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Turn and Helical Peptide Handedness Governed Exclusively by Side-Chain Chiral Centers

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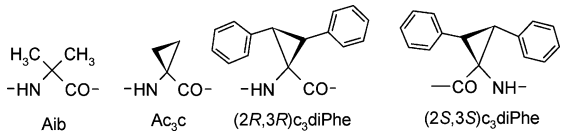
All protein α-amino acids, except Gly, are characterized by an asymmetric α-carbon. There is ample evidence in the literature that the configuration at the amino acid α-carbon dictates the preferred handedness of the turns and helices that are formed.[1] In most cases the L configuration preferentially induces right-handed folded structures. Two protein amino acids, Ile and Thr, bear an additional chiral center (at the β-carbon). To our knowledge, no significant results have been published to date on the conformational effect of Ile (or Thr) side-chain chirality.

We describe here the first step in our research on the influence of amino acid side-chain configuration on the preferred conformation of peptide molecules. In the past few years two independent syntheses[2] of the non-natural α-amino acid 1-amino-*c*-2,*t*-3-diphenylcyclopropane-*r*-1-carboxylic acid have been reported. This amino acid (abbreviated as $c_3$diPhe) bears two phenyl β-substituents in a trans relative disposition. It lacks an asymmetric center in the backbone, but it exhibits well-determined chiralities at the two β-carbon atoms, with two enantiomeric forms (2*R*,3*R*) and (2*S*,3*S*) being possible. Experimental[3a] and theoretical[3a,b] conformational analyses on diamide derivatives of this stereochemically unusual amino acid have been performed. Some of us have also investigated[3c] the behavior of Pro-$c_3$diPhe dipeptides. The former $c_3$diPhe derivatives are too short to form any commonly found hydrogen-bonded folded structure, that is, β-turn[4] or 3_{10}- (or α-) helix[5] conformation, whereas in the latter peptides $c_3$diPhe is combined with Pro, which is known to possess by itself a strong conformational bias.[6] In any case, these studies evidenced that $c_3$diPhe may easily accommodate into folded conformations (albeit with some distortion).

These results were not surprising in view of the following considerations: (i) $c_3$diPhe is a member of the class of the C^{α}-tetrasubstituted α-aminoisobutyric acid (Aib) residue, which is known to be a strong turn and helix promoter[7] and (ii) $c_3$diPhe is a side-chain β,β'-disubstituted derivative of 1-aminocyclopropanecarboxylic acid (Ac_3c), known to prefer the *bridge* region of the ϕ, ψ space,[7c,8] which is close to that where the turn- and helix-forming amino acid residues are found.[9]

In the present work, we decided to take advantage of $c_3$diPhe to investigate the relationship, if any, between α-amino acid side-chain chirality and the screw sense of its turn and helical conformations in the absence of any potentially overlapping influence that might arise from the asymmetric α-carbon.

To this end, we synthesized a series of terminally protected (2*R*,3*R*)$c_3$diPhe homopeptides to the tetramer level, free of any bias from other chiral amino acids, and long enough to fold into multiple β-turn conformations and even into short 3_{10}- or α-helices. Starting from Boc-(2*R*,3*R*)$c_3$diPhe-OH,[2b] we prepared the sterically demanding homodimer, trimer, and tetramer amides Boc-[(2*R*,3*R*)$c_3$diPhe]$_n$−NH*i*Pr (n = 2−4; Boc, *tert*-butyloxycarbonyl; *i*Pr, isopropyl) in 61−84% yield by activating the amino acid carboxyl function with HOAt (7-aza-1-hydroxy-1,2,3-benzotriazole)/HATU (HOAt uronium salt derivative)[10] in dry methylene chloride in the presence of *N*,*N*-diisopropylethylamine (for details of peptide synthesis and characterization see the Supporting Information).



We were able to grow a single crystal suitable for X-ray diffraction analysis from Boc-[(2*R*,3*R*)$c_3$diPhe]$_2$−NH*i*Pr (Supporting Information). Figure 1 shows one of the three independent molecules (molecule **A**) in the asymmetric unit of the homodipeptide, the conformations of the other two molecules (**B** and **C**) being distinct from but quite close to that of molecule **A**. All molecules are folded in a right-handed, slightly distorted, helical type-III β-turn conformation. The turn handedness is uniquely assessed by X-ray diffraction analysis since the peptide has asymmetric centers the chirality of which is known. The ranges of the backbone ϕ, ψ torsion angles[11] are very narrow: ϕ_1 −72.0(6)° ÷ −67.1(6)°, ψ_1 −22.9(7)° ÷ −17.6(7)°, ϕ_2 −53.4 (7)° ÷ −50.4(7)°, ψ_2 −41.2-(6)° ÷ −31.9(7)°. Theoretical ϕ, ψ values for a regular, right-handed type-III β-turn are −60°, −30°,[4] and experimental average values for a right-handed 3_{10}-helix are −57°, −30°.[5a] The folded conformation of the homodipeptide is stabilized by an intramolecular $C_0{=}O_0\cdots H{-}N_T$ H-bond. In the three molecules, the N···O distances are in the range 2.987(6)−3.004(6) Å.[12] In each $c_3$diPhe residue there are two types of average values for the side-chain χ^1 torsion angles: $\chi^{1'}$ 137.0(5)° ± 5.8°, toward the carbonyl, and $\chi^{1''}$ −6.0(8)° ± 4.7°, toward the nitrogen. Not surprisingly, they are quite different from that most frequently reported for Phe in peptides and proteins (g^- or −60°).[13] As that found for the Ac_3c residues in peptides,[7c,8] the average value for the conformationally sensitive exocyclic τ (N−C^{α}−C′) bond angle of each $c_3$diPhe residue is very large, 116.1(4)° ± 1.4°, for a regular tetrahedral value (109.5°).

