Amino Acids (2012) 43:649–655
DOI 10.1007/s00726-011-1112-y

ORIGINAL ARTICLE

Resolution of protected silaproline for a gram scale preparation

C. Martin · N. Vanthuyne · H. Miramon · J. Martinez · F. Cavelier

Received: 13 May 2011 / Accepted: 26 September 2011 / Published online: 15 October 2011

Abstract Silaproline is an analogue of proline, which exhibits similar conformational properties. Moreover, the presence of dimethylsilyl group confers to silaproline a higher lipophilicity as well as an improved resistance to biodegradation. This report describes the comparison of two routes to obtain Fmoc-(L) Sip-OH on the gram scale using chiral HPLC resolution.

Keywords Silaproline · Chiral HPLC resolution · Absolute configuration · Enantiomers

Introduction

Proline plays an essential role in the three-dimensional structure of peptides and proteins (Rizo and Gierasch 1992), especially for inducing reverse turns. In our laboratory, an original proline analogue has been synthesized: the 4,4-dimethylsilaproline, denoted silaproline (Vivet et al. 2000a) (Sip) (Fig. 1). Introduction of silylated amino acids in peptides increases lipophilicity (Cavelier et al. 2002, 2008; Mortensen et al. 2009; Bains and Tacke 2003; Marchand et al. 2008). The octanol–water partition coefficient of Fmoc-Sip-OH has been experimentally determined to be 14 times greater than that of Fmoc-Pro-OH (Cavelier et al. 2002). Increased lipophilicity may therefore facilitate membrane crossing. Sip used as a source of amphipaticity led to a 20-fold increase in the cellular up-take of a Pro-rich cell penetrating peptide (Pujals et al. 2006). Reduced sensitivity to enzymatic degradation may also arise from substitution of Pro by Sip in peptides. Moreover, the similarity of the Sip and Pro rings results in similar conformational properties for analogous Sip- and Pro-containing peptides (Cavelier et al. 2002, 2004, 2006; Vivet et al. 2000b).

Silaproline was synthesized successfully via asymmetric synthesis by the Schöllkopf method (Vivet et al. 2000a). However, poor yields, instability of the intermediates and difficulties in purification prevented the scale-up of this silaproline synthesis. Thus, a racemic method was carried out for the gram scale synthesis of enantiomerically pure Sip, requiring resolution of the enantiomers. We chose to perform separation by chiral high performance liquid chromatography (HPLC).

This report describes the synthesis of different protected versions of racemic silaproline, and their conditions of separation for gram scale synthesis of enantiomerically pure-protected silaproline derivatives.

This study shows that the best synthetic route depends on the needed N-protection. Boc- and Z- protected silaproline were synthesized as racemic version and then separated by preparative chiral HPLC. Fmoc-Sip-OH resulted from protection change of previously obtained enantiopure Boc-Sip-OtBu.

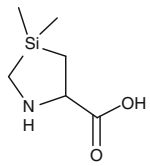
Materials and methods

All reactions involving air-sensitive reagents were performed under nitrogen or argon. Solvents and reagents were purchased from Aldrich and Fluka. THF was freshly distilled from benzophenone/sodium prior to use. Merck silica gel 60 F-254 plates were used for TLC.

C. Martin · H. Miramon · J. Martinez · F. Cavelier (✉)
IBMM, UMR-CNRS-5247, Universités Montpellier I and II, Place Eugène Bataillon, Montpellier 34095, France
e-mail: florine@univ-montp2.fr

N. Vanthuyne
UPCAM, ISm2, UMR-6263, Université Paul Cézanne, Av. Escadrille Normandie, 13397 Marseille, France

Fig. 1 Silaproline (Sip) structure



Purifications were performed with column chromatographies using silica gel (Merck 60, 230–400 mesh) or with a Biotage instrument Isolera 4 using SNAP KP-SIL flash cartridges. Proton nuclear magnetic resonance (Rizo and Gierasch 1992) ^{1}H-NMR and carbon nuclear magnetic resonance (Roussel et al. 2004) ^{13}C-NMR spectra were recorded on a Bruker spectrometer advance 300 at 300 and 75 MHz, respectively. All chemical shifts were recorded as values (ppm) relative to internal tetramethylsilane when $CDCl_3$ was used as solvent. Low resolution electrospray ionization (ESI) mass spectra were recorded on a micromass platform electrospray mass spectrometer. Spectra were recorded in the positive mode (ESI^+). HRMS were recorded in positive mode using NBA (3-nitrobenzyl alcohol) or GT (glycerol/thioglycerol) as matrix. Optical rotation values were measured on a Perkin-Elmer 341 (20°C, sodium ray).

