Water-Stable Foldamers

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Unique α,β- and α,α,β,β-Peptide Foldamers Based on *cis*-β-Amino-cyclopentanecarboxylic Acid**

Łukasz Berlicki, Ludwig Pilsl, Edit Wéber, István M. Mándity, Chiara Cabrele, Tamás A. Martinek, Ferenc Fülöp, and Oliver Reiser**

Non-natural oligomers with well-defined three-dimensional structures (foldamers) raise great interest owing to their ability to mimic and expand the world of biopolymers.[1] Their successful application in several fields has been shown, for example as antimicrobial compounds,[2] protein–protein interaction inhibitors,[3] or gelating agents.[4] The most widely studied foldamers are built from β- or γ-amino acids subunits, as well as from their combination with natural α-amino acids, and various types of periodic structures have been already described.[5] With the aim to move from structure to function, foldamers must satisfy at least two criteria: being stable under physiological conditions and bearing functional groups typical of the side chains of proteinogenic amino acids.[6] Although the application of structurally constrained β-amino acids is highly interesting owing to the superior conformational stability of the resulting peptides, the incorporation of proteinogenic side chains, which should provide the desired functional properties, into such non-natural building blocks is rather inconvenient.[7] Thus, the combination of the latter with natural α-amino acids has emerged as a promising approach towards the development of functional foldamers.[5e,8]

Herein we present helical peptide foldamers with defined spatial distributions of their functional groups in polar solvents including water. They contain α-amino and *cis*-β-aminocyclopentanecarboxylic acids (*cis*-ACPC) that have been combined following the stereochemical patterning approach by Fülöp and co-workers[9] (see the Supporting Information). Such an approach correlates the stereochemistry of the backbone atoms with the signs of the ψ and ϕ dihedral angles flanking each amide bond (indicated with][), which in the case of a helical fold must be both + or − and exhibit a periodic pattern along the backbone, to obtain a helical fold (Figure 1).

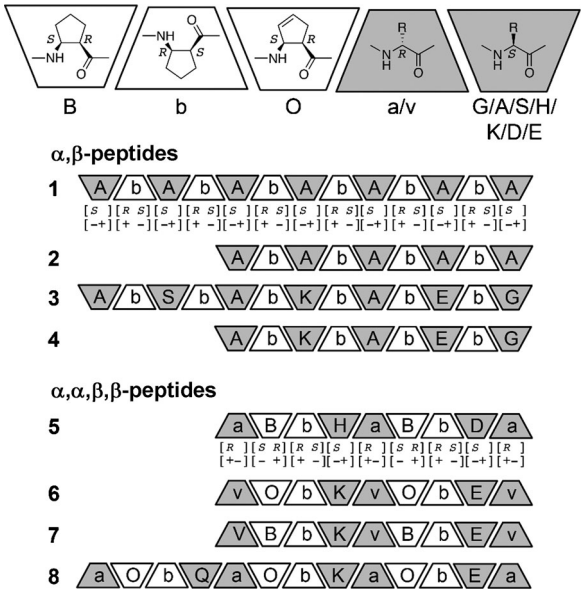

Figure 1. Sequences of α,β- (**1**–**4**) and α,α,β,β-peptide foldamers (**5**–**8**). The preferred signs of the dihedral angles flanking each amide bond (ψ][ϕ) are indicated for the first peptide of each series. All peptides are C-terminally amidated. α,β-Peptides (**1**–**4**) are N-terminally acetylated.

The first type of foldamers consists of 9- and 13-mers containing alternated L-α-amino acids (A, G, E, K, S) and (*S*-α,*R*-β)-*cis*-ACPC units (**1**–**4**; Figure 1 and Supporting Information, Figure S1). The second type of foldamers consists of 9- and 13-mers with the backbone pattern α-α-β-β, in which the two adjacent residues α-α and β-β display opposite stereochemistry (**5**–**8**; Figure 1 and Supporting Information, Figure S2). Peptides **6** and **8** contain some partially unsaturated *cis*-ACPC units, which were introduced to prevent

