Isomerization

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Tuning the *cis*/*trans* Conformer Ratio of Xaa–Pro Amide Bonds by Intramolecular Hydrogen Bonds: The Effect on PPII Helix Stability**

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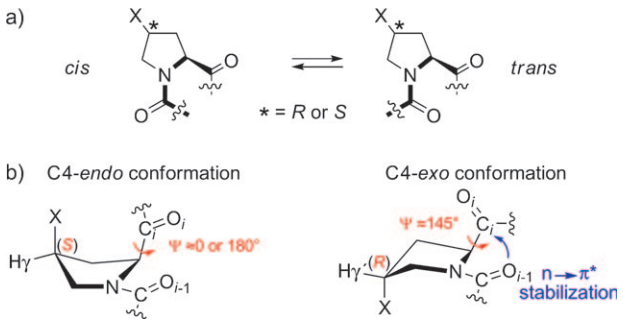
Dedicated to Professor Bernd Giese on the occasion of his 70th birthday

Isomerizations between *cis* and *trans* conformers in Xaa–Pro amide bonds (Xaa = any amino acid, Pro = proline) are crucial in many natural processes such as protein folding and signal transduction.[1] Both the understanding of the factors that determine the *cis*/*trans* conformer ratio and the development of tools that allow for the tuning of this equilibrium is therefore important.[2] Proline derivatives with a substituent in the γ position (C4) have proven useful in both respects (Scheme 1 a).[3–5] Conformational studies have shown that the nature of the substituent at C4 and the absolute configuration at this center critically influence both the pyrrolidine ring pucker and the *cis*/*trans* conformer ratio of the amide bond in Xaa–Pro bonds. In all of these proline derivatives, a correlation between the ring pucker and the *cis*/*trans* conformer ratio has been observed: A C4-*exo* ring pucker favors the *trans* conformation, whereas a C4-*endo* ring pucker leads to a higher population of the *cis* conformer.[3–6] This observation has been attributed to a stronger non-covalent interaction between adjacent amide bonds through an n→π* interaction in the C4-*exo* compared to C4-*endo*-puckered proline derivatives (Scheme 1 b).[6,7]



Scheme 1. a) *cis*/*trans* amide conformers in Xaa–Pro bonds. b) C4-*endo* and C4-*exo* puckering. X represents electron-withdrawing groups such as F or N_3.

As a result, a variety of proline derivatives are available as tools to stabilize the *trans* conformer of Xaa–Pro bonds in which the proline ring adopts a C4-*exo* ring pucker.[3–5] However, within many peptides and proteins the preferred ring pucker of proline with *trans* amide bonds is not C4-*exo* but C4-*endo*.[8] Thus, proline derivatives that preferentially adopt a C4-*endo* conformation and favor the *trans* conformer are important alternative probes and have not been developed to date. Herein we present C4-*endo* ring-puckered proline derivatives in which the *trans* conformer is favored because of a hydrogen-bond-donating substituent in the γ position. Furthermore, we demonstrate the versatility of these derivatives to stabilize *trans* amide bonds within longer peptides.

We started our investigations by an analysis of the relative orientation of the carbonyl groups in acetylated methyl esters of proline derivatives with substituents at C4, which have proven as valuable model compounds for Xaa–Pro bonds.[3–5,9] This orientation is crucial because the n→π* interaction that stabilizes the *trans* conformer requires a Bürgi–Dunitz trajectory between the oxygen atom of the acetyl group (O_{i-1}) and the carbonyl group of the methyl ester (C_i=O_i) as well as a short distance between O_{i-1} and C_i (Scheme 1 b, right).[7,10] The geometry for this n→π* interaction is ideal when the methyl ester is pointing to the inside of the pyrrolidine ring (angle Ψ (N_i-C2-C_i-OCH_3) ≈ 145°). This orientation is observed in *exo*-puckered proline derivatives, in particular those with electron-withdrawing groups (EWGs) at C4 and a 4*R*-configuration (Scheme 1 b, right).[2a,4,5] In contrast, in *endo*-puckered derivatives, the carbonyl group of the methyl ester is turned away from the ring center (Ψ ≈ 0° or 180°, Scheme 1 b, left).[2a,4,5] On the basis of this conformational analysis, we hypothesized that an inward orientation of the methyl ester is disfavored in *endo*-puckered proline derivatives because of a repulsion between the substituent at C4 and the oxygen of the methyl ester (Scheme 2, left).

