A Computational Study of the Isomerization of Prolyl Amides As Catalyzed by Intramolecular Hydrogen Bonding

Kathryn N. Rankin and Russell J. Boyd*

Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J3

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The cis-trans isomerization of *N*-acetylproline methylamide has been investigated by density functional theory. The introduction of an electron-withdrawing group at the C$^{\gamma}$ of proline has notable geometrical and energetic consequences on the cis-trans isomerization. In the presence of the fluoro and hydroxy substituents, the preferred mode of rotation of the acetyl group is in a counterclockwise direction, assisting in the formation of an N···HN intramolecular hydrogen bond in the transition structures and enhancing the pyramidalization of the prolyl nitrogen. This study illustrates the important role the intramolecular N···HN hydrogen bond plays in the cis-trans isomerization of proline-containing molecules and the catalytic possibilities of hydrogen bonding.

Introduction

As the basic building block in a variety of biologically important polymers, the amide group is one of the most significant functional groups in biology and chemistry. Of the 20 amino acids in naturally occurring polypeptides that are subject to nondegenerate cis and trans rotamers, proline is unique in that it has the potential to establish an imidic peptide bond through its prolyl nitrogen. Although the cis and trans rotamers of amides N-terminal to proline are energetically similar[1] (Figure 1), the cis-trans isomer producing rotation about the C−N bond involving proline residues is the slow step in the folding of peptides[2−4] and has significant implications for the secondary structure of proteins. Thus, the catalysis of the amide bond isomerization is an area of scientific interest with important biological and chemical implications.

The cis-trans isomerization of the amide bonds in peptides and proteins of biological systems is catalyzed by a class of enzymes referred to as peptidyl-prolyl isomerases[5] (PPIases). This group of enzymes represents the only type of biocatalyst whose sole function is conformational interconversion. Although the exact mechanism by which the PPIases catalyze the cis-trans interconversion is yet to be elucidated, it has been proposed[6−8] that intramolecular catalysis of the amide isomerization, by the formation of a weak hydrogen bond between the prolyl nitrogen acting as a hydrogen bond acceptor and the NH unit of the proline residue, is an important part of the mechanism and a common feature in structural protein chemistry.[8] In fact, previous theoretical studies have shown that the formation of hydrogen bonds may accelerate the rate of chemical transformations.[9,10]

Experimental[11−14] and theoretical[6,15−17,25−27] studies often employ *N*-acetylproline methylamide as a model for the study of the cis-trans isomerization of proline-containing residues. These studies have shown that the rate of isomerization may be accelerated through the addition of substituents on various positions of the proline ring. However, a recent spectroscopic and kinetic study[8] of substituted prolines indicated the existence of an intramolecular N−H···N hydrogen bond, which catalyzed the amide isomerization. Similar intramolecular hydrogen bonds have more recently been proposed to stabilize the transition structures, thereby lowering the rotation barrier for amide isomerization, in proline dipeptide,[6] 5-methylated proline dipeptide,[16] and pseudoprolines.[17]

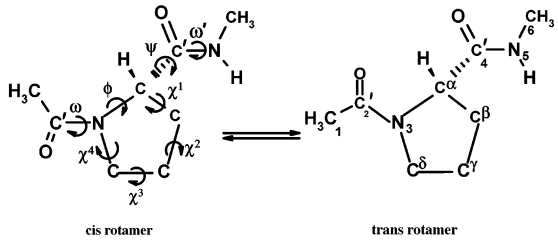

Figure 1. The numbering of the atoms in the isomerization of *N*-acetylproline methylamide.

In the present study, density functional theory is employed in order to investigate conformational preferences in transition structures during isomerization and the role of intramolecular hydrogen bonding in the model system of *N*-acetylproline methylamide. The effects of electron-withdrawing fluoro {Ac-Flp-NHMe} and hydroxy {Ac-Hyp-NHMe} subsitutents at the C$^{\gamma}$ in *N*-acetylproline methylamide on the barrier to isomerization and the formation of an intramolecular hydrogen bond are also investigated. Solvent effects are not considered in this study, as the role of solvent on the rotational barrier for the amide bond is well documented.[15,18]

Computational Details

Density functional theory calculations were carried out using the Gaussian 98[19] suite of programs. All geometry optimizations were performed using the B3LYP functional and the 6-31G(d,p) basis set. The B3LYP functional is composed of Becke's three-parameter hybrid exchange functional (B3),[20,21] as implemented in Gaussian 98,[22] and the correlation functional of Lee, Yang, and Parr (LYP).[23] Harmonic vibrational frequencies and zero-point vibrational energy (ZPVE) corrections were calculated at the same level of theory. Relative energies, obtained by subsequent single point calculations performed at the B3LYP/6-311+G(2df,p) level using the above geometries, were corrected with the appropriate ZPVE, i.e., B3LYP/6-311+G(2df,p)//

* To whom correspondence should be addressed. Email: russell.boyd@dal.ca.

