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Stereochemistry of reactions of the inhibitor/substrates L- and D-β-chloroalanine with β-mercaptoethanol catalysed by L-aspartate aminotransferase and D-amino acid aminotransferase respectively†

Benjamin Adams, Kreingkrai Lowpetch, Faye Thorndycroft, Sheena M. Whyte and Douglas W. Young*

Department of Chemistry, University of Sussex, Falmer, Brighton, UK BN1 9QJ

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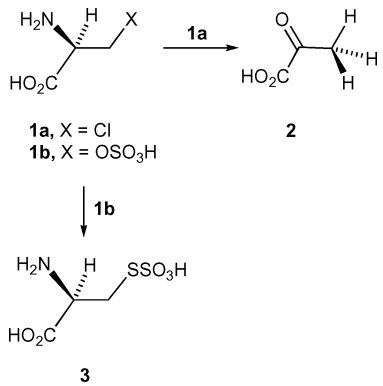
Two members of the α-family of PLP-dependent enzymes, L-aspartate aminotransferase and D-amino acid aminotransferase, have been shown to catalyse β-substitution of L- and D-β-chloroalanine respectively with β-mercaptoethanol, reactions typical of the β-family of PLP-dependent enzymes. The reaction catalysed by L-aspartate aminotransferase has been shown to occur with retention of stereochemistry, a typical outcome for reactions catalysed by β-family enzymes. There are also indications that the reaction catalysed by D-amino acid aminotransferase may involve retention of stereochemistry. Both enzymes have been shown to catalyse exchange at C-3 when the appropriate enantiomer of β-chloroalanine is the substrate.

Introduction

Pyridoxal phosphate (PLP) dependent enzymes catalyse a wide variety of different reactions, mainly involving amino acids as substrates. Theories to generalise and explain the mechanistic basis for these widely differing reactions were proposed at an early date[2–4] and have largely been confirmed by subsequent experiments. Dunathan proposed that the family of PLP-dependent enzymes evolved from a common ancestor protein.[5] However it has been recognised from profile analysis that the enzymes can be characterised as belonging to one of three different families of homologous proteins.[6] These were named the α-, β-, and γ-families and they specifically catalyse reactions at the α-centre, the β-centre and the γ-centre respectively of amino acids.[6] X-Ray crystal structures[7,8] of several PLP-mediated enzymes are available and the three dimensional structures of the α-family are organised according to the same basic pattern.

L-Aspartate aminotransferase (EC 2.6.1.1) is an enzyme of the α-family, catalysing the transamination of L-aspartic acid to α-ketoglutaric acid to afford oxaloacetic and glutamic acids. β-Chloro-L-alanine **1a** been shown not only to inhibit this enzyme,[9] but also to be converted to pyruvic acid **2**[10] by it, as shown in Scheme 1. It has been observed that introduction of thiosulfate during the inactivation of L-aspartate aminotransferase by the inhibitor L-serine-*O*-sulfate **1b** reversed inhibition and caused production of L-cysteine-*S*-sulfonate **3**.[11] Further, inhibition of D-amino acid aminotransferase (EC 2.6.1.21) by β-bromo-D-alanine **4a** was reversed by addition of thiols and, when β-mercaptoethylamine was used, *S*-(β-aminoethyl)-D-cysteine **5** and Δ^1-thiomorpholine-2-carboxylic acid **6** were products as shown in Scheme 2.[12] These α-family enzymes have, therefore, catalysed β-substitution reactions typical of an enzyme of the β-family, rather than the more usual transamination reactions. These results would suggest that the first-formed Schiff base intermediate **7** in Scheme 3 gives the quinonoid form **8** as in the transamination reaction. However, instead of protonation at C-4′ by Lys258 expected in the transamination reaction, the leaving group X allows for conversion to the imine **9** in the inhibition reaction. This may be processed to pyruvate **2** or to an inhibitor complex in the absence of a thiol. In the presence of a thiol, however, the reaction presumably proceeds as shown in Scheme 3 to give intermediate **10**, the precursor of the final cysteine derivative **12**.

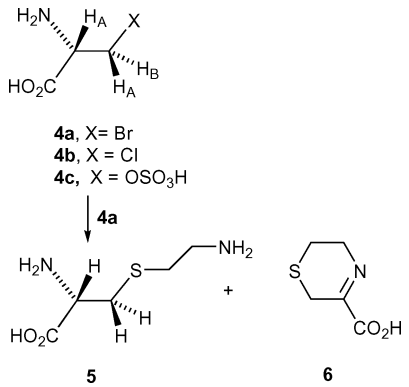


1a, X = Cl
1b, X = OSO_3H

2

3

Scheme 1



4a, X= Br
4b, X = Cl
4c, X = OSO_3H

5 **6**

Scheme 2

We have previously investigated the stereochemistry of the overall reaction of the inhibitors β-chloro-D-alanine **4b** and D- and L-serine-*O*-sulfate, **4c** and **1b** respectively, to pyruvic acid **2** catalysed by D-amino acid aminotransferase and L-aspartate aminotransferase respectively.[13] This work implied that an intermediate in the enzyme catalysed reaction is protonated partially outside of the active site of D-amino acid aminotransferase and completely outside the active site of L-aspartate aminotransferase. It also showed that the D-enzyme catalyses protonation with overall retention of stereochemistry.[13] The stereospecifically labelled samples of both D- and L-amino acids and of the corresponding enzyme inhibitors required for

† See ref. 1.

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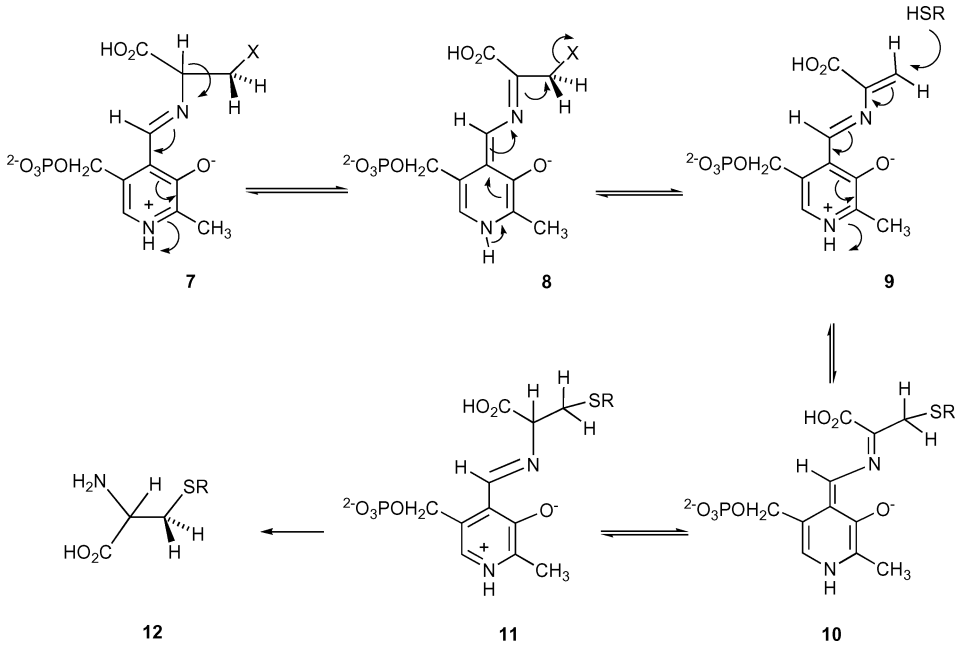


Scheme 3

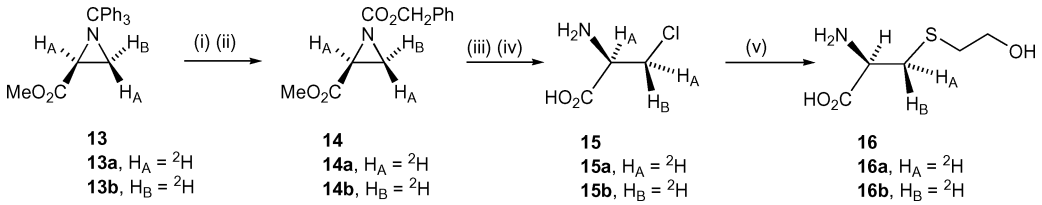
this work were prepared using our general and versatile chemico-enzymatic synthesis[14,15] and we have more recently improved on this synthesis by developing a shorter and completely chemical synthesis in which stereospecific labelling was solely in the β-position of the amino acid.[16] Although the new synthesis gave ee's of 81–86% whereas the original synthesis was entirely stereoselective, its convenience and the fact that singly labelled products were obtained made its use attractive.

