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Influence of Silaproline on Peptide Conformation and Bioactivity

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Abstract: The analogue γ-(dimethylsila)-proline, denoted silaproline (Sip), was synthesized in both enantiomerically pure forms by diastereoselective alkylation of a chiral glycine equivalent with use of Schöllkopf's bis-lactim ether method. The effect of replacing a proline residue in model peptides by this new proline surrogate has been examined in the crystal state by X-ray diffraction and in solution by IR absorption and NMR techniques. Silaproline and proline-containing sequences exhibit very similar conformational properties. Silaproline was also substituted for proline in a neurotensin (8−13) analogue that retained biological activity and exhibited enhanced resistance to biodegradation.

Introduction

Proline plays an essential role in the three-dimensional structure of peptides and proteins, especially for inducing reverse turns.[1] To modulate the structural properties of proline, several analogues have been proposed in the literature. For example, C^{β}-substituted prolines have the advantage of orienting the substituent in a given direction depending on their cis or trans stereochemistry.[2] Azaproline (AzPro), where the α-carbon is replaced by a nitrogen, has been found to highly favor the cis-isomer conformation of the azaproline-preceding amide bond, therefore inducing typeVI β-turn folding of the Xaa-AzPro sequence.[3] Imidazolidine-2-carboxylic acid differs from proline by a nitrogen in the β-position.[4] The so-called pseudo-prolines ΨPro,[5] containing an oxazolidine or thiazolidine ring, have been developed as a protection of serine, threonine, or cysteine side chains that may increase peptide solubility and reduce their polarity and side reactions such as α-epimerization. The cis−trans ratio for the ΨPro-preceding amide bond depends strongly on the degree of substitution and chirality of the δ-carbon. In particular, unlike proline and δunsubstituted ΨPro, δ-disubstituted ΨPro favors the cis conformer while unsubstitution results in the same preference as proline.[6] Influence of steric interactions on the cis−trans conformation of the Pro-preceding amide has also been studied with δ-*tert*-butylproline.[7] Another sterically hindered residue, the δ,δ-dimethylproline, has been developed as a substitute to lock proline in the cis conformation in peptides.[8]

We have now prepared γ-(dimethylsila)-proline, denoted silaproline (Sip), a new proline analogue in which the dimethylsilyl group is substituted for its γ-methylene carbon. The modification of the γ-position is expected to minimally interact with the proline conformation.[9] Replacement of Sip for Pro in peptides should increase lipophilicity because the octanol−water partition coefficient of Sip was experimentally determined to be 14 times greater than that of Pro. Increased lipophilicity may therefore facilitate membrane permeability. Reduced sensitivity to enzymatic degradation may also arise from substitution of Sip for Pro in peptides. Moreover, the similarity of the Sip and Pro rings should result in similar conformational properties for analogous Sip- and Pro-containing peptides.

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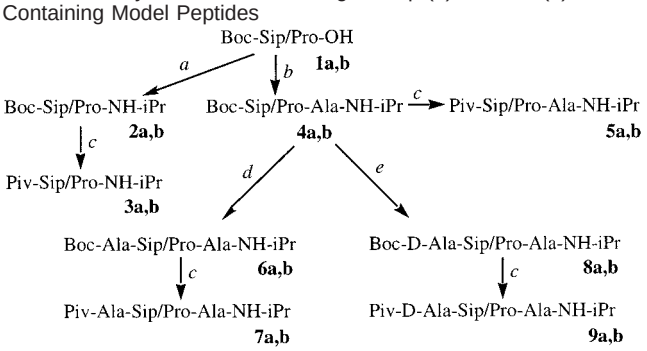
Scheme 1. Synthesis of the Analogous Sip (a) and Pro (b) Containing Model Peptides



[a] H_2NiPr/BOP/DIEA/DMF. [b] HCl·H-Ala-NHiPr/BOP/DIEA/DMF. [c] (1) TFA/CH_2Cl_2, (2) PivCl/DIEA/CH_2Cl_2. [d] (1) TFA/CH_2Cl_2, (2) Boc-Ala-OH/BOP/DIEA/DMF. [e] (1) TFA/CH_2Cl_2, (2) Boc-D-Ala-OH/BOP/DIEA/DMF.

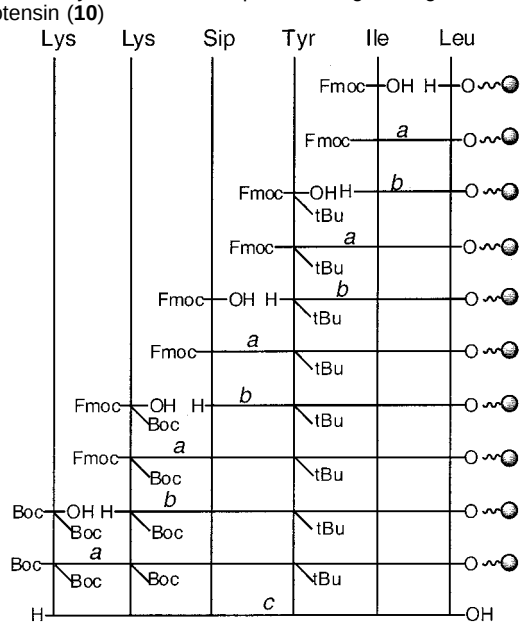
Syntheses of the highly lipophilic silaproline in both enantiomerically pure forms with Boc and Fmoc *N*-protections have been described.[10] In the present paper, we report the incorporation of silaproline into model peptides to determine its conformational impact compared to proline. Silaproline has also been substituted for proline in the neurotensin (8−13) analogue H-Lys-Lys-Pro-Tyr-Ile-Leu-OH. The neuropeptide neurotensin (NT) elicits hypothermic and naloxone-insensitive analgesic responses after brain injection.[11] Structure−activity studies have demonstrated that the minimal sequence requested for full biological activity is the C-terminal hexapeptide NT(8−13). However, NT is quickly digested by enzymes, mainly around the proline residue, such that NT must be injected with a cocktail of enzyme inhibitors to be active.[12] To assess the benefit in bioresistance obtained with silaproline, we have replaced proline by silaproline in NT(8−13) and compared its stability and receptor affinity with those of various NT(8−13) analogues.

