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Building β-Peptide H10/12 Foldamer Helices with Six-Membered Cyclic Side-Chains: Fine-Tuning of Folding and Self-Assembly

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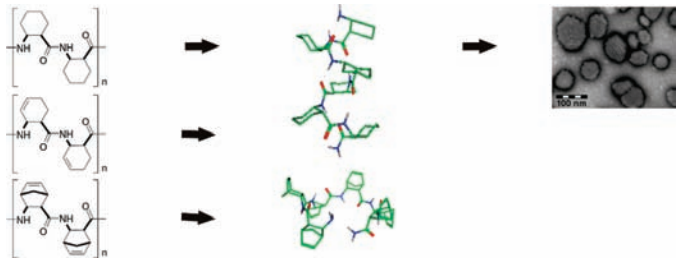
ABSTRACT



The ability of the β-peptidic H10/12 helix to tolerate side-chains containing six-membered alicyclic rings was studied. *cis*-2-Aminocyclohex-3-ene carboxylic acid (*cis*-ACHEC) residues afforded H10/12 helix formation with alternating backbone configuration. Conformational polymorphism was observed for the alternating *cis*-ACHC hexamer, where chemical exchange takes place between the major left-handed H10/12 helix and a minor folded conformation. The hydrophobically driven self-assembly was achieved for the *cis*-ACHC-containing helix which was observed as vesicles ∼100 nm in diameter.

The β-peptide foldamers are among the most interesting artificial polymers.[1] The importance of β-peptides in chemistry and biology is due to their promising biological applications.[2] The stable secondary structure patterns available to β-peptides are efficiently controllable via the side-chain chemistry,[3] and it is possible to construct helices that mimick the overall geometry of the natural α-helix: H14,[4] H12,[5] the alternating H10/12,[6] and the *de novo* designed

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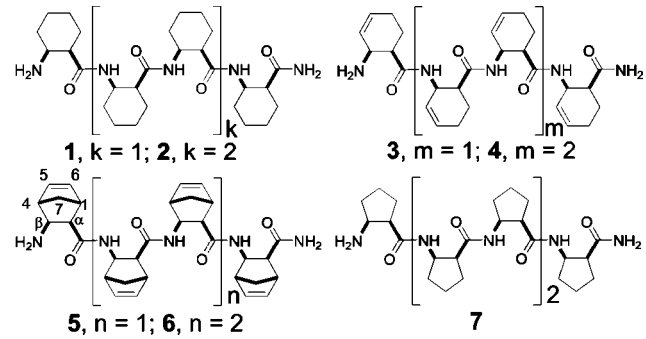
H14/16 helices.[7] The controlled self-assembly of these foldameric helices leads to helix bundles,[8] vesicle-forming membranes of vertically amphiphilic helices,[9] and lyotropic liquid crystals,[10] all of importance for future applications. These self-assembling phenomena have been reported only for H14 helices, and in the solution phase, only for those containing segments of *trans*-2-aminocyclohexanecarboxylic acids (*trans*-ACHC). While the H10/12 helix has been stated to be inherently stable,[11] its controlled self-assembly has not been observed. Even H10/12 helices formed by the enatiomeric pairs of *cis*-2-aminocyclopentanecarboxylic acids (*cis*-ACPC) escape self-association in solution. Following the design principle of the alternating backbone configuration, Boc-protected β-peptide tetramers and hexamers were synthetized through use of a bulky and strongly hydrophobic *cis*-ACPC derivative, *diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-carboxylic acid enantiomers (*diexo*-ABHEC).[12] In contrast with the expected H10/12 helix, this oligomer did not exhibit a helical fold; its secondary structure is banana-shaped and aggregation in a polar solvent was not reported.

The present work focuses on the ability of the H10/12 helix to tolerate side-chains containing six-membered alicyclic rings. While the helix-stabilizing effect of the ACHC residues for the H14 helix[4] and the H12 helix formation of the *trans*-ACPC oligomers[5] are well-known, the role of the six membered side-chain in the alternating H10/12 helix has not been studied. Through the increased hydrophobicity of the side chain, a further aim was the induction of the self-assembly for the β-peptide H10/12 helix.

Enantiopure *cis*-1*R*,2*S*-ACHC, *cis*-1*S*,2*R*-ACHC, *cis*-1*R*,2*S*-ACHEC and *cis*-1*S*,2*R*-ACHEC were utilized as building blocks.[14] The effects of N- and C-terminal protecting groups on the self-assembly β-peptides have been described;[4c] therefore, free β-peptide amides were synthetized by using the strongly hydrophobic bicyclic *diexo*-3*S*,4*R*-ABHEC and *diexo*-3*R*,4*S*-ABHEC monomers. The structures studied are shown in Scheme 1.

