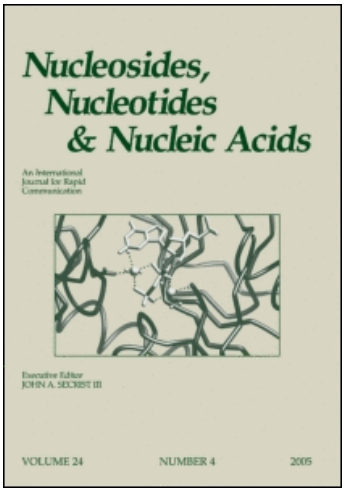

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Kanda S. Ramasamy[a]; Devron Averett[a]
[a] ICN Pharmaceuticals, Inc., Costa Mesa, CA

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A MODIFIED SYNTHESIS OF TIAZOFURIN

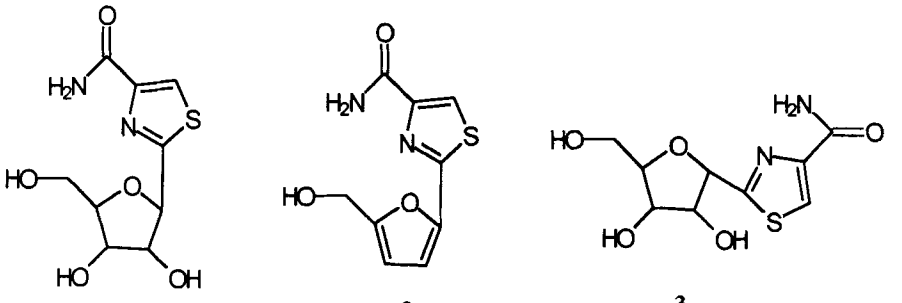
Kanda S. Ramasamy* and Devron Averett

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

ABSTRACT: An improved synthesis of tiazofurin is described from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose.

Tiazofurin[1] [1, 2-(β-D-ribofuranosyl)thiazole-4-carboxamide)] is a C-nucleoside that possesses significant activity against both human lymphoid,[2] lung tumor cell lines[3] and murine-implanted human ovarian cancers.[4] Tiazofurin also demonstrated efficacy in the treatment of acute myeloid leukemia.[5] In addition, recent findings have brought interest in tiazofurin as a possible treatment for patients with chronic myeloid leukemia (CML) in blast crisis.[6] Other studies have shown[7] that tiazofurin is converted to its active metabolite, thiazole-4-carboxamide adenine dinucleotide (TAD) which inhibits IMP dehydrogenase and as a result depletes guanosine nucleotide pools. Thus, tiazofurin exhibits potent antitumor activity.

Although tiazofurin has been known for over 15 years, and is currently under phase II/III trials in humans, there is no suitable synthetic method available for large scale production. Tiazofurin was first synthesized independently by Srivastava *et al.*[8] and Fuertes *et al.*[9] in low yields. In both methods the authors obtained side products (*i.e.* compounds **2** and **3**) along with the required product. Hennen *et al.*[10] developed a somewhat different route to tiazofurin in 19% yield. Recently, Bimwala *et al.*[11] reported nine steps synthesis to tiazofurin in 25% yield. More recently, Humber *et al.*[12] worked out a procedure for tiazofurin starting from benzyl(2,3,5-tri-*O*-benzoyl-β-D-ribo-furanosyl)penicillinate. The only known method that is suitable for large scale



