Versatile Synthesis of Stereospecifically Labelled D-Amino Acids *via* Labelled Aziridines—Preparation of (2*R*,3*S*)-[3-$^2H_1$]- and (2*R*,3*R*)-[2,3-$^2H_2$]-Serine; (2*S*,2'*S*,3*S*,3'*S*)-[3,3'-$^2H_2$]- and (2*S*,2'*S*,3*R*,3'*R*)-[2,2',3,3'-$^2H_4$]-Cystine; and (2*S*,3*S*)-[3-$^2H_1$-] and (2*S*,3*R*)-[2,3-$^2H_2$]-β-Chloroalanine[1]

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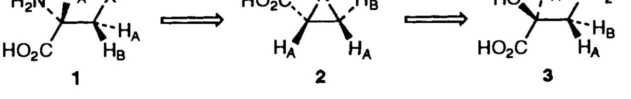
Stereospecifically β-labelled protected 2-carboxyaziridines **2**, with the stereochemistry of a D-amino acid at C-2, have been prepared by a chemicoenzymic synthesis. Preparation of the labelled malates **5**, by hydration of fumaric acid using the enzyme fumarase or by amination with aspartase followed by nitrosation, was followed by conversion into the isoserines **3**, by a process involving Curtius rearrangement with retention of stereochemistry at the chirally labelled primary centre. Protection and ring closure gave the aziridines **2**, which, on ring opening with the appropriate nucleophiles and deprotection, gave stereospecifically labelled samples of D-serine **16**, D-cystine **20** and β-chloro-D-alanine **22**.

Naturally occurring amino acids overwhelmingly exist as the L-enantiomers, although D-amino acids do occur, with the L-enantiomers, in bacteria.[2] Because D-amino acids, except in rare instances,[3] do not exist in mammals, enzymes which metabolize D-amino acids are seen as targets for antibacterial drugs. The mechanism of action of such enzymes is therefore of great interest for the design of inhibitors which may be of medicinal interest.

Elucidation of the mechanism of action of enzymes which metabolise L-amino acids has been greatly advanced by studies[4] of the stereochemical consequences of the enzymic reactions at the β-carbon atom of the substrate. Similar information on the corresponding reactions of D-amino acids **1** is, however, relatively rare.[5] We have, therefore, devised a versatile synthesis of D-amino acids which are stereospecifically labelled at the β-centre. This should allow the stereochemistry of metabolic reactions of D-amino acids to be elaborated.

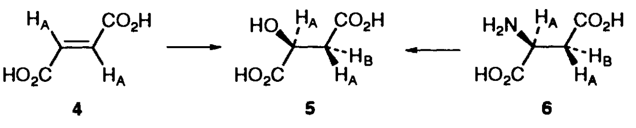
From the literature on the reactions of protected 2-carboxyaziridines **2** with heteronucleophiles, it appeared that, if we could prepare a suitably protected 2-carboxyaziridine **2** with D-amino acid stereochemistry at C-2 and stereospecific labelling at C-3, then a general synthesis of stereospecifically labelled D-amino acids **1** could be developed. Nucleophilic substitution of the aziridine **2** should occur with inversion of stereochemistry at the labelled primary centre C-3. Since the aziridines **2** might be accessed from labelled samples of (2*S*)-isoserine **3** by protection and cyclisation with inversion of stereochemistry at the centre C-2, synthesis of the labelled (2*S*)-isoserines **3** became our first synthetic goal.



1 **2** **3**

It has long been known[6] that the commercially available enzyme fumarase (EC 4.2.1.2) will catalyse the conversion of fumaric acid **4** into (2*S*)-malic acid **5** with *anti* addition of water. We therefore incubated unlabelled fumaric acid **4** with the enzyme in the minimum amount of ^2H_2O which would effect reaction to obtain (2*S*,3*R*)-[3-$^2H_1$]malic acid, **5** $H_B = {}^2H$, in ~ 50% yield. Incubation of [2,3-$^2H_2$]fumaric acid, **4** $H_A = {}^2H$,[7] in water gave (2*S*,3*S*)-[2,3-$^2H_2$]malic acid, **5** $H_A = {}^2H$ (Scheme 1).

Since yields were not high, and, occasionally led to mixtures containing unchanged fumaric acids which were difficult to remove, we found that an alternative method of preparation of the labelled samples of malic acid was preferable to using fumarase. Here samples of (2*S*,3*R*)-[3-$^2H_1$]- and (2*S*,3*S*)-[2,3-$^2H_2$]-aspartic acid, **6** $H_B = {}^2H$ and **6** $H_A = {}^2H$, respectively, were prepared[8] in excellent yield using immobilised *Escherichia coli* by the method of Woodard.[9] These were treated with nitrous acid to yield the corresponding malic acids **5** in ~ 70% yield.



4 **5** **6**

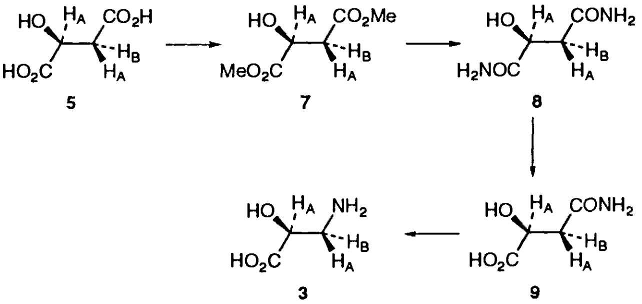
Scheme 1

Our first approach to labelled samples of isoserine **3** was to prepare the labelled monoamides, (2*S*)-β-malamic acids **9**, by methods which had been successful for the preparation of the unlabelled material[10,11] and to subject these, *via* the *O*-acetyl derivatives, to a Hofmann rearrangement which should, by precedent,[12] occur with retention of stereochemistry at the stereospecifically labelled primary carbon atom.

We therefore prepared the diesters **7** from malic acid **5** by reaction with methanol and HCl. Care had to be taken during isolation of the product to prevent its thermal elimination to dimethyl fumarate. The diamides **8** were prepared from the diesters **7** in good yield and the ^1H NMR spectra were indicative that the stereospecificity of labelling was intact.

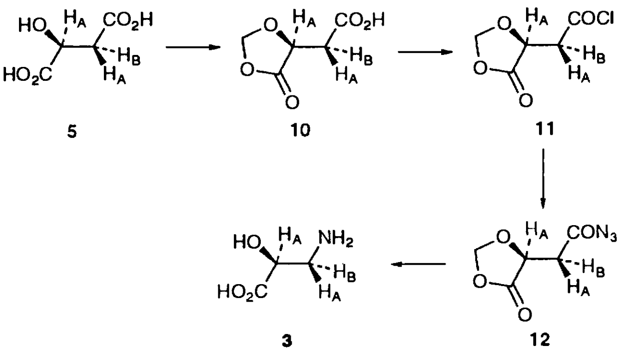
Initially, these spectra were run in 10% $NaO^2H/^2H_2O$ until it was realised that they were in fact identical with the spectra obtained for the β-malamic acids **9**. It was seen that the spectra changed to those of the monoamides very quickly and so hydrolysis of the diamides **8** to the monoamides **9** was extremely rapid under these conditions. The ^1H NMR spectra of the diamides were therefore routinely run in $(CD_3)_2SO$ ([$^2H_6$]DMSO).

The diamides **8** were now hydrolysed to the monoamides **9** by using 1 mol aq. KOH. Since we found it to be extremely important in the subsequent Hofmann rearrangement step that there be no anionic contaminant in the substrate it was essential to purify the monoamides **9** by careful ion-exchange chromatography. The monoamides were then converted into the corresponding *O*-acetates and subjected *in situ* to Hofmann rearrangement by the method of Andruszkiewicz[13] using freshly prepared[14] [bis(trifluoroacetoxy)iodo]benzene. The labelled isoserines **3** were obtained on hydrolysis (Scheme 2). Although the overall yield from the labelled malates was reasonable, the process involved several difficult and tedious steps. Thus, publication of a procedure involving the Curtius rearrangement and not requiring purification of intermediate products[15] presented an attractive alternative, the Curtius rearrangement being expected to proceed with retention of stereochemistry at the migrating primary centre.[16]



Scheme 2

The samples of labelled malic acid were therefore converted into the dioxolidinones **10** by reaction with paraformaldehyde and catalytic quantities of toluene-*p*-sulfonic acid (PTSA) (Scheme 3). These were converted without purification, *via* the acid chlorides **11** and azides **12**, into labelled samples of isoserine **3** which had identical spectroscopic properties to the samples obtained by the Hofmann route. The Curtius and Hofmann rearrangements had therefore proceeded with the same stereochemical outcome at the migrating labelled primary centre. Overall yields were of the order of 30–39% for the four steps.



Scheme 3

Having achieved a reliable synthesis of stereospecifically labelled samples of our first target compound, we now investigated their conversion into the protected aziridines **2**. The samples of isoserine **3** were therefore esterified to yield the corresponding methyl esters **13** in nearly quantitative yields by using thionyl dichloride and methanol and these were then converted into the *N*-trityl derivatives **14** by using 1 mole equivalent of trityl chloride with triethylamine in chloroform. These derivatives were treated with toluene-*p*-sulfonyl chloride in pyridine to yield the corresponding tosyl esters, which were cyclised without further purification to the *N*-tritylaziridines, **15** $H_A = {}^2H$ and **15** $H_B = {}^2H$, respectively (Scheme 4). The ^1H and ^2H NMR spectra of these compounds are shown in Fig. 1, indicating complete stereospecificity in every stage of the synthesis to this point.

