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Molecular Recognition in Methanol: The First Example of Hydrogen-Bond-Mediated Self-Association of a Calix[4]arene in Polar, Protic Solvent

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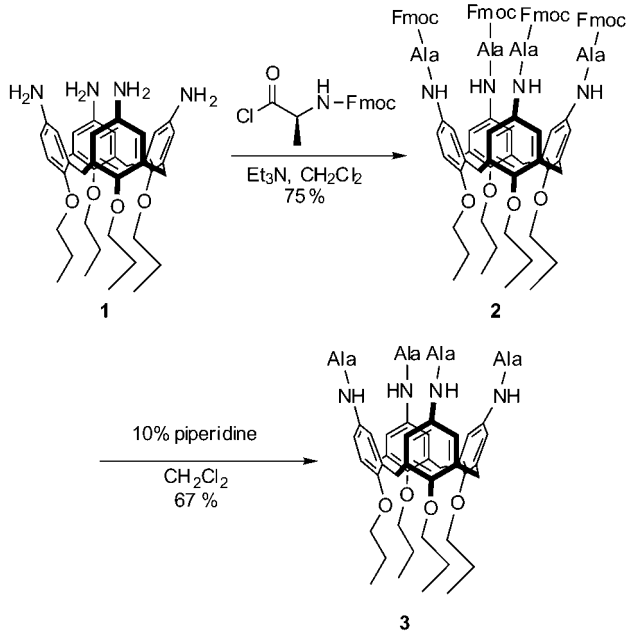
The design of organic compounds that form defined structures through noncovalent association is important for the development of new molecules for information storage, catalysis, molecular transport, sensing, and the assembly of supramolecular structures, among other things. Many examples of compounds that assemble by hydrogen bonding in apolar, noncompetitive solvent have been reported, but molecules that form hydrogen-bonded complexes in polar solvent are much less common.[1,2] It is desirable to prepare systems that will associate in polar solvent, especially for the development of compounds that will be compatible with a biological environment and have applications in drug transport and delivery, biocatalysis, and the binding and detection of biomolecules. Calix[4]arenes substituted with aryl[3] or peptidyl[4] ureas have been studied extensively for their ability to undergo hydrogen-bond-mediated dimerization in apolar solvent. This phenomenon is not observed in polar solvent, however, and typically the addition of only a few percent of polar solvent will result in complete disassembly to monomers.[3e,f,i,4b] Here we report the first calix[4]arene derivative that undergoes hydrogen-bond-mediated self-association in polar, protic solvent.

Calixarenes have become an important class of host molecules, and appropriately functionalized calixarenes have been used for binding a variety of compounds, from small molecules[5,6] to protein surfaces.[7] Peptide-substituted calixarenes, in particular, are attractive as hosts for molecular recognition because amino acids encompass a diversity of functionality, techniques for the synthesis of peptides have been extensively developed, and their chirality can influence the stereoselectivity of binding. A number of calixarenes substituted with amino acids at the upper rim have been reported.[5,7] However, with only one exception that we are aware of,[8] these approaches utilize carboxyl functionality at the upper rim and require coupling to the amino terminus of an amino acid or peptide. Our approach involves the coupling of the carboxyl termini of amino acids to an aminocalixarene, which can be accomplished through standard Fmoc peptide synthesis, as we recently reported for the solid-phase synthesis of a calix[4]arene substituted with tripeptides at the upper rim.[9] This was also the first example of a tetrapeptidocalix[4]arene in which the amino acids are attached to the upper rim through the carboxyl termini. We are interested in the recognition properties of this new class of peptidocalixarenes, both in their ability to dimerize and to bind small molecules, for application to molecular recognition and catalysis.

The synthesis of alanine-substituted calix[4]arene from the known aminocalixarene **1**[10] is shown in Scheme 1. The aminocalixarene

Scheme 1



was treated with the acid chloride of Fmoc-alanine to provide the desired product, **2**, in 75% yield. Treatment with piperidine removed the protecting groups to furnish **3** in 67% yield.

