photosensitizing activities, which are given as ΔA_{440} relative to that of 8-MOP (100%) were as follows: **4a**, 86; **4d**, 170; **4h**, 150; **5c**, 68; and **5k**, 80. The values of **4a**, **5c**, and **5k** were insignificantly different from the blind values obtained upon irradiation of the solution containing no psoralens.

Acknowledgment. Photohemolysis determinations were kindly provided by M.Sci. Carsten Midskov, A/S Dumex, Copenhagen. The work was supported by a grant from the Danish Medical Research Council to J.B.H. and by a generous gift of 8-methoxypsoralen from A/S Pharma-medica, Copenhagen. P.E.N. is a recipient of a Niels Bohr Fellowship through the Egmont Foundation.

Registry No. 1, 298-81-7; **2**, 43111-03-1; **2**-T, 96616-33-0; **2**-T·$(CH_2)_6N_4$, 96632-97-2; **3**, 96616-34-1; **3**-T, 96616-35-2; **4a**, 78827-46-0; **4a**·HCl, 78827-47-1; **4a**-T, 96616-36-3; **4b**, 96616-37-4; **4b**·HCl, 96616-38-5; **4c**, 96616-39-6; **4c**-T, 96616-40-9; **4d**, 96616-41-0; **4e**, 96616-421; **4e**·HCl, 96616-43-2; **4f**, 73166-85-5; **4f**·HCl, 96616-44-3; **4g**, 96616-45-4; **4g**·HCl, 96616-46-5; **4h**, 96616-47-6; **4h**·HCl, 96616-48-7; **4i**, 73166-84-4; **4i**·HCl, 96616-49-8; **4j**, 96616-50-1; **4j**·HCl, 96616-51-2; **4l**, 43111-10-0; **4l**-T, 96616-52-3; **4m**, 50639-52-6; **4n**, 86863-32-3; **5c**, 96616-53-4; **5c**-T, 96616-54-5; **5j**, 86863-15-2; **5k**, 85079-39-6; **5p**, 96616-55-6; **6**, 86863-17-4; **7**, 96616-56-7; **8**, 96616-57-8; **10**, 43111-11-1; $ClCH_2OCH_3$, 107-30-2; $Br(CH_2)_3Br$, 109-64-8; $HO(CH_2)_3OH$, 504-63-2; 5-(azidomethyl)-8-methoxypsoralen, 78827-45-9; 5-[[(3-hydroxypropyl)oxy]methyl]-8-methoxypsoralen, 86863-32-3.

Synthesis and Biological Activity of 6-Azacadeguomycin and Certain 3,4,6-Trisubstituted Pyrazolo[3,4-*d*]pyrimidine Ribonucleosides[1,2]

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Several 3,4,6-trisubstituted pyrazolo[3,4-*d*]pyrimidine ribonucleosides were prepared and tested for their biological activity. High-temperature glycosylation of 3,6-dibromoallopurinol (**6**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose in the presence of BF_3·OEt_2, followed by ammonolysis, provided 6-amino-3-bromo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**7a**). Similar glycosylation of either 3-bromo-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidin-6-yl methyl sulfoxide (**8**) or 6-amino-3-bromopyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**11**), and subsequent ammonolysis, also gave **7a**. The structural assignment of **7a** was on the basis of spectral studies, as well as its conversion to the reported guanosine analogue **1d**. Application of this glycosylation procedure to 6-(methylthio)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**14**) gave the corresponding N-1 glycosyl derivative (**16a**). Dethiation and debenzoylation of **16a** provided an alternate route to the recently reported 3-carbamoylallopurinol ribonucleoside (**17**), thus confirming the structural assignment of **16a** and the nucleosides derived therefrom. Oxidation of **16a** and subsequent ammonolysis afforded 6-amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**15a**). Alkaline treatment of **15a** gave 6-azacadeguomycin (**18**). Acetylation of **15a**, followed by dehydration with phosgene, provided the versatile intermediate 6-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (**19**). Deacetylation of **19** gave 6-amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (**20a**). Reaction of **19** with H_2S gave 6-amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-thiocarboxamide (**20b**). All of these compounds were tested in vitro against certain viruses and tumor cells. Among these compounds, the guanosine analogues **7a** and **20a** showed significant activity against measles in vitro and were found to exhibit moderate antitumor activity in vitro against L1210 and P388 leukemia. 6-Azacadeguomycin (**18**) and all other compounds were inactive against the viruses and tumor cells tested in vitro.

Pyrazolo[3,4-*d*]pyrimidines have received renewed attention in recent years owing to the discovery of certain derivatives possessing antiparasitic activity. Since there is an absence of de novo purine biosynthesis in most parasites,[3-8] these organisms are wholly dependent on the salvage pathway for purine nucleoside metabolism and will accept certain pyrazolo[3,4-*d*]pyrimidines in place of purines.[3] Allopurinol (pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one, **1a**)[9] was the first such analogue shown to be active against several leishmania[10-14] and trypanosoma[13-15] species in vitro. In both leishmania[12] and trypanosoma[16] species, allopurinol is converted to allopurinol ribonucleoside 5′-phosphate (HPPR-MP) by a unique enzyme of the parasite, nucleoside phosphoribosyltransferase. Sequential conversion of HPPR-MP by the parasite enzymes adenylosuccinate synthetase and succino-AMP lyase gives 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP) ribonucleoside

(1) This work is taken, in part, from the Ph.D. Dissertation of H.B.C., Brigham Young University, 1983.
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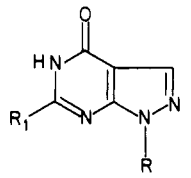
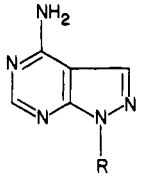
5′-phosphate, which is eventually incorporated into the cellular RNA of the parasite as the 5′-triphosphate, resulting in lethality to the parasite.[17,18] This conversion is analogous to the conversion of IMP to AMP in mammalian cells.[8,19] The incorporation of 4-APP-TP into the RNA of the parasite is unique, since mammalian cells do not show either this conversion or the incorporation of allopurinol or its metabolic products into RNA.[12,13] These unusual metabolic transformations of HPPR-MP reveal significant biochemical differences between the host and the parasite, which offer considerable potential for fruitful chemotherapeutic exploitation.[20]



1a, R=R$_1$=H
b, R=H; R$_1$=NH$_2$
c, R$_1$=H; R=β-D-ribofuranose
d, R$_1$=NH$_2$; R=β-D-ribofuranose

