Effect of Mixed Pendent Groups on the Solution and Catalytic Properties of Europium(III) Macrocyclic Complexes: Bifunctional and Monofunctional Amide and Alcohol Pendents in Septadentate or Octadentate Ligands

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Five new Eu(III) macrocyclic complexes have been prepared and their solution and catalytic properties studied. The Eu(III) complexes with septadentate ligands TRED and NB-TRED dissociate rapidly at pH 7.4, 37 °C (TRED = 1,4,7-tris(hydroxyethyl)-1,4,7,10-tetraazacyclododecane, NB-TRED = 1-(nitrobenzyl)-4,7,10-tris(hydroxyethyl)-1,4,7,10-tetraazacyclododecane). Dissociation rates as determined in the presence and absence of strongly binding competing ligands suggest that under most conditions the Eu(III) complexes of ATHC, ABHC, and CNPHC are more kinetically inert to dissociation than is the Eu(III) complex containing all hydroxyethyl groups (ATHC = 1-(carbamoylmethyl)-4,7,10-tris(hydroxyethyl)-1,4,7,10-tetraazacyclododecane, ABHC = 1,7-bis(carbamoylmethyl)-4,10-bis(hydroxyethyl)-1,4,7,10-tetraazacyclododecane, CNPHC = 1-(1-carboxamido-3-(4-nitrophenyl)propyl)-4,7,10-tris(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane). Laser-induced luminescence excitation spectra of Eu(III) complexes of ABHC, ATHC, CNPHC, THED, and *S*-THP suggest that there is a single major species in solution at pH 6.3 and a second species that appears at more basic pH values (THED = 1,4,7,10-tetrakis-(hydroxyethyl)-1,4,7,10-tetraazacyclododecane, *S*-THP = 1*S*,4*S*,7*S*,10*S*-tetrakis(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane). The species present at basic pH is proposed to be an alkoxide or hydroxide complex; pK_a values as determined by potentiometric titrations are 7.5 and 8.1 for Eu(CNPHC)$^{3+}$ and Eu(ABHC)$^{3+}$, respectively. Eu(CNPHC)$^{3+}$, Eu(ATHC)$^{3+}$, and Eu(ABHC)$^{3+}$ promote transesterification of the hydroxypropyl ester of 4-nitrophenyl phosphate with pseudo-first-order rate constants at pH 7.3, 37 °C, and 1.00 mM complex of 1.4×10^{-5}, 9.3×10^{-6}, and 1.0×10^{-6} s^{-1}, respectively. Both Eu(CNPHC)$^{3+}$and Eu(ABHC)$^{3+}$ promote attack of an hydroxyethyl group of the macrocycle on bis(4-nitrophenyl) phosphate with pseudo-first-order rate constants at pH 7.3, 37 °C, and 1.00 mM complex of 1.5×10^{-4} and 3.5×10^{-5} s^{-1}, respectively. In general, an increase in the number of amide groups on the macrocycle of the Eu(III) complex decreases the rate of both intramolecular or intermolecular phosphate diester transesterification reactions.

Introduction

Cyclen derivatives containing four neutral pendent groups are effective ligands for the trivalent lanthanides.[1−6] Many of the lanthanide(III) complexes of these neutral octadentate ligands are inert to lanthanide(III) ion release in water and are catalysts for RNA transesterification[7−9] or for cleavage of phosphate esters[10] or phosphoric anhydrides.[11] To date our studies on these complexes have focused on macrocycles that contain a single type of pendent group. However, in many applications it would be advantageous to have two different types of pendent groups. For example alkylation of cyclen with a pendent group that is bifunctional[12] is an effective route to prepare complexes for attachment to oligonucleotides. Mixing pendent groups with different properties has potential for fine-tuning the properties of the metal ion complex. Macrocyclic ligands with mixed pendent groups have been used effectively as ligands for Gd-(III) MRI agents.[13−16]

The focus of the study here was to prepare new lanthanide-(III) complexes of octadentate or septadentate neutral macrocycles that would have catalytic or solution properties superior to those reported previously or would be suitable for attachment to oligonucleotides. We have prepared macrocycles with mixed

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amide and hydroxyethyl pendent groups including a pendent group bearing a functionality for both lanthanide ion binding and covalent attachment to an oligonucleotide. Both septadentate and octadentate macrocycles have been prepared. The Eu(III) complexes of these macrocycles are prepared in order to exploit the luminescent properties of the Eu(III) ion for studying the solution chemistry and speciation of the complexes by laser-induced luminescence spectroscopy. In addition, stability constants are higher and dissociation rates are lower for the middle lanthanide ion complexes of octadentate cyclen ligands with neutral pendent groups.[17]

Materials and Methods

All reagents were of reagent grade and were used without further purification, unless otherwise noted. Millipore MILLI-Q purified water was used in all experiments. HPNP was prepared as reported previously.[18] $Eu(CF_3SO_3)_3$ was generated by treating Eu_2O_3 with concentrated trifluoromethanesulfonic acid.[19] Ethanol, dried over Mg turnings and distilled, was used for the syntheses of all Eu(III) complexes. All glassware used in the syntheses of the Eu(III) complexes was oven dried and cooled under N_2(g). The free base form of cyclen (1,4,7,10-tetraazacyclododecane) was generated by dissolution of the tetrahydrochloride salt (Parish Chemicals) or the dihydrogen sulfate salt (Aldrich) in water (1.5 g/8 mL) and adjustment of the pH to 12.5 with NaOH pellets. The aqueous solution was extracted with $CHCl_3$ (6 × 100 mL), and evaporation of the $CHCl_3$ yielded the free base form of cyclen (93% (Parish) or 85% (Aldrich) recovery). Amylene-stabilized $CHCl_3$ (Aldrich) was used in the synthesis of NBC and CNPC. All reactions were carried out under an N_2(g) atmosphere unless noted otherwise. TLC plates (Aldrich) were silica gel $60F_{254}$, 0.2 mm thickness. Merck grade 9385, 230−400 silica gel, 60 Å (Aldrich), was used in all chromatography, except for NB-TRED and CNPHC. Solvent systems for TLC and column chromatography were as follows: solvent system 1, $CHCl_3$/MeOH/concentrated NH_4OH (12:4:1); solvent system 2, $CHCl_3$/MeOH/concentrated NH_4OH (12:3:0.5); solvent system 3, CH_2Cl_2/MeOH (9:1); solvent system 4, $CHCl_3$/MeOH/concentrated NH_4OH (12:5:1). Diol-derivatized silica gel (J. T. Baker) was used in the purification of NB-TRED and CNPHC.

^{1}H NMR spectra were recorded on a Varian XL-400 spectrometer. ^{13}C NMR spectra were recorded on a Gemini-300 or 500 spectrometer. A VG 70-SE mass spectrometer with fast atom bombardment and chemical ionization was utilized. Orion research digital ion analyzer 510 equipped with a temperature compensation probe was used for all pH measurements. A Hewlett-Packard diode array 8452A spectrophotometer with a thermostated cell compartment was used for all UV−vis spectra and kinetic measurements.

Synthesis. 1-(4-Nitrobenzyl)-1,4,7,10-tetraazacyclododecane (NBC). A previously reported procedure was modified.[20,21] To a 25 mL round-bottom flask equipped with a magnetic stir bar and gas inlet tube was added cyclen (500 mg, 2.90 mmol), $CHCl_3$ (7 mL), and 4-nitrobenzyl bromide (419 mg 1.94 mmol). The reaction mixture became cloudy within 10 min and appeared to be complete as determined by TLC analysis (solvent system 1). The reaction was stirred for an additional 3.5 h and monitored by TLC. The volume was reduced in vacuo to 1 mL. This slurry was applied to a silica gel column (2.5 × 20 cm) and eluted with solvent system 1. Small fractions (1 mL) were collected and analyzed by TLC (trisalkylated product, $R_f = 0.72$; bisalkylated product, $R_f = 0.68$; NBC, $R_f = 0.56$). Fractions containing NBC were combined and the solvents removed in vacuo. The material was vacuum-dried at 50 °C overnight to give NBC (376 mg, 63%) as a yellow powder: ^{1}H NMR (400 MHz, CD_3CN) δ 2.2 (br, 3H, NH), 2.46 (t, 4H, cyclen $-CH_2-$), 2.51 (m, 4H, cyclen $-CH_2-$), 2.59 (t, 4H, cyclen $-CH_2-$), 2.69 (t, 4H, cyclen $-CH_2-$), 3.66 (s, 2H, $-CH_2$-Ar), 7.56 (d, 2H, Ar), 8.16 (d, 2H, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 44.93, 46.14, 46.97, 51.57 (cyclen $-CH_2-$), 58.74 ($-CH_2$Ar), 123.83, 129.64, 147.44 (Ar); FAB MS *m*/*e* 308.2 {NBC + H^+}. Anal. Calcd for $C_{15}H_{25}N_5O_2$: C, 58.61; H, 8.19; N, 22.78. Found: C, 58.39; H, 8.29; N, 23.06.

1-(4-Nitrobenzyl)-4,7,10-tris(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (NB-TRED). To a 250 mL round-bottom flask chilled in an ice bath was added NBC (250.5 mg, 0.815 mmol), H_2O (25 mL), and ethylene oxide (approximately 3.4 mL, 80 equiv). The reaction mixture, a pale yellow slurry, was stirred for 2 h. The ethylene oxide was removed in vacuo and the aqueous layer extracted with $CHCl_3$ (2 × 40 mL). A yellow solid was recovered after evaporation of $CHCl_3$, and it was applied to a column of diol silica gel (4.32 g silica gel, 1 cm diameter). The first yellow band was collected with $CHCl_3$ elution. Combined fractions were reduced to dryness and dissolved in minimal $CHCl_3$ (<1 mL), and Et_2O was added (5 mL) to induce precipitation. After chilling in the freezer, the solid was collected by suction filtration. The material was vacuum-dried at 40 °C to give NB-TRED (252.0 mg, 70%) as a yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.45 (t, 4H, cyclen $-CH_2-$), 2.50−2.64 (m, 12H, cyclen $-CH_2-$, N*C*$H_2$$CH_2$-OH), 3.63 (m, 6H, NCH_2C*H*$_2$OH), 5.2 (br, 3H, −OH), 7.58 (d, 2H, Ar), 8.19 (d, 2H, Ar); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 51.10, 51.74, 52.14, 52.38 (cyclen $-CH_2-$), 56.14, 56.80 (NCH_2CH_2OH), 58.74 ($-C$*H*$_2$Ar), 59.4, 59.60 (NCH_2C*H*$_2$OH), 123.58, 129.65, 146.49, 147.09 (Ar); FAB MS *m*/*e* 440.5 {NB-TRED + H^+}.

