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Model dipeptides incorporating the *trans* cyclohexane analogues of phenylalanine: further evidence of the relationship between side-chain orientation and β-turn type

Marta Lasa,[a] Ana I. Jiménez,[a] María M. Zurbano[b] and Carlos Cativiela[a,*]

[a]*Departamento de Química Orgánica, Instituto de Ciencia de Materiales de Aragón, Universidad de Zaragoza—CSIC, 50009 Zaragoza, Spain*
[b]*Departamento de Química, Universidad de La Rioja, UA—CSIC, 26006 Logroño, Spain*

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Dedicated to Professor Joaquín Plumet on the occasion of his 60th birthday

Abstract—In order to study the influence of the side-chain orientation on the peptide backbone conformation we have synthesised the model dipeptides *t*-BuCO-L-Pro-(1*S*,2*R*)-c_6Phe-NHMe and *t*-BuCO-L-Pro-(1*R*,2*S*)-c_6Phe-NHMe, incorporating each enantiomer of the *trans* cyclohexane analogue of phenylalanine (*trans*-1-amino-2-phenylcyclohexanecarboxylic acid). The orientation of the aromatic side-chain determines the β-turn type accommodated by these peptides to the point that the (1*S*,2*R*)-c_6Phe derivative retains the type I β-turn in the crystalline state, in contrast to the behaviour exhibited by the natural counterpart *t*-BuCO-L-Pro-L-Phe-NHMe.

An important aspect of peptide structure is the interrelationship between main-chain and side-chain conformation, a situation that becomes evident from the statistical analysis of crystallised proteins.[1,2] The side-chains of proteinogenic amino acids primarily adopt staggered conformations. It is clear that the (ϕ,ψ) values adopted by the main-chain may affect the equilibrium distribution among the three staggered C^{α}–C^{β} rotamers to minimise unfavourable contacts. The converse situation can also be considered: is the side-chain of a residue fixed in a definite orientation (at a certain χ^1 value) able to impart a particular conformation to the backbone?

Terminally blocked dipeptides RCO-L-Pro-Xaa-NHR′ are appropriate systems to study the interdependence between side-chain and backbone conformation. The presence of L-proline in the $i+1$ position means that these peptides are prone to adopt β-turn conformations of type I or II.[1,3,4] For L-Pro-D-Xaa dipeptides the βII-turn is systematically favoured.[5–7] In comparison, L-Pro-L-Xaa sequences exhibit a higher conformational freedom and the β-turn type depends both on the environment and on the L-Xaa side-chain: in poorly solvating media the βI-turn is largely preferred but for most L-Xaa residues the βII form becomes more stable in the crystalline state.[5–7] This βI-to-βII transition is due to the higher accessibility of the L-Xaa NH site in the βII disposition, which allows the engagement of this NH in an intermolecular hydrogen bond with an adjacent molecule in the crystal.[7] Of the proteinogenic L amino acids, only those bearing a highly polar side-chain that is able to form an intramolecular hydrogen bond with the central amide group (such as serine,[8] threonine[9] or histidine[10]) have been shown to retain the type I β-turn in the solid state.[6–10]

In agreement with the behaviour described above, dipeptide Piv-L-Pro-L-Phe-NHMe (Piv = *t*-BuCO) accommodates a βI-turn in chlorinated solvents.[7,11] It has been proposed[7] that this conformation is stabilised by a weak interaction between the L-Phe aromatic side-chain and NH, which accounts for the predominance of the *gauche*(+) orientation for the benzylic substituent—despite this being the most sterically

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* Corresponding author. Tel./fax: +34 976 761210; e-mail: cativiela@unizar.es

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disfavoured staggered disposition available to the L-Phe side-chain (Fig. 1). This interaction is not strong enough to compete with intermolecular hydrogen-bonding in the solid state, where the molecule changes to a βII-turn and the aromatic side-chain exhibits the sterically more favourable *gauche*(−) disposition ($\chi^1 = -42°$).[7] The conformational preferences of the backbone and the aromatic side-chain in the Piv-L-Pro-L-Phe-NHMe dipeptide therefore appear to be strictly related.

