

cis*-2-Aminocyclopentanecarboxylic Acid Oligomers Adopt a Sheetlike Structure: Switch from Helix to Nonpolar Strand*

Tamás A. Martinek, Gábor K. Tóth, Elemér Vass, Miklós Hollósi, and Ferenc Fülöp*

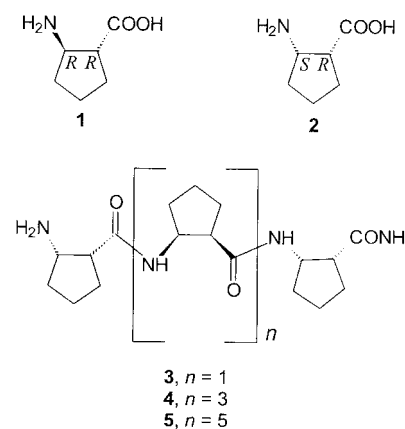
It is now well known that biological molecules (peptides and RNA) are not alone in forming periodic secondary structures.^[1] The extremely rapidly growing area of non-natural foldamers has provided a number of examples of synthetic molecular chains, and especially β -peptides, which adopt well-defined three-dimensional structures.^[2–4] Interest has recently turned towards the residue-based control of secondary structure formation, and it was demonstrated that the substituent pattern and the stereochemistry do indeed exert certain effects on the β -peptide folding.^[5] The conformational control over the secondary structure continues to attract considerable interest,^[6, 7] and the induced switch between a stable helix and a stable nonpolar strand with a propensity to form a pleated sheet remains an unresolved problem. Here we show that, by inversion of the relative configuration of conformationally restricted cyclic β -amino acid residues, the preferred periodic structure can be switched from a helix to a single nonpolar strand. These results may furnish a general tool for foldamer designers, thereby promoting the synthesis of future tertiary structures of β -peptides.

β -Oligopeptides consisting of *trans*-2-aminocyclohexanecarboxylic acid and *trans*-2-aminocyclopentanecarboxylic acid (*trans*-ACPC, **1**) monomers (*anti*-disubstituted in the aldol convention) have been reported to exhibit an especially high propensity to form helical structures^[8] and the rational residue-based control of helix shape could be achieved by the application of these fascinating molecules. After the first successes, the natural question emerged of whether the 1*R*,2*S*-disubstitution of the cyclic β -amino acid monomers leads to a stable secondary structure or to a random coil conformation.

We used homo-oligomers composed of (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid/(1*R*,2*S*)-*cis*-ACPC **2** as a model system to test the effect of the inversion of the relative configuration. The generally accepted rules of thumb for the effects of the stereochemistry of the β -amino acid residues

would anticipate an extended polar strand for 1*R*,2*S*-disubstitution;^[9] however, the NH- C^β - C^α -CO torsion in *cis*-ACPC is restrained in the *gauche* position, which facilitates either the helix or nonpolar strand conformation. Our preliminary modeling results do not allow the possible helix structures to be excluded from the analysis since the inter-residue steric repulsion^[10] is not a real issue in the modeled helices of the *cis*-ACPC oligomers. This therefore facilitates study of the general effects of the stereochemistry on the preferences for the periodic structure formation of β -peptides with cyclic monomers.

Enantiomerically pure (1*R*,2*S*)-*cis*-ACPC oligomers **3–5** (Scheme 1) were prepared by solid-phase synthesis techniques, with *tert*-butoxycarbonyl chemistry.^[11] The couplings were performed with *N,N'*-dicyclohexylcarbodiimide (DCC) without difficulty. The crude peptides were purified by reversed-phase HPLC and the compounds were characterized by mass spectrometry.



Scheme 1. Chemical structures of *trans*- and *cis*-ACPC, and the studied oligomers.

