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Published on Web 08/18/2006

Catalysis of Diribonucleoside Monophosphate Cleavage by Water Soluble Copper(II) Complexes of Calix[4]arene Based Nitrogen Ligands

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Abstract: Calix[4]arenes functionalized at the 1,2-, 1,3-, and 1,2,3-positions of the upper rim with [12]ane-N_3 ligating units were synthesized, and their bi- and trimetallic zinc(II) and copper(II) complexes were investigated as catalysts in the cleavage of phosphodiesters as RNA models. The results of comparative kinetic studies using monometallic controls indicate that the subunits of all of the zinc(II) complexes and of the 1,3-distal bimetallic copper(II) complex **7**−Cu_2 act as essentially independent monometallic catalysts. The lack of cooperation between metal ions in the above complexes is in marked contrast with the behavior of the 1,2-vicinal bimetallic copper(II) complex **6**−Cu_2, which exhibits high catalytic efficiency and high levels of cooperation between metal ions in the cleavage of HPNP and of diribonucleoside monophosphates *NpN*′. A third ligated metal ion at the upper rim does not enhance the catalytic efficiency, which excludes the simultaneous cooperation in the catalysis of the three metal ions in **8**−Cu_3. Rate accelerations relative to the background brought about by **6**−Cu_2 and **8**−Cu_3 (1.0 mM catalyst, water solution, pH 7.0, 50 °C) are on the order of 10^4-fold, largely independent of the nucleobase structure, with the exception of the cleavage of diribonucleoside monophosphates in which the nucleobase *N* is uracil, namely UpU and UpG, for which rate enhancements rise to 10^5-fold. The rationale for the observed selectivity is discussed in terms of deprotonation of the uracil moiety under the reaction conditions and complexation of the resulting anion with one of the copper(II) centers.

Many enzymes that catalyze the hydrolytic cleavage of phosphodiesters incorporate two or three metal ions in their active site.[1] In the aim at mimicking the extraordinary catalytic activity of such enzymes, di- and trinuclear complexes of di- and trivalent metal ions have been synthesized and shown to be better catalysts than analogous mononuclear complexes in the cleavage of di- and oligoribonucleotides, as well as of RNA model compounds.[2]

Our previous studies in the field have shown that the calix-[4]arene scaffold, blocked in the *cone* conformation by proper alkylation of the lower rim hydroxyls, is suitable for the design of di- and trimetallic phosphodiesterase models.[2g,3] Efficient catalysis by synergic action of the metal centers was observed, for instance, for the zinc(II) complexes of 2,6-bis[(dimethylamino)methyl]pyridine (BAMP) functionalized calix[4]arenes **1**−**3**. Analogous copper(II) complexes were investigated, but much poorer results were obtained.[4] A major drawback of the BAMP functionalized catalysts was the limited solubility in water which required the use of mixed aqueous−organic solvents. Another limitation, a more serious one, was the moderate affinity of BAMP for zinc(II). The binding constant $K \approx 1 \times 10^5\ M^{-1}$ in CH_3CN/H_2O 1:1, pH 7.0,[5] which is large enough for studies carried out at 1 mM catalyst concentration, drops to $1.1 \times 10^3\ M^{-1}$ in water at the same pH.[6]

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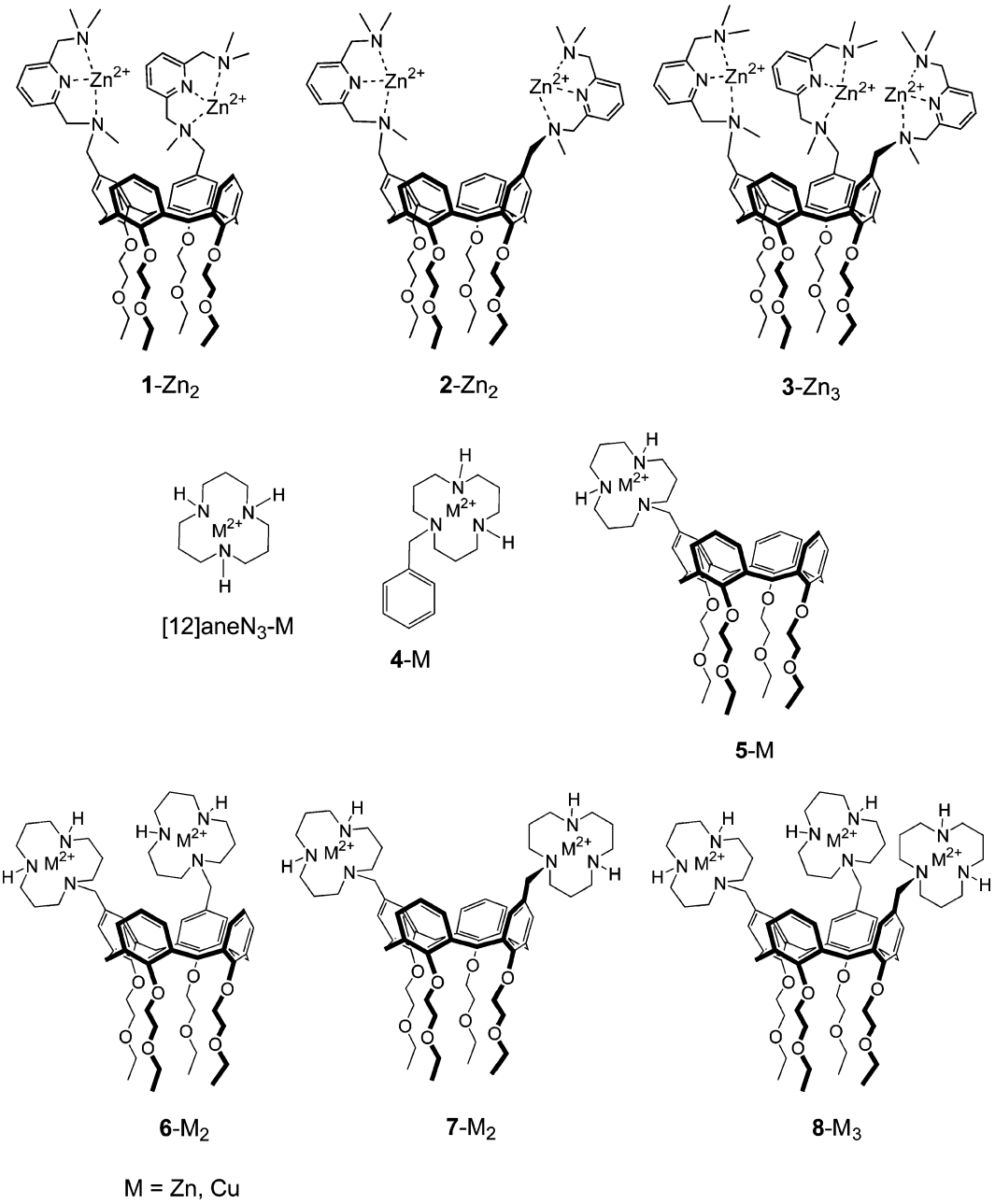
(6) From spectrophotometric titration at 266 nm of 0.20 mM BAMP with Zn-$(ClO_4)_2$ in H_2O, 20 mM HEPES, pH 7.0, 25 °C (this work).

10.1021/ja0632106 CCC: $33.50

Chart 1



To investigate further the potential of calix[4]arene-based artificial phosphodiesterases, and with the specific aim at developing metal catalysts soluble enough in water to avoid the use of organic cosolvents, we turned our attention to 1,5,9-triazacyclododecane ([12]aneN$_3$) as a ligating unit for the construction of di- and tritopic ligands. Zinc(II) and copper(II) complexes of [12]aneN$_3$ and other polynitrogen macrocycles have well documented catalytic properties in phosphodiester cleavage.[2a−j,7] In water solvent [12]aneN$_3$ has a very high affinity for zinc(II) (log K = 8.41 at 25 °C),[8] and even higher is the affinity for copper(II) under the same conditions (log K = 12.63).[9] This ensures extensive binding of the metal ion to the ligand, on condition that the pH is not too low.[10]

Herein, we describe the synthesis of calix[4]arenes functionalized with [12]aneN$_3$ ligating units and the results of a kinetic investigation of the catalytic activity of their zinc(II) and copper(II) complexes in the cleavage of RNA model compound 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNP). The copper(II) complexes were further investigated as catalysts of the cleavage of a number of diribonucleoside 3′,5′-monophosphates that more closely mimic the structure of RNA.

