ChemPubSoc Europe

DOI: 10.1002/chem.201404820

CHEMISTRY A European Journal Communication

Peptidomimetics

Silaproline Helical Mimetics Selectively Form an All-*trans* PPII Helix

Charlotte Martin,[a] Baptiste Legrand,[a] Aurélien Lebrun,[a] Dorothée Berthomieu,[b] Jean Martinez,[a] and Florine Cavelier*[a]

Abstract: The polyproline II helix (PPII) is increasingly recognized as an important element in peptide and protein structures. The discovery of pertinent PPII peptidomimetics is of great interest to tune physical properties of the targeted structure. A series of silaproline oligomers from dimer to pentamer were synthesized. CD studies, NMR spectroscopy and molecular modeling revealed that the ribbon preferentially populates the polyproline type II secondary structure in both [D]chloroform and [D_4]MeOH. The characteristics of this new lipophilic PPII-like helix were determined.

Proline residue promotes the formation of helical extended secondary structures in proline-rich region (PRR) such as the polyproline type II helix (PPII).[1] Although only 10% of all protein adopt this type of conformation, they are essential to biological activity.[2] Most PPII helices contain four residues and are present in about half of the proteins of known structure.[3] PPII helices are directly involved in a wide range of molecular functions including signaling, transcription, and immune response, as examples. The major and best known functional role of PPII structures is to mediate protein interactions.[4] The most commonly studied example is the binding of PRR by SH3 domains.[5] Another important role of PPII is related to cell penetrating peptides (CPPs) that appear as promising tools for cellular targeting.[6]

The typical PPII is a left-handed helix with all peptide bonds in *trans* configuration ($\omega = 180^\circ$) and dihedral angles (ϕ, ψ) = (-75°, $+145^\circ$), leading to a semi-extended structure, with a highly solvated backbone and no intramolecular hydrogen bonds.[7] Although the PPII helix conformation was first identified in polyproline,[7a] peptides that do not contain proline residues but share similar ϕ, ψ and ω angles are defined as a PPII helixes. Thus, the designation "PPII helix" is somewhat misleading, since proline may be absent from this structure[8] and amino acids such as glycine, asparagine, alanine, glutamine, valine, aspartic acid, histidine and lysine contribute to PPII helical conformations.[9] PPII structure contains three residues per turn aligning every third residue on the same face of the helix, with a pitch of approximately 10 Å per turn. A few crystal structures are available for oligoproline and side chain derivatives, therefore most structural information on such secondary structures come from studies by CD[10] and NMR spectroscopy in solution.[11] Nevertheless, polyproline sequences can also adopt an unusual right-handed helix, named PPI, with all amide bonds in the *cis* configuration. This type of structure (ϕ, ψ) = (-75°, $+160^\circ$) is energetically unfavorable in aqueous medium and is not encountered in a biological context.[7b,12] Moreover, owing to its cyclic structure, proline mediates slow isomerization between *cis/trans* conformations depending on the environment (solvent, temperature)[13] and *cis* conformations are often encountered.

The biological properties of such structure have recently stimulated chemists to synthesize PPII mimics as potential chemotherapeutic agents.[14] While α-helices or β-turn mimics have been largely studied in the past, few approaches have been described that emulate the PPII structure.[15] Polyimide-based foldamers,[16] Ser-Pro dipeptide oligomer mimic[17] or tricyclic Pro-Pro mimic,[18] triproline mimics[19] and PTAAs[20] are among current PPII structural mimics, designed to modulate physical properties such as water solubility. In this context, a few years ago, we developed a silicon-containing proline surrogate, silaproline (Sip)[21] that promotes higher lipophilicity with an octanol-water coefficient of Sip 14 times greater than that of Pro. The introduction of silaproline in model peptide sequences induced similar conformational properties to that of proline.[22] We also showed that the envelope conformation is of C^β-*endo* type in solid state within the Sip-Ala dipeptide, while the C^β-*exo* puckering of the Sip ring is favored in solution due to the absence of intermolecular packing forces. The five membered ring of silaproline assumes a skewed conformation, which is uncommon in the proline pyrrolidine ring.[23] Replacement of proline by silaproline in bioactive peptides resulted in analogues with similar receptor affinity and in vivo bioactivity while improving resistance to enzymatic degradation.[22,24] On the other hand, replacement of Pro by Sip in the hydrophobic face of a Pro-rich amphipathic CPP peptide did not disturb the secondary structure and greatly enhanced the cellular uptake of the peptide by 20-fold.[25] Recently, we described the synthe-

