Toward an Artificial Acetylcholinesterase

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Abstract: The methanolysis of choline *p*-nitrophenylcarbonate in chloroform containing 1% methanol is catalyzed with turnover by ditopic receptors **1** and **2**, consisting of a calix[6]arene connected to a bicyclic guanidinium by means of a short spacer. The calix[6]arene subunit strongly binds to the trimethylammonium head group through cation–π interactions, whereas the guanidinium moiety is deputed to stabilize through hydrogen bonding reinforced by electrostatic attraction the anionic tetrahedral intermediate resulting from methoxide addition to the ester carbonyl. The observed cholinesterase activity had been anticipated on the basis of the ability of the ditopic receptors **1** and **2** to bind strongly to the choline phosphate DOPC, which is a transition state analogue for the $B_{Ac}2$-type cleavage of choline esters.

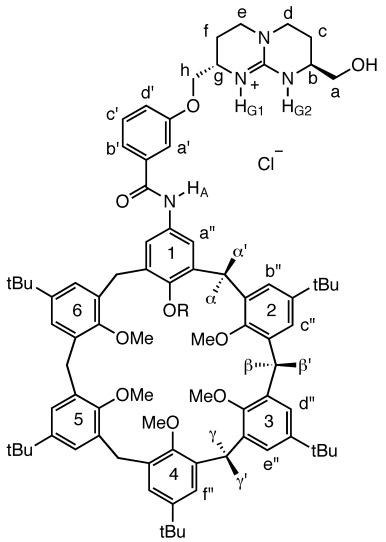
Keywords: acetylcholinesterase mimetics · calixarenes · molecular recognition · supramolecular catalysis · transition state analogue

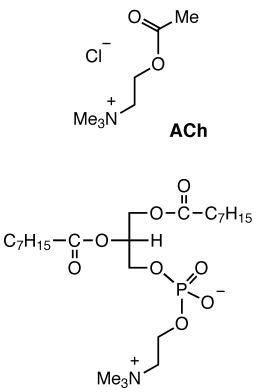
Introduction

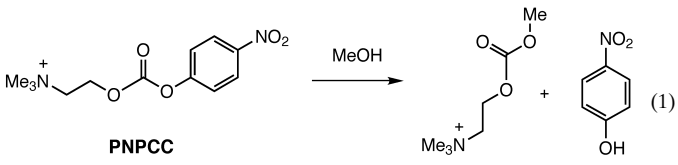
Phosphate diesters are well known transition state analogues for the basic hydrolysis of esters and carbonates.[1] This concept provided the basis for Schultz's pioneering study of chemical catalysis by the MOPC167 antibody, which binds *p*-nitrophenylphosphocholine with high affinity, and catalyzes the hydrolysis of *p*-nitrophenylcholine carbonate (PNPCC).[2] We reasoned therefore that synthetic receptors capable of binding specifically to dioctanoyl-L-α-phosphatidylcholine (DOPC) would also be expected to catalyze the basic hydrolysis of acetylcholine (ACh) and choline esters under appropriate conditions, as well as other reactions of the same substrates occurring via a $B_{Ac}2$ mechanism.

The ditopic receptor **1** has been recently shown[3] to mimic the phosphocholine binding site of the McPC603 antibody,[4] because it binds strongly and specifically to DOPC in chloroform solution. The complex is stabilized both by ion-pairing and hydrogen bonding between the phosphate monoanion and the guanidinium moiety.[5] Encapsulation of the choline trimetylammonium group in the calix[6]arene subunit, governed by cation–π interactions, provides an additional source of stability.[6]

In this article we report that **1** and its more preorganized cyclohexylmethyl derivative **2** promote the cleavage of PNPCC [Eq. (1)] under slightly basic conditions with high rate enhancements and catalytic turnover.[7]







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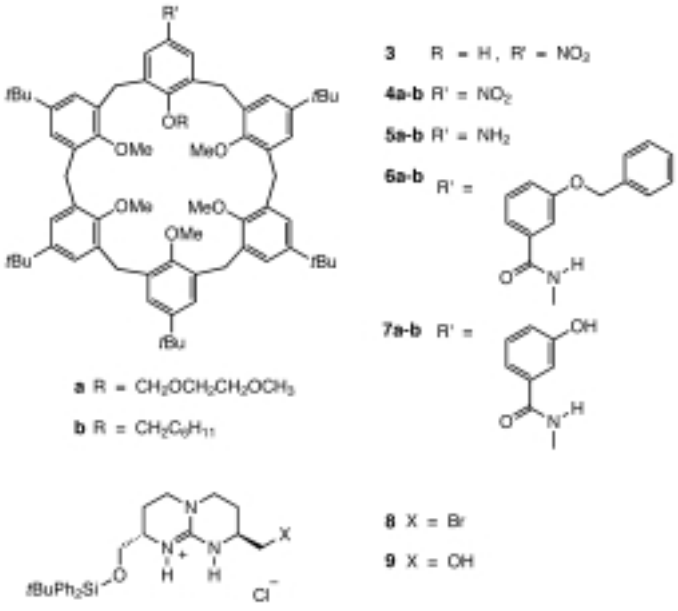
Results and Discussion

Synthesis: Receptors **1** and **2** were assembled in five steps from readily available mononitrocalix[6]arene **3**.[8] Free phenol was protected either by methoxyethoxymethyl (MEM) or by cyclohexylmethyl, affording **4a** and **4b** in 85 and 78% yields, respectively. Reduction of nitro group (H_2/PtO_2) gave amines **5a–b** quantitatively. The amide spacer was then introduced by reaction with *m*-benzyloxybenzoyl chloride (**6a–b**, 88 and 80%) and deprotected to yield **7a** and **7b** (H_2/10% Pd/C, 85 and 100%). Finally, receptors **1** and **2** resulted in 48 and 32% yields, respectively, from the reaction of **7a–b** with the (*S,S*)-guanidinium bromo-derivative **8**.[9]

Structural studies: Although receptors **1** and **2** are rather flexible molecules, their most stable conformations in chloroform or dichoromethane solutions were established unambiguously by NMR spectroscopy. ^{1}H-NMR spectra of **1** and **2** are consistent with the presence of an average symmetry plane bisecting the calixarene macrocycle. The bridging methylene protons are shown as singlets in **1**; this indicates a rapid inversion of the cone at room temperature. On the contrary, however, methylene protons appear as three AX-systems in receptor **2**, suggesting a less flexible conformation for the calixarene. The data are fully consistent with those described by Gutsche for related compounds.[10]

Abstract in Spanish: *Los receptores ditópicos **1** y **2**, constituidos por una subunidad de calix[6]areno unida, mediante un corto espaciador, a un grupo guanidinio bicíclico, catalizan la metanolisis del p-nitrofenilcarbonato de colina en cloroformo con un 1% de metanol. Se consiguen repetir varios ciclos catalíticos (turnover). La unidad de calix[6]areno compleja el grupo trimetilamonio mediante interacciones catión–π, mientras que el guanidinio estabiliza, por medio de enlaces de hidrógeno y atracción electrostática, el intermedio tetrahédrico formado tras el ataque nucleófilo del metóxido sobre el carbonilo del éster. La actividad de colinesterasa observada es consecuencia de la capacidad que tienen los receptores ditópicos **1** y **2** para complejar fosfato de dioctanoilcolina (DOPC), un análogo del estado de transición por el que transcurre la ruptura, de tipo $B_{Ac}2$, de los ésteres de colina.*

Abstract in Italian: *La metanolisi del p-nitrophenyl carbonato di colina in cloroformio contenente l% di metanolo viene catalizzata con turnover da recettori ditopici **1** e **2**, formati da un calix[6]arene collegato ad un guanidinio biciclico mediante un breve spaziatore. La subunità calix[6]arenica complessa fortemente il gruppo trimetilammonio mediante interazioni catione–π, mentre al gruppo guanidinio è affidato il compito di stabilizzare mediante legame idrogeno, rinforzato da attrazione elettrostatica, l'intermedio tetraedrico anionico che si forma a seguito della addizione di metossido al carbonile estereo. Questa attività catalitica di tipo colinesterasico era stata prevista sulla base della capacità dei recettori ditopici **1** e **2** di legarsi fortemente al fosfato di colina DOPC, che è un analogo dello stato di transizione per le scissioni di tipo $B_{Ac}2$ degli esteri della colina.*



The predominant cone conformation, required for the encapsulation of trimethylammonium, was also indicated by the ROE cross-peaks between aromatic protons in neighboring rings and by the ^{13}C NMR methylene signals at $\delta = 31-32$.[11] The slower cone inversion in **2** was also revealed by the ROE cross-peaks between aromatic protons of rings carrying the methoxy groups ($H_{b''}-H_{f''}$) and both protons of the adjacent methylene groups. In addition, cross-peaks between ring 1 protons ($H_{a''}$) and only the equatorial protons of the neighboring methylenes ($H_{\alpha'}$) show that this ring is not inverting.