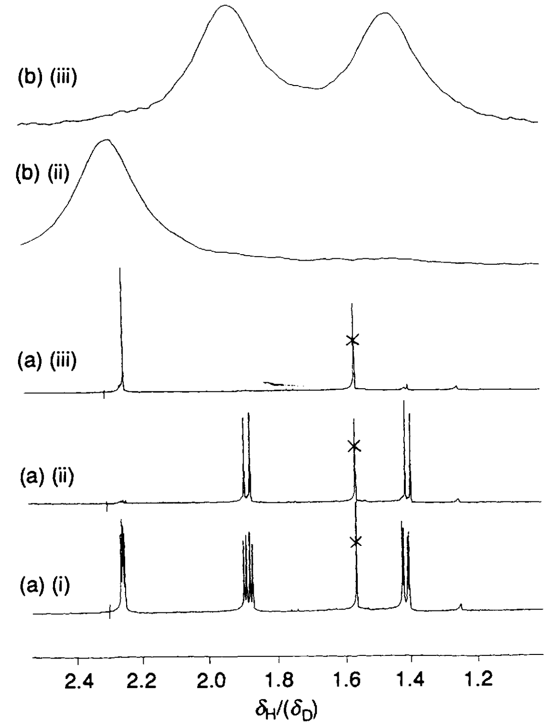
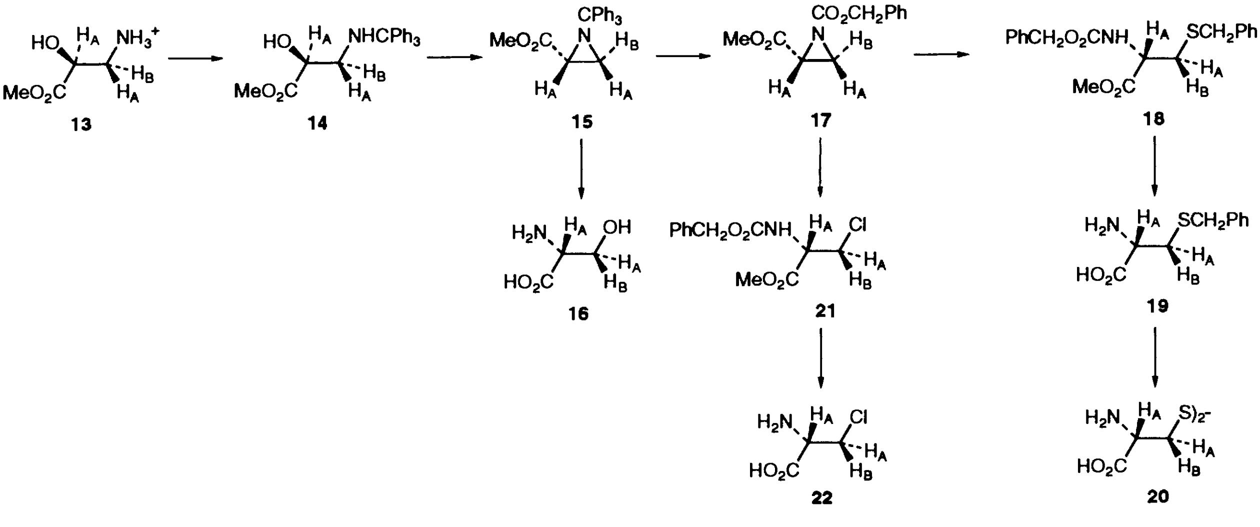


Fig. 1 Part of (a) the ^1H NMR spectrum in $CDCl_3$, and (b) the ^2H NMR spectrum in $CHCl_3$ of (i) methyl (2*R*)-*N*-tritylaziridine-2-carboxylate **15**; (ii) methyl (2*R*,3*R*)-*N*-trityl-[3-$^2H_1$]aziridine-2-carboxylate **15** $H_B = {}^2H$; and (iii) methyl (2*R*,3*S*)-*N*-trityl-[2,3-$^2H_2$]aziridine-2-carboxylate **15** $H_A = {}^2H$

When the *N*-tritylaziridines **15** were heated to reflux with 20% aq. perchloric acid for 30 h, nearly quantitative yields of the corresponding samples of the labelled serine **16** were obtained. Since we had previously synthesized samples of (2*S*,3*R*)-[3-$^2H_1$]- and (2*S*,3*S*)-[2,3-$^2H_2$]-serine[17] we were now in a position to confirm the stereochemistry assigned to the samples prepared *via* our aziridine synthesis. The ^1H NMR spectra are shown in Fig. 2, and it can be seen that the spectrum of (2*S*,3*R*)-[3-$^2H_1$]serine is identical with that of (2*R*,3*S*)-[3-$^2H_1$]serine, the optical rotations being numerically equal but of opposite sign. Similarly the spectra of (2*S*,3*S*)-[2,3-$^2H_2$]- and (2*R*,3*R*)-[2,3-$^2H_2$]-serine were identical and their rotations were those expected of enantiomers. This confirms the assumption that our synthesis involves retention of stereochemistry at the labelled primary centre in the Curtius rearrangement step, **12**→**3**; inversion of stereochemistry at C-2 in the aziridine ring closure step, **14**→**15**; and inversion of stereochemistry in the nucleophilic ring-opening step, **15**→**16**.

The *N*-tritylaziridines **15** were not reactive enough with other nucleophiles and so they were converted into the *N*-benzyloxycarbonyl derivatives **17** by deprotection using trifluoroacetic acid (TFA) in methanol–chloroform and then reaction with benzyl chloroformate under Schotten–Baumann conditions. The labelled aziridines **17** $H_A = {}^2H$ and **17** $H_B = {}^2H$ were treated with benzyl mercaptan and boron trifluoride–diethyl ether to yield the adducts **18** $H_A = {}^2H$ and **18** $H_B = {}^2H$, respectively in ~40% yield. When these were deprotected by using refluxing 6 mol dm^{-3} HCl, labelled samples of *S*-benzylcysteine **19** $H_A = {}^2H$ and **19** $H_B = {}^2H$ were obtained. The *S*-benzyl protecting group was removed by using sodium in liquid ammonia to give reasonably clean labelled samples of cysteine before purification. Since this was partly oxidised



Scheme 4

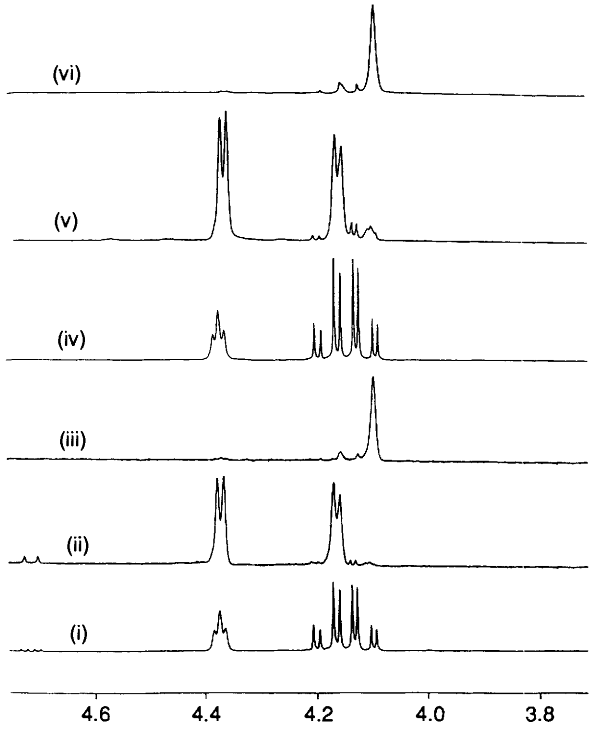


Fig. 2 ^1H NMR spectra in 20% DCl/D_2O of (i) (2*R*)-serine **16**; (ii) (2*R*,3*S*)-[3-$^2H_1$]serine **16** H_B = ^2H; (iii) (2*R*,3*R*)-[2,3-$^2H_2$]serine **16** H_A = ^2H; (iv) (2*S*)-serine; (v) (2*S*,3*R*)-[3-$^2H_1$]serine; and (vi) (2*S*,3*S*)-[2,3-$^2H_2$]serine

during careful ion-exchange purification, however, we found it easier to oxidise the product by passing oxygen through the column fractions. Very clean samples of stereospecifically labelled D-cysteine **20** were obtained, in good yield.

Our final target was to prepare stereospecifically labelled samples of the enzyme inhibitor β-chloroalanine **22**. Previous work[18,19] indicated that ring opening of 3-unsubstituted 2-carboxyaziridine derivatives with HCl was not entirely regiospecific, attack being at both α- and β-carbon atoms and, indeed, reaction of our *N*-tritylaziridine, **15**, with HCl in ethanol and diethyl ether followed by deprotection gave a mixture of α- and β-chloroalanine. Change of *N*-protecting group and solvent altered the α/β ratio but failed to yield β-chloroalanine **22** as the sole product. The best conditions for production of the isomer using HCl were sonication in 6 mol dm^{-3} HCl but, when this reaction was carried out on the stereospecifically labelled aziridines **15** H_A = ^2H and **15** H_B = ^2H then the ^1H NMR spectra of the products indicated loss of stereochemical integrity in the products of both β- and α-attack.

We were finally able to achieve synthesis of stereospecifically labelled samples of β-chloroalanine **22** by reaction of the *N*-benzyloxycarbonyl derivatives **17** with $TiCl_4$ in dichloromethane–chloroform at −78 °C, when only ~8% of α-attack was observed and the spectra of the esters **21** indicated that labelling was stereospecific. The stereospecifically labelled samples of the inhibitor β-chloroalanine were then obtained by hydrolysis in refluxing 4 mol dm^{-3} aq. sulfuric acid.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations (given in units of 10^{-1} deg cm^2 g^{-1}) were measured on a Perkin-Elmer PE241 polarimeter using a 1 dm pathlength micro cell. IR spectra were recorded on a Perkin-Elmer 1720 Fourier-transform instrument and UV spectra were recorded on a Philips PU8720 spectrophotometer. Mass spectra were recorded by Mr. A. Greenway using Kratos MS25 and Kratos MS80 instruments and on KS50 and VG7070 instruments by Dr. S. Chotai at the Wellcome Research Laboratories, Beckenham. 3-NBA refers to 3-nitrobenzyl alcohol. All ^1H NMR spectra were recorded on a Bruker WM360 instrument (360 MHz), ^{13}C NMR spectra (^1H-decoupled) were recorded by Dr. A. G. Avent on a Bruker AMX 500 instrument (125.8 MHz), and ^2H NMR spectra were recorded on a Bruker A-C 250SY instrument (38.4 MHz) by Mr. C. M. Dadswell. *J*-Values are given in Hz. 3-(Trimethylsilyl)propane-1-sulfonic acid (DSS), located at δ 0.0, was used as the internal standard for samples run in 20% DCl in D_2O. For all other NMR spectra, the residual solvent peak was used as reference. TLC was carried out on Merck Kieselgel 60 F_{254} precoated silica gel plates of thickness 0.2 mm (ART 5554 and ART 5714). Column chromatography was performed using Merck Kieselgel 60 (230–400 mesh—ART 9385). Ion-exchange resins were purchased in the chloride form from Aldrich (Dorset) and converted into the required form by passage of at least a five-fold excess of the relevant ion through a column of the resin, followed by washing with distilled water. Microanalyses were performed by Miss M. Patel, Sussex University, and by Mrs. P. Firmin, Wellcome Research Laboratories, Beckenham.

