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Synthesis of (2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-valine and (2*S*,3*S*)-4-fluorovaline †

Jean-Damien Charrier, Peter B. Hitchcock and Douglas W. Young*
Sussex Centre for Biomolecular Design and Drug Discovery, Department of Chemistry, University of Sussex, Falmer, Brighton, UK BN1 9QJ

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A new synthesis of (2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-valine **2** has been completed and (2*S*,3*S*)-4-fluorovaline **4** has been synthesised for the first time. Both compounds have been prepared by routes involving stereoselective addition to the (*S*)-pyroglutamate derivative **5** and are available for studies in several areas of bio-organic chemistry.

Introduction

The synthesis of amino acids in which prochiral atoms or groups are stereospecifically labelled has enabled the mechanisms of metabolic reactions to be studied in great detail.[1] Further, subtle aspects of protein structure and molecular recognition may be studied using such compounds. Thus we have incorporated a sample of (2*S*,4*R*)-[5,5,5-$^2H_3$]-leucine, **1**, specifically labelled in the pro-*R* methyl group with deuterium, into the protein dihydrofolate reductase. This allowed assignment of the diastereotopic methyl groups of all but one of the thirteen leucine residues in the ^{1}H-NMR spectrum of this enzyme bound to the cancer drug, methotrexate.[2] In order that studies in these areas may be extended, we now report a new synthesis of (2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-valine **2**. Samples of (2*RS*,3*S*)-,[3] (2*R*,3*S*)-,[4] (2*S*,3*R*)-[5] and (2*S*,3*S*)-[5a,6] [3′,3′,3′-$^2H_3$]-valines have already been synthesised by a variety of methods but this new and relatively high yielding method should be useful for incorporation into proteins.

The possibility of using fluorine as a reporter group in protein folding studies encouraged us to synthesise the epimeric (2*S*)-5-fluoroleucines[7] and we have incorporated the (2*S*,4*S*)-epimer **3** into all of the leucine residues of dihydrofolate reductase.[7] We have also achieved selective strategic incorporation of this fluorinated amino acid into the leucine residues 50 and 67 of the protein ubiquitin, which are close neighbours in the hydrophobic core.[8] In order that these studies may be extended, we now report the stereoselective synthesis of (2*S*,3*S*)-4-fluorovaline **4**.

Results and discussion

The starting point for our synthesis of (2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-valine **2** was (5*S*)-*N*-*tert*-butoxycarbonyl-1*H*-5-*tert*-butyldiphenylsiloxymethyl-2(5*H*)-pyrrol-2-one **5**[9] which has been shown to react with methylcuprate entirely from the less hindered face at C-4.[10] We therefore reacted this compound with [$^2H_3$]-methylmagnesium iodide and copper bromide–dimethyl sulfide in tetrahydrofuran, as shown in Scheme 1, to give (4*S*,5*S*)-[4,4,4-$^2H_3$]-*N*-*tert*-butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-methylpyrrolidin-2-one **6** in 89% yield. The stereochemistry was defined by the NOE experiments shown in Fig. 1 since irradiation of H-6A at 3.4 ppm caused enhancement of H-4 at 1.82 ppm (3.4%). Irradiation of H-6B at 3.88 ppm caused enhancement of H-4 at 1.82 ppm (0.4%) and to H-3*S* at 2.63 ppm (0.7%), thus defining the stereochemistry of the latter peak. Irradiation of H-3*S* at 2.63 ppm caused enhancements to H-4 at 1.82 ppm (6.4%), H-3*R* (28.8%) and H-6B (0.5%). Irradiation of H-4 at 1.82 ppm caused enhancement to H-3*S* (2.9%) and H-6A (1.8%). This stereochemical assignment was confirmed by an X-ray crystal structure of the deuteriated compound (Fig. 2), the structure of the unlabelled compound having previously been reported.[11]

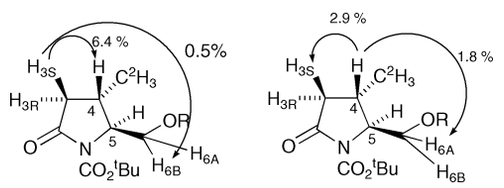

Fig. 1 NOE enhancements on compound **6**.

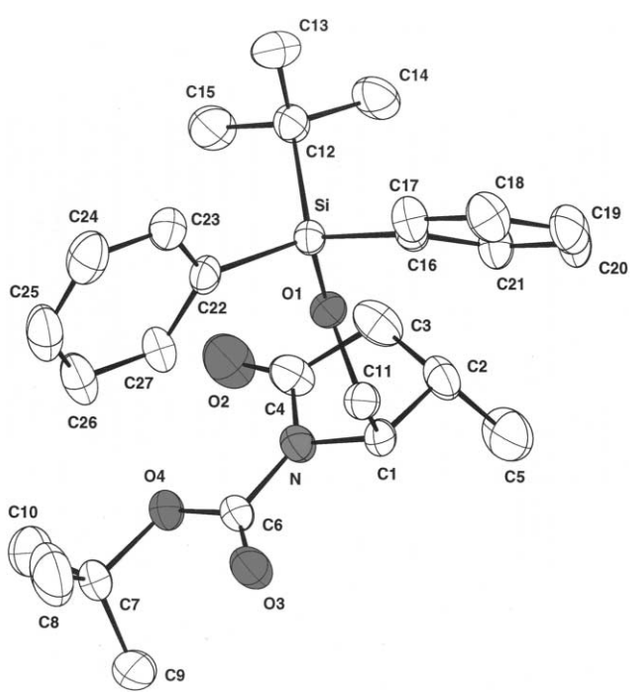

Fig. 2 X-ray structure of compound **6**.

