Total synthesis of certain 2-, 6-mono- and 2,6-disubstituted-tubercidin derivatives. Synthesis of tubercidin via the sodium salt glycosylation procedure

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Received 15 November 1983; Accepted 29 November 1983

ABSTRACT

Direct glycosylation of the sodium salt of 4,6-dichloro- or 4,6-dibromo-2-methylthiopyrrolo[2,3-d]pyrimidine (6 and 7) with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide gave good yield of the corresponding N₇-glycosylated pyrrolo[2,3-d]pyrimidine. The intermediate 4-amino-6-chloro-2-methylthio-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (10) provided a new synthetic route to tubercidin, via 6-chlorotubercidin (12). 6-Chloro-2-methoxytubercidin was also obtained from 10 via the methylsulfone (14). Application of this glycosylation procedure to 4,6-dichloro- or 4,6-dibromo-2-methylpyrrolo[2,3-d]pyrimidine (17 and 18) also furnished the corresponding N₇-glycosyl derivatives with β-configuration, 21 and 22. Dehalogenation of 21 gave 2-methyltubercidin and bromination with bromine in a buffered solution gave 5,6-dihalo-2-methyltubercidin (23). Several new 2,6-disubstituted tubercidin derivatives were prepared from these glycosyl intermediates. This new sodium salt glycosylation procedure was found to be superior to other procedures for the total synthesis of these halogenated 7-deazapurine nucleosides.

INTRODUCTION

Pyrrolo[2,3-d]pyrimidine (7-deazapurine) nucleosides continue to be of considerable interest both from chemical and biological points of view. Because of the structural resemblance to purine nucleosides and the unusual biological properties of naturally occurring nucleoside antibiotics¹ tubercidin (1), toyocamycin (2), sangivamycin (3) and cadeguomycin (4), there has been an ongoing effort by many investigators to develop synthetic procedures for the

preparation of the nucleoside derivatives of the pyrrolo[2,3-d]pyrimidine ring system.

The adenosine analog tubercidin (1) exhibits a profound cytotoxic effect on a variety of mammalian cell strains in vitro and shows significant antitumor activity in vivo.²⁻⁸ Tubercidin also affects multiplication of both DNA and RNA viruses⁹ and inhibits the growth of a variety of microorganisms.¹⁰ Sangi-vamycin (3) has demonstrated¹¹ potent activity against L1210 leukemia in mice, cytotoxicity against HeLa cells, and in a phase I toxicity study¹² on humans produced no evidence of toxicity at maximally tolerated doses. Although the antitumor activity of toyocamycin (2) is significant,^{6,9,13} its clinical use has been precluded due to severe toxicity, suggesting that structural modifications might possibly reduce the toxicity within the acceptable limits.

Considerable effort has been expended in the development of methods for the chemical synthesis of pyrrolo[2,3-d]pyrimidine nucleosides related to tubercidin. Mizuno et al.^{14,15} synthesized α - and β -anomers of 7-deazainosine by the condensation of 4-amino-5-(2,2-diethoxyethyl)-6-pyrimidone with 2,3,4-tri-O-acetyl-5-O-trityl-D-ribose. The chloromercuri procedure has been successfully employed for the preparation of 4'-thio analogs of toyocamycin.¹⁶ Robins and co-workers developed two separate routes for the synthesis of pyrrolo[2,3-d]pyrimidine nucleosides. The first one involved direct fusion (acid catalyzed as well as noncatalyzed) of an appropriate 7-deazapurine and 1,2,3,5-tetra-O-acetyl- β -D-ribofuranose.^{17,18} However, the yield of the nucleoside product was rather low. The second route utilized a trimethylsilylated 7-deazapurine and acylated sugar halide,^{19,20} by which the yield was much improved. Recently, a phase-transfer glycosylation procedure using a preformed 7-deazapurine and 2,3,5-tri-O-benzyl-D-ribofuranosyl bromide has been developed by Seela and co-workers.²¹⁻²³ However, this procedure cannot generally be used for a halogenated aglycon. The yields are usually low and the removal of the blocking groups troublesome.

We have now developed a new procedure in which a halogenated pyrrolo-[2,3-d]pyrimidine can be successfully used for glycosylation studies. In this paper we describe a method for the rapid synthesis of 4,6-dihalogenated pyrrolo[2,3-d]pyrimidine nucleosides with a β -configuration using the sodium salt of a preformed aglycon (generated in situ), and 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide. Seela et al.²⁴ have previously reported the use of sodium hydride for the glycosylation of 4-chloro-5-methyl-2-methylthiopyrrolo[2,3-d]pyrimidine, but no yield of the isolated protected nucleoside was reported. Goto and co-workers²⁵ also reported the use of sodium hydride in the glycosyla-

tion of 4-methoxy-5-methyl-2-methylthiopyrrolo[2,3-d]pyrimidine with 2,3,5-tri-O-benzyl-D-ribofuranosyl bromide, which gave a complex anomeric mixture and an abnormal by-product. The desired 4-methoxy-5-methyl-2-methylthio-7-(2,3,5-tri-O-benzyl- β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine was isolated in less than 25% yield.

