

Synthesis of (2*S*)-*O*-phosphohomoserine and its C-2 deuteriated and C-3 chirally deuteriated isotopomers: probes for the pyridoxal phosphate-dependent threonine synthase reaction¹

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A short efficient synthesis of the threonine synthase substrate (2*S*)-*O*-phosphohomoserine and its C-2 deuteriated and C-3 chirally deuteriated isotopomers is described. The synthetic route also provides access to (2*S*)-homoserine and its C-2 deuteriated and C-3 chirally deuteriated isotopomers in high yield. Preliminary deuterium isotope effect determinations performed using the deuteriated (2*S*)-*O*-phosphohomoserines and threonine synthase from *E. coli* indicate that the removal of protons from both C-2 and C-3 is kinetically important.

Introduction

Pyridoxal 5'-phosphate (PLP)-dependent threonine synthase catalyses the conversion of (2*S*)-*O*-phosphohomoserine **1** into (2*S*)-threonine **2** and inorganic phosphate in the final step of threonine biosynthesis in bacteria and plants.² Compounds which can block the action of threonine synthase in plants are of herbicidal or fungicidal potential and, therefore, there is considerable interest in the structure and mechanism of the enzyme.³



Threonine synthase from several sources has now been sequenced.⁴ There is a small region of strong homology about Lys-124 (yeast enzyme numbering) which is believed to form the internal aldimine linkage with the coenzyme; see structure **3**, Scheme 1. However, for the remaining parts of the protein the homology between enzyme from different sources is poor, even for different bacterial enzymes. Details of the mechanism of the threonine synthase reaction are sparse. By analogy to the mode of action of other PLP-dependent systems it is believed that the conversion of the external aldimine **4** into the quinonoid intermediate **5** increases the acidity of the 3-*pro-S* proton of the substrate,⁵ as determined for the yeast enzyme, such that the β,γ -elimination of phosphoric acid can occur. [Note that these studies used tritiated substrates (90% homochiral at C-3) in which the phosphate group was introduced using adenosine triphosphate (ATP) and homoserine kinase, *vide infra*.] If threonine synthase shows the same stereochemical imperative as other PLP-dependent enzymes in utilising the 4'-*si*-face of the coenzyme for its reactions,⁶ the elimination would occur in a *syn*-fashion to give the conjugated enamine **6** as shown in Scheme 1. Protonation at C-4 of the imino acid moiety in **6** would give enamine **7** and allow attack by water at C-3 on the 4'-*si*-face of the coenzyme to give product quinonoid **8**. Protonation at C-2 would then furnish the product aldimine which, upon transaldimination, would yield (2*S*,3*R*)-threonine **2**. In an interesting side reaction threonine synthase can convert phosphohomoserine into an α -oxobutyric acid and ammonia. Presumably these products form when the enamine **7** undergoes premature transaldimination to give the holoenzyme **3** and the enamine, 2-aminobut-3-enoic acid, which would hydrolyse non-enzymically in the aqueous environment of the enzyme.

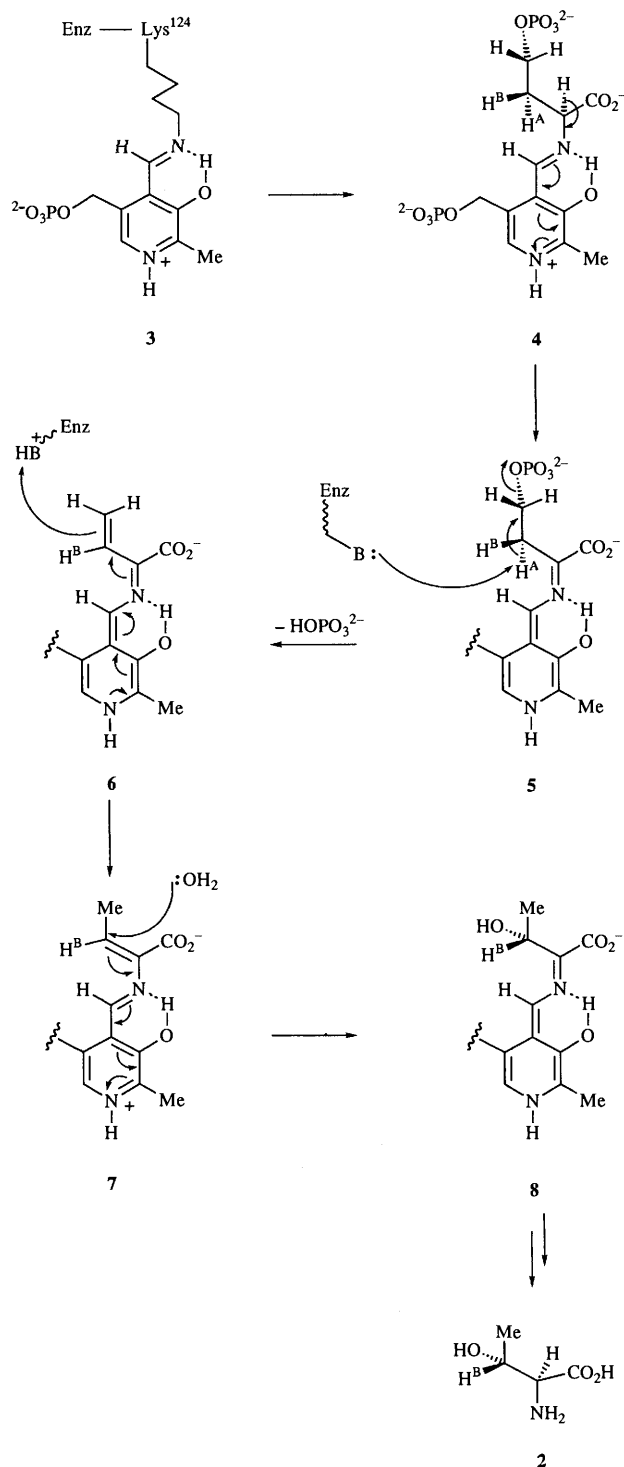
Results and discussion

In order to examine the kinetics and mechanism of the reaction and to allow the determination of the size of the primary deuterium isotope effect for the elimination process and the effects of product inhibition, quantities of pure (2*S*)-*O*-phosphohomoserine and its C-2 deuteriated and C-3 chirally monodeuteriated isotopomers were required. Since neither of the established methods for the preparation of (2*S*)-phosphohomoserine [based upon (i) the use of *Bacillus subtilis* mutants lacking threonine synthase which accumulate the compound,⁷ or (ii) the enzymic phosphorylation of homoserine with ATP and yeast homoserine kinase,⁸ or (iii) the phosphorylation of *N*-benzyloxycarbonylhomoserine *p*-nitrobenzyl ester with diphenylphosphoryl chloride⁹] were either efficient, convenient or suitable for our requirements, a new synthesis was devised starting from the appropriately labelled aspartic acids.

(2*S*)-Aspartic acid **9** was converted into the *N*-(trifluoroacetyl)aspartic anhydride through treatment with trifluoroacetic anhydride (TFAA) in tetrahydrofuran (THF), Scheme 2. The selective protection of the α -carboxy group through treatment of the anhydride with methanol was reported to give the desired α -methyl ester β -acid in 75–80% yield.¹⁰ Performing the reaction at lower temperatures did not improve the ratio in favour of the α -ester over the β -ester, nor did treatment with ethanol. However, treatment with propan-2-ol at 0 °C gave >96% of the desired α -isopropyl ester β -acid **10**, as judged by examination of the ¹H NMR spectrum of the crude material, which could be obtained in 92% yield after one recrystallisation.

