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Efficient Synthesis of Nicotianamine and Non-Natural Analogues

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An efficient synthesis of nicotianamine has been achieved by using a new strategy based on *N*-alkylation. Sulfonamide activation proved to be necessary for the alkylation of the primary amine and the 2-(trimethylsilyl)ethanesulfonyl group was easily introduced and found to provide the best compromise for the *N*-alkylation and deprotection reactions. This new strategy enabled the preparation of several unnatural analogues including original molecules that will be useful as tools for plant physiology studies.

Introduction

Nicotianamine (NA, **1**), which was first isolated from the leaves of *Nicotiana tabacum L.*,[1] is a key biosynthetic precursor of phytosiderophores (Figure 1). This ubiquitous compound is found in all higher plants and is involved in the homeostasis of such essential metal ions as Fe, Zn and Cu.[2] In gramineous plant species, NA is a precursor of the phytosiderophores, the main role of which is in iron uptake. In plants, NA is characterized by high mobility and is also found at low concentrations (micromolar) in the circulating saps of xylem and phloem.[3] NA has a role in the loading of Cu in xylem for translocation from the roots to the shoots and also for the unloading of the Fe in the leaves from the vascular system. NA is also involved in the loading of Fe by seeds. Its chelation ability has been studied in vitro with several metals including Fe, Zn, Ni, Cu and Mn.[4] Interestingly, it has been discovered that NA exhibits antihypertensive activity in humans by inhibiting the angiotensin I converting enzyme.[5]

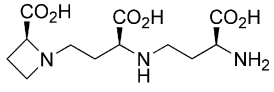

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Figure 1. Structure of nicotianamine (NA).

The importance of NA in plant physiology has prompted synthetic investigations. Two main strategies for the synthesis of NA have been described in the literature:[6] reductive amination of intermediate protected aldehydes, either from the left to the right part[7] or from the right to the left part[8] of the molecule. More recently, the reduction of amides through thioamide bonds followed by subsequent desulfurization has been developed.[9] Owing to the importance of NA it is desirable to develop new efficient methods for the synthesis of NA suitable for large-scale production.

The purpose of this research was to develop a new synthetic strategy based on nucleophilic substitution by an amine as the key reaction.

Results and Discussion

This novel approach to NA involved different building blocks: a free amine for one moiety and a good leaving group on the other part with suitable protecting groups on all other functions. In this way building blocks can be used twice to construct NA from the left to the right part of the molecule.

Two successive *N*-alkylation steps were necessary to assemble NA, with noticeable differences between the two, the first one involving a secondary amine, the second one a primary amine, which was much more difficult to alkylate (Scheme 1).

For the first alkylation step, we compared several leaving groups under standard conditions (Scheme 2, Table 1). We selected iodine as the most efficient leaving group (entry 4). Then we optimized the synthesis of the intermediate bearing iodine as the leaving group and chose suitable protections for the other reactive functions. Then we optimized the *N*-alkylation conditions.

tert-Butyl ester and benzyl ester (Z) protection were chosen for the iodine-containing compound. *tert*-Butoxycarbonyl (*S*)-2-(benzyloxycarbonylamino)-4-iodobutanoate (**4**), the key precursor, was prepared in three steps by using L-*tert*-butyl aspartate (**2**) as the starting material (Scheme 3).

Treatment of compound **2** with benzyl chloroformate under the usual conditions ($NaHCO_3$, H_2O/dioxane) yielded the Z derivative, which was readily reduced under McGeary

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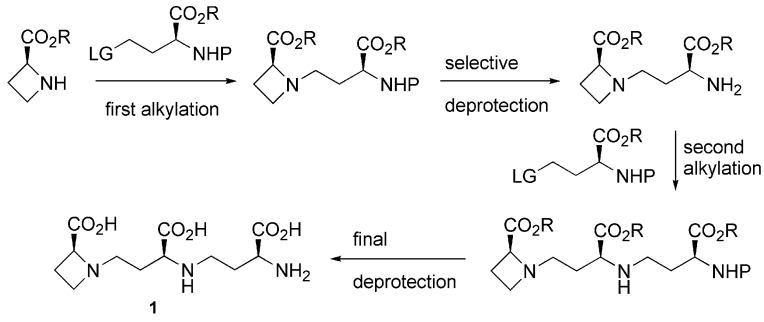
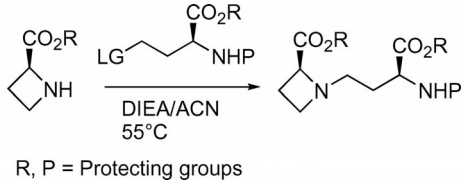


Scheme 1. General strategy for the synthesis of NA by *N*-alkylation.



Scheme 2. Comparison between leaving groups on a model substrate.

Table 1. Comparison between leaving groups in the *N*-alkylation reaction.

Entry	Leaving group	*N*-Alkylation yields [%][a]
1	OMs	32
2	OTs	25
3	Br	69
4	I	78

[a] Yields of the isolated product.

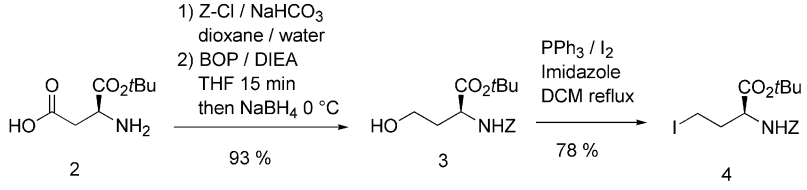
conditions[10] (preactivation of the acid with BOP then reduction with $NaBH_4$) to afford the desired alcohol **3** in 93% yield. The use of the *tert*-butyl ester as protecting group significantly increased the yield of the desired alcohol by preventing lactonization. Then the alcohol **3** was converted into the iodide **4** by using iodine, triphenylphosphane and imidazole.[11]

In the first alkylation step, *tert*-butyl L-azetidine-2-carboxylate[12] (**5**) was successfully *N*-alkylated with the iodide derivative **4**. The optimized alkylation conditions, DIEA (2 equiv.) in acetonitrile at 55 °C, provided **6** in good yield. Selective deprotection of the N-terminal amino group by hydrogenolysis of **6** using 10% Pd/C and H_2 afforded the free amine **7** (Scheme 4).

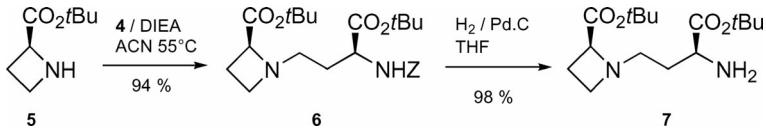
However, the second direct alkylation of the resulting primary amine with the iodine derivative did not afford the desired product, but gave only the starting material, thus proving the poor reactivity of this amine in this reaction and the need for specific activation. For this experiment, we used sulfonamide as temporary protection.

In a first attempt, we introduced onto the free amine the *p*-nitrophenylsulfonyl group (nosyl)[13] widely used in polyamine synthesis.[14] This activated intermediate was obtained in 78% yield and was subsequently alkylated with compound **4**. The reaction was followed by LC–MS: after 6 hours the expected compound was detected with a conversion of 60%. After 12 hours we observed total conversion with the appearance of a side-product with a mass of 64 units less than the expected product. A rearrangement, already observed in the literature,[15] resulted in the loss of SO_2. Under optimized conditions we successfully obtained the desired compound in 65% yield. The same rearrangement occurred in the deprotection step and cannot be avoided when an electron-withdrawing group flanks the nosyl sulfonamide.