CHEMISTRY

6-Halogensubstituted pyrrolo[2,3-d]pyrimidine nucleosides are not known, except for a recent report by Bergstrom and Brattesani,²⁶ who brominated tubercidin directly with N-bromosuccinimide in DMF buffered with potassium acetate to obtain 6-bromotubercidin. In the present work we elected to use 2-methyl- or 2-methylthio-4,6-dihalopyrrolo[2,3-d]pyrimidine for glycosylation studies to obtain N₇-glycosyl intermediates which could eventually be converted into various desired 6-halotubercidin derivatives. Several general glycosylation procedures were initially tried which included: (a) acid catalyzed fusion procedures, (b) Lewis acid (stannic chloride and trimethylsilyl trifluoromethanesulfonate) catalyzed glycosylation and (3) trimethylsilyl procedure with or without iodine as the catalyst. None of these glycosylation procedures was satisfactory.

In an effort to prepare 4,6-dichloro-2-methylthiopyrrolo[2,3-d]pyrimidine (6), diethyl α -cyanosuccinate²⁷ was converted to the intermediate imino-ether with anhydrous hydrogen chloride gas in ethanol which, without isolation, was ring-closed with 2-methyl-2-thiopseudourea in the presence of sodium ethoxide to give 2-methylthiopyrrolo[2,3-d]pyrimidin-4,6(3H,5H)-dione (5) (Scheme I). Chlorination of 5 with phosphorus oxychloride in the presence of N,N-dimethylaniline afforded 6. A similar halogenation of 5 with phosphorus oxybromide also gave 4,6-dibromo-2-methylthiopyrrolo[2,3-d]pyrimidine (7) in rather low yield. Both 6 and 7 served as versatile starting materials for these glycosylation studies. 2,3,5-Tri-O-benzoyl-D-ribofuranosyl bromide was freshly prepared from 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose according to the method of Fletcher and co-workers.²⁸ The sodium salt of 6, produced *in situ* by sodium hydride in dioxane, was treated with the ribosyl bromide at 70°C. A relatively clean reaction mixture was obtained, which was purified on an open bed silica gel column to give 4,6-dichloro-2-methylthio-7-(2,3,5-tri-O-benzoyl- β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (8) as the major product in 67.8% yield. No attempt was made to isolate the minor products. Compound 8 was found to be sensitive to moisture and our attempts to crystallize it failed. Therefore, without further characterization, compound 8 was treated

Scheme I

with methanolic ammonia at 120–125°C. Debenzoylation of 8 with concomitant nucleophilic displacement of the 4-chloro function to an amino group occurred to give 4-amino-6-chloro-2-methylthio-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (10), which was fully characterized. Treatment of 10 with Raney nickel readily gave 4-amino-6-chloro-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (6-chlorotubercidin, 12). Dehalogenation of 12 with Pd/C in a hydrogen atmosphere provided tubercidin (1), thereby proving the site of glycosylation and the anomeric configuration of 10 and the nucleosides derived from it. This constitutes a new route to the synthesis of tubercidin and is superior to previously published procedures.^{29,30}

Oxidation of 10 with *m*-chloroperoxybenzoic acid in ethanol at room temperature provided the sulfone 14. In the ¹H NMR (DMSO-d₆) of 14, all the protons were shifted downfield as compared to those of 10. The SO₂CH₃ protons were shifted 0.82 ppm, whereas C₁H, C₅H and C₄-NH₂ were shifted by 0.06, 0.18 and 0.66 ppm, respectively. The downfield shift of 14 would be expected

Scheme II

due to the bulky sulfonyl group. Nucleophilic displacement of the methylsulfonyl function of 14 by treatment with sodium methoxide in methanol at reflux temperature gave 4-amino-6-chloro-2-methoxy-7- β -D-ribofuranosylpyrrolo[2,3-d]pyrimidine (15). Bromination of 6-chlorotubercidin (12) with bromine in a buffered acetic acid solution readily gave 4-amino-5-bromo-6-chloro-7- β -D-ribofuranosylpyrrolo[2,3-d]pyrimidine (13) in 89% yield. Such electrophilic halogenation reactions with 7-deazapurine nucleosides have been documented in the literature.³¹ The site of bromination in 13 was determined on the basis of ¹H NMR, which revealed the absence of a peak at δ 6.68 for the C₅H proton. Further structural proof came from elemental analysis.