A range of methods and conditions for reducing the β -carboxy group to the corresponding alcohol were examined. Finally, under optimised conditions, the alcohol **11** was prepared by adding the mixed β -aspartic isobutylcarbonic anhydride derived from compound **10** to a solution of sodium boranuide in THF. The desired *N*-(trifluoroacetyl)homoserine ester **11** was obtained as an oil in 92% yield and showed the expected spectral and analytical data. Base-catalysed hydrolysis of the trifluoroacetyl and isopropyl protecting groups in aq. ethanol afforded (2*S*)-homoserine **13**, identical in all respects with an authentic sample { $[\alpha]_D -24.4$ (*c* 10, water); lit.,¹¹ $[\alpha]_D -24.5$ (*c* 10, water)}, in 95% yield (85% from aspartic acid). This compares favourably with other syntheses starting from aspartic acid.¹²

In order to prepare samples of *O*-phosphohomoserine, all that remained was to convert the alcohol **11** into a phosphate ester derivative and to remove the protecting groups. A range of phosphorylating reagents were considered and because phosphate esters are known to be labile under acidic conditions,

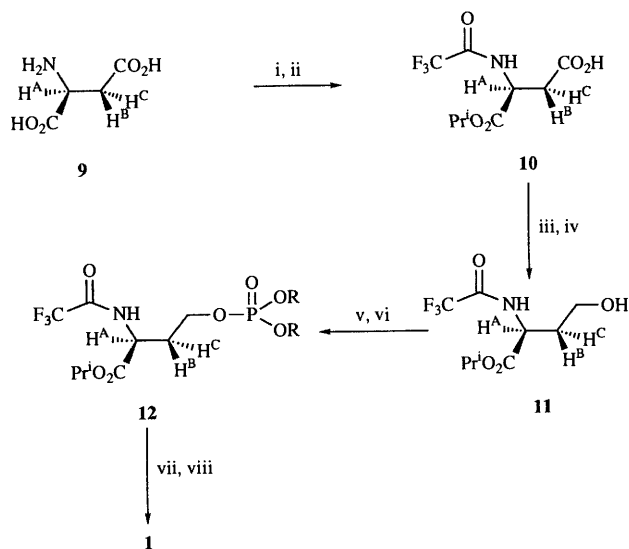


Scheme 1

we opted to use base-labile cyanoethyl phosphate protecting groups in the first instance, which should cleave *via* β -elimination. It was hoped that such a strategy might allow the removal of all of the protecting groups in one step.

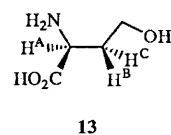
Accordingly, the alcohol 11 was treated with bis-cyanoethyl phosphoramidite to give the phosphate triester, which was oxidised *in situ* using *m*-chloroperbenzoic acid (MCPBA) in dichloromethane. The resulting phosphate triester (12; R = CH₂CH₂CN) was obtained in good yield (87%) but proved very difficult to purify. All attempts to deprotect the crude material resulted in substantial dephosphorylation although (2S)-phosphohomoserine 1 could be purified from the hydrosylate by ion-exchange chromatography in ~10% yield.

Treatment of the alcohol 11 with dibenzyl *N,N*-diisopropyl-



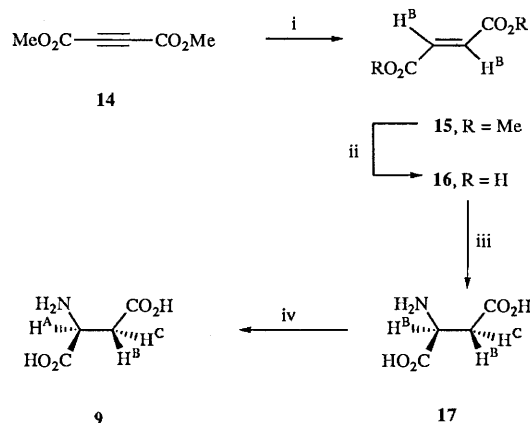
Scheme 2 Reagents and conditions: i, TFAA, THF, 0 °C, 3 h; ii, PrⁱOH, 0 °C, 48 h, 95% over 2 steps; iii, NMM, Bu^tOCCl, THF, -40 °C, 15 min; iv, NaBH₄, THF, -40 °C, 4 h, 92% over 2 steps; v, Prⁱ₂NP(OBn)₂, 1*H*-tetrazole, CH₂Cl₂, 20 °C, 45 min; vi, MCPBA, CH₂Cl₂, 0 °C, 92% over 2 steps; vii, Pd/C, H₂, MeOH, 20 °C, 24 h; viii, KOH, EtOH, 20 °C, 3 h; then Dowex 50W (H⁺), 80% over 2 steps

phosphoramidite followed by oxidation of the resulting dibenzyl phosphite with MCPBA, as outlined in Scheme 2, gave the phosphate triester (12; R = CH₂Ph) in excellent yield (92%). The phosphate triester was then smoothly converted in two steps into (2S)-*O*-phosphohomoserine 1, *via* catalytic hydrogenolysis of the *O*-benzyl protecting groups followed by base-catalysed hydrolysis of the carboxylic ester and amide groups. The title compound 1 was obtained in 80% yield (63% from aspartic acid) after purification on Dowex 50W and showed the expected spectral and analytical data.



In order to prepare the deuteriated phosphohomoserines 1 (H^A = ²H, H^B = H^C = H; H^A = H^B = H, H^C = ²H; and H^A = H^C = H, H^B = ²H), (2S)-[2-²H]aspartic acid and (2S,3*R*)- and (2S,3*S*)-[3-²H₁]aspartic acid¹³ were prepared as described below and as outlined in Scheme 3 and were taken through the steps outlined in Scheme 2.

(2S)-[2-²H₁]Aspartic acid 9 (H^A = ²H, H^B = H^C = H) was prepared by incubating (2S)-aspartic acid with aspartate



Scheme 3 Reagents and conditions: i, Ph₃P, ²H₂O, THF; ii, 12% NaOH, 41% over 2 steps; iii, β-methylaspartase, NH₃, K⁺, Mg²⁺, water, 60%; iv, AAT, PLP, water, 78%

aminotransferase (AAT) and PLP in deuterated water, step iv, Scheme 3, as described previously.¹⁴ (2*S*,3*R*)-[3-²H₁]Aspartic acid **9** (H^A = H^B = H, H^C = ²H) was prepared by treatment of fumaric acid **16** (H^B = H) with deuterated ammonia in deuterium oxide in the presence of methylaspartase and the necessary metal ion cofactors as described previously.¹³ In order to prepare (2*S*,3*S*)-[3-²H₁]aspartic acid **9** (H^A = H^C = H, H^B = ²H), dimethyl acetylenedicarboxylate **14** was reductively deuterated according to the method of Richards *et al.*¹⁵ and the resulting fumarate ester **15** (H^B = ²H) was saponified. The dideuterated fumaric acid **16** (H^B = ²H) was then aminated using methylaspartase¹³ and the resulting aspartic acid **17** (H^B = ²H, H^C = H) was treated with aspartate aminotransferase in diprotium oxide to exchange the C-2 deuterium atom and give isotopomer **9** (H^A = H^C = H, H^B = ²H). Each of the aspartic acids was then taken through the steps outlined in Scheme 2 to give the (2*S*)-homoserine and (2*S*)-phosphohomoserine derivatives. For the phosphohomoserines the overall yields from the precursor aspartic acids ranged from 40–60% and the ¹H NMR spectra for the compounds showed the expected spectral omissions for the C-2 or C-3 hydrogen atoms. The diastereotopic C-3 hydrogen atoms of phosphohomoserine occur as well separated multiplets and can be assigned from the known absolute configurations of the precursor aspartic acids. The signals for the 3-*pro-R* and 3-*pro-S* hydrogens resonate at $\delta \sim 2.35$ and 2.05, respectively in deuterium oxide solution.

