show activity when tested at concentrations up to 100 μg/mL were 5-(2,2-dichloro-1-fluorovinyl)-2′-deoxyuridine (12), which showed an ID_{50} of 26 μg/mL against the HFEM strain of the virus, and (*E*/*Z*)-5-(2-chloro-1,2-difluorovinyl)-2′-deoxyuridine (15 and 16), which showed an ID_{50} of 20–24 μg/mL against KOS, SC16, and HFEM strains. At none of the concentrations tested did either of these compounds cause destruction of the cell monolayer. Separation of the *E* and *Z* isomers (15 and 16) was accomplished on a small scale. One isomer showed an ID_{50} of 24 μg/mL against the HFEM strain of the virus, whereas the other isomer showed no activity even at 100 μg/mL. Simultaneously, standard compounds were assayed against the HFEM strain to give the following values for ID_{50}: arabinosyladenine, 21 μg/mL; acycloguanosine, 0.80 μg/mL; 2′-deoxy-5-iodouridine, 4.4 μg/mL; and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine, 0.07 μg/mL.

The mixed isomers 15 and 16 were tested for inhibition of the multiplication of rapidly dividing Vero cells and MRC-5 (human diploid fibroblast) cells. With Vero cells an 18% reduction in cell growth was observed at 100 μg/mL, and with MRC-5 cells there was no observable effect upon cell proliferation at concentrations up to 200 μg/mL.

Acknowledgment. The authors thank Dr. M. J. Browne and M. Conlin for performing the biological tests and Dr. M. Brightwell for HPLC separations. Thanks are also due to the Science Research Council of the United Kingdom, for a CASE research studentship (to S.A.N.) and to Beecham Pharmaceuticals for supplies of materials and services.

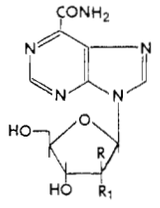
Synthesis and Antiviral Activity of Certain Carbamoylpyrrolopyrimidine and Pyrazolopyrimidine Nucleosides

Richard J. Goebel,† Alexander D. Adams,† Patricia A. McKernan,† Byron K. Murray,† Roland K. Robins,† Ganapathi R. Revankar,*,† and Peter G. Canonico‡

Cancer Research Center, Department of Chemistry, Brigham Young University, Provo, Utah 84602, and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21701. *Received April 9, 1982*

Following our recent discovery that 9-β-D-ribofuranosylpurine-6-carboxamide (1) exhibits potent antiviral activity, we were prompted to synthesize certain pyrrolopyrimidine and pyrazolopyrimidine nucleosides containing a carbamoyl function (**7a,b** and **13**). The key precursor, 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-carbonitrile (**8a**), required for the synthesis of **7a** was prepared from the corresponding 4-chloro analogue (**4a**). Reaction of **4a** with methanethiol, followed by oxidation, gave the 4-methylsulfonyl derivative (**6a**), which with NaCN in DMF gave **8a**. Alkaline hydrolysis of **8a** provided **7a**. Similarly, **7b** was prepared from 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*] pyrimidine (**4b**) via the carbonitrile intermediate **8b**. Starting with thioformycin B or 7-chloro-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (**10**) and following the similar sequence of reactions, we obtained compound **13**. The in vitro antiviral studies of these carbamoyl and certain related nucleosides indicated **7a** to be a potent antiviral agent against vaccinia virus, whereas **13** was moderately active. 4-Chloro-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine was found to be one of the most active compounds against RVF, PICH, YF, and SF viruses in culture.

The introduction of a carbamoyl function at the 6-position of certain purine nucleosides resulted in compounds with significant antiviral efficacy.[1] 9-β-D-Ribofuranosylpurine-6-carboxamide (1) was found to be a po-



1: R = H, R_1 = OH

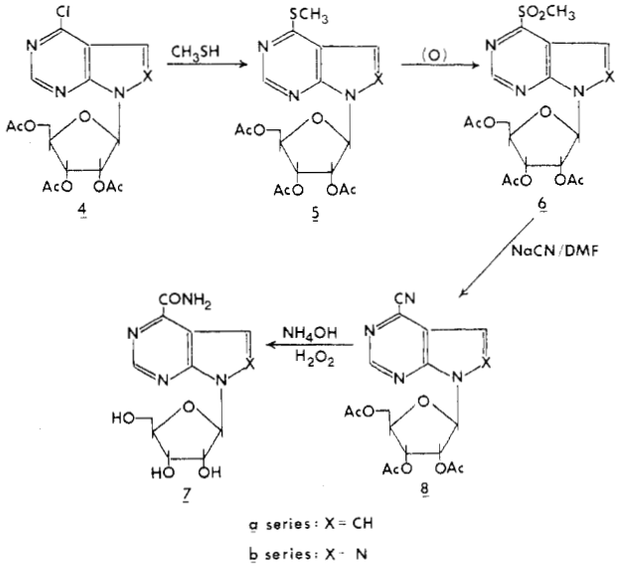
2: R = OH, R_1 = H

3: R = R_1 = H

tent, broad-spectrum antiviral agent.[1] Compound 1 inhibited the growth of Rift Valley fever (RVF) virus and Pichinde (PICH) virus to the extent of 90% at 250 μg/mL, and inhibition was nearly complete at 500 μg/mL. In an attempt to gain further insight into the nature of this antiviral potency, we recently reported[2] on the synthesis and biological activity of β-D-arabinosyl (2) and 2′-deoxy-β-D-ribosyl (3) analogues of 1. This report describes the synthesis and antiviral activity of certain analogues of 1 modified at the aglycon portion.

