An electronic effect on protein structure

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Abstract

The well-known preference of the peptide bond for the *trans* conformation has been attributed to steric effects. Here, we show that a proline residue with an *N*-formyl group (H_{i-1}–$C'_{i-1}=O_{i-1}$), in which H_{i-1} presents less steric hindrance than does O_{i-1}, likewise prefers a *trans* conformation. Thus, the preference of the peptide bond for the *trans* conformation cannot be explained by steric effects alone. Rather, an $n \rightarrow \pi^*$ interaction between the oxygen of the peptide bond (O_{i-1}), and the subsequent carbonyl carbon in the polypeptide chain (C'_i) also contributes to this preference. The O_{i-1} and C'_i distance and $O_{i-1}\cdots C'_i=O_i$ angle are especially favorable for such an $n \rightarrow \pi^*$ interaction in a polyproline II helix. We propose that this electronic effect provides substantial stabilization to this and other elements of protein structure.

Keywords: Bürgi–Dunitz trajectory; collagen; hyperconjugation; peptide bond; polyproline II helix; proline isomerization; stereoelectronic effect; steric effect

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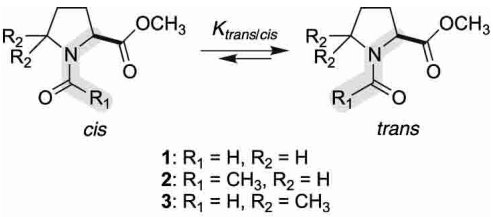
In folded proteins, only 0.03% of Xaa_{i-1}–$nonPro_i$ peptide bonds are in the *cis* conformation (Stewart et al. 1990; Weiss et al. 1998; Jabs et al. 1999). This prevalence is 5.2% for Xaa_{i-1}–Pro_i peptide bonds. The greater stability of *trans* peptide bonds is assumed to arise solely from a steric effect (Schulz and Schirmer 1979; Creighton 1993; Kyte 1995; Fischer 2000). Simply put, the C^{α} substituents of each amino acid are forced into clashing proximity in the *cis* isomer, whereas this steric strain is relieved in the more pervasive *trans* isomer. The isomers of a prolyl peptide bond are more nearly isoenergetic, presumably because the C^{δ} protons of the pyrrolidine ring provide nearly as much steric encumbrance as do the C^{α} substituents.

Are steric effects alone responsible for the observed *trans*:*cis* ratio of prolyl peptide bonds? To answer this question, we synthesized *N*-formyl-L-proline methyl ester (FmProOMe; **1**; Scheme 1). The amide bond in amide **1** is isologous to a prolyl peptide bond, except that the



Scheme 1

steric effects in amide **1** are reversed, favoring the *cis* rather than the *trans* isomer. This reversal occurs because the van der Waals radius of oxygen is greater than that of hydrogen and the $C'_{i-1}=O_{i-1}$ bond is longer than the C'_{i-1}–H_{i-1} bond (Fig. 1A). The synthesis of amide **1** as a methyl ester rather than a secondary amide avoids intramolecular hydrogen bonding to form a γ-turn, as has been observed in *N*-acetylproline (Madison and Schellman 1970; DeTar and Luthra 1977) and *N*-acetylproline *N*-methylamide (Matsuzaki and Iitaka 1971; Higashijima et al. 1977; Liang et al. 1992; Benzi et al. 2002).

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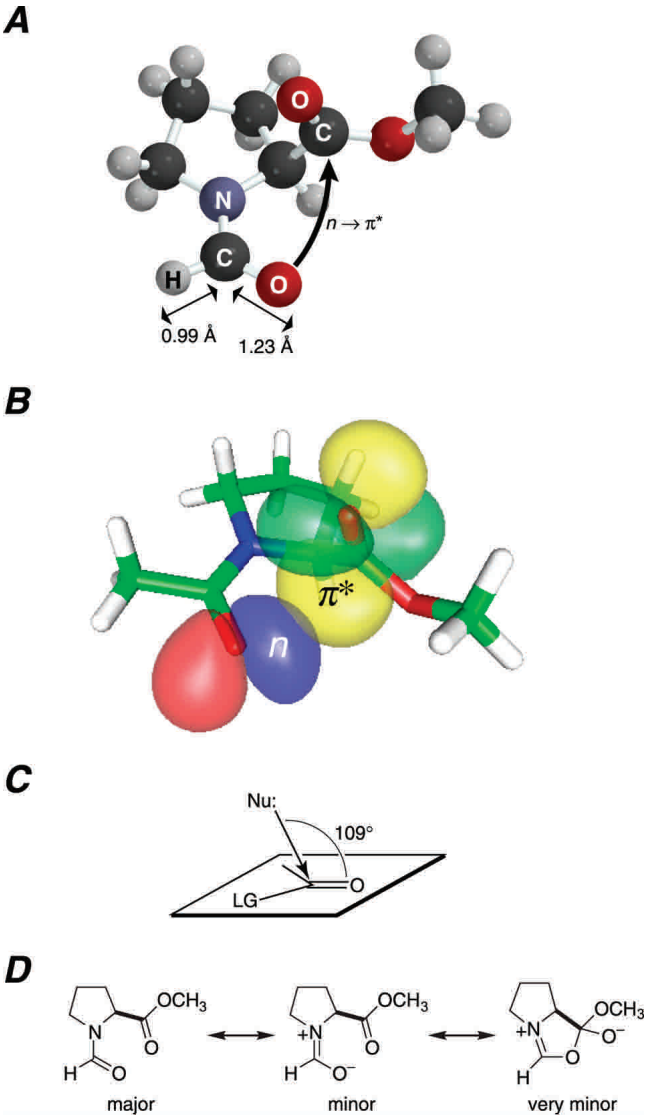