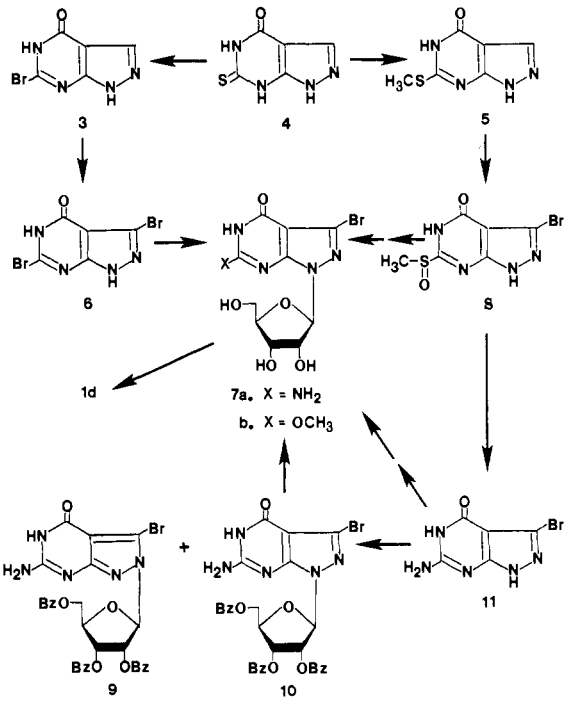


2a, R=H
b, R=β-D-ribofuranose

Recently, 4-APP (**2a**)[9] has been shown to be effective in the treatment of experimental Chagas disease in mice.[21,22] The beneficial results obtained recently with allopurinol on human cutaneous leishmaniasis suggest that this compound may be a candidate for a successful treatment of this disease.[22] Moreover, the ribonucleoside derivatives of allopurinol (**1c**) and 4-APP (**2b**) were shown to be several-fold more active than allopurinol against promastigotes of the isolates of American *Leishmania brazilienses* and *Leishmania mexicana*[23] and *Leishmania tropica*[24] in vitro.

The guanine analogue 6-aminopyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (6-aminoallopurinol, **1b**) has recently been shown to be significantly active against *T. cruzi* epimastigotes in vitro.[22] We have recently reported a convenient synthesis of 6-amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]-pyrimidin-4(5*H*)-one (**1d**)[25] and several 3-substituted allopurinol ribonucleosides.[26] As a part of this ongoing program, we have now prepared several 3-substituted 6-aminoallopurinol ribonucleosides (substituted guanosine analogues) including 6-azacadeguomycin (**18**). Cadeguomycin is a novel nucleoside antibiotic isolated[27] recently from an actinomycete strain IM7912T and identified[28,29]

Scheme I



as 2-amino-7-β-D-ribofuranosyl-4(3*H*)-oxopyrrolo[2,3-*d*]-pyrimidine-5-carboxylic acid. This antibiotic was reported[27] to have inhibitory effects on transplantable animal tumors but no significant activity against bacteria and fungi.

Chemistry. Our synthetic approach (Scheme I) starts with the readily available 6-thioxo-1,5,6,7-tetrahydropyrazolo[3,4-*d*]pyrimidin-4-one[9] (**4**) and involves a replacement of the mercapto group by a bromine atom to yield 6-bromoallopurinol (**3**). This type of reaction has previously been reported from our laboratory[30] for the synthesis of 2-bromopurines from 2-mercaptopurines. The next step involved a simple electrophilic aromatic bromination of **3** at the C-3 position to produce the 3,6-dibromopyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**6**). When a high-temperature glycosylation procedure[26] using 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose and BF_3·OEt_2 as a catalyst was applied to **6**, the guanosine analogue 6-amino-3-bromo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**7a**) was obtained in 46% yield (overall yield from **6**), after ammonolysis of the blocked intermediate. The structural assignment of **7a** was accomplished by debromination of **7a** to provide the guanosine analogue **1d**, whose structure has been established previously from our laboratory.[25]

In an effort to develop a synthetic procedure that would lead to guanosine analogues on a scale more suitable for preparative work, the use of other pyrazolo[3,4-*d*]pyrimidine bases for glycosylation studies was investigated. Thus, when 6-(methylthio)pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one[9] (**5**) was heated with bromine–water in the presence of sodium acetate (used as a buffer), 3-bromo-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidin-6-yl methyl sulfoxide (**8**) was obtained in 85% yields. Compound **8** was glycosylated by the BF_3·OEt_2-catalyzed procedure and the resulting intermediate product was deprotected by two different methods. When the intermediate was deblocked

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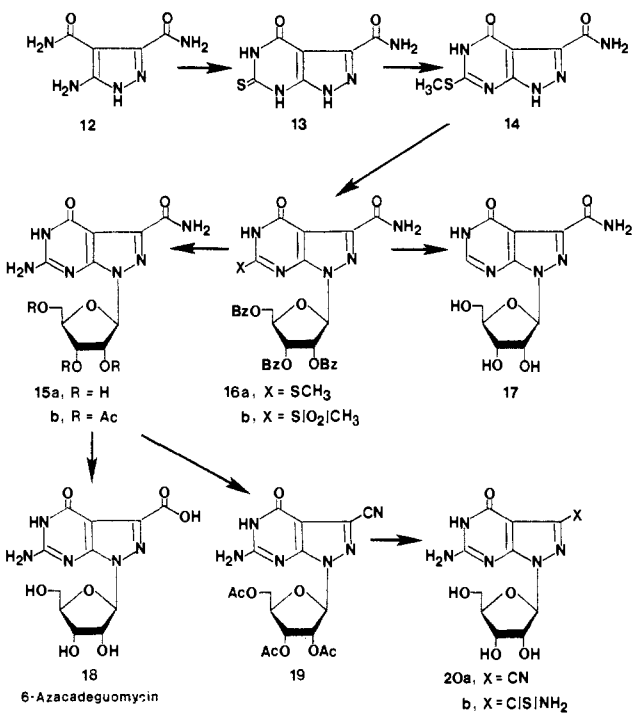
Scheme II



with CH_3OH/NH_3, compound **7a** was produced. However, treatment of the intermediate with $NaOCH_3$ in CH_3OH gave 3-bromo-6-methoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**7b**). The versatility of the methylsulfinyl group at the C-6 position for use in nucleophilic displacement reactions was thus illustrated by this deblocking study.

The other heterocycle that was used for the glycosylation studies was the substituted guanine analogue itself. Treatment of 3-bromo-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidin-6-yl methyl sulfoxide (**8**) with concentrated NH_4OH at 120 °C for 16 h gave 6-amino-3-bromopyrazolo[3,4-*d*]-pyrimidin-4(5*H*)-one (**11**). Glycosylation of **11** in the presence of $BF_3 \cdot OEt_2$ gave a mixture of two isomeric blocked nucleosides, which were separated on a flash silica gel column and identified as 6-amino-3-bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**10**) and the corresponding N-2 glycosyl isomer (**9**). Deprotection of **10** with $NaOCH_3/CH_3OH$ provided a third method of preparing the guanosine analogue **7a**.

