Axial proton c is geminal to equatorial proton f and axial proton d is geminal to equatorial proton g.

Two sharp resonances at 60.75 and 53.80 ppm and one broad resonance at 50.80 ppm are observed in the ^{13}C NMR spectrum of La(THED)$(CF_3SO_3)_3$ at 25 °C in d^4-methanol. The resonance at 50.80 ppm becomes less broad at 50 °C as the fast-exchange limit is approached. Upon cooling of the sample to −20 °C, the resonance at 50.80 ppm splits into two peaks at 52.05 and 49.25 ppm. This dynamic behavior, similar to that observed for the DOTA complexes of the lanthanides,[4,12] leads us to assign the resonances at the 52.05 and 49.25 ppm to the two carbons in the cyclen ring. ^{13}C resonances at 60.75 and 53.80 ppm are assigned to the carbons of the hydroxyethyl groups. An activation energy of 52 ± 0.7 kJ mol^{-1} is calculated for the ring carbon exchange process at four temperatures from rate constants estimated[13] at the coalescence temperature (−3.0 °C, $k_{ex} = \pi(\Delta\nu_{AB})/2^{1/2}$, at slow exchange (−20 and −30 °C, $k_{ex} = \pi(\Delta\omega)$), and at fast exchange (25 °C, $k_{ex} = \pi(\Delta\nu_{AB})^2/2(\Delta\omega)$).

A ^{139}La NMR spectrum of La(THED)$(CF_3SO_3)_3$ in water exhibits a single broad resonance at 230 ppm downfield of La$(NO_3)_3$, consistent with a single lanthanum(III) complex in solution. The line width at half-peak height ($\Delta\nu_{1/2}$) is 2.3 kHz, similar to other reported ^{139}La resonances of lanthanum(III) complexes.[14] A second resonance is observed upon addition of La$(NO_3)_3$ to the sample and has no effect on the peak attributed to the complex.

Eight ^{1}H NMR resonances are observed for the europium complex at 17 °C in d^4-methanol. (A similar ^{1}H NMR spectrum is observed in D_2O.) All resonances are broad ($\Delta\nu_{1/2}$ = 300 Hz). Resonances narrowed slightly upon cooling to 0 °C, but no coupling could be resolved. The ^{13}C NMR of the complex at 17 °C exhibits resonances at 88.6 ppm ($\Delta\nu_{1/2}$ = 34 Hz) and 76.4 ppm ($\Delta\nu_{1/2}$ = 17 Hz) and one broad resonance at 84.2 ppm ($\Delta\nu_{1/2}$ = 180 Hz). The resonance at 84.2 ppm broadened further upon cooling the sample to 0 °C. By analogy to the lanthanum complex, the resonance at 84.2 ppm is assigned to the ring carbons of the macrocycle broadened by a dynamic process. The remaining two ^{13}C resonances are assigned to carbons of the hydroxyethyl groups.

La(THED)$(CF_3SO_3)_3$ is stable in aqueous solution, pD 8.0, room temperature for a few hours as studied by use of ^{1}H NMR. Resonances attributed to free ligand are observed after approximately 6 h at either pD 7.0 or 8.0. After 2 h at room temperature, traces of free ligand are noted in solutions containing DTPA (diethylenetriaminepentaacetic acid) and La(THED)$(CF_3SO_3)_3$. Twenty-four hours after the addition of DTPA, most of the lanthanum is bound to DTPA and only a trace of complex remains. The europium complex is difficult to study by NMR because its ^{1}H resonances are broad and mask other peaks. However, concentrated samples in aqueous solution show no trace of precipitate formation when left for several days. Solutions 1 mM in europium complex do not form precipitates even at pH 11.

