Dinuclear and Trinuclear Zn(II) Calix[4]arene Complexes as Models for Hydrolytic Metallo-Enzymes. Synthesis and Catalytic Activity in Phosphate Diester Transesterification

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Calix[4]arenes modified with two or three Zn(II)-2,6-bis(aminomethyl)pyridyl groups, **3**-$[Zn]_2$ and **5**-$[Zn]_3$, respectively, were investigated as models for dinuclear and trinuclear metallo-enzymes that catalyze the cleavage of phosphate diesters. Under neutral conditions, 0.48 mM of **3**-$[Zn]_2$ causes a rate acceleration of 23 000 in the transesterification of the RNA model substrate 2-hydroxyproyl-*p*-nitrophenyl phosphate (HPNP, 0.19 mM). Comparison with the activities of a mononuclear complex **2**-[Zn] and a reference complex lacking the calix[4]arene backbone **1**-[Zn] shows that the catalysis is due to cooperative action of the Zn(II) centers and indicates that hydrophobic effects contribute to the catalysis. Saturation kinetics and pH variation studies demonstrate that the high catalytic activity of the flexible complex **3**-$[Zn]_2$ originates from a very high substrate binding affinity, affording a Michaelis−Menten complex in which the substrate is converted with a relatively moderate rate. A rigid analogue **4**-$[Zn]_2$ exhibits both a lower substrate binding strength and a lower catalytic rate. This demonstrates the importance of a certain flexibility between the cooperating catalytic centers. The trinuclear complex **5**-$[Zn]_3$ induces a rate acceleration of 32 000 times, and shows a decreased substrate binding and an increased catalytic rate compared to its dinuclear analogue **3**-$[Zn]_2$. In a possible mechanism two Zn(II) ions activate the phosphoryl group and another activates the β-hydroxyl group of HPNP.

Introduction

Hydrolytic enzymes that cleave phosphate ester bonds often have in their active site two transition metal ions such as Zn(II), Mg(II), Mn(II), Ni(II), or Fe(III) that act cooperatively as Lewis acid sites in the catalytic process.[1] The mode of catalysis of phosphate ester hydrolysis has been studied with various synthetic model compounds in which two metal ions[2] like Zn(II),[3,4,5] Cu(II),[3b,6,7] Co(III),[8] or lanthanides(III)[9] are held apart by appropriate ligands.[10] The metal ions are proposed to activate the phosphate group and a nucleophilic water molecule and to stabilize the pentacoordinate phosphorus transition state and possibly the leaving group by cooperative action. A specific subclass of these metallo-hydrolases possesses even a third metal ion in the active site. Examples include phospholipase C and P1 nuclease which use three Zn(II) ions to catalyze the hydrolytic cleavage of phosphate diester bonds in phosphatidylcholine and in nucleotides such as RNA and DNA, respectively.[1] However, the function of a third metal ion in close proximity of a dinuclear metal cluster in enzymes is yet not fully understood, and only one example of a biomimetic study has been reported.[5]

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(1) For recent reviews, see (a) Sträter, N.; Lipscomb, W. N.; Klablunde, T.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2024. (b) Wilcox, D. E. *Chem. Rev.* **1996**, *96*, 2435.

(2) For a recent review, see Chin, J. *Curr. Opin. Chem. Biol.* **1997**, *1*, 514.

(3) (a) Breslow, R.; Singh, S. *Bioorg. Chem.* **1988**, *16*, 408. (b) Clewley, R. G.; Slebocka-Tilk, H.; Brown, R. S. *Inorg. Chim. Acta* **1989**, *157*, 233. (c) Chapman, W. H., Jr.; Breslow, R. *J. Am. Chem. Soc.* **1995**, *117*, 5462. (d) Yashiro, M.; Ishikubo, A.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1995**, 1793. (e) Koike, T.; Inoue, M.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 3091. (f) Bazzicalupi, C.; Bencini, A.; Bianchi, A.; Fusi, V.; Giorgi, C.; Paoletti, P.; Valtancoli, B.; Zanchi, D. *Inorg. Chem.* **1997**, *36*, 2784.

(4) Preliminary results have been published: Molenveld, P.; Kapsabelis, S.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1997**, *119*, 2948.

(5) Yashiro, M.; Ishikubo, A.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1997**, 83.

(6) Molenveld, P.; Engbersen, J. F. J.; Kooijman, H.; Spek, A. L.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1998**, *120*, 6726.

(7) (a) Wall, M.; Hynes, R. C.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1633. (b) Young, M. J.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 10577. (c) Liu, S.; Hamilton, A. D. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1779. (d) Liu, S.; Luo, Z.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2678.

(8) (a) Williams, N. H.; Chin, J. *J. Chem. Soc., Chem. Commun.* **1996**, 131. (b) Wahnon, D.; Lebuis, A.-M.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2412. (c) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 12165. (d) Chung, Y.; Akkaya, E. U.; Venkatachalam, T. K.; Czarnik, A. W. *Tetrahedron Lett.* **1990**, *31*, 5413. (e) Seog Seo, J.; Sung, N.-D.; Hynes, R. S.; Chin, J. *Inorg. Chem.* **1996**, *35*, 7472. (f) Williams, N.; Cheung, W.; Chin, J. *J. Am. Chem. Soc.* **1998**, *120*, 8079.

(9) (a) Ragunathan, K. G.; Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1219. (b) Bruice, T. C.; Tsubouchi, A.; Dempcy, R. O.; Olson, L. P. *J. Am. Chem. Soc.* **1996**, *118*, 9867.

(10) Artificial dinuclear metal complexes have also been reported to promote the hydrolysis of amides, amino acid esters, and guanidiniums: (a) Karet, G. B.; Kostic, N. M. *Inorg. Chem.* **1998**, *37*, 1021. (b) Yamaguchi, K.; Koshino, S.; Akagi, F.; Suzuki, M.; Uehara, A.; Suzuki, S. *J. Am. Chem. Soc.* **1997**, *119*, 5752. (c) Scrimin, P.; Tecilla, P.; Tonellato, U. *Eur. J. Org. Chem.* **1998**, 1143. (d) Frey, S. T.; Murthy, N. N.; Weintraub, S. T.; Thompson, L. K.; Karlin, K. D. *Inorg. Chem.* **1997**, *36*, 956. (e) He, C.; Lippard, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 105.

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Chart 1

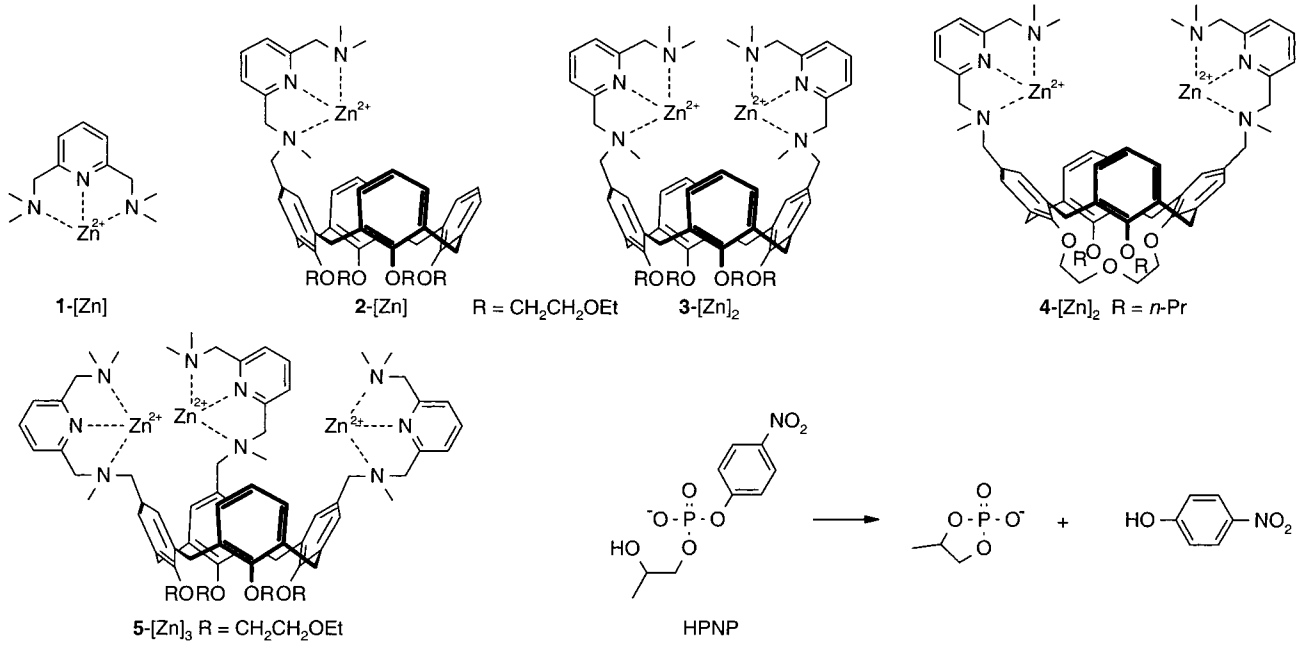
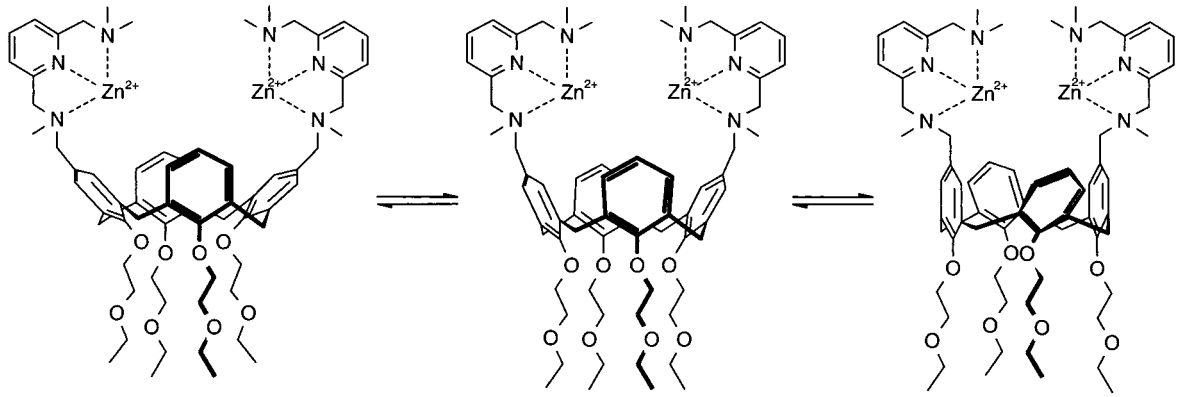


Chart 2



In previous papers,[4,6] we have shown that calix[4]-arenes[11] are suitable building blocks for the design of mimics for dinuclear metallo-hydrolases and that high-rate enhancements can be achieved in the catalytic cleavage of phosphate diesters. The observed synergy in catalysis by two Zn(II) or Cu(II) ions was attributed to an enzyme-like dynamic binding of the substrate and transition state,[12,13] facilitated by low-energy conformational changes of the flexible calix[4]arene backbone.[14−16] Here, we report a detailed study of the catalytic activity of various calix[4]arene-based Zn(II) complexes in phosphate diester transesterification (Chart 1).[4] The effect of cooperative action is demonstrated by comparison of the catalytic activity of dinuclear complex **3**-$[Zn]_2$ with mononuclear complexes **2**-[Zn] and **1**-[Zn]. The importance of flexibility in catalysis is demonstrated by comparison with dinuclear complex **4**-$[Zn]_2$ which is rigidified by modification of the calix[4]arene lower rim with a crown ether bridge. In this way, the common interconversion between calix[4]arene conformations with two diverged (flattened) or parallel (pinched) opposing aromatic units, via a symmetrical cone-shaped intermediate (Chart 2), is inhibited.[15] Furthermore, we have enlarged the dinuclear calix[4]arene system **3**-$[Zn]_2$ with an additional Zn(II) center to trinuclear complex **5**-$[Zn]_3$, mimicking the active site of trinuclear metallo-hydrolases.