The glycosylation studies were further extended to 4,6-dibromo-2-methylthiopyrrolo[2,3-d]pyrimidine (7). Direct glycosylation of the sodium salt of 7 with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide gave a complex reaction mixture from which the protected nucleoside 4,6-dibromo-2-methylthio-7-(2,3,5-tri-O-benzoyl- β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (9) was isolated as the major product in 80% yield. As observed in the case of 8, compound 9 was also found to be susceptible to moisture. When 9 was treated with methanolic ammonia at 100°C, 4-amino-6-bromo-2-methylthio-7- β -D-ribofuranosylpyrrolo[2,3-d]pyrimidine (11) was formed. The structural assignment of 11 was by analogy to compound 10, where very similar ultraviolet and ¹H NMR spectral pat-

terns were observed.

For the synthesis of 2-methyltubercidin (25) (Scheme II), 4,6-dihalo-2-methylpyrrolo[2,3-d]pyrimidines were found to be suitable starting materials. Chlorination of 2-methylpyrrolo[2,3-d]pyrimidin-4,6(3H,5H)-dione (16), according to the procedure of Földi et al.³² gave 4,6-dichloro-2-methylpyrrolo[2,3-d]pyrimidine (17). A similar halogenation of 16 with phosphorus oxybromide in the presence of N,N-dimethylaniline gave 4,6-dibromo-2-methylpyrrolo[2,3-d]pyrimidine (18) in 50% yield. Direct glycosylation of the sodium salt of 17 with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide in anhydrous dioxane at 60°C gave 4,6-dichloro-2-methyl-7-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (19) as the sole product in 91% yield, after silica gel column chromatography. Like the nucleoside 8, compound 19 was also found to be unstable. Therefore, without extensive purification, 19 was treated with methanolic ammonia at 100°C, which gave 4-amino-6-chloro-2-methyl-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (21). A similar methodology was used to obtain the corresponding 6-bromo derivative (22) from 18. Dehalogenation of either 21 or 22 with Pd/C in a hydrogen atmosphere provided the desired 2-methyltubercidin (25). Bromination of 21 with bromine in a buffered acetic acid solution gave a 64% yield of 4-amino-5-bromo-6-chloro-2-methyl-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (23). A similar bromination with 22 gave 4-amino-5,6-dibromo-2-methyl-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (24) in 61% yield.

The essentially identical ultraviolet absorption spectra of 2-methyltubercidin and tubercidin (see experimental) indicated the site of glycosylation in 25 to be N₇. The anomeric configuration was assumed to be β in view of the small coupling constant of the anomeric proton³³ in 2-methyltubercidin. This assumption was substantiated by the appearance of the C₁H proton of 25 at δ 5.90 ppm in the ¹H NMR spectrum, which is very similar to that reported²⁵ for 5-methyltubercidin (δ 5.96).

EXPERIMENTAL SECTION

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (¹H NMR) spectra were determined at 90 MHz with JEOL FX-90Q spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of H₂O as indicated by elemental analyses was verified by ¹H NMR. Infrared spectra (IR) were obtained on a Beckman acculab 2 spectrophotometer and ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses

were performed by Robertson Laboratory, Florham Park, NJ, and the results are within 0.4% of the theoretical values. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 (EM Reagents) plates. J. T. Baker silica gel (70-230 mesh) was used for column chromatography. All solvents were reagent grade. Detection of components on TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30°C.

2-Methylthiopyrrolo[2,3-d]pyrimidin-4,6(3H,5H)-dione, 5. An ice-cooled solution of diethyl α -cyanosuccinate²⁷ (65.7 g, 0.33 mol) in absolute ethanol (15.2 g, 0.33 mol) was saturated with anhydrous hydrogen chloride gas (12.5 g). The resulting mixture was allowed to stand at 0-5°C for 48 hours, during which time the imino-ether formed as a thick syrup. Sodium ethoxide in ethanol, prepared by dissolving sodium (30.4 g, 1.32 mol) in absolute ethanol (800 ml), was added dropwise over a one-hour period to the cooled (0-5°C) and stirred mixture of the above imino-ether and 2-methyl-2-thiopseudourea sulfate (45.9 g, 0.165 mol). The reaction mixture was stirred at room temperature for 2 hours and then refluxed for 2 hours. After cooling the reaction mixture to room temperature, the solid that separated was collected by filtration and dissolved in hot water (300 ml). The red solution was treated with decolorizing carbon, filtered and acidified with conc. hydrochloric acid. The title compound 5 precipitated as a white chromatographically pure solid to yield 10.5 g (16.1%); mp > 300°C; UV λ_{max} (MeOH) 237 nm (ϵ 18,400), 298 (5,300). Anal. Calcd. for $\text{C}_7\text{H}_7\text{N}_3\text{O}_2\text{S}$: C, 42.63; H, 3.58; N, 21.31. Found: C, 42.47; H, 3.60; N, 21.56.

