Synthesis and Biological Activity of Certain 3,4-Disubstituted Pyrazolo[3,4-*d*]pyrimidine Nucleosides[1]

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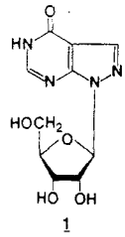
A number of 3,4-disubstituted pyrazolo[3,4-*d*]pyrimidine ribonucleosides were synthesized and tested for their biological activity. Glycosylation of persilylated as well as nonsilylated 3-bromoallopurinol with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**4**) provided the key intermediate 3-bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**5a**). Similar glycosylations of 3-cyanoallopurinol and 3-(methylthio)allopurinol furnished the corresponding protected N-1 glycosyl derivatives (**5b** and **5c**). Debenzoylation of these nucleosides (**5a–c**) gave the corresponding 3-bromo-, 3-cyano-, and 3-(methylthio)allopurinol nucleosides (**6a–c**). The site of glycosylation and anomeric configuration of **6a** and **6c** were assigned on the basis of spectral studies as well as conversion to allopurinol ribonucleoside, whereas the structural assignment of **6b** was made by single-crystal X-ray analysis. Conventional functional group transformation of **5a** and **5b** provided a number of novel 3-substituted allopurinol nucleosides, which included **10a** and **18a–d**. Glycosylation of 4-amino-3-bromopyrazolo[3,4-*d*]pyrimidine (**14**) with **4** and subsequent debenzoylation gave 3-bromo-4-aminopyrazolo[3,4-*d*]pyrimidine ribonucleoside (**13a**) from which 3,4-diamino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**13b**) was obtained by amination. Thiation of **5b**, followed by deblocking, gave 3-cyanothiopurinol ribonucleoside (**20**). All of these compounds were tested in vitro against certain viruses, tumor cells, and the parasite *Leishmania tropica*. Among the 3-substituted allopurinol nucleosides, **18b** and **18c** showed significant activity against Para 3 virus and were found to be potent inhibitors of growth of L1210 and P388 leukemia. Compound **20** exhibited the most significant broad-spectrum in vitro antiviral and antitumor activity. 3-Bromoallopurinol ribonucleoside (**6a**) was found to be more active than allopurinol ribonucleoside against *Leishmania tropica* within human macrophages in vitro.

Allopurinol ribonucleoside [1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one, **1**] has been shown to be 10-fold more active against *Leishmania braziliensis* and 300-fold more active against *Leishmania donovani* than the aglycon allopurinol in inhibiting the growth of *Leishmania* promastigotes in vitro.[2,3] Both allopurinol and **1** are equally effective in preventing the transformation of the intracellular form (amastigote) of *L. donovani* to the extracellular promastigote form.[2] In both *Leishmania*[2] and *Trypanosoma*[4] species, allopurinol is converted to allopurinol ribonucleoside 5′-phosphate by a unique enzyme of the parasite, nucleoside phosphoribosyltransferase. Selective amination of allopurinol ribonucleoside 5′-phosphate by adenylosuccinate synthetase and succino-AMP lyase produced the 5′-phosphate of 4-aminopyrazolo[3,4-*d*]pyrimidine nucleoside (**2**), which is eventually incorporated into RNA of the parasite as the 5′-triphosphate, resulting in cytotoxicity to the parasite.[5] This conversion is analogous to the conversion of IMP to AMP in mammalian cells.[6,7] The incorporation of 4-aminopyrazolo[3,4-*d*]pyrimidine ribonucleoside 5′-triphosphate into the RNA of the parasite is unique, since mammalian cells show neither this conversion nor the incorporation of allopurinol and its metabolic products into RNA.[2,3,8] These unusual metabolic transformations of allopurinol ribonucleoside 5′-phosphate reveal significant biochemical differences between the host and the parasite, which provide great potential for chemotherapeutic exploitation.[9] Studies of the amastigote form of *L. donovani* show that **1** is also metabolized to 4-aminopyrazolo[3,4-*d*]pyrimidine ribonucleotides,[8] similar to the promastigote form.[2]



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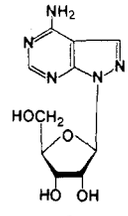

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Recently, the antileishmanial effect of allopurinol against promastigotes of the isolates of American *L. braziliensis* and *L. mexicana* has been confirmed.[10] However, both 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP) and its ribonucleoside **2** were found to be several-fold more active than allopurinol against these isolates.[10] In light of these antiparasitic and other biological properties of allopurinol, 4-APP, and their corresponding nucleosides, we initiated a program to synthesize certain nucleosides of the pyrazolo[3,4-*d*]pyrimidine ring. Recently, we reported[11] the synthesis of several 4,6-disubstituted pyrazolo[3,4-*d*]pyrimidine nucleosides. We now report the chemical synthesis and in vitro biological activity of certain hitherto unreported 3,4-disubstituted pyrazolo[3,4-*d*]pyrimidine nucleosides. Although the syntheses of several 3,4-disubstituted pyrazolo[3,4-*d*]pyrimidine nucleosides related to the nucleoside antibiotics toyocamycin and sangivamycin have been reported,[12,13] the corresponding 3-substituted

(1) Portions of this work have been presented: Cottam, H. B.; Petrie, C. R.; Revankar, G. R.; Robins, R. K. "Abstracts of Papers", the 9th International Congress of Heterocyclic Chemistry; Tokyo, Japan, August 1983; Abstr G-117. This work is taken, in part, from the Ph.D. Dissertation of H.B.C., Brigham Young University, 1983.
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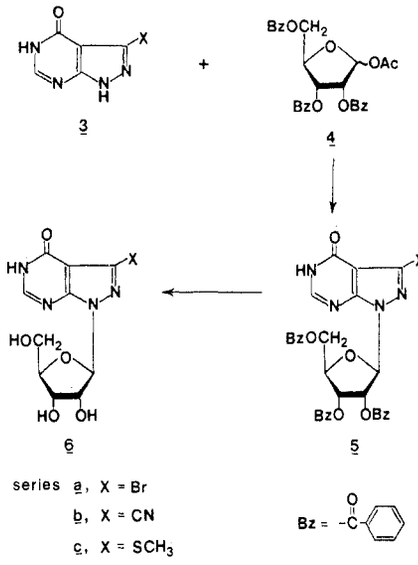
Scheme I



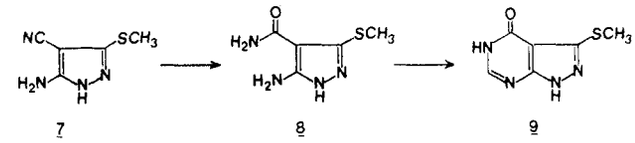
allopurinol ribonucleoside derivatives have not been documented in the chemical literature. It is of particular interest that introduction of certain substituents such as cyano or carbamoyl function at position 3 of 4-APP ribonucleoside led to a dramatic increase in antitumor activity against L1210 and P388 leukemias in mice, in comparison to the parent nucleoside.[14] The 3-thiocarbamoyl derivative of 4-APP ribonucleoside was also found to be cytotoxic to the growth of mouse fibroblasts.[13]

Chemistry. The synthesis of 3-substituted allopurinol ribonucleosides was approached by the direct glycosylation of preformed 3-bromoallopurinol (**3a**).[15] Two different methods of glycosylation were employed. The first method was a modification of the procedure of Lichtenthaler and Cuny[16] for synthesis of allopurinol ribonuclsodie via the Vorbrüggen procedure.[17] Glycosylation of the trimethylsilyl derivative of **3a** was carried out with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (4) in the presence of the catalyst, (trimethylsilyl)trifluoromethanesulfonate (TMS-triflate).[18] The major product formed within the first few hours of the reaction (as determined by TLC) was presumed to be the N-5 glycosyl isomer, which, upon heating for a week, rearranged to the more thermodynamically stable 3-bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**5a**) (Scheme I). However, the isolated yield of **5a** by this method was only 29%. In the second method of glycosylation, nonsilylated **3a** was reacted with **4** in the presence of the catalyst BF_3-Et_2O in a boiling polar aprotic solvent such as nitromethane or benzonitrile for 90 min. Under these conditions, no formation of N-5 glycosyl isomer was observed and only a minor amount of N-2 isomer was detected by TLC. The yield of the desired versatile intermediate **5a** was increased to 45% by this method.

