Synthesis of L-Serine Stereospecifically Labelled at C-3 with Deuterium [1]

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(2*S*,3*R*)-[3-$^2H_1$]- and (2*S*,3*S*)-[2,3-$^2H_2$]-Serines have been synthesised from the corresponding labelled aspartic acids. The synthesis involves a Baeyer-Villiger oxidation in which a migrating primary chiral centre rearranges with retention of stereochemistry.

The amino acid L-serine is involved in many biological reactions in which substitution or elimination occurs at C-3.[2] The availability of L-serine, chirally labelled at C-3 with isotopic hydrogen would allow the stereochemistry of these enzymic reactions to be assessed. Syntheses of L-serine stereospecifically labelled at C-3 with tritium [3] or deuterium [3c,4] have been reported but these have limitations for producing the deuteriated amino acid either in large amounts or in high stereochemical purity. A further synthesis [5] of stereospecifically deuteriated L-serines was reported at the same time as the publication of the preliminary account of the present work.[1] After a final resolution, this gave the labelled L-amino acids in yields of 8.5 to 12%.

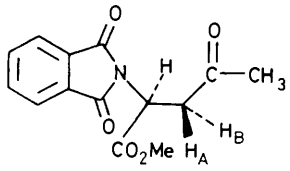
Because of our interest in biological one-carbon transfer reactions [6] and in using labelled L-serine as a synthon for other labelled compounds of biological importance, we decided to develop a new route to samples of L-serine stereospecifically labelled at C-3 with deuterium. As a starting point, we chose (2*S*,3*R*)-[3-$^2H_1$]- and (2*S*,3*S*)-[2,3-$^2H_2$]-aspartic acids, (2; H_B = ^2H) and (2; H_A = ^2H) respectively which we had found [7] could be prepared readily from fumaric acid (1) and [2,3-$^2H_2$]fumaric acid (1; H_A = ^2H)[7,8] respectively by an adaptation of the method of Krasna [9] using commercially available L-aspartase (EC 4.3.1.1). Higher deuterium incorporations in the [3-$^2H_1$]aspartate were achieved compared to our earlier work [7] by changing the buffer and exchanging out H_2O before the enzymic incubation.

Since we had already converted the labelled aspartic acids (2) into the diazoketones (3; R = Et) [7] and since Weygand [10] had reduced the unlabelled diazoketone (3; R = Et) to the corresponding methyl ketone (4; R = Et), it seemed that we had but to prepare the labelled ketones (4; R = Et) and Baeyer-Villiger oxidation, followed by hydrolysis, would yield the desired labelled serines. When the labelled diazoketone (3; R = Et, H_B = ^2H) was hydrogenated under the conditions of Weygand [10] or indeed under milder catalytic conditions, the deuterium label was exchanged by hydrogen. We were, however, able to prepare the desired stereospecifically labelled compound (4; R = Et, H_B = ^2H) either by photolysis of the diazoketone (3; R = Et, H_B = ^2H) in isopropyl alcohol in the presence of Michler's ketone or by reduction with hydrogen iodide in chloroform. A 220 MHz ^1H n.m.r. spectrum showed that the ABX system for C-2 and C-3 in the unlabelled compound became an AX system in the labelled compound (4; R = Et, H_B = ^2H). This compound was also shown to be optically active. The reduction had therefore left the stereochemistry at both chiral centres undisturbed.

Having obtained the desired ketone (4; R = Et), we now found that it could not be induced to undergo Baeyer-Villiger oxidation under any of a wide variety of conditions which were used. In the hope that the *N*-phthaloyl analogue (5) might prove more amenable to Baeyer-Villiger oxidation, we elected to prepare this compound from aspartic acid.

L-Aspartic acid (2) was readily converted into the *N*-phthaloyl derivative (6; R^1 = R^2 = H) using *N*-ethoxycarbonylphthalimide in sodium carbonate. Specific protection of the α-carboxy group was achieved either by careful titration with diazomethane to effect esterification of the more acidic (α) carboxylic acid, or by diesterification followed by selective demethylation of the β-ester using trimethylsilyl iodide. The monoester (6; R^1 = Me, R^2 = H) was treated with ethyl chloroformate in the presence of triethylamine and the resultant mixed anhydride was treated *in situ* with diazomethane to yield the crude diazoketone (7). This was reduced with hydrogen iodide in chloroform to yield the desired ketone (5). When this ketone was subjected to Baeyer-Villiger oxidation using trifluoroperoxyacetic acid, the crude product showed a methyl singlet at slightly higher field than the CH_3CO singlet of the starting ketone. This suggested that the Baeyer-Villiger reaction had finally been successful when the methyl ester (5) was used as substrate. Unfortunately, difficulties in purification and deprotection meant that this route was not viable for the synthesis of stereospecifically labelled L-serines. If the Baeyer-Villiger reaction were equally successful with the methyl ester (4; R = Me) however, then there would be no difficulty in deprotecting the resultant acetate (8; R = Me) to yield L-serine which could then be readily purified.

Aspartic acid (2) was therefore treated with trifluoroacetic anhydride and the intermediate anhydride (9) was treated with methanol *in situ*. In our earlier work,[7] we had followed the method of Weygand,[10] using ethanol in this reaction. Like Weygand, we had assumed the reaction to be totally regiospecific giving the α-ethyl ester (10; R^1 = Et, R^2 = OH) as the sole product. The ^1H n.m.r. spectrum of the product of the reaction of the anhydride (9) with methanol however showed two distinct methoxy singlets. This suggested the presence of both the α- and the β-monoesters, (10; R^1 = Me, R^2 = OH) and (10; R^1 = H, R^2 = OMe), respectively. The α-ester accounted for 80% of the total mixture. The monoesters were converted into a mixture of the corresponding acid chlorides which, on crystallisation from benzene, gave the pure acid chloride (10; R^1 = Me, R^2 = Cl) in 75% yield. A sample of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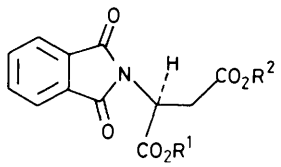
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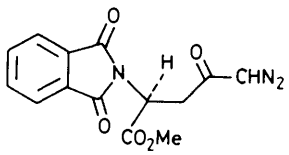
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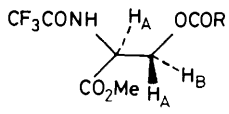
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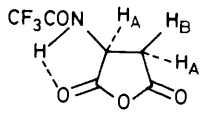
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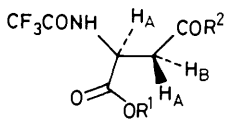
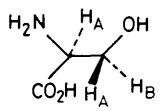

