Choline Recognition in Water

Acetylcholine Recognition by a Deep, Biomimetic Pocket**

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Synthetic receptors for small targets relevant to biology often mimic the molecular recognition features of naturally occurring proteins. For the target molecule acetylcholine (**1**$^+$), X-ray structural information is available for its cognate esterase. Negatively charged Asp and Glu residues on the enzyme

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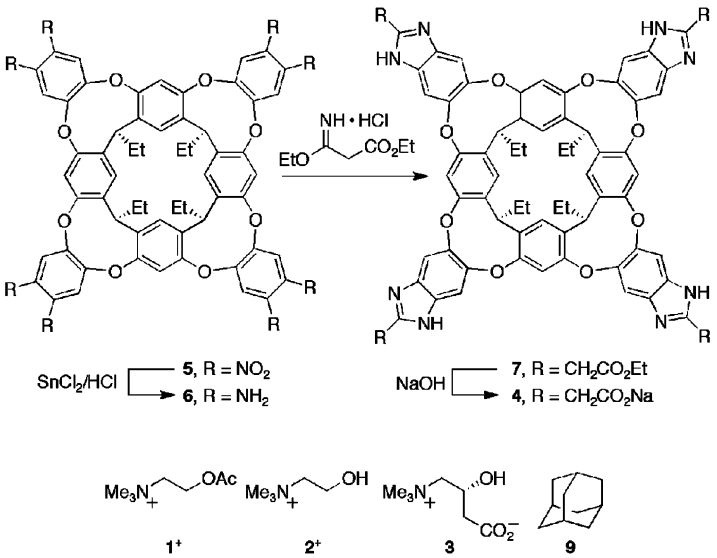
surround the mouth of a deep (ca. 20 Å) active-site gorge.[1] Once the substrate enters the gorge it is guided to the active site and there secured almost exclusively by aromatic residues.[2,3] The quaternary ammonium function of acetylcholine does not permit the use of conventional hydrogen bonds or salt bridges; rather, the ion presents a thin layer of positive charge on its surface. These features of the target and enzyme[4] have inspired the synthesis of synthetic receptors for quaternary ammonium ions with negative charges on concave aromatic surfaces. The first examples were cyclophanes appended with polar solubilizing groups.[5–9] Receptors based on clefts,[10–14] porphyrins,[15–17] calixarenes/resocinarenes,[18–23] and metal–ligand clusters[24] have followed, and yielded an assortment of thermodynamically stable host–guest complexes. Examples of good binding selectivity[5,12] and kinetic stability[24] in water remain elusive, but some success has been achieved in organic media.[25] In general, the concavity of these receptors is modest—they surround only a small fraction of the target molecules. We describe here a deep pocket synthetic receptor that binds acetylcholine ($\mathbf{1}^+$) and choline ($\mathbf{2}^+$) in water with high thermodynamic and kinetic stability. Selectivity over L-carnitine (**3**) is established by the positioning of negative charges at the mouth of the deep pocket.

The receptor **4** is prepared from the known octanitrocavitand **5**[26] in three steps (Scheme 1). Reduction of **5** to the octaamine **6** with $SnCl_2$ and subsequent heating in the presence of the appropriate imidate led to the formation of the tetrabenzimidazole tetraester **7** in 62% yield.[27] The ester is hydrolyzed with NaOH to provide cavitand **4** as the tetrasodium salt. The negative charges found on the upper rim of **4** enable its solubility in water at pH/pD 7.8 at levels up to 10 mM.



Scheme 1. Synthesis of receptor **4**, and the structures of some guests that form kinetically stable complexes in water.

A 1 mM sample of **4** in D_2O provides an NMR spectrum that has sharp signals and shows the characteristics and symmetry expected for a time-averaged C_{4v} conformation (Figure 1a). Rapid tautomerization of the benzimidazoles occurs on the NMR time scale, facilitated by the water molecules that complete the seam of hydrogen bonds. These water molecules are known to stabilize the pocketlike conformation of structurally related cavitands in organic solvents.[28] The methine protons are observed at $\delta = 5.5$ ppm, which indicates that the host exists in a vase-shaped conformation.[29] In solution this preformed hydrophobic cavity is

