Diastereoselective Synthesis of the Antibiotic L-Azatyrosine

Steven R. Schow,* Susan Quinn DeJoy, Michael M. Wick, and S. S. Kerwar

American Cyanamid Company, Medical Research Division, Lederle Laboratories, Middletown Road, Pearl River, New York 10965

Received May 12, 1994

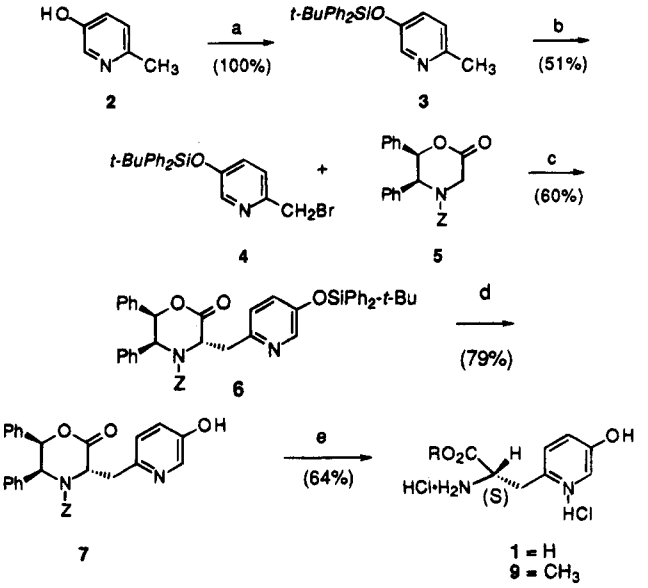
The antibiotic, L-azatyrosine (**1**) [L-β-(5-hydroxy-2-pyridyl)alanine],[1] has been shown to inhibit oncogenic *ras*-induced maturation of Xenopus oocytes,[2a,b] revert oncogenic *ras* and *raf* transformed mouse and human cell lines to a normal phenotype,[2c] and suppress growth of oncogenic *ras* transformed mouse embryos[2d] and *ras*-induced neurite formation in PC12 cells.[2e] In addition, **1** inhibits 7,12-dimethylbenz[*a*]anthracene- or methylnitrosourea-induced carcinogenesis in mice harboring a normal c-Ha *ras* gene.[2f]

In order to examine the effects of L-azatyrosine in mice implanted with oncogenic *ras* transformed tumors, significant quantities of L-azatyrosine were required. Although D/L-azatyrosine has been synthesized from kojic acid,[3] L-azatyrosine has only been isolated from natural sources.[1] We devised a diastereoselective synthesis of L-azatyrosine to provide sufficient quantities of this unusual amino acid analog for our studies, as outlined in Scheme 1.

Commercially available pyridine **2** was protected with a *tert*-butyldiphenylsilyl group. Benzylic bromination of **3** yielded the unstable bromide **4**, which was purified by chromatography on silica gel and stored as a dilute ethereal solution in a freezer. The major byproduct of the bromination reaction was identified as the silanol resulting from cleavage of the hydroxy protecting group.

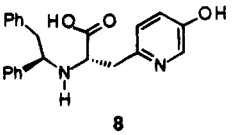
Alkylation of the enolate of the chiral glycine equivalent **5** with **4** using the methodology of Williams[4] yielded diastereomerically pure protected azatyrosine **6**. Subsequent proton NMR studies on both **6** and **7** involved heating solutions of **6** and **7** in DMSO in order to collapse rotamer signals. Analysis of the simplified spectra suggested that both **6** and **7** were single diastereomers. Similar carbon NMR studies on **7** gave ^{13}C spectra which displayed single resonances for each of the aliphatic carbon atoms. Whether or not **6** had been generated as a single diastereomer during the alkylation step was not ascertained. Preliminary chromatographic purification of **6** in order to remove starting materials which constitute the primary bulk contamination may have also removed any minor isomeric impurities.

Scheme 1[a]



[a] Reagents: (a) Ph_2tBuSiCl/Imidazole/DMF; (b) NBS, AIBN, CCl_4; (c) $NaN(SiMe_3)_2$, THF; (d) TBAF, THF; (e) $Pd(OH)_2/C$, H_2/50 psi, THF; Pd/C, H_2/50 psi, HCl/H_2O.

