Two separate and distinct syntheses of stereospecifically deuteriated samples of (2*S*)-proline†

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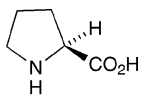
Received 23rd January 2006, Accepted 14th February 2006
First published as an Advance Article on the web 9th March 2006
DOI: 10.1039/b601097k

Two distinct syntheses of samples of the amino acid L-proline which are stereospecifically deuteriated on the β-carbon atom are reported. In the first of these, the labelled diazoketones **6**, prepared by a chemico-enzymatic synthesis, have been photolysed in alkaline conditions to give the corresponding labelled methyl pyroglutamates **10** *via* hydrolysis and intramolecular trapping of the resultant ketene intermediates **9**. These were then converted into (2*S*,3*S*)-[3-$^2H_1$]- and (2*S*,3*R*)-[2,3-$^2H_2$]-proline, **1a** and **1b** respectively. The second synthesis provides (2*S*)-[3,3-$^2H_2$]-, (2*S*,3*S*)- and (2*S*,3*R*)-[3-$^2H_1$]-proline, **1d**, **1a** and **1c** respectively, and has as its key step the highly stereoselective hydrolysis of the silylenol ethers **14** and **14a** respectively in which deuteriation or protonation occurs from the *re*-face of the enol ether.

Introduction

The use of stereospecifically labelled amino acids in metabolic studies[2] and, in combination with multidimensional NMR spectroscopy, in obtaining detailed protein solution structures[3–5] makes the ready availability of such compounds of considerable importance. The amino acid L-proline **1** and its post-translationally modified derivatives are constituents of antibiotics and are important for conformational constraint in proteins. Several syntheses of stereospecifically labelled samples of this amino acid have been completed by ourselves[1] and others[2,6] and we now wish to report two straightforward syntheses of samples of L-proline which are labelled with deuterium in the 3-*pro*-*R* or the 3-*pro*-*S* position.



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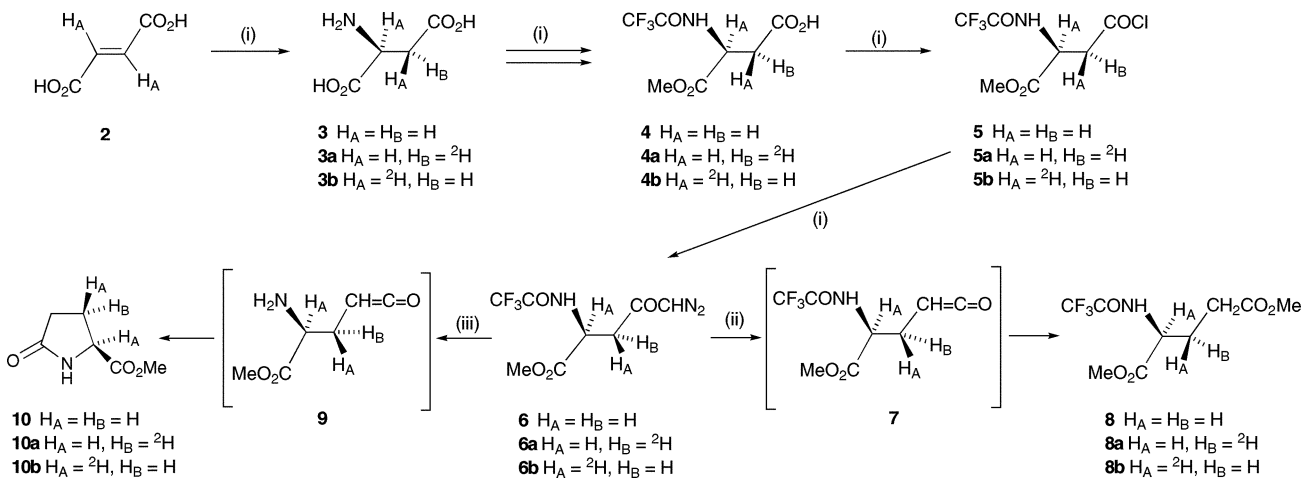
† See ref. 1.

Results and discussion

As the first step in our synthesis of stereospecifically labelled L-amino acids[7] and β-amino acids,[8] we have used the well-known[2,9] *anti*-addition of ammonia to the double bond of fumaric acid **2** by the enzyme L-aspartase (EC 4.3.1.1) to obtain samples of (2*S*,3*R*)-[3-$^2H_1$]- and (2*S*,3*S*)-[2,3-$^2H_2$]-aspartic acid, **3a** and **3b** respectively, as shown in Scheme 1. We subsequently found that using this enzyme in a preparation of *Escherichia coli* immobilised on polyacrylamide gel[10] was more effective for accessing these labelled samples **3** of aspartic acid which were used in our general synthesis of stereospecifically labelled amino acids.[11] In our synthesis of labelled glutamic acids,[7a,b] we treated the labelled aspartic acids **3** with trifluoroacetic anhydride, followed by methanol to obtain a mixture containing *ca.* 80% of the trifluoroacetyl-β-acid-α-ester **4** together with *ca.* 20% of its isomer, the α-ester-β-acid. The mixed esters were converted into a mixture of the acid chloride **5** and its isomer which were originally[7b] separated by recrystallisation from benzene. The isomer **5** was then converted into the diazoketone **6**. In the current synthesis, we have carried through the mixtures of regioisomers to the diazoketones **6**, **6a**, and **6b** which have been



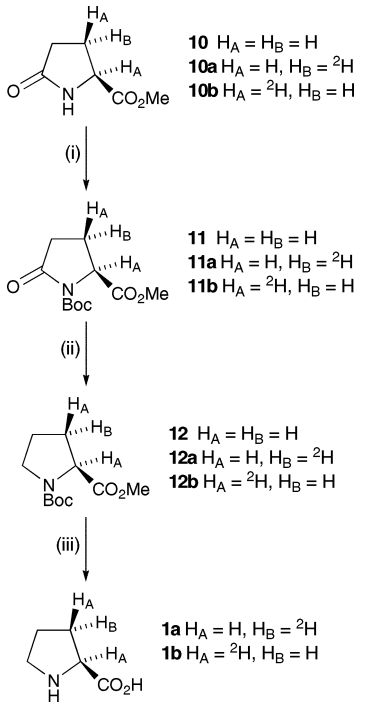
Scheme 1 *Reagents and conditions*: (i) see ref. 7*b*; (ii) *hν*, MeOH, 3 h (96%); (iii) *hν*, MeOH, aq. $NaHCO_3$, 3 h (quantitative).

purified by recrystallisation from dichloromethane and petroleum ether.

In our synthesis of labelled samples of glutamic acid,[7a,b] the diazoketones **6** were photolysed in redistilled methanol to give labelled samples of dimethyl *N*-trifluoroacetylglutamate **8** *via* rearrangement to the ketenes **7** which reacted with the solvent methanol. However, when we carried out the photolysis of the unlabelled diazoketone **6** in methanol which had previously been dried by distillation from magnesium methoxide or calcium hydride, a new product was obtained. This was identified as methyl pyroglutamate **10** by spectroscopic comparison with an authentic sample. When the reaction was repeated using the labelled diazoketones, **6a** and **6b**, stereospecifically labelled samples of methyl pyroglutamate **10a** and **10b** were obtained. The most reasonable explanation for the new course of the reaction was that the solution had become slightly alkaline, leading to hydrolysis of the trifluoroacetate to give the primary amine **9**. The amine would then undergo intramolecular nucleophilic addition to the ketene function, yielding the pyroglutamate **10**. In neutral conditions the unhydrolysed trifluoroacetamide moiety would not be sufficiently nucleophilic to compete with methanol for the ketene in the intermediate **7** and so the protected dimethyl glutamate **8** would be formed. To test this hypothesis, the diazoketone **6** was hydrolysed to the corresponding free amino acid by adaptation of a literature method.[12] Photolysis of the crude amino acid diazoketone in methanol gave (2*S*)-pyroglutamic acid. However, when this sequence of reactions was conducted using the stereospecifically labelled samples of diazoketone, **6a** and **6b**, the 3*R* and 3*S* deuterium labels (but not the C-2 label) were exchanged. Photolysis in methanol containing sodium methoxide caused exchange of labels both at C-2 and at C-3. Eventually, conditions were found which reliably and consistently gave the stereospecifically labelled methyl pyroglutamates **10a** and **10b** in quantitative yield. This involved photolysis in methanol containing 1 equivalent of 6% w/v aqueous sodium bicarbonate.

The samples of methyl pyroglutamate, **10a** and **10b**, obtained by this latter method, were converted into the corresponding *tert*-butoxycarbonyl protected derivatives **11a** and **11b** by reaction with di-*tert*-butyl dicarbonate and DMAP in acetonitrile, as shown in Scheme 2. Reduction using borane dimethylsulfide then gave the protected proline derivatives **12a** and **12b**. The ^{1}H NMR spectra of the products **12** were complicated by the well-known[13] conformational isomerism found in *N*-acylproline derivatives but, when the products were deprotected using refluxing 6 M aqueous HCl, good yields of the labelled prolines **1a** and **1b** were obtained. The ^{1}H NMR spectra of these compounds, shown in Fig. 1, indicated stereospecific labelling, and so synthesis of (2*S*,3*S*)-[3-^{2}H$_1$]-proline **1a** and (2*S*,3*R*)-[2,3-^{2}H$_2$]-proline **1b** had been achieved.

