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Upper Rim Guanidinocalix[4]arenes as Artificial Phosphodiesterases

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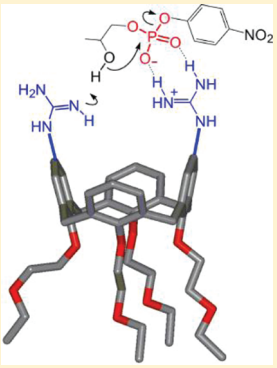
Supporting Information

ABSTRACT: Calix[4]arene derivatives, blocked in the *cone* conformation and functionalized with two to four guanidinium units at the upper rim were synthesized and investigated as catalysts in the cleavage of the RNA model compound 2-hydroxypropyl *p*-nitrophenyl phosphate. When compared with the behavior of a monofunctional model compound, the catalytic superiority of the calix[4]arene derivatives points to a high level of cooperation between catalytic groups. Combination of acidity measurements with the pH dependence of catalytic rates unequivocally shows that a necessary requisite for effective catalysis is the simultaneous presence, on the same molecular framework, of a neutral guanidine acting as a general base and a protonated guanidine acting as an electrophilic activator. The additional guanidinium (guanidine) group in the diprotonated (monoprotonated) trifunctional calix[4]arene acts as a more or less innocent spectator. This is not the case with the tetrasubstituted calix[4]arene, whose mono-, di-, and triprotonated forms are slightly less effective than the corresponding di- and triguanidinocalix[4]arene derivatives, most likely on account of a steric interference with HPNP caused by overcrowding.



INTRODUCTION

In recent years many research groups have achieved significant results in the synthesis and study of simple, nonpeptidic molecules that mimic structural and functional aspects of enzymes.[1−3] In this context artificial catalysts that efficiently cleave DNA and RNA fragments have received considerable attention[4−19] because phosphodiester bonds are extremely reluctant to undergo hydrolysis[20] and because cleavage agents of these substrates have a potential application in health-related goals, such as the antisense therapy.[21,22]

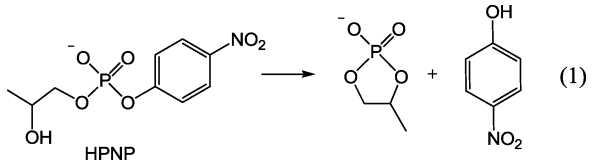
In artificial enzymes a primary role is played by the molecular scaffold. An efficient scaffold should keep the active functions at the proper distance as a result of a good compromise between preorganization and flexibility. Previous studies in the field have shown that the calix[4]arene scaffold, blocked in the *cone* conformation by proper alkylation of the lower rim hydroxyl groups, is suitable for the design of catalysts active in the cleavage of carboxylic and phosphoric esters.[4,9,23−29]

Bi- and trimetallic derivatives of calix[4]arenes decorated with suitable ligating units proved to be highly efficient catalysts in phosphodiester hydrolysis, thanks to a high degree of cooperation between the metal centers[4,9,27] which can act as binding centers as well as activating units.

The guanidinium unit also has a great importance as an activating and/or anchoring group in hydrolytic reactions. Guanidinium plays an important role in nature as it is present in many proteins. The active site of *staphylococcal nuclease* contains two arginine units, which, in conjunction with a calcium ion, lead to an electrophilic activation of phosphodiesters for hydrolysis.[30] Studies are also available of the catalytic activity of artificial systems based on one or more guanidinium units.[15,31−35] or on a guanidinium unit in conjunction with another active unit such as a metal center,[36−39] a hydroxyl group,[40] or another unit acting as a general base.[41,42]

In the past years the guanidinium unit has been successfully used in the design of molecular receptors.[43−46] Guanidinocalixarenes were synthesized for the purpose of binding to negatively charged substrates through electrostatic interactions, and they were initially used to build up supramolecular assemblies on surfaces.[47,48] Thanks to their remarkable affinity for nucleic acid strands,[49] more recently they were successfully used to vehicle DNA through cell membranes, thus inducing transfection.[45,46]

In this paper we report on the synthesis and kinetic investigation of the catalytic activity of guanidinocalix[4]arenes **1**−**4** in the transesterification of the RNA model compound 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNP), (eq 1).



(1)

These catalysts are based on the calix[4]arene scaffold blocked in the *cone* conformation by alkylation of the phenolic OH groups with ethoxyethyl chains for the purpose of increasing the solubility in polar solvents and getting rid of the self-aggregating properties typical of amphiphilic *p*-guanidinocalixarenes endowed with lipophilic chains at the lower rim.[45] The

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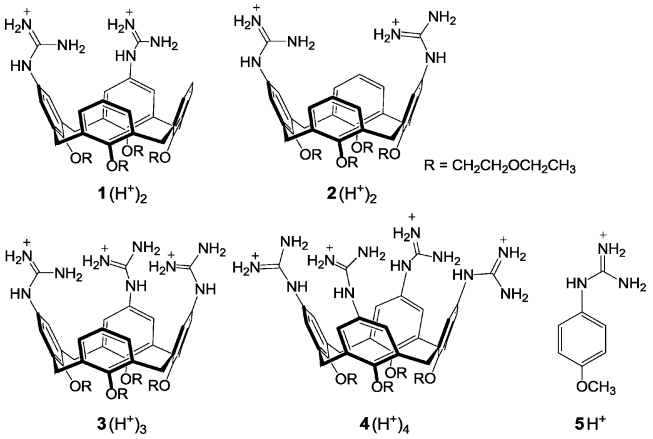
upper rim is functionalized with two to four guanidinium units. In view of the well established mechanism of general acid−base catalysis in the cleavage of HPNP, involving the synergic action of neutral and protonated forms of guanidine,[35] compounds **1**−**4** offer wide opportunities for achieving guanidine−guanidinium combinations in varying proportions, depending on pH. As a control, the monofunctional compound **5** was also synthesized and investigated under the same conditions.