The protecting groups on **6** were removed in a two-step sequence. TBAF cleavage of the silyl group yielded phenol **7**. Reductive removal of the carbobenzyloxy and diphenylethylene groups proved to be problematical. Hydrogenation of **7** over Pearlman's catalyst (24 h) yielded a variable mixture of azatyrosine and what appears to be intermediate **8**. Extended hydrogenation



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yielded over-reduced products. This problem was circumvented by treating the mixture of azatyrosine and **8** with HCl and continuing hydrogenation in water over 5% Pd/C (~30 min). The pure product was isolated as the trihydrochloride salt. The specific rotation of **1** proved to be identical to the natural product.[1] In addition, methyl ester **9** was prepared from **1** by treatment with saturated methanolic HCl. Ester **9** demonstrated a specific rotation identical to that which was reported in the literature.[1,5] Biological studies with **1** are in progress.[6,7]

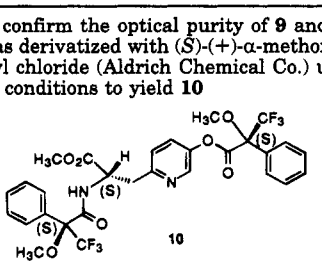
(1) Inouye, S.; Shomura, T.; Tsuruoka, T.; Ogawa, Y.; Watanabe, H.; Yoshida, J.; Niida, T. *Chem. Pharm. Bull.* **1975**, *23*, 2669.

(2) (a) Chung, D. L.; Brandt-Rauf, P.; Murphy, R. B.; Nishimura, S.; Yamaizumi, Z.; Weinstein, I. B.; Pincus, M. R. *Anticancer Res.* **1991**, *11*, 1373. (b) Campa, M. J.; Glickman, J. F.; Yamamoto, K.; Chang, K-J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7654. (c) Shindo-Okada, N.; Makabe, O.; Nagahara, H.; Nishimura, S. *Mol. Carcinog.* **1989**, *2*, 159. (d) Nomura, T.; Ryoyama, K.; Okada, G.; Matano, S.; Nakamura, S.; Kameyama, T. *Jpn. J. Cancer Res.* **1992**, *83*, 851. (e) Fujita-Yoshigaki, J.; Yokoyama, S.; Shindo-Okada, N.; Nishimura, S. *Oncogene* **1992**, *7*, 2019. (f) Izawa, M.; Takayama, S.; Shindo-Okada, N.; Doi, S.; Kimura, M.; Katsuki, M.; Nishimura, S. *Cancer Res.* **1992**, *52*, 1628.

(3) Norton, S.; Skinner, C. G.; Shive, W. *J. Org. Chem.* **1961**, *26*, 1495.

(4) William, R. M.; Im, M.-N. *Tetrahedron Lett.* **1988**, *29*, 6075; *J. Am. Chem. Soc.* **1991**, *113*, 9276.

(5) In order to confirm the optical purity of **9** and by inference **1**, methyl ester **9** was derivatized with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (Aldrich Chemical Co.) under Schotten–Bauman reaction conditions to yield **10**



10

[mass spectra: CI (*m*/*e*) 629 MH^+]. ^1H, ^{13}C and ^{19}F NMR spectrascopic studies on Mosher derivative **10** clearly demonstrate that it is a single diastereomer. Specifically, only two methoxy carbon peaks (δ 54.9 and δ 55.7) and only two fluorine peaks (δ −69.1and δ −71.3) were observed in the respective NMR spectra. Thus, both **1** and **9** are inferred to be in optically pure form.