The analytical chiral HPLC experiments were performed on a unit composed of a Merck D-7000 system manager, Merck-Lachrom L-7100 pump, Merck-Lachrom L-7360 oven, Merck-Lachrom L-7400 UV-detector, and on-line Jasco OR-1590 polarimeter. Hexane, ethanol and isopropanol, HPLC grade, were degassed and filtered on a 0.45 µm membrane before use. Chiral columns (250×4.6 mm) tested are: Chiralcel OD-H, OJ, OC, OB-H, Chiralpak AS-H, IC and AD-H from Chiral Technologies Europe (Illkirch, France), Whelk O1 (*S*,*S*) and Ulmo (*S*,*S*) from Regis Technologies (Morton Grove, USA), Sumichiral OA-2500 from Sumika (Japan) and Lux-Cellulose-2 from Phenomenex. Semi-preparative separations were performed on a Knauer unit with pump, UV detector and a software to collect the different fractions.

Bis-(iodomethyl)dimethylsilane **(3)**

Bis-(chloromethyl)dimethylsilane (6.06 mL, 41.82 mmol) was dissolved in dry acetone (227 mL), sodium iodide (25.07 g, 167.28 mmol) was added and the mixture was refluxed for 3 h. After filtration, the solvent was removed by distillation. After addition of Et_2O to the remaining oil, the NaCl was filtered off. Et_2O was evaporated in vacuo and the residue was distilled to give 13.02 g of **3** (91%), oil—$R_f = 0.9$ (cyclohexane/ethyl acetate 8/2)—^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 0.35 (s, 6 H, $Si(CH_3)_2$), 2.15 (s, 4 H, $Si(CH_2I)_2$).

Typical procedure for the preparation of protected Sip from protected Gly

Boc-Gly-OtBu (2 g, 8.66 mmol) or *Z*-Gly-OtBu (2.3 g, 8.66 mmol) was dissolved in anhydrous THF (86 mL) under argon atmosphere and cooled to −10°C. 60% sodium hydride in mineral oil (1 g, 26 mmol) was added in small portions under vibro-mixing and allowed to warm up at room temperature. After 20 min, the *bis*-(iodomethyl) dimethylsilane (5.9 g, 17.3 mmol) was added and the reaction mixture was stirred for 72 h. The mixture was cooled to 0°C before adding 100 mL of water to neutralize excess of hydride. After THF evaporation, the remaining aqueous phase was extracted with AcOEt. Then the organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated. After column chromatography (cyclohexane/ethyl acetate 9/1) the pure product was obtained, 85% for Boc and 80% for Z protection.

Characterisation of Boc-(D/L)Sip-OtBu **(4)**

Solid—$R_f = 0.55$ (cyclohexane/ethyl acetate 8/2). Two conf.: ^{1}H-NMR ($CDCl_3$, 300 MHz): $\delta = 0.20$ (s, 6 H, $Si(CH_3)_2$), 1.00–1.30 (m, 2H, $SiCH_2CH_\alpha$), 1.45 (2 s, 18 H, Boc and OtBu), 2.70–2.90 (m, 2 H, $SiCH_2N$), 4.52 (d, 0.65 H, J =10 Hz, $CH_{\alpha'}$), 4.65 (d, 0.35 H, J = 10 Hz, $CH_{\alpha'}$); ^{13}C-NMR ($CDCl_3$, 75 MHz): $\delta = -1.58$ (s, $Si(CH_3)_2$), 16.85 and 17.79 (2 s, $SiCH_2CH$), 28.54 (s, $C(CH_3)_3$), 34,98 and 35.29 (2 s, $SiCH_2N$), 61.20 and 61.06 (2 s, CH_α), 81.51 (s, $C(CH_3)_3$), 156.77 and 157.43 (2 s, *C*=O urethane), 174.22 (s, *C*=O ester). HRMS (ESI^+): *m/z* calcd for $C_{15}H_{30}NO_4Si$ $(M+H)^+$ 316.1944, found 316.1935. Analytical chiral HPLC (1 mL/min, UV 220 nm, polarimeter): Chiralpak AD-H (hexane/2-PrOH 95/5,): R_t(D,+) = 4.47, R_t(L,−) = 7.04, k(D) = 0.44, k(L) = 1.27, $\alpha = 2.90$ and $R_s = 7.15$ or on Chiralpak IC (hexane/2-PrOH 9/1): R_t(L,−) = 6.19, R_t(D,+) = 9.01, k(L) = 1.00, k(D) = 1.91, $\alpha = 1.91$ and $R_s = 5.77$. For the (L)-enantiomer, $[\alpha]_D^{20} = -34$ (c 1, $CHCl_3$).

