Synthesis of 5-Methyltubercidin and Its α -Anomer via Condensation of the Anion of 4-Methoxy-5-methyl-2-methylthiopyrrolo [2, 3-d] pyrimidine and 2, 3, 5-Tri-O-benzyl-D-ribofuranosyl Bromide

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5-Methyltubercidin and its α -anomer were synthesized from 4-methoxy-5-methyl-2-methylthio-7-(2,3,5-tri-O-benzyl- α - and β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidines, which in turn prepared by condensation of anion of 4-methoxy-5-methyl-2-methylthiopyrrolo[2,3-d]pyrimidine with 2,3,5-tri-O-benzyl-D-ribofuranosyl bromide. The anomeric configuration was rigorously established by deriving 5-methyltubercidin to the quaternary cyclonucleoside.

Three 7-deazapurine nucleoside antibiotics, tubercidin,^{1,2)} toyocamycin,³⁾ and sangivamycin,⁴⁾ have been isolated from strains of *Streptomyces*. They have antitumor activity. Recently nucleoside Q was isolated from the first position of anticodon of *Escherichia coli* tRNA^{Tyr}, tRNA^{His}, tRNA^{Asn}, and tRNA^{Asp},⁵⁾ and its structure elucidated to be **1**,⁶⁾ which belongs to the 7-deazapurine nucleosides. A modified nucleoside Q, Q*, was also found in tRNA's of various animals⁷⁾ and its structure determined.⁸⁾

Several methods for the synthesis of 7-deazapurine nucleosides have been developed. Mizuno *et al.*²⁾ synthesized α - and β -7-deazainosine through condensation of 4-amino-5-(2,2-diethoxyethyl)-6-hydroxypyrimidine with 2,3,4-tri-O-acetyl-5-O-trityl-D-ribose. Tolman *et al.* developed two routes of synthesis of the nucleosides: one⁹⁾ involved direct fusion of a 7-deazapurine and tri-O-benzoyl-D-ribofuranosyl acetate, though the yield is very low, and the other¹⁰⁾ was using a silylated 7-deazapurine as the reactant, by which the yield is much improved. These methods, however, gave only β -nucleosides.

Toyocamycin and sangivamycin as well as the nucleoside Q have one carbon atom at the 5-position of pyrrolo[2,3-d]pyrimidine nucleus. As common intermediates for the synthesis of these nucleosides, we selected

FIG. 1.

appropriately protected 4-methoxy-5-methyl-2-methylthio-7-(α - and β -D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidines. In this report we describe the synthesis of 5-methyltubercidin (**2**) and its α -anomer (**3**) via this common intermediates, which were prepared by condensation of the anion of 4-methoxy-5-methyl-2-methylthiopyrrolo[2,3-d]pyrimidine (**8**) and 2,3,5-tri-O-benzyl-D-ribofuranosyl bromide (**9**). This method gave satisfactory yields of both α - and β -nucleosides. Anomeric configuration of these anomers was rigorously established (*vide infra*). Nucleoside Q has been synthesized from the intermediate (**10**) as is reported elsewhere.

Cyanoacetal **4** was condensed with thiourea in the presence of sodium ethoxide¹¹⁾ and then treated with hydrochloric acid to give 2-mercapto-7-methyl-7-deazahypoxanthine (**5**). Methylation of **5** with methyl iodide and sodium hydroxide afforded S-methyl derivative **6** in 97% yield. Chlorination of **6** with phosphorus oxychloride and dimethylaniline afforded **7** in a nearly quantitative yield. Displacement of chlorine of **7** with methoxy group was achieved by refluxing with sodium methoxide in methanol giving **8** in 74% yield.

2,3,5-Tri-O-benzyl-D-ribofuranosyl bromide (**9**) was prepared from 2,3,5-tri-O-benzyl-1-O-*p*-nitrobenzoyl- β -D-ribose according to the method of Fletcher *et al.*¹²⁾ just before use. Anion of the deazapurine (**8**) produced by sodium hydride was condensed with the ribofuranosyl bromide (**9**) and the product was chromatographed to give three compounds: β -nucleoside **10**, α -nucleoside **11**, and abnormal by-product **12** in 25%, 25%, and 9% yield, respectively. The β -nucleoside (**10**) was converted to 7-methyltubercidin (**2**) by a series of reactions: desulfurization of **10** with Raney nickel followed by hydrolysis with hydrochloric acid gave the benzylated 7-methyl-7-deazainosine (**17**), which was debenzylated by catalytic hydrogenation to **19**. After the ribose moiety of **19** was protected by isopropylidena-tion and acetylation, 7-deazahypoxanthine

FIG. 2.

FIG. 3.

group was converted to 7-deazaadenine group by chlorination followed by ammonolysis. Hydrolysis of the protecting group of **27** with aq. trifluoroacetic acid afforded 7-methyltubercidin (**2**). The overall yield of **2** from 7-methyl-7-deazainosine (**19**) was *ca.* 21%. The α -anomer (**3**) was also prepared similarly from the α -nucleoside **11**.

