Synthesis and Characterization of Photolabile Compounds Releasing Noracetylcholine in the Microsecond Time Range

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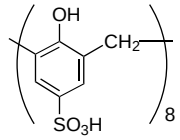
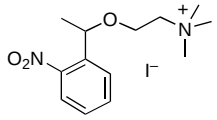
Mutually Induced Formation of Host–Guest Complexes between *p*-Sulfonated Calix[8]arene and Photolabile Cholinergic Ligands**

Alexandre Specht, Philippe Bernard, Maurice Goeldner,* and Ling Peng*

Molecular recognition is a fundamental phenomenon in biology that has inspired many chemists to design artificial receptors and enzymes.[1] These biomimetic systems are mostly preorganized and are relatively rigid receptors capable of forming host–guest complexes with small molecules. A few flexible receptors were exploited for their biomimetic properties.[2] Here we report an original example of adaptive host–guest systems which employs the mutually induced fit between conformationally flexible molecules, namely *p*-sulfonated calix[8]arene (**A**) and photolabile cholinergic ligands (**1**–**3**) as host and guests, respectively (Figure 1).



A



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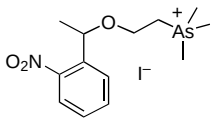

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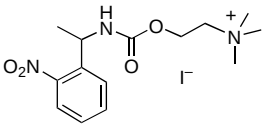

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The calixarene family plays an important role in host–guest chemistry. While calix[4]arenes and calix[6]arenes have been widely studied, calix[8]arenes were exploited much less because of their more challenging chemistry and higher conformational flexibility.[3] Water-soluble *p*-sulfonated calix[4]arene and calix[6]arene, which bind cholinergic ligands such as choline and acetylcholine, can be considered as simple prototypes for an artificial cholinergic receptor.[4] While bridged calix[8]arenes have been involved in alkali-cation-induced "conformational templatation",[5] we reasoned that the large annular cavity of *p*-sulfonated calix[8]arene **A** with nonrestricted conformational flexibility would be suitable for displaying a mutually induced fit (Figure 1) with flexible guests such as photolabile cholinergic ligands **1**–**3**.[6,7]

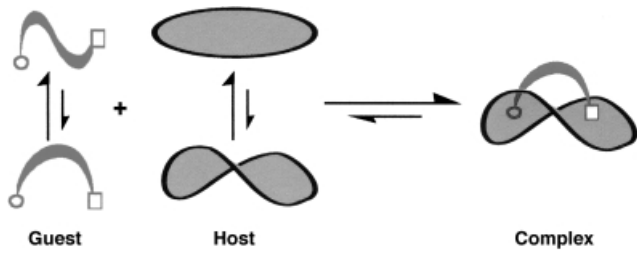

Figure 1. Mutually induced fit in a host–guest complex.

Compounds **1**–**3** are inhibitors of cholinesterases (ChEs) such as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Upon photolysis, these ligands release choline,[6a] arsenocholine,[6b] and carbamylcholine, respectively.[6c–d] They are thus ideal probes for time-resolved crystallographic studies on cholinesterases. Our previous studies showed that *p*-sulfonated calix[4]arene formed host–guest complexes with **1** or **3** at neutral pH by specific inclusion of their ammonium group to give monotopic binding complexes.[8] Here we show that *p*-sulfonated calix[8]arene **A** forms host–guest systems with **1**–**3** by ditopic binding of both the aromatic ring and the cationic ammonium moiety of the guest. Interestingly, these complexes are formed through mutually induced fit, thus mimicking more closely the binding occurring in the active site of ChEs.

The complexes between **A** and **1**–**3** were formed in 0.1M phosphate buffer at pD = 7.3, as indicated by ^{1}H NMR analysis (Figure 2). Progressive addition of **A** to a solution containing the guest molecule led to increasing upfield chemical shifts ($\Delta\delta$) of both the aromatic protons and the ammonium group of **1**–**3** (Figure 2a). These shifts suggest an inclusion of both the aromatic ring and the ammonium group

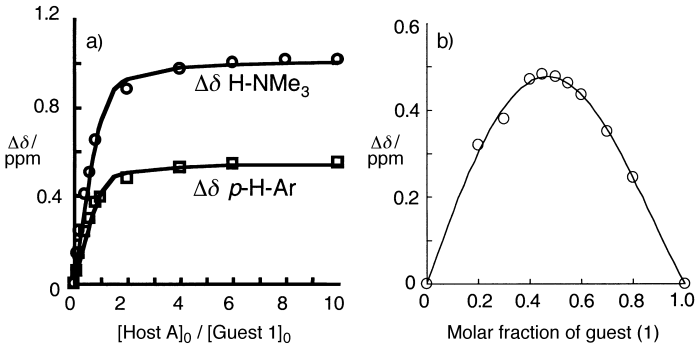

Figure 2. NMR analysis of the complex [**A**-**1**]. a) The observed (symbols) and calculated (curves) upfield shifts $\Delta\delta$ (ppm) of the N-CH_3 (○) and *p*-H-Ar (□) signals of **1** obtained by progressive addition of **A** to 3.10^{-4}M **1** in 0.1M phosphate buffer pD 7.3, 20°C. b) Job plot.

