PhD Thesis

Tailoring β-peptide foldamers by backbone stereochemistry and side-chain topology

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A. Introduction and aims

Foldamers, the non-natural self-organizing biomimicking systems show similar properties to the proteins, e. g. they have a tendency to fold into the specific periodic compact structures. Foldamers have the potential for reaching similar structural versatility as natural proteins, consequently a numerous promising biological applications have been established where the tailored 3D structure of designed foldamers was crucial. Foldamers, based on the structural features and pharmaceutical applications, can be designated as protein mimetics. Foldamers play roles as novel class of drug scaffolds with tailored molecular shape and surface. This fact raises the need for more versatile frameworks with the aim to design foldamers that bind to almost any surface. The most thoroughly studied representatives of this field are the β-peptides consisting of β-amino acids. They populate a wide range of tunable secondary structures (numerous helices, sheet forming strands and turn motifs) with a propensity to associate into tertiary structure like motifs. Novel secondary structural motifs can be gained (*i*) by the use of new non-natural amino acids and derivatives or (*ii*) by the understanding of the structure promoting effect of the stereochemistry.

Our major aim was to harness the latter one to gain new structures with designed specific backbone configuration. The basic secondary structures for β-peptide foldamers have been established mainly for uniformly substituted β-amino acids leading to uniformly substituted backbone pattern. We aimed to combine the alternating design principle with the stereochemical configuration pattern of β-peptide oligomers and planned to create alternating heterochiral homooligomers.

The controlled self-assembly of foldameric helices leads to helix bundles, vesicle-forming membranes of vertically amphiphilic helices and lyotropic liquid crystals, all of importance for future applications. We aimed to investigate the existing secondary structure and self-association in the function of different six membered alicyclic side-chain shapes with alternating heterochiral homooligomers.

The prevailing 3D structure of a foldamer is determined by many factors, such as the residue type, the side-chain topology and chemistry, etc. An extension of the alternating backbone configuration to several residues along the backbone and simultaneous variation of the residue types (α- and β-residues) can lead to novel periodic secondary structures. With the study of the existing secondary structures as the function of backbone configurations, we intended to establish an intuitive foldamer design tool, a simple stereochemical patterning

approach (SPA), with which the prevailing 3D structure, can be predicted even for non-homogeneous back-bone.

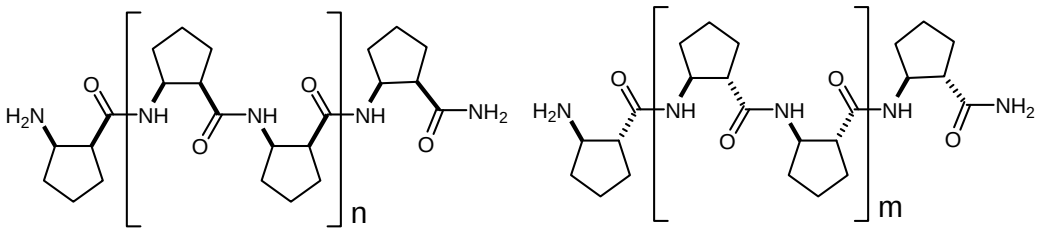
B. Methods

The peptides were synthesized by using SPPS with the Fmoc or Boc technique. The peptides were purified by RP-HPLC and characterized by ESI-MS. Structures were determined by the use of molecular modeling, various NMR techniques, ECD and VCD. Particle size was measured via DLS and TEM measurements.

C. Results and discussion

1. On the basis of earlier results, we performed the molecular modeling of various peptides with heterochiral backbone and different side-chains. After careful selection of the resulting conformational ensemble we synthesized the compounds with ordered structures. Peptides **1**-**10**, **12**, **13**, **15** and **16** were prepared by Boc or Fmoc techniques. This was followed by structure determination with different analytical techniques.
2. The combination of the alternating design principle with the heterochiral sequences led to foldamers with novel 3D structures and the accessible conformational space of foldameric secondary and tertiary structures was enlarged.
3. The alternating heterochiral ACPC sequences (Scheme 1) show self-organization.