Characterisation of Z-(D/L)Sip-OtBu **(5)**

Oil—$R_f = 0.6$ (cyclohexane/ethyl acetate 9/1). Two conf.: ^{1}H-NMR ($CDCl_3$, 300 MHz): $\delta = 0.20$ (s, 6H, $Si(CH_3)_2$), 1.10–1.35 (m, 2H, $SiCH_2CH$), 1.45 (s, 9H, OtBu), 2.80–3.10 (m, 2 H, $SiCH_2N$), 4.65–4.85 (m, 1 H, $CH\alpha$), 5.05–5.25 (m, 2 H, CH_2 benzyl), 7.30–7.45 (m, 5 H, *CH*arom); ^{13}C-NMR ($CDCl_3$, 75 MHz): $\delta = -1.64$ (s, $Si(CH_3)_2$), 16.90 and 17.77 (2 s, $SiCH_2CH$), 28.59 (s, $C(CH_3)_3$), 34,95 and 35.62 (2 s, $SiCH_2N$), 60.95 and 61.41 (2 s, CH_α), 67.70 (s, CH_2 benzyl), 81.81 (s, $C(CH_3)_3$), 128.47, 129.09, 137.54, 137.80 (s, *C*H arom), 157.20, 157.76 (2 s, *C*=O urethane), 173.78 (s, *C*=O ester). HRMS

(ESI$^+$): *m/z* calcd for $C_{18}H_{28}NO_4Si$ $(M+H)^+$ 350.1788, found 350.1786. Analytical chiral HPLC (1 mL/min, UV 254 nm, polarimeter) : Chiralpak AD-H (hexane/2-PrOH 9/1): R_t(D,+) = 6.17, R_t(L,−) = 8.72, k(D) = 0.99, k(L) = 1.82, $\alpha = 1.83$ and $R_s = 5.08$, on Chiralpak IC (hexane/2-PrOH 8/2): R_t(L,−) = 7.66, R_t(D,+) = 11.82, k(L) = 1.47, k(D) = 2.81, $\alpha = 1.91$ and $R_s = 6.45$, on Chiralpak AS-H (hexane/2-PrOH 9/1): R_t(L,−) = 5.01, R_t(D,+) = 5.63, k(L) = 0.62, k(D) = 0.82, $\alpha = 1.33$ and $R_s = 1.54$, on Lux-Cellulose-2 (hexane/2-PrOH 9/1): R_t(L,−) = 7.81, R_t(D,+) = 17.57, k(L) = 1.53, k(D) = 4.67, $\alpha = 3.05$ and $R_s = 9.70$ and on Chiralcel OD-H (hexane/2-PrOH 9/1): R_t(L,−) = 4.75, R_t(D,+) = 6.68, k(L) = 0.53, k(D) = 1.16, $\alpha = 2.17$ and $R_s = 4.37$. For the (L)-enantiomer, $[\alpha]_D^{20} = -35$ (c 1, $CHCl_3$).

Synthesis of Fmoc-Sip-OH **(7)** from H-Sip-OH **(6)**

Boc-(D/L)Sip-OtBu (500 mg, 1.59 mmol) was dissolved in 6 N HCl to pH 2–3 at room temperature, to afford **6** with quantitative yield.

After concentration in vacuo H-Sip-OH (**6**) (107 mg, 0.67 mmol) was dissolved in 9% sodium carbonate solution (1.5 mL) and cooled in an ice-bath, a solution of Fmoc-Cl (207.8 mg, 0.80 mmol) in dioxane (3 mL) was added at 0°C under stirring, which was continued at room temperature for 24 h. The mixture was diluted with water, and extracted with ether and ethyl acetate. The remaining aqueous phase was cooled and acidified with concentrated hydrochloric acid to pH 2. The aqueous phase was extracted with ethyl acetate. The organic phase was washed with saturated sodium chloride solution and water, then dried over anhydrous $MgSO_4$, filtered and concentrated to afford the Fmoc-(D/L)Sip-OH (213 mg, 83%).

Oil—$R_f = 0.5$ ($CHCl_3$/MeOH/AcOH: 120/10/5). Two conf.: ^{1}H-NMR ($CDCl_3$, 300 MHz): $\delta = 0.20$ and 0.30 (2 s, 6 H, Si(CH_3)$_2$), 0.9–1.40 (m, 2 H, SiCH_2CH), 2.80–3.10 (m, 2 H, SiCH_2N), 4.20–4.60 (m, 3 H, CH_2CH fluorene), 473–4.85 (2dd, $J = 3$ Hz, $J = 10$ Hz, 1 H, CH_α), 7.20–7.80 (m, 8 H, arom H), 10.50 (s, 1 H, CO_2H); ^{13}C-NMR ($CDCl_3$, 75 MHz): $\delta = -2.35$ and −2.05 (2 s, Si(CH_3)$_2$), 16.22 and 17.55 (2 s, SiCH_2CH), 34.68 and 35.18 (2 s, SiCH_2N), 47.67 (s, $CHCH_2$ fluorene), 59.43 and 60.23 (2 s, CH_α), 68.28 (s, $CHCH_2$ fluorene), 120.39, 125.40, 125.60, 127;47, 128.05, 141.67, 144.29, 144.51 (8 s, arom *C*), 156.87 and 157.91 (2 s, *C*=O urethane), 179.10 and 179.80 (2 s, *C*=O acid). Analytical chiral HPLC (1 mL/min, UV 254 nm, polarimeter) : Chiralpak IC (hexane/ethanol/TFA 9/1/0.1): R_t(L,−) = 10.61, R_t(D,+) = 13.44, k(L) = 2.48, k(D) = 3.41, $\alpha = 1.37$ and $R_s = 3.35$. For the (L)-enantiomer, $[\alpha]_D^{20} = -45$ (c 1, $CHCl_3$).

