RNA Cleavage and Phosphate Diester Transesterification by Encapsulated Lanthanide Ions: Traversing the Lanthanide Series with Lanthanum(III), Europium(III), and Lutetium(III) Complexes of 1,4,7,10-Tetrakis(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane

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Lanthanide(III) complexes of two related ligands, 1,4,7,10-tetrakis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (THED) and (1*S*,4*S*,7*S*,10*S*)-1,4,7,10-tetrakis(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane (S-THP) promote transesterification of the 4-nitrophenyl phosphate ester of propylene glycol (**1**) and cleavage of RNA oligomers A_{12}-A_{18}. The pH-rate profiles for the transesterification of **1** or cleavage of RNA oligomers by lanthanide(III) complexes suggest that a lanthanide-bound hydroxide ($Ln(OH)^{2+}$) or lanthanide-bound alkoxide ($Ln(OR)^{2+}$) complex is the catalytically active species. Second-order rate constants for the transesterification of **1** by $Ln(OH)^{2+}$ or $Ln(OR)^{2+}$ at 37 °C are 7.2 (±0.5) × 10^{-2} M^{-1} s^{-1}, 6.3 (±0.3) × 10^{-2} M^{-1} s^{-1} and 1.4 (±0.2) × 10^{-2} M^{-1} s^{-1} for the THED complex of europium(III) and for the S-THP complexes of lanthanum(III) and europium(III), respectively. A second-order rate constant of 1.1 M^{-1} s^{-1} is obtained for the cleavage of RNA oligomers by the $Eu(OH)^{2+}$ or $Eu(OR)^{2+}$ form of the europium(III) THED complex. At higher concentrations of lanthanide(III) complex (>1.00 mM) at pH values where $Ln(OH)^{2+}$ or $Ln(OR)^{2+}$ are the predominent species, saturation kinetics are observed for the transesterification of **1** by Eu(THED)$(CF_3SO_3)_3$, Eu(S-THP)$(CF_3SO_3)_3$, or La(S-THP)$(CF_3SO_3)_3$; binding constants and first-order catalytic rate constants are, respectively, 62 (±9) M^{-1} and 9.5 (±0.9) × 10^{-4} s^{-1}, 68 (±9) M^{-1} and 1.8 (±0.4) × 10^{-4} s^{-1}, and 130 (±24) M^{-1} and 6.6 (±1.0) × 10^{-4} s^{-1}. As determined by potentiometric titrations, pK_a values for lanthanide-bound water or lanthanide-bound hydroxyalkyl decrease on traversing the lanthanide series: La(S-THP)$(CF_3SO_3)_3$, 8.40 (±0.05); Eu(THED)$(CF_3SO_3)_3$, 7.50 (±0.05); Eu(S-THP)$(CF_3SO_3)_3$ 7.80 (±0.1); Lu(S-THP)$(CF_3SO_3)_3$, 6.40 (±0.1) and 9.30 (±0.1). Conductivity measurements suggest that the europium(III) complexes exist as 3:1 electrolytes in water at pH 6.5.

Introduction

Lanthanide ions promote rapid phosphate ester transesterification, hydrolysis, and RNA cleavage under mild conditions.[1-12] For transesterification of phosphate esters, simple trivalent lanthanide salts are more effective than any of the divalent cations (except Pb^{2+}).[4,11] Work is underway in our laboratory to construct robust complexes of the trivalent lanthanides that are good catalysts for phosphate ester hydrolysis and transesterification. The development of lanthanide(III) complexes that are active catalysts is crucial if the catalytic activity of a trivalent lanthanide ion is to be harnessed for specific cleavage of nucleic acids.[13,14] Toward this goal, macrocyclic Schiff-base lanthanide(III) complexes have been shown to efficiently catalyze RNA cleavage.[12]

Our recent work has focused on the use of octadentate ligands that virtually encapsulate a lanthanide ion; some of these complexes are inert to lanthanide ion dissociation.[15,16] Part of this work has been directed toward the synthesis of new ligands that form inert complexes with all trivalent lanthanide ions. We had anticipated that certain trivalent lanthanide ions would be more active catalysts than others. In addition, we hoped to utilize the spectroscopic, electrochemical, and radiochemical properties of different lanthanides to elucidate the solution chemistry of these complexes and to track the complexes in biological systems.[17] Our recent efforts have produced two octadentate ligands, 1,4,7,10-tetrakis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (THED) and the *S*,*S*,*S*,*S* stereoisomer of 1,4,7,10-tetrakis(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane (S-THP), that form lanthanide(III) complexes which are inert to dissociation in water at 37 °C, at neutral pH.[15,18] The S-THP macrocycle, is unusual in that it forms robust complexes of early, middle, and late lanthanides (lanthanum(III), europium(III), and lutetium(III)). Other macrocycles that we have