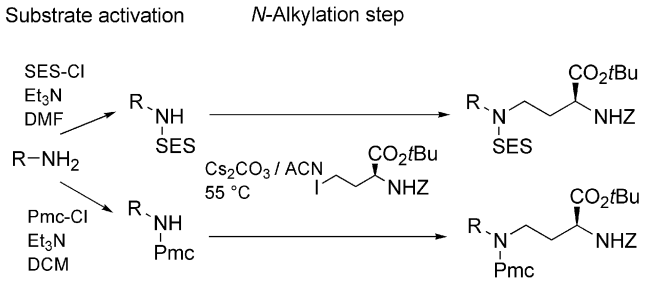
We therefore tried different sulfonamide protecting groups, that is, 2-(trimethylsilyl)ethanesulfonyl (SES)[16] and 2,2,5,7,8-pentamethylchromane-6-sulfonyl (Pmc)[17] and we evaluated the efficiency of the activation and the *N*-alkylation reactions with several substrates (Scheme 5, Table 2).



Scheme 3. Synthesis of *tert*-butoxycarbonyl (*S*)-2-(benzyloxycarbonylamino)-4-iodobutanoate (**4**).



Scheme 4. Synthesis of the free amine **7**.



Scheme 5. Comparison of the efficacy of the SES and Pmc protecting groups with a model substrate.

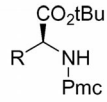
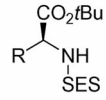
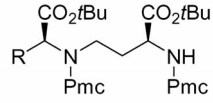
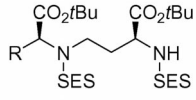
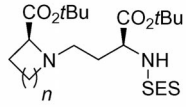
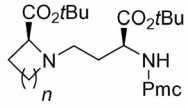
With monomeric (entries 1 and 2) and dimeric linear substrates (entries 3 and 4), Pmc provided better yields for both reactions, but to a higher extent during the activation than in the *N*-alkylation. However, with ring-containing substrates (entry 6) we were unable to isolate the alkylation product due to purification difficulties. In addition, the Pmc protecting group proved to be resistant to final HF deprotection.

For these reasons we used the SES group for the synthesis. After introducing the SES protecting group, which afforded the sulfonamide **8**, the *N*-alkylation step gave an optimal yield when using caesium carbonate (2 equiv.) in acetonitrile at 55 °C. Higher temperatures and a large excess of caesium carbonate led to the decomposition of **4**. The targeted compound **9** was obtained in 55% yield. Removal of the SES, Z and *tert*-butyl protecting groups proceeded without difficulty on HF treatment to afford NA after lyophilization as a white powder in high purity (Scheme 6).

We then extended this strategy to produce three synthetic analogues (Figure 2) by replacing the carboxy-azetidine ring with proline (**10**), silaproline (**11**)[18] and pyroglutamic acid (**12**), thus modifying the physicochemical properties of the molecules. These compounds were used as probes in plant experiments.[19] By using the same operational conditions, the NA analogues **10**[20] and **11** were synthesized with overall yields in the same range as that of **1** (Scheme 7).

For the synthesis of the analogue **12**, *tert*-butyl pyroglutamate (**21**) was alkylated in the presence of the iodide derivative and sodium hydride in DMF at 0 °C to afford the tertiary amide **22**. The pyroglutamate analogue **12** was then obtained by following the same procedure as described for the synthesis of **1** (Scheme 8).

Table 2. Comparison of the efficacy of the Pmc and SES protecting groups in the activation and *N*-alkylation steps with the iodine derivative **4**.

Entry	Substrate	Activation Yield (%)[a]	*N*-Alkylation Yield (%)[b]
1		91	80
2		53	73
3		86	62
4		56	54
5		65	55
6		90	n.d.

[a] Sulfonamide introduction, yields of the isolated products. [b] Formation of N–C bonds, yields of the isolated products. R = any alkyl group, *n* = 1 or 2.

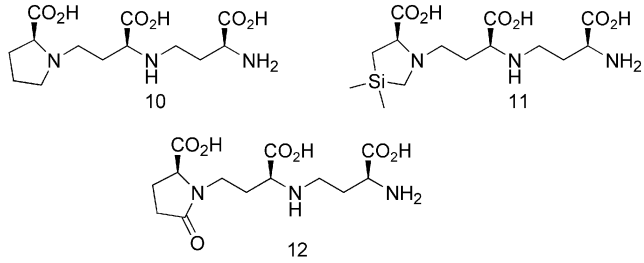
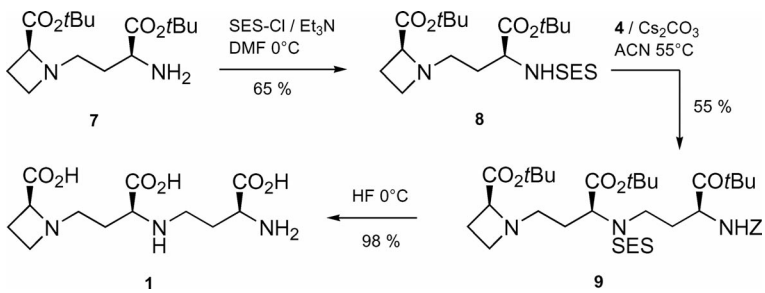
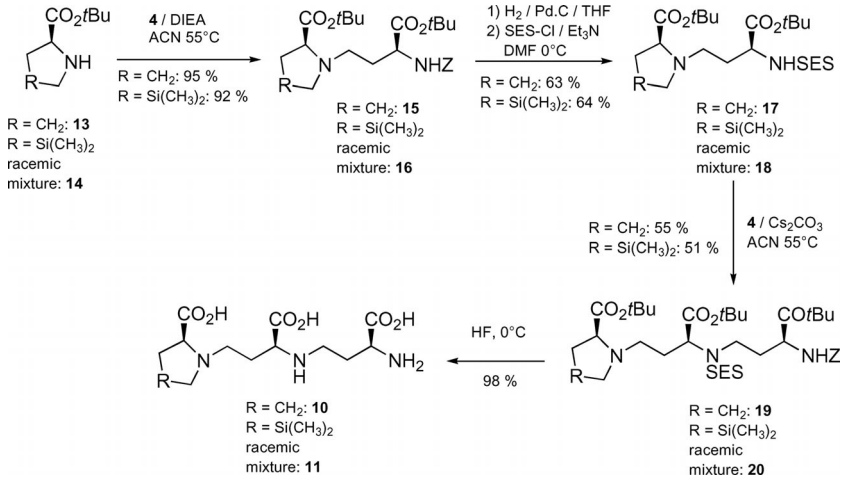


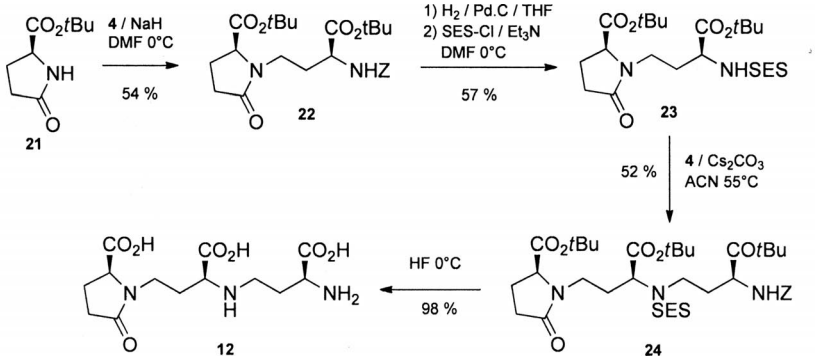
Figure 2. Unnatural nicotianamine analogues.