High-resolution three-dimensional structure analysis was performed in the solution phase by utilizing NMR cross-relaxation data. All NH, C^α H, and C^β H resonances were assigned for pentamer **4** by means of standard NMR methods (TOCSY^[12] and ROESY^[13]). The linewidths and chemical shifts seen in the NMR spectra as well as the ROESY cross-peak pattern of **4** were identical for 1 mM and 8 mM samples, thus demonstrating the absence of any uncontrolled aggregation in [D₆]DMSO. The studied compounds, **4** and **5**, displayed very similar ROESY cross-peak patterns, but the unsatisfactory chemical shift resolution for the heptamer led to the high-resolution conformational analysis being carried out for the pentamer only.

The low-intensity ROESY cross-peaks defining four long-range $NH_i-C^\beta H_{i+1}$ and $NH_i-C^\beta H_{i-1}$ interactions could readily be assigned in terms of monomers with $i = 1, 2$, and 3 (Figure 1a). The low chemical shift resolution of the $C^\beta H_i$ protons for monomers with $i = 3, 4$, and 5 did not allow direct assignment and integration of the expected signals, but the multiplet pattern and the increased intensity of the short-range $NH_i-C^\beta H_i$ cross-peaks forecast the presence of further valuable long-range $NH_i-C^\beta H_j$ NOE interactions as signal overlaps. To obtain direct experimental evidence, a series of

[*] Prof. Dr. F. Fülöp, Dr. T. A. Martinek
Institute of Pharmaceutical Chemistry
University of Szeged
Eötvös 6, 6720 Szeged (Hungary)
Fax: (+36) 62-545-564
E-mail: fulop@pharma.szote.u-szeged.hu

Dr. G. K. Tóth
Department of Medical Chemistry
University of Szeged
Dóm tér 10, 6720 Szeged (Hungary)
Dr. E. Vass, Prof. Dr. M. Hollósi
Department of Organic Chemistry
Eötvös Loránd University
Pázmány P. 11/17, 1117 Budapest (Hungary)

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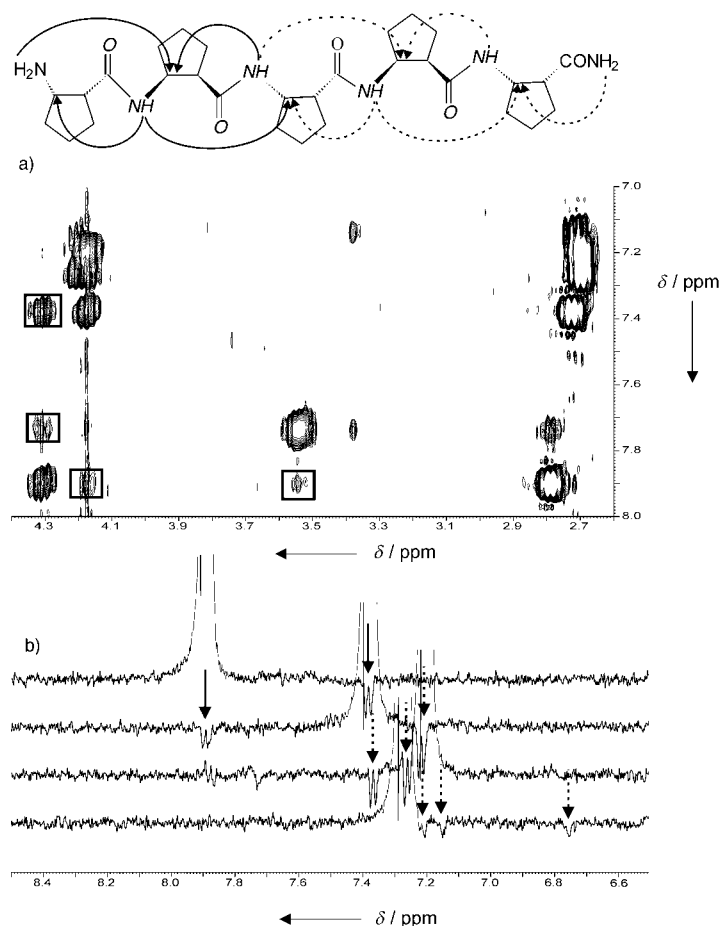


