Selective Hydrolysis of Phosphate Esters, Nitrophenyl Phosphates and UpU, by Dimeric Zinc Complexes Depends on the Spacer Length

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Abstract: Zn(II) complexes of monomers and dimers derived from 1,4,7-triazacyclododecane and 1,5,9-triazacyclotetradecane were examined as catalysts for the hydrolyses of *p*-nitrophenyl phosphate and bis(*p*-nitrophenyl) phosphate and for the cyclizations of *p*-nitrophenyl 2-hydroxypropyl phosphate and 3′,5′-uridyluridine (UpU). The dimers with 1,4-phenyl and 1,3-phenyl linkers were more effective than were monomers or a longer dimer—with a 4,4′-biphenyl linker—in the hydrolysis of *p*-nitrophenyl phosphate, suggesting that two Zn(II) ions coordinate to the phosphate group, as in the enzyme alkaline phosphatase. However, for the hydrolysis or cyclization of the phosphate diesters, the longer biphenyl linker was preferred. In this case one Zn(II) coordinates to the phosphate group while the other delivers a nucleophilic oxide anion. Bell-shaped pH vs rate profiles were seen in both cases, but with different p*K*'s related to the specific mechanisms in the two cases.

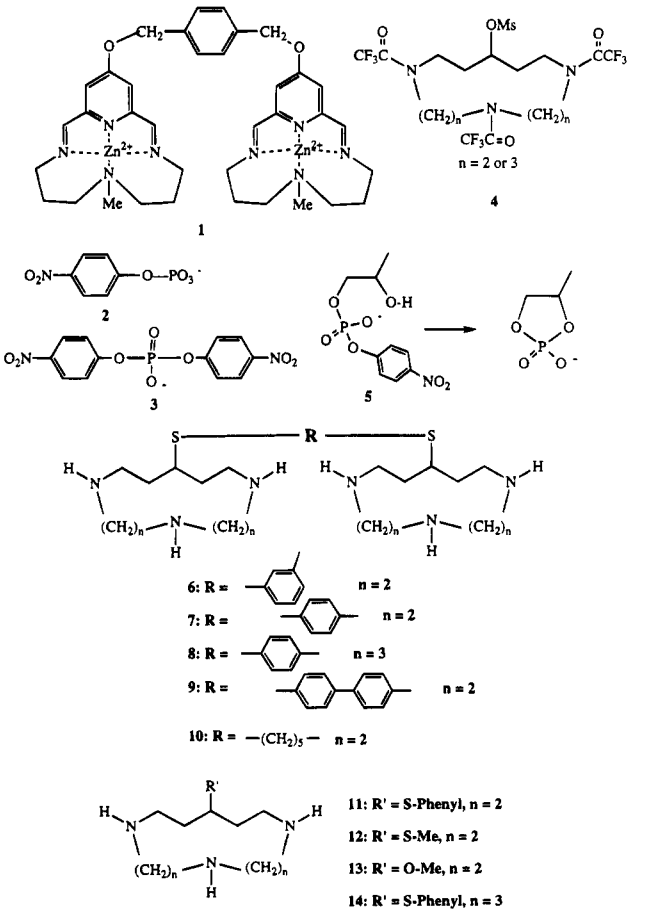
Introduction

Many enzymes, including those that hydrolyze phosphate esters, use two metal ions in a bifunctional catalytic mechanism.[1] For hydrolysis, the metal ions are often Zn(II).[2] We have described earlier[3] a catalyst (**1**) in which two bound Zn(II) ions cooperate in the cleavage of a phosphate triester or carboxylic ester. We saw a 5-fold increase in the rate with *p*-nitrophenyl diphenyl phosphate and a 7-fold increase with p-nitrophenyl acetate, compared to the rate with a single ligand group of **1**.

Others have described some systems in which two Cu(II) or La(III) can cooperate in the hydrolysis of anionic phosphate esters.[4] Czarnik has done two studies of bis-Co(III) complexes and saw that a flexible dimer showed no rate advantage over the monomeric complex.[5] A rigid dimer showed a 10-fold rate improvement over monomer in the catalyzed hydrolysis of *p*-nitrophenyl phosphate (**2**) but a rate disadvantage relative to the monomer for the hydrolysis of bis(*p*-nitrophenyl) phosphate (**3**).[6]

We have now prepared a set of catalysts in which two Zn(II) complexes of a macrocyclic ligand (e.g., **11**) are linked by spacers of differing length and geometry. We find that one of our molecules shows optimal bifunctional catalysis for cleavage reactions of phosphate diesters but that a different geometry is preferred for the bifunctional cleavage of a phosphate monoester.

Chart 1



Results

Our building blocks[7] are the triazamacrocycles **4**, with three nitrogen ligands for Zn(II) and a displaceable group—on the

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(1) Cf.: Steitz, T. A.; Steitz, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6498.

(2) (a) *Zinc Enzymes*; Spiro, T. G., Ed.; John Wiley & Sons: New York, 1983. (b) Kim, E. E.; Wyckoff, H. W. *J. Mol. Biol.* **1991**, *218*, 449. (c) Hough, E.; Hansen, L. K.; Birknes, B.; Jynge, K.; Hansen, S.

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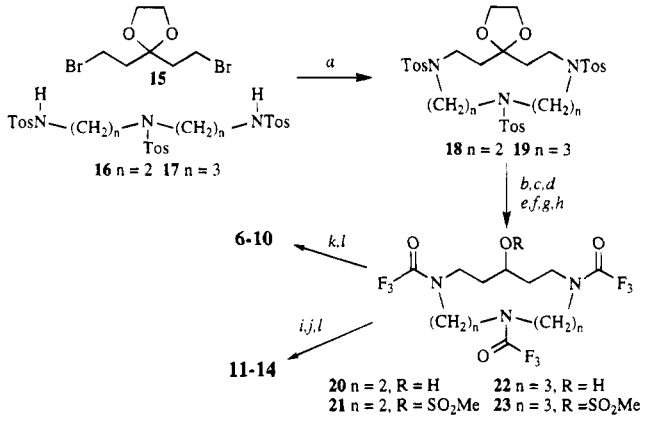

Figure 1. Synthesis of compounds **6**–**14**. (*a*) Cs_2CO_3, DMF, 90–110 °C, 15 h, 45%. (*b*) $HClO_4$, CH_2Cl_2, room temperature (RT), 30 min. (*c*) $NaBH_4$, MeOH (from **18**, 83%), EtOH (from **19**, 91%), RT, 15 min. (*d*) (1) NaH, 2–5 mol % imidazole, THf; reflux, 1 h; (2) MeI, reflux, 3 h. (*e*) Li/NH_3, −78 °C to RT, 3 h, 45–82% through steps *d* and *e*. (*f*) Trifluoroacetic anhydride, (*i*-Pr)$_2$NEt, CH_2Cl_2, 0 °C to RT, 30 min, 71–79%. (*g*) BBr_3, CH_2Cl_2, 0 °C to RT, 20 min, 81%. (*h*) MesCl, (*i*-Pr)$_2$NEt, CH_2Cl_2, 0 °C, 15 min, 82%. (*i*) Protected **14** from **22**, Ph_3P, DIAD, THF, thiophenol, 0 °C to RT, overnight, 43%. (*j*) Protected **11** (22%) and **12** (46%) from **21**, sodium salt of the appropriate thiol in dimethylimidazolone (DMI), 45 °C, overnight. (*k*) Protected **6**–**10** from **21** and **23**, bis-sodium salt of the appropriate dithiol in DMI, 45 °C overnight, 30–70%. (*l*) NH_3, MeOH, RT, overnight.

five-carbon chain—that was used to link the macrocycles. The Zn(II) complexes of monomers and dimers **6**–**14** were examined as catalysts for the hydrolysis of *p*-nitrophenyl phosphate (**2**) and of bis(*p*-nitrophenyl) phosphate (**3**). We also examined the cyclization–cleavage of compound **5**, a model for the chemistry in RNA cleavage, and of 3′,5′-uridyluridine (UpU, cf. Figure 7), a true RNA dinucleotide. Molecular models show that, with the short spacers, two Zn(II) ions bound to ligands **6**, **7**, and **8** can both bind to a phosphate dianion but that this is difficult with the long spacer of compound **9**. As monomeric controls, compounds **11**–**14** were also examined.