[a] Dr. C. Martin, Dr. B. Legrand, A. Lebrun, Prof. J. Martinez, Dr. F. Cavelier
Institut des Biomolécules Max Mousseron
UMR 5247, CNRS-UM2-UM1-ENSCM
Place Eugène Bataillon, CC1703, 34095 Montpellier (France)
E-mail: florine@univ-montp2.fr

[b] Dr. D. Berthomieu
Institut Charles Gerhardt de Montpellier, UMR 5253
CNRS-UM2-UM1-ENSCM, Place Eugène Bataillon
CC1701, 34095 Montpellier (France)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201404820.

sis of polydisperse homopolysilaprolines of different lengths by ring opening polymerization of *N*-carboxyanhydride with Pro-OBn as initiator, where the benzyl group serves as an NMR reference.[26] We now report the synthesis and characterization of short L-silaproline oligomers using CD and NMR spectroscopy, and molecular modeling and ab initio calculations to describe polysilaproline helical mimetics.

Oligomers **2**–**5** (Figure 1) were synthesized in solution using a step-by-step strategy from the appropriate *N*-Boc-protected monomer **1**. To be consistent with the previously synthesized polydisperse polymers,[26] Pro was chosen as C-terminal residue and OBn as capping group.

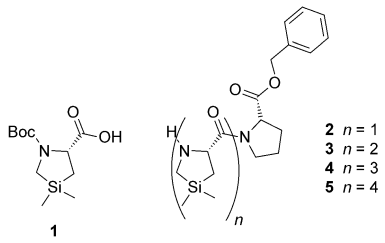

Figure 1. Silaproline building block and oligomers **2**–**5**.

Dimer **2** was obtained in solution by coupling equimolar amounts of *N*-Boc silaproline **1** with proline benzyl ester hydrochloride salt in the presence of triethylamine. Chain extension was achieved by selective Boc-deprotection of the dimer and coupling with monomer **1** to afford trimer **3** after N-deprotection. Oligomers **4** and **5** were synthesized in a similar fashion (Figure 2).

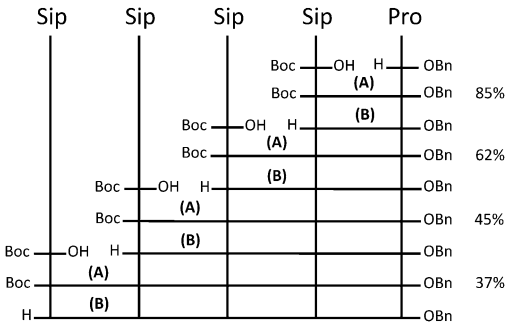

Figure 2. Strategy of oligomer syntheses. A) HBTU/TEA/DMF; B) TFA.

The presence of the Boc group caused a rotamer effect that resulted in the presence of conformers for the N-protected monomer **1** as observed in the NMR spectra both in chloroform and in methanol. Recording 1D NMR spectra at various temperatures in [D_6]DMSO emphasized this phenomenon (see the Supporting Information). As such, structural studies were restricted to the free amine oligomers **2**–**5**.

We first started our investigations by recording the far-UV (190–260 nm) CD spectra of oligomers **2**–**5** in methanol. These data indicated a change in conformational preferences while increasing oligomer length (Figure 3 A). Overall they shared similar shapes with negative maxima and positive maxima in the 203–208 and 220–230 nm ranges, respectively. The per residue molar ellipticity of these extrema increased and was red-shifted with the oligomer's length. Pentamer **5** exhibited a typical PPII signature with a negative maximum at 207 nm and a positive maximum at 229 nm while proline oligomers mainly adopted PPI helical folds in methanol, with two negative maxima at 200 and 232 nm and a positive maximum at 215 nm. Increasing the temperature from 20 to 55 °C did not modify the shape of the CD spectrum of oligomer **4** in methanol with only a very slight decrease of the extrema showing the high stability of silaproline oligomer structures (Figure 3 B).

