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General Base–Guanidinium Cooperation in Bifunctional Artificial Phosphodiesterases

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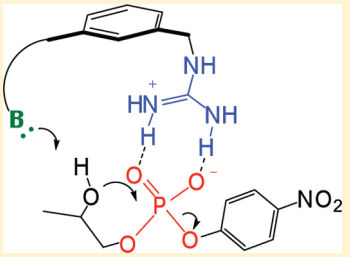
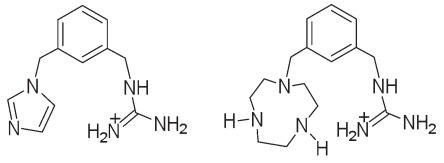
Supporting Information

ABSTRACT: Artificial phosphodiesterases that combine a guanidinium unit with a general base connected by a *m*-xylylene linker catalyze the transesterification of the RNA model compound 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNP). The bifunctional catalysts presented in this work show varying extents of cooperation between catalytic units and a rate enhancement of 4×10^4 in the most favorable case.



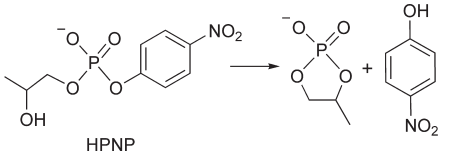
In the past two decades, simple, nonpeptidic molecules that mimic structural and functional aspects of natural enzymes have received considerable attention.[1−3] The extreme reluctance of phosphodiester bonds to undergo hydrolysis[4] has challenged many research groups to design and synthesize artificial catalysts capable of cleaving DNA, RNA, and their model compounds.[5−15] These studies are interesting not only in their own right but also in the prospect of health-related goals, such as the antisense therapy based on the coupling of RNA hydrolytic agents to antisense DNA.[16]



The guanidinium unit, which plays a key role in enzymes such as staphylococcal nuclease,[17] has been extensively used as an activating and/or anchoring group in the design of hydrolytic catalysts.[9,18−28] These catalysts contain one or more guanidinium units, or a guanidinium unit in conjunction with another active unit such as a metal center,[23−25] a hydroxyl group,[26] or a free base in solution.[27,28]

As a first part of a program devoted to the investigation of guanidinium units in the development of artificial phosphodiesterases, we report here a kinetic investigation of the cleavage of the RNA model compound HPNP (eq 1) in the presence of $\mathbf{1}H^+$ and of the Cu^{II} and Zn^{II} complexes of $\mathbf{2}H^+$. The use of a *m*-xylylene spacer as a convenient platform for the connection of catalytic groups is well precedented.[21,29−31] Compounds **1** and **2** were synthesized as described in Scheme 1 and Scheme 2, respectively.

Catalytic reactions were carried out in DMSO/H_2O 4:1 (v/v), hereafter referred to as 80% DMSO, which is a solvent mixture highly suitable for potentiometric pK_a determination[32] and for investigation of the kinetics of hydrolytic reactions of phosphodiesters.[22,33,34] Furthermore, the interaction of guanidinium with phosphate via a two-point hydrogen bonding motif is stronger in DMSO/water mixtures than in pure water.[35,36] As a consequence, a catalyst featuring a guanidinium unit as an anchoring and/or activating group is potentially favored in a less aqueous solvent mixture.



(1)

The imidazole in catalyst $\mathbf{1}H^+$ is expected to act as a general base, whereas intramolecular attack of the HPNP activated hydroxyl on phosphate should benefit from electrophilic activation promoted by the neighboring guanidinium. As for the metal-based catalysts, there are strong indications that the cleavage of HPNP and related compounds catalyzed by metal complexes in water involves reversible deprotonation of the substrate hydroxyl, followed by rate-limiting intramolecular nucleophilic attack.[37−39] However, Sánchez-Lombardo and Yatsimirsky[40] convincingly argued that in 80% DMSO the general base-assisted intramolecular cyclization of HPNP is more favorable because of the extremely low acidity of the substrate hydroxyl in this medium. Although a specific base catalytic mechanism cannot be ruled out with any certainty, we assume that the hydroxo forms of Cu^{II} and Zn^{II} complexes of $\mathbf{2}H^+$ will act as general bases, in keeping with Sánchez-Lombardo and Yatsimirsky's conclusions.

