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β-Peptide foldamer helices with tailored diameters

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A. Introduction and aims

Foldamers are regarded as synthetic polymer architectures that have the feature of adopting well-defined periodic compact structures, and could therefore be rivals of the natural polymer systems both in their functions and applications. The most significant representatives of these non-natural self-organized biomimicking systems are the β-peptides, which are built up from β-amino acid units. The different types and derivatives of β-amino acids occupy a specific field in synthetic chemistry. The β-peptides are the closest relatives of the natural α-peptides, but the insertion of a methylene group between the peptide bonds enhances the conformational freedom of these systems and allows the existence of numerous programmable secondary structures (a wide range of helices, strands and turn motifs). The most thoroughly studied secondary structure motif of the β-peptides is the helix.

Three helical structures, the H14, H12 and H10/12 play crucial roles regarding biological activity as displaying a close resemblance to the α-helix. They can be induced by a special backbone substitution and a specific backbone stereochemical pattern. The H14 helix is the most throughly studied helical structure and there are many ways to stabilize it. In contrast with the H14 helix, the H12 helix can be obtained only through the incorporation of a sufficient number of the five-membered ring-containing cyclic β-amino acid residues (ACPC) with *trans* relative backbone configuration. This helix type is the best mimic of the α-helix, and one of our major aims was therefore to find another method to stabilize the H12 helix. For this purpose, we used β-amino acid derivatives with special side-chain shapes.

Conformational polymorphism, such as the random coil → helix or the helix → helix transition, is a very important feature in the natural protein systems, which is mostly connected to a certain biological function. The control over the 3_{10}-helix → α-helix equilibria takes place in a concentration-dependent way and with the change of the solvent polarity, but chain-length-dependent transition is also known. Conformational transitions have also been described for peptidic foldamers. The transition from the H10 helix to the H14 helix occurs with the increase of the peptidic chain length for oligomers having *trans*-ACHC in the sequence. Our aim was to prove the existence of the helix transition and its influencing factors for foldameric oligomers containing the ACHC analogue *trans*-ABHC and β^3-hSer residues in various patterns.

The β-peptide foldamers have the propensity to adopt higher-ordered structure motifs, like helical bundles of α-peptides. It has been shown that β and αβ-peptidic sequences are able to form quaternary structures with self-assembling helical building blocks. We planned to

investigate the folding and the possibility of higher-ordered structure formation in alternating heterochiral *trans*-ACPC-containing β-peptide foldamer systems.

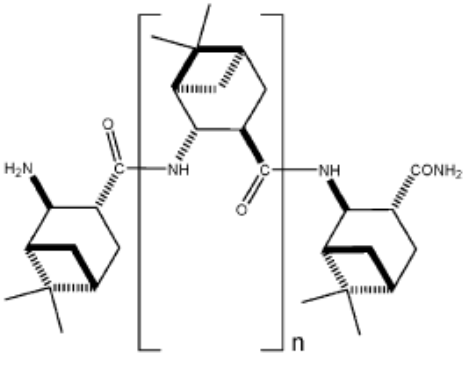
B. Methods

The peptides were synthesized on a solid support with Fmoc methodology. The peptides were purified by HPLC and characterized by ESI-MS. Structures were investigated by using molecular modelling, various NMR techniques and ECD. Higher-order structures of the peptides were measured by the use of DOSY-NMR and concentration-dependent ECD.

C. Results and discussion

1. Various helically ordered β-peptides were designed to map different structuring effects on the preferred secondary structure and reveal the possibility of higher-order motifs. Peptides **1**-**14** were synthesized by Fmoc techniques, followed by estimation of the helix type with molecular modelling. Structure characterization was carried out with different analytical methods.
2. The long-range side-chain steric repulsion concept proved to be a novel route for the shaping of the desired helical secondary structure.
3. The *trans*-ABHC (bicyclic analogue of ACHC) containing sequences (Scheme 1) were constructed.

