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The "Azido Gauche Effect"—Implications for the Conformation of Azidoprolines

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Abstract: The "azido gauche effect" was examined both experimentally and theoretically and was found to determine the conformation of, for example, (4*R*)- and (4*S*)-azidoproline (Azp) derivatives. For (4*R*)Azp derivatives, the azido gauche effect induces a preferred C(4)-exo conformation of the pyrrolidine ring, which leads to stabilization of the s-trans amide conformer of, e.g., Ac-(4*R*)Azp-OCH_3 (**5R**) via an n→π^* interaction between the nonbonding electrons of the oxygen of the acetyl group and the carbonyl group of the ester. For (4*S*)Azp derivatives, the azido gauche effect results in a C(4)-endo conformation of the pyrrolidine ring that does not allow for this stabilizing n→π^* interaction of the s-trans conformer. Consequently, a significantly higher s-trans:s-cis amide conformer ratio is observed for (4*R*)Azp compared to (4*S*)Azp derivatives (e.g., 6.1:1 versus 2.6:1 in D_2O for Ac-(4*R*)Azp-OCH_3 (**5R**) compared to Ac-(4*S*)Azp-OCH_3 (**5S**)). These conformational preferences are reflected in the higher tendency of (4*S*)Azp-containing peptides to form cyclic peptides with all-cis amide bonds compared to (4*R*)Azp derivatives. Ab initio calculations demonstrate that the strength of the azido gauche effect is comparable to that of the well-known "fluorine gauche effect". For azidoethane derivatives $N_3-CH_2CH_2-X$ (X = N_3, NHCOH, NHAc, or $N(CH_3)Ac$), the ab initio calculations revealed energy differences of 5−13 kJ mol^{-1} between the anti and gauche conformations in favor of the gauche conformer. Calculations were also performed for the (4*R*)Azp and (4*S*)Azp derivatives **5R** and **5S**, supporting the experimentally observed data.

Introduction

The conformation of flexible molecules is typically determined by a complex balance of different factors, such as noncovalent interactions (e.g., ionic interactions, H-bonding), steric hindrance, and stereoelectronic effects. In the absence of stronger factors, even comparatively weak stereoelectronic effects, like the "gauche effect", can have a large influence on the conformation of molecules. The gauche effect describes the preference for the gauche conformer over the corresponding anti conformer and is well known for 1,2-difluoroethane and many other derivatives X−C−C−Y, where at least X or Y is an electron-withdrawing substituent.[1−16] 1,2-Difluoroethane has also been the subject to a multitude of theoretical studies for which, for example, Møller−Plesset or coupled cluster methods predict energy differences between the gauche and the anti conformers on the order of 2−4 kJ mol^{-1}.[15−24] The gauche effect has been found to determine the conformation of small molecules but also those of larger molecules, like 9,10-difluorostearic acid, 4-fluoroproline, and fluoroproline-containing peptides and proteins.[25−33]

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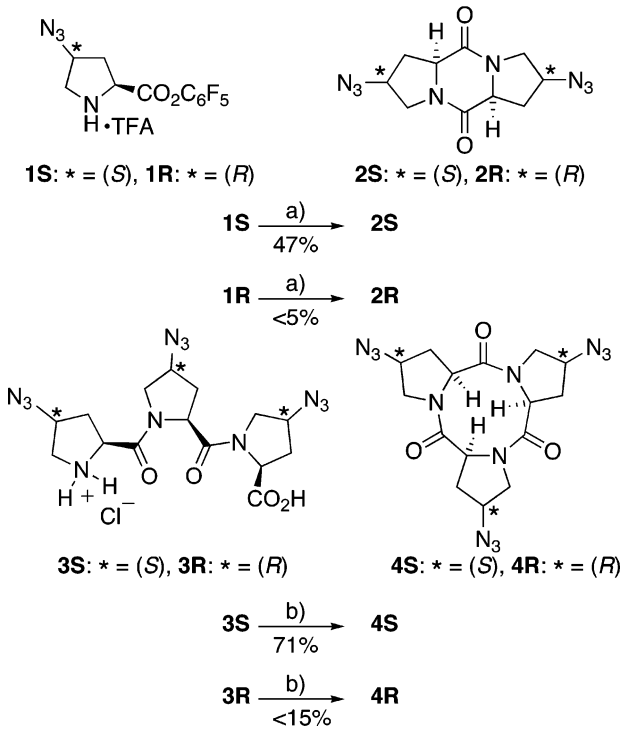
In comparison to the "fluorine gauche effect", little is known about the gauche effect exerted by azido substituents. Although organic and aliphatic azides have been studied both experimentally and theoretically, we are not aware of any systematic studies on the gauche effect in conjunction with azido substituents in organic compounds. There are only a few reports on investigations of the conformation of azido-substituted cycloalkanes, mainly based on computational analyses.[34−36]