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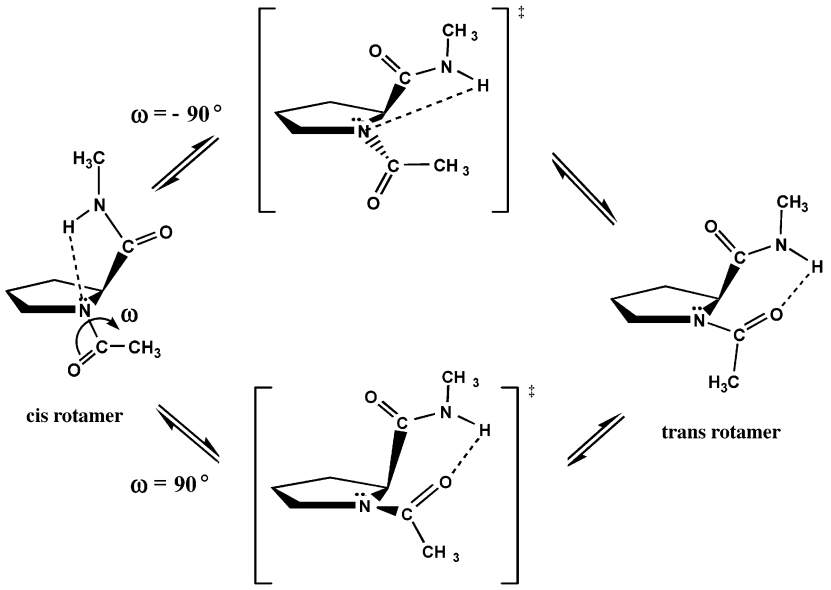

Figure 2. Schematic illustration of the possible reaction pathways for cis-trans isomerization of *N*-acetylproline methylamide.

B3LYP/6-31G(d,p) + ZPVE. Optimized structures, total energies, and charges on atoms in the structures (from Mulliken population analysis) are summarized in Tables S1 and S2 and Figure S1, respectively, of the Supporting Information.

The proline ring may adopt two distinct puckered conformations that are distinguished by the displacement of C^{γ} relative to the plane of the ring[24,25] and are referred to as "up" and "down" or endo and exo, respectively. Consequently, the conformational space of proline analogues has numerous possible low-energy structures.[26] The initial backbone torsional angles for the cis and trans rotamers of *N*-acetylproline methylamide (Figure 1) were taken from Kang and Jhon,[15] who used the HF/6-31+G(d) optimized structures for their DFT calculation. The optimized conformation of *cis*-*N*-acetylproline methylamide with $\omega = 90°$ or $\omega = -90°$ for the imide torsion was used as a starting point for the optimization of the transition state. The initial orientation of the OH group was chosen to minimize the repulsive interactions between neighboring H atoms and was allowed to move during minimization as previous studies[26] on hydroxyproline analogues offer no insight into the optimal orientation of the OH group.

Results

The cis rotamer of *N*-acetylproline methylamide is expected to contain a long intramolecular $N_3 \cdots HN_5$ hydrogen bond between the NH group of the methylamide side chain and the prolyl nitrogen. In the trans rotamer, however, the carbonyl group in the acetyl unit is in close proximity to the amide group, and, thus, the formation of the $O \cdots HN_5$ bond may be structurally favored over the $N_3 \cdots HN_5$ bond. In order for *N*-acetylproline methylamide to undergo conversion between the cis and trans rotamers, there exist two mutually exclusive, structurally accessible, pathways as illustrated in Figure 2. These two pathways differ in the direction by which the acetyl ($COCH_3$) group rotates, as described by the angle ω.

