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(4*R*)- and (4*S*)-Azidoprolines – Conformation Directing Amino Acids and Sites for Functionalization

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§SCS Poster Prize Winner

Abstract: An 'azido *gauche* effect' determines the conformation of (4*S*)- and (4*R*)-azidoproline (Azp) derivatives and affects the *s-cis*:*s-trans* conformer ratio of Xaa-Azp bonds. The article summarizes our research on the conformational analysis of monomers as well as oligomers derived from (4*S*)Azp and (4*R*)Azp. We show that (4*S*)Azp and (4*R*)Azp can be used to tune the stability of the polyproline II (PPII) helix. In addition we demonstrate that Azp containing oligoprolines are attractive molecular scaffolds with a well-defined helical conformation that can be readily further functionalized using *e.g.* click chemistry.

Keywords: Azidoproline · Collagen · *Gauche* effect · Peptides · Polyproline II helix

1. Introduction

Proline and proline derivatives are unique amongst the natural amino acids due to their cyclic structure bearing a secondary amine. The secondary amine causes a significant content of *s-cis* conformers in Xaa-Pro amide bonds. The conformational rigidity of the pyrrolidine ring leads to the preferred formation of the polyproline II (PPII) conformation of proline rich peptides. In nature, *s-cis*:*s-trans* isomerizations around Xaa-Pro amide bonds are involved in many important processes such as signalling and protein folding.[1] Likewise, the PPII structure is an important secondary structure occurring in collagen and many segments of proteins.[2] Thus, understanding the factors that determine the *s-cis*:*s-trans* conformer ratio and the stability of the PPII structure is important. Proline derivatives bearing a substituent at the γ-carbon (C(4)) are an important tool in this respect since their *s-cis*:*s-trans* conformer ratios often differ from that of unsubstituted proline.[3,4]

We became interested in (4*R*)- and (4*S*)-azidoproline (Azp) as versatile amino acids that allow for further functionalization. Our research started by utilizing the diketopiperazine **1R** derived from (4*R*)Azp as a template for two-armed peptide receptors (Fig. 1).[5,6] These diketopiperazine receptors are highly versatile molecular hosts binding to peptides and other small molecules with high selectivity and binding affinities of up to $\Delta G \leq 6$ kcal/mol.[5a,5d]

Differences in the synthesis of the cyclic dipeptides (and later tripeptides[7]) derived from (4*R*)-Azp and (4*S*)-Azp led us to examine their conformational properties in more detail. Here we summarize our insights into the conformation-directing effect of the azido-substituent on monomeric and oligomeric Azp derivatives. In addition, we demonstrate how Azp-containing polyprolines enable further functionalization and use as molecular scaffolds.

2. Conformational Analysis of Ac-(4*R*)Azp-OCH_3 (2R) and Ac-(4*S*)Azp-OCH_3 (2S)

To analyze the conformational differences between (4*R*)- and (4*S*)-configured azidoprolines we initially used the acetylated methylesters Ac-(4*R*)Azp-OCH_3 (**2R**) and Ac-(4*S*)Azp-OCH_3 (**2S**) as simple model compounds.[4] Conformational analysis by ^{1}H NMR spectroscopy revealed considerable differences in their *s-cis*:*s-trans* conformer ratios and the conformation of the pyrrolidine rings. Regardless of the solvent, the *s-trans* conformer was the major conformer in both diastereoisomers, however, in the spectra of the (4*R*)-configured stereoisomer a significantly higher portion of the *s-trans* conformer was observed. For example, Ac-(4*R*)Azp-OCH_3 (**2R**) and Ac-(4*S*)Azp-OCH_3 (**2S**) have *s-cis*:*s-trans* conformer ratios of 1:6.1 and 1:2.6, respectively, in deuterated water (Fig. 2).[4] In comparison, the *s-cis*:*s-trans* conformer ratio of unsubstituted Ac-Pro-OCH_3 is 1:4.9 in D_2O.

The pyrrolidine ring of Pro can adopt essentially two main conformations, a C(4)-*endo* or a C(4)-*exo* conformation.