(11) (a) Gutsche, C. D. Calixarenes. In *Monographs in Supramolecular Chemistry*, Vol. 1; Stoddart, J. F., Ed.; The Royal Society of Chemistry: Cambridge, England, 1989. (b) Vicens, J.; Böhmer, V., Eds.; *Calixarenes: A Versatile Class of Macrocyclic Compounds*; Kluwer Academic Publishers: Dordrecht, 1991. (c) For recent reviews, see Böhmer, V. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 713; Takeshita, M.; Shinkai, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1088.

(12) (a) Koshland, D. E., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 98. (b) Koshland, D. E., Jr. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2375. (c) Jorgenson, W. L. *Science* **1991**, *254*, 954. (d) Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman and Company: New York, 1984. (e) Klibanov, A. M. *Nature* **1995**, *374*, 596.

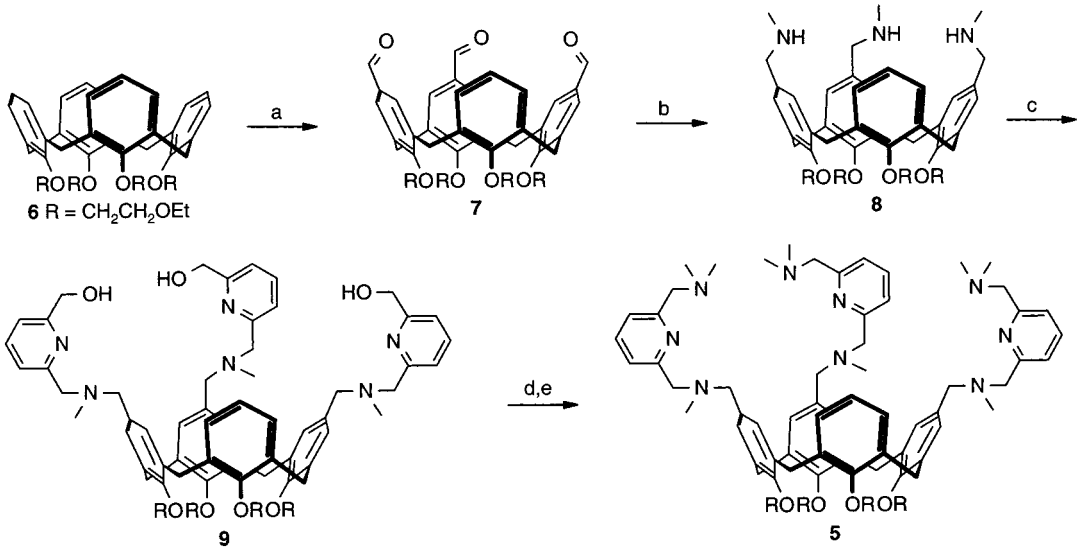
(13) (a) Kirby, A. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 706. (b) Kirby, A. J. *Acc. Chem. Res.* **1997**, *30*, 290. (c) Sanders, J. K. M. *Chem. Eur. J.* **1998**, *4*, 1378. (d) Rowan, S. J.; Sanders, J. K. M. *Curr. Opin. Chem. Biol.* **1997**, *1*, 483. (e) Eblinger, F.; Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 826.

(14) (a) Grootenhuis, P. D. J.; Kollman, P. A.; Groenen, L. C.; Reinhoudt, D. N.; van Hummel, G. J.; Ugozzoli, F.; Andreetti, G. D. *J. Am. Chem. Soc.* **1990**, *112*, 4165. (b) Harada, T.; Rudzinsky, J. M.; Osawa, E.; Shinkai, S. *Tetrahedron* **1993**, *49*, 5941. (c) Scheerder, J.; van Duynhoven, J. P. M.; Engbersen, J. F. J.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1090. (d) Scheerder, J.; Vreekamp, R. H.; Engbersen, J. F. J.; Verboom, W.; van Duynhoven, J. P. M.; Reinhoudt, D. N. *J. Org. Chem.* **1996**, *61*, 3476. (e) Soi, A.; Bauer, W.; Mauser, H.; Moll, C.; Hampel, F.; Hirsch, A. *J. Chem. Soc., Perkin Trans.* **1998**, 1471.

(15) Arduini, A.; Fabbi, M.; Mantovani, M.; Mirone, L.; Pochini, A.; Ungaro, R. *J. Org. Chem.* **1995**, *60*, 1454.

(16) Arduini, A. Fanni, S.; Manfredi, G.; Pochini, A.; Ungaro, R.; Sicuri, A. R.; Ugozzoli, F. *J. Org. Chem.* **1995**, *60*, 1448.

Scheme 1. Synthesis of a Calix[4]arene-Based Trinucleating 2,6-Bis(aminomethyl)Pyridyl Ligand[a]



(a) Hexamethylenetetraamine, TFA, 55%; (b) 33% $MeNH_2$ in EtOH, H_2, 10% Pd−C, 96%; (c) 2-bromomethyl-6-hydroxymethylpyridine, K_2CO_3, THF, 65%; (d) $SOCl_2$, CH_2Cl_2; (e) 33% Me_2NH in EtOH, 77% over two steps.

Results and Discussion[4]

In this paper, we describe the synthesis of the ligands **1**−**5**, the formation of the corresponding Zn(II) complexes, and their catalytic activity in the cleavage of the phosphate diester bond of an RNA model substrate. The rate of phosphate diester transesterification was studied as a function of the Zn(II) concentration, the pH of the reaction medium, and the substrate concentration, respectively. The catalytic activity of the investigated complexes follows the order **5**-$[Zn]_3$ > **3**-$[Zn]_2$ > **4**-$[Zn]_2$ > **2**-[Zn] > **1**-[Zn] and is explained in terms of cooperativity between the Zn(II) centers with respect to substrate binding and conversion.

Synthesis. The reference 2,6-bis(aminomethyl)pyridine ligand **1** was synthesized by an improved literature procedure[17] in which 2,6-bis(chloromethyl)pyridine was reacted with excess of dimethylamine, giving the product in quantitative yield. The flexible calix[4]arene ligands **2**, **3**, and **5** are substituted at the lower rim with four ethoxyethyl groups.[18] These ethoxyethyl groups prevent inversion of the aromatic units through the annulus of the macrocycle and bring the calix[4]arene skeleton in a rapid equilibrium between cone and flattened/pinched cone conformations (Chart 2). In contrast, the rigidified calix[4]arene ligand **4** was at the lower rim modified with a crown ether bridge. This modification inhibits interconversion between two flattened/pinched cone conformations and fixes the calix[4]arene skeleton in a conformation in which the upper rim-functionalized aromatic nuclei diverge from the cavity (Chart 1).[15]

The stepwise synthesis of the 2,6-bis(aminomethyl)-pyridyl ligand system in calix[4]arenes **2**−**5** was started from the formyl derivatives, as is depicted for the trinucleating ligand **5** in Scheme 1. Whereas for the synthesis of the mono- and disubstituted analogues **2**, **3**, and **4** the aldehyde functionalities were introduced by Gross formylation[6,16] using $SnCl_4$ and Cl_2CHOMe in $CHCl_3$, the aldehydes in the triformyl derivative **7** were introduced by a Duff reaction using hexamethylenetetraamine in trifluoroacetic acid.[19] This method gives a higher yield and is less laborious than the reported procedure with $SnCl_4$ and Cl_2CHOMe in $CHCl_3$.[16] The aldehyde functionalities were converted in high yield to secondary amines (**8**) by reductive amination using methylamine under hydrogenation conditions. Originally, the characterization of the crude reductive amination product of the diformyl derivative **10** was hampered by the presence of broad peaks in the ^1H NMR spectrum. Since this aminomethyl calix[4]arene (**12**) could not be purified by common methods such as crystallization or chromatography, it was temporary derivatized to the Boc-protected amine (**11**). Purification and deprotection with trifluoroacetic acid yielded the aminomethyl calix[4]arene (**12**) in 74%. The ^1H NMR spectrum of **12**·2TFA showed for the six protons of the two N−CH_3 groups one singlet at 2.40 ppm, whereas in the free amine **12** these protons appear as two singlets at 2.62 and 2.26 ppm integrating for 4.5 and 1.5 protons, respectively. The same holds for the benzylic protons of **12** appearing as two singlets at 3.46 and 3.40 ppm, which indicates a hindered rotation of the two N−CH_3 groups in **12**. This phenomenon was not observed for the tri-, mono-, and rigidified disubstituted aminomethyl calix[4]arenes **8**, **17**, and **20** of which the crude reductive amination product was pure enough and could be well characterized. The 2-hydroxymethylpyridyl moieties in **9** were introduced by reacting the secondary amine **8** with exactly 3 equiv of 2-bromomethyl-6-hydroxymethylpyridine,[20] avoiding in that way overalkylation of the resulting tertiary amines. Conversion of the hydroxyl groups in **9** to the chlorides proceeded easily with $SOCl_2$ affording the tris(2-chloromethylpyridine) derivative as a hydrochloride in almost quantitative yield. The corresponding free amine appeared to be

(17) Markies, B. A.; Wijkens, P.; Boersma, J.; Kooijman, H.; Veldman, N.; Spek, A. L.; van Koten, G. *Organometallics* **1994**, *13*, 3244.

(18) Arduini, A.; Casnati, A.; Fabbi, M.; Minari, P.; Pochini, A.; Sicuri, A. R.; Ungaro, R. *Supramol. Chem.* **1993**, *1*, 235.

(19) (a) Smith, W. E. *J. Org. Chem.* **1972**, *37*, 3973. (b) Linnane, P.; James, T. D.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1995**, 1997. (c) Dondoni, A.; Marra, A.; Scherrmann, M.-C.; Casnati, A.; Sansone, F.; Ungaro, R. *Chem. Eur. J.* **1997**, *3*, 1774.

(20) Newcomb, M.; Timko, J. M.; Walba, D. M.; Cram, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 6392.

Table 1. Protonation Constants of Ligand 1 and the Corresponding Zn(II) and Cu(II) Complexes Determined by Potentiometric pH Titrations[a]

log K_a[b]	**1**	**1**-[Zn][c]	**1**-[Cu][d]
log K_1	7.50 ± 0.04		
log K_2	8.32 ± 0.02		
log K_3 (M)		5.56 ± 0.02	12.20 ± 0.04
log K_4 (MOH_2)		7.94 ± 0.1	8.11 ± 0.09
log K_5 (MOH_2)		9.63 ± 0.09	11.59 ± 0.19

[a] In 0.1 M KNO_3 in 35% EtOH/H_2O (v/v), at 20 °C. [b] $K_1 = [H_2\mathbf{1}]/[H\mathbf{1}][H^+]$; $K_2 = [H\mathbf{1}]/[\mathbf{1}][H^+]$; $K_3 = [\mathbf{1}M]/[\mathbf{1}][M]$; $K_4 = [\mathbf{1}M(H_2O)_n]/[\mathbf{1}M(OH)(H_2O)_{n-1}][H^+]$; $K_5 = [\mathbf{1}M(OH)(H_2O)_{n-1}]/[\mathbf{1}M(OH)_2(H_2O)_{n-2}][H^+]$. [c] M = Zn(II). [d] M = Cu(II).

unstable and was therefore not isolated and characterized. Hence, the tris(2-chloromethylpyridine) hydrochloride was immediately reacted with excess of dimethylamine yielding the trinucleating ligand **5** in 77%. This highly polar and basic compound could easily be purified by chromatography using aluminum oxide.