Materials and Methods

Synthesis. BOP, HBTU, HOBt, DIEA, and isopropylamine were purchased from Aldrich. CH_2Cl_2 was dried overnight over $CaCl_2$, then distilled on K_2CO_3 and stored away from bright light in a brown bottle. Water was obtained from the Milli-Q plus system (Millipore) and acetonitrile and trifluoroacetic acid (TFA) from Merck. TLC was performed on silica gel plates Merck $60F_{254}$, visualized by ultraviolet light or by staining with phosphomolybdic acid. Flash chromatography was performed on Merck silica gel 60 (230−400 mesh). Mass spectra were obtained by electron spray ionization (ESI-MS) on a Micromass Platform II quadrupole mass spectrometer (Micromass) fitted with an electrospray source coupled with an HPLC Waters or with a JEOL SX 102 apparatus, using Xenon in the FAB mode, in glycerol/thioglycerol (50/50, GT) or nitrobenzyl alcohol (NBA). HPLC runs were performed on Waters equipment using columns packed with Nucleosil 300 Å 5 μm C_{18} particles unless otherwise stated. The analytical column (50 × 4.6 mm) operated at 1 mL/min, with a photodiode array detector 996, wavelength 214 nm. Solvent A consisted of 0.1% TFA in H_2O and solvent B of 0.1% TFA in acetonitrile.

Silaproline and proline were incorporated into model di- and tripeptides (Scheme 1). Treatment of Boc-Xaa-OH **1a**,**b** with isopropylamine using the BOP[13] reagent and DIEA as a base gave compounds **2a**,**b** in quantitative yield. Switching from the Boc to the Piv group was performedby treatment with TFA, followed by pivaloyl chloride in dichloromethane. Coupling steps were carried out in DMF using BOP and DIEA. The yields in Sip-Ala and Ala-Sip bond formation were of the same range as those for proline, indicating that the amine and carboxyl termini in both residues had the same reactivity. All model peptides were purified on silica gel by column chromatography. Contrary to proline, silaproline generally resulted in oily model peptides probably due to unfavorable molecular packing of the bulky dimethylsilyl group in the solid state.

Scheme 2. Synthesis of the Sip-Containing Analogue of Neurotensin (**10**)



[a] HBTU/HOBT/DIEA/DMF. [b] Piperidine/DMF. [c] TFA/anisole.

Sip is about 14 times more lipophilic than Pro, ascertained by the higher octanol−water partition coefficient, log $P = 1.3$ for Fmoc-Sip-OH and 0.094 Fmoc-Pro-OH. The Sip-containing peptides are actually more lipophilic than their proline counterparts, as shown by HPLC retention times for **7a**/**7b** and **9a**/**9b** under the same elution conditions. Silaproline was also introduced in place of proline in [Sip[10]]NT(8−13) **10**, TMSAla was introduced in place of Ile in neurotensin analogue **11**, and both mutations are combined in analogue **12**. These peptides were synthesized by the solid-phase method (Scheme 2 for the synthesis of peptide **10** as an example) on a Perkin-Elmer ABI433A automatic synthesizer on a 0.25 mmol scale with Wang resin. The α-amino protection was achieved with the Fmoc group and the coupling reagent was a 0.45 M solution of HBTU/HOBt.[14] Deprotection cycles were carried out in piperidine/DMF (20/80) and monitored by conductimetry. Elongation was effected by single 30-min couplings in DMF with DIEA as a base. Final cleavage was carried out with 80% TFA and 20% anisole for 3 h. Peptide resins were washed extensively with DMF, then dried in vacuo, dissolved in an acetonitrile−water mixture, and freeze dried.

Piv-Sip-NHiPr (3a): oil. R_f (EtOAc/hexane: 3/7) = 0.30. $t_R = 12.9$ min (0−90% B, 15 min, C_{18}). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.26 and 0.38 (2s, 6H, Sip-Si$^{\gamma}$(CH_3)$_2$); 0.90−1.40 (m, 2H, Sip-C$^{\beta}H_2$); 1.06 and 1.12 (2d, 6H, $J = 6.6$ Hz, ^{i}Pr-CH_3); 1.24 (s, 9H, tBu); 2.57 and 3.31 (2d, 2H, $J = 13.9$ Hz, Sip-C$^{\delta}H_2$); 3.98 (m, 1H, ^{i}Pr-CH); 5.28 (bd, 1H, $J = 9.0$ Hz, Sip-C$^{\alpha}H$); 6.45 (b, 1H, NH^iPr). ESI-MS: 285.1 [M +

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H]$^+$, 307.0 [M + Na]$^+$, 569.7 [2M + H]$^+$, 591.0 [2M + Na]$^+$. HRMS (FAB+): Calcd. for $C_{14}H_{29}N_2O_2Si$ [M + H]$^+$, 285.1998; found, 285.2022.

Piv-SipAla-NHiPr (5a): oil. R_f (EtOAc/hexane: 5/5) = 0.25. t_R = 12.4 min (0−90% B, 15 min, C_{18}). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.24 and 0.37 (2s, 6H, Sip-Si$^\gamma$(CH_3)$_2$); 0.90−1.40 (m, 2H, Sip-C$^\beta H_2$); 1.02 and 1.15 (2d, 6H, J = 6.6 Hz, ^{i}Pr-CH_3); 1.33 (s, 9H, tBu); 1.33 (d, 3H, J = 7.5 Hz, Ala-C$^\beta H_3$); 2.72 and 3.33 (2d, 2H, J = 13.8 Hz, Sip-C$^\delta H_2$); 4.04 (m, 1H, ^{i}Pr-CH); 4.32 (m, 1H, Ala-C$^\alpha H$); 5.18 (dd, 1H, J = 2.7 and 10.0 Hz, Sip-C$^\alpha H$); 6.10 (d, 1H, J = 7.8 Hz, NH^iPr); 6.63 (d, 1H, J = 7.8 Hz, Ala-NH). ESI-MS: 356.1 [M + H]$^+$, 378.4 [M + Na]$^+$, 711.6 [2M + H]$^+$, 733.5 [2M + Na]$^+$. HRMS (FAB+): Calcd. for $C_{17}H_{34}N_3O_3Si$ [M + H]$^+$, 356.2369; found, 356.2393.