The syntheses of **6**–**14** are outlined in Figure 1 (intermediates numbered **24**–**39** are involved in these syntheses, and described in the Experimental Section, but are not shown in this figure). The carbon-functionalized 1,4,7-triazacyclododecane (**18**) and 1,5,9-triazacyclotetradecane (**19**) rings were constructed by the Cs_2CO_3-mediated cyclization of dibromide **15** with sulfonamide **16** or **17** (step *a*).[8] The dioxolane ring was converted to a methyl ether (*b*–*d*) and the *N*-protecting groups were removed by reduction with Li in NH_3. Trifluoroacetylation (*f*) of the free amine followed by demethylation of the ether (*g*) left the alcohols **20** and **22**. Compound **14** was prepared by displacement of the hydroxyl group from **22** under Mitsunobu conditions.[9] The protected precursors of **6**, **7**, and **9**–**11** were prepared by displacement of the mesylate group from **21** or **23** with the appropriate thiophenoxide; they were purified by flash chromatography, and the trifluoroacetamide groups were removed with NH_3 in MeOH. Samples of the ligands of known mass (for preparing solutions of known concentration) were prepared by weighing samples of the protected macrocycles (~50 mg), deprotection with NH_3, and removal of the trifluoroacetamide byproduct by evaporation.

Under essentially our conditions, Kimura has found that such ligands are completely complexed by Zn(II).[7] We have

(8) Cf.: (a) Chavez, F.; Sherry, A. D. *J. Org. Chem.* **1989**, *54*, 2990 and ref 8 therein. (b) Richmann, J. E.; Atkins, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 2268. (c) Vriesema, B. K.; Butler, J.; Kellogg, R. M. *J. Org. Chem.* **1984**, *49*, 110.

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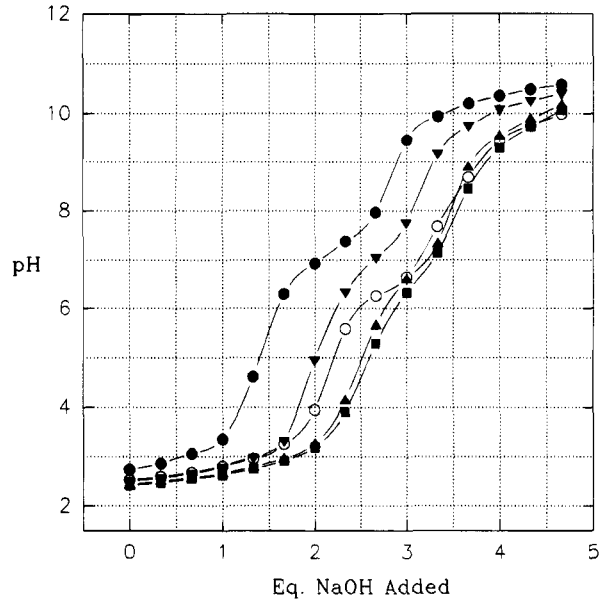

Figure 2. Potentiometric titrations of the ligands alone, as described in the Experimental Section: (●) 1,5,9-triazacyclododecane, (▼) phenylthio ether **11**, (■) 1,3-phenyl dimer **6**, (▲) 1,4-phenyl dimer **7**, (○) 4,4′-biphenyl dimer **9**.

examined the ^1H NMR spectrum of ligand **12** in D_2O/DMSO-d_6 with Tris-d_{11} buffer (50 mM, titrated to 30% protonation with $DClO_4$) under the conditions of our catalytic reactions. We find that the methyl group of the free macrocycle (singlet at 2.28 ppm) disappears and a new singlet appears (2.32 ppm) on addition of Zn(II) and that this change is complete on addition of 1 equiv of Zn(II). Furthermore, the free macrocycle singlet did not reappear even after dilution of the **12**–Zn(II) complex solution to 46 μM. From this data, the formation constant (K_a) for the **12**–Zn(II) complex is calculated to be $>2 \times 10^6$ M^{-1} (90% association at 46 μM). For comparison, $K_a = 1 \times 10^7$ M^{-1} for the corresponding unfunctionalized case (1,4,7-triazacyclododecane–Zn(II)).[7]

In addition, we have done potentiometric titrations of the ligands in the presence and absence of Zn(II) and find curves consistent with complete Zn(II) binding under our operating conditions. The titration curves are shown in Figures 2 and 3. From these curves it can be seen that the addition of Zn(II) to the ligand solutions leads to an additional 2.0–2.3 titratable protons up to pH 8.0 for all the ligands based on 1,4,7-triazacyclododecane, and somewhat more (2.9 protons) for 1,5,9-triazacyclododecane. The ligands are mostly diprotonated, but partially triprotonated, at the starting pH of the titrations, and a one-proton titration of the ligand itself occurs in the pH 7.0–8.0 region. Our curves show that at pH 7.0 the Zn(II) has displaced an additional 1.5 or so protons from the ligands, beyond those that would already be titrated at that pH. This indicates complete binding of the Zn(II) to the ligands at this pH and thus complete Zn(II) binding under our operating kinetic condition, as expected from prior work.

In the pH region from 7.0 to 9.0, an additional proton is fully titratable in the presence of Zn(II). This shows that the pK of the $ZnOH_2$ is in this region for our systems **6**, **7**, **9**, and **11**. Rate vs pH studies, described below, show kinetic pK's that are consistent with these titration pK's.

For rate studies, reaction mixtures were prepared by the addition of concentrated solutions of the macrocycles (25 mM dimers **6**–**10** and 50 mM monomers **10**–**14** in MeOH), $Zn(ClO_4)_2(H_2O)_6$ (50 mM in MeOH) and the substrate (50 mM in H_2O or DMSO) in this order to a mixture of DMSO (20%

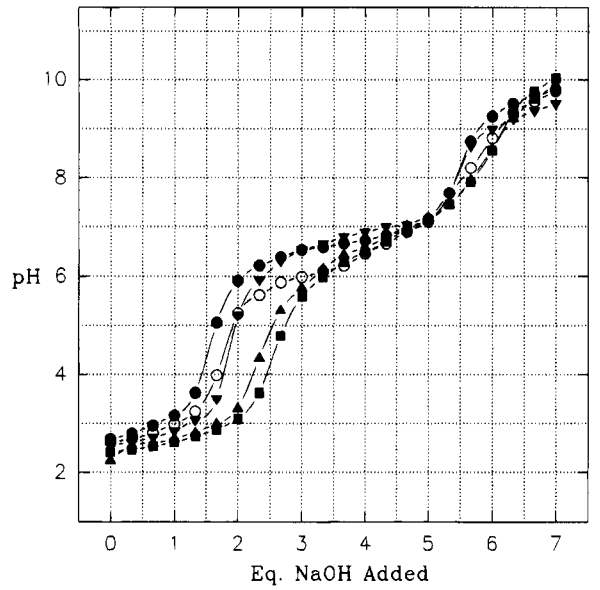

Figure 3. Potentiometric titrations in the presence of 1 equiv of $ZnCl_2$ per macrocycle, as described in the Experimental Section: (●) 1,5,9-triazacyclododecane, (▼) phenylthio ether **11**, (■) 1,3-phenyl dimer **6**, (▲) 1,4-phenyl dimer **7**, (○) 4,4′-biphenyl dimer **9**.

by volume) and buffered water (50 mM Tris (pH 8.36) or HEPES (pH 8.13), titrated with 70% $HClO_4$ or 1 M NaOH). Final concentrations were 227 mM in dimer and substrate and 454 mM in all other species. The mixtures were homogeneous after heating to 55 °C and remained this way throughout the reaction. The rate of formation of the nitrophenylate ion was followed at 400 nm. The catalytic rate constants (k_{cat}) were calculated by the initial slopes method (to avoid complications from product inhibition) and are presented in Table 1.

We determined the dependence of rate on pH for the catalyzed hydrolysis of the phosphate diester **3** by the extended dimeric catalyst **9** and for the hydrolysis of phosphate monoester **2** by catalyst **6**. In the latter case, we could cover the pH region of interest using the single buffer EPPS (4-(2-hydroxyethyl)-1-piperazinepropane), while for the greater pH range in the former case, we used three different buffers: EPPS, CAPS (3-(cyclohexylamino)-1-propanesulfonic acid), and CHES (2-(cyclohexylamino)ethanesulfonic acid). These are all buffers known to minimize interactions with metal ions. The results are shown in Figures 4 and 5; the conditions are indicated in the figure legends.

Discussion

The appropriate dimer catalysts are superior to the monomers for all four substrates studied. The distance between the metal binding sites determines the catalysts' selectivity—the short phenyl spacer (**6** and **7**) is better for the hydrolysis of monoester **2** (Table 1, entries 1 and 2 compared with 4), whereas the longer biphenyl spacer (**9**) favors the hydrolysis of diester **3** (entry 11 compared with 9 and 10). Double binding of a single phosphate group by the two metals is favored by the phenyl spacers and is essentially impossible with the biphenyl spacer.

