a gift from J. Prior. dUMP, FdUMP, and *dl*,L-H_4folate were the preparations previously described.[18,23] *Ara*-U, *ara*-T, *ara*-5-EtU, and corresponding 5′-monophosphates were prepared according to the method of Nakayama et al.[32] *Ara*-FU and 2,2′-anhydro-*ara*-FU were prepared as reported.[36] *Ara*-FUMP was prepared by phosphorylation and hydrolysis of 2,2-anhydro-*ara*-FUMP:[32] 2,2′-anhydro-5-fluorouridine (20 mg) in 1 mL of acetonitrile was treated with tetrachloropyrophosphate (66 μL) at room temperature for 10 h. The acetonitrile was evaporated in vacuo and water (ca. 1 mL) was added to the residue. The aqueous solution was heated at 90 °C for 3 h and was concentrated by evaporation under reduced pressure. The concentrated solution was applied to Toyo filter paper (Toyo 52) and developed with the solvent system 1-propanol–concentrated NH_4OH–0.2 M boric acid, 6:1:3. The UV-absorbing band (R_f 0.25) corresponding to *ara*-FUMP was excised and eluted by water: yield 300 ODU (41%); UV λ_{max} (0.1 N HCl) 270 nm [ϵ(P) = 9400]; paper chromatography R_f 0.28 (isobutyric acid–0.5 M ammonia water, 66:34), 0.25 (1-propanol–concentrated NH_4OH–0.2 M boric acid, 6:1:3); paper electrophoresis, mobility of UMP, 6.5 cm; *ara*-FUMP, 6.5 cm (600 V, 40 min, 30 mM sodium acetate, pH 4.0); CD (molecular ellipticity) +22 600 (271 nm). The product was converted to *ara*-FU upon treatment with alkaline phosphatase, and was resistant to P_1 nuclease–3′-nucleotidase. HPLC using Lichrosorb C_{18} (4.0 × 250 mm) verified the purity of all compounds used in this study. For nucleosides, the eluant was 1% CH_3CN in water; for nucleotides, 5 mM $Bu_4N^+HSO_4^-$, 5 mM $KHPO_4$ (pH 7.1) containing 1% MeOH was used. Using these systems, arabinofuranosyl analogues are well separated from their ribo and 2′-deoxyribosyl counterparts.

Kinetic assays of dTMP synthetase activity were performed spectrophotometrically at 30 °C under conditions previously described.[19] The assay mixture (1.2 mL) contained 50 mM *N*-methylmorpholine hydrochloride (pH 7.4), 25 mM $MgCl_2$, 1.0 mM EDTA, 75 mM 2-mercaptoethanol, 0.20 mM *dl*,L-CH_2-H_4folate, 2.5 mM H_2CO, 3.5–20 μM dUMP, specified amounts of the inhibitor (0.01 to 0.2 mM), and ca. 2 nM dTMP synthetase; reactions were initiated by the addition of enzyme and initial velocities were determined over the first 1–3 min. For experiments on time-dependent inactivation of dTMP synthetase, a solution (ca. 1 mL) containing 0.1 μM dTMP synthetase, 50 mM NMM·HCl (pH 7.4), 0.1 mM CH_2-H_4folate, and specified amounts of the nucleotide to be tested was incubated at 0 or 30 °C. At specified intervals, 100 μL aliquots of this solution were added to the standard assay mixture, and the initial velocity was determined. Assays of dThd and Urd phosphorylase were performed using the standard assay conditions and spectrophotometric assay previously reported for dThd phosphorylase.[21]

Ultraviolet difference spectra were obtained by a modification of the procedure described previously.[23] Two cuvettes containing in 0.9 mL 50 μmol of NMM·HCl (pH 7.4), 10 μmol of $MgCl_2$, 1 μmol of EDTA, 5 μmol of DTT, 26 nmol of dTMP synthetase, 30 nmol of (±)-H_4folate, and 0.5 μmol of H_2CO were placed in the reference and sample compartments (30 °C) of a Cary 118 recording spectrophotometer. After the base line was recorded, 100 μL of 10 μM *ara*-FUMP (10 nmol) was added to the sample cuvette and 100 μL of water to the reference cuvette, and the difference spectra of the native complex was recorded. For difference spectra of the denatured complex, 75 μL of 30% $NaDodSO_4$ was added to each of the above cuvettes, and repetitive scans of the spectrum were obtained until no further changes occurred. For the titration of the enzyme, an analogous procedure was used, except that aliquots of the enzyme and CH_2-H_4folate were 13 nmol and 0.23 μmol, respectively; spectra were recorded after the addition of 5-nmol increments of *ara*-FUMP from 5 to 60 μmol, and appropriate corrections were made for dilutions.

Acknowledgment. This investigation was supported by Grant CA 14394 from the National Cancer Institute, DHEW.

(35) H. Machida, S. Sakata, A. Kuninaka, H. Yoshino, C. Nakayama, and M. Saneyoshi, *Antimicrob. Agents Chemother.*, **16**, 158 (1979).

(36) M. Saneyoshi, M. Inomata, and F. Fukuoka, *Chem. Pharm. Bull.*, **26**, 2990 (1978).

Pyrazolopyrimidine Nucleosides. 12. Synthesis and Biological Activity of Certain Pyrazolo[3,4-*d*]pyrimidine Nucleosides Related to Adenosine[1]

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Received April 24, 1981

The chemical synthesis of certain 4-substituted pyrazolo[3,4-*d*]pyrimidine nucleosides is described. Using 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4-one (**1**) as the starting material, the reactive intermediate 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**2**) was prepared in excellent yield. Compound **2** served as a versatile precursor for the synthesis of a number of 4-substituted pyrazolo[3,4-*d*]pyrimidine nucleosides. In antitumor studies of these nucleosides, in vitro and in vivo, it was found that any alteration of the 4-amino substituent of 4-amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**3**) was accompanied by a significant decrease or loss of antitumor activity. On the other hand, introduction of certain substituents at the 3 position of **3** (synthesis reported previously) led to a dramatic increase in antitumor activity in comparison to the parent compound.

Several 6-substituted adenosine derivatives have been reported[2] to possess antitumor activity. On the other hand, there has been a paucity of studies[3] involving the synthesis and biological evaluation of the structurally related 4-substituted derivatives of 4-amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (4-APPR). The adenosine

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(1) For part 11, see Crabtree, G. W.; Agarwal, R. P.; Parks, R. E., Jr.; Lewis, A. F.; Wotring, L. L.; Townsend, L. B. *Biochem. Pharmacol.* **1979**, *28*, 1491.

(2) Fleysher, M. H.; Bernacki, R. J.; Bullard, G. A. *J. Med. Chem.* **1980**, *23*, 1448, and references cited therein.

