

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

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The long-range side-chain repulsion between the (1R,2R,3R,5R)-2-amino-6,6-dimethyl-bicyclo[3.1.1]-heptane-3-carboxylic acid (*trans*-ABHC) residues stabilize the H12 helix in β -peptide oligomers.

Synthetic oligomers with distinct conformational preferences (foldamers) are highly intriguing compounds at the interface of covalent and supramolecular (noncovalent) chemistry.¹ Among foldamers based on remote intrastrand interactions, peptides containing β -amino acid residues are probably the most thoroughly studied systems.² Their versatile secondary structure patterns and the side-chain controllable conformational preferences are attracting increasing interest.³ β -Peptide oligomers with designed molecular interaction fields have gained a number of biological applications, such as rationally designed antiviral H12 helices.⁴ In these applications, the helical secondary structures (H14, H12 and H10/12) play crucial roles with regard to the biological activity; they are induced by the special substitution and stereochemical pattern of the β -peptide backbone, while the side-chain chemistry is varied independently. The stabilization effects of β -amino acid residues with cyclic side-chains are indispensable in the β -peptide helix design. The H12 helix, which is a good mimic of the α -helix in terms of overall shape, can be stabilized by the incorporation of a sufficient number of *trans*-2-aminocyclopentanecarboxylic acid (*trans*-ACPC) or the structurally related *trans*-amino-pyrrolidinecarboxylic acid (*trans*-APC) residues in the sequence.⁵ Two-thirds of the residues are required to have the side chain topology of a five-membered ring with a *trans* relative backbone configuration in order to gain a reasonably stable H12 helix, which imposes considerable limitations on the chemical diversity of the side-chain pattern along these β -peptide helices. Independent methods to stabilize the H12 helix are therefore still sought.⁶

In the present work, a novel approach is proposed whereby to induce formation of the H12 helix. The steric repulsion between the side-chains in positions *i* and (*i* + 3) was utilized to bias the potential energy landscape, which

eventually destabilizes the H14 helix in favor of the H12 helix. We demonstrate here that bulky *trans*-ABHC residues retain the helical organization of the peptide chain, and the designed interactions facilitate formation of the H12 helix.

Construction of a H14 helix, the β -peptide secondary structure most often utilized in biomedical studies, can readily be achieved by using β_3 -substituted β -amino acids in combination with *trans*-2-aminocyclohexanecarboxylic acids (*trans*-ACHCs).⁷ The cyclic side-chain of the latter lends extra stability to the secondary structure through the restricted conformational space and the *i*-(*i* + 3) hydrophobic stacking interactions between the cyclohexane rings.⁸ Recent results led to the successful synthesis of monoterpene-derived β -amino acids with an apopinane skeleton (2-amino-6,6-dimethyl-bicyclo[3.1.1]heptane-3-carboxylic acid; ABHC).⁹ The apopinane moiety is a dimethyl-substituted, methylene-bridged analog of ACHC and, as such, further enhances the conformational preorganization of the cyclohexane ring, but the bulky substituent certainly disfavors the *i*-(*i* + 3) hydrophobic stacking interactions in H14 (Fig. 1).

To test the effect of the *i*-(*i* + 3) steric clash on the preferred secondary structure, oligomers of *trans*-ABHC were constructed (Scheme 1). Conformational sampling was carried out on **4** by using a hybrid molecular dynamics (MD)–Monte Carlo (MC) method and MMFF94x force field.¹⁰ The lowest energy conformational family was the H12 helix (32% of the conformers, average cluster energy relative to the lowest energy conformation: 18.55 kJ mol⁻¹). Interestingly, the H16 helix fold was also populated (21%, 74.32 kJ mol⁻¹), however with higher conformational energies. The H14 helix was only a low-population high-energy cluster (0.3%, 138.37 kJ mol⁻¹). Helical structures with combinations of 12-membered and 16-membered H-bonded rings were identified in 22% of the conformers. Other regular helical fold was not found. All the geometrically possible helices for **1–4**, H8, H10, H12, H14, and H16 were optimized at the *ab initio* quantum chemistry level (Fig. 2).¹¹ The HF/3-21G//B3LYP/6-311G** levels of theory *in vacuo* were utilized, as this has been reported to provide a good approximation for the β -peptides.¹² The structures converged to the corresponding local minimum of the potential energy

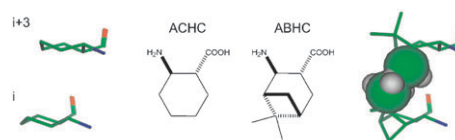
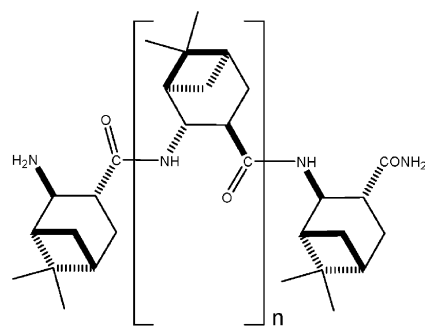


Fig. 1 The steric interactions between side-chains in positions *i*-(*i* + 3) in the ideal H14 helix for ACHC (no clash) and ABHC (predicted clash) oligomers.

