A Concise Synthesis of the Differentiating Antibiotic L-Azatyrosine

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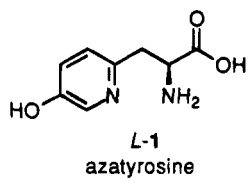
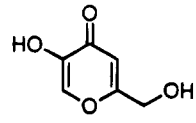
The treatment of cancers has traditionally relied on cytotoxic agents directed against processing of the DNA or RNA which is requisite for mitogenesis. Agents operating by these mechanisms frequently exhibit undesired collateral toxicity against normal proliferating tissues such as blood progenitors, hair, and gastrointestinal linings. More recently it has become clear that aberrations in cellular signal transduction can cause or contribute to a variety of diseases, including several cancers. Efforts are therefore underway to develop new anticancer drugs directed against pathogenic cell signaling as potentially more selective and less toxic alternative therapies.[1] The Ras family of guanine nucleotide-binding proteins are key mediators in a variety of growth factor and cytokine-dependent signaling pathways, and in mutated form they play major roles in human carcinogensis. Therefore, Ras proteins have become important targets of anticancer drug development.[2,3]

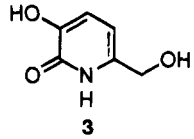
Azatyrosine (L-β-(5-hydroxy-2-pyridyl)alanine) (**1**) is an antibiotic isolated from *Streptomyces chibanesis*[4] which has been shown to promote permanent reversion of *ras*-dependent transformed cells to the normal phenotype in culture[5] and to inhibit chemical induction of carcinogenesis in transgenic mice bearing oncogenic human *ras*.[6] Azatyrosine has been previously prepared as a racemate in six to eight steps starting with kojic acid (**2**),[7-9] and in five steps from 3-hydroxy-6-(hydroxymethyl)-2(1*H*)-pyridone (**3**) (derived in two steps from the antibiotic nojirimycin).[4] Since enantiomeric discrimination is frequently observed in biological contexts, the unnatural D-azatyrosine would be expected to potentially have reduced potency relative to its L-counterpart. Indeed, synthetic DL-azatyrosine was reported to exhibit only 50% of the antibacterial potency of the L-isomer.[4] We therefore developed an enantiospecific synthesis of L-azatyrosine using a convergent route which is amenable to large scale preparation of this valuable antibiotic in optically pure form. During the writing of this manuscript a diastereoselective synthesis of L-azatyrosine appeared utilizing different chemistry.[10] The method presented herein affords an alternate approach to this valuable amino acid analogue.



L-**1**
azatyrosine



2
kojic acid



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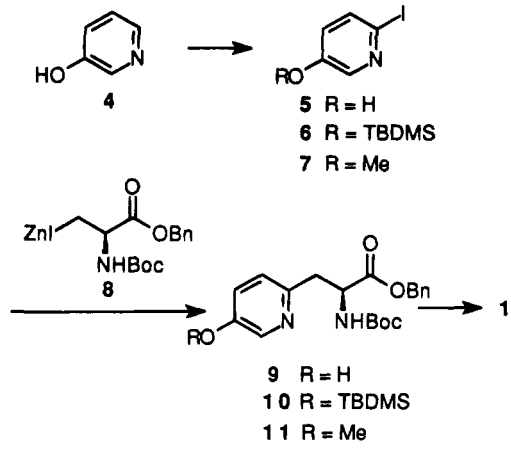
Scheme 1



