Synthesis of the Trifluoroacetate Salt of Aspartic Acid β-Semialdehyde, an Intermediate in the Biosynthesis of L-Lysine, L-Threonine, and L-Methionine

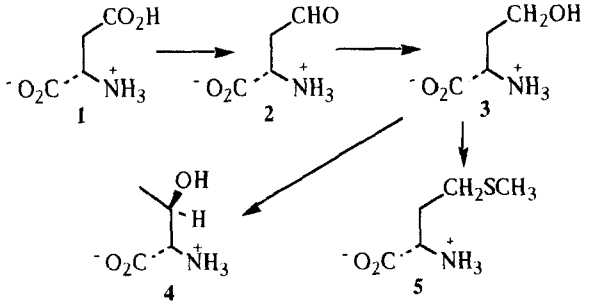
David W. Tudor,[a] Terence Lewis,[b] David J. Robins*[a]

[a] Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland
[b] I.C.I. Agrochemicals (Zeneca Ltd.), Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, England

Received 10 May 1993; revised 7 June 1993

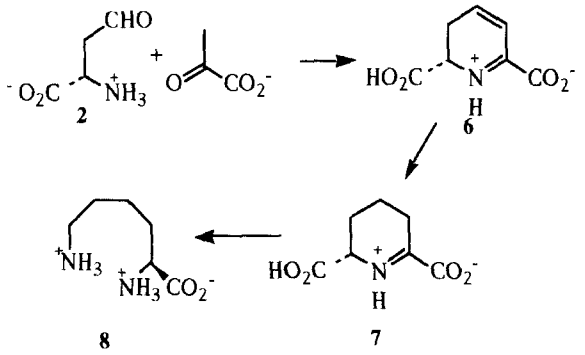
The trifluoroacetate salt of aspartic acid β-semialdehyde, an important intermediate in the biosynthesis of L-lysine, L-threonine, and L-methionine has been prepared in DL-, L-, and D-forms as stable solid hydrates **13** in 43% overall yield by ozonolysis of doubly protected allylglycine, followed by careful deprotection with trifluoroacetic acid.

Aspartic acid β-semialdehyde **(2)** is derived biosynthetically from L-aspartic acid **(1)** by formation of L-aspartic acid β-phosphate catalysed by aspartate kinase,[1] followed by reduction to **2** in a pyridine nucleotide-linked reaction.[2] L-Aspartic acid β-semialdehyde **(2)** is involved in the biosynthesis of several important amino acids.[3] Thus, the biosynthesis of L-threonine **(4)** and L-methionine **(5)** proceeds via homoserine **(3)** after reduction of **2** with homoserine dehydrogenase (Scheme 1).[4] Furthermore, in the diaminopimelate (DAP) pathway to L-lysine **(8)** which occurs in bacteria and higher plants (Scheme 2)[5] the most intriguing step is the condensation of L-aspartic acid β-semialdehyde **(2)** with pyruvate catalysed by dihydrodipicolinic acid (DHDPA) synthase to give L-2,3-dihydrodipicolinic acid **(6)**. A reductase then acts on **6** to generate L-2,3,4,5-tetrahydrodipicolinic acid (THDPA) **(7)**. Further enzyme-catalysed reactions proceeding from THDPA **(7)** lead to L-lysine **(8)**. We recently reported the preparation of THDPA **(7)** as a potassium salt which can be isolated, characterized and stored as a stable solid.[6] Production of aspartic acid β-semialdehyde is usually carried out by the ozonolysis of DL-allylglycine **(9)** in 1 M HCl at 0 °C.[4] Aspartic acid β-semialdehyde produced in this way has a variable purity, which can be estimated by conversion of aspartic acid β-semialdehyde into homoserine **(3)** using homoserine dehydrogenase,[2,7] and **2** is only stable if kept cold in acid solution. We now report the synthesis of D, L, and DL-aspartic acid β-semialdehyde in convenient stable solid forms as their trifluoroacetate salts.



