SHORT PAPER

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A revised synthesis of the antitumour antibiotic L-azatyrosine *via* 2-iodo-5-methoxypyridine

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A revised synthesis of the antitumour antibiotic L-azatyrosine is reported, the main features of which are the unambiguous synthesis of the previously misinterpreted 2-iodo-5-methoxypyridine and subsequent palladium-catalysed coupling with an iodoalanine-derived zinc reagent.

Keywords: antitumour agents, amino acids, palladium-coupling, zinc

L-β-(5-Hydroxy-2-pyridyl)alanine, or L-azatyrosine (**1**) (Fig. 1), continues to attract attention as an anticancer lead compound[1] due to its unique ability *in vitro* to induce reversion of *Ras* or c-*erbB*-2 transformed cells to the apparently normal phenotype and its inhibitory activity on tumour formation *in vivo*.[2] The continued interest in the biological properties of this unnatural amino acid has resulted in a number of notable synthetic approaches,[3–7] including our own[3] which made use of the α-chymotrypsin catalysed resolution of a protected α-amino ester as a key step. Due to our interest in the biological properties of L-azatyrosine and the preparation of more potent azatyrosine analogues, we required a concise, practical and high-yielding synthetic route; clearly our enzymatic resolution process was limited in this respect due to production of the inactive D-isomer.

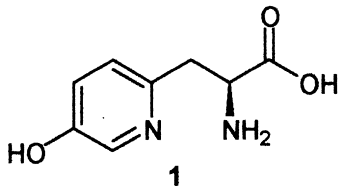

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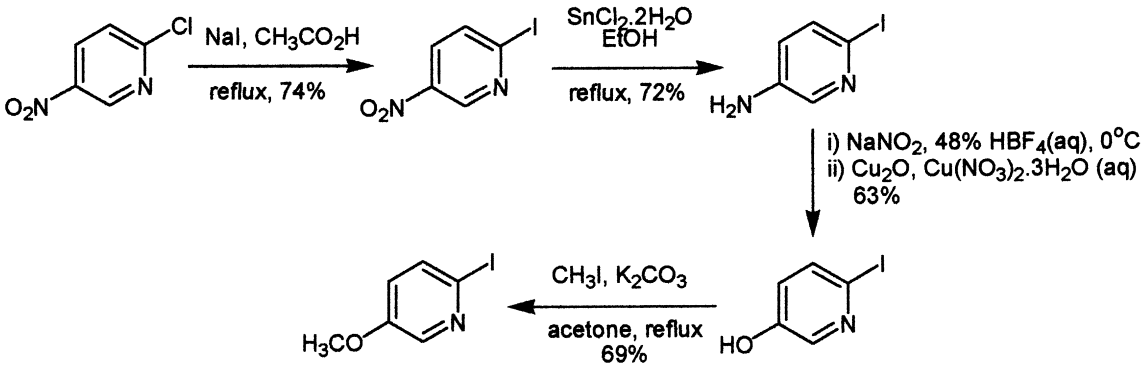
Fig. 1 L-Azatyrosine

Our attention turned to a report by Ye and Burke[6] regarding the synthesis of L-azatyrosine *via* the palladium-catalysed coupling of 2-iodo-5-methoxypyridine with an iodoalanine-derived zinc reagent using methodology extensively developed by Jackson and co-workers.[8] This route appeared to represent a concise method for azatyrosine synthesis since the protected iodoalanine component is commercially available and the protecting groups can be easily removed after the coupling reaction. The other coupling partner, however, reported as 2-iodo-5-methoxypyridine (*via* iodination of 3-hydroxypyridine) was misinterpreted[6,9] and was in fact the isomeric 2-iodo-3-methoxypyridine. More recently another report detailing the erroneous synthesis of 2-iodo-5-methoxypyridine has appeared.[10] This structural misinterpretation was pointed out by Sheldrake and co-workers[11] who then proceeded to synthesise and purify authentic 5-hydroxy-2-iodopyridine as a minor regioisomer obtained *via* iodination of 3-hydroxypyridine in just 2% yield. In view of the importance of L-azatyrosine and in order to clear up the confusion surrounding the synthesis of 5-hydroxy-2-iodopyridine we have devised a synthesis of this key intermediate and its subsequent elaboration to L-azatyrosine, the results of which are reported in this paper.

We have now synthesised 5-hydroxy-2-iodopyridine in three steps from commercially available 2-chloro-5-nitropyridine and protected the hydroxy group as the methyl ether, as shown in Scheme 1. Surprisingly this represents the first viable synthetic approach to this deceptively simple compound. Displacement of the halogen in 2-chloro-5-nitropyridine with sodium iodide in refluxing acetic acid[12] gave the corresponding iodide. This was reduced to 5-amino-2-iodopyridine using stannous chloride in refluxing ethanol.[13] One-pot



Scheme 1 Synthesis of 2-iodo-5-methoxypyridine.