Spectrophotometric Titrations. The addition of Zn-$(ClO_4)_2$ to the ligands in 50% CH_3CN/20 mM HEPES pH 7.0, the conditions for the catalysis experiments (vide infra),[21] results in an increase in UV absorbance of the pyridine groups at $\lambda = 266$ nm with isosbestic points at 254 and 282 nm in the overlap spectra at different concentrations of $Zn(ClO_4)_2$. Upon titration of the ligands (**1** and **2**, 0.2 mM; **3**, **4**, and **5**, 0.05 mM) with $Zn(ClO_4)_2$, the increase in absorbance shows saturation curves according to the formation of mononuclear complexes **1**-[Zn] and **2**-[Zn], dinuclear complexes **3**-$[Zn]_2$ and **4**-$[Zn]_2$, and trinuclear complex **5**-$[Zn]_3$. Fitting of the titration curves by a nonlinear least-squares method[22] yielded association constants larger than 10^5 M^{-1} for binding of Zn(II) to the 2,6-bis(aminomethyl)pyridyl groups. The titration curve for **5**-$[Zn]_3$ demonstrates that more than 85% of the Zn(II) ions are bound during the catalysis studies.

Potentiometric Titrations. To understand the pH behavior of the metal complexes in catalysis, we have determined the protonation constants of the mononucleating ligand **1** and the deprotonation constants of the water molecules ligated to the corresponding complexes **1**-[Zn] and **1**-[Cu] by potentiometric pH titrations (Table 1).[23] The titrations were performed in 0.1 M KNO_3 in 35% EtOH/H_2O.[24] Titration of **1**·3HNO_3 in the absence (Figure 1a) and in the presence of equimolar amounts of $Zn(NO_3)_2$ (b), or $Cu(NO_3)_2$ (c), with 3 equiv base show titration curves corresponding to the formation of **1**-[Zn] and **1**-[Cu] in the pH regions 5.5−7.2 and 3.6−6.6, respectively. Upon further titration of the complexes with base, an additional acidic group, which must be a metal-bound water molecule, deprotonates with an apparent pK_a of 7.9 for **1**-[Zn] and 8.1 for **1**-[Cu]. The stability constants

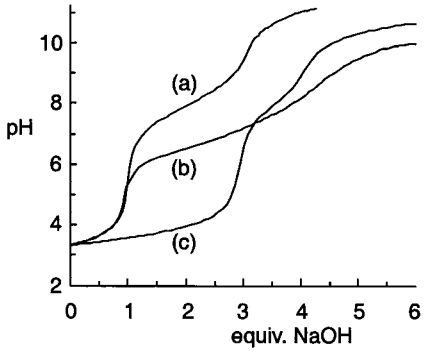


Figure 1. Experimental titration curves for ligand **1**·3HNO_3 in 0.1 M KNO_3 in 35% EtOH/H_2O at 20 °C, in the absence (a) and in the presence of 1 equiv $Zn(NO_3)_2$ (b) or $Cu(NO_3)_2$ (c), respectively.

of **1**-[Zn] ($10^{5.6}$ M^{-1}) and **1**-[Cu] ($10^{12.2}$ M^{-1}) and the pK_as of the metal-bound water molecules are in the same range as those found for similar 2,6-bis(aminomethyl)-pyridine metal complexes in the literature.[3b,25] The slightly lower pK_a of **1**-[Zn]OH_2 compared to **1**-[Cu]OH_2 might be due to a different coordination number,[25c,26] or due to the lower stability of **1**-[Zn] as is observed for the pK_as of a series of triamine complexes with decreasing stability constants.[27]

Groves[25c] and Coates[28] have already shown that small increases in hydrophobicity can effect a dramatic increase in the acidity of a metal-bound water molecule. Recently, we have reported[6] that the proximity of a hydrophobic calix[4]arene surface lowers the pK_a of a Cu(II) complex, mimicking in this way the hydrophobic cavity of metalloenzymes.[28] This effect is also expected in the Zn(II) and Cu(II) complexes of calix[4]arenes **2**−**5** (vide infra). Also, the presence of 50% CH_3CN in the reaction mixtures used for the catalysis experiments may increase the acidity of the metal-bound water molecules to a pK_a value lower than 7.9 or 8.1 which were determined for **1**-[Zn] and **1**-[Cu] in 35% EtOH.[24] In conclusion, the spectrophotometric and potentiometric titrations reveal the formation of sufficiently stable Zn(II) and Cu(II) complexes which possess a metal-bound hydroxide around neutral conditions.

X-ray Crystallography. To demonstrate the mode of Zn(II) binding by a 2,6-bis(aminomethyl)pyridyl ligand, we have prepared a crystalline Zn(II) complex of **1**.[29] Slow diffusion of Et_2O to an equimolar solution of **1** and Zn-$(CH_3COO)_2$·2H_2O in CH_3CN afforded crystals of (**1**)Zn-$(CH_3COO)_2$·H_2O, suitable for X-ray structure analysis. In the solid state, the Zn(II) center is coordinated by the

(21) Because the calix[4]arene Zn(II) complexes are insoluble in pure water, we selected CH_3CN as a cosolvent. The pH discussed in the text refers to the pH of the aqueous portion of the reaction mixture before dilution with CH_3CN up to 50% (v/v). For this method, see also Gellman, S. H.; Petter, R.; Breslow, R. *J. Am. Chem. Soc.* **1986**, *108*, 2288.

(22) de Boer, J. J. A.; Reinhoudt, D. N.; Harkema, S.; van Hummel, G. J.; de Jong, F. *J. Am. Chem. Soc.* **1982**, *104*, 4073.

(23) Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1985**, 1195.

(24) Besides titrations in 0.1 M KNO_3 in 35% EtOH/H_2O we have also performed titrations in 0.1 M $NaClO_4$ in 50% CH_3CN/H_2O. Although these different conditions show almost equal titration curves the pK_a determinations were calculated from the titrations in 35% EtOH since possible hydration of CH_3CN may give irreproducible results. See: Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145.

(25) (a) Kady, I. O.; Tan, B. *Tetrahedron Lett.* **1995**, *36*, 4031. (b) Kady, I. O.; Tan, B.; Ho, Z.; Scarborough, T. *J. Chem. Soc., Chem. Commun.* **1995**, 1137. (c) Groves, J. T.; Olson, J. R. *Inorg. Chem.* **1985**, *24*, 2715. (d) Groves, J. T.; Chambers, R. R. *J. Am. Chem. Soc.* **1984**, *106*, 630. (e) Bunton, C. A.; Scrimin, P.; Tecilla, P. *J. Chem. Soc., Perkin Trans.* **1996**, 419.

(26) (a) Courtney, R. C.; Gustafson, R. L.; Chaberek, S., Jr.; Martell, A. E. *J. Am. Chem. Soc.* **1958**, *80*, 2121. (b) Wooley, P. *Nature* **1975**, *258*, 677.

(27) (a) Kimura, E.; Shiota, T. Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805. (b) Itoh, T.; Fujii, Y.; Tada, T.; Yoshikawa, Y.; Hisada, H. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1265. (c) Bazzicalupi, C.; Bencini, A.; Bencini, A.; Bianchi, A.; Corana, F.; Fusi, V.; Giorgi, C.; Paoli, P.; Paoletti, P.; Valtancoli, B.; Zanchini, C. *Inorg. Chem.* **1996**, *35*, 5540.

(28) Coates, J. H.; Gentle, G. J.; Lincoln, S. F. *Nature* **1974**, *249*, 773.

(29) Unfortunately, we were not successful in growing suitable crystals of the calix[4]arene Zn(II) complexes.

three nitrogens of the tridentate ligand **1** and by two oxygens of two monodentate acetates. The τ-descriptor[30] for the complex is 0.36, meaning that the coordination geometry around Zn(II) is intermediate between the idealized square pyramidal ($\tau = 0$) and trigonal-bipyramidal ($\tau = 1$) extremes. The asymmetric unit contains besides one Zn(II) complex also one water molecule. This water molecule donates hydrogen bonds to a coordinating (O20) and a noncoordinating (O18) acetate oxygen, forming an infinite chain of hydrogen-bonded molecules parallel to the *a*-axis. Presumably, this hydrogen bonding causes the difference in acetate-Zn(II) coordination angles and distances.

The X-ray structure of (**1**)Zn$(CH_3COO)_2$ demonstrates that the 2,6-bis(aminomethyl)pyridyl ligand occupies in the solid state, and presumably also in solution, three of five available coordination sites on Zn(II). This indicates that two cis-oriented coordination sites on each Zn(II) center in **1**-[Zn], **2**-[Zn], **3**-$[Zn]_2$, **4**-$[Zn]_2$, and **5**-$[Zn]_3$ are available for the binding of a substrate and a water molecule in the catalysis. Crystalline Cu(II) complexes of **1** display an almost idealized square pyramidal geometry.[31]

Catalysis. The catalytic activity of the complexes **1**-[Zn], **2**-[Zn], **3**-$[Zn]_2$, **4**-$[Zn]_2$, and **5**-$[Zn]_3$, generated in situ by adding stoichiometric amounts of $Zn(ClO_4)_2$ to the ligands, was studied toward the transesterification of the RNA model substrate[32] 2-hydroxypropyl-*p*-nitrophenyl phosphate[33] (HPNP, Chart 1) in 50% CH_3CN/20 mM aqueous buffer at 25 °C.[21] The complexes showed good pseudo-first-order kinetics. The initial rate constants for both the catalyzed (k_{obs}) and uncatalyzed (k_{uncat}) reactions were calculated from the increase in absorbance at $\lambda =$ 400 nm due to the release of *p*-nitrophenolate (Table 2). In a preliminary communication,[4] we have already reported the enormous rate acceleration,[34] compared to the uncatalyzed reaction, of a factor 23 000 that is induced by 0.48 mM of the dinuclear complex **3**-$[Zn]_2$ at pH 7.0.[21] Comparison with the catalytic activities of mononuclear complexes **1**-[Zn] and **2**-[Zn], which are a factor 300 and 50 less active, respectively, shows that the high rate acceleration is due to synergetic action of the two Zn(II) centers in **3**-$[Zn]_2$. Furthermore, the fact that the mononuclear calix[4]arene complex **2**-[Zn] is a factor 6 more active than reference complex **1**-[Zn], lacking the calix[4]arene backbone, indicates that hydrophobic effects play a role in the catalytic process.

The conformationally rigid dinuclear complex **4**-$[Zn]_2$ cleaves HPNP also by the cooperative action of the Zn(II) centers, but is a factor 8 less efficient than **3**-$[Zn]_2$. The trinuclear complex appeared to be the most efficient catalyst; at 0.48 mM of **5**-$[Zn]_3$, at pH 7.0, a rate acceleration of 32 000 times was observed. This corresponds to a reduction of the half-life from approximately 300 days for the uncatalyzed reaction to 13 min. Compared with the dinuclear complex **3**-$[Zn]_2$ the third Zn(II) center in **5**-$[Zn]_3$ effects a 40% higher rate enhancement in HPNP cleavage.

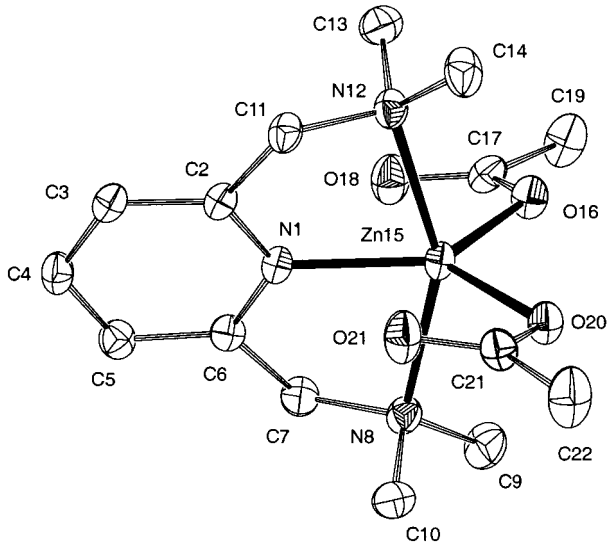


Figure 2. X-ray crystal structure of (**1**)Zn$(CH_3COO)_2 \cdot H_2O$ drawn as anisotropic displacement ellipsoid plot (at 50% probability level). Hydrogen atoms and the water solvate molecule are omitted for clarity.