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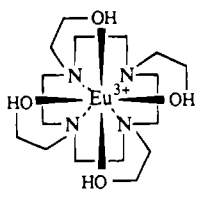
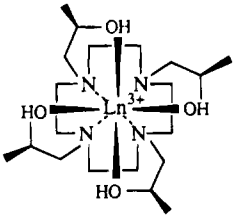
examined such as a hexadentate Schiff-base macrocycle and THED form inert complexes solely with the middle lanthanide ions.[12,15]

The coordination chemistry of trivalent lanthanide ions changes gradually as one traverses the series from lanthanum to lutetium;[19] such changes may modulate the catalytic properties of the lanthanide ion. For example, the decrease in ionic radius from La^{3+} to Lu^{3+} may modify the Lewis acidity and the resultant pK_a values of bound water molecules as well as the coordination number of the lanthanide ion.[20] Earlier work demonstrated surprisingly little difference in the rate of phosphate ester transesterification by seven trivalent lanthanide ions (La, Nd, Eu, Gd, Tb, Yb, Lu).[4,11] Other studies using high concentrations of lanthanide salts at alkaline pH have reported a greater difference (100-fold) between the rate of dinucleoside cleavage by early and late lanthanide ions.[6]

Here we report kinetic studies of phosphate ester transesterification and RNA cleavage by a new class of lanthanide catalysts: the S-THP complexes of lanthanum(III), europium(III), and lutetium(III) and a THED complex of europium(III). Changes in coordination number and variation in the Lewis acidity of the lanthanide(III) complexes (as measured by the pK_a of lanthanide-bound water or lanthanide-bound hydroxyalkyl) are important properties to consider in choosing a trivalent lanthanide ion as a catalyst.



$Eu(THED)^{3+}$



$Ln(S\text{-}THP)^{3+}$
Ln = La, Eu, Lu

Experimental Section

The 4-nitrophenylphosphate ester of propylene glycol (**1**) was synthesized according to literature procedures.[21] A single ^{31}P resonance was observed at −4.07 ppm in D_2O. ^{31}P NMR spectra were obtained at 169 MHz by use of a Varian 400 XL spectrometer. The external reference was 85% phosphoric acid and chemical shifts downfield of the standard are positive. Solutions of **1** were analyzed by spectrophotometric measurement of the amount of 4-nitrophenolate produced upon complete base hydrolysis. Adenylic acid oligomers A_{12}−A_{18} were purchased from Pharmacia. The lanthanide complexes $Ln(THP)(CF_3SO_3)_3$ and $Ln(THED)(CF_3SO_3)_3$ were synthesized as reported previously.[15,18]

Hepes (*N*-(2-hydroxyethyl)piperazine-N′-ethanesulfonic acid), Ches (2-(*N*-cyclohexylamino)ethanesulfonic acid), and Mes (2-morpholinoethanesulfonic acid) buffers were reagent grade and were purchased from Sigma Chemicals. All solutions were made with Milli-Q water. Solution pHs were measured at 37 °C by use of an Orion digital pH meter equipped with temperature compensation probe. Either Hepes, Mes, or Ches buffer was used to maintain solution pH and reaction solutions were tested at the end of kinetic runs to insure that the pH had remained constant (±0.03 pH).

Conductivity Measurements. Measurements were made with aqueous solutions of $Eu(THED)(CF_3SO_3)_3$ and $Eu(THP)(CF_3SO_3)_3$ in the concentration range 0.30−1.0 mM at 25 °C on a YSI model 31. The cell was calibrated with a 0.020 M solution of KCl. Solution conductance was plotted versus the square root[22-24] of the concentration of complex.

Potentiometric Titrations. Potentiometric titrations were carried out at 37 °C under an atmosphere of nitrogen. Solutions were 1.0 mM in complex with 0.1 M $NaNO_3$ added to maintain a constant ionic strength. The pH of the solution (50 mL total) was adjusted to 10.0 by addition of NaOH, followed by titration with a solution of 0.0100 M HCl (Baker Chemicals "dilute it" ampule).

