Crystal structure analysis of N-acetylated proline and ring size analogs

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Crystal structure analysis of *N*-acetylated proline and ring size analogs

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Crystal structures of *N*-acetylated proline and homologs with four- and six-membered rings (azetidine carboxylic acid and piperidine carboxylic acid) were obtained and compared. The distinctly different conformations of the four-, five-, and six-membered rings reflect Bayer strain, $n \rightarrow \pi^*$ interaction, and allylic strain, and result in crystal lattices with a zigzag structure.

KEYWORDS
amino acids, conformation, crystals, proline

1 | INTRODUCTION

The amino acid proline (Pro) often induces rigid segments, turns, or both, in peptides and proteins.[1–3] Examples range from natural collagen[4,5] and gluten[6] to synthetic oligoprolines[7] and peptide catalysts.[8,9] Analyses of Pro-containing peptides and proteins showed that Pro adopts either a C^{γ}-*endo* or a C^{γ}-*exo* pucker.[5,10,11] The tertiary Xaa–Pro (Xaa: any amino acid) amide bond adopts a *cis*- or *trans*-conformation. Both conformers are typically populated, which is in contrast to secondary amide bonds between non-cyclic amino acids that adopt almost exclusively the *trans*-conformation.[1,2] These unique conformational properties allow Pro to play an important role in the structure and function of peptides and proteins.[1,3]

Substituents at the pyrrolidine ring of Pro influence the ring pucker, the *trans*/*cis* amide bond equilibrium, and thus the peptide conformation and function, through steric*[12–16] or stereoelectronic effects,†[16–23] or transannular hydrogen bonding (H-bonding).‡[16,24–27] Homologs of Pro with four- and six-membered rings (azetidine carboxylic acid [H–Aze–OH] and piperidine carboxylic acid [H–Pip–OH]) have also become useful tools to alter the properties of a peptide when installed in place of a Pro residue. For example, the incorporation of Aze was found to increase the flexibility of Pro-containing peptides.[28–30] Yeast cells that grew in an Aze-containing medium produced misfolded proteins.[31] The higher homolog Pip is common in biologically active peptides including protein inhibitors,[32,33] antibiotics,[34,35] and immunosuppressants.[36] Pip has been shown to affect the feeding/sleeping rhythm of neonatal chicks and to activate γ-aminobutyric acid receptors.[37] We used Pip to enhance the reactivity and stereoselectivity of a peptide catalyst.[38–42] Furthermore, we and others showed that both, Aze and Pip destabilize the collagen triple helix.[43–45] These effects arise from the structural properties of Aze and Pip, but whereas a lot is known about Pro derivatives,[1,2,16] only a few experimental studies explored the conformation of Aze and Pip derivatives.[38,43,46–51] Studies in solution revealed that the *trans*/*cis* ratio of Xaa–Aze and Xaa–Pip amide bonds can differ significantly from that of Xaa–Pro bonds.[38,43,47–49]

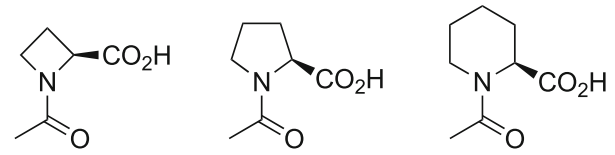
Here, we present crystal structures of *N*-acetylated Aze, Pro, and Pip (Ac–Aze–OH **1**, Ac–Pro–OH **2**, Ac–Pip–OH **3**; Figure 1).

In contrast to previous crystal structures of **2**§[52] and **3**,¶[53] the molecules crystallized with a *trans*-configured amide bond and without

In memory of Prof. Dr. Ulf Diederichsen.

*For examples of steric effects that guide proline conformation, see ref[12–16]
†For examples of stereoelectronic effects that guide proline conformation, see ref[16–23]
‡For examples of transannular hydrogen bonds that guide proline conformation, see ref[16,24–27]

§For a crystal structure of Ac–Pip–OH with a *cis*-amide bond, see Rae et al.[52]
¶For a crystal structure of Ac–Pro–OH with a co-crystalized water molecule, see Rajalakshmi et al.[53]



Ac-Aze-OH (**1**) Ac-Pro-OH (**2**) Ac-Pip-OH (**3**)

FIGURE 1 Acetylated azetidine carboxylic acid (**1**), proline (**2**), and piperidine carboxylic acid (**3**).

a co-crystallized water molecule. Thus, these structures allow for a comparison of the conformational properties of the ring-size analogs.