Chemistry. Because of the natural occurrence of the 7-deazapurine ring system, e.g., in tubercidin, toyocamycin, sangivamycin, nucleoside Q and Q*, and their unusual biological properties, there has been a great deal of interest

Scheme I



a series: X = CH

b series: X = N

† Brigham Young University.

‡ U.S. Army Medical Research Institute of Infectious Diseases.

(1) J. D. Westover, G. R. Revankar, R. K. Robins, R. D. Madsen, J. R. Ogden, J. A. North, R. W. Mancuso, R. J. Rousseau, and E. L. Stephen, *J. Med. Chem.*, **24**, 941 (1981).

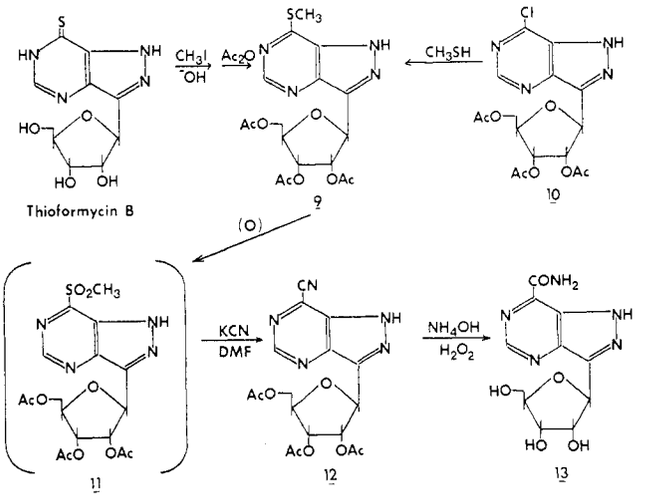
(2) P. C. Srivastava, G. R. Revankar, R. K. Robins, and R. J. Rousseau, *J. Med. Chem.*, **24**, 393 (1981).

0022-2623/82/1825-1334$01.25/0

in this ring system. We have now prepared 7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine-4-carboxamide (**7a**) (Scheme I) starting with 4-chloro-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**4a**).[3] Direct treatment of **4a** with methanethiol in the presence of a stoichiometric amount of KOBu^t provided 4-(methylthio)-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**5a**) as a chromatographically pure gum. The $KMnO_4$ oxidation of **5a** in acetic acid gave the corresponding 4-methylsulfonyl derivative (**6a**). In the ^1H NMR ($CDCl_3$) of **6a**, all the protons, except acetyls and $C_{4'}$ H and $C_{5'}$ H_2, were shifted downfield as compared to those of **5a**. The SO_2CH_3 protons had a shift of 0.67 ppm, whereas C_2 H, C_5 H, C_6 H, and $C_{1'}$ H were shifted by 0.37, 0.70, 0.42, and 0.12 ppm, respectively. The downfield shift in **6a** would be expected due to the sulfonyl group. Our attempts to crystallize **6a** were unsuccessful, and it was analyzed as a syrup. Treatment of **6a** with NaCN in DMF provided crystalline 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine-4-carbonitrile (**8a**). Compound **8a** exhibited a weak absorption at 2230 cm^{-1} for the nitrile band in the IR spectrum and was completely characterized by UV, ^1H NMR, and elemental analysis. Once isolated in pure form, compound **8a** was found to be stable at room temperature for several months. Reaction of **8a** with NH_4OH in presence of H_2O_2 provided the desired **7a**, as water soluble needles. The structure of **7a** was determined on the basis of ^1H NMR, which showed, in addition to other protons at appropriate positions, peaks at δ 8.40 and 7.90 as broad singlets for the $CONH_2$ protons. Further structural proof came from elemental analysis, UV spectrum, and mass spectrometry, which exhibited a molecular ionic peak at 294.

As a part of this program we have now synthesized 1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4-carboxamide (**7b**) from 4-(methylthio)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**5b**). Treatment of allopurinol riboside triacetate[4] with $SOCl_2$ and DMF in $CHCl_3$ gave crystalline 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**4b**).[5] During the course of the present work, Townsend and co-workers[6] reported a similar chlorination procedure (they used CH_2Cl_2 instead of $CHCl_3$) to obtain **4b**. Compound **4b** was found to be unstable at room temperature. Direct treatment of **4b** with methanethiol in presence of a stoichiometric amount of KOBu^t gave **5b**. The ^1H NMR spectrum of **5b** revealed three sharp singlets between δ 2.12 and 2.20 (9 protons, 3 OAc groups) and a singlet at δ 2.75 (SCH_3), indicating that the nucleophilic displacement of the 4-chloro group of **4b** has occurred retaining the blocking acetyl groups. The oxidation of **5b** with *m*-chloroperoxybenzoic acid in anhydrous ethyl ether provided the crude oxidation product **6b**, which was found to be very susceptible to moisture. Therefore, without further purification, product **6b** was treated with NaCN in DMF, which provided the desired 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-4-carbonitrile (**8b**) after column chromatographic purification. Compound **8b** was found to be quite stable but did not exhibit appreciable absorption in the IR for the nitrile band. Treatment of **8b** with NH_4OH in the presence of H_2O_2 under carefully controlled conditions provided the desired **7b** as water-soluble needles. The structure of **7b** was determined on the basis of the ^1H NMR spectrum, which exhibited, in addition to other protons at appropriate positions, the peaks at δ 8.17 and 8.53 as broad singlets for $CONH_2$ protons, which were exchangeable by D_2O. Further structural proof came from elemental analysis.