Since dinuclear Cu(II) complexes have been reported as efficient catalysts in phosphate diester cleavage,[6,7] we have also measured the catalytic activities of **3**-$[Cu]_2$ and **5**-$[Cu]_3$. However, these complexes show only a very low activity similar to the activity of the mononuclear Zn(II) complex **2**-[Zn]. Since the pK_a of a Cu(II)-bound water molecule in **1**-[Cu] was found to be in the same range as the pK_a of a Zn(II)-bound water in **1**-[Zn], it is not expected that the dramatic rate differences between the calix[4]arene Cu(II) and Zn(II) complexes is only caused by differences in protonation state at a specific pH. The lack of efficient catalytic power of the Cu(II) complexes may be explained by the different Cu(II) coordination geometry (see X-ray Crystallography) disfavoring cooperative action in catalysis and by the known low binding affinity of Cu(II) to anionic phosphate ligands.[6,35]

Turnover Catalysis. In experiments with the substrate HPNP present in 4-fold excess over the catalyst **3**-$[Zn]_2$ or **5**-$[Zn]_3$, we observed turnover conversion to completion. The loss of activity during the course of a multiple turnover reaction was determined by (i) measuring the initial rate of a mixture of equimolar amounts HPNP and catalyst; (ii) continuing the reaction to total conversion; (iii) adding a new equivalent of HPNP; (iv) measuring the initial rate, up to conversion of 4 equiv of HPNP. Whereas these experiments showed for the dinuclear complex **3**-$[Zn]_2$ only 10% loss of activity after each catalytic cycle, the trinuclear complex **5**-$[Zn]_3$ loses 40% of its activity after each turnover. Product inhibition is most probably the reason for the loss of activity since the addition of equimolar amounts of diethyl phosphate to a mixture of catalyst and HPNP also caused a decrease in rate of approximately 10% for **3**-$[Zn]_2$ and 40% for **5**-$[Zn]_3$. This indicates an enhanced binding of the cyclic phosphate diester product to the Zn(II) centers in **5**-$[Zn]_3$ compared to those in **3**-$[Zn]_2$.

(30) Addison, A. W.; Rao, T. N.; Reedijk, J.; van Rijn, J.; Verschoor, G. C. *J. Chem. Soc., Dalton Trans.* **1984**, 1349.

(31) Unfortunately, we were not successful in growing suitable crystals of (**1**)Cu$(CH_3COO)_2$. A search on (derivatives of) **1**-[Cu(II)] in the Cambridge Structural Database afforded limited information on X-ray structures of (**1**)$CuBr_2$ and (**1**)$Cu(SCN)_2$: Day, V. W.; Fredrich, M. F.; Dabestani, S.; Bryan, P. S. *Am. Cryst. Assoc., Ser. 2* **1977**, *5*, 23.

(32) Perreault, D. M.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 433.

(33) Brown, D. M.; Usher, D. A. *J. Chem. Soc.* **1965**, 6558.

(34) For a discussion about rate enhancements, see Morrow, J. R.; Trogler, W. C. *Inorg. Chem.* **1988**, *27*, 3387.

(35) Martell, A. E.; Smith, R. M., Eds.; *Critical Stability Constants*; Plenum: New York, 1974; Vol. 2.

Table 2. Observed Pseudo-First-Order Rate Constants and Relative Rate Accelerations for the Transesterification of HPNP Catalyzed by Zn(II) and Cu(II) Complexes[a]

catalyst	$k_{obs} \times 10^{-4}$ (s^{-1})	relative rate acceleration
none[b]	2.7×10^{-4}	1
1-[Zn][c]	0.045	170
2-[Zn][c]	0.25	900
3-$[Zn]_2$[d]	6.26	23 000
3-$[Cu]_2$[d]	0.18	700
4-$[Zn]_2$[d]	0.82	3000
5-$[Zn]_3$[d]	8.55	32 000
5-$[Cu]_3$[d]	0.16	600

[a] In 50% CH_3CN/20 mM HEPES buffer pH 7.0, at 25 °C. [b] Measured from a 2.0 mM HPNP solution. [c] Catalyst concentration 0.98 mM. [d] Catalyst concentration 0.48 mM.

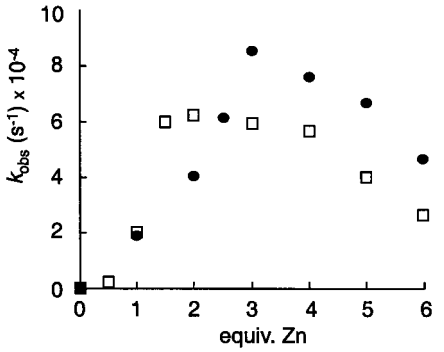


Figure 3. Dependence of k_{obs} on the amount of $Zn(ClO_4)_2$ with respect to dinucleating ligand **3** [(□) 0.48 mM] and trinucleating ligand **5** [(●) 0.48 mM] for the cleavage of HPNP (0.19 mM) in 50% CH_3CN/20 mM HEPES buffer pH 7.0, at 25 °C.

Effect of Zn(II) Concentration. To demonstrate cooperative catalytic action of the two Zn(II) centers in **3**-$[Zn]_2$ and **4**-$[Zn]_2$ and the three Zn(II) centers in **5**-$[Zn]_3$, we have determined the rate as a function of the ratio ligand:[Zn]. This shows for **3** and **4** an optimum at 2 equiv of $Zn(ClO_4)_2$ and for **5** an optimum at 3 equiv of $Zn(ClO_4)_2$ (Figure 3). The decrease in rate that is observed at high concentrations of $Zn(ClO_4)_2$ can be explained by competitive binding of HPNP to free Zn(II) in solution. These studies confirm that the substrate HPNP is indeed cleaved by **3**-$[Zn]_2$, **4**-$[Zn]_2$, and **5**-$[Zn]_3$ and not by the ligands or by free Zn(II) ions, which was already apparent from the spectrophotometric titrations of the ligands with $Zn(ClO_4)_2$.

Effect of pH. The pH−rate profiles for the transesterification of HPNP catalyzed by the dinuclear and trinuclear complexes are bell shaped in the pH region 6.0−8.0 with the optimum for **3**-$[Zn]_2$[4] and **4**-$[Zn]_2$ at pH 7.4 and for **5**-$[Zn]_3$ at pH 7.0 (Figure 4). For the dinuclear complexes **3**-$[Zn]_2$ and **4**-$[Zn]_2$, the optimum activity is reached by acid-base transitions with kinetic pK_as of approximately 7.1 and 7.8, whereas for the trinuclear complex **5**-$[Zn]_3$, these pK_as are approximately 6.5 and 7.5. According to the potentiometric titrations, these two acid-base transitions must correspond to the subsequent deprotonation of two water molecules bound to the Zn(II) centers. This indicates that the catalytically most active form of calix[4]arene complexes **3**-$[Zn]_2$, **4**-$[Zn]_2$, and **5**-$[Zn]_3$ possesses only one Zn(II)-bound hydroxide ion. Bell-shaped pH−rate profiles at low concentrations of phosphate diester substrates[3c,4,6,7b−d] can result from opposing pH effects on the formation of a catalyst−substrate complex and conversion of the substrate within this complex. Efficient binding of the substrate to the catalyst requires the displacement of one or more Zn(II)-bound Lewis bases, e.g., weakly bound water molecules at low pH and tightly bound hydroxide ions at high pH. Moreover, the bound hydroxides lower the catalysts substrate binding affinity by diminishing the Lewis acidity of the Zn(II) centers. On the other hand, conversion of the bound substrate by transesterification requires a general base. This can be buffer ions at low pH or hydroxide ions, either bound to Zn(II) or free in solution, at higher pH. Reference complex **1**-[Zn], lacking the calix[4]arene backbone, shows a sigmoidal pH−rate profile (Figure 4) with the inflection point at pH 7.5, which is in agreement with the pK_a determined by the potentiometric titrations. The activity of the mononuclear calix[4]arene analogue **2**-[Zn] increases strongly from pH 6.8−7.4 with a kinetic pK_a of 7.1 and decreases slowly to 80% of the optimum activity at pH 8.2. Apparently, the strength of substrate binding plays a crucial role in the catalysis and is for the calix[4]arene-based complexes efficient around neutral pH.[4]

Substrate Binding and Conversion. The HPNP binding affinities of the calix[4]arene Zn(II) catalysts were studied by measuring the rate of transesterification as a function of HPNP concentration at a fixed catalyst concentration. This results in saturation curves due to the formation of a catalyst−substrate complex with a stability constant K_{ass} and conversion of the substrate within this complex with a catalytic rate constant k_{cat} (Figure 5). The kinetic data for this Michaelis−Menten process were obtained by analyzing the experimental data with Eady−Hofstee plots and nonlinear least-squares curve fitting[22] and are summarized in Table 3. Previously, we have reported that the HPNP binding affinity of dinuclear calix[4]arene **3**-$[Zn]_2$ is extremely high in the pH region 7.0−7.6 and decreases strongly at more alkaline pH.[4] At pH 7.0, the binding constant and the catalytic rate constant are a factor 70 and 40 smaller, respectively, for the mononuclear complex **2**-[Zn]. This demonstrates the cooperative catalytic action of the Zn(II) centers in the flexible calix[4]arene **3**-$[Zn]_2$. The rigidified complex **4**-$[Zn]_2$ is a factor 8 less efficient in both HPNP binding (K_{ass}) and conversion (k_{cat}), which shows the importance of a certain flexibility in the cooperating Zn(II) centers during the catalytic process.[6,10c,12,13] The 6 times higher activity and the slightly different pH behavior of the mononuclear calix[4]arene complex **2**-[Zn] over the reference complex **1**-[Zn] points to an enhanced binding of the substrate, probably due to favorable hydrophobic interactions with the calix[4]arene surface.

The trinuclear complex **5**-$[Zn]_3$ shows saturation kinetics, but the rate of transesterification decreases at high HPNP concentrations (Figure 5). This decrease is probably caused by inhibition due to binding of more than one molecule of HPNP to this catalyst. Analysis of the experimental data at low HPNP concentrations indicates a binding affinity which is 45 times lower than for the receptor **3**-$[Zn]_2$. However, the catalytic power (k_{cat}) of **5**-$[Zn]_3$ is 3 times larger, as may be expected on the basis of three catalytic Zn(II) centers which are in close proximity to each other. Apparently, there is a kinetic barrier for complexation of the substrate, probably caused by steric hindrance by the three 2,6-bis(aminomethyl)-pyridyl groups. Once the substrate is bound, it is rapidly

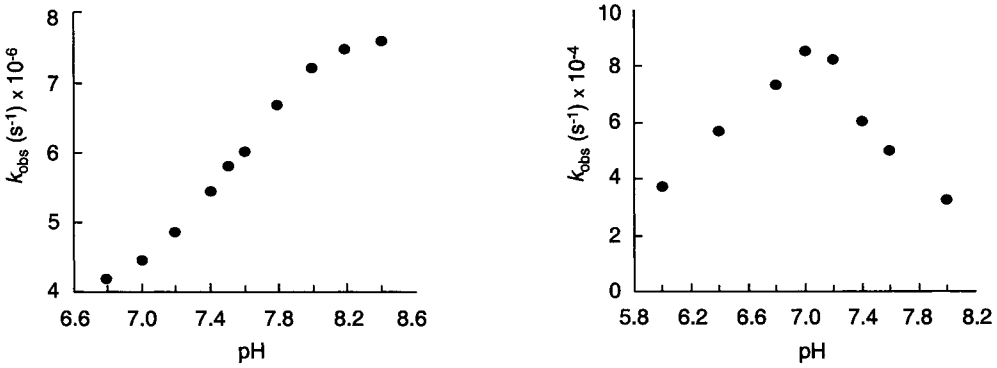


Figure 4. Dependence of k_{obs} on pH for the transesterification of HPNP (0.19 mM) catalyzed by **1**-[Zn] (0.98 mM, left) and **5**-$[Zn]_3$ (0.48 mM, right) in 50% CH_3CN/20 mM buffer, at 25 °C. Buffers: pH 6.0−7.0, MES; pH 7.0−8.2, HEPES; pH 8.2−8.6, EPPS.

