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A new cyclic tetrapeptide composed of alternating L-proline and 3-aminobenzoic acid subunits

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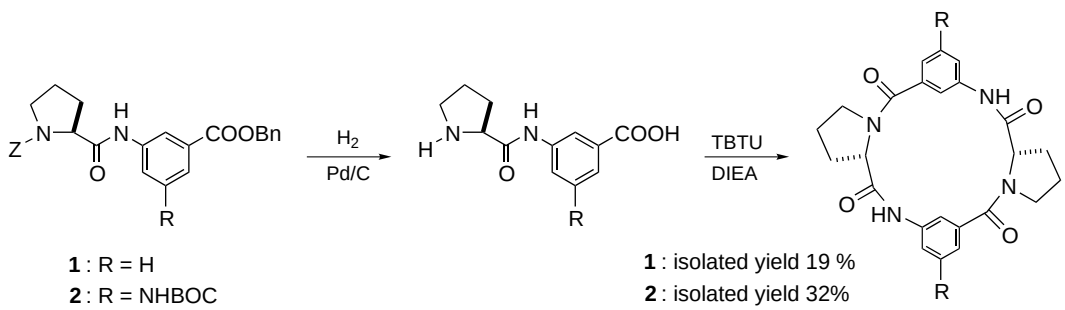
Abstract—A cyclic tetrapeptide composed of alternating L-proline and 3-aminobenzoic acid subunits has been synthesized and its structure determined in solution and in the solid state. The cyclopeptide possesses a significantly smaller cation affinity than the corresponding hexapeptide. Derivatives with suitable substituents on the aromatic subunits can be used as tweezer-type receptors.

Cyclic peptides make attractive candidates for artificial receptors when they adopt conformations in solution with well defined cavities in which guest molecules can be included. One way to control the conformational flexibility of cyclopeptides is the introduction of rigid, non-natural amino acids in the ring. A subunit that has received some attention in this regard is 3-aminobenzoic acid (Aba). This aromatic amino acid not only adds rigidity to the cyclic product, but can also carry various additional functional groups with which guest molecules can interact. Cyclopeptides composed of alternating natural amino acids and Aba subunits were first introduced by Ishida and co-workers as artificial receptors for monophosphate esters[1a] and serine protease mimics.[1b] The anion affinity of these peptides is due to the cyclic arrangement of NH groups around the cavity. Besides these amide groups, the Aba subunits of such peptides can also contribute to guest binding. We have recently shown that a cyclic hexapeptide containing alternating L-proline and Aba subunits preferentially adopts a conformation in solution that is comparable with the *cone* conformation of calixarenes.[2] In this conformation, the aromatic peptide subunits line the wall of a shallow cavity and are able to cooperatively interact with positively charged guests such as quaternary ammonium ions by cation–π interactions. The natural amino acid proline adds additional conformational stability to this hexapeptide by virtue of its cyclic structure.

A factor that also determines the binding properties of macrocyclic receptors is ring size. So far, we have focussed our attention on cyclic hexapeptides, but other groups have used larger peptides containing Aba sub-



Scheme 1.

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units, e.g. as scaffolds for the assembly of four α-helix bundles.[3] Peptides smaller than a hexapeptide, however, have not been described yet. We were therefore interested whether cyclic *tetrapeptides* with L-proline and Aba subunits are also accessible and what their conformational and receptor properties might be. Here we report the results of these investigations.

We usually prepare our cyclic peptides by macrocyclization of the corresponding completely deprotected linear precursors.[2] A cyclic hexapeptide, for example, can be obtained from a linear hexapeptide. However, cyclopeptide synthesis can alternatively be carried out with linear peptides that only correspond to the shortest repeating unit in the cyclic product. In this case, chain elongation and cyclization proceed successively in solution, and an isolation of intermediates is not necessary. Thus, the unprotected dipeptide (L)-Glu(O*i*Pr)-Aba, for example, affords both the corresponding cyclic hexapeptide and octapeptide as the major cyclic products under the usual cyclization conditions. To our surprise, we found that in the case of the proline containing dipeptide (L)-Pro-Aba, the cyclic tetrapeptide **1** is formed primarily (Scheme 1). In spite of the rigidity of the subunits, the cyclization of the linear tetrapeptide precursor is clearly relatively efficient, which can most probably be attributed to the presence of tertiary amides which are generally known to facilitate cyclization because of their reduced rotational energy barrier in comparison to secondary amides.