Interestingly, we showed in a previous study[12] that replacement of L-Phe in this system by a constrained analogue with χ^1 fixed in the *gauche*(+) orientation results in the retention of the βI-turn in the crystal. This side-chain constrained surrogate is (1*S*,2*S*)-c_6Phe [(1*S*,2*S*)-1-amino-2-phenylcyclohexanecarboxylic acid], the cyclohexane analogue of L-Phe with the amino and phenyl groups in a *cis* relative orientation. In order to further explore the possible correlation between restriction of the L-Phe χ^1 dihedral angle as *gauche*(+) and retention of the βI-turn, we decided to synthesise and study the conformational tendencies of the *trans* cyclohexane analogues of Phe, namely (1*S*,2*R*)- and (1*R*,2*S*)-c_6Phe. Rotation about C^α–C^β in c_6Phe is inhibited by covalent constraints and the orientation of the phenyl ring is given by the stereochemistry and conformational preferences of the six-membered system. Assuming that the cyclohexane ring adopts a chair conformation, only two χ^1 regions are allowed for each c_6Phe stereoisomer, with one of them being largely preferred by the propensity of the bulky aromatic substituent to occupy an equatorial position. For both *cis* (1*S*,2*S*)-c_6Phe and *trans* (1*S*,2*R*)-c_6Phe derivatives the lowest-energy chair fixes the phenyl ring at χ^1 near 60° (Fig. 1) and these compounds can thus be viewed as frozen *gauche*(+) analogues of L-Phe (note that the L and *S* configurations for the α or 1 positions are equivalent). It should be noted that the different stereochemistry at C^β (position 2) of these L-Phe analogues results in a different orientation of the cyclohexane ring with respect to the main-chain (Fig. 1).

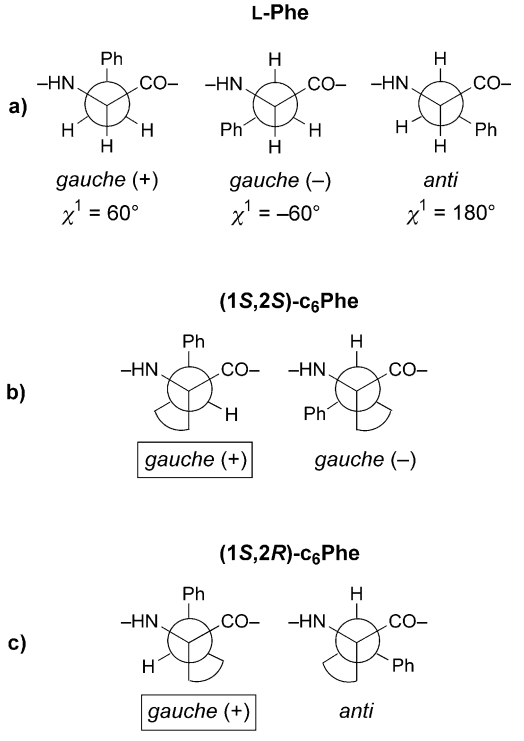
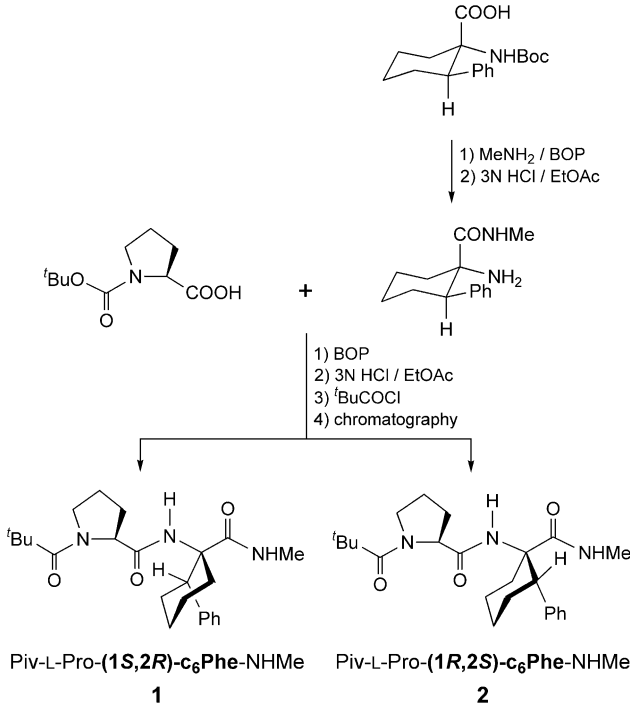