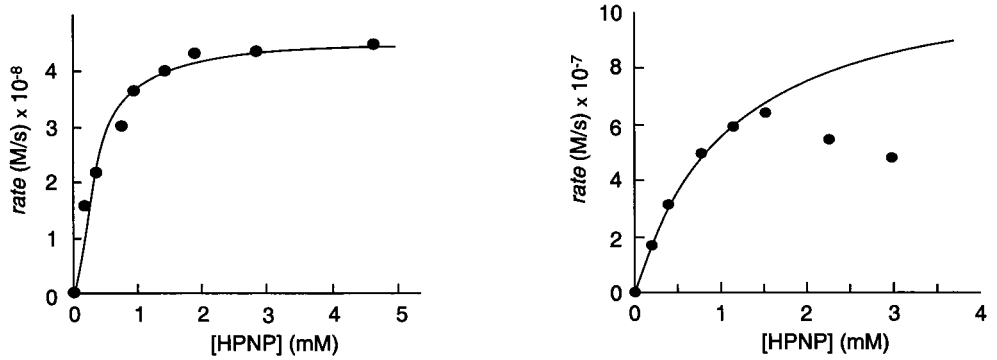


Figure 5. Saturation kinetics curves for the transesterification of HPNP in 50% CH_3CN/20 mM HEPES pH 7.0, at 25 °C and 0.48 mM catalyst. (Left) **4**-$[Zn]_2$; the experimental data points are fitted with $K_{ass} = 7000\ M^{-1}$ and $k_{cat} = 0.95 \times 10^{-4}\ s^{-1}$. (Right) **5**-$[Zn]_3$; the experimental data points are fitted to a 1:1 binding equation at low concentrations of HPNP with $K_{ass} = 1200\ M^{-1}$ and $k_{cat} = 24 \times 10^{-4}\ s^{-1}$ (obtained by means of an Eady−Hofstee plot).

Table 3. Kinetic Data for the Transesterification of HPNP Catalyzed by Calix[4]arene Zn(II) Complexes[a]

catalyst	k_2[b] ($M^{-1}\ s^{-1}$)	k_{cat}[c,d] $\times 10^{-4}$ (s^{-1})	K_{ass}[c] $\times 10^2$ (M^{-1})	K_m[e] (mM)
2-[Zn]	0.015	0.19	7.5	1.3
3-$[Zn]_2$	43	7.7	550	0.018
4-$[Zn]_2$	0.68	0.95	70	0.14
5-$[Zn]_3$	2.9	24	12	0.83

[a] In 50% CH_3CN/20 mM HEPES pH 7.0, at 25 °C. [b] Calculated by $k_2 = k_{cat}/K_m$. [c] Determined by curve fitting. [d] Determined by means of an Eady−Hofstee plot. [e] Calculated by $K_m = 1/K_{ass}$.

converted to the products, and the products are slowly released as was pointed out by the turnover experiments.

Mechanism of Catalysis. A generally proposed mechanism for phosphate ester cleavage catalyzed by two metal ions concerns double Lewis acid activation by bridging of the phosphoryl group between the two metal centers to form a catalyst−substrate complex.[2,3c−f,7,8] Subsequent in-line nucleophilic attack by either a (metal-bound) hydroxide ion or a substrate β-hydroxyl group results in leaving group expulsion through a pentacoordinate trigonal bipyramidal transition state. In contrast to transesterification of the RNA model substrate HPNP, dinuclear complex **3**-$[Zn]_2$ is hardly active in the hydrolysis of diethyl *p*-nitrophenyl phosphate, ethyl *p*-nitrophenyl phosphate, and *p*-nitrophenyl phosphate. As is known that double Lewis acid activation of the phosphoryl group induces cleavage of these substrates, it is unlikely that the phosphoryl group of HPNP bridges the two Zn(II) centers in **3**-$[Zn]_2$. The experimental data can be reconciled with the mechanism shown in Figure 6.[4] Herein, catalyst **3**-$[Zn]_2$ is proposed to bind HPNP by two-point coordination, one Zn(II) center activating the phosphoryl group and the other activating the β-hydroxyl group, subsequently followed by general base-promoted cyclization.[3c,7c] Also **4**-$[Zn]_2$ shows a distinct cooperativity in the catalytic cleavage of HPNP. Whereas from CPK models the two-point binding mode seems possible for the rigidified complex **4**-$[Zn]_2$, it is more likely that one Zn(II) center in **4**-$[Zn]_2$ delivers a bound hydroxide that can deprotonate HPNP (not shown).

In the trinuclear complex **5**-$[Zn]_3$, three Zn(II) centers are cooperatively involved in the catalysis. The enhanced k_{cat} in HPNP transesterification indicates double Lewis acid activation of the phosphoryl group by two neighboring Zn(II) centers (Figure 6). The third Zn(II) center situated at further distance can possibly facilitate deprotonation of the β-hydroxyl group of HPNP, either by Lewis acid activation similar to catalysis by **3**-$[Zn]_2$ or via a bound hydroxide ion.

Conclusions

The transesterification of the RNA model substrate HPNP is efficiently catalyzed by dinuclear and trinuclear calix[4]arene complexes, exhibiting rate accelerations over the uncatalyzed reaction of a factor 23 000 and 32 000, at 0.48 mM **3**-$[Zn]_2$ and **5**-$[Zn]_3$, respectively. The high catalytic activity of dinuclear complex **3**-$[Zn]_2$ results from a very high substrate binding affinity (K_{ass}), affording a Michaelis−Menten complex in which the substrate is subsequently converted with a relatively moderate rate (k_{cat}). Generally, binding interactions of a substrate and a transition state with the active site of an enzyme

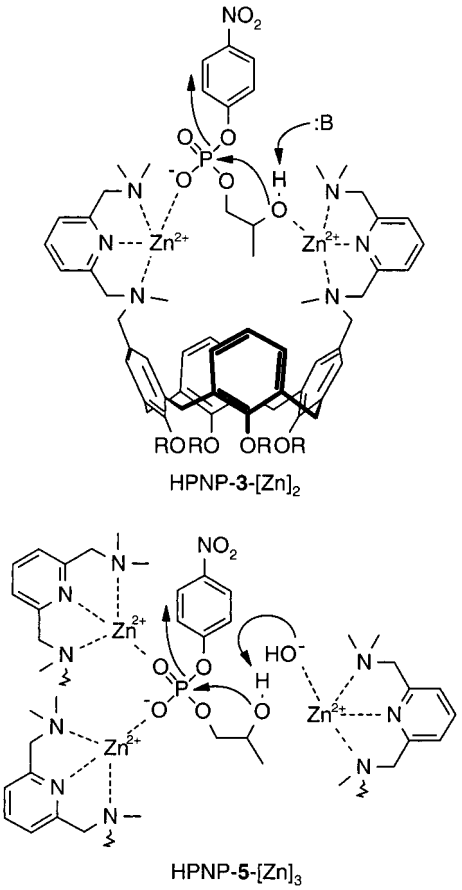


Figure 6. Schematic representations of possible mechanisms for HPNP cleavage catalyzed by **3**-$[Zn]_2$ and **5**-$[Zn]_3$.

involve an induced fit, in which the enzyme undergoes conformational changes.[12,13,36] A similar dynamic process might explain the enzyme-like substrate-binding affinity of the flexible enzyme model **3**-$[Zn]_2$, since the rigidified analogue **4**-$[Zn]_2$ demonstrates a lower binding strength as well as a lower catalytic rate.[4,6] Also in the hydrolysis of amino acid esters catalyzed by dinuclear Cu(II) complexes, the group of Tonellato[10c] concluded that flexibility of the catalyst is better than rigidity.[13] The presence of a third Zn(II) center in close proximity of the dinuclear Zn(II) cluster, e.g., complex **5**-$[Zn]_3$, results in a decreased substrate affinity and an increased catalytic rate. This implies that **5**-$[Zn]_3$ binds the pentacoordinate phosphorus transition state better than **3**-$[Zn]_2$. Participation of three Zn(II) ions in the catalytic cleavage of phosphate esters was already proposed for the trinuclear enzymes phospholipase C and P1 nuclease,[1] and the efficiency is now confirmed by using artificial trinuclear Zn(II) complexes.[5]

In conclusion, we have shown that calix[4]arenes functionalized with Lewis acidic Zn(II) centers can mimic properties of natural metallo-phosphodiesterases. For the design of artificial enzymes with other catalytic functions, calix[4]arenes might be suitable building blocks since multiple catalytic groups can be preorganized dynamically, similar to the amino acid residues and cofactors in the polypeptide backbone of enzymes.

(36) For a recent report concerning guest-induced structural changes in artificial receptors, see Hayashi, T.; Asai, T.; Borgmeier, F. M.; Hokazono, H.; Ogoshi, H. *Chem. Eur. J.* **1998**, *4*, 1266.

Experimental Section

General Information. THF was freshly distilled from sodium/benzophenone, CH_2Cl_2 from $CaCl_2$, and $SOCl_2$ from linseed oil. Other solvents and chemicals were of reagent grade and were used as received from commercial sources. Column chromatography was performed with silica gel (SiO_2, 0.040−0.063 mm, 230−400 mesh). Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with Me_4Si as internal standard. FAB-MS spectra were recorded with *m*-NBA as a matrix. Tetrakis(2-ethoxyethoxy)calix[4]-arene **6**,[18] diformyl-tetrakis(2-ethoxyethoxy)calix[4]arene **10**,[6,16] formyl-tetrakis(2-ethoxyethoxy)calix[4]arene **16**,[16] diformyl-di-*n*-propoxycalix[4]arene-crown-3 **20**,[15] and 2-bromomethyl-6-hydroxymethylpyridine[20] were synthesized according to literature procedures. The pH meter used for adjustment of buffered solutions was calibrated daily. UV−vis spectra were measured with a diode array spectrophotometer equipped with a thermostated cuvette holder (7 cuvettes, 1.0 cm path length) and a sample transport accessory.

2,6-Bis[(dimethylamino)methyl]pyridine (1). This compound was synthesized by an improved literature procedure.[17] A solution of 2,6-pyridine dimethanol (3.00 g, 21.6 mmol) in $SOCl_2$ (100 mL) was stirred for 3 h, and subsequently concentrated under reduced pressure. The residue was dissolved in EtOH (20 mL), added to a stirred 33% dimethylamine solution in EtOH (100 mL), and stirred for 18 h. The solution was concentrated in vacuo, the residue was taken up in EtOAc/saturated Na_2CO_3 solution (100/100 mL), and the aqueous phase was extracted with EtOAc (4 × 100 mL). The combined organic phases were dried over K_2CO_3, filtered, and concentrated in vacuo to give the pure product as a yellow liquid (4.18 g, 100%) with the same analytical data as reported in the literature.