Scheme 1. The studied structures



1, n = 1; **2**, n = 2; **3**, n = 3, **4**, n = 4

4. The bulky *trans*-ABHC monomers with their special side-chain shape caused steric clashes between positions (*i*) – (*i* + 3) in the peptide sequence, which disfavours the H14 helical fold and induces the formation of the H12 helix (Figure 1). The chain-length-dependent investigations revealed that oligomers containing up to five trans-ABHC residues (**3** and **4**) unambiguously stabilize the H12 helix.

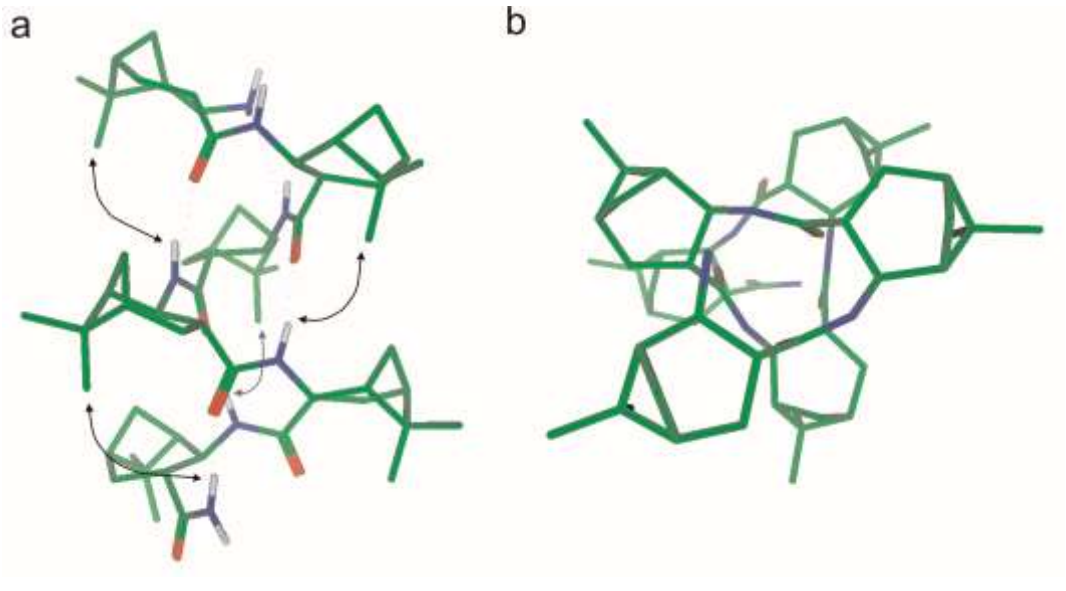
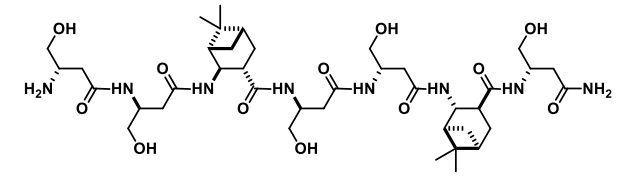


Figure 1. Side view (a) and top view (b) of the NMR-derived H12 helix formed by **4**. Arrows indicate the very characteristic Me_i-NH-$_{i+3}$ NOEs observed in the ROESY spectrum of **4** (see Fig. 1)

5. The bicyclic-residue based β-peptidic H12 helix tolerates open-chain β^3-amino acid residues incorporated in the sequence. Oligomers with different patterns of *trans*-ABHC and β^3-hSer residues (**5-9)** were investigated in the heptamer-nonamer range (Scheme 2). At least two repulsive contacts of the *trans*-ABHC pairs are necessary in positions (*i*) – (*i* + 3) to prevent the formation of the H14 helix in the heptameric sequences.

