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# A Crystal Structure of an Oligoproline PPII-Helix, at Last

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Supporting Information

**ABSTRACT:** The first crystal structure of an oligoproline adopting an *all-trans* polyproline II (PPII) helix is presented. The high-resolution structure provides detailed insight into the dimensions and conformational properties of oligoprolines that are important for, e.g., their use as "molecular rulers" and "molecular scaffolds". The structure also showed that the amides interact with each other within a PPII helix and that water is not necessary for PPII helicity.

Polyproline II (PPII) helices are, together with $\alpha$-helices and $\beta$-sheets, the most abundant secondary structures in peptides and proteins.[1] The structural protein collagen and many domains within proteins that play important roles in biological processes adopt PPII helices.[1−3] Oligo-L-prolines are the progenitors of this left-handed helix, which they adopt already at chain lengths as short as six residues.[4] Due to their well-defined and rigid conformation oligoprolines are often used as "molecular rulers" and "molecular scaffolds".[5,6] Whereas crystal structures of collagen and several proteins with PPII helical domains have been obtained, crystallization of oligoprolines has so far been elusive.[7] Only crystal structures of proline di-, tri-, and tetramers are known, which did, however, not crystallize as PPII helices.[8,9] Powder diffraction studies of oligoprolines long ago provided valuable low resolution structural insight into the PPII helix.[10] They revealed the basic parameters of this secondary structure, for example, that all amide bonds are in *trans* conformations and every third residue is stacked on top of each other. Other experimental structure information on oligoprolines with PPII helicity stems from CD, IR, and Raman spectroscopy, along with FRET and NMR studies, which provided information on their dynamic behavior.[4,11−13] Numerous molecular details, *e.g.*, the dimensions of oligoprolines, that are important for their use as molecular rulers, have, however, remained uncertain. The lack of a crystal structure of the parent peptide of the PPII helix has also led to a debate about the factors that are critical for this secondary structure. In particular, the role of hydration and $n \rightarrow \pi^*$ interactions between adjacent carbonyl groups has been controversially discussed.[3,14,15] Herein we present the first crystal structure of an oligoproline in a PPII helical conformation. The high-resolution structure provided detailed insight into the molecular parameters and distances within oligoprolines. In addition, the study revealed that interactions between neighboring amide groups are present and likely contribute to the stability of the PPII helix whereas water was not recruited into the crystal.

Our interest in functionalized oligoprolines as molecular scaffolds led us to revisit the crystallization of oligoprolines.[6,16] Initial attempts to crystallize N-terminally acetylated and C-terminally amidated oligoprolines of different lengths in a range of different aqueous and nonaqueous solvents were unsuccessful and underlined the difficulty in obtaining crystals suitable for X-ray analysis.[7] Crystallization was finally achieved with hexaproline **1** bearing a *p*-bromobenzoyl moiety at the N-terminus and a carboxylic acid group at the C-terminus (Figure 1a).

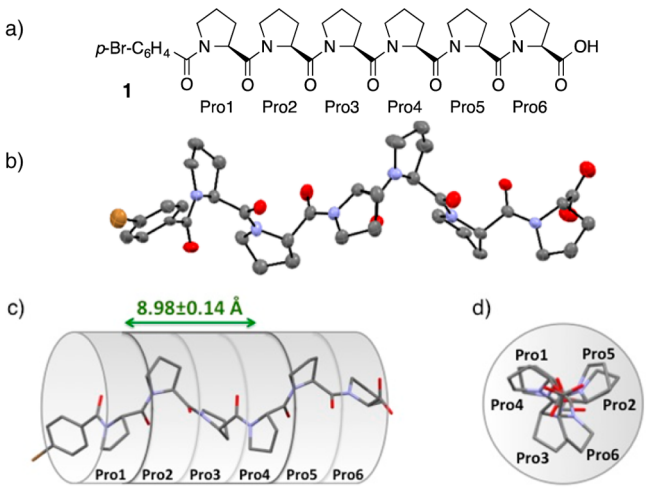


**Figure 1.** (a) Hexaproline *p*-Br-$C_6H_4$-$Pro_6$-OH (**1**). (b) Crystal structure of **1** (ORTEP). (c) Segmental side view. (d) View along the axis.

After numerous attempts using different solvents and conditions, single crystals of **1** suitable for X-ray crystallographic analysis were obtained by vapor diffusion using acetonitrile as solvent and tetrahydropyran as cosolvent.[17] Both solvents were not dried but contained residual water and were exposed to air.[18] The obtained crystals belong to the monoclinic space group $P2_1$ ($\beta$ = 91.901(5)°, $V$ = 1943.8(2) Å$^3$), and the structure was solved by direct methods to atomic resolution (Figure 1b).[17] The peptide cocrystallized with a molecule of $CH_3CN$ that forms a side-on close contact[19] to the bromide of the N-terminal benzoyl group and has a short contact to the C-terminal carboxylic acid group of a neighboring molecule.[17] This coordination might have helped the crystallization of **1**. The only close contacts, which might

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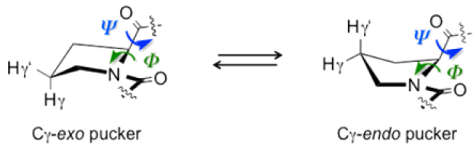
disturb the intrinsic conformational properties of **1**, involve the carbonyl groups of Pro3 and Pro5 that are close (2.3−2.6 Å) to two neighboring molecules.[17] Aside, there are no obvious packing effects that might override the conformation of the peptide.