(10)

the pure α-methyl ester (10; R^1 = Me, R^2 = OH) could be obtained by hydrolysis of the acid chloride and the diesters (10; R^1 = Me, R^2 = OMe) and (10; R^1 = Me, R^2 = OEt) could be obtained by treating the acid chloride with methanol and ethanol respectively. When the product from the reaction of the anhydride (9) with ethanol [7] was treated with diazomethane, the ^{1}H n.m.r. spectrum of the crude product showed two methoxy singlets at δ 3.62 and 3.72 in the ratio 7 : 3 so that it was evident that a mixture containing *ca.* 70% of the diester (10; R^1 = Me, R^2 = OEt) and *ca.* 30% of the diester (10; R^1 = Et, R^2 = OMe) had been formed. It is evident, therefore, that while the reactions of the anhydride (9) with methanol and ethanol are largely regiospecific, they are by no means as exclusively so as we [7] and others [10] once thought.

We were now able to prepare the pure acid chlorides (10; R^1 = Me, R^2 = Cl) stereospecifically labelled at H_A and H_B respectively with deuterium, using the aspartic acids (2) prepared by the L-aspartase reaction. All subsequent steps in the synthesis were carried out using the unlabelled and the two stereospecifically labelled series of compounds.

The acid chlorides (10; R^1 = Me, R^2 = Cl) were treated with diazomethane to yield the diazoketones (10; R^1 = Me, R^2 = CHN_2). Reduction of these with hydrogen iodide in chloroform gave the methyl ketones (4; R = Me). Baeyer-Villiger oxidation using trifluoroperoxyacetic acid now gave a product which, when starting ketone was removed using Girard's reagent P, was shown by g.c./mass spec. to consist of three products: the desired acetate (8; R = Me), the trans-esterification product (8; R = CF_3) and the diester (10; R^1 = Me, R^2 = OMe). The latter product was evidently the result of methyl migration in the Baeyer-Villiger reaction. This was unexpected in view of the generalisation [11] that methyl groups have the lowest migratory aptitudes of all alkyl groups in Baeyer-Villiger reactions. It may, however, reflect the effect of distant electron-withdrawing groups in decreasing the migratory aptitude of the methylene centre C-3. Recent work has shown [12,13] that distant electron-withdrawing groups can decrease migratory aptitude in Baeyer-Villiger reactions. Equally surprising was the fact that, while the ethyl ester (4; R = Et) would not undergo Baeyer-Villiger reaction under any conditions, the methyl ester (4; R = Me) did, in fact, do so.

The mixture of products from the Baeyer-Villiger reaction was hydrolysed directly with 6M-hydrochloric acid at reflux. Separation of the hydrolysate on Amberlite IR45 gave pure L-serine in 18.7% yield, and L-aspartate in 32% yield. The overall yield from L-aspartate was therefore 8.6% *not* allowing for recovered L-aspartate. Since Baeyer-Villiger reactions are expected to proceed with retention of stereochemistry at the migrating centre [11,14] the samples of deuteriated L-serine would be expected to be labelled as shown in formula (11).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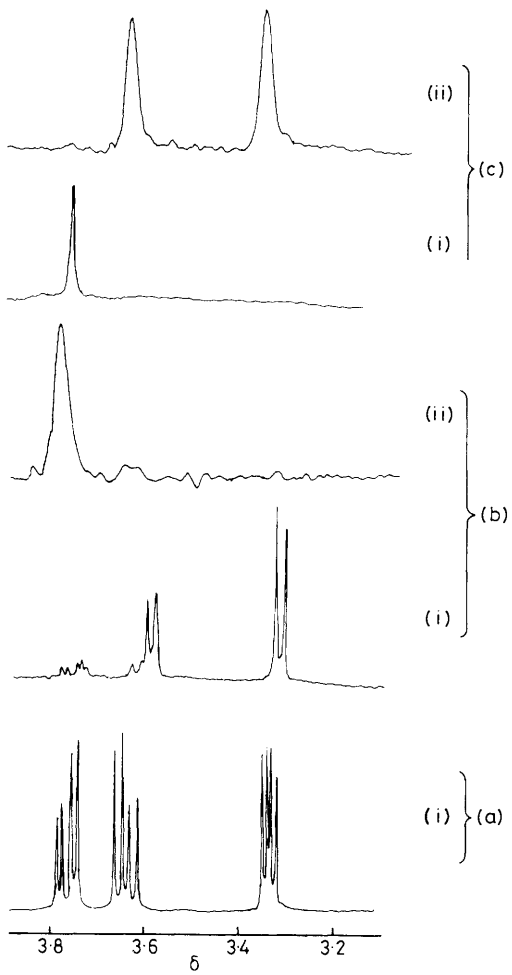

Figure. (i) 360 MHz ^{1}H N.m.r. spectra in 10% $NaO^2H/^2H_2O$; (ii) 55.28 MHz ^{2}H n.m.r. spectra in 10% $NaOH/H_2O$ of (a) L-serine (11); (b) (2*S*,3*R*)-[3-$^2H_1$]serine (11; H_B = ^{2}H); and (c) (2*S*,3*S*)-[2,3-$^2H_2$]serine (11; H_A = ^{2}H)