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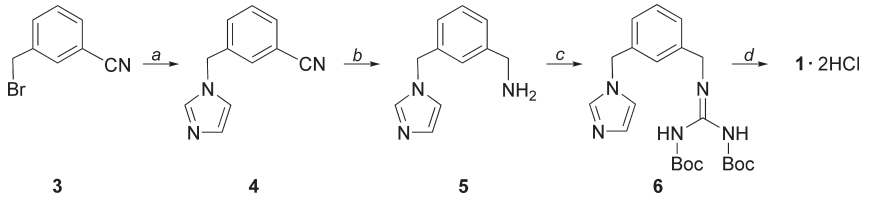
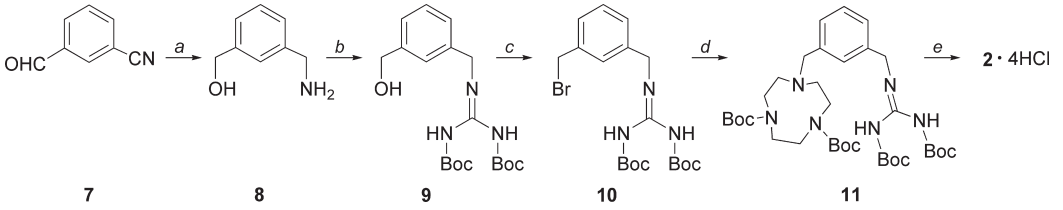
Scheme 1. Synthesis of 1[a]



[a] *a*: Imidazole, K_2CO_3; CH_3CN, reflux. *b*: H_2, Pd−C; MeOH, rt. *c*: *N*,*N*′-Bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine; CH_2Cl_2, rt. *d*: 0.1 M aqueous HCl/dioxane 1:1, rt.

Scheme 2. Synthesis of 2[a]



[a] *a*: $LiAlH_4$; THF, reflux. *b*: *N*,*N*′-Bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine; CH_2Cl_2, rt. *c*: PBr_3, 2,6-di-*tert*-butylpyridine; toluene, rt. *d*: *N*,*N*′-Bis(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane, 2,6-di-*tert*-butylpyridine; CH_3CN, rt. *e*: 0.5 M aqueous HCl/dioxane 1:1, rt.

Table 1. Pseudo-First-Order Rate Constants[a] and Acceleration Factors Relative to Background for the HPNP Cleavage in 80% DMSO in the Presence of Additives

conditions	entry	additives	k_{obs} (s^{-1})	k_{obs}/k_{bkg}[b]
pH 7.0, 50 °C[c,d]	1	guanidine·HCl	4.8×10^{-8}	0.94
	2	**12**	8.2×10^{-8}	1.6
	3	$\mathbf{1}H^+$	5.2×10^{-7}	10
pH 9.0, 25 °C[e]	4	guanidine·HCl	1.4×10^{-8}[d]	1.1
	5	TACN + $CuCl_2$	1.3×10^{-5}	980
	6	$2H^+$ + $CuCl_2$	5.1×10^{-4}	39000
pH 9.8, 25 °C[e]	7	guanidine·HCl	8.1×10^{-8}[d]	1.0
	8	TACN + $ZnCl_2$	1.8×10^{-4}	2300
	9	$2H^+$ + $ZnCl_2$	1.0×10^{-3}	13000

[a] k_{obs} calculated as $v_o/[HPNP]$, where v_o is the initial rate of *p*-nitrophenol liberation; [additive] = 5.0 mM; [HPNP] = 0.20 mM, unless otherwise stated. [b] $k_{bkg} = 5.1 \times 10^{-8}$ s^{-1} at pH 7.0, 50 °C; $k_{bkg} = 1.3 \times 10^{-8}$ s^{-1} at pH 9.0, 25 °C; $k_{bkg} = 7.9 \times 10^{-8}$ s^{-1} at pH 9.8, 25 °C. [c] 0.10 M HEPES buffer. [d] [HPNP] = 3.0 mM. [e] 0.10 M *N*,*N*′-diisopropyl-*N*-ethanolamine buffer.

In 80% DMSO, the autoprotolysis of water is strongly suppressed (pK_w = 18.4 at 25 °C),[41] but the pK_a values of nitrogen bases are hardly affected.[42] For guanidinium chloride, we measured a pK_a value of 13.7 at 25 °C (Figure 2S, Supporting Information), very close to the value of 13.6 at the same temperature in water.[43] Titration of **1**•2HCl revealed two pK_a values of 4.8[44] and 13.4 (Figure 3S, Supporting Information), which were assigned to the imidazole and guanidine groups in the given order. The influence of $\mathbf{1}H^+$ on rates of transesterification of HPNP was investigated at pH 7.0, 50 °C. At this pH, the imidazole unit is >99% in the neutral form, whereas the guanidine unit is fully protonated. Rate data listed in Table 1 show an insignificant influence of guanidinium (entry 1) and a very modest rate enhancement brought about by **12** (entry 2) relative to background hydrolysis (k_{bkg}). The superiority of the bifunctional