For investigation of the dimerization and binding properties of **3** by NMR, the alaninecalixarene was dissolved in MeOH-d_4 containing 4% D_2O. The resonances for the alanine methyl protons shift upon dilution (see Figure 1), consistent with the formation of a dimer. In addition, mass spectroscopy of an 8 mM solution indicates the presence of the dimer. Mass spectroscopy also confirms the presence of multiply charged species, in agreement with the expectation that at least some of the terminal amines are protonated. This protonation likely contributes to the strong association of the dimer, since the strength of hydrogen bonding increases with increasing acidity of the H-bond donor.[11] From the dilution data, the association constant, K_a, was calculated to be 29 000 M^{-1} in 24:1 CD_3OD/D_2O.[12] The association constants of only one peptidocalixarene homodimer have been reported, a peptidylureidocalixarene with a K_a of 20 M^{-1} in CD_2Cl_2 and 5100 M^{-1} in toluene.[4a] Remarkably, the K_a of **3** in methanol is over 5 times as large as that of the previously reported homodimer in the *apolar* solvent toluene! This is the first example of a calixarene dimer that is thermodynamically stable in polar, protic solvent. While several examples of dimers that are kinetically stable due to

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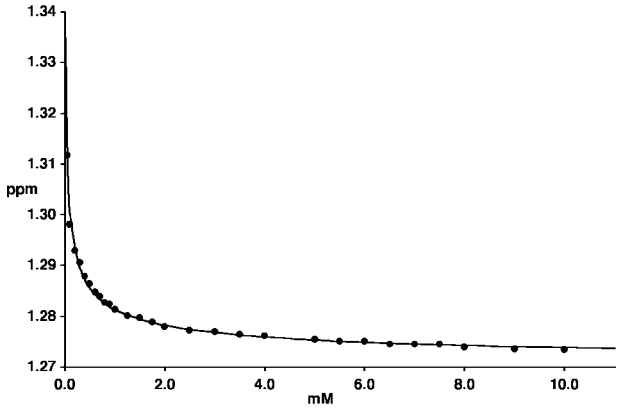

Figure 1. Chemical shift versus calixarene concentration. The closed circles are the experimental data points, and the line is the theoretical curve based on the calculated values for K_a, δ_{dimer}, and $\delta_{monomer}$.

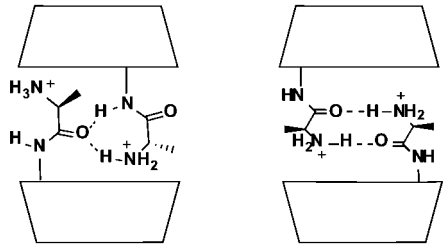

Figure 2. Proposed interactions in the tetraalaninecalixarene dimer.

the presence of bulky substituents have been reported, these dimers eventually dissociate in polar solvent.[13]

The peptidylureidocalixarenes that have been reported self-associate through hydrogen bonding of the urea moieties, with the amino acids participating only in secondary interactions.[4a] In contrast, hydrogen bonding in the dimerization of **3** must take place through the amino acids. Two modes of interaction of the alanines in the dimer are shown in Figure 2. In model A there are a total of 8 hydrogen bonds in the dimer, while in B there are 16, which suggests that B should be more favorable.

To determine if **3** binds amino acids, NMR spectra were recorded of 2 mM solutions of the calixarene containing 2 mM concentrations of each of the following: arginine, asparagine, aspartic acid, glycine, lysine, phenylalanine, serine, and valine. Significant changes in the chemical shift of the alanine α and β protons were observed upon the addition of arginine and lysine. In the mass spectrum of a solution of the calixarene and amino acid, there is a peak corresponding to the 1:1 complex and no peaks corresponding to the 2:1 or 1:2 complexes. This suggests that the addition of arginine or lysine to the calixarene solution results in the formation of 1:1 inclusion complexes of the amino acid with the calixarene and dissociation of the calixarene dimer, rather than the encapsulation of the amino acid within the dimer. This phenomenon of the addition of a guest molecule resulting in the disassembly of calixarene dimers has not been reported previously.

In conclusion, we present here the first example of a calix[4]-arene derivative that self-associates in polar, protic solvent. The association constant, K_a, for this dimerization is 29 000 M^{-1} in 24:1 MeOH:H_2O, which is higher than the K_a value that has been reported for a peptidylureidocalixarene in *apolar* solvent.[4a] Upon addition of arginine or lysine, a 1:1 complex is formed between the calixarene and the amino acid at the expense of the homodimer. The preparation of a peptidocalixarene that associates in polar solvent opens new doors for the use of calixarenes for molecular recognition in biologically relevant environments. We are currently preparing calixarenes substituted with other amino acids and peptides to determine the effect of the length and composition of the peptide chain on dimerization. In addition, we are investigating the binding of amino acids by these peptidocalixarenes, and we are determining the structures of the dimers and complexes to better understand the forces that govern the associations.

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Supporting Information Available: Experimental procedures and spectral data for **2** and **3** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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