The change in role from being a catalyst for transamination to being a catalyst for β-substitution exhibited by aspartate aminotransferase and D-amino acid aminotransferase discussed above suggested that these enzymes of the α-family of PKLP dependent proteins were acting as enzymes of the β-family and so we decided to examine the overall stereochemistry of the β-substitution reactions in the presence of thiol to ascertain whether this was in keeping with the overall retention of stereochemistry generally shown by reactions catalysed by the enzymes whose normal role this was.[17]

Results and discussion

In our first experiments we have prepared stereospecifically labelled samples of β-chloro-L-alanine **1a** using labelled samples of methyl (2*S*)-*N*-tritylaziridine-2-carboxylate **13a** and **13b** prepared by our chemico-enzymatic route as shown in Scheme 4.[14,15] This was achieved by reacting these compounds with trifluoroacetic acid in chloroform to remove the trityl group, followed immediately by reaction with benzyl chloroformate under Schotten Baumann conditions. β-Substitution with a chloride ion was then carried out with inversion of stereochemistry at the labelled β-atom by reaction with $TiCl_4$ in CH_2Cl_2 and $CHCl_3$ as in the synthesis of the corresponding D-isomers.[14] Deprotection using refluxing 4 M H_2SO_4 then gave the enzyme inhibitors **15**. The ^{1}H- and ^{2}H-NMR spectra indicated that the inhibitors were unique diastereoisomers and, since substitution is accompanied by inversion of stereochemistry at the labelled β-position, they were assigned as (2*R*,3*R*)-[2,3-$^2H_2$]- and (2*R*,3*S*)-[3-$^2H_1$]-β-chloroalanine, **15a** and **15b** respectively.

The enzyme L-aspartate aminotransferase (EC 2.6.1.1) was isolated by a standard purification protocol[18] from *E. coli* TY103[19] which was transformed with plasmid pKDHE19/AspC obtained as a gift from Professor H. Kagamiyama.[20] It had specific activity of 36.57 units/mg and appeared as one major band of molecular weight 43000 Da on SDS-PAGE. Incubation of the enzyme with (2*R*,3*R*)-[2,3-$^2H_2$]-β-chloroalanine **15a** prepared by the chemico-enzymatic synthesis in the presence of a variety of small thiols and under various conditions was now undertaken. The best results were obtained at pH 8.4 when β-mercaptoethanol was employed as in Scheme 4 and samples of labelled 3-(2-hydroxyethyl)-cysteine **16** were obtained in which the α-deuterium atom had exchanged as expected from the mechanism in Scheme 3. We noticed (Fig. 1b) that the β-deuterium atom also exchanged on prolonged incubation. This result was not unexpected since, although this enzyme does not exchange the β-hydrogens of aspartate, it has been shown to exchange these hydrogens in other substrates.[21,22] We were able to minimise β-exchange by stopping the incubation after three hours when, after purification, a sample of (2*R*)-3-(2-hydroxyethyl)-cysteine **16a** was obtained with a ^{1}H-NMR spectrum (Figs. 1a and 2e) which, although showing the presence of impurities, showed loss of one of the protons for H-3 and the remaining H-3 as a doublet, coupling to H-2 (which had exchanged due to transamination) with $J_{2,3}$ 7.3 Hz. There was evidently stereospecific labelling at C-3. Incubation with unlabelled (2*R*)-β-chloroalanine gave a product with a ^{1}H-NMR spectrum (Fig. 2d) similar to that of a synthetic sample of



Scheme 4 *Reagents and conditions*: (i) F_3CCO_2H, 0 °C, 4 h; (ii) $ClCO_2CH_2Ph/NaHCO_3/EtOAc/H_2O$, rt, 20 h (96% **14**, 99% **14a**, 90% **14b** over steps i and ii); (iii) $TiCl_4/CH_2Cl_2/CHCl_3$, −78 °C, 11 h (72–99%); (iv) 4 M aq H_2SO_4, reflux, 3.5 h (69% **15**, 61% **15a**, 58% **15b**); L-aspartate aminotransferase/arsenate buffer, 27 °C, 3 h.

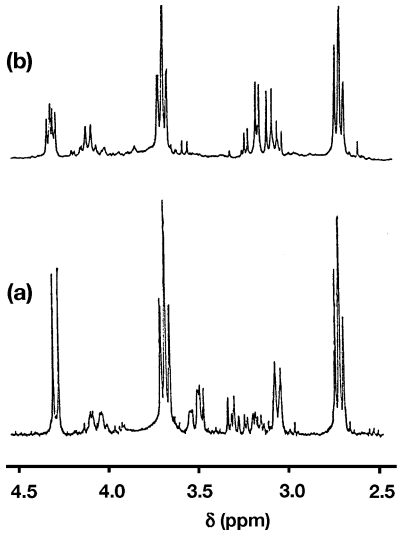
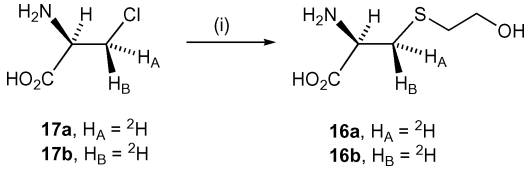


Fig. 1 ^{1}H-NMR spectra of the incubation of (2*R*,3*R*)-[2,3-$^2H_2$]-β-chloroalanine **15a** with L-aspartate aminotransferase and β-mercaptoethanol—(a) 3 h incubation; (b) 6 h incubation.

(2*R*)-β-hydroxyethylcysteine (Fig. 2a) and a specific rotation compatible with it being the (2*R*)-enantiomer. When (2*R*,3*S*)-[3-$^2H_1$]-3-β-chloroalanine **15b**, prepared by the chemico-enzymatic synthesis, was incubated for three hours with the enzyme and β-mercaptoethanol, the ^{1}H-NMR spectrum (Fig. 2f) of the purified product **16b** indicated that it was the C-3 epimer and $J_{2,3}$ was 4.4 Hz.

The samples of (2*R*,3*S*)-[3-$^2H_1$]-β-chloroalanine **17b** and (2*R*,3*R*)-[3-$^2H_1$]-β-chloroalanine **17a** prepared by our chemical synthesis[16] were also incubated with the enzyme in the presence of β-mercaptoethanol as shown in Scheme 5, giving purified products **16** with the ^{1}H-NMR spectra shown in Figs. 3b and 3c respectively. The results, although not as clean as those from the experiments using the samples prepared by the chemico-enzymatic method, were compatible with them both in terms of the chemical shift of the remaining label at H-3 and of $J_{2,3}$, indicating that, in spite of the fact that the samples from the chemical synthesis had been prepared in ee of 81–86%, they could be used to assess the stereochemistry at the β-centre during the enzyme catalysed reactions.



17a, H_A = ^{2}H
17b, H_B = ^{2}H

16a, H_A = ^{2}H
16b, H_B = ^{2}H

Scheme 5 *Reagents and conditions*: (i) L-aspartate aminotransferase/arsenate buffer, 27 °C, 3 h.

It was now evident that the β-substitution reaction was stereospecific and so it was necessary to deduce the absolute stereochemistry of the overall reaction. This was achieved by independent synthesis of samples of (2*R*)-3-(2-hydroxyethyl)-cysteine **18** which were stereospecifically labelled with deuterium at C-3 in an unambiguous manner. This synthesis is shown in Scheme 6, the labelled carbobenzyloxyaziridines **14a** and **14b** being reacted with β-mercaptoethanol containing a catalytic quantity of boron trifluoride etherate. Inversion of stereochemistry at the labelled atom, C-3, is expected and ^{1}H- and ^{2}H-NMR spectra showed that the labelled products were obtained from the aziridines as single diastereoisomers. Hydrolysis in refluxing 4 M H_2SO_4 then gave the free amino acids **18a** and **18b**, the ^{1}H-NMR spectra of which are shown in Figs. 2b and 2c respectively.