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† Electronic supplementary information (ESI) available: Experimental details, NMR signal assignments and ³J(NH_i–C^βH_i) values in CD₃OH and DMSO-*d*₆, ROESY, TOCSY and DOSY spectra for **4**, *ab initio* energies and structures, and complete ref. 16. See DOI: 10.1039/b812114a



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

Scheme 1 The studied ABHC oligomers.

surface, except for the H10 helices, which finally converged to H14.

The modeling indicated that H12 and H16 are the most likely secondary structures for 3 and 4. For 1 and 2, H8 is a low energy fold too. It should be noted that the final *ab initio* structures of the H12 helix optimized *in vacuo* exhibited a 6-membered H-bonded pseudo ring at the N-terminal residue (terminal-N...HN₂), though this was not corroborated by the NMR results (see later). These findings support the side-chain repulsion concept for shaping the desired secondary structure.

Model compounds 1–4 were synthesized. The Fmoc-(1*R*,2*R*,3*R*,5*R*)-ABHC stereoisomer was prepared by using literature methods.⁹ The chain assembly was carried out on a solid support, with Fmoc chemistry. The final products were characterized by means of MS and various NMR methods, including COSY, TOCSY and ROESY, in 4 mM CD₃OH and DMSO-*d*₆. In water, severe solubility problems were experienced. The NMR signal dispersions were very good, resonance broadening was not observed, and thus the resonance assignment was achievable.

The time dependence of the residual NH signal intensities in the N¹H–N²D exchange experiments for 4 point to the corresponding atoms being considerably shielded from the solvent, due to H-bonding interactions. The proton resonances relating to the terminal amine and the C-terminal amide disappeared immediately after dissolution, whereas the other signals remained for a longer time. The exchange pattern observed is in good agreement with the theoretically predicted H-bonding network of the H12 helix. For 3, a similar exchange pattern was detected, but the exchange rates were higher, suggesting a less-ordered structure.

For 4, ROESY experiments were run in CD₃OH and DMSO-*d*₆. In DMSO, the characteristic C^βH_{*i*}–C^αH_{*i*+2},

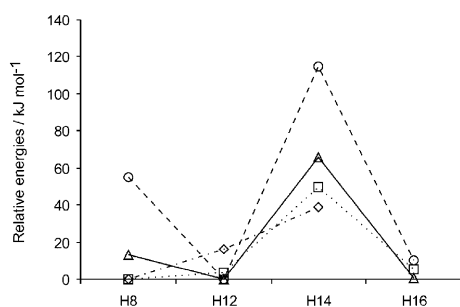


Fig. 2 Relative B3LYP/6-311G** energies (actual energy–lowest energy) of 1 (◇), 2 (□), 3 (△) and 4 (○) for the *ab initio* calculated helical secondary structures.

C^βH_{*i*}–NH_{*i*+2} and Me_{*i*}–NH_{*i*+3} long-range NOE interactions could readily be observed, which unequivocally proves the predicted H12 conformation (Fig. 3 and 4). Hybrid MD-MC modeling with NMR restrains for 4 exclusively resulted in the H12 helix ruling out unfolded structures or other helix types as a predominant conformation. The NOEs between the axial Me groups and amide protons are only due the H12 helix; NOEs of aggregation origin would not be so specific. Although a sufficient number of characteristic NOEs were observed in CD₃OH, some of the long-range ROESY cross-peaks could not be resolved because of the poorer resonance dispersion for the C^βH_{2–4} protons. The C^βH₁–NH₃ interaction could not be observed, but a weak C^βH₁–NH₄ cross-peak was detected in both solvents, in full agreement with the H12 helix formed by the *trans*-ABHC homo-oligomers. For 3, the long-range NOE interactions characteristic of the H12 helix could be observed, but 2 and 1 did not exhibit any helix-related signal.

For 3 and 4, scalar couplings measured on the amide protons indicated values well above 8.5 Hz in DMSO-*d*₆, except for the N-terminal residues, which is in line with the predominantly antiperiplanar NH–C^βH orientation necessary for the helical structure. For 2, the couplings were decreased significantly, but indicate a prevailing antiperiplanar arrangement for the nonterminal residues, while 1 exhibits couplings pointing to random NH–C^βH_{*i*} dihedrals. In CD₃OH, a similar coupling pattern was obtained.

