Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 21, pp. 2613-2616, 1996

0960-894X/96 $15.00 + 0.00

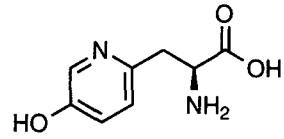
PII: S0960-894X(96)00480-5

A CONCISE SYNTHESIS OF EITHER ENANTIOMER OF AZATYROSINE

Margaret S. Cooper, Alison W. Seton, Malcolm F. G. Stevens* and Andrew D. Westwell
Cancer Research Laboratories, Department of Pharmaceutical Sciences,
University of Nottingham, Nottingham, NG7 2RD, U.K.

Abstract: A facile route to either enantiomer of azatyrosine is reported, utilising an efficient enzymic resolution of protected azatyrosine ethyl ester by α-chymotrypsin as a key step.

The antibiotic L-azatyrosine (**1**), first isolated in optically pure form from *Streptomyces chibanesis*,[1] has in recent years been found to possess important antitumour properties. For example, Shindo-Okada and co-workers[2] reported that L-azatyrosine induces permanent reversion of activated c-Ha-*ras*-transformed NIH3T3 cells to the apparently normal phenotype, without significantly affecting the growth of cells possessing normal *ras* genes. *Ras* proteins are important components of cellular signalling pathways, and in their mutated form play a major role in the cell proliferation and differentiation associated with human carcinogenesis.[3] Further *in vivo* studies[4] have shown that L-azatyrosine has a dramatic effect on reducing the size and incidence of skin and forestomach papillomas in transgenic mice harbouring human oncogenic *ras* .

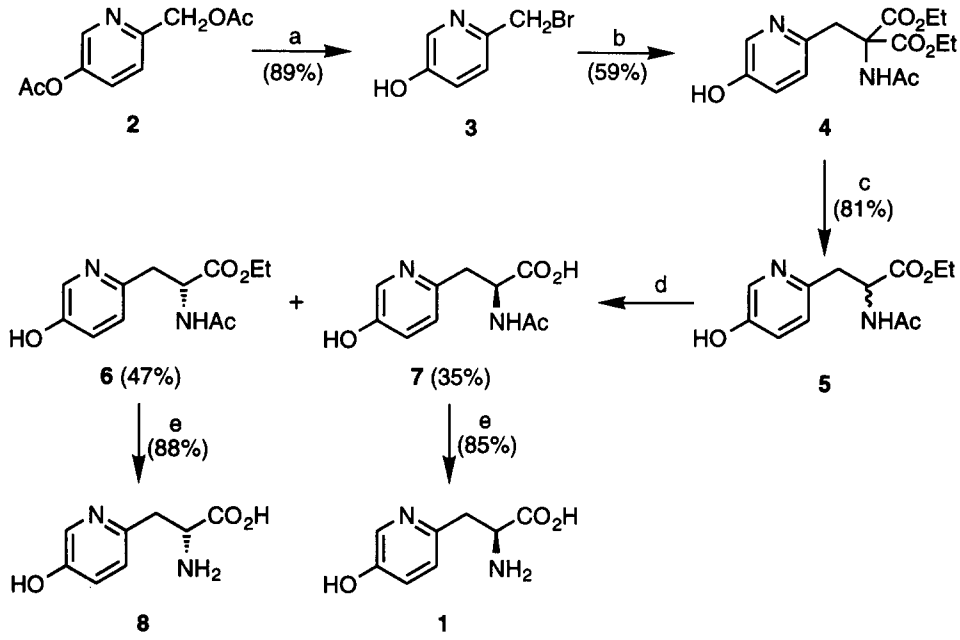


1

In view of the limited availabilty of L-azatyrosine and its intriguing biological activity and potential as a lead compound in anticancer research, the requirement for good synthetic routes to (**1**) is apparent. Three recent syntheses of L-azatyrosine have been reported,[5,6,7] prompting us to report our own synthetic route to either enantiomer of azatyrosine in a concise and reliable procedure, making use of a novel enzymatic resolution of protected azatyrosine ethyl ester by α-chymotrypsin as a key step.