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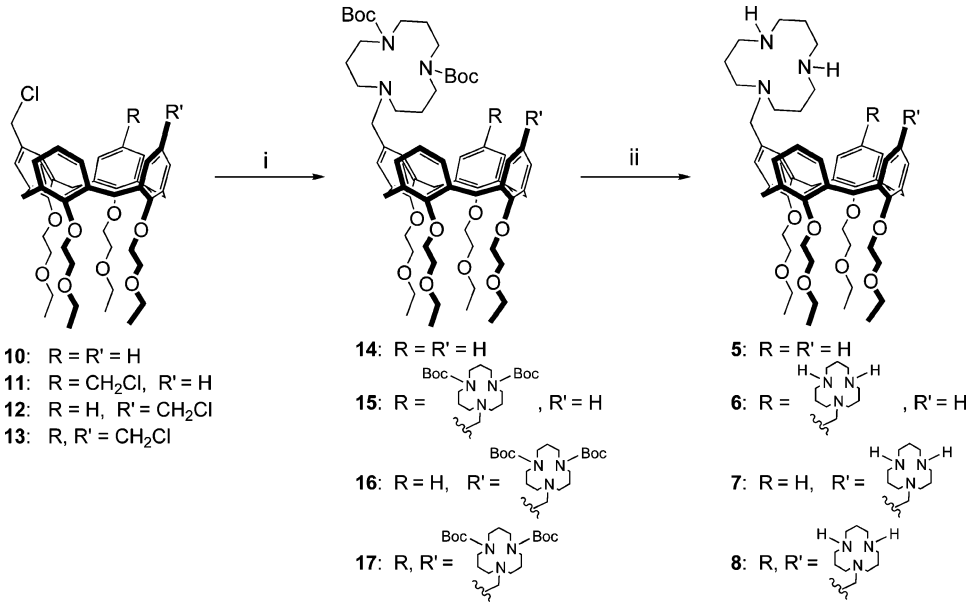
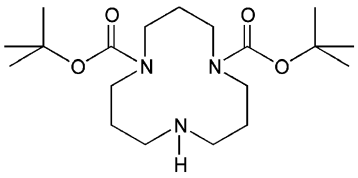
Scheme 1[a]



[a] Reagents and conditions: (i) bis-Boc-protected [12]aneN$_3$ **9**, K_2CO_3, CH_3CN; (ii) (a) CF_3COOH, CH_2Cl_2, rt, 2 h; (b) Li_2CO_3.

Results and Discussion

Ligand Syntheses. To avoid complications arising from undesired polysubstitution of [12]aneN$_3$, we developed a synthetic procedure for the preparation of calix[4]arenes **5**−**8** (Scheme 1) which involved alkylation of bis-Boc-protected [12]-aneN$_3$ **9**.[11] This strategy offered the additional advantage of an



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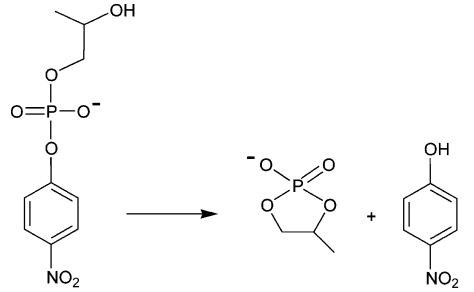
easier purification of the protected calix[4]arene intermediates. Introduction of [12]aneN$_3$ units at the upper rim was carried out by reaction of the chloromethylated calix[4]arenes **10**−**13** with **9** in dry acetonitrile, in the presence of K_2CO_3. The reaction rate of the latter process was quite low, reasonably due to steric hindrance in the nucleophile. The monofunctionalized compound **14** was obtained in 70% yield after two days of reaction, whereas 5 days were necessary to obtain good yields of the di- and trifunctionalized compounds **15**−**17**. Pure products **5**−**8** were obtained in quantitative yield upon removal of Boc protecting groups by reaction with trifluoroacetic acid in dichloromethane. The trifunctionalized derivative **8** resulted in being water soluble up to 0.5 mM showing a well resolved ^{1}H NMR spectrum (see Supporting Information).

Table 1. HPNP Transesterification Catalyzed by Mono-, Di-, and Trinuclear Zinc(II) Complexes of [12]aneN$_3$-Based Systems, in 50% CH_3CN/H_2O, 20 mM HEPES, pH 7.0, 25.0 °C[a]

catalyst	k_{obs}[b] (s^{-1})	k_{obs}/k_{bg}[c]
[12]aneN$_3$−Zn	8.6×10^{-6}	450
4−Zn	3.7×10^{-6}	190
6−Zn$_2$	6.3×10^{-6}	330
7−Zn$_2$	6.0×10^{-6}	320
8−Zn$_3$	2.1×10^{-5}	1100

[a] UV−vis monitoring of reaction progress in runs carried out on 0.15 mM substrate solutions, in the presence of 0.20 mM catalyst. [b] Initial rate method, error limit ±10%. [c] $k_{bg} = 1.9 \times 10^{-8}$ s^{-1}, from ref 3.

HPNP Cleavage. Contrary to our hopes, the zinc(II) complexes of ligands **5**−**8** were not soluble enough in water for catalytic studies of phosphodiester cleavage. Nevertheless, complexes **6**-Zn$_2$, **7**-Zn$_2$, and **8**-Zn$_3$ were tested as catalysts of HPNP transesterification (eq 1) in 50% aqueous CH_3CN at pH 7.0 for comparison with analogous BAMP-based complexes.[2g,3]



HPNP (1)

Since **5**-Zn turned out to be insoluble also in 50% aqueous CH_3CN, it was replaced by the *N*-benzylated model **4**-Zn. Kinetic data (Table 1) are expressed as pseudo-first-order rate constants for the spectrophotometrically monitored liberation of *p*-nitrophenol under a standard set of experimental conditions. The results of the catalytic experiments reveal a disappointing lack of synergism between metal ions, which is in marked contrast with the results previously obtained in the transesteri-

(10) Combination of the log *K* values for binding of zinc(II) and copper(II) to [12]aneN$_3$ with a value of 12.6 for the pK_a of the ligand (from ref 8) shows that, at pH 7.0 and 1 mM ligand and metal ion, the amount of ligated metal ion is >99% in the case of copper(II) but drops to less than 50% for zinc(II). However, because of the insolubility of zinc(II) complexes in water (see further on), catalytic experiments were carried out in 50% aqueous CH_3CN. If the binding affinity of [12]aneN$_3$ for zinc(II) on going from pure water to 50% aqueous CH_3CN experiences the same enhancement as that of BAMP, namely, 2 orders of magnitude, then the amount of zinc(II) bound to [12]aneN$_3$ rises to about 90%.

(11) Brandes, S.; Gros, C.; Denat, F.; Pullumbi, P.; Guilard, R. *Bull. Soc. Chim. Fr.* **1996**, *133*, 65−73.

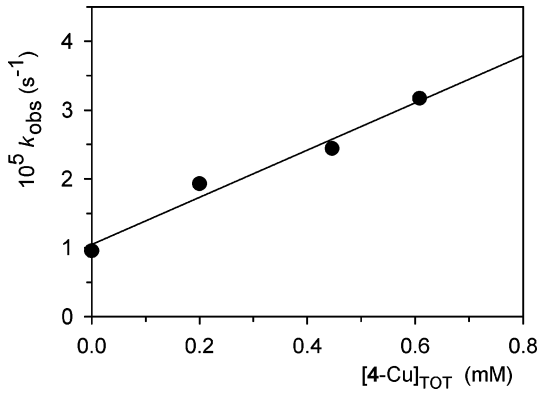


Figure 1. Effect of added **4**−Cu on the hydrolysis of 0.1 mM *p*-nitrophenyl acetate (water, pH 7.0, 20 mM HEPES, 25.0 °C; time-course experiments).

fication of HPNP catalyzed by the zinc(II) complexes of the BAMP functionalized calix[4]arene ligands.[2g,3] In that case a high degree of synergism between metal centers was observed for both dinuclear complexes **1**-Zn_2 and **2**-Zn_2, with 61- and 380-fold rate-enhancements of dinuclear over mononuclear catalyst, respectively. It is worth noting that [12]aneN_3-Zn is about 13 times more efficient a catalyst than BAMP−Zn;[12] yet metal complexes **6**-Zn_2, **7**-Zn_2, and **8**-Zn_3 exhibit no significant synergism between metal centers. These findings clearly indicate that promising arrangements of catalytically effective ligated metal ions on a molecular platform by no means guarantee efficient catalysis. Such a situation is disappointing but not unprecedented. A number of examples are available in the literature in which the efficiency of bimetallic complexes is similar to, or even lower than, that of analogous monometallic complexes.[7b,13]

We had better luck with the copper(II) complexes. With the sole exception of **5**−Cu, they were soluble enough in water for kinetic measurements, the highest solubility being experienced by **8**−Cu_3 (ca. 5 mM).

