Acceleration of *p*-Nitrophenyl Ester Cleavage by Zn(II)-Organized Molecular Receptors

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Tris(2-aminoethyl)amine (TREN) has been functionalized by introducing phenolic residues in the tripodal ligand side arms. The resulting functionalized ligands **1**−**5** form stable complexes with Zn(II) ions at pH > 6−6.5. The conformation of the Zn(II) complexes is such to form an ill-defined cavity with the metal ion occupying its bottom and the aromatic residues defining its hydrophobic walls. In these Zn(II) complexes one of the phenolic hydroxyls is, depending on the structure of the ligand, up to 1.3 pK_a units more acidic than that of phenol itself. This enhanced acidity is attributed to second sphere coordination to the metal center. The complexes, particularly **1**·Zn(II), behave as molecular receptors of *p*-nitrophenyl esters of carboxylic acids with binding constants ≥300 M^{-1} for those substrates capable of coordination to the Zn(II) ion (*p*-nitrophenyl nicotinate, PNPN, *p*-nitrophenyl isonicotinate, PNPIN and *p*-nitrophenyl urocanoate, PNPU). At pH 8.3 they also accelerate the cleavage of these esters with rate accelerations with respect to the uncatalyzed hydrolysis of up to 60 times, depending on the structure of the substrate. The kinetic analysis of the process shows that the rate effects are due to two independent mechanisms: a bimolecular process that does not comprise binding of the substrate and a pseudointramolecular process within the supramolecular complex made of ligand, metal ion, and substrate. In both cases the nucleophile is one of the phenolic hydroxyls of the functionalized side arms of the TREN-based complex which, in the first step, is acylated by the substrate and eventually slowly hydrolyzes turning over the catalyst. Determination of second-order rate constants shows that the nucleophilicity of the phenolic hydroxyls is higher than that of a substituted phenol of the same pK_a. Comparison of the metalloreceptor **1**·Zn(II) with cyclodextrins allowed one to highlight similarity and differences between the two receptors.

Introduction

The Zn(II) ion in biological systems plays two relevant roles:[1] (i) stabilization of structures of proteins and (ii) participation in the catalytic site of several enzymes. Examples are provided by zinc finger[2] proteins and carboxypeptidase A.[3] Systems are also known (liver alcohol dehydrogenase, for instance) in which the metal ion plays both roles within the same protein.

Synthetic systems exploiting these aspects of coordination chemistry of Zn(II) (and other transition metal ions as well) have been reported by several laboratories. If we focus on the aspect of the stabilization of specific conformations of structures we may cite several examples.[4] For instance, Schneider[5] synthesized a diphenylmethane-based molecule which forms a macrocycle as a Cu(II) complex and we studied[6] a pyridine-based, tweezers-like ligand which forms a pseudomacrocycle upon binding two Cu(II) ions. Furthermore self-assembling supramolecular structures have been obtained from properly designed ligands and different metal ions. These range from helices (Lehn,[7] Constable[8]), catenanes (Sauvage[9]), macrocycles (Stang,[10] Hunter,[11] Fujita[12]), and peptide helices bundles (Ghadiri[13]).

Our idea was to synthesize a ligand with properly appended functional groups which, upon addition of Zn(II) ions, could (a) assume a specific conformation suitable for binding of selected substrates and (b) act as a catalyst of a transacylation reaction. A reaction mechanism in which binding is portal to successive pseudo-

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Chart 1

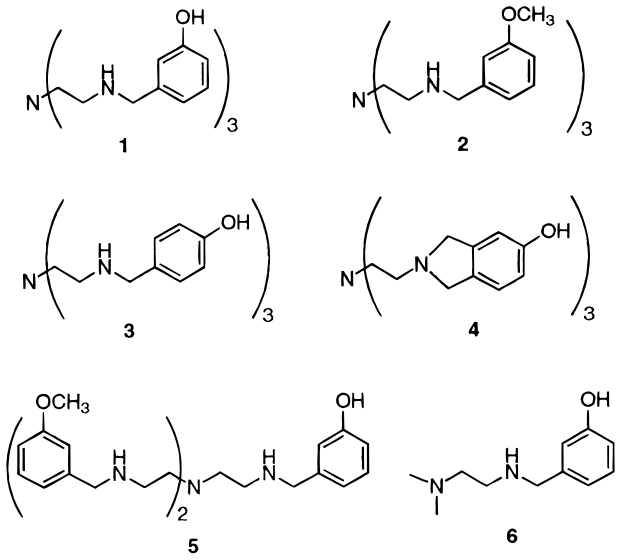
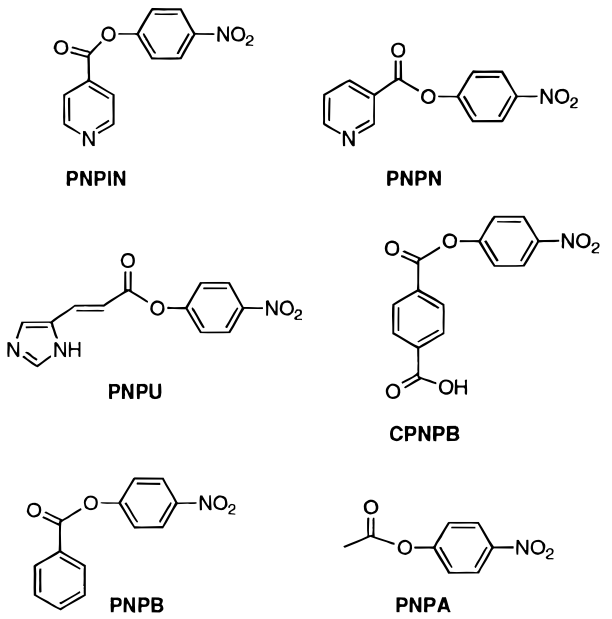


Chart 2



intramolecular reactivity is a rule in enzymatic reactions[14] and our system was conceived with this in mind. The considerable interest in supramolecular systems[15] stems also from the search for simple molecules that, operating on the basis of principles similar to those of enzymes, may allow one to develop new and fully synthetic catalysts. We focused our attention on tris(2-aminoethyl)amine (TREN), a strong ligand for several transition metal cations; thus, for the formation constant of the 1:1 complex with Zn(II) a log $K_b \approx 15$ was reported.[16] An appealing aspect of the Zn(II)·TREN complex was that being the typical coordination geometry a trigonal bipyramid,[16a] the coordination of the four nitrogens of the ligand leaves a fifth position available for binding of substrates functionalized with suitable donor groups. Eventually we finalized our endeavors toward the synthesis of ligand **1** and similarly functionalized ligands **2**−**5** (Chart 1) on the assumption that a phenolic group would be an acceptable nucleophile to test the validity of our hypothesis. A preliminary investigation of compounds **1** and **2** gave promising results.[17] This paper reports the full analysis of the TREN-based phenolic receptors **1**−**5** as transacylation catalysts of the *p*-nitrophenyl ester of carboxylic acids illustrated in Chart 2.