Scheme 6. Synthesis of nicotianamine (**1**) from the free amine **7**.



Scheme 7. Synthesis of nicotianamine analogues **10** and **11**.



Scheme 8. Synthesis of nicotianamine analogue **12**.

Conclusions

An efficient synthesis of NA based on *N*-alkylation as the key reaction has been developed. Different sulfonamide activations were compared and the SES protecting group was finally chosen on the basis of the best compromise for its introduction and the *N*-alkylation and deprotection reactions. This new strategy enabled the preparation of several unnatural analogues, including novel structures, that will be useful tools for plant physiology studies. Their biological evaluation in plants and in in vitro metal chelation is currently under progress.

Experimental Section

General: 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. Column chromatography was performed by using silica gel (Merck 60, 230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded 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. The ^{13}C standard in D_2O was calculated from the chemical shift of water in ^{1}H NMR and the apparatus frequency (SF1 = BF1). Low-resolution mass spectrometry (MS) was performed with a Micromass Platform II spectrometer by using electrospray ionization (ESI). HRMS were recorded with a JEOL JMS-SX-102A spectrometer. High-pressure liquid chromatography (HPLC) was performed with a Waters Alliance 2796 apparatus with a Chromolith SpeedROD (50 × 4.6 mm) column and a diode array detector. A linear gradient of H_2O to ACN (0.1% TFA) in 3 min was used as eluent in the HPLC analysis. Melting points were measured with a Büchi instrument. Optical rotations values were measured with a Perkin–Elmer 341 apparatus (20 °C, sodium discharge lamp).

***tert*-Butyl (*S*)-2-(Benzyloxycarbonylamino)-4-hydroxybutanoate (3):** Sodium hydrogen carbonate (13 g, 159 mmol) was added to a solution of compound **2** (10 g, 53 mmol) in water/dioxane (2:1; 170 mL) and over a period of 2 h, a solution of benzyl chloroformate (11.8 mL, 69 mmol) in dioxane (70 mL) was added. The reaction mixture was stirred overnight, then washed with ethyl acetate (3 × 200 mL) and the pH was lowered to 2 with 6 N hydrochloric acid solution at 0 °C. The aqueous phase was extracted with ethyl acetate (3 × 200 mL). The organic phases were combined and washed successively with brine (200 mL) and distilled water (200 mL), then dried with anhydrous magnesium sulfate and concentrated to obtain a colourless oil (16.7 g, 97%). R_f ($CHCl_3$/MeOH/AcOH 37%) = 0.68. HPLC: t = 1.56 min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.45 (s, 9 H), 2.95 (dd, J = 4.5, 14 Hz, 2 H), 4.55 (q, J = 4.3 Hz, 1 H), 5.10 (s, 2 H), 5.8 (d, J = 8 Hz, 1 H),

7.35 (m, 5 H), 10.7 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 27.8, 36.7, 50.8, 67.1, 82.8, 128.1, 128.2, 128.5, 136.1, 156.06, 169.5, 176.3 ppm. MS: m/z = 324.1 [M + H]$^+$, 268.1 [M + H – *t*Bu]$^+$.

DIEA (10.5 mL, 60.4 mmol) was added to a suspension of the resulting oil (15 g, 46.4 mmol) and BOP (27 g, 60.4 mmol) in anhydrous THF was added. The reaction mixture was stirred for 10 min until it turned yellow, and cooled down to 0 °C for 15 min. Then $NaBH_4$ (2.3 g, 60.4 mmol) was added portionwise. The reaction mixture was stirred overnight. The solvent was evaporated in vacuo and the crude was dissolved in ethyl acetate (250 mL). The organic layer was washed successively with a 10% citric acid solution (3 × 100 mL), a saturated solution of sodium hydrogen carbonate (3 × 100 mL) and distilled water (2 × 100 mL). The organic layer was dried with anhydrous magnesium sulfate and concentrated to afford a colourless oil. Compound **3** was purified by chromatography on silica gel (petroleum ether/diethyl ether, 3:7) to afford a colourless oil (13.95 g, 96%).

R_f (petroleum ether/diethyl ether, 3:7) = 0.42. HPLC: t = 1.45 min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.43 (s, 9 H), 2.09 (dd, J = 4.6, 15.2 Hz, 2 H), 3.48 (s, 1 H), 3.8 (m, 2 H), 4.45 (m, 1 H), 5.10 (s, 2 H), 5.87 (d, J = 8 Hz, 1 H), 7.35 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 27.9, 35.5, 52.0, 58.4, 67.0, 82.4, 128.07, 128.1, 128.3, 129.0, 136,1, 156.8, 169.5, 171.7 ppm. MS: m/z = 310.2 [M + H]$^+$, 254.1 [M + H – *t*Bu]$^+$.

***tert*-Butyl (*S*)-2-(Benzyloxycarbonylamino)-4-iodobutanoate (4):** Iodine (14.3 g, 56.4 mmol), triphenylphosphane (14.8 g, 56.4 mmol) and, after 10 min, imidazole (9.6 g, 141 mmol) were added to a solution of compound **3** (5.8 g, 18.8 mmol) in anhydrous DCM (260 mL) under argon. The reaction mixture was heated at reflux for 3 h. The reaction mixture was cooled to room temperature and washed successively with a molar solution of potassium sulfate (3 × 150 mL), a saturated solution of sodium hydrogen carbonate (3 × 150 mL), a 5% sodium thiosulfate solution (3 × 150 mL) and with brine (3 × 150 mL). The organic layer was dried with anhydrous magnesium sulfate and concentrated to afford a yellow oil. Compound **4** was obtained after silica gel chromatography (petroleum ether/ethyl acetate, 10:0 to 8:2) as a yellow oil (6.2 g, 78%). R_f (petroleum ether/ethyl acetate, 8:2) = 0.65. HPLC: t = 2.42 min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.45 (s, 9 H), 2.15–2.45 (2 m, 2 H), 3.15 (m, 2 H), 4.35 (m, 1 H), 5.10 (s, 2 H), 5.87 (d, J = 8 Hz, 1 H), 7.35 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 16.1, 28.4, 38.5, 54.2, 56.2, 67.9, 83.6, 128.95, 129.03, 129.2, 129.3, 129.4, 137.0, 156.7, 171.0 ppm. MS: m/z = 442.0 [M + Na]$^+$, 420.1 [M + H]$^+$, 364.2 [M + H – *t*Bu]$^+$.