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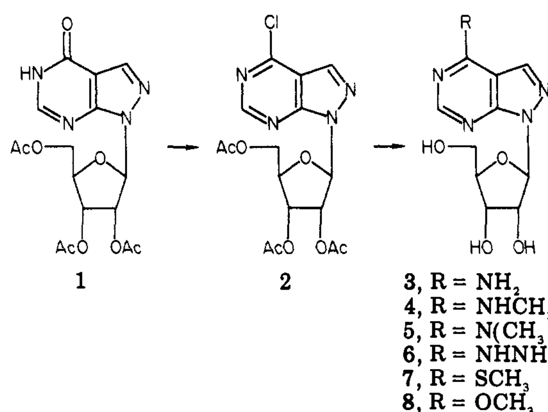
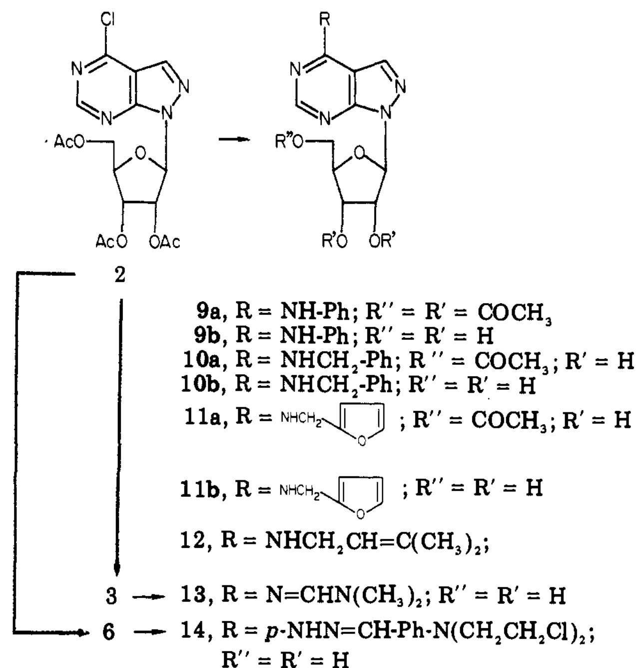
Scheme I



Scheme II



analogue 4-APPR has shown some modest antitumor activity.[3e] Therefore, it was deemed of interest to prepare some specific derivatives of 4-APPR for the evaluation of their biological activity.

In a recent investigation,[4] we described the syntheses of certain 4-substituted pyrazolo[3,4-*d*]pyrimidine nucleosides from 4-(methylthio)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**7**). For the current study, we elected to use 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**2**) as the precursor for the desired N^4-substituted derivatives, since milder reaction conditions could be employed. The preparation of **2** via the silyl fusion glycosylation method was reported[3d] earlier from our laboratories. However, this procedure was found to be unsuitable for the large-scale production of **2** which we needed for the current investigation. We have now found that **2** can be prepared in sufficient quantity and in an excellent yield from 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4-one[4] (**1**) using dimethylchloromethylammonium chloride (Scheme I).[5] This mild chlorinating agent was generated in situ from thionyl chloride and dimethylformamide. Unlike the 4-(methylthio) analogue **7**, the 4-chloro derivative **2** required cold storage (deep freeze) under anhydrous conditions because of its instability at room temperature. Once these conditions were met, we found that **2** could be kept in a pure state for months.

Chemistry. The reactivity of the 4-chloro substituent toward nucleophilic displacement surpassed that of the 4-(methylthio) group. The relative ease with which this substituent underwent nucleophilic displacement provided a second and much more facile route for the preparation of **3**–**7**. In addition to these nucleosides, treatment of **2** with methoxide furnished 4-methoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine in good yield.

The N^4-substituted pyrazolo[3,4-*d*]pyrimidine nucleosides of particular interest were the 4-(benzylamino)- (**10b**), 4-(furfurylamino)- (**11b**), and 4-anilino- (**9b**) derivatives, since the corresponding purines had all displayed[2] an increase in the survival time of tumor-bearing mice. The synthesis of these derivatives (Scheme II) subsequently provided further insight into the reactivity of the 4-chloro group. Treatment of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**2**) with an excess of benzylamine afforded a mixture of two nucleosides (TLC) which could be easily separated by column chromatography. Based on spectral data (UV, IR, and ^{1}H NMR) and elemental analyses, the two nucleosides were assigned the structures 4-(benzylamino)-1-(5-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**10a**) and 4-(benzylamino)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**10b**). An initial assignment of the *O*-acetyl group to the 5′ position on the β-D-ribofuranosyl moiety was established by comparing the ^{1}H NMR spectra of **10a** and **10b**. The 5′-methylene protons of **10b** were observed at δ 3.6, whereas in the spectrum of **10a** they were observed downfield at δ 4.07. Presumably, this is a result of the deshielding effect of the acetyl group.[6] Additional evidence for this assignment was provided by the mass spectra of the trimethylsilyl derivatives of **10a** and **10b**. The characteristic $CH_2{=}O^+Me_3Si$ fragment[7] (m/e 103), which arises from the cleavage of the C4′–C5′ bond of the carbohydrate moiety, appeared in the spectrum of **10b** but was absent in the spectrum of **10a**. Also, the B + 116 ion[7b] at m/e 340 was present in both spectra, indicating that the 2′-hydroxyl group of the β-D-ribofuranosyl moiety was unsubstituted. Removal of the 5′-*O*-acetyl group of **10a** could be accomplished by treating this nucleoside with methanolic ammonia at 5 °C.

A similar situation was observed when furfurylamine was allowed to react with **2**. Once again, two nucleosides were obtained and separated by column chromatography. Like the aforementioned **10a** and **10b**, they were found (^{1}H NMR, MS, UV, IR and elemental analyses) to be the 5′-*O*-acetyl and completely deblocked analogues **11a** and **11b**,

(3) (a) Earl, R. A.; Townsend, L. B. *J. Heterocycl. Chem.* **1974**, *11*, 1033. (b) Davoll, J. A.; Kerridge, K. A. *J. Chem. Soc.* **1961**, 2589. Montgomery, J. A.; Clayton, S. J.; Fitzgibbon, W. E. Jr. *J. Heterocycl. Chem.* **1964**, *1*, 215. (c) Krenitsky, T. A.; Elion, G. B.; Strelitz, R. A.; Hitchings, G. H. *J. Biol. Chem.* **1967**, 212, 2675. (d) Revankar, G. R.; Townsend, L. B. *J. Chem. Soc. C* **1961**, 2440. (e) Wotring, L. L.; Townsend, L. B. *Cancer Res.* **1979**, *39*, 3018.

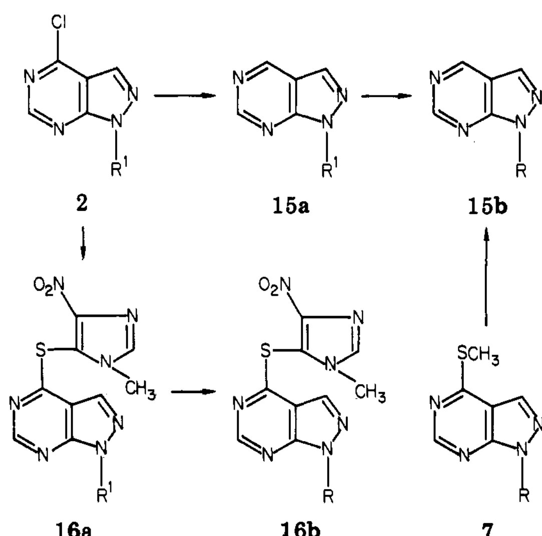
(4) Montero, J.-L. G.; Bhat, G. A.; Panzica, R. P.; Townsend, L. B. *J. Heterocycl. Chem.* **1977**, *14*, 483.

