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Simultaneous Interaction with Base and Phosphate Moieties Modulates the Phosphodiester Cleavage of Dinucleoside 3′,5′-Monophosphates by Dinuclear Zn^{2+} Complexes of Di(azacrown) Ligands

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Abstract: Five dinucleating ligands (**1**−**5**) and one trinucleating ligand (**6**) incorporating 1,5,9-triazacyclododecan-3-yloxy groups attached to an aromatic scaffold have been synthesized. The ability of the Zn^{2+} complexes of these ligands to promote the transesterification of dinucleoside 3′,5′-monophosphates to a 2′,3′-cyclic phosphate derived from the 3′-linked nucleoside by release of the 5′-linked nucleoside has been studied over a narrow pH range, from pH 5.8 to 7.2, at 90 °C. The dinuclear complexes show marked base moiety selectivity. Among the four dinucleotide 3′,5′-phosphates studied, viz. adenylyl-3′,5′-adenosine (ApA), adenylyl-3′,5′-uridine (ApU), uridylyl-3′,5′-adenosine (UpA), and uridylyl-3′,5′-uridine (UpU), the dimers containing one uracil base (ApU and UpA) are cleaved up to 2 orders of magnitude more readily than those containing either two uracil bases (UpU) or two adenine bases (ApA). The trinuclear complex (**6**), however, cleaves UpU as readily as ApU and UpA, while the cleavage of ApA remains slow. UV spectrophotometric and ^{1}H NMR spectroscopic studies with one of the dinucleating ligands (**3**) verify binding to the bases of UpU and ApU at less than millimolar concentrations, while no interaction with the base moieties of ApA is observed. With ApU and UpA, one of the Zn^{2+}−azacrown moieties in all likelihood anchors the cleaving agent to the uracil base of the substrate, while the other azacrown moiety serves as a catalyst for the phosphodiester transesterification. With UpU, two azacrown moieties are engaged in the base moiety binding. The catalytic activity is, hence, lost, but it can be restored by addition of a third azacrown group on the cleaving agent.

Introduction

Cleavage of RNA phosphodiester bonds by dinuclear 3d transition metal ion complexes has received considerable interest[1] because many phosphoesterases contain two metal ions in their catalytic center.[2] Since the intermetallic distance in phosphoesterases is of the order of 4 Å, it has been reasoned that binding of two metal ions to a ligand that affords a 3−5 Å distance between the metal centers is optimal for their cooperative action. In fact, studies with substitution by inert Co^{3+} complexes have lent support to this reasoning. Two Co^{3+} ions at a distance of 2.9 Å have been shown to result in a 4×10^5-fold rate acceleration in the hydroxide-ion-catalyzed hydrolysis of 2-hydroxypropyl 4-nitrophenyl phosphate (HPNP) by binding simultaneously to both of the nonbridging phosphoryl oxygen atoms.[3] Equally high rate accelerations have not been obtained with substitution-labile divalent metal ions. The dinuclear Zn^{2+} and Cu^{2+} complexes are typically 1 or 2 orders of magnitude more efficient catalysts than their mononuclear counterparts when HPNP is used as a substrate. Dinuclear complexes containing a bridging alkoxide group that keeps the two metal ions in close proximity have been shown to exhibit up to 100-fold rate enhancements over the corresponding mononuclear complexes.[4,5] The acceleration has been attributed to increased acidity of the metal aquo ion, increased affinity to phosphate, and additional transition-state stabilization.[5] Additional examples of synergic action of two Zn^{2+} ions are offered by a dinuclear complex of a cyclic β-sheet peptide, gramicidin S,[6] and a dinuclear complex of calix[4]arene bearing two 2,6-bis(dialkylaminomethyl) moieties as nucleating centers.[7] Care should,

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however, be exercised on interpreting the rate accelerations by bimetallic systems, since considerable rate enhancements may also result from interaction between an aromatic ligand and the departing 4-nitrophenoxide group.[8]

The results obtained with simple model esters containing no nucleic acid base cannot be directly extrapolated to the cleavage of RNA. First, the mechanism of transesterification of the unactivated phosphodiester bonds in RNA is, in all likelihood, different from that utilized by the highly labile 4-nitrophenyl esters. With aryl phosphodiesters, the formation rather than cleavage of the phosphorane intermediate obtained by the attack of the neighboring hydroxyl group on the phosphorus atom is rate-limiting, while with alkyl esters, such as RNA, the breakdown of the phosphorane constitutes the slow step.[9] Second, the plethora of donor atoms in RNA may result in formation of nonproductive complexes that compete with the desired species, benefiting from double Lewis acid activation. For example, a dinuclear Zn^{2+} complex, where the intermetallic distance had been adjusted to 4.5 Å by steric gearing, exhibited an 80-fold catalytic advantage over the mononuclear complex when HPNP was used as a substrate, but resulted in no additional acceleration on the cleavage of uridylyl-3′,5′-uridine (UpU).[10] Nevertheless, clear evidence for double Lewis acid activation has been obtained even with dinucleoside 3′,5′-monophosphates. The dinuclear Cu^{2+} complex of 1,8-bis(1,4,7-triazacyclononan-1-ylmethyl)naphthalene cleaves adenylyl-3′,5′-adenosine (ApA) 500 times more readily than the mononuclear Cu^{2+} complex of 1,4,7-triazacyclononane.[11] More modest rate accelerations have been observed with other bimetallic agents. The dinuclear Zn^{2+} complexes of *N*,*N*,*N*′,*N*′-tetrakis(pyridin-2-ylmethyl)-1,3-diaminopropan-2-ol[12] and *N*,*N*,*N*′,*N*′-tetrakis(pyridin-2-ylmethyl)-*m*- and -*p*-xylyldiamine[12,13] cleave the internucleosidic phosphodiester bond 2−15 times as fast as the mononuclear complex of bis(pyridin-2-ylmethyl)amine, without any marked selectivity toward the base moiety. Among complexes of macrocyclic ligands, the dinuclear Zn^{2+} complex of bisdien (1,13-dioxa-4,7,10,16,19,22-hexaazacyclotetracosane) cleaves UpU 1 order of magnitude faster than the mononuclear complex of 1,5,9-triazacyclododecane ([12]aneN_3),[14] and the dinuclear Zn^{2+} complex of 1,4,7,16,19,22-hexaaza-10,13,25,28-tetraoxacyclotriacontane cleaves ApA more rapidly than its mononuclear counterparts.[15] This kind of modest rate acceleration may, however, reflect enhanced binding rather than real double Lewis acid activation.

Binding of dinuclear cleaving agents to the base moiety of ribonucleoside 3′-phosphodiesters has not received much attention. Only in a few cases has the effect of the base moiety structure on the catalytic activity been systematically studied. The only clear indication of base selectivity has been obtained with a dinuclear Cu^{2+} complex containing terpyridine and bipyridine as nucleating moieties.[16] This complex cleaves ApA, adenylyl-3′,5′-cytidine (ApC), and cytidylyl-3′,5′-adenosine (CpA) approximately 1 order of magnitude more rapidly than dimers containing no adenine base. The observed selectivity has been attributed to stacking between the aromatic ligand and the adenine base. Another example of marked base selectivity is offered by the trinuclear Zn^{2+} (and Cu^{2+}) complex of calix[4]arene, bearing three 2,6-bis(aminomethyl)pyridine coordination sites.[17] This complex cleaves guanylyl-3′,5′-guanosine (GpG) more than 1 order of magnitude faster than other dinucleoside monophosphates. The trinuclear Zn^{2+} complex of 1,3,5-tris[di(pyridin-2-ylmethyl)aminomethyl]benzene has been shown to cleave UpU much more readily than its 2′,5′-isomer, while with the corresponding adenosine diesters, the situation is the opposite.[18] However, no clear selectivity toward the base moiety has been observed.

While the previous studies on cleavage of RNA phosphodiester bonds by dinuclear transition metal complexes have mainly concerned the interaction of metal ions with the phosphoryl oxygens and/or the entering and departing nucleophile, the present work is aimed at elucidating the effects that anchoring of a dinuclear complex to the base moiety may have on its catalytic activity. The Zn^{2+} chelates of small azacrowns have been established to bind very tightly to the deprotonated *N*3 atom of uracil and thymine bases.[19] In addition, ditopic complexes containing two Zn^{2+}−azacrown moieties attached to an aromatic scaffold have been shown to exhibit simultaneous interaction with the base and phosphate moieties of thymidine 5′-phosphate.[19g,j] Accordingly, such dinuclear complexes show potential for development of cleaving agents that simultaneously interact with the base and phosphate moiety, resulting in base-selective cleavage. As an indication of the feasibility of this approach, we have previously shown that the heterodinuclear Ni^{2+},Zn^{2+} complex of a spiro di(azacrown) ligand, 2,6,10,14,-18,22-hexaazaspiro[11.11]tricosane, cleaves UpU and adenylyl-3′,5′-uridine (ApU) much faster than uridylyl-3′,5′-adenosine (UpA) and exerts no cooperative acceleration on the cleavage of ApA.[20] Recently, the dinuclear Zn^{2+} complex of 1,3-bis(1,4,7-triazacyclononan-1-yl)-2-propanol has been shown to cleave UpU more efficiently than HPNP, but the more efficient catalysis has been attributed to stabilization of the transition state by interaction of the cleaving agent with the C5′-oxyanion.[21] To learn more about the selectivity of various

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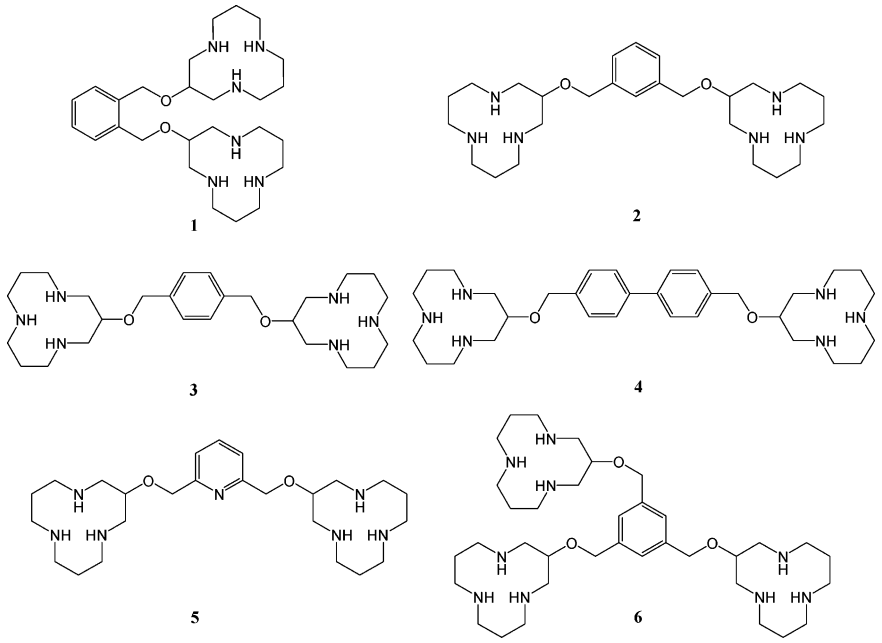
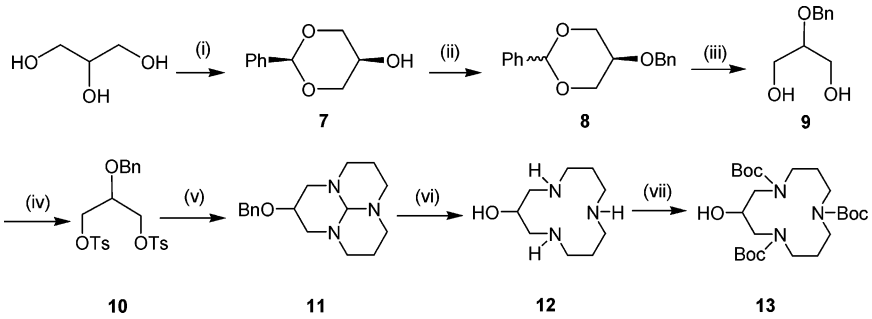
Chart 1. The Ligands Studied



Scheme 1 [a]



[a] Reagents and conditions: (i) $PhCHO$, H_2SO_4; (ii) $PhCH_2Cl$, KOH, benzene, reflux; (iii) HCl, MeOH(aq); (iv) TsCl, THF(aq), NaOH, 3−5 °C; (v) 1. TBD, KOH, dioxane, reflux, 48 h, 2. $NaBH_4$, dioxane, rt, 2 d; (vi) 4 mol L^{-1} HCl(aq), reflux, 4 d; (vii) Boc_2O, ^{t}BuOH(aq), NaOH, 40 °C, 2 d.

di(azacrown) complexes as cleaving agents, five dinucleating ligands (**1**−**5**) and one trinucleating ligand (**6**) incorporating two and three 1,5,9-triazacyclododecan-3-yloxy groups, respectively, as coordination sites (Chart 1) have been synthesized and studied in the presence of Zn^{2+} ion as catalysts for the cleavage of the phosphodiester bond of ApA, ApU, UpA, and UpU. The ligands employed allow the intermetallic distance to vary within rather wide limits and enable bridging between the base and phosphate moiety. Efficient cleavage and high base moiety selectivity have been obtained with these complexes.