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PPII = polyproline II, Pro = proline, Xaa = any amino acid.

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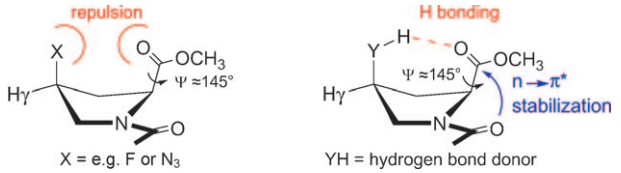


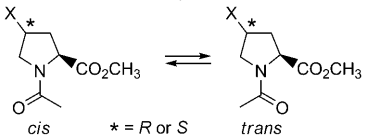
Scheme 2. C4-*endo* conformations of 4*S*-configured derivatives at Ψ angles of approximately 145° without (left) and with (right) H-bond donors at C4.

The ideal orientation of the carbonyl groups for an n→π* interaction should, however, be attainable also in an *endo* ring pucker by an attractive interaction, for example, by H-bonding or electrostatic interactions between the substituent at C4 and the carbonyl group of the methyl ester (Scheme 2, right).[11] As a result, the stabilizing n→π* interaction should be possible and therefore a favorisation of the *trans* amide in such *endo*-puckered proline derivatives.

To test this hypothesis, we prepared and analyzed the conformation of (4*S*)-configured acetylated methyl esters of proline derivatives that bear H-bond-donating groups at C4. In addition, the respective (4*R*)-configured diastereoisomers were studied for comparison. Specifically, derivatives with ammonium (**1S**, **1R**), acetamides (**2S**, **2R**), *tert*-butyl carbamate (NHBoc; **3S**, **3R**), and hydroxy (**4S**, **4R**) moieties at C4 were examined and compared to those of (4*R*)- and (4*S*)-azidoproline derivatives **5S** and **5R**.[5] NMR spectroscopic analyses were performed both in D_2O and the aprotic solvent $CDCl_3$, in which the envisioned attractive interaction was expected to be stronger because an aprotic solvent cannot compete with H-bonding by solvating H-bond donor sites.

The NMR spectroscopic analyses strongly support our hypotheses: 1) Concerning the ring pucker, analysis of the ^1H–^1H vicinal coupling constants showed that all of the (4*S*)-configured derivatives preferentially adopt C4-*endo* ring puckers, whereas those with a (4*R*)-configuration prefer C4-*exo* conformations.[12] 2) With respect to *cis*/*trans* conformer ratios all spectra showed two sets of signals corresponding to the major *trans* and the minor *cis* conformers in different ratios for the (4*S*)- and (4*R*)-configured diastereoisomers (Table 1).[13] Remarkably, for the ammonium-functionalized prolines **1S** and **1R**, a higher *trans*/*cis* conformer ratio of $K_{trans/cis} = 5.7$ is observed in D_2O for the (4*S*) stereoisomer adopting a C4-*endo* ring pucker compared to $K_{trans/cis} = 3.5$ for the (4*R*) isomer adopting a C4-*exo* ring pucker (Table 1, entries 1 and 2). The same preference for the *trans* conformer in *endo* ring puckers compared to *exo* ring puckers is observed also for the other proline derivatives with H-bond-donating substituents at C4 in $CDCl_3$ (Table 1, entries 3–8). As expected, the effect is less pronounced in water, where hydration competes with intramolecular H-bonding. Nevertheless, the differences in the *trans*/*cis* conformer ratios are significantly smaller for compounds **2** and **3** compared to those observed with EWGs that bear no H-bond donor sites at C4 such as (4*R*)-azidoproline **5R** and (4*S*)-azidoproline **5S**. Here, a twofold greater preference for the *trans* isomer is observed for the (4*R*)-configured compound with an *exo* pucker, compared to the *endo*-puckered (4*S*) derivative ($K_{trans/cis} = 6.1$ versus 2.6, Table 1, entries 9 and 10).[5] These

