Stereospecific Synthesis of α-Deuteriated α-Amino Acids: Regiospecific Deuteriation of Chiral 3-Isopropyl-2,5-dimethoxy-3,6-dihydropyrazines[1]

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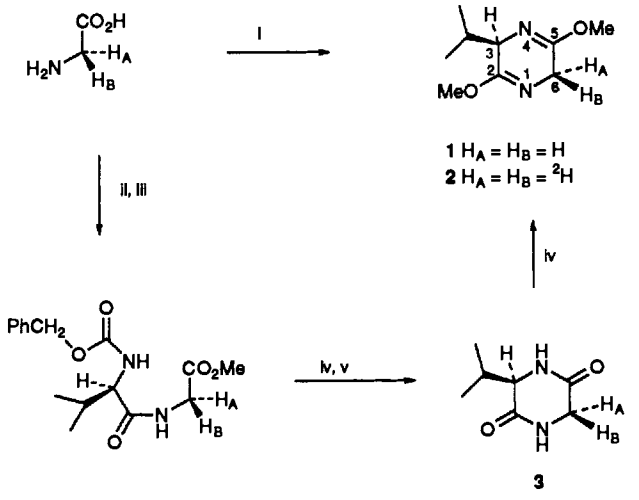
Base-catalysed deuteriation of (3*R*)- or (3*S*)-3-isopropyl-2,5-dimethoxy-3,6-dihydropyrazines in refluxing CH_3O^2H–^2H_2O gives the [6-$^2H_2$]-isotopomer in excellent yields without disturbing the stereogenic centre at C-3. These compounds provide convenient and efficient access to a range of (*R*)- and (*S*)-α-deuteriated α-amino acids, including serine, aspartic acid, allylglycine and phenylalanine, *via* alkylation of the butyllithium generated C-6 anion.

The availability of labelled amino acids in enantiomerically pure form is of prime importance in many biosynthetic and metabolic studies and also in the delineation of enzyme mechanism. Access to some C^{α}-deuteriated and tritiated (2*S*)-α-amino acids, for example, chiral glycine, (2*S*)-aspartic acid and (2*S*)-glutamic acid, has been facilitated through the use of enzymes in appropriately labelled buffer solution.[2,3] The preparation of labelled (2*R*)-amino acids, however, has been much more difficult. Some (2*R*)-α-amino acids [as well as the (*S*)-antipodes] have been prepared through the acylase-catalysed kinetic resolution of racemic mixtures of *N*-acetylated amino acids.[4,5] Here, deuterium or tritium can be introduced by exchanging the acidic hydrogen of the azlactone intermediate during acetylation.[3] Unfortunately, the differential rates for the deacylation vary considerably for the two antipodes of different amino acids, so that enantiomeric excesses can be variable particularly, for the D-isomers.[4,5] Furthermore, some common *N*-acyl amino acids are poor substrates or are not substrates.[5]

Schollkopf's bis-lactam ether methodology has proved to be of enormous utility in the preparation of a wide range of (2*R*)- and (2*S*)-α-amino acids[6,7] and the chiral dihydropyrazine precursors are now commercially available, for example compound 1. Nevertheless, to introduce deuterium at C^{α} of the amino acid product it has been hitherto required to synthesise the [$^2H_2$]dihydropyrazine **2** from [$^2H_2$]glycine, (Scheme 1), a rather expensive and inefficient practice, see below. Here we describe a facile and efficient method of preparing the [$^2H_2$]dihydropyrazine in excellent yield directly from the unlabelled material and demonstrate its use in the preparation of α-deuteriated α-amino acids.

(3*R*)-3-Isopropyl-2,5-dimethoxy-3,6-dihydropyrazine **1** was prepared from glycine and (2*R*)-valine in up to a maximum overall yield of 62% from glycine (Scheme 1; see Experimental section). The conversion of the diketopiperazine **3** into the bis-lactim ether **1** gave variable yields (66–89%) and was wasteful of trimethyloxonium tetrafluoroborate which was required in large excesses (> 3.5 equiv.) to achieve the best yields. Thus, the synthesis of the deuteriated analogue in this manner would require large quantities of deuteriated glycine, and would be very costly. Accordingly, methods for introducing deuterium directly into the molecule at C-6 were considered using the known kinetic preference for C-6 proton abstraction.[6,7] Since both antipodes of 3-isopropyl-2,5-dimethoxy-3,6-dihydropyrazine were commercially available and since solvents containing acidic deuterons are the cheapest source of deuterium, this strategy offered considerable promise in providing access to α-deuteriated amino acids.

Methods based upon repeatedly quenching the BuLi generated anion of the dihydropyrazine **1** with ^2H_2O or



Scheme 1 *Reagents and conditions:* i, As per refs. 5 and 6; ii, $SOCl_2$ (1.2 equiv.), MeOH, 0 °C, then reflux 30 min; iii, isobutyl chloroformate (1 equiv.), *N*-methylmorpholine (2 equiv.), EtOAc–DMF, *N*-benzyloxycarbonyl-(2*R*)-valine (1 equiv.), stir, room temp., 16 h, 87%; iv, H_2, Pd/C, CH_2Cl_2–MeOH (3 : 1); v, PhMe, reflux, 16 h, 45% over 2 steps; vi, $[Me_3O]^+BF_4^-$ (3.5 equiv.), CH_2Cl_2, 66%

MeO^2H were quickly abandoned owing to the formation of an array of by-products after the first cycle. This method also suffered from the potential problem of low selectivity for removing protium from the singly deuteriated compound in the second cycle of anion formation.

Stirring compound **1** in ^2H_2O in the presence of KOH under a variety of conditions also proved to be of little or no utility. At room temperature, the exchange of the C-6 protons was almost undetectable after 3 h, and at higher temperatures, several by-products were formed. However, under optimised conditions in which a 1 mol dm^{-3} solution of the 3-isopropyl-2,5-dimethoxy-3,6-dihydropyrizine **1** was refluxed in MeO^2H–^2H_2O (10 : 1, v/v) containing 1 equiv. of KO^2H, the C-6 deuteriation proceeded smoothly, without the formation of side products or C-3 deuteriated material, and was complete within 3 h.

This pleasing selectivity for C-6 anion formation over C-3 anion formation was further probed by refluxing compound **1** under the optimised exchange conditions for 6 h, a period twice as long as that required for the complete exchange of hydrogen at C-6. Very little deuterium (< 10 atom%) was incorporated at C-3 as judged by ^1H NMR spectroscopy and mass spectrometry. Note that 6-alkyldihydropyrazines (the alkylated products, *vide infra*) were resistant to base-catalysed

deuteriation at C-6 under the same conditions, in accord with expectations.

To confirm that the chiral centre at C-6 of the dideuteriated material **2** was intact, since measuring the extent of deuterium at C-3 (which is subject to a primary isotope effect) might underestimate the extent of racemisation, the dihydropyrazine ring was cleaved under acidic conditions (0.1 mol dm^{-3} HCl, 16 h) to give the valine and glycine methyl esters. These were separated, the valine methyl ester was hydrolysed (5 mol dm^{-3} HCl, reflux, 3 h) and then converted into its free base form with propylene oxide. The optical rotation of the recrystallised valine was −24.2 (*c* 1.02 in 5 mol dm^{-3} HCl). This value compares favourably to that obtained for an authentic sample of (2*R*)-valine [−24.95 (*c* 1.09 in 5 mol dm^{-3} HCl)], and indicates that the enantiomeric excess of the deuterium exchanged material **2** is ≥97%. Thus, the deuteriation had proceeded highly selectively and without disturbing the stereogenic centre at C-3.

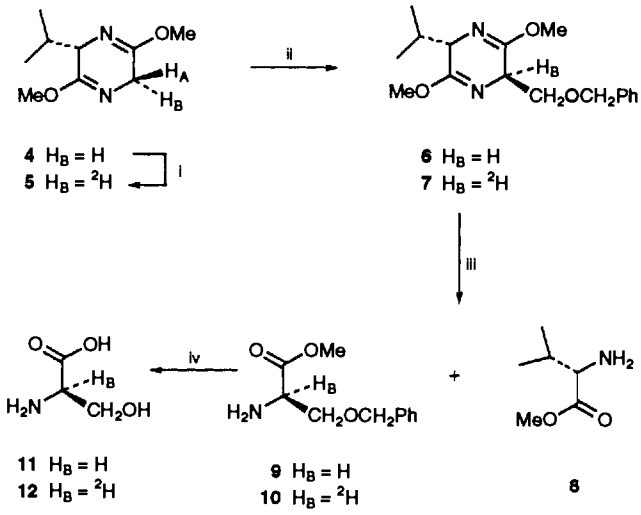
The utility of the enantiomers of the [$^2H_2$]dihydropyrazine **2** in the synthesis of α-deuteriated (2*R*)-serine (Scheme 2) and (2*S*)-serine, and some other (2*S*)-amino acids (Scheme 3) is summarised in Table 1. It was found that in most cases, the alkylation of the lithiated anion of compound **2** proceeded smoothly. As expected, long straight-chain alkyl halides required longer reaction times (up to 24 h) than benzyl chloromethyl ether (16 h). The reaction of the anion with ethyl bromoacetate required only 5 h to complete. The acid hydrolysis (0.2 mol dm^{-3} HCl) of the alkylated dihydropyrazine products to give a mixture of the corresponding amino acid and valine methyl ester, in each case, proceeded cleanly. The separation of the amino acid esters was achieved either by flash chromatography on silica or by distillation.

All of the deuteriated amino acids prepared from 3-isopropyl-2,5-dimethoxy-3,6-dihydropyrazines using these methods [*viz* (2*R*)- and (2*S*)-[2-^{2}H]serine **31**, (2*S*)-[2-^{2}H]phenylalanine **33**, (2*S*)-[2-^{2}H]allylglycine **35** and (2*S*)-[2-^{2}H]aspartic acid **37**] were obtained in moderate to good yield. In all cases, the extent of C^α-deuteriation was >95% and enantiomeric excesses were of the order of 95%. In particular, these protocols were far superior to others employed in an earlier synthesis of (2*S*)-[2-^{2}H]serine in our laboratory starting from (2*S*)-aspartic acid (Scheme 4). These improved methods have been successfully employed in the preparation of both enantiomers of [2-^{2}H]serine *O*-sulphate. These compounds were required to support kinetic and mechanistic studies on the suicide inhibition of *E. coli* glutamic acid and have been described elsewhere.[8]

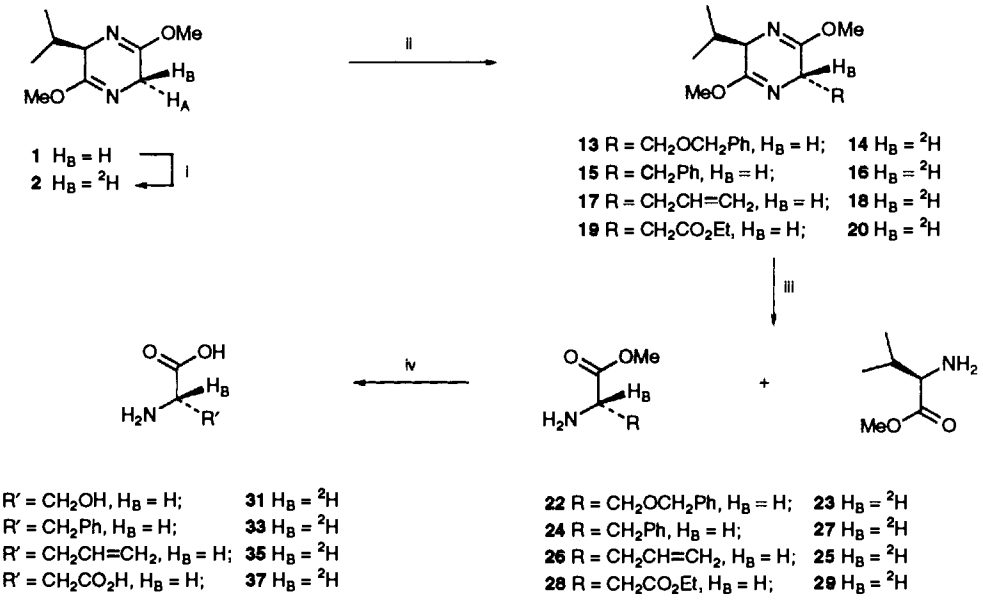
The protocols described herein are suitable for the preparation of a vast array of single enantiomers of natural and unnatural C^α-deuteriated and tritiated amino acids and can easily be extended to provide access to multiply labelled compounds.

