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Molecular Recognition of Pyridine *N*-Oxides in Water Using Calix[4]pyrrole Receptors

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Calix[4]pyrroles,[1] are macrocyclic species having an array of four NHs that act as a binding site for anionic and electron-rich neutral guests in organic solvents.[2] In nonpolar organic solvents, the formation of the complex is usually accompanied by a change from the preferred 1,3-alternate conformation of the free calix[4]pyrrole core to the cone conformation.[3] Aryl extended calix[4]pyrroles bear an aryl group substituent at each of the four *meso*-carbons.[4] In the cone conformation, the α,α,α,α isomers of aryl extended calix[4]pyrroles present a deep aromatic cavity with a functionalized closed end which is suitable for including electron-rich neutral molecules and anions.[5] To date and to the best of our knowledge, there are no examples reported in the literature of water-soluble aryl extended calix[4]pyrroles. These types of receptors, however, are attractive candidates to achieve selective binding in an aqueous solution of neutral aromatic guests having electron-rich sites by a combination of hydrogen bonding, $\pi-\pi$, CH$-\pi$, and hydrophobic interactions. This arrangement of intermolecular forces is commonly observed in protein binding pockets. In contrast, the number of examples of synthetic receptors where hydrogen bonding sites have been used in conjunction with hydrophobic binding to provide selective binding in water is very limited.[6] In this communication we report our synthetic results in producing water-soluble aryl extended calix[4]pyrroles **1b**−**c** and preliminary complexation studies with pyridine *N*-oxides in water (Figure 1). *N*-Oxides are interesting targets for molecular recognition studies due to their pronounced biological activity.[7]

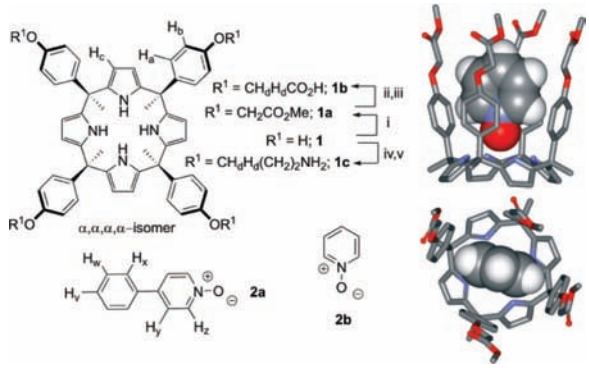

Figure 1. (Left) Chemical structures of the receptors and the pyridine N-oxides used in the study. Reaction conditions: (i) $BrCH_2COOMe$, K_2CO_3, DMF; (ii) LiOH, H_2O; (iii) H^+; (iv) 3-bromopropylphthalamide, NaH; (v) NH_2NH_2. (Right) X-ray crystal structure (side and top views) of the inclusion complex **2b**⊂**1a**. Hydrogen atoms of **1a** are omitted for clarity.

To gain access to water-soluble aryl extended calix[4]pyrroles, we appended ionizing and water solubilizing groups such as those in carboxy **1b** and amino **1c** using the hydroxyl functionalities already present in the upper rim of compound **1**.[8]

Reaction of **1** with methyl bromoacetate in similar conditions to those reported by Gale afforded tetraester **1a**[8] which was hydrolyzed with LiOH followed by acidification with concentrated HCl to provide the tetracid **1b** in 71% yield. The tetraamino receptor **1c** was synthesized by alkylation of **1** with 3-bromopropylphthalimide and NaH in dry DMF, followed by hydrazinolysis of the phthalimido group.[9] Compound **1c** was isolated and stored as the tetrahydrochloride salt by precipitation from a dioxane solution saturated with HCl. The charges that can be generated on the upper rims of **1b** and **1c** promote water solubility at a wide range of pH's and at concentrations up to 10 mM. While screening a variety of neutral aromatic compounds as potential guest for the water-soluble calix[4]pyrroles **1b**−**c**, we came across pyridine *N*-oxides.

1 mM D_2O solutions of either **1b** or **1c** at pH ≈ 7 show ^{1}H NMR spectra with sharp signals and the characteristics and symmetry of a time-averaged C_{4v} cone conformation (Figure 2). The β-pyrrolic protons for both receptors appear as a sharp singlet at $\delta = 5.8$ ppm, which is indicative of the host adopting the cone conformation (*vide infra*). The preformed aromatic cavity must be occupied by an unknown amount of water molecules.