Figure 1. $n \rightarrow \pi^*$ Interaction between O_{i-1} and C'_i. (*A*) Structure of the *trans* isomer of *N*-formyl-L-proline methyl ester (**1**) in its C^γ-*exo* conformation. The C'_{i-1}–H and $C'_{i-1}=O_{i-1}$ bond lengths are from the structure of crystalline dimethyl formamide (Borrmann et al. 2000). (*B*) Depiction of the *n* and π^* natural bond orbitals of the *trans* isomer of *N*-acetyl-L-proline methyl ester (**2**) in its C^γ-*exo* conformation. The $O_{i-1}\cdots C'_i$ distance is δ_{BD} = 2.87 Å and the $O_{i-1}\cdots C'_i=O_i$ angle is τ_{BD} = 99.35° (DeRider et al. 2002). (*C*) Bürgi–Dunitz trajectory for the attack of a nucleophile on a carbonyl group to displace a leaving group (LG; Bürgi et al. 1973, 1974a,b; Bürgi and Dunitz 1983; Eliel and Wilen 1994). (*D*) Major and minor resonance forms of amide **1** (Pauling 1960) and a very minor structure that arises from the hyperconjugative delocalization of an $n \rightarrow \pi^*$ interaction.

In addition to diminishing steric effects, we also sought to enhance those effects by increasing the steric bulk on either side of the amide bond of amide **1**. Specifically, replacing the formyl proton with a methyl group yields *N*-acetyl-L-proline methyl ester (AcProOMe; **2**; Scheme 1). Replacing the two C^δ protons with methyl groups yields *N*-formyl-5,5-dimethyl-L-proline methyl ester (Fm[dmPro]OMe; **3**). We synthesized amides **2** and **3**, and compared their values of $K_{trans/cis}$ = [*trans*]/[*cis*] to that for amide **1**. The resulting data reveal the manifestation of a previously unappreciated electronic effect on protein structure.

Results and Discussion

Existence of an electronic effect

Steric repulsion is perhaps the most well-known factor in molecular conformational stability. Based on steric considerations alone, the value of $K_{trans/cis}$ for amide **1** should be <1. Yet, we find that the value of $K_{trans/cis}$, as determined by ^{1}H-NMR spectroscopy, is actually >1 in water, dioxane, and chloroform (Table 1, Fig. 2). This result provides the first experimental evidence that steric effects alone are not the sole determinant of the preference of a peptide bond for the *trans* conformation.

What is the explanation for the value of $K_{trans/cis}$ for amide **1** being >1? In a peptide bond, the oxygen (O_{i-1}) bears a partial negative charge, and the carbon (C'_i) bears a partial positive charge (Pauling 1960; Momany et al. 1975; Zimmerman and Scheraga 1976). The favorable Coulombic interaction between O_{i-1} and C'_i could, of course, increase the value of $K_{trans/cis}$ (Zimmerman and Scheraga 1976). The true picture is, however, more complex.

No less important than steric effects in dictating molecular conformation are the stabilizing effects of hyperconjugative delocalization (Cramer 1998; Weinhold 2001). A familiar manifestation of hyperconjugation in a biomolecule is the "anomeric effect," which arises from the delocalization of a nonbonding pair of electrons (*n*) from the ring oxygen of pyranose sugars to the σ^* orbital of the adjacent C–O bond (Deslongchamps 1983; Petillo and Lerner 1993). This $n \rightarrow \sigma^*$ interaction stabilizes the α anomer.

We have analyzed in detail the conformational energetics of amides like **1** with density functional theory calculations

Table 1. *Values of* $K_{trans/cis}$ *for amides 1–3*

	Solvent		
Amide	D_2O	Dioxane-d_8	$CDCl_3$
FmProOMe (**1**)	1.8	1.4	1.4
AcProOMe (**2**)	5.3	4.2	4.0
Fm(dmPro)OMe (**3**)	25	9	13

Values of $K_{trans/cis}$ (±20%) were determined by integration of ^{1}H-NMR spectra obtained at 25°C. Resonances of the *trans* and *cis* isomers were assigned by using NOESY 1D spectroscopy with observed NOEs between the formyl (C'_{i-1}–H) or acetyl (C^α_{i-1}–H) and α (C^α_i–H) or δ (C^δ_i–H) protons.