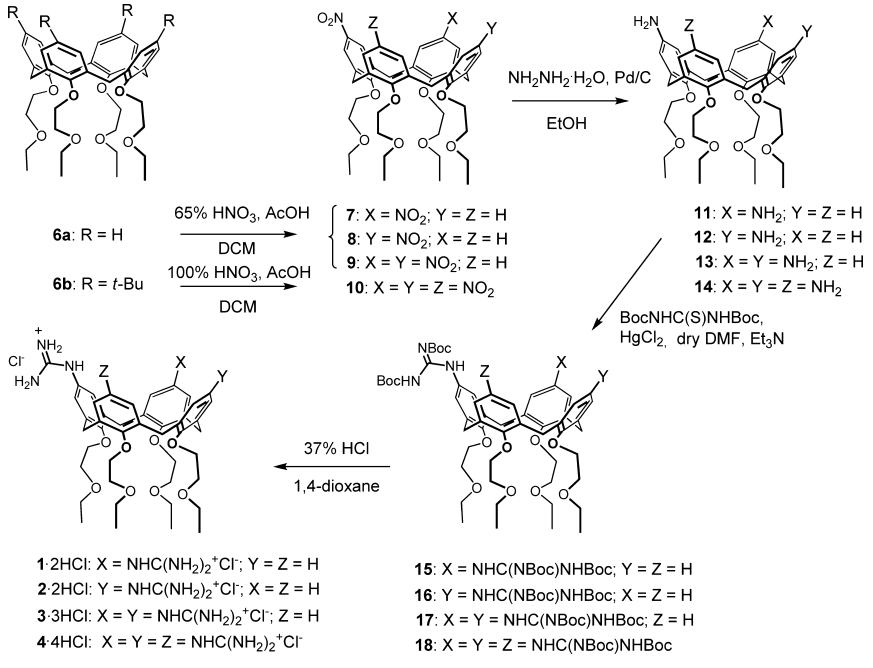


RESULTS AND DISCUSSION

Catalyst Synthesis. The synthesis of guanidinylated tetrakis(2-ethoxyethoxy)calix[4]arenes **1**−**4** was carried out according to Scheme 1. It is known that the 1,3-distal dinitro derivative **8** can be efficiently obtained by a selective *ipso*-nitration of a 5,17-bis-*tert*-butylcalix[4]arene derivative,[50] but since the 1,2-vicinal dinitro derivative and the trinitro derivatives **7** and **9** were also needed, we preferred to carry out the nitration[51] of tetrakis(2-ethoxyethoxy)calix[4]arene **6a**, followed by separation of the various substitution products. Indeed the nitration reaction showed a rather poor selectivity, yielding a mixture of mononitro-, dinitro-, and trinitrocalixarenes. While the 1,3-distal dinitrocalix[4]arene **8**[50] could be obtained by crystallization of the crude reaction mixture from MeOH, the 1,2-vicinal dinitro- and the trinitro derivatives **7** and **9**, respectively, were isolated after a careful chromatographic separation. The structures of the isomeric derivatives **7** and **8** were assigned on the basis of the highly diagnostic patterns of the ^{1}H NMR signals of $ArCH_2Ar$, well precedented for other upper rim substituted calix[4]arenes.[52] The 1,3-distal dinitro derivative **8** originates a pair of doublets at 4.50 and 3.40 ppm, while the proximal 1,2-vicinal dinitro derivative **7** gives rise to three pairs of doublets (1:2:1 ratio) at 4.66 and 3.28, 4.56 and 3.23, 4.45 and 3.16 ppm. The tetranitro derivative **10** was obtained by *ipso*-nitration of the *p*-*tert*-butylated ether **6b** in the *cone* structure.[50] The four nitro compounds **7**−**10** were then reduced with hydrazine hydrate and Pd/C to the corresponding amino derivatives **11**−**14** in 70−79% yields. Particular care was paid in the workup and storage of these multivalent aminocalixarenes **11**−**14** because they may absorb atmospheric CO_2, giving rise to carbamates.[53] The guanidinylation reaction, carried out by using bis-Boc-thiourea and $HgCl_2$, gave the expected di- (**15** and **16**) and triguanidinylated (**17**) products in 45−54% isolated yields after column chromatography. As previously reported for other upper rim functionalized calixarenes,[45,49] the low yields of isolated products were ascribed to partial Boc removal and consequent loss of material during the chromatographic purification process. The tetraguanidinylated compound **18** was instead isolated by crystallization in moderate yield (59%). The deprotection reactions of **15**−**18** with hydrochloric acid proceeded smoothly and afforded compounds **1**−**4** as hydrochlorides in quantitative yields.

Potentiometric Titrations. Knowledge of the acidity constants of the hydrochlorides of **1**−**4** and of the monofunctional model compound **5** is a prerequisite for a meaningful investigation of their catalytic properties. A mixture of DMSO/H_2O, 80:20 (v/v), hereafter referred to as 80% DMSO, was used as reaction medium for titration experiments, as well as for kinetic analysis. This mixture is well-known to be suitable for pK_a determination[54,55] and for investigation of the kinetics of hydrolytic reactions of phosphodiesters.[35,39,56−58] The binding

Scheme 1. Synthesis of Calix[4]arene Derivatives 1−4



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of guanidinium to phosphate in 80% DMSO is stronger than in pure water[59−61] and, consequently, in this solvent mixture a catalyst based on guanidinium as binding/activating group is potentially favored. In 80% DMSO the pK_w for water autoprotolysis rises to 18.4,[62] but the pK_a values of amines and other nitrogen bases are approximately the same or slightly lower than those in water.[63]

Acidity constants of protonated bases were determined by standard potentiometric titrations of their chloride salts with Me_4NOH in 80% DMSO. All titrations were carried out on 2 mM solutions of guanidinocalix[4]arenes. A typical titration plot is reported in Figure 1. Figure 2 shows the distribution diagrams of the species for **2**−**4**.

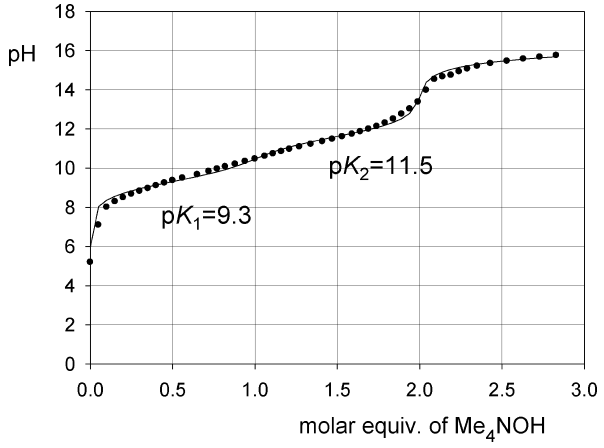

Figure 1. Titration of **2**·2HCl (2 mM) with Me_4NOH in 80% DMSO, 25 °C, in the presence of 10 mM Et_4NBr.

The acidity constants of guanidinocalix[4]arenes (Table 1) range over almost 3 orders of magnitude. A number of factors contribute to determine the variations in the acidity of the various guanidinium units on the calixarene scaffold relative to that of the monofunctional compound $\mathbf{5}H^+$. First of all statistical factors must be taken into account. As shown by the statistically corrected pK_i values given in parentheses in Table 1, statistical corrections are sizable in a number of cases, but do not alter to a significant extent the general picture. More importantly, as one would expect from electrostatic considerations, repulsion between two or more guanidinium groups facilitates the departure of a proton from one of the groups. The operation of this strong acidity-enhancing effect makes the pK_i values of each one of the polysubstituted guanidinocalix[4]arenes increase markedly on increasing *i*. It might seem odd, however, that the statistically corrected pK_1 values of the diprotic, triprotic, and tetraprotic acids are remarkably similar to one another, showing an apparent insensitivity to the varying number of acidity-enhancing positive groups. Similar conclusions are drawn when the pK_2 and pK_3 of the triprotic acid $\mathbf{3}(H^+)_3$ are compared with the corresponding values of the tetraprotic acid $\mathbf{4}(H^+)_4$. The apparent insensitivity to the action of multiple positive poles finds an at least partial explanation in the operation of medium effects on the stability of multiply charged ions (see Supporting Information, Appendix 1).

