Synthesis of (2*S*,4*S*)- and (2*S*,4*R*)-5,5′-dihydroxy[5,5-$^2H_2$]leucine by two independent routes[1]

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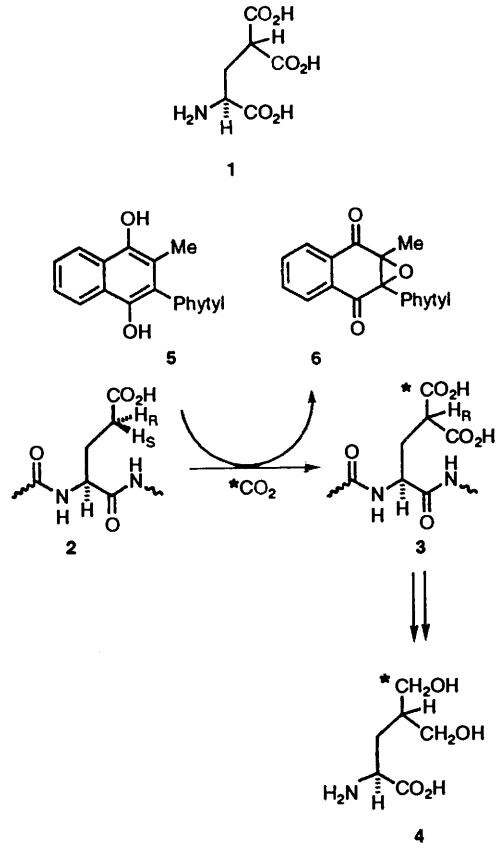
In studies directed at discovering the absolute stereochemistry of the reaction catalysed by the enzyme glutamate γ-carboxylase, two independent stereoselective syntheses of (2*S*,4*S*)-5,5′-dihydroxy[5,5-$^2H_2$]-leucine 4a and its (2*S*,4*R*)-diastereoisomer 4b have been developed. The first synthesis uses (2*S*)-pyroglutamic acid as starting material and provides (2*S*,4*S*)-5,5′-dihydroxy[5,5-$^2H_2$]leucine 4a, while the second starts with (2*S*,4*R*)-4-hydroxyproline and provides (2*S*,4*R*)-5,5′-dihydroxy[5,5-$^2H_2$]leucine 4b.

The unusual proteinogenic amino acid residue γ-carboxyglutamic acid **1** was not discovered until 1974 because of the lability of the terminal malonate moiety to decarboxylation. Two research groups found this amino acid to be present as residues in normal prothrombin [2,3] whereas the corresponding residues in abnormal prothrombin, obtained from patients treated with anticoagulants, were glutamic acid residues. γ-Carboxyglutamic acid was later discovered to be present in other proteins of the blood clotting cascade. This amino acid occurs most abundantly at the amino terminal end of these proteins and it arises through post-translational modification of glutamate residues **2** in precursor proteins as shown in Scheme 1. The enzyme responsible for the γ-carboxylation of glutamate residues is unusual, being linked to the oxidation of the hydroquinone **5** of vitamin K to yield vitamin K epoxide **6**.[4] Inhibitors of this enzyme are potential anti-thrombotic drugs. The mechanism of the reaction has excited much speculation [5] and elucidation of the stereochemistry of the process is important in understanding the mechanism of action of the enzyme and in designing enzyme inhibitors. Marquet, Azerad and co-workers have shown [6–9] that the hydrogen, 4-H_S, is abstracted in the carboxylation process and they have implied that the *4-pro-R* carboxy group in the non-fluorinated product is derived from CO_2, using a pentapeptide containing (4*S*)-4-fluoroglutamate.[10] This suggests inversion of stereochemistry in the enzyme catalysed reaction.

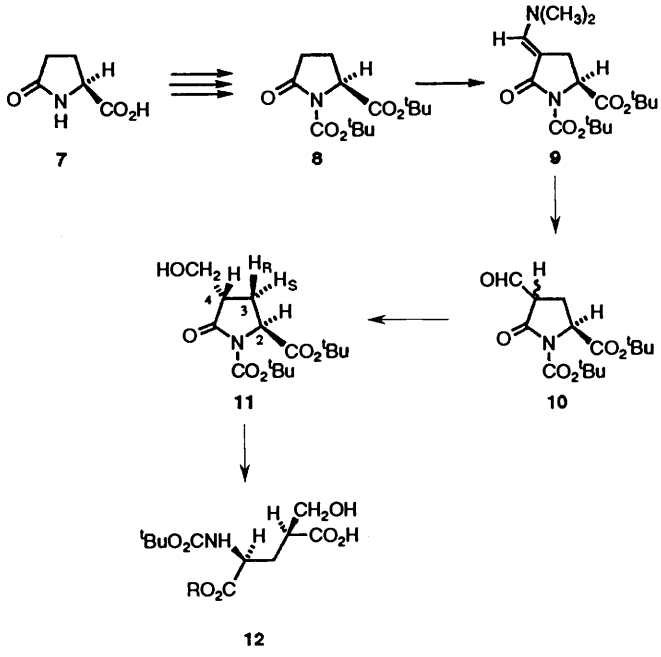
In our own studies in this area, we noted that reduction of peptide bound γ-carboxyglutamate with diborane followed by hydrolysis had been reported [11] to yield 5,5′-dihydroxyleucine **4** so that, if ^{13}C-labelled carbon dioxide were used in the enzyme catalysed reaction, we might obtain a sample of dihydroxyleucine **4** labelled in one of the two diastereotopic CH_2OH groups. Thus synthesis of a sample of this compound labelled in an unambiguously defined manner and comparison of its ^{13}C NMR spectrum with that of an enzymically derived sample would define the stereochemistry of the enzymic reaction. Since deuterium labelling will allow the resonances in the ^{13}C NMR spectrum to be defined, we opted to synthesise samples of 5,5′-dihydroxyleucine **4** labelled with deuterium specifically in one of the diastereotopic CH_2OH groups so that the synthesis would define the chirality of the labelled compound.

For our first synthesis of stereospecifically labelled dihydroxyleucine **4**, we chose (2*S*)-pyroglutamic acid **7** as a chiral template. We had already prepared the enaminone **9** [12] from this commercially available amido acid and had used it in our synthesis of stereospecifically labelled leucine,[12] and in the synthesis of various non-proteinogenic amino acids [13–15] and glutamate antagonists.[16] We expected that hydrolysis of the enaminone **9** to the aldehyde **10** might involve stereochemical control from the bulky ester group at C-2, and that reduction of the aldehyde **10** would lead to the required hydroxymethyl group as in compound **11** (Scheme 2). Hydrolytic ring opening would then afford the acid **12**, R = Bu^t, and subsequent reduction of the carboxyl group in this compound with deuteriated reagents would lead to the second, labelled hydroxymethyl group of the target compound. Hydrolysis of the ester at C-2 of the pyroglutamate derivative **11** during the ring opening process might lead to problems of regioselectivity in reactions of the diacid **12**, R = H, but we expected to avoid this problem by using the *tert*-butyl ester in the synthesis.

We therefore hydrolysed the enaminone **9** [12] at pH 4.5 in methanolic HCl until the UV spectrum indicated that the chromophore due to the enaminone at λ_{max} 313 nm had been

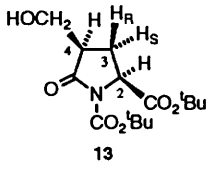


Scheme 1

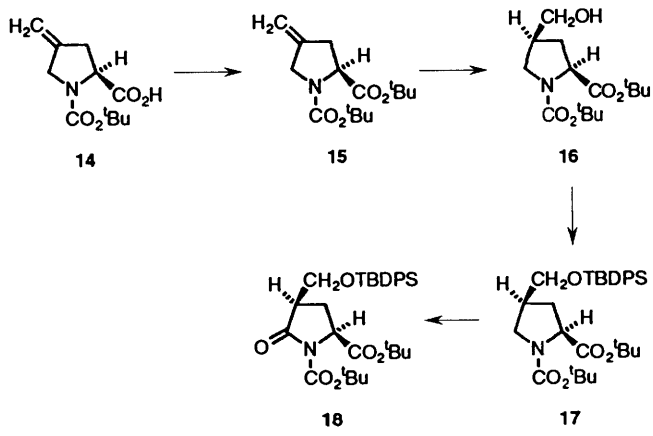


Scheme 2

replaced by that due to the enol of aldehyde **10** at λ_{max} 257 nm. We then added $NaB(CN)H_3$ to the solution *in situ*, keeping the pH constant at 4.5 to avoid deprotection. When the chromophore due to the aldehyde **10** was no longer present in the UV spectrum, the product was isolated. The product was evidently a mixture of the *trans* and *cis* alcohols **11** and **13** respectively in a ratio of 5:2.[17] The isomers were separated and their stereochemistry was deduced by ^{1}H NMR spectroscopic experiments. Irradiation of the absorbance at δ 2.47 corresponding to H-3*S* of the *cis* isomer **13** showed NOE at *both* δ 4.41 for H-2 *and* δ 2.76 for H-4. The *trans* isomer **11** showed *separate* enhancements in the ^{1}H NMR spectrum between H-4 at δ 2.85 and H-3*R* at δ 2.10, and between H-2 at δ 4.46 and H-3*S* at δ 2.24. These results are in keeping with the thermodynamically more stable *trans* diastereoisomer of the aldehyde **10** being formed in the hydrolysis step. A small amount of the unsubstituted compound **8** was also obtained from this reaction, presumably resulting from retro-aldol reaction of the alcohols **11** and **13**.