Scheme 2. The studied *trans*-ABHC-containing structures



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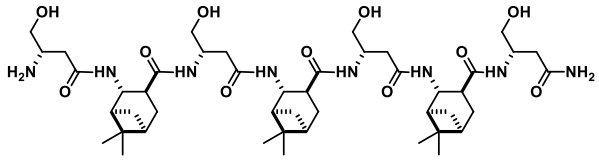

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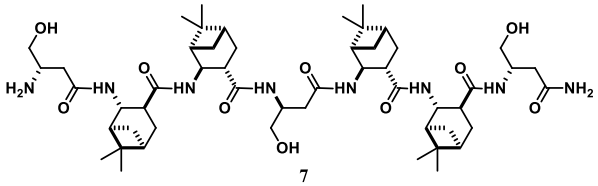

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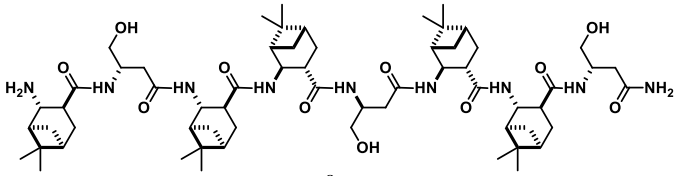

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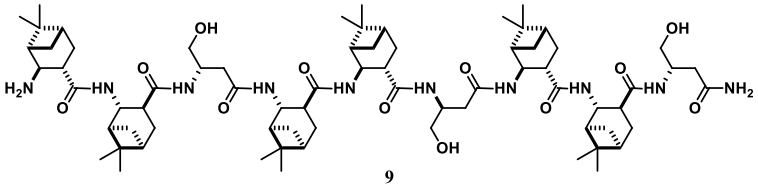

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6. We further proved that the H12 helix constructed from the *trans*-ABHC residues is capable of chain-length-dependent self-association.
7. Solvent-dependent conformational polymorphism was observed for the octamer **8** and nonamer **9** sequences, supported by NMR. In [D_6]DMSO, the H12 helix remained a stable conformation, whereas a large-diameter H18 helix was clearly detected in polar medium. Head-to-tail long-range NOE interactions were also detected for compound **9**, which indicated higher-order structural forms in the solution phase. Theoretical calculations confirmed the new foldameric H18 helix too (Figure 2).

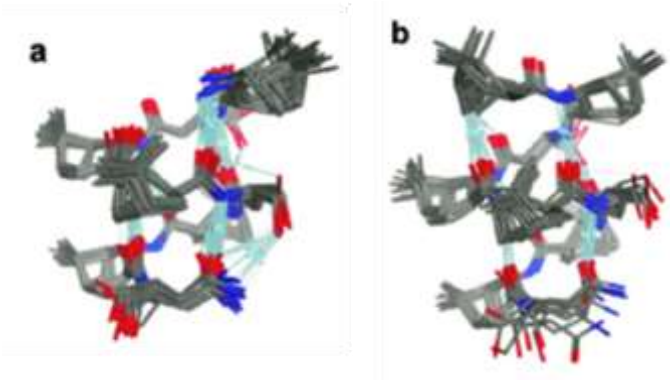
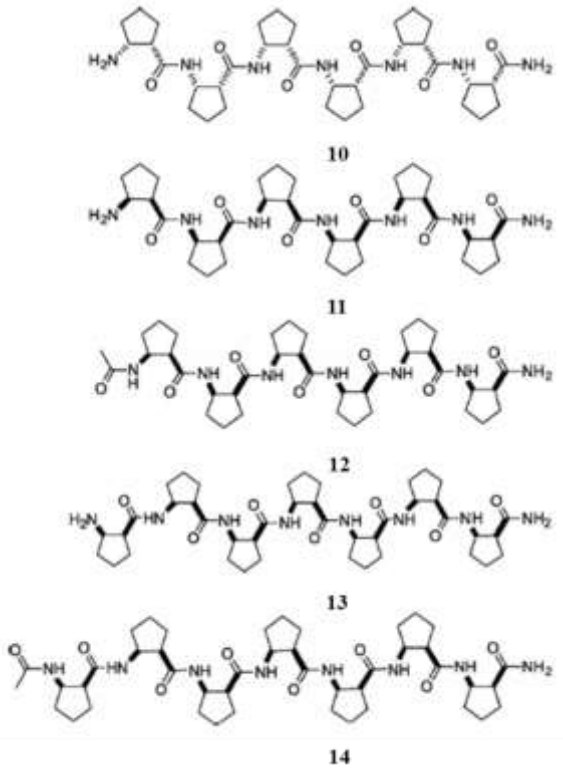