Kinetic Measurements. The catalytic activity of calix[4]arene derivatives **1**−**4** in the transesterification of the RNA model compound HPNP (eq 1) was investigated in the same solvent mixture used for the potentiometric measurements, namely, 80% DMSO at 25.0 °C. In a recent study of the

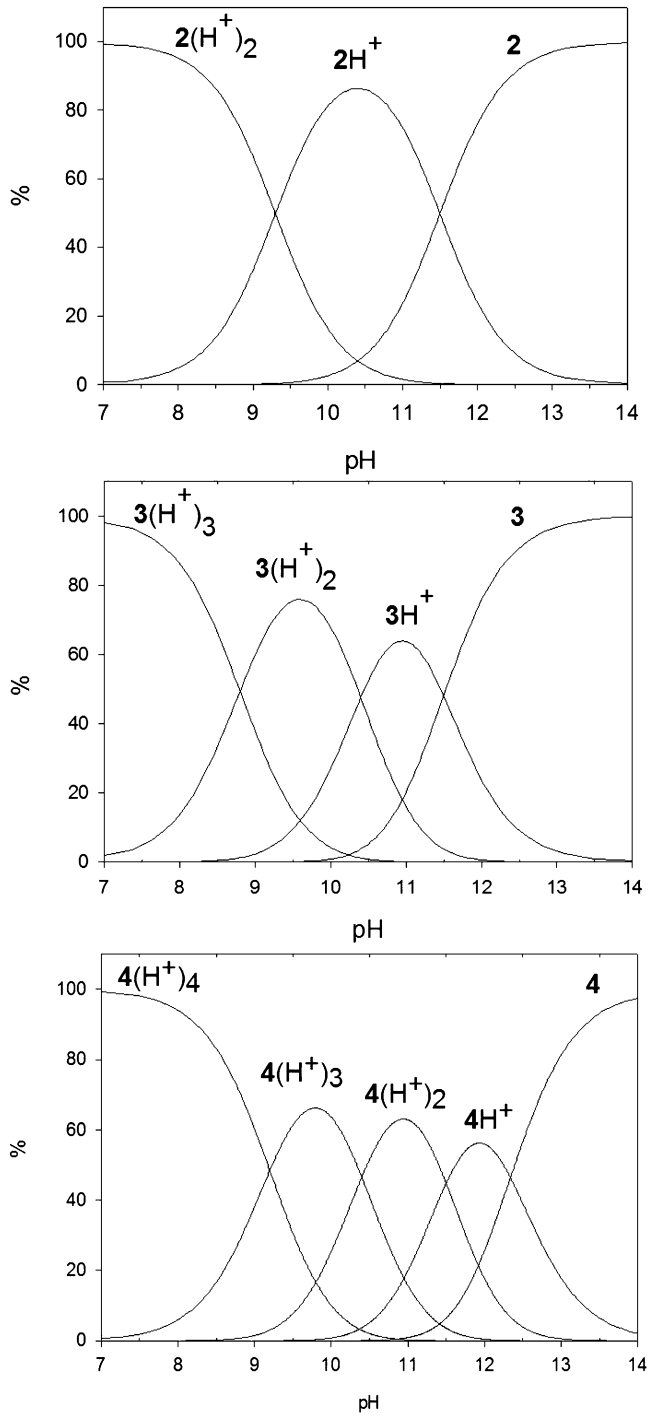

Figure 2. Distribution diagrams of the species for **2** (top), **3** (middle), and **4** (bottom) as a function of pH under the conditions of the titration experiments in Table 1.

kinetics of HPNP transesterification in 80% DMSO at 37 °C in guanidine buffers, Yatsimirsky et al. established the rate law of eq 2 (B = guanidine),

$$k_{obs} = k_1[B] + k_2[B][BH^+] \quad (2)$$

showing contributions from simple general base catalysis (k_1 = 0.025 M^{-1} s^{-1}), and a reaction involving concerted action of neutral and protonated forms of the buffer (k_2 = 1.68 M^{-2} s^{-1}).[35] On the basis of a proton inventory study, these authors convincingly argued that the protonated buffer component acts via

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Table 1. Acidity Constants of Guanidinocalix[4]arenes 1−4 and of the Model Compound 5 in 80% DMSO, 25 °C[a,b]

entry	species	pK_1	pK_2	pK_3	pK_4
1	**5**·HCl	11.5			
2	**1**·2HCl	9.3 (9.6)	11.6 (11.3)		
3	**2**·2HCl	9.3 (9.6)	11.5 (11.2)		
4	**3**·3HCl	8.8 (9.3)	10.4 (10.4)	11.5 (11.0)	
5	**4**·4HCl	9.0 (9.6)	10.3 (10.5)	11.5 (11.3)	12.3 (11.7)

[a] pK_i data from potentiometric titrations carried out on 2 mM substrate solutions in the presence of 10 mM Et_4NBr. Experimental uncertainty = ±0.1 pK units or less. Statistically corrected values are in parentheses. [b] Under the same conditions, the pK of guanidine·HCl is 13.7.

electrostatic transition state stabilization, rather than proton transfer.

Preliminary to the kinetic investigation of the catalytic efficiency of di-, tri-, and tetrafunctional calix[4]arene derivatives **1**−**4**, the transesterification of HPNP was investigated in 80% DMSO, 25 °C, in *N*-(4-methoxyphenyl)-guanidine (**5**) buffer (50% free base). The results are reported in Figure 3. The plot does not approach zero at low buffer

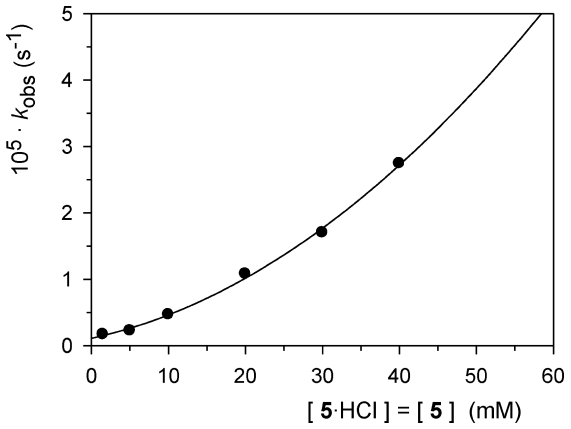

Figure 3. Catalysis of transesterification of HPNP by *N*-(4-methoxyphenyl)guanidine (**5**) buffer (50% free base, pH = 11.5) in 80% DMSO at 25 °C. $k_{obs} = v_o/[HPNP]$, where v_o is the spectrophotometrically determined initial rate of *p*-nitrophenol liberation. The ionic strength was adjusted to 0.1 M by the addition of Et_4NBr. The line is a plot of eq 3, with the best fit parameters given in the text.

concentrations, indicating that the rate of background reaction at pH 11.5, albeit very small, is not negligible. A nonlinear least-squares procedure was used to fit data points to the parabolic eq 3, with the following values of best fit parameters: $k_o = (0.11 \pm 0.05) \times 10^{-5}$ s^{-1}; $k_1 = (2.5 \pm 0.7) \times 10^{-4}$ M^{-1} s^{-1}, $k_2 = (1.0 \pm 0.2) \times 10^{-2}$ M^{-2} s^{-1}.

$$k_{obs} = k_o + k_1[B] + k_2[B][BH^+] \quad (3)$$

Comparison of the best fit specific rate of background reaction k_o with the value 0.17×10^{-5} s^{-1} measured for the rate of HPNP transesterification in phosphate buffer at pH 11.5 (see Supporting Information, Table 3S in Appendix 2) shows that a major component of the background reaction is due to hydroxide catalysis. The numerical values of k_1 and k_2 are 2 orders of magnitude lower than the corresponding values of the reaction catalyzed by guanidine buffers,[35] which is easily understandable on the basis of the lower basicity of *N*-(4-methoxyphenyl)guanidine (**5**) relative to guanidine (Table 1, entry 1 and footnote *b*), and of the different temperatures of the two sets of experiments, (25 °C vs 37 °C). These findings clearly indicate that the mechanisms of HPNP transesterification are essentially the same in both cases, i.e. general base catalysis in combination with general-base/general-acid catalysis in which the protonated form of the catalyst stabilizes the anionic transition state via electrostatic interaction, most likely reinforced by a bidentate hydrogen-bonding interaction, as schematically depicted in Figure 4.