The ^{1}H-NMR spectra of the synthetic samples of (2*R*,3*S*)-[3-$^2H_1$]-3-(2-hydroxyethyl)-cysteine **18b** (Fig. 2c) and (2*R*,3*R*)-

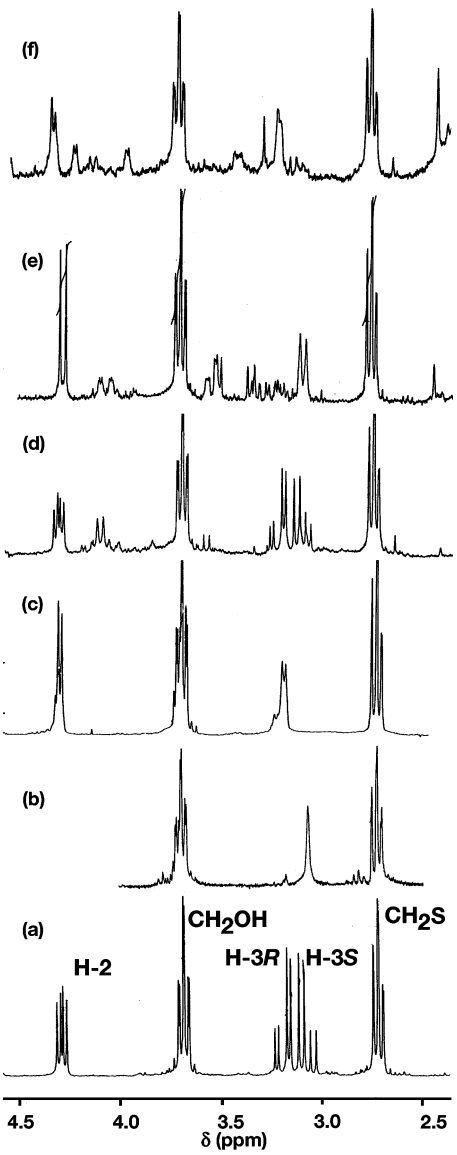
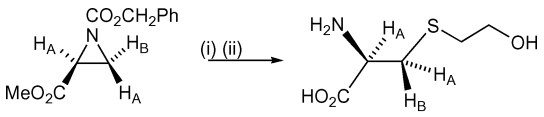


Fig. 2 ^{1}H-NMR spectra in 10% ^{2}HCl–^2H_2O of (a) synthetic (2*R*)-β-hydroxyethylcysteine **18**; (b) synthetic (2*R*,3*R*)-[2,3-$^2H_2$]-β-hydroxyethylcysteine **18a**; (c) synthetic (2*R*,3*S*)-[3-$^2H_1$]-β-hydroxyethylcysteine **18b**; (d) incubation of (2*R*)-β-chloroalanine **15** with L-aspartate aminotransferase and β-mercaptoethanol; (e) incubation of (2*R*,3*R*)-[2,3-$^2H_2$]-β-chloroalanine **15a** from synthesis A with L-aspartate aminotransferase and β-mercaptoethanol; (f) incubation of (2*R*,3*S*)-[3-$^2H_1$]-β-chloroalanine **15b** from synthesis A with L-aspartate aminotransferase and β-mercaptoethanol.



14
14a, H_A = ^{2}H
14b, H_B = ^{2}H

18
18a, H_A = ^{2}H
18b H_B = ^{2}H

Scheme 6 *Reagents and conditions*: (i) $HOCH_2CH_2SH/BF_3$, rt, 46 h (20–61%); (ii) 4 M aq H_2SO_4, reflux, 3.5 h (45% **18**, 42% **18a**, 50% **18b**).

[2,3-$^2H_2$]-3-(2-hydroxyethyl)-cysteine **18a** (Fig. 2b) allowed the chemical shifts for the 3-*pro*-*S* and 3-*pro*-*R* protons in the spectrum of (2*R*)-3-(2-hydroxyethyl)-cysteine **16** to be assigned and therefore the absolute stereochemistry of the incubation products to be deduced. The coupling constants were and $J_{2,3S}$ 7.3 Hz and $J_{2,3R}$ 4.4 Hz. It was evident that the product from the incubation using (2*R*,3*S*)-[3-$^2H_1$]-3-β-chloroalanine **15b**/**17b** (Figs. 2f and 3b) was (2*R*,3*S*)-[3-$^2H_1$]-3-(2-hydroxyethyl)-cysteine **16b** and that the product of incubation

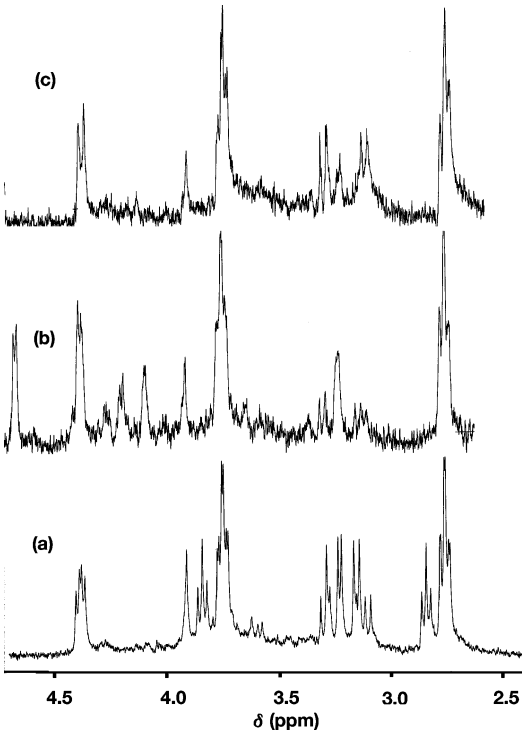


Fig. 3 ^{1}H-NMR spectra in 10% ^{2}HCl–^2H_2O of incubation of samples from total synthesis[16] (a) (2*R*)-β-chloroalanine **16** with L-aspartate aminotransferase and β-mercaptoethanol; (b) (2*R*,3*S*)-[3-$^2H_1$]-β-chloroalanine **16b** with L-aspartate aminotransferase and β-mercaptoethanol; (c) (2*R*,3*R*)-[3-$^2H_1$]-β-chloroalanine **16a** with L-aspartate aminotransferase and β-mercaptoethanol.

using (2*R*,3*R*)-[2,3-$^2H_2$]-3-β-chloroalanine **15a** or (2*R*,3*R*)-[3-$^2H_1$]-β-chloroalanine **17a** (Figs. 2e and 3c) was (2*R*,3*R*)-[3-$^2H_1$]-3-(2-hydroxyethyl)-cysteine **16a**.

These results imply that the β-replacement reaction, which in this case is catalysed by an enzyme of the α-family whose normal function is transamination, occurs with overall retention of stereochemistry. This is the general expectation[17] for the PLP-mediated enzymes of the β-family where β-replacement reactions are the norm and so our results may imply a closer relationship between families than homology[6] suggests. X-Ray structures are available for aspartate aminotransferase[23] and other α-family enzymes, and the tertiary structure of tryptophan synthase (EC 4.2.1.20), an enzyme of the β-family, has been defined by X-ray crystallography.[24] It is interesting to note that, in the presence of thiols, tryptophan synthase has been shown to catalyse both transamination and β-replacement of L-serine by mercaptoethanol.[25] Further, the Lys87Thr mutant of tryptophan synthase will not turn-over the natural substrate serine in the absence of NH_4^+ but it will turn-over β-chloro-L-alanine which has a better leaving group at C-3.[26]

Having shown that L-aspartate aminotransferase acts as a typical β-family enzyme, given the right substrate, we now turned our attention to D-amino acid aminotransferase (EC 2.6.1.21). This was over-expressed in *E. coli* DH5-α cells which we transformed using plasmid pICT113[27] obtained as a gift from Professor K. Soda, Institute for Chemical Research, Kyoto University and purified by modification of the existing method.[28] It ran as one band on SDS-PAGE gel and had a specific activity of 49.3 units/mg. Incubation of the enzyme with (2*S*)-β-chloroalanine and β-mercaptoethanol gave a product with a positive rotation, suggesting that it had (2*S*) stereochemistry, as expected. The ^{1}H-NMR spectrum (Fig. 4a) was also clearly that expected for (2*S*)-β-hydroxyethylcysteine. (2*S*,3*S*)-[3-$^2H_1$]-β-Chloroalanine **19b** and (2*S*,3*R*)-[3-$^2H_1$]-β-chloroalanine **19a** from the chemical synthesis[16] were also incubated with the enzyme to give products, the ^{1}H-NMR spectra of which are shown in Figs. 4b and 4c respectively (Scheme 7). These appear to indicate that the β-substitution reaction has occurred but unfortunately the region for the signals due to H-3 could not be interpreted due to the presence of impurity and/or exchange at C-3. However in the experiment where (2*S*,3*S*)-[3-$^2H_1$]-β-chloroalanine was the substrate, the chemical shift for H-2 appeared as a doublet, $J_{2,3}$ 7 Hz. Since $J_{2,3S}$ for the (2*R*)-isomer should be the same as $J_{2,3R}$ for the enantiomeric (2*S*)-isomer, this suggests that the product was more likely to be (2*S*,3*S*)-[3-$^2H_1$]-β-hydroxyethylcysteine, the result of substitution with retention of stereochemistry.