Addition of one equivalent of DOPC to a $CDCl_3$ solution of **1** or **2** resulted in significant changes in the NMR spectra. Inclusion of the trimethylammonium head into the calix[6]-arene cavity was in all cases shown by significant upfield shifts of the trimethylammonium protons and the methylene protons bound to the quaternary nitrogen ($-\Delta\delta = 0.51$ and 0.35, respectively, for **1**, $-\Delta\delta = 0.20$ and 0.11 for **2**). On the other hand, important downfield shifts of the amide proton H_A ($\Delta\delta = 1.76$ for **1**, 1.08 for **2**) and the guanidine NH proton (H_{G1}) close to the linker ($\Delta\delta = 0.81$ for **1**, 0.89 for **2**) indicate strong hydrogen bonds. Somewhat surprisingly, the signal of the other guanidinium NH proton (H_{G2}) was shifted upfield in the complexes (by -0.51 in **1**, -0.16 in **2**). This accounts not only for the nonparticipation of the distal guanidinium NH proton in the phosphate complexation, but also for the effects of counterions on the chemical shifts of these protons, the upfield shift being probably the result of the missing chloride counterion.[12] Inclusion was confirmed by the observation of a rotating frame NOE between the methyl group of choline and one of the aromatic calixarene protons (namely $H_{a''}$) in DOPC-**1** (CD_2Cl_2).

Cone inversion is slower in DOPC-**1** than in the free receptor, allowing the observation of an AB system for the methylene group between rings 2 and 3. However, the fact that the remaining methylene groups appear as singlets and that weak ROEs are observed between methoxy protons in ring 4 and one of the aromatic protons in rings 3 and 5 suggests that some flexibility persists in this complex.

Complexation of DOPC by **2** did not result in significant changes on the 2D-NMR spectra, in agreement to the more preorganized host structure. Similar in the free receptor, NOE contacts between most calixarene aromatic protons ($H_{b''}-H_{f''}$) and both protons at the adjacent methylene groups were observed. For $H_{b''}$, the NOE is more intense with $H_{\alpha'}$ than with H_{α}. Proton $H_{a''}$, at the ring 1 carrying the bulkier substituent, shows NOE contacts with only the equatorial methylene proton ($H_{\alpha'}$). This is fully consistent with a lower flexibility in the region of rings 1, 2 and 6.

The linker also appears to be more structured in the complexes. In particular, proton $H_{a'}$ shows intense ROEs to the amide proton H_A and to the methylene group directly attached to the bicyclic guanidine (H_h), indicating a folding of the side-arm that results in the simultaneous participation of both the guanidinium and amide protons in hydrogen bonding to the guest phosphate group.

Extensive molecular modelling studies were performed for the DOPC-**1** complex. AMBER 4.0 force field was employed for the molecular mechanics calculations.[3] In full agreement with the NMR data, unrestrained molecular dynamics at 298 K in chloroform showed that the calix[6]arene moiety displays also a cone conformation in the calculated structure, with the choline head remaining inside the cavity, while the phosphate group interacts through strong hydrogen bonds with proximal guanidinium (H_{G1}) and amide (H_A) protons. Structures computed for DOPC-**1** complex in chloroform are fully compatible with the relative intensities of the observed NOEs.

Binding studies: In neat chloroform, equilibrium constants of $73\,000 \pm 5000\,\text{M}^{-1}$ and $730 \pm 30\,\text{M}^{-1}$ have been reported for the complexation of **1** with DOPC and ACh chloride, respectively.[3] For **2**, these constants are slightly higher, namely $95\,000 \pm 3000\,\text{M}^{-1}$ and $860 \pm 80\,\text{M}^{-1}$. We found that complexation is weaker, but still significant, in the mixture of solvents employed for the catalysis studies (see below), namely $CHCl_3/CH_3OH$ 99:1 (*v*/*v*). ^{1}H-NMR titrations in 99:1 $CDCl_3/CD_3OD$ showed that the equilibrium constants for binding of **1** and **2** to DOPC are about 6000 and $3000\,\text{M}^{-1}$, respectively, error limits being too high to allow accurate measurements, and that the corresponding values with ACh are 130 ± 10 and $170 \pm 20\,\text{M}^{-1}$.

Catalysis: Cleavage of PNPCC (iodide salt) [Eq. (1)] was studied in $CHCl_3/CH_3OH$ 99:1 (*v*/*v*) under slightly basic conditions. The tiny amount of methanol in the mixed solvent was dictated by the need of containing within acceptable limits the adverse effect of a polar, protic solvent on the relevant binding interactions.[13]

The methanolysis of PNPCC proceeded smoothly and quantitatively under buffered conditions. A first set of kinetic experiments is summarized in Table 1. The spectrophotometrically determined liberation of *p*-nitrophenol showed a clean first order time dependence both in the absence and presence of additives. Modest rate accelerations are seen in the presence of calix[6]arene model compounds **6c** (R = H) and **6b**, which lack the guanidinium "catalytic site" (entries 2 and 5). More significant is the nine-fold rate enhancement brought about by model compound **9**[5] (entry 1). This clearly

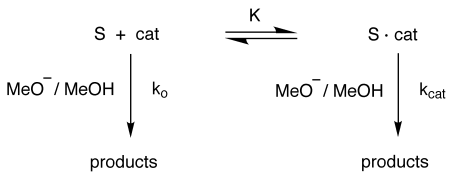
Table 1. Effect of 1.0 mM additives on the rate of methanolysis of 0.050 mM PNPCC in $CHCl_3/CH_3OH$ 99:1 (*v*/*v*) in the presence of 25 mM diisopropylethylamine/0.50 mM perchlorate salt buffer at 25 °C.

Entry	Additive(s)	k_{obs} [s^{-1}][a]	k_{obs}/k_o[b]
1	**9**	1.67×10^{-3}	9.0
2	**6c**	3.80×10^{-4}	2.0
3	**6c**+**9**	1.64×10^{-3}	9.0
4	**1**	1.41×10^{-2}	76
5	**6b**	2.66×10^{-4}	1.4
6	**6b**+**9**	1.43×10^{-3}	7.7
7	**2**	2.75×10^{-2}	149

[a] Errors in the order of ± 3–5%. [b] In the absence of additives $k_o = 1.85 \times 10^{-4}\,s^{-1}$ (background methanolysis).

demonstrates the ability of the guanidinium subunit to stabilize the negatively charged $B_{Ac}2$-type transition state. The largest rate accelerations are observed with ditopic receptors **1** and **2**. Comparison of these rate accelerations with those brought about by equimolar mixtures of the disconnected subunits (compare entries 4 with 3, and 7 with 6) shows that the two subunits in **1** and **2** bind the transition state with a considerable degree of synergism.