Electronic CD measurements were carried out to gain further evidence in support of the presence of the helical fold determined by NMR. Measurements were carried out in MeOH only because the ECD spectra in DMSO are not useful for the secondary structural analysis of peptides and proteins, due to interfering absorption from the solvent below 268 nm. In water, solubility problems prevented the measurement. In view of the literature results on the extreme sensitivity of the ECD to small changes in the foldamer geometry,¹³ and of the UV absorbance of the asymmetric cyclobutane moiety in the range 190–250 nm,¹⁴ the direct comparison with the literature ECD fingerprints of the H14 and H12 helices obtained for various UV inactive side-chains is not possible.¹⁵ Unfortunately, CD result was not published for the only known β-peptide helix having cyclobutane fragment in the side-chain.⁶

The ECD spectra revealed a significant chain length-dependence (Fig. 5). The positive lobes display a maximum at around 220 nm for 1 and 2, while the band is shifted to 210 nm and 205 nm for 3 and 4, respectively. The position of the negative band remains constant at around 195 nm. The continuous hypsochromic displacement of the positive band and the increasing symmetry of the

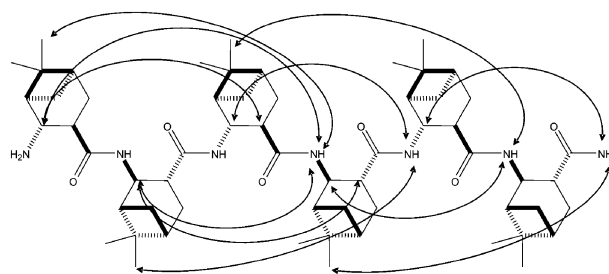


Fig. 3 Resolvable long-range NOE interactions detected in the ROESY spectrum of 4 in DMSO-*d*₆.

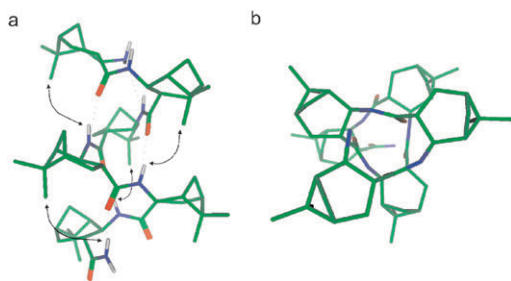


Fig. 4 Side view (a) and top view (b) of the NMR-derived H12 helix formed by **4**. Arrows indicate only the $\text{Me}_i\text{-NH}_{i+3}$ NOEs out of the many long-range interactions observed in the ROESY spectra of **4** (see Fig. 3).

positive couplet unambiguously indicate that the folding takes place in a chain length-dependent fashion, which is an essential feature of the helix formation. Foldamers **2** and **3** display higher intensities at short wavelengths, which might indicate a partial population of an elongated structure such as H8 helix.¹⁶

Experimental¹⁷ and theoretical¹⁸ studies and our own earlier results¹⁹ indicate that self-association in the solution phase is an inherent feature of the horizontally or vertically amphiphilic β -peptide foldamers. To test this phenomenon, DOSY NMR spectra were run and the aggregation numbers were determined in CD_3OD from the measured diffusion constants for **3** and **4** in the same way as described earlier.²⁰ In the present work, glucose was utilized as external reference. The average aggregation numbers for **3** and **4** were 8.6 and 13.9, respectively. Since no head-to-tail NOE was detected for these foldamers, the side-by-side helix bundle type association is likely. This result reveals that the H12 helix constructed from the *trans*-ABHC residues is capable of self-association, in agreement with the slowness of the NH/ND exchange due to the extra shielding.

In the present work, a newly synthesized apopinene-derived β -amino acid monomer (*trans*-ABHC) was utilized, which has a special side-chain shape preventing $i\text{-(}i+3\text{)}$ hydrophobic side-chain stacking interactions. The NMR, ECD and *ab initio* modeling evidences unequivocally prove that the H12 helix is the prevailing conformation for these residues, and the folding takes place in a chain length-dependent manner. Diffusion-ordered NMR spectroscopy demonstrated that the secondary structure formation in MeOH is accompanied by a self-association phenomenon, driven by hydrophobic interactions.

We believe that the *trans*-ABHC residues incorporated in $i\text{-(}i+3\text{)}$ positions in the β -peptide sequence should be able to

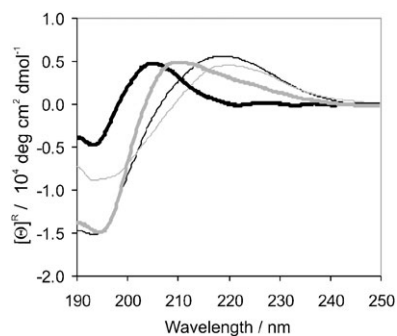


Fig. 5 ECD curves measured for **1** (thin gray), **2** (thin black), **3** (thick gray), and **4** (thick black).

force formation of the H12 helix even if open-chain β_3 -amino acid residues are present in the structure, which would thereby afford a novel route to the building of this intriguing helix type. A study to test this feature is in progress.

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