(5) Zemlicka, J.; Owens, J. "Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods and Techniques", Part 2; Townsend, L. B.; Tipson, R. S.; Eds.; Wiley-Interscience: New York, 1978; p 989.

(6) Leutzinger, E. E.; Meguro, T.; Townsend, L. B.; Shuman, D. A.; Schweizer, M. P.; Stewart, C. M.; Robins, R. K. *J. Org. Chem.* **1972**, *37*, 3695.

(7) (a) McCloskey, J. A. *Basic Princ. Nucleic Acid Chem.* **1974**, *1*. (b) McCloskey, J. A.; Lawson, A. M.; Tsuboyama, K.; Krueger, P. M.; Stillwell, R. N. *J. Am. Chem. Soc.* **1968**, *90*, 4184.

Scheme III[a]



[a] R = β-D-Ribofuranosyl; R^1 = 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl.

respectively. Treatment of **11a** with sodium methoxide in methanol furnished **11b**.

Benzylamine and furfurylamine are of comparable basicity, and the 4-chloro group on **2** was rapidly displaced (TLC) by either amine. However, deacetylation of the β-D-ribofuranosyl moiety occurred at a much slower rate than the displacement. With aniline, an amine that is less basic than benzylamine or furfurylamine (several orders of magnitude), only a displacement of the chloro group was observed. The sole product from this reaction was 4-anilino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**9a**). Thus, as one decreases the basicity of the nucleophile, deacetylation ceases, yet replacement of the chloro group still occurs. Conversion of **9a** to **9b** was achieved by treatment with methanolic ammonia.

Several other N^4-substituted 4-APP ribosides of possible therapeutic interest were also prepared in this series. The reaction of **2** with 3-methyl-2-butenylamine hydrochloride[8] in *n*-butyl alcohol containing triethylamine furnished a moderate yield of the nucleoside **12**. Treatment of 4-APP riboside (**3**) with dimethylformamide dimethyl acetal[9] in dimethylformamide afforded a good yield of 4-[[(dimethylamino)methylene]amino]-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**13**). Unlike **3**, this derivative is very soluble in aqueous solution. The nitrogen mustard derivative **14** was prepared by a condensation of **6** with *p*-[*N,N*-bis(2-chloroethyl)amino]benzaldehyde.[10]

In order to determine what effect the absence of a N^4 substituent would have on the biological activity, we synthesized the nebularine analogue 1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**15b**) (Scheme III). This nucleoside was prepared via two routes. The first route involved a catalytic dehalogenation of **2** in the presence of 10% Pd/C. This furnished the acetylated intermediate **15a**, which on treatment with methanolic ammonia provided **15b**. The second pathway involved a catalytic dethiation of the 4-(methylthio) analogue **7** with Raney nickel to provide **15b**. It would seem worthwhile to emphasize once again the reactivity of a 4-substituent toward nucleophilic displacement. This can be illustrated as follows: a reaction of **2** with the ammonium salt of 1-methyl-4-nitro-5-mercaptoimidazole afforded the imuran analogue **16a**; however, attempts to deacetylate this derivative with methanolic ammonia gave the aforementioned imidazole and 4-APP riboside instead of the expected **16b**.

Antitumor Results and Discussion

The results of testing a number of 4-substituted pyrazolo[3,4-*d*]pyrimidine ribosides for antitumor activity in vitro and in vivo are shown in Table II. Of all the analogues tested, the parent compound 4-APPR (**3**) and the 4-[(dimethylamino)methylene] derivative **13** showed the greatest inhibition of growth of L1210 mouse leukemic cells in vitro (ID_{50} = $\sim 5 \times 10^{-7}$ M). Derivatization of the 4-amino with other groups or a conversion to OH, SH, SCH_3, or H resulted in a markedly diminished growth inhibitory potency ($ID_{50} \geq 10^{-5}$ M). The 4-[[(dimethylamino)methylene]amino] substituent of **13** should be readily hydrolyzed to the free amino group in the culture medium, liberating 4-APPR (**3**). Significant hydrolysis of **13** to **3** has been reported[11a] to occur in aqueous solution, within 2 h at room temperature. In aqueous solution, the heterocyclic portion of **13**, 4-[[(dimethylamino)methylene]amino]pyrazolo[3,4-*d*]pyrimidine, was hydrolyzed to 4-aminopyrazolo[3,4-*d*]pyrimidine.[11b] Therefore, the growth-inhibitory activity of **13** is assumed to be due to the production of **3** by hydrolysis in the aqueous culture medium. Thus, the amino group appears to be required for the antitumor activity of **3**. The results of in vivo antitumor testing against leukemias in mice also support this conclusion. Compound **3** was the only 4-substituted pyrazolo[3,4-*d*]pyrimidine riboside that showed significant antitumor activity. The failure of **13** to prolong the life span of tumor-bearing mice may have been due to changes in the pharmacokinetics of the drug in mice that resulted from derivatization of the NH_2.

Host toxicity was observed for compounds **4**, **6**, **7**, **10b**, **13**, **15b**, and **19–21**. The mechanisms of host toxicity are unknown, but one might speculate that the mechanism for the 4-(hydroxyamino) derivative (**21**) might involve methemoglobin formation and hemolysis, as has been previously observed[12] in vivo for 6-(hydroxyamino)-9-β-D-ribofuranosylpurine, a derivative of adenosine. Methemoglobin formation was also observed[13] when 4-(hydroxyamino)-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine, a derivative of tubercidin, was incubated with human erythrocytes in vitro.

An interesting biochemical property of compound **9b**, the N^4-phenyl derivative of 4-APPR, is that it appears to be an inhibitor of adenosine kinase. In studies to be re-

(8) Lewis, A. F.; Townsend, L. B. *J. Heterocycl. Chem.* **1974**, *11*, 71 and references cited therein.

(9) (a) Zemlicka, J.; Holy, A. *Collect. Czech. Chem. Commun.* **1967**, *32*, 3159. (b) Zemlicka, J. "Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods and Techniques", Part 2; Townsend, L. B.; Tipson, R. S., Eds.; Wiley-Interscience: New York, 1978; p 709.

(10) (a) Elderfield, R. C.; Covey, I. S.; Geiduschek, J. B.; Meyer, W. L.; Ross, A. B.; Ross, J. H. *J. Org. Chem.* **1958**, *23*, 1749. (b) Fleysher, M. H. "Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods and Techniques", Part 2; Townsend, L. B.; Tipson, R. S., Eds., Wiley-Interscience: New York, 1978; p 667.

(11) (a) Panzica, R. P.; Bhat, G. A.; Earl, R. A.; Montero, J.-L. G.; Roti Roti, L. W.; Townsend, L. B. "Chemistry and Biology of Nucleosides and Nucleotides"; Harmon, R. E.; Robins, R. K.; and Townsend, L. B.; Eds.; Academic Press; New York, 1978; p 121. (b) Panzica, R. P.; Townsend, L. B. "Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods and Techniques", Part 1; Townsend, L. B.; Tipson, R. S., Eds.; Wiley-Interscience: New York, 1978; p 111.

