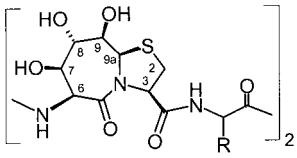

1 *cyclo*[-BicA-*D*-Phe-BicB-Gly-]
2 *cyclo*[-BicA-*L*-Phe-BicB-Gly-]

Peptidomimetics

Coupled Hydrogen-Bonding Networks in Polyhydroxylated Peptides**

*Peter Tremmel and Armin Geyer**

In memory of Murray Goodman

The active sites of proteins contain networks of hydrogen bonds which are functionally coupled to perform the enzyme function.[1] The side chains of short peptides cannot align in comparable microenvironments because they are too flexible to form more than transient hydrogen bonds. Dipeptides which are highly decorated with ring-constrained hydrogen-bond donors and acceptors can be assembled to oligocyclic peptidomimetics which are then confined to only a few modes of flexibility. Two such peptides are presented herein which have stable hydrogen-bonding networks involving several side-chain hydroxy groups and backbone amide groups. The side-chain hydroxy groups are pushed against each other to cooperatively align in a chain of hydrogen bonds bridging two backbone amide groups which are 7 Å apart. The inversion of the chirality of a single amino acid results in a flip of the entire hydrogen-bonding network with an exchange of proton donor and acceptor groups. Both peptides are characterized by crystal structures and by NMR spectroscopy. The conformational homogeneity and the existence of a hydrogen-bonding network in solution is corroborated by the dispersion of NMR spectroscopic data.

The synthesis of the bicyclic dipeptide (Bic) starting from D-glucurono-3,6-lactone was described recently.[2] C-terminal coupling with L-Phe, D-Phe, or Gly yielded three tripeptides which were coupled to hexapeptides and finally cyclized to either **1** or **2**.

[*] Dipl.-Chem. P. Tremmel, Prof. Dr. A. Geyer
Fachbereich Chemie
Philipps-Universität Marburg
35032 Marburg (Germany)
Fax: (+49) 6421-2822021
E-mail: geyer@staff.uni-marburg.de

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In a first approximation, cyclic hexapeptides can be regarded as the antiparallel combination of two β turns (Figure 1a) or two tripeptides (Figure 1b).[3] This differentiation makes sense when asking for the preferred positions of bicyclic dipeptides in a cyclic hexapeptide. The dipeptide can end up either in the central positions of the β turn as traditionally proposed for so-called β-turn mimics[4] or in the long side of the antiparallel mini β sheet. Crystal structures of the β-turn dipeptide (BTD)[4b] or of analogues[5c] are known but no crystal structures of unprotected oligopeptides containing bicyclic dipeptides are published to date. The concept of β-turn mimics was challenged by detailed experimental analyses[5] and modeling studies[6] and therefore the more general label bicyclic dipeptide mimetics is used herein. Oligomers of Bic assume an extended polyproline-II-helical structure[2c] indicating that Bic will prefer the long side of a cyclic hexapeptide (Figure 1b) irrespective of the chiralities of the other amino acids.

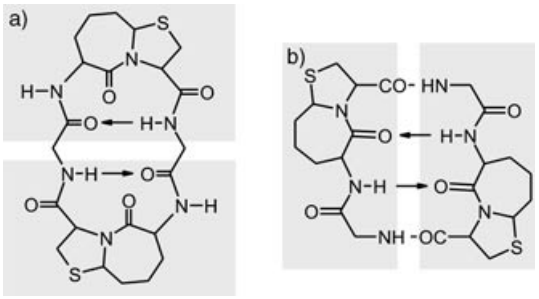

Figure 1. Schematic representations of two possible conformations of C_2-symmetric cyclic hexapeptides with arrows indicating antiparallel hydrogen bonds of the central mini β sheet. The 7,5-bicyclic dipeptide mimetics can occupy the central $i+1$ and $i+2$ positions of the β turns (a) or occupy the i and $i+1$ positions (b).

The crystals of cyclopeptides **1** and of **2** show an overall C_2-symmetric rectangular backbone structure with Bic occupying the i to $i+1$ positions of βI and βII turns, respectively (Figure 2).[7] Thus, **1** and **2** fit the general structure of Figure 1b. In peptide **1**, D-Phe is found in the $i+2$ position of βII turn and Gly in the $i+2$ position of a βI turn. Although only one out of 13 stereocenters was changed in peptide **2**, a mirrored backbone structure is observed with L-Phe occupying the $i+2$ position of a βI turn while Gly is found in the $i+2$ position of βII turn. Four side chain hydroxy groups (7-OH and 9-OH of BicA and BicB) are in close contact in each cyclopeptide. They are aligned in a zipperlike fashion forming a chain of hydrogen bonds connecting the two central amide bonds of the β turns. The inversion of the chirality of the Phe

