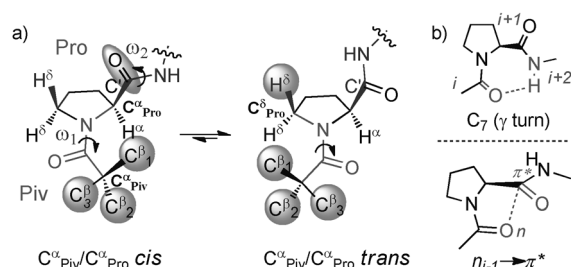


Crystal-Structure Analysis of *cis*-X-Pro-Containing Peptidomimetics: Understanding the Steric Interactions at *cis* X-Pro Amide Bonds**

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The *cis* conformer of the Piv-Pro (Piv = pivaloyl) tertiary amide bond (Scheme 1 a) is representative of a large conformational space in peptide science that could not be accessed. It was believed that van der Waals distance clashes between the *t*Bu group of the Piv group and the carbonyl



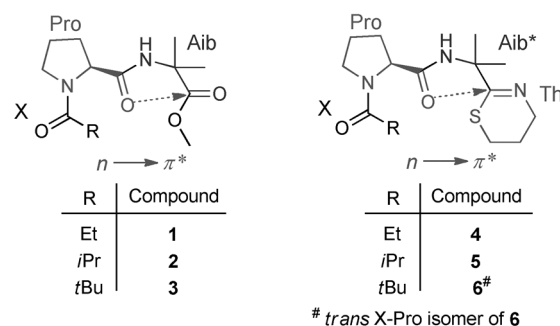
Scheme 1. a) Steric interactions in the *cis* and *trans* isomers of the Piv-Pro tertiary amide bond. b) Electronic interactions that stabilize the *trans* isomer of the X-Pro tertiary amide bond.

substituent on the C_{Pro}^α atom are unavoidable in this conformer.^[1] The failure to observe the *cis* Piv-Pro conformer in solution or in crystal structures of any peptides^[2] thus far has generally precluded the verification of this hypothesis, but has indirectly supported it. In this study, we accessed the *cis* conformer of the Piv-Pro tertiary amide bond in the crystalline state. We show that even the constrained molecule Piv-Pro-Aib-OMe (**3**) containing the stereochemically restricted Pro-Aib sequence, which imparts rigidity through its β -bend ribbon conformation^[3] to several biologically relevant antibiotics,^[4] ionophores,^[5] and action-potential inducers in lipid membranes,^[6] is sufficiently flexible to endure bond distortions so as to avoid unfavorable van der Waals contacts in the *cis* Piv-Pro rotamer. It is rather the mandatory distortion of the peptide bond to the carbonyl group of Pro and the loss in resonance energy associated with this distortion that destabilizes the *cis* Piv-Pro conformer. In peptides in which this peptide-bond distortion is accommo-

dated, the *cis* conformer of any X-Pro tertiary amide bond is accessible.

Only two isomeric states, namely, the *cis* and the *trans* states, are detectable for the X-Pro tertiary amide bond (Scheme 1 a). It is known that short-range electronic effects centered at the Pro residue, such as $i+2 \rightarrow i$ (C_7 or γ turn) hydrogen-bonding interactions^[1e,h] and $n_{i-1} \rightarrow \pi^*$ interactions^[7] (Scheme 1 b), contribute to the selective stabilization of the X-Pro tertiary amide bond in the *trans* isomer. We hypothesized that we could augment the chances of crystallizing the *cis* isomer by designing peptides in which such *trans*-stabilizing electronic interactions are disrupted and hence the fraction of the peptide that exists as the *trans* isomer is minimized.