The bromo group in **7a** was found to be quite unreactive toward conventional nucleophilic displacement reactions. Attempts to introduce a carbonitrile function directly into the position 3 of **7a** by nucleophilic displacement reactions failed. Therefore, direct glycosylation of 6-(methylthio)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**14**) was considered. Ring closure of 5-aminopyrazole-3,4-dicarboxamide[31] (**12**) with potassium ethyl xanthate gave 3-carbamoyl-6-thioxo-1,5,6,7-tetrahydropyrazolo-[3,4-*d*]pyrimidin-4-one (**13**), which on methylation with methyl iodide furnished **14** (Scheme II). Direct glycosylation of the nonsilylated **14** with the blocked benzoyl sugar in the presence of the catalyst $BF_3 \cdot OEt_2$ in a boiling polar aprotic solvent such as nitromethane gave a nucleoside product, identified as 6-(methylthio)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**16a**). No formation of other isomeric nucleosides was observed. Dethiation of **16a** with Raney nickel, followed by debenzoylation, readily gave 4(5*H*)-oxo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**17**), virtually identical with 3-carbamoyllallopurinol ribonucleoside (**17**), prepared and recently reported from our laboratory.[26] This confirmed the structural assignment of **16a** and the subsequent nucleosides derived therefrom.

Oxidation of **16a** with *m*-chloroperoxybenzoic acid in CH_2Cl_2 readily gave the corresponding methyl sulfonyl derivative **16b**. In the ^1H NMR ($CDCl_3$) of **16b**, all the protons, except benzoyl and C_4-H and C_5-H_2, were shifted downfield as compared to those of **16a**. The SO_2CH_3 protons had a considerable shift of 0.79 ppm, whereas the anomeric proton of **16b** shifted by 0.24 ppm. This downfield shift in **16b** would be expected due to the sulfonyl group. Treatment of **16b** with liquid NH_3 at 90 °C for 48 h gave a good yield of 6-amino-1-β-D-ribofuranosyl-4-(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**15a**). When compound **15a** was heated under gentle reflux in 6 N NaOH, 6-azacadeguomycin (6-amino-1-β-D-ribofuranosyl-4(5H)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxylic acid, **18**) was formed, which was isolated in more than 73% yield. Crystallization from aqueous acetic acid gave an analytical sample, and all the spectroscopic data are in agreement with the assigned structure (see Experimental Section). Further, acetylation of **15a** with acetic anhydride in the presence of 4-(dimethylamino)pyridine (DMAP) in DMF at low temperature gave the corresponding tri-*O*-acetyl derivative **15b**, which on dehydration with phosgene furnished the key intermediate 6-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (**19**).

Compound **19** was employed for further functional group transformation studies (Scheme II). Careful deacetylation of **19** with $NaOCH_3/CH_3OH$ gave 6-amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (**20a**) in excellent yield. Reaction of **19** with liquid H_2S in the presence of DMAP and subsequent deprotection gave the 3-thiocarbamoylguanosine analogue 6-amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-thiocarboxamide (**20b**).

Biological Evaluations

A. Antiviral Activity. The pyrazolo[3,4-*d*]pyrimidine derivatives synthesized during this study were tested against herpes simplex type 2 (HSV-2), vaccinia (VV), parainfluenza type 3 (Para 3), and measles viruses in vitro in parallel with ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide)[32] and selenazofurin (2-β-D-ribofuranosylselenazole-4-carboxamide)[33] (Table I). Of all the compounds tested, **7a**, **20a**, and **20b** exhibited significant potent activity and low toxicity against measles; and the activity was comparable to that of ribavirin and selenazofurin. Compound **7a** was also significantly active against VV in vitro. The aglycon **14** showed marked activity against HSV-2 and moderate activity against Para 3, measles, and VV. Similarly, **20a** exhibited moderate activity against Para 3, VV, and HSV-2 without appreciable toxicity. Compounds **6**, **7a**, **13**, and **15a** all showed moderate activity against HSV-2 in vitro, as did ribavirin. The moderate activity of **3**, **6**, and **8** against VV is accompanied by slight toxicity, whereas **13** and **15a** were nontoxic. Because of the low solubility, compounds **9** and **11** could

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Table I. Comparative in Vitro Antiviral Activity of Ribavirin, Selenazofurin, and Certain 3,4,6-Trisubstituted Pyrazolo[3,4-*d*]pyrimidine Derivatives

compd	ED_{50},[a] M				toxic level, M
	para 3	measles	VV	HSV-2	
1d	$>5.0 \times 10^{-3}$	*b*	1.0×10^{-3}	1.6×10^{-3}	none
3	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	4.3×10^{-4}	1.6×10^{-3}	1.6×10^{-3}
6	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	2.4×10^{-4}	$>5.0 \times 10^{-4}$	5.0×10^{-3}
8	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	2.6×10^{-4}	1.2×10^{-3}	5.0×10^{-4}
7a	$>5.0 \times 10^{-3}$	2.0×10^{-5}	7.6×10^{-5}	1.8×10^{-4}	1.6×10^{-3}
7b	$>2.5 \times 10^{-3}$	$>2.5 \times 10^{-3}$	3.5×10^{-3}	1.6×10^{-3}	2.5×10^{-3}
13	3.9×10^{-4}	$>5.0 \times 10^{-4}$	$>5.0 \times 10^{-4}$	1.8×10^{-4}	none
14	$>5.0 \times 10^{-4}$	$>5.0 \times 10^{-4}$	3.3×10^{-4}	8.5×10^{-5}	5.0×10^{-4}
15a	$>5.0 \times 10^{-4}$	$>5.0 \times 10^{-4}$	$>5.0 \times 10^{-4}$	$>5.0 \times 10^{-4}$	none
18	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	none
20a	2.2×10^{-4}	5.4×10^{-5}	1.4×10^{-4}	1.0×10^{-4}	none
20b	5.9×10^{-4}	6.5×10^{-5}	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	none
ribavirin[32]	1.6×10^{-4}	3.3×10^{-5}	8.5×10^{-5}	1.9×10^{-4}	none
selenazofurin[33]	3.2×10^{-6}	3.2×10^{-6}	9.8×10^{-6}	1.4×10^{-5}	none

[a] The concentration of compound that resulted in a 50% reduction of viral CPE as compared with nondrug controls. [b] Not tested.

not be tested. 6-Azacadeguomycin (**18**) and all other compounds were inactive against the viruses used. As a result of these in vitro antiviral studies, compounds **7a** and **20a** appear to be the potent agents against measles.