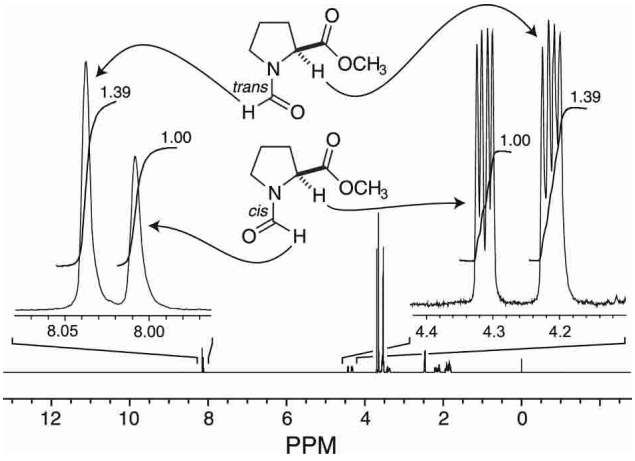


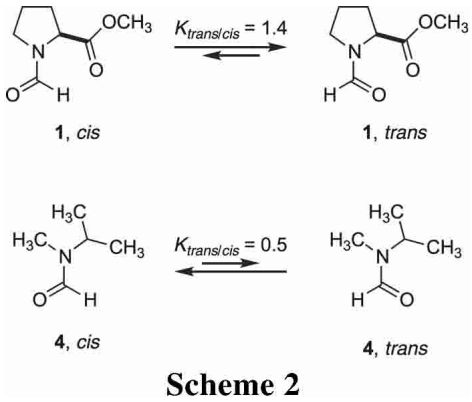
Figure 2. ^{1}H NMR spectrum of *N*-formyl-L-proline methyl ester (**1**) in dioxane-d_8 at 25°C. Values of $K_{trans/cis}$ were determined from integration of the indicated resonances.

using the natural bond orbital (NBO) paradigm (DeRider et al. 2002). These previous calculations reveal that the interaction between O_{i-1} and C'_i has a large dependence on the $O_{i-1}\cdots C'_i=O_i$ angle as well as the $O_{i-1}\cdots C'_i$ distance, and is better described by quantum mechanics than by simple electrostatics. More precisely, the increase in the value of $K_{trans/cis}$ arises from extensive hyperconjugative delocalization of a nonbonding pair of electrons (*n*) from the amide oxygen to the π^* orbital of the ester carbon (Fig. 1B).

The $n \rightarrow \pi^*$ interaction in amide **1** is reminiscent of the Bürgi–Dunitz trajectory of organic chemistry, which describes the most favorable approach of a nucleophile (e.g., O_{i-1}) to the carbon of a carbonyl group (e.g., $C'_i=O_i$) during an acyl transfer reaction (Fig. 1C; Bürgi et al. 1973, 1974a,b; Bürgi and Dunitz 1983). Accordingly, the hyperconjugative delocalization that arises from an $n \rightarrow \pi^*$ interaction between O_{i-1} and C'_i can be depicted as a very minor resonance form of amide **1** (Fig. 1D). A similar structure is a likely intermediate during the formation of a 2-oxazolin-5-one, or "azlactone," which is a cleavage product that can arise during the chemical synthesis of peptides (Dakin and West 1928a,b,c; Kemp 1979).

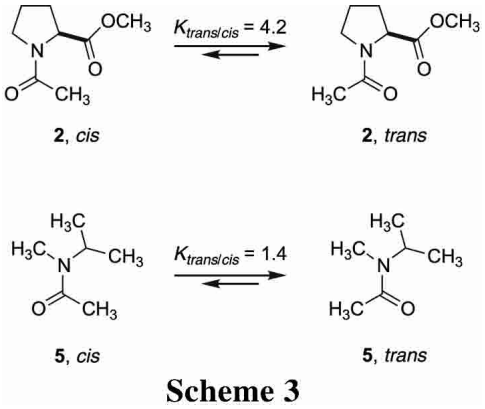
Strength of n → π* *interaction*

The strength of the $n \rightarrow \pi^*$ interaction in amides **1** and **2** can be estimated from $K_{trans/cis}$ values for isologous amides that lack a $C'_i=O_i$ group. The isopropyl and methyl groups of *N*-isopropyl-*N*-methylformamide present steric hindrance to O_{i-1} and H_{i-1} that are similar to those in amide **1** (Scheme 2). In contrast to amide **1**, the *trans* isomer of *N*-isopropyl-*N*-methylformamide (**4**)



