Highly Efficient Phosphate Diester Transesterification by a Calix[4]arene-Based Dinuclear Zinc(II) Catalyst

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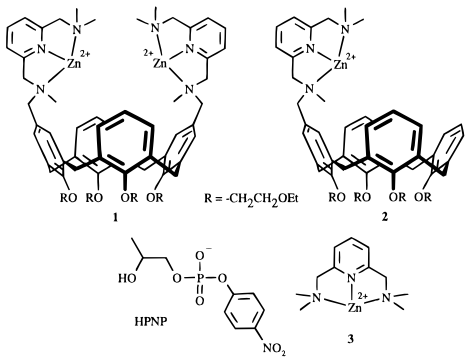
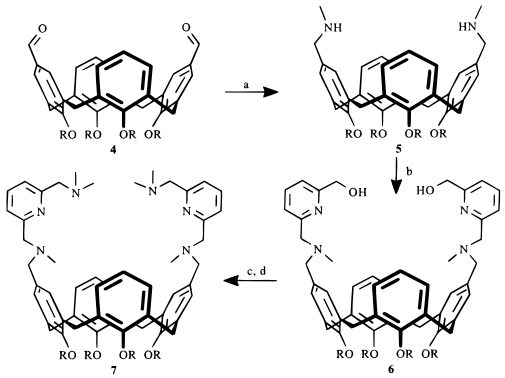
Various enzymes,[1] including P1 nuclease, DNA polymerase I, phospholipase C, and alkaline phosphatase, use the synergic action of two metal centers for the hydrolytic cleavage of phosphate ester bonds. Several research groups have taken the challenge to mimic this process with relatively simple model compounds in which two metal centers have been linked by a spacer group (*i.e.*, Co(III) by Chin[2,3] and Czarnik,[4,5] Cu(II) by Chin,[6,7] Zn(II) by Breslow,[8] Kimura,[9] and Komiyama,[10] or lanthanide(III) by Schneider[11]). Although occasionally substantial rate accelerations for phosphate ester hydrolysis have been observed, low substrate binding and lack of turnover are general problems encountered with simple model systems. Calix[4]arenes[12−14] have been recognized for many years as very suitable building blocks for the construction of multifunctional enzyme models,[12,15,16] because of the possibility of spatial preorganization of catalytic groups and substrate binding sites. However, although many examples of calix[4]arene-based supramolecular *receptors* are known,[13,14] the only example of a calix[4]arene-based enzyme model[17] has been reported by Mandolini *et al.*,[16] who showed that a calix[4]arene modified at the lower rim with a crown ether Ba(II) complex exhibits transacylase activity.

In this paper we present calix[4]arene **1**, functionalized with two Zn(II) centers at the distal positions of the upper rim, as the first example of a dinuclear complex which shows both strong binding to a phosphate diester substrate and high catalytic activity. The presence of 0.48 mM of **1** induces a 23000-fold rate enhancement in the catalytic cyclization of the RNA model substrate 2-(hydroxypropyl)-*p*-nitrophenyl phosphate (HPNP,[18] pH 7, 25 °C). This is the largest catalytic rate acceleration reported for nuclease mimics using this substrate. Comparison of **1** with monofunctionalized calix[4]arene **2** and reference pyridine complex **3** shows that the high catalytic activity of **1** can be attributed to *a favorable contribution of the calix[4]-arene moiety* in substrate binding and catalytic synergic action of the two Zn(II) centers.



Scheme 1. Synthesis of a Calix[4]arene-Based Dimeric Ligand (R = CH_2CH_2OEt)[a]



[a] Key: 33% $MeNH_2$ in EtOH, H_2, 10% Pd/C, 71%; (b) 2-(bromomethyl)-6-(hydroxymethyl)pyridine,[20] K_2CO_3, CH_3CN, 64%; (c) $SOCl_2$, CH_2Cl_2, 100%; (d) $Me_2NH{\cdot}HCl$, K_2CO_3, CH_3CN, 65%.

