A New Synthetic Methodology for Tiazofurin

Kanda S. Ramasamy,* Rajanikanth Bandaru, and Devron Averett

ICN Pharmaceuticals, Inc., 3300 Hyland Avenue, Costa Mesa, California 92626

kramasamy@icnpharm.com

Received March 28, 2000

Tiazofurin[1] [**1**, 2-(β-D-ribofuranosyl)thiazole-4-carboxamide)] (see Figure 1) is a C-nucleoside that attracted considerable attention because of its significant activity against both human lymphoid,[2] and lung tumor cell lines,[3] and murine-implanted human ovarian cancers.[4] Its biological effects also include its efficacy in the treatment of acute myeloid leukemia.[5] In addition, recent studies of tiazofurin have generated even more interest as a possible treatment for patients with chronic myeloid leukemia (CML) in blast crisis.[6] The biological activities of tiazofurin are due to the inhibition of inosine monophosphate dehydrogenase (IMPDH), which induces the shutdown of guanine nucleotide synthesis.[7]

Although several methods of synthesis of tiazofurin have been published,[8−14] most of them suffer from low yield, give a mixture of products (**2** and **3**, Figure 1), and utilize hydrogen sulfide gas, which is environmentally unsafe on large-scale production. The problems associated with the reported methods and the biological importance of tiazofurin encouraged us to seek a new synthetic methodology for the preparation of tiazofurin. Herein, we report a new and novel method of synthesis for tiazofurin, which eliminates the use of H_2S gas and the byproducts.

Our strategy was to form thiazoline intermediate **6** by the cyclocondensation[15] of cysteine ethyl ester hydrochloride (**5**) with nitrile **4**,[16] which on dehydrogenation, followed by removal of the protecting groups, should provide the desired nucleoside **1**. Accordingly (see Scheme 1), 1-cyano-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**4**)[16] was allowed to react with cysteine ethyl ester hydrochloride in the presence of triethylamine at room temperature. After 2 h of stirring, the reaction mixture was evaporated to dryness and extracted with dichloromethane, and the extract was evaporated to dryness to give an oily residue. The ^{1}H NMR of the product showed signals at δ 3.61 and 5.08 which are characteristic of Δ^2-thiazolines.[17] The thiazoline intermediate **6** on heating with activated MnO_2[18] readily provided a product. ^{1}H NMR spectrum of the product showed the presence of one benzoyl group, one ethyl ester group, and three methine protons, which was consistent with the reported data for **8**.[8] In addition, TLC and proton spectrum of **8** matched very well with that reported in the literature.[8] Interestingly, MnO_2 oxidation of **6** not only converted the thiazoline ring to a thiazole ring but also concomitantly eliminated the 2′,3′-benzoate groups to form a furan ring. Similar elimination of benzoate groups has been reported.[19] When we tried the dehydrogenation of **6** with *N*-bromosuccinimide, followed by DBU, we also obtained compound **8**. All our effort to dehydrogenate **6** with other known[20] methods was unsuccessful. We believe that the formation **8** from **6** may have occurred through radical intermediate. To our knowledge, this is the first report where elimination of benzoate groups under radical condition is shown.

Having no success, with the available methods known in the literature, to oxidize **6**, we conceived that the formation of **8** from **6** could be avoided by using a protecting group that is stable under radical reaction conditions for the 2′,3′-hydroxyl groups of **6**. Thus, we prepared the known 1-cyano-2,3-*O*-isopropylidene-5-*O*-benzoyl-β-D-ribofuranose (**9**)[19] from **4**. Reaction of **9** with cysteine ethyl ester hydrochloride in the presence of triethylamine at room temperature gave readily thiazoline intermediate **10** in 90% yield (Scheme 2). ^{1}H NMR spectrum of **10** showed the presence of a thiazoline methylene group and a methine proton, which was comparable to **6**. As anticipated, oxidation of **10** with activated MnO_2 in benzene afforded a clean product **11**. Observation of the NMR spectrum of **11** indicated the absence of the methylene and the methine protons of the thiazoline ring and the presence of a vinyl proton, which accounted for the dehydrogenation of **10**. Significantly, the oxidation of **10** proceeded only with freshly activated MnO_2. Other oxidizing reagents were also tried, but MnO_2 appeared to be the best for this particular reaction. Exposure of **11** to 90% TFA for 1 h at room temperature gave ethyl 2-(5′-*O*-benzoyl-β-D-ribofuranosyl)thiazole-4-carboxylate (**12**) in 98% yield. Deprotection of the isopropylidene group in **11** was also achieved with iodine