Experimental

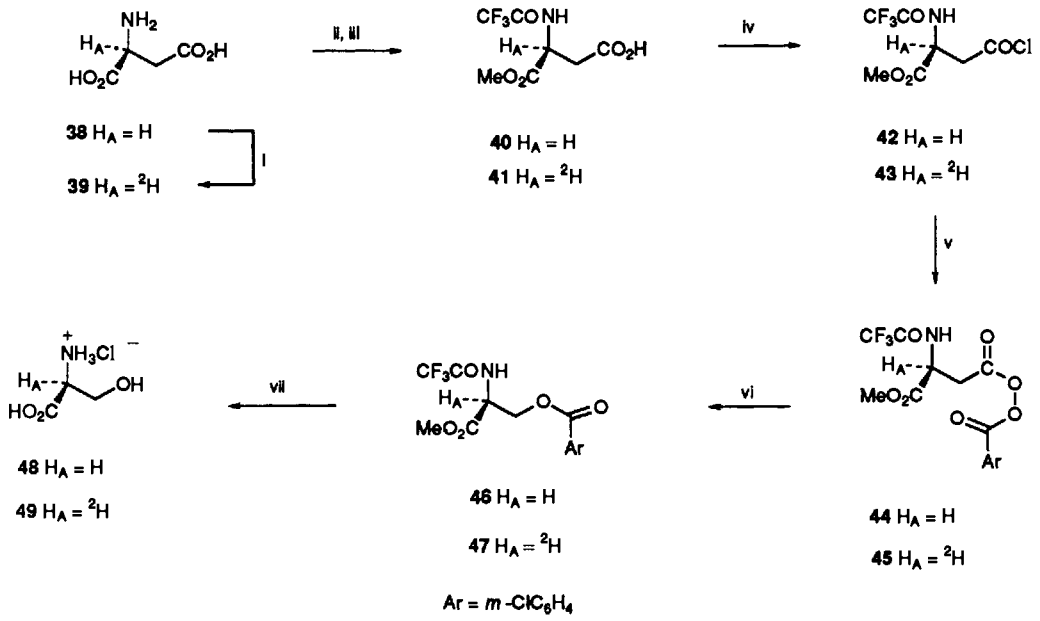
Elemental microanalyses were performed in the departmental microanalytical laboratory. NMR spectra were recorded on a Bruker AM-300 (300 MHz; f.t. ^{1}H NMR, and 74.76 MHz; ^{13}C NMR), or a Varian gemini 200 (200 MHz; f.t. ^{1}H NMR and



Scheme 2 *Reagents and conditions:* i, MeO^{2}H–^2H_2O (10:1, v/v), KOH (1 equiv.), reflux, 3 h, 80%; ii, BuLi (1 equiv.), THF, −90 °C to −60 °C, then $PhCH_2OCH_2Br$ (1.5 equiv.), THF, −90 °C, stir, 16 h, 56%; iii, 0.2 mol dm^{-3} HCl (2 equiv.), room temp., stir, 16 h; then separation on flash silica (19:1, Et_2O–EtOH), 70%; iv, 5 mol dm^{-3} HCl, reflux, 3 h; then EtOH, propylene oxide, reflux, 15 min, 89%



Scheme 3 *Reagents and conditions:* i, MeO^{2}H–^2H_2O (10:1, v/v), KOH (1 equiv.), reflux, 3 h; ii, BuLi (1 equiv.), THF, −90 °C to −60 °C, then RBr (1.5 equiv.), THF, −90 °C, stir, 5–20 h; iii, 0.2 mol dm^{-3} HCl (2 equiv.), room temp., stir, 16 h; then separation (chromatography for R = CH_2OCH_2Ph, $CH_2CH{=}CH_2$, CH_2CO_2Et, distillation for R = CH_2Ph); iv, 5 mol dm^{-3} HCl, reflux, 3 h; then EtOH, propylene oxide, reflux, 15 min, 89%



Scheme 4 *Reagents and conditions:* i, ^2H_2O, PLP, ATT, pH 7.25, 37 °C, 3 days, 72%; ii, TFAA (10 equiv.), THF, 0 °C to room temp., 2 h; iii, MeOH, 0 °C to room temp., 20 min, 85% of the α and β esters over two steps; iv, $SOCl_2$, reflux, 2 h, 59%; v, *m*CPBA, pyridine (1.5 equiv.), ether, 0 °C, 4 h, 40%; vi, CCl_4, 6 days, 27%; vii, 5 mol dm^{-3} HCl, 2 h, 100%

Table 1

Compound (stereochemistry and label at C^α)	Overall yield (%) (from *R*- or *S*-**1** or **2**)	$[\alpha]_D^{23}$
Serine		
11 (*R*-, H)	20	−13.6 (*c* 1.02 in 1 mol dm^{-3} HCl)
12 (*R*-, ^2H)	35	−13.5 (*c* 1.025 in 1 mol dm^{-3} HCl)
30 (*S*-, H)	25	+13.2 (*c* 1 in 1 mol dm^{-3} HCl)
31 (*S*-, ^2H)	20	+12.7 (*c* 1.01 in 1 mol dm^{-3} HCl)
Phenylalanine		
32 (*S*-, H)	30	−30.9 (*c* 2.035 in H_2O)
33 (*S*-, ^2H)	35	−28.2 (*c* 1.5 in H_2O)
Allylglycine		
34 (*S*-, H)	15	−5.7 (*c* 2.0 in 5 mol dm^{-3} HCl)
35 (*S*-, ^2H)	20	−4.2 (*c* 1.87 in 5 mol dm^{-3} HCl)
Aspartic acid		
36 (*S*-, H)	15	+21.8 (*c* 0.495 in 5 mol dm^{-3} HCl)
37 (*S*-, ^2H)	15	+12.7 (*c* 0.495 in 5 mol dm^{-3} HCl)

50.31 MHz; ^{13}C NMR) spectrometers. ^1H NMR spectra recorded at 400 MHz were obtained from the SERC NMR service at Warwick University. ^1H NMR spectra were referenced internally on ^2HOH (4.68 p.p.m.), $CHCl_3$ (7.27 p.p.m.) or DMSO (2.47 p.p.m.). ^{13}C NMR spectra were referenced on CH_3OH (49.9 p.p.m.), C^2HCl_3 (77.5 p.p.m.), or DMSO (39.70 p.p.m.). IR spectra were recorded on a Perkin-Elmer 1710 FT IR spectrometer. The samples were prepared as Nujol mulls, solutions in chloroform or thin films between sodium chloride discs. Mass spectra and accurate mass measurements were recorded on a VG 70-250 SE, a Kratos MS-50 or by the SERC service at Swansea using a VG AZB-E. Fast atom bombardment (FAB) spectra were recorded using glycerol as a matrix. Major peaks are given as percentages of the base peak intensity (100%). Melting points were taken on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured at 23 °C on an Optical Activity AA-100 polarimeter using 10 or 20 cm path length cells and are given in units of 10^{-1} deg cm^2 g^{-1}. Flash chromatography was performed according to the method of Still *et al.*[9] using Sorbsil C 60 (40–60 μm mesh) silica gel. Analytical thin layer chromatography was carried out on 0.25 mm precoated silica gel plates (Macherey-Nagel SIL g/UV_{254}) and compounds were visualised using UV fluorescence, iodine vapour, ethanolic phosphomolybdic acid, or ninhydrin. Amino acid substrates, buffers, salts and deuterium oxide were obtained from Sigma Chemical Co. (Poole, Dorset, UK). All other chemicals were of analytical grade or were recrystallised or redistilled before use.

N-Benzyloxy-carbonyl-(2R)-valinylglycine Methyl Ester.—*N*-Benzyloxycarbonyl-(2*R*)-valine (9.4 g, 37.4 mmol) was dissolved in dry THF (125 cm^3) and the solution cooled to −5 °C in an ice–salt bath. *N*-Methylmorpholine (4.1 cm^3, 37.4 mmol) and isobutyl chloroformate (4.8 cm^3, 37.4 mmol) were added to the stirred solution followed after 3 min, by a suspension of glycine methyl ester hydrochloride salt (4.68 g, 37.4 mmol) in DMF (20 cm^3) and *N*-methylmorpholine (4.8 cm^3, 37.4 mmol). The reaction mixture was stirred overnight and then diluted with water (100 cm^3). The solution was concentrated to 100 cm^3 under reduced pressure and, with time, provided a white precipitate. This was filtered off and recrystallised from dichloromethane–light petroleum to give white crystals of the protected dipeptide (10.44 g, 87%), m.p. 139–140 °C (lit.,[10,11] 130–132, 151–153 °C; $[\alpha]_D^{23}$ +6.8 (*c* 1.0 in CH_2Cl_2); ν_{max}(Nujol)/cm^{-1} 3290 (NH str), 2800–3000 (CH str), 1750 (ester C=O), 1685 (urethane C=O) and 1645 and 1535 (amide); δ_H(200 MHz; C^2HCl_3) 0.93, 0.97 (6 H, 2 d, *J* 7, 2 × Pr^i CH_3), 2.1 (1 H, m, *J* 7, Pr^i CH), 3.7 (3 H, s, 1-OCH_3), 3.9–4.3 (3 H, m, 2′-CH and 2-CH_2), 5.1 (2 H, s, PhCH_2), 5.6 and 6.9 (2 × NH) and 7.35 (5 H, m, ArH); δ_C(50 MHz; C^2HCl_3) 17.69, 19.12 (2 × Pr^i CH_3), 31.04 (Pr^i CH), 41.01 (2-CH_2), 52.26 (1-OCH_3), 60.21 (2′-CH), 67.00 (PhCH_2), 127.92, 128.1, 128.47, 136.18 (ArC) and 156.18 (urethane C=O); *m*/*z* (FAB) 323 ($[M + H]^+$, 100%).