Scheme II



Formycin B has shown significant activity against influenza A_1 virus.[7] In our laboratory, thioformycin B was found to be highly active against the myxovirus group in vitro.[8] Of considerable interest is the fact that formycin B and thioformycin B have low toxicity in animals,[7,9] in contrast to formycin. In view of these observations, the synthesis of 3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine-7-carboxamide (**13**) is of special interest (Scheme II). The key precursor needed for the synthesis of **13** was 7-(methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[4,3-*d*]pyrimidine (**9**), which could be obtained either from thioformycin B or from 7-chloro-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (**10**).[10] The previously described procedure[10] for the preparation of thioformycin B from formycin is fairly lengthy, involving five steps. However, in the present study, sulfhydrolysis[11-13] of formycin with liquid H_2S in pyridine gave thioformycin B in comparable yield. Methylation of thioformycin B with CH_3I in the presence of NH_4OH, followed by acetylation, furnished **9**. Alternatively, direct nucleophilic displacement of the 7-chloro group in **10** by treatment with methanethiol in the presence of a stoichiometric amount of KOBu^t also gave **9**. The conversion of **9** to crystalline 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine-7-carbonitrile (**12**) by following the sequence of reactions as described for **8b** using *m*-chloroperoxybenzoic acid oxidation and treatment

(3) J. F. Gerster, B. Carpenter, R. K. Robins, and L. B. Townsend, *J. Med. Chem.*, **10**, 326 (1967).
(4) Henning Berlin GimbH, German Patent 2 226 673 (1973).
(5) G. R. Revankar and L. B. Townsend, *J. Chem. Soc. C*, 2440 (1971).
(6) G. A. Bhat, J.-L. G. Montero, R. P. Panzica, L. L. Wotring, and L. B. Townsend, *J. Med. Chem.*, **24**, 1165 (1981).
(7) T. Takeuchi, J. Iwanga, T. Coyagi, M. Murase, T. Sawa, and H. Umezawa, *J. Antibiot.*, **20**, 297 (1967).
(8) J. R. Ogden, R. D. Madsen, and J. A. North "Abstracts, Annual Meeting of the American Society for Microbiology", Los Angeles, CA, 1979, American Society for Microbiology, Washington, DC, 1979, Abstr. A47, p 8.
(9) T. Takeuchi, J. Iwanga, T. Coyagi, and H. Umezawa, *J. Antiobiot.*, **19**, 286 (1966).
(10) R. A. Long, A. F. Lewis, R. K. Robins, and L. B. Townsend, *J. Chem. Soc. C*, 2443 (1971).
(11) T. Ueda, M. Imazawa, K. Miura, R. Iwata, and K. Odajima, *Tetrahedron Lett.*, 2507 (1971).
(12) K. Miura and T. Ueda, *Chem. Pharm. Bull.*, **23**, 2064 (1975).
(13) R. B. Meyer, Jr., T. E. Stone, F. P. Heinzel, *J. Heterocycl. Chem.*, **15**, 1511 (1978).

with KCN/DMF was accomplished. Compound **12** exhibited a weak absorption at 2220 cm^{-1} for the nitrile band in the IR spectrum. Subsequent treatment of **12** with H_2O_2 in an ammoniacal solution provided the desired **13**. The structural assignment and the purity of **13** were assured by ^1H NMR spectroscopy and by elemental analysis.

The site of ribosylation and the β configuration of all the nucleosides synthesized in this study were confirmed, since the structures of the starting nucleosides **4a, b** and **10** were already established.

Antiviral Evaluation. Inhibition of the virus-induced cytopathic effect (CPE) was used as the initial indicator of antiviral activity. CPE was observed in African green monkey kidney (Vero, V) and human laryngeal epithelioma (HEp-2, H) cells after infection with herpes simplex type 1 (HSV 1), vaccinia (VV), and parainfluenza type 3 (Para 3) viruses. In this system, monolayers (18 h) of cells were exposed to 320 $TCID_{50}$ (tissue culture 50% infective dose) of virus, and concentrations of each compound in one-half log dilutions ranging from 1000 to 1 $\mu g/mL$ were added within 15 to 30 min. The degree of CPE inhibition was observed microscopically after 72 h of incubation at 37 °C in 5% CO_2 and scored numerically in order to calculate a virus rating (VR) as previously reported.[14] Significance of antiviral activity in terms of VRs has been assigned as follows: 0.5, slight or no activity; 0.5–0.9, moderate activity; ≥1.0, marked activity. Most of the compounds synthesized during this study have been tested against the above viruses. Several purine nucleosides are also included in this study for comparison. 4-(Methylthio)-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine, 6-(methylthio)-9-β-D-ribofuranosylpurine, 7-(methylthio)-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine, and **9** showed significant antiviral activity, depending on the cell line, and the results of a single experiment in parallel with the synthetic broad-spectrum antiviral agent 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin)[15] are shown in Table I. 6-(Methylthio)-9-β-D-ribofuranosylpurine has been shown previously to be significantly active against Friend leukemia virus.[16] Among the newly synthesized nucleoside carboxamides, **7a** exhibited potent antiviral activity against VV, and **13** showed only moderate activity.