(1) Tiazofurin is the generic name approved by the United States Adopted Name Council.

(2) Earle, M. F.; Glazer, R. I. *Cancer Res.* **1983**, *43*, 133.
(3) Carnex, D. N.; Abluwalia, G. S.; Jayaram, H. N.; Cooney, D. A.; Johns, D. G. *J. Clin. Invest.* **1985**, *75*, 175.
(4) Micha, J. P.; Kucera, P. R.; Preve, C. N.; Rettenmaier, M. A.; Stratton, J. A.; DiSaia, P. J. *Gynecol. Oncol.* **1985**, *21*, 351.
(5) Tricot, G. T.; Jayaram, H. N.; Nichols, C. R.; Pennington, K.; Lapis, E.; Weber, G.; Hoffman, R. *Cancer Res.* **1997**, *47*, 4988.
(6) Weber, G. U.S. Pat. 5, 405, 837 (1995).
(7) Olah, E.; Natusmeda, Y.; Ikegami, T.; Kote, Z.; Horanyi, M.; Szelenye, J.; Paulik, E.; Kremmer, T.; Hollan, S. R.; Sugar, J.; Weber, G. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6533.
(8) Srivastava, P. C.; Pickering, M. V.; Allen, L. B.; Streeter, D. G.; Campbell, M. T.; Witkowski, J. T.; Sidwell, R. W.; Robins, R. K. *J. Med. Chem.* **1977**, *20*, 256.
(9) Fuertes, M.; Garcia-Lopez, T.; Garcia-Munoz, G.; Stud, M. *J. Org. Chem.* **1976**, *41*, 4074.
(10) Hennen, W. J.; Hinshaw, B. C.; Riely, T. A.; Wood, S. G.; Robins, R. K. *J. Org. Chem.* **1985**, *50*, 1741.
(11) Bimwala, R. M.; Vogel, P. *Helv. Chim. Acta* **1989**, *72*, 1825.
(12) Humber, D. C.; Mulholland, K. R.; Stoodley, R. J. *J. Chem. Soc., Perkin Trans. 1* **1990**, 283.
(13) Parsons, J. L.; Vizine, D.; Summer, M.; Marathe, S.; Dubicki, H. U.S. Pat. 4,451,648 (1984).
(14) Ramasamy, K. S.; Averett, D. *Nucleosides Nucleotides* **1999**, *18*, 2425.
(15) Baganz, H.; Domaschke, L. *Chem. Ber.* **1962**, *95*, 1842.
(16) Cook, P. D.; McNamara, D. J. *J. Heterocycl. Chem.* **1986**, *23*, 155.
(17) Minster, D. K.; Jordis, U.; Evans, D. L.; Hecht, S. M. *J. Org. Chem.* **1978**, *43*, 1624.
(18) Ishihara, H.; Shimura, K. *FEBS Lett.* **1988**, *226*, 319.
(19) Albrecht, H. P.; Repke, D. B.; Moffatt, J. G. *J. Org. Chem.* **1973**, *38*, 1836.
(20) (a) Meyers, A. I.; Tavares, F. X. *J. Org. Chem.* **1996**, *61*, 8207. (b) Barrish, J. C.; Singh, J.; Spergel, S. H.; Han, W.-C.; Kissick, T. P.; Kronenthal, D. R.; Mueller, R. H. *J. Org. Chem.* **1993**, *58*, 4494.