Results

Syntheses of Ligands 1−6. Scheme 1 depicts the synthesis of *N-tert*-butoxycarbonyl (Boc)-protected 1,5,9-triazacyclododecan-3-ol (**13**) used to construct the di- and tri(azacrown) ligands studied (**1**−**6**). Glycerol was first reacted with benzaldehyde to obtain *cis*-5-hydroxy-2-phenyl-1,3-dioxane (**7**), as described previously.[22] This was converted to a mixture of cis and trans benzyl ethers (**8**) and hydrolyzed to 2-(benzyloxy)propane-1,3-diol (**9**). The hydroxyl functions were tosylated (**10**), and the tosyloxy groups were displaced with 1,5,7-triazabicyclo[4.4.0]-dec-5-ene (TBD) to obtain a tricyclic orthoamide (**11**), as described originally by Alder et al.[23] and Kim et al.[24] Prolonged treatment (4 d) of **11** with 4 mol L^{-1} aqueous hydrogen chloride under reflux gave 1,5,9-triazacyclododecan-3-ol trihydrochloride (**12**), which was converted to the *N*-Boc-protected form (**13**) by conventional methods.

Ligands **1**−**6** were obtained in *N*-Boc-protected form (**14**−**19**) by treating an appropriate bis(bromomethyl)benzene, 4,4′-bis(chloromethyl)biphenyl, 2,6-(tosyloxy)pyridine,[25] or 1,3,5-tris(bromomethyl)benzene[26] in dimethylformamide (DMF) with the oxyanion of **13** (Scheme 2). The Boc groups were removed by acid-catalyzed hydrolysis, which gave **1**−**6** as hydrochloric salts. The free bases were obtained by passing the aqueous solutions of the hydrochloric salts through a strong anion-exchange resin (Dowex 1X2, OH^- form). The products were

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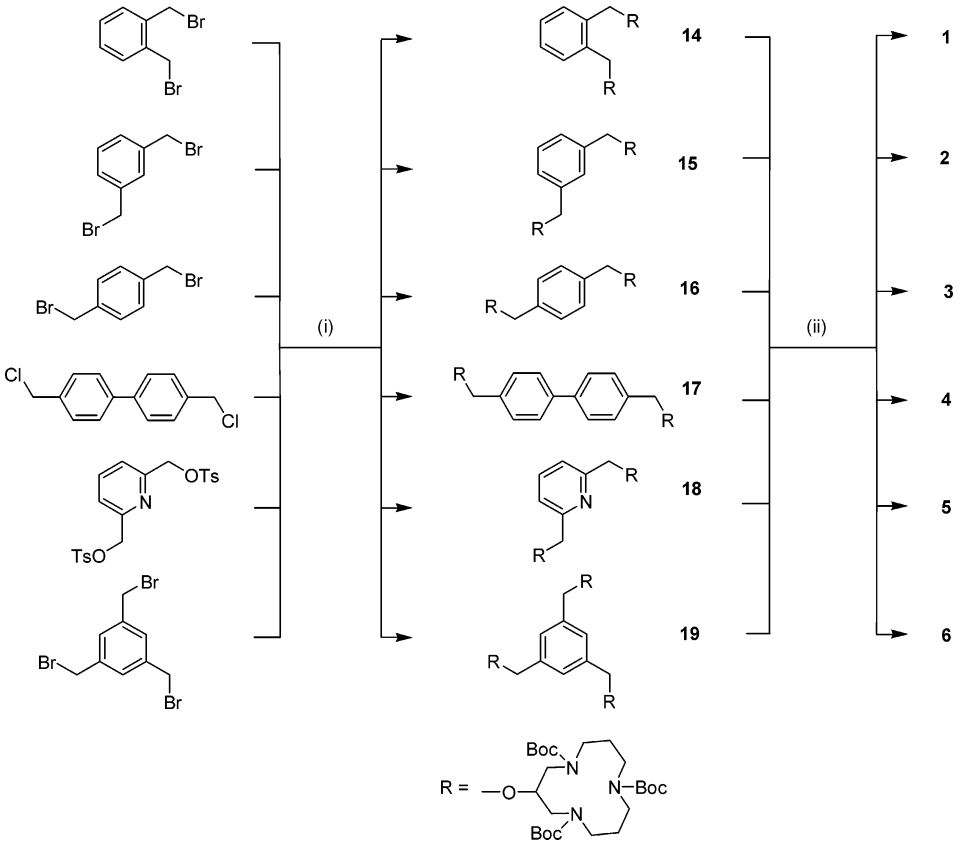
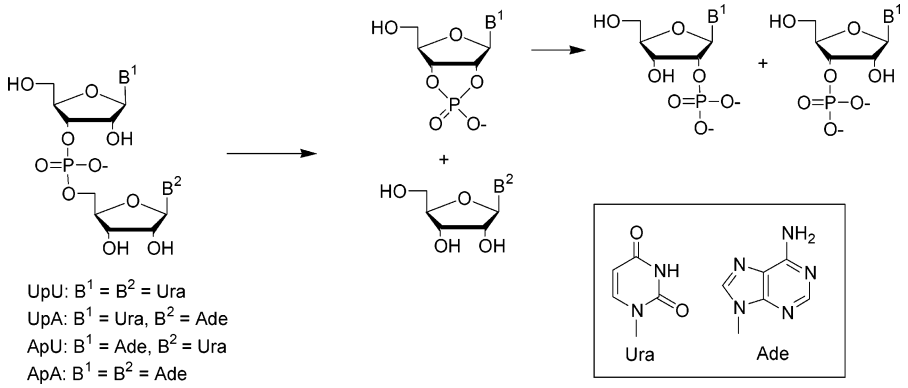
Scheme 2 [a]



[a] Reagents and conditions: (i) **13**, NaH, DMF; (ii) 1. HCl(aq), 2. Dowex 1X2, OH^- form.

Scheme 3



characterized by ^1H and ^{13}C NMR spectroscopy and high-resolution mass spectroscopy.

It is worth noting that the 1,5,9-triazacyclododecanyl groups in ligands **1**−**6** are bonded to the aromatic scaffold through a carbon atom (C3), not through a nitrogen atom as in many previously reported constructs.[5,11,19g,j] All the ring nitrogen atoms, hence, retain their ability to serve as hydrogen bond donors, which has been suggested to play a role in binding of azacrown chelates to nucleic acid bases.[19]

Preparation of the Reaction Solutions. The reaction solutions were prepared as follows. A buffer solution (HEPES, 0.1 mol L^{-1}, $I = 0.1$ mol L^{-1} with $NaNO_3$) having an exactly known pH value in the pH range 6.5−8.0 (at 25 °C) was first prepared. Appropriate amounts of the ligand and $Zn(NO_3)_2$ were then added to give the desired final concentration for each. The solutions were allowed to stand a few hours, and their pH was then checked. In case any deviation from the initial pH was observed, the pH was adjusted back to the original value. No precipitation of zinc hydroxide was observed. The pH values measured at 25 °C were extrapolated to the temperature of the kinetic measurements (90 °C) with the aid of the known temperature dependence of the pK_a value of HEPES.[27]

Kinetic Measurements. The progress of the Zn^{2+}-complex-promoted cleavage of dinucleoside 3′,5′-monophosphates was followed by withdrawing aliquots from the reaction solutions at suitable intervals and analyzing their composition by RP-HPLC. The compounds studied were UpU, UpA, ApU, and ApA. The products were identified by spiking with authentic compounds, viz. adenosine and uridine and their 2′,3′-cyclic phosphates and 2′- and 3′-monophosphates. The rate constants

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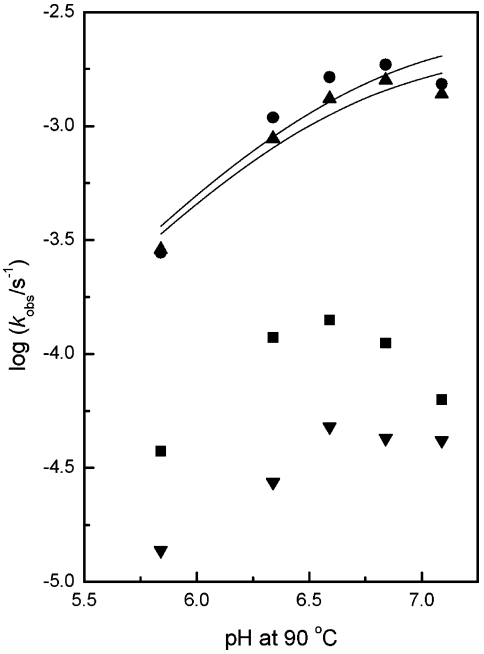

Figure 1. pH−rate profiles for the cleavage of ApU (●), UpA (▲), ApA (■), and UpU (▼) (0.05 mmol L^{-1}) in an excess of 1,2-bis[(1,5,9-triazacyclododecan-3-yl)oxymethyl]benzene (**1**, 0.5 mmol L^{-1}) and Zn^{2+} (1.0 mmol L^{-1}) at 90 °C (I = 0.1 mol L^{-1}).

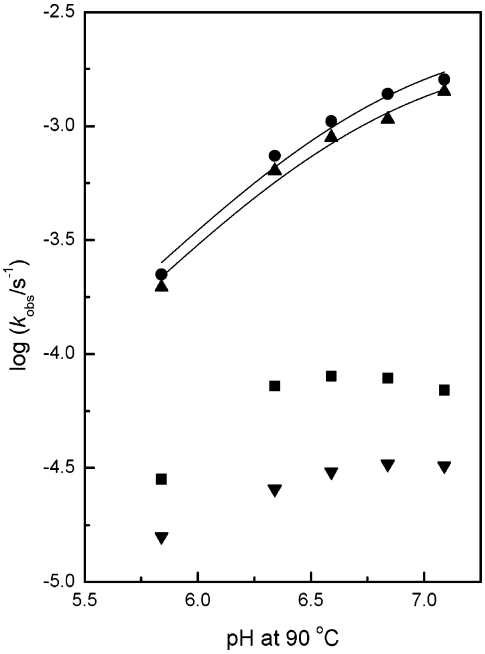

Figure 2. pH−rate profiles for the cleavage of ApU (●), UpA (▲), ApA (■), and UpU (▼) (0.05 mmol L^{-1}) in an excess of 1,3-bis[(1,5,9-triazacyclododecan-3-yl)oxymethyl]benzene (**2**, 0.5 mmol L^{-1}) and Zn^{2+} (1.0 mmol L^{-1}) at 90 °C (I = 0.1 mol L^{-1}).

were calculated from the time-dependent diminution of the concentration of the starting material. In each case, the 3′-linked nucleoside was expectedly released as a 2′,3′-cyclic phosphate and the 5′-linked nucleoside as a free alcohol (Scheme 3). The cyclic phosphate was not, however, markedly accumulated, but it was hydrolyzed to a 2:1 mixture of 2′- and 3′-phosphates. The maximal mole fraction of the cyclic phosphate during a kinetic run ranged from 0.01 to 0.05. The kinetics of the hydrolysis of cyclic phosphate to phosphomonoesters was not studied.