Figure 2. The H18 helix obtained from the NMR structure refinement for **8** (a) and **9** (b)

8. Concentration-dependent ECD and DOSY-NMR measurements confirmed the self-association process in MeOH for compounds **8** and **9**, which correlated well with the H12 → H18 helix transition. The self-assembly was also detected for the heptamer **7** sequence, but was unable to change its overall H12 helical structure. These results supported that foldameric helix refolding promoted higher-older packing of the helices in MeOH.
9. The chain-lengthening approach was extended to alternating heterochiral *trans*-ACPC sequences (**11**-**14**) (Scheme 3).

Scheme 3. Structures studied for their helicity



10. We proved that manipulation of the N-terminus of **10** is able to determine the transfer of chiral information along the helix by changing the helicity of the chain.
    We showed that the alternating heterochiral *cis*-ACPC-containing hexameric sequences (**10** and **11**) and N-acetylated sequences (**12** and **14**) formed the well-known H10/12 helix (Figure 3).

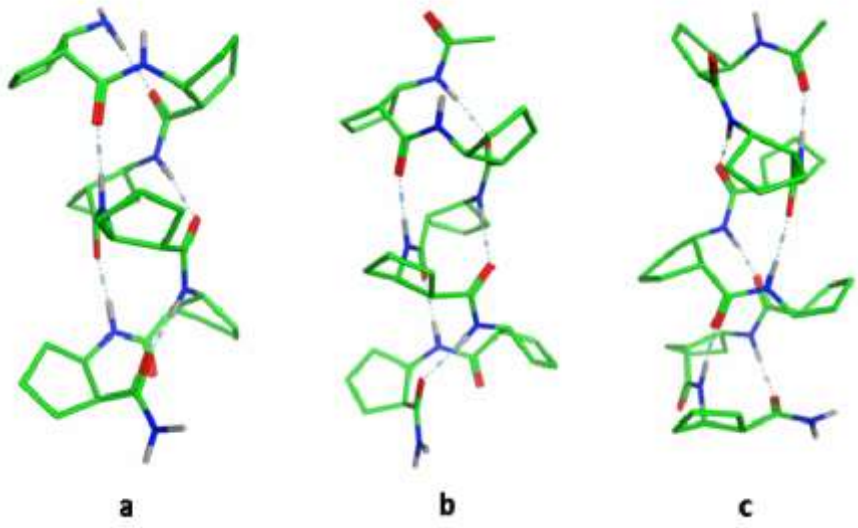


Figure 3. *Ab initio* geometries of **11** (a), **12** (b) and **14** (c)

11. The heptameric chain **13** exhibits a dramatic change in the helix diameter, theoretical calculations and the NMR structure refinement revealed a novel, concentration-dependent mixed H18/20 helical conformation, stabilized by intramolecular (*i)* – (*i* + 4) hydrogen-bonding contacts, The manifestation of the H18/20 was found to be solvent-dependent, and **13** has opposite handedness relative to the other peptides (**11**, **12** and **14**).
12. The folding and self-association processes with axial helix-helix interactions provide an alternative route for higher-order structure formation for the large-diameter foldameric helices. The NMR and ECD results strongly suggested that mixed H18/20 helix is stabilized by intermolecular head-to-tail contacts (Figure 4).