When 0.5 equiv of 4-phenylpyridine *N*-oxide **2a** were added to a 1 mM solution of **1b** in D_2O (pH ≈ 7), a separate set of host resonances appears and new proton signals corresponding to the two pyridyl protons of included **2a** at $\delta = 4.6$ ppm and $\delta = 6.8$ ppm also became evident (Figure 2). The intense upfield shift ($\Delta\delta = -3.5$ ppm) experienced by the pyridyl protons alpha to the *N*-oxide nitrogen in the bound guest indicates that the *N*-oxide functionality is included deeply into the cavity and that **2a** is

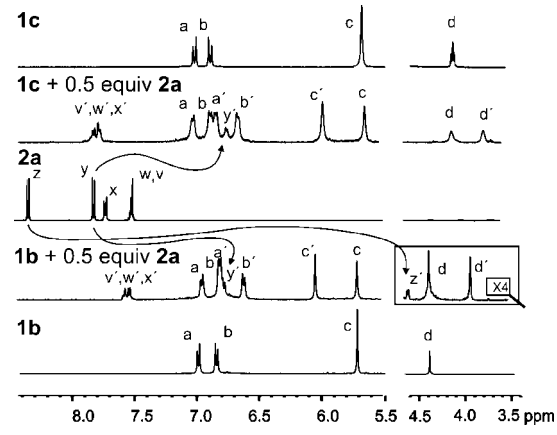

Figure 2. Changes in the ^{1}H NMR spectra during the titration of **1b** and **1c** with **2a** in water-d_2 at pD = 7.3. See Figure 1 for proton assignments. Primed letters are assigned to the proton signals in the 1:1 complex.

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surrounded by the four aromatic walls. Furthermore, the downfield shift ($\Delta\delta = 1.1$ ppm) for the signal due to the NH pyrrole protons of **1b**, observed in a titration experiment in H_2O-D_2O (9:1), shows that they form H-bonds with the oxygen atom of guest **2a**. Integration of the ^{1}H NMR signals of **1b** at different concentrations of **2a** provided an association constant of $(2.4 \pm 1.3) \times 10^3$ M^{-1} for the **2a**⊂**1b** complex in D_2O (Table 1). It is worth noting that

Table 1. Binding Constants (M^{-1}) for 1:1 Complexes of Pyridine *N*-Oxides **2a** and **2b** with Receptors **1a**, **1b**, and **1c** Determined in D_2O and CD_3CN

receptor	K_a [M^{-1}] in CD_3CN	K_a [M^{-1}] in D_2O
Guest	**2a**; 4-Phenylpyridine *N*-Oxide	
1a	$>10^4$ [a]/$(1 \pm 0.4) \times 10^4$ [b]	−[c]
1b	$(2.9 \pm 0.3) \times 10^3$ [a]/$(2 \pm 0.4) \times 10^3$ [b]	$(2.4 \pm 1.3) \times 10^3$ [a]
1c	−[c]	$(1.5 \pm 0.2) \times 10^3$ [a]
Guest	**2b**; Pyridine *N*-Oxide	
1a	$>10^4$ [a]/$(2.9 \pm 0.2) \times 10^4$ [b]	−[c]
1b	$>10^4$ [a]/$(2.5 \pm 0.2) \times 10^4$ [b]	$>10^4$ [a]/$(1.6 \pm 0.2) \times 10^4$ [b]
1c	−[c]	$>10^4$ [a]/$(2.0 \pm 0.2) \times 10^4$ [b]

[a] ^{1}H NMR titration. [b] UV−vis titration. [c] Not soluble.

