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Synthesis of (2*S*,3*S*)-3′-fluoroisoleucine

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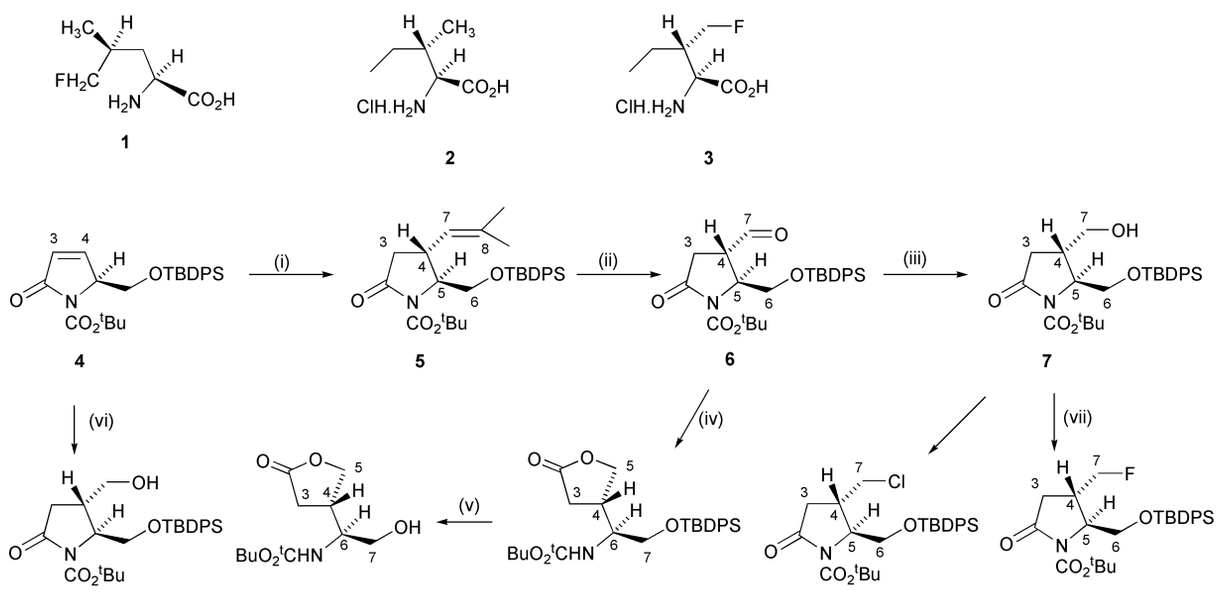
The fluorinated amino acid, (2*S*,3*S*)-3′-fluoroisoleucine, **3**, has been synthesised by a route involving stereoselective cuprate or photochemical addition to the compound **4**, derived in turn from (2*S*)-pyroglutamic acid. This provides a reporter group for the hydrophobic amino acid (2*S*)-isoleucine for use in protein studies.

Introduction

Our interest in the use of fluorinated amino acids as reporter groups for protein studies was stimulated by our work on the binding of substrates and inhibitors to the anti-cancer drug target enzyme, dihydrofolate reductase.[1] As part of these studies, we prepared a sample of the enzyme in which all thirteen leucine residues were substituted by (2*S*,4*S*)-5-fluoroleucine, **1**.[2] The biological methods used gave protein in which *every* leucine residue was labelled and there was incomplete incorporation at each position. Use of fluorinated amino acids as reporter groups in studies of protein interactions would ideally require incorporation at specific strategically relevant positions and with complete incorporation at these positions. We therefore turned to solid-state protein synthesis to achieve this goal. The small, highly conserved protein, ubiquitin, important in a number of cell regulatory mechanisms including cell cycle control, was chosen as our template, and we completed a synthesis of a ubiquitin "mutant" in which the leucine residues 50 and 67, close neighbours in the hydrophobic core, were replaced by (2*S*,4*S*)-5-fluoroleucine, **1**.[3] This allowed us to complete a variety of studies on the mutant and to show its promise for the study of protein folding.[3] After leucine, the most common hydrophobic aliphatic amino acid in ubiquitin is isoleucine **2**[4] and the availability of a sample of this amino acid containing a fluorine reporter group will enable the use of fluorinated amino acids in studying a variety of protein interactions to be expanded. We therefore decided to initiate a synthesis of (2*S*,3*S*)-3′-fluoroisoleucine, **3**, having the stereochemistry found in the natural amino acid.

Results and discussion

The starting point for our synthesis was (5*S*)-*N*-*tert*-butoxycarbonyl-1*H*-5-*tert*-butyldiphenylsiloxymethylpyrrol-2(5*H*)-one **4** which had been shown to react with alkyl cuprates at C-4 entirely from the less hindered face.[5] We therefore prepared (5*S*)-*N*-*tert*-butoxycarbonyl-5-*tert*-butyldiphenylsiloxymethylpyrrol-2(5*H*)-one **4** from (2*S*)-pyroglutamic acid by the method of Shimamoto[6] and reacted it with 2-methyl-1-propenylmagnesium bromide and copper bromide–dimethyl sulfide complex to obtain the adduct **5** as a single diastereoisomer in 68% yield as shown in Scheme 1. Ozonolysis to the aldehyde **6** in 84% yield followed by reduction using sodium borohydride in methanol gave the alcohol **7** in 75% yield. On one occasion reduction of the aldehyde using sodium borohydride in methanol gave a product in 45% yield which was evidently not the alcohol **7** but which had an exchangeable one proton doublet at 4.78 ppm characteristic of ring opening of the lactam. The other spectra were consistent with the lactone structure **8** which would have been formed by intramolecular nucleophilic reaction of the alcohol **7** with the reactive amide/urethane



Scheme 1 *Reagents and conditions* (i) $Me_2C{=}CHMgBr/CuBr{\cdot}SMe_2/THF/-78$ °C, 68%; (ii) (a) $O_3/CH_2Cl_2/-78$ °C, (b) Zn/HOAc, 84%; (iii) $NaBH_4$/MeOH, 75%; (iv) $NaBH_4$/MeOH, 45%; (v) Bu_4NF/THF, quant; (vi) *hν*/$Ph_2C{=}O$/MeOH, 68%; (vii) Et_2NSF_3/CH_2Cl_2, 61%.

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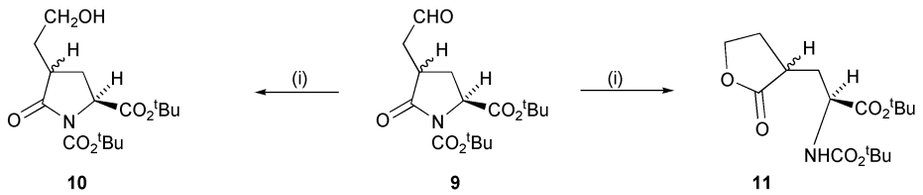


Scheme 2 *Reagents and conditions* (i) ref. 7.

system in the basic conditions of the reaction. This irreproducibility and the γ-lactone side-product were reminiscent of our attempts to reduce the aldehyde **9** consistently to the alcohol **10** with $NaB(CN)H_3$ when the lactone **11** was obtained in some instances (Scheme 2).[7] In both reactions, the alcohol group of the expected product was separated from the electrophilic ring carbonyl atom by three carbon atoms making γ-lactone formation possible. We confirmed the structure of the oily product **8** by deprotection using tetrabutylammonium fluoride to obtain the crystalline alcohol **12** in quantitative yield (Scheme 1). The X-ray crystal structure (Fig. 1) of this compound was as expected.