The calix[4]arene-based dimeric ligand **7** was prepared stepwise from diformyltetrakis(ethoxyethyl)calix[4]arene **4**[19] and 2-(bromomethyl)-6-(hydroxymethyl)pyridine[20] according to Scheme 1. The monomeric ligand was prepared analogously, starting from monoformyltetrakis(ethoxyethyl)calix[4]arene,[19] and the ligand of reference complex **3** was obtained by reaction of bis(bromomethyl)pyridine[20] with dimethylamine. The corresponding Zn(II) complexes (**1**−**3**) were generated *in situ* at 0.48 mM in acetonitrile/20 mM HEPES buffer 1:1 (v/v) at 25 °C, the reaction conditions for the catalysis experiments.[21] The association constants for Zn(II) complexation were determined by UV spectrometry and are *ca.* 1×10^5 M^{-1}, which implies that under the reaction conditions 85% of the Zn(II) is bound to the (aminomethyl)pyridine ligands.[22]

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(17) *p*-Sulfonatocalix[6]arenes (ref 17a) and *p*-(carboxyethyl)calix[*n*]-arenes ($n = 5-8$, ref 17b) have shown to be catalytically active in the acid-catalyzed hydration of 1-benzyl-1,4-dihydronicotinamide: (a) Shinkai, S.; Mori, S.; Koreishi, H.; Tsubaki, T.; Manabe, O. *J. Am. Chem. Soc.* **1986**, *108*, 2409. (b) Gutsche, C. D.; Alam, I. *Tetrahedron* **1988**, *44*, 4689.
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(21) In a typical kinetic experiment, the ligand **7** (20 μL, 50 mM) and the metal perchlorate (40 μL, 50 mM) were added to 2 mL of acetonitrile/20 mM HEPES 1:1 (v/v) at 25 °C. After a couple of minutes equilibration time, HPNP[18] (4 μL, 100 mM) was injected into the cuvette. The observed first-order rate constant k_{obsd} (s^{-1}) was calculated with the extinction coefficient of *p*-nitrophenolate at 400 nm by the initial slope method (<5% conversion).
(22) All solutions remained clear during the time of the kinetic experiments. In the absence of ligand, precipitation of polymeric Zn(II) hydroxide took place.

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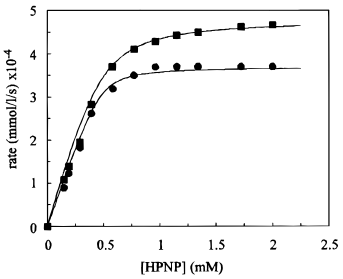


Figure 1. Dependency of the transesterification rate catalyzed by **1**, on HPNP concentration at pH 7.0 (●) and 7.4 (■) ([**1**] = 0.48 mM, acetonitrile/20 mM HEPES 1:1 (v/v), 25 °C).

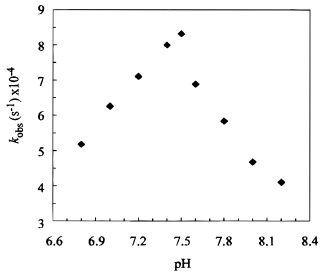


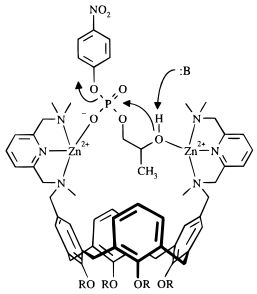
Figure 2. pH vs rate profile for transesterification of HPNP (0.19 mM) catalyzed by **1** (0.48 mM) in acetonitrile/20 mM HEPES 1:1 (v/v) at 25 °C.

