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Synthesis of (2*S*,4*S*)- and (2*S*,4*R*)-5-fluoroleucine and (2*S*,4*S*)-[5,5-$^2H_2$]-5-fluoroleucine

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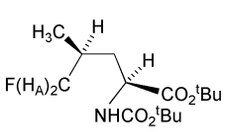
Received 21st November 2003, Accepted 16th December 2003
First published as an Advance Article on the web 28th January 2004

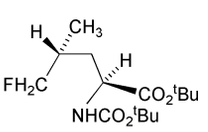
Syntheses of (2*S*,4*S*)- and (2*S*,4*R*)-5-fluoroleucine, **1a** and **2**, and of (2*S*,4*S*)-[5,5-$^2H_2$]-5-fluoroleucine, **1b**, have been completed. The methodology allows these compounds to be prepared in sufficient quantities for incorporation by solid-state protein synthesis into strategic sites in proteins for folding studies. X-ray structures of the epimers **1a** and **2** have been obtained and show the presence of conformational isomerism. The torsion angles between the F–C bond and the main chain are compared with values found in a mutant of the protein ubiquitin in which (2*S*,4*S*)-5-fluoroleucine replaces leucine residues 50 and 67 in the native protein.

Introduction

Although fluoroaromatic and derivatised fluorinated amino acids have been used as reporter groups for NMR spectroscopic investigations of proteins for some time,[1] the first study with fluorinated aliphatic amino acids appeared only in 1996, when we reported incorporation of (2*S*,4*S*)-5-fluoroleucine **1a** in place of all thirteen leucine residues in the enzyme dihydrofolate reductase using auxotrophic bacteria.[2] The biological methods used gave protein with all leucine residues labelled and with incomplete incorporation at each position. Protein folding is currently a major research challenge and we wished to evaluate the use of ^{19}F-NMR spectroscopy in its solution by incorporating strategically selected fluorine reporter groups into the hydrophobic core of a protein. An alternative method of incorporation was indicated and solid-state protein synthesis was selected as an unambiguous means of achieving our goal. Because there were many steps in our original synthesis of (2*S*,4*S*)-5-fluoroleucine **1a**,[2,3] it was difficult to produce the multi-gram quantities required for the solid-state approach and so we needed to improve this synthesis. We now report our new synthesis, the product of which we have used to prepare a 'mutant' of the protein ubiquitin in which the leucine residues Leu50 and Leu67, close neighbours in the hydrophobic core, have been replaced by (2*S*,4*S*)-5-fluoroleucine residues.[4] We showed by X-ray crystallographic, CD, ^{1}H-NMR spectroscopic and calorimetric studies that this Fleu50/67-mutant adopts the required native-like fold and ^{19}F-NMR spectroscopic studies showed the promise of such mutants for use in protein folding studies.[4] We also report the synthesis of the isotopomer (2*S*,4*S*)-[5,5-$^2H_2$]-5-fluoroleucine **1b** which should lead to simpler spectra.



1a, H_A = H
1b H_A = ^{2}H



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The two fluoroleucine residues in our mutant ubiquitin were uniquely aligned with respect to each other (Fig. 1) and so it was of interest to prepare the epimer, (2*S*,4*R*)-5-fluoroleucine **2**, so that the interaction of close-neighbour fluoroleucine residues in proteins might be studied in greater detail.

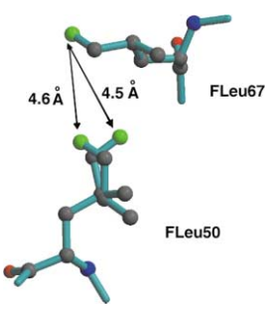
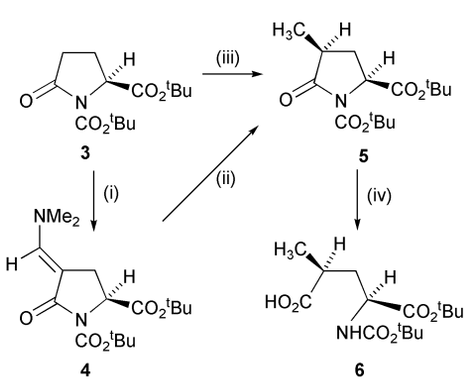


Fig. 1 Relationship of the (2*S*,4*S*)-5-fluoroleucine residues 50 and 67 in the hydrophobic core of the Fleu50/67 ubiquitin mutant from the X-ray structure reported in reference 4.

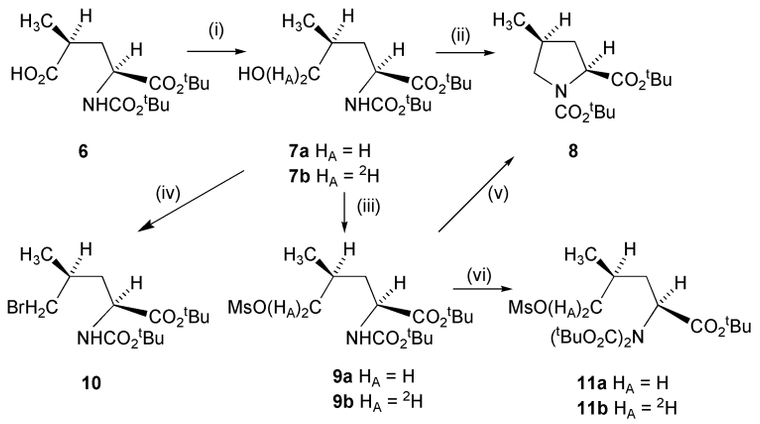
Results and discussion

The starting point for our synthesis of the (2*S*,4*S*)-isomers **1** was *tert*-butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-methylpyroglutamate **5** which we had prepared as shown in Scheme 1, either by stereoselective reduction of the enaminone **4**,[5] or by direct alkylation of *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **3** followed by protonation of the anion of the product with the sterically hindered acid 2,6-di-*tert*-butylphenol.[6] This was hydrolysed to give the acid **6** using aqueous LiOH in tetrahydrofuran[5] and formation of a mixed anhydride followed by reduction with either $NaBH_4$ or NaB^2H_4 gave the alcohols **7a**[2,3] and **7b**[5] respectively as shown in Scheme 2. In our



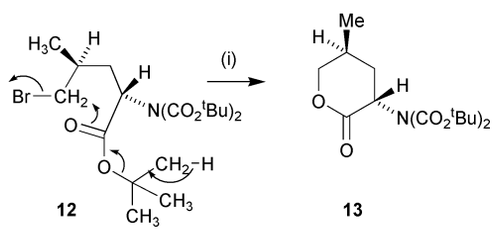
Scheme 1 *Reagents and conditions*: (i) ^{t}BuOCH(NMe$_2$)$_2$/dimethoxymethane/Δ, 83%; (ii) H_2/10% Pd–C/EtOAc, 78%; (iii) (a) LiHMDS/THF/−78 °C, $MeOSO_2CF_3$, 75%; (b) LiHMDS, THF −78 °C/2,6-di-*tert*-butylphenol, 89%; (iv) LiOH/H_2O/THF/0 °C, 94%.

DOI: 10.1039/b314933a



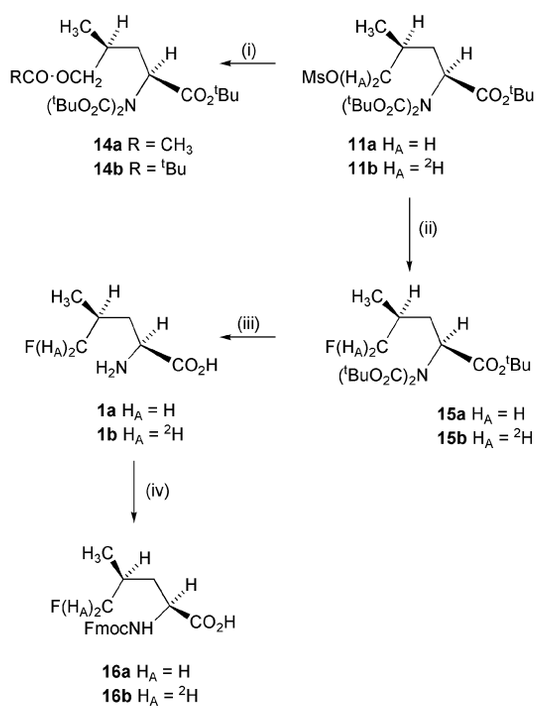
Scheme 2 *Reagents and conditions*: (i) (a) NEt_3/THF/$^tBuO_2CCl$/−40 °C; (b) for **7a** $NaBH_4/H_2O$/THF/0 °C, 84%; for **7b** $NaB^2H_4/^2H_2O$/THF, 0 °C, 75%; (ii) DAST/THF/−40 °C, 63%; (iii) $MeSO_2Cl$/pyridine/CH_2Cl_2, 96%; (iv) $Ph_3P/CBr_4/CH_3CN$, 0 °C, 47%; (v) TBAF/THF, 95%; (vi) $(Boc)_2O$/DMAP/CH_3CN, 93%.

original synthesis of (2*S*,4*S*)-5-fluoroleucine **1a**, reaction of the alcohol **7a** with diethylaminosulfur trifluoride resulted in an intramolecular reaction to give the 4-methylproline derivative **8**. An attempt to avoid this cyclisation by direct bisprotection using di-*tert*-butyl dicarbonate, led to reaction at the alcohol group and so we resorted to the lengthy, time-consuming and inelegant course of protection of the alcohol, formation of the bisurethane and deprotection of the alcohol group which was then reacted with diethylaminosulfur trifluoride without the danger of intramolecular cyclisation. In order to avoid the alcohol protection/deprotection sequence, we decided to investigate the possibility of converting the alcohol to a leaving group which might not immediately cyclise to the methylprolinate **8**. We were able to prepare the mesylate **9a** in 96% yield, without cyclisation, by reaction of the alcohol **7a** with methanesulfonyl chloride in dichloromethane containing triethylamine. We were also able to prepare the bromide **10** from the alcohol **6a** in 47% yield using carbon tetrabromide and triphenylphosphine in acetonitrile. When the mesylate **9a** was treated with tetrabutylammonium fluoride or the bromide **10** was treated with silver fluoride, cyclisation to the prolinate **8** ensued and so bisprotection of the nitrogen was required. The mesylate **9a** and the bromide **10** were therefore treated with di-*tert*-butyl dicarbonate in acetonitrile containing a catalytic quantity of DMAP to give the bis-urethanes **11a** and **12** respectively. Reaction of the mesylate **11a** with tetrabutylammonium fluoride in tetrahydrofuran at reflux slowly gave the corresponding fluoride as a mixture of diastereoisomers. It was evident that the basic fluoride anion had caused the reactive centre C-2 to epimerise. Interestingly, reaction of the bromide **12** with the less basic reagent, silver fluoride, in wet acetonitrile gave the lactone **13** in 75% yield, presumably by the mechanism shown in Scheme 3.