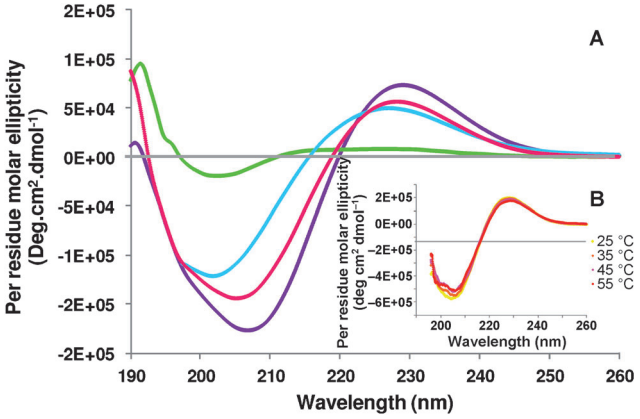

Figure 3. A) CD spectra in MeOH (0.1 mM) for oligomers **2** (green), **3** (blue), **4** (pink), **5** (violet). Molar ellipticity was normalized for concentration and per residue at 20 °C. B) CD signals of tetramer **4** at variable temperature (25, 35, 45 and 55 °C).

NMR studies were performed in [D]chloroform and in [D_4]MeOH for oligomers **2**–**5**. Combining COSY and ROESY, ^{13}C HSQC and ^{13}C HMBC experiments allowed assignments of all ^{1}H and ^{13}C resonances. The best solubility and NMR spectra resolution of oligomers were observed in [D_4]MeOH. In both solvents, we observed strong NOEs between the $^{\alpha}$CH(i) and $^{\delta}$CH($i+1$) proton for all oligomers (Figure 4). This correlation is characteristic of the *trans* conformation of the peptide bond ($\omega \sim 180°$) of the PPII secondary structure. Except for dimer **2**, a single "*trans* conformer" was detectable on the ^{1}H NMR spectra of the longer oligomers both in chloroform and methanol. Dimer **2** exhibited a *cis/trans* isomerization of the amide bond between silaproline and proline. About 7% of the *cis* isomer was found as measured by ^{1}H and ^{29}Si NMR in both solvents (Figure 4). In methanol, silaproline in H-Sip-Pro-OBn induced more *trans* isomerism of the peptide bond than proline in H-Pro-Pro-OBn (93 and 85%, respectively). While for high molecular weight proline oligomers ($n = 3$–5) the percentage of peptide bonds in *trans* conformation remained quasi-constant (90%), the *cis* conformer was not detected in silaproline oligomers starting from the trimer.[27] Despite many efforts, crystallization assays were not successful, consistent with difficulties in obtaining crystals of polyproline helices by others.[28]

In this context, NOEs were used as restraints for NMR solution structure calculations using a typical simulated annealing

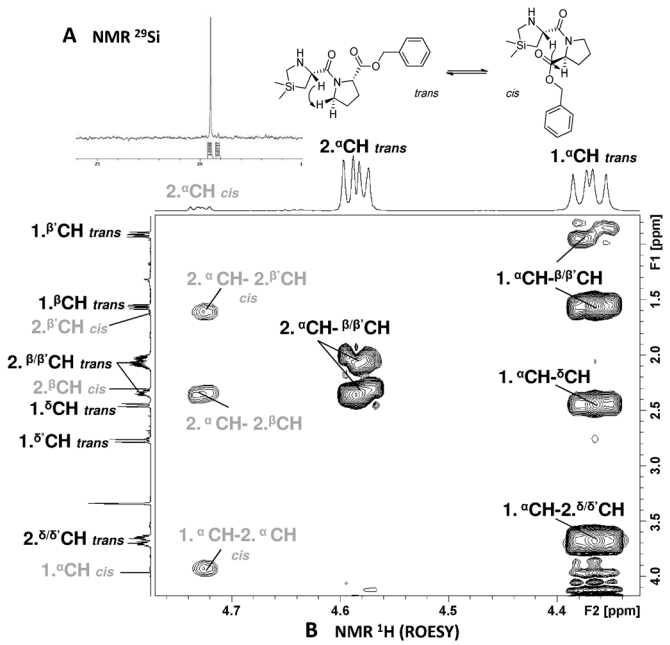

Figure 4. A) ^{18}Si NMR spectrum of dimer **2** in CD_3OD at 298 K. B) ROESY spectrum of dimer **2** in CD_3OD at 298 K. NOE correlations between $^{\alpha}CH(i)$ and $^{\alpha}CH(i+1)$ in the *cis* conformer (600 MHz spectrometer, mixing time = 300 ms).