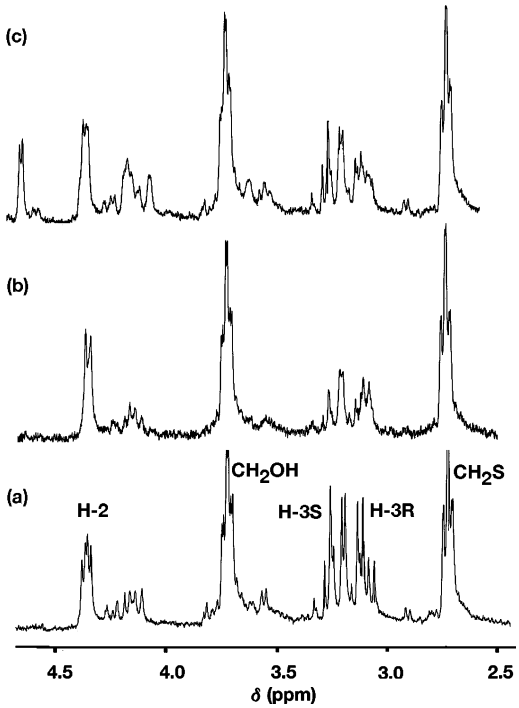
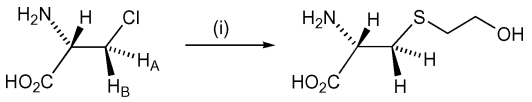


Fig. 4 ^{1}H-NMR spectra in 10% ^{2}HCl–^2H_2O of (a) (2*S*)-β-hydroxyethylcysteine **20** from incubation of (2*S*)-β-chloroalanine **19** with D-amino acid aminotransferase and β-mercaptoethanol; (b) β-hydroxyethylcysteine from incubation of (2*S*,3*S*)-[3-$^2H_1$]-β-chloroalanine **19a** with D-amino acid aminotransferase and β-mercaptoethanol; (c) β-hydroxyethylcysteine from incubation of (2*S*,3*R*)-[3-$^2H_1$]-β-chloroalanine **19b** with D-amino acid aminotransferase and β-mercaptoethanol.



19
19a, H_A = ^2H
19b, H_B = ^2H

20

Scheme 7 *Reagents and conditions*: (i) D-amino acid aminotransferase/arsenate buffer, 27 °C, 3 h.

Conclusions

We have shown that L-aspartate aminotransferase (EC 2.6.1.1), which is an enzyme of the α-family of PLP-dependent proteins, will catalyse a β-substitution reaction when L-β-chloroalanine is the substrate and that the overall stereochemistry of the reaction is one of retention of stereochemistry. In this respect, the reaction follows the stereochemical preference of reactions catalysed by enzymes of the β-family of PLP-dependent proteins. We have also shown that D-amino acid aminotransferase (EC 2.6.1.21) catalyses a similar β-substitution reaction and, although the results are less clear-cut than those for L-aspartate aminotransferase, there is a suggestion that this reaction may also proceed

with retention of stereochemistry. These results indicate that these members of the α-family of PLP-dependent enzymes can catalyse a β-substitution reaction with the same stereochemical outcome as enzymes of the β-family of PLP-dependent proteins.

Experimental

Materials and methods

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations (in units of 10^{-1} deg cm^2 g^{-1}) were recorded using a Perkin Elmer PE241 polarimeter with a 1 dm pathlength cell. IR spectra were recorded using a Perkin Elmer 1710 Fourier transform spectrometer. UV spectra recorded in assay of L-aspartate aminotransferase were recorded on a Varian Carey 210 spectrophotometer. ^{1}H-NMR spectra were recorded using Bruker DPX 300 (300 MHz), WM360 (360 MHz) or A–C 250SY (250 MHz) instruments. ^{2}H-NMR spectra were recorded on a Bruker A–C 250SY instrument (38.4 MHz). ^{13}C-NMR spectra (^{1}H decoupled) were recorded using a Bruker DPX 300 (75.5 MHz) Fourier transform instrument. NMR chemical shifts are given in ppm using residual undeuteriated solvent peaks, tetramethylsilane (TMS), 3-trimethylsilylpropanesulfonic acid (DSS) or acetic acid as standards. The ^{1}H-NMR spectra of the samples of β-hydroxyethylcysteine were standardised to a CH_2S shift of 2.74 ppm. All coupling constants (*J*) are given in Hz. All NMR spectra were recorded at 25 °C. Low resolution mass spectra were recorded by Dr A. Sada (Sussex) on a Kratos MS-80 instrument, and high resolution and electrospray mass spectra by the EPSRC National Mass Spectrometry Service at Swansea. Microanalyses were carried out by Medac Ltd. Column chromatography was performed using Merck Kieselgel 60 (230–400 mesh - ART 9385) or Davisil® Silica 60A, 35–70 mesh silica gel. Ion exchange resins were purchased from Aldrich and converted into the required form by passage of an excess of the relevant ion through the resin followed by washing with distilled water. Petroleum ether refers to that fraction of hexanes of boiling point 60–80 °C.

L-Aspartate aminotransferase (EC 2.6.1.1) was purified by the protocol of Herold and Kirschner[18] from *E. coli* TY103[19] which was transformed with plasmid pKDHE19/AspC,[20] both of which were gifts of Professor H. Kagamiyama, Department of Biochemistry, Osaka Medical College, Takatsuki, Osaka 569–8686, Japan. The enzyme was assayed by the method described by Herold and Kirschner[18] and protein concentration was obtained by the method of Warberg and Christian.[30]

D-Amino acid aminotransferase (EC 2.6.1.21) was purified using a modification of the method of Soda *et al.*[28] using *E. coli* DH5-α cells which we transformed with the plasmid pICT113, a gift from Professor K. Soda, Laboratory of Microbial Research, Institute for Chemical Research, Kyoto University, Uji, Kyto-Fu 611.[27] After cell lysis, the pellet was discarded, 1% aqueous streptomyces sulfate was added and the solution was incubated on ice for 1 h. Precipitated DNA was removed by centrifugation (Sorvall- RC-5B, 15 000 rpm, 30 min, 4 °C) and the solution containing protein was heated to 55 °C for 20 min and spun (Sorvall RC-5B, 15 000 rpm, 30 min, 4 °C) to remove precipitated proteins. The supernatant was extensively dialysed against 10 mM phosphate buffer containing 2 mM ethylenediaminetetraacetate (EDTA) and 1 mM phenylmethylsulfonyl fluoride (PMSF). The dialysed sample was filtered through a disposable filter unit of pore size 0.2 μl and subjected to FPLC using a MONO-Q ion exchange column using 10 mM phosphate buffer containing 1 M α-ketoglutarate to elute D-amino acid aminotransferase. Powdered ammonium sulfate was added to give a concentration of 25% w/v and the sample was filtered through a disposable filter unit of pore size 0.2 μl and loaded onto a phenyl superose column equilibrated with 10 mM phosphate buffer pH 7.4 containing 25% w/v ammonium sulfate. D-Amino acid aminotransferase eluted in 10 mM phosphate buffer pH 7.4 and was stored at −80 °C until needed. The enzyme ran as one band on SDS-PAGE and assayed as 49.3 units per mg protein, using the assay of Bhatia *et al.*[29] which coupled the production of pyruvate from D-alanine with lactate dehydrogenase and loss of the chromophore due to NADH, and the protein estimation of Warburg and Christian.[30]