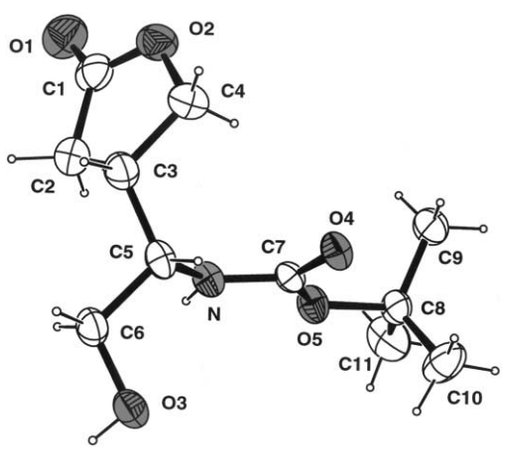


Fig. 1 X-ray structure of compound **12**.

Because of the problems associated with reduction of the aldehyde **6**, we investigated application of a direct photochemical addition of methanol to the enone **4** discovered by Mann *et al.*[8] Irradiation of a solution of the dihydropyrrolone **4** in methanol containing benzophenone as photoexitant gave a 68% yield of the alcohol **7**, identical in all respects with the sample prepared by reduction of the aldehyde **6**. The alcohol was now treated with diethylaminosulfur trifluoride in dichloromethane which had been passed through a column of basic alumina prior to use, to give the fluoride **13** in 61% yield. Failure to use the basic alumina step resulted in production of the chloride **14** in 87% yield. The stereochemistry of the fluoride **13** was now ascertained using X-ray crystallography (Fig. 2), thus confirming the stereochemistry both of the cuprate addition and of the photochemical addition of methanol to the enone **3**.

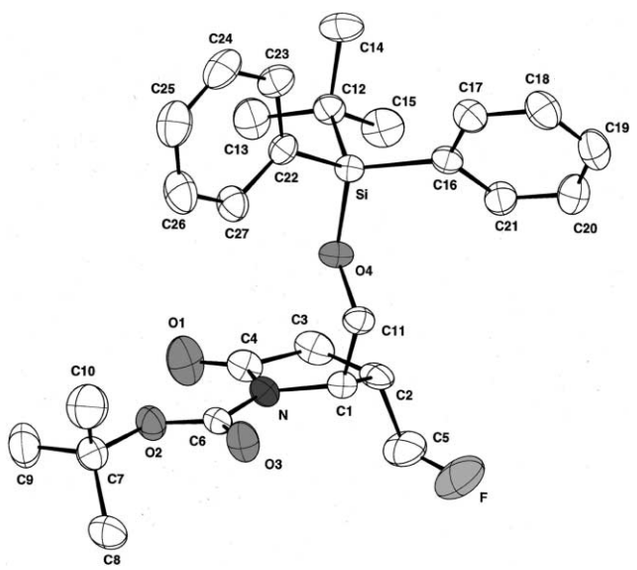
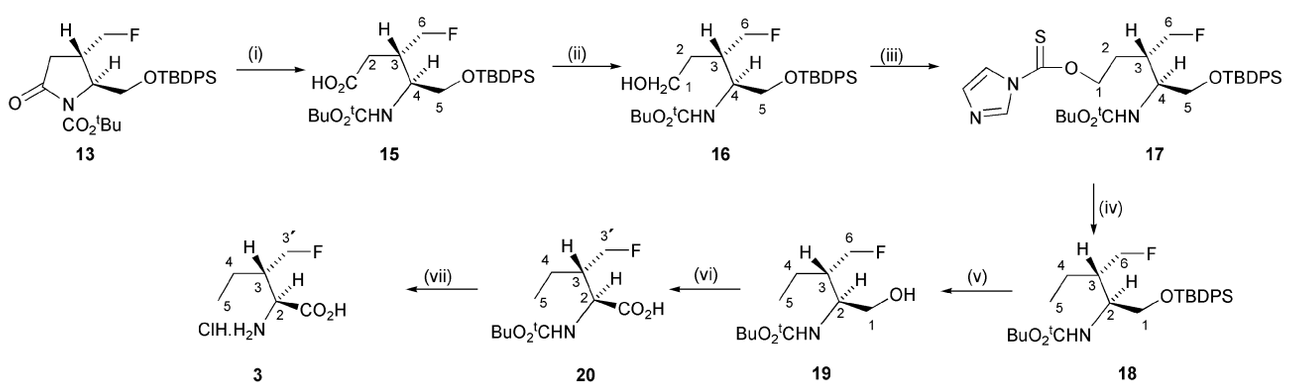


Fig. 2 X-ray structure of compound **13**.

Hydrolysis of the lactam-urethane **13** with aqueous lithium hydroxide in tetrahydrofuran as shown in Scheme 3 gave the acid **15** in quantitative yield, and this was reacted with *iso*-butyl chloroformate to give the mixed anhydride which was reduced *in situ* to the alcohol **16** in 83% yield. Barton, McCombie radical deoxygenation was now employed to complete the fluoroisoleucine side chain. The imidazolethiocarbamate **17** was prepared in quantitative yield and this was reduced with tri-n-butyltin hydride and 2,2′-azobis(2-methylpropionitrile) to yield the protected alcohol **18** in 65% yield.

It now remained to convert the protected alcohol to an acid. This was achieved by first deprotecting the silyl ether **18** in quantitative yield using tetrabutylammonium fluoride in tetrahydrofuran. The resultant alcohol **19** was now oxidised with



Scheme 3 *Reagents and conditions* (i) 1 M aq LiOH/THF/0 °C, quant.; (ii) (a) $i BuO_2CCl/Et_3N/THF$ −40 °C, (b) $NaBH_4/THF/H_2O$, 83%; (iii) thiocarbonyldiimidazole/CH_2Cl_2, quant.; (iv) $Bu_3SnH/AIBN/\Delta$, 65%; (v) Bu_4NF/THF, quant; (vi) $RuCl_3/NaIO_4/CH_3CN/CCl_4/H_2O$, 84%; (vii) 6 M aq HCl/40 °C, 94%.

ruthenium tetroxide, generated *in situ* from ruthenium trichloride and sodium periodate, to give the acid **20** in 84% yield. This was deprotected without further purification using 6 M hydrochloric acid to yield (2*S*,3*S*)-3′-fluoroisoleucine **3** in 94% yield.