B. Antitumor Activity. The 3,4,6-trisubstituted pyrazolo[3,4-*d*]pyrimidine derivatives synthesized during this study were tested against L1210 and P388 leukemia in vitro (Table II). The guanosine analogues **7a** and **20a** were found to be moderately active against these cell lines and other compounds under study were devoid of antitumor activity in vitro.

The new compounds synthesized during this study did not show significant antileishmanial activity in vitro.

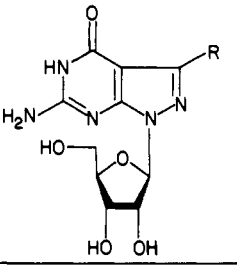
Experimental Section

General Procedures. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H NMR) spectra were determined at 89.6 MHz with a JEOL FX 90Q spectrometer. The chemical-shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of solvent as indicated by elemental analysis was verified by ^1H NMR. Infared spectra (IR) were obtained on a Beckman Acculab 2 spectrophotometer and ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Robertson Labs, Florham Park, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM Reagents). E. Merek silica gel (230–400 mesh) was used for flash column chromatography. Preparative liquid chromatography (LC) was run on the Waters Prep 500 LC system. 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.

6-Bromopyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (6-Bromoallopurinol, 3). Finely ground 6-thioxo-1,5,6,7-tetrahydropyrazolo[3,4-*d*]pyrimidin-4-one[9] (**4**; 5.0 g, 30 mmol) was combined with 48% aqueous HBr (50 mL) and the suspension was cooled to 5 °C. Bromine (16.0 g, 100 mmol) was then added dropwise over a period of 45 min while the temperature was maintained between 0 and 10 °C. The resulting thick yellow suspension was stirred for 90 min (0–5 °C) and then stored in the freezer (–10 °C) overnight. The beige suspension was filtered cold and the filter cake was dissolved in aqueous NaOH (1 N, 150 mL) and the pH adjusted to 3 with HBr. The resulting colorless precipitate was collected, washed with cold water (3 × 10 mL), and air-dried to yield 3.0 g of impure material, which was purified by continuous extraction with EtOAc to obtain 2.0 g of pure material.

The original filtrate from the reaction mixture was cooled to –20 °C and neutralized with 50% NaOH, and after the pH was adjusted to 3 with HBr, there was obtained 1.5 g of pure **3**. The yield of combined pure material was 54.7%; mp >300 °C; IR (KBr) ν 1690 (C=O) cm^{-1}; UV λ_{max} (pH 1) 252 nm (ϵ 6600); UV λ_{max} (pH 7 and 11) 260 nm (ϵ 7300), isosbestic point λ_{max} 244 nm (ϵ 6000); ^1H NMR (Me_2SO-d_6) δ 8.20 (s, 1, C_3-H). Anal. ($C_5H_3BrN_4O$) C, H, N, Br.

Table II. Comparative in Vitro Antitumor Activity of Allopurinol-3-thiocarboxamide Ribonucleoside and Certain 3-Substituted 6-Amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]-pyrimidin-4(5*H*)-ones



compd	R	ID_{50},[a] M	
		L1210	P388
1d	H	*b*	*b*
7a	Br	3.6×10^{-5}	3.6×10^{-5}
15a	$CONH_2$	30% inhibn at 10^{-4}	22% inhibn at 10^{-4}
18	COOH	*b*	*b*
20a	CN	2.2×10^{-5}	2.1×10^{-5}
20b	$C(=S)NH_2$	43% inhibn at 10^{-4}	*b*
allopurinol-3-thiocarboxamide ribonucleoside[2b]		3.6×10^{-7}	6.0×10^{-7}

[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] Compounds **1d** and **18**, as well as **3**, **6**, **7a**, **8**, **9**, **11**, **13**, and **14**, are inactive at 10^{-4} M.

3,6-Dibromopyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (6). Finely ground **3** (1.38 g, 6.4 mmol) was suspended in water (75 mL) and bromine (2 mL, 40 mmol) was added. The suspension was stirred at room temperature for 1 h and then heated on a steam bath for 1 h with occasional stirring. On cooling, a fluffy white solid separated, which was collected by filtration and dried to yield 1.62 g (86%) of **6**; mp >300 °C; IR (KBr) ν 1690 (C=O) cm^{-1}; UV λ_{max} (pH 1) 215 nm (ϵ 18 500), 252 (6900); UV λ_{max} (pH 7) 223 nm (sh) (ϵ 11 900), 267 (6600); UV λ_{max} (pH 11) 229 nm (sh) (ϵ 13 400), 253 (sh) (6300), 275 (sh) (4100). Anal. ($C_5H_2Br_2N_4O \cdot H_2O$) C, H, N, Br.

3-Bromo-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidin-6-yl Methyl Sulfoxide (8). 6-(Methylthio)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one[9] (**5**; 1.0 g, 5.5 mmol) was suspended in water (75 mL) to which was added sodium acetate trihydrate (2 g) and then bromine (3 mL, 60 mmol). The reaction mixture was warmed to 40–50 °C for 30 min and then cooled in ice–water. The filtered product was washed with water (3 × 25 mL) and dried to yield 1.3 g (85%) of the title compound. A small amount was crystallized from water for analytical purposes; mp >300 °C; IR (KBr)

ν 1075 (S=O), 1670–1690 (C=O) cm^{-1}; UV λ_{max} (pH 1 and 7) 268 nm (sh) (ϵ 5800); UV λ_{max} (pH 11) 235 nm (sh) (ϵ 12200), 285 (sh) (3300); ^1H NMR (Me_2SO-d_6) δ 3.01 (s, 3, $SOCH_3$), 10.86 (s, 1, N_1-H), 11.59 (s, 1, N_5-H). Anal. ($C_6H_5BrN_4O_2S$) C, H, N, Br, S.

6-Amino-3-bromopyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (11). Compound 8 (2.0 g, 7.2 mmol) was combined with concentrated aqueous NH_4OH (75 mL) and heated in a steel bomb at 120 °C for 16 h. The contents were transferred to a flask and boiled to remove dissolved NH_3, and then the pH of the solution was adjusted to 5 with glacial acetic acid. After the solution cooled for (0–5 °C) several h, the white solid that separated was collected, washed with water (2 × 25 mL), and dried to yield 1.22 g of 11 (73%); mp >300 °C; IR (KBr) ν 1690 (C=O), 3140–3360 (NH_2, NH) cm^{-1}; UV λ_{max} (pH 1) 218 nm (ϵ 21400), 243 (9900); UV λ_{max} (pH 7) 220 nm (ϵ 21900), 247 (10100); UV λ_{max} (pH 11) 255 nm (ϵ 7900). Anal. ($C_5H_4BrN_5O$) C, H, N, Br.