Figure 1. Spectra used for the assignment of the long-range NOE interactions: a) 2D ROESY experiment, b) selective 1D TOCSY (30 ms)/ROESY (400 ms) experiments. The solid lines show the assignments resulting from spectrum shown in (a), while the dashed lines designate the interactions proved by the series of spectra shown in (b).

selective 1D experiments was performed (Figure 1b), which involved selective excitation of the amide proton signals, subsequent short (30 ms) homonuclear Hartmann–Hahn mixing, and a final ROESY spin-lock (400 ms). The magnetization exclusively transferred by the cross-relaxation between the NH_i and C^βH_i protons could be detected as a negative phase signal in the amide region, since no direct interaction is observed between the amide protons. As a result, we obtained the full periodic pattern of the long-range $\text{NH}_i\text{--C}^\beta\text{H}_{i+1}$ and $\text{NH}_i\text{--C}^\beta\text{H}_{i-1}$ NOE interactions, which rules out any helical or random-coil conformation, and can thus be assigned to a predominantly populated strandlike secondary structure. The analysis of the corrected short-range $\text{NH}_i\text{--C}^\beta\text{H}_i$ and $\text{NH}_i\text{--C}^\alpha\text{H}_{i-1}$ cross-peak intensities provided further evidence of the highly ordered strand conformation.

To retain as much quantitative information as possible from the cross-peak integrals we calculated the intensity ratio from Equation (1), where I designates the integrated and offset-compensated ROESY cross-peak intensities. The intensity

$$R = I_{\text{NOE}}(\text{NH}_i\text{--C}^\alpha\text{H}_{i-1})/I_{\text{NOE}}(\text{NH}_i\text{--C}^\beta\text{H}_i) \quad (1)$$

ratios were compared with those calculated from the structures obtained by using the molecular mechanical force-

field,^[14] literature data on torsions of accessible periodic conformations, and H-bond restraints. We applied the isolated spin-pair approximation for the calculation.^[15] The R value is unique for each possible secondary structure; it is in the proximity of the theoretical maximum for the six-strand conformation (Figure 2). The experimental values ($R=5.6$, 5.9, 5.2, and 6.2 for $i=2, 3, 4$, and 5, respectively) clearly point to the predominance of the six-strand conformation.

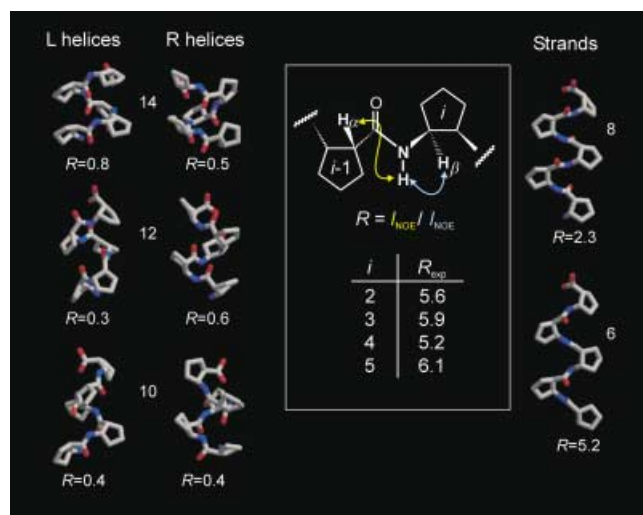


Figure 2. Calculated R values for the theoretically possible periodic conformations and the experimental values. The estimated value of R for a random-coil conformation is 2.1. See the text for the details of the calculations.

We carried out a standard restrained simulated annealing conformational search protocol by using the internuclear distances estimated from the 2D ROESY spectrum and selective ROESY experiments to reinforce our results. Each final low-energy structure displayed a mean restraint violation of less than 0.03 Å and a highest restraint violation of 0.06 Å (Figure 3). The conformational family obtained has a strand-like structure stabilized by the intra-residue electrostatic attraction between amide oxygen atoms and protons, which may form weak H-bonds in an appropriate solvent and lead to six-membered H-bonded rings. Within experimental error, the measured coupling constants $^3J(\text{NH}_i\text{--C}^\beta\text{H}_i)$ are uniformly 8.2 Hz, which likewise points to the presence of a periodically ordered structure and supports the result of the conformation selection protocol applied.