We have therefore prepared the target amino acid (2*S*,3*S*)-3′-fluoroisoleucine **3** making it available for incorporation into strategic positions of proteins. This will allow ^{19}F-NMR spectroscopy to be used in the study of a variety of protein interactions.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotation measurements (in units of 10^{-1} deg cm^2 g^{-1}) were obtained on a Perkin Elmer PE241 polarimeter using a 1 dm path length micro cell. IR spectra were recorded on a Perkin Elmer 1720 Fourier Transform spectrometer. ^{1}H-NMR spectra were recorded on Bruker DPX 300 (300 MHz) and AMX 500 (500 MHz) Fourier-transform instruments. ^{13}C-NMR spectra were recorded on a Bruker DPX 300 (75.5 MHz) Fourier-transform instrument. DEPT experiments were used to assign ^{13}C-NMR spectra where necessary. ^{19}F-NMR spectra were recorded on a Bruker DPX 300 (282 MHz) Fourier-transform instrument and were referenced to $CFCl_3$ (0.00 ppm). All ^{1}H- and ^{13}C-spectra were recorded using TMS, 3-trimethylsilylpropanesulfonic acid (DSS) (0.00 ppm) or residual solvent peaks as internal references. δ are given in ppm and J in Hertz (Hz). Low-resolution mass spectra were recorded on Kratos MS80F and MS25 double focusing spectrometers. High-resolution mass measurements were recorded by the EPSRC National Mass Spectrometry Service (Swansea). Microanalyses were performed at Medac Ltd. Solvents were freshly distilled before use. Column chromatography was performed using Merck Kieselgel 60 (230–400 mesh) – Art 9385 and Sorbisil C60 40/60A. Petroleum ether refers to that fraction of hexanes of bp 60–80 °C.

(4*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-(2-methylprop-1-enyl)pyrrolidin-2-one (5)

2-Methyl-1-propenylmagnesium bromide (0.5 M in tetrahydrofuran, 44.4 ml, 22.2 mmol) was added to a solution of copper bromide–dimethyl sulfide complex (2.28 g, 11.1 mmol) in dry tetrahydrofuran (20 ml) at 15 °C. After 15 min the mixture was cooled to −78 °C. A solution of (5*S*)-*N*-*tert*-butoxycarbonyl-1*H*-5-*tert*-butyldiphenylsiloxymethylpyrrol-2(5*H*)-one **4**[6] (1 g, 2.22 mmol) and trimethylsilyl chloride in (1 M in tetrahydrofuran, 4.43 ml, 4.43 mmol) in tetrahydrofuran (10 ml) was added dropwise over 15 min. After 1 h, aqueous ammonium chloride (20 ml) was added and the mixture was allowed to warm to room temperature. The brown mixture was filtered through a pad of Celite® and the filtrate was extracted with ethyl acetate. The organic layers were washed with aqueous ammonium chloride until the aqueous layer was no longer blue. The organic layer was dried ($MgSO_4$) and the solvents were removed *in vacuo*. The residue was chromatographed on silica gel using petroleum ether : ethyl acetate (9 : 1) as eluent to afford *(4R,5S)-N-tert-butoxycarbonyl-5-tert-butyldiphenylsilyloxymethyl-4-(2-methylprop-1-enyl)pyrrolidin-2-one* **5** as a colourless oil (757 mg, 68%), $[\alpha]^{22}_D$ −42.76 (*c* 0.72, $CHCl_3$); *m*/*z* [FAB, PEG/NBA] Found: 530.2720 ([M + Na]$^+$), [$C_{30}H_{41}NO_4Si$ + Na] requires: 530.2703; *m*/*z* [+ve FAB, NBA] 530 ([M + Na]$^+$); ν_{max} (KBr)/cm^{-1} 1782 and 1752 (urethane) and 1719; δ_H (300 MHz, $C_6^2H_6$) 1.13 [9H, s, $SiC(CH_3)_3$], 1.36 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.45 [9H, s, $OC(CH_3)_3$], 2.00 (1H, dd, $J_{3A,3B}$ 17.2, $J_{3A,4}$ 3.1, H-3A), 2.78 (1H, dd, $J_{3B,3A}$ 17.2, $J_{3B,4}$ 8.8, H-3B), 3.03 (1H, m, H-4), 3.69 (1H, dd, $J_{6A,6B}$ 10.3, $J_{6A,5}$ 2.1, H-6A), 3.93 (1H, m, H-5), 4.06 (1H, dd, $J_{6B,6A}$ 10.3, $J_{6B,5}$ 4.7, H-6B), 4.92 (1H, d, $J_{7,4}$ 9.4, H-7), 7.16–7.26 (6H, m, aromatics) and 7.70–7.72 (4H, m, aromatics); δ_C (75.5 MHz, $C_6^2H_6$) 17.9 (CH_3), 19.4 [Si*C*$(CH_3)_3$], 25.4 (CH_3), 27.0 [SiC-(*C*$H_3)_3$], 28.1 [OC(*C*$H_3)_3$], 32.4 (C-4), 39.4 (C-3), 63.9 (C-6), 65.7 (C-5), 82.1 [O*C*$(CH_3)_3$], 127.2 (C-7), 128.1 and 130.1 (aromatics), 133.0 (C-8), 133.4, 133.5 and 136.0 (aromatics), 151.2 (urethane) and 172.1 (lactam).

(4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-2-oxopyrrolidine-4-carbaldehyde (6)

(4*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-(2-methylprop-1-enyl)pyrrolidin-2-one **5** (4.20 g, 8.3 mmol) was dissolved in dichloromethane (100 ml) under nitrogen and the solution was cooled to −78 °C. Nitrogen was displaced by oxygen over 15 min and ozone was passed through the solution until it became blue. Excess ozone was removed by passing a stream of nitrogen through the solution. Zinc dust (5.73 g, 87.6 mmol) and acetic acid (5.02 ml, 87.7 mmol) were added and the mixture was allowed to warm to room temperature and stirred for a further 1 h. The mixture was filtered through a pad of Celite® and the solvents were removed *in vacuo*. The residue was chromatographed on silica gel using petroleum ether : ethyl acetate (7 : 3) as eluent to afford *(4S,5S)-N-tert-butoxycarbonyl-5-tert-butyldiphenylsilyloxymethyl-2-oxopyrrolidin-4-carbaldehyde* **6** as a colourless oil (3.36 g, 84%), $[\alpha]^{22}_D$ +1.49 (*c* 0.97, $CHCl_3$); *m*/*z* [+ve FAB, NBA] 504 ([M + Na]$^+$) and 404 ([M − Boc + Na]$^+$); ν_{max} (KBr)/cm^{-1} 1782 (urethane) and 1719 (aldehyde); δ_H (300 MHz, $C_6^2H_6$) 1.08 [9H, s, $SiC(CH_3)_3$], 1.38 [9H, s, $OC(CH_3)_3$], 2.34 (1H, d, $J_{4,3B}$ 9.1, H-4), 2.50 (1H, d, $J_{3A,3B}$ 16.1, H-3A), 2.61 (1H, dd, $J_{3B,3A}$ 16.1, $J_{3B,4}$ 9.1, H-3B), 3.41 (1H, d, $J_{6A,6B}$ 10.4, H-6A), 4.03 (1H, dd, $J_{6B,6A}$ 10.4, $J_{6B,5}$ 2.2, H-6B), 4.41 (1H, d, $J_{5,6B}$ 2.2, H-5), 7.16–7.25 (6H, m, aromatics), 7.68–7.71 (4H, m, aromatics) and 8.86 (1H, s, H-7); δ_C (75.5 MHz, $C_6^2H_6$) 19.3 [Si*C*$(CH_3)_3$], 26.9 [SiC(*C*$H_3)_3$], 28.0 [OC(*C*$H_3)_3$], 32.2 (C-3), 45.6 (C-4), 57.7 (C-5), 65.0 (C-6), 82.6 [O*C*$(CH_3)_3$], 128.0, 130.3, 133.0, 133.3 and 135.9 (aromatics), 150.6 (urethane), 170.4 (lactam) and 197.8 (aldehyde).