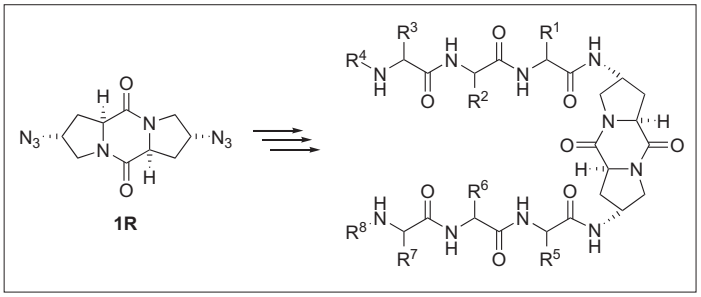

Fig. 1. General structure of diketopiperazine receptors and cyclic dipeptide **1R**.

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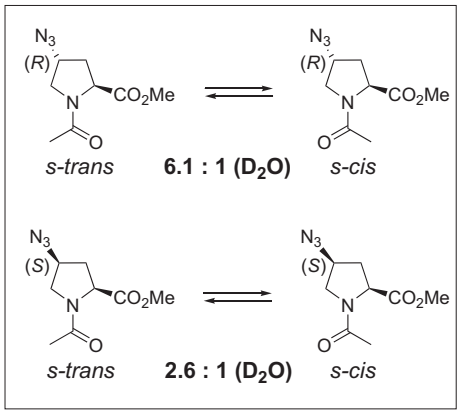


Fig. 2. *s-cis*:*s-trans* isomerization of azidoprolines **2R** (top) and **2S** (bottom).

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$_3$ (**2R**) adopt C(4)-*exo* conformations whereas both conformers of Ac-(4*S*)Azp-OCH$_3$ (**2S**) adopt C(4)-*endo* conformations. The common denominator of these preferred conformations is a pseudo-axial positioning of the azido-substituent that is thereby *gauche* with respect to the N-acetyl group (Fig. 3).[4]

These conformational analyses by ^{1}H NMR spectroscopy are supported by a crystal structure of **2R** and *ab initio* calculations for **2R** and **2S**. Both the calculated lowest energy conformations and the crystal structure show the pseudo-axial position of the azido groups and their *gauche* relationship to the N-acetyl group (Fig. 4). These results suggest that a stereoelectronic 'azido *gauche* effect' determines the overall conformation of the pyrrolidine ring of azidoprolines. Further *ab initio* calculations on simple ethane derivatives demonstrated that the strength of this 'azido *gauche* effect' is comparable to that of the well-known 'fluorine *gauche* effect'.[4]

To understand the differences in the *s-cis*:*s-trans* conformer ratios of **2R** and **2S**, a closer analysis of their lowest energy structures as well as the crystal structure of **2R** was most revealing. Within the preferred C(4)-*exo* conformation of **2R**, the angle between the oxygen of the N-acetyl group and the carbon of the methyl ester is 98° and thereby reminiscent of the Bürgi–Dunitz trajectory with which a nucleophile approaches a carbonyl group. This suggests a stabilizing interaction between these two functional groups which is further supported by the short distance of 2.8 Å between them (Fig. 4). Thus, a n→π* interaction[3h] stabilizes the *s-trans* conformer of (4*R*)-configured azidoprolines.[4]

In contrast, this stabilizing interaction is not possible in the conformation of the (4*S*)-configured diastereomer **2S**. These results demonstrate not only that the pyrrolidine ring conformation of azidoprolines is governed by the 'azido *gauche* effect' but that this effect also determines the relative population of the *s-cis* and *s-trans* conformers by allowing a stabilizing n→π* interaction in the case of the *s-trans* conformer of **2R** but not in the case of **2S**. As a result, a higher content of the *s-trans* conformer of **2R** is observed.[4]

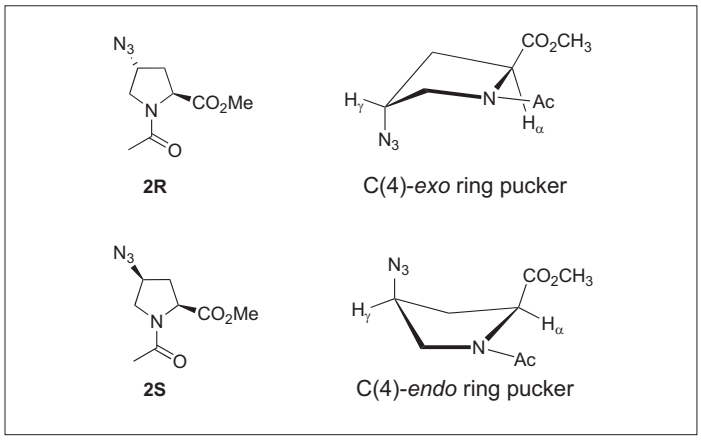


Fig. 3. C(4)-*exo* and C(4)-*endo* conformations of **2R** and **2S**.