Synthesis of stereospecifically deuteriated samples of L-β-chloroalanine

Methyl (2*S*)-*N*-carbobenzyloxyaziridine-2-carboxylate (14). Trifluoroacetic acid (24 ml, 323 mmol) was added over a period of 10 min to a solution of methyl (2*S*)-*N*-triphenylmethylaziridine-2-carboxylate **13**[14,15] (6.6 g, 19.1 mmol) in a mixture of chloroform (24 ml) and methanol (24 ml). The mixture was cooled to 0 °C and stirred under nitrogen for 4 h. The solvents were removed *in vacuo* at 0 °C, the last traces of trifluoroacetic acid being removed by azeotroping with diethyl ether (3 × 30 ml). The residue was partitioned between diethyl ether (30 ml) and water (30 ml). The ether layer was extracted with water (3 × 30 ml) and the aqueous extracts were made basic with solid sodium bicarbonate (10.0 g). Ethyl acetate (150 ml) was added to the aqueous layers and the mixture was cooled to 0 °C. Benzyl chloroformate (2.8 ml, 19.6 mmol) was added and the mixture was stirred vigorously under nitrogen at room temperature for 20 h. The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 50 ml). The combined organic layers were washed with brine (2 × 50 ml) and dried (Na_2SO_4). Removal of the solvent *in vacuo* yielded methyl (2*S*)-*N*-carbobenzyloxyaziridine-2-carboxylate **14** as a colourless oil (4.35 g, 96%); $[\alpha]_D^{25}$ −38.4 (*c* 0.7, $CHCl_3$) [lit[14] (2*R*) enantiomer $[\alpha]_D^{23}$ +38.56 (*c* 0.7, $CHCl_3$)]; *m/z* [+ve FAB (3-NBA)] 236 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1746 (ester); δ_H (360 MHz, C^{2}HCl$_3$) 2.5 (1H, dd, $J_{3R,3S}$ 1.2, $J_{3R,2}$ 5.4, H-3*R*), 2.6 (1H, dd, $J_{3S,3R}$ 1.2, $J_{3S,2}$ 3.1, H-3*S*), 3.1 (1H, dd, $J_{2,3S}$ 3.1, $J_{2,3R}$ 5.4, H-2), 3.7, (3H, s, OCH_3), 5.2 (2H, s, OCH_2Ar) and 7.4 (5H, s, ArH).

Methyl (2*S*,3*R*)-[3-^{2}H$_1$]-*N*-carbobenzyloxyaziridine-2-carboxylate (14b) by the chemico-enzymatic route. This was prepared as described above using methyl (2*S*,3*R*)-[3-^{2}H$_1$]-*N*-triphenylmethylaziridine-2-carboxylate **13b**[15] (460 mg, 1.34 mmol) to yield the product **14b** (284 mg, 90%); $[\alpha]_D^{25}$ −38.7 (*c* 0.7, $CHCl_3$) [lit[14] (2*R*,3*R*) [3-^{2}H$_1$]-isomer $[\alpha]_D^{23}$ +40.63 (*c* 0.7, $CHCl_3$)]; *m/z* [+ve FAB (3-NBA)] 237 ([M + H]$^+$), 259 ([M + Na]$^+$); ν_{max} (film)/cm^{-1} 1733 (ester); δ_H (300 MHz, C^{2}HCl$_3$) 2.52 (1H, d, $J_{3S,2}$ 3.1, H-3*S*), 3.1 (1H, d, $J_{2,3S}$ 3.1, H-2), 3.7 (3H, s, OCH_3), 5.2 (2H, s, OCH_2Ar) and 7.4 (5H, s, ArH); δ_D (38.4 MHz, $CHCl_3$) 2.49 (1^{2}H, s, ^{2}H-3*R*); δ_C (75.5 MHz, C^{2}HCl$_3$) 31.5 (t, C-3), 35.2 (C-2), 53.1 (CH_3O), 69.1 (CO_2CH_2), 128.9–129.0 (Ar), 135.7 (*ipso* Ar), 161.2 (urethane) and 169.1 (ester).

Methyl (2*S*,3*S*)-[2,3-^{2}H$_2$]-*N*-carbobenzyloxyaziridine-2-carboxylate (14a). This was prepared as described above using methyl (2*S*,3*S*)-[2,3-^{2}H$_2$]-*N*-triphenylmethylaziridine-2-carboxylate **13a** (250 mg, 0.72 mmol) to yield the product **14a** (170 mg, 99%); $[\alpha]_D^{25}$ −38.4 (*c* 0.7, $CHCl_3$) [lit[14] (2*R*,3*S*)-[2,3-^{2}H$_2$]-isomer $[\alpha]_D^{23}$ +42.46 (*c* 0.7, $CHCl_3$)]; *m/z* [+ve FAB (3-NBA)] 238 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1735 (ester); δ_H (300 MHz, C^{2}HCl$_3$) 2.42 (1H, s, H-3*R*), 3.63 (3H, s, OCH_3), 5.02 (2H, s, OCH_2Ar) and 7.34 (5H, s, ArH); δ_D (38.4 MHz, $CHCl_3$) 2.61 (1^{2}H, s, ^{2}H-3*S*) and 3.11 (1^{2}H, s, ^{2}H-2); δ_C (75.5 MHz, C^{2}HCl$_3$) 32.9 (t, C-3), 36.4 (t, C-2), 54.5 (CH_3O), 70.5 (OCH_2Ar), 127.7–130.4 (Ar), 137.2 (*ipso* Ar), 162.6 (urethane) and 170.5 (ester).

Methyl (2*R*)-*N*-carbobenzyloxy-β-chloroalaninate. Titanium tetrachloride (1.67 ml, 15.20 mmol) was added dropwise with vigorous stirring over 10 min to a solution of methyl (2*S*)-*N*-carbobenzyloxyaziridine-2-carboxylate **14** (1.35 g, 5.75 mmol) in dichloromethane–chloroform (1 : 1; 30 ml) under argon at −78 °C. The yellow solution was stirred under argon for 11 h, maintaining a constant temperature, and quenched at −78 °C

by adding water (32 ml) dropwise. The reaction was allowed to warm slowly to room temperature, with stirring. The aqueous layer was extracted using dichloromethane (3 × 12 ml) and the combined organic layers were dried (Na_2SO_4). The solvent was removed *in vacuo* to yield an opaque oil which showed a single spot by thin layer chromatography. This solidified overnight to give a white solid which was recrystallised from chloroform–petroleum ether to yield methyl (2*R*)-*N*-carbobenzyloxy-β-chloroalaninate as white needles, (1.55 g, 99%); mp 54.2–54.9 °C; $[a]_D^{30}$ +35.6 (*c* 1.5, $CHCl_3$); (Found: C, 53.2; H, 5.2; N, 5.1. $C_{12}H_{14}NO_4Cl$ requires C, 53.05; H, 5.2; N, 5.15%); *m/z* [+ve FAB (3-NBA) 272 and 274 (ratio 3 : 1); $[M + H]^+$); ν_{max} (film)/cm^{-1} 1752 (ester); δ_H (360 MHz, C^2HCl_3) 3.83 (3H, s, OMe), 3.88 (1H, *A*BX, $J_{3S,3R}$ 11.3, $J_{3S,2}$ 3.4, H-3*S*), 4.0 (1H, A*B*X, $J_{3R,3S}$ 11.3, $J_{3R,2}$ 3.0, H-3*R*), 4.77 (1H, m, H-2), 5.14 (2H, s, OCH_2Ar), 5.68 (1H, d, *J* 7.3, NH) and 7.37 (5H, m, ArH).