(4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-hydroxymethylpyrrolidin-2-one (7)

Method A. A solution of (4*S*,5*S*)-*N*-*tert*-butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-2-oxopyrrolidine-4-carbaldehyde **6** (3.658 g, 7.60 mmol) in methanol (100 ml) was cooled to 0 °C and sodium borohydride (316 mg, 8.36 mmol) was added in small portions. The solution was stirred for 25 min at room temperature. The solvent was removed *in vacuo*, the residue was extracted with ethyl acetate and the extracts were washed with aqueous ammonium chloride. The organic layer was dried ($MgSO_4$) and the solvents were removed *in vacuo*. The residue was chromatographed on silica gel using petroleum ether : ethyl acetate (6 : 4) as eluent to afford *(4S,5S)-N-tert-butoxycarbonyl-5-tert-butyldiphenylsilyloxymethyl-4-hydroxymethylpyrrolidin-2-one* **7** as a colourless oil which crystallised as a white solid on standing (2.77 g, 75%), mp 132–134 °C; $[\alpha]^{22}_D$ −21.3 (*c* 1.0, $CHCl_3$); *m*/*z* [FAB, PEG/NBA] Found: 506.2331 ([M + Na]$^+$), [$C_{27}H_{37}NO_5Si$ + Na] requires: 506.2339); *m*/*z* [+ve FAB, NBA] 506 ([M + Na]$^+$) and 384 ([M − Boc + H]$^+$); ν_{max} (KBr)/cm^{-1} 3472 (OH), 1775 and 1736 (urethane) and 1697; δ_H (300 MHz, $C_6^2H_6$) 1.11 [9H, s, $SiC(CH_3)_3$], 1.42 [9H, s, $OC(CH_3)_3$], 1.78 (1H, br s, OH), 2.12 (1H, dd, $J_{3A,3B}$ 17.8, $J_{3A,4}$ 1.7, H-3A), 2.20 (1H, m, H-4), 2.82 (1H, dd, $J_{3B,3A}$ 17.6, $J_{3B,4}$ 9.2, H-3B), 3.07 (2H, d, $J_{7,4}$ 6.5, H-7), 3.59 (1H, dd, $J_{6A,6B}$ 11.3, $J_{6A,5}$ 3.2, H-6A), 4.11 (2H, m, H-6B + H-5), 7.16–7.29 (6H, m, aromatics) and 7.67–7.72 (4H, m, aromatics); δ_C (75.5 MHz, $C_6^2H_6$) 19.4 [Si*C*$(CH_3)_3$], 27.0 [SiC(*C*$H_3)_3$], 28.1 [OC(*C*$H_3)_3$], 36.0 (C-3), 36.2 (C-4), 61.6 (C-5), 64.7 (C-6), 65.4 (C-7), 82.2 [O*C*$(CH_3)_3$], 128.2, 130.1, 133.3, 133.6 and 135.9 (aromatics), 151.0 (urethane) and 173.4 (lactam).

Method B. (5*S*)-*N*-*tert*-Butoxycarbonyl-1*H*-5-*tert*-butyldiphenylsilyloxymethylpyrrol-2(5*H*)-one **4** (2.50 g, 5.54 mmol) and benzophenone (1.01 g, 5.52 mmol) were dissolved in methanol (2 l) in a Pyrex vessel. The solution was degassed by passing nitrogen through it for 1.5 h and irradiated for 24 h, at room temperature, using a medium pressure immersion mercury vapour lamp fitted with a Pyrex filter. The solvents were removed *in vacuo* to yield a clear yellow oil. Flash column chromatography on silica gel using petroleum ether : ethyl acetate (6 : 4) as eluent gave *(4S,5S)-N-tert-butoxycarbonyl-5-tert-butyldiphenylsilyloxymethyl-4-hydroxymethylpyrrolidin-2-one* **7** as a clear colourless oil which crystallised on standing (1.82 g, 68%); mp 133–134 °C; $[\alpha]_D^{24}$ −22.0 (*c* 1.0, $CHCl_3$) with identical spectra to those for the sample prepared by method A above.

(1′*S*,4*S*)-4-(1-*N*-*tert*-Butoxycarbonylamino-2-*tert*-butyldiphenylsilyloxyethyl)-dihydrofuran-2(3*H*)-one (8)

Sodium borohydride (790 mg, 20.83 mmol) was added to a solution of (4*S*,5*S*)-*N*-*tert*-butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-2-oxopyrrolidine-4-carbaldehyde **6** (5.01 g, 10.42 mmol) in methanol (100 ml) cooled to 0 °C. The solution was stirred for 2 h at room temperature. The solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate (100 ml) and washed with saturated aqueous ammonium chloride (2 × 25 ml). The organic layer was dried ($MgSO_4$) and the solvents were removed *in vacuo*. The product was purified by flash column chromatography on silica gel using petroleum ether : ethyl acetate (3 : 1) as eluent to afford *(1′S,4S)-4-(1-N-tert-butoxycarbonylamino-2-tert-butyldiphenylsilyloxyethyl)-dihydrofuran-2(3H)-one* **8** as a clear colourless oil which crystallised on standing (2.26 g, 45%); mp 70–72 °C; $[\alpha]_D^{27}$ +14.1 (*c* 1.0, $CHCl_3$); (Found: C, 67.05; H, 7.8; N, 2.8. $C_{27}H_{37}NO_5Si$ requires: C, 67.1; H, 7.7; N, 2.9%); *m*/*z* [+ve FAB (3-NBA)] 506 $[M + Na]^+$ and 484 $[M + H]^+$; ν_{max} (KBr) /cm^{-1} 3520 (NH), 1779 (lactone) and 1675 (urethane); δ_H (500 MHz, C^2HCl_3) 7.63–7.6 (4H, m, aromatics), 7.48–7.37 (6H, m, aromatics), 4.78 (1H, d, $J_{NH,6}$ 9.1, NH), 4.20 (1H, dd, $J_{5A,5B}$ 8.6, $J_{5A,4}$ 8.5, H-5A), 3.94 (1H, dd, $J_{5B,5A}$ 8.6, $J_{5B,4}$ 8.5, H-5B), 3.77 (1H, m, H-6), 3.67 (1H, dd, $J_{7A,7B}$ 10.6, $J_{7A,5}$ 4.3, H-7A), 3.56 (1H, dd, $J_{7B,7A}$ 10.6, $J_{7B,5}$ 3.5, H-7B), 2.84 (1H, m, H-4), 2.52 (1H, dd, $J_{3A,3B}$ 17.7, $J_{3A,4}$ 8.7, H-3A), 2.47 (1H, dd, $J_{3A,3B}$ 17.7, $J_{3B,4}$ 9.8, H-3B), 1.44 (9H, s, $C(CH_3)_3$) and 1.07 (9H, s, $OSiC(CH_3)_3$).