2 | MATERIALS AND METHODS

Single crystals of **1**–**3** were obtained by solvent evaporation from chloroform. Suitable crystals were selected and measured on a Rigaku Synery S/Cu microfocus Radiation/Dectris P300K detector. The crystals were kept at 100.0(1) K during data collection. Using Olex2,[54] the structures were solved with the XT[55] structure solution program using charge flipping and refined with the XL[56] refinement package using least squares minimization. Deposit numbers in CCSD: **1**, 2195811; **2**: 2195807, **3**: 2195812. Our crystallization studies also provided us with the crystal structure of *cis*-configured Ac–Pip–OH (racemic; CCSD: 2202936), which is included in Data S1.

2.1 | Synthesis of Ac–Aze–OH 1

A solution of Fmoc–Aze–OH (897 mg, 1.85 mmol, 1.5 equiv.) and iPr$_2$NEt (645 µl, 3.70 mmol, 2 equiv.) in anhydrous CH_2Cl_2 (~5 ml) was added to a suspension of 2-chlorotrityl chloride resin (loading: 1.62 mmol/g; 1.41 g; pre-swollen in anhydrous CH_2Cl_2). The reaction mixture was agitated for 1 h and washed with a mixture of CH_2Cl_2/MeOH/iPr$_2$NEt (17:2:1, 5×), CH_2Cl_2 (5×), DMF (5×), and CH_2Cl_2 (5×). For Fmoc deprotection, a solution of 20% piperidine in DMF (~5 ml) was added to the resin, and the reaction mixture was agitated for 10 min, drained, and the piperidine treatment was repeated for 10 min. The resin was washed with DMF (3×) and CH_2Cl_2 (3×). The resin-bound amine was acetylated by the addition of a solution of CH_2Cl_2/Ac_2O/NEt_3 17:2:1 (1 h), followed by washing with CH_2Cl_2 (3×). The acetylated amino acid was cleaved from the resin by shaking in a mixture of CH_2Cl_2/hexafluoroisopropanol (7:3; ~5 ml) for 1 h. The filtrate was collected, and the cleavage procedure was repeated with shaking for 30 min. Pooling of the filtrates and removal of all volatiles under reduced pressure followed by precipitation and thorough washing with Et_2O yielded **1** (148 mg, 56% yield) as a colorless solid. ^{1}H NMR (500 MHz, D_2O) signals of the *trans* conformer: $\delta = 4.83$ (dd, $J = 9.6$, 5.5 Hz, 1H; Hα), 4.25 (m, 2H; Hγ), 2.71 (m, 1H; Hβ), 2.32 (m, 1H; Hβ), 1.94 (s, 3H; Ac). The NMR spectroscopic data are in agreement with previous reports.[47]

2.2 | Ac–Pro–OH 2

The compound was purchased from Chem Impex.

2.3 | Synthesis of Ac–Pip–OH 3

Ac–Pip–OH was prepared following the same protocol as described for Ac–Aze–OH (**1**). Purification by flash chromatography (silica, 0–20% MeOH in CH_2Cl_2) yielded **3** (156 mg, 46% yield) as a colorless solid. ^{1}H NMR (400 MHz, D_2O) $\delta = 5.19$ (d, $J = 3.1$ Hz, 1H, Hα), 3.95–3.75 (m, 1H, Hε), 3.27–3.16 (m, 1H, Hε), 2.28–2.17 (m, 1H, Hβ), 2.17 (s, 3H, Ac), 1.85–1.60 (m, 2H, Hγ, Hδ), 1.60–1.46 (m, 1H, Hβ), 1.46–1.30 (m, 2H, Hγ, Hδ). The NMR spectroscopic data are in agreement with previous reports.[47]