Kinetic Measurements. The rates of transesterification of **1** by metal ions were measured spectrophotometrically by following the increase at 400 nm due to the production of 4-nitrophenol species. A Hewlett-Packard 5420 diode array UV−vis spectrophotometer equipped with a thermostated cell was used for measurements. Typically, the reaction was initiated by injection of 30 uL of a concentrated solution of **1** into 5 mL of a buffered reaction solution containing 0.10 M $NaNO_3$ maintained at 37 °C. Concentrations of **1** ranged from 1×10^{-4} to 2.0×10^{-4} M. Reactions with 1.00 mM complex showed good pseudo-first-order kinetics for greater than 4 half-lives. Pseudo-first-order rate constants were calculated by using an iterative curve fit (Hewlett-Packard Kinetics software package) to the following equation: $A = A_\infty + K(e^{-(k_{obs})(t)})$. Pseudo-first-order rate constants for the lutetium(III) complex were measured from the slopes of linear plots of absorbance against time by converting to concentration units (ϵ = 18 500) and dividing by the initial concentration of **1**. The pK_a of 4-nitrophenol at 37 °C (6.90) with 0.10 M $NaNO_3$ was used to correct for the concentration of 4-nitrophenolate present at pH 6.5−8.5. Control experiments containing **1**, 10 mM buffer, and 0.10 M $NaNO_3$ were run alongside experiments with lanthanide(III) catalysts. Conditional first-order rate constants for the transesterification of **1** in the absense of metal complexes (buffer and hydroxide) were measured and, when not negligible, were subtracted from the pseudo-first-order rate constants for the metal complex catalyzed reactions.

Pseudo-first-order rate constants for the cleavage of A_{12}−A_{18} were determined as reported previously[14] by use of a Waters 600E HPLC equipped with a 490E programmable multiwavelength UV−vis detector. Reactions were analyzed on a 7 M Nucleogen DEAE 60−7 column. For most experiments, solutions contained 5.0 mM Hepes, pH 7.60, 0.20 mM complex, and 0.080 mM RNA (adenylic acid concentration). For this analysis, the method of initial rates was used. Controls containing 5.0 mM buffer and 0.080 mM RNA showed negligible cleavage over the time period (30−40 min) where RNA cleavage by the lanthanide complexes was monitored. All standard precautions were taken to avoid ribonuclease contamination. Solutions were made fresh and were autoclaved, and gloves were worn in all stages of experiments.

Results

The lanthanide complexes $Eu(THED)(CF_3SO_3)_3$, $La(S\text{-}THP)(CF_3SO_3)_3$, $Eu(S\text{-}THP)(CF_3SO_3)_3$, $Lu(S\text{-}THP)(CF_3SO_3)_3$ and $Eu(THP)(CF_3SO_3)_3$ are synthesized[15,18] from their respective lanthanide salts ($Ln(CF_3SO_3)_3$)[25] and the free base form of the macrocycle. (The S-THP complexes have the S configuration at the chiral α-carbons of all pendent hydroxypropyl groups; THP complexes are synthesized from THP containing a mixture of stereoisomers arising from chirality at the α-carbons such as the diastereomers *S,S,S,S*, *R,S,S,S*, *R,R,S,S* etc.). All complexes are remarkably inert to dissociation in water at 37 °C at neutral pH.[15,18] For 0.1 mM complex at 37 °C and pH 6.0, half-lives for dissociation of the lanthanum(III), europium(III) and lutetium(III) S-THP complexes are 73, 100, and 53 days, respectively. The europium(III) THED complex has half-lifes of 11 and 18 days at pH 6.0 and 6.8, respectively. Dissociation of these complexes is little affected by strong ligands such as DTPA (diethylenetriaminepentaacetic acid) or by buffer.

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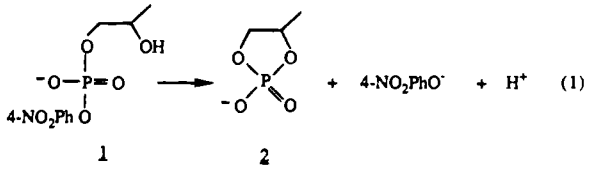
In general, synthesis of the THED and THP complexes yields crystalline products in high yield that give good analytic data. However, because trivalent lanthanide ions promote transesterification approximately 10-fold more rapidly[11] than do the lanthanide(III) complexes here, it is important to rule out catalysis by small amounts of free lanthanide ions. Lanthanide EDTA complexes are inactive in the transesterification of **1** or in RNA cleavage.[12,26] Addition of an equivalent of EDTA to a reaction solution of **1** and a free lanthanide ion arrests lanthanide ion promoted transesterification of **1**. Thus, experiments were carried out with and without the addition of a small amount of EDTA (15%) to reaction solutions to trap free lanthanide ions. For all of the complexes, no appreciable change (<5%) in the rate of transesterification of **1** was observed upon addition of EDTA.