***tert*-Butyl (*S*)-1-[(*S*)-3-(Benzyloxycarbonylamino)-4-*tert*-butoxy-4-oxobutyl]azetidine-2-carboxylate (6):** A solution of compound **4** (2.68 g, 6.4 mmol) in anhydrous acetonitrile (2 mL) was added to compound **5** (1 g, 6.4 mmol) dissolved in anhydrous acetonitrile (2 mL). The reaction mixture was stirred for 30 min under argon. Freshly distilled DIEA (2.8 mL, 16 mmol) was added and the reaction mixture was diluted with anhydrous acetonitrile (13 mL). The reaction mixture was heated to 55 °C and stirred vigorously for 16 h. The mixture was concentrated and the crude was dissolved in ethyl acetate (25 mL). The organic layer was washed successively with a 5% hydrochloric acid solution (2 × 15 mL), brine (15 mL) and distilled water (20 mL). The organic phase was dried with magnesium sulfate, filtered and concentrated to afford a yellow oil. The compound was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 8:2 to 0:1) to afford a yellow oil (2.3 g, 94%). R_f (petroleum ether/ethyl acetate, 8:2) = 0.3. HPLC: t = 2.17 min. $[\alpha]_D^{24}$ = –10.4 (c = 4.55, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.45 (s, 9 H), 1.47 (s, 9 H), 1.65–1.78 (m, 1 H), 1.80–1.97 (m, 1 H), 2.14–2.35 (2 m, 2 H), 2.39–2.47 (m, 1 H), 2.71–2.82 (m, 2 H), 3.28–3.32 (m, 1 H), 3.34–3.5 (m, 1 H), 4.17–4.28 (dd, J = 7.5, 12.5 Hz, 1 H), 5.12 (s, 2 H), 6.21 (d, J = 8.5 Hz, 1 H), 7.32 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 18.9, 21.1, 28.0, 29.3, 29.7, 50.8, 51.1, 53.8, 55.1, 58.4, 65.9, 66.6, 81.1, 81.7, 127.95, 127.99, 128.2, 128.4, 128.6, 136.7, 156.2, 171.2, 171.8 ppm. MS (ES$^+$): m/z = 449.2 [M + H]$^+$, 393.2 [M – *t*Bu]$^+$, 337.0 [M – 2 *t*Bu]$^+$.

***tert*-Butyl (*S*)-1-[(*S*)-3-Amino-4-*tert*-butoxy-4-oxobutyl]azetidine-2-carboxylate (7):** Palladium on charcoal (400 mg, 20% weight) was added to compound **6** (2 g, 4.46 mmol) in anhydrous THF (35 mL). The air in the apparatus was eliminated and replaced by argon through three cycles of vacuum/argon and then by hydrogen with three cycles of vacuum/hydrogen. The reaction mixture was stirred vigorously for 10 h, filtered through a pad of Celite and concentrated to afford a pale-brown oil (1.38 g, 98%). R_f ($CHCl_3$/MeOH/AcOH 37%, 24:2:1) = 0.3. $[\alpha]_D^{24}$ = –9.7 (c = 4.4, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.45 (s, 9 H), 1.47 (s, 9 H), 1.51–1.62 (m, 1 H), 1.80–1.93 (m, 1 H), 2.14–2.35 (m, 2 H), 2.42–2.55 (m, 1 H), 2.71–2.82 (m, 2 H), 3.35–3.52 (m, 3 H), 3.64 (br. s, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.3, 27.99, 28.03, 28.3, 31.1, 50.8, 53.3, 55.2, 62.0, 65.2, 81.0, 81.3, 172.1, 174.2 ppm. MS (ES$^+$): m/z = 315.3 [M + H]$^+$, 337.2 [M + Na]$^+$.

***tert*-Butyl (*S*)-1-{(*S*)-4-*tert*-Butoxy-4-oxo-3-[2-(trimethylsilyl)ethylsulfonamido]butyl}azetidine-2-carboxylate (8):** The free amine **7** (1.3 g, 4.14 mmol) was dissolved in anhydrous dimethylformamide (15 mL) and then TEA (1.74 mL, 12.14 mmol) was added and the mixture was cooled to 0 °C. A solution of 2-(trimethylsilyl)ethanesulfonic chloride (1.3 g, 6.6 mmol) in anhydrous dimethylformamide (5 mL) was added over a 10 min period. The reaction mixture was stirred overnight. The reaction mixture was concentrated and dissolved in ethyl acetate (35 mL), washed with brine (20 mL) and distilled water (20 mL), then dried on anhydrous magnesium and concentrated. Compound **7** was obtained after silica gel chromatography (petroleum ether/ethyl acetate, 8:2 to 7:3) as a yellow oil (1.25 g, 65%). R_f (petroleum ether/ethyl acetate, 6:4) = 0.52. $[\alpha]_D^{24}$ = –81.4 (c = 6.7, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.00 (s, 9 H), 0.91–1.27 (m, 2 H), 1.42 (s, 9 H), 1.44 (s, 9 H), 1.65–1.89 (m, 2 H), 2.05–2.35 (m, 2 H), 2.42–2.55 (m, 1 H), 2.65–2.83 (m, 2 H), 2.84–3.01 (m, 2 H), 3.36–3.51 (m, 2 H), 4.00–4.15 (m, 1 H), 6.56 (d, J = 8.07 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = –0.3, 0.0, 0.3, 12.1, 23.0, 29.8, 30.0, 30.1, 32.2, 52.0, 52.7, 55.4, 56.2, 57.2, 67.5, 83.0, 84.0, 173.1, 173.8 ppm. MS (ES$^+$): m/z = 479.2 [M + H]$^+$. HRMS (ES$^+$): calcd. for $C_{21}H_{43}N_2O_6SSi$ 479.2611; found 479.2592.

***tert*-Butyl (*S*)-1-[(*S*)-3-{*N*-[(*S*)-3-(Benzyloxycarbonylamino)-5,5-dimethyl-4-oxohexyl]-2-(trimethylsilyl)ethylsulfonamido}-4-*tert*-butoxy-4-oxobutyl]azetidine-2-carboxylate (9):** Caesium carbonate (411 mg, 1.26 mmol) was added to a solution of **8** (300 mg, 0.63 mmol) in anhydrous acetonitrile (14 mL). The reaction mixture was heated at 55 °C for 30 min whilst vigorously being stirred. Then compound **4** (264 mg, 0.63 mmol) in anhydrous acetonitrile (5 mL) was added dropwise through a syringe pump. The reaction mixture was stirred overnight at 55 °C. The reaction mixture was concentrated and dissolved in ethyl acetate (25 mL). The organic layer was successively washed with brine (15 mL) and distilled water (15 mL), then dried with anhydrous magnesium sulfate and concentrated. Compound **9** was obtained after silica gel chromatography (petroleum ether/ethyl acetate, 8:2 to 7:3) as a yellow oil (266 mg, 55%). R_f (petroleum ether/ethyl acetate, 6:4) = 0.62. $[\alpha]_D^{24}$ = –71.4 (c = 6.7, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.00 (s, 9 H), 0.91–1.27 (m, 2 H), 1.43 (s, 27 H), 1.56–1.80 (m,

1 H), 1.92–2.05 (m, 1 H), 2.09–2.15 (m, 3 H), 2.16–2.19 (m, 1 H), 2.4 (dt, J = 4.1, 20.4 Hz, 1 H), 2.6–2.95 (m, 4 H), 3.20–3.31 (m, 2 H), 3.51 (t, J = 8.4 Hz, 1 H), 4.05–4.33 (m, 2 H), 5.08 (s, 2 H), 5.48 (d, J = 7.07 Hz, 1 H), 7.28–7.38 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = –0.3, –0.0, 0.3, 12.1, 23.3, 29.9, 29.95, 30.04, 33.9, 35.7, 45.1, 50.8, 52.7, 54.8, 57.5, 61.7, 67.7, 68.8, 82.9, 84.2, 84.3, 129.9, 130.0, 130.1, 131.00, 138.3, 158.0, 172.3, 172.8, 173.9 ppm. MS (ES^+): m/z = 771.4 $[M + H]^+$. HRMS (ESI^+): calcd. for $C_{37}H_{64}N_3O_{10}SSi$ 770.4082; found 770.4099.

