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Stabilisation of the type I β-turn conformation by a bicyclic analogue of proline

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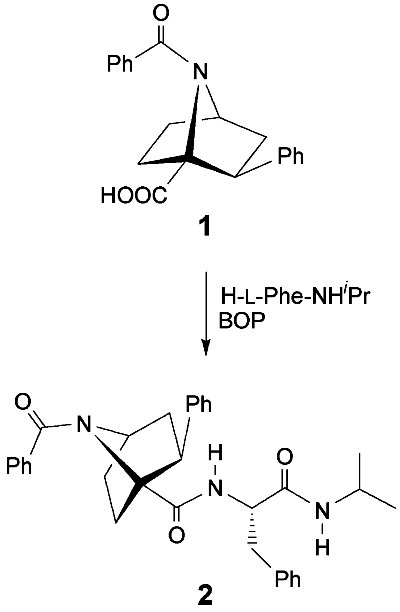
Abstract—A highly constrained analogue of L-proline, (1*S*,2*S*,4*R*)-2-phenyl-7-azabicyclo[2.2.1]heptane-1-carboxylic acid, has been incorporated into a model dipeptide. X-Ray diffraction analysis has shown that, in the solid state, this constrained peptide adopts a type I β-turn whereas the analogous dipeptide sequence incorporating L-proline has been shown to accommodate a βII-turn disposition. Attractive interactions involving the middle NH group and either the aromatic rings or the pyramidalised bicycle nitrogen seem to play a role in the stabilisation of the βI-turn conformation observed.

β-Turns[1,2] are elements of primary importance in the structure and function of peptides and proteins. They involve four consecutive residues and are classified according to the (ϕ,ψ) dihedral angles associated with the central *i*+1 and *i*+2 positions. Among the variety of β-bends, types I and II are the most widely distributed. These two turns are characterised by all *trans* peptide bonds and are generally stabilised by an intramolecular *i*+3 to *i* hydrogen bond, whereas they differ in the orientation of the middle amide group.[†]

Due to its intrinsic tendency to adopt ϕ values in the −60° region,[3] proline is frequently found in the *i*+1 position of types I and II β-turns.[1–3] In such L-Pro-Xaa sequences, the preference for the βI or the βII conformation depends both on the structure of the Xaa residue (L/D configuration, side chain nature) and on the environment (solid or solution state, solvent polarity).[1,4,5]

We have evaluated the relative stability of the βI- and βII-turn conformations in model peptides RCO-L-Pro-L-Phe-NHR′ in which phenylalanine has been replaced by different constrained derivatives.[6–8] We report here the structural consequences arising from the replacement of the proline residue in this sequence by an analogue of norbornane structure. In this proline surrogate, the flexibility of the pyrrolidine ring has been



Scheme 1. Synthesis of dipeptide **2**, incorporating the bicyclic analogue of L-proline in position *i*+1.

Keywords: constrained amino acid; constrained proline; 7-azanorbornane; crystal structure; peptide conformation; β-turn; nonplanar amide; pyramidalisation of amide nitrogen; amide-aromatic interaction.

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[†] 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: $(\phi,\psi)_{i+1}=(-60,120)$; $(\phi,\psi)_{i+2}=(80,0)$.

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frozen by linking the α- and δ-carbons through an ethylene bridge, and an additional phenyl substituent, which can interact with the backbone both sterically and electronically, has been incorporated at the β-position.

Enantiomerically pure (1*S*,2*S*,4*R*)-*N*-benzoyl-2-phenyl-7-azabicyclo[2.2.1]heptane-1-carboxylic acid (**1**) was obtained by combining a racemic synthesis and a chromatographic resolution procedure.[9] Condensation with L-phenylalanine isopropylamide was carried out through activation with (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP)[10] to afford the corresponding dipeptide (**2**) in good yield (Scheme 1).