(2*S*,3*R*)-[3-$^2H_1$]Serine (11; H_B = ^{2}H) would be derived ultimately from (2*S*,3*R*)-[3-$^2H_1$]aspartic acid (2; H_B = ^{2}H) and (2*S*,3*S*)-[2,3-$^2H_2$]serine (11; H_A = ^{2}H) would be derived ultimately from (2*S*,3*S*)-[2,3-$^2H_2$]aspartic acid (2; H_A = ^{2}H). That this was indeed the case could be seen from the ^{1}H and ^{2}H n.m.r. spectra of the labelled serines (see Figure) where the chemical shifts are in good agreement with the values of *ca.** δ 3.71 p.p.m. for the 3-*pro-R* hydrogen, *ca.** δ 3.63 for the 3-*pro-S*

* Averaged values.

hydrogen and δ 3.314 for the 2-hydrogen of L-serine reported by Benkovic.[5]

Experimental

M.p.s were determined on a Kofler hot-stage apparatus. I.r. spectra were recorded on Perkin-Elmer 257, 457, and 477 instruments. ^{1}H N.m.r. spectra were recorded on Perkin-Elmer R12 (60 MHz), and R32 (90 MHz) instruments and by P.C.M.U., Harwell (220 MHz). 360 MHz ^{1}H- and 55.28 MHz ^{2}H-N.m.r. spectra were obtained by Dr. I. Sadler, Edinburgh University using a Bruker WH 360 instrument. Mass spectra (E.I.) were obtained using AEI-MS30 and Kratos MS 25 instruments. Specific rotations were recorded on a Perkin-Elmer PE241 polarimeter using a 1-dm path length cell.

*Ethyl 4-Oxo-*N*-trifluoroacetyl-(2S)-norvalinate* (4; R = Et) *and its* (2S,3R)-[3-^{2}H$_1$]-*Derivative* (4; R = Et, H_B = ^{2}H).—*Method* (*a*).* Ethyl 5-diazo-4-oxo-*N*-trifluoroacetyl-(2*S*)-norvalinate (3; R = Et) (200 mg, 0.712 mmol) [7] and Michler's ketone (0.7 mg) were dissolved in distilled isopropyl alcohol (400 ml). The solution was degassed with nitrogen for 1 h and photolysed using a 125-W medium-pressure lamp and a Pyrex filter for 1.5 h. The solvent was removed under reduced pressure and the product purified by sublimation at 40 °C and 4 mmHg (160 mg, 89%), m.p. 77—79 °C (lit.,[10] 79 °C); $[\alpha]_D$ −17.1° (*c* 1.123, $CHCl_3$) (lit.,[10] −17°); δ(C^2HCl_3), 1.27 (3 H, t, *J* 6.5 Hz, CH_2CH_3), 2.20 (3 H, s, $COCH_3$), 3.05 (1 H, ABq, J_{AB} 18 Hz, J_{AX} 4 Hz, 3*R*-H), 3.33 (1 H, ABq, J_{AB} 18 Hz, J_{BX} 4 Hz, 3*S*-H), 4.25 (2 H, q, *J* 6.5 Hz, CH_2CH_3), 4.74 (1 H, m, 2-H) and 7.41br (1 H, NH, exchangeable in ^2H_2O); $\nu_{max.}$ (Nujol) 1 735 (ester) and 1 708 cm^{-1} (ketone and trifluoroacetamide). The (2*S*,3*R*)-[3-^{2}H$_1$]-derivative (4; R = Et, H_B = ^{2}H) was prepared in 87% yield from the diazoketone (3; R = Et, H_B = ^{2}H) [7] (250 mg, 0.89 mmol) using the same method. The spectra were similar but the ^{1}H n.m.r. spectrum had no absorption at δ 3.05 and a broad singlet at δ 3.30. Mass spectrometry indicated that the compound was *ca.* 77% monodeuteriated.

Method (*b*).* Constant boiling aqueous hydrogen iodide (55%, 3 ml) was carefully added to a solution of ethyl 5-diazo-4-oxo-*N*-trifluoroacetyl-(2*S*)-norvalinate (3; R = Et) (1 g, 3.56 mmol) in chloroform (20 ml) in a separating funnel. The mixture was shaken carefully until nitrogen evolution had ceased (*ca.* 2 min) after which the chloroform layer was washed with water and aqueous sodium thiosulphate solution until colourless. After a final wash with water, the organic layer was dried (Na_2SO_4) and the solvent was removed under reduced pressure to give a pale yellow solid which was purified by sublimation at 40 °C and 4 mmHg (700 mg, 78%), m.p. 75—77 °C (lit.,[10] 79 °C), $[\alpha]_D$ −16.4° (*c* 0.95, $CHCl_3$) (lit.,[10] −17°). The (2*S*,3*R*)-[3-^{2}H$_1$]-derivative (4; R = Et, H_B = ^{2}H) was prepared from the diazoketone (3; R = Et, H_B = ^{2}H) [7] (1 g, 3.56 mmol) in 82% yield. Both the undeuteriated and the deuteriated compounds had identical spectra to the corresponding compounds prepared by method (*a*) above.

N-*Phthaloyl*-L-*aspartic Acid* (6; R^1 = R^2 = H).—L-Aspartic acid (2 g, 15 mmol) and Na_2CO_3 (anhyd, 1.7 g, 16 mmol) were dissolved in water (20 ml) and *N*-ethoxycarbonylphthalimide (3.6 g, 16 mmol) was added. The mixture was stirred at room temperature for 1 h by which time most of the *N*-ethoxycarbonylphthalimide had dissolved. The solution was filtered, carefully acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The extracts were dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a foam which was crystallised from ethyl acetate–light petroleum (b.p. 60—80 °C) (3.6 g, 91%), m.p. 220—222 °C (lit.,[15] for DL 220—223 °C); $[\alpha]_D$ −53° (*c* 3.06, EtOH) (lit.,[16] −58°); $\nu_{max.}$ (Nujol) 1 720br cm^{-1} (carbonyl); δ (10% NaO^2H in ^2H_2O) 3.17br (2 H, d, 2 × 3-H), and 7.82br (4 H, s, aromatics). The ^{2}HOH signal obscured the CH–N absorbance.

