inoculated with 24 test organisms by means of a multiple inoculation consisting of 1-mm diameter loops. The inocula, which consisted of 18-h nutrient broth cultures, suitably diluted to yield approximately 500 colonies, were streaked along 1 cm. After 18 h of incubation at 37 °C, the MIC was read as the lowest concentration which caused 80% inhibition, as judged by eye, of the control growth on the medium containing no test compound. As a standard, trimethoprim (1) was included in each daily series of tests.

In Vivo Antibacterial Assays. (1) Infections. Groups of six mice (18–20 g) were infected ip with 10–100 lethal doses of the infecting organisms. The test substances, suspended in 0.5% carboxymethylcellulose, were administered po immediately after infection and 6-h later; on the following days the number of doses depended on the acuteness of the infection. In the case of *Klebsiella pneumoniae* and *Proteus vulgaris*, the drugs were given once on the following day, and in the case of *Escherichia coli*, twice on that day. With the staphylococcal infections, the drugs were given twice on each of the following two days, and with the streptococcal infections, the dosing was twice on the 2nd day and once on the 3rd.

The test substances were given in graded doses, differing in 2/3 intervals, and the ED_{50} was determined by the method of Reed and Muench.[40]

(2) Blood Concentrations. A single dose of the test substance was given po to groups of three mice (18–20 g), and then at intervals ~0.2 mL of blood was drawn from the supra orbital plexus of each mouse and pooled. The serum was separated and the concentration of the test substance was determined microbiologically using *Bacillus pumilus* as the test organism.

Acknowledgment. The authors are grateful for the continued encouragement of Dr. George H. Hitchings during the course of this investigation.

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Synthesis and Antiviral Activity of Certain 9-β-D-Ribofuranosylpurine-6-carboxamides

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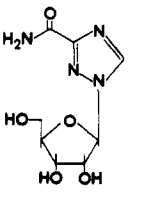
To examine the structural parameters necessary for antiviral efficacy of certain purine nucleosides, several 9-β-D-ribofuranosylpurine-6-carboxamides have been synthesized. Glycosylation of the Me_3Si derivative of purine-6-carboxamide with protected ribofuranose in the presence of a Lewis acid gave the blocked nucleoside which on deprotection furnished 9-β-D-ribofuranosylpurine-6-carboxamide (**6a**). Alternatively, **6a** was synthesized via the nucleophilic displacement of 9-β-D-ribofuranosyl-6-iodopurine with cyanide ion. Certain 2-amino- and 2-methyl-9-β-D-ribofuranosylpurine-6-carboxamides have also been prepared. 8-Carbamoylguanosine (**16**) has been prepared by homolytic acylation of the parent nucleoside. These compounds were tested against several RNA and DNA viruses in cell culture. 9-β-D-Ribofuranosylpurine-6-carboxamide (**6a**), the corresponding 6-thiocarboxamide (**7b**), and 4-amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (**8**) showed significant in vitro antiviral activity at nontoxic dosage levels. **6a** employed in the treatment of Rift Valley fever virus infected mice at 50 (mg/kg)/day gave a 55% survival rate on day 21 compared to a 30% survival in the controls.

The role of viruses in chronic and degenerative diseases is a subject of considerable interest. A tremendous amount of evidence is accumulating which implicates viral etiology in diseases such as arthritis, diabetes, multiple sclerosis, mental retardation, and infectious mononucleosis.[2] The progress made in the synthesis and development of antiviral agents has recently been reviewed.[3-5] Approval of 9-β-D-arabinofuranosyladenine (*ara*-A) for use against herpes infection is indeed a step forward.[6] The broad-spectrum activity of synthetic 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (**1**, ribavirin) against influenza,



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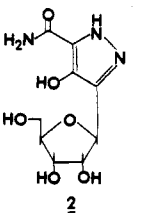

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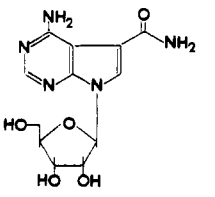

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hepatitis, herpes, and vaccinia viral infections in vivo is