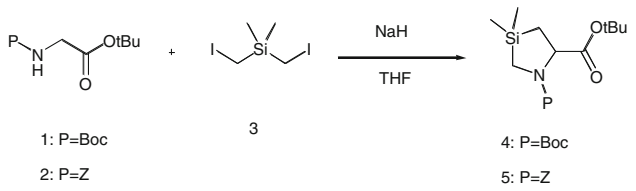
Synthesis of Fmoc-Sip-OtBu **(9)** from H-Sip-OtBu **(8)**

A solution of HCl/dioxane (4 mL, 4 M) in a round-bottom flask equipped with a magnetic stir-bar was cooled by an ice-water bath under argon. Boc-(D/L)Sip-OtBu (63 mg, 0.2 mmol) was added in one portion under stirring. The ice-bath was removed and the mixture was kept stirred. After 30 min, the reaction mixture was condensed by rotary evaporation in vacuo at room temperature. The residue H-Sip-OH **(8)** was then dissolved in dioxane. The apparent pH of the mixture was maintained at 8–9 during the reaction by adding triethylamine when necessary. A solution of Fmoc-Cl (68 mg, 0.26 mmol) in dioxane was added. The reaction mixture was stirred for 24 h. The mixture was concentrated in vacuo, and the remaining residue was dissolved in ethyl acetate. The organic layer was extracted three times with a saturated solution of potassium hydrogenocarbonate, and then with water. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated to afford the Fmoc-(D/L)Sip-OtBu (80%).

Oil—$R_f = 0.42$ (cyclohexane/ethyl acetate 8/2). Two conf.: ^{1}H-NMR ($CDCl_3$, 300 MHz): $\delta = 0.18$ (s, 6 H, Si(CH_3)$_2$), 1.04–1.28 (m, 2 H, SiCH_2CH), 1.38 (s, 9 H, OtBu), 2.74–2.95 (m, 2 H, SiCH_2N), 4.10–4.35 (m, 3 H, CH_2CH fluorene), 4.66 (dd, $J = 3$ Hz, $J = 10$ Hz, 1 H, CH_α), 7.18–7.70 (m, 8 H, *H* arom); ^{13}C-NMR ($CDCl_3$, 75 MHz): $\delta = -3.59$ and −3.50 (2 s, Si(CH_3)$_2$), 14.93 and 16.05 (2 s, SiCH_2CH), 26.76 (s, OtBu), 33.05 and 33.66 (2 s, SiCH_2N), 46.07 (1 s,$CHCH_2$ fluorene), 58.98 and 59.38 (2 s, CH_α), 66.29 and 66.60 (2 s, $CHCH_2$ fluorene), 79.90 and 80.11 (2 s, $C(CH_3)_3$), 118.66, 123.92, 125.77, 140.03, 142.87 (8 s, *C* arom), 155.24 and 155.76 (2 s, *C*=O urethane), 171.72 and 171.79 (2 s, *C*=O ester). HRMS (ESI$^+$): *m/z* calcd for $C_{25}H_{32}NO_4Si$ $(M+H)^+$ 438.2101, found 438.2097. Analytical chiral HPLC (1 mL/min, UV 254 nm, polarimeter) , Chiralpak AD-H (hexane/2-PrOH 9/1), R_t(D,+) = 11.34, R_t(L,−) = 12.88, k(D) = 2.78, k(L) = 3.29, $\alpha = 1.19$ and $R_s = 1.32$, on Lux-Cellulose-2 (hexane/2-PrOH 9/1): R_t(L,−) = 11.24, R_t(D,+) = 14.93, k(L) = 2.75, k(D) = 3.98, $\alpha = 1.45$ and $R_s = 3.25$ or on Chiralpak IC (hexane/2-PrOH 7/3): R_t(L,−) = 6.65, R_t(D,+) = 9.85, k(L) = 1.22, k (D) = 2.28, $\alpha = 1.87$ and $R_s = 5.63$.