(*S*)-1-{(*S*)-3-[(*S*)-3-Amino-3-carboxypropylamino]-3-carboxypropyl}azetidine-2-carboxylic Acid (1): Hydrofluoric acid (1 mL, large excess) was added with the help of a Teflon® apparatus to a Teflon® vessel containing **9** (140 mg, 0.18 mmol). The reaction mixture was stirred at 0 °C for 1.5 h. The hydrofluoric acid was evaporated and the remaining was neutralized by addition of potassium hydroxide. The crude was dissolved in distilled water (3 mL) and the aqueous phase washed with diethyl ether (3 × 3 mL). After freeze-drying, compound **1** was obtained as a pure pale-brown solid (49 mg, 98%); m.p. 218–220 °C. $[\alpha]_D^{24}$ = –55.6 (c = 0.8, H_2O) {ref.[9b] $[\alpha]_D^{34}$ = –56.4 (c = 0.81, H_2O)}. ^{1}H NMR (D_2O, 300 MHz): δ = 1.95–2.28 (m, 4 H), 2.35–2.52 (m, 1 H), 2.6 (qd, J = 9.1, 4.2 Hz, 1 H), 3.1–3.4 (m, 4 H), 3.69 (dd, J = 4.5, 8.2 Hz, 1 H), 3.73 (m, 1 H), 3.85 (t, J = 9.6 Hz, 1 H), 3.96 (dt, J = 9.6, 6.6 Hz, 1 H), 4.92 (td, J = 9.6, 6.5 Hz, 1 H) ppm. ^{13}C NMR (D_2O, 75 MHz): δ = 16.3, 17.8, 21.0, 26.9, 42.5, 44.0, 54.4, 57.9, 65.6, 170.6, 171.4, 173.0 ppm. MS (ES^+): m/z = 304.2 $[M + H]^+$, 203.1 $[M – AzeOH]^+$. HRMS (FAB^+): calcd. for $C_{12}H_{22}N_3O_6$ 304.1509; found 304.1502.

***tert*-Butyl (*S*)-1-[(*S*)-3-(Benzyloxycarbonylamino)-4-*tert*-butoxy-4-oxobutyl]pyrrolidine-2-carboxylate (15):** Compound **4** (2 g, 4.78 mmol) was added to **13** (0.98 g, 5.75 mmol) dissolved in anhydrous acetonitrile (4 mL). The reaction mixture was kept under argon and stirred for 30 min. Anhydrous DIEA (2 mL, 11.95 mmol) was added and the reaction mixture was diluted with anhydrous acetonitrile (12 mL). The reaction mixture was heated to 55 °C and vigorously stirred for 16 h. The mixture was concentrated and the crude was dissolved in ethyl acetate (25 mL). The organic layer was successively washed with a 5% hydrochloric acid solution (2 × 15 mL), brine (15 mL) and distilled water (20 mL). The organic phase was dried with magnesium sulfate, filtered and concentrated to afford a yellow oil. The compound was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 8:2 to 0:1) to afford a yellow oil (2.15 g, 95%). R_f (petroleum ether/ethyl acetate, 7:3) = 0.74. HPLC: t = 2.27 min. $[\alpha]_D^{24}$ = –27.9 (c = 1.95, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.35 (s, 18 H), 1.6–1.84 (m, 4 H), 1.85–2.05 (m, 2 H), 2.13–2.29 (m, 1 H), 2.33–2.48 (m, 1 H), 2.69–2.80 (m, 1 H), 2.89–2.98 (m, 1 H), 3.00–3.11 (m, 1 H), 4.17–4.28 (dd, J = 7.5, 13.5 Hz, 1 H), 5.04 (s, 2 H), 6.4 (d, J = 8.6 Hz, 1 H), 7.29 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 23.1, 27.7, 27.9, 29.3, 30.2, 51.0, 53.0, 53.8, 66.3, 66.5, 81.1, 81.6, 126.7, 127.3, 127.8, 127.9, 128.1, 128.3, 136.7, 156.2, 171.2, 173.2 ppm. MS (ES^+): m/z = 463.2 $[M + H]^+$. HRMS (ES^+): calcd. for $C_{25}H_{39}N_2O_6$ 463.2848; found 463.2825.

***tert*-Butyl 1-[(*S*)-3-(Benzyloxycarbonylamino)-4-*tert*-butoxy-4-oxobutyl]-3,3-dimethyl-1,3-azasilolidine-5-carboxylate (16):** Compound **4** (2 g, 4.78 mmol) was added to **14** (1.23 g, 5.74 mmol) dissolved in anhydrous acetonitrile (2 mL). The reaction mixture was kept under argon and stirred for 30 min. Anhydrous DIEA (2 mL, 11.95 mmol) was added and the reaction mixture was diluted with anhydrous acetonitrile (12 mL). The reaction mixture was heated to 55 °C and vigorously stirred for 16 h. The mixture was concentrated and the crude was dissolved in ethyl acetate (25 mL), successively washed with a 5% hydrochloric acid solution (2 × 15 mL), brine (15 mL) and distilled water (20 mL). The organic phase was dried with magnesium sulfate, filtered and concentrated to afford a yellow oil. The compound was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 8:2 to 0:1) to afford a yellow oil (1.98 g, 92%). R_f (petroleum ether/ethyl acetate, 7:3) = 0.74. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = –0.35–0.10 (m, 6 H), 0.56–0.80 (m, 2 H), 1.35 (s, 18 H), 1.85–2.05 (m, 2 H), 1.92–2.00 (m, 2 H), 2.13–2.29 (m, 1 H), 2.33–2.48 (m, 1 H), 2.83–3.00 (m, 1 H), 3.75–4.17 (m, 1 H), 5.25 (s, 2 H), 6.50 (d, J = 9.8 Hz, 1 H), 7.19 (m, 5 H) ppm. MS (ES^+): m/z = 507.2 $[M + H]^+$. HRMS (ES^+): calcd. for $C_{26}H_{43}N_3O_6Si$ 507.2890; found 507.2883.

***tert*-Butyl (*S*)-1-{(*S*)-4-*tert*-Butoxy-4-oxo-3-[2-(trimethylsilyl)ethylsulfonamido]butyl}pyrrolidine-2-carboxylate (17):** Palladium on charcoal (300 mg, 20% weight) was added to compound **15** (1.5 g, 3.25 mmol) dissolved in anhydrous THF (17 mL). The air in the apparatus was eliminated and replaced by argon with three cycles of vacuum/argon and then by hydrogen with three cycles of vacuum/hydrogen. The reaction mixture was vigorously stirred for 10 h, filtered through a pad of Celite and concentrated to afford the free amino compound as a brown oil (1.04 g, 98%). R_f ($CHCl_3$/MeOH/AcOH 37%, 24:2:1) = 0. $[\alpha]_D^{24}$ = –5.3 (c = 2.2, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.42 (s, 18 H), 1.51–1.65 (m, 1 H), 1.69–2.05 (m, 7 H), 2.19–2.46 (m, 2 H), 2.75–2.88 (m, 1 H), 2.91–3.05 (m, 1 H), 3.09–3.17 (m, 1 H), 3.41–3.49 (m, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 22.9, 28.01, 28.07, 28.3, 29.2, 33.5, 51.1, 53.1, 53.3, 66.5, 80.4, 80.8, 173.1, 175.2 ppm. MS (ES^+): m/z = 329.5 $[M + H]^+$, 273.3 $[M – t\text{Bu}]^+$, 217.2 $[M – 2t\text{Bu}]^+$.