The catalytic activities of the Zn(II) complexes toward transesterification of HPNP were studied in the pH range of 6.8−8.2 of the aqueous buffer portion of the reaction mixture.[21] In the absence of metallocatalysts, HPNP reacts extremely slowly; the initial rate at pH 7.0 indicates a half-life for the *p*-nitrophenol release of approximately 300 days ($k_{obsd} = 2.7 \times 10^{-8}$ s^{-1}). However, the reaction rate increases dramatically (23 000 times) upon the addition of 0.48 mM of **1** to the solution (half-life 18 min, $k_{obsd} = 6.3 \times 10^{-4}$ s^{-1}).[23] In a separate experiment using a 4-fold excess of HPNP, **1** shows turnover without significant loss of activity during completion of the reaction, which demonstrates that no product inhibition occurs. The catalytic activity of the mononuclear calix[4]arene Zn(II) complex **2** is 50 times lower than that of **1**, emphasizing the importance of synergism of the two Zn(II) centers in **1**. The fact that mononuclear calix[4]arene complex **2** is still 6 times more active than reference complex **3** indicates that the calix[4]arene moiety contributes to the catalysis, probably by enhancing the formation of the catalyst−substrate complex.

Assuming product formation by a two-step pathway *viz.*, (i) rapid pre-equilibration of the catalyst−substrate complex (K_{assn}) followed by (ii) rate-determining conversion of the substrate within the complex (k_{cat}), saturation kinetics must be observed when binding of the substrate to the catalyst is sufficiently high. Measurement of the rate as a function of substrate concentration (Figure 1) reveals that catalyst **1** has a very high affinity for the substrate. At pH 7.0, already 80% of the substrate is bound to catalyst **1** at equimolar concentrations (0.48 mM), and saturation is readily reached at higher substrate concentration. Fitting of the experimental data according to steady state pseudo-first-order kinetics gave values for K_{assn} of 5.5×10^4 M^{-1} and for k_{cat} of 7.7×10^{-4} s^{-1}. The unusually high binding constant may be the result of the synergic action and the directional preorganization of the two metallo binding sites[24] on the calix[4]arene as well as the capacity to adjust the receptor site by low-energy conformational changes of the calix[4]arene moiety.[19,25−27] As is shown in Figure 1, increase of the pH to 7.4 results in higher reaction rates and a higher substrate concentration before saturation is reached ($K_{assn} = 1.7 \times 10^4$ M^{-1}, $k_{cat} = 10 \times 10^{-4}$ s^{-1}).

Further analysis of the catalytic activity of **1** yields a bell-shaped dependency of the pH (Figure 2). This behavior[6,8] can be explained by opposing pH effects on catalyst−substrate complex formation (K_{assn}) and conversion of the substrate within this complex (k_{cat}). Binding of the substrate to the Zn(II) centers requires the displacement of either a Zn(II) bound water molecule or a more tightly bound hydroxide ion. At higher pH, the fraction of catalyst with Zn(II) bound hydroxide ions is increased and consequently K_{assn} decreases. On the other hand, the effective nucleophile concentration increases due to deprotonation of Zn(II) bound water molecules (or substrate hydroxyl group), and this increases k_{cat}.[28,29] The optimum rate at pH 7.5 reveals the relatively low pK_a of water coordinated to Zn(II) in **1**,[30] which may be due to the close proximity of the hydrophobic calix[4]arene moiety and the presence of 50% acetonitrile in the reaction mixture. Coates *et al.* already showed that the pK_a of water coordinated to Zn(II) and Cu(II) is lowered in a hydrophobic environment.[31]

Calix[4]arene **1** is not catalytically active in the hydrolysis of diethyl *p*-nitrophenyl phosphate, ethyl *p*-nitrophenyl phosphate, or *p*-nitrophenyl phosphate, which shows that the *β*-hydroxyl group of HPNP is essential in the catalytic process. Therefore, the most likely mode of catalysis is a bifunctional mechanism in which one of the Zn(II) centers serves as Lewis acid activator of the phosphate group and the other is involved in activation of the nucleophilic *β*-hydroxyl group giving rise to relatively fast intramolecular reaction.[8]



In conclusion, the nuclease mimic **1**, possessing two Zn(II) centers at the upper rim of a flexible calix[4]arene, exhibits fast and strong binding of HPNP, followed by efficient intramolecular transesterification, overall resulting in fast catalytic turnover under very mild conditions.

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(23) The catalytic activity of different calix[4]arene-based dinuclear metal complexes was found to follow the order $Zn^{2+} \gg Co^{2+} > Ni^{2+} \approx Cu^{2+}$.

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