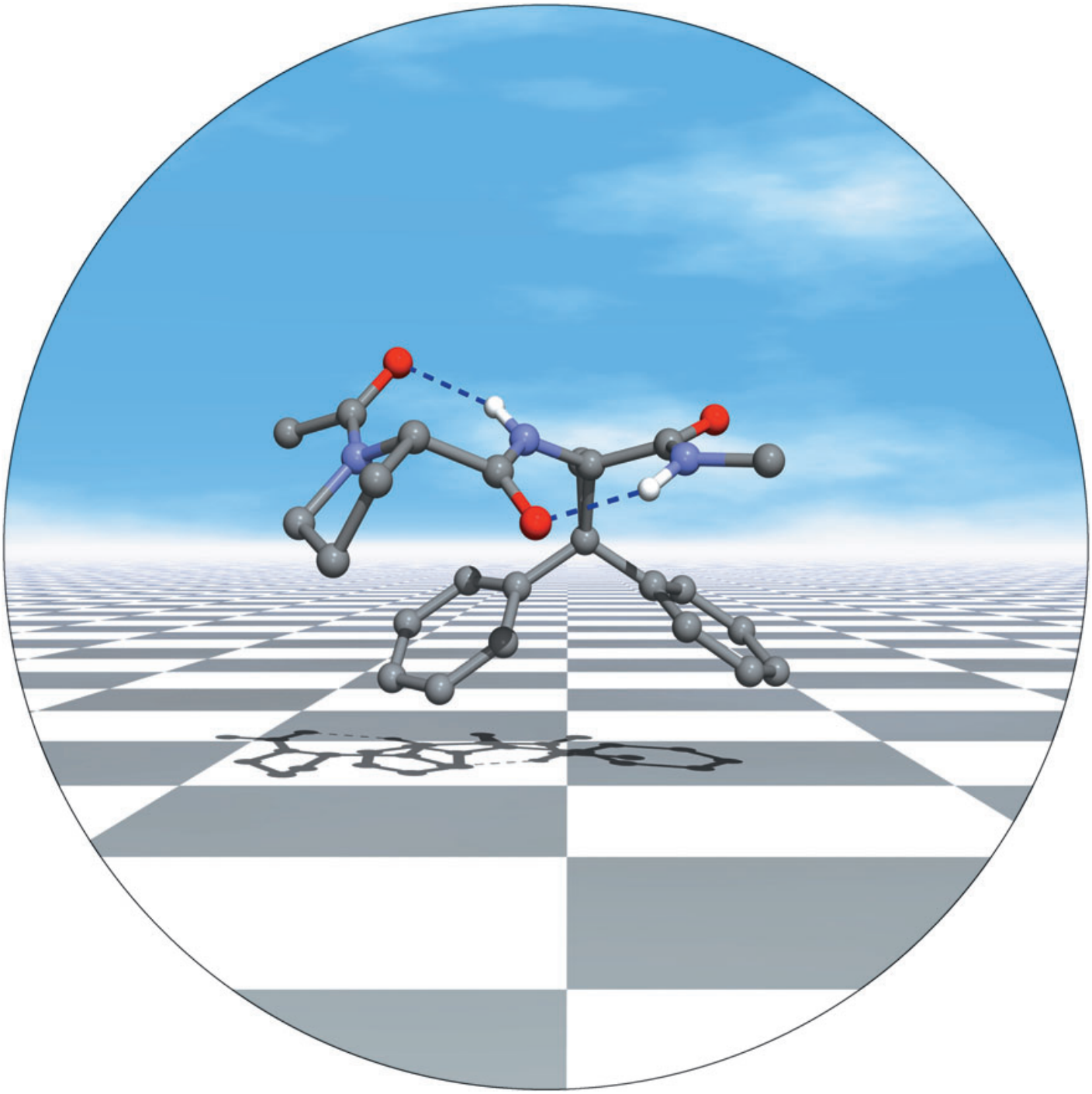
Zuschriften



Das Dipeptid Ac-L-Pro-D-c_3Dip-NHME, das eine Cyclopropanaminosäure enthält, nimmt eine Konformation mit zwei aufeinander folgenden, durch intramolekulare H-Brücken stabilisierten γ-Turns an. Details zu dieser Konformation, die in solchen Peptiden noch nicht beobachtet worden war, sind der Zuschrift von A. I. Jiménez et al. auf den folgenden Seiten zu entnehmen.

