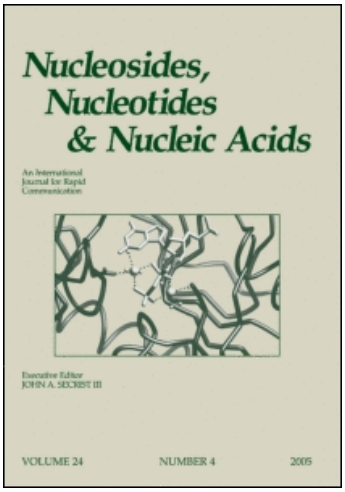

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Synthesis of 1-methyl-3-β-d-ribofuranosylpyrazolo[4, 3,-D]-pyrimidin-7(6h)-selone and Certain Related Nucleosides and Nucleotides

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SYNTHESIS OF 1-METHYL-3-β-D-RIBOFURANOSYLPYRAZOLO[4,3-d]-PYRIMIDIN-7(6H)-SELONE AND CERTAIN RELATED NUCLEOSIDES AND NUCLEOTIDES

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ABSTRACT: 1-Methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7(6H)-selone (6) and the corresponding thione analog (5) have been synthesized for the first time from 1-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidin-7(6H)-one (12). Thiation and deacetylation of 12 gave 5. Compound 6 was prepared from 12 via the chloro intermediate (13) and selenourea followed by deacetylation. A convenient, high yield procedure for the preparation of 1-methylformycin B (4) from 1-methylformycin (7) is described. Phosphorylation of 7 provided 1-methylformycin 5'-phosphate (17). Compounds 6 and 17 were found to be potent inhibitors of growth of L1210 and P388 leukemia.

Pyrazolo[4,3-d]pyrimidine 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[1] of the naturally occurring nucleoside antibiotics formycin[2,3] and formycin B[4] (1), there has been an ongoing effort by many investigators[5-16] to synthesize nucleoside derivatives of the pyrazolo[4,3-d]pyrimidine ring system. Formycin B (3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7(6H)-one, 1),[17] an inosine analog with a stable carbon-carbon glycosidic linkage, is a potent inhibitor of promastigote and amastigote forms of *Leishmania*[18-21] and is remarkably nontoxic to animals.[22] Formycin B has recently been shown to be a potent inhibitor of the growth of *Trypanosoma cruzi* (the causative agent of Chagas' disease) epimastigotes in culture.[23] Thioformycin B (3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7(6H)-thione, 2)[24] has also shown significant activity (ED_{50} 3.6 μM) against *Leishmania tropica* in human monocyte-derived macrophages *in vitro*[25] and is much less toxic than formycin B.

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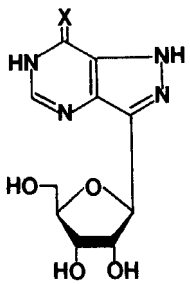
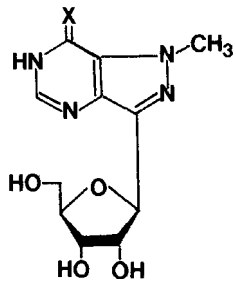