In addition to the above viruses, certain of these nucleosides were tested at the U.S. Army Medical Research Institute of Infectious Disease, Fort Detrick, MD, against Rift Valley fever (RVF), Venezuelan equine encephalitis (VEE), Pichinde (PICH), yellow fever (YF), and Sandfly fever (SF) viruses in vitro in vero cells. Viruses were adsorbed for 1 h at 37 °C in 24-well monolayer cell cultures, overlaid with agar containing appropriate dilutions of compounds, and incubated again at 37 °C in a CO_2 incubator. Plaque were enumerated after staining with crystal violet stain, and the inhibition of viral growth in cell culture is shown in Table I. The results indicate formycin B is much more effective than ribavirin against PICH. Both thioformycin B and **9** exhibited good activity against YF. In addition to YF, thioformycin B was active against SF in vitro. Several 7-deazapurine nucleosides, such as 7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidin-4-one and 4-chloro-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine, showed significant antiviral activity against RVF, PICH, YF, and SF in vitro, indicating that derivatives of the 7-deazapurine ring may be much more efficient in inhibiting the above viruses than the pyrazolopyrimidine ring. 4-Chloro-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine was found to be superior to ribavirin against the above viruses in vitro but was found to be quite toxic. Because of the paucity of compounds **7a, b** and **13**, they were not tested on these viruses.

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 Varian EM-390 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 as indicated by elemental analyses was verified by ^1H NMR. Infrared spectra (IR) were obtained on a Perkin-Elmer 257 spectrophotometer, and ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, 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. ICN Woelm silica gel (70–230 mesh) was used for column chromatography. All solvents used 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.

4-(Methylthio)-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (5a). To a cold (0–5 °C) solution of 4-chloro-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine[3] (**4a**; 4.11 g, 10 mmol) in absolute ethanol (100 mL) was added a cold ethanolic solution (100 mL) of CH_3SH (CH_3SH bubbled in for 5 min), followed by $KOBu^t$ (1.23 g, 11 mmol). The mixture was stirred at 0 °C for 2.5 h. The solvent was evaporated, and the residue was extracted with $CHCl_3$ (250 mL). The organic layer was washed with water (2 × 50 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was purified by passing through a silica gel column (3 × 35 cm, $CHCl_3$) to obtain colorless gum: yield 4.0 g (95%); IR (KBr) ν 1370 (SCH_3), 1740 (OAc) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.0–2.15 (2 s, 9, 3 OAc), 2.66 (s, 3, SCH_3), 6.43 (d, 1, J = 4.5 Hz, $C_{1'}$ H), 6.56 (d, 1, J = 3.0 Hz, C_5 H), 7.23 (d, 1, J = 3.0 Hz, C_6 H), 8.63 (s, 1, C_2 H), and other sugar protons. Anal. ($C_{18}H_{21}N_3SO_7$) C, H, N, S.

4-(Methylsulfonyl)-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6a). To a stirred and cold (0–5 °C) solution of **5a** (1.40 g, 3.3 mmol) in acetic acid (35 mL) and water (35 mL) was added powdered $KMnO_4$ (1.32 g, 8.35 mmol) over a period of 30 min. The reaction mixture was stirred at ice-bath temperature for an additional 1 h and then 30% H_2O_2 was added until the solution became colorless. The mixture was extracted with $CHCl_3$ (3 × 50 mL), and the combined organic layer was washed with saturated aqueous $NaHCO_3$ solution (2 × 50 mL). The dried (Na_2SO_4) organic layer was evaporated to dryness. The residual syrup was purified by passing through a silica gel column (2.5 × 25 cm, $CHCl_3$) to obtain a colorless gum: yield 0.48 g (31.8%); IR (KBr) ν 1310 (SO_2CH_3), 1740 (OAc) cm^{-1}; UV λ_{max} (MeOH) 227 nm (ϵ 27 300), 284 (4500), 295 sh (4100); ^1H NMR ($CDCl_3$) δ 2.05–2.20 (2 s, 9, 3 OAc), 3.33 (s, 3, SO_2CH_3), 6.55 (d, 1, J = 4.5 Hz, $C_{1'}$ H), 7.26 (d, 1, J = 3.0 Hz, C_5 H), 7.65 (d, 1, J = 3.0 Hz, C_6 H), 9.00 (s, 1, C_2 H) and other sugar protons. Anal. ($C_{18}H_{21}N_3SO_9 \cdot H_2O$) C, H, N, S.