Table 1: Equilibrium constants $K_{trans/cis}$ of **1**–**5**.[a]



Entry	X	abs. conf. at C4	$K_{trans/cis}$ (D_2O)	$K_{trans/cis}$ ($CDCl_3$)
1	NH_3^+	**1S**	5.7[b]	n.d.[c]
2		**1R**	3.5[b]	n.d.[c]
3	NHAc	**2S**	4.3	5.8
4		**2R**	5.8	5.2
5	NHBoc	**3S**	3.8	5.5
6		**3R**	5.2	3.5
7	OH	**4S**	2.4[d]	4.7
8		**4R**	6.1[d]	4.2
9	N_3	**5S**	2.6[e]	1.9[e]
10		**5R**	6.1[e]	3.9[e]

[a] Determined by ^1H NMR spectroscopic analysis at a concentration of 80 mM. [b] Spectra recorded in 1 M trifluoroacetic acid were identical to those of the Cl^- and the $CF_3CO_2^-$ salts of **1S** and **1R** in D_2O. [c] Not determined because of poor solubility of **1S** and **1R** in $CDCl_3$. [d] From reference [4e]. [e] From reference [5].

observations show that the strongest effect is exerted by the ammonium substituent at C4 followed by the amide, carbamate, and hydroxy moieties. Most importantly, the results demonstrate that the *trans* amide conformer in Xaa–Pro bonds can be populated to a significant extent in proline derivatives with a C4-*endo* ring pucker.

To further support the hypothesis that the preference of the *trans* amide bond in an *endo*-ring pucker is caused by an interaction between the H-bond-donating substituents at C4 and the methyl ester and thereby an enhanced n→π* interaction, ab initio calculations and IR spectroscopic studies were performed. In the lowest-energy conformation of Ac-[(4*S*)NHAc-Pro]-OCH_3 (**2S**) predicted by quantum chemical calculations,[14,15] a C4-*endo* ring pucker and a hydrogen bond between the acetamide at C4 and the carbonyl group of the methyl ester is observed (Figure 1). As predicted, this hydrogen bond allows for the inward orientation of the methyl ester with a dihedral angle Ψ (N_i-C2-C_i-OCH_3) of 138°, which is ideal for an n→π* interaction.[7] This beneficial arrangement for an n→π* interaction is reflected in the angle of 104°

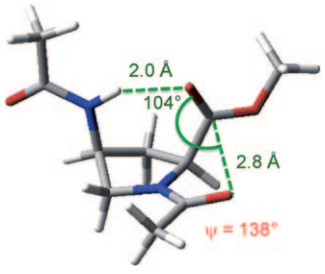


Figure 1. Lowest-energy conformation of Ac-[(4*S*)NHAc-Pro]-OCH_3 (**2S**) obtained from ab initio calculations.

between the oxygen of the acetyl group and the carbonyl group of the methyl ester, as well as the O_{i-1}–C_i distance of 2.8 Å, which is within the van der Waals radii of the two centers.

The energy differences between the *trans* and *cis* conformers ($\Delta E_{trans-cis}$) were computed in the gas phase at the RI-MP2/TZVP level based on B3LYP/6-31G** optimized structures.[14–17] The calculated energies support a stronger preference of the *trans* conformer for **2S** compared to the corresponding (4*R*)-configured derivative **2R** (Table 2, column 1). Similar conformations and energy differences are predicted for the hydroxyproline (Hyp) derivatives **4S** and **4R** in the gas phase. For the respective azidoprolines **5S** and **5R**, the ab initio studies predict a higher preference of the *trans* conformer for the (4*R*)-configured derivative compared to the (4*S*)-configured isomer; this computational result is also in agreement with the experimental data.[5] For the ammonium-functionalized prolines **1S** and **1R**, in addition to the hydrogen bond an electrostatic interaction between the positively charged ammonium group and the methyl ester is suggested by the quantum chemical calculations. This additional stabilization supports the experimentally observed high *trans*/*cis* conformer ratio of the ammonium derivative **1S** compared to those of the amide and carbamate derivatives **2S** and **3S**.