Cyclo-[(2R)-val-gly] **3**.—*N*-Benzyloxycarbonyl-(2*R*)-valinylglycine methyl ester (2.21 g, 6.8 mmol) was dissolved in dry methanol (12 cm^3) and dry dichloromethane (36 cm^3) and 10% Pd/C (100 mg) were added to the solution. Hydrogen gas was bubbled through the solution until the starting material was no longer detected by TLC. After *ca.* 24 h, the solution was filtered

through Celite and was concentrated to dryness under reduced pressure. The residue was dissolved in dry toluene (35 cm^3) and refluxed for 12 h to complete cyclisation. The resulting suspension was cooled to 0 °C and the precipitated white solid was filtered off, washed on the pad with diethyl ether and recrystallised from hot water (0.48 g, 45% [first crop]), m.p. 257–259 °C; $[\alpha]_D^{23}$ −25.3 (*c* 0.9 in H_2O) [lit.,[12] $[\alpha]_D$ 20.2 (*c* 0.9 in H_2O) for the (3*S*)-isomer]; ν_{max}(Nujol)/cm^{-1} 3180 (NH str), 2800–3000 (CH str) and 1670 (amide C=Os); δ_H(200 MHz; ^2H_2O) 0.87, 0.96 (6 H, dd, *J* 7, 2 × Pr^i CH_3), 2.2 (1 H, dsept. *J* 7, Pr^i CH), 4.09 (2 H, dd, *J* 17, 6-CH_2) and 4.11 (1 H, d, *J* 3, 3-H); δ_C(75 MHz; ^2H_2O) 18.47, 20.70 (2 × Pr^i CH_3), 35.72 (Pr^i CH), 46.54 (6-CH_2), 62.79 (3-CH), 171.72 and 173.13 (C=Os); *m/z* (CI) 157 ([M + H]$^+$, 100%) and 114 (82.5 [M − $CH(CH_3)_2$ + H]$^+$). A second crop of crystals (0.21 g, 20%) of similar purity was obtained from the mother liquor over a prolonged period of time.

(3R)-3-*Isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **1**.—A suspension of *cyclo*-[(2*R*)-val-gly] **3** (400 mg, 2.56 mmol) and trimethyloxonium tetrafluoroborate (950 mg, 6.41 mmol) in dry dichloromethane (10 cm^3) was stirred vigorously under argon. After 24 h a further portion of trimethyloxonium tetrafluoroborate (380 mg, 2.56 mmol) was added to the reaction mixture which was then stirred for a further 48 h. To the resulting mixture a solution of disodium hydrogen phosphate (8.5 g) and dihydrogen sodium phosphate (2.25 g) in water (40 cm^3) was added. The organic phase was separated, and the aqueous phase was extracted with dichloromethane (3 × 10 cm^3). The pooled extracts were dried ($MgSO_4$) and evaporated under reduced pressure to yield a yellow oil (0.31 g, 66%); *m/z* (Found: M$^+$, 184.1212. $C_9H_{16}N_2O_2$ requires 184.1212); $[\alpha]_D^{23}$ −120.7 (*c* 1.0 in ethanol) [lit.,[7] $[\alpha]_D^{20}$ 106.3 (*c* 1.0 in ethanol) for the (6*S*)-isomer]; ν_{max}(neat)/cm^{-1} 2940 (CH) and 1680 (C=N); δ_H(200 MHz; C^2HCl_3) 0.75, 1.03 (6 H, 2 d, *J* 7, 2 × Pr^i CH_3), 2.22 (1 H, dsp, Pr^i CH), 3.68, 3.71 (6 H, 2 s, 2 and 5-OCH_3), 4.0 (1 H, m, 3-CH) and 4.0 (2 H, m, 6-CH_2); δ_C(75 MHz; C^2HCl_3) 16.9, 18.2 (2 × Pr^i CH_3), 32.42 (Pr^i CH), 46.54 (6-CH_2), 52.47, 52.51 (2 and 5-OCH_3), 61.02 (3-CH), 162.28 and 164.85 (2 and 5-C); *m/z* (EI) 185 ([M + H]$^+$, 30%) and 171 (100, [M − CH]$^+$).

Higher yields were obtained by using greater excesses of trimethyloxonium tetrafluoroborate.

(3R)-[6-$^2H_2$]-2,5-*Dimethoxy*-3-*isopropyl*-3,6-*dihydropyrazine* **2**.—The dihydropyrazine **1** (1.09 g, 5.9 mmol) was dissolved in $C^2H_3O^2H$ (5 cm^3) and KO^2H (5.9 mmol; preformed from KOH and ^2H_2O) in ^2H_2O (0.5 cm^3) was added to the solution. The solution was refluxed under nitrogen for 3 h and then cooled and treated with water (10 cm^3) containing HCl (1 equiv.). The solution was then extracted with dichloromethane (3 × 10 cm^3) and the extract dried ($MgSO_4$) and concentrated under reduced pressure to give the dideuteriated isotopomer (0.8 g, 80%), $[\alpha]_D^{23}$ −74.2 (*c* 1.02 in ethanol); ν_{max}(neat)/cm^{-1} 2800–3000 (CH str) and 1697 (C=N); δ_H(200 MHz; C^2HCl_3) 0.78, 1.01 (6 H, 2 d, *J* 7, 2 × Pr^i CH_3), 2.1–2.3 (1 H, m, Pr^i CH), 3.69, 3.73 (6 H, 2s, 2 and 5-OCH_3) and 3.97 (1 H, d, *J* 3.8, 3-CH); δ_C(50 MHz; C^2HCl_3) 16.91, 18.96 (2 × Pr^i CH_3), 32.37 (Pr^i CH), 52.42 (2 and 5-OCH_3), 60.99 (3 CH), 162.26 and 164.81 (2 and 5-C); δ_D(61.4 MHz; $CHCl_3$) 3.97 and 3.93 (6-C^2H_2); *m/z* (CI) 187 (M$^+$, 100%). The material was at least 95% dideuteriated as judged by comparison of the NMR and mass spectra for the labelled and unlabelled materials. Hydrolysis of the compound under acidic conditions (0.1 mol dm^{-3} HCl, 16 h) to give the valine and glycine methyl esters, followed by separation and hydrolysis (5 mol dm^{-3} HCl, reflux, 3 h) of the valine methyl ester to give valine·HCl, followed by conversion into its free base form with propylene oxide and recrystallisation, gave valine $[\alpha]_D^{23}$ −24.2 (*c* 1.01 in 5 mol dm^{-3} HCl). An authentic sample of (2*R*)-valine gave an $[\alpha]_D^{23}$ value of −24.95 (*c* 1.09 in 5 mol dm^{-3} HCl).

(3S)-[6-$^2H_2$]-3-*Isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **5**.—This compound was prepared in a manner identical with that for the 3,6-dihydropyrazine **2**, using commercial (3*S*)-3-isopropyl-2,5-dimethoxy-3,6-dihydropyrazine **4**, in 94% yield, $[\alpha]_D^{23}$ +65.6 (*c* 1.38 in ethanol); ν_{max}(neat)/cm^{-1} 2800–3000 (CH str) and 1697 (C=N); δ_H(360 MHz; C^2HCl_3) 0.75, 1.03 (6 H, 2 d, *J* 7, 2 × Pr^i CH_3), 2.23 (1 H, m, Pr^i CH), 3.68, 3.72 (6 H, 2 s, 2 and 5-OCH_3) and 3.99 (1 H, d, *J* 3.8, 3-CH); δ_C(90 MHz; C^2HCl_3) 16.92, 18.92 (2 × Pr^i CH_3), 32.31 (Pr^i CH), 46.23 (t, *J* 21.42, 6-C^2H), 52.43, 52.37 (2 and 5-OCH_3), 61 (3-CH), 162.27 and 164.84 (2 and 5-C); *m/z* (CI) 187 ([M + H]$^+$, 100%).

(3R,6S)-6-*Benzyloxymethyl*-3-*isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **13**.—BuLi (2 mol dm^{-3} in hexane; 750 mm^3, 1.474 mmol) was added to a stirred solution of dihydropyrazine **1** (0.25 g, 1.34 mmol) in dry THF (3.5 cm^3) at −80 °C. The solution was allowed to warm to −60 °C, to allow the formation of the anion, and a solution of benzyl chloromethyl ether (0.315 g, 2.01 mmol) in dry THF (750 mm^{-3}) at −70 °C was added dropwise to it with stirring at −70 °C. After 10 h, the reaction mixture was allowed to warm to room temperature after which it was evaporated under reduced pressure. The residual oil was stirred with 100 mmol dm^{-3} potassium phosphate solution (pH 7; 5 cm^3) and the suspension was extracted with diethyl ether (4 × 5 cm^3). The extracts were pooled, dried ($MgSO_4$), concentrated under reduced pressure and then purified by flash chromatography on silica eluting with petroleum–ethyl acetate (9 : 1) to give a pale yellow oil (250 mg, 60%) (Found: C, 67.25; H, 8.1; N, 9.5. Calc. for $C_{17}H_{24}N_2O_3$: C, 67.1; H, 7.95; N, 9.2%); *m/z* (Found: [M + H]$^+$ 305.18651. $C_{17}H_{25}N_2O_3$ requires 305.18651); $[\alpha]_D^{23}$ +2.6 (*c* 0.8 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 2800–3000 (CH str), 1685 (C=N), 1230, 1065 [C(R)OMe] and 1120 (CH_2OCH_2Ph); δ_H(200 MHz; C^2HCl_3) 0.71, 1.08 (6 H, 2 d, *J* 6.8, 2 × Pr^i CH_3), 2.32 (1 H, m, Pr^i CH), 3.72 (6 H, 2 s, 2 and 5-OCH_3), 3.7–4.2 (4 H, m, 3′-CH_2, 3-CH, 6-CH), 4.55 (2 H, s, PhCH_2O) and 7.3 (5 H, s, ArH); δ_C(75 MHz; C^2HCl_3) 16.47, 19.02 (2 × Pr^i CH_3), 31.37 (Pr^i CH), 52.53, 52.41 (2-OCH_3 and 5-OCH_3), 56.78 (6-CH), 60.69 (3-CH), 71.29, 73.08 (CH_2OCH_2Ph), 127.29, 128.11, 138.45 (ArC), 161.49 and 164.81 (2 and 5-C); *m/z* (EI) 305 ([M + H]$^+$, 100) and 91 (30, [$PhCH_2$]$^+$).