Scheme 1. Structures with Alternating Back-Bone Configurations Made from the *cis*-ACHC (**1**, **2**), *cis*-ACHEC (**3**, **4**), *diexo*-ABHEC (**5**, **6**), and *cis*-ACPC (**7**)



The foldamers were synthetized on a solid support by means of Fmoc technology, leading to unprotected sequences. The saturated oligomers **1** and **2** were prepared in two ways: by the catalytic reduction of **3** and **4** in an H-Cube apparatus[15] and by direct coupling of the Fmoc-*cis*-ACHC monomers. The foldamers were characterized through the use of HPLC, ESI-MS, and various NMR methods, with different solvents: 4 mM solutions in CD_3OH, DMSO-d_6, and water (H_2O/D_2O 90:10). The NMR signal dispersions were good for most of the compounds in these solvents; no signal broadening was observed and signal assignment could be performed. Interestingly, significant signal broadening was detected for **2** in all solvents. Cooling the sample to 245 K in $CDCl_3$ furnished well-resolved signals where a new set of signals was frozen out and signal assignment was achievable for the major conformer. This finding suggests that **2** has two stable conformers that undergo chemical exchange.

For the hexameric sequences **2**, **4**, and **6**, NH/ND exchange studies in CD_3OD resulted in decay plots with low slopes. For **4** and **6**, the proton resonances relating to the terminal nitrogen, the amide NH_2 and the C-terminal amide disappeared immediately after dissolution, while the remainder of the signals persisted even 1 h after dissolution (see Supporting Information). Despite the phenomenon of chemical exchange at an intermediate rate, the average signal intensity measured for the amide protons indicated similar decay for **2**. The corresponding amide hydrogens of the hexamers are significantly shielded from the solvent in consequence of H-bonding interactions, which may be an indication of the existing folded secondary structure. The shorter sequences **1**, **3**, and **5** exhibited considerably faster exchange; all the signals were practically lost within 40 min.

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This finding suggests that increasing chain length leads to increased order.

Electronic circular dichroism (ECD) fingerprints were recorded at room temperature in methanol and water (Figure 1). The ECD spectra of **2** and **4** in methanol exhibit features

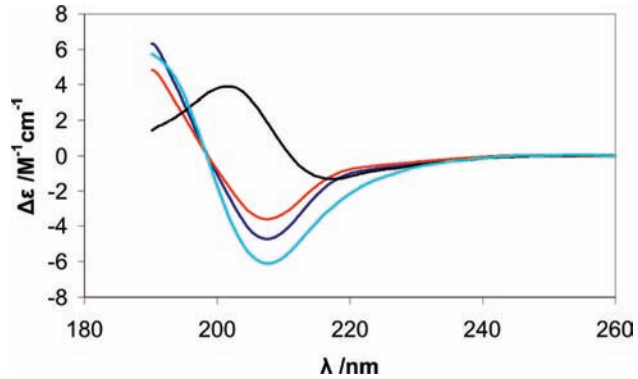

Figure 1. ECD curves of 1 mM solutions of **2** (red), **4** (blue), **6** (black), and **7** (cyan, taken from ref 6e and corrected for opposite handedness) in methanol. Data are normalized to unit chromophore.

similar to those of **7**, which has been assigned as a H10/12 helix earlier.[6e] The signal intensities for **2** and **4** are lower indicating a destabilizing effect of the 6-membered side chains. The ECD curve of **2** in water exhibited a significant drift, possibly due to the light scattering of the large aggregates (see DLS and TEM results), and thus this data set was not interpretable. The H10/12 helical feature was in part observed for **4** in water as well (see Supporting Information). For **6**, the ECD curve in methanol displayed a minimum at ~215 nm and a maximum at ~200 nm. This clear difference from the typical ECD curve of the H10/12 helix and also from the known ECD patterns of β-peptide strand-like structures[6e,16] supports the view that **6** does not fold into the H10/12 helix in the solution phase, but the overall structure exhibits helicity. The ECD curve of **6** in water demonstrated a maximum at ~210 nm and a minimum at ~190 nm; these significant differences from the results in methanol indicating a solvent-driven structural change.

