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Azidoproline Containing Helices: Stabilization of the Polyproline II Structure by a Functionalizable Group

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The polyproline II (PPII) helix is a common secondary structure within natural proteins and plays important roles in many biological processes.[1] The development of molecular probes that allow for influencing the conformational stability of the PPII structure is therefore an important goal.[1−3] Likewise, conformationally well-defined molecular scaffolds that allow for facile functionalization have become increasingly important for applications ranging from the generation of functional materials to the development of cell-penetrating compounds, antibiotics, and inhibitors for specific protein−protein interactions.[4,5] Here we demonstrate that 4-azidoprolines can be used both as conformation directing elements of the PPII structure and as functionalizable sites for the development of proline-based molecular scaffolds.

Short proline oligomers adopt the PPII conformation in aqueous solution, and changing to a less polar solvent, like aliphatic alcohols, can induce a different secondary structure, the polyproline I (PPI) helix. The PPII conformation is a left-handed helix with all amide bonds in *s*-trans conformations and every third residue stacked on top of each other in a lateral distance of 9.4 Å.[6,7] The PPI helix is right-handed and more compact (helical pitch of 5.6 Å per turn) with all amide bonds in *s*-cis conformations and 3.3 proline residues per turn.[6,7] The characteristic well-defined conformation of oligoprolines in water, combined with the possibility of switching from one conformation to another,[7] render oligoprolines interesting as molecular scaffolds, if attachment sites can be introduced that allow for site-specific placement of functionalized side chains.

Previous studies on (4*R*)- or (4*S*)-azidoprolines (Azp) had revealed that their conformation is governed by an "azido gauche effect" which leads to significantly different *s*-trans/*s*-cis isomer ratios around their amide bonds (Figure 1).[8] For Ac-(4*R*)Azp-OCH_3 **1R** and Ac-(4*S*)Azp-OCH_3 **1S** *s*-trans/*s*-cis ratios of 6.1:1 and 2.6:1, respectively, were observed in D_2O compared to a ratio of 4.6:1 for Ac-Pro-OCH_3. The higher *s*-trans:*s*-cis isomer ratio observed for the (4*R*)Azp derivative was rationalized on the basis of a stabilizing n → π* interaction between the oxygen of the acetyl group with the carbonyl group of the methylester of the *s*-trans conformer of **1R**.[2,8]

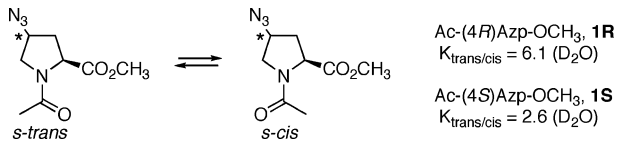

Figure 1. *s*-Trans/*s*-cis isomer ratios of **1R** and **1S**.

These results suggest that (4*R*)Azp and (4*S*)Azp could be used to tune the stability of the PPII conformation. In addition, the azido groups of Azp containing oligoprolines would provide versatile sites for functionalization of the polyproline scaffold.

To test the influence of (4*R*)Azp and (4*S*)Azp on the PPII conformation, we synthesized the 9-mers Ac[(4*R*)Azp]$_9$OH **2R** and Ac[(4*S*)Azp]$_9$OH **2S** and studied their conformations in aqueous phosphate buffer (10 mM, pH 7.2), *n*-PrOH, and mixtures thereof by CD-spectroscopy (Figure 2).

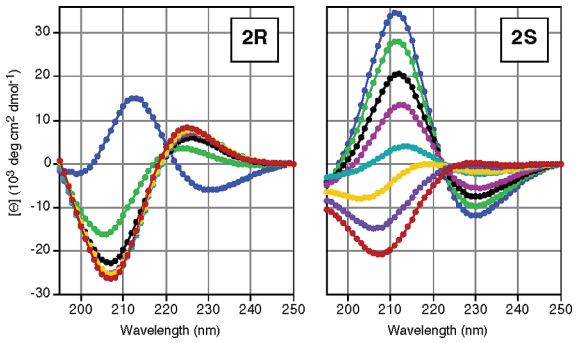

Figure 2. CD-spectra of Ac-[(4*R*)Azp]$_9$-OH **2R** (left) and Ac-[(4*S*)Azp]$_9$-OH **2S** (right) in aqueous phosphate buffer (10 mM, pH 7.2) (red), 25% vol/vol *n*-PrOH in buffer (dark purple), 50% *n*-PrOH (yellow), 75% *n*-PrOH (aqua), 85% *n*-PrOH (light purple), 90% *n*-PrOH (black), 95% *n*-PrOH (green), and *n*-PrOH (blue). Spectra were recorded at concentrations of 6×10^{-4} M per residue at 25 °C.