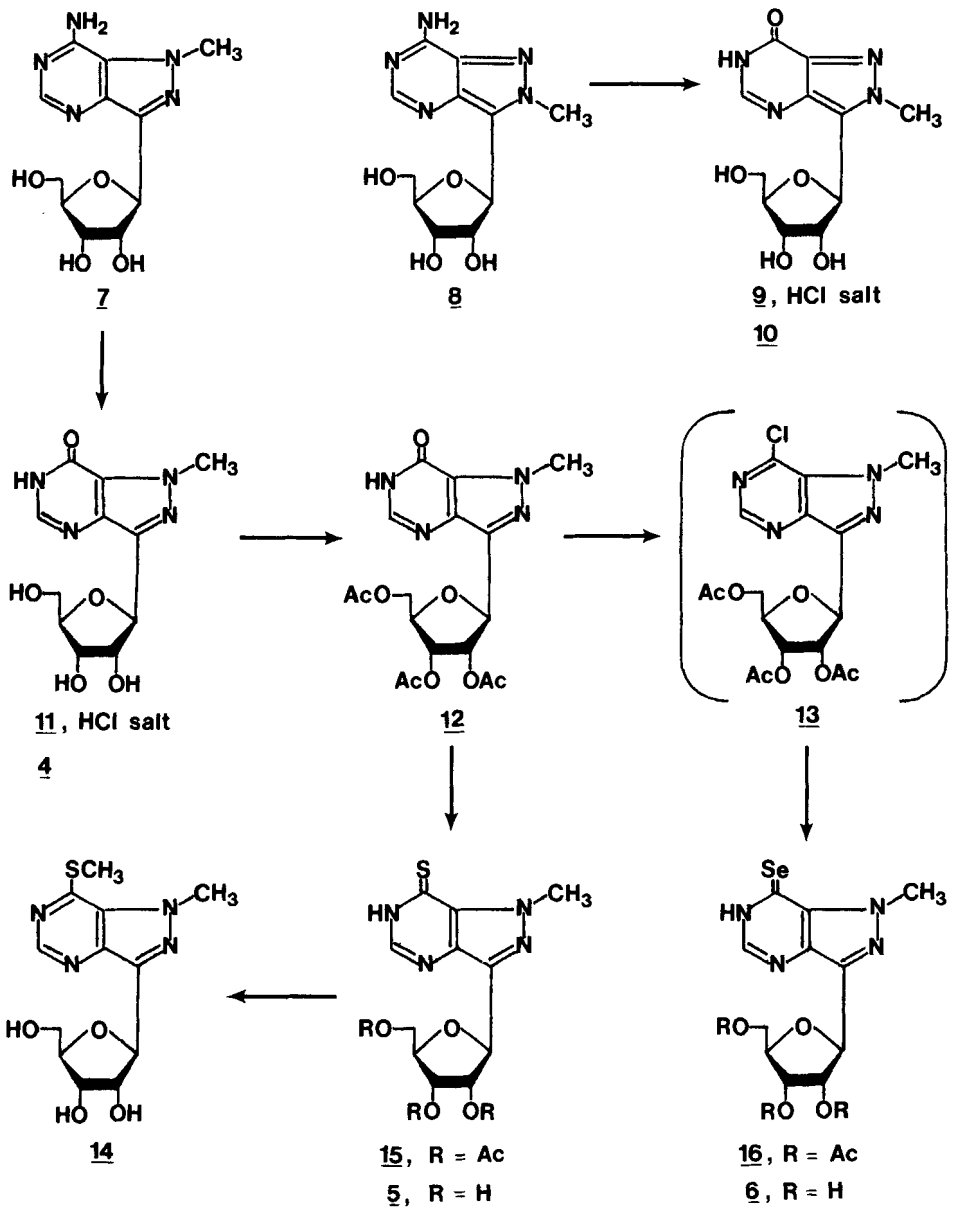
1, X = O
2, X = S
3, X = Se



4, X = O
5, X = S
6, X = Se

Recently, Santi and co-workers[26] have found that selenoformycin B (3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7(6H)-selone, 3)[27] is more active than thioformycin B, but less active than formycin B against L. tropica promastigotes in vitro with an ED_{50} of 0.2 μM. Although the reported EC_{50} value (concentration of drug that inhibits the growth rate of cells by 50%) of allopurinol riboside (1-β-D-ribofuranosylpyrazolo-[3,4-d]pyrimidin-4(5H)-one)[28] is similar to that of formycin B (7.5 μM)[23] for T. cruzi epimastigotes, the observed EC_{50} value of 1-methylformycin B (4) is 0.6 μM.[26] In view of these findings, the synthesis of hitherto unreported sulfur and selenium derivatives of 1-methylformycin B containing the exocyclic thione and selone function at position 7 was undertaken.

The selenopurine nucleosides are generally synthesized from the corresponding halo-nucleosides with either sodium hydrogen selenide[29-32] or selenourea.[27,33,34] Recently, the synthesis of several 6-selenopurine nucleosides has been reported by Shiue and Chu[35] employing adenosine and H_2Se in aqueous pyridine in a sealed tube. For our purposes, the halonucleoside-selenourea procedure proved to be the most satisfactory. The starting materials 1-methylformycin (7)[11] and 2-methylformycin (8)[8] were prepared as reported. Deamination of 1-methylformycin (7) with liquid nitrosyl chloride in DMF gave 4 in essentially quantitative yield, which is significantly superior to the yield (<10%) previously reported[15] (SCHEME 1). The intermediate 1-methylformycin B monohydrochloride (11) has been isolated for the first time and fully characterized. 2-Methylformycin B (10) has also previously been prepared by chemical[8]