Scheme 2

lacks any stabilization from an $n \rightarrow \pi^*$ interaction, and its value of $K_{trans/cis}$ is threefold lower than that of amide **1** (Laplanche and Rogers 1963; Stewart and Siddall III 1970). A comparison of *N*-isopropyl-*N*-methylacetamide and amide **2** yields a similar result (Scheme 3). From these data,



Scheme 3

we estimate that the $n \rightarrow \pi^*$ interaction contributes approximately 0.7 kcal/mole ($= RT\ln 3$) at 25°C to the stability of the *trans* isomer of amides **1** and **2**. This value is in gratifying agreement with the previous density functional theory calculations (DeRider et al. 2002), which indicate that $n \rightarrow \pi^*$ delocalization provides 0.42 kcal/mole of stabilization energy when the pyrrolidine ring of amide **2** is in the C^γ-*endo* (major: 66%) pucker and 1.29 kcal/mole in the C^γ-*exo* (minor: 34%; Fig. 1B) pucker. These calculations likewise assign a value of 0.7 kcal/mole (= 0.42 kcal/mole × 66% + 1.29 kcal/mole × 34%) to the strength of the $n \rightarrow \pi^*$ interaction. It is also noteworthy that the preference for a *trans* isomer of amide **2** has an almost entirely enthalpic origin (Eberhardt et al. 1993), which is consistent with the enthalpic contribution expected from an $n \rightarrow \pi^*$ interaction as well as steric effects. Finally, we recognize that an amide carbon is somewhat less electron-deficient than is an ester carbon. As a consequence, the conformational stability provided by an $n \rightarrow \pi^*$ interaction will be lower, but still

manifested, in an amide. That stability could be similar in magnitude to that from a cation–π interaction (Shi et al. 2002a), another nonclassical noncovalent interaction (Dougherty 1996).

Replacing the formyl or C^{δ} protons of amide **1** with bulkier methyl groups increases steric effects in a manner that should favor the *trans* isomer (Scheme 1). The value of $K_{trans/cis}$ does indeed increase in the order **1** < **2** < **3** (Table 1). We conclude that steric effects are manifested in amides **1**–**3**. Likewise, the identity of the residue preceding a proline residue is a primary determinant of the prevalence of a *trans* prolyl peptide bond (Grathwohl and Wüthrich 1981; Yao et al. 1994), as increasing steric hindrance tends to increase the relative stability of the *trans* isomer in peptides. For example, Gly_{i-1}–Pro_i and Ala_{i-1}–Pro_i peptide bonds have $K_{trans/cis}$ values in water of 6.2 and 12, respectively (Reimer et al. 1998). Finally, 5,5-dimethylproline is known to provide a conformational lock for the *cis* conformation in peptides (An et al. 1999). Clearly, steric effects make a dominant contribution to the preference for the *trans* isomer of peptide bonds.

Implications of n → π* *interaction*

Proline has a unique influence on protein structure (MacArthur and Thornton 1991; Reiersen and Rees 2001). This influence arises largely from the constraint imposed by the pyrrolidine ring on its ϕ torsion angle, which tends to range from −75° in the C^{γ}-*endo* pucker to −60° in the C^{γ}-*exo* pucker (Vitagliano et al. 2001). This constraint preorganizes proline for a favorable $n \rightarrow \pi^*$ interaction (DeRider et al. 2002). Moreover, the subtle modulation of this interaction, as occurs in diastereomers of 4-hydroxyproline and 4-fluoroproline, has been shown to affect the value of $K_{trans/cis}$ (Bretscher et al. 2001; DeRider et al. 2002). An $n \rightarrow \pi^*$ interaction is also likely to increase the value of $K_{trans/cis}$ for Xaa_{i-1}–$nonPro_i$ peptide bonds, albeit to a lesser extent.

The influence of an $n \rightarrow \pi^*$ interaction extends beyond being a determinant of the value of $K_{trans/cis}$. A meaningful $n \rightarrow \pi^*$ interaction can be defined as one in which the $O_{i-1} \cdots C'_i$ distance is $\delta_{BD} \leq 3.2$ Å and the $O_{i-1} \cdots C'_i = O_i$ angle is $99° \leq \tau_{BD} \leq 119°$, which is ±10° of the Bürgi–Dunitz trajectory (Fig. 1C). Such an optimal $n \rightarrow \pi^*$ interaction can exist in a polyproline II helix (P_{II}), right-handed α-helix (α_R), and left-handed α-helix (α_L; Fig. 3A). Each of these secondary structures could gain stability from this electronic effect.