4,6-Dichloro-2-methylthiopyrrolo[2,3-d]pyrimidine, 6. A mixture of dry 5 (8.2 g, 0.041 mol), phosphorus oxychloride (80 ml) and freshly distilled N,N-dimethylaniline (18 ml) was heated under reflux for an hour with the exclusion of moisture. The dark reaction mixture was evaporated to one-third the volume and poured with stirring into crushed ice (~ 500 g). The resulting dark red solution was extracted with chloroform (5 X 100 ml). The combined organic layers were dried (Na_2SO_4) and evaporated to an oil. The oil was dissolved in ethanol (150 ml), treated with decolorizing carbon, filtered and the filtrate was diluted with water (300 ml). The desired compound 6 precipitated as a light yellow solid to yield 4.2 g (43%). An analytical sample was obtained by crystallization from methanol; mp 220°C; UV λ_{max} (MeOH) 226 nm (ϵ 8,400), 248 (11,200), 278 (7,400), 314 (7,500); ^1H NMR ($\text{DMSO}-d_6$) δ 2.70 (s, 3, SCH_3), 6.65 (s, 1, C_5H). Anal. Calcd. for $\text{C}_7\text{H}_5\text{Cl}_2\text{N}_3\text{S}$: C, 35.91; H, 2.15; N, 17.95; Cl, 30.29. Found: C, 35.86; H, 2.20; N, 17.75; Cl, 30.13.

4,6-Dibromo-2-methylthiopyrrolo[2,3-d]pyrimidine, 7. A mixture of dry 5 (4.0 g, 0.02 mol), phosphorus oxybromide (27 g) and N,N-dimethylaniline (3 ml) was heated at 130°C (bath temperature) for 15 minutes under anhydrous conditions. After cooling to room temperature, the reaction mixture was poured into ice-water (~ 200 g). The pH of the aqueous solution was adjusted to 4 with concentrated ammonium hydroxide. The black precipitate that separated was collected by filtration and purified several times on a silica gel column, using toluene as the solvent to yield 1.3 g (20%) of pure 7; mp 228°C. An analytical sample was obtained by crystallization from aqueous acetone, mp 230°C; UV λ_{max} (MeOH) 229 nm (ϵ 13,200), 249 (16,300), 318 (8,000); ^1H NMR (DMSO- d_6) δ 2.56 (s, 3, SCH_3), 6.64 (s, 1, C_5H). Anal. Calcd. for $\text{C}_7\text{H}_5\text{Br}_2\text{N}_3\text{S}$: C, 26.03; H, 1.56; N, 13.01; Br, 49.48. Found: C, 25.86; H, 1.67; N, 12.99; Br, 49.64.

4-Amino-6-chloro-2-methylthio-7- β -D-ribofuranosylpyrrolo[2,3-d]pyrimidine, 10.

To a suspension of 6 (2.34 g, 0.01 mol) in dry dioxane (30 ml) was added sodium hydride (50% in oil, 0.50 g, 0.0104 mol) and the mixture was stirred at room temperature in an atmosphere of dry nitrogen for 20 minutes. A solution of 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (prepared from 6.0 g of 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose) in dry dioxane (30 ml) was added dropwise with stirring. The mixture was stirred at room temperature for an hour and at 70°C for an hour before it was filtered to remove the insoluble material. The filtrate was adsorbed onto silica gel (~ 20 g) and evaporated to dryness. Coevaporation with toluene (3 X 50 ml) gave a dry residue, which was placed on top of a silica gel column (2.5 X 40 cm) prepacked in toluene. Elution with toluene gave chromatographically homogeneous 4,6-dichloro-2-methylthio-7-(2,3,5-tri-O-benzoyl- β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (8) as a foam, 4.6 g (67.8%). A solution of 8 (4.5 g) in methanolic ammonia (saturated at 0°C, 65 ml) was heated in a steel bomb at 120–125°C for 24 hours and the resulting brown solution was evaporated to dryness. The residue was dissolved in methanol (50 ml) and adsorbed onto silica gel (~ 50 g). Coevaporation with methanol (3 X 50 ml) gave a dry residue, which was placed on top of a silica gel column (3 X 45 cm) prepacked in chloroform. The column was eluted with a gradient of chloroform-methanol. The desired nucleoside 10 was eluted at 15% methanolic chloroform and crystallized from 50% aqueous ethanol to yield 0.78 g (34%) as fine needles; mp 228–230°C; UV λ_{max} (pH 1) 283 nm (ϵ 13,800); UV λ_{max} (pH 7 and 11) 235 nm (ϵ 16,200), 285 (13,700); ^1H NMR (DMSO- d_6) δ 2.46 (s, 3, SCH_3), 5.84 (d, 1, $J = 6.5$ Hz, C_1H), 6.66 (s, 1, C_5H), 7.22 (br, s, 2, NH_2 , exchanged with D_2O) and other sugar protons. Anal. Calcd. for $\text{C}_{12}\text{H}_{15}\text{ClN}_4\text{O}_4\text{S}$: C, 41.56; H, 4.36; N, 16.15; Cl, 10.22. Found:

C, 41.38; H, 4.37; N, 16.09; Cl, 10.27.

