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12-Helix Folding of Cyclobutane β-Amino Acid Oligomers

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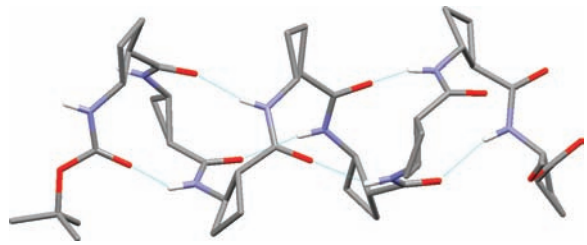
ABSTRACT



The hexamer and octamer of *trans*-2-aminocyclobutane carboxylic acid were prepared and their conformational preferences studied experimentally and using molecular modeling. All observations suggest a marked preference for the folding of these oligomers into a well-defined 12-helical conformation, in both solution and the solid state.

There is considerable interest in synthetic oligomers which have well-defined folding preferences (foldamers).[1] β-Peptides are among the most studied class of compounds in this respect and offer an array of possibilities for regular folding through hydrogen bond formation.[2] Some ground rules have emerged for the folding of oligomers which contain β-amino acids (β-AAs): the 14-helix is a preferred conformation in oligomers of β-AAs with a six-membered ring constraint, such as *trans*-2-aminocyclohexane carboxylic acid,[3] while oligomers of *trans*-2-aminocyclopentane carboxylic acid (*trans*-ACPC) and related five-membered heterocyclic β-AAs have distinct 12-helix preferences.[4] Mixed α/β-peptides incorporating carbocyclic β-AAs display other conformational preferences.[5]

The folding propensities of oligomers constructed from four-membered cyclic β-AA building blocks are less clear-

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cut. Fleet[6] described an oxetane-based *cis*-β-AA hexamer which adopted a 10-helix conformation, while Ortuño observed 14-helical folding for a tetramer containing in alternation two *cis*-cyclobutane β-AA (*cis*-ACBC) residues and two β-alanines.[7] Homo-oligomers composed uniquely of *cis*-ACBC units (2−8 residues) showed a conformational bias derived essentially from six-membered intramolecular hydrogen bonds, which facilitated supramolecular interactions and self-assembly, producing nanosized fibers or gels.[8] However, *trans*-ACBC units have only been studied in dipeptides: a preference for the formation of eight-membered hydrogen-bond rings was noted for (*trans,cis*) and (*trans,trans*) combinations,[9] provoking the question of whether this structural feature might prevail in longer oligomers of *trans*-ACBC. It thus seemed opportune to examine the conformational behavior of such β-peptides.

The preparation of the target oligomers was not a trivial task since the parent β-AA is only moderately stable[10] and has only recently been described in enantiomerically pure form.[11] However, solution-phase oligomer assembly was achieved successfully and is summarized as follows. Dimer **1** was obtained by coupling equimolar amounts of the *N*-Boc and the methyl ester derivatives of (1*R*,2*R*)-*trans*-ACBC. This is the only step requiring any particular care: the free amine of the methyl ester of (1*R*,2*R*)-*trans*-ACBC is liberated from its stable hydrochloride immediately prior to coupling. Subsequent chain extension procedures were free of difficulty: selective deprotection and coupling of two dimer units gave the tetramer **2**. Analogous operations were performed to combine **1** and **2** to provide hexamer **3** and to combine 2 equiv of **2** to give octamer **4** (full details are given in the Supporting Information file). In contrast to the free monomer, all of these terminally protected oligomers are stable, and their structures are shown in Figure 1.

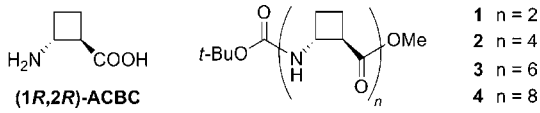

Figure 1. Parent *trans*-β-amino acid building block and the series of oligomers.