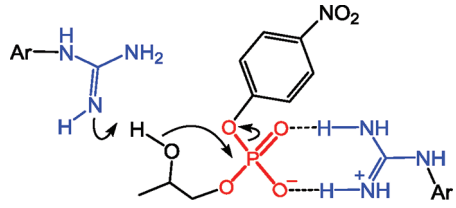

Figure 4. General-acid/general-base catalytic mechanism of HPNP transesterification in *N*-(4-methoxyphenyl)guanidine (**5**) buffer.

A different kinetic behavior was found when free and protonated guanidine units are bound to the same molecular framework. Half-neutralization of the bifunctional precatalyst **2**·2HCl with one molar equivalent of Me_4NOH gave buffer solutions of **2**·H^+ (pH = 10.4) which were used for the transesterification of HPNP. A strictly linear dependence of k_{obs} vs buffer concentration was observed (Figure 5, eq 4), showing,

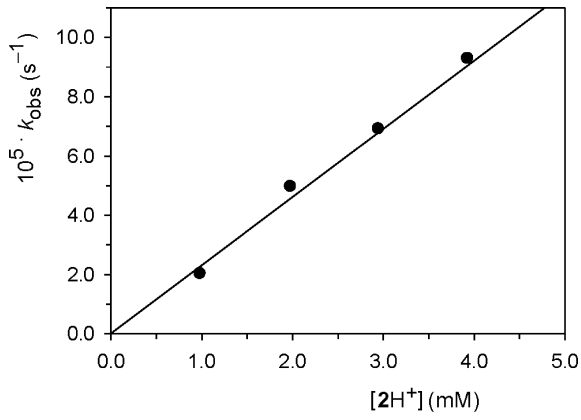

Figure 5. Plot of k_{obs} for the liberation of *p*-nitrophenol from 0.1 mM HPNP vs the concentration of **2**H^+. Buffer solutions obtained by mixing equimolar amounts of **2**·2HCl and Me_4NOH, pH = 10.4 in DMSO 80% at 25 °C. From the slope of the straight line: $k_{cat} = (2.3 \pm 0.05) \times 10^{-2}$ M^{-1} s^{-1}. (The value $k = 1.6 \times 10^{-7}$ s^{-1} calculated for background reaction of HPNP at pH 10.4 ($k_{bg} = 10^{(pH-17.2)}$; Supporting Information, Appendix 2) was used as the intercept value with the *y*-axis).

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inter alia, that in the investigated concentration range the catalyst works

$$k_{obs} = k_{cat}[2H^+] \quad (4)$$

under subsaturating conditions, with a second-order catalytic rate constant $k_{cat} = (2.30 \pm 0.05) \times 10^{-2}$ M^{-1} s^{-1}.

Compared with the reaction carried out in the presence of the monofunctional control **5**, the efficiency of the bifunctional catalyst is so high as to obscure contributions from background reaction ($k_{bg} = 1.6 \times 10^{-7}$ s^{-1} at pH 10.4; from $k_{bg} = 10^{(pH-17.2)}$, Supporting Information, Appendix 2). As to the simple general-base catalyzed mechanism, the second-order rate constant k_1 for the reaction of **5** (eq 3) is 2 orders of magnitude lower than k_{cat}, in spite of the fact that **5** is 2 orders of magnitude more basic than $2H^+$ (Table 1). We conclude therefore that the catalysis in buffer solutions of $2H^+$ is entirely due to a mechanism involving concerted actions of the neutral and protonated forms of the guanidine moieties in **2**, as shown in Figure 6. In other

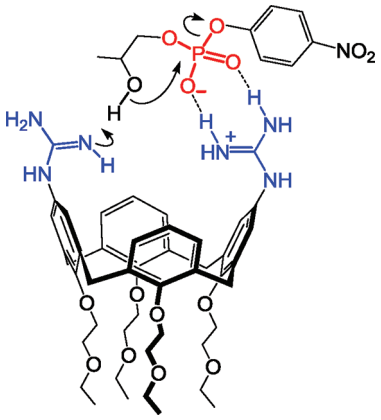

Figure 6. Bifunctional general acid–base catalysis of $2H^+$ in the transesterification of HPNP.

words, this mechanism is the intramolecular counterpart of the general-base/general-acid mechanism depicted in Figure 4. The ratio $k_{cat}/k_2 = 2.3$ M is the effective molarity (EM) of the reaction undergoing intramolecular acid–base catalysis. This means that 2.3 M concentrations of **5** and $5H^+$ display the same catalytic rate as that of a 2.3 M solution of $2H^+$ or, more realistically, that at millimolar catalyst concentrations the reaction catalyzed by $2H^+$ is 3 orders of magnitude faster than that catalyzed by the 1:1 mixture of **5** and $5H^+$. Thus, an EM value as high as 2.3 M provides a measure of the high degree of synergism displayed by neutral and protonated guanidinium units of $2H^+$ in the stabilization of the transition state of the HPNP transesterification.

In order to carry out a comparison of the catalytic efficiencies of the various calix[4]arene derivatives in the transesterification of HPNP, a set of rate measurements was carried out in buffer solutions obtained by partial neutralization of 3 mM solutions of guanidinocalix[4]arene precatalysts with varying amounts of Me_4NOH. The results are collected in Table 2. HPNP transesterifications in the presence of guanidinocalix[4]arene catalysts were in all cases faster than background transesterification, with rate enhancements as high as 3 orders of magnitude in a number of cases (entries 1, 4, 7, and 8). In three cases only a minor correction for background was required (entries 3, 6, and 13), as the background contributions were larger than the 5% estimated uncertainties in k_{obs}.

Table 2. Transesterification of HPNP Catalyzed by Guanidinocalix[4]arene Derivatives in 80% DMSO at 25.0 °C[a]

entry	precatalyst	Me_4NOH (mol equiv)	pH	$10^5 \times k_{obs}$ (s^{-1})
1	**1**·2HCl	0.5	9.3	1.9
2		1.0	10.4	3.3
3		1.5	11.5	2.0[b]
4	**2**·2HCl	0.5	9.3	4.6
5		1.0	10.4	6.9
6		1.5	11.5	5.0[b]
7	**3**·3HCl	1.0	9.7	4.9
8		1.5	10.4	9.7
9		2.0	11.1	11.4
10		2.5	11.5	9.6
11	**4**·4HCl	1.0	9.8	1.0
12		2.0	10.9	3.2
13		3.0	11.8	2.5[b]

[a]Pseudo-first-order specific rates k_{obs} calculated as v_o/[HPNP], where v_o is the spectrophotometrically determined initial rate of *p*-nitrophenol liberation in 0.1 mM HPNP solutions in the presence of 3 mM catalyst. The rate constant (k_{bg}, s^{-1}) for the hydroxide-catalyzed reaction as a function of pH is given by the following expression: $k_{bg} = 10^{(pH-17.2)}$ (Supporting Information, Appendix 2). [b]Corrected for the background reaction. $k_{bg} = 0.2 \times 10^{-5}$ s^{-1} at pH 11.5; $k_{bg} = 0.4 \times 10^{-5}$ s^{-1} at pH 11.8.