4-Amino-6-bromo-2-methylthio-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine. 11.

To a solution of 7 (2.6 g, 0.008 mol) in dry dioxane (30 ml) was added sodium hydride (50% in oil, 0.45 g, 0.009 mol) and the mixture was stirred with slight warming (~ 40°C) for 30 minutes. A solution of 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl bromide (prepared from 5.1 g of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose) in anhydrous dioxane (20 ml) was added dropwise over a 20 minute period and the stirring was continued at room temperature for an hour. The insoluble material was removed by filtration and the filtrate was evaporated to an oil, which was chromatographed on a silica gel column (2.5 X 40 cm) prepacked in toluene. Elution with toluene gave 5.5 g (80%) of the benzoylated nucleoside 9 as a foam.

Compound 9 (5.1 g) and methanolic ammonia (saturated at 0°C, 60 ml) were placed in a steel bomb. The bomb was heated at 100°C for 6 hours. The dark green solution was evaporated to dryness and the residue was purified on a silica gel column (3 X 40 cm) using a gradient of chloroform-methanol. The title compound was eluted at 15% methanolic chloroform and crystallization from water gave 0.48 g (18.5%) as needles; mp 225-227°C; UV λ_{max} (pH 1) 285 nm (ϵ 18,900); UV λ_{max} (pH 7 and 11) 236 nm (ϵ 21,700), 288 (18,400); ^1H NMR (DMSO- d_6) δ 2.52 (s, 3, SCH_3), 5.84 (d, 1, $J = 6.0$ Hz, C_1H), 6.80 (s, 1, C_5H), 7.28 (br, s, 2, NH_2 , exchanged with D_2O) and other sugar protons. Anal. Calcd. for $\text{C}_{12}\text{H}_{15}\text{BrN}_4\text{O}_5\text{S}$: C, 36.84; H, 3.86; N, 14.32; Br, 20.42. Found: C, 36.94; H, 3.97; N, 14.27; Br, 20.41.

4-Amino-6-chloro-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (6-Chlorotubercidin, 12). To a solution of 10 (0.34 g, 0.001 mol) in *n*-propanol (20 ml) was added Raney nickel (wet weight, 2 g) and the mixture was heated under reflux for 5 hours. After cooling to room temperature, the mixture was filtered through a Celite pad and the filtrate was evaporated to dryness. The residue was purified on a preparative silica gel tlc plate using CHCl_3 :MeOH (8:2) as the solvent. A faster moving compound with $R_f = 0.46$ was isolated and identified as 6-chlorotubercidin. After crystallization from water, the title compound was obtained as needles, 0.12 g (40%); mp 182°C; UV λ_{max} (pH 1) 226 nm (ϵ 17,500), 273 (13,700); UV λ_{max} (pH 7 and 11) 274 nm (ϵ 14,100); ^1H NMR (DMSO- d_6) δ 5.86 (d, 1, $J = 6.0$ Hz, C_1H), 6.68 (s, 1, C_5H), 7.24 (br, s, 2, NH_2 , exchanged with D_2O), 8.24 (s, 1, C_2H) and other sugar protons. Anal. Calcd. for $\text{C}_{11}\text{H}_{13}\text{ClN}_4\text{O}_4\cdot\text{H}_2\text{O}$: C, 41.45; H, 4.74; N, 17.57; Cl, 11.12. Found: C, 41.42; H, 4.90; N, 17.45; Cl, 11.20.

A slower moving compound with $R_f = 0.25$ was also isolated and identified

as tubercidin, yield 0.025 g (9%); mp 245-247°C.