Piv-Ala-Sip-Ala-NHiPr (7a): oil. R_f (EtOAc) 0.20. t_R = 11.5 min (0−90% B, 15 min, C_{18}). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.27 and 0.36 (2s, 6H, Sip-Si$^\gamma$(CH_3)$_2$); 0.90−1.40 (m, 2H, Sip-C$^\beta H_2$); 1.14 and 1.15 (2d, 6H, J = 6.6 Hz, ^{i}Pr-CH_3); 1.21 (s, 9H, tBu); 1.32 (d, 3H, J = 6.9 Hz, Ala^3-C$^\beta H_3$); 1.34 (d, 3H, J = 6.9 Hz, Ala^1-C$^\beta H_3$); 3.07 and 2.73 (2d, 2H, J = 13.5 Hz, Sip-C$^\delta H_2$); 4.03 (m, 1H, ^{i}Pr-CH); 4.29 (m, 1H, Ala^3-C$^\alpha H$); 4.92 (m, 1H, Ala^1-C$^\alpha H$); 5.08 (dd, 1H, J = 2.5 and 9.9 Hz, Sip-C$^\alpha H$); 5.85 (d, 1H, J = 6.9 Hz, NH^iPr); 6.55 (d, 1H, J = 6.9 Hz, Ala^3-NH); 6.68 (d, 1H, J = 6.7 Hz, Ala^1-NH). ESI-MS: 427.3 [M + H]$^+$, 449.5 [M + Na]$^+$, 853.8 [2M + H]$^+$, 875.9 [2M + Na]$^+$. HRMS (FAB+): Calcd. for $C_{20}H_{39}N_4O_4Si$ [M + H]$^+$, 427.2741; found, 427.2735.

Piv-Ala-Pro-Ala-NHiPr (7b): mp 162 °C. R_f (^{i}PrOH/EtOAc 1/3) = 0.58. t_R = 8.2 min (0−100% B, 15 min, C_{18}). ^{1}H NMR (200 MHz, $CDCl_3$, major trans conformer) δ 1.14 and 1.16 (2d, 6H, J = 6.6 Hz, ^{i}Pr-(CH_3)$_2$); 1.21 (s, 9H, tBu); 1.35 (d, 3H, J = 7.3 Hz, Ala^3-C$^\alpha H_3$); 1.39 (d, 3H, J = 7.0 Hz, Ala^1-C$^\alpha H_3$); 1.97−2.27 (m, 4H, Pro-C$^\beta H_2$ + Pro-C$^\gamma H_2$); 3.50−3.80 (m, 2H, Pro-C$^\delta H_2$); 4.04 (m, 1H, ^{i}Pr-CH); 4.34 (m, 1H, Ala^3-NH); 4.53 (m, 1H, Pro-C$^\alpha H$); 4.71 (m, 1H, Ala^1-C$^\alpha H$); 6.01 (d, 1H, J = 7.6 Hz, NH^iPr); 6.52 (d, 1H, J = 6.6 Hz, Ala^1-NH); 6.82 (d, 1H, J = 7.6 Hz, Ala^3-NH). ESI-MS: 383.1 [M + H]$^+$, 405.2 [M + Na]$^+$. HRMS (FAB+): Calcd. for $C_{19}H_{35}N_4O_4$ [M + H]$^+$, 383.2658; found, 383.2648.

Piv-D-Ala-Sip-Ala-NHiPr (9a): oil. R_f (EtOAc) 0.20. t_R = 11.9 min (0−90% B, 15 min, C_{18}). ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.28 and 0.30 (2s, 6H, Sip-Si$^\gamma$(CH_3)$_2$); 1.10−1.65 (m, 2H, Sip-C$^\beta H_2$); 1.12 and 1.13 (2d, 6H, J = 6.6 Hz, ^{i}Pr-(CH_3)$_2$); 1.19 (s, 9H, tBu); 1.37 (d, 3H, J = 6.9 Hz, Ala^1-C$^\beta H_3$); 1.44 (d, 3H, J = 7.3 Hz, Ala^3-C$^\beta H_3$); 3.19 and 2.86 (2d, 2H, J = 13.5 Hz, Sip-C$^\delta H_2$); 3.93 (m, 1H, ^{i}Pr-CH); 4.35 (m, 1H, Ala^3-C$^\alpha H$); 4.71 (m, 1H, Ala^1-C$^\alpha H$); 5.03 (dd, 1H, J = 1.8 and 10.6 Hz, Sip-C$^\alpha H$); 6.10 (d, 1H, J = 4.4 Hz, Ala^1-NH); 6.39 (d, 1H, J = 6.9 Hz, NH^iPr); 7.13 (d, 1H, J = 8.0 Hz, Ala^3-NH). ESI-MS: 427.3 [M + H]$^+$, 449.3 [M + Na]$^+$, 853.8 [2M + H]$^+$, 875.9 [2M + Na]$^+$. HRMS (FAB+): Calcd. for $C_{20}H_{39}N_4O_4Si$ [M + H]$^+$, 427.2741; found, 427.2763.