Circular dichroism data gave the first indication of a change in conformational preferences with increasing oligomer length (Figure 2). In MeOH solution, a marked evolution in behavior was observed progressing from dimer **1** through tetramer **2** to hexamer **3**. The spectra of hexamer **3** and octamer **4** were very similar, with a maximum near 209 nm and a minimum near 227 nm. While it has been pointed out that CD data on its own are insufficient for drawing firm conclusions on the conformational preferences of β-peptides,[12] we noted that the data for **3** and **4** resembled a slightly red-shifted version of the characteristic spectral signature of a 12-helix in the same solvent.[4]

The best solubility and NMR spectral resolution of **3** and **4** were secured in pyridine-d_5, which was the only solvent

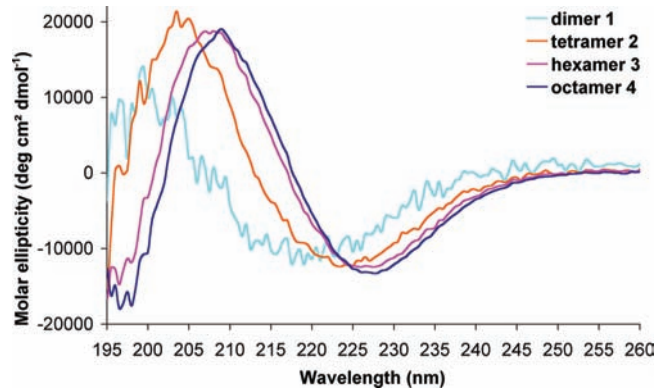

Figure 2. Circular dichroism spectra in MeOH (0.2 mM) for dimer **1**, tetramer **2**, hexamer **3**, and octamer **4**. Molar ellipticity has been normalized for concentration and per-residue.

which allowed complete assignment of all backbone ^{1}H signals, a necessary prerequisite for the intended NOE experiments. The two N-terminal amide and carbamate protons were deshielded, which can be explained as a result of these hydrogens being less involved in intramolecular C═O···H−N hydrogen bonding, leaving them more solvent-exposed and forming hydrogen bonds with the solvent. The temperature coefficients for the ^{1}H NMR amide (or carbamate) signals of oligomers **3** and **4** were consistent with this postulate: in each case, the values for the protons of residues 1 and 2 (−15 and −12 ppb/°C, respectively) were considerably larger than all others (<−6 ppb/°C).

ROESY spectral data were then used to identify nonadjacent NOE interactions, and the three correlation types observed in both **3** and **4** ($C_\beta H_i$-NH_{i+2}, $C_\beta H_i$-$C_\alpha H_{i+2}$, $C_\beta H_i$-NH_{i+3}) are characteristic of a 12-helix.[4] Correlations are summarized in Figure 3 for hexamer **3**.

NMR data obtained for **3** were used in molecular modeling studies. Molecular dynamics calculations were carried out

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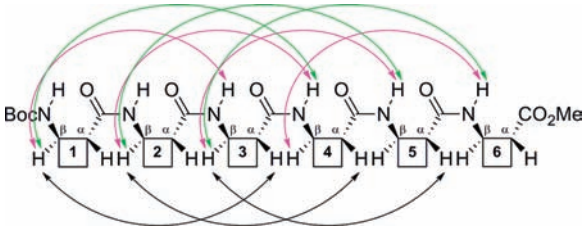

Figure 3. Graphical summary of nOes observed for hexamer **3**. Analogous correlations were observed for octamer **4**. Conditions: 10 mM solution in pyridine-d_5, 298 K, mixing time 300 ms.

on hexamer **3** in a pyridine continuum using SYBYL 7.0 software and the MMFFs94 force field, initially without restraints. Five low-energy conformer families emerged, and each was subjected to an ab initio geometrical optimization using the B3LYP STO-3G base. This refinement provided only two accessible conformer families, from which the lowest potential energy corresponded to a 12-helix.[13] Restrained molecular dynamics calculations were then carried out in a similar manner, including energy terms for the ROESY distance restraints and dihedral angle restraints based on $NH_i-C_\beta H_i$ coupling constants. Only 12-helical structures were obtained; the minimum energy conformer is shown in Figure 4.