Consistent with the distribution diagram in Figure 2, the maximum catalytic activity in the reaction catalyzed by the 1,3-distal bifunctional catalyst **2** is achieved at pH 10.4 (compare entries 4 and 6 with entry 5), which provides a definite confirmation that the catalytically active species is the monoprotonated form $2H^+$. Similar considerations apply to the 1,2-vicinal regioisomer **1** (entries 1–3), whose acid–base behavior is very similar to that of **2** (Table 1, entries 2 and 3). In previous studies, homobimetallic complexes of calix[4]arenes decorated at the upper rim with suitable ligating units were investigated as catalysts for the transesterification of HPNP. It was found that the degrees of synergism critically depend on the nature of the catalytic units and on whether the substitution pattern is 1,2-vicinal or 1,3-distal,[25,27] and that two effective catalytic groups at the upper rim of a calix[4]arene backbone do not necessarily make a good catalyst.[27] It is remarkable, therefore, to find that both 1,2-vicinal $1H^+$ and 1,3-distal $2H^+$ exhibit a high degree of synergism between catalytic groups, the 1,3-distal bifunctional catalyst being slightly more than twice as effective as its 1,2-vicinal regioisomer.

As to the trifunctional catalyst **3**, comparison of the distribution diagram in Figure 2 with kinetic data in Table 2 (entries 7–10) shows that the species $3H^+$, featuring one guanidinium and two guanidine groups, is catalytically more effective than the doubly protonated species $3(H^+)_2$.[64] The former is also slightly more effective than $2H^+$, which is easily ascribed to statistical factors,[28] thus showing that the inherent catalytic effectiveness of 1,2-vicinal or 1,3-distal guanidine–guanidinium pairs is not affected to a significant extent by the presence of an additional guanidine moiety, which, as a matter of fact, behaves as an innocent spectator. This is clearly not the case with the tetrafunctional catalyst **4**, for which all the species obtained by partial neutralization with 1, 2, and 3 mol equiv of Me_4NOH, i.e., $4(H^+)_3$, $4(H^+)_2$, and $4H^+$ are somewhat less effective than $3H^+$ (Table 2, compare entries 11–13 with entry 9). It appears, therefore, that the extra guanidine moiety in **4** no longer behaves as an innocent spectator but destabilizes the transition state, presumably through a moderate

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steric interference with the altered substrate in the transition state. In other words, the steric overcrowding caused by the presence of two extra guanidine or guanidinium moieties, in addition to the guanidine−guanidinium pair directly involved in the acid−base catalysis, causes a modest steric repulsion with the substrate in the activation process. Such an interference is apparently avoided in the reaction catalyzed by $3H^+$.

To sum up, the data reported in this work, when combined with analogous data from previous studies, strongly reinforce the notion that *cone*-calix[4]arenes are quite useful for the design of efficient catalysts. The catalytic efficiency results from a proper organization of catalytic groups brought about by the calix[4]arene platform, coupled with a high level of adaptability resulting from the conformational flexibility of the platform itself.

A still open question is whether *cone*-calix[4]arenes are suitable for the construction of multifunctional catalysts whose catalytic performance results from the cooperative action of three or more catalytic groups. Some evidence was obtained of the operation of trimetallic catalysis of zinc(II) and copper(II) complexes in the cleavage of carboxylate and phosphate esters.[4,25,26,28] On the contrary, there seems to be no sign of the operation of multifunctional catalysis in the transesterification of HPNP catalyzed by calix[4]arene derivatives **3** and **4**, which behave as bifunctional catalysts, with no evidence of additional stabilization of the transition state arising from functional groups other than the guanidine−guanidinium pair involved in the general acid−base catalysis.

EXPERIMENTAL SECTION

Instruments. NMR spectra were recorded on either a 400- or 300 MHz spectrometer. Partially deuterated solvents were used as internal standards to calculate the chemical shifts (δ values in ppm). Mass spectra were obtained on an electrospray ionization (ESI) time-of-flight spectrometer.

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

Materials. All reactions were carried out under nitrogen atmosphere. Dry DMF was prepared according to standard procedures and stored over molecular sieves. Anhydrous CH_2Cl_2 was obtained by distillation over $CaCl_2$. DMSO, purged 30 min with argon, and mQ water were used in the preparation of 80% DMSO used in kinetic and potentiometric experiments. All the other solvents and reagents were used as commercially available without any further purification. Flash chromatography purifications were carried out on 230−240 mesh silica gel. HPNP[65] and tetrakis(2-ethoxyethoxy)calix[4]arene **6a**[51] were prepared according to literature procedures. The 5,11,17,23-tetraamino-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene **14** was prepared according to a literature procedure.[66] Aminocalixarenes **11**−**14** were stored under nitrogen atmosphere to avoid the formation of their carbamate derivatives.[53]

Nitration of 25,26,27,28-Tetrakis(2-ethoxyethoxy)calix[4]arene (6a). To a stirring solution of 25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene **6a** (2.0 g, 2.8 mmol) in DCM (95 mL) and glacial acetic acid (5.6 mL) was added HNO_3 (65%, 93.3 mL). The reaction turned purple and then dark brown and was quenched after 135 min by adding H_2O (60 mL). The organic phase was separated and washed with a 5% K_2CO_3 aqueous solution until basic pH. The solvent was removed under reduced pressure to afford an orange residue, from which most of the 1,3-distal dinitro derivative (**8**) was obtained through crystallization from MeOH. The solvent was removed from the mother liquor and the residue chromatographed (SiO_2, petroleum ether/AcOEt, 7:3; petroleum ether/AcOEt, 2:3; and AcOEt/MeOH, 98:2) to afford, in the given order, the mononitro derivative, a mixture of 1,2-vicinal dinitro (**7**) and 1,3-distal dinitro (**8**) compounds, and finally the trinitro derivative (**9**).

5,11-Dinitro-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (7). A pure sample of compound **7** was obtained by two consecutive flash column chromatography separations on SiO_2 gel. The first column in DCM/AcOEt (96:4) gave enriched fractions of compound **7** which was further purified by using a second column in hexane/acetone (8:2) to obtain 300 mg of a yellow oil (13% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (s, 2H), 7.47 (s, 2H), 6.68−6.50 (m, 6H), 4.66 (d, J = 14.0 Hz, 1H), 4.56 (d, J = 14.0 Hz, 2H), 4.45 (d, J = 14.0 Hz, 1H), 4.31−4.20 (m, 2H), 4.21−4.00 (m, 6H), 3.88−3.68 (m, 8H), 3.58−3.42 (m, 8H), 3.28 (d, J = 14.0 Hz, 1H), 3.23 (d, J = 14.0 Hz, 2H), 3.16 (d, J = 14.0 Hz, 1H), 1.18 and 1.15 (2t, J = 7.0 Hz, 6H each). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.3, 156.2, 142.6, 137.0, 135.5, 135.3, 133.7, 129.0, 128.1, 124.3, 123.3, 122.6, 74.0, 73.4, 69.8, 69.5, 66.4, 31.0, 30.8, 15.3, 15.2. MS (ESI): m/z 825.3 $[M + Na]^+$. Anal. Calcd for $C_{44}H_{54}N_2O_{12}$: C, 65.82; H, 6.78; N, 3.49. Found: C, 65.75; H, 6.89; N, 3.58.