As outlined in Scheme 1, our synthesis of L-azatyrosine (**1**) relied on the transition metal-catalyzed coupling of an aryl iodide with the organozinc reagent **8** derived from suitably protected iodoalanine. This approach has been elegantly applied by Jackson to the synthesis of other amino acid analogues[11] and has more recently been utilized in our laboratory for the chiral synthesis of L-difluorouromethyl phenylalanine (L-F_2Pmp) derivatives.[12] Our synthesis started with commercially available 3-hydroxypyridine (**4**) which was converted into 2-iodo-5-hydroxypyridine **5** in 83% yield according to known methods.[13] Initial coupling of free phenolic **5** with organozinc compound **8**[14] provided low yields (33%) of desired **9** which were not satisfactory for large scale preparation of L-azatyrosine. Prior protection of **5** as its TBDMS derivative **6** resulted in variable coupling yields (40–80%) of TBDMS-protected product **10** contaminated with partially deprotected product **9** (4–40%). Additionally, desilylation of **10** with tetrabutylammonium fluoride (TBAF) at room temperature failed. While increasing the temperature to 60–70 °C for 2–3 hours brought the reaction to completion, the optical rotation of the resulting **9** ($[\alpha]_D = -2.0°$) was lower than **9** obtained by the route described below ($[\alpha]_D = -2.83°$). This indicated that partial racemization may have occurred. We therefore resorted to alternate phenolic protection strategies and eventually found methylation to be satisfactory.

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Synthesis of the required 2-iodo-5-methoxypyridine (**7**) from **5** using diazomethane (85% yield) was superior to using iodomethane (50% yield). Coupling of **7** with **8** proceeded smoothly to give fully protected product **11** in 74% yield. Treatment of **11** with 3 mol equiv of boron tribromide resulted in complete removal of all protecting groups, providing L-azatyrosine **1** in 90% yield.

In conclusion, we report a total synthesis of L-azatyrosine in four steps from commercially available 3-hydroxypyridine in 47% overall yield. Since the limiting reagent (in terms of cost) is the iodozinc reagent **8**, the reaction may be viewed more practically as a two-step process in 67% effective overall yield. This represents an attractive alternative to the recently reported synthesis of the same compound.

Experimental Section

Melting points were determined on a Mel Temp II melting point apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA, and fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. ^{1}H NMR data were obtained on a Bruker AC250 (250 MHz) instrument. Optical rotations were measured on Perkin-Elmer 240 Polarimeter and infrared spectra were acquired on a Perkin-Elmer 1600 series FTIR instrument. Solvent was removed by rotory evaporation under reduced pressure and silica gel chromatography was performed using Merck silica gel 60 with a particle size of 40−63 μm. Anhydrous solvents were obtained commercially and used without further drying.

5-[[Dimethyl(1,1-dimethylethyl)silyl]oxy]-2-iodopyridine (6). To a stirred solution of 5-hydroxy-2-iodopyridine[13] (**5**) (3.10 g, 14 mmol) in dry DMF (8 mL) was added TBDMS chloride (2.82 g, 18.8 mmol) in one portion at room temperature under argon. After 10 min, imidazole (2.86 g, 42 mmol) in dry DMF (4 mL) was added via syringe, and the mixture was stirred overnight. The mixture was diluted with ethyl acetate (50 mL), washed with brine (5 mL), dried ($MgSO_4$), and evaporated to give crude product. Silica gel chromatography [hexane/ethyl acetate (98:2)] afforded **6** as an oil (4.36 g, 93%): ^{1}H NMR ($CDCl_3$) δ 7.99 (1H, dd, J = 1.6, 4.5 Hz); 7.09 (1H, dd, J = 4.5, 8 Hz); 6.98 (1H, dd, J = 1.6 8 Hz); 1.04 (9H, s); 0.26 (6H, s); FABMS m/z $(M + H)^+$.