The method employed is as indicated in the scheme. Conversion of 2-acetoxymethyl-5-acetoxypyridine (**2**)[8] to 2-bromomethyl-5-hydroxypyridine (**3**), as the hydrobromide salt, was achieved by treatment with 45%HBr in acetic acid. Bromide (**3**) proved difficult to crystallise but could be used in its crude form in the next step of the synthesis, in which reaction of (**3**) with diethyl acetamidomalonate anion in ethanol, utilising Sorenson methodolgy[9] gave ready access to diethyl 2-acetamido-2-(5-hydroxypyridin-2-ylmethyl)malonate (**4**). Partial saponification of the malonate in aqueous sodium hydroxide (2.5 equivalents) followed by decarboxylation gave ethyl (D,L)-2-acetamido-3-(5-hydroxypyridin-2-yl)propanoate (**5**) in 81% yield.

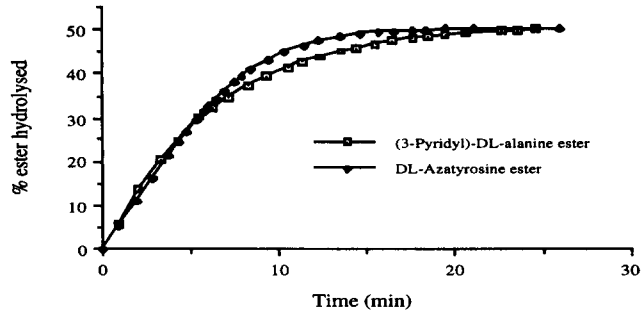
E-Mail: Malcolm.Stevens@nottingham.ac.uk Fax: +44 (0) 115 951 3412



Reagents and conditions: (a) 45% HBr in acetic acid, 120°C, 3h (b) Na, $CH(CO_2Et)_2NHAc$, EtOH / THF, reflux, 18h (c) 6M NaOH, EtOH, 2h then dioxane, reflux, 3h (d) α-chymotrypsin, NH_4OAc, DMF / H_2O, 1M NH_4OH to maintain pH 6.9 (e) 5M HCl, reflux, 3.5h

Resolution of the racemic ester (**5**) with α-chymotrypsin in aqueous DMF was very successful, resulting in the isolation of optically pure unhydrolysed ethyl (D)-2-acetamido-3-(5-hydroxypyridin-2-yl)propanoate (**6**) in 47% yield and (L)-2-acetamido-3-(5-hydroxypyridin-2-yl)propanoic acid (**7**) in 35% yield after two hours at 25°C. The pH of the solution was kept constant at 6.9, using an autotitrator, by the addition of 1M NH_4OH. The L-enantiomer of racemic ester (**5**) was found to be a very good substrate for α-chymotrypsin, suggesting that azatyrosine bears a close structural resemblance to tyrosine; the related N^{α}-acetyltyrosine ethyl ester is a known substrate for this enzyme. The hydrolysis rates for of β-(3-pyridyl)-DL-alanine[10] and azatyrosine esters, measured by consumption of 1M NH_4OH to maintain the pH at 6.9 at 25°C, are seen to be nearly identical and are shown in the Figure below.

Figure. Kinetic data for hydrolysis of β-(3-pyridyl)-DL-alanine and DL-azatyrosine ethyl esters.



Treatment of the protected amino acid (**7**) with 5M HCl under refluxing conditions[11] gave optically pure L-azatyrosine (**1**) ($[\alpha]_D$ +62.3 (c 1.0, 1N HCl), lit.[1] +55 (c 1.1, 1N HCl)) whereas treatment of the D-ester (**6**) resulted in formation of D-azatyrosine (**8**) ($[\alpha]_D$ -61.3 (c 1.0, 1N HCl).

In conclusion, we have devised a concise and reliable route to either enantiomer of azatyrosine which is highly competitive with previously reported syntheses of L-azatyrosine. Studies on azatyrosine analogues and derivatives are currently underway, and will be reported in due course.

Experimental Section

2-Bromomethyl-5-hydroxypyridine hydrobromide (3). A solution of 2-acetoxymethyl-5-acetoxypyridine (**2**) (2.0g, 9.6mmol) in 45% hydrobromic acid in acetic acid (15mL, 83mmol) was heated under reflux for 3h. After cooling the solvent was removed *in vacuo* and the resulting dark oil covered with diethyl ether. After several days a brown solid had formed and this was recrystallised from ethanol/diethyl ether to give **3** (2.29g, 89%) as a brown crystalline solid; m.p. 131-132°C; ^{1}H NMR (DMSO-d_6) 11.1 (1H, br s), 9.7 (1H, br s), 8.34 (1H, d, J 2.8Hz, H6), 7.82 (1H, d, J 8.8Hz, H3), 7.74 (1H, dd, 8.8, 2.8Hz, H4), 4.82 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6) 155.9 (C5), 142.6 (C2), 131.6, 131.5, 128.4, 27.9 (CH_2); IR cm^{-1} (KBr disc) 3019, 1614, 1574, 1409, 1307, 1248, 890, 861.