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Peptide Structures

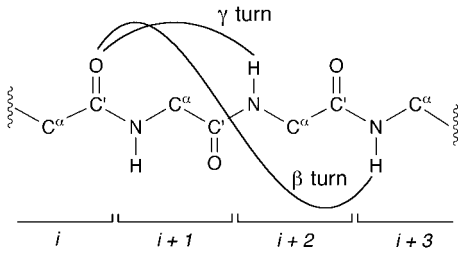
First Observation of Two Consecutive γ Turns in a Crystalline Linear Dipeptide**

Ana I. Jiménez, Gema Ballano, and Carlos Cativiela*

Dedicated to Dr. Michel Marraud

Reverse turns (or bends) are elements of primary importance both in the structure and function of peptides and proteins.[1] They give rise to a sharp reversal in the polypeptide chain, thus conferring on proteins their globular character. Turns have been suggested to play a role in the initial stages of protein folding and to be propitious sites for molecular recognition and receptor binding. Many naturally occurring oligopeptides have been proposed to adopt turns in their bioactive conformation.[1,2]

Different types of bends have been recognized according to the number and spatial arrangement of the residues involved. The most common is the β turn,[1,3] which involves four consecutive amino acids (*i* to *i*+3, Figure 1) and is generally stabilized by an intramolecular hydrogen bond between the CO group at position *i* and the NH group at *i*+3. Several subclasses of β turns are further distinguished on the basis of the backbone dihedral angles (φ,ψ) associated with the central *i*+1 and *i*+2 positions. Types I and II (Figure 1) are the most widely distributed and differ essentially in the orientation of the middle peptide group. The other main category of chain reversal is the so-called γ turn,[1,4] which is tighter and less common than the β bend. The γ turn is centered at a single residue (*i*+1, Figure 1), usually with a hydrogen bond between the CO and NH groups at positions *i* and *i*+2, respectively.

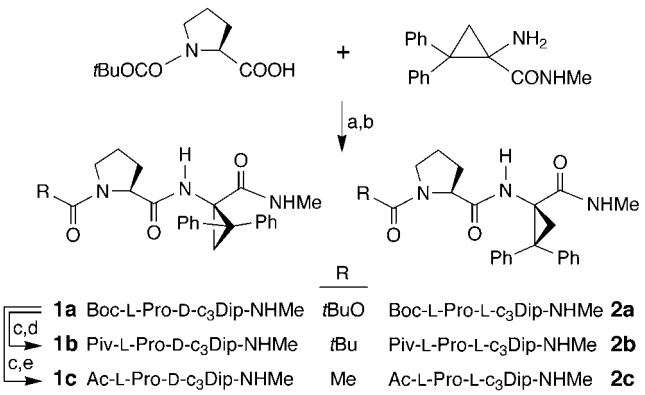


	ϕ	ψ	ϕ	ψ
βI turn	–60	–30	–90	0
βII turn	–60	120	80	0
γ turn	–75	60		

Figure 1. Schematic representation of the intramolecular hydrogen bond that stabilizes the β turn (*i*+3→*i*) and the γ turn (*i*+2→*i*) in a peptide chain. The ideal torsion angles of the central residues for the γ turn and the most common types of β turn are indicated.

We are involved in a research project aimed at evaluating the relative stability of the βI and βII turns in model peptides RCO-L-Pro-Xaa-NHR′ that incorporate different constrained α-amino acids at the *i*+2 position (Xaa).[5] Such terminally protected dipeptides constitute the smallest systems able to accommodate the β-turn conformation. As part of these investigations we undertook the study of the L-Pro-c_3Dip sequence, where c_3Dip denotes each enantiomer of α,β-methanodiphenylalanine,[6] a cyclopropane analogue of phenylalanine. The results of the solid-state conformational analysis, of high structural relevance, are reported herein.

Racemic *N*′-methyl-2,2-diphenyl-1-aminocyclopropanecarboxamide[7] (H-c_3Dip-NHMe) was coupled to *N*-*tert*-butoxycarbonyl-L-proline by the mixed-anhydride method (Scheme 1). Column chromatography allowed the separation of the resulting diastereomeric dipeptides (**1a**, **2a**), which were isolated in optically pure form. The difficulties encountered in growing single crystals from these compounds prompted us to replace the N-terminal urethane protecting group with different amide groups. Single crystals suitable for X-ray diffraction analysis were finally obtained from the pivaloyl derivative **2b** and the acetyl derivative **1c**.