Debenzoylation of **5a** with methanolic ammonia gave 3-bromoallopurinol ribonucleoside (**6a**), which, on dehalogenation with Pd/C, readily gave allopurinol ribonucleoside (1), thus confirming the structural assignment of **5a** and the nucleosides derived from it.

A similar glycosylation of the trimethylsilyl derivative of 3-cyanoallopurinol (**3b**)[19] by the first method gave a 46% yield of the protected 3-cyanoallopurinol ribonucleoside (**5b**). However, under the conditions of the second method, the yield of **5b** was increased to 52% and the reaction was essentially complete within 15 min. Deprotection of **5b** with sodium methoxide gave 4(5*H*)-oxo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (**6b**). The absolute structural assignment of **6b** was made on the basis of single-crystal X-ray crystallographic studies.

Since the methylthio group could not be substituted directly into **6a** by a nucleophilic displacement reaction to obtain 3-methylthioallopurinol ribonucleoside (**6c**), direct glycosylation of the preformed 3-(methylthio)allopurinol (**9**) was considered. Hydrolysis of 5-amino-3-(methylthio)pyrazole-4-carbonitrile (**7**)[20] with polyphosphoric acid gave the corresponding carboxamide **8** which, on subsequent ring closure with formamide, produced **9**. Glycosylation of **9** by the silyl-TMS-triflate



procedure gave the blocked nucleoside **5c**, which was deblocked with sodium methoxide to yield **6c**. Dethiation of **6c** with Raney nickel readily gave 1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one, identical in all respects with allopurinol ribonucleoside (1), thus confirming the structural assignment of **6c**.

The C_3-Br group in **5a** was found to be quite unreactive toward conventional nucleophilic displacement reactions. The use of copper compounds as catalysts in nucleophilic aromatic substitution reactions are reported in the literature.[21,22] Although this type of catalysis is generally well-known in the homoaromatic series, relatively little has been reported in the heteroaromatic series. Under the catalysis of a metal halide, particularly CuCl, the 3-bromo group in **5a** was found to be activated toward certain nucleophilic substitutions. Thus, treatment of **5a** with methanolic ammonia in the presence of CuCl at elevated temperatures and pressure gave an 85% yield of 3-aminoallopurinol ribonucleoside (**10a**) (Scheme II). A similar treatment with methylamine solution in the presence of CuCl gave a good yield of 3-(methylamino)-allopurinol ribonucleoside (**10b**). Likewise, the 3-(dimethylamino) derivative of allopurinol ribonucleoside was obtained. While the amination reactions generally proceeded in good yield, attempts to displace the bromo group with other nucleophiles were unsuccessful.

In an effort to deaminate compound **10a**, treatment in aqueous acetic acid with an excess of $NaNO_2$ gave a product identified as the N-2 nitrosated nucleoside **16** instead of the desired 1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3,4(2*H*,5*H*)-dione (**17**). Furthermore, the deaminated product **17** could not be obtained by controlling the amount of $NaNO_2$ under varied experimental

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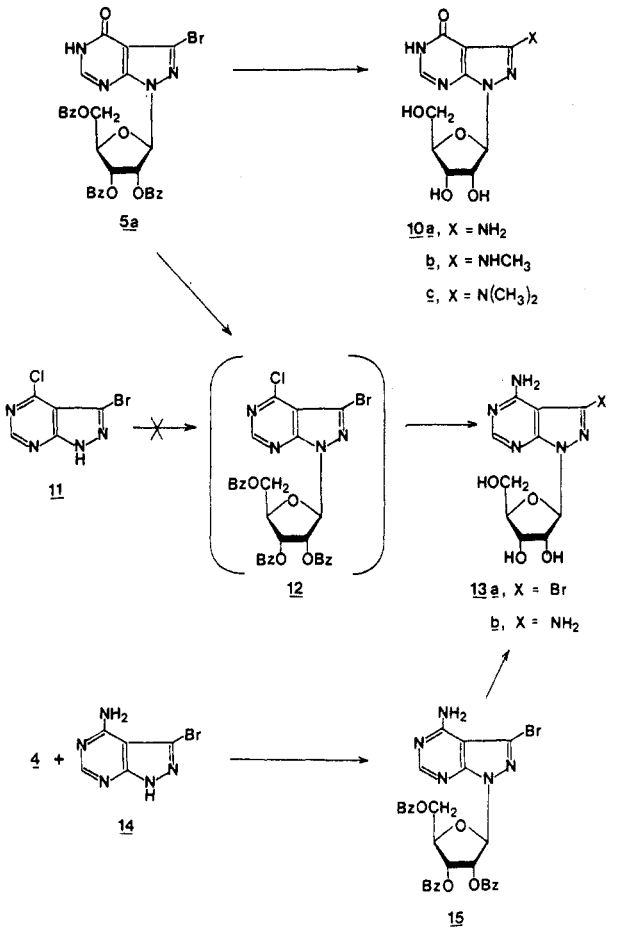
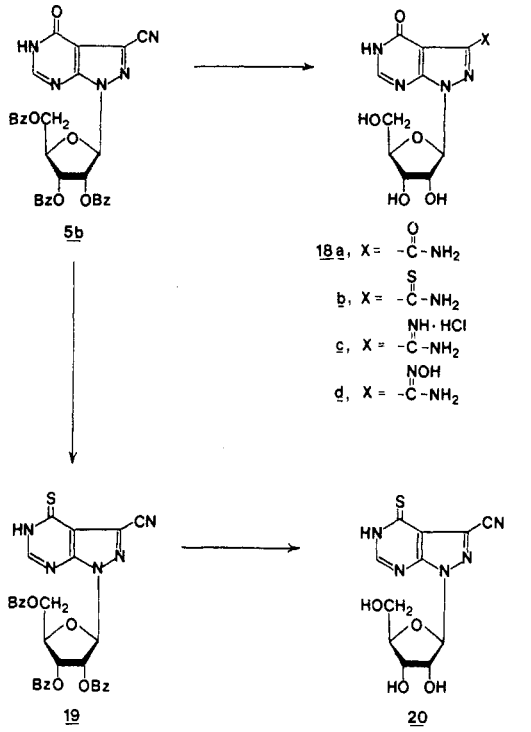
(22) Bacon, R. G. R.; Hill, H. A. O. *Q. Rev., Chem. Soc.* **1965**, *19*, 95–125.

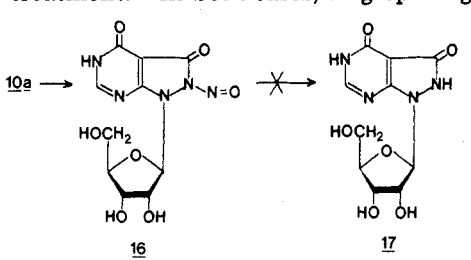
Scheme II



Scheme III



conditions. There appears to be a high propensity toward N-nitrosation. Documented examples[23] of the ease with which fused pyrazolone derivatives undergo N-nitrosation have been found. In addition to these findings, it was observed that **16** could not be successfully reduced to yield **17** by either Raney nickel reduction or by sodium dithionite treatment. In both cases, ring opening of the



pyrazole ring took place. Indeed, literature examples[24,25] suggest that fused pyrazolone compounds are quite susceptible to reductive ring cleavage.

In an effort to prepare 3-substituted 4-APP ribonucleosides (**13**), the glycosylation of 3-bromo-4-chloropyrazolo[3,4-*d*]pyrimidine (**11**) was initially attempted. Several general glycosylation procedures were tried, which included acid-catalyzed fusion procedure and silyl-TMS-triflate procedure. None of these procedures gave the required glycosylated product. However, treatment of **5a** with the mild chlorinating agent dimethyl(chloromethylene)ammonium chloride, generated in situ from thionyl chloride and DMF, gave essentially quantitative yield of 3-bromo-4-chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**12**), which was found to be rather unstable and required cold storage under anhydrous conditions. Treatment of **12** with metanolic ammonia at room temperature readily gave 4-amino-3-bromo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**13a**). However, treatment of either **12** or **13a** with methanolic ammonia in the presence of CuCl at 100 °C gave 3,4-diamino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**13b**). The nucleoside **13b** was also prepared by the direct glycosylation of 4-amino-3-bromopyrazolo[3,4-*d*]pyrimidine (**14**)[26] with **4** in the presence of $BF_3 \cdot Et_2O$ in boiling nitromethane, which gave the protected nucleoside **15**. Treatment of **15** with methanolic ammonia in the presence of CuCl gave the required **13b**.