Figure 4. H18/20*p* mixed helix head-to-tail dimer of **13** obtained by NMR structure refinement in CD_3OH and a final *ab initio* geometry optimization[222] at the B3LYP/6-311G** level of theory

D. Scientific publications and lectures

Full papers related to the thesis

1. Hetényi, Z. Szakonyi, I. M. Mándity, É. Szolnoki, G. K. Tóth, T. A. Martinek and F. Fülöp:
Sculpting the β-peptide foldamer H12 helix via a designed side-chain shape
Chem. Commun. **2009**, 177-179. IF.: 5.504*

2. É. Szolnoki, A. Hetényi, T. A. Martinek, Z. Szakonyi and F. Fülöp:
Self-association-driven transition of the β-peptidic H12 helix to the H18 helix
Org. Biomol. Chem. **2012**, *10,* 255-259. IF.: 3.568

3. É. Szolnoki, A. Hetényi, I. M. Mándity, F. Fülöp and T. A. Martinek:
Foldameric β-H18/20P mixed helix stabilized by head-to-tail contacts: a way to higher-order structures
Eur. J. Org. Chem. **2013**, *17,* 3555-3559. IF.: 3.065

Other publications

4. T. A. Martinek, É. Szolnoki, Z. Zalán and F. Fülöp:
Synthesis and steric structure of pyrrolidine- and piperidine-fused 1,3,4,2-oxadiazaphosphinanes
Arkivoc **2007**, (v), 202-209. IF.: 1.253

5. E. Wéber, A. Hetényi, B. Váczi, É. Szolnoki, R. Fajka-Boja, V. Tubak, É. Monostori and T. A. Martinek:
Galectin-1-Asialofetuin interaction is inhibited by peptides containing the Tyr-Xxx-Tyr motif acting on the glycoprotein
ChemBioChem **2010**, *11*, 228-234. IF.: 3.945

*The impact factors for the year of publication are given.

Scientific lectures related to the thesis

1. Szolnoki Éva
β-peptid H12 hélix stabilizálása tervezett oldallánc kölcsönhatásokkal
MTA Peptidkémiai Munkabizottság Ülése
Balatonszemes, 2008. május 14-16.

2. Szolnoki Éva
β-peptid H12 hélix stabilizálása tervezett oldallánc kölcsönhatásokkal
XXXI. Kémiai Előadói Napok
Szeged, 2008. október 27-29.

3. Szolnoki Éva
Biciklusos oldalláncok hatása α-hélix mimetikumok stabilizálására és önrendeződésére
MTA Peptidkémiai Munkabizottság Ülése
Balatonszemes, 2009. május 26-28.

4. Éva Szolnoki, Zsófia Hegedüs, Zsolt Szakonyi, Tamás A. Martinek, Ferenc Fülöp:
Effects of bicyclic residues on β-peptide secondary structures
COST, Foldamers: Building blocks, structure and function
Szeged, Hungary, September 24-26, 2009, Abstr.: P02

5. Szolnoki Éva, Hegedüs Zsófia, Martinek Tamás, Szakonyi Zsolt, Fülöp Ferenc
Biciklusos oldalláncok hatása β-peptidek helikális másodlagos szerkezeteire
Congressus Pharmaceuticus Hungaricus XIV.

Budapest, 2009. november 13-15. Abstr.: P-10

6. Éva Szolnoki, Zsófia Hegedüs, Tamás A. Martinek, Zsolt Szakonyi, Ferenc Fülöp
β-Peptide helical structures induced by bicyclic amino acid residues
COST, Foldamers: From design to protein recognition
Bordeaux-Pessac, France, January 25-28, 2010, Abstr.: p. 16.

7. Éva Szolnoki, A. Hetényi, Tamás A. Martinek, Zsolt Szakonyi, Ferenc Fülöp
β-Peptidic H18 helices
COST, Foldamers: Synthesis and structure of functional materials
Bellaterra (Barcelona), Spain, April 7-9, 2011, Abstr.: OC18