Coupling Reaction of Compound 5 with Compound 8: Synthesis of Phenylmethyl 2(*S*)-[[[(1,1-Dimethylethyl)oxy]carbonyl]amino]-3-[5′-hydroxy-2′-pyridyl)propionate (9). To phenylmethyl 2(*R*)-[[[1,1-dimethylethyl)oxy]carbonyl]amino]-3-iodopropionate[11,14] (810 mg, 2.0 mmol) in anhydrous *N,N*-dimethylacetamide (DMAC, 2 mL) and THF (2 mL) was added freshly activated zinc dust (130 mg, 2 mmol) in one portion at room temperature under argon. The mixture was then warmed to 65−70 °C and stirred until all zinc had dissolved (1−1.5 h), providing iodozinc intermediate **8**. The reaction mixture was cooled to room temperature, and $PdCl_2(PPh_3)_2$ (70 mg, 0.1 mmol) was added quickly in one portion. Compound **5** (221 mg, 1.0 mmol) in DMAC (1 mL) and THF (1 mL) was added to the reaction mixture via cannula and allowed to react at 65−70 °C for 5 h. The reaction mixture was then cooled to 0 °C, quenched with saturated ammonium chloride (10 mL), extracted with ethyl acetate (3 × 40 mL), washed with brine (10 mL), dried ($MgSO_4$), and then evaporated to give crude product. Silica gel chromatography (hexane-ethyl acetate in a gradient from 9:1 to 1:1) afforded product **9** as an oil (125 mg, 33%) along with recovered starting **5** (160 mg): $[\alpha]_D$ = −2.83° (c = 1.13, $CHCl_3$); IR (film) 3357, 2977, 1745, 1715, 1503, 1367, 1023, 801 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.94 (1H, brm); 7.26 (5H, brm); 7.12 (1H, brd, J = 7.8 Hz); 7.0 (1H, brm); 6.02 (1H, brm); 5.10 (2H, s); 4.74 (1H, brm); 3.34 (2H, brm); 1.35 (9H, s); FABMS m/z 373 $(M + H)^+$. Anal. Calcd for $C_{20}H_{24}N_2O_5 \cdot {}^1/_2H_2O$: C, 62.98; H, 6.61; N, 7.34. Found: C, 63.27; H, 6.49; N, 6.99. High resolution mass spectrum calcd for $C_{20}H_{24}N_2O_5$ 373.1764, found 373.1768.

Coupling Reaction of Compound 6 with Compound 8: Synthesis of Phenylmethyl 2(*S*)-[[[(1,1-Dimethylethyl)oxy]carbonyl]amino]-3-[5′-[[dimethyl(1,1-dimethylethyl)silyl]oxy]-2′-pyridyl]propionate (10). Coupling of silyl-protected **6** (335 mg, 1 mmol) to **8** was carried out in a manner identical to that described for the coupling of **5** to **8**, affording TBDMS-protected product **10** as an oil (260 mg, 54%) along with desilylated compound **9** (103 mg, 28%). Compound **10**: $[\alpha]_D$ = +10.9° (c = 1.0, $CHCl_3$); IR (film) 3459, 2980, 1738, 1726, 1512, 1368, 1087, 821 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.9 (1H, m); 7.40−7.20 (6H, brm); 7.01 (1H, d, J = 2.5 Hz); 5.97 (1H, brm); 5.12 (2H, m); 4.74 (1H, m); 3.38 (1H, dd, J = 6.3, 15.6 Hz); 3.24 (1H, dd, J = 4.1, 15.6 Hz); 1.39 (9H, s); 0.96 (9H, s); 0.19 (3H, s); 0.18 (3H, s); FABMS m/z 487 $(M + H)^+$. Anal. Calcd for $C_{26}H_{38}N_2O_5Si$: C, 64.17, H, 7.87; N, 5.76. Found: C, 63.96; H, 8.23; N, 5.69.

2-Iodo-5-methoxypyridine (7). To a stirred solution of 5-hydroxy-2-iodopyridine (**5**) (200 mg, 0.91 mmol) in ether (5 mL) at 0 °C was added dropwise diazomethane (0.6 M in ether, 2.5 mL) and the solution stirred at 0 °C (30 min). Ether was removed under reduced pressure and crude product was purified by silica gel chromatography [hexane-ethyl acetate (9:1)] affording product **7** as an oil (179 mg, 84.8%): IR (film) 2950, 2870, 1580, 1475, 1367, 1010, 890 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.99 (1H, dd, J = 1.5, 4.6 Hz); 7.19 (1H, dd, J = 4.6, 8.1 Hz); 6.98 (1H, dd, J = 1.5, 8.1 Hz); 3.91 (3H, s); FABMS m/z 236 $(M + H)^+$.