An $n \rightarrow \pi^*$ interaction is likely to be of particular importance to the conformational stability of a P_{II} helix. An ideal P_{II} helix has main-chain dihedral angles ($\phi = -75°$, $\psi = +145°$) that produce a favorable $n \rightarrow \pi^*$ interaction, with $\delta_{BD} = 3.2$ Å and $\tau_{BD} = 103°$ (Fig. 3B). Unlike an α-helix, a P_{II} helix lacks intrastrand hydrogen bonds. Yet, the P_{II} helix is prevalent in polyproline (Isemura et al. 1968; Tiffany and Krimm 1968), has been observed in Lys_7 and Ala_7 peptides (Rucker and Creamer 2002; Shi et al. 2002b), and is a common motif in folded proteins (Siligardi and Drake 1995; Kleywegt and Jones 1996). An $n \rightarrow \pi^*$ inter-

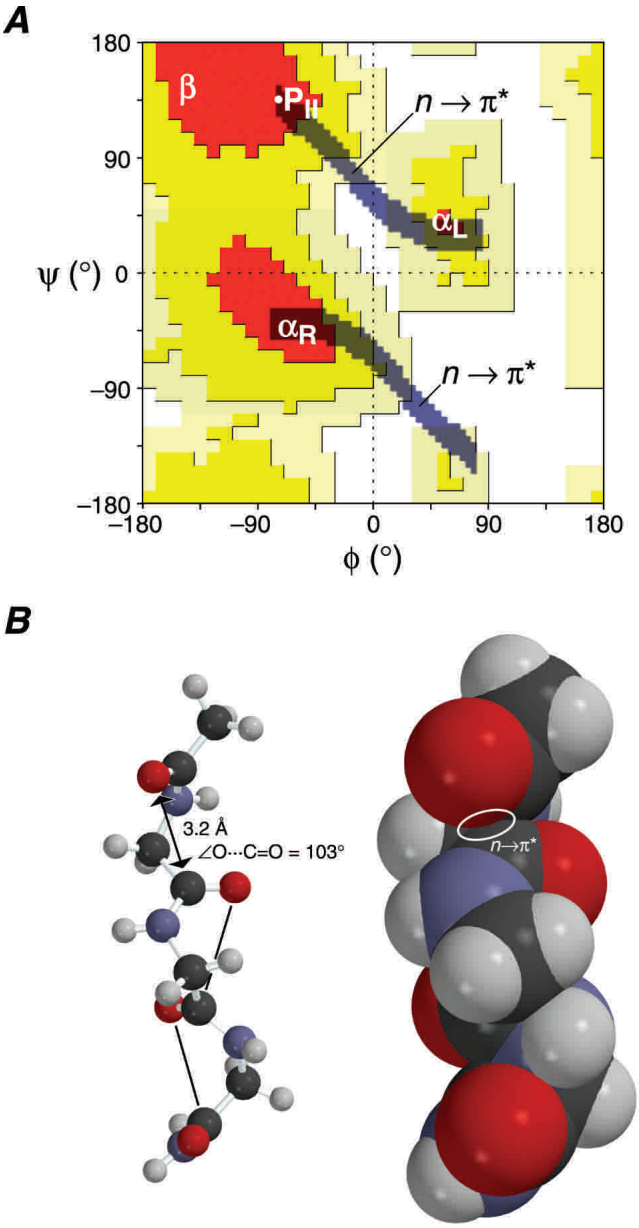


Figure 3. Implications of the $n \rightarrow \pi^*$ interaction between O_{i-1} and C'_i. (*A*) Ramachandran plot (Ramachandran and Sasisekharan 1968; Richardson 1981) showing the two "$n \rightarrow \pi^*$" regions of the *trans* isomer of $AcGlyNH_2$. In these regions, the $O_{i-1} \cdots C'_i$ distance is $\delta_{BD} \leq 3.2$ Å and the $O_{i-1} \cdots C'_i = O_i$ angle is $99° \leq \tau_{BD} \leq 119°$. The white dot indicates the ϕ and ψ angles for an ideal polyproline II helix (*B*). (*B*) Energy-minimized structure of $AcGly_3NH_2$ in the conformation of a polyproline II helix with $\phi = -75°$ and $\psi = +145°$. The structure is depicted as a ball-and-stick (*left*) or space-filling (*right*) model. The $O_{i-1} \cdots C'_i$ distance ($\delta_{BD} = 3.2$ Å) and $O_{i-1} \cdots C'_i = O_i$ angle ($\tau_{BD} = 103°$) is indicated in the ball-and-stick model.

action between O_{i-1} and C'_i does not rely on solvation. Likewise, specific hydration is not critical for the conformational stability of a P_{II} helix, which can form in methanol, trifluoroethanol, propionic acid, and benzyl alcohol (Knof and Engel 1974; Antonyraj et al. 1998; Nakamura et al. 2001). Analogous solvent-independent stereoelectronic effects enhance the conformational stability of the collagen triple helix (Engel and Prockop 1998; Holmgren et al. 1998; Bretscher et al. 2001; Jenkins and Raines 2002), in which each residue has ϕ and ψ torsion angles that are similar to those in a P_{II} helix (Bella et al. 1994).