The lanthanide(III) complexes were characterized by potentiometric titrations and by conductivity experiments. Potentiometric titration of 1.0 mM solutions of the lanthanide(III) complexes at 37 °C with 0.1 M $NaNO_3$ gave the following pK_a values: 7.50 (±0.05), Eu(THED)$(CF_3SO_3)_3$; 8.40 (±0.05), La(S-THP)$(CF_3SO_3)_3$; 7.80 (±0.1), Eu(S-THP)$(CF_3SO_3)_3$; 6.40, 9.30 (±0.1), Lu(S-THP)$(CF_3SO_3)_3$. Conductivity measurements on Eu(THED)$(CF_3SO_3)_3$ and Eu(THP)$(CF_3SO_3)_3$ at pH 6.5 gave molar conductivities of 2500 and 1300 $mol^{-3/2}$ $L^{1/2}$ cm^2 Ω^{-1}, respectively, well within the range anticipated for 3:1 electrolytes.[24]

The kinetics of transesterification of the 4-nitrophenylphosphate ester of propylene glycol (**1**) at 37 °C, by the lanthanide(III) complexes (eq 1) was monitored by following the



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production of the 4-nitrophenol species by use of UV−vis spectroscopy. The rate expression for the transesterification of **1** by the lanthanide(III) complexes ($Ln(HOR)^{3+}$) as in eq 2 is given in eq 3. Formation of the cyclic phosphate ester was

$$Ln(HOR)^{3+} \overset{-H^+, K_a}{\rightleftharpoons} Ln(OR)^{2+} \overset{1, K}{\rightleftharpoons} Ln(OR)(\mathbf{1})^{+} \xrightarrow{k_{cat}} Ln(HOR)^{3+} + \mathbf{2} + \text{4-nitrophenolate} \quad (2)$$

R = H, hydroxyethyl or hydroxypropyl

$$k_{obs} = \frac{k_{cat}K[Ln]_T K_a/(K_a + [H^+])}{1 + K[Ln]_T K_a/(K_a + [H^+])} \quad (3)$$

confirmed by use of ^{31}P NMR spectroscopy. This rate expression is derived for conditions where there is a large excess of lanthanide(III) complex relative to **1** and the amount of lanthanide(III) complex bound to **1** is negligible relative to the concentration of free lanthanide(III) complex. As discussed below, R is either a proton and the acidic group is a bound water molecule or R is an alkyl group and the acidic group is a bound hydroxyalkyl group of the THP or THED macrocycle. K is the association constant for the phosphate diester complex and k_{cat} is the first-order catalytic rate constant.

The transesterification of **1**, initially first order in lanthanide(III) complex concentration in the 0.400−1.00 mM concentration range becomes independent of lanthanide(III) complex concentration at higher concentrations of complex (>1.00 mM).

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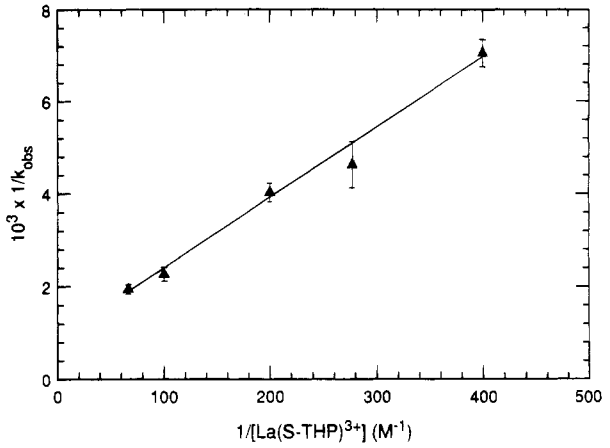

Figure 1. Plot of kinetic data at saturating concentrations of La(S-THP)$(CF_3SO_3)_3$ according to a form of eq 4 for the transesterification of **1** at pH 9.0, 37 °C.

Table 1. Kinetic Data for the Transesterification of **1** and Cleavage of RNA by Lanthanide Complexes at 37 °C

complex	pK_a[a]	10^2k_2 (M^{-1} s^{-1})[a]	10^4k_{cat} (s^{-1})[b]	K (M^{-1})[b]
Eu(THED)$^{3+}$	7.0, 7.1[c]	7.2 (±0.5) 110 (±7)[c]	9.5 (±0.9)	62 (±9)
La(S-THP)$^{3+}$	8.1	6.3 (±0.3)	6.6 (±1.0)	130 (±24)
Eu(S-THP)$^{3+}$	7.4	1.4 (±0.2)	1.8 (±0.4)	68 (±9)
Lu(S-THP)$^{3+}$		0.088 (±0.007)[d]		

[a] From pH-rate profile for the transesterification of **1**, 10.0 mM buffer, 0.10 M $NaNO_3$. [b] From fit of kinetic data to eq 4 for the transesterification of **1** at pH 8.0, 9.0, and 8.5 (for complexes as listed) at high concentrations of lanthanide complex (>1.00 mM). [c] From pH-rate profile for cleavage of A_{12}−A_{18}, 5.0 mM buffer, 0.10 M $NaNO_3$. [d] From data at pH 7.5.