(2S,3R)-[3-^{2}H$_1$]*Malic Acid* **5** H$_B$ = ^{2}H.—*Method A*. Fumaric acid **4** (15 g, 129 mmol) and dipotassium hydrogen phosphate (22 g, 126 mmol) were dissolved in water (1 dm^3) and the pH of the solution was adjusted to 7.4 with 3 mol dm^{-3} sodium hydroxide. The solution was lyophilised, and the residue was redissolved in ^{2}H$_2$O and relyophilised. The lyophilisation procedure was repeated twice to ensure maximum exchange by deuterium. The residue was finally dissolved in ^{2}H$_2$O (100 cm^3) to give a pD of 7.7 ± 0.2. Fumarase (Sigma, 500 units) was added and the reaction mixture was incubated at 28 °C for 5 days, the progress of the reaction being followed by observing the disappearance of λ 290 nm in the UV spectrum. The enzyme was denatured by immersion in boiling water for 30 min. The precipitated protein was removed by filtration, and the filtrate was titrated with 0.1 mol dm^{-3} sodium hydroxide to the phenolphthalein end point and concentrated under reduced pressure to a volume of 10–20 cm^3.

The product was purified on a Dowex 1X2-200 (formate) ion-exchange column. Inorganic salts were eluted with water and (2*S*,3*R*)-[3-^{2}H$_1$]malic acid, **5** H$_B$ = ^{2}H, was recovered by elution with 6% aq. formic acid. The product was a solid (9.3 g, 54%), m.p. 97–98 °C (lit.,[20] 100 °C); $[\alpha]_D^{23}$ −6.06 (*c* 1.6, MeOH). The ^{1}H NMR spectrum was identical with that described in Method B below, but in some incubations ^{1}H NMR spectroscopic analysis showed the product to be contaminated with significant quantities of fumaric acid.

Method B. Freshly prepared 30% aq. NaNO$_2$ (112.8 cm^3) was added over a period of 20 min to a stirred solution of (2*S*,3*R*)-[3-^{2}H$_1$]aspartic acid **6** H$_B$ = ^{2}H (10 g, 75 mmol) in 0.5 mol dm^{-3} H$_2$SO$_4$ (376 cm^3) at room temperature. The reaction mixture was stirred at room temperature for 2 h, the volume was reduced under reduced pressure to ~100 cm^3, and Celite was added until a thick sludge was obtained. This was placed in an extraction thimble and extracted in a Soxhlet apparatus with diethyl ether during 48 h. The solvent was removed under reduced pressure to yield (2*S*,3*R*)-[3-^{2}H$_1$]malic acid **5** H$_B$ = ^{2}H as a pale yellow solid (7.4 g, 73%), m.p. 97.5–99.0 °C (lit.,[20] 100 °C); $[\alpha]_D^{21.0}$ −6.83 (*c* 1.6, MeOH); *m*/*z* [+ve FAB (glycerol)] 136 ([M + H]$^+$), ν_{max}(KBr)/cm^{-1} 3435br (OH), 3000–2600br (CO*OH*) and 1732 (*CO*OH); δ_H(10% NaOD in D$_2$O) 1.93 (1 H, d, $J_{3S,2}$ 9.3, 3*S*-H) and 3.81 (1 H, d, $J_{2,3S}$ 9.3, 2-H); δ_C(10% NaOD in D$_2$O) 183.53 and 182.25 (2 × CO$_2$H), 72.65 (C-2) and 44.86 (t, C-3).

(2S,3S)-[2,3-^{2}H$_2$]*Malic Acid* **5** H$_A$ = ^{2}H.—*Method A*. The dideuteriated compound was prepared as above in 46% yield by using [2,3-^{2}H$_2$]fumaric acid **4** H$_A$ = ^{2}H, in water. The spectra were identical with those reported below for method B.

Method B. The product was prepared as above in 67% yield by using (2*S*,3*S*)-[2,3-^{2}H$_2$]aspartic acid **6** H$_A$ = ^{2}H, and was a solid, m.p. 97–99.5 °C; $[\alpha]_D^{22}$ −6.81 (*c* 1.6, MeOH); *m*/*z* [EI] 137 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3446br (OH), 3000–2600 (CO*OH*) and 1724 (*CO*OH); δ_H(10% NaOD in D$_2$O) 2.15 (1 H, s, 3*R*-H); δ_C(10% NaOD in D$_2$O) 183.52 and 182.28 (2 × CO$_2$H), 72.35 (t, C-2) and 44.78 (t, C-3).

Dimethyl (2S)-*Malate* **7**.—Malic acid **5** (20 g, 149 mmol) was dissolved in methanol (250 cm^3). The solution was cooled to 0 °C in an ice-bath and was saturated with dry HCl gas. The reaction mixture was left at room temperature for 2 days, and the solvent was removed under reduced pressure to yield dimethyl (2*S*)-malate **7** as a pale green oil. If any solid starting material was present at this stage, the residue was redissolved in methanol, and the solution was resaturated with HCl gas and left for a further period until reaction was complete. Owing to a tendency for the product to dehydrate to fumaric acid, it was not purified further (21.8 g, 90%); $[\alpha]_D^{22.5}$ −9.86 (*c* 1.5, MeOH) (lit.,[20] −6.85); *m*/*z* [EI] 163 ([M + H]$^+$), 131 ([M − OCH$_3$]$^+$) and 103 ([M − CO$_2$CH$_3$]$^+$); ν_{max}(film)/cm^{-1} 3473br (OH) and 1741 (ester); δ_H(CDCl$_3$) 2.80 (1 H, dd, $J_{3S,2}$ 6.4, $J_{3S,3R}$ 16.4, 3*S*-H), 2.90 (1 H, dd, $J_{3R,2}$ 4.5, $J_{3R,3S}$ 16.4, 3*R*-H), 3.72 (3 H, s, CO$_2$Me), 3.80 (3 H, s, CO$_2$Me) and 4.56 (1 H, dd, $J_{2,3S}$ 6.4, $J_{2,3R}$ 4.5, 2-H).

Dimethyl (2*S*,3*R*)-[3-^{2}H$_1$]malate **7** H$_B$ = ^{2}H was prepared as above in 85% yield by using (2*S*,3*R*)-[3-^{2}H$_1$]malic acid **5** H$_B$ = ^{2}H, and had $[\alpha]_D^{23.0}$ −9.63 (*c* 1.5, MeOH); *m*/*z* [EI] 164 ([M + H]$^+$), 132 ([M − OCH$_3$]$^+$) and 104 ([M − CO$_2$CH$_3$]$^+$); ν_{max}(film)/cm^{-1} 3465br (OH) and 1741 (ester); δ_H(CDCl$_3$) 2.80 (1 H, d, $J_{3S,2}$ 6.4, 3*S*-H), 3.72 (3 H, s, CO$_2$Me), 3.80 (3 H, s, CO$_2$Me) and 4.56 (1 H, d, $J_{2,3S}$ 6.4, 2-H).

Dimethyl (2*S*,3*S*)-[2,3-^{2}H$_2$]malate **7** H$_A$ = ^{2}H was prepared as above in 88% yield by using (2*S*,3*S*)-[2,3-^{2}H$_2$]malic acid **5** H$_A$ = ^{2}H, and had $[\alpha]_D^{24.0}$ −10.5 (*c* 1.5, MeOH); *m*/*z* [EI] 165 ([M + H]$^+$), 133 ([M − OCH$_3$]$^+$) and 105 ([M − CO$_2$CH$_3$]$^+$); ν_{max}(film)/cm^{-1} 3424br (OH) and 1737 (ester); δ_H(CDCl$_3$) 2.90 (1 H, s, 3*S*-H), 3.72 (3 H, s, CO$_2$Me) and 3.81 (3 H, s, CO$_2$Me).

(2S)-*Malamide* **8**.—Dimethyl (2*S*)-malate **7** (20 g, 123.45 mmol) was dissolved in methanol (200 cm^3) and the solution was cooled to 0 °C in an ice-bath. Liquid ammonia was added to the constantly stirred reaction mixture until the liquid volume had approximately doubled. The reaction mixture was then stirred for 2 h at room temperature to allow evaporation of the excess of ammonia, and was then left overnight in a refrigerator. The resulting crystals were filtered off, washed with cold water, and recrystallised from methanol–water (13 g, 80%), m.p. 156–157 °C (lit.,[20] 157 °C); $[\alpha]_D^{23}$ −34.8 (*c* 1.5, water) (lit.,[20] −37.9); *m*/*z* [+ve CI (NH$_3$)] 133 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3413 (OH), 3373 and 3207 (NH) and 1657 (amide); δ_H([^{2}H$_6$]DMSO) 2.20 (1 H, dd, $J_{3S,2}$ 9.5, $J_{3S,3R}$ 14.8, 3*S*-H), 2.41 (1 H, dd, $J_{3R,2}$ 2.9, $J_{3R,3S}$ 14.8, 3*R*-H), 4.14 (1 H, m, 2-H), 5.56 (1 H, d, exch., $J_{OH,2}$ 5.8, OH) and 6.87, 7.15, 7.22 and 7.31 (4 × 1 H, 4 s, exch., 2 × CONH$_2$).