The overall mechanism of catalysis probably involves reversible formation of a substrate–catalyst complex (S·cat), followed by rate-limiting reaction with a solvent component (k_{cat}), as shown in Scheme 1. Consistent with a



Scheme 1. Overall mechanism of the catalysis.

rapid and reversible complexation between substrate and catalyst are the ^{1}H NMR titration data obtained with model compounds DOPC and ACh. Assuming that PNPCC is bound by **1** and **2** with equal affinities as ACh,[14] the fraction of complexed substrate under the conditions of the experiments listed in Table 1 is 0.12 with receptor **1** and 0.15 with receptor **2**. These figures, combined with the pertinent k_{obs}/k_o ratios translate into k_{cat}/k_o values of 600 for **1** and 1000 for **2**. Interestingly, these estimates compare very well with the k_{cat}/k_o ratio of 770 reported by Schultz et al.[2] for the hydrolysis of PNPCC catalyzed by MOPC167 antibody.

Turnover catalysis and competitive inhibition by DOPC was demonstrated by a second set of kinetic experiments, in which the substrate concentration was increased to 0.40 mM and the catalyst was the less concentrated component in the reaction mixture. The reaction progress in typical runs is shown in Figure 1. The sigmoid shape of the profile related to background methanolysis clearly reveals a kinetic complication which was absent in the more dilute substrate solution. Furthermore, the initial rate of $4.1 \times 10^{-8}\,\text{M}\,s^{-1}$ translates into a first order specific rate of $1.0 \times 10^{-4}\,s^{-1}$, that deviates markedly from the value of $1.85 \times 10^{-4}\,s^{-1}$ obtained at lower concentrations (Table 1). Figure 1 shows that in the presence

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of **1** or a mixture of **1** and DOPC, more regular profiles are obtained, but the corresponding first-order plots (not shown here) still exhibit significant deviations from linearity. A similar behaviour was observed in the presence of **2**.

In the basic hydrolysis of ACh the positive trimethylammonium stabilizes the negative charge developing at the carbonyl oxygen during the activation process.[2, 15] Since this cationic group is expected to be anion paired in $CHCl_3$/CH_3OH 99:1, the observed kinetic complications may be attributed, at least in part, to variations in concentrations and proportions of the several counteranions involved, namely Cl^- in **1** and **2**, ClO_4^- in the buffer, and I^- in PNPCC. An additional complication arises from the production of *p*-nitrophenoxide ion as the reaction proceeds. In very dilute substrate solutions (Table 1), the produced *p*-nitrophenoxide does not significantly alter the anion composition. In the more concentrated substrate solutions (Table 2 and Figure 1) the amount of *p*-nitrophenoxide becomes comparable to that of other anions. This might explain the lack of first-order time dependence in the background methanolysis (Figure 1a). When the trimethylammonium head is included into the calix[6]arene cavity, the importance of both the intramolecular assistance and ion pairing decreases, and the kinetics (Figure 1b and 1c) approach first-order. These considerations imply that the mechanism of the uncatalyzed and catalyzed reactions may not be strictly the same.[2]

Table 2. Competitive inhibition by DOPC in the methanolysis of 0.40 mM PNPCC catalyzed by **1** and **2**.[a]

Entry	Catalyst [mM]	DOPC [mM]	$10^7\ v_{obs}$[b] [Ms^{-1}]	$10^7\ (v_{obs} - v_o)$[c] [Ms^{-1}]
1	**1**, 0.10	none	5.1	4.7
2	**1**, 0.10	0.40	1.9	1.5
3	**2**, 0.10	none	5.3	4.9
4	**2**, 0.10	0.40	3.4	3.0
5	**2**, 0.02	none	1.5	1.1

[a] Reaction conditions as in Table 1. [b] Initial rate of liberation of *p*-nitrophenol; errors ± 10%. [c] In the absence of additives $v_o = 4.1 \times 10^{-8}\ M\,s^{-1}$ (background methanolysis).

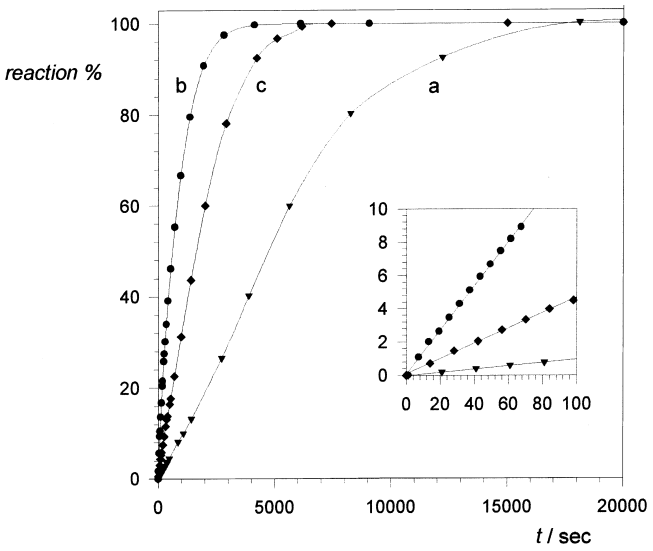

Figure 1. Reaction progress as a function of time in the methanolysis of 0.40 mM PNPCC. Curve a: background methanolysis. Curves b and c correspond to entries 1 and 2, respectively, in Table 2. The inset shows the early stages of reaction, from which initial rates were evaluated.

Because of the above kinetic complications initial rates rather than rate constants are reported for this set of experiments (Table 2). For the same reason, the adherence of the catalyzed reactions to Michaelis–Menten kinetics could not be tested. Nevertheless, these experiments demonstrate that receptors **1** and **2** are genuine turnover catalysts, in that in their presence PNPCC was completely reacted at rates that were significantly higher than background, even with a substrate to catalyst ratio of 20:1 (entry 5). Furthermore, if one assumes that the drop in initial rate (corrected for background) caused by the inhibitor (compare entries 2 with 1 and 4 with 3) reflects the diminution of catalyst available to the substrate, *K* values of $6400 \pm 1800\ M^{-1}$ and $1800 \pm 1000\ M^{-1}$ for the binding of DOPC to **1** and **2**, are calculated. Thus, the extent of inhibition is well commensurate to the extent of binding, as estimated from ^{1}H-NMR titrations.

Conclusion

Ditopic receptors **1** and **2** mimic not only the phosphocholine binding site of the McPC603 antibody, but also the catalytic site of the MOPC167 antibody. Despite the complications of detail, the essence of the catalysis is the possibility of using the calix[6]arene subunit as a recognition element for the non-reacting part of the substrate (choline moiety) and the guanidinium subunit for the specific recognition of the altered substrate in the transition state. Although the rate enhancements shown by compounds **1** and **2** are far below the remarkable accelerations furnished by acetylcholinesterase,[16] we believe that the results described in this article constitute a first, definite step toward the construction of artificial versions of the natural enzyme.[17]

Experimental Section

^{1}H-NMR spectra were recorded with Bruker AC-200 (200 MHz), AC-300 (300 MHz) or DRX-500 (500 MHz) spectrometers. ^{13}C-NMR spectra were recorded with a Bruker AC-300 (75 MHz) spectrometer. Mass spectra were recorded with a VG-AutoSpec instrument using a FAB^+ technique (NBA: *m*-nitrobenzyl alcohol). Elemental analyses were carried out with a Perkin–Elmer 2400 CHN analyzer. Spectrophotometric measurements were carried out in the thermostated cell compartment of a Hewlett–Packard 8452 A diode array instrument.