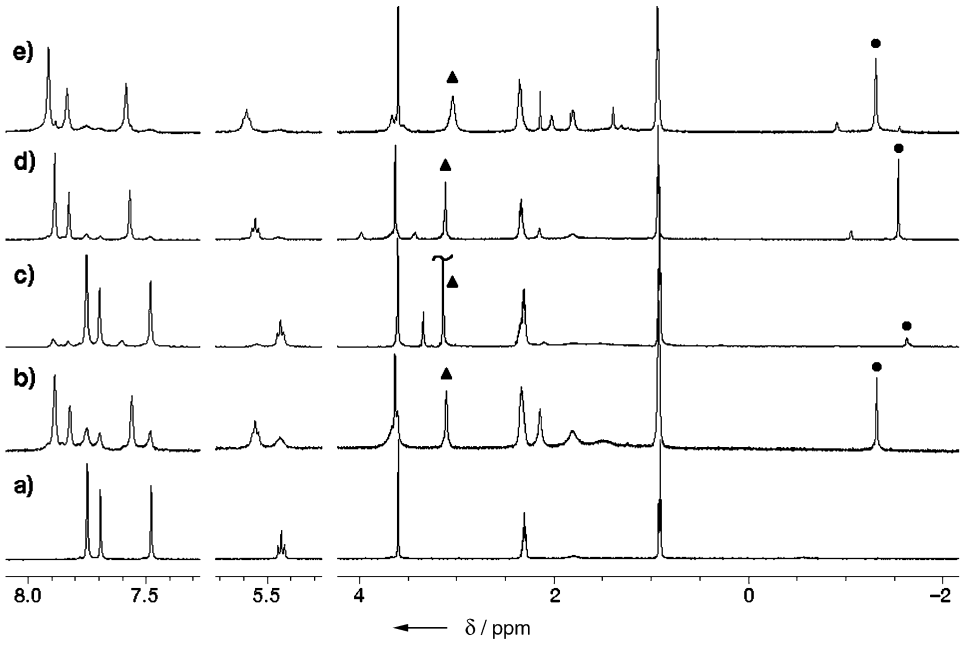


Figure 1. ^{1}H NMR spectra of host **4** at 1 mM in D_2O: a) before the addition of guest, and in the presence of about one equivalent of: b) Me_4NBr, c) L-carnitine, d) choline chloride, and e) acetylcholine chloride. Signals arising from free and bound guest protons are marked with (▲) and (●), respectively.

occupied by one encapsulated THF molecule, and likely accompanied by an unknown amount of water. The adventitious THF resists removal even when solid **4** is heated at 100 °C under high vacuum. When one equivalent of tetramethylammonium bromide ($\mathbf{8}^+Br^-$) is added to a solution of **4** in D_2O, the THF is released and a separate set of host resonances appears: A new signal corresponding to encapsulated $\mathbf{8}^+$ appears at $\delta = -1.35$ ppm, which is shifted $\delta = 4.4$ ppm upfield of the free guest (Figure 2c). The large anisotropy experienced by the bound guest shows it is included deep within the pocket, surrounded by aromatic walls. NMR titrations provide an association constant of $4300\,M^{-1}$ for $\mathbf{8}^+$ with **4** in D_2O (Table 1). Larger cations such as tetrapropylammonium and tetrabutylammonium show no evidence of complex formation; they cannot fit within the binding pocket. The host also demonstrates functional group selectivity—primary amines (for example, glycine methyl ester hydrochloride) and heterocycles (for example, imidazole, pyridine, and methylpyridinium salts) do not form stable complexes with **4**.

Similar 1:1 host–cation complexes are formed with acetylcholine chloride ($\mathbf{1}^+Cl^-$) and choline chloride ($\mathbf{2}^+Cl^-$), with association constants greater than $10^4\,M^{-1}$ (the limit of the NMR determinations). Direct competition of $\mathbf{1}^+$ with $\mathbf{2}^+$ reveals a slight (5:4) preference for the binding of choline. The structurally related, but zwitterionic guest, L-carnitine (**3**) shows a binding constant of only $140\,M^{-1}$. For $\mathbf{1}^+$, $\mathbf{2}^+$, and **3** the NMR spectra of each host–guest complex indicates that the trimethylammonium residue of each guest is bound deep within the aromatic pocket ($\Delta\delta = 4.4$–4.9 ppm). The binding of L-carnitine in this mode positions the guest's carboxylate group in proximity to the four carboxylate residues that decorate the opening of the host's pocket (Figure 2). The resulting electrostatic repulsion is likely to be responsible for the host's rejection of L-carnitine relative to choline and acetylcholine.

Isothermal titration calorimetry (ITC) was carried out to measure the enthalpy and entropy of complex formation for each guest. In general, the calorimetric data gives K_a values that agree with those obtained by NMR titrations (Table 1). The enthalpy of binding contributes significantly to the thermodynamic stability of each host–guest complex—it is likely that some portion of this enthalpy arises from complementary electrostatic interactions. The complexation of choline shows the most favorable enthalpy of binding, with a compensating unfavorable entropy term. For all other guests the entropy of binding is favorable, and constitutes a significant portion of the free energy of complexation. The binding of guests within the cavity causes the release of ordered THF and water molecules from the preformed cavity—a hydrophobic effect that is expected to be responsible for the favorable entropy of binding. The origins of the unfavorable entropy of complexation for choline are less clear. Additional evidence for a hydrophobic driving force is provided by the study of adamantane (**9**), a neutral hydrocarbon. Sonication of a D_2O solution of **4** in the presence of solid adamantane (**9**) results in the extraction of the insoluble solid into water. ^1H NMR studies show that this uncharged guest forms a 1:1 complex similar to those formed by charged guests (**1**–**3** and **8**).

