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Programmed Enzyme-Mimic Hydrolysis of a Choline Carbonate by a Metal-Free 2-Aminobenzimidazole-Based Cavitand

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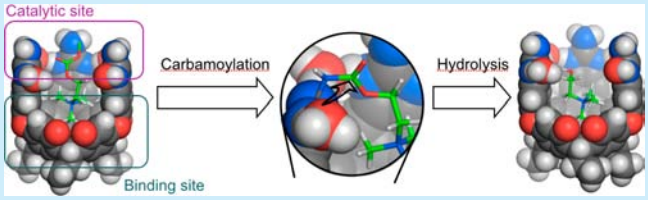
Supporting Information

ABSTRACT: The hydrolysis of a choline carbonate through a metal-free, enzyme-like mechanism has been achieved using a 2-aminobenzimidazole-based deep cavitand as catalyst. The supramolecular catalysis involves three steps: host−guest binding, carbamoylation and enzyme-like hydrolysis. Interestingly the rate-determining step proceeds through a programmed hydrolysis of carbamoylcholine-cavitand intermediate that may be driven by water molecules surrounding the benzimidazole walls of the cavity.



The development of artificial enzymes has recently attracted a lot of attention due to their applications in chemistry, biology, and medicine.[1] It is well-known that natural enzymes bind their substrates through weak interactions and selectively place the appropriate functional groups to achieve the catalysis. This approach helps chemists to design supramolecular systems that are able to simulate specific enzymatic roles. A number of supramolecular catalysts based on polymers,[2] cages,[3] cavities,[4] and macrocycles,[5] among others, have demonstrated enzyme-like activity in a wide variety of reactions. However, only a few examples of supramolecular catalysis that mimic the enzymatic mechanisms and kinetics have been reported.[6] Herein, we report the metal-free, enzyme-like hydrolysis of *p*-nitrophenylcholine carbonate (PNPCC) catalyzed by the supramolecular cavitand **1** (Figure 1). This reaction involves three steps, i.e. host−guest binding, covalent bonding, and a rate-determining hydrolysis that proceeds in days. It is remarkable that the final hydrolysis may proceed by the nucleophilic attack of the hydrogen bonded water molecules located between the 2-aminobenzimidazole (2-ABI) walls of the cavity.

Resorcin[4]arene cavitands having extended aromatic walls have been shown to be effective receptors for improved binding to charged and neutral small guests.[7] Work done mainly by Rebek et al. have demonstrated that deep cavitands decorated with a variety of functional groups at the upper rim fulfill specialized functions, such as encapsulation,[8] catalysis,[9] or as nanocontainers designed to stabilize unstable intermediates.[10] In particular, benzimidazole-based cavitands are very effective as hosts for alkylammonium compounds[11] and hydrocarbons[12] of suitable size and shape. The reason for the notable binding capabilities of benzimidazole cavities lies in their stable vase conformations under humid conditions due to the presence of hydrogen bonded water molecules bridging two consecutive benzimidazole walls that fix the vase conformation (Figure 1b).

Our intention here is to use a benzimidazole-based cavitand as the catalyst for a stepwise hydrolysis of choline carbonates. The new extended resorcinarene **1**, bearing (2-ABI) groups, was designed to offer an appropriate environment to accommodate the ammonium group of the choline derivative inside the cavity owing to the stable vase conformation of the cavitand. Concomitantly, the 2-ABI moieties and their hydrogen bonded structural water molecules may promote the hydrolysis of choline carbonates in the upper rim of the supramolecular pocket (see abstract image).

Previously, the hydrolysis of PNPCC was achieved through supramolecular catalysts based on cavitands or cyclodextrin bearing Zn(II) as an active Lewis acid. In these complexes, the metal center was key to modulating both the binding event and the hydrolysis.[6c,13] However, natural cholinesterases do not include any metal ion in the active center.

In this work, we prepared a metal-free cavitand **1**, which was obtained in moderate yields by a stepwise synthetic procedure (see the Supporting Information). The ^{1}H NMR of **1** showed the diagnostic methine proton as a sharp triplet at 5.52 ppm in D_2O/MeCN-d_3, indicating the existence of **1** in the vase (C_{4v}) form (Figure 3a). The addition of choline (Cho) or acetylcholine (ChoAc) to **1** induces their complexation. The large upfield shifts observed for the $N(CH_3)_3$ and NCH_2 protons and ^{1}H−^{14}N HSQC experiments prove that the trimethylammonium groups of choline derivatives were located deep inside the cavity.[11a] The affinity of **1** for choline derivatives was assessed by integration of the resonances of free and bound guests. Both complexes, namely **1**·Cho$^+$ and **1**·ChoAc$^+$, afforded values well above 10^4 M^{-1} that preclude their exact determination by ^{1}H NMR titrations. These complexes were also observed by MALDI-TOF mass spectrometry at *m*/*z*