Pseudo-first-order rate constants for the cleavage of the four dinucleoside 3′,5′-monophosphates were determined in an excess of the cleaving agent over a narrow pH range, viz. pH 5.8−7.2 (T = 90 °C, I = 0.1 mol L^{-1} with $NaNO_3$). The kinetic data obtained with ligands **1**−**5**, containing two azacrown moieties, are presented in Figures 1−5. All these ligands exhibit a common feature in the presence of 2 equiv of Zn^{2+}: with ApU and UpA, the cleavage is up to 2 orders of magnitude faster than the cleavage promoted by the mononuclear Zn^{2+} chelate of 1,5,9-triazacyclododecane ([12]aneN_3]), whereas no such acceleration is observed with ApA or UpU. Figure 6 shows similar data for the cleavage in an excess of a trinucleating ligand (**6**) and Zn^{2+}. Now the cleavage of all uracil-containing dimers (UpU, UpA, ApU) is markedly accelerated, while the cleavage of ApA still remains slow. Table 1 summarizes the rate constants at pH 6.84 (refers to 90 °C), where the cleavage rate is maximal. Table 2 records the rate constants obtained at two different concentrations of one of the dinucleating ligands (**3**) and the trinucleating ligand (**6**). For comparative purposes, the rate constants for the cleavage promoted by Zn^{2+}[12]aneN_3 and free Zn^{2+}−aquo ion are also included.

UV Spectrophotometric Titrations. To elucidate the stability of the mixed-ligand Zn^{2+} complex of UpU and a binucleating azacrown ligand, a 50 μmol L^{-1} solution of UpU was titrated at pH 7.5, 7.0, and 6.5 (T = 25 °C, I = 0.1 mol L^{-1}) with a 1:2

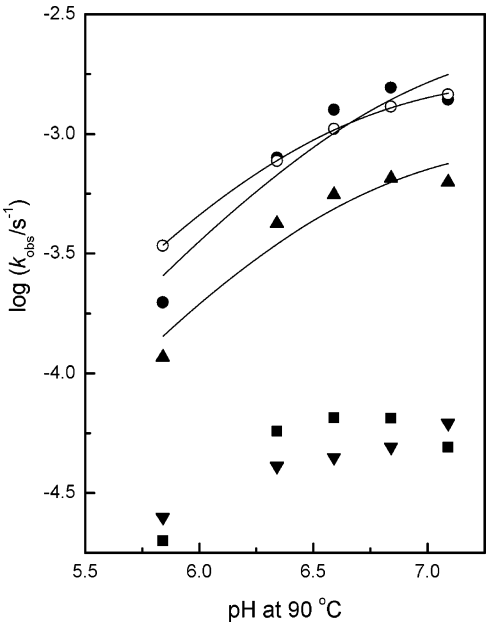

Figure 3. pH−rate profiles for the cleavage of ApU (●), UpA (▲), ApA (■), and UpU (▼) (0.05 mmol L^{-1}) in an excess of 1,4-bis[(1,5,9-triazacyclododecan-3-yl)oxymethyl]benzene (**3**, 0.5 mmol L^{-1}) and Zn^{2+} (1.0 mmol L^{-1}) at 90 °C (I = 0.1 mol L^{-1}), and the pH−rate profile for the cleavage of ApU (○) at [**3**] = 2.0 mmol L^{-1} and [Zn^{2+}] = 4.0 mmol L^{-1}.

mixture of ligand **3** and Zn^{2+}. Comparative measurements were carried out with ApU and ApA. The concentration of the dinucleoside monophosphates studied was the same as that used in kinetic experiments. The pH values at 25 °C correspond to 0.8 unit lower values at 90 °C, since the ionic product of water at 90 °C is 1.6 logarithmic units lower than that at 25 °C.[28] Figures 7 and 8 show the results obtained with UpU and ApA

(28) Marshall, W. L.; Franck, E. U. *J. Phys. Chem. Ref. Data* **1981**, *10*, 295−304.

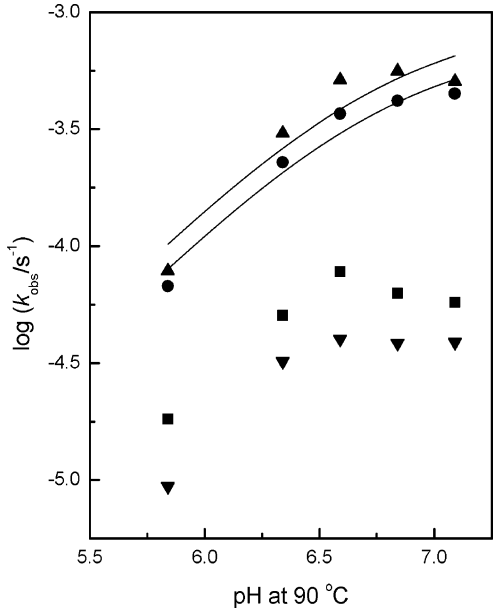

Figure 4. pH−rate profiles for the cleavage of ApU (●), UpA (▲), ApA (■), and UpU (▼) (0.05 mmol L^{-1}) in an excess of 4,4′-bis[(1,5,9-triazacyclododecan-3-yl)oxymethyl]biphenyl (**4**, 0.5 mmol L^{-1}) and Zn^{2+} (1.0 mmol L^{-1}) at 90 °C (I = 0.1 mol L^{-1}).

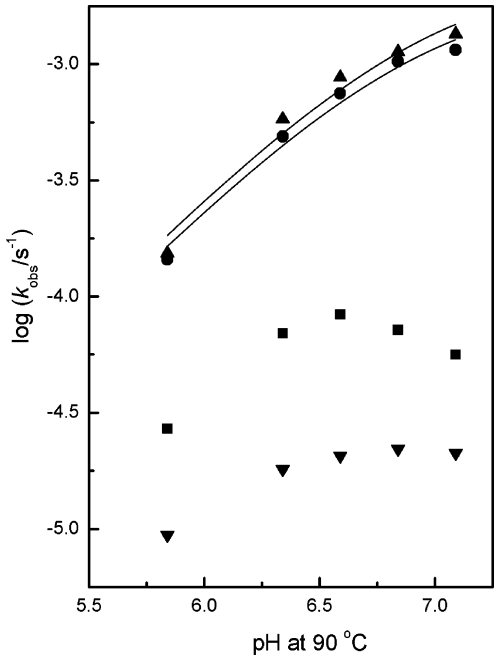

Figure 5. pH−rate profiles for the cleavage of ApU (●), UpA (▲), ApA (■), and UpU (▼) (0.05 mmol L^{-1}) in an excess of 2,6-bis[(1,5,9-triazacyclododecan-3-yl)oxymethyl]pyridine (**5**, 0.5 mmol L^{-1}) and Zn^{2+} (1.0 mmol L^{-1}) at 90 °C (I = 0.1 mol L^{-1}).

at pH 7.5. The decrease in the absorbance at the wavelength of the absorption maximum is plotted in Figure 9 as a function of the composition of the reaction mixture. With UpU at pH 7.5, the absorption decreases linearly upon addition of ligand **3** and Zn^{2+} (in 1:2 molar ratio) until 1 equiv of **3** has been added and remains constant thereafter. At pH 7.0, the break is not as sharp, and a plateau is reached at [**3**]:[UpU] ≈ 1.6; at pH 6.5, only a modest continuous decrease of absorption takes place. The UV spectrum of ApA, in turn, remains almost unchanged upon similar additions of **3** and Zn^{2+}. The behavior

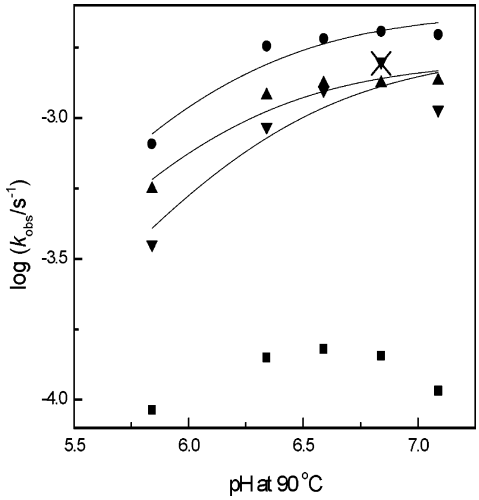

Figure 6. pH−rate profiles for the cleavage of ApU (●), UpA (▲), ApA (■), and UpU (▼) (0.05 mmol L^{-1}) in an excess of 1,3,5-tris[(1,5,9-triazacyclododecan-3-yl)oxymethyl]benzene (**6**, 0.5 mmol L^{-1}) and Zn^{2+} (1.5 mmol L^{-1}) at 90 °C (I = 0.1 mol L^{-1}). In addition, the rate constant for the cleavage of UpU at [**6**] = 1.5 mmol L^{-1} and [Zn^{2+}] = 4.5 mmol L^{-1} is indicated (×).

Table 1. Pseudo-First-Order Rate Constants for the Cleavage of Dinucleoside 3′,5′-Monophosphates (0.05 mmol L^{-1}) in Excess of Ligands **1**−**6** (0.5 mmol L^{-1}) and Zn^{2+} (1.0 mmol L^{-1} with **1**−**5** and 1.5 mmol L^{-1} with **6**) at pH 6.84 (90 °C, I = 0.1 mol L^{-1})

	$k_{obs}/10^{-3}$ s^{-1} [a]			
complex	ApU	UpA	ApA	UpU
$(Zn^{2+})_2$-**1**	1.86 ± 0.03	1.59 ± 0.02	0.112 ± 0.003	0.043 ± 0.001
$(Zn^{2+})_2$-**2**	1.38 ± 0.01	1.07 ± 0.01	0.078 ± 0.001	0.033 ± 0.001
$(Zn^{2+})_2$-**3**	1.56 ± 0.02	0.66 ± 0.05	0.065 ± 0.002	0.049 ± 0.002
$(Zn^{2+})_2$-**4**	0.42 ± 0.01	0.56 ± 0.01	0.063 ± 0.001	0.038 ± 0.001
$(Zn^{2+})_2$-**5**	1.03 ± 0.01	1.13 ± 0.03	0.072 ± 0.001	0.022 ± 0.001
$(Zn^{2+})_3$-**6**	2.03 ± 0.07	1.33 ± 0.03	0.143 ± 0.002	1.58 ± 0.02

[a] The errors indicated are standard deviations of the mean of single kinetic runs.

of ApU resembles that of UpU, although the change in absorption is smaller, since only one of the base moieties (uracil) participates in complex formation, and the plateau is reached at [**3**]:[ApU] ≈ 1.6. ApA does not exhibit marked interaction with $(Zn^{2+})_2$-**3**.

NMR Spectroscopic Titrations. To obtain additional evidence for the high stability of the mixed-ligand complex UpU-$(Zn^{2+})_2$-**3**, the changes in the ^{1}H NMR spectrum of UpU upon addition of **3** and Zn^{2+} (in a 1:2 molar ratio) were studied. The *H*6 protons of UpU were observed to exhibit two doublets at 7.8 ppm, referring to the 3′- and 5′-linked nucleosides. When **3** and Zn^{2+} were added, the signals gradually disappeared, and two new resonances at 7.5 and 7.6 ppm appeared. Upon addition of 1 equiv of **3** and 2 equiv of Zn^{2+} to a 4 mmol L^{-1} solution of UpU in D_2O, this change was quantitative (Figure 10). For comparison, the *H*2 and *H*8 signals of ApA did not respond on a similar addition, suggesting that no interaction existed between the adenine bases and the Zn^{2+} complex.