Scheme 1

The ozonolysis of DL-allylglycine **(9)** in acid solution at 0 °C resulted in a complex mixture of products including aspartic acid β-semialdehyde, aspartic acid, and formic acid (NMR data). Our attempts to purify this solution

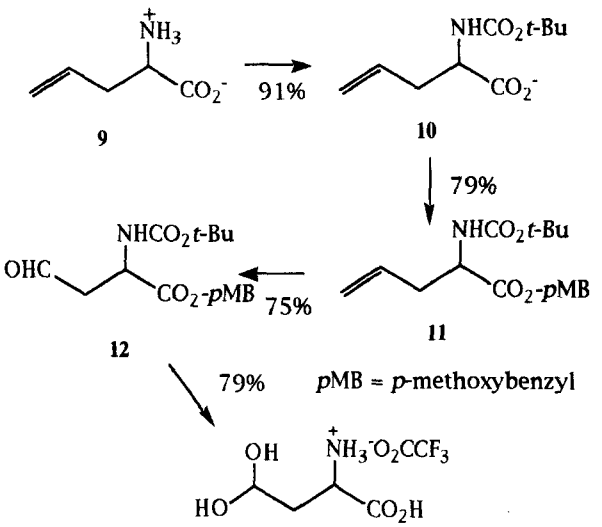


Scheme 2

containing aspartic acid β-semialdehyde **(2)** using ion exchange chromatography were unsuccessful, largely due to the instability of the aspartic acid β-semialdehyde. We decided to investigate other methods for the formation of aspartic acid β-semialdehyde. Previously, Baldwin and Flinn prepared aspartic acid β-semialdehyde doubly protected on the amino and carboxy groups from L-methionine via L-homoserine in their route to more complex amino acids but did not carry out a deprotection step.[8] A synthesis of methyl *N*-acetylaspartate β-semialdehyde has been reported by Turner and Whitesides.[9] We decided to prepare doubly protected aspartic acid β-semialdehyde by ozonolysis of doubly protected allylglycine.

DL-Allylglycine was converted into the *N*-*tert*-butoxycarbonyl derivative **10** using di-*tert*-butyl dicarbonate under basic conditions (Scheme 3). The carboxyl group was protected as the *p*-methoxybenzyl ester using *p*-methoxybenzyl chloride in DMF. The doubly protected material **11** was subjected to ozonolysis and the ozonide was decomposed with triethylamine. Purification by flash column chromatography afforded the aldehyde **12**. Finally the protecting groups were removed by treatment of **12** with trifluoroacetic acid in CH_2Cl_2 under nitrogen for two hours at room temperature and the product **13** was isolated in 43% overall yield from allylglycine **(9)**. The ^1H and ^{13}C NMR data indicated that the product exists as a hydrate, i.e. no aldehyde proton was observed and there was a signal at δ 5.1 for the 4-H and at δ 89.4 for C-4. This material is stable for several months if kept dry at 0 °C. The procedure was repeated for D- and L-allylglycine. The L-isomer of **13** had $[\alpha]_D^{16}$ + 3.33 °C and mp 63–64 °C, whereas the D-isomer had $[\alpha]_D^{14}$ − 3.15 °C and mp 63–65 °C. The CD spectra of the D- and L-isomers were mirror images.

Finally the trifluoroacetate salts of DL- and L-aspartic acid β-semialdehyde were shown to be substrates for homoserine dehydrogenase (isolated and partially purified using the method of Bachi and Cohen[7]) by incubation



Scheme 3

with the enzyme and observation of the disappearance of NADPH. These salts were also shown to act as substrates for the DHDPA synthase reaction. In order to carry out enzyme assays on DHDPA synthase, aspartic acid β-semialdehyde is condensed with pyruvate and the product **6** oxidises spontaneously in air to afford dipicolinic acid which is estimated spectrophometrically.[10] The D-isomers did not act as substrates for either enzymic reaction. The spectroscopic and enzymic data are convincing evidence for the formation of the trifluoroacetate salt of aspartic acid β-semialdehyde. This compound is now available as a substrate for the study of these two enzyme reactions.