Scheme 1. The studied alternating heterochiral β-peptide sequences



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

The alternating heterochiral *cis*-ACPC hexameric **2** formed the H10/12 helix (Figure 1a). For the alternating heterochiral *cis*-ACPC oligomers, no self-assembly was observed.

4. For the alternating heterochiral *trans*-ACPC sequences, molecular modeling and ECD results indicated a polar-strand conformation (Figure 1 b,c), which was supported by the fact that self-assembly into nanostructured fibrils was observed in water for the hexameric

structure **4**. The self-assembly was chain-length-dependent, since tetramer **3** did not exhibit self-association.

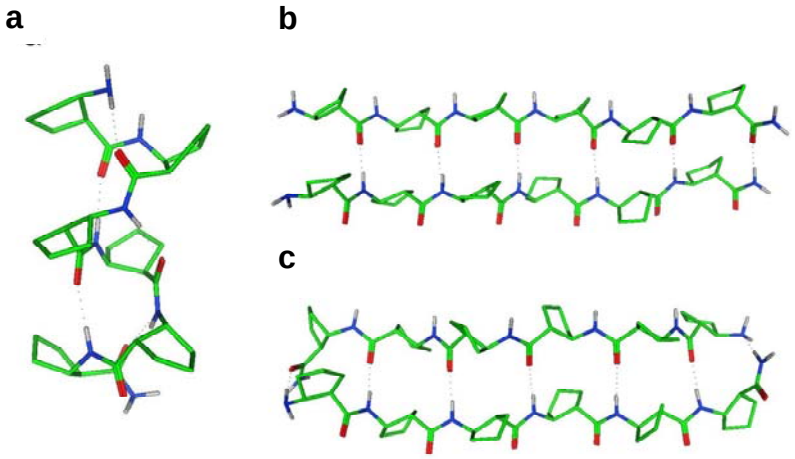
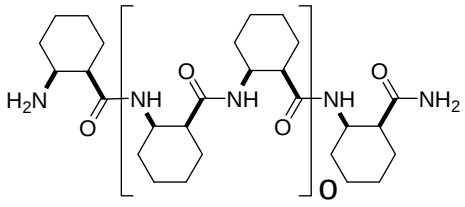


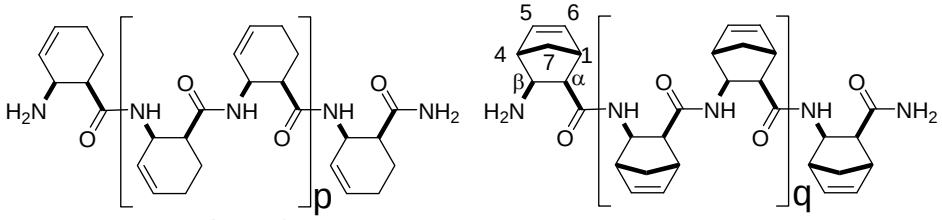
Figure 1. *Ab initio* structure of **2** (a) calculated in vacuum at the B3LYP/6-311G** level of theory and geometry for the parallel (b) and antiparallel (c) dimer of **4**, showing the polar strand geometry, calculated by means of molecular mechanics (MMFF94x with implicit water)

5. The β-peptidic alternating H10/12 helix tolerates the 6-membered side-chain topology (Scheme 2).

Scheme 2. The structures with alternating backbone configurations with different six-membered cyclic side-chains



5, o=1; **6**, o=2



7, p=1; **8**, p=2 **9**, q=1; **10**, q=2

Both the *cis*-ACHC hexamer **6** and the *cis*-ACHEC hexamer (**8**) afforded the H10/12 helix (Figure 2a). The ECD results indicated that the 6-membered ring topology slightly destabilizes the H10/12 helix as compared with the alternating *cis*-ACPC oligomers.