the stability of the **2a**⊂**1b** complex is unaffected by pH changes in the range 5−11. Stepwise addition of **2a** resulted in an increase of the proton signals assigned to bound host **1b** at the expenses of the signals of the free receptor. When more than 1 equiv of **2a** is present in solution, a new set of proton signals corresponding to the free guest can also be observed. The phenyl protons of bound **2a** exhibited a moderate upfield shift indicative that this residue is not included into the aromatic cavity and only experiences the magnetic anisotropy due to the substituents in the upper rim. Similar 1:1 complexes are formed with the tetraamine receptor **1c** (Figure 2) or when using pyridine *N*-oxide **2b** as guest. The **2b**⊂**1b**−**c** complexes are kinetically less stable that their **2a**⊂**1b**−**c** counterparts. Most likely, the dissociation energy barrier is reduced for guest **2b**, lacking the phenyl substituent, due to the more favorable solvation of the transition state. In water, the binding affinities of receptors **1b**−**c** for guests **2** are considerable, $K_a = 10^3-10^4$ M^{-1} (Table 1). In addition, the 1:1 inclusion complexes **2a**⊂**1b**−**c** display a high kinetic stability. Considering the values measured for the complexes' stability constants and the fact that, in the cone conformation, the open end of the aromatic cavity seems to be unobstructed for the guest release (Figure 1 right), the observed slow exchange constitutes a surprising result.

To further characterize the kinetic stability, we performed a 2D EXSY experiment at 298 K on the **2a**⊂**1b** complex in the presence of excess guest **2a**. Based on the integration of the diagonal and the cross peaks due to the chemical exchange of free and bound guest, we calculated the exchange rate constant $k_{out} = 8.1$ s^{-1} for the guest exiting the host corresponding to the free energy barrier $\Delta G^{\ddagger 298K}_{diss} = 16.2$ kcal mol^{-1}. The high dissociation energy barrier determined for the exchange of the guest suggests a pathway requiring a conformational change of the bound receptor (from cone to partial-cone or alternate). This type of exchange process is closely related to the exchange mechanisms described by Rebek for deep cavitands derived from resorcin[4]arene.[10]

The complexation behavior of receptors **1a** and **1b** with *N*-oxides **2** was also probed in acetonitrile solution using ^{1}H NMR experiments (Table 1). The addition of incremental amounts of **2a** (25.1 mM) to a solution (3.35 mM) of **1a**−**b** in CD_3CN alters the host and guest proton signals analogously to the case described for its complexation in water, suggesting that the structures of the complexes are identical in both solvents. Remarkably, the stability constant values for **2**⊂**1b** are not affected in water. Most likely, the increase in solvent competition for the hydrogen bonding sites is compensated by the release to the bulk solvent of solvating water molecules. The inclusion geometry assigned to the complexes **2**⊂**1a**−**c** in solution is also observed in the solid state. The X-ray structures of single crystals obtained by slow evaporation of acetonitrile solutions containing the tetraester **1a** and an excess of *N*-oxides **2a** or **2b** show that the receptor adopts a cone conformation with the *N*-oxide included deep in the cavity (Figure 1 and Supporting Information). The oxygen of the *N*-oxide is symmetrically bound to the four pyrrolic NH groups, with an average distance of 2.92 Å for the N···O hydrogen bonds. Furthermore, the *meso* phenyl substituents and the pyridine core are at an interacting distance for *offset* π-stacking (3.8 Å centroid-to-centroid) and CH−π (2.34 Å H-to-centroid) interactions to be also operative in the complex (Figure 1).

In conclusion, we report the synthesis of two new water-soluble calix[4]pyrroles **1b** and **1c** containing four carboxylic and four amino groups, respectively. The complexation studies of **1b** and **1c** with *N*-oxides **2a**−**b** carried out using ^{1}H NMR and UV−vis spectroscopy reveal that both receptors are able to form 1:1 inclusion complexes in water. The resulting complexes are highly stable kinetically and thermodynamically. The combination of hydrophobic binding with the formation of hydrogen bonds provide the major driving forces for the complexation of pyridine *N*-oxides in water yielding a complex geometry in which the polar group is buried deep inside the aromatic cavity. A similar binding behavior is observed in acetonitrile. The geometry assigned to the inclusion complexes in solution has also been determined by X-ray analysis of single crystals of the **2**⊂**1a** complexes grown in acetonitrile solution.

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Supporting Information Available: Experimental details of syntheses, EXSY of **2a**⊂**1b**, potentiometric measurements for **1b**−**c**, ^{1}H NMR and UV−vis titration experiments. X-ray crystallographic files (CIF) of the acetonitrile solvate of **1a** and the complexes **2a**⊂**1a** and **2b**⊂**1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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