10.1021/jo000460a CCC: $19.00
Published on Web 08/10/2000

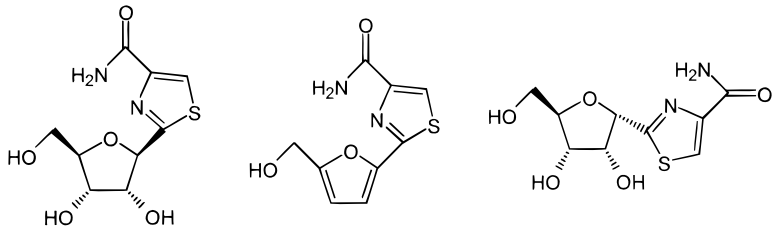
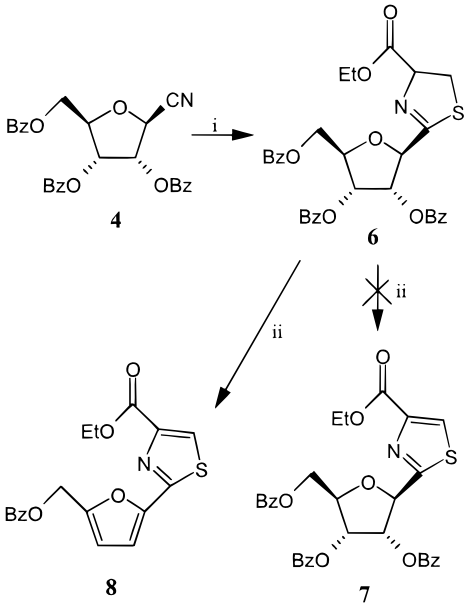


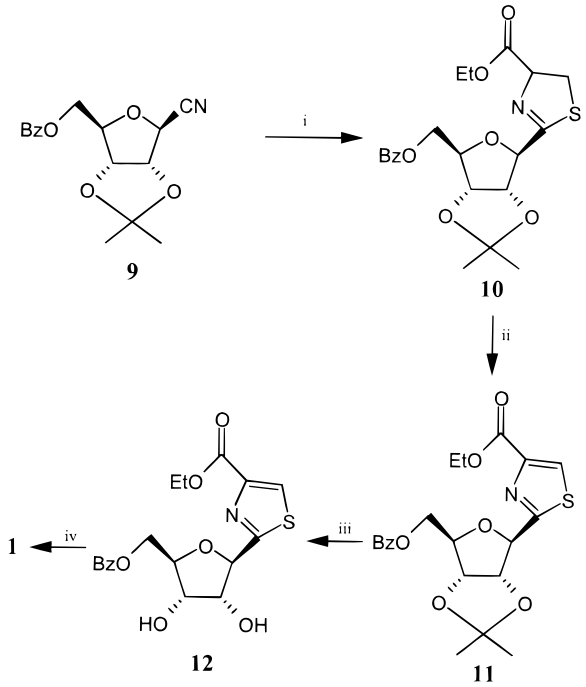
Figure 1.

Scheme 1[a]



[a] Reagents and conditions: (i) cysteine ethyl ester hydrochloride/TEA; (ii) MnO_2/benzene.

Scheme 2[a]



[a] Reagents and conditions: (i) cysteine ethyl ester hydrochloride/TEA; (ii) MnO_2/benzene/reflux; (iii) 90% TFA; (iv) MeOH/NH_3.

in methanol[21] to provide **12**. Finally, treatment of **12** with methanolic ammonia at room temperature for 12 h afforded tiazofurin in 90% yield.

There are four major merits to the present method. First, it avoided the use of toxic mercuric salt and hydrogen sulfide, which are environmentally unsafe. Second, the formation of the side products **2** and **3** were eliminated, and the yield of tiazofurin is substantially improved over previous methods. Third, the present method does not require column chromatographic purification, thereby reducing the cost of production of the drug. Fourth, the same methodology could be used to construct other five-membered C-nucleosides by replacing sulfur in **1** with heteroatoms such as oxygen, selenium, or nitrogen. The synthesis of other such C-nucleosides are in progress, and their results will be reported elsewhere.