We see very poor catalysis by an analog of our catalytic metal dimers with a flexible pentamethylene chain linker (**10**) instead of our rigid spacers (entry 5 compared to 1 and 2, entry 12 compared to 11 and 20 compared to 19). The results show the importance of rigidity in catalytic systems of this class. One can expect even better catalyses and greater selectivity with more rigidly held dimers.

The cyclization of the glycol phosphate **5** was found to be catalyzed by the monomers as well as the dimers. Among the monomers, the thiophenyl-substituted **11** is more effective than the others, including the ligand **14** with a larger macrocycle.

Table 1. Kinetic Data for Zn(II) Complexes **6**–**14**[a]

entry	ligand	dimer spacer	substrate	$k_{obs} \times 10^5$ s^{-1} [b]	k_{obs}/k_{uncat} [c]
1	**6**	1,3-Ph	monoester **2**[d]	0.22 ± 0.02	4.7
2	**7**	1,4-Ph	monoester **2**[d]	0.28 ± 0.02	6.0[f]
3	**8**	1,4-Ph	monoester **2**[d]	0.04	0.9
4	**9**	4,4′-BP	monoester **2**[d]	0.06 ± 0.01	1.3
5	**10**	1,5-pentane	monoester **2**[d]	0.12	2.6
6	**11**	monomer	monoester **2**[d]	0.04 ± 0.01	0.9
7	**14**	monomer	monoester **2**[d]	0.04	0.9
8	none		monoester **2**[d]	0.06 ± 0.02	1.3
9	**6**	1,3-Ph	diester **3**[d]	0.09 ± 0.01	273
10	**7**	1,4-Ph	diester **3**[d]	0.34 ± 0.01	1030
11	**9**	4,4′-BP	diester **3**[d]	0.64 ± 0.01	1937
12	**10**	1,5-pentane	diester **3**[d]	0.09 ± 0.02	273
13	**11**	monomer	diester **3**[d]	0.12 ± 0.02	364
14	**14**	monomer	diester **3**[d]	0.08	242
15	none		diester **3**[d]	0.05 ± 0.01	152
16	**6**	1,3-Ph	glycol ester **5**[e]	8.1 ± 0.3	450
17	**7**	1,4-Ph	glycol ester **5**[e]	10.0	556
18	**8**	1,4-Ph	glycol ester **5**[e]	0.90	50
19	**9**	4,4′-BP	glycol ester **5**[e]	19.3 ± 0.1	1072
20	**10**	1,5-pentane	glycol ester **5**[e]	8.0	444
21	**11**	monomer	glycol ester **5**[e]	3.5 ± 0.2	194
22	**14**	monomer	glycol ester **5**[e]	0.8	44
23	none		glycol ester **5**[e]	1.20 ± 0.01	67
24	**6**	1,3-Ph	UpU[g,h]	0.009 ± 0.003	9.2
25	**7**	1,4-Ph	UpU[g,h]	0.018	18
26	**9**	4,4′-BP	UpU[g,h]	0.038 ± 0.004	39
27	**11**	monomer	UpU[g,h]	0.009 ± 0.004	9.2
28	none		UpU[g,h]	0.015 ± 0.004	15

[a] Run in 80% 50 mM Tris buffer (pH = 8.36) and 20% DMSO. [b] Pseudo-first-order rate constants with 227 mM dimer and 454 mM monomer. Entries with a listed error are the average of two runs. [c] **2**: $(k)_{uncat} = 4.70 \times 10^{-7}$ s^{-1}. **3**: $k_{uncat} = 3.3 \times 10^{-9}$ s^{-1}. **5**: $k_{uncat} = 1.8 \times 10^{-7}$ s^{-1}. [d] Run at 55 °C. [e] Run at 30 °C. [f] In 50 mM HEPES (pH = 8.13). $k_{obs}/k_{uncat} = 9.4$. [g] UpU: $k_{uncat} = 9.8 \times 10^{-9}$ s^{-1}, both from a run at 41 °C and from Arrhenius extrapolation of other runs at higher temperatures, as described in the Experimental Section. [h] Run at 41 °C.

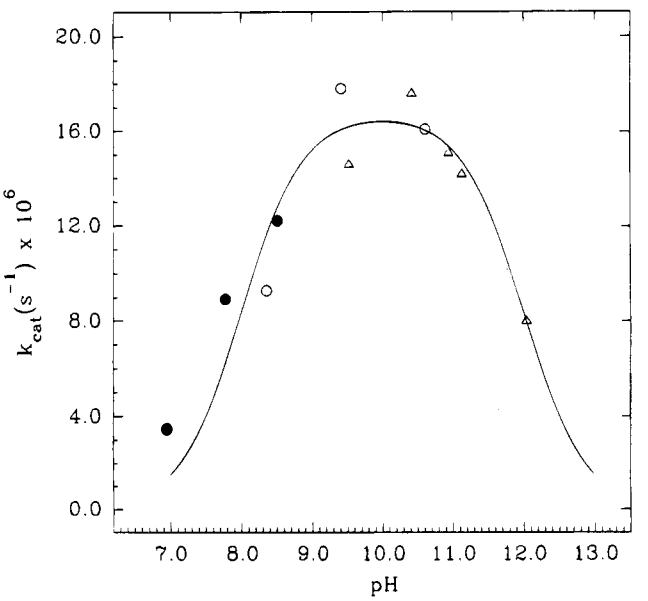

Figure 4. Plot of the rate of hydrolysis of bis(*p*-nitrophenyl) phosphate (**3**) (2.3×10^{-4} M) catalyzed by the Zn(II) complex of **9** (2.3×10^{-4} M) at various pH's in EPPS (●), CHES (○), and CAPS (△) buffer (10 mM) in water containing 20% DMSO by volume and 50 mM $Me_4N^+ClO_4^-$. The background rates in the absence of catalyst are subtracted. The line is calculated as two titration curves with $pK_1 = 8$ and $pK_2 = 12$.

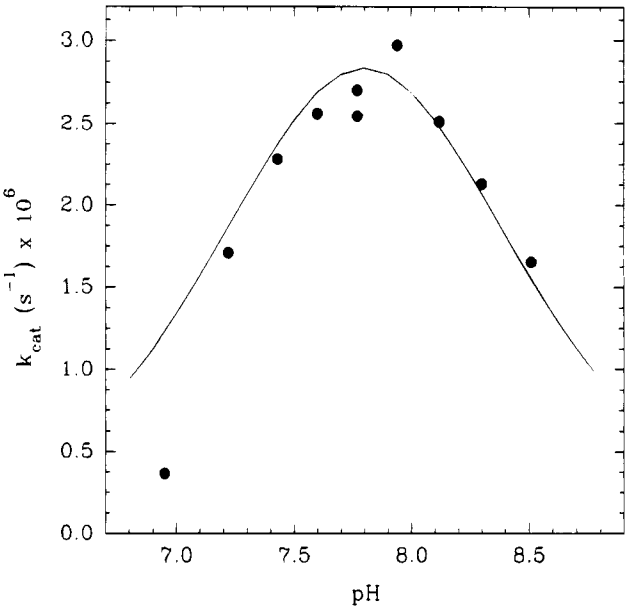

Figure 5. Plot of the rate of hydrolysis of *p*-nitrophenyl phosphate (**2**) (2.3×10^{-4} M) catalyzed by the Zn(II) complex of **6** (2.3×10^{-4} M) at various pH's in EPPS buffer (10 mM) in water containing 20% DMSO by volume and 50 mM $Me_4N^+ClO_4^-$. The line is calculated as two titration curves with $pK_1 = pK_2 = 7.8$.

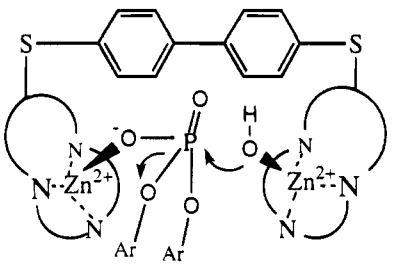

Figure 6. Proposed mechanism for the bifunctional catalysis of the hydrolysis of diester **3** by the bis-zinc complex of ligand **9**. For the cyclization–cleavage of compound **5** and of the dinucleotide UpU, the nucleophile is a coordinated alkoxide ion.

The dimers **6**, **7**, and **9** show similar trends in reactivity toward **5** and toward phosphate ester **3**, suggesting that the mechanism of reaction of both substrates is similar.