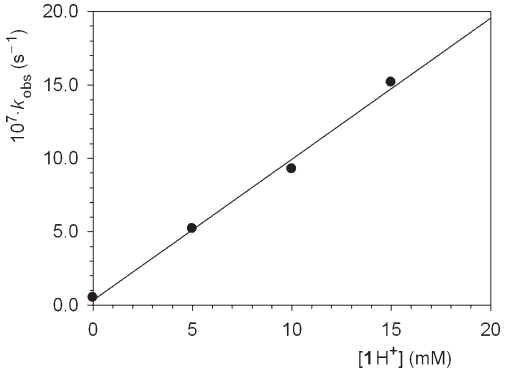
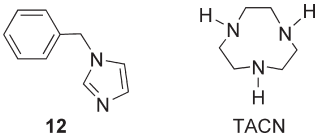


Figure 1. Transesterification of 3.0 mM HPNP catalyzed by $\mathbf{1}H^+$ in 80% DMSO, pH 7.0, 50 °C. Pseudo-first-order rate constant vs catalyst concentration.

catalyst $\mathbf{1}H^+$ (entry 3) compared with monofunctional models reveals a modest, but clearly detectable, degree of synergism between functional groups. These data are consistent with the expected mechanism in which the imidazole unit acts as a general base and the negative charge developing on the altered phosphate in the transition state is stabilized by the neighboring guanidinium, possibly via a two-point hydrogen bonding motif. Figure 1 shows that k_{obs} values for HPNP transesterification exhibit a strictly linear dependence on catalyst



concentration, which implies that *K* in eq 2 is too small to affect the kinetics ($K < 10$ M^{-1}). In other words, there is significant

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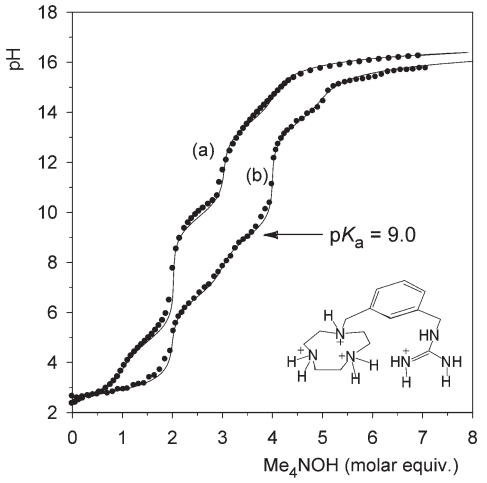

Figure 2. Titration of 2.0 mM **2**·4HCl with Me_4NOH in 80% DMSO in the absence (*a*) and presence (*b*) of 1 equiv of Cu^{II}. Data points are experimental, and the lines are calculated.

binding of HPNP to $\mathbf{1H}^+$ in the transition state, but not in the reactant state.

$$\text{cat} + \text{HPNP} \overset{K}{\rightleftarrows} \text{cat}\cdot\text{HPNP} \rightarrow \text{products} \qquad (2)$$

Titration of **2**·4HCl (Figure 2, curve *a*) reveals, as expected, four titratable protons (pK_a values: <2, 4.9, 9.8, 13.1). The largest pK_a value belongs to the guanidinium moiety, whereas the other three values compare well with the pK_a values of TACN·3HCl (<2, 6.0, 10.6; Figure 4S, Supporting Information), when allowance is made for the acidity enhancing effect of the positively charged guanidinium. Addition of 1 mol equiv of $CuCl_2$ caused a deep modification of the titration curve (Figure 2, curve *b*). The number of titratable protons was raised to five, with pK_a values of <2, <2, 6.5, 9.0, and 13.4. The three most acidic protons were assigned to the fully protonated triazacyclononane unit. The increased apparent acidity is a result of the strong binding of Cu^{II} to the triazacyclononane ligand (log *K* 8.6), whereas the acidity of the guanidinium moiety is affected to a moderate extent. The pK_a value of 9.0 was attributed to a Cu^{II}-bound water molecule that turns out to be 10.4 pK_a units more acidic than bulk water in 80% DMSO. Quite unexpectedly, the metal-bound water molecule in the complex between Cu^{II} and TACN turned out to be more acidic (pK_a = 8.4; Figure 6S, Supporting Information) than in the corresponding complex with $\mathbf{2H}^+$, but the reason for this enhanced acidity is unclear.

Catalytic experiments in the presence of equimolar amounts (5.0 mM) of $\mathbf{2H}^+$ and $CuCl_2$ were carried out at pH 9.0. At the given pH, the triazacyclononane ligand is fully bound to Cu^{II}; the guanidine unit is still fully protonated; and the metal-bound water molecule is 50% deprotonated.

