A Versatile Synthesis of Stereospecifically Labelled D-Amino Acids and Related Enzyme Inhibitors

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Stereospecifically deuteriated isoserines **4**, formed from enzymically prepared 3-deuteriated malic acids **2** (X = OH) by Curtius rearrangement, have been converted to the deuteriated aziridines **7** and **9** which, on ring-opening and deprotection, yielded samples of the amino acids D-serine and D-cystine and the enzyme inhibitor–substrates D-β-chloroalanine and D-serine *O*-sulphate which are labelled stereospecifically at C-3 with deuterium.

Except in rare instances,[1] D-amino acids are not present in mammals but are extremely common in bacteria.[2] Bacterial enzymes which metabolise D-amino acids have, therefore, long been seen as targets for antibacterial drugs. An understanding of the mechanism of action of these enzymes is important in the design of inhibitors as potential antibacterial drugs.

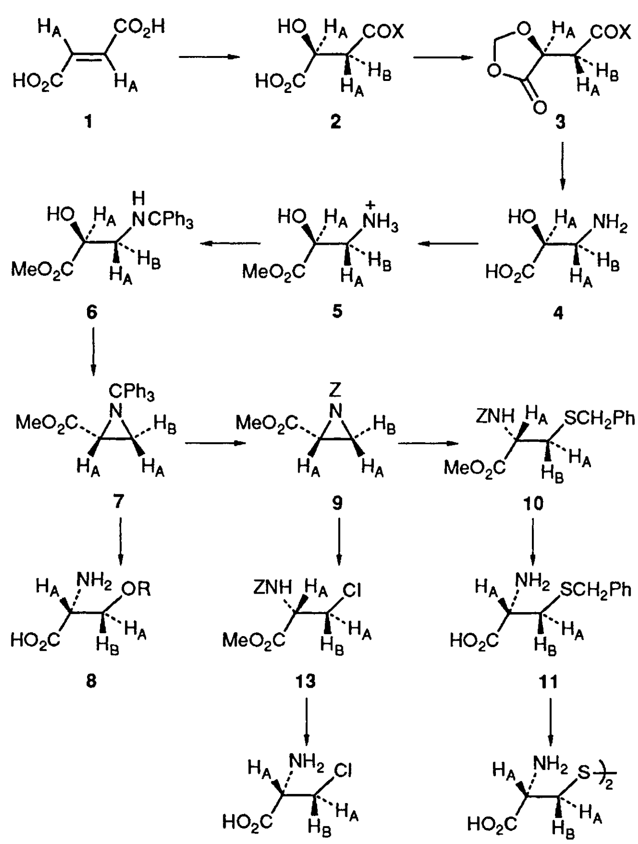
Much detail has been obtained on the mechanism of action of enzymes which metabolise L-amino acids by studying the stereochemistry of their reactions at the β-carbon atom of the amino acid substrate.[3] Similar information on the corresponding reactions of D-amino acids is, however, relatively rare.[4] We have, therefore, undertaken a general synthesis of D-amino acids stereospecifically labelled with deuterium at C-3 and, using it, have prepared stereospecifically labelled samples of the aminoacids D-serine **8** (R = H, H_A = ^2H) and **8** (R = H, H_B = ^2H) and D-cystine **12** (H_A = ^2H) and **12** (H_B = ^2H), and the enzyme inhibitor–substrates D-serine *O*-sulphate **8** (R = SO_3H, H_A = ^2H) and **8** (R = SO_3H, H_B = ^2H) and D-β-chloroalanine **14** (H_A = ^2H) and **14** (H_B = ^2H).