Piv-D-Ala-Pro-Ala-NHiPr (9b): mp 199 °C. R_f (^{i}PrOH/EtOAc 1/3) = 0.52. t_R = 8.7 min (0−100% B, 15 min, C_{18}). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.12 and 1.14 (2d, 6H, J = 6.6 Hz, ^{i}Pr-(CH_3)$_2$); 1.19 (s, 9H, tBu); 1.40 (d, 3H, J = 7.0 Hz, Ala^1-NH); 1.46 (d, 3H, J = 7.3 Hz, Ala^3-NH); 2.04 (m, 2H, Pro-C$^\gamma H_2$); 2.21 (m, 2H, Pro-C$^\beta H_2$); 3.52 and 4.07 (2m, 2H, Pro-C$^\delta H_2$); 3.95 (m, 1H, ^{i}Pr-CH); 4.28−4.48 (m, 2H, Ala^1-NH + Ala^3−NH); 4.52 (dd, 1H, J = 3.7 and 7.7 Hz, Pro-C$^\alpha H$); 6.03 (d, 1H, J = 4.0 Hz, Ala^1-NH); 6.65 (d, 1H, J = 7.7 Hz, NH^iPr); 7.18 (d, 1H, J = 8.0 Hz, Ala^3-NH). ESI-MS: 383.1 [M + H]$^+$, 405.1 [M + Na]$^+$. HRMS (FAB+): Calcd. for $C_{19}H_{35}N_4O_4$ [M + H]$^+$, 383.2658; found, 383.2657.

[Sip^{10}]NT(8−13) (10): The crude peptide was purified by semipreparative HPLC. Mp 130 °C. t_R = 17.25 min (20−50% B, 30 min, C_{18}). Purity >98% (Figure 1). ESI-MS: 403.4 [M + 2H]$^{2+}$, 805.7 [M + H]$^+$. HRMS (FAB+): Calcd. for $C_{39}H_{69}N_8O_8Si$ [M + H]$^+$, 805.5007; found, 805.5074.

[TMSAla^{12}]NT(8−13) (11): The crude peptide was purified by semipreparative HPLC. Mp 130 °C. t_R = 17.25 min (20−50% B, 30 min, C_{18}). Purity >99%. ESI-MS: 396.4 [M + 2H]$^{2+}$, 791.6 [M + H]$^+$. HRMS (FAB+): Calcd. for $C_{38}H_{67}N_8O_8Si$ [M + H]$^+$, 791.4851; found, 791.4856.

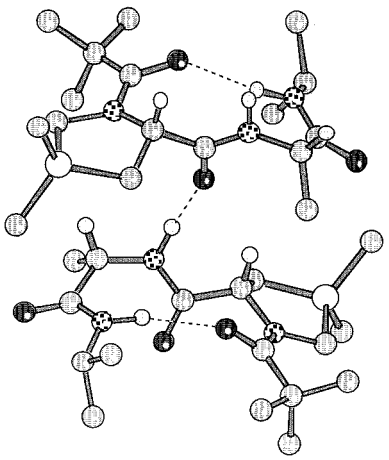

Figure 1. X-ray molecular structure of **5a** showing the two independent A (down) and B (up) βII-folded molecules connected by the intermolecular Ala-NH to Sip-CO H-bond. Only the NH and C$^\alpha$ hydrogens are shown.

[Sip^{10}, TMSAla^{12}]NT(8−13) (12): The crude peptide was purified by semipreparative HPLC. Mp 130 °C. t_R = 17.25 min (20−50% B, 30 min, C_{18}). Purity >99% (Figure 1). ESI-MS: 418.5 [M + 2H]$^{2+}$, 835.6 [M + H]$^+$. HRMS (FAB+): Calcd. for $C_{39}H_{71}N_8O_8Si_2$ [M + H]$^+$, 835.4933; found, 835.4941.

Experimental deTermination of the Octanol−Water Partition Coefficient *P*. The compound to be analyzed (1.5 mg) was dissolved in a solution (2.2 mL) of 1-octanol previously saturated with 0.02 M phosphate buffer. A 1 mL sample of this solution was mixed with phosphate buffer (1 mL). The resulting mixture was shaken for 10 min, submitted to ultrasounds for 10 min, shaken for another 10 min, and then centrifuged for 10 min at 5000 r/min. Both layers were analyzed by HPLC and P was calculated according to the following formula: $P = C_o/C_b = (A_o/A_b)(V_b/V_o)$, where A_o, A_b, V_o, and V_b stand for peak area in octanol, peak area in buffer, injected volume of octanol, and injected volume of buffer, respectively.

X-ray Diffraction. By slow evaporation of an ^{i}Pr$_2$O/EtOAc solution, one of the Sip-containing peptides, Piv-Sip-Ala-NHiPr **5a**, gave single crystals suitable for X-ray diffraction.[15] Details on the crystal and molecular structures have been published elsewhere.[16]

IR Absorption Spectroscopy. We have considered the analogous Sip- and Pro-containing tripeptides **7a**,**b** and **9a**,**b** where the Piv group confers a trans peptide-like character on the Piv-Ala amide bond, and a better solubility in most organic solvents than the acetyl group.[17] IR absorption spectra were scanned on a Bruker IFS-25 apparatus using a cell path length of 0.5 mm to investigate the N−H (3200−3500 cm^{-1}) stretching frequencies. The peptide concentration was 0.005 M in CH_2Cl_2 and in dimethyl sulfoxide (DMSO), and further dilution confirmed the absence of molecular aggregation. The N−H stretching frequencies were assigned on the basis of previous studies on related peptides.[17] In particular, the free amide N−H gives a sharp absorption in the 3425−3445 cm^{-1} region in CH_2Cl_2, and is shifted up to about 3465 cm^{-1} in the case of a pivaloylamide.[18] An N−H to C=O H-bond results in a shift to low frequencies of the N−H stretching depending on the strength of the interaction. The shift is smaller (about 25 cm^{-1}) for the

(15) $P2_1$; a = 9.665(1) Å, b = 19.662(2) Å, c = 11.123(1) Å, β = 89.60(1)°; Z = 4, 2 molecules of A and B per asymmetric unit; $d_{calcd.}$ = 1.117 g·cm^{-3}; 3536 reflections; R = 0.054.