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of **1**–**3** into the cavity of **A**. Moreover, the curves $\Delta\delta = f$[host]/[guest] (Figure 2a) indicate the formation of 1:1 complexes, which was confirmed by a Job plot (Figure 2b). The association constants of **A** for **1**–**3** were determined by ^{1}H NMR spectroscopy,[9] and similar values for both the aromatic ring and the ammonium group were obtained (Figure 2a). These association constants are in the same range for all three ligands (Table 1) and approach their binding affinities for AChE and BuChE.

Table 1. Binding affinities ($10^3\,M^{-1}$) of **1**–**3** for **A**, AChE, and BuChE.

	1	**2**	**3**
K_A, **A** ($10^3\,M^{-1}$)	34.4	21.7	21.3
$1/K_I$ AChE ($10^3\,M^{-1}$)	77.0	59.0	22.7
$1/K_I$ BuChE ($10^3\,M^{-1}$)	91.0	55.5	12.8

To analyze the supramolecular nature of the complex more accurately, we studied the complex [**A-1**] at lower pH values (pD = 0.4) where all the phenol groups in **A** are protonated, and thus favor inclusion of the aromatic group.[10] However, no pronounced pH preference was observed for the upfield chemical shifts and the binding affinities of either the aromatic protons or the ammonium group of **1** in the complex [**A-1**] in going from pD 7.3 to 0.4 (data not show). Considering the large annular cavity of **A** and the previous findings, we propose that a ditopic binding occurs in the complex [**A-1**] with concomitant inclusion of both aromatic and ammonium groups of the guest into the cavity of **A**, rather than monotopic binding with a fast equilibrium between two complexes hosting either the ammonium or the aromatic group of **1**. The latter case is observed when **1** is complexed by *p*-sulfonated calix[4]arene at pD 0.4, which demonstrates that complexation shows a clear pH dependence (data not show).

To gain more insight into the formation of these complexes, we studied separately the binding features of **A** with the two essential parts of guest **1**, namely 2-nitrobenzyl alcohol and choline, respectively. While 2-nitrobenzyl alcohol showed a very poor binding affinity with **A** ($K_A < 10^3\,M^{-1}$), choline formed a stable complex. However, we could not establish a defined stoichiometry for this latter complex, which might be a mixture of 1:1 and 1:2 complexes, as suggested by the Job plot (data not shown). Even though a precise binding constant for choline could not be determined from these experiments, it forms a much weaker complex with **A** than does the bifunctional guest **1**, thus emphasizing the ditopic binding feature of [**A-1**].

Complex [**A-1**] could exist as a bimolecular 1:1 host:guest complex or higher order aggregation. No dependency of the chemical shifts of [**A-1**] on concentration was observed upon dilution,[11] which suggests that [**A-1**] has no propensity to aggregate. Moreover, a series of NMR titration experiments with different concentrations of guest were undertaken for [**A-1**]. The experimental data matched perfectly with the curves fitted for a 1:1 bimolecular complex, and led to the same binding constants and the same maximal chemical shifts.[11] All these results are in agreement with [**A-1**] being a bimolecular complex with ditopic binding of both the aromatic and ammonium groups of **1** into the cavity of **A**.

The ditopic binding mode of [**A-1**] was further confirmed by NOESY experiments. A specific NOE signal was observed between the methyl protons of the ammonium group and the aromatic proton in the position *para* to the side chain of **1** in [**A-1**] (Figure 3a). A distance of 4.0 Å between these two groups could be deduced within this complex (Figure 3b) at both pD 7.3 and pD 0.4. However, this particular NOE signal

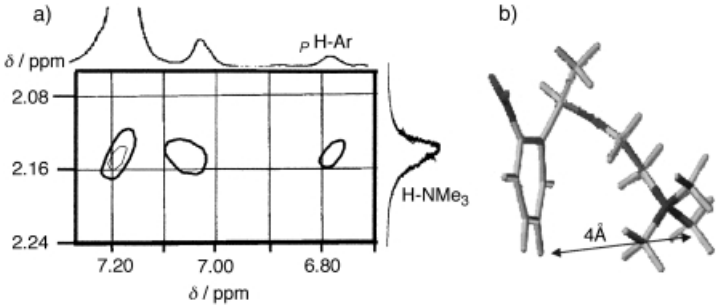

Figure 3. a) Specific NOE signal between the ammonium moiety and and aromatic proton of **1** in [**A-1**]. b) Intramolecular distance constraint deduced from the NOE data.

was not observed when **1** was either free or complexed with *p*-sulfonated calix[4]arene. Thus, when **1** was complexed by **A**, it adopted a constrained conformation by bringing the ammonium moiety and the aromatic ring closer (to a distance of 4.0 Å) so that it could to be accommodated concomitantly in the cavity of **A**. When **1** was either free or complexed with *p*-sulfonated calix[4]arene, it was in more relaxed conformations, with the ammonium moiety and the aromatic ring further apart. These results suggest that the cavity of **A** directs the flexible guest **1** to adopt a bent conformation for a favorable fit in the complex.