The blocked 3-cyanoallopurinol ribonucleoside (**5b**) was employed for further functional group transformation studies (Scheme III). In an attempt to prepare 4(5*H*)-oxo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**18a**), compound **5b** was treated with ammonium hydroxide containing H_2O_2, which gave a complex reaction mixture. The isolation of the desired **18a** from the mixture was extremely difficult. However, when the deblocked nucleoside **6b** was utilized directly in this reaction, a clean reaction mixture was obtained, from which **18a** was isolated in 90% yield. Reaction of **5b** with H_2S in the presence of 4-(dimethylamino)pyridine and subsequent deprotection afforded 3-thiocarbamoylallopurinol ribonucleoside (**18b**). Heating the blocked nucleoside **5b** in liquid ammonia containing ammonium chloride at 120 °C resulted in 4(5*H*)-oxo-1-β-D-ribofuranosylpyrazolo-[3,4-*d*]pyrimidine-3-carboxamidine (**18c**), isolated as the hydrochloride salt. Treatment of **6b** with crystalline hydroxylamine in ethanol readily gave the 3-*N*-hydroxycarboxamidine derivative of allopurinol ribonucleoside (**18d**) in excellent yield. Thiation of **5b** with phosphorus pentasulfide, followed by debenzoylation of the interme-

(23) Bunnett, J. F.; Zahler, R. E. *Chem. Rev.* **1951**, *49*, 273–412.
(24) Taylor, E. C.; Barton, J. W.; Osdene, R. S. *J. Am. Chem. Soc.* **1958**, *80*, 421–427.
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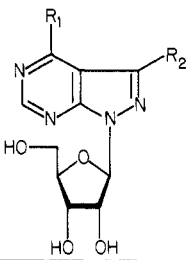
Table I. Comparative in Vitro Antiviral Activity of Allopurinol Ribonucleoside (1) and Certain 3,4-Disubstituted 1-β-Ribofuranosylpyrazolo[3,4-*d*]pyrimidines



compd	R_1	R_2	toxic level	virus rating[a]			
				Para 3	VSV	VV	HSV1
6a	OH	Br	5×10^{-3} M	0.03	*b*	0.03	0.20
6b	OH	CN	nontoxic at 5×10^{-3} M	0.32	0	0.55	0.25
6c	OH	SCH_3	5×10^{-3} M	0.11	*b*	0	0.16
10a	OH	NH_2	5×10^{-3} M	0.30	0	0.45	0.15
10b	OH	$NHCH_3$	1.6×10^{-3} M	0.10	*b*	0.20	0
13a	NH_2	Br	5×10^{-4} M	0.20	0	0.10	0.40
13b	NH_2	NH_2	nontoxic at 5×10^{-3} M	0.45	0.25	0.40	1.30
18a	OH	$C(=O)NH_2$	5×10^{-3} M	0.30	0	0.40	0
18b	OH	$C(=S)NH_2$	5×10^{-3} M	1.15	0	0.40	0
18c	OH	$C(=NH)NH_2 \cdot HCl$	5×10^{-6} M	1.10	0	0.31	0.20
18d	OH	$C(=NOH)NH_2$	nontoxic at 5×10^{-3} M	0.20	0.35	0.12	0.10
20	SH	CN	nontoxic at 5×10^{-3} M	1.10	0.56	1.24	1.00
16	*c*	*c*	5×10^{-3} M	0.45	0	0.80	0.35
1	OH	H	nontoxic at 5×10^{-3} M	0.20	0.30	0.20	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] Not tested. [c] 2-Nitroso-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3,4(5*H*)-dione.

diate **19**, furnished 1-β-D-ribofuranosyl-4(5*H*)-thioxopyrazolo[3,4-*d*]pyrimdine-3-carbonitrile (**20**) in good yield.

X-ray Crystallographic Study. Since a crystal of **6b** suitable for single-crystal X-ray analysis was not available, a derivative of **6b**, i.e., 4(5*H*)-oxo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-thiocarboxamide (**18b**), was used. Slow crystallization of **18b** from aqueous methanol gave X-ray quality crystals. A suitable single crystal was mounted on a Nicolet P3 autodiffractometer, and the diffraction data were collected, using Mo Kα radiation (λ = 0.71073 Å) with a graphite monochrometer. Two molecules are found in each unit cell. The unit cell parameters were determined by a least-squares procedure using 15 centered 2θ values. The compound crystallizes in the monoclinic space group $P2_1$ with the cell dimensions $a = 7.111$ (6) Å, $b = 10.122$ (7) Å, $c = 9.780$ (7) Å; $\beta = 99.15$ (6) Å. A total of 2099 unique reflection data were obtained to a 2θ limit of 60° ($\sin \theta/\lambda = 0.70$). Of these, 135 were considered unobserved with $I < 2\sigma(I)$. The structure was solved by using direct methods and refined by using a full-matrix least-squares procedure to a final R value of 0.0380 and $R_w = 0.0327$. A perspective view of the molecule, drawn with the aid of the ORTEP program,[27] is shown in Figure 1 with numbering of the atoms and bond lengths (angstroms).

The results of this structure determination study confirmed the β-anomeric configuration and the site of glycosylation as N-1 in **18b** and consequently its precursor **6b**.

Biological Evaluations. A. Antiviral Activity. Most of 3,4-disubstituted pyrazolo[3,4-*d*]pyrimidine nucleosides synthesized during this study were tested against herpes simplex type 1 (HSV1), vaccinia (VV), parainfluenza type 3 (Para 3), and vesicular stomatitis (VSV) viruses in vitro (Table I). Of all the compounds tested,

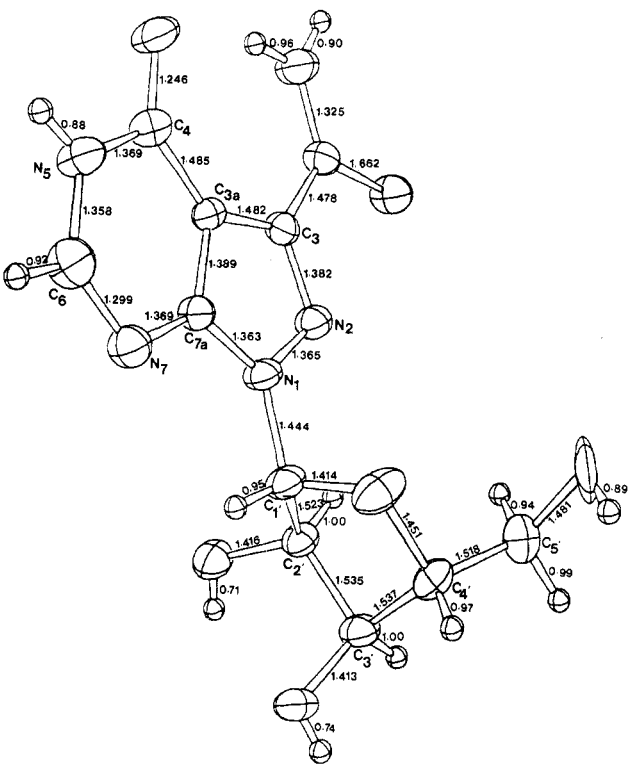
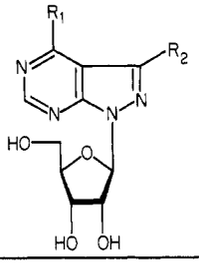


Figure 1. ORTEP drawing of **18b** with numbering of the atoms and bond length (angstroms).

1-β-D-ribofuranosyl-4(5*H*)-thioxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (**20**) exhibited very potent activity against HSV1, Para 3, and VV viruses. 3,4-Diaminopyrazolo[3,4-*d*]pyrimidine nucleoside (**13b**) showed significant activity only against HSV1, whereas compounds **18b** and **18c** were found to inhibit the growth of Para 3 significantly, in vitro. Both 3-cyanoallopurinol ribonucleoside (**6b**) and 3-cyanothiopurinol ribonucleoside (**20**)

(27) ORTEP program: Johnson, C. K. ORTEP Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, TN, 1965.

