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Cyclopropane analogue of valine: influence of side chain orientation on peptide folding

Ana I. Jiménez,[a,*] Michel Marraud[b] and Carlos Cativiela[a]

[a]*Department of Organic Chemistry, ICMA, University of Zaragoza-CSIC, 50009 Zaragoza, Spain*
[b]*Laboratory of Macromolecular Physical Chemistry, UMR CNRS-INPL 7568, ENSIC, BP 451, 54001 Nancy, France*

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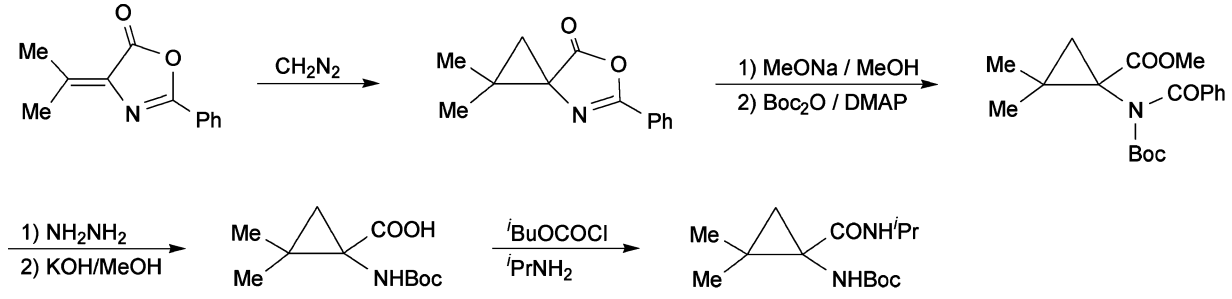
Abstract—The cyclopropane analogue of valine (1-amino-2,2-dimethylcyclopropanecarboxylic acid, c_3Val) has been synthesised and incorporated into the model peptides ^{t}BuCO-L-Pro-L-c_3Val-NH^{i}Pr and ^{t}BuCO-L-Pro-D-c_3Val-NH^{i}Pr. In the solid state, both dipeptides accommodate a type II β-turn stabilised by an NH^{i}Pr to ^{t}BuCO hydrogen bond. Remarkably, the peptide incorporating L-c_3Val also exhibits a distorted γ-turn around the cyclopropane residue, with Pro-CO and NH^{i}Pr intramolecularly hydrogen-bonded.

The introduction of conformational constraints constitutes a widely used approach in the search of peptide analogues with more favourable pharmacological properties. In this context, extensive efforts have been devoted to the design of peptides with well-defined backbone conformations,[1–3] while the geometry of the side chain moieties has received much less attention. Side chains are, however, directly involved in the molecular recognition processes and their three-dimensional arrangement is essential for adequate peptide–receptor interaction.[4] Moreover, the conformation of the backbone may be modulated, to a certain extent, by interactions with the side chains either of steric or electronic nature.[5,6] The dependence of the main chain conformation on the side chain disposition becomes evident from the statistical analysis of protein crystalline structures.[6]

Cyclopropane α-amino acids are a unique class of side chain constrained residues. The orientation of the substituents is fixed by the stereochemistry of the rigid three-membered ring (χ^1 can adopt values only in the 0° and ±140° regions), and this offers the possibility to evaluate accurately the influence of the side chain orientation on the conformation adopted by the peptide backbone. The great potential of the cyclopropane system for this kind of structural studies has actually been evidenced in model[7–9] as well as in biologically active[10] peptides that incorporate cyclopropane analogues of phenylalanine. However, very little work has been



Scheme 1. Synthesis of the cyclopropane analogue of valine (c_3Val). Abbreviations: Boc, *tert*-butyloxycarbonyl; DMAP, 4-(dimethylamino)pyridine.

Keywords: 2,3-methanovaline; constrained amino acid; cyclopropane amino acid; X-ray diffraction; crystal structure; peptide conformation; peptide structure; peptide turn; beta-turn; gamma-turn.