Conversely, guest **1** is able to select a suitable conformation of **A** for an optimal fitting, as supported by molecular modeling studies.[12] Both the pleated loop conformation[13] and the pinched conformation[14] of calix[8]arene were used as starting points to minimize the energy of **A**. Without the guest, the pleated loop conformation of **A** is 10 kcal mol^{-1} lower in energy than the pinched one. However, when **A** was docked with **1**, a stable complex was obtained only with the pinched conformer of **A**. In this complex the aromatic part and the ammonium moiety of **1** were 3.7 Å apart and inserted simultaneously into the two aromatic pockets of **A** (Figure 4). This is in excellent agreement with the results obtained from NOE experiments. Moreover, the aromatic ring of **1** stacked with one of the phenol units of **A**, and the ammonium moiety of **1** formed a π–cation interaction with another phenol unit of

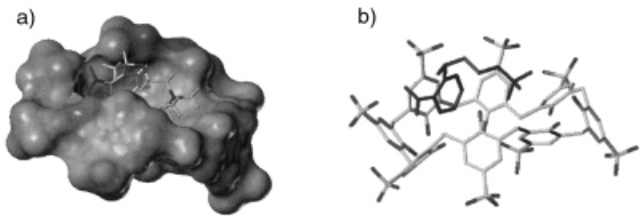

Figure 4. Molecular modeling studies of [**A-1**]. **1** is shown in stick presentation and **A** is shown in space-filling a) and stick presentation b).

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A (Figure 4). Thus, [**A**-**1**] is stabilized by both π–π and π–cation interactions. Furthermore, the binding of the guest would displace the water molecules in the cavity of **A**, thus providing a favorable entropic gain in [**A**-**1**].

In conclusion, we report an original example of adaptive host–guest systems which employs mutually induced fit between the conformationally flexible host **A** and guests **1**–**3**. Both the host and the guests adapted to each other and selected the higher energy but correct geometric conformers so that the guest could fit favorably into the cavity of the host to give ditopic binding. While mutually induced fit has been demonstrated mostly in biological macromolecules interactions, that is, in protein/DNA,[15] protein/RNA,[16] protein/tRNA complexes,[17] it has been recently depicted within a neocarzinostatin–chromophore/DNA complex.[18] Recent development in drug design has considered mutual adaptability between a ligand and a receptor as a key element in molecular recognition[19] and has prompted the outlining of the "relaxed-complex" method as a novel dynamic computational model to take into account the flexibility of receptors.[20] Therefore, our system represents an original example of adaptive supramolecular biomimetic chemistry.

Experimental Section

General: NMR spectra were recorded on a Bruker VPC 300 spectrophotometer unless otherwise mentioned. Solutions of complexes for NMR analysis were prepared in 0.1M deuterated phosphate buffer at pD 7.3 and 0.4. The molecular modeling study was performed by using Sybyl 6.8 on a Silicon Graphics Octane 2XR10000 station.

NMR titration: Experiments were carried out by keeping the concentration of guest (**1**–**3**) constant while varying that of the host (**A**) from 0.1 equiv to 10 equiv. Association constants K_A of the complexes were calculated with a nonlinear least-squares method.[9]

2D NMR analysis: Solutions for 2D NMR analysis were prepared with 20 mM of **1** and 40 mM of the host, which ensured over 99% complex formation between the guest and the host. 2D NMR spectra were recorded by using a NOESY sequence from Bruker (Noesyprtp) and a mixing time of 400 ms. Experiments were performed on **1**, [**A**-**1**], and the complex of *p*-sulfonated calix[4]arene with **1**.

Molecular modeling studies: The starting structure **1** was constructed from the coordinates of the crystal structure of an analogue.[6b] For **A**, two crystal structures of calix[8]arene (Dovhif[13] and Foztix[14]) were used as starting points to construct the structure and led to the pleated loop conformation and the pinched conformation, respectively. For the complex [**A**-**1**], **1** was manually docked with each conformer of **A**. All the starting structures of **1**, **A**, and [**A**-**1**] were optimized by a molecular mechanics algorithm using the Tripos force field with a convergence criterion of 0.01 kcal mol^{-1}. The electrostatic component was applied by means of the Gasteiger–Hückel charges and a dielectric function equal to 1.

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New Ferrocenyl Ligands with Broad Applications in Asymmetric Catalysis**

Matthias Lotz, Kurt Polborn, and Paul Knochel*

The development of new ligands for asymmetric catalysis is an highly active field of research.[1] Although many chiral ligands have been prepared, only a few, such as binaphthyl-,[2] salen-,[3] pybox-,[4] and DuPHOS-type[5] ligands have found broad applications in various asymmetric reactions. The ferrocenyl unit has proven to allow excellent spatial recognition,[6] and we recently reported new ferrocenyl diphosphanes of type **1** (taniaphos),[7] which gave excellent results in

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