Diethyl 2-acetamido-2-(5-hydroxypyridin-2-ylmethyl)malonate (4). Addition of the sodium salt of diethyl acetamidomalonate using the well known Sorenson methodology[9] gave **4** as a white crystalline solid in 59% yield; m.p. 152-153°C, lit. 150-153°C;[11] having spectroscopic data in agreement with literature values.

Ethyl (DL)-2-acetamido-3-(5-hydroxypyridin-2-yl)propanoate (5) A solution of **4** (3.24g, 10mmol) in ethanol (60mL) was stirred at room temperature with 6M NaOH (4.2mL, 25mmol) for 1.5h. The precipitate was dissolved in water and the solution acidified using 10M HCl (2.5mL, 25mmol). After concentration *in vacuo* and drying over silica gel overnight, the residue was refluxed with dry dioxane (50mL) for 1.5h. The reaction mixture was allowed to cool then the solvent removed *in vacuo* and the solid residue mixed with water. The pH was adjusted to 7 using 6M NaOH and the solvent removed *in vacuo*. The product was extracted using hot, dry ethyl acetate (3x60mL) and the combined extracts concentrated *in vacuo*. Drying over silica gel followed by purification by flash column chromatography (10% MeOH/CH_2Cl_2) gave **5** (2.04g, 81%) as a white solid; m.p. 177-178°C; ^{1}H NMR (DMSO-d_6) d 9.79 (1H, s, OH), 8.27 (1H, d, J 7.7Hz, NH), 8.03 (1H, d, J 1.7Hz, H6), 7.05 (2H, d, J 1.7Hz, H3, H4), 4.55 (1H, m, H^{α}), 4.02 (2H, q, J 7.1Hz, OCH_2), 3.00 (1H, dd, J 7.8, 6.0Hz, one of CH_2), 2.90 (1H, dd, J 7.8, 5.3Hz, other CH_2), 1.78 (3H, s, CH_3CO), 1.10 (3H, t, J 7.1Hz, CH_3); ^{13}C NMR (DMSO-d_6) 174.7 (C=O), 172.1 (C=O), 155.0 (C2), 149.9 (C5), 139.9, 126.7, 125.2, 63.1 (OCH_2), 55.3 (C^{α}), 40.9 (CH_2), 25.0 ($\underline{C}H_3CO$), 16.7 ($\underline{C}H_3CH_2$); IR cm^{-1} ($CHCl_3$ solution) 2357, 1734 (C=O), 1669, 1492, 1228; MS (electrospray) *m/e* 253 (M^++1 (64)), 232 (5), 217 (7), 209 (8), 137 (100), 135 (12).

Ethyl (D)-2-acetamido-3-(5-hydroxypyridin-2-yl)propanoate (6) A solution of **5** (1.5g, 5.6mmol) in DMF (5mL) was added dropwise to a stirred solution of α-chymotrypsin (15mg) and ammonium acetate (29mg, 0.38mmol) in water (40mL) at room temperature, the pH being kept constant at 6.9, using an autotitrator, by the addition of 1M ammonium hydroxide. The reaction mixture was stirred for a further 2h and then the solvents removed *in vacuo*. The crude product was passed through a pad of flash silica (20% MeOH/CH_2Cl_2), and then extracted using hot, dry ethyl acetate (3x50mL) and the combined ethyl acetate extracts concentrated *in vacuo*. Purification by flash column chromatography (10% MeOH/CH_2Cl_2) gave **6** (0.71g, 47%) as a white solid. Analytical data as above.