* Corresponding author. Fax: (34) 976761210; e-mail: anisjim@posta.unizar.es

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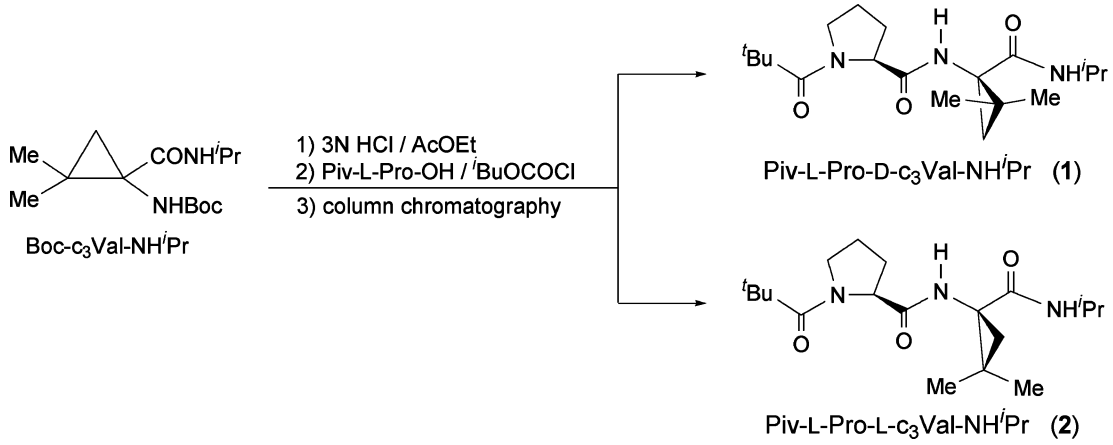
carried out with cyclopropane derivatives of other protein-ogenic amino acids[11,12] (obviously, the unsubstituted 1-aminocyclopropanecarboxylic acid, Ac_3c, analogue of alanine, has no application in this field). We report here the synthesis and structural study in the crystalline state of the model dipeptides Piv-L-Pro-L-c_3Val-NH^{i}Pr and Piv-L-Pro-D-c_3Val-NH^{i}Pr, incorporating the cyclopropane analogue of L- and D-valine, respectively.

Racemic c_3Val was prepared starting from 4-isopropylidene-2-phenyl-5(4*H*)-oxazolone[13] (Scheme 1). Its exocyclic double bond was reacted with diazomethane to afford the corresponding spirocyclopropane derivative. Methanolysis of the oxazolone ring followed by amide/urethane exchange[14] and saponification of the methyl ester group provided the *N*-Boc-protected amino acid in high overall yield. Condensation with isopropylamine was then achieved via formation of the intermediate isobutyl chloroformate mixed anhydride.[15]

After Boc removal by acidolysis (Scheme 2), the resulting amino terminus was coupled to Piv-L-Pro-OH (*N*-pivaloyl-L-proline) by the mixed anhydride method.[15] The diastereomeric dipeptides formed were separated by column chromatography on silica gel and isolated in optically pure form. Both **1**† and **2**‡ yielded single crystals, which were subjected to X-ray diffraction analysis.§ Taking L-proline as a reference, this allowed us to establish the absolute configuration of the c_3Val residue as *R* (or D) in **1** and *S* (or L) in **2**.

Model dipeptides RCO-Xaa-Ybb-NHR′ constitute the minimum sequence able to adopt the β-turn structure.[16] When position *i*+1 is occupied by L-proline (Xaa = L-



Scheme 2. Synthesis of the diastereoisomeric dipeptides **1** and **2**. Abbreviations: Boc, *tert*-butyloxycarbonyl; c_3Val, 1-amino-2,2-dimethylcyclopropanecarboxylic acid; Piv, pivaloyl (*tert*-butylcarbonyl); Pro, proline.

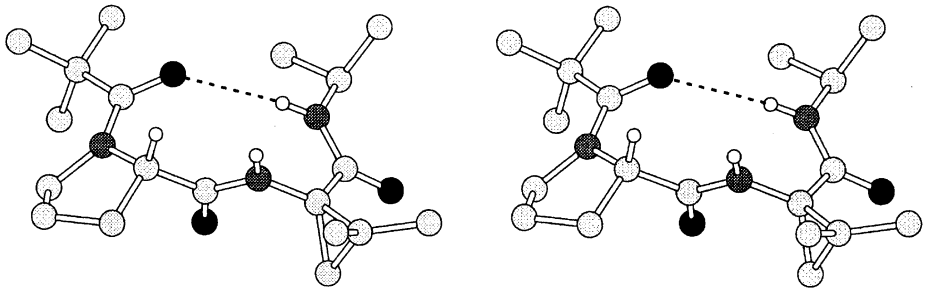


Figure 1. Stereoview of the crystal molecular structure of Piv-L-Pro-D-c_3Val-NH^{i}Pr (**1**) showing a βII-turn conformation (torsion angles: Pro-$\phi,\psi = -61°$, 143°; c_3Val-$\phi,\psi = 75°$, 9°). The intramolecular hydrogen bond is represented as a dashed line. Most hydrogen atoms are omitted for clarity.

† Single crystals of **1** were grown by slow evaporation from a dichloromethane/diisopropyl ether solution. Orthorhombic, $P2_12_12$; $a = 15.9654(12)$, $b = 20.9507(16)$, $c = 5.9824(5)$ Å; $Z = 4$; $D_{calcd} = 1.167$ g cm^{-3}; 12218 reflections collected, 4584 unique ($R_{int} = 0.034$); final *R* indices [3994 observed reflections, $I > 2\sigma(I)$]: $R_1 = 0.038$, $wR_2 = 0.071$; final *R* indices (all data): $R_1 = 0.044$, $wR_2 = 0.073$.