Scheme 1. Synthesis of L-Pro-c_3Dip dipeptides. Reagents and conditions: a) *i*BuOCOCl, NMM, THF, −15 °C; b) column chromatography (CH_2Cl_2/*i*PrOH 93/7); c) 3 N HCl/EtOAc, RT; d) *t*BuCOCl, NMM, $CHCl_3$, 0 °C; e) Ac_2O, NMM, $CHCl_3$, RT. NMM = *N*-methylmorpholine.

The crystalline structure of dipeptide **2b** (Figure 2) revealed an L (or *S*) configuration for the c_3Dip residue. The molecule adopts a β-turn conformation with the terminal pivaloyl CO and methylamide NH groups intramolecularly hydrogen bonded (N···O: 2.97 Å; N–H···O: 156°). The (φ,ψ) torsion angles of the L-Pro (−63,133) and L-c_3Dip (69,−2) residues correspond to a β bend of type II (Figure 1). It should be noted that the βI turn is energetically more favorable for L-Pro-L-Xaa dipeptides in low-polarity solvents[5a,8] but, in general, this conformation is not retained in

[*] Dr. A. I. Jiménez, G. Ballano, Dr. C. Cativiela
Departamento de Química Orgánica, ICMA
Universidad de Zaragoza-CSIC
50009 Zaragoza (Spain)
Fax: (+34) 976-761-210
E-mail: anisjim@unizar.es

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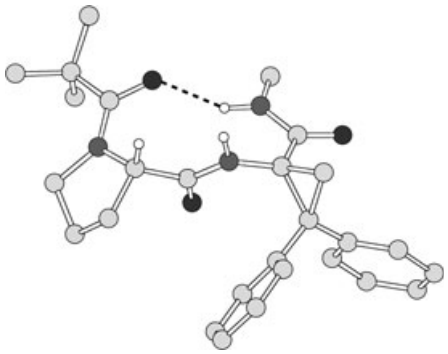

Figure 2. X-ray diffraction structure of Piv-L-Pro-L-c_3Dip-NHMe (**2b**) accommodating a βII turn. The intramolecular $i+3\rightarrow i$ hydrogen bond is indicated by a dashed line. Only the proline C^{α} and the amide hydrogen atoms are shown.

the solid state, where the βII turn becomes preferred because it allows the Xaa NH group to participate in intermolecular contacts.[5a,d,8] The L-c_3Dip NH site in **2b** is actually hydrogen bonded to a carbonyl group of a neighboring molecule. In comparison, RCO-L-Pro-D-Xaa-NHR′ dipeptides are more prone to βII folding under all environmental conditions and have been found to invariably crystallize in the βII form.[5,8]

On the basis of the above discussion, Ac-L-Pro-D-c_3Dip-NHMe (**1c**) was expected to accommodate a βII turn in the solid state. Surprisingly, a conformation consisting of two consecutive γ turns was found (Figure 3). The (φ,ψ) torsion

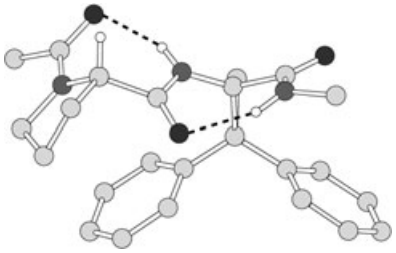

Figure 3. X-ray diffraction structure of Ac-L-Pro-D-c_3Dip-NHMe (**1c**) exhibiting two consecutive γ turns, each stabilized by an intramolecular $i+2\rightarrow i$ hydrogen bond (dashed lines). Only the proline C^{α} and the amide hydrogen atoms are shown.

angles for both L-Pro (−82,61) and D-c_3Dip (−72,47) lie close to the typical values (−75,60),[1] with the larger deviation being observed for the ψ angle of the D-c_3Dip residue. However, the excellent agreement between the observed ψ value and that predicted from theoretical calculations[9] for the γ-turn minimum energy conformation of cyclopropane residues bearing a phenyl substituent *cis* to the CO terminus ($\psi = 47°$) is remarkable, and appears to reflect a balance between steric and hyperconjugative effects. Whatever the origin of this deviation, it does not seem to impede the appropriate alignment of the L-Pro CO and methylamide NH sites, which form a strong intramolecular hydrogen bond (N···O: 2.81 Å, N−H···O: 147°), as do the acetyl CO and D-c_3Dip NH groups (N···O: 2.78 Å, N−H···O: 152°).