Hydrolysis of the lactam **6** was achieved using aqueous lithium hydroxide in tetrahydrofuran, giving the acid **7** in 98% yield.

The valine side chain was now accessed by decarboxylation of the acid **7** using methodology developed by Barton *et al.*[12] Thus the acid **7** was converted into the mixed anhydride using *iso*-butyl chloroformate, and this was reacted with *N*-hydroxy-2-thiopyridone followed by addition of *tert*-butyl-

† Electronic supplementary information (ESI) available: copies of all ^{1}H and ^{13}C spectra, except for those already shown in Fig. 3. See http://www.rsc.org/suppdata/ob/b4/b401646g/

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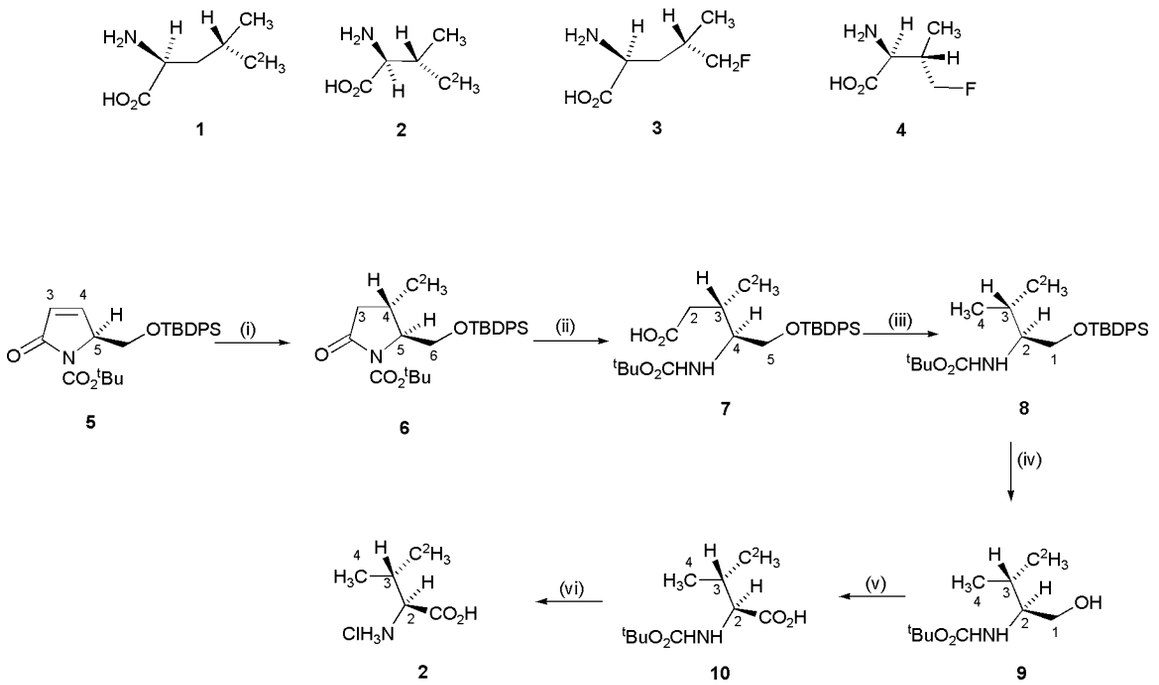
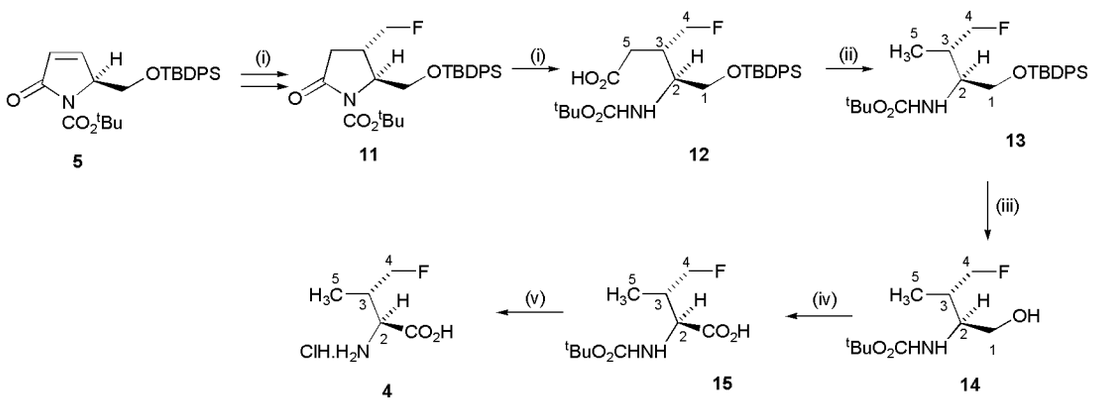


Scheme 1 *Reagents and conditions*: (i) $^{2}H_3CMgI/CuBr{\cdot}SMe_2/THF/-78$ °C, 89%; (ii) aq. LiOH/THF, 98%; (iii) (a) *i*BuO_2CCl/*N*-methylmorpholine/THF, (b) *N*-hydroxythiopyridone/Et_3N, (c) *t*BuSH/*hv*, 82%; (iv) Bu_4NF/THF, 85%; (v) $RuCl_3/NaIO_4/CH_3CN/CCl_4/H_2O$, 77%; (vi) 6 M aq. HCl, 99%.