(3R,6S)-[6-^{2}H]-6-*Benzyloxymethyl*-3-*isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **14**.—This was prepared in a manner identical with that for the 3,6-dihydropyrazine **13**, from 3,6-dihydropyrazine **2**, in 60% yield; *m/z* (Found: [M + H]$^+$, 306.1930. $C_{17}H_{24}N_2O_3{}^2H$ requires 306.1928); $[\alpha]_D^{23}$ +2.52 (*c* 0.396 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 2800–3000 (CH str), 1670 (C=N) and 1230 [C(R)OMe]; δ_H(200 MHz; C^2HCl_3) 0.70, 1.01 (6 H, 2 d, *J* 6.8, 2 × Pr^i CH_3), 2.15–2.4 (1 H, m, Pr^i CH), 3.6–3.9 (8 H, s and d, d, 2-OCH_3, 5 OCH_3 and CH_2OCH_2Ph), 4.02 (1 H, d, *J* 3.4, 3-CH), 4.54 (2 H, s, CH_2Ph) and 7.2–7.4 (5 H, m, ArH); δ_C(75 MHz; C^2HCl_3) 16.54, 19.13 (2 × Pr^i CH_3), 31.41 (Pr^i CH), 52.43, 52.54 (2 and 5-OCH_3), 56.40 (t, *J* 21, 6-C^2H), 60.75 (3-CH), 71.3, 73.16 (CH_2OCH_2Ph), 127.36, 128.19, 138.53 (ArC), 161.54 and 164.95 (2 and 5-C); δ_D (61.4 MHz; $CHCl_3$) 4.08 (6-C^2H).

(3S,6R)-6-*Benzyloxymethyl*-3-*isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **6**.—This compound was prepared in a manner identical with that for the dihydropyrazine **13**, from the dihydropyrazine **4**, in 56% yield; *m/z* (Found: [M + H]$^+$, 305.187. $C_{17}H_{25}N_2O_3$ requires 305.1865); $[\alpha]_D^{23}$ −3.0 (*c* 0.7 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 2900–3000 (CH str), 1690 (C=N) and

[C(R)OMe]; δ_H(200 MHz; C^2HCl_3) 0.7, 1.08 (6 H, 2 d, *J* 7.5, 2 × Pr^i CH_3), 2.27 (1 H, m, Pr^i CH), 3.7 (6 H, s, 2 and 5-OCH_3), 3.8 (2 H, m, CH_2OCH_2Ph), 4.03, 4.08 (2 H, 2 m, 3-CH and 6-CH), 4.55 (2 H, s, OCH_2Ph) and 7.3 (5 H, m, ArH); δ_C(50 MHz; C^2HCl_3) 16.54, 19.11 (2 × Pr^i CH_3), 31.43 (Pr^i CH), 52.39, 52.48 (2 and 5-OCH_3's), 56.87 (6-CH), 60.76 (3-CH), 71.36, 73.15 (CH_2OCH_2Ph), 127.35, 128.18, 138.53 (ArC), 161.55 and 164.88 (2- and 5-C).

(3S,6R)[6-^{2}H]-6-*Benzyloxymethyl*-3-*isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **7**.—This compound was prepared in a manner identical with that for the dihydropyrazine **13**, from the dihydropyrazine **5**, in 54% yield; $[\alpha]_D^{23}$ −2.7 (*c* 0.75 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 2800–3000 (CH str), 1698 (C=N) and 1240 [C(R)OMe]; δ_H(360 MHz; C^2HCl_3) 0.67, 1.07 (6 H, 2 d, *J* 7, 2 × Pr^i CH_3), 2.27 (1 H, m, Pr^i CH), 3.81 (6 H, 2 s, 2 and 5-OCH_3), 3.7–3.85 (2 H, d, d, CH_2OCH_2Ph), 4.0 (1 H, d, *J* 1.6, 3-CH), 4.53 (2 H, s, CH_2Ph) and 7.22–7.34 (5 H, m, ArH); δ_C(90 MHz; C^2HCl_3) 16.5, 19.1 (2 × Pr^i CH_3), 31.4 (Pr^i CH), 52.4 (2 and 5-OCH_3), 56.4 (t, *J* 21, 6-C^2H), 60.80 (3-CH), 71.3, 73.2 (CH_2OCH_2Ph), 127.4, 128.2, 139 (ArC), 161.5 and 165 (2 and 5-C); *m/z* (EI) 306 ([M + H]$^+$, 100%).

(2S)-O-*Benzylserine Methyl Ester* **22**.—The dihydropyrazine **13** (0.19 g, 0.625 mmol) was added to a 0.1 mol dm^{-3} solution of hydrochloric acid (12.5 cm^3) and the mixture was stirred at room temperature overnight. The aqueous solution was extracted with diethyl ether (3 × 10 cm^3) and then concentrated under reduced pressure. The residue was dissolved in water (2 cm^3) and aqueous 10 mol dm^{-3} ammonia solution (0.2 cm^3) and then extracted with diethyl ether. The ether extract was dried ($MgSO_4$) and concentrated to dryness under reduced pressure and the (2*R*)-valine and (2*S*)-*O*-benzylserine methyl esters were separated by flash silica chromatography eluting with diethyl ether–ethanol (19:1), to give (2*S*)-*O*-benzylserine methyl ester as a colourless oil (40 mg, 31%), *m/z* (Found: [M + H]$^+$, 210.1130. $C_{11}H_{16}NO_3$ requires 210.1130); ν_{max}(Nujol)/cm^{-1} 3200 (NH str), 2800–3000 (CH str and Nujol) and 1730 (C=O); $[\alpha]_D^{23}$ +1.24 (*c* 1.21 in CH_2Cl_2) [lit.,[13] $[\alpha]_D^{20}$ −2.8 (*c* 1.0 in EtOH) for (2*R*)-*O*-benzylserine methyl ester)]; δ_H(200 MHz; C^2HCl_3) 1.95 (2 H, br s, NH_2), 3.64–3.74 (6 H, m, CO_2CH_3, 2-CH, 3-CH_2), 4.53 (2 H, s, OCH_2Ph) and 7.32 (5 H, m, ArH); δ_C(50 MHz; C^2HCl_3) 52.09 (CH), 54.80 (CO_2CH_3), 71.83, 73.20 (3-CH_2 and 3'-CH_2), 127.55, 127.67, 128.33, 137.76 (ArC) and 174.07 (ester C=O).

(2S)-[2-^{2}H]-O-*Benzylserine Methyl Ester* **23**.—This compound was prepared in a manner identical with that for the ester **22**, starting from the dihydropyrazine **14**, in 51% yield; *m/z* (Found: [M + H]$^+$, 211.1193. $C_{11}H_{15}NO_3{}^2H$ requires 211.1193); $[\alpha]_D^{23}$ +0.83 (*c* 1.02 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 2800–3000 (CH str) and (C=O); δ_H(200 MHz; C^2HCl_3) 1.95 (br s, NH_2), 3.7 (5 H, m, CH_2 and CO_2CH_3), 4.55 (2 H, s, CH_2Ph) and 7.3 (5 H, m, ArH); δ_C(50 MHz; C^2HCl_3) 51.82 (OCH_3), 54.22 (t, *J* 20.55, 2-C^2H), 71.59, 72.95 (3-CH_2 and CH_2Ph), 127.34, 127.45, 128.12, 137.59 (ArC) and 173.9 (C=O); δ_D(61.4 MHz; $CHCl_3$) 3.58 (2-C^2H).

(2R)-O-*Benzylserine Methyl Ester* **9**.—This compound was prepared in a manner identical with that for the ester **22**, from the dihydropyrazine **6**, in 61% yield; *m/z* (Found: [M + H]$^+$, 210.1130. $C_{11}H_{16}NO_3$ requires 210.1130); $[\alpha]_D^{23}$ −2.22 (*c* 0.9 in CH_2Cl_2) [lit.,[13] $[\alpha]_D^{20}$ −2.8 (*c* 1.0 in EtOH)]; ν_{max}(neat)/cm^{-1} 3600br (NH_2 str), 2800–3000 (CH str) and 1742 (CO_2Me); δ_H(200 MHz; C^2HCl_3) 2.15 (2 H, br s, NH_2), 3.7 (6 H, m, OCH_3, 2-CH, 3-CH_2), 4.5 (2 H, s, $PhCH_2O$) and 7.3 (5 H, m, ArH); δ_C(50 MHz; C^2HCl_3) 51.92 (OCH_3), 54.56 (2-CH), 71.58 (3-CH_2), 73.00 ($PhCH_2$), 127.38, 127.51, 128.16, 137.59 (ArC) and 173.9 (C=O).

(2R)-[2-^{2}H]-O-*Benzylserine Methyl Ester* **10**.—This compound was prepared in a manner identical with that for the ester **22**, from the dihydropyrazine **7**, in 70% yield; *m/z* (Found: [M + H]$^+$, 211.1193. $C_{11}H_{15}NO_3{}^2H$ requires 211.1193); $[\alpha]_D^{23}$ −0.32 (*c* 0.932 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 2800–3000 (CH str) and 1740 (C=O); δ_H(300 MHz; C^2HCl_3) 1.56 (br s, NH_2), 3.7 (5 H, m, CH_2 and CO_2CH_3), 4.53 (2 H, s, CH_2Ph) and 7.35 (5 H, m, ArH); δ_C(75 MHz; C^2HCl_3) 51.97 (OCH_3), 54.34 (t, *J* 21, 2-C^2H), 71.68, 73.08 (3-CH_2 and CH_2Ph), 127.43, 127.56, 128.22, 137.65 (ArC) and 174 (C=O).

(2S)-*Serine* **30**.—The ester **22** (0.36 g, 1.72 mmol) was added to 6 mol dm^{-3} HCl (10 cm^3) and the mixture was refluxed for 3 h. The solvents were removed under reduced pressure, and the residue was dried over phosphorus pentoxide then dissolved in dry ethanol (30 cm^3). Propylene oxide (30 cm^3) was added to the solution and the mixture was then refluxed for 15 min. After this the solvents were removed under reduced pressure, and the residue was recrystallised from aqueous ethanol to give a white crystalline solid (0.1 g, 55%), m.p. 214 °C (decomp) [lit.,[14] 228 °C (decomp.)] (Found: C, 34.2; H, 6.4; N, 13.05. Calc. for $C_3H_7NO_3$: C, 34.3; H, 6.7; N, 13.35%); $[\alpha]_D^{23}$ +13.2 (*c* 1.0 in 1 mol dm^{-3} HCl) [lit.,[15,16] $[\alpha]_D^{23}$ +14.5 (*c* 1.0 in 1 mol dm^{-3} HCl)]; ν_{max}(Nujol)/cm^{-1} 3400 (NH_2 str), 3000–2800 (CH str) and 1590 (CO_2^-); δ_H(200 MHz; ^2H_2O–NaO^2H) 3.19 (1 H, t, *J* 5, 2 CH) and 3.57 (2 H, m, 3 CH_2); δ_C(75 MHz; ^2H_2O–NaO^2H–MeOH) 58.58 (2 CH), 65.76 (3 CH_2) and 181.65 (C=O); *m/z* (CI) 106 ([M + H]$^+$, 100%), 88 (35, [M − OH]$^+$) and 44 (50, CO_2^+).