In a study of phosphodiester hydrolysis catalyzed by the copper(II) complex of 1,4,7-triazacyclononane (water solution, pH 7.24), Burstyn and Deal[7k] reported that the catalytically active monomeric form is in equilibrium with an inactive dimer, as indicated by the clean $^1/_2$-order dependence on the total copper(II) complex concentration. Furthermore, the catalytic inertness of copper(II) complexes of ditopic polynitrogen ligands was ascribed by Mancin et al.[13b] to the formation of intracomplex μ-hydroxo-bridged dinuclear species. Therefore, the [12]aneN_3−copper(II) system was tested for the possible formation of unreactive dimeric species. As a convenient test reaction, we have investigated the rate of *p*-nitrophenyl acetate hydrolysis in the presence of increasing amounts of **4**−Cu at pH 7.0. The linear dependence of the pseudo-first-order rate constant on the total concentration of **4**−Cu (Figure 1) excludes any significant involvement of dimeric species in the investigated concentration range.

(12) The value $k_{obs}/k_{bg} = 180$ reported in ref 3 for the transesterification of 0.2 mM HPNP promoted by 1.0 mM BAMP−Zn has been corrected taking into account the different catalyst concentration and assuming subsaturating conditions.

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Table 2. HPNP Transesterification Catalyzed by Cu^{2+} Complexes of [12]aneN_3-Based Systems in H_2O, 20 mM HEPES, pH 7.0, 25.0 °C[a]

catalyst	k_{obs}[b] (s^{-1})	k_{obs}/k_{bg}[c]
[12]aneN_3−Cu	3.3×10^{-6}	15
4−Cu	5.8×10^{-6}	26
6−Cu_2	2.4×10^{-4}	1090
7−Cu_2	8.7×10^{-6}	40
8−Cu_3	1.3×10^{-4}	590

[a] UV−vis monitoring of reaction progress in runs carried out on 0.1 mM substrate solutions, in the presence of 0.20 mM catalyst. [b] Initial rate method, error limit ±10%. [c] $k_{bg} = 2.2 \times 10^{-7}\ s^{-1}$, from transesterification of a 2.0 mM HPNP solution.

Complexes **6**−Cu_2, **7**−Cu_2, and **8**−Cu_3 and mononuclear controls [12]aneN_3−Cu and **4**−Cu were analyzed for catalysis of the transesterification of HPNP in water solution, pH 7.0. The results are collected in Table 2. Comparison with the data in Table 1 shows that mononuclear copper(II) complexes are significantly less efficient than the corresponding zinc(II) complexes. The different catalytic activity is most likely related to the acidity of the water coordinated to the [12]aneN_3−ligated metal ion, which is much higher for the zinc(II) complex ($pK_a = 7.3$)[8] than for the copper(II) complex ($pK_a = 8.4$).[7i] However in this case the different substitution pattern of metal ion binding sites in **6**−Cu_2 and **7**−Cu_2 has a marked influence on catalytic activity, the 1,2-vicinal bimetallic catalyst **6**−Cu_2 being much more effective than its 1,3-distal regioisomer **7**−Cu_2. The catalytic rate enhancement brought about by the latter is about twice as great as those exhibited by mononuclear complexes, showing that there is no rate acceleration per metal center. In contrast, cooperativity between metal ions is observed in the reaction catalyzed by **6**−Cu_2. Here the rate enhancement relative to a monometallic analogue is either 70-fold or 40-fold, depending on whether [12]aneN_3−Cu or **4**−Cu is taken as reference.

No evidence of trimetallic catalysis is seen in the reaction catalyzed by the trinuclear complex **8**−Cu_3, whose catalytic efficiency turns out to be slightly lower than that of **6**−Cu_2. Therefore it appears that **8**−Cu_3 uses only two vicinal metal centers in the catalytic mechanism, thus behaving as a 1,2-vicinal bimetallic catalyst, whose efficiency is somewhat lowered by the third ligated metal ion, presumably because of steric hindrance.

In line with expectations, a plot of initial rate vs substrate concentration for the **8**−Cu_3 catalyzed transesterification of HPNP showed the downward curvature typical of saturation kinetics (Figure 2). Fitting of the data to the Michaelis−Menten equation gave a value of 2.0 mM for K_M and one of $7.6 \times 10^{-4}\ s^{-1}$ for k_{cat}. Thus, the kinetics are consistent with a two-step catalytic mechanism in which a reversibly formed, moderately strong catalyst−substrate complex ($K = 1/K_M = 500\ M^{-1}$) decomposes into products with a first-order rate constant equal to k_{cat}. Any significant formation of unproductive 1:2 catalyst−substrate complex must be ruled out, since a bell-shaped concentration−rate profile should have been observed in such a case.[14] A likely mode of binding of HPNP to the catalyst involves bridging[4] of the phosphate moiety to the ligated copper(II) ions in 1,2-vicinal position. Accordingly, in line with previous suggestions,[4] the high rate accelerations observed for

(14) Cacciapaglia, R.; Di Stefano, S.; Kelderman, E.; Mandolini, L. *Angew. Chem., Int. Ed.* **1999**, *38*, 348−351.

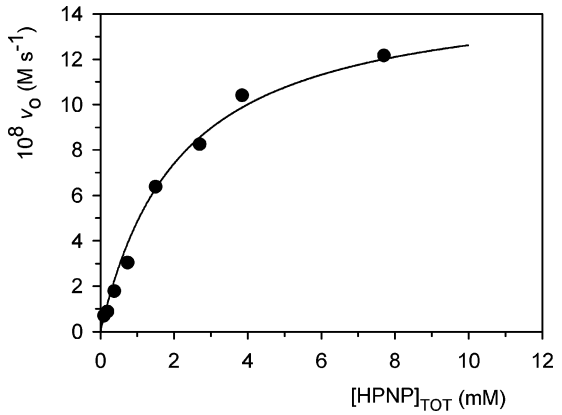
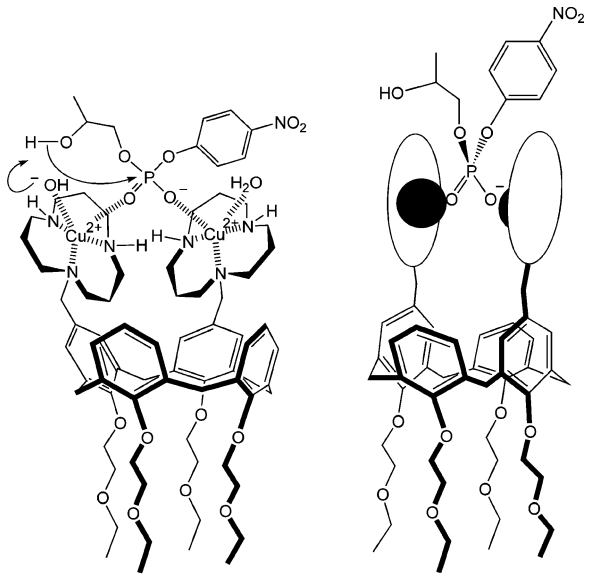


Figure 2. Initial rate of HPNP transesterification vs substrate concentration for the reaction carried out in the presence of 0.20 mM **8**−Cu_3, in water, pH 7.0 (20 mM HEPES), 25.0 °C. The points are experimental, and the curve is a plot of the Michaelis−Menten equation with best fit values K_M = 2.0 mM and $k_{cat} = 7.6 \times 10^{-4}$ s^{-1}.

the transesterification catalyzed by **6**−Cu_2 and **8**−Cu_3 are due to double Lewis acid activation, with the putative involvement of the metal hydroxide as an intramolecular general base (**I**).



I **II**

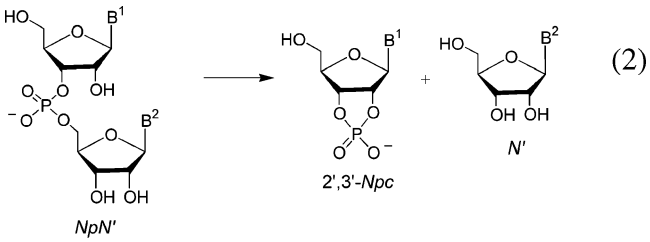
Inspection of a CPK molecular model of the complex of HPNP with the 1,3-distal regioisomer **7**−Cu_2 shows that phosphate bridging to the copper(II) ions requires a *pinched-cone* conformation in which the two [12]aneN_3 units are brought in a nearly parallel position, thereby defining a narrow corridor in which the HPNP substrate is hosted (**II**). It is difficult to judge whether such a catalyst−substrate complex has a definite stability, since the existence of, say, 2−3 kcal/mol of strain energy arising from eclipsing and/or nonbonded interactions can hardly be estimated on the basis of a CPK model. But what is clearly suggested by inspection of the model is that one of the [12]aneN_3 units prevents the 2-hydroxypropyl chain from assuming the pseudocyclic conformation required by OH addition to the phosphorus. This is tantamount to saying that **7**−Cu_2 is not a good host for the transition state of the HPNP transesterification because of steric repulsion between the 2-hydroxypropyl chain and one of the ligating units. We speculate therefore that the sterically demanding [12]aneN_3 ligand is most responsible for the lack of cooperativity between metal ions in **7**−Cu_2. In contrast, a CPK model of the HPNP complex with **6**−Cu_2 reveals a much more open structure, in which the average planes of the ligating units are far from parallel, and no steric hindrance to the motion of the 2-hydroxypropyl chain is apparent.