1,4,7-Tris(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (TRED). To a 50 mL two-necked round-bottom flask was added 10% Pd/C (267.8 mg), and MeOH (18 mL) was slowly added while N_2(g) was being flushed through the system. *Care must be taken to avoid flaming.* NB-TRED (299.7 mg, 0.682 mmol) and cyclohexene (5 m L) were added. The mixture was heated at reflux for 2 h. The Pd/C was removed by filtering the reaction mixture through a fine fritted glass funnel and was washed with MeOH (30 mL). The filtrate was evaporated to dryness in vacuo. To the residue was added H_2O (3 mL) and the pH adjusted to 12.5 by the addition of concentrated NH_4-OH. The aqueous layer was extracted with hexanes (3 × 10 mL) to remove the 4-aminotoluene. The pH was readjusted to 12.5 and the aqueous layer extracted with $CHCl_3$ (3 × 20 mL) to recover the TRED ligand. The combined $CHCl_3$ layers were reduced to dryness and the residue transferred with minimal $CHCl_3$ to a tared vial. The $CHCl_3$ was evaporated in a stream of N_2(g) and Et_2O (2−3 mL) added. A white precipitate formed and was allowed to settle in the freezer. The Et_2O was decanted, and the white solid was vacuum-dried at 40 °C to give TRED (145.8 mg, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.2 (br, 1H, NH), 2.45 (t, 4H, N$CH_2$$CH_2$OH), 2.5−2.7 (m, 18H, N$CH_2$$CH_2$OH and cyclen $-CH_2-$), 3.60 (t, 4H, NCH_2C*H*$_2$OH), 3.68 (t, 2H, NCH_2C*H*$_2$-OH), 4.9 (br, 1H, OH), 5.65 (br, 2H, OH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 44.83, 50.64, 51.49, 53.33 (cyclen $-CH_2-$), 56.24, 58.48, 58.56, 59.43 (NCH_2CH_2OH). FAB MS *m*/*e* 305.2 {TRED + H^+}, 327.2 {TRED + Na^+}.

1-(Carbamoylmethyl)-4,7,10-tris(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (ATHC). To a 25 mL round-bottom flask was added TRED (112.1 mg, 0.368 mmol), $CHCl_3$ (4 mL), Et_3N (0.104 mL, 0.746 mmol), and 2-bromoacetamide (90.2 mg, 0.654 mmol). The mixture was stirred at room temperature under a N_2(g) atmosphere and monitored by ^{1}H NMR ($CDCl_3$) spectroscopy. After 2 h, additional 2-bromoacetamide (30.4 mg) was added, since TRED signals were still present in the ^{1}H NMR spectrum. After 23 h the solvent was removed in vacuo. The residue was dissolved in H_2O (3 mL), and the pH was adjusted to 12.5 with concentrated NH_4OH. The aqueous layer was extracted with $CHCl_3$ (3 × 20 mL), and the combined $CHCl_3$ layers were reduced to dryness. Minimal $CHCl_3$ (approximately 1 mL) was added to the material recovered, and Et_2O (10 mL) was added. The precipitate was collected by suction filtration and further vacuum-dried at 40 °C to give ATHC (119.0 mg, 89.5%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49, 2.6 (two br, 22 H, cyclen $-CH_2-$ and N$CH_2$$CH_2$OH), 3.08 (s, 2H, NCH_2C(O)NH_2), 3.6 (series of t, 6H,

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NCH_2CH_2OH), 5.0−5.4 (br, 3H, OH), 5.4 (br, 1H, NH), 7.9 (br, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.06, 51.23, 51.29, 52.08 (cyclen $-CH_2-$), 54.82, 57.17 (NCH_2CH_2OH), 58.53, 58.66 (NCH_2CH_2OH), 60.36 ($NCH_2C(O)NH_2$), 173.8 (C(O)); FAB MS *m*/*e* 362.3, {ATHC + H^+} 384.3 {ATHC + Na^+}.

1,7-Ditosyl-1,4,7,10-tetraazacyclododecane (1,7-Ts_2-cyclen). A procedure reported previously was modified.[22,23] To a 50 mL round-bottom flask chilled in an ice bath was added pyridine (5 mL) and tosyl chloride (499.6 mg, 2.62 mmol). Cyclen (226 mg, 1.31 mmol) in pyridine (2.5 mL) was added to the flask over 5 min. The bright yellow mixture was stirred for 4 h, during which time a yellow precipitate formed. The pyridine was removed by vacuum distillation. Water (2 mL) was added to the yellow residue, and the slurry was stirred for 4 h. The yellow solid was collected by suction filtration and washed with water (10 mL), with a saturated sodium bicarbonate solution (7 mL), and finally with water (15 mL). The solid was vacuum-dried at 60−70 °C overnight to yield 1,7-Ts_2-cyclen as a yellow powder (526.7 mg, 84% yield). Before the material was used in the next step it was applied to a silica gel column and eluted with solvent system 1. TLC analysis was done on all fractions and those containing 1,7-Ts_2-cyclen (R_f = 0.84) were combined: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 6H, $-CH_3$), 2.62 (br, 2H, NH), 2.86 (t, 8H, cyclen $-CH_2-$), 3.18 (t, 8H, cyclen $-CH_2-$), 7.37 (d, 4H, Ar), 7.75 (d, 4H, Ar).

1,7-Ditosyl-4,10-bis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (Ts_2BHC). A procedure reported previously[22,23] was modified. To a 15 mL round-bottom flask chilled in an ice bath was added $LiClO_4$ (227 mg, 2.15 mmol), CH_3CN (1 mL), ethylene oxide (0.5 mL, 10.2 mmol), and 1,7-Ts_2-cyclen (498.9 mg, 1.04 mmol). The flask was stoppered and the yellow-orange mixture stirred in an ice bath for 4 h. The mixture was allowed to stir overnight at room temperature, and over this time period a precipitate formed. The solvent was removed in vacuo. The reaction mixture was applied to a 2.5 × 22 cm column of silica gel and eluted with solvent system 2. Fractions containing the product were combined and the solvent removed. The residue was dissolved in $CHCl_3$ (1 mL), and Et_2O (15 mL) was added. The yellow precipitate was collected over suction filtration. The solid was vacuum-dried overnight to yield Ts_2BHC as a yellow powder (367.4 mg, 62% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 6H, $-CH_3$), 2.52 (t, 4H, NCH_2CH_2OH), 2.87 (t, 8H, $-CH_2-$ cyclen), 3.19 (t, 8H, $-CH_2-$ cyclen), 3.69 (t, 4H, NCH_2CH_2OH), 4.82 (br, 2H, −OH), 7.33 (d, 4H, Ar), 7.67 (d, 4H, Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.42 ($-CH_3$), 50.34, 55.72, (cyclen $-CH_2-$), 56.98, (NCH_2CH_2OH), 59.20 (NCH_2CH_2OH), 127.76, 129.86, 133.94, 143.84 (Ar); FAB MS *m*/*e* 569.3 {Ts_2BHC + H^+}.

1,7-Bis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (BHC). To a 25 mL two-necked round-bottom flask was added Ts_2BHC (404 mg, 0.710 mmol), anhydrous Na_2HPO_4 (946.6 mg), 2% sodium amalgam (19.7 g), and MeOH that was stored over 3 Å sieves (12 mL). The mixture was heated at reflux and stirred under nitrogen for 20 h. During this time the mixture became white and fizzy and liquid mercury formed. The reaction mixture was allowed to cool and the mercury settle. The reaction mixture was decanted away from the mercury and dropped into stirring water (30 mL) to quench the reaction. The flask was rinsed with additional portions of MeOH (2 × 5 mL), and this was added to the water. The MeOH was removed in vacuo to avoid an emulsion in the extraction step. The aqueous layer was extracted with $CHCl_3$ (3 × 60 mL), and the combined $CHCl_3$ layers were reduced to dryness. The white residue was dissolved in minimal $CHCl_3$, and Et_2O (10 mL) was added. The solid was collected by suction filtration and vacuum-dried to give BHC as a white powder (114.4 mg, 62% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.52−2.80 (br and t, 20H, cyclen and NCH_2CH_2OH), 3.59 (t, 4H, NCH_2CH_2OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 45.38, 52.82 (cyclen $-CH_2-$), 57.36 (NCH_2CH_2OH), 59.56 (NCH_2CH_2OH); FAB MS *m*/*e* 261.3 {BHC + H^+}.

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1,7-Bis(carbamoylmethyl)-4,10-bis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (ABHC). To a 15 mL round-bottom flask was added BHC (101.8 mg, 0.391 mmol), $CHCl_3$ (4 mL), Et_3N (0.3 mL, 2.16 mmol), and 2-bromoacetamide (152.5 mg, 1.11 mmol). The reaction was stirred for 20 h and became cloudy. The $CHCl_3$ was removed in vacuo, and water (3 mL) was added. The pH of the aqueous layer was adjusted and maintained at 12−13 by the addition of concentrated NH_4OH during the $CHCl_3$ extractions (6 × 20 mL). The combined $CHCl_3$ layers were reduced to dryness, and to the residue was added $CHCl_3$ (1 mL) and Et_2O (10 mL). The solid was collected by suction filtration and vacuum-dried to give ABHC as a white powder (113.4 mg, 77.5% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.52−2.80 (br, 20H, cyclen and NCH_2CH_2OH), 3.10 (s, 4H, $CH_2C(O)NH_2$), 3.57 (br, 4H, NCH_2CH_2OH), 5.42 (br, 2H, −OH), 5.47 (br, 2H, NH), 8.10 (br, 2H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.71, 51.81 (cyclen $-CH_2-$), 54.44, (NCH_2CH_2OH), 58.14 (NCH_2CH_2OH), 60.64 ($-CH_2C(O)NH_2$), 173.9 (C(O)); FAB MS *m*/*e* 375.3 {A_2BHC + H^+}, 397.3 {A_2BHC + Na^+}.