The position of attachment of the sugar moiety on the 7-deazapurine of the β - and α -nucleosides, **10** and **11**, was determined as follows: that 4-methoxy-5-methyl-2-methylthiopyrrolo[2,3-d]pyrimidine has the 7H-structure was evident from its NMR spectrum, on which H-6 signal was spin-coupled with N-H signal (2.2 Hz). Since UV spectra of **10** and **11** (λ_{max}^{DMSO} 288 nm, ϵ 12700) was practically identical with the deazapurine (**8**) (λ_{max}^{DMSO} 287 nm, ϵ 11000), the sugar moiety of the nucleosides must be attached at 7-position. Anomeric configuration of the nucleosides, **10** and **11**, was established by the method of Clark *et al.*¹³⁾ Thus, the nucleosides were converted to the corresponding 3',4'-acetone-5'-*p*-toluenesulfonates, **29** and **30**. One nucleoside, **29**, was

very unstable and could be converted easily to the quaternary salt (31), whereas the other (30) was recovered unchanged by heating. The cyclonucleoside *p*-toluenesulfonate (31) was transformed to its iodide (32). NMR spectra of the *p*-toluenesulfonate (31) and the iodide (32) were different in deuteriochloroform solution, but superimposable (except the signals of *p*-toluenesulfonic acid) to each other in deuterium oxide. In the former solution, the salts formed an ion pair and gave different spectra. The quaternary nature of the cyclonucleoside (31) was further confirmed by paper electrophoresis: at pH 7.9 the cyclonucleoside moved toward cathod, whereas no movement was observed with 7-methyltubercidin acetone (27). Thus, nucleoside 2 and 3 have β - and α -configuration, respectively.

The tentative structure 12 was assigned to the abnormal by-product of the condensation from the following evidence: the compound has only two benzyl groups as evident from its NMR spectrum and that the anomeric hydroxyl group is free was shown by treatment with Dowex 50 (H^+ type) in methanol affording its methyl glycoside (13). Detailed analysis of NMR spectra of the methyl glycoside (13) and the 1-acetate (14) suggested structure 12 of the compound, but some ambiguity remains on the configuration at 2-position.

EXPERIMENTAL

All melting points are uncorrected. The spectra were recorded on the following instruments: IR spectra, JASCO IR-G; UV spectra, Hitachi EPS-3T; $[\alpha]_D$, JASCO DIP-4; NMR spectra, JEOL JNM-FX 100 (FT 100 MHz); mass spectra, JEOL D-100 and SG-1 (high resol.).

5-Methyl-2-mercaptopyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (5)

Thiourea (12.5 g) was dissolved in ethanol (355 ml) containing 0.5 *N* sodium ethoxide. To this solution was added the cyanoacetal (4)⁽¹¹⁾ (33 g) dissolved in ethanol (30 ml), and the mixture was refluxed for 80 min. After evaporation *in vacuo*, the solid residue was stirred with 2 *N* HCl (650 ml) at room temp. for 150 min to give white precipitates (28 g), which were collected and crystallized from aq. ethanol to give prisms, mp above

300°C, in nearly quantitative yield. NMR (DMSO- d_6) δ : 2.16 (3H, d, $J=1$ Hz), 6.47 (1H, sextet, H-6, $J=1$ and 2 Hz), 10.89, 11.71 and 13.05 (3H, exchangeable H); UV λ_{max}^{MeOH} nm (ϵ): 244 (6570), 303 (10500); IR ν_{max}^{KBr} cm^{-1} : 3200, 1670, 1605, 1160, 1110; MS m/e : 181 (M^+). Found: C, 45.70; H, 3.93; N, 22.71. Calcd. for $C_7H_7N_3OS$: C, 46.39; H, 3.89; N, 23.19%.

5-Methyl-2-methylthiopyrrolo[2,3-*d*]pyrimidin-4-one (6)

To a solution of the thiol 5 (4.09 g) in 1 *N* NaOH (37.3 ml) and methanol (8.6 ml) was added dropwise methyl iodide (8.6 ml) and the mixture was stirred at room temp. for 45 min. After evaporation to half of the volume, the solution was diluted with 2 *N* HCl (30 ml) giving white precipitates (4.23 g), mp above 300°C. NMR (DMSO- d_6) δ : 2.23 (3H, d, $J=1$ Hz), 2.49 (3H, s), 6.62 (1H, sextet, H-6, $J=1$ and 2 Hz), 11.37 (exchangeable H); UV λ_{max}^{MeOH} nm (ϵ): 223 (8850), 290 (6200); IR ν_{max}^{KBr} cm^{-1} : 2900, 1665, 1590; MS m/e : 195 (M^+).

4-Chloro-5-methyl-2-methylthiopyrrolo[2,3-*d*]pyrimidine (7)

A mixture of the pyridone 6 (8.0 g), $POCl_3$ (80 ml) and *N,N*-dimethylaniline (8 ml) was refluxed for 38 min. After cooling the mixture was poured on ice-water, stirred for 60 min at room temp. and extracted with CH_2Cl_2 . Usual work-up of the extract gave a solid product, which was crystallized from benzene as colorless needles (7.9 g), mp 211~212°C (sealed tube). NMR (DMSO- d_6) δ : 2.36 (3H, d, $J=1$ Hz), 2.54 (3H, s), 7.23 (1H, sextet, H-6), 12.02 (exch. H); UV λ_{max}^{MeOH} nm (ϵ): 224 (9500), 255 (18700), 280sh (4420), 315 (4020); IR ν_{max}^{KBr} cm^{-1} : 3100, 1615, 1210, 865; MS m/e : 213 and 215 (M^+). Found: C, 44.72; H, 3.73; N, 19.96. Calcd. for $C_8H_8N_3S$: C, 44.96; H, 3.77; N, 19.66%.

