

Energetics of an $n \rightarrow \pi^*$ Interaction that Impacts Protein Structure

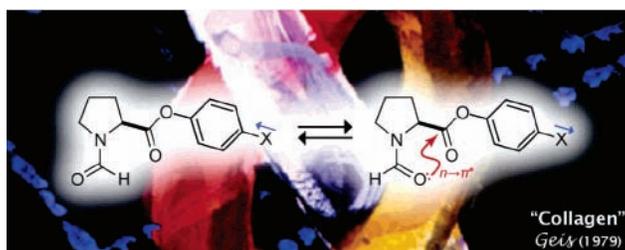
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ABSTRACT



The trans/cis ratio of the amide bond in *N*-formylproline phenylesters correlates with electron withdrawal by a para substituent. The slope of the Hammett plot ($\rho = 0.26$) is indicative of a substantial effect. This effect arises from a favorable $n \rightarrow \pi^*$ interaction between the amide oxygen and ester carbonyl. In a polypeptide chain, an analogous interaction can stabilize the conformation of trans peptide bonds, α -helices, and polyproline type-II helices.

Certain noncovalent interactions are known to direct a polypeptide chain to assume a folded structure. Those interactions include the hydrophobic effect, hydrogen bonding, Coloumbic forces, van der Waals forces, and cation– π interactions.¹ Recently, we suggested that $n \rightarrow \pi^*$ interactions should be added to this list.^{2,3} In an $n \rightarrow \pi^*$ interaction (which is not to be confused with an $n \rightarrow \pi^*$ electronic transition), the oxygen of a peptide bond (O_{i-1}) donates electron density from one of its lone pairs into the antibonding orbital of the

carbon in the subsequent peptide bond ($C'_i=O_i$). This $O_{i-1} \cdots C'_i=O_i$ interaction, which mimics the approach of a nucleophile to the electrophilic carbon of an acyl group, is strongest when O_{i-1} is positioned proximally and along the Bürgi–Dunitz trajectory to $C'_i=O_i$. In polypeptides, this geometry occurs in the α -helix and polyproline type-II (PPII) helix,^{2c} which is the structure assumed by the strands of the prevalent collagen triple helix.^{3,4}

The $n \rightarrow \pi^*$ interaction can occur only if the peptide bond containing O_{i-1} is trans (i.e., *Z*), as opposed to cis (*E*). Accordingly, the trans/cis ratio of an Xaa_{i-1} – Pro_i peptide bond can report on the strength of an $n \rightarrow \pi^*$ interaction. We had demonstrated previously that the $n \rightarrow \pi^*$ interaction contributes to the endogenous preference for trans peptide bonds.^{2c} Here, we present a simple model system that uses the trans/cis ratio of a peptide bond to report on the energetics of an $n \rightarrow \pi^*$ interaction.

We reasoned that the strength of an $n \rightarrow \pi^*$ interaction could be altered by using para-substituted phenyl esters of

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N-formyl-L-proline to modulate the electrophilicity of the carbonyl ($C_i=O_i$). Our expectation was that the trans/cis ratio would correlate with that electrophilicity. We chose *N*-formylproline because its trans/cis ratio is close to unity and can thus serve as the basis for a highly sensitive assay.^{2c} The esters were synthesized by condensation of *N*-formylproline with the corresponding phenol using PyBOP activation and were purified by flash chromatography.

We used ¹H NMR spectroscopy to measure the trans/cis ratio of each ester (90–170 mM) in CDCl₃. We chose chloroform as the solvent to minimize effects from differential solvation of the trans and cis isomers. Because the isomerization of the peptide bond is slow on the NMR time scale, two sets of signals were observed in the spectrum of each ester. Though most of the resonances exist as multiplets and overlap with those from their isomer, the resonance of the formyl proton appears as two singlets—one for each isomer. The larger, upfield resonance arises from the trans isomer. Integration of the two signals provides the trans/cis ratio at the amide bond.

We find that more electron-withdrawing para substituents correlate strongly with larger trans/cis ratios (Table 1).

Table 1. Trans/Cis Ratios (CDCl₃) and σ_p Values of X in Fm-Pro-OC₆H₄-*p*-X at 25 °C

X	σ_p^a	$K_{\text{trans/cis}}$	ΔG (kcal/mol) ^b
NO ₂	0.78	4.13 ± 0.05	−0.84
CN	0.66	3.95 ± 0.03	−0.81
H	0.00	2.12 ± 0.01	−0.45
OMe	−0.27	2.01 ± 0.04	−0.41
NMe ₂	−0.83	1.67 ± 0.02	−0.30

^a From ref 6. ^b $\Delta G = -RT \ln K_{\text{trans/cis}}$.