3 | RESULTS AND DISCUSSION

Single crystals of Ac–Aze–OH (**1**), Ac–Pro–OH (**2**), and Ac–Pip–OH (**3**) suitable for X-ray crystallographic analysis were obtained by slow evaporation from chloroform. The acetylated amino acids crystallized without solvent or water molecules in the crystal lattice (Figure 2). Ac–Aze–OH (**1**) crystallized in a monoclinic crystal system with the space group $P2_1$, and the homologs **2** and **3** in an orthorhombic crystal system with the space group $P2_12_12_1$. Each compound crystallized with a *trans*-amide bond. In the crystal lattice, each of the acetylated amino acids forms long zigzag-shaped strands with intermolecular H-bonds between the carboxylic acid and the acetyl groups of neighboring molecules (Figure 2A,B plane). Because the crystal structures of each ring-size analog feature similar intermolecular contacts, we used them to analyze and compare the conformational features of **1**–**3**.

The proline derivative Ac–Pro–OH (**2**) crystallized with a twisted C^{β}-*endo*–C^{γ}-*exo* pucker and the carboxylic acid substituent in a *pseudo*-equatorial position ($\phi = -49°$ and $\psi = 143°$; Figure 3A).[#57] The dihedral angle ϕ is smaller compared to that observed for C^{γ}-*exo* puckering in the crystal structure of an oligoproline with a polyproline II (PPII) helical conformation ($\phi \approx -67°$ and $\psi \approx 140°$; Figure 3B).[10] The distance between the oxygen atom of the acetyl group and the carbon atom of the carboxylic acid is 2.71 Å, and the angle between the oxygen of the acetyl group and the C═O moiety of the carboxylic acid is 92° (Figure 3A). These geometries are indicative of an $n \rightarrow \pi^*$ interaction (Figure 3C) between the amide and carboxylic acid moieties.[21,58] This interaction between neighboring amide bonds involves the delocalization of the non-bonding electrons of O_{i-1} into the π^* orbital of the C_i═O_i bond. The interaction results in an O_{i-1}—C_i═O_i distance that is less than their van der Waals radii (d < 3.22 Å) and an angle that reflects the Bürgi–Dunitz trajectory for the approach of a

[#]Of note, the crystal structure of Ac–Pro–OMe exhibits a C^{γ}-*endo* pucker and thus also different phi and psi angles, for example, see Kubyshkin et al.[47]