The results indicate that double metal binding to a phosphate group increases the rate of hydrolysis of monoester **2** and that a different mechanism is operative in the case of diester **3**. From our results and molecular models, we propose the diester mechanism in Figure 6; one Zn(II) binds to the phosphate and acts as a Lewis acid, while the other delivers a nucleophilic-bound OH^-. With substrate **5** and with UpU (Figure 7), the bound nucleophile is the internal alkoxide ion. With poor leaving groups, a Lewis acid stabilization of the leaving group is also possible, as has been proposed for enzymatic cases.[1]

As expected from this mechanism, we see (Figure 4) a bell-shaped pH vs rate profile, with the ascending leg corresponding to a pK_a of 8 and the descending leg to a pK_a of 12. The lower one is presumably the titration of a water molecule on the Zn(II) that does not bind to the phosphate group, whose pK_a is essentially the same as that for the catalyst without bound substrate. The second one must correspond to the binding of an OH^- to the second Zn(II), that carries the bound phosphate. Thus the kinetic p*K* is displaced upward. This OH^- probably displaces the substrate, but if not, it will certainly diminish the Lewis acidity of that Zn. In either case it will lead to loss of catalysis, as is observed.

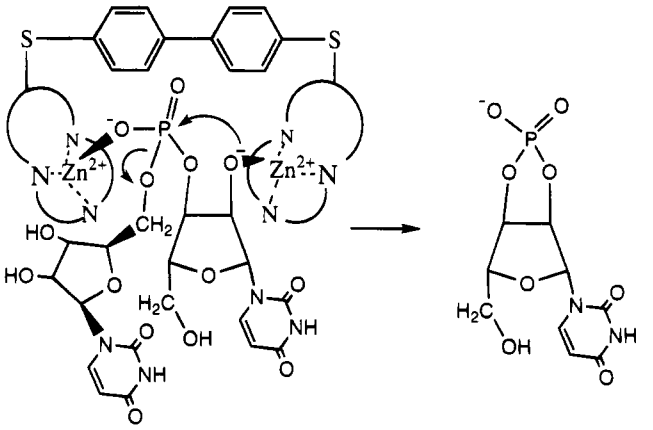

Figure 7. Proposed mechanism for the bifunctional catalysis of the hydrolysis of 3′,5′-uridyluridine (UpU) by the bis-zinc complex of ligand **9**. The attack by the coordinated 2′ oxide anion may lead to a phosphorane intermediate, with eventual displacement of the 5′ oxygen of the second uridine unit.

We have previously described[10] the cyclization reaction of **5** catalyzed by Zn(II) and imidazole and saw that this combination also catalyzed the cyclization–cleavage of the ribodinucleotide 3′,5′-UpU. As expected from this comparison, and from the selectivity observed in the catalysis of **5**, we now see that the cyclization–cleavage of UpU is catalyzed most effectively by the Zn(II) complex of the elongated dimer **9**, not by the shorter dimer **6**. The results with UpU are also listed in Table 1. This reaction is much slower than that of the other substrates, so the control reaction in the absence of catalyst was performed also at several higher temperatures, where multiple reliable data points were more easily obtained. The values at temperatures of 91, 81, and 71 °C all fit an Arrhenius plot along with the determined value at 41 °C. Furthermore, the value obtained is consistent with that we reported elsewhere for the uncatalyzed rate of UpU cleavage.[10] As we saw before,[10] the metal-ion-catalyzed cleavage of 3′,5′-UpU is *not* accompanied by its isomerization to 2′,5′-UpU, in contrast to the situation[11] with simple imidazole catalysis.

The rate vs pH results with the phosphate monoester **2** are interesting. Catalysis by the dimer catalyst 6, that has minimum Zn–Zn separation, shows a bell-shaped pH vs rate profile as well (Figure 5), but with data that fit a plot for a pK_a of 7.8 for *both* legs of the curve. The rise in rate with increasing pH—in a region outside the titration range of the substrate—indicates that here too there is attack by an OH^- on the phosphate, presumably an OH^- bound to a Zn(II). The preference for catalyst **6** must indicate that the phosphate of **2** binds to *both* Zn's, but then the low pK_a for binding OH^- to the Zn(II) that also carries a phosphate group requires explanation.

One possibility is that this OH^- binds to both Zn's also, perhaps unsymmetrically, and that this makes up for the raising of the p*K* of a bound water that should occur when phosphate dianion is also bound (which however holds the two Zn's close together). The loss of catalysis when a second OH^- interacts with the system can then indicate what we discussed above, probably displacement of the substrate but possibly simple diminution of the Lewis acidity of a Zn ion. However, why is this second p*K* also so low, in contrast with the situation in Figure 4? The only reasonable idea seems again to be an interaction with *both* Zn(II)'s. The two OH^- groups each interact with the two Zn(II)'s as shown in Figure 8, by

(10) (a) Breslow, R.; Huang, D.-L.; Anslyn, E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1746. (b) Breslow, R.; Huang, D.-L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4080.

(11) Breslow, R. *Acc. Chem. Res.* **1991**, *24*, 317.

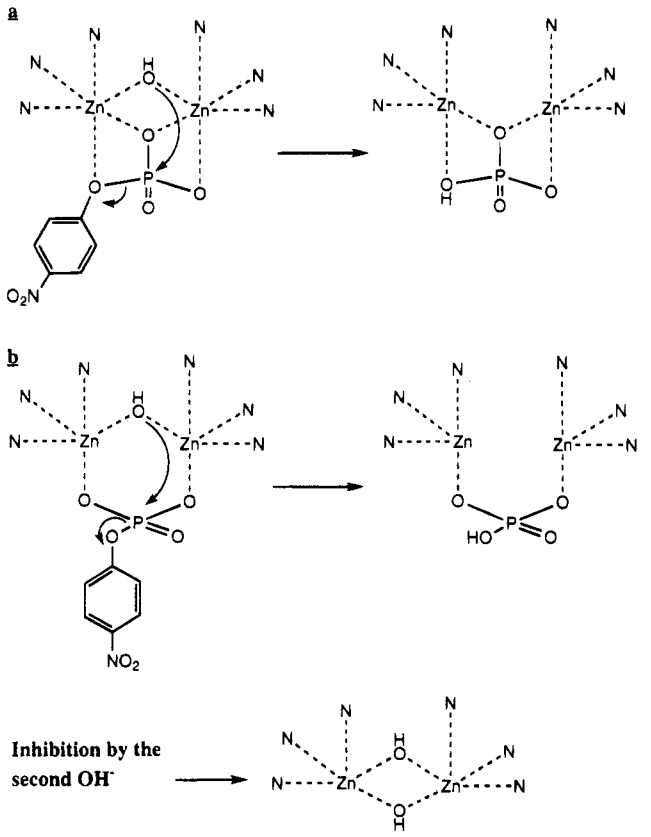

Figure 8. Two alternative mechanisms for the hydrolysis of *p*-nitrophenyl phosphate (**2**) catalyzed by the double Zn(II) complex of ligand **6**. Mechanism a is analogous to that proposed for the action of alkaline phosphatase, based on X-ray structures,[2b] while mechanism b is based on the finding[2b] that inorganic phosphate binds to the two Zn(II) atoms of alkaline phosphatase using two different oxygen atoms. Both mechanisms can fit our (somewhat flexible) catalyst, from molecular models, and our data. To avoid clutter in the drawings, the charges on the Zn and O atoms are omitted. Also shown is one way in which a second OH^- could inhibit the catalysis, by displacing the substrate. Alternative mechanisms for catalysis and inhibition are also possible.

symmetrical or unsymmetrical bridging. A less likely possibility is that the second OH^- deprotonates the first bound OH to form a μ-oxo ligand between the two Zn's.

There is ambiguity about the cleavage mechanism, so we propose two alternatives in Figure 8. One (mechanism a) is related to that invoked for alkaline phosphatase enzyme, based on X-ray evidence,[2b] in which a *single* phosphate oxygen bridges between the two Zn(II) ions. However, in the enzyme, the other two anionic phosphate oxygens are bound to a guanidinium ion of arginine, but not in our case. Thus, in our alternative (mechanism b), we bridge between the two Zn(II) ions using *two* phosphate oxygen atoms, in line with the X-ray evidence[2b] on the mode of binding of inorganic phosphate ion to alkaline phosphatase. Either alternative would fit our observations.

In common with the evidence on the enzyme, our evidence rules out a unimolecular metaphosphate cleavage mechanism (Figure 9) for the hydrolysis of **2** by **6**. Substrate **2** uses a metaphosphate fragmentation mechanism for hydrolysis in simple slightly acid solution,[12] but this mechanism is ruled out

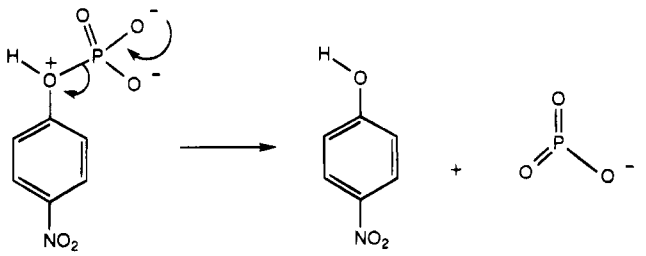

Figure 9. Metaphosphate mechanism for cleavage of *p*-nitrophenyl phosphate. This mechanism is preferred[12] under normal slightly acidic conditions but is suppressed in favor of the OH^- displacement mechanism of Figure 8 by Zn(II) binding to the phosphate.

in our case by the observed acceleration with the first hydroxide ion. Binding to Zn(II) blocks this fragmentation while promoting the mechanism actually observed.