Dimethyl N-*Phthaloyl*-L-*aspartate* (6; R^1 = R^2 = Me).—Excess of ethereal diazomethane [17] was added to a solution of *N*-phthaloyl-L-aspartic acid (600 mg, 2.28 mmol) in tetrahydrofuran (80 ml) at 0 °C. After 10 min, excess of diazomethane was removed in a stream of nitrogen and the solvent was removed under reduced pressure to yield an oil (660 mg, 99%) which crystallised on prolonged storage; m.p. 69—71 °C; $[\alpha]_D^{22}$ −44.6° (*c* 1.427, $CHCl_3$) (Found: C, 57.9; H, 5.0; N, 4.8. $C_{14}H_{13}NO_6$ requires C, 57.7; H, 4.5; N, 4.8%); *m*/*z* 291 (M^+); $\nu_{max.}$ (Nujol) 1 772—1 710br cm^{-1}; δ(C^2HCl_3) 3.16 (2 H, *ABX*, J_{AB} 15, J_{AX} 9, J_{BX} 7 Hz, 3-H), 3.63 and 3.71 (2 × 3 H, s, CO_2Me), 5.25 (1 H, q, J_{AX} 9, J_{BX} 7 Hz, 2-H) and 7.62 (4 H, m, aromatics).

α-*Methyl* N-*Phthaloyl*-L-*aspartate* (6; R^1 = Me, R^2 = H).—*Method* (*a*). *N*-Phthaloyl-L-aspartic acid (1.24 g, 4.71 mmol) was dissolved in tetrahydrofuran (50 ml) at −80 °C and slowly treated with a cold ethereal solution of diazomethane [17] until a yellow colour persisted for several seconds. The solvent was removed under reduced pressure and the resultant oil was dissolved in chloroform (50 ml) and extracted with aqueous sodium hydrogencarbonate. The aqueous phase was acidified and extracted with chloroform (3 × 20 ml). These extracts were dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield the ester as an oil (795 mg, 61%); $[\alpha]_D^{22}$ −47.3° (*c* 0.595, $CHCl_3$) (Found: C, 54.5; H, 4.5; N, 4.9. $C_{13}H_{11}NO_6 \cdot \frac{1}{2}H_2O$ requires C, 54.5; H, 4.2; N, 4.9%); *m*/*z* 277 (M^+); $\nu_{max.}$ (Nujol) 1 777, 1 742—1 707 (phthalimide and ester), 1 666 cm^{-1} (CO_2H); δ(C^2HCl_3) 3.19 (2 H, *ABX*, J_{AB} 16, J_{AX} 8, J_{BX} 6 Hz, 3-H), 3.66, (3 H, s, OCH_3), 5.21 (1 H, q, J_{AX} 8, J_{BX} 6 Hz) and 7.59 (4 H, m, aromatics). For full characterisation a *dicyclohexylamine salt* was prepared by dissolving the ester (6; R^1 = Me, R^2 = H) (30 mg, 0.11 mmol) with dicyclohexylamine (20 mg, 0.11 mmol) in benzene (2 ml). After 2 h, light petroleum (b.p. 40—60 °C) was added to yield the salt (42 mg, 83%), m.p. 154—156 °C (Found: C, 65.1; H, 7.6; N, 6.0. $C_{25}H_{34}N_2O_6$ requires C, 65.5; H, 7.4; N, 6.1%).

Method (*b*). Dimethyl *N*-phthaloyl-L-aspartate (6; R^1 = R^2 = Me) (140 mg, 0.48 mmol) was dissolved in C^2HCl_3 (0.5 ml) and trimethylsilyl iodide [18] (289 mg, 1.4 mmol) was added. The ^{1}H n.m.r. spectrum showed steady cleavage of the β-methyl ester and after 2 weeks at room temperature chloroform (10 ml) was added and the solution was washed with aqueous sodium thiosulphate and water and dried (Na_2SO_4). The solvent was removed under reduced pressure to yield an oil (105 mg, 79%) identical in all respects with the compound prepared by method (*a*).

*Methyl 5-Diazo-4-oxo-*N*-phthalimido-(2S)-norvalinate* (7).—α-Methyl *N*-phthaloyl-L-aspartate (1.5 g, 5.42 mmol) was dissolved in tetrahydrofuran (30 ml) with triethylamine (0.54 g, 5.4 mmol) and the solution was cooled to −30 °C. Ethyl chloroformate (0.6 g, 5.53 mmol) was added dropwise with stirring during 5 min and after 40 min the solution was filtered directly into a cold stirred solution of ethereal diazomethane [17] (excess) during *ca.* 20 min. After 3 h, the excess diazomethane was removed in a stream of nitrogen and the solvent was removed under reduced pressure to yield the crude diazoketone (7) (1.57 g, 96%), δ(C^2HCl_3) 3.21 (2 H, m, 3-H), 3.68 (3 H, s, OCH_3), 5.22 (1 H, s, CHN_2), 5.48 (1 H, q,

* This experiment was performed by Dr. S. J. Field.

2-H), and 7.66 (4 H, m, aromatics). Other absorbances were present in this spectrum.

Methyl 4-*Oxo*-N-*phthalimido*-(2S)-*norvalinate* (5).—The crude diazoketone (7) (1.3 g, 4.3 mmol) was dissolved in chloroform (100 ml) and constant boiling aqueous hydrogen iodide (55%, excess) was carefully added with shaking. The chloroform layer was washed with aqueous sodium thiosulphate (until colourless) and water (2 × 10 ml) and then dried (Na_2SO_4). The solvent was removed under reduced pressure to yield the ketone (5) as an oil (1.1 g, 92%), $[\alpha]_D^{22}$ −57.1° (*c* 1.203, $CHCl_3$) (Found: C, 58.5; H, 4.7; N, 4.8%; *m/z* 275.0806. $C_{14}H_{13}NO_5$ requires C, 61.1; H, 4.8; N, 5.1%, *m/z* 275.0794), $\nu_{max.}$ (Nujol) 1 775 and 1 760—1 710 cm^{-1}; $\delta(C^2HCl_3)$ 2.22 (3 H, s, $COCH_3$), 3.38 (2 H, *AB*X, J_{AB} 18, J_{AX} 8, J_{BX} 7 Hz, 3-H), 3.72 (3 H, s, OCH_3), 5.48 (1 H, t, $J_{AX} = J_{BX} = 7$ Hz, 2-H), and 7.79 (4 H, m, aromatics).