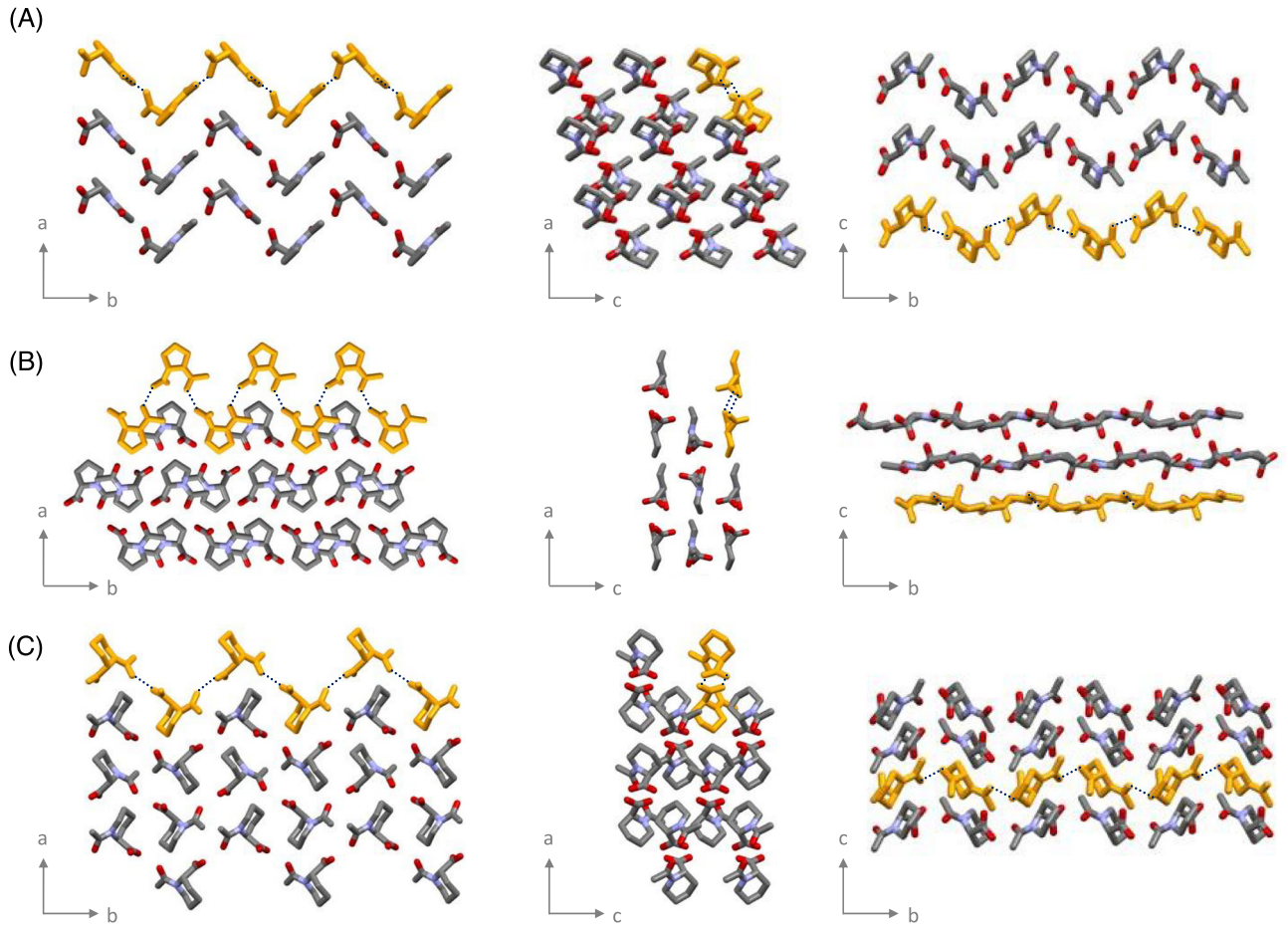
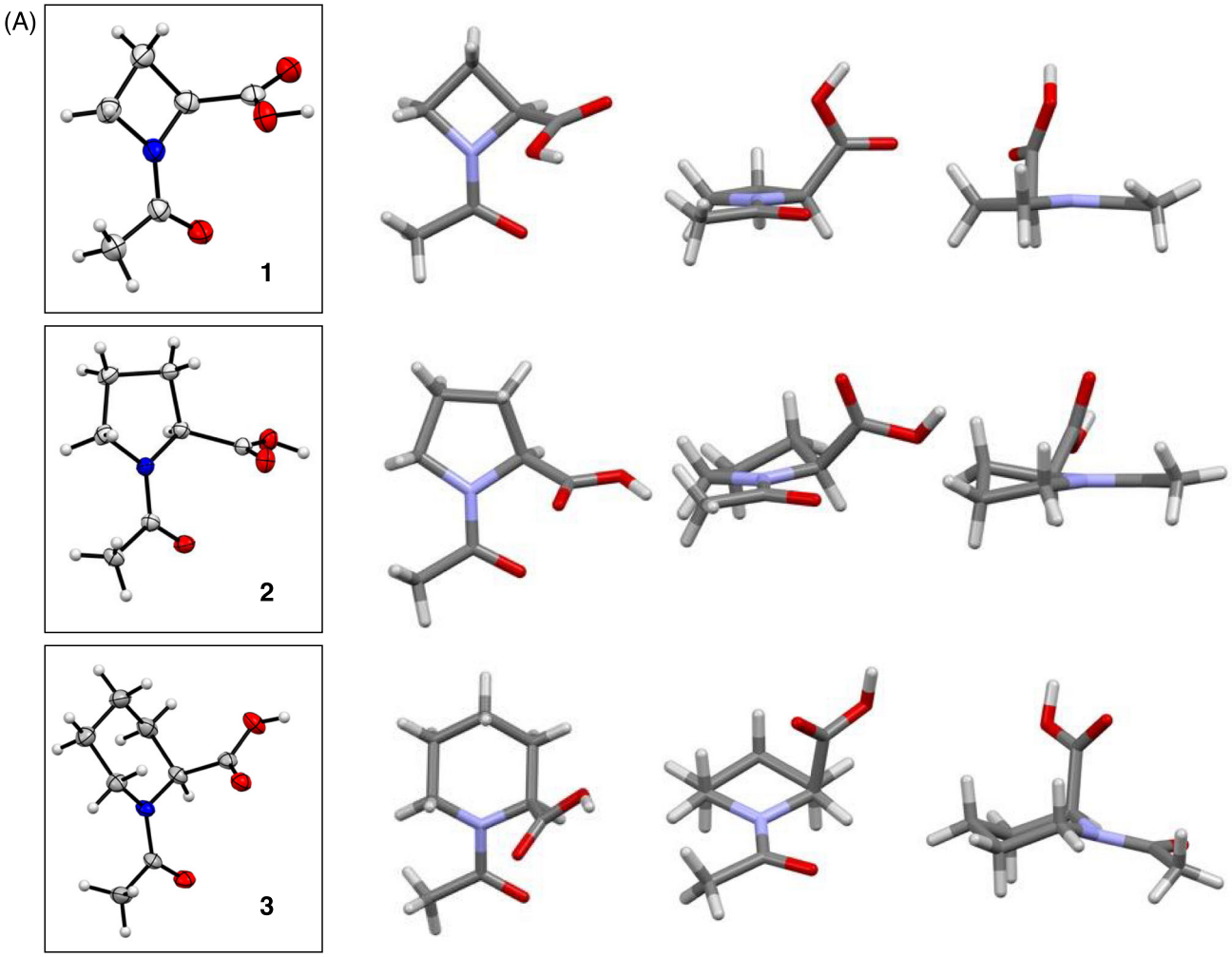