Table II. Comparative in Vitro Antitumor Activity of 4-Amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-carbonitrile and Certain 3,4-Disubstituted 1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidines



compd	R_1	R_2	ID_{50},[a] M			
			L1210	P388	Lewis Lung	B16 Melanoma
6a	OH	Br	25% inhib at 10^{-4}	28% inhib at 10^{-4}	*c*	*c*
6b	OH	CN	2.0×10^{-7}	4.6×10^{-7}	2.5×10^{-6}	7.0×10^{-6}
6c	OH	SCH_3	*b*	16% inhib at 10^{-4}	*c*	*c*
10a	OH	NH_2	*b*	*b*	*b*	*b*
10b	OH	$NHCH_3$	*b*	20% inhib at 10^{-4}	*c*	*c*
13a	NH_2	Br	28% inhib at 10^{-4}	*b*	*c*	*c*
13b	NH_2	NH_2	1×10^{-4}	*b*	*c*	*c*
18a	OH	$C(=O)NH_2$	1.3×10^{-5}	1.8×10^{-5}	2.0×10^{-5}	37% inhibition at 10^{-4}
18b	OH	$C(=S)NH_2$	3.6×10^{-7}	6.0×10^{-7}	2.4×10^{-6}	1.6×10^{-5}
18c	OH	$C(=NH)NH_2 \cdot HCl$	2.3×10^{-7}	3.7×10^{-7}	6.4×10^{-6}	3.8×10^{-6}
18d	OH	$C(=NOH)NH_2$	*b*	*b*	*c*	*c*
20	SH	CN	1.4×10^{-7}	3.2×10^{-7}	*c*	*c*
16	*d*	*d*	4.3×10^{-7}	1.6×10^{-6}	2.5×10^{-6}	8.0×10^{-5}
4-amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-carbonitrile[14]			7.9×10^{-8}			

[a] Inhibitory dose 50 (ID_{50}) is the concentration of the compound in the culture media that produced 50% inhibition of the tumor cell growth as compared to the untreated controls. [b] Inactive at 10^{-4} M. [c] Not tested. [d] 2-Nitroso-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3,4(5*H*)-dione.

showed moderate activity against VV and VSV, respectively. All other compounds were inactive against the viruses used.

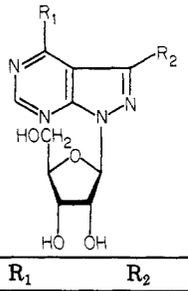
B. Antitumor Activity. The 3,4-disubstituted pyrazolo[3,4-*d*]pyrimidine nucleosides synthesized during this study were also tested against L1210 leukemia, P388, Lewis Lung, and B16 Melanoma in vitro (Table II). Compounds **6b**, **16**, and **18a–c** were all found to be potent inhibitors of the growth of all the cell lines used. Of considerable interest is the pronounced activity exhibited by 1-β-D-ribofuranosyl-4(5*H*)-thioxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (**20**) against L1210 leukemia and P388. Compound **13b** showed slight activity against L1210 leukemia, whereas the other compounds under study were inactive in vitro.

C. Antileishmanial Activity. The 50% effective doses (ED_{50}) for the elimination of *Leishmania* amastigotes from infected macrophages were exposed to several pyrazolo[3,4-*d*]pyrimidine ribonucleosides synthesized during this study (Table III). The ED_{50}s for these nucleosides had to be estimated owing to the flatness of the dose–response curves. The estimated ED_{50} for 1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thione (thiopurinol ribonucleoside) was similar to that for allopurinol ribonucleoside (**1**). No more than 60% of organisms were eliminated by 1 or thiopurinol ribonucleoside at the highest concentrations tested (72–190 μM). 3-Aminoallopurinol ribonucleoside (**10a**), as well as the nitroso compound (**16**), have similar ED_{50}s. However, 3-bromoallopurinol ribonucleoside (**6a**) was found to be more active ($ED_{50} > 15$ μM) than allopurinol ribonucleoside against *Leishmania tropica* in vitro.

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

Table III. In Vitro Antileishmanial Activity of Certain 1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidines



compd	R_1	R_2	ED_{50},[a] μM
1	OH	H	76–190
	SH	H	72[30]
6a	OH	Br	>15
10a	OH	NH_2	>177
16	*b*	*b*	>160

[a] Effective dose 50 (ED_{50}) is the concentration of the compound in the culture media that produced 50% inhibition of the parasitic growth (6-day period) as compared to the untreated controls. The percentage of macrophage-contained *Leishmania* spp. surviving exposure to several doses of a pyrazolo[3,4-*d*]pyrimidine nucleoside was determined in two to five experiments for each compound. The number of organisms per 100 macrophages in non-drug-treated controls was 805 ± 224 (n = 25). Allopurinol ribonucleoside (**1**) was used as a positive control in each experiment. Since the dose–response curves for these compounds were relatively flat, the ED_{50}s were estimated by inspection of the data. [b] 2-Nitroso-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3,4(5*H*)-dione.

analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and Robertson Labs, Florham Park, NJ. 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.

3-Bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (5a). Method 1. 3-Bromoallopurinol[15] (**3a**; 21.5 g, 100 mmol) was combined with hexamethyldisilazane (HMDS, 100 mL), pyridine (20 mL), and $(NH_4)_2SO_4$ (50 mg) and allowed to reflux for 4 h with the exclusion of moisture. The clear solution was then evaporated to a thick syrup, which was subjected to high vacuum at room temperature for 2 h. The residual syrup was dissolved in anhydrous acetonitrile (500 mL), and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**4**; 50.4 g, 100 mmol) was added. To this solution was added (trimethylsilyl)trifluoromethanesulfonate (TMS-triflate; 33.3 g, 27.3 mL, 150 mmol) and the solution was refluxed for a week (this length of time is necessary to effect the rearrangement of the rapidly formed N-5 isomer to the desired N-1 isomer). The solution was evaporated to a dry foam, which was dissolved in ethyl acetate (700 mL) and washed with saturated aqueous $NaHCO_3$ (1 × 500 mL) and then with water (1 × 500 mL) and dried over Na_2SO_4. The solvent was removed to yield a brown foam, which was applied to a Prep LC system using 5% acetone in dichloromethane as the eluting solvent. The unreacted sugar eluted first, followed by the fraction containing the N-1 isomer (contaminated with a small amount of N-5 isomer). The syrup obtained from the N-1 fraction was crystallized from methanol to yield 19.1 g (29%). A small sample was recrystallized from MeOH for analytical purposes: mp 221 °C; IR (KBr) ν 1720, 1730 (C=O of esters) cm^{-1}; UV λ_{max} (95% EtOH) 224 nm (ϵ 20 400), 263 (sh, 3600); NMR (Me_2SO-d_6) δ 6.65 (d, 1, J = 1.8 Hz, $C_{1'}$ H), 7.50 and 7.91 (2 m, 15 benzoyl aromatics), 8.20 (s, 1, C_6 H), and other sugar protons. Anal. ($C_{31}H_{23}BrN_4O_8$) C, H, N, Br.

Method 2. Compounds **3a** (10.0 g, 46.5 mmol) and **4** (35.0 g, 69.7 mmol) were added to dry nitromethane (125 mL) and brought to reflux temperature. BF_3·Et_2O (9.9 g, 8.8 mL, 69.7 mmol) was added. The suspension became clear within 3 min, and refluxing was continued for a total of 90 min. The solvent was evaporated to yield a brown foam, which was worked up and purified as described in method 1 above. Crystallization from methanol gave 13.8 g (45%) of **5a**: mp 217–219 °C. This product was found to be identical with that prepared by method 1.

3-Bromo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (6a). Compound **5a** (1.5 g, 2.4 mmol) was combined with methanolic ammonia (70 mL, saturated at 0 °C), placed in a steel bomb, and heated at 90–100 °C for 5 h. The solvent was evaporated and the residue treated with boiling benzene (3 × 25 mL) to remove benzamide. The benzene-insoluble solid was then crystallized from water to yield colorless needles (540 mg, 65%): mp 240–242 °C; IR (KBr) ν 1695 (C=O), 3400 (NH, OH) cm^{-1}; UV λ_{max} (pH 1 and 7) 215 nm (ϵ 23 000), 230 (sh, 9400), 252 (7800); UV λ_{max} (pH 11) 225 nm (ϵ 9400), 276 (11 800); NMR (Me_2SO-d_6) δ 6.10 (d, 1, J = 4.5 Hz, $C_{1'}$ H) 8.20 (s, 1, C_6 H), and other sugar protons. Anal. ($C_{10}H_{11}BrN_4O_5$) C, H, N.