5,11,17-Triformyl-25,26,27,28-tetrakis(2-ethoxyethoxy)-calix[4]arene (7). To a solution of tetrakis(2-ethoxyethoxy)-calix[4]arene **6**[18] (1.00 g, 1.40 mmol) in trifluoroacetic acid (30 mL) was added hexamethylenetetraamine (3.14 g, 22.4 mmol). The reaction mixture was refluxed for 18 h, cooled to room temperature, and poured out in ice water (150 mL). The aqueous suspension was extracted with CH_2Cl_2 (3 × 50 mL), after which the combined organic phases were washed with saturated Na_2CO_3 solution (75 mL) and water (75 mL). The solution was dried with $MgSO_4$ and filtered, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/EtOAc, 9/1) to give the product as a colorless oil (610 mg, 55%). The analytical data were the same as reported in the literature.[16]

5,11,17-Tris(methylaminomethyl)-25,26,27,28-tetrakis-(2-ethoxyethoxy)calix[4]arene (8). Triformyl calix[4]arene **7** (510 mg, 0.641 mmol) was dissolved in a 33% methylamine solution in EtOH (50 mL), after which 10% Pd−C (50 mg) was added. The reaction mixture was stirred for 16 h in a hydrogen atmosphere, filtered over Hyflo, and evaporated till dryness. The residue was taken up in saturated Na_2CO_3 solution containing 5% of EDTA (40 mL) and was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic phases were dried over K_2CO_3 and evaporated to dryness to give the crude secondary amine (620 mg, 96%), which was pure enough for further modification. ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 6.73 (s, 4 H), 6.43−6.51 (m, 3 H), 6.40 (s, 2 H), 4.48 and 3.12 (2 AB q, 8 H J = 13.2 Hz), 4.13 (t, 4 H, J = 5.7 Hz), 4.05 (t, 4 H, J = 5.0 Hz), 3.88−3.83 (m, 8 H), 3.59−3.51 (m, 8 H), 3.49 (s, 4 H), 3.30 (s, 2 H), 2.36 (s, 6 H), 2.19 (s, 3 H), 1.24−1.16 (m, 12 H). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 155.8, 155.7, 154.7, 135.3, 135.2, 134.4, 134.2, 133.5, 128.2, 128.1, 127.9, 127.7, 122.2, 121.9, 73.3, 72.9, 69.6, 66.3, 66.2, 55.8, 55.5, 35.9, 35.8, 30.8, 15.3 (2×). FAB-MS: m/z 842.4 ([M + H]$^+$, calcd 842.5).

5,11,17-Tris[((6-hydroxymethyl)pyridin-2-yl-methyl)-*N*-methylaminomethyl]-25,26,27,28-tetrakis(2-ethoxy-ethoxy)calix[4]arene (9). A mixture of calix[4]arene **8** (510 mg, 0.641 mmol), K_2CO_3 (265 mg, 1.92 mmol), and 2-bromomethyl-6-hydroxymethylpyridine[20] (389 mg, 1.92 mmol) was stirred for 2 days in THF (30 mL). The solvent was removed under reduced pressure, the residue was taken up in EtOAc/

saturated Na_2CO_3 solution (50/50 mL), and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N, 90/9/1) to give **9** as a colorless oil (500 mg, 65%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 7.76−7.54 (m, 3 H), 7.25−7.12 (m, 6 H), 6.76 (s, 2 H), 6.73 (s, 2 H), 6.53 (s, 2 H), 6.52 (d, 2 H, J = 7.3 Hz), 6.26 (t, 2 H, J = 7.3 Hz), 5.33 (br s, 3 H), 4.73 (s, 6 H), 4.48 and 3.13 (AB q, 8 H, J = 13.0 Hz), 4.18−4.08 (m, 8 H), 3.90−3.83 (m, 8 H), 3.60−3.50 (m, 8 H), 3.43 (s, 4 H), 3.24 (s, 2 H), 3.10 (s, 4 H), 3.07 (s, 2 H), 2.06 (s, 6 H), 1.73 (s, 3 H), 1.25−1.17 (m, 12 H). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 159.4, 159.3, 158.2, 155.7, 155.1, 137.2, 135.0 (2×), 134.6, 134.3, 131.5, 129.2, 129.0, 127.9, 121.4, 121.2, 119.3, 119.1, 73.3, 73.0, 69.7, 66.4, 66.3, 64.7, 64.4, 62.0, 61.1, 46.0, 42.3, 41.7, 30.8, 15.3, 8.8. FAB-MS: *m/z*, 1205.6 ([M + H]$^+$, calcd 1205.7).

5,11,17-Tris[((6-*N*,*N*-dimethylaminomethyl)pyridin-2-yl-methyl)-*N*-methylamino-methyl]-25,26,27,28-tetrakis-(2-ethoxyethoxy)calix[4]arene (5). To a solution of calix-[4]arene **9** (500 mg, 0.415 mmol) in CH_2Cl_2/toluene (40 mL/5 mL) was added $SOCl_2$ (0.91 mL, 12.4 mmol), and the solution was stirred for 4 h. The solvent was removed under reduced pressure to give the crude tris(2-chloromethylpyridine) hydrochloride. The tris(2-chloromethylpyridine) hydrochloride was dissolved in CH_2Cl_2 (20 mL) and was added dropwise to a 33% Me_2NH solution in EtOH (30 mL). The mixture was stirred for 16 h and concentrated in vacuo. Saturated Na_2CO_3 solution (40 mL) and CH_2Cl_2 (40 mL) was added, the aqueous layer was extracted with CH_2Cl_2 (3 × 40 mL), and the combined organic phases were dried over K_2CO_3. Filtration and evaporation of the solvent gave the crude product which was purified by column chromatography (basic Al_2O_3, CH_2Cl_2/MeOH, 99/1) to give **5** as a colorless oil (410 mg, 77%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 7.56 (t, 2 H, J = 7.7 Hz), 7.43 (t, 1 H, J = 7.6 Hz), 7.30 (d, 2 H, J = 7.7 Hz), 7.18 (d, 2 H, J = 7.7 Hz), 7.11 (d, 1 H, J = 7.6 Hz), 6.96 (d, 1 H, J = 7.6 Hz), 6.89 (s, 2 H), 6.83 (s, 2 H), 6.30 (s, 2 H), 6.18 (d, 2 H, J = 7.5 Hz), 5.99 (t, 1 H, J = 7.5 Hz), 4.40 and 3.07 (2 AB q, 8 H, J = 13.1 Hz), 4.15 (t, 4 H, J = 6.2 Hz), 3.90 (t, 4 H, J = 4.7 Hz), 3.82 (t, 4 H, J = 6.2 Hz), 3.73 (t, 4 H, J = 4.7 Hz), 3.57 (s, 4 H), 3.50 (s, 4 H), 3.45 (s, 2 H), 3.53−3.39 (m, 8 H), 3.34 (s, 4 H), 3.17 (s, 2 H), 2.89 (s, 2 H), 2.20 (s, 12 H), 2.17 (s, 6 H), 2.10 (s, 6 H), 1.69 (s, 3 H), 1.15 (t, 6 H, J = 7.0 Hz), 1.09 (t, 6 H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 159.5, 159.3, 158.3, 157.9, 156.3, 155.1, 154.2, 136.7, 136.4, 135.7, 135.6, 133.8, 133.5, 132.2, 132.1, 129.3, 129.2, 128.2, 127.6, 122.1, 121.1, 121.0, 73.6, 72.5, 69.6, 66.4, 66.2, 65.9, 63.2, 63.0, 61.8, 61.5, 45.7, 45.6, 42.3, 42.2, 30.8, 15.3 (2×). FAB-MS: *m/z* 1285.3 ([M]$^+$, calcd 1285.8).

5,17-Bis[(*N*-Boc-*N*-methyl)aminomethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (11). Diformyl-tetrakis(2-ethoxyethoxy)calix[4]arene **10**[6,16] (6.43 g, 8.36 mmol) was dissolved in a 33% methylamine solution in EtOH (300 mL) after which 10% Pd−C (2.00 g) was added. The reaction mixture was stirred for 16 h in a hydrogen atmosphere, filtered over Hyflo, and evaporated till dryness to give the crude secondary amine. This was dissolved in MeOH (300 mL) after which $(Boc)_2O$ (9.13 g, 41.8 mmol) and Et_3N (5.80 mL, 41.8 mmol) were added. The reaction mixture was stirred for 20 h and subsequently evaporated till dryness. The residue was taken up in CH_2Cl_2 (250 mL), washed with 0.1 M HCl (2 × 200 mL) and water (200 mL), and dried with $MgSO_4$. The mixture was filtered and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc, 9/1) to give **11** as a colorless oil (6.23 g, 74%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 6.68 (br s, 4 H), 6.44 (br s, 6 H), 4.49 and 3.11 (AB q, 8 H, J = 13.3 Hz), 4.19−4.04 (m, 12 H), 3.81−3.88 (m, 8 H), 3.55 (q, 4 H, J = 7.0 Hz), 3.53 (q, 4 H, J = 7.1 Hz), 2.70 (br s, 6 H), 1.50 (s, 18 H), 1.22 (t, 6 H, J = 7.0 Hz), 1.19 (t, 6 H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 156.2, 155.6, 135.5, 134.3, 131.2, 127.9, 122.4, 79.4, 73.4, 72.9, 69.7, 69.6, 66.4, 66.3, 66.2, 33.5, 30.9, 28.5, 15.3. FAB-MS: *m/z* 999.6 ([M + H]$^+$, calcd 999.6), 897.8 ([M-*t*-BuOCO]$^+$, calcd 897.5). Anal. calcd for $C_{58}H_{82}N_2O_{12}$: C, 69.71; H, 8.27; N, 2.80. Found: C, 69.80; H, 8.43; N, 2.74.

5,17-Bis(methylaminomethyl)-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (12). The Boc protected amine **11** (6.23 g, 6.23 mmol) was dissolved in CH_2Cl_2 (300 mL) after which trifluoroacetic acid (15 mL, excess) was added. The reaction mixture was stirred for 16 h and evaporated till dryness. The residue was dissolved in a minimum amount of CH_2Cl_2, and hexane was added until a precipitate formed. Filtration gave **12**·4CF_3COOH·H_2O as a sticky solid material (3.25 g, 41%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 12.10 (br s, 4 H), 8.18 (br s, 4 H), 6.95 (d, 4 H, J = 7.2 Hz), 6.81 (t, 2 H, J = 7.2 Hz), 6.56 (s, 4 H), 4.51 and 3.16 (AB q, 8 H, J = 13.0 Hz), 4.32 (t, 4 H, J = 6.1 Hz), 4.01−3.94 (m, 8 H), 3.78 (t, 4 H, J = 4.7 Hz), 3.67 (br s, 4 H), 3.62−3.50 (m, 8 H), 2.40 (s, 6 H), 1.23 (t, 6 H, J = 7.0 Hz), 1.19 (t, 6 H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 161.0, 160.4, 156.4, 156.3, 135.3, 135.2, 129.4, 128.8, 124.0, 123.2, 117.8, 113.2, 74.2, 72.2, 69.5, 66.5, 66.4, 52.4, 32.4, 30.7, 15.1, 15.0. FAB-MS: *m/z* 999.3 ([M + H]$^+$, calcd 999.5). Anal. calcd for $C_{48}H_{66}N_2O_8$·4CF_3COOH·H_2O: C, 52.09; H, 5.78; N, 2.17. Found: C, 52.02; H, 5.78; N, 2.18. The mother liquor was concentrated in vacuo, taken up in saturated Na_2CO_3 solution (200 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried over K_2CO_3 and filtered, and the solvent was evaporated under reduced pressure to give the free amine **12** as a colorless oil (2.89 g, 58%), pure enough for further modification (overall yield 99%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 6.68 (s, 2 H), 6.51 (s, 4 H), 6.63−6.49 (m, 4 H), 4.48 and 3.12 (AB q, 8 H, J = 13.3 Hz), 4.13 (t, 4 H, J = 5.8 Hz), 4.08 (t, 4 H, J = 5.8 Hz), 3.87−3.81 (m, 8 H), 3.59−3.49 (m, 8 H), 3.46 and 3.40 (s, 4 H), 2.62 and 2.26 (s, 6 H), 1.51 (br s, 2 H), 1.26−1.14 (m, 12 H).