Aib is a constrained amino acid that promotes 3_{10} - and α -helical conformations and strong $n_{i-1} \rightarrow \pi^*$ interactions about itself.^[3,4c,8] We reasoned that the formation of an $n_{i-1} \rightarrow \pi^*$ interaction between the lone electron pair (n) on the oxygen atom of Pro (O_{Pro}) and the π^* orbital of the carbonyl group of Aib (C_{Aib}') in the molecules R-CO-Pro-Aib-OMe **1–3** (Scheme 2) would distort the Pro-Aib peptide group from



Scheme 2. Structures of peptides **1–3** and peptidomimetics **4–6**.

the ideal geometries required for the formation of *trans*-X-Pro-stabilizing electronic interactions. The Aib ester carbonyl group is a good electrophile and can accept strong $n_{i-1} \rightarrow \pi^*$ interactions.^[7a,8c-e,9] Three other peptidomimetics, **4–6**, with the structure R-CO-Pro-Aib*-Thi (Thi = 5,6-dihydro-4H-1,3-thiazine; Scheme 2) were also studied; the Thi group is an isostere of an ester group, but its carbonyl group is a weaker electron acceptor.

Compounds **1–3** indeed crystallized from a mixture of EtOAc and hexanes (3:1). In the crystal structures,^[10] the X-Pro tertiary amide bond is in the $C_X^\alpha/C_{\text{Pro}}^\alpha$ *cis* form in all compounds, including **3** (Figure 1). There are several similarities between the crystal structures of **1** and **3** (Scheme 3 and Figure 2 a). The C_X^β atom is approximately eclipsed with the

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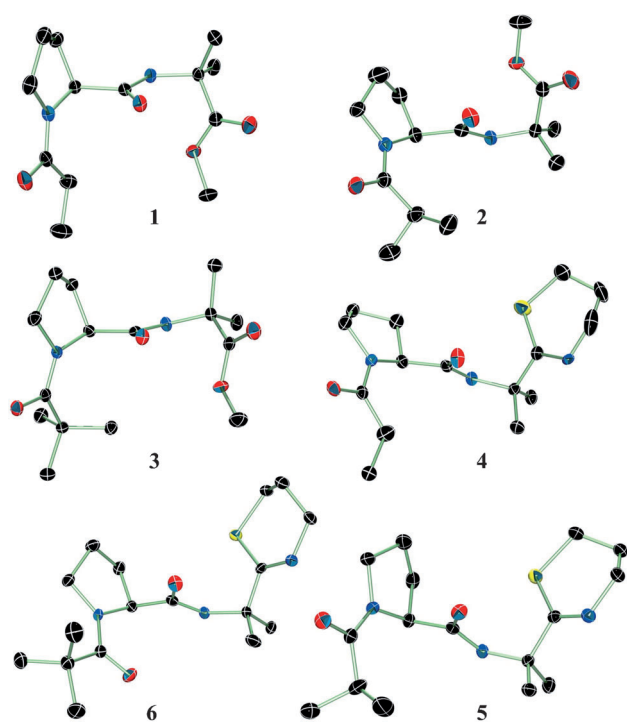
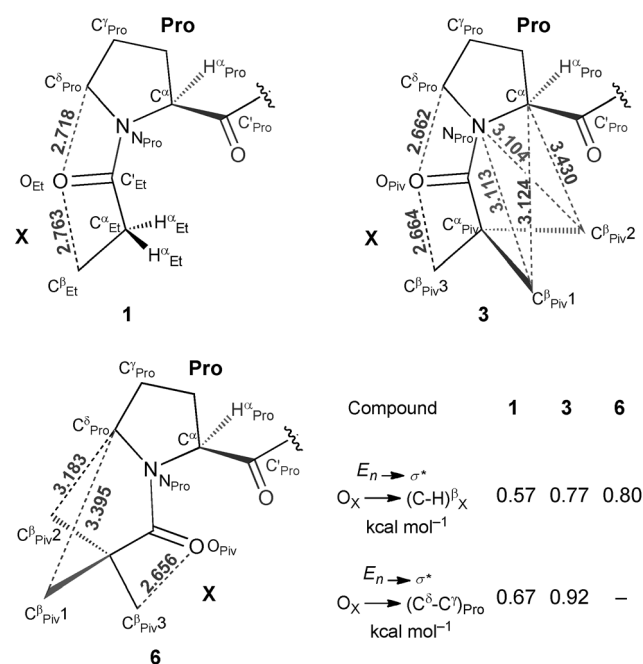


Figure 1. ORTEP/POV-Ray rendition of the crystal structures of compounds **1–6** (C black, N blue, O red with blue, S yellow with blue).