(1′*S*,4*S*)-4-(1-*N*-*tert*-Butoxycarbonylamino-2-hydroxyethyl)-dihydrofuran-2(3*H*)-one (12)

Tetrabutylammonium fluoride (1.0 M in tetrahydrofuran, 0.10 ml, 0.10 mmol) was added to a solution of (1′*S*,4*S*)-4-(1-(*N*-*tert*-butoxycarbonylamino-2-*tert*-butyldiphenylsilyloxyethyl)-dihydrofuran-2(3*H*)-one **8** (50 mg, 0.10 mmol) in tetrahydrofuran (1 ml) at room temperature. The reaction was stirred at room temperature under nitrogen for 16 h. Saturated aqueous ammonium chloride (1 ml) was carefully added and the crude product was extracted into ethyl acetate (3 × 5 ml). The organic extracts were washed with water (2 ml) and brine (2 ml). The organic layer was dried ($MgSO_4$) and the solvents were removed *in vacuo*. The crude product was purified by flash column chromatography on silica gel using petroleum ether : ethyl acetate (1 : 1) as eluent, followed by recrystallisation from petroleum ether to give *(1′S,4S)-4-(1-N-tert-butoxycarbonylamino-2-hydroxyethyl)-dihydrofuran-2(3H)-one* **12** as a white crystalline solid (25 mg, quantitative), mp 76–77 °C, $[\alpha]_D^{22}$ +9.1 (*c* 1.0, $CHCl_3$); (Found: C, 53.6; H, 7.9; N, 5.7. $C_{11}H_{19}NO_5$ requires: C, 53.9; H, 7.8; N, 5.7%); *m*/*z* $[EI]^+$ 246 $[M + H]^+$ and 146 $[M - Boc]^+$; ν_{max} (KBr) /cm^{-1} 3346 (OH), 1768 (lactone) and 1690 (urethane); δ_H (300 MHz, C^2HCl_3) 5.02 (1H, d, $J_{NH,6}$ 8.0, NH), 4.44 (1H, dd, $J_{5A,5B}$ 9.0, $J_{5A,4}$ 8.5, H-5A), 4.11 (1H, dd, $J_{5B,5A}$ 9.0, $J_{5B,4}$ 8.4, H-5B), 3.72 (1H, m, H-6), 3.65 (2H, m, H-7), 2.89 (1H, m, H-4), 2.59 (1H, dd, $J_{3A,3B}$ 17.7, $J_{3A,4}$ 8.7, H-3A), 2.49 (1H, dd, $J_{3B,3A}$ 17.7, $J_{3B,4}$ 9.8, H-3B), 2.30 (1H, br s, OH) and 1.45 (9H, s, $C(CH_3)_3$); δ_C (75.5 MHz, C^2HCl_3) 176.8 (lactone), 156.3 (urethane), 80.4 ($OC(CH_3)_3$), 70.5 (C-5), 63.6 (C-7), 53.1 (C-6), 38.0 (C-4), 31.3 (C-3) and 28.3 ($C(CH_3)_3$).

Crystal data: compound 12†

$C_{11}H_{19}NO_5$, M = 245.27, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 6.4668(5), b = 10.4584(4), c = 19.4553(12) Å, V = 1315.8(1) Å^3, Z = 4, D_{calc} = 1.24 Mg m^{-3}, $F(000)$ = 528, μ(Mo-Kα) = 0.10 mm^{-1}, T = 173(2) K, 6633 total reflections measured, 2280 independent reflections collected on a Nonius Kappa CCD diffractometer (R_{int} = 0.060) using Mo-Kα radiation (λ = 0.71073 Å). Refinement using SHELXL-97. Final residues were $R1 = 0.040$, $wR2 = 0.102$ (for 2115 reflections with $I > 2\sigma(I)$), $R1 = 0.044$, $wR2 = 0.105$ for all reflections.

(4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-fluoromethylpyrrolidin-2-one (13)

Diethylaminosulfur trifluoride (1.09 ml, 8.28 mmol) was added to a solution of (4*S*,5*S*)-*N*-*tert*-butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-hydroxymethylpyrrolidin-2-one **7** (1.333 g, 2.76 mmol) in dichloromethane (35 ml) and the solution was stirred for 2 h at room temperature. Saturated aqueous sodium bicarbonate was added and the mixture was extracted with dichloromethane. The organic extracts were washed with saturated aqueous sodium bicarbonate and dried ($MgSO_4$). The solvents were removed *in vacuo* at room temperature. The residue was chromatographed on silica gel using petroleum ether : ethyl acetate (4 : 1) as eluent to afford *(4S,5S)-N-tert-butoxycarbonyl-5-tert-butyldiphenylsilyloxymethyl-4-fluoromethylpyrrolidin-2-one* **13** as a colourless oil which crystallised as a white solid on standing (817 mg, 61%), mp 76–78 °C; $[\alpha]^{22}_D$ −32.77 (*c* 1.0, $CHCl_3$); (Found : C, 66.5; H, 7.3; N, 2.8. $C_{27}H_{36}NO_4FSi$ requires: C, 66.8; H, 7.5; N, 2.9%); *m*/*z* [+ve FAB, NBA] 508 ($[M + Na]^+$); ν_{max} (KBr)/cm^{-1} 1754 (urethane) and 1707; δ_H (300 MHz, $C_6{}^2H_6$) 1.09 [9H, s, $SiC(CH_3)_3$], 1.43 [9H, s, $OC(CH_3)_3$], 1.87 (1H, dd, $J_{3A,3B}$ 17.8, $J_{3A,4}$ 1.8, H-3A), 2.21 (1H, m, H-4), 2.69 (1H, dd, $J_{3B,3A}$ 17.8, $J_{3B,4}$ 9.3, H-3B), 3.49 (1H, dd, $J_{6A,6B}$ 10.3, $J_{6A,5}$ 1.3, H-6A), 3.62 (2H, dd, $J_{7,F}$ 47.0, $J_{7,4}$ 6.8, H-7), 4.02 (1H, m, H-5), 4.06 (1H, dd, $J_{6B,6A}$ 10.3, $J_{6B,5}$ 3.3, H-6B), 7.16–7.29 (6H, m, aromatics) and 7.64–7.69 (4H, m, aromatics); δ_C (75.5 MHz, $C_6{}^2H_6$) 19.3 [$SiC(CH_3)_3$], 27.0 [$SiC(CH_3)_3$], 28.1 [$OC(CH_3)_3$], 34.3 (d, $^2J_{CF}$ 26.5, C-4), 34.4 (C-3), 60.4 (d, $^3J_{5,F}$ 5.1, C-5), 65.0 (C-6), 82.3 [$OC(CH_3)_3$], 84.2 (d, $^1J_{7,F}$ 172.3, C-7), 128.2, 130.2, 133.1, 133.4 and 135.9 (aromatics), 150.9 (urethane) and 171.2 (lactam); δ_F (282 MHz, $C_6{}^2H_6$) −221.6 (td, $J_{F,H7}$ 47.0, $J_{F,H4}$ 17.0).