Moreover, a linear relationship exists between the log of the trans/cis ratio and the Hammett parameter (σ_p) of the substituent (Figure 1A), which is indicative of an electronic effect.⁵ The values of $K_{\text{trans/cis}}$ and σ_p are related by $\rho = \partial \log(K_X/K_H) / \partial \sigma_p = 0.26$. This ρ -value signifies that the electronic effect of X on $K_{\text{trans/cis}}$ is substantial, especially given that the interconversion of the cis and trans isomers of Fm-Pro-OC₆H₄-*p*-X does not involve a change in covalent bonding.

Density functional theory (DFT) calculations predicted a similar correlation between $K_{\text{trans/cis}}$ and σ_p (Table 2; Figure 1B) and enabled a detailed analysis. Electron-withdrawing substituents on a phenyl ring are known to increase the electrophilicity⁷ (and reactivity⁵) of aryl esters. The electronics of an ester can be described by three major resonance forms: **A–C** (Scheme 1). As the phenyl substituent becomes more electron withdrawing, forms **B** and **C** become dis-

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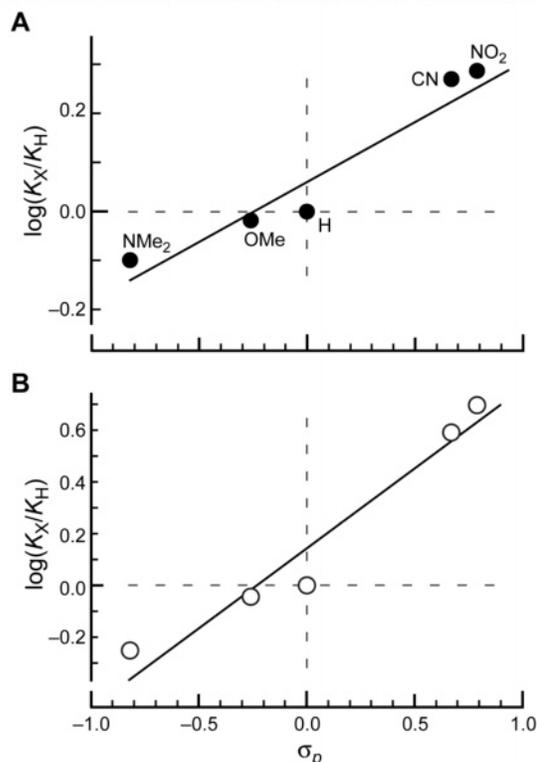
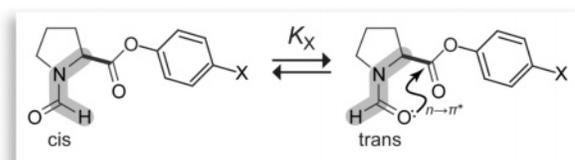


Figure 1. Relationship between the trans/cis ratio and the electron-withdrawing ability of X in Fm-Pro-OC₆H₄-*p*-X. (A) Values of K_X are from experimental data (Table 1) and give $\rho = 0.26$. (B) Values of K_X are from density functional theory calculations on the trans isomer with C^γ -exo pyrrolidine ring pucker (Table 2) and give $\rho = 0.60$.

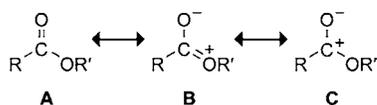
avored⁸ due to π -polarization.⁹ The diminished delocalization decreases the energy of the $C_i=O_i$ π^* -orbital, making $C_i=O_i$ more electrophilic, and leads to an increased contribution from resonance form **A**, which results in greater

Table 2. Results of Density Functional Theory Calculations (B3LYP/6-31+G*) and Natural Bond Order Analysis on the Trans and Cis Isomers of Fm-Pro-OC₆H₄-*p*-X with C^γ -exo Pyrrolidine Ring Pucker

X	ΔE (kcal/mol) ^a	$\nu_{C_i=O_i}$ (cm ^{−1}) ^{b,c}	$E_{\pi^*,C_i=O_i}$ (Hartrees) ^c	$E_{n \rightarrow \pi^*}$ (kcal/mol) ^d	$r_{O_{i-1} \cdots C_i}$ (Å) ^d
NO ₂	2.22	1837	−0.02994	1.45	2.88
CN	2.08	1835	−0.02723	1.39	2.89
H	1.27	1829	−0.01240	0.86	2.98
OMe	1.22	1826	−0.00962	0.83	2.99
NMe ₂	0.94	1824	−0.00422	0.68	3.02

^a In Figure 1B, $K_{\text{trans/cis}} = e^{-\Delta E/RT}$. ^b Unscaled. ^c Cis isomer. ^d Trans isomer.