Single crystals of **2** were obtained from a dichloromethane/hexane solution. Crystallographic analysis by X-ray diffraction[‡] showed that the molecule adopts a β-folded conformation that is stabilised by an intramolecular hydrogen bond between the benzoyl oxygen and the isopropylamide hydrogen, with an N···O distance of 2.89 Å and an N–H···O angle of 159° (Fig. 1). The backbone torsion angles correspond to a type I β-turn [$(\phi,\psi)=(-46,-31)$ and $(-73,-12)$ for the proline surrogate and phenylalanine, respectively]. In comparison, the analogous dipeptide ^{t}BuCO-L-Pro-L-Phe-NHMe has been shown to accommodate a βII-turn disposition in the solid state.[5] Remarkably, the latter compound also exhibits a higher preference for βI-folding in low-polarity solvents,[5,6] but the βII becomes more stable in the crystalline state.[5] It should be emphasised that in the crystal structure of **2** the phenylalanine amide proton is not involved in any intermolecular contact, whereas in the L-Pro-containing dipeptide this middle NH is hydrogen-bonded to a carbonyl group of a neighbouring molecule.[5] These observations are in line with the known lower accessibility of the Xaa-NH site in βI-folded L-Pro-L-Xaa sequences.[5–7] This result provides evidence that the bicyclic proline system exhibits a higher propensity for βI-folding than proline itself. Several factors can contribute to the stabilisation of the type I β-turn observed in the crystalline structure of **2**.

All the amide bonds in Figure 1 assume a *trans* disposition, with ω deviating by less than 5° from the standard ±180°. However, the nitrogen atom in the 7-position of the norbornane moiety exhibits a significant distortion from planarity, lying at a distance of 0.38 Å from the plane defined by the three carbon atoms bonded to it. In fact, the pyramidal character of this nitrogen has been highlighted as an intrinsic feature of the tensioned 7-azanorbornane system.[11,12] Indeed, to the best of our knowledge, all the crystalline structures reported for *N*-acyl-7-azabicyclo[2.2.1]heptane derivatives[12–17] exhibit a non-planar geometry at the nitrogen atom. In the case of dipeptide **2**, this pyramidalisation is particularly strong, as revealed by the sum of the valence angles around the nitrogen (339°), which is closer to the value predicted for trimethylamine (333°)[18] than to that expected for a planar trigonal arrangement (360°). In comparison, the sum of the bond angles at the nitrogen atom in *N*-benzoyl-7-azabicyclo[2.2.1]heptane is 350°.[12]

The marked pyramidal geometry of the tertiary nitrogen in **2** confers it as a pronounced *sp*^{3} character and hampers delocalisation of the lone pair to the adjacent carbonyl group. This is reflected by a significant lengthening of the N–C(O) bond (1.38 Å) with respect to the standard amide value (1.33 Å).[19] At the same time, the higher accessibility of the lone pair allows the nitrogen to participate in hydrogen bonding interactions as an acceptor site. This is actually the case in the crystalline structure of **2**, where the small ψ value adopted by the azanorbornane residue orients the phenylalanine amide hydrogen towards the lone pair of the pyramidalised