6. The *cis*-ACHC-containing hexamer (**6**) with alternating backbone configuration exhibited conformational polymorphism with two folded conformational states that underwent chemical exchange (Figure 2a,b). This behavior was not observed for the double bond-containing *cis*-ACHEC hexamer (**8**). An apparently subtle change in the hybridization of a carbon atom pair in the side-chain can tune the conformational preferences of β-peptide oligomers.
7. The bicyclic *diexo*-ABHEC prevented the formation of a small-diameter helix; the experimental results pointed to a circle-like fold for the hexamer (**10**), which was sufficiently stable in MeOH to maintain considerably shielded H-bonds. (Figure 2c).

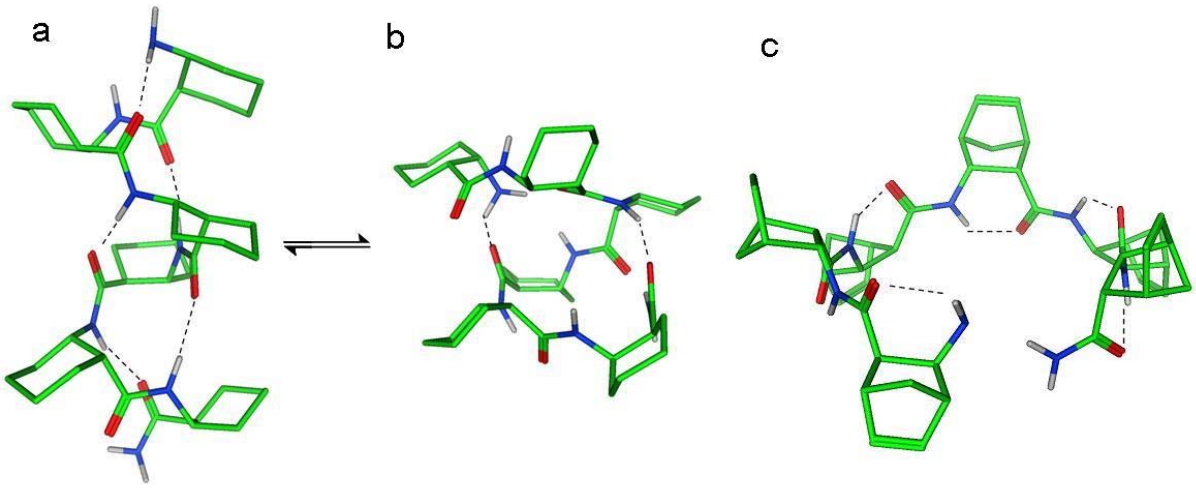


Figure 2. The H10/12 helix geometry for **6** (a), the H18/20 helix geometry for **6** (b) and the circular fold for **10** (c), obtained through NMR restrained structure refinement and by final optimization at the B3LYP/6-311G** level of theory

8. Our results reveal that, for the β-peptide H10/12 helix studied, minor changes in the side-chain topology, size and shape resulted in large effects on the self-assembly process. The H10/12 helix is capable of self-association in a polar medium if the helix is built up from *cis*-ACHC residues and the chain length is as long as the hexamer. We showed that the hydrophobically driven helix association is a result of the combination of the hydrophobic nature of the side-chains and the presence of a stable secondary structure.
9. We analyzed various secondary structures of different foldameric and α-peptidic structures as a function of the back-bone stereochemical pattern. The results showed that the homochiral and the alternating heterochiral systems do not cover all the possibilities to create periodic secondary structures.
10. The absolute configurations can be regarded as the basic instruction set in the assembly language of the peptidic foldamer sequences, and an SPA was established.