7-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine-4-carbonitrile (8a). To a solution of **6a** (0.90 g, 1.9 mmol) in anhydrous DMF (7 mL) was added granular NaCN (0.17 g, 3.47 mmol). The reaction mixture was stirred at room temperature for 2 h to provide a dark-brown solution. The solvent was evaporated and the residue was partitioned with $CHCl_3$ (50 mL) and water (35 mL). The organic phase was washed with cold saturated aqueous $NaHCO_3$ solution (2 × 50 mL), followed by

(14) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971).
(15) "Ribavirin—A Broad Spectrum Antiviral Agent", R. A. Smith and W. Kirkpatrick, Eds., Academic Press, New York, 1980.
(16) R. W. Sidwell, G. J. Dixon, S. M. Sellers, and F. M. Schabel, Jr., *Cancer Chemother. Rep.*, **50**, 299 (1966).
(17) J.-L. G. Montero, G. A. Bhat, R. P. Panzica, and L. B. Townsend, *J. Heterocycl. Chem.*, **14**, 483 (1977).
(18) Y. Mizuno, M. Ikehara, K. Watanabe, and S. Suzaki, *Chem. Pharm. Bull.*, **11**, 1091 (1963).
(19) M. Hori, E. Ito, T. Takita, G. Koyama, and H. Umezawa, *J. Antibiot.*, **17A**, 96 (1964).

Table I. Comparative in Vitro Antiviral Activity of Ribavirin and Certain Purine, Pyrrolopyrimidine, and Pyrazolopyrimidine Nucleosides

compd	cell line/ toxic level[d]	virus ratings[a]							
		HSV1	VV	para 3	RVF	VEE	PICH	YF	SF
6-chloro-9-β-D-ribofuranosylpurine	V/1000	0.4	1.8	0.4					
4-chloro-7-β-D-ribofuranosylpyrrolo[2,3-*d*]-pyrimidine[e]	V/320	0.2	*c*	0.1	++++[b]	0	++++	++++	+++
7-chloro-3-β-D-ribofuranosylpyrazolo-[4,3-*d*]pyrimidine[f]	V/32	0	1.0	1.3					
9-β-D-ribofuranosylpurine-6-thione	V/1000	0.8	1.1	1.2					
7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine-4-thione[e]	V/>1000	0.2	0.5	1.1					
1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4-thione[g]	V/>1000	0	0.9	0.7					
3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine-7-thione[h]	V/1000	0	1.0	1.1	+	0	+	+++	+++
6-(methylthio)-9-β-D-ribofuranosylpurine	V/320	1.7	2.0	2.2					
4-(methylthio)-7-β-D-ribofuranosylpyrrolo-[2,3-*d*]pyrimidine[e]	V/>1000	0.5	1.0	0.9					
	H/100	1.3	1.4	1.5					
4-(methylthio)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**5b**)	V/320	0	0.5	0	+	0		+	0
4-(methylthio)-1-β-D-ribofuranosylpyrazolo-[3,4-*d*]pyrimidine[g]	V/>1000	0.2	0.2	1.0					
7-(methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (**9**)	V/all doses	0.6	1.2	1.0	0	0	0	+++	0
7-(methylthio)-3-β-D-ribofuranosylpyrazolo-[4,3-*d*]pyrimidine[f]	V/all doses	0.8	1.4	1.3					
9-β-D-ribofuranosylpurine-6-carboxamide (**1**)[i]	V/>1000	0.6	1.5	0					
	H/320	0.6	1.1	1.4					
7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine-4-carboxamide (**7a**)	V/1000	0.4	1.3	0.4					
	H/100	0	1.2	0.2					
1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyramidine-4-carboxamide (**7b**)	V/>1000	0	0	0					
	H/320	0	0.2	0					
3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyramidine-7-carboxamide (**13**)	V/100	0	0.6	0					
	H/100	0	0.8	0.4					
1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide[j]	V/>1000	0.6	1.2	1.3	++	+	+++	+++	+++
	H/>1000	1.2	1.7	1.7					
7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidin-4-one[k]	V/32	0	*c*	0	+++	0	+++	+++	+
1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-one[l]	V/>1000	0	*c*	*c*	0	0	++	0	0
3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7-one[m]	V/10	0.1	*c*	*c*	0	0	++++	0	0

[a] The virus rating (VR) was determined by comparing CPE development in drug-treated cells (T) and virus control cells (C). The CPE value (0–4) assigned to T for each drug level was subtracted from that of C, and the differences (C – T) were totaled. If partial toxicity was evident at any drug level, the C – T of that level was divided by 2. The sum of all C – T values was then divided by 10 times the number of test cups used per drug level. [b] +, 10–30% plaque reduction, at drug concentrations of 250–500 μg/mL; ++, 31–60% plaque reduction, at drug concentrations of 100–250 μg/mL; +++, 61–90% plaque reduction, at drug concentrations of 25–100 μg/mL; ++++, >90% plaque reduction, at drug concentrations < 25 μg/mL. [c] Not determined. [d] In micrograms per milliliter. [e] Reference 3. [f] Reference 10. [g] Reference 17. [h] Thioformycin B. [i] Reference 1. [j] Ribavirin; ref 15. [k] Reference *k*. [l] Reference *l*. [m] Formycin B; ref 19.

water (2 × 50 mL), before it was dried (Na_2SO_4). $CHCl_3$ was evaporated, and the residue was chromatographed over a silica gel column (2.5 × 25 cm, $CHCl_3$) with 5% acetone in $CHCl_3$ as eluant. The homogeneous fractions were pooled and evaporated to yield light yellow crystals. Recrystallization from 10% MeOH in diethyl ether provided an analytical sample: yield 0.62 g (78%); mp 98–99 °C; IR (KBr) ν 1750 (OAc), 2230 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.03–2.20 (3 s, 9, 3 OAc), 6.46 (d, 1, J = 4.5 Hz, $C_{1'}$ H), 6.83 (d, 1, J = 3.0 Hz, C_5 H), 7.60 (d, 1, J = 3.0 Hz, C_6 H), 8.98 (s, 1, C_2 H), and other sugar protons. Anal. ($C_{18}H_{18}N_4O_7$) C, H, N.

