Noncovalent interactions define and modulate biomolecular structure, function, and dynamics.\textsuperscript{1} A fundamental feature of noncovalent interactions with short contacts is electronic delocalization. For example, a hydrogen bond involves delocalization of the lone pair (n) of the acceptor atom over the antibonding orbital (\(n^*\)) of the donor.\textsuperscript{2} We discovered an interaction in proteins, termed the \(n\textasciitilde\pi^*\) interaction, with similar electronic delocalization.\textsuperscript{3–6} In this interaction, a lone pair (n) of the oxygen (O\textsubscript{i-1}) of a peptide bond overlaps with the antibonding orbital (\(\pi^*\)) of the carbonyl group (C\textsubscript{i}=O\textsubscript{i}) of the subsequent peptide bond (Figure 1A). This interaction underlies the adoption of the Bürgi–Dunitz trajectory for nucleophilic additions to the carbonyl group.\textsuperscript{3,4,7} The stereochemical constraints required for an energetically meaningful \(n\textasciitilde\pi^*\) interaction are met in several fundamental structural elements in proteins, including α-helices,\textsuperscript{5,8,9} 3\text{10} helices,\textsuperscript{10} and polyproline II-type (PPII) helices,\textsuperscript{9,11} as well as within the backbone of peptides.\textsuperscript{12} Hence, the \(n\textasciitilde\pi^*\) interaction could be one of the most prevalent noncovalent interactions in proteins and their congeners.

A signature of the \(n\textasciitilde\pi^*\) interaction is a short O\textsubscript{i-1}⋯C\textsubscript{i} contact. Some recent work has attributed such proximity to an attractive interaction that arises from the orthogonal orientation of carbonyl dipoles (Figure 1B).\textsuperscript{13} We were skeptical of this interpretation for several reasons. First, the energetically most favorable trajectory of approach of a nucleophile to the carbonyl group (Bürgi–Dunitz trajectory) is not orthogonal to the carbonyl group but is inclined at an obtuse angle, presumably to facilitate the overlap between the lone pair of the nucleophile and the antibonding orbital of the carbonyl group.\textsuperscript{6,7} Nucleophilic attack on alkenes, which lack carbonyl-like dipoles, employs a similar Bürgi–Dunitz angle.\textsuperscript{14} Second, this short contact occurs in small molecules and in proteins even when the two carbonyl dipoles are not aligned favorably. For example, O\textsubscript{i-1} and C\textsubscript{i} are proximal in an α-helix,\textsuperscript{5,9} even though the interaction between adjacent carbonyl dipoles in the α-helix backbone is repulsive.\textsuperscript{15} In common protein secondary structures, carbonyl groups that display such short contacts are typically not oriented orthogonally (see: Supporting Information, Figure S1). These observations cast doubt on whether these short contacts can be attributed to dipole–dipole interactions. A third possible origin for the proximity of the amide carbonyl groups is a simple Coulombic attraction between the negatively polarized O\textsubscript{i-1} and positively polarized C\textsubscript{i} (Figure 1C).\textsuperscript{16} Here, we report on the nature of the intimate interaction between adjacent amide groups.

We designed a simple system with which to reveal the origin of the C\textasciitildeX\textsubscript{i-1}⋯C\textsubscript{i}=O interaction (Figure 2).\textsuperscript{17} Regardless of its origin, this interaction stabilizes the trans conformation in compounds 1–6 preferentially over the cis conformation. Thus, the value of K\textsubscript{trans\textasciitildecis} reports on the strength of the C\textasciitildeX\textsubscript{i-1}⋯C\textsubscript{i}=O interaction. This value can be measured in water by using \(^1\text{H}\) NMR spectroscopy.