In our second synthesis, where stereoselectively β-monodeuteriated products were required, we used the silylenol ether **14**, prepared during our work on the synthesis of analogues of kainic acid[14] as starting material. When this silylenol ether was prepared from a solution of the ketone **13**[14] using excess trimethylsilyl chloride and the product was treated *in situ* with ^{2}H$_2$O (Scheme 3), we observed remarkable stereoselectivity in deuteriation at C-3 in the resultant ketone **13a**, since, although the ^{1}H NMR spectrum of the unpurified product was complicated by the conformational isomerism[13] shown by *N*-acylprolines,



Scheme 2 *Reagents and conditions*: (i) O(CO$_2$^{t}Bu)$_2$, DMAP, CH$_3$CN, rt, overnight (71% **11**, 24% **11a**, 26% **11b**); (ii) H$_3$B·SMe$_2$, THF, 0 °C, 18.5 h (65% **12**, 66% **12a**, 56% **12b**); (iii) 6 M HCl, reflux, 2 h (55% **1**, 78% **1a**, 94% **1b**).

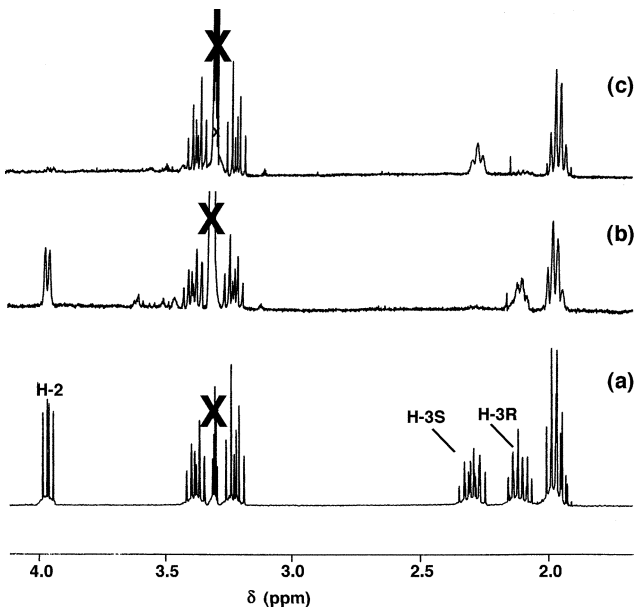
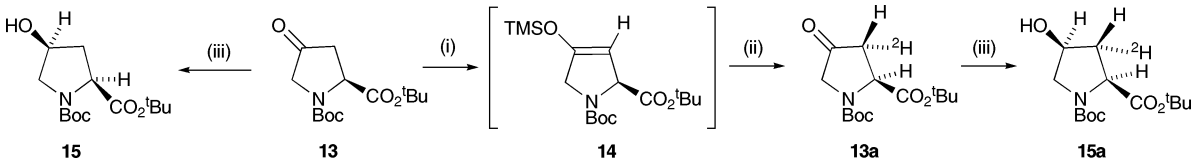


Fig. 1 360 MHz ^{1}H NMR spectra in C^{2}H$_3$O^{2}H of (a) (2*S*)-proline **1**; (b) (2*S*,3*S*)-[3-^{2}H$_1$]-proline **1a**; and (c) (2*S*,3*R*)-[2,3-^{2}H$_2$]-proline **1b**.

only one of the two multiplets at δ 2.50 and 2.89 ppm (due to the diastereotopic protons at C-3) was absent in the ^{1}H NMR spectrum of the ketone **13a**. Since the signal at δ 2.89 ppm showed a 4.5% enhancement upon irradiation of the signal for H-2 at *ca.* δ 4.6 ppm, it was evidently due to H-3*R* and the signal at δ 2.50 ppm was therefore assigned as being due to H-3*S*. Deuteriation had evidently occurred from the *re*-face of the silylenol ether **14**. Attempts to purify the compound by chromatography on silica



Scheme 3 *Reagents and conditions*: (i) (a) LDA, THF, −78 °C; (b) Me_3SiCl, −78 °C; (ii) ^2H_2O, rt (quantitative); (iii) $NaBH_4$, MeOH, Et_2O, 0 °C, 5 min, then rt, 30 min (98% **15** from **13**, 85% **15a** from **13a**).

gel 'washed out' the label. The unpurified compounds **13** and **13a** were reduced directly to the stable alcohols **15** and **15a** respectively using sodium borohydride in methanol and diethyl ether at 0 °C, as shown in Scheme 3. This allowed the stereoselectivity to be confirmed using a purer and more stable compound. The ^1H NMR spectrum of the purified unlabelled compound **15** in C^2HCl_3 was complicated by conformational isomerism but a simpler spectrum was obtained for a solution in C^2H_3CN at 60 °C. NOE experiments in C^2HCl_3, summarised in Fig. 2, allowed assignment of the signals due to the hydrogens H-3*R* and H-3*S*, since irradiation at δ 4.2 ppm (H-2) gave a considerably larger enhancement to the signal for H-3 at δ 2.3 ppm than to that at δ 2.0 ppm. The former signal was therefore assigned as being due to H-3*R*. Irradiation at the signal at δ 4.3 ppm (H-4) gave a large enhancement to the signal for H-3*R*, thus confirming the stereochemistry at C-4. The ^1H NMR spectrum of the labelled alcohol **15a** exhibited a broad one-proton singlet for H-3*S* at δ 2.03 ppm and a residual multiplet (*ca.* 20%) for H-3*R* at δ 2.3 ppm. The stereoselectivity of hydrolysis of the silylenol ether **14** was thus confirmed. No deuterium was present in H-3*S* and the small amount of protium at H-3*R* was presumably the result of an isotope effect.

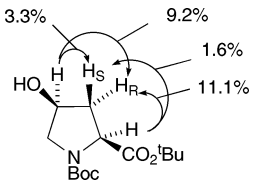
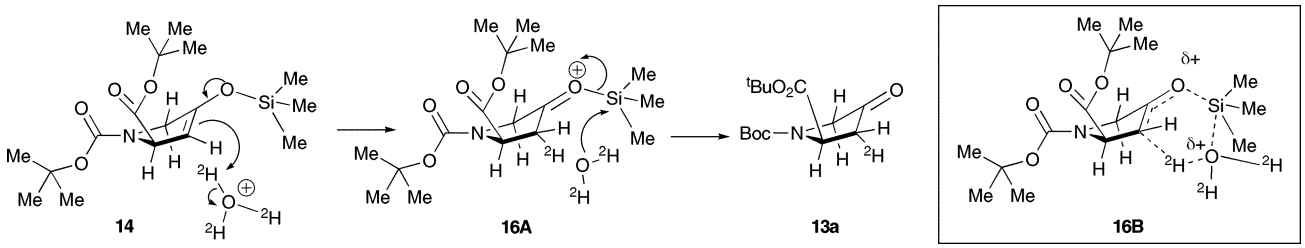


Fig. 2 NOE experiments on the alcohol **15**.

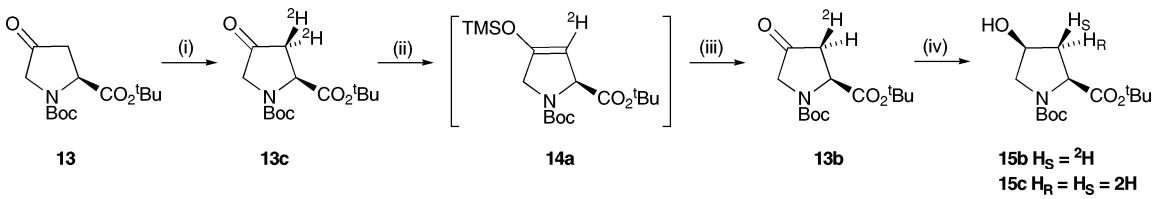
The high stereoselectivity found in this reaction was somewhat surprising and it is tempting to suggest that the mechanism shown in Scheme 4 could be partly synchronous giving the chair-like transition state **16B** shown. Interaction of the bulky trimethylsilyl and *tert*-butoxycarbonyl groups in **16B** would result in deuteriation occurring from the *re*-face as shown.

Reasoning that we might obtain the isotopomer **13b** by protonation of the deuteriated silylenol ether **13a**, we decided to prepare the [3,3-$^2H_2$]-ketone **13c**. We therefore treated the ketone **13** with lithium diisopropylamide (LDA) in tetrahydrofuran and quenched the solution with ^2H_2O. The ^1H NMR spectrum of the product indicated that any label introduced at C-3 had been 'washed out' upon purification using silica gel chromatography but that there was evidently considerable deuteriation (*ca.* 75%) at C-5. We therefore stirred the ketone **13** in ^2H_2O containing silica gel which had been washed with ^2H_2O (Scheme 5). After 10 d at room temperature, complete exchange was obtained and the product **13c** was converted to a solution of the silylenol ether **14a** by reaction with LDA followed by treatment with excess trimethylsilyl chloride. Addition of H_2O to the solution gave the ketone **13b**. The ^1H NMR spectrum of this compound exhibited a signal at δ 2.89 ppm for H-3*R* but not at δ 2.50 ppm for H-3*S*.