Results and Discussion

Syntheses. The synthesis of ligands **1**−**3** was neatly performed by condensation of TREN with three equivalents of the proper aldehyde and subsequent reduction of the imine with $NaBH_4$. Compound **5** was obtained by condensation of two equivalents of 3-methoxybenzaldehyde with Boc-monoprotected TREN, reduction, deprotection, and condensation with 3-hydroxybenzaldehyde followed again by reduction. Single-arm ligand **6** was analogously obtained by condensation/reduction of *N*,*N*-dimethyl-1,2-diaminoethane with 3-hydroxybenzaldehyde. Derivative **4** was obtained by reaction of 4-acetoxyphthalic anhydride with TREN and subsequent reduction of phthalimide derivative with $LiAlH_4$. Details of the syntheses and spectroscopic characterization of new ligands are in the Experimental Section.

The synthesis of all substrates but 4-carboxy-*p*-nitrophenyl benzoate (CPNPB) has been already reported. CPNPB was obtained by converting commercially available 4-carboxybenzaldehyde into the corresponding *p*-nitrophenyl ester and oxidizing the aldehyde to the carboxylic acid.

Complex Formation with Zn(II). Because of the competition with protons, the complete formation of the 1:1 complexes with Zn(II) does not occur below pH 6−6.5, depending on the ligands. This is clearly highlighted in the pH titration curves for ligands **1** and **2** (see Figure S1 of the Supporting Information) by the buffer region between pH 5.8 and 6.3 where three OH^- equivalents are taken up with the loss of three protons of the three protonated secondary amines. At the concentration employed for these titrations the complexes formed are not soluble at pH > 8.5 and, hence, we cannot evaluate the pK_a of the phenolic functions from the titration experiments. For this reason we also measured the spectroscopic changes, as a function of pH, in the 285−295 nm wavelength region where the strong absorbance of the phenolate anion allows one to determine the pK_a of the phenols of the complexes. These are reported in Table 1 along with those of simple phenols obtained from the literature.[18] It should be noted that because of the broad pH interval associated with the change of absorbance due to the formation of phenolates, the pK_a values are less precise than those obtained for simple phenols.

From the analysis of Table 1 we note that (a) one of the three phenolic −OH groups of complexes **1**·Zn(II) and **3**·Zn(II) is more acidic than the other two and more acidic than phenol itself by more than one pK_a unit; (b) the above effect is more pronounced for **1**·Zn(II) and vanishes in the case of complex **4**·Zn(II); (c) the acidity of the single phenolic −OH of **5**·Zn(II) is also lower than that of phenol although to a lower extent than that of the most acidic

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Table 1. Apparent pK_a[a] of the Phenolic Groups of Complexes 1·Zn(II) and 3−5·Zn(II) and Those of Simple Phenols

system	pK_a (number of protons) spectroscopic[b]	kinetic[c]
1·Zn(II)	8.5(1); 9.3(2)	8.4
3·Zn(II)	8.7(1); 9.6(2)	
4·Zn(II)	9.6(3)	
5·Zn(II)	8.9	8.8
phenol[d]	9.81	
3,4-dichlorophenol[d]	8.44	
3,5-dichlorophenol[d]	8.03	

[a] See text for comments. [b] [ligand] = [$Zn(NO_3)_2$] = 0.1 mM; I = 0.05 ($NaNO_3$); 25 °C. [c] From the rate vs pH profiles of Figure 4. [d] Data from ref 18.

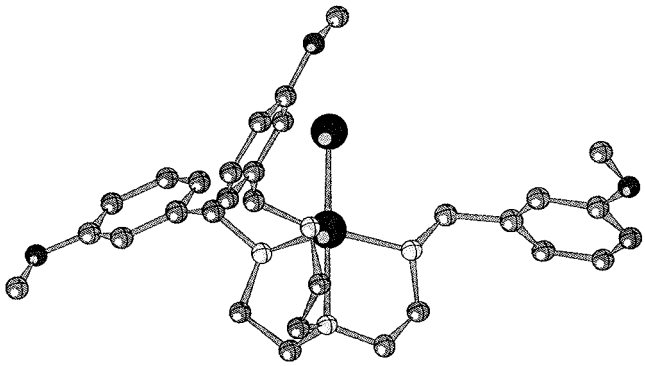

Figure 1. X-ray structure of complex **2**·Zn(II), 2 Cl^-. One Cl^- is omitted, while the other is coordinated to the Zn(II) ion.

−OH of **1**·Zn(II). From these data it is apparent that the Zn(II) ion in the TREN cavity of the complexes influences the acidity of the phenols. Direct coordination of the phenolic −OH to the metal center is not allowed for geometric reasons and this effect is likely due to second-sphere (or indirect) coordination.[4] The fact that one group is more acidic than the other two may be explained with electrostatic interactions with the first phenolate anion. In the rigid complex **4**·Zn(II) the −OH groups cannot get close enough to the metal center and, consequently, their pK_a is very similar to that of phenol.

The structure of **2**·Zn(II) obtained by X-ray diffractometry,[17] shown in Figure 1, indicates that the TREN-based ligands form complexes the conformation of which provide a hydrophobic cavity. On the bottom of this ill-defined cavity is located the Zn(II) ion and the walls are constituted by the mobile aromatic groups. However, in aqueous solution, because of hydrophobic interaction, the aromatic groups may be forced to approach each other to give a more closed, calyx-like conformation.

Kinetics. Table 2 shows the pseudo-first-order rate constants for the cleavage of esters shown in Chart 2 in the presence of the Zn(II)-complexes of ligands **1**−**6**, as determined in aqueous solutions of pH 8.3 (0.05 M EPPS buffer) by following spectrophotometrically at 400 nm the release of *p*-nitrophenolate. This pH was the highest attainable without formation of precipitates with all complexes. This pH is also fairly close to the pK_a of the most acidic phenolic groups linked to the TREN platform thus permitting advantage to be taken of a sizeable concentration of phenolate, the expected nucleophile in the process.

Figure 2 shows the k_ψ vs [**1**·Zn(II)] profile obtained for the cleavage of *p*-nitrophenyl isonicotinate (PNPIN). The curvature of the plot suggests binding of the substrate

Table 2. Rate Constants[a] for the Cleavage of the Different *p*-Nitrophenyl Esters with Zn(II) Complexes of Ligands 1−6

entry	ligand[b]	substrate	$10^4 k_\psi$[c], s^{-1}	k_ψ/k_o	$10^3 k_{lim}$, s^{-1} (K_b, M^{-1})	k_{lim}/k_o
1	none[d]	PNPIN	6.3	1	−	
2	**1**	PNPIN	51.4	8.2	14.5 (380 ± 45)	23
3	**2**	PNPIN	8.2	1.3	*e*	
4	**3**	PNPIN	19.5	3.1	*e*	
5	**4**	PNPIN	1.7[f]	6.8[f]	*e*	
6	**5**	PNPIN	22.5	3.6	*e*	
7	**6**	PNPIN	17.0	2.7	*g*	
8	TREN	PNPIN	6.3	1	−	
9	TREN[h]	PNPIN	16.0	2.5	−	
10	none	PNPN	0.97	1	−	
11	**1**	PNPN	7.8	8.0	5.8 (300 ± 50)	60
12	none	PNPU	0.081	1	−	
13	**1**	PNPU	0.41	5.1	0.089 (560 ± 90)	11
14	none	CPNPB	0.24	1	−	
15	**1**	CPNPB	4.1	17.1	*e*	
16	none	PNPB	0.071	1	−	
17	**1**	PNPB	0.59	8.3	*e*	
18	none	PNPA	0.25	1	−	
19	**1**	PNPA	2.5	10	*e*	