Control experiments (Table 1) confirmed the insignificant influence of guanidinium (entry 4) but showed that the Cu^{II} complex of TACN, that is 80% in the hydroxo form at pH 9.0, strongly enhances the transesterification rate of HPNP compared with background (entry 5). Still larger was the rate enhancement brought about by the Cu^{II} complex of the bifunctional catalyst $\mathbf{2H}^+$ (entry 6). Its catalytic efficiency is 40 times higher than that of the monofunctional control[45] and indicates a large extent of cooperation of the hydroxo complex of the ligated Cu^{II} cation

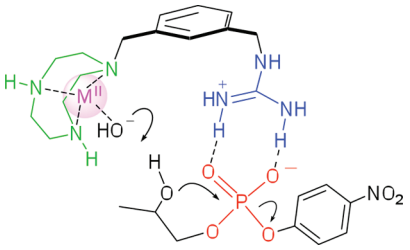

Figure 3. Suggested mechanism of HPNP cleavage catalyzed by the metal complexes of $\mathbf{2H}^+$ (M^{II} = Cu^{II}, Zn^{II}).

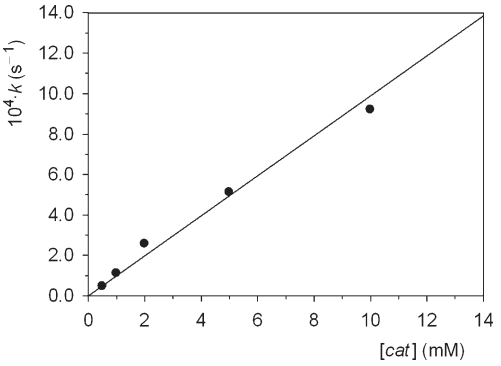

Figure 4. Plot of k_{obs} for the cleavage of 0.20 mM HPNP in 80% DMSO vs the concentration of the Cu^{II} complex of $\mathbf{2H}^+$, pH 9.0, 25 °C. From the slope of the straight line $k_2 = 9.5 \times 10^{-2}$ s^{-1} M^{-1}.

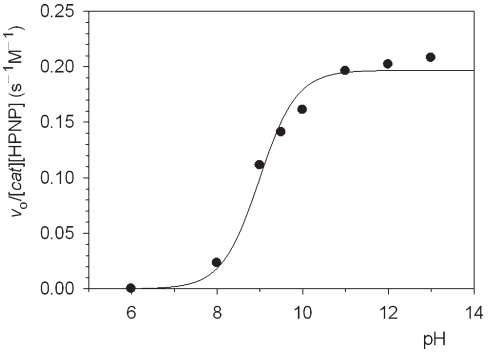

Figure 5. pH-rate profile for the cleavage of 0.2 mM HPNP catalyzed by 1.0 mM $\mathbf{2H}^+Cu^{II}$ in 80% DMSO, 25.0 °C. The data at pH 12 and 13 are corrected for the background hydrolysis ($k_{bkg} = 5.0 \times 10^{-6}$ s^{-1} at pH 12 and $k_{bkg} = 7.6 \times 10^{-5}$ s^{-1} at pH 13).

with the neighboring guanidinium (Figure 3). Here again a linear dependence of k_{obs} vs catalyst concentration was observed (Figure 4), showing that in the investigated concentration range the catalyst works under subsaturating conditions, with a second-order rate constant $k_2 = 9.5 \times 10^{-2}$ s^{-1} M^{-1}, at pH 9.0.

The catalytic efficiency of the Cu^{II} complexes of $\mathbf{2H}^+$ was also investigated at different pH values. The sigmoidal shape of the pH-rate profile (Figure 5) is typical of a kinetic titration in which an unreactive species is transformed into a reactive one at higher pH values. Data points nicely fit to eq 3, where k_2 is the second-order rate constant for the reaction of the conjugate base of an acid whose acidity constant is K_a. A least-squares fitting procedure gave pK_a = 8.94 ± 0.12 and

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$k_2 = (1.97 \pm 0.06) \times 10^{-1}\ M^{-1}\ s^{-1}$.

$$\frac{v_o}{[cat][HPNP]} = \frac{k_2 K_a}{[H^+] + K_a} \quad (3)$$

The very good agreement with the potentiometric pK_a value of 9.0 (Figure 2, curve *b*) strongly reinforces the view that the reactive species is the hydroxo complex of the ligated Cu^{II} cation. Furthermore the limiting value of the second-order rate constant is almost exactly twice as large as the value of $9.5 \times 10^{-2}\ M^{-1}s^{-1}$ determined at pH 9.0 (Figure 4).

Titration of **2**·4HCl in the presence of 1 mol equiv of $ZnCl_2$ (Figure 5S, Supporting Information) showed similarities with the analogous titration in the presence of $CuCl_2$ (Figure 2, curve *b*) but also significant differences. The first 3 equiv of added base was used up as before for deprotonating the triprotonated ligand unit. Addition of the fourth equivalent of base revealed a proton with a pK_a = 9.8 which was assigned to a Zn^{II} complexed water molecule, to be compared with the pK_a value of 9.0 of the Cu^{II} complex. Complexation of the triazacyclononane unit with Zn^{II} (log K = 6.8) is weaker than complexation with Cu^{II} (log K = 8.6), yet strong enough to ensure complete binding of the metal under the conditions of the catalytic runs, namely, equimolar amounts (5 mM) of $\mathbf{2}H^+$ and $ZnCl_2$ at pH 9.8.[46]

Here again, the metal-bound water molecule in the Zn^{II} complex with TACN turned out to be more acidic (pK_a = 8.6; Figure 7S, Supporting Information) than in the corresponding complex with $\mathbf{2}H^+$.