0022-3263/94/1959-6850$04.50/0

In conclusion, a rapid, convenient diastereoselective synthesis of the antibiotic, L-azatyrosine, has been devised. This procedure may be used to provide significant quantities of this interesting tyrosine analog for biological evaluation as well as provide a basis for analog development.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Chemical ionization (CI) mass spectra were determined on a Finnigan MAT-90 mass spectrometer. The high-resolution FAB mass spectra were determined on a Fisons VG ZAB-SE mass spectrometer. IR spectra were recorded on a Nicole 20SXB FT-IR spectrometer. ^{1}H NMR spectra were determined at 300 MHz, and ^{13}C NMR spectra were determined at 75 MHz, using a Nicolet QE-300 WB spectrometer; chemical shifts (δ) are in parts per million relative to tetramethylsilane. Apparent couplings are given in hertz. Specific rotations were recorded on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ. Unless otherwise noted, all reagents and solvents obtained from commercial suppliers were of the highest possible purity and used without further purification. All nonaqueous reactions were performed in dry glassware under an inert atmosphere of dry argon. Tetrahydrofuran was freshly distilled from sodium benzophenone ketyl. Benzyl (2*R*,3*S*)-(−)-6-oxo-2,3-diphenyl-4-morpholinecarboxylate (**5**) was obtained from GLYTECH, Inc., P.O. Box 19, Ft. Collins, CO 80522.

5-[[(1,1-Dimethylethyl)diphenylsilyl]oxy]-2-methylpyridine (3). To a solution of 2.0 g of 5-hydroxy-2-methylpyridine (**2**) and 1.24 g of imidazole in 15 mL of DMF at room temperature was added 5.0 g of *tert*-butyldiphenylsilyl chloride. The reaction mixture was stirred for 12 h. The solution was diluted with 50 mL of water, and the resulting mixture was extracted with 3 × 50 mL of ether. The ether extracts were combined and washed with 2 × 20 mL of water and 50 mL of brine. The ether solution was dried over magnesium sulfate and concentrated *in vacuo*. Flash chromatography of the crude product on silica gel using a gradient of 100%−50% hexane in ethyl acetate yielded 6.40 g (100%) of pure product. ^{1}H NMR ($CDCl_3$) δ: 1.11 (s, 9H); 2.40 (s, 3H); 6.80−6.88 (m, 2H); 7.26−7.46 (m, 6H); 7.66−7.71 (m, 4H); 8.07−8.08 (m, 1H). IR (neat): 2840 (m); 1480 (m); 1260 (m); 910 (m); 820 (m); 710 cm^{-1} (s). Mass spectra CI (*m*/*e*): 348 MH$^+$. C,H,N: C, 75.74; H, 7.19; N, 3.99. Found: C, 76.03; H, 7.25; N, 4.03 theory.

2-(Bromomethyl)-5-[[(1,1-dimethylethyl)diphenylsilyl]oxy]pyridine (4). To a solution of 3.66 g of compound **3** in carbon tetrachloride was added 2.3 g of NBS and 200 mg of AIBN. The reaction was warmed to 50 °C for 24 h, cooled, and filtered. The solution was concentrated *in vacuo* and chromatographed on silica gel using a gradient of 100%−50% hexane in ethyl acetate. Purified bromide **4** (2.3 g, 51%) was recovered upon evaporation. This compound was unstable to concentration and thus was used immediately. ^{1}H NMR ($CDCl_3$) δ: 1.12 (s, 9H); 4.42 (s, 2H); 6.93−7.83 (m, 12H); 8.20−8.27 (m, 1H).

Phenylmethyl [*S*-(3α,5β,6β)]-3-[5-[[(1,1-Dimethylethyl)diphenylsilyl]oxy]-2-pyridinyl]-2-oxo-5,6-diphenyl-4-morpholinecarboxylate (6). To a solution of 4.9 g of oxazinone **5** in 150 mL of THF at −100 °C was added 12 mL of 1 M sodium bis(trimethylsilyl)amide−THF solution. The reaction was stirred for 1 h, and then 2.7 g of bromide **4** in 10 mL of THF was rapidly added to the solution. The reaction was warmed to −78 °C and then stirred for 5 h. Water (50 mL) quench followed by ether extraction (2 × 100 mL) yielded a crude product solution. The ethereal extracts were combined, washed with brine (50 mL), and dried over magnesium sulfate. The solvent was removed *in vacuo*, and the residue was chromatographed on silica gel using a gradient of 100%−100% hexane in ethyl acetate. Product (2.81 g, 60%) was recovered upon solvent removal. The adduct was crystallized from ether−hexane. Mp: 68−71 °C sinter; 72 °C melt. $[\alpha]^{25}_D$ = +49 ± 1 (1.0% in $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 1.10 (s, 9H); 3.47−3.70 (m, 2H); 4.85−5.13 (m, 4H); 5.31−5.34 (m, 1H); 6.48−7.68 (m, 27H); 8.15−8.16 (m, 1H). IR (KBr): 1760 (s); 1710 (s); 1480 (s); 1270 (s); 1120 (s); 700 cm^{-1} (s). Mass spectra CI (*m*/*e*): 733 MH$^+$, 495 MH$^+$ − $SiPh_2tBu$. High-resolution mass spectrum FAB (*m*/*e*): 733.3089 (MH$^+$) found, 733.3098 theory. C,H,N: C, 74.76; H, 6.07; N, 3.52. Found: C, 75.38; H, 6.05; N, 3.82 theory.