Since the ketone **13** had been shown to be so acidic, readily exchanging at C-3, the labelled sample **13b** was not further purified, but reduced immediately with sodium borohydride to give the alcohol **15b**. The ^1H NMR spectrum of the purified alcohol **15b** showed a one-proton multiplet at δ 2.28 ppm for H-3*R* and *ca.* 30% residual protium at δ 2.0 ppm for H-3*S*. The residual protium presumably reflected incomplete deuteriation in the initial dideuteriated compound.

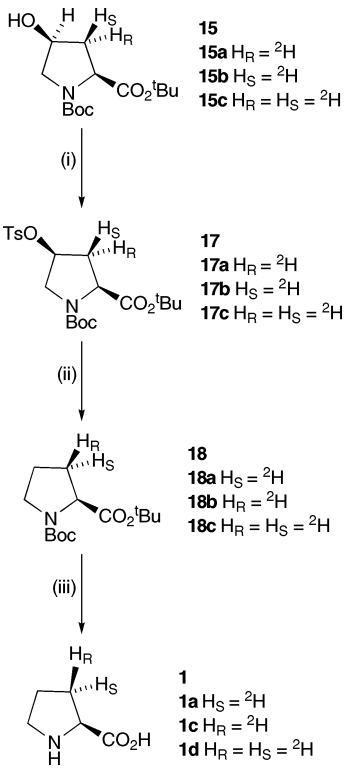


Scheme 4



Scheme 5 *Reagents and conditions*: (i) ^2H_2O, silica gel, rt, 10 d (93%); (ii) (a) LDA, THF, −78 °C, (b) Me_3SiCl, −78 °C; (iii) H_2O, rt (quantitative); (iv) $NaBH_4$, MeOH, Et_2O, 0 °C, 5 min, then rt, 30 min (40% **15b** from **13b**; 89% **15c** from **13c**).

Conversion of the unlabelled and labelled alcohols **15** to the corresponding samples of proline **1** proceeded as described in Scheme 6. The alcohols **15** were first converted into the *para*-toluenesulfonates **17** using *para*-toluenesulfonyl chloride in pyridine at room temperature. These were then reduced to the protected proline derivatives **18** by heating at 85 °C with $NaBH_4$ in DMSO for 6.5 h. Deprotection using 6 M aqueous hydrochloric acid for 2 h at room temperature gave the samples of proline, **1**, **1a**, **1c** and **1d**. The ^1H NMR spectra of these compounds are shown in Fig. 3, the sample of (2*S*,3*S*)-[3-$^2H_1$]-proline **1a** having 22% protium at H-3*S*, and that of (2*S*,3*R*)-[3-$^2H_1$]-proline **1c** having *ca.* 33% protium at H-3*R*. The same synthetic route was used to convert (2*S*)-[3,3-$^2H_2$]-4-ketoproline **15c** to (2*S*)-[3,3-$^2H_2$]-proline **1d**.



Scheme 6 *Reagents and conditions*: (i) TsCl, pyridine, rt, 20 h (58% **17**, 33% **17a**, 71% **17b**, 50% **17c**); (ii) $NaBH_4$, DMSO, 85 °C, 6.5 h (94% **18**, 87% **18a**, 59% **18b**, 89% **18c**); (iii) 6 M HCl, rt, 2 h (88% **1**, 27% **1a**, 71% **1c**, 53% **1d**).

Conclusions

Two distinct and separate syntheses of samples of L-proline which are stereospecifically deuteriated in the β-position have been completed. The first, a chemico-enzymatic synthesis, provided samples of (2*S*,3*S*)-[3-$^2H_1$]- and (2*S*,3*R*)-[2,3-$^2H_2$]-proline, **1a** and **1b**, while the second, a chemical synthesis from a homochiral 4-ketoproline derivative, provided samples of (2*S*,3*S*)- and (2*S*,3*R*)-[3-$^2H_1$]-proline, **1a** and **1c**.

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 1dm path length micro cell. IR spectra were recorded on a Perkin Elmer 1710 Fourier transform instrument. ^1H NMR spectra were recorded on Bruker WM360 (360 MHz), AMX500 (500 MHz) and AC-P250 (250 MHz) instruments. ^{13}C NMR spectra were recorded on Bruker AMX 500 (125.8 MHz) WM360 (90.6 MHz) and AC-P250 (62.9 MHz) instruments. *J* values are given in Hz. Residual solvent peaks were used as internal references in all NMR spectra. Low resolution mass spectra were recorded at Sussex University using Kratos MS80RF and Fisons VG Autospec instruments by Mr A. M. Greenway and Dr A. Abdul Sada, and at the Wellcome Research Laboratories, Beckenham using Kratos Concept 1S and MS50, and Fisons BIO Q instruments by Dr S. Chotai. High resolution mass measurements were performed by Dr S. Chotai at the Wellcome Research Laboratories, Beckenham using a Kratos Concept 1S instrument. Microanalyses were performed by Miss M. Patel at Sussex University, and by Ms C. Lawless and W. C. Man at the Wellcome Research Laboratories, Beckenham. Column chromatography was carried out using Merck Kieselgel 60 (230–400 mesh-Art 9385). Unless otherwise indicated, petroleum ether refers to that fraction of hexanes of bp 60–80 °C.

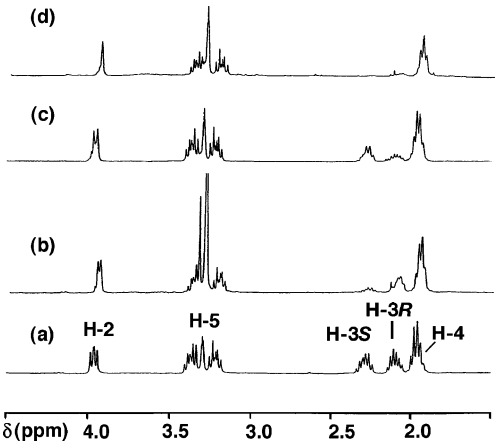


Fig. 3 360 MHz ^1H NMR spectra in $C^2H_3O^2H$ of (a) (2*S*)-proline **1**; (b) (2*S*,3*S*)-[3-$^2H_1$]-proline **1a**; (c) (2*S*,3*R*)-[3-$^2H_1$]-proline **1c**; and (d) (2*S*)-[3,3-$^2H_2$]-proline **1d**.

Dimethyl (2*S*)-*N*-trifluoroacetylglutamate (8)

Methyl (2*S*)-5-diazo-4-oxo-trifluoroacetamidopentanoate **6**[7b] (500 mg, 1.87 mmol) was dissolved in methanol (200 ml) that had been purified by fractional distillation. The solution was degassed under a rapid stream of dry nitrogen for 30 min and then irradiated using a 125 W medium pressure Hanovia UV lamp fitted with a quartz filter. The reaction was shown to be complete after 3 h by UV spectroscopy. The solvent was removed *in vacuo* to give dimethyl (2*S*)-*N*-trifluoroacetylglutamate **8** (460 mg, 96%) as a brown oil with identical spectra to those of an authentic sample.

Methyl (2*S*)-pyroglutamate (10)

Methyl (2*S*)-5-diazo-4-oxo-2-trifluoroacetamidopentanoate **6**[7b] (3 g, 11.2 mmol) was added to a mixture of methanol (100 ml) and aqueous sodium hydrogencarbonate (6% w/v, 1.56 ml). Further

methanol (500 ml) was added and the solution was degassed using a rapid stream of dry argon with continuous stirring for 45 min. Precipitated bicarbonate had dissolved after this time and the mixture was irradiated using a 125 W Hanovia medium pressure UV lamp fitted with a quartz filter until no starting material could be detected by UV spectroscopy (*ca.* 3 h). The solvent was removed *in vacuo* to give methyl (2*S*)-pyroglutamate **10** as a viscous yellow oil after thorough drying (1.6 g, quant.). A sample was purified by column chromatography on silica gel by sequential elution with ethyl acetate–petroleum ether (1 : 2); then ethyl acetate; then ethyl acetate–methanol (9 : 1) to yield methyl (2*S*)-pyroglutamate **10** as a pale yellow oil; $[\alpha]_D^{22}$ −2.3 (*c* 1, CH_2Cl_2) [lit.[15] −6.95 (*c* 1, CH_2Cl_2)]; ν_{max} (film)/cm^{-1} 3300 (br, NH), 1742 (ester) and 1698 (lactam); δ_H (360 MHz, C^2HCl_3) 2.18–2.39 (4H, m, H-3 and H-4), 3.76 (3H, s, OCH_3), 4.26 (1H, dd, $J_{2,3R}$ 5, $J_{2,3S}$ 9, H-2) and 6.40 (1H, br s, NH, exch. in ^2H_2O); δ_C (90.6 MHz, $C^2H_3O^2H$) 26.2 (C-3), 30.6 (C-4), 53.4 (OCH_3), 57.3 (C-2), 174.9 (ester) and 181.0 (lactam).