11,17,23,29,35-Penta-*tert*-butyl-37,38,39,40,41-pentamethoxy-42-(methoxyethyloxymethyloxy)-5-nitrocalix[6]arene (4a): A suspension of nitrocalix[6]arene **3**[8] (3.0 g, 2.9 mmol) and K_2CO_3 (485 mg, 4.9 mmol) in acetonitrile (300 mL) was stirred at room temperature under argon for 2 h. Methoxyethoxymethyl chloride (MEMCl) (0.6 mL, 5.25 mmol) was added and the mixture was stirred at room temperature for 24 h. Then a solution of NH_4OH (30%) (10 mL) was added and the mixture was stirred for 30 min. The solution was concentrated in vacuo, CH_2Cl_2 (100 mL) was added and the precipitate was filtered off. The organic solution was washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was solved in acetonitrile and allowed to precipitate, affording **4a** as a white solid (2.75 g, 85%). M.p. 168–169 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C):

$\delta = 7.57$ (s, 2H), 7.22 (d, ^4J(H,H) = 2.6 Hz, 2H), 7.21 (d, ^4J(H,H) = 2.6 Hz, 2H), 7.13 (s, 2H), 6.78 (d, ^4J(H,H) = 2.4 Hz, 2H), 6.62 (d, ^4J(H,H) = 2.4 Hz, 2H), 5.23 (s, 2H), 3.4–4.5 (brs, 12H), 4.02 (m, 2H), 3.6 (m, 2H), 3.52 (s, 6H), 3.39 (s, 3H), 2.63 (s, 3H), 2.51 (s, 6H), 1.34 (s, 18H), 1.25 (s, 9H), 0.89 (s, 18H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$, 25 °C, DEPT): $\delta = 158.6$, 154.22, 154.18, 153.1, 146.5, 145.9, 145.6, 144.0, 136.8, 133.6, 133.5, 133.4, 131.6, 128.3, 127.0, 126.9, 124.6, 124.5, 122.4, 98.9, 71.7, 69.6, 59.9, 59.8, 59.1, 34.2, 34.1, 33.9, 31.5, 31.4, 31.2, 30.9, 30.2, 30.1; HR-MS (FAB, NBA matrix): *m/z* (%): calcd for $C_{71}H_{93}NO_{10}$: 1120.68777; found 1120.69168 (100) $[M+1]^+$; $C_{71}H_{93}NO_{10}\cdot H_2O$ (1137.6): calcd C 74.90, H 8.41, N 1.23; found C 75.07, H 7.97, N 1.11.

5,11,17,23,29-Penta-*tert*-butyl-37-cyclohexylmethyloxy-38,39,40,41,42-pentamethoxy-35-nitrocalix[6]arene (4b): A suspension of calix[6]arene **3**[8] (4.0 g, 3.8 mmol) and K_2CO_3 (1.12 g, 8.1 mmol, 2.1 equiv) in acetonitrile (400 mL) was heated at 70 °C under argon for 2 h. Cyclohexylmethyl tosylate (4.3 g, 16 mmol) was added and the mixture was heated at 70 °C for 4 d. The solution was concentrated in vacuo. Water (150 mL) and CH_2Cl_2 (300 mL) were added and the organic layer was separated and washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was triturated in methanol to afford **4b** as a white solid (3.3 g, 78%). M.p. 238 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.58$ (s, 2H), 7.25 (d, ^4J(H,H) = 2.5 Hz, 2H), 7.20 (d, ^4J(H,H) = 2.5 Hz, 2H), 7.11 (s, 2H), 6.81 (d, ^4J(H,H) = 2.5 Hz, 2H), 6.64 (d, ^4J(H,H) = 2.5 Hz, 2H), 4.45 (d, AB system, 2H), 4.15 (d, AB system, 4H), 3.72 (d, AB system, 4H), 3.73 (d, ^3J(H,H) = 7 Hz, 2H), 3.55 (d, AB system, 2H), 3.47 (s, 6H), 2.68 (s, 3H), 2.54 (s, 6H), 2.00–1.60 (m, 5H), 1.34 (s, 18H), 1.24 (m, 6H), 1.23 (s, 9H), 0.91 (s, 18H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 159.7$, 154.2, 154.17, 153.2, 146.5, 145.9, 145.6, 143.6, 136.8, 133.7, 133.6, 133.5, 133.4, 131.8, 128.1, 126.9, 126.7, 124.7, 122.5, 78.7, 56.0, 59.9, 38.8, 34.2, 34.1, 33.9, 31.5, 31.4, 31.0, 30.6, 30.3, 30.1, 29.9, 26.4, 25.8; MS (FAB, NBA matrix): *m/z* (%): 1127.8 (100)$[M]^+$; $C_{74}H_{97}NO_8\cdot CH_3OH$ (1159.7): calcd C 77.60, H 8.78, N 1.21; found: C 77.19, H 8.70, N 1.23.

5-Amino-11,17,23,29,35-penta-*tert*-butyl-37,38,39,40,41-pentamethoxy-42-(methoxyethyloxymethyloxy)calix[6]arene (5a): A suspension of calix[6]-arene **4a** (295 mg, 0.264 mmol) and PtO_2 (34 mg, 0.149 mmol) in THF (23 mL) was bubbled with a hydrogen stream at room temperature for 30 min. The mixture was stirred under hydrogen for 24 h. The mixture was filtered through Celite and the filtrate was evaporated to dryness to afford **5a** as a white solid (287 mg, 100%). M.p. 138–140 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 7.13$ (d, ^4J(H,H) = 2.5 Hz, 2H), 7.12 (d, ^4J(H,H) = 2.5 Hz, 2H), 6.98 (brs, 2H), 6.85 (d, ^4J(H,H) = 2.2 Hz, 2H), 6.78 (d, ^4J(H,H) = 2.2 Hz, 2H), 5.86 (s, 2H), 5.08 (s, 2H), 4.01 (m, 2H), 3.95 (brs, 8H), 3.88 (brs 4H), 3.62 (m, 2H), 3.49 (s, 6H), 3.40 (s, 3H), 2.87 (s, 3H), 2.75 (brs, 2H), 2.73 (s, 6H), 1.28 (s, 18H), 1.10 (s, 9H), 1.02 (s, 18H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$, 25 °C, DEPT): $\delta = 154.3$, 153.9, 153.6, 145.9, 145.6, 145.3, 142.4, 135.2, 134.1, 133.4, 133.2, 133.16, 127.1, 126.9, 126.4, 125.2, 124.8, 113.7, 98.6, 71.9, 69.1, 60.0, 59.9, 59.0, 34.1, 34.0, 31.5, 31.3, 31.2, 31.0, 30.6, 30.3; MS (FAB, NBA matrix): *m/z* (%): 1090.7 (100) $[M+1]^+$; $C_{71}H_{95}NO_8$ (1089.7): C 78.18, H 8.78, N 1.28; found C 77.86, H 8.55, N 1.15.

35-Amino-5,11,17,23,29-penta-*tert*-butyl-38,39,40,41,42-pentamethoxy-37-cyclohexylmethyloxycalix[6]arene (5b): This compound was prepared as **5a** from calix[6]arene **4b** in quantitative yield. M.p. 228–230 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.18$ (d, ^4J(H,H) = 2.5 Hz, 2H), 7.11 (d, ^4J(H,H) = 2.5 Hz, 2H), 7.00 (s, 2H), 6.88 (d, ^4J(H,H) = 2.5 Hz, 2H), 6.80 (d, ^4J(H,H) = 2.5 Hz, 2H), 5.93 (s, 2H), 4.15 (brs, 8H), 3.87 (brs, 4H), 3.65 (d, ^3J(H,H) = 7 Hz, 2H), 3.40 (s, 6H), 2.89 (s, 3H), 2.72 (s, 6H), 2.3–1.8 (m, 5H), 1.28 (s, 18H), 1.20 (m, 6H), 1.12 (s, 9H), 1.04 (s, 18H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): $\delta = 154.2$, 153.8, 153.5, 146.7, 145.7, 145.68, 145.4, 141.7, 135.2, 134.1, 133.4, 133.1, 126.8, 126.2, 125.2, 125.0, 113.8, 78.3, 60.0, 59.8, 59.7, 38.8, 34.0, 33.96, 31.4, 31.3, 31.2, 30.6, 30.4, 30.3, 30.1, 26.5, 26.0; MS (FAB, NBA matrix): *m/z* (%): 1098.9 (100)$[M+1]^+$; $C_{74}H_{99}NO_6\cdot 2H_2O$ (1133.8): calcd C 78.32, H 9.16, N 1.23; found C 78.68, H 9.05, N 1.22.