The results of the catalytic experiments (Table 1) confirm once more the ineffectiveness of the guanidinium control (entry 7) and show that the hydroxy derivative of the Zn^{II} complex of TACN is 14 times more effective a catalyst than the corresponding Cu^{II} complex in terms of k_{obs} and 2.3 times more effective in terms of rate enhancement relative to background (compare entry 8 with entry 5). Comparison of the catalytic efficiency of the Zn^{II} complex of the bifunctional catalyst $\mathbf{2}H^+$ with that of the control complex with TACN (compare entry 9 with entry 8)[45] reveals a modest degree of cooperation of the guanidinium unit with the metal center, which is significantly lower than that found in the corresponding Cu^{II} complex. The net result is that the hydroxo derivative of the Zn^{II} complex of $\mathbf{2}H^+$ is twice as effective as the corresponding Cu^{II} complex in terms of absolute rates but three times less effective when rates relative to background are compared.

In summary, the structurally simple *m*-xylylene spacer induces fair to good contacts between catalytic groups and altered substrate in the transition state. Investigations of more elaborate, hopefully more efficient spacers, are in progress. The good catalytic performance of the Cu^{II} containing bifunctional catalyst encourages application to the cleavage of ribonucleotides.

EXPERIMENTAL SECTION

Instruments and General Methods. NMR spectra were recorded on either a 300 or 200 MHz spectrometer. Chemical shifts are reported as δ values in parts per million from tetramethylsilane added as an internal standard. Mass spectra were performed by an Electrospray Ionization Time-of-Flight spectrometer.

Materials. Dry CH_3CN and CH_2Cl_2 were obtained by distillation over CaH_2 and $CaCl_2$, respectively. THF and toluene were dried by distillation over Na. DMSO purged 30 min with argon and mQ water were used in the preparation of 80% DMSO. HPNP was prepared as reported in the literature.[47] Other solvents and reagents were commercially available and used without any further purification.

3-((Imidazol-1-yl)methyl)benzonitrile (4). Imidazole (2.95 g, 43.3 mmol) and anhydrous K_2CO_3 (8 g, 58 mmol) were added to a solution of 3-(bromomethyl)benzonitrile (**3**) (2.26 g, 11.5 mmol) in acetonitrile (100 mL). The reaction mixture was kept at reflux under stirring for 30 min and then, after cooling, filtered to eliminate K_2CO_3 and evaporated under reduced pressure. A saturated Na_2CO_3 aqueous solution (50 mL) and dichloromethane (50 mL) were added to the residue. The organic phase was separated, washed with a saturated Na_2CO_3 aqueous solution (50 mL × 3), and dried over $MgSO_4$. The salt was separated by filtration and the solvent evaporated under reduced pressure to give **4** as a pale yellow solid (1.52 g, 8.30 mmol; 72% yield): mp 58−62 °C. ^{1}H NMR (200 MHz; $CDCl_3$): δ 5.12 (s, 2H), 6.84 (s, 1H), 7.04 (s, 1H), 7.21−7.59 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 49.6, 113.0, 118.0, 119.0, 129.8, 130.2, 130.3, 131.2, 131.7, 137.3, 137.8. ES-MS: m/z 184.12 ($M + H^+$), 206.14 ($M + Na^+$). Anal. Calcd for $C_{11}H_9N_3$: C, 72.11; H, 4.95; N, 22.94. Found: C, 71.97; H, 5.09; N, 22.79.

(3-((Imidazol-1-yl)methyl)phenyl)methylamine (5). 10% Pd−C (0.25 g) was added to a solution of the nitrile **4** (1.52 g, 8.31 mmol) in methanol (50 mL). The resulting mixture was stirred for 12 h at room temperature in a hydrogen atmosphere and then filtered through Celite under reduced pressure. The solvent was evaporated, and the crude product was purified by column chromatography (basic Al_2O_3; CH_2Cl_2/CH_3OH from 99:1 to 95:5), giving the amine **5** as a colorless oil (0.212 g, 1.13 mmol; 14% yield). ^{1}H NMR (200 MHz, CD_3OD): δ 3.87 (s, 2H), 5.23 (s, 2H), 6.94−7.44 (m, 6H), 7.74 (s, 1H). ^{13}C NMR (50 MHz, CD_3OD): δ 45.7, 51.5, 120.9, 128.0, 128.3, 128.7, 129.3, 130.4, 138.6, 138.7, 141.7. ES-MS: m/z 188.12 ($M + H^+$), 210.10 ($M + Na^+$). Anal. Calcd for $C_{11}H_{13}N_3$: C, 70.56; H, 7.00; N, 22.44. Found: C, 70.38; H, 7.15; N, 22.17.