Methyl (2*R*,3*S*)-[3-$^2H_1$]-*N*-carbobenzyloxy-β-chloroalaninate. This was prepared as described above using methyl (2*R*,3*S*)-[3-$^2H_1$]-*N*-carbobenzyloxyaziridine-2-carboxylate **14b** (250 mg, 1.06 mmol) to yield the product (211.3 mg, 73%); mp 54.5–55.7 °C; $[a]_D^{31}$ +35.8 (*c* 1.5, $CHCl_3$) [lit[14] (2*S*,3*S*)-[3-$^2H_1$]-isomer $[a]_D^{22}$ +35.9 (*c* 1.3, $CHCl_3$)]; *m/z* [+ve FAB (3-NBA)] 273 and 275 (intensity ratio 3 : 1; $[M + H]^+$); ν_{max} (film)/cm^{-1} 1725 (ester); δ_H (300 MHz, C^2HCl_3) 3.77 (3H, s, OCH_3), 3.80 (1H, m, H-3*R*), 4.71 (1H, m, H-2), 5.05 (2H, s, OCH_2Ar), 5.62 (1H, br d, *J* 6.8, NH) and 7.25 (5H, m, ArH); δ_D (38.4 MHz, $CHCl_3$) 4.00 (1^2H, s, ^{2}H-3*S*); δ_C (75.5 MHz, C^2HCl_3) 46.6 (t, C-3), 54.0 (CH_3O), 56.5 (C-2), 69.1 (OCH_2Ar), 129.1–129.5 (Ar), 137.7 (*ipso* Ar), 157.4 (urethane) and 171.0 (ester).

Methyl (2*R*,3*R*)-[2,3-$^2H_2$]-*N*-carbobenzyloxy-β-chloroalaninate. This was prepared as described above using methyl (2*R*,3*R*)-[3-$^2H_2$]-*N*-carbobenzyloxyaziridine-2-carboxylate **14a** (85 mg, 0.36 mmol) to yield the product (71 mg, 72%); mp 54.7–55.7 °C; $[a]_D^{30}$ +34.9 (*c* 1.5, $CHCl_3$) [lit[14] (2*S*,3*R*)-[2,3-$^2H_2$]-isomer $[a]_D^{22}$ −35.5 (*c* 1.5, $CHCl_3$)]; *m/z* [+ve FAB (3-NBA)] 274 and 276 (ratio 3 : 1; $[M + H]^+$); ν_{max} (film)/cm^{-1} 1752 (ester); δ_H (300 MHz, C^2HCl_3) 3.73 (3H, s, OCH_3), 3.89 (1H, s, H-3*S*), 5.06 (2H, s, OCH_2Ar), 5.60 (1H, br d, *J* 6.8, NH) and 7.28 (5H, m, ArH); δ_D (38.4 MHz, $CHCl_3$) 3.90 (1^2H, s, ^{2}H-3*R*) and 4.79 (1^2H, s, ^{2}H-2); δ_C (75.5 MHz, C^2HCl_3) 45.4 (t, C-3), 53.5 (CH_3O), 55.0 (t, C-2), 67.2 (OCH_2Ar), 128.3–129.0 (Ar), 136.3 (*ipso* Ar), 156.0 (urethane) and 169.6 (ester).

(2*R*)-β-Chloroalanine (15). Methyl (2*R*)-*N*-carbobenzyloxy-β-chloroalaninate (200 mg, 0.74 mmol) was suspended in 4 M aqueous sulfuric acid under nitrogen and heated at reflux for 3.5 h. The solution was allowed to cool and neutralised with 4 M aqueous ammonium hydroxide. The solvent was removed *in vacuo* until solid began to form in the flask. Sufficient water was added to redissolve the solid residue and the pH was adjusted to 7. The resulting solution was chromatographed on an anion exchange column (Dowex 1 × 2–200 (OH^-), 2.5 × 15 cm), eluting first with water until the pH of the eluant was seen to change from basic to neutral and then with 5% aqueous acetic acid to elute the amino acid. Confirmed ninhydrin-positive fractions were collected and lyophilised, and the residue was recrystallised from ethanol–water to give (2*R*)-β-chloroalanine **15** as white needles, (70 mg, 69%); mp 166–167 °C (decomp.); $[a]_D^{30}$ −6.5 (*c* 0.5, H_2O); (Found: C, 22.4; H, 4.4; N, 8.8. $C_3H_6NO_2Cl$ requires C, 22.5; H, 4.4; N, 8.75%); *m/z* [+ve FAB (glycerol + water)] 124 and 126 (ratio 3 : 1; $[M + H]^+$); ν_{max} (KBr)/cm^{-1} 2634 (br, NH, OH) and 1598 (CO_2H); δ_H (300 MHz, 10% ^{2}HCl in ^2H_2O) 3.98 (1H, *AB*X, $J_{3R,2}$ 3.4, $J_{3R,3S}$ 12.9, H-3*R*), 4.05 (1H, *AB*X, $J_{3R,3S}$ 12.9, $J_{3S,2}$ 4.4, H-3*S*) and 4.52 (1H, AB*X*, $J_{2,3R}$ 3.4, $J_{2,3S}$ 4.4, H-2).

(2*R*,3*S*)-[3-$^2H_1$]-β-Chloroalanine (15b). This was prepared as described above using methyl (2*R*,3*S*)-[3-$^2H_1$]-*N*-carbobenzyloxy-β-chloroalaninate (154 mg, 0.57 mmol) to yield the product **15b** (46 mg, 58%); mp 166–167 °C (decomp.); [lit[14] (2*S*,3*S*)-[3-$^2H_1$]-isomer mp 167–168 (decomp)]; $[a]_D^{30}$ −7.1 (*c* 0.5, H_2O); [lit[14] (2*S*,3*S*)-[3-$^2H_1$]-isomer $[a]_D^{23}$ +6.1 (*c* 0.54, H_2O)]; *m/z* [+ve FAB (glycerol + water)] 125 and 127 (intensity ratio 3 : 1; $[M + H]^+$); ν_{max} (KBr)/cm^{-1} 2634 (br, NH, OH) and 1745 (CO_2H); δ_H (300 MHz, 10% ^{2}HCl in ^2H_2O) 3.98 (1H, d, $J_{3R,2}$ 3.1, H-3*R*) and 4.52 (1H, d, $J_{2,3R}$ 3.1, H-2); δ_D (38.4 MHz, H_2O) 4.13 (1^2H, s, ^{2}H-3*S*).

(2*R*,3*R*)-[2,3-$^2H_2$]-β-Chloroalanine (15a). This was prepared as described above using methyl (2*R*,3*R*)-[2,3-$^2H_2$]-*N*-carbobenzyloxy]-β-chloroalaninate (60 mg, 0.22 mmol) to yield the product **15a** (18.7 mg, 61%); mp 165–167 °C (decomp.); $[a]_D^{29}$ −6.8 (*c* 0.5, H_2O); [lit[14] (2*S*,3*R*)-[2,3-$^2H_2$]-isomer $[a]_D^{23}$ +7.1 (*c* 0.57, H_2O)]; *m/z* [+ve FAB (glycerol + water)] 126 and 128 (ratio 3 : 1; $[M + H]^+$); ν_{max} (KBr)/cm^{-1} 2634 (br, NH, OH) and 1745 (CO_2H); δ_H (300 MHz, 10% ^{2}HCl in ^2H_2O) 4.14 (1H, s, H-3*S*); δ_D (38.4 MHz, H_2O) 4.00 (1^2H, s, ^{2}H-3*R*) and 4.53 (1^2H, s, ^{2}H-2); δ_C (75.5 MHz, 10% ^{2}HCl in ^2H_2O) 40.4 (t, C-3), 52.4 (t, C-2) and 167.0 (acid).

Incubation of stereospecifically labelled samples of L-β-chloroalanine with L-aspartate aminotransferase and β-mercaptoethanol

Incubation of L-aspartate aminotransferase with (2*R*)-β-chloroalanine (15) and β-mercaptoethanol. 100 ml Sodium arsenate buffer containing β-mercaptoethanol (12 mg, 0.154 mmol) was adjusted to pH 8.4 and L-aspartate aminotransferase was added to give a final concentration of 40 units/ml in a total reaction volume of 2 ml. L-β-Chloroalanine **15**[14] (10 mg, 0.072 mmol) was added and the reaction was incubated at 27 °C with stirring for 3 h. Chloroform (1 litre) was used in portions to wash the solution and remove excess β-mercaptoethanol and the resulting aqueous layer was adjusted to pH 7. This was passed through an ion exchange column (Dowex 1 × 8–200, 2.5 × 5 cm, OH^- form). Impurities were eluted with water and β-hydroxyethylcysteine was eluted with 5% aqueous acetic acid. The presence of β-hydroxyethylcysteine was detected using chromatography and ninhydrin spray. Lyophilisation yielded a white solid which had the ^{1}H-NMR spectrum shown in Fig. 2d. Further cation exchange chromatography on Dowex 50 W × 8–200, 2.5 × 5 cm, H+ form), eluting the amino acid with water, gave the same ^{1}H-NMR spectrum and $[a]_D^{34}$ −58.1 (*c* 0.1, H_2O).