In the analysis above, we have treated the $C'_i(O_i)$–N_{i+1} amide as merely a ketone. Of course, the immonium resonance form $C'_i(O_i^-) = N_{i+1}{}^+$, which has been estimated to contribute as much as 40% to the structure of an amide (Pauling 1960), must also play a role in determining the most favorable position for O_{i-1} (Baldwin 1976). Ideally, the $O_{i-1}\cdots C'_i = N_{i+1}{}^+$ angle, like the $O_{i-1}\cdots C'_i = O_i$ angle, would be near 109°. Remarkably, the $O_{i-1}\cdots C'_i = N_{i+1}{}^+$ angle in a P_{II} helix is identical to the $O_{i-1}\cdots C'_i = O_i$ angle (Fig. 3B), as both are $\tau_{BD} = 103°$. Thus, a P_{II} helix is stabilized by hyperconjugative delocalization of n from O_{i-1} to the π^* orbitals of both $C'_i = O_i$ and $C'_i = N_{i+1}{}^+$.

A favorable Coulombic interaction between O_{i-1} and C'_i has been proposed to enhance the stability of the right-handed twist of β-strands (Maccallum et al. 1995). The $O_{i-1}\cdots C'_i$ distance ($\delta_{BD} = 3.45$ Å) and $O_{i-1}\cdots C'_i = O_i$ angle ($\tau_{BD} = 125°$) in a right-twisted β-strand ($\phi = -90°$, $\psi = +125°$) are, however, inappropriate for a strong $n \rightarrow \pi^*$ interaction (Fig. 3A). We therefore believe that an interaction between O_{i-1} and C'_i is unable to provide substantial stability to the right-handed twist of β-strands.

Finally, we note that the contribution of an $n \rightarrow \pi^*$ interaction to conformational stability can be cooperative. Both the negative charge on O_i and the $C'_i = O_i$ bond length increase as a result of an $n \rightarrow \pi^*$ interaction between O_{i-1} and C'_i (DeRider et al. 2002). These effects can in turn serve to increase the stabilization provided by an $n \rightarrow \pi^*$ interaction between O_i and C'_{i+1}.

Materials and methods

General

Chemicals and solvents were from Aldrich. Reactions were monitored by thin-layer chromatography using TLC plates (AL SIL G/UV) from Whatman, with visualization by illumination with ultraviolet light or staining with I_2. NMR spectra were obtained with Bruker AC-250, Bruker AC-300, and Varian UNITY-500 spectrometers. Mass spectra were obtained with electrospray ionization (ESI) techniques at the University of Wisconsin Biotechnology Center.

Synthesis of N-*formyl-*L*-proline methyl ester (**1**)*

L-Proline (2.0 g, 17.4 mmole) was dissolved in formic acid (25 mL), and the resulting solution was cooled to 0°C. The cooled solution was added to a mixture of acetic anhydride (20 mL, 218 mmole) in formic acid (25 mL), which was also at 0°C. The resulting solution was allowed to warm to room temperature overnight. The solvent was then removed under reduced pressure. The residue was purified by flash chromatography (30 g silica gel, 5% v/v methanol in chloroform). Fractions containing *N*-formyl-L-proline were pooled, and the solvent was removed under reduced pressure to yield *N*-formyl-L-proline (1.73 g, 63%) as a clear oil. $R_f = 0.6$ (10% v/v methanol in chloroform). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 11.7 (s, 1H), 8.34 (s, 0.6H), 8.27 (s, 0.4H), 4.45–4.52 (m, 1H), 3.63–3.77 (m, 1H), 3.55 (apparent triplet, $J = 7$ Hz, 1H) 2.22–2.38 (m, 1H), 1.89–2.19 (m, 3H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 173.7, 173.6, 163.1, 162.2, 58.9, 56.6, 46.8, 44.1, 29.4, 29.0, 23.6, 22.5. MS (ESI) *m/z* 166.0 ($M + Na^+$).