Scheme 2 *Reagents and conditions*: (i) ref. 14; (ii) (a) *i*BuO_2CCl/*N*-methylmorpholine/THF, (b) *N*-hydroxythiopyridone/Et_3N, (c) *t*BuSH/*hv*, 73%; (iii) Bu_4NF/THF, 86%; (iv) $RuCl_3/NaIO_4/CH_3CN/CCl_4/H_2O$, 74%; (v) 6 M aq. HCl, 95%.

thiol and irradiation with visible light. The product **8**, which was obtained in 82% yield, was deprotected using tetrabutylammonium fluoride to give the alcohol **9** in 85% yield. Oxidation using ruthenium chloride and sodium periodate gave the protected labeled valine in 77% yield. Deprotection using 6 M aqueous hydrochloric acid finally gave the target (2*S*,3*R*)-[3′,3′,3′-$^{2}H_3$]-valine **2** as the hydrochloride. Literature values for the ^{1}H-[5] and ^{13}C-[13] NMR spectra were in keeping with those found (Fig. 3), the *pro-S* methyl signal in $^{2}H_2O/NaO^{2}H$ being to higher field in each case.

Our synthetic route to (2*S*,3*S*)-4-fluorovaline **4** has also made use of stereoselective addition to the conjugated system of the compound **5**. In previous work,[14] we converted this compound into the acid **12** as shown in Scheme 2. Application of the Barton methodology, used in our synthesis of the labelled valine above, allowed us to convert this into the fluoro-compound **13** in 73% yield. Deprotection using tetrabutylammonium fluoride gave the alcohol **14** in 86% yield and this was oxidised using ruthenium chloride and sodium periodate to give the protected fluorovaline **15** in 74% yield. Deprotection using 6 M hydrochloric acid gave (2*S*,3*S*)-4-fluorovaline **4** in 95% yield.

We have therefore succeeded in completing a new synthesis of (2*S*,3*R*)-[3′,3′,3′-$^{2}H_3$]-valine **2**, making it available for a variety of studies in bio-organic chemistry. We have also prepared (2*S*,3*S*)-4-fluorovaline **4**, thus widening the possibility of strategic substitution of hydrophobic amino acids in proteins by amino acids bearing a fluorine reporter group. This will expand the use of ^{19}F-NMR spectroscopy in the study 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 a Bruker DPX 300 (300 MHz) Fourier-transform instrument. ^{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). All ^{1}H- and ^{13}C-NMR spectra were recorded using TMS, DSS (0.00 ppm) or residual solvent peaks as internal references. δ are in ppm and *J* in Hertz (Hz). Low-resolution mass spectra were recorded on Kratos MS80F and MS25 double focusing spectrometers. High-resolution mass

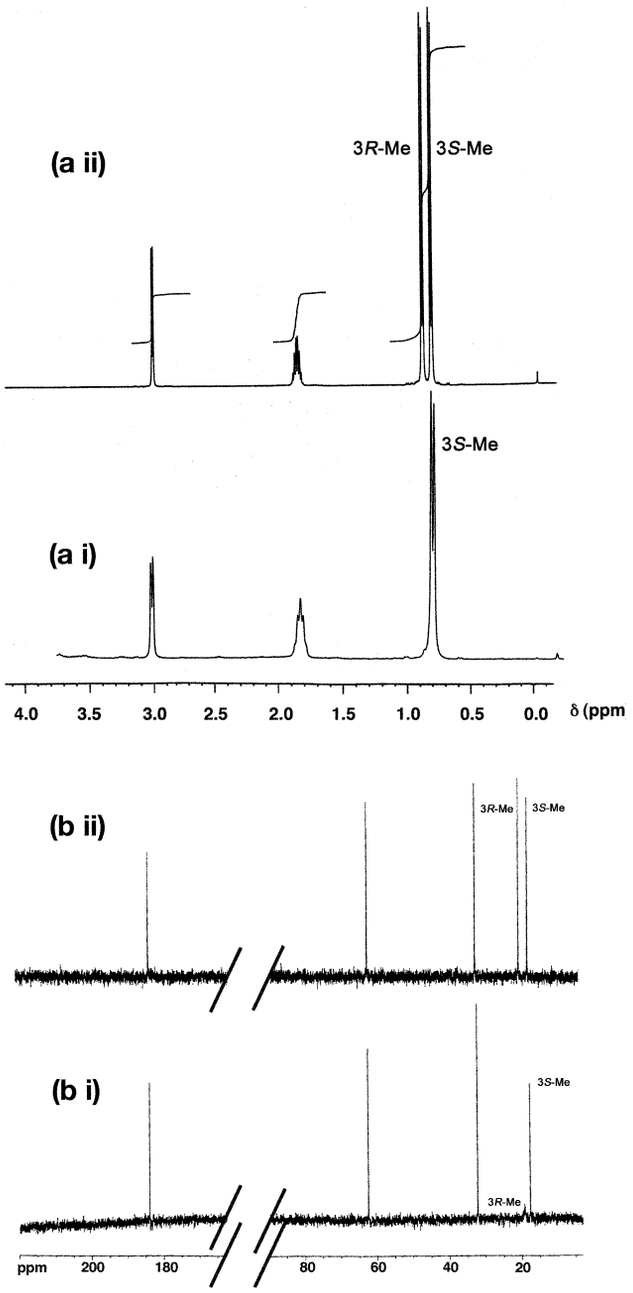


Fig. 3 NMR spectra in $^2H_2O/NaO^2H$: (a) ^1H; (b) ^{13}C; of (i) (2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-valine, **2**, and (ii) (2*S*)-valine.