35-*N*-(3-Benzyloxybenzoyl)amino-5,11,17,23,29-penta-*tert*-butyl-38,39,40,41,42-pentamethoxy-37-(methoxyethyloxymethyloxy)calix[6]arene (6a): A suspension of *m*-benzyloxybenzoic acid (175.6 mg, 0.77 mmol) in $SOCl_2$ (3 mL) was heated at 45 °C under argon for 1 h. The solution was concentrated in vacuo. The residue was disolved in THF (11 mL) and was slowly added into a solution of **5a** (760 mg, 0.7 mmol) in anhydrous THF (10 mL) and NEt_3 (0.5 mL). The mixture was refluxed for 16 h, the resulting solid was filtered and the solvent was removed in vacuo. The residue was triturated in methanol/water 1:1 to afford pure **6a** as a white solid (795 mg, 88%). M.p. 110–112 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 7.50$–7.28 (m, 9H), 7.22 (d, ^4J(H,H) = 2.5 Hz, 2H), 7.20 (m, 1H), 7.08 (d, ^4J(H,H) = 2.5 Hz, 2H), 7.06 (s, 2H), 6.99 (s, 2H), 6.88 (d, ^4J(H,H) = 2.5 Hz, 2H), 6.78 (d, ^4J(H,H) = 2.5 Hz, 2H), 5.14 (s, 2H), 5.08 (s, 2H), 4.00 (m, 2H), 3.90 (brs, 12H), 3.67 (m, 2H), 3.40 (s, 3H), 3.25 (s, 6H), 2.81 (s, 3H), 2.72 (s, 6H), 1.28 (s, 18H), 1.21 (s, 9H), 1.00 (s, 18H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$, 25 °C, DEPT): $\delta = 164.9$, 158.9, 154.2, 154.0, 153.6, 150.0, 146.1, 145.7, 145.5, 136.7, 136.5, 135.7, 133.9, 133.6, 133.5, 133.47, 133.3, 133.0, 129.5, 128.6, 128.1, 127.5, 127.0, 126.4, 125.3, 125.1, 120.2, 119.1, 118.2, 113.5, 98.7, 71.8, 70.1, 69.3, 60.1, 59.9, 59.87, 59.1, 34.2, 34.1, 34.0, 31.5, 31.2, 30.8, 30.4; HR-MS (FAB, NBA matrix): *m/z* (%): calcd for $C_{85}H_{105}NO_{10}$: 1299.77385, found 1299.77240 (100) $[M]^+$.

35-*N*-(3-Benzyloxybenzoyl)amino-5,11,17,23,29-penta-*tert*-butyl-38,39,40,41,42-pentamethoxy-37-cyclohexylmethyloxycalix[6]arene (6b): A suspension of *m*-benzyloxybenzoic acid (135 mg, 0.59 mmol) in $SOCl_2$ (4 mL) was heated at 45 °C under argon for 20 min. The solution was concentrated in vacuo. A solution of calix[6]arene **5b** (584 mg, 0.53 mmol) in anhydrous THF (15 mL) and Et_3N (0.5 mL) was added and the mixture was heated at 60 °C under argon for 24 h. The solution was concentrated in vacuo and the residue was disolved in CH_2Cl_2 (60 mL) and washed with 1N HCl (60 mL) and brine, dried (Na_2SO_4), and evaporated in vacuo. Trituration in cold methanol afforded pure **6b** as a white solid (560 mg, 80%). M.p. 156–160 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.40$ (m, 5H), 7.37 (s, 1H), 7.28 (d, ^4J(H,H) = 2.5 Hz, 2H), 7.23 (m, 4H), 7.06 (d, ^4J(H,H) = 2.5 Hz, 2H), 7.03 (s, 2H), 6.96 (s, 2H), 6.89 (d, ^4J(H,H) = 2.5 Hz, 2H), 6.79 (d, ^4J(H,H) = 2.5 Hz, 2H), 5.08 (s, 2H), 4.35 (d, AB system, 2H), 3.98 (d, AB system, 4H), 3.84 (d, AB system, 4H), 3.67 (d, ^3J(H,H) = 7 Hz, 2H), 3.58 (d, AB system, 2H), 3.19 (s, 6H), 2.86 (s, 3H), 2.74 (s, 6H), 2.00–1.57 (m, 5H), 1.27 (s, 18H), 1.20 (m, 6H), 1.18 (s, 9H), 1.01 (s, 18H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta =$ 165.0, 158.9, 154.1, 154.0, 153.6, 151.4, 146.1, 145.6, 145.5, 136.7, 136.5, 135.6, 133.9, 133.6, 133.4, 133.3, 133.2, 132.9, 129.5, 128.5, 128.0, 127.5, 127.0, 126.9, 126.2, 125.3, 125.2, 120.5, 119.1, 118.1, 113.4, 78.4, 70.1, 60.1, 60.0, 59.8, 38.9, 34.1, 34.06, 34.0, 31.5, 31.4, 31.2, 30.8, 30.6, 30.4, 30.1, 26.5, 26.0; MS (FAB, NBA matrix): *m/z* (%): 1309.1 (100)$[M+1]^+$; $C_{88}H_{109}NO_8\cdot 2CH_3OH$ (1371.9): calcd C 78.72, H 8.60, N 1.02; found C 78.66, H 8.37, N 1.11.

35-*N*-(3-Benzyloxybenzoyl)amino-5,11,17,23,29-penta-*tert*-butyl-37-hydroxy-38,39,40,41,42-pentamethoxycalix[6]arene (6c): A solution of **6a** (200 mg, 0.15 mmol) in THF (25 mL), was stirred at room temperature with conc. HCl in water (2 mL) for 12 h. The solution was concentrated in vacuo and the residue was dissolved in CH_2Cl_2 (25 mL), washed with brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by flash chromatography (hexane/THF 9:1) to afford an oil which was triturated in a mixture MeOH/H_2O 95:5 to give **6c** (150 mg, 57%) as a white solid. M.p. 132–138 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 7.67$ (s, 1H), 7.45–7.33 (m, 8H), 7.18 (s, 2H), 7.12 (m, 1H), 7.09 (d, ^4J(H, H) = 2.5 Hz, 2H), 7.07 (d, ^4J(H, H) = 2.5 Hz, 2H), 7.00 (d, ^4J(H, H) = 2.3 Hz, 2H), 6.96 (d, ^4J(H, H) = 2.3 Hz, 2H), 6.93 (s, 2H), 5.11 (s, 2H), 3.92 (s, 8H), 3.84 (s, 4H), 3.45 (s, 3H), 3.27 (s, 6H), 2.98 (s, 6H), 1.17 (s, 18H), 1.16 (s, 18H), 1.06 (s, 9H); HR-MS (FAB, NBA matrix): *m/z* (%): calcd for $C_{81}H_{98}NO_8$: 1212.72924, found: 1212.7299 (100) $[M+1]^+$; $C_{81}H_{97}NO_8\cdot \frac{1}{2}H_2O$ (1220.7): C 79.64, H 8.08, N 1.15; found: C 79.49, H 7.81, N 1.02.