Figure 1. Newman projection through the C^α–C^β bond for: (a) the three low-energy side-chain rotamers of L-phenylalanine; (b) the two possible side-chain conformations for the *cis* cyclohexane analogue of L-Phe (previously studied: Ref. 12); (c) the two possible side-chain conformations for the *trans* cyclohexane analogue of L-Phe (studied in this work). For the c_6Phe derivatives, the most favoured chair bearing the phenyl ring in an equatorial orientation is indicated.

The synthesis of *trans* 1-(*N*-*tert*-butoxycarbonyl)amino-2-phenylcyclohexanecarboxylic acid in racemic form has already been reported.[13] The methylamide derivative of this compound was formed through activation with BOP[14] (Scheme 1). After removal of the Boc protecting group, the resulting amino terminus was condensed with *N*-*tert*-butoxycarbonyl-L-proline using the same activating agent. Subsequent Boc/Piv exchange afforded the diastereomeric dipeptides Piv-L-Pro-(1*S*,2*R*)-c_6Phe-NHMe (**1**)[15] and Piv-L-Pro-(1*R*,2*S*)-c_6Phe-NHMe (**2**),[16] which were separated by column chromatography on silica gel. The absolute configuration of the c_6Phe residues was assigned on the basis of X-ray diffraction analysis on single crystals of both dipeptides,[17–19] taking the known configuration of L-proline as a reference.

In CH_2Cl_2 solution both dipeptides adopt a β-turn conformation, with the terminal pivaloyl CO and methylamide NH intramolecularly hydrogen-bonded, as



Scheme 1. Synthesis of dipeptides **1** and **2**, incorporating the *trans* cyclohexane analogues of phenylalanine in the *i*+2 position. Abbreviations: BOP = (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; Boc = *tert*-butoxycarbonyl; Piv = pivaloyl (*tert*-butylcarbonyl).

evidenced by their low IR stretching frequencies in comparison with those expected for free groups.[7,11,12,20] The ^{1}H NMR spectra in $CDCl_3$ exhibit a large vicinal coupling constant (about 14 Hz) for the benzylic proton in both **1** and **2**, which indicates the axial arrangement of this proton and therefore confirms the equatorial orientation of the aromatic ring. These side-chain dispositions correspond to *gauche*(+) for (1*S*,2*R*)-c$_6$Phe (Fig. 1) and *gauche*(−) for (1*R*,2*S*)-c$_6$Phe.

The βI- and βII-turns differ essentially in the orientation of the plane containing the middle amide group,[5,7,21] which is rotated by almost 180°. In CH_2Cl_2 solution both types of turns can be unambiguously distinguished[7,11,12] on the basis of the IR frequencies characterising the L-Pro-*CO* and Piv-*CO* vibrators. In dipeptide **1** these carbonyl groups appear, respectively, at 1688 and 1612 cm^{-1}, whereas they shift to 1696 and 1603 cm^{-1} in compound **2**, and this observation is consistent with a βI-turn disposition for the former and βII-folding for the latter. This behaviour parallels that observed[7,11] in the same solvent for the analogous dipeptides containing L- and D-Phe, respectively.