Saturation kinetics for the transesterification of **1** by La(S-THP)-$(CF_3SO_3)_3$, Eu(S-THP)$(CF_3SO_3)_3$ and Eu(THED)$(CF_3SO_3)_3$ were examined at alkaline pH where k_{obs} is independent of pH (pH = 9.0, 8.5, and 8.0, respectively). All complexes are present predominantly in deprotonated form as $Ln(OR)^{2+}$ or $Ln(OH)^{2+}$ and eq 3 with $K_a \gg [H^+]$ becomes eq 4. The

$$k_{obs} = k_{cat}K[Ln]_T/(1 + K[Ln]_T) \quad (4)$$

observed kinetics for the transesterification of **1** by these three complexes can be described by the preequilibrium formation of a reactive complex (eq 2). Data were fit to a linear form of eq 4 ($1/k_{obs}$ versus $1/[Ln]_T$, correlation coefficients >0.995) to determine values for K and k_{cat}. A typical example is shown in Figure 1. Values for K and k_{cat} are reported in Table 1. Similar saturation kinetics observed for the europium salt (Eu-$(CF_3SO_3)_3$) gave the following values for K and k_{cat}: 140 (±30) M^{-1}; 1.5 (±0.3) × 10^{-3} s^{-1}.

Transesterification of **1** showed a first-order dependence on complex concentration for all complexes in the concentration range 0.400−1.00 mM. For the La(S-THP)$(CF_3SO_3)_3$, Eu(S-THP)$(CF_3SO_3)_3$, and Eu(THED)$(CF_3SO_3)_3$ complexes, pseudo-first-order rate constants for the transesterification of **1** as a function of pH are shown in Figure 2. Under these conditions, $K[Ln]_T K_a/(K_a + [H^+]) \ll 1$ and eq 3 becomes eq 5. A

$$k_{obs} = k_2 K_a[Ln]_T/([H^+] + K_a) \qquad k_2 = k_{cat}K \quad (5)$$

computer-assisted fitting of the data gave K_a values of 7.9 (±0.8) × 10^{-9}, 4.0 (±0.5) × 10^{-8}, and 1.0 (±0.1) × 10^{-7} for the La(S-THP)$(CF_3SO_3)_3$, Eu(S-THP)$(CF_3SO_3)_3$, and Eu(THED)$(CF_3SO_3)_3$ complexes, respectively. Note that k_2 should be the product of K and k_{cat}. The values of k_2 from the fits were in

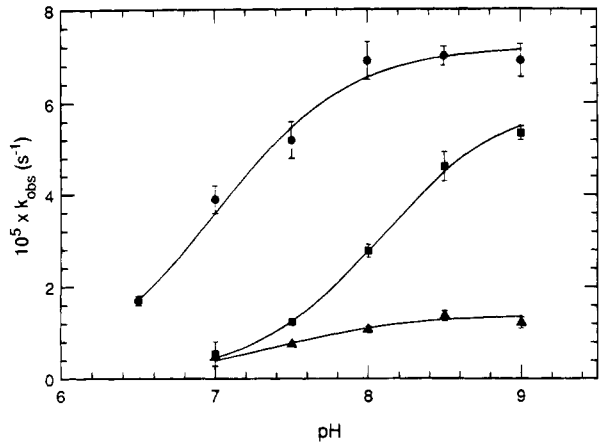

Figure 2. pH dependence of the pseudo-first-order rate constants for the transesterification of **1** by lanthanum(III) and europium(III) S-THP complexes and by the europium(III) complex of THED with 1.00 mM complex, 37 °C. Data are fit to a theoretical curve produced by eq 5 with the rate constants and K_as given in the text and in Table 1. Key: Eu(THED)$(CF_3SO_3)_3$ (●); Eu(S-THP)$(CF_3SO_3)_3$ (▲); La(S-THP)$(CF_3SO_3)_3$ (■).