From a synthetic viewpoint, the azide is a versatile functional group for a wide range of transformations.[37] For example, the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition, the "click reaction", allows for facile further functionalization of azides with alkynes under mild conditions in the presence of a wide range of other functional groups.[38,39]

Recently, we have used diketopiperazines **2S** and **2R**, derived from (4*R*)Azp and (4*S*)Azp, as templates for two-armed receptors that bind peptides with high sequence selectivity.[40,41] Furthermore, we prepared tripodal templates based on the cyclotriazidoproline derivatives **4S** and **4R**, derived from (4*R*)-Azp and (4*S*)Azp, respectively.[42] For both the cyclic di- and tripeptides, significant differences in the cyclization yield were observed, depending on the configuration at C(4) of the Azp derivatives (Scheme 1): The diketopiperazine and cyclotriproline derivatives **2S** and **4S**, with (4*S*)-configuration, were obtained in good yields of 47% and 71%, respectively, whereas the diastereomers **2R** and **4R**, with (4*R*)-configuration, formed in less than 5% and 15% yield, respectively, under identical reaction conditions.[43]

Thus, the configuration at C(4) exerts a significant effect on the cyclization tendency of Azp derivatives. These observations led us to study the conformation of (4*R*)Azp and (4*S*)Azp derivatives in more detail with a combination of NMR and IR spectroscopic studies, crystal structure analysis, and ab initio calculations. We will show that an "azido gauche effect" determines the conformation of (4*R*)Azp and (4*S*)Azp derivatives and ultimately influences their different cyclization tendencies. Furthermore, we demonstrate that the strength of the azido gauche effect is comparable to that of the well-known fluorine gauche effect and can therefore be used as a conformation-directing element.

Scheme 1. Different Cyclization Tendencies of Azp Derivatives Depending on the Configuration at C(4)[a]



[a] Reagents: (a) 2 equiv of $^{i}Pr_2NEt$, THF or DMF, 0.3 M; (b) 3 equiv of HATU, 9 equiv of $^{i}Pr_2NEt$, DMF, 8 mM.

Results and Discussion

The formation of cyclic di- and tripeptides requires all amide bonds of the linear precursors to adopt s-cis conformations. Thus, the observed higher cyclization tendencies of the (4*S*)-Azp derivatives **1S** and **3S** compared to the (4*R*)-configured diastereomers **1R** and **3R** suggest that this should correlate with a higher s-cis:s-trans conformer ratio of the proline amide bond of (4*S*)-azidoprolines. To test this hypothesis, we initially analyzed the conformation of Ac-(4*S*)Azp-OCH_3 (**5S**) and Ac-(4*R*)Azp-OCH_3 (**5R**) as simple model systems (Figure 1). Their comparatively low complexity allowed for conformational analysis not only by spectroscopic means but also by ab initio calculations. Since **5R** and **5S** have the same functional groups as the putative intermediates H-(4*R*)Azp-(4*R*)Azp-OC_6F_5 and H-(4*S*)Azp-(4*S*)Azp-OC_6F_5 for the formation of the diketopiperazines **2R** and **2S** (a nonreactive methyl ester in place of the activated pentafluorophenyl ester and an acetyl group instead of the second Azp residue), they can also be regarded as simple models for these intermediates.

s-cis:s-trans Ratio of Ac-(4*R*)Azp-OCH_3 (5R) and Ac-(4*S*)-Azp-OCH_3 (5S). ^{1}H NMR spectra of **5R** and **5S** were recorded in a range of different solvents at a concentration of 80 mM. All spectra showed two eight-spin systems, corresponding to the s-trans and the s-cis amide conformers. The s-trans isomer

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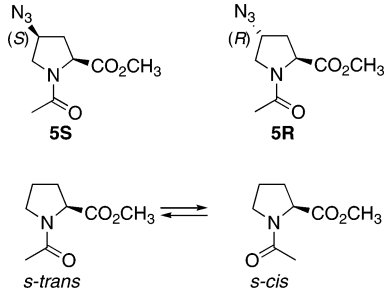

Figure 1. Ac-(4*S*)Azp-OCH_3 (**5S**), Ac-(4*R*)Azp-OCH_3 (**5R**), and the s-trans and s-cis conformations around the proline amide bond.

Table 1. s-cis:s-trans Conformer Ratios of **5S** and **5R** As Determined by ^{1}H NMR Spectroscopy in Different Solvents[a]

		s-cis:s-trans	
entry	solvent	**5S**	**5R**
1	D_2O	1:2.6	1:6.1
2	d_7-DMF	1:1.6	1:3.8
3	d_5-pyridine	1:2.0	1:4.5
4	CD_3OD	1:1.7	1:4.0
5	d_6-acetone	1:1.6	1:3.7
6	$CDCl_3$	1:1.9	1:3.9
7	d_8-dioxane	1:2.0	1:4.2

[a] All samples were measured at 295 K at a concentration of 80 mM.