(2S)-[2-^{2}H]-*Serine* **31**.—This compound was prepared in a manner identical with that for (2*S*)-serine **30**, using the ester **23**, in 72% yield (95% deuteriated by ^{1}H NMR); m.p. 220–223 °C [lit.,[14] 228 °C (decomp.) for (2*S*)-serine] (Found: C, 34.25; H, 5.95; N, 13.35%; M$^+$, 106.0489. Calc. for $C_3H_6NO_3{}^2H$: C, 33.95; H, 5.7; N, 13.2%; *M*$^+$, 106.0489); $[\alpha]_D^{23}$ +12.72 (*c* 1 in 1 mol dm^{-3} HCl) [lit.,[15,16] $[\alpha]_D^{23}$ +14.5 (*c* 1.0 in 1 mol dm^{-3} HCl) for (2*S*)-serine)]; ν_{max}(Nujol)/cm^{-1} 3400 (NH_2 str), 3000–2800 (CH str) and 1597 (CO_2^-); δ_H(400 MHz; ^2H_2O–NaO^2H) 3.51, 3.33 (2 H, 2 d, *J* 11, 3-CH_2); δ_C(100 MHz; ^2H_2O–NaO^2H) 59.10 (t, *J* 20.85, 2-C^2H), 66.19, (3-CH_2) and 182.32 (C=O); δ_D(61.4 MHz; H_2O–NaOH) 3.24 (2-C^2H); *m/z* (CI) 107 ([M + H]$^+$, 100%), 61 (80, [M − CO_2H]$^+$) and 45 (30, CO_2H^+).

(2R)-*Serine* **11**.—This compound was prepared in a manner identical with that for (2*S*)-serine **30**, from the ester **9**, in 20% yield; m.p. 221–223 °C (decomp.) [lit.,[14] 228 °C (decomp.)] (Found: C, 34.4; H, 6.8; N, 13.1. Calc. for $C_3H_7NO_3$: C, 34.3; H, 6.7; N, 13.35%); *m/z* (Found: [M + H]$^+$, 106.050. $C_3H_8NO_3$ requires 106.050); $[\alpha]_D^{23}$ −13.6 (*c* 1.02 in 1 mol dm^{-3} HCl) [lit.,[15,16] $[\alpha]_D^{23}$ +14.5 (*c* 1.0 in 1 mol dm^{-3} HCl) for (2*S*)-serine]; ν_{max}(Nujol)/cm^{-1} 3459 (NH/OH str), 2800–3000 (CH str) and 1597 (CO_2^-); δ_H(200 MHz; ^2H_2O–NaO^2H) 3.22 (1 H, t, *J* 5, 2-CH) and 3.60 (2 H, m, 3-CH_2); δ_C(50 MHz; ^2H_2O–NaO^2H) 58.12 (2-CH), 64.17 (3-CH_2) and 178.75 (CO_2H); *m/z* (CI) 106 ([M + H]$^+$, 100%), 60 (42, [M − CO_2H]$^+$) and 44 (12, CO_2^+).

(2R)-[2-^{2}H]-*Serine* **12**.—This compound was prepared in a manner identical with that for (2*S*)-serine **30**, using the ester **6**, in 89% yield (95% deuteriated by ^{1}H NMR spectroscopy); m.p. 194 °C (Found: C, 33.95; H, 6.0; N, 13.05%; M$^+$, 106.0489. Calc. for $C_3H_6NO_3{}^2H$: C, 33.95; H, 5.7; N, 13.2%; *M*$^+$, 106.0489); $[\alpha]_D^{23}$ −13.5 (*c* 1.03 in 1 mol dm^{-3} HCl); ν_{max}(Nujol)/cm^{-1} 3200–2400 (NH_2, OH and CH str) and 1661 (amino acid CO_2H); δ_H(400 MHz; ^2H_2O) 3.47, 3.29 (2 H, 2 d, *J* 11, 3 CH_2) and 3.04 (0.05 H, m, 2-CH undeuteriated); δ_C(100

MHz; ^2H_2O–NaO^2H) 58.81 (t, *J* 20, 2-C^2H), 65.89 (3-CH_2) and 182.02 (C=O); δ_D(61.4 MHz; H_2O–NaOH) 3.21 (2-C^2H); *m/z* (CI) 107 ($[M + H]^+$, 100%), 61 (60, $[M - CO_2H]^+$) and 45 (20, CO_2H^+).

(3R,6S)-6-*Benzyl*-3-*isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **15**.—The dihydropyrazine **1** (250 mg, 1.36 mmol) was dissolved in dry THF (3 cm^3) and the solution cooled to −80 °C. To this was added dropwise 2 mol dm^{-3} BuLi (0.68 cm^3, 1.36 mmol) followed, after 30 min, by a solution of benzyl bromide (349 mg, 2.04 mmol) in dry THF (3 cm^3). The mixture was stirred at −80 °C overnight after which it was concentrated to dryness under reduced pressure and treated with potassium phosphate buffer (100 mmol dm^{-3}, pH 7; 20 cm^3). The suspension was extracted with diethyl ether (3 × 20 cm^3) and the combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure to provide a residue. This was purified by flash chromatography on silica eluting with petroleum–diethyl ether (9:1) to give a colourless oil (260 mg, 70%), *m/z* (Found: $[M + H]^+$, 275.176. $C_{16}H_{23}N_2O_2$ requires 275.1756); $[\alpha]_D^{23}$ +65.4 (*c* 1.6 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 2944, 2871 (CH str), 1698 (C=N) and 1495 (Ph); δ_H(200 MHz; C^2HCl_3) 0.61, 0.95 (6 H, 2 d, *J* 6.8, 2 × Pr^i CH_3), 2.15 (1 H, dsp, Pr^i CH), 3.10 (2 H, d, *J* 4.6, CH_2Ph), 3.27 (1 H, dd, *J* 3.4, 3-CH), 3.70, 3.73 (6 H, 2 s, 2 and 5-OCH_3), 4.35 (1 H, dd, *J* 4.6, 6-CH) and 7.07–7.23 (5 H, m, ArH); δ_C(75 MHz; C^2HCl_3) 16.39, 18.93 (2 × Pr^i CH_3), 31.1 (Pr^i CH), 39.95 (CH_2Ph), 52.07, 52.31 (2 and 5-OCH_3), 56.59 (6-CH), 60.16 (3-CH), 126.24, 127.76, 129.95, 137.26 (ArC), 162.38 and 163.91 (2 and 5-C); *m/z*(EI) 275 ($[M + H]^+$, 100%), 183 (20, $[M - PhCH_2]^+$) and 141 (30, $[M - PhCH_2 - CH(CH_3)_2 + H]^+$).

(3R,6S)-[6-^{2}H]-6-*Benzyl*-3,6-*dihydropyrazine*-3-*isopropyl*-2,5-*dimethoxy* **16**.—This compound was prepared in a manner identical with that for the dihydropyrazine **15**, starting from the dihydropyrazine **2**, in 88% yield; *m/z* (Found: $[M + H]^+$, 276.1822. $C_{16}H_{22}N_2O_2{}^2H$ requires 276.1822); ν_{max}(neat)/cm^{-1} 2944 (CH str), 1696 (C=N) and 1496 (Ph); $[\alpha]_D^{23}$ +40.4 (*c* 1.55 in CH_2Cl_2); δ_H(200 MHz; C^2HCl_3) 0.64, 0.97 (6 H, 2 d, *J* 6.8, 2 × Pr^i CH), 2.18 (1 H, dsp, Pr^i CH), 3.11 (2 H, s, CH_2Ph) 3.28 (1 H, d, *J* 3.2, 3-CH), 3.70, 3.74 (6 H, 2 s, 2 and 5-OCH_3) and 7.1–7.3 (5 H, m, ArH); δ_C(50 MHz; C^2HCl_3) 16.31, 18.95 (2 × Pr^i CH_3), 30.98 (Pr^i CH), 39.81 (CH_2Ph), 51.98, 52.23 (2 and 5-OCH_3), 56.0 (t, *J* 22, 6-CH), 60.07, (3-CH), 126.19, 127.70, 129.89, 137.17 (ArC), 162.38 and 163.31 (2 and 5-C); δ_D(61.4 MHz; $CHCl_3$) 4.31 (6-C^2H); *m/z* (CI) 276 ($[M + H]^+$, 100), 184 (20, $[M - PhCH_2]^+$) and 142 (32, $[M - PhCH_2 - CH(CH_3)_2 + H]^+$).

(2S)-*Phenylalanine Methyl Ester* **24**.—The dihydropyrazine **15** (750 mg, 2.74 mmol) was stirred at room temperature in 0.1 mol dm^{-3} HCl (55 cm^3) for 12 h after which the mixture was extracted with diethyl ether to remove the unchanged starting material. The aqueous residue was concentrated under reduced pressure and then dissolved in water (2 cm^3). The pH of the solution was adjusted to 9 with concentrated ammonia solution and then extracted with diethyl ether (3 × 30 cm^3). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure and the (2*R*)-valine and (2*S*)-phenylalanine methyl esters were separated by distillation at 1 mmHg. The lower boiling fraction containing (2*S*)-phenylalanine methyl ester was collected (0.64 g, 70%) and used for the next step without further purification; δ_H(200 MHz; C^2HCl_3) 1.76 (2 H, br s, NH_2), 2.87 (1 H, ABX, J_{AB} 13.4, J_{AX} 5.2, benzyl CH_A), 3.11 (1 H, ABX, j_{AB} 13.4, j_{BX} 7.9, benzyl CH_B), 3.7–3.8 (4 H, m, OCH_3 and 2-CH) and 7.15–7.4 (5 H, m, ArH); δ_C(75 MHz; C^2HCl_3) 40.97 (3-CH_2), 51.92 (1′-CH_3), 55.71 (2-CH), 126.77, 128.49, 129.19, 137.13 (ArC) and 175.26 (CO_2Me).

(2S)-[2-^{2}H]*Phenylalanine Methyl Ester* **25**.—This compound was prepared in a manner identical with that for the ester **24**, using dihydropyrazine **16**, in 82% yield, and was used for the next step without further purification; δ_H(200 MHz; C^2HCl_3) 1.76 (2 H, br s, NH_2), 2.87 (1 H, d, *J* 14, benzyl CH_A), 3.11 (1 H, d, *J* 14, benzyl CH_B), 3.73 (3 H, s, OCH_3) and 7.1–7.35 (5 H, m, ArH).