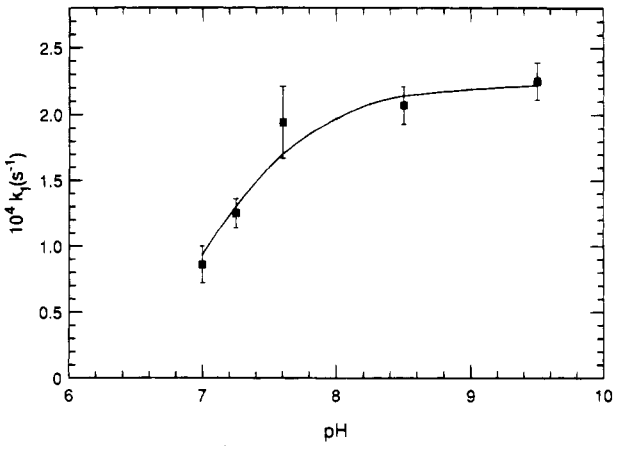

Figure 3. pH dependence of the pseudo-first-order rate constant for the cleavage of A_{12}–A_{18} by Eu(THED)$(CF_3SO_3)_3$ (0.200 mM), 37 °C. Data are fit to a theoretical curve produced by eq 5 with k_2 = 1.1 (±0.07) M^{-1} s^{-1} and $K_a = 7.9$ (±0.9) × 10^{-8}.

fact in good agreement with the product of K and k_{cat} as obtained from the saturation kinetic data at high pH. As a check, the data shown in Figure 2 was also fit to eq 3 with the formation constants calculated above and a $[Ln]_T$ concentration of 1.00 mM. This did not markedly change the parameters K_a or k_2 for any of the complexes. The pseudo-first-order rate constant for the transesterification of **1** at pH 7.50 by 1.00 mM Lu(S-THP)$^{3+}$ (Table 1) was much smaller than that for the other complexes and was independent of pH in the pH region 6.8 to 7.5. More extensive studies were not carried out with the lutetium complex because it was such a poor promoter. Rate constants (k_2) and pK_a values are listed in Table 1. For comparison, the second-order rate constant for transesterification by NaOH at 37 °C is 8.1 (±0.3) × 10^{-2} M^{-1} s^{-1} for concentrations of NaOH ranging from 0.2 to 1.0 mM and 0.10 M $NaNO_3$.

Pseudo-first-order rate constants for the cleavage of oligomers of adenylic acid (A_{12}–A_{18}) by the europium(III) THED complex and by the S-THP complexes of europium(III) and lanthanum(III) at 37 °C, pH 7.60, with 0.200 mM complex are 19 (±3) × 10^{-5} s^{-1}, 4.2 (±0.5) × 10^{-5} s^{-1}, and 7.1 (±0.8) × 10^{-5} s^{-1}, respectively. Cleavage of RNA by the lutetium(III) complex of S-THP was negligible under similar conditions over a period of 1 h. RNA cleavage products include 2′,3′-cyclic adenosine monophosphate, consistent with cleavage by transesterification. High salt concentrations (0.5 M NaCl) decreased the pseudo-first-order rate constant for cleavage by 0.200 mM Eu(THED)$^{3+}$ to 6.4 × 10^{-5} s^{-1}. For Eu(THED)$^{3+}$, the reaction is nearly first order in complex; decreasing the concentration of complex to 0.100 mM gave a pseudo-first-order rate constant of 1.1 × 10^{-4} s^{-1}. A pH–rate profile for the cleavage of A_{12}–A_{18} by Eu(THED)$^{3+}$ is shown in Figure 3. Least-squares fitting of the data in Figure 3 to the expression above (eq 5) gave $K_a = 7.9 \pm 0.9 \times 10^{-8}$ (pK_a = 7.1 ± 0.05) and k_2 = 1.1 (±0.07) M^{-1} s^{-1}.

Discussion

Designing a ligand that binds trivalent lanthanide ions strongly yet does not inhibit catalysis has proven to be a challenge. Commonly used anionic ligands with high formation constants for the trivalent lanthanides such as EDTA or NTA (nitrilotriacetate) form complexes that do not cleave RNA.[26] In addition, lanthanide complexes of linear chelates such as EDTA or DTPA are exchange labile and tend to dissociate readily in competition with other metal ions.[27] Accordingly, our efforts have focused on the design of macrocyclic lanthanide(III) complexes that are kinetically inert to lanthanide ion release.[12] The tetraazacyclododecane derivatives reported here are an attractive choice as ligands because of their ease of functionalization for attachment to biomolecules[28] and the large choice of pendent groups for modification of macrocycle properties.[29]

Potentiometric titration data may be accommodated by deprotonation of a lanthanide-bound water or, alternately, deprotonation of a lanthanide-bound hydroxyethyl or hydroxypropyl group. The pK_a values for water molecules bound to trivalent lanthanide ions are in the range of those reported here.[20] However, metal-bound hydroxyethyl groups are acidic, and we cannot rule out deprotonation of one of the bound hydroxyalkyl groups. Initially, we had wondered whether an hydroxyalkyl group of the complex might already be deprotonated at neutral pH. This was ruled out by the potentiometric data and by conductivity measurements that were consistent with the europium(III) complexes existing predominantly as Eu(THED)$^{3+}$ and Eu(THP)$^{3+}$ in water at pH 6.5. Generally, as one progresses from La^{3+} to Lu^{3+}, the Lewis acidity of the lanthanide ion increases.[19,20] Here, as expected, the pK_a values of the S-THP complexes increase in the following order: lutetium(III) < europium(III) < lanthanum(III). That Lu(S-THP)$(CF_3SO_3)_3$ has a second pK_a in the pH range of our titrations may be attributed to the greater Lewis acidity of the lutetium ion. This second pK_a may be due to deprotonation of a either a lutetium-bound hydroxypropyl or a lutetium-bound water.

