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An Oligomeric Ser-Pro Dipeptide Mimetic Assuming the Polyproline II Helix Conformation

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Peptide sequences assuming a left-handed helical polyproline II (PPII) conformation are observed at protein−protein interfaces where they play an important role in the recognition process.[1] Short PPII helical peptides and peptidomimetics are interesting synthetic targets because of their potential for probing such recognition events in protein chemistry.[2] However, short *all*-L-configured peptides are not expected to significantly populate left-handed helical conformations unless local covalent restraints freeze the extended helical structure by excluding other backbone rotamers. We describe the synthesis and the structural analysis of a hexapeptide surrogate displaying all characteristic features of the PPII helix. The sugar-derived peptide mimetics have the amphiphilic character of Ser-Pro dipeptide units as they occur in proline-rich regions of several biologically important proteins.[3]

The bicyclic thiazolidinlactam **1** is obtained in a single step from the condensation of commercial D-γ-glucuronolactone with the L-cysteine methylester (Scheme 1).[4] Triflation of **1**, yielding **2** proceeds regioselectively at the α-hydroxy group without the necessity of protecting the residual secondary hydroxyls. Subsequent azide exchange furnishes **3** with retention of configuration. Reduction of **3** followed by Boc-protection of the amino terminus yields the polyhydroxylated dipeptide mimetic **4**.

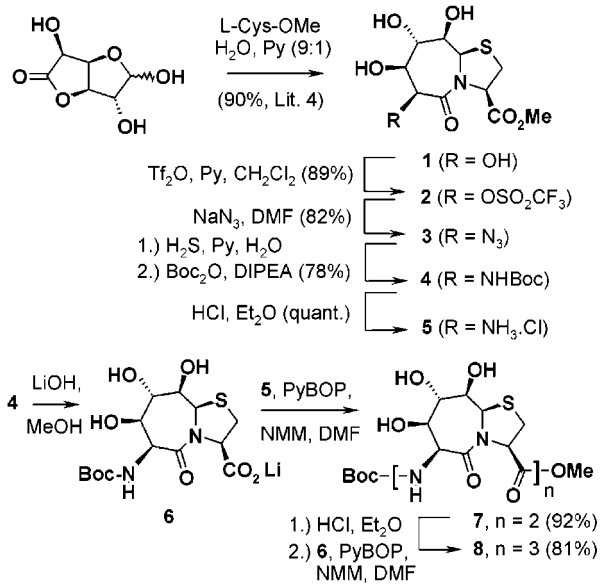
The amino terminus is set free with hydrochloric acid (**4** → **5**). Saponification of **4** yields the salt **6**. The dimer **7** is obtained by peptide coupling of **5** and **6** with PyBOP. Repetition of the deprotection coupling sequence finally leads to the hexapeptide mimetic **8**.

Small-ring cyclizations efficiently select single peptide backbone and side chain rotamers.[5] Bicyclic rings bridging the amide bond add enzymatic stability and exclude *cis*-amide rotamers about the peptide bond,[6] thus avoiding the well-known resistance of oligoprolines against NMR investigations. Additionally, the conformation of **8** is stabilized by intramolecular hydrogen bonds as described below.

A preferred overall conformation of a homooligomeric peptide is accompanied by well-separated resonance signals in the ^{1}H NMR spectrum as observable for **8** (Figure 1).[7]

The strongest dependence between the degree of oligomerization of **4** and the chemical shift is observed for the O*H*(9) resonances which experience downfield shifts of up to 1.5 ppm in **8**. O*H*(9) is trapped within an intramolecular hydrogen bond toward the carboxyl oxygen of the subsequent amide or ester group, respectively (Figure 2). **8** forms three pairs of hydrogen bonds of the type O(9)−H···O═C. Hydrogen bonds to the bicyclic ring system can be identified in DMSO, in protic solvents, and in the crystal structures of **1**[4a] and **4**.[8] A side chain-to-backbone hydrogen bond enclosing a similar ring size is observed in PPII helical structures for Gln which has a high propensity of forming *i*, *i* + 1 side chain-to-backbone interaction as shown in Figure 2.[9]

Scheme 1



In oligoamide **8,** nine out of the twelve ϕ and ψ torsions of the hexapeptide backbone are constrained within covalent rings rigidified by hydrogen bonds. The remaining three ϕ torsions about the N(6)−C(6) bonds are experimentally characterized by ^{3}J(N*H*,H$^\alpha$) coupling constants of 7.6−7.8 Hz and by medium-sized NOEs between N*H*(6) and C*H*(7).

The thioproline rings populate half-chair conformations. C(2) is the out-of-plane atom exhibiting conformational averaging about the plane spanned by the other four ring atoms which are fixed to a torsion angle of 0° (±10°) by the fused ring system. The thioproline ring puckering is experimentally characterized from the average values of the $^3J_{2H,3H}$ coupling constants and NOEs. A well-defined chair conformation is assumed by the seven-membered rings which is documented among other criteria by the virtual absence of $^3J_{6H,7H}$ and $^3J_{9H,9aH}$ couplings in **8**.