This early work establishes some geometric factors to be incorporated into phosphate ester hydrolysis catalysts and shows that the geometric requirements are different for phosphate monoesters and diesters. One must wonder why the two different classes of substrates—the phosphate monoester and the phosphate diesters—have different geometric preferences when they could have used similar mechanisms. The two differences are (1) the diesters are more hindered than the monoester and (2) the monoester dianion is more basic than the diester monoanion. The steric factor could favor the more open geometry of the mechanism in Figures 6 and 7, while the extra basicity of the phosphate dianion might be needed to promote the double metal ion binding in the two alternative mechanisms of Figure 8. Both factors favor what we have observed: the phosphate monoester prefers that the two metals be close, while the diesters prefer a greater metal–metal separation.

Experimental Section

Synthesis. All reactions were run in oven-dried glassware under an atmosphere of Ar gas. Anhydrous solvents used in reactions either were purchased in anhydrous form (DMF, Aldrich) or were distilled from a desiccant prior to use (THF from sodium–benzophenone, CH_2Cl_2 from CaH_2). 1,3-Dimethyl-2-imidazolidinone (DMI, Aldrich) was dried overnight over 4 Å molecular sieves and was vacuum distilled, discarding the first fraction.

The organic phase from all liquid–liquid extractions was dried over Na_2SO_4 unless specified otherwise. Flash chromatography[14] was performed on 40–63 μm silica gel (EM #60) with certified ACS solvents (Fisher Scientific). All reported compounds were single spots by thin layer chromatography (precoated 0.25 mm silica plates from Merck).

All NMR spectra were recorded with a Varian VXR 200, 300, or 400 spectrometer (the model number refers to the proton frequency) and are reported in δ units. ^1H and ^{13}C (75 MHz) spectra were referenced to the solvent peak (D_3COD, CD_2Cl_2) or TMS ($CDCl_3$). ^{31}P spectra (122 MHz) were referenced to neat H_3PO_4 sealed in a melting point capillary tube. Assignments made by the use of homonuclear decoupling are indicated as HND. Mass spectra were recorded at the Columbia University MS facility by Vinka Parmakovich or Slavica Sporer with a Nermag R-10-10 (CI) or a JOEL DX303HF (FAB) spectrometer. IR spectra were recorded of samples ground and pressed with KBr with a Perkin-Elmer 1600 FT instrument.

Ketal 18. A solution of *N,N',N'''*-tris(*p*-tolylsulfonyl)diethylenetriamine (**16**) (50 g, 88.5 mmol) in 1.5 L of anhydrous DMF was mechanically stirred at 100 °C with Cs_2CO_3[12] (106 g, 325 mmol) for 3 h. 2,2-Bis(2-bromoethyl)-1,3-dioxolane[13] (**15**) (25.5 g, 88.5 mmol) in 35 mL of DMF was added via syringe pump over 19 h.[8] The DMF was evaporated, and the residue was partitioned between CH_2Cl_2 and 5% NaOH. The organic phase was separated, and the aqueous phase was washed twice with CH_2Cl_2. Evaporation of the solvent left a white

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(13) Dibromide **15** was prepared from dimethylacetonedicarboxylate with the following sequence of reagents: (a) ethylene glycol, TosOH, reflux; (b) $LiAlH_4$, THF, RT; (c) MesCl, Et_3N, CH_2Cl_2; (d) LiBr, THF, reflux. Rizzo, C.; Breslow, R. Unpublished results. Cf.: Wasylishen, R. E.; Rice, K. E.; Weiss, U. *Can. J. Chem.* **1975**, *53*, 414.

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foam. The product (22.3 g, 36.5% yield) was recrystallized from toluene. Mp = 196−197 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): 2.05 (broad t, J = 6.1 Hz, 4 H), 2.44 (s, 3 H), 2.46 (s, 6 H), 3.18 (broad, 4 H), 3.44 (broad, 4 H), 3.49 (broad, 4 H), 3.95 (s, 4 H), 7.2−7.4 (m, 6 H), 7.48 (d, J = 8.3 Hz, 2 H), 7.71 (d, J = 8.3 Hz, 4 H). ^{13}C NMR ($CDCl_3$, 75 MHz): 21.4 (I = 47), 32.4 (I = 46), 45.8 (I = 28), 47.8 (I = 18), 53.0 (I = 50), 64.4 (I = 55), 109.6 (I = 16), 127.6 (I = 168), 127.7 (I = 99), 129.7 (I = 209), 133.1 (I = 5), 135.0 (I = 6), 143.5 (I = 20), 144.0 (16). MS (CI − NH_3): 709 (M^+ + NH_4, 100%), 692 (M^+ + 1, 20%).

Ketal 19 was prepared from the tris-sulfonamide **17** as is described for ketal **18**. The product was purified by flash chromatography with 5% EtOAc in CH_2Cl_2 as the eluent (37% yield). ^{1}H NMR ($CDCl_3$, 200 MHz): 2.02 (m, 8 H), 2.44 (s, 6 H), 2.46 (s, 3 H), 3.08−3.30 (m, 12 H), 3.92 (s, 4 H), 7.32 (d, J = 7.7 Hz, 4 H), 7.34 (d, J = 8.1 Hz, 2 H), 7.66 (d, J = 8.1 Hz, 2 H), 7.69 (d, J = 7.7 Hz, 4 H). ^{13}C NMR ($CDCl_3$, 75 MHz): 21.4 (I = 87), 29.3 (I = 54), 36.1 (I = 55), 45.2 (I = 56), 48.1 (I = 58), 48.3 (I = 60), 64.5 (I = 87), 108.6 (I = 33), 127.2 (I = 230), 129.6 (I = 224), 134.9 (I = 45), 135.5 (I = 20), 143.4 (I = 45), 143.5 (I = 24). MS (CI − NH_3): 737 (M^+ + NH_4, 100%), 720 (M^+ + 1, 14%).

1,4,7-Tris(*p*-tolylsulfonyl)-1,4,7-triazacyclododec-10-one (24). $HClO_4$ (15 mL, 69−72%) was added dropwise to an ice−water-cooled solution of the spiroketal (**18**, 15 g, 21.7 mmol) in CH_2Cl_2 (200 mL) in *ca.* 2 min.[15] After 2 h of stirring, the lower, bright yellow acid layer was separated and the organic phase was washed once with saturated $NaHCO_3$. Evaporation of the solvent left the ketone as a white foam (92% yield) and was used without further purification. ^{1}H NMR ($CDCl_3$, 200 MHz): 2.45 (s, 9 H), 2.86 (m, 4 H, coupled to m at 3.40), 3.13 (t, J = 5.9 Hz, 4 H), 3.34 (t, J = 5.9 Hz, 4 H), 3.40 (m, J = 4.8 Hz, 4 H), 7.30 (d, J = 8.1 Hz, 2 H), 7.33 (d, J = 8.3 Hz, 4 H), 7.65 (d, J = 8.3 Hz, 4 H), 7.78 (d, J = 8.1 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 75 MHz): 21.5 (I = 48), 44.1 (I = 63), 44.9 (I = 53), 48.2 (I = 48), 50.5 (I = 50), 127.5 (I = 163), 129.7 (I = 65), 129.9 (I = 166), 130.0 (I = 36), 134.6 (I = 8), 136.5 (I = 5), 143.4 (I = 11), 143.8 (I = 23), 209.3 (I = 10). IR (KBr): 1710 cm^{-1} (C=O).

1,5,9-Tris(*p*-tolylsulfonyl)-1,5,9-triazacyclododec-12-one (25) was prepared from ketal **19** as is described for ketone **24** in 96% yield. ^{1}H NMR ($CDCl_3$, 200 MHz): 1.86 (m, 4 H), 2.42 (s, 9 H), 2.94 (m, 4 H), 3.10 (m, 8 H), 3.30 (m, 4 H), 7.30 (d + d, 6 H), 7.65 (d + d, 6 H).