(2S,3R)-[3-$^2H_1$]*Aspartic Acid*.—Fumaric acid (16.14 g, 0.14 mol), $MgSO_4 \cdot 7H_2O$ (AnalaR) (3.45 g, 0.012 mol) and tris(hydroxymethyl)methylamine (AnalaR) (4.25 g, 0.035 mol) were added to ^2H_2O (99.7%, 60 ml). The solution was heated for 10 min, and the solvent removed under reduced pressure. Ammonium chloride (AnalaR) (14.97 g, 0.27 mol) was similarly exchanged with ^2H_2O (99.7%, 20 ml). The mixture containing fumaric acid was dissolved in ^2H_2O (99.7%, 100 ml), and a solution of the exchanged ammonium chloride in ^2H_2O (99.7%, 50 ml) was added. The solution was made up to 230 ml with ^2H_2O (99.7%), the pH adjusted to 8.1, and L-aspartase (Sigma, 12 units) added in a minimum of ^2H_2O at 30 °C. The reaction was incubated at 30 °C for 10 days when the O.D. at 240 nm for fumarate was at a minimum. The enzyme was denatured by boiling for 30 min and an excess of saturated aqueous copper sulphate was added. The solution was left overnight at 4 °C after which the copper aspartate was filtered off, washed with water (4.0 ml), and suspended in water (200 ml). Hydrogen sulphide was passed through the stirred solution for 20 min and the black precipitate was filtered off with the aid of Celite. The filtrate was treated with absolute ethanol until turbid. After some time two crops of (2*S*,3*R*)-[3-$^2H_1$]-aspartic acid (7.17 g, 39%) were obtained. Spectra were as expected.[7]

(2S,3S)-[2,3-$^2H_2$]*Aspartic Acid*.—This was prepared from [2,3-$^2H_2$]fumaric acid [7] as above, using H_2O instead of ^2H_2O; yields of labelled aspartate were routinely 40—50%.

α-*Methyl* N-*Trifluoroacetyl*-L-*aspartate* (10; R^1 = Me, R^2 = OH).—Trifluoroacetic anhydride (80 g, 381 mmol) was added to a stirred suspension of L-aspartic acid (6 g, 45.1 mmol) in dry tetrahydrofuran (150 ml) at 0 °C during 30 min in an atmosphere of nitrogen. The reaction was allowed to warm to room temperature during 2 h when the solution became homogeneous. The solvent was removed under reduced pressure and the resultant solid thoroughly dried. Cold dry methanol (50 ml) was added and the solution was left for 20 min to warm to room temperature. The solvent was removed under reduced pressure to give a solid in quantitative yield. This showed two methoxy singlets in its ^1H n.m.r. spectrum at δ 3.64 and 3.73 in a ratio of 1 : 4 by integration. The pure α-methyl ester (10; R^1 = Me, R^2 = OH) was obtained by leaving the pure acid chloride (10; R^1 = Me, R^2 = Cl) prepared as described below (85 mg, 0.325 mmol) in water (5 ml) at room temperature. Lyophilisation gave the acid (10; R^1 = Me, R^2 = OH) (78 mg, 99%) as white crystals, m.p. 102—103 °C, $[\alpha]_D^{35}$ +27.9° (*c* 0.623, $CHCl_3$–MeOH, 9 : 1) (Found: C, 34.6; H, 3.8; N, 5.8. $C_7H_8F_3NO_5$ requires C, 34.6; H, 3.3; N, 5.8%), *m/z* 198 ($M^+ - CO_2H$) and 184 ($M^+ - CO_2Me$); $\nu_{max.}$ (Nujol) 1 748—1 705br cm^{-1}; δ (C^2HCl_3) 3.04 (2 H, m, 3-H), 3.73 (3 H, s, OMe), 4.80 (1 H, m, 2-H) and exchangeable protons. A dicyclohexylamine salt was prepared by dissolving the ester (300 mg, 1.23 mmol) in dry benzene (5 ml). Dicyclohexylamine (225 mg, 1.24 mmol) was added and after 10 min light petroleum (b.p. 40—60 °C) was added dropwise with gentle warming. The salt crystallised out (310 mg, 59%), m.p. 158—159 °C, $[\alpha]_D^{27}$ −2.43° (*c* 0.782, CH_2Cl_2) (Found: C, 53.7; H, 7.1; N, 6.6%. $C_{19}H_{31}F_3N_2O_5$ requires C, 53.8; H, 7.3; N, 6.6%).

α-*Methyl* (2S,3R)-*N*-*Trifluoroacetyl*[3-$^2H_1$]*aspartate* (10; R^1 = Me, R^2 = OH, H_B = ^2H).—This was prepared from (2*S*,3*R*)-[3-$^2H_1$]aspartic acid in an identical manner to the unlabelled compound above, m.p. 102—104 °C, $[\alpha]_D^{35}$ +26.1° (*c* 0.519, $CHCl_3$–MeOH; 9 : 1). Spectra were similar to those of the unlabelled compound except that only *one* of the protons at position 3 was apparent at δ 3.05 in the ^1H n.m.r. spectrum, and the mass spectral fragment ions were one mass number higher at *m/z* 199 and 185, with incorporation estimated at *ca.* 88% $^2H_1$.