Table 2: Calculated energy differences $\Delta E_{trans-cis}$ between the lowest energy *trans* and *cis* conformers.[a]

	$\Delta E_{trans-cis}$ [kJ mol^{-1}]		
	2 (NHAc)	**4** (OH)	**5** (N_3)
(4*S*)	−11.2	−15.5	1.1[b]
(4*R*)	−4.0	−6.6	−6.7[b]

[a] Values calculated at the RI-MP2/TZVP//B3LYP/6-31G** level. A negative sign denotes a preference for the *trans* conformation. [b] From reference [5].

IR spectroscopic studies further support this analysis (Table 3). H-bonding combined with the n→π* interaction should lead to a significantly reduced bond order of the ester C=O bond. Indeed, stretching vibrations of the ester (ν_{ester}) of the (4*S*)-configured isomers **2S**–**4S** in $CHCl_3$ are 10–20 cm^{-1} lower compared to those of the (4*R*)-configured isomers (Table 3). In contrast, for the respective azido derivatives, the stretching vibration for the (4*R*) derivative is only 4 cm^{-1} lower than that for the (4*S*) derivative (Table 3). The larger differences observed for the proline derivatives **2**–**4** further support the contributions from both H-bonding and n→π* interactions. In addition, as expected for the formation of a *trans*-annular hydrogen bond, the N–H stretching vibration of, for example, the NHAc moiety in **2S** is lower (3316 cm^{-1}) compared to that of **2R** (3439 cm^{-1}).[3h,18]

Table 3: Ester carbonyl stretching vibrations ν_{ester} of **2**–**5** in cm^{-1} determined by IR spectroscopy.[a]

	ν_{ester} [cm^{-1}]			
	2 (NHAc)	**3** (NHBoc)	**4** (OH)	**5** (N_3)
(4*S*)	1733	1737	1725[b]	1749[c]
(4*R*)	1748	1746	1745[b]	1745[c]

[a] IR spectra were recorded at a concentration of 10 mM in $CHCl_3$ at 25 °C. [b] Data is consistent with reported results in reference [4e]. [c] From reference [5].

Finally, we evaluated whether the observed effects can be used to stabilize *trans* amide bonds within a larger peptide. As an example we used the solvent-induced conformational switch of oligoprolines between polyproline II (PPII) and polyproline I (PPI) helices.[19,20] The PPII helix with all-*trans* amide bonds predominates in water, whereas the PPI helix with all-*cis* amide bonds is favored in more hydrophobic solvents such as *n*-PrOH. The amount of *n*-PrOH necessary to switch from the PPII to the PPI helix is therefore a measure for the conformational stability of the PPII helix with *trans* amide bonds.[19,20] Thus, we studied the conformational properties of the (4*S*)- and (4*R*)-ammoniumproline (Amp)-containing oligoprolines Ac-[(4*S*)Amp-Pro-(4*S*)Amp]$_3$-OH (**6S**) and Ac-[(4*R*)Amp-Pro-(4*R*)Amp]$_3$-OH (**6R**), which were prepared by standard solid-phase peptide synthesis.[15]

CD spectroscopic studies of oligoprolines **6S** and **6R** in different ratios of phosphate buffer and *n*-PrOH show that the PPII helix with all-*trans* amide bonds is stabilized by the (4*S*)-configured Amp residues, as was expected from the studies on the monomers. Even in pure *n*-PrOH, a solvent that is known to favor the PPI helix, the CD spectrum of **6S** is still indicative of a PPII helix (Figure 2, right). In contrast, the isomer with (4*R*)Amp residues **6R** switches to the PPI helix with *all-cis* amide bonds upon addition of *n*-PrOH (Figure 2, left). These results reflect the preference for the *trans* and *cis* conformers of the monomers **1S** and **1R** and demonstrate the value of the described derivatives to tune the *cis*/*trans* conformer ratio of Xaa–Pro amide bonds within peptides.