The kinetics of dissociation of lanthanide ions from the Ln(THED)$(CF_3SO_3)_3$ complexes are studied in the presence of Cu^{2+} by monitoring the formation of the Cu^{2+} complex of THED. The copper ion serves the dual purpose of trapping the macrocycle and as an indicator of the reaction. The Cu(THED)$^{2+}$ complex has an absorbance band at 312 nm that follows Beer's law and is conveniently used to measure free THED. This method could be used at copper concentrations of 1 mM at pH 3.0–6.0 or 0.20 mM Cu^{2+} at pH 6.8. Absorbance bands of free Cu^{2+} masked the 312-nm absorbance of the Cu(THED)$^{2+}$ complex at more basic pH. Experiments containing Cu^{2+} and THED alone indicate that complexation of Cu^{2+} by THED is several hundred-fold more rapid than dissociation of the lanthanum complex. A first-order rate constant of 9.2 (±0.5) × 10^{-6} s^{-1} is calculated for dissociation of La^{3+} from the THED complex at pH 6.0. First-order rate constants for the dissociation of Eu^{3+} from the THED complex are 1.6 (±0.2) × 10^{-6} s^{-1}, 7.8 (±0.4) × 10^{-7} s^{-1}, 7.1 (±0.4) × 10^{-7} s^{-1}, and 4.4 (±0.3) × 10^{-7} s^{-1} at pH 3.0, 5.0, 6.0, and 6.8, respectively.

Discussion

The procedures used for the synthesis of the lanthanum(III) and europium(III) complexes of THED are similar to those employed for synthesis of the lanthanide(III) cryptates[15] or crown ether complexes.[16] Treatment of La$(CF_3SO_3)_3$ or Eu$(CF_3SO_3)_3$ with the free-base form of THED under anhydrous conditions gave the complexes in good yield. Because of our interest in the conjugation of lanthanide complexes to biopolymers, we studied the formation of complex in water as well. Attempts to form the lanthanum complex in water under conditions where lanthanum hydroxides do not precipitate out of solution were unsuccessful. However after 30 min, 27% of the europium THED complex is formed with the remainder of the Eu^{3+} bound to the Arzenazo III dye. After 8 h, the amount of Eu^{3+} bound to THED is unchanged. These studies suggest that complex formation is not highly favorable at pH 7.4, where two of the four amine groups are protonated and the lanthanide ion is coordinated to water. In this respect, the THED complexes are similar to the lanthanide(III)–[2.2.1]cryptate complexes that are readily synthesized in organic solvents and inert to metal ion release in water but do not show a large extent of complex formation at pH 7 in water.[17] For the lanthanum complex, we cannot rule out very slow kinetics of La^{3+} complexation by THED. However in view of the fact that the Eu^{3+} complex is formed at shorter times under similar conditions, this explanation is unlikely. Complexation of Lu^{3+} by DOTA under similar conditions to ours is 50% complete in approximately 2 h.[11] Kinetics of metal macrocycle formation is, however, dependent on pendent groups.[9] If differences in THED complexation of Eu^{3+} and La^{3+} do not have a kinetic origin, then the greater extent of formation of the europium complex compared to the lanthanum complex may be attributed to a larger formation constant for the europium complex.

At neutral or slightly basic pH La(THED)$(CF_3SO_3)_3$ is resistant to dissociation as monitored by use of ^{1}H NMR. Because

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(12) Aime, S.; Botta, M.; Ermondi, G. *Inorg. Chem.* **1992**, *31*, 4291–4299.

(13) Faller, J. W. *Adv. Organomet. Chem.* **1977**, *16*, 211.

(14) Frechette, M.; Butler, I. R.; Hynes, R.; Detellier, C. *Inorg. Chem.* **1992**, *31*, 1650–1656.

(15) (a) Pruett, D. J. Thesis, Michigan State University, East Lansing, MI, 1978. (b) Gansow, O. A.; Kausar, A. R.; Triplett, K. M.; Weaver, M. J.; Yee, E. L. *J. Am. Chem. Soc.* **1977**, *99*, 7087–7089. (c) Ciampolini, M.; Dapporto, P.; Nardi, N. *J. Chem. Soc., Dalton Trans.* **1979**, 974–977.

(16) Desreux, J. F.; Duyckaerts, G. *Inorg. Chim. Acta* **1979**, *35*, L313–L315.