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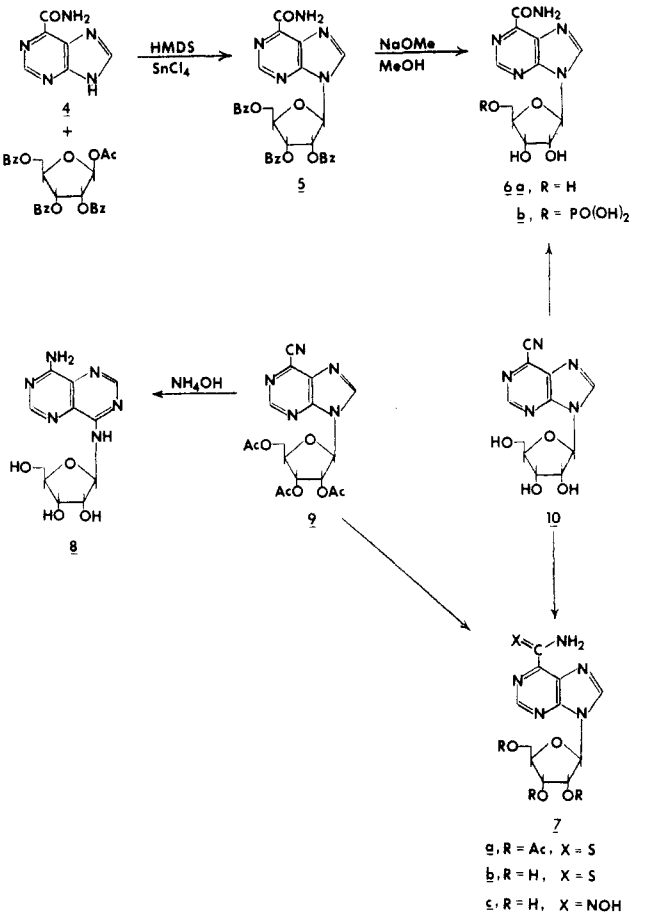
especially encouraging.[7,8] The pronounced in vivo activity of ribavirin against influenza when administered orally[9-12] or by aerosol spray[13,14] is quite remarkable. Further, ribavirin is particularly effective against certain RNA viruses having substantial implications from a global epidemiologic standpoint (Rift Valley fever, Lassa fever, Bolivian hemorrhagic fever, and others).[8] One of the most interesting features of ribavirin is the presence of a hydrogen-bonding carboxamide function, which is absolutely essential for its antiviral activity.[15] Indeed, the carboxamide function is an integral part of several naturally occurring nucleoside antibiotics such as pyrazofurin (2) and sangivamycin (3). To examine the structural parameters necessary for antiviral potency, we initiated a program to prepare and evaluate new and novel nucleosides containing a carboxamide function. We now report our results on the synthesis and antiviral activity of certain 9-β-D-ribofuranosylpurine-6-carboxamides.

Chemistry. The formation of the carbon–carbon bond at the 6 position of purine nucleoside through the nucleophilic displacement of the corresponding chloropurine nucleoside has been generally unsuccessful. The carbon-substituted purine nucleosides at the 6 position have been previously prepared by the direct glycosylation of the appropriate purine base.[16-18] In the present study we examined the above approaches to the preparation of 9-β-D-ribofuranosylpurine-6-carboxamides. During the course of the present work, Japanese workers[19-22] reported yet another procedure for the synthesis of 6-carbon substituted purines by the nucleophilic displacement of the corresponding 6-(methylsulfonyl) derivative.[23]

Treatment of purine-6-carboxamide (4)[24] with hexamethyldisilazane gave the trimethylsilyl derivative, which was then coupled with 1 equiv of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of 1.4 molar equiv of $SnCl_4$ in $ClCH_2CH_2Cl$ at ambient temperature. Under these conditions, an 83% yield of 9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)purine-6-carboxamide (**5**) was obtained (Scheme I). Careful investigation of the mother liquor furnished chromatographic evidence of the formation of other nucleoside material in very minor amount, presumably the positional isomer. Debenzoylation of **5** with MeOH/NaOMe at ambient temperature gave 9-β-D-ribofuranosylpurine-6-carboxamide (**6a**). Direct phosphorylation[25] of unprotected **6a** with $POCl_3$ in trimethyl phosphate at 0–5 °C, followed by hydrolysis, furnished 9-β-D-ribofuranosylpurine-6-carboxamide 5′-phosphate (**6b**). The purity and structure of **6a** and **6b** were confirmed by elemental and ^1H NMR analyses.

The synthesis of **6a** was also approached by an alternate route for which 9-β-D-ribofuranosylpurine-6-carbonitrile (**10**) was found to be a versatile intermediate. When 9-β-D-ribofuranosyl-6-iodopurine[26] was treated with CuCN in pyridine at reflux temperature, a 60% yield of crystalline

Scheme I



Scheme II

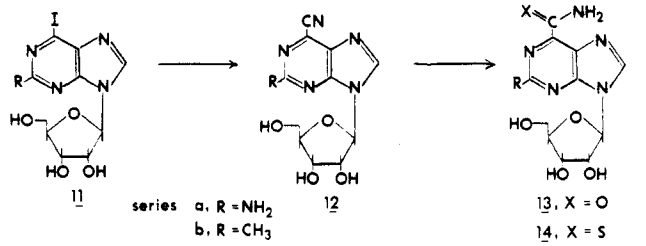


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Table I. Comparative in Vitro Antiviral Activity of Ribavirin and Certain 9-β-D-Ribofuranosylpurine-6-carboxamides

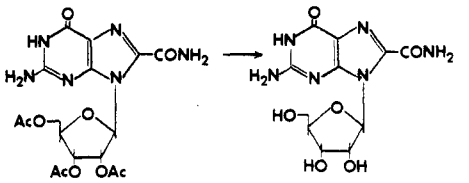
no.	compd	cell line/ toxic level	virus ratings[a] HSV1	VV	Para 3	VSV	Cox B1
6a	9-β-D-ribofuranosylpurine-6-carboxamide	V/none	0.6	1.5	0.0	0.0	0.4
		H/320	0.6	1.1	1.4	1.2	0.9
7b	9-β-D-ribofuranosylpurine-6-thiocarboxamide	V/320	1.1	0.8	b	0.5	1.0
		H/100	0.4	1.2	1.3	0.7	1.1
8	4-amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-d]pyrimidine	V/320	0.8	1.4	0.7	0.6	0.6
		H/320	1.6	1.3	1.5	1.5	1.0
10	9-β-D-ribofuranosyl-6-cyanopurine	V/1000	0.4	1.0	0.0	0.0	0.0
		H/320	0.8	1.0	1.2	0.8	1.0
	9-β-D-ribofuranosyl-6-iodopurine	V/1000	1.0	1.2	0.7	0.0	0.1
		H/320	1.6	0.8	0.6	0.9	1.0
	9-β-D-ribofuranosyl-6-chloropurine	V	0.9	1.5	b	0.5	0.4
		H/1000	0.6	0.8	1.3	0.3	1.0
1	1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin)	V/none	0.6	1.2	1.3	0.8	0.6
		H/none	1.2	1.2	1.4	1.6	1.1

[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 ten times the number of test cups used per drug level. [b] Not determined.