Table 3. Cleavage of Diribonucleoside 3′,5′-Monophosphates *NpN′* in the Presence of Trinuclear Catalyst **8**−Cu_3[a]

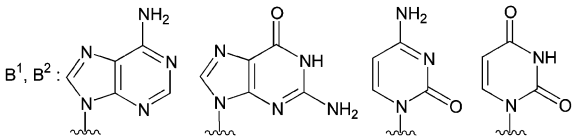
NpN′	k_{obs}[b] (s^{-1})	k_{rel}	k_{obs}/k_{bg}[c]
CpA	$<2 \times 10^{-7}$[d]	<0.14	$<3 \times 10^2$
GpA	1.4×10^{-6}	1.0	
CpG	2.1×10^{-6}	1.5	0.6×10^4
CpC	2.5×10^{-6}	1.8	0.6×10^4
ApG	2.7×10^{-6}	1.9	1.3×10^4
GpG	2.8×10^{-6}	2.0	1.3×10^4
GpU	3.7×10^{-6}	2.6	
UpG	4.2×10^{-5}	30	
UpU	5.5×10^{-5}	39	1.4×10^5

[a] Reaction conditions: [**8**−Cu_3] = 1.0 mM, [*NpN′*] = 0.10 mM, water, pH 7.0 (HEPES 20 mM), 50.0 °C. [b] Error limits ±10%. [c] 10^{10} k_{bg} (pH 7.0, 50.0 °C) (s^{-1}): CpA, 6.1; CpC, 4.3; UpU, 3.9; CpG, 3.5; GpG, 2.2; ApG, 2.1. From data reported in ref 17 after extrapolation (see ref 18) to pH 7.0. [d] No reaction after 24h.

Diribonucleoside Monophosphate Cleavage. It is well-known that investigations of phosphodiesters with good leaving groups may not give conclusions which apply to phosphodiesters with bad leaving groups, because species that cleave effectively *p*-nitrophenyl phosphates are not necessarily good catalysts for the cleavage of unactivated phosphate esters, and vice versa.[13c,15] It was therefore of interest to investigate the catalytic activity of our copper(II) complexes in the cleavage of diribonucleoside 3′,5′-monophosphates *NpN′*. The reaction involves intramolecular nucleophilic attack of the 2′-OH group and liberation of a nucleoside and of a nucleoside 2′,3′-cyclic monophosphate (eq 2).[15,16] The lot of investigated substrates listed in Table 3 is an



(2)



arbitrary choice of 9 out of the 16 possible diribonucleoside 3′,5′-monophosphates but sufficient to the purpose of disclosing nucleobase selective cleavage, if any. A first set of catalytic experiments was carried out in water solution, pH 7.0 at 50.0 °C, in the presence of **8**−Cu_3, which is the complex with the highest solubility in water. The kinetics were monitored by HPLC. Typical chromatograms are shown in Figure 3. Initial rates of nucleoside formation were translated into the pseudo-

(15) Oivanen, M.; Kuusela, S.; Lönnberg, H. *Chem. Rev.* **1998**, *98*, 961−990.
(16) (a) Cassano, A. G.; Anderson, V. E.; Harris, M. E. *Biopolymers* **2004**, *73*, 110−129. (b) Perreault, D. M.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 432−450.

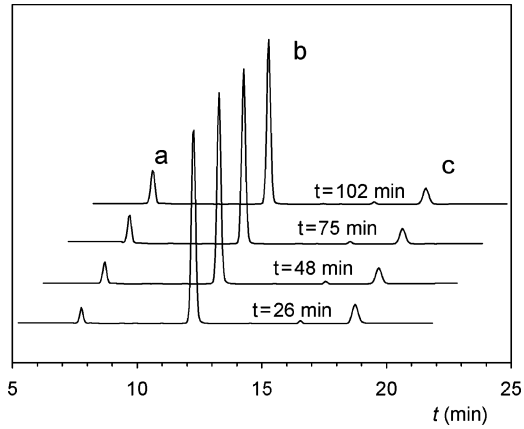


Figure 3. HPLC traces of the cleavage of 0.10 mM UpU promoted by 1.0 mM **8**−Cu_3 (water, 20 mM HEPES, pH 7.0, 50.0 °C). Chromatograms of the reaction mixture quenched at different time intervals showing chromatographic peaks of uridine (a), UpU (b), and internal standard (c). The axis refers to the bottom trace. Other traces are offset with respect to the next one by 1.0 min.

Table 4. CpA and UpU Cleavage in the Presence of Cu^{2+} Complexes of [12]aneN$_3$ and **6**−**8**[a]

	CpA			UpU		
catalyst	k_{obs}[b] (s^{-1})	k_{rel}	k_{obs}/k_{bg}[c]	k_{obs} (s^{-1})	k_{rel}	k_{obs}/k_{bg}[c]
[12]aneN$_3$−Cu	6.6×10^{-7}	1.0	1.1×10^3	2.7×10^{-7}	1.0	6.9×10^2
6−Cu_2	3.7×10^{-6}	5.6	6.1×10^3	4.2×10^{-5}	160	1.1×10^5
7−Cu_2	1.3×10^{-6}	2.0	2.1×10^3	3.8×10^{-7}	1.4	9.7×10^2
8−Cu_3[d]	$<2 \times 10^{-7}$	<0.3	$<3 \times 10^2$	5.5×10^{-5}	200	1.4×10^5

[a] Reaction conditions: [sub.] = 0.10 mM, [cat.] = 1.0 mM (water, pH 7.0, HEPES 20 mM, 50.0 °C). [b] Error limits ±10%. [c] See footnote *c* to Table 3. [d] Data from Table 3.

first-order rate constants summarized in Table 3. With the sole exception of CpA, for which no reaction was observed after 24 h, **8**−Cu_3 effectively cleaves all of the investigated diribonucleoside monophosphates, with a remarkable preference for UpU and UpG, that are cleaved 39 and 30 times, respectively, more rapidly than GpA. Rates of transesterification of CpA and UpU, the least and the most reactive diribonucleoside monophosphate, respectively, in the **8**−Cu_3 catalyzed reaction, were also investigated in the presence of [12]aneN$_3$−Cu, **6**−Cu_2, and **7**−Cu_2. The results (Table 4) indicate that there are considerable analogies with the order of catalytic efficiency observed in the cleavage of HPNP (Table 2). First, in both CpA and UpU cleavage reactions catalyzed by the 1,3-distal bimetallic catalyst **7**−Cu_2, there is no rate acceleration per metal center, as shown by the behavior of the same substrates in the presence of the mononuclear catalyst [12]aneN$_3$−Cu. Second, cooperativity is found between metal centers in the 1,2-vicinal bimetallic catalyst **6**−Cu_2. It is remarkably large in the cleavage of UpU and much smaller, but still appreciable, in the cleavage of CpA. Finally, the dinuclear complex **6**−Cu_2 catalyzes the cleavage of UpU with an efficiency quite similar to that of **8**−Cu_3, thus reinforcing the view that the trinuclear catalyst uses only two vicinal metal centers, whereas the third metal ion acts as a more or less innocent spectator.

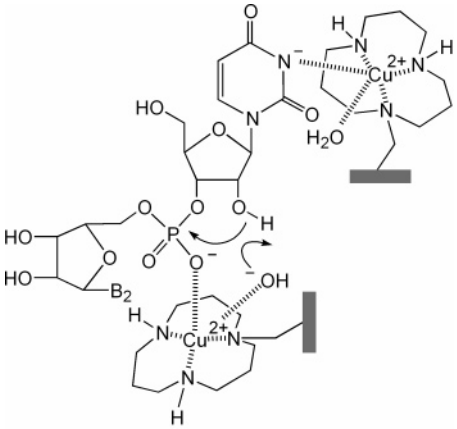
The background rate of cleavage of CpA is very similar to that of other diribonucleoside monophosphates (see below). Hence, the surprising inertness of **8**−Cu_3 in CpA cleavage is neither due to an inherently low reactivity of the substrate nor to its insensitivity to copper(II) catalysis, as clearly shown by the finding that CpA is cleaved by [12]aneN$_3$−Cu and by **7**−Cu_2 about 3 times more rapidly than UpU (Table 4). The lack of activity of **8**−Cu_3 toward CpA must be due to the formation of a presumably strong, nonproductive substrate−catalyst complex, where an improper substrate orientation is imposed by the third ligated metal ion.