Hexaproline **1** crystallized with an almost ideal $C_3$-symmetry along the screw axis, one of the hallmarks of the PPII helix, with respect to the backbone atoms N, $C^{\alpha}$, and $C_i$. The only residue that is significantly off the symmetry axis is Pro6 with its C-terminal carboxylic acid group (Figure 1d). Whereas the powder diffractogram showed distances of 9.36 Å between every third residue,[10] the distance between every third residue in the oligoproline crystal is on average only 8.98 ± 0.14 Å.[20] Thus, one proline residue contributes 3.0 Å, which provides a predictive value for the length of oligoprolines that is essential for their use as molecular rulers and scaffolds.

The slight distortions of the backbone atoms N, $C_i^{\alpha}$, and $C_i$ from the ideal $C_3$-symmetry along the central screw axis are due to differences in the dihedral angles $\phi$ and $\psi$ and the puckering of the six proline residues. Whereas residues Pro3-Pro5 adopt twisted $C^{\gamma}$-*endo* ring puckers, residues Pro1 and Pro2 are $C^{\gamma}$-*exo* puckered and Pro6 adopts a twisted $C^{\gamma}$-*exo* ring pucker (Figure 1, Table 1). Such a distribution of different puckers is not

**Table 1. Ring Puckering and $\phi$ and $\psi$ Angles of Pro1-Pro6**




| residue | $\phi^a$ | $\psi^a$ | ring pucker |
|---|---|---|---|
| Pro1 | −67.1° | +143.6° | $C^{\gamma}$-*exo* |
| Pro2 | −65.7° | +138.0° | $C^{\gamma}$-*exo* |
| Pro3 | −73.1° | +163.9° | twisted $C^{\beta}$-*exo*−$C^{\gamma}$-*endo* |
| Pro4 | −72.8° | +151.0° | twisted $C^{\beta}$-exo−$C^{\gamma}$-*endo* |
| Pro5 | −72.5° | +165.2° | twisted $C^{\beta}$-*exo*−$C^{\gamma}$-*endo* |
| Pro6 | −69.0° | +150.3° | twisted $C^{\beta}$-*endo*−$C^{\gamma}$-*exo* |

[a] $\phi$ angles: ($C_{i-1}$−N−$C_i^{\alpha}$−$C_i$). $\psi$ angles: ($N_i$−$C_i^{\alpha}$−$C_i$−$N_{i+1}$)

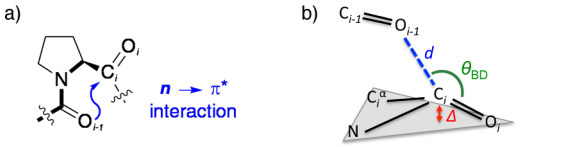
surprising in light of the small energy difference between them.[21−23] The occurrence of different puckers in the crystal allowed for differentiating between conformational properties that are typical for *exo* and *endo* ring puckers.

Analysis of the dihedral angles showed a clear correlation between the $\phi$ and $\psi$ angles and the ring pucker: the closer $\phi$ and $\psi$ are to −65° and +140°, respectively, the more pronounced the $C^{\gamma}$-*exo* ring pucker becomes (Table 1). This correlation is particularly evident for residues Pro1 and Pro2 whereas it is the least pronounced for Pro6, the residue with a carboxylic acid instead of an amide at the C-terminal site. Conversely, $\phi$ and $\psi$ angles around −73° and +155°, respectively, are realized in the $C^{\gamma}$-*endo* puckers. These values are all in good agreement with a statistical database analysis of proline residues in protein crystals where a similar correlation between the values of backbone and endocyclic torsion angles was found.[23]

Next, we analyzed which interactions are present in the PPII helical structure. Several previous studies, among them theoretical calculations,[24] had suggested the importance of coordinating water molecules for the stability of the PPII helix.[3,14] In the absence of coordinating water molecules, a PPI helix with *all-cis* amide bonds has been predicted to be most stable.[25] Other studies had proposed that interactions between adjacent amide groups are responsible for the stability of PPII helices.[15,16] Within the crystal structure no water molecules have been recruited despite the use of wet solvents for the crystallization.[18] The cocrystallized $CH_3CN$ molecule is only in close contact with the N- and C-termini of **1** but not with the amide groups. This lack of coordinating water in the crystal structure clearly shows that hydration is not a prerequisite for PPII helicity. These findings naturally do not exclude hydration and a stabilizing effect of water on PPII helical oligoprolines and other peptides in aqueous solution.