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Table 1. Backbone and Side-Chain Torsion Angles in the Crystal Molecular Structure of **5a**

angles	molecule A	molecule B
	backbone	
ω_0	178.4	178.4
ω'_0	−20.1	−17.6
ϕ_1	−49.8	−51.2
ψ_1	136.6	137.7
ω_1	169.8	169.7
ϕ_2	63.4	63.6
ψ_2	20.2	20.7
ω_2	−177.6	−178.2
	Sip ring[a]	
χ^1	−33.3	−33.5
χ^2	25.1	29.7
χ^3	−11.3	−14.7
χ^4	−6.8	−3.1
θ	27.1	24.6

[a] The torsional angles are defined as for the Pro ring.[22]

bent $i \rightarrow i$ interaction (extended C_5 conformer) than for the nearly linear $i + 2 \rightarrow i$ (γ-turn) and $i + 3 \rightarrow i$ (β-turn) interactions.[17a,19]

NMR Spectroscopy. ^{1}H NMR spectra were run for the analogous tripeptides **7a**,**b** and **9a**,**b** on a Bruker AC-200P apparatus with Me_4Si as an internal reference, and the spin systems were solved by COSY and HOHAHA experiments. The solvent accessibility and therefore the extent of the free or hydrogen-bonded character for the amide protons in these small molecules were investigated in two ways: by considering the shifts $\Delta\delta$ from $CDCl_3$ to DMSO-d_6,[17,20] and the temperature coefficients $\Delta\delta/\Delta T$ in DMSO-d_6 for the NH resonances.[21] The signal of a hydrogen-bonded, solvent-shielded N*H* is only slightly sensitive to the solvent or the temperature, whereas the signal of a free, solvent-exposed N*H* shows a higher $\Delta\delta$ shift and a $\Delta\delta/\Delta T$ value as low as −6 ppb/K in DMSO-d_6. In the case of a rapid equilibrium between an extended (solvent-exposed NH) and a folded (solvent-protected NH) conformer, the average $\Delta\delta$ and $\Delta\delta/\Delta T$ values are correlated with the relative percentages of these conformers.[21,22]

Biological Tests. Binding experiments were carried out on COS-7 cell membrane fractions 70 h after transfection with NTRL cDNA. Cell membranes (25 μg) were incubated in 250 μL of 50 mM Tris−HCl, pH 7.5, containing 0.1% bovine serum albumin and 0.8 mM 1,10-phenanthroline (binding buffer) with 0.4 nM ^{125}I-Tyr^3-NT and increasing concentrations of neurotensin analogues. After 20 min at 25 °C, incubation media were filtered through cellulose acetate filters. Filters were washed twice with 2 mL of ice-cold binding buffer and counted in a Parker γ counter. Ki values were determined from inhibition curves as the concentration of unlabeled ligand inhibiting 50% of ^{125}I-Tyr^3-NT specific binding.

Results and Discussion

X-ray Diffraction Structure of Piv-Sip-Ala-NHiPr, 5a. Two independent molecules A and B having quite similar structures and dimensions are found in the asymmetric unit.[16] Both are folded by an NH to CO H-bond of the $i + 3 \rightarrow i$ type [N···O = 3.12 (A) and 3.13 (B) Å], closing a 10-membered cycle, and typical of a β-turn.[1] As already found for similar model dipeptides containing the Pro-Xaa sequence,[17] the ϕ and Ψ dihedral angles (Table 1) indicate a type-II β-turn. Although the alternative type-I β-turn is known to be favored for homochiral dyads in the proteins,[1] and for homochiral model dipeptides in solution,[17a] the participation of the Ala−NH bond in an intermolecular H-bond to Sip-CO [N(A)···O(B) = 2.83 Å and N(B)···O(A) = 2.84 Å] imposes the orientation of the middle amide plane typical of the type-II β-turn (Figure 1).[17]

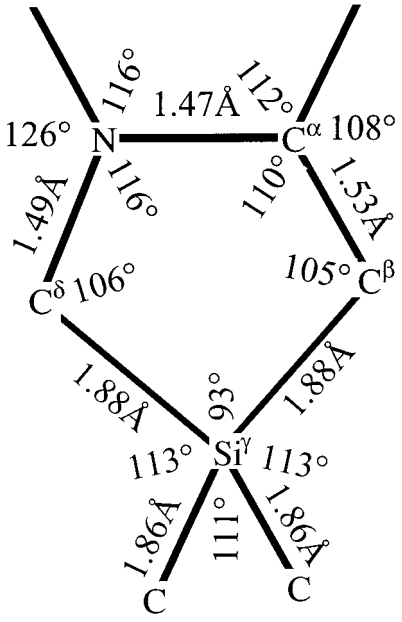

Figure 2. Average dimensions of the Sip ring in the crystal structure of molecules A and B of **5a**.

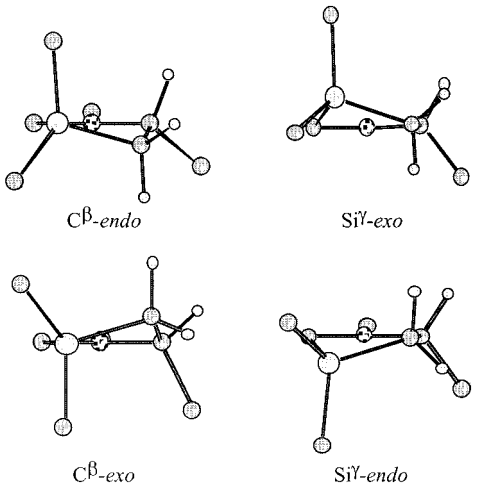

Figure 3. The four possible envelope conformations of the Sip ring with the relative orientation of the vicinal protons in $C^\alpha H$-$C^\beta H_2$. The C^β-endo structure is present in the crystal and molecular structure of **5a**. The C^β-exo structure is adopted by all the investigated model peptides on the basis of the $J_{\alpha\beta}$ vicinal proton coupling constants.