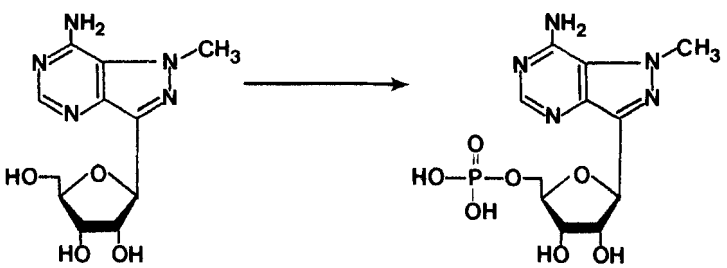


SCHEME 1

and enzymatic[36] deamination of 8. But the isolated yield of 10 from the alkaline hydrolysis of 8 was only 16%.[8] In the present study, deamination of 8 with nitrosyl chloride under the conditions used to prepare 4 gave again an almost quantitative yield of 2-methylformycin B hydrochloride (9) which, on neutralization with Dowex 1-X8 OH^- resin, gave 10.

Acetylation of 4 with acetic anhydride in the presence of 4-N,N-dimethylaminopyridine (DMAP) gave an excellent yield of 1-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidin-7(6H)-one (12). Treatment of 12 with phosphoryl chloride at reflux temperature gave essentially a quantitative yield of 7-chloro-1-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine (13), which was found to be rather unstable and required cold storage under anhydrous conditions. Subsequent reaction of 13 with selenourea in ethanol at room temperature gave crystalline 1-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidin-7(6H)-selone (16). Deacetylation of 16 with sodium methoxide in methanol under controlled conditions gave the desired 1-methyl-3-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-7(6H)-selone (6). The ultraviolet absorption spectrum of 6 exhibited a large bathochromic shift as compared to 1-methylformycin B (see experimental), which is due[33] to the presence of -NH-C=Se function. Thiation of 12 with purified phosphorus pentasulfide in the presence of DMAP gave the crystalline 1-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidin-7(6H)-thione (15) in an 81% yield. Deacetylation of 15 with sodium methoxide in methanol readily gave 1-methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7(6H)-thione (5). Methylation of 5 with methyl iodide in the presence of sodium methoxide furnished 1-methyl-7-methylthio-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (14).

In view of the fact that the nucleosides most often exert their biological activities after conversion in the cell to the corresponding



7 17

nucleotides,[37] we have now prepared the 5'-phosphate ester of 1-methylformycin (17). Phosphorylation of unprotected 1-methylformycin (7) with phosphoryl chloride in trimethylphosphate[38] gave 7-amino-1-methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine 5'-phosphate (17), isolated in the free acid form. The purity of 17 was confirmed by spectral studies and elemental analysis.

The structural assignment of all the nucleosides and the nucleotide synthesized in this study were confirmed, since the structure of the starting nucleosides 7 and 8 were already established.[8,11]

All the compounds synthesized during this study have been tested against herpes simplex type 2 (233), vaccinia, parainfluenza type 3 and measles viruses, as well as L1210 leukemia and P388 *in vitro*.[39] These compounds are devoid of any significant antiviral activity against the viruses tested *in vitro*. However, compounds 6 and 17 were found to be potent inhibitors of growth of L1210 leukemia (ID_{50} of 2.7 X 10^{-7}M) and P388 leukemia (ID_{50} of 3.2 X 10^{-7}M) *in vitro*. All other compounds tested were not active. These nucleosides and nucleotides have been submitted to Walter Reed Army Institute of Research for antiparasitic evaluation.

EXPERIMENTAL SECTION

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on either a Varian EM-390 or on a Jeol FX-90 Q spectrometer. The chemical-shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of H_2O or other solvents as indicated by elemental analyses was verified by NMR. Infrared spectra (IR) were obtained on a Beckman Acculab 2 spectrophotometer and are expressed in reciprocal centimeters. Ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee, and Robertson Labs, Florham Park, New Jersey. Analytical results indicated by element symbols were 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. 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.