7-β-D-Ribofuranosylpyrrolo[2,3-*d*]pyrimidine-4-carboxamide (7a). To a cold (0–5 °C) solution of **8a** (0.50 g, 1.25 mmol) in MeOH (15 mL) and water (15 mL) was added H_2O_2 (30%, 0.3 mL), and the solution was adjusted to pH 9 with concentrated NH_4OH. The mixture was stirred at ice-bath temperature for 5 h and then allowed to stand in the refrigerator overnight. The solvent was evaporated, and the residue was chromatographed on a silica gel column (1.5 × 20 cm) with EtOAc/H_2O/1-PrOH, 4:2:1, upper phase, as the elution solvent. The homogeneous product was crystallized from aqueous ethanol to provide an analytical sample: yield 0.15 g (41%); mp 171–172 °C (sintered at 85–100 °C); IR (KBr) ν 1670 (CO of $CONH_2$), 3300–3400 (OH, NH_2) cm^{-1}; UV λ_{max} (pH 1) 231 nm (ε 20 300), 288 (3200); UV λ_{max} (pH 7) 227 nm (ε 22 300), 295 (4100); UV λ_{max} (pH 11) 294 nm (ε 4100); ^1H NMR (Me_2SO-d_6) δ 6.30 (d, 1, J = 4.0 Hz, $C_{1'}$ H), 7.20 (d, 1, J = 2.5 Hz, C_5 H), 8.10 (d, 1, J = 2.5 Hz, C_6 H), 7.90 and 8.40 (2 s, 2, $CONH_2$, exchanged with D_2O, and other sugar protons. Anal. ($C_{12}H_{14}N_4O_5$) C, H, N.

4-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine (4b). This procedure is essentially similar to the one described by Townsend and co-workers.[6] However, we feel the following workup is relatively simple and less time consuming.

To a solution of 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidin-4-one[4] (3.95 g, 10 mmol) in anhydrous $CHCl_3$ (100 mL) was added, dropwise, a mixture of DMF (6.0 mL) and freshly distilled $SOCl_2$ (24.0 mL) in $CHCl_3$ (50 mL). After the addition was complete, the mixture was heated under reflux for 3 h under anhydrous conditions and then evaporated to dryness. The residue was coevaporated with dry benzene (3 × 50 mL) before it was added to crushed ice (100 mL). The pH of the aqueous solution was kept neutral by adding $NaHCO_3$, which was then extracted with $CHCl_3$ (3 × 100 mL). The combined organic layer was washed with cold water (2 × 50 mL) and dried over Na_2SO_4. Evaporation of $CHCl_3$ gave a syrup, which

crystallized after triturating with ethanol: yield 3.70 g (90%); mp 115–116 °C (lit.[6] mp. 115–116 °C); IR (KBr) ν 740 (C–Cl), 1750 (OAc) cm^{-1}; UV λ_{max} (pH 1) 255 nm (ϵ 4750); UV λ_{max} (pH 7) 252 nm (ϵ 5600); UV λ_{max} (pH 11) 255 nm (ϵ 4750); ^1H NMR ($CDCl_3$) δ 2.10–2.20 (3 s, 9, 3 OAc), 6.65 (d, 1, J= 3.0 Hz, $C_{1'}$ H), 8.30 (s, 1, C_6 H), 8.85 (s, 1, C_3 H), and other sugar protons. Anal. ($C_{16}H_{17}ClN_4O_7$) C, H, N, Cl.