2-Bromo-4-(4-nitrophenyl)butanamide (BNBA). To a 50 mL two-necked round-bottom flask equipped with a magnetic stir bar, reflux condenser, and gas inlet tube was added 4-(4-nitrophenyl)butanoic acid (1.5015 g, 7.18 mmol), CCl_4 (0.7 mL), and $SOCl_2$ (2.1 mL, 28.8 mmol). The reaction mixture was heated at reflux for 1 h. The reflux was halted, and to the clear yellow reaction mixture was added *N*-bromosuccinimide (1.5456 g, 8.68 mmol), CCl_4 (4 mL), and 48% HBr(aq) (2 small drops). The reaction mixture was heated at reflux for 40 min and turned from a bright orange to a dark green-brown. $SOCl_2$, CCl_4, and Br_2 were removed by vacuum distillation at room temperature. The green-brown oil and the succinimide produced during the bromination reaction remained in the flask. CH_2Cl_2 (15 mL) was placed in a two-necked round-bottom flask and chilled in an ice bath, and NH_3(g) was bubbled through the CH_2Cl_2 for 5 min. To the green-brown oil was added CH_2Cl_2 (5 mL), and this mixture was added to the CH_2Cl_2/NH_3 solution. The reaction mixture turned to a light blue-green slurry which was left to stand chilled in the ice bath for 1 h. The reaction mixture was filtered by suction filtration and washed with CH_2Cl_2 (20 mL). The solid collected in the funnel was very pale blue-green, and the solid recovered from the filtrate was yellow. The pale blue-green solid was applied as a slurry to a silica gel column (3.5 × 12 cm) and eluted with solvent system 3. All fractions were tested by TLC (BNBA, R_f = 0.90; 4-(4-nitrophenyl)butanamide, R_f = 0.83; succinimide, R_f = 0.66), and like fractions were combined. Most of the fractions contained succinimide in addition to BNBA. The yellow solid, recovered from the filtrate of the reaction mixture, was applied to a separate silica gel column (3.5 × 18 cm) as a slurry and eluted with solvent system 3. Fractions contained BNBA and 4-(4-nitrophenyl)butanamide were chromatographed again on a silica gel column (3.5 × 12 cm) using solvent system 3. Material that was still yellow was slurried in EtOAc/hexanes (1:1) and filtered. This process was repeated with the solid recovered from the filtrate until the material was white. All materials recovered from all columns that contained only succinimide and BNBA were placed in a large sublimator. Succinimide was removed from BNBA by sublimation in vacuo at 70−80 °C overnight. The unsublimed material was vacuum-dried overnight at 50 °C to give BNBA (1.2099 g, 58.7%) as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 (m, 1H, $-CHC(Br)-$), 2.48 (m, 1H, $-CHC(Br)-$), 2.93 (m, 2H, $-CH_2Ar$), 4.26 (m, 1H, $-CH(Br)-$), 5.72 (br, 1H, CONH), 6.36 (br, 1H, CONH), 7.39 (d, 2H, Ar), 8.17 (d, 2H, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 33.3 ($-CH_2Ar$), 36.8 ($-CH_2CH(Br)$), 49.2 ($-CH(Br)-$), 124.5, 130.0, 147.5, 148.4 (Ar), 171.0 ($CONH_2$); CI MS *m*/*e* 287 and 289 {BNBA + H^+}, approximately 1:1 intensity.

1-(1-Carboxamido-3-(4-nitrophenyl)propyl)-1,4,7,10-tetraazacyclododecane (CNPC). To a 100 mL flask equipped with a magnetic stir bar and gas inlet tube was added cyclen (1.0029 g, 5.82 mmol) and amylene-stabilized $CHCl_3$ (40 mL). The solution was warmed to 35 °C in an oil bath, and BNBA (491.1 mg, 1.71 mmol) was added. The temperature was increased to 45 °C, and after 25 min all materials dissolved. A second portion of BNBA (491.2 mg, 1.71 mmol) was added, the temperature was increased to 50 °C, and the mixture was stirred until all materials dissolved. The progress of the reaction was monitored by TLC (solvent system 4, BNBA, R_f = 0.91; bisalkylated

product, $R_f = 0.80$; CNPC, $R_f = 0.68$). After 2 h from the last addition of BNBA, the volume of the reaction was reduced to 20 mL in vacuo at room temperature. Material began to precipitate but redissolved upon warming to 50 °C. After 10 h the volume was reduced to 8 mL, and the reaction mixture was now a clear orange. After 25 h the volume was reduced to 6 mL, and the reaction was allowed to stir an additional 2.5 h at 50 °C. The volume was reduced to approximately 2 mL, and 0.5 mL of solvent system 4 was added. This was applied to a silica gel column (3.5 × 25 cm) and eluted with solvent system 4. All fractions were tested by TLC, and like fractions were combined. Those fractions that contained a mixture of the bis- and monoalkylated products were applied to a second column using the same conditions. All fractions that contained CNPC were combined, reduced to dryness, and dissolved to H_2O (8 mL). The pH of this solution was adjusted to 12.5 by addition of a concentrated NaOH solution. The cloudy yellow aqueous layer was extracted with $CHCl_3$ (6 × 50 mL), and the combined $CHCl_3$ layers were reduced to dryness in vacuo. The yellow solid recovered was vacuum-dried overnight at 35 °C to yield CNPC (0.8613 g, 66.6%) as a light orange solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.88 (m, 1H, $-CHCH_2Ar$), 2.23 (m, 1H, $-CHCH_2Ar$), 2.2−2.5 (br, 3H, NH amine), 2.5−2.9 (m, 17H, cyclen $-CH_2-$ and $-CHAr$), 3.04 (m, 1H, $-CHAr$), 3.25 (m, 1H, CHC(O)), 5.82 (br, 1H, CONH), 7.44 (d, 2H, Ar), 7.49 (br, 1H, CONH), 8.14 (d, 2H, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 27.6, 34.1 (amide arm), 45.7, 46.4, 47.5, 49.1 (cyclen $-CH_2-$), 62.7 (amide arm), 123.8, 129.5, 146.7, 150.1 (Ar), 176.2 ($CONH_2$); FAB MS *m*/*e* 379 {CNPC + H^+}.

1-(1-Carboxamido-3-(4-nitrophenyl)propyl)-4,7,10-tris-(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane (CNPHC). To a 100 mL round-bottom flask equipped with a stir bar and chilled in an ice bath was added CNPC (199.7 mg, 0.528 mmol), water (10 mL), and ethylene oxide (2 mL, 41 mmol). The flask was stoppered in air, and the clear yellow solution was stirred in an ice bath (T < 10 °C) for 2 h. The ethylene oxide was removed in vacuo, and a precipitate formed. The cloudy yellow aqueous layer was extracted with $CHCl_3$ (3 × 40 mL). The $CHCl_3$ layer was evaporated to dryness, and this material was applied to a column of diol-derivatized silica gel (2.84 g, 1 cm diameter). Pure CNPHC was collected in the first mobile yellow band with $CHCl_3$ elution. The first fraction was reduced to dryness and redissolved in a minimal amount of $CHCl_3$ (<1 mL), and Et_2O (4 mL) was added to induce precipitation. The precipitate was collected by suction filtration and vacuum-dried at 40 °C for 1 d to yield CNPHC (117.6 mg, 43%) as a pale yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.79 (quart, 1H, $-CHCH_2Ar$), 2.15 (m, 9H, cyclen $-CH_2-$ and $-CHCH_2Ar$), 2.27 (d, 3H, NCH_2CH_2OH), 2.63 (t, 3H, NCH_2CH_2OH), 2.73 (t, 5H, cyclen $-CH_2-$ and $-CHC(O)-$), 2.90 (quint 4H, cyclen $-CH_2-$), 3.22 (m, 2H, $-CH_2(Ar)$), 3.52 (t, 3H, NCH_2CH_2OH), 3.61, 3.72 (two t, 3H, NCH_2CH_2OH) 5.12 (br, 3H, −OH), 5.55 (s, 1H, CONH), 7.40 (d, 2H, Ar), 7.90 (s, 1H, CONH), 8.15 (d, 2H, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 25.22, 34.82 (amide arm), 46.75, 50.55, 51.56, 52.35 (cyclen), 54.95, 57.37, 58.55, 58.61 (NCH_2CH_2OH), 62.71 (amide arm), 123.76, 129.57, 146.66, 150.46 (Ar), 175.98 ($CONH_2$); FAB MS *m*/*e* 511 {CNPHC + H^+}. Anal. Calcd for $C_{24}H_{42}N_6O_6$: C, 56.46; H, 8.28; N, 16.46. Found: C, 56.20; H, 8.48; N, 16.40.

The lanthanide complexes [Eu(NB-TRED)]$(CF_3SO_3)_3$, [Eu(TRED)]$(CF_3SO_3)_3$, [Eu(ATHC)]$(CF_3SO_3)_3$, [Eu(ABHC)]$(CF_3SO_3)_3$, and [La(ABHC)]$(CF_3SO_3)_3$ were prepared by the following procedure. An example is given here for the preparation of [Eu(NB-TRED)]$(CF_3SO_3)_3$. To a 25 mL two-necked round-bottom flask was added $Eu(CF_3SO_3)_3$ (98.0 mg, 0.164 mmol) and NB-TRED (71.9 mg, 0.164 mmol). The flask was flushed with N_2(g), and dry ethanol (7 mL) was added by syringe. Precipitate formed within 15 min after ethanol was added, and the mixture was stirred at room temperature for 15 h. The volume was reduced to 1−2 mL, 2 mL of 2-propanol was added, and the mixture was chilled in the freezer. The 2-propanol was decanted, and the solid was vacuum-dried at 50 °C overnight. The complex was recovered as a white powder (82.9 mg, 48.8%). FAB MS (*m*/*e*) for [Eu(1-NB-TrHED)]$(CF_3SO_3)_3$: 892.1, 890.1 {[Eu(1-NB-TrHED](CF$_3$SO$_3$)$_2^+$}, 741.6, 739.6 {[Eu(1-NB-TrHED](CF$_3$SO$_3$)$^{2+}$ − H^+}, 590.3, 588.3{[Eu(1-NB-TrHED)$^{3+}$ − 2H^+}.