4-Amino-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (Tubercidin, 1). To a solution of 12 (0.06 g, 0.0002 mol) in aqueous n-propanol (1:9, 25 ml) containing potassium carbonate (30 mg) was added Pd/C (5%, 100 mg) and the mixture was hydrogenated at 2 atm for 8 hours. The mixture was filtered through a Celite pad and the filtrate was evaporated to dryness. Crystallization of the residue from a small volume of water provided 42 mg (79%) of tubercidin as needles; mp 246-248°C (Lit.¹⁰ mp 247-248°C). UV $\lambda_{\max}$ (pH 1) 226 nm (ϵ 22,700), 270.5 (12,000); UV $\lambda_{\max}$ (pH 7 and 11) 270 nm (ϵ 11,900). [Lit.¹⁰ UV $\lambda_{\max}$ (0.01N HCl) 227 nm, 272 (ϵ 12,200); UV $\lambda_{\max}$ (0.01N NaOH) 270 nm (ϵ 12,100)]; ¹H NMR (DMSO-d₆) δ 6.02 (d, 1, J = 5.5 Hz, C₁H), 6.61 (d, 1, J = 3.5 Hz, C₅H), 7.035 (br, s, 2, NH₂), 7.39 (d, 1, J = 3.5 Hz, C₆H), 8.10 (s, 1, C₂H) and other sugar protons.

4-Amino-5-bromo-6-chloro-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine, 13. To a stirred solution of 12 (0.06 g, 0.0002 mol) and sodium acetate (0.04 g, 0.0005 mol) in glacial acetic acid (3 ml) was added a solution of bromine (0.04 g, 0.00025 mol) in acetic acid (1 ml) dropwise over a period of 10 minutes. After stirring for an additional 10 minutes, the mixture was evaporated to dryness and the residue was coevaporated with water (2 X 10 ml). The resulting residue was triturated with cold water (2 ml) and the precipitate that separated was collected by filtration. Crystallization from aqueous ethanol gave the title compound as a monohydrate, 0.071 g (89%); mp 210-212°C. UV $\lambda_{\max}$ (MeOH) 284 nm (ϵ 10,400); ¹H NMR (DMSO-d₆) δ 5.96 (d, 1, J = 6.0 Hz, C₁H), 7.00 (br, s, 2, NH₂, exchanged with D₂O), 8.14 (s, 1, C₂H) and other sugar protons. Anal. Calcd. for C₁₁H₁₂BrClN₄O₄·H₂O: C, 33.22; H, 3.55; N, 14.09. Found: C, 33.08; H, 3.78; N, 14.30.

4-Amino-6-chloro-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine-2-methylsulfone, 14. To a solution of 10 (0.345 g, 0.001 mol) in ethanol (14 ml) was added m-chloroperoxybenzoic acid (0.45 g, 0.0022 mol) portionwise over 4 hours. After the addition was complete, the mixture was stirred for an additional hour and then poured into anhydrous ether (50 ml). The pale yellow precipitate that separated was collected by filtration, washed with ether (2 X 25 ml) and crystallized from aqueous ethanol to yield 0.28 g (75%); mp 233-236°C (dec. > 220°C); UV $\lambda_{\max}$ (MeOH) 273 nm (ϵ 9,600), 299 (9,700); ¹H NMR (DMSO-d₆) δ 3.28 (s, 3, SO₂CH₃), 5.90 (d, 1, J = 6.0 Hz, C₁H), 6.84 (s, 1, C₅H), 7.88 (br, s, 2, NH₂, exchanged with D₂O) and other sugar protons. Anal. Calcd. for C₁₂H₁₅ClN₄O₆S: C, 38.05; H, 3.99; N, 14.79; S, 8.46. Found: C, 38.27; H,

4.23; N, 14.59; S, 8.64.

4-Amino-6-chloro-2-methoxy-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine, 15.

To a solution of 14 (0.20 g, 0.00053 mol) in anhydrous methanol (5 ml) was added sodium methoxide in methanol (prepared by dissolving 23 mg of sodium in 5 ml of methanol) over a period of one hour and the resulting solution was heated under reflux for an hour. After cooling to room temperature, the mixture was neutralized with acetic acid, evaporated to dryness and the residue was crystallized twice from 50% aqueous ethanol to obtain 0.95 g (54.5%) of the title compound as a hemihydrate; mp 222°C; UV $\lambda_{\max}$ (pH 1) 225 nm (ϵ 24,000), 260 (10,700), 292 (8,300); UV $\lambda_{\max}$ (pH 7 and 11) 264 nm (ϵ 12,000), 275 sh (6,000); ^1H NMR (DMSO- d_6) δ 3.42 (s, 3, OCH₃) 5.76 (d, 1, J = 6.0 Hz, C₁H), 6.60 (s, 1, C₅H), 7.22 (br, s, 2, NH₂, exchanged with D₂O) and other sugar protons. Anal. Calcd. for C₁₂H₁₅ClN₄O₅·0.5H₂O: C, 42.42; H, 4.74; N, 16.49. Found: C, 42.33; H, 4.70; N, 16.46.