As expected, the Si−C bonds in the five-membered ring of silaproline (Figure 2) are longer by about 0.35 Å than the C−C bonds in proline, and the intracyclic C−Si−C angle is significantly smaller (about 93°) than the analogous C−C−C angle in proline (105°). The five-membered ring of silaproline assumes a skewed conformation of the C^β-endo type[23] (Figure 3) which is uncommon for the Pro-pyrrolidine ring,[24] except in constrained 2,5-dioxopiperazines.[25] Due to the steric hindrance of the tBu group of the Piv moiety, the amide bond N-terminal to Sip deviates significantly out of planarity, as denoted by the C−CO−N−C$^\delta$ (ω'_0) angle (Table 1).

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Table 2. NMR Data for the Analogous Sip- and Pro-Containing Tripeptides[a]

	cis (%)		Ala[1]-NH		Ala[3]-NH		NH(iPr)	
compd	$CDCl_3$	DMSO-d_6	$\Delta\delta^b$	$\Delta\delta/\Delta T^c$	$\Delta\delta^b$	$\Delta\delta/\Delta T^c$	$\Delta\delta^b$	$\Delta\delta/\Delta T^c$
7a	nd[d]	15	**0.75**	−6.1, −5.0	1.16	−5.8, −6.4	1.68	−4.8, nd
7b	7	14	**0.72**	−6.5, −4.4	1.30	−5.1, −5.2	1.45	−4.2, −6.2
9a	nd[d]	15	1.56	−6.7, −5.2	**0.45**	**−3.1**, −6.6	**0.70**	**−1.6**, −4.6
9b	nd[d]	17	1.83	−6.4, −6.7	**0.36**	**−1.7**, −6.3	**0.43**	**−1.5**, −6.4

[a] Bold: solvent-protected NH. [b] NH chemical shift difference ($CDCl_3 \rightarrow$ DMSO-d_6) for the major trans conformer. [c] NH temperature coefficient (ppb/K) in DMSO-d_6 for the major trans (left value) and minor cis (right value) conformers. [d] nd = not detected.

Conformational Analysis in Solution. The compared conformational preferences of the analogous Sip- and Pro-containing derivatives having the *N*-terminal Piv group were investigated by IR absorption and NMR spectroscopies. Two main points were examined: the cis−trans isomer equilibrium of the Pro- or Sip-preceding amide bond and the occurrence of a folded conformation involving an intramolecular H-bond.

The percentage of the cis-conformer for the Sip- or Pro-preceding amide bond has been estimated from the intensities of the NH proton resonances in the NMR spectra in $CDCl_3$ and in DMSO-d_6 (Table 2). In $CDCl_3$, there is little to no detectable cis conformer. In DMSO-d_6, the percentage of cis-isomer for the analogous tripeptides did not differ significantly and was usually measured at about 15%. Consequently, Sip has practically the same preferences as Pro, thus confirming that γ-modification has a weak influence on the proline conformational preferences.[9]

The NH proton shifts $\Delta\delta$ in $CDCl_3$ and DMSO-d_6 and the temperature coefficients $\Delta\delta/\Delta T$ in DMSO-d_6 for the analogous Pro- and Sip-derivatives did not differ significantly, indicating similar conformations (Table 2). Except for the Ala[3]-NH and NH(iPr) amide protons of the trans conformer of peptides **9a** and **9b** which were solvent protected in DMSO-d_6, the $\Delta\delta/\Delta T$ values suggested open conformers, with solvated NHs in DMSO. In $CDCl_3$, only the trans conformers were examined. The Ala[1]-NH proton in **7a** and **7b** and both Ala[3]-NH and NH(iPr) protons in **9a** and **9b** exhibited temperature coefficients characteristic of intramolecular H-bonding, which was confirmed by IR absorption spectroscopy**.** Due to the high $\Delta\delta$ value of 1.03 and 1.48 ppm for **3a** and **5a**, respectively, it follows that the folded conformations in **3a** (γ-turn) and **5a** (β-turn) are the minor conformers.

The N−H stretching frequencies for the analogous Sip- and Pro-containing model peptides are listed in Table 3. They have been attributed on the basis of previous studies on similar model peptides and of the influence of the tBu group on the Piv amide IR contributions.[17,18] Again, the Sip and Pro derivatives exhibit similar profiles and therefore similar conformational properties.

The N−H stretching for **3a** and **5a** in dichloromethane (DCM) is composed of a major high-frequency absorption denoting free NHs, and a minor low-frequency absorption denoting a smaller proportion of H-bonded NHs. In DMSO, a broad absorption at a very low frequency (about 3250 cm^{-1}) was indicative of the solvation of the NHs, and the lack of folded structure.

Table 3. NH Stretching Frequencies for the Analogous Sip- and Pro-Containing Peptides in CH_2Cl_2[a]

compd		Ala[1]-NH	Ala[3]-NH	NHiPr
Piv-Sip-NHiPr	**3a**			3426[m]
				3338[w]
Piv-Pro-NHiPr	**3b**			3426[m]
				3338[w]
Piv-Sip-Ala-NHiPr	**5a**			3426[s]
				3345[bm]
Piv-Pro-Ala-NHiPr	**5b**		3426[s]	
				3345[bm]
Piv-Ala[1]-Sip-Ala[3]-NHiPr	**7a**	3461[vw]	3425[s]	
		3425[s]	**3359**[bw]	**3328**[bvw]
Piv-Ala[1]-Pro-Ala[3]-NHiPr	**7b**	3461[vw]	3425[s]	
		3425[s]	**3355**[bm]	**3330**[bw]
Piv-D-Ala[1]-Sip-Ala[3]-NHiPr	**9a**	3461[s]	3425[w]	
			3342[vs]	
Piv-D-Ala[1]-Pro-Ala[3]-NHiPr	**9b**	3461[s]	3425[m]	
			3342[s]	

[a] Concentration: 0.01 M. The bold characters denote H-bonding. Profile: b broad, m medium, s strong, vs very strong, vw very weak, w weak absorption.