measurements were recorded by the EPSRC National Mass Spectrometry Service (Swansea). 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*S*,5*S*)-[4′,4′,4′-$^2H_3$]-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-methylpyrrolidin-2-one (6)

[$^2H_3$]-Methylmagnesium iodide (1 M in diethyl ether, 100 ml, 100 mmol) was added to copper bromide–dimethyl sulfide complex (10.28 g, 50 mmol) in dry tetrahydrofuran (90 ml) at −15 °C. After 15 min the mixture was cooled to −78 °C. (5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-2(5*H*)-pyrrol-2-one **5**[9] (4.51 g, 10 mmol) and trimethylsilyl chloride (1 M in tetrahydrofuran, 20 ml, 20 mmol) in tetrahydrofuran (45 ml) were added dropwise over 15 min. After 1 h, aqueous ammonium chloride (100 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 layer was 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 *(4S,5S)-[4,4,4-$^2H_3$]-N-tert-butoxycarbonyl-5-tert-butyldiphenylsilyloxymethyl-4-methylpyrrolidin-2-one* **6** as a colourless oil which was crystallised from ethanol/water as a white solid (4.21 g, 89%), mp 77 °C; $[\alpha]^{22}_D$ −15.16 (*c* 1.0, $CHCl_3$); *m/z* [FAB, PEG/NBA] Found 493.2580 ($[M+Na]^+$), [$C_{27}H_{34}{}^2H_3NO_4Si$ + Na] requires 493.2578; *m/z* [+ve FAB, NBA] 963 ($[2M+Na]^+$) and 493 ($[M+Na]^+$); ν_{max} (KBr)/cm^{-1} 1748 and 1711; δ_H (300 MHz, $C_6{}^2H_6$) 0.98 [9H, s, $SiC(CH_3)_3$], 1.33 [9H, s, $OC(CH_3)_3$], 1.60 (1H, dd, $J_{3R,3S}$ 17.3, $J_{3R,4}$ 1.8, H-3*R*), 1.82 (1H, dd, $J_{4,3S}$ 8.6, $J_{4,3R}$ 1.8, H-4), 2.63 (1H, dd, $J_{3S,3R}$ 17.3, $J_{3S,4}$ 8.6, H-3*S*), 3.40 (1H, dd, $J_{6A,6B}$ 10.3, $J_{6A,5}$ 2.0, H-6A), 3.53 (1H, m, H-5), 3.88 (1H, dd, $J_{6B,6A}$ 10.4, $J_{6B,5}$ 4.1, H-6B), 7.08–7.15 (6H, m, aromatics) and 7.54–7.59 (4H, m, aromatics); δ_C (75.5 MHz, $C_6{}^2H_6$) 19.4 [Si*C*$(CH_3)_3$], 20.0 (m, C^2H_3), 27.0 [SiC(CH_3)$_3$], 28.2 [OC(CH_3)$_3$+ C-4], 40.5 (C-3), 64.5 (C-6), 65.7 (C-5), 81.9 [O*C*$(CH_3)_3$], 128.1, 130.1, 133.3, 133.6 and 135.9 (aromatics), 151.3 (urethane) and 172.1 (C-2).

Crystal data – compound 6‡

$C_{27}H_{34}{}^2H_3NO_4Si$, M = 470.7, monoclinic, space group $P2_1$ (No. 4), a = 9.767(5), b = 13.052(4), c = 11.178(4) Å, β = 105.26(3)°, V = 1375(1) Å^3, Z = 2, D_{calc} = 1.14 Mg m^{-3}, $F(000)$ = 504, μ(Cu-Kα) = 0.99 mm^{-1}, T = 293(2) K, 2272 total reflections measured, 2145 independent reflections collected on a Nonius CAD4 diffractometer (R_{int} = 0.040) using Cu-Kα radiation (λ = 1.5418 Å). Refinement using SHELXL-97. Final residues were $R1$ = 0.048, $wR2$ = 0.117 (for 1819 reflections with $I > 2\sigma(I)$), $R1$ = 0.060, $wR2$ = 0.128 for all reflections.

(3*S*,4*S*)-[3′,3′,3′-$^2H_3$]-4-*tert*-Butoxycarbonylamino-5-*tert*-butyldiphenylsilyloxy-3-methylpentanoic acid (7)