4,6-Dibromo-2-methylpyrrolo[2,3-d]pyrimidine, 18. A mixture of 2-methylpyrrolo[2,3-d]pyrimidin-4,6(3H,5H)-dione³² (16, 4.0 g, 0.024 mol), phosphorus oxybromide (25 g) and N,N-dimethylaniline (3 ml) was heated at 130°C (bath temperature) for 15 minutes. After cooling to room temperature, the reaction mixture was poured into crushed ice (~250 g) and the aqueous solution was adjusted to pH 4 with 1 N NaOH. The dark-grey product that separated was collected by filtration, dissolved in chloroform:acetone (300 ml, 1:1, v/v) and filtered. The filtrate was evaporated to dryness and the residue was purified on a silica gel column (3 X 45 cm) prepacked in toluene. Elution with 1% acetone in toluene gave 3.5 g (50%) of the title compound. A small sample was crystallized from aqueous acetone for analysis; mp 253°C; UV $\lambda_{\max}$ (MeOH) 227 nm (ϵ 7,700), 292 (4,400); ^1H NMR (DMSO- d_6) δ 2.60 (s, 3, CH₃), 6.56 (s, 1, C₅H). Anal. Calcd. for C₇H₅Br₂N₃: C, 28.89; H, 1.73; N, 14.44; Br, 54.92. Found: C, 28.73; H, 1.60; N, 14.37; Br, 54.73.

4-Amino-6-chloro-2-methyl-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine, 21. A mixture of 4,6-dichloro-2-methylpyrrolo[2,3-d]pyrimidine³² (17, 2.02 g, 0.01 mol) and sodium hydride (50% in oil, 0.52 g, 0.0108 mol) in anhydrous dioxane was stirred at ambient temperature for 30 minutes in an atmosphere of dry nitrogen. A solution of 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (prepared from 5.8 g of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose) in anhydrous dioxane (30 ml) was added over 20 minutes. After stirring at room temperature for one hour and at 60°C for an additional hour, the reaction mixture was worked up as described for 10 to obtain 5.9 g (91%) of the protected nucleo-

side 19 as an amorphous foam.

A solution of 19 (5.70 g) in methanolic ammonia (saturated at 0°C, 60 ml) was heated at 100°C for 10 hours in a steel bomb (125 ml). Methanolic ammonia was evaporated and the residue was purified on a silica gel column (3 X 50 cm) prepacked in chloroform. Elution with chloroform:methanol gradient gave a homogeneous product which, after crystallization from 50% aqueous ethanol, gave 21 as needles, 1.01 g (35%); mp 228-230°C; UV λ_{max} (pH 1) 226 nm (ϵ 20,000), 272 (14,300); UV λ_{max} (pH 7 and 11) 274 nm (ϵ 15,200); ^1H NMR (DMSO- d_6) δ 2.38 (s, 3, CH_3), 5.90 (d, 1, $J = 6.5$ Hz, C_1H), 6.88 (s, 1, C_5H), 7.20 (br, s, 2, NH_2 , exchanged with D_2O) and other sugar protons. Anal. Calcd. for $\text{C}_{12}\text{H}_{15}\text{ClN}_4\text{O}_4$: C, 45.79; H, 4.80; N, 17.81; Cl, 11.26. Found: C, 45.86; H, 4.87; N, 17.82; Cl, 11.47.

4-Amino-6-bromo-2-methyl-7- β -D-ribofuranosylpyrrolo[2,3-d]pyrimidine, 22. To a solution of 18 (2.70 g, 0.0093 mol) in anhydrous acetonitrile (25 ml) was added sodium hydride (50% in oil, 0.50 g, 0.0104 mol) and the mixture was stirred at room temperature for 20 minutes in an atmosphere of dry nitrogen. A solution of 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (prepared from 6.0 g of 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose) in dry acetonitrile (20 ml) was added over 30 minutes. After stirring at room temperature for 1.5 hours, the reaction mixture was worked up as described for 10 to obtain 5.9 g (86%) of the protected nucleoside 20 as an amorphous foam.

A solution of 20 (8.0 g) in methanolic ammonia (saturated at 0°C, 80 ml) was heated at 100°C for 10 hours in a steel bomb (200 ml). The methanolic ammonia was evaporated and the residue was co-evaporated with water (3 X 50 ml) before it was purified on a silica gel column (2.5 X 50 cms) prepacked in chloroform. The column was eluted with chloroform-methanol gradient and the title compound eluted at 20% methanolic chloroform. Crystallization of the homogeneous product from ethyl acetate gave 1.38 g (36%) of 22 as needles; mp 219-221°C; UV λ_{max} (pH 1) 227 nm (ϵ 20,100), 275 (15,000); UV λ_{max} (pH 7 and 11) 277 nm (ϵ 15,100); ^1H NMR (DMSO- d_6) δ 2.36 (s, 3, CH_3), 5.86 (d, 1, $J = 6.5$ Hz, C_1H), 6.76 (s, 1, C_5H), 7.22 (br, s, 2, NH_2) and other sugar protons. Anal. Calcd. for $\text{C}_{12}\text{H}_{15}\text{BrN}_4\text{O}_4$: C, 40.13; H, 4.21; N, 15.60; Br, 22.25. Found: C, 40.35; H, 4.33; N, 15.49; Br, 22.32.