[a] Conditions: EPPS buffer 0.05 M, pH 8.3; [substrate] = 2−3 × 10^{-5} M; 25 °C. [b] 1:1 complex with $Zn(NO_3)_2$. [c] Pseudo-first-order rate constants at [complex] = 1 × 10^{-3} M. [d] Also without $Zn(NO_3)_2$. [e] No appreciable curvature in the k_ψ vs [receptor] plot; see text. [f] Kinetics were run in 1:1 (v/v) DMSO/H_2O due to the low solubility of the complex in water; k_o (in the absence of complex) is 2.5 × 10^{-5} s^{-1} under this conditions. [g] Complex forms dimers, see text. [h] In the presence of three equivalents of phenol.

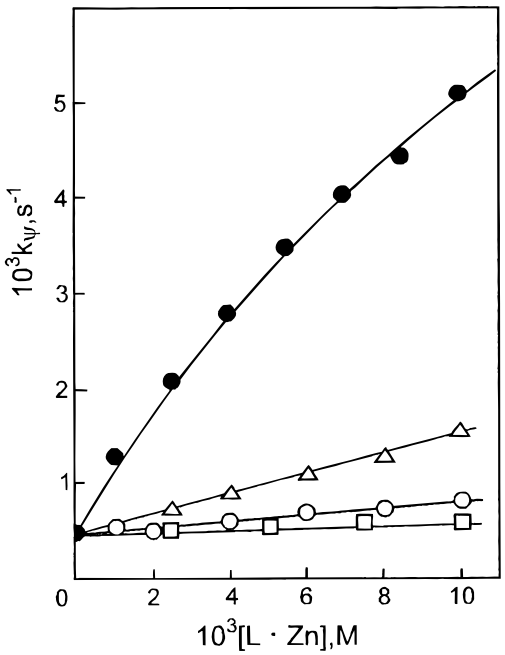

Figure 2. Rate constant vs complex concentration profiles for the cleavage of PNPIN by different complexes in 0.05 M HEPES buffer, pH 8.3, 25 °C. Ligands (L) are: ●, **1**; △, TREN, and 3 equiv of phenol; ○, **2**; □, TREN.

to the Zn(II) complex which behaves like a molecular receptor. Conventional analysis[19] of this curve for a 1:1 complex substrate/receptor gives a binding constant, K_b, of 380 ± 45 M^{-1} and a value of the observed rate constant when all substrate is bound to the metal receptor, k_{lim}, of 1.45 × 10^{-2} s^{-1}. However, not all substrates and Zn(II) complexes show sizeable curvatures in analogous plots, thus preventing such analysis. For this reason Table 2

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shows rate constants determined at [complex] = 1×10^{-3} M, and k_{lim} and K_b only for those systems giving reliable values. It should be pointed out, however, that because of the very limited concentration interval we could explore (for the low solubility of the complexes, see above), $K_b < 80$ M^{-1} could not be determined with confidence from the plots k_ψ vs [complex] because the curves may be fitted in some cases equally well as straight lines or saturation curves. Analysis of Table 2 allows the following observations: (a) complex **1**·Zn(II) is the most efficient system (compare entries 2 and 7); (b) methylation of the three phenolic hydroxyls of **1** almost completely suppresses the reactivity (compare entries 2 and 3); (c) complexes **5**·Zn(II) and **6**·Zn(II) bearing one phenolic −OH are only moderately reactive (entries 6 and 7). They differ considerably, however, in the tendency to form binuclear complexes. Dimerization of 1,2-diaminoethane-based complexes is, in fact, well known[20] and its adverse effect on their kinetic efficiency has been recently reported;[21] (d) TREN·Zn(II) alone is totally ineffective; (e) TREN·Zn(II) and three equivalents of phenol slightly accelerate the rate but at a much lower extent than **1**·Zn(II) (entry 9); (f) the reactivity decreases as the phenolic −OH is moved from the *meta* to the *para* position or the complex is made more rigid as in **4** (compare entries 2, 4, and 5); (g) significant binding of the substrate to the metal complexes is only present with **1**·Zn(II) and esters bearing a nitrogen donor atom (PNPIN, *p*-nitrophenyl nicotinate (PNPN), and *p*-nitrophenyl urocanoate (PNPU)). Surprisingly in the case of ester 4-carboxy-*p*-nitrophenyl benzoate (CPNPB), which features a free carboxylate suitable for binding to a Zn(II)·TREN derivative, as reported,[22] kinetic data do not indicate a K_b large enough to be appreciated.

The above observations rule out an involvement of a Zn(II)-bound hydroxyl of a deprotonated water molecule as the nucleophile in this process and point to one of the phenolic groups of the complex as the nucleophilic species. This is particularly evidenced by the lack of reactivity of the methylated complex **2**·Zn(II).

Competitive Inhibition. In order to establish whether binding of the substrates to the Zn(II) complexes is a key step in the mode of cleavage of the esters we ran kinetics in the presence of a potential inhibitor, i.e., a molecule able to coordinate to Zn(II) and hence to displace the substrate from the complex. Figure 3 shows the effect on the rate of release of *p*-nitrophenol by PNPIN and *p*-nitrophenyl benzoate (PNPB) with 1×10^{-3} M **1**·Zn(II) upon addition of increasing amounts of 4-methylpyridine. We clearly observe a decrease of the rate in the case of PNPIN. However, and quite unexpectedly, we observe *acceleration* in the case of PNPB. Control experiments in the presence of TREN·Zn(II) and phenol showed that 4-methylpyridine does not affect the rate of cleavage of PNPIN (Figure 3) and PNPB (data not shown). Analysis of k_ψ vs [4-methylpyridine] gives $K_i = 45 \pm 12$ M^{-1} and 48 ± 8 M^{-1} for the binding of this molecule to **1**·Zn(II) from the rate profile of PNPIN and PNPB, respectively. These K_i values are in fair agreement with the reported[23] value of 23 M^{-1} for its binding to Zn(II) alone when