(17) (a) Yee, E. L.; Gansow, O. A.; Weaver, M. J. *J. Am. Chem. Soc.* **1980**, *102*, 2278–2285. (b) Torres, R. A.; Baisden, P. A. *Inorg. Chem.* **1989**, *28*, 2807–2810.

of our interest in utilizing these lanthanide complexes to cleave RNA under physiological conditions, we were interested in developing an alternative method to detect dissociation of the lanthanide complexes under a variety of conditions. The kinetics of dissociation of the europium and lanthanum complexes of THED complexes in the presence of Cu^{2+} are studied by following the production of $Cu(THED)^{2+}$ over time. For the lanthanum complex at pH 6.0, the reaction is first order in $La(THED)(CF_3SO_3)_3$ and shows no dependence on the concentration of copper. A simple kinetic scheme consistent with these results has La^{3+} dissociating from the macrocycle with Cu^{2+} trapping the free macrocycle (Scheme I). A slight pH dependence of the rate constant for dissociation of the europium complex is observed in the pH range 3.0–6.8 with dissociation approximately 3.6-fold more rapid at pH 3.0 than at pH 6.8. The increase in the rate constant at lower pH may be due to the onset of acid-catalyzed dissociation as observed for similar macrocyclic complexes[5a] such as Gd(DOTA).

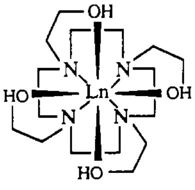
Scheme I

$$La(THED)^{3+} \rightarrow La^{3+} + THED \qquad (k_1)$$

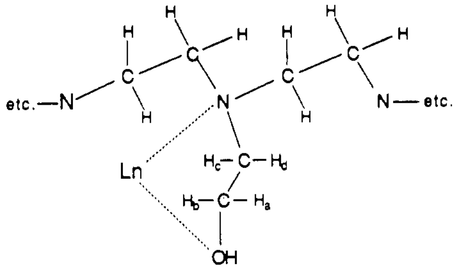
$$THED + Cu^{2+} \rightarrow Cu(THED)^{2+} \qquad (\text{rapid})$$

Dissociation of the $Eu(THED)(CF_3SO_3)_3$ complex proceeds much more slowly than the lanthanum complex. At pH 6.0, the europium complex has a half-life of 270 h compared to a half-life of 21 h for the lanthanum complex under similar conditions. It is not unusual for the inertness of lanthanide macrocycle complexes to vary dramatically with lanthanide ion.[3] Properties such as coordination number, ionic radius, and Lewis acidity may all influence the inertness of a macrocycle complex to metal ion release. A better fit of the smaller Eu^{3+} to the macrocycle may be responsible for the greater kinetic inertness of the europium complex to metal ion release. In addition, the difference in ionic radius between La^{3+} and Eu^{3+} may result in a different coordination number for the complexes in water.[18] At present, we have no information about the number of coordination sites occupied by water in $La(THED)(CF_3SO_3)_3$ or in $Eu(THED)(CF_3SO_3)_3$. Analogous DOTA complexes of Eu^{3+} or Gd^{3+} bind one water molecule in the solid state[19,20] or in solution.[20] Further experiments to probe the coordination sphere of the lanthanum(III) and europium(III) THED complexes may elucidate these differences in reactivity. Our studies suggest that the inertness of the europium complex to dissociation is reminiscent of the [2.2.1]cryptate complex of Eu^{3+}.[15b] Only lanthanide DOTA complexes are substantially more inert to dissociation.[5a]