Phenylmethyl 2(*S*)-[[[(1,1-Dimethylethyl)oxy]carbonyl]amino]-3-[5′-methoxy-2′-pyridyl)propionate (11). To compound **8** (10.5 mmol) prepared as described above was added $PdCl_2(PPh_3)_2$ (367 mg, 0.52 mmol) quickly in one portion at room temperature. To this was added compound **7** (1.23 g, 5.2 mmol) in DMAC (3 mL) and THF (3 mL) via cannula and the reaction stirred at 65−70 °C (5 h). The reaction mixture was cooled to 0 °C, quenched with saturated ammonium chloride (20 mL), extracted with ethyl acetate (3 × 50 mL), washed with brine (10 mL), and dried ($MgSO_4$) and solvent removed. Purification by silica gel chromatography (hexane-ethyl acetate in a gradient from 99:1 to 95:5) afforded product **11** (1.49 g, 74%): $[\alpha]_D$ = +8.11° (c = 1.22, $CHCl_3$); IR (film) 3636, 2976, 1711, 1702, 1587, 1454, 1366, 1217, 1049, 862 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 8.01 (1H, d, J = 2.8 Hz); 7.38−7.21 (5H, m); 7.14−7.01 (2H, m); 5.91 (1H, d, J = 8.8 Hz); 5.13 (1H, d, J = 12.2 Hz); 5.06 (1H, d, J = 12.2 Hz); 4.76 (1H, m); 3.78 (3H, s); 3.45 (1H, dd, J = 6.4, 15.5 Hz); 3.24 (1H, dd, J = 4.5, 15.5 Hz); 1.39 (9H, s); FABMS m/z 387 $(M + H)^+$. Anal. Calcd for $C_{21}H_{26}N_2O_5$: C, 65.27; H, 6.78; N, 7.25. Found: 65.26; H, 6.80; N, 7.18.

Synthesis of L-Azatyrosine (1). To compound **11** (646 mg, 1.67 mmol) in dichloromethane (120 mL) at −78 °C under argon was added boron tribromide (1.26 g, 5.02 mmol) in dichloromethane (20 mL) over 30 min. After an additional 20 min at −78 °C the reaction mixture was allowed to warmed to room temperature, stirred overnight, cooled to 0 °C, and quenched with water (10 mL). Solvent was removed and the residue passed through a cation-exchange column (Dowex 50W-X4, 50−100 mesh) using 0.1 N aqueous ammonium hydroxide as eluent. Lyophylization yielded L-azatyrosine (**1**) a white solid (274 mg, 90 %): IR (KBr) 3300−2200 (br), 1622, 1597, 1480, 1345, 844 cm^{-1}; ^{1}H NMR (DMSO) δ 7.94 (1H, dd, J = 1.2, 3.1 Hz); 7.17 (1H, dd, J = 1.2, 8 Hz); 7.11 (1H, dd, J = 3.1, 8 Hz); 4.0 (1H, m); 3.28 (1H, dd, J = 4.8, 16.4 Hz); 3.07 (1H, dd, J = 6.9, 16.4 Hz); FABMS m/z 183 $(M + H)^+$. For combustion analysis a sample was dissolved in H_2O and decolorized with activated charcoal, and then charcoal was removed by filtration through a short C_{18} pad. Lyophylization provided an analytical sample of **1** as a snow-white solid: mp 210−211 °C. (Note: The following range of mp have been reported for D,L-**1**; 209−210 °C,[7] 240−241 °C,[8] and 272−273 °C,[9] and for L-**1**; 262−263 °C.[4]) $[\alpha]_D$ = −29.2° [c = 1.0, H_2O; lit.[4] −33° (c = 1.0, H_2O)]. Anal. Calcd for $C_8H_{10}N_2O_3{}^3/_4H_2O$: C, 49.10; H, 5.88; N, 14.32. Found: 49.09; H, 5.88; N, 14.33.

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