Methyl (2*S*,3*S*)-[3-$^2H_1$]-pyroglutamate (10a)

This was prepared from methyl (2*S*,3*R*)-[3-$^2H_1$]-5-diazo-4-oxo-2-trifluoroacetamidopentanoate **6a**[7b] (3.1 g, 11.6 mmol) as described above (1.67 g, quant.). A sample was purified by chromatography as described above; ν_{max} (film)/cm^{-1} 3380 (br, NH), 1730 (br, ester) and 1686 (lactam); δ_H (360 MHz, C^2HCl_3) 2.18–2.37 (3H, m, H-3*R* and H-4), 3.75 (3H, s, OCH_3), 4.23 (1H, d, $J_{2,3R}$ 5, H-2) and 6.60 (1H, br s, NH); δ_C (62.9 MHz, $C^2H_3O^2H$) 26.0 (t, C-3), 30.6 (C-4), 53.4 (OCH_3), 57.4 (C-2), 174.9 (ester) and 181.5 (lactam).

Methyl (2*S*,3*R*)-[2,3-$^2H_2$]-pyroglutamate (10b)

This was prepared from methyl (2*S*,3*S*)-[2,3-$^2H_2$]-5-diazo-4-oxo-2-trifluoroacetamidopentanoate **6b**[7b] (3.6 g, 13.4 mmol) as described above (1.88 g, 97%). A sample was purified by chromatography as described above; ν_{max} (film)/cm^{-1} 3380 (br, NH), 1730 (ester) and 1681 (lactam); δ_H (360 MHz, C^2HCl_3) 2.30–2.50 (3H, m, H-3 and H-4), 3.77 (3H, s, OCH_3) and 6.1 (1H, br s, NH); δ_C (90.6 MHz, $C^2H_3O^2H$) 25.8 (t, C-3), 30.6 (C-4), 53.3 (OCH_3), 57.0 (t, C-2), 174.9 (ester) and 181.4 (lactam).

Methyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate (11)

Crude methyl (2*S*)-pyroglutamate **10** from photolysis of the diazoketone **6** (1.6 g, 11.2 mmol) was dissolved in acetonitrile (50 ml) at 0 °C under nitrogen. DMAP (122 mg, 1 mmol) was added and the solution was stirred at room temperature for 10 min. Di-*tert*-butyl dicarbonate (3.17 g, 14.5 mmol) was added and stirring was continued overnight at room temperature. The solvent was removed *in vacuo* to afford an orange gum which was dissolved in ethyl acetate (50 ml). The organic layer was washed with saturated aqueous sodium hydrogen carbonate (2 × 20 ml), 10% aqueous citric acid (2 × 20 ml) and saturated aqueous sodium chloride (20 ml), and dried ($MgSO_4$). The solvent was removed *in vacuo* to give an orange solid which was purified by column chromatography on silica gel, eluting with diethyl ether to yield methyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **11** as a white solid (2.73 g, 71%); mp 69 °C (lit.[16] 72.5–73.5 °C); $[\alpha]_D^{22}$ −30 (*c* 0.36, $CHCl_3$) [lit.[16] −30.4 (*c* 1.0, $CHCl_3$)]; (Found C, 54.3; H, 7.0; N, 5.7. $C_{11}H_{17}NO_5$ requires C, 54.3; H, 7.0; N, 5.8%); *m*/*z* [+ve FAB, ($CHCl_3$/NBA)], 244 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1760 (imide), 1741 (ester) and 1705 (urethane); δ_H (360 MHz, C^2HCl_3) 1.42 [9H, s, $C(CH_3)_3$], 1.90 (1H, dddd, $J_{3R,3S}$ 13.3, $J_{3R,2}$ 3.0, $J_{3R,4R}$ 10.0, $J_{3R,4S}$ 3.7, H-3*R*), 2.30 (1H, m, $J_{3S,3R}$ 13.3, $J_{3S,2} \approx J_{3S,4R} \approx J_{3S,4S} \approx$ 9.4, H-3*S*), 2.40 (1H, ddd, $J_{4S,4R}$ 17.5, $J_{4S,3S}$ 9.4, $J_{4S,3R}$ 3.7, H-4*S*), 2.60 (1H, m, $J_{4R,4S}$ 17.5 $J_{4R,3S} \approx J_{4R,3R} \approx$ 10.0, H-4*R*), 3.72 (3H, s, OCH_3) and 4.55 (1H, dd, $J_{2,3S}$ 9.4, $J_{2,3R}$ 3.0, H-2); irradiation at δ 4.55 ppm (H-2) gave 3.5% enhancement at δ 2.30 ppm (H-3*S*) and 1% enhancement at δ 1.90 ppm (H-3*R*); δ_C (125.8 MHz, C^2HCl_3) 21.5 (C-3), 27.9 [$C(CH_3)_3$], 31.1 (C-4), 52.5 (OCH_3), 58.8 (C-2), 83.6 [$OC(CH_3)_3$], 149.3 (urethane), 171.8 (ester) and 172.9 (lactam).

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

This was prepared by as described above from methyl (2*S*,3*S*)-[3-$^2H_1$]-pyroglutamate **10a** (4.3 g, 29.8 mmol) (1.73 g, 24%); mp 68–70 °C; $[\alpha]_D^{22}$ −31.2 (*c* 0.35, $CHCl_3$); *m*/*z* [+ve FAB, ($CHCl_3$/NBA)], 245 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1759 (imide), 1742 (ester) and 1704 (urethane); δ_H (360 MHz, C^2HCl_3) 1.49 [9H, s, $C(CH_3)_3$], 2.0 (1H, br d, $J_{3R,4R}$ 9.6, H-3*R*), 2.50 (1H, dd, $J_{4S,4R}$ 17.5, $J_{4S,3R}$ 3.6, H-4*S*), 2.60 (1H, dd, $J_{4R,4S}$ 17.5, $J_{4R,3R}$ 9.6, H-4*R*), 3.79 (3H, s, OCH_3) and 4.60 (1H, d, $J_{2,3R}$ 2.8, H-2); δ_C (125.8 MHz, C^2HCl_3) 21.1 (t, C-3), 27.8 [$C(CH_3)_3$], 30.9 (C-4), 52.4 (OCH_3), 58.7 (C-2), 83.5 [$OC(CH_3)_3$], 149.2 (urethane), 171.8 (ester) and 172.9 (lactam).

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

This was prepared as described above from methyl (2*S*,3*R*)-[2,3-$^2H_2$]-pyroglutamate **10b** (5.59 g, 38.5 mmol) as a solid (2.45 g, 26%); mp 69–70 °C; $[\alpha]_D^{22}$ −31.5 (*c* 0.42, $CHCl_3$); *m*/*z* [+ve FAB, ($CHCl_3$/NBA)], 246 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1759 (imide), 1740 (ester) and 1704 (urethane); δ_H (360 MHz, C^2HCl_3) 1.47 [9H, s, $C(CH_3)_3$] 2.20 (1H, br t, *J* 10, H-3*S*), 2.50 (1H, dd, $J_{4S,4R}$ 17.5, $J_{4S,3S}$ 9.4, H-4*S*), 2.60 (1H, dd, $J_{4R,4S}$ 17.5, $J_{4R,3S}$ 10.2, H-4*R*) and 3.77 (3H, s, OCH_3); δ_C (125.8 MHz, C^2HCl_3) 21.1 (t, C-3), 27.8 [$C(CH_3)_3$], 30.9 (C-4), 52.3 (OCH_3), 58.4 (t, C-2), 83.4 [$OC(CH_3)_3$], 149.2 (urethane), 171.7 (ester) and 172.9 (lactam).

Methyl (2*S*)-*N*-*tert*-butoxycarbonylprolinate (12)

Methyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **11** (180 mg, 0.74 mmol) was dissolved in dry tetrahydrofuran (10 ml) and the solution was cooled to 0 °C. Borane dimethylsulfide (2 M in tetrahydrofuran, 0.7 ml, 1.4 mmol) was added *via* syringe and the mixture was stirred for 18.5 h. The solvent was removed *in vacuo* and the crude oily product was dissolved in ethyl acetate (20 ml). The organic layer was washed with water (2 × 10 ml) and saturated aqueous sodium chloride (10 ml), dried ($MgSO_4$), filtered and the solvent was removed *in vacuo* to yield methyl (2*S*)-*N*-*tert*-butoxycarbonylprolinate **12** as a clear oil (110 mg, 65%); $[\alpha]_D^{22}$ −61.7 (*c* 1.15, MeOH) [lit.[17] −68.3 (*c* 1.08, MeOH) for the [4,4-$^2H_2$]-labelled compound]; *m*/*z* [FAB/3-NBA] 252 ([M + Na]$^+$) and 230 ([M + H]$^+$); *m*/*z* [EI], 170 ([M − CO_2CH_3]$^+$); ν_{max} (film)/cm^{-1} 1751 (ester) and 1703 (urethane); δ_H (360 MHz, C^2HCl_3) 1.36 and 1.42 [9H, 2s, $C(CH_3)_3$], 1.80 (3H, m, H-3*R* and H-4), 2.20 (1H, m, H-3*S*), 3.40 (2H, m, H-5), 3.70 (3H, s, OCH_3), and 4.18 and 4.28 (1H, 2dd, H-2); δ_C (125.8 MHz, C^2HCl_3) 23.6 and 24.2 (C-4), 28.2 and 28.3 [$C(CH_3)_3$], 29.8 and 30.8 (C-3), 46.2 and 46.5 (C-5), 52.0

(OCH_3), 58.6 and 59.0 (C-2), 79.7 and 79.9 [O*C*(CH_3)$_3$], 153.7 and 154.3 (urethane), and 173.4 and 173.7 (ester).

Methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonylprolinate (12a)

This was prepared as above from methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonylpyroglutamate **11a** (74 mg, 0.30 mmol) and purified by column chromatography on silica gel, eluting with 1 : 1 ethyl acetate–petroleum ether (40–60 °C) (46 mg, 66%); $[\alpha]_D^{23}$ −58.2 (*c* 0.98, MeOH); *m*/*z* [EI], 171 [M − CO_2CH_3]$^+$; ν_{max} (film)/cm^{-1} 1751 (ester) and 1703 (urethane); δ_H (360 MHz, C^2HCl_3) 1.37 and 1.43 [9H, 2s, C(CH_3)$_3$], 1.80 (3H, m, H-3*R* and H-4), 3.40 (2H, m, H-5), 3.69 (3H, s, OCH_3) and 4.28 and 4.31 (1H, 2br s, H-2); δ_C (125.8 MHz, C^2HCl_3) 23.5 and 24.1 (C-4), 28.2 and 28.3 [C(*C*H_3)$_3$], 29.0 and 30.0 (t, C-3), 46.2 and 46.5 (C-5), 52.0 (OCH_3), 58.6 and 59.0 (C-2), 79.7 and 79.9 [O*C*(CH_3)$_3$], 153.7 and 154.4 (urethane) and 173.4 and 173.7 (ester).

Methyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonylprolinate (12b)

This was prepared as above from methyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonylpyroglutamate **11b** (80 mg, 0.326 mmol) and purified by column chromatography on silica gel, eluting with 1 : 1 ethyl acetate–petroleum ether (40–60 °C) (42 mg, 56%); $[\alpha]_D^{25}$ −56.5 (*c* 0.96, MeOH); *m*/*z* [EI], 172 ([M − CO_2CH_3]$^+$); ν_{max} (film)/cm^{-1} 1751 (ester) and 1703 (urethane); δ_H (360 MHz, C^2HCl_3) 1.39 and 1.44 [9H, 2s, C(CH_3)$_3$], 1.80 (2H, m, H-4), 2.20 (1H, m, H-3*S*), 3.40 (2H, m, H-5) and 3.70 (3H, s, OCH_3); δ_C (125.8 MHz, C^2HCl_3) 23.5 and 24.2 (C-4), 28.2 and 28.4 [C(*C*H_3)$_3$], 29.1 and 30.4 (t, C-3), 46.2 and 46.5 (C-5), 52.0 (OCH_3), 58.6 (m, C-2), 79.7 and 79.8 [O*C*(CH_3)$_3$], 153.7 and 154.4 (urethane), and 173.5 and 173.7 (ester).

(2*S*)-Proline (1)

Methyl (2*S*)-*N*-*tert*-butoxycarbonylprolinate **12** (98 mg, 0.427 mmol) was heated at reflux with 6 M aqueous HCl (2 ml) for 2 h. The product was dried by lyophilisation and excess water was removed by vacuum desiccation. The residue was dissolved in water, the pH was adjusted from 2 to 7 with 2 M aqueous ammonium hydroxide, and the solution was loaded onto a Dowex 50X8 ion exchange column. The column was washed with water until the eluant was no longer acidic. The product **1** was eluted with 2 M aqueous ammonium hydroxide, dried by lyophillisation and obtained as an off-white solid (30 mg, 55%); mp 229 °C (decomp.); (lit.[15] 227–229 °C); $[\alpha]_D^{25}$ −82.0 (*c* 0.72, H_2O) [lit.[15] −83 (*c* 1, H_2O)]; *m*/*z* [+ve FAB (thioglycerol)], 231 ([2M + H]$^+$), 138 ([M + Na]$^+$) and 116 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3400 (br, NH and OH) and 1622 (br, carboxylate); δ_H (360 MHz, $C^2H_3O^2H$) 1.96 (2H, m, H-4), 2.13 (1H, m, H-3*R*), 2.28 (1H, m, H-3*S*), 3.23 (1H, m, H-5A), 3.36 (1H, m, H-5B) and 3.95 (1H, dd, $J_{2,3R}$ 6.2, $J_{2,3S}$ 8.7, H-2); δ_C (62.9 MHz, $C^2H_3O^2H$) 25.1 (C-4), 30.4 (C-3), 47.0 (C-5), 62.6 (C-2) and 174.2 (acid).

(2*S*,3*S*)-[3-$^2H_1$]-Proline (1a)

This was prepared as above from methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonylprolinate **12a** (46 mg, 0.20 mmol) (20 mg, 78%); mp 230 °C (decomp.); $[\alpha]_D^{25}$ −83.2 (*c* 0.1, H_2O); *m*/*z* [+ve FAB (thioglycerol)], 117 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3400 (br, NH and OH) and 1626 (carboxylate); δ_H (360 MHz, $C^2H_3O^2H$) 1.95 (2H, m, H-4), 2.20 (1H, m, H-3*R*), 3.23 (1H, m, H-5A), 3.36 (1H, m, H-5B) and 3.94 (1H, d, $J_{2,3R}$ 6.2, H-2); δ_C (62.9 MHz, $C^2H_3O^2H$) 25.4 (C-4), 30.3 (m, C-3), 47.4 (C-5), 63.0 (C-2) and 174.0 (acid).

(2*S*,3*R*)-[2,3-$^2H_2$]-Proline (1b)

This was prepared as above from methyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-*tert*-butoxycarbonylprolinate **12b** (42 mg, 0.18 mmol) (22 mg, 94%); mp 230 °C (decomp.); $[\alpha]_D^{26}$ −85.2 (*c* 0.20, H_2O); *m*/*z* [+ve FAB (thioglycerol)] 140 ([M + Na]$^+$) and 118 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3400 (br, NH and OH) and 1626 (carboxylate); δ_H (360 MHz, $C^2H_3O^2H$) 1.95 (2H, m, H-4), 2.20 (1H, br t, H-3*S*), 3.23 (1H, m, H-5A) and 3.36 (1H, m, H-5B); δ_C (62.9 MHz, $C^2H_3O^2H$) 24.0 (C-4), 28.9 (t, C-3), 46.0 (C-5) and 172.9 (acid). The signal for C-2 was too weak to detect.

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-oxoprolinate (13)

This was prepared as described in ref. 14; δ_H (360 MHz, C^2HCl_3), 1.42 [18H, 2s, C(*C*H_3)$_3$], 2.5 (1H, d, $J_{3S,3R}$ 18.8, H-3*S*), 2.89 (1H, m, $J_{3R,2}$ 10.4, $J_{3R,3S}$ 18.8, H-3*R*), 3.85 (2H, m, H-5) and 4.55 and 4.63 (1H, 2 × br d, $J_{2,3R}$ 10.4, H-2); δ_C (125.8 MHz, C^2HCl_3), 27.88 and 28.23 [C(*C*H_3)$_3$], 40.87 and 41.40 (C-3), 52.51 and 52.95 (C-5), 56.56 and 57.03 (C-2), 81.03 and 82.36 [O*C*(CH_3)$_3$], 153.65 and 154.34 (urethane), 170.84 and 170.92 (ester) and 208.18 and 209.0 (ketone). Irradiation at the two doublets for H-2 at δ 4.55 and 4.63 ppm gave a 4.5% enhancement to the multiplet at δ 2.89 ppm, thus defining this as the signal due to H-3*R*.

tert-Butyl (2*S*,3*R*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-oxoprolinate (13a)

Diisopropylamine (2.9 ml, 20.7 mmol) was dissolved in dry tetrahydrofuran (50 ml) and cooled to −78 °C under nitrogen. *n*-Butyl lithium (1.6 M in hexane, 11.6 ml, 18.56 mmol) was added and the solution was stirred for 30 min. A solution of *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-oxoprolinate **13** (5 g, 17.5 mmol) in dry tetrahydrofuran (50 ml) was added and the mixture was stirred for 1 h at −78 °C. Chlorotrimethylsilane (3.8 ml, 297 mmol) was added slowly over a period of 5 min. The mixture was allowed to warm to room temperature, stirred for 1 h and deuterium oxide (15 ml) was added. The organic solvents were removed *in vacuo* and the aqueous residue was extracted with ethyl acetate (3 × 50 ml) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give *tert*-butyl (2*S*,3*R*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-oxoprolinate **13a** (5.27 g, quant.) as a pale yellow oil which was reduced without further purification as described below; ν_{max} (film)/cm^{-1} 1769 (ketone), 1742 (ester) and 1708 (urethane); δ_H (360 MHz, C^2HCl_3) 1.42 [18H, br s, C(CH_3)$_3$], 2.5 (1H, s, H-3*S*), 3.8 (2H, m, H-5) and 4.52 and 4.6 (1H, 2s, H-2).