α-*Methyl* (2S,3S)-N-*Trifluoroacetyl*[2,3-$^2H_2$]*aspartate* (10; R^1 = Me, R^2 = OH, H_A = ^2H).—This was prepared from (2*S*,3*S*)-[2,3-$^2H_2$]aspartic acid in a manner identical with that used for the unlabelled compound above; it had m.p. 101—103 °C, $[\alpha]_D^{35}$ +22.9° (*c* 0.912; $CHCl_3$–MeOH, 9 : 1). Spectra were similar to those of the unlabelled compound except that 2-H was missing and there was only *one* proton at position 3 (δ 2.84) in the ^1H n.m.r. spectrum. The mass spectral fragment ions were two mass numbers higher at *m/z* 200 and 186 with incorporation estimated at *ca.* 90% $^2H_2$, 9% $^2H_1$.

α-*Methyl* N-*Trifluoroacetyl*-L-*aspartyl* β-*Chloride* (10; R^1 = Me, R^2 = Cl).—The mixture of α- and β-monoesters from the above reaction (5.23 g, 21.5 mmol) was heated to reflux in redistilled thionyl chloride (60 ml) for 1 h. The solvent was removed under reduced pressure to yield a pale yellow solid which was recrystallised from benzene (40 ml). The pure β-chloride crystallised out (4.21 g, 75%), m.p. 114—115 °C, $[\alpha]_D^{27}$ +62.6° (*c* 1.830, $CHCl_3$) (Found: C, 32.6; H, 3.1; N, 5.4. $C_7H_7ClF_3NO_4$ requires C, 32.1; H, 2.7; N, 5.4%), *m/z* 198 ($M^+ - COCl$); $\nu_{max.}$ (Nujol) 1 795 (COCl), 1 738 (ester), and 1 713 cm^{-1} (trifluoroacetamide); $\delta(C^2HCl_3)$ 3.50 (2 H, m, 3-H), 3.73 (3 H, s, OMe), and 4.64 (1 H, m, 2-H).

α-*Methyl* (2S,3R)-N-*Trifluoroacetyl*[3-$^2H_1$]*aspartyl* β-*Chloride* (10; R^1 = Me, R^2 = Cl, H_B = ^2H).—This was prepared from α-methyl (2*S*,3*R*)-*N*-trifluoroacetyl[3-$^2H_1$]aspartate in a manner identical with that used for the unlabelled compound above; it had m.p. 113—115 °C. Spectra were similar to those of the unlabelled compound except that only one proton for the 3-position was apparent at δ 3.67 and the mass spectral fragment ion *m/z* 199 was one mass number higher. Incorporation was estimated to be *ca.* 87% $^2H_1$.

α-*Methyl* (2S,3S)-N-*Trifluoroacetyl*[2,3-$^2H_2$]*aspartyl* β-*Chloride* (10; R^1 = Me, R^2 = Cl, H_A = ^2H).—This was prepared from α-methyl (2*S*,3*S*)-*N*-trifluoroacetyl[2,3-$^2H_2$]-aspartate in a manner identical with that used for the unlabelled compound above; it had m.p. 113—115 °C. Spectra were similar to those of the unlabelled compound except that 2-H was missing and there was only one proton for the 3-position at δ 3.48. The mass spectral fragment ion, *m/z* 200, was two mass numbers higher than in the unlabelled compound and showed incorporation to be *ca.* 92% $^2H_2$, 7% $^2H_1$.

Dimethyl N-*Trifluoroacetyl*-L-*aspartate* (10; R^1 = Me, R^2 = OMe).—The acid chloride (10; R^1 = Me, R^2 = Cl) (80 mg, 0.31 mmol) was dissolved in methanol (5 ml) and left at room temperature for 2 h. The solvent was removed under reduced pressure and the residual oil azeotropically dried with chloroform. The product crystallised with time and sublimed at room temperature *in vacuo* (73 mg, 93%); it had m.p. 36—37 °C, $[\alpha]_D^{35}$ +42.62° (*c* 1.44, $CHCl_3$) (Found: C, 36.5; H, 4.1; N, 5.45. $C_8H_{10}F_3NO_5$ requires C, 37.35; H, 3.9; N, 5.5%), *m/z* 257 (M^+); $\nu_{max.}$ (Nujol) 1 730br cm^{-1} (CO); δ(C^2HCl_3) 2.76 (2 H, m, 3-H), 3.48 and 3.56 (2 × 3 H, s, OMe), 4.61 (1 H, m, 2-H), and 7.32br (1 H, d, *J* 7 Hz, NH).

α-*Methyl* β-*Ethyl* N-*Trifluoroacetyl*-L-*aspartate* (10; R^1 = Me, R^2 = OEt).—The acid chloride (10; R^1 = Me, R^2 = Cl) (200 mg, 0.765 mmol) was dissolved in ethanol (5 ml) and left at room temperature for 30 min. The solvent was removed under reduced pressure and the residual oil dried azeotropically with chloroform to yield an oil (200 mg, 96%), *m/z* 271 (M^+); $\nu_{max.}$ (Nujol) 1 727br cm^{-1} (CO); δ(C^2HCl_3) 1.22 (3 H, t, *J* 7 Hz, CH_2CH_3), 3.0 (2 H, m, 3-H), 3.75 (3 H, s, OCH_3), 4.13 (2 H, q, *J* 7 Hz, CH_2CH_3), 4.80 (1 H, m, 2-H), and 7.68br (1 H, d, *J* 8 Hz, NH).