10 was obtained. The synthesis of **10** from 9-β-D-ribofuranosyl-6-(methylsulfonyl)purine has recently been described;[22] however, the yield obtained was only 6%. Compound **10** was found to be quite stable, and the IR spectrum revealed a weak nitrile band at 2240 cm^{-1}. Treatment of **10** with cold H_2O_2 in alkaline media (pH 8.5) gave **6a**. The identity of this compound was confirmed by rigorous comparison of the physicochemical data obtained for **6a** prepared from **5**. When 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-6-carbonitrile (**9**)[27,28] was treated with H_2S in pyridine, 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-purine-6-thiocarboxamide (**7a**) was obtained, which on subsequent deacetylation furnished 9-β-D-ribofuranosylpurine-6-thiocarboxamide (**7b**). The ^1H NMR spectrum of **7b** revealed the two thiocarboxamide resonances as more downfield (δ 10.1 and 10.5) than the carboxamide resonances (δ 8.06 and 8.38) of **6a**. When **10** was allowed to react with free NH_2OH in EtOH, a nearly quantitative yield of 9-β-D-ribofuranosylpurine-6-carboxamidoxime (**7c**) was obtained. The IR spectrum of **7a–c** did not show any absorption in the 2220-cm^{-1} region. Treatment of **9** with a large excess of NH_4OH at room temperature gave the rearrangement product 4-amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-d]pyrimidine (**8**),[28] the structure of which has recently been confirmed by X-ray crystallographic studies.[29]

Studies were extended to provide several 2-substituted 9-β-D-ribofuranosylpurine-6-carboxamide derivatives (Scheme II). Treatment of 2-amino-9-β-D-ribofuranosyl-6-iodopurine (**11a**)[26] with CuCN in boiling pyridine gave 2-amino-9-β-D-ribofuranosylpurine-6-carbonitrile (**12a**). Reaction of **12a** with alkaline H_2O_2 or H_2S in pyridine readily gave 2-amino-9-β-D-ribofuranosylpurine-6-carboxamide (**13a**) and the corresponding thiocarboxamide (**14a**), respectively. Similar reactions starting with 2-methyl-9-β-D-ribofuranosyl-6-iodopurine (**11b**), which in turn was obtained from 2-methyl-9-β-D-ribofuranosyl-6-chloropurine,[30] led to the isolation of 2-methyl-9-β-D-ribofuranosylpurine-6-carboxamide (**13b**) and the thiocarboxamide (**14b**).

Scheme III



15 16

Homolytic acylation[31] of 2′,3′,5′-tri-O-acetylguanosine with formamide in 3 N H_2SO_4 using ammonium persulfate as the radical source gave good yield of fluorescent 2′,3′,5′-tri-*O*-acetyl-8-carbamoylguanosine (**15**) (Scheme III). Deacetylation of **15** with methanolic ammonia gave 8-carbamoylguanosine (**16**). The structure of **16** was assigned by the ^1H NMR spectrum, which showed the expected new peaks for the $CONH_2$ function in addition to the loss of the C_8 proton.

The site of ribosylation and β configuration of all nucleosides synthesized in this study were confirmed, since the structure of the starting purine nucleosides was 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), parainfluenza type 3 (Para 3), vesicular stomatitis (VSV), and Coxsackie type B1 (Cox B1) viruses. In this system, monolayers (18 h) of cells were exposed to 320 $CCID_{50}$ of virus, and concentrations of each compound in one-half log dilutions ranging from 1000 to 1 μg/mL were added within 15 to 30 min. The degree of CPE inhibition and compound cytotoxicity were observed microscopically after 72 h of incubation at 37 °C in 5% CO_2 and scored numerically in order to calculate a virus

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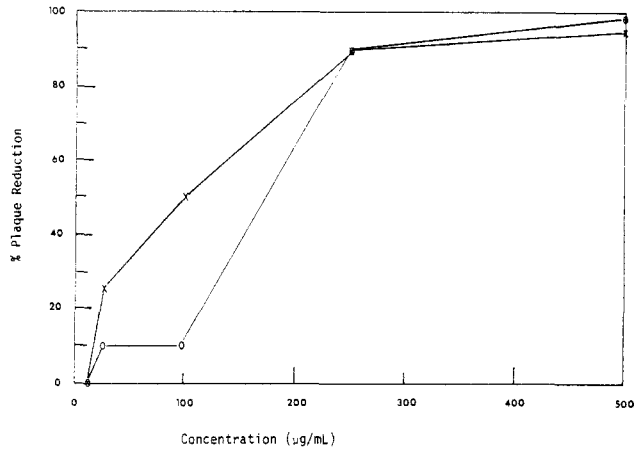

Figure 1. Inhibition of viral growth by 9-β-D-ribofuranosylpurine-6-carboxamide: (O) Rift Valley fever virus (RVFV) in Vero cells; (x) Pichinde virus (PICH) in Vero cells.