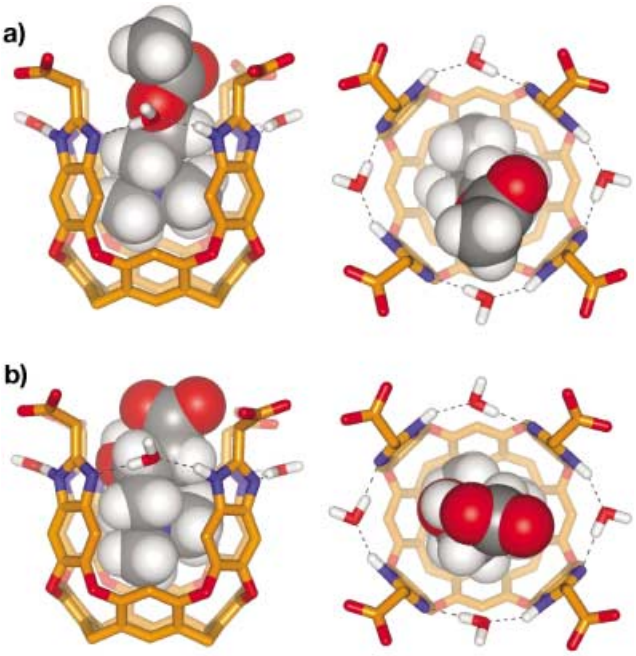


Figure 2. Molecular models of a) acetylcholine and b) L-carnitine complexed with host **4** clearly show the origin of the host's low affinity for the latter: the binding of trimethylammonium groups within the aromatic cavity results in the placement of the carboxylate group of carnitine near the host's negatively charged rim. Only the four water molecules that are thought to form the host's hydrogen bonding seam have been included in the models. Some protons and pendant alkyl groups have been omitted for clarity.

Table 1: Thermodynamic parameters for the binding of guests by **4** in water.[a]

Guest	NMR[b] K_a (M^{-1})	ITC[c] K_a (M^{-1})	$-\Delta H$ (kcal mol^{-1})	ΔS (cal K^{-1} mol^{-1})
Me_4N^+	4300 ± 600	3800 ± 600	2.4 ± 0.1	8.3
choline	$>10^4$	25900 ± 700	9.0 ± 0.1	−10.0
acetylcholine	$>10^4$	14600 ± 1200	4.1 ± 0.1	5.3
L-carnitine	140 ± 10	150 ± 10	2.1 ± 0.1	2.9

[a] pH/pD of each solution is 7.8. [b] NMR titrations were carried out in D_2O at 298 K using a constant host concentration of 1 mM. The limit for accurate determinations using this method is $10^4\,M^{-1}$. [c] Isothermal titration calorimetry was carried out in H_2O at 298 K using starting host concentrations between 0.1 and 5 mM. The enthalpy (ΔH) is directly measured, and values for K_a and ΔS are determined by fitting the data to a binding isotherm. For the fitting procedure the host–guest ratio was constrained between 0.90:1 and 1.10:1, except for choline for which a satisfactory fit was only available using a ratio of 0.63:1.

In addition to the observed thermodynamic stability, these complexes exhibit unusually high kinetic stability. Previously reported receptors for acetylcholine in water (with association constants as high as $10^5\,\text{M}^{-1}$) do not demonstrate slow exchange of free and bound guest on the NMR time scale.[6,19,22] In contrast, complexes of host **4** and its guests give rise to separate NMR signals for the free and bound species in each case. To further characterize the kinetic stability of this system, the rates of guest exchange were determined from 2D NOESY experiments on the complex of **4** with $\mathbf{8}^+$. Integration of the cross-peaks gives kinetic parameters for the exchange process;[30] the release rate of the guest ion at 298 K is $8.2\,\text{s}^{-1}$, which corresponds to an energy barrier for guest exchange of 16 kcal mol^{-1}.

The similarities between acetylcholinesterase and **4** begin and end with binding—the synthetic host shows no signs of catalytic activity. But the monofunctionalization of related cavitands[31] suggests that catalytically useful groups can eventually be directed into the binding pocket. In the meantime, the synthetic receptor described here represents an unprecedented combination of affinity and kinetic stability for recognition of acetylcholine and choline in water.

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