Synthesis of L-*O*-Phosphohomoserine and its C-3 Chirally Deuteriated Isotopomers: Probes for the Pyridoxal Phosphate Dependent Threonine Synthase Reaction

Fiona Barclay,[a] Ewan Chrystal[b] and David Gani*[a]

[a] *School of Chemistry, The Purdie Building, The University, St. Andrews, Fife, UK KY16 9ST*
[b] *Zeneca Agrochemicals, Jealott's Hill, Bracknell, Berkshire, UK RG12 6EY*

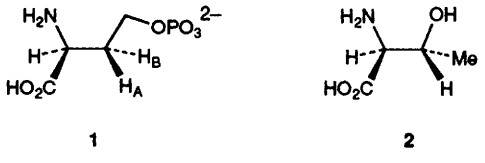
A short efficient synthesis of the threonine synthase substrate L-*O*-phosphohomoserine and its C-3 chirally deuteriated isotopomers is described; the route also provides access to L-homoserine and its C-3 chirally deuteriated isotopomers in high yield.

Pyridoxal 5'-phosphate (PLP) dependent threonine synthase catalyses the conversion of L-*O*-phosphohomoserine **1** to L-threonine **2** and inorganic phosphate in the final step of threonine biosynthesis in bacteria and plants.[1] 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.[2]

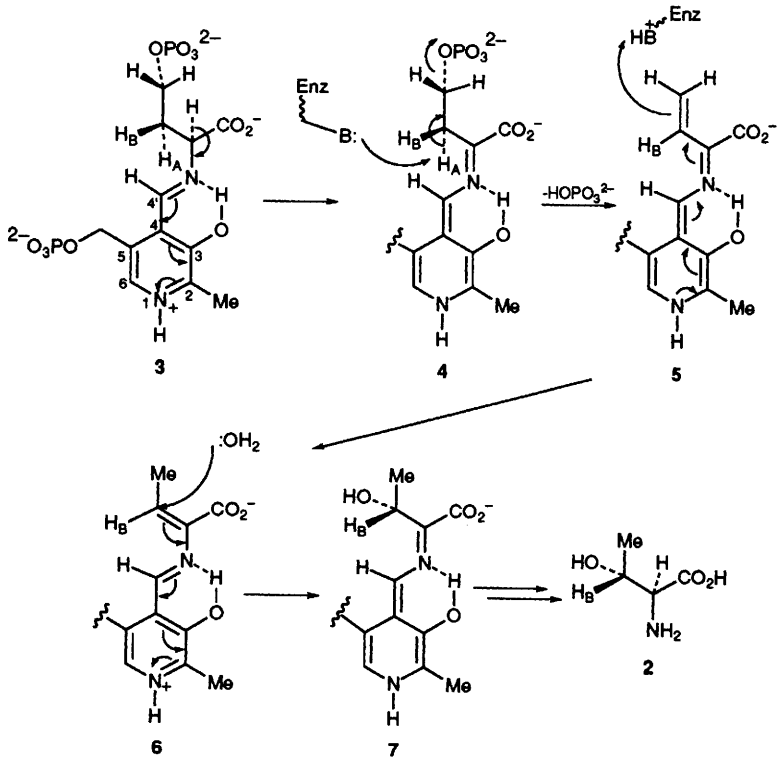
Although threonine synthase from several sources has now been sequenced,[3] details of the mechanism of the reaction remain sparse. By analogy to the mode of action of other PLP dependent systems, however, it is believed that the conversion of the external aldimine **3** to the quinonoid intermediate **4** increases the acidity of the 3-*pro*-S proton of the substrate[4] such that the β,γ-elimination of phosphoric acid can occur. 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,[5] the elimination would occur in a *syn*-fashion to give the conjugated enamine **5** as shown in Scheme 1. Protonation at C-4 of the imino acid moiety in **5** would give enamine **6** and allow attack by water at C-3 on the 4'-*si*-face of the coenzyme to give product quinonoid **7**. Protonation at C-2 would then furnish the product aldimine which, upon transaldimination, would yield (2*S*, 3*R*)-threonine **2**.

In order to examine the kinetics and mechanism of the reaction including the determination of the size of the primary deuterium isotope effect for the elimination process and effect of product inhibition, quantities of pure (2*S*)-*O*-phosphonomoserine and its C-3 chirally deuteriated 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,[6] or; (*ii*) the enzymic phosphorylation of homoserine with ATP and yeast homoserine kinase,[7] or (*iii*) the phosphorylation of *N*-benzyloxycarbonylhomoserine α-*p*-nitrobenzyl ester with diphenylphosphoryl chloride[8]] 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 **8** was converted to the *N*-trifluoroacetylaspartic anhydride (TFAA) through treatment with trifluoroacetic anhydride in THF, Scheme 2. The selective protection of the α-carboxy group through treatment of the anhydride of methanol was reported to yield 75–80% of the desired α-methyl ester β-acid.[9] Performing the reaction at lower temperatures did not improve the ratio in favour of the



1 **2**



Scheme 1

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 **9**, as judged by examination of the ^{1}H NMR spectrum of the crude material, which could be obtained in 92% yield after one recrystallisation {mp 89 °C, $[\alpha]_D$ −40.7 (*c* 1.0, MeOH)}.