There is ample evidence that the nucleobase structure has only a modest influence on the background cleavage of the phosphodiester bond of diribonucleoside monophosphates, under both acid and basic conditions.[15] Most relevant to the present study is a set of specific rates (10^6 k_{bg}, s^{-1}) reported by Komiyama[17] for the cleavage at pH 11.08 and 50.0 °C: CpA, 7.3; CpC, 5.2; UpU, 4.7; CpG, 4.2; GpG, 2.7; ApG, 2.5. Extrapolation[18] of such data to pH 7.0 gave the values reported in footnote *c* of Table 3, from which catalytic rate enhancements k_{obs}/k_{bg} were calculated (Tables 3 and 4). Complexes **6**−Cu_2 and **8**−Cu_3 that provide ca. 1×10^4 rate enhancements (at 1.0 mM catalyst) for cleaving a large number of diribonucleoside monophosphates (Tables 3 and 4) give ca. 10^3 rate enhancements (at 0.2 mM catalyst) for cleaving HPNP (Table 2), which indicates that replacement of a good leaving group with a bad leaving group has no adverse effect on catalytic efficiency. In fact, the monometallic [12]aneN$_3$−Cu complex is much more effective in the cleavage of diribonucleoside monophosphates than in the cleavage of HPNP, which implies leaving group activation through coordination of the poor leaving group oxygen to the metal.[2i] The good catalytic efficiency of **6**−Cu_2 and **8**−Cu_3 in the cleavage of RNA dinucleoside monophosphates is at variance with a recent report by Anslyn et al.[13c] that a bis-zinc(II) complex cleaves effectively HPNP with a remarkable cooperativity between the two metals but shows no cooperativity in the cleavage of UpU.

A final comment is devoted to the most reactive diribonucleoside monophosphates UpU and UpG. The 10^5-fold rate accelerations provided by **6**−Cu_2 and **8**−Cu_3 are comparable to those reported for the best synthetic copper(II)- and zinc-(II)-based di- and trimetallic phosphodiesterases.[2i,19] Furthermore, the high selectivity for UpU and UpG reported in this work is quite remarkable, in view of the findings that in the cleavage of 3′,5′-*NpN*′substrates a number of copper(II) complexes of pyridine-based ligands[20] are highly selective for adenine bases vs uracil bases. As shown by Kimura et al.,[21] the acidity of uracil (pK_a = 9.9 for 3-methyluracil) increases to pK_a = 7.14 when it is linked to a doubly protonated [12]aneN$_4$ moiety, as a result of the +2 electrostatic effect on the uracil NH. Even higher is the acidity enhancement resulting from zinc-(II) complexation to the [12]aneN$_4$ moiety (pK_a < 5). In line with the above findings, we suggest that the uracil moiety at the 5′-hydroxyl terminus of UpU and UpG is deprotonated and

(17) Komiyama, M. *Carbohydr. Res.* **1989**, *192*, 97−102.
(18) Analysis of pH−rate profiles related to the cleavage of diribonucleoside monophosphates (Järvinen, P.; Oivanen, M.; Lönnberg, H. *J. Org. Chem.* **1991**, *70*, 5396−5401) has shown that at pH > 7, the dominant reaction path involves a monoanionic diester having additionally the 2′-hydroxyl deprotonated. Accordingly, extrapolation of the background rate constants from pH 11.08 to pH 7.0 was carried out as follows: k_{bg} (pH 7.0) = 0.83 × 10^{-4} k_{bg} (pH 11.08).
(19) Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *Angew. Chem., Int. Ed.* **1999**, *38*, 3189−3192.
(20) (a) Liu, S. H.; Hamilton, A. D. *Chem. Commun.* **1999**, 587−588. (b) Komiyama, M.; Kina, S.; Matsumura, K.; Sumaoka, J.; Tobey, S.; Lynch, V. M.; Anslyn, E. *J. Am. Chem. Soc.* **2002**, *124*, 13731−13736.
(21) Kimura, E.; Kitamura, H.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 10909−10919.

the resulting uracil anion forms a stable copper(II) complex.[22] In this way one of the copper(II) centers in **6**−Cu_2 and **8**−Cu_3 acts as an anchoring site for a nonreacting part of the substrate (**III**), whereas a second metal center in the 1,2-vicinal position enables the entire catalytic job—nucleophile activation and delivery to a metal activated phosphoryl group and, possibly, leaving group activation.[23]



III

In conclusion, we have reported an additional example of the successful use of the calix[4]arene scaffold in the design and synthesis of efficient dinuclear metallo-phosphodiesterases. Combination of the present data with analogous data from previous investigations shows that the catalytic efficiency of calix[4]arene-based bimetallic complexes is the result of a subtle interplay between the identity of the metal ion and the structure of the ligating unit and critically depends on whether the substitution pattern of ligated metal ions is 1,2-vicinal or 1,3-distal. It also shows that the role of a third metal ion ranges from strongly rate enhancing (GpG in the presence of **3**−Zn_3)[19] to strongly rate retarding (CpA in the presence of **8**−Cu_3, Table 3), thus offering a varied phenomenological picture. In most cases the synergic action of two metal centers provides ca. 10^4-fold rate accelerations, which rise to 10^5-fold in the cleavage of UpU and UpG, showing an unprecedented selectivity in comparison with other base-selective artificial nucleases.

The good catalytic efficiency and selectivity of the copper(II) complexes described in this work, coupled with their water solubility, encouraged further studies in the cleavage of RNA oligonucleotides, which are currently carried out in our laboratories.

Experimental Section

Instruments and General Methods. NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts are reported as δ values in ppm from tetramethylsilane added as the internal standard. Mass spectra obtained by electrospray ionization (ESI) and chemical ionization (CI) methods were recorded on a Micromass ZMD and on a Finnigan Mat SSQ710 spectrometer, respectively.

Materials. HPNP was available from previous investigations.[3] [12]aneN$_3$ was commercially available and used without further purification. *N*-Benzyl-1,5,9-triazacyclododecane **4** was synthesized according to a literature procedure.[24] Diribonucleoside 3′,5′-monophosphates UpU, GpG, and ApG were purchased from Sigma-Aldrich, while UpG, CpC, GpA, GpU, CpA, and CpG were from Dharmacon Research (Lafayette, CO). Pure samples of *NpN*′ and their aqueous solutions were stored at −4 °C.

5-[5,9-Bis(*tert*-butoxycarbonyl)-1,5,9-triazacyclododec-1-ylmethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (14). K_2CO_3 (23 mg, 0.17 mmol) and bis-Boc-protected [12]aneN$_3$ **9** (85 mg, 0.229 mmol) were added to a solution of chloromethyl calix[4]arene **10** (115 mg, 0.152 mmol) in dry acetonitrile (4 mL), and the reaction mixture was stirred under nitrogen for 2 days. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2 (40 mL) and washed with a saturated $NaHCO_3$ aqueous solution (40 mL). The aqueous phase was extracted again with dichloromethane (40 mL), and the combined organic layers were evaporated under vacuum. The crude product was purified by column chromatography (SiO_2; Et_2O/Hex, 1/1), giving calix[4]arene **14** (118 mg) as a colorless oil. Yield 70%. ^{1}H NMR (300 MHz; $CDCl_3$): δ 6.73−6.70 (m, Ar*H*, 4H), 6.62 (d, Ar*H*, J = 7.4 Hz, 2H), 6.58 (d, Ar*H*, J = 7.0 Hz, 2H), 6.48 (t, Ar*H*, J = 7.0 Hz, 1H), 6.43 (s, Ar*H*, 2H), 4.50 (d, ArCH_2Ar ax, J = 13.2 Hz, 2H), 4.49 (d, ArCH_2Ar ax, J = 13.2 Hz, 2H), 4.19 (t, ArOCH_2, J = 6.0 Hz, 4H), 4.07 (t, ArOCH_2, J = 5.6 Hz, 4H), 3.88 (t, ArOCH$_2$CH_2, J = 6.0 Hz, 4H), 3.83 (t, ArOCH$_2$CH_2, J = 5.6 Hz, 4H), 3.54 (q, OCH_2CH$_3$, J = 7.0 Hz, 4H), 3.53 (q, OCH_2CH$_3$, J = 7.0 Hz, 4H), 3.36 (t, BocNCH_2-CH$_2$CH_2NBoc, J = 6.8 Hz, 4H), 3.21 (t, NHCH$_2$CH$_2$CH_2NBoc, J = 5.9 Hz, 4H), 3.16 (s, ArCH_2N, 2H), 3.14 (d, ArCH_2Ar eq, J = 13.2 Hz, 2H), 3.11 (d, ArCH_2Ar eq, J = 13.2 Hz, 2H), 2.09 (t, ArCH$_2$-NHCH_2, J = 5.7 Hz, 4H), 1.90 (quin, BocNCH$_2$CH_2CH$_2$NBoc, J = 6.8 Hz, 2H), 1.67 (quin, NHCH$_2$CH_2CH$_2$NBoc, J = 5.9 Hz, 4H), 1.47 (s, C(CH_3)$_3$, 18H), 1.21 (t, OCH$_2$CH_3, J = 7.0 Hz, 6H), 1.20 (t, OCH$_2$CH_3, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz; $CDCl_3$): δ 156.5, 156.2, 155.9, 154.8, 135.3, 134.6, 134.1, 131.5, 128.6, 128.2, 128.1, 128.0, 122.2, 122.0, 79.1, 73.3, 72.9, 69.6, 66.3, 66.2, 56.4, 49.8, 45.3, 43.4, 30.9, 30.8, 28.5, 26.9, 26.5, 15.2. MS (ES+) *m*/*z* (%): 1096.3 (30) [M + H]$^+$, 1118.0 (100) [M + Na]$^+$, 570.6 (80) [M + 2Na]$^{2+}$. Anal. Calcd for $C_{64}H_{93}N_3O_{12}$ (1096.46): C, 70.18; H, 8.55; N, 3.83. Found: C, 70.04; H, 8.68; N, 3.77.