(3-(Imidazol-1-yl)methyl)benzyl-*N*,*N*′-bis(*tert*-butoxycarbonyl)guanidine (6). *N*,*N*′-Bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine (0.48 g, 1.23 mmol) was added under stirring to a solution of the amine **5** (0.212 g, 1.13 mmol) in anhydrous dichloromethane (30 mL). The reaction mixture was kept under stirring at room temperature for 24 h and then extracted with a saturated $NaHCO_3$ aqueous solution (50 mL). The organic phase was separated, washed with a saturated NaCl aqueous solution, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography (SiO_2; CH_2Cl_2/CH_3OH from 99:1 to 95:5), giving the compound **6** as a pale yellow oil (0.175 g, 0.407 mmol; 36% yield). ^{1}H NMR (200 MHz, CD_3OD): δ 1.44 (s, 9H), 1.49 (s, 9H), 4.53 (s, 2H), 5.17 (s, 2H), 6.97 (s, 1H), 7.05−7.38 (m, 5H), 7.72 (s, 1H). ^{13}C NMR (50 MHz, CD_3OD): δ 28.1, 28.2, 45.0, 51.6, 80.5, 84.6, 121.0, 127.7, 127.8, 128.3, 128.9, 130.3, 138.5, 140.0, 152.5, 154.1, 157.5. Anal. Calcd for $C_{22}H_{31}N_5O_4$: C, 61.52; H, 7.27; N, 16.31. Found: C, 61.62; H, 7.10; N, 16.20.

(3-(Imidazol-1-yl)methyl)benzylguanidine (1). Compound **6** (0.175 g, 0.407 mmol) was dissolved in a 1:1 mixture (100 mL) of 0.1 M aqueous HCl/dioxane. The reaction was kept under stirring at room temperature for 70 h. Afterward the solvent was evaporated under reduced pressure and the residue dried under high vacuum for 8 h to give **1**·HCl as a colorless oil (0.10 g, 0.331 mmol; 81% yield). ^{1}H NMR (200 MHz, D_2O): δ 4.49 (s, 2H), 5.49 (s, 2H), 7.35−7.58 (m, 7H). ^{13}C NMR (50 MHz, D_2O): δ 44.8, 53.1, 120.7, 122.5, 127.4, 128.3, 128.6, 130.5, 135.1, 135.2, 137.9, 157.5. ES-MS: m/z 230.10 ($\mathbf{1}H^+$), 213.09 ($\mathbf{1}H^+ - NH_3$). Anal. Calcd for $C_{12}H_{17}Cl_2N_5$: C, 47.69; H, 5.67; N, 23.17. Found: C, 47.42; H, 5.79; N 23.12.

3-(Aminomethyl)phenylmethanol (8). $LiAlH_4$ 1 M in THF (62 mL, 62 mmol) was added dropwise under an argon atmosphere to a solution of 3-formylbenzonitrile (**7**) (2.31 g, 17.6 mmol) in anhydrous THF (50 mL). The mixture was stirred for 30 min at room temperature and then heated up to reflux for 8 h. After cooling, the reaction was

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quenched with NaOH 3 M in water (20 mL), and the mixture was extracted with chloroform (90 mL × 3). The collected organic phases were dried over $MgSO_4$ anhydrous, and the solvent was removed at reduced pressure to give the aminoalcohol **8** as a pale yellow oil (1.4 g, 10.2 mmol; 58% yield). ^{1}H NMR (200 MHz, CD_3OD): δ 3.78 (s, 2H), 4.60 (s, 2H), 7.15−7.36 (m, 4H). ^{13}C NMR (50 MHz, CD_3OD): δ 46.6, 65.2, 126.6, 127.0, 127.4, 129.6, 143.0, 143.6. ES-MS: m/z 138.11 ($M + H^+$), 160.09 ($M + Na^+$). Anal. Calcd for $C_8H_{11}NO$: C, 70.04; H, 8.08; N, 10.21; Found: C, 70.43; H, 7.72; N, 10.03.