11. We tested the SPA experimentally in terms of sequences with novel backbone stereochemical pattern (Figure 3).

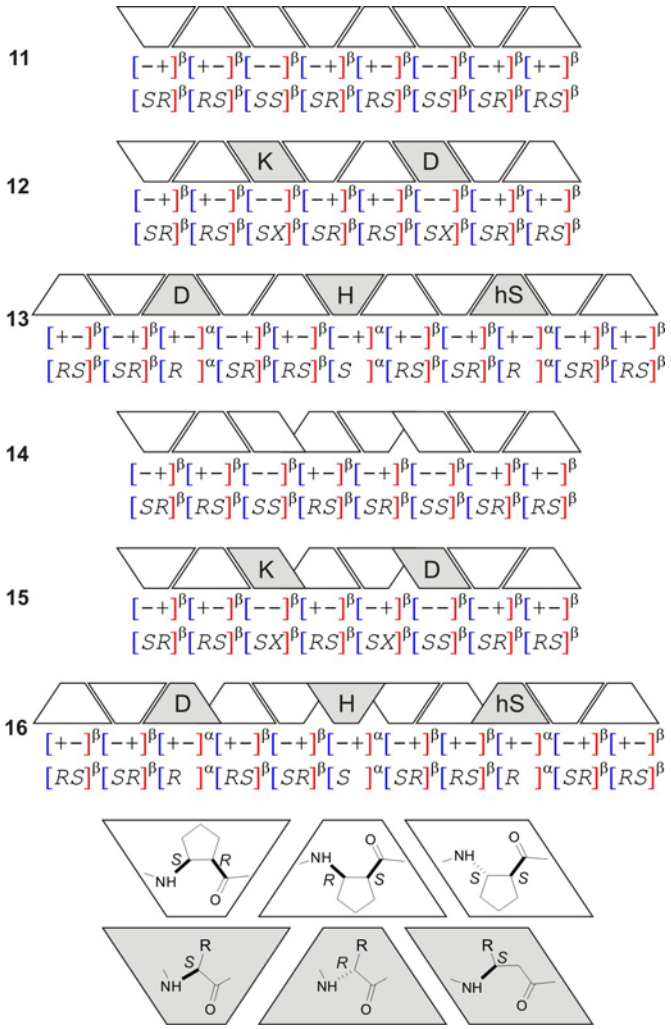


Figure 3. The *de novo* designed β- and α/β-peptide sequences based on the SPA (**11-13**) and their quasi-randomized controls (**14-16**). The side-chains are indicated by the single letter codes (hS: homo-serine). The configurations are indicated in the CIP system with R = methyl

The sequence **12** formed an H14/16 helix while oligomer **13** created an H9-12 helix (Figure 4). These are *de novo* designed helices. Further evidence in connection with the relevance of the SPA is the two distorted structures (**15** and **16**) with the swapped backbone pattern, because they do not form helical structures. Their secondary structure is loop-like.

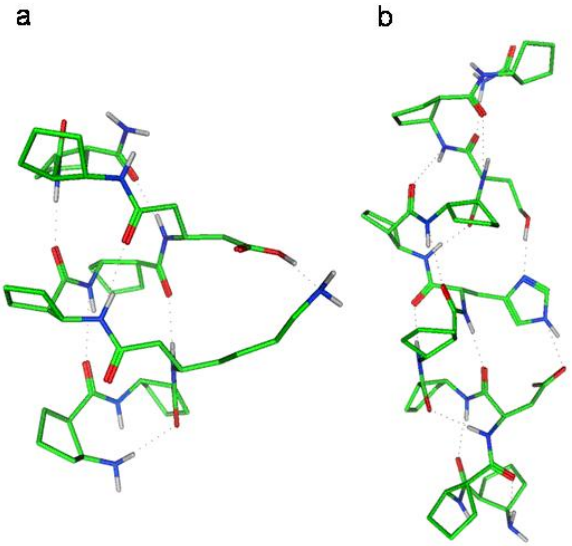


Figure 4. (a) The H14/16 helix obtained by molecular modeling for **12**; (b) the H9-12 helical conformation gained for **13**