[Eu(TRED)]$(CF_3SO_3)_3$. The complex was recovered as a white powder (74.7 mg, yield 35%): FAB MS *m*/*e* 755.0 {[Eu(TRED)](CF$_3$SO$_3$)$_2^+$}, 606.0, 604.0 {[Eu(TRED](CF$_3$SO$_3$)$^{2+}$ − H^+}, 455.1, 453 {[Eu(TRED)$^{3+}$ − 2H^+}. Anal. Calcd for $C_{21}H_{41}N_4O_{13}F_9S_3Eu$: C, 22.06; H, 3.57; N, 6.20. Found: C, 22.80; H, 3.37; N, 6.25.

[Eu(ATHC)]$(CF_3SO_3)_3$. The complex was recovered as a white powder (176.3 mg, yield 66.3%): FAB MS *m*/*e* 663.5, 661.5 {[Eu(ATHC](CF$_3$SO$_3$)$^{2+}$ − H^+}, 512.3, 510.3 {[Eu(ATHC)]$^{3+}$ − 2H^+}, 362.3 {ATHC + H^+}. Anal. Calcd for $C_{21}H_{41}N_5O_{14}F_9S_3Eu$: C, 25.06; H, 4.10; N, 6.96. Found: C, 25.03; H, 4.24; N, 6.93.

[Eu(ABHC)]$(CF_3SO_3)_3$. The complex was recovered as a white powder (178.7 mg, yield 85.7%): FAB MS *m*/*e* 825.9, 823.9 {[Eu(ABHC)](CF$_3$SO$_3$)$_2^+$}, 676.5, 674.5 {[Eu(A_2BHC)](CF$_3$SO$_3$)$^+$ − H^+}, 525.3, 523.3 {[Eu(A_2BHC)$^{3+}$ − 2H^+}. Anal. Calcd for $C_{21}H_{40}N_6O_{14}F_9S_3Eu$: C, 24.73; H, 3.95; N, 8.24. Found: C, 24.83; H, 3.99; N, 7.81.

[La(ABHC)]$(CF_3SO_3)_3$. The complex was recovered as a white powder (163 mg, yield 68.4%): FAB MS *m*/*e* 810.89 {[La(A_2BHC)](CF$_3$SO$_3$)$_2^+$}, 661.20 {[La(A_2BHC)](CF$_3$SO$_3$)$^+$ − H^+}, 511.20 {[LaA_2BHC)$^{3+}$ − 2H^+}, 375.32 {A_2BHC + H^+}.

[Eu(CNPHC)]$(CF_3SO_3)_3$. To a two-necked round-bottom flask equipped with a stir bar and gas inlet tube was added CNPHC (42.8 mg, 0.0838 mmol) and $Eu(CF_3SO_3)_3$ (50.2 mg, 0.0838 mmol). The flask was flushed with N_2(g), and dry EtOH (13 mL) was added by syringe. The reaction mixture was stirred at room temperature. Progress of the reaction was monitored by ^{1}H NMR (D_2O) spectroscopy, and the disappearance of the ligand phenyl resonances was followed. After 23 h, the solvent was removed in vacuo. The residue was dissolved in CH_3CN (1 mL), and Et_2O was added until cloudiness was observed. The mixture was chilled in the freezer, and solid formed on the bottom of the flask. The Et_2O was decanted, and the residue was vacuum-dried. The complex (59.5 mg, 64.0%) was recovered as a white powder: FAB MS *m*/*e* 813.2, 811.2 {[Eu(CNPHC)](CF$_3$SO$_3$)$^{2+}$ − H^+}, 662.0, 660.0 {[Eu(CNPHC)]$^{3+}$ − 2H^+}, 511.9 {CNPHC − H^+}. Anal. Calcd for $C_{27}H_{42}F_9N_6O_{15}S_3Eu$: C, 29.22; H, 3.81; N, 7.57. Found: C, 28.97; H, 3.63; N, 7.38.

[La(CNPHC)]$(CF_3SO_3)_3$. To a 25 mL two-necked round-bottom flask was added $La(CF_3SO_3)_3$ (41.2 mg, 0.070 mmol) and CNPHC (35.9 mg, 0.070 mmol). The flask was flushed with N_2(g), and dry EtOH (6 mL) was added by syringe. The reaction mixture was stirred at room temperature for 20 h. The solvent was removed in vacuo. The residue was dissolved in CH_3CN (1 mL), and Et_2O was added until cloudiness was observed. The mixture was chilled in the freezer, and solid formed on the bottom of the flask. The Et_2O was decanted, the residue was vacuum-dried, and the complex (52.1 mg, 67.6%) was recovered as a pale yellow powder: FAB MS *m*/*e* 797.0 {[La(CNPHC)](CF$_3$SO$_3$)$^{2+}$ − H^+}, 647.2 {[La(CNPHC)$^{3+}$ − 2H^+}, 511.3 511.3{CNPHC + H^+}.

[Nd(CNPHC)]$(CF_3SO_3)_3$. This complex was prepared as described above for the La(III) derivative. [Nd(CNPHC)]$(CF_3SO_3)_3$ (59.5 mg, 64.0%) was recovered as a white powder: FAB MS *m*/*e* 800.8, 801.8, 802.8, 803.8, 804.8 {[Nd(CNPHC)](CF$_3$SO$_3$)$^{2+}$ − H^+}, 650.6, 651.6, 652.6, 653.6, 654.6 {[Nd(CNPHC)$^{3+}$ − 2H^+}, {CNPHC + H^+}, 511.6.

Potentiometric Titrations. Potentiometric titrations were carried out under an atmosphere of nitrogen. Solutions were 1.00 mM in Eu(macrocycle)$^{3+}$ (ca. 0.025 mmol triflate salt of Eu(III) complex) with 0.1 M NaCl added to maintain constant ionic strength. The pH, initially around 7.0, was adjusted to below 3.0 by the addition of 0.2 M HCl (approximately 100 μL). The Eu(macrocycle)$^{3+}$ solution was titrated with 0.1 M NaOH (Baker Chemicals "Dilute it" ampule).

Kinetics. The rate of dissociation of Eu^{3+} from the Eu(III) ATHC and ABHC macrocyclic complexes at 37 °C in the presence of Cu^{2+} was monitored by following the increase in absorbance at 312 nm. Beer's law plots were with varying concentration of the Cu(macrocycle)$^{2+}$ complexes (0.04−0.12 mM) and the following extinction coefficients (M^{-1} cm^{-1}) were determined at pH = 6.0 at 312 nm: 6700, [Cu(ATHC)]$^{2+}$; 5900 [Cu(ABHC)]$^{2+}$. Solutions for the experiments to monitor the rate of dissociation of Eu^{3+} contained 0.1 mM Eu(III) complex, 1.0 mM Cu^{2+}, and 10 mM Mes buffer, pH = 6.0 at 37 °C. The method of initial rates was employed. To check that the Cu(macrocycle)$^{2+}$ complexes would form completely in competition with Eu(III), the absorbance at 312 nm of solutions containing 0.10 mM macrocycle, 0.10 mM Eu^{3+}, and 1.0 mM Cu^{2+} was monitored.

Under these conditions, formation of the [Cu(macrocycle)]$^{2+}$ complexes were complete within a few minutes.

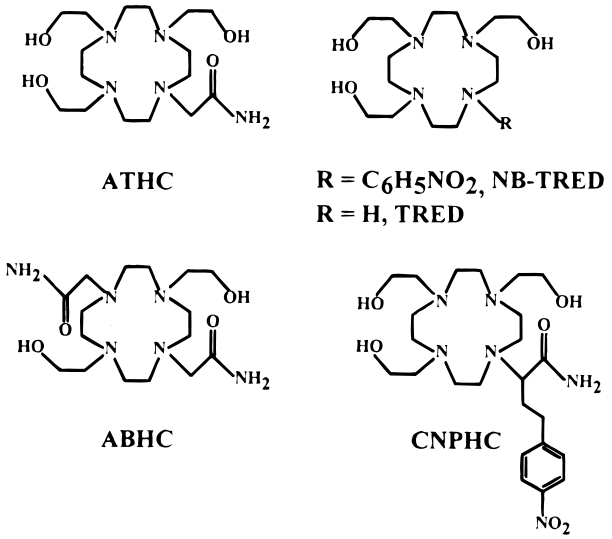
The rates of transesterification of HPNP by the Eu(III) complexes of CNPHC, ATHC, and ABHC were measured spectrophotometrically by following the increase in absorbance at 400 nm due to the production of 4-nitrophenolate. The reaction was initiated by injection of a stock solution of HPNP (3 μL) into 2 mL of a reaction solution buffered to pH = 7.3 with 10 mM HEPES (Sigma) and containing 0.10 M $NaNO_3$, 1.0 mM [Eu(macrocycle)]$(CF_3SO_3)_3$, and 0.10 mM EDTA maintained at 37 °C. Experiments were run in the presence of 0.10 mM EDTA to trap any free Eu(III) ion and to rule out catalysis by free metal ion. The concentration of HPNP in the reaction solution was determined at the end of each run by total hydrolysis in concentrated NaOH (3 M). Plots of absorbance versus time were converted to concentration units (ϵ = 18500) and divided by the concentration of HPNP to obtain first-order rate constants. The pK_a value for 4-nitrophenol at 37 °C is 6.90, and this value was used to correct for the degree of 4-nitrophenolate ionized at pH = 7.3. The first-order rate constant of HPNP transesterification under these conditions but in the absence of Eu(macrocycle)$^{3+}$ was measured to be 1.6×10^{-7} s^{-1}. This value was subtracted from the pseudo-first-order rate constant for the metal complex catalyzed reaction where not negligible.