Unsubstituted Isomerization. The cis rotamer of *N*-acetylproline methylamide contains an $N_3 \cdots HN_5$ intramolecular hydrogen bond (2.277 Å) and is energetically less stable than the trans rotamer by 13.0 kJ mol^{-1}. The calculated backbone torsional angles (ω, ϕ, ψ, ω') obtained for the cis and trans rotamers of *N*-acetylproline methylamide are (12.7°, −93.1°, −1.7°, −179.5°) and (−172.1°, −84.2°, 70.5°, −177.2°),

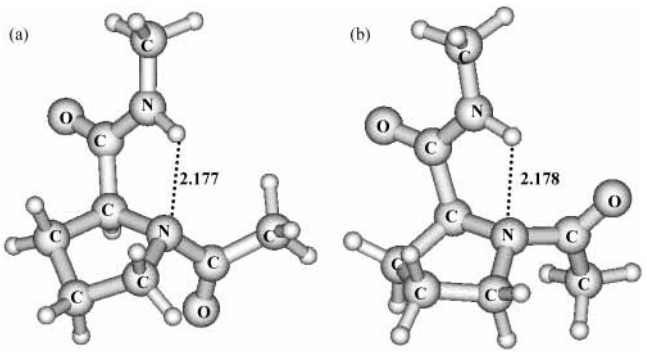

Figure 3. Transition structures obtained in the cis-trans isomerization of *N*-acetylproline methylamide for (a) counterclockwise ($\omega = -90°$) rotation of the acetyl group (TS **1a**) and (b) clockwise ($\omega = 90°$) rotation of the acetyl group (TS **1b**).

respectively. These values are similar to those obtained in previous theoretical studies.[15−17,26,27] To undergo isomerization from the cis to the trans rotamer, the acetyl ($COCH_3$) group may rotate in a clockwise or counterclockwise direction. Counterclockwise rotation ($\omega = -90°$) results in the formation of TS **1a**, whose backbone torsional angles are (120.5°, −108.3°, −7.1°, −176.6°), lying 70.4 kJ mol^{-1} higher than the cis rotamer. Clockwise ($\omega = 90°$) rotation of the acetyl group generates transition structure (TS) **1b,** lying 70.5 kJ mol^{-1} higher than the cis structure, with backbone torsional angles of (−65.1°, −97.6°, 6.9°, 177.3°). In both the aforementioned TSs, rotation of the acetyl group enables the intramolecular $N_3 \cdots HN_5$ hydrogen bond to shorten to 2.177 and 2.178 Å in TS **1a** and TS **1b**, respectively (Figure 3).

Although the two reaction pathways are energetically similar, the transition structures are significantly different. As the acetyl moiety rotates to form the transition structures, deformation of the planarity of the proline ring results due to the puckering of C^{γ} either exo (away from) or endo (toward) with respect to the methylamide group of proline. In addition, the geometry of the prolyl nitrogen (N3) changes from a nearly planar sp^2 to essentially tetrahedral sp^3 hybridization. This deformation of the N3 geometry is associated with C2, the carbon of the acetyl moiety, moving away from (exo) or closer to (endo) C4, the carbon in the methylamide unit, and is described by a virtual dihedral angle η. This parameter, which describes the imide

TABLE 1: Calculated Torsional Angles of *N*-Acetylproline Methylamide[a]

parameter[b]	cis	trans	TS **1a**	TS **1b**
χ^0	−13.6	−13.9	6.2	2.3
χ^1	31.7	31.8	−28.4	22.0
χ^2	−38.2	−38.2	39.0	−37.4
χ^3	29.8	29.4	−35.9	38.6
χ^4	−10.1	−9.8	18.6	−25.7

[a] Angles are in degrees. [b] Torsional angles are defined as χ^0(C^δ−N1−C^α−C^β), χ^1(N1−C^α−C^β−C^γ), χ^2(C^α−C^β− C^γ−C^δ), χ^3(C^β− C^γ−C^δ−N1), and χ^4(C^γ−C^δ−N1−C^α).

TABLE 2: Summary of the Barriers to cis-trans Isomerization (kJ mol^{-1}) for *N*-Acetylproline Methylamide with Substituents on C$^\gamma$ of the Proline Ring cis to the Methylamide Moiety

substituent	$\omega = 90°$	$\omega = -90°$
unsubstituted	70.5	70.4
fluorine	83.1	66.7
hydroxy	67.3	64.9

nitrogen pyramidalization, is defined by the atoms (C^α−N3−C^δ−C2) and takes on values of $\eta = \pm 180°$ for a planar nitrogen and $\eta = \pm 120°$ for a tetrahedral nitrogen.