5,11-Bis[5,9-bis(*tert*-butoxycarbonyl)-1,5,9-triazacyclododec-1-ylmethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (15). Compound **15** was synthesized according to the preparation of **14**, starting from 1,2-dichloromethyl calix[4]arene **11** and using a 1.2 equiv excess of K_2CO_3 and 1.35 equiv excess of bis-Boc-protected [12]aneN$_3$ **9** per chloromethyl group. Reaction time: 3 days. The crude was purified by column chromatography (Hex/EtOAc/Et_2O, 6/4/1), giving calix[4]arene **15** as a colorless oil. Yield 45%. ^{1}H NMR (300 MHz; $CDCl_3$): δ 6.67 (dd, Ar*H*, J^3 = 7.6 Hz, J^4 = 1.3 Hz, 2H), 6.63 (dd, Ar*H*, J^3 = 7.6 Hz, J^4 = 1.3 Hz, 2H), 6.55 (d, Ar*H*, J = 1.0 Hz, 2H), 6.53 (t, Ar*H*, J = 7.6 Hz, 2H), 6.46 (d, Ar*H*, J = 1.0 Hz, 2H), 4.50 (d, ArCH_2Ar ax, J = 13.0 Hz, 1H), 4.48 (d, ArCH_2Ar ax, J = 13.2 Hz, 2H), 4.45 (d, ArCH_2Ar ax, J = 13.0 Hz, 1H), 4.11 (t, ArOCH_2, J = 5.6 Hz, 8H), 3.85 (t, ArOCH$_2$CH_2, J = 5.6 Hz, 8H), 3.54 (q, OCH_2CH$_3$, J = 7.1 Hz, 4H), 3.53 (q, OCH_2CH$_3$, J = 7.1 Hz, 4H), 3.35 (bs, BocNCH_2-CH$_2$CH_2NBoc, 8H), 3.24 (t, NCH_2CH$_2$CH_2NBoc, J = 6.7 Hz, 8H), 3.20 (s, ArCH_2N, 4H), 3.15 (d, ArCH_2Ar eq, J = 13.0 Hz, 1H), 3.11 (d, ArCH_2Ar eq, J = 13.2 Hz, 2H), 3.07 (d, ArCH_2Ar eq, J = 13.0 Hz, 1H), 2.15 (bs, ArCH$_2$NCH_2, 8H), 1.90 (quin, BocNCH$_2$CH_2CH$_2$-NBoc, J = 6.7 Hz, 4H), 1.69 (bs, ArCH$_2$NCH$_2$CH_2, 8H), 1.45 (s, C(CH_3)$_3$, 36H), 1.20 (t, OCH$_2$CH_3, J = 7.1 Hz, 6H), 1.19 (t, OCH$_2$CH_3, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz; $CDCl_3$): δ 156.2, 155.2, 135.0, 134.6, 134.4, 131.7, 128.7, 128.6, 128.5, 128.1, 128.0, 127.7, 122.1, 79.1, 73.1, 69.7, 66.3, 49.8, 45.3, 43.5, 30.9, 29.7, 28.5, 26.5, 15.3.

(22) Anchoring of the azacrown-ligated zinc(II) ion of a bimetallic zinc(II)−nickel(II) cleaving agent to deprotonated N(3) of the uracil moiety of the 3′-hydroxyl terminus of 3′,5′-UpU has been previously suggested. See: Wang, Q.; Mikkola, S.; Lönnberg, H. *Chem. Biodiv.* **2004**, *1*, 1316−1322.

(23) Unfortunately, it was impossible to analyze catalytic data in terms of the Michaelis−Menten parameters k_{cat} and K_M, because of complications in the kinetic experiments arising from aggregation/precipitation phenomena in the region of high catalyst concentrations.

(24) Hubsch-Weber, P.; Youinou, M.-T. *Tetrahedron Lett.* **1997**, *38*, 1911−1914.

MS (ES+) *m*/*z* (%): 1479.9 (10) $[M + H]^+$, 1502.4 (35) $[M + Na]^+$, 762.4 (100) $[M + 2Na]^{2+}$. Anal. Calcd for $C_{84}H_{130}N_6O_{16}$ (1479.99): C, 68.17; H, 8.85; N, 5.68. Found: C, 68.05; H, 8.99; N, 5.51.

5,17-Bis[5,9-bis(*tert*-butoxycarbonyl)-1,5,9-triazacyclododec-1-ylmethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (16). Compound **16** was synthesized according to the preparation of **14**, starting from 1,3-dichloromethyl calix[4]arene **12** and using a 1.2 equiv excess of K_2CO_3 and 1.35 equiv excess of bis-Boc-protected [12]aneN$_3$ **9** per chloromethyl group. Reaction time: 4 days. The crude was purified by column chromatography (Et_2O/Hex, 4/1), giving calix[4]arene **16** as a colorless oil. Yield 54%. ^{1}H NMR (300 MHz; $CDCl_3$): δ 6.85 (s, Ar*H*, 4H), 6.34−6.22 (m, Ar*H*, 6H), 4.48 (d, ArCH_2Ar ax, J = 13.2 Hz, 4H), 4.22 (t, ArOCH_2, J = 5.4 Hz, 4H), 3.98 (t, ArOCH_2, J = 5.7 Hz, 4H), 3.87 (t, ArOCH$_2$CH_2, J = 5.4 Hz, 4H), 3.80 (t, ArOCH$_2$CH_2, J = 5.7 Hz, 4H), 3.57 (q, OCH_2CH$_3$, J = 6.9 Hz, 4H), 3.50 (q, OCH_2CH$_3$, J = 7.2 Hz, 4H), 3.39 (s, 4H, ArCH_2N), 3.38−3.29 (m, BocNCH_2CH$_2$CH_2NBoc + NHCH$_2$CH$_2$CH_2NBoc, 16H), 3.10 (d, ArCH_2Ar eq, J = 13.2 Hz, 4H), 2.41 (t, ArCH$_2$NHCH_2, J = 6.0 Hz, 8H), 1.93 (quint, BocNCH$_2$CH_2CH$_2$NBoc, J = 6.0 Hz, 4H), 1.80 (quint, NHCH$_2$CH_2CH$_2$NBoc, J = 6.0 Hz, 8H), 1.45 (s, C(CH_3)$_3$, 36H), 1.23 (t, OCH$_2$CH_3, J = 6.9 Hz, 6H), 1.17 (t, OCH$_2$CH_3, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz; $CDCl_3$): δ 156.5, 156.2, 155.1, 135.8, 133.8, 132.2, 129.4, 127.6, 122.2, 79.2, 73.6, 72.6, 69.7, 69.6, 66.4, 66.2, 57.9, 50.2, 45.1, 43.6, 30.9, 28.5, 27.1, 26.8, 15.3, 15.2. MS (MALDI-TOF) *m*/*z* (%): 1480.5 (100) $[M + H]^+$. Anal. Calcd for $C_{84}H_{130}N_6O_{16}$ (1479.99): C, 68.17; H, 8.85; N, 5.68. Found: C, 68.29; H, 9.01; N, 5.50.