1-Methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7(6H)-one (4). Liquid nitrosyl chloride (1 ml) was added to a cooled (ice-salt bath) and stirred suspension of 1-methylformycin[11] (7, 2.81 g, 10 mmol) in dry DMF (35 ml). After 15 min additional nitrosyl chloride (1 ml) was added and the stirring was continued for further 30 min. The reaction mixture was allowed to warm to room temperature before it was purged with nitrogen. The contents of the flask were poured over ice-water (50 ml) and evaporated to dryness. The residual solid was crystallized from ethanol to yield 3.0 g (98%) of the hydrochloride salt 11; mp 216-217°C; IR (KBr) ν 1710 (C=O), 3120-3450 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 225 nm (ε 12,000), 280 (6,000); UV λ_{max} (pH 7) 212 nm (ε 15,300), 280 (7,600); UV λ_{max} (pH 11) 290 nm (ε 9,900); NMR (Me_2SO-d_6) δ 4.20 (s, 3, CH_3), 4.90 (d, 1, $J_{1',2'}$ = 6.2 Hz, $C_1'H$), 7.60 (broad s, 1, HCl), 8.15 (s, 1, C_5H), and other sugar protons. Anal. Calcd for $C_{11}H_{14}N_4O_5 \cdot HCl$: C, 41.45; H, 4.74; N, 17.58; Cl, 11.12. Found: C, 41.36; H, 4.84; N, 17.33; Cl, 11.35.

A solution of 11 (2.80 g, 3.78 mmol) in water (50 ml) was neutralized with Dowex 1-X8 OH^- resin. The resin was removed by filtration, washed repeatedly with water and the combined filtrate and washings were evaporated to obtain a solid, which was crystallized from methanol-water (1:1) to yield 2.40 g (97%) of 4; mp 208°C (Lit[15] mp 189-192°C); IR (KBr) ν 1690 (C=O), 3200-3400 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 222 nm (ε 13,800), 280 (7,100); UV λ_{max} (pH 7) 222 nm (ε 14,400), 280 (7,400); UV λ_{max} (pH 11) 227 sh, nm (ε 9,300), 290 (10,500), 305 sh (5,600); NMR (Me_2SO-d_6) δ 4.18 (s, 3, CH_3), 4.85 (d, 1, $J_{1',2'}$ = 6.8 Hz, $C_1'H$), 7.90 (s, 1, C_5H), and other sugar protons. Anal. Calcd for $C_{11}H_{14}N_4O_5$: C, 46.81; H, 5.00; N, 19.85. Found: C, 46.58; H, 4.97; N, 19.62.

2-Methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7(6H)-one (10). 2-Methylformycin[8] (8, 2.81 g, 10 mmol) was deaminated with liquid nitrosyl chloride (2 ml) in anhydrous DMF (35 ml) as described for 11. Crystallization of the residual solid with absolute ethanol gave the hydrochloride salt 9, 3.0 g (98%); mp 209-210°C; IR (KBr) ν 1740 (C=O), 3100-3350 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 222 nm (13,700), 280 (8,600); UV λ_{max} (pH 7) 220 nm (ε 15,600), 280 (9,200); UV λ_{max} (pH 11) 290 sh, nm (ε 11,500), 300 (12,300), 312 sh (8,000); NMR (Me_2SO-d_6) δ 4.10 (s,

3, $C\underline{H}_3$), 5.15 (d, 1, $J_{1',2'}$ = 6.2 Hz, $C_1'\underline{H}$), 8.10 (s, 1, $C_5\underline{H}$), and other sugar protons. Anal. Calcd for $C_{11}H_{14}N_4O_5 \cdot HCl$: C, 41.45; H, 4.74; N, 17.58; Cl, 11.12. Found: C, 41.52; H, 4.82; N, 17.29; Cl, 11.38.

Neutralization of an aqueous solution of 9 (2.80 g, 8.78 mmol) with Dowex 1-X8 OH^- resin and work up as described for 4 gave the title compound 10, 2.40 g (97%), mp 213°C (Lit[8] mp 213-215°C); IR (KBr) ν 1680 (C=O), 3100-3410 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 220 nm (ε 13,500), 283 (8,200); UV λ_{max} (pH 7) 218 nm (ε 14,400), 282 (8,000); UV λ_{max} (pH 11) 283 sh, nm (ε 10,400), 297 (10,700), 310 sh (6,200); NMR (Me_2SO-d_6) δ 4.20 (s, 3, $C\underline{H}_3$), 4.85 (d, 1, $J_{1',2'}$ = 6.8 Hz, $C_1'\underline{H}$), 7.90 (s, 1, $C_5\underline{H}$) and other sugar protons. Anal. Calcd for $C_{11}H_{14}N_4O_5$: C, 46.81; H, 5.00; N, 19.85. Found: C, 46.68; H, 4.97; N, 19.80.