Scheme 3. Relevant interatomic distances in the crystal structures of **1**, **3**, and **6**. The table shows the energies of the significant $n \rightarrow \sigma^*$ interactions in **1**, **3**, and **6**, as calculated by using the NBO paradigm.

O_X atom in both **1** and **3**. The *cis* X-Pro tertiary amide bonds in **1** and **3** are distorted similarly away from the ideal resonance plane (by -8.26° and -10.81° , respectively).^[11] The $(\phi, \psi)_{Pro}$ dihedral angles in **1** (-73.7° , 166.1°) and **3** (-86.7° , 162.9°) are comparable to those found for Pro in the $i+2$

position of type VIb β turns (-75° , 160°),^[12] and the pyrrolidine rings adopt a C^β_{exo} puckered conformation;^[13] these geometries all favor the *cis* X-Pro rotamer.^[14] The *cis* tertiary amide bonds are stabilized by continuous intermolecular hydrogen bonds between the O_X and $(NH)_{Aib}$ atoms of neighboring molecules.^[15] The Aib moiety adopts a left-handed α -helical conformation. Hence, if it is assumed that the differences in the crystal-packing energies are negligible, the structural differences between **1** and **3** can be taken to directly reflect the consequences of steric effects resulting from the replacement of the two hydrogen atoms H^α_{Et} of **1** with two methyl (C^β_{Piv}, H_3) groups in **3**. On the other hand, peptide **2** contains Aib in a right-handed α -helical conformation, which precludes such direct structural correlations with **3**.

In **3**, all bonds are distorted within allowed limits, and the bond lengths are longer than in **1**. The turn in the backbone conformation of these molecules is wider in **3**, as indicated by the significant increase in the $C^\alpha_X \cdots O_{Est}$ distance (Est = ester) to 5.01 Å in **3** (3.98 Å in **1**; Table 1). As a result, all bond

Table 1: Relevant interatomic distances in compounds **1**, **3**, and **6**.

Atoms	1 [Å]	3 [Å]	6 [Å]
$C^\alpha_X \cdots C^\alpha_{Pro}$	2.888	3.119	3.845
$C^\alpha_X \cdots C^\alpha_{Pro}$	3.163	3.337	2.953
$C^\alpha_X \cdots O_{Est}$	3.983	5.097	–
$O_{Pro} \cdots C^\alpha_{Aib}$	2.777	2.658	3.036

angles facing the inside of the turn increase in **3** (relative to those in **1**) by up to about 4.1° , and those along the outside of the turn decrease by up to 5° .^[15] The prolyl tertiary-amide oxygen atom (O_X) is more proximal to both C^β_X and C^δ_{Pro} in **3** than in **1** (Scheme 3). DFT calculations based on the natural bond orbital (NBO) paradigm^[16] indicate that the n orbital of O_X is involved in improved $n \rightarrow \sigma^*$ interactions^[15] with adjacent σ^* orbitals (of the $(C-H)^\beta_X$ and $(C^\delta-C^\gamma)_{Pro}$ bonds) in **3** relative to the equivalent interactions in **1**.