(12) Dollinger, M. R.; Krakoff, I. H. *Clin. Pharmacol. Ther.* **1975**, *17*, 57.

(13) Crabtree, G. W.; Parks, R. E., Jr., unpublished observations.

Table II. In Vitro Cytotoxicity and in Vivo Antitumor Testing against L1210 and P388 Leukemias in Mice for 4-Substituted 1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidines

compd	R	in vitro ID_{50},[a] M	in vivo[b]: tumor	dose per injection,[c] mg/kg	days	ILS[d]	cures	host toxicity at higher doses[e]
3	NH_2	4×10^{-7}	L1210	133	1	48	1/6	n
				9.3	1-9	24		y
			P388	25	1-9	54		n
4	$NHCH_3$	$>10^{-4}$	L1210	50	1-5	4		y
5	$N(CH_3)_2$	-	L1210	200	1-5	0		nt
6	$NHNH_2$	6×10^{-5}	L1210	200	1-9	12		y
7	SCH_3	9×10^{-6}	L1210	200	1-5	19		y
8	OCH_3	-	L1210	400	1-5	12		nt
9b	NH-phenyl	-	L1210	400	1-5	−4		nt
10b	$NHCH_2$-phenyl	-	L1210	25	1-5	3		y
11b	$NHCH_2$-furyl	$>10^{-4}$	L1210	400	1-9	6		nt
13	$N{=}CHN(CH_3)_2$	6×10^{-7}	L1210	50	1-5	12		y
14	*p*-$NHN{=}CH$-Ph-$N(CH_2CH_2Cl)_2$	$\sim 10^{-5}$	L1210	400	1-9	−7		nt
15b	H	$>10^{-4}$	L1210	200	1-9	−3		y
16a	O_2N-imidazolyl(N-CH_3)-S-[f]	3×10^{-5}	L1210	100	1-9	0		y
17	OH[g]	-	L1210	400	1-5	2		nt
18[h]	SH[g]	-	L1210	400	1-5	−6		nt
19[h]	*p*-SCH_2-Ph-NO_2	$\sim 10^{-4}$	L1210	200	1-5	1		y
20[h]	$SCH_2CH{=}CH_2$	-	L1210	100	1-5	2		y
21[h]	NHOH	1×10^{-5}	L1210	12.5	1-5	14		y

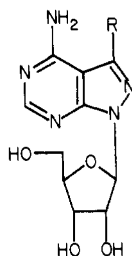
[a] ID_{50} is the concentration required to reduce the growth rate of L1210 cells in culture to half of the control rate. A hyphen indicates that 10^{-4} M compound had no effect on cell growth. [b] Data are presented for the protocol that gave optimal activity, or for inactive compounds (ILS < 25%) the protocol using the highest nontoxic dose tested for each tumor. [c] Administered to tumor-bearing mice on days indicated after inoculation of the animals with tumor cells.[23] [d] ILS is the increase in life span for drug-treated animals as compared to control, untreated animals, expressed as a percentage of the life span of the untreated tumor-bearing animals. Cures represent animals surviving 30 days without tumor, expressed as number of cured animals per total number of animals in group.[23] [e] Occurrence of drug-induced shortening of life span resulting from treatment with higher doses on the same schedule: y = yes; n = no; nt = no higher dose tested. [f] 2′,3′,5′-Tri-*O*-acetyl derivative. [g] Ketone substituents are shown as OH and thione substituents as SH for convenience in presenting the structures. [h] Synthesis reported in ref 4.

ported[14] elsewhere, this possibility was investigated by testing the ability of **9b** and N^6-phenyladenosine to prevent inhibition of the growth of L1210 cells by 6-(methylmercapto)-9-β-D-ribofuranosylpurine (MMPR), an adenosine analogue. Both compounds were found to be about equally effective in preventing the MMPR-induced inhibition of growth.[14] N^6-Phenyladenosine has been previously shown[15] to be an inhibitor of adenosine kinase partially purified from Sarcoma 180 cells. Thus, it appears that by inhibiting phosphorylation of MMPR by adenosine kinase,[16] N^6-phenyladenosine or **9b** prevented formation of the 5′-phosphate derivative, the active metabolite, and the growth inhibitory activity of MMPR was blocked. The change from a purine ring to the pyrazolo[3,4-*d*]pyrimidine ring system does not seem to have significantly altered the adenosine kinase inhibitory activity.

Modification of 4-APPR (**3**) by the introduction of a cyano (**29**), carboxamide (**24**), and other related substituents at the 3 position[3a,17] has resulted in a marked enhancement of the antitumor activity. Since 4-APPR can be viewed as a ring-modified analogue of the nucleoside antibiotic tubercidin, **29** and **24** can be viewed as ring-modified analogues of the nucleoside antibiotics toyocamycin and sangivamycin, respectively. The antitumor evaluation of these compounds is shown in Table III. The

(14) Wotring, L. L.; Townsend, L. B., manuscript in preparation.
(15) Divekar, A. Y.; Hakala, M. T. *Mol. Pharmacol.* **1971**, *7*, 663.
(16) Schnebli, H. P.; Hill, D. L.; Bennett, L. L., Jr. *J. Biol. Chem.* **1967**, *242*, 1997.
(17) Hecht, S. M.; Frye, R. B.; Werner, D.; Fukui, T.; Hawrelak, S. D. *Biochemistry* **1976**, *15*, 1005.

Table III. In Vitro Cytotoxicity and in Vivo Antitumor Testing against L1210 and P388 Leukemias in Mice for 3-Substituted 4-Amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidines



			in vivo[b]					
compd	R	in vitro ID_{50},[a] M	tumor	dose per injection,[c] mg/kg	days	ILS[d]	cures	host toxicity at higher doses[e]
22	$C(=NH)NH_2$	nt[f]	L1210	25	1-5	98	2/12	y
			P388	40	1-9	29		y
23	COOH	-						
24	$C(=O)NH_2$	1×10^{-7}	L1210	200	1-5	136	2/6	nt
			P388	6	1-9	90		y
25	$C(=NH)OCH_3$	nt	L1210	400	1-5	64		nt
26	$C(=S)NH_2$	nt	L1210	3.12	1-9	46		y
			P388	3	1-9	34		y
27	$C(=NOH)NH_2$	1.8×10^{-7}	L1210	25	1-5	78	1/12	y
			P388	100	1, 5, 9	33		y
28	$C(=NHNH_2)NH_2$	nt	L1210	166	1-5	31		n
			P388	320	1-5	4		nt
29	C≡N	7.9×10^{-8}	L1210	100	1-9	27		nt

[a-e] See corresponding footnotes in Table II. [f] Not tested.

ID_{50} values for inhibition of growth of L1210 cells were approximately 10^{-7} M for compounds **24**, **27**, and **29**, indicating a slightly greater potency[18] for the 3-substituted derivatives than for the parent compound **3**. The antileukemic activity in vivo was even more enhanced. The most active compound in this series was 4-amino-3-carboxamido-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**24**), with values for ILS of L1210-bearing mice as high as 136% with two animals (out of a group of six) surviving 30 days and being considered cured. This compound also exhibited significant antileukemic activity in P388-bearing mice, with values for ILS as high as 90%. These results, showing two- to threefold prolongation of the life span of leukemic mice, constitute a strikingly greater antileukemic activity than was observed for the parent compound, 4-APPR (**3**), for which the maximal ILS obtained was 48% (Table II).