Having prepared the (2*S*)-phosphohomoserine, we wished to use the compound to develop a protein purification protocol for threonine synthase from *E. coli* strain Tir 8.³ The synthetic (2*S*)-*O*-phosphohomoserines were successfully converted into threonine and inorganic phosphate by the enzyme as determined using a phosphomolybdate assay for inorganic phosphate¹⁶ and the compounds are, therefore, suitable for use as mechanistic probes for the enzyme.

From a partially purified preparation of *E. coli* threonine synthase and unlabelled substrate a K_m value of $44 \pm 8 \mu\text{mol dm}^{-3}$ was obtained at pH 7.5 and at 37 °C.¹⁷ In accord with expectations, addition of the allosteric activator *S*-adenosyl-methionine (SAM) caused an increase in V_{max} and also an increase in the value of K_m for the substrate.¹⁸ In the absence of SAM and at high concentrations of the substrate, both (2*S*)-[2-²H₁]- and (2*S*,3*S*)-[3-²H₁]phosphohomoserine displayed reduced reaction rates whereas (2*S*,3*R*)-[3-²H₁]phosphohomoserine did not. Full details of the enzyme-purification protocols, the enzyme assay, and the kinetic experiments will be reported in due course together with a detailed analysis of the primary deuterium isotope effects.

The availability of the previously elusive natural product, (2*S*)-*O*-phosphohomoserine, from aspartic acid will also facilitate studies of its metabolism to α -oxobutyrates.

Experimental

NMR Spectra were recorded on a Bruker AM-300 (300 MHz; FT ¹H NMR, 74.76 MHz; ¹³C NMR and 121.5 MHz; ³¹P NMR), a Varian Gemini 200 (200 MHz; ¹H NMR, and 50.3 MHz; ¹³C NMR) and a JEX GX 400 (400 MHz; FT ¹H NMR, 100.6 MHz; ¹³C NMR) spectrometers. ¹H NMR spectra were referenced on ²H₂O (δ 4.68), CHCl₃ (δ 7.27) or dimethyl sulfoxide (DMSO) (δ 2.47). ¹³C spectra were referenced on CH₃OH (δ_c 49.9), CHCl₃ (δ_c 77.5) or DMSO (δ_c 39.70) and ³¹P spectra on external H₃PO₄. NMR spectral signals are described in parts per million downfield shift from SiMe₄ and are reported consecutively as position (δ_H or δ_C), relative integral, multiplicity, coupling constant ($J_{X,Y}$ Hz if applicable) and assignment.

IR spectra were taken on a Perkin-Elmer 1420 recording spectrometer and a Perkin-Elmer 1710 FT IR spectrometer. The samples were prepared as Nujol mulls or thin films between

sodium chloride discs. The frequencies (ν) as absorption maxima are given in wavenumbers (cm⁻¹) relative to a polystyrene standard. Mass spectra were recorded on a Kratos MS50, a JEOL DX 303, a VG TRIO 1 and obtained on a SERC service basis at the University of Swansea using a VG ZAB E. Major fragments are given as percentages of the base-peak intensity. GC-MS spectra were recorded on a Hewlett Packard 5890A 6C. Elemental microanalyses were performed in the departmental microanalytical laboratory. Flash chromatography was performed according to the procedure of Still *et al.*¹⁹ using Sorbisil C60 (40–60 μm mesh) silica gel. Analytical TLC was carried out on 0.25 mm precoated silica plates (Macherey-Nagel SIL g/UV₂₅₄) or on 0.1 mm precoated cellulose plates (CEL MN 300–10/UV₂₅₄), and compounds were visualised by UV fluorescence, iodine vapour, ethanolic phosphomolybdic acid, aq. potassium permanganate, acidic palladium(II) chloride or ninhydrin.

Mps were taken on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were taken at 23 °C on an Optical Activity AA-1000 polarimeter using 10 cm pathlength cells. $[\alpha]_D$ -Values are given in 10⁻¹ deg cm² g⁻¹. All pH values were measured on a WPA CD620 Digital pH meter using a glass electrode.

The solvents used were either distilled or of analar quality, and light petroleum refers to that portion boiling between 40 and 60 °C. Solvents were dried according to literature procedures.²⁰

Dideuteriofumaric acid **16** (H^B = ²H)

This was prepared following the original method of Richards *et al.*¹⁵ and the protocols of Field and Young²¹ starting from dimethyl acetylenedicarboxylate **14** (5 g, 35 mmol). The intermediate dimethyl dideuteriofumarate **15** was obtained as crystals after sublimation and recrystallisation from methanol (2.11 g, 41%), mp 98–100 °C (lit.,¹⁵ 98–101 °C) (Found: C, 49.45; H, 5.15. Calc. for C₆H₆²H₂O₄: C, 49.3; H, 5.15%); ν_{max} (Nujol)/cm⁻¹ 2260 (C–²H), 1730 (ester C=O) and 1610 (C=C); δ_H (200 MHz; ²H₂O, 5% NaO²H) 3.81 (6 H, s, 2 × CH₃); δ_C (50.3 MHz; ²H₂O, 5% NaO²H) 51.6 (OCH₃), 137.0 (t, *J* 12, alkenic C) and 177.49 (CO₂Me); m/z (EI) 146 (2%, M⁺) and 59 (75, CO₂Me⁺).

This material (2 g, 13.7 mmol) was saponified following published procedures. Acidification of the sodium salt gave the free diacid **16** (H^B = ²H) (1.19 g, 66%), mp 297–300 °C, (lit.,¹⁵ 299–300 °C); ν_{max} (Nujol)/cm⁻¹ 2400–3400 (acid OH), 2260 (C–²H) and 1680 (acid C=O); δ_C (50.3 MHz; ²H₂O, 5% NaO²H) 133.10 (t, *J* 25, C=C) and 172.88 (CO₂H); m/z (EI) 118 (38%, M⁺) and 101 (41, [M – OH]⁺).