5,17-Dinitro-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (8). This compound (270 mg; 12% yield) showed the same physicochemical and spectroscopic properties as those reported in the literature.[51]

5,11,17-Trinitro-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (9). A sample was obtained as a slightly pink solid (280 mg; 10% yield): mp 147−150 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83−7.80 (m, 4H), 7.20 (s, 2H), 6.33 (s, 3H), 4.66 and 4.55 (2d, J = 13.8 Hz, 2H each), 4.40−4.20 (m, 4H), 4.14 and 3.99 (2t, J = 5.6 Hz, 2H each), 3.81−3.72 (m, 8H), 3.56−3.42 (m, 8H), 3.32 and 3.27 (2d, J = 13.8 Hz, 2H each), 1.18 and 1.12 (2t, J = 7.0 Hz, 6H each). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.0, 161.0, 155.3, 143.0, 142.6, 137.5, 136.2, 135.0, 132.8, 128.4, 124.6, 123.7, 123.6, 123.0, 74.4, 74.0, 73.9, 69.8, 69.4, 66.6, 66.5, 66.3, 31.0, 30.9, 29.7, 15.2. ES-MS: m/z 870.2 $(M + Na)^+$. Anal. Calcd (%) for $C_{44}H_{53}N_3O_{14}$: C, 62.33; H, 6.30; N, 4.96. Found: C, 62.37; H, 6.42; N, 5.02.

5,11-Diamino-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (11). A sample of 1,2-vicinal dinitro compound **7** (0.15 g, 0.187 mmol) was dissolved in absolute EtOH (10 mL), and hydrated hydrazine (98%, 0.1 mL, 2.12 mmol) and a catalytic amount of Pd/C were added. The reaction mixture was refluxed overnight under nitrogen. After cooling, the catalyst was removed by filtration and the solvent evaporated to dryness to obtain compound **11** as a colorless oil (0.11 g; 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.72−6.50 (m, 6H), 6.01 (d, J = 2.5 Hz, 2H), 5.97 (d, J = 2.5 Hz, 2H), 4.50 (d, J = 13.4 Hz, 1H), 4.42 (d, J = 13.4 Hz, 2H), 4.33 (d, J = 13.4 Hz, 1H), 4.09 (t, J = 5.8 Hz, 4H), 4.00 (t, J = 6.0 Hz, 4H), 3.89−3.75 (m, 8H), 3.54 (q, J = 6.8 Hz, 8H), 3.15 (d, J = 13.4 Hz, 1H), 3.05 (bs, 4H), 3.03 (d, J = 13.4 Hz, 2H), 2.90 (d, J = 13.4 Hz, 1H), 1.24−1.14 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$); δ 156.6, 149.5, 140.6, 135.5, 135.1, 128.2, 128.1, 121.8, 115.4, 73.1, 69.7, 69.6, 66.4, 30.9, 15.3. ES-MS: m/z 765.34 $(M + Na)^+$, m/z 743.38 $(M + H)^+$. Anal. Calcd for $C_{44}H_{58}N_2O_8$: C, 71.13; H, 7.87; N, 3.77. Found: C, 71.03; H, 7.95: N, 3.91.

5,17-Diamino-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (12). The reaction was carried out as for the preparation of compound **11**, starting from the 1,3-distal dinitro derivative **8** (0.17 g, 0.212 mmol). Compound **12** was obtained as a reddish oil (0.116 g; 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.73 (d, J = 7.2 Hz, 4H), 6.63 (t, J = 7.2 Hz, 2H), 5.98 (s, 4H), 4.44 (d, J = 13.2 Hz, 4H), 4.12 (t, J = 5.5 Hz, 4H), 4.03 (t, J = 5.3 Hz, 4H), 3.92 (m, 8H), 3.57 and 3.55 (2q, J = 7.3 Hz, 8H), 3.19 (s, 4H), 3.06 (d, J = 13.2 Hz, 4H), 1.23 and 1.21 (2t, J = 7.3 Hz, 6H each). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.6, 149.5, 140.7, 135.5, 135.2, 128.2, 122.0, 115.7, 73.2, 73.0, 69.7, 69.6, 66.4, 66.3, 30.9, 15.3. ES-MS: m/z 743.38 $(M + H)^+$. Anal. Calcd for $C_{44}H_{58}N_2O_8$: C, 71.13; H, 7.87; N 3.77. Found: C, 71.06; H, 7.92; N, 3.88.

5,11,17-Triamino-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (13). The reaction was carried out as for the preparation of compound **11**, starting from trinitro compound **9** (0.235 g, 0.278 mmol). Compound **13** was obtained as a dark-red oil (0.147 g; 70% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.75 (d, J = 6.9 Hz, 2H), 6.65 (t, J = 6.9 Hz, 1H), 6.09 (s, 2H), 6.00 and 5.95 (2d, 2H each, J = 2.1 Hz), 4.42 and 4.32 (2d, J = 13.2 Hz, 2H each), 4.10 (t, J = 5.7 Hz, 2H), 4.05−3.90 (m, 6H), 3.90−3.70 (m, 8H), 3.50 (q, J = 6.8 Hz, 8H), 3.27 (s, 6H), 3.03 and 2.90 (2d, J = 13.2 Hz, 2H each), 1.20 and 1.18 (2t, J = 6.8 Hz, 6H each). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.1,

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150.2, 140.4, 138.7, 138.5, 138.4, 136.0, 135.5, 135.4, 128.4, 122.0, 116.5, 115.7, 73.3, 72.9, 69.7, 69.6, 66.4, 66.3, 30.9, 29.7, 15.3. ES-MS: m/z 780.3 $(M + Na)^+$, 758.3 $(M + H)^+$. Anal. Calcd for $C_{44}H_{59}N_3O_8$: C, 69.72; H, 7.85; N, 5.54. Found: C, 69.64; H, 7.97; N, 5.65.

5,11-Bis[(*N,N*′-di-Boc)guanidine]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (15). A stirring solution of the 1,2-vicinal diaminocalix[4]arene **11** (0.1 g, 0.135 mmol) and bis-Boc thiourea (0.088 g, 0.314 mmol) in dry DMF (6 mL) was cooled to 0 °C with an ice-bath and then Et_3N (0.131 mL, 0.94 mmol) and $HgCl_2$ (0.085 g, 0.314 mmol) were added. After 2 h, the reaction was quenched by adding AcOEt (15 mL) and filtering the HgS precipitate. The solvents were removed from the collected filtrate at the rotavapor and the resulting reddish foam chromatographed (SiO_2; hexane/AcOEt, 3:1). Compound **15** was obtained as a white solid (0.089 g; 54% yield): mp 104−106 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.58 (s, 2H), 9.79 (s, 2H), 6.74 (s, 2H), 6.73 (s, 2H), 6.62 (s, 6H), 4.49 (d, J = 12.8 Hz, 1H), 4.45 (d, J = 12.8 Hz, 2H), 4.41 (d, J = 12.8 Hz, 1H), 4.08 and 4.04 (2t, J = 6.1 Hz, 4H each), 3.81 and 3.79 (2t, J = 6.1 Hz, 4H each), 3.53 (q, J = 7.2 Hz, 8H), 3.14 (d, J = 12.8 Hz, 1H), 3.13 (d, J = 12.8 Hz, 3H), 1.51 and 1.47 (2s, 18H each), 1.25−1.12 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.6, 156.5, 153.9, 153.4, 153.2, 135.4, 135.1, 134.8, 130.6, 128.3, 122.7, 122.6, 122.1, 83.3, 79.2, 73.1, 69.7, 69.4, 66.4, 30.9, 28.2, 28.1, 15.3. ES-MS: m/z 1249.5 $(M + Na)^+$, 1227.7 $(M + H)^+$. Anal. Calcd for $C_{66}H_{94}N_6O_{16}$: C, 64.58; H, 7.72; N, 6.85. Found: C, 64.63; H, 7.81; N, 6.95.