The commercially available enzyme fumarase (EC 4.2.1.2) is known[5] to convert fumaric acid **1** into (2*S*)-malic acid **2** (X = OH) with *anti*-addition of water and so, using [2,3-$^2H_2$]-fumaric acid **1** (H_A = ^2H),[6] we were able to prepare (2*S*, 3*S*)-[2,3-$^2H_2$]malic acid **2** (X = OH, H_A = ^2H)† in 69% yield. (2*S*, 3*R*)-[3-$^2H_1$]Malic acid **2** (X = OH, H_B = ^2H)† could also be prepared using this enzyme but we have found[7] it more efficient to synthesise this compound by nitrosation[8] of (2*S*, 3*R*)-[3-$^2H_1$]aspartic acid, prepared from fumaric acid **1** using immobilised *Escherichia coli*.[9]

The next phase of the synthesis was to convert these samples of malic acid into the labelled isoserines **4**. This was achieved by conversion into the β-malamic acids **2** (X = NH_2)† using methods developed for the unlabelled compounds[10] and then using bis(trifluoroacetoxy)phenyl iodide to effect Hofmann rearrangement with retention of stereochemistry at the migrating chirally labelled centre. Yields were variable, however, and we found it more reliable to use a 'one-pot' Curtius procedure.[11] Reaction of the samples of malic acid **2** (X = OH) with paraformaldehyde and catalytic amounts of acid gave the protected compounds **3** (X = OH) which were converted without purification, *via* the acid chlorides **3** (X = Cl) and azides **3** (X = N_3) into the labelled samples of isoserine **4** (H_A = ^2H)† and **4** (H_B = ^2H)† in overall yields of 30–39% from the labelled samples of malic acid. The Curtius rearrangement was expected[12] to proceed with retention of stereochemistry at the migrating stereospecifically labelled centre and indeed the ^1H NMR spectra of the products indicated that labelling was stereospecific.

The samples of isoserine **4** were now converted into the esters **5** (H_A = ^2H)† and **5** (H_B = ^2H)† in nearly quantitative yield using thionyl chloride and methanol and tritylation gave the *N*-trityl derivatives **6** (H_A = ^2H)† and **6** (H_B = ^2H)† in excellent yields. These derivatives were converted into the tosylates using toluene-*p*-sulphonyl chloride in pyridine and reaction with triethylamine in tetrahydrofuran at reflux then afforded the aziridines **7** (H_A = ^2H)† and **7** (H_B = ^2H)† in overall yields of *ca.* 50% from the *N*-trityl-esters **6**. The ^1H and ^2H NMR spectra of the aziridines are shown in Fig. 1. Reaction of the *N*-tritylaziridines **7** with refluxing 20% aqueous perchloric acid for 30 h gave nearly quantitative yields of (2*R*, 3*R*)-[2,3-$^2H_2$]serine **8** (R = H, H_A = ^2H)† and (2*R*, 3*S*)-[3-$^2H_1$]serine **8** (R = H, H_B = ^2H)†.

Our synthesis implies retention of stereochemistry at the β-carbon in the Curtius step **3** (X = N_3) → **4**; inversion at the α-carbon in the aziridine ring closure step **6** → **7**; and inversion at the β-carbon in the ring-opening step **7** → **8**. We were now in a position to confirm these assumptions since we had previously prepared samples of (2*S*, 3*S*)-[2,3-$^2H_2$]- and (2*S*, 3*R*)-[3-$^2H_1$]-serine.[13] The ^1H NMR spectra of these samples were the same as those of the samples of (2*R*, 3*R*)-[2,3-$^2H_2$]-and (2*R*, 3*S*) [3-$^2H_1$]-serine **8** (R = H, H_A = ^2H) and **8** (R = H, H_B = ^2H) respectively. The (2*S*)- and (2*R*)-samples, however, had numerically equal specific rotations of opposite sign. All four stereospecifically labelled samples of serine were converted to the corresponding samples† of the inhibitor–substrate D-serine *o*-sulphate (**8** R = SO_3H) using the method developed by Previero *et al.*[14] to prepare the unlabelled compound.



† The samples all had the expected spectroscopic data and absences in the ^1H NMR spectra indicated that labelling was stereospecific.