TEA was added (1.8 mL, 12.8 mmol) to a solution of the resulting amino compound (1.4 g, 4.26 mmol) in anhydrous dimethylformamide (14 mL). The reaction mixture was cooled to 0 °C. A solution of 2-(trimethylsilyl)ethanesulfonyl chloride (1.4 g, 7.26 mmol) in anhydrous dimethylformamide (5 mL) was added over a 10 min period. The reaction mixture was stirred overnight at 0 °C. The dimethylformamide was evaporated and the crude was partitioned between ethyl acetate (15 mL) and water (20 mL). The aqueous phase was extracted with ethyl acetate (2 × 10 mL). The combined organic phases were washed with brine (20 mL), dried with anhydrous sodium sulfate, filtered and concentrated to afford a yellow oil, which was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 8:2 then 7:3) to afford **17** as a yellow oil (1.34 g, 64%). R_f (petroleum ether/ethyl acetate, 6:4) = 0.52. $[\alpha]_D^{24}$ = –35.4 (c = 1.53, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.00 (s, 9 H), 0.91–1.22 (m, 2 H), 1.43 (s, 18 H), 1.65–1.89 (m, 4 H), 1.91–2.09 (m, 2 H), 2.29 (q, J = 8.01 Hz, 1 H), 2.45–2.56 (m, 1 H), 2.74–2.85 (m, 1 H), 2.87–2.96 (m, 2 H), 2.99–3.08 (m, 1 H), 3.12–3.21 (m, 1 H), 4.05–4.15 (m, 1 H), 6.59 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = –0.3, –0.0, 0.3, 12.2, 25.2, 29.94, 30.05, 31.5, 31.6, 32.3, 32.4, 52.1, 53.2, 54.9, 58.3, 68.5, 82.7, 83.8, 173.1, 175.2 ppm. MS (ES^+): m/z = 493.26 $[M + H]^+$.

***tert*-Butyl 1-{(*S*)-4-*tert*-Butoxy-4-oxo-3-[2-(trimethylsilyl)ethylsulfonamido]butyl}-3,3-dimethyl-1,3-azasilolidine-5-carboxylate (18):** Palladium on charcoal (300 mg, 20% weight) was added to a solution of **16** (1.5 g, 3.25 mmol) in anhydrous THF (30 mL). The air in the apparatus was eliminated and replaced by argon with three cycles of vacuum/argon and then by hydrogen with three cycles of vacuum/hydrogen. The reaction mixture was stirred vigorously for 10 h, filtered through a pad of Celite and concentrated to afford the free amino compound as a pale-brown oil (1.04 g, 98%). R_f ($CHCl_3$/MeOH/AcOH 37%, 24:2:1) = 0.41. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = –0.31–0.15 (m, 6 H), 0.61–0.83 (m, 2 H), 1.35 (s, 18 H), 1.85–2.05 (m, 2 H), 1.92–2.00 (m, 2 H), 2.13–2.29 (m, 1 H), 2.33–2.48 (m, 1 H), 2.83–3.00 (m, 1 H), 3.35–3.61 (m, 1 H), 4.97

(sl, 2 H) ppm. MS (ES^+): *m*/*z* = 373.25 [M + H]$^+$, 317.25 [M – *t*Bu]$^+$, 261.24 [M – 2 *t*Bu]$^+$.

TEA was added (1.45 mL, 10.47 mmol) to a solution of the resulting free amino compound (1.3 g, 3.49 mmol) in anhydrous dimethylformamide (14 mL). The reaction mixture was cooled to 0 °C in an ice bath. A solution of 2-(trimethylsilyl)ethanesulfonyl chloride (1.12 g, 5.58 mmol) in anhydrous dimethylformamide (5 mL) was added over a 10 min period. The reaction mixture was stirred overnight at 0 °C. The dimethylformamide was evaporated and the crude was partitioned between ethyl acetate (15 mL) and water (20 mL). The aqueous phase was extracted with ethyl acetate (2 × 10 mL). The combined organic phases were washed with brine (20 mL), dried with anhydrous sodium sulfate, filtered and concentrated to afford a yellow oil, which was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 8:2 then 7:3) to afford **18** as a yellow oil (1.25, 65%). R_f (petroleum ether/ethyl acetate, 6:4) = 0.56. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = –0.10 (s, 9 H), 0.05–0.35 (m, 6 H), 0.81–1.20 (m, 4 H), 1.42 (s, 18 H), 1.75–2.05 (m, 4 H), 2.51–3.00 (m, 4 H), 3.31–3.51 (m, 1 H), 4.05–4.18 (m, 1 H), 6.75 (br. s, 1 H) ppm. MS (ES^+): *m*/*z* = 537.1 [M + H]$^+$.

***tert*-Butyl (*S*)-1-[(*S*)-3-{*N*-[(*S*)-3-(Benzyloxycarbonylamino)-5,5-dimethyl-4-oxohexyl]-2-(trimethylsilyl)ethylsulfonamido}-4-*tert*-butoxy-4-oxobutyl]pyrrolidine-2-carboxylate (19):** Caesium carbonate (166 mg, 1.62 mmol) was added to a solution of **17** (400 mg, 0.81 mmol) in anhydrous acetonitrile (15 mL). The reaction mixture was heated at 55 °C for 30 min whilst being stirred vigorously. A solution of **4** (339 mg, 0.81 mmol) in anhydrous acetonitrile (5 mL) was then added dropwise through a syringe pump. The reaction mixture was stirred overnight at 55 °C. The reaction mixture was concentrated and the crude was partitioned between ethyl acetate (12 mL) and distilled water (17 mL). The aqueous layer was extracted with ethyl acetate (3 × 7 mL). The combined organic layers were successively washed with brine (15 mL) and distilled water (15 mL), then dried with anhydrous magnesium sulfate and concentrated. Compound **19** was obtained after silica gel chromatography (petroleum ether/ethyl acetate, 8:2 to 7:3) as a yellow oil (349 mg, 55%). R_f (petroleum ether/ethyl acetate, 6:4) = 0.62. HPLC 2.95 min. $[\alpha]_D^{24}$ = –13.4 (*c* = 1.25, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.00 (s, 9 H), 0.91–1.09 (m, 2 H), 1.41 (s, 27 H), 1.62–1.90 (m, 4 H), 1.92–2.20 (m, 4 H), 2.26–2.35 (m, 1 H), 2.39–2.48 (m, 1 H), 2.6–2.95 (m, 4 H), 3.20–3.31 (m, 2 H), 3.45 (t, *J* = 8.4 Hz, 1 H), 4.05–4.33 (m, 2 H), 5.08 (s, 2 H), 5.48 (d, *J* = 7.07 Hz, 1 H), 7.28–7.38 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = –0.3, –0.0, 0.3, 12.1, 23.3, 29.87, 29.95, 30.04, 33.9, 35.7, 45.1, 50.8, 52.7, 54.8, 57.5, 61.7, 67.7, 68.8, 82.9, 84.2, 84.3, 129.9, 130.0, 130.01, 131.0, 138.3, 158.0, 17.3, 172.8, 173.9 ppm. MS (ES^+): *m*/*z* = 784.4 [M + H]$^+$, 728.8 [M – *t*Bu]$^+$, 672.6 [M + 2 *t*Bu]$^+$, 616.5 [M – 3 *t*Bu]$^+$. HRMS (ES^+): calcd. for $C_{38}H_{66}N_3O_{10}SSi$ 784.4238; found 784.4256.