5,11,17-Tris[5,9-bis(*tert*-butoxycarbonyl)-1,5,9-triazacyclododec-1-ylmethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (17). Compound **17** was synthesized according to the preparation of **14**, starting from trichloromethyl calix[4]arene **13** and using a 1.15 equiv excess of K_2CO_3 and bis-Boc-protected [12]aneN$_3$ **9** per chloromethyl group. Reaction time: 5 days. The crude was purified by column chromatography using Et_2O as eluent, giving calix[4]arene **17** as a colorless oil. Yield 60%. ^{1}H NMR (300 MHz; $CDCl_3$): δ 6.82 (d, Ar*H*, J = 1.0 Hz, 2H), 6.74 (d, Ar*H*, J = 1.0 Hz, 2H), 6.25 (s, Ar*H*, 3H), 6.09 (s, Ar*H*, 2H), 4.45 (d, ArCH_2Ar ax, J = 13.2 Hz, 2H), 4.42 (d, ArCH_2Ar ax, J = 13.2 Hz, 2H), 4.21 (t, ArOCH_2, J = 6.2 Hz, 4H), 3.92 (t, ArOCH_2, J = 5.2 Hz, 4H), 3.84 (t, ArOCH$_2$CH_2, J = 6.2 Hz, 4H), 3.75 (t, ArOCH$_2$CH_2, J = 5.2 Hz, 4H), 3.52 (q, OCH_2CH$_3$, J = 7.0 Hz, 4H), 3.44 (q, OCH_2CH$_3$, J = 7.0 Hz, 4H), 3.35 (s, ArCH_2N, 4H), 3.33−3.26 (m, CH_2NBocCH_2, 20H), 3.08 (bs, NCH$_2$CH_2CH$_2$NBoc, 4H), 3.06 (d, ArCH_2Ar eq, J = 13.2 Hz, 2H), 3.02 (d, ArCH_2Ar eq, J = 13.2 Hz, 2H), 2.94 (s, ArCH_2N, 2H), 2.35 (bs, ArCH$_2$NCH_2, 8H), 1.94−1.78 (bs, ArCH$_2$NCH_2 + BocNCH$_2$CH_2CH$_2$NBoc, 10H), 1.75 (m, ArCH$_2$NCH$_2$CH_2, 8H), 1.52 (bs, ArCH$_2$NCH$_2$CH_2, 4H), 1.40 (s, C(CH_3)$_3$, 54H), 1.27 (t, OCH$_2$CH_3, J = 7.0 Hz, 6H), 1.19 (t, OCH$_2$CH_3, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz; $CDCl_3$): δ 156.4, 156.2, 156.1, 155.1, 154.0, 135.8, 135.7, 133.7, 133.2, 132.1, 131.4, 129.3, 128.1, 127.5, 125.4, 122.0, 79.2, 79.1, 73.7, 72.4, 69.7, 69.6, 66.4, 66.1, 57.9, 49.9, 49.5, 45.4, 45.1, 43.5, 43.4, 30.9, 30.3, 29.6, 28.5, 27.2, 27.1, 26.7, 26.4, 15.3, 15.2. MS (FAB+) *m*/*z* (%): 1863.3 (100) $[M + H]^+$. Anal. Calcd for $C_{104}H_{167}N_9O_{20}$ (1863.53): C, 67.03; H, 9.03; N, 6.76. Found: C, 67.20; H, 9.19; N, 6.67.

General Procedure for the Boc Deprotection. Boc protecting groups were removed from compounds **14**−**17** by the following procedure. The Boc-protected calix[4]arene was dissolved in CH_2Cl_2 (2 mL), and trifluoroacetic acid (1 mL) was added. The solution was stirred for 2 h and then evaporated under reduced pressure. The residue was taken up with dichloromethane (20 mL) and a Li_2CO_3 aqueous saturated solution (20 mL). The organic phase was separated, the aqueous one was extracted again with CH_2Cl_2 (2 × 20 mL), and the organic fractions were combined and evaporated under vacuum, to give the corresponding deprotected products **5**−**8** in quantitative yield.

5-(1,5,9-Triazacyclododec-1-ylmethyl)-25,26,27,28-tetrakis-(2-ethoxyethoxy)calix[4]arene (5). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.05−7.01 (m, Ar*H*, 4H), 6.82 (t, Ar*H*, J = 7.5 Hz, 2H), 6.56 (t, Ar*H*, J = 7.5 Hz, 1H), 6.41 (d, Ar*H*, J = 7.5 Hz, 2H), 6.25 (s, Ar*H*, 2H), 4.56 (d, ArCH_2Ar ax, J = 13.1 Hz, 2H), 4.49 (d, ArCH_2Ar ax, J = 12.8 Hz, 2H), 4.40 (dt, ArOCH_AH$_B$, J^2 = 11.0 Hz, J^3 = 6.3 Hz, 2H), 4.31 (dt, ArOCH$_A$$H_B$, J^2 = 11.0 Hz, J^3 = 6.3 Hz, 2H), 3.99−3.90 (m, ArOCH_2 + ArOCH$_2$CH_2, 12H), 3.80−3.73 (m, ArOCH$_2$CH_2, 4H), 3.70 (s, ArCH_2N, 2H), 3.57 (q, OCH_2CH$_3$, J = 7.0 Hz, 2H), 3.55 (q, OCH_2CH$_3$, J = 6.9 Hz, 2H), 3.52 (q, OCH_2CH$_3$, J = 6.9 Hz, 4H), 3.15 (d, ArCH_2Ar eq, J = 12.8 Hz, 2H), 3.14 (d, ArCH_2Ar eq, J = 13.1 Hz, 2H), 3.08 (bs, HNCH_2CH$_2$CH_2NH, 4H), 2.95 (bs, NCH$_2$CH$_2$CH_2NH, 4H), 2.12 (bs, ArCH$_2$NCH_2, 2H), 1.90 (bs, ArCH$_2$NCH_2, 2H), 1.66 (bs, NCH$_2$CH_2CH$_2$NH, 4H), 1.51 (bs, NHCH$_2$CH_2CH$_2$NH, 2H), 1.24 (t, OCH$_2$CH_3, J = 7.0 Hz, 3H), 1.23 (t, OCH$_2$CH_3, J = 6.9 Hz, 3H), 1.18 (t, OCH$_2$CH_3, J = 6.9 Hz, 6H). ^{13}C NMR (75 MHz; $CDCl_3$): δ 156.9, 155.7, 154.7, 136.7, 136.4, 134.5, 133.6, 131.5, 128.8, 128.2, 127.4, 122.9, 122.4, 119.9, 74.4, 74.3, 72.3, 69.6, 69.4, 66.4, 66.3, 66.1, 52.1, 48.0, 47.4, 44.0, 30.7, 23.2, 21.9, 15.3,15.1. MS (ES+) *m*/*z* (%): 896.7 (30) $[M + H]^+$, 918. 5 (100) $[M + Na]^+$. Anal. Calcd for $C_{54}H_{77}N_3O_8$ (896.23): C, 72.37; H, 8.66; N, 4.69. Found: C, 72.21; H, 8.76; N, 4.78.

5,11-Bis(1,5,9-triazacyclododec-1-ylmethyl)-25,26,27,28-tetrakis-(2-ethoxyethoxy)calix[4]arene (6). Mp (Et_2O) = 61−62 °C. ^{1}H NMR (300 MHz; $CDCl_3$): δ 6.66−6.63 (m, Ar*H*, 4H), 6.56−6.52 (m, Ar*H*, 4H), 6.46 (s, Ar*H*, 2H), 4.51 (d, ArCH_2Ar ax, J = 13.1 Hz, 2H), 4.49 (d, ArCH_2Ar ax, J = 13.1 Hz, 2H), 4.15 (t, ArOCH_2, J = 5.5 Hz, 4H), 4.13 (t, ArOCH_2, J = 5.5 Hz, 4H), 3.86 (t, ArOCH$_2$CH_2, J = 5.5 Hz, 4H), 3.85 (t, ArOCH$_2$CH_2, J = 5.5 Hz, 4H), 3.54 (q, OCH_2CH$_3$, J = 6.9 Hz, 4H), 3.53 (q, OCH_2CH$_3$, J = 6.9 Hz, 4H), 3.30 (d, ArCH_AH$_B$N, J = 14.0 Hz, 2H), 3.24 (d, ArCH$_A$$H_B$N, J = 14.0 Hz, 2H), 3.15 (d, ArCH_2Ar eq, J = 13.1 Hz, 1H), 3.12 (d, ArCH_2Ar eq, J = 13.1 Hz, 2H), 3.08 (d, ArCH_2Ar eq, J = 13.1 Hz, 1H), 2.97 (t, HNCH_2CH$_2$CH_2NH, J = 4.8 Hz, 8H), 2.72 (t, NCH$_2$CH$_2$CH_2NH, J = 4.8 Hz, 8H), 2.18 (bs, ArCH$_2$NCH_2, 8H), 1.88 (bs, HNCH$_2$CH_2CH$_2$NH, 4H), 1.67 (bs, NCH$_2$CH_2CH$_2$NH, 8H), 1.22 (t, OCH$_2$CH_3, J = 6.9 Hz, 6H), 1.20 (t, OCH$_2$CH_3, J = 6.9 Hz, 6H). ^{13}C NMR (75 MHz; $CDCl_3$): δ 156.2, 155.2, 135.1, 134.7, 134.5, 130.9, 129.2, 128.9, 128.1, 122.2, 73.2, 69.7, 69.6, 66.3, 55.2, 51.8, 49.9, 49.5, 47.1, 31.0, 30.8, 29.7, 27.7, 25.2, 15.3. MS (ES+) *m*/*z* (%): 540.2 (100) $[M + 2H]^{2+}$. Anal. Calcd for $C_{64}H_{98}N_6O_8$ (1079.52): C, 71.21; H, 9.15; N, 7.78. Found: C, 71.38; H, 9.29; N, 7.59.

