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

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Abstract: An ‘azido gauche effect’ determines the conformation of (4S)- and (4R)-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 (4S)Azp and (4R)Azp. We show that (4S)Azp and (4R)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 (4R)- and (4S)-azidoproline (Azp) as versatile amino acids that allow for further functionalization. Our research started by utilizing the diketopiperazine 1R derived from (4R)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 ∆G ≤ 6 kcal/mol.[5a,5d]

Differences in the synthesis of the cyclic dipeptides (and later tripeptides[7]) derived from (4R)-Azp and (4S)-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-(4R)Azp-OC=OCH3 (2R) and Ac-(4S)Azp-OC=OCH3 (2S)

To analyze the conformational differences between (4R)- and (4S)-configured azidoprolines we initially used the acetylated methylesters Ac-(4R)Azp-OC=OCH3 (2R) and Ac-(4S)Azp-OC=OCH3 (2S) as simple model compounds.[8] Conformational analysis by 1H 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 (4R)-configured stereoisomer a significantly higher portion of the s-trans conformer was observed. For example, Ac-(4R)Azp-OC=OCH3 (2R) and Ac-(4S)Azp-OC=OCH3 (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-OC=OCH3 is 1:4.9 in D2O.

The pyrrolidine ring of Pro can adopt essentially two main conformations, a C(4)-endo or a C(4)-exo conformation.
Analysis of the vicinal $^1$H,$^1$H-coupling constants revealed that both the $s$-cis and the $s$-trans conformers of Ac-(4R)Azp-OCH$_3$ (2R) adopt C(4)-exo conformations whereas both conformers of Ac-(4S)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 indicative of a PPI helix with all amide bonds in $s$-cis conformations is predominant. In nature, the highly symmetric PPI helix where every third residue is stacked on top of the other (Fig. 5) is widespread and plays important roles in many biological processes.[24] For example, the single strands of collagen adopt this PPII conformation.

To test whether (4R)Azp and (4S)Azp influence the conformational preferences of oligoprolines, we prepared the 9-mers Ac-[(4R)Azp]$_9$-OH (3R) and Ac[(4S)Azp]$_9$-OH (3S) and studied their conformational properties by CD spectroscopy. A maximum at 226 nm and an intense minimum at 206 nm are 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 PPII 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 (4R)Azp oligomer 3R was expected to stabilize the PPII helix with all-trans amide bonds whereas oligomer 3S consisting of (4S)Azp was expected to favor the PPII helix with all-cis amide bonds. This expectation proved true: The spectra of 3R are similar to that of 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 PPII helix (Fig. 6, right). In contrast, the conformation of 3S changes drastically towards PPII 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 $n$–$\pi^*$ 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]
conformational stability is in between those of 3R and 3S. These results demonstrate that (4R)Azp stabilizes whereas (4S) 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, providing 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-(4R)Azp-Pro]-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-(4R) 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

(4R)- and (4S)-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.

1) Fmoc-Pro-(4R)Azp-Pro-OH
2) DMF/PrOH (4:1) Pr2NEt
3) repeat 1)
4) repeat 2) with Ph
5) repeat 3)


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.