The test for the possible weak H-bonds stabilizing the strand was carried out by using infrared spectroscopy in the intramolecular H-bond-promoting solvent CH_2Cl_2 (Figure 4) and in the H-bond-breaking solvent DMSO at a concentration of 2 mg mL^{-1} . The strongest band in the amide I region of the FTIR spectra of *cis*-ACPC oligomers **3–5** in CH_2Cl_2 appears at about 1645 cm^{-1} . In CH_2Cl_2 , this band is missing only from the spectrum of trimer **3**; instead, a shoulder is seen at about 1653 cm^{-1} . In DMSO, the band at approximately 1645 cm^{-1} in the spectrum of pentamer **4** is replaced by a shoulder at about 1653 cm^{-1} , and in the spectrum of heptamer **5** it appears with a decreased relative intensity. The strongest band occurs at approximately 1670 cm^{-1} in the FTIR spectra of the pentamer

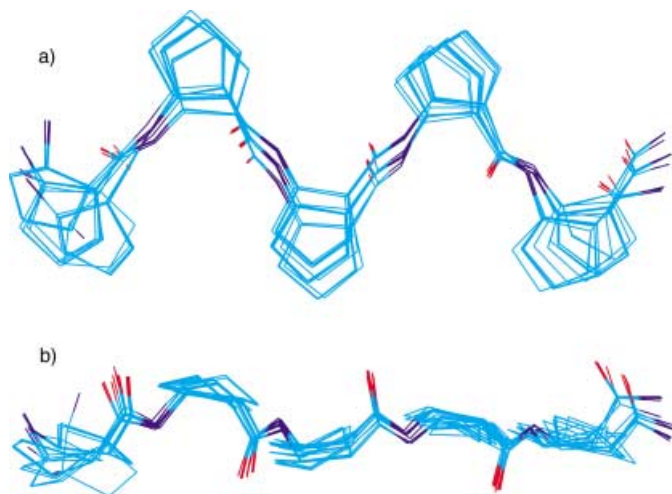


Figure 3. Superposition of the 30 lowest energy structures of pentamer **4** determined by NMR distance restraints and molecular mechanics: a) top view, b) side view. Molecular mechanics simulations were carried out on a SGI Origin 2000 using Accelrys' CDiscover in an Insight 2000 environment. For the energy calculations, the CVFF force field was used, with a 12-Å cutoff for van der Waals and Coulomb interactions. Before the restrained simulated annealing (RSA), a random structure set of 100 molecules was generated by saving the conformations during a 100-ps dynamics simulation at 1000 K in every 1000 steps. The RSA was performed for each structure with an exponential temperature profile in 75 steps and a total duration of 25 ps. Minimization was applied after every RSA in a cascade manner.