5,17-Bis(1,5,9-triazacyclododec-1-ylmethyl)-25,26,27,28-tetrakis-(2-ethoxyethoxy)calix[4]arene (7). ^{1}H NMR (300 MHz; $CDCl_3$): δ 6.75 (s, Ar*H*, 4H), 6.32−6.22 (m, Ar*H*, 6H), 4.45 (d, ArCH_2Ar ax, J = 13.5 Hz, 4H), 4.15 (t, ArOCH_2, J = 6.0 Hz, 4H), 3.95 (t, ArOCH_2, J = 5.1 Hz, 4H), 3.80 (t, ArOCH$_2$CH_2, J = 6.0 Hz, 4H), 3.76 (t, ArOCH$_2$CH_2, J = 5.1 Hz, 4H), 3.50 (q, OCH_2CH$_3$, J = 6.9 Hz, 4H), 3.45 (q, OCH_2CH$_3$, J = 7.2 Hz, 4H), 3.33 (s, ArCH_2N, 4H), 3.04 (d, ArCH_2Ar eq, J = 13.5 Hz, 4H), 2.97 (bs, HNCH_2CH$_2$CH_2NH, 8H), 2.73 (bs, CH_2NHCH$_2$CH$_2$CH$_2$NHCH_2, 8H), 2.42 (bs, ArCH$_2$NCH_2, 8H), 1.85 (bs, NHCH$_2$CH_2CH$_2$NH, 4H), 1.75 (bs, ArCH$_2$NCH$_2$CH_2, 8H), 1.16 (t, OCH$_2$CH_3, J = 6.9 Hz, 6H), 1.10 (t, OCH$_2$CH_3, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz; $CDCl_3$): δ 156.7, 155.3, 135.9, 133.9, 130.6, 129.4, 127.6, 122.3, 73.6, 72.9, 69.8, 69.6, 66.4, 66.2, 55.2, 51.9, 50.0, 46.8, 31.0, 23.9, 15.3, 15.2. MS (FAB+) *m*/*z* (%): 1079.6 (100) $[M + H]^+$. Anal. Calcd for $C_{64}H_{98}N_6O_8$ (1079.52): C, 71.21; H, 9.15; N, 7.78. Found: C, 71.39; H, 9.27; N, 7.68.

5,11,17-Tris(1,5,9-triazacyclododec-1-ylmethyl)-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (8). Mp (Et_2O) = 59−60 °C. ^{1}H NMR (300 MHz; $CDCl_3$): δ 6.86 (d, 2H, Ar*H*, J = 1.1 Hz), 6.82 (d, Ar*H*, J = 1.1 Hz, 2H), 6.29 (s, Ar*H*, 3H), 6.14 (s, Ar*H*, 2H), 4.43 (d, ArCH_2Ar ax, J = 12.8 Hz, 2H), 4.41 (d, ArCH_2Ar ax, J = 13.2 Hz, 2H), 4.20 (t, ArOCH_2, J = 6.2 Hz, 4H), 3.92 (t, ArOCH_2, J = 5.1 Hz, 4H), 3.84 (t, ArOCH$_2$CH_2, J = 6.2 Hz, 4H), 3.74 (t, ArOCH$_2$CH_2, J = 5.1 Hz, 4H), 3.50 (q, OCH_2CH$_3$, J = 6.9 Hz, 4H), 3.40 (q, OCH_2CH$_3$, J = 6.9 Hz, 4H), 3.34 (d, ArCH_AH$_B$N, J = 13.2 Hz, 2H), 3.26 (d, ArCH$_A$$H_B$N, J = 13.2 Hz, 2H), 3.07 (d, ArCH_2Ar eq, J = 12.8 Hz, 2H), 3.05 (d, ArCH_2Ar eq, J = 13.2 Hz, 2H), 3.00 (s, ArCH_2N, 2H),

2.76 (t, HNCH_2CH$_2$CH_2NH, J = 4.8 Hz, 8H), 2.69 (t, HNCH_2CH$_2$CH_2-NH, J = 4.8 Hz, 4H), 2.63 (t, NCH$_2$CH$_2$CH_2NH, J = 5.1 Hz, 8H), 2.48 (t, NCH$_2$CH$_2$CH_2NH, J = 5.2 Hz, 4H), 2.41 (bs, ArCH$_2$NCH_2, 8H), 1.92 (bs, ArCH$_2$NCH_2, 4H), 1.61 (bs, NCH$_2$CH_2CH$_2$NHCH$_2$CH_2, 14H), 1.37 (bs, NCH$_2$CH_2CH$_2$NH, 4H), 1.17 (t, OCH$_2$CH_3, J = 7.0 Hz, 3H), 1.14 (t, OCH$_2$CH_3, J = 7.0 Hz, 3H), 1.10 (t, OCH$_2$CH_3, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz; CDCl$_3$): δ 156.4, 155.1, 154.2, 135.7, 133.9, 133.3, 131.6, 129.9, 129.4, 129.3, 127.6, 122.3, 73.7, 72.6, 69.7, 69.6, 66.4, 66.2, 55.9, 52.3, 51.2, 49.9, 49.5, 47.1, 47.0, 31.0, 30.3, 29.7, 25.6, 25.2, 25.1, 24.9, 15.3, 15.2. MS (ES+) m/z (%): 1262.8 (30) $[M + H]^+$, 632.0 (100) $[M + 2H]^{2+}$. Anal. Calcd for $C_{74}H_{119}N_9O_8$ (1262.82): C, 70.38; H, 9.50; N, 9.98. Found: C, 70.25; H, 9.41; N, 9.83.

Kinetic Measurements. Spectrophotometric measurements were carried out on either a double beam or on a diode array spectrophotometer. HPLC analyses were performed on a liquid chromatograph fitted with a UV−vis detector operating at 254 nm. The samples were analyzed on a Supelcosil LC-18 DB column (25 cm × 4.6 mm I.D., particle size 5 μm).

The aqueous solutions were prepared from deionized (Millipore) distilled water.

Metal complexes were formed in situ by addition of the calculated stoichiometric amount of the aqueous solution of the metal salt (Zn(ClO$_4$)$_2$ or CuCl$_2$) to the buffered reaction mixture. Due to the slow formation of the complexes,[25] the solutions were incubated for 2 h before the start of the kinetic run by fast addition of a small volume of the substrate solution.

Nonlinear least-squares calculations of kinetic data were carried out using the program SigmaPlot 2002 for Windows, version 8.0 (SPSS, Inc.).

(25) Riedo, T. J.; Kaden T. A. *Helv. Chim. Acta* **1979**, *62*, 1089−1096.

HPNP Transesterification. Kinetic measurements of transesterification of HPNP were monitored by UV−vis spectrophotometry at 400 nm. Rate constants were obtained by an initial rate method, error limit on the order of ±10%.

Diribonucleoside 3′,5′-Monophosphate Cleavage. Cleavage of diribonucleoside 3′,5′-monophosphates *NpN*′ was monitored by HPLC analyses of aliquots of the reaction mixture withdrawn at appropriate time intervals. Reactions were carried out at 50.0 °C on 0.1 mM *NpN*′ and 1 mM catalyst solutions in water, 20 mM HEPES, pH 7.0. In a typical experiment the ligand **8** (10 μL, 50 mM in ethanol) and CuCl$_2$ (50 μL, 30 mM in water) were added to 430 μL of the buffered water solution (20 mM HEPES, pH 7.0) thermostated at 50.0 °C. After 2 h *NpN*′ (10 μL, 5 mM in water) was added. At proper time intervals aliquots (80 μL) of the reaction mixture were withdrawn and quenched with 80 μL of a saturated water solution of EDTA. After addition of the internal standard (50 μL of 0.16 mM *p*-hydroxybenzoic acid), the solution was filtered and subjected to HPLC analysis by elution with H$_2$O (0.1% trifluoroacetic acid)/MeCN, linear gradient from 100:0 to 85:15 in 20 min, flow 0.9 mL/min.

Acknowledgment. Financial contribution from MIUR COFIN 2003 Progetto Dispositivi Supramolecolari is acknowledged for the work carried out in Parma and Roma. The Centro Interfacoltà di Misure (CIM) of the University of Parma is also acknowledged for the use of NMR and MS facilities.

Supporting Information Available: ^{1}H NMR spectrum of compounds **5**−**8** in CDCl$_3$ and of compound **8** in D$_2$O (PDF). This material is available free of charge via the Internet at http://pubs.ac.org.

JA0632106