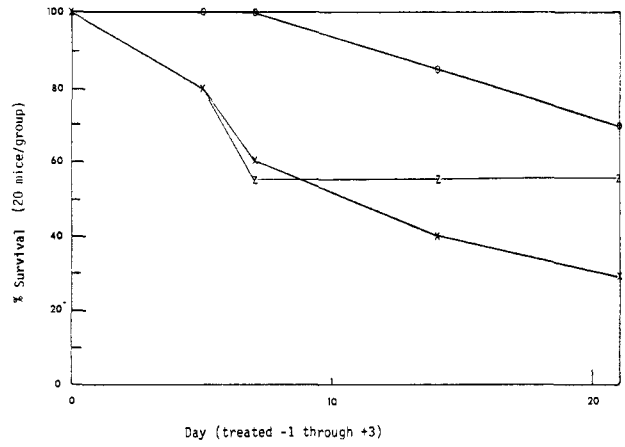

Figure 2. Effect of 9-β-D-ribofuranosylpurine-6-carboxamide and 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin) on Rift Valley fever virus (RVFV) in mice: (x) control; (z) 9-β-D-ribofuranosylpurine-6-carboxamide, 50 (mg/kg)/day; (O) 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, 100 (mg/kg)/day.

rating (VR) as previously reported.[32] 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. All the compounds synthesized during this study have been tested against the above viruses. Only **6a**, **7b**, **8**, **10**, and 9-β-D-ribofuranosyl-6-chloropurine showed significant antiviral activity, and the results of a single experiment in parallel with 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin) are shown in Table I. In particular **6a**, **7b**, and **8** exhibited marked antiviral activity against both RNA and DNA viruses in cell culture, depending on the cell line.

In addition to the above viruses, 9-β-D-ribofuranosylpurine-6-carboxamide (**6a**) has been tested at the U.S. Army Medical Research Institute of Infectious Disease, Fort Detrick, MD, against Rift Valley fever (RVF) and Pichinde (PICH) 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 **6a**, and incubated again at 37 °C in a CO_2 incubator. Plaque were enumerated after staining with crystal violet stain. The inhibition of viral growth by **6a** at various concentrations in cell culture is shown in Figure 1. It inhibited the RVF virus growth to the extent of 90% at 250 µg/mL, and inhibition was nearly complete at 500 µg/mL. Similarly, **6a** also inhibited PICH virus to the extent of 90% at 250 µg/mL. In a preliminary study of **6a** employed in the treatment of RVF virus infected mice at 50 (mg/kg)/day given for 5 days beginning 1 day prior to virus inoculation subcutaneously, survival rate was 55% on day 21 compared to 30% in sham-treated controls. Ribavirin at 100 (mg/kg)/day in the same experiment (Figure 2) gave a 70% survival rate on day 21. Pichinde virus infection is not lethal in weanling mice and was, therefore, not evaluated.

Previous experiments[8] using ribavirin have shown the value of a more protracted regimen of therapy. Mice did not begin to die (Figure 2) until after the cessation of treatment.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H 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 or EtOH as indicated by elemental analyses was verified by ^{1}H NMR. Infrared spectra (IR) were obtained on a Perkin-Elmer 257 spectrophotometer (KBr pellets), 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 and EtOAc/H_2O/1-PrOH, 4:2:1, upper phase, as the elution solvent. 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.

9-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)purine-6-carboxamide (5). A mixture of dry purine-6-carboxamide (**4**;[24] 2.44 g, 15 mmol, dried at 110 °C over P_2O_5 under vaccum, overnight), hexamethyldisilazane (HMDS, 15.0 mL), and $(NH_4)_2SO_4$ (25 mg) was heated at reflux temperature for 18 h under anhydrous conditions. The excess HMDS was removed by distillation, and the residual solid was presumed to be the trimethylsilyl derivative and was used without further purification. To a solution of the Me_3Si derivative in dry 1,2-dichloroethane (100 mL) was added 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (7.56 g, 15 mmol), followed by anhydrous $SnCl_4$ (5.47 g, 21 mmol). The reaction mixture was protected from moisture and stirred at ambient temperature for 24 h. The mixture was poured into a cold mixture of 5% aqueous $NaHCO_3$ (600 mL) and $CHCl_3$ (250 mL) and stirred for 2 h. The resulting emulsion was filtered through a Celite pad, and the filter cake was washed with $CHCl_3$ (5 × 50 mL). The combined organic layer was washed with 5% aqueous $NaHCO_3$ (2 × 100 mL), followed by water (2 × 100 mL), before it was dried over anhydrous Na_2SO_4. Removal of $CHCl_3$ gave a solid residue, which was triturated with ether, filtered, and air-dried. Crystallization from EtOH gave 7.58 g (83.2%) of pale yellow needles: mp 194–195 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.82 (d, 1, J = 5.0 Hz, C_1'H), 8.03 and 8.36 (2 s, 2, $CONH_2$), 8.93 and 9.06 (2 s, C_2H and C_8H), and other sugar protons; IR 1690 (amide C=O), 1720 (C=O), 3300 and 3400 (amide NH_2) cm^{-1}; UV λ_{max} (pH 1) 235 nm (ε 30 300), 277 (12 700); UV λ_{max} (pH 7) 235 nm (ε 27 700), 277 (12 700); UV λ_{max} (pH 11) 233 nm (ε 34 000), 277 (11 200). Anal. ($C_{32}H_{25}N_5O_8$) C, H, N.