Initial rates experiments for the hydrolysis of BNPP by the Eu(III) complexes of CNPHC and ABHC were measured spectrophotometrically by following the increase in absorbance at 400 nm due to the production of 4-nitrophenolate. The reaction mixture (total volume 2.0 mL) contained 1.0 mM Eu(III) complex, 0.1 mM EDTA, 0.1 M NaCl, 10 mM HEPES (pH = 7.4) or 10 mM EPPS (pH = 8.0), and 0.11 mM BNPP. Plots of absorbance versus time were converted to concentration units (ϵ = 18 500) and divided by the concentration of BNPP to obtain first-order rate constants. The pK_a value for 4-nitrophenol at 37 °C is 6.90, and this value was used to correct for the degree of 4-nitrophenolate ionized at pH = 7.3 and pH = 8.0. Rate constants were determined for both complexes at pH = 7.3 and at 8.0 for Eu(ABHC)$^{3+}$ by the method of initial rates. The cleavage of BNPP by Eu(CNPHC)$^{3+}$ at pH 7.3 was also monitored under the same conditions for over 3 half-lives and the rate constant calculated by a plot of time vs $\ln[(A - A_t)/(A - A_0)]$. Control experiments run in the absence of metal complex showed no measurable hydrolysis of BNPP over the time period that the metal complex promoted reactions were measured.

Reactions monitored by ^{31}P NMR contained 10 mM HPNP, 10 mM Eu(ABHC)$^{3+}$ or Eu(CNPHC)$^{3+}$, 122 mM HEPES (pH = 7.4, 37 °C), 0.1 M $NaNO_3$, 1.0 mM EDTA, and 20% D_2O (v/v) (total volume = 600 μL). For BNPP hydrolysis, the reactions contained 10 mM BNPP, 10 mM La(ABHC)$^{3+}$ or La(CNPHC)$^{3+}$, 136 mM buffer, 0.1 M NaCl, and 20% D_2O (v/v) (total volume = 600 μL). HEPES (pH = 7.4, 37 °C) was used in the reaction of La(CNPHC)$^{3+}$ with BNPP, and EPPS (pH = 8.0, 37 °C) was used in the reaction of La(ABHC)$^{3+}$ with BNPP. As the reactions progressed, the BNPP resonance decreased and new resonances increased in intensity at −5.5 and −6.2 ppm for the reaction with La(ABHC)$^{3+}$ and at −5.0 and −5.2 ppm for La(CNPHC)$^{3+}$.

Luminescence Spectroscopy. Instrumentation and experimental conditions for laser-induced luminescence studies were similar to those reported previously.[5,24] The dissociation of the Eu(III) complexes (0.2 mM Eu(macrocycle)$^{3+}$) was studied in water at 37 °C with solutions containing 20 mM HEPES, pH = 7.3 at 37 °C and 0.1 M NaCl, with and without 1.0 mM competing ligand, either DTPA or EDTA. Samples were incubated at 37 °C and frozen at specified time intervals. The percent dissociation of the complexes was determined by monitoring the appearance of the peak for the Eu(III) complex of the competing ligand as a function of time. For samples incubated without competing ligand, DTPA or EDTA was added just before the spectra were collected to determine the amount of dissociated Eu(III) ion. The number of water molecules bound to the Eu(III) in the various complexes was determined by luminescence lifetime measurements in H_2O and D_2O in the pH range 6.39−8.59. Energy transfer experiments were carried out in 20 mM buffer and 0.1 M NaCl with 10 μM Eu(CNPHC)$^{3+}$ and 20−100 μM Nd(CNPHC)$^{3+}$.

Chart 1



Results

Macrocycle Synthesis. Five new macrocyclic ligands were prepared (Chart 1). Three of the ligands are octadentate ligands for Eu(III) and contain mixed amide and hydroxyethyl pendent groups. Two additional ligands are heptadentate ligands for Eu(III) and have three hydroxyethyl groups. The CNPHC and NB-TRED ligands contain a 4-nitrobenzyl group. Conversion of the 4-nitrobenzyl group to an isothiocyanate group is useful for the attachment of lanthanide(III) complexes to oligonucleotides.[25,26]

The key steps in the syntheses of these macrocycles were the selective monoalkylation and dialkylation of cyclen (Schemes 1−3). The first step in the syntheses of ATHC, CNPHC, NB-TRED, and TRED was monoalkylation of the cyclen macrocycle which was accomplished by using a protocol similar to that developed by Kruper and co-workers.[20,21] The ATHC, NB-TRED, and TRED ligands were prepared from 4-nitrobenzyl-1,4,7,11-tetraazacyclododecane (NBC) as shown in Scheme 1. The NBC macrocycle was treated with ethylene oxide and was chromatographed on a diol-derivatized silica gel to purify the NB-TRED macrocycle from products containing partially alkylated macrocycle. Removal of the 4-nitrobenzyl group from NB-TRED was accomplished by using transfer hydrogenation.[27] The remaining site on TRED was alkylated with 2-bromoacetamide in the presence of Et_3N in $CHCl_3$ to give ATHC. ATHC was not readily prepared by direct monoalkylation of cyclen with bromoacetamide due to the insolubility of the amide macrocycle in most solvents.

The new pendent group BNBA was prepared from 2-bromo-4-(4-nitrophenyl)butanoyl chloride,[20,21] followed by conversion of the acid chloride to the amide by reaction with ammonia. From this reaction, pure BNBA was recovered after chromatography and sublimation of the succinimide produced in the bromination. The alkylation of cyclen with BNBA (Scheme 2) was carried out using conditions similar to those described for the preparation of NBC except that, due to solubility

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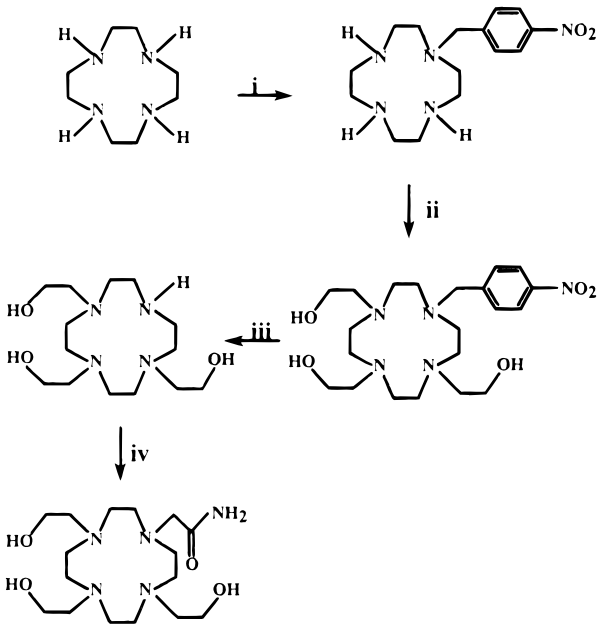
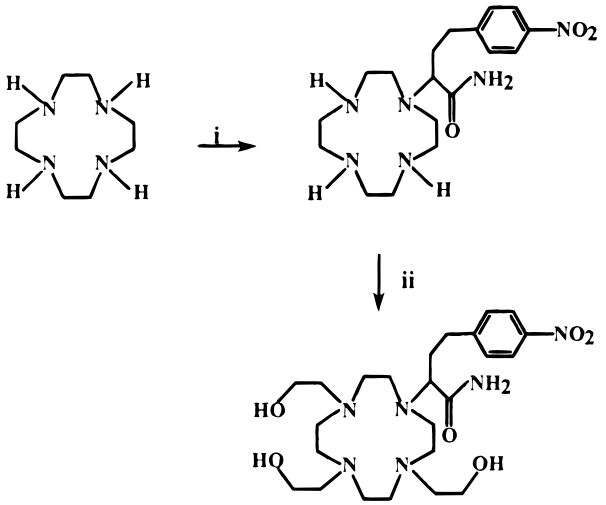
Scheme 1



i. 4-nitrobenzylbromide ii. ethylene oxide
iii. 10% Pd/C, cyclohexene
iv. 2-bromoacetamide /triethylamine.

Scheme 2



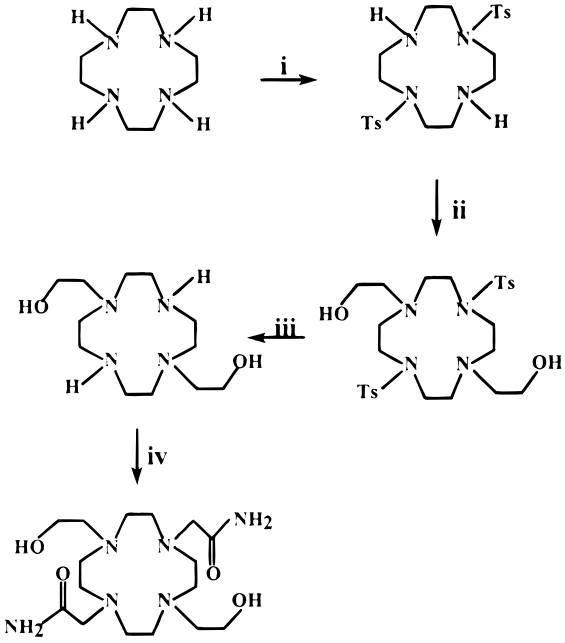
i. 2-bromo-4-(4-nitrophenyl)butanamide
ii. ethylene oxide

limitations, a larger volume of solvent was employed. This increased dilution slowed the reaction, and the solutions had to be heated and the volume reduced as the reaction progressed. This macrocycle was treated with ethylene oxide, and the CNPHC macrocycle was purified by column chromatography.

The initial steps in the synthesis of ABHC (Scheme 3) were carried out according to the procedure by Desreux and coworkers,[22,23] and the product was purified by column chromatography. Removal of the protecting groups[28] followed by treatment with 2-bromoacetamide produced the ABHC ligand.