5,17-Bis[((6-hydroxymethyl)pyridin-2-yl-methyl)-*N*-methylaminomethyl]-25,26,27,28-tetrakis(2-ethoxy-ethoxy)calix[4]arene (13). A mixture of calix[4]arene **12** (2.26 g, 2.83 mmol), K_2CO_3 (783 mg, 5.67 mmol), and 2-bromomethyl-6-hydroxymethylpyridine[20] (1.15 g, 5.67 mmol) was stirred for 16 h in CH_3CN (150 mL). The solvent was removed under reduced pressure, the residue was taken up in saturated Na_2CO_3 solution (125 mL) and the aqueous phase was extracted with EtOAc (3 × 100 mL). The combined organic phases were dried over K_2CO_3, filtered, and concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 9/1) to give **13** as a colorless oil (1.89 g, 64%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 7.62 (t, 2 H, J = 7.6 Hz), 7.17 (d, 2 H, J = 7.6 Hz), 7.10 (d, 2 H, J = 7.6 Hz), 6.74 (d, 4 H, J = 7.3 Hz), 6.63 (t, 2 H, J = 7.3 Hz), 6.50 (s, 4 H), 4.76 (s, 4 H), 4.48 and 3.13 (AB q, 8 H, J = 13.1 Hz), 4.19 (t, 4 H, J = 6.0 Hz), 4.05 (t, 4 H, J = 5.4 Hz), 3.90 (t, 4 H, J = 6.0 Hz), 3.82 (t, 4 H, J = 5.4 Hz), 3.54 (q, 8 H, J = 7.0 Hz), 3.33 (s, 4 H), 2.81 (s, 4 H), 1.99 (s, 6 H), 1.20 (t, 12 H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 158.6, 156.4, 137.2, 135.4, 134.2, 128.7, 128.2, 122.2, 121.7, 119.5, 73.4, 72.9, 69.7, 66.3, 64.8, 62.2, 60.7, 42.2, 30.8, 15.3 (2×). FAB-MS: *m/z* 1039.8 ([M−H]$^-$, calcd 1039.6), 1042.1 ([M + H]$^+$, calcd 1041.6).

5,17-Bis[((6-*N*,*N*-dimethylaminomethyl)pyridin-2-yl-methyl)-*N*-methylamino-methyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (3). To a solution of calix[4]-arene **13** (640 mg, 0.644 mmol) in CH_2Cl_2 (60 mL) was added $SOCl_2$ (0.47 mL, 6.4 mmol), and the solution was stirred for 5 h. The solvent was removed under reduced pressure to give the bis(2-chloromethylpyridine) **14** as the bis(hydrochloride) (694 mg, 100%), which was pure enough for further modification. An analytical sample of **14**, free from hydrochloric acid, was obtained by extraction from saturated $NaHCO_3$ solution with CH_2Cl_2, subsequently followed by drying over Na_2SO_4, filtering, and concentration in vacuo. ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 7.68 (t, 2 H, J = 7.7 Hz), 7.38 (d, 2 H, J = 7.8 Hz), 7.33 (d, 2 H, J = 7.7 Hz), 6.82 (s, 4 H), 6.42−6.31 (m, 6 H), 4.66 (s, 4 H), 4.47 and 3.13 (AB q, 8 H, J = 13.3 Hz), 4.17 (t, 4 H, J = 6.1 Hz), 4.04 (t, 4 H, J = 5.5 Hz), 3.81−3.88 (m, 8 H), 3.57 (s, 4 H), 3.56 (q, 4 H, J = 7.0 Hz), 3.52 (q, 4 H, J = 7.0 Hz), 3.32 (s, 4 H), 2.16 (s, 6 H), 1.22 (t, 6 H, J = 7.0 Hz), 1.17 (t, 6 H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm)

159.8, 156.1, 155.7, 137.3, 135.3, 134.4, 134.3, 133.3, 131.9, 129.1, 128.8, 127.8, 122.2, 120.8, 73.4, 72.8, 69.6, 66.4, 66.2, 62.7, 61.7, 46.9, 46.5, 42.5, 30.8, 15.3. FAB-MS: *m/z* 1042.3 ([M-Cl]$^+$, calcd 1041.5). To a solution of calix[4]arene bis-(hydrochloride) **14** (694 mg, 0.644 mmol) in CH_3CN (60 mL) was added $Me_2NH\cdot HCl$ (1.04 g, 12.8 mmol) and K_2CO_3 (1.77, 12.8 mmol). The mixture was stirred for 16 h and concentrated in vacuo. Saturated Na_2CO_3 solution (40 mL) was added, the aqueous layer was extracted with CH_2Cl_2 (3 × 40 mL), and the combined organic phases were dried over K_2CO_3. Filtration and evaporation of the solvent gave the crude product which was purified by column chromatography (basic Al_2O_3, CH_2Cl_2/MeOH, 99/1) to give **3** as a colorless oil (460 mg, 65%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 7.64 (t, 2 H, J = 7.7 Hz), 7.36 (d, 2 H, J = 7.7 Hz), 7.25 (d, 2 H, J = 6.5 Hz), 6.88 (s, 4 H), 6.32 (s, 6 H), 4.47 and 3.13 (AB q, 8 H, J = 13.3 Hz), 4.19 (t, 4 H, J = 6.1 Hz), 4.01 (t, 4 H, J = 5.3 Hz), 3.80−3.89 (m, 8 H), 3.62 (s, 4 H), 3.58 (s, 4 H), 3.51 (q, 8 H, J = 7.0 Hz), 3.38 (s, 4 H), 2.29 (s, 12 H), 2.18 (s, 6 H), 1.23 (t, 6 H, J = 7.0 Hz), 1.17 (t, 6 H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 159.3, 158.0, 156.4, 155.3, 136.8, 135.6, 134.0, 132.0, 129.3, 127.7, 122.2, 121.2, 73.5, 72.7, 69.6, 66.4, 66.2, 65.7, 63.0, 61.8, 45.6, 42.4, 30.8, 15.3. FAB-MS: *m/z* 1096.5 ([M + H]$^+$, calcd 1095.7).

5-[(*N*-Boc-*N*-methyl)aminomethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (15). This compound was synthesized according to the synthesis of the bis-derivative **11**, starting from formyl-tetrakis(2-ethoxyethoxy)calix[4]arene **16**.[16] The crude product was purified by column chromatography (CH_2Cl_2/EtOAc, 95/5) to give **15** as a colorless oil (83%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 6.71−6.61 (m, 6 H), 6.49 (br s, 3 H), 6.36 (br s, 2 H), 4.50 and 3.14 (AB q, 4 H, J = 13.3 Hz), 4.49 and 3.11 (AB q, 4 H, J = 13.3 Hz), 4.20−4.15 (m, 4 H), 4.08−4.04 (m, 4 H), 3.98 (br s, 2 H), 3.90−3.80 (m, 8 H), 3.60−3.50 (m, 8 H), 2.50 and 2.31 (br s, 1 H, 2 H), 1.46 (br s, 9 H), 1.24−1.17 (m, 12 H). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 156.7, 155.9, 155.2, 135.4, 134.6, 134.5, 131.2, 128.3, 128.0, 127.4, 122.3, 122.1, 79.2, 73.4, 73.0, 69.7, 66.4, 66.3, 33.3, 30.9, 28.5, 15.4, 15.3. FAB-MS: *m/z* 856.4 ([M + H]$^+$, calcd 856.5).

5-(Methylaminomethyl)-25,26,27,28-tetrakis(2-ethoxyethoxy)-calix[4]arene (17). This compound was synthesized according to the synthesis of the bis-derivative **12**. After stirring in trifluoroacetic acid, the reaction mixture was concentrated in vacuo to give crude **17**·3CF_3COOH (99%), which was pure enough for further modification. ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 11.68 (br s, 2 H), 8.08 (br s, 2 H), 6.88−6.71 (m, 5 H), 6.47−6.39 (m, 4 H), 4.50 and 3.15 (AB q, 4 H, J = 13.5 Hz), 4.48 and 3.13 (AB q, 4 H, J = 13.3 Hz), 4.25−4.19 (m, 4 H), 4.07−4.00 (m, 4 H), 3.91−3.80 (m, 8 H), 3.62−3.51 (m, 10 H), 2.18 (s, 3 H), 1.25−1.03 (m, 12 H). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 156.9, 156.6, 155.8, 135.8, 135.6, 135.1, 134.6, 134.1, 129.6, 128.7, 128.5, 127.9, 123.5, 122.6, 121.9, 73.6, 73.5, 72.9, 69.6, 66.4, 66.3, 51.9, 31.4, 31.1, 30.8, 30.7, 15.3. FAB-MS: *m/z* 756.2 ([M + H]$^+$, calcd 756.4). The free amine **17** was obtained by extraction with CH_2Cl_2 from saturated Na_2CO_3 solution, followed by drying over K_2CO_3.^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 6.47−6.67 (m, 11 H), 4.50 and 3.13 (AB q, 4 H, J = 13.3 Hz), 4.48 and 3.11 (AB q, 4 H, J = 13.3 Hz), 4.06−4.15 (m, 8 H), 3.83−3.87 (m, 8 H), 3.50−3.58 (m, 8 H), 3.38 (s, 2 H), 2.26 (s, 3 H), 1.40 (br s, 1 H), 1.12−1.26 (m, 12 H). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 156.4, 156.3, 155.3, 135.2, 135.1, 135.0, 134.8, 133.3, 128.3, 128.2, 128.1, 128.0, 122.2, 121.9, 73.2, 73.1, 69.7, 66.4, 55.6, 35.8, 31.5, 30.9, 15.3. FAB-MS: *m/z* 756.9 ([M + H]$^+$, calcd 756.4).

5-[(6-Hydroxymethyl)pyridin-2-yl-methyl)-*N*-methylaminomethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)-calix[4]arene (18). This compound was synthesized according to the synthesis of the bis-derivative **13**. The crude product was purified by column chromatography (CH_2Cl_2/MeOH, 9/1) to give **18** as a colorless oil (65%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 7.64 (t, 1 H, J = 7.7 Hz), 7.11 (d, 2 H, J = 7.7 Hz), 6.77−6.60 (m, 6 H), 6.55 (s, 2 H), 6.43−6.35 (m, 2 H), 6.30−6.24 (m, 1 H), 4.72 (s, 2 H), 4.50 and 3.15 (AB q, 8 H, J = 13.3 Hz), 4.48 and 3.11 (AB q, 8 H, J = 13.2 Hz), 4.21−4.16 (m, 4 H), 4.08−4.02 (m, 4 H), 3.91−3.80 (m, 8 H), 3.59−3.50 (m, 8 H), 3.33 (s, 4 H), 3.23 (s, 2 H), 2.01 (s, 3 H), 1.25−1.14 (m, 12 H). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 158.6, 158.2, 156.6, 155.9, 155.1, 137.0, 135.4, 134.5, 134.3, 131.6, 128.6, 128.3 (2×), 128.0, 122.2, 122.1, 121.6, 118.4, 73.4, 72.9, 69.7, 66.4, 66.3, 64.0, 62.2, 61.3, 42.5, 30.9, 15.3. FAB-MS: *m/z* 877.4 ([M + H]$^+$, calcd 877.5).