Although a good yield of the mixture was obtained, separation of the isomers in useful amounts proved an obstacle to progress and so we sought an alternative route. We were encouraged by an early report[18,19] that hydroboronation of 4-methylideneproline derivatives with disiamylborane followed by oxidation with aqueous hydrogen peroxide gave *cis*-4-hydroxymethylproline derivatives in good yield. (2*S*)-*N*-*tert*-Butoxycarbonyl-4-methylideneproline **14** was therefore prepared by the method of Herdewijn *et al.*[20] from (2*S*,4*R*)-4-hydroxyproline *via* the protected 4-ketone[21] and Wittig reaction. This compound was crystalline rather than an oil as reported.[20] It was converted into the *tert*-butyl ester **15** in *ca.* 82% yield using either di-*tert*-butyl dicarbonate, DMAP and triethylamine or *tert*-butyl alcohol, DMAP and dicyclohexylcarbodiimide. Reaction with disiamylborane followed by oxidation using aqueous hydrogen peroxide gave the protected alcohol **16** (Scheme 3). The ^{1}H NMR spectrum was complicated by the

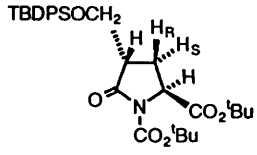


Scheme 3

well known[22] rotational isomerism of proline amides and urethanes and so an estimate of the stereoselectivity of the reaction could not be made by NMR spectroscopic methods. Fractional crystallisation from light petroleum gave a pure sample of the *cis* isomer, *tert*-butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-hydroxymethylprolinate **16** which showed a coalescence temperature of 333 K on variable temperature ^{1}H NMR spectroscopy in [$^2H_6$]DMSO.

To proceed to ring opened compounds, it was necessary to convert the proline derivative into a pyroglutamic acid derivative. This had been achieved for simpler compounds by oxidation with ruthenium tetraoxide[23] and we decided to protect the alcohol as the TBDPS derivative before attempting this step. Protection was achieved and the TBDPS ether **17** was obtained in 79% yield using *tert*-butyldiphenylsilyl chloride and imidazole in dimethylformamide. The protected compound was reacted with RuO_2–$NaIO_4$ to yield the corresponding pyroglutamic acid derivative in 39% yield together with a 20% yield of two products which were not fully characterised but the ^{1}H NMR spectra of which suggested that, although oxidation to a pyroglutamate had proceded normally, the protecting group had been modified. The pyroglutamic acid derivatives had simpler NMR spectra than the corresponding proline derivatives and so analysis of the spectra of the oxidised products allowed us to show that the hydroboronation step had given a mixture containing *ca.* 80% of the *cis* isomer **16** and *ca.* 20% of the corresponding *trans* isomer. The stereochemistry of the products was confirmed by NOE studies in the ^{1}H NMR spectra of the products **18** and **19**. Interestingly, when the hydroboronation step was accomplished using BH_3–Me_2S, the ratio of the isomers **18**:**19** in the final product was *ca.* 3:2. Most usefully, we now found that the *tert*-butyldiphenylsilyl ethers, unlike the corresponding alcohols, could be separated chromatographically in excellent yield. We therefore converted the alcohols **11** and **13** obtained by reduction of the aldehydes **10** into the *tert*-butyldiphenylsilyl ethers which separated chromatographically giving a 34% yield of the *trans* isomer **19** and a 12% yield of the *cis* isomer **18**. The route from pyroglutamic acid therefore gave predominantly the *trans* isomer **19** whilst the route from 4-hydroxyproline gave predominantly the *cis* isomer **18**.

The remainder of the synthesis now involved ring opening and further elaboration of the molecule to obtain separate samples of the target compounds (2*S*,4*S*)- and (2*S*,4*R*)-5,5′-dihydroxy[5,5-$^2H_2$]leucine so that the ^{13}C NMR spectrum could be assigned for studies on the enzyme glutamate γ-carboxylase. Hydrolytic ring opening of the 4-methylpyroglutamate **20** using aqueous LiOH in tetrahydrofuran had proved successful in other work in our laboratory[12] but this reaction could not be successfully applied to the TBDPS ether **19**. We therefore investigated ring opening of the silyl ether **19** using methanol containing a catalytic quantity of triethylamine. The



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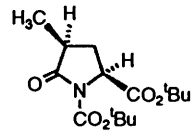

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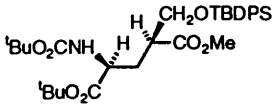

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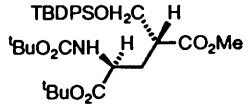

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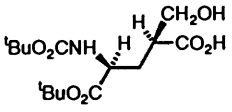

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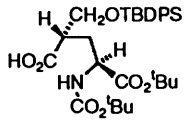

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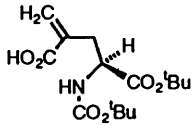

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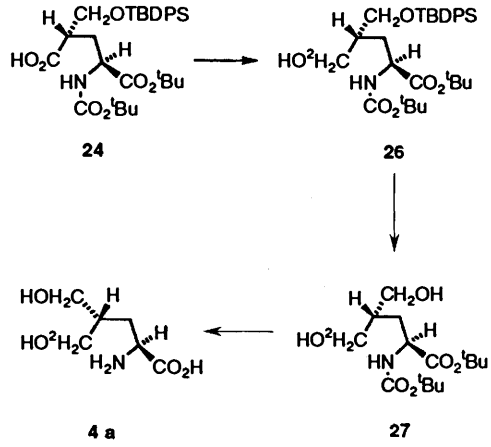
product was evidently a mixture of the diastereoisomers **21** and **22** in a ratio of 4:1 and so epimerisation had occurred either at C-4 or at C-2 under the conditions used for ring opening. In order to define the centre at which epimerisation had occurred, the reaction was repeated using methan[$^2H_1$]ol. The ^2H NMR spectrum of the crude product showed a single resonance at δ 2.73 which corresponded to incorporation of the label at C-4 and there was no labelling at C-2. Epimerisation had therefore occurred solely at C-4. When the unprotected alcohol **11** was reacted with aqueous lithium hydroxide in tetrahydrofuran, then ring opening proceeded normally without epimerisation to yield the substituted glutamic acid **23**. When the silyl ether **19** was used and the solvent was changed to tetrahydrofuran or acetonitrile, then ring opening using either aqueous LiOH or KOH gave a partially purified compound from the alkali soluble fraction. The ^1H NMR spectrum of this crude product indicated the presence not only of the desired product **24** in less than 20% yield but also of the olefin **25** resulting from elimination of the *tert*-butyldiphenylsilyl ether group, with olefinic singlets at δ 5.81 and 6.44. We evidently needed to alter the ratio of basicity to nucleophilicity in our reagent for the ring opening reaction to occur, and so lithium hydroperoxide in aqueous tetrahydrofuran was chosen to effect this reaction. With the *trans* lactam **19**, this gave a clean product **24** in 57% yield.

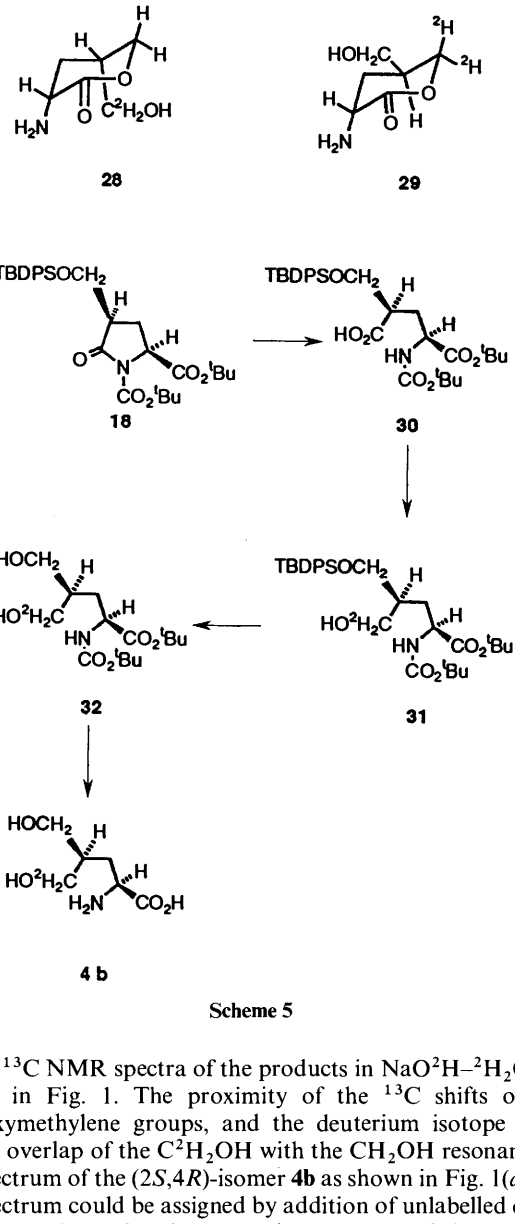
To prepare our target (2*S*,4*S*)-5,5′-dihydroxy[5,5-$^2H_2$]leucine **4a**, we now proceeded as outlined in Scheme 4. The acid **24** was first converted into the mixed anhydride with isobutyl chloroformate and this was reduced *in situ* with NaB^2H_4 in ^2H_2O to give the labelled alcohol **26** in 68% yield after purification. Deprotection was now effected in two stages, first reacting the compound **26** with ammonium fluoride in methanol to obtain the diol **27** in 72% yield. The final hydrolysis of the diol **27** using trifluoroacetic acid was complicated by cyclisation of the product to diastereoisomeric lactones **28** and **29** but these could be hydrolysed to the sodium salt of the acid **4a** with sodium hydroxide. Use of $NaBH_4$ in the synthesis gave unlabelled 5,5′-dihydroxyleucine **4**, the ^1H and ^{13}C NMR



24 26 27 4 a

Scheme 4

spectra of which were in keeping with those reported[24] for a sample obtained by an alternative synthesis. The diastereoisomerically labelled alcohol **4b** was prepared by the same route, as shown in Scheme 5, using the lactam **18** as starting material.