[*] Dr. Ł. Berlicki, L. Pilsl, Prof. Dr. O. Reiser
Universität Regensburg, Institut für Organische Chemie
Universitätsstrasse 31, 93053 Regensburg (Germany)
E-mail: oliver.reiser@chemie.uni-regensburg.de
Homepage: http://www-oc.chemie.uni-regensburg.de/reiser/
Dr. Ł. Berlicki
Department of Bioorganic Chemistry
Wrocław University of Technology, 50-370 Wrocław (Poland)
Dr. E. Wéber, Dr. I. M. Mándity, Prof. Dr. T. A. Martinek, Prof. Dr. F. Fülöp
Institute of Pharmaceutical Chemistry
University of Szeged, 6720 Szeged (Hungary)
E-mail: martinek@pharm.u-szeged.hu
Prof. Dr. C. Cabrele
Faculty of Chemistry and Biochemistry
Ruhr University Bochum, 44801 Bochum (Germany)
Prof. Dr. C. Cabrele
Paris Lodron University Salzburg
Department of Molecular Biology
Billrothstrasse 11, 5020 Salzburg (Austria)

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signal overlap in the NMR spectra. The α-amino acid composition was varied among the peptide series to obtain sequences with different degrees of hydrophobicity/hydrophilicity. Moreover, in some cases pairs of oppositely charged residues were also introduced because they may enhance the stability of secondary structures provided that they are close enough to form salt bridges. Accordingly, K-E and H-D with different bridge lengths and ionization properties were chosen. The α,β- and α,α,β,β-peptides were expected to differ in the spatial arrangement of their α-residue side chains; moreover, the presence of D-α-amino acids in the second type of foldamers should improve their proteolytic stability.

All of the peptides were successfully obtained by solid-phase peptide synthesis (SPPS) using Fmoc chemistry, followed by preparative HPLC. Peptide **7** was obtained by catalytic hydrogenation[10] of **6**. Hydrogenation of **8** to the corresponding saturated 13-mer failed, most probably because of strong peptide adsorption to the charcoal of the catalyst system.

Circular dichroism (CD) spectra were recorded in methanol and water (Figure 2). Furthermore, peptides **1**–**4** were measured in acetonitrile and 2,2,2-trifluoroethanol (Supporting Information, Figure S3), whereas the α,α,β,β-peptides **5**–**8**, which showed significant CD signals in water, were measured in phosphate buffer at pH 7.4 to mimic physiological conditions. All of the spectra were normalized by the peptide concentration and the number of amide bonds. The CD intensity of the longer peptides is much higher than that of the shorter analogues (**1** vs. **2**, **3** vs. **4**, and **8** vs. **5**–**7**), which indicates that the increase in the peptide length positively affects the conformational stability.[11] In general, the CD signals in methanol are considerably more intense than in water or buffer (except for **5**). However, in contrast to α,β-peptides **1**–**4**, α,α,β,β-peptides **5**–**8** show strong CD signals in aqueous solutions as well (Figure 2).

The CD spectra of the α,β-peptides **1**–**4** have a strong maximum near 200 nm (Figure 2 and Supporting Information, Figure S3), being consistent for both right-handed[5d] and left-handed[2k] α,β-helices. However, the NMR data described below clearly support a right-handed helix for **1**–**4**.

The α,α,β,β-peptides **5**–**8** are characterized by a positive band with a maximum in the range of 198–206 nm, and by a negative band in the range 188–191 nm. The similarity of this CD pattern to that of the above-described α,β-peptides **1**–**4** and of other β-residue-containing peptide helices[5d] supports a helical fold. The stability of helices in aqueous solution, being rare in short linear peptides,[12] is of particular importance.

In contrast to **6**, which is apparently unaffected by a change of pH, the conformation of foldamers **5**, **7**, and **8** is more stable at physiological pH than in unbuffered water. Nevertheless, even in the latter environment, peptide **8** shows remarkable stability against thermal unfolding, as indicated by a loss of folded structure only up to 35% at 90°C (Supporting Information, Figure S8). In the case of the 9-mer **5**, a red-shift of the CD curve from methanol to aqueous media as well as the superior CD intensity in buffer over water and methanol should also be noted. This behavior is remarkable and might reflect the presence of a slightly