To evaluate the extent of interactions between the amide moieties, we started by analyzing the distances between the carbonyl oxygens and the carbonyl carbons of neighboring residues ($O_{i-1}\cdots C_i$) (Table 2). All $O_{i-1}\cdots C_i$ distances are below

**Table 2. Trajectory Angles $O_{i-1}\cdots C_i{=}O_i$ ($\theta_{BD}$), Distances $O_{i-1}\cdots C_i$ ($d$), and Pyramidalization of $C_i$ ($\Delta$) in 1**




| residue | $\theta_{BD}$ | $d$ [Å] | $\Delta$ [Å] |
|---|---|---|---|
| Pro1 | 102.9° | 2.958 | 0.040 |
| Pro2 | 106.5° | 2.923 | 0.023 |
| Pro3 | 89.8° | 3.072 | 0.005 |
| Pro4 | 98.9° | 3.063 | 0.022 |
| Pro5 | 88.2° | 3.173 | 0.010 |
| Pro6 | 98.2° | 3.047 | nd[a] |

[a] The high anisotropy of the O atoms within the carboxylic acid did not allow for determining a meaningful $\Delta$ value.

3.2 Å and therefore within the sum of the van der Waals radii of these atoms. Such short distances are indicative of $n \rightarrow \pi^*$ interactions between neighboring carbonyl groups, which involve delocalization of the nonbonding electrons of $O_{i-1}$ into the $\pi^*$ orbital of the $C_i{=}O_i$ bond (Table 2).[15,26,27] Additional indicators of $n \rightarrow \pi^*$ interactions are the degree of pyramidalization ($\Delta$) of $C_i$ and the trajectory angle $O_{i-1}\cdots C_i{=}O_i$ between the adjacent carbonyl groups (Table 2).[15,26] The more pyramidalized $C_i$, the shorter $O_{i-1}\cdots C_i$, and the closer the trajectory angle is to the Bürgi−Dunitz angle,[26] with which a nucleophile approaches a carbonyl group, the more $n \rightarrow \pi^*$ character the interaction has.

Ideal trajectory angles $O_{i-1}\cdots C_i{=}O_i$ for an $n \rightarrow \pi^*$ interaction of ∼104° and the highest degrees of pyramidalization of $C_i$ ($\Delta$ = 0.040 and 0.023 Å) occur in the $C^{\gamma}$-*exo* ring puckered residues Pro1 and Pro2 with the shortest $O_{i-1}\cdots C_i$ distances (Table 2).[28] In fact these are the highest yet observed pyramidalizations for interactions between two amides and among the highest for carboxylic acid derivatives in general. Comparable or larger $\Delta$ values have only been observed for interactions between thioesters and oxoesters that are significantly more nucleophilic and electrophilic moieties compared to amides.[29] The degree of the interaction is smaller for the twisted $C^{\gamma}$-*exo* puckered Pro6 residue where an amide is adjacent to the C-terminal carboxylic acid moiety, which is less electrophilic compared to amide groups (Table 2). A significant degree of pyramidalization ($\Delta$ = 0.022 Å) of $C_i$ and a trajectory angle reminiscent of the Bürgi−Dunitz angle are also observed for the twisted $C^{\gamma}$-*endo* puckered residue Pro4 (Table 2). In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contrast, the $C_i$ carbons of the two other twisted $C^{\gamma}$-*endo* puckered residues Pro3 and Pro5 are hardly pyramidalized ($\leq$0.01 Å) and also the trajectory angles of ~89° are not indicative of electron delocalization. This suggests that $n \rightarrow \pi^*$ interactions are favored by $C^{\gamma}$-*exo* and disfavored by $C^{\gamma}$-*endo* puckering. However, as noted above, the amide moieties of Pro3 and Pro5 are in the vicinity of two neighboring molecules in the unit cell, which might compromise their ability to engage in $n \rightarrow \pi^*$ interactions. Overall, the crystallographic data demonstrate that the amide bonds within the oligoproline helix interact with each other and that the degree of interaction is largest in the case of $C^{\gamma}$-*exo* ring puckered residues. Further investigations into the exact nature of these interactions are currently underway.

In conclusion, the first crystal structure of a PPII helical oligoproline clarified uncertainties regarding the structural features of the PPII helix. It revealed that $n \rightarrow \pi^*$ interactions occur in PPII helices whereas hydration is not a prerequisite for PPII helicity. Moreover, the study provided reliable dimensions of oligoproline-based PPII helices. Since oligoprolines have become increasingly popular for applications as molecular rulers and scaffolds in medicinal and material science,[5,6] this knowledge is important for the molecular design of functionalized derivatives with defined spatial orientations of their substituents.

## ■ ASSOCIATED CONTENT

### Supporting Information

CCDC-1014542 contains the supplementary crystallographic data. They can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. The experimental and analytical details of **1** are also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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