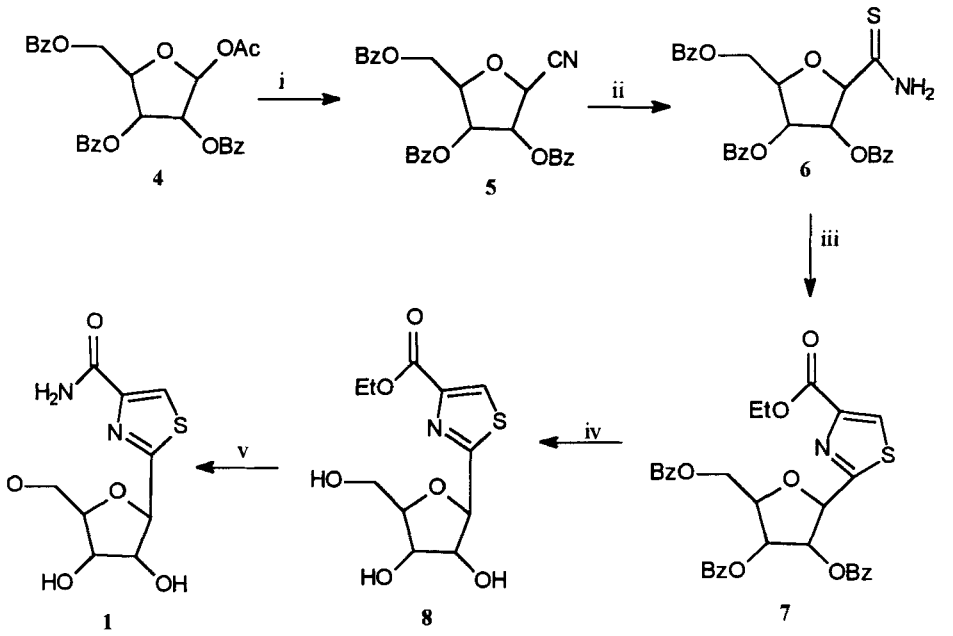
1 2 3

FIG. 1

production is by Parsons *et al.*[13] Unfortunately, the Parsons method uses mercury cyanide and also gives a mixture of α and β products. The problems associated with the reported methods and our need of tiazofurin for clinical studies led us to solve the problems, which impede the easy access to the compound. Herein, we report a practical method for preparation of tiazofurin in large quantities.

We elected to use 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**4**) as our starting material. Reaction of **4** with trimethylsilylcyanide and stannic chloride in anhydrous CH_2Cl_2 gave 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosylcyanide (**5**) in 75% yield.[14] Exposure of **5** to hydrogen sulfide gas and N,N-dimethylaminopyridine in dry EtOH for 2 h followed by stirring the reaction mixture at room temperature for 16 h afforded crystalline 2,5-anhydro-3,4,6-tri-*O*-benzoyl-β-D-allonthioamide (**6**)[13] in 97% yield. The ring closure of **6** with ethylbromopyruvate gives not only the required thiazole product (**7**) but also varying amounts of the furan derivative **2**[8,9] and α isomer **3**.[13] The formation of these unwanted by-products makes the reported procedure unattractive for larger scale production of tiazofurin. Several reports are known for the construction of thiazole ring from thiocarboxamide **6**.[15-17] However, most of the reaction conditions did not eliminate the formation of **2** and **3** satisfactorily.[15a] Thus, the formation of side products and the low yield obtained for the thiazole **7** prompted a search for improved conditions to the key step, the ring closure of **6**.

After careful study of the literature and taking into account the generally accepted mechanism for the Hantzsch reaction,[18] a modified Hantzsch thiazole synthesis



Scheme 1: i. TMSCN/$SnCl_4$; ii.H_2S/DMAP/EtOH; iii. (a) Ethyl bromopyruvate/ $NaHCO_3$/DME;(b) TFAA/2,6-lutidine/DME; iv. NaOEt/EtOH; v. NH_3/MeOH.

procedure[19] was used for the ring closure of **6**. Accordingly, treatment of **6** with ethyl bromopyruvate and solid $NaHCO_3$ in dry 1,2-dimethoxyethane followed by addition of a mixture of trifluoroacetic anhydride and 2,6-lutidine in dry 1,2-dimethoxyethane afforded exclusively ethyl 2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazole-4-carboxylate (7) in quantitative yield. Furthermore, the reaction was found to be solvent and base specific. When the reaction was carried out in dry THF, we did see the formation of furan derivative **2** in 10 to 20% yield. Changing the base from 2,6-lutidine to pyridine or TEA or some other bases also caused the formation **2**. Hydrolysis of the benzoyl protecting groups of the crude **7** with sodium ethoxide in dry ethanol followed by neutralization of the reaction mixture with Dowex H^+ resin gave ethyl 2-(β-D-ribofuranosyl)thiazole-4-carboxylate (**8**) in 83% yield. Exposure of the intermediate **8** to methanolic ammonia at room temperature for 12 h provided tiazofurin in 87% yield.

Several advantages are notable in the present modified method. First, it avoided the use of toxic mercuric salt, which is environmentally unsafe. Second, it eliminated

the formation of side products **2** and **3**, 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.

In summary, we have developed a safe, convenient and higher yielding process to the important antitumor agent tiazofurin.

Experimental

Melting points were taken on a Haake Buchler capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian Mercury 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 (5x10 cm; EM Science) using different solvents prepared freshly. ICN silica gel 18-32 (60 A) was used for flash column chromatography. All solvents used were reagent grade. Most of the dry solvents were purchased from Fluka and used as such without further purification. Most of the reactions were conducted under argon atmosphere. Evaporations were carried out under reduced pressure with the bath temperature below 35°C.

2,5-Anhydro-3,4,6-tri-*O*-benzoyl-D-allonthioamide (6): Hydrogen sulfide was passed through a cold (8-10°C) stirred suspension of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl cyanide[14] (**5**, 50 g, 106 mmol) in dry EtOH (900 mL) for 5 min, then N,N-dimethylaminopyridine (1.2 g, 10 mmol) was added in one portion. Hydrogen sulfide was slowly passed through the stirred reaction mixture for 2 h (the outlet tube from the reaction flask was bubbled through bleach solution made in 5% NaOH). After 2 h the flask was sealed and stirring continued below 25°C for 16 h. Argon was passed through the reaction mixture for 1 h to remove the last traces of H_2S. The suspension was stirred at 0°C for 2 h and solid separated was filtered, washed with cold dry EtOH and dried over P_2O_5 under vacuum. Yield 52 g (97%); mp 133-135°C (lit.,[13] 131-133°C). ^{1}H NMR ($CDCl_3$): δ 4.72 (m, 2H) 4.74 (m, 1H), 5.12 (d, 1H), 5.71 (t, 1H), 5.98 (t, 1H), 7.30-7.60 (m, 10H), 7.86 (d, 2H), 8.14 (m, 4H) and 8.46 (bs, 1H).