In summary, we have developed a safe, convenient, and higher yielding new methodology for the synthesis of the important antitumor agent tiazofurin.

Experimental Section

General. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on 300 MHz spectrometer. The chemical shifts are expressed in δ values (ppm) relative to tetramethylsilane as internal standard. Thin-layer chromatography (TLC) was performed on plates of silica gel $60F_{254}$ coated on aluminum sheets (5 × 10 cm) using different solvents prepared freshly. All solvents used were reagent grade. Most of the reactions were conducted under argon atmosphere. Evaporations were carried out under reduced pressure with the bath temperature below 35 °C.

Ethyl 2-(2′,3′,5′-Tri-*O*-benzoyl-β-D-ribofuranosyl)thiazoline-4-carboxylate (6). To a stirred solution of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-1-carbonitrile[16] (**4**, 4.71 g, 10.0 mmol) in dry methanol (150 mL) at room temperature under argon atmosphere was added cysteine ethyl ester hydrochloride (2.78 g, 15.0 mmol) followed by TEA (1.5 g, 15 mmol). The reaction mixture was stirred at room temperature under argon atmosphere for 3 h and evaporated to dryness. The residue was dissolved in methylene chloride and washed with water, 5% $NaHCO_3$ solution, and brine. The CH_2Cl_2 extract was dried over anhydrous $MgSO_4$, filtered, and washed with CH_2Cl_2. The combined filtrate was evaporated to dryness, and the residue was used as such for the next reaction. A small amount of the crude product was purified by flash chromatography over silica gel using hexane → ethyl acetate as eluent: ^{1}H NMR ($CDCl_3$) δ 1.24 (m, 3 H), 3.61 (m, 2 H), 4.25 (m, 2 H), 4.65 (m, 2 H), 5.08 (m, 1 H), 5.21 (m, 1 H), 5.98 (d, 1 H), 6.12 (d, 1 H), 7.40−7.50 (m, 6 H), 7.60 (m, 4 H), 8.04 (m, 4 H), 8.12 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 14.2, 35.3, 58.2, 61.8, 63.8, 78.2, 84.7, 104.2, 105.0, 112.6, 115.7, 128.2, 129.6, 133.0, 133.2, 147.6, 152.2, 154.4, 159.8, 161.2, 165.6, 169.7, 170.2; IR (film) 1025, 1096, 1270, 1721, 2980 cm^{-1}. Anal. Calcd for $C_{32}H_{29}NO_9S$: C, 63.67; H, 4.84; N, 2.32; S, 5.31. Found: C, 63.80; H, 4.73; N, 2.37; S, 5.39.

(21) Ramasamy, K. S.; Bandaru, R.; Averett, D. *Synth. Commun.* **1999**, *29*, 2881.

2-(4-Carboethoxythiazol-2-yl)-5-((benzoyloxy)methyl)furan (8). To a stirred solution of **6** (0.587 g, 1.0 mmol) in benzene (25 mL) at room temperature was added activated MnO_2 (1.0 g). The reaction mixture was heated at reflux for 12 h and filtered. The catalyst was washed with CH_2Cl_2, and the filtrate was evaporated to dryness. The residue was purified by flash chromatography over silica gel using hexane → ethyl acetate as eluent to give 0.3 g (85%) of **8** as crystalline material: mp 97−100 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (t, 3 H), 4.34 (m, 2 H), 5.30 (s, 2 H), 6.60 (d, 1 H), 7.08 (d, 1 H), 7.36 (t, 2 H), 7.46 (t, 1 H), 8.00 (d, 2 H), 8.06 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.2, 58.1, 61.4, 110.9, 113.1, 126.2, 128.1, 129.5, 132.9, 147.6, 148.2, 151.1, 157.8, 160.6, 165.7. Anal. Calcd for $C_{18}H_{15}NO_5S$: C, 60.50; H, 4.23; N, 3.92; S, 8.95. Found: C, 60.69; H, 4.13; N, 3.90; S, 8.99.