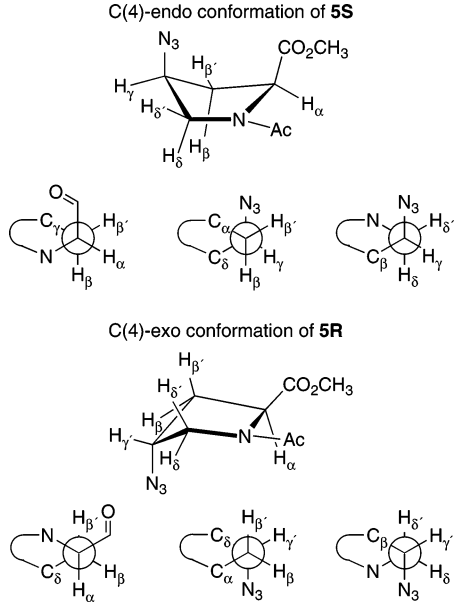

Figure 2. C(4)-exo and C(4)-endo conformations with Newman projections of the dihedral angles. For clarity, the dihedral angles are shown fully staggered rather than with ideal angles.

was assigned on the basis of a nuclear Overhauser effect (NOE) between the protons of the acetyl group and those at C(5); the s-cis isomers showed NOEs between the protons of the acetyl group and the proton at C(2).

In each spectrum, the s-trans conformer was the major conformer; however, the s-cis:s-trans ratios differed significantly, depending on the solvent and particularly the configuration at C(4). As expected from the synthesis, the (4*R*)-configured diastereomer **5R** favors the s-trans conformer over the s-cis conformer more than the (4*S*)-configured diastereomer **5S** (Table 1), reflecting the lower cyclization tendency of the (4*R*)-configured azidoprolines **1R** and **3R** compared to their (4*S*)-configured diastereomers **1S** and **3S**. In comparison to Ac-Pro-OCH_3 (s-trans:s-cis 4.6:1 in D_2O), the proportion of the s-trans conformer is higher for **5R** and lower for **5S**.

These results support the hypothesis that the s-cis:s-trans conformer ratio is closely linked to the cyclization tendency. To understand how the configuration at C(4) governs the s-cis:s-trans conformer ratio of azidoprolines, we analyzed the conformation of **5S** and **5R** in more detail.

Conformational Analysis of the Pyrrolidine Rings of 5R and 5S. The pyrrolidine ring of Azp can adopt essentially two main conformations, a C(4)-endo or a C(4)-exo conformation (Figure 2).[44] Depending on which conformation is adopted, the azido substituent occupies either the pseudoaxial or the pseudoequatorial position.

Analysis of the vicinal ^{1}H,^{1}H coupling constants revealed that both the s-cis and the s-trans conformers of Ac-(4*R*)Azp-OCH_3 (**5R**) adopt C(4)-exo conformations, while both conformers of Ac-(4*S*)Azp-OCH_3 (**5S**) adopt C(4)-endo conformations. Most indicative of the C(4)-endo conformation of **5S** are the small coupling constants observed between Hα and Hβ', between Hβ' and Hγ, and between Hγ and Hδ' (Table 2). Judged by the Karplus curve, these values are indicative of torsion angles close to 90°, which can be realized in a C(4)-endo but not a C(4)-exo conformation.[45] Furthermore, the long-range ^4J coupling between Hβ' and Hδ' demonstrates their pseudoequatorial positions, which is only possible in a C(4)-endo or related conformation.[44]

The conformation of **5R** is revealed by the small coupling constants between Hβ and Hγ' and between Hγ' and Hδ, which are only in agreement with a predominant C(4)-exo conformation (Table 3). Also, the long-range ^4J coupling between Hβ and Hδ, indicating their pseudoequatorial positions, is only possible in a C(4)-exo conformation. The common factor of both the C(4)-exo conformation of **5R** and the C(4)-endo conformation of **5S** is the pseudoaxial positioning of the azido substituents that are thereby in a gauche conformation relative to the *N*-acetyl groups.

This conformational analysis by ^{1}H NMR spectroscopy is supported by a crystal structure of **5R** as well as ab initio calculations for **5R** and **5S** (Figure 3). Both the lowest energy conformations obtained by ab initio calculations (vide infra) and the crystal structure show the pseudoaxial position of the azido groups and their gauche relationship to the *N*-acetyl group. These results suggest that an azido gauche effect determines the overall conformation of the pyrrolidine ring of azidoprolines.

The "Azido Gauche Effect". To analyze and quantify the azido gauche effect observed in **5S** and **5R**, we performed ab initio calculations with simple azidoethane derivatives and azidoprolines **5S** and **5R**. The corresponding fluoroethane derivatives were calculated for comparison.

(a) Ethane Derivatives. Calculations were performed on the 1,2-disubstituted ethane derivatives X$-$$CH_2CH_2-$Y (**6**$-$**13**), with X being either N_3 or F (Figure 4). These ethane derivatives

(44) Due to the facile rotation of five-membered rings, the C(4)-endo conformation is very similar to other conformations, like the C(3)-exo, the C(5)-exo, and twist conformations thereof, which will not be distinguished here. Likewise, the C(4)-exo conformation is similar to the C(3)-endo, the C(5)-endo, and twist conformations thereof.