The CD-spectrum of **2R** in phosphate buffer is typical for a PPII helix[6] (maximum at 225, minimum at 207 nm) and hardly any conformational change occurs up to a content of 90% vol/vol of *n*-PrOH in buffer. Only in pure *n*-PrOH is the CD spectrum of **2R** indicative of a PPI helix (minimum at 230 nm, maximum at 212 nm). In contrast, the conformation of **2S** changes drastically toward PPI when *n*-PrOH is present in aqueous buffer (Figure 2). Analysis of the unmodified 9-mer of proline, Ac-[Pro]$_9$-OH, revealed that its conformational stability is in between those of **2R** and **2S** (see Supporting Information). These results demonstrate that (4*R*)Azp stabilizes whereas (4*S*)Azp destabilizes the PPII conformation.[9] The data suggest that the stabilizing effect of (4*R*)Azp is due to an enhancement of the n → π* interactions which have been proposed to stabilize the PPII conformation.[2] Comparable observations have been made by Raines et al. with 4-fluoroprolines,[10] suggesting that the effect of an azide is comparable to that of a fluorine. In contrast to fluorine, azido groups allow for further functionalization.[11]

To evaluate the functionalizability of Azp containing oligoprolines, we prepared oligoproline Ac-[Pro-(4*R*)Azp-Pro]$_6$-OH **3R** with Azp residues in every third position. **3R** was used as a model compound, designed to have all Azp residues stacked on top of each other at one edge of the PPII helix (Figure 3A). Conformational analyses of **3R** in aqueous solution support this geometry. The CD-spectrum in phosphate buffer is typical of a PPII conformation and ^{1}H and ^{13}C NMR spectra show essentially one set of signals for each, the Azp and the two flanking Pro residues. In *n*-PrOH, the CD-spectrum of **3R** is typical of a PPI-helix.

Functionalization of 18-mer **3R** was accomplished using Huisgen's 1,3-dipolar cycloaddition ("click reaction").[12] Reaction of the six azido groups with methyl propiolate, $CuSO_4 \cdot 5H_2O$, and sodium ascorbate led to the desired hexatriazole **4R** which was isolated by simple extraction and precipitation in 40% yield (Figure 3B).

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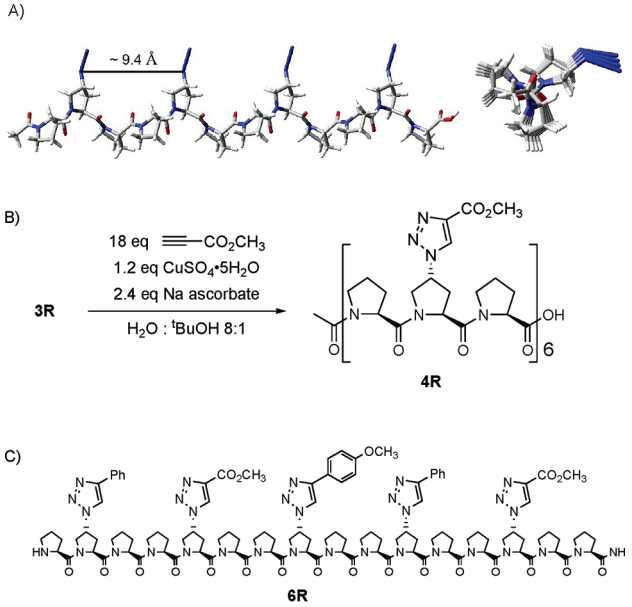

Figure 3. (A) Model of an oligoproline PPII-helix with Azp residues in every third position. (B) Functionalization of the 18-mer **3R** using "click chemistry". (C) The 16-mer **6R** prepared by successive peptide coupling and cycloaddition steps.

Derivatization of the Azp containing oligoprolines can be expected to influence the stability of the PPII and PPI conformations. CD-spectra of **4R** in aqueous buffer and *n*-PrOH are indicative of PPII and PPI conformations, respectively, demonstrating that triazole substituents still allow for adopting both helical structures (see Supporting Information).

For many applications, functionalization of the polyproline scaffold with different functional groups will be desirable. Thus, we next focused on differential functionalization of oligoprolines by successive peptide coupling and cycloaddition steps (Figure 3C). Fmoc-Pro-(4*R*)Azp-Pro-OH **5R** was coupled onto proline-functionalized rink amide resin followed by a "click-reaction" using methyl propiolate. After a second coupling of **5R**, a cycloaddition using phenylacetylene was performed. Third, fourth, and fifth cycles followed with 4-ethynylanisole, phenylacetylene, and methyl propiolate as alkynes. The "click chemistry" steps were monitored by IR spectroscopy and were complete within 12 h, except for the cycloaddition of 4-ethynylanisole, which had to be repeated twice. The 16-mer **6R** was removed from the resin with a purity of the crude product of ~60% (after 12 synthesis steps) as judged by HPLC. CD-Spectroscopic analysis of purified **6R** showed spectra indicative of a PPII-conformation in aqueous buffer and a PPI conformation in *n*-PrOH (see Supporting Information).

In summary, the presented work is the first study where azido groups are used both as conformation directing elements and as sites for further functionalization. Incorporation of (4*R*)Azp into oligoprolines stabilizes, whereas (4*S*)Azp destabilizes the PPII conformation. (4*R*)Azp and (4*S*)Azp might therefore serve as valuable tools to tune the conformational stability of the PPII helix for studying biological processes where the PPII conformation plays a role. Furthermore, we have demonstrated that Azp containing polyprolines can be efficiently functionalized in a differential way using "click chemistry". Upon functionalization the well-defined PPII conformation is retained, thereby allowing for the positioning of functional groups at desired sites. In light of the increasing importance of molecular scaffolds,[4,5] the presented Azp-containing oligoprolines might serve as interesting new leads.

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Supporting Information Available: Experimental details on the syntheses and analyses of compounds **2S** and **2R**−**6R**. This material is available free of charge via the Internet at http://pubs.acs.org.

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