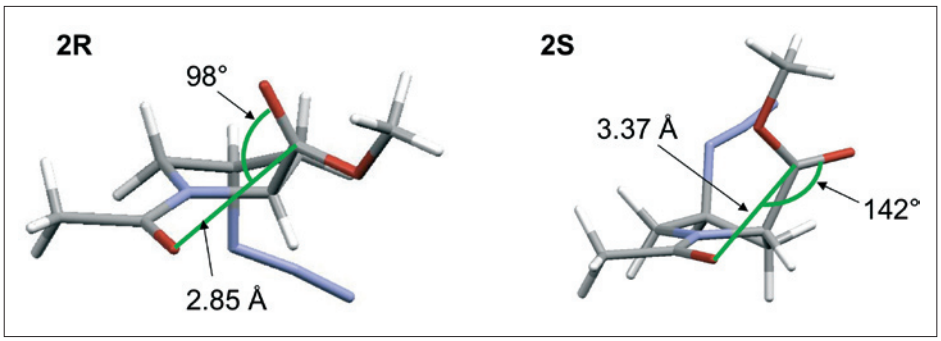


Fig. 4. Crystal structure of **2R** with the n→π* interaction stabilizing the *s-trans* conformer (left). Lowest energy conformation derived from *ab initio* calculations of **2S** (right).

3. Conformational Analysis of (4*R*) Azp and (4*S*)Azp Oligomers

Energetically the differences in the *s-cis*:*s-trans* equilibrium constants between the diastereoisomers **2R** and **2S** are small ($K_{t/c}$ (D_2O) = 6.1 and 2.6, respectively). To test whether these small differences in energy are sufficient to influence the *s-cis*:*s-trans* conformer ratio when (4*R*)Azp and (4*S*)Azp are incorporated in a larger peptide, oligoprolines were used as model systems. This choice was guided by the fact that oligoprolines are known to adopt two distinctly different helical secondary structures depending on the solvent.[8] The left-handed polyproline II (PPII) helix, with all amide bonds in *s-trans* conformations is adopted by oligoprolines in aqueous environment. In more hydrophobic solvents like aliphatic alcohols the right-handed polyproline I (PPI) helix with all amide bonds in *s-cis* conformations is predominant. In nature, the highly symmetric PPII helix where every third residue is stacked on top of the other (Fig. 5) is widespread and plays important roles in many biological processes.[2,8] For example, the single strands of collagen adopt this PPII conformation.

To test whether (4*R*)Azp and (4*S*)Azp influence the conformational preferences of oligoprolines, we prepared the 9-mers Ac-[(4*R*)Azp]$_9$-OH (**3R**) and Ac[(4*S*)Azp]$_9$-OH (**3S**) and studied their conformational properties by CD spectroscopy in phosphate buffer (10 mM, pH 7.2), n-PrOH and mixtures of these two solvents.[9] The two helical conformations have characteristic and well distinguishable CD spectra. A maximum at 226 nm and an intense minimum at 206 nm are indicative of the PPII structure, whereas spectra of PPI helices exhibit maxima at 215 nm and minima around 232 nm.[8] Based on the differences in the *s-cis*:*s-trans* conformer ratios observed for the monomers **2R** and **2S**, the (4*R*)Azp oligomer **3R** was expected to stabilize the PPII helix with all-*trans* amide bonds whereas oligomer **3S** consisting of (4*S*)Azp was expected to favor the PPI helix with all-*cis* amide bonds. This expectation proved true: The spectra of **3R** are typical for a PPII helix up to a content of 95% n-PrOH in aqueous buffer. Only in pure n-PrOH is the CD spectrum of **3R** indicative of a PPI helix (Fig. 6, right). In contrast, the conformation of **3S** changes drastically towards PPI when n-PrOH is present in aqueous buffer (Fig. 6, left).