6-Amino-3-bromo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (7a). Method 1. Compound 6 (1.5 g, 5.1 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (3.9 g, 7.7 mmol) were combined with dry CH_3NO_2 (100 mL) and brought to reflux. To this suspension was added freshly distilled BF_3·OEt_2 (2.13 g, 1.9 mL, 7.7 mmol) through the condenser by syringe. The resulting clear solution was refluxed for 20 min and then evaporated to dryness. The residue was dissolved in EtOAc (200 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 100 mL), followed by water (2 × 100 mL). After drying over Na_2SO_4, the solvent was evaporated to yield 5.1 g of an amber foam, which was used directly for amination-deblocking reaction. This was accomplished by combining the above foam (5.0 g) with methanolic ammonia (75 mL, saturated at 0 °C) and heating in a steel bomb for 16 h at 120 °C. The contents of the bomb, after cooling, yielded a brown solid, which was crystallized from aqueous EtOH to give an off-white solid, 0.85 g (46% based on 6); mp 255 °C dec; IR (KBr) ν 1685 (C=O) cm^{-1}; UV λ_{max} (pH 1 and 7) 252 nm (ϵ 11400); UV λ_{max} (pH 11) 261 nm (ϵ 9200); ^1H NMR (Me_2SO-d_6) δ 5.82 (d, 1, J = 4.75 Hz, C_1-H), 6.85 (s, 2, NH_2), 10.77 (br s, 1, N_5-H), and other sugar protons. Anal. ($C_{10}H_{12}BrN_5O_5$) C, H, N, Br.

Method 2. 3-Bromo-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidin-6-yl methyl sulfoxide (8; 1.0 g, 3.6 mmol) was suspended in CH_3NO_2 (100 mL) and brought to reflux. BF_3·OEt_2 was added by syringe until all the heterocycle dissolved (required 3 mL, 12.2 mmol). The benzoyl blocked sugar (2.72 g, 5.4 mmol) was added and the reaction mixture was refluxed for 30 min and worked up as described in method 1 to yield 3.0 g of a two-component mixture as a syrup. The crude material was purified by flash column chromatography on silica gel with 2% CH_3OH in CH_2Cl_2 (v/v). The first nucleoside material to be eluted was found to be unstable to deblocking conditions with either sodium methoxide or ammonia and amounted to 0.90 g. The second product eluted to yield 1.0 g of a colorless syrup, which was deblocked with CH_3OH/NH_3 as described in method 1 above. The crude product was crystallized from aqueous EtOH to yield 0.20 g (15% based on 8) of analytically pure 7a; mp 254 °C dec. This compound was identical in all respects with **7a** prepared by method 1.

Method 3. Compound 11 (1.0 g, 4.35 mmol) was combined with benzoyl-blocked sugar (3.3 g, 6.52 mmol) in dry CH_3NO_2 (100 mL) and brought to reflux temperature. BF_3·OEt_2 (0.93 g, 0.82 mL, 6.52 mmol) was added by syringe and the mixture was refluxed for 1 h. After workup as described in method 1, the residual amber foam (3.2 g) was applied to a flash silica gel column and eluted with CH_2Cl_2/CH_3OH (20:1, v/v). The first nucleoside product to elute crystallized from the fractions as a colorless solid (1.3 g, 44%); mp 300 °C dec. This product was identified as the N-2 glycosyl isomer 6-amino-3-bromo-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (9); IR (KBr) ν 705 (CBr), 1690 (C=O of heterocycle), 1725 (C=O of benzoyl esters), 3200–3400 (NH) cm^{-1}; UV λ_{max} (pH 1) 228 nm (ϵ 37800); UV λ_{max} (EtOH) 228 nm (ϵ 54000), 257 (sh) (4700); UV λ_{max} (pH 11) 252 nm (sh) (ϵ 8800), 285 (sh) (4700). Anal. ($C_{31}H_{24}BrN_5O_8$) C, H, N, Br.

The second product to elute yielded 0.80 g of a colorless syrup (10), which was deblocked by dissolving in CH_3OH (50 mL) and adding commercial $NaOCH_3$ until pH 10 and allowing the solution to stir for 16 h at room temperature. The solution was neutralized with glacial acetic acid (to pH 5), adsorbed onto silica gel, and subjected to silica gel flash chromatography with EtOAc/EtOH (5:1, v/v) to give pure **7a** (0.30 mg, 19% based on 11); mp 255 °C dec. This material was found to be identical with that prepared by method 1.

3-Bromo-6-methoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (7b). A portion of the second blocked nucleoside intermediate product of reaction 8 (method 2) (1.0 g, 1.4 mmol) was dissolved in CH_3OH (50 mL) and $NaOCH_3$ was added to pH 10. The mixture was stirred for 16 h at room temperature and neutralized with Dowex-50 H^+ resin and the solvent evaporated. The residue was crystallized from CH_3OH to yield 0.24 g (45%) of **7b**; mp 235 °C dec; IR (KBr) ν 1685 (C=O), 3400 (OH, NH) cm^{-1}; UV λ_{max} (pH 1 and 7) 246 nm (ϵ 8900); UV λ_{max} (pH 11) 260 nm (ϵ 9200); ^1H NMR (Me_2SO-d_6) δ 4.02 (s, 3, OCH_3), 6.10 (d, 1, J = 4.23 Hz, C_1-H), and other sugar protons. Anal. ($C_{11}H_{13}BrN_4O_6$) C, H, N, Br.

6-Amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (1d). To a solution of **7a** (0.50 g, 1.38 mmol) in 50% aqueous EtOH (50 mL) was added Pd/C (5%, 50 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 water gave white needles, 0.30 g (77%); mp 264–265 °C. This compound was identical in all respects with **1d**, prepared and reported recently from our laboratory.[25]

3-Carbamoyl-6-thioxo-1,5,6,7-tetrahydropyrazolo[3,4-*d*]pyrimidin-4-one (13). A mixture of 5-aminopyrazole-3,4-dicarboxamide[31] (12; 2.0 g, 12 mmol) and potassium ethyl xanthate (3.8 g, 24 mmol) in DMF (180 mL) was heated under reflux for 2 h. The reaction mixture was cooled in an ice bath and the precipitated product was collected by filtration. The solid was dissolved in 1 N NaOH, treated with charcoal, and filtered and the filtrate acidified with AcOH. The light yellow solid was collected, washed with cold water (3 × 25 mL), and dried (at 100 °C under vacuum) to yield 2.1 g (82%) of the title compound; mp >300 °C; IR (KBr) ν 1250 (C=S) 1600, 1650 (C=O), cm^{-1}; UV λ_{max} (pH 1) 272 nm (ϵ 31600); UV λ_{max} (pH 7) 240 nm (ϵ 21000), 284 (30000); UV λ_{max} (pH 11) 264 nm (ϵ 29600); ^1H NMR (Me_2SO-d_6) δ 8.16 and 9.12 (2 br s, $CONH_2$), 12.45 (br s, 2 NH). Anal. ($C_6H_5N_5O_2S$·$^1/_4H_2O$) C, H, N, S.