(2*S*,3*R*)-[3-^{2}H$_1$]Malamide **8** H$_B$ = ^{2}H was prepared as above in 76% yield from dimethyl (2*S*,3*R*)-[3-^{2}H$_1$]malate **7** *H*$_B$ = ^{2}H, and had m.p. 155.5–156.5 °C; $[\alpha]_D^{23}$ −38.3 (*c* 1.5, water); *m*/*z* [+ve CI (NH$_3$)] 134 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3403 (OH), 3370 and 3200 (NH) and 1657 (amide); δ_H([^{2}H$_6$]DMSO) 2.18 (1 H, d, $J_{3S,2}$ 9.5, 3*S*-H), 4.14 (1 H, dd, $J_{2,3S}$ 9.5, $J_{2,OH}$ 5.8, 2-H), 5.56 (1 H, d, $J_{OH,2}$ 5.8, OH) and 6.88, 7.16, 7.22 and 7.32 (4 × 1 H, 4 s, exch., 2 × CONH$_2$).

(2*S*,3*S*)-[2,3-^{2}H$_2$]Malamide **8** H$_A$ = ^{2}H was prepared as above in 76% yield by using dimethyl (2*S*,3*S*)-[2,3-^{2}H$_2$]malate **7** H$_A$ = ^{2}H, and had m.p. 155–157 °C; $[\alpha]_D^{23}$ −35.8 (*c* 1.2, water); *m*/*z* [+ve CI (NH$_3$)] 135 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3404 (OH), 3390 and 3200 (NH) and 1656 (amide); δ_H([^{2}H$_6$]DMSO) 2.39 (1 H, s, 3*R*-H), 5.56 (1 H, br s, OH) and 6.88, 7.16, 7.24 and 7.36 (4 × 1 H, 4 s, exch., 2 × CONH$_2$).

(2S)-β-*Malamic Acid* **9**.—(2*S*)-Malamide **8** (12 g, 90.91 mmol) was dissolved in 1 mol dm^{-3} potassium hydroxide (91 cm^3, 91 mmol) and the solution was heated to reflux for 3 h. Conc. hydrochloric acid (12 cm^3) was added to the cooled reaction mixture, which was then left in a refrigerator overnight. Crystals precipitated. These were filtered off, and washed with ice-cold water. The yield at this stage was 8.7 g (72%). The product was pure except for the presence of chloride ions. The crystalline product was dissolved in water (10 cm^3) and applied to a column of Dowex 1X2-200 (OH$^-$) ion-exchange resin. The column was eluted with water until no chloride ions could be detected in the eluent (silver nitrate–nitric acid). The product was eluted with 1% aq. acetic acid and was recrystallised from water (7.52 g, 62%); m.p. 148–149 °C (lit.,[11] 149 °C); $[\alpha]_D^{23}$ −9.4 (*c* 1.2, water) (lit.,[11] −9.33); *m*/*z* [+ve CI (NH$_3$)] 134 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3404 (OH), 3250 and 3240 (NH), 2500–3000 (CO*OH*), 1720 (acid) and 1676 (amide); δ_H(10% NaOD in D_2O) 2.36 (1 H, dd, $J_{3S,2}$ 9.2, $J_{3S,3R}$ 15.3, 3*S*-H), 2.57 (1 H, dd, $J_{3R,2}$ 3.6, $J_{3R,3S}$ 15.3, 3*R*-H) and 4.21 (1 H, m, $J_{2,3S}$ 9.3, $J_{2,3R}$ 3.6, 2-H).

(2*S*,3*R*)-[3-$^2H_1$]-β-Malamic acid **9** H_B = ^2H was prepared as above, by using (2*S*,3*R*)-[3-$^2H_1$]malamide **8** H_B = ^2H, in 66% yield, m.p. 148–149 °C; $[\alpha]_D^{23}$ −9.50 (*c* 1.4, water); *m/z* [+ve CI (NH_3)] 135 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3398 (OH), 3250 and 3240 (NH), 2500–3000 (CO*OH*) and 1721 (acid); δ_H(10% NaOD in D_2O) 2.34 (1 H, d, $J_{3S,2}$ 9.1, 3*S*-H) and 4.21 (1 H, d, $J_{2,3S}$ 9.1, 2-H).

(2*S*,3*S*)-[2,3-$^2H_2$]-β-Malamic acid **9** H_A = ^2H was prepared as above, by using (2*S*,3*S*)-[2,3-$^2H_2$]malamide **8** H_A = ^2H, in 66% yield, m.p. 148–149 °C; $[\alpha]_D^{23}$ −9.30 (*c* 1.0, water); *m/z* [+ve CI (NH_3)] 136 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3402 (OH), 3300 and 3200 (NH), 2500–3000 (CO*OH*), 1736 (acid) and 1655 (amide); δ_H(10% NaOD) 2.57 (1 H, s, 3*R*-H).

(2S)-*Isoserine* **3**.—*Method A*. (2*S*)-β-Malamic acid **9** (3 g, 22.56 mmol) and pyridine (18 cm^3, 225 mmol) were dissolved in constantly stirred acetonitrile (100 cm^3) at 0 °C in an ice-bath. Acetic anhydride (2.3 cm^3, 25 mmol) was added dropwise as the system was purged with nitrogen. The reaction mixture was stirred for 1 h at room temperature and was then diluted with water (100 cm^3). Freshly prepared [bis(trifluoroacetoxy)iodo]-benzene (14.63 g, 33 mmol) was added and the reaction mixture was stirred for an additional 4 h at room temperature. The solvents were removed by heating (60 °C) under reduced pressure to yield an oil, which was dissolved in water (100 cm^3) and extracted with diethyl ether (3 × 200 cm^3). The aqueous phase was diluted with acetone (100 cm^3)–conc. hydrochloric acid (50 cm^3) and was heated to reflux for 2 h. The solvents were removed under reduced pressure by heating (70 °C) to yield an oily residue, which was dissolved in water (10 cm^3) and separated on a column of Dowex 1X2-200 (OH$^-$). The pyridine-derived contaminants were eluted with water and then (2*S*)-isoserine was recovered by elution with 5% aq. acetic acid. The solvent was removed from the relevant fractions (as determined by TLC) under reduced pressure to yield a solid, which was recrystallised from methanol–water (1.56 g, 66%), m.p. 187–188 °C (lit.,[13] 188–189 °C); $[\alpha]_D^{23}$ −31.4 (*c* 1.4, water) (lit.,[13] −32.2). Spectra were identical with those for the product prepared using Method B below.

Method B. (2*S*)-Malic acid **5** (5.36 g, 40 mmol), para-formaldehyde (1.60 g, 53 mmol) and PTSA (40 mg; 0.23 mmol) were added to chloroform (40 cm^3) and the reaction mixture was heated at reflux with azeotropic trapping of water for 4 h. The solvent was removed under reduced pressure. The remaining residue was heated to reflux with $SOCl_2$ (10 cm^3, 137 mmol) for 1 h. After removal of the excess of $SOCl_2$ under reduced pressure, CCl_4 (20 cm^3) was added and the solvent was removed under reduced pressure. The latter procedure was repeated several times to remove last traces of $SOCl_2$.

The residue was dissolved in acetone (40 cm^3), cooled to −15 °C using a salt–ice-bath, and added to a solution of sodium azide (3.5 g, 53.9 mmol) in water (12 cm^3) at 0 °C. The reaction mixture was stirred at −15 °C for 30 min. The acetone was removed under reduced pressure at 0 °C and the mixture was extracted with toluene (2 × 20 cm^3). The organic phase was dried ($MgSO_4$), and concentrated under reduced pressure to ~20 cm^3. The mixture was heated to 60 °C, whereupon nitrogen was given off. After evolution of nitrogen had ceased, the mixture was heated at reflux for 15 min and the solvent was then removed under reduced pressure. 5 mol dm^{-3} Hydrochloric acid (20 cm^3) was added, and the reaction mixture was heated at reflux for 30 min. The solvent was removed under reduced pressure. Water (50 cm^3) was added and the solvent was again removed under reduced pressure. The residue was dissolved in water (10 cm^3) and applied to a Dowex 1X2-200 (OH$^-$) ion-exchange column. The column was eluted with water until the pH of the eluted water was neutral. The product was then recovered by elution with 5% aq. acetic acid. The solvent was removed from the relevant column fractions (as determined by TLC) under reduced pressure to yield (2*S*)-isoserine **3** as an orange solid (1.64 g, 39%), m.p. 188.5–190.5 °C (lit.,[13] 188–189 °C); $[\alpha]_D^{25.5}$ −26.8 (*c* 1, water) (lit.,[13] −32.2); *m/z* [+ve FAB (glycerol)] 106 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3246 (OH), 3200 (NH), 3000–2500 (CO*OH*) and 1657 (acid); δ_H(D_2O) 2.90 (1 H, dd, $J_{3S,2}$ 8.4, $J_{3S,3R}$ 13.1, 3*S*-H), 3.13 (1 H, dd, $J_{3R,2}$ 4.1, $J_{3R,3S}$ 13.1, 3*R*-H) and 4.01 (1 H, dd, $J_{2,3S}$ 8.4, $J_{2,3R}$ 4.1, 2-H).

(2*S*,3*R*)-[3-$^2H_1$]Isoserine **3** H_B = ^2H was prepared by Method A above in 62% yield by using (2*S*,3*R*)-β-malamic acid **9** H_B = ^2H, or by using Method B from (2*S*,3*R*)-[3-$^2H_1$]malic acid **5** H_B = ^2H in 30% overall yield, m.p. 188–190 °C; $[\alpha]_D^{26.0}$ −26.4 (*c* 1, water); *m/z* [+ve FAB (glycerol)] 107 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3400 (OH), 3070 (NH), 3000–2500 (CO*OH*) and 1650 (*CO*OH); δ_H(D_2O) 2.90 (1 H, d, $J_{3S,2}$ 8.4, 3*S*-H) and 4.03 (1 H, d, $J_{2,3S}$ 8.4, 2-H).

(2*S*,3*S*)-[2,3-$^2H_2$]Isoserine **3** H_A = ^2H was prepared by Method A above in 63% yield by using (2*S*,3*S*)-β-malamic acid **9** H_A = ^2H, or by Method B by using (2*S*,3*S*)-[2,3-$^2H_2$]malic acid **5** H_A = ^2H, in 34% overall yield, m.p. 188–190 °C; $[\alpha]_D^{26.5}$ −26.9 (*c* 1, water); *m/z* [+ve FAB (glycerol)] 108 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3246 (OH), 3050 (NH), 3000–2500 (CO*OH*) and 1656 (*CO*OH); δ_H(D_2O) 3.12 (1 H, s, 3*R*-H).