Ethyl 2-(5′-*O*-Benzoyl-2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)thiazoline-4-carboxylate (10). To a stirred solution of 5-*O*-benzoyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl-1-carbonitrile[19] (**9**, 4.71 g, 15.55 mmol) in dry methylene chloride (150 mL) at room temperature under argon atmosphere were added cysteine ethyl ester hydrochloride (1.49 g, 8 mmol) and TEA (0.81 g, 8 mmol) at 0, 2, 4, and 6 h periods. The reaction mixture was stirred at room temperature under argon atmosphere for 24 h. The reaction was diluted with methylene chloride and washed with water and brine. The CH_2Cl_2 extract was dried over anhydrous $MgSO_4$, filtered, and washed with CH_2Cl_2. The combined filtrate was evaporated to dryness and the residue was used as such for the next reaction. A small amount of the crude product was purified by flash chromatography over silica gel using hexane → ethyl acetate as eluent: ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3 H), 1.35 (s, 3 H), 1.52 (s, 3 H), 3.40 (m, 2 H), 4.20 (m, 2 H), 4.42 (m, 3 H), 4.80 (m, 2 H), 5.12 (m, 2 H), 7.42 (m, 2 H), 7.58 (m, 1 H), 8.08 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 14.1, 25.4, 26.7, 27.1, 53.2, 61.9, 64.6, 65.2, 81.3, 83.6, 83.8, 83.9, 113. 7, 114.1, 128.3, 128.5, 129.2, 129.6, 133.1, 133.3, 166.1, 167.2, 169.3, 169.5, 170.1; IR (film) 1026, 1095, 1269, 1721, 2984 cm^{-1}. Anal. Calcd for $C_{21}H_{25}NO_7S$: C, 57.92; H, 5.78; N, 3.22; S, 7.36. Found: C, 58.03; H, 5.91; N, 3.39; S, 7.43.

Ethyl 2-(5′-*O*-benzoyl-2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)thiazole-4-carboxylate (11). Method A: To a vigorously stirred solution of the crude ethyl 2-(5′-*O*-benzoyl-2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)thiazoline-4-carboxylate (**10**, 7.0 g) in methylene chloride (300 mL) was added activated manganese dioxide (27.8 g) at room temperature. The reaction mixture was stirred at room temperature for 24 h, filtered through Celite, and washed with acetone. The filtrates were combined and evaporated to dryness to give 5.9 g (88% from the cyano sugar **9**) of an oily residue. A small amount of the crude product was purified by flash chromatography over silica gel using CH_2Cl_2 → ethyl acetate as eluent: ^{1}H NMR ($CDCl_3$) δ 1.39 (t, 6 H), 1.63 (s, 3 H), 4.39 (m, 3 H), 4.60 (m, 2 H), 4.84 (m, I H), 5.26 (m, 1 H), 5.40 (d, 1 H), 7.40 (m, 2 H), 7.52 (m, 1 H), 7.89 (m, 2 H), 8.02 (s, 1 H); IR (film) 1025, 1091, 1265, 1715, 2986, 3116, 3442 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 14.3, 25.4, 27.2, 61.3, 64.6, 81.9, 83.7, 84.6, 85.6, 114.1, 127.8, 128.2, 129.1, 129.3, 132.9, 146.9, 160.3, 165.3, 171.3. Anal. Calcd for $C_{21}H_{23}NO_7S$: C, 58.19; H, 5.35; N, 3.23; S, 7.38. Found: C, 58.31; H, 5.23; N, 3.41; S, 7.46.

Method B: A mixture of the crude **10** (7.0 g) and activated manganese dioxide (27.8 g) in dry benzene (150 mL) was heated at 80 °C for 2 h. The reaction mixture was filtered through Celite and washed with acetone. The filtrates were combined and evaporated to dryness to give an oily residue: yield 6.0 g (89% from the cyano sugar **9**). A small amount of the crude product was purified by flash chromatography over silica gel using CH_2Cl_2 → ethyl acetate as eluent. The product obtained by this method was found to be identical in all respects with the product obtained from the previous method.