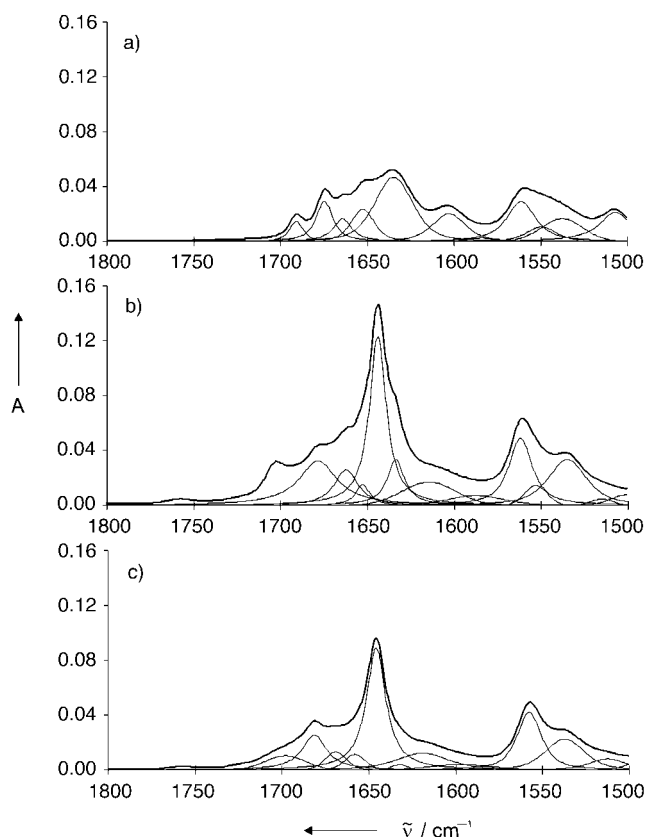


Figure 4. Comparison of the IR spectra of *cis*-ACPC oligomers recorded in CH_2Cl_2 : a) trimer, b) pentamer, c) heptamer. The thick curve represents the measured data, while the thin curves show the component bands obtained from nonlinear fitting.

and heptamer in DMSO. Shoulders or weak bands are present near or above 1680 cm^{-1} in the spectra measured in CH_2Cl_2 or DMSO. DMSO is a solvent which is well known to destroy weak intramolecular H-bonds. On the basis of its absence or decreased intensity in the FTIR spectra measured in DMSO, the band at about 1645 cm^{-1} can be assigned to repeats of C_6 intramolecular H-bonds. The high-frequency ($>1660\text{ cm}^{-1}$) component bands in the curve-fitted spectra, which in many cases appear as shoulders in the experimental spectra obtained in CH_2Cl_2 or DMSO, belong to amide CO groups not involved as acceptors in H-bonding. In DMSO, the NH part of these amides probably forms a H-bond with the acceptor SO group of DMSO. Bands (or component bands) near or below 1635 cm^{-1} are indicative of the presence of strong intermolecular H-bonds. In agreement with this assignment, the band at 1635 cm^{-1} is most intense in the opaque solution of trimer **3** in CH_2Cl_2 , and it is replaced only by a weak shoulder (at 1637 cm^{-1}) in DMSO, which is known to dissolve aggregates.

Our conclusion from the IR measurements is that pentamer **4** and heptamer **5** display the same characteristic features in regard to the H-bonding pattern, which reinforces the results obtained from the comparison of the ROESY cross-peak patterns. The component bands with decreased relative intensity for the heptamer point to a more stable strand conformation; however, we expected the same spectral features to be present in the CD spectra of both compounds. The CD spectra were recorded both in methanol and in trifluoroethanol (TFE) at a concentration of 1 mM. The curves obtained in methanol and normalized for the residue concentration (Figure 5) point to the same spectroscopic behavior of both compounds, except for the higher intensity of the

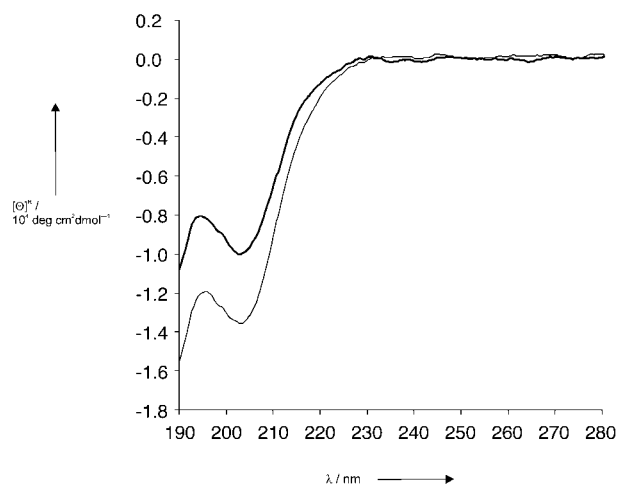


Figure 5. Circular dichroism spectra of **4** and **5** in methanol. The thick curve corresponds to the pentamer and the thin curve to the heptamer. The mean residue ellipticity is displayed.

negative band at 203 nm in the spectrum of the heptamer. The IR results obtained in TFE exhibit the same characteristics as those obtained in methanol. These observations suggest stabilization of the strand conformation in the heptamer as a result of the longer chain and the smaller relative contribution of the less-fixed terminal residues.