***tert*-Butyl 1-[(*S*)-3-{*N*-[(*S*)-3-(Benzyloxycarbonylamino)-5,5-dimethyl-4-oxohexyl]-2-(trimethylsilyl)ethylsulfonamido}-4-*tert*-butoxy-4-oxobutyl]-3,3-dimethyl-1,3-azasilolidine-5-carboxylate (20):** Caesium carbonate (166 mg, 0.51 mmol) was added to a solution of **18** (180 mg, 0.34 mmol) in anhydrous DMF (3 mL). The reaction mixture was heated at 55 °C for 30 min whilst being stirred vigorously. A solution of **4** (172 mg, 0.41 mmol) in anhydrous DMF (1 mL) was then added dropwise through a syringe pump. The reaction mixture was stirred overnight at 60 °C then concentrated and taken up in ethyl acetate (10 mL). The organic layer was successively washed with brine (7 mL) and distilled water (7 mL), then dried with anhydrous magnesium sulfate and concentrated. Compound **20** was obtained after silica gel chromatography (petroleum ether/ethyl acetate, 9:1 to 8:2) as a yellow oil (145 mg, 51%). R_f (petroleum ether/ethyl acetate, 8:2) = 0.44. HPLC: *t* = 2.85 min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = –0.10 (s, 9 H), 0.10–0.39 (2 m, 6 H), 0.75–1.20 (m, 4 H), 1.42 (s, 27 H), 1.75–2.39 (m, 6 H), 2.51–3.00 (m, 5 H), 3.21–3.50 (m, 3 H), 4.05–4.18 (m, 1 H), 5.05 (s, 2 H), 5.50 (d, *J* = 7.40 Hz, 1 H), 7.28–7.38 (m, 5 H) ppm. MS (ES^+): *m*/*z* = 829.4 [M + H]$^+$.

(*S*)-1-{(*S*)-3-[(*S*)-3-Amino-3-carboxypropylamino]-3-carboxypropyl}pyrrolidine-2-carboxylic Acid (10): Hydrofluoric acid (2 mL, large excess) was added with the help of a Teflon® apparatus to a Teflon® vessel containing **19** (140 mg, 0.18 mmol). The reaction mixture was stirred at 0 °C for 1.5 h. The hydrofluoric acid was evaporated and the remaining neutralized by addition of potassium hydroxide. The crude was dissolved in distilled water (5 mL) and the aqueous phase washed with diethyl ether (3 × 5 mL). After freeze-drying, compound **10** was obtained as a pure white solid (39 mg, 98%); m.p. 203–205 °C. ^{1}H NMR (D_2O, 300 MHz): δ = 1.95–2.28 (m, 6 H), 2.35–52 (m, 1 H), 2.6 (qd, *J* = 9.1, 4.2 Hz, 1 H), 3.1–3.4 (m, 4 H), 3.69 (dd, *J* = 4.5, 8.2 Hz, 1 H), 3.73 (m, 1 H), 3.85 (t, *J* = 9.6 Hz, 1 H), 3.96 (dt, *J* = 9.6, 6.6 Hz, 1 H), 4.92 (td, *J* = 9.6, 6.5 Hz, 1 H) ppm. ^{13}C NMR (D_2O, 75 MHz): δ = 22.4, 25.1, 26.7, 28.5, 43.5, 51.5, 55.1, 59.2, 68.8, 171.6, 172.4, 173.0 ppm. MS (ES^+): = 318.2 [M + H]$^+$, 220.1 [M – Pro]$^+$. HRMS (FAB^+): calcd. for $C_{13}H_{24}N_3O_7$ 318.1665; found 318.1675.

1-{(*S*)-3-[(*S*)-3-Amino-3-carboxypropylamino]-3-carboxypropyl}-3,3-dimethyl-1,3-azasilolidine-5-carboxylic Acid (11): Hydrofluoric acid (2 mL, large excess) was added with the help of a Teflon® apparatus to a Teflon® vessel containing **20** (140 mg, 0.17 mmol). The reaction mixture was stirred at 0 °C for 1.5 h. The hydrofluoric acid was evaporated and the remaining neutralized by addition of potassium hydroxide. The crude was dissolved in distillate water (5 mL) and the aqueous phase washed with diethyl ether (3 × 5 mL). After freeze-drying, compound **11** was obtained as a pure white solid (59 mg, 98%); m.p. 176–178 °C. ^{1}H NMR (D_2O, 300 MHz): δ = 0.26–0.42 (m, 6 H), 0.78–1.78 (m, 2 H), 2.12–2.51 (m, 5 H), 2.97–3.28 (m, 4 H), 3.35–3.62 (m, 1 H), 3.66–3.97 (m, 3 H) ppm. MS (ES^+): *m*/*z* = 362.0 [M + H]$^+$, 384.0 [M + Na]$^+$, 203.5 [M – Sip]$^+$. HRMS (ES^+): calcd. for $C_{14}H_{28}N_3O_6Si$ 362.1747; found 362.1770.

***tert*-Butyl (*S*)-1-[(*S*)-3-(Benzyloxycarbonylamino)-4-*tert*-butoxy-4-oxobutyl]-5-oxopyrrolidine-2-carboxylate (22):** Sodium hydride at 60% in mineral oil (1.45 g, 3.6 mmol) was added portionwise to a solution of **21** (670 mg, 3.6 mmol) in anhydrous dimethylformamide (2 mL) at 0 °C under argon. After 30 min of vigorous stirring, a solution of **4** (1 g, 2.4 mmol) in dimethylformamide (2 mL) was added dropwise over a 15 min period. The reaction mixture was stirred vigorously under argon at room temperature overnight. After evaporation of the dimethylformamide, the crude was taken up in ethyl acetate (10 mL) and washed with a 5% hydrochloric acid solution (2 × 5 mL), brine (10 mL) and distilled water (10 mL). The organic phase was dried with magnesium sulfate, filtered and concentrated. Compound **22** was obtained after silica gel flash chromatography (petroleum ether/ethyl acetate, 7:3 to 6:4) as a white solid (628 mg, 54%). R_f (petroleum ether/ethyl acetate, 6:4) = 0.34. $[\alpha]_D^{24}$ = –1.4 (*c* = 1.5, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.39 (s, 18 H), 1.75–2.05 (m, 3 H), 2.08–2.23 (m, 2 H), 2.25–2.48 (m, 1 H), 2.86–3.04 (m, 1 H), 3.50–3.19 (m, 1 H), 3.89–4.09 (m, 1 H), 4.11–4.3 (m, 1 H), 5.06 (s, 2 H), 5.41 (d, *J* = 8.5 Hz, 1 H), 7.32 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 19.2, 23.1, 27.90, 27.93, 29.4, 29.6, 31.6, 45.5, 52.3, 60.8, 63.7, 67.1, 68.6, 71.1, 82.3, 127.95, 127.99, 128.2, 128.4, 128.6, 136.3, 155.8, 170.8, 171.1, 175.4 ppm. MS (ES^+): *m*/*z* = 477.1 [M + H]$^+$, 421.1 [M +

H – *t*Bu]$^+$, 377.1 [M + H – CO_2*t*Bu]$^+$, 499.1 [M + Na]$^+$. HRMS (ES$^+$): calcd. for $C_{25}H_{37}N_2O_7$ 477.2601; found 477.2603.