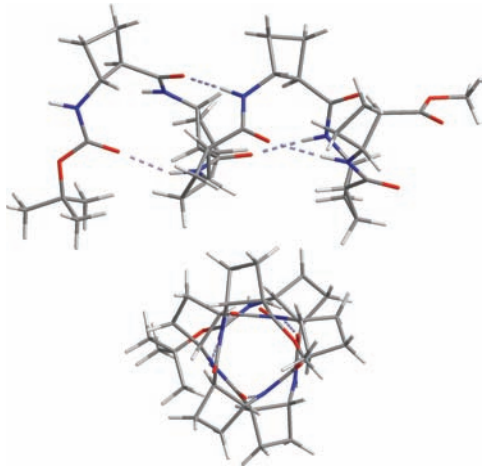

Figure 4. Minimum energy structure for hexamer **3** showing the hydrogen bonds implicated in the 12-helix structure (top) and looking down the helix axis (bottom).

Crystals of octamer **4** grown from $CHCl_3/Et_2O$ solution were suitable for X-ray diffraction. A well-defined 12-helical structure is adopted by **4** in the solid state (Figure 5). The average backbone torsion angle is −95.7°, with little distortion (max. −100.1° at residue 4, min. −89.7° at residue 5). Six standard hydrogen bonds form the basis of the helical structure, with C═O···H−N distances within the range 2.03−2.22 Å and O···H−N angles falling in the range 133.9−160.1°. The cyclobutane ring conformation is highly uniform all along the oligomer chain, with a puckering angle in the range 25.6−29.4°, with exceptions at residue 5 and 8, where puckering of 33.2° and 31.8°, respectively, are observed. The amine and carbonyl moieties occupy pseudoequatorial positions. Figure 5 shows a typical constituent *trans*-ACBC moiety (residue 3).

Four molecules are present in the asymmetric unit of the crystal, and their conformations are very similar. Two molecules are aligned end-to-end on the same axis, but no hydrogen bond exists between them; the other two molecules are also in an antiparallel orientation but on a different axis. Intermolecular hydrogen bonds are formed between helices which are on different axes but having the same global orientation. In addition to the six intramolecular hydrogen bonds that maintain the helix, each helix forms four intermolecular hydrogen bonds with the closest neighbors; all hydrogen-bonding sites are thus satisfied. The second pair of helices in the asymmetric unit pack via the same set of intermolecular hydrogen bonds but are in an opposite global orientation, thus creating layers of helices; there is no apparent interaction between these layers. Likewise, there is little side-to-side intermolecular contact between the cyclobutane rings in neighboring layers.

It is interesting to compare the 12-helices adopted by the *trans*-ACBC oligomers in the crystalline state and the modeled solution state. Figure 6 shows the superposition of two comparable internal segments from the minimum energy conformer for **3** and the crystal structure for **4**. Overall, there is a good overlap of the structural features, including the conformation of the cyclobutane rings. In the solid state, the backbone torsion angles are marginally larger, leading to a slight elongation of the 12-helix.

The solid state conformation of the backbone of molecule **4** is very similar to that of a protected octamer of *trans*-ACPC,[4c] while more subtle differences appear in the behavior of the peripheral parts of the respective carbocycles. There is some lateral interaction between cyclopentane rings of neighboring *trans*-ACPC molecules, whereas the rigidity of the cyclobutane rings in **4** appears to preclude such interactions.

In summary, the hexamer and octamer of *trans*-ACBC do not fold around short-range (eight-membered ring) hydrogen bonds but show instead a clear preference for longer-range hydrogen bonding interactions leading to a stabilized 12-helix. This structure is an important manifold for the elaboration of functional foldamers: antibiotic and antiviral activities[14] and γ-secretase inhibition[15] have been reported for rationally designed 12-helix oligomers, and efforts are made to secure new ways of stabilizing this conformational

(13) In response to a request by a reviewer, the lowest-energy conformer was reoptimized at the 6-31G(d,p) level, with no discernable difference in the core helical structure. See the Supporting Information for details.