The trends for transesterification of **1** by the lanthanide complexes are similar to those observed for cleavage of A_{12}–A_{18} (Table 1). Eu(THED)$(CF_3SO_3)_3$ is a better promoter than any of the S-THP complexes. To our surprise, the S-THP complexes of lanthanum(III) and europium(III) exhibited similar pseudo-first-order rate constants for transesterification of **1** and for RNA cleavage at near-neutral pH, although the pK_a values of the complexes, a measure of Lewis acidity, differ. In contrast, the lutetium(III) complex is inactive in promoting transesterification of **1** or cleavage of RNA oligomers. To investigate

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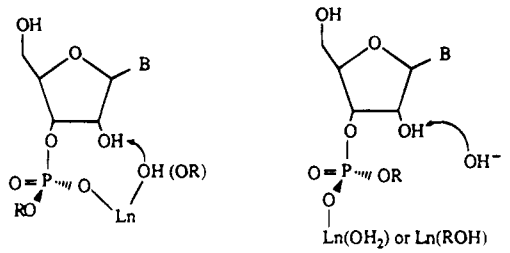
Scheme 1



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this further, the pH−rate profiles for transesterification of **1** by the complexes were studied.

The sigmoidal pH−rate profiles observed for Eu(THED)$(CF_3SO_3)_3$, La(S-THP)$(CF_3SO_3)_3$ or Eu(S-THP)$(CF_3SO_3)_3$ promoted transesterification of **1** and the Eu(THED)$(CF_3SO_3)_3$ promoted cleavage of $A_{12}-A_{18}$ support a mechanism where a lanthanide-bound hydroxide (Ln−OH) or a lanthanide-bound alkoxide (Ln−OR) complex is the active catalytic species and participates in catalysis presumably as a general base (Scheme 1i). Alternately, the kinetically equivalent mechanism has OH^- as a base and the acid form of the lanthanide complex as the catalyst (Scheme 1ii). An exact agreement between potentiometric measurements and the kinetic pK_a values is not observed. In general the kinetic pK_a value is 0.3−0.5 pH units less than the pK_a for the complexes measured by use of potentiometric titrations. However, experimental conditions used for kinetics experiments were slightly different than those for the potentiometric titrations. Most notably, kinetics experiments contained large concentrations of buffers which have been shown to modify the rate constants for metal ion catalyzed transesterification of **1**.[11]

For complexes of zinc(II) and copper(II), pH−rate profiles for RNA cleavage that are indicative of a metal-bound hydroxide as the active catalytic species have been reported.[31,32] It is unlikely that copper(II), zinc(II), or lanthanide(III) complexes act simply as general bases in promoting phosphate ester transesterification. Organic bases such as imidazole with a pK_a of approximately 7 promote RNA cleavage several orders of magnitude more slowly than do the metal complexes.[33] The apparent second-order rate constant for transesterification of **1** by hydroxide ($k_{OH} = 8.1 \times 10^{-2}$ M^{-1} s^{-1}) is similar in magnitude to the apparent second-order rate constant for transesterification of **1** by the lanthanide(III) complexes ($(1.4-7.2) \times 10^{-2}$ M^{-1} s^{-1}) even though the hydroxide ion is a stronger base than the complexes by many orders of magnitude. Thus, for metal complexes, catalysis by electrophilic activation of the phosphate ester must also be important. As Lewis acids, metal ions may act as catalysts by binding to the phosphate ester and making it more susceptible to nucleophilic attack by the hydroxyl group or by binding to the leaving group. Stabilization of a hypervalent phosphorus transition state or intermediate is another alternative.[11,34]