N-Formyl-L-proline was converted to amide **1** by esterification with diazomethane. Diazomethane was generated in situ with an Aldrich minidiazald reactor. (CAUTION! Accumulated diazomethane is highly explosive. See Aldrich Technical Information Bulletin No. AL-180.) Diazald (1.5 g, 7.0 mmole) was mostly dissolved in ether (10 mL). Residual solids were removed by decanting the yellow solution into a dropping funnel with clear-seal glass joints. At a rate of ~1 drop every 2–3 sec, the diazald solution was added to the diazald reactor, which contained KOH (1.00 g, 17.9 mmole) dissolved in water/ethanol (40:60 v/v, 2.5 mL) heated to 70°C. The ethereal diazomethane immediately distilled over to the second chamber and condensed against a coldfinger at −78°C. The yellow diazomethane droplets then dripped into a solution of *N*-formyl-L-proline (0.50 g, 3.2 mmole) in ether/acetonitrile (50:50 v/v, 25 mL) cooled to 0°C. The reaction continued until the product solution turned light yellow. The reaction was then quenched with glacial acetic acid. The product was isolated by removal of the volatile components under reduced pressure. The residue was purified by flash chromatography (30 g silica gel, ethyl acetate/hexanes [50:50–100:0 v/v]). Fractions containing amide **1** were pooled, and the solvent was removed under reduced pressure to yield amide **1** as a pale yellow oil (0.53 g, 97%). $R_f = 0.45$ (ethyl acetate/hexane [80:20 v/v], I_2 staining). ^{1}H-NMR (D_2O, 500 MHz) δ 8.28 (s, 0.64H), 8.23 (s, 0.36H), 4.76 (dd, $J = 8$, 3 Hz, 0.36H) 4.52 (m, 0.64H), 3.70–3.85 (m, 4.3H). 3.55 (m, 0.7H), 2.22–2.46 (m, 1.3H), 1.98–2.12 (m, 2.6H). ^{13}C-NMR (dioxane-d_8, 125 MHz) δ 172.9, 172.4, 161.3, 160.5, 58.7, 56.5, 52.2, 51.8, 46.1, 43.8, 29.8, 29.6, 24.3, 23.1. MS (ESI) *m/z* 180.0 ($M + Na^+$). IR Data: (neat) υ_{max} 1742.8 (s), 1672.1 (s).

*Synthesis of acetyl-*L*-proline methyl ester (**2**)*

Amide **2** was synthesized as described previously (Panasik Jr. et al. 1994).

Synthesis of N-*formyl-5,5-dimethyl-*L*-proline methyl ester (**3**)*

2-(3-Methyl-3-nitro-butyl)-[1,3]dioxolane was prepared as described previously (Bonnett et al. 1959). 2-(3-Methyl-3-nitro-butyl)-[1,3]dioxolane (68 g, 0.36 mole) was dissolved in methanol (0.25 L). Raney nickel was added to this solution, and the flask was filled with H_2(g) from a balloon. (CAUTION! Raney nickel is highly pyrophoric and will ignite methanol vapors if dry.) Periodically, the reaction was assayed for completion by ^{13}C-NMR spectroscopy. Completion times ranged from overnight to 10 days, depending on the scale and catalyst loading. Upon completion, the catalyst was carefully filtered away. The solvent was removed under reduced pressure to yield 3-[1,3]dioxolan-2-yl-1,1-dimethylpropylamine as a pale yellow oil (56.5 g, 99%). ^{1}H-NMR ($CDCl_3$,

300 MHz) δ 4.84 (t, J = 5 Hz, 1H), 3.82–3.98 (m, 4H), 1.65–1.75 (m, 2H), 1.42–1.51 (m, 2H), 1.10 (s, 6H). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) 104.0, 64.1, 48.3, 38.1, 29.67, 28.5. MS (ESI) *m/z* 160.2 ($M + H^+$).

3-[1,3]Dioxolan-2-yl-1,1-dimethyl-propylamine (15.3g, 96 mmole) was dissolved in hot water (40 mL) and the pH lowered to ~3 with 2 N HCl. The resulting solution was heated at reflux for 30 min, and then made basic by the addition of 6 N KOH and extracted (4×) with chloroform. The organic layer was dried over $MgSO_4$(s), and the solvent was removed under reduced pressure to yield a black oil. The black oil was purified by distillation under reduced pressure to yield 2,2-dimethyl-3,4-dihydro-2H-pyrrole as a clear pungent oil (7.5 g, 80%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.33 (broad s, 1H), 2.52 (t, J = 7 Hz, 2H), 1.56 (t, J = 7 Hz, 2H), 1.2 (s, 6H). ^{13}C-NMR ($CDCl_3$, 75.4 MHz) δ 163.1, 72.9, 36.7, 34.3, 28.5.

2,2-Dimethyl-3,4-dihydro-2H-pyrrole (12.4 g, 128 mmole) was dissolved in water (70 mL) at 0°C. KCN (16.3 g, 251 mmole) was added to the solution, and the pH was lowered from ~13.4 to ~6 over 2 h by the addition of 2 N HCl (128 mL, 256 mmole). The pH continued to rise, but was maintained near 6 by the addition of 2 N HCl. After 3 h at 0°C, the solution was made basic by the addition of 2 N NaOH, and extracted (4×) with chloroform. The combined organic extract was dried with $MgSO_4$(s), and the solvent removed under reduced pressure. The residue was purified by flash chromatography (0.4 kg silica gel, ethyl acetate/hexanes [65: 35 v/v]). Fractions containing 5,5-dimethyl-pyrrolidine-2-carbonitrile were pooled, and the solvent was removed under reduced pressure to yield 5,5-dimethyl-pyrrolidine-2-carbonitrile as a pale yellow oil (7.47 g, 46%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 4.07–4.12 (apparent dd, J = 8, 5 Hz, 1H), 2.12–2.36 (m, 2H), 1.79–1.90 (m, 2H), 1.61–1.72 (m, 1H), 1.30 (s, 3H), 1.17 (s, 3H). ^{13}C-NMR ($CDCl_3$, 75.4 MHz) δ 122.0, 59.5, 46.5, 38.3, 30.9, 29.0, 28.8. ^{13}C-DEPT-135 δ 46.5, 29.0, 28.8 positive (CH or CH_3), 38.3, 30.9 negative (CH_2). MS (ESI) *m/z* 125.2 ($M + H^+$).