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Table 2. Vicinal Coupling Constants of the s-trans and s-cis Conformers of Ac-(4*S*)Azp-OCH_3 (**5S**) in Different Solvents[a]

		D_2O		DMF		CD_3OD		$CDCl_3$	
entry	^3J(H,H)	s-trans	s-cis	s-trans	s-cis	s-trans	s-cis	s-trans	s-cis
1	Hα−Hβ	9.6	7.2	9.1	9.0	9.2	8.5	8.9	6.1
2	Hα−Hβ'	2.5	3.1	4.2	1.5	3.7	1.5	4.3	4.1
3	Hβ−Hγ	5.2	nd	6.0	5.2	5.7	4.8	nd	nd
4	Hβ'−Hγ	2.4	nd	4.7	3.0	3.8	1.6	4.4	nd
5	Hγ−Hδ	5.3	5.3	6.1	5.4	5.9	5.5	6.1	nd
6	Hγ−Hδ'	1.6	<1	4.0	1.5	3.5	<1	4.2	1.3
7	Hβ'−Hδ' [b]	1.5	nd	<1	<1	0.7	1.6	nd	nd

[a] "nd" indicates that the coupling constant could not be determined due to overlapping signals. [b] The ^4J(H,H) coupling between Hβ' and Hδ' indicates their pseudoequatorial positions at C(3) and C(5).

Table 3. Vicinal Coupling Constants of the s-trans and s-cis Conformers of Ac-(4*R*)Azp-OCH_3 (**5R**) in Different Solvents[a]

		D_2O		DMF		CD_3OD		$CDCl_3$	
entry	^3J(H,H)	s-trans	s-cis	s-trans	s-cis	s-trans	s-cis	s-trans	s-cis
1	Hα−Hβ	8.0	8.6	8.2	7.5	8.2	8.5	8.2	8.3
2	Hα−Hβ'	8.3	nd	7.1	6.7	7.5	6.0	6.4	5.8
3	Hβ−Hγ'	3.3	4.7	4.4	nd	4.1	5.0	5.0	5.1
4	Hβ'−Hγ'	5.3	nd	5.6	nd	5.5	5.9	6.1	6.1
5	Hγ'−Hδ	2.3	nd	3.5	nd	3.2	1.5	3.8	4.8
6	Hγ'−Hδ'	5.9	5.5	5.4	5.7	5.2	5.7	5.6	5.8
7	Hβ−Hδ[b]	1.5	nd	1.2	nd	1.4	1.4	1.0	1.2

[a] "nd" indicates that the coupling constant could not be determined due to overlapping signals. [b] The ^4J(H,H) coupling between Hβ and Hδ indicates their pseudoequatorial positions at C(3) and C(5).

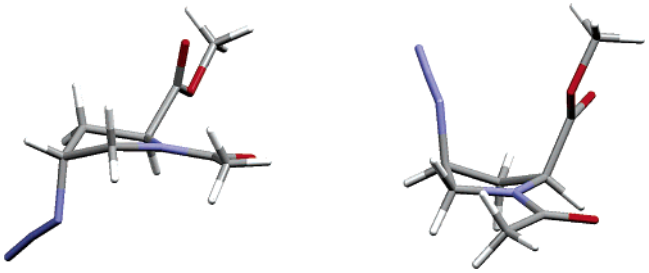


Figure 3. Structures of **5R** (left) and **5S** (right). For **5S**, the lowest energy conformation as calculated at the RI-MP2/TZVP//B3LYP/6-31G** level is shown. For **5R**, the crystal structure is shown. The calculated lowest energy conformation of **5R** is in good agreement with the crystal structure, except for the flexible torsion angle of the azido group (C−C(4)−N−N); see Supporting Information.

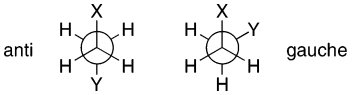
6 X = Y = N_3
7 X = N_3, Y = NHCOH
8 X = N_3, Y = NHAc
9 X = N_3, Y = N(CH_3)Ac
10 X = Y = F
11 X = F, Y = NHCOH
12 X = F, Y = NHAc
13 X = F, Y = N(CH_3)Ac

Figure 4. Azido- and fluoroethane derivatives **6**−**13**.

allowed for an analysis of the azido gauche effect without influence from other effects like ring torsions.

The ethane derivatives with amide substituents as Y were chosen as fragments of acetylated 4-azidoprolines. For all derivatives, systematic conformational searches were performed using molecular mechanics calculations with the force field MMFF94.[46] All conformers were then optimized at the HF/6-31G** level. The lowest energy structure was used as the initial structure for a second conformational search. Thereafter, the conformers were optimized again at the HF/6-31G** level. The energetically most favored conformer was optimized at both the B3LYP[47,48] and RI-MP2[49−51] levels using the basis sets 6-31G** [52,53] and SVP,[54] respectively. In addition, single-point calculations were performed at the MP2 level using larger basis sets. Table 4 shows the energy differences between the anti and gauche conformers obtained by using the MP2 method and a QZ2P basis[54] for structures optimized at the RI-MP2/SVP level.