Crystal data: compound 13†

$C_{27}H_{36}FNO_4Si$, M = 485.66, monoclinic, space group $P2_1$ (No. 4), a = 9.811(7), b = 13.067(6), c = 11.121(5) Å, β = 104.99(4)°, V = 1377(1) Å^3, Z = 2, D_{calc} = 1.17 Mg m^{-3}, $F(000)$ = 520, μ(Cu-Kα) = 1.06 mm^{-1}, T = 293(2) K, 2277 total reflections measured, 2152 independent reflections collected on a Nonius CAD4 diffractometer (R_{int} = 0.024) using Cu-Kα radiation (λ = 1.5418 Å). Refinement using SHELXL-93. Final residues were $R1 = 0.064$, $wR2 = 0.163$ (for 1848 reflections with $I > 2\sigma(I)$), $R1 = 0.074$, $wR2 = 0.175$ for all reflections.

(4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-chloromethylpyrrolidin-2-one (14)

(4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-hydroxymethylpyrrolidin-2-one **7** (5.01 g, 10.4 mmol) was dissolved in dichloromethane (75 ml) under nitrogen and cooled to 0 °C. Diethylaminosulfur trifluoride (1.53 ml, 11.5

† CCDC reference numbers 227306 (**12**) and 227307 (**13**). See http://www.rsc.org/suppdata/ob/b3/b316219b/ for crystallographic data in.cif or other electronic format.

mmol) was added to the mixture which was stirred at room temperature for 12 h. Methanol was added and the crude product was extracted into ethyl acetate (3 × 50 ml). The organic layers were washed with brine (25 ml) and water (25 ml) and dried ($MgSO_4$). The solvents were removed *in vacuo* to give a clear colourless oil. Column chromatography on silica gel using petroleum ether : ethyl acetate (3 : 1), followed by recrystallisation from petroleum ether gave *(4S,5S)-N-tert-butoxycarbonyl-5-tert-butyldiphenylsilyloxymethyl-4-chloromethylpyrrolidin-2-one* **14** as a white crystalline solid (4.52 g, 87%), mp 76–77 °C, $[a]_D^{27}$ +30.8 (*c* 1.0, acetone); (Found: C, 64.5; H, 7.3; N, 2.8. $C_{27}H_{36}NO_4SiCl$ requires: C, 64.6; H, 7.2; N, 2.8%); *m/z* [+ve FAB (3-NBA)] 526 and 524 (1 : 3) $[M + Na]^+$ and 504 and 502 (1 : 3) $[M + H]^+$; ν_{max} (KBr)/cm^{-1} 3482 (NH) and 1751 (urethane); δ_H (300 MHz, C^2HCl_3) 7.68–7.45 (4H, m, aromatics), 7.40–7.22 (6H, m, aromatics), 4.01 (1H, m, H-5), 3.86 (1H, dd, $J_{6A,6B}$ 10.6, $J_{6A,5}$ 3.6, H-6A), 3.65 (1H, dd, $J_{6B,6A}$ 10.6, $J_{6B,5}$ 1.6, H-6B), 3.46 (2H, d, $J_{7,4}$ 7.1, H-7), 2.96 (1H, dd, $J_{3A,3B}$ 18.0, $J_{3A,4}$ 9.3, H-3A), 2.63 (1H, m, H-4), 2.27 (1H, m, $J_{3B,3A}$ 18.0, H-3B), 1.35 (9H, s, $C(CH_3)_3$) and 1.00 (9H, s, $OSiC(CH_3)_3$); δ_C (75.5 MHz, C^2HCl_3) 173.0 (lactam), 150.0 (urethane), 135.9, 133.2, 132.7, 130.4 and 128.3 (aromatics), 83.6 ($OC(CH_3)_3$), 65.1 (C-7), 62.6 (C-5), 47.7 (C-6), 37.3 (C-3), 36.5 (C-4), 28.4 ($C(CH_3)_3$), 27.2 ($OSiC(CH_3)_3$) and 19.6 ($OSiC(CH_3)_3$).

(3*S*,4*S*)-4-*tert*-Butoxycarbonylamino-5-*tert*-butyldiphenylsilyloxy-3-fluoromethylpentanoic acid (15)

(4*S*,5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-flurooromethylpyrrolidin-2-one **13** (97 mg, 0.20 mmol) was dissolved in tetrahydrofuran (2 ml) and cooled to 0 °C. 1 M Aqueous lithium hydroxide (0.24 ml, 0.24 mmol) was added dropwise to the solution. The reaction was stirred for 1 h at 0 °C and a further 2 h at room temperature. Ethyl acetate (5 ml) and saturated aqueous sodium hydrogen carbonate (5 ml) were added and the reaction was stirred for 1 h. The organic layer was separated and the aqueous layer was further extracted with ethyl acetate (3 × 5 ml). The aqueous layer was cooled to 0 °C and acidified with 1 M hydrochloric acid. The acidified aqueous layer was extracted with ethyl acetate (5 × 5 ml). The organic layers were combined, washed with water (10 ml) and brine (10 ml) and dried ($MgSO_4$). The residual colourless oil, *(3S,4S)-4-tert-butoxycarbonylamino-5-tert-butyldiphenylsilyloxy-3-fluoromethylpentanoic acid* **15** was used for further reactions without further purification (100 mg quantitative), $[a]_D^{23}$ +19.0 (*c* 1.0, MeOH); *m/z* [+ve FAB (3-NBA)] 526 $[M + Na]^+$, 504 $[M + H]^+$ and 404 $[M - Boc]^+$; ν_{max} (film) /cm^{-1} 3325 (NH and OH) and 1712 (C=O); δ_H (300 MHz, $C^2H_3O^2H$) 7.70–7.65 (4H, m, aromatics), 7.46–7.36 (6H, m, aromatics), 4.95 (1H, br s, NH), 4.42 (2H, dd, $J_{H,F}$ 47.4, $J_{6,3}$ 4.6, H-6), 3.83 (1H, m, H-4), 3.68 (2H, m, H-5), 2.54 (1H, m, H-3), 2.44 (1H, m, H-2A), 2.29 (1H, dd, $J_{2B,2A}$ 11.6, $J_{2B,3}$ 8.9, H-2B), 1.44 (9H, s, $C(CH_3)_3$) and 1.05 (9H, s, $OSiC(CH_3)_3$); δ_C (75.5 MHz, C^2HCl_3) 176.1 (acid), 158.5 (urethane), 137.2, 134.6, 131.5 and 129.4 (aromatics), 86.2–84.0 (d, $^1J_{6,F}$ 159.8, C-6), 80.7 ($OC(CH_3)_3$), 65.6 (C-5), 54.5 (C-4), 39.2 (C-3), 33.4 (C-2), 29.4 ($C(CH_3)_3$), 27.9 ($OSiC(CH_3)_3$) and 20.6 ($OSiC(CH_3)_3$); δ_F (282 MHz, C^2HCl_3) −228.2 (dt, $J_{F,H6}$ 47.5, $J_{F,H3}$ 25.0).