1,4,7-Tris(*p*-tolylsulfonyl)-1,4,7-triazacyclododec-10-ol (26). The ketone (**24**, 12.9 g, 19.9 mmol) was added portionwise to a solution of $NaBH_4$ (24 mmol) in MeOH (100 ml). After 15 min, the MeOH was evaporated and the residue was partitioned between CH_2Cl_2 and water. The organic phase was separated and dried over Na_2SO_4. Solvent evaporation left a white foam (88% yield) which was used without further purification. ^{1}H NMR ($CDCl_3$, 200 MHz): 1.75 (m, 2 H), 2.0 (m, 2 H), 2.43 (s, 9 H), 2.9−3.8 (m, 12 H), 4.30 (p, J = 5.3 Hz, 1 H), 7.30 (d + d, 6 H), 7.52 (d, J = 8.1 Hz, 2 H), 7.69 (d, J = 8.3 Hz, 4 H). IR (KBr): 3600 cm^{-1} (broad, OH).

1,5,9-Tris(*p*-tolylsulfonyl)-1,5,9-triazacyclododecan-12-ol (27) was prepared by the reduction of the ketone **25** with $NaBH_4$ in EtOH as is described for **26**. The product was isolated as a white foam (91% yield from **25**) and was used in the next step without further purification.

10-Methoxy-1,4,7-tris(*p*-tolylsulfonyl)-1,4,7-triazacyclododecane (28). To a THF (150 mL) solution of the alcohol **26** (14 g, 21.7 mmol) was added NaH (1.04 g, 26 mmol, 60% dispersion in mineral oil) and a catalytic amount of imidazole (5 mg, 0.07 mmol). After refluxing for 2 h, MeI (2.68 mL, 43 mmol) was added and refluxing was continued for 3 h. The mixture was diluted with H_2O, and the THF phase was separated, washed with saturated aqueous NaCl, and dried over Na_2SO_4. Solvent evaporation left a white foam which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 200 MHz): 1.85 (m, 4 H), 2.44 (s, 9 H), 2.7−3.2 (m, 6 H), 3.32 (s, 3 H), 3.4−3.8 (m, 7 H), 7.3 (d + d, 6 H), 7.46 (d, J = 8.1 Hz, 2 H), 7.69 (d, J = 8.1 Hz, 4 H).

12-Methoxy-1,5,9-tris(*p*-tolylsulfonyl)-1,5,9-triazacyclododecane (29) was prepared from alcohol **27** is as described for methyl ether **28**. ^{1}H NMR ($CDCl_3$, 200 MHz): 1.86 (m, 4 H), 1.94 (m, 4 H), 2.43 (s, 9 H), 2.8−3.1 (m, 4 H), 3.1−3.3 (m, 8 H), 3.36 (s, 3 H), 3.57 (p, J = 5.4 Hz, 1 H), 7.32 (d, J = 8.2 Hz, 6 H), 7.66 (d, J = 8.2 Hz, 6 H).

10-Methoxy-1,4,7-triazacyclododecane (13). A solution of sulfonamide **28** (2.6 g, 3.92 mmol) in THF (20 mL) was added dropwise to Li (823 g, 118 mmol, 99.9%, ~0.01% Na) dissolved in NH_3 (*ca.* 200 mL cooled to −78 °C) at a rate which did not discharge the reaction's color (*ca.* 1 h). After the addition was complete, the mixture was heated to reflux, cooled to −78 °C, and quenched by the dropwise addition of MeOH (25 mL, Ar saturated). After warming to room temperature, H_2O (25 mL) was added and the solution was concentrated to a clear, colorless solution (~25 mL). The product was extracted six times with CH_2Cl_2 (100 mL portions), and the extract dried over K_2CO_3. Solvent evaporation left a semisolid which was further purified by flash chromatography, eluting with 15−20% *ca.* 7 N NH_3 in MeOH. The yield of clear, colorless oil was 45%. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.58 (m, 2 H; $H_{9,11}$), 1.71 (m, 2 H; $H_{9,11}$), 2.60 (m, 2 H; d with HND at 1.7 ppm, J = 12 Hz; $H_{8,12}$), 2.6−2.8 (m, 8 H, $H_{2,3,5,6}$), 2.88 (m, 2 H; d with HND at 1.7 ppm, J = 12 Hz; $H_{8,12}$), 3.38 (s 3 H, $-CH_3$), 3.94 (p, J = 6 Hz, 1 H; H_{10}). ^{13}C NMR (CD_2Cl_2, 300 MHz): 32.6 (I = 137, $C_{9,11}$), 44.1 (I = 121; $C_{8,12}$), 46.8 (I = 196; $C_{2,3,5,6}$), 56.5 (I = 23, C_{10}), 75.8 (I = 43, $-CH_3$). HRMS (+ FAB): 202.1910 found, M^+ + 1 calculated to be 202.1919 with the formula $C_{10}H_{24}N_3O$.

12-Methoxy-1,5,9-triazacyclotetradecane (30) was prepared from **29** as is described for **13**. The product (79% yield) was found to crystallize on standing, Mp = 53−55 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): 1.5−1.9 (m, 8 H), 2.1−2.9 (m, 12 H), 3.37 (s, 3 H), 3.63 (p, J = 6.5, 1 H). MS (CI−NH_3): 230 (M^+ + 1). ^{13}C NMR (D_3COD, 75 MHz): 28.6 (I = 147), 31.8 (I = 120), 46.5 (I = 180), 48.0 (I = 150), 49.2 (I = 166), 56.8 (I = 31), 77.5 (I = 66).

10-Methoxy-1,4,7-tris(trifluoroacetyl)-1,4,7-triazacyclododecane (31). Trifluoroacetic anhydride (1.47 mL, 10.44 mmol) was added dropwise in 5 min to an ice−water-cooled solution of macrocycle **13** (350 mg, 1.74 mmol) and diisopropylethylamine (1.82 mL, 10.44 mmol) in CH_2Cl_2 (5 mL). The cooling bath was removed, and the solution was stirred for 30 min. The reaction was quenched with saturated aqueous $NaHCO_3$, and the product was extracted three times with CH_2Cl_2. After drying over $MgSO_4$, the volatiles were evaporated to a white foam. The crude product was further purified by flash chromatography with 30% EtOAc in CH_2Cl_2 (73% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): 1.8−2.4 (broad, 4 H), 3.43 (s, 3 H), 3.3−4.3 (broad, 13 H). Deprotection of the product with NH_3 (*ca.* 7 N in MeOH) yielded **13** by TLC.

12-Methoxy-1,5,9-tris(trifluoroacetyl)-1,5,9-triazacyclotetradecane (32) was prepared from **30** as is described for **31**. ^{1}H NMR ($CDCl_3$, 200 MHz): 1.7−2.4 (broad, 8 H), 3.1−3.8 (broad, 13 H), 3.38 (s, 3 H). Deprotection with NH_3 yielded **30** by TLC.

1,5,9-Tris(trifluoroacetyl)-1,5,9-triazacyclotetradecan-10-ol (22). Methyl ether **32** (300 mg, 0.58 mmol) was demethylated with BBr_3 (3.9 mL, 1 M in CH_2Cl_2) in CH_2Cl_2 at room temperature.[15,16] The reaction was quenched after the addition was complete (15 min) with saturated $NaHCO_3$, and the product was extracted with CH_2Cl_2. The product, a white foam, was purified by flash chromatography with 35% EtOAc in CH_2Cl_2 (81% yield). ^{1}H NMR ($CDCl_3$, 200 MHz): 1.5−2.4 (broad, 8 H), 2.9−4.3 (broad, 14 H).

1,4,7-Tris(trifluoroacetyl)-1,4,7-triazacyclododecan-10-ol (20) was prepared from **31** as is described for **22**. ^{1}H NMR ($CDCl_3$, 200 MHz): 1.6−2.3 (broad, 4 H), 2.9−4.5 (broad, 14 H).

10-((Methylsulfonyl)oxy)-1,4,7-tris(trifluoroacetyl)-1,4,7-triazacyclododecane (21). Methanesulfonyl chloride (33 microl, 0.42 mmol) was added dropwise in 5 min to an ice-cooled CH_2Cl_2 (4 mL) solution

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of the alcohol **20** (100 mg, 0.21 mmol) and diisopropylethylamine (73 microl, 0.42 mmol). The reaction was quenched with H_2O after 15 min, and the product was extracted with CH_2Cl_2. Solvent evaporation left a white foam which was further purified by flash chromatography with 25% EtOAc in CH_2Cl_2 as the eluent (82% yield). ^1H NMR ($CDCl_3$, 400 MHz): 1.8−2.4 (broad m, 4 H), 3.17 (s, 3 H), 3.2−4.2 (broad m, 12 H), 5.0 (broad, 1 H). MS (CI − NH_3): 571 ($M^+ + NH_4$).