To estimate the stability of the binuclear Zn^{2+} complexes of **1**−**5**, the changes in the ^{1}H NMR spectrum of ligand **3** upon addition of Zn^{2+} were studied in D_2O at 35 °C (pD 7.1) and 90 °C (pD 6.5). The protons at C7 and C11 resonated at 1.88 ppm (quintet). Upon addition of Zn^{2+}, this resonance was decreased with concomitant appearance of two new multiplets at 1.69 and 2.04 ppm. Figure 11 shows the mole fraction of the uncom-

Table 2. Comparison of the Cleavage Rate of Dinucleoside 3′,5′-Monophosphates at Two Concentrations of Dinucleating Ligand **3** and Trinucleating Ligand **6** to the Rate of the Cleavage Promoted by Zn^{2+}[12]aneN$_3$ and Free Zn^{2+}−Aquo Ion at pH 6.84 (90 °C, I = 0.1 mol L^{-1})

		$k_{obs}/10^{-3}$ s^{-1} [a]			
complex	c/mmol L^{-1}	ApU	UpA	ApA	UpU
$(Zn^{2+})_2$-**3**	0.5	1.56 ± 0.02	0.66 ± 0.05	0.065 ± 0.002	0.049 ± 0.002
$(Zn^{2+})_2$-**3**	2.0	1.35 ± 0.02	0.76 ± 0.01	0.127 ± 0.004	0.158 ± 0.002
$(Zn^{2+})_3$-**6**	0.5	2.03 ± 0.07	1.33 ± 0.03	0.143 ± 0.002	1.58 ± 0.02
$(Zn^{2+})_3$-**6**	2.0	2.10 ± 0.02	1.53 ± 0.02	0.30 ± 0.01	1.56 ± 0.01
Zn^{2+}[12]aneN$_3$	1.0	0.027 ± 0.001	0.060 ± 0.001	0.054 ± 0.001	0.026 ± 0.001
Zn^{2+}[12]aneN$_3$	4.0	0.067 ± 0.001	0.181 ± 0.001	0.155 ± 0.001	0.072 ± 0.01
Zn^{2+}	1.0	0.024[b]	0.034[b]	0.036[b]	0.030[b]

[a] The errors indicated are standard deviations of the mean of single kinetic runs. [b] Extrapolated from the data in ref 32 on the basis of first-order dependence of the cleavage rate on [Zn^{2+}] and [OH^-].

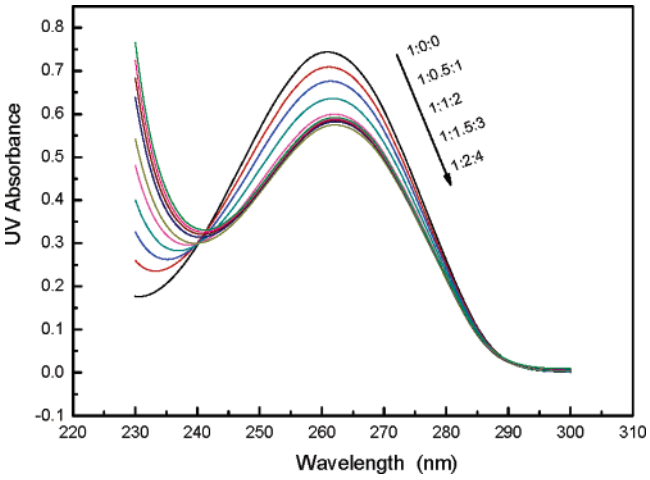

Figure 7. UV spectra of UpU (50 μmol L^{-1} aqueous solution) at various concentrations of **3** and Zn^{2+} at 25 °C (pH 7.5, I = 0.1 mol L^{-1} with $NaNO_3$). Notation: the molar ratios of UpU, **3**, and Zn^{2+} indicated by the arrow refer to the curves from the top to bottom (at 260 nm).

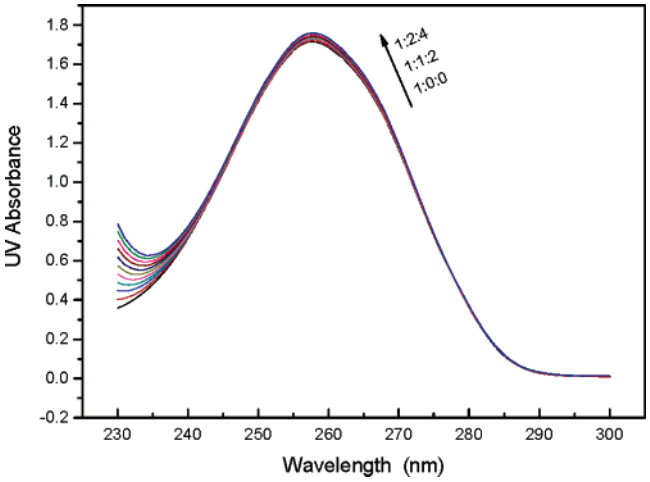

Figure 8. UV spectra of ApA (50 μmol L^{-1} aqueous solution) at various concentrations of **3** and Zn^{2+} at 25 °C (pH 7.5, I = 0.1 mol L^{-1} with $NaNO_3$). Notation: the molar ratios of ApA, **3**, and Zn^{2+} indicated by the arrow refer to the curves from the bottom to top (at 258 nm).

plexed [12]aneN$_3$ moieties as a function of the overall Zn^{2+} concentration at [**3**] = 2.0 mmol L^{-1}. Interestingly, the complexing efficiency is independent of temperature. Evidently, the temperature dependence of protonation of **3** is very similar to that of complexation of Zn^{2+} with neutral ligand, and hence, the Zn^{2+} ion competes for **3** with proton in a temperature-independent manner. The other ligands gave similar results. On

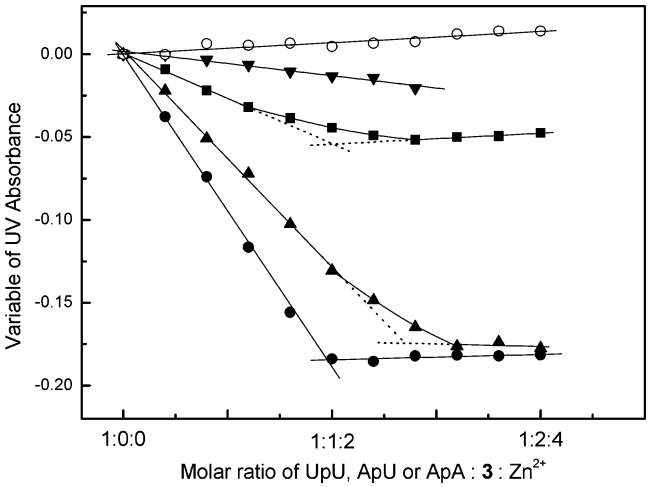

Figure 9. Changes observed in the absorbance of 50 μmol L^{-1} solutions of UpU (261 nm), ApU (260 nm), and ApA (258 nm) (T = 25 °C, I = 0.1 mol L^{-1}) as a function of the molar ratio of NpN (N = U or A), **3**, and Zn^{2+}. The contribution of the absorbance of **3** has been subtracted. Notation: UpU at pH 7.5 (●), UpU at pH 7.0 (▲), UpU at pH 6.5 (▼), ApU at pH 7.5 (■), and ApA at pH 7.5 (○).

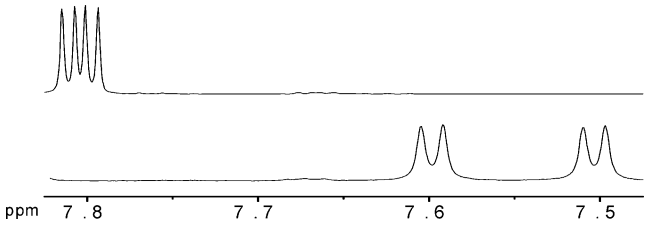

Figure 10. *H*6 NMR signals of the uracil bases of UpU (4 mmol L^{-1}) in the absence and in the presence of 1 equiv of **3** and 2 equiv of Zn^{2+} at pD 7.1 (T = 25 °C).

mixing a dinucleating ligand (**1**−**5**, 2.0 mmol L^{-1}, pD 7.1) with 2 equiv of Zn^{2+}, the mole fraction of the uncomplexed azacrown moiety at 35 °C was 0.36 with **1**, 0.17 with **2**, 0.45 with **3**, 0.59 with **4**, and 0.26 with **5**. In a 1:3 mixture of ligand **6** (2.0 mmol L^{-1}) and Zn^{2+}, the mole fraction of the uncomplexed azacrown moiety was 0.24.

The data presented in Figures 9 and 10 suggest that the complex UpU-$(Zn^{2+})_2$-**3** is more stable than the complex $(Zn^{2+})_2$-**3**. To obtain further evidence for this argument, binding of Zn^{2+} to ligand **3** was studied by NMR spectroscopy in the presence of UpU. Figure 12 shows the H7,H11 resonance of the azacrown moieties of ligand **3** ([**3**] = 2.0 mmol L^{-1}). Upon addition of 2 equiv of Zn^{2+}, the H7,H11 resonance of the free ligand was weakened and two new multiplets appeared at 1.69 and 2.04 ppm, referring to the Zn^{2+}-complexed azacrown

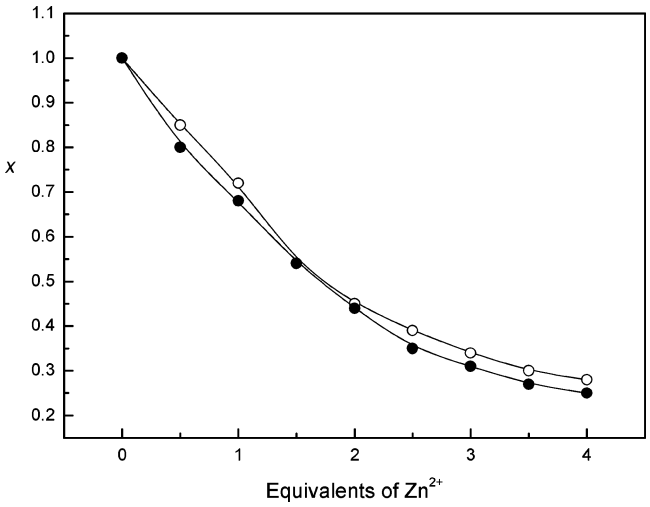

Figure 11. Titration of 1,4-bis[(1,5,9-triazacyclododecan-3-yl)oxymethyl]-benzene (**3**) with Zn^{2+} at 35 °C (●) and 90 °C (○). Mole fraction of uncomplexed azacrown moieties (determined by ^1H NMR) plotted against the number of equivalents of Zn^{2+} compared to **3**.

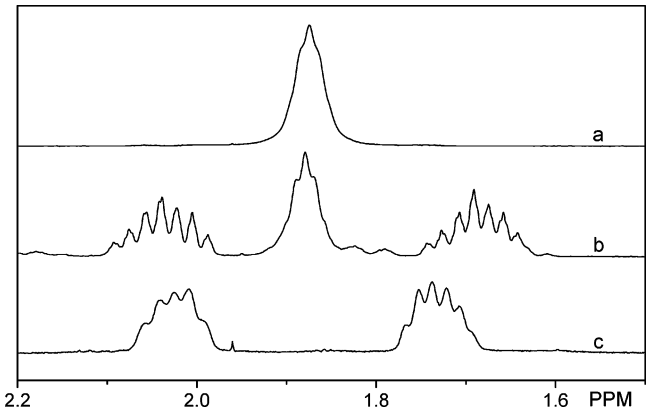

Figure 12. ^1H NMR signal of H7,H11 protons of ligand **3** in D_2O at pD 7.1 and 35 °C. (a) Ligand **3** (2.0 mmol L^{-1}); (b) ligand **3** (2.0 mmol L^{-1}) + Zn^{2+} (4.0 mmol L^{-1}); and (c) ligand **3** (2.0 mmol L^{-1}) + Zn^{2+} (4.0 mmol L^{-1}) + UpU (2.0 mmol L^{-1}).

moieties. However, 45% of the azacrown moieties remained uncomplexed. Addition of 1 equiv of UpU then resulted in complete disappearance of the signal of uncomplexed [12]aneN$_3$.