We reasoned that the replacement of O\textsubscript{i-1} with sulfur (S\textsubscript{i-1}) would distinguish between a charge–charge interaction and \(n\textasciitilde\pi^*\) electronic delocalization. A charge–charge interaction would be attenuated because sulfur is less negatively polarized than oxygen (see: Supporting Information, Table S1).\textsuperscript{18} On the other hand, this substitution would strengthen \(n\textasciitilde\pi^*\) electronic delocalization because sulfur is a softer base than oxygen and thus a better electron-pair donor. Accordingly, a decrease in K\textsubscript{trans\textasciitildecis} upon this isosteric substitution would indicate that charge–charge attraction dominates this interaction, whereas an increase in K\textsubscript{trans\textasciitildecis} would implicate \(n\textasciitilde\pi^*\) electronic delocalization.

Replacing an amide donor (1) with a thioamide donor (4) led to a marked increase in the value of K\textsubscript{trans\textasciitildecis} (Table 1). Thus, the stabilization of the trans conformation is not due to a charge–charge interaction. Still, its origin could be a dipole–dipole interaction, as thioamides have a larger dipole moment than do amides.\textsuperscript{19} To discern whether the stabilization of the trans conformation is due to a dipole–dipole interaction or \(n\textasciitilde\pi^*\) electronic delocalization, we employed a subtle means to alter the distance between the donor and acceptor.

The gauche effect arising from a 4S electron-withdrawing group (EWG) is known to enforce a C\textsubscript{i}–endo ring pucker upon the pyrrolidine ring of a proline residue.\textsuperscript{4} In the C\textsubscript{i}–endo pucker, the O\textsubscript{i-1} or S\textsubscript{i-1} donor and C\textsubscript{i}=O acceptor will likely be too far apart for appreciable orbital overlap. In contrast, they are close enough for a dipole–dipole interaction (E \(\alpha\ r^{-3}\)). If the interaction between adjacent carbonyl groups is dipolar in nature, then the isosteric

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substitution of an amide (2) with a thioamide (5) would result in a substantial increase in $K_{trans/cis}$ because of the larger dipole moment of thioamides.

The value of $K_{trans/cis}$ for amide 2 and thioamide 5 were within experimental error (Table 1). Accordingly, a dipole–dipole interaction cannot stabilize the trans conformation significantly. We next modified our model system to verify that enhanced orbital overlap does indeed correlate with an increase in the stability of the trans conformation. Antithetical to a $4\pi$–exo ring pucker, as expected, the value of $K_{trans/cis}$ for thioamide 6 is significantly greater than that of thioamide 4 (Table 1).

Next, we employed X-ray diffraction analysis of thioamides 4–6 to validate our assumptions (Figure 3). Electronic delocalization requires that the distance between the donor atom (S$_2$–O$_2$) and acceptor atom (C$_3$) be less than the sum of their van der Waals radii ($r_3 + r_6 < 3.5$ Å). In agreement with our predictions, the distances between the donor and acceptor atoms in 4 and 6 are $<3.5$ Å, whereas that in 5 is $>3.5$ Å (Table 1). None of these thioamides display orthogonal orientation carbonyl dipoles.

The X-ray diffraction analyses provide another informative parameter. The ester carbon, C$_6$, of compounds 3–6 is at the apex of a pyramid that is directed toward X$_{trans}$ (Table 1). A charge–charge interaction would predict a higher degree of pyramidalization for amides than thioamides, which is inconsistent with the experimental data. A dipole–dipole interaction would pyramidalize in the opposite direction, as the positive pole of the thioamide dipole is closer to C$_6$ than the negative pole. Rather, both the direction and the extent of the apicality of C$_6$ is consistent with an n–π* interaction, which can be envisaged as a small step along the Bürgi–Dunitz trajectory. Most interestingly, the degree of pyramidalization correlates with a measure of the strength of the n–π* interaction, the value of $K_{trans/cis}$ (Figure 4).

To interpret these experimental data further, we resorted to hybrid density functional theory (DFT) and Natural Bond Orbital (NBO) analysis. Geometry optimizations, frequency calculations, and NBO analyses were performed at the B3LYP/6-311+G(2d,p) level of theory on preferred conformations of 1–3 and four conformations of 4–6 (see Supporting Information, Table S1). The stabilization afforded by n–π* electronic delocalization was estimated by using second-order perturbation theory as implemented in NBO 5.0 and deletion analysis.