There are three methyl substituents on the C^α atom of the Piv group in **3** (Scheme 3). One ($C^\beta_{Piv,3}$) is eclipsed with the Pro C^δ atom (as in **1**), one ($C^\beta_{Piv,2}$) is oriented in the same direction as the Pro H^α atom, and one ($C^\beta_{Piv,1}$) is oriented in the same direction as the larger Pro carbonyl group. Examination of the nonbonding distances in **3** reveals that the carbonyl substituent on the Pro C^α atom exerts no greater steric influence on the $C^\beta_{Piv,1}$ group than does the Pro H^α atom on the $C^\beta_{Piv,2}$ group. Duly, the $C^\beta_{Piv,1} \cdots C^\alpha_{Pro}$ distance (3.124 Å) is shorter than the $C^\beta_{Piv,2} \cdots C^\alpha_{Pro}$ distance (3.430 Å), and $C^\beta_{Piv,1}$ and $C^\beta_{Piv,2}$ are at similar distances from N_{Pro} .^[15] Thus, the 1,2-torsional and the 1,3-steric strain exerted by the methyl substituents of Piv on N_{Pro} and C^α_{Pro} , respectively, do not significantly influence the steric effects in the *cis* rotamer of the Piv-Pro tertiary amide bond.

Comparison of the crystal structures of **3** and **6**, in which the Piv-Pro tertiary amide bond is in the *cis* and the *trans* form, respectively, shows similarities between the nonbonding distances $C^\alpha_{Piv} \cdots C^\delta_{Pro}$ and $C^\beta_{Piv} \cdots N_{Pro}$ in **6** and $C^\alpha_{Piv} \cdots C^\alpha_{Pro}$ and $C^\beta_{Piv} \cdots N_{Pro}$ in **3**.^[15] In other words, the relative positions of the

methyl substituents of the Piv group with respect to the Pro ring are similar in the *cis* and *trans* forms. In fact, the overall bond distortions are similar in the *cis* and *trans* Piv-Pro rotamers. Hence, the consequence of the steric strain in the X-Pro tertiary amide bond is that the bond geometries in the molecule undergo distortions to maximize the interatomic distances in general and not those between specific atoms in the vicinity of the strain. So what is the steric effect that differentiates the stabilities of the *cis* and *trans* Piv-Pro rotamers?

A distinct structural feature in these *cis* X-Pro conformers is that the Pro-Aib peptide bond, which is near-planar in **1** (177.7°), is increasingly distorted out of the ideal resonance plane in **2** (173.2°) and in **3** (166.7° ; Figure 2a). The lengthening of the $C'_{\text{Pro}}-N_{\text{Aib}}$ amide bond by $0.013 \text{ \AA}$ in **2** and $0.017 \text{ \AA}$ in **3**, relative to that in **1**, is in agreement with such distortions. A 90° rotation about the amide $C'-N$ bond from its ideal plane is known to lengthen it by approximately $0.08 \text{ \AA}$, with an associated destabilization energy of

$-21.0 \text{ kcal mol}^{-1}$ in *N,N*-dimethylformamide.^[17] Large distortions are not uncommon for the torsion angle ω of Pro-Aib peptide bonds,^[3] as seen in the well-characterized crystal structures of terminally blocked (Pro-Aib)_{*n*} sequential peptides.^[15] In this study, the formation of two intramolecular $N-H\cdots O=C$ ($i+2\rightarrow i$ or $i+3\rightarrow i$) hydrogen bonds assist in constraining these high-energy folds in γ - or β -turn conformations, respectively, in which the X-Pro tertiary amide bond adopts the *trans* conformation.^[11 f,g, 3, 4c, 9b-f, k, 18] Identical hydrogen-bond assistance is not structurally possible when the X-Pro tertiary amide bond is in the *cis* conformation, especially in **3**, which contains a C-terminal methyl ester rather than an amide. More notably, in the *cis* X-Pro conformation, the $O_{\text{Pro}}\cdots C'_{\text{Piv}}$ hard-sphere repulsion^[19] also contributes to the Pro-Aib peptide-bond distortions. Whereas this mandatory repulsion destabilizes the *cis* Piv-Pro rotamers in most peptides, it is accommodated in **1–3**.