α-*Ethyl* β-*Methyl* N-*Trifluoroacetyl*-L-*aspartate* (10; R^1 = Et, R^2 = OMe).—The presumed pure [7] monoester (10; R^1 = Et, R^2 = OH) (120 mg, 0.467 mmol) was dissolved in chloroform–methanol (5 : 1, 6 ml). Excess of ethereal diazomethane [17] was added and after 10 min at room temperature the excess of diazomethane was removed in a stream of nitrogen. The solvents were removed under reduced pressure to yield an oil (120 mg, 94%) which showed two methoxy singlets at δ 3.62 and 3.72 in a 7 : 3 ratio in the ^1H n.m.r. spectrum in C^2HCl_3. Purer α-ethyl β-methyl diester was obtained by recrystallisation from chloroform–light petroleum (b.p. 40—60 °C) as yellow flakes (71 mg, 56%), m.p. 42—45 °C; $[\alpha]_D^{35}$ +33.2° (*c* 2.85, $CHCl_3$); *m/z* 271 (M^+); $\nu_{max.}$ (Nujol) 1 734 (ester) and 1 710 cm^{-1} (trifluoroacetamide); δ (C^2HCl_3) 1.23 (3 H, t, *J* 7 Hz, CH_2CH_3), 2.92 (2 H, m, 3-H), 3.61 (3 H, s, OCH_3), 4.12 (2 H, q, *J* 7 Hz, CH_2CH_3), 4.69 (1 H, m, 2-H), and 7.30br (1 H, NH). The β-ethyl α-methyl diester was evidently still present as a contaminant.

Methyl 5-*Diazo*-4-*oxo*-N-*trifluoroacetyl*-(2S)-*norvalinate* (3; R = Me).—α-Methyl *N*-trifluoroacetyl-L-aspartyl β-chloride (10; R^1 = Me, R^2 = Cl) (3 g, 11.43 mmol) dissolved in diethyl ether (40 ml) and tetrahydrofuran (10 ml) was added dropwise during 30 min to an excess of ethereal diazomethane [17] (200 ml) with stirring. The solution was allowed to warm to room temperature during 90 min after which the excess of diazomethane was removed under reduced pressure to yield the diazoketone (3.04 g, 100%). A sample was recrystallised from chloroform–light petroleum (b.p. 40—60 °C) as rosettes, m.p. 104—105 °C, $[\alpha]_D$ −15.0° (*c* 0.746, MeOH) (Found: C, 36.25; H, 3.65; N, 14.9. $C_8H_8F_3N_3O_4$ requires C, 36.0; H, 3.0; N, 15.7%), *m/z* 239 ($M^+ - N_2$); $\nu_{max.}$ (Nujol) 2 105 (N≡N), and 1 755—1 706br cm^{-1} (CO); δ(C^2HCl_3) 2.97 (2 H, *ABX*, J_{AB} 16, $J_{AX} = J_{BX}$ = 4 Hz, 3-H), 3.72 (3 H, s, OMe), 4.75 (1 H, m, 2-H), 5.28 (<1 H, s, CHN_2) and 7.50br (1 H, NH).

Methyl (2S,3R)-5-*Diazo*-4-*oxo*-N-*trifluoroacetyl*[3-$^2H_1$]*norvalinate* (3; R = Me, H_B = ^2H).—This was prepared from the (2*S*,3*R*)-[3-$^2H_1$]acid chloride (10; R^1 = Me, R^2 = Cl, H_B = ^2H) in a manner identical with that of the unlabelled compound above; it had m.p. 105—106 °C, $[\alpha]_D^{35}$ −17.1° (*c* 0.911, MeOH). Spectra were similar to those of the unlabelled compound except that there was but one proton at the 3-position as a broad singlet at δ 3.04 in the ^1H n.m.r. spectrum. The fragment ion *m/z* 240 was one mass number higher than in the unlabelled sample.

Methyl (2S,3S)-5-*Diazo*-4-*oxo*-N-*trifluoroacetyl*[2,3-$^2H_2$]*norvalinate* (3; R = Me, H_A = ^2H).—This was prepared from the (2*S*,3*S*)-[2,3-$^2H_2$]acid chloride (10; R^1 = Me, R^2 = Cl, H_A = ^2H) in a manner identical with that of the unlabelled compound above; it had m.p. 103—105 °C, $[\alpha]_D$ −13.2° (*c* 0.851, MeOH). The ^1H n.m.r. spectrum showed no absorption for 2-H and but one proton for the 3-position as a broad singlet δ 2.82. The fragment ion *m/z* 241 was two mass numbers higher than in the unlabelled sample.

Methyl 4-*Oxo*-N-*trifluoroacetyl*-(2S)-*norvalinate* (4; R = Me).—Aqueous HI (55%; excess) was added dropwise with shaking to a solution of the diazoketone (3; R = Me) (1.45 g, 5.43 mmol) in chloroform (80 ml). After 5 min the organic layer was washed with water (2 × 5 ml), dilute aqueous sodium thiosulphate (until colourless), and water (5 ml) and then dried (Na_2SO_4). The solvent was removed under reduced pressure to yield the crystalline ketone (1.03 g, 79%). A sample was recrystallised from diethyl ether–light petroleum (b.p. 40—60 °C) and had m.p. 67—68 °C, $[\alpha]_D^{27}$ −43.1° (*c* 0.972, MeOH), $[\alpha]_D^{25}$ +53.0° (*c* 0.544; $CHCl_3$) (Found: C, 39.6; H, 4.2; N, 5.8. $C_8H_{10}F_3NO_4$ requires C, 39.8; H, 4.15; N, 5.8%), *m/z* 241 (M^+); $\nu_{max.}$ (Nujol) 1 743 (ester) and 1 710 cm^{-1} (amide and ketone); δ(C^2HCl_3) 2.13 (3 H, s, $COCH_3$), 3.09 (2 H, m, 3-H), 3.72 (3 H, s, OMe), 4.60 (1 H, m, 2-H), and 7.3br (1 H, s, NH).

Methyl (2S,3R)-4-*Oxo*-N-*trifluoroacetyl*[3-$^2H_1$]*norvalinate* (4; R = Me, H_B = ^2H).—This was prepared from the diazoketone (3; R = Me, H_B = ^2H) in a manner identical with that of the unlabelled compound above; it had m.p. 67—68 °C, $[\alpha]_D^{26}$ −43.2° (*c* 1.102, MeOH). Spectra were similar to those of the unlabelled compound except that there was but one proton for the 3-position at δ 3.26 in the ^1H n.m.r. spectrum. The parent ion *m/z* 242 was one mass number higher than in the unlabelled compound and showed incorporation to be *ca.* 87% $^2H_1$.

