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Chiral Dimeric Capsules from *N,C*-Linked Peptidocalix[4]arenes Self-Assembled through an Antiparallel *β*-Sheetlike Motif

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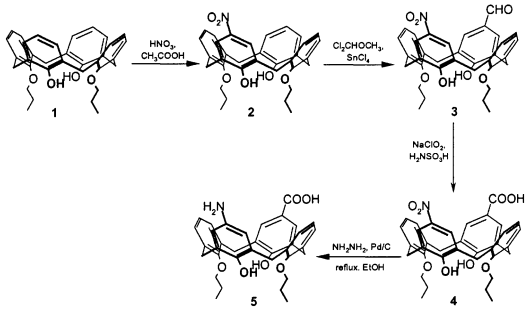
Self-assembly of molecular containers such as calixarene or resorcinarene derivatives has been widely studied in recent years to obtain interesting supramolecular structures.[1] The *self*-assembly process has been induced by hydrogen bonding,[2] ion pairing,[3] metal coordination,[4] and solvophobic forces.[5] Among the hydrogen-bonded systems, particularly studied are the calixarene tetraurea derivatives, which give rise to the formation of dimeric *self*-assembled capsules able to complex, in a reversible fashion, neutral organic molecules and organic cations. It is well-known that amino acids and peptides are involved in the formation of several *self*-assembled structures such as α-helices, *β*-sheets, and nanotubes.[6] However, they have never been used as promoters of *self*-assembling capsules from molecular cavities.[7] Only two examples are known in which amino acid derivatives have been used to adorn calix[4]arene tetraurea capsules to (i) increase their stability via peripheral hydrogen bonds[8] or (ii) transfer chiral information through noncovalent molecular assemblies.[9] Recently, we have been engaged in the study of *N*- and *C*-linked peptidocalix[4]arenes which have shown interesting supramolecular properties.[10]

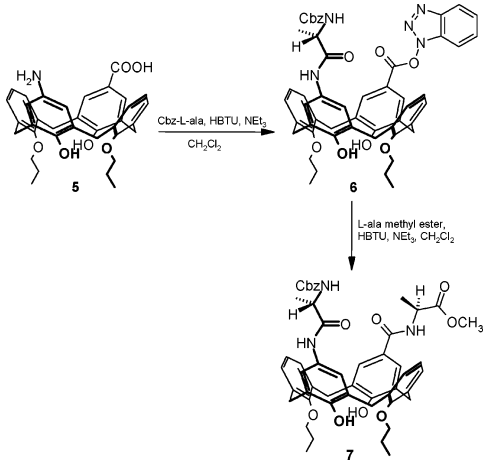
In this communication we report the first synthesis of a calixarene amino acid (**5**) and the unique *self*-assembly properties of the pseudopeptides in which this macrocyclic unit has been incorporated. To the best of our knowledge calix[4]arene amino acids, having an amino group on one aromatic nucleus and a carboxylic group on another, are unknown, since their synthesis is rather difficult. After several unsuccessful attempts, we finally synthesized compound **5** (Scheme 1), passing through derivative **3**[11] which was

Scheme 1. Synthesis of the Calix[4]arene Amino Acid **5**

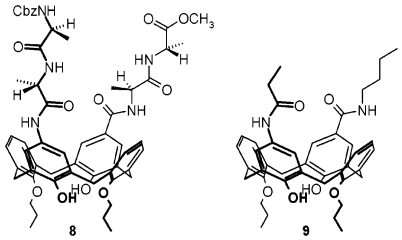


first oxidized to the corresponding carboxylic acid derivative **4** and then reduced to amino acid. The treatment of **5** (Scheme 2) with *N*-Cbz-L-alanine in the presence of HBTU and NEt_3 gave compound **6** with the terminal carboxylic group already activated as benzotriazolyl ester. This compound was condensed with L-alanine methyl ester to yield the *N,C*-linked peptidocalix[4]arene **7** (63% yield from **5**).

Scheme 2. Synthesis of the *N,C*-Linked Peptidocalix[4]arene **7**



Using similar procedures, the longer *N,C*-linked peptidocalix[4]arene **8** and the simple mixed diamide derivative **9** were synthesized. All calixarene derivatives exhibit *cone* conformation as evidenced by the typical geminal coupling constants (12.3−13.9 Hz) and $\Delta\delta$ values (0.6−1.0 ppm) between the *axial* and *equatorial* protons of the methylene bridge.



The three compounds **7**−**9** show ^{1}H NMR spectra in $CDCl_3$, which are strongly dependent on concentration. For example, by diluting a 10^{-2} M solution of **8** down to 5×10^{-5} M, a 1.5 ppm upfield shift of the NH proton linked to the aromatic nucleus (NHar) is observed. At the same time, the NH signal of the alanine methyl ester undergoes an upfield shift of 0.8 ppm, those of the unsubstituted aromatic rings a 0.7−0.9 ppm downfield shift, while shifts of 0.1−0.6 ppm are experienced by the OHs and the other NH protons. A similar behavior is shown by compounds **7** and **9**. In all cases the spectra remain sharp, which rules out the formation of large, disordered aggregates and suggests the formation of well-defined supramolecular species. ESI-MS experiments using a 10^{-4}