Scheme 3 *Reagents and conditions*: (i) $AgF/CH_3CN/H_2O/\Delta$, 75%.

In order to moderate the basic properties of tetrabutylammonium fluoride, we now reacted it with the mesylate **11a** in refluxing tetrahydrofuran in the presence of added acetic acid (Scheme 4). The product **14a** was obtained in 74% yield and so the more hindered trimethylacetic acid was used. Unfortunately nucleophilic attack again occurred, the product **14b** being obtained in 78% yield. Although the carboxylic acid had

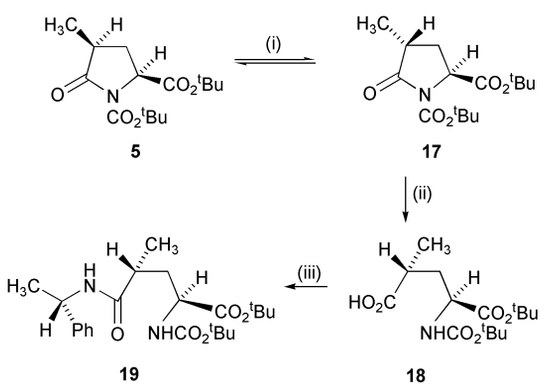


Scheme 4 *Reagents and conditions*: (i) for **14a** TBAF/$(CH_3)_3CCO_2H$/THF/Δ, 74%; for **14b** TBAF/$(CH_3)CCO_2H$/THF/Δ, 78%; (ii) TBAF/2-mesitylenesulfonic acid/THF/Δ, 69%; (iii) 6 M aq HCl, 96%; (iv) FmocOSu/Na_2CO_3/acetone, 96%.

behaved as a nucleophile in each case, there was no sign of the presence of a second diastereoisomer in either product and so part of our strategy had been achieved. 2-Mesitylenesulfonic acid has a less nucleophilic conjugate base and so it was now used to moderate the basicity of tetrabutylammonium fluoride in its reaction with the mesylate **11a**. The fluoride **15a** was obtained from this reaction in 69% yield. The ^{19}F-NMR spectrum of the product indicated that there was only 2% of the epimeric compound present. Deprotection using 6 M aqueous HCl gave (2*S*,4*S*)-5-fluoroleucine **1a** in 96% yield. The overall yield in the new synthesis was 30% over 9 steps from (2S)-pyroglutamic acid, compared to 12% over 11 steps in our previous synthesis. The Fmoc protected amino acid **16a** was prepared in 96% yield by reaction with 9-fluorenylmethoxycarbonyl-*N*-hydroxysuccinamide. Use of the alcohol **7b**[5] in the synthesis gave (2*S*,4*S*)-[5,5-$^2H_2$]-5-fluoroleucine **1b** as reported in the experimental section and this was also converted into the Fmoc derivative **16b**.

The epimer **2** was our next target and, although we had been able to synthesise *tert*-butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-methylpyroglutamate **5** stereoselectively by two different routes,

we were not able to develop a method to prepare the (2*S*,4*R*)-epimer **17** directly as a single diastereoisomer. Coudert *et al.*[7] have reported that treatment of the (2*S*,4*S*)-isomer with KCN in DMF at room temperature for 5 days gave a 3 : 1 mixture of the (2*S*,4*R*)- and (2*S*,4*S*)- isomers. To avoid the use of cyanide, we decided to investigate the use of tetrabutylammonium fluoride as base. We therefore heated a solution of *tert*-butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-methylpyroglutamate **5** in tetrahydrofuran containing one equivalent of tetrabutylammonium fluoride for 1.5 hours at reflux. A mixture of the epimeric products was obtained from which the (2*S*,4*S*)-epimer was obtained in 15% yield and the (2*S*,4*R*)-epimer in 62% yield by chromatography on silica gel, as shown in Scheme 5. To confirm that we had, in fact, epimerised the centre C-4 and not the centre C-2, we now hydrolysed the (2*S*,4*R*)-isomer using aqueous lithium hydroxide in THF at 0 °C. The acid **18** was obtained in quantitative yield and this was converted to the mixed anhydride with *iso*-butyl chloroformate and triethylamine in THF at −40 °C and reacted *in situ* with (*S*)-α-methylbenzylamine. The resultant amide **19**, obtained in 92% yield, was recrystallised and subjected to X-ray crystal structure analysis. The structure, shown in Fig. 2, indicated (2*S*,4*R*,7*S*)-stereochemistry, confirming that we had prepared the expected stereoisomer.



Scheme 5 *Reagents and conditions* (i) TBAF/THF/Δ, 62% **17** + 15% **5**; (ii) LiOH/H_2O/THF/0 °C, 97%; (iii) (a) $^tBuO_2CCl/NEt_3$/THF −40 °C, (b) (*S*)-methylbenzylamine *in situ*/0 °C, 92%.

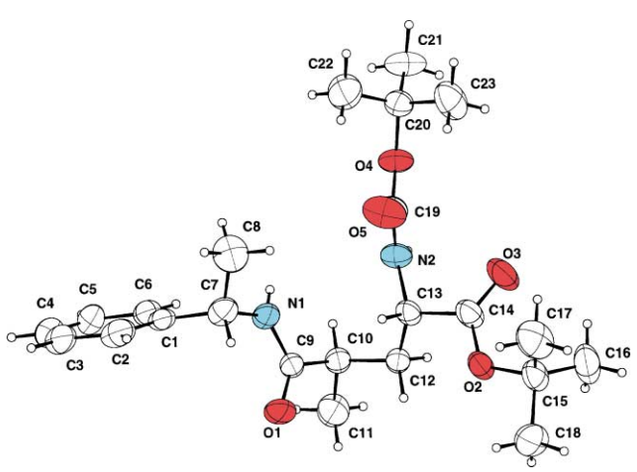
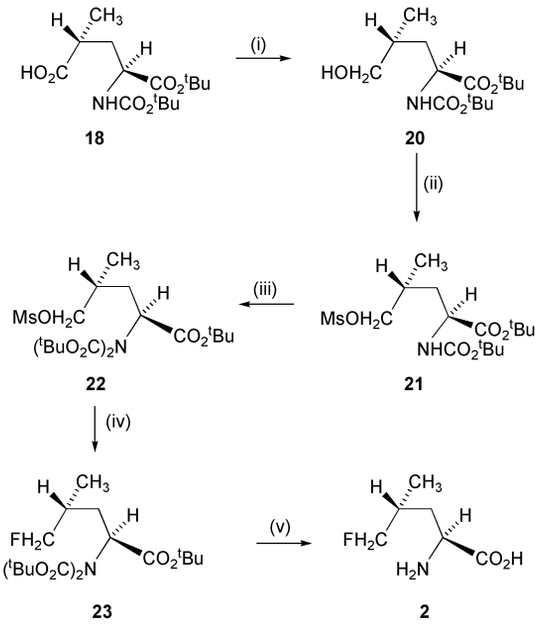


Fig. 2 X-ray structure of the amide **19**.

The acid **18** was again converted to the mixed anhydride with *iso*-butyl chloroformate and this was reacted *in situ* with sodium borohydride in aqueous tetrahydrofuran to give the alcohol **20** in 87% yield (Scheme 6). Conversion to the mesylate **21** in 93% yield followed by bisurethenation to give the product **22** in 72% yield was carried out using the method which had been successful in the synthesis of the epimeric compounds. Conversion to the fluoride **23** was accompanied by minimal epimerisation (ratio 13 : 1). The by-product had identical spectra to the



Scheme 6 *Reagents and conditions* (i) (a) NEt_3/THF/$^tBuO_2CCl$/−40 °C; (b) $NaBH_4/H_2O$/THF/0 °C, 87%; (ii) $MeSO_2Cl$/pyridine/CH_2Cl_2, 93%; (iii) $(Boc)_2O$/DMAP/CH_3CN, 72%; (iv) TBAF/2-mesitylenesulfonic acid/THF/Δ, 70%; (v) 6 M aq HCl, 80%.

epimer **15a** and the major product **23** was obtained in 70% yield after purification. This had identical spectra to the by-product from the reaction in the epimeric series. Finally, deprotection using 6 M aqueous hydrochloric acid at 40 °C for 3 days gave (2*S*,4*R*)-5-fluoroleucine **2**.

Both epimers of 5-fluoroleucine, **1a** and **2**, were subjected to X-ray crystallographic analysis (Figs. 3 and 4) confirming the structures. For the (2*S*,4*S*)-epimer **1a** (Fig. 3a) there was one independent molecule with the fluorine disordered over two conformations with different torsion angles. The major conformer (56%) had a torsion angle of 180° (*anti-periplanar*) for the C–F bond to the main chain whilst the minor one (44%) had a torsion angle of 68° (*gauche*) for C–F to the main chain. These are the general conformations found for FLeu50 in our ubiquitin mutant[4] (Fig. 1) which is shown for comparison in

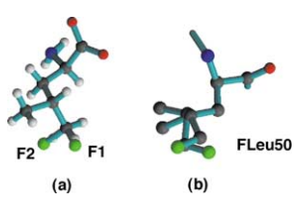


Fig. 3 (a) X-ray structure of (2*S*,4*S*)-5-fluoroleucine showing the major conformer (56%) with the F1–C bond *anti-periplanar* to Cβ overlapped with the minor conformer (44%) with the F2–C bond *gauche* to Cβ; (b) the two conformations of the (2*S*,4*S*)-5-fluoroleucine residue 50 from the Fleu50/67 mutant of ubiquitin are shown overlapped for comparison.