^1H and ^{13}C NMR studies in d^4-methanol indicate that $La(THED)(CF_3SO_3)_3$ and $Eu(THED)(CF_3SO_3)_3$ may have solution structures similar to those of the lanthanide(III) DOTA complexes as shown schematically in **1**. For lanthanide(III) DOTA complexes, solution structures are similar to solid-state structures[4,19,20] and feature a lanthanum ion lying above the plane of the cyclen ring with all four acetates bound. The geometry about the lanthanide ion is best described as a distorted square antiprism capped by a water ligand. Many of the unusual NMR spectral properties of these complexes arise from the high degree of ligand rigidity.[4] Very similar NMR properties are observed for the THED complexes of La^{3+} and Eu^{3+}. At −40 °C the ethylenic carbons of the cyclen ring are locked into a staggered conformation making the two carbons inequivalent. That there are two ^{13}C resonances for carbons in the cyclen ring at low temperatures indicates the presence of a C_4 axis of symmetry. Similar conformational rigidity of the ethylene groups of cyclen is observed for lanthanide DOTA complexes[4,12] and lanthanide crown ether complexes.[16] For $La(THED)(CF_3SO_3)_3$, macrocycle rigidity gives rise to four signals in the ^{13}C NMR spectrum of the complexes at low temperature, two for cyclen ring carbons and two for the hydroxyethyl groups. In structure **1**, there are eight unique protons giving rise to eight resonances in the ^1H NMR spectrum (for the lanthanum complex two resonances are overlapping). In the cyclen ring there are two types of axial protons and two types of equatorial protons, similar to that observed for lanthanide(III) DOTA complexes.[4,12] The four protons in a hydroxyethyl group are all inequivalent at low temperature. This is consistent with an asymmetric environment about each nitrogen. As shown schematically in structure **2**, a nitrogen is bound to the metal, a hydroxyethyl group, and carbons of two different cyclen ethylenic groups. Thus, NMR data and the robust nature of the complexes support octadentate coordination of the THED ligand to La^{3+} or Eu^{3+}. All four cyclen nitrogens and all four hydroxyethyl groups are coordinated with all hydroxyethyl groups lying on the same side of the cyclen ring shown schematically in **1**.



1



2

In D_2O, the ^1H NMR spectra of lanthanum and europium complexes of THED closely resemble those of the complexes in d^4-methanol, suggesting a similar solution structure in water and in methanol. For the lanthanum complex in water a single ^{139}La resonance was observed, consistent with the presence of a single lanthanum complex in solution. No exchange with free La^{3+} was observed.

For lanthanide(III) DOTA complexes a dynamic process interconverts cyclen ring carbons through changes in conformation of the ethylenic groups.[4,12] This process also averages protons of the acetate groups because the quaternary nitrogen loses its asymmetry. A similar dynamic process is consistent with our results here. Carbons in the cyclen ring of $La(THED)(CF_3SO_3)_3$ undergo an exchange process as observed by ^{13}C NMR spectroscopy. This process should also exchange two pairs of protons in the cyclen ring and two pairs of protons of the hydroxyethylene groups. By ^1H NMR we observe that the (hydroxyethyl) methylene protons furthest downfield appear as a triplet at 50 °C. The high-temperature fast-exchange limiting spectrum was not recorded for the remaining protons because of solvent temperature limitations. An activation energy of 52 ± 0.7 kJ mol^{-1} was calculated from the ^{13}C NMR data at several temperatures. Comparison to the activation energy for La-

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(18) Choppin, G. R. In *Lanthanide Probes in Life, Chemical and Earth Sciences, Theory and Practice*; Bunzli, J.-C. G., Choppin, G. R., Eds.; Elsevier: New York, 1989; Chapter 1.

(19) Spirlet, M. R.; Rebizant, J.; Desreux, J. F.; Loncin, M.-F. *Inorg. Chem.* **1984**, *23*, 359–363.

(20) Aime, S.; Anelli, P. L.; Botta, M.; Fedeli, F.; Grandi, M.; Paoli, P.; Uggeri, F. *Inorg. Chem.* **1992**, *31*, 2422–2428.

$(DOTA)^-$ (60.7 ± 1.2 kJ mol^{-1})[14] indicates that the THED ligand when bound to La^{3+} is less rigid than DOTA bound to La^{3+}. This suggests that pendent groups contribute to the overall rigidity of the macrocycle ring. These differences reflect how strongly the pendent group coordinates the lanthanide ion and possibly the conformational flexibility of the pendent group. Carboxylate groups in polyamino carboxylate ligands are good donor groups for many metal ions, including the lanthanides.[21] There is less information for coordination of lanthanides by hydroxyethyl groups; available formation constants suggest that hydroxyalkyl or hydroxybenzyl groups coordinate strongly to La^{3+} (log K of nitriloacetic acid, $N(CH_2CH_2CO_2H)_3$ = 10.4; log K of DL-*N*-(2,3-dihydroxypropyl)iminodiacetic acid, $N(CH_2CH_2CO_2H)_2(CH_2CH(OH)CH_2OH)$, = 7.52; log K of *N*-(2-hydroxybenzyl)iminodiacetic acid, $N(CH_2CH_2CO_2H)_2(CH_2C_6H_4OH)$, = 11.57).