9-β-D-Ribofuranosylpurine-6-carboxamide (6a). Method 1. To a solution of **5** (6.07 g, 10 mmol) in anhydrous MeOH (100 mL) was added NaOMe until the pH was 8.5, and the mixture was stirred at ambient temperature for 15 h with the exclusion of moisture. The reaction mixture was neutralized with Amberlite IRC-50. The resin was removed by filtration and washed with hot MeOH (3 × 35 mL). The combined filtrates were evaporated to dryness. The residue was dissolved in the minimum amount

(32) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971).

of water and precipitated by the addition of EtOH. Crystallization of the precipitate from a large excess of EtOH gave colorless crystals: yield 2.8 g (94.8%); mp 110–112 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 6.12 (d, 1, J = 5.5 Hz, $C_{1'}$ H), 8.06 and 8.38 (2 s, 2, $CONH_2$, exchanged with D_2O), 9.02 and 9.06 (2 s, C_2 H and C_8 H), and other sugar protons; IR 1690 (amide C=O), 3350 (amide NH_2, OH) cm^{-1}; UV λ_{max} (pH 1, 7, and 11) 282 nm (ε 7900). Anal. ($C_{11}H_{13}N_5O_5 \cdot H_2O$) C, H, N.

Method 2. To a suspension of 9-β-D-ribofuranosylpurine-6-carbonitrile (**10**; 0.5 g, 1.8 mmol) in 50% aqueous MeOH (50 mL), cooled to −10 °C, was added concentrated NH_4OH (or 10% NaOH) until the pH was 8.5. H_2O_2 (30%; 0.4 mL, 3.6 mmol) was added and stirred for 2 h. The mixture was evaporated to dryness and purified by column chromatography to yield 0.30 g (56.5%). This material was identical with **6a** prepared by method 1.

9-β-D-Ribofuranosylpurine-6-carboxamide 5′-Phosphate (6b). A solution of phosphoryl chloride (1.0 mL) in trimethyl phosphate (20 mL) was cooled to 0 °C, and **6a** (1.0 g, 3.38 mmol) was added with stirring. The mixture was protected from moisture and stirred for 5 h at 0 °C until phosphorylation was complete, as shown by TLC of a hydrolyzed aliquot on silica gel with CH_3CN–0.1 N NH_4Cl (7:3) as developer. The solution was poured into ice–water (40 mL), and the pH was adjusted to 2 with 2 N NaOH. The solution was extracted with $CHCl_3$ (2 × 50 mL) to remove trimethyl phosphate. The aqueous solution was applied to a column of activated charcoal (30 g), and the column was washed with H_2O until the eluate was salt free. The nucleotide was eluted with a mixture of EtOH–H_2O–concentrated NH_4OH (10:10:1, v/v). The solvent was removed and the residue was dissolved in H_2O (20 mL). This aqueous solution was passed through a column of Bio-Rad AG 50W-X2 (H^+). Elution with H_2O afforded the nucleotide. The solvent was removed, and the residue was dissolved in H_2O (10 mL), frozen, and lyophilized to yield 0.66 g (52.0%) of the nucleotide: mp 118–125 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 6.12 (d, 1, J = 6.0 Hz, $C_{1'}$ H), 8.03 and 8.33 (2 br s, $CONH_2$, exchanged with D_2O), 8.90 and 9.03 (2 s, C_2 H and C_8 H), and other sugar proton; IR 1685 (amide C=O), 3310–3400 (OH) cm^{-1}; UV λ_{max} (pH 1) 279 nm (ε 7500); UV λ_{max} (pH 7 and 11) 282 nm (ε 7600). Anal. ($C_{11}H_{14}N_5PO_8 \cdot H_2O$) C, H, N, P.

9-β-D-Ribofuranosylpurine-6-carbonitrile (10). A mixture of CuCN (5.0 g, 55 mmol) and dry pyridine (75 mL) was heated under reflux with the exclusion of moisture. The solution was cooled to room temperature as 9-β-D-ribofuranosyl-6-iodopurine[26] (5.0 g, 13.2 mmol) was added. The mixture was heated again to reflux temperature for 5 min and then evaporated to dryness. The black residual solid was triturated with H_2O (3 × 50 mL), followed by EtOH (3 × 50 mL). The combined H_2O and EtOH extracts were evaporated to dryness along with silica gel (10 g). The crude product which had been absorbed onto the silica gel was loaded onto a 3 × 25 cm silica gel column packed in EtOAc and eluted with EtOAc/H_2O/1-PrOH (4:2:1, upper phase). The appropriate fractions were collected, and the solvent was evaporated to yield 2.2 g (60.0%) of tan-colored solid. Crystallization from EtOH gave the product as light tan-colored needles: mp 198–199 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 6.08 (d, 1, J = 6.0 Hz, $C_{1'}$ H), 9.12 and 9.15 (2 s, C_2 H and C_8 H), and other sugar protons; IR 2240 (C≡N, very weak), 3380 (OH) cm^{-1}; UV λ_{max} (pH 1 and 7) 286 nm (ε 9700); UV λ_{max} (pH 11) 284 nm (ε 7800), 320 sh (3000). Anal. ($C_{11}H_{11}N_5O_4$) C, H, N.