5,11,17,23,29-Penta-*tert*-butyl-35-*N*-(3-hydroxybenzoyl)amino-38,39,40,41,42-pentamethoxy-37-(methoxyethyloxymethyloxy)calix[6]arene (7a): A suspension of calix[6]arene **6a** (400 mg, 0.305 mmol) and 10% Pd/C (327 mg, 0.305 mmol) in THF (50 mL) was bubbled with a hydrogen stream at room temperature for 15 min. The mixture was stirred under H_2 for 24 h, filtered through Celite and the filtrate was evaporated. The residue was triturated in MeOH to afford pure **7a** as a white solid (313 mg, 85%). M.p. 181–183 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 7.54$ (s, 1H), 7.21 (brs, 1H), 7.19 (d, ^4J(H,H) = 2.4 Hz, 2H), 7.16 (brs, 1H), 7.14 (d, ^4J(H,H) = 2.5 Hz, 2H), 7.05 (d, ^4J(H,H) = 2.4 Hz, 2H), 6.97 (s, 2H), 6.94 (m, 3H), 6.89 (m, 3H), 5.15 (s, 2H), 4.4–4.1 (brs, 2H), 3.90–3.60 (brs, 2H), 4.03 (m, 2H), 3.92 (brs, 8H), 3.64 (m, 2H), 3.41 (s, 3H), 3.13 (s, 6H), 3.01 (s, 3H), 2.9 (s, 6H), 1.24 (s, 18H), 1.07 (s, 27H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$, 25 °C, DEPT): $\delta = 165.6$, 156.8, 154.0, 153.8, 153.6, 149.9, 146.2, 145.9, 145.7, 136.2, 135.6, 133.9, 133.6, 133.4, 133.2, 133.21, 133.0, 129.6, 126.8, 126.0, 125.6, 125.5, 120.8, 118.8, 118.3, 114.3, 98.7, 71.8, 69.4, 60.2, 60.0, 59.9, 59.0, 34.1, 34.0, 31.4, 31.3, 31.2, 31.1, 30.9, 30.5; HR-MS (FAB, NBA matrix): *m/z* (%): calcd for $C_{78}H_{100}NO_{10}$: 1210.73472, found 1210.73267 (100) $[M+1]^+$; $C_{78}H_{99}NO_{10}\cdot MeOH$ (1241.7): C 76.34, H 8.36, N 1.13; found C 75.95, H 7.90, N 1.01.

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5,11,17,23,29-Penta-*tert*-butyl-35-*N*-(3-hydroxybenzoyl)amino-38,39,40,41, 42-pentamethoxy-37-cyclohexylmethyloxycalix[6]arene (7b): Prepared as **7a** in quantitative yield. M.p. 176–180 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.35$ (s, 1H), 7.23 (d, $^4J(H,H) = 2.5$ Hz, 2H), 7.16 (m, 4H), 7.05 (d, $^4J(H,H) = 2.5$ Hz, 2H), 6.93 (d, $^4J(H,H) = 2.5$ Hz, 4H), 6.90 (d, $^4J(H,H) = 2.5$ Hz, 4H), 6.14 (brs, 1H), 4.28 (d, AB system, 2H), 3.96 (d, AB system, 2H), 3.91 (d, AB system, 4H), 3.79 (d, AB system, 2H), 3.67 (d, $^3J(H,H) = 7$ Hz, 2H), 3.64 (d, AB system, 2H), 3.12 (s, 6H), 3.02 (s, 3H), 2.93 (s, 6H), 2.10–1.65 (m, 5H), 1.24 (s, 18H), 1.20 (m, 6H), 1.07 (s, 9H), 1.04 (s, 18H); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): $\delta = 166.0$, 156.8, 154.0, 153.7, 151.5, 146.2, 145.7, 135.5, 134.0, 133.7, 133.3, 132.7, 129.6, 126.8, 125.8, 125.6, 121.1, 118.9, 118.2, 114.4, 78.6, 60.2, 60.0, 59.8, 38.9, 34.1, 34.0, 31.4, 31.3, 30.8, 30.6, 30.4, 30.1, 26.6, 26.0; MS (FAB, NBA matrix): *m*/*z* (%): 1218.9 (100)$[M+1]^+$; $C_{81}H_{103}NO_8 \cdot 2CH_3OH$ (1281.8): calcd C 77.70, H 8.73, N 1.09; found C 77.20, H 8.60, N 1.13.

Compound 1: A suspension of calix[6]arene **7a** (652 mg, 0.540 mmol) and Cs_2CO_3 (442 mg, 1.35 mmol) in anhydrous acetonitrile (30 mL) was heated at 70 °C under argon for 1 h and then a solution of bromoguanidinium **8**[9] (410 mg, 0.76 mmol) in anhydrous acetonitrile (10 mL) was added. The mixture was heated at 70 °C for 3 d. After cooling at room temperature conc. HCl (35%, 16 mL) was added and the solution was stirred for 20 h. Water was added (10 mL) and the organic solvent was evaporated. The aqueous layer was extracted with $CHCl_3$, the organic extract was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (CH_2Cl_2/methanol 30:1 to 10:1) to afford pure **1**. (347 mg, 48%). M.p. 199–200 °C; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta =$ 9.06 (s, 1H, H_{G2}), 8.22 (s, 1H, H_A), 8.18 (s, 1H, H_{G1}), 7.62 (s, 1H, $H_{a'}$), 7.47 (s, 1H, OH), 7.42 (d, $^4J(H,H) = 7.5$ Hz, 1H, $H_{b'}$), 7.39 (s, 2H, $H_{a''}$), 7.32 (t, $^4J(H,H) = 8.0$ Hz, 1H, $H_{c'}$), 7.10 (d, $^4J(H,H) = 2.0$ Hz, 2H, $H_{e''}$), 7.07 (s, 1H, $H_{d'}$), 7.04 (d, $^4J(H,H) = 2$ Hz, 2H, $H_{b''}$), 6.96 (d, $^4J(H,H) = 2.5$ Hz, 2H, $H_{d''}$), 6.92 (d, $^4J(H,H) = 2.0$ Hz, 2H, $H_{c''}$), 6.91 (s, 2H, $H_{f'}$), 4.16 (m, 1H, H_h), 3.97 (m, 1H, H_h), 3.91 (brs, 4H, H_γ), 3.89 (brs, 4H, H_β), 3.84 (m, 1H, H_g), 3.81 (brs, 4H, H_α), 3.78 (m, 1H, H_a), 3.57 (m, 1H, H_a), 3.49 (brs, 1H, H_b), 3.46 (s, 3H, MeO ring 4), 3.35 (m, 2H, H_e), 3.31 (m, 2H, H_d), 3.28 (s, 6H, MeO rings 2, 6), 3.24 (m, 1H, H_d), 2.90 (s, 6H, MeO rings 3, 5), 2.11 (m, 1H, H_f), 1.98 (m, 1H, H_f), 1.90 (m, 1H, H_c), 1.82 (m, 1H, H_c), 1.17 (s, 18H, *t*Bu rings 3, 5), 1.10 (s, 18H, *t*Bu rings 2, 6), 1.05 (s, 9H, *t*Bu ring 4); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$, 25 °C, HMQC): $\delta = 127.0$, 126.5, 126.2, 125.8, 121.5 (ArCH), 71.0 (CH_2), 68.0 (CH), 64.0 (CH_2), 60.2, 60.1 (CH_3O), 44.0 (CH_2), 32.0, 31.0, 30.0 ($ArCH_2Ar$), 31.5, 31.4, 31.3 (($CH_3)_3C$), 22.5 (CH_2); HR-MS (FAB, NBA matrix): *m*/*z* (%): calcd for $C_{83}H_{107}N_4O_9$: 1303.80381, found: 1303.80240 (100) $[M]^+$; $C_{83}H_{107}N_4O_9Cl.MeOH$ CH_2Cl_2 (1454.7): C 70.11, H 7.83, N 3.85; found: C 70.09, H 7.71, N 4.04.