(2S)-*Phenylalanine* **32**.—A suspension of the ester **24** (0.82 g, 4.6 mmol) in 2 mol dm^{-3} HCl (50 cm^3) was refluxed under nitrogen for 2 h after which it was evaporated under reduced pressure and the residue was dried over phosphorus pentoxide. The crude hydrochloride salt followed by propylene oxide (20 cm^3) was added to dry ethanol (20 cm^3), and the mixture was refluxed for 15 min. The precipitated free base was filtered off, dried and recrystallised from aqueous ethanol to afford white crystals (0.76 g, 60%), m.p. 275 °C [lit.,[11] 283 °C (decomp.)] (Found: C, 65.65; H, 6.4; N, 8.5. Calc. for $C_9H_{11}NO_2$: C, 65.45; H, 6.7; N, 8.5%); *m/z* (Found: $[M + H]^+$, 166.0868. $C_9H_{12}NO_2$ requires 166.0868); $[\alpha]_D^{23}$ −30.9 (*c* 2.035 in H_2O) {lit.,[17] $[\alpha]_D^{20}$ −32.5 (*c* 2.0 in H_2O)}; ν_{max}(Nujol)/cm^{-1} 2800–3000 (CH str) and 1559 (CO_2^-); δ_H(200 MHz; ^2H_2O–NaO^2H) 2.7 (1 H, ABX, J_{BX} 7.4, J_{AB} 13.4, 3-CH_B), 2.85 (1 H, ABX, J_{AX} 5.5, J_{AB} 13.4, 3-CH_A), 3.36 (1 H, ABX, 2-CH) and 7.1–7.3 (5 H, m, ArH); δ_C(75 MHz; ^2H_2O–NaO^2H) 41.79 (3-CH_2), 58.46 (2-CH), 127.63, 129.59, 130.43, 139.32 (ArC) and 183.41 (CO_2H); *m/z* (EI) 166 ($[M + H]^+$, 100%) and 120 (20, $[M - CH_2H]^+$).

(2S)-[2-^{2}H]-*Phenylalanine* **33**.—This was prepared in a manner identical with that for (2*S*)-phenylalanine **32**, starting from the ester **25**, in 60% yield, m.p. 264 °C [lit.,[11] 283 °C (decomp.) for (2*S*)-phenylalanine] (Found: C, 64.85; H, 5.7; N, 8.25. Calc. for $C_9H_{10}NO_2{}^2H$: C, 65.05; H, 6.0; N, 8.45%); *m/z* (Found: $[M + H]^+$, 167.0931 $C_9H_{11}NO_2{}^2H$ requires 167.0931); $[\alpha]_D^{23}$ −28.2 (*c* 1.5 in H_2O) [lit.,[17] $[\alpha]_D^{20}$ −32.5 (*c* 2.0 in H_2O) for (2*S*)-phenylalanine]; ν_{max}(Nujol)/cm^{-1} 2800–3000 (CH str) and 1559 (CO_2^-); δ_H(200 MHz; ^2H_2O–NaO^2H) 2.69 (1 H, d, *J* 13.2, 3-CH_A), 2.84 (1 H, d, *J* 13.2, 3-CH_B) and 7.1–7.3 (5 H, m, ArH); δ_C(75 MHz; ^2H_2O–NaO^2H) 41.79 (3-CH_3), 58.55 (2-CH), 127.68, 129.66, 130.50, 139.48 (ArC) and 183.52 (CO_2H); δ_D(61.4 MHz; H_2O–NaOH) 3.36 (2-C^2H).

(3R,6S)-6-*Allyl*-3-*isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **17**.—The dihydropyrazine **1** (250 mg, 1.36 mmol) was dissolved in dry THF (3 cm^3) and the solution cooled to −80 °C. To this was added dropwise 1.9 mol dm^{-3} BuLi (0.72 cm^3, 1.36 mmol) followed, after 30 min, by a solution of allyl bromide (329 mg, 2.72 mmol) in dry THF (3 cm^3). The mixture was stirred at −80 °C overnight after which it was concentrated to dryness under reduced pressure. The residue was treated with potassium phosphate buffer (100 mmol dm^{-3}, pH 7; 20 cm^3) and the suspension extracted with diethyl ether (3 × 20 cm^3). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure and the residue was purified by flash chromatography on silica eluting with petroleum–ethyl acetate (7:3) to give a colourless oil (220 mg, 73%) (Found: M^+, 224.1525. $C_{12}H_{20}N_2O_2$ requires *M*, 224.1525); $[\alpha]_D^{23}$ +13.9 (*c* 2.8 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 3078, 2945, 2872 (CH str), 1699 (C=N) and 1642 (C=C); δ_H(200 MHz; C^2HCl_3) 0.68, 1.05 (6 H, 2 d, *J* 7.6, 2 × Pr^i CH_3), 2.27 (1 H, dsp, Pr^i CH), 2.55 (2 H, m, 3′-CH_2), 3.7 (6 H, 2 s, 2 and 5-OCH_3), 3.9 (1 H, t, *J* 3.4, 3-CH), 4.1 (1 H, dd, 6-CH), 5.0–5.15 (2 H, m, 1′-CH_2) and 5.7 (1 H, m, 2′-CH); δ_C(50 MHz; C^2HCl_3) 16.47, 19.03 (2 × Pr^i CH_3), 31.56 (Pr^i CH), 38.43 (3′-CH_2), 52.25, 52.39 (2 and 5-OCH_3), 55.41 (6-CH), 60.65 (3-CH), 117.77 (1′-CH_2), 133.78 (2′-CH_2), 163.06 and 163.77 (2 and 5-C); *m/z* (EI) 225 ($[M + H]^+$, 100%), 183

(50, $[M - CH_2CHCH_2]^+$) and 141 (43, $[M - CH_2CHCH_2 - CH(CH_3)_2 + H]^+$).

(3R,6S)-[6-^{2}H]-6-*Allyl*-3-*isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazine* **18**.—This compound was prepared in a manner identical with that for the dihydropyrazine **17**, starting from the dihydropyrazine **2**, in 65% yield; *m/z* (Found: M^+, 225.1590. $C_{12}H_{19}N_2O_2{}^2H$ requires 225.1586); $[\alpha]_D^{23}$ +1.11 (*c* 2.88 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 3000–2800 (CH str), 1696 (C=N) and 1641 (C=C); δ_H(200 MHz; C^2HCl_3) 0.68, 1.02 (6 H, 2 d, *J* 6.8, 2 × Pr^i CH_3), 2.25 (1 H, dsp, Pr^i CH), 2.52 (2 H, d, *J* 7, 3'-CH_2), 3.7 (6 H, 2 s, 2 and 5-OCH_3), 3.92 (1 H, d, *J* 3.2, 3-CH), 4.99–5.1 (2 H, m, 1'-CH_2) and 5.7 (1 H, m, 2'-CH); δ_C(50 MHz; C^2HCl_3) 16.48, 19.06 (2 × Pr^i CH_3), 31.54 (Pr^i CH), 38.37 (3'-CH_2), 52.28, 52.43 (2 and 5-OCH_3), 55.4 (t, *J* 20, 6-C^2H), 60.65 (3-CH), 117.83 (1'-CH_2), 133.79 (2'-CH_2), 163.09 and 163.85 (2 and 5-C); δ_D(61.4 MHz; $CHCl_3$) 4.06 (6-C^2H); *m/z* (CI) 226 ($[M + H]^+$, 100%), 212 (35, $[M - CH_2 + H]^+$), 184 (120, $[M - CH_2CHCH_2]^+$) and 142 (15, $[M - CH_2CHCH_2 - CH(CH_3)_2 + H]^+$).

(2S)-*Allylglycine Methyl Ester* **26**.—The dihydropyrazine **17** (1.3 g, 5.8 mmol) was stirred in 0.2 mol dm^{-3} HCl (58 cm^3) at room temperature for 12 h after which the mixture was extracted with diethyl ether to remove the unchanged starting material. The aqueous residue was concentrated under reduced pressure and then dissolved in water (5 cm^3). The solution was adjusted to pH 9 with concentrated ammonia solution and then extracted with diethyl ether (3 × 30 cm^3). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure to yield a mixture of (2*R*)-valine and (2*S*)-allylglycine methyl esters. These were separated by flash column chromatograhy on silica (eluting with diethyl ether and one drop of ammonia solution) to give allylglycine methyl ester as a colourless oil (0.2 g, 39%); *m/z* (Found: $[M + H]^+$, 130.087. $C_6H_{12}NO_2$ requires 130.0868); $[\alpha]_D^{23}$ +3.2 (*c* 0.8 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 3382 (NH_2 str), 3079 (C=C–H str), 2800–3000 (CH str), 1740 (CO_2CH_3) and 1642 (C=C); δ_H(200 MHz; C^2HCl_3) 1.60 (2 H, s, NH_2), 2.36 (2 H, m, 3-CH_2), 3.5 (1 H, 2 d, *J* 5, 2-CH), 3.66 (3 H, s, OCH_3), 5.04 (1 H, d, *J* 1.2, 5-CH *cis*), 5.11 (1 H, d, *J* 5.8, 5-CH *trans*) and 5.6 (1 H, m, 4-CH); δ_C(75 MHz; C^2HCl_3) 38.9 (3-CH_2), 51.69 (OCH_3), 53.66 (2-CH), 118.36 (5-CH_2), 133.17 (4-CH) and 175.37 (C=O); *m/z* (EI) 130 ($[M + H]^+$, 100%), 70 (58, $[M - CO_2CH_3]^+$) and 44 (20, $CO_2{}^+$).

(2S)-[2-^{2}H]*Allylglycine Methyl Ester* **27**.—This compound was prepared in a manner identical with that of the ester **26**, starting with dihydropyrazine **18**, in 40% yield; *m/z* (Found: $[M + H]^+$, 131.093. $C_6H_{11}NO_2{}^2H$ requires 131.0931); $[\alpha]_D^{23}$ +2.6 (*c* 0.93 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 3381 (NH_2 str), 3079 (C=C–H str), 2800–3000 (CH str), 1735 (CO_2CH_3) and 1642 (C=C); δ_H(200 MHz; C^2HCl_3) 1.55 (2 H, s, NH_2), 2.4 (2 H, m, 3-CH_2), 3.67 (3 H, s, OCH_3), 5.1 (2 H, m, 5-CH_2) and 5.7 (1 H, m, 4-CH); δ_C(75 MHz; C^2HCl_3) 38.86 (3-CH_2), 51.68 (OCH_3), 53.34 (t, *J* 21.6, 2-C^2H), 118.35 (5-CH_2), 133.19 (4-CH) and 175.41 (C=O); δ_D(61.4 MHz; $CHCl_3$) 3.53 (2-C^2H); *m/z* (EI) 131 ($[M + H]^+$ 100%) and 71 (36, $[M - CO_2CH_3]^+$).