Phenylmethyl [*S*-(3α,5β,6β)]-3-(5-Hydroxy-2-pyridinyl)-2-oxo-5,6-diphenyl-4-morpholinecarboxylate (7). To a solution of 2.8 g of protected phenol **6** in 20 mL of THF at room temperature was added 4 mL of 1 M tetra-*n*-butylammonium fluoride−THF solution. The reaction was stirred for 15 min and then evaporated and chromatographed on silica gel using a gradient of 100%−100% hexane in ethyl acetate. A yield of 1.5 g (79%) of deprotected product **7** was isolated. This material could be recrystallized from methylene chloride/ether. Mp: 153−155 °C sinter; 156 °C melt. $[\alpha]^{25}_D$ = +24 ± 1 (1.0% in $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 3.41−3.66 (m, 2H); 4.85−5.28 (m, 3H); 5.30−5.45 (m, 2H); 6.50−7.24 (m, 17H); 7.34 (bs, 1H); 8.18−8.22 (m, 1H). ^{13}C NMR (partial/DMSO/85 °C) δ: 57.3 ($CHCH_2$); 59.7 (*C*HN); 66.4 (OCH_2); 77.4 (*C*HO). IR (KBr): 1750 (s); 1700 (s); 1400 (s); 1270 (s); 1110 (m); 700 cm^{-1} (s). Mass spectra CI (*m*/*e*): 495 MH$^+$. C,H,N: C, 72.62; H, 5.43; N, 5.58. Found: C, 72.86; H, 5.30; N, 5.66 theory.

(*S*)-α-Amino-5-hydroxy-2-pyridinepropanoic Acid Trihydrochloride (1). To a solution of 3.2 g of oxazinone **7** in 50 mL of a 1:1 mixture of 2-propanol−THF was hydrogenated at 50 psi hydrogen over 200 mg of palladium hydroxide (30% on carbon) for 18 h. The mixture was filtered and evaporated. The residue was dissolved in 5 mL of concentrated hydrochloric acid. The acidic solution was evaporated, and then the residue was redissolved in 100 mL of water. The aqueous solution was then hydrogenated at 50 psi hydrogen over 500 mg of 5% palladium-on-carbon for 30 min. The solution was filtered and decolorized with carbon. The residue was washed with ether (3 × 50 mL). Removal of the water yielded 1.4 g (64%) of pure L-azatyrosine hydrochloride salt. This trihydrochloride salt was isolated as a highly hygroscopic foam. $[\alpha]^{25}_D$ = +28 ± 2 (.06% in 1 N HCl). ^{1}H NMR (D_2O) δ: 3.58 (d, J = 7 Hz, 2H); 4.39 (t, J = 7 Hz, 1H); 7.87 (d, J = 9 Hz, 1H); 8.01 (dd, J = 9, 3 Hz, 1H); 8.29 (d, J = 3 Hz, 1H). ^{13}C NMR (D_2O) δ: 35.7 (CH_2); 55.4 (*C*HN); 131.9 (*C*H); 132.5 (*C*H); 136.7 (*C*HN); 144.0 (*C*N); 158.1 (*C*O); 173.6 (*C*=O). IR (KBr): 3300 (s); 1740 (s); 1620 (s); 1560 cm^{-1} (s). Mass spectra CI (*m*/*e*): 183 MH$^+$. C,H,N,Cl: C, 28.88; H, 5.48; N, 8.34; Cl, 31.10. Found: C, 28.55; H, 5.39; N, 8.32; Cl, 31.59 theory. Calcd for $C_8H_{12}O_3N_2{\cdot}3HCl{\cdot}2.5H_2O$.