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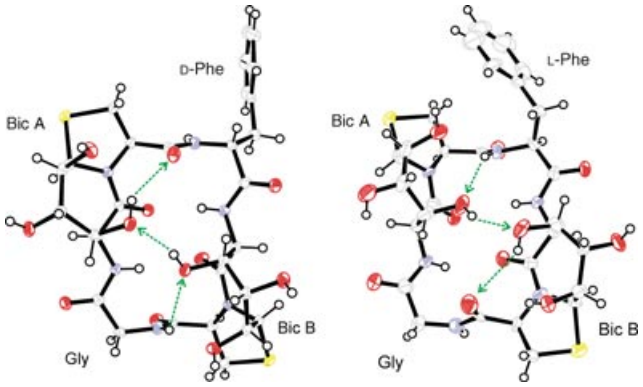

Figure 2. Crystal structures of peptides **1** (left) and **2** (right). Green arrows indicate the chains of intramolecular hydrogen bonds connecting the central amide bonds of the two β turns (red O, blue N, yellow S).[7,9]

residue is accompanied by an 180° flip of the neighboring amide bond between BicA and L-Phe. As a consequence, the two cyclopeptides exhibit a reversed hydrogen-bonding network with exchanged donor and acceptor groups. Several water molecules and many intermolecular hydrogen bonds are found in both crystals.[7] To separate potential influences of the crystal environment on the peptide conformations, **1** and **2** were investigated in isotropic solution, too.

In both peptides, the two hydrogen bonds spanning the antiparallel mini β sheet (BicA-6N*H*–BicB-5C*O* and BicB-6N*H*–BicA-5C*O*) are protected from chemical exchange and therefore no cross-peaks between the two NH protons and the water signal are detectable in the expansion from the ROESY spectrum of **1** (Figure 3).[8] All residual protons are accessible to water. The mini β sheet is retained in the organic solvent [D_6]DMSO, as shown by the temperature dependence of the ^{1}H NMR spectroscopy chemical shifts ($\Delta\delta/\Delta T$) of the amide protons. They are close to zero for the amide protons involved in hydrogen bonds **1**BicA-6N*H* (+0.2 ppb/K), **1**BicB-N*H* (−1.3 ppb/K), **1**Gly-N*H* (−0.6 ppb/K), **2**BicA-6N*H* (−1.0 ppb/K), **2**BicB-N*H* (−0.1 ppb/K), and **2**Phe-N*H* (−0.5 ppb/K). They are significantly larger for the residual amide protons **1**Phe-N*H* (−5.7 ppb/K) and **2**Gly-N*H* (−4.1 ppb/K). Similar to the crystal structures, the exchange of the $i+2$ amino acid D-Phe against L-Phe is accompanied by a 180° flip of the amide bond preceding this amino acid. Rotating-frame NOEs and ^3J coupling constants unequivocally identify the main backbone conformations of **1** and of **2** which match the crystal structures. Even the preferred side-chain rotamers of L-Phe and D-Phe in solution are the same as in the crystals, as determined from the $^3J_{H\alpha,H\beta}$ coupling constants. Hydrophobic stacking between the aromatic side chain and the neighboring thiaproline ring may contribute to stabilize the observed rotamers.

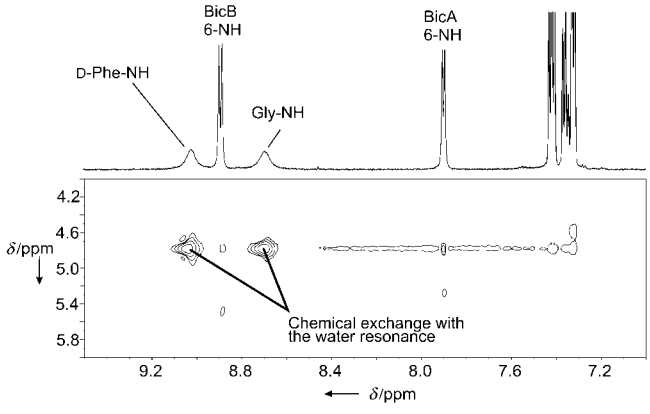

Figure 3. ROESY spectrum (watergate, 600 MHz, 300 K, H_2O/D_2O, 10:1). Expansion of the NH region. All the protons of the hydrogen-bonding network are exchange broadened at room temperature. Both 6N*H* protons are protected from chemical exchange and therefore no cross-peaks with the solvent signal are observed. D-PheN*H* and Gly-N*H* are solvent accessible and show intense cross-signals with water together with chemical exchange broadening.