3-(Hydroxymethyl)benzyl-*N*,*N*′-bis(*tert*-butoxycarbonyl)-guanidine (9). The aminoalcohol **8** (0.390 g, 2.85 mmol) and *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine (0.55 g, 1.4 mmol) were dissolved in anhydrous dichloromethane (100 mL). After 1 h a further portion of 1.4 mmol of the latter reagent was added to the solution. The reaction mixture was left under stirring for 16 h at room temperature, extracted with a 2 M $NaHSO_4$ aqueous solution (50 mL), separated, and washed with a saturated $NaHCO_3$ water solution (50 mL). The aqueous phase was extracted with dichloromethane (30 mL × 3), and the combined organic phases were washed with a saturated NaCl aqueous solution, dried over $MgSO_4$, and evaporated. The crude material was purified by column chromatography (SiO_2; Hex/AcOEt from 20:1 to 4:1), giving the alcohol **9** as a colorless oil (0.730 g, 1.92 mmol; 67% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.48 (s, 9H), 1.52 (s, 9H), 1.74 (br s, 1H), 4.63 (d, 2H, J = 5.2 Hz), 4.70 (s, 2H), 7.21−7.42 (m, 4H), 8.59 (s, 1H), 11.54 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.2, 28.4, 45.0, 65.1, 79.6, 83.3, 126.3, 126.5, 127.1, 129.0, 137.6, 141.6, 153.2, 156.2, 163.6. ES-MS: m/z 380.17 ($M + H^+$). Anal. Calcd for $C_{19}H_{29}N_3O_5$: C, 60.14; H, 7.70; N, 11.07. Found: C, 59.79; H, 7.58; N, 11.37.

3-(Bromomethyl)benzyl-*N*,*N*′-bis(*tert*-butoxycarbonyl)-guanidine (10). PBr_3 (0.038 mL, 0.405 mmol) was added to a solution cooled to −10 °C of the alcohol **9** (0.460 g, 1.21 mmol) and 2,6-di-*tert*-butylpyridine (0.272 mL, 1.21 mmol) in dry toluene (7 mL). The reaction was stirred at room temperature for 16 h under an argon atmosphere. The mixture was then filtered and the solvent removed by evaporation at reduced pressure to give the bromide **10** as a yellow oil (0.53 g, 1.20 mmol; 99% yield), pure enough to be used in the following step without any further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.49 (s, 9H), 1.53 (s, 9H), 4.49 (s, 2H), 4.72 (d, 2H, J = 5.3 Hz), 7.11−7.38 (m, 4H), 8.79 (s, 1H), 11.53 (s, 1H). ES-MS: m/z 442.18 ($M + H^+$), 464.18 ($M + Na^+$).

3-((*N*,*N*′-Bis(*tert*-butoxycarbonyl)triazacyclononane)methyl)-benzyl-*N*,*N*′-bis(*tert*-butoxycarbonyl)guanidine (11). A solution of the bromide **10** (0.420 g, 0.949 mmol), *N*,*N*′-bis(*tert*-butoxycarbonyl)triazacyclononane (0.195 g, 0.592 mmol), and 2,6-di-*tert*-butylpyridine (0.546 mL, 2.43 mmol) in anhydrous acetonitrile (5 mL) was stirred at room temperature for 30 h under an argon atmosphere. The solvent was then removed by evaporation at reduced pressure, and the residue was dissolved in dichloromethane (20 mL). The mixture was filtered, and the solution was washed with a saturated $NaHCO_3$ aqueous solution (30 mL). The organic phase was separated, dried over $MgSO_4$, and evaporated at reduced pressure. The crude product was purified by column chromatography (SiO_2; from Hex to Hex/AcOEt 4:1), to give **11** as a colorless oil (0.213 g, 0.308 mmol; 52% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.48 and 1.51 (s, 36H), 2.71 (m, 4H), 3.26−3.58 (m, 8H), 3.70 (s, 2H), 4.60 (d, 2H, J = 5.1 Hz), 7.15−7.33 (m, 4H), 8.51 (s, 1H), 11.51 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 27.9, 28.2, 28.4, 28.5, 44.9, 48.6, 49.1, 49.7, 50.3, 51.5, 52.8, 53.6, 54.1, 60.6, 79.2, 83.0, 126.3, 128.1, 128.3, 128.5, 136.9, 140.5, 153.0, 155.3, 155.6, 155.9, 163.5. ES-MS: m/z 691.46 ($M + H^+$), 713.47 ($M + Na^+$). Anal. Calcd for $C_{35}H_{58}N_6O_8$: C, 60.85; H, 8.46; N, 12.16. Found: C, 60.78; H, 8.31; N, 12.29.

(3-(1,4,7-Triazacyclononan-1-yl)methyl)benzylguanidine (2). An amount of 0.130 g (0.188 mmol) of **11** was dissolved in 6 mL of a 1:1 mixture of 0.5 M aqueous HCl/dioxane and stirred at room temperature for 70 h. Afterward the solvent mixture was evaporated under reduced pressure and the residue dried under high vacuum for 8 h, giving the tetrahydrochloride **2**·4HCl as a white solid (0.080 g, 0.183 mmol; 97% yield): mp 157−158 °C. ^{1}H NMR (300 MHz, D_2O): δ 2.95 (t, 4 H, J = 3.1 Hz), 3.15 (t, 4H, J = 3.1 Hz), 3.39 (s, 4H), 3.56 (s, 4H), 3.85 (s, 2H), 4.38 (s, 2H), 7.25−7.45 (m, 4H). ^{13}C NMR (75 MHz, D_2O): δ 42.7, 44.2, 44.9, 48.1, 59.3, 127.4, 129.0, 129.9, 130.3, 136.5, 137.3, 157.5. ES-MS: m/z 291.41 (**2**H^+). Anal. Calcd for $C_{15}H_{30}Cl_4N_6$: C, 41.30; H, 6.93; N, 19.26. Found: C, 41.23; H, 7.18; N, 19.22.