4-Methoxy-5-methyl-2-methylthiopyrrolo[2,3-*d*]pyrimidine (8)

The chloride 7 (2.3 g) was refluxed with 0.5 *N* sodium methoxide in methanol (150 ml) for 36 hr. After cooling, the resultant crystals were collected and recrystallized from methanol to give colorless prisms (1.65 g), mp 181~183°C. NMR (DMSO- d_6) δ : 2.27 (3H, d, $J=1$ Hz), 2.36 (3H, s), 4.00 (3H, s), 6.89 (1H, sextet, H-6); UV λ_{max}^{MeOH} nm (ϵ): 242 (11800), 286 (8080); IR ν_{max}^{KBr} cm^{-1} : 3100, 2900, 1610, 1560, 1090; MS m/e : 209 (M^+). Found: C, 50.89; H, 5.29; N, 19.90. Calcd. for $C_9H_{11}N_3OS$: C, 51.65; H, 5.30; N, 20.08%.

Condensation of 4-methoxy-5-methyl-2-methylthiopyrrolo[2,3-*d*]pyrimidine (8) with 2,3,5-tri-*O*-benzyl-*D*-ribofuranosyl bromide (9)

The bromide 9 was prepared from 2,3,5-tri-*O*-

benzyl-1-O-*p*-nitrobenzoyl- β -D-ribose (16.4 g, 0.0288 mol) according to the method of Fletcher *et al.*¹⁸⁾ just before use. CH_2Cl_2 solution of the bromide was dried up below 0°C *in vacuo* and the residue was dissolved in dimethoxyethane (40 ml). To a stirred suspension of deazapurine **8** (5.97 g, 0.0285 mol) in dimethoxyethane (42 ml) at -15°C was added 50% NaH dispersion in mineral oil (1.48 g, 0.030 mol) in small portions during 20 min. Then, dry dimethylformamide (14 ml) was added to the mixture and stirring was continued at room temp. for further 30 min. To this mixture was added dropwise during 45 min the dry ice-cooled solution of 2,3,5-tri-O-benzylribofuranosyl bromide (40 ml) prepared before. The reaction mixture was evaporated and the residue was treated with a mixture of CH_2Cl_2 and water. The unchanged deazapurine (**8**) was recovered as insoluble precipitates. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to dryness. The residue was chromatographed on a silica gel column by elution with benzene to give the β -nucleoside (**10**) (4.35 g, 25%), α -nucleoside (**11**) (4.35 g, 25%), and an abnormal by-product (**12**) (1.33 g, 9%).

4-Methoxy-5-methyl-2-methylthio-7-(2,3,5-tri-O-benzyl- β -D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (10**):** needles from isopropanol, mp 105°C; UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 245 (17100), 285 (11000); NMR (CDCl_3) δ : 2.14 (3H, d, $J=1$ Hz), 2.55 (3H, s), 3.67 and 3.80 (each 1H, dd, H-5', $J_{5'6'}=10.5$ Hz, $J_{4'5'}=2.7$ Hz), 4.05 (3H, s), 4.08~4.40 (3H, H-2', 3' and 4'), 4.40~4.68 (6H, PhCH_2), 6.40 (1H, d, H-1', $J=2.5$ Hz), 6.76 (1H, br.s, H-6), 7.16~7.28 (15H, arom); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 2900, 1590, 1450, 1235, 1030; $[\alpha]_D^{25}+99.5^\circ$ ($c=0.2$, CHCl_3); MS m/e : 611 (M^+). Found: C, 68.86; H, 6.07; N, 6.86. Calcd. for $\text{C}_{36}\text{H}_{37}\text{N}_5\text{O}_6\text{S}$: C, 68.71; H, 6.10; N, 6.87%.

4-Methoxy-5-methyl-2-methylthio-7-(2,3,5-tri-O-benzyl- α -D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (11**):** syrup; NMR (CDCl_3) δ : 2.29 (3H, s), 2.58 (3H, s), 3.40~3.72 (2H, m, H-5'), 4.04 (3H, s), 4.12~4.92 (9H, H-2', 3', and 4', and PhCH_2), 6.65 (1H, d, H-1', $J_{1'2'}=5$ Hz), 6.92~7.36 (16H, m, arom); $[\alpha]_D^{25}+4.5^\circ$ ($c=0.2$, CHCl_3); MS m/e : 611 (M^+); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 245, 285.

3,5-Di-O-benzyl-2-deoxy-2-(2-methylthio-4-methoxy-5-methylpyrrolo-[2,3-*d*]pyrimidin-7-yl)-D-arabinose (?) (12**):** (the abnormal by-product): syrup; NMR (CDCl_3) δ : 2.26 (3H, s), 2.58 (3H, s), 3.40~3.70 (3H, H-2' and 5'), 4.06 (3H, s), 4.40~4.70 (6H, H-1' and 4', and PhCH_2), 5.52 (1H, H-3), 6.40 (1H, H-6), 7.20 (10H, arom); MS calcd. for $\text{C}_{28}\text{H}_{31}\text{N}_5\text{O}_6\text{S}$: 521.1984, found: 521.1977.