(2*S*,3*S*)-2-*N*-*tert*-Butoxycarbonylamino-1-*tert*-butyldiphenylsilyloxy-3-fluoromethyl-5-hydroxypentane (16)

(3*S*,4*S*)-4-*tert*-Butoxycarbonylamino-5-*tert*-butyldiphenylsilyloxy-3-fluoromethylpentanoic acid **15** (3.70 g, 7.37 mmol) was dissolved in tetrahydrofuran (40 ml) under nitrogen and the solution was cooled to −40 °C. Triethylamine (1.23 ml, 8.84 mmol) and *iso*-butyl chloroformate (1.11 ml, 8.48 mmol) were added and the reaction was stirred at − 40 °C for 1 h. The reaction was allowed to warm to 0 °C. Sodium borohydride (840 mg, 22.1 mmol) in a mixture of tetrahydrofuran (60 ml) and water (15 ml) was added. The reaction was stirred for a further 1 h at 0 °C, quenched by careful addition of saturated aqueous ammonium chloride (50 ml) and allowed to warm to room temperature. The crude product was extracted into ethyl acetate (3 × 50 ml) and washed with water (50 ml) and brine (50 ml). The organic layer was dried ($MgSO_4$) and the solvents were removed *in vacuo* to give a clear pink oil. Purification by column chromatography on silica gel using petroleum ether : ethyl acetate (3 : 1) as eluent afforded *(2S,3S)-2-N-tert-butoxycarbonylamino-1-tert-butyldiphenylsilyloxy-3-fluoromethyl-5-hydroxypentane* **16** as a clear colourless oil (2.99 g, 83%); $[a]_D^{25}$ −0.1 (*c* 1.0, $CHCl_3$); *m/z* (ES^+) Found: 490.2799 ($[M + H]^+$), $[C_{27}H_{40}NO_4SiF + H]$ requires: 490.2789; *m/z* [+ve FAB (3-NBA)] 490 $[M + H]^+$ and 390 $[M - Boc]^+$; ν_{max} (film) /cm^{-1} 3428 (OH) and 1694 (urethane); δ_H (500 MHz, C^2HCl_3) 7.66–7.63 (4H, m, aromatics), 7.46–7.40 (6H, m, aromatics), 4.89 (1H, d, $J_{NH,2}$ 8.2, NH), 4.54 (1H, ddd, $J_{6A,F}$ 47.0, $J_{6A,6B}$ 9.7, $J_{6A,3}$ 5.3, H-6A), 4.44 (1H, ddd, $J_{6B,F}$ 47.0, $J_{6B,6A}$ 9.7, $J_{6B,3}$ 5.3, H-6B), 3.83 (1H, m, H-2), 3.74 (4H, m, H-5 and H-1), 2.20 (1H, m, H-3), 1.99 (1H, br s, OH), 1.63 (2H, m, H-4), 1.44 (9H, s, $C(CH_3)_3$) and 1.08 (9H, s, $OSiC(CH_3)_3$); δ_C (75.5 MHz, C^2HCl_3) 159.0 (urethane), 136.0, 133.4, 130.3 and 128.2 (aromatics), 83.0–81.5 (d, $^1J_{6,F}$ 164.4, C-6), 80.0 ($OC(CH_3)_3$), 64.6 (C-5), 61.0 (C-1), 55.0 (C-2), 35.8 (d, $^2J_{3,F}$ 19.0, C-3), 30.3 (C-4), 28.8 ($C(CH_3)_3$), 27.3 ($C(CH_3)_3$) and 19.6 ($OSiC(CH_3)_3$); δ_F (282 MHz) −225.15 (td, $J_{F,H6}$ 47.0, $J_{F,H3}$ 26.0).

(3*S*,4*S*)-*O*-(4-*tert*-Butoxycarbonylamino-5-*tert*-butyldiphenylsilyloxy-3-fluoromethylpentyl) 1*H*-imidazole-1-carbothioate (17)

Thiocarbonyldiimidazole (1.20 g, 6.73 mmol) was added to a solution of (2*S*,3*S*)-2-*N*-*tert*-butoxycarbonylamino-1-*tert*-butyldiphenylsilyloxy-3-fluoromethyl-5-hydroxypentane **16** (2.99 g, 6.11 mmol) in dichloromethane (50 ml). The mixture was stirred under nitrogen at room temperature for 12 h. The solvents were removed *in vacuo* to give a clear orange oil. The crude products were purified by flash column chromatography on silica gel using petroleum ether : ethyl acetate (3 : 1) as eluent. *(3S,4S)-O-(4-tert-Butoxycarbonylamino-5-tert-butyldiphenylsilyloxy-3-fluoromethylpentyl)-1H-imidazole–1-carbothioate* **17** was obtained as a clear colourless oil (3.66 g, quantitative); *m/z* (ES^+) Found: 600.2731 ($[M + H]^+$), $[C_{31}H_{42}N_3O_4SiFS + H]$ requires: 600.2727; *m/z* [+ve FAB (3-NBA)] 622 $[M + Na]^+$ and 600 $[M + H]^+$; ν_{max} (film) /cm^{-1} 3405 (NH) and 1703 (C=O); δ_H (300 MHz, C^2HCl_3) 8.26 (1H, br s, imidazole), 7.60–7.50 (4H, m, aromatics), 7.40–7.29 (6H, m, aromatics), 6.95 (1H, br s, imidazole), 4.77 (1H, d, $J_{NH,5}$ 8.5, NH), 4.64 (2H, t, $J_{1,2}$ 6.8, H-1), 4.55–4.25 (2H, dm, $J_{6,F}$ 47.4, H-6), 3.77–3.67 (1H, m, H-4), 3.66–3.59 (2H, m, H-5), 2.20–2.00 (1H, m, H-3), 1.36 (9H, s, $C(CH_3)_3$) and 1.00 (9H, s, $OSiC(CH_3)_3$); δ_C (75.5 MHz, C^2HCl_3) 184.4 (C=S), 156.0 (urethane), 135.9 and 133.1 (aromatics), 131.2 (imidazole), 130.4 and 128.3 (aromatics), 118.2 (imidazole), 84.1 (d, $^1J_{6,F}$ 166.2, C-6), 80.1 ($OC(CH_3)_3$), 72.2 (C-1), 64.0 (C-5), 52.9 (C-4), 38.8 (d, $^2J_{3,F}$ 18, C-3), 28.8 ($C(CH_3)_3$), 27.3 ($OSiC(CH_3)_3$), 26.7 (C-2) and 19.7 ($OSiC(CH_3)_3$); δ_F (282 MHz) −225.22 (td, $J_{F,H6}$ 47.0, $J_{F,H3}$ 26.0).

(2*S*,3*S*)-1-(*O*-*tert*-Butyldiphenylsilyl)-2-*N*-*tert*-butoxycarbonylamino-3-fluoromethylpentan-1-ol (18)