Methyl (2S)-*Isoserinate Hydrochloride* **13**.—(2*S*)-Isoserine **3** (1.32 g, 12.6 mmol) was added to a constantly stirred solution of methanol (20 cm^3) and $SOCl_2$ (2.5 cm^3, 34.3 mmol) at 0 °C. When the starting material had dissolved, the reaction mixture was allowed to reach room temperature and was stirred for 22 h. The solvent was removed under reduced pressure, CCl_4 (10 cm^3) was added and the solvent was again removed under reduced pressure. The latter procedure was repeated several times to remove last traces of $SOCl_2$. Methyl (2*S*)-isoserinate hydrochloride **13** was obtained as a pale brown solid (1.95 g, 99%), m.p. 104–105 °C; $[\alpha]_D^{21.0}$ −19.2 (*c* 1, water); *m/z* [+ve FAB (glycerol)] 120 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3537 (OH), 3059 (NH) and 1746 (ester); δ_H(^2H_2O) 3.10 (1 H, dd, $J_{3S,2}$ 8.4, $J_{3S,3R}$ 13.3, 3*S*-H), 3.31 (1 H, dd, $J_{3R,2}$ 4.1, $J_{3R,3S}$ 13.3, 3*R*-H), 3.67 (3 H, s, CO_2Me) and 4.44 (1 H, dd, $J_{2,3S}$ 8.4, $J_{2,3R}$ 4.1, 2-H).

Methyl (2*S*,3*R*)-[3-$^2H_1$]isoserinate hydrochloride **13** H_B = ^2H was prepared as above in 100% yield by using (2*S*,3*R*)-[3-$^2H_1$]isoserine **3** H_B = ^2H. The product was a solid, m.p. 101.5–103.5 °C; $[\alpha]_D^{21.5}$ −18.2 (*c* 1, water); *m/z* [+ve FAB (glycerol)] 121 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3503 (OH), 3050 (NH) and 1746 (ester); δ_H(D_2O) 3.01 (1 H, d, $J_{3S,2}$ 8.4, 3*S*-H), 3.60 (3 H, s, CO_2Me) and 4.37 (1 H, d, $J_{2,3S}$ 8.4, 2-H).

Methyl (2*S*,3*S*)-[2,3-$^2H_2$]isoserinate hydrochloride **13** H_A = ^2H was prepared as above in 97% yield by using (2*S*,3*S*)-[2,3-$^2H_2$]isoserine **3** H_A = ^2H. The product was a solid, m.p. 102–104 °C; $[\alpha]_D^{22.5}$ −16.6 (*c* 1, water); *m/z* [+ve FAB (glycerol)] 122 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3504 (OH), 3051 (NH) and 1744 (ester); δ_H(^2H_2O) 3.25 (1 H, s, 3*R*-H) and 3.62 (3 H, s, CO_2Me).

Methyl (2S)-N-*Tritylisoserinate* **14**.—Methyl (2*S*)-isoserinate hydrochloride **13** (1.95 g, 12.5 mmol) was dissolved in chloroform (10 cm^3)–triethylamine (3.85 cm^3, 27.6 mmol) at room temperature. The mixture was cooled to 0 °C and a solution of trityl chloride (3.49 g, 12.5 mmol) in chloroform (10 cm^3) was added dropwise over a period of 30 min. The reaction mixture was stirred at 0 °C under nitrogen for 24 h. It was washed successively with 10% aq. citric acid (2 × 15 cm^3) and water (2 × 15 cm^3), dried over Na_2SO_4, and the solvent was removed under reduced pressure to yield the *product* as an off-white foam (4.08 g, 90%); $[\alpha]_D^{24.0}$ +17.2 (*c* 1, $CHCl_3$) (Found: C, 76.4; H, 6.5; N, 3.8. $C_{23}H_{23}NO_3$ requires C, 76.4; H, 6.4; N, 3.9%); *m/z* [+ve CI (NH_3)] 284 ($[M - Ph]^+$); ν_{max}(film)/cm^{-1} 3485 (OH), 3317 and 3021 (NH) and 1709 (ester); δ_H($CDCl_3$) 2.45 (1 H, dd, $J_{3R,2}$ 3.7, $J_{3R,3S}$ 12.1, 3*R*-H), 2.51 (1 H, dd, $J_{3S,2}$ 4.7, $J_{3S,3R}$ 12.1, 3*S*-H), 3.84 (3 H, s, CO_2Me), 4.26 (1 H, t, 2-H) and 7.13–7.43 (15 H, m, CPh_3).

Methyl (2*S*,3*R*)-*N*-trityl-[3-$^2H_1$]isoserinate **14** H_B = ^2H was prepared as above in 92% yield by using methyl (2*S*,3*R*)-[3-$^2H_1$]isoserinate **13** H_B = ^2H. The product was a foam, $[\alpha]_D^{24.5}$ +15.6 (*c* 1, $CHCl_3$); *m/z* [+ve CI (NH_3)] 285 ($[M - Ph]^+$); ν_{max}(film)/cm^{-1} 3485 (OH), 3317 and 3021 (NH) and 1713 (ester); δ_H($CDCl_3$) 2.48 (1 H, d, $J_{3S,2}$ 4.8, 3*S*-H), 3.84 (3 H, s, CO_2Me), 4.26 (1 H, d, $J_{2,3S}$ 4.8, 2-H) and 7.14–7.43 (15 H, m, CPh_3).

Methyl (2*S*,3*S*)-*N*-trityl-[2,3-$^2H_2$]isoserinate **14** H_A = ^2H was prepared in 97% yield by using methyl (2*S*,3*S*)-[2,3-$^2H_2$]isoserinate hydrochloride **13** H_A = ^2H. The product was a foam, $[\alpha]_D^{24.5}$ +15.2 (*c* 1, $CHCl_3$); *m/z* [+ve CI (NH_3)] 286 ($[M - Ph]^+$); ν_{max}(film)/cm^{-1} 3486 (OH), 3317 and 3021 (NH) and 1709 (ester); δ_H($CDCl_3$) 2.45 (1 H, s, 3*R*-H), 3.86 (3 H, s, CO_2Me) and 7.16–7.44 (15 H, m, CPh_3).

Methyl (2R)-N-*Tritylaziridine-2-carboxylate* **15**.—Methyl (2*S*)-*N*-tritylisoserinate **14** (2.30 g, 6.37 mmol) was dissolved in pyridine (8 cm^3, 99 mmol) and the solution was cooled to −15 °C in a salt–ice-bath. Toluene-*p*-sulfonyl chloride (3.98 g, 20.9 mmol) was added over a period of 30 min and the reaction mixture was stirred at 0 °C under nitrogen for 22 h. The solvent was removed under reduced pressure and the gummy residue was partitioned between ethyl acetate (20 cm^3) and water (20 cm^3). The organic phase was washed successively with 10% aq. citric acid (20 cm^3) and water (20 cm^3), dried over Na_2SO_4, and the solvent was removed under reduced pressure to yield the intermediate tosyl compound as a gum.

The gum was dissolved in a mixture of tetrahydrofuran (15 cm^3) and triethylamine (2.5 cm^3, 17.9 mmol) and heated at reflux under nitrogen for 22 h. The solvent was removed under reduced pressure and the resulting product was dissolved in ethyl acetate (15 cm^3) and washed successively with 10% aq. citric acid (2 × 10 cm^3), 1 mol dm^{-3} aq. sodium hydrogen carbonate (2 × 10 cm^3) and water (2 × 10 cm^3). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield a dark red/brown solid.

The crude product was dissolved in chloroform (10 cm^3) and purified by silica gel column chromatography with (2:1) chloroform–light petroleum (60–80 °C) as eluent. The solvent was removed from the relevant column fractions (as determined by TLC) under reduced pressure to yield the *title compound* **15** as a solid, which was recrystallised from methanol (1.03 g, 47%), m.p. 127–129 °C; $[\alpha]_D^{20.0}$ +102.4 (*c* 0.7, MeOH) (Found: C, 80.1; H, 6.2; N, 4.1. $C_{23}H_{21}NO_2$ requires C, 80.4; H, 6.1; N, 4.2%); *m/z* [+ve FAB (3-NBA)] 366 ($[M + Na]^+$), 344 ($[M + H]^+$) and 266 ($[M - Ph]^+$); ν_{max}(KBr)/cm^{-1} 1744 (ester); δ_H($CDCl_3$) 1.41 (1 H, dd, $J_{3S,2}$ 6.2, $J_{3S,3R}$ 1.6, 3*S*-H), 1.89 (1 H, dd, $J_{2,3S}$ 6.2, $J_{2,3R}$ 2.7, 2-H), 2.25 (1 H, dd, $J_{3R.2}$ 2.7, $J_{3R,3S}$ 1.6, 3*R*-H), 3.76 (3 H, s, CO_2Me) and 7.21–7.51 (15 H, m, CPh_3); δ_C($CDCl_3$) 171.85 (CO_2Me), 143.62 (*ipso* arom. C), 129.32 and 127.62 (*ortho* and *meta* arom. C), 126.91 (*para* arom. C), 74.42 (Ph_3CN), 52.00 (MeO), 31.73 (C-2) and 28.63 (C-3).

Methyl (2*R*,3*R*)-*N*-trityl-[3-$^2H_1$]aziridine-2-carboxylate **15** H_B = ^2H was prepared from methyl (2*S*,3*R*)-*N*-trityl-[3-$^2H_1$]-isoserinate **14** H_B = ^2H in 49% yield by using the above method. The product was a solid, m.p. 126–129 °C; $[\alpha]_D^{23.0}$ + 101.6 (*c* 0.7, MeOH); *m/z* [+ve FAB (3-NBA)] 367 ($[M + Na]^+$) and 345 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 1744 (ester); δ_H($CDCl_3$) 1.40 (1 H, d, $J_{3S,2}$ 6.2, 3*S*-H), 1.88 (1 H, d, $J_{2,3S}$ 6.2, 2-H), 3.76 (3 H, s, CO_2Me) and 7.20–7.51 (15 H, m, CPh_3); δ_D(38.4 MHz; $CHCl_3$) 2.28 (1 D, br s, 3*R*-D); δ_C($CDCl_3$) 171.85 (CO_2Me), 143.63 (*ipso* arom. C), 129.32 and 127.63 (*ortho* and *meta* arom. C), 126.91 (*para* arom. C), 74.39 (Ph_3CN), 52.00 (MeO), 31.64 (C-2) and 28.33 (t, C-3).