In the solid state the two compounds under investigation maintain their respective βI- and βII-turn conformations. The crystalline structure of dipeptide **1** is shown in Figure 2, with the proline C$^\alpha$–H and C=O bonds in a *syn* orientation as corresponding to the βI-turn. The equatorial aromatic side-chain actually falls in the *gauche*(+) region ($\chi^1 = 62°$ in molecule A and 64° in molecule B). The βII-turn accommodated by dipeptide **2** and characterised by the *anti* arrangement of the proline C$^\alpha$–H and C=O bonds is shown in Figure 3. In this case, the phenyl ring occupies an axial position ($\chi^1 = -161°$ in molecule A and −157° in molecule B, *anti* conformer) instead of the expected equatorial [*gauche*(−)] arrangement, an effect most probably due to the packing forces operating in the crystal.

The retention of the βI-turn in the crystal of the (1*S*,2*R*)-c$_6$Phe-containing dipeptide (**1**) is remarkable and provides further evidence of the direct relationship between the *gauche*(+) orientation of the aromatic L-Phe side-chain and the preference of this amino acid for the *i*+2 position of a βI-turn. Steric and electronic effects similar to those invoked[12] in the previously investigated *cis* (1*S*,2*S*)-c$_6$Phe stereoisomer seem to operate in **1** to stabilise the βI-turn conformation.

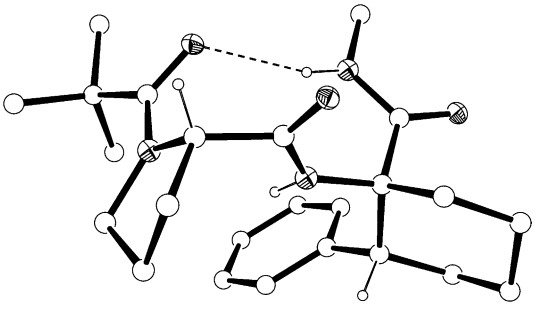


Figure 2. Crystal molecular structure of the (1*S*,2*R*)-c$_6$Phe-containing dipeptide (**1**) accommodating a βI-turn (molecule A). Most H atoms have been omitted for clarity. The N and O atoms are shown as thermal ellipsoids. The intramolecular hydrogen bond is represented as a dashed line. Torsion angles: Pro-(ϕ,ψ) = (−72, −28) in mol. A, (−57, −37) in mol. B; (1*S*,2*R*)-c$_6$Phe-(ϕ,ψ) = (−80, −5) in mol. A, (−62, −23) in mol. B.

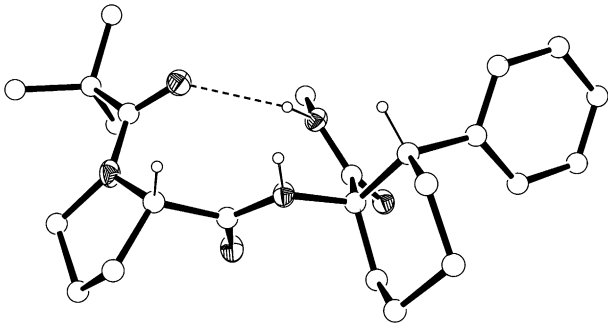


Figure 3. Crystal molecular structure of the (1*R*,2*S*)-c$_6$Phe-containing dipeptide (**2**) accommodating a βII-turn (molecule A). Most H atoms have been omitted for clarity. The N and O atoms are shown as thermal ellipsoids. The intramolecular hydrogen bond is represented as a dashed line. Torsion angles: Pro-(ϕ,ψ) = (−64, 132) in mol. A, (−66, 146) in mol. B; (1*R*,2*S*)-c$_6$Phe-(ϕ,ψ) = (51, 40) in mol. A, (53, 39) in mol. B.

The results described provide evidence for the possibility of modulating the backbone conformation in a peptide by restricting the mobility of the side substituents. It is also a clear example of the enormous utility that synthetic side-chain constrained amino acids may have in the investigation of this little explored field of peptide structure.