5-[(6-*N*,*N*-Dimethylaminomethyl)pyridin-2-yl-methyl)-*N*-methylaminomethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (2). This compound was synthesized according to the synthesis of the bis-derivative **3**. The crude product was purified by column chromatography (basic Al_2O_3, CH_2Cl_2) to give **2** as a colorless oil (83%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 7.63 (t, 1 H, J = 7.7 Hz), 7.24 (d, 1 H, J = 7.7 Hz), 7.14 (d, 1 H, J = 7.6 Hz), 6.74−6.58 (m, 8 H), 6.43−6.37 (m, 2 H), 6.31−6.25 (m, 1 H), 4.51 and 3.15 (AB q, 8 H, J = 13.3 Hz), 4.49 and 3.12 (AB q, 8 H, J = 13.2 Hz), 4.19−4.14 (m, 4 H), 4.09−4.03 (m, 4 H), 3.90−3.82 (m, 8 H), 3.58−3.50 (m, 10 H), 3.37 (s, 2 H), 3.21 (s, 2 H), 2.27 (s, 6 H), 2.05 (s, 3 H), 1.25−1.15 (m, 12 H). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 159.6, 158.0, 156.5, 155.9, 155.1, 136.6, 135.3 (2×), 134.6, 134.3, 132.3, 128.5, 128.3, 128.2, 128.0, 122.2, 122.1, 121.3, 121.0, 73.3, 72.9, 69.7, 66.4, 66.3, 65.9, 62.9, 61.6, 45.7, 42.6, 31.5, 30.8, 15.3. FAB-MS, *m/z* 904.5 ([M + H]$^+$, calcd 904.5).

11,23-Bis(methylaminomethyl)-26,28-di-*n*-propoxycalix-[4]arene-25,27-crown-3 (19). This compound was synthesized according to the synthesis of the tris-derivative **8**, starting from diformyl-di-*n*-propoxycalix[4]arene-crown-3 **20**.[15] The crude reaction product was pure enough for further modification (yield 94%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.08 (s, 4 H), 6.17 (t, 2 H, J = 7.6 Hz), 5.98 (d, 4 H, J = 7.6 Hz), 4.35 and 3.16 (AB q, 8 H, J = 13.5 Hz), 4.20 (t, 4 H, J = 4.0 Hz), 3.99 (t, 4 H, J = 4.0 Hz), 3.76 (s, 4 H), 3.66 (t, 4 H, J = 6.8 Hz), 2.51 (s, 6 H), 1.88−1.79 (m, 4 H), 1.57 (br s, 2 H), 1.09 (t, 6 H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 157.1, 154.3, 136.4, 133.2, 132.6, 128.6, 126.6, 121.6, 76.3, 72.6, 69.7, 55.4, 35.6, 30.3, 23.0, 10.4. FAB-MS: *m/z* 664.3 ([M]$^+$, calcd 664.4), 634.5 ([M − $NHCH_3$]$^+$, calcd 634.4).

11,23-Bis[((6-hydroxymethyl)pyridin-2-yl-methyl)-*N*-methylaminomethyl]-26,28-dipropoxycalix[4]arene-25,27-crown-3 (21). This compound was synthesized and purified according to the synthesis and purification of the tris-derivative **9** (yield 70%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) 7.64 (t, 2 H, J = 7.7 Hz), 7.46 (d, 2 H, J = 7.0 Hz), 7.18−7.12 (m, 6 H), 6.37−5.93 (m, 6 H), 4.75 (s, 4 H), 4.36 and 3.17 (AB q, 8 H, J = 13.5 Hz), 4.19 (br s, 4 H), 4.01 (br s, 4 H), 3.75 (s, 4 H), 3.62 (s, 4 H), 2.33 (s, 6 H), 1.83 (m, 4 H), 1.09 (t, 6 H, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 158.2, 157.7, 154.8, 137.2, 137.0, 136.8, 133.1, 130.1, 127.1, 122.1, 121.5, 118.6, 76.9, 73.1, 70.2, 64.0, 62.8, 61.9, 53.5, 42.6, 30.8, 23.5, 11.0. FAB-MS: *m/z* 907.5 ([M + H]$^+$, calcd 907.5).

11,23-Bis[(6-*N*,*N*-dimethylaminomethyl)pyridin-2-yl-methyl)-*N*-methylaminomethyl]-26,28-dipropoxy-calix[4]arene-25,27-crown-3 (4). This compound was synthesized according to the synthesis of the tetrakis(2-ethoxy-ethoxy) derivative **5**. The crude product was purified by column chromatography (basic Al_2O_3, CH_2Cl_2) to give **4** as a colorless oil (62%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.67 (t, 2 H, J = 7.7 Hz), 7.49−7.45 (m, 2 H), 7.29 (d, 2 H, J = 7.2 Hz), 7.17 (s, 4 H), 6.13−5.94 (m, 6 H), 4.37 and 3.18 (AB q, 8 H, J = 13.7 Hz), 4.19 (br s, 4 H), 4.02 (br s, 4 H), 3.77 (s, 4 H), 3.68 (t, 4 H, J = 6.6 Hz), 3.61 (s, 8 H), 2.31 (s, 18 H), 1.96−1.82 (m, 4 H), 1.11 (t, 6 H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 250 MHz): δ (ppm) 158.9, 157.7, 157.1, 155.8, 154.3, 136.3, 136.2, 132.6, 129.5, 126.5, 121.5, 120.7, 120.6, 76.3, 72.6, 69.7, 65.4, 62.7, 61.4, 45.1, 42.1, 30.3, 23.0, 10.4. FAB-MS: *m/z* 961.7 ([M + H]$^+$, calcd 961.6).

Spectrophotometric Titrations. To a cuvette containing 2 mL of 50% CH_3CN/20 mM aqueous HEPES solution pH 7.0 (v/v, see kinetics),[21] was added 8 μL of a 50 mM stock solution of ligand **1** or **2** in EtOH (2 μL 50 mM for **3**, **4**, and **5**). The increase in absorbance at λ = 266 nm at 25 °C by the ligand **1** or **2** (ϵ_{266}: **1**, 4.0 × 10^3 M^{-1} cm^{-1}; **2**, 4.9 × 10^3 M^{-1} cm^{-1}; **3**, 5.9 × 10^3 M^{-1} cm^{-1}; **4**, 6.0 × 10^3 M^{-1} cm^{-1}; **5**, 12 × 10^3 M^{-1}

cm^{-1}) was followed upon the stepwise addition of 2 μL of 50 mM $Zn(ClO_4)_2 \cdot 6H_2O$ in water up to 3 equiv of $Zn(ClO_4)_2 \cdot 6\ H_2O$ (6 equiv for **3**, **4**, and **5**). The concentration of the ligands before titration was 0.2 mM for **1** and **2**, and 0.05 mM for **3**, **4**, and **5**, respectively. The absorbance was corrected for both dilution upon titration and for the weak absorbance of free $Zn(ClO_4)_2$ ($\epsilon_{266} = 70\ M^{-1}\ cm^{-1}$). Stability constants for Zn(II) complexation were estimated by nonlinear least-squares fitting[22] of the titration curves to a 1:1 model using the absorption end values at 266 nm.

Potentiometric pH Titrations. Solutions (50 mL) of ligand **1** (0.4 mM) in 0.1 M KNO_3 in 35% EtOH/H_2O (v/v), acidified with HNO_3 (1.2 mM), were titrated under N_2 at 20.0 °C in the presence and absence of 1 equiv of $Zn(NO_3)_2$ or $Cu(NO_3)_2$, respectively. The titrations were carried out at fixed titrant increments of 50 μL NaOH solution (0.020 M). The titration apparatus was calibrated with appropriate buffers immediately before use and checked by the pK_a determination of acetic acid. Three independent titrations were always made for pK_a determinations. For calculation of deprotonation constants and Zn(II)/Cu(II) association constants from the titration data a multiparameter curve fitting program based on SUPERQUAD was used.[23] The resulting equilibrium constants could be interpreted as conditional constants at $I = 0.1$ M. The value for K_w (=$[H^+][OH^-]$) at 20 °C was $10^{-14.38}$.

X-ray Crystallography. To a solution of **1** (100 mg, 0.517 mmol) and $Zn(CH_3COO)_2 \cdot 2\ H_2O$ (113 mg, 0.517 mmol) in acetonitrile (2 mL) was added Et_2O (5 mL). The obtained white crystals were dissolved in acetonitrile (2 mL) and recrystalized by slow vapor diffusion of Et_2O to the solution, yielding crystals of (**1**)$Zn(CH_3COO)_2$ suitable for X-ray crystal structure analysis. Crystal data for (**1**)$Zn(CH_3COO)_2 \cdot H_2O$: $C_{15}H_{25}N_3O_4Zn \cdot H_2O$, $M_r = 394.79$, colorless, block-shaped crystal (0.2 × 0.2 × 0.3 mm), monoclinic, space group $P2_1/c$ with $a = 9.0875(15)$, $b = 16.725(3)$, $c = 14.255(2)$ Å, $\beta = 120.11(8)°$, $V = 1874.2(16)$ Å^3, $Z = 4$, $D_x = 1.399$ g cm^{-3}, μ(MoKα) = 13.4 cm^{-1}, 27 324 reflections measured, 4293 independent, $R_{int} = 0.0461$, ($1.5° < \theta < 28.5°$, $T = 150$ K, MoKα radiation, graphite monochromator, $\lambda = 0.710\ 73$ Å), on a Nonius κ-CCD diffractometer on rotating anode. Data were corrected for Lp effects but not for absorption. The structure was solved by automated direct methods (SHELXS86). Refinement F^2 was carried out by full-matrix least-squares techniques (SHELXL-97) for 230 parameters; no observance criterion was applied during refinement. Refinement converged at a final wR_2 value of 0.0831, $R_1 = 0.0344$ [for 3610 reflections with $I > 2\sigma(I)$], $S = 1.025$. Hydrogen atom coordinates were included as parameters in the refinement. A final difference Fourier showed no residual density outside −0.61 and 0.31 e Å^{-3}.

Kinetics. Solutions for spectrophotometric titrations and kinetic measurements were made by adding CH_3CN (spectrophotometric grade) up to 50% (v/v) to a 20 mM aqueous buffer solution adjusted with NaOH to the desired pH. Buffers (MES, pH 6.0−7.0; HEPES, pH 7.0−8.2; EPPS, pH 8.2−8.6) were obtained from commercial sources and used without further purification in deionized distilled water. HPNP was prepared according to the literature.[33] Stock solutions were freshly prepared before performing the kinetic measurements. In a typical experiment, the ligand **5** (20 μL, 50 mM in EtOH) and $Zn(ClO_4)_2 \cdot 6H_2O$ (60 μL, 50 mM in water) were added to a cuvette containing 2 mL of 50% CH_3CN/20 mM buffer solution (v/v) and thermostated at 25 °C. After a couple of minutes equilibration time, HPNP (4 μL, 100 mM in water) was injected and the increase in UV absorption at $\lambda = 400$ nm due to the release of *p*-nitrophenolate was recorded every 30 s. Final concentrations were 0.98 mM for complexes **1**-[Zn] and **2**-[Zn], and 0.48 mM for complexes **3**-$[Zn]_2$, **4**-$[Zn]_2$, and **5**-$[Zn]_3$, 0.19 mM in HPNP, and 10 mM in buffer. All solutions remained clear during the time of the kinetic measurements. In the absence of ligand precipitation of polymeric Zn(II) hydroxide took place. The observed pseudo-first-order rate constants k_{obs} (s^{-1}) were calculated with the extinction coefficient of *p*-nitrophenolate at $\lambda = 400$ nm by an initial slope method (<4% conversion). All rate constants were obtained by averaging three kinetic measurements. The pseudo-first-order rate constants for uncatalyzed reactions (k_{uncat}, s^{-1}) were measured from a 2.0 mM HPNP solution by following the increase in absorbance at 400 nm due to the release of *p*-nitrophenolate for 1 week.

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Supporting Information Available: Further details of the X-ray structure determination, including atomic coordinates, bond lengths and angles and thermal parameters, and ^{1}H NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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