Methyl 3,5-di-O-benzyl-2-deoxy-2-(2-methylthio-4-methoxy-5-methylpyrrolo[2,3-*d*]pyrimidin-7-yl)-D-arabino-side (13**) ?**

A solution of **12** (100 mg) in methanol (5 ml) was refluxed in the presence of Dowex 50 (H^+) (59 mg) for 12 hr. After removal of the resin, the solution was

evaporated and the residue was separated by tlc using ethyl acetate-benzene (1:20) to afford the methyl glycoside (**13**) (54 mg) as a syrup. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 247, 288; NMR (CDCl_3) δ : 2.23 (3H, d, $J=1$ Hz), 2.52 (3H, s), 3.46 (3H, s), 3.65 (2H, H-5'), 4.06 (3H, s), 4.24 (1H, q, H-2', $J_{1'2'}=3.5$ Hz), 4.3~4.7 (5H, H-4 and PhCH_2), 5.09 (1H, d, H-1', $J_{1'2'}=1$ Hz), 5.22 (1H, q, H-3', $J_{2'3'}=3.5$ Hz, $J_{3'4'}=8.5$ Hz), 6.67 (1H, q, H-6, $J=1$ Hz), 7.0~7.8 (arom); MS m/e : 537.

1-O-Acetyl-3,5-di-O-benzyl-2-deoxy-2-(2-methylthio-4-methoxy-5-methylpyrrolo[2,3-*d*]pyrimidin-7-yl)-D-arabinose (14**) ?**

Acetylation of **12** (23.3 mg) with acetic anhydride-pyridine afforded the acetate (**14**) (13.6 mg) as a syrup which was isolated by tlc [ethyl acetate-benzene (1:6)]. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 247, 288; NMR [CDCl_3 - C_6D_6 (2:1)] δ : 1.92 (3H, s), 2.28 (3H, d, $J=1$ Hz), 2.44 (3H, s), 3.53 (2H, m, H-5', $J_{5'6'}=12$ Hz), 3.94 (3H, s), 4.3~4.5 (4H, PhCH_2), 4.60 (1H, q, H-2', $J_{1'2'}=2$ Hz, $J_{2'3'}=5$ Hz), 4.70 (1H, q, H-4', $J_{3'4'}=8.5$ Hz, $J_{4'5'}=4$ Hz), 5.17 (1H, q, H-3', $J_{2'3'}=5$ Hz, $J_{3'4'}=8.5$ Hz), 6.40 (1H, d, H-1', $J_{1'2'}=2$ Hz), 6.50 (1H, q, H-6, $J=1$ Hz), 6.8~7.4 (arom); IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1748; MS m/e : 565.

4-Methyl-5-methyl-7-(2,3,5-tri-O-benzyl- β -D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine(15**) and its α -anomer (**16**)**

A solution of **10** (237 mg) in ethanol (10 ml) and benzene (5 ml) containing Raney nickel (W-2, 1.4 g) was refluxed with vigorous stirring for 4 hr. After cooling, the mixture was filtered and the filtrate was evaporated to dryness. The crystalline residue (221 mg) was recrystallized from isopropanol to give **15** as needles, mp 105~106°C. NMR (CDCl_3) δ : 6.90 (1H, q, H-6, $J=1$ Hz), 8.39 (1H, s, H-2); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 265 (8430), 278 (8430). Found: C, 72.20; H, 6.29; N, 7.48. Calcd. for $\text{C}_{34}\text{H}_{35}\text{N}_5\text{O}_6$: C, 72.19; H, 6.24; N, 7.43%.

Reduction of **11** (1.0 g) with Raney nickel gave **16** as syrup (830 mg), which is pure on NMR spectrum. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 259 (5400), 265 (5500), 283 (5820); NMR (CDCl_3) δ : 8.35 (1H, s, H-2).

5-Methyl-7-(2,3,5-tri-O-benzyl- β -D-ribofuranosyl)pyrrolo [2,3-*d*]pyrimidin-4-one (17**) and its α -anomer (**18**)**

A solution of **15** (179 mg) in dioxane (8 ml) and 0.5 N HCl (2 ml) was refluxed under N_2 for 15 hr and then evaporated *in vacuo* below 50°C to dryness. The residue was taken into CH_2Cl_2 and worked up as usual to give crystalline **17**, mp 150°C (164 mg), which was recrystallized from isopropanol as needles, mp 151°C. NMR (CDCl_3) δ : 2.25 (3H, d, $J=1$ Hz), 6.38 (1H, d, $J_{1'2'}=3$ Hz), 6.75 (1H, q, H-6, $J=1$ Hz), 7.80 (1H, s, H-2); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 264 (8540), 280 (7840).

Hydrolysis of **16** (765 mg) gave syrupy **18** (736 mg),

which gave a single spot on tlc. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 265 (8630), 285 (8170); $\lambda_{\text{max}}^{\text{MeOH-NaOH}}$ 282 (10200); NMR (CDCl_3) δ : 2.44 (3H, br.s), 6.61 (1H, d, H-1', $J_{1,2'}=5$ Hz), 7.83 (1H, s, H-2).