4-Amino-2-methyl-7- β -D-ribofuranosylpyrrolo[2,3-d]pyrimidine (2-Methyltubercidin, 25). To a solution of 22 (0.40 g, 0.0011 mol) in 80% aqueous *n*-propanol (20 ml) containing potassium carbonate (80 mg) was added Pd/C (10%, 60 mg) and the mixture was hydrogenated at 2 atm for 5 hours. The mixture was filtered through a Celite pad and the filtrate was evaporated to dryness. Crystalliza-

tion of the residue from water gave 0.28 g (91%) of the title compound as needles; mp 246–248°C; UV $\lambda_{\max}$ (pH 1) 227 nm (ϵ 17,300), 271 (8,200); UV $\lambda_{\max}$ (pH 7 and 11) 271 nm (ϵ 9,200); ^1H NMR ($\text{DMSO}-d_6$) δ 2.36 (s, 3, CH_3), 5.90 (d, 1, J = 6.5 Hz, C_1H), 6.50 (d, 1, J = 3.5 Hz, C_5H), 6.98 (br, s, 2, NH_2 , exchanged with D_2O), 7.20 (d, 1, J = 3.5 Hz, C_6H) and other sugar protons. Anal. Calcd. for $\text{C}_{12}\text{H}_{16}\text{N}_4\text{O}_4$: C, 51.42; H, 5.75; N, 19.91. Found: C, 51.53; H, 5.67; N, 19.97.

4-Amino-5-bromo-6-chloro-2-methyl-7- β -D-ribofuranosylpyrrolo[2,3-d]pyrimidine, 23. To a solution of 21 (0.315 g, 0.001 mol) in glacial acetic acid (12 ml) containing sodium acetate (0.245 g, 0.003 mol) was added a solution of bromine (0.18 g, 0.0011 mol) in acetic acid (2 ml), dropwise. The mixture was stirred at room temperature for 15 minutes before it was evaporated to dryness. The residue was co-evaporated with water (2 X 10 ml), dissolved in minimum volume of water (~ 8 ml) and neutralized with concentrated ammonium hydroxide. The product that separated was collected by filtration and crystallized from 50% aqueous ethanol to yield 0.25 g (64%); mp 217–220°C (dec.); UV $\lambda_{\max}$ (MeOH) 281 nm (ϵ 13,200); ^1H NMR ($\text{DMSO}-d_6$) δ 2.40 (s, 3, CH_3), 5.94 (d, 1, J = 6.5 Hz, C_1H), 6.92 (br, s, 2, NH_2) and other sugar protons. Anal. Calcd. for $\text{C}_{12}\text{H}_{14}\text{BrClN}_4\text{O}_4$: C, 36.61; H, 3.58; N, 14.23. Found: C, 36.50; H, 3.60; N, 14.08.

4-Amino-5,6-dibromo-2-methyl-7- β -D-ribofuranosylpyrrolo[2,3-d]pyrimidine, 24. To a solution of 22 (0.25 g, 0.0007 mol) in glacial acetic acid (7 ml) containing sodium acetate (0.245 g, 0.003 mol) was added a solution of bromine (0.14 g, 0.009 mol) in acetic acid (1 ml), dropwise. After stirring at room temperature for 15 minutes, the reaction mixture was worked up as described for 23 to obtain 0.20 g (61%) of the title compound as the monohydrate; mp 208–210°C; UV $\lambda_{\max}$ (pH 1) 235 nm (ϵ 19,700), 285 (11,700); UV $\lambda_{\max}$ (pH 7 and 11) 283 nm (ϵ 11,900); ^1H NMR ($\text{DMSO}-d_6$) δ 2.40 (s, 3, CH_3), 5.92 (d, 1, J = 6.5 Hz, C_1H), 6.94 (br, s, 2, NH_2 , exchanged with D_2O) and other sugar protons. Anal. Calcd. for $\text{C}_{12}\text{H}_{14}\text{Br}_2\text{N}_4\text{O}_4 \cdot \text{H}_2\text{O}$: C, 31.60; H, 3.53; N, 12.28. Found: C, 31.77; H, 3.53; N, 12.13.

ACKNOWLEDGMENT

This investigation was supported by Grant Number R01 AI 15324-04, awarded by the National Cancer Institute, DHEW.

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