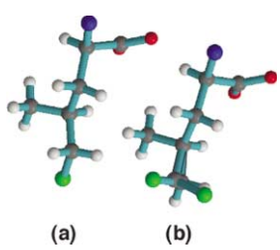


Fig. 4 X-ray structure of (2*S*,4*R*)-5-fluoroleucine showing the two molecules in the unit cell; (a) exists in the single conformation shown with the F–C bond *anti-periplanar* to Cβ; (b) exists in the two conformations shown overlapped, the major (76%) with the F–C bond *gauche* to Cβ and the minor (24%) with this torsion angle 25°.

Fig. 3b, FLeu67 in ubiquitin (Fig. 1) existing solely in the *gauche* conformation. For the (2*S*,4*R*)-epimer **2** there were two independent molecules. The first had a torsion angle of 179° (*anti-periplanar*) for the C–F bond to the main chain and the second had two orientations for C–F, one (76%) was −62° (*gauche*) to the main chain and the other was 25° to the main chain as shown in Fig. 4.

We have therefore prepared three compounds by a route which will afford them in quantities sufficient for solid state synthesis of proteins where they can act as reporter groups in protein folding studies. X-ray studies on two of these compounds have been completed.

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 Bruker DPX 300 (75.5 MHz) and AMX 500 (125.8 MHz) Fourier-transform instruments. 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). ^{2}H NMR spectra (proton decoupled) were recorded on a Bruker AMX 500 (76.8 MHz), using the solvent as an internal reference. All ^{1}H and ^{13}C spectra were recorded using TMS, 3-trimethylsilylpropanesulfanoic 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.

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine (9a)

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-5-hydroxyleucine **7a**[2,3] (8.243 g, 27.2 mmol) was dissolved in dichloromethane (160 ml) under nitrogen. Methanesulfonyl chloride (4.21 ml, 54.41 mmol) and pyridine (8.80 ml, 0.109 mol) were added and the reaction was stirred overnight at room temperature. The solvents were removed *in vacuo* and the residue was extracted with ethyl acetate. The extracts were washed with 0.5 M aqueous HCl, saturated aqueous ammonium chloride and dried ($MgSO_4$). The solvents were removed *in vacuo* and *tert-butyl (2S,4S)-N-tert-butoxycarbonyl-5-methylsulfonyloxyleucine* **9a** was purified by chromatography on silica gel using petroleum ether : ethyl acetate (6 : 4) as eluent as a colourless oil which crystallised as a white solid on standing (9.945 g, 96%), mp 44–46 °C; $[\alpha]^{22}_D$ −6.39 (*c* 1.02, $CHCl_3$); *m/z* [FAB (PEG/NBA)] Found: 381.1834 ([M]$^+$), [$C_{16}H_{31}NO_7S$] requires 381.1821; *m/z* [+ve FAB, NBA] 404 ([M + Na]$^+$) and 382 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3383 (NH), 1734 (ester) and 1709 (urethane); δ_H (300 MHz, $C_6{}^2H_6$) 0.78 (3H, d, $J_{Me,4}$ 6.6, CH_3), 1.31 (9H, s, $OC(CH_3)_3$), 1.41 (9H, s, $OC(CH_3)_3$), 1.51 (2H, m, H-3), 1.82 (1H, m, H-4), 2.22 (3H, s, CH_3SO_2), 3.69 (2H, m, H-5), 4.41 (1H, m, H-2) and 4.99 (1H, d, $J_{NH,2}$ 8.7, NH); δ_C (75.5 MHz, $C_6{}^2H_6$) 15.8 (CH_3), 27.8 ($OC(CH_3)_3$), 28.3 ($OC(CH_3)_3$), 30.3 (C-4), 36.1 (C-3), 36.5 (CH_3SO_2), 52.4 (C-2), 73.7 (C-5), 79.4 ($OC(CH_3)_3$), 81.5 ($OC(CH_3)_3$), 155.8 (urethane) and 172.1 (ester).

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-[5,5-$^2H_2$]-5-methylsulfonyloxyleucine (9b)

tert-Butyl (2*S*,4*S*)-[5,5-$^2H_2$]-*N*-*tert*-butoxycarbonyl-5-hydroxyleucine **7b**[5] (7.27 g, 23.84 mmol) was dissolved in dichloromethane (140 ml) under nitrogen. Methanesulfonyl chloride (3.69 ml, 47.67 mmol) and pyridine (7.71 ml, 95.34 mmol) were added and the reaction was stirred overnight at room temperature. The solvents were removed *in vacuo* and the residue was extracted with ethyl acetate, washed with 0.5 M aqueous HCl, saturated aqueous ammonium chloride and dried ($MgSO_4$). The solvents were removed *in vacuo*. *tert-Butyl (2S,4S)-N-tert-butoxycarbonyl-[5,5-$^2H_2$]-5-methylsulfonyloxyleucine* **9b** was purified by chromatography on silica gel using petroleum ether : ethyl acetate (7 : 3) as eluent as a colourless oil which crystallised as a white solid on standing (8.832 g, 97%), mp 45–47 °C; $[\alpha]^{22}_D$ −0.43 (*c* 1.0, $CHCl_3$); *m/z* [FAB (PEG/NBA)] Found: 384.2038 [(M + H]$^+$), [$C_{16}H_{29}{}^2H_2NO_7S$ + H] requires 384.2025; *m/z* [+ve FAB, NBA] 406 ([M + Na]$^+$) and 384 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3388 (NH) and 1724 (br); δ_H (300 MHz, $C_6{}^2H_6$) 0.77 (3H, d, $J_{Me,4}$ 6.6, CH_3), 1.31 (9H, s, $OC(CH_3)_3$), 1.41 (9H, s, $OC(CH_3)_3$), 1.50 (2H, m, H-3), 1.79 (1H, m, H-4), 2.21 (3H, s, CH_3SO_2), 4.41 (1H, m, H-2) and 4.95 (1H, d, $J_{NH,2}$ 8.7, NH); δ_{2H} (76.8 MHz, $C_6{}^2H_6$) 3.72 (^{2}H-5); δ_C (75.5 MHz, $C_6{}^2H_6$) 15.8 (CH_3), 27.8 ($OC(CH_3)_3$), 28.3 ($OC(CH_3)_3$), 30.1 (C-4), 36.1 (C-3), 36.5 (CH_3SO_2), 52.4 (C-2), 79.4 ($OC(CH_3)_3$), 81.5 ($OC(CH_3)_3$), 155.8 (urethane) and 172.1 (ester).

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-5-bromoleucine (10)

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-5-hydroxyleucine **7a** (1.851 g, 6.11 mmol) was dissolved in acetonitrile (15 ml) under nitrogen. Triphenylphosphine (1.68 g, 6.41 mmol) was added and the mixture was cooled to 0 °C under nitrogen. Carbon tetrabromide (2.13 g, 6.41 mmol) in acetonitrile (5 ml) was added and the reaction was stirred overnight at room temperature. The solvents were removed *in vacuo* and the residue was purified by chromatography on silica gel using dichloromethane as eluent. *tert-Butyl (2S,4S)-N-tert-butoxycarbonyl-5-bromoleucine* **10** was obtained as a white solid (1.045 g, 47%), mp 57–58 °C; $[\alpha]^{22}_D$ +0.08 (*c* 0.5, $CHCl_3$); *m/z* [ES+] Found: 366.1271 ([M + H]$^+$), [$C_{15}H_{28}NO_4{}^{79}Br$ + H] requires 366.1280; *m/z* [+ve FAB, NBA] 366 and 368 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3331 (NH), 1732 (ester) and 1683 (urethane); δ_H (300 MHz, C^2HCl_3) 0.92 (3H, d, $J_{Me,4}$ 6.5, CH_3), 1.26 (9H, s, $OC(CH_3)_3$), 1.29 (9H, s, $OC(CH_3)_3$), 1.49 (2H, m, H-3), 1.79 (1H, m, H-4), 3.21 (2H, d, $J_{5,4}$ 5.4, H-5), 4.02 (1H, m, H-2) and 5.07 (1H, d, $J_{NH,2}$ 8.7, NH); δ_C (75.5 MHz, C^2HCl_3) 18.1 (CH_3), 27.6 ($OC(CH_3)_3$), 28.0 ($OC(CH_3)_3$), 31.9 (C-4), 37.7 (C-3), 40.5 (C-5), 51.9 (C-2), 79.2 ($OC(CH_3)_3$), 81.4 ($OC(CH_3)_3$), 155.3 (urethane) and 171.8 (ester).

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-methylprolinate (8)

Method A: *tert*-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine **9a** (211 mg, 0.554 mmol) was dissolved in tetrahydrofuran (5 ml) under nitrogen. Tetrabutylammonium fluoride (1.1 M in tetrahydrofuran, 1.51 ml, 1.66 mmol) was added with stirring. The mixture was stirred overnight at room temperature and extracted with ethyl acetate. The organic layers were washed with saturated aqueous ammonium chloride and dried ($MgSO_4$). The solvents were removed *in vacuo* and the residue was purified by chromatography on silica gel using petroleum ether : ethyl acetate (17 : 3) as eluent to afford *tert-butyl (2S,4S)-N-tert-butoxycarbonyl-4-methylprolinate* **8** as a colourless oil (150 mg, 95%) with spectral data identical to those of a sample prepared previously.[2,3]

Method B: Silver fluoride (89 mg, 0.698 mmol) was suspended in acetonitrile (2 ml) under nitrogen in a flask protected from light. A solution of *tert*-butyl (2*S*,4*S*)-*N*-*tert*-butoxy-

carbonyl-5-bromoleucine **10** (111 mg, 0.303 mmol) in acetonitrile (3 ml) containing a drop of water was added dropwise and the mixture was stirred overnight at room temperature. The reaction was filtered through a pad of Celite® which was rinsed with ethyl acetate. The solvents were removed *in vacuo* and the residue was purified by chromatography on silica gel using petroleum ether : ethyl acetate (17 : 3) as eluent to afford *tert-butyl (2S,4S)-N-tert-butoxycarbonyl-4-methylprolinate* **8** as a colourless oil (85 mg, 98%) with spectral data identical to those of a sample prepared previously.[2,3]

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine (11a)