For the unsubstituted isomerization, counterclockwise rotation results in C$^\gamma$ of the proline ring distorting to adopt an exo conformation. Conversely, clockwise rotation does not perturb the prolyl ring as significantly, as illustrated by the torsional angles in Table 1. In the TSs for both modes of rotation, the C−N bond to the acetyl group is elongated from 1.379 Å in the cis conformer to 1.457 and 1.441 Å in TSs **1a** and **1b**, respectively. This results in variations in the bond angles around the prolyl nitrogen. Specifically, the ∠C^δN3C^α, ∠C^δN3C′, and ∠C^αN3C′ change from (112.1°, 118.6°, 126.3°) in the cis rotamer to (110.2°, 116.9°, 118.3°) and (107.6°, 111.3°, 112.3°) for TS **1a** and TS **1b**, respectively. As the C−N(sp^2) bond lengths (1.36−1.39 Å) are considerably shorter than the C−N(sp^3) bond lengths (1.45−1.47 Å), this, in addition to the increased negative charge of the prolyl nitrogen (see Supporting Information) and the variation in the aforementioned bond angles, is consistent with the change in the geometry of the prolyl nitrogen from sp^2 to sp^3 hybridization.

Replacement of the H at the C$^\gamma$ by various electron-donating and electron-withdrawing groups can be situated either cis or trans to the methylamide moiety on C$^\alpha$. The orientation of these groups will have markedly different effects not only on the N···HN intramolecular hydrogen bond but also on the barrier to rotation and the puckering of the proline ring. The backbone torsion angles (ω, ϕ, ψ, ω') and prolyl ring torsion angles for Ac-Flp-NHMe and Ac-Hyp-NHMe are summarized in Table S3 of the Supporting Information.

cis Substitution on C$^\gamma$. Table 2 summarizes the barriers to isomerization**.** for the *N*-acetylproline methylamide with electron-withdrawing fluoro and hydroxy substituents on C$^\gamma$ of the proline ring. In both cases, clockwise rotation of the acetyl group ($\omega = 90°$) produces transition states (TSs) that are of higher energy than those obtained by counterclockwise rotation ($\omega = -90°$) of the acetyl group. The TSs resulting from clockwise rotation of the acetyl group contain an intramolecular O···HN hydrogen bond whereas the analogous TSs obtained by counterclockwise rotation of the acetyl group contain an intramolecular N···HN hydrogen bond. As the barrier to rotation for the unsubstituted *N*-acetylproline methylamide is larger than that obtained upon replacement of the hydrogen at C$^\gamma$ by electron-withdrawing fluoro and hydroxy substituents, this implies an accelerated rate of prolyl peptide isomerization accompanies the formation of the intramolecular N···HN hydrogen bond. The transition structures obtained from $\omega = -90°$ rotation of the acetyl group are illustrated in Figure 4.

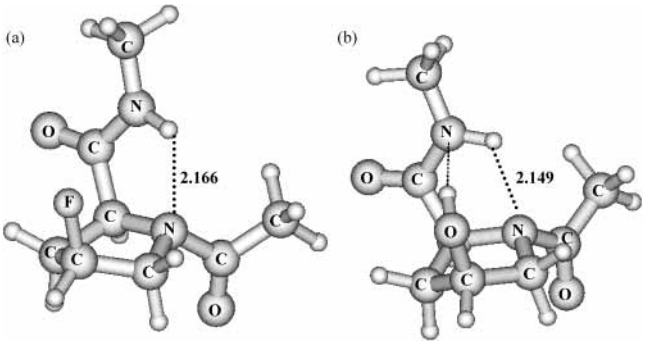

Figure 4. Transition structures obtained for counterclockwise rotation of the acetyl group of *N*-acetylproline methylamide containing a (a) 4-fluoro substitutent cis to the methylamide unit (TS **2a**) and (b) a 4-hydroxy substitutent cis to the methylamide unit (TS **3a**).