1 M Aqueous lithium hydroxide (6.38 ml, 6.38 mmol) was added to a solution of (4*S*,5*S*)-[4′,4′,4′-$^2H_3$]-*N*-*tert*-butoxycarbonyl-5-*tert*-butyldiphenylsilyloxymethyl-4-methyl-pyrrolidin-2-one **6** (1 g, 2.13 mmol) in tetrahydrofuran (10 ml) at room temperature. The mixture was stirred overnight and extracted with ethyl acetate and aqueous sodium bicarbonate. The combined aqueous phases were acidified to pH 4–4.5 with 10% aqueous citric acid and extracted with ethyl acetate. The combined organic layers were dried ($MgSO_4$) and the solvents were removed *in vacuo*. The residue was chromatographed on silica gel using petroleum ether : ethyl acetate (3 : 1) as eluent to afford *(3S,4S)-[3′,3′,3′-$^2H_3$]-4-tert-butoxycarbonylamino-5-tert-butyldiphenylsilyloxy-3-methylpentanoic acid* **7** as a pale yellow oil (1.02, 98%) which was used directly in further reactions, $[\alpha]^{22}_D$ −12.30 (*c* 1.0, $CHCl_3$); *m/z* [+ve FAB, NBA] 511 ($[M+Na]^+$) and 489 ($[M+H]^+$); ν_{max} (film)/cm^{-1} 3325 (NH) and 1713 (acid); δ_H (300 MHz, C^2HCl_3) 0.99 [9H, s, $SiC(CH_3)_3$], 1.35 [9H, s, $OC(CH_3)_3$], 2.07 (1H, dd, $J_{2A,2B}$ 15.1, $J_{2A,3}$ 8.1, H-2A), 2.21 (1H, dd, $J_{3,2A}$ 8.1, $J_{3,2B}$ 4.2, H-3), 2.48 (1H, dd, $J_{2B,2A}$ 15.1, $J_{2B,3}$ 4.2, H-2B), 3.37 (1H, m, H-5A), 3.62 (2H, m, H-5B + H-4), 4.79 (1H, d, $J_{NH,4}$ 9.6, NH), 7.14–7.32 (6H, m, aromatics), 7.55–7.62 (4H, m, aromatics) and 9.59 (1H, br s, COOH); δ_C (75.5 MHz, C^2HCl_3) 19.4 (m, C^2H_3), 19.3 [Si*C*$(CH_3)_3$], 26.9 [SiC(CH_3)$_3$], 28.3 [OC(CH_3)$_3$], 31.5 (C-3), 38.3 (C-2), 56.1 (C-4), 63.8 (C-5), 79.5 [O*C*$(CH_3)_3$], 127.7, 129.8, 133.0, 133.1 and 135.5 (aromatics), 155.9 (urethane) and 178.8 (acid).

‡ CCDC reference number 232480. See http://www.rsc.org/suppdata/ob/b4/b401646g/ for crystallographic data in.cif or other electronic format.

(2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-2-*tert*-Butoxycarbonylamino-*O*-*tert*-butyldiphenylsilyl-3-methylbutan-1-ol (8)

iso-Butyl chloroformate (0.487 ml, 3.76 mmol) was added at −15 °C with stirring to a solution of (3*S*,4*S*)-[3′,3′,3′-$^2H_3$]-4-*tert*-butoxycarbonylamino-5-*tert*-butyldiphenylsilyloxy-3-methylpentanoic acid **7** (1.666 g, 3.41 mmol) in dry tetrahydrofuran (15 ml) containing *N*-methylmorpholine (0.413 ml, 3.76 mmol). After 15 min, the flask was protected from light, triethylamine (0.619 ml, 4.44 mmol) and *N*-hydroxy-2-thiopyridone (493 mg, 3.88 mmol) were added and the mixture was stirred for 1 h in the dark. *tert*-Butylthiol (3.85 ml, 34.14 mmol) was added and the mixture was irradiated with two desk lamps (100 watt) for 1 h at room temperature. A slight decolourization was observed. Ethyl acetate was added and the solution was washed with 0.5 M aqueous hydrochloric acid, brine and water. 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 (19 : 1) as eluent to afford *(2S,3R)-[3′,3′,3′-$^2H_3$]-2-tert-butoxycarbonylamino-O-tert-butyldiphenylsilyl-3-methylbutan-1-ol* **8** as a colourless oil (1.247 g, 82%), $[\alpha]^{22}_D$ −17.08 (*c* 0.1, $CHCl_3$); *m/z* [FAB, PEG/NBA] Found 445.2967 ([M+H]$^+$), [$C_{26}H_{36}{}^2H_3NO_3Si$ + H] requires 445.2966; *m/z* [+ve FAB, NBA] 467 ([M+Na]$^+$) and 445 ([M+H]$^+$); ν_{max} (film)/cm^{-1} 3448 (NH) and 1702 (urethane); δ_H (300 MHz, $C_6{}^2H_6$) 0.76 (3H, d, $J_{Me,3}$ 6.6, CH_3), 1.13 [9H, s, $SiC(CH_3)_3$], 1.47 [9H, s, $OC(CH_3)_3$], 1.73 (1H, m, H-3), 3.58 (1H, d, $J_{1A,1B}$ 10.0, H-1A), 3.60 (1H, d, $J_{1B,1A}$ 10.0, H-1B), 3.65 (1H, m, H-2), 4.55 (1H, d, $J_{NH,2}$ 8.7, NH), 7.20–7.31 (6H, m, aromatics) and 7.64–7.79 (4H, m, aromatics); δ_C (75.5 MHz, $C_6{}^2H_6$) 18.4 (C-4), 18.9 (m, C^2H_3), 19.5 [$SiC(CH_3)_3$], 27.1 [$SiC(CH_3)_3$], 28.6 [$OC(CH_3)_3$], 29.2 (C-3), 57.4 (C-2), 64.7 (C-1), 78.4 [$OC(CH_3)_3$], 128.1, 130.0, 133.8 and 136.0 (aromatics) and 155.8 (urethane).