Methyl (2*R*,3*S*)-*N*-trityl-[2,3-$^2H_2$]aziridine-2-carboxylate **15** H_A = ^2H was prepared in 50% yield by the above method using methyl (2*S*,3*S*)-*N*-trityl-[2,3-$^2H_2$]isoserinate **14** H_A = ^2H. The product was a solid, m.p. 126–130 °C; $[\alpha]_D^{23.5}$ +103.0 (*c* 0.7 MeOH); *m/z* [+ve FAB (3-NBA)] 368 ($[M + Na]^+$) and 346 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 1744 (ester); δ_H($CDCl_3$) 2.24 (1 H, s, 3*R*-H), 3.76 (3 H, s, CO_2Me) and 7.22–7.51 (15 H, m, CPh_3); δ_D($CHCl_3$) 1.45 (1 D, br s, 3*S*-D) and 1.92 (1 D, br s, 2-D); δ_C($CDCl_3$) 171.84 (CO_2Me), 143.64 (*ipso* arom. C), 129.31 and 127.63 (*ortho* and *meta* arom. C), 126.91 (*para* arom. C), 74.37 (Ph_3CN), 51.99 (MeO), 31.39 (t, C-2) and 28.29 (t, C-3).

(2R)-*Serine* **16**.—Methyl (2*R*)-*N*-tritylaziridine-2-carboxylate **15** (450 mg, 1.31 mmol) was suspended in 20% aq. perchloric acid (11.25 cm^3) and the mixture was heated at reflux for 30 h. The reaction mixture was cooled to room temperature, neutralised with ammonium hydroxide, and extracted with chloroform (3 × 50 cm^3). The aqueous phase (pH 7) was applied to a Dowex 1X2-200 (OH^-) ion-exchange column. Inorganic contaminants were eluted with water and (2*R*)-serine **16** was recovered by elution with 5% aq. acetic acid. The solvent was removed from the relevant fractions (as determined by TLC) by lyophilisation to yield the product as a solid (133 mg, 97%), m.p. 225–228 °C (lit.,[21] 228 °C); $[\alpha]_D^{21.5}$ −14.8 (*c* 0.75, 1 mol dm^{-3} HCl) (lit.,[22] −14.32); *m/z* [+ve FAB (glycerol)] 106 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 3452 (OH), 3094 (NH), 3000–2500br (COO*H*) and 1599 (*C*OOH); δ_H(20% DCl in D_2O) 4.12 (1 H, dd, $J_{3S,2}$ 3.5, $J_{3S,3R}$ 12.6, 3*S*-H), 4.19 (1 H, dd, $J_{3R,2}$ 4.2, $J_{3R.3S}$ 12.6, 3*R*-H) and 4.37 (1 H, t, 2-H); δ_C(20% DCl in D_2O) 171.39 (CO_2H), 61.41 (C-3) and 56.88 (C-2).

(2R,3S)-[3-$^2H_1$]*Serine* **16** H_B = ^2H was prepared by the above method in 98% yield by using methyl (2*R*,3*R*)-*N*-trityl-[3-$^2H_1$]aziridine-2-carboxylate **15** H_B = ^2H. The product was a solid, m.p. 224.5–229 °C; $[\alpha]_D^{22.0}$ −15.1 (*c* 0.75, 1 mol dm^{3} HCl); *m/z* [+FAB (glycerol)] 107 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 3441 (OH), 3093 (NH), 3000–2500br (COO*H*) and 1598 (*C*OOH); δ_H(20% DCl in D_2O) 4.17 (1 H, d, $J_{3R.2}$ 4.2, 3*R*-H) and 4.37 (1 H, d, $J_{2,3R}$ 4.2, 2-H); δ_C(20% DCl in D_2O) 171.40 (CO_2H), 61.22 (t, C-3) and 56.84 (C-2).

(2R,3R)-[2,3-$^2H_2$]*Serine* **16** H_A = ^2H was prepared by the above method in 100% yield by using methyl (2*R*,3*S*)-*N*-trityl-[2,3-$^2H_2$]aziridine-2-carboxylate **15** H_A = ^2H. The product was a solid, m.p. 225–229 °C; $[\alpha]_D^{22.0}$ −15.0 (*c* 0.75, 1 mol dm^{-3} HCl); *m/z* [+ve FAB (glycerol)] 108 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 3451 (OH), 3089 (NH), 3000–2500br (COO*H*) and 1597 (*C*OOH); δ_H(20% DCl in D_2O) 4.10 (1 H, s, 3*S*-H); δ_C(20% DCl in D_2O) 171.35 (CO_2H), 61.04 (t, C-3) and 56.52 (t, C-2).

1-*Benzyl* 2-*Methyl* (2R)-*Aziridine*-1,2-*dicarboxylate* **17**.—Methyl (2*R*)-*N*-tritylaziridine-2-carboxylate **15** (250 mg, 0.73 mmol) was dissolved in a mixture of chloroform (0.9 cm^3) and methanol (0.9 cm^3) and cooled to 0 °C in an ice-bath. TFA (0.9 cm^3, 12.7 mmol) was added dropwise over a period of 1–2 min and the reaction mixture was stirred at 0 °C under nitrogen for 2.5 h. The solvents were removed under reduced pressure at 0 °C and the resulting solid residue was azeotroped five times with diethyl ether (5 × 2 cm^3) at 0 °C and partitioned between diethyl ether (15 cm^3) and water (15 cm^3). The ether layer was washed with water (10 cm^3) and the combined aqueous fractions were basified with sodium hydrogen carbonate (250 mg, 2.98 mmol). The aqueous fractions were diluted with ethyl acetate (25 cm^3) and the mixture was cooled to 0 °C in an ice-bath. Benzyl chloroformate (124 mg, 0.73 mmol) was added and the reaction mixture was stirred vigorously at room temperature for 1.5 h. The layers were separated and the aqueous layer was washed with ethyl acetate (20 cm^3). The combined organic fractions were washed with brine (30 cm^3), then dried over Na_2SO_4, and the solvent was removed under reduced pressure to yield the product as a gum (161 mg, 94%); $[\alpha]_D^{23.0}$ +38.56 (*c* 0.7, $CHCl_3$); *m/z* [+ve FAB (thioglycerol)] 236 ([M + H]$^+$); ν_{max}(film)/cm^{-1} 1729 (ester); δ_H($CDCl_3$) 2.48 (1 H, dd, $J_{3S,2}$ 5.4, $J_{3S,3R}$ 1.3, 3*S*-H), 2.60 (1 H, dd, $J_{3R,2}$ 3.2, $J_{3R,3S}$ 1.3, 3*R*-H), 3.10 (1 H, dd, $J_{2,3S}$ 5.4, $J_{2,3R}$ 3.2, 2-H), 3.71 (3 H, s. CO_2Me), 5.14 (2 H, s, CO_2CH_2Ph) and 7.36 (5 H, s, CO_2CH_2Ph); δ_C($CDCl_3$) 168.55 (CO_2Me), 160.67 (CO_2CH_2Ph), 135.26 (*ipso* arom. C), 128.48–128.05 (*ortho*, *meta* and *para* arom. C), 68.55 (CO_2CH_2Ph), 52.57 (MeO), 34.76 (C-2) and 31.28 (C-3).

1-Benzyl 2-methyl (2*R*,3*R*)-[3-$^2H_1$]aziridine-1,2-dicarboxylate **17** H_B = ^{2}H was prepared as above in 99% yield by using methyl (2*R*,3*R*)-*N*-trityl-[3-$^2H_1$]aziridine-2-carboxylate **15** H_B = ^{2}H. The product was a gum, $[\alpha]_D^{24.5}$ +40.63 (*c* 0.7, $CHCl_3$); *m/z* [+ve FAB (thioglycerol)] 237 ([M + H]$^+$); ν_{max}(film)/cm^{-1} 1731 (ester); δ_H($CDCl_3$) 2.48 (1 H, d, $J_{3S,2}$ 5.4, 3*S*-H), 3.11 (1 H, d, $J_{2,3S}$ 5.4, 2-H), 3.71 (3 H, s, CO_2Me), 5.14 (2 H, s, CO_2CH_2Ph) and 7.36 (5 H, s, CO_2CH_2Ph); δ_C($CDCl_3$) 168.55 (CO_2Me), 160.67 (CO_2CH_2Ph), 135.28 (*ipso* arom. C), 128.49–128.37 (*ortho*, *meta* and *para* arom. C), 68.56 (CO_2CH_2Ph), 52.57 (MeO), 34.69 (C-2) and 31.02 (t, C-3).

1-Benzyl 2-methyl (2*R*,3*S*)-[2,3-$^2H_2$]aziridine-1,2-dicarboxylate **17** H_A = ^{2}H was prepared as above in 100% yield by using methyl (2*R*,3*S*)-*N*-trityl-[2,3-$^2H_2$]aziridine-2-carboxylate **15** H_A = ^{2}H. The product was a gum, $[\alpha]_D^{23.5}$ +42.46 (*c* 0.7, $CHCl_3$); *m/z* [+ve FAB (thioglycerol)] 238 ([M + H]$^+$); ν_{max}(film)/cm^{-1} 1729 (ester); δ_H($CDCl_3$) 2.59 (1 H, s, 3*R*-H), 3.71 (3 H, s, CO_2Me), 5.15 (2 H, s, CO_2CH_2Ph) and 7.36 (5 H, s, CO_2CH_2Ph); δ_C($CDCl_3$) 168.56 (CO_2Me), 160.67 (CO_2CH_2Ph), 135.30 (*ipso* arom. C), 128.51–128.40 (*ortho*, *meta* and *para* arom. C.), 68.58 (CO_2CH_2Ph), 52.59 (MeO), 34.45 (t, C-2) and 30.95 (t, C-3).