Methyl (2S,3S)-4-*Oxo*-N-*trifluoroacetyl*[2,3-$^2H_2$]*norvalinate* (4; R = Me, H_A = ^2H).—This was prepared from the diazoketone (3; R = Me, H_A = ^2H) in a manner identical with that of the unlabelled compound above; it had m.p. 66—68 °C, $[\alpha]_D^{26}$ −41.9° (*c* 0.751, MeOH). Spectra were similar to those of the unlabelled compound except that 2-H was missing and there was but one proton for the 3-position as a broad singlet at δ 2.95 in the ^1H n.m.r. spectrum (C^2HCl_3). The parent ion *m/z* 243 in the mass spectrum was two mass units higher than in the unlabelled sample and showed an incorporation of *ca.* 91% $^2H_2$, 8% $^2H_1$.

L-*Serine* (11).—The ketone (4; R = Me) (2.0 g, 8.3 mmol) was dissolved in freshly prepared trifluoroperoxyacetic acid [from 85% H_2O_2 (4 ml) in dichloromethane (27 ml) vigorously stirred at 0 °C whilst trifluoroacetic anhydride (26 ml) was added during 30 min]. The solution was heated to reflux for 8 h. The solvent was removed at low temperature and freshly prepared trifluoroperoxyacetic acid (an equivalent amount to that first added) was added at 0 °C. The solution was heated to reflux for a further 14 h after which the solvent was removed. The residue was heated for 30 min with an 8% solution of Girard's reagent P in 10% methanolic acetic acid (40 ml) and allowed to cool during 2 h. The solution was diluted to 200 ml with water containing sodium hydroxide (1 g) and the pH was carefully adjusted to 6.8 using 2M-aqueous sodium hydroxide. The solution was extracted with chloroform to obtain non-

ketonic material. The aqueous solution was acidified to pH *ca.* 2 with 2M-HCl and after being kept for 1 h at 40 °C was extracted exhaustively with chloroform. These latter extracts were dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield unchanged ketone (219 mg). The solvent was removed from the non-ketonic fraction yielding a colourless oil (1.47 g, 70% or 77.4% based on unchanged starting ketone).

The oil above (1.35 g) was dissolved in 6M-hydrochloric acid (60 ml) and heated to reflux for 2 h. The solution was lyophilised and the dark residue was dissolved in water (20 ml) and centrifuged at 8 000 r.p.m. for 20 min. The supernatant solution was diluted to 40 ml and applied to a column of Amberlite IR45 weakly basic anion-exchange resin (30 ml made up as per ref. 19). Elution with water (120 ml at 1 drop/3 s) and freeze drying gave serine (103 mg, 18.7%), m.p. 201—203 °C (decomp.); $[\alpha]_D^{35}$ +14.8° (*c* 0.750, 1M HCl) {lit.,[20] m.p. 228 °C (decomp.); lit.,[21] $[\alpha]_D$ in 1M-HCl + 15.9°} (Found: C, 34.6; H, 6.6; N, 13.4. $C_3H_7NO_3$ requires C, 34.3; H, 6.7; N, 13.3%), *m/z* of tris-trimethylsilyl derivative (mass spec./g.c.) 306 ($M^+ - CH_3$); $\nu_{max.}$ (Nujol) 2 950 (*COOH*), 1 645—1 595br cm^{-1} (CO); δ(10% NaO^2H–^2H_2O) [see Figure (i)(a)] 3.34 (1 H, d × d, *J* 4.2 and 6.3 Hz, 2-H), 3.64 (1 H, d × d, J_{AB}, 11.1, J_{AX} 6.3 Hz, 3*S*-H), and 3.76 (1 H, d × d, J_{AB} 11.1, J_{BX} 4.2 Hz, 3*R*-H).

Further elution of the column with 0.05M-aqueous acetic acid gave a 32% yield of aspartic acid, identical in all respects with an authentic sample.

(2S,3R)-[3-$^2H_1$]*Serine* (11; $H_B = {}^2H$).—This was prepared from the ketone (4; R = Me, $H_B = {}^2H$) in a manner identical with that of the unlabelled compound above; it had m.p. 200—202 °C (decomp.); $[\alpha]_D^{35}$ +18.2° (*c* 0.572, 1M-HCl). Spectra were similar to those of the unlabelled compound above except that the ^1H n.m.r. spectrum showed 3*S*-H as a one proton doublet (*J* 6.4 Hz) at δ 3.59 and 2-H as a one proton doublet δ 3.32 (*J* 6.4 Hz) [Figure (i)(b)]. The ^2H n.m.r. spectrum [Figure (ii)(b)] showed 3*R*-H as a broad singlet δ 3.77. The mass spectrum (E.I.; g.c./mass spec.) of the tris-trimethylsilyl derivative had a fragment ion, *m/z* 307, one mass unit higher than in the unlabelled compound and showed incorporation to be *ca.* 90% $^2H_1$.

(2S,3S)-[2,3-$^2H_2$]*Serine* (11; $H_A = {}^2H$).—This was prepared from the ketone (4; R = Me, $H_A = {}^2H$) in a manner identical with that of the unlabelled compound above; it had m.p. 199—202 °C, $[\alpha]_D^{35}$ +13.1° (*c* 0.731, 1M-HCl). Spectra were similar to those of the unlabelled compound except that the ^1H n.m.r. spectrum [Figure (i)(c)] showed 3*R*-H as a singlet (δ 3.75). The ^2H n.m.r. spectrum [Figure (ii)(c)] showed 2-H and 3*S*-H as broad singlets at δ 3.34 and 3.62 respectively. The mass spectrum (E.I., g.c./mass spec.) of the tris-trimethylsilyl derivative had a fragment ion, *m/z* 308, two mass units higher than in the unlabelled compound and showed incorporation of *ca.* 93% $^2H_2$, 6% $^2H_1$.

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