5-Methyl-7-(β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one (19) and its α -anomer (20)

The benzyl ether **17** (330 mg) was hydrogenated in dioxane (4 ml) and methanol (8 ml) in the presence of 10% Pd-C (543 mg) under H_2 at room temp. for 2 days. After filtration, the solution was evaporated *in vacuo* to dryness. The residue was crystallized from isopropanol to give **19** (140 mg), mp 249~250.5°C. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 266 (8070), 280 (7860); $\lambda_{\text{max}}^{\text{MeOH-NaOH}}$ 278 (9750); NMR (DMSO-d_6) δ : 2.27 (3H, d, $J=1$ Hz), 3.55 (2H, H-5'), 3.82 (1H, q, H-4', $J_{3,4'}=3.5$ Hz, $J_{4,5'}=3.5$ Hz), 4.02 (1H, q, H-3', $J_{2,3'}=5$ Hz, $J_{3,4'}=3.5$ Hz), 4.24 (1H, q, H-2', $J_{1,2'}=5.5$ Hz, $J_{2,3'}=5$ Hz), 5.96 (1H, d, H-1', $J_{1,2'}=5.5$ Hz), 7.06 (1H, q, H-6, $J=1$ Hz), 7.82 (1H, br. d, H-2, $J_{2,\text{NH}}=3.5$ Hz), 11.80 (1H, br.s, NH). Found: C, 50.99; H, 5.32; N, 14.27. Calcd. for $\text{C}_{12}\text{H}_{16}\text{N}_8\text{O}_5$: C, 51.24; H, 5.37; N, 14.94%.

Hydrogenation of **18** (123 mg) gave **20**, which was crystallized from isopropanol-ether, mp 231°C. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 222 (16000), 267 (6600), 283 (6600); $\lambda_{\text{max}}^{\text{MeOH-NaOH}}$: 278 (8120); NMR (DMSO-d_6) δ : 2.27 (3H, br.s), 6.34 (1H, d, H-1', $J_{1,2'}=4.5$ Hz), 7.13 (1H, br.s, H-6), 7.81 (1H, br.s, H-2); $[\alpha]_D^{25}+25.6^\circ$ (H_2O , $c=0.19$). Found: C, 51.04; H, 5.39; N, 14.87. Calcd. for $\text{C}_{12}\text{H}_{16}\text{N}_8\text{O}_5$: C, 51.24; H, 5.37; N, 14.94%.

5-Methyl-7-(2,3-O-isopropylidene- β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one (21) and its α -anomer (22)

A mixture of **19** (73.8 mg), 2,2-dimethoxypropane (0.57 ml), acetone (2 ml), acetonitrile (2 ml), and camphorsulfonic acid (5 mg) was stirred at room temp. until the starting material disappeared on a tlc plate for monitoring. Acetic acid (20%, 4 ml) was added and the mixture allowed to stand at room temp. for 30 min. Then, it was neutralized with conc. ammonia and evaporated to dryness. Extraction of the residue with CH_2Cl_2 gave crystalline **21** (76.1 mg), which was recrystallized from isopropanol-ether, mp 214°C. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 218 (17500), 265 (7760), 280 (7250); $\lambda_{\text{max}}^{\text{MeOH-NaOH}}$: 277 (9450); NMR (CDCl_3) δ : 1.38 (3H, s), 1.68 (3H, s), 2.38 (3H, d, $J=1$ Hz), 5.70 (1H, d, H-1', $J=4$ Hz), 6.62 (1H, q, H-6, $J=1$ Hz), 7.78 (1H, s, H-2). Found: C, 55.32; H, 5.96; N, 12.55. Calcd. for $\text{C}_{18}\text{H}_{20}\text{N}_8\text{O}_5$: C, 56.06; H, 5.96; N, 13.08%.

Isopropyridenation of **20** (396 mg) gave **22**, which was continuously extracted from the neutralized mixture with ether to give crystals (recrystallized from isopropanol, 278 mg), mp 191°C. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 222 (15700), 267 (6740), 282 (6580); $\lambda_{\text{max}}^{\text{MeOH-NaOH}}$: 278 (8190); NMR (DMSO-d_6) δ : 1.25 (3H, s), 1.41 (3H, s),

2.27 (3H, d, $J=1$ Hz), 3.58 (2H, d, H-5', $J=3.5$ Hz), 4.21 (1H, t, H-4', $J_{3,4'}\sim 0$ Hz, $J_{4,5'}=3.5$ Hz), 4.8~4.9 (2H, m, H-2' and 3'), 6.50 (1H, d, H-1', $J=3.5$ Hz), 6.91 (1H, q, H-6, $J=1$ Hz), 7.82 (br.d, H-2). Found: C, 55.61; H, 6.03; N, 12.62. Calcd. for $\text{C}_{18}\text{H}_{18}\text{N}_8\text{O}_5$: C, 56.06; H, 5.96; N, 13.08%.