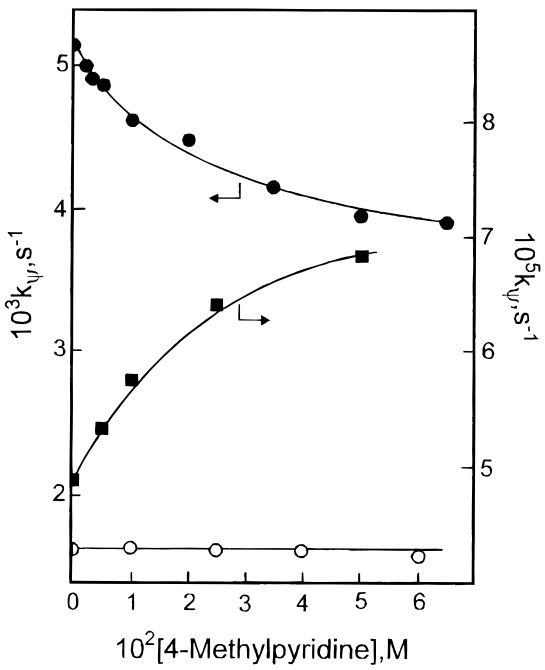

Figure 3. Effect of the addition of 4-methylpyridine on the rate constant of cleavage of PNPIN (●) and PNPB (■) by complex **1**·Zn(II). Conditions: [**1**·Zn(II)] = 1×10^{-3} M, 0.05 M HEPES buffer, pH 8.3, 25 °C. The white circles represent the effect observed in the absence of the complex.

allowance is made for hydrophobic contributions and the fact that Zn(II) is already coordinated to the TREN platform.[24] Furthermore the limiting value of k_ψ obtained for fully bound 4-methylpyridine in the case of PNPIN (3.5×10^{-3} s^{-1}) reveals that a considerable amount of the rate acceleration is not attributable to a mechanism that comprises binding of this substrate to **1**·Zn(II). With this ester the cleavage process associated with binding appears to be more efficient than that occurring without complexation to **1**·Zn(II) while the contrary holds true for PNPB. A similar behavior has been reported recently by Tee and Hoeven[25] to explain the reactivity of β-cyclodextrin with *p*-nitrophenyl acetate.

pH Dependence of the Rate of Cleavage of PNPIN. We have shown above that some of the phenolic groups in our complexes, notably **1**·Zn(II) and, to a lesser extent **5**·Zn(II), are particularly acidic. If those phenolates are the nucleophilic species in the cleavage process we would expect a first-order dependence on hydroxide ion of the rate of *p*-nitrophenol release up to a pH at which total ionization of the nucleophile has occurred. For this reason we run kinetics of cleavage of substrate PNPIN in the presence of 1×10^{-3} M **1**·Zn(II) and **5**·Zn(II) as well as in the absence of any metal complex. The data are shown in Figure 4. The basic hydrolysis of the ester exhibits the expected unity slope in the log k_ψ vs pH profile in the pH interval 8.6−11.0. Below pH 8.6 the slope decreases suggesting a change of mechanism in the hydrolytic process. In the presence of **1**·Zn(II) and **5**·Zn(II) the plots are linear with slope close to unity up to pH ∼ 8.3 for **1**·Zn(II) and ∼ 8.7 for **5**·Zn(II). Above these pHs the slopes of the plots decrease until at pH close to 11 the rate becomes only slightly faster than that

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(24) The first effect increases while the second decreases its binding constant to the metal ion.
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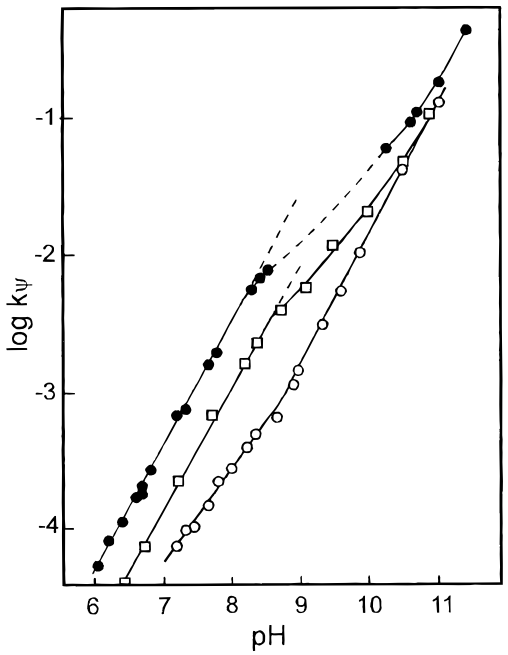

Figure 4. log k_ψ vs pH profiles for the cleavage of PNPIN at 25 °C. ○, No additives; □, with [**5**·Zn(II)] = 1×10^{-3} M; ●, with [**1**·Zn(II)] = 1×10^{-3} M. Buffers used were: MES, pH 6.0−6.7; HEPES, pH 6.7−7.7; EPPS, pH 7.7−8.5; CHES, pH 8.7−9.9; CAPS, pH 10.3−11.2.

observed for the OH^- catalyzed hydrolysis. Clearly above this pH the hydrolytic process driven by aqueous OH^- becomes dominant as this species outnumbers the phenolate ions present in solution. From the curvatures of the plots we may calculate the pK_a of the nucleophile responsible for the rate acceleration in the cleavage of PNPIN. Unfortunately, in the pH interval 8.5−10.2 solutions of **1**·Zn(II) were slightly turbid and the accuracy of the kinetic experiments in that pH interval (dashed region of upper curve of Figure 4) was less than desirable. Nevertheless, we estimate an apparent pK_a of 8.4 and 8.8 for **1**·Zn(II) and **5**·Zn(II), respectively. These values are quite close to the pK_a determined spectrophotometrically for the most acidic phenol of **1**·Zn(II) and for that of **5**·Zn(II) (see Table 1); we may conclude that one of the deprotonated phenols of the Zn(II) complex is the actual nucleophile.

Turnover. If this is the case, we should expect the formation of an acylated intermediate which may eventually hydrolyze, turning over the nucleophile. Under our kinetic conditions with substrate PNPIN we failed to detect such an intermediate. However, carrying out the reaction in an NMR tube using a 1:1 mixture CD_3CN/D_2O (pD of the D_2O component 8.3, unbuffered) we clearly detected the formation of the product of acylation (see Figure S2 of the Supporting Information). Such an intermediate hydrolyzes (at pH 8.3, 0.05 M EPPS buffer) with $k_\psi = 3.7 \times 10^{-4}$ s^{-1}, a rate substantially slower than k_{lim} for the transacylation process when the substrate is fully bound to **1**·Zn(II) (8.2×10^{-2} s^{-1}). Thus, our complex is not a real catalyst, in analogy with what has been observed for the cleavage of esters with cyclodextrins.[26] The slow rate of the hydrolytic cleavage provides indirect evidence that the Zn(II)-bound H_2O in this complex is a rather poor nucleophile at this pH and this is probably related to the fact that because its pK_a[16b] is >9, only a small fraction is deprotonated.