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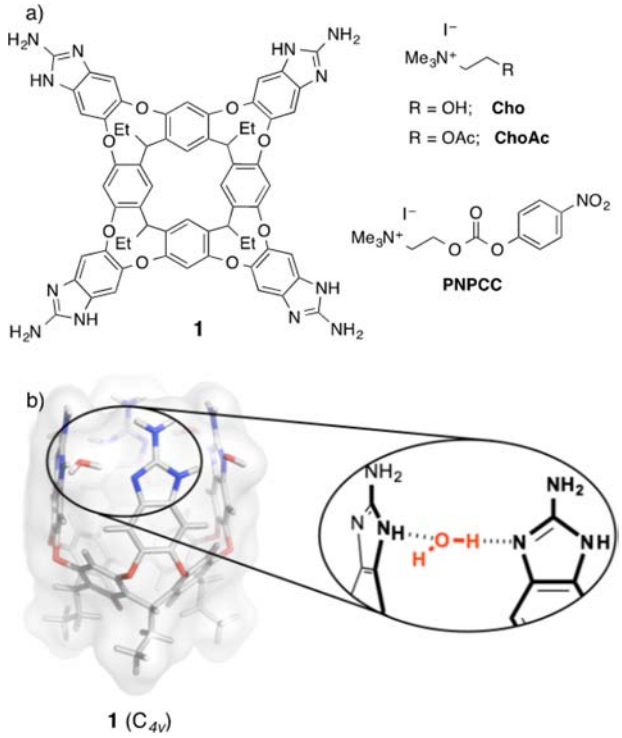

Figure 1. (a) Chemical structures of 2-aminobenzimidazole-based cavitand **1**, Choline (Cho), Acetylcholine (ChoAc), and *p*-nitrophenylcholine carbonate (PNPCC) iodide salts. (b) Energy-minimized (PM3) perspective side view of cavitand **1** (C_{4v}) including four hydrogen bonded water molecules on the upper rim.

1220.56 and 1262.56 assigned to caviplexes **1**·Cho$^+$ and **1**·ChoAc$^+$, respectively, thus confirming that cavitand **1** forms tight complexes with choline derivatives (see the Supporting Information).

Kinetic studies of PNPCC hydrolysis with **1** were performed in acetonitrile−water mixtures by adaptation of reported procedures.[6c,14] Hydrolysis of PNPCC under basic conditions generally affords choline, CO_2, and *p*-nitrophenol (Figure 2a) which can be easily monitored by UV spectroscopy. Reactions of cavitand **1** with PNPCC were carried out in MeCN/H_2O (99:1 v/v) buffered with EtN(*i*- Pr)$_2$/CF_3CO_2H at different concentrations of **1**, and the formation of the *p*-nitrophenolate anion was followed at 405 nm by UV measurements. The experimental kinetic curves obtained for different concentrations of cavitand are shown in Figure 2b. Pseudo-first-order rate constants (k_{obs}) evaluated from the initial slopes were in line with previous reports.[15] Table 1 shows that the amount of cavitand has a marked effect on the initial rates of the reaction (entries 1−5). The linear dependence of the pseudo-first-order rate constants with the concentration of cavitand indicates its participation in the reaction. The apparent second-order rate constant was 79.3 ± 0.2 M^{-1} mn^{-1}. It is noteworthy that the effectiveness of cavitand **1** is higher than that of 2-ABI alone (entry 6). 2-ABI also catalyzes the reaction of PNPCC, though less effectively, thus confirming the active role of the 2-aminobenzimidazole units of **1** in the event.

It was expected that the catalytic hydrolysis of PNPCC afforded Cho, CO_2, and *p*-nitrophenol (Figure 2a). However, ^{1}H NMR experiments ran on mixtures of **1** and PNPCC reveals that the reaction presented here does not proceed through a single reaction step. Instead, a stepwise mechanism involving