1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (1). From 6a. To a solution of **6a** (100 mg, 0.29 mmol) in 50% aqueous EtOH (15 mL) was added Pd/C (10%, 20 mg), and the mixture was hydrogenated at 40 psi at room temperature for 2 h. The reaction mixture was filtered through a Celite pad, and the filtrate was evaporated to dryness. Crystallization of the residual solid from EtOH gave colorless crystals (56 mg, 72%): mp 170 °C, resolidifies, melts 205–206 °C. This compound was identical in all respects with 1, prepared and reported previously.[16]

From 6c. To a solution of **6c** (50 mg, 0.16 mmol) in 50% aqueous EtOH (15 mL) was added Raney nickel (0.5 g, wet weight) and the mixture was refluxed for 1 h. The reaction mixture was filtered hot and the filter cake was washed with boiling water (2 × 5 mL). The combined filtrate and washings were evaporated to dryness, and the residual solid was crystallized from EtOH to yield 25 mg (59%) of colorless material: mp 169–170 °C, resolidifies, melts 205–206 °C. This material was identical in all respects with 1, prepared and reported previously.[16]

4(5*H*)-Oxo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (5b). Method 1. A suspension of 3-cyanoallopurinol[19] (**3b**; 4.0 g, 25 mmol) in HMDS (15 mL) was refluxed for 4 h. The excess HMDS was removed to give a crystalline residue. The residue was dissolved in dry acetonitrile (100 mL) and cooled to −20 °C. TMS-triflate catalyst (8.3 g, 6.8 mL, 37.5 mmol) was added in one portion, followed by slow addition of **4** (18.9 g, 37.5 mmol) in acetonitrile (50 mL). The addition was complete in 4 h and the reaction mixture was allowed to warm slowly to room temperature. The solution was then refluxed for 1 week and evaporated to dryness, and the residue dissolved in dichloromethane (300 mL). After washing with saturated aqueous $NaHCO_3$ (1 × 200 mL) and then with water (2 × 150 mL), the organic layer was dried over Na_2SO_4 and evaporated to a syrup. The syrup was triturated with MeOH to give a crystalline product (6.9 g, 46%): mp 240–242 °C; IR (KBr) ν 1710 (C=O of heterocycle), 2240 (CN), 3420 (OH, NH) cm^{-1}; UV λ_{max} (MeOH) 262 nm (ϵ 12 400); NMR (Me_2SO-d_6) δ 3.36 (s, 1, H_2O), 6.79 (d, 1, J = 2.7 Hz, $C_{1'}$ H), 7.50–8.10 (m, 15, benzoyl aromatics), 8.31 (s, 1, C_6 H), 12.92 (s, 1, N_5 H), and other sugar protons. Anal. ($C_{32}H_{23}N_5O_8 \cdot {}^1/_2H_2O$) C, H, N.

Method 2. To a suspension of **3b** (1.0 g, 6.3 mmol) in benzonitrile (50 mL) was added BF_3·Et_2O (0.56 g, 0.5 mL, 4.0 mmol). After a few seconds all of the base had dissolved and the solution was heated to 160–180 °C. To the hot solution was added **4** (4.7 g, 9.4 mmol) and after 15 min the dark solution was cooled to room temperature. The reaction mixture was slowly poured into hexane (1 L) with stirring, whereupon a dark oil separated. The hexane was decanted and the oil was dissolved in acetone (30 mL) and subjected to silica gel column chromatography using ether as eluent. The nucleoside fractions were combined and evaporated to a syrup, which, on trituration with MeOH, gave a crystalline solid: yield 2.1 g (52%). This product was identical with that obtained by method 1.

4(5*H*)-Oxo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (6b). To a suspension of **5b** (710 g, 11.5 mmol) in MeOH/acetonitrile (250 mL, 3:1, v/v) was added freshly prepared sodium methoxide in methanol (1 N) until "pH 9" was reached. After 18 h, TLC monitoring indicated no further reaction and Dowex-50 H^+ resin was added to pH less than 5. The resin was removed by filtration and the MeOH solution was evaporated to dryness. Crystallization of the residue from water gave 3.0 g (89%) of the title compound: mp 230–232 °C; IR (KBr) ν 1690 (C=O), 2260 (CN), 3250 (NH, OH) cm^{-1}; UV λ_{max} (pH 1 and 7) 227 nm (ϵ 10 400), 261 (8400); UV λ_{max} (pH 11) 230 nm (ϵ 10 100), 281 (9300); NMR (Me_2SO-d_6) δ 3.35 (s, 2, H_2O), 6.11 (d, 1, J = 4.41 Hz, $C_{1'}$ H), 8.28 (s, 1, C_6 H), and other sugar protons. Anal. ($C_{11}H_{11}N_5O_5 \cdot H_2O$) C, H, N.

5-Amino-3-(methylthio)pyrazole-4-carboxamide (8). 5-Amino-3-(methylthio)pyrazole-4-carbonitrile[20] (**7**; 2.0 g, 11.8 mmol) was dissolved in polyphosphoric acid (7.5 g) and the mixture was heated at 115–120 °C for 45 min. After cooling, ice water (100 mL) was added, and the solution was neutralized with 2 N NaOH and then extracted with ethyl acetate (5 × 90 mL). The combined organic layers were washed with water (1 × 200 mL) and dried over Na_2SO_4 before evaporating to dryness. The resulting solid was suspended in ether, filtered, and dried to yield 1.6 g (72%) of the title compound: mp 158–160 °C; IR (KBr) ν 1650 (C=O), 3300–3400 (NH_2) cm^{-1}; UV λ_{max} (pH 1, 7, and 11) 256 nm (ϵ 6600); NMR (Me_2SO-d_6) δ 2.45 (s, 3, SCH_3), 6.10 and 6.78 (2 s, 4, NH_2 and $CONH_2$, exchanged with D_2O), 11.90 (s, 1, N_1 H, exchanged with D_2O). Anal. ($C_5H_8N_4OS$) C, H, N, S.

3-(Methylthio)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (9). Compound **8** (5.0 g, 29 mmol) was heated in dry formamide (15 mL) at 180 °C for 30 min. After the mixture cooled to 90 °C, water was added (75 mL), the solution was decolorized with Norit A, and upon further cooling, the colorless product separated. Recrystallization from water with a small amount of EtOH gave colorless needles: yield 4.0 g (76%); mp 286–287 °C; IR (KBr) ν 1680 (C=O), 3100 and 3400 (NH's) cm^{-1}; UV λ_{max} (pH 1 and 7) 228 nm (ϵ 11 400), 242 (sh, 8800); UV λ_{max} (pH 11) 232 nm (11 600), 275 (sh, 4200); NMR (Me_2SO-d_6) δ 2.60 (s, 3, SCH_3), 8.10 (s, 1, C_6 H), 11.5 (br s, 1, N_5 H). Anal. ($C_6H_6N_4OS \cdot {}^1/_2H_2O$) C, H, N, S.

3-(Methylthio)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (5c). Compound **5c** was prepared by method 1, described for **5a**, using compounds **9** (2.0 g, 11 mmol), **4** (5.54 (g, 11 mmol), and TMS-triflate catalyst (3.7 g, 3.0 mL, 16.5 mmol): yield 1.0 g (15%); mp 260–261 °C; IR (KBr) ν 1720 and 1730 (C=O of esters), 1680 (C=O of heterocycle) cm^{-1}; NMR (Me_2SO-d_6) δ 2.48 (s, 3, SCH_3), 6.53 (d, 1, J = 2.8 Hz, $C_{1'}$ H), 7.39–7.95 (m, 15, benzoyl aromatics), 8.08 (s, 1, C_6 H), and other sugar protons. Anal. ($C_{32}H_{26}N_4O_8S$) C, H, N, S.