(2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-2-*tert*-Butoxycarbonylamino-3-methylbutan-1-ol (9)

Tetrabutylammonium fluoride (1 M in tetrahydrofuran, 10 ml, 10 mmol) was added to a solution of (2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-2-*tert*-butoxycarbonylamino-*O*-*tert*-butyldiphenylsilyl-3-methylbutan-1-ol **8** (1.12 g, 2.52 mmol) in tetrahydrofuran (40 ml). The mixture was stirred for 5 days at room temperature when TLC showed the reaction to be complete. The mixture was diluted with ethyl acetate and washed with aqueous ammonium chloride (2×). The organic layer was dried ($MgSO_4$) and the solvents were removed *in vacuo*. The residue was purified on silica gel using petroleum ether : ethyl acetate (3 : 1) as eluent to afford *(2S,3R)-[3′,3′,3′-$^2H_3$]-2-tert-butoxycarbonylamino-3-methylbutan-1-ol* **9** as a colourless oil (443 mg, 85%), $[\alpha]^{22}_D$ −17.44 (*c* 1.0, $CHCl_3$); *m/z* [FAB, PEG/NBA] Found 207.1795 ([M+H]$^+$), [$C_{10}H_{18}{}^2H_3NO_3$ + H] requires 207.1788; *m/z* [+ve FAB, NBA] 207 ([M+H]$^+$); ν_{max} (film)/cm^{-1} 3356 (NH) and 1686 (urethane); δ_H (300 MHz, $C^2H_3O^2H$) 0.78 (3H, d, $J_{Me,3}$ 6.8, CH_3), 1.34 [9H, s, $OC(CH_3)_3$], 1.71 (1H, dq, $J_{3,Me} = J_{3,2}$ = 6.8, H-3), 3.25 (1H, m, H-1A) and 3.43 (2H, m, H-1B + H-2); δ_C (75.5 MHz, $C^2H_3O^2H$) 18.5 (C-4), 19.2 (m, C^2H_3), 28.8 [$OC(CH_3)_3$], 29.9 (C-3), 58.9 (C-2), 63.3 (C-1), 79.7 [$OC(CH_3)_3$] and 158.5 (urethane).

(2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-*N*-*tert*-Butoxycarbonylvaline (10)

(2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-2-*tert*-Butoxycarbonylamino-3-methylbutan-1-ol **9** (136 mg, 0.66 mmol) was dissolved in a mixture of acetonitrile (3 ml) and carbon tetrachloride (3 ml). Sodium periodate (706 mg, 3.3 mmol), water (4.5 ml) and ruthenium chloride hydrate (14 mg, 0.066 mmol) were added and the mixture was stirred for 1.5 h at room temperature. Dichloromethane was added and the solution was filtered through a pad of Celite®. The filtrate was dried ($MgSO_4$) and the solvents were removed *in vacuo* to afford a colourless oil (111 mg, 77%). Chromatography on silica gel using ethyl acetate as eluent gave *(2S,3R)-[3′,3′,3′-$^2H_3$]-N-tert-butoxycarbonylvaline* **10** as a colourless oil, $[\alpha]^{22}_D$ −5.63 (*c* 1.0, $CHCl_3$); *m/z* [+ve FAB (glycerol, H_2O)] 221 ([M+H]$^+$); ν_{max} (film)/cm^{-1} 3320 (NH) and 1719 (acid + urethane); δ_H (300 MHz, $C^2H_3O^2H$) 0.82 (3H, d, $J_{Me,3}$ 6.9, CH_3), 1.35 [9H, s, $OC(CH_3)_3$], 2.00 (1H, m, H-3) and 3.90 (1H, d, $J_{2,3}$ 5.5, H-2); δ_C (75.5 MHz, $C^2H_3O^2H$) 18.1 (C-4), 19.0 (m, C^2H_3), 28.7 [$OC(CH_3)_3$], 31.5 (C-3), 60.2 (C-2), 80.4 [$OC(CH_3)_3$], 158.2 (urethane) and 175.5 (C-1).

(2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-Valine hydrochloride (2)

A solution of (2*S*,3*R*)-[3′,3′,3′-$^2H_3$]-*N*-*tert*-butoxycarbonylvaline **10** (56 mg, 0.254 mmol) in 6 M aqueous HCl (2.5 ml) was stirred for 4 days at room temperature. The solvent was removed *in vacuo* and the residue was azeotroped with diethyl ether (3×) to eliminate residual HCl. *(2S,3R)-[3′,3′,3′-$^2H_3$]-Valine hydrochloride* **2** was precipitated in diethyl ether as white crystals (39 mg, 99%), mp 238–240 °C; $[\alpha]^{22}_D$ +10.35 (*c* 0.66, H_2O); *m/z* [EI+] Found 75.1007 ([M − CO_2H]$^+$), [$C_5H_8{}^2H_3NO_2$ − CO_2H] requires 75.1002; *m/z* [+ve FAB (glycerol, H_2O)] 121 ([M+H]$^+$); ν_{max} (KBr)/cm^{-1} 3320 (NH) and 1731 (acid); δ_H (300 MHz, ^2H_2O, NaO^2H) 0.97 (3H, d, $J_{Me,3}$ 6.8, CH_3), 2.00 (1H, m, H-3) and 3.16 (1H, d, $J_{2,3}$ 5.2, H-2); δ_C (125.9 MHz, ^2H_2O, NaO^2H) 17.7 (C-4), 19.2 (m, C^2H_3), 32.2 (C-3), 62.4 (C-2) and 183.9 (acid); δ_H (300 MHz, $C^2H_3O^2H$) 0.88 (3H, d, $J_{Me,3}$ 7.0, CH_3), 2.08 (1H, m, H-3) and 3.64 (1H, d, $J_{2,3}$ 4.2, H-2); δ_C (74.5 MHz, $C^2H_3O^2H$) 18.1 (C-4), 30.5 (C-3), 59.3 (C-2) and 171.2 (C-1).