(2*S*,3*S*)-[2-²H₁,3-²H₁]Aspartic acid **17** (H^B = ²H, H^C = H)

Following the method of Akhtar *et al.*,¹³ dideuteriofumaric acid **16** (H^B = ²H) (2 g, 16.9 mmol) was suspended in water (20 cm³) and the pH was adjusted to 9 with 35 mol dm⁻³ aq. ammonia. The solution was concentrated under reduced pressure, the diammonium fumarate was redissolved in water (20 cm³), and magnesium chloride hexahydrate (40 mg, 10 mmol) and potassium chloride (7 mg, 4.5 mmol) were added. The pH was again adjusted to 9 with 35 mol dm⁻³ aq. ammonia and 3-methylaspartase (30 units) was added. The reaction mixture was incubated at 30 °C for 72 h, when no further decrease in the absorbance at 240 nm occurred. The solution was heated at 100 °C for 2 min and was then filtered through a Celite pad. The filtrate was acidified to pH 1 with 12 mol dm⁻³ HCl and was extracted with diethyl ether (2 × 10 cm³). The aqueous layer was adjusted to pH 4 and the aspartic acid precipitated out upon addition of ethanol. Recrystallisation from aq. ethanol gave the required aspartic acid **17** (H^B = ²H, H^C = H) as crystals (1.39 g, 60%), mp 300 °C (decomp.); m/z (Found: [M + H]⁺, 136.0579. Calc. for C₄H₆²H₂NO₄: m/z , 136.0577); $[\alpha]_D + 24.7$ (c 0.6, 6 mol dm⁻³ HCl) {lit.,²¹ $[\alpha]_D$

+22.7 (*c* 0.74, 1 mol dm⁻³ HCl); δ_c (50.3 MHz; ²H₂O, 5% NaO²H) 46.5 (t, *J* 20, C^B), 56.0 (t, *J* 19, C^A), 183.21 (β -CO₂H) and 185.33 (α -CO₂H); *m/z* (EI) 135 (3%, M⁺) and 44 (100, CO₂⁺). Other spectral data were identical with those reported for the compound synthesized using L-aspartase.²²

(2S,3S)-[3-²H₁]Aspartic acid 9 (H^A = H^C = H, H^B = ²H)

Following the method of Rose *et al.*,¹⁴ (2S,3S)-[2-²H₁,3-²H₁]aspartic acid 17 (H^B = ²H, H^C = H) (2.66 g, 20 mmol) was dissolved in water (50 cm³) and the pH was adjusted to 7.25 with 35 mol dm⁻³ aq. ammonia. AAT (200 units) and PLP (5 mg) were added and the reaction mixture was incubated at 37 °C. The progress of the C ^{α} -H exchange reaction was followed by removing aliquots for examination by ¹H NMR spectroscopy and after 72 h, when no further reaction was evident, the incubation solution was boiled for 2 min. The denatured protein was removed by filtration and the filtrate was concentrated under reduced pressure. The solid residue was recrystallised from dil. hydrochloric acid to yield deuteriated aspartic acid 9 (H^A = H^C = H, H^B = ²H) as fine crystals (2.09 g, 78%), mp > 300 °C; *m/z* (Found: [M + H]⁺, 135.0518). Calc. for C₄H₇²HNO₄: *m/z*, 135.0514; [α]_D +24.1 (*c* 0.6, 6 mol dm⁻³ HCl); δ_H (200 MHz; ²H₂O, 5% NaO²H) 2.58 (1 H, d, *J* 2.4, C^B-H) and 3.50 (1 H, d, *J* 2.4, C^A-H); δ_c (50.3 MHz; ²H₂O, 5% NaO²H) 45.8 (t, *J* 17, C^B), 56.8 (C^A), 183.2 (β -CO₂H) and 185.3 (α -CO₂H); *m/z* (CI) 135 (100%, [M + H]⁺), 88 (7, [M - CH₂O₂]⁺) and 45 (34, [CHO₂]⁺).

(2S,3R)-[3-²H₁]Aspartic acid 9 (H^A = H^B = H, H^C = ²H) (\equiv 17; H^B = H, H^C = ²H)

This compound was prepared through the enzymic amination of fumaric acid (2 g, 17.2 mmol), as described above, using deuterium oxide as the solvent¹³ to give fine crystals (1.55 g, 67%); mp 290 °C (decomp.) {lit.,¹³ 290 °C (decomp.)}; *m/z* (Found: M⁺, 135.0498. Calc. for C₄H₆²HNO₄: *M*, 135.0516); [α]_D +23.9 (*c* 0.6, 6 mol dm⁻³ HCl) {lit.,¹³ +23.9 (*c* 0.6, 6 mol dm⁻³ HCl) lit.,²⁰ +19.4 (*c* 0.918, 1 mol dm⁻³ HCl)}; δ_c (50.3 MHz; ²H₂O, 5% NaO²H) 45.0 (t, *J* 15, C^B), 56.47 (C^A), 182.79 (β -CO₂H) and 184.10 (α -CO₂H); *m/z* (FAB) 269 (10%, 2 × [M + H]⁺) and 135 (100, [M + H]⁺). All other spectral data were identical with those reported.^{13,20}

(2S)-[2-²H₁]Aspartic acid 9 (H^A = ²H, H^B = H^C = H)

This compound was prepared by exchanging the C ^{α} -hydrogen atom of (2S)-aspartic acid as previously described.¹⁴

1-Isopropyl (2S)-N-(trifluoroacetyl)aspartate 10

TFAA (80 g, 381 mmol) was added dropwise over a period of 30 min to a stirred suspension of (2S)-aspartic acid (6 g, 45.1 mmol) in dry THF (150 cm³) at 0 °C under nitrogen. The reaction mixture was allowed to warm to room temperature during 2 h, when it became homogeneous. The solvent was removed under reduced pressure and the resulting solid was thoroughly dried under high vacuum. Cold, dry propan-2-ol (50 cm³) was added to the cyclic anhydride and the reaction was allowed to warm to room temperature over a period of several hours while being stirred. After 24 h the solvent was removed under reduced pressure to yield an oil which solidified upon storage. Recrystallisation from diethyl ether–light petroleum gave the required α -ester as crystals (11.2 g, 92%), mp 98 °C (Found: C, 39.9; H, 4.65; N, 5.25. C₉H₁₂F₃NO₅ requires C, 39.9; H, 4.45; N, 5.15%); [α]_D -40.7 (*c* 1.0, MeOH); ν_{\max} (Nujol)/cm⁻¹ 3311 (N-H), 1740 (F₃CC=O), 1708 and 1707 (superimposed CO₂Prⁱ and CO₂H), 1280 (C-O) and 1185–1106 (CF₃); δ_H (200 MHz; C²HCl₃) 1.27 (6 H, dd, *J* 4.4 and 17.5, CHMe₂), 3.23 (2 H, ABX, *J*_{AX} = *J*_{BX} = 4.3, *J*_{AB} 17.7, C^B-H₂), 4.77 (1 H, dd, *J* 3.9 and 7.6, C^A-H), 5.11 (1 H, sp, *J* 4.5, Me₂CH) and 7.39 (1 H, d, *J* 7.6, NH); δ_c (50.3 MHz; C²HCl₃) 21.8 and 22.0 (CHMe₂), 35.7 (C^B), 49.4 (C^A), 71.5 (CHMe₂), 116.0 (q, *J* 287, F₃C), 157.6 (q, *J* 37.9, F₃CCO), 169.0 (CO₂Prⁱ) and 175.86

(CO₂H); *m/z* (EI) 272 (1%, [M + H]⁺), 96 (21, F₃CCO⁺) and 43 {100, [(CH₃)₂CHOH]⁺}.