Discussion

Cleavage of UpU. As seen from Table 2, the rate constant for the cleavage of UpU by the monomeric Zn^{2+} complex of [12]aneN$_3$ is 2.6×10^{-5} s^{-1} under the experimental conditions of the present work ([Zn^{2+}] = 1.0 mmol L^{-1}, T = 90 °C, pH 6.84, I = 0.1 mol L^{-1}),[20,29] representing a 20-fold rate acceleration compared to the metal-ion-independent cleavage.[30] The cleavage rate obtained in this work with bifunctional ligands **1**−**5** at the same Zn^{2+} concentration is only slightly higher (Table 1). Accordingly, incorporation of two [12]aneN$_3$ moieties into a single ligand structure does not result in any marked change in the cleavage rate. However, the situation is dramatically changed upon addition of a third [12]aneN$_3$ moiety. The trifunctional ligand **6** at the overall Zn^{2+} concentration of 1.5

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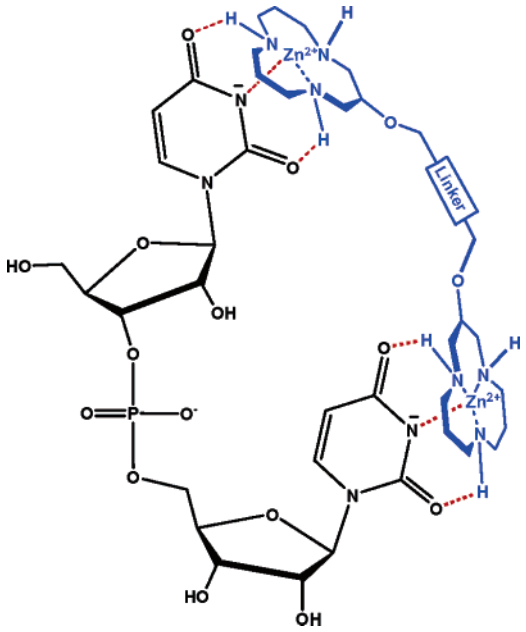
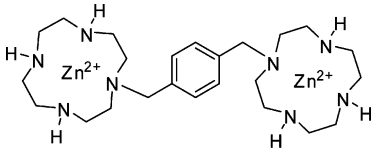


Figure 13. Assumed structure of the dinuclear mixed-ligand Zn^{2+} complexes of UpU and ligands **1**−**5**.

mmol L^{-1} accelerates the cleavage of UpU 50 times as efficiently as its difunctional counterpart (**2**) at [Zn^{2+}] = 1.0 mmol L^{-1} (1000-fold acceleration compared to the metal-ion-independent reaction). Since it has been shown that the dinuclear Zn^{2+} complex of a closely related di(azacrown) ligand (**20**) binds



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under neutral conditions at micromolar concentrations to thymidylyl-3′,5′-thymidine by displacement of the *N*3 proton of both base moieties,[19e] it appears reasonable to assume that ligands **1**−**6** in the presence of Zn^{2+} form a similar mixed-ligand complex with UpU. The assumed structure of the complexes obtained with ligands **1**−**5** is depicted in Figure 13. Formation of this kind of complex does not accelerate the cleavage, since both azacrown moieties are engaged in the base moiety binding. The observed slow cleavage evidently is of the same origin as the cleavage promoted by Zn^{2+}[12]aneN$_3$ or Zn^{2+}−aquo ion, involving binding of the catalytically active metal ion only to the scissile monoanionic phosphodiester linkage.[9b,c] An aquo ligand of the phosphate-bound Zn^{2+} then serves as an intramolecular general acid, protonating the departing oxygen in concert with the PO bond cleavage. Evidently, binding of another dinuclear complex to the base moieties does not prevent this reaction. The trinuclear complex of ligand **6**, in turn, undergoes a high-affinity pre-equilibrium binding to the substrate. Two of the azacrown moieties anchor as Zn^{2+} chelates the cleaving agent to the base moieties of UpU, while the third participates as an intracomplex catalyst in the cleavage of the phosphodiester linkage. The mechanism of the actual cleavage step is, in all likelihood, similar to that described above, viz. a pre-equilibrium formation of a dianionic phosphorane intermediate followed by rate-limiting proton transfer from an aquo ligand of the phos-

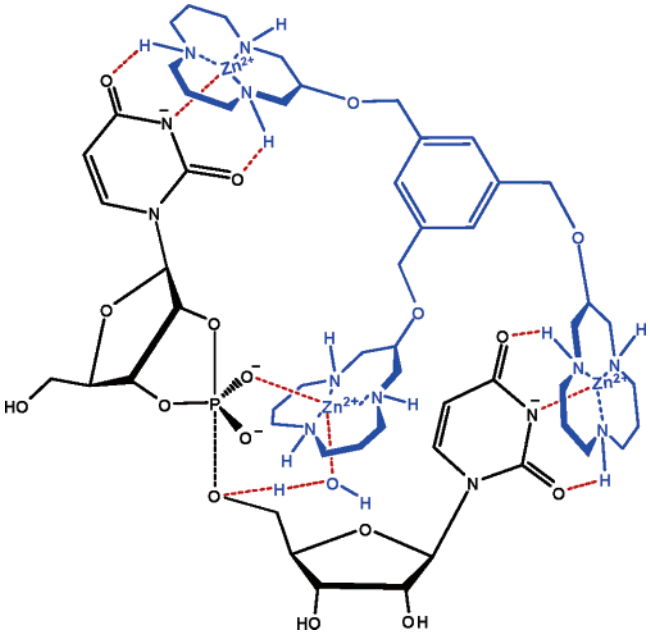

Figure 14. Assumed transition-state structure for the cleavage of the trinuclear mixed-ligand Zn^{2+} complex of UpU and ligand **6**.

phorane-bound metal ion to the departing 5′-oxyanion. Figure 14 shows the assumed transition-state structure for the cleavage reaction.

The UV and ^{1}H NMR spectroscopic studies on a ternary system consisting of UpU, ligand **3**, and Zn^{2+} verify that the complex UpU-$(Zn^{2+})_2$-**3** (cf. Figures 10 and 12) really is stable at millimolar concentrations. The ^{1}H NMR spectroscopic measurements show that both of the uracil bases are involved in metal ion binding: upon addition of **3** and Zn^{2+} ([**3**]:[Zn^{2+}] 1:2), the *H*6 proton doublets at 7.8 ppm disappear and new signals at 7.5 and 7.6 ppm appear. For comparison, the *H*2 and *H*8 signals of ApA do not respond to addition of **3** and Zn^{2+}. UV spectrophotometric titrations, in turn, allow estimation of the stability of the UpU-$(Zn^{2+})_2$-**3** complex. The UV absorption of a 50 μmol L^{-1} solution of UpU (pH 7.5, T = 25 °C, I = 0.1 mol L^{-1}) decreases linearly upon addition of ligand **3** and Zn^{2+} ([**3**]:[Zn^{2+}] 1:2) until the molar ratio of UpU, **3**, and Zn^{2+} reaches the value 1:1:2, and remains constant thereafter (Figure 9). At pH 7.0, **3** has to be present in 70% excess to reach the plateau value (at a molar ratio of 1:1.6:3.2). On the basis of the latter observation, the complex UpU-$(Zn^{2+})_2$-**3** may be estimated to be half dissociated when **3** and Zn^{2+} are present in 15 and 30 μmol L^{-1} excess, respectively, at pH 7.0, referring to pH 6.2 at 90 °C. For comparison, the dissociation constant for the complex TpT-$(Zn^{2+})_2$-**20** has been reported to be 0.6 μmol L^{-1} at pH 7.3.[19e] On going to lower pH, the stability of the complex UpU-$(Zn^{2+})_2$-**3** is dramatically decreased: at pH 6.5 (5.84 at 90 °C), the absorption experiences only a modest continuous decrease upon addition of **3** and Zn^{2+}. This is expected since the Zn^{2+} ions have to compete with protons for both the uracil bases and the azacrown ligands. The pK_a value of a uracil moiety in a dinucleoside 3′,5′-monophosphate is 9.0 (25 °C, I = 0.1 mol L^{-1}),[31] and the pK_a values of ligand **3** expectedly are somewhat lower than those reported for [12]aneN$_3$ (12.6 and 7.6 at 25 °C, I = 0.1 mol L^{-1}).[32]

According to ^{1}H NMR spectroscopic measurements, the dinuclear complex $(Zn^{2+})_2$-**3** is considerably less stable than the complex UpU-$(Zn^{2+})_2$-**3**. While addition of 1 equiv of ligand **3** and 2 equiv of Zn^{2+} to a solution of UpU (4 mmol L^{-1}, pD 7.1) is sufficient to convert UpU entirely to UpU-$(Zn^{2+})_2$-**3**, as evidenced by complete disappearance of the *H*6 resonances at 7.8 ppm and appearance of new signals at 7.4 and 7.5 ppm (Figure 10), addition of 2 equiv of Zn^{2+} to a solution of **3** (2 mmol L^{-1}, pD 7.1) still leaves 40% of the azacrown moieties uncomplexed (Figure 11). Interestingly, the situation is very similar at both 35 and 90 °C. Addition of 1 equiv of UpU to this mixture, however, results in quantitative binding of Zn^{2+} to ligand **3** (Figure 12).

Since the kinetic experiments of UpU cleavage have been carried out in excess of the ligand and Zn^{2+} ([**6**]:[Zn^{2+}] 1:3), the reaction solutions contain uncomplexed Zn^{2+} in addition to UpU-$(Zn^{2+})_3$-**6**. As mentioned above, the first-order rate constant for the cleavage of UpU by Zn^{2+}[12]aneN$_3$ is 2.6 × 10^{-5} s^{-1} under the experimental conditions of the present work ([Zn^{2+}] = 1 mmol L^{-1}, pH 6.84). Catalysis by Zn^{2+}−aquo ion has previously been studied at pH < 6 and shown to be first-order in hydroxide ion concentration.[33] Extrapolation to pH 6.84 shows the rate constant for the Zn^{2+}-promoted cleavage to be 3 × 10^{-5} s^{-1} at [Zn^{2+}] = 1.0 mmol L^{-1}. In the pH range used in the present study, both reactions are approximately first-order in hydroxide ion concentration. Since the rate constant for the cleavage reaction promoted by the Zn^{2+} complex of **6** varies from 3.5 × 10^{-4} s^{-1} at pH 5.8 to 1.6 × 10^{-3} s^{-1} at pH 6.8, the contribution of uncomplexed Zn^{2+} ion to the observed rate constants may be neglected. Consistent with this argument, the rate constants obtained at pH 6.84 for the cleavage of UpU at 0.5 and 1.5 mmol L^{-1} concentrations of **6** ([Zn^{2+}] = 1.5 and 4.5 mmol L^{-1}, respectively) are equal within the limits of experimental errors: (1.58 ± 0.02) × 10^{-3} and (1.56 ± 0.03) × 10^{-3} s^{-1} (Table 2). Evidently, at this pH, UpU is quantitatively engaged in the complex UpU-$(Zn^{2+})_3$-**6**. The pH−rate profile is, hence, quite similar to that reported previously for the Zn^{2+}[12]aneN$_3$,[28] leveling off to constant value on passing the pK_a value of the chelated Zn^{2+}−aquo ion (with Zn^{2+}[12]-aneN$_3$, 7.5 at 25 °C, I = 0.1 mol L^{-1})[31] that participates in the catalysis. By contrast, the rate constants obtained with ligands **1**−**5** undoubtedly contain contributions of uncomplexed Zn^{2+} ion and various Zn^{2+} complexes; hence, the pH−rate profiles cannot be analyzed on the basis any single reaction.