1-Methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]-pyrimidin-7(6H)-one (12). A mixture of 4 (5.64 g, 20 mmol), acetic anhydride (200 ml) and 4-N,N-dimethylaminopyridine (0.20 g, 1.6 mmol) was stirred at room temperature for 16 hr with the exclusion of moisture. Evaporation of the mixture gave a syrupy residue, which was dissolved in ethyl acetate (200 ml). The organic phase was washed with water (2 X 100 ml), dried over Na_2SO_4 and then evaporated to dryness. Crystallization of the residue from methanol gave the title compound, 7.85 g (96%); mp 140°C; IR (KBr) ν 1690 (C=O), 1730 ($-COCH_3$), 3200 (NH) cm^{-1}; UV λ_{max} (pH 1) 222 nm (ε 13,500), 276 (12,700); UV λ_{max} (pH 7) 220 nm (ε 13,100), 276 (12,300); UV λ_{max} (pH 11) 289 nm (ε 15,500); NMR ($CDCl_3$) δ 2.1 and 2.15 (2s, 9, $3COC\underline{H}_3$), 4.30 (s, 3, $C\underline{H}_3$), 5.40 (d, 1, $J_{1',2'}$ = 6.5 Hz, $C_1'\underline{H}$), 7.90 (s, 1, $C_5\underline{H}$), and other sugar protons. Anal. Calcd for $C_{17}H_{20}N_4O_8 \cdot \frac{1}{2}H_2O$: C, 48.92; H, 5.07; N, 13.42. Found: C, 49.06; H, 4.86; N, 13.31.

1-Methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]-pyrimidin-7(6H)-thione (15). A mixture of 12 (4.0 g, 10 mmol), purified P_2S_5 (4.44 g, 20 mmol) and 4-N,N-dimethylaminopyridine (0.20 g, 1.6 mmol) in anhydrous dioxane (100 ml) was heated under gentle reflux for 30 min. An additional amount of P_2S_5 (2.22 g, 10 mmol) was added and heating was continued for another 50 min. The reaction mixture was cooled and poured into ice-water (1 lit) and stirred vigorously for 1 hr. The solution was extracted with $CHCl_3$ (3 X 100 ml) and the combined $CHCl_3$ layers were washed with aqueous saturated NaCl solution (2 X 50 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The

residual foam was crystallized from methanol to yield 3.44 g (81%) of 15; mp 163-164°C; IR (KBr) ν 1580 (C=S), 1740 (-$COCH_3$), 3240 (NH) cm^{-1}; UV λ_{max} (pH 1 and 7) 225 sh, nm (ε 7,400), 344 (17,800), 360 sh (14,900); UV λ_{max} (pH 11) 226 sh, nm (ε 10,600), 337 (16,000); NMR ($CDCl_3$) δ 2.1 and 2.15 (2s, 9, $3COCH_3$), 4.40 (s, 3, CH_3), 5.40 (d, 1, $J_{1',2'}$ = 6.8 Hz, $C_1'H$), 7.80 (s, 1, C_5H), 10.90 (broad s, 1, NH), and other sugar protons. Anal. Calcd for $C_{17}H_{20}N_4O_7S$: C, 48.11; H, 4.75; N, 13.20; S, 7.55. Found: C, 47.92; H, 5.03; N, 13.08; S, 7.59.