Solution Properties of Complexes. The kinetics of dissociation of the Eu(III) complexes of ATHC and ABHC were studied at 37 °C and pH = 6.0 in the presence of a 10-fold excess of Cu^{2+} as a trapping agent. The absorbance at 312 nm for $Cu(ATHC)^{2+}$ and $Cu(ABHC)^{2+}$ was monitored by UV−vis spectroscopy. For $Eu(ATHC)^{3+}$ and $Eu(ABHC)^{3+}$, the first-order rate constants were $6.2(\pm1.0) \times 10^{-7}$ s^{-1} (half-life of 13 d) and $5.8(\pm0.30) \times 10^{-7}$ s^{-1} (half-life of 14 d), respectively. Dissociation was independent of Cu^{2+} concentration (0.5−1.0 mM), consistent with a rate-determining dissociation process that is not assisted by Cu^{2+}.[24] For $Eu(TRED)^{3+}$ (0.1 mM) at pH = 6.0, 37 °C, nearly complete dissociation was observed after 20 h in the presence of Cu^{2+} (1.0 mM). Laser-induced luminescence spectroscopy was used to monitor the decomposition of the various mixed-pendent group macrocyclic Eu(III) complexes while in the presence or absence of competing ligands including DTPA and EDTA (DTPA = diethylenetriaminepentaacetic acid and EDTA = ethylenediaminetetraacetic acid). In this study the percent dissociation was measured by monitoring the excitation peaks for the complexes of interest and the excitation peaks for the DTPA or EDTA complexes of Eu(III) which increased over time. For $Eu(CNPHC)^{3+}$, $Eu(ATHC)^{3+}$, and $Eu(ABHC)^{3+}$, no dissociation was observed after 3 d when these complexes (0.20 mM Eu(III) complex) were incubated at 37 °C, 20 mM HEPES, pH = 7.3, and 0.1 NaCl in the absence of competing ligand. For $Eu(THED)^{3+}$, 18% dissociation was observed after 3 d under the same conditions. Dissociation of all complexes was more rapid in the presence of EDTA or DTPA under otherwise similar conditions. In the presence of a 20-fold excess of DTPA, $Eu(CNPHC)^{3+}$ remained the most intact, with 41% dissociation after 3 d. $Eu(THED)^{3+}$ and $Eu(ATHC)^{3+}$ were both 80% dissociated, and $Eu(ABHC)^{3+}$ was 57% dissociated after 3 d. $Eu(THED)^{3+}$ was 30% dissociated while $Eu(CNPHC)^{3+}$, $Eu(ABHC)^{3+}$, and $Eu(ATHC)^{3+}$ were 59%, 73%, and 81% dissociated, respectively, after 3 d in the presence of a 20-fold excess of EDTA (Supporting Information Tables S6−S9). The $Eu(TRED)^{3+}$ and $Eu(NB\text{-}TRED)^{3+}$ complexes dissociated rapidly in the absence of competing ligand and were nearly

Scheme 3



i. tosyl chloride/pyridine
ii. ethylene oxide/$LiClO_4$/acetonitrile
iii. sodium amalgam/NaH_2PO_4/methanol
iv. 2-bromoacetamide

(28) Sessler, J. L.; Silbert, J. W.; Burrell, A. K.; Lynch, V.; Markert, J. T.; Wooten, C. L. *Inorg. Chem.* **1993**, *32*, 4277.

Table 1. Laser-Induced Luminescence Data for Eu(III) Macrocyclic Complexes

complex	pH	excitation band (nm)	q^a
Eu(ABHC)$^{3+}$	6.39	579.73, 580.04	1.14
Eu(ABHC)$^{3+}$	8.19	578.36, 579.72, 580.03	1.44
Eu(ATHC)$^{3+}$	6.39	579.61	1.23
Eu(ATHC)$^{3+}$	8.19	578.04, 579.64	1.70
Eu(CNPHC)$^{3+}$	6.39	579.64	1.23
Eu(CNPHC)$^{3+}$	8.19	578.12, 579.62	1.59
Eu(THED)$^{3+}$	6.33	579.60	1.19
Eu(THED)$^{3+}$	8.60	577.95	1.83
Eu(*S*-THP)$^{3+}$	6.33	579.32	1.18
Eu(*S*-THP)$^{3+}$	8.60	579.32, 577.51	2.19

[a] The number of coordinated water molecules, *q*, is calculated with the assumption that each hydroxyethyl group contributes one O−H oscillator and *q* is adjusted for a contribution from each N−H amide oscillator as described previously[29] and in text (eq 1).

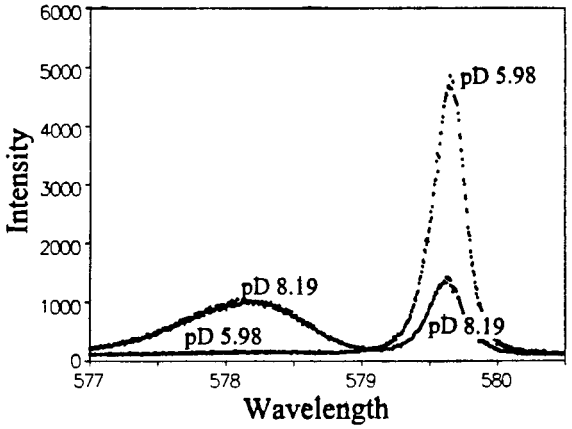


Figure 1. $^7F_0 \rightarrow {}^5D_0$ excitation spectra (λ_{em} = 614 nm) for a 0.01 mM solution of Eu(CNPHC)$^{3+}$ at pD 5.98 and pD 8.19, 20 mM buffer, and 0.1 M NaCl.

completely dissociated in minutes when in the presence of EDTA or DTPA.

Laser-induced excitation luminescence spectroscopy was used to study the solution chemistry of the Eu(III) complexes of CNPHC, ATHC, ABHC, THED, and *S*-THP. All complexes except Eu(ABHC)$^{3+}$ had a single excitation peak ($^7F_0 \rightarrow {}^5D_0$) at pH 6.33 (Table 1, Figure 1, Supporting Information Figures S1−S4). The excitation spectrum of Eu(ABHC)$^{3+}$ showed a major peak at 579.73 nm and a minor peak at 580.04 nm. As the pH was increased, a higher energy peak appeared for all complexes. The intensity of the peak increased with a concomitant decrease in the intensity of the original peak present at low pH values (Figure 1 and Supporting Information).

Luminescence lifetime measurements in H_2O and D_2O were carried out to determine the number of coordinated water molecules[24] for Eu(CNPHC)$^{3+}$, Eu(ATHC)$^{3+}$, Eu(ABHC)$^{3+}$, and, for comparison, Eu(THED)$^{3+}$ and Eu(S-THP)$^{3+}$. Although there were two excitation peaks in the spectra of all complexes at neutral to basic pH, a single luminescence lifetime was observed consistent with rapid exchange of the two species in solution. The contribution of N−H and O−H oscillators in close proximity to Eu(III) to the nonradiative deexcitation of the Eu(III) excited state has been described.[24,29] By taking each contribution into account, one can estimate the number of water molecules (*q*) bound to Eu(III). Each hydroxyethyl group will contribute one O−H oscillator worth 0.45 s^{-1}, and each N−H oscillator of the amide groups will contribute a smaller amount

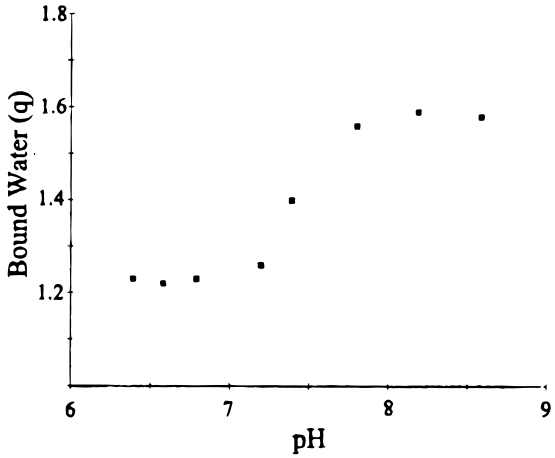


Figure 2. pH dependence of *q*, the number of bound water molecules, for Eu(CNPHC)$^{3+}$.

(0.053 s^{-1}).[29] This is expressed in eq 1, where *a* is the number

$$q = 1.05(\tau^{-1}(H_2O) - \tau^{-1}(D_2O) - 0.45a - 0.053b) \quad (1)$$

of alcohol OH bonds, *b* is the number of amide NH bonds, and τ^{-1} is the reciprocal excited-state lifetime in ms^{-1}. The major remaining contribution is from O−H oscillators of bound water molecules. Our *q* values (Table 1 and Supporting Information Tables S1−S4) also contain an unknown contribution from unbound closely diffusing water molecules. The value of *q* increases with increasing pH (Table 1 and Supporting Information Tables S1−S4) for all complexes. From a plot of *q* for Eu(CNPHC)$^{3+}$, Eu(ATHC)$^{3+}$, or Eu(ABHC)$^{3+}$ as a function of pH, the midpoint for this change is approximately 7.4, 7.4, and 7.9 for the three complexes, respectively (Figure 2). These values are in reasonable agreement with those determined by use of potentiometric titrations. The pK_a values as determined by potentiometric titration are 7.5, 7.8, 7.5, and 8.1 for Eu(THED)$^{3+}$, Eu(*S*-THP)$^{3+}$,[7] Eu(CNPHC)$^{3+}$, and Eu(ABHC)$^{3+}$, respectively.