5,17-Bis[(*N,N*′-di-Boc)guanidine]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (16). The reaction was carried out as for the preparation of compound **15**, starting from 1,3-distal diaminocalix[4]arene **12** (0.12 g, 0.16 mmol). A pure sample of **16** was obtained by flash column chromatography (SiO_2, cyclohexane/AcOEt, 15:4) as a white solid (0.086 g; 45% yield): mp 157−160 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.65 (s, 2H), 10.24 (s, 2H), 7.29 (s, 4H), 6.32−6.19 (m, 6H), 4.45 (d, J = 13.2 Hz, 4H), 4.24 (t, J = 6.6 Hz, 4H), 3.95−3.81 (m, 8H), 3.76 (t, J = 5.0 Hz, 4H), 3.56 and 3.51 (2q, J = 7.2 Hz, 4H each), 3.12 (d, J = 13.2 Hz, 4H), 1.54 and 1.49 (2s, 18H each), 1.23 and 1.18 (2t, J = 7.2 Hz, 6H each). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.7, 155.0, 154.7, 153.4, 153.3, 136.9, 133.0, 130.7, 127.8, 122.6, 122.4, 83.5, 79.4, 73.9, 72.5, 69.6, 69.5, 66.5, 66.2, 30.8, 28.2, 28.1, 15.4, 15.3. ES-MS: m/z 1227.6 $(M + H)^+$. Anal. Calcd (%) for $C_{66}H_{94}N_6O_{16}$: C, 64.58; H, 7.72; N, 6.85. Found: C, 64.51; H, 7.83; N, 6.93.

5,11,17-Tris[(*N,N*′-di-Boc)guanidine]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (17). The reaction was carried out according to the preparation of compound **15**, starting from triamino compound **13** (0.147 g, 0.194 mmol), 3 equiv of bis-Boc-thiourea and $HgCl_2$ and 9 equiv of Et_3N. A pure sample of **17** was obtained by flash column chromatography (SiO_2; hexane/AcOEt, 3:1) as a slightly pink oil (0.145 g; 50% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.67 (bs, 2H), 11.55 (bs, 1H), 10.27 (bs, 2H), 9.31 (bs, 1H), 7.33 and 7.28 (2d, 2H each, J = 2.3 Hz), 6.37 (t, 1H, J = 7.6 Hz), 6.22 (d, 2H, J = 7.6 Hz), 6.17 (s, 2H), 4.45 and 4.41 (2d, J = 13.5 Hz, 2H each), 4.20 (t, J = 5.9 Hz, 4H), 3.60−3.47 (m, 12H), 3.60−3.46 (m, 8H), 3.13 and 3.11 (2d, J = 13.5 Hz, 2H each), 1.53, 1.49, 1.47, and 1.42 (4s, 54H), 1.22 and 1.16 (2t, J = 6.9 Hz, 6H each). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.6, 155.1, 154.9, 153.8, 153.4, 153.2, 153.0, 152.7, 136.9, 136.7, 133.6, 133.2, 130.8, 130.4, 127.8, 123.3, 122.5, 122.4, 122.1, 83.4, 83.1, 79.4, 79.0, 73.8, 72.5, 69.6, 69.5, 69.4, 66.6, 66.5, 66.2, 31.0, 28.2, 28.1, 15.4, 15.2. ES-MS: m/z 1484.6 $(M + H)^+$, 1506.6 $(M + Na)^+$. Anal. Calcd for $C_{77}H_{113}N_9O_{20}$: C, 62.29; H, 7.67; N, 8.49. Found: C, 62.18; H, 7.73; N, 8.56.

5,11,17,23-Tetrakis[(*N,N*′-di-Boc)guanidine]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (18). The reaction was carried out according to the preparation of compound **15**, starting from tetraamino compound **14** (0.650 g, 0.84 mmol), 4.5 equiv of bis-Boc-thiourea and $HgCl_2$ and 12 equiv of Et_3N in dry DMF (30 mL). After 18 h, DCM was added and HgS filtered off. After removal of DCM at the rotavapor, the DMF solution was left standing, and a white solid precipitated, was filtered off, and washed with hexane. The solvents were completely removed from the filtrate at the rotavapor, and the residue was uptaken in AcOEt (15 mL) and washed with H_2O (15 mL). The organic phase was separated and dried over Na_2SO_4 and the filtrate evaporated under reduced pressure. The residue was recrystallized from Et_2O/hexane to obtain pure compound **18** (0.44 g; 59% yield): mp > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.59 (s, 4H), 9.83 (s, 4H), 6.94 (s, 8H), 4.46 (d, J = 13 Hz, 4H), 4.09 (t, J = 5.6 Hz, 8H), 3.83 (t, J = 5.6 Hz, 8H), 3.53 (q, J = 7 Hz, 8H), 3.15 (d, J = 13 Hz, 4H), 1.47 and 1.44 (2s, 36H each), 1.20 (t, J = 7 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.6, 153.6, 153.0, 134.7, 131.0, 123.2, 83.0, 79.1, 73.3, 69.4, 66.4, 31.2, 28.2, 28.1, 15.3. ES-MS: m/z 893.46 $(M + 2Na)^{2+}/2$, 1763.85 $(M + Na)^+$.

5,11-Diguanidine-25,26,27,28-tetrakis(2-ethoxyethoxy)-calix[4]arene Bis-hydrochloride (1·2HCl). To a stirring solution of the 1,2-vicinal derivative **15** (0.083 g, 0.068 mmol) in 1,4-dioxane (6.8 mL), 37% HCl (0.23 mL, 2.76 mmol) was added. After 5 days at room temperature, the solvent was removed at the rotavapor; pure compound **1**·2HCl was obtained in quantitative yield as a white solid (0.061 g; 99% yield): mp 136−139 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 6.78−6.60 (m, 10H), 4.62 (d, J = 13.5 Hz, 1H), 4.60 (d, J = 13.5 Hz, 2H), 4.57 (d, J = 13.5 Hz, 1H), 4.28−4.09 (m, 8H), 4.00−3.82 (m, 8H), 3.62 and 3.57 (2q, J = 7.0 Hz, 4H each), 3.25 (d, J = 13.5 Hz, 1H), 3.23 (d, J = 13.5 Hz, 2H), 3.20 (d, J = 13.5 Hz, 1H), 1.22 and 1.21 (2t, J = 7.0 Hz, 6H each). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.5, 156.3, 156.0, 137.3, 136.4, 135.3, 134.4, 128.3, 127.9, 125.5, 125.0, 122.3, 73.8, 73.3, 70.0, 69.7, 66.2, 66.0, 30.4, 30.3, 14.3, 14.2. ES-MS: m/z 849.32 (**1** + Na)$^+$, 827.35 (**1** + H)$^+$: HR ES-MS: m/z Calcd for $C_{46}H_{63}N_6O_8^+$ 827.4707 (**1** + H)$^+$, found 827.4693.