Acknowledgements

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15. Piv-L-Pro-(1*S*,2*R*)-c$_6$Phe-NHMe (**1**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.02 (s, 9H), 1.35–1.67 (m, 4H), 1.87–2.15 (m, 5H), 2.34 (m, 1H), 2.51 (m, 1H), 2.55 (m, 1H), 2.65 (d, $J = 4.7$ Hz, 3H), 3.19 (ddd, $J = 3.0$, 3.6, 13.7 Hz, 1H), 3.56 (m, 1H), 3.71 (m, 1H), 4.26 (dd, $J = 5.5$, 7.5 Hz, 1H), 6.26 (br s, 1H), 6.54 (br q, $J = 4.7$ Hz, 1H), 7.20–7.30 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.62, 25.98, 26.01, 26.14, 27.18, 27.68, 30.07, 35.48, 38.98, 48.60, 54.05, 62.44, 64.44, 127.47, 128.62, 128.84, 141.21, 171.07, 172.16, 178.89.
16. Piv-L-Pro-(1*R*,2*S*)-c$_6$Phe-NHMe (**2**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.17 (s, 9H), 1.39–1.67 (m, 4H), 1.81–2.19 (m, 5H), 2.41 (m, 1H), 2.51–2.64 (m, 2H), 2.59 (d, $J = 4.7$ Hz, 3H), 3.24 (ddd, $J = 3.1$, 3.7, 13.7 Hz, 1H), 3.68 (m, 2H), 3.96 (dd, $J = 6.0$, 7.7 Hz, 1H), 6.00 (br s, 1H), 7.03 (br q, $J = 4.7$ Hz, 1H), 7.19–7.32 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.57, 26.00, 26.26, 27.09, 27.82, 29.44, 29.65, 35.20, 38.68, 48.64, 53.65, 62.43, 63.49, 127.67, 128.64, 128.78, 140.55, 171.82, 172.15, 177.44.
17. Single crystals of dipeptide **1** were grown by slow evaporation from an ethyl acetate/hexanes solution. X-ray data ($C_{24}H_{35}N_3O_3$): orthorhombic, space group $P2_12_12_1$, $a = 10.3036(7)$ Å, $b = 16.3420(11)$ Å, $c = 26.8134(19)$ Å; $Z = 8$; $D_{calcd} = 1.217$ g cm^{-3}; 54,942 reflections collected, 10,359 unique ($R_{int} = 0.045$); final R indices (9852 observed reflections, $I > 2\sigma I$): $R_1 = 0.047$, $wR_2 = 0.106$; final R indices (all data): $R_1 = 0.050$, $wR_2 = 0.107$.
18. Single crystals of dipeptide **2** were grown by slow evaporation from a methanol solution. X-ray data ($C_{24}H_{35}N_3O_3$): monoclinic, space group $P2_1$, $a = 13.1850(3)$ Å, $b = 10.7340(3)$ Å, $c = 17.2630(5)$ Å, $\beta = 111.1241(10)°$; $Z = 4$; $D_{calcd} = 1.205$ g cm^{-3}; 15,771 reflections collected, 7873 unique ($R_{int} = 0.076$); final R indices (5943 observed reflections, $I > 2\sigma I$): $R_1 = 0.059$, $wR_2 = 0.128$; final R indices (all data): $R_1 = 0.087$, $wR_2 = 0.142$.
19. Crystallographic data (excluding structure factors) for the structures in this letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 279937 (**1**) and 279938 (**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].
20. The free Me*NH* and Piv-*CO* contributions are expected at 3450 and 1619 cm^{-1}, respectively. In both dipeptides the former group appears at about 3370 cm^{-1}. The frequency observed for the Piv-*CO* group is explained in the text.
21. Torsion angles for an ideal βI-turn: $(\phi,\psi)_{i+1} = (-60,-30)$; $(\phi,\psi)_{i+2} = (-90,0)$. Torsion angles for an ideal βII-turn: $(\phi,\psi)_{i+1} = (-60,120)$; $(\phi,\psi)_{i+2} = (80,0)$. Note that they differ mainly in the i+1 ψ and the i+2 ϕ angles, as a consequence of a 180° flip of the plane defined by the central amide group.