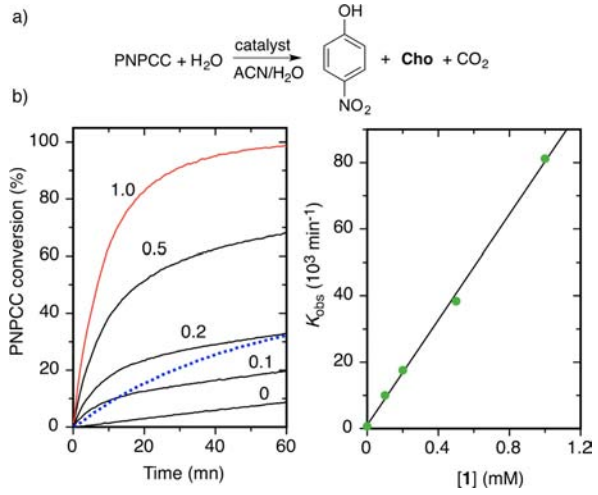

Figure 2. (a) Ideal catalyzed hydrolysis of PNPCC. (b) Real-time UV−vis kinetic conversion curves at 298 K of a solution of PNPCC (0.1 mM) in MeCN/H_2O (99:1 v/v) buffered with 10 mM EtN(*i*-Pr)$_2$ and 5 mM CF_3CO_2H containing cavitand **1** at different molar ratios: 0, 0.1, 0.2, 0.5, and 1.0 with respect to PNPCC. Blue dashed line: time course for 2-aminobenzimidazole (2-ABI) (0.1 mM). (b) Dependence of the apparent first-order rate constant on cavitand concentration (0.01−0.1 mM). The data were fitted to a straight line (R^2 = 0.998), with the second-order rate constant of hydrolysis defined by the slope of the line.

Table 1. Kinetic Parameters for the Reaction of PNPCC (0.1 mM) in the Presence of Variable Amounts of Cavitand 1 or 2-ABI

entry	catalyst	[PNPCC]/[**1**]	$k_{obs} \times 10^{-3}$ mn^{-1}	k_{obs}/k_{uncat}
1	none	–	0.7 ± 0.2	–
2	**1**	0.1	10.0 ± 2.1	14
3	**1**	0.2	17.6 ± 5.4	25
4	**1**	0.5	38.4 ± 13.5	55
5	**1**	1	81.2 ± 24.3	116
6	2-ABI	–	21.5 ± 6.4	30

the formation and subsequent hydrolysis of a 1-cholinecarbamate intermediate takes place under these conditions. The time course of a solution of **1** (0.5 mM) and PNPCC (0.5 mM) in unbuffered MeCN−D_2O (95:5 v/v) was followed by ^{1}H NMR spectroscopy, and the representative results are shown in Figure 3. To slow down the reaction between **1** and PNPCC and to detect the formation of **1**·PNPCC$^+$, the spectrum at *t* = 10 min was registered at 278 K. Figure 3b shows the characteristic upfield signal at −1.28 ppm corresponding to the ($^+$NMe$_3$) of bound PNPCC deep inside of **1**. The coexisting methine protons of **1**·PNPCC$^+$ and unbound **1** were also observed at 5.63 and 5.53 ppm, respectively. Formation of the **1**·PNPCC$^+$ complex was confirmed by MALDI-TOF mass spectrometry, with *m*/*z* 1385.423 assigned to [**1**·PNPCC]$^+$. Thereafter, the extensive carbamoylation of **1** to give the *N*-benzimidazoylcarbamate **2** was evident, and it was complete after 18 h (Figure 3c). ^{1}H NMR spectra taken during this period shows the progressive loss of the C_{4v} symmetry characteristic of **1** and **1**·PNPCC. MALDI-TOF mass spectrometry also revealed the majority presence of a peak at *m*/*z* 1246.502 corresponding to **2**. The broadened ^{1}H NMR spectra and the absence of any significant signal at negative shifts indicate that the trimethylammonium "knob" is outside the aromatic pocket. Interestingly, the cavitand portion remains

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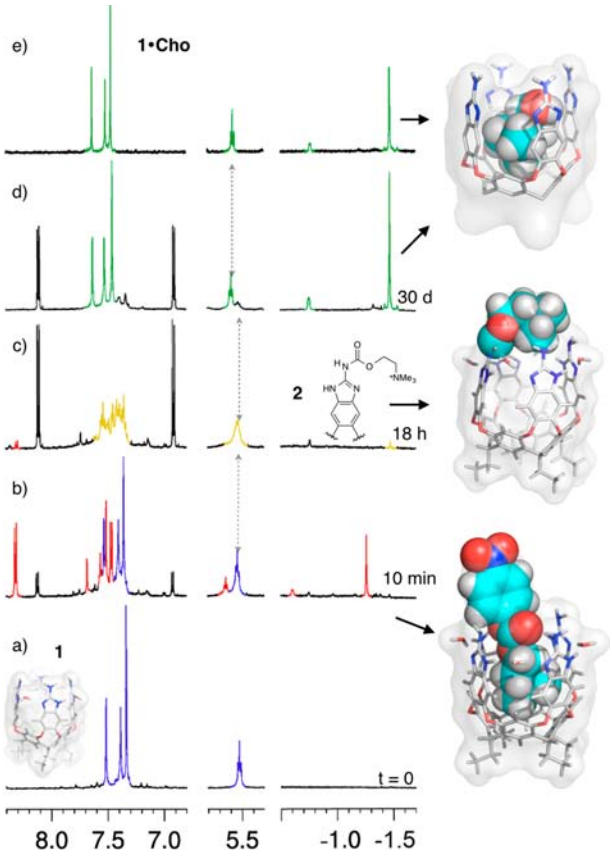