3-(Methylthio)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (6c). Compound **5c** (670 mg, 1.07 mmol) was dissolved in anhydrous MeOH (15 mL) and commercial sodium methoxide was added until "pH 10" was reached. The solution was stirred overnight (16 h), neutralized with Dowex-50 H^+ resin, filtered, and evaporated to dryness to yield a crude solid, which was crystallized from a small amount of water to give colorless plates: 300 mg (89%); mp 218–219 °C; IR (KBr) ν 1680 (C=O), 3250 and 3400 (NH, OH) cm^{-1}; UV λ_{max} (pH 1 and 7) 228 nm (ϵ 36 100), 243 (sh, 23 300); UV λ_{max} (pH 11) 250 nm (ϵ 21 700), 273 (16 300); NMR (Me_2SO-d_6) δ 2.52 (s, 3, SCH_3), 5.95 (d, 1, J = 4.0 Hz, $C_{1'}$ H), 8.08 (s, 1, C_6 H), and other sugar protons. Anal. ($C_{11}H_{14}N_4O_5S$) C, H, N, S.

3-Amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (10a). To a suspension of **5a** (15.0 g, 23 mmol) in methanolic ammonia (125 mL, saturated at 0 °C) were added cuprous chloride (330 mg, 3.3 mmol) and copper turnings (380 mg, 6 mmol). The mixture was heated in a steel bomb for 16 h at 100 °C. The contents were evaporated to dryness onto silica gel (25 g) and placed on top of a silica gel column (4 × 25 cm, prepacked in EtOAc). The column was eluted with EtOAc/95% EtOH (1:1, v/v). The appropriate fractions were combined and evaporated to dryness, and the residue was crystallized from 95% EtOH (Norit A) to yield colorless needles: 5.5 g (85%); mp 230–232 °C; IR (KBr) ν 1680 (C=O), 3340 and 3460 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1) 223 nm (ϵ 22 500), 279 (sh, 4400); UV λ_{max} (pH 7) 223 nm (ϵ 25 200) 279 (sh, 5000); UV λ_{max} (pH 11) 217 nm (ϵ 25 800), 279 (7100); NMR (Me_2SO-d_6) δ 5.54 (s, 2, NH_2), 5.93 (d, 1, J = 4.0 Hz, $C_{1'}$ H), 8.00 (s, 1, C_3 H), 11.90 (br s, 1, N_5 H), and other sugar protons. Anal. ($C_{10}H_{13}N_5O_5$) C, H, N.

3-(Methylamino)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (10b). To a suspension of **5a** (6.0 g, 9.1 mmol) in 40% aqueous methylamine solution (100 mL) were added CuCl (150 mg) and Cu turnings (200 mg). The mixture was heated in a steel bomb for 16 h at 100 °C. The reaction mixture was worked up as described for **10a** to yield 2.5 g (91%), which was crystallized from CH_3CN/MeOH: mp 130 °C; IR (KBr) ν 1680 (C=O), 3200 and 3400 (NH, OH) cm^{-1}; UV λ_{max} (pH 1 and 7) 227 nm (ϵ 12 200), 243 (ϵ 5900); UV λ_{max} (pH 11) 223 nm (ϵ 13 900), 242 (6800); NMR (Me_2SO-d_6) δ 2.71 (s, 3, NCH_3), 5.91 (d, 1, J = 3.73 Hz, $C_{1'}$ H), 8.00 (s, 1, C_6 H), 12.00 (br s, 1, N_5 H), and other sugar protons. Anal. ($C_{11}H_{15}N_5O_5 \cdot CH_3OH$) C, H, N.

3-(Dimethylamino)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (10c). To a suspension of **5a** (6.0 g, 9.1 mmol) in 40% aqueous dimethylamine solution (75 mL) was added CuCl (150 mg) and Cu turnings (200 mg). The mixture was heated at 100 °C for 3 days in a steel bomb. The mixture was worked up and purified as described for **10a** to yield 0.96 g (34%). An analytical sample was obtained by preparative TLC techniques: IR (KBr) ν 1680 (C=O), 3200 and 3450 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 220 nm (sh, ϵ 16 600), 242 (sh, 11 700); UV λ_{max} (pH 7) 227 nm (ϵ 22 700), 244 (sh, 16 800); UV λ_{max} (pH 11) 217 nm (ϵ 22 100), 240 (sh, 13 100), 270 (7500); NMR (Me_2SO-d_6) δ 2.70 [s, 6, $N(CH_3)_2$], 5.97 (d, 1, J = 3.73 Hz, $C_{1'}$ H), 7.97 (s, 1, C_6 H), and other sugar protons. Anal. ($C_{12}H_{17}N_5O_5 \cdot 1^1/_2H_2O$) C, H.

3-Bromo-4-chloropyrazolo[3,4-*d*]pyrimidine (11). 3-Bromoallopurinol[15] (**3a**; 20.0 g, 93 mmol) was combined with phosphorus oxychloride (250 mL) and *N,N*-diethylaniline (25 mL, mono-free) and brought to reflux. Reflux was continued for a total of 2 h and the excess $POCl_3$ was removed. The thin amber syrup was stirred into crushed ice (500 mL) for 15 min. The aqueous mixture was extracted with ether (3 × 350 mL), and the combined ether layers were washed with ice-cold 2% $NaHCO_3$ solution (1 × 300 mL), followed by water (1 × 300 mL). The dried (Na_2SO_4) organic phase was evaporated to yield **11** as an off-white solid. If further purification is desired, this compound may be applied to a Prep LC system using 15% acetone in hexane as the eluent. **11**: yield 18.2 g (84%); mp 181–183 °C; MS, *m/e* 232 (76.8%, M^+), 233 (5.9%, M + 1), 234 (100%, M + 2), 235 (7.0%, M + 3), 236 (23.3%, M + 4); IR (KBr) ν 1230, 1550, 1600 cm^{-1}; UV λ_{max} (EtOH) 262 nm (ϵ 4200), 286 (sh, 3000); NMR (Me_2SO-d_6) δ 8.30 (s, 1, C_6 H). Anal. ($C_5H_2BrClN_4$) C, H, N, Cl.

3-Bromo-4-chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (12). Compound **5a** (5.0 g, 8 mmol) was dissolved in dry dichloromethane (100 mL) and to this was added a solution of freshly distilled thionyl chloride (15 mL) and DMF (7.5 mL) in dichloromethane (50 mL) dropwise over a period of about 2 h. The reaction was kept at reflux (60 °C) under a slow stream of dry nitrogen for 16 h. The mixture was carefully poured into ice and saturated $NaHCO_3$ solution (200 mL) and stirred for 20 min. The layers were separated, and the aqueous layer was extracted with additional dichloromethane (2 × 150 mL) and combined with the original organic layer. The combined organic layers were washed with water (1 × 250 mL), dried over Na_2SO_4, evaporated to dryness, and subjected to high vacuum at room temperature for 2 h; yield of pure material (as a yellow foam) 5.4 g (100%). This compound was used immediately due to its instability and a satisfactory elemental analysis could not be obtained.

4-Amino-3-bromo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (13a). Method 1. Compound **12** (2.8 g, 4.37 mmol) was combined with methanolic ammonia (150 mL, saturated at 0 °C) and stirred at room temperature for 24 h. Evaporation of the solvent and treatment of the residue with boiling benzene (2 × 30 mL), followed by crystallization from ethanol, gave colorless needles: 1.1 g (73%); mp 200 °C; IR (KBr) ν 1550, 1630 cm^{-1}; UV λ_{max} (pH 1) 226 nm (ϵ 18 000), 255 (6200); UV λ_{max} (pH 7 and 11) 230 nm (6100), 260 (5500), 280 (7400) 290 (sh, 5000); NMR (Me_2SO-d_6) δ 6.09 (d, 1, J = 4.50 Hz, $C_{1'}$ H) 7.60 (s, 2, NH_2), 8.33 (s, 1, C_6 H), and other sugar protons. Anal. ($C_{10}H_{12}BrN_5O_4$) C, H, N.

Method 2. To a solution of **15** (3.4 g, 5.2 mmol) in methanol (100 mL) was added sodium methoxide until "pH 10" was reached. The mixture was stirred at room temperature for 20 h and the resulting solution was filtered to remove a small amount of white precipitate. The filtrate was neutralized with Dowex-50 H^+ resin and worked up as described for **6c** to yield 1.50 g (84%): mp 200 °C. This product was found to be identical with that prepared by method 1.