As mentioned above, **2** exhibited good signal dispersion in $CDCl_3$ at 245 K, where a mixture of two conformers was observed. For the major conformer, the NOESY spectrum at 245 K exhibited a long-range NOE pattern characteristic of the left-handed H10/12 helix: NH_1-$C^{\beta}H_3$, $C^{\beta}H_2$-NH_4, NH_3-$C^{\beta}H_5$ and $C^{\beta}H_4$-NH_6 (Figure 2a). On the basis of the NMR restraints, structure refinement was performed. The resulting low-energy conformational cluster corresponds to the left-handed H10/12 helical structure. The lowest energy conformation was subjected to *ab initio* quantum chemical optimization. At both the RHF/3-21G and B3LYP/6-311G** levels of theory, the left-handed H10/12 proved stable (Figure

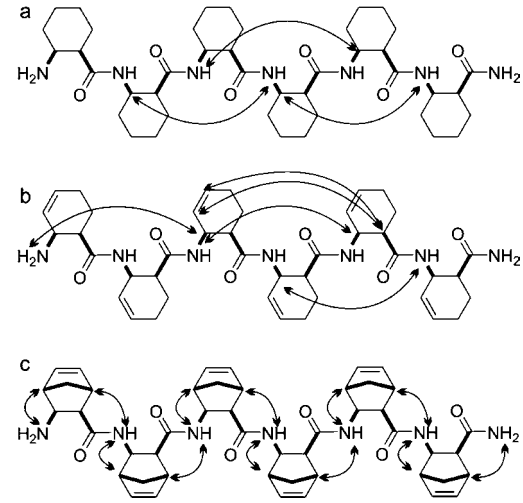

Figure 2. Long-range NOE interactions for (a) **2** in $CDCl_3$ and for (b) **4** and (c) **6** found in DMSO and CD_3OH.

3a).[17] The secondary structure of the minor conformer of **2** could not be assigned experimentally because of its low

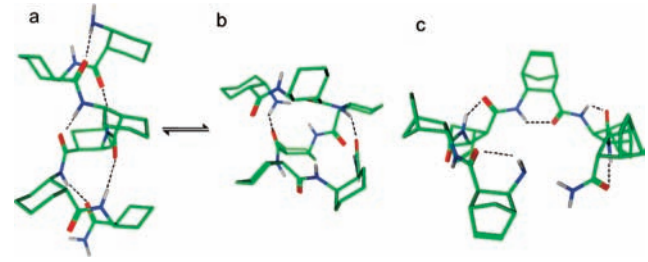

Figure 3. (a) H10/12 helix geometry for **2**, (b) the H18/20 helix geometry for **2**, (c) and the circular fold for **6**, obtained through NMR restrained structure refinement and by final optimization at the B3LYP/6-311G** level of theory.

relative abundance (~6%). The low overall NH/ND exchange rate for **2** indicates that the conformational equilibrium can not involve an unfolded minor conformer or a significant fraction of unfolded intermediate states. Molecular modeling at the B3LYP/6-311G** level suggested that the H-bond-stabilized conformation closest in energy is the left handed H18/20 ($\Delta E = 4$ kcal mol^{-1}) (Figure 3b). The left-handed H18/20 helix is proposed, which can occur through a rapid shift between the H-bonds, without the overall disassembly of the helical structure. For longer sequences this helix could be the dominant conformation.

Importantly, for **4** the C^{β}H-NH vicinal couplings exhibited an alternating pattern (values given for CD_3OH): 9.8 Hz for NH_3-$C^{\beta}H_3$ and NH_5-$C^{\beta}H_5$, and 8.7 Hz for NH_2-$C^{\beta}H_2$, NH_4-

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$C^{\beta}H_4$ and NH_6-$C^{\beta}H_6$. For **4**, long-range NOEs were recorded from the ROESY spectra in CD_3OH and DMSO-d_6. These NOE interactions are typical for the H10/12 helix and the structure refinement resulted in the left-handed H10/12 in the same way as found for **2** (Figure 2b) which is in accordance with the $C^{\beta}H$-NH vicinal couplings as well.

In order to test the secondary structure of **6**, ROESY measurements were run in CD_3OH, DMSO-d_6 and water (H_2O/D_2O 90:10). A series of NOEs were observed in these solvents for the pairs NH_i-C^4H_i and NH_i-C^1H_{i-1} (Figure 2c), but $i - i+2$ interactions could not be detected, which is evidence against the formation of the alternating H10/12 helix and lends support to a bent conformation.[12] For **6**, the $C^{\beta}H$-NH vicinal couplings did not show an alternating pattern. The same structure refinement based on NMR-derived distance restraints, converged to a circle-like fold (Figure 3c). This conformation was corroborated by the absence of C^7H_i-C^1H_{i-1} and C^7H_i-C^4H_{i+1} NOE interactions in the ROESY spectra, which should appear for an elongated strand.