tert-Butyl (2*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-oxoprolinate (13c)

Deuterium oxide (15 ml) and silica gel prewashed with deuterium oxide (3 × 10 ml) were added to a solution of *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-oxoprolinate **13** (6 g, 21 mmol) in dry tetrahydrofuran (15 ml). The mixture was stirred at room temperature for 10 days under nitrogen, filtered, and the filtrate

was concentrated *in vacuo*. The aqueous phase was extracted with ethyl acetate (3 × 50 ml) and the combined organic layers were washed with saturated aqueous sodium chloride (50 ml) and dried ($MgSO_4$). The solvent was removed *in vacuo* to afford *tert*-butyl (2*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-oxoprolinate **13c** as a colourless oil (5.6 g, 93%) which was not further purified; δ_H (360 MHz, C^2HCl_3) 1.46 and 1.47 [18H, 2s, $C(CH_3)_3$], 3.86 (2H, m, H-5) and 4.57 (50%) and 4.65 (50%) (1H, 2 br s, H-2); δ_C (125.8 MHz, C^2HCl_3) 27.85 [$C(CH_3)_3$], 28.19 [$C(CH_3)_3$], 40.93 (m, C-3), 52.49 and 52.92 (C-5), 56.44 and 56.90 (C-2), 80.97 and 82.31 [$OC(CH_3)_3$], 153.63 and 154.31 (urethane), 170.82 and 170.91 (ester) and 208.23 and 209.0 (ketone).

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

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-oxoprolinate **13** (4.8 g, 17 mmol) was dissolved in distilled methanol (51 ml) and dry diethyl ether (66 ml) and the solution was cooled to 0 °C under nitrogen. Sodium borohydride (1.32 g, 35 mmol) in distilled methanol (51 ml) and dry diethyl ether (66 ml) was added and the reaction was stirred at 0 °C for 5 min and allowed to warm to room temperature for 30 min. The solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate (60 ml) and washed with saturated aqueous sodium hydrogen carbonate (2 × 60 ml), water (2 × 60 ml) and saturated aqueous sodium chloride (2 × 60 ml) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give *tert*-butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-hydroxyprolinate **15** as a white solid (4.77 g, 98%); mp 53–55 °C; $[\alpha]_D^{34}$ −2.52 (*c* 0.5, $CHCl_3$); (Found C, 58.6; H, 9.0; N, 4.85. $C_{14}H_{25}NO_5$ requires C, 58.5; H, 8.8; N, 4.9%); *m*/*z* [+ve FAB (3-NBA)] 288 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1745 (ester) and 1674 (urethane); δ_H (500 MHz, C^2HCl_3) 1.45 [18H, 3s, $C(CH_3)_3$], 2.0 (1H, 2d, $J_{3S,3R}$ 11.5, H-3*S*), 2.3 (1H, m, $J_{3R,4}$ 9.6, $J_{3R,2}$ 9.8, $J_{3R,3S}$ 11.5, H-3*R*), 3.47–3.75 (3H, m, 1H exch., $J_{5R,4}$ 4.6, $J_{5R,5S}$ 11.9, $J_{5S,5R}$ 11.9, OH and H-5), 4.16 (63%) and 4.22 (37%) (1H, 2d, $J_{2,3R}$ 9.8, H-2) and 4.3 (1H, m, $J_{4,5R}$ 4.6, $J_{4,3R}$ 9.6, H-4); irradiation at δ 4.16 and 4.22 ppm (H-2) caused an 11.1% enhancement at δ 2.3 ppm and a 1.6% enhancement at δ 2.0 ppm, indicating that the former was due to H-3*R*; irradiation at δ 4.3 ppm (H-4) caused a 9.2% enhancement at δ 2.3 ppm (H-3*R*) and a 3.3% enhancement at δ 2.0 ppm (H-3*S*), confirming the stereochemistry at C-4; δ_H (360 MHz, C^2H_3CN, 60 °C) 1.45 [18H, 2s, $C(CH_3)_3$], 1.89 (1H, m, $J_{3S,5A}$ 1.3, $J_{3S,4}$ 3.5, $J_{3S,2}$ 3.4 $J_{3S,3R}$ 13.6, H-3*S*), 2.35 (1H, m, $J_{3R,4}$ 5.4, $J_{3R,2}$ 9.4 $J_{3R,3S}$ 13.6, H-3*R*), 3.08 (1H, d, exch., $J_{OH,4}$ 6.3, OH), 3.28 (1H, ddd, $J_{5A,3S}$ 1.3, $J_{5A,4}$ 3.1, $J_{5A,5B}$ 11.4, H-5A) 3.55 (1H, dd, $J_{5B,4}$ 5.3, $J_{5B,5A}$ 11.4, H-5B), 4.13 (1H, dd, $J_{2,3S}$ 3.5, $J_{2,3R}$ 9.4, H-2) and 4.28 (1H, m, H-4); δ_C (125.8 MHz, C^2HCl_3) 27.80 and 27.87 [$C(CH_3)_3$], 28.30 and 28.45 [$C(CH_3)_3$], 37.67 and 38.64 (C-3), 55.67 and 56.08 (C-5), 58.72 and 58.75 (C-2), 70.33 and 71.31 (C-4), 80.08 and 80.24 [$OC(CH_3)_3$], 82.32 and 82.50 [$OC(CH_3)_3$], 153.82 and 154.33 (urethane) and 174.3 and 174.53 (ester).

tert-Butyl (2*S*,3*R*,4*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-hydroxyprolinate (15a)

This was prepared from *tert*-butyl (2*S*,3*R*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-oxoprolinate **13a** (4.8 g, 17 mmol) as described above to afford a white solid (4.17 g, 85%). A small amount was purified for analysis by column chromatography on silica gel, eluting with ethyl acetate–petroleum ether (1 : 1); mp 55–57 °C; $[\alpha]_D^{32}$ −4.38 (*c* 0.5, $CHCl_3$); *m*/*z* [+ve FAB (EtOAc/3-NBA)] 289 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1748 (ester) and 1654 (urethane); δ_H (360 MHz, C^2HCl_3) 1.48 [18H, 3s, $C(CH_3)_3$], 2.03 (1H, br s, H-3*S*), 3.46–3.79 (3H, m, 1H exch., OH and H-5), 4.19 (1H, 2s, H-2) and 4.3 (1H, br d, H-4); δ_C (125.8 MHz, C^2HCl_3) 27.79 and 27.85 [$C(CH_3)_3$], 28.29 [$C(CH_3)_3$], 38.3 (m, C-3), 55.61 and 56.02 (C-5), 58.67 and 58.3 (C-2), 70.21 and 71.2 (C-4), 80.05 and 80.22 [$OC(CH_3)_3$], 82.27 and 82.45 [$OC(CH_3)_3$], 153.82 and 154.31 (urethane) and 174.22 and 174.45 (ester).

tert-Butyl (2*S*,3*S*,4*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-hydroxyprolinate (15b)

Diisopropylamine (2.19 ml, 16 mmol) was dissolved in dry tetrahydrofuran (38 ml) and cooled to −78 °C under nitrogen. *n*-Butyl lithium (1.6 M in hexane; 8.76 ml, 14 mmol) was added and the solution was stirred for 30 min. A solution of *tert*-butyl (2*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-oxoprolinate **13c** (3.9 g, 14 mmol) in dry tetrahydrofuran (38 ml) was added and the mixture was stirred for 1 h at −78 °C. Chlorotrimethylsilane (2.87 ml, 22 mmol) was added slowly over a period of 5 min. The reaction was allowed to warm to room temperature, stirred for 1 h and poured into saturated aqueous ammonium chloride (50 ml). The solvent was concentrated *in vacuo*. The aqueous layer was extracted with ethyl acetate (3 × 50 ml) and dried ($MgSO_4$). The solvent was removed *in vacuo* to produce (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-oxoprolinate **13b** (3.9 g, quant.) as a pale yellow oil which was reduced without purification using the method described above. The product was purified by column chromatography on silica gel, eluting with ethyl acetate–petroleum ether (1 : 1) to give *tert*-butyl (2*S*,3*S*,4*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-hydroxyprolinate **15b** as a colourless oil (1.59 g, 40%); $[\alpha]_D^{32}$ −6.32 (*c* 0.5, $CHCl_3$); *m*/*z* [ES+] 327 ([M + K]$^+$), 311 ([M + Na]$^+$) and 289 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1745 (ester) and 1674 (urethane); δ_H (360 MHz, C^2HCl_3) 1.46 [18H, 3s, $C(CH_3)_3$], 2.28 (1H, m, H-3*R*), 3.47–3.75 (3H, m, 1H exch., OH and H-5), 4.1–4.3 (H-2 and H-4); δ_C (125.8 MHz, C^2HCl_3) 27.92 [$C(CH_3)_3$], 28.34 [$C(CH_3)_3$], 38.2 (m, C-3), 55.79 and 56.9 (C-5), 58.75 (C-2), 70.38 and 71.37 (C-4), 80.3 and 80.29 [$OC(CH_3)_3$], 82.39 and 82.58 [$OC(CH_3)_3$], 153.87 (urethane) and 174.36 (ester).