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine **9a** (1.808 g, 4.74 mmol) was dissolved in acetonitrile (10 ml) under nitrogen with stirring. Dimethylaminopyridine (58 mg, 0.475 mmol) was added followed by a solution of di-*tert*-butyl dicarbonate (3.10 g, 14.2 mmol) in acetonitrile (5 ml). The mixture was stirred overnight at room temperature. As the reaction progressed, the clear colourless solution turned orange. Further di-*tert*-butyl dicarbonate (3.10 g, 14.2 mmol) was added in small portions to complete the reaction. The solvent was removed *in vacuo* to give an orange oil which was purified by chromatography on silica gel using dichloromethane : ethyl acetate (19 : 1) as eluent. *tert-Butyl (2S,4S)-N,N-di-tert-butoxycarbonyl-5-methylsulfonyloxyleucine* **11a** was obtained as a pale yellow oil (2.12 g, 93%), $[\alpha]^{22}_{D}$ −10.29 (*c* 1.0, $CHCl_3$); *m/z* [FAB (PEG)] Found: 504.2241 ($[M + Na]^+$), $[C_{21}H_{39}NO_9S + Na]$ requires 504.2243; *m/z* [+ve FAB, NBA] 504 ($[M + Na]^+$); ν_{max} (film)/cm^{-1} 1787 (bisurethane), 1736 (ester) and 1702; δ_H (300 MHz, C^2HCl_3) 0.98 (3H, d, $J_{Me,4}$ 6.1, CH_3), 1.38 (9H, s, $OC(CH_3)_3$), 1.44 (18H, s, $OC(CH_3)_3$), 1.82 (2H, m, H-3A + H-4), 2.01 (1H, t, $J_{3B,3A}$ = $J_{3B,2}$ = 10.2, H-3B), 2.93 (3H,s, CH_3SO_2), 3.99 (2H, d, $J_{5,4}$ 5.5, H-5) and 4.75 (1H, dd, $J_{2,3B}$ 10.2, $J_{2,3A}$ 3.4, H-2); δ_C (75.5 MHz, C^2HCl_3) 15.8 (CH_3), 27.8 ($OC(CH_3)_3$), 27.9 ($OC(CH_3)_3$), 30.3 (C-4), 32.0 (C-3), 37.1 (CH_3SO_2), 56.4 (C-2), 74.2 (C-5), 81.4 ($OC(CH_3)_3$), 83.0 ($OC(CH_3)_3$), 152.4 (urethane) and 169.7 (ester).

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-[5,5-$^2H_2$]-5-methylsulfonyloxyleucine (11b)

tert-Butyl (2*S*,4*S*)-[5,5-$^2H_2$]-*N*-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine **9b** (8.832 g, 23.06 mmol) was dissolved in acetonitrile (50 ml) under nitrogen with stirring. Dimethylaminopyridine (281 mg, 2.3 mmol) was added followed by di-*tert*-butyl dicarbonate (15.08 g, 69.2 mmol) dissolved in acetonitrile (25 ml). The mixture was stirred overnight at room temperature. As the reaction progressed, the clear colourless solution turned orange. Further di-*tert*-butyl dicarbonate (15.08 g, 69.2 mmol) was added in small portions to complete the reaction. The solvent was removed *in vacuo* to give an orange oil which was purified by chromatography on silica gel using petroleum ether : ethyl acetate (3 : 1) as eluent. *tert-Butyl (2S,4S)-N,N-di-tert-butoxycarbonyl-[5,5-$^2H_2$]-5-methylsulfonyloxyleucine* **11b** was obtained as a pale yellow oil (9.439 g, 85%); $[\alpha]^{22}_{D}$ −14.34 (*c* 1.0, $CHCl_3$); *m/z* [FAB (PEG)] Found: 484.2567 ($[M + H]^+$), $[C_{21}H_{37}{}^2H_2NO_9S + H]$ requires 484.2549; *m/z* [+ve FAB, NBA] 506 ($[M + Na]^+$) and 484 ($[M + H]^+$); ν_{max} (film)/cm^{-1} 1790 (bisurethane), 1739 (ester) and 1704; δ_H (300 MHz, $C_6{}^2H_6$) 0.80 (3H, d, $J_{Me,4}$ 6.5, CH_3), 1.39 (9H, s, $OC(CH_3)_3$), 1.42 (18H, s, $OC(CH_3)_3$), 1.84 (1H, m, H-4), 1.96 (1H, ddd, $J_{3A,3B}$ 14.0, $J_{3A,4}$ 9.9, $J_{3A,2}$ 4.5, H-3A), 2.17 (3H, s, CH_3SO_2), 2.30 (1H, ddd, $J_{3B,3A}$ 14.0, $J_{3B,2}$ 10.6, $J_{3B,4}$ 3.4, H-3B) and 5.07 (1H, dd, $J_{2,3B}$ 10.6, $J_{2,3A}$ 4.5, H-2); δ_{2H} (76.8 MHz, $C_6{}^2H_6$) 3.75 (s, ^{2}H-5); δ_C (75.5 MHz, $C_6{}^2H_6$) 15.7 (CH_3), 27.9 ($OC(CH_3)_3$), 28.0 ($OC(CH_3)_3$), 30.6 (C-4), 32.5 (C-3), 36.4 (CH_3SO_2), 57.0 (C-2), 80.9 ($OC(CH_3)_3$), 82.5 ($OC(CH_3)_3$), 153.2 (urethane) and 169.7 (ester).

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-bromoleucine (12)

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-5-bromoleucine **10** (489 mg, 1.34 mmol) was dissolved in acetonitrile (5 ml) under nitrogen with stirring. Dimethylaminopyridine (16 mg, 0.131 mmol) was added followed by a solution of di-*tert*-butyl dicarbonate (580 mg, 2.67 mmol) in acetonitrile (5 ml). The mixture was stirred overnight at room temperature. As the reaction progressed, the clear colourless solution turned orange. Further di-*tert*-butyl dicarbonate (580 mg, 2.67 mmol) was added in small portions to complete the reaction. The solvent was removed *in vacuo* to give an orange oil which was purified by chromatography on silica gel using dichloromethane as eluent. *tert-Butyl (2S,4S)-N,N-di-tert-butoxycarbonyl-5-bromoleucine* **12** was obtained as a white solid (423 mg, 68%), mp 58–59 °C; $[\alpha]^{22}_{D}$ −16.97 (*c* 0.3, $CHCl_3$); *m/z* [FAB (PEG/NBA)] Found: 466.1765 ($[M + H]^+$), $[C_{20}H_{37}NO_6{}^{79}Br + H]$ requires 466.1804; *m/z* [+ve FAB, NBA] 466 and 468 ($[M + H]^+$); ν_{max} (film)/cm^{-1} 1726 (ester) and 1686; δ_H (300 MHz, C^2HCl_3) 1.07 (3H, d, $J_{Me,4}$ 6.0, CH_3), 1.45 (9H, s, $OC(CH_3)_3$), 1.52 (18H, s, $OC(CH_3)_3$),1.85 (2H, m, H-3A + H-4), 2.16 (1H, t, $J_{3B,3A}$ = $J_{3B,2}$ 10.2, H-3B), 3.35 (2H, d, $J_{5,4}$ 5.3, H-5) and 4.80 (1H, dd, $J_{2,3B}$ 10.2, $J_{2,3A}$ 3.8, H-2); δ_C (75.5 MHz, C^2HCl_3) 18.0 (CH_3), 27.6 ($OC(CH_3)_3$), 27.7 ($OC(CH_3)_3$), 32.5 (C-4), 34.0 (C-3), 40.6 (C-5), 56.6 (C-2), 81.0 ($OC(CH_3)_3$), 82.6 ($OC(CH_3)_3$), 152.2 (urethane) and 169.5 (ester).

(3*S*,5*S*)-3-Di-*tert*-butoxycarbonylamino-5-methyltetrahydropyran-2-one (13)

Silver fluoride (249 mg, 1.96 mmol) was suspended in acetonitrile (5 ml) under nitrogen in a flask protected from light. A solution of *tert*-butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-bromoleucine **12** (228 mg, 0.489 mmol) in acetonitrile (5 ml) containing a drop of water was added dropwise and the mixture was heated to reflux overnight. The reaction was cooled to room temperature and filtered through a pad of Celite® which was rinsed with ethyl acetate. The solvents were removed *in vacuo* and the residue was purified by chromatography on silica gel using petroleum ether : ethyl acetate (3 : 1) as eluent to afford *(3S,5S)-3-di-tert-butoxycarbonylamino-5-methyltetrahydropyran-2-one* **13** as a white solid (120 mg, 75%), mp 65–67 °C; $[\alpha]^{22}_{D}$ −7.20 (*c* 1.0, $CHCl_3$); *m/z* [FAB (PEG/NBA)] Found: 330.1902 ($[M + H]^+$). $[C_{16}H_{27}NO_6 + H]$ requires 330.1917; *m/z* [+ve FAB, NBA] 681 ($[2M + Na]^+$), 352 ($[M + Na]^+$) and 330 ($[M + H]^+$); ν_{max} (film)/cm^{-1} 1762 (lactone) and 1709; δ_H (300 MHz, $C_6{}^2H_6$) 0.52 (3H, d, $J_{Me,5}$ 6.9, CH_3), 1.42 (18H, s, $OC(CH_3)_3$), 1.48–1.74 (2H, m, H-4), 2.14 (1H, m, H-5), 3.35 (1H, dd, $J_{6A,6B}$ 10.8, $J_{6A,5}$ 6.4, H-6A), 3.86 (1H, dd, $J_{6B,6A}$ 10.8, $J_{6B,5}$ 3.1, H-6B) and 5.24 (1H, t, $J_{3,4}$ 9.2, H-3); δ_C (75.5 MHz, $C_6{}^2H_6$) 15.5 (CH_3), 28.0 ($OC(CH_3)_3$ + C-5), 33.2 (C-4), 52.4 (C-3), 73.1 (C-6), 82.9 ($OC(CH_3)_3$), 152.7 (urethane) and 168.6 (lactone).