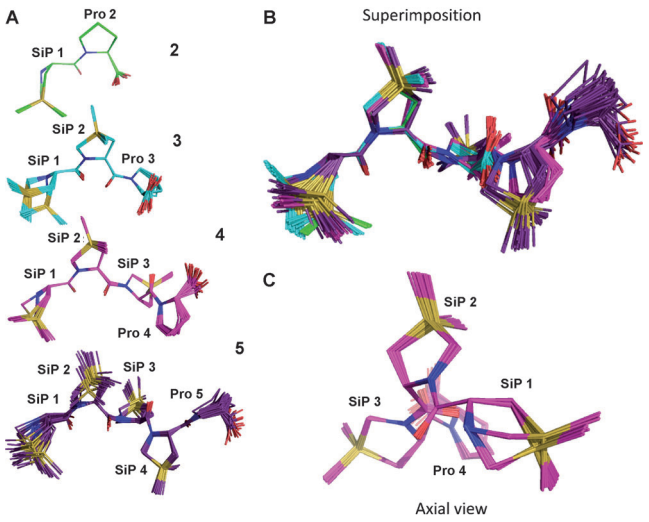

Figure 5. A) NMR solution structures of dimer **2** (green), trimer **3** (cyan), tetramer **4** (pink) and pentamer **5** (purple). B) Overlay of the structures of compounds **2**–**5** optimized by DFT. C) Axial view of the tetramer **4** PPII helical structure. Hydrogen atoms and the disordered OBn C-terminal moiety are omitted for clarity.

protocol with AMBER 11.[29] The solution structures of **2**, **3**, **4** and **5** in methanol were solved using 4, 12, 18 and 24 unambiguous restraint distances, respectively. Figure 5 shows the 20 lowest-energy NMR structures calculated for each compound. As expected, the Sip oligomers converged towards PPII structures. The root mean square deviations (RMSD) on all heavy atoms were 0.03, 0.31, 0.18 and 0.40 Å for **2**, **3**, **4** and **5**, respectively, when the OBn capping group was omitted. Average values of the backbone dihedral angles for the Sip residue in the polysilaproline helix were $\phi = -74.5(\pm 8.9)^{\circ}$ and $\psi = 143.6(\pm 13)^{\circ}$ after optimization of the NMR structures using the B3LYP/6-31+G(d,p) method. This PPII helix is a left-handed helix with an axial translation of 3.2 Å composed of three residues per turn, with all peptide bonds in *trans* configuration ($\omega = 170$–175°).

To conclude, we successfully synthesized silaproline oligomers in solution using a step-by-step strategy. We showed that they adopt a PPII helical structure both in chloroform and in methanol. Interestingly, only 7% of *cis* isomer for the Sip-Pro dipeptide was observed in methanol and was not detectable for longer oligomers. Considering the interesting biological properties previously observed when Sip residues were incorporated in peptide sequences (e.g., cellular uptake enhancement, resistance to proteolysis), short silaproline PPII helical moieties may represent promising drug delivery systems.

Experimental Section

Peptide synthesis

Materials and reagents were of the highest grade commercially available and were used without further purification. Reactions were monitored by thin-layer chromatography using TLC Silicagel 60 F_{254} from Merck. Compounds were visualized by UV, phosphomolybdic acid (PMA), and ninhydrin. Flash chromatography was performed using SiliaFlash P60.

NMR spectrometry

NMR samples contained 10 mM of compounds **2**–**5** dissolved in $CDCl_3$, in CD_3OD and in [D_6]DMSO for the monomer. All spectra were recorded on a Bruker AVANCE III 600 MHz spectrometer equipped with a 5 mm quadruple-resonance probe (^{1}H, ^{13}C, ^{15}N, ^{31}P). Homonuclear 2D spectra DQF-COSY and ROESY were typically recorded in the phase-sensitive mode using the States-TPPI method as data matrices of 128–512 real (t_1) × 2048 (t_2) complex data points; 16–64 scans per t_1 increment with 1.5 s recovery delay and spectral width of 6009 Hz in both dimensions were used. The mixing time was 300 ms for the ROESY experiments. In addition, 2D heteronuclear spectra ^{13}C HSQC and ^{13}C HMBC were acquired to fully assign the oligomers (8–32 scans, 128–256 real (t_1) × 1024–2048 (t_2) complex data points). Spectra were processed and visualized with Topspin 3.0 (Bruker Biospin) on a Linux station. Matrices were zero-filled to 1024 (t_1) × 2048 (t_2) points after apodization by shifted sine-square multiplication and linear prediction in the F1 domain. Chemical shifts were referenced to the solvent.