Although in principle cyclic tetrapeptides are able to adopt conformations with four *trans* amide bonds in the ring,[4] structures in which *cis* amides at the tertiary amide groups of proline or sacrosine subunits are present are more common. X-Ray crystallography[5,6] shows that both proline amides in **1** adopt the *cis* conformation in the crystal (Fig. 1). Thus, an alternating sequence of *cis* and *trans* amide groups (*ctct*) results, a pattern that is in fact frequently found in many cyclotetrapeptides.[7]

Peptide **1** crystallizes from aqueous methanol with two molecules of water per peptide unit in an exact C_2 symmetrical structure. Whereas C_2 symmetrical cyclotetrapeptides with four L-α-amino acids and a *ctct* conformation of the amide bonds usually exhibit up–down–up–down arrangement of the carbonyl groups,[8] in **1** all four carbonyl groups point toward the same side of the ring because of the presence of the two γ-amino acid subunits. This arrangement is similar to the one found in *cyclo*-β-tetrapeptides with *S* configuration at the four amino acid subunits.[9] Peptide **1** furthermore appears to possess no defined cavity since the aromatic rings make an angle of 109° to each other.

In solution, characteristic elements of this conformation are retained. The ^{1}H and ^{13}C NMR spectra of **1** in DMSO-d_6 and DMSO-d_6/$CDCl_3$ mixtures are consistent with averaged C_2 symmetrical conformations. The chemical shifts of C(β) (31.3 ppm) and C(γ) (22.9 ppm) in the ^{13}C NMR spectrum indicate the presence of *cis* amide bonds at the proline subunits.[10] In the NOESY NMR spectrum, cross peaks are visible between H(4) of the Aba subunits and the NH groups, as well as between the NH groups and H(α) of the proline subunits. However, there is also a cross peak visible between H(2) of the aromatic subunits and NH. This indicates that, as we have found in the larger hexapeptide, the secondary amide groups of **1** are able to rotate in solution.[2b]

We also prepared the tetrapeptide with BOC-protected amino groups in the 5-position of the aromatic subunits (**2**). Spectroscopic investigations indicate that the additional substituents have no significant influence on the conformation of **2**, and on average **2** adopts a conformation in solution similar to that of **1**. The crystal structure of the monohydrate of **2** is given in Fig. 2.[6,11] At first sight the conformations of **1** and **2** appear to be completely different, but a closer inspection reveals that the *ctct* sequence of the amide bond conformations and the relative orientation of the H(4), H(α), and NH protons are retained in both structures. The major difference lies in the torsion angles of the four independent non-amide rotatable bonds (**1** (**2**): C1–N1–C11–C12 = 43° (−9°); N2*–C6–C7–C12 = 51° (120°); N1–C1–C2–N2 = 167° (136°); C1–C2–N2–C6* = −91° (−51°)). This has the result that in **2** the angle between the aromatic rings is reduced to 2°. Thus, **2**

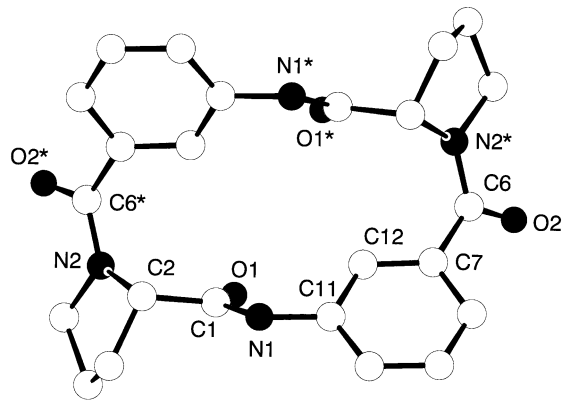

Figure 1. Crystal structure of cyclotetrapeptide **1**.

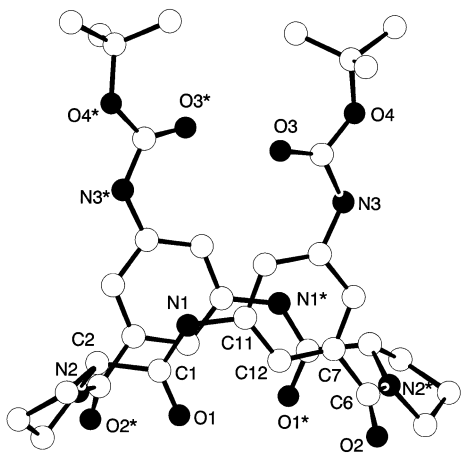

Figure 2. Crystal structure of cyclotetrapeptide **2**.

adopts a folded peptide conformation in the crystal with a slightly tilted, face-to-face arrangement of the two aromatic rings, whereas in **1** the structure is much more open.

At room temperature, both tetrapeptides can be expected to equilibrate between folded and open conformations in solution. At −50°C, the signals in the ^{1}H NMR spectrum of **1** in DMF-d_7 are somewhat broadened but even at this temperature the conformational equilibrium is still too fast for individual conformers to be resolved.