‡ Single crystals of **2** were grown by slow evaporation from a dichloromethane/diisopropyl ether solution. Orthorhombic, $P2_12_12_1$; $a = 9.0027(7)$, $b = 12.3632(9)$, $c = 18.7698(14)$ Å; $Z = 4$; $D_{calcd} = 1.118$ g cm^{-3}; 11567 reflections collected, 3693 unique ($R_{int} = 0.039$); final *R* indices [2729 observed reflections, $I > 2\sigma(I)$]: $R_1 = 0.043$, $wR_2 = 0.089$; final *R* indices (all data): $R_1 = 0.059$, $wR_2 = 0.095$.

§ Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-201547 (**1**) and CCDC-201548 (**2**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

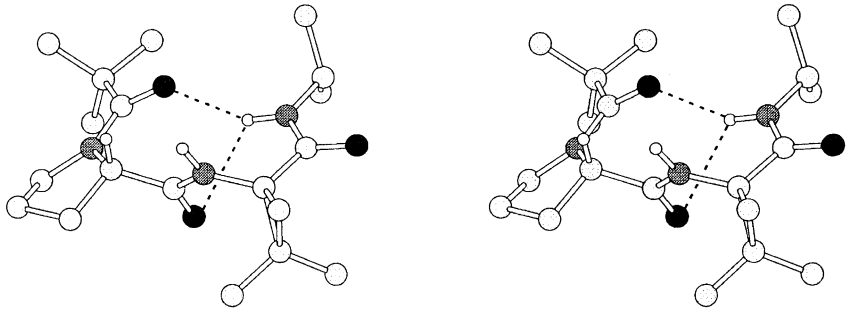


Figure 2. Stereoview of the crystal molecular structure of Piv-L-Pro-L-c_3Val-NH^{i}Pr (**2**) showing a βII-turn and a distorted γ-turn (torsion angles: Pro-$\phi,\psi = -57°, 141°$; c_3Val-$\phi,\psi = 82°, -19°$). The intramolecular hydrogen bonds are represented as dashed lines. Most hydrogen atoms are omitted for clarity.

Pro) β-turns of types I or II are accommodated depending both on the nature of the *i*+2 residue and on the environment.[16–18] In the solid state, the βII-turn is largely preferred, and type I β-turns are adopted almost exclusively when Ybb is an L-residue with a highly polar side chain.

Figures 1 and 2 show the crystalline structures of **1** and **2**, respectively. As expected, both molecules adopt a β-folded conformation, with the *anti* disposition of the proline C=O and C^{α}–H bonds corresponding to the type II β-turn.[16] In both cases, the folded structure is stabilised by an intramolecular hydrogen bond between the Piv-*C′O* and *NH*(^{i}Pr) terminal groups (dipeptide **1**: N···O distance = 3.26 Å, N–H···O angle = 158°; dipeptide **2**: N···O distance = 3.07 Å, N–H···O angle = 153°), that closes a ten-membered cycle.

In addition to the classical βII-turn arrangement, a remarkable structural feature is observed in Figure 2. The *NH*(^{i}Pr) group in dipeptide **2** is not only involved in the $i+3 \rightarrow i$ hydrogen bond typical of the β-turn structure, but is also intramolecularly hydrogen-bonded to the proline carbonyl (N···O distance = 3.25 Å, N–H···O angle = 126°). The latter interaction closes a seven-membered ring, stabilising a γ-turn[16,19] around the L-c_3Val residue. This result is noteworthy since the γ-turn (also called C7 structure) is very infrequent in crystalline small linear peptides.[2,16,18,20,21] Moreover, this finding confirms that the γ-turn conformation observed in the crystalline structure of Piv-L-Pro-(2*R*,3*R*)$c_3$diPhe-NH^{i}Pr[9] ($c_3$diPhe stands for 1-amino-2,3-diphenylcyclopropanecarboxylic acid) was not a consequence of the crystal packing forces but an intrinsic characteristic of this phenylalanine cyclopropane analogue.

The ψ angle assumed by the L-c_3Val residue in **2** ($\psi = -19°$) deviates significantly from the value expected for an ideal γ-turn ($\psi \approx -70°$).[16,19] However, it is almost identical to that adopted by (2*R*,3*R*)$c_3$diPhe in the dipeptide sequence aforementioned ($\psi = -20°$)[9] and also shows an excellent agreement with the small ψ value predicted theoretically for the C7 minimum energy conformation of cyclopropane residues ($\psi \approx -30°$)[22–24] and that has been ascribed to hyperconjugative effects between the three-membered ring and the adjacent C=O bond. It should be noted that L-c_3Val and (2*R*,3*R*)$c_3$diPhe share a substituent situated in an L position and *cis* with respect to the carbonyl function. The close proximity of this substituent (a methyl and a phenyl group, respectively) to the cyclopropane carbonyl oxygen is responsible for the deviation of the ψ torsion angle to negative values, thus allowing the formation of the hydrogen bond that stabilises the C7 conformation around the cyclopropane residue.

The present results constitute an experimental probe of the influence that the side chain orientation can exert on the conformation accommodated by the peptide backbone, and evidences the potential value of cyclopropane amino acids as γ-turn inductors.

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