28 29 18 30 32 31 4 b

Scheme 5

The ^{13}C NMR spectra of the products in NaO^2H–^2H_2O are shown in Fig. 1. The proximity of the ^{13}C shifts of the hydroxymethylene groups, and the deuterium isotope shift, caused overlap of the C^2H_2OH with the CH_2OH resonance in the spectrum of the (2*S*,4*R*)-isomer **4b** as shown in Fig. 1(*a*) but the spectrum could be assigned by addition of unlabelled diol **4** to the sample, as in Fig. 1(*c*). The spectrum of the (2*S*,4*S*)-isomer **4a**, shown in Fig. 1(*b*), was unambiguous. From the

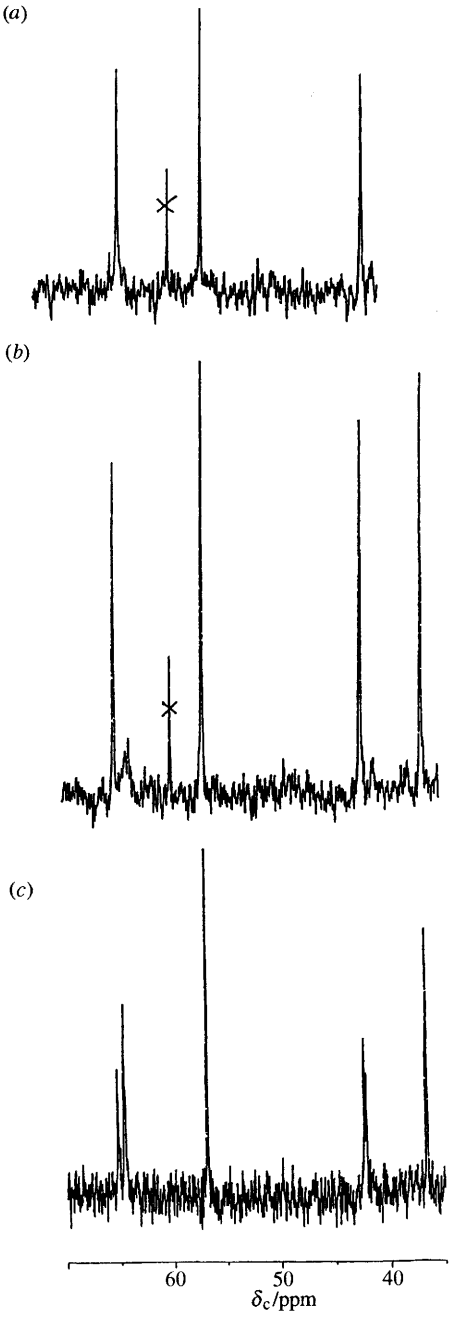


Fig. 1 Broad band ^{1}H-decoupled ^{13}C NMR spectra in 10% NaO^2H–^2H_2O of: (*a*) (2*S*,4*R*)-5,5′-dihydroxy[5,5-$^2H_2$]leucine **4b**, (*b*) (2*S*,4*S*)-5,5′-dihydroxy[5,5-$^2H_2$]leucine **4a** and (*c*) **4b** mixed with unlabelled 5,5′-dihydroxyleucine **4**

spectra, it is evident that the higher field absorption can be assigned to the 4-*pro*-*S* hydroxymethyl group and the lower field absorption to the 4-*pro*-*R* hydroxymethyl group.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations (given in units of 10^{-1} deg cm^{-2} g^{-1}) were measured 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 instrument, and UV spectra on a Phillips PU8720 UV/VIS scanning spectrophotometer. ^{1}H NMR spectra were recorded on Bruker WM 360 (360 MHz) and AMX 500 (500 MHz) Fourier transform instruments. *J* Values are given in Hz. ^{13}C NMR spectra (broad band ^{1}H decoupled) were recorded on Bruker WM 360 (90.6 MHz), AMX 500 (125.8 MHz) and AC-P 250 (62.9 MHz) Fourier transform instruments. INEPT experiments were used to help assign ^{13}C NMR resonances where necessary. ^{2}H NMR spectra were recorded on a Bruker AC-P 250 (38.4 MHz) Fourier transform instrument. Unless otherwise stated, residual solvent peaks were used as an internal reference in the NMR spectra. Mass spectra were recorded on Kratos MS80RF, MS50 and MS25 and Fisons/VG Autospec spectrometers and the accurate mass measurements were recorded on a Fisons VG Autospec by Dr A. Abdul-Sada. Microanalyses were performed by Mrs P. Firmin (Wellcome Research Laboratories), and Miss K. Plowman and Miss M. Patel (Sussex). Thin layer chromatography was performed using Merck Kieselgel 60 F_{254} pre-coated silica gel plates of thickness 0.2 mm (ART 5554) and column chromatography was performed using Merck Kieselgel 60 (230–400 mesh, ART 9385).

tert-Butyl (2*S*,4*RS*)-*N*-*tert*-butoxycarbonyl-4-hydroxymethylpyroglutamate, 11 and 13

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-(*N*,*N*-dimethylaminomethylidene)pyroglutamate, **9** (6.0 g, 18 mmol) was dissolved in methanol (140 cm^3) and aq. hydrochloric acid (0.2 mol dm^{-3}; 90 cm^3) was added dropwise with stirring at room temperature. TLC analysis showed complete hydrolysis of the enaminone **9** to the enol of the aldehyde **10** within 45 min. Sodium cyanoborohydride (2.22 g, 35 mmol) was added in portions while maintaining the pH at 4.0–4.5 by automated addition of aq. hydrochloric acid (0.2 mol dm^{-3}) using a pH-stat. The reaction mixture was stirred for 48 h at room temperature to afford a pale green solution. The solvent was removed *in vacuo* to afford an aqueous layer to which ethyl acetate (100 cm^3) and saturated aq. sodium chloride (100 cm^3) were added. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 80 cm^3). The organic layers were combined, washed with saturated aq. sodium chloride (50 cm^3), 10% aq. citric acid (50 cm^3) and water (50 cm^3) and dried (Na_2SO_4). The solvent was removed *in vacuo* to afford a pale yellow foam (4.94 g, ~86% crude recovery) which contained the *trans* and *cis* alcohols, **11** and **13** in a ratio of 5:2. The crude foam was purified by column chromatography on silica gel, using ethyl acetate–light petroleum (2:1, 40–60 °C) as eluent, to afford fractions of the diastereoisomeric alcohols in various ratios (~46% yield). Column fractions containing 80–90% of the *trans* isomer and 80–90% of the *cis* isomer respectively were recrystallised from ethyl acetate–light petroleum (60–80 °C) to yield pure samples. The major product was tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*hydroxymethylpyroglutamate* **11** (444 mg), mp 100–102 °C, $[\alpha]_D^{23}$ −19.2 (*c* 1.97 in $CHCl_3$) (Found: C, 57.0; H, 8.1; N, 3.9. $C_{15}H_{25}NO_6$ requires C, 57.1; H, 7.9; N, 4.4%); *m*/*z* [+ve FAB (NBA)] 316 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 3497 (OH), 1772 (imide) and 1740 (ester); δ_H(360 MHz, C^2HCl_3–5% ^2H_2O) 4.46 (1 H, dd, $J_{2,3S}$ 9.6, $J_{2,3R}$ 1.2, H-2), 3.94 (1 H, dd, $J_{6A,6B}$ 11.3, $J_{6A,4}$ 4.1, C*H*OH), 3.70 (1 H, dd, $J_{6B,6A}$ 11.3, $J_{6B,4}$ 5.6, C*H*OH), 2.85 (1 H, m, $J_{4,3S}$ 11.7, $J_{4,3R}$ 9.0, $J_{4,6A}$ 4.1, H-4), 2.24 (1 H, ddd, $J_{3S,3R}$ 13.2, $J_{3S,2}$ 9.6, $J_{3S,4}$ 11.7, H-3*S*), 2.10 (1 H, ddd, $J_{3R,3S}$ 13.2, $J_{3R,2}$ 1.5, $J_{3R,4}$ 9.0, H-3*R*), 1.50 [9 H, s, $OC(CH_3)_3$] and 1.48 [9 H, s, $OC(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 174.8 and 170.2 (C=O), 149.2 (urethane), 83.6 [O*C*$(CH_3)_3$], 82.4 [O*C*$(CH_3)_3$], 61.5 (CH_2OH), 58.1 (C-2), 43.9 (C-4), 28.0 [C(CH_3)$_3$] and 24.8 (C-3). The minor component was tert-*butyl* (2S,4R)-N-tert-*butoxycarbonyl*-4-*hydroxymethylpyroglutamate* **13** (92 mg), mp 102–103 °C, $[\alpha]_D^{22}$ −74 (*c* 0.73 in $CHCl_3$) (Found: C, 57.3; H, 8.2; N, 4.2. $C_{15}H_{25}NO_6$ requires C, 57.1; H, 7.9; N, 4.4%); *m*/*z* [+ve FAB (NBA)] 316 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 3479 (OH), 1772 and 1705 (imide); δ_H(360 MHz, C^2HCl_3–5% ^2H_2O) 4.41 (1 H, dd, $J_{2,3S}$ 9.3, $J_{2,3R}$ 6.3, H-2), 3.83 (1 H, dd, $J_{6A,6B}$ 11.2, $J_{6A,4}$ 5.2, C*H*OH), 3.73 (1 H, dd, $J_{6B,6A}$ 11.2, $J_{6B,4}$ 6.1, C*H*OH), 2.76 (1 H, m, $J_{4,3S}$ 7.5, $J_{4,3R}$ 9.4, H-4), 2.47 (1 H, dtd, $J_{3S,3R}$ 13.4, $J_{3S,4}$ 7.5, $J_{3S,2}$ 9.3, H-