TABLE 3: Summary of the Barriers to cis-trans Isomerization (kJ mol^{-1}) of *N*-Acetylproline Methylamide with Substituents on C$^\gamma$ of the Proline Ring trans to the Methylamide Moiety

substituent	$\omega = 90°$	$\omega = -90°$
unsubstituted	70.5	70.4
fluorine	72.5	71.8
hydroxy	71.5	65.8

As the acetyl moiety of *N*-acetyl-4-fluoroproline methylamide rotates by $\omega = -90°$ from the cis to trans rotamer, the C$^\gamma$ of the proline ring puckers to adopt an endo conformation in TS **2a**, lying 66.7 kJ mol^{-1} higher than the cis rotamer. This is accompanied by a shortening of the N_3···HN_5 hydrogen bond from 2.311 Å in the cis structure to 2.166 Å in the TS. Similarly, the ∠C^δN3C^α, ∠C^δN3C′, and ∠C^αN3C′ prolyl bond angles change from (112.1°, 118.6°, 126.8°) in the cis rotamer to (108.2°, 112.2°, 113.0°) in TS **2a**. This rotation also yields noticeable elongations of the N3−C′, N3−C^α, and N3−C^δ bond distances of 0.026 Å (1.499 Å), 0.077 Å (1.458 Å), and 0.015 Å (1.493 Å), respectively, and an increase in the negative charge of the prolyl nitrogen, all indicative of an enhancement in the pyramidalization of the prolyl nitrogen ($\eta = -125.0°$).

Similarly, rotation of the acetyl moiety by $\omega = -90°$ in *N*-acetyl-4-hydroxyproline methylamide generates TS **3a** in which the N_3···HN_5 bond is shortened by 0.142 Å to 2.149 Å relative to that present in the cis structure. Unlike that observed for replacement of the H at C$^\gamma$ by a fluorine atom, replacing the H atom by a hydroxy substituent results in the formation of a long and weak NH···O hydrogen bond between the OH group and the N of the methylamide unit in the cis (2.312 Å) structure and TS **3a** (2.353 Å). This secondary hydrogen bond may be important to encourage the formation of the intramolecular N···HN bond. As observed for *N*-acetyl-4-fluoroproline methylamide, the C$^\gamma$ of proline is in an endo conformation, and N3 has deformed to attain a more tetrahedral geometry ($\eta = -124.8°$) as indicated by the variation in the ∠C^δN3C^α, ∠C^δN3C′, and ∠C^αN3C′ prolyl bond angles from (112.7°, 118.8°, 125.9°) in the cis rotamer to (108.2°, 112.2°, 113.9°) in TS **3a** and the elongation of the N3−C′, N3−C^α, and N3−C^δ bond distances by 0.026 Å (1.499 Å), 0.077 Å (1.458 Å), and 0.015 Å (1.493 Å) in TS **3a** relative to that observed in the corresponding cis rotamer.

trans Substitution on C$^\gamma$. In Table 3, the barriers to isomerization are summarized for *N*-acetylproline methylamide

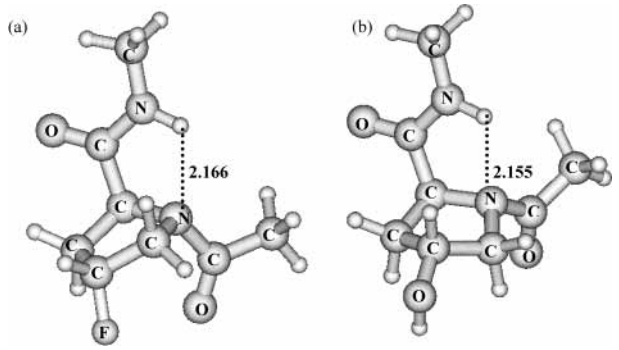

Figure 5. Transition structures obtained for counterclockwise rotation of the acetyl group of *N*-acetylproline methylamide containing a (a) 4-fluoro substitutent trans to the methylamide unit (TS **4a**) and (b) a 4-hydroxy substitutent trans to the methylamide unit (TS **5a**).

with the electron-withdrawing substituents on C^{γ} of proline trans to the methylamide unit. As was observed for the substituents on C^{γ} cis to the amide unit, the preferred mode of rotation for the acetyl group is counterclockwise ($\omega = -90°$). Figure 5 schematically illustrates the transition structures obtained by counterclockwise rotation for trans-substituted proline.