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-acetyloxyleucine (14a)

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine **11a** (223 mg, 0.464 mmol) was dissolved in tetrahydrofuran (3 ml) under nitrogen. Tetrabutylammonium fluoride (1 M in tetrahydrofuran, 1.39 ml, 1.39 mmol) was added followed by acetic acid (0.029 ml, 0.510 mmol). The mixture was heated at reflux overnight and extracted with ethyl acetate. The organic layers were washed with saturated aqueous ammonium chloride and dried ($MgSO_4$). The solvents were removed *in vacuo* and the residue was purified by chromatography on silica gel using petroleum ether : ethyl acetate (9 : 1) as eluent to afford *tert-butyl (2S,4S)-N,N-di-tert-butoxycarbonyl-5-acetyloxyleucine* **14a** as a colourless oil (152 mg, 74%), $[\alpha]^{22}_{D}$ −14.38 (*c* 1.0, $CHCl_3$); *m/z* [CI] Found: 446.2755

([M + H]$^+$), [$C_{22}H_{39}NO_8$ + H] requires 446.2754; *m/z* [+ve FAB, NBA] 468 ([M + Na]$^+$) and 446 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1733 and 1701; δ_H (300 MHz, C^2HCl_3) 0.95 (3H, d, $J_{Me,4}$ 6.0, CH_3), 1.47 (9H, s, $OC(CH_3)_3$), 1.52 (18H, s, $OC(CH_3)_3$), 1.79 (2H, m, H-4 + H-3A), 2.03 (3H, s, CH_3), 2.09 (1H, m, H-3B), 3.89 (2H, m, H-5) and 4.82 (1H, dd, $J_{2,3}$ 10.4 and 4.1, H-2); δ_C (75.5 MHz, C^2HCl_3) 15.8 (CH_3), 20.6 (CH_3), 27.8 ($OC(CH_3)_3$), 27.9 ($OC(CH_3)_3$), 29.9 (C-4), 32.5 (C-3), 56.5 (C-2), 69.3 (C-5), 81.2 ($OC(CH_3)_3$), 82.7 ($OC(CH_3)_3$), 152.1 (urethane), 170.1 (ester) and 179.5 (ester).

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-trimethylacetyloxyleucine (14b)

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine **11a** (195 mg, 0.405 mmol) was dissolved in tetrahydrofuran (5 ml) under nitrogen. Tetrabutylammonium fluoride (1 M in tetrahydrofuran, 0.81 ml, 0.81 mmol) was added followed by trimethylacetic acid (0.046 ml, 0.40 mmol). The mixture was heated at reflux overnight and extracted with ethyl acetate. The organic layers were washed with saturated aqueous ammonium chloride and dried ($MgSO_4$). The solvents were removed *in vacuo* and the residue was purified by chromatography on silica gel using petroleum ether : ethyl acetate (9 : 1) as eluent to afford *tert-butyl (2S,4S)-N,N-di-tert-butoxycarbonyl-5-trimethylacetyloxyleucine* **14b** as a colourless oil (154 mg, 78%), $[\alpha]^{22}_D$ −17.75 (*c* 1.0, $CHCl_3$); *m/z* (ES+) Found: 488.3225 ([M + H]$^+$), [$C_{25}H_{45}NO_8$ + H] requires 488.3223; *m/z* [+ve FAB, NBA] 510 ([M + Na]$^+$) and 488 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1735 and 1702; δ_H (300 MHz, C^2HCl_3) 0.99 (3H, d, $J_{Me,4}$ 6.0, CH_3), 1.20 (9H, s, $OC(CH_3)_3$), 1.45 (9H, s, $OC(CH_3)_3$), 1.51 (18H, s, $OC(CH_3)_3$),1.84 (2H, m, H-4 + H-3A), 2.04 (1H, t, $J_{3B,3A} = J_{3B,2}$ 10.5, H-3B), 3.91 (2H, d, $J_{5,4}$ 5.3, H-5) and 4.83 (1H, dd, $J_{2,3B}$ 10.5,$J_{2,3A}$ 4.2, H-2); δ_C (75.5 MHz, C^2HCl_3) 15.9 (CH_3), 27.1 ($OC(CH_3)_3$), 27.8 ($OC(CH_3)_3$), 27.9 ($OC(CH_3)_3$), 29.7 (C-4), 32.6 (C-3), 38.7 ($OC(CH_3)_3$), 56.6 (C-2), 69.5 (C-5), 81.1 ($OC(CH_3)_3$), 82.7 ($OC(CH_3)_3$), 152.4 (urethane), 169.9 (ester) and 178.3 (ester).

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-fluoroleucine (15a)

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine **11a** (4.16 g, 8.65 mmol) was dissolved in tetrahydrofuran (80 ml) under nitrogen. 2-Mesitylenesulfonic acid dihydrate (2.66 g, 11.24 mmol) was added followed by tetrabutylammonium fluoride (1 M in tetrahydrofuran, 25.9 ml, 25.9 mmol). The mixture was heated for 3 days at between 50 and 52 °C and extracted with ethyl acetate. The organic layers were washed with saturated aqueous ammonium chloride and dried ($MgSO_4$). The solvents were removed *in vacuo* and the residue was purified by chromatography on silica gel using petroleum ether : ethyl acetate (19 : 1) as eluent to afford *tert-butyl (2S,4S)-N,N-di-tert-butoxycarbonyl-5-fluoroleucine* **15a** as a colourless oil. Unlike the sample from our previous synthesis,[2,3] this crystallised on standing as a white solid (2.408 g, 69%), mp 47–48 °C, $[\alpha]^{22}_D$ −23.9 (*c* 0.34, $CHCl_3$), [lit.[2,3] $[\alpha]^{25}_D$ −17.6 (*c* 0.34 $CHCl_3$)] but otherwise the spectra were identical to those of the sample obtained from our previous synthesis.[2,3] Based on recovered unreacted starting material (1.02 g), the yield was 91%.

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-[5,5-$^2H_2$]-5-fluoroleucine (15b)

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-[5,5-$^2H_2$]-5-methylsulfonyloxyleucine **11b** (1.615 g, 3.34 mmol) was dissolved in tetrahydrofuran (25 ml) under nitrogen. 2-Mesitylenesulfonic acid (1.027 g, 4.35 mmol) was added followed by tetrabutylammonium fluoride (1 M in tetrahydrofuran, 10.0 ml, 10.0 mmol). The mixture was heated for 3 days at between 50 and 52 °C and extracted with ethyl acetate. The organic layers were washed with saturated aqueous ammonium chloride and dried ($MgSO_4$). The solvents were removed *in vacuo* and the residue was purified by chromatography on silica gel using petroleum ether : ethyl acetate (19 : 1) as eluent to afford *tert-butyl (2S,4S)-N,N-di-tert-butoxycarbonyl-[5,5-$^2H_2$]-5-fluoroleucine* **15b** as a colourless oil (844 mg, 62%), which solidified on standing, mp 44–45 °C; $[\alpha]^{22}_D$ −22.46 (*c* 1.0, $CHCl_3$); *m/z* [CI] Found: 408.2733 ([M + H]$^+$), [$C_{20}H_{34}{}^2H_2NO_6F$ + H] requires 408.2730; *m/z* [+ve FAB, NBA] 430 ([M + Na]$^+$) and 408 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1740 (ester) and 1698; δ_H (300 MHz, $C_6{}^2H_6$) 0.85 (3H, d, $J_{Me,4}$ 6.6, CH_3), 1.39 (9H, s, $OC(CH_3)_3$), 1.41 (18H, s, $OC(CH_3)_3$),1.88 (1H, m, H-4), 1.99 (1H, ddd, $J_{3A,3B}$ 14.2, $J_{3A,4}$ 10.1, $J_{3A,2}$ 4.6, H-3A), 2.37 (1H, ddd, $J_{3B,3A}$ 14.2, $J_{3B,2}$ 10.6, $J_{3B,4}$ 3.4, H-3B) and 5.13 (1H, dd, $J_{2,3B}$ 10.6, $J_{2,3A}$ 4.6, H-2); δ_{2H} (76.8 MHz, $C_6{}^2H_6$) 4.02 (d, $J_{2H,F}$ 7.1, C^2H-5); δ_C (125.8 MHz, $C_6{}^2H_6$) 15.0 (d, $^3J_{Me,F}$ 6.7, CH_3), 28.0 ($OC(CH_3)_3$), 28.0 ($OC(CH_3)_3$), 31.5 (d, $^2J_{C4,F}$ 18.3, C-4), 32.3 (d, $^3J_{C3,F}$ 5.0, C-3), 57.1 (C-2), 80.8 ($OC(CH_3)_3$), 82.3 ($OC(CH_3)_3$), 153.1 (urethane) and 169.8 (ester); δ_F (282 MHz, $C_6{}^2H_6$) −229.3 (dt, $J_{F,4H}$ 23.6, $J_{F,5H}$ 6.5). The yield based on recovered starting material (541 mg) was 93%.

(2*S*,4*S*)-5-Fluoroleucine hydrochloride (1a)

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-fluoroleucine **15a** (1.054 g, 2.60 mmol) was stirred at room temperature in 6 M aqueous HCl (20 ml) for four days. The solvent was removed *in vacuo* to afford a white solid which was purified by flash chromatography on silica gel using dichloromethane : methanol : water : acetic acid (7 : 3 : 0.6 : 0.3) as eluent to afford *(2S,4S)-5-fluoroleucine hydrochloride* **1a** as a white solid (464 mg, 96%), mp 188–190 °C, $[\alpha]^{22}_D$ −2.1 (*c* 2.0, 3 M HCl), (lit.[2,3] mp 188–190 °C, $[\alpha]^{18}_D$ −1.8 (*c* 2.0, 3 M HCl)]. The spectra were identical with those of the sample from our previous synthesis.

Crystal data for compound 1a †

$C_6H_{12}FNO_2$, M = 149.17, monoclinic, space group $C2$ (No.5), a = 9.6878(16), b = 5.3021(6), c = 15.229(3) Å, β = 107.292(5)°, V = 746.9(2) Å^3, Z = 4, D_{calc} = 1.33 Mg m^{-3}, μ(Mo-Kα) = 0.11 mm^{-1}, T = 173(2) K, 4317 total reflections measured, 1290 independent reflections collected on a Nonius Kappa CCD diffractometer (R_{int} = 0.057) using Mo-Kα radiation (λ = 0.71073 Å). The F atom was disordered over two positions and one H atom could not be located. Refinement using SHELXL-97. Final residues were $R1$ = 0.067, $wR2$ = 0.174 (for 1089 reflections with $I > 2\sigma(I)$), $R1$ = 0.080, $wR2$ = 0.183 for all reflections.