Computational methods

NOE cross-peaks were integrated and assigned within the NMRView software.[30] The volume of a ROE between methylene pair protons was used as a reference of 1.8 Å. The lower bound for all restraints was fixed at 1.8 Å and upper bounds at 2.7, 3.3 and 5.0 Å, for strong, medium and weak correlations, respectively. Pseudo-atom corrections of the upper bounds were applied for unresolved aromatic, methylene and methyl proton signals as described previously.[31] Structure calculations were performed with AMBER 11[29] in three stages: cooking, simulated annealing in vacuum. The cooking stage was performed at 1000 K to generate 100 initial random structures. SA calculations were carried out for 20 ps (20 000 steps, 1 fs long). First, temperature was raised quickly

and maintained at 1000 K for the first 5000 steps, then the system was gradually cooled from 1000 to 100 K from step 5001 to 18000 and finally the temperature was brought to 0 K during the 2000 remaining steps. For the 3000 first steps, the force constant of the distance restraints was increased gradually from 2.0 to 20 kcal mol^{-1} Å. For the rest of the simulation (step 3001 to 20000), the force constant was kept at 20 kcal mol^{-1} Å. The 20 lowest energy structures with no violations >0.3 Å were considered as representative of the peptide structure. Representation and quantitative analysis were carried out using Ptraj, MOLMOL[32] and PyMOL (Delano Scientific). DFT geometry optimizations (see the Supporting Information) were carried out within the ab initio Gaussian code,[33] using the hybrid B3LYP functional, and an all-electron Gaussian basis set 6–31g(d,p). SCF convergence was set to 10^{-8}.

CD spectroscopy

Samples were dissolved in a spectrophotometric grade MeOH at 100–200 μm. CD experiments were carried out using a Jasco J815 spectropolarimeter. Spectra were recorded in MeOH using a 1 mm path length CD cuvette, over a wavelength range of 190–260 nm, at 20–55 °C. Continuous scanning mode was used, with a response of 4.0 s with 0.05 nm steps and a scan speed of 100 nm min^{-1}. The signal-to-noise ratio was improved by acquiring each spectrum over an average of two scans. Baseline was corrected by subtracting background from the sample spectrum.

Preparation of Boc-(L)Sip-OH from H-(L)Sip-OH

To a solution of H-(L)Sip-OH·HCl (391 mg, 2 mmol) dissolved in a 1:1 mixture of THF/water (12 mL), $NaHCO_3$ (336 mg, 4 mmol) and Boc_2O (373 mg, 4 mmol) were added consecutively at 0 °C. After 30 min, the solution was stirred, overnight, at room temperature. The resulting mixture was extracted with ether. The aqueous layer was acidified to pH 4–5 by addition of 10% acid citric solution at 0 °C and then extracted with CH_2Cl_2. The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to yield Boc-(L)Sip-OH as a colorless oil (92% yield); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 298 K): $\delta=4.51$ (d, $J=10.4$ Hz, 1H; Hα), 2.76/2.62 (dd, $J=14.4$ Hz, 2H; Hδ/δ′), 1.39 (s, 3H; Boc), 1.28 (dd, $J=10.4$ Hz, $J=15$ Hz 1H; Hβ), 1.04 (dd, $J=2.8$ Hz, $J=15$ Hz 1H; Hβ′), 0.21 (s, 3H; CH_3(Si)), 0.20 ppm (s, 3H; CH_3(Si)); ^{13}C NMR (150 MHz, [D$_6$]DMSO, 298 K): $\delta=174.6$, 155.1, 78.2, 58.7, 34.1, 27.7, 15.9, −3.1 ppm; HRMS (ESI-MS): *m/z* 282.1138 calcd for $C_{11}H_{21}NO_4NaSi$ $[M+Na]^+$; found 282.1136; $[\alpha]_D^{20}=-28$ ($c=0.5$, $CHCl_3$).

Preparation of Boc-(L)Sip-(L)Pro-OBn

Boc-(L)Sip-OH (259 mg, 1 mmol) was dissolved in DMF (10 mL) and HBTU (417 mg, 1.1 mmol) was added. Then H-(L)Pro-OBn·HCl (241.5 mg, 1 mmol) was added to the solution. Finally triethylamine (1.1 mmol) was added dropwise to the solution (pH 8–9). The resulting mixture was stirred at room temperature, overnight. DMF was concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (20 mL) and then the solution was washed successively with 10% citric acid solution (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/cyclohexane 7:3) to afford colorless oil (378 mg, 0.85 mmol, 85%).

General procedure for N-deprotection

TFA (2 mL) was added to the peptide and the solution was stirred for 5 min. The excess TFA was then concentrated in vacuo and the resulting TFA salt was directly used for the next coupling as described for the synthesis of Boc-(L)Sip-(L)Pro-OBn.

Dimer (2)

This was synthesized according to the procedure described for the preparation of Boc-(L)Sip-(L)Pro-OBn.