3,4-Diamino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (13b). Method 1. Compound **12** (2.0 g, 3.1 mmol) was combined with methanolic ammonia (75 mL, saturated at 0 °C), CuCl (43 mg, 0.43 mmol), and Cu turnings (50 mg, 0.80 mmol). The mixture was heated in a steel bomb at 100 °C for 16 h and then worked up and purified as described for the preparation of **10a**. Crystallization of the solid from aqueous EtOH gave 0.2 g (23%) of off-white material: mp 154 °C dec; IR (KBr) ν 1550, 1600, 3200, 3350 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1) 237 nm (ϵ 21 400), 282 (2800); UV λ_{max} (pH 7 and 11) 224 nm (ϵ 15 500), 285 (4000); NMR (Me_2SO-d_6) δ 5.10–4.60 (br s, C_3 NH_2 and H_2O, OH's), 5.92 (d, 1, J = 3.9 Hz, $C_{1'}$ H), 7.63 (s, 2, C_4 NH_2), 8.08 (s, 1, C_6 H), and other sugar protons. Anal. ($C_{10}H_{14}N_6O_4 \cdot 1^1/_2H_2O$) C, H, N.

Method 2. Compound **13a** (100 mg, 0.29 mmol) was combined with MeOH/NH_3 (25 mL, saturated at 0 °C) and a catalytic amount (~5 mg) of CuCl, and the mixture was heated in a steel bomb at 110–120 °C for 16 h. The reaction mixture was worked up as described for **10a** to yield 45 mg (55%) of the title compound, mp 153–154 °C dec, and was identical with the product prepared by method 1.

4-Amino-3-bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (15). Compound **15** was prepared by method 2, described for **5a**, utilizing 4-amino-3-bromopyrazolo[3,4-*d*]pyrimidine[26] (**14**; 5.0 g, 23.4 mmol), **4** (17.5 g, 35 mmol), and $BF_3 \cdot Et_2O$ (4.4 mL, 35 mmol): yield 13.4 g (87%). An analytical sample was obtained by slow evaporation of a methanolic solution: mp 154–156 °C; IR (KBr) ν 710 (CBr), 1735 (C=O of benzoyls), 3350 and 3430 (NH_2) cm^{-1}; UV λ_{max} (EtOH) 228 nm (ϵ 7200), 271 (15 300), 288 (sh, 8600). Anal. ($C_{31}H_{24}BrN_5O_7$) C, H, N, Br.

2-Nitroso-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3,4(5*H*)-dione (16). To a solution of **10a** (2.0 g, 7 mmol) in water

(50 mL, cooled to 5 °C) was added sodium nitrite (3.4 g, 50 mmol). Glacial acetic acid was then dripped in (5 mL) with stirring while the temperature was maintained below 10 °C. The reddish-brown solution was allowed to stir for 30 min and the mixture was evaporated to dryness on silica (10 g) and packed on a short column, eluted with EtOAc/EtOH (3:1, v/v). The residual solid, after evaporation of the solvent, was crystallized from water (Norit A) to yield 1.3 g (59%) of microneedles: mp 234–235 °C; MS, *m/e* 181 (100%, base minus sugar fragment); IR (KBr) ν 1340 (NN=O), 1690 (C=O), 3200, 3480 and 3600 (NH, OH) cm^{-1}; UV λ_{max} (pH 1 and 7) 250 nm (ϵ 8200); UV λ_{max} (pH 11) 226 nm (sh, 8200), 264 (7200); NMR (Me_2SO-d_6) δ 6.18 (d, 1, J = 4.50 Hz, $C_{1'}$ H), 8.30 (s, 1, C_6 H), and other sugar protons. Anal. ($C_{10}H_{11}N_5O_7 \cdot H_2O$) C, H, N.

4(5*H*)-Oxo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-carboxamide (18a). To a solution of **6b** (410 mg, 1.4 mmol) in water (25 mL) was added 28% NH_4OH (25 mL) and 30% H_2O_2 (5 mL). After 30 min, TLC showed no starting material remained and the solution was evaporated to dryness. The residue was suspended in hot MeOH (25 mL) and water was added dropwise until all the material dissolved. On standing, a crystalline solid formed: yield of analytically pure **18a**, 385 mg (90%); mp >260 °C dec; IR (KBr) ν 1680 (C=O), 3400 (NH, OH) cm^{-1}; UV λ_{max} (pH 1 and 7) 257 nm (ϵ 7300); UV λ_{max} (pH 11) 275 nm (ϵ 8100); NMR (Me_2SO-d_6) δ 6.13 (d, 1, J = 4.40 Hz, $C_{1'}$ H), 7.87 and 9.58 (2 s, 2, $CONH_2$), 8.26 (s, 1, C_6 H), and other sugar protons. Anal. ($C_{11}H_{13}N_5O_6$) C, H, N.

4(5*H*)-Oxo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-thiocarboxamide (18b). A mixture of **5b** (500 mg, 0.82 mmol), liquid H_2S (15 mL), and 4-(dimethylamino)pyridine (5 mg) was heated in a steel bomb at 120 °C for 5 h. The bomb was cooled in ice–water and the H_2S allowed to evaporate. The solid residue was chromatographed on a silica gel column using 40% acetone in hexane as eluent. The appropriate fractions were pooled and evaporated to provide 345 mg (65%) of blocked material, mp 180–182 °C dec.

A solution of this material (325 mg) in $MeOH/CH_3CN$ (10:1, v/v, 20 mL) was treated with sodium methoxide in methanol (1 N) to "pH 9". After 18 h at room temperature, the reaction was quenched by addition of Dowex-50 H^+ resin to "pH 5". After stirring with the resin for several minutes, a fine crystalline solid formed. The solid and supernate were decanted from the residue, and the resin was washed several times with MeOH. The solid product was filtered, and the filtrate and resin washings were combined and evaporated to dryness. The solid residue was crystallized from aqueous MeOH and then combined with the original solid product and recrystallized from aqueous MeOH to give analytically pure **18b**: yield 159 mg (59% overall); mp 250–252 °C dec; IR (KBr) ν 1650 (C=O), 3360 (NH, OH) cm^{-1}; UV λ_{max} (pH 1 and 7) 245 nm (ϵ 13 000), 259 (sh, 13 000), 308 (4800); UV λ_{max} (pH 11) 257 nm (sh, ϵ 12 000), 269 (12 100), 317 (sh, 12 100); NMR (Me_2SO-d_6) δ 6.15 (d, 1, J = 4.1 Hz, C_1, H), 8.28 (s, 1, C_6 H), 10.25 and 11.17 (2 s, 2, $CSNH_2$), and other sugar protons. Anal. ($C_{11}H_{13}N_5O_5S$) C, H, N, S.

4(5*H*)-Oxo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-carboxamidine Hydrochloride (18c). A mixture of **5b** (250 mg, 0.41 mmol), liquid ammonia (20 mL), and ammonium chloride (20 mg) was heated in a bomb at 90–100 °C for 18 h. The bomb was cooled in a dry ice/acetone bath and the ammonia allowed to evaporate. The resulting solid was crystallized from 95% EtOH containing a drop of concentrated HCl. Yield of **18c** was 101 mg (71%). A second crystallization gave analytically pure material: mp 243 °C dec; IR (KBr) ν 1660 (C=O), 3300 (NH, OH) cm^{-1}; UV λ_{max} (pH 1 and 7) 233 nm (ϵ 13 500), 261 (9000); UV λ_{max} (pH 11) 236 nm (ϵ 11 600), 274 (8000); NMR (Me_2SO-d_6) δ 6.16 (d, 1, J = 4.40 Hz, $C_{1'}$ H), 8.39 (s, 1, C_6 H), 9.55–9.93 (m, 4, amidine–HCl), and other sugar protons. Anal. ($C_{11}H_{14}N_6O_5 \cdot HCl$) C, H, N, Cl.

***N*-Hydroxy-4(5*H*)-oxo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (18d).** To a suspension of **6b** (200 mg, 0.68 mmol) in ethanol (50 mL) was added crystalline hydroxylamine (100 mg) and the mixture was refluxed for 1 h. After stirring at room temperature overnight, the mixture was cooled and filtered and the residue was washed with ethanol (2 × 15 mL). Crystallization of the residue with water gave an analytical sample: yield 210 mg (94%); mp >263 °C dec; IR (KBr) ν 1665 (C=O), 3180–3470 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 233 nm (ϵ 14 200), 253 (sh, 11 700); UV λ_{max} (pH 7) 253 nm (ϵ 10 800); UV λ_{max} (pH 11) 273 nm (ϵ 12 400); NMR (Me_2SO-d_6) δ 6.11 (d, 1, J = 4.07 Hz, $C_{1'}$ H), 6.59 (s, 2, NH_2), 8.20 (s, 1, C_6 H), 10.00–13.00 (2 s, 2, N_5 H and NOH), and other sugar protons. Anal. ($C_{11}H_{14}N_6O_6 \cdot {}^1/_4H_2O$) C, H, N.