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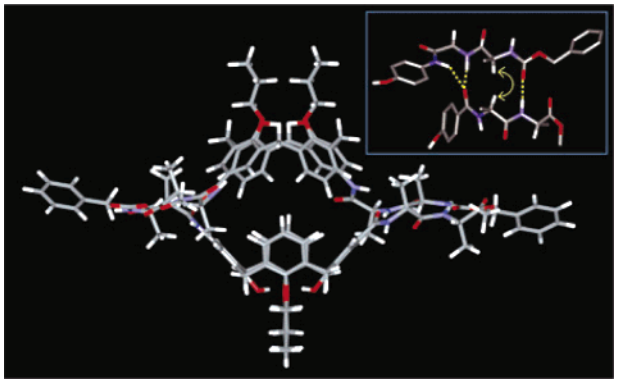

Figure 1. Minimized structure for the dimer of the *N*,*C*-linked peptidocalix[4]arene **8**, showing (see box) the hydrogen bonds and the NOE contact between the peptide chains.

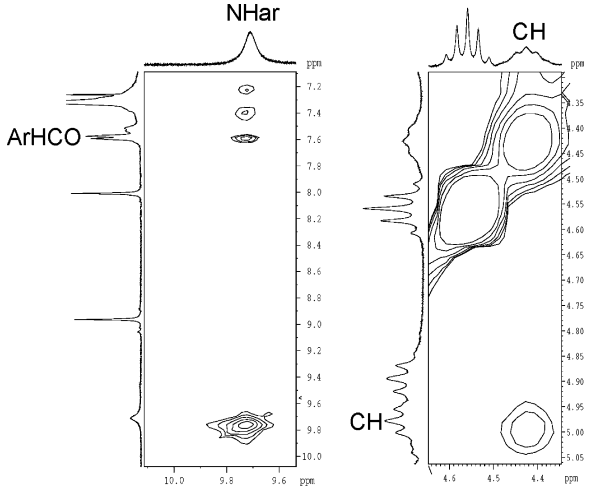

Figure 2. Expansions of the NOESY map of peptidocalix[4]arene **8** ($CDCl_3$, concentration: 4.0×10^{-2} M, 300 MHz, 300 K).

M methanol solution of the receptors show the presence of a molecular ion corresponding to the dimer, with a relative abundance with respect to the monomer of 15% (**8**), 12% (**7**), and 10% (**9**). NMR dilution experiments allowed us to evaluate[12] the association constants for the dimerization process (K_{dim}), which increase in the order: **9** (74 M^{-1}) < **7** (105 M^{-1}) < **8** (776 M^{-1}), following the increase in the number of the potential hydrogen-bonding donor and acceptor groups. To have more insight into the structure of this dimeric species, we performed molecular mechanics calculations on compound **8** which show (see Figure 1) the formation of a dimeric capsule with a volume of ca. 150 Å^3 (see Supporting Information), held together by an array of hydrogen bonds which resembles an antiparallel β-sheet motif.[13]

This structure is also present in $CDCl_3$ solution. In fact, the NOESY NMR spectrum of compound **8** at a high concentration (>10^{-2} M) shows (see Figure 2) the presence of a cross-peak between NHar and the aromatic protons (ArHCO) in ortho position to the *N*-linked dipeptide and a cross-peak between two CH-alanine protons, one belonging to the *N*-linked and the other to the *C*-linked peptide (Figure 1, box). A similar behavior is also shown by the other two derivatives **7** and **9** (see Supporting Information). The concentration-dependent NMR spectra observed for compounds **7**−**9** rule out the formation of intramolecular hydrogen bonding, as indeed observed with the *C*-linked peptidocalix[4]arenes.[14] Only at very low concentration ($\leq 5.0 \times 10^{-5}$ M), where the receptors **7**−**9** exist in a monomeric form, an equilibrium between *open* and *closed* conformations may be possible, as suggested by the broadness of some signals in the corresponding ^{1}H NMR spectra (see Supporting Information).

It seems that the minimum requirement for the formation of a *self*-assembled capsule is the simultaneous presence of a *N*-linked and a *C*-linked amide group on the diametral positions of a *cone* calix[4]arene. This novel hydrogen-bonding motif for the *self*-assembly of calix[4]arene dimeric capsules is attractive for a number of reasons: (i) the robustness of the cage can be modulated by changing length, nature, and number of the peptide chains up to the formation of α-helices; (ii) the capsular system obtained with **7**−**9** resembles a hemicarcerand type of molecule. However, the size of the portals can be varied by introducing substituents on the remaining aromatic nuclei, which could lead to a completely closed system similar to the covalently assembled Cram's carcerands. Studies are in progress in our laboratory to evaluate the scope of these novel *self*-assembled dimeric capsules in supramolecular chemistry and nanoscience.

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Supporting Information Available: Experimental procedures and spectroscopic data of all new compounds, 1D- and 2D-NMR and MS spectra of compounds **7**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) The observed shifts (δ_{obs}) were fitted to the equation $\delta_{obs} = \delta_{dimer} + \{-(\delta_{monomer} - \delta_{dimer})[(-1 + (1 + 8K_{dim})^{1/2}]/(4K_{dim}C)\}$ using the program HypNMR (Frassineti, C.; Ghelli, S.; Gans, P.; Sabatini, A.; Moruzzi, M. S.; Vacca, A. *Anal. Biochem.* **1995**, *231*, 374−382). *C* is the analytical calixarene concentration and K_{dim}, $\delta_{monomer}$, and δ_{dimer} are the calculated parameters.

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