We also performed NMR analyses (Supporting Information) on the Boc-[(2*R*,3*R*)$c_3$diPhe]$_{2,4}$−NH*i*Pr homopeptides. In particular, the data on the longest and structurally more significant oligomer (the tetramer) indicates that it adopts a right-handed 3_{10}-helical conformation. Its ROESY ^{1}H NMR spectrum shows a complete set of $d_{NN}(i,i+1)$ NOE cross-peaks indicative of a helical structure. The presence of an NOE between a C^{β}H proton of residue 1 and the NH proton of residue 4 (Figure 2A), together with the observation that the chemical shifts of N_3H, N_4H, and N_TH protons are strongly solvent-shielded (while N_1H and N_2H protons are more

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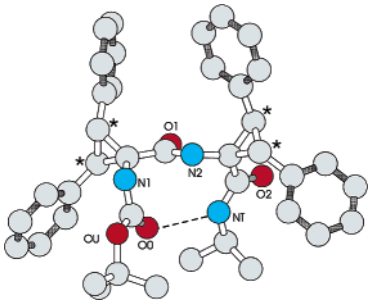

Figure 1. X-ray diffraction structure of molecule **A** in the asymmetric unit of Boc-[(2*R*,3*R*)$c_3$diPhe]$_2$−NH*i*Pr with heteroatoms colored and numbered. The intramolecular C═O···H−N hydrogen bond is represented by a dashed line. The structures of the two other independent molecules **B** and **C** (deposited) are not shown as they are very close to that of molecule **A**. Side-chain chiral carbons are starred.

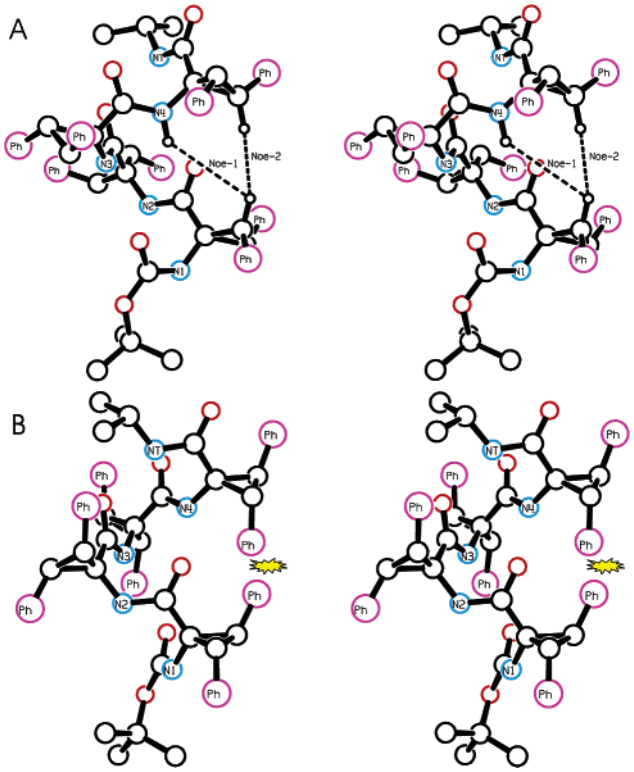

Figure 2. (A) Stereomodel of the right-handed 3_{10}-helix (ref 5) of Boc-[(2*R*,3*R*)$c_3$diPhe]$_4$−NH*i*Pr highlighting the $C_1{}^{\beta}H\cdots N_4H$ cross-peak (NOE-I) indicative of the helical structure and the $C_1{}^{\beta}H\cdots C_4{}^{\beta}H$ cross-peak (NOE-II) supporting the right-handed screw sense. (B) Stereomodel of the left-handed 3_{10}-helix (ref 5) of the same tetrapeptide highlighting the unfavorable contact between a phenyl group of residue 1 and a phenyl group of residue 4. The nitrogen atoms are light blue and numbered, while the oxygen atoms are red.

solvent exposed), as apparent from a temperature study, indicates the most populated helix is the 3_{10}-type. The right-handedness of the helix was deduced from the observation of an NOE cross-peak between the same $C^{\beta}H$ proton of residue 1 and a $C^{\beta}H$ proton of residue 4 (Figure 2A). In the left-handed 3_{10}-helical conformation (Figure 2B), steric repulsion between two side-chain phenyl groups disfavors a close contact between these two $C^{\beta}H$ protons.

Collectively, our X-ray diffraction and NMR data show that the ϕ, ψ angles of the (2*R*,3*R*)$c_3$diPhe homooligomers are confined to negative values (right-handedness). Interestingly, in previous computational analyses of simple (2*S*,3*S*)$c_3$diPhe diamides,[3a,b] similar results, namely energy minima corresponding to the left-handedness, were found.

In summary, we have reported unambiguous proofs that the screw sense of peptide turns and helices may be dictated not only by the amino acid asymmetric α-carbons but by the topological (chirality) properties[14] of their side-chain β-carbons as well. The peptides based on $c_3$diPhe studied in this work are related to those formed by the binaphthyl α-amino acid (Bin) previously described by some of us,[15] in the sense that both residues lack an asymmetric center in the main chain. However, $c_3$diPhe bears chiral carbons in its side chains, while Bin, devoid of any side-chain chiral carbon, is overall dissymmetric (axially chiral). Our next step in this research on the role of side-chain chiral centers in 3D structure will be a comparison between peptides based on diastereomeric L-Ile versus L-alloIle residues.

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Supporting Information Available: Experimental details on the synthesis, characterization, and NMR data of the three homooligomers, and X-ray diffraction data of Boc-[(2*R*,3*R*)$c_3$diPhe]$_2$−NH*i*Pr (CCDC 254024) (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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