Methyl (2S)-S-*Benzyl*-N-*benzyloxycarbonylcysteinate* **18**.—1-Benzyl 3-methyl (2*R*)-aziridine-1,2-dicarboxylate **17** (143 mg, 0.61 mmol) was dissolved in chloroform (3 cm^3) and a solution of benzyl mercaptan (1.43 g, 11.5 mmol) in chloroform (14.5 cm^3) was added. Boron trifluoride–diethyl ether (5 drops) was added, the flask was purged with nitrogen, and the mixture was stirred at room temperature for 3 days. The solvent was removed from the reaction mixture under reduced pressure. The resulting oil was dissolved in ethyl acetate (15 cm^3), washed with 1 mol dm^{-3} aq. sodium hydrogen carbonate (35 cm^3), dried over Na_2SO_4, and the solvent was removed under reduced pressure.

The crude product was dissolved in chloroform (5 cm^3) and purified by silica gel column chromatography. Unchanged benzyl mercaptan was eluted by using 10% chloroform–90% light petroleum (60–80 °C). *Methyl* (2S)-S-*benzyl*-N-*benzyloxycarbonylcysteinate* **18** was then recovered with ethyl acetate as eluent. The solvent was removed from the relevant column fractions (as determined by TLC) under reduced pressure to yield an oily residue. Recrystallisation from ethyl acetate–light petroleum ether (60–80 °C) yielded the product as long, needle-like crystals (79 mg, 36%), m.p. 56–58 °C; $[\alpha]_D^{21}$ +50.5 (*c* 0.5, MeOH) (Found: C, 62.7; H, 5.9; N, 3.7. $C_{19}H_{21}NO_4S$ requires C, 63.5; H, 5.9; N, 3.9%); *m/z* [+ve FAB (thioglycerol)] 360 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3342 (NH), 1748 (ester) and 1689 (urethane); δ_H($CDCl_3$) 2.84 (1 H, dd, $J_{3R,2}$ 5.6, $J_{3R,3S}$ 13.8, 3*R*-H), 2.91 (1 H, dd, $J_{3S,2}$ 4.9, $J_{3S,3R}$ 14.0, 3*S*-H), 3.70 (2 H, s, SCH_2Ph), 3.74 (3 H, s, CO_2Me), 4.59 (1 H, m, 2-H), 5.12 (2 H, s, CO_2CH_2Ph), 5.52 (1 H, exch. br d, $J_{N-H,2}$ 7.6, NH) and 7.22–7.38 (10 H, m, CO_2CH_2Ph and SCH_2Ph); δ_C($CDCl_3$) 171.20 (CO_2Me), 155.70 (CO_2CH_2Ph), 137.57 and 136.19 (2 × *ipso* arom. C), 129.82–127.26 (*ortho*, *meta* and *para* arom. C), 67.14 (CO_2CH_2Ph), 53.52 (C-2), 52.60 (MeO), 36.70 (SCH_2Ph) and 33.64 (C-3).

Methyl (2*S*,3*S*)-*S*-benzyl-*N*-benzyloxycarbonyl-[3-$^2H_1$]cysteinate **18** H_B = ^{2}H was prepared as above in 40% yield from 1-benzyl 2-methyl (2*R*,3*R*)-[3-$^2H_1$]aziridine-1,2-dicarboxylate **17** H_B = ^{2}H. The product was a solid, m.p. 58–60 °C; $[\alpha]_D^{21}$ +50.1 (*c* 0.5, MeOH); *m/z* [+ve FAB (thioglycerol)] 361 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3342 (NH), 1748 (ester) and 1689 (urethane); δ_H($CDCl_3$) 2.82 (1 H, d, $J_{3R,2}$ 5.3, 3*R*-H), 3.69 (2 H, s, SCH_2Ph), 3.74 (3 H, s, CO_2Me), 4.57 (1 H, dd, $J_{2,3R}$ 5.7, J_{2N-H} 7.8, 2-H), 5.12 (2 H, s, CO_2CH_2Ph), 5.52 (1 H, br d, $J_{N-H,2}$ 7.0, NH) and 7.21–7.37 (10 H, m, CO_2CH_2Ph and SCH_2Ph); δ_C($CDCl_3$) 171.20 (CO_2Me), 155.71 (CO_2CH_2Ph), 137.58 and 136.18 (2 × *ipso* arom. C), 128.91–127.26 (*ortho*, *meta* and *para* arom. C), 67.13 (CO_2CH_2Ph), 53.45 (C-2), 52.59 (MeO), 36.64 (SCH_2Ph) and 33.36 (t, C-3).

Methyl *S*-benzyl-*N*-benzyloxycarbonyl-(2*S*,3*R*)-[2,3-$^2H_2$]-cysteinate **18** H_A = ^{2}H, was prepared in 40% yield from 1-benzyl 2-methyl (2*R*,3*S*)-[2,3-$^2H_2$]aziridine-1,2-dicarboxylate **17** H_A = ^{2}H. The product was a solid, m.p. 58–59 °C; $[\alpha]_D^{21.5}$ +50.5 (*c* 0.5, MeOH); *m/z* [+ve FAB (thioglycerol)] 362 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3342 (NH), 1747 (ester) and 1691 (urethane); δ_H($CDCl_3$) 2.87 (1 H, s, 3*S*-H), 3.69 (2 H, s, SCH_2Ph), 3.74 (3 H, s, CO_2Me), 5.12 (2 H, s, CO_2CH_2Ph), 5.50 (1 H, br s, NH) and 7.23–7.37 (10 H, m, CO_2CH_2Ph and SCH_2Ph); δ_C($CDCl_3$) 171.20 (CO_2Me), 155.70 (CO_2CH_2Ph), 137.57 and 136.18 (2 × *ipso* arom. C), 128.91–127.26 (*ortho*, *meta* and *para* arom. C), 67.13 (CO_2CH_2Ph), 53.14 (t, C-2), 52.59 (MeO), 36.64 (SCH_2Ph) and 33.26 (t, C-3).

(2S)-S-*Benzylcysteine* **19**.—Methyl (2*S*)-*S*-benzyl-*N*-benzyloxycarbonylcysteinate **18** (50 mg, 0.14 mmol) was suspended in 6 mol dm^{-3} aq. hydrochloric acid (6 cm^3) and the mixture was heated at reflux and stirred under nitrogen for 16 h. The solvent was removed under reduced pressure to yield the product as an off-white solid (29.4 mg, 100%), m.p. 208–212 °C (lit.,[21] 208–211 °C); $[\alpha]_D^{23.5}$ −27.4 (*c* 0.3, water) (lit.,[23] −25); *m/z* [+ve FAB (glycerol)] 212 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3426 (NH), 3000–2500 (COO*H*) and 1703 (*C*OOH); δ_H(20% DCl in D_2O) 3.05 (1 H, dd, $J_{3R,2}$ 8.0, $J_{3R,3S}$ 15.1, 3*R*-H), 3.15 (1 H, dd, $J_{3S,2}$ 4.4, $J_{3S,3R}$ 15.0, 3*S*-H), 3.89 (2 H, s, SCH_2Ph), 4.23 (1 H, dd, $J_{2,3R}$ 8.0, $J_{2,3S}$ 4.4, 2-H) and 7.36–7.55 (5 H, m, SCH_2Ph).

(2*S*,3*S*)-*S*-Benzyl-[3-$^2H_1$]cysteine **19** H_B = ^{2}H was prepared as above in 100% yield by using methyl (2*S*,3*S*)-*S*-benzyl-*N*-benzyloxycarbonyl-[3-$^2H_1$]cysteinate **18** H_B = ^{2}H. The product was a solid, m.p. 207–210 °C; $[\alpha]_D^{24}$ −18.6 (*c* 0.3, water); *m/z* [+ve FAB (glycerol)] 213 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3245 (NH), 3000–2500 (br, COO*H*) and 1703 (*C*OOH); δ_H(20% DCl in D_2O) 3.04 (1 H, d, $J_{3R,2}$ 7.9, 3*R*-H), 3.89 (2 H, s, SCH_2Ph), 4.24 (1 H, d, $J_{2,3R}$ 7.4, 2-H) and 7.43–7.50 (5 H, m, SCH_2Ph).

(2*S*,3*R*)-*S*-Benzyl-[2,3-$^2H_2$]cysteinate **19** H_A = ^{2}H was prepared in 96% yield by using methyl (2*S*,3*R*)-*S*-benzyl-*N*-benzyloxycarbonyl-[2,3-$^2H_2$]cysteinate **18** H_A = ^{2}H. The product was a solid, m.p. 210–213 °C; $[\alpha]_D^{24}$ −20.1 (*c* 0.3, water); *m/z* [+ve FAB (glycerol)] 214 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3425 (NH), 3000–2500br (COO*H*) and 1687 (*C*OOH); δ_H(20% DCl in D_2O) 3.14 (1 H, s, 3*S*-H), 3.89 (2 H, s, SCH_2Ph) and 7.43–7.49 (5 H, m, SCH_2Ph).

(2S)-*Cystine* **20**.—(2*S*)-*S*-Benzylcysteine **19** (25 mg, 0.12 mmol) was dissolved in stirred liquid ammonia (~10 cm^3) at −78 °C. Small lumps of metallic sodium, which had been washed in light petroleum (60–80 °C), were added until a permanent dark blue colour remained and the reaction mixture was then stirred at −78 °C for 1.5 h. The reaction was stirred overnight at room temperature under nitrogen to allow the ammonia to evaporate. The ^{1}H NMR spectrum of the crude product indicated the presence of a reasonably clean sample of (2*S*)-cysteine, δ_H(10% NaOD in D_2O) 1.81 (1 H, dd, J_{AX} 9.1, J_{AB} 12.6, 3-H), 2.18 (1 H, dd, J_{AB} 12.6, J_{BX} 3.6, 3-H) and 2.44 (1 H, dd, J_{AX} 9.1, J_{XB} 3.6, 2-H).