5-Methyl-7-(5-O-acetyl-2,3-O-isopropylidene- β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one (23) and its α -anomer (24)

A mixture of **21** (66 mg), acetic anhydride (1 ml) and pyridine (2 ml) was allowed to stand at room temp. for 15 hr and then diluted with absolute methanol (2 ml). After evaporation, the residue was crystallized from ether-isopropanol to give **23** (63.7 mg), mp 156°C. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 265 (8490), 280 (7910); $\lambda_{\text{max}}^{\text{MeOH-NaOH}}$: 276 (10800); NMR (CDCl_3) δ : 2.08 (3H, s), 4.90 (1H, q, H-3', $J_{2,3'}=10$ Hz, $J_{3,4'}=3.5$ Hz), 5.12 (1H, q, H-2', $J_{1,2'}=2.4$ Hz, $J_{2,3'}=10$ Hz), 6.17 (1H, d, H-1', $J=2.4$ Hz). Found: C, 56.28; H, 5.88; N, 11.43. Calcd. for $\text{C}_{17}\text{H}_{21}\text{N}_8\text{O}_8$: C, 56.19; H, 5.83; N, 11.56%.

Acetylation of **22** (112 mg) gave **24**, which was crystallized from isopropanol-ether as plates (117 mg), mp 234~235°C. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 220 (9000), 267 (7140), 283 (7000); $\lambda_{\text{max}}^{\text{MeOH-NaOH}}$: 278 (8930); NMR [CDCl_3 - CD_3OD (3:1)] δ : 2.16 (3H, s), 4.26 (2H, d, H-5', $J=4.5$ Hz), 4.55 (1H, t, H-4', $J_{3,4'}\sim 0$ Hz, $J_{4,5'}=4.5$ Hz), 4.84 (2H, m, H-2' and 3'), 6.54 (1H, d, H-1', $J=3$ Hz). Found: C, 56.03; H, 5.87; N, 11.46. Calcd. for $\text{C}_{17}\text{H}_{21}\text{N}_8\text{O}_8$: C, 56.19; H, 5.83; N, 11.56%.

4-Chloro-5-methyl-7-(5-O-acetyl-2,3-O-isopropylidene- β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (25) and its α -anomer (26)

The acetate **23** (55 mg) was refluxed in POCl_3 (2 ml) containing dimethylaniline (0.2 ml) for 8 hr, and the reaction mixture was evaporated *in vacuo*. After usual work-up, the residue was applied on preparative silica gel tlc and developed with acetone-benzene (1:12) to give **25** as an amorphous solid (49 mg). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 231, 273, 296; NMR (CDCl_3) δ : 1.38 (3H, s), 1.62 (3H, s), 2.05 (3H, s), 2.46 (3H, d, $J=1$ Hz), 4.1~4.6 (3H, m, H-4' and 5'), 4.88 (1H, q, H-3', $J_{2,3'}=10$ Hz, $J_{3,4'}=3.5$ Hz), 5.24 (1H, q, H-2', $J_{1,2'}=2.5$ Hz, $J_{2,3'}=10$ Hz), 6.22 (1H, d, H-1', $J_{1,2'}=2.5$ Hz), 7.07 (1H, q, H-6, $J=1$ Hz), 8.58 (1H, s, H-2); MS m/e : calcd. for $\text{C}_{17}\text{H}_{20}\text{N}_8\text{O}_5\text{Cl}$: 381.1091; found: 381.1092.

Chlorination of **24** (104 mg) gave **26** (61 mg). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 232 (28800), 273 (3820), 300 (3620); NMR (CDCl_3) δ : 1.32 (3H, s), 1.55 (3H, s), 2.16 (3H, s), 2.48 (3H, d, $J=1$ Hz), 4.26 (2H, d, H-5', $J_{4,5'}=4.5$ Hz), 4.54 (1H, t, H-4', $J_{3,4'}\sim 0$ Hz, $J_{4,5'}=4.5$ Hz), 4.85 (2H, H-2' and 3'), 6.70 (1H, d, H-1', $J=3.5$ Hz), 7.33 (1H, q, H-6, $J=1$ Hz), 8.54 (1H, s, H-2); MS calcd. for $\text{C}_{17}\text{H}_{20}\text{N}_8\text{O}_5\text{Cl}$: 381.1091; found m/e : 381.1092.

4-Amino-5-methyl-7-(2,3-O-isopropylidene- β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (27) and its α -anomer (28)

A solution of **25** (40 mg) in absolute methanol (3 ml) was placed in a syringe and saturated with anhydrous ammonia at -40°C . The syringe was sealed and heated at 120°C for 18 hr. After cooling, the mixture was evaporated and the residue extracted with CH_2Cl_2 . From the extract was obtained solid **27**, which was crystallized from ether-isopropanol as needles (24 mg), mp $151\sim 152^{\circ}\text{C}$. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 234 (24900), 280 (8680), $\lambda_{\text{max}}^{\text{MeOH-HCl}}$: 285 (8080); NMR (CDCl_3) δ : 1.36 (3H, s), 1.62 (3H, s), 2.39 (3H, d, $J=1$ Hz), 3.6~4.1 (2H, m, H-5'), 4.46 (1H, q, H-4', $J_{3/4'}=1.2$ Hz, $J_{4'/5'}=1.2$ Hz), 5.08 (1H, q, H-3', $J_{2/3'}=5.9$ Hz, $J_{3/4'}=1.2$ Hz), 5.18 (1H, q, H-2', $J_{1/2'}=4.9$ Hz, $J_{2/3'}=5.9$ Hz), 5.65 (1H, d, H-1', $J=4.9$ Hz), 6.73 (1H, q, H-6, $J=1$ Hz), 8.18 (1H, s, H-2); MS m/e : 320 (M^+). Found: C, 56.07; H, 6.26; N, 17.31. Calcd. for $\text{C}_{18}\text{H}_{20}\text{N}_4\text{O}_4$: C, 56.24; H, 6.29; N, 17.49%.