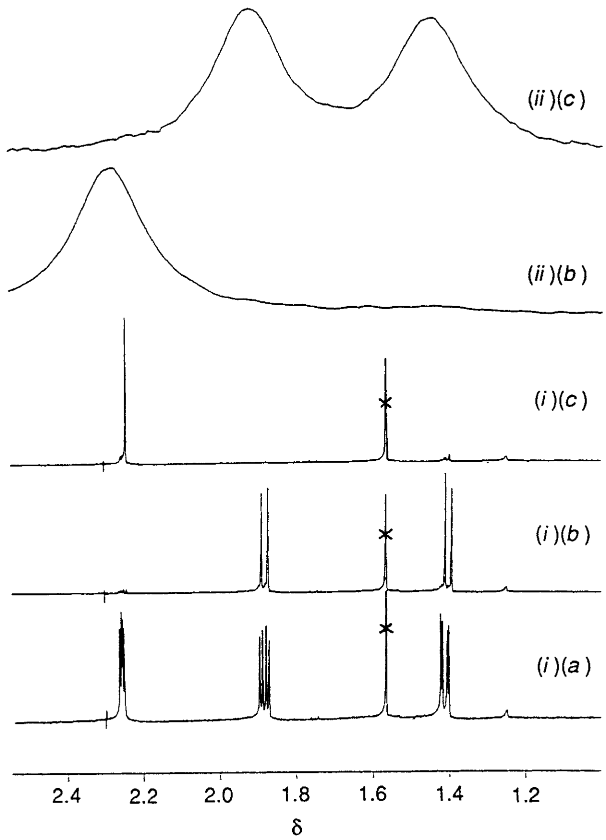


Fig. 1 Part of (*i*) the 360 MHz ^1H NMR spectra in C^2HCl_3 and (*ii*) the 38.4 MHz ^2H NMR spectra in $CHCl_3$ of (*a*) unlabelled *N*-tritylaziridine 7; (*b*) (2*R*, 3*R*)-[3-$^2H_1$]-**7** and (*c*) (2*R*, 3*S*)-[2,3-$^2H_2$]-**7**. Coupling constants are J_{AB} = 1.6 Hz, $J_{AX(cis)}$ = 6.2 Hz and $J_{BX(trans)}$ = 2.7 Hz for the aziridine protons.

It was necessary to convert the *N*-tritylaziridines **7** to the corresponding *N*-benzyloxycarbonyl compounds **9** to complete the synthesis of other amino acids. This was achieved in nearly quantiative yield by first deprotection using trifluoroacetic acid in methanol and chloroform and then reaction with benzyl chloroformate under Schotten–Baumann conditions. The labelled aziridines **9** (H_A = ^2H)† and **9** (H_B = ^2H)† were treated with benzyl mercaptan and boron trifluoride–diethyl ether to yield the adducts **10** (H_A = ^2H)† and **10** (H_B = ^2H)† respectively in *ca.* 40% yield. These were deprotected in two steps. Refluxing 6 mol dm^{-3} HCl first gave the amino acids **11** (H_A = ^2H)† and **11** (H_B = ^2H)† and further treatment with sodium in liquid ammonia gave the cysteines which were oxidised to (2*S*, 3*R*)-[2,3-$^2H_2$]- and (2*S*, 3*S*)-[3-$^2H_1$]-cystine **12** (H_A = ^2H)† and **12** (H_B = ^2H)† respectively.

Initial attempts to prepare labelled samples of the enzyme inhibitor–substrate β-chloroalanine by treatment of the aziridines **7** with HCl resulted in non-regiospecific and non-stereospecific ring opening. Reaction of the aziridines **9** with $TiCl_4$ in $CHCl_3$–CH_2Cl_2 (1:1), however, gave the protected β-chloroalanines **13** (H_A = ^2H)† and **13** (H_B = ^2H)†. Hydrolysis in refluxing 2 mol dm^{-3} sulphuric acid then afforded (2*S*, 3*R*)-[2,3-$^2H_2$]- and (2*S*, 3*S*) [3-$^2H_1$]-β-chloroalanines **14** (H_A = ^2H)† and **14** (H_B = ^2H).†

We thank the SERC for studentships (to K. J. O'T. and P. A. S.), the Swedish Natural Science Research Council for a Fellowship (to B. S. A.) and Dr Ryan A. August for preliminary experiments.

Received, 3rd May 1991; Com. 1/02129J

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