(2S)-*Allylglycine* **34**.—The ester **26** (0.27 g, 2.09 mmol) was refluxed in 2 mol dm^{-3} HCl (10 cm^3) for 2 h after which it was concentrated under reduced pressure and the residue was dried over phosphorus pentoxide. Dry ethanol (10 cm^3) and propylene oxide (10 cm^3) were added to the residue and the mixture was refluxed under nitrogen for 15 min. The suspension was cooled and the precipitated solid was filtered off and dried and recrystallised from ethanol (0.2 g, 83%), m.p. 254 °C (decomp.) (Found: C, 52.2; H, 8.1; N, 12.15. Calc. For $C_5H_9NO_2$: C, 52.15; H, 7.90; N, 12.15%); *m/z* (Found: $[M + H]^+$, 116.071. $C_5H_{10}NO_2$ requires 116.0712); $[\alpha]_D^{23}$ −5.7 (*c* 2.0 in 5 mol dm^{-3} HCl) {lit.,[18] $[\alpha]_D^{24}$ +5.7 (*c* 2.0 in 5 mol dm^{-3} HCl) for (2*R*)-allylglycine)}; ν_{max}(Nujol)/cm^{-1} 3200–2600 (CH str and OH str) and 1586 ($CO_2{}^-$); δ_H(300 MHz; ^2H_2O) 2.5 (2 H, m, 3-CH_2), 3.7 (1 H, dd, 2-CH), 5.15 (2 H, m, 5-CH_2) and 5.7 (1 H, m, 4-CH); δ_C(75 MHz, ^2H_2O–dioxane) 35.55 (3-CH_2), 54.66 (2-CH), 121.21 (5-CH_2), 132.06 (4-CH) and 174.69 (C=O); *m/z* (EI) 116 ($[M + H]^+$, 100%) and 70 (15, CO_2H^+).

(2S)-[2-^{2}H]*Allylglycine* **35**.—This compound was prepared in a manner identical with that of (2*S*)-allylglycine **34**, starting from the ester **27**, in 59% yield; m.p. 246–248 °C (decomp.) (Found: C, 51.4; H, 6.75; N, 11.95. Calc. for $C_5H_8NO_2{}^2H$: C, 51.7; H, 6.95; N, 12.05%) (Found: $[M + H]^+$, 117.077. $C_5H_9NO_2{}^2H$ requires 117.0774); $[\alpha]_D^{23}$ −4.2 (*c* 1.87 in 6 mol dm^{-3} HCl) {lit.,[128] $[\alpha]_D^{24}$ +5.7 (*c* 2.0 in 5 mol dm^{-3} HCl) for (2*R*)-allylglycine}; ν_{max}(Nujol)/cm^{-1} 3200–2600 (Nujol, CH str and OH str) and 1581 ($CO_2{}^-$); δ_H(300 MHz; ^2H_2O) 2.49 (1 H, d, *J* 4, 3-CH_A), 2.52 (1 H, d, *J* 3, 3-CH_B), 5.15 (2 H, m, 5-CH_2) and 5.6 (1 H, m, CH); δ_C(75 MHz; ^2H_2O) 35.43 (3-CH_2), 54.36 (t, *J* 22, 2-C^2H), 121.20 (5-CH_2), 132.03 (4-CH) and 174.73 (C=O); δ_D(61.4 MHz; H_2O) 3.54 (2-C^2H); *m/z* (EI) 117 ($[M + H]^+$, 100%) and 71 (20, CO_2H^+).

Ethyl (3R,6S)-3-*Isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazin*-6-*ylacetate* **19**.—The dihydropyrazine **1** (1 g, 5.49 mmol) was dissolved in dry THF (25 cm^3) and the solution cooled to −80 °C; the anion was then generated as described above. After 30 min at −60 °C, the temperature of the reaction mixture was reduced to −90 °C and a cold solution of ethyl bromoacetate (0.92 g, 5.49 mmol) in dry THF (2 cm^3) was added to it. The reaction mixture was stirred at −90 °C for 5 h and, after it had been allowed to warm to room temperature, was evaporated under reduced pressure. Potassium phosphate buffer (100 mmol dm^{-3}, pH 7; 20 cm^3) was added to the residue and the suspension was extracted with diethyl ether (3 × 20 cm^3). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The ^{1}H NMR spectrum of the residue contained a quartet in the region 3.5–4.0 ppm characteristic of the β-aspartic acid protons. Since purification of the residue by flash column chromatograhy on silica proved to be unhelpful, the crude material was used directly in the next step.

4-*Ethyl* 1-*Methyl* (2S)-*Aspartate* **28**.—The crude ester **19** (1.85 g, 6.9 mmol) was stirred in 0.2 mol dm^{-3} HCl (69 cm^3, 13.8 mmol) overnight after which the solution was extracted with diethyl ether (15 cm^3) and the ethereal extract discarded. The aqueous layer was concentrated to dryness under reduced pressure and the residue was dissolved in water (2 cm^3). The solution was adjusted to pH 9 with 10 mol dm^{-3} ammonia solution and then extracted with diethyl ether (3 × 10 cm^3). The combined extracts were dried ($MgSO_4$) and concentrated to dryness under reduce pressure to give a mixture of the (2*S*)-aspartate and (2*R*)-valine methyl esters. These were separated and purified by flash column chromatography on silica eluting with diethyl ether–ethanol (19:1, containing one drop of conc. ammonia solution) to give the aspartate diester as colourless oil (0.32 g, 39%); δ_H(200 MHz; C^2HCl_3) 1.25 (3 H, t, *J* 7.6, ethyl CH_3), 1.85 (2 H, br s, NH_2), 2.7 (2 H, ABX, J_{AB} 12.6, J_{AX} 3, J_{BX} 9.6, 3-CH_2), 3.7 (3 H, s, OCH_3), 3.8 (1 H, ABX, J_{AX} 3, J_{BX} 9.6, 2-CH) and 4.15 (2 H, q, *J* 7.6, ethyl CH_2); δ_C(50 MHz; C^2HCl_3) 14.05 (ethyl CH_2), 38.88 (3-CH_2), 51.08 (OCH_3), 52.24 (2-CH), 60.72 (ethyl CH_2), 171.07 and 174.59 (ester C=O).

(2S)-*Aspartic Acid* **36**.—The ester **28** (0.32 g, 2.67 mmol) was refluxed in 5 mol dm^{-3} HCl (10 cm^3) for 2 h after which the solvent was removed under reduced pressure and the residue

was dried over phosphorus pentoxide and then dissolved in dry ethanol (10 cm^3). Propylene oxide (10 cm^3) was added to the solution which was refluxed for 15 min and then allowed to cool. The precipitated amino acid was filtered off and recrystallised from aqueous ethanol to give a white crystalline solid (20 mg, 11% from the initial bis-lactim ether). On allowing partial evaporation of the mother liquor further crops of crystals were obtained to give a total yield of 37% from compound **19**; m.p. 290 °C (decomp.) (lit.,[19] 270–271 °C) (Found: C, 36.35; H, 5.55; N, 10.25. Calc. for $C_4H_7NO_4$: C, 36.1; H, 5.3; N, 10.5%); *m/z* (Found: $[M + H]^+$, 134.046. $C_4H_8NO_4$ requires 134.0453); $[\alpha]_D^{23}$ +21.8 (*c* 0.495 in 5 mol dm^{-3} HCl) {lit.,[15] $[\alpha]_D^{23}$ +24.1 (*c* 0.56 in 5 mol dm^{-3} HCl)}; ν_{max}(Nujol)/cm^{-1} 3000–2800 (CH, NH), 1692, 1644 (CO_2^-) and 1599 (NH_3^+ deformations); δ_H(200 MHz; ^2H_2O–NaO^2H) 2.21 (1 H, ABX, J_{AX} 9.9, J_{AB} 15.8, 3-CH_A), 2.55 (1 H, ABX, J_{BX} 3.5, J_{AB} 15.8, 3-CH_B) and 3.48 (1 H, ABX, J_{AX} 9.9, J_{BX} 3.5, 2-CH); δ_C(50 MHz; ^2H_2O–NaO^2H) 42.46 (3-CH_2), 54.30 (2-CH), 180.32 and 181.13 (1 and 4-CO_2H); *m/z* (CI) 134 ($[M + H]^+$, 100%) and 44 (53, CO_2^+).

Ethyl (3R,6S)-[6-^{2}H]-3-*Isopropyl*-2,5-*dimethoxy*-3,6-*dihydropyrazin*-6-*ylacetate* **20**.—This compound was prepared in a manner identical with that of the ester **19**, using dihydropyrazine **2**; the crude material was used directly in the next step.

4-*Ethyl* 1-*Methyl* (2S)-[2-^{2}H]*Aspartate* **29**.—This compound was prepared in a manner identical with that of the ester **28**, starting from the crude ester **20**, in 41% yield; δ_H(200 MHz; C^2HCl_3) 1.25 (3 H, t, *J* 7.6, ethyl CH_3), 1.85 (2 H, br s, NH_2), 2.7 (2 H, dd, *J* 17.7, 3-CH_2), 3.7 (3 H, s, OCH_3) and 4.15 (2 H, q, *J* 7.6, ethyl CH_2).

(2S)-[2-^{2}H]*Aspartic Acid* **37**.—This compound was prepared in a manner identical with that of the acid **36**, using the ester **29**, in 32% yield from the bis-lactim ether **20**; m.p. >300 °C (Found: C, 35.6; H, 4.55; N, 10.3. Calc. for $C_4H_6NO_4{}^2H$: C, 35.8; H, 4.5; N, 10.45%); *m/z* (Found: $[M + H]^+$, 135.0516. $C_4H_7NO_4{}^2H$ requires 135.0516); $[\alpha]_D^{23}$ +19.5 (*c* 0.495 in 5 mol dm^{-3} HCl); ν_{max}(Nujol)/cm^{-1} 3000–2800 (CH str, NH str), 1690, 1643 (CO_2^-) and 1591 (NH_3^+ deformations); δ_H(200 MHz; ^2H_2O–NaO^2H) 2.24 (1 H, d, J_{AB} 16, 3-CH_b); δ_C(100 MHz; ^2H_2O–NaO^2H) 43.91 (3-CH_2), 54.41 (t, *J* 20.7, 2-C^2H), 180.93 and 183.09 (1 and 4 CO_2H); δ_D(61.4 MHz; H_2O–NaOH) 3.46 (2-C^2H).

1-*Methyl* N-*Trifluoroacetyl*-(2S)-*aspartate* **40**.—This compound was prepared according to the method of Gani and Young[20] in 88% yield; m.p. 101–103 °C (lit.,[20] m.p. 101–102 °C); ν_{max}(Nujol)/cm^{-1} 1745 (ester C=O), 1730 (acid C=O) and (trifluoroacetyl C=O); δ_H(90 MHz; C^2HCl_3), 3.3 (2 H, m, 3-CH_2), 4.05 (3 H, s, OCH_3) and 5.1 (1 H, m, 2-CH); *m/z* (CI) 261 ($[M + NH_4]^+$, 100%) and 35 (35, Cl^+).