Characterisation of **10**

^{1}H-NMR (D_2O, 300 MHz): $\delta = 0.20$ (s, 6H, Si(CH_3)$_2$), 1.18 (d, $J = 6$ Hz, 2H, SiCH_2CH), 2.65 (s, 3 H, CH_3N), 3.60 (dd, $J = 3$ Hz, $J = 6$ Hz 1 H, $CH\alpha$), MS–ESI$^+$ (CAD): 178 $[M+H]^+$, 160 $[M-(H_2O)+H]^+$, 129 $[M-(CH_3NH_2)+H]^+$.



Scheme 1 Synthesis of protected racemic silaproline (Z and Boc)

Results and discussion

Different protected versions of racemic silaproline were prepared as shown in Scheme 1. This synthesis was performed starting from protected glycine and *bis*-(iodomethyl)dimethylsilane, in anhydrous THF under basic conditions (NaH).

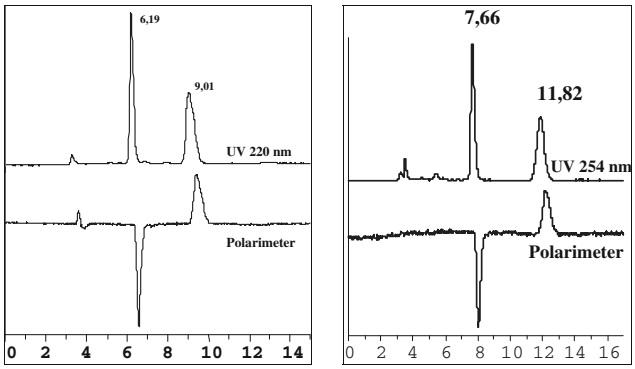
Z-(D/L)Sip-OtBu and Boc-(D/L)Sip-OtBu were easily synthesized, these protecting groups being stable under basic conditions. However Fmoc-Sip-OtBu could not be obtained directly from Fmoc-Gly-OtBu; successive deprotection and protection steps were necessary.

Comparison of Z-(D/L)Sip-OtBu and Boc-(D/L)-Sip-OtBu synthese

Z-(D/L)Sip-OtBu offers the possibility of orthogonality of protecting groups. However, the starting material Z-(D/L)Gly-OtBu is not commercially available. Furthermore, the reaction yield was found to be 71% only due to some difficulties to purify the product (presence of by-products). On the other hand, Boc(D/L)-Sip-OtBu was synthesized with a good yield (85%) in one step from commercially available Boc-(D/L)Gly-OtBu.

Resolution of Z-(D/L)Sip-OtBu and Boc-(D/L)Sip-OtBu

We compared the separation of the enantiomers of two versions of racemic silaproline Boc-(D/L)Sip-OtBu (**4**) and Z-(D/L)Sip-OtBu (**5**). Attempts to separate **4** and **5** on chiral stationary phases were found to be successful. Compound **4** was dissolved in a mixture of 90/10 hexane/isopropanol and then injected into different columns. Boc-Sip-OtBu was detected at 220 nm and also with a polarimeter. Among 11 chiral columns tested, only 2 of them showed a good separation of the enantiomers with a return to the baseline ($R_s > 1.25$). The separation obtained with the Chiralpak IC column was the most advantageous ($R_s = 5.77$). It was transposed in semi-preparative conditions with Chiralpak IC column (250×10 mm). Chromatograms (Fig. 2a) showed the separation of enantiomers obtained under these conditions. Compound **5** was dissolved in a mixture of 90/10 hexane/isopropanol, and then injected into different columns. Z-Sip-OtBu was detected at 254 nm and also with a polarimeter. From the eleven



(a) Boc-(D/L) Sip-OtBu **(b)** Z-(D/L) Sip-OtBu

Fig. 2 Separation of enantiomers of Boc-(D/L)Sip-OtBu **(a)** and Z-(D/L)Sip-OtBu **(b)**

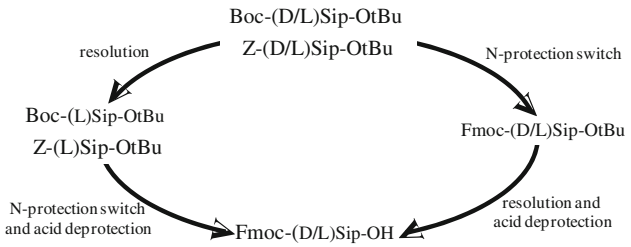
chiral columns tested, 5 successfully performed the separation of the enantiomers ($R_s > 1.25$). The Chiralpak IC column was used for the semi-preparative separation with hexane/isopropanol (80/20) as eluent. The chromatograms (Fig. 2b) showed the separation of the two enantiomers obtained under these conditions.

Using automatic successive injections on a semi-preparative Chiralpak IC column (250 × 10 mm), 50 mg of racemic mixture was injected every 12 min. After about 40 h, 5 g of each enantiomer of **4** and **5** was recovered with an ee >99%.