In summary, we have introduced proline derivatives with a C4-*endo* ring pucker that favor *trans* amide bonds as tools to tune the *cis*/*trans* amide conformer ratio in Xaa–Pro bonds. In addition, we showed that these derivatives stabilize the PPII conformation. The work also provides deeper insight into the factors that determine the conformation of proline residues,

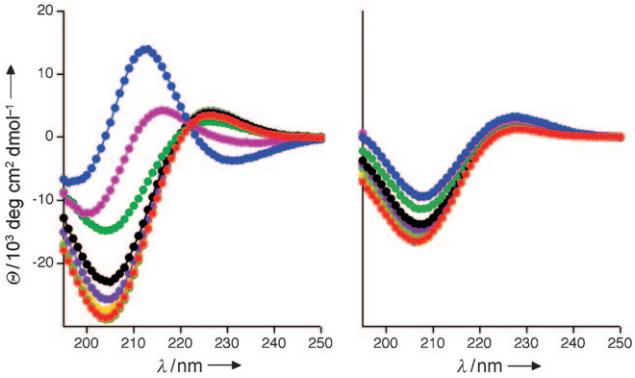


Figure 2. CD spectra of Ac-[(4*R*)Amp-Pro-(4*R*)Amp]$_3$-OH **6R** (left) and Ac-[(4*S*)Amp-Pro-(4*S*)Amp]$_3$-OH **6S** (right) in aqueous phosphate buffer (10 mM, pH 2) (red), 25% v/v *n*-PrOH in buffer (light blue), 50% *n*-PrOH (yellow), 75% *n*-PrOH (light green), 85% *n*-PrOH (purple), 90% *n*-PrOH (black), 95% *n*-PrOH (dark green), 99% *n*-PrOH (magenta) and *n*-PrOH (dark blue). Spectra were recorded at concentrations of 70 μM at 25 °C.

and demonstrates that the ring pucker is not the only factor that influences the amide bond conformer ratio. In addition, noncovalent interactions between the substituent at C4 of the proline ring and the amide backbone are key for favoring or disfavoring the *trans* conformer in Xaa–Pro bonds. Thus, the presented proline derivatives will not only be useful for the study of biological processes in which *cis*/*trans* isomerizations are involved, but also for the design of new probes.

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[12] This result demonstrates that all of the examined substituents at C4 prefer a gauche rather than an anti orientation towards the N-acetyl group as previously observed for other electron-withdrawing substituents such as F and N_3 (refs. [3–5]).

[13] The *trans* and *cis* isomers were assigned based on NOEs between the protons of the acetyl group and those at Cδ and Cα, respectively.

[14] Conformational searches and ab initio calculations were performed by using the Spartan′02 package (Spartan′02, Wavefunction, Inc., Irvine, CA) and a development version of the Q-Chem program package (http://www.q-chem.com), respectively.

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[18] The existence of the hydrogen bond in **2S**–**4S** is further supported by the larger vicinal NH–Hγ coupling constants in the *trans* conformer of **2S**–**4S** (7.9–8.6 Hz) compared to those observed for the *cis* conformer (5.4–6.5 Hz) as well as those of **2R**–**4R** (6.5–6.8 Hz, both conformers). A large coupling constant is expected for a C4-*endo* conformation with a fixed dihedral angle Hγ-C-N-H close to 180° (see Scheme 2, right). Crystal structures of **1S** and **2S** are controlled by intermolecular hydrogen bonds and therefore not representative of their conformations in solution phase.

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