Mechanism. The apparently complicated set of kinetic results reported above may be simply explained by assuming that two distinct modes of action are involved: a) a bimolecular process (equation 1) and (b) a pseudointramolecular process occurring when the substrate is bound to the complex (eq 2). In the process depicted in eq (1) we assume that the "inhibitor" does not affect the reactivity of the metalloreceptor and the substrate no longer binds to the catalyst−inhibitor complex. These assumptions are reasonable if we consider that the effect on the acidity of the phenol is not due to direct coordination to the metal ion and the rather weak binding of the substrates when coordination to the Zn(II) is prevented.[27]

$$\text{substrate} + \text{Zn(II)-complex} \xrightarrow{k_2} \text{P} \qquad (1)$$

$$\text{substrate} + \text{Zn(II)-complex} \overset{K_b}{\rightleftharpoons} \text{substrate·Zn(II)-complex} \xrightarrow{k_b} \text{P} \qquad (2)$$

When all substrate is bound to the metal complex the only mechanism that operates is pseudointramolecular. Under this condition k_b is k_{lim} in Table 2. If binding of the substrate to the molecular receptor (the metal complex in our case) leads to a supramolecular complex in which the pseudointramolecular reaction between nucleophile (the phenolate ion) and ester is not easily attainable for geometric reasons without at least partial decomplexation of the substrate, it may well occur that the bimolecular process is more efficient than the pseudointramolecular one. This appears to be the case of PNPB, for which addition of the "inhibitor" 4-methylpyridine *increases* the observed rate constant (see Figure 3); in such a case the reaction outside the cavity of the complex (with no binding) is faster than that inside (when bound). On the contrary with substrate PNPIN the process indicated in eq (2) is more efficient than that of eq (1) and addition of 4-methylpyridine inhibits the reaction (see Figure 3) as expected. Coordination of the pyridine nitrogen to the metal center likely results in a geometry of binding more appropriate for the subsequent interaction of the ester group with the phenolate ion. Obviously this control of the geometry of binding is not attainable with PNPB. However, even with PNPIN there is a sizeable contribution of the purely bimolecular process (eq 1) as the rate acceleration is not completely suppressed by addition of the inhibitor. This dichotomy of behavior should not be surprising although it is often not considered in studies of catalysis through complexation.[28] The two competing mechanisms are sketched in Figure 5. Similar mechanisms appear to operate, for instance, in the cleavage of *p*-nitrophenyl acetate by β-cyclodextrin, as mentioned above.[25,29]

The "inhibition" experiments allow one to estimate, from the limiting value of k_ψ obtained for fully bound

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(27) Note that data by Tee and Bozzi (*J. Am. Chem. Soc.* **1990**, *112*, 7815) suggest that in the case of cyclodextrin there may be binding of the substrate and the inhibitor with formation of a ternary complex. However, one can hardly think that this system, in which the hydrophobic contribution to the binding is much lower than that of cyclodextrins, can really bind both guest molecules.

(28) This has been recently pointed out by Williams, too: Pirrincioglu, N.; Zaman, F.; Williams, A. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2561.

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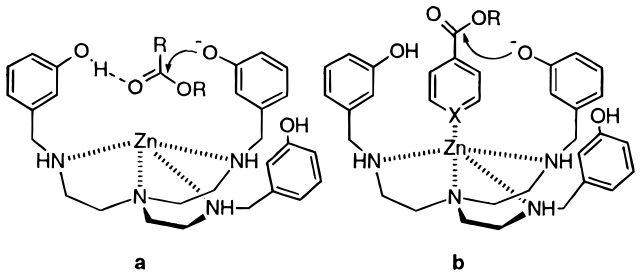

Figure 5. Bimolecular (a) and pseudo-intramolecular (b) modes of nuclephilic attack of the tripodal complex **1**·Zn(II) to the ester substrates.

4-methylpyridine, the second-order rate constants, k_2, for the bimolecular process for the two substrates PNPIN and PNPB: they are 9.07 and 1.99 × 10^{-1} M^{-1} s^{-1}, respectively, when correction for the partial formation of phenolate ion is made.[30] A Brønsted-type correlation for these two substrates using the three phenolic derivatives reported in Table 1 is illustrated in Figure 6. This plot can be used to compare the nucleophilicity of the phenolate anion of **1**·Zn(II) with that of a phenol of the same pK_a. Quite clearly the phenolate ion of our complex is more nucleophilic than predicted by its acidity (see open symbols in Figure 6) and this indicates that in the transacylation process with the metal complex there is some sort of assistance in the formation of the product. Assistance may be provided by hydrogen bonding with a Zn(II)-bound H_2O or with one of the other two phenolic groups of the metal complex. This latter hypothesis is supported by the fact that the log k_2 value obtained for **5**·Zn(II), devoid of any other free phenolic −OH, fits very nicely in the Brønsted-type plot with no evidence of extra nucleophilicity.

As for the pseudointramolecular process, the k_{lim} values of Table 2 evaluated for **1**·Zn(II) (entries 2, 11, and 13) allow one to compare the effectiveness of this mechanism with the three substrates PNPIN, PNPN, and PNPU. The best substrate appears to be PNPN with a ratio between the catalyzed transacylation process and the uncatalyzed hydrolysis (k_{lim}/k_o) of 60. This ratio goes down to 23 with PNPIN and 11 with PNPU. Inspection of molecular models suggests that the ester group in *meta* position with respect to the pyridine nitrogen coordinated to Zn(II) in the supramolecular complex is closer to the phenolate ion in the case of PNPN than in that of PNPIN, while in the case of PNPU the spacer between the imidazole and the ester is too extended for an effective interaction with the nucleophile. The effective molarities,[31] EM, i.e., the nominal concentration of a phenol of the same pK_a necessary to obtain a k_ψ matching the k_{lim} in the pseudo-intramolecular process, are 1.3 × 10^{-2} and 3.3 × 10^{-2} M for PNPIN and PNPN, respectively; rather modest if compared with those of real intramolecular processes.[31] However, similar values of EM have been found for the cleavage of simple esters by cyclodextrins[31] while much higher values have been found for substrates properly designed to match the rigid cavity of these molecular receptors and interact with their nucleophilic rim.[32]

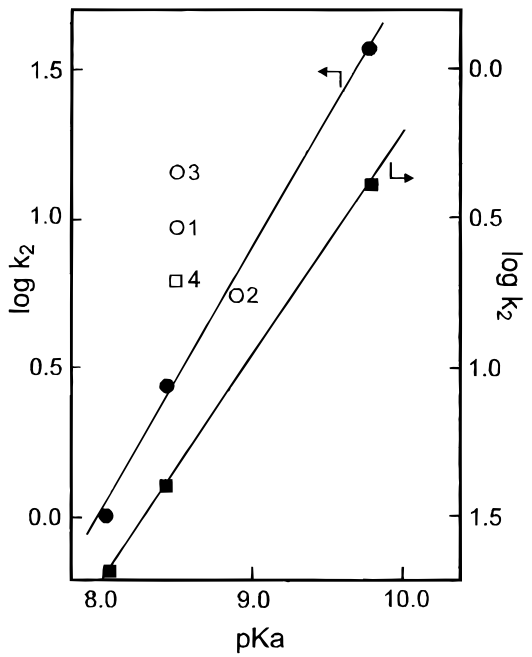

Figure 6. Brønsted-type plots for the cleavage of PNPIN (circles, $\beta = 0.85$) and PNPB (squares, $\beta = 0.75$) by phenols of different pK_a (filled symbols). Open symbols refer to second-order rate constants for the bimolecular process with metal complexes **1**·Zn(II), 1 and 4, and **5**·Zn(II), 2. Point 3 is the value obtained from $k_{lim} \times K_b$ for **1**·Zn(II). See the text for details.