1-Isopropyl (2S,3R)-N-(trifluoroacetyl)-[3-²H₁]aspartate 10 (H^A = H^B = H, H^C = ²H)

This compound was prepared in a manner identical with that for the unlabelled material 10 by using (2S,3R)-[3-²H₁]aspartic acid 9 (H^A = H^B = H, H^C = ²H) (1.0 g, 7.5 mmol) to give the required ester as crystals (1.84 g, 90%), mp 97–98 °C; ν_{\max} (Nujol)/cm⁻¹ 3311 (N-H), 1740 (F₃CC=O), 1708 and 1707 (superimposed CO₂Prⁱ and CO₂H), 1280 (C-O) and 1185–1106 (CF₃); δ_H (200 MHz; C²HCl₃) 1.28 (6 H, t, *J* 6.0, CHMe₂), 3.16 (1 H, d, *J* 4.3, C^B-H), 4.83 (1 H, dd, *J* 3.9 and 6.2, C^A-H), 5.11 (1 H, m, *J* 6.3, CHMe₂), 7.56 (1 H, d, *J* 7.7, NH) and 10.21 (1 H, br s, CO₂H); δ_c (50.3 MHz; C²HCl₃) 21.8 and 22.0 (CHMe₂), 35.7 (t, *J*_{C²H} 6.6, C^B), 49.3 (C^A), 71.4 (CHMe₂), 123.1 (q, *J* 288, F₃C), 158.0 (q, *J* 37.8, F₃CCO), 169.0 (CO₂Prⁱ) and 176.0 (CO₂H); *m/z* (EI) 227 (7%, [M - CO₂H]⁺), 213 {21, [M - OCH(CH₃)₂]⁺}, 140 {45, [M - CO₂CH(CH₃)₂]⁺} and 43 {100, [CH(CH₃)₂]⁺}.

1-Isopropyl (2S,3S)-N-(trifluoroacetyl)-[3-²H₁]aspartate 10 (H^A = H^C = H, H^B = ²H)

This compound was prepared in a manner identical with that for the unlabelled material 10 by using (2S,3S)-[3-²H₁]aspartic acid 9 (H^A = H^C = H, H^B = ²H) (1.50 g, 11.2 mmol) to give the required ester as crystals (2.78 g, 91%), mp 96–98 °C; ν_{\max} (Nujol)/cm⁻¹ 3311 (N-H), 1740 (F₃CC=O), 1708 and 1707 (superimposed CO₂Prⁱ and CO₂H), 1280 (C-O) and 1185–1106 (CF₃); δ_H (200 MHz; C²HCl₃) 1.28 (6 H, t, *J* 6.0, CHMe₂), 2.93 (1 H, d, *J* 4.3, C^B-H), 4.82 (1 H, dd, *J* 3.9 and 7.7, C^A-H), 5.12 (1 H, m, *J* 6.3, CHMe₂), 7.60 (1 H, d, *J* 7.7, NH) and 8.43 (1 H, br s, CO₂H); δ_c (50.3 MHz; C²HCl₃) 21.84 and 21.96 (CHMe₂), 49.40 (t, *J* 5.6, C^B), 71.47 (CHMe₂), 116.0 (q, *J* 287, F₃C), 157.61 (q, *J* 37.9, F₃CCO), 169.04 (CO₂Prⁱ) and 175.86 (CO₂H); *m/z* (EI) 227 (10%, [M - CO₂H]⁺), 213 {25, [M - OCH(CH₃)₂]⁺}, 185 {40, [M - CO₂H - CH(CH₃)₂]⁺}, 167 {22, [M - CO₂CH(CH₃)₂ - OH₂]⁺} and 43 {100, [CH(CH₃)₂]⁺}.

1-Isopropyl (2S)-N-(trifluoroacetyl)-[2-²H₁]aspartate 10 (H^A = ²H, H^B = H^C = H)

This compound was prepared in a manner identical with that for the unlabelled material 10 by using (2S)-[2-²H₁]aspartic acid 9 (H^A = ²H, H^B = H^C = H) (1.15 g, 8.58 mmol) to give the required ester as crystals (2.03 g, 87%), mp 95–87 °C; ν_{\max} (Nujol)/cm⁻¹ 3311 (N-H), 1740 (F₃CC=O), 1708 and 1707 (superimposed CO₂Prⁱ and CO₂H), 1280 (C-O) and 1185–1106 (CF₃); δ_H (200 MHz; C²HCl₃) 1.22 and 1.25 (6 H, d, *J* 5.9, CHMe₂), 3.04 (2 H, q, *J* 18.7, C^B-H₂), 5.07 (1 H, sp, *J* 5.9, CHMe₂), 7.55 (1 H, d, *J* 7.9, NH) and 10.08 (1 H, br s, CO₂H); δ_c (50.3 MHz; C²HCl₃) 21.65 and 21.79 (CHMe₂), 35.6 (t, *J* 9.2, C^A), 49.2 (C^B), 71.5 (CHMe₂), 116 (q, *J* 287, F₃C), 157.6 (q, *J* 38.1, F₃CCO), 169.0 (CO₂Prⁱ) and 175.9 (CO₂H); *m/z* (EI) 227 (5%, [M - CO₂H]⁺), 213 {15, [M - OCH(CH₃)₂]⁺}, 185 {48, [M - CO₂H - CH(CH₃)₂]⁺}, 167 {22, [M - CO₂CH(CH₃)₂ - OH₂]⁺}, 140 {45, [M - CO₂CH(CH₃)₂]⁺} and 43 {100, [CH(CH₃)₂]⁺}.

Isopropyl (2S)-N-(trifluoroacetyl)homoserine 11

1-Isopropyl (2S)-N-(trifluoroacetyl)aspartate 10 (1 g, 3.9 mmol) was dissolved in dry THF (15 cm³), the solution was cooled to -50 °C and *N*-methylmorpholine (NMM) (0.39 g, 3.9 mmol) and isobutyl chloroformate (0.506 g, 3.9 mmol) were added. A precipitate formed immediately and after 5 min the solution was filtered into a stirred solution of NaBH₄ (0.10 g, 2.7 mmol) in THF (10 cm³) at -20 °C. The mixture was stirred for 4 h and then water (10 cm³) was added. The THF was removed under reduced pressure and the aqueous residue was extracted with diethyl ether (3 × 15 cm³). The combined ethereal layers were washed with brine, dried (MgSO₄), and

concentrated under reduced pressure. The residual pale yellow oil was purified by flash chromatography on silica, and eluted with ethyl acetate–light petroleum–diethyl ether (40:40:20) containing a few drops of glacial acetic acid, to yield the alcohol **11** as an oil (0.86 g, 92%) (Found: C, 42.45; H, 5.15; N, 5.45. $C_9H_{14}F_3NO_4$ requires C, 42.05; H, 5.5; N, 5.45%); m/z (Found: $[M - C_3H_7O]^+$, 198.0363. $C_6H_7F_3NO_3$ requires m/z , 198.0378); $[\alpha]_D -48.2$ (c 1.75, Et_2O); ν_{max} (Nujol)/ cm^{-1} 3400–3000 (OH) and 1720 (ester C=O); δ_H (200 MHz; C^2HCl_3) 1.28 (6 H, m, $CHMe_2$), 1.9 (1 H, m, C^B-H^B), 2.17 (1 H, m, C^B-H^A), 4.67 (1 H, dd, J_{XA} 4.32, J_{XB} 7.7, $C^{\alpha}-H$), 5.08 (1 H, sp, J 6.27, $CHMe_2$) and 7.64 (1 H, d, J 2.1, NH); δ_C (50.3 MHz; C^2HCl_3) 20.0 and 20.1 ($CHMe_2$), 34.5 (C^B), 51.6 (C^{α}), 59.2 (C^{γ}) 70.8 ($CHMe_2$), 126.0 (q, J 287, F_3C), 158.0 (q, J 37.9, F_3CCO) and 170.8 (CO_2Pr^+); m/z (CI) (60%, $[M + NH_4]^+$) and (100, $[M - C_3H_7OH]^+$).