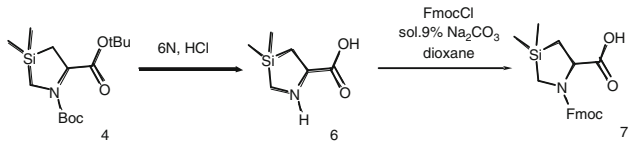
Synthesis of Fmoc-Sip-OH

In solid phase peptide synthesis, amino acids are commonly used as Fmoc protected version. Fmoc-Sip-OH (**7**) was obtained from compounds **4** and **5** previously synthesized. Two strategies were considered as depicted in Scheme 2. The best method was to introduce the Fmoc group on enantiomerically pure silaproline resulting from a resolution of the Boc version. Indeed, this pathway saved one step, and prevented the loss of the D amino acid already bearing the expensive Fmoc protection.

The first method was to synthesize Fmoc-Sip-OH (**7**) and then to separate the enantiomers. The compound **7** could be obtained from Boc-(D/L)Sip-OtBu, by total deprotection with 6 N HCl, giving **6**. Then, H-(D/L)Sip-OH



Scheme 2 Strategies to obtain Fmoc-(L)Sip-OH



Scheme 3 Synthesis of Fmoc-(D/L)Sip-OH

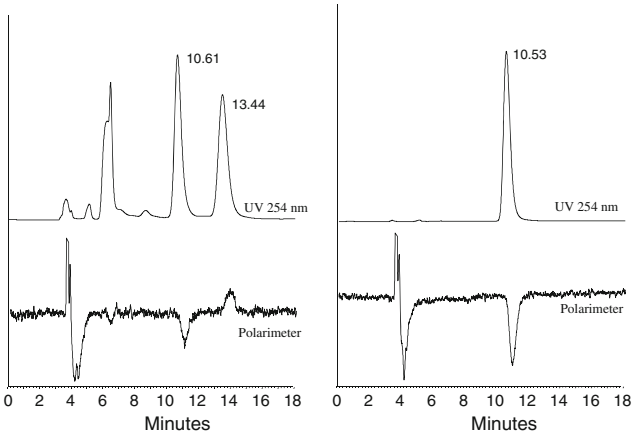


Fig. 3 Determination of absolute configuration of Fmoc-Sip-OH enantiomers

was protected with Fmoc-Cl (Lapatsanis et al. 1983) in dioxane in the presence of 9% Na_2CO_3 solution to afford Fmoc-(D/L)Sip-OH (**7**) (Scheme 3).

The absolute configuration of each enantiomer (Roussel et al. 2004) could be assigned by comparison of chromatograms (Fig. 3).

The Fmoc-(D/L)Sip-OH and Fmoc-(L)Sip-OH resulting from enantioselective synthesis using the Schöllkopf's method were injected into a Chiralpak IC column and elution was performed with hexane/ethanol/TFA (90/10/0.1). The chromatogram b) showed that Fmoc-(L)Sip-OH was enantiomerically pure with a negative sign on the polarimeter. This was confirmed by the value of its specific optical rotation $[\alpha]_D^{20} = -45$ (c = 1, $CHCl_3$). Thus, for the racemic mixture of Fmoc-Sip-OH (a), the first eluted enantiomer was Fmoc-(L)Sip-OH with a negative sign, the second one was Fmoc-(D)Sip-OH with a positive sign. The same elution order, (L) first eluted, was found for Fmoc-(D/L)-Pro-OH under the same analytical conditions.

Enantiomers of **7** were successfully separated under analytical conditions, on a Chiralpak IC column, with hexane/ethanol (90/10)+TFA as mobile phase. However, the use of TFA in the mobile phase did not allow the scale up to semi-preparative columns, because it will damage the column resulting in a loss of enantioselectivity.

Therefore another strategy had to be envisaged. Fmoc-Sip-OtBu (**9**) was synthesized using Boc-Sip-OtBu (**4**) or Z-Sip-OtBu (**5**) as starting material. H-Sip-OtBu (**8**) was obtained in two different ways. Either Boc-Sip-OtBu was selectively deprotected (Han et al. 2001) on the amino group by anhydrous HCl/dioxane 4 N for 30 min or the Z group was removed (Scheme 4).

From Z protected Sip, the deprotection of the amine fonction was carried out under classical conditions such as hydrogenolysis. The tested conditions are gathered in Table 1.

Whatever the catalyst and the solvent, the reaction did not go to completion. Moreover, an example of serendipity was observed in ethanol, since an unexpected compound (**10**) was formed by the ring opening of silaproline, probably caused by traces of water in the solvent. Indeed, when hydrogenolysis was carried out in isopropanol (entry 7), no ring opening was observed, but when the reaction was performed in isopropanol containing 20% of water (entry 9) or in ethanol with 1% water (entry 11), ring opening was detected. We assumed that coordination of the silicon atom to the close nitrogen and the oxygen of water caused Si–C bond activation, which then can be hydrogenolysed under such conditions (Castillo and Tilley 2001). The resulting Si–H bond can further be oxidised by water in the presence of catalyst (Chauhan et al. 2009), giving rise to the silanol **10** (Fig. 4) (see characterisation in experimental part).