D. List of publications and lectures

Full papers related to the thesis

I. Tamás A. Martinek, **István M. Mándity**, Lívia Fülöp, Gábor K. Tóth, Elemér Vass, Miklós Hollósi, Enikő Forró, Ferenc Fülöp:
Effects of the alternating backbone configuration on the secondary structure and self-assembly of β-peptides
J. Am. Chem. Soc. **2006**, *128*, 13539-13544. IF.: 8.091*

II. **István M. Mándity**, Edit Wéber, Tamás A. Martinek, Gábor Olajos, Gábor K. Tóth, Elemér Vass, Ferenc Fülöp:
Design of peptidic foldamer helices: A stereochemical patterning approach
Angew. Chem. Int. Ed. **2009**, *48*, 2171-2175. IF.: 10.879

III. **István M. Mándity**, Livia Fülöp, Elemér Vass, Gábor K. Tóth, Tamás A. Martinek, Ferenc Fülöp
Building β-peptide H10/12 foldamer helices with six-membered cyclic side-chains: fine-tuning of folding and self-assembly
Org. Lett., **2010**, *12*, 5584-5587. IF.: 5.128

Other full papers

1. **István M. Mándity**, Gábor Paragi, Ferenc Bogár, Imre G. Csizmadia:
A conformational analysis of histamine, and its protonated or deprotonated forms: an

ab initio study

J. Mol. Struct. (THEOCHEM) **2003**, *666*, 143. IF.: 1.167*

2. Anasztázia Hetényi, **István M. Mándity**, Tamás A. Martinek, Gábor K. Tóth, Ferenc Fülöp:

   Chain-lenght-dependent helical motifs and self-association of β-peptides with constrained side chains

   J. Am. Chem. Soc. **2005**, *127*, 547. IF.: 8.091

3. Tamás A. Martinek, Anasztázia Hetényi, Lívia Fülöp, **István M. Mándity**, Gábor K. Tóth, Imre Dékány, Ferenc Fülöp:

   Secondary structure dependent self-assembly of β-peptides into nanosized fibrils and membranes

   Angew. Chem. Int. Ed. **2006**, *45*, 2396. IF.: 10.879

4. Péter Csomós, Lajos Fodor, **István M. Mándity**, Gábor Bernáth:

   An efficient route for the synthesis of 2-arylthiazino[5,6-*b*]indole derivatives

   Tetrahedron **2007**, *63*, 4983. IF.: 2.897

5. Anasztázia Hetényi, Zsolt Szakonyi, **István M. Mándity**, Éva Szolnoki, Gábor K. Tóth, Tamás A. Martinek, Ferenc Fülöp:

   Sculpting the β-peptide foldamer H12 helix *via* a designed side-chain shape

   Chem. Commun. **2009**, 177. IF.: 5.340

6. **István M. Mándity**, Tamás A. Martinek, Ferenc Darvas, Ferenc Fülöp:

   A simple, efficient, and selective deuteration via a flow chemistry approach

   Tetrahedron Lett. **2009**, *50*, 4372. IF.: 2.538

7. Brigitta Kazi, Loránd Kiss, Enikő Forró, **István M. Mándity**, Ferenc Fülöp:

   Synthesis of conformationally constrained, orthogonally protected 3-azabicyclo-[3.2.1]octane β-amino esters

   Arkivoc **2010**, 31. IF.: 1.377

8. Elemér Vass, Ulf Strijowski, Katarina Wollschläger, **István M. Mándity**, Gábor Szilvágyi, Mark Jewgiński, Katarina Gaus, Silvia Royo, Zsusza Majer, Norbert Sewald, Miklós Hollósi:

   VCD studies on cyclic peptides assembled from L-β-amino acids and a *trans*-2-aminocyclopentane- or *trans*-2-aminocyclohexane carboxylic acid

   J. Pept. Sci. **2010**, 16, 613. IF.: 1.654

9. Sándor B. Ötvös, **István M. Mándity**, Ferenc Fülöp:
   Highly selective deuteration of pharmaceutically relevant nitrogen-containing heterocycles: a flow chemistry approach
   Mol. Div.. **2010**, DOI: 10.1007/s11030-010-9276-z IF.: 2.071
   *The impact factors for the year 2009 are given