A range of methods and conditions for reducing the β-carboxy group to the corresponding alcohol were examined. Finally, under optimised conditions, the alcohol **10** was prepared by adding the mixed β-aspartic isobutylcarbonic anhydride derived from compound **9** to a solution of sodium borohydride in THF. The desired *N*-trifluoroacetylhomoserine ester **10** was obtained as an oil in 93% yield {$[\alpha]_D$ −48.2 (*c* 1.75, Et_2O)} and showed the expected spectral and analytical data. Base-catalysed hydrolysis of the trifluoroacetyl and isopropyl protecting groups in aqueous ethanol afforded (2*S*)-homoserine, identical in all respects to an authentic sample {$[\alpha]_D$ −24.4 (*c* 10, water), lit.[10] $[\alpha]_D$ −24.5 (*c* 10, water)}, in 95% yield (85% from aspartic acid). This compares favourably with other syntheses starting from aspartic acid.[11] The C-3 chirally deuteriated isotopomers of (2*S*)-homoserine were prepared in similar manner and in similar yield, starting from the appropriately labelled aspartic acid,[12] *vide infra*.

In order to convert **10** into its phosphate ester derivative, a range of phosphorylating reagents were considered. Phosphate esters are labile under acidic conditions and, hence, we opted to use base-labile cyanoethyl phosphate protecting groups which should cleave *via* β-elimination.

Accordingly, the alcohol **10** was treated with *N,N*-diisopropyl biscyanoethyl phosphoramidite to give the phosphite triester which was oxidised *in situ* using *m*-CPBA in dichloromethane (DCM). The resulting phosphate triester (**11**, R = CH_2CH_2CN) was obtained in good yield (87%) but proved difficult to purify. All attempts to deprotect the crude material resulted in substantial dephosphorylation although (2*S*)-phosphohomoserine could be purified from the hydrosylate by ion-exchange chromatography in *ca.* 10% yield.

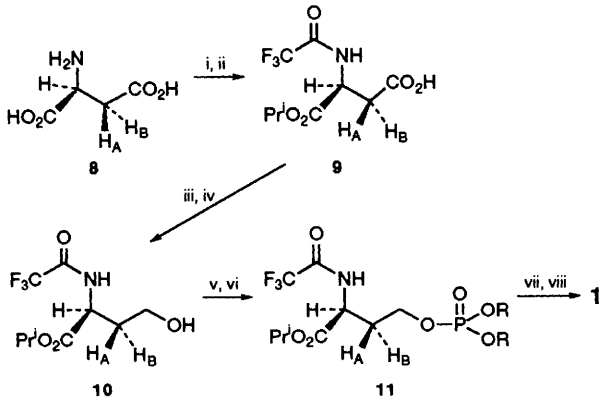
Treatment of **10** with *N,N*-diisopropyl dibenzyl phosphoramidite followed by oxidation of the resulting dibenzyl phosphite with *m*-CPBA, as described above, gave the phosphate triester (**11**, R = CH_2Ph)† in excellent yield (92%). The phosphate triester was then converted smoothly in two steps to (2*S*)-*O*-phosphohomoserine **1**, *via* catalytic hydrogenolysis of the *O*-benzyl 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 desalting on Dowex 50W and showed the expected spectral and analytical data.‡

In order to prepare the chirally deuteriated enantiomers (**1**, H_A = H, H_B = ^{2}H and H_A = ^{2}H, H_B = H), (2*S*,3*R*)- and (2*S*,3*S*)-[3-$^2H_1$]aspartic acid[12] were prepared from the appropriate fumaric acid[9] as described previously and were taken through the steps outlined in Scheme 2. Each of the C-3 chirally deuteriated (2*S*)-*O*-phosphohomoserines was obtained in *ca.* 60% overall yield from aspartic acid and showed the expected spectral omissions for the C-3 hydrogen atoms in their ^{1}H NMR spectra.

The synthetic (2*S*)-*O*-phosphohomoserines were successfully converted to threonine and inorganic phosphate by threonine synthase from *Escherichia coli* strain Tir 8[3] and the compounds are, therefore, suitable for use as mechanistic probes for the enzyme. The availability of this previously elusive natural product from aspartic acid will also facilitate studies of its metabolism to ketobutyrate.