We may get a further estimate of the overall efficiency of our catalyst, including the binding step, by determining $k_{lim} \times K_b$ which is the apparent second-order rate constant of the fully bound substrate. This value is 14.2 s^{-1} M^{-1} for **1**·Zn(II) and PNPIN when correction is introduced for the percentage of phenolate ion at the pH of the kinetic experiments. It is reported in Figure 6 so that a comparison can be made between the second-order rate constant of the pseudointramolecular and bimolecular processes involving **1**·Zn(II) (eqs 1 and 2) and the bimolecular process involving a phenol of the same pK_a. The pseudointramolecular process involving **1**·Zn(II) is roughly one order of magnitude more efficient than a bimolecular process involving a simple phenolate ion but only slightly better than the bimolecular process involving **1**·Zn(II) itself. This is consistent with our finding of competition between the two mechanisms under the conditions employed for our kinetic experiments.

Comparison with Cyclodextrins. For many aspects our metalloreceptors, **1**·Zn(II) in particular, show a mechanistic behavior very similar to that of cyclodextrins (CDs) as has already been pointed out several times in the discussion. Table 3 compares the kinetics and binding constants of **1**·Zn(II) with those of CDs.[33] Binding constants are higher for **1**·Zn(II). With these substrates the driving force for binding likely switches from purely hydrophobic (as for CDs) to metal coordination and, only to a lesser extent, hydrophobic with the metalloreceptor. The interaction with the metal center is important in our system as can be appreciated by comparing the binding constants of PNPIN and PNPN (380 and 300 M^{-1}, respectively) with that of PNPB (<80 M^{-1}). However, in CDs the binding occurs with insertion of the *p*-nitrophenyl moiety as highlighted by the very small difference in kinetic effects between PNPIN and

(30) Since one of the three phenols is considerably more acidic than the other two no correction was made for their contribution to the rate acceleration.

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(33) Daniele, A.; Fornasier, R.; Tonellato, U. unpublished results.

Table 3. Comparison of Binding and Rate Effects by 1·Zn(II), α- and *β*-Cyclodextrin[a] (α-, *β*-CD) on the Cleavage of Selected Carboxylate Esters[b]

substrate	catalyst	k_{lim}/k_o	K_b, M^{-1}
PNPIN	α-CD[c]	3.6	110
PNPIN	*β*-CD[c]	7.5	170
PNPIN	**1**·Zn(II)[d]	23	380
PNPN	α-CD[c]	3.7	90
PNPN	*β*-CD[c]	7.5	170
PNPN	**1**·Zn(II)[d]	60	300
PNPU	α-CD[e]	0.12	90
PNPU	*β*-CD[e]	5.8	210
PNPU	**1**·Zn(II)[d]	11	560
MNPIN[f]	α-CD[c]	110	90
MNPIN[f]	*β*-CD[c]	65	160

[a] Data from ref 33. [b] In aqueous solutions at 25 °C. [c] pH 9.5. [d] pH 8.3, this work. [e] pH 10.5. [f] *m*-Nitrophenyl isonicotinate.

PNPN paralleled by the very large difference between PNPIN and *m*-nitrophenyl isonicotinate (MNPIN). The selectivity of the cyclodextrins toward the geometry of the substrate appears to be much higher than that of **1**·Zn(II). The ratios between k_{lim}/k_o for the two substrates PNPIN and MNPIN are 30.5 and 8.7 for α- and *β*-CD, respectively, while that between PNPIN and PNPN with **1**·Zn(II) is only 2.6. This is consistent with the much higher rigidity of the conformation of CDs compared with that of our metalloreceptor.

Conclusion

We have synthesized a series of TREN-based ligands functionalized with different phenolic groups. Coordination of Zn(II) imparts a restricted conformation to the complexes and affects the acidity of the phenolic hydroxyls as a function of their proximity to the metal center. These complexes, **1**·Zn(II) in particular, act as metalloreceptors of *p*-nitrophenyl esters of carboxylic acids, at least of those which are able to coordinate to Zn(II) ion.

The **1**·Zn(II) complex, which we have studied in more detail, catalyzes the cleavage of the substrates with formation of the product of acylation of one of the phenolic hydroxyls followed by a slower hydrolysis. The transacylation process may occur via a pseudointramolecular attack within the supramolecular complex or via a simple bimolecular mechanism. Kinetic benefits are apparent from both mechanisms: in the bimolecular mechanism because of assistance by one of the phenolic hydroxyls in the transacylation, in the associative mechanism because of the lower entropic price to be paid for the nucleophilic attack. The mechanistic behavior of these metalloreceptors is reminiscent of that found with cyclodextrins although the latter show an higher selectivity which may be ascribed to their much lower conformational flexibility.

As pointed out recently by Schneider[34] "supramolecular catalytic systems lack the effectiveness but not the complexity of an enzymatic process". As a consequence they must be analyzed very carefully in order to dissect the different parameters that contribute to the observed rate enhancements. In the present case we have shown that a metal center *not directly involved in the process* studied may in fact influence many important parameters (e.g., conformation of the receptor, acidity of the nucleophilic groups, recognition of the substrate) that eventually result in the kinetic phenomena experimentally observed.

Experimental Section

General Considerations. ^{1}H-NMR spectra were recorded at 200 or 250 MHz using $(CH_3)_4Si$ as internal standard. Melting points are uncorrected. Elemental analyses were performed by the "Laboratorio di microanalisi" of the Department of Organic Chemistry in Padova. Kinetic traces were recorded on multicell instruments equipped with thermostated cell holders. Temperature control was ±0.1 °C.

Materials. $Zn(NO_3)_2$ was analytical grade commercial product. Metal ion stock solutions were titrated against EDTA following standard procedure.[35] Buffer solutions were prepared using Milli-Q water. The buffer components[36] were used as supplied by the manufacturers. Abbreviations used are as follows: MES, 4-morpholineethanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EPPS, 4-(2-hydroxyethyl)piperazine-4-(3-propanesulfonic acid); CHES, 2-(cyclohexylamino)ethanesulfonic acid; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid. PNPIN,[37] PNPN,[37] and PNPU[33] were synthesized according to reported procedures.