In the S-THP series, increasing Lewis acidity of the lanthanide(III) complexes (as indicated by pK_a) does not correlate to increasing efficiency of the complex in promoting transesterification. One might have anticipated that the lutetium(III) complex, as the best Lewis acid, would be the best promoter. However, the reverse order La(S-THP)$(CF_3SO_3)_3$ > Eu(S-THP)$(CF_3SO_3)_3$ > Lu(S-THP)$(CF_3SO_3)_3$ appears to hold, in accord with behavior of the lanthanide(III)-bound hydroxide or alkoxide complexes as general bases. One property that may differ between the lanthanide(III) complexes that may also account for the order observed is the coordination number of the complex and the corresponding number of available sites for catalysis. The solid state structure of [Eu(THP)(H_2O)][$CF_3SO_3]_3$·EtOH·$^1/_2H_2O$ has an octadentate THP ligand with one water bound to the $Eu(THP)^{3+}$ cation.[18] No structural data is available for the $Lu(S\text{-}THP)^{3+}$ or $La(S\text{-}THP)^{3+}$ cations. However, the solid state structure for a lanthanum(III) complex with a similar tetraazacyclododecane macrocycle containing pendent groups that form five-membered chelate rings ([La(TCMC)(CF_3SO_3)(EtOH)][$CF_3SO_3]_2$) features a ten-coordinate lanthanum cation with an octadentate TCMC ligand and two coordination sites not occupied by the macrocycle.[35] (TCMC is 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane). Hence, because of the larger size of the lanthanum(III) ion, it likely that the coordination number in the S-THP complex is greater than that for the analogous europium(III) or lutetium(III) complexes. This difference may be important for catalysis and may contribute to the larger k_2 observed for the lanthanum(III) complex.

How many coordination sites are necessary for catalysis? Two coordination sites are required for simultaneously binding an hydroxide and a phosphate ester as in pathway i (Scheme 1). However, as noted above, the europium(III) THP complex binds a single water molecule in the solid state. It has been proposed that for similar complexes with octadentate ligands such as $Eu(DOTA)^-$ or $Eu(DOTMA)^-$, a small percentage of the complexes may exist in solution with a dissociated acetate pendent group and an additional bound water molecule.[36,37] (DOTA is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracetic acid and DOTMA is α,α′,α″,α‴-tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracetic acid). Alternatively, if an hydroxyethyl or hydroxypropyl group in the lanthanide(III) S-THP or THED complexes is deprotonated, then one coordination site would be available for binding the phosphate ester and a deprotonated coordinated hydroxypropyl or hydroxyethyl group may participate as a general base catalyst.

For RNA cleavage and for transesterification of **1**, the Eu(THED)$(CF_3SO_3)_3$ complex is the best promoter. There is little difference between association constants for the formation of a reactive complex between **1** and the two europium(II) complexes: S-THP (68 ± 9 M^{-1}) and THED (62 ± 9 M^{-1}). (As usual, we do not know whether we have measured the association constant for the reactive complex or for a complex not in the reaction pathway). Association constants are similar to those of Eu^{3+} (140 ± 30 M^{-1}), La^{3+} (74 M^{-1})[11], and La(S-THP)$(CF_3SO_3)_3$ (130 ± 24 M^{-1}). Association constants for most divalent transition metal ions with phosphate diesters (except Cu^{2+} and Pb^{2+}) are smaller than these. For example, association constants for Mn^{2+} and a phosphate diester are 11 ± 3 M^{-1} and 20 ± 6 M^{-1}, as measured by kinetic methods and EPR titrations, respectively.[34] The major difference in the kinetics of transesterification of **1** by the europium(III) THED and S-THP complexes lies in the magnitude of k_{cat} (9.5×10^{-4} s^{-1} and 1.8×10^{-4} s^{-1}, respectively). From comparison of pK_a values, the europium(III) THED complex is a slightly better

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Lewis acid than the S-THP complex. Differences may also arise from the very rigid nature of the bound S-THP macrocycle.[18] That the S-THP complexes are all highly inert to dissociation has been attributed to the rigidity of the S-THP ligand. If further coordination sites are required for catalysis, a very rigid ligand may inhibit catalysis in this series of complexes.

In conclusion, lanthanide(III) complexes that promote phosphate ester transesterification and RNA cleavage have been constructed. Binding constants for **1** and trivalent lanthanide ions or for **1** and the lanthanide(III) complexes presented here are not unusually large compared to other metal cations.[11] For trivalent lanthanide cations, coordination numbers are high (8–12) and geometric requirements (as seen from the large number of different coordination geometries observed) are flexible.[19] This may be important if more than one coordination site is utilized to bind the phosphate ester or to stabilize a hypervalent phosphorus transition state or intermediate. More important, the large number of coordination sites of the lanthanide(III) ions makes it possible to design complexes that do not readily dissociate in water yet retain open coordination sites for catalysis. The lanthanide(III) ions of the THED and THP complexes are nearly encapsulated by the macrocyclic ligand, yet the complexes promote rapid RNA cleavage and phosphate ester transesterification.

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