Cleavage of ApU and UpA. In striking contrast to UpU, dinucleoside 3′,5′-monophosphates containing only one uracil base, viz. ApU and UpA, are cleaved by the Zn^{2+} complexes of the dinucleating ligands (**1**−**5**) up to 2 orders of magnitude more rapidly than by Zn^{2+}[12]aneN$_3$. Interestingly, a similar 100-fold acceleration has been obtained by tethering [12]aneN$_3$ to a 2′-*O*-methylribo oligonucleotide complementary to the target oligoribonucleotide.[34] Evidently, a 100-fold acceleration may be attributed to a proximity effect, i.e., to increased concentration of the cleaving agent in the vicinity of the scissile bond. On using an oligonucleotide conjugate, the proximity effect is achieved by hybridization. On using $(Zn^{2+})_2$-**1**, a similar effect appears to be obtained by anchoring one of the Zn^{2+} chelate moieties of the cleaving agent to the uracil base. The

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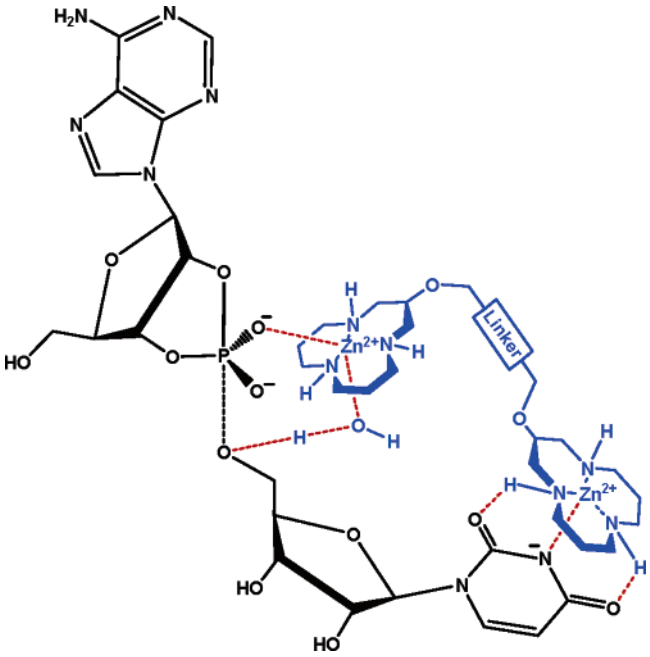

Figure 15. Assumed transition-state structure for the cleavage of ApU by dinuclear Zn^{2+} complexes of ligands **1**−**5**.

remaining Zn^{2+} chelate moiety is then able to bind to the phosphodiester linkage and facilitate the departure of the 5′-linked nucleoside as an intracomplex general acid. A hypothetical structure of the transition state is depicted in Figure 15. Evidently, binding to a single uracil moiety is sufficiently strong to ensure quantitative complex formation in an excess of a dinucleating ligand and Zn^{2+}, since the pH−rate profiles (Figure 3) obtained with ApU at [**3**] = 0.5 and 2.0 mmol L^{-1} ([Zn^{2+}] = 1.0 and 4.0 mmol L^{-1}, respectively) are almost identical (Figure 3). Only at pH 5.8, a moderate rate acceleration takes place upon increasing the concentration of **3**. Evidently, the pre-equilibrium anchoring is not any more quantitative at such a low pH when the concentration of **3** is 0.5 mmol L^{-1}. The situation is similar with UpA: only a slight rate acceleration with increasing catalyst concentration is observed (Table 2). The cleavage by uncomplexed Zn^{2+} or mononuclear Zn^{2+} complexes is too slow to compete with the intracomplex reaction around neutrality (Table 2). Accordingly, the pH−rate profiles again resemble those obtained with Zn^{2+}[12]aneN$_3$. The trinucleating ligand (**6**) cleaves ApU and UpA approximately as efficiently as UpU. Most likely, one of the azacrown moieties is engaged in base moiety binding, and only one of the remaining coordination sites binds Zn^{2+}. The latter assumption is based on the observation that, at pH 7.5 (6.84 at 90 °C), the Zn^{2+}−azacrown complex is considerably less stable than the mixed-ligand complex with the uracil base and azacrown.

The argument that one uracil base is sufficient to anchor the cleaving agent to ApU receives support from the UV spectrophotometric titrations. At pH 7.5, 2 equiv of ligand **3** and 4 equiv of Zn^{2+} are sufficient to convert ApU at a concentration of 50 μmol L^{-1} to ApU-(Zn^{2+})$_n$-**3**. The complex is half-dissociated when **3** and Zn^{2+} are present in 15 and 30 μmol L^{-1} excess, respectively. Whether the complex contains one or two Zn^{2+} ions cannot be deduced from the spectrophotometric data. Obviously, only the dinuclear complex can be catalytically active.

Cleavage of ApA. ApA is cleaved by the Zn^{2+} complexes of **1**−**6** only slightly more rapidly than by Zn^{2+}[12]aneN$_3$ (Table 2). With ApA, the only feasible interaction between the cleaving agent and the base moieties is a stacking interaction, and this interaction is too weak to result in a marked proximity effect. While with ApU and UpA the cleavage rate is independent of the concentration of (Zn^{2+})$_2$-**3**, consistent with quantitative formation of the catalytically active mixed-ligand complex, the cleavage rate of ApA expectedly depends on the catalyst concentration, analogously to Zn^{2+}[12]aneN$_3$-promoted cleavage. The somewhat higher catalytic activity of the Zn^{2+} complex of **6** may be attributed to the fact that, with this ligand, the overall Zn^{2+} concentration is 1.5 mmol L^{-1}, compared to the 1.0 mmol L^{-1} concentration used with the other ligands. It is also worth noting that, since a stable complex between ApA, Zn^{2+}, and the azacrown-derived ligand is not formed and the ligands employed do not bind Zn^{2+} quantitatively over the entire pH range studied, the rate constants most likely include contributions of various metal-ion-containing species.

Dependence of the Cleavage Rate on pH and the Ligand Structure. As discussed above, the pH−rate profiles for the cleavage of ApU and UpA with **1**−**6** and UpU with **6** resemble the profile reported previously for the Zn^{2+}[12]aneN$_3$-promoted cleavage.[28] In other words, the observed rate constant levels off to a constant value on passing the pK_a value of the chelated Zn^{2+}−aquo ion (with Zn^{2+}[12]aneN$_3$, 7.5 at 25 °C, I = 0.1 mol L^{-1})[31] that participates in the catalysis. At pH less than the pK_a of the chelated Zn^{2+}−aquo ion, one proton is lost on going from the initial state to the transition state indicated in Figure 13, since the attacking 2′-OH has to be deprotonated to obtain the dianionic phosphorane. At pH greater than the pK_a of the chelated Zn^{2+}−aquo ion, the initial state and transition state are tautomeric structures: in the initial state, 2′-OH bears a proton and one of the Zn^{2+} ligands is deprotonated, whereas on going to the transition state, 2′-OH undergoes deprotonation and the Zn^{2+}−hydroxo ligand protonation. Accordingly, rate equation (1) is obeyed. Here, K_a is the acidity constant of the

$$k_{obs} = k\frac{K_a}{K_a + [H^+]} \qquad (1)$$

catalytically active chelated Zn^{2+}−aquo ion and k is the first-order rate constant for the cleavage of the complexed dinucleoside monophosphate. Figures 1−6 show the curves obtained by least-squares fitting for the cleavage of ApU, UpA, and UpU (only on using **6** as a cleaving agent). While this equation in many cases fits excellently to the experimental rate constants, some of the curves tend to exhibit more marked curvature than predicted by eq 1. Evidently, the complex formation is not always quantitative over the whole pH range studied. The pK_a values obtained for the chelated Zn^{2+}−aquo ion, serving as an intracomplex catalyst, and the first-order rate constants, k, for the cleavage of the fully complexed dinucleoside monophosphates are given in Table 3. The cleavage of UpU and ApA is not markedly accelerated by the dinucleating ligands (**1**−**5**), and the rate constants obtained undoubtedly contain contributions of uncomplexed Zn^{2+} ion and various Zn^{2+} complexes; hence, the pH−rate profiles cannot be analyzed on the basis any single reaction.

As seen from Table 1, the cleaving activities of all five dinuclear complexes (**1**−**5**) studied are surprisingly similar. Only

Table 3. Kinetic Parameters for the Cleavage of ApU, UpA, and UpU When Engaged in a Mixed-Ligand Complex NpN-$(Zn^{2+})_2$-L (L = **1**−**5**) or NpN-$(Zn^{2+})_3$-**6**[a]

nucleoside monophosphate	ligand	pK_a	$k/10^{-3}$ s^{-1}
ApU	**1**	6.7 ± 0.3	2.8 ± 0.9
	2	6.8 ± 0.1	2.7 ± 0.5
	3	6.8 ± 0.2	2.7 ± 1.1[b]
		6.5 ± 0.1	1.9 ± 0.1[c]
	4	6.8 ± 0.2	0.77 ± 0.02
	5	6.9 ± 0.1	2.1 ± 0.5
	6	6.1 ± 0.2	2.4 ± 0.2
UpA	**1**	6.6 ± 0.4	2.3 ± 0.5
	2	6.8 ± 0.1	2.2 ± 0.3
	3	6.6 ± 0.2	1.0 ± 0.2
	4	6.6 ± 0.2	1.0 ± 0.4
	5	6.9 ± 0.2	2.6 ± 0.7
	6	6.1 ± 0.2	1.6 ± 0.2
UpU	**6**	6.3 ± 0.2	1.7 ± 0.4

[a] pK_a values refer to the chelated Zn^{2+}−aquo ion serving as an intracomplex catalyst and the first-order rate constants, k, to the cleavage of a fully complexed dinucleoside monophosphate at 90 °C (I = 0.1 mol L^{-1}). [b] At [**3**] = 0.5 mmol L^{-1} and [Zn^{2+}] = 1.0 mmol L^{-1}. [c] At [**3**] = 2.0 mmol L^{-1} and [Zn^{2+}] = 4.0 mmol L^{-1}.

the 4,4′-biphenyl-bridged complex $(Zn^{2+})_2$-**4** is somewhat less efficient than the others, the cleaving activity toward ApU and UpA being 22% and 35% of that of the most efficient cleaving agent, the *o*-phenylene-bridged complex $(Zn^{2+})_2$-**1**. The *p*-phenylene-bridged complex $(Zn^{2+})_2$-**3** is, in turn, the only one that exhibits marked selectivity between ApU and UpA, the former being cleaved almost 3 times as fast as the latter. In other words, the proximity effect is rather insensitive to the structure of the scaffold that links the two nucleating moieties to each other. Evidently, the freely rotating −CH_2O− bridge between the aromatic nucleus and the azacrown moieties allows the cleaving agent to find a conformation where simultaneous binding to the base and phosphate moieties is possible. Both of the latter interactions are of electrostatic origin and, hence, as long-range interactions are able to guide the cleaving agents to a proper conformation.

Conclusions

Dinuclear complexes containing two Zn^{2+}[12]aneN_3 moieties attached to an aromatic core show potential as base-selective cleaving agents of RNA phosphodiester bonds. With dinucleoside 3′,5′-monophosphates containing only one uracil base, one of the Zn^{2+}[12]aneN_3 moieties anchors the cleaving agent to the substrate, while the other Zn^{2+} chelate moiety serves as an intracomplex catalyst for the cleavage of the neighboring phosphodiester bond. A 100-fold rate acceleration, compared to that obtained with a monomeric Zn^{2+}[12]aneN_3, is achieved at 0.5 mmol L^{-1} concentration of the cleaving agent. Substrates containing two uracil bases are not cleaved, since both Zn^{2+}-[12]aneN_3 moieties are engaged in uracil binding. The catalytic activity is, however, restored by addition of a third azacrown group on the cleaving agent.

Experimental Section

General. All reagents employed were of reagent grade, and they were used without further purification. All nucleotides and nucleosides were products of Sigma. They were used as received after checking the purity by HPLC.