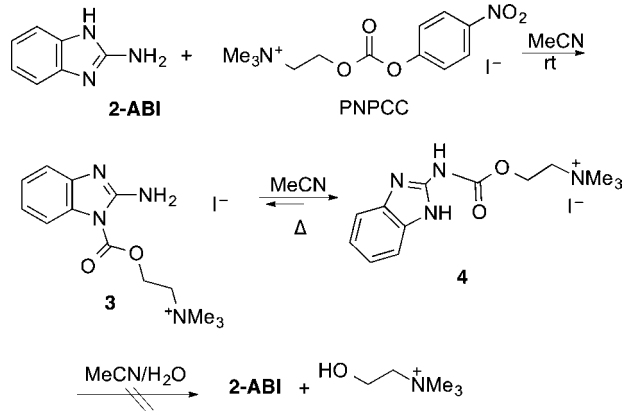
Figure 3. Representative ^{1}H NMR spectra (600 MHz) of selected regions of a stoichiometric solution containing **1** (0.5 mM) and PNPCC in MeCN-d_3/D_2O (95:5 v/v) recorded at different time intervals. Colored resonances are diagnostic signals for free **1** (a, blue), **1**·PNPCC$^+$ caviplex (b, red), carbamoylated cavitand (c, khaki), and bound choline **1**·Cho$^+$ (d, green). The spectrum of **1**·Cho$^+$, prepared independently, is included for comparison (e, green).

always unchanged in vase form as indicated by a permanent diagnostic signal at around 5.6 ppm. This intermediate slowly hydrolyzes affording the final complex **1**·Cho$^+$ which recovers the symmetric appearance expected for a cavitand−choline complex (Figure 3d,e). It is relevant that the complete decarbamoylation of **2** proceeds in a period of around 30 days at 298 K.

Remarkably, carbamoylation followed by slow decarbamoylation is the mechanism of inhibition operating in certain cholinergic drugs used for treatment of dementia, Parkinson's, and Alzheimer's diseases.[16] Rivastigmine is a paradigmatic example of this behavior. Inhibition of acetylcholinesterases (AcChE) with rivastigmine occurs by a mechanism that includes (i) initial formation of a complex with AChE prior to carbamoylation, (ii) stoichiometric carbamoylation of a histidine residue in the catalytic triad of AChE, and (iii) very slow (hours to days) decarbamoylation of the enzyme.[16] Due to the slow decarbamoylation process, rivastigmine is classified as a "pseudoirreversible" inhibitor of AChE. These results closely parallel those of PNPCC with cavitand **1**.

The reaction of PNPCC with 2-aminobenzimidazole (2-ABI) and the subsequent hydrolysis were also investigated for comparison (Scheme 1). Depending on the experimental conditions, the addition of PNPCC to a solution of 2-ABI in MeCN−D_2O (95:5 v/v) gave the benzimidazole carbamates **3** and **4** (see the Supporting Information).[17] However, in striking

Scheme 1. Reaction of PNPCC with 2-ABI



contrast with the carbamoyl-cavitand intermediate **2** these carbamates, obtained by direct condensation of 2-ABI and PNPCC, do not hydrolyze even after extended periods at room temperature.

These results suggest that hydrolysis of carbamate intermediate **2** does not proceed by simple nucleophilic attack of the water molecules in the solvent matrix. Hence, decarbamoylation of **2** to afford **1**·Cho$^+$ may be enabled by water molecules located near the upper rim of the cavitand. Molecular modeling of **2** reveals that the trimethylammonium group is outside the cavity and that the carbamoyl group adopts a planar conformation. In this conformation, the carbonyl group of the carbamate is susceptible to hydrolysis by pseudo-intramolecular nucleophilic attack of water molecules lying in the interstice between benzimidazole walls.

In conclusion, this new metal-free cavitand provides the appropriate environment to promote the stepwise PNPCC hydrolysis in an enzyme-like process. It is of special interest that the hydrolysis of PNPCC takes place slowly due to the combined effect of the supramolecular recognition event and nucleophilic attack of water molecules confined at the outer rim of the carbamoylated cavitand **2**. This is a unique example of quasi-irreversible catalyst inhibition found in cavitands.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and characterization data for all new compounds. 1D, 2D NMR and mass spectra of complexes. 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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