Scheme 1



double-bond character and higher $C_i'=O_i$ vibrational frequency.⁸ These effects are observed theoretically in the cis isomer, which represents the ester electronics in a state unperturbed by the $n \rightarrow \pi^*$ interaction. In the cis isomer, the calculated vibrational frequency ($\nu_{C_i'=O_i}$) of the carbonyl increases with the electron-withdrawing ability of the para substituent (Table 2). Moreover, natural bond order (NBO¹⁰) analysis of the cis isomer demonstrates a decrease in the ester π^* -orbital energy ($E_{\pi^*,C_i'=O_i}$) with increasing electron withdrawal (Table 2). These two effects are indicative of the increased electrophilicity of $C_i'=O_i$ that results from electron-withdrawing groups.

The strength of the $n \rightarrow \pi^*$ interaction in the trans isomer was examined directly with NBO analysis. Second-order perturbation theory indicates that as the trans/cis ratio increases so does the energy ($E_{n \rightarrow \pi^*}$) of the interaction between the lone pair on the amide carbonyl oxygen and the π^* -orbital of the ester (Table 2). The increasing strength of the $n \rightarrow \pi^*$ interaction is reflected in the decreasing distance ($r_{O_i \cdots C_i'}$) between the amide oxygen and the ester carbon (Table 2). The value of $\rho = 0.60$ for the theoretical data (Figure 1B) is greater than that for the experimental data (Figure 1A). The pyrrolidine ring was held in the C^y -exo conformation during the theoretical analyses, as this ring pucker allows for much greater $n \rightarrow \pi^*$ interaction than the C^y -endo conformation.^{2b,11} The pyrrolidine ring has, however, a slight preference for the C^y -endo pucker.¹² Hence, the effect of the para substituent on the trans/cis ratio is amplified in the calculations.

The link between the electron-withdrawing ability of the remote para substituent and the trans/cis ratio supports the

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(12) The pyrrolidine ring of Ac–Pro–OMe has 66% C^y -endo pucker and 34% C^y -exo pucker in dioxane at 25 °C (ref 2b).

role of an electronic $n \rightarrow \pi^*$ interaction in the conformational preference of the amide bond. This effect cannot be steric. Located in the para position, the aryl substituent is remote from the amide bond. Moreover, if the effect was steric, then the trans/cis ratios would fall in line with the steric bulk of the substituent. Such a correlation was not observed, as the $K_{\text{trans/cis}}$ value of the smallest substituent, a proton, is between those of the largest substituents, dimethylamino and nitro.¹³

We conclude that an $n \rightarrow \pi^*$ interaction occurs between the amide and ester groups in this model system. In addition, we propose that this interaction has important ramifications for protein structure. An $n \rightarrow \pi^*$ interaction could stabilize not only a trans peptide bond but also an α -helix and a PPII helix.^{2c} The α -helix,¹⁴ like the β -sheet,¹⁵ is stabilized by $C_i'=O_i \cdots H-N$ hydrogen bonds between main-chain atoms.^{1b,16} The $C_i'=O_i$ bonds in α -helices are, however, demonstrably longer than those in β -sheets.¹⁷ This greater bond length is consistent with the manifestation of an $n \rightarrow \pi^*$ interaction in an α -helix. In contrast to the α -helix and β -sheet, the PPII helix does not contain any hydrogen bonds between its main-chain atoms. Yet, recent data indicate a notable prevalence of the PPII helix in both folded proteins and unfolded polypeptide chains.¹⁸ We propose that the $n \rightarrow \pi^*$ interaction stabilizes the PPII helix, contributing significantly to its prevalence. Accordingly, the $n \rightarrow \pi^*$ interaction is a noncovalent interaction, like the hydrophobic effect, hydrogen bonding, Coloumbic forces, van der Waals forces, and cation– π interactions,¹ that directs a polypeptide chain to assume a folded structure.

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Supporting Information Available: Procedures for the preparation of *N*-formylproline phenylesters and related analytical data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(13) In contrast to para substituents, ortho substituents can have adverse steric consequences. For example, DFT analysis of the highly electron-withdrawing pentafluorophenyl ester showed a lower trans/cis ratio than in the unsubstituted phenyl ester, presumably due to a steric interaction between the *ortho*-fluoro groups and the amide oxygen (data not shown).

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