Incubation of (2*R*,3*R*)-[2,3-$^2H_2$]-β-chloroalanine (15a) prepared by the chemico-enzymatic synthesis with L-aspartate aminotransferase in the presence of β-mercaptoethanol.

Attempt A-6 h. The reaction was repeated as described above up to lyophilisation of the solid from the anion exchange column using (2*R*,3*R*)-[2,3-$^2H_2$]-β-chloroalanine **15a** for an incubation time of 6 h; δ_H (250 MHz, 10% ^{2}HCl in^2H_2O, Fig. 1b) 2.74 (2H, t, $J_{2',1'}$ 5.8, CH_2S), 3.15 (2H, *AB*X, H-3), 3.71 (2H, t, $J_{1',2'}$ 5.8, CH_2OH) and 4.33 (1H, AB*X*, H-2).

Attempt B-3 h. The reaction was repeated as described above using (2*R*,3*R*)-[2,3-$^2H_2$]-β-chloroalanine **15a** for an incubation time of 3 h; δ_H (250 MHz, 10% ^{2}HCl in ^2H_2O, Fig. 1a) 2.74 (2H, t, $J_{2',1'}$ 5.7, CH_2S), 3.05 (1H, d, $J_{3R,2}$ 7.3, H-3*R*), 3.68 (2H, t, $J_{1',2'}$ 5.7, CH_2OH) and 4.28 (1H, d, $J_{2,3R}$ 7.3, H-2).

Incubation of (2*R*,3*S*)-[3-$^2H_1$]-β-chloroalanine (15b) prepared by the chemico-enzymatic synthesis with L-aspartate aminotransferase in the presence of β-mercaptoethanol. The reaction was repeated as described above using (2*R*,3*S*)-[3-$^2H_1$]-β-chloroalanine **15b** for an incubation time of 3 h; δ_H (250 MHz, 10% ^{2}HCl in^2H_2O, Fig. 2f) 2.74 (2H, t, $J_{2',1'}$ 5.8, CH_2S), 3.26 (2H, d, $J_{3S,2}$ 4.4, H-3*S*), 3.71 (2H, t, $J_{1',2'}$ 5.8, CH_2OH) and 4.33 (1H, d, $J_{2,3S}$ 4.4, H-2).

Incubation of (2*R*,3*R*)-[3-$^2H_1$]-β-chloroalanine (17a) prepared by total synthesis with L-aspartate aminotransferase in the presence of β-mercaptoethanol. This was carried out as above using

L-aspartate aminotransferase (40 units/ml) and (2*R*,3*R*)-[3-$^2H_1$]-β-chloroalanine **17a**[16] (10 mg, 0.072 mmol); δ_H (300 MHz, 10% ^{2}HCl in ^2H_2O, Fig. 3c) 2.74 (2H, br t, $J_{2',1'}$ 6.1, CH_2S), 3.09 (1H, d, $J_{3S,2}$ 7.4, H-3*S*), 3.71 (2H, dt, $J_{1',2'}$ 6.1, $J_{1',OH}$ 2.2, CH_2OH) and 4.33 (1H, d, $J_{2,3S}$ 7.4, H-2).

Incubation of (2*R*,3*S*)-[3-$^2H_1$]-β-chloroalanine (17b) prepared by total synthesis with L-aspartate aminotransferase in the presence of β-mercaptoethanol. This was carried out as above using L-aspartate aminotransferase (40 unit/ml) and (2*R*,3*S*)-[3-$^2H_1$]-β-chloroalanine **17b**[16] (10 mg, 0.072 mmol); δ_H (300 MHz, 10% ^{2}HCl in ^2H_2O, Fig. 3b) 2.74 (2H, unresolved dt, CH_2S), 3.21 (1H, br s, H-3*R*), 3.73 (2H, unresolved dt, CH_2OH) and 4.34 (1H, d, $J_{2,3R}$ 4.3, H-2).

Independent synthesis of stereospecifically labelled samples of (2*R*)-β-hydroxyethylcysteineate

Methyl (2*R*)-*N*-carbobenzyloxy-β-hydroxyethylcysteineate. Boron trifluoride diethyletherate (7 drops) was added dropwise to a suspension of methyl (2*S*)-*N*-carbobenzyloxyaziridine-2-carboxylate **14** (2.11 g, 8.99 mmol) in β-mercaptoethanol (5.8 ml) with vigorous stirring under nitrogen at room temperature, and stirring was continued for 46 h. Chloroform (50 ml) was added to the solution which was washed with 10% aqueous sodium bicarbonate (2 × 50 ml), water (6 × 50 ml) and brine (50 ml) and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield a colourless gum which was chromatographed on silica gel, eluting with petroleum ether–ethyl acetate (11 : 9). Chromatography was repeated twice to remove a close-running impurity. This yielded methyl (2*S*)-*N*-carbobenzyloxy-β-hydroxyethylcysteineate as a colourless oil, (1.71 g, 61%); $[\alpha]_D^{25}$ +9.05 (*c* 1, $CHCl_3$); *m/z* (CI) Found 314.1065. $C_{14}H_{19}NO_5S + H^+$ requires 314.1062; *m/z* [+ve FAB (3-NBA)] 314 ([M + H]$^+$ and 336 ([M + Na]$^+$); ν_{max} (film)/cm^{-1} 3358–3063 (OH, NH), 1735 (ester), 1719 (urethane) and 1697 (amide); δ_H (250 MHz, C^2HCl_3) 2.71 (2H, m, CH_2S), 2.98 (2H, *AB*X, $J_{3R,3S}$ 14.0, $J_{3R,2}$ 5.0, $J_{3S,2}$ 5.7, H-3), 3.69 (2H, m, CH_2OH), 3.76 (3H, s, OCH_3), 4.61 (1H, m, H-2), 5.11 (2H, OCH_2Ar), 5.70 (1H, d, *J* 5.13, NH) and 7.32–7.35 (5H, s, ArH); δ_C (75.5 MHz, C^2HCl_3) 34.7 (C-3), 36.1 (SCH_2), 52.7 (OCH_3), 54.0 (C-2), 60.8 (CH_2OH), 67.1 (OCH_2Ar), 128.1–128.5 (Ar), 136.1 (*ipso* Ar), 155.9 (urethane) and 171.1 (ester).

Methyl (2*R*,3*S*)-[3-$^2H_1$]-*N*-carbobenzyloxy-β-hydroxyethylcysteineate. This was prepared as described above using methyl (2*S*,3*R*)-[3-$^2H_1$]-*N*-carbobenzyloxyaziridine-2-carboxylate **14b** (85 mg, 0.36 mmol) to yield the product (45 mg, 40%); $[\alpha]_D^{25}$ +9.05 (*c* 1, $CHCl_3$); *m/z* [+ve FAB (3-NBA)] 315 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3354 (br, OH) and 1718 (urethane); δ_H (300 MHz, C^2HCl_3) 2.73 (2H, m, CH_2S), 3.02 (1H, d, $J_{3R,2}$ 4.3, H-3*R*), 3.71 (2H, m, *C*H_2OH), 3.78 (3H, s, OCH_3), 4.63 (1H, m, H-2), 5.14 (2H, OCH_2Ar), 5.73 (1H, d, *J* 6.5, NH) and 7.36–7.38 (5H, s, ArH); δ_D (38.4 MHz, $CHCl_3$) 3.00 (1^2H, s, ^{2}H-3*S*); δ_C (75.5 MHz, C^2HCl_3) 36.9 (t, C-3) 38.6 (SCH_2), 55.2 (CH_3O), 56.4 (C-2), 63.2 (CH_2OH), 69.7 (OCH_2Ar), 131.1–130.6 (Ar), 138.5 (*ipso* Ar), 158.3 (urethane) and 173.5 (ester).