tert-Butyl (2*S*,4*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-hydroxyprolinate (15c)

tert-Butyl (2*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-oxoprolinate **13c** (2.55 g, 8.9 mmol) was reduced according to the method described above to yield *tert*-butyl (2*S*,4*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-hydroxyprolinate **15c** as a colourless oil (2.28 g, 89%), *m*/*z* [+ve FAB (3-NBA)] 290 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3430 (OH), 1745 (ester) and 1703 (br, urethane); δ_H (360 MHz, C^2HCl_3) 1.5 [18H, 3s, $C(CH_3)_3$], 3.49–3.77 (3H, m, 1H exch., OH and H-5), 4.21 (1H, 2s, H-2) and 4.3 (1H, m, H-4); δ_C (125.8 MHz, C^2HCl_3) 27.90 and 28.3 [$C(CH_3)_3$], 38.4 (m, C-3), 55.67 and 56.08 (C-5), 58.72 and 58.75 (C-2), 70.33 and 71.31 (C-4), 80.08 and 80.24 [$OC(CH_3)_3$], 82.32 and 82.50 [$OC(CH_3)_3$], 153.82 and 154.33 (urethane) and 174.3 and 174.53 (ester).

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-*O*-*para*-toluenesulfonyloxyprolinate (17)

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-hydroxyprolinate **15** (4.5 g, 15.7 mmol) was dissolved in pyridine (21 ml, 260 mmol) and cooled to −15 °C under nitrogen. *para*-Toluenesulfonyl chloride (10.11 g, 53 mmol) was added over 30 min and the mixture was stirred at room temperature for 20 h. The solvent was removed *in vacuo* and the resultant solid was partitioned between ethyl acetate (60 ml) and water (60 ml). The organic phase was washed with 10% aqueous citric acid (60 ml), water (60 ml) and saturated aqueous sodium chloride (30 ml) and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel, eluting with ethyl acetate–petroleum ether (1 : 2) to afford *tert*-butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-*O*-*para*-toluenesulfonyloxyprolinate **17** as a colourless oil which solidified on standing to give a white solid (3.98 g, 58%); mp 67–70 °C; $[\alpha]_D^{34}$ −28.34 (*c* 0.5, $CHCl_3$); (Found C, 57.0; H, 7.2; N, 3.1. $C_{21}H_{31}NO_7S$ requires C, 57.1; H, 7.1; N, 3.2%); *m*/*z* [+ve FAB (ethyl acetate/3-NBA)] 442 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1707 (urethane); δ_H (360 MHz, C^2HCl_3) 1.5 [18H, 4s, $C(CH_3)_3$], 2.4 (2H, m, H-3), 2.45 (3H, s, CH_3), 3.5 (1H, t, $J_{5A,5B}$ 13.0, H-5A), 3.65 (1H, m, $J_{5B,4}$ 5.5, $J_{5B,5A}$ 13.0, H-5A), 4.2 (54%) and 4.3 (46%) (1H, 2 dd, $J_{2,3S}$ 2.8, $J_{2,3R}$ 9.07, H-2), 5.0 (1H, m, $J_{4,5R}$ 5.5, H-4), 7.35 (2H, 2d, J_{ortho} 8.1, ArH) and 7.76 (1H, 2 d, J_{ortho} 8.1, ArH); δ_C (125.8 MHz, C^2HCl_3) 21.04 and 21.65 (CH_3), 27.79, 28.25 and 28.30 [$C(CH_3)_3$], 36.03 and 37.18 (C-3), 51.81 and 52.25 (C-5), 57.93 and 58.18 (C-2), 78.95 (C-4), 80.21 and 80.31 [$OC(CH_3)_3$], 81.69 and 81.74 [$OC(CH_3)_3$], 127.74–145.08 (Ar), 153.42 and 153.59 (urethane) and 170.09 and 170.40 (ester).

tert-Butyl (2*S*,3*R*,4*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-*O*-*para*-toluenesulfonyloxyprolinate (17a)

This was prepared from *tert*-butyl (2*S*,3*R*,4*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-hydroxyprolinate **15a** (3.96 g, 14 mmol) as described above (2.04 g, 33%); mp 69–71 °C; $[\alpha]_D^{32}$ −23.68 (*c* 0.5, $CHCl_3$); (*m*/*z* [EI] Found 442.1911 $C_{21}H_{30}{}^2HNO_7S$ requires 442.1884); ν_{max} (KBr)/cm^{-1} 1742 (ester) and 1706 (urethane); δ_H (360 MHz, C^2HCl_3) 1.45 [18H, 4s, $C(CH_3)_3$], 2.36 (1H, m, H-3*S*), 2.45 (3H, s, CH_3), 3.6 (2H, m, H-5), 4.2 (54%) and 4.3 (46%) (1H, 2s, H-2), 5.0 (1H, br s, H-4), 7.35 (2H, d, J_{ortho} 7.5, ArH) and 7.76 (1H, d, J_{ortho} 7.5, ArH); δ_C (125.8 MHz, C^2HCl_3) 21.64 (CH_3), 27.81, 28.26 and 28.31 [$C(CH_3)_3$], 37.18 (m, C-3), 51.81 and 52.25 (C-5), 57.89 and 58.14 (C-2), 77.93 and 78.74 (C-4), 80.22 and 80.32 [$OC(CH_3)_3$], 81.74 [$OC(CH_3)_3$], 127.75–145.07 (Ar), 153.44 and 153.61 (urethane) and 170.11 and 170.41 (ester).

tert-Butyl (2*S*,3*S*,4*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-*O*-*para*-toluenesulfonyloxyprolinate (17b)

This was prepared from *tert*-butyl (2*S*,3*S*,4*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-hydroxyprolinate **15b** (1.5 g, 5.2 mmol) as described above to give the product as a colourless oil (1.64 g, 71%); $[\alpha]_D^{34}$ −21.48 (*c* 0.25, $CHCl_3$); *m*/*z* [EI] 442 ([M]$^+$) ν_{max} (KBr)/cm^{-1} 1700 (br, urethane); δ_H (360 MHz, C^2HCl_3) 1.5 [18H, 4s, $C(CH_3)_3$], 2.45 (4H, s + m, CH_3 and H-3*R*), 3.53 (1H, m, $J_{5A,4}$ 2.25 $J_{5A,5B}$ 13.0, H-5A), 3.59 (1H, m, $J_{5B,4}$ 5.64, $J_{5B,5A}$ 13.0, H-5B), 4.21 (67%) and 4.33 (33%) (1H, 2d, $J_{2,3R}$ 9.06, H-2), 5.0 (1H, br s, H-4), 7.35 (2H, 2d, J_{ortho} 8.0, ArH) and 7.8 (1H, 2d, J_{ortho} 8.0, ArH); δ_C (125.8 MHz, C^2HCl_3) 21.64 (CH_3), 27.81, 28.26 and 28.31 [$C(CH_3)_3$], 37.18 (m, C-3), 51.81 and 52.25 (C-5), 57.95 and 58.14 (C-2), 77.93 and 78.88 (C-4), 80.22 and 80.32 [$OC(CH_3)_3$], 81.69 and 81.74 [$OC(CH_3)_3$], 127.75–145.07 (Ar), 153.43 and 153.61 (urethane) and 170.1 and 170.4 (ester).

tert-Butyl (2*S*,4*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-*O*-*para*-toluenesulfonyloxyprolinate (17c)

This was prepared from *tert*-butyl (2*S*,4*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-hydroxyprolinate **15c** (2.28 g, 7.9 mmol) as described above (1.75 g, 50%); mp 67–70 °C; *m*/*z* [ES+] 444 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1747 (urethane); δ_H (360 MHz, C^2HCl_3) 1.5 [18H, 4s, $C(CH_3)_3$], 2.45 (3H, s, CH_3), 3.6 (2H, m, H-5), 4.25 (1H, 2s, H-2), 5.0 (1H, br s, H-4), 7.35 (2H, 2d, J_{ortho} 7.5, ArH) and 7.76 (1H, 2d, J_{ortho} 7.5, ArH); δ_C (125.8 MHz, C^2HCl_3) 21.64 (CH_3), 27.80 [$C(CH_3)_3$], 28.25 [$C(CH_3)_3$], 36.0 (C-3), 51.81 and 52.25 (C-5), 57.81 and 58.06 (C-2), 77.89 and 78.83 (C-4), 80.26 and 80.35 [$OC(CH_3)_3$], 81.77 [$OC(CH_3)_3$], 127.75–145.09 (Ar), 153.46 (urethane) and 170.43 (ester).