(2*S*,3*S*)-2-*tert*-Butoxycarbonylamino-*O*-*tert*-butyldiphenylsilyl-3-fluoromethylbutan-1-ol (13)

iso-Butyl chloroformate (0.332 ml, 2.56 mmol) was added to a solution of (3*S*,4*S*)-4-*tert*-butoxycarbonylamino-5-*tert*-butyldiphenylsilyloxy-3-fluoromethylpentanoic acid **12**[14] (1.169 g, 2.32 mmol) in dry tetrahydrofuran (10 ml) containing *N*-methylmorpholine (0.281 ml, 2.56 mmol) at −15 °C with stirring. After 15 min the flask was protected from light and triethylamine (0.421 ml, 3.02 mmol) and *N*-hydroxy-2-thiopyridone (336 mg, 2.64 mmol) were added. The mixture was stirred for 1 h in the dark and *tert*-butylthiol (2.62 ml, 23.2 mmol) was added. The mixture was irradiated with two desk lamps (100 watt) for 1 h at room temperature. Slight decolourization was observed. The mixture was diluted with ethyl acetate, washed with 0.5 M aqueous hydrochloric acid, brine and water. 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 (19 : 1) as eluent to afford *(2S,3S)-2-tert-butoxycarbonylamino-O-tert-butyldiphenylsilyl-3-fluoromethylbutan-1-ol* **13** as a colourless oil (783 mg, 73%), $[\alpha]^{22}_D$ −14.11 (*c* 1.0, $CHCl_3$); *m/z* [FAB, PEG/NBA] Found 460.2672 ([M+H]$^+$), [$C_{26}H_{38}{}^2H_3NO_3SiF$ + H] requires 460.2683; *m/z* [+ve FAB, NBA] 460 ([M+H]$^+$); ν_{max} (film)/cm^{-1} 3369 (NH) and 1703 (urethane); δ_H (300 MHz, $C_6{}^2H_6$) 0.66 (3H, d, $J_{Me,3}$ 6.9, CH_3), 1.10 [9H, s, $SiC(CH_3)_3$], 1.43 [9H, s, $OC(CH_3)_3$], 1.96 (1H, m, H-3), 3.52 (2H, d, $J_{1,2}$ 5.0, H-1), 3.95 (1H, m, H-2), 4.05 (1H, ddd, $J_{4A,F}$ 47.7, $J_{4A,4B}$ 8.8, $J_{4A,3}$ 6.2, H-4A), 4.18 (1H, ddd, $J_{4B,F}$ 48.7, $J_{4B,4A}$ 8.8, $J_{4B,3}$ 6.0, H-4B), 4.50 (1H, d, $J_{NH,2}$ 9.3, NH), 7.21–7.23 (6H, m, aromatics) and 7.68–7.71 (4H, m, aromatics); δ_C (75.5 MHz, $C_6{}^2H_6$) 11.6 (d, $^3J_{CF}$ 6.2, CH_3), 19.4 [$SiC(CH_3)_3$], 27.0 [$SiC(CH_3)_3$], 28.5 [$OC(CH_3)_3$], 35.9 (d, $^2J_{CF}$ 17.7, C-3), 53.1 (d, $^3J_{CF}$ 4.4, C-2), 64.4 (C-1), 78.9 [$OC(CH_3)_3$], 85.9 (d, $^1J_{CF}$ 169.4, C-4), 128.1, 130.1, 133.6 and 135.9 (aromatics) and 155.7 (urethane); δ_F (282 MHz, $C_6{}^2H_6$) −219.4 (td, $J_{F,4}$ 47.6, $J_{F,3}$ 19.5).

(2*S*,3*S*)-2-*tert*-Butoxycarbonylamino-3-fluoromethylbutan-1-ol (14)

Tetrabutylammonium fluoride (1 M in tetrahydrofuran, 5.84 ml, 5.84 mmol) was added to a solution of (2*S*,3*S*)-*O*-*tert*-butyldiphenylsilyl-2-*tert*-butoxycarbonylamino-3-fluoromethylbutan-1-ol **13** (670 mg, 1.46 mmol) in tetrahydrofuran (20 ml). The mixture was stirred for 5 days at room temperature until the reaction was shown to be complete by TLC. Ethyl acetate was added and the solution was washed with aqueous ammonium chloride (2×). 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 (3 : 1) as eluent to afford *(2S,3S)-2-tert-butoxycarbonylamino-3-fluoromethylbutan-1-ol* **14** as a colourless oil (277 mg, 86%), $[a]^{22}_D$ −14.00 (*c* 1.0, $CHCl_3$); *m/z* [FAB, PEG/NBA] Found 222.1499 ($[M+H]^+$), [$C_{10}H_{20}NO_3F$ + H] requires 222.1505; *m/z* [+ve FAB, NBA] 244 ($[M+Na]^+$) and 222 ($[M+H]^+$); ν_{max} (film)/cm^{-1} 3347 (NH) and 1694 (urethane); δ_H (300 MHz, $C^2H_3O^2H$) 0.94 (3H, d, $J_{Me,3}$ 6.9, CH_3), 1.44 [9H, s, $OC(CH_3)_3$], 2.10 (1H, m, H-3), 3.55 (2H, d, $J_{1,2}$ 5.8, H-1), 3.67 (1H, m, $J_{2,1} = J_{2,3}$ = 5.8, H-2), 4.29 (1H, ddd, $J_{4A,F}$ 47.8, $J_{4A,4B}$ 9.1, $J_{4A,3}$ 6.0, H-4A) and 4.37 (1H, ddd, $J_{4B,F}$ 47.5, $J_{4B,4A}$ 9.1, $J_{4B,3}$ 5.9, H-4B); δ_C (75.5 MHz, $C^2H_3O^2H$) 11.6 (d, $^3J_{CF}$ 5.7, CH_3), 28.7 [$OC(CH_3)_3$], 36.4 (d, $^2J_{CF}$ 18.1, C-3), 54.1 (d, $^3J_{CF}$ 5.0, C-2), 63.0 (C-1), 79.9 [$OC(CH_3)_3$], 86.7 (d, $^1J_{CF}$ 167.8, C-4) and 158.2 (urethane); δ_F (282 MHz, C^2HCl_3) −228.8 (td, $J_{F,4H}$ 47.7, $J_{F,3H}$ 21.5).