Compound 2: Prepared similarly, from calix[6]arene **7b** (661 mg, 0.542 mmol), Cs_2CO_3 (442 mg, 1.35 mmol), and bromoguanidinium **8**[9] (410 mg, 0.76 mmol). Light yellow solid (254 mg, 32%). M.p. 184–186 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 8.69$ (s, 1H, H_{G2}), 8.12 (s, 1H, H_{G1}), 7.86 (s, 1H, H_A), 7.39 (s, 1H, $H_{a'}$) 7.24 (m, 4H, $H_{b'}$,$H_{c'}$,$H_{b''}$), 7.11 (m, 3H, $H_{d'}$), 7.01 (d, $^4J(H,H) = 2.5$ Hz, 4H, $H_{c''}$), 7.00 (d, $^4J(H,H) = 1.8$ Hz, 1H, $H_{e''}$), 6.98 (s, 2H, $H_{f''}$), 6.84 (s, 2H, $H_{d''}$), 4.61 (brs, 1H, OH), 4.29 (d, AB system, 2H, H_α), 4.06 (m, 1H, H_h), 4.01 (m, 1H, H_h), 4.01 (d, AB system, 2H, H_γ), 3.90 (d, AB system, 2H, H_β), 3.83 (d, AB system, 2H, H_g, $H_{\beta'}$), 3.74 (m, 1H, H_a) 3.73 (d, AB system, 2H, $H_{\gamma'}$), 3.64 (d, 2H, OCH_2cychx), 3.59 (d, AB system, 2H, $H_{\alpha'}$), 3.55 (m, 1H, H_a), 3.50 (m, 1H, H_b) 3.30 (m, 4H, H_d, H_e), 3.20 (s, 3H, MeO ring 4), 3.00 (s, 6H, MeO rings 3, 5), 2.82 (s, 6H, MeO rings 2, 6), 2.12 (m, 1H, H_f), 1.98 (m, 1H, H_f), 1.96 (m, 1H, H_c), 2.10–1.60 (m, 5H, cychx), 1.85 (m, 1H, H_c), 1.3–1.0 (m, 6H, cychx), 1.22 (s, 18H, *t*Bu rings 2, 6), 1.15 (s, 9H, *t*Bu ring 4), 1.04 (s, 18H, *t*Bu rings 3, 5); ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz): $\delta = 165.0$, 158.1, 154.1, 153.8, 153.7, 151.4, 145.9, 145.6, 145.5, 136.6, 135.3, 133.8, 133.5, 133.4, 133.3, 133.2, 129.6, 126.8, 126.6, 125.7, 125.5, 120.7, 119.9, 118.2, 113.6, 78.5, 69.6, 64.0, 60.2, 59.9, 59.8, 50.7, 47.6, 45.7, 44.9, 38.8, 34.1, 34.0, 31.4, 31.2, 30.8, 30.6, 30.0, 26.5, 25.9, 22.9, 22.8, 22.5; MS (FAB, NBA matrix): *m*/*z* (%): 1400.05 $[M - Cl]^+$; HR-MS (FAB, NBA matrix): *m*/*z* (%): calcd for $C_{90}H_{119}N_4O_9$: 1399.8977, found 1399.8950 (100) $[M+1]^+$.

Preparation of the complexes DOPC-1 and DOPC-2: Complexes DOPC-**1** and DOPC-**2** were prepared adding 6.0×10^{-3} M (0.6 mL) solutions in $CDCl_3$ of each receptor over DOPC (3.05 mg, 6.00 mmol).

DOPC-1: ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 9.98$ (brs, 1H, H_A), 9.09 (brs, 1H, H_{G1}), 8.55 (brs, 1H, H_{G2}), 7.93 (s, 1H, $H_{a'}$), 7.83 (s, 1H, ArOH), 7.60 (d, $^3J(H,H) = 7.5$ Hz, 1H, $H_{b'}$), 7.43 (s, 2H, $H_{a''}$), 7.31 (t, $^3J(H,H) = 8.0$ Hz, 1H, $H_{c'}$), 7.18 (brs, 4H, $H_{b''}$, $H_{e''}$), 7.03 (d, $^4J(H,H) = 2.0$ Hz, 2H, $H_{d''}$), 6.99 (m, 1H, $H_{d'}$), 6.87 (s, 2H, $H_{f''}$), 6.71 (s, 2H, $H_{c''}$), 4.42 (m, 1H, H_h), 4.06 (m. 1H, H_h), 3.92 (br d, 2H, H_β), 3.88 (brs, 4H, H_γ, $H_{\gamma'}$), 3.88 (m, 1H, H_g), 3.82 (s, 4H, H_α, $H_{\alpha'}$), 3.80 (d, 2H, $H_{\beta'}$), 3.63 (s, 3H, MeO ring 4), 3.63 (m, 1H, H_a), 3.54 (m, 1H, H_b), 3.50 (m, 1H, H_d), 3.47 (m, 1H, H_a), 3.45 (m, 1H, H_e), 3.33 (s, 6H, MeO rings 2, 6), 3.30 (m, 1H, H_e), 3.20 (m, 1H, H_d), 3.01 (brs, 6H, MeO rings 2, 6), 2.15 (m, 1H, H_f), 2.03 (m, 1H, H_f), 1.85 (m, 1H, H_c), 1.72 (m, 1H, H_c), 1.24 (s, 18H, *t*Bu rings 3, 5), 1.11 (s, 18H, *t*Bu rings 2, 6), 1.04 (s, 9H, *t*Bu ring 4); MS (FAB, NBA matrix): *m*/*z* (%): 1813.6 (70) $[M]^+$.

DOPC-2: ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 9.01$ (s, 1H, H_{G1}), 8.94 (brs, 1H, H_A), 8.53 (s, 1H, H_{G2}), 7.62 (s, 1H, $H_{a'}$), 7.39 (d, $^3J(H,H) = 7.5$ Hz, 1H, $H_{b'}$), 7.30 (t, $^3J(H,H) = 8.0$ Hz, 1H, $H_{c'}$), 7.24, (brs, 2H, $H_{b''}$), 7.11 (brs, 2H, $H_{a''}$), 7.09 (brs, 2H, $H_{e''}$), 7.05 (brs, 1H, $H_{d'}$), 7.04 (brs, 2H, $H_{c''}$), 6.90 (s, 4H, $H_{d''}$, $H_{f''}$), 4.30 (brs, 2H, H_α), 4.19 (m, 1H, H_h), 4.17 (brs, 2H, H_γ), 4.06 (m, 1H, H_h), 3.99 (brs, 2H, H_β), 3.88 (m, 1H, H_g), 3.73 (brs, 2H, $H_{\beta'}$), 3.68 (brs, 1H, H_α), 3.63 (brs, 2H, $H_{\gamma'}$), 3.59 (brs, 2H, OCH_2cychx), 3.57 (brs, 2H, $H_{\alpha'}$) 3.52 (brs, 2H, H_a, H_b), 3.40 (m, 2H, H_e), 3.47 (brs, 1H, H_d), 3.30 (brs, 1H, H_d), 3.16 (s, 9H, MeO rings 3–5), 2.81 (s, 6H, MeO rings 2, 6), 2.17 (m, 1H, H_f), 2.00 (m, 1H, H_f), 1.93 (m, 1H, H_c), 1.9–1.0 (m, 11H, cychx), 1.83 (m, 1H, H_c), 1.25 (s, 18H, *t*Bu rings 2, 6), 1.16 (s, 18H, *t*Bu rings 3, 5), 1.04 (s, 9H, *t*Bu ring 4).