1-β-D-Ribofuranosyl-4(5*H*)-thioxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (20). A mixture of **5b** (4.60 g, 7.8 mmol), phosphorus pentasulfide (4.6 g), and 4-(dimethylamino)pyridine (100 mg) in dioxane (100 mL) was refluxed for 2 h. The mixture was poured into ice–water (200 mL) and stirred for 30 min. The solution was extracted with EtOAc (3 × 100 mL), and the combined organic layers were evaporated to dryness. The residue was purified by flash chromatography using 25% acetone in hexane to give 4.0 g (85%) of **19**. This material was used without further purification for the deblocking reaction.

To a solution of **19** (0.50 g) in methanol (30 mL) was added sodium methoxide until "pH 10", and the mixture was stirred at room temperature for 18 h. After neutralization with Dowex-50 H^+ resin, the mixture was filtered and the filtrate evaporated to dryness. Two crystallizations of the residue from aqueous methanol gave 220 mg (89%) of analytically pure **20**: mp 125–127 °C; IR (KBr) ν 1200 (C=S), 2240 (CN), 3400 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 248 nm (ϵ 7100), 330 (12 200); UV λ_{max} (pH 7) 248 nm (ϵ 7100), 333 (10 700); UV λ_{max} (pH 11) 248 nm (ϵ 7100), 336 (10 500); NMR (Me_2SO-d_6) δ 3.38 (br s, 1, H_2O), 6.15 (d, 1, J = 3.6 Hz, $C_{1'}$ H), 8.39 (s, 1, C_6 H), 11.18 (s, 1, N_5 H), and other sugar protons. Anal. ($C_{11}H_{11}N_5O_4S \cdot {}^1/_2H_2O$) C, H, N, S.

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) cells after infection with herpes simplex type 1 (HSV 1, KOS), vaccinia (VV), parainfluenza type 3 (Para 3), and vesicular stomatitis (VSV) viruses. In this system, monolayers (18 h) of cells were exposed to the following $TCID_{50}$ (tissue culture 50% infective dose) units of virus; HSV 1 (63), VV (200), Para 3 (56), VSV (3), and concentrations of each compound in one-half log dilutions ranging from 1000 to 1 μg/mL were added within 15–30 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.[28] 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.

Antitumor Evaluation. A. L1210 and P388 Leukemias. L1210 leukemia and P388 lymphoid neoplasm were maintined in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum. For determination of cell growth inhibition, L1210 and P388 cells were seeded in 13 × 100 tubes at 5 × 10^4 cells/mL (2 mL/tube). Cells were grown in the presece of the compound of interest, at 4–5 log doses, for 48 h at 37 °C. Cell growth assessed by cell count, using a Coulter cell counter. Cell growth at each dose level was expressed as a percentage of growth in control tubes and dose resulting in 50% inhibition of growth was determined.

B. B16 Melanoma and Lewis Lung Carcinoma. Lewis Lung carcinoma was maintained as a monolayer in RPMI-1640 medium supplemented with 5% heat-inactivated fetal calf serum, whereas B16 Melanoma was maintained as a monolayer in Eagle's minimum essential medium (MEM) containing 10% heat inactivated fetal calf serum, 0.1 mM MEM nonessential amino acids, 1 mM sodium pyruvate and MEM vitamin solution. For determination of cell growth inhibition using either cell line, cells were seeded at 2.5 × 10^4 cells per well in 24 tissue culture plates. Cells were grown 24 h at 37 °C in 5% CO_2, and then growth medium was replaced with medium containing the compound of interest at 4 or 5 log dose levels. After an additional 72-h incubation, cells were washed twice with normal saline and growth was assessed by determination of cell protein by using the Lowry method.[29]

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Cell growth at each dose level was expressed as a percentage of growth (protein) in control wells and the dose resulting in 50% inhibition of growth was determined.

Antileishmanial Evaluation. Human macrophage cultures were derived from the monocytes of the peripheral blood of normal human volunteers by methods previously described.[31] After being infected with amastigotes of *Leishmania tropica* WR 401 (NIH 173), infected macrophage cultures in 0.1 mL of culture medium were exposed to a constant dose of pyrazolo[3,4-*d*]pyrimidine nucleoside for 6 days. The culture medium used was RPMI-1640 (GIBCO Laboratories, Grand Island, NY) containing 10% heat-activated fetal calf serum (GIBCO Laboratories), penicillin (50 U/mL), and streptomycin (50 μg/mL). After 6 days the number of amastigotes per 100 macrophages in control (non-drug-treated) cultures and experimental cultures was determined by counting 100–200 Giemsa-stained macrophages in each culture. The number of macrophages per culture was estimated by counting 20 representative fields for each culture. In initial experiments, drug doses of 0.01–1.0 μM were employed. Generally, the drug dosage was increased in subsequent experiments until macrophage toxicity (see below) or a dose of at least 70 μM was achieved.

Enumeration of Data. The number of *Leishmania* amastigotes per 100 macrophages surviving in drug-treated cultures was expressed as a percentage of the number in simultaneously cultivated controls. The concentration of drug calculated to eliminate 50% of amastigotes compared to controls (the 50% effective dose [ED_{50}]) was determined by nonlinear regression analysis[32] of the results of each experiment. For drugs for which the dose–response curve was so flat that statistical analysis could not be performed, the ED_{50} was estimated by inspection of the data.

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Synthesis and Antihypertensive Activity of 6,7-Disubstituted *trans*-4-Amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ols

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A series of novel 6,7-disubstituted *trans*-3,4-dihydro-2,2-dimethyl-4-pyrrolidino-(or piperidino)-2*H*-1-benzopyran-3-ols was prepared and tested for antihypertensive activity in the conscious spontaneously hypertensive rat (SHR) and compared with certain of their monosubstituted analogues. The potent blood pressure lowering activity of the 6-monosubstituted compounds was enhanced by incorporation of an acetylamino or amino group at C(7) and that of the 7-nitro-substituted compound by incorporation of an amino (but not an acetylamino group) at C(6). The combination of 6-nitro or 6-cyano with 7-(acetylamino) or 7-amino groups and 6-amino with 7-nitro groups in *trans*-4-pyrrolidino- or -4-piperidino-2,2-dimethyl-2*H*-1-benzopyranols conferred superior antihypertensive activity to hydralazine and to the calcium antagonist, nifedipine, in SHR. The synthetic route to these compounds involves the conversion of 2*H*-1-benzopyrans to bromohydrins that were treated with pyrrolidine or piperidine. Preparation of the 6-cyano-7-amino analogue was accomplished when 6-cyano-7-[(trifluoroacetyl)amino]-2,2-dimethylbenzopyran was used as starting material.

In a series of monosubstituted *trans*-4-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ols,[1] two structural features were found to maximize antihypertensive activity. The first of these was a strong electron-withdrawing aromatic substituent, such as nitro or cyano located at either C(6) or C(7), and the second that the 4-amino group was incorporated in a pyrrolidine or piperidine ring. An extension to that work was the synthesis of *trans*-3,4-dihydro-2,2-dimethyl-4-(1-pyrrolidinyl)-2*H*-1-benzopyran-3-ols and *trans*-3,4-dihydro-2,2-dimethyl-4-(1-piperidinyl)-2*H*-1-benzopyran-3-ols containing substituents located at both C(6) and C(7). Several disubstituted compounds were thus prepared and evaluated in the spontaneously hypertensive rat (SHR) as this model was subsequently found to be more sensitive to the monosubstituted aminobenzopyranols (**1–6**, see Table I) than the deoxycorticosterone acetate (DOCA)/saline treated hypertensive rat used in the initial study.[1] Hydralazine and nifedipine were included as standard antihypertensive agents since in the previous study[1] the monosubstituted aminobenzopyranols were shown to have a vasodilator action.

Chemistry. Convenient starting materials for the synthesis of the 6,7-disubstituted compounds described in Table II are the 2,2-dimethyl-2*H*-1-benzopyrans that stem

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