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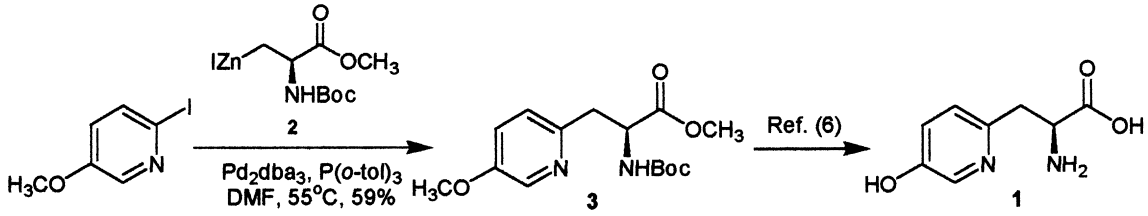
† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*



Scheme 2 Coupling of 2-iodo-5-methoxypyridine with iodoalanine-derived zinc reagent **2** and deprotection to yield L-azatyrosine.

formation of the diazonium tetrafluoroborate salt followed by its addition at room temperature to a solution of aqueous copper (II) nitrate containing a catalytic amount of copper (I) oxide according to the method of Cohen *et al.*[14] gave the desired 5-hydroxy-2-iodopyridine in 34% yield over the three steps. Protection of the phenolic group prior to palladium-catalysed coupling was accomplished using iodomethane and potassium carbonate in refluxing acetone.[15]

Initially, we employed the conditions reported by Ye and Burke[6] in their synthesis of (what later transpired to be) the azatyrosine isomer L-β-(3-hydroxy-2-pyridyl)alanine, for the key coupling reaction between 2-iodo-5-methoxypyridine and protected iodoalanine-derived zinc reagent **2**. In our hands, however, these conditions ($PdCl_2(PPh_3)_2$ in DMAC / THF) did not produce the desired protected L-azatyrosine. Instead use of the coupling conditions more recently reported by Dexter and Jackson for the synthesis of β- and γ-amino acids achieved the desired coupling.[16] Thus reaction of 2-iodo-5-methoxypyridine with zinc reagent **2** (prepared using zinc dust activated according to the Knochel procedure[17]) using the catalyst mixture *tris*(dibenzylideneacetone)-*di*-palladium and tri-*o*-tolylphosphine in dry N,N-dimethylformamide gave protected azatyrosine **3** in 59% yield. Deprotection under the conditions reported by Ye and Burke[6] (BBr_3 in CH_2Cl_2) yielded L-azatyrosine **1** with spectroscopic data identical to literature values[18] (Scheme 2).

In conclusion, we have synthesised the antitumour antibiotic L-azatyrosine *via* the unambiguous synthesis and coupling of 2-iodo-5-methoxypyridine with an iodoalanine-derived zinc reagent under palladium (0) catalysis. We believe this revised route to L-azatyrosine to be extremely competitive with previously published routes to this important antitumour lead compound.

Experimental

A: Synthesis of 2-Iodo-5-methoxypyridine

2-Iodo-5-nitropyridine:[12] Sodium iodide (23.6 g, 158 mmol) was added to a solution of 2-chloro-5-nitropyridine (5.00 g, 32 mmol) in glacial acetic acid (100 ml) and the mixture heated under reflux for two hours. After cooling the mixture was poured onto crushed ice (125 g) and the resulting precipitate collected by filtration and recrystallised from benzene and petroleum ether (60–80°C) to give the product as a brown crystalline solid (5.92 g, 24 mmol) in 74% yield, m.p. 165–166°C (lit.[12] 163–164°C).

5-Amino-2-iodopyridine:[13] A mixture of 2-iodo-5-nitropyridine (4.5 g, 18 mmol) and tin (II) chloride dihydrate (20.3 g, 90 mmol) in ethanol (60 ml) was heated under reflux for four hours. After cooling ethanol was removed *in vacuo* and the residue extracted with ethyl acetate (3 × 100 ml). The combined organic extracts were washed with 2M sodium hydroxide (aq) (2 × 100 ml) and water (100 ml) then dried ($MgSO_4$), filtered and concentrated *in vacuo*. Recrystallisation from benzene gave the product as a pale brown crystalline solid (2.85 g, 13 mmol) in 72% yield, mp 130–131°C (lit.[19] 132°C).