9-β-D-Ribofuranosylpurine-6-carboxamidoxime (7c). A solution of **10** (1.0 g, 3.6 mmol) and free NH_2OH (0.59 g, 18 mmol) in absolute EtOH (35 mL) was heated under reflux with the exclusion of moisture. Within 10 min the product precipitated out. Heating was continued for a further 30 min and, after cooling to room temperature, the product was filtered off. Crystallization from aqueous EtOH gave 1.1 g (98.3%) of cream-colored crystals: mp 219–220 °C dec; ^{1}H (Me_2SO-d_6) δ 6.15 (d, 3, $C_{1'}$ H and NH_2, exchanged with D_2O to doublet with J = 5.0 Hz, for $C_{1'}$ H), 8.86 and 8.96 (2 s, C_2 H and C_8 H), and other sugar protons; IR 935, 1630, 3460 cm^{-1} (C=NOH), no absorption in 2220-cm^{-1} region: UV λ_{max} (pH 1) 286 nm (ε 9000); UV λ_{max} (pH 7) 276 nm (ε 7700), 297 sh (6000); UV λ_{max} (pH 11) 275 nm (ε 6500), 297 sh (4800). Anal. ($C_{11}H_{14}N_6O_5$) C, H, N.

9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)purine-6-thiocarboxamide (7a). H_2S gas was bubbled through a solution of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-6-carbonitrile (**9**;[27] 4.03 g, 10 mmol) in anhydrous pyridine (250 mL) at room temperature, with stirring, until the deep color which had formed began fading. Stirring was continued for a further 2 h before the amber-colored solution was flushed with N_2. Evaporation of the mixture gave a syrup, which on crystallization with 2-propanol containing a few drops of petroleum ether (30–60 °C) afforded 3.87 g (88.5%) of light orange crystals: mp 154.5–155 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.43 (d, 1, J = 6.0 Hz, $C_{1'}$ H), 8.93 and 9.06 (2 s, C_2 H and C_8 H), 10.03 and 10.60 (2 s, $CSNH_2$, exchanged with D_2O), and other sugar protons; IR 1220 [C(=S)N], 1740 (C=O), 3300 (amide NH) cm^{-1}; UV λ_{max} (pH 1 and 7) 280 nm (ε 8000); UV λ_{max} (pH 11) 273 nm (ε 7600). Anal. ($C_{17}H_{19}N_5SO_7$) C, H, N, S.

9-β-D-Ribofuranosylpurine-6-thiocarboxamide (7b). A solution of **7a** (1.0 g, 2.28 mmol) in cold (0 °C) MeOH (80 mL) was saturated with NH_3 and allowed to stand in a pressure bottle at 5 °C for 20 h. The MeOH/NH_3 was evaporated to dryness, and the residual solid was triturated with boiling benzene (5 × 25 mL). The benzene-insoluble solid was crystallized from 2-propanol to yield 0.35 g (49.3%) of light tan needles: mp 167–169 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 6.50 (d, 1, J = 5.0 Hz, $C_{1'}$ H), 8.8 and 9.0 (2 s, C_2 H and C_8 H), 10.1 and 10.5 (2 s, $CSNH_2$, exchanged with D_2O), and other sugar protons; UV λ_{max} (pH 1) 280 nm (ε 10 000); UV λ_{max} (pH 7) 280 nm (ε 8500); UV λ_{max} (pH 11) 272 nm (ε 7800). Anal. ($C_{11}H_{13}N_5SO_4$) C, H, N.

4-Amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (8). A solution of **9** (6.0 g, 14.8 mmol) in concentrated NH_4OH (200 mL) was stirred at room temperature for 8 h and then allowed to stand at 4 °C for 16 h. The mixture was evaporated to dryness. The crude product which had been absorbed onto silica gel (10 g) was loaded on a 3.5 × 35 cm silica gel column packed in EtOAc. The column was eluted with EtOAc/H_2O/1-PrOH (4:2:1, upper phase). The appropriate fractions were pooled, and the solvent was evaporated to yield 3.3 g (80%) of white solid. Crystallization from H_2O gave thin fan-shaped plates: mp 214–216 °C; ^{1}H NMR (Me_2SO-d_6) δ 5.88 (quartet, 1, $C_{1'}$ H, which collapsed to a doublet at δ 5.88 after deuteration),[28] 7.80 (br s, 2, NH_2, exchanged with D_2O), 8.4 and 8.5 (2 s, C_2 H and C_6 H), and other sugar protons; UV λ_{max} (pH 7) 292 nm (ε 16 000), 303 (14 100), 319 (12 400), 334 (8500). Anal. ($C_{11}H_{14}N_6O_4$) C, H, N.

2-Amino-9-β-D-ribofuranosylpurine-6-carbonitrile (12a). A mixture of CuCN (5.0 g, 55 mmol) and dry pyridine (100 mL) was heated under reflux until all the CuCN dissolved. The solution was cooled to room temperature as 2-amino-6-iodo-9-β-D-ribofuranosylpurine (**11a**;[26] 5.0 g, 12.7 mmol) was added, and the mixture was heated at 130–135 °C for 10 min. The mixture was evaporated to dryness, and the black residue was extracted with hot CH_3CN (3 × 250 mL). The combined CH_3CN extracts evaporated, and the residual solid was reextracted with hot 2-propanol (3 × 200 mL). The 2-propanol extract was evaporated to yield 6.0 g of tan solid. Purification by silica gel column chromatography gave pure sample: yield 2.6 g (70.0%). Crystallization from EtOH gave light yellow crystals: mp 131–132 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 5.97 (d, 1, J = 6.0 Hz, $C_{1'}$ H), 7.18 (s, 2, NH_2, exchanged with D_2O), 8.61 (s, 1, C_8 H), and other sugar protons; IR 2240 (C≡N), 3200–3320 (OH, NH_2) cm^{-1}; UV λ_{max} (pH 1 and 7) 227 nm (ε 21 600), 266 sh (1200), 350 (5500); UV λ_{max} (pH 11) 227 nm (22 200), 266 sh (1200), 350 (5500). Anal. ($C_{11}H_{12}N_6O_4$) C, H, N.