Table 4. Energy Differences (in kJ mol^{-1}) between Anti and Gauche Conformers of Ethane Derivatives **6**−**13**[a]



		$\Delta E = E_{anti} - E_{gauche}$ (kJ mol^{-1})	
entry	Y	X = F	X = N_3
1	F	3.7[b]	nd[d]
2	N_3	nd[d]	5.5[c]
3	NHCOH	6.0	5.3
4	NHAc	6.0	6.8
5	N(CH_3)Ac	7.0	13.9

[a] The calculations were performed at the MP2/QZ2P level, based on RI-MP2/SVP-optimized structures. The ZPE corrections were computed at the B3LYP/6-31G** level. [b] ZPE-corrected value: 4.0 kJ mol^{-1}. [c] ZPE-corrected value: 4.6 kJ mol^{-1}. [d] Not determined.

Vibrational frequencies were calculated at the B3LYP/6-31G** level for diazido- and difluoroethane (**6** and **10**) based on the B3LYP/6-31G**-optimized structures. The zero-point energies (ZPEs) differ by 0.9 kJ mol^{-1} at most.

These data demonstrate that the gauche conformer is more stable compared to the anti conformer for all azido- and

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Table 5. Energy Differences (in kJ mol^{-1}) between Anti and Gauche Conformers of the Azp Derivatives **5R** and **5S** and between s-trans and s-cis Conformers of the Gauche Conformers[a]

		$\Delta E = E_{anti} - E_{gauche}$ (kJ mol^{-1})		$\Delta E = E_{s-trans} - E_{s-cis}$ (kJ mol^{-1})
entry	compound	s-trans	s-cis	gauche conformer
1	5R	3.0 (3.3)	6.3 (7.0)	−6.7 (−6.1)
2	5S	2.9 (3.2)	13.6 (14.4)	1.1 (1.9)

[a] The calculations were performed at the RI-MP2/TZVP level based on B3LYP/6-31G**-optimized structures. Zero-point-corrected energy differences are listed in parentheses. The ZPE corrections were computed at the B3LYP/6-31G** level.

fluoroethane derivatives studied. The gauche effect exerted by the azido group is either comparable to the fluorine gauche effect (entries 1−4) or slightly stronger (entry 5).

To check the accuracy of the relative energies, additional single-point calculations were performed for 1,2-difluoroethane (**10**). The influence of using different cc-pV*X*Z basis sets (X = 4, 5, 6)[55−58] at the MP2 level for the RI-MP2/SVP structures is rather small, with variations as compared to the MP2/QZ2P value of 0.3 kJ mol^{-1} at most. Further calculations were carried out at both the CCSD and CCSD[T] levels,[59] which indicate a preference for the gauche conformation by 2.9 kJ mol^{-1} (CCSD/QZ2P//RI-MP2/SVP) and 3.2 kJ mol^{-1} (CCSD[T]/QZ2P//RI-MP2/SVP), respectively. This is in line with earlier quantum chemical investigations for difluoroethane.[15−24] For *N*-acetyl-2-fluoroethylamine (**12**), ab initio studies at the DFT and MP2 levels found energy differences in favor of the gauche conformer of 7.5 and 7.1 kJ mol^{-1}, respectively, which is also in agreement with our studies.[16,60]

(b) Azidoprolines 5S and 5R. To analyze the azido gauche effect on the conformation of azidoprolines **5S** and **5R**, calculations of their s-cis and s-trans conformers were performed. Systematic conformational searches with the MMFF94 force field were performed, and each conformer was optimized at the B3LYP/6-31G** level. More accurate energies were obtained by using single-point RI-MP2 calculations with a TZVP basis.[54]

For the energetically most stable structures at the RI-MP2/TZVP level, stationary points were confirmed as minima by calculations of vibrational frequencies at the B3LYP/6-31G** level based on the B3LYP/6-31G**-optimized structures. Table 5 shows the resulting energy differences between the anti and gauche conformers with regard to the relative position of the azido and *N*-acetyl groups, as well as between the s-trans and s-cis amide conformers. Here "gauche" denotes the C(4)-exo conformation of **5R** and the C(4)-endo conformation of **5S**, whereas "anti" denotes the C(4)-endo conformation of **5R** and the C(4)-exo conformation of **5S**.