5-Hydroxy-2-iodopyridine:[14] 5-Amino-2-iodopyridine (2.00 g, 9.1 mmol) was dissolved in 48% aqueous tetrafluoroboric acid (12 ml) and the solution cooled in an ice bath. An ice-cooled solution of sodium nitrite (0.69 g, 10 mmol) in water (2 ml) was then added dropwise with stirring, maintaining the solution below 5°C. The reaction mixture was stirred in the ice bath for a further hour then the precipitated diazonium salt (1.82 g) collected by vacuum filtration. The crude diazonium salt was then added to a mixture of copper (I) oxide (0.77 g, 5.4 mmol) and copper (II) nitrate trihydrate (250 g, 1.1 mol) in water (450 ml) at 25°C with stirring. The reaction mixture was stirred for a further hour then cooled (ice bath) and neutralised using saturated sodium carbonate (aq) then filtered. The filtrate was extracted using ethyl acetate (3 × 200 ml) and the combined organic extracts dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (40% ethyl acetate / hexane) to give the product as a tan solid (1.27 g, 5.7 mmol) in 63% yield, m.p. 148–150°C (lit.[11] 149-151°C). ^{1}H NMR (DMSO-d_6) δ 10.23 (1H, broad s, OH), 7.95 (1H, d, *J* 2.5 Hz, H6), 7.59 (1H, d, *J* 7.5 Hz, H3), 6.95 (1H, dd, *J* 7.5, 2.5 Hz, H4).

2-Iodo-5-methoxypyridine:[15] Iodomethane (0.46 ml, 7.5 mmol) was added to a mixture of 5-hydroxy-2-iodopyridine (1.10 g, 5.0 mmol) and potassium carbonate (0.69 g, 5.0 mmol) in acetone (25 ml) and the reaction mixture heated under reflux for 18 hours. After cooling, acetone was removed *in vacuo* and the residue purified by flash column chromatography (25% ethyl acetate / hexane) to give the product (0.81 g, 3.4 mmol) as a pale brown solid in 69% yield, m.p. 59–60°C. ^{1}H NMR ($CDCl_3$) δ 8.04 (1H, d, *J* 3.1 Hz, H6), 7.53 (1H, d, *J* 8.5 Hz, H3), 6.87 (1H, dd, *J* 8.5, 3.1 Hz, H4), 3.79 (3H, s, CH_3); IR (KBr disc) 2934, 1559, 828 cm^{-1}; *m/z* (EI) 235 (M^+), 108; Anal. calcd. for C_6H_6INO: C, 30.66; H, 2.57; N, 5.96; found C, 30.54; H, 2.49; N, 5.69.

B: Methyl (L)-2-[(tert-butoxycarbonyl)amino]-3-(5-methoxypyridin-2-yl)propanoate (**3**)

Activation of zinc[17] 1,2-Dibromoethane (20 μl, 0.23 mmol) was added to a suspension of zinc dust (0.30 g, 4.5 mmol) in anhydrous DMF (0.5 ml) under nitrogen. The suspension was briefly heated to 80°C (5 min.) then allowed to cool. Trimethylsilyl chloride (6 μL, 0.05 mmol) was added and the mixture stirred vigorously at 25°C for 30 min.

Coupling reaction[16] A solution of N-(tert-butoxycarbonyl)-3-iodo-L-alanine methyl ester (0.247 g, 0.75 mmol) in anhydrous DMF (0.5 ml) was added to the suspension of activated zinc and the mixture stirred vigorously under nitrogen at 25°C for two hours. 2-Iodo-5-methoxypyridine (0.23 g, 0.98 mmol), $Pd_2(dba)_3$ (20 mg, 0.019 mmol) and tri-*o*-tolylphosphine (22 mg, 0.076 mmol) were then added and the reaction mixture heated at 50°C for 5 hours. After cooling, the mixture was filtered through Celite and then partitioned between ethyl acetate (50 ml) and water (25 ml). The layers were separated and the organic layer washed with brine (25 ml) and water (25 ml) before being dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude product. Purification by flash column chromatography (2% MeOH / CH_2Cl_2) gave the product **3** (0.179 g, 0.57 mmol) as a colourless oil in 59% yield, $[\alpha]_D$ +20.0 (c 2.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.22 (1H, d, *J* 2.9 Hz, H6), 7.13 (1H, dd, *J* 8.4, 2.9 Hz, H4), 7.05 (1H, d, *J* 8.4 Hz, H3), 5.85 (1H, d, *J* 8.0 Hz, NH), 4.65 (1H, m, H^α), 3.84 (3H, s, OCH_3), 3.70 (3H, s, CH_3CO_2), 3.23 (2H, m, CH_2), 1.43 (9H, s, $C(CH_3)_3$); IR (CH_2Cl_2 solution) 2963, 1744 (C = O), 1711 (C = O), 806 cm^{-1}; *m/z* (EI) 310 (M^+), 123; Accurate mass (ES) *m/z* calc. mass 333.1426 ([M+Na]$^+$), observed mass 333.1429 ([M+Na]$^+$).

Deprotection of coupled product **3** under the conditions previously reported[6] (BBr_3 in CH_2Cl_2) gave L-azatyrosine **1** as a single enantiomer with spectroscopic data corresponding to that previously reported, m.p. 265–266°C, lit.[18] 262–263°C; $[\alpha]_D$ +58.2 (c 1.0, 1N HCl), lit. +55 (c 1.1, 1N HCl).

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