In the crystal structures of peptides **1** and **3**, these peptide-bond distortions are stabilized by $n_{i-1}\rightarrow\pi^*$ interactions at Aib. The carbonyl oxygen atom (O_{Pro}) of the distorted Pro-Aib peptide bonds and the Aib carbonyl carbon atom (C'_{Aib}) are at distances well within the Bürgi–Dunitz trajectory,^[20] as prescribed to be essential for efficient $O_{\text{Pro}}\rightarrow C'_{\text{Aib}}$ ($n_{i-1}\rightarrow\pi^*$) interactions. The $O_{\text{Pro}}-C'_{\text{Aib}}$ distance is shorter in **3** ($2.658 \text{ \AA}$) than in **1** ($2.777 \text{ \AA}$; Table 1), which indicates a stronger interaction in **3** than in **1**. NBO analysis was in agreement with these conclusions drawn from the crystal structures and revealed the $n_{i-1}\rightarrow\pi^*$ interaction energy in **3** ($3.53 \text{ kcal mol}^{-1}$) to be more than double that in **1** ($1.68 \text{ kcal mol}^{-1}$). Moreover, the ϕ_{Aib} dihedral angles are more significantly distorted from the ideal α -helical values ($(\phi, \psi)_{\text{Aib}} = 57^\circ, 47^\circ$) in **3** ($43.6^\circ, 50.4^\circ$) than in **1** ($52.2^\circ, 39.8^\circ$). The $O_{\text{Pro}}\rightarrow C'_{\text{Aib}}$ interaction appears to be central to the accommodation of such large distortions in ϕ_{Aib} and in the Pro-Aib peptide plane (ω_2) in **3**.

It is interesting that distortions in the Pro-Aib peptide plane favor higher-energy $n_{i-1}\rightarrow\pi^*$ interactions in **3**. These interactions distinctly facilitate the crystallization of these peptides with the X-Pro tertiary amide bond in the *cis* form: they enable the accommodation of the distortions in the Pro-Aib peptide bond, which are essential structural artifacts of the *cis* conformer, and the $n_{i-1}\rightarrow\pi^*$ interaction centered at Aib engages the Pro-Aib peptide group and hence competes against and disrupts the *trans*-stabilizing C_7 hydrogen bond and the $n_{i-1}\rightarrow\pi^*$ interactions centered at Pro. Consequently, crystallization of the *cis* X-Pro form must be favored in all molecules in which such Pro-NHR' peptide-bond distortion is accommodated through similar electronic effects.

The analogous peptidomimetics **4** and **5** (Scheme 2) also crystallized in the *cis* form,^[21] with similar intermolecular hydrogen-bonding interactions, distorted Pro-Aib* peptide bonds, and intramolecular ($O_{\text{Pro}}\rightarrow C'_{\text{Thi}}$) $n_{i-1}\rightarrow\pi^*$ interactions centered at Aib*.^[15] The energy of the $n_{i-1}\rightarrow\pi^*$ interaction centered at Aib* in **4** ($1.36 \text{ kcal mol}^{-1}$), however, is slightly less than that of the equivalent interaction centered at Aib in **1** ($1.68 \text{ kcal mol}^{-1}$), owing to the weaker electrophilicity of the Thi carbonyl group relative to that of the ester carbonyl group. For the same reason, the corresponding energy in **5** ($0.49 \text{ kcal mol}^{-1}$) is far less than that in the ester **2** ($2.19 \text{ kcal mol}^{-1}$). More notably, **6** crystallized only in the *trans*