5,5-Dimethyl-pyrrolidine-2-carbonitrile was hydrolyzed to form 5,5-dimethylproline as described previously (Bonnett et al. 1959). Racemic 5,5-dimethylproline was resolved with D-tartrate as described previously (An et al. 1999) to yield 5,5-dimethyl-L-proline in greater than 97% ee. 5,5-Dimethyl-L-proline was converted to *N*-formyl-5,5-dimethyl-L-proline methyl ester by the procedure used to convert L-proline to *N*-formyl-L-proline methyl ester (*vide supra*). ^{1}H NMR (D_2O, 500 MHz) δ 8.25 (s, 0.96H), 8.10 (s, 0.04H), 4.48–4.58 (m, 1H), 3.76 (s, 3H), 2.2–2.45 (m, 1H), 1.85–2.15 (m, 3H), 1.47 (s, 3H), 1.42 (s, 3H). ^{1}H-NMR (dioxane-d_8, 500 MHz) δ 8.22 (s, 0.89H), 8.09 (s, 0.11H), 4.37–4.45 (m, 1H), 3.71 (s, 0.45H), 3.65 (s, 2.55H), 2.12–2.23 (m, 1H), 1.84–1.93 (m, 1.8H), 1.75–1.83 (m, 1.2H), 1.46–1.49 (2 singlets, 3H total), 1.35–1.38 (2 singlets, 3H total). ^{13}C-NMR (dioxane-d_8, 125 MHz) δ 173.4, 172.5, 161.2, 159.8, 61.6, 61.0, 52.2, 51.8, 40.6, 40.0, 28.9, 28.8, 27.3, 26.6, 24.9. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 8.30 (s, 0.92H), 8.22 (s, 0.8H), 4.39–4.59 (m, 1H), 3.78 (s, 0.33H), 3.74 (s, 2.67H), 2.15–2.35 (m, 0.96H), 1.78–2.08 (m, 3.04H), 1.54–1.6 (pair of singlets, 3H total), 1.41–1.44 (pair of singlets, 3H total). ^{13}C-NMR ($CDCl_3$, 62.9 MHz) δ 171.8, 161.2, 160.0, 60.5, 58.1, 52.0, 39.3, 29.1, 28.7, 26.3. ^{13}C NMR DEPT-135 δ 160.0, 58.1, 52.0, 29.1, 28.7 are positive (CH or CH_3). δ 39.3, 26.3 are negative (CH_2). MS (ESI) *m/z* 208.1 ($M + Na^+$), 160.1.

Determination of $K_{trans/cis}$ *values*

cis and *trans* resonances of amides **1**–**3** were identified by using NOESY1D spectroscopy as described in the supporting information. Values of $K_{trans/cis}$ were determined by integration of ^{1}H NMR spectra obtained at 25°C.

Depiction of natural bond orbitals

The n and π^* NBOs of the *trans* isomer of AcProOMe (**2**) in its C^γ-*exo* conformation were depicted in Figure 1B with the program gOpenMol, version 2.2 (Laaksonen 1992; Bergman et al. 1997) by using NBO 4.0 output from the B3LYP/6-311+G(2d,p) level of theory (DeRider et al. 2002).

Calculation of $O_{i-1}\cdots C'_i$ *distances and* $O'_i\cdots C'_i{=}O_i$ *angles*

The ϕ (C'_{i-1}–N_i–C^α_i–C'_i) and ψ (N_i–C^α_i–C'_i–N_{i+1}) torsion angles in the *trans* (ω = 180°) isomer of $AcGlyNH_2$ were varied in 5°-increments before energy minimization with the program MacSpartanPro, version 1.0.4 (Wavefunction). The $O_{i-1}\cdots C'_i$ distance (δ_{BD}) and $O_{i-1}\cdots C'_i{=}O_i$ angle (τ_{BD}) were recorded in each energy-minimized structure. Those structures with $O_{i-1}\cdots C'_i$ distances $\delta_{BD} \leq 3.2$ Å and $O_{i-1}\cdots C'_i{=}O_i$ angles $99° \leq \tau_{BD} \leq 119°$ were depicted in Figure 3A on a Ramachandran plot (Ramachandran and Sasisekharan 1968; Richardson 1981).

Electronic supplemental material

NOESY 1D, ^{1}H-, and ^{13}C-NMR spectra and IR spectra are available for amides **1**–**3**.

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