The N−H stretching for **9a** in DCM exhibits an intense low-frequency absorption, in good agreement with the fact that a D-residue often favors a folded structure stabilized by intramolecular H-bonds.[1,7b,17,18,26] The high $\Delta\delta$ value and high stretching frequency for Ala[1]-NH indicated a solvent exposed amide proton. In contrast, the small $\Delta\delta$ shifts for both Ala[3]-NH and NH(iPr) (Table 2) and the strong absorption at 3342 cm^{-1} may be assigned to the simultaneous occurrence in **9a** of Ala[3]-NH to Piv-CO and NH(iPr) to Ala[1]-CO H-bonds, which may suggest consecutive β-turns. Taking into consideration that Sip imposes a ϕ^2 angle of about −60°, and that the $J_{N\alpha}$ vicinal coupling constants of 4.5 Hz for Ala[1] and 8.8 Hz for Ala[3] correspond to a f^1 angle of about 60° and a f^3 angle of about −80°, respectively,[27] a conformation may be adopted possessing type-II′ β-turn folded D-Ala-Sip and βI-folded Sip-Ala sequences (Figure 4). A small population of open conformer is denoted by the weak absorption at about 3425 cm^{-1} that may be due to a small percentage of free Ala[3]-NH and NH(iPr). The occurrence in DMSO of a strong, rather sharp absorption centered at about 3310 cm^{-1}, which differs significantly from the broad absorption around 3250 cm^{-1} for DMSO-solvated NHs, corroborates the retention of the folded conformation in this strong solvating medium.

The homochiral tripeptide **7a** exhibits a much less intense N−H absorption in the low-frequency domain, and therefore essentially adopts an open conformation in CH_2Cl_2. However, both the occurrence of a weak shoulder at 3461 cm^{-1} and the low $\Delta\delta/\Delta T$ value for Ala[1]-NH (Table 2) indicate that this NH

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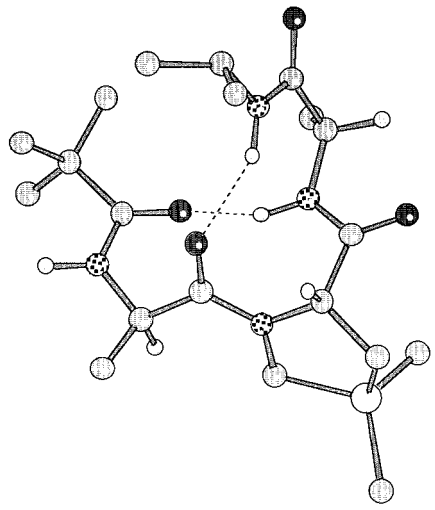

Figure 4. Folded structure of **9a** in $CHCl_3$ showing two consecutive turns of the βII′ and βI-type. Only the NH and C^{α} hydrogens are shown.

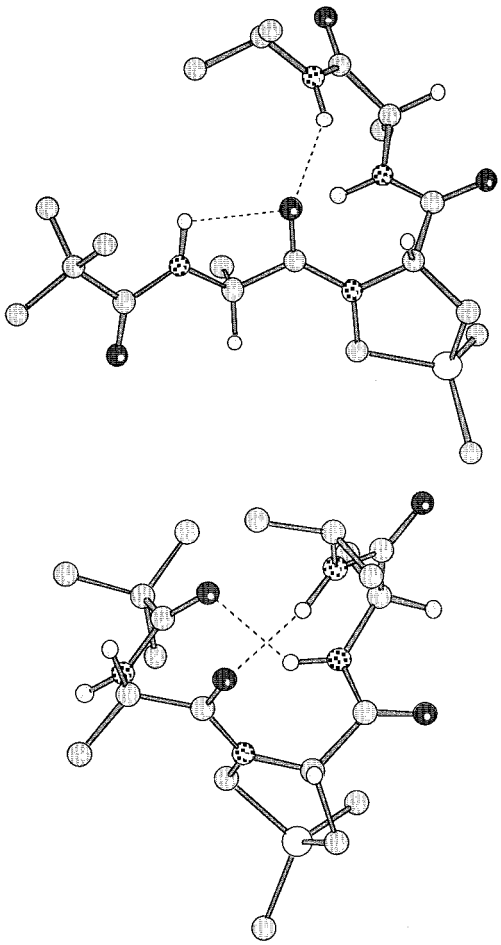

Figure 5. Flexibility of **7a** where the Ala^1 residue is involved in either an extended (major conformer, up) or βI-folded (minor conformer, down) structure, and the C-terminal dipeptide is partly βI-folded. Only the NH and C^{α} hydrogens are shown.

may be engaged in a H-bond that is partly responsible for the strong absorption at 3425 cm^{-1}, a frequency that is typical of an H-bond of the $i \rightarrow i$ type.[20] The low-frequency absorption domain is composed of two broad, overlapping contributions: the major one at 3359 cm^{-1}, and the minor one at 3328 cm^{-1}. On the basis of the medium $\Delta\delta/\Delta T$ value for Ala^3-NH (Table 2), the major contribution suggests the participation of this NH in a type-I β-turn centered at the Ala^1-Sip sequence. The minor contribution may denote the occurrence of a small percentage of type-I β-turn folded Sip-Ala^3 sequence with the H-bonded NH(^{i}Pr). In conclusion, **7a** is a flexible molecule (Figure 5) where the Ala^1 fragment is essentially extended and partly engaged in the type-I β-turn folded Ala^1-Sip sequence. Both structures are also compatible with the type-I β-turn folded Sip-Ala^3 sequence. This flexibility in CH_2Cl_2 is reinforced in DMSO where all the NHs were solvated and gave rise to a broad absorption centered at about 3275 cm^{-1}.