10-((Methylsulfonyl)oxy)-1,5,9-tris(trifluoroacetyl)-1,5,9-triazacyclotetradecane (23) was prepared as is described for mesylate **21**. ^1H NMR ($CDCl_3$, 200 MHz): 1.8−2.4 (broad, 8 H), 2.99 (s, 3 H), 3.2−3.8 (broad, 12 H), 4.78 (broad, 1 H).

12-(Phenylthio)-1,5,9-tris(trifluoroacetyl)-1,5,9-triazacyclotetradecane (33). Triphenylphosphine (168 mg, 0.640 mmol) and diisopropylazodicarboxylate (DIAD, 125 microl, 0.635 mmol) were added in this order to a THF (5 mL) solution of the alcohol **22** (214 mg, 0.425 mmol). Thiophenol (65 microl, 0.633 mmol) was added dropwise to the mixture in 5 min. After stirring for 30 min, additional portions of triphenylphosphine (168 mg), DIAD (125 microl), and thiophenol (65 microl) were added. After 1 h, the volatiles were evaporated and the semisolid product mixture was subjected to chromatography, with 5% EtOAc in CH_2Cl_2. The product (46% yield) was found to be a white foam. ^1H NMR ($CDCl_3$, 200 MHz): 1.7−2.4 (broad, 8 H), 3.1−3.8 (broad, 13 H), 7.3−7.6 (m, 5 H). MS (CI − NH_3): 613 ($M^+ + NH_4$).

***S,S'*-Bis(10-(1,4,7-tris(trifluoroacetyl)-1,4,7-triazacyclododecyl))-4',4''-dithiobiphenyl (34).** 4,4'-Biphenyldithiol (22 mg, 0.101 mmol) was deprotonated with NaH (8 mg, 0.55 mmol) in dimethylimidazolone (DMI) (1 mL). A solution of the mesylate **21** (110 mg, 0.199 mmol) in DMI (1 mL) was added to the thiolate solution, and the mixture was heated to 45 °C overnight. After 11 h, the solvent was evaporated and the product was isolated as a white foam (67% yield) by flash chromatography with 20% EtOAc in CH_2Cl_2 as the eluent. ^1H NMR ($CDCl_3$, 400 MHz): 1.7−2.4 (broad, 8 H), 3.0−4.3 (broad, 26 H), 7.55 (broad s, 8 H). MS (+ FAB): 1132 (M^+).

10-(Phenylthio)-1,4,7-tris(trifluoroacetyl)-1,4,7-triazacyclododecane (35) was prepared in 43% yield from thiophenol and mesylate **21** as is described for **34**. ^1H NMR ($CDCl_3$, 400 MHz): 1.7−2.4 (broad m, 4 H), 3.0−4.2 (broad m, 13 H), 7.35 (m, 3 H), 7.47 (m, 2 H). MS (CI − NH_3): 585 ($M^+ + NH_4$).

10-(Methylthio)-1,4,7-tris(trifluoroacetyl)-1,4,7-triazacyclododecane (36) was prepared in 73% yield by the reaction of sodium thiomethoxide with the mesylate **21** in DMI. ^1H NMR ($CDCl_3$, 400 MHz): 1.7−2.4 (broad m, 7 H), 2.75 (broad, 1H), 3.1−4.2 (broad, 12 H). MS (CI − NH_3): 523 ($M^+ + NH_4$).

***S,S'*-Bis(10-(1,4,7-tris(trifluoroacetyl)-1,4,7-triazacyclododecyl))-1',4'-dithiobenzene (36)** was prepared in 37% yield by the reaction of a 1,4-benzenedithiolate[23] with the mesylate **20**, as is described for **33**. ^1H NMR ($CDCl_3$, 400 MHz): 1.7−2.4 (broad, 8 H), 3.1−4.3 (broad, 26 H), 7.4 (broad, 4 H). MS (CI − NH_3): 1074 ($M^+ + NH_4$).

***S,S'*-Bis(12-(1,5,9-tris(trifluoroacetyl)-1,5,9-triazacyclotetradecyl)-1',4'-dithiobenzene (38)** was prepared in 62% yield by the reaction of a 1,4-benzenedithiolate[23] with the mesylate **23**, as is described for **34**. ^1H NMR ($CDCl_3$, 200 MHz): 1.7−2.4 (broad, 16 H), 3.1−3.8 (broad, 26 H), 7.3−7.5 (broad, 4 H). MS (CI − NH_3): 1130 ($M^+ + NH_4$).

***S,S'*-Bis(10-(1,4,7-tris(trifluoroacetyl)-1,4,7-triazacyclododecyl))-1',5'-dithiopentane (39)** was prepared in 23% yield by the reaction of 1,5-dimercaptopentane with the mesylate **21**, as is described for **34**. ^1H NMR ($CDCl_3$, 200 MHz): 1.6 (broad, 6H), 2.0 (broad, 8H), 2.5 (broads, 4H), 2.8 (broad, 2H), 3.3−4.1 (broad, 24H). MS (+ FAB): 1051 ($M^+ + 1$).

***S,S'*-Bis(10-(1,4,7-tris(trifluoroacetyl)-1,4,7-triazacyclododecyl))-1',3'-dithiobenzene (38)** was prepared by the reaction of 1,3-benzenedithiol with the mesylate **20**, as described for **33**. ^1H NMR ($CDCl_3$, 200 MHz): 1.7−2.3 (broad, 8H), 3.1−4.3 (broad, 26H), 7.36 (broad, 4H), 7.6 (broad, 1H). MS (CI − NH_3): 1074 (M + NH_4).

The trifluoroacetamides were weighed (*ca.* 20.00 mg), dissolved in NH_3−MeOH solution (7 N), and allowed to sit overnight. The next day, the solvent was evaporated to an oil. The samples were freed of the side product trifluoroacetamide by the evaporation of three volumes (*ca.* 5 mL) of methanol from the sample, followed by vacuum drying at <0.1 mmHg after each evaporation. From the weight of the purified product, the deprotections were seen to be complete and all trifluoroacetamide was removed from the sample. Ligands **7**, **9**, and **11**−**13** were further purified by flash chromatography (5%−30% NH_3 in MeOH). The purified and unpurified macrocycles **9**, however, were not distinguishable by our kinetic assay.

***S,S'*-Bis(10-(1,4,7-triazacyclododecyl))-1',3'-dithiobenzene (6).** ^1H NMR (CD_2Cl_2, 400 MHz): 1.60 (m, 4 H, coupled to p at 4.06 ppm, $H_{9,11}$), 1.69 (m, 4 H, coupled to p at 4.06 ppm, $H_{9,11}$), 2.61 (m, 4 H, d with HND at 1.7 ppm, J = 13.3 Hz, $H_{8,12}$), 2.72 (m, 6 H, $H_{2,3,5,6}$), 2.9 (m, 14 H, $H_{2,3,5,6}$), 2.95 (broad, 3 H, N−H), 4.06 (p, J = 6.6 Hz, 2 H, H_{10}), 7.19 (s, 3 H), 7.46 (s, 1 H). MS (CI − NH_3): 481 ($M^+ + 1$).

***S,S'*-Bis(10-(1,4,7-triazacyclododecyl))-1',4'-dithiobenzene (7).** ^1H NMR (CD_2Cl_2, 400 MHz): 1.62 (m, 4 H, coupled to p at 4.09 ppm, $H_{9,11}$), 1.70 (m, 4 H, coupled to p at 4.09 ppm, $H_{9,11}$), 2.60 (m, 8 H, d with HND at 1.7 ppm, J = 12.7 Hz, $H_{8,12}$), 2.75 (m, 10 H, $H_{2,3,5,6}$), 2.85 (m, 6 H, $H_{2,3,5,6}$), 2.94 (m, 4 H, d with HND at 1.7 ppm, J = 12.7 Hz, $H_{8,12}$), 3.0 (broad, 6 H, N−H), 4.09 (p, J = 6.8 Hz, 2 H, H_{10}), 7.32 (s, 4 H). HRMS (+ FAB): 480.3069 ± 0.0006 (average of two determinations). M^+ is calculated to be 480.3069 with the formula $C_{24}H_{44}N_6S_2$.

***S,S'*-Bis(12-(1,5,9-triazacyclododecyl))-1',4'-dithiobenzene (8).** ^1H NMR (CD_2Cl_2, 200 MHz): 1.7 (m, 16 H), 2.51 (m, 4 H), 2.74 (m, 20 H), 2.90 (broad, 6 H), 3.69 (p, J = 6.9 Hz, 2 H), 7.40 (s, 4 H). MS (CI − NH_3): 537 ($M^+ + 1$).