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonylprolinate (18)

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-*O*-*para*-toluenesulfonyloxyprolinate **17** (3.55 g, 8.05 mmol) was dissolved in dry dimethyl sulfoxide (60 ml). Sodium borohydride (1.87 g, 49 mmol) was added and the mixture was heated under nitrogen at 85 °C for 6.5 h. The mixture was cooled, diluted with water (100 ml) (effervescence) and the aqueous phase was extracted with diethyl ether (3 × 100 ml). The combined organic phases were washed with saturated aqueous sodium chloride (50 ml) and dried ($MgSO_4$). The solvent was removed *in vacuo* to afford *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonylprolinate **18** as a colourless oil (2.04 g, 94%) which was used without further purification. A small amount was purified for analysis by column chromatography on silica gel, eluting with ethyl acetate–petroleum ether (1 : 4); $[\alpha]_D^{22}$ −50.2 (*c* 1, $CHCl_3$); (*m*/*z* [EI] Found 271.17718, $C_{14}H_{25}NO_4$ requires 271.17838); *m*/*z* [ES+] 272 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1742 (ester) and 1703 (urethane); δ_H (250 MHz, C^2HCl_3) 1.45 [18H, 2s, $C(CH_3)_3$], 1.85 (3H, m, H-3*R* and H-4), 2.19 (1H, m, H-3*S*), 3.45 (2H, m, H-5) and 4.15 (1H, m, H-2); δ_C (125.8 MHz, C^2HCl_3) 23.37 and 24.16 (C-4), 27.96 and 28.32 [$C(CH_3)_3$], 28.38 and 29.84 [$C(CH_3)_3$], 30.84 (C-3), 46.26 and 46.47 (C-5), 59.66 (C-2), 79.38 and 79.58 [$OC(CH_3)_3$], 80.78 [$OC(CH_3)_3$], 153.95 and 154.28 (urethane) and 172.29 (ester).

tert-Butyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonylprolinate (18a)

This was prepared from *tert*-butyl (2*S*,3*R*,4*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-*O*-*para*-toluenesulfonyloxyprolinate **17a** (2 g, 4.52 mmol) as described above (1.07 g, 87%); $[\alpha]_D^{33}$ −44.94 (*c* 0.95, $CHCl_3$); *m*/*z* [ES+] 273 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1739 (ester) and 1703 (urethane); δ_H (250 MHz, C^2HCl_3) 1.45 [18H, 2s, $C(CH_3)_3$], 1.85 (3H, m, H-3*R* and H-4), 3.45 (2H, m, H-5) and 4.15 (1H, 2s, H-2).

tert-Butyl (2*S*,3*R*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonylprolinate (18b)

This was prepared from *tert*-butyl (2*S*,3*S*,4*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonyl-4-*O*-*para*-toluenesulfonyloxyprolinate **17b** (1.6 g,

3.6 mmol) as described above (580 mg, 59%); $[\alpha]_D^{33}$ −47.87 (*c* 1, $CHCl_3$); *m/z* [EI] 272 ($[M]^+$); ν_{max} (film)/cm^{-1} 1742 (ester) and 1703 (urethane); δ_H (250 MHz, C^2HCl_3) 1.45 [18H, 2s, $C(CH_3)_3$], 1.85 (2H, m, H-4), 2.19 (1H, m, H-3*S*), 3.45 (2H, m, H-5) and 4.15 (1H, 2d, H-2); δ_C (125.8 MHz, C^2HCl_3) 23.37 and 24.16 (C-4), 27.96 and 28.32 [$C(CH_3)_3$], 28.38 and 29.84 [$C(CH_3)_3$], 30.72 (m, C-3), 46.26 and 46.47 (C-5), 59.66 (C-2), 79.38 and 79.58 [$OC(CH_3)_3$], 80.78 [$OC(CH_3)_3$], 153.95 and 154.28 (urethane) and 172.29 (ester).

tert-Butyl (2*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonylprolinate (18c)

This was prepared from *tert*-butyl (2*S*,4*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonyl-4-*O*-*para*-toluenesulfonyloxyprolinate **17c** (1.75 g, 3.95 mmol) as described above (960 mg, 89%), *m/z* [EI] 273 $[(M)^+]$; ν_{max} (film)/cm^{-1} 1740 (ester) and 1702 (urethane); δ_H (250 MHz, C^2HCl_3) 1.45 [18H, 2s, $C(CH_3)_3$], 1.85 (2H, m, H-4), 3.45 (2H, m, H-5) and 4.15 (1H, 2s, H-2).

(2*S*)-Proline (1)

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonylprolinate **18** (720 mg, 2.66 mmol) was stirred with 6 M aqueous hydrochloric acid (12 ml) at room temperature for 2 h. The solvent was removed *in vacuo* and the residue was dissolved in water (5 ml). The pH was adjusted from pH 2 to 6 using 2 M aqueous ammonium hydroxide and the solution was loaded on to a Dowex 50X8 (H^+) ion exchange column. The column was eluted with water until the eluant was no longer acidic and the product was eluted with 2 M aqueous ammonium hydroxide. The solvent was removed *in vacuo*. The residue was dissolved in water (5 ml) and lyophilised to afford (2*S*)-proline **1** as an off-white solid which was recrystallised from diethyl ether and ethanol (300 mg, 88%); mp 223 °C (decomp.); (lit.[15] 227–229 °C); $[\alpha]_D^{23}$ −61.48 (*c* 1.33, MeOH) [lit.[15] −83 (*c* 1, H_2O)]; *m/z* [ES+] 116 $([M + H]^+)$; ν_{max} (KBr)/cm^{-1} 1624 (acid); δ_H (500 MHz, $C^2H_3O^2H$) 1.96 (2H, m, H-4), 2.1 (1H, m, H-3*R*), 2.29 (1H, m, H-3*S*), 3.25 (1H, m, H-5A), 3.38 (1H, m, H-5B) and 4.0 (1H, dd, $J_{2,3R}$ 6.23, $J_{2,3S}$ 8.74, H-2); δ_C (125.8 MHz, C^2HCl_3) 25.63 (C-4), 30.91 (C-3), 47.53 (C-5), 63.16 (C-2) and 174.55 (acid).

(2*S*,3*S*)-[3-$^2H_1$]-Proline (1a)

This was prepared from *tert*-butyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonylprolinate **18a** (1 g, 3.68 mmol) as described above and was purified by dissolving in methanol and boiling with decolourising charcoal (126 mg, 27%), mp 225 °C (decomp.); $[\alpha]_D^{28}$ −47.2 (*c* 0.25, MeOH); *m/z* [ES+] 117 $([M + H]^+)$; ν_{max} (KBr)/cm^{-1} 1624 (acid); δ_H (360 MHz, $C^2H_3O^2H$) 1.96 (2H, m, H-4), 2.1 (1H, m, H-3*R*), 3.25 (1H, m, H-5A), 3.38 (1H, m, H-5B) and 4.0 (1H, d, $J_{2,3R}$ 5.8, H-2); δ_C (125.8 MHz, C^2HCl_3) 25.53 (C-4), 30.8 (m, C-3), 48.99 (C-5), 63.09 (C-2) and 174.54 (acid).

(2*S*,3*R*)-[3-$^2H_1$]-Proline (1c)

This was prepared from *tert*-butyl (2*S*,3*R*)-[3-$^2H_1$]-*N*-*tert*-butoxycarbonylprolinate **18b** (580 mg, 2.13 mmol) as described above (195 mg, 71%); mp 225 °C (decomp.); $[\alpha]_D^{32}$ −57.36 (*c* 1.1, MeOH); *m/z* [+ve FAB (thioglycerol)] 139 $([M + Na]^+$ and 117 $([M + H]^+)$; ν_{max} (film)/cm^{-1} 1626 (acid); δ_H (360 MHz, $C^2H_3O^2H$) 1.96 (2H, m, H-4), 2.29 (1H, m, H-3*S*), 3.25 (1H, m, H-5A), 3.38 (1H, m, H-5B) and 4.0 (1H, d, $J_{2,3S}$ 8.48, H-2); δ_C (125.8 MHz, C^2HCl_3) 25.53 (C-4), 30.8 (m, C-3), 47.52 (C-5), 63.10 (C-2) and 174.47 (acid).

(2*S*)-[3,3-$^2H_2$]-Proline (1d)

This was prepared from *tert*-butyl (2*S*)-[3,3-$^2H_2$]-*N*-*tert*-butoxycarbonylprolinate **18c** (960 mg, 3.51 mmol) as described above and the product was purified by dissolving in methanol and boiling with decolourising charcoal (240 mg, 53%); mp 227 °C (decomp.); $[\alpha]_D^{33}$ −63.53 (*c* 1, MeOH) *m/z* [+ve FAB (thioglycerol)] 118 $([M + H]^+)$; ν_{max} (film)/cm^{-1} 1624 (acid); δ_H (360 MHz, $C^2H_3O^2H$) 1.96 (2H, m, H-4), 3.25 (1H, m, H-5A), 3.38 (1H, m, H-5B) and 4.0 (1H, s, H-2); δ_C (125.8 MHz, C^2HCl_3) 25.45 (C-4), 30.6 (m, C-3), 47.54 (C-5), 63.05 (C-2) and 174.55 (acid).

Acknowledgements

We thank the EPSRC for studentships (to P. D. and C. A. S.)

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