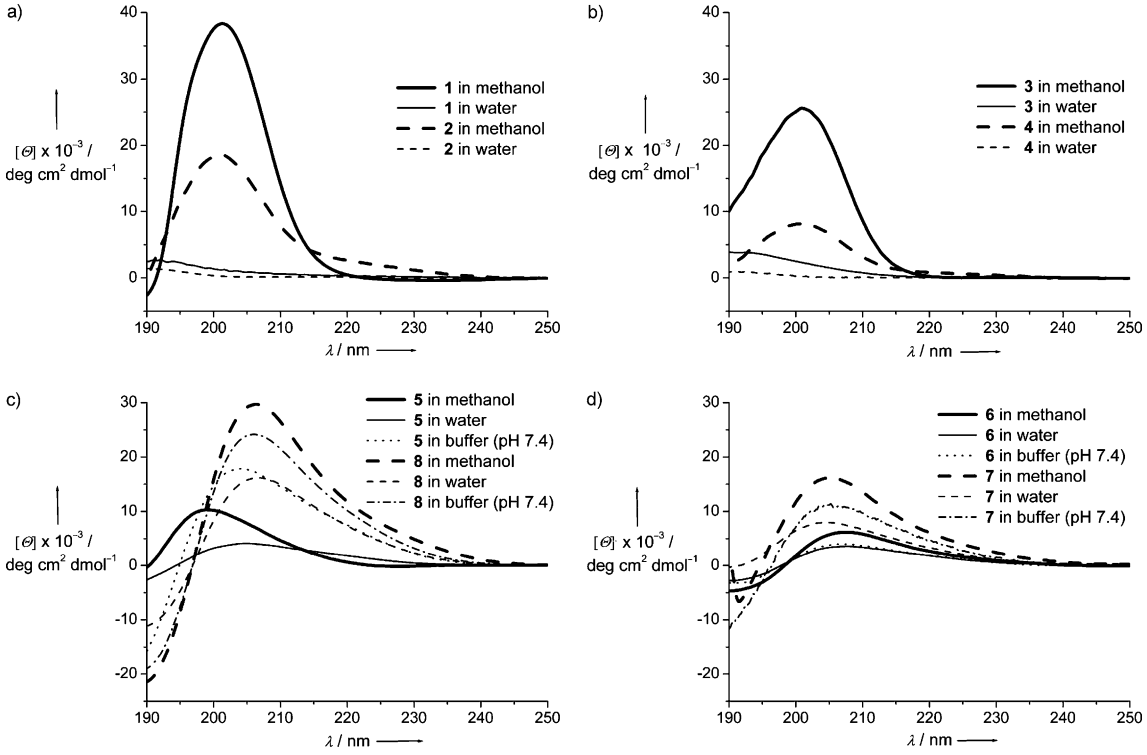

Figure 2. CD spectra of α,β-peptides **1**–**4** (a, b) and α,α,β,β-peptides **5**–**8** (c, d). Spectra were recorded at room temperature and the CD intensity is expressed as mean-residue molar ellipticity.

different structure in aqueous media compared to that in methanol.

The CD spectrum of the partially unsaturated peptide **6** is less intense than that of the fully saturated analogue **7** (Figure 2d). This is more likely due to the high aggregation propensity of **6** rather than to the different saturation of the ACPC rings. Indeed, peptide **6** was the only one of all peptides investigated to be poorly soluble in the solvents used (DMSO, acetonitrile, methanol, water, and phosphate buffer).

NMR spectra of the α,β-peptides were recorded in [D_3]methanol at different temperatures. Almost all signals could be assigned unambiguously (Supporting Information, Table S2a). The temperature-dependent shifts of the amide proton signals suggest the presence of backbone hydrogen bonding and secondary structure formation (Supporting Information, Table S4a).[13] Remarkably low absolute values of the temperature coefficients were obtained for residues 3–11 of peptide **3** (in the range from -1.08 to -4.38 ppb K^{-1}).

Several ROESY cross-peaks were detected for the 13-mers **1** and **3**. Sequential ROEs were of the type NH_i–$C_\alpha H_{i-1}$, whereas medium-range contacts HN_{i+4}–$C_\alpha H_i$ for α residues and HN_i–$C_\beta H_{i+4}$ for β residues were found (Figure 3a). Futhermore, amide ^3J coupling constants in the range 8.4–9.4 Hz for β residues and 7.3–7.8 Hz for α residues (excluding terminal residues) were observed (Supporting Information, Table S3a). These ^3J values, together with the well-dispersed proton chemical shifts, indicate the presence of ordered structures. Using the experimental NMR restraints, the structure refinement of the α,β-peptides by Monte Carlo (MC)/molecular dynamics (MD) conformational sampling through molecular mechanics resulted in a well-defined structure (Figure 4). Surprisingly, an unprecedented right-handed 16/18 helix was found (that had however been previously computationally predicted by Hofmann and co-workers[14]), which is the widest peptide foldamer helix reported to date.[8a] This structure is characterized by $i,i-3$ and $i,i+5$ C=O···HN interactions (Figure 3b). Dihedral angle analysis showed mean values of $\psi=-176°$, $\phi=-129°$ for α residues and $\psi=-96°$, $\phi=108°$, $\theta=9°$ for the β residues, which are uncommon for helical structures: Indeed, they indicate that the α residues are in extended conformation, whereas the *cis*-ACPC units form turns, leading to a hybrid structure (Figure 4).