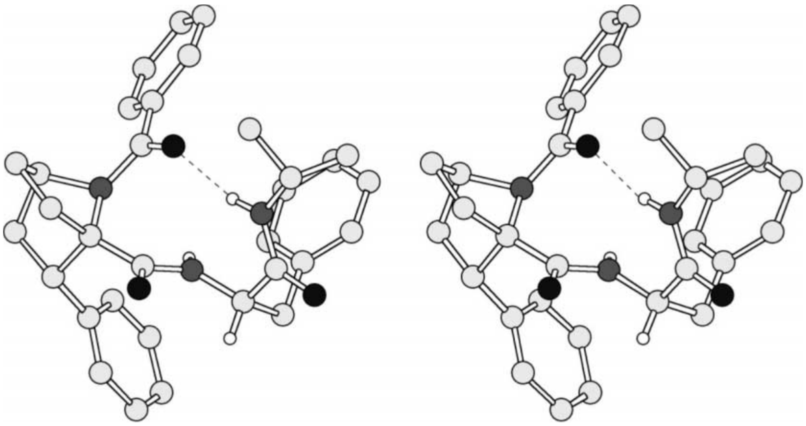

Figure 1. Stereoview of the βI-folded molecular structure of dipeptide **2** obtained by X-ray diffraction analysis. Only the C$^\alpha$ and amide hydrogen atoms are shown. The intramolecular *i*+3 to *i* hydrogen bond is represented as a dashed line.

[‡] Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 211369. 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]. Tetragonal, $P4_12_12$; $a=b=12.3287(6)$ Å, $c=37.151(4)$ Å; $Z=8$; $D_{\text{calcd}}=1.199$ g cm^{-3}; 33645 reflections collected, 5541 unique ($R_{\text{int}}=0.104$); final R indices [3086 observed reflections, $I>2\sigma(I)$]: $R_1=0.038$, $wR_2=0.042$; final R indices (all data): $R_1=0.080$, $wR_2=0.047$.

nitrogen (H···N = 2.59 Å, N···N = 2.92 Å). Similar N–H···N attractive contacts have been observed in other model peptides containing non-planar tertiary amide bonds.[20–22] Moreover, such an interaction has been proposed[23–25] as a factor that promotes the *cis–trans* isomerisation of the amide bond preceding proline by stabilising the lone pair of the pyramidalised proline nitrogen in the transition state. In a similar way, the non-planar character inherent in the bicyclic nitrogen atom in **2** could be enhanced by its engagement in a stabilising interaction with the proximal phenylalanine amide proton. This could be the reason for the out-of-plane deviation of this nitrogen atom being more pronounced in **2** than in similar systems.

It should be noted that ψ values for the proline surrogate in the 130° region (corresponding to position i+1 of a βII-turn) would preclude the existence of this N–H···N interaction in **2**. Moreover, rotation of the middle amide plane by about 150°, as associated with the βI-to-βII transition, would place the pseudoproline carbonyl oxygen in the neighbourhood of the pyramidalised nitrogen lone pair and would therefore introduce a repulsive interaction.

An additional source of stabilisation of the βI-turn conformation by the bicyclic analogue of proline stems from the presence of the extra β-phenyl substituent. In dipeptide **2**, this *exo*-oriented phenyl ring lies in close proximity to the phenylalanine amide hydrogen, giving rise to an attractive interaction of the N–H···π type. Amide-aromatic interactions have been frequently cited as stabilising factors in the structure of peptides and proteins.[5–7,22,26–28] Again, such an interaction would not be possible had **2** accommodated a βII-folded structure.

Another remarkable feature in Figure 1 is the *gauche*(+) disposition adopted by the phenylalanine side chain ($\chi^1 = 58°$). This orientation corresponds to the sterically most disfavoured staggered rotamer, with the phenyl ring being flanked by the amino and carbonyl substituents. However, this situation allows the existence of an additional amide-aromatic interaction between the phenylalanine amide hydrogen and phenyl side chain. As a matter of fact, such an interaction stabilises the βI-turn conformation for ^{t}BuCO-L-Pro-L-Phe-NHMe in low-polarity solvents ($\chi^1 \approx 60°$ for L-Phe) but is not retained in the solid state, where it is compensated by intermolecular hydrogen-bonding and the benzylic side chain assumes the sterically more favourable *gauche*(−) orientation.[5]

The βI-folded structure adopted by **2** in the crystalline state seems to indicate that the extra attractive intramolecular interactions involving the middle amide hydrogen and the norbornane proline analogue compete successfully with the intermolecular hydrogen bond that stabilises the βII conformation for the L-Pro-L-Phe sequence. Further investigations will be necessary to evaluate the contribution of the different factors discussed above to the stabilisation of the βI-turn structure.

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