The compounds with 3-substituents of carboxamidine (**22**), methyl imidate (**25**), and carboxamidoxime (**27**) were only slightly less active than the 3-carboxamide (**24**), and those with 3-substituents of cyano (**29**), carboxamidrazone (**28**), and thiocarboxamide (**26**) showed slight in vivo antitumor activity (Table III). However, as has been found previously for sangivamycin,[19,20] hydrolysis of the 3-substituent to COOH (compound **23**) resulted in total loss of antitumor activity, as indicated by the observation that the growth of L1210 cells was unaffected by this compound at 10^{-4} M (Table III).

(18) This result with L1210 cells appears to be in contrast to the report of Hecht et al.[17] on the effects of 4-APPR and 3-$CONH_2$-4-APPR on mouse fibroblasts 3T6. The 3T6 cells were reported to be much more sensitive to APPR than to APPCR. This apparent discrepancy may be at least partially accounted for by differences between the cell lines and the procedures and criteria used in evaluation of cytotoxicity.

(19) Rao, K. V. *J. Med. Chem.* **1968**, *11*, 939.

(20) Wotring, L. L.; and Townsend, L. B., unpublished observations.

The increase of in vitro inhibition of cell growth that is observed when 4-APPR (**3**) is derivatized with certain substituents at the 3 position is not great enough to satisfactorily account for the striking increase in antitumor activity in vivo. Furthermore, the 3-cyano derivative (**29**) is highly active as an inhibitor of growth of L1210 cells in vitro ($ID_{50} = 7.9 \times 10^{-8}$ M) but shows only very slight antitumor activity in vivo (ILS ≤ 27%). Therefore, we investigated the possibility that some other factors might enter into the enhancement of antitumor activity in vivo. The effect of growth inhibition by 4-APPR (**3**), 3-CN-4-APPR (**29**), and 3-$CONH_2$-4-APPR (**24**) on the viability of L1210 cells was determined. After 20 h of treatment with one of the three analogues at equitoxic growth-inhibiting concentrations, the analogue-containing medium was removed and the cells were returned to normal medium. Their subsequent growth was monitored for 5 days, and that fraction of the cell population which was viable at the time the drug was removed was estimated by growth curve back-extrapolation.[3e] When the cells were treated with 4-APPR (2×10^{-5} M) or 3-CN-4-APPR (10^{-5} M), a major fraction (≥50%) of the cell population was viable and able to resume cell division after removal of the analogue. However, when the cells were treated with 3-$CONH_2$-4-APPR (10^{-4} M), no significant increase in cell number could be detected during the 5-day period of observation, indicating that the L1210 cells treated with this analogue were reproductively dead and could not resume cell division. Thus, it appears that 3-$CONH_2$-4-APPR (**24**), which showed the greatest activity as an antitumor agent in vivo, had a lethal effect on the cells.[3e] This property was not shared by 4-APPR (**3**) or 3-CN-4-APPR (**29**), since both of these agents had less antitumor activity in vivo. This lethal effect of 3-$CONH_2$-4-APPR appears to provide at least a partial explanation for its superior antitumor activity in vivo.

Another factor which may contribute to the superior antitumor activity of 3-$CONH_2$-4-APPR is its resistance

toward deamination.[3e,21] 4-APPR, on the other hand, can be deaminated by adenosine deaminase,[3e,21,22] and this inactivation may limit its effectiveness in vivo. However, in a more detailed study[3e] on the cellular effects of 4-APPR and 3-$CONH_2$-4-APPR, the difference in susceptibility to deamination has been shown not to be the basis for the difference in the effect on cellular viability between 4-APPR and 3-$CONH_2$-4-APPR.

In summary, compound **24**, the 3-carboxamide derivative of 4-APPR (**3**), shows great promise as an antitumor agent as indicated by its antileukemic activity in mice, its lethal effect on L1210 cells in vitro, and its resistance toward deamination.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and were uncorrected. ^{1}H NMR spectra were measured with a Varian EM-390 90 MHz spectrometer. Chemical shifts are given in parts per million on a δ scale using DDS as an internal standard; coupling constants are expressed in hertz. UV absorption spectra were measured on a Beckman Acta CIII spectrometer. Optical rotations were obtained with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Mass spectra were obtained with the LKB 9000S instrument and Varian MAT 112S/SS100 C data system. Thin-layer chromatography was carried out on glass plates coated (250 μm) with SilicAR-7GF (Mallinckrodt). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

4-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine (2). 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4-one[4] (**1**; 23.6 g, 60 mmol) was dissolved in dry methylene chloride (400 mL) in a 1-L, three-necked flask fitted with a condenser, dropping funnel, and gas inlet tube. A solution of freshly distilled thionyl chloride (50.4 mL, 700 mmol) and dry dimethylformamide (25.2 mL) in dry methylene chloride (200 mL) was added dropwise to the original solution by use of a dropping funnel, over a period of 2 h. During the addition, the reaction solution was maintained at a gentle reflux (oil bath 60 °C) and under a dry nitrogen atmosphere. After the addition was complete, the solution was stirred and heated (60 °C, N_2 atmosphere) for an additional 14 h. The mixture was cooled to room temperature and then filtered to remove a small amount of material which had precipitated during the 14-h period. The filtrate was then slowly poured into a saturated sodium bicarbonate solution (300 mL) which contained ice, and the mixture was vigorously stirred for 15 min. The layers were separated, and the aqueous phase was extracted (1 × 100 mL) with methylene chloride. The combined organic layer was then washed with a saturated sodium bicarbonate solution (2 × 200 mL) and cold water (3 × 200 mL) and dried over anhydrous magnesium sulfate. The organic phase was then evaporated under reduced pressure to provide a syrupy liquid, which gave a white crystalline compound on triturating with ethanol (25 mL). The crystalline material was filtered, dried under vacuum in a dessicator at room temperature (22.9 g, 97%), and then stored at −20 °C (under anhydrous conditions): mp 115–116 °C, $[\alpha]^{26}_D$ −36.5° (*c* 1.04, $CHCl_3$), −33.5° (*c* 1.00, MeOH); ^{1}H NMR (Me_2SO-d_6) δ 8.9 and 8.67 (2 s, 2, H_3 and H_6), 6.55 (d, 1, $J_{1',2'}$ = 3 Hz, Hl'), 2.12, 2.06, and 2.0 (3 s, 9, $COCH_3$). Anal. ($C_{16}H_{17}N_4O_7Cl$) C, H, N.