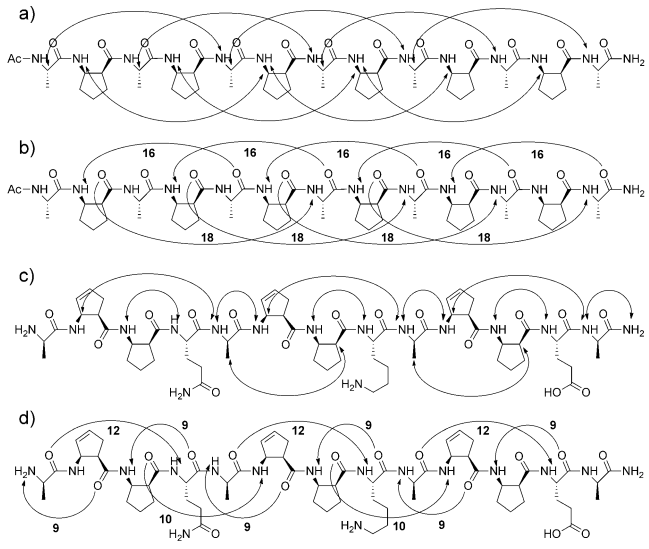

Figure 3. Characteristic sequential and medium-range interactions as well as hydrogen-bonding patterns for **1** (a,b) and **8** (c,d).

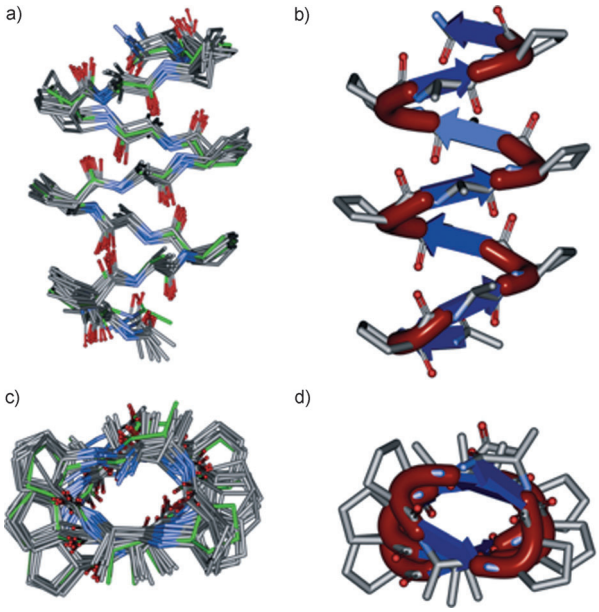

Figure 4. NMR solution structure of peptide **1** in [D_3]methanol. Ten superimposed low-energy structures (a,c) and a ribbon representation of one of them (b,d) are shown. All hydrogen atoms were omitted for clarity.

NMR spectra of the α,α,β,β-peptides were recorded in [D_3]methanol and phosphate buffer (20 mM, pH 7.4, with 10% D_2O). Peptide **8** was additionally measured in [D_6]DMSO. The NMR signals were well-dispersed, which allowed their almost full assignment (Supporting Information, Table S2b,c) as well as the calculation of the temperature coefficients of the backbone amide protons. The latter indicated the presence of hydrogen-bonded amide protons (Supporting Information, Table S4b–d). In the case of peptide **5** in [D_3]methanol, the temperature coefficients of residues 4–7 were in the range from -3.5 to -5.3 ppb K^{-1} (Supporting Information, Table S4b).[13] In the case of peptide **8** in [D_3]methanol, the temperature coefficients of the Ala residues were much larger than those of the hydrophilic α residues, which suggests that the Ala residues were solvent-exposed. Moreover, almost every second unit, being Gln4, *cis*-ACPC6, Lys8, *cis*-ACPC10, and Glu12, has low absolute values (in a range from -1.5 to -4.7 ppb K^{-1}), which may indicate hydrogen bonding (Supporting Information, Table S4b).[13] Such an alternating pattern of the backbone amide temperature coefficients suggests the formation of a periodic, that is helical, structure.