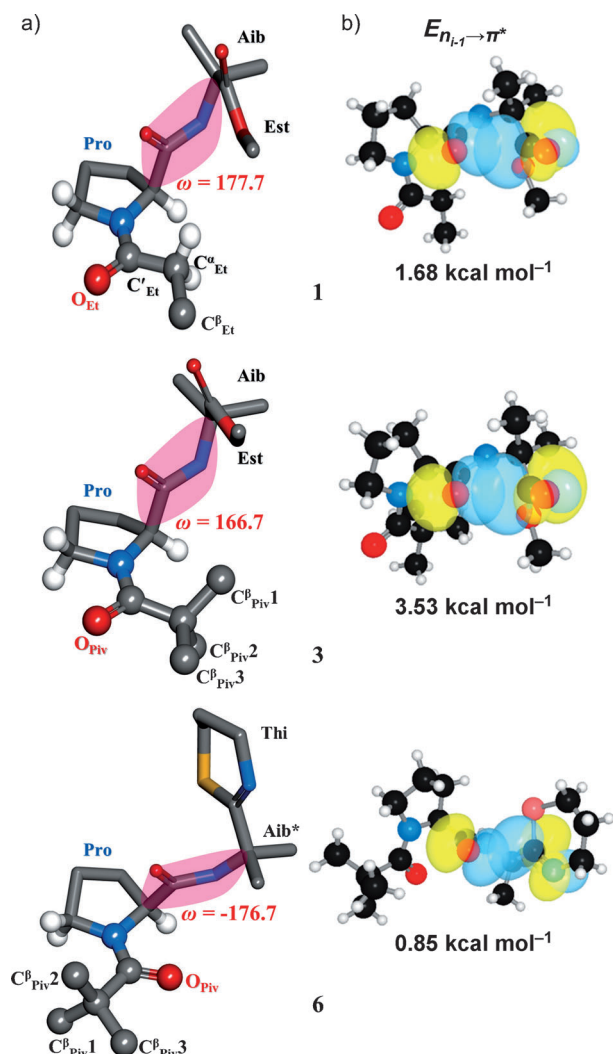


Figure 2. a) Ball-and-stick representation of compounds **1**, **3**, and **6**; the torsion angles of the Pro-Aib peptide bond are highlighted. b) NBO View 1.0 rendering of the $n_{i-1}\rightarrow\pi^*$ ($O_{\text{Pro}}\rightarrow C'_{\text{Aib}}$) interactions in **1**, **3**, and **6**.

conformation, which indicates that the $n_{i-1} \rightarrow \pi^*$ interactions in the *cis* form of **6** is absent or not strong enough to counter the *cis*-destabilizing steric effects involving the bulky *t*Bu group. The correlation between the accommodation of distortions in the Pro–NHR peptide bond and the manifestation of the *cis* X-Pro conformer in these crystal structures identifies these noncovalent intramolecular interactions involving the carbonyl group of Pro ($O_{\text{Pro}} \rightarrow C'_{\text{Thr}}$) as new control elements for accessing the *cis* X-Pro (including Piv-Pro) conformers.

In conclusion, we have shown through systematic crystal-structural correlations that the steric interactions in *cis* X-Pro conformers are similar to those in the *trans* conformers and involve the optimization of interatomic distances in the peptide. It is the large distortion of the Pro–NHR peptide bond, which occurs selectively in the *cis* X-Pro conformer owing to hard-sphere $C_{\alpha}^{\text{X}} \cdots O_{\text{Pro}}$ repulsions, that mainly differentiates the steric interactions in the *cis* and the *trans* conformers. This mandatory peptide-bond distortion cannot be alleviated by simple bond distortions in the peptide. However, electronic interactions that can compensate for the associated loss in resonance energy provide access to the *cis* conformers of X-Pro (including Piv-Pro) tertiary amide bonds. This finding lays the foundation for accessing the *cis* conformers of sterically hindered tertiary amide bonds, such as Piv-Pro, and is a considerable step forward in foldamer control and for peptide-drug design, especially since the Piv-Pro tertiary amide bond is a close mimic of the Aib-Pro tertiary amide bond, which is found extensively in biologically relevant peptides.^[3–6, 9b, f, 18, 22] Furthermore, this study provides clear insight into the fundamental characteristics of steric interactions in the *cis* and *trans* rotamers of X-Pro tertiary amide bonds and a way to control these interactions.

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