The data clearly demonstrate that a gauche conformation of the azido substituent relative to the *N*-acetyl group is energetically more favorable compared to an anti conformation for the s-cis as well as the s-trans amide isomers of both **5R** and **5S**. Thus, in the lowest energy conformations, the s-cis and s-trans conformers of **5R** adopt a C(4)-exo conformation and the conformers of **5S** a C(4)-endo conformation (Figure 3 and Supporting Information). For these preferred conformations, the calculations indicate an energetic preference for the s-trans amide conformer by **5R** and almost equal energies for the s-cis and s-trans conformers for **5S** (Table 5, column on the right). Although these predictions from the ab initio calculations were obtained in the gas phase, they are in line with the s-cis:s-trans ratios observed in solution by ^{1}H NMR spectroscopic studies (Table 1). Here, a stronger preference for the s-trans amide conformer by **5R** compared to **5S** is observed.

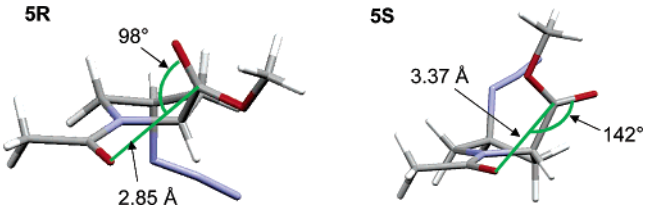

Figure 5. n→π* interaction in the s-trans conformer of **5R** (left), whereas the s-trans conformer of **5S** is not stabilized by an n→π* interaction (right).

The studies so far clearly demonstrate that an azido gauche effect determines the conformation of the pyrrolidine ring of the azidoproline derivatives. To rationalize how this gauche effect leads to a higher s-trans:s-cis amide conformer ratio of the (4*R*)-configured azidoproline **5R** compared to the (4*S*)-configured diastereomer **5S**, a closer analysis of the lowest energy structures of the ab initio studies as well as the crystal structure of **5R** was most revealing.

Stabilization of the s-trans Conformer of (4*R*)Azp by an n→π* Interaction. In the crystal structure and the lowest energy conformation from the ab initio studies of the s-trans amide conformer of **5R**, the oxygen of the acetyl group is at an angle of 98° and 101°, respectively, to the carbonyl group of the methyl ester. This angle is typical for the trajectory (Bürgi−Dunitz trajectory) at which a nucleophile is known to approach a carbonyl group.[61] The distance between the oxygen of the acetyl group and the carbonyl carbon of the methyl ester is 2.85 Å (crystal structure) and 2.86 Å (ab initio structure), demonstrating the good agreement between experiment and theory. Thus, the angle between O- - -C═O and the distance between O- - -C, which is well within the van der Waals radii, indicate a stabilizing n→π* interaction of the s-trans conformation.[28,30,62] The n→π* interaction is only observed in the s-trans conformation of (4*R*)-azidoproline **5R** (Figure 5, left). The C(4)-endo conformation of the (4*S*)-diastereomer **5S** does not allow for such a stabilization of the s-trans conformer since the distance between the oxygen of the acetyl group and the carbonyl group of the methyl ester is too large and the angle does not fit, either (Figure 5, right).

These results demonstrate not only that the pyrrolidine ring conformation of azidoprolines is governed by the azido gauche effect but also that this effect furthermore determines the relative

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Table 6. IR Spectroscopic Data (in cm^{-1}) of the Methyl Ester C═O Bond of **5R** and **5S**[a]

compound	in $CHCl_3$	in 1,4-dioxane
5R	1745	1748
5S	1749	1752

[a] Spectra were recorded at a concentration of 10 mM^{-1} at 298 K.

population of the s-cis and s-trans conformers by allowing for a stabilizing n→π* interaction in the case of the s-trans conformer of **5R** but not in the case of **5S**. As a result, a higher content of the s-trans conformer of **5R** is observed.

IR spectroscopic studies provided further evidence for this stabilizing n→π* interaction of the s-trans conformer of **5R**. Spectra of **5R** and **5S** in $CHCl_3$ and 1,4-dioxane revealed vibrational stretching frequencies for the methyl ester C═O bond of **5R** at slightly lower wavenumbers (difference of ~4 cm^{-1}) compared to those of **5S** (Table 6). Lower wavenumbers indicate lower bond orders, which is in agreement with a stabilizing n→π* interaction between the acetyl group and the methyl ester.

Conclusions

Our results demonstrate that the "azido gauche effect" can be used as a structure-directing element. We have shown how the azido gauche effect determines the conformation of 4-azidoproline derivatives and leads to a higher s-trans:s-cis amide conformer ratio for (4*R*)Azp compared to (4*S*)Azp derivatives. The higher s-cis content of (4*S*)Azp derivatives can also be used to explain their higher tendency to form cyclic di- and tripeptides compared to (4*R*)Azp derivatives. It is clear that the gauche effect is energetically a relatively small effect, so that other factors, such as kinetics, may be of importance for the different cyclization tendencies as well. Nevertheless, in the absence of stronger noncovalent interactions, the azido gauche effect will be the decisive element.