(3*S*,4*S*)-*O*-(4-*tert*-Butoxycarbonylamino-5-*tert*-butyldiphenylsilyloxy-3-fluoromethylpentyl) 1*H*-imidazole–1-carbothioate **17** (200 mg, 0.33 mmol) was dissolved in toluene (5 ml) in a two-necked round bottomed flask. 2,2′-Azobis(2-methylpropionitrile) (1 mg, 0.007 mmol) was added to the solution and the mixture was heated to reflux under nitrogen. Tri-n-butyltin hydride (1.0 M in toluene, 0.11 ml, 0.43 mmol) was added to the refluxing mixture. The reaction was heated at reflux for a further 2 h under nitrogen. The solvents were removed *in vacuo* to give a pale yellow oil. Purification of the product by flash column chromatography on silica gel using petroleum ether :

ethyl acetate (3 : 1) as eluent gave *(2S,3S)-1-(O-tert-butyldiphenylsilyl)-2-N-tert-butoxycarbonylamino-3-fluoromethylpentan-1-ol* **18** as a clear colourless oil (100 mg, 65%); $[a]_D^{27}$ −11.6 (*c* 1.0, $CHCl_3$); *m/z* [+ve FAB (3-NBA)] 496 [M + Na]$^+$and 474 [M + H]$^+$; ν_{max} (film) /cm^{-1} 3437 (NH) and 1703 (urethane); δ_H (300 MHz, C^2HCl_3) 7.55 (4H, m, aromatics), 7.40–7.25 (6H, m, aromatics), 4.73 (1H, d, $J_{NH,2}$ 8.0, NH), 4.60 (2H, dm, $J_{6,F}$ 48.0, H-6), 3.73 (1H, m, H-2), 3.61 (2H, m, H-1), 1.80 (1H, m, H-3), 1.39 (9H, s, $C(CH_3)_3$), 1.00 (9H, s, $OSiC(CH_3)_3$), 0.85 (3H, t, $J_{5,4}$ 7.4, H-5) and 0.75 (2H, m, H-4); δ_C (75.5 MHz, C^2HCl_3) 156.0 (urethane), 136.0, 133.5, 130.2 and 128.2 (aromatics), 84.1 (d, $^1J_{6,F}$ 164.0, C-6), 79.6 ($OC(CH_3)_3$), 64.1 (C-1), 53.0 (C-2), 43.0 (d, $^2J_{3,F}$ 19.3, C-3), 29.8 (C-4), 28.8 ($C(CH_3)_3$), 27.3 ($OSiC(CH_3)_3$), 19.7 ($OSiC(CH_3)_3$) and 12.3 (C-5); δ_F (282 MHz, C^2HCl_3) −226.7 (dt, $J_{F,H6}$ 47.5, $J_{F,H3}$ 26.0).

(2*S*,3*S*)-2-*N*-*tert*-Butoxycarbonylamino-3-fluoromethylpentan-1-ol (19)

Tetrabutylammonium fluoride (1 M in tetrahydrofuran, 0.25 ml, 0.25 mmol) was added to a solution of (2*S*,3*S*)-1-(*O*-*tert*-butyldiphenylsilyl)-2-*N*-*tert*-butoxycarbonylamino-3-fluoromethylpentan-1-ol **18** (97 mg, 0.21 mmol) in tetrahydrofuran (2 ml) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 12 h. A further portion of tetrabutylammonium fluoride (1 M in tetrahydrofuran, 0.11 ml, 0.11 mol) was added and the reaction was stirred for a further 5 h at room temperature. The reaction was quenched by careful addition of saturated aqueous ammonium chloride (2 ml). The crude product was extracted into ethyl acetate (3 × 5 ml) and washed with water (2 ml) and brine (2 ml). The organic layer was dried ($MgSO_4$) and the solvents were removed *in vacuo*. The crude product was purified using preparative thin layer chromatography using petroleum ether/diethyl ether (9 : 1) as eluent. The desired product *(2S,3S)-2-N-tert-butoxycarbonylamino-3-fluoromethylpentan-1-ol* **19** was obtained as a white crystalline solid (48 mg, quantitative); mp 96–98 °C; $[a]_D^{27}$ −11.6 (*c* 1.0, $CHCl_3$); *m/z* (ES$^+$) Found: 236.1667 ([M + H]$^+$), [$C_{11}H_{21}NO_3F$ + H] requires: 236.1662; *m/z* [+ve FAB (3-NBA)] 258 [M + Na]$^+$ and 236 [M + H]$^+$; ν_{max} (film) /cm^{-1} 3430 (OH) and 1691 (urethane); δ_H (300 MHz, C^2HCl_3) 5.00–4.90 (1H, br s, OH), 4.49 (1H, ddd, $J_{6A,F}$ 47.3, $J_{6A,6B}$ 9.7, $J_{6A,4}$ 3.5, H-6A), 4.42 (1H, ddd, $J_{6B,F}$ 47.9, $J_{6B,6A}$ 9.7, $J_{6B,4}$ 5.8, H-6B), 3.62 (3H, m, H-2 and H-1), 2.50 (1H, br s, OH), 1.80 (1H, m, H-3), 1.30 (2H, m, H-4), 1.35 (9H, s, $C(CH_3)_3$) and 0.92 (3H, t, $J_{5,4}$ 7.4, H-5); δ_C (75.5 MHz, C^2HCl_3) 156.7 (urethane), 84.0 (d, $^1J_{6,F}$ 164.0, C-6), 80.1 ($OC(CH_3)_3$), 63.3 (C-1), 54.1 (C-2), 43.0 (d, $^2J_{3,F}$ 22.0, C-3), 28.8 ($C(CH_3)_3$), 20.4 (C-4) and 12.2 (C-5); δ_F (282 MHz, C^2HCl_3) −226.0 (dt, $J_{F,H6}$ 47.4, $J_{F,H3}$ 29.4).

(2*S*,3*S*)-3′-Fluoroisoleucine hydrochloride (3)

(2*S*,3*S*)-2-*N*-*tert*-Butoxycarbonylamino-3-fluoromethylpentan-1-ol **19** (20 mg, 0.09 mmol) was dissolved in carbon tetrachloride (1 ml). Acetonitrile (1 ml) and water (1.5 ml) were added and the mixture was cooled to 0 °C. Sodium periodate (75 mg, 0.35 mmol) and ruthenium chloride hydrate (5 mg, 0.024 mmol) were added sequentially and the mixture was stirred at room temperature for 2 h. Diethyl ether (5 ml) was added and the mixture was stirred for 30 min. The mixture was dried ($MgSO_4$) and filtered through a pad of Celite®. The solid residue was washed with diethyl ether (3 × 5 ml). The organic layers were combined and the solvents were removed *in vacuo* to give *(2S,3S)-2-N-tert-butoxycarbonylamino-3′-fluoroisoleucine* **20** as a clear colourless oil (18 mg, 84%) which was suspended in 6 M aqueous hydrochloric acid (2 ml). The mixture was heated to 40 °C for 2 days. The solvents were removed *in vacuo* to afford a white powder. Recrystallisation from methanol gave *(2S,3S)-3′-fluoroisoleucine* **3** (10 mg, 94%; overall 82%); mp 163–165 °C; $[a]_D^{22}$ −3.0 (*c* 0.1, MeOH); *m/z* [FAB, CsI/glycerol] Found: 150.0926 ([M + H]$^+$), [$C_6H_{11}NO_2F$ + H] requires: 150.0930; *m/z* [+ve FAB (3-NBA)] 150 [M + H]$^+$; ν_{max} (KBr) /cm^{-1} 3415 (NH and OH) and 1638 (acid); δ_H (500 MHz, $^2H_2O/^2HCl$ (20%)) 5.10–4.83 (2H, br m, $J_{3',F}$ 48, H-3′), 4.66 (1H, m, H-2), 2.75 (1H, m, H-3), 1.79 (2H, m, H-4) and 1.24 (3H, m, H-5); δ_C (75.5 MHz, $C^2H_3O^2H$)) 170.0 (acid), 84.8 (d, $^1J_{3',F}$ 164, C-3′), 55.6 (C-2), 42.0 (d, $^2J_{3,F}$ 16.5, C-3), 19.5 (d, $^3J_{4,F}$ 6.1, C-4) and 11.9 (C-5).

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