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Calix[4]arene-constrained cyclopeptides, a novel type of macrocyclic host molecule

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Abstract

Two calix[4]arene cyclopeptides were synthesized by reacting 1,3-di(chlorocarbonyl-methoxy)-*p-tert*-butylcalix[4]arene with cystine dimethyl ester dihydrochloride. One of them was found to be a good receptor for phosphomonoester.

Keywords: calixarene; cyclic peptide; molecular recognition.

Cyclic peptides have reduced conformational flexibility compared to their parent linear molecules, a feature that makes them of great interest. Apart from being a useful tool for the study of protein secondary structure and of the structure–activity relationships of bioactive peptides,[1] this type of compound has also been found to be important in ion transport across biological membranes,[2] in self-assembling size-adjustable nanotubes,[3] and in serving as enzyme models[4] or as artificial receptors.[5,6]

Despite the relatively strong topological constraint in their skeletons, cyclic peptides still possess quite large conformational flexibility. Thus, several constraints such as non-natural amino acids,[7] S–S linkages,[8–10] and semi-rigid aromatic units[9–11] have been incorporated into the cyclic backbone. The convenience and efficiency of these approaches in controlling structural freedom have already been demonstrated.[6b,12] It is envisaged that if a rigid host molecule is used as a constraint in modifying a cyclopeptide, it should not only reduce conformational freedom, but may also provide additional binding sites, and thus lead to improved or specialized properties, for example, in molecular recognition. In this regard, calixarenes (especially calix[4]arenes),[13,14] the newly developed class of cyclic host molecules of rigid structure, are thought to be good candidates. In fact, a few calixarenes bearing amino acid or peptide (including cyclic peptide) units have been synthesized.[15] However, only one report on calixarene–cyclopeptide conjectures bridged at the upper rim has recently appeared.[16] In the present work, we wish to report the first synthesis of the lower-rim-linked calix[4]arene-bearing cyclic peptides **2** and **3** and the preliminary investigation of the recognition property of **2**.

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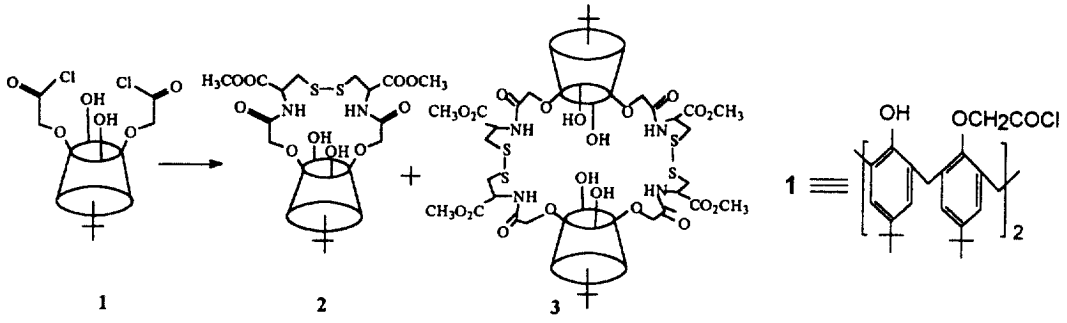
A simple multistep route shown in Scheme 1 was chosen for the synthesis of calix[4]arene-bearing cyclopeptides. Reaction of **1**[17] with an equimolar amount of L-cystine dimethyl ester dihydrochloride, in the presence of excess Et_3N, in highly dilute solution provided calix[4]arene cyclopeptide **2** and its dimer **3** in 28% and 3% yields, respectively, after column chromatography (silica gel, with dichloromethane/ethyl acetate as eluent).



Scheme 1. Reaction conditions. L-Cystine dimethyl ester dihydrochloride, $N(C_2H_5)_3$, CH_2Cl_2

Formation of the two calix[4]arene cyclic peptides was confirmed by MS, NMR and IR measurements.[23] FD-MS spectra show that peptides **2** and **3** correspond to '1+1' and '2+2' cyclization products, respectively. The ^1H NMR spectrum reveals that the H_A and H_B protons of the $ArCH_AH_BAr$ moiety in **2** appear as two sets of two doublets (δ 4.34, 3.29, J=12.7 Hz, δ 4.04, 3.48, J=14.6 Hz; respectively), the $-OCH_2$ protons are two doublets (δ 4.97, 4.27, J=15.5 Hz), and the aromatic protons are four doublets (δ 7.11, 7.05, 6.80, 6.71, J=1.68, 1.94, 1.98, 1.94 Hz, respectively). These suggest that **2** should be in a cone conformation and the splitting pattern be attributed to the presence of chiral centres within **2**. Similar splitting was observed in other chiral calix[4]arenes.[15] Peptide **3** shows a similar NMR spectrum.