Furthermore, our ab initio calculations suggest that the strength of the azido gauche effect is comparable to that of the well-known "fluorine gauche effect". This is in line with the fact that the conformations of 4-azidoprolines are very similar to those described by Raines for the corresponding 4-fluoroprolines.[26−28] Thus, the azido group can be used as a conformation-directing element which allows for, in contrast to fluorine, facile functionalization by, for example, "click chemistry". In light of the importance of cis−trans isomerism around the Xxx−Pro bond in natural peptides and proteins,[63] 4-azidoprolines might serve as interesting new tools as well.

Experimental Section

Syntheses of 5S, 5R, 14S, and 14R. Materials and reagents were of the highest grade commercially available and used without further purification. Reactions were monitored by thin-layer chromatography using Merck silica gel 60 F_{254} plates. Compounds were visualized by UV, ceric ammonium molybdate (CAM), and ninhydrin. Flash chromatography was performed using Merck silica gel 60, particle size 40−63 μm. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX 400 and Bruker DPX 500 spectrometers. Chemical shifts are reported in ppm. Infrared spectra were obtained on a Perkin-Elmer FT-IR Spectrum spectrometer; peaks are reported in cm^{-1}. Finnigan MAT LCQ and TSQ 700 instruments were used for electrospray ionization (ESI) mass spectrometry.

Ac-(4*S*)Azp-OCH_3 (5S). Boc-(4*S*)Azp-OCH_3 (340 mg, 1.258 mmol) was dissolved in 4 M HCl in dioxane (3.5 mL) and stirred at room temperature for 1 h. After removal of all volatiles at reduced pressure, the residue was triturated with Et_2O (3 × 10 mL), providing the HCl salt as a white solid. The crude product was reacted with acetic anhydride (0.24 mL, 2.52 mmol) and triethylamine (0.52 mL, 3.77 mmol) in dry CH_2Cl_2 (2 mL). The reaction was monitored by thin-layer chromatography (TLC). After completion, 1 M HCl (1 mL) was added, and the mixture was extracted twice with EtOAc (2.5 mL). The combined organic layers were dried, and the solvent was removed in vacuo. After flash chromatography on silica gel (CH_2Cl_2:CH_3OH 98:2 to 97:3), the acetylated methyl ester **5S** (253 mg, 1.19 mmol, 95%) was isolated as a colorless oil. ^{1}H NMR (500 MHz, DMF-d_7, 25 °C): δ (s-trans conformer) = 4.53 (m, 1H; Hγ), 4.49 (dd, J = 9.2 Hz, 4.3 Hz, 1H; Hα), 3.97 (dd, J = 11.0 Hz, 6.1 Hz, 1H; Hδ), 3.68 (s, 3H; OCH_3), 3.54 (dd, J = 11.0 Hz, 3.9 Hz, 1H; Hδ'), 2.62 (ddd, J = 13.5 Hz, 9.2 Hz, 6.0 Hz, 1H; Hβ), 2.03 (dt, J = 13.5 Hz, 4.3 Hz, 1H; Hβ'), 2.04 (s, 3H; Ac); δ (s-cis conformer) = 4.84 (dd, J = 9.1 Hz, 1.5 Hz, 1H; Hα), 4.53 (m, 1H; Hγ), 3.77(s, 3H; OCH_3), 3.69 (dd, J = 13.0 Hz, 5.5 Hz, 1H; Hδ), 3.41 (dt, J = 13.0 Hz, 1.5 Hz, 1H; Hδ'), 2.62 (m, J = 9.1 Hz, 5.2 Hz, 1H; Hβ), 2.38 (dddd, J = 13.7 Hz, 3.2 Hz, 1.5 Hz, 0.4 Hz, 1H; Hβ'), 1.98 (s, 3H; Ac). ^{13}C NMR (125 MHz, DMF-d_7, 25 °C): δ (s-trans conformer) = 172.3, 169.4, 57.7, 60.2, 52.3, 52.7, 34.9, 22.1; δ (s-cis conformer) = 172.7, 170.1, 59.1, 59.1, 52.8, 51.7, 36.7, 22.1. IR (10 mM $CHCl_3$): ν = 3021, 2108, 1749, 1652, 1420. ESI-MS: m/z (relative intensity) = 235.0 (100) $[M + Na]^+$, 447.0 (60) $[2M + Na]^+$. Anal. Calcd for $C_8H_{12}N_4O_3$ (212.09): C, 45.28; H, 5.70; N, 26.40. Found: C, 45.21; H, 5.60; N, 26.28.