Ethyl 2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thiazole-4-carboxylate (7): To a stirred mixture of compound **6** (10.12 g, 20 mmol) and solid $NaHCO_3$, (16.8 g, 200

mmol) in dry 1,2-dimethoxyethane (60 mL) at 0°C under argon atmosphere was added ethyl bromopyruvate (7.8 g, 40 mmol) during 10 min period. After the addition, the reaction mixture was stirred at 0°C under argon for 6 h. TLC indicated complete conversion of the starting material into a single product (Hex: EtOAc, 7:3). The reaction was cooled to -15°C in dry ice/CCl_4 under argon. A solution of trifluoroacetic anhydride (12.6 g, 60 mmol) and 2,6-lutidine (12.8 g, 120 mmol) dissolved in dry 1,2-dimethoxyethane (20 mL) was added slowly over 15 min. After the addition, the reaction was stirred at -15°C for 2 h under an argon atmosphere. The reaction mixture was filtered, washed with dry methylene chloride (100 mL) and the combined filtrates taken to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (200 mL) and the pH was adjusted to 7 with sat. $NaHCO_3$ solution. The organic extract was washed with 1N HCl (100 mL), sat. $NaHCO_3$ (200 mL) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, washed with CH_2Cl_2 (100 mL) and evaporated to dryness. The crude material was used as such for further reaction. A small quantity was purified by flash chromatography over silica gel using hexane-ethyl acetate as the eluent. ^1H NMR ($CDCl_3$): δ 1.36 (t, 1H) 4.40 (m, 2H), 4.62 (dd, 1H), 4.74 (m, 1H), 4.86 (dd, 1H), 5.74 (d, 1H), 5.84 (m, 2H), 7.30-7.60 (m, 9H), 7.91 (d, 2H), 7.98 (d, 2H), 8.08 (m, 2H) and 8.12 (s, 1H).

Ethyl 2-(β-D-ribofuranosyl)thiazole-4-carboxylate (8): The crude compound 7 (15.00 g) was dissolved in dry ethanol (100 mL) and treated with sodium ethoxide powder (1.36 g, 20 mmol) under argon atmosphere. The reaction mixture was stirred at room temperature for 12 h under argon. The solution was neutralized with Dowex-X8 H^+ resin, filtered and washed with methanol (100 mL). The filtrate was evaporated to dryness. The residue was partitioned between water (100 mL) and chloroform (150 mL). The aqueous layer was washed with chloroform (100 mL) and evaporated to dryness. The residue was dissolved in methanol (100 mL), silica gel (15 g) was added and evaporated to dryness. The dried compound adsorbed silica gel was placed on top of the silica column (5 x 20 cm) packed in CH_2Cl_2. The column was eluted with CH_2Cl_2/acetone (7:3; 500 mL) followed by CH_2Cl_2/methanol (95:5; 1000 mL). The CH_2Cl_2/methanol fractions were combined and evaporated to give pure **8**. A small amount was crystallized from 2-propanol/ether as colorless product. Yield 4.8 g (83%); mp 62-64°C (lit.,[10] 78-79.5 °C). ^1H NMR (DMSO-d_6): δ 1.36 (t, 3H), 3.52 (m, 2H), 3.84

(m, 2H), 4.06 (m, 1H), 4.28 (m, 2H), 4.94 (t, 1H), 4.98 (d, 1H), 5.08 (d, 1H), 5.46 (d, 1H) and 8.52 (s, 1H).

2-β-D-Ribofuranosylthiazole-4-carboxamide (Tiazofurin) (1): The crude compound **8** (4.6 g, 15.9 mmol) was placed in a steel bomb and mixed with freshly prepared methanolic ammonia (saturated at 0°C, 70 mL). The reaction mixture was stirred at room temperature for 12 h. The steel bomb was cooled, opened carefully and the content evaporated to dryness. The residue was triturated with dry ethanol (60 mL) and evaporated to dryness. The residue was treated with dry ethanol (60 mL) which on trituration gave light yellow solid. The solid was filtered, washed with ethyl acetate and dried. The solid was recrystallized from ethanol/ethyl acetate to give pure product. Yield 3.6 g (87%); mp 142-144°C (lit.,[8] 144-145°C). ^{1}H NMR (DMSO-d_6): δ 3.57 (m, 2H), 3.89 (bs, 2H), 4.06 (m,1H), 4.84 (t, 1H), 4.93 (d,1H), 5.06 (m, 1H), 5.37 (d, 1H), 7.57 (s,1H), 7.69 (s, 1H) and 8.21 (s, 1H).

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