The solid-state conformation exhibited by compound **1c** is of extraordinary structural significance. Although much less frequent than β bends, γ turns are found to be abundant in crystallized proteins,[10] where they are stabilized or induced by long-range interactions. Such a source of stabilization is not possible in small peptides and γ turns are observed almost exclusively in poor-solvating media,[1] that is, in the absence of competing intermolecular hydrogen-bonding interactions either with the solvent or with other peptide molecules in the crystal. A few examples of X-ray diffraction structures exhibiting a γ turn have been reported for cyclic oligopeptides—mostly pentapeptides with proline at the turn center—while this conformation is extremely uncommon in acyclic sequences.[1,8b,11] The double γ turn encountered in **1c** is, therefore, unique among crystalline short linear peptides. Moreover, a survey of the Cambridge Structural Database[12] indicates[13] that this is the first report in which proline accommodates a γ-turn conformation in the solid state in the absence of the constraints imposed by backbone cyclization. Proline is, in fact, the proteinogenic amino acid with the highest folding propensity[10b,14] and has been shown to adopt the γ-turn conformation in low-polarity solvents,[1b,c,15] a capacity that is not maintained in the solid state, where proline is overwhelmingly found in the (−60,−30) or (−60,140) region of the (φ,ψ) map.[1b,5,8,10b,14,16]

Accordingly, the γ-turn disposition accommodated by proline in **1c** can be ascribed to the presence of the contiguous D-c_3Dip residue, whose conformational tendencies appear to prevail over those of proline. The latter consideration is of special relevance given that proline is the proteinogenic amino acid with the strongest conformational preferences.[10b,14] Cyclopropane α-amino acids have been suggested as promising candidates for γ-turn stabilization on the basis of theoretical and experimental studies.[5c,d,9,17] However, the fact that theoretical calculations on small peptides generally overestimate the stability of γ-folded conformers,[18] together with the limited experimental evidence available to date, made it premature to proclaim the capacity of such residues to promote γ-turn conformations.

The result reported here constitutes the first unequivocal evidence of the ability of a cyclopropane α-amino acid to adopt a γ-turn disposition in a nonpropitious environment and to influence the overall folding of the peptide chain. Clearly, much work remains to be done to determine the precise conditions under which c_3Dip is able to nucleate γ turns and, thereafter, to establish its scope as a γ-turn inductor. The finding of an α-amino acid with a clear preference for the γ-turn conformation would be of enormous help in the stabilization of this structural motif in bioactive peptides, as well as in the construction of appropriate models to study the factors that govern γ folding.

Experimental Section

2b: m.p. 228 °C (iPr$_2$O/CH$_2$Cl$_2$); $[\alpha]_D^{26} = +56.1$ ($c = 0.42$ in MeOH); IR (Nujol): $\tilde{\nu} = 3377$, 3317, 1691, 1641, 1608 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.18$–1.29 (m, 1H), 1.31 (s, 9H), 1.39–1.52 (m, 1H), 1.63–1.74 (m, 2H), 2.00 (d, $J = 5.6$ Hz, 1H), 2.62 (d, $J = 5.6$ Hz, 1H), 2.78 (d, $J = 4.8$ Hz, 3H), 3.42 (m, 1H), 3.57 (m, 1H), 4.09 (dd, $J = 8.3$, 5.0 Hz, 1H), 6.01 (brs, 1H), 7.16–7.29 (m, 6H), 7.37–7.43 (m, 4H), 7.45 ppm (brq, $J = 4.8$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta =$ 24.71, 25.73, 26.93, 27.10, 27.31, 39.10, 44.05, 45.44, 48.64, 63.20,

126.74, 126.77, 128.12, 128.31, 128.93, 129.38, 140.42, 140.84, 168.94, 172.62, 178.20 ppm; elemental analysis (%) calcd for $C_{27}H_{33}N_3O_3$: C 72.46, H 7.43, N 9.39; found: C 72.61, H 7.37, N 9.47.

1c: m.p. 180 °C (Et_2O); $[\alpha]_D^{21} = -218.3$ ($c = 0.27$ in MeOH); IR (Nujol): $\tilde{\nu} = 3369$, 3347, 1676, 1660, 1629 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 1.51$–1.59 (m, 1H), 1.64–1.80 (m, 3H), 1.76 (s, 3H), 2.21 (m, 1H), 2.56 (d, $J = 5.6$ Hz, 1H), 2.64 (d, $J = 4.8$ Hz, 3H), 2.87 (m, 1H), 3.03 (m, 1H), 4.25 (dd, $J = 7.8$, 1.3, 1H), 7.04–7.18 (m, 6H), 7.34–7.39 (m, 4H), 7.86 (br s, 1H), 7.90 ppm (br q, $J = 4.8$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 22.38$, 23.38, 24.72, 26.55, 26.75, 46.28, 46.31, 48.09, 59.26, 126.67, 126.94, 128.00, 128.27, 129.16, 129.44, 140.22, 140.96, 169.10, 171.21, 173.43 ppm; elemental analysis (%) calcd for $C_{24}H_{27}N_3O_3$: C 71.09, H 6.71, N 10.36; found: C 70.89, H 6.66, N 10.39.