5,17-Diguanidine-25,26,27,28-tetrakis(2-ethoxyethoxy)-calix[4]arene Bis-hydrochloride (2·2HCl). The reaction was carried out according to the preparation of compound **1**, stirring for 4 days a mixture of compound **16** (0.086 g, 0.07 mmol), 0.23 mL of 37% HCl (2.76 mmol), and 5 mL of 1,4-dioxane. Compound **2**·2HCl was obtained in quantitative yield as a pale-yellow oil (0.077 g; 99% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.00 (d, J = 7.2 Hz, 4H), 6.83 (t, J = 7.2 Hz, 2H), 6.42 (s, 4H), 4.62 (d, J = 13.0 Hz, 4H), 4.33 (t, J = 6.0 Hz, 4H), 4.09 (t, J = 5.0 Hz, 4H), 3.97 (t, J = 6.0 Hz, 4H), 3.91 (t, J = 5.0 Hz, 4H), 3.64 and 3.57 (2q, J = 7.0 Hz, 4H each), 3.25 (d, J = 13.0 Hz, 4H), 1.27 and 1.20 (2t, J = 7.0 Hz, 6H each).^{13}C NMR (75 MHz, CD_3OD): δ 158.2, 157.8, 156.5, 137.7, 136.7, 130.0, 129.5, 126.1, 124.0, 75.4, 74.2, 71.2, 67.6, 67.3, 31.8, 15.72, 15.68. ES-MS: m/z 414.1 $[(2 + 2H)^{2+}]/2$, 827.4 (**2** + H)$^+$; HR ES-MS m/z Calcd for $C_{46}H_{63}N_6O_8^+$ 827.4707 (**2** + H)$^+$, found 827.4738.

5,11,17-Triguanidine-25,26,27,28-tetrakis(2-ethoxyethoxy)-calix[4]arene Tris-hydrochloride (3·3HCl). The reaction was carried out according to the preparation of compound **1**, stirring for 4 days a mixture of the trifunctional derivative **17** (0.1 g, 0.068 mmol), 37% HCl (0.335 mL, 4.0 mmol), and 6.7 mL of 1,4-dioxane. The reaction was quenched, removing the solvent at the rotavapor, and pure compound **3**·3HCl was obtained in quantitative yield as a pale-yellow oil (0.077 g; 99% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 6.83 (d, J = 7.2 Hz, 2H), 6.79−6.71 (m, 3H), 6.55 (s, 4H), 4.61 and 4.58 (2d, J = 13.3 Hz, 2H each), 4.25 (t, J = 3.9 Hz, 2H), 4.19 (t, J = 5.1 Hz, 2H), 4.20−4.05 (m, 4H), 3.99−3.81 (m, 8H), 3.72−3.50 (m, 8H), 3.26 and 3.23 (2d, J = 13.3 Hz, 2H each), 1.26−1.11 (m, 12H). ^{13}C NMR (75 MHz, CD_3OD) δ: 157.87, 157.82, 157.76, 157.58, 156.9, 138.1, 138.0, 137.3, 136.1, 129.7, 127.0, 126.6, 126.2, 124.0, 75.3, 75.1, 74.5, 71.4, 71.3, 71.0, 67.6, 67.4, 31.8, 31.7, 15.72, 15.68. ES-MS: m/z 442.8 $[(3 + 2H)^{2+}]/2$, 295.6 $[(3 + 3H)^{3+}]/3$. HR ES-MS m/z Calcd for $C_{47}H_{67}N_9O_8^{2+}$ 442.7556 $[(3 + 2H)^{2+}]/2$, found 442.7570.

5,11,17,23-Tetraguanidine-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene Tetrakis-hydrochloride (4·4HCl). To a stirring solution of compound **18** (0.425 g, 0.24 mmol) in 1,4-dioxane (30 mL) at rt were added a 37% HCl aqueous solution (3.5 mL) and triethylsilane (TES) (0.39 mL, 2.44 mmol). After 24 h, the solvent was evaporated and the residue dissolved in water and washed with Et_2O (2 × 10 mL). The aqueous layer was lyophilized, thus leaving a sample of pure compound **4**·4HCl (0.250 g; 96% yield): mp > 300 °C. ^{1}H NMR (300 MHz, D_2O): δ 6.78 (s, 8H), 4.53 (d, J = 13.5, 4H), 4.25 (t, J = 4.5 Hz, 8H), 3.99 (t, J = 4.5 Hz, 8H), 3.66 (q, J = 7 Hz, 8H), 3.37 (d, J = 13.5, 4H), 1.20 (t, J = 7 Hz, 12H). ^{13}C NMR (100 MHz, D_2O/MeOD, 7/3): δ 156.8, 156.5, 137.2, 129.1,

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126.1, 74.15, 70.8, 67.2, 31.0, 14.9. ES-MS: m/z 941.9 $(4 + H)^+$, 471.5 $[(4 + 2H)^{2+}]/2$. HR ES-MS m/z Calcd for $C_{48}H_{69}N_{12}O_8^+$ 941.5361 $(4 + H)^+$, found 941.5352.

***N*-(4-Methoxyphenyl)-*N*,*N*′-bis-(*tert*-butoxycarbonyl)-guanidine (19).** *N*,*N*′-Bis-(*tert*-butoxycarbonyl)-*N*″-triflyl-guanidine (304 mg, 0.77 mmol), and *p*-methoxyaniline (333 mg, 2.7 mmol) were dissolved in anhydrous DCM (100 mL). The reaction mixture was kept under stirring at room temperature for 40 h and then was washed with $KHSO_4$ (1 M, 50 mL) and a saturated solution of $NaHCO_3$ (50 mL). The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2; hexane/AcOEt (9:1) eluent), and active carbon was added to the eluted fractions containing the product. After filtration over Celite and evaporation of the solvent, compound **19** was obtained as a pale-yellow oil (229 mg, 0.63 mmol; 82% yield). ^{1}H NMR (300 MHz; $CDCl_3$): δ 1.53 (s, 9H), 1.49 (s, 9H), 3.79 (s, 3H), 6.86 (d, 2H, J = 9 Hz), 7.47 (d, 2H, J = 9 Hz). ^{13}C NMR (75 MHz; $CDCl_3$) 28.1, 28.2, 55.5, 114.0, 129.9, 127.6, 129.7, 153.6, 156.8. Anal. Calcd for $C_{18}H_{27}N_3O_5$: C, 59.16; H, 7.45; N, 11.50. Found: C, 58.90; H, 7.69; N, 11.42.

***N*-(4-Methoxyphenyl)guanidine Hydrochloride (5·HCl).** In 20 mL of a 1:1 mixture of dioxane and HCl 0.1 M in water were dissolved 229 mg (0.627 mmol) of compound **19**. The solution was kept under stirring at room temperature for 2 days. The solvent mixture was removed by evaporation, obtaining compound **5**·HCl as a white solid (115 mg, 0.571 mmol; 91% yield): mp 108−110 °C. ^{1}H NMR (300 MHz; CD_3OD): δ 3.75 (s, 3H), 6.96 (d, 2H, J = 9 Hz); 7.17 (d, 2H, J = 9 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 56.2, 115.8, 124.8, 127.5, 128.9, 159.3. Anal. Calcd for $C_8H_{12}ClN_3O$: C, 47.65; H, 6.00; N, 20.84. Found: C, 47.27; H, 6.09; N, 20.50.

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 = 10 mM (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 $-\log c_{H}^{+} = a + b \times pH_{read}$, and best fit values a = −0.7852 ± 1.5%, and b = 0.965 ± 5%. The pK_w values determined in several titrations coincided, within experimental errors, with the value reported in the literature.[62] 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.[67,68]

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 on 0.1 mM substrate solutions in the presence of 3 mM catalyst by UV−vis monitoring of *p*-nitrophenol liberation at 400 nm. Hydrochlorides were neutralized by the addition to the reaction mixture of the calculated amount of Me_4NOH before kinetic runs. 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.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra, plots of potentiometric acid−base titrations, and medium effects on acid−base equilibrium and transesterification rates. Background rates of HPNP transesterification in 80% DMSO, 25 °C. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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