Scientific lectures related to the thesis

1. **Mándity István**:
   Ciklusos oldalláncú β-aminosav oligomerek helikális konformációinak és stabilitásának lánchosszfüggése
   Szegedi Tudományegyetem Szent-Györgyi Albert Orvos- és Gyógyszerésztudományi Centrum és Egészségügyi Főiskolai Kar Tudományos Diákköri Konferenciája
   Szeged, 2005. febr. 3-5. Abstr.: 093, 119.
2. **Mándity István**:
   További lépések a β-peptidek harmadlagos szerkezete felé
   XXVII. Országos Tudományos Diákköri Konferencia, Orvostudományi Szekció
   Szeged, 2005. márc. 21-23. Abstr.: GY07, 187.
3. **Mándity István**, Martinek Tamás, Fülöp Lívia, Tóth Gábor, Vass Elemér, Hollósi Miklós, Forró Enikő, Fülöp Ferenc:
   Alternáló kiralitású β-peptid oligomerek szintézise és szerkezete
   Magyar Tudomány Ünnepe
   Szeged, 2006. november 8.
4. **Mándity István**, Martinek Tamás, Tóth K. Gábor, Fülöp Ferenc:
   Alternáló kiralitású norbornénvázas β-peptidek önrendeződésének és asszociációjának vizsgálata
   Centenáriumi Vegyészkonferencia
   Sopron, 2007. május 29-június 1. Abstr.: SZ-P-35, 353.
5. **István Mándity**, Edit Wéter, Tamás A. Martinek, Gábor K. Tóth, Elemér Vass, Ferenc Fülöp:
   Building β-peptide foldamers via stereochemical LEGO approach
   30th European Peptide Symposium
   Helsinki, Finland, August 31-September 5, 2008, Abstr.: P20121-021, p. 86.

6. **Mándity István**:
   Új típusú foldamerek létrehozása sztereokémiai mintázat változtatásával
   Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány 8. tudományos előadóülése
   Szeged, 2008. április 16.
7. **Mándity István**, Olajos Gábor, Fülöp Lívia, Vass Elemér, Tóth K. Gábor, Martinek Tamás, Fülöp Ferenc:
   Béta-peptidek finoman hangolt önasszociációja, a sztereokémia és oldallánc topológia hatása
   MTA Peptidkémiai Munkabizottság Ülése
   Balatonszemes, 2009. május 26-28.
8. **István M. Mándity**, Tamás A. Martinek, Ferenc Darvas, Ferenc Fülöp:
   Simple, efficient and selective deuteration with a flow chemistry approach
   16th European Symposium on Organic Chemistry
   Prague, Czech Republic, July 12-16, 2009, Abstr.: P2.015, p. 368.
9. **István M. Mándity**, Tamás A. Martinek, Pirjo Vainiotalo, Ferenc Fülöp, Janne Jänis:
   Probing secondary structure of β-peptide foldamers using gas-phase H/D exchange reactions
   COST, Foldamers: from design to protein recognition
   Bordeaux, France, January 25-28, 2010, Abstr.: p. 4.
10. **Mándity István**, Karsai János, Martinek Tamás, Janis Janne, Fülöp Ferenc:
    Különböző másodlagos szerkezetű béta-peptid foldamerek önrendeződésének vizsgálata gáz fázisú H/D csere reakcióval
    MTA Peptidkémiai Munkabizottság ülése
    Balatonszemes, 2010. május 26-28.
11. **Mándity István**, Karsai János, Martinek Tamás, Janne Jänis, Fülöp, Ferenc:
    Különböző másodlagos szerkezetű β-peptid foldamerek önrendeződésének vizsgálata gáz fázisú H/D csere reakcióval
    MKE Vegyészkonferencia
    Hajdúszoboszló, 2010. június 30-július 2. Abstr.: O-35, 68.