Analysis of the unmodified 9-mer of proline, Ac-[Pro]$_9$-OH, revealed that its

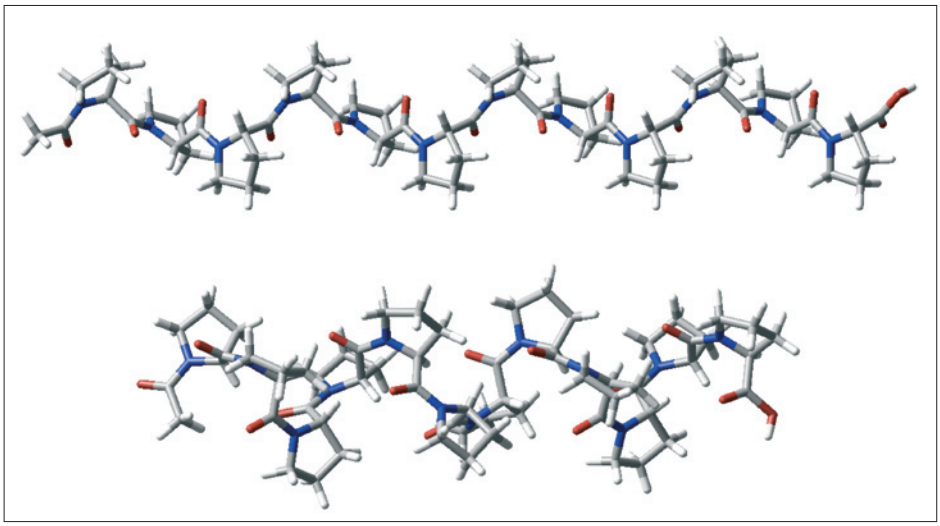


Fig. 5. Models of oligoprolines adopting PPII (top) and PPI (bottom) conformations.

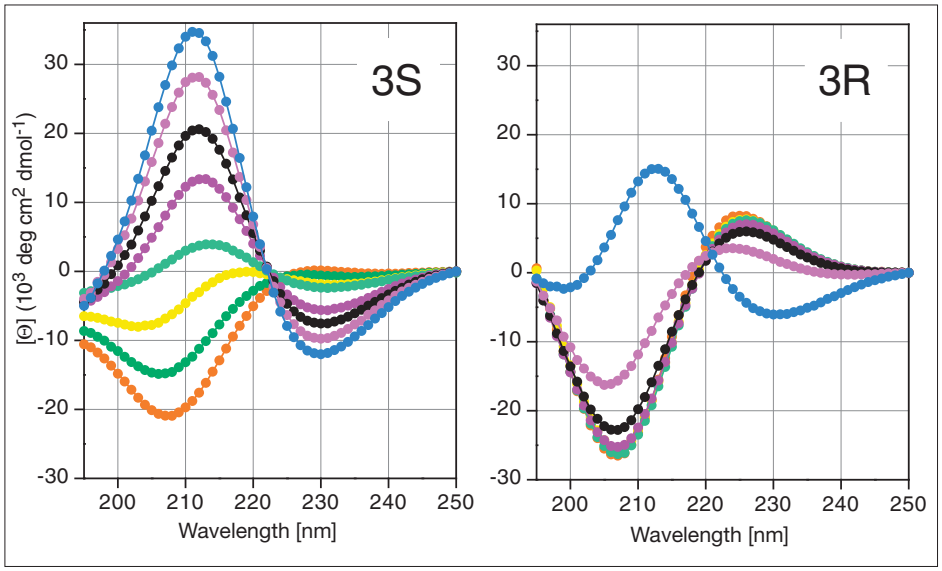
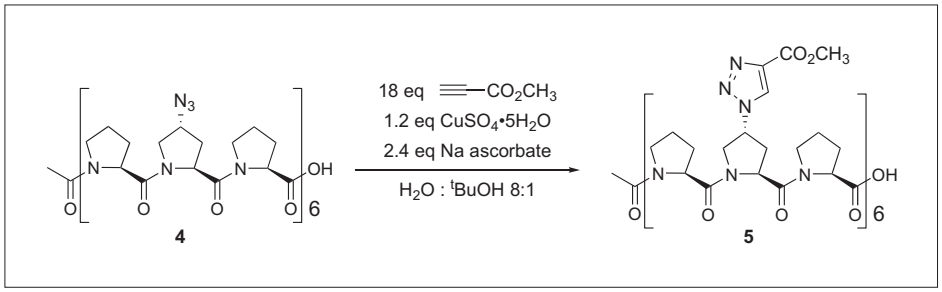


Fig. 6. CD-spectra of **3S** (left) and **3R** (right) recorded in aqueous phosphate buffer pH 7.2 (red), n-PrOH (magenta), 25% vol/vol n-PrOH in buffer (dark green), 50% n-PrOH (yellow), 75% n-PrOH (green), 85% n-PrOH (dark pink), 90% n-PrOH (black), 95% n-PrOH (pink).