1-Methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7(6H)-thione (5). A solution of 15 (3.0 g, 7.0 mmol) in methanol (75 ml) was adjusted to pH 9 with $NaOCH_3$ and stirred at room temperature for 16 hr under anhydrous conditions. The mixture was neutralized with Dowex-50 H^+ resin and the resin was removed by filtration. Evaporation of the filtrate gave a solid which was crystallized from methanol-water (1:1) to yield 1.9 g (97%); mp 207-208°C; IR (KBr) ν 1585 (C=S), 3190-3350 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 225 sh, nm (ε 10,100), 272 (5,800), 345 (21,200), 365 (17,000); UV λ_{max} (pH 7) 225 sh, nm (ε 9,500), 272 (4,800), 345 (19,700), 365 (15,500); UV λ_{max} (pH 11) 225 sh, nm (ε 9,200), 340 (13,300); NMR (Me_2SO-d_6) δ 4.38 (s, 3, CH_3), 4.80 (d, 1, $J_{1',2'}$ = 7.2 Hz, $C_1'H$), 8.00 (s, 1, C_5H), 13.50 (broad s, 1, NH), and other sugar protons. Anal. Calcd for $C_{11}H_{14}N_4O_4S$: C, 44.29; H, 4.73; N, 18.78; S, 10.75. Found: C, 43.99; H, 4.80; N, 18.55; S, 10.66.

1-Methyl-7-methylthio-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (14). A solution of 5 (0.07 g, 0.23 mmol) in methanol (15 ml) was adjusted to pH 9 with $NaOCH_3$. Methyl iodide (0.1 ml) was added and the mixture was stirred at room temperature for 2 hr. The reaction mixture was acidified (pH 5) with dilute acetic acid and the solid that precipitated was collected by filtration. Crystallization of the solid with methanol-water (1:1) gave 0.05 g (69%) of the title compound; mp 205-206°C; IR (KBr) ν 1305 (-SCH_3), 3130-3400 (OH) cm^{-1}; UV λ_{max} (pH 1) 220 nm (ε 18,900), 278 (9,800); UV λ_{max} (pH 7) 220 nm (ε 19,800), 278 (10,200); UV λ_{max} (pH 11) 280 nm (ε 12,500); NMR (Me_2SO-d_6) δ 2.60 (s, 3, SCH_3), 4.20 (s, 3, NCH_3), 4.81 (d, 1, $J_{1',2'}$ = 6.8 Hz, $C_1'H$), 8.10 (s, 1, C_5H), and other sugar protons. Anal. Calcd for $C_{12}H_{16}N_4O_4S$: C, 46.15; H, 5.16; N, 17.94; S, 10.20. Found: C, 45.93; H, 5.26; N, 17.82; S, 9.85.

1-Methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]-pyrimidin-7(6H)-selone (16). A mixture of 12 (6.6 g, 16.1 mmol) and

phosphoryl chloride (100 ml) was heated under reflux for 45 min and excess $POCl_3$ was evaporated. The residual syrup was poured over crushed ice (200 g) and stirred vigorously for 30 min. The aqueous mixture was extracted with CH_2Cl_2 (4 X 100 ml) and the combined organic phase was washed with cold water until the washings were neutral. The organic layer was dried (Na_2SO_4) and evaporated to provide 7-chloro-1-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine (13) as a pale yellow syrup. The compound 13 was used immediately due to its instability and a satisfactory elemental analysis could not be obtained.

To a solution of the above syrupy 13 in absolute ethanol (150 ml) was added selenourea (3.0 g, 24 mmol) and the mixture was stirred at room temperature for 2 hr. The precipitated selenium was filtered off and washed with ethanol (25 ml). Evaporation of the combined filtrates gave a solid which was crystallized from methanol to yield 5.85 g (77%) of 16; mp 165°C; IR (KBr) ν 1585 (C=Se), 1740 ($COCH_3$), 3270 (NH) cm^{-1}; UV λ_{max} (pH 1) 236 sh, nm (ε 5,700), 376 (16,500); UV λ_{max} (pH 7) 235 sh, nm (ε 7,100), 370 (13,200); UV λ_{max} (pH 11) 235 sh, nm (ε 12,700), 356 (16,500); NMR ($CDCl_3$) δ 2.01 and 2.10 (2s, 9, $3COCH_3$), 4.53 (s, 3, CH_3), 5.23 (d, 1, $J_{1',2'}$ = 6.5 Hz, $C_{1'}H$), 8.06 (s, 1, C_5H), and other sugar protons. Anal. Calcd for $C_{17}H_{20}N_4O_7Se$: C, 43.32; H, 4.28; N, 11.89; Se, 16.75. Found: C, 43.03; H, 4.33; N, 11.64; Se, 17.05.