We thank the SERC and Zeneca Agrochemicals for the CASE Studentship to F. B. and Stewart Askew, Janet Rose and Basil Hartzoulakis for preliminary experiments. We also thank Dr T. Lewis (Zeneca) for useful discussions and Dr C. Parsot (Institute Pasteur, Paris) for providing the *E. coli* strain Tir 8 for the preparation of the enzyme.

Received, 13th December 1993; Com. 3/07337H



Scheme 2 *Reagents and conditions*: i, TFAA, THF, 0 °C, 3 h; ii, Pr^iOH, 0 °C, 48 h; iii, NMM, Bu^iOCOCl, THF, −40 °C, 15 min; iv, $NaBH_4$, THF, −40 °C, 4 h; v, $Pr^i_2NP(OBn)_2$, 1-*H* tetrazole, CH_2Cl_2, 20 °C, 45 min; vi, *m*-CPBA, DCM, 0 °C; vii, Pd/C, H_2, MeOH, 20 °C, 24 h; viii, KOH, EtOH, 20 °C, 3 h; Dowex 50 (H^+); NMM = *N*-methylmorpholine

Footnotes

† *Selected spectral data* for **11**: *m/z* (Found: $[M + H]^+$ 518.1556. $C_{23}H_{28}F_3NO_7P$ requires 518.1555); $[\alpha]_D$ +3.04 (*c* 2.7, Et_2O); δ_H (200 MHz, C^2HCl_3) 7.63 (1H, d, *J* 7.6 Hz, NH), 7.35 and 7.34 (10H, s, 2 × *Ph*CH_2), 5.0 (5H, m, Me_2CH, 2 × PhCH_2), 4.33 (1H, dd, J_{AX} 6.6, J_{BX} 13.5 Hz, 2-H), 4.0 (2H, m, 4-H_2), 2.18 (2H, m, 3-H_2) and 1.22 (6H, m, Me_2CH); δ_C (50.3 MHz, C^2HCl_3) 169.9 (CO_2Pr^i), 129.2, 129.1 and 128.5 (2 × *Ph*CH), 70.8 (Me_2CH), 70.1 and 70.0 ($PhCH_2$), 64.1 (d, J_{PC} 22.3 Hz, 4-C), 50.7 (2-C), 31.8 (d, J_{PC} 27.5 Hz, 3-C), 22.1 and 22.0 (Me_2CH); δ_P −0.55 (121.5 MHz, C^2HCl_3).

‡ *Selected spectra data* for **1**: *m/z* (Found: $[M + H]^+$ 200.0329. $C_4H_{11}NO_6P$ requires 200.0324); mp 170 °C; δ_H (200 MHz, ^2H_2O) 4.12 (1 H, dd, J_{AX} 12.4, J_{BX} 4.9 Hz, 2-H), 4.01 (2H, dt, J_{HP} 5.49, *J* 5.7 Hz, d, 4-H_2) and 2.23 (2 H, m., 3-H_2); δ_C (50.3 MHz, ^2H_2O) 186.2 (CO_2H), 64.3 (d, J_{PC} 4.9 Hz, 4-C), 56.3 (2-C) and 38.7 (d, J_{PC} 7.4 Hz, 3-C); δ_P (121.5 MHz, ^2H_2O) 0.5; $[\alpha]_D$ + 4.19 (*c* 2.4, H_2O), {lit.[10] $[\alpha]_D$ 4.21 (*c* 2.4, H_2O)}.

References

1 S. E. Rognes, in *Methods in Plant Biochemistry*, Academic Press, 1990, vol. 3, ch. 19, p. 315.
2 S. L. Shames, D. E. Ash, F. C. Wedler and J. J. Villafranca, *J. Biol. Chem.*, 1984, **259**, 15331.
3 C. Parsot, P. Cossart, I. Saint-Girons and G. N. Cohen, *Nucleic. Acid. Res.*, 1983, **11**, 7331.
4 C. Fuganti, *J. Chem. Soc., Chem. Commun.*, 1979, 337.
5 K. Tilley, M. Akhtar and D. Gani, *J. Chem. Soc., Chem. Commun.*, 1992, 68; N. R. Thomas, J. E. Rose and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 1993, 2933.
6 M. T. Skarstedt and S. B. Greer, *J. Biol. Chem.*, 1973, **248**, 1032.
7 M. Flavin and C. Slaughter, *J. Biol. Chem.*, 1960, **235**, 1103.
8 J. Schnyder and M. Rottenberg, *Helv. Chim. Acta*, 1975, **58**, 518.
9 D. Gani and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2393.
10 K. Ramalingam and R. W. Woodard, *J. Org. Chem.*, 1988, **53**, 1900.
11 B. Gong and D. G. Lynn, *J. Org. Chem.*, 1990, **55**, 4763.
12 M. Akhtar, N. P. Botting, M. Cohen and D. Gani, *Tetrahedron*, 1987, **43**, 5899