FIGURE 2 Crystal lattices of (A) **1**, (B) **2**, and (C) **3** in different orientations with H-bonds indicated as dotted lines.

nucleophile onto a carbonyl group ($\theta \approx 105°$).[21,58] The $n \rightarrow \pi^*$ interaction is further supported by a pyramidalization of the carbon of the carboxylic acid moiety of $\Delta = 0.023$ Å (Figure 3A). This pyramidalization is greater compared with those reported for other carboxylic acids (e.g., acetic acid: $\Delta = 0.004$ Å)[59] and similar to the values observed in crystal structures of PPII helical peptides that are stabilized by $n \rightarrow \pi^*$ interactions.[10]

Ac–Aze–OH (**1**) crystallized with an almost planar azetidine ring and endocyclic torsion angles of about 3° (Figure 3). This planarity indicates an interplay of Baeyer strain,[60] which is typical for four-membered rings and the strain induced by the endocyclic amide bond with a partial double bond character. The ring strain causes a greater pyramidalization of the amide nitrogen, $\Delta(N) = 0.074$ Å, compared to that of Pro derivative **2**, $\Delta(N) = 0.024$ Å. The planar geometry of the azetidine ring constrains the positions of the substituents and results in symmetric τ angles of $\tau^1 \approx \tau^2 \approx 60°$ relative to the ring plane (Figure 3D). These conformational restrictions are reflected in dihedral angles of $\phi = -70°$ and $\psi = -24°$ that deviate significantly from those of **2** and do not allow for an $n \rightarrow \pi^*$ interaction between the acetyl group and the carboxylic acid (d [C═$\mathbf{O}_{Ac}$··· **C**═O_{COOH}] = 3.28 Å, θ [C═$\mathbf{O}_{Ac}$··· **C**═$\mathbf{O}_{COOH}$] = 124°, Δ[$\mathbf{CO}_2$H] = 0.017 Å).