Ethyl 2-(5′-*O*-Benzoyl-β-D-ribofuranosyl)thiazole-4-carboxylate (12). Method A: A solution of the crude ethyl 2-(5′-*O*-benzoyl-2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)thiazole-4-carboxylate (**11**, 4.5 g, 10.39 mmol) in a mixture of trifluoroacetic acid:tetrahydrofuran:water (30:20:6 mL) was allowed to stir at room temperature for 1 h. The reaction mixture was evaporated to dryness. The residue was suspended in methylene chloride, cooled, and neutralized with saturated (sat.) $NaHCO_3$ solution. The aqueous solution was extracted with CH_2Cl_2 and washed with sat. $NaHCO_3$ solution, water, and brine. The organic extract was dried over $MgSO_4$, filtered, and washed with CH_2Cl_2, and the filtrate was evaporated to dryness. The residue that obtained was crystallized from ethanol/water (1:1) to give 4.0 g (98%) of colorless crystals. The solid was filtered and dried over P_2O_5 under vacuum: mp 82−85 °C; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, 3 H), 4.31 (m, 4 H), 4.45 (m, 3 H), 4.55 (m, 1 H), 4.74 (m, 1 H), 5.32 (d, 1 H), 7.37 (m, 2 H), 7.51 (m, 1 H), 7.99 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 14.4, 61.7, 64.3, 72.1, 77.2, 82.3, 82.9, 127.5, 128.3, 129.6, 129.7, 133.0, 146.6, 161.1, 166.2, 173.6; IR (KBr) 713, 1023, 1090, 1240, 1291, 1697, 1718, 2953, 3131, 3459 cm^{-1}. Anal. Calcd for $C_{18}H_{19}NO_7S$: C, 54.95; H, 4.87; N, 3.56; S, 8.13. Found: C, 55.02; H, 4.73; N, 3.70; S, 8.19.

Method B: A solution of the crude ethyl 2-(5′-*O*-benzoyl-2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)thiazole-4-carboxylate (**11**, 4.33 g, 10.0 mmol) and iodine (1.26 g, 10 mmol) in methanol (100 mL) was refluxed for 1 h. The reaction mixture was cooled, neutralized with sodium thiosulfate solution, and evaporated to dryness. The residue was dissolved in methylene chloride and washed with brine. The organic extract was dried over $MgSO_4$, filtered, and washed with CH_2Cl_2, and the filtrate was evaporated to dryness. The residue that obtained was crystallized from ethanol:water (1:1) to give 3.6 g (92%) of colorless crystals. The product obtained by this procedure was found to be identical with the product obtained by the method A.

2-β-D-Ribofuranosylthiazole-4-carboxamide (Tiazofurin) (1). Ethyl 2-(5′-*O*-benzoyl-β-D-ribofuranosyl)thiazole-4-carboxylate (**12**, 3.7 g, 942 mmol) was placed in a steel bomb and mixed with freshly prepared cold methanolic ammonia (70 mL, saturated at 0 °C). The mixture was protected from moisture and stirred at room temperature for 12 h. The reaction vessel was cooled to 0 °C and opened carefully, and the solution was evaporated to a sticky foam. The residue was triturated with dry toluene, and the toluene layer was discarded. The residue that obtained was treated with anhydrous ethanol and triturated to give light yellow solid. The solid obtained was filtered, washed with ethyl acetate, and dried. The solid was crystallized from ethanol/ethyl acetate to provide 2.25 g (90%) of the pure product: mp 145−147 °C; ^{1}H NMR (DMSO-d_6) δ 3.57 (m, 2 H), 3.89 (s, 2 H), 4.07 (m, 1 H), 4.83 (t, 1 H), 4.92 (d, 1 H), 5.05 (d, 1 H), 5.36 (d, 1 H), 7.56 (s, 1 H), 7.69 (s, 1 H), 8.20 (s,1 H).

JO000460A