Isopropyl (2S,3R)-N-(trifluoroacetyl)-[3- 2H_1]homoserine 11
($H^A = H^B = H$, $H^C = ^2H$)

This compound was prepared in a manner identical with that for the unlabelled material **11** by using 1-isopropyl (2S,3R)-N-(trifluoroacetyl)-[3- 2H_1]aspartate **10** ($H^A = H^B = H$, $H^C = ^2H$) (0.57 g, 2.09 mmol) to give the required alcohol as an oil (0.469 g, 86%); ν_{max} (Nujol)/ cm^{-1} 3400–3000 (OH) and 1720 (ester C=O); δ_H (200 MHz; C^2HCl_3) 1.18 (6 H, m, $CHMe_2$), 1.93 (1 H, m, C^B-H^B), 3.67 (2 H, m, $C^{\gamma}-H_2$), 4.57 (1 H, t, J 7.3, $C^{\alpha}-H$), 4.98 (1 H, sp, J 6.3, $CHMe_2$) and 8.06 (1 H, d, J 6.9, NH); δ_C (50.3 MHz; C^2HCl_3) 22.0 and 22.1 ($CHMe_2$), 33.6 (t, J 4.7, C^B), 51.6 (C^{α}), 59.2 (C^{γ}), 70.7 ($CHMe_2$), 126.0 (q, J 287, F_3C), 158.0 (q, J 37.9, F_3CCO) and 170.6 (CO_2Pr^+); m/z (EI) 259 (45%, $[M + H]^+$), 241 (15, $[M - OH]^+$), 199 {70, $[M - OCH(CH_3)_2]^+$ }, 171 {35, $[M - CO_2CH(CH_3)_2]^+$ }, 141 {60, $[M - CO_2CH(CH_3)_2 - HCOH]^+$ }, 57 {75, $[OCH(CH_3)_2]^+$ } and 43 {100, $[CH(CH_3)_2]^+$ }.

Isopropyl (2S,3S)-N-(trifluoroacetyl)-[3- 2H_1]homoserine 11
($H^A = H^C = H$, $H^B = ^2H$)

This compound was prepared in a manner identical with that for the unlabelled material **11** by using 1-isopropyl (2S,3S)-N-(trifluoroacetyl)-[3- 2H_1]aspartate **10** ($H^A = H^C = H$, $H^B = ^2H$) (0.65 g, 2.34 mmol) to give the required alcohol as an oil (0.462 g, 75%); ν_{max} (Nujol)/ cm^{-1} 3400–3000 (OH) and 1720 (ester C=O); δ_H (200 MHz; C^2HCl_3) 1.18 (6 H, m, $CHMe_2$), 2.17 (1 H, m, C^B-H^A), 3.67 (2 H, m, $C^{\gamma}-H_2$), 4.57 (1 H, dd, J 4.2 and 6.4, $C^{\alpha}-H$), 5.09 (1 H, sp, J 6.3, $CHMe_2$) and 7.71 (1 H, d, J 6.9, NH); δ_C (50.3 MHz; C^2HCl_3) 22.1 and 22.2 ($CHMe_2$), 33.6 (t, J 7.2, C^B), 51.6 (C^{α}), 59.2 (C^{γ}), 70.7 ($CHMe_2$), 126.3 (q, J 287, F_3C), 158.0 (q, J 37.8, F_3CCO) and 171.2 (CO_2Pr^+); m/z (EI) 259 (40%, $[M + H]^+$), 241 (18, $[M - OH]^+$), 199 {60, $[M - OCH(CH_3)_2]^+$ }, 171 {45, $[M - CO_2CH(CH_3)_2]^+$ }, 141 {50, $[M - CO_2CH(CH_3)_2 - HCOH]^+$ }, 57 {80, $[OCH(CH_3)_2]^+$ } and 43 {100, $[CH(CH_3)_2]^+$ }.

Isopropyl (2S)-N-(trifluoroacetyl)-[2- 2H_1]homoserine 11
($H^A = ^2H$, $H^B = H^C = H$)

This compound was prepared in a manner identical with that for the unlabelled material **11** by using 1-isopropyl (2S)-N-(trifluoroacetyl)-[2- 2H_1]aspartate **10** ($H^A = ^2H$, $H^B = H^C = H$) (0.83 g, 3.05 mmol) to give the required alcohol as an oil (0.59 g, 75%); ν_{max} (Nujol)/ cm^{-1} 3400–3000 (OH) and 1720 (ester C=O); δ_H (200 MHz; C^2HCl_3) 1.17 (6 H, m, $CHMe_2$), 1.93 (2 H, m, C^B-H_2), 3.67 (2 H, m, $C^{\gamma}-H_2$), 5.11 (1 H, sp, J 6.3, $CHMe_2$) and 7.75 (1 H, d, J 6.9, NH); δ_C (50.3 MHz; C^2HCl_3) 22.0 and 22.1 ($CHMe_2$), 33.7 (C^B), 51.7 (t, J 4.7, C^{α}), 59.2 (C^{γ}), 70.7 ($CHMe_2$), 125.9 (q, J 287, F_3C), 158.0 (q, J 37.8, F_3CCO) and 170.7 (CO_2Pr^+); m/z (EI) 259 (35%, $[M + H]^+$), 241 (25, $[M - OH]^+$), 199 {65, $[M - OCH(CH_3)_2]^+$ }, 171 {45, $[M - CO_2CH(CH_3)_2]^+$ }, 141 {75, $[M - CO_2CH(CH_3)_2 - HCOH]^+$ }, 57 {60, $[OCH(CH_3)_2]^+$ } and 43 {100, $[CH(CH_3)_2]^+$ }.

(2S)-Homoserine 13

Isopropyl (2S)-N-(trifluoroacetyl) homoserine **11** (1 g, 3.89 mmol) was dissolved in ethanol (20 cm^3) and 1 mol dm^{-3} KOH (20 cm^3) was added slowly dropwise. After 2 h the solution was neutralised (to pH 7) with 1 mol dm^{-3} HCl and the solvents were removed under reduced pressure. The yellow residue was applied to a 20 × 2 cm column of Dowex 50W (H^+ -form) and, after being washed with water (100 cm^3), the column was eluted with 0.1 mol dm^{-3} KOH. The ninhydrin-positive fractions were pooled and were concentrated under reduced pressure. The residue was recrystallised from aq. ethanol to yield (2S)-homoserine as crystals (0.43 g, 95%), mp 202–203 °C (decomp.) [lit.,²³ 203 °C (decomp.)]; $[\alpha]_D +21.2$ (c 0.5, 1 mol dm^{-3} HCl) {lit.,²⁴ +20.4 (c 0.5, 1 mol dm^{-3} HCl)}; ν_{max} (Nujol)/ cm^{-1} 3420 (NH), 3400–2400 (acid OH), 1640 (acid C=O) and 1230 (C–O); δ_H (200 MHz; 2H_2O) 1.62 (2 H, m, C^B-H_2), 3.14 (1 H, dd, $C^{\alpha}-H$) and 3.49 (2 H, t, $C^{\gamma}-H_2$); δ_C (50.3 MHz; 2H_2O) 34.57 (C^B), 56.12 (C^{α}), 61.40 (C^{γ}) and 177.18 (CO_2H).