The experimental data are reflected in the computations. Thioamides are indeed better than amides as electron-pair donors (Table 1). The SCF energy of the system increases upon deletion of the n–π* electronic delocalization, a result inexplicable by consideration of a classical dipole–dipole interaction. In addition, our computational analyses echo both the observed effect of a C$_3$ EWG on pyrrolidine ring pucker and that of ring pucker on the extent of n–π* electronic delocalization (Table S1). In particular, values of $K_{trans/cis}$ and relevant overlap between n and π* orbitals increases in the order 3 > 1 > 2 among the amides and 6 > 4 > 5 among the thioamides (Figure 5).

Our experimental and computational analyses indicate that the interaction between adjacent carbonyl groups in proteins has substantial resonance character. The stereochemical criteria necessary for these n–π* interactions are met in common secondary structures of proteins. Moreover, some of the conformational stability of the PPII helix, a structure adopted by individual strands in the collagen triple helix and by unfolded polypeptide chains, has already been attributed in part to n–π* interactions. Likewise, the presence of n–π* interactions in α-helices is consistent with the C=O bonds in α-helices being longer than those in β-sheets.

### Table 1. Conformational Parameters for Amides 1–3 and Thioamides 4–6

<table>
<thead>
<tr>
<th>compound</th>
<th>$K_{trans/cis}$</th>
<th>ring pucker</th>
<th>$d$ (Å)</th>
<th>$\Delta$ (Å)</th>
<th>$\Theta$ (deg)</th>
<th>$\Delta$ (Å)</th>
<th>$\Theta$ (deg)</th>
<th>$E_{trans}$ (kcal/mol)</th>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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</tr>
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<td>102.30</td>
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<td>endo</td>
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<td>0.038</td>
<td>4.18</td>
<td>101.59</td>
<td>0.038</td>
</tr>
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</table>

* From ref 4. In D$_2$O at 25 °C; values are ±10%. From X-ray diffraction analysis of the crystalline compound in the trans conformation (ref 20; Figure 3). Parameters are defined in Figures 3 and 4. Mean values are listed for amide 3 and thioamide 6, which have two independent molecules in the asymmetric unit. From the preferred conformation. From second-order perturbation theory. From deletion analysis.

### Figure 3. Crystalline structures of thioamides 4–6 depicting the Bürgi–Dunitz parameters, d and θ. Pyrrolidine ring pucker and values of d and θ are listed in Table 1. The crystalline structures of amides 1 (cis) and 3 (trans) were reported previously.

### Figure 4. Relationship between the degree of ester pyramidalization in compounds 1–6 and the value of $K_{trans/cis}$ in D$_2$O at 25 °C (Table 1). Closed symbols, experimental values; open symbols, computational values.
Although the contribution of each individual $n \rightarrow \pi^*$ interaction is small—the range of $K_{\text{francis}}$ values herein corresponds to 0.8 kcal/mol at 25 °C—their contribution to protein structure is cumulative (and could be cooperative).34

Finally, we note that $n \rightarrow \pi^*$ electronic delocalization likely plays an important role in many protein–ligand interactions.28,41 Our data indicate that the isosteric substitution of an amide donor with a thioamide could increase ligand affinity as a result of enhanced $n \rightarrow \pi^*$ electronic delocalization. Accordingly, this tenet bears on the approach of lead optimization in medicinal chemistry.

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Supporting Information Available: Procedures for syntheses and analyses reported herein and additional experimental and computational data on compounds 1–6. This material is available free of charge via the Internet at http://pubs.acs.org.

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Figure 5. Overlap between $n$ and $\pi^*$ orbitals in the preferred conformations of amides 1–3 and thioamides 4–6. The overlap integrals are 1, 0.0749; 2, 0.0493; 3, 0.1073; 4, 0.1031; 5, 0.0814; 6, 0.1314. Depictions were generated with NBOView 1.1.20

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