***cis*- and *trans*-5-(Benzyloxy)-2-phenyl-1,3-dioxane (8).** A mixture of *cis*-2-phenyl-1,3-dioxan-5-ol (**7**)[35] (0.113 mol, 20.95 g), benzyl chloride (0.233 mol, 29.5 g), and powdered KOH (0.243 mol, 13.62 g) in dry benzene (600 mL) was heated in a Dean−Stark apparatus until the separation of water was complete (12 h), as described previously for the preparation of closely related compounds.[36] The solution was cooled to room temperature, washed with water, 5% $NaHCO_3$ aqueous solution, and water, and then dried with Na_2SO_4. Evaporation of the solvent under reduced pressure gave a yellow solid, which was further purified on a silica gel column, eluting first with neat dichloromethane (DCM) and then with a 5:95 (v/v) mixture of MeOH and DCM. Although the starting material (**7**) was a pure cis isomer, compound **8** was obtained as a mixture of cis and trans isomers. Facile isomerization of this compound in $CDCl_3$ has been previously reported.[37] The overall yield was 99% (31.0 g). ^{1}H NMR ($CDCl_3$), trans isomer δ 7.41 (m, 10H), 5.43 (s, 1H), 4.64 (s, 2H), 4.39 (dd, $J_{4eq5ax} = J_{6eq5ax}$ = 5.0 Hz, $J_{4eq4ax} = J_{6eq6ax}$ = 11.1 Hz, 2H), 3.83 (tt, $J_{4eq5ax} = J_{6eq5ax}$ = 5.0 Hz, $J_{4ax5ax} = J_{6ax5ax}$ = 10.1 Hz, 1H), 3.69 (t, J = 11.0 Hz, 2H); cis isomer δ 7.44 (m, 10H), 5.59 (s, 1H), 4.74 (s, 2H), 4.41 (dd, $J_{4eq5eq} = J_{6eq5eq}$ = 1.3 Hz, $J_{4eq4ax} = J_{6eq6ax}$ = 12.6 Hz, 2H), 4.08 (dd, $J_{4ax5eq} = J_{6ax5eq}$ = 1.7 Hz, $J_{4eq4ax} = J_{6eq6ax}$ = 12.6 Hz, 2H), 3.38 (quint., J = 1.7 Hz, 1H). ^{13}C NMR ($CDCl_3$): trans isomer δ 137.89, 137.64, 129.01, 128.59, 128.31, 128.08, 127.79, 126.06, 101.31, 71.87, 70.23, 67.93; cis isomer δ 138.14, 138.11, 128.93, 128.47, 128.22, 127.78, 127.73, 126.22, 101.42, 70.32, 69.20, 69.04.

2-(Benzyloxy)propane-1,3-diol (9). 5-(Benzyloxy)-2-phenyl-1,3-dioxane (**8**) (55.6 mmol, 15.0 g) was dissolved in MeOH (300 mL), and 1 mol L^{-1} HCl (50 mL) was added. The mixture was heated for 1 h under reflux. All volatiles were removed under reduced pressure, and the residue was subjected to a similar treatment. Compound **9** was obtained as a colorless oil in quantitative yield (10.1 g) and used without purification for the next reaction. ^{1}H NMR ($CDCl_3$): δ 7.37 (m, 5H), 4.69 (s, 2H), 3.83 (dd, J = 4.4, 11.7 Hz, 2H), 3.76 (dd, J = 5.0, 11.7 Hz, 2H), 3.64 (quint., J = 4.7 Hz, 1H), 2.02 (br s, 2H). ^{13}C NMR ($CDCl_3$): δ 137.99, 128.64, 128.06, 127.86, 79.18, 72.02, 62.39.

2-(Benzyloxy)propane-1,3-diyl Bis(4-methylbenzenesulfonate) (10). A mixture of 2-(benzyloxy)propane-1,3-diol (**9**) (55.6 mmol, 10.1 g) in THF (250 mL) and aqueous sodium hydroxide (100 mL of a 2.2 mol L^{-1} solution) was placed in a flask equipped with a dropping funnel and a thermometer. The mixture was cooled on an ice−salt bath with vigorous stirring, and *p*-toluenesulfonyl chloride (122 mmol, 23.4 g) in THF (150 mL) was added dropwise during 1 h, as described in the literature for preparation of similar tosylates.[38] The temperature was kept between 3 and 5 °C during the addition. Stirring at 3−5 °C was continued, and the progress of the reaction was monitored by TLC every 10−15 min. Upon completion of the reaction, concentrated aqueous HCl was added to quench the reaction. THF was removed under reduced pressure, and the residue was extracted with DCM (5 × 60 mL). All extracts were combined, dried with Na_2SO_4, and evaporated to dryness under reduced pressure. Recrystallization from ethanol gave the pure product in 85% yield (23.2 g). ^{1}H NMR ($CDCl_3$): δ 7.75 (d, J = 8.3 Hz, 4H), 7.33 (m, 7H), 7.20 (m, 2H), 4.51 (s, 2H), 4.06 (m, J = 5.0, 10.7 Hz), 3.82 (quint., J = 5.0 Hz, 1H), 2.47 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 145.17, 136.97, 132.38, 129.97, 128.46, 128.06, 127.98, 127.80, 73.83, 72.53, 67.71, 21.71.

2-(Benzyloxy)hexahydro-3a,6a,9a-triazaphenalene (11). A mixture of ditosylate **10** (20.4 mmol, 10 g), 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD, 20.4 mmol, 2.84 g), and powdered KOH (82 mmol, 4.6 g) in dry 1,4-dioxane (300 mL) was added to a flask. The mixture was heated for 48 h under reflux. The completeness of the reaction was verified by LC/ESI-MS (m/z 286 [M]$^+$). The mixture was then cooled

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to room temperature, and sodium borohydride (0.10 mol, 3.8 g) was slowly added. Stirring was continued for 48 h at room temperature, and the formation of **11** was checked by LC/ESI-MS (*m*/*z* 288 $[M + H]^+$ and 310 $[M + Na]^+$). The solvent was removed under reduced pressure. Water (100 mL) was added to the residue, and the aqueous phase was extracted with DCM (5 × 50 mL). The combined DCM extracts were dried with Na_2SO_4 and evaporated to dryness under reduced pressure. The crude product was used for the next step without purification.

1,5,9-Triazacyclododecan-3-ol (12). The crude product of **11** was heated for 4 d in 4 mol L^{-1} aqueous HCl (600 mL) under reflux and evaporated to dryness under reduced pressure. Addition of MeOH (80 mL) to the residue gave **12** as a pure trihydrochloride in 93% yield (5.63 g) (calculated by comparison to **10**). ^{1}H NMR (D_2O): δ 4.25 (quint., J = 5.3 Hz, 1H), 3.37 (m, 12H), 2.16−2.11 (quint., J = 6.6 Hz, 4H). ^{13}C NMR (D_2O): δ 61.65, 47.93, 43.20, 41.00, 19.24. HR-MS for $C_9H_{21}N_3O$: requires 187.1685, found 187.1683.

Tri-*tert*-butyl 3-Hydroxy-1,5,9-triazacyclododecane-1,5,9-tricarboxylate (13). 1,5,9-Triazacyclododecan-3-ol trihydrochloride (19.0 mmol, 5.63 g) was dissolved in a mixture of *tert*-butyl alcohol (120 mL) and aqueous sodium hydroxide (80 mL of 2.9 mol L^{-1} solution). Di-*tert*-butyl dicarbonate (Boc_2O, 62.8 mmol, 13.7 g) in *tert*-butyl alcohol (50 mL) was added to this solution with vigorous stirring. The stirring was continued at room temperature overnight and then at 40−50 °C for 2 d. Formation of **13** was followed by LC/ESI-MS (*m*/*z* 488 $[M + H]^+$, 510 $[M + Na]^+$). Because the reactivity of the hydroxyl group turned out to be only moderately lower than that of the ring nitrogen atoms, Boc_2O could not be used in excess, and still ester formation could not be entirely avoided. The 2 days of incubation at 40−50 °C, however, resulted in isomerization to the desired product. The solvent was removed under reduced pressure, water (50 mL) was added, and the product was extracted to DCM (5 × 40 mL). The combined extracts were dried with Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography (silica gel, 5% MeOH in DCM). Yield: 7.7 g (83.2%). ^{1}H NMR ($CDCl_3$, 50 °C): δ 4.04 (quint., J = 5.8 Hz, 1H), 3.44 (m, 12H), 1.85 (quint., J = 6.7 Hz, 4H), 1.50 (s, 18H), 1.48 (s, 9H). ^{13}C NMR ($CDCl_3$, 50 °C): δ 155.77, 80.65, 79.55, 73.55, 53.56, 47.72, 43.43, 28.44, 28.36. HR-MS for $(C_{24}H_{45}N_3O_7 + H)^+$: requires 488.3336, found 488.3328.

General Procedure for Preparation of the Boc-Protected Ligands (14−19). α,α′-Bis(halomethyl)arene (0.72 mmol, to obtain **1**−**4**), pyridine-2,6-diyl ditosylate (0.72 mmol, to obtain **5**), or 1,3,5-tris(bromomethyl)benzene (0.48 mmol, to obtain **6**) was dissolved in dry DMF (20 mL), and tri-*tert*-butyl 1,5,9-triazacyclododecan-3-ol-1,5,9-tricarboxylate (**13**) (1.44 mmol, 0.7 g) was added. Dry sodium hydride (2.1 mmol, 50 mg) was added, and the mixture was stirred for 1 h at room temperature. The unreacted sodium hydride was destroyed with MeOH (2 mL), and the volatiles were removed under reduced pressure. The residue was dissolved in water (40 mL) and extracted with DCM (5 × 20 mL). The combined extracts were dried with Na_2SO_4 and evaporated to dryness under reduced pressure. The product was purified by column chromatography (silica gel, 25−40% ethyl acetate in DCM).

Hexa-*tert*-butyl 3,3′-[1,2-Phenylenebis(methylene)bis(oxy)]bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate) (14). 1,2-Bis(bromomethyl)benzene (0.72 mmol, 0.19 g) was used as a starting material. Compound **14** was obtained in 76% yield (0.59 g). ^{1}H NMR ($CDCl_3$, 50 °C): δ 7.37 (m, 2H), 7.24 (m, 2H), 4.67 (s, 4H), 3.84 (quint., J = 4.9 Hz, 2H), 3.59 (dd, J = 6.1, 14.8 Hz, 4H), 3.42 (m, 12H), 3.11 (m, 8H), 2.01 (m, 4H), 1.74 (m, 4H), 1.45 (s, 54H). ^{13}C NMR ($CDCl_3$, 50 °C): δ 156.22, 155.95, 136.42, 128.64, 127.59, 79.79, 79.54, 75.33, 69.13, 49.53, 47.02, 44.79, 29.43, 28.43, 28.40. HR-MS for $(MH - C_5H_8O_2)^+$, $C_{56}H_{96}N_6O_{14}$: requires 977.6538, found 977.6503.

Hexa-*tert*-butyl 3,3′-[1,3-Phenylenebis(methylene)bis(oxy)]bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate) (15). 1,3-Bis(bromomethyl)benzene (0.72 mmol, 0.19 g) was used. Compound **15** was obtained in 83% yield (0.64 g). ^{1}H NMR ($CDCl_3$, 50 °C): δ 7.24 (m, 4H), 4.60 (s, 4H), 3.86 (m, 2H), 3.38 (m, 24H), 2.01 (m, 4H), 1.76 (m, 4H), 1.46 (s, 36H), 1.44 (s, 18H). ^{13}C NMR ($CDCl_3$, 50 °C): δ 156.20, 155.94, 138.67, 128.28, 126.78, 126.55, 79.77, 79.53, 75.68, 71.78, 49.70, 46.89, 44.98, 29.40, 28.40. HR-MS for $(MH - C_5H_8O_2)^+$, $C_{56}H_{96}N_6O_{14}$: requires 977.6538, found 977.6503.