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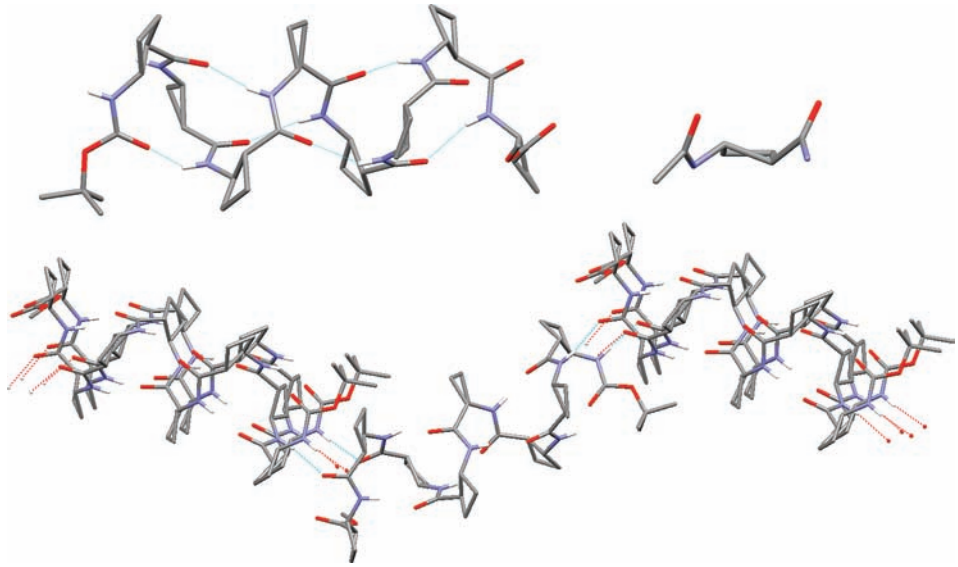

Figure 5. Crystal structure images of octamer **4**. For clarity, all hydrogen atoms have been removed except those involved in hydrogen bonds. Top left: a single molecule is shown with the six H-bonds which stabilize the 12-helix. Top right: an enlargement of one of the β-amino acid units (residue 3), highlighting the cyclobutane ring conformation. Bottom: Intermolecular interactions of a selected molecule with four neighbors: the NHs of residues 1 and 2 interact with the COs of residues 6 and 7 (respectively) of the two closest helices in adjacent planes; likewise, the COs of residues 6 and 7 interact with the NHs of residues 1 and 2 of the two nearest helices, also in adjacent planes.

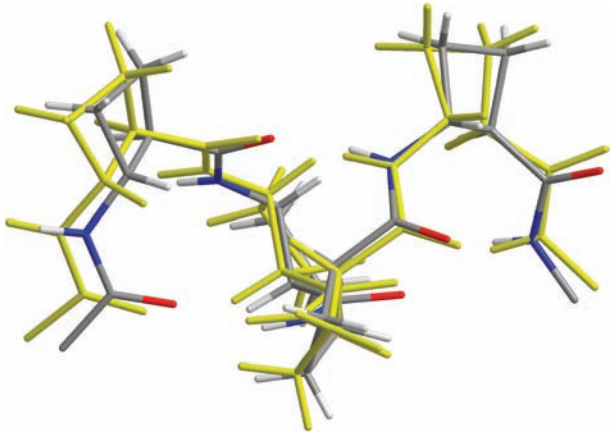

Figure 6. Superposition of comparable segments of modeled solution state (multicolored) and solid state (yellow) 12-helix structures. The solution state segment is residues 2−5 of hexamer **3**, and the solid state segment is residues 3−6 of octamer **4**.

feature.[16] *trans*-ACBC-based peptides are similar, but not identical, to *trans*-ACPC oligomers and thus enlarge the set of basic structures available for foldamer development and design.

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Supporting Information Available: Preparative procedures, copies of NMR spectra, chemical shift assignments, details of ROESY experiments, molecular modeling, and crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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