(2*S*,4*S*)-[5,5-$^2H_2$]-5-Fluoroleucine hydrochloride (1b)

tert-Butyl (2*S*,4*S*)-*N*,*N*-di-*tert*-butoxycarbonyl-[5,5-$^2H_2$]-5-fluoroleucine **15b** (1.825 g, 4.48 mmol) was stirred at room temperature in 6 M aqueous HCl (35 ml) for four days. The solvent was removed *in vacuo* to afford a white solid which was purified by flash chromatography on silica gel using dichloromethane : methanol : water : acetic acid (7 : 3 : 0.6 : 0.3) as eluent to afford *(2S,4S)-[5,5-$^2H_2$]-5-fluoroleucine hydrochloride* **1b** as a white solid (719 mg, 85%), mp 187–190 °C; $[\alpha]^{22}_D$ +3.39 (*c* 1.0, MeOH); *m/z* Found: [EI] 106.0997 ([M − CO_2H]$^+$), [$C_6H_{10}{}^2H_2NO_2F$ − CO_2H] requires 106.1001; *m/z* [+ve FAB, glycerol/1 M HCl] 303 ([2M + H]$^+$), 244 ([M + Na]$^+$) and 152 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3423 (NH), and 1741 (br); δ_H (300 MHz, $C^2H_3O^2H$) 1.04 (3H, dd, $J_{Me,4}$ 6.7, $J_{Me,F}$ 1.0, CH_3), 1.86 (1H, ddd, $J_{3A,3B}$ 14.5, $J_{3A,4}$ 8.6, $J_{3A,2}$ 6.1, H-3A), 1.94 (1H, ddd, $J_{3B,3A}$ 14.1, $J_{3B,2}$ 8.3, $J_{3B,4}$ 5.7, H-3B), 2.09 (1H, m, H-4) and 4.03 (1H, dd, $J_{2,3B}$ 8.3, $J_{2,3A}$ 6.1, H-2); δ_{2H} (76.8 MHz, CH_3OH) 4.22

† CCDC reference numbers 224897 (**1a**), 224898 (**19**) and 224899 (**2**). See http://www.rsc.org/suppdata/ob/b3/b314933a/ for crystallographic data in.cif or other electronic format.

(t, $J_{2H5A,F} = J_{2H5B,F}$ 6.7, C^2H-5); δ_C (125.8 MHz, $C^2H_3O^2H$) 14.5 (d, $^3J_{Me,F}$ 6.9, CH_3), 30.7 (d, $^2J_{4,F}$ 18.7, C-4), 33.8 (d, $^3J_{3,F}$ 5.1, C-3), 50.9 (C-2) and 171.0 (acid); δ_F (282 MHz, $C^2H_3O^2H$) −237.6 (dt, $J_{F,H4}$ 19.4, $J_{F,2H5}$ 6.7).

(2*S*,4*S*)-*N*-9-Fluorenylmethoxycarbonyl-5-fluoroleucine (16)

(2*S*,4*S*)-5-Fluoroleucine hydrochloride **1a** (1.24 g, 6.69 mmol) was dissolved in water (26 ml) and acetone (14 ml). Sodium carbonate (2.13 g, 20.07 mmol) and 9-fluorenylmethoxycarbonyl-*N*-hydroxysuccinimide (FmocOSu) (2.37 g, 7.02 mmol) were added and the clear solution was stirred overnight at room temperature. The solution was extracted with ethyl acetate. The aqueous layer was acidified to pH 1.5 with 2 M aqueous HCl and extracted with ethyl acetate. The combined organic layers were dried ($MgSO_4$) and the solvent was removed *in vacuo* to afford a white solid which was purified by flash chromatography on silica gel using chloroform : methanol : acetic acid (91 : 8 : 1) as eluent to afford *(2S,4S)-N-9-fluorenylmethoxycarbonyl-5-fluoroleucine* **16** as a white solid (2.38 g, 96%), mp 151–153 °C; $[\alpha]^{22}_D$ −19.13 (*c* 0.34, $CHCl_3$); (Found: C, 66.4; H, 5.8; N, 3.65. $C_{21}H_{22}FNO_4 \cdot 0.5\ H_2O$ requires C, 66.3; H, 6.1; N, 3.7%); *m/z* [+ve FAB, NBA] 394 ($[M + Na]^+$); ν_{max} (film)/cm^{-1} 3338 (NH), 3150, 1721 (acid) and 1677 (urethane); δ_H (500 MHz, $C_6{}^2H_6$, 323 K) 0.69 (3H, dd, $J_{Me,4}$ 6.3, $J_{Me,F}$ 1.1, CH_3), 1.37–1.67 (3H, m, H-3 + H-4), 3.83 (2H, ddd, $J_{5,F}$ 47.5, $J_{5,4}$ 5.4 and 3.8, CH_2F), 3.99 (1H, t, $J_{2,3}$ 6.6, H-2), 4.42 (3H, d, *J* 6.2, H-7 + H-8), 4.65 (1H, br s, NH) and 7.13–7.57 (8H, 3m, ArH); δ_C (125.8 MHz, C^2HCl_3) 14.9 (d, $^3J_{Me,F}$ 6.9, CH_3), 30.9 (d, $^2J_{4,F}$ 18.0, C-4), 35.3 (C-3), 47.1 (C-8), 51.7 (C-2), 67.1 (C-7), 87.7 (d, $^1J_{5,F}$ 170.6, C-5), 120.0, 125.0, 127.1, 127.7, 141.3, 143.5 and 143.8 (7 × CHAr), 156.3 (C-6) and 177.6 (acid); δ_F (282 MHz, $C_6{}^2H_6$) −222.2 (td, $J_{F,H5}$ 47.5, $J_{F,H4}$ 18.7).

(2*S*,4*S*)-*N*-9-Fluorenylmethoxycarbonyl-[5,5-$^2H_2$]-5-fluoroleucine (16b)

(2*S*,4*S*)-[5,5-$^2H_2$]-5-fluoroleucine hydrochloride **1b** (499 mg, 2.66 mmol) was dissolved in water (10 ml) and acetone (6 ml). Sodium carbonate (846 mg, 7.98 mmol) and 9-fluorenylmethoxycarbonyl-*N*-hydroxysuccinimide (FmocOSu) (988 mg, 2.93 mmol) were added and the clear solution was stirred overnight at room temperature. The solution was extracted with ethyl acetate. The aqueous layer was acidified to pH 1.5 with 2 M aqueous HCl and extracted with ethyl acetate. The combined organic layers were dried ($MgSO_4$) and the solvent was removed *in vacuo* to afford a white solid which was purified by flash chromatography on silica gel using chloroform : methanol : acetic acid (95 : 4 : 1) as eluent to afford *(2S,4S)-N-9-fluorenylmethoxycarbonyl-[5,5-$^2H_2$]-5-fluoroleucine* **16b** as a white solid (883 mg, 89%), mp 152–154 °C; $[\alpha]^{22}_D$ −17.83 (*c* 1.0, MeOH); *m/z* [EI+] Found: 373.1649 ($[M]^+$), $[C_{21}H_{20}{}^2H_2NO_4F]$ requires 373.1658; *m/z* [+ve FAB, NBA] 396 ($[M + Na]^+$) and 374 ($[M + H]^+$); ν_{max} (KBr)/cm^{-1} 3335 (NH), 3136, 1719 (acid) and 1675 (urethane); δ_H (300 MHz, $C^2H_3O^2H$) 0.98 (3H, d, $J_{Me,4}$ 6.7, CH_3), 1.67 (1H, ddd, $J_{3A,3B}$ 14.3, *J* 10.2, *J* 4.3, H-3A), 1.78 (1H, ddd, $J_{3B,3A}$ 14.3, *J* 11.2, *J* 4.0, H-3B), 1.89 (1H, m, H-4), 4.23 (2H, m, H-2 + H-8), 4.36 (2H, d, $J_{7,8}$ 7.0, H-7), 4.93 (exchangeable, OH + NH), 7.27–7.41, 7.59–7.69 and 7.78–7.80 (8H, 3m, HAr); δ_{2H} (76.8 MHz, MeOH) 4.18 (d, $J_{2H,F}$ 5.9, C^2H-5); δ_C (75.5 MHz, $C^2H_3O^2H$) 15.0 (d, $^3J_{Me,F}$ 6.1, CH_3), 32.1 (d, $^2J_{4,F}$ 18.6, C-4), 35.3 (d, $^3J_{3,F}$ 5.4, C-3), 47.6 (C-8), 53.0 (C-2), 67.9 (C-7), 120.9, 126.3, 128.8, 130.1, 142.6, 145.1 and 145.4 (7 × CHAr), 158.8 (C-6) and 173.3 (acid); δ_F (282 MHz, $C_6{}^2H_6$) −230.5 (dt, $J_{F,H4}$ 19.7, $J_{F,2H5}$ 6.4).

Epimerisation of *tert*-butyl *N*-*tert*-butoxycarbonyl-(2*S*,4*S*)-4-methylpyroglutamate (5)

tert-Butyl *N*-*tert*-butoxycarbonyl-(2*S*,4*S*)-4-methylpyroglutamate **5** (5.00 g, 17 mmol) was dissolved in tetrahydrofuran (75 ml) under an atmosphere of nitrogen. Tetrabutylammonium fluoride (1.0 M in tetrahydrofuran, 18.4 ml, 18 mmol) was added and the mixture was stirred at reflux for 1.5 h. The reaction was left to cool to room temperature and saturated aqueous ammonium chloride (75 ml) was added. The solution was extracted with ethyl acetate (3 × 30 ml). The organic layers were washed with water (25 ml) and brine (25 ml) and dried ($MgSO_4$). The solvents were removed *in vacuo* to give a clear dark orange oil which was purified by flash column chromatography on silica gel using petroleum ether : ethyl acetate (85 : 15) as eluent to give *tert-butyl N-tert-butoxycarbonyl-(2S,4S)-4-methylpyroglutamate* **5** as a white crystalline solid (750 mg, 15%) and *tert-butyl N-tert-butoxycarbonyl-(2S,4R)-4-methylpyroglutamate* **17** as a white crystalline solid (3.10 g, 62%). Both compounds had identical spectroscopic properties to those previously reported.[5,6]