Ac-(4*R*)Azp-OCH_3 (5R). 5R was synthesized according to the same procedure as described for **5S** and obtained in 96% yield (36 mg) as a white solid. ^{1}H NMR (500 MHz, DMF-d_7, 25 °C): δ (s-trans conformer) = 4.53 (m, J = 9.3 Hz, 5.1 Hz, 3.9 Hz, 1H; Hγ), 4.38 (dt, J = 7.7 Hz, 1H; Hα), 3.89 (dd, J = 11.1 Hz, 5.3 Hz, 1H; Hδ'), 3.66 (m, 1H; Hδ), 3.65 (s, 3H; OCH_3), 2.38 (dddd, J = 12.0 Hz, 8.2 Hz, 4.4 Hz, 1.2 Hz, 1H; Hβ), 2.25 (dd, J = 7.1 Hz, 5.6 Hz, 1H; Hβ') 2.03 (s, 3H; Ac); δ (s-cis conformer) = 4.78 (dd, J = 8.0 Hz, 6.4 Hz, 1H; Hα), 4.43 (m, J = 9.2 Hz, 5.4 Hz, 1H; Hγ), 3.75 (s, 3H; OCH_3), 3.66 (m, 1H; Hδ), 3.56 (dd, J = 12.3 Hz, 5.7 Hz, 1H; Hδ'), 2.48 (m, 1H; Hβ'), 2.23 (dd, J = 7.1 Hz, 5.6 Hz, 1H; Hβ), 1.91 (s, 3H; Ac). ^{13}C NMR (125 MHz, DMF-d_7, 25 °C): δ (s-trans conformer) = 172.8, 169.3, 60.7, 57.9, 53.1, 52.3, 35.3, 22.1; δ (s-cis conformer) = 173.1, 169.8, 59.0, 58.9, 52.92, 51.2, 37.0, 21.6. IR (10 mM $CHCl_3$): ν = 3021, 2106, 1745, 1651, 1420. ESI-MS: m/z (relative intensity) = 235.0 (100) $[M + Na]^+$, 447.0 (60) $[2M + Na]^+$. Anal. Calcd for $C_8H_{12}N_4O_3$ (212.09): C, 45.28; H, 5.70; N, 26.40. Found: C, 45.27; H, 5.64; N, 26.36.

Crystal Structure Determination of 5R. Crystals of **5R** were obtained by crystallization from acetone. Crystals of the compound under investigation were mounted on a Nonius KappaCCD. Data collection was carried out using the Nonius collect suite.[64] The structures were solved by using direct methods with the program SIR92.[65] Least-squares refinement was carried out using the program CRYSTALS.[66] Plots were produced using ORTEP3 for Windows.[67]

The asymmetric unit within the unit cell of **5R** consists of two molecules, one of which is in a C(4)-exo s-trans conformation (Figures 3 and 5), and the other is in a C(3)-exo s-cis conformation. Judged by the Karplus curve,[45] the dihedral angles of the C(3)-exo conformation are not in agreement with the vicinal ^{1}H,^{1}H coupling constants observed in the solution phase. Thus, this conformer is probably stabilized in

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the solid state by packing effects within the crystal lattice, and such stabilization occurs in the solution phase only to a minor extent. The dihedral angles of the molecule that adopts a C(4)-exo conformation in the crystal are in good agreement with the torsion angles suggested by the vicinal ^{1}H,^{1}H coupling constants observed in solution, as judged by the Karplus curve (see Results and Discussion).

Experimental details of the structure determination of **5R** are compiled in the Supporting Information. CCDC-615190, deposited with the Cambridge Crystallographic Data Centre, contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.

Computational Details. Force field calculations were carried out with the Spartan '02 package[68] and the force field MMFF94.[46] The ab initio calculations were performed using the program packages Q-Chem[69] and TURBOMOLE.[70] The following basis sets were employed: 3-21G,[71] 6-31G**,[52,53] SVP,[54] TZVP,[54] QZ2P,[54] cc-pVQZ,[55] cc-pV5Z,[58] and cc-pV6Z.[56−58] Basis set contraction patterns: SVP, (4s1p)/[2s1p] for H, (7s4p1d)/[3s2p1d] for C, N, O, and F; TZVP, (5s1p)/[3s1p] for H, (11s6p1d)/[5s3p1d] for C, N, O, and F; QZ2P, (7s2p1d)/[4s2p1d] for H, (11s7p2d1f)/[6s4p2d1f] for C, N, O, and F; cc-pVQZ, (6s,3p,2d,1f)/[4s,3p,2d,1f] for H, (12s,6p,3d,2f,1g)/[5s,4p,3d,2f,1g] for C and F; cc-pV5Z, (8s,4p,3d,2f,1g)/[5s,4p,3d,2f,1g] for H, (14s,8p,4d,3f,2g,1h)/[6s,5p,4d,3f,2g,1h] for C and F; cc-pV6Z, (10s,5p,4d,3f,2g,1h)/[6s,5p,4d,3f,2g,1h] for H, (16s,10p,5d,4f,3g,2h,1i)/[7s,6p,5d,4f,3g,2h,1i] for C and F. The density functional theory calculations for the ethane derivatives were performed using the standard grid as implemented in Q-Chem.[69] Difluoroethane and diazidoethane were additionally optimized using a grid with 110 radial points and 590 angular points. The frequency calculations and all calculations concerning the proline derivatives were performed using a grid with 110 radial points and 590 angular points.

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Supporting Information Available: Complete ref 69; ^{1}H and ^{13}C NMR spectra of **5R** and **5S**, details on the crystal structure analysis of **5R** and on the ab initio calculations (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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