An energy-minimized structural model of **8** identifies a left-handed helix with backbone torsions corresponding to that of the PPII helix. One pair of ϕ, ψ torsions is constrained within the annelated ring system to the values of −80° and 160°. The amino-terminal ϕ torsion is less severly restricted to values around −90°, and the carboxy terminal ψ torsion of the bicyclic dipeptide unit is tethered by the hydrogen bond to 140°. Values which deviate from the idealized PPII torsions (−78°, 146°)[10] uncoil the extended helix slightly toward the β-sheet conformation, while all other regions of the Ramachandran plot are not accessible for **8**.

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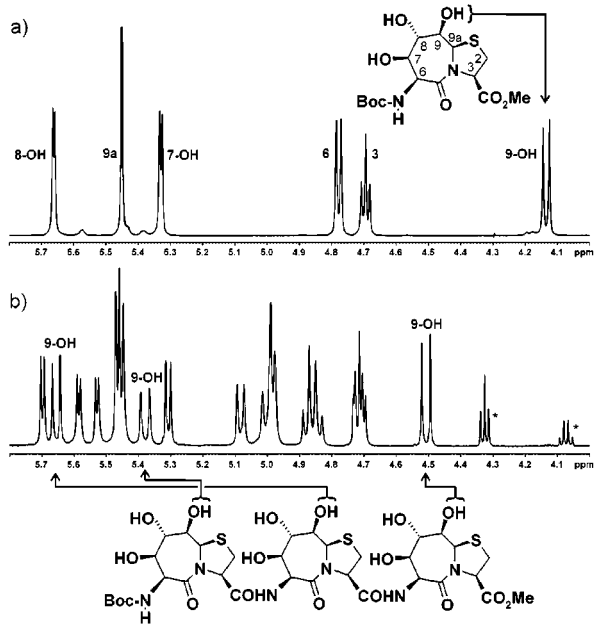

Figure 1. ^{1}H NMR of monomer **4** (a) and trimer **8** (b) in DMSO-d_6, (400 MHz, 300 K). O*H*(9) of **4** resonates as a doublet at 4.13 ppm (**4**, $^3J = 9.7$ Hz, +0.7 ppb/K). The three O*H*(9) protons of the trimer **8** resonate at 5.65 ppm (**8**, second unit, $^3J = 9.6$ Hz, −0.4 ppb/K), 5.38 ppm (**8**, first unit, $^3J = 10.7$ Hz, −0.7 ppb/K), and 4.50 ppm (**8**, third unit, $^3J = 10.5$ Hz, −1.1 ppb/K). The large coupling constants correlate with trans orientations of C*H*(9) and O*H*(9). The minimal temperature dependences of the O*H*(9) resonances prove their internal orientation and solvent shielding, while the other N*H* and O*H* signals are oriented toward the solvent and exhibit temperature dependences between −4 and −6 ppb/K (*EtOH).

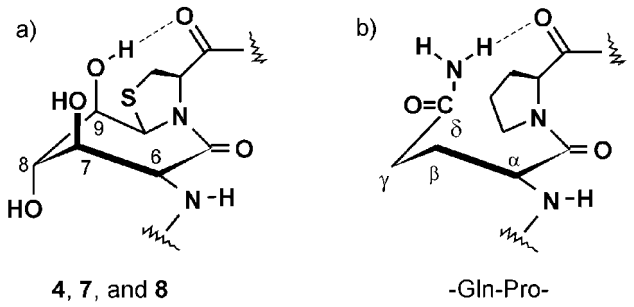

Figure 2. (a) Bicyclic thiazolidinlactam assumes a chair conformation, fixing the hydroxy groups 7, 8, and 9 to axial orientations and stabilizing the hydrogen bond O(9)−H···O═C. (b) Side chain-to-backbone hydrogen bond of the same ring size is observed in PPII helical conformations as shown for a Gln-Pro dipeptide sequence.

NMR spectroscopy characterizes the solution conformation of **8** from local parameters such as *J*-couplings and NOEs. CD spectroscopy on the other hand identifies helical structures from the overall ordering of chromophores. The carbonyl groups of a PPII helix assume perpendicular orientations to the helix axis, and the resulting CD pattern differs from α-helical or from random coil structures by a strong negative band at approximately 205 nm.[11] The CD spectrum of **8** measured in water corresponds to the CD spectrum observed for poly-L-proline by showing a strong minimum at 205 nm. The very weak positive band of poly-L-proline at 235 nm is not observed for **8**.

In conclusion, a rigid left-handed helix with a three-fold axis of rotation results for the trimer **8** which resembles one full turn of the helix equivalent to two full turns of the PPII helical amide backbone. Amino acids with side chain hydrogen bond donors such as Gln can actively stabilize the PPII helix and therefore have high propensities for PPII helices,[9] making the side chain-to-backbone hydrogen bond a characteristic feature of this type of secondary structure. Peptide copolymers of **4** can give further insight into the stabilization of the PPII helix which was not accessible to detailed NMR studies before. Derivatives of **4** may also provide the basis for the development of PPII helical protein ligands with the heteroatomic side chains providing potential for further synthetic modifications.

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Supporting Information Available: X-ray crystallographic file of **4** (CIF). Synthetic procedures and reproductions of homo- and heteronuclear NMR spectra including signal assignments of **4** and **8** and the CD spectrum of **8**, energy minimized conformation of **8** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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