As the acetyl group in the cis rotamer of *N*-acetyl-4-fluoroproline methylamide rotates counterclockwise, the $\angle C^{\delta}$N3C$^{\alpha}$, $\angle C^{\delta}$N3C′, and $\angle C^{\alpha}$N3C′ prolyl bond angles and the $N_3 \cdots HN_5$ hydrogen bond change from (112.2°, 118.6°, 126.3°) and 2.281 Å in the cis rotamer to (104.2°, 111.8°, 113.2°) and 2.166 Å in TS **4a,** lying 71.8 kJ mol^{-1} higher in energy with C^{γ}-exo. Similarly, $\omega = -90°$ rotation of the acetyl group in *N*-acetyl-4-hydroxyproline methylamide generates TS **5a** which lies 65.8 kJ mol^{-1} higher than the cis structure. As observed for with the fluoro substituent, counterclockwise rotation of the acetyl group from the cis rotamer results in the N3—C′, N3—C$^{\alpha}$, and N3—C$^{\delta}$ bonds elongating by 0.078 Å (1.458 Å), 0.029 Å (1.504 Å), and 0.015 Å (1.487 Å), the $N_3 \cdots HN_5$ hydrogen bond shortening by 0.192 Å (2.155 Å), and the $\angle C^{\delta}$N3C$^{\alpha}$, $\angle C^{\delta}$N3C′, and $\angle C^{\alpha}$N3C′ prolyl bond angles contracting by (4.5°, 6.5°, 13.1°) to (108.1°, 112.3°, 112.9°) in TS **5a**, with C^{γ} -endo. Thus, geometrical changes consistent with a more pyramidalized structure for the prolyl nitrogen, which assists in the formation of the NH···N hydrogen bond, for both trans fluoro and hydroxy substitution ($\eta = -122.6°$ and $\eta = -124.9°$, respectively) are evident.

Unlike the lower barriers to isomerization observed in the case of substitution of electron-withdrawing subsitutents cis to the methylamide moiety, the fluoro substitution trans to the methylamide unit increases the barriers to isomerization while the corresponding hydroxy substitution yields lower barriers to isomerization. The marginal increase in the barrier to isomerization for the cis *N*-acetyl-4-fluoroproline methylamide may be due to a Coulombic repulsion between the lone pairs on the fluorine substitutents and the carbonyl oxygen in the acetyl moiety. Such an effect has previously[6] been proposed to account for an analogous reduction in the rate of prolyl isomerization.

Conclusions

The cis-trans isomerization of *N*-acetylprolyl methylamide and its derivatives containing electron-withdrawing groups on the C^{γ} of proline have been investigated using density functional theory. Relative to the unsubstituted isomerization of *N*-acetylmethyl prolylamide, the presence of electron-withdrawing fluoro and hydroxy groups on the C^{γ} of proline results in a preferential rotation of the acetyl group in a counterclockwise direction to generate TSs with lower barriers to isomerization. This preferred direction of rotation enables the formation of an N···HN intramolecular hydrogen bond in the TSs and enhances the pyramidalization of the prolyl nitrogen, as is evident by the trend in the virtual dihedral angle (η) toward 120° and the elongation of the C—N bond length. These observations are consistent with that observed experimentally,[11] and have been attributed to an inductive effect by the electron-withdrawing substitutents.

The effect of electron-withdrawing substituents on the overall barriers to isomerization is dependent upon the orientation of the substituents relative to the prolylamide group. The addition of fluoro and hydroxy subsitutents cis to the prolylamide group decreases the barrier to isomerization. However, the addition of the aforementioned substituents trans to the methylamide unit increases the barrier to isomerization for fluoro substitution while hydroxy substitution substantially decreases the barrier to isomerization.

Thus, the presence of electron-withdrawing groups on the proline ring has significant geometrical and energetic consequences for the cis-trans isomerization. This study illustrates the important role the intramolecular N···HN hydrogen bond plays in the cis-trans isomerization of proline-containing molecules and the catalytic possibilities of hydrogen bonding.

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Supporting Information Available: Archive entries of the B3LYP/6-31G(d,p) optimized structures (Table S1), the total electronic energies of all species in the study (Table S2), the torsional angles of all structures (Table S3), and charges on the heavy atoms in the various transition structures from Mulliken population analyses (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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