4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (3). A mixture of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**2**; 2.06 g, 5 mmol) and ammonium hydroxide (28%, 60 mL) was heated at reflux with stirring for 2 h. The solid which separated on cooling was filtered and washed with water–alcohol (5 mL, 1:1, v/v). Crystallization of the solid from methanol gave 1.2 g (90%) of pure compound: mp 258–259 °C; $[\alpha]^{23}_D$ −78.1° (*c* 1.00, DMF); ^{1}H NMR (Me_2SO-d_6) δ 8.33 (br s, 2, H_3 and H_6), 7.93 (br s, 2, NH_2), 6.19 (d, 1, $J_{1',2'}$ = 4.5 Hz, Hl'). This compound was identical in all respects with an authentic sample.[3d]

4-(Methylamino)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (4). 4-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine (**2**; 2 g, 4.8 mmol) was suspended in 40% aqueous methylamine (50 mL), and the suspension was heated at reflux for 1 h. Excess solvent was removed in vacuo, and the resulting solid was recrystallized from ethanol to provide **4** as white crystals (1.23 g, 90%). This nucleoside was identical, in all respects, with that reported earlier.[4]

4-(Dimethylamino)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]-pyrimidine (5). To a 40% aqueous dimethylamine solution (50 mL) was added 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyranozolo[3,4-*d*]pyrimidine (**2**; 2 g, 4.85 mmol), and the mixture was heated at reflux for 1 h on a steam bath. After the mixture cooled, the excess solvent was removed in vacuo, and the resulting solid was crystallized from 90% ethanol to provide **5** as white crystals (1.36 g, 92%). This nucleoside was identical, in all respects, with that reported earlier.[4]

4-Hydrazino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (6). 4-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine (**2**; 1.24 g, 3 mmol) was dissolved in absolute ethanol, and to this solution was added 97% hydrazine (0.4 g, 12 mmol). The reaction mixture was heated at reflux for 3 h. During the 3-h time period, a light-pink crystalline precipitate formed. After the reaction mixture cooled to room temperature, the crystalline material was collected by filtration and washed with aqueous ethanol (2 × 10 mL). Recrystallization of the product from aqueous methanol gave 0.6 g of pure **6** (70.8%), mp 220–221 °C. This material was identical in all respects with that reported earlier.[4]

4-(Methylthio)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (7). Sodium metal (0.74 g, 32 mmol) was dissolved in absolute ethanol (100 mL) and the solution was saturated with methyl mercaptan (Matheson) at room temperature and with the exclusion of moisture. The nucleoside **2** (8.25 g, 20 mmol) was added to this solution[24] and the mixture was heated at reflux and stirred for 18 h. After cooling, the solution was neutralized with dry ice to a pH of 6. The solvent was removed under diminished pressure and furnished crude **7**. This material was recrystallized from methanol and afforded pure **7** (4.46 g, 75%), mp 169–170 °C, which was identical, in all respects, with the material obtained from our previous investigation.[4]

4-Methoxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (8). 4-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo-[3,4-*d*]pyrimidine (**2**; 3 g, 7.27 mmol) was added to a solution of dry absolute methanol (100 mL) which contained AR sodium methoxide (3.93 g, 72.7 mmol), and the mixture was stirred at room temperature with the exclusion of moisture for 6 h. The resulting clear solution was neutralized with Amberlite IRC-50 (10 mL) to pH 6. The resin was removed by filtration and washed with hot methanol (3 × 10 mL). The filtrate and washings were combined and evaporated under diminished pressure to provide a white solid. This solid was recrystallized from methanol to furnish **8** (1.95 g, 95%): mp 182–183 °C; $[\alpha]^{26}_D$ −80.6° (*c* 1.01, MeOH); ^{1}H NMR (Me_2SO-d_6) δ 8.61 and 8.31 (2 s, 2, H_3 and H_6), 6.16 (d, 1, $J_{1',2'}$ = 5 Hz, Hl'), 4.15 (s, 3, OCH_3). Anal. ($C_{11}H_{14}N_4O_5$) C, H, N.

4-Anilino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine (9a). To a solution of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**2**; 1.238 g, 3 mmol) in ethanol (50 mL) was added freshly distilled aniline (1.86 g, 20 mmol), and the mixture was refluxed for 6 h. Solvent was removed under reduced pressure, and the residue was extracted with chloroform (3 × 50 mL). The chloroform layer

(21) Agarwal, R. P.; Crabtree, G. W.; Agarwal, K. C.; Parks, R. E., Jr.; Townsend, L. B. *Proc. Am. Assoc. Cancer Res.* **1976**, *17*, 214.

(22) Bennett, L. L., Jr.; Allan, P. W.; Smithers, D.; Vail, M. H. *Biochem. Pharmacol.* **1969**, *18*, 725.

(23) Geran, R. I.; Greenberg, W. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 1.

(24) If the reaction is stirred at room temperature (≃4 h), 4-(methylthio)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo-[3,4-*d*]pyrimidine can be isolated as an intermediate: ^{1}H NMR ($CDCl_3$) δ 8.72 and 8.12 (2 s, 2, H_3 and H_6), 6.60 (d, 1, $J_{1',2'}$ = 4 Hz, Hl'), 2.72 (s, 3, SCH_3), 2.15, 2.12, and 2.08 (3 s, 9, $COCH_3$).

was washed with water (2 × 25 mL) and dried over anhydrous magnesium sulfate. The volume of the dried chloroform phase was reduced to ca. 20 mL and was applied to the top of a silica gel CC7 dry packed column (150 g). Elution of the column with chloroform–methanol (19:1, v/v) furnished a syrup which was crystallized with aqueous ethanol to provide **9a** as a white solid (1.0 g, 71%): mp 138–139 °C, $[\alpha]^{26}_D$ −33.6° (*c* 1.0, MeOH); ^1H NMR (Me_2SO-d_6) δ 10.32 (br s, 1, *H*N-Ph), 8.55, 7.93, and 7.11-7.57 (2, s and m, 7, aromatic protons), 6.54 (d, 1, $J_{1',2'}$ = 3.0 Hz, Hl'), 2.1 [d, 9, $(CH_3CO_2)_3$]. Anal. ($C_{22}H_{23}N_5O_7$) C, H, N.

4-Anilino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (9b). The nucleoside **9a** (0.469 g, 1 mmol) was added to a saturated solution of methanolic ammonia (15 mL), and the mixture was allowed to stand at 3 °C for 24 h. Removal of solvent in vacuo was followed by crystallization of the residue from alcohol to afford compound **9b** (0.3 g, 87.5%): mp 163–164 °C; $[\alpha]^{26}_D$ −64° (*c* 1.03, MeOH); ^1H NMR (Me_2SO-d_6) δ 10.2 (s, 1, *H*N-Ph), 8.5, 8.4, 7.7–7.16 (2 s and m, 7, aromatic protons), 6.26 (d, 1, $J_{1',2'}$ = 4.5 Hz, Hl'). Anal. ($C_{16}H_{17}N_5O_4$) C, H, N.

4-(Benzylamino)-1-(5-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (10a) and 4-(Benzylamino)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (10b). To a solution of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**2**; 1.24 g, 3 mmol) in ethanol (50 mL) was added freshly distilled benzylamine (3.21 g, 30 mmol), and the mixture was heated at reflux for 18 h. The excess solvent was evaporated under reduced pressure, and the residue was extracted with ethyl acetate (3 × 50 mL) and then filtered to remove benzylamine hydrochloride. The filtrate was evaporated in vacuo. The resulting syrup was dissolved in chloroform (25 mL) and chromatographed using a silica gel CC7 column (150 g) prepacked in benzene. The fraction which eluted with chloroform–methanol (9.7:0.3, v/v) crystallized from ethyl acetate to give **10a** (0.45 g, 37.5%): mp 116–117 °C; $[\alpha]^{26}_D$ −39.0° (*c* 0.97, MeOH); ^1H NMR (Me_2SO-d_6) δ 8.9 (t, 1, *H*NCH_2-Ph), 8.33 (2 s, 2, H_3 and H_6), 7.43 (br s, 5, aromatic), 6.21 (d, 1, $J_{1',2'}$ = 4.2 Hz, Hl'), 2.06 (s, 3, 5'-$OCOCH_3$), 4.07 (m, 2, 5'-CH_2). Anal. ($C_{19}H_{21}N_5O_5$) C, H, N.