***p*-Nitrophenylcholine carbonate iodide (PNPCC)**:[18] This compound was prepared in two steps. *p*-Nitrophenyl chloroformate was treated with *N,N*-dimethylethanolamine followed by quaternization with methyl iodide in acetonitrile. M.p. 156–158 °C from acetonitrile/hexane/toluene 2:2:1 (lit.[18] 157–158 °C); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 8.45$ (d, $^2J(H,H) = 9$ Hz, 2H), 7.65 (d, $^2J(H,H) = 9$ Hz, 2H), 4.82 (m, 2H), 3.98 (m, 2H), 3.38 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 154.8$, 152.2, 146.3, 125.9, 123.0, 64.7, 62.9, 54.6; MS (FAB, NBA matrix): *m*/*z* (%): 269.1 (100) $[M+1-I]^+$; $C_{12}H_{17}N_2O_5I$ (396.2): calcd C 36.36, H 4.33, N 7.07; found C 36.35, H 4.39, N 7.22.

^{1}H-NMR titrations: Titration curves were obtained in $CDCl_3/CD_3OD$ 99:1 at 25 °C, by adding variable amounts of guest (DOPC or ACh) to a constant concentratrion (5.0 mM) of host (**1** or **2**). Plots of the variations of the chemical shift of the aromatic hydrogen $H_{a'}$ upon addition of the titrant were registered. Titration data were then treated according to a standard binding isotherm for the case of 1:1 association. At lower host concentration ranges signal $H_{a'}$ was too small to allow accurate location along the titration procedure.

Kinetics: Spectrophotometric grade chloroform was used without further purification. Dry methanol was prepared and handled as previously reported.[19] The reaction progress was followed spectrophotometrically by monitoring the liberation of *p*-nitrophenoxide at 420 nm. Because of the low solubility of PNPCC in CH_3Cl/CH_3OH 99:1 the reaction was started by adding to the mixture a small amount of a concentrated stock solution of PNPCC in CH_3CN.

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[1] L. Stryer, *Biochemistry*, 3rd ed., W. H. Freeman, New York, **1998**, p. 228.

[2] S. J. Pollack, J. W. Jacobs, P. G. Schultz, *Science* **1986**, *234*, 1570–1573. For additional examples see: D. Y. Jackson, J. R. Prudent, E. P. Baldwin, P. G. Schultz, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 58–62 and references therein.

[3] J. O. Magrans, A. R. Ortiz, A. Molins, P. H. P. Lebouille, J. Sánchez-Quesada, P. Prados, M. Pons, F. Gago, J. de Mendoza, *Angew. Chem.* **1996**, *108*, 1816–1819; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1712–1715.

[4] a) D. M. Segal, E. A. Padlan, G. H. Cohen, S. Rudikoff, M. Potter, D. R. Davies, *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 4298–4302; b) Y. Satow, G. H. Cohen, E. A. Padlan, D. R. Davies, *J. Mol. Biol.* **1986**, *190*, 593–604.

[5] a) F. P. Schmidtchen, *Tetrahedron Lett.* **1989**, *30*, 4493 – 4496; b) C. Andreu, A. Galán, K. Kobiro, J. de Mendoza, T. K. Park, J. Rebek Jr., A. Salmerón, N. Ussman, *J. Am. Chem. Soc.* **1994**, *116*, 5501 – 5502, and references therein; c) P. Schiessl, F. P. Schmidtchen, *J. Org. Chem.* **1994**, *59*, 509 – 511; d) V. Alcázar, M. Segura, P. Prados, J. de Mendoza, *Tetrahedron Lett.* **1998**, *39*, 1033 – 1036.

[6] a) D. A. Dougherty, D. A. Stauffer, *Science* **1990**, *250*, 1558 – 1560; b) J. C. Ma, D. A. Dougherty, *Chem. Rev.* **1997**, *97*, 1303 – 1324.

[7] For other examples of synthetic catalysts designed according to the above principles, see: a) E. V. Anslyn, R. Breslow, *J. Am. Chem. Soc.* **1989**, *111*, 5972 – 5973; b) V. Jubian, R. P. Dixon, A. D. Hamilton, *J. Am. Chem. Soc.* **1992**, *114*, 1120 – 1121; c) C. Raposo, M. Almaraz, M. Crego, M. L. Mussons, N. Pérez, C. Caballero, J. R. Morán, *Tetrahedron Lett.* **1994**, *35*, 7065 – 7068; d) R. P. Bonar-Law, L. G. Mackay, C. J. Walter, V. Marvaud, J. K. M. Sanders, *Pure Appl. Chem.* **1994**, *66*, 803 – 810; e) V. Alcázar, J. R. Morán, J. de Mendoza, *Tetrahedron Lett.* **1995**, *36*, 3941 – 3944, and references therein. For the use of guanidines as catalysts in the addition of nitromethane to aldehydes (Henry reaction), see: f) R. Chinchilla, C. Nájera, P. Sánchez-Agulló, *Tetrahedron: Asymmetry* **1994**, *5*, 1393 – 1402.

[8] a) J. de Mendoza, M. Carramolino, F. Cuevas, P. Nieto, P. Prados, D. N. Reinhoudt, W. Werboom, R. Ungaro, A. Casnati, *Synthesis* **1994**, 47 – 50; b) A. Casnati, L. Damiano, A. Pochini, R. Ungaro, M. Carramolino, J. O. Magrans, J. López Prados, P. Prados, J. de Mendoza, R. G. Janssen, W. Verboom, D. N. Reinhoudt, *Tetrahedron* **1995**, *51*, 12 699 – 12 720.

[9] a) W. Peschke, P. Schiessl, F. P. Schmidtchen, P. Bissinger, A. Schier, *J. Org. Chem.* **1995**, *60*, 1039 – 1043; b) J. Sánchez-Quesada, PhD thesis, Universidad Autónoma de Madrid (Spain), **1996**.

[10] S. Kanamathareddy, C. D. Gutsche, *J. Org. Chem.* **1994**, *59*, 3871 – 3879.

[11] C. Jaime, J. de Mendoza, P. Prados, P. M. Nieto, C. Sánchez, *J. Org. Chem.* **1991**, *56*, 3372 – 3376.

[12] For the downfield shift effect of chloride anions on bicyclic guanidinium protons, see [5b.]

[13] For previous examples of use of a mixed solvent containing a small amount of protic solvent, see: a) Y. Chao, D. J. Cram, *J. Am. Chem. Soc.* **1976**, *98*, 1015 – 1017; b) Y. Chao, G. R. Weisman, G. D. Y. Sogah, D. J. Cram, *J. Am. Chem. Soc.* **1979**, *101*, 4948 – 4958.

[14] The solubility of PNPCC was too low for equilibrium measurements.

[15] T. Bruice, S. J. Benkovic, *Bioorganic Chemistry*, *Vol. 1*, Benjamin, New York, **1965**, p. 134; b) H.-J. Schneider, U. Schneider, *J. Org. Chem.* **1987**, *52*, 1613 – 1615, and references therein.

[16] The catalytic accelerations of the basic and acid hydrolysis of ACh are in the order of 10^8 and 10^{13}, respectively, see: R. L. Schowen in *Transition State of Biochemical Processes* (Eds.: R. D. Gandour, R. L. Schowen), Plenum, New York, **1978**, p. 85.

[17] For previous attempts, see: a) J. M. Harrowfield, W. R. Richmond, A. N. Sobolev, *J. Inclusion Phenom. Mol. Recognit. Chem.* **1994**, *19*, 257 – 275; b) H.-J. Schneider, U. Schneider, *Chem. Ber.* **1994**, *127*, 2455 – 2469.

[18] J. Stadlmüller, PhD thesis, Ludwig Maximilians Universität München (Germany), **1991**.

[19] R. Cacciapaglia, S. Lucente, L. Mandolini, A. P. van Doorn, D. N. Reinhoudt, W. Verboom, *Tetrahedron* **1989**, *45*, 5293 – 5304.

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