The X-ray crystal structures of **2b** and **1c** were solved by direct methods using SHELXS-97.[19a] Refinement was performed using SHELXL-97[19b] by the full-matrix least-squares technique. Hydrogen atoms were located by calculation, with the exception of the cyclopropane and NH protons, which were found on the E map. CCDC-243527 (**2b**) and -243526 (**1c**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

X-ray data for **2b** ($C_{27}H_{33}N_3O_3 \cdot 3/2\,H_2O$): $M_r = 474.59$, orthorhombic, space group $P2_12_12$, $a = 11.0690(10)$, $b = 23.498(2)$, $c = 10.1750(10)$ Å, $V = 2646.5(4)$ Å^3, $Z = 4$; $\rho_{calcd} = 1.191$ g cm^{-3}, $\mu(Mo_{K\alpha}) = 0.081$ mm^{-1}, $T = 293(2)$ K, $F(000) = 1020$, $2\theta_{max} = 50.1°$; 3449 reflections collected, of which 3241 unique ($R_{int} = 0.0308$). Final R indices (2380 observed reflections, $I > 2\sigma I$): $R_1 = 0.0529$, $wR_2 = 0.1257$; final R indices (all data): $R_1 = 0.0784$, $wR_2 = 0.1409$. Highest residual electron density 0.19 e Å^{-3}.

X-ray data for **1c** ($C_{24}H_{27}N_3O_3$): $M_r = 405.49$, orthorhombic, space group $P2_12_12_1$, $a = 10.4010(2)$, $b = 12.6760(2)$, $c = 15.8660(3)$ Å, $V = 2091.82(7)$ Å^3, $Z = 4$; $\rho_{calcd} = 1.288$ g cm^{-3}, $\mu(Mo_{K\alpha}) = 0.086$ mm^{-1}, $T = 173(2)$ K, $F(000) = 864$, $2\theta_{max} = 53°$; 13835 reflections collected, of which 4276 unique ($R_{int} = 0.0559$). Final R indices (3738 observed reflections, $I > 2\sigma I$): $R_1 = 0.0409$, $wR_2 = 0.0855$; final R indices (all data): $R_1 = 0.0501$, $wR_2 = 0.0892$. Highest residual electron density 0.15 e Å^{-3}.

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[1] a) C. Toniolo, *Crit. Rev. Biochem.* **1980**, *9*, 1; b) G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Protein Chem.* **1985**, *37*, 1; c) E. Vass, M. Hollósi, F. Besson, R. Buchet, *Chem. Rev.* **2003**, *103*, 1917.

[2] A. F. Spatola in *Bioorganic Chemistry: Peptides and Proteins* (Ed.: S. M. Hecht), Oxford University Press, New York, **1998**, pp. 367–394.

[3] C. M. Venkatachalam, *Biopolymers* **1968**, *6*, 1425.

[4] G. Némethy, M. P. Printz, *Macromolecules* **1972**, *5*, 755.

[5] a) A. I. Jiménez, C. Cativiela, A. Aubry, M. Marraud, *J. Am. Chem. Soc.* **1998**, *120*, 9452; b) A. I. Jiménez, C. Cativiela, J. Gómez-Catalán, J. J. Pérez, A. Aubry, M. París, M. Marraud, *J. Am. Chem. Soc.* **2000**, *122*, 5811; c) A. I. Jiménez, C. Cativiela, M. Marraud, *Tetrahedron Lett.* **2000**, *41*, 5353; d) A. I. Jiménez, M. Marraud, C. Cativiela, *Tetrahedron Lett.* **2003**, *44*, 3147.

[6] For a given α-amino acid Xaa, we designate its cyclopropane analogue, obtained by linking the α and β carbon atoms with a methylene group, as c_3Xaa. β,β-Diphenylalanine is a phenylalanine derivative known in the abbreviated form as Dip and, accordingly, we have named its cyclopropane analogue as c_3Dip.