Methyl (*S*)-α-Amino-5-hydroxy-2-pyridinepropionate Dihydrochloride (9). A solution of 0.40 g of acid **1** in 50 mL of methanol was saturated with HCl gas and then stirred for 18 h at room temperature. The solution was evaporated to near dryness, and then ~70 mL of ethyl ether was added. The slurry was filtered, and the residue was washed with ether (3 × 50 mL). The pure L-azatyrosine methyl ester hydrochloride salt was dried *in vacuo* at ambient temperature (yield: 0.30 g/94%). Material was recrystallized from methanol and ethanol. A sample of prerecrystallized ester **9** was utilized in the preparation of Mosher ester **10**.[5] Mp: 218−219 °C sinter; 220−222 °C melt with gas evolution and dec. $[\alpha]^{25}_D$ = +36 ± 2 (.398% in H_2O). ^{1}H NMR (D_2O) δ: 3.62 (d, J = 7 Hz, 2H); 3.80 (s, 3H); 4.63 (t, J = 7 Hz, 1H); 7.84 (d, J = 9 Hz, 1H); 7.98 (dd, J = 9, 3

(6) L-Azatyrosine synthesized by the above procedure exhibited the following effect on cellular metabolism and growth: at concentrations ranging from 10 to 30 ng, it inhibited geminal vesicle breakdown in oocytes injected with mutant Val^{12} H-*ras*. L-Azatyrosine inhibited the growth of *ras* and *raf* transformed 3T3 cells: the IC_{50} for *ras* transformed cells was 30−60 μg/mL, for *raf* transformed cells it was 60−120 μg/mL. L-Azatyrosine inhibited the *in vitro* synthesis of CAT (chloramphenicol acetyl transferase) when tested in a reticulocyte lysate system. L-Azatyrosine inhibited the synthesis of tyr-tRNA^{tyr} suggesting competition with L-tyrosine for aminoacylation. These results are consistent with those reported for L-azatyrosine isolated and purified from fermentation broths (see ref 7).

(7) L-Azatyrosine isolated from *Streptomyces chilbanensis* causes oncogenic *ras*-transformed NIH 3T3 cells to revert to a normal phenotype (see 2c). Synthetic L-azatyrosine exhibited similar properties. Oncogenic *ras*-transformed NIH 3T3 cells were incubated for 7 days with L-azatyrosine (500 μg/mL). At this dose, L-azatyrosine exhibited significant cytotoxicity, but clones that were isolated from surviving cells had diminished ability to form colonies in soft agar. These L-azatyrosine revertant cells also produced fewer tumors in nude mice than the parental *ras*-transformed cell line (20/49 compared to 23/24), and the tumors that formed were substantially smaller (0.06 ± 0.02 g compared to 1.25 ± 0.24 g). These revertant cells still contained the mutant Val^{12} *ras* gene and synthesized mutant *ras* protein (Southern blot analysis and an ELISA assay).

Hz, 1H); 8.30 (d, J = 3 Hz, 1H). ^{13}C NMR (D_2O) δ: 33.4 (CH_2); 52.7 (CH_3); 54.8 (CHN); 129.6 (CH); 131.0 (CH); 134.0 (CHN); 141.4 (CN); 156.1 (CO); 169.4 (C=O). IR (KBr): 3300–2600 (s); 1746 (s); 1623 (s); 1563 cm^{-1} (s). Mass spectra CI (m/e): 197 MH^+. C,H,N,Cl: C, 40.24; H, 5.33; N, 10.35; Cl, 26.37. Found: C, 40.17; H, 5.24; N, 10.41; Cl, 26.35 theory. Calcd for $C_9H_{12}O_3N_2 \cdot 2HCl$.

Acknowledgment. The authors wish to thank the Analytical Services Group at American Cyanamid for providing analytical and spectral data for this paper. Special appreciation is extended to G. Morton for the extensive NMR studies performed in support of this work.