(3S)-3-*Methoxycarbonyl*-3-*trifluoroacetylaminopropanoyl Chloride* **42**.—This compound, prepared according to the method of Gani and Young[20] as a pale yellow solid, was recrystallised from dry diethyl ether–petroleum to afford the pure acid chloride as a white crystalline solid (1.21 g, 57%), m.p. 116–117 °C (lit.,[20] m.p. 114–115 °C); ν_{max}(Nujol)/cm^{-1} 1798 (acid chloride C=O), 1740 (ester C=O) and 1709 (trifluoroacetyl C=O); δ_H(90 MHz; C^2HCl_3) 3.65 (2 H, dd, 3-CH_2), 3.85 (3 H, s, methyl ester) and 4.79 (1 H, m, 2-CH); *m/z* (CI) 279 ($[M + NH_4]^+$, 35%), 261 (40, M^+), 260 (100, $[M - H]^+$), 243 (65, $[M + NH_3 - Cl]^+$) and 35 (35, Cl^+).

m-*Chlorobenzoyl* (3S)-3-*Methoxycarbonyl*-3-*trifluoroacetylaminopropanoyl Peroxide* **44**.—The acid chloride **42** (1.5 g, 6 mmol) and 85% *m*-chloroperbenzoic acid (1.03 g, 6 mmol) were added to dry diethyl ether (45 cm^3) and the solution was stirred under nitrogen in an ice–salt bath. Pyridine (800 mm^3, 9 mmol) in dry diethyl ether (3 cm^3) was added dropwise to the mixture and stirring was continued for a further 4 h at 0 °C. The solution was then filtered, washed with water and aqueous 1 mol dm^{-3} sodium carbonate and dried (Na_2SO_4), and evaporated under reduced pressure to give a white solid which was recrystallised from diethyl ether–petroleum to yield the pure product (0.5 g, 21%), m.p. 106–107 °C (Found: C, 42.45; H, 2.55; N, 3.55. Calc. for $C_{14}H_{11}ClF_3NO_7$: C, 42.3; H, 2.8; N, 3.5%); $[\alpha]_D^{23}$ +68 (*c* 0.69 in $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3269 (amide NH), 1795, 1758 (peroxyanhydride C=O), 1751 (ester C=O) and 1714 (trifluoroacetyl C=O); δ_H(90 MHz; C^2HCl_3) 3.25 (2 H, m, 3-CH_2), 3.88 (3 H, s, OMe), 5.00 (1 H, m, 2-CH) and 7.35–8.00 (5 H, ArH and NH br); δ_C(22.5 MHz; C^2HCl_3) 32.35 (3-CH_2), 48.34 (OCH_3), 54.43 (2-CH) and 127.4–135.4 (ArC), 157.53, 162.32, 166.90 and 169.23 (4 × C=O); *m/z* (CI), 415 ($[M + NH_4]^+$, 5%), (6, $[M + NH_4 - CO_2H]^+$), 261 (100, $[M - C_7H_4O_3]^+$), 217 (30, $[M - C_8H_4O_5]^+$) and 35 (60, Cl^+).

Methyl (2S)-3-(m-*Chlorobenzoyloxy*)-N-*trifluoroacetylalaninate* **46**.—The peroxide **44** (500 mg, 1.2 mmol) was heated to reflux in carbon tetrachloride (40 cm^3) under nitrogen for 6 days and then evaporated under reduced pressure to yield a gummy solid. Purification after this by column chromatography on silica (petroleum–ethyl acetate 7:3) gave the product which was then recrystallised from diethyl ether–petroleum to afford a white crystalline solid (110 mg, 30%), m.p. 77–78 °C (Found: C, 44.25; H, 2.95; N, 3.75. Calc. for $C_{13}H_{11}ClF_3NO_5$: C, 44.15; H, 3.15; N, 3.95%); $[\alpha]_D^{23}$ +50.3 (*c* 0.295 in $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 3245 (NH str), 1740 (aliphatic ester C=O), 1730 (aromatic ester C=O) and 1700 (trifluoroacetyl C=O); δ_H(200 MHz; C^2HCl_3) 3.85 (3 H, s, CO_2CH_3), 4.75 (2 H, m, 3-CH_2), 4.97 (1 H, m, 2-CH) and 7.35–8.00 (>5 H, ArH and NH); δ_C(50 MHz; C^2HCl_3) 52.87 (2-CH), 54 (CO_2CH_3), 64.19 (3-CH_2), 128–134 (ArC), 157.08 (CF_3CO), 164.95, 166.16 and 168.36 (3 × C=Os); *m/z* (CI) 371 ($[M + NH_4]^+$, 100%), 307 (50, $[M - CO_2H - H]^+$), 261 (38, $[M - C_6H_4O]^+$) and 35 (50, Cl^+).

(2S)-*Serine Hydrochloride* **48**.—The alaninate **46** (63.7 mg, 0.18 mmol) was refluxed in 5 mol dm^{-3} HCl (10 cm^3) under nitrogen for 2 h after which the solution was cooled and washed with chloroform (3 × 10 cm^3). The aqueous layer was then lyophilised to give a white solid (10 mg, 32%), m.p. 140 °C (decomp.); $[\alpha]_D^{23}$ +11.5 (*c* 0.5 in H_2O) {lit.,[21] $[\alpha]_D^{20}$ +7.2 (*c* 1.2 in H_2O), lit.,[22] $[\alpha]_D$ +8.2 (*c* 1.9 in H_2O)}; ν_{max}(Nujol)/cm^{-1} 3815 (NH), 3500–3000 (OH), 3000–2800 (CH), 1760 (acid CO_2H monomer) and 1590 (CO_2^-); δ_H(200 MHz; ^2H_2O–NaO^2H) 3.32 (1 H, t, *J* 5, 2-CH) and 3.65 (2 H, m, 3-CH_2); δ_C(50 MHz; ^2H_2O) 57.67 (3-CH_2), 62.27 (2-CH) and 173.22 (CO_2H); *m/z* (CI) 106 ($[M - Cl]^+$, 100%), 88 (30, $[M - OH]^+$), 60 (50, $[M - CO_2H - H]^+$) and 44 (50, CO_2^+).

(2S)-[2-^{2}H]*Aspartic Acid* **39**.—(2*S*)-Aspartic acid (3 g, 22.5 mmol) was added to deuterium oxide (50 cm^3) and the solution adjusted to pH 7.25 with ammonia solution. Pyridoxal phosphate (5 mg, 20 μmol) and aspartate aminotransferase (AAT) (1 mg, 300 unit) were added to the solution which was then incubated at 37 °C. The reaction progress was monitored by ^{1}H NMR spectroscopy. After 72 h the pH was readjusted to 7.25 and the solution was boiled, filtered to remove the denatured protein, and concentrated to dryness under reduced pressure. The off-white solid was recrystallised from hot water (pH 4)–ethanol to give the free amino acid (2.19 g, 72%), m.p. >300 °C; $[\alpha]_D^{23}$ +21.05 (*c* 0.95 in 6 mol dm^{-3} HCl); ν_{max}(Nujol)/cm^{-1} 3000–2500 (OH stretching), 1688 and 1643

(carboxylate anion) and 1586 (N–H deformations); δ_H(270 MHz; ^2H_2O–^2HCl) 3.12 (2 H, d, J 2.7, 3-CH_2) and 4.4 (1/13H, t, 2-CH, 7% undeuteriated amino acid); δ_C(67.5 MHz; ^2H_2O) 34.32 (3-CH_2), 49.68 (2-C^2H), 171.44 and 173.80 (carboxylate carbons); m/z (FAB) 135 ($[M + H]^+$, 70%), 134 (30, M^+) and 74 (52, $[M - CO_3]^+$).

1-*Methyl* N-*Trifluoroacetyl*-(2S)-[2-^{2}H]*aspartate* **41**.—This compound was prepared in a manner identical with that described for the ester **40**, using the acid **39**. The 2:1 mixture of the α- and β-esters was obtained in 100% yield; δ_H(200 MHz; C^2HCl_3) 2.85–3.25 (m, 3-CH_2 in α- and β-esters), 3.75 (s, β-methyl ester), 3.85 (s, α-methyl ester), 7.45 and 7.55 (2 br s, NH of both esters).

(3S)-3-*Methoxycarbonyl*-3-*trifluoroacetylamino*[3-^{2}H]*propanoyl Chloride* **43**.—This compound was prepared in a manner identical with that of the acid chloride **42**, using the ester **41**, in 59% yield; m.p. 112–113 °C; $[\alpha]_D^{23}$ +66.3 (*c* 0.395 in chloroform); δ_H(200 MHz; C^2HCl_3) 3.62 (2 H, d, J 5.2, 3-CH_2), 3.9 (3 H, s, CO_2CH_3) and 7.25 (1 H, br s, NH); m/z (EI) 227 ($[M - Cl]^+$, 10%), 203 (70, $[M - CO_2CH_3]^+$) and 199 (35, $[M - COCl]^+$).

m-*Chlorobenzoyl* (3S)-3-*Methoxycarbonyl*-3-*trifluoroacetylamino*[3-^{2}H]*propanoyl Peroxide* **45**.—This compound was prepared in a manner identical with that of the peroxide **44**, using the acid chloride **43**, in 40% yield; m.p. 102–103 °C; $[\alpha]_D^{23}$ +49.5 (*c* 0.66 in chloroform); ν_{max}(Nujol)/cm^{-1} 3260 (NH), 1790, 1770 (peroxy anhydride C=O), 1750 (ester C=O) and 1710 (trifluoroacetyl C=O); δ_H(200 MHz; C^2HCl_3) 3.25 (2 H, dd, J 16, 3-CH_2), 3.9 (3 H, s, CO_2CH_3) and 7.4–8.1 (5 H, m, 4 ArH and NH); δ_C(50 MHz; C^2HCl_3) 32.58 (CH_2CO), 49.35 (t, J 21, C^2H), 54.21 (CO_2CH_3), 126.98, 128.36, 130.27, 130.78, 135.20, 135.67 (ArC), 157.28, 162.08, 166.65 and 168.97 (4 × C=O); m/z (CI) 416 ($[M - NH_4]^+$, 100%) and 262 (35, $[M - C_7H_4O_3]^+$).

Methyl (2S)-3-(m-*Chlorobenzoyloxy*)-N-*trifluoroacetyl*[2-^{2}H]*alaninate* **47**.—This compound was prepared in a manner identical with that of the alaninate **46**, using the peroxide **45**, in 27% yield; m.p. 73–74 °C; $[\alpha]_D^{23}$ +79.3 (*c* 0.295 in chloroform); ν_{max}(Nujol)/cm^{-1} 3245 (NH str), 3000–2800 (CH str), 1750 (aliphatic ester C–O), 1730 (aromatic ester C=O) and 1705 (trifluoroacetyl C=O); δ_H(200 MHz; C^2HCl_3) 3.88 (3 H, s, CO_2CH_3), 4.72 (2 H, s, 3-CH_2), 7.25 (1 H, br s, NH) and 7.4–7.95 (ArH); δ_C(75 MHz; C^2HCl_3), 52.1 (t, J 20, C^2H) 53.6 (CO_2CH_3), 63.7 (CH_2O), 115.5 (q, J 285, CF_3), 127.8, 129.79, 130.6, 133.7, 134.8 (ArC), 157.1 (q, J 38, CF_3CO), 164.9, 168.3 (2 × C=O); m/z (CI) 372 ($[M + NH_4]^+$, 100%).

(2S)-[2-^{2}H]*Serine Hydrochloride* **49**.—This compound was prepared in a manner identical with that of the hydrochloride **48**, using alaninate **47**, in quantitative yield (4.6% from aspartic acid). Spectral data were identical with those for compound **31** except that compound **48** contained *ca.* 10 atom% of protium at C-2.

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