Potassium Salt of DL-*N*-*tert*-Butoxycarbonylallylglycine (10):
To a solution of DL-allylglycine (1 g, 8.69 mmol) in water (25 mL) was added dioxane (12 mL), $KHCO_3$ (957 mg, 1.1 equiv) and di-*tert*-butyl dicarbonate (2 mL, 1 equiv.) with continuous stirring at r. t. for 18 h. The solvents were removed with EtOH in vacuo to give a white solid, 2.0 g (91% yield).

IR (KBr disc): ν = 3360, 2980, 1675, 1595, 1530 cm^{-1}.

^1H NMR (200 MHz, D_2O): δ = 1.26 (9 H, s, 3 × Me), 2.25 (2 H, m, 3-H_2), 2.78 (1 H, m, 2-H), 4.97 (2 H, m, 5-H_2), 5.60 (1 H, m, 4-H).

^{13}C NMR (50 MHz, D_2O): δ = 28.6 (3 × Me), 37.3 (C-3), 56.5 (C-2), 81.9 (C–O), 118.9 (C-5), 134.9 (C-4), 166.5 (CONH), 180.2 (C-1).

MS: *m/z* (%) = 214 (M^+, 0.3%), 112,59 (100%), 41.

DL-*N*-*tert*-Butoxycarbonylallylglycine *p*-Methoxybenzyl Ester (11):
To a solution of **10** (1.50 g, 5.92 mmol) in DMF (10 mL) was added *p*-methoxybenzyl chloride (0.9 mL, 1.04 equiv) with continuous stirring for 48 h. DMF was removed with xylene in vacuo and the resultant residue was partitioned between CH_2Cl_2 (20 mL) and aq Na_2CO_3 solution (20 mL). The organic portion was washed with water (2 × 10 mL), dried ($MgSO_4$) and the solvent was removed in vacuo to give a yellow oil. Purification was achieved on a silica gel column eluting with 60% Et_2O in hexane to give a clear oil, 1.56 g (79% yield), R_F 0.37 (50% Et_2O/hexane).

IR ($CHCl_3$): ν = 3440, 3020, 2980, 1715, 1620, 1515, 1500 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ = 1.43 (9 H, s, 3 × Me), 2.51 (2H, dd, 3-H_2), 3.80 (3 H, s, MeO), 4.39 (1 H, m, 2-H), 5.10 (5 H, m, 5-H_2, CH_2Ar and NH), 5.66 (1 H, m, 4-H), 6.89, 7.29 (4 H, almost A_2B_2 system, J = 8 Hz).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.3 (3 × Me), 36.8 (C-3), 53.0 (C-2), 55.3 (MeO), 66.9 (CH_2Ar), 79.8 (C–O), 113.8 (Ar s), 113.9 (2 × Ar d), 119.1 (C-5), 127.5 (Ar d), 130.2 (2 × Ar d), 132.2 (C-4), 159.8 (CONH), 171.9 (C-1).

MS: *m/z* (%) = 335 (M^+, 1.0%), 279, 170, 121 (100%), 70, 57.

HRMS: Found: M^+, 335.1718; $C_{18}H_{25}NO_5$ requires M, 335.1732.

DL-*N*-*tert*-Butoxycarbonylaspartic Acid β-Semialdehyde *p*-Methoxybenzyl Ester (12):
A solution of **11** (1.20 g, 3.58 mmol) in CH_2Cl_2 was ozonized at −78 °C and excess ozone was removed with N_2. The ozonide was decomposed with Et_3N (2 equiv) at −78 °C, and then the mixture was stirred at r. t. for 5 h. CH_2Cl_2 was removed in vacuo and Et_2O (25 mL) was added to precipitate triethylamine *N*-oxide. Purification was achieved on a silica gel column eluting with Et_2O to give a clear oil, 900 mg (75% yield), R_F 0.36 (Et_2O).