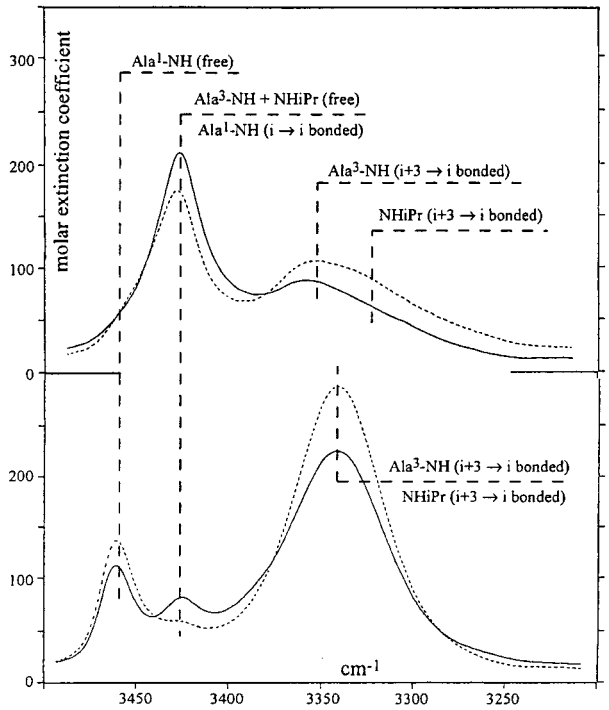

Figure 6. Superimposed NH stretching absorption of the analogous tripeptides **7a**,**b** (up) and **9a**,**b** (down) in CH_2Cl_2 showing the higher propensity to folding for the Pro-tripeptides (dashed line) compared to the Sip-tripeptides (solid line).

The superimposed N−H absorptions for the analogous derivatives **7a** and **7b** on one hand and **9a** and **9b** on the other hand revealed an interesting difference: in both cases the low frequency absorption was significantly more intense for the Pro-containing tripeptides than for the Sip derivatives, while the opposite was observed in the high-frequency absorption domain (Figure 5). Since the low-frequency domain denoted some H-bonding, the percentage of folded conformations was a little higher for Pro- than for Sip-containing sequences.

Another difference concerns the rather rigid conformation of the Sip ring at variance with the flexibility of the Pro ring. Proline may adopt various continuously interchanging conformations[23] depending on the conformation of the backbone, and this fact was denoted by different sets of the two vicinal coupling constants $J_{\alpha\beta}$ of small, medium, or high intensity in the $C^{\alpha}H$-$C^{\beta}H_2$ fragment.[24] In contrast, the Sip $C^{\alpha}H$-$C^{\beta}H_2$ fragment gave rise to a single set of one small (1.8−2.5 Hz) and one high (9.9−10.6 Hz) $J_{\alpha\beta}$ for all the molecules investigated in the present work. This pattern was not consistent with the Sip C^{β}-endo conformation found in the crystal structure of **5a**, which would give two high $J_{\alpha\beta}$ values, but with the Si^{γ}-endo or C^{β}-exo conformation (Figure 4). The latter ring puckering was the

Table 4. Binding of Neurotensin Analogue[a]

	IC_{50} (nM)		IC_{50} hNTR1/
	hNTR1	hNTR2	IC_{50} hNTR2
neurotensin (NT)	0.16	1.10	0.14
H-Lys-Lys-Pro-Tyr-Ile-Leu-OH (NT(8−13))	0.08	0.47	0.17
H-Lys-Lys-**Sip**-Tyr-Ile-Leu-OH (**10**)	17.5	5.0	3.50
H-Lys-Lys-Pro-Tyr-**TMS**-**Ala**-Leu-OH (**11**)	146.0	133.0	1.10
H-Lys-Lys-**Sip**-Tyr-**TMS**-**Ala**-Leu-OH (**12**)	920.0	238.0	3.87

[a] TMS-Ala: α-trimethylsilylalanine.

only one compatible with the folded structure of **9a** in solution in which the type-II′ β-turn imposes a rather high Sip-ϕ angle.[26,28] Because the same NMR pattern was observed in all cases, we believe that the C^{β}-exo puckering of the Sip ring is favored in the absence of intermolecular packing forces.

Bioactivity and Biostability of [Sip[10]]NT(8−13). The affinity of NT(8−13) analogues possessing Sip and Pro residues was next compared at the hNTR1 and hNTR2 NT receptors (Table 4).

First, we observed that [Sip[10]]NT(8−13) maintained activity under conditions in which the natural peptide is rapidly degradated. Indeed, NT had to be injected along with enzyme inhibitors to express its activity.[12] On the other hand, [Sip[10]]-NT(8−13) retained activity in the absence of enzyme inhibitors, suggesting that Sip effectively reduced the biodegradation of NT analogues. The second result concerned the binding affinity of Sip-NT with NT receptors. Replacing proline with silaproline resulted in a 100-fold decreased affinity for hNTR1, and 5-fold decreased affinity for hNTR2, compared with the natural neurotensin. Binding of the same magnitude may reflect the retention of the active conformation, which is consistent with our NMR findings on the model peptides. It is worth noting that replacing isoleucine by the unnatural silylated amino acid TMS-Ala led to practically inactive compounds (Table 4).

Conclusion

Boc-γ-(dimethylsila)-proline (Boc-Sip-OH) is easily obtained in both enantiomeric forms from Schöllkopf's bislactim ether, and can be introduced in a peptide sequence by using the conventional methods. In model di- and tripeptides, silaproline exhibits similar conformational properties as proline. Moreover, the presence of the dimethylsilyl group confers on silaproline a high lipophilicity and improved resistance to biodegradation. This point was verified by the synthesis of an analogue of the C-terminal segment NT(8−13) of neurotensin which required injection with a cocktail of protease inhibitors to be active, whereas [Sip[10]]NT(8−13) was active by itself. The retention of receptor affinity for [Sip[10]]NT(8−13) is encouraging for the use of silaproline as a proline surrogate.

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Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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