Hexa-*tert*-butyl 3,3′-[1,4-Phenylenebis(methylene)bis(oxy)]bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate) (16). 1,4-Bis(bromomethyl)benzene (0.72 mmol, 0.19 g) was used. Compound **16** was obtained in 85% yield (0.66 g). ^{1}H NMR ($CDCl_3$, 50 °C): δ 7.28 (s, 4H), 4.61 (s, 4H), 3.88 (m, 2H), 3.41 (m, 24H), 2.04 (m, 4H), 1.79 (m, 4H), 1.48 (s, 36H), 1.47 (s, 18H). ^{13}C NMR ($CDCl_3$, 50 °C): δ 156.25, 155.97, 137.93, 127.49, 79.82, 79.59, 75.67, 71.72, 49.69, 47.00, 45.01, 29.45, 28.43, 28.42. HR-MS for $(MH - C_5H_8O_2)^+$, $C_{56}H_{96}N_6O_{14}$: requires 977.6538, found 977.6520.

Hexa-*tert*-butyl 3,3′-[Biphenyl-4,4′-diylbis(methylene)bis(oxy)]bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate) (17). 4,4′-Bis(chloromethyl)biphenyl (0.72 mmol, 0.18 g) was used. Compound **17** was obtained in 82% yield (0.68 g). ^{1}H NMR ($CDCl_3$): δ 7.51 (d, J = 8.1 Hz, 4H), 7.37 (d, J = 8.1 Hz, 4H), 4.64 (s, 4H), 3.87 (quint., J = 5.9 Hz, 2H), 3.60 (dd, J = 5.9, 14.9 Hz, 4H), 3.40 (m, 12H), 3.16 (m, 8H), 2.01 (m, 4H), 1.74 (m, 4H), 1.46 (s, 36H), 1.42 (s, 18H). ^{13}C NMR ($CDCl_3$): δ 156.29, 156.01, 140.28, 137.50, 128.11, 127.01, 79.91, 79.66, 75.30, 71.55, 49.50, 47.05, 44.93, 29.46, 28.43. HR-MS for $(MH - C_5H_8O_2)^+$, $C_{62}H_{100}N_6O_{14}$: requires 1053.6851, found 1053.6873.

Hexa-*tert*-butyl 3,3′-[Pyridine-2,6-diylbis(methylene)]bis(oxy)bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate) (18). 2,6-Bis(tosyloxymethyl)pyridine[24] (0.72 mmol, 0.32 g) was used. Compound **18** was obtained in 76% yield (0.59 g). ^{1}H NMR ($CDCl_3$, 50 °C): δ 7.56 (t, J = 7.7 Hz, 1H), 7.26 (d, J = 7.7 Hz, 2H), 4.60 (s, 4H), 3.84 (quint., J = 5.8 Hz, 2H), 3.54 (dd, J = 6.2, 14.9 Hz, 4H), 3.35 (m, 12H), 1.94 (m, 4H), 1.70 (m, 4H), 1.36 (s, 36H), 1.35 (18H). ^{13}C NMR ($CDCl_3$, 50 °C): δ 157.76, 156.10, 155.84, 137.08, 119.84, 79.72, 79.45, 76.43, 72.59, 49.30, 46.96, 44.76, 29.39, 28.34, 28.31. HR-MS for $(C_{55}H_{95}N_7O_{14} + H)^+$: requires 1078.7015, found 1078.6977.

Nona-*tert*-butyl 3,3′,3″-[Benzene-1,3,5-triyltris(methylene)]tris(oxy)tris(1,5,9-triazacyclododecane-1,5,9-tricarboxylate) (19). 1,3,5-Tris(bromomethyl)benzene[25] (0.48 mmol, 0.17 g) was used. Compound **19** was obtained in 80% yield (0.56 g). ^{1}H NMR ($CDCl_3$, 50 °C): δ 7.07 (s, 3H), 4.49 (s, 6H), 3.77 (quint., J = 5.9 Hz, 3H), 3.49 (dd, J = 6.1, 14.9 Hz, 6H), 3.32 (m, 18H), 3.07 (m, 12H), 1.92 (m, 6H), 1.68 (s, 6H), 1.35 (s, 54H), 1.34 (s, 27H). ^{13}C NMR ($CDCl_3$, 50 °C): δ 156.05, 155.80, 138.78, 125.69, 79.61, 79.36, 75.79, 71.71, 49.62, 46.85, 44.82, 29.36, 28.34, 28.32. HR-MS for $(MH - C_5H_8O_2)^+$, $C_{81}H_{141}N_9O_{21}$: requires 1476.9796, found 1476.9840.

General Procedure for Preparation of Ligands 1−6. Compounds **14**−**19** were dissolved in MeOH (30 mL). Concentrated aqueous HCl (0.8 mL) was added. The mixture was incubated at 40−45 °C for 2 h. Upon the solution cooling to 0 °C, the deprotected product precipitated as a hydrochloride. The hydrochlorides were converted to free bases by passing their aqueous solutions through a strong anion-exchange resin (Dowex 1X2, OH^- form).

1,2-Bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]benzene Hexahydrochloride (1). Yield: 94%. ^{1}H NMR (D_2O): δ 7.42 (m, 2H), 7.34 (m, 2H), 4.73 (s, 4H), 4.04 (m, 2H), 3.23 (m, 24H), 2.03 (m, 8H). ^{13}C NMR (D_2O): δ 135.13, 129.38, 128.90, 70.26, 68.80, 47.06, 44.05, 41.63, 19.97. HR-MS for $(C_{26}H_{48}N_6O_2 + H)^+$: requires 477.3917, found 477.3892.

1,3-Bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]benzene Hexahydrochloride (2). Yield: 92%. ^{1}H NMR (D_2O, 50 °C): δ 7.68 (m, 4H), 4.93 (s, 4H), 4.31 (m, 2H), 3.68 (dd, J = 5.4, 13.8 Hz, 4H), 3.51 (m, 16H), 3.35 (m, 4H), 2.33 (m, 8H). ^{13}C NMR (D_2O, 50 °C): δ 138.05, 129.77, 128.69, 128.45, 71.75, 71.37, 48.94, 45.41, 43.50, 21.52. HR-MS for $(C_{26}H_{48}N_6O_2 + H)^+$: requires 477.3917, found 477.3897.

1,4-Bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]benzene Hexahydrochloride (3). Yield: 96%. ^{1}H NMR (D_2O, 50 °C): δ 7.70 (s, 4H),

4.93 (s, 4H), 4.25 (m, 2H), 3.50 (m, 24H), 2.32 (m, 8H). ^{13}C NMR (D_2O, 50 °C): δ 137.75, 128.98, 71.65, 71.45, 49.73, 45.73, 44.14, 22.09. HR-MS for $(C_{26}H_{48}N_6O_2 + H)^+$: requires 477.3917, found 477.3899.

4,4′-Bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]biphenyl Hexahydrochloride (4). Yield: 89%. ^{1}H NMR (D_2O, 50 °C): δ 7.97 (d, J = 8.1 Hz, 4H), δ 7.78 (d, J = 8.1 Hz, 4H), 4.98 (s, 4H), 4.30 (m, 2H), 3.51 (m, 24H), 2.33 (m, 8H). ^{13}C NMR (D_2O, 50 °C): δ 140.60, 137.25, 129.43, 127.71, 71.81, 71.51, 50.05, 45.95, 44.43, 22.34. HR-MS for $(C_{32}H_{52}N_6O_2 + H)^+$: requires 553.4230, found 553.4218.

2,6-Bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]pyridine Heptahydrochloride (5). Yield: 91%. ^{1}H NMR (D_2O): δ 8.47 (t, J = 8.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 2H), 5.04 (s, 4H), 4.10 (m, 2H), 3.55 (dd, J = 4.6, 13.3 Hz, 4H), 3.25 (m, 12H), 2.97 (m, 4H), 2.05 (m, 8H). ^{13}C NMR (D_2O): δ 151.99, 147.07, 124.63, 72.66, 66.60, 48.90, 44.62, 43.08, 21.35. HR-MS for $(C_{25}H_{47}N_7O_2 + H)^+$: requires 478.3869, found 478.3848.

1,3,5-Tris[(1,5,9-triazacyclododecan-3-yloxy)methyl]benzene Nonahydrochloride (6). Yield: 93%. ^{1}H NMR (D_2O, 50 °C): δ 8.11 (s, 3H), 4.97 (s, 6H), 4.31 (m, 3H), 3.71 (dd, J = 5.2, 13.7 Hz, 6H), 3.54 (m, 24H), 3.36 (m, 6H), 2.36 (m, 12H). ^{13}C NMR (D_2O, 50 °C): δ 138.36, 128.16, 71.27, 71.25, 48.77, 45.12, 43.25, 21.33. HR-MS for $(C_{36}H_{69}N_9O_3 + H)^+$: requires 676.5602, found 676.5587.

Kinetics Experiments. The pH of the reaction solutions was adjusted with a HEPES buffer (0.1 mol L^{-1}) to a desired value at 25 °C (I = 0.1 mol L^{-1} with $NaNO_3$). Sufficient amounts of the ligand and $Zn(NO_3)_2$ were added to give the final concentration of 0.5 mmol L^{-1} for the complex (in some cases 1.5 or 2.0 mmol L^{-1}). In other words, the concentration of the ligand (**1**−**6**) was 0.5 mM, and the concentration of Zn^{2+} was 1.0 mmol L^{-1} in solutions of **1**−**5** and 1.5 mmol L^{-1} in the solution of **6**. The solutions were allowed to stand for a few hours to be sure that the equilibrium had settled. The pH was then checked and, when needed, adjusted back to the original value with sodium hydroxide. No precipitation of zinc hydroxide was observed, even at pH < 7, indicating that the binding of Zn^{2+} to the ligand was virtually quantitative. The pH values at 90 °C were obtained by extrapolation based on the known temperature dependence of the pK_a value of HEPES.[26]

Reactions were carried out in sealed tubes immersed in a water bath, the temperature of which was adjusted to 90.0 ± 0.1 °C. The aliquots withdrawn at appropriate intervals during 3 half-lives of the disappearance of the starting material were immediately cooled in an ice−water bath and stored in a freezer before analysis. The composition of the aliquots was analyzed by RP-HPLC on a Hypersil ODS column C18 (250 × 4.6 mm, particle size 5 μm) or a Waters Atlantis column dC18 (250 × 4.6 mm, particle size 5 μm). The samples were eluted with a linear gradient from 0.1 mol L^{-1} KH_2PO_4/KOH buffer (pH 6.0, EDTA 2.0 mmol L^{-1}) to the same buffer containing 8% MeCN. The detection wavelength was 260 nm, and the bandwidth was 4 nm throughout the work. The initial concentration of the starting material was 50 μmol L^{-1}. In other words, the cleaving agent was present in 10-fold excess (or more). The pseudo-first-order rate constants of the disappearance of the starting material were calculated by means of the integrated first-order rate law.

UV Absorption Titration. Two milliliters of a 50 μmol L^{-1} solution of either ApA, ApU, or UpU (HEPES buffer, 0.1 mol L^{-1}, I = 0.1 mol L^{-1}, pH 7.5, 7.0, or 6.5) was placed in a cell having an optical path of 1 cm. The temperature of the cell housing block was adjusted to 25.0 ± 0.1 °C. A much more concentrated solution of **3** (7.19 mmol L^{-1}) and Zn^{2+} (14.4 mmol L^{-1}) was added portionwise, and the UV spectrum was measured immediately after each addition.

^{1}H NMR Spectroscopy Experiments. 3′,5′-ApA or 3′,5′-UpU was dissolved in D_2O to give a concentration of 4.0 mmol L^{-1}, and the pD was adjusted to 7.1 with NaOD in D_2O. The ^{1}H NMR spectrum was recorded at 600 MHz. Ligand **3** and Zn^{2+} in D_2O were added to the solution in a concentration ratio of 1:2. The pH was adjusted to pD 7.1, and the ^{1}H NMR spectrum was again measured. The additions and adjustment of pH were repeated until 1 equiv of **3** had been added.

A similar technique was used to study the ability of ligands **1**−**6** to bind Zn^{2+}. The measurements were carried out at 35 °C and pD 7.1 and with ligand **3** additionally at 90 °C. The concentration of **3** was 2 mmol L^{-1}, and altogether 4 equiv of Zn^{2+} compared to **3** were added.

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