The second nucleoside was isolated from the fraction which was eluted with chloroform–methanol (19:1, v/v) and crystallized from alcohol to give 0.45 g (42%) of **10b**: mp 185–186 °C; $[\alpha]^{26}_D$ −64.4° (*c* 0.96, MeOH); ^1H NMR (Me_2SO-d_6) δ 8.93 (t, 1, *H*NCH_2-Ph), 8.37 (br s, 2, H_3 and H_6), 7.4 (br s, 5, aromatic), 6.18 (d, $J_{1',2'}$ = 5.5 Hz, Hl') 3.6 (m, 2, 5'-CH_2).

Deacetylation of 4-(Benzylamino)-1-(5-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine to 10b. Compound **10a** (0.15 g, 0.38 mmol) was added to a saturated methanolic ammonia solution (10 mL) and allowed to stand at 5 °C for 60 h. The excess solvent was removed under reduced pressure, and the residue was crystallized from alcohol to yield 0.1 g of **10b**. This was identical in all respects with compound **10b** as described above. Anal. ($C_{17}H_{19}N_5O_4$) C, H, N.

4-(Furfurylamino)-1-(5-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (11a) and 4-(Furfurylamino)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (11b). 4-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**2**; 3.71 g, 9 mmol) and freshly distilled furfurylamine (7.76 g, 80 mmol) were added to absolute ethanol (60 mL), and the solution heated at reflux for 20 h. The excess solvent was evaporated in vacuo, the residue was then extracted with methylene chloride (3 × 100 mL) and filtered. The filtrate was evaporated to dryness under reduced pressure, and the resulting oil was dissolved in methanol (15 mL) and applied to a silica gel CC7 column (200 g) which had been prepacked in benzene. The fraction which eluted with chloroform–methanol (19:1, v/v) was crystallized from alcohol to furnish 1.3 g (34%) of **11a**: mp 139–140 °C; $[\alpha]^{26}_D$ −35.2° (*c* 0.97, MeOH); ^1H NMR (Me_2SO-d_6) δ 8.76 (t, 1, *H*NCH_2-R), 8.25 (s, 2, H_3 and H_5), 7.58 and 6.36 (2 br s, 3, aromatic), 6.14 (d, 1, $J_{1',2'}$ = 3 Hz, Hl'), 2.08 (s, 3, 5'-$OCOCH_3$). Anal. ($C_{17}H_{19}N_5O_6$) C, H, N.

The second nucleoside (**11b**) was isolated from the fraction which eluted with chloroform–methanol (9:1, v/v) and was crystallized from aqueous methanol to afford 1.2 g (42%) of **11b**: mp 124–125 °C; $[\alpha]^{26}_D$ −59.6° (*c* 1.01, MeOH); ^1H NMR (Me_2SO-d_6) δ 8.82 (br s, 1, *H*NCH_2-R), 8.38 (s, 2, H_3 and H_6), 7.68 and 6.44 (2 br s, 3, aromatic), 6.20 (d, 1, $J_{1',2'}$ = 4.5 Hz, Hl'), 3.53 (m, 2, 5'-CH_2). Anal. ($C_{15}H_{17}N_5O_5$·$0.5H_2O$) C, H, N.

Deacetylation of 4-(Furfurylamino)-1-(5-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (11a) to Furnish 11b. The nucleoside **11a** (1.2 g) was dissolved in methanol (50 mL), and AR sodium methoxide (0.170 g) was added. The reaction mixture was then stirred at room temperature for 2.5 h. The excess solvent was evaporated to dryness in vacuo, and the residue was triturated with cold water (15 mL) and filtered. The solid was recrystallized from chloroform–methanol (99:1, v/v) to yield 1.0 g of **11b**. This nucleoside was identical in all respects with **11b** described above.

1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidine (15b). Method A. A mixture of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**2**; 2.476 g, 6 mmol) in absolute ethanol (100 mL), palladium on charcoal (10%, 0.2 g), and triethylamine (0.51 mL) were hydrogenated with stirring at room temperature for 3 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness in vacuo. The residue was extracted with methylene chloride (2 × 60 mL) and washed with cold water (2 × 25 mL). The methylene chloride phase was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness to afford 1.8 g (79.3%) of the blocked nucleoside **15a**. This blocked nucleoside (1.8 g) was then treated with saturated methanolic ammonia solution (50 mL) and kept at 5 °C for 48 h. The solvent was removed in vacuo, and the residue was crystallized from alcohol to provide 0.9 g (81.8%) of **15b**: mp 225–226 °C, ^1H NMR (Me_2SO-d_6) δ 9.43, 9.13, and 8.57 (3 s, 3, aromatic protons), 6.38 (d, 1, $J_{1',2'}$ = 4.2 Hz, Hl'). Anal. ($C_{10}H_{12}N_4O_4$) C, H, N.

Method B. 4-(Methylthio)-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**7**; 0.298 g, 1 mmol) was dissolved in ethanol (30 mL) and then Raney nickel[25] and 1 drop of ammonium hydroxide were added. The mixture was then heated in a steel bomb (oil bath temperature 135 °C) for 96 h. After cooling, the reaction mixture was filtered, and the filtrate was evaporated to dryness in vacuo. The residue was crystallized twice from alcohol to afford 0.1 g of **15b**. (The mother liquid contained some unreacted starting material as confirmed by TLC.) This product was identical in all respects with the material obtained from method A.

4-[(3-Methyl-2-butenyl)amino]-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (12). A mixture of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**2**; 1.238 g, 3 mmol), 3-methyl-2-butenylamine hydrochloride (0.423 g, 3.5 mmol), triethylamine (1.5 mL), and 1-butanol (20 mL) was heated at reflux for 2 h. The mixture was cooled to room temperature and a 1 N sodium hydroxide solution (2.9 mL) which had been diluted to 10 mL with distilled water was added. The mixture was then evaporated to dryness in vacuo. The residue was stirred with ice–water (30 mL), extracted with methylene chloride (2 × 100 mL), dried over anhydrous magnesium sulfate, and filtered. The filtrate was evaporated under reduced pressure, and the residue was dissolved in chloroform (15 mL) and applied to a silica gel CC7 (100 g) column in chloroform. The fraction which was eluted with chloroform–methanol (9.8:0.2, v/v) was then crystallized from alcohol to provide 0.5 g (36%) of **12**: mp 124–125 °C; $[\alpha]^{25}_D$ −37.55° (*c* 1.0, MeOH).