This solid was dissolved in water (2 cm^3) and applied to a Dowex 50X8-100 ion-exchange column. Inorganic contaminants were eluted with water and the product was recovered by elution with 4 mol dm^{-3} aq. NH_4OH. The relevant column fractions (as determined by TLC) were combined and aerated for several hours. Subsequent lyophilisation yielded (2*S*)-cystine **20** as a pale yellow solid (10.8 mg, 75%), m.p. 236–241 °C (lit.,[21] 247–249 °C); $[\alpha]_D^{20.5}$ +190.7 (*c* 0.2, 1 mol dm^{-3} HCl) (lit.,[21] +232); *m/z* [+ve FAB (glycerol)] 241 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3441 (NH), 3000–2500br (CO*OH*) and 1622 (*CO*OH); δ_H(10% NaOD in D_2O) 2.27 (1 H, dd, $J_{3R,2}$ 7.6, $J_{3R,3S}$ 13.6, 3*R*-H), 2.48 (1 H, dd, $J_{3S,2}$ 4.7, $J_{3S,3R}$ 13.6, 3*S*-H) and 2.93 (1 H, dd, $J_{2,3R}$ 7.5, $J_{2,3S}$ 4.7, 2-H).

(2*S*,2′*S*,3*S*,3′*S*)-[3,3′-$^2H_2$]Cystine **20** H_B = ^{2}H was prepared as above in 75% yield from (2*S*,3*S*)-*S*-benzyl-[$^2H_1$]cysteine **19** H_B = ^{2}H. The product was a solid, m.p. 234–239 °C; $[\alpha]_D^{21.5}$ +185.0 (*c* 0.2, 1 mol dm^{-3} HCl); *m/z* [+ve FAB (glycerol)] 243 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3441 (NH), 3000–2500br (CO*OH*) and 1619 (*CO*OH); δ_H(10% NaOD in D_2O) 2.24 (1 H, d, $J_{3R,2}$ 7.6, 3*R*-H) and 2.91 (1 H, d, $J_{2,3S}$ 7.6, 2-H).

(2*S*,2′*S*,3*R*,3′*R*)-[2,2′,3,3′-$^2H_4$]Cystine **20** H_A = ^{2}H was prepared as above in 79% yield from (2*S*,3*R*)-*S*-benzyl-[2,3-$^2H_2$]cysteine **19** H_A = ^{2}H. The product was a solid, m.p. 232–238 °C; $[\alpha]_D^{22.5}$ +189.3 (*c* 0.2, 1 mol dm^{-3} HCl); *m/z* [+ve FAB (glyercol)] 245 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3434 (NH), 3000–2500br (CO*OH*) and 1631 (*CO*OH); δ_H(10% NaOD in D_2O) 2.42 (1 H, s, 3*S*-H).

Methyl (2R)-N-*Benzyloxycarbonyl*-β-*chloroalaninate* ent-**21**.*— 1-Benzyl 2-methyl (2*S*)-aziridine-1,2-dicarboxylate *ent*-**17** (236 mg, 1.00 mmol) was dissolved in dichloromethane–chloroform (5 cm^3; 1:1) in a flask equipped with a rubber septum and the solution was cooled to −78 °C. $TiCl_4$ (300 mm^3, 2.73 mmol) was added and the mixture was stirred for 12 h. Ice–water (5 cm^3) was added and the mixture was allowed to reach room temperature. The aqueous phase was extracted with dichloromethane (3 × 4 cm^3) and the pooled organic phases were dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was chromatographed on silica gel, and eluted with ethyl acetate–light petroleum (2:3). The fractions of R_f 0.42 on TLC were pooled to yield a gum (205 mg, 75%) which contained ~8% of an impurity by GLC and NMR analysis, *m/z* (+ve FAB, Xe) 272 and 274 ([M + H]$^+$); ν_{max}(film)/cm^{-1} 1750 (ester); δ_H($CDCl_3$) 3.82 (3 H, s, OMe), 3.88 (1 H, *A*BX, $J_{3R,3S}$ 11.3, $J_{3S,2}$ 3.4, 3*S*-H), 4.0 (1 H, A*B*X, $J_{3R,3S}$ 11.3, $J_{3R,2}$ 3.0, 3*R*-H), 4.78 (1 H, m, 2-H), 5.14 (2 H, s, C*H*$_2$Ph), 5.69 (1 H, d, *J* 7.32, NH) and 7.37 (5 H, m, Ph).*

Methyl(2S,3R)-N-*Benzyloxycarbonyl*-β-*chloro*[2,3-$^2H_2$]*alaninate* **21** H_A = ^{2}H.—1-Benzyl 2-methyl (2*R*,3*S*)-[2,3-$^2H_2$]aziridine-1,2-dicarboxylate **17** H_A = ^{2}H (350 mg, 1.5 mmol) was dissolved in dichloromethane–chloroform (1:1; 10 cm^3). The solution was cooled to −78 °C, $TiCl_4$ (700 mm^3, 6.4 mmol) was added, and the mixture was stirred at this temperature for 7 h. Ice–water (10 cm^3) was added and the mixture was allowed to reach room temperature. The aqueous phase was extracted with dichloromethane and the pooled organic phases were dried (Na_2SO_4). The solvent was removed under reduced pressure to yield a gum, which was purified by chromatography on silica gel and eluted with ethyl acetate–light petroleum, and fractions of R_f 0.48 on TLC were collected. The product was a gum (309 mg, 76%); $[\alpha]_D^{22}$ −35.5 (*c* 1.5, $CHCl_3$); *m/z* (+ve FAB, Xe) 274 and 276 ([M + H]$^+$); ν_{max}(film)/cm^{-1} 1750 (ester); δ_H($CDCl_3$) 3.79 (3 H, s, OMe), 3.85 (1 H, s, 3*S*-H), 5.19 (2 H, s, C*H*$_2$Ph), 5.71 (1 H, s, NH) and 7.36 (5 H, m, Ph). Gas chromatographic analysis showed the presence of 8% of an isomeric compound, assumed to be the product of α-nucleophilic attack on the aziridine.

Methyl (2*S*,3*S*)-*N*-Benzyloxycarbonyl-β-chloro-[3-$^2H_1$]alaninate **21** H_B = ^{2}H was prepared from 1-benzyl 2-methyl (2*R*,3*R*)-[3-$^2H_1$]aziridine-1,2-dicarboxylate **17** H_B = ^{2}H in 90% crude yield and was used in the next step without purification, $[\alpha]_D^{22}$ −35.9 (*c* 1.03, $CHCl_3$); *m/z* (+ve FAB, Xe) 273 and 275 ([M + H]$^+$); ν_{max}(film)/cm^{-1} 1750 (ester); δ_H($CDCl_3$) 3.80 (3 H, s, OMe), 3.96 (1 H, d, $J_{2,3R}$ 3.3, 3*R*-H), 4.76 (1 H, dd, $J_{2,3R}$ 3.2, $J_{NH,2}$ 7.9, 2-H), 5.13 (2 H, s, C*H*$_2$Ph), 5.72 (1 H, d, *J* 7.4, NH) and 7.36 (5 H, m, Ph). There were traces of a by-product in this spectrum.

β-*Chloroalanine* ent-**22**.*—Methyl (2*R*)-*N*-benzyloxycarbonyl-β-chloroalanine *ent*-**21** (32 mg, 0.12 mmol) was heated to reflux in 4 mol dm^{-3} aq. H_2SO_4 for 8 h. The solution was neutralised with 4 mol dm^{-3} aq. NH_3 and the solvent was removed until crystals started to form. Water was added to redissolve the crystals, the pH was adjusted to 7, and the solution was chromatographed on Dowex 1X2-200 (OH^-) (3 g), eluting first with water (6 cm^3) and then with 5% aq. acetic acid. The residue was crystallised from ethanol–water and had an identical IR spectrum with that of an authentic sample (9 mg, 61%); *m/z* [+ve FAB (3-NBA)] 124 and 126 (intensity ratio 3:1; [M + H]$^+$); δ_H(20% DCl in D_2O) 3.98 (1 H, *AB*X, $J_{3S,3R}$ 12.9, $J_{3S,2}$ 3.4, 3*S*-H), 4.05 (1 H, ABX, $J_{3S,3R}$ 12.9, $J_{3R,2}$ 4.4, 3*R*-H) and 4.52 (1 H, dd, $J_{2,3S}$ 3.4, $J_{2,3R}$ 4.4, 2-H).*

(2*S*,3*R*)-β-Chloro-[2,3-$^2H_2$]alanine **22** H_A = ^{2}H was prepared in 30% yield by the above method by using methyl (2*S*,3*R*)-*N*-benzyloxycarbonyl-β-chloro-[2,3-$^2H_2$]alanine **21** H_A = ^{2}H, and had m.p. 164–165 °C (decomp.); $[\alpha]_D^{26}$ +7.1 (*c* 0.57, water); *m/z* [+ve FAB (3-NBA)] 126 and 128 (intensity ratio 3:1; [M + H]$^+$); δ_H(20% DCl in D_2O) 3.97 (1 H, s, 3*S*-H).

(2*S*,3*S*)-β-Chloro-[3-$^2H_1$]alanine **22** H_B = ^{2}H was prepared in 22% yield by the above method by using methyl (2*S*,3*S*)-*N*-benzyloxycarbonyl-β-chloro-[3-$^2H_1$]alanine **21** H_B = ^{2}H, and had m.p. 167–168 °C (decomp.); $[\alpha]_D^{26}$ +6.1 (*c* 0.54, water); *m/z* [+ve FAB (3-NBA)] 125 and 127 (intensity ratio 3:1; [M + H]$^+$); δ_H(20% DCl in D_2O) 4.16 (1 H, d, $J_{3R,2}$ 4.4, 3*R*-H) and 4.64 (1 H, d, *J* 4.4, 2-H).

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* The unlabelled samples in the β-chloroalanine series were prepared as the L- (2*R*)-isomers from 1-benzyl 2-methyl (2*S*)-aziridine-1,2-dicarboxylate. This was prepared exactly as reported here for the (2*R*)-isomer, by using methyl (2*S*)-*N*-tritylaziridine-2-carboxylate, in turn prepared from methyl L-serinate by the method of Nakajima.[24] The ^{1}H NMR spectra are analysed as if for the D- (2*S*)-isomers for ease of comparison with the spectra of the labelled compounds.

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