Ammonolysis of **26** (87 mg) gave **28** (66 mg), which was crystallized from ethyl acetate, mp 153°C . UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 281 (8890); $\lambda_{\text{max}}^{\text{MeOH-HCl}}$: 236 (29500), 286 (8640); NMR (CDCl_3) δ : 1.34 (3H, s), 1.57 (3H, s), 2.42 (3H, d, $J=1$ Hz), 3.84 (2H, m, H-5'), 4.36 (1H, t, H-4', $J_{3/4'}\sim 0$ Hz, $J_{4'/5'}=4$ Hz), 4.92 (2H, m, H-2' and 3'), 5.29 (br.s, exchangeable H), 6.72 (1H, d, H-1', $J=2.5$ Hz), 7.13 (1H, q, H-6, $J=1$ Hz), 8.17 (1H, s, H-2). Found: C, 55.18; H, 6.42; N, 17.00. Calcd. for $\text{C}_{18}\text{H}_{20}\text{N}_4\text{O}_4\cdot 1/2 \text{H}_2\text{O}$: C, 54.70; H, 6.43; N, 17.01%.

5-Methyltubercidin (2) and its α -anomer (3)

A solution of **27** (6.6 mg) in trifluoroacetic acid-water (19:1) (0.5 ml) was allowed to stand at room temp. for 10 min and then evaporated *in vacuo*. The residue was crystallized from ethyl acetate to give 5-methyltubercidin (**2**) trifluoroacetate (4 mg), mp 264°C (dec). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 225sh (14200), 281 (7560); $\lambda_{\text{max}}^{\text{MeOH-HCl}}$: 235 (22500), 285 (7210); NMR (D_2O , *t*-BuOH as internal standard δ 1.23) δ : 2.41 (3H, d, $J=1$ Hz), 4.1~4.3 (2H, m, H-5'), 4.64 (1H, q, H-4', $J_{3/4'}=3.5$ Hz, $J_{4'/5'}=3.5$ Hz), 4.79 (1H, q, H-3', $J_{2/3'}=5.5$ Hz, $J_{3/4'}=3.5$ Hz), 5.05 (1H, q, H-2', $J_{1/2'}=6$ Hz, $J_{2/3'}=5.5$ Hz), 6.20 (1H, d, H-1', $J=6$ Hz), 7.34 (1H, q, H-6, $J=1$ Hz), 8.23 (1H, s, H-2). Found: C, 42.41; H, 4.34; N, 14.00. Calcd. for $\text{C}_{15}\text{H}_{16}\text{N}_4\text{O}_4\cdot \text{CF}_3\text{COOH}$: C, 42.64; H, 4.34; N, 14.21%. The salt was treated in water with Amberlite IR-45 resin to give 5-methyltubercidin (**2**), which was crystallized from methanol, mp $262\sim 263^{\circ}\text{C}$ (dec). NMR ($\text{DMSO}-d_6$) δ : 2.33 (3H, d, $J=1$ Hz), 3.5~4.5 (5H, m, H-2', 3', 4' and 5'), 5.96 (1H, d, H-1', $J=6$ Hz), 6.5 (br.s, exchangeable H), 7.05 (1H, q, H-6, $J=1$ Hz), 7.98 (1H, s, H-2).

Hydrolysis of the acetonide **28** (6 mg) with trifluoroacetic acid gave trifluoroacetate of **3**, which was crystallized from ethyl acetate (3 mg), mp $151\sim 153^{\circ}\text{C}$.

UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 225 (17400), 283 (9000); $\lambda_{\text{max}}^{\text{MeOH-HCl}}$: 236 (29400), 287 (9000); NMR (D_2O) δ : 2.34 (3H, d, $J=1$ Hz), 3.72 and 3.88 (2H, m, H-5', $J_{5/6'}=12.5$ Hz), 4.37 (2H, m, H-2' and 3'), 4.52 (1H, t like, H-4'), 4.47 (1H, d, H-1', $J=4.5$ Hz), 7.35 (1H, q, H-6, $J=1$ Hz), 8.12 (1H, s, H-2). Found: C, 41.67; H, 4.28; N, 13.79. Calcd. for $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}_4\cdot \text{CF}_3\text{COOH}\cdot 1/2 \text{H}_2\text{O}$: C, 41.69; H, 4.49; N, 13.89%. Free nucleoside **3** was obtained as a syrup by treatment with Amberlite IR-45 in water. NMR ($\text{DMSO}-d_6$) δ : 2.34 (3H, d, $J=1$ Hz), 6.40 (1H, d, H-1', $J=5$ Hz), 6.54 (1H, br.s, NH), 7.17 (1H, q, H-6, $J=1$ Hz), 7.99 (1H, s, H-2).