Isopropyl (2S)-N-(trifluoroacetyl)-phosphohomoserine bis-(2-cyanoethyl) ester 12 (R = CH_2CH_2CN)

By use of a modification of the procedure of Cole *et al.*,²⁵ isopropyl (2S)-N-(trifluoroacetyl)homoserine **11** (100 mg, 0.4 mmol) and 1H-tetrazole (63 mg, 0.9 mmol) were added to THF (3 cm^3) under argon. Bis-(2-cyanoethyl) N,N-diisopropylphosphoramidite²⁶ (136 mg, 0.5 mmol) was then added to the solution and the reaction was followed by TLC [(50:50) ethyl acetate–light petroleum; developing with iodine]. After 1 h the solution was cooled in an ice-bath and MCPBA (114 mg, 0.66 mmol) as a solution in dichloromethane (2 cm^3) was added dropwise. The reaction mixture was stirred for 45 min, was then diluted with diethyl ether (5 cm^3) and was washed successively with 10% aq. Na_2SO_3 , 1 mol dm^{-3} HCl, saturated aq. $NaHCO_3$ and 100 mmol dm^{-3} monopotassium phosphate buffer (pH 7). The organic fraction was dried (Na_2SO_4) and the solvent was removed under reduced pressure to give the crude product **12** as a gum (154 mg, 87%); δ_H (200 MHz; C^2HCl_3) 1.22 (6 H, m, $CHMe_2$), 2.23 (2 H, m, C^B-H_2), 2.78 (4 H, t, J 7.5, H_2CCN), 4.26 (6 H, m, $C^{\gamma}-H_2$ and 2 × $POCH_2$), 4.66 (1 H, dd, J_{XA} 4.3, J_{XB} 7.7, $C^{\alpha}-H$), 5.05 (1 H, sp, J 6.7, $CHMe_2$) and 7.5 (1 H, d, J 2.1, NH); δ_C (50.3 MHz; C^2HCl_3) 20.21 (d, J 7.3, POC), 22.09 ($CHMe_2$), 32.20 (d, J_{PC} 5.3, C^B), 50.27 (C^{α}), 63.10 (t, J 5.1, 2 × CCN), 65.14 (d, J_{PC} 5.8, C^{γ}), 71.02 ($CHMe_2$), 116.15 (q, J_{FC} 290, F_3C), 117.11 (CN), 158.54 (q, J_{CF} 38, F_3CCO) and 170.01 (CO_2Pr^+). This compound was ~90% pure as judged by NMR spectroscopy and was not purified further, but was used in trial hydrolysis reactions to remove the protecting groups; see results and discussion.

Isopropyl (2S)-N-(trifluoroacetyl)-phosphohomoserine dibenzyl ester 12 (R = CH_2Ph)

By use of a modification of the procedure of Cole *et al.*,²⁵ a mixture of isopropyl (2S)-N-(trifluoroacetyl)homoserine **11** (100 mg, 0.4 mmol) and 1H-tetrazole (84 mg, 1.2 mmol) in anhydrous dichloromethane (10 cm^3) was treated with dibenzyl N,N-diisopropylphosphoramidite²⁷ (127 mg, 0.4 mmol). The reaction mixture was stirred at room temperature for 2 h, then was cooled to –40 °C and a solution of MCPBA (138 mg, 0.8 mmol) in dichloromethane (5 cm^3) was added dropwise. The resulting solution was stirred at 0 °C for 45 min, diluted with dichloromethane (40 cm^3), and was washed successively with aq. Na_2SO_3 (10%; 2 × 20 cm^3), aq. $NaHCO_3$ (2 × 15 cm^3), water (15 cm^3) and brine (15 cm^3). The solution was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude product was then purified by flash chromatography on silica with 25% ethyl acetate in light petroleum as the eluent to give the required phosphate triester **12** (R = CH_2Ph) as an oil (190 mg, 92%); m/z (Found: $[M + H]^+$, 518.1556. $C_{23}H_{27}F_3NO_7P$ requires m/z 518.1555); $[\alpha]_D +3.04$ (c 2.7, Et_2O); δ_H (200 MHz; C^2HCl_3) 1.22 (6 H, m, $CHMe_2$), 2.18 (2 H,

m, C^β-H₂), 4.0 (2 H, td, *J*_{HP} 6.3, C^γ-H₂), 4.33 (1 H, dd, *J*_{AX} 6.6, *J*_{BX} 13.5, C^α-H), 5.0 (5 H, m, CHMe₂ and 2 × benzyl CH₂), 7.34 and 7.35 (10 H, ArH) and 7.63 (1 H, d, *J*_{NH,X} 7.6, NH); δ_C(50.3 MHz; C²HCl₃) 22.0 and 22.1 (CHMe₂), 31.8 (d, *J*_{PC} 27, C^β), 50.7 (C^α), 64.1 (d, *J*_{PC} 22, C^γ), 70.0 and 70.1 (benzyl CH₂), 70.8 (CHMe₂), 116 (q, *J*_{CF} 290, F₃C), 128.5, 129.1 and 129.2 (aromatics), 143.2 (benzyl quaternary C), 158.1 (q, *J*_{CF} 38, F₃CCO) and 169.9 (CO₂Prⁱ); δ_P(121.5 MHz; C²HCl₃) -0.55.

Isopropyl (2*S*,3*R*)-*N*-(trifluoroacetyl)-[3-²H₁]phosphohomoserine dibenzyl ester 12 (R = CH₂Ph, H^A = H^B = H, H^C = ²H)
This compound was prepared in a manner identical with that for the unlabelled compound 12 (R = CH₂Ph) by using isopropyl (2*S*,3*R*)-*N*-(trifluoroacetyl)-[3-²H₁]homoserine 11 (H^A = H^B = H, H^C = ²H) (0.50 g, 1.94 mmol), in 85% yield (0.855 g); δ_H(200 MHz; C²HCl₃) 1.24 (6 H, m, CHMe₂), 2.16 (1 H, m, C^β-H), 4.15 (2 H, m, C^γ-H₂), 4.55 (1 H, t, *J* 7.2, C^α-H), 5.0 (5 H, m, CHMe₂ and 2 × benzyl CH₂), 7.33 and 7.36 (10 H, ArH) and 7.94 (1 H, d, *J* 7.7, NH); δ_C(50.3 MHz; C²HCl₃) 22.1 and 22.0 (CHMe₂), 31.5 (t, *J* 4.6, C^β), 50.5 (C^α), 64.2 (d, *J*_{PC} 21, C^γ), 70.0 and 70.1 (benzyl CH₂), 70.7 (CHMe₂), 115.0 (q, *J*_{FC} 288, F₃C), 128.6, 129.1 and 129.2 (aromatics), 142.9 (benzyl quaternary C), 159.0 (q, *J*_{FC} 39, F₃CCO) and 169.8 (CO₂Prⁱ); δ_P(121.5 MHz; C²HCl₃) -0.57.

Isopropyl (2*S*,3*S*)-*N*-(trifluoroacetyl)-[3-²H₁]phosphohomoserine dibenzyl ester 12 (R = CH₂Ph, H^A = H^C = H, H^B = ²H)
This compound was prepared in a manner identical with that for the unlabelled compound 12 (R = CH₂Ph) by using isopropyl (2*S*,3*S*)-*N*-(trifluoroacetyl)-[3-²H₁]homoserine 11 (H^A = H^C = H, H^B = ²H) (0.425 g, 1.65 mmol), in 87% yield (0.742 g); δ_H(200 MHz; C²HCl₃) 1.24 (6 H, m, CHMe₂), 2.16 (1 H, m, C^β-H), 4.15 (2 H, m, C^γ-H₂), 4.55 (1 H, t, *J* 7.2, C^α-H), 5.0 (5 H, m, CHMe₂ and 2 × PhCH₂), 7.33 and 7.36 (10 H, ArH) and 7.94 (1 H, d, *J* 7.7, NH); δ_C(50.3 MHz; C²HCl₃) 22.1 and 22.0 (CHMe₂), 31.5 (t, *J* 4.6, C^β), 50.5 (C^α), 64.2 (d, *J*_{PC} 21, C^γ), 70.0 and 70.1 (PhCH₂), 70.7 (CHMe₂), 115.0 (q, *J*_{FC} 289, F₃C), 128.6, 129.1 and 129.2 (aromatics), 143.0 (benzyl quaternary C), 158.8 (q, *J*_{FC} 39, F₃CCO) and 170.1 (CO₂Prⁱ); δ_P(121.5 MHz; C²HCl₃) -0.53.