Several metal complexes of THED,[8,22] of THP-12ane-N_4[23] (1,4,7,10-tetrakis(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane), and of the 14-membered macrocycle THEC[9,24–26] (1,4,8,11-tetrakis(2-hydroxyethyl)-1,4,8,11-tetraazacyclotetradecane) are known. THEC complexes of Ni^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, K^+, and Na^+, THED complexes of Li^+, K^+, Na^+, Hg^{2+}, and Cd^{2+}, and THP-12ane-N_4 complexes of Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Ba^{2+}, and Sr^{2+} have been studied. For smaller metal ions such as Cu^{2+}, Ni^{2+}, and Zn^{2+}, all four hydroxyethyl groups of THEC or THP-12ane-N_4 cannot coordinate to the metal ion if the metal ion coordinates to the nitrogens of the cyclen ring. In contrast, larger metal ions may form complexes where all oxygen and nitrogen donor atoms are coordinated such as the Pb^{2+} complex of THP-12ane-N_4.[23] Our results here indicate that the relatively large Eu^{3+} and La^{3+} ions also form THED complexes with all nitrogens and hydroxyethyl groups coordinated in a manner reminiscent of lanthanide DOTA complexes. It would be of interest to compare the stability constants of the trivalent lanthanide THED complexes with those of the analogous DOTA complexes. Formation constants for the lanthanide complexes of THED are not available; however, formation constants[23] for the Pb^{2+} complex of DOTA and for the Pb^{2+} complex of THP (log K of DOTA = 19.9; log K of THP = 15.07) would suggest that acetate pendent groups are superior in the formation of highly stable complexes with large metal ions such as Pb^{2+}.

An important consideration in the synthesis of lanthanide(III) tetraazamacrocyclic complexes that are kinetically inert to metal ion release is the size of the macrocycle ring. For example lanthanide(III) DOTA complexes are more stable and less prone to lanthanide dissociation than are lanthanide(III) TETA complexes (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid) containing a 14-membered tetraazamacrocycle.[27] Attempts to synthesize lanthanum(III) complexes of THEC by a method similar to that used here to synthesize La(THED)$(CF_3SO_3)_3$ failed to produce complexes that were inert in water.[28] Thus, use of the sterically efficient cyclen framework may be crucial in the design of kinetically inert complexes. Ligands such as THED encapsulate lanthanide ions, forming complexes that are inert to dissociation in water. Further work is underway to synthesize lanthanide complexes of cyclen derivatives containing other neutral pendent groups such as amides.[29] Further work will examine the effect that various pendent groups have on the inertness of a complex to metal ion release and on the ability of these complexes to catalyze the hydrolysis of small molecules and biopolymers.

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(21) *Critical Stability Constants*; Martell, A. E., Smith, R. M., Eds.; Plenum: New York, 1988; Vol. 6.

(22) Pittet, P.-A.; Laurence, G. S.; Lincoln, S. F.; Turonek, M. L.; Wainwright, K. P. *J. Chem. Soc., Chem. Commun.* **1991**, 1205–1206.

(23) Hancock, R. D.; Shaikjee, M. S.; Dobson, S. M.; Boeyens, J. C. A. *Inorg. Chim. Acta* **1988**, *154*, 229–238.

(24) Hay, R. W.; Clark, D. M. S. *Inorg. Chim. Acta* **1984**, *83*, L23–L24.

(25) Clarke, P.; Lincoln, S. F.; Wainwright, K. P. *Inorg. Chem.* **1991**, *30*, 134–139.

(26) Clarke, P.; Hounslow, A. M.; Keough, R. A.; Lincoln, S. F.; Wainwright, K. P. *Inorg. Chem.* **1990**, *29*, 1793–1797.

(27) Loncin, M. F.; Desreux, J. F.; Merciny, E. *Inorg. Chem.* **1986**, *25*, 2646–2648.

(28) Chin, K. O. A.; Morrow, J. R. Unpublished observations.

(29) Morrow, J. R.; Amin, S.; Churchill, M. R.; Lake, C. Submitted for publication.