4-Carboxy-*p*-Nitrophenyl Benzoate (CPNPB). 4-Carboxybenzaldehyde (300 mg, 2 mmol) was suspended in benzene (10 mL) containing 8 mmol of $SOCl_2$ and the slurry refluxed until all of the solution became limpid (ca. 3 h). Benzene was then evaporated and the solid dissolved in CH_2Cl_2 (10 mL), to which *p*-nitrophenol (278 mg, 2 mmol) and Et_3N (0.28 mL) were added. After the mixture was stirred at room temperature for 2 h, TLC (CH_2Cl_2) revealed the complete formation of the ester. The solution was washed with water (3 × 10 mL) and CH_2Cl_2 evaporated to leave a white solid that was recrystallized from CH_2Cl_2/Et_2O. The pure CPNPB (400 mg) has a mp 190−191 °C. ^{1}H-NMR (200 MHz, $CDCl_3$), *δ*: 7.35, 8.27 (AA′BB′, 4H); 7.93, 8.24 (AA′BB′, 4H); 10.05 (s, 1H).

The above material (200 mg, 1.22 mmol) was dissolved in 5 mL of dry pyridine to which 220 mg of $KMnO_4$ was added and let to stir overnight at room temperature. After evaporation of the solvent at reduced pressure, the solid was treated with CH_2Cl_2, washed with 0.1 M HCl, and the organic layer filtered through a celite pad. The crude material obtained after evaporation of the solvent was chromatographed down a SiO_2 column ($CHCl_3$/EtOH, 95:5) to give 40 mg of pure CPNPB with mp 211 °C dec. ^{1}H-NMR (200 MHz, CD_3CN), *δ*: 7.43, 8.27 (AA′BB′, 4H); 8.12, 8.20 (AA′BB′, 4H); 8.9 (brs, 1H). Anal. Calcd for $C_{14}H_9NO_6$: C, 58.54; H, 3.16; N, 4.88. Found: C, 58.46; H, 3.21; N, 4.79.

General Procedure for the Synthesis of Compounds 1 and 3. TREN (3.42 mmol) and 3- or 4-hydroxybenzaldehyde (10.26 mmol) were dissolved in acetonitrile (20 mL) and vigorously stirred at rt. After 30 min a white solid started to formed. After 1.5 h the solid was filtered off, washed with acetonitrile, and dried in vacuo. ^{1}H-NMR analysis of the material revealed the disappearance of the signal of the proton of the aldehyde and appearance of a new signal (imine) at ca. 8.1 *δ*. The tris-imine derivative was then added to a solution of 500 mg (13.2 mmol) of $NaBH_4$ in ethanol and the reaction let to stir overnight at rt. Work up with concentrated HCl, filtration of the precipitate, and evaporation of the solvent afforded a crude material which was purified twice by elution through a Sephadex G-75 column using water as eluent. Lyophilization of the proper fractions gave the expected compounds as hydrochloride salts.

***N*,*N*,*N*-Tris{2-[((3-hydroxyphenyl)methyl)amino]-ethyl}amine−3.5HCl (1·3.5HCl)**: 556 mg; ^{1}H-NMR (250 MHz, D_2O) *δ* 2.66 (t, J = 6.3 Hz, 6H); 2.94 (t, J = 6.3 Hz, 6H), 3.99 (s, 6H), 6.7−6.9 (m, 9H); 7.18 (t, J = 7.75 Hz, 3H). Anal.

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Calcd for $C_{27}H_{36}O_3N_4 \cdot 3.5HCl \cdot 1.5H_2O$: C, 52.37; H, 6.92; N, 9.05; Cl, 20.04. Found: C, 52.20; H, 6.72; N, 8.93; Cl, 20.57.

***N,N,N*-Tris{2-[((4-hydroxyphenyl)methyl)amino]-ethyl}amine−3.5HCl (1·3.5HCl)**: 440 mg; ^{1}H-NMR (200 MHz, D_2O) δ 2.62 (t, J = 6 Hz, 6H), 2.88 (t, J = 6 Hz, 6H), 3.94 (s, 6H), 6.73, 6.77, 7.14, 7.17 (AA′BB′, 12H). Anal. Calcd for $C_{27}H_{36}O_3N_4 \cdot 3.5HCl \cdot 1.5H_2O$: C, 52.37; H, 6.92; N, 9.05; Cl, 20.04. Found: C, 51.93; H, 6.70; N, 8.89; Cl, 20.26.

***N,N,N*-Tris{2-[((3-methoxyphenyl)methyl)amino]-ethyl}amine−3HCl (2·3HCl).** The preparation of compound **2** differs from the above general procedure for the workup after reduction and successive purification. Thus, the reduction mixture was treated with H_2O (10 mL), extracted with CH_2Cl_2 (3 × 40 mL), and the organic solution dried over Na_2SO_4. The crude material obtained after evaporation of CH_2Cl_2 was chromatographed through a column of SiO_2 (CH_2Cl_2/MeOH/NH_3(aq), 90:9:1) to give 1.3 g of pure material. This was then converted in the hydrochloride salt by treatment of an Et_2O solution with Dioxane saturated with dry HCl. The white salt that precipitates was collected by filtration. ^{1}H-NMR (250 MHz, D_2O), δ: 2.71 (t, J = 7.3 Hz, 6H); 2.99 (t, J = 7.3, 6H); 3.70 (s, 9H); 4.06 (s, 6H), 6.85−7.0 (m, 9H); 7.29 (t, J = 9.5 Hz, 3H). Anal. Calcd for $C_{30}H_{42}N_4O_3 \cdot 3HCl$: C, 58.49; H, 7.36; N, 9.09. Found: C, 58.38; H, 7.31; N, 8.95.

***N,N,N*-Tris{2-(6-hydroxyisoindolinyl)ethyl}amine−3.5HCl (4·3.5HCl).** 5-Acetoxyphthalic anhydride (2.5 g, 11.25 mmol) was dissolved in 30 mL of xylene with 510 mg (3.47 mmol) of TREN and the mixture refluxed for 1 h. After the mixture cooled, a dark oily material separated on the bottom of the flask. This was decanted and the residual solvent evaporated at reduced pressure. The oily material obtained was purified by column chromatography (SiO_2, $CHCl_3$/EtOAc, 9:1) to give 480 mg (18.2% yield) of trisphthalimido derivative: ^{1}H-NMR (200 MHz, $CDCl_3$), δ: 2.33 (s, 9H), 3.02 (brs, 6H), 3.82 (brs, 6H), 7.37 (d, J = 7.5 Hz, 3H), 7.47 (s, 3H), 7.68 (d, J = 7.5 Hz, 3H).

This material was then suspended in 20 mL of dry tetrahydrofuran (THF) and, to the slurry, 10 mL of a 1 M solution of $LiAlH_4$ in THF was added and the reaction mixture refluxed overnight over N_2. After the mixtured cooled, it was worked up with 2 mL of 1 M NaOH (CAUTION!), evaporated of the solvent, and the solid continuosly extracted with ethanol. Evaporation of the solvent gave the expected compound contaminated with some impurities. These were removed by treating the crude with a pH 1 aqueous solution followed by continuous extraction with $CHCl_3$. After evaporation of the water the residue was chromatographed twice over a Sephadex G15 column and, subsequently, over a Sephadex G10 column using water as eluent. The final amount of pure material collected was 150 mg. ^{1}H-NMR (200 MHz, D_2O), δ: 3.01 (brt, J = 6.5 Hz, 6H), 3.53 (brt, J = 6.5 Hz, 6H), 4.65 (s, 12H), 6.7−6.85 (m, 6H), 7.13 (d, J = 7 Hz, 3H). Anal. Calcd for $C_{30}H_{36}O_3N_4 \cdot 3.5HCl \cdot 0.5H_2O$: C, 56.54; H, 6.41; N, 8.79; Cl, 19.47. Found: C, 57.02; H, 6.51; N, 8.51; Cl, 19.56.