Isopropyl (2*S*)-*N*-(trifluoroacetyl)-[2-²H₁]phosphohomoserine dibenzyl ester 12 (R = CH₂Ph, H^A = ²H, H^B = H^C = H)
This compound was prepared in a manner identical with that for the unlabelled compound 12 (R = CH₂Ph) by using isopropyl (2*S*)-*N*-(trifluoroacetyl)-[2-²H₁]homoserine 11 (H^A = ²H, H^B = H^C = H) (0.675 g, 2.6 mmol), in 76% yield (1.030 g); δ_H(200 MHz; C²HCl₃) 1.23 (6 H, m, CHMe₂), 2.16 (1 H, m, C^β-H), 4.04 (2 H, dq, *J* 7.1, 9.3, C^γ-H₂), 5.03 (5 H, m, CHMe₂ and 2 × PhCH₂), 7.32, 7.34 and 7.36 (10 H, ArH) and 7.65 (1 H, d, *J* 6.9, NH); δ_C(50.3 MHz; C²HCl₃) 22.1 and 22.0 (CHMe₂), 31.5 (C^β), 50.5 (t, *J* 4.8, C^α), 64.2 (d, *J*_{PC} 21, C^γ), 70.0 and 70.1 (PhCH₂), 70.7 (CHMe₂), 115.0 (q, *J*_{FC} 288, F₃C), 128.6, 129.1 and 129.2 (aromatics), 142.9 (benzyl quaternary C), 159.0 (q, *J*_{FC} 39, F₃CCO) and 169.8 (CO₂Prⁱ); δ_P(121.5 MHz; C²HCl₃) -0.54.

(2*S*)-*O*-Phosphohomoserine 1

To a stirred solution of isopropyl *N*-(trifluoroacetyl)phosphohomoserine dibenzyl ester 12 (R = CH₂Ph) (500 mg, 10 mmol) in methanol (15 cm³) was added 10% palladium on charcoal (50 mg). The mixture was purged with nitrogen and then hydrogen gas, and hydrogen was bubbled through the suspension for 6 h. The mixture was filtered through a Celite pad, and the filtrate was evaporated to dryness. The residue was dissolved in ethanol (10 cm³) and, with stirring, 1 mol dm⁻³ KOH (10 cm³) was added, when the solution immediately turned yellow. The mixture was stirred for 3 h and the solution was concentrated under reduced pressure. The yellow residue was purified by ion-exchange chromatography (Dowex 50-W 200-400 mesh,

8% cross-linked) with water as eluent and fractions which contained phosphohomoserine, as judged by TLC on cellulose with propan-2-ol-water-conc. aq. ammonia (26:6:5) and development with ninhydrin were collected. The pooled product-containing fractions were lyophilised to yield (2*S*)-*O*-phosphohomoserine 1 as a solid (155 mg, 80%), mp 170 °C; *m/z* (Found: [M + H]⁺, 200.0328. Calc. for C₄H₁₁NO₆P: *m/z*, 200.0324); [α]_D +4.19 (c 2.4, water) {lit.⁹ [α]_D +4.21 (c 2.4, water)}; δ_H(200 MHz; ²H₂O) 2.23 (2 H, m, C^β-H₂), 4.01 (2 H, t, *J*_{AB,X} 11.4, d, *J*_{AB,P} 5.49, C^γ-H₂) and 4.12 (1 H, dd, *J*_{AX} 12.4, *J*_{BX} 4.9, C^α-H); δ_C(50.3 MHz; ²H₂O) 38.7 (d, *J*_{PC} 7.4, C^β), 56.3 (C^α), 64.3 (d, *J*_{PC} 4.9, C^γ) and 186.2 (CO₂H); δ_P(121.5 MHz; ²H₂O) 0.245; *m/z* (FAB) 200 (20%, [M + H]⁺).

(2*S*,3*R*)-[3-²H₁]-*O*-Phosphohomoserine 1 (H^A = H^B = H, H^C = ²H)

This compound was prepared in a manner identical with that for compound 1, by using isopropyl (2*S*,3*R*)-*N*-(trifluoroacetyl)-[3-²H₁]phosphohomoserine dibenzyl ester 12 (R = CH₂Ph, H^A = H^B = H, H^C = ²H) (0.785 g, 1.5 mmol) as the starting material, in 83% yield (0.250 g), mp 168-169 °C; δ_H(200 MHz; ²H₂O) 2.03 (1 H, m, C^β-H) and 4.0 (3 H, m, C^α-H and C^γ-H₂); δ_C(50.3 MHz; ²H₂O) 34.3 (t, *J*_{C²H} 4.7, C^β), 55.3 (C^α), 65.0 (d, *J*_{PC} 5.0, C^γ) and 181.3 (CO₂H); δ_P(121.5 MHz; ²H₂O) 0.251.

(2*S*,3*S*)-[3-²H₁]-*O*-Phosphohomoserine 1 (H^A = H^C = H, H^B = ²H)

This compound was prepared in a manner identical with that for compound 1, by using isopropyl (2*S*,3*S*)-*N*-(trifluoroacetyl)-[3-²H₁]phosphohomoserine dibenzyl ester 12 (R = CH₂Ph, H^A = H^C = H, H^B = ²H) (0.685 g, 0.13 mmol) as the starting material, in 81% yield (0.213 g), mp 167-169 °C; δ_H(200 MHz; ²H₂O) 2.34 (1 H, m, C^β-H), 4.06 (2 H, m, C^γ-H₂) and 4.16 (1 H, t, *J* 4.5, C^α-H); δ_C(50.3 MHz; ²H₂O) 34.2 (t, *J*_{C²H} 4.6, C^β), 54.9 (C^α), 64.8 (d, *J*_{PC} 5.0, C^γ) and 182.4 (CO₂H); δ_P(121.5 MHz; ²H₂O) 0.240.

(2*S*)-[2-²H₁]-*O*-Phosphohomoserine 1 (H^A = ²H, H^B = H^C = H)

This compound was prepared in a manner identical with that for the unlabelled compound 1, by using isopropyl (2*S*)-*N*-(trifluoroacetyl)-[2-²H₁]phosphohomoserine dibenzyl ester 12 (R = CH₂Ph, H^A = ²H, H^B = H^C = H) (0.855 g, 1.6 mmol) as the starting material, in 78% yield (0.257 g), mp 168-169 °C; δ_H(200 MHz; ²H₂O) 2.34 (2 H, m, C^β-H₂) and 4.06 (2 H, m, C^γ-H₂); δ_C(50.3 MHz; ²H₂O) 34.2 (C^β), 54.9 (t, *J* 4.8, C^α), 64.8 (d, *J*_{PC} 5.0, C^γ) and 182.4 (CO₂H); δ_P(121.5 MHz; ²H₂O) 0.239.

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