In the crystal structure of Ac–Pip–OH (**3**), the piperidine ring adopts a chair-like conformation with characteristic endocyclic torsion angles of about 55°. The carboxylic acid substituent is in an axial position, which avoids allylic strain between the amide oxygen and the carboxylic acid moiety at C^α (Figure 3D).[61,62] Such allylic strain cannot be avoided in the case of Aze due to the planarity of the four-membered ring. In Pro derivatives, a balance between allyl strain and stabilization through the $n \rightarrow \pi^*$ interaction is realized with angles of $\tau^1 = -47°$ and $\tau^2 = 64°$. The conformation of the piperidine ring with dihedral angles of $\phi = -89°$ and $\psi = 169°$ does not allow for an $n \rightarrow \pi^*$ interaction (d = 3.34 Å, $\theta \approx 83°$, $\Delta = -0.016$ Å). The piperidine ring affects the pyramidalization of the amide nitrogen, which is significantly higher $\Delta(N) = -0.119$ Å and in the opposite direction compared with that of the other analogs. The nitrogen lone-pair points away from the carboxylic acid substituents, presumably reducing the strain between the nitrogen lone pair and the carboxylic acid substituent.



	ω [°]	ϕ [°]	ψ [°]	θ [°]	d [Å]	Δ(CO$_2$H) [Å]	Δ(N) [Å]	τ^1 [°]	τ^2 [°]
Ac-Aze-OH **1**	174	–70	–24	124	3.28	0.017	0.074	–56	60
Ac-Pro-OH **2**	178	–48	143	92	2.71	0.023	0.024	–47	64
Ac-Pip-OH **3**	177	–89	–169	83	3.34	0.016	–0.119	–83	23

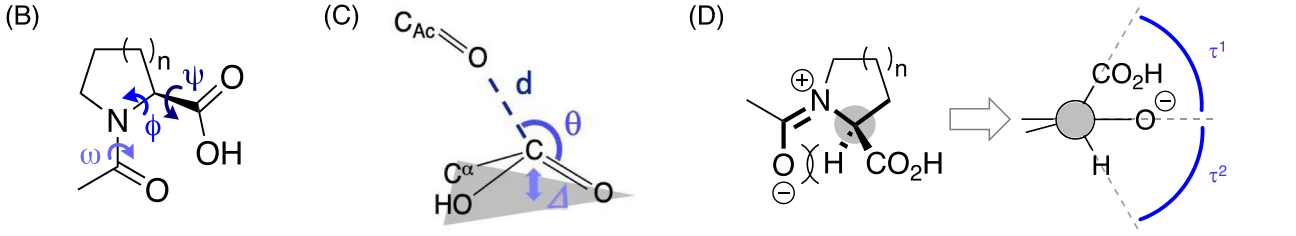


FIGURE 3 (A) Crystal structures of compounds **1**–**3**. Left: ORTEP representations (50% electron probability); right: capped stick representation of **1**–**3** in three different orientations. Below: Summary of distances, angles, and torsions found in the crystal structures of **1**–**3**. Illustration of the dihedral angles ϕ, ψ, and ω (B) and the key indicators of an $n \rightarrow \pi^*$ interaction (C) and allylic strain (D).

4 | CONCLUSIONS

The crystal structures of Ac–Aze–OH **1**, Ac–Pro–OH **2**, and Ac–Pip–OH **3** provided insight into the conformation of proline and its larger and smaller ring-size homologs. All three amino acids crystallized as *trans*-conformers with a related intermolecular H-bonding network, thus allowing for comparisons of their structures. The results show that the geometry of these cyclic amino acids is controlled by a balance between Baeyer strain, allylic strain, and the $n \rightarrow \pi^*$ interaction. Baeyer strain and allylic strain, respectively, are the predominant

forces that control the conformation of the four- and six-membered Aze and Pip derivatives. The five-membered ring of Pro is the least constrained and allows for stabilization of the *trans*-conformer through the $n \rightarrow \pi^*$ interaction, which compensates for unfavorable allylic strain. This flexibility is likely the reason why nature evolved proline, but not Aze or Pip, into an essential proteinogenic amino acid.

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CONFLICT OF INTEREST

The authors declare no competing interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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