Methyl (2*R*,3*R*)-[2,3-$^2H_2$]-*N*-carbobenzyloxy-β-hydroxyethylcysteineate. This was prepared as described above using methyl (2*S*,3*S*)-[2,3-$^2H_2$]-*N*-carbobenzyloxyaziridine-2-carboxylate **14a** (50 mg, 0.21 mmol) to yield the product (16.2 mg, 20%); $[\alpha]_D^{30}$ +9.01 (*c* 1, $CHCl_3$); *m/z* [+ve FAB (3-NBA)] 316 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3354 (OH, br) and 1718 (urethane, amide); δ_H (300 MHz, C^2HCl_3) 2.69 (2H, m, CH_2S), 3.01 (1H, s, H-3*S*), 3.69 (2H, m, CH_2OH), 3.76 (3H, s, OCH_3), 5.11 (2H, OCH_2Ar), 5.70 (1H, s, NH) and 7.33–7.34 (5H, s, ArH); δ_D (38.4 MHz, $CHCl_3$) 2.92 (1^2H, s, ^{2}H-3*R*) and 4.60 (1^2H, s, ^{2}H-2); δ_C (75.5 MHz, C^2HCl_3) 34.9 (t, C-3) 36.8 (SCH_2), 53.4 (OCH_3), 54.6 (t, C-2), 61.3 (CH_2OH), 67.8 (OCH_2Ar), 128.8–129.2 (Ar), 136.6 (*ipso* Ar), 156.5 (urethane) and 171.7 (ester).

(2*R*)-β-Hydroxyethylcysteine (18). Methyl (2*R*)-*N*-carbobenzyloxy-β-hydroxyethylcysteineate (100 mg, 0.32 mmol) was heated to reflux in 4 M aqueous sulfuric acid (3 ml) for 4 h. The solution was allowed to cool, washed with dichloromethane (2 × 5 ml), and neutralised with aqueous ammonium hydroxide. This solution was concentrated *in vacuo* until solid began to precipitate. Water was added until the residue redissolved and the pH of the resultant solution was adjusted to 7. The solution was chromatographed on an anion exchange column (Dowex 1 × 8–200, 2.5 × 15 cm, OH^- form) by passing water until the pH of the eluant became neutral, and eluting the amino acid with 5% aqueous acetic acid (50 ml). The solvent was removed *in vacuo* to yield (2*R*)-β-hydroxyethylcysteine **18** as a white solid (26 mg, 45%); mp 188–189 °C; $[\alpha]_D^{32}$ −51.2 (*c* 2, H_2O) [lit[31] $[\alpha]_D$ −53.3 (*c* 2, H_2O)]; *m/z* [+ve FAB (glycerol)] 166 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3412 (br, OH, NH) and 1732 (acid); δ_H (300 MHz,^2H_2O, Fig. 2a) 2.74 (2H, dt, $J_{2',1'}$ 5.8, $J_{2',OH}$ 2.0, CH_2S), 3.10 (1H, dd, $J_{3S,3R}$ 15, $J_{3S,2}$ 7.3, H-3*S*), 3.23 (1H, dd, $J_{3R,3S}$ 15, $J_{3R,2}$ 4.5, H-3*R*), 3.71 (2H, dt, $J_{1',2'}$ 5.8, *J* 2.3, CH_2OH) and 4.33 (1H, AB*X*, $J_{2,3R}$ 4.5, $J_{2,3S}$ 7.3, H-2); δ_C (75.5 MHz, ^2H_2O) 33.1 (C-3), 35.8 (SCH_2), 54.1 (C-2), 62.0 (CH_2OH) and 170.5 (acid).

(2*R*,3*S*)-[3-$^2H_1$]-β-Hydroxyethylcysteine (18b). This was prepared as described above using methyl (2*S*,3*S*)-[3-$^2H_1$]-*N*-carbobenzyloxy-β-hydroxyethylcysteineate (6 mg, 0.022 mmol) to yield the product **18b** (2 mg, 50%); mp 188–189 °C; $[\alpha]_D^{30}$ −50.9 (*c* 0.05, H_2O); *m/z* [+ve FAB (glycerol + H_2O)] 167 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3412 (br, OH, NH,) and 1732 (acid); δ_H (250 MHz, 10% ^{2}HCl in ^2H_2O, Fig. 2c) 2.74 (2H, t, $J_{2',1'}$ 5.7, CH_2S), 3.21 (1H, d, $J_{3R,2}$ 4.3, H-3*R*), 3.75 (2H, t, $J_{1',2'}$ 5.7, CH_2OH) and 4.31 (1H, d, $J_{2,3R}$ 4.3, H-2); δ_D (38.4 MHz, 10% HCl in H_2O) 3.18 (1^2H, s, ^{2}H-3*S*); δ_C (75.5 MHz, 10% ^{2}HCl in ^2H_2O) 31.4 (t, C-3), 34.0 (SCH_2), 52.3 (C-2), 60.2 (CH_2OH) and 170.4 (acid).

(2*R*,3*R*)-[2,3-$^2H_2$]-β-Hydroxyethylcysteine (18a). This was prepared as described above using methyl (2*S*,3*R*)-[2,3-$^2H_2$]-*N*-carbobenzyloxy-β-hydroxyethylcysteineate (8 mg, 0.029 mmol) to yield the product **18a** (2.2 mg, 42%); mp 185–188 °C; $[\alpha]_D^{30}$ −51.4 (*c* 0.05, H_2O); *m/z* [+ve FAB (glycerol + H_2O)] 168 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3410 (br, OH, NH) and 1730 (acid); δ_H (250 MHz, 10% ^{2}HCl in ^2H_2O, Fig. 2b) 2.74 (2H, t, $J_{2',1'}$ 5.9, CH_2S), 3.08 (2H, s, H-3*S*) and 3.71 (2H, t, $J_{1',2'}$ 5.9, CH_2OH); δ_D (38.4 MHz, 10% HCl in H_2O) 3.18 (1^2H, s, ^{2}H-3*R*) and 4.12 (1^2H, s, ^{2}H-2).

Incubation of stereospecifically labelled samples of D-β-chloroalanine with D-amino acid aminotransferase and β-mercaptoethanol

Incubation of (2*S*)-β-chloroalanine (19) with D-amino acid aminotransferase in the presence of β-mercaptoethanol. This was carried out following the method for the L-aspartate aminotransferase incubations but using D-amino acid aminotransferase (40 units/ml) and (2*S*)-β-chloroalanine **19**[32] (10 mg, 0.072 mmol). The optical rotation of the white solid was measured to give the value $\alpha_D^{28.5}$ +0.021; δ_H (300 MHz, 10% ^{2}HCl in ^2H_2O, Fig. 4a) 2.74 (2H, dt, $J_{2',1'}$ 6.1, $J_{2',OH}$ 1.5, CH_2S), 2.90 (1H, dd, $J_{3R,2}$ 7.4, $J_{3R,3S}$ 15.2, H-3*R*), 3.11 (1H, dd, $J_{3S,2}$ 4.5, $J_{3S,3R}$ 15.0, H-3*S*), 3.61 (2H, dt, $J_{1',2'}$ 6.1, $J_{1',OH}$ 2.2, CH_2OH) and 4.23 (1H, dd, $J_{2,3R}$ 7.4, $J_{2,3S}$ 4.5, H-2).

Incubation of (2*S*,3*S*)-[3-$^2H_1$]-β-chloroalanine (19b) with D-amino acid aminotransferase in the presence of β-mercaptoethanol. This was carried out as above using (2*S*,3*S*)-[3-$^2H_1$]-β-chloroalanine **19b**[16] (10 mg, 0.072 mmol) prepared by total synthesis; δ_H (300 MHz, 10% ^{2}HCl in ^2H_2O, Fig. 4b) 2.74 (2H, unresolved dt, CH_2S), 3.07 (1H, m, H-3*R*), 3.82 (2H, dt, $J_{1',2'}$ 6.0, $J_{1',OH}$ 2.1, CH_2OH) and 4.23 (1H, d, $J_{2,3R}$ 7.0, H-2).

Incubation of (2*S*,3*R*)-[3-$^2H_1$]-β-chloroalanine (19a) with D-amino acid aminotransferase in the presence of β-mercaptoethanol. This was carried out as above using (2*S*,3*R*)-[3-$^2H_1$]-β-chloroalanine **19a**[16] (10 mg, 0.072 mmol) prepared by total synthesis; δ_H (300 MHz, 10% ^{2}HCl in ^2H_2O, Fig. 4c) 2.74 (2H, unresolved dt, CH_2S), 3.20 (1H, m, H-3*S*) 3.72 (2H, dt, $J_{1',2'}$ 5.9, $J_{1',OH}$ 2.1, CH_2OH) and 4.32 (1H, d, $J_{2,3S}$ 4.3, H-2).

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