6-(Methylthio)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (14). To a solution of 13 (2.1 g, 10 mmol) in 1 N NaOH (50 mL) was added methyl iodide (1.7 g, 12 mmol) and the mixture was stirred vigorously for 2 h at room temperature. The precipitate that separated on acidification (AcOH, pH 5) was collected by filtration, washed with cold water (2 × 50 mL), and dried to yield 2.1 g (93%); mp >300 °C; IR (KBr) ν 1360 (SCH_3), 1650, 1680 (C=O) cm^{-1}; UV λ_{max} (pH 1) 238 nm (ϵ 19800), 266 (14500); UV λ_{max} (pH 7) 239 nm (ϵ 19300), 265 (13200); UV λ_{max} (pH 11) 249 nm (ϵ 22500), 283 (sh) (8700); ^1H NMR (Me_2SO-d_6) δ 2.59 (s, 3, SCH_3), 7.89 and 9.38 (2 br s, $CONH_2$), 12.81 and 14.31 (2 br s, NH). Anal. ($C_7H_7N_5O_2S$) C, H, N, S.

6-(Methylthio)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (16a). In the same manner as for **7a**, the title compound was prepared with use of 14 (10.0 g, 44 mmol), 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (33.0 g, 67 mmol) and BF_3·OEt_2 (3 mL) in CH_3NO_2 (200 mL). The product was purified on a Prep LC system using 25% acetone in toluene as the eluting solvent. The unreacted sugar eluted first, followed by **16a**, yield 21.1 g (66%); mp 131–132 °C (sinters), >160 °C dec; IR (KBr) ν 1345 (SCH_3), 1670, 1710 (C=O) cm^{-1}; UV ν_{max} (MeOH) 230 nm (ϵ 62000), 273 (16100); ^1H NMR ($CDCl_3$) δ 2.63 (s, 3, SCH_3), 6.69 (d, 1, J = 2.5 Hz, C_1-H), 7.40 and 7.95 (m, 17, 3 COC_6H_5, $CONH_2$), 10.0 (br s, 1, N_5-H), and other sugar protons. Anal. ($C_{33}H_{27}N_5O_9S$) C, H, N, S.

4(5*H*)-Oxo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-3-carboxamide (17). A mixture of **16a** (0.67 g, 1 mmol) and Raney nickel (W-4, 1.5 g, wet weight) in EtOH (25 mL) was heated under reflux for 24 h. The mixture was filtered hot through a Celite pad to remove the catalyst, which was washed with hot EtOH (3 × 10 mL). The combined filtrate and washings were evaporated to dryness. The residue was dissolved in MeOH (50 mL) and adjusted to pH 10 with NaOMe. After stirring at room temperature for 24 h, the solution was neutralized with Dowex-50 H^+ resin. The mixture was filtered and the filtrate was evaporated to dryness. The residue was suspended in hot MeOH (15 mL)

and water was added dropwise until all the material dissolved. After the solution was allowed to stand overnight, a crystalline solid formed: overall yield of analytically pure 17, 0.24 g (78%); mp >200 °C dec. This compound was identical in all respects with 17, prepared and reported by us previously.[26]

6-(Methylsulfonyl)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (16b). To a solution of **16a** (2.0 g, 3 mmol) in CH_2Cl_2 (50 mL) was added *m*-chloroperoxybenzoic acid (2.1 g, 12 mmol). After stirring at room temperature for 18 h, the solution was evaporated to dryness. The residue was triturated with ether (3 × 50 mL) and the ether-insoluble solid was crystallized from a mixture of EtOH/hexane to yield 1.95 g (93%) of the title compound; mp 115–116 °C (sinters), >150 °C dec; IR (KBr) ν 1120 and 1320 (SO_2CH_3), 1730 (C=O) cm^{-1}; UV λ_{max} (MeOH) 229 nm (ε 63 100), 282 (14 700); ^1H NMR ($CDCl_3$) δ 3.42 (s, 3, SO_2CH_3), 6.93 (d, 1, J = 2.5 Hz, C_1'-H), 7.41 and 8.01 (m, 17, 3 COC_6H_5, $CONH_2$), and other sugar protons. Anal. ($C_{33}H_{27}N_5O_{11}S\cdot H_2O$) C, H, N, S.

6-Amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (15a). A mixture of **16b** (6.5 g, 9 mmol) and liquid NH_3 (75 mL) was heated in a steel bomb at 90 °C for 48 h. The bomb was cooled in a dry ice–acetone bath and the NH_3 was allowed to evaporate. The residual solid was suspended in MeOH (50 mL), boiled for a few minutes, and then filtered. The solid was again suspended in 0.1 N AcOH (50 mL), boiled for a few minutes, and filtered to give 2.6 g (86%) of off-white solid: mp >300 °C; IR (KBr) ν 1675 (C=O), 3200–3400 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1) 230 nm (ε 21 900), 254 (sh) (10 800), 282 (5200); UV λ_{max} (pH 7) 230 nm (ε 22 500), 254 (sh) (10 500), 284 (5400); UV λ_{max} (pH 11) 230 nm (ε 26 600), 254 (sh) (9500), 286 (6850); ^1H NMR (Me_2SO-d_6) δ 3.38 (s, H_2O), 5.92 (d, 1, J = 4.5 Hz, C_1'-H), 6.92 (br s, 2, NH_2), 7.74 and 9.51 (2 br s, $CONH_2$), 11.23 (br s, 1, N_5-H), and other sugar protons. Anal. ($C_{11}H_{14}N_6O_6\cdot{}^1/_4H_2O$) C, H, N.