***tert*-Butyl (*S*)-1-{(*S*)-4-*tert*-Butoxy-4-oxo-3-[2-(trimethylsilyl)-ethylsulfonamido]butyl}-5-oxopyrrolidine-2-carboxylate (23):** Palladium on charcoal (110 mg, 20% weight) was added to a solution of **22** (550 mg, 1.16 mmol) in anhydrous ethyl acetate (15 mL). The air in the apparatus was eliminated and replaced by argon with three cycles of vacuum/argon and then by hydrogen with three cycles of vacuum/hydrogen. The reaction mixture was stirred vigorously for 10 h, filtered through a pad of Celite and concentrated to afford the free amino compound as a colourless oil (388 mg, 98%). R_f ($CHCl_3$/MeOH/AcOH 37%, 24:2:1) = 0.25. $[\alpha]_D^{24}$ = –2.9 (*c* = 1.5, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.39 (s, 18 H), 1.50–1.69 (m, 3 H), 1.75–2.03 (m, 2 H), 2.11–2.45 (m, 3 H), 2.86–3.04 (m, 1 H), 3.11–3.14 (m, 1 H), 3.68–3.81 (m, 1 H), 3.89–4.09 (m, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 22.98, 23.08, 27.8, 27.9, 29.4, 31.9, 32.2, 38.6, 39.2, 52.3, 60.8, 61.2, 80.9, 82.3, 171.8, 174.1, 175.4 ppm. MS (ES$^+$): *m*/*z* = 343.1 [M + H]$^+$, 287.0 [M – *t*Bu]$^+$.

TEA was added (0.4 mL, 2.97 mmol) to a solution of the resulting amino compound (369 mg, 1.1 mmol) in anhydrous dimethylformamide (7 mL). The reaction mixture was cooled to 0 °C. A solution of 2-(trimethylsilyl)ethanesulfonyl chloride (396 mg, 1.76 mmol) in anhydrous dimethylformamide (2 mL) was added over a 10 min period. The reaction mixture was stirred overnight at 0 °C. The dimethylformamide was evaporated and the crude was partitioned between ethyl acetate (5 mL) and water (10 mL). The aqueous phase was extracted with ethyl acetate (2 × 5 mL). The combined organic phases were washed with brine (10 mL), dried with anhydrous sodium sulfate, filtered and concentrated to afford a yellow oil, which was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 8:2 then 7:3) to afford **23** as a yellow oil (323 mg, 58%). R_f (petroleum ether/ethyl acetate, 6:4) = 0.52. $[\alpha]_D^{24}$ = –5.4 (*c* = 3.24, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = –0.10 (s, 9 H), 0.89–1.15 (m, 2 H), 1.39 (s, 9 H), 1.45 (s, 9 H), 1.80–2.08 (m, 3 H), 2.13–2.49 (m, 3 H), 2.76–2.97 (m, 2 H), 2.99–3.15 (m, 1 H), 3.05–3.21 (m, 1 H), 3.80–3.98 (m, 1 H), 4.01–4.13 (m, 1 H), 5.35 (d, *J* = 9.01 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = –0.34, –0.00, 0.34, 12.26, 25.08, 29.80, 29.93, 31.44, 32.59, 40.65, 51.31, 56.77, 62.66, 84.32, 84.93, 173.09, 173.13, 177.40 ppm. MS (ES$^+$): *m*/*z* = 507.2 [M + H]$^+$, 529.0 [M + Na]$^+$, 451.0 [M – *t*Bu]$^+$. HRMS (ES$^+$): calcd. for $C_{22}H_{43}N_2O_7SSi$ 507.2560; found 507.2558.

***tert*-Butyl (*S*)-1-[(*S*)-3-{*N*-[(*S*)-3-(Benzyloxycarbonylamino)-5,5-dimethyl-4-oxohexyl]-2-(trimethylsilyl)ethylsulfonamido}-4-*tert*-butoxy-4-oxobutyl]-5-oxopyrrolidine-2-carboxylate (24):** Caesium carbonate (147 mg, 0.45 mmol) was added to a solution of **23** (150 mg, 0.31 mmol) in anhydrous acetonitrile (4 mL). The reaction mixture was heated at 55 °C for 30 min whilst being stirred vigorously. A solution of **4** (151 mg, 0.36 mmol) in anhydrous acetonitrile (2 mL) was then added dropwise through a syringe pump. The reaction mixture was stirred overnight at 55 °C. The reaction mixture was concentrated and the crude was partitioned between ethyl acetate (4 mL) and distilled water (6 mL). The aqueous layer was extracted with ethyl acetate (3 × 3 mL). The organic layers were combined and successively washed with brine (6 mL) and distilled water (6 mL), then dried with anhydrous magnesium sulfate and concentrated. Compound **24** was obtained after silica gel chromatography (petroleum ether/diethyl ether, 4:6) as a yellow oil (129 mg, 52%). R_f (petroleum ether/ethyl acetate, 6:4) = 0.62. HPLC: *t* = 3.18 min. $[\alpha]_D^{24}$ = –17.4 (*c* = 1.9, DCM). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.00 (s, 9 H), 0.91–1.27 (m, 2 H), 1.43 (s, 27 H), 1.56–1.80 (m, 1 H), 1.92–2.05 (m, 1 H), 2.09–2.15 (m, 2 H), 2.16–2.19 (m, 1 H), 2.40–2.51 (m, 1 H), 2.75–2.99 (m, 4 H), 3.20–3.31 (m, 2 H), 3.51–3.79 (m, 1 H), 3.81–4.35 (m, 2 H), 4.39–4.55 (m, 2 H), 5.08 (d, *J* = 5.11 Hz, 2 H), 5.48 (d, *J* = 7.07 Hz, 1 H), 7.28–7.38 (m, 5 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 0.0, 12.1, 24.0, 25.3, 29.9, 30.7, 31.5, 31.6, 32.7, 36.9, 41.6, 45.1, 50.8, 54.7, 60.5, 62.75, 68.8, 84.3, 84.77, 84.83, 129.8, 130.0, 130.1, 138.3, 158.01, 171.5, 172.3, 173.8, 177.9 ppm. MS (ES$^+$): *m*/*z* = 798.3 [M + H]$^+$, 820.2 [M + Na]$^+$, 742.2 [M – *t*Bu]$^+$, 686.1 [M – 2 *t*Bu]$^+$, 630.0 [M – CO_2*t*Bu – *t*Bu]$^+$. HRMS (ES$^+$): calcd. for $C_{38}H_{64}N_3O_{11}SSi$ 798.4031; found 798.4038.

(*S*)-1-{(*S*)-3-[(*S*)-3-Amino-3-carboxypropylamino]-3-carboxypropyl}-5-oxopyrrolidine-2-carboxylic Acid (12): Hydrofluoric acid (2 mL, large excess) was added with the help of a Teflon® apparatus to a Teflon® vessel containing **24** (110 mg, 0.14 mmol). The reaction mixture was stirred at 0 °C for 1.5 h. The hydrofluoric acid was evaporated and the remaining neutralized by addition of potassium hydroxide. The crude was dissolved in distilled water (5 mL) and the aqueous phase washed with diethyl ether (3 × 5 mL). After freeze-drying, compound **12** was obtained as a pure white solid (45 mg, 98%); m.p. 210–212 °C. $[\alpha]_D^{24}$ = –35.4 (*c* = 1.2, H_2O). ^{1}H NMR (D_2O, 300 MHz): δ = 1.95–2.28 (m, 4 H), 2.35–2.52 (m, 3 H), 3.05–3.29 (m, 3 H), 3.51–3.71 (m, 3 H), 3.79–3.90 (m, 1 H), 4.20–4.39 (m, 1 H) ppm. ^{13}C NMR (D_2O, 75 MHz): δ = 22.4, 26.6, 27.0, 29.5, 38.1, 43.5, 52.2, 59.6, 61.6, 172.6, 177.4, 179.0, 179.8 ppm. MS (ES$^+$): *m*/*z* = 332.2 [M + H]$^+$. HRMS (FAB$^+$): calcd. for $C_{13}H_{22}N_3O_7$ 332.1458; found 332.1447.

Supporting Information (see also the footnote on the first page of this article): ^{1}H and ^{13}C NMR and mass spectra of all described compounds.

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