^{1}H NMR (600 MHz, [D$_4$]MeOD, 298 K): δ (*trans* conformer) = 5.17/5.13 (d, $J=12.1$ Hz, 2H; OCH_2Bn), 4.56 (dd, $J=5.2$ Hz, 8.7 Hz, 1H; Hα Pro2), 4.35 (dd, $J=7.3$ Hz,10.9 Hz, 1H; Hα Sip1), 3.68 (ddd, $J=6.4$ Hz,9.9 Hz,16.3 Hz, 1H; Hδ Pro2), 3.62 (ddd, $J=6.9$ Hz,9.9 Hz,16.3 Hz, 1H; Hδ′ Pro2), 2.42/2.75 (d, $J=14.3$ Hz, 2H; Hδ/δ′ Sip1), 2.31 (m, 1H; Hβ Pro2), 2.05 (m, 2H; Hγ/γ′ Pro2), 1.99 (m, 1H; Hβ′ Pro2), 1.54 (dd, $J=7.3$ Hz, 14.9 Hz, 1H; Hβ Sip1), 0.88 (dd, $J=10.9$ Hz,14.9 Hz, 1H; Hβ′ Sip1), 0.38/0.34 ppm (s, 6H; Si CH_3 Sip1); δ (*cis* conformer) = 5.26/5.23 (d, $J=12.1$ Hz, 2H; OCH_2Bn), 4.71 (dd, $J=3.7$ Hz,6.4 Hz, 1H; Hα Pro2), 3.90 (dd, $J=6.9$ Hz, 11.3 Hz, 1H; Hα Sip1), 3.62/3.51 (m, 2H; Hδ/δ′ Pro2), 2.42/2.75 (d, $J=14.6$ Hz, 2H; Hδ/δ′ Sip1), 2.36 (m, 2H; Hβ/β′ Pro2), 1.96 (m, 1H; Hγ Pro2), 1.79 (m, 1H; Hγ′ Pro2), 1.59 (dd, $J=6.9$ Hz, 14.8 Hz, 1H; Hβ Sip1), 0.91 (dd, $J=11.3$ Hz,14.8 Hz, 1H; Hβ′ Sip1), 0.36/0.30 ppm (s, 6H; Si CH_3 Sip1); ^{13}C NMR (150 MHz, MeOD-*d4*, 298 K): $\delta=172.9$, 169.6, 68.0, 61.0, 60.6, 48.0, 34.1, 29.9, 25.9, 15.2, −3.1 ppm; HRMS (ESI-MS): *m/z* 347.1791 calcd for $C_{18}H_{27}N_2O_3Si$ $[M+H]^+$; found 347.1771.

Tetramer (4)

This was synthesized according to the procedure described for the preparation of Boc-(L)Sip-(L)Pro-OBn.

^{1}H NMR (600 MHz, [D$_4$]MeOD, 298 K): $\delta=5.34$ (dd, $J=3.4$ Hz,10.8 Hz, 1H; Hα Sip2), 5.16/5.12 (d, $J=12.2$ Hz, 2H; OCH_2Bn), 4.98 (dd, $J=3.7$ Hz, 10.8 Hz, 1H; Hα Sip3), 4.50 (dd, $J=7.0$ Hz, 10.8 Hz, 1H; Hα Sip1), 4.44 (dd, $J=4.1$ Hz, 8.6 Hz, 1H; Hα Pro4), 3.75 (ddd, $J=7.2$ Hz,9.9 Hz, 16.0 Hz, 1H; Hδ Pro4), 3.68 (ddd, $J=6.6$ Hz,9.8 Hz, 16.0 Hz, 1H; Hδ′ Pro4), 3.15/3.00 (d, $J=13.3$ Hz, 2H; Hδ/δ′ Sip3), 3.00/2.95 (d, $J=13.2$ Hz, 2H; Hδ/δ′ Sip2), 2.76/2.42 (d, $J=14.5$ Hz, 2H; Hδ/δ′ Sip1), 2.23 (m, 1H; Hβ Pro4), 2.03 (m, 2H; Hγ/γ′ Pro4), 1.98 (m, 1H; Hβ′ Pro4), 1.66 (dd, $J=7$ Hz, 15.0 Hz, 1H; Hβ Sip1), 1.33 (dd, $J=10.8$ Hz, 15.0 Hz, 1H; Hβ Sip2), 1.26 (dd, $J=10.8$ Hz, 15.1 Hz, 1H; Hβ Sip3), 1.14 (dd, $J=3.4$ Hz, 15.0 Hz, 1H; Hβ′ Sip2), 1.00 (dd, $J=3.7$ Hz, 15.1 Hz, 1H; Hβ′ Sip3), 0.94 (dd, $J=10.8$ Hz, 15.0 Hz, 1H; Hβ′ Sip1), 0.39/0.35 (s, 6H; Si CH_3 Sip1), 0.28/0.25 ppm (s, 12H; Si CH_3 Sip2, Sip3); ^{13}C NMR (150 MHz, [D$_4$]MeOD, 298 K): $\delta=176.3$, 174.1, 173.3, 171.8, 67.9, 61.3, 60.3, 60.0, 47.9, 36.6, 36.7, 34.1, 30.0, 25.9, 14.9, 14.5, 14.2, −2.5, −2.7, −3.0, −3.1 ppm; HRMS (ESI-MS): *m/z* 629.3011 calcd for $C_{30}H_{49}N_4O_5Si_3$ $[M+H]^+$; found 629.3023.