1-Methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidin-7(6H)-selone (6). A solution of 16 (5.4 g, 11.46 mmol) in methanol (120 ml) was adjusted to pH 9 with $NaOCH_3$ and stirred at room temperature for 16 hr. The precipitated selenium was filtered off and the filtrate was neutralized with Dowex-50 H^+ resin. The resin was removed by filtration and the filtrate was evaporated to dryness. Crystallization of the residual semi-solid from methanol gave 2.0 g (50%) of the title compound; mp 194-195°C; IR (KBr) ν 1590 (C=Se), 3180-3400 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 235 sh, nm (ε 7,300), 377 (17,100); UV λ_{max} (pH 7) 235 sh, nm (ε 7,300), 370 (12,500); UV λ_{max} (pH 11) 235 sh, nm (ε 10,500), 356 (13,400); NMR (Me_2SO-d_6) δ 4.52 (s, 3, CH_3), 4.92 (d, 1, $J_{1',2'}$ = 6.5 Hz, $C_{1'}H$), 8.05 (s, 1, C_5H), and other sugar protons. Anal. Calcd for $C_{11}H_{14}N_4O_4Se \cdot H_2O$: C, 36.37; H, 4.44; N, 15.42; Se, 21.74. Found: C, 36.53; H, 4.26; N, 15.46; Se, 21.55.

7-Amino-1-methyl-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine 5'-phosphate (1-Methylformycin 5'-phosphate, 17). A solution of phosphoryl

chloride (2 ml) in trimethyl phosphate (25 ml) was cooled to 0°C, and dry powdered 1-methylformycin (7, 2.0 g, 7.1 mmol) was added with stirring. The mixture was protected from moisture and stirred at 0°C for 5 hr. Complete dissolution was obtained within one hr. TLC [aliquot hydrolyzed with water, silica gel, CH_3CN:0.1 M NH_4Cl, 7:3] indicated a complete conversion of the nucleoside to the nucleotide. The solution was poured into ice-water (100 ml) and stirred for 30 min. The pH of the aqueous solution was adjusted to 2 with 2 N NaOH and extracted with ether (2 X 100 ml). The aqueous solution was applied to a column of activated charcoal (60 g), and the column was washed with water until the eluate was salt-free. The nucleotide was eluted with a mixture of ethanol-water-concentrated ammonium hydroxide (10:10:1, v/v). The homogeneous fractions containing the nucleotide were concentrated (∿50 ml) and passed through a column of Dowex-50 H^+ resin (2 X 25 cm). The column was eluted with water (2 lit). The solution was concentrated to ∿100 ml, filtered through a membrane filter and the filtrate was frozen and lyophilized to obtain the title compound as homogeneous powder, yield 1.6 g (64%); mp 215°C; IR (KBr) ν 3050-3400 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1) 240 nm (ε 10,500), 300 (12,300); UV λ_{max} (pH 7) 230 sh, nm (ε 8,700), 295 (11,600); UV λ_{max} (pH 11) 230 sh, nm (ε 8,700), 295 (10,500); NMR (Me_2SO-d_6) δ 4.25 (s, 3, CH_3), 4.95 (d, 1, $J_{1',2'}$ = 7.0 Hz, $C_1'H$), 7.65 (broad s, 2, NH_2), 8.22 (s, 1, C_5H), and other sugar protons. Anal. Calcd for $C_{11}H_{16}N_5O_7P \cdot H_2O$: C, 34.83; H, 4.78; N, 18.46; P, 8.16. Found: C, 34.95; H, 4.60; N, 18.13; P, 8.47.

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REFERENCES

1. Suhadolnik, R. J. "Nucleosides as Biological Probes" (1979), Wiley-Interscience, New York, NY, pp. 169-183.
2. Hori, M.; Ito, E.; Takita, T.; Koyama, G.; Takeuchi, T.; Umezawa, H. J. Antibiot. Ser. A (1964), 17, 96.
3. Koyama, G.; Maeda, K.; Umezawa, H.; Iitaka, Y. Tetrahedron Lett. (1966) 597.