Only hydrogenolysis in acetic acid (entry 10) afforded the expected H-Sip-OtBu without ring opening. However, removal of acetic acid proved to be difficult and successive handling of this compound was not possible.

Scheme 4 Synthesis of Fmoc-Sip-OH by protection exchanges

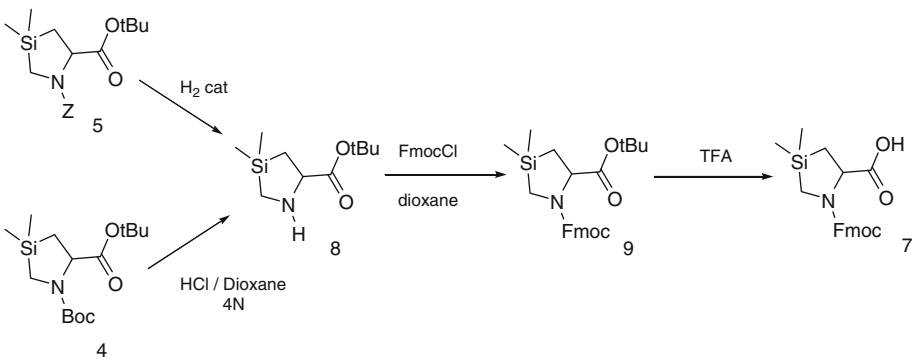
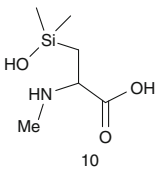


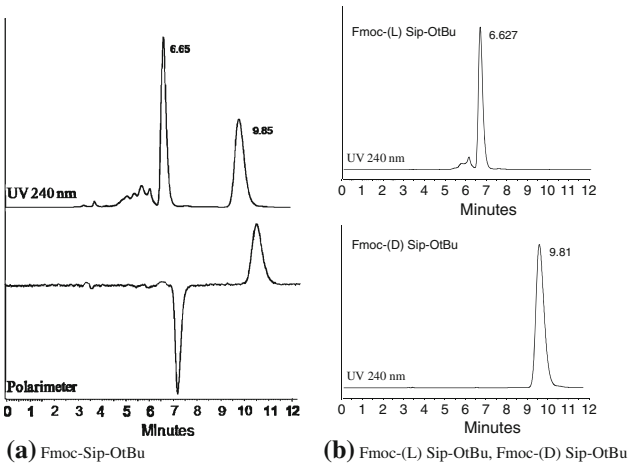
Table 1 Selective Z removal of Z-Sip-OtBu

Entry	Conditions	Product
1	H_2, THF, Pd/C	No reaction
2	H_2, AcOEt, acetic acid, Pd/C	No reaction
3	H_2, AcOEt, acetic acid, $Pd(OH)_2/C$	Incomplete reaction[a]
4	H_2, EtOH, Pd/C	Ring opening
5	H_2, EtOH, $Pd(OH)_2/C$	Ring opening
6	H_2, MeOH, Pd/C	N-methylation
7	H_2, isopropanol, Pd/C	Incomplete reaction[a]
8	H_2, isopropanol, acetic acid, Pd/C	Incomplete reaction[a]
9	H_2, isopropanol, 20% water, Pd/C	Ring opening
10	H_2, acetic acid, Pd/C	Compound 8
11	H_2, EtOH, 1% water, Pd/C	Ring opening

[a] Expected product with unreacted starting material

Fig. 4 Stucture of the undesired compound **10**





(a) Fmoc-Sip-OtBu **(b)** Fmoc-(L) Sip-OtBu, Fmoc-(D) Sip-OtBu

Fig. 5 Separation of two enantiomers of Fmoc-Sip-OtBu (**a**) and Chromatograms of Fmoc-(L)Sip-OtBu and Fmoc-(D)Sip-OtBu (**b**)

Then, H-Sip-OtBu was protected using Fmoc-Cl. Fmoc-Sip-OtBu (**9**) enantiomers were separated with a semi-preparative Chiralpak IC column using a mixture of hexane/isopropanol (70/30) as mobile phase. Two hundred milligrams of each enantiomers of **9** were recovered with an ee >99% (Fig. 5).

After separation, deprotection of the *tert*-butyl ester of Fmoc-(L)Sip-OtBu was performed with TFA in dichloromethane in a quantitative yield (100%) within 30 min. Both Fmoc-(L)Sip-OH and Fmoc-(D)Sip-OH were isolated.