Intermolecular energy transfer experiments were carried out with the Eu(CNPHC)$^{3+}$ complex (10 μM) with 25−100 μM Nd(CNPHC)$^{3+}$ added as an energy acceptor. These experiments were carried out to determine whether there was appreciable self-association of hydroxalkyl Eu(III) complexes in solution.[30,31] In this experiment, dynamic or collisional quenching is distinguished from static quenching (donor−acceptor association) by comparing lifetime quenching and intensity quenching data. Such donor−acceptor association in solution will affect luminescence intensity but not luminescence lifetime. Quenching by association is more effective in deexcitation of the donor than is dynamic quenching, and this may lead to large differences in Stern−Volmer quenching constants ($K_{SV}^{\phi} \gg K_{SV}^{\tau}$). The Stern−Volmer intensity quenching constant (K_{SV}^{ϕ}) was obtained as the slope of a plot of the luminescence emission intensity of Eu(CNPHC)$^{3+}$ as a function of concentration of Nd(CNPHC)$^{3+}$ quencher (eq 2, Supporting Information Figures

$$(I_0 - I)/I = K_{SV}[Q] \quad (2)$$

S5−S8).[30,31] The Stern−Volmer lifetime quenching constant (K_{SV}^{τ}) was obtained from plots of luminescence lifetime as a function of Nd(CNPHC)$^{3+}$ quencher concentration. Stern−

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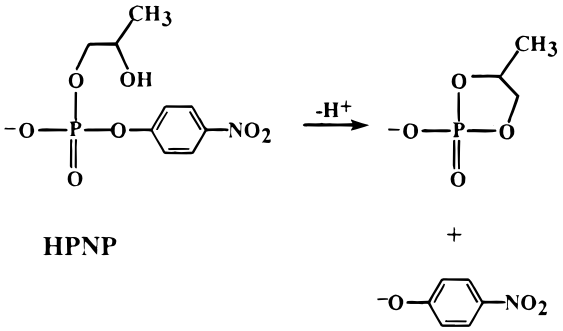
Scheme 4

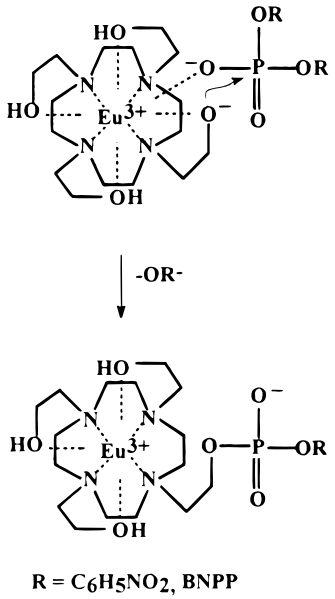


Volmer constants of $K_{SV}^{\phi} = 600$ and 910 M^{-1} and $K_{SV}^{\tau} = 240$ and 210 M^{-1} were determined at pH 7.3 and 8.6, respectively. From these data formation constants of 1200 and 350 M^{-1} at pH 7.30 and 8.60, respectively, were calculated.[32] Thus under these conditions, self-association of $Eu(CNPHC)^{3+}$ (5.2 and 2.2% association at pH 7.30 and 8.60) is not substantial at either pH value.

Transesterification. The rate of transesterification of the RNA model compound 2-hydroxypropyl 4-nitrophenyl phosphate, HPNP (Scheme 4), by the Eu(III) complexes of CNPHC, ATHC, and ABHC was measured spectrophotometrically by following the increase in absorbance at 400 nm due to 4-nitrophenolate species. The pseudo-first-order rate constants for transesterification of HPNP by $Eu(ATHC)^{3+}$, $Eu(CNPHC)^{3+}$, and $Eu(ABHC)^{3+}$ in solutions buffered at pH = 7.3 were 0.93(±0.06) × 10^{-5} s^{-1}, 1.4(±0.09) × 10^{-5} s^{-1}, and 0.10(±0.03) × 10^{-5} s^{-1}, respectively. Production of the cyclic phosphate ester was confirmed by use of ^{31}P NMR spectroscopy.

The rate of cleavage of bis(4-nitrophenyl) phosphate (BNPP) by the Eu(III) complexes of CNPHC and ABHC was measured spectrophotometrically by following the increase in absorbance at 400 nm for 4-nitrophenolate species. The pseudo-first-order rate constants for 4-nitrophenolate production upon treatment of BNPP with $Eu(CNPHC)^{3+}$ or $Eu(ABHC)^{3+}$ at pH = 7.3 were 1.5(±0.18) × 10^{-4} s^{-1} and 3.5(±0.4) × 10^{-6} s^{-1}, respectively. At pH 8.0, the pseudo-first-order rate constant for cleavage of BNPP by $Eu(ABHC)^{3+}$ increased to 1.4(±0.08) × 10^{-5} s^{-1}. As anticipated, 4-nitrophenol was a major product but the phosphate ester that would result from hydrolysis of BNPP, 4-nitrophenyl phosphate (NPP), was not produced as determined by use of HPLC. These products are consistent with attack of an hydroxyethyl group of the Eu(III) macrocyclic complexes on BNPP as we have observed previously with similar complexes[10] including $La(THED)^{3+}$ and $Eu(THED)^{3+}$ (Scheme 5). Further studies were carried out to analyze the phosphorus-containing products. Solutions containing 10 mM BNPP and 10 mM $La(ABHC)^{3+}$ (pH = 8.0) or $La(CNPHC)^{3+}$ (pH = 7.3) were monitored by use of ^{31}P NMR spectroscopy. No NPP was detected in these experiments. The ^{31}P resonances that appear (−5.0 and −5.2 ppm for $La(CNPHC)^{3+}$ and −5.5 and −6.2 ppm for $La(ABHC)^{3+}$) have chemical shifts that are similar to the phosphate diester products formed from treatment of BNPP with $La(THED)^{3+}$ (−5.6 and −4.8 ppm).[10] Removal of the La^{3+} ion by treatment of reaction solutions with EDTA resulted in the appearance of a single new ^{31}P NMR resonance for the free macrocycle containing a phosphate diester. Thus the two original ^{31}P NMR resonances probably arise from two La(III) complexes which have different macrocycle conformations similar to that observed for Eu(III) tetraamide macrocyclic complexes.[5]

Scheme 5



R = $C_6H_5NO_2$, BNPP

Discussion

Five new Eu(III) macrocyclic complexes containing alcohol pendent groups were prepared; three of these complexes also contained amide pendent groups (Chart 1). Hydroxyethyl pendent groups are activated by metal ion coordination to become potent nucleophiles[10,33−35] while amide groups impart kinetic inertia toward metal ion dissociation.[4,5] ATHC and ABHC were prepared to study the effects of mixed amide and hydroxyethyl groups on kinetic inertia and catalytic properties of lanthanide(III) macrocyclic complexes. NB-TRED and TRED were prepared as new examples of septadentate ligands for Eu(III) since lanthanide(III) complexes of septadentate ligands are more efficient catalysts than their octadentate analogues.[12] CNPHC and NB-TRED were of interest as macrocycles that, after conversion of the nitro group to an isothiocyanato group, may be attached to oligonucleotides.

Lanthanide(III) complexes of the new macrocycles are of interest as catalysts for the transesterification of RNA[3−9] and for the cleavage of the 5′-cap of mRNA.[11] The cleavage of two different phosphate diesters, one with an internal hydroxyl nucleophile (HPNP) and one without an internal nucleophile (BNPP), was studied by several of the Eu(III) macrocyclic complexes (Schemes 4 and 5). Laser-induced luminescence spectroscopy was used to characterize the solution properties of the Eu(III) complexes of the new macrocycles. Properties of interest which are important in catalysis include kinetic inertia of the complexes to Eu(III) ion dissociation, the pK_a values of bound water molecules or hydroxyalkyl groups, the number of bound water molecules, and self-association properties of the complexes.

Luminescence. The excitation spectra are remarkably similar for all five complexes including the $Eu(S\text{-}THP)^{3+}$ and $Eu(THED)^{3+}$ complexes prepared previously and the $Eu(CNPHC)^{3+}$, $Eu(ABHC)^{3+}$, and $Eu(ATHC)^{3+}$ complexes reported here. The only complex with a significantly different spectrum

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(33) Kimura, E.; Kodama, Y.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1995**, *117*, 8304.

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is the Eu(ABHC)$^{3+}$ complex which has an additional minor excitation peak present at both acidic and basic pH values (Table 1). This feature is similar to that observed for the Eu(TCMC)$^{3+}$ complex which has a second minor isomer in solution (TCMC = 1,4,7,10-tetrakis(carbamoyl)-1,4,7,10-tetraazacyclododecane).[5] For all other complexes there is a single excitation peak at acidic pH values. For all complexes a new excitation peak grows in as the pH is increased. These data in conjunction with potentiometric titrations suggest that the new species which is the predominant one at basic pH is either an Eu(III) hydroxide or an alkoxide complex. Interestingly, the excitation spectrum and the pH-dependent behavior observed here is remarkably similar to that of the Eu(III) oncomodulin CD site which contains a serine residue.[36] Studies are underway to identify the species present at basic pH values.

The number of O−H oscillators for the Eu(III) complexes varied with pH in a quite unexpected fashion. The *q* value in Table 1 was calculated by subtracting the contribution of each hydroxethyl group O−H oscillator and each amide N−H oscillator. At pH 6.3 all complexes have close to a single bound water molecule. That all values are greater than 1 is attributed to a contribution from unbound water molecules which is on the order of 0.1−0.3.[29] Thus, at acidic pH each Eu(III) ion is nine-coordinate with one bound water molecule and the macrocycle occupies the remaining eight coordination sites. The fact that there is an increase in the number of O−H oscillators with increasing pH is not in agreement with simple deprotonation of a water or hydroxyethyl pendent group. With deprotonation of a water molecule, *q* values decrease since an O−H oscillator is removed.[12] Instead, *q* values increased by 0.30, 0.36, 0.64, and 1.0 for Eu(ABHC)$^{3+}$, Eu(CNPHC)$^{3+}$, Eu(THED)$^{3+}$, and Eu(*S*-THP)$^{3+}$, respectively, from pH 6.3 to 8.6 and by 0.47 for Eu(ATHC)$^{3+}$ from pH 6.3 to 8.2. Outer-sphere water molecules contribute to depopulation of the Eu(III) excited state,[29] and deprotonation of an hydroxyethyl group or bound water could influence the number of closely associated water molecules. However, for Eu(THED)$^{3+}$ and Eu(*S*-THP)$^{3+}$, the change is quite large. If an O−H oscillator is removed by deprotonation of water or hydroxyethyl, then there is an additional 2−3 O−H oscillators in close proximity to Eu(III) at pH 8.6. One interpretation of the data is that deprotonation is accompanied by binding of an additional water molecule to the Eu(III) center. To examine whether the Eu(III) species are dinuclear complexes, energy transfer experiments were carried out with Eu(CNPHC)$^{3+}$ as an example.[30,31] These experiments suggest that monomeric complexes are the predominant species at pH 7.30 and 8.60.