Transmission electron microscopy (TEM) and dynamic light scattering (DLS) were employed to analyze the self-association phenomena. For a 4 mM solution of **2** in water, vesicles with an average diameter of 100 nm were observed in the TEM images immediately after dissolution, sonication and filtration. The vesicles tended to associate into clusters. This result was supported by the DLS measurements, indicated particles with diameters in the range 100−1000 nm. For **1** and **3**−**6**, no self-association was observed under the same conditions (Figure 4). The morphology of the associates did not change after 1 week of incubation.

Small changes in the side-chain chemistry resulted in significantly different behavior in the association properties of the β-peptide foldamers studied. Solvophobically driven self-assembly of the H10/12 helix was observed only the cyclohexane side-chain (**2**). This suggests that the pronounced hydrophobic nature of the cyclohexane side-chain promotes self-assembly, given the tendency of the H10/12 helix to fold. On the other hand, the strongly hydrophobic *cis*-ABHEC residue in itself is not sufficient to initiate aggregation, indicating the necessity of folding into the compact helical structure. These findings indicate that the interplay of the stable compact helical conformation and the adequately hydrophobic side-chain is necessary for the creation of self-assembling foldameric helices.

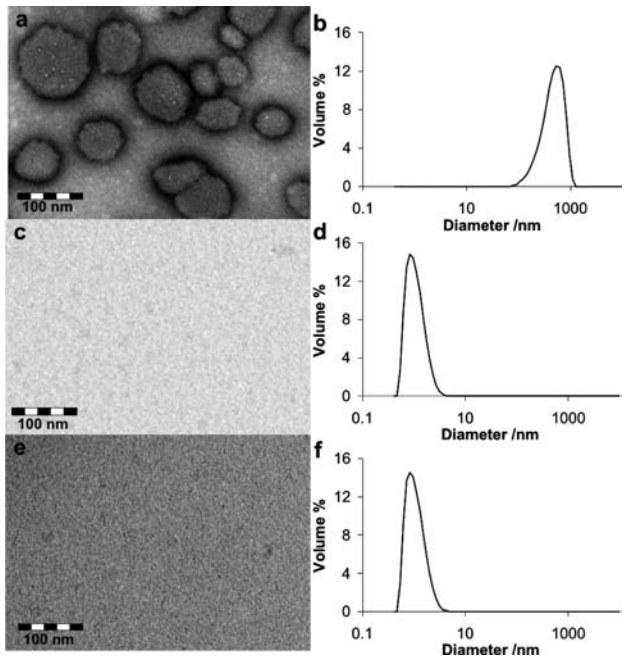

Figure 4. TEM images of (a) **2**, (c) **4**, and (e) **6** and DLS curves for (b) **2**, (d) **4**, and (f) **6** as 4 mM solutions in water.

For an assessment of the hydrophilic/hydrophobic character of the studied foldamers in their folded state (lowest energy *ab initio* geometries), hydrophobic and hydrophilic surface areas were calculated (see Table S4, Supporting Information). The values indicate that the self-assembling foldamer **2** exhibits the largest hydrophilic/hydrophobic surface ratio in its folded state.

The β-peptidic alternating H10/12 helix tolerates the six-membered side-chain topology. Both the *cis*-ACHC and the *cis*-ACHEC afforded the H10/12 helix. Based on the ECD results, we can conclude that the six membered ring topology slightly destabilizes the H10/12 helix as compared with the alternating *cis*-ACPC oligomers. Moreover, the *cis*-ACHC-containing foldamer **2** exhibited conformational polymorphism with two folded conformational states that underwent chemical exchange. A noteworthy finding was that an apparently subtle change in the hybridization of a carbon atom pair in the side-chain can tune the conformational behavior to such an extent. The bicyclic *diexo*-ABHEC prevented the formation of a small-diameter helix; the experimental results pointed to a circle-like fold which was sufficiently stable in methanol to maintain considerably shielded H-bonds. For the β-peptide helices studied, minor changes in the side-chain topology, size and shape resulted in large effects on the self-assembly process. It emerged, that the H10/12 helix is capable of self-association in a polar medium if the helix is built up from *cis*-ACHC residues. Our observations strongly suggest 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. These results serve as an important starting point for the design of helix assemblies involving the H10/12 helix.

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Supporting Information Available: NMR signal assignments, NMR spectra, NH/ND exchange, ECD, MS, HPLC characterization and modeling data. This material is available free of charge via the Internet at http://pubs.acs.org.

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