(2*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-4-fluorovaline (15)

(2*S*,3*S*)-2-*tert*-Butoxycarbonylamino-3-fluoromethylbutan-1-ol **14** (68 mg, 0.31 mmol) was dissolved in a mixture of acetonitrile (1.5 ml) and carbon tetrachloride (1.5 ml). Sodium periodate (329 mg, 1.54 mmol), water (2 ml) and ruthenium chloride hydrate (7 mg, 0.031 mmol) were added and the mixture was stirred 1.5 h at room temperature. Dichloromethane was added and the solution was filtered through a pad of Celite® and dried ($MgSO_4$). The solvents were removed *in vacuo* to afford *(2S,3S)-N-tert-butoxycarbonyl-4-fluorovaline* **15** as a colourless oil which crystallized on long standing as a white solid (53 mg, 74%), mp 69–71 °C; $[a]^{22}_D$ −11.92 (*c* 0.36, MeOH); *m/z* [+ve FAB] 258 ($[M+Na]^+$) and 236 ($[M+H]^+$); ν_{max} (film)/cm^{-1} 3331 (NH) and 1719 (urethane/acid); δ_H (300 MHz, $C^2H_3O^2H$) 0.90 (3H, dd, $J_{Me,3}$ 7.1, $J_{Me,F}$ 1.0, CH_3), 1.43 [9H, s, $OC(CH_3)_3$], 2.45 (1H, m, H-3), 4.30 (2H, dt, $J_{4,F}$ 47.4, $J_{4,3}$ 7.8, H-4) and 4.36 (1H, d, $J_{2,3}$ 3.6, H-2); δ_C (75.5 MHz, $C^2H_3O^2H$) 9.9 (d, $^3J_{CF}$ 7.5, CH_3), 27.7 [$OC(CH_3)_3$], 36.5 (d, $^2J_{CF}$ 18.7, C-3), 54.3 (C-2), 79.7 [$OC(CH_3)_3$], 84.7 (d, $^1J_{CF}$ 168.9, C-4), 157.3 (urethane) and 174.0 (acid); δ_F (282 MHz, C^2HCl_3) −227.0 (td, $J_{F,4H}$ 47.3, $J_{F,3H}$ 15.4).

(2*S*,3*S*)-4-Fluorovaline hydrochloride (4)

A solution of (2*S*,3*S*)-*N*-*tert*-butoxycarbonyl-4-fluorovaline **15** (34 mg, 0.145 mmol) in 6 M aqueous HCl (1.5 ml) was stirred for 4 days at room temperature. The solvent was removed *in vacuo* and the residue was azeotroped with diethyl ether (3×) to eliminate residual HCl. Precipitation using diethyl ether gave *(2S,3S)-4-fluorovaline hydrochloride* **4** as white crystals (24 mg, 95%), mp 145–147 °C, $[a]^{22}_D$ +6.85 (*c* 1.2, MeOH); *m/z* [EI+] Found 90.0720 ($[M - CO_2H]^+$), [$C_5H_{10}NO_2F - CO_2H$] requires 90.0719; *m/z* (EI) 90 ($[M - CO_2H]^+$); ν_{max} (KBr)/cm^{-1} 3392 (NH), 2972 (OH) and 1736 (acid); δ_H (300 MHz, $C^2H_3O^2H$) 1.10 (3H, d, $J_{Me,3}$ 6.4, CH_3), 2.65 (1H, m, H-3), 4.16 (1H, s, H-2), 4.47 (1H, dd, $J_{4A,F}$ 47.0, $J_{4A,4B}$ 6.8, H-4A) and 4.62 (1H, dd, $J_{4B,F}$ 46.4, $J_{4B,4A}$ 6.7, H-4B); δ_C (75.5 MHz, [$^2H_4$]-MeOH) 11.0 (d, $^3J_{MeF}$ 6.9, CH_3), 36.4 (d, $^2J_{CF}$ 18.9, C-3), 57.3 (C-2), 85.6 (d, $^1J_{CF}$ 168.6, C-4) and 171.3 (acid); δ_F (282 MHz, C^2HCl_3) −224.9 (td, $J_{F,4H}$ 46.7, $J_{F,3H}$ 19.5).

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