For the samples of **5**–**8** in buffer it was not possible to precisely determine all temperature coefficients because of signal overlapping (Supporting Information, Table S4 d). However, in the case of peptide **8**, four out of the eight values were between 1.3 and 4.4 ppb K^{-1}. Similarly, three amide protons of peptides **6** and **7** are characterized by low-temperature coefficients (from 2.0 to 4.6 ppb K^{-1} for **6** and from 1.4 to 4.2 ppb K^{-1} for **7**).[13]

ROESY cross-peaks of type $i,i+1$ were detected for the backbone atoms in **5**–**8** (Figure 3 c). Moreover, $i,i+2$ interactions between backbone and side chains were observed, supporting the presence of a helical structure. Among them, $C_\beta H_i$–$C_\alpha H_{i+2}$ or $C_\gamma H_i$–$C_\alpha H_{i+2}$ interactions for internal Ala and Val side chains, respectively, were also observed for peptides **5**–**8**, but not for the N-terminal residues. This suggests that the N-terminal α-residues (Ala/Val) of the α,α,β,β-peptides maintain significant flexibility.

The structure refinement of α,α,β,β-peptides reveals a right-handed helix, which is stabilized by an interlinked series of H-bonded 9/12/9/10-membered rings ($i,i-1$ and $i,i+3$ C=O···HN interactions, Figure 3 d). The helix of peptide **8** is subjected to compression upon changing the solvent from methanol to water (Figure 5), which is apparently forced by a favorable electrostatic interaction between the side chains of Lys8 and Glu12 as well as by hydrophobic interactions of the ACPC-rings in position 2, 6, and 10.

A weak $C_\alpha H_1$-$C_\alpha H_4$ ROE was observed for **5**, which is inconsistent with the proposed helix. One possible explanation might be the presence of a second structure population, in which the α protons of residues 1 and 4 are proximal to each other (for example with a hydrogen-bonded 18-membered ring between C=O(1) and NH(6)). This would also explain the CD spectrum changes observed for **5** when dissolved in methanol rather than in aqueous media, as the one ROE inconsistency was especially evident in methanol.

Unfortunately, the aggregation and the corresponding low signal intensity of **6** prevented the accurate integration of the cross-peaks; however, the presence of the key long-range interactions and the high homology with **8** strongly support the proposed 9/12/9/10-helical structure.

In summary, we have successfully applied the stereochemical patterning approach to design stable helical structures based on *cis*-ACPC and α-amino acid units. The use of α,β and α,α,β,β sequential motifs has allowed obtaining unprecedented 16/18 and 9/12/9/10 helices, respectively. We believe that these two novel structures have potential for applications in medicinal and biological chemistry, for at least three reasons: 1) the high content of α-amino acid residues in both helix types makes it possible to easily introduce several functional groups; 2) the two types of foldamers differ in their side-chain arrangement, which offers the possibility to decorate helical surfaces with distinguished patterns of functional groups; and 3) the short α,α,β,β-peptides are attractive for their ability to adopt a highly ordered secondary structure in aqueous media.

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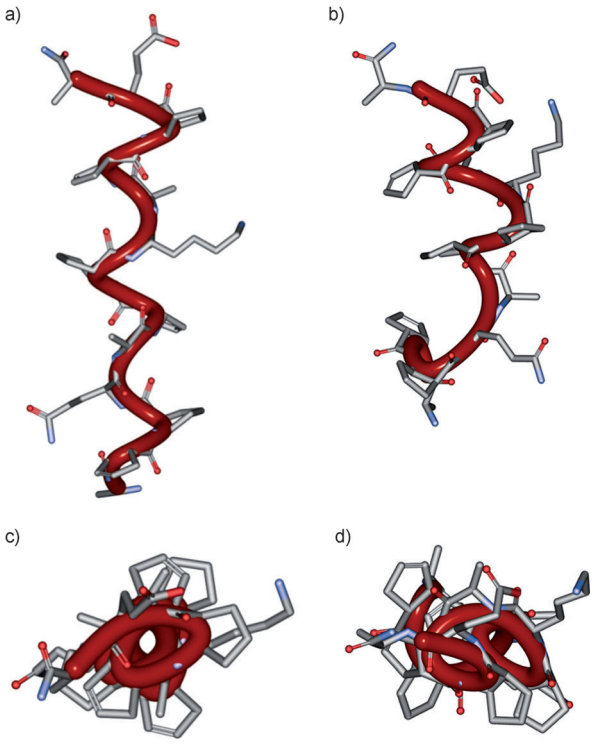

Figure 5. NMR solution structure of peptide **8** in [D_3]methanol (a, c) and water (b, d). See also the Supporting Information for the overlay of the ten lowest energy structures, for which RMSD values of 1.8 Å and 4.1 Å in methanol and water, respectively, were calculated.

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