In spite of Z-Sip-OtBu presenting orthogonality of its protecting groups, the difficulties observed during the synthesis and the deprotection of Z group forced us to investigate a different strategy. Boc-Sip-OtBu possesses two acid sensitive groups. The experiments proved nonetheless that selective deprotection could be performed under controlled reaction times and optimized conditions to avoid, after *N*-protection with a Fmoc group the expected product. This route afforded Fmoc-(D/L)Sip-OtBu. Then the racemic mixture of **9** was separated to afford Fmoc-(L)Sip-OH after deprotection. Finally, another route consisted in resolution of Boc-Sip-OtBu, the next part of synthesis was performed using Boc-(L)Sip-OtBu to afford Fmoc-(L)Sip-OH, after total deprotection, followed by Fmoc introduction.

Conclusion

HPLC chiral resolution allowed overcoming the difficulties of enantioselective synthesis. Several grams of each protected version of silaproline have been obtained. When the synthesis was immediate for Boc and Z protections, an exchange of protection was necessary for the Fmoc version. Since separation on preparative column was possible only with the ester form of silaproline. The best method to obtain enantiomerically pure Fmoc-(L)(-)Sip-OH is the chromatographic resolution of racemic Boc-(D/L)Sip-OtBu followed by partial deprotection of Boc-(L)(-)Sip-OtBu to form H-(L)Sip-OtBu. Introduction of the Fmoc group produced Fmoc-(L)(-)Sip-OtBu, the final step was the liberation of the acid function.

References

Bains W, Tacke R (2003) Silicon chemistry as a novel source of chemical diversity in drug design. Curr Opin Drug Discov Dev 6:526–543

Castillo I, Tilley TD (2001) Organolutetium complexes in sigma-bond metathesis reactions involving silicon. Catalysts for the hydrogenolysis of Si-C bonds. Organometallics 20:5598–5605

Cavelier F, Vivet B, Martinez J, Aubry A, Didierjean C, Vicherat A, Marraud M (2002) Influence of silaproline on peptide conformation and bioactivity. J Am Chem Soc 124:2917–2923

Cavelier F, Marchand D, Martinez J, Sagan S (2004) Biological activity of silylated amino acid containing substance P analogues. J Pept Res 63:290–296

Cavelier F, Marchand D, Mbassi P, Martinez J, Marraud M (2006) Conformational studies of proline-, thiaproline- and dimethylsilaproline-containing diketopiperazines. J Pept Sci 12:621–625

Cavelier F, Marchand D, Martinez J (2008) alpha,alpha '-disubstituted amino acids with silylated side chains as lipophilic building blocks for the synthesis of peptaibol analogues. Chem Biodivers 5:1279–1287

Chauhan BPS, Sarkar A, Chauhan M, Roka A (2009) Water as green oxidant: a highly selective conversion of organosilanes to silanols with water. Appl Organomet Chem 23:385–390

Han G, Tamaki M, Hruby V (2001) Fast, efficient and selective deprotection of the tert-butoxycarbonyl (Boc) group using HCl/dioxane (4 M). J Pept Res 58:338–341

Lapatsanis L, Milias G, Froussios K, Kolovos M (1983) Synthesis of N-2,2,2-(trichloroethoxycarbonyl)-L-amino acids and N-(9-fluorenylmethoxycarbonyl)-L-amino acids involving succinimidoxy anion as a leaving group in amino-acid protection. Synthesis-Stuttgart 671–673

Marchand D, Martinez J, Cavelier F (2008) Straightforward synthesis of chiral silylated amino acids through hydrosilylation. Eur J Org Chem 3107–3112

Mortensen M, Husmann R, Veri E, Bolm C (2009) Synthesis and applications of silicon-containing alpha-amino acids. Chem Soc Rev 38:1002–1010

Pujals S, Fernandez-Carneado J, Kogan MJ, Martinez J, Cavelier F, Giralt E (2006) Replacement of a proline with silaproline causes a 20-fold increase in the cellular uptake of a pro-rich peptide. J Am Chem Soc 128:8479–8483

Rizo J, Gierasch LM (1992) Constrained peptides: models of bioactive peptides and protein substructures. Annu Rev Biochem 61:387–418

Roussel C, Del Rio A, Pierrot-Sanders J, Piras P, Vanthuyne N (2004) Chiral liquid chromatography contribution to the determination of the absolute configuration of enantiomers. J Chromatogr A 1037:311–328

Vivet B, Cavelier F, Martinez J (2000a) Synthesis of silaproline, a new proline surrogate. Eur J Org Chem 807–811

Vivet B, Cavelier F, Martinez J, Didierjean C, Marraud M, Aubry A (2000b) A silaproline-containing dipeptide. Acta Crystallogr Sect C-Cryst Struct Commun 56:1452–1454