IR ($CHCl_3$): ν = 3440, 3030, 2980, 1715, 1620, 1515 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ = 1.42 (3 × Me), 3.03 (2 H, t, 3-H_2), 3.80 (3 H, s, MeO), 4.58 (1 H, m, 2-H), 5.10 (2 H, s, CH_2Ar), 5.42 (1 H, br d, NH), 6.89, 7.26 (4 H, almost A_2B_2 system, J = 8 Hz), 9.69 (1 H, s, 4-H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.2 (3 × Me), 46.0 (C-3), 48.8 (C-2), 55.3 (MeO), 67.5 (C-5), 80.2 (C–O), 114.0 (2 × Ar d), 115.1 (Ar s), 128.6 (Ar s), 130.2 (2 × Ar d), 159.8 (CONH), 171.1 (C-1), 199.4 (C-4).

MS: *m/z* (%) = 337 (M^+, 0.7%), 281, 202, 137, 121 (100%), 72, 57.

HRMS Found: M^+, 337.1538; $C_{17}H_{23}NO_6$ requires M, 337.1525.

DL-Aspartic Acid β-Semialdehyde Hydrate Trifluoroacetate (13):
A solution of **12** (103 mg, 0.30 mmol) in trifluoroacetic acid (2 mL) and CH_2Cl_2 (2 mL) was stirred under N_2 at r. t. for 2 h. The solvent was removed in vacuo to give an oily residue. This was partitioned between water (10 mL) and EtOAc (10 mL). The aqueous layer was separated and washed with EtOAc (2 × 10 mL). Removal of the water in vacuo gave a yellow solid, 60 mg (79% yield).

IR (KBr disc): ν = 3420, 2925, 1675, 1630, 1400 cm^{-1}.

^1H NMR (200 MHz, D_2O): δ = 2.01 (2 H, m, 3-H_2), 3.80 (1 H, dd, 2-H), 5.18 (1 H, m, 4-H).

^{13}C NMR (50 MHz, D_2O): δ = 37.8 (C-3), 51.8 (C-2), 89.7 (C-4), 117 (CF_3C), 161 (CCF_3), 174.2 (C-1).

MS: *m/z* (%) = 137 (MH^+, 17.5%), 119, 107, 93, 86, 69, 44 (100%).

HRMS Found: MH^+, 137.0442; $C_4H_{11}NO_4$ requires M, 137.0431.

The L-isomer had $[\alpha]_D^{16}$ + 3.33° (*c* = 1.5, H_2O), mp 63–64 °C; CD $\Delta\varepsilon_{202}$ + 1.0 (*c* = 0.05, H_2O).

The D-isomer had $[\alpha]_D^{14}$ − 3.15° (*c* = 1.5, H_2O), mp 63–65 °C; CD $\Delta\varepsilon_{202}$ − 1.0 (*c* = 0.086, H_2O).

We are grateful to Emma Borthwick, Susanne Connell and Professor J. R. Coggins for assistance with carrying out the enzymic experiments. Thanks are due to Dr. A. F. Drake, Birkbeck College, University of London, for running the CD spectra. We thank Dr. E. J. T. Chrystal, ICI, for useful discussions and ICI Agrochemicals for financial support.

(1) Black, S.; Wright, N. G. *J. Biol. Chem.* **1954**, *213*, 27.
(2) Black, S.; Wright, N. G. *J. Biol. Chem.* **1954**, *13*, 39.
(3) Goodwin, T. W.; Mercer, E. I. *Introduction to Plant Biochemistry*; 2nd Edn., Pergamon: 1983.
(4) Black, S.; Wright, N. G. *J. Biol. Chem.* **1954**, *213*, 51.
(5) Patte, J.-C. In *Amino Acids Biosynthesis and Genetic Regulation*; Herrmann, K. M.; Somerville, R. L. Eds.; Addison-Wesley: Reading, Mass., 1983; Chap. 12.
(6) Couper, L.; Robins, D. J.; Chrystal, E. J. T. *Tetrahedron Lett.* **1992**, *33*, 2717.
(7) Bachi, P. T.; Cohen, C. N. *Methods Enzymol.* **1969**, *17A*, 694.
(8) Baldwin, J. E.; Flinn, A. *Tetrahedron Lett.* **1987**, *28*, 3605.
(9) Turner, N. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 624.
(10) Borthwick, E. B.; Coggins, J. R.; Couper, L.; Tudor, D. W.; Robins, D. J.; Shneier, A.; Abell, C. *Biochem. J.*, submitted for publication.