3*S*), 1.86 (1 H, dtd, $J_{3R,3S}$ 13.4, $J_{3R,4}$ 9.4, $J_{3R,2}$ 6.3, H-3*R*), 1.48 [9 H, s, $OC(CH_3)_3$] and 1.45 [9 H, s, $OC(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 175.0 and 170.4 (C=O), 149.3 (urethane), 83.7 [$OC(CH_3)_3$], 82.4 [$OC(CH_3)_3$], 62.3 (CH_2O), 58.3 (C-2), 44.8 (C-4), 27.9 [$C(CH_3)_3$] and 24.1 (C-3). tert-*Butyl* (2S)-N-tert-*butoxycarbonylpyroglutamate* **8** was also obtained as a colourless oil during column chromatography (870 mg, 17%) and had a ^1H NMR spectrum identical with that of an authentic sample.[12]

(2*S*)-*N*-*tert*-Butoxycarbonyl-4-methylideneproline 14

Prepared using the method of Herdewijn *et al.*[20] but proved to be a crystalline solid and not an oil as reported. The product was recrystallised from diethyl ether in 83% yield, mp 108–110 °C, $[\alpha]_D^{24}$ −55.3 (*c* 0.52 in $CHCl_3$) (Found: C, 58.0; H, 7.4; N, 6.0. $C_{11}H_{17}NO_4$ requires C, 58.1; H, 7.5; N, 6.2%); *m*/*z* [+ve FAB, NBA] 228 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 1742 (urethane); δ_H(500 MHz, C^2HCl_3, two rotational isomers) 9.00 (1 H, br s, COOH), 5.02 (2 H, s, olefinics), 4.52 and 4.40 (1 H, 2dd, $J_{2,3S}$ 2.2, $J_{2,3R}$ 9.4, H-2), 4.08 (s, one rotational isomer) and 4.05 and 4.01 (2 × AB, J_{AB} 15.0, other rotational isomer) (total 2 H, H-5), 3.01 and 2.93 (1 H, 2 × dd, $J_{3R,2}$ 9.4, $J_{3R,3S}$ 16.3, H-3*R*), 2.78 and 2.69 (1 H, 2 × br d, $J_{3S,2}$ 2.2, $J_{3R,3S}$ 16.3, H-3*S*) and 1.42 and 1.47 [9 H, 2 × s, $(CH_3)_3C$]; δ_C(125.8 MHz, C^2HCl_3, two rotational isomers) 178.01 and 176.02 (C=O), 155.37 and 153.83 (urethane), 142.80 and 142.04 (C-4), 108.15 (=CH_2), 81.12 and 80.66 [$OC(CH_3)_3$], 58.83 and 58.62 (C-2), 50.89 and 50.50 (C-5), 36.63 and 35.29 (C-3) and 28.34 and 28.22 [$C(CH_3)_3$].

***tert*-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylideneprolinate 15**

Method A. *tert*-Butyl alcohol (0.30 cm^3, 3 mmol), DMAP (0.013 g, 0.1 mmol) and dicyclohexylcarbodiimide (230 mg, 1.1 mmol) were added successively at 0 °C to (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylideneproline **14** (0.227 g, 1 mmol) dissolved in dry dichloromethane (10 cm^3). The mixture was stirred overnight at room temperature and filtered. The filtrate was washed with 0.05% aq. citric acid (10 cm^3) and water (10 cm^3) and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was chromatographed on silica gel using dichloromethane–diethyl ether (13:1) as eluent, to yield tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-*methylideneprolinate* **15** as a colourless oil (230 mg, 82%), $[\alpha]_D^{25}$ −20.9 (*c* 1.01 in $CHCl_3$); (Found: C, 63.7; H, 9.6; N, 5.0. $C_{15}H_{25}NO_4$ requires C, 63.6; H, 8.9; N, 4.9%); *m*/*z* [FAB] 284 $[M + H]^+$; ν_{max}(film)/cm^{-1} 1739 (ester) and 1692 (urethane); δ_H(360 MHz, C^2HCl_3; two rotational isomers) 5.01 and 4.98 (2 H, 2 × br s, olefinic), 4.37 and 4.27 (1 H, 2 × dd, $J_{2,3S}$ 2.5, $J_{2,3R}$ 9.5, H-2), 4.07 and 4.02 (2 H, 2 × br s, H-5), 2.97 and 2.91 (1 H, 2 × m, $J_{3R,2}$ 9.5, $J_{3R,3S}$ 16.3, H-3*R*), 2.56 (1 H, br d, $J_{3S,2}$ 2.5, $J_{3S,3R}$ 16.3, H-3*S*) and 1.47, 1.45 and 1.44 [18 H, 3 × s, $C(CH_3)_3$]; δ_C(62.9 MHz, C^2HCl_3, two rotational isomers) 171.76 (C=O), 143.87 (urethane), 142.74 (C-4), 107.63 and 107.37 (=CH_2), 81.22 [$OC(CH_3)_3$], 79.93 and 79.81 [$OC(CH_3)_3$], 59.66 and 59.37 (C-2), 50.78 and 50.57 (C-5), 36.89 and 36.14 (C-3) and 28.38, 28.30 and 27.92 [$C(CH_3)_3$].

Method B. Di-*tert*-butyl dicarbonate (330 mg, 1.5 mmol), triethylamine (0.21 cm^3, 1.5 mmol) and DMAP (13 mg, 0.1 mmol) were added successively at 0 °C to (2*S*)-*N*-*tert*-butyloxycarbonyl-4-methylideneproline **14** (227 mg, 1 mmol) dissolved in acetonitrile (10 cm^3, dried over CaH_2) and the solution was stirred overnight at room temperature. The solvent was removed *in vacuo* and the residue was purified by chromatography on silica gel using light petroleum (60–80 °C)–ethyl acetate (4:1) as eluent. The product tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-*methylideneprolinate* **15** was a colourless oil (238 mg, 84%) with spectra identical to those of the sample prepared by method A.

***tert*-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-hydroxymethylprolinate 16**

Method A—using disiamylborane. 2-Methylbut-2-ene in tetrahydrofuran (2 mol dm^{-3}; 3 cm^3, 6 mmol) and B_2H_6 in tetrahydrofuran (1 mol dm^{-3}; 3 cm^3, 3 mmol) were added dropwise over a period of 5 min at −5 °C to a 100 cm^3 three-necked flask under a slight positive pressure of nitrogen. After stirring for 2 h at 0 °C, a solution of *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylideneprolinate **15** (283 mg, 1 mmol) in dry tetrahydrofuran (5 cm^3) was added dropwise over a period of 5 min. The solution was stirred for 24 h at 20 °C and the excess of disiamylborane was destroyed by addition of water (0.5 cm^3). When hydrogen was no longer evolved at 0 °C, the reaction was oxidised by addition of aq. NaOH (3 mol dm^{-3}; 0.35 cm^3, 1 mmol) followed by aq. H_2O_2 (30%; 0.35 cm^3, 5.25 mmol). After stirring for 30 min at 20 °C, water (20 cm^3) was added and the solution was extracted with diethyl ether. The organic layer was washed with water and dried (Na_2SO_4) and the solvent was removed *in vacuo* to give a colourless oil (555 mg). Purification by chromatography on silica gel using diethyl ether as eluent afforded tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-*hydroxymethylprolinate* **16** (157 mg, 52%). This was used in the next step without further purification but the *cis* isomer, tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*hydroxymethylprolinate* **16** could be obtained by fractional crystallisation using light petroleum (40–60 °C) and was isolated as white crystals, mp 40.5–41.5 °C, $[\alpha]_D^{25}$ −59.2 (*c* 0.515 in $CHCl_3$) (Found: C, 58.0; H, 9.1; N, 4.5. $C_{15}H_{27}NO_5$ requires C, 59.8; H, 9.0; N, 4.65%); *m*/*z* [+ve FAB, NBS] 302 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 1744 (ester) and 1672 (carbamate); δ_H(360 MHz, $C^2H_3COC^2H_3$, two rotational isomers) 4.08 (m, H-2 of one rotational isomer), 3.78 (t, $J_{2,3}$ 5.3, H-2 of other rotational isomer), 3.61 (1 H, dd, $J_{5R,4}$ 7.8, $J_{5R,5S}$ 10.4, H-5*R*), 3.52 (2 H, t, $J_{6,4}$ 6.0, CH_2OH), 3.14 and 3.10 (1 H, 2 × t, $J_{5S,5R;5S,4}$ 9.4, H-5*S*), 2.81 and 2.78 (1 H, 2 × s, OH), 2.36 (2 H, m, H-3), 1.66 (1 H, m, H-4) and 1.42 and 1.39 [18 H, 2 × s, $C(CH_3)_3$]. The temperature of coalescence of the two rotational isomers was found to be 333 K in DMSO by variable temperature ^1H NMR spectroscopy.

Method B—using borane dimethylsulfide. Borane dimethylsulfide in tetrahydrofuran (2 mol dm^{-3}; 3 cm^3, 6 mmol) was added dropwise to *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylideneprolinate **15** (556 mg, 2 mmol) in dry tetrahydrofuran (10 cm^3) over a period of 8 min at 0 °C under a positive pressure of nitrogen. The solution was stirred for 20 h at 20 °C. After cooling at 0 °C, ethanol (1 cm^3) was added dropwise and the solution was stirred for 1 h until there was no further effervescence of hydrogen. Aq. sodium hydroxide (3 mol dm^{-3}; 0.7 cm^3, 2 mmol) was added, followed by aq. H_2O_2 (30%; 0.7 cm^3, 10.5 mmol) and the solution was stirred for 2 h at 20 °C. After addition of water (50 cm^3), the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was purified by chromatography on silica gel using light petroleum (60–80 °C)–ethyl acetate (1:1) as eluent to yield tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-*hydroxymethylprolinate* **16** (341 mg, 57%) with the same spectroscopic properties as the sample obtained by method A.

***tert*-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethylprolinate 17**

Dry dimethylformamide (1 cm^3), imidazole (200 mg, 2.9 mmol) and *tert*-butyldiphenylsilyl chloride (0.4 cm^3, 1.5 mmol) were added to the mixed diastereoisomers of *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-hydroxymethylprolinate **16** (345 mg, 1.14 mmol) at 0 °C. The solution was stirred for 48 h at room temperature under nitrogen and, after addition of ethyl acetate (100 cm^3), was washed with brine, 10% aq. citric acid and brine and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was purified by chromatography on silica gel using light petroleum (60–80 °C)–dichloromethane (3:7) as eluent. The first fraction (Ph_2Bu^tSiOH) was isolated as white crystals and the second fraction afforded tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-tert-*butyldiphenylsilyloxymethylprolinate* **17**

as a colourless oil (486 mg, 79%). Use of the pure (2*S*,4*S*)-alcohol **16** in the reaction gave tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-tert-*butyldiphenylsilyloxymethylprolinate* **17** as an oil, $[\alpha]_D^{27}$ −35.8 (*c* 0.355 in $CHCl_3$) (Found: C, 67.8; H, 8.2; N, 2.2. $C_{31}H_{45}NO_5Si$ requires C, 69.0; H, 8.4; N, 2.6%); ν_{max}(film)/cm^{-1} 1743 (ester) and 1704 (carbamate); δ_H(500 MHz, C^2HCl_3, two rotational isomers) 7.64 and 7.36 (10 H, 2 × m, aromatics), 4.17 and 4.12 (1 H, 2 × t, $J_{2,3}$ 7.9, H-2), 3.79 and 3.70 (1 H, 2 × dd, $J_{5R,4}$ 7.2, $J_{5R,5S}$ 10.5, H-5*R*), 3.64 (2 H, m, CH_2OSi), 3.24 and 3.17 (1 H, 2 × dd, $J_{5S,4}$ 8.9, $J_{5S,5R}$ 10.5, H-5*S*), 2.41 (1 H, m, H-3*S*), 2.34 (1 H, m, H-3*R*), 1.76 (1 H, m, H-4), 1.47 and 1.45 and 1.45 and 1.44 [18 H, 4 × s, $(CH_3)_3C$], 1.06 and 1.05 [2 × s, 9 H, $(CH_3)_3CSi$]; δ_C(125.8 MHz, C^2HCl_3) 172.26 (C=O), 153.90 (urethane), 135.52–127.69 (aromatics), 80.84 [$(CH_3)_3CO$], 79.79 and 79.50 [$(CH_3)_3CO$], 64.83 and 64.59 (CH_2OSi), 59.70 (C-2), 49.41 and 49.27 (C-5), 40.86 and 40.04 (C-4), 33.11 and 32.20 (C-3), 28.42, 28.36, 27.99 and 27.92 [$C(CH_3)_3$], 26.80 [$(CH_3)_3CSi$] and 19.22 [$(CH_3)_3CSi$]. Irradiation of δ 1.76 (H-4) gave a 21% NOE at δ 2.34 (H-3*R*), 0.7% at δ 3.24 (H-5*S*) and 1.5% at δ 3.64 (CH_2O). Irradiation of δ 4.15 (H-2) gave a 1% NOE at δ 2.41 (H-3*S*) and 6% at δ 2.34 (H-3*R*).

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethylpyroglutamate 18

Method A, from the proline route. A solution of *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethylprolinate **17** (602 mg, 1.12 mmol) in ethyl acetate (12 cm^3) was added to a mixture of $RuO_2 \cdot H_2O$ (60 mg, 0.45 mmol) and aq. $NaIO_4$ (10%; 24 cm^3). The solution was stirred vigorously overnight at room temperature. The layers were separated and the organic solution was treated with propan-2-ol (1 cm^3). Ethyl acetate (80 cm^3) was added and the solution was washed with brine and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel using light petroleum (60–80 °C)–ethyl acetate (17:3) as eluent. Starting material (48 mg) was recovered, followed by tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-tert-*butyldiphenylsilyloxymethylpyroglutamate* **18** and **19** (221 mg, 39%) as two separate diastereoisomers. The *trans* isomer, tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-tert-*butyldiphenylsilyloxymethylpyroglutamate* **19** was recrystallised from light petroleum (40–60 °C) as white crystals (88 mg, 14%), mp 97–99 °C; $[\alpha]_D^{24}$ −36.7 (*c* 0.4 in $CHCl_3$) (Found: C, 67.1; H, 7.9; N, 2.4. $C_{31}H_{43}NO_6Si$ requires C, 67.2; H, 7.8; N, 2.5%); *m*/*z* [EI] 496 [M − C_4H_9]$^+$; ν_{max}(KBr)/cm^{-1} 1754 and 1714 (imide) and 1732 (ester); δ_H(360 MHz, C^2HCl_3) 7.70–7.30 (10 H, m, aromatics), 4.50 (1 H, dd, $J_{2,3S}$ 9.7, $J_{2,3R}$ 2.9, H-2), 4.05 (1 H, dd, $J_{6A,6B}$ 10.2, $J_{6A,4}$ 4.7, CHOSi), 3.75 (1 H, dd, $J_{6B,6A}$ 10.2, $J_{6B,4}$ 3.4, CHOSi), 2.77 (1 H, m, $J_{4,6A}$ 4.7, $J_{4,6B}$ 3.4, $J_{4,3R}$ 9.3, $J_{4,3S}$ 9.7, H-4), 2.42 (1 H, ddd, $J_{3S,3R}$ 13.2, $J_{3S,2}$ 9.7, $J_{3S,4}$ 9.7, H-3*S*), 2.10 (1 H, ddd, $J_{3R,3S}$ 13.2, $J_{3R,2}$ 2.9, $J_{3R,4}$ 9.3, H-3*R*), 1.51 [9 H, s, $OC(CH_3)_3$], 1.48 [9 H, s, $OC(CH_3)_3$] and 1.03 [9 H, s, $SiC(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 173.4 and 170.6 (C=O), 149.3 (urethane), 127.7–135.7 (aromatics), 83.2 and 82.2 [$OC(CH_3)_3$], 62.7 (CH_2OSi), 58.2 (C-2), 44.4 (C-4), 27.9 and 26.7 [$C(CH_3)_3$], 25.2 (C-3) and 19.2 [$SiC(CH_3)_3$]. Irradiation at the resonance at δ 2.42 (H-3*S*) resulted in a 10% NOE at δ 4.50 (H-2), whereas irradiation at δ 2.10 (H-3*R*) resulted in a 17% NOE at δ 2.77 (H-4). The *cis* isomer, tert-*butyl* (2S,4R)-N-tert-*butyloxycarbonyl*-4-tert-*butyldiphenylsilyloxymethylpyroglutamate* **18** was obtained as white crystals, (133 mg, 21%), mp 100–102 °C, $[\alpha]_D^{25}$ −12.2 (*c* 0.43 in $CHCl_3$); *m*/*z* [EI] 496.216 462, $C_{27}H_{34}NO_6Si$ requires 496.215 542 [M − Bu^t]$^+$; *m*/*z* [+ve FAB, NBA] 576 [M + Na]$^+$ and 554 [M + H]$^+$; ν_{max}(KBr)/cm^{-1} 1789 (imide), 1743 (ester) and 1722 (urethane); δ_H(360 MHz, C^2HCl_3) 7.65 and 7.40 (10 H, 2 × m, aromatics), 4.40 (1 H, dd, $J_{2,3S}$ 7.3, $J_{2,3R}$ 9, H-2), 3.93 (1 H, dd, $J_{6B,4}$ 6.5, J_{AB} 10.3, CHOSi), 3.89 (1 H, dd, $J_{6A,4}$ 4.7, J_{AB} 10.3, CHOSi), 2.77 (1 H, dddd, $J_{6A,4}$ 4.7 $J_{6B,4}$ 6.5, $J_{4,3S}$ 8.4, $J_{4,3R}$ 9, H-4), 2.46 (1 H, dt, $J_{3R,4;3R,2}$ 9, $J_{3R,3S}$ 13.2, H-3*R*), 2.13 (1 H, ddd, $J_{3S,2}$ 7.3, $J_{3S,4}$ 8.4, $J_{3R,3S}$ 13.2, H-3*S*), 1.51 and 1.46 [18 H, 2 × s, $(CH_3)_3C$] and 1.05 [9 H, s, $(CH_3)_3C$]; δ_C(125.8 MHz, C^2HCl_3) 173.03 and 170.63 (C=O), 149.74 (urethane), 135.9–127.9 (aromatics), 83.62 [$(CH_3)_3CO$], 82.29 [$(CH_3)_3CO$], 62.78 (CH_2OSi), 58.35 (C-2), 45.80 (C-4), 28.15 and 27.70 [$(CH_3)_3C$], 24.70 (C-3) and 19.53 [$(CH_3)_3CSi$]. Two other products (77 mg) eluted from the column. The first showed δ_H(360 MHz, C^2HCl_3) 7.61 and 7.31 (5 H, 2 × m, aromatics), 4.41 (1 H, dd, $J_{2,3A}$ 2.2, $J_{2,3B}$ 9.8, H-2), 4.25 (1 H, dd, $J_{6A,4}$ 3.5, J_{AB} 10.8, CHOSi), 3.87 (1 H, dd, $J_{6B,4}$ 3.5, J_{AB} 10.8, CHOSi), 3.3 (1 H, br, OH), 2.81 (1 H, m, H-4), 2.4 (1 H, m, H-3A), 2.07 (1 H, m, H-3B) and 1.48, 1.45 and 0.95 [27 H, 3 × s, $C(CH_3)_3$].

Method B, from the pyroglutamate route. The diastereoisomeric mixture of *tert*-butyl (2*S*,4*RS*)-*N*-*tert*-butoxycarbonyl-4-hydroxymethylpyroglutamate **11** and **13** (8.61 g, 27.3 mmol) was dissolved in dichloromethane (175 cm^3) and DMAP (700 mg, 5.7 mmol) and triethylamine (9.7 cm^3) were added at 0 °C with stirring. After 15 min, *tert*-butylchlorodiphenylsilane (8.52 cm^3, 32.8 mmol) was added under nitrogen and the reaction mixture was stirred for 2 days. The mixture was concentrated *in vacuo* and dichloromethane (215 cm^3) was added. The solution was washed with aq. hydrochloric acid (0.05 mol dm^{-3}; 80 cm^3). The aqueous layer was extracted with dichloromethane (3 × 200 cm^3) and the organic layers were washed with water (250 cm^3) and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield a pale orange oil which was purified by chromatography on silica gel using light petroleum (40–60 °C)–ethyl acetate (17:3) as eluent. The major component, tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-tert-*butyldiphenylsilyloxymethylpyroglutamate* **19** was a solid, (5.15 g, 34%) mp 88–89 °C, with spectra identical with those of the sample independently prepared by method A above. The minor component, tert-*butyl* (2S,4R)-N-tert-*butoxycarbonyl*-4-tert-*butyldiphenylsiloxymethylpyroglutamate* **18** was a solid, mp 96–98 °C, (1.8 g, 12%) with spectra identical to those of the sample prepared by method A above.

1-*tert*-Butyl 5-methyl (2*S*,4*RS*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethylglutamate 21 and 22

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethylpyroglutamate **19** (150 mg, 0.271 mmol) was dissolved in methanol (10 cm^3) and triethylamine (4.10 mg, 0.041 mmol) was added. The reaction was stirred for 6 days at room temperature and the solvents were removed *in vacuo* to afford a pale yellow oil which was purified by column chromatography on silica gel, using diethyl ether–light petroleum (40–60 °C) as eluent, to yield three separate compounds as colourless oils. The major component was a 4:1 mixture of the diastereoisomeric esters **21** and **22** (114 mg, 72%) (Found: C, 65.4; H, 8.2; N, 2.1. $C_{32}H_{47}NO_7Si$ requires C, 65.6; H, 8.0; N, 2.4%); *m*/*z* [EI] 528 [M − C_4H_9]$^+$; ν_{max}(KBr)/cm^{-1} 1718 (ester); δ_H(500 MHz, C^2HCl_3) 7.80–7.30 (10 H, m, aromatics), 5.05 and 5.00 [1 H, 2 × br m, (2*S*,4*R*)- and (2*S*,4*S*)-NH], 4.25 [1 H, m, (2*S*,4*RS*)-2-CH], 3.90–3.75 [2 H, m, (2*S*,4*RS*)-CH_2OSi], 3.71 and 3.68 [3 H, 2 × s, (2*S*,4*S*)- and (2*S*,4*R*)-OCH_3], 2.71 [1 H, m, (2*S*,4*RS*)-H-4], 2.35 [0.8 H, dtd, (2*S*,4*S*)-H-3A], 2.15 and 2.00 [0.4 H, 2 × m, (2*S*,4*R*)-H-3A and H-3B], 1.75 [0.8 H, dtd, (2*S*,4*S*)-H-3B], 1.40–1.50 [18 H, overlapping singlets, (2*S*,4*RS*)-$OC(CH_3)_3$] and 1.08 and 1.05 [9 H, (2*S*,4*RS*)-$SiC(CH_3)_3$]; selective irradiation of the multiplet at δ 2.71 (H-4) led to simplification of the C-3 protons of the major (2*S*,4*S*) diastereoisomer **21** (δ 2.35, dtd and δ 1.75, dtd) to doublets of doublets, and of the multiplet at δ 3.80 (CH_2O); δ_C(125.8 MHz, C^2HCl_3, major diastereoisomer only—minor diastereoisomer not observed above the background noise) 174.0 and 171.5 (C=O), 155.2 (urethane), 140.0–130.0 (aromatics), 82.0 and 79.6 [$OC(CH_3)_3$], 64.8 (CH_2OSi), 52.6 (C-2), 51.8 (OCH_3), 44.6 (C-4), 31.3 (C-3), 28.3 and 28.0 [$C(CH_3)_3$], 26.7 [$SiC(CH_3)_3$] and 19.2 [$SiC(CH_3)_3$]. The

minor component, tert-*butyl* (2S,4S)-4-tert-*butyldiphenylsilyloxymethylpyroglutamate* was obtained as a colourless oil (18 mg, 15%); $[\alpha]_D^{26}$ −3.25 (*c* 2.18 in $CHCl_3$) (Found: C, 68.3; H, 7.9; N, 3.2. $C_{26}H_{35}NO_4Si$ requires C, 68.8; H, 7.7; N, 3.1%); *m/z* [+ve CI (NH_3)] 454 $[M + H]^+$; ν_{max}(film)/cm^{-1} 3250 (NH, br d), 1738 (ester) and 1708 (lactam); δ_H(360 MHz, C^2HCl_3) 7.69–7.35 (10 H, m, aromatics), 4.13 (1 H, dd, $J_{2,3S}$ 9.1, $J_{2,3R}$ 4.4, H-2), 3.99 (1 H, dd, $J_{6A,6B}$ 10.1, $J_{6A,4}$ 4.8, CHOSi), 3.80 (1 H, dd, $J_{6B,6A}$ 10.1, $J_{6B,4}$ 3.3, CHOSi), 2.62 (1 H, m, $J_{4,3R}$ 9.2, $J_{4,6A}$ 4.8, $J_{4,6B}$ 3.3, H-4), 2.53 (1 H, ddd, $J_{3S,2}$ 9.1, $J_{3S,3R}$ 13.1, $J_{3S,4}$ 6.9, H-3*S*), 2.31 (1 H, ddd, $J_{3R,2}$ 4.4, $J_{3R,3S}$ 13.1, $J_{3R,4}$ 9.2, H-3*R*), 1.48 [9 H, $OC(CH_3)_3$] and 1.04 [9 H, $SiC(CH_3)_3$]; δ_C(90.6 MHz, C^2HCl_3) 177.3 and 171.4 (C=O), 135.7–127.7 (aromatics), 82.3 [$OC(CH_3)_3$], 63.3 (CH_2OSi), 54.8 (C-2), 42.9 (C-4), 28.2 (C-3), 28.0 [$OC(CH_3)_3$], 26.9 [$SiC(CH_3)_3$] and 19.3 [$SiC(CH_3)_3$].

1-*tert*-Butyl 5-methyl (2*S*,4*RS*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethyl[4-^{2}H]glutamate 21 and 22

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethylpyroglutamate **19** (35 mg, 0.063 mmol) was dissolved in [*O*-^{2}H]methanol (3 cm^3) with triethylamine (9.60 mg, 0.095 mmol). The reaction was stirred for 5 days at room temperature and the solvents were removed *in vacuo* to afford a colourless oil which was dissolved in ethyl acetate (30 cm^3). The organic layer was washed with aq. sodium chloride (10%; 20 cm^3) and the aqueous layer was extracted with ethyl acetate (2 × 30 cm^3). The organic layers were combined and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield the product as a colourless oil (36 mg) which was shown to contain a mixture of products by ^{1}H NMR spectroscopic comparison of the crude product with that of authentic samples. These were unchanged *tert*-butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethylpyroglutamate **19** (~8%), *tert*-butyl (2*S*,4*S*)-4-*tert*-butyldiphenylsilyloxymethylpyroglutamate (~16%) and 1-tert-*butyl* 5-*methyl* (2S,4RS)-N-tert-*butoxycarbonyl*-4-tert-*butyldiphenylsilyloxymethyl*[4-^{2}*H*]-*glutamate* **21** and **22** in a 2.3:1 ratio of diastereoisomers and ~76% yield. From the difference in the integration of the C-4 and C-3 protons of the crude product, it was evident that ~20% deuterium had been incorporated at the C-4 position; δ_D(38.4 MHz, $CHCl_3$) 7.26 (C^2HCl_3) and 2.73 (^{2}H-4); δ_D(38.4 MHz, $CHCl_3$ + 5% CH_3O^2H) 7.26 (C^2HCl_3), 2.78 (^{2}H-4) and 1.48 (CH_3O^2H).

1-*tert*-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-hydroxymethylglutamic acid 23

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-hydroxymethylpyroglutamate **11** (176 mg, 0.56 mmol) was dissolved in tetrahydrofuran (7 cm^3) at 0 °C and aq. lithium hydroxide (1 mol dm^{-3}; 0.73 cm^3) was added dropwise with vigorous stirring over a period of 5 min. Stirring was continued for a further 20 min at 0 °C, ethyl acetate (25 cm^3) and aq. sodium chloride (10%; 10 cm^3) were added to the reaction mixture, and the organic layer was separated. The aqueous layer was carefully acidified to pH 4–4.5 at 0 °C with stirring by the careful dropwise addition of 10% aq. citric acid. The aqueous layer was extracted with ethyl acetate (3 × 25 cm^3) and the organic layers were combined, washed with 10% aq. sodium chloride and dried (Na_2SO_4). The solvent was removed *in vacuo* to afford a white foam (98 mg, 53%) which was crystallised from ethyl acetate–light petroleum (60–80 °C) to yield 1-tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*hydroxymethylglutamic acid* **23** as a white solid (62 mg, 33%), mp 128–130 °C; $[\alpha]_D^{22}$ −32.3 (*c* 0.44 in MeOH); *m/z* [+ve FAB, NBA] 334 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 3200–3400 (NH, br d) and 1719 (acid); δ_H(360 MHz, $C^2H_3O^2H$) 4.02 (1 H, dd, $J_{2,3A}$ 10.3, $J_{2,3B}$ 4.4, H-2), 3.52 (2 H, m, CH_2OH), 2.60 (1 H, m, H-4), 2.12 (1 H, dtd, $J_{3A,3B}$ 13.9, $J_{3A,2}$ 10.3, $J_{3A,4}$ 3.8, H-3A), 1.68 (1 H, dtd, $J_{3B,3A}$ 13.9, $J_{3B,2}$ 4.3, $J_{3B,4}$ 10.0, H-3B), 1.44 [9 H, s, $C(CH_3)_3$] and 1.41 [9 H, s, $C(CH_3)_3$]; δ_H(360 MHz, C^2HCl_3) showed the presence of a –N*H* doublet δ 5.50 (1 H, $J_{NH,2}$ 7.3) which exchanged upon addition of ^2H_2O, and a C-2 proton at δ 4.21; δ_C(125.8 MHz, $C^2H_3O^2H$) 177.1 and 173.5 (C=O), 158.0 (urethane), 82.7 and 80.5 [$OC(CH_3)_3$], 64.3 (CH_2OH), 54.4 (C-2), 46.2 (C-4), 31.5 (C-3) and 28.7 and 28.3 [$OC(CH_3)_3$].

tert-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethylglutamic acid 24

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethylpyroglutamate **19** (600 mg, 1.08 mmol) was dissolved in tetrahydrofuran (stabilised with BHT, 16.2 cm^3) and water (5.4 cm^3). The solution was treated at 0 °C with hydrogen peroxide (60% w/v; 0.123 cm^3, 3.69 mmol) followed by lithium hydroxide (91.2 mg, 2.17 mmol) and stirred at 0 °C for 15 min to give a purple solution which was stirred for a further 3.5 h at room temperature. The reaction was quenched at 0 °C by addition of aq. sodium sulfite (1.5 mol dm^{-3}; 1.6 cm^3) over a period of 20 min. The organic solvent was removed *in vacuo* to afford an aqueous layer which was acidified to pH 3 by dropwise addition of aq. hydrochloric acid (0.2 mol dm^{-3}) and extracted with ethyl acetate (3 × 60 cm^3). The organic layers were combined and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield tert-*butyl* (2S,4R)-N-tert-*butoxycarbonyl*-4-tert-*butyldiphenylsilyloxymethylglutamic acid* **24** as a colourless oil (403 mg) which was crystallised from light petroleum (60–80 °C) (355 mg, 57%); mp 125–127 °C; $[\alpha]_D^{25}$ −2.52 (*c* 0.5 in $CHCl_3$) (Found: C, 64.4; H, 7.9; N, 2.7. $C_{31}H_{45}NO_7Si$ requires C, 65.1; H, 7.9 N, 2.45%); *m/z* [+ve FAB, NBA] 572 $[M + H]^+$; ν_{max} 1730 (ester) and 1712 (acid); δ_H(360 MHz, C^2HCl_3) 7.63 and 7.40 (10 H, 2 × m, aromatics), 5.35 (1 H, d, $J_{NH,2}$ 7.7, NH), 4.25 (1 H, m, H-2), 3.90 (1 H, dd, $J_{6A,4}$ 6.31, $J_{6A,6B}$ 9.55, CHOSi), 3.73 (1 H, dd, $J_{6B,4}$ 6.33, $J_{6B,6A}$ 9.55, CHOSi), 2.71 (1 H, m, H-4), 2.18 (1 H, ddd, $J_{3A,2}$ 10.33, $J_{3A,3B}$ 10.9, H-3A), 1.78 (1 H, ddd, $J_{3A,3B}$ 10.9, $J_{3B,2}$ 11.4, H-3B), 1.45 [18 H, 2 × s, $C(CH_3)_3$] and 1.04 [9 H, s, $SiC(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 176.07 and 171.03 (C=O), 156.43 (urethane), 135.53–127.73 (aromatics), 82.61 and 80.68 [$OC(CH_3)_3$], 64.34 (CH_2O), 52.5 (C-2), 44.8 (C-4), 32.72 (C-3), 28.2 [$C(CH_3)_3$], 26.75 [$C(CH_3)_3$], 26.54 [$SiC(CH_3)_3$] and 19.22 [$SiC(CH_3)_3$].

tert-Butyl (2*S*,4*S*)-*tert*-butoxycarbonylamino-5-hydroxy-4-*tert*-butyldiphenylsilyloxymethyl[5,5-$^2H_2$]pentanoate 26

tert-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-*tert*-butyldiphenylsilyloxymethylglutamic acid **24** (100 mg, 0.175 mmol) was dissolved in tetrahydrofuran (0.81 cm^3) and the solution was cooled to −40 °C. Triethylamine (0.032 cm^3, 0.23 mmol) was added, followed by dropwise addition of isobutyl chloroformate (0.027 cm^3, 0.2 mmol) under nitrogen. A white sediment was formed in the reaction which was stirred for 1.5 h at −40 °C. The mixture was filtered under nitrogen and a mixture of sodium borodeuteride (NaB^2H_4; 22 mg, 0.58 mmol) in tetrahydrofuran (0.55 cm^3) and ^2H_2O (0.064 cm^3) was added dropwise to the filtrate at 0 °C with stirring. Effervescence was observed and a white sediment was obtained in the reaction mixture. Stirring was continued at room temperature for 1 h. The reaction was cooled to 0 °C and ethyl acetate (1 cm^3) and aq. sodium chloride (10%; 0.3 cm^3) were added. The organic layer was washed with ice-cold aq. citric acid (10%; 0.5 cm^3) and aq. sodium chloride (10%; 0.5 cm^3) and dried (Na_2SO_4). The solvents were removed *in vacuo* to afford a white foam (130 mg). On addition of ethyl acetate, a polymer was formed and the solution was centrifuged and chromatographed on silica gel, using light petroleum (40–60 °C)–ethyl acetate (1 : 1) as eluent to yield tert-*butyl* (2S,4S)-2-tert-*butoxycarbonylamino*-5-*hydroxy*-4-tert-*butyldiphenylsilyloxymethylpentanoate* **26** as a colourless oil (66.3 mg, 68%) (Found: C, 67.0; H, 7.9; N, 2.5. $C_{31}H_{45}{}^2H_2NO_6Si$ requires C, 66.5 H, 8.1; N, 2.5%); *m/z* [+ve FAB, NBA] 560 $[M + H]^+$; δ_H(360 MHz, C^2HCl_3) 7.66 and 7.42 (10 H, 2 × m, aromatics), 5.18 (1 H, d,

$J_{NH,2}$ 7.46, NH), 4.26 (1 H, m, H-2), 3.73 (1 H, m, $J_{6A,6B}$ 10.06, CHOSi), 3.65 (1 H, dd, $J_{6B,6A}$ 10.06, $J_{6B,4}$ 5.79, CHOSi), 1.87 (2 H, m, H-4 and H-3A), 1.53 (1 H, m, H-3B), 1.44 [18 H, 2s, $C(CH_3)_3$] and 1.07 [9 H, s, $SiC(CH_3)_3$]; δ_H(360 MHz, $C_6{}^2H_6$) 7.73 and 7.24 (10 H, 2 × m, aromatics), 5.34 (1 H, d, $J_{NH,2}$ 7.29, NH), 4.55 (1 H, m, H-2), 3.63 (2 H, m, CHOSi), 1.93 (1 H, m, H-4), 1.87 (1 H, m, H-3A), 1.53 (1 H, m, H-3B), 1.40 [9 H, s, $C(CH_3)_3$], 1.29 [9 H, s, $C(CH_3)_3$] and 1.12 [9 H, s, $SiC(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 172.03 (C=O), 155.72 (urethane), 135.55–128.74 (aromatics), 81.8 [$OC(CH_3)_3$], 79.76 [$OC(CH_3)_3$], 66.24 (CH_2O), 63.6 (m, C^2H_2OH), 52.62 (C-2), 39.19 (C-4), 32.18 (C-3), 28.28 [$C(CH_3)_3$], 27.94 [$C(CH_3)_3$], 25.97 [$SiC(CH_3)_3$] and 19.19 [$SiC(CH_3)_3$].

tert-Butyl (2*S*,4*R*)-2-_tert_-butoxycarbonylamino-5-hydroxy-4-_tert_-butyldiphenylsilyloxymethylpentanoate

Prepared as described for the labelled compound above, using sodium borohydride to reduce the mixed anhydride of the acid **24**; *m/z* [+ve FAB, NBA] 558 $[M + H]^+$; δ_H(360 MHz, C^2HCl_3) 7.67 and 7.4 (10 H, m, aromatics), 5.17 (1 H, d, $J_{NH,2}$ 7.7, NH), 4.26 (1 H, br, H-2), 3.76 (2 H, m, H-5), 3.73 (1 H, AB, J_{AB} 10.15, CHOH), 3.65 (1 H, ABX, J_{AB} 10.15, $J_{4,5}$ 5.68, C*H*OH), 1.87 (2 H, br, H-3A and H-4), 1.5 (1 H, br, H-3B), 1.44 [18 H, s, $C(CH_3)_3$] and 1.07 [9 H, s, $SiC(CH_3)_3$]; δ_H(360 MHz, $C_6{}^2H_6$) 7.74 and 7.24 (10 H, m, aromatics), 5.30 (1 H, d, $J_{NH,2}$ 8, NH), 4.55 (1 H, m, H-2), 3.65 (4 H, m, 2 × CH_2O), 2.09 (1 H, m, H-4), 1.91 (2 H, m, H-3) and 1.39, 1.28 and 1.12 [27 H, 3 × s, $C(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 172.04 (C=O), 155.72 (urethane), 135.55–127.75 (aromatics), 81.81 and 79.75 [$OC(CH_3)_3$], 66.29 (CH_2OSi), 64.37 (CH_2OH), 52.58 (C-2), 39.46 (C-4), 32.21 (C-3), 28.27, 26.83 and 25.96 [$C(CH_3)_3$] and 19.18 [$SiC(CH_3)_3$].

tert-Butyl (2*S*,4*R*)-2-_tert_-butoxycarbonylamino-5-hydroxy-4-_tert_-butyldiphenylsilyloxymethyl[5,5-$^2H_2$]pentanoate 31

The acid **30** was prepared from the *cis*-silyl ether **18** as described above for the *trans*-acid **24**. The product **30**, *m/z* [+ve FAB, NBA] 572 $[M + H]^+$; δ_H(360 MHz, C^2HCl_3) 7.6–7.3 (10 H, m, aromatics), 5.14 (1 H, d, $J_{NH,2}$ 8, NH), 4.23 (1 H, br, H-2), 3.88 (2 H, m, CH_2O), 2.73 (1 H, m, H-4), 2.21 and 2.05 (2 H, 2 × m, H-3), 1.45 and 1.41 [18 H, 2 × s, $C(CH_3)_3$] and 1.03 [9 H, s, $SiC(CH_3)_3$], was reduced using NaB^2H_4 by the method used for the *trans* series to give the alcohol **31**, *m/z* [+ve FAB, NBA] 560 $[M + H]^+$; δ_H(360 MHz, C^2HCl_3) 7.7–7.4 (10 H, m, aromatics), 5.21 (1 H, br d, $J_{NH,2}$ 7.5, NH), 4.18 (1 H, m, H-2), 3.74 (2 H, d, *J* 3.97, CH_2O), 1.9 (2 H, m, H-4 and H-3A), 1.61 (1 H, m, H-3B), 1.46 [9 H, s, $C(CH_3)_3$], 1.41 [9 H, s, $C(CH_3)_3$] and 1.0 [9 H, s, $SiC(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 172.07 (C=O), 155.52 (urethane), 135.55–119.64 (aromatics), 81.97 and 80.0 [$OC(CH_3)_3$], 65.06 (CH_2O), 64.0 (m, C^2H_2O), 52.26 (C-2), 39.38 (C-4), 31.25 (C-3), 28.86, 28.28 and 27.98 [3 × $C(CH_3)_3$] and 19.29 [$SiC(CH_3)_3$].

tert-Butyl (2*S*,4*S*)-2-_tert_-butoxycarbonylamino-5-hydroxy-4-hydroxymethyl[5,5-$^2H_2$]pentanoate 27

tert-Butyl (2*S*,4*R*)-2-*tert*-butoxycarbonylamino-5-hydroxy-4-*tert*-butyldiphenylsilyloxymethyl[5,5-$^2H_2$]pentanoate **26** (50 mg, 0.9 mmol) was dissolved in methanol (1.8 cm^3) and ammonium fluoride (45.7 mg, 12.3 mmol) was added. The mixture was heated to reflux at 60 °C for 3.25 h. Water (2 cm^3) was added and the solution was extracted with chloroform (3 × 10 cm^3). The organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield an oil which was purified by silica gel column chromatography using ethyl acetate as eluent to yield a white solid (28.6 mg, 72%). This solid was recrystallised from ethyl acetate–light petroleum mp 79–81 °C; $[\alpha]_D^{27}$ +8.71 (*c* 1 in $CHCl_3$) (Found: C, 56.1; H, 9.15; N, 4.2. $C_{15}H_{27}{}^2H_2NO_6$ requires C, 56.1; H, 9.65; N, 4.4%); *m/z* [+ve FAB, NBA] 322 $[M + H]^+$; δ_H(360 MHz, C^2HCl_3) 5.33 (1 H, d, $J_{NH,2}$ 7.88, NH), 4.20 (1 H, m, H-2), 3.74 (2 H, br, CH_2OH), 1.83 (1 H, m, H-4), 1.77 (1 H, m, H-3A), 1.64 (1 H, m, H-3B), 1.46 [9 H, s, $C(CH_3)_3$] and 1.43 [9 H, s, $C(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 171.96 (C=O), 155.78 (urethane), 82.2 [$OC(CH_3)_3$], 80.05 [$OC(CH_3)_3$], 64.7 (CH_2OH), 64.19 (m, C^2H_2OH), 52.28 (C-2), 39.05 (C-4), 31.86 (C-3) and 28.3 and 27.87 [$C(CH_3)_3$].

tert-Butyl (2*S*)-2-_tert_-butoxycarbonylamino-5-hydroxy-4-hydroxymethylpentanoate

Prepared from the unlabelled compound using the method described above for the preparation of compound **27**, $[\alpha]_D^{27}$ +6.08 (*c* 1 in $CHCl_3$); *m/z* [+ve FAB, NBA] 320 $[M + H]^+$; δ_H(360 MHz, C^2HCl_3) 4.22 (1 H, d, $J_{2,3}$ 5.65, H-2), 3.79 (1 H, dd, $J_{5A,4}$ 4.1, $J_{5A,5B}$ 10.85, H-5A), 3.76 (2 H, br, CH_2OH), 3.71 (1 H, dd, $J_{5B,4}$ 5.85, $J_{5B,5A}$ 10.85, H-5B), 3.03 (1 H, br d, OH) and 3.00 (1 H, br d, OH), 1.84 (1 H, m, H-4), 1.77 (1 H, m, H-3A), 1.65 (1 H, m, H-3B) and 1.46 and 1.43 [18 H, 2 × s, $C(CH_3)_3$]; δ_C(125.8, C^2HCl_3) 171.93 (C=O), 155.78 (urethane), 82.23 and 82.08 [$OC(CH_3)_3$], 64.96 and 64.85 (2 × CH_2OH), 52.28 (C-2), 39.25 (C-4), 31.96 (C-3) and 28.30 and 27.97 [$C(CH_3)_3$].

tert-Butyl (2*S*,4*R*)-2-_tert_-butoxycarbonylamino-5-hydroxy-4-hydroxymethyl[5,5-$^2H_2$]pentanoate 32

Prepared from the (2*S*,4*R*)-silyl ether **31** as described for the (2*S*,4*S*) compound **27** above; δ_H(360 MHz, C^2HCl_3) 5.30 (1 H, d, $J_{NH,2}$ 5.49, NH), 4.22 (1 H, d, *J* 4.64, H-2), 3.80 (1 H, dd, $J_{5A,4}$ 4.12, $J_{5A,5B}$ 10.84, H-5A), 3.71 (1 H, dd, $J_{5B,4}$ 5.76, $J_{5B,5A}$ 10.84, H-5B), 2.41 (2 H, br d, 2 × CH_2OH), 1.80 (1 H, m, H-3A), 1.64 (1 H, m, H-3B) and 1.47 and 1.44 [18 H, 2 × s, $C(CH_3)_3$]; δ_C(128.5 MHz, C^2HCl_3) 171.91 (C=O), 155.79 (urethane), 82.27 and 80.10 [$OC(CH_3)_3$], 65.03 (CH_2O) and 63.88 (m, C^2H_2OH), 52.16 (C-2), 39.05 (C-4), 31.99 (C-3) and 28.29 and 27.96 [$C(CH_3)_3$].

Sodium (2*S*,4*S*)-5,5′-dihydroxy[5,5-$^2H_2$]leucinate 4a

tert-Butyl (2*S*,4*S*)-2-*tert*-butoxycarbonylamino-5-hydroxy-4-hydroxymethyl[5,5-$^2H_2$]pentanoate **27** (50 mg, 0.16 mmol) was dissolved in methanol (0.2 cm^3) and trifluoroacetic acid (1 cm^3) was added. The solution was stirred at room temperature overnight. The solvent was removed *in vacuo* to afford a colourless oil which was dried by azeotropic removal of residual trifluoroacetic acid with diethyl ether (8 × 10 cm^3), (25 mg, 95%), δ_H(360 MHz, 10% NaO^2H–^2H_2O, referenced on H-3B) 3.21 (2 H, m, CH_2OH), 2.92 (1 H, m, H-2), 1.38 (1 H, m, H-4), 1.23 (1 H, m, H-3A) and 1.08 (1 H, m, H-3B); δ_C(125.8 MHz, 10% NaO^2H–^2H_2O, normalised on C-3) 186.55 (C=O), 65.90 (CH_2OH), 64.45 (m, C^2H_2OH), 57.37 (C-2), 42.04 (C-4) and 37.25 (C-3).

Sodium (2*S*)-5,5′-dihydroxyleucinate 4

Prepared from the unlabelled alcohol *tert*-butyl (2*S*)-2-*tert*-butoxycarbonylamino-5-hydroxy-4-hydroxymethylpentanoate using the method described above for the labelled compound **4a**, *m/z* [FAB, H_2O–glycerol] 164 $[M + H]^+$; δ_H(360 MHz, 10% NaO^2H–^2H_2O, unreferenced) 3.20 (4 H, m, 2 × CH_2OH), 2.90 (1 H, m, H-2), 1.38 (1 H, m, H-4), 1.23 (1 H, m, H-3A) and 1.08 (1 H, m, H-3B); δ_C(125.8 MHz, 10% NaO^2H–^2H_2O, normalised on C-3) 186.75 (C=O), 65.72 (CH_2OH), 65.13 (CH_2OH), 57.46 (C-2), 42.93 (C-4) and 37.25 (C-3) [lit.,[24] δ_C(Na salt in ^2H_2O, DSS), 65.0 and 64.42 (CH_2OH), 56.88 (C-2), 42.33 (C-4) and 37.25 (C-3)].

Sodium (2*S*,4*R*)-5,5′-dihydroxy[5,5-$^2H_2$]leucinate 4b

Prepared from the diol **32** using the method described above for the labelled compound **4a**, δ_H(360 MHz, 10% NaO^2H–^2H_2O, referenced on H-3B) 3.26 (2 H, m, H-6), 2.93 (1 H, m, H-2), 1.40 (1 H, m, H-4), 1.29 (1 H, m, H-3A) and 1.08 (1 H, m, H-3B); δ_C(128.5 MHz, 10% NaO^2H–^2H_2O, normalised on C-3) 186.64 (C=O), 65.20 (CH_2OH), 57.33 (C-2), 47.23 (C-4) and 7.25 (C-3).

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