We wondered what effect the reduced ring size and the particular conformational flexibility would have on the ability of **1** to interact with cations. Peptide **1** does possess a certain cation affinity because the typical upfield shifts of the guest protons that usually accompany cation–π interactions between a cation and an artificial receptor are indeed observed when *N*-methylpyridinium (MPy^+) picrate or *n*-butyltrimethylammonium ($BTMA^+$) picrate is added to a 1 mM solution of **1** in 2.5% DMSO-d_6/$CDCl_3$.[12] These shifts are small, however (ca. −0.03 ppm for the *N*-methyl protons of MPy^+ and −0.02 ppm for those of $BTMA^+$) and the saturation curves obtained from NMR titrations indicate only weak binding. The low solubility of **1** in 2.5% DMSO-d_6/$CDCl_3$ at higher concentrations prevents an accurate determination of the stability constants of the two cation complexes, but the K_a for both systems are certainly <100 M^{-1}. Under the same conditions, the stability constants of the corresponding cyclic hexapeptide complexes are 1480±130 M^{-1} with $\Delta\delta_{max}=-0.43$ ppm for $BTMA^+$ picrate and 2300±200 M^{-1} with $\Delta\delta_{max}=-0.56$ ppm for MPy^+ picrate at 298 K. The geometry and the cavity size of the hexapeptide are clearly much better suited for the binding of quaternary ammonium ions than those of the smaller tetrapeptide.

The conformational properties of the cyclotetrapeptide indicate, however, that it would function well as a molecular hinge in which the peptide ring controls the relative orientation of the two aromatic subunits. Such a behavior would be advantageous for the design of tweezer-type receptors. Binding sites that are necessary for this type of artificial receptor can easily be introduced by attaching substituents, e.g. via amide or urea linkages to the deprotected aromatic amino groups of **2**. A variety of different molecular scaffolds have already been used for similar structures,[13] but our cyclotetrapeptides have the advantage that their flexibility makes them better adaptable to the structure of a potential guest molecule. We have recently synthesized tetrapeptide derivatives based on **2** with urea residues for the complexation of anions, and cholic acid residues for the complexation of carbohydrates. Typical downfield shifts of the urea NH protons are observed in the ^{1}H NMR spectrum of the phenylurea substituted cyclotetrapeptide in DMSO-d_6 upon addition of, e.g. iodide, nitrate or acetate salts, which indicate an interaction of this peptide with the anions. We are currently investigating these effects and shall report quantitative results in due course.

Acknowledgements

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5. Crystal data for **1**·2(H_2O): $C_{24}H_{24}N_4O_4$·2(H_2O), M_w=468.5, colorless prism 0.10×0.16×0.17 mm, tetragonal $P4_12_12$ [no. 92], a=9.5406(4), c=24.405(1) Å, U=2221.4(2) Å^3, T=100 K, Z=4, D_x=1.401 g cm^{-3}, λ=0.71073 Å, μ=1.02 cm^{-1}, Nonius Kappa CCD diffractometer, θ_{max}=27.48°, 5690 measured reflections, 2532 independent, 1638 with $I>2\sigma(I)$. Structure solved by direct methods (SHELXS-97) and refined by least-squares (SHELXL-97) using Chebyshev weights on F_o^2 to R_1=0.069 [$I>2\sigma(I)$], wR_2=0.175 (all data), 165 parameters, H atoms on peptide constrained, H atoms on one solvate water molecule not located, S=0.984, residual electron density 0.38 e Å^{-3} (1.01 Å from O1).
6. Crystallographic data (excluding structure factors) for the structures have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 163975 and 163976. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambrige CB2 1EZ, UK [fax: (internat.) +44-1223/336-033; e-mail: deposit@ccdc.cam.ac.uk].
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11. Crystal data for **2**·H_2O: $C_{34}H_{42}N_6O_8 \cdot H_2O$, $M_w = 680.8$, colorless prism 0.16×0.16×0.36 mm, tetragonal $P4_32_12$ [no. 96], $a = 12.5767(2)$, $c = 21.4083(7)$ Å, $U = 3386.2(1)$ Å^3, $T = 100$ K, $Z = 4$, $D_x = 1.335$ g cm^{-3}, $\lambda = 0.71073$ Å, $\mu = 0.98$ cm^{-1}, Nonius Kappa CCD diffractometer, $\theta_{max} = 30.18°$, 17664 measured reflections, 4645 independent, 2644 with $I > 2\sigma(I)$. Structure solved by direct methods (SHELXS-97) and refined by least-squares (SHELXL-97) using Chebyshev weights on F_o^2 to $R_1 = 0.051$ [$I > 2\sigma(I)$], $wR_2 = 0.095$ (all data), 226 parameters, H atoms on peptide constrained, H atom of water solvate molecule refined with isotropic adp ($U_H = 0.043(8)$ Å^2), $S = 0.870$, residual electron density 0.24 e Å^{-3} (1.0 Å from O1).
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