***N*-Benzylimidazole (12).** K_2CO_3 (1 g, 7.2 mmol) and imidazole (1.25 g, 18.4 mmol) were added to a solution of benzyl bromide (0.3 mL, 2.35 mmol) in anhydrous acetonitrile (50 mL). The mixture was refluxed for 30 min and then filtered. The solvent was removed under vacuum and the residue dissolved in CH_2Cl_2 (60 mL). The organic phase was washed with a saturated Na_2CO_3 aqueous solution (50 mL × 3), dried over $MgSO_4$, and evaporated to give **12** as a colorless oil (0.296 g, 1.87 mmol; 80% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.10 (s, 2H), 6.89 (s, 1H), 7.08 (m, 1H), 7.09−7.19 (m, 2H), 7.27−7.37 (m, 3H), 7.57 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 50.7, 119.2, 127.1, 128.1, 128.8, 129.5, 136.0, 137.2. ES-MS: m/z 159.12 ($M + H^+$), 181.11 ($M + Na^+$). Anal. Calcd for $C_{10}H_{10}N_2$: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.45; H, 6.39; N, 17.82.

Potentiometric Titrations. Potentiometric titrations were performed by an automatic titrator equipped with a combined microglass pH electrode. The electrode was calibrated using standard $HClO_4$ and Me_4NOH solutions at different concentrations, I = 0.1 (Et_4NBr). The time required to obtain a stable pH reading increased from 1 min in acid medium up to 6 min at pH above 9. The calibration plot of calculated $-\log c_{H}^{+}$ values vs experimental pH readings was linear in the range 2−17, with $-\log c_{H}^{+} = a + b \cdot pH_{read}$, and best fit values $a = -0.7852 \pm 1.5\%$ and $b = 0.965 \pm 5\%$. The pK_w values determined in several titrations coincided, within experimental errors, with the value reported in the literature.[36,41] Potentiometric titrations were carried out under a nitrogen atmosphere, on 6 mL of 1−3 mM solutions of the compound, in the presence of 0.1 M Et_4NBr (80% DMSO, 25 °C). A 0.05−0.2 M Me_4NOH solution in 80% DMSO was added to the titration vessel in small increments. Analysis of titration plots was carried out by the program HYPERQUAD 2000.[48,49]

Kinetic Measurements. Spectrophotometric measurements were carried out on either a double beam or on a diode array spectrophotometer.

Kinetic measurements of HPNP transesterification were carried out by UV−vis monitoring of *p*-nitrophenol liberation at 400 nm. Rate constants were obtained by an initial rate method, error limit on the order of ±10% for rate constants of 10^{-8}−10^{-7} s^{-1} and ±5% for faster reactions.

Hydrochlorides were neutralized, with the exception of the guanidinium proton, by the addition to the reaction mixture of the calculated amount of Me_4NOH before kinetic runs.

Metal complexes were formed in situ by addition of the calculated stoichiometric amount of $ZnCl_2$ or $CuCl_2$ to the buffered reaction mixture. Due to the slow formation of the complexes,[50] the solutions were incubated for 1 h before the start of the kinetic run by fast addition of a small volume of the substrate solution.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra, plots of potentiometric acid−base titrations, and kinetic data on the effect of chloride on TACN−Cu^{II} catalyzed cleavage of HPNP. This material is available free of charge via the Internet at http://pubs.acs.org.

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(44) The pK_a value of **12**H$^+$ is 5.0 under the same conditions, see Figure 1S, Supporting Information.
(45) A correction for the different concentrations of the hydroxo complexes was not carried out because the likely reduction in reactivity of the more abundant, less basic hydroxo complex cannot be quantified.
(46) The most significant difference between the Zn^{II} complexes and the Cu^{II} complexes is that the number of titratable protons raised to six in the former case. In the high pH region (Figure 5S, Supporting Information), corresponding to the titration of the fifth (pK_a = 12.3) and sixth (pK_a = 14.2) proton, a dull titration profile was observed, characterized by a monotonic approach of the pH to the limiting value of about 16, that was reached in the presence of excess base. Whereas the least acid proton was assigned to the guanidinium unit, the pK_a value of 12.3 was tentatively interpreted as due to a second water molecule bound to Zn^{II}. In any event, any proton transfer process taking place in the high pH region has no influence on catalytic runs carried out at pH 9.8.
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