6-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxamide (15b). A mixture of **15a** (1.0 g, 3 mmol), acetic anhydride (10 mL), and DMAP (50 mg) in dry DMF (50 mL) was stirred for 8 h, below 10 °C. Methanol (25 mL) was added and the mixture was evaporated to dryness and the residue was purified on a flash silica gel column (2 × 25 cm) with $CHCl_3/CH_3OH$ (6:1) as the solvent to yield 1.20 g (86%) of the title compound; mp >260 °C dec; IR (KBr) ν 1670 and 1740 (C=O), 3330 (NH_2) cm^{-1}; UV λ_{max} (MeOH) 227 nm (ε 19 000), 258 (10 400), 284 (sh) (5200); ^1H NMR (Me_2SO-d_6) δ 2.10 and 2.13 (2 s, 9, 3 $COCH_3$), 3.37 (s, H_2O), 6.21 (d, 1, J = 2.5 Hz, C_1'-H), 7.04 (br s, 2, NH_2), 7.83 and 9.52 (2 br s, $CONH_2$), 11.35 (br s, 1, N_5-H), and other sugar protons. Anal. ($C_{17}H_2N_6O_9$) C, H, N.

6-Amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxylic Acid (6-Azacadeguomycin, 18). A solution of **15a** (0.50 g, 1.5 mmol) in 6 N NaOH (20 mL) was heated under gentle reflux for 30 min. The reaction mixture was cooled to room temperature and then poured over crushed ice (50 g). The resultng solution was neutralized with Dowex-50 H^+ resin, filtered, and lyophilized. The resulting solid was crystallized from aqueous acetic acid to yield 0.36 g (73%) of the title compound; mp 271 °C dec; IR (KBr) ν 1660, 1670 (C=O, COOH), 3320–3400 (OH, NH_2) cm^{-1}; UV λ_{max} (pH 1) 227 nm (ε 16 300), 255 (sh) (8300), 286 (4600); UV λ_{max} (pH 7) 222 nm (sh) (ε 15 500), 255 (11 300); UV λ_{max} (pH 11) 220 nm (sh) (ε 24 700), 263 (8800); ^1H NMR (Me_2SO-d_6) δ 5.92 (d, 1, J = 4.5 Hz, C_1'-H), 7.08 (br s, 2, NH_2), 12.26 (br s, 1, N_5-H), 13.15 (br s, 1, COOH), and other sugar protons. Anal. ($C_{11}H_{13}N_5O_7$) C, H, N.

6-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (19). To an ice-cold solution of **15b** (0.30 g, 0.7 mmol) in CH_2Cl_2 (25 mL) and pyridine (5 mL) was added 12% phosgene in benzene (0.83 g, 1 mmol), slowly with stirring. After the addition was complete (15 min), the reaction mixture was allowed to warm to room temperature. After stirring at room temperature for 2 h, the solution was poured onto ice (100 g) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with water (2 × 20 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was purified on a silica gel column with acetone/hexane (1:1 v/v) as the solvent. The homogeneous product was crystallized from CH_3OH to yield 0.25 g (87%) of the title compound; mp 250–255 °C dec; IR (KBr) ν 1690 and 1740 (C=O), 2240 (C≡N), 3350 (NH_2) cm^{-1}; UV λ_{max} (MeOH) 226 nm (ε 32 800), 261 (19 300), 284 (10 900); ^1H NMR (Me_2SO-d_6) δ 2.023, 2.068, and 2.095 (3 s, 9, 3 $COCH_3$), 3.32 (s, H_2O), 6.15 (d, 1, J = 2.5 Hz, C_1'-H), 7.10 (br s, 2, NH_2), 11.23 (br s, 1, N_5-H), and other sugar protons. Anal. ($C_{17}H_{18}N_6O_8$) C, H, N.

6-Amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-carbonitrile (20a). To a solution of **19** (0.43 g, 1 mmol) in CH_3OH (25 mL) was added $NaOCH_3$ 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 isopropyl alcohol gave 2.60 g (83%) of analytically pure **20a**; mp >235 °C dec; IR (KBr) ν 1690 (C=O), 2260 (C≡N), 3350–3420 (OH, NH_2) cm^{-1}; UV λ_{max} (pH 1) 230 nm (ε 20 900), 259 (10 800), 285 (6500); UV λ_{max} (pH 7) 230 nm (ε 21 400), 259 (10 500), 285 (6800); UV λ_{max} (pH 11) 230 nm (30 200), 255 (7100), 292 (8600); ^1H NMR (Me_2SO-d_6) δ 5.90 (d, 1, J = 2.5 Hz, C_1'-H), 7.04 (br s, 2, NH_2), 11.10 (br s, 1, N_5-H), and other sugar protons. Anal. ($C_{11}H_{12}N_6O_5\cdot{}^1/_4H_2O$) C, H, N.

6-Amino-1-β-D-ribofuranosyl-4(5*H*)-oxopyrazolo[3,4-*d*]pyrimidine-3-thiocarboxamide (20b). A mixture of **19** (0.43 g, 1 mmol), liquid H_2S (15 mL), and DMAP (75 mg) was heated in a steel bomb at 80 °C for 60 h. The bomb was cooled and the H_2S allowed to evaporate. The residual solid was chromatographed on a flash silica gel column (2 × 20 cm) with $CHCl_3/CH_3OH$ (9:1, v/v) as eluent. The appropriate fractions were pooled and evaporated to dryness. A solution of the residue in CH_3OH (25 mL) was adjusted to pH 9 with CH_3ONa. After stirring for 18 h at room temperture, the reaction mixture was neutralized with Dowex-50 H^+ resin. The resin was removed by filtration and the filtrate evaporated to dryness. The residual solid was crystallized from water to give 0.17 g (49.6%); mp >250 °C dec; IR (KBr) ν 1250 (C=S), 1625 (C=O) 3360 (OH, NH_2) cm^{-1}; UV λ_{max} (pH 1) 246 nm (ε 13 100), 295 (4800); UV λ_{max} (pH 7) 246 nm (ε 12 400), 295 (4400); UV λ_{max} (pH 11) 234 nm (ε 10 300), 243 (sh) (9600), 293 (5600); ^1H NMR (Me_2SO-d_6) δ 5.94 (d, 1, J = 4.5 Hz, C_1'-H), 6.96 (br s, 2, NH_2), 10.09 and 11.24 (2 s, 2, $CSNH_2$), and other sugar protons. Anal. ($C_{11}H_{14}N_6O_5S$) C, H, N, S.

Antiviral Evaluation. Test compounds were evaluated for their ability to inhibit virus-induced cytopathic effect (CPE) produced by measles, herpes simplex virus type 2 (HSV-2,233), vaccinia virus (VV), and parainfluenza virus type 3 (para-3) in African green monkey (Vero) cells (American Type Culture Collection, Rockville, MD). Vero cells were maintained in antibiotic free Eagle minimum essential medium (EMEM) with Earle's salts supplemented with 10% heat inactivated newborn bovine serum (Grand Island Biolgocial Co., Grand Island, NY). For antiviral experiments, cells were inoculated into 96-well tissue culture plates (Corning Glassworks, Corning, NY) at a concentration of 4×10^4 cells/0.2 mL per well and cultured for 24 h at 37 °C in 5% CO_2 to confluency.