4-(Mercapto-1-methyl-4-nitroimidazol-5-yl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (16a). A mixture of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**2**; 2.06 g, 4.99 mmol) and the ammonium salt of 1-methyl-4-nitro-5-mercaptoimidazole (0.9 g, 5.1 mmol) was added to 150 mL of ethanol. The mixture was stirred for 18 h at room temperature. After the reaction was complete (TLC), the solvent was evaporated in vacuo. The residue was dissolved in chloroform and applied to a silica gel column prepacked with benzene/chloroform (1:1) and then eluted with $CHCl_3$. Evaporation of the solvent in vacuo gave **16a** (2.55 g) as a colorless foam (95.4%): $[\alpha]^{25}_D$ +38.56° (*c* 1.01, MeOH); ^1H NMR ($CDCl_3$) δ 7.76, 8.03, and 8.56 (3 s, 3 aromatic protons), 6.56 (d, 1, $J_{1',2'}$ = 3.9 Hz, 1H'). Anal. ($C_{20}H_{21}N_7O_9S$) C, H, N.

4-[[(Dimethylamino)methylene]amino]-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (13). A mixture of 4-

(25) Raney nickel no. 28 from Grace Chemical Co.

amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**3**; 1.238 g, 3 mmol), dimethylformamide dimethyl acetal (1.785 g, 15 mmol), and dimethylformamide (18 mL) was stirred for 48 h at room temperature. The solvent was evaporated in vacuo at room temperature and the syrupy intermediate **15** was dissolved in dioxane (14 mL). The solution was stirred with excess dry ice and then with water (14 mL). The resulting solution was stirred for an additional hour, and the solvent was evaporated to dryness in vacuo. The oily product was crystallized from alcohol to provide 1.2 g (80%) of **13**: mp 158–159 °C dec; $[\alpha]^{26}_D$ –74.7° (*c* 1.004, MeOH); ^{1}H NMR (Me_2SO-d_6) δ 9.03, 8.53, and 8.26 (3 s, 3, N=C*H*N, H_3 and H_6), 6.21 (d, 1, $J_{1',2'}$ = 5.5 Hz, H1′), 3.23 [d, 6, $N(CH_3)_2$]. Anal. ($C_{13}H_{18}N_6O_4$) C, H, N.

***p*-[Bis(2-chloroethyl)amino]benzaldehyde (1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-yl)hydrazone (14).** 4-Hydrazino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**6**; 1.415 g, 5 mmol) was suspended with stirring in absolute *N*,*N*-dimethylformamide (50 mL), and *p*-[*N*,*N*-bis(2-chloroethyl)-amino]benzaldehyde (1.35 g, 5.5 mmol) was added. After the mixture was stirred at room temperature for 1 h, molecular sieves (5 Å, 25 g) were added and stirring was continued overnight. Another fresh quantity (11 g) of molecular sieves was then added and stirring continued for another 60 h (the reaction was monitored by TLC). The reaction mixture was filtered, and the residue was washed with dimethylformamide (4 × 50 mL). The combined filtrate and washings were evaporated in vacuo at <40 °C to provide a gum. Absolute ethanol (200 mL) was added to the gum to precipitate the crude product (1.6 g), mp 195–200 °C dec. For purification, this product was dissolved in dimethylformamide (80 mL), the solution was filtered through Celite, and absolute methanol (≃200 mL) was added to the filtrate to induce a cloud point. The solution was then allowed to stand at room temperature for 18 h. The solid which had separated was collected by filtration and dried over P_2O_5 in a vacuum dessicator at room temperature to afford 2 g (78.5%) of **14**: mp 225–226 °C dec; $[\alpha]^{25}_D$ –1.4° (*c* 1.043, DMF); ^{1}H NMR (Me_2SO-d_6) δ 11.93 (s, 1, NH), 8.53, 8.4, 8.23 (3 s, 3, H_3, H_6, and aldehyde C*H*), 7.72 and 6.91 (2 d, 4 aromatic protons), 6.27 (d, 1, $J_{1',2'}$ = 4.5 Hz, H1′). Anal. ($C_{21}H_{25}N_7O_4Cl_2$) C, H, N.

Antitumor Studies. The in vitro cytotoxicity against L1210 was evaluated as described previously.[3e] L1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the presence of various concentrations of the test compound. The ID_{50} was defined as the concentration required to reduce the growth rate to 50% of the control.

The in vivo antitumor data was furnished by the Division of Cancer Treatment using standard National Cancer Institute protocols for evaluation of compounds against the mouse leukemias L1210 and P388.[23]

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Supplementary Material Available: Table I listing ultraviolet spectral data for certain pyrazolo[3,4-*d*]pyrimidines (2 pages). Ordering information is given on any current masthead page.

Synthesis and Biological Activity of Certain Derivatives of Oxazinomycin and Related Oxadiazole Nucleosides

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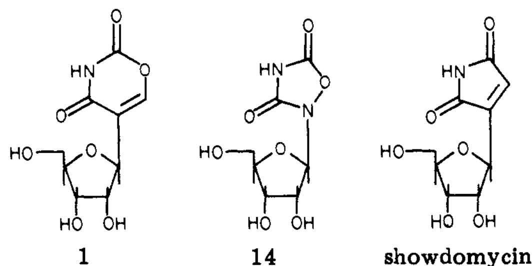
Oxazinomycin was converted into 2′,3′,5′-tri-*O*-acetyloxazinomycin (**2**) and 2′,3′-*O*-isopropylideneoxazinomycin (**3**), respectively. Compound **3** was iodinated and reduced to provide 5′-deoxy-2′,3′-*O*-isopropylideneoxazinomycin (**5**) which, after acid hydrolysis, provided 5′-deoxyoxazinomycin (**6**). Alternatively, the iodination of oxazinomycin followed by catalytic hydrogenation also provided **6**. Oxazinomycin was treated with 2-acetoxybenzoyl chloride to provide 3′-*O*-acetyl-2′-chloro-2′-deoxyoxazinomycin (**8**) which, after reduction with tributyltin hydride, provided 3′-*O*-acetyl-2′-deoxyoxazinomycin (**9**). Oxazinomycin was also converted into oxazinomycin 5′-phosphate (**10**) and into O^4,2′-anhydrooxazinomycin (**11**). 1,2,4-Oxadiazole-3,5-dione (**12**) was glycosylated to provide 2-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-oxadiazole-3,5-dione (**13**) which, after deacetylation, provided 2-β-D-ribofuranosyl-1,2,4-oxadiazole-3,5-dione (**14**). Similarly, **12** provided 2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3,5-dione (**17**); **14** was also converted into the corresponding 2′,3′-*O*-isopropylidene derivative **15**. Compound **14** showed significant antiviral activity against herpes simplex virus type 1, in vitro.

Various pyrimidine analogue nucleosides, such as cytosine arabinoside (*ara*-C), 3-deazauridine, 5-azacytidine, and 5-azacytosine arabinoside, have shown great promise in cancer chemotherapy.[1] A close examination would reveal that much of the interest in the synthetic nucleosides is due to their varied biological activities, which result from the close structural relationship of these synthetic molecules to the "natural" nucleoside metabolites. Deletion, introduction, or exchange of a hetero atom in aglycon and a modification in the sugar moiety could have interesting effects on the biological activity. Oