Synthesis of (2*S*,3*S*)-[3-$^2H_1$]-4-methyleneglutamic acid and (2*S*,3*R*)-[2,3-$^2H_2$]-4-methyleneglutamic acid

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(2*S*,3*S*)-[3-$^2H_1$]-4-Methyleneglutamic acid **1a** and (2*S*,3*R*)-[2,3-$^2H_2$]-4-methyleneglutamic acid **1b** have been synthesised for use in biosynthetic and metabolic studies.

Introduction

L-4-Methyleneglutamic acid **1** was first isolated as a product of germinated peanuts[1] and then found in a variety of other plants.[2] Its biosynthesis in tissue cultures of peanut seeds has been shown to be inhibited by auxins.[3] It has been reported to exhibit strong CNS inhibitory action,[4] and, in peptides, to inhibit the vitamin K mediated γ-carboxylation of glutamic acid residues, a process which is important in blood clotting.[5] We have synthesised this amino acid, among others, in our versatile and general amino acid synthesis[6] and now report a synthesis of samples of this compound which are stereoselectively deuteriated at C-3. This will allow the stereochemistry of the hydroxylation, which has been suggested[7] to yield (2*S*,3*S*)-3-hydroxy-4-methyleneglutamic acid **2** in *Leguminosae*, to be investigated in addition to other metabolic and biosynthetic processes involving this amino acid. Since deamination of these products will allow appropriately labelled samples of 2-methyleneglutaric acid **3** to be obtained, the synthesis had application to the study of the stereochemistry of the reaction catalysed by 2-methyleneglutarate mutase (EC 5.4.99.4), as shown in Scheme 1. However, subsequent to our commencing this aspect of the work, it has been reported[8] that this biological reaction is non-stereospecific.

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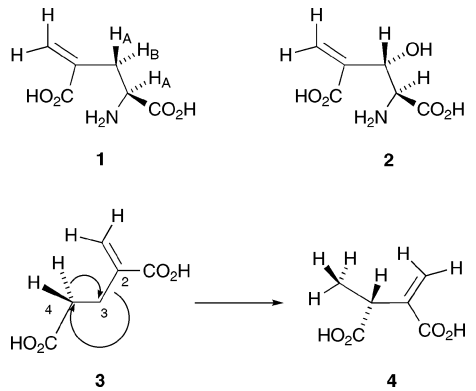
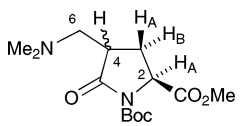
Results and discussion

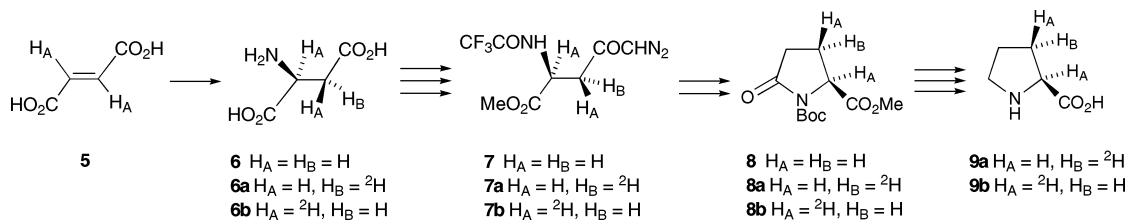
In our chemico-enzymatic synthesis of labelled L-amino acids[9] we used the well-known[10] *anti*-addition of ammonia to the double bond of fumaric acid **5** either by the enzyme L-aspartase (EC 4.3.1.1) or by using this enzyme in a preparation of *Escherichia coli* immobilised on polyacrylamide gel to obtain samples of (2*S*,3*R*)-[3-$^2H_1$]- and (2*S*,3*S*)-[2,3-$^2H_2$]-aspartic acid, **6a** and **6b** respectively, as shown in Scheme 2. For the synthesis of stereospecifically labelled samples of L-proline **9**,[11] we converted the labelled aspartic acids **6** into the diazoketones **7**, which were photolysed in methanol containing aqueous sodium bicarbonate and then reacted with di-*tert*-butyl dicarbonate. This gave the stereospecifically labelled methyl pyroglutamate urethanes **8a** and **8b** in quantitative yield.

The unlabelled urethane ester **8** was now heated to 75 °C with *tert*-butoxy bis(dimethylamino)methane (Bredereck's reagent) in 1,2-dimethoxyethane to give the enaminone **10** in 93% yield (Scheme 3) and this was treated with DIBAL-H in tetrahydrofuran to yield the olefin **11** in 55% yield together with the diastereoisomeric amines **12** in 45% yield. The yield of the olefin

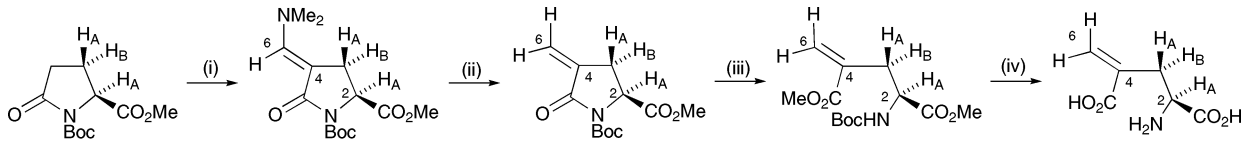


Scheme 1





Scheme 2



8 $H_A = H_B = H$
8a $H_A = H$, $H_B = {}^2H$
8b $H_A = {}^2H$, $H_B = H$

10 $H_A = H_B = H$
10a $H_A = H$, $H_B = {}^2H$
10b $H_A = {}^2H$, $H_B = H$

11 $H_A = H_B = H$
11a $H_A = H$, $H_B = {}^2H$
11b $H_A = {}^2H$, $H_B = H$

13 $H_A = H_B = H$
13a $H_A = H$, $H_B = {}^2H$
13b $H_A = {}^2H$, $H_B = H$

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

Scheme 3 *Reagents and conditions*: (i) (^{t}BuO)CH(NMe_2)$_2$, $MeOCH_2CH_2OMe$, 75 °C, 12 h (93% **10**, 71% **10a**, 72% **10b**); (ii) DIBAL-H, hexane, THF, −78 °C, 1 h, then rt, 1 h (64% **11**, 62% **11a**, 66% **11b**); (iii) LiOMe, MeOH, THF, −40 °C, 15 min (76% **13**, 78% **13a**, 88% **13b**); (iv) 9 M aq. HBr, reflux, 2 h (quantitative **1**, 97% **1a**, quantitative **1b**).

11 could be increased to 64% on a small scale but a 19% yield of the amine mixture **12** was still obtained. The amines **12** were converted into the olefin **11** in 85% yield by Hoffman elimination using methyl iodide followed by reaction with triethylamine. The labelled amines **12a** and **12b** and enones **11a** and **11b** were obtained in good yield and without loss of label from the enaminones **10a** and **10b** when the urethane esters **8a** and **8b** were used in these experiments. The next stage was to open the pyroglutamate ring by solvolysis of the reactive lactam-urethanes **11**. This was achieved using methanolic lithium methoxide in tetrahydrofuran at −40 °C since Michael addition of methanol occurred at higher temperatures, lowering the yield. In this way, good yields of the diesters **13**, **13a**, and **13b** were obtained. These were converted into the desired amino acid hydrobromides **1**, **1a** and **1b**, by heating at reflux with 9 M aqueous HBr. The ^{1}H NMR spectra of these compounds are shown in Fig. 1 and indicate that the stereoselective synthesis of (2*S*,3*S*)-[3-$^2H_1$]-4-methyleneglutamic acid **1a** and (2*S*,3*R*)-[2,3-$^2H_2$]-4-methyleneglutamic acid **1b** has been successful.

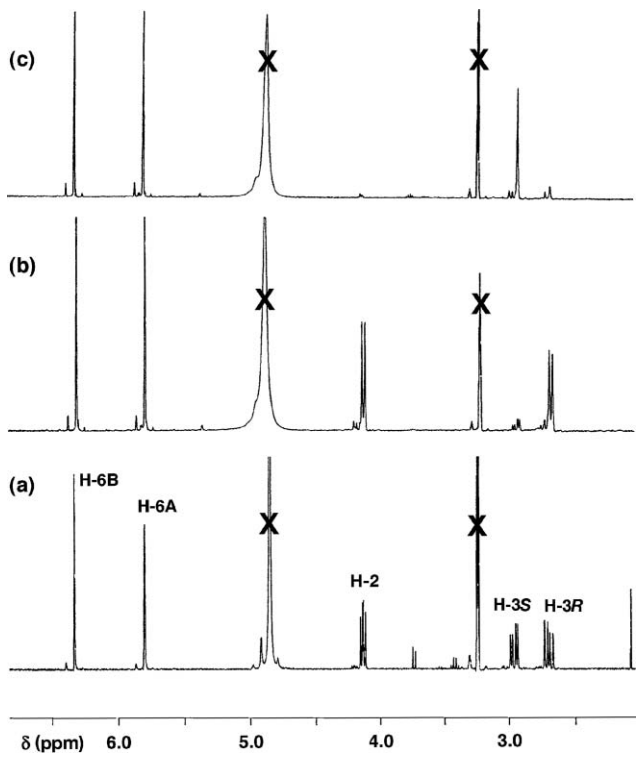


Fig. 1 360 MHz ^{1}H NMR spectra in $C^2H_3O^2H$ of (a) 4-methyleneglutamic acid **1**; (b) (2*S*,3*S*)-[3-$^2H_1$]-4-methyleneglutamic acid **1a**; and (c) (2*S*,3*R*)-[2,3-$^2H_2$]-4-methyleneglutamic acid **1b**.

Conclusions

We have completed the synthesis of (2*S*,3*S*)-[3-$^2H_1$]-4-methyleneglutamic acid **1a** and (2*S*,3*R*)-[2,3-$^2H_2$]-4-methyleneglutamic acid **1b** for use in metabolic and biosynthetic studies.

Experimental

Melting points were determined on a Kofler hot-stage 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 Philips PU8720 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker WM 360 (360 MHz) Fourier transform instrument. *J* values are given in Hz. ^{13}C NMR spectra were recorded on a Bruker AMX 500 (125.9 MHz) Fourier transform instrument. INEPT experiments were used to help assign ^{13}C NMR signals where necessary. Residual solvent peaks were used as internal references in the NMR spectra. Mass spectra were recorded on Kratos MS80RF and MS25 double focussing spectrometers by Mr A. M. Greenway at the University of Sussex and on a Kratos MS50 spectrometer by Dr S. Chotai at the Wellcome Research Laboratories, Beckenham. Microanalyses were performed by Miss M. Patel. Column chromatography was performed using Merk Kieselgel 60 (230–400 mesh-ART 9385).

Methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylenepyroglutamate (10)

tert-Butoxy bis(dimethylamino)methane (Bredereck's reagent) (22.27 g, 0.128 mol) was added to a solution of methyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **8**[11] (20.72 g, 85 mmol) in 1,2-dimethoxyethane (200 ml). The mixture was heated at 75 °C for 12 h and the solvent was removed *in vacuo* to give a dark solid which was recrystallised from diethyl ether and petroleum ether (40–60 °C) containing a small amount of dichloromethane. Methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylenepyroglutamate **10** was obtained as a pale yellow solid (23.78 g, 93%); mp 127 °C; $[a]_D^{18}$ −44 (*c* 0.77, $CHCl_3$); (Found C, 56.4; H, 7.4; N, 9.15. $C_{14}H_{22}N_2O_5$ requires: C, 56.4; H, 7.4; N, 9.4%); *m*/*z* [+ve FAB, (EtOAc/NBA)] 299 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1766 (urethane-lactam), 1730 (ester) and 1682 (lactam-urethane); λ_{max} (MeOH)/nm 313 (ε 22 000); δ_H (360 MHz,

C^2HCl_3) 1.46 [9H, s, $C(CH_3)_3$], 2.88 (1H, dd, $J_{3R,3S}$ 15.3, $J_{3R,2}$ 3.5, H-3*R*), 2.99 [6H, s, $N(CH_3)_2$], 3.22 (1H, dd, $J_{3S,3R}$ 15.3, $J_{3S,2}$ 10.7, H-3*S*), 3.73 (3H, s, OCH_3), 4.52 (1H, dd, $J_{2,3S}$ 10.7, $J_{2,3R}$ 3.5, H-2) and (1H, br s, H-6); δ_C (125.9 MHz, C^2HCl_3) 26.2 (C-3), 27.9 [$C(CH_3)_3$], 41.9 [$N(CH_3)_2$], 52.2 (OCH_3), 55.9 (C-2), 82.2 [$OC(CH_3)_3$], 90.8 (C-4), 146.4 (C-6), 150.4 (urethane), 169.4 (ester) and 172.5 (lactam).

Methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylenepyroglutamate (10a)

This was prepared as the above using methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonylpyroglutamate **8a**[11] (1.73 g, 7.1 mmol). Methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylenepyroglutamate **10a** was obtained as a solid (1.5 g, 71%); mp 124–127 °C; $[\alpha]_D^{19}$ −43.3 (*c* 0.77, $CHCl_3$); *m*/*z* [+ve FAB, (EtOAc/NBA)] 300 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1767 (urethane-lactam), 1730 (ester) and 1682 (lactam-urethane); δ_H (360 MHz, C^2HCl_3) 1.47 [9H, s, $C(CH_3)_3$], 2.87 (1H, br s, H-3*R*), 3.00 [6H, s, $N(CH_3)_2$], 3.74 (3H, s, OCH_3), 4.53 (1H, d, $J_{2,3R}$ 3.6, H-2) and 7.12 (1H, d, $J_{6,3R}$ 1.5, H-6); δ_C (125.9 MHz, C^2HCl_3) 25.7 (t, C-3), 28.0 [$C(CH_3)_3$], 41.9 (NMe_2), 52.3 (OCH_3), 55.8 (C-2), 82.2 [$OC(CH_3)_3$], 90.8 (C-4), 146.5 (C-6), 150.5 (urethane), 169.4 (ester) and 172.6 (lactam).

Methyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylenepyroglutamate (10b)

This was prepared as above from methyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonylpyroglutamate **8b**[11] (2.45 g, 10 mmol). Methyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylenepyroglutamate **10b** was obtained as a solid (2.15 g, 72%); mp 123–127 °C; $[\alpha]_D^{18}$ −44.4 (*c* 0.77, $CHCl_3$); *m*/*z* [+ve FAB, (EtOAc/NBA)] 301 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1765 (urethane-lactam), 1730 (ester) and 1682 (lactam-urethane); δ_H (360 MHz, C^2HCl_3), 1.47 [9H, s, $C(CH_3)_3$], 3.00 [6H, s, $N(CH_3)_2$], 3.22 (1H, s, H-3*S*), 3.79 (3H, s, OCH_3) and 7.11 (1H, d, $J_{6,3S}$ 1.7, H-6); δ_C (125.9 MHz, C^2HCl_3) 26.0 (t, C-3), 28.4 [$C(CH_3)_3$], 42.4 [$N(CH_3)_2$], 52.7 (OCH_3), 55.8 (t, C-2), 82.6 [$OC(CH_3)_3$], 91.2 (C-4), 146.9 (C-6), 150.9 (urethane), 169.4 (ester) and 172.9 (lactam).

Methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate (11) from the enaminone 10 (method A)

Methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylenepyroglutamate **10** (1 g, 3.35 mmol) was dissolved in tetrahydrofuran (200 ml) and cooled to −78 °C. A 1.0 M solution of DIBAL-H in hexane (5.53 ml, 5.53 mmol) was added and the mixture was kept at −78 °C for 1 h, allowed to warm to room temperature and stirred for 1 h. Saturated aqueous ammonium hydroxide solution (*ca.* 20 ml) was added and the mixture was stirred at room temperature overnight. The mixture was dissolved in ethyl acetate (40 ml) and 10% aqueous citric acid (20 ml) and the layers were separated. The organic layer was further washed with saturated aqueous sodium chloride (20 ml), saturated aqueous sodium hydrogencarbonate (20 ml) and saturated aqueous sodium chloride (20 ml). The organic layer was dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate **11** (550 mg, 64%) as a clear, viscous oil; $[\alpha]_D^{19}$ −13.2 (*c* 1.79, $CHCl_3$); *m*/*z* [+ve FAB, (ethyl acetate/NBA)] 156 ([M − $CO_2C_4H_9$ + 2H]$^+$); *m*/*z* [EI] 240 ([M − CH_3]$^+$); ν_{max} (film)/cm^{-1} 1787 (urethane-lactam), 1751 (ester) and 1721 (lactam-urethane); λ_{max} (MeOH)/nm 217 (br, ε 7600); δ_H (360 MHz, C^2HCl_3) 1.48 [9H, s, $C(CH_3)_3$], 2.65 (1H, tdd, $J_{3R,3S}$ 17.5, $J_{3R,2}$ 3.2, $J_{3R,6}$ 2.3, H-3*R*), 2.99 (1H, tdd, $J_{3S,3R}$ 17.5, $J_{3S,2}$ 10.1, H-3*S*), 3.73 (3H, s, OCH_3), 4.60 (1H, dd, $J_{2,3S}$ 10.1, $J_{2,3R}$ 3.2, H-2), 5.49 (1H, t, $J_{6A,3S} \approx J_{6A,3R} \approx$ 2.3, H-6A) and 6.20 (1H, t, $J_{6B,3S} \approx J_{6B,3R} \approx$ 3.0, H-6B); δ_C (125.9 MHz, C^2HCl_3) 27.7 (C-3), 27.8 [$C(CH_3)_3$], 52.5 (OCH_3), 55.5 (C-2), 83.7 [$OC(CH_3)_3$], 120.8 (C-6), 136.4 (C-4), 149.7 (urethane), 165.3 (ester) and 171.4 (lactam).

The acidic layer was treated with saturated aqueous sodium hydrogen carbonate to pH 8–9 and extracted with ethyl acetate (3 × 40 ml). The organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield methyl (2*S*,4*RS*)-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylpyroglutamate **12** (190 mg, 19%), a clear oil which solidified after long drying; mp 57–60 °C; (Found: C, 56.25; H, 7.95; N, 8.8. $C_{14}H_{24}N_2O_5$ requires: C, 56.0; H, 8.05; N, 9.3%); *m*/*z* [EI] 300 ([M]$^+$); ν_{max} (KBr)/cm^{-1} 1784 (urethane-lactam), 1740 (ester) and 1704 (lactam-urethane); δ_H (360 MHz, C^2HCl_3, mixed diastereoisomers) 1.43 [9H, br s, $C(CH_3)_3$], 1.90 (1H, m, H-3*R*) 2.16 and 2.17 [6H, 2s, $N(CH_3)_2$], 2.20–2.80 (4H, m, H-3*S*, H-4 and H-6), 3.71 (3H, 4s, OCH_3) and 4.50 (1H, 2dd, H-2); δ_C (125.9 MHz, C^2HCl_3, mixed diastereoisomers) 26.2 (C-3), 27.7 [$C(CH_3)_3$], 40.6 and 41.5 (C-4), 45.3 and 45.5 [$N(CH_3)_2$], 52.3 and 52.4 (OCH_3), 57.0 and 57.4 (C-2), 59.7 and 59.9 (C-6), 83.4 and 83.5 [$OC(CH_3)_3$], 149.1 and 149.2 (urethane), 171.1 and 171.9 (ester), and 173.9 and 174.1 (lactam).

Methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate (11) from the amine 12 (method B)

Methyl (2*S*,4*RS*)-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylpyroglutamate **12** (200 mg, 0.66 mmol) was dissolved in methyl iodide (5 ml) and stirred at room temperature in the dark under argon overnight. The methyl iodide was removed *in vacuo* and the amine salt was suspended in dichloromethane (5 ml). Triethylamine (1 ml, 7.2 mmol) was added and the mixture was stirred at room temperature for 7 h. The solvents were removed *in vacuo* and and the residue was dissolved in ethyl acetate (30 ml). The organic layer was washed with 10% aqueous citric acid (10 ml), saturated aqueous sodium thiosulfate (10 ml), saturated aqueous sodium chloride (10 ml) and dried (Na_2SO_4). Further drying *in vacuo* provided the product **11** as a clear oil (140 mg, 83%) with identical spectra to those described for the sample prepared by method A.

Methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate (11a)

This was prepared by the two methods described above for the unlabelled compound **11**, using methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylenepyroglutamate **10a**. Methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate **11a** was obtained (62% by method A and 75% by method B); $[\alpha]_D^{20}$ −15.0 (*c* 1.34, $CHCl_3$); *m*/*z* [+ve FAB, (EtOAc/NBA)] 157 ([M − $CO_2C_4H_9$ + 2H]$^+$); ν_{max} (film)/cm^{-1} 1785 (urethane-lactam), 1750 (ester) and 1719 (lactam-urethane); δ_H (360 MHz, C^2HCl_3) 1.48 [9H, s, $C(CH_3)_3$], 2.67 (1H, br s, H-3*R*), 3.74 (3H, s, OCH_3), 4.60 (1H, d, $J_{2,3R}$ 3.0, H-2), 5.50 (1H, d,

$J_{6A,3R}$ 2.1, H-6A) and 6.20 (1H, d, $J_{6B,3R}$ 2.3 H-6B); δ_C (125.9 MHz, C^2HCl_3), 27.0 (t, C-3), 27.5 [$C(CH_3)_3$], 52.5 (OCH_3), 55.5 (C-2), 84.0 [$OC(CH_3)_3$], 121.0 (C-6), 136.5 (C-4), 149.5 (urethane), 165.5 (ester) and 171.5 (lactam).

Methyl (2*S*,3*S*,4*RS*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylpyroglutamate **12a** was isolated as a yellow oil (23% by method A); ν_{max} (film)/cm^{-1} 1789 (urethane-lactam), 1751 (ester) and 1718 (lactam-urethane); δ_H (360 MHz, C^2HCl_3, mixed diastereoisomers) 1.46 [9H, br s, $C(CH_3)_3$], 1.94 (1H, m, H-3*R*), 2.20 and 2.21 [6H, 2s, $N(CH_3)_2$], 2.27–2.81 (3H, m, H-4 and H-6), 3.73–3.76 (3H, 4s, OCH_3) and 4.50–4.60 (1H, m, H-2).

Methyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate (11b)

This was prepared by the two methods described above for the unlabelled compound **11**, using methyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylenepyroglutamate **10b**. Methyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate **11b** was obtained (66% by method A, 71% by method B); $[\alpha]_D^{22}$ −13.6 (*c* 1.59, $CHCl_3$); *m*/*z* [+ve FAB, ($CHCl_3$/NBA)] 158 ([M − $CO_2C_4H_9$ + 2H]$^+$); ν_{max} ($CHCl_3$)/cm^{-1} 1785 (urethane-lactam), 1748 (ester) and 1718 (lactam-urethane); δ_H (360 MHz, C^2HCl_3) 1.48 [9H, s, $C(CH_3)_3$], 3.00 (1H, br s, H-3*S*), 3.74 (3H, s, OCH_3), 5.50 (1H, d, $J_{6A,3S}$ 2.7, H-6A) and 6.20 (1H, d, $J_{6B,3S}$ 3.2, H-6B); δ_C (125.9 MHz, C^2HCl_3) 26.5 (t, C-3), 26.9 [$C(CH_3)_3$], 51.5 (OCH_3), 54.3 (t, C-2), 82.8 [$OC(CH_3)_3$], 119.9 (C-6), 135.5 (C-4), 148.8 (urethane), 164.4 (ester) and 170.5 (lactam).

Methyl (2*S*,3*R*,4*RS*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-*N*,*N*-dimethylaminomethylpyroglutamate **12b** was isolated as a yellow oil (25% from method A); ν_{max} (film)/cm^{-1} 1789 (urethane-lactam), 1749 (ester) and 1718 (lactam-urethane); δ_H (360 MHz, C^2HCl_3, mixed diastereoisomers) 1.46 [9H, br s, $C(CH_3)_3$], 2.21 and 2.22 [6H, 2s, $N(CH_3)_2$], 2.27–2.82 (4H, m, H-3*S*, H-4 and H-6) and 3.74–3.75 (3H, 4s, OCH_3).

Dimethyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate (13)

Methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate **11** (1.53 g, 6 mmol) was dissolved in dry freshly distilled tetrahydrofuran (30 ml) and the mixture was cooled to −40 °C. Lithium methoxide (1.0 M in MeOH, 7.2 ml, 7.2 mmol) was added in portions *via* syringe (0.5 ml) and the solution was stirred for 15 min. Ethyl acetate (50 ml) and water (50 ml) were added, the layers were separated and the aqueous layer was re-extracted with ethyl acetate (50 ml). The organic layers were combined, washed with saturated aqueous sodium chloride (30 ml), dried (Na_2SO_4) and the solvent was removed *in vacuo*. Column chromatography on silica gel, eluting with diethyl ether–petroleum ether (30–40 °C) (1 : 1) gave dimethyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate **13** as a clear oil (1.3 g, 76%); $[\alpha]_D^{20}$ +15.2 (*c* 1.2, $CHCl_3$); *m*/*z* [+ve FAB, (ethyl acetate/NBA)] 288 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1756 (sh, urethane-lactam) and 1718 (urethane/ester); δ_H (360 MHz, C^2HCl_3) 1.40 [9H, s, $C(CH_3)_3$], 2.65 (1H, dd, $J_{3R,3S}$ 14.0, $J_{3R,2}$ 8.0, H-3*R*), 2.80 (1H, dd, $J_{3S,3R}$ 14.0, $J_{3S,2}$ 5.8, H-3*S*), 3.71 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 4.46 (1H, dd, $J_{2,3S}$ 5.8, $J_{2,3R}$ 8.0, H-2), 5.10 (1H, br d, $J_{NH,2}$ 7.4, NH, ^2H_2O exchangeable), 5.64 (1H, s, H-6A) and 6.20 (1H, s, H-6B); δ_C (125.9 MHz, C^2HCl_3) 28.6 [$C(CH_3)_3$], 35.2 (C-3), 52.4 (OCH_3), 52.5 (OCH_3), 53.3 (C-2), 80.1 [$OC(CH_3)_3$], 128.9 (C-6), 136.0 (C-4), 155.5 (urethane), 167.4 (ester) and 172.7 (ester).

Dimethyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate (13a)

This was prepared as for the unlabelled compound **13** using methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate **11a** (310 mg, 1.21 mmol). Dimethyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate **13a** was obtained as an oil (270 mg, 78%); $[\alpha]_D^{21}$ +16.9 (*c* 1.3, $CHCl_3$); *m*/*z* [+ve FAB, (ethyl acetate/NBA)] 289 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1756 (sh) and 1718 (urethane-ester); δ_H (360 MHz, C^2HCl_3) 1.37 [9H, s, $C(CH_3)_3$], 2.61 (1H, d, $J_{3R,2}$ 7.5, H-3*R*), 3.67 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 4.42 (1H, t, $J_{2,3R} \approx J_{2,NH} \approx$ 7.9, H-2), 5.20 (1H, br d, $J_{NH,2} \approx$ 7.6, NH), 5.61 (1H, s, H-6A) and 6.22 (1H, s, H-6B); δ_C (125.9 MHz, C^2HCl_3) 27.3 [$C(CH_3)_3$], 33.6 (t, C-3), 51.1 (OCH_3), 51.2 (OCH_3), 51.9 (C-2), 78.8 [$OC(CH_3)_3$], 127.6 (C-6), 134.6 (C-4), 154.2 (urethane), 166.1 (ester) and 171.3 (ester).

Dimethyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate (13b)

This was prepared as for the unlabelled compound **13** above using methyl *N*-(2*S*,3*R*)-[2,3-$^2H_2$]-*tert*-butoxycarbonyl-4-methylenepyroglutamate **11b** (330 mg, 1.28 mmol). Dimethyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate **13b** was obtained as an oil (330 mg, 88%); $[\alpha]_D^{21}$ +18.0 (*c* 1.7, $CHCl_3$); *m*/*z* [+ve FAB, (NBA)] 290 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1756 (sh) and 1718 (urethane-ester); δ_H (360 MHz, C^2HCl_3) 1.39 [9H, s, $C(CH_3)_3$], 2.77 (1H, s, H-3*S*), 3.70 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 5.28 (1H, br s, NH), 5.63 (1H, s, H-6A) and 6.24 (1H, s, H-6B); δ_C (125.9 MHz, C^2HCl_3) 27.3 [$C(CH_3)_3$], 33.5 (t, C-3), 51.1 (OCH_3), 51.2 (OCH_3), 51.6 (t, C-2), 78.7 [$OC(CH_3)_3$], 127.6 (C-6), 134.6 (C-4), 154.8 (urethane), 166.1 (ester) and 171.4 (ester).

(2*S*)-4-Methyleneglutamic acid hydrobromide (1)

Dimethyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate **13** (1.31 g, 4.56 mmol) was heated at reflux with 9 M aqueous hydrobromic acid (30 ml) for 2 h. The solvent was removed *in vacuo* and the oily product was azeotroped with methanol to yield pale orange crystals. A sample was recrystallised from 9 M aqueous hydrobromic acid to give the product **1** as off-white crystals (1.08 g, quantitative); mp ≈ 200 °C (decomp.); $[\alpha]_D^{17}$ +12 (*c* 1.0, 3 M HCl) [lit.[2c] +12.8 (11% w/v HCl)]; (Found C, 30.3; H, 4.1; N, 5.8. $C_6H_9NO_4$·HBr requires: C, 30.0; H, 4.2; N, 5.8%); *m*/*z* [+ve FAB, (EtOH/glycerol)] 160 (free amino acid [M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3600–2800 (br, NH, OH) and 1739 (br, carboxylate); δ_H (360 MHz, $C^2H_3O^2H$) 2.76 (1H, dd, $J_{3R,2}$ 8.1, $J_{3R,3S}$ 14.3, H-3*R*), 3.00 (1H, dd, $J_{3S,2}$ 5.6, $J_{3S,3R}$ 14.3, H-3*S*), 4.20 (1H, dd, $J_{2,3S}$ 5.6, $J_{2,3R}$ 8.1, H-2), 5.86 (1H, d, $J_{6A,3R}$ 1.0, H-6A) and 6.39 (1H, d, $J_{6B,3R}$ 1.0, H-6B); δ_C (125.9 MHz, $C^2H_3O^2H$) 35.0 (C-3), 53.8 (C-2), 131.8 (C-4), 136.5 (C-6), 169.8 (acid) and 171.8 (acid).

(2*S*,3*S*)-[3-$^2H_1$]-4-Methyleneglutamic acid hydrobromide (1a)

This was prepared as for **1** above using dimethyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate **13a** (95 mg, 0.33 mmol). (2*S*,3*S*)-[3-$^2H_1$]-4-Methyleneglutamic acid hydrobromide **1a** was a solid (77 mg, 97%); mp ≈ 200 °C (decomp.); $[\alpha]_D^{20}$ +10

(c 0.46, 3 M HCl); m/z [+ve FAB, (EtOH/ethyl acetate/glycerol)] 161 (free amino acid $[M + H]^+$); ν_{max} (KBr)/cm^{-1} 3600–2800 (br, NH, OH) and 1737 (br, carboxylate); δ_H (360 MHz, $C^2H_3O^2H$) 2.74 (1H, d, $J_{3R,2}$ 8.3, H-3*R*), 4.21 (1H, d, $J_{2,3R}$ 8.3, H-2), 5.86 (1H, d, $J_{6A,3R}$ 1.0, H-6A) and 6.39 (1H, d, $J_{6B,3R}$ 1.0, H-6B); δ_C (125.9 MHz, $C^2H_3O^2H$) 34.7 (t, C-3), 53.8 (C-2), 131.6 (C-4), 136.5 (C-6), 169.8 (acid) and 171.5 (acid).

(2*S*,3*R*)-[2,3-$^2H_2$]-4-Methyleneglutamic acid hydrobromide (1b)

This was prepared in the same way as **1** above using dimethyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate **13b** (110 mg, 0.38 mmol). (2*S*,3*R*)-[2,3-$^2H_2$]-4-Methyleneglutamic acid hydrobromide **1b** was a solid (92 mg, quantitative); mp $\approx$ 200 °C; $[a]_D^{20}$ +10 (c 0.72, 3 M HCl); m/z [+ve FAB, (EtOH/glycerol)] 162 (free amino acid $[M + H]^+$); ν_{max} (KBr)/cm^{-1} 3600–2800 (br, NH, OH) and 1736 (carboxylate); δ_H (360 MHz, $C^2H_3O^2H$) 2.99 (1H, s, H-3*S*), 5.86 (1H, s, H-6A) and 6.39 (1H, s, H-6B); δ_C (125.9 MHz, $C^2H_3O^2H$) 34.6 (t, C-3), 53.5 (t, C-2), 131.6 (C-4), 136.5 (C-6), 169.8 (acid) and 171.5 (acid).

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