4-(Methylthio)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine (5b). Starting with **4b** (5.90 g, 14.3 mmol) and $KOBu^t$ (1.45 g, 12.9 mmol) in absolute ethanol (175 mL) containing CH_3SH and following the procedure as described for **5a**, we obtained the title compound as needles: yield 3.52 g (58%); mp 88–90 °C; IR (KBr) ν 1370 (SCH_3), 1740 (OAc) cm^{-1}; UV λ_{max} (pH 1) 225 nm sh (ϵ 7200), 294 (14800); UV λ_{max} (pH 7 and 11) 225 nm sh (ϵ 9800), 292 (15700); ^1H NMR ($CDCl_3$) δ 2.12–2.20 (3 s, 9, 3 OAc), 2.75 (s, 3, SCH_3), 6.65 (d, 1, J = 3.0 Hz, $C_{1'}$ H) 8.15 (s, 1, C_6 H), 8.80 (s, 1, C_3 H), and other sugar protons. Anal. ($C_{17}H_{20}N_4SO_7$) C, H, N, S.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]-pyrimidine-4-carbonitrile (8b). To a cold (0–5 °C) solution of **5b** (1.14 g, 2.7 mmol) in ethyl ether (75 mL) was added *m*-chloroperoxybenzoic acid[20] (80–90%, 1.50 g, 8.7 mmol), and the mixture was stirred for 5 h under anhydrous conditions at ice-bath temperature. The solvent was removed, and the residue was dissolved in dry DMF (50 mL) containing KCN (0.31 g, 4.76 mmol). The mixture was stirred at room temperature for 18 h with exclusion of moisture. The dark brown solution was evaporated, and the residue was taken in $CHCl_3$ (100 mL). The $CHCl_3$ portion was washed with cold 5% $NaHCO_3$ solution (2 × 50 mL), followed by water (2 × 50 mL), and dried over anhydrous Na_2SO_4. The $CHCl_3$ was evaporated to provide crude **8b**, which was purified by silica gel column chromatography (2.5 × 25 cm, $CHCl_3$) to yield 0.24 g (22%) of pure **8b**: mp 166–167 °C; IR (KBr) ν 1740 (OAc), 2260 (very weak, CN) cm^{-1}; UV λ_{max} (pH 1) 275 nm (ϵ 2000); UV λ_{max} (pH 11) 270 nm (ϵ 2600); ^1H NMR (Me_2SO-d_6) δ 1.98–2.10 (3 s, 9, 3 OAc), 6.60 (d, 1, J = 3.0 Hz, $C_{1'}$ H), 8.92 (s, 1, C_6 H), 9.30 (s, 1, C_3 H), and other sugar protons. Anal. ($C_{17}H_{17}N_5O_7$) C, H, N.

1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4-carboxamide (7b). To a cold (0–5 °C) solution of **8b** (0.50 g, 1.24 mmol) in 50% aqueous MeOH (50 mL) was added H_2O_2 (30%, 0.4 mL) and the solution was adjusted to pH 9 with concentrated NH_4OH. The mixture was stirred at ice-bath temperature for 4 h and then allowed to stand in the refrigerator overnight. The solvent was evaporated, and the residue was chromatographed on a silica gel column (1.5 × 20 cm) with EtOAc/H_2O/1-PrOH, 4:2:1, upper phase, as the elution solvent, to obtain an analytical sample (crystallized from water) as colorless needles: yield 0.22 g (60%); mp 211–212 °C; IR (KBr) ν 1675 (C=O of $CONH_2$), 3300–3400 (OH, NH_2) cm^{-1}; UV λ_{max} (pH 1 and 11) 274 nm (ϵ 4700); UV λ_{max} (pH 7) 274 nm (ϵ 5000); ^1H NMR (Me_2SO-d_6) δ 6.38 (d, 1, J = 3.5 Hz, $C_{1'}$ H), 8.17 and 8.53 (2 s, 2, $CONH_2$, exchanged with D_2O), 8.76 (s, 1, C_6 H), 9.21 (s, 1, C_3 H), and other sugar protons. Anal. ($C_{11}H_{13}N_5O_5$) C, H, N.

3-β-D-Ribofuranosylpyrazolo[4,3-*d*]pyrimidine-7-thione (Thioformycin B). A 250-mL high-pressure stainless-steel reaction vessel was charged with a solution of formycin (10.0 g, 3.7 mmol) in water (55 mL) and pyridine (3.7 mL). The vessel was cooled to −70 °C (dry ice–acetone bath), and pyridine/liquid H_2S (1:1, v/v, 30 mL) was added. the vessel was sealed and heated at 85–90 °C for 10 days. After venting excess of H_2S (through aqueous sodium hypochlorite), the mixture was filtered and evaporated to dryness. The residue was coevaporated with water (2 × 100 mL). The residue was again dissolved in hot water (25 mL) and adsorbed onto ~15 g of silica gel. The excess solvent was evaporated, and coevaporation with MeOH (4 × 50 mL) gave dry solid mass, which was loaded onto a silica gel column (4 × 60 cm, EtOAc). The column was eluted with EtOAc/H_2O/1-PrOH, 4:2:1, upper phase. The homogeneous fractions were pooled and evaporated to dryness. The residue was crystallized from water as light yellow needles: yield 2.90 g (27.3%); mp 234–235 °C (lit.[10] 226–228 °C); IR (KBr) ν 1075, 1110 (>C=S), 3180 (OH) cm^{-1}; UV λ_{max} (pH 1) 219 nm sh (ϵ 4600), 275 (3800), 339 (20000), 352 sh (16000); UV λ_{max} (pH 7) 270 nm (ϵ 4000), 340 (19000), 354 sh (14500); UV λ_{max} (pH 11) 228 nm (ϵ 13500), 272 (4000), 332 (18000); ^1H NMR (Me_2SO-d_6) δ 4.90 (d, 1, J = 5.0 Hz, $C_{1'}$ H), 8.10 (s, 1, C_5 H), 13.80 and 14.20 (2 s, 2, N_1H and N_6 H, exchanged with D_2O), and other sugar protons. Anal. ($C_{10}H_{12}N_4SO_4$) C, H, N, S.