Acknowledgements

DFT calculations were performed using HPC resources from GENCI-[CCRT/CINES/IDRIS] (Grant x2013085119). Computations were carried out also on the IBM SP4 computers of the CINES (Centre Informatique National de l'Enseignement Supérieur) in Montpellier (France).

Keywords: helix · molecular modeling · NMR spectroscopy · peptidomimetics · silaproline

[1] a) A. A. Adzhubei, M. J. E. Sternberg, A. A. Makarov, *J. Mol. Biol.* **2013**, *425*, 2100 – 2132; b) M. D. Zotti, F. Formaggio, M. Crisma, C. Peggion, A. Moretto, C. Toniolo, *J. Pept. Sci.* **2014**, *20*, 307 – 322.
[2] B. Bochicchio, A. M. Tamburro, *Chirality* **2002**, *14*, 782 – 792.
[3] B. J. Stapley, T. P. Creamer, *Protein Sci.* **1999**, *8*, 587 – 595.
[4] L. J. Ball, R. Kühne, J. Schneider-Mergener, H. Oschkinat, *Angew. Chem.* **2005**, *117*, 2912 – 2930; *Angew. Chem. Int. Ed.* **2005**, *44*, 2852 – 2869.
[5] B. K. Kay, M. P. Williamson, M. Sudol, *FASEB J.* **2000**, *14*, 231 – 241.
[6] a) Y. A. Fillon, J. P. Anderson, J. Chmielewski, *J. Am. Chem. Soc.* **2005**, *127*, 11798 – 11803; b) D. S. Daniels, A. Schepartz, *J. Am. Chem. Soc.* **2007**, *129*, 14578 – 14579.
[7] a) P. M. Cowan, S. McGavin, *Nature* **1955**, *176*, 501 – 503; b) F. Rabanal, M. D. Ludevid, M. Pons, E. Giralt, *Biopolymers* **1993**, *33*, 1019 – 1028.
[8] B. W. Chellgren, T. P. Creamer, *Biochemistry* **2004**, *43*, 5864 – 5869.
[9] a) M. A. Kelly, B. W. Chellgren, A. L. Rucker, J. M. Troutman, M. G. Fried, A.-F. Miller, T. P. Creamer, *Biochemistry* **2001**, *40*, 14376 – 14383; b) M. Moradi, V. Babin, C. Sagui, C. Roland, *J. Phys. Chem. B* **2011**, *115*, 8645 – 8656; c) Y. Mansiaux, A. P. Joseph, J.-C. Gelly, A. G. de Brevern, *PLoS One* **2011**, *6*, e18401.
[10] R. W. Woody, *J. Am. Chem. Soc.* **2009**, *131*, 8234 – 8245.
[11] Z. Shi, C. A. Olson, G. D. Rose, R. L. Baldwin, N. R. Kallenbach, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 9190 – 9195.
[12] W. Traub, U. Shmueli, *Nature* **1963**, *198*, 1165 – 1166.
[13] W. J. Wedemeyer, E. Welker, H. A. Scheraga, *Biochemistry* **2002**, *41*, 14637 – 14644.
[14] M. Kümin, L.-S. Sonntag, H. Wennemers, *J. Am. Chem. Soc.* **2007**, *129*, 466 – 467.
[15] a) C. G. Overberger, Y. S. Jon, *Polym. Chem. Ed.* **1977**, *15*, 1413 – 1421; b) E. Beausoleil, W. D. Lubell, *Biopolymers* **2000**, *53*, 249 – 256.
[16] a) F. Bernardi, M. Garavelli, M. Scatizzi, C. Tomasini, V. Trigari, M. Crisma, F. Formaggio, C. Peggion, C. Toniolo, *Chem. Eur. J.* **2002**, *8*, 2516 – 2525; b) C. Tomasini, G. Angelici, N. Castellucci, *Eur. J. Org. Chem.* **2011**, 3648 – 3669; c) C. Tomasini, V. Trigari, S. Lucarini, F. Bernardi, M. Garavelli, C. Peggion, F. Formaggio, C. Toniolo, *Eur. J. Org. Chem.* **2003**, 259 – 267.