[7] This compound was obtained as a racemate by reaction of diphenyldiazomethane with an α,β-dehydroalanine derivative and subsequent elaboration of the protecting groups. G. Ballano, A. I. Jiménez, C. Cativiela, unpublished results.

[8] a) A. Aubry, M. T. Cung, M. Marraud, *J. Am. Chem. Soc.* **1985**, *107*, 7640; b) M. Marraud, A. Aubry, *Biopolymers* **1996**, *40*, 45.

[9] a) C. Alemán, A. I. Jiménez, C. Cativiela, J. J. Perez, J. Casanovas, *J. Phys. Chem. B* **2002**, *106*, 11849; b) J. Casanovas, A. I. Jiménez, C. Cativiela, J. J. Pérez, C. Alemán, *J. Org. Chem.* **2003**, *68*, 7088.

[10] a) K. Guruprasad, S. Rajkumar, *J. Biosci.* **2000**, *25*, 143; b) P. Chakrabarti, D. Pal, *Prog. Biophys. Mol. Biol.* **2001**, *76*, 1.

[11] a) E. Benedetti, *Biopolymers* **1996**, *40*, 3; b) C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, *Biopolymers* **2001**, *60*, 396.

[12] The CSD covers peptides of up to 24 residues. a) F. H. Allen, *Acta Crystallogr. Sect. B* **2002**, *58*, 380; b) I. J. Bruno, J. C. Cole, P. R. Edington, M. Kessler, C. F. Macrae, P. McCabe, J. Pearson, R. Taylor, *Acta Crystallogr. Sect. B* **2002**, *58*, 389.

[13] The November 2003 version (2 updates) of the CSD was reviewed. The proline -CO-Pro-NH- fragment was searched, considering both L and D configurations and torsion angles within the ranges $(\varphi,\psi) = (-75 \pm 30, 60 \pm 30)$ (or their enantiomeric values). Only proline residues included in cyclic peptides were found to fulfill these requirements (refcodes: CGPGAP10, ICOGAI, PAPGAP, SIKMUU10, WAWQOA, DHCMYD10, HEBLIJ).

[14] M. W. MacArthur, J. M. Thornton, *J. Mol. Biol.* **1991**, *218*, 397.

[15] a) G. Boussard, M. Marraud, A. Aubry, *Biopolymers* **1979**, *18*, 1297; b) V. Madison, K. D. Kopple, *J. Am. Chem. Soc.* **1980**, *102*, 4855; c) G. B. Liang, C. J. Rito, S. H. Gellman, *Biopolymers* **1992**, *32*, 293; d) E. Beausoleil, W. D. Lubell, *J. Am. Chem. Soc.* **1996**, *118*, 12902; e) M. Miyazawa, K. Inouye, T. Hayakawa, Y. Kyogoku, H. Sugeta, *Appl. Spectrosc.* **1996**, *50*, 644.

[16] Note that these regions correspond to the $i+1$ position of a βI or a βII turn, respectively (Figure 1).

[17] a) K. Burgess, K.-K. Ho, B. M. Pettitt, *J. Am. Chem. Soc.* **1994**, *116*, 799; b) V. N. Balaji, K. Ramnarayan, M. F. Chan, S. N. Rao, *Pept. Res.* **1994**, *7*, 60; c) K. Burgess, C.-Y. Ke, *J. Org. Chem.* **1996**, *61*, 8627; d) C. Alemán, J. Casanovas, S. E. Galembeck, *J. Comput.-Aided Mol. Des.* **1998**, *12*, 259; e) D. Moye-Sherman, S. Jin, S. Li, M. B. Welch, J. Reibenspies, K. Burgess, *Chem. Eur. J.* **1999**, *5*, 2730; f) C. Peggion, F. Formaggio, M. Crisma, C. Toniolo, A. I. Jiménez, C. Cativiela, B. Kaptein, Q. B. Broxterman, M. Saviano, E. Benedetti, *Biopolymers* **2003**, *68*, 178.

[18] a) H.-J. Böhm, S. Brode, *J. Am. Chem. Soc.* **1991**, *113*, 7129; b) H. S. Shang, T. Head-Gordon, *J. Am. Chem. Soc.* **1994**, *116*, 1528.

[19] a) G. M. Sheldrick, SHELXS-97, Program for the Solution of Crystal Structures, University of Göttingen, Göttingen (Germany), **1997**; b) G. M. Sheldrick, SHELXL-97, Program for the Refinement of Crystal Structures, University of Göttingen, Göttingen (Germany), **1997**.