4. Koyama, G.; Umezawa, H. J. Antibiot. Ser. A (1965) 18, 175.
5. Long, R. A.; Lewis, A. F.; Robins, R. K.; Townsend, L. B. J. Chem. Soc. C. (1971) 2443.
6. Robins, M. J.; McCarthy, Jr., J. R.; Jones, R. A.; Mengel, R. Can. J. Chem. (1973) 51, 1313.
7. Jain, T. C.; Russell, A. F.; Moffatt, J. G. J. Org. Chem. (1973) 38, 3179.
8. Townsend, L. B.; Long, R. A.; McGraw, J. P.; Miles, D. W.; Robins, R. K.; Eyring, H. J. Org. Chem. (1974) 39, 2023.
9. Robins, M. J.; Naik, S. R.; Lee, A. S. K. J. Org. Chem. (1974) 39, 1891.
10. Acton, E. M.; Fujiwara, A. N.; Goodman, L.; Henry, D. W. Carbohydr. Res. (1974) 33, 135.
11. Zemlicka, J. J. Am. Chem. Soc. (1975) 97, 5896.
12. Huynh-Dinh, T.; Kolb, A.; Gouyette, C.; Igolen, J.; Tran-Dinh, S. J. Org. Chem. (1975) 40, 2825.
13. Chung, H. L.; Zemlicka, J. J. Heterocycl. Chem. (1977) 14, 135.
14. Makabe, O.; Miyadera, A.; Kinoshita, M.; Umezawa, S.; Takeuchi, T. J. Antibiot. (1978) 31, 456.
15. Lewis, A. F.; Townsend, L. B. J. Am. Chem. Soc. (1980) 102, 2817.
16. Lewis, A. F.; Townsend, L. B. J. Am. Chem. Soc. (1982) 104, 1073.
17. Robins, R. K.; Townsend, L. B.; Cassidy. F. C.; Gerster, J. F.; Lewis, A. F.; Miller, R. L. J. Heterocycl. Chem. (1966) 3, 110.
18. Carson, D. A.; Chang, K.-P. Biochem. Biophys. Res. Commun. (1981) 100, 1377.
19. Berman, J. D. J. Inf. Dis. (1982) 145, 279.
20. Nelson, D. J.; Lafon, S. W.; Jones, T. E.; Spector, T.; Berens, R. L.; Marr, J. J. Biochem. Biophys. Res. Commun. (1982) 108, 349.
21. Rainey, P.; Santi, D. V. Proc. Natl. Acad. Sci. USA (1983) 80, 288.
22. Ishizuka, M.; Sawa, T.; Hori, S.; Takazama, H.; Takeuchi, T.; Umezawa, H. J. Antibiot. (1968) 21, 5.
23. Rainey, P.; Garrett, C. E.; Santi, D. V. Biochem. Pharmacol. (1983) 32, 749.
24. Goebel, R. J.; Adams, A. D.; McKernan, P. A.; Murray, B. K.; Robins, R. K.; Revankar, G. R.; Canonico, P. G. J. Med. Chem. (1982) 25, 1334.

25. Berman, J. D.; Lee, L. S.; Robins, R. K.; Revankar, G. R. Antimicrob. Agents Chemother. (1983) 24, 233.
26. Santi, D. V. Department of Biochemistry, University of California, San Francisco, CA. Private communication.
27. Milne, G. H.; Townsend, L. B. J. Chem. Soc. Perkin I. (1972) 2677.
28. Marr, J. J.; Berens, R. L.; Nelson, D. J. Science (1978) 201, 1018.
29. Townsend, L. B.; Milne, G. H. J. Heterocycl. Chem. (1970) 7, 753.
30. Chu, S-H. J. Med. Chem. (1971) 14, 254.
31. Chu, S-H.; Davidson, D. D. J. Med. Chem. (1972) 15, 1088.
32. Chu, S-H; Shiue, C-Y.; Chu, M-Y. J. Med. Chem. (1974) 17, 406.
33. Townsend, L. B.; Milne, G. H. Ann. N.Y. Acad. Sci. (1975) 255, 91.
34. Witczak, Z. J. Nucleosides Nucleotides (1983) 2, 295.
35. Shiue, C-Y.; Chu, S-H. J. Chem. Soc. Chem. Commun. (1975) 319.
36. Giziewicz, J.; Shugar, D. Acta Biochim. Pol. (1977) 24, 231.
37. Robins, R. K. Pharmaceutical Res. (1984) 11.
38. Yoshikawa, M.; Kato, T.; Takenishi, T. Tetrahedron Lett. (1967) 5065.
39. In vitro antiviral and antitumor data were obtained by using standard assays as described in: Ugarkar, B. G.; Cottam, H. B.; McKernan, P. A.; Robins, R. K.; Revankar, G. R. J. Med. Chem. (1984) 27, in press.

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