2-Amino-9-β-D-ribofuranosylpurine-6-carboxamide (13a). To a solution of **12a** (0.8 g, 2.7 mmol) in 50% aqueous MeOH (50 mL), cooled to −10 °C, was added concentrated NH_4OH until the pH was 8.5. H_2O_2 (30%; 0.5 mL, 4.4 mmol) was added and stirred for 1 h as it warmed to room temperature. The mixture was evaporated to dryness, and the crude product was purified by silica gel column chromatography to yield 0.58 g (68.3%): mp 189–190 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.02 (d, 1, J = 5.5 Hz, $C_{1'}$ H), 6.87 (s, 2, NH_2, exchanged with D_2O), 8.02 and 8.32 (2 s, $CONH_2$, exchanged with D_2O), 8.56 (s, 1, C_8 H), and other sugar protons; IR 1685 (amide C=O), 3200, 3370 (OH, NH_2) cm^{-1}; UV λ_{max} (pH 1) 220 nm sh (ε 20 700), 247 sh (7500), 275 (4900), 345 (5300); UV λ_{max} (pH 7 and 11) 247 nm sh (ε 7700), 277 (5900), 337 (6200). Anal. ($C_{11}H_{14}N_6O_5 \cdot 0.5H_2O$) C, H, N.

2-Amino-9-β-D-ribofuranosylpurine-6-thiocarboxamide (14a). Dry H_2S gas was bubbled through a solution of **12a** (1.0

g, 3.4 mmol) in anhydrous pyridine (125 mL) at room temperature for 15 h. The mixture was stirred for a further 2 h before the solution was flushed with N_2. Evaporation of the mixture gave a solid residue, which on crystallization with H_2O gave 0.70 g (66.7%) of yellow crystals: mp 207–210 °C dec; ^1H NMR (Me_2SO-d_6) δ 5.88 (d, 1, J = 6.0 Hz, $C_{1'}$ H), 6.70 (s, 2, NH_2, exchanged with D_2O), 8.43 (s, 1, C_8 H), 9.95 and 10.37 (2 s, $CSNH_2$, exchanged with D_2O), and other sugar protons; IR 1200, 1575 [C(=S)N), 3340 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1) 227 nm (ϵ 16 300), 345 (3600); UV λ_{max} (pH 7) 226 nm (ϵ 16 600), 246 sh (6500), 323 (5900); UV λ_{max} (pH 11) 223 nm (ϵ 18 600), 246 sh (8200), 318 (6600). Anal. ($C_{11}H_{14}N_6SO_4$) C, H, N, S.

2-Methyl-9-β-D-ribofuranosyl-6-iodopurine (11b). To HI (47%, 30 mL), which had been cooled to −60 °C, was added 2-methyl-9-β-D-ribofuranosyl-6-chloropurine[30] (3.0 g, 10 mmol). The temperature was maintained at −60 °C until solution was complete and was then allowed to slowly rise to −5 °C over the next hour, by which time a yellow precipitate had formed. Stirring was continued for another 20 min before the mixture was cooled to −60 °C. Concentrated NH_4OH (~15 mL) was slowly added until pH 8 was achieved. The mixture was allowed to warm to 0 °C. The white precipitate was collected by filtration and washed with cold H_2O, EtOH, and ether. The air-dried product was crystallized from MeOH as needles: yield 3.0 g (76.5%); mp 173–175 °C dec; ^1H NMR (Me_2SO-d_6) δ 2.73 (s, 3, CH_3), 6.04 (d, 1, J = 6.0 Hz, $C_{1'}$ H), 8.86 (s, 1, C_8 H), and other sugar protons; IR 830 (CI), 2950 (CH_3), 3340 (OH) cm^{-1}; UV λ_{max} (pH 1) 258 nm sh (ϵ 5500), 278 (10 200); UV λ_{max} (pH 7 and 11) 258 nm sh (ϵ 5900), 278 (11 400). Anal. ($C_{11}H_{13}IN_4O_4$) C, H, N, I.

2-Methyl-9-β-D-ribofuranosylpurine-6-carbonitrile (12b). A mixture of CuCN (1.0 g, 5.5 mmol) and dry pyridine (10 mL) was heated, with stirring, until the CuCN dissolved. After the mixture cooled slightly, **11b** (1.0 g, 2.5 mmol) was added and the mixture was heated quickly to reflux for 2 min. Excess pyridine was removed by evaporation, and the dark residue was extracted with CH_3CN (4 × 25 mL). Evaporation of the CH_3CN extracts, reextraction of the residue with 2-propanol (4 × 25 mL), and evaporation gave the crude product. Purification by silica gel column chromatography, followed by crystallization from EtOH, gave 0.43 g (57.9%): mp 199–201 °C dec; ^1H NMR (Me_2SO-d_6) δ 2.81 (s, 3, CH_3), 6.10 (d, 1, J = 6.0 Hz, $C_{1'}$ H), 9.1 (s, 1, C_8 H), and other sugar protons; IR 2240 (C≡N, very weak), 2950 (CH_3), 3340 and 3470 (OH) cm^{-1}; UV λ_{max} (pH 1) 295 nm (ϵ 4400); UV λ_{max} (pH 7) 295 nm (ϵ 4700); UV λ_{max} (pH 11) 295 nm (ϵ 5000). Anal. ($C_{12}H_{13}N_5O_4$) C, H, N.