***S,S'*-Bis(10-(1,4,7-triazacyclododecyl))-4',4''-dithiobiphenyl (9).** ^1H NMR ($CDCl_3$, 400 MHz): 1.65 (m, 4H, coupled to p at 3.98 ppm, $H_{9,11}$), 1.78 (m, 4 H, coupled to p at 3.98 ppm, $H_{9,11}$), 2.68 (m, 4 H; d with HND at 1.7 ppm, J = 13 Hz; $H_{8,11}$), 2.75 (m, 16 H, $H_{2,3,5,6}$), 2.89 (m, 4 H; d with HND at 1.7 ppm, J = 13 Hz; $H_{8,11}$), 3.98 (p, J = 6.7 Hz, 2 H, H_{10}), 7.49 (d, J = 2.1 Hz, 4 H), 7.54 (d, J = 2.1 Hz, 4 H). ^{13}C NMR (CD_2Cl_2, 300 MHz): 33.9 (I = 140), 40.9 (I = 48), 44.4 (I = 165), 45.9 (I = 88), 46.2 (I = 92), 127.5 (I = 188), 131.6 (I = 98), 135.8 (I = 7), 138.6 (I = 10). MS (CI − NH_3): 557 ($M^+ + 1$).

***S,S'*-Bis(10-(1,4,7-triazacyclododecyl))-1',5'-dithiopentane (10).** ^1H NMR (D_3COD, 400 MHz): 1.5 (m, 2 H), 1.6 (m, 8 H), 1.72 (m, 4 H), 2.52 (t, J = 7 Hz, 4 H), 2.6−2.9 (m, 24 H), 3.26 (p, J = 6.8 Hz, 2 H). MS (CI − NH_3): 475 ($M^+ + 1$).

10-(Phenylthio)-1,4,7-triazacyclododecane (11). ^1H NMR ($CDCl_3$, 400 MHz): 1.65 (m, 2 H, coupled to p at 4.03 ppm, $H_{9,11}$), 1.70 (m, 2 H, coupled to p at 4.03 ppm, $H_{9,11}$), 2.20 (m, 3 H, N−H), 2.64 (m, 2 H; d with HND at 1.7 ppm, J = 13 Hz; H_{8+12}), 2.71 (m, 4 H, $H_{2,3,5,6}$), 2.92 (m, 4 H, $H_{2,3,5,6}$), 2.95 (m, 2 H; d with HND at 1.7 ppm, J = 13 Hz, $H_{8,12}$), 4.03 (p, J = 6.7 Hz, 1 H), 7.24 (m, 4 H), 7.4 (m, 1 H). MS (CI − NH_3): 280 ($M^+ + 1$).

10-(Methylthio)-1,4,7-triazacyclododecane (12). ^1H NMR ($CDCl_3$, 400 MHz): 1.58 (m, 2 H, coupled to p at 4.03 ppm, $H_{9,11}$), 1.65 (m, 2 H, coupled to p at 4.03 ppm, $H_{9,11}$), 2.04 (s, 3 H, CH_3), 2.68 (m, 2 H; d with HND at 1.7 ppm, J = 13 Hz; $H_{9,11}$), 2.65−2.88 (m, 8 H, $H_{2,3,5,6}$), 2.92 (m, 2 H; d with HND at 1.7 ppm, J = 13 Hz; $H_{9,11}$), 3.32 (p, J = 6.8 Hz, 1 H, H_{10}). MS (CI − NH_3): 218 ($M^+ + 1$).

10-(Phenylthio)-1,5,9-triazacyclododecane (14). ^1H NMR (D_3COD, 400 MHz): 1.68 (m, 6 H), 1.83 (m, 2 H), 2.2−3.0 (b, 3H), 2.50 (m, 2 H), 2.68 (m, 8 H), 2.85 (m, 2 H), 3.45 (p, J = 7.2 Hz, 1 H), 7.18−7.34 (m, 3 H), 7.48 (m, 2 H). ^{13}C NMR (D_3COD, 300 MHz): 29.9 (I = 127.5), 35.5 (I = 130), 45.1 (I = 50), 48.0 (I = 156), 49.3 (I = 139), 50.7 (I = 157), 129.5 (I = 58), 131.3 (I = 82), 135.3 (I = 81), 138.3 (I = 8). Ms (CI − NH_3): 308 ($M^+ + 1$).

Kinetic Measurements. All buffers were prepared with deionized (Millipore) distilled H_2O and were sparged with Ar. The pH of all aqueous solutions was measured with an Orion combination electrode (Ag/AgCl reference) and an Orion 701A digital meter at room temperature before addition of DMSO (spectrophotometric grade). The free base of Trisma (also commonly named Tris) buffer solution (50 mmol) was titrated to the desired pH with $HClO_4$ (69−72%). HEPES (50 mM) was titrated with NaOH (1 M). EPPS, CAPS, and CHES (10 mM each) were titrated Me_4NOH (2.74 M). The buffers were filtered through a 0.2 μm nylon membrane filter (Gelman) immediately before use.

All absorbance traces were recorded with a Cary-Varian (model 1e) dual-beam absorbance spectrometer fitted with a thermostated cuvet holder and a sample transport accessory. Samples were prepared in

(23) Newman, M. S.; Karnes, H. A. *J. Org. Chem.* **1966**, *31*, 3980.

10 cm path length glass microcuvettes. The buffer (1.1 mL) was measured into the cuvette followed by the macrocycle of choice (10 μL, 2.5 mM dimers or 50 mM monomers in MeOH) and $Zn(ClO_4)_2$ (10 μL, 50 mM in MeOH). The cuvettes were sealed with Teflon stoppers and were brought to the desired temperature in the cell block of the absorbance spectrometer. After a *ca.* 30 min equilibration time, the substrate (10 μL, 50 mM in DMSO (**3**) or H_2O (**2** and **5**)) was injected into the cell and the solutions were mixed thoroughly with the tip of a Pasteur pipet. The increase in concentration of nitrophenylate (l = 400 nm) was measured every 2–5 min. The initial slope (<5% conversion) of a plot of the measured absorbance vs time was determined (correlation coefficient >0.99), and the tabulated rate constants calculated with the extinction coefficient of nitrophenylate (ϵ(400 nm) = 1.88×10^4 M^{-1} cm^{-1}).

For the cyclization–hydrolysis of UpU, reactions were performed with 1.5 mM catalysts and 0.16 mM 3′,5′-UpU in 20 vol % DMSO in H_2O with 50 mM Tris buffer at pH 8.36 and 41 °C. The formation of uridine was followed by HPLC using an internal standard, as we have described previously,[17-21] but reactions with our catalysts were followed using an autosampler and temperature was controlled by the autosampler compartment. The control reaction for UpU cleavage in the absence of catalysts—to determine k_{uncat}—was performed with 20 mM HEPES buffer (pH = 8.35). Reactions were performed at 91, 81, 71, and 41 °C. The data fit an Arrhenius plot, with r^2 = 0.994. The value at 41 °C was used to calculate the values of k_{cat}/k_{uncat} that are listed in Table 1.

Identification of the Products of a Catalyzed Reaction. The products of the hydrolysis of **3** (500 mM) catalyzed by the dimer **9**–Zn(II) complex (250 mM BP ligand, 500 mM $Zn^{II}(ClO_4)_4$) were determined by ^{31}P and ^1H NMR. The reaction mixture (80% 3 mM HEPES, 20% DMSO, pH 8.55, 140 mL per run) was heated to 55 °C for 23 h. After this time, the degree of conversion was found to be 48% by absorbance (400 nm). The product mixture was desalted with the basic form of a strongly acidic cation exchange resin (Amberlite, IR-120 Na^+, 10 g), and the solvent was evaporated under reduced pressures. Analysis of the residue by ^{31}P NMR (50:50 D_2O:DMSO) revealed 7% phosphate (1 ppm), 49% **2** (−5 ppm), and 44% **3** (−12 ppm). ^1H NMR spectra of the same sample revealed 35% nitrophenol and 65% **2** + **3** by integration of the upfield aromatic peaks (the two phosphates were not well resolved). Evaporation of the solvent from the reaction mixture without desalting left an insoluble yellow solid. Treatment of an authentic mixture of **2** and **3** (250 mM each in 80% mM HEPES and 20% DMSO) with IR-120 Na^+ and evaporation of the solvent did not disturb the ratio of the two phosphates by ^{31}P NMR.

Potentiometric Titration of the Ligands. Acidified mixtures of the macrocycles (1 mM, 0.5 mL, 4.0 equiv of HCl) were titrated with NaOH (100 mM, 1.6 μL per addition) in 50 mM Me_4NClO_4. The base was delivered to the solution via a gas tight syringe whose plunger was driven by a micrometer. The titration apparatus was calibrated by relating the weight of water delivered by the syringe to the change in the micrometer setting. The pH after each addition of NaOH was measured with a solid state microelectrode (model PHR-146 from Lazer Research).

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