Dissociation. The rate constants for dissociation of the Eu(III) complexes indicate that additional amide pendent groups helped to slow Eu(III) ion dissociation from the macrocycle in the absence of competing ligands, although the change is not as pronounced as might be expected. The half-lives for Eu(ATHC)$^{3+}$ and Eu(ABHC)$^{3+}$ ($t_{1/2}$ = 13 and 14 d), respectively, fall between those found for Eu(THED)$^{3+}$ ($t_{1/2}$ = 11 d) and Eu(TCMC)$^{3+}$ (no dissociation) under similar conditions. However, the rates of dissociation for both Eu(ATHC)$^{3+}$ and Eu(ABHC)$^{3+}$ are much closer to that of Eu(THED)$^{3+}$ and the difference in the rates between the two mixed-pendent group macrocyclic Eu(III) complexes is small. The decomposition experiments followed by laser-induced luminescence spectroscopy were also informative. In the absence of competing ligand (DTPA or EDTA), no dissociation was observed after 3 d for the mixed-pendent group macrocyclic Eu(III) complexes. This is an improvement over Eu(THED)$^{3+}$ for which 18% dissociation was observed after 3 d under the same conditions. It is interesting that Eu(CNPHC)$^{3+}$ and Eu(ABHC)$^{3+}$ dissociation is promoted more rapidly by EDTA than by DTPA as the competing ligand. This is the reverse of the order for the stability constants for Eu(III) ion with EDTA and DTPA.[37] In contrast, DTPA promotes more rapid dissociation of Eu(THED)$^{3+}$ while dissociation of Eu(ATHC)$^{3+}$ is similar in the presence of EDTA or DTPA. This dependence on competing ligand suggests that the dissociation of the Eu(III) ion from the macrocycle is aided by the competing ligand.[12]

The Eu(CNPHC)$^{3+}$ complex is more resistant to dissociation in the presence of competing ligands compared to the Eu(ATHC)$^{3+}$ complex. This difference is most marked in the presence of DTPA where the Eu(CNPHC)$^{3+}$ complex is 3.9% dissociated after 3 h whereas the Eu(ATHC)$^{3+}$ complex is 53% dissociated under similar conditions. One explanation is that the functionalized amide pendent group imparts rigidity to the Eu(III) complex to slow dissociation. A Gd(III) complex of a macrocycle similar to CNPHC demonstrated increased rigidity.[38] The macrocycle is a DOTA derivative with one of the acetate pendent groups having a 4-nitrophenyl group α to the carboxylate. This group locks the orientation of the other three pendent groups into place. Another possibility is that the steric bulk of the amide pendent group interferes with the incoming ligand associating with the Eu(CNPHC)$^{3+}$ complex.

A series of ligands synthesized by Sherry and co-workers are a useful comparison to those synthesized here.[39] Unfortunately these studies do not include the determination of the dissociation rates of the complexes; therefore, a direct comparison to the data presented here is not possible. Their studies involve mixed-pendent group macrocycles, combining acetate, 2-hydroxyethyl, 2-hydroxylpropyl, ethylphosphonate, and ethylphosphinate pendent groups. Substitution of one or two of the acetate groups by another of these pendents resulted in a decrease in the conditional stability constants, K_c, with a $\Delta\log K_c$, relative to that of Gd(DOTA)$^-$.[39] One specifically interesting case was a macrocycle containing two acetate groups, "trans" to each other, and two ethylphosphonate or two ethylphosphinate pendent groups. These Gd(III) complexes were found to be far less stable than Gd(DOTA)$^-$ ($\Delta\log K_c$ = 7.9 and 7.3) and even less stable than the Gd(DO2A)$^+$ complex which contains two pendent acetate groups in a hexadentate macrocycle. The explanation given points to the conflicting geometric requirements for these two pendent groups as based on the crystal structures of Gd(DOTA)$^-$ and Gd(DOTEP)$^-$, where all four pendents are ethylphosphinate groups. Similarly, the Eu(ATHC)$^{3+}$ and Eu(ABHC)$^{3+}$ complexes contain two types of pendent groups that have different geometric requirements, and this may make the complexes less kinetically inert to dissociation than would be anticipated from the dissociation properties of the two Eu(III) parent macrocycles which contain four identical pendent groups.

Septadentate ligands with three 2-hydroxyethyl pendent groups were not good ligands for the Eu^{3+} ion and dissociated rapidly in aqueous solution. The Eu(TRED)$^{3+}$ complex dissociated even in the absence of competing ligands with nearly compete dissociation after 20 h at pH 6.0 in the presence of

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1.0 M Cu^{2+}. Septadentate ligands with three acetate pendent groups ligated to cyclen (DO3A) or three amide groups (NBAC) were also found to form less kinetically inert complexes with the early and middle lanthanides than their octadentate analogues, although the Ln(III) complexes of DO3A and NBAC are far more kinetically stable to dissociation than either of the hydroxyethyl macrocyclic complexes studied here.[12,40]

Transesterification. Kinetic experiments with Eu(III) complexes of CNPHC, ATHC, and ABHC demonstrate that these complexes promote transesterification of the RNA model compound HPNP. The pseudo-first-order rate constant of 1.4×10^{-5} s^{-1} for $Eu(CNPHC)^{3+}$ is close to that for $La(TCMC)^{3+}$ (1.6×10^{-5} s^{-1}) and 12 times less than for $Eu(THED)^{3+}$ (1.7×10^{-4} s^{-1}), under similar conditions.[4,7] The $Eu(ATHC)^{3+}$ complex has catalytic activity similar to that of $Eu(CNPHC)^{3+}$ while a further decrease in activity is observed for $Eu(ABHC)^{3+}$. The pK_a of $Eu(ABHC)^{3+}$ (8.1) is higher than that of $Eu(THED)^{3+}$ (7.5) or $Eu(CNPHC)^{3+}$ (7.5) suggesting that the bis(amide) complex is a poorer Lewis acid. However, our work with lanthanide(III) complexes has shown that the pK_a of lanthanide(III) bound ligands is not the sole indicator of catalytic activity for RNA transesterification. The Ln^{3+} center must be accessible to bind or interact with the phosphate diester.[5] On the basis of the luminescence lifetime measurements for $Eu(CNPHC)^{3+}$, $Eu(ATHC)^{3+}$, and $Eu(ABHC)^{3+}$, there should be at least one open coordination site at pH = 7.4 for each of these complexes. Another consideration is macrocycle rigidity. As demonstrated with the Eu(III) complexes of THED and *S*-THP, the Eu(III) complex of the more rigid *S*-THP ligand is a poorer transesterification catalyst for RNA.[7]

The hydroxyethyl pendent groups of the CNPHC, ATHC, and ABHC ligands when complexed to Eu(III) ions can undergo the same type of intermolecular transesterification reactions with BNPP as observed for $Eu(THED)^{3+}$ (Scheme 5).[10,11] Alcohol pendent groups in ligands in Zn(II) complexes,[33] Ln(III) complexes,[10,11,34] and Cu(II) complexes[35] act as nucleophiles toward BNPP and other phosphate esters that do not contain internal nucleophiles. Of these, $Eu(THED)^{3+}$ has one of the highest rate constants, 1.9×10^{-4} s^{-1} at 1 mM complex. The first-order rate constant for BNPP cleavage by $Eu(CNPHC)^{3+}$ is similar ($1.5(\pm 0.18) \times 10^{-4}$ s^{-1}). The $Eu(ABHC)^{3+}$ complex also promoted BNPP cleavage, although at a slower rate, $3.5(\pm 0.4) \times 10^{-6}$ s^{-1}. This is consistent with the higher pK_a of the $Eu(ABHC)^{3+}$ complex since the active catalyst in this reaction is formed by deprotonation of one of the hydroxyethyl pendent groups[10] to form an alkoxide nucleophile. Consistent with this interpretation, the pseudo-first-order rate constant for cleavage of BNPP by $Eu(ABHC)^{3+}$ increased by 4-fold at pH 8.0. Reaction products as studied by use of ^{31}P NMR spectroscopy and by HPLC analysis are consistent with attack of an hydroxyethyl pendent group on BNPP to form a new phosphate ester attached to the macrocycle.

Summary. Three new macrocyclic complexes of Eu(III) with mixed amide and hydroxyethyl pendent groups and two new septadentate macrocycles with hydroxyethyl pendent groups have been prepared. The solution speciations of the Eu(III) mixed amide hydroxyethyl macrocyclic complexes are remarkably similar to those of the $Eu(THED)^{3+}$ complex. Dissociation rates of the Eu(III) ion from the macrocycles in the absence of other good ligands for Eu(III) are retarded in complexes containing amide pendent groups relative to those containing only hydroxypropyl groups under most conditions. However, the effect is not as pronounced as would have been predicted from dissociation rates of macrocycles containing all amide pendent groups. The efficiency with which the Eu(III) complexes promote transesterification of a model RNA substrate decreases by approximately a factor of 10 upon replacement of an hydroxyethyl group with an amide group. In contrast, reactions where the hydroxyalkyl pendent group of the Eu(III) macrocyclic complex is itself a nucleophile are not as sensitive to the presence of other pendent groups on the macrocycle. For these reactions, it will be advantageous to use complexes containing amide groups such as $Eu(CNPHC)^{3+}$ due to the greater resistance of the amide complexes to dissociation.

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Supporting Information Available: Tables listing luminescence lifetime data and dissociation data and figures of luminescence excitation spectra (17 pages). Ordering information is given on any current masthead page.

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