A comparison of the ^1H NMR spectrum of compound **2** with that of a structurally similar calixazacrown[18] indicates that the amide protons appearing at δ 9.06 ppm are likely involved in intramolecular hydrogen bonding with neighbouring OH oxygen atoms. This is supported by NH stretch at 3327 cm^{-1} in the IR spectrum in dichloromethane,[19] and by an H/D exchange investigation where the OH protons were found to be completely exchanged within minutes whereas the NH protons remained for an hour. This may further suggest that the N–H bonds have oriented towards the calixarene cavity, a feature that good H-bonding donors usually possess.

Preliminary binding experiments with phosphomonoester were carried out in order to test the potential of compound **2** in molecular recognition. Upon addition of **2** to the DMSO solution containing disodium 4-nitrophenyl phosphate **4** (Fig. 1) (4.08×10^{-5} M, 299 K), the absorbance of **4** at 314 nm increased and the one at 436 nm decreased, with an isobestic point at 371 nm. A similar spectral change was reported when cyclodepsipeptides were used as hosts and was believed to be due to hydrogen bonding to the phosphate oxygen atoms.[6b] An analysis on the changes of the absorbance at 436 nm suggested a 1:1 complex formation and allowed the binding constant to be calculated as $(3.9\pm0.4)\times10^3$ M^{-1}, on the basis of the modified Hildebrand–Benesi method.[20] For comparison, binding constants with three reference receptors, i.e., cyclopeptide **5**,[21] calix[4]arene **6a**[21] and **6b**[22] (Fig. 1), were determined, giving binding constants of $(2.8\pm0.3)\times10^2$ M^{-1}, $(4.2\pm0.4)\times10^2$ M^{-1} and $(2.6\pm0.3)\times10$ M^{-1}, respectively. It is clear that cyclopeptide **2** is a much more efficient receptor than the three references, demonstrating that the introduction of a calix[4]arene unit substantially enhances molecular recognition for a phosphate group, and that the amide groups are mainly responsible for the recognition.

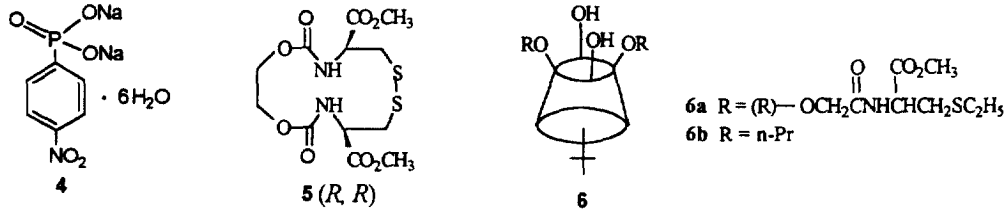


Figure 1.

Evidence showing good association between the phosphate and the host can also be found in the NMR spectrum of this supramolecular system, especially on the side of the phosphate anion. In Me_2SO-d_6, the aromatic protons of the anion appearing at δ 6.24 ppm are downshifted substantially by 0.56 ppm in the presence of peptide **2**, indicating clearly that electrons in the phosphate phenyl ring are being removed by the host, presumably through H-bonded complexation as shown in the UV experiments (vide supra). It is noted, however, that the chemical shifts of the NH and OH protons in **2** are small. This is probably because the intermolecular hydrogen bonding between **2** and **4** has reached a comparable level to that of the original hydrogen bonding in **2**. All the above data showed that cyclic peptide **2** binds with the phosphomonoester via hydrogen bonding mainly to the amide hydrogen atoms.

In summary, we have designed and successfully synthesized two cyclic peptides of novel structure (**2** and **3**) and found that one of them (i.e., **2**) is an efficient receptor for the biologically important phosphate molecule whose neutral receptors are only scarcely reported.[6b] The present result clearly demonstrates for the first time that introducing rigid host molecules such as calix[4]arene as constraints to the backbone of cyclic peptides improves the properties of cyclopeptides, e.g., in molecular recognition.

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