2-Methyl-9-β-D-ribofuranosylpurine-6-carboxamide (13b). To a cold (0 °C) solution of **12b** (0.75 g, 2.5 mmol) in concentrated NH_4OH (10 mL) was added 30% H_2O_2 (0.3 mL) with stirring. After 5 min the mixture was evaporated to dryness and the residue was purified by silica gel column chromatography followed by crystallization from aqueous ethanol to yield 0.55 g (69.0%): mp 114 °C dec; ^1H NMR (Me_2SO-d_6) δ 2.78 (s, 3, CH_3), 6.07 (d, 1, J = 6.0 Hz, $C_{1'}$ H) , 8.00 and 8.30 (2 s, 2, $CONH_2$, exchanged with D_2O), 8.85 (s, 1, C_8 H), and other sugar protons; IR 1690 (amide C=O), 2940 (CH_3), 3360 (OH) cm^{-1}; UV λ_{max} (pH 1) 283 nm (ϵ 7100); UV λ_{max} (pH 7 and 11) 287 nm (ϵ 6500). Anal. ($C_{12}H_{15}N_5O_5 \cdot H_2O$) C, H, N.

2-Methyl-9-β-D-ribofuranosylpurine-6-thiocarboxamide (14b). H_2S gas was bubbled through a solution of **12b** (0.75 g, 2.5 mmol) in anhydrous pyridine (50 mL) at room temperature for 2 h. The mixture was stirred for a further 24 h before the solution was flushed with N_2. Evaporation of the mixture gave a dark brown product, which on purification by silica gel column chromatography followed by crystallization from H_2O gave 0.2 g (25.2%) of orange-colored crystals: mp 96–101 °C dec; ^1H NMR (Me_2SO-d_6) δ 2.73 (s, 3, CH_3), 6.06 (d, 1, J = 6.0 Hz, $C_{1'}$ H), 8.83 (s, 1, C_8 H), 9.97 and 10.43 (2 s, 2, $CSNH_2$, exchanged with D_2O), and other sugar protons; IR 1200, 1575 [C(=S)N], 2940 (CH_3), 3320 (OH) cm^{-1}; UV λ_{max} (pH 1) 290 nm (ϵ 6200); UV λ_{max} (pH 7) 287 nm (ϵ 7200); UV λ_{max} (pH 11) 283 nm (ϵ 6500). Anal. $C_{12}H_{15}N_5SO_4 \cdot 0.4EtOH$) C, H, N, S.

2′,3′,5′-Tri-*O*-acetyl-8-carbamoylguanosine (15). To a stirred solution of 2′,3′,5′-tri-*O*-acetylguanosine (4.09 g, 10 mmol) in H_2O (250 mL) was added AcOH (250 mL), 3 N H_2SO_4 (25 mL), and formamide (50 mL). After the solution cooled to 10 °C, $FeSO_4 \cdot 7H_2O$ (50.0 g) was added. A solution of $(NH_4)_2S_2O_8$ (41.1 g) in H_2O (150 mL) was then added dropwise over 45 min, followed by stirring for an additional 30 min. The solution was diluted with H_2O (150 mL) and extracted with $CHCl_3$ (3 × 100 mL). The organic phase was washed with 10% $NaHCO_3$ solution (2 × 50 mL), followed by H_2O (2 × 50 mL). Evaporation of the dried (Na_2SO_4) $CHCl_3$ solution gave a white solid which on recrystallization from EtOH afforded white platelets: yield 2.85 g (63.0%); mp 161–163 °C; ^1H NMR (Me_2SO-d_6) δ 6.74 (s, 2, NH_2, exchanged with D_2O), 7.00 (d, 1, J = 5.0 Hz, $C_{1'}$ H), 7.60 and 8.10 (2 s, 2, $CONH_2$, exchanged with D_2O), 10.65 (br s, 1, NH), and other sugar protons; UV λ_{max} (pH 1) 276 nm (ϵ 10 600), 300 (10 400); UV λ_{max} (pH 7) 276 nm (ϵ 11 800), 300 (11 800); UV λ_{max} (pH 11) 311 nm (ϵ 10 400). Anal. ($C_{17}H_{20}N_6O_9$) C, H, N.

8-Carbamoylguanosine (16). A solution of **15** (1.0 g) in MeOH/NH_3 (50 mL, saturated at 0 °C) was allowed to stand in a pressure bottle overnight. The solution was concentrated to ~15 mL and cooled, and the solid that separated was collected and crystallized from H_2O to yield 0.60 g (83.3%): mp 190–195 °C dec; ^1H NMR (Me_2SO-d_6) δ 6.70 (s, 2, NH_2, exchanged with D_2O), 6.85 (d, 1, J = 6.0 Hz, $C_{1'}$ H), 7.65 and 8.12 (2 s, 2, $CONH_2$, exchanged with D_2O), and other sugar protons; UV λ_{max} (pH 1) 276 nm (ϵ 13 200), 294 (12 000); UV λ_{max} (pH 7) 276 nm (ϵ 12 700), 299 (12 400); UV λ_{max} (pH 11) 310 nm (ϵ 11 200). Anal. $C_{11}H_{14}N_6O_6 \cdot 0.5H_2O$) C, H, N.

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