Scheme 1. Functionalization of **4** to hexatriazole **5**.

conformational stability is in between those of **3R** and **3S**. These results demonstrate that (4*R*)Azp stabilizes whereas (4*S*) Azp destabilizes the PPII conformation. Furthermore, the observed stabilities of the helical conformations of the oligomers **3R** and **3S** reflect the *s-cis*:*s-trans* conformer ratios of their respective monomers **2R** and **2S**.[9]

4. Azidoproline Containing Oligoprolines as Functionalizable Molecular Scaffolds

The well-defined helical conformation of oligoprolines in water, combined with the possibility of switching from one conformation to another, render oligoprolines interesting as molecular scaffolds, provided that attachment sites are present that allow for site-specific functionalization. We therefore explored whether Azp-containing oligoprolines can be functionalized, for example, by Huisgen's 1,3-dipolar cycloadditions ('click chemistry')[10] and prepared the 18-mer Ac-[Pro-(4*R*)Azp-Pro]$_6$-OH (**4**) with Azp residues in every third position.[9] Within peptide **4** all azido groups were designed to be positioned on one edge of the PPII helix in aqueous solution as supported by CD and NMR spectroscopy. Under typical click chemistry conditions, the six azido groups within **4** reacted readily with, for example, methyl propiolate to the desired hexatriazole **5** (Scheme 1). CD spectra of **5** in n-PrOH and aqueous buffer are indicative of PPI and PPII helices, respectively, demonstrating that triazole substituents on the oligoprolines still allow both helical structures to be adopted.

Azp-containing oligoprolines can also be readily functionalized with different alkynes when peptide coupling and cycloaddition reactions are alternated (Scheme 2). To showcase this strategy, the trimeric building block Fmoc-Pro-(4*R*) Azp-Pro-OH was coupled followed by a 'click-chemistry' step with different terminal alkynes. In five cycles 16-mer **6** bearing five triazoles was prepared by solid phase synthesis.

5. Conclusion and Outlook

(4*R*)- and (4*S*)-azidoprolines are useful probes to tune the *s-cis*:*s-trans* ratio of Xaa-Pro amide bonds. A stereoelectronic 'azido *gauche* effect' determines the preferred conformation of Azp residues. When incorporated into oligoprolines, Azp residues can be readily and differentially functionalized using *e.g.* 'click chemistry'. Upon functionalization the well-defined PPII conformation is retained, thereby allowing functional groups to be positioned at desired sites and rendering Azp-containing oligoprolines interesting as molecular scaffolds. Thus, azides are both highly versatile sites for further functionalization and conformation-directing functional groups. We are currently extending our research to the incorporation of Azp residues into collagen model peptides to study their effect on the stability of collagen and use the functionalizability of the azido group for interstrand cross-linking.[11]

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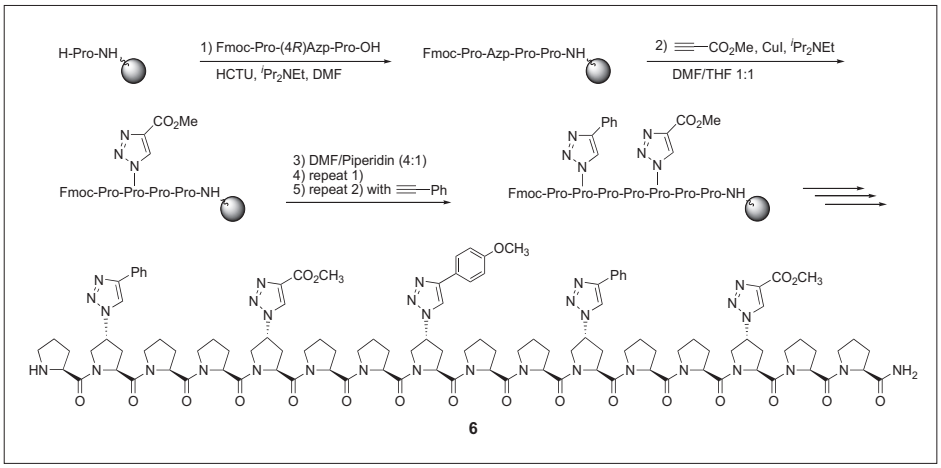

Scheme 2. Synthesis of differentially functionalized oligoprolines by sequential peptide coupling and click chemistry steps.

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[11] Towards this goal we have established the silyl-protected building block Fmoc-Pro-Hyp(TBDPS)-Gly-OH, see R. S. Erdmann, H. Wennemers, *Synthesis* **2009**, 143.