Monolayers were inoculated with a predetermined number of $TCID_{50}$ (50% tissue culture infective dose) units of virus that will produce complete destruction of the cell monolayer in 72 h. The number of $TCID_{50}$ units in 0.1 mL/well were as follows: measles, 80; HSV-2, 100; VV, 200; para-3, 60. After 30-min adsorption at 37 °C, test compounds were added (0.1 mL/well) in seven 0.5 log concentrations ranging from 1×10^{-5} to 1×10^{-2} M, resulting in final well concentrations of 5×10^{-6} to 5×10^{-3} M. At each concentration, duplicate wells were used for evaluation of antiviral activity and single uninfected wells for cytotoxicity evaluation.

The degree of inhibition of viral-induced CPE and compound cytotoxicity were observed microscopically after 72-h incubation at 37 °C in 5% CO_2. CPE was scored numerically from 0 (normal control cells) to 4 (100% cell destruction as in virus controls) and the dose of test compound that inhibits viral CPE by 50% (ED_{50}) was calculated.

Antitumor Evaluation. Compounds were evaluated for their ability to inhibit growth of murine leukemia L1210 and lymphoid neoplasm P388 (American Type Culture Collection, Rockville, MD) maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Grand Island Biological Co., Grand Island, NY) and 20 mM Hepes buffer. For growth experiments, cells were adjusted to 1×10^5 cells/mL and distributed into 13 × 100 mm

culture tubes (1 mL/tube). Test compounds were dissolved in growth medium, sterilized by passage through a 0.22-μm membrane filter and added to tubes of cells (1 mL/tube). Compounds were tested in duplicate at log concentrations ranging from 1×10^{-7} to 1×10^{-4} M. Following 48-h incubation at 37 °C, cell counts were determined with a Coulter Model ZF cell counter. Cell growth in the presence of test compounds was expressed as a percentage of growth in untreated control tubes and the concentration of compound producing 50% inhibition of cell growth was determined (ID_{50}).

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Registry No. **1d**, 85426-74-0; **3**, 96555-35-0; **4**, 24521-76-4; **5**, 5334-26-9; **6**, 96555-36-1; **7a**, 96555-37-2; **7b**, 96555-38-3; **8**, 96555-39-4; **9**, 96555-40-7; **10**, 96555-41-8; 11, 96575-35-8; **12**, 90586-01-9; **13**, 96555-42-9; 14, 96555-43-0; **15a**, 96555-44-1; **15b**, 96555-45-2; **16a**, 96555-46-3; **16b**, 96555-47-4; 17, 90914-46-8; 18, 96555-48-5; **19**, 96575-36-9; **20a**, 96555-49-6; **20b**, 96555-50-9; 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose, 6974-32-9.

Syntheses and Evaluation as Antifolates of MTX Analogues Derived from 2,ω-Diaminoalkanoic Acids

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Methotrexate (MTX) analogues **27a–c** bearing 2,ω-diaminoalkanoic acids (ornithine and its two lower homologues) in place of glutamic acid were synthesized by routes proceeding through N^2-[4-(methylamino)benzoyl]-N^ω-[(1,1-dimethylethoxy)carbonyl]-2,ω-diaminoalkanoic acid ethyl esters (**12a,b**) and N^2-[4-(methylamino)benzoyl]-N^5-[(1,1-dimethylethoxy)carbonyl]-2,5-diaminopentanoic acid (**13**) followed by alkylation with 6-(bromomethyl)-2,4-pteridinediamine hydrobromide. Reactions at the terminal amino group of **27**-type analogues or of appropriate precursors led to other MTX derivatives whose side chains terminate in ureido (**23a,b**), methylureido (**24**), *N*-methyl-*N*-nitrosoureido (**30**), *N*-(2-chloroethyl)-*N*-nitrosoureido (**31**), and 4-chlorobenzamido (**28a–c**) groups. Also prepared were unsymmetrically disubstituted ureido types resulting from addition of ethyl isocyanatoacetate and diethyl 2-isocyanatoglutarate to the ethyl esters of **27a,b**. Of these ureido adducts (**32a,b** and **33a,b**, respectively), only **33a** was successfully hydrolyzed to the corresponding pure acid, in this instance the tricarboxylic acid **34**, a pseudo-peptide analogue of the MTX metabolite MTX-γ-Glu. Biological evaluations of the prepared compounds affirmed previous findings that the γ-carboxyl is not required for tight binding to dihydrofolate reductase (DHFR) but is operative in the carrier-mediated transport of classical antifolates through cell membranes. High tolerance levels observed in studies against L1210 leukemia in mice suggest the reduced potency may be due not only to lower transport efficacy but also to loss of the function of intracellular γ-polyglutamylation. The *N*-nitrosoureas **30** and **31** showed appreciable activity in vivo vs. L1210, but the activity did not appear to be due to antifolate action as evidenced by their poor inhibition of both L1210 DHFR and cell growth in vitro.

The venerable anticancer agent methotrexate (MTX) remains the only folic acid antimetabolite currently in established clinical use. Continuing studies of structure–activity relationships have produced information on modifications that may be made on the MTX structure that have little or no effect on inhibition of the intracellular target enzyme [dihydrofolate reductase (DHFR), EC 1.5.1.3] while, in some instances, favorably influencing aspects of membrane transport in normal vs. malignant mammalian cells.[1-10] Such studies have led to promising new MTX analogues now in clinical trials.[7-10]

The findings that MTX analogues modified in the region of the γ-carboxyl group still bind strongly to DHFR[11,12] prompted interest in analogues in which glutamic acid has been replaced by 2,ω-diaminoalkanoic acids (as in structural type **27** of Scheme III). The terminal amino group of these analogues allows opportunity to prepare varied derivatives. Rosowsky et al. prepared the lysine analogue of MTX via coupling of 4-[[(2,4-diamino-6-pteridinyl)-methyl]methylamino]benzoic acid with an appropriately protected derivative of the diamino acid.[13] Kempton et al. prepared both the ornithine and lysine analogues in similar fashion.[14] The products from the coupling reaction were subsequently deprotected and then converted to N^ω-substituted derivatives for further studies. Rosowsky et al. converted the lysine analogue to its N^6-iodoacetyl derivative for study as a potential active site directed irreversible inhibitor[13] and also added fluorescein isothiocyanate to the N^6-amino group to give a fluorescent de-

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