The results of CD spectroscopy accord well with the amide proton/deuterium exchange NMR measurements performed in $[D_4]$ methanol, where **4** lost its amide signal intensity in 15 minutes, while the amide protons of **5** survived for more than 1 hour. The lower exchange rate for heptamer **5**, together with the IR and CD results, supports the view that the folding occurs in a cooperative manner as the chain length increases.

Herein we have shown that the 1*R*,2*S*-ACPC pentamer and heptamer adopt a self-stabilizing six-strand secondary structure in the solution phase. From earlier results, we know that the model system composed of (1*R*,2*R*)-*trans*-ACPC monomers adopts the highly stable 12-helix conformation^[16] (Figure 6). The change in the relative configuration of the ACPC residues in these models provides an efficient control over the

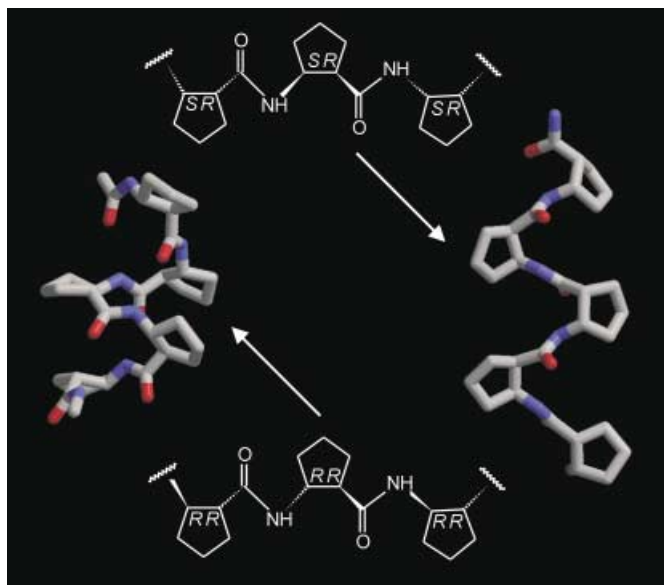


Figure 6. Comparison of the secondary structures induced by the different relative configurations of the ACPC residues.

secondary structures of the helix and strand. The application of the 2-aminocycloalkanecarboxylic acids as a general backbone in the controlled design of β -peptide foldamers is supported by the relatively easy synthetic availability of this family of compounds.^[17] This type of chirality-based selection between regular secondary structures may become a general tool in the design of β -peptide foldamers and may facilitate the construction of stable tertiary structures.

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Chiral Transmission between Amino Acids: Chirally Selective Amino Acid Substitution in the Serine Octamer as a Possible Step in Homochirogenesis**

Kim J. Koch, Fabio C. Gozzo, Sergio C. Nanita, Z. Takats, Marcos N. Eberlin, and R. Graham Cooks*

Pasteur's discovery^[1] that tartaric acid can occur in two enantiomeric forms raised still unanswered questions^[2–6] concerning the origin of chirality in biological systems (homochirality). Homochirogenesis, the set of events leading to the almost exclusive preference for one enantiomeric form over the other in the suite of biological compounds that make up living organisms, can be considered to have involved three steps: 1) symmetry breaking, 2) chiral enrichment, and 3) chiral transmission.

[*] Prof. R. G. Cooks, K. J. Koch, S. C. Nanita, Z. Takats
 Purdue University
 Department of Chemistry
 1393 Brown Laboratory
 West Lafayette, IN 47907 (USA)
 Fax: (+1) 765-494-9421
 E-mail: cooks@purdue.edu
 F. C. Gozzo, Prof. M. N. Eberlin
 Institute of Chemistry
 State University of Campinas
 CP6154 Campinas (Brazil)

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