1-*tert*-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-methylglutamic acid (18)

tert-Butyl *N*-*tert*-butoxycarbonyl-(2*S*,4*R*)-4-methylpyroglutamate **17** (5.00 g, 17 mmol) was dissolved in tetrahydrofuran (50 ml) and the solution was cooled to 0 °C. 1 M Aqueous lithium hydroxide (20 ml, 20 mmol) was added dropwise and the mixture was stirred vigorously for 45 min. Ethyl acetate (50 ml) and saturated aqueous sodium hydrogen carbonate (50 ml) were added and the mixture was stirred at 0 °C for 1 h. The organic layer was discarded and the aqueous layer was washed with ethyl acetate (3 × 50 ml). The aqueous layer was cooled to 0 °C and acidified with 1 M aqueous hydrochloric acid. The acidified aqueous layer was extracted with ethyl acetate (5 × 50 ml). The organic layers were combined, washed with water (100 ml) and brine (100 ml) and dried ($MgSO_4$). The solvents were removed *in vacuo* to give *1-tert-butyl (2S,4R)-N-tert-butoxycarbonyl-4-methylglutamic acid* **18** as a clear colourless oil which crystallised upon standing (5.24 g, 97%). An analytically pure sample was obtained by recrystallisation from petroleum ether; mp 100–101 °C; $[\alpha]_D{}^{23}$ −29.2 (*c* 1.0, $CHCl_3$); (Found: C, 56.8; H, 8.6; N, 4.3. $C_{15}H_{27}NO_6$ requires: C, 56.8; H, 8.6; N, 4.4%); *m/z* [+ve FAB, 3-NBA] 657 ($[2M + Na]^+$), 635 ($[2M + H]^+$), 340 ($[M + Na]^+$) and 318 ($[M + H]^+$); ν_{max} (KBr)/cm^{-1} 3416 and 3244 (OH and NH), 1739 (ester), 1716 (acid) and 1651 (urethane); δ_H (500 MHz, C^2HCl_3) 5.32 (1H, d, $J_{NH,2}$ 7.8, NH), 4.24 (1H, dd, $J_{2,3A}$ 8.8, $J_{2,3B}$ 5.5, H-2) 2.59 (1H, m, H-4), 2.21 (1H, ddd, $J_{3B,3A}$ 14.1, $J_{3B,4}$ 8.7, $J_{3B,2}$ 5.5, H-3B), 1.65 (1H, ddd, $J_{3A,3B}$ 14.1, $J_{3A,2}$ 8.8, $J_{3A,4}$ 5.3, H-3A), 1.47 (9H, s, $C(CH_3)_3$), 1.45 (9H, s, $C(CH_3)_3$) and 1.24 (3H, d, $J_{Me,4}$ 7.0, CH_3); δ_C (75.5 MHz, C^2HCl_3) 179.5 (acid), 171.2 (ester), 156.2 (urethane), 82.6 ($OC(CH_3)_3$), 80.6 ($OC(CH_3)_3$), 52.5 (C-2), 37.5 (C-3), 36.2 (C-4), 28.3 ($C(CH_3)_3$), 28.0 ($C(CH_3)_3$) and 17.6 (C-6).

tert-Butyl 2(*S*)-*tert*-butoxycarbonylamino-4(*R*)-[1(*S*)-phenylethylcarbamoyl]-pentanoate (19)

1-*tert*-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-methylglutamic acid **18** (130 mg, 0.411 mmol) was dissolved in tetrahydrofuran (2 ml) and the solution was cooled to −40 °C. Triethylamine (0.074 ml, 0.534 mmol) and *iso*-butyl chloroformate (0.063 ml, 0.493 mmol) were added and the mixture was stirred at −40 °C for 1 h. (*S*)-Methylbenzylamine (75 mg, 0.616 mmol) was added and the mixture was stirred for 1 h at 0 °C and 1 h at room temperature. Saturated aqueous sodium chloride (2 ml) was added and the mixture was allowed to warm to room temperature and extracted with ethyl acetate (5 × 5 ml). The organic layers were washed with water (5 ml) and brine (5 ml) and dried ($MgSO_4$). The solvents were removed *in vacuo* to give *tert*-butyl 2(*S*)-*tert*-butoxycarbonylamino-4(*R*)-[1(*S*)-phenylethylcarbamoyl]-pentanoate **19** as a clear colourless oil which crystallised upon standing (160 mg, 92%); mp 109–111 °C; $[\alpha]_D{}^{24}$ −36.6 (*c* 1.5, $CHCl_3$); (Found: C, 65.6; H, 8.7; N, 6.6. $C_{23}H_{36}N_2O_5$ requires C, 65.7; H, 8.6; N, 6.7%); *m/z* [+ve FAB,

3-NBA] 443 ([M + Na]$^+$) and 421 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1715 (ester), 1701 (urethane) and 1644 (amide); δ_H (300 MHz, C^{2}HCl$_3$) 7.52 (1H, d, $J_{NH,7}$ 7.7, NH), 7.41–7.23 (5H, m, ArH), 5.30 (1H, d, $J_{NH,2}$ 8.3, NH), 5.16 (1H, m, H-7), 4.25 (1H, m, H-2), 2.30 (1H, m, H-4), 2.18 (1H, m, H-3A), 1.45 (3H, d, $J_{8,7}$ 6.9, Me), 1.46 (1H, m, H-3B), 1.47 (18H, s, 2 × C(CH$_3$)$_3$) and 1.12 (3H, d, $J_{5,4}$ 6.6, CH$_3$); δ_C (75.5 MHz, C^{2}HCl$_3$) 174.0 (amide), 171.6 (ester), 156.5 (urethane), 128.6, 127.3 and 127.1 (Ar), 126.3 (Ar), 82.5 (O*C*(CH$_3$)$_3$), 80.4 (O*C*(CH$_3$)$_3$), 52.7 (C-2), 49.1 (C-7), 40.1 (C-3), 37.1 (C-4), 28.3 (C(*C*H$_3$)$_3$), 28.0 (C(*C*H$_3$)$_3$), 22.6 (C-8) and 18.1 (C-5).

Crystal data for compound 19†

$C_{23}H_{36}N_2O_5$, M = 420.5, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 9.005(2), b = 16.541(5), c = 16.841(4) Å, V = 2508.5(11) Å^3, Z = 4, D_{calc} = 1.11 Mg m^{-3}, μ(Mo-Kα) = 0.08 mm^{-1}, T = 293(2) K, 6576 total reflections measured, 6028 independent reflections collected on a Nonius CAD4 diffractometer (R_{int} = 0.034) using Mo-Kα radiation (λ = 0.71073 Å). Refinement using SHELXL-97 with non-H atoms anisotropic, H atoms on N(1) and N(2) freely refined and other H atoms in riding mode. Final residues were $R1$ = 0.067 and $wR2$ = 0.127 (for 2531 reflections with $I > 2\sigma(I)$, $R1$ = 0.179, $wR2$ = 0.167 for all reflections.

***tert*-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-5-hydroxyleucine (20)**

1-*tert*-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-methylglutamic acid **18** (5.20 g, 16 mmol) was dissolved in tetrahydrofuran (75 ml) and the solution was cooled to −40 °C. Triethylamine (2.97 ml, 21 mmol) and *iso*-butyl chloroformate (2.50 ml, 20 mmol) were added and the mixture was stirred at −40 °C for 1 h. The solution became bright yellow and a white sediment formed. Sodium borohydride (1.82 g, 48 mmol) in a mixture of tetrahydrofuran (53 ml) and water (7 ml) was added to the mixture at 0 °C. Effervescence was observed and the mixture turned pale red. The mixture was allowed to warm to room temperature over 2 h, saturated aqueous ammonium chloride (50 ml) was added and the solution was extracted into ethyl acetate (3 × 75 ml). The organic layers were washed with 10% aqueous citric acid (50 ml), water (50 ml) and brine (50 ml) and dried ($MgSO_4$). The solvents were removed *in vacuo* to give a clear pink oil which was purified by column chromatography on silica gel using petroleum ether : ethyl acetate (6 : 4) as eluent to give *tert-butyl (2S,4R)-N-tert-butoxycarbonyl-5-hydroxyleucine* **20** as a clear colourless oil (4.32 g, 87%); $[\alpha]_D^{27}$ +11.1 (*c* 0.9, $CHCl_3$); *m*/*z* (ES+) Found: 304.2119 ([M + H]$^+$), [$C_{15}H_{29}NO_5$ + H] requires 304.2124; *m*/*z* [+ve FAB, 3-NBA] 326 ([M + Na]$^+$) and 304 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3372 (OH and NH) and 1717 (br, C=O); δ_H (300 MHz, C^{2}HCl$_3$) 5.24 (1H, d, $J_{NH,2}$ 8.0, NH), 4.51 (1H, m, H-2), 3.27 (1H, dd, $J_{5A,5B}$ 10.5, $J_{5A,4}$ 5.1, H-5A), 3.16 (1H, dd, $J_{5B,5A}$ 10.5, $J_{5B,4}$ 6.0, H-5B), 1.92 (1H, br s, O*H*), 1.90–1.73 (1H, m, H-4), 1.65–1.58 (1H, m, H-3A), 1.45–1.35 (1H, m, H-3B), 1.30 (9H, s, C(CH$_3$)$_3$), 1.19 (9H, s, C(CH$_3$)$_3$) and 0.70 (3H, d, $J_{6,4}$ 6.8, CH$_3$); δ_C (75.5 MHz, C^{2}HCl$_3$) 172.8 (ester), 156.5 (urethane), 81.4 (O*C*(CH$_3$)$_3$), 79.5 (O*C*(CH$_3$)$_3$), 67.6 (C-5), 53.3 (C-2), 37.8 (C-3), 32.8 (C-4), 28.6 (C(*C*H$_3$)$_3$), 28.1 (C(*C*H$_3$)$_3$) and 17.8 (C-6).

***tert*-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine (21)**

tert-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-5-hydroxyleucine **20** (4.50 g, 15 mmol) was dissolved in dichloromethane (60 ml) under an atmosphere of nitrogen. Pyridine (4.70 ml, 59 mmol) and methanesulfonyl chloride (2.30 ml, 30 mmol) were added and the mixture was stirred at room temperature for 18 h. The solvents were removed *in vacuo* and the residue was dissolved in ethyl acetate (100 ml). The solution was washed with saturated aqueous ammonium chloride (50 ml), water (50 ml) and brine (50 ml). The organic layer was dried ($MgSO_4$) and the solvents were removed *in vacuo* to give *tert-butyl (2S,4R)-N-tert-butoxycarbonyl-5-methylsulfonyloxyleucine* **21** as a clear colourless oil which crystallised upon standing (5.26 g, 93%). An analytically pure sample was obtained by recrystallisation from petroleum ether; mp 45–46 °C; $[\alpha]_D^{27}$ +4.1 (*c* 1.1, $CHCl_3$); (Found: C, 50.4; H, 8.4; N, 3.7. $C_{16}H_{31}NO_7S$ requires: C, 50.4; H, 8.2; N, 3.7%); *m*/*z* [+ve FAB (3-NBA)] 404 ([M + Na]$^+$) and 382 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3382 (NH), 1744 (ester) and 1705 (urethane); δ_H (300 MHz, C^{2}HCl$_3$) 5.08 (1H, d, $J_{NH,2}$ 7.9, NH), 4.25–4.15 (1H, m, H-2), 4.13 (2H, m, H-5), 3.03 (3H, s, H-7), 2.16 (2H, m, H-3A and H-4), 1.87 (1H, m, H-3B), 1.47 (9H, s, C(CH$_3$)$_3$), 1.42 (9H, s, C(CH$_3$)$_3$) and 1.08 (3H, d, $J_{6,4}$ 6.9, CH$_3$); δ_C (75.5 MHz, C^{2}HCl$_3$) 171.6 (ester), 156.9 (urethane), 82.4 (O*C*(CH$_3$)$_3$), 79.9 (O*C*(CH$_3$)$_3$), 73.7 (C-5), 51.9 (C-2), 37.1 (C-7), 36.6 (C-3), 29.9 (C-4), 28.3 (C(*C*H$_3$)$_3$), 27.8 (C(*C*H$_3$)$_3$) and 17.0 (C-6).

***tert*-Butyl (2*S*,4*R*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine (22)**

tert-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-5-mesyloxyleucine **21** (5.0 g, 13 mmol) was dissolved in acetonitrile (75 ml). 4-Dimethylaminopyridine (320 mg, 0.26 mmol) and di-*tert*-butyl dicarbonate (8.59 g, 39 mmol) were added and the mixture was stirred at room temperature under nitrogen for 16 h. The solvent was removed *in vacuo* to give a pale yellow oil which was purified by flash column chromatography on silica gel using petroleum ether : ethyl acetate (3 : 1) as eluent giving *tert-butyl (2S,4R)-N,N-di-tert-butoxycarbonyl-5-methylsulfonyloxyleucine* **22** as a clear colourless oil (4.55 g, 72%); $[\alpha]_D^{24}$ −21.9 (*c* 10.7, $CHCl_3$); *m*/*z* (ES+) Found: 482.2433 ([M + H]$^+$), [$C_{21}H_{39}NO_9S$ + H] requires 482.2424; *m*/*z* [EI] 504 ([M + Na]$^+$) and 482 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1790 (bisurethane) and 1737 (ester); δ_H (500 MHz, C^{2}HCl$_3$) 4.77 (1H, m, H-2), 4.17 (1H, dd, $J_{5A,5B}$ 9.6, $J_{5A,4}$ 4.7 H-5A), 4.14 (1H, dd, $J_{5B,5A}$ 9.6, $J_{5B,4}$ 4.7 H-5B), 3.02 (3H, s, CH_3SO_2), 2.20 (1H, m, H-4), 1.98 (1H, m, H-3A), 1.75 (1H, ddd, $J_{3B,3A}$ 14.4, *J* 8.9, *J* 5.9, H-3B), 1.51 (18H, s, C(CH$_3$)$_3$), 1.50 (9H, s, C(CH$_3$)$_3$) and 1.06 (3H, d, $J_{6,4}$ 6.8, CH$_3$); δ_C (75.5 MHz, C^{2}HCl$_3$) 169.5 (ester), 152.4 (urethane), 83.1 (2 × O*C*(CH$_3$)$_3$), 81.5 (O*C*(CH$_3$)$_3$), 73.6 (C-5), 56.6 (C-2), 37.1 (C-7), 32.5 (C-3), 30.5 (C-4), 28.0 (2 × C(*C*H$_3$)$_3$), 27.9 (C(*C*H$_3$)$_3$) and 17.3 (*C*-6).

***tert*-Butyl (2*S*,4*R*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-fluoroleucine (23)**

tert-Butyl (2*S*,4*R*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-methylsulfonyloxyleucine **22** (1.04 g, 2.17 mmol) was dissolved in tetrahydrofuran (15 ml) under nitrogen. 2-Mesitylenesulfonic acid dihydrate (615 mg, 2.60 mmol) and tetrabutylammonium fluoride (1.0 M in tetrahydrofuran, 6.50 ml, 6.50 mmol) were added and the mixture was stirred at 50–52 °C for 3 days. The solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate (50 ml) and washed with water (20 ml) and brine (20 ml). The organic layer was dried ($MgSO_4$) and the solvents were removed *in vacuo* to give a clear colourless oil which was purified by column chromatography on silica gel using petroleum ether : ethyl acetate (3 : 1) as eluent to give unchanged *tert-butyl (2S,4R)-N,N-di-tert-butoxycarbonyl-5-mesyloxyleucine* **22** (250 mg, 24%) as a clear colourless oil, and a mixture of *tert*-butyl (2*S*,4*R*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-fluoroleucine **23** and *tert-butyl (2R,4R)-N,N-di-tert-butoxycarbonyl-5-fluoroleucine* **23b** as a white crystalline solid. Purification of the desired epimer was achieved by recrystallisation from petroleum ether to give *tert-butyl (2S,4R)-N,N-di-tert-butoxycarbonyl-5-fluoroleucine* **23** as a white crystalline solid (610 mg, 70%); mp 52–54 °C; $[\alpha]_D^{25}$ −32.6 (*c* 1.5, $CHCl_3$); (Found: C, 59.2; H, 8.8; N, 3.3. $C_{20}H_{36}NO_6F$ requires C, 59.2; H, 9.0; N, 3.5%); *m*/*z* [+ve FAB (3-NBA)] 833 ([2M + Na]$^+$), 428 ([M + Na]$^+$) and 406 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1731 (ester) and 1699 (urethane); δ_H (300 MHz, C^{2}HCl$_3$) 4.78 (1H, m, H-2), 4.30 (2H, dm, $J_{H,F}$ 48.0, H-5), 2.32 (1H, m, H-3A), 1.90 (1H, m, H-4), 1.70

(1H, m, H-3B), 1.52 (18H, s, 2 × $C(CH_3)_3$), 1.43 (9H, s, $C(CH_3)_3$) and 1.01 (3H, d, $J_{6,4}$ 7.0, CH_3); δ_C (75.5 MHz, C^2HCl_3) 169.8 (ester), 152.4 (urethane), 88.5–86.2 (d, $^1J_{5,F}$ 181.0, C-5), 83.0 (2 × $OC(CH_3)_3$), 81.5 ($OC(CH_3)_3$), 57.0 (C-2), 32.1 (C-3), 31.4 (C-4), 28.0 (3 × $C(CH_3)_3$) and 16.9 (C-6); δ_F (282 MHz, C^2HCl_3) −225.15 (t × d, $J_{F,H5}$ 47.5, $J_{F,H4}$ 21.6, CH_2F).

(2*S*,4*R*)-5-Fluoroleucine hydrochloride (2)

tert-Butyl (2*S*,4*R*)-*N*,*N*-di-*tert*-butoxycarbonyl-5-fluoroleucine **23** (102 mg, 0.252 mol) was suspended in 6 M aqueous HCl (2 ml) and the mixture was heated at 40 °C for 3 days. The solvents were removed *in vacuo* to afford a white powder. Recrystallisation from methanol gave (2*S*,4*R*)-5-fluoroleucine hydrochloride (**2**) as a white crystalline solid (38 mg, 80%); mp 210–212 °C; $[\alpha]_D^{19}$ −8.6 (*c* 0.3, $CHCl_3$); *m*/*z* [+ve FAB (3-NBA)] 150 ($[M + H]^+$); ν_{max} (KBr)/cm^{-1} 3413 (NH and OH) and 1690 (br, C=O); δ_H (300 MHz, $C^2H_3O^2H$) 4.32–4.09 (2H, d × m, $J_{H,F}$ 48.0, H-5), 3.50 (1H, m, H-2), 2.0 (1H, m, H-3A), 1.5 (1H, m, H-4), 1.05 (1H, m, H-3B) and 0.95 (3H, d, $J_{6,4}$ 6.7, CH_3); δ_C (75.5 MHz, $C^2H_3O^2H$) 170.8 (acid), 88.7 (d, $^1J_{5,F}$ 169.3, C-5), 57.1 (C-2), 32.1 (C-3), 31.4 (C-4) and 16.9 (C-6); δ_F (282 MHz, $C^2H_3O^2H$)) −228.4 (dt, $J_{F,H5}$ 47.6, $J_{F,H4}$ 19.4, CH_2F).

Crystal data for compound 2†

$C_6H_{12}FNO_2$, M = 149.17, monoclinic, space group $P2_1$ (No.4), a = 9.637(4), b = 5.314(3), c = 14.574(7) Å, β = 93.10(3)°, V = 745.3(6) Å^3, Z = 4, D_{calc} = 1.33 Mg m^{-3}, μ(Mo-Kα) = 0.11 mm^{-1}, T = 173(2) K, 3162 total reflections measured, 1844 independent reflections collected on a Nonius Kappa CCD diffractometer (R_{int} = 0.175) using Mo-Kα radiation (λ = 0.71073 Å). There are two independent molecules both of which showed large ADPs for the F end of the molecule. H atoms were not located for the NH_2 group and were omitted for the disordered region. Refinement using SHELXL-97. Final residues were $R1$ = 0.108, $wR2$ = 0.226 (for 853 reflections with $I > 2\sigma(I)$), $R1$ = 0.215, $wR2$ = 0.285 for all reflections.

Acknowledgements

We thank the BBSRC and EPSRC (BMS committee) for funding, Professor R. Ramage and Dr L. Sawyer for useful discussions, Dr A. G. Avent for NMR spectra, Dr A. Abdul-Sada for mass spectra and the EPSRC Central Mass Spectroscopy Service, Swansea for high resolution mass spectra.

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