(L)-2-Acetamido-3-(5-hydroxypyridin-2-yl)propanoic acid (7) The solid residue remaining from hot ethyl acetate extraction of **6** as described above was dissolved in water (10mL) and the pH adjusted to 3.8 using 10% aqueous acetic acid. The solution was lyophilized (x2) and the residue extracted using absolute ethanol (2x50mL). The combined ethanol extracts were then concentrated *in vacuo* then the crude product recrystallised from ethyl acetate to give **7** (0.46g, 36%) as a white solid; m.p. 180-182°C (dec.); ^{1}H NMR (DMSO-d_6) d 9.77 (1H, s, OH), 8.13 (1H, d, J 8.0Hz, NH), 8.03 (1H, d, J 1.7Hz, H6), 7.07 (1H, m, H4), 7.06 (1H, d, J 8.0Hz, H3), 4.52 (1H, m, H$^{\alpha}$), 3.04 (1H, dd, J 8.8, 5.2Hz, one of CH_2), 2.87 (1H, dd, J 8.8, 4.9Hz, other CH_2), 1.76 (3H, s, CH_3CO); ^{13}C NMR (DMSO-d_6) 174.2 (C=O), 170.0 (C=O), 152.9, 148.5, 137.9, 124.7, 123.3, 53.2 (C$^{\alpha}$), 38.9 (CH_2), 23.2 (CH_3CO); IR cm^{-1} ($CHCl_3$ solution) 3327, 1628, 1544, 1439, 1288, 1133; MS (electrospray) *m/e* 225 (M^++1 (56)), 217 (16), 138 (67), 137 (100), 110 (39), 54 (52).

β-(5-Hydroxy-2-pyridyl)-D-alanine (D-azatyrosine) (8) Amino ester **(6)** (0.30g, 1.2mmol) was dissolved in 5M HCl (10mL) and the solution heated under reflux for 3.5h. The solvent was removed *in vacuo* then the residue dissolved in water (2mL) and brought to pH5 using conc. ammonia. An equal volume of acetone was added and the solution chilled overnight. Amino acid **8** (0.191g, 88%) crystallised as white prisms; m.p. 269-270°C (dec.); $[\alpha]_D$ -61.3 (c 1.0, 1N HCl); ^{1}H NMR (D_2O) 8.16 (1H, d, J 2.8Hz, H6), 7.39 (1H, dd, J 8.5, 2.8Hz, H4), 7.32 (1H, d, J 8.5Hz, H3), 4.14 (1H, dd, J 7.8, 5.3Hz, H$^{\alpha}$), 3.40 (1H, dd, J 15.2, 5.3Hz, one of CH_2), 3.26 (1H, dd, J 15.2, 8.0Hz, other CH_2); MS (electrospray) *m/e* 183 (M^++1 (100)), 137 (91), 110 (69).

β-(5-Hydroxy-2-pyridyl)-D-alanine (L-azatyrosine) (1) A similar procedure to the one described above using protected amino acid **(7)** (0.25g, 1.2mmol) gave the free amino acid **1** (0.173g, 85%) as white prism crystals; m.p. 269-270°C (dec.), lit. 262-263°C (dec.)[1] $[\alpha]_D$ +62.3 (c 1.0, 1N HCl), lit. +55 (c 1.1, 1N HCl).[1]

Acknowledgements We thank the Cancer Research Campaign for financial support of this work.

References and Notes

1. Inouye, S.; Shomura, T.; Tsuruoka, T.; Ogawa, Y.; Watanabe, H.; Yoshida, J.; Niida, T. *Chem. Pharm. Bull.* **1975**, 23, 2669.
2. Shindo-Okada, N.; Makabe, O.; Nagahara, H.; Nishimura, S. *Mol. Carcinog.* **1989**, 2, 159.
3. For a review on the role of *ras* proteins in signal transduction, see Egan, S. E.; Weinberg, R. A. *Nature* **1993**, 365, 781.
4. Izawa, M.; Takayama, S.; Shindo-Okada, N.; Doi, S.; Kimura, M.; Katsuki, M.; Nishimura, S. *Cancer Res.* **1992**, 52, 1628.
5. Schow, S. R.; DeJoy, S. Q.; Wick, M. M.; Kerwar, S. S. *J. Org. Chem.* **1994**, 59, 6850.
6. Ye, B.; Burke, Jr., T. R. *J. Org. Chem.* **1995**, 60, 2640.
7. (a) Myers, A. G.; Gleason, J. L. *J. Org. Chem.* **1996**, 61, 813. (b) Myers, A. G.; Gleason, J. L.; Yoon, T. *J. Am. Chem. Soc.* **1995**, 117, 8488.
8. Hass, W.; Konig, W. A. *Liebigs Ann. Chem.*, **1982**, 1615.
9. Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; Vol. 1, pp 709-712.
10. Folkers, K.; Kubiak, T.; Stepinski, J. *Int. J. Peptide Protein Res.*, **1984**, 24, 197.
11. Harris, R. L. N.; Teitei, T. *Aust. J. Chem.*, **1977**, 30, 649.

(*Received in Belgium* 20 *August* 1996; *accepted* 7 *October* 1996)