The organic solvent [D_6]DMSO slows down chemical exchange and therefore the ^{1}H NMR spectroscopy resonance signals of the hydroxy protons are better resolved than in water. As a consequence, even ^4J couplings become observable for **1**: $^4J_{7\text{-OH},6\text{-CH}} = 1.4$ Hz in BicA and $^4J_{9\text{-OH},9a\text{-CH}} = 1.0$ Hz in BicB. $^4J_{H,H}$ long-range couplings are immediately lost upon deviation from the fixed W-arrangement of the four atoms involved and therefore $^4J_{H,H}$ long-range couplings can be regarded as another indicator of conformational homogeneity. The δ_H chemical shifts, the ^3J coupling constants, the rotating-frame NOEs, the temperature gradients ($\Delta\delta_H/\Delta T$) of the 7-OH, 9-OH, and NH protons distinguish the network of hydrogen bonds of **1** from that of **2**. Differences between crystal structure and solution structure are detected for the 9-OH moiety of BicA in **1** which forms an intermolecular hydrogen bond in the crystal but finds a different proton acceptor in solution. The $^3J_{9\text{-CH,OH}}$ coupling constant of 12.4 Hz identifies the inward orientation of BicA 9-OH towards the central carbonyl group of the βII turn. This type of hydrogen bond has been observed in the linear oligomers of Bic.[2c] Two views of **1** from opposite directions showing all relevant hydrogen-bonding contacts are given in Figure 4. Clearly, all four 7-OH and 9-OH hydroxy groups and two amides are linked by hydrogen bonds and polar contacts in solution. The solution structure of **2** resembles its crystal structure with BicB 7-OH group donating a proton to the carbonyl of the βII-turn and BicA 7-OH accepting a proton from the NH of the βI-turn. Conformational averaging can be excluded for both cyclic hexapeptides in water and in [D_6]DMSO solution, too. Diastereomers **1** and **2** differ in only a single stereocenter but exhibit remarkably stable and significantly different conformations. The overall C_2 symmetry of the cyclic hexapeptides **1** and **2** is broken by the benzyl side chains of the phenylalanines, a minor disturbance which locks the overall conformations through the alignment of all amide and OH groups except for the 8-OH groups which are directed outwards (Figure 2; the numbering of the OH groups corresponds to the numbering of the carbon atoms to which they are bound, see the formula of Bic).

In conclusion, the crystal structures of bicyclic dipeptide analogues within unprotected oligopeptides are described for the first time. The bicyclic dipeptides can occupy the i to $i+1$ position of either βI or βII turns. The rigidified amino acids derived from sugars are able to form stable hydrogen-bonding

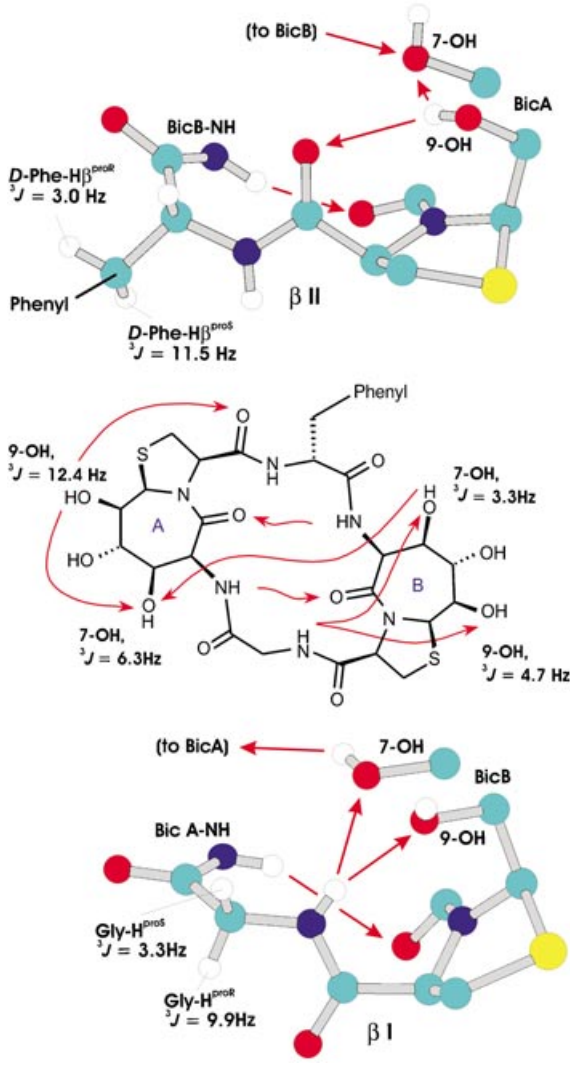

Figure 4. Solution structure of **1** in [D_6]DMSO. Two views from opposite directions perpendicular to the central amide bond of each β turn (cyan C, red O, blue N, yellow S). For clarity only selected atom positions are shown. Red arrows indicate hydrogen bonds and polar contacts. The schematic representation in the middle shows the entire hydrogen bonding network. Each of the two bifurcated hydrogen bonds cannot be distinguished from fast averaging between one hydrogen bond together with a polar contact to the second proton acceptor. The model (HyperChem 7.0) is based on NOEs and ^3J coupling restraints. The geometry of the two Bic-6N*H*–Bic-5C*O* hydrogen bonds was taken from the crystal structure.

networks which can transfer structural information along a chain of ambident proton donors and acceptors over several Å. Extended and functionally coupled hydrogen-bonding networks which can switch between different states play an important role in proteins. The structural diversity of secondary hydroxy groups is characterized by the dispersion of NMR parameters.

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[7] CCDC-241421 (**1**) and CCDC-241422 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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[9] The asymmetric unit of **1** contains one cyclopeptide and four water molecules. The asymmetric unit of **2** contains four cyclopeptides and 25 water molecules. The four cyclopeptides of **2** have identical βI, βII arrangements, only one is shown in Figure 2.