4-Amino-5-methyl-7-(2,3-O-isopropylidene-5-O-p-toluenesulfonyl- β -D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (29) and its α -anomer (30)

A solution of **27** (2.25 mg) and freshly prepared *p*-toluenesulfonyl chloride (18.3 mg) in anhydrous pyridine (0.1 ml) was prepared at 0°C and allowed to stand at room temp. for 1 hr. Aq. NaHCO_3 was added with vigorous stirring to the solution and the mixture was extracted with CH_2Cl_2 . The extract was evaporated and the residue separated by silica gel tlc developed with methanol- CH_2Cl_2 (1:20) to give the tosylate **29** (2.0 mg) which is unstable at room temp. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 276; NMR (CDCl_3) δ : 1.35 (3H, s), 1.57 (3H, s), 2.41 (3H, s), 2.41 (3H, d, $J=1$ Hz), 4.2 (2H, m, H-5'), 4.31 (1H, H-4') 4.97 (1H, q, H-3', $J_{2/3'}=7$ Hz, $J_{3/4'}=3.5$ Hz), 5.16 (s, NH), 5.19 (1H, q, H-2', $J_{1/2'}=2.5$ Hz, $J_{2/3'}=7$ Hz), 6.12 (1H, d, H-1', $J=2.5$ Hz), 6.77 (1H, q, H-6, $J=1$ Hz), 7.22 and 7.67 (4H, arom), 8.13 (1H, s, H-2).

The nucleoside **28** (2.5 mg) was tosylated and purified by tlc using acetone-benzene (1:2) to give the tosylate **30**. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 278; $\lambda_{\text{max}}^{\text{MeOH-HCl}}$: 285; NMR (CDCl_3) δ : 1.32 (3H, s), 1.55 (3H, s), 2.41 (3H, d, $J=1$ Hz), 2.41 (3H, s), 4.08 and 4.20 (2H, m, H-5', $J_{4'/5'}=4$ Hz, $J_{5/6'}=11$ Hz), 4.41 (1H, t, H-4', $J_{3/4'}\sim 0$ Hz, $J_{4'/5'}=4$ Hz), 4.88 (2H, m, H-2' and 3'), 5.1 (br.s, exchangeable H), 6.56 (1H, d, H-1', $J=3.5$ Hz), 7.01 (1H, q, H-6, $J=1$ Hz), 7.30 and 7.87 (arom), 8.23 (1H, s, H-2); MS calcd. for $\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}_6\text{S}$: 474.1573, found m/e : 474.1558. This compound was stable by heating in acetone at 100°C for 10 hr.

Cyclonucleoside of 5-methyltubercidin acetonide *p*-toluenesulfonate (31)

A solution of **29** (2.0 mg) in acetone (0.7 ml) was heated at 60°C for 3 hr (actually only brief heating is enough) and then dried up to give the cyclonucleoside *p*-toluenesulfonate (**31**). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 218, 243, 275, 303; NMR (CDCl_3) δ : 1.18 (3H, s), 1.49 (3H, s), 2.33 (3H, s), 2.42 (3H, d, $J=1$ Hz), 4.06 (1H, d, H-2' or 3', $J_{2/3'}=3$ Hz), 4.36 (1H, br.d, H-5', $J_{5/6'}=12$ Hz), 4.82 (1H, br.s, H-4'), 5.01 (1H, d, H-2' or 3', $J_{2/3'}=3$ Hz), 5.30 (1H, br.d, H-5', $J_{5/6'}=12$ Hz), 5.86 (1H, s, H-1'), 6.71 (1H, q, H-6, $J=1$ Hz), 7.13 and 7.75 (4H, arom),

9.28 (1H, br.s, H-2); MS calcd. for $C_{15}H_{15}N_4O_3$: 302.1379; found m/e : 302.1364.

Cyclonucleoside of 5-methyltubercidin acetonide iodide (32)

The cyclonucleoside *p*-toluenesulfonate **31** (7.8 mg) was dissolved into acetone (0.3 ml) containing NaI (2.5 mg), when sodium *p*-toluenesulfonate was immediately precipitated. After filtration, the filtrate was evaporated *in vacuo* and the residue was extracted with CH_2Cl_2 and crystallized from ethyl acetate to give the cyclonucleoside iodide (**32**) (4.5 mg), mp 297°C (dec). UV λ_{max}^{MeOH} nm (ϵ): 218 (32200), 275 (7820), 303 (8140); $\lambda_{max}^{MeOH-NaOH}$: 274 (9120), 300 (6510); NMR ($CDCl_3$) δ : 1.34 (3H, s), 1.53 (3H, s), 2.45 (3H, d, $J=1$ Hz), 4.46 (1H, d, H-3', $J_{2'3'}=6$ Hz), 4.50 (1H, br.d, H-5', $J_{5'5'}=12$ Hz), 4.90 (1H, br.s, H-4'), 5.22 (1H, br.d, H-5', $J_{5'5'}=12$ Hz), 5.59 (1H, d, H-2', $J_{2'3'}=6$ Hz), 5.92 (1H, s, H-1'), 6.81 (1H, q, H-6, $J=1$ Hz), 7.6 (br, NH), 9.54 (1H, br.s, H-2). Found: C, 41.53; H, 4.43; N, 12.96. Calcd. for $C_{15}H_{15}N_4O_3I$: C, 41.87; H, 4.45; N, 12.96%.

Paper electrophoresis of cyclonucleoside 31 and 5-methyltubercidin acetonide (27)

The electrophoresis of **27** and **31** was carried out in 0.05 M Tris buffer, pH 7.9, for 3 hr at 300 V using Toyo No. 51A filter paper (37.5 cm) at room temp. Mobility: **31**, 4.5 cm toward cathod; **27**, 0.5 cm toward cathode.

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