7-(Methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[4,3-*d*]pyrimidine (9). Method 1. To a cold (0–5 °C) solution of 7-chloro-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl-pyrazolo[4,3-*d*]pyrimidine[11] (**10**; 3.55 g, 8.6 mmol) in absolute ethanol (80 mL) was added a cold ethanolic solution (80 mL) of CH_3SH (CH_3SH bubbled in for 3 min), followed by $KOBu^t$ (0.77 g, 7 mmol). The mixture was stirred at 0 °C for 6 h and then purged with N_2 for 30 min. The solvent was evaporated, and the residue was extracted with $CHCl_3$ (200 mL). The organic layer was washed with water (3 × 50 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was purified by silica gel column chromatography with 10% acetone in $CHCl_3$ as the eluant to obtain a colorless foam: yield 3.30 g (90.4%); IR (KBr) ν 1370 (SCH_3), 1740 (OAc) cm^{-1}; UV λ_{max} (pH 1) 214 nm (ϵ 11900), 252 (3000), 313 (10000), 323 sh (7800); UV λ_{max} (pH 7) 217 nm (ϵ 23300), 252 (6400), 325 (25000); UV λ_{max} (pH 11) 222 nm (ϵ 18700), 252 (4900), 286 (5900), 327 (4700); ^1H NMR ($CDCl_3$) δ 2.10–2.20 (2 s, 9, 3 OAc), 2.70 (s, 3, SCH_3), 5.50 (d, 1, J = 3.0 Hz, $C_{1'}$ H), 8.80 (s, 1, C_5 H), 11.50 (br s, 1, N_1 H), and other sugar protons. Anal. ($C_{17}H_{20}N_4SO_7$) C, H, N, S.

Method 2. A suspension of 7-(methylthio)-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine[10] (1.0 g, 3.35 mmol) and a catalytic amount of 4-(dimethylamino)pyridine (25 mg) in acetic anhydride (5 mL) was stirred at room temperature for 2 h with the exclusion of moisture. A clear solution was obtained, which was evaporated to dryness. The residue was dissolved in $CHCl_3$ (25 mL) and washed successively with water (50 mL), aqueous saturated $NaHCO_3$ (25 mL), and water (50 mL). The $CHCl_3$ portion was dried (Na_2SO_4) and evaporated to provide pure foam: yield 0.95 g (66.9%). This material was found to be identical with **9** prepared by method 1.

3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]-pyrimidine-7-carbonitrile (12). To a cold solution of **9** (3.0 g, 7.07 mmol) in ethyl ether (175 mL) was added *m*-chloroperoxybenzoic acid (4.0 g, 23.18 mmol), and the mixture was stirred for 5 h with the exclusion of moisture at ice-bath temperature. The solvent was removed to obtain crude methyl sulfone (**11**), which was immediately reacted with KCN (0.81 g, 12.4 mmol) in DMF (100 mL) at room temperature for 18 h. The product was isolated exactly as described for **8b** and was crystallized from CH_2Cl_2/hexane to obtain analytically pure **12** as microneedles: yield 0.23 g (8%); mp 171–172 °C; IR (KBr) ν 1740 (OAc), 2220 (very weak, CN) cm^{-1}; UV λ_{max} (pH 1) 217 nm (ϵ 17900), 277 (4000), 333 (5800); UV λ_{max} (pH 7) 217 nm (ϵ 17300), 283 (3200), 334 sh (2200); UV λ_{max} (pH 11) 233 nm (ϵ 18300), 294 (3200); ^1H NMR (Me_2SO-d_6) δ 2.0–2.14 (2 s, 9, 3 OAc), 5.48 (d, 1, J = 5.0 Hz, $C_{1'}$ H), 9.30 (s, 1, C_5 H), and other sugar protons. Anal. ($C_{17}H_{17}N_5O_7$) C, H, N.

3-β-D-Ribofuranosylpyrazolo[4,3-*d*]pyrimidine-7-carboxamide (13). To a cold solution of **12** (0.50 g, 1.24 mmol) in 50% aqueous MeOH (50 mL) was added H_2O_2 (30%, 0.4 mL), and the solution was adjusted to pH 9 with concentrated NH_4OH. The mixture was stirred at 0 °C for 4 h and then allowed to stand in the refrigerator overnight. After evaporation, the residue was purified as described for **7b**, and the title compound was obtained as off-white crystals after crystallization from water: yield 0.24 g (63.6%); mp 196–197 °C; IR (KBr) ν 1675 (C=O of $CONH_2$), 3240–3360 (OH, NH_2) cm^{-1}; UV λ_{max} (pH 1) 218 nm (ϵ 10500), 274 (2700), 328 (3900); UV λ_{max} (pH 7) 217 nm (ϵ 13800), 274 (3250), 328 (4700); UV λ_{max} (pH 11) 320 nm (ϵ 13600), 289 (2500); ^1H NMR (Me_2SO-d_6) δ 5.20 (d, 1, J = 4.5 Hz, $C_{1'}$ H), 8.18 and 8.55 (2 s, $CONH_2$, exchanged with D_2O), 9.20 (s, 1, C_5 H), and other sugar protons. Anal. ($C_{11}H_{13}N_5O_5 \cdot 0.5H_2O$) C, H, N.

Acknowledgment. This work was supported in part by Contract DAMD-17-79-C-9046 with the U.S. Army Research and Development Command, Washington, DC. The authors thank Jan M. Ashdown for large-scale syntheses of some intermediate compounds.

(20) Commercially available from Aldrich Chemical Co.