***N*-{2-[((3-Hydroxyphenyl)methyl)amino]ethyl}-*N,N*-{2,-[((3-methoxyphenyl)methyl)amino]ethyl}amine (5).** TREN (1.46 g, 10 mmol) was dissolved in 150 mL of dry CH_2Cl_2, the flask cooled to −79 °C and di-*tert*-butyldicarbonate (440 mg, 2 mmol) dissolved in 50 mL of CH_2Cl_2 was added dropwise in 30 min. When the addition was complete, the solution was let to warm to rt and stirred for 12 h more. The crude material obtained after evaporation of the solvent was purified by chromatography (SiO_2, $CHCl_3$/MeOH/NH_3(aq), 10:4:1). The monoprotected product (R_f 0.33, 430 mg) was an oily material used without further purification in the subsequent step. ^{1}H-NMR (250 MHz, CD_3OD), δ: 1.48 (s, 9H), 2.56 (m, 6H), 2.73 (t, 4H), 3.16 (t, 2H).

The above compound was dissolved in 60 mL of acetonitrile, 4 Å molecular sieves (10 g), and 3-methoxybenzaldehyde (2 equivalents) were then added and the slurry stirred overnight. Filtration and evaporation of the solvent gave the imine derivative in quantitative yield. ^{1}H-NMR (250 MHz, CD_3OD), δ: 1.49 (s, 9H), 2.68 (t, 2H), 3.65 (t, 4H), 3.8 (s, 6H), 5.25 (brm, 1H), 6.85 (d, 2H), 7.2 (d, 2H), 7.25 (t, 2H), 8.23 (s, 2H).

The above imine was added to a solution of $NaBH_4$ (186 mg) in 50 mL of ethanol and let to react overnight. Water (30 mL) was added to the reaction mixture, the ethanol evaporated, and the aqueous solution extracted with $CHCl_3$ (30 × 3 mL). Evaporation of the organic layer gave a crude that was purified by column chromatography (SiO_2, $CHCl_3$/MeOH, 10:1 then $CHCl_3$/MeOH/NH_3(aq), 10:4:1) to give 800 mg of pure material. ^{1}H-NMR (250 MHz, $CDCl_3$), δ: 1.42 (s, 9H), 2.59 (m, 10H), 3.14 (brm, 2H), 3.74 (s, 4H), 3.79 (s, 6H), 5.73 (brm, 1H), 6.77 (d, 2H), 6.86 (s, 2H), 6.88 (d, 2H), 7.21 (t, 2H).

The monoprotected bis(methoxy) derivative was then dissolved in CH_2Cl_2 (4 mL) and treated with 2 mL of trifluoroacetic acid. After 1 h the solvent was evaporated under reduced pressure, the crude treated with 20 mL of a 10% aqueous solution of Na_2CO_3 and extracted with $CHCl_3$ (20 × 3 mL). After the material was dried and the solvent was evaporated, 580 mg of deprotected compound was obtained. ^{1}H-NMR (250 MHz, $CDCl_3$), δ: 2.48 (t, 2H), 2.7 (m, 10H), 3.74 (s + m, 10H), 6.85 (m, 6H), 7.23 (t, 2H).

This compound was treated, as above for the Boc-monoprotected TREN, with 3-hydroxybenzaldehyde to give the imine derivative which was analogously reduced with $NaBH_4$ to the crude final product. This was purified by elution through a SiO_2 column ($CHCl_3$/MeOH/NH_3(aq), 100:20:0.5) to give 450 mg of pure **5**. ^{1}H-NMR (250 MHz, $CDCl_3$), δ: 2.50 (m, 8H), 2.72 (t, J = 6 Hz, 4H), 3.70 (s, 4H), 3.74 (s, 6H), 3.79 (s, 2H), 6.61 (d, J = 7.5 Hz, 1H), 6.68 (d, J = 8 Hz, 1H), 6.77 (dd, J = 8.2 Hz and 2.5 Hz, 2H), 6.88 (m, 4H), 6.99 (s, 1H), 7.14 (t, J = 8 Hz, 1H), 7.20 (t, J = 8.2 Hz, 2H). ^{13}C-NMR (62.5 MHz, $CDCl_3$), δ: 45.05, 46.05, 51.65, 52.66, 53.33, 54.15, 55.16, 112.9, 113.67, 114.01, 114.53, 118.61, 120.75, 129.5, 129.74, 139.91, 140.11, 158.22, 159.75. FAB-MS (MNBA matrix): m/z calcd for $C_{29}H_{40}N_4O_3$, 492; found, 493 ($M + H^+$).

***N,N*-Dimethyl-*N′*-[(3-hydroxyphenyl)methyl]ethane-1,2-diamine−2HCl (6·2HCl).** 3-Hydroxybenzaldehyde (1.18 g, 9.66 mmol) and 1,2-diamino-*N,N*-dimethylethane (10 mmol) were dissolved in 30 mL of CH_2Cl_2 containing freshly activated 4 Å molecular sieves and let to stir overnight. Evaporation of the solvent gave an oily material that was dissolved in a 40-mL solution of $NaBH_4$ (730 mg, 19 mmol) in ethanol. After the solution was stirred for 12 h, it was made acidic by adding concentrated HCl and, subsequently, the solvent was stripped off. Recrystallization from ethanol gave 1.1 g of pure **6**·2HCl with mp 188−189 °C. ^{1}H-NMR (250 MHz, CD_3OD), δ: 3.02 (s, 6H), 3.35 (s, 4H), 4.26 (s, 2H), 6.91 (m, 1H), 7.03 (m, 2H), 7.33 (t, J = 8 Hz, 1H). Anal. Calcd for $C_{11}H_{12}N_2O \cdot 2HCl$: C, 49.45; H, 7.54; N, 10.48. Found: C, 49.34; H, 7.62; N, 10.25.

Kinetics. Kinetics were started by addition of 20 μL of a 2 × 10^{-3} M solution of the substrate in acetonitrile to 2 mL of buffered solution containing the metal complexes in the appropriate concentration and followed at 400 nm (pH ≥ 6.5) or 317 nm (pH < 6.5) by recording the increase of absorbance due to *p*-nitrophenolate or *p*-nitrophenol release. Rate constants were determined by nonlinear fitting of the absorbance vs time data.[38] Reproducibility of independent runs was within 2.5%.

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Supporting Information Available: Figures S1 and S2 reporting potentiometric titrations of complexes **1**·Zn(II), **2**·Zn(II), and **5**·Zn(II) and time course of the transacylation experiment of PNPI with **1**·Zn(II) (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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