[17] P. Tremmel, A. Geyer, *J. Am. Chem. Soc.* **2002**, *124*, 8548 – 8549.
[18] J. Zaminer, C. Brockmann, P. Huy, R. Opitz, C. Reuter, M. Beyermann, C. Freund, M. Müller, H. Oschkinat, R. Kühne, H.-G. Schmalz, *Angew. Chem.* **2010**, *122*, 7265 – 7269; *Angew. Chem. Int. Ed.* **2010**, *49*, 7111 – 7115.
[19] B. Raghavan, K. J. Skoblenick, S. Bhagwanth, N. Argintaru, R. K. Mishra, R. L. Johnson, *J. Med. Chem.* **2009**, *52*, 2043 – 2051.
[20] a) R. Zhang, F. Brownewell, J. S. Madalengoitia, *J. Am. Chem. Soc.* **1998**, *120*, 3894 – 3902; b) R. Zhang, C. K. Nickl, A. Mamai, S. Flemer, A. Natarajan, W. R. Dostmann, J. S. Madalengoitia, *J. Pept. Res.* **2005**, *66*, 151 – 159; c) S. Flemer, A. Wurthmann, A. Mamai, J. S. Madalengoitia, *J. Org. Chem.* **2008**, *73*, 7593 – 7602.
[21] a) B. Vivet, F. Cavelier, J. Martinez, *Eur. J. Org. Chem.* **2000**, 807 – 811; b) C. Martin, N. Vanthuyne, H. Miramon, J. Martinez, F. Cavelier, *Amino Acids* **2012**, *43*, 649 – 655.
[22] F. Cavelier, B. Vivet, J. Martinez, A. Aubry, C. Didierjean, A. Vicherat, M. Marraud, *J. Am. Chem. Soc.* **2002**, *124*, 2917 – 2923.
[23] B. Vivet, F. Cavelier, J. Martinez, C. Didierjean, M. Marraud, A. Aubry, *Acta Crystallogr. Sect. C* **2000**, *56*, 1452 – 1454.
[24] F. Cavelier, D. Marchand, J. Martinez, S. Sagan, *J. Pept. Res.* **2004**, *63*, 290 – 296.
[25] S. Pujals, J. Fernández-Carneado, M. J. Kogan, J. Martinez, F. Cavelier, E. Giralt, *J. Am. Chem. Soc.* **2006**, *128*, 8479 – 8483.
[26] C. Martin, A. Lebrun, J. Martinez, F. Cavelier, *J. Polym. Sci. Part A* **2013**, *51*, 3103 – 3109.
[27] R. Zhang, J. S. Madalengoitia, *Tetrahedron Lett.* **1996**, *37*, 6235 – 6238.
[28] M. P. Williamson, *Biochem. J.* **1994**, *297*, 249 – 260.
[29] T. A. D. D. A. Case, T. E. Cheatham III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, R. C. W. Luo, W. Zhang, K. M. Merz, B. Roberts, B. Wang, S. Hayik, A. Roitberg, I. K. G. Seabra, K. F. Wong, F. Paesani, J. Vanicek, J. Liu, X. Wu, S. R. Brozell, H. G. T. Steinbrecher, Q. Cai, X. Ye, J. Wang, M.-J. Hsieh, G. Cui, D. R. Roe, D. H. Mathews, M. G. Seetin, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko, P. A. Kollman, AMBER 11, San Francisco, University of California, **2010**.
[30] B. Johnson, R. Blevins, *J. Biomol. NMR* **1994**, *4*, 603 – 614.
[31] K. Wüthrich, *NMR of Proteins and Nucleic Acids*, Wiley, **1986**.
[32] R. Koradi, M. Billeter, K. Wüthrich, *J. Mol. Graphics* **1996**, *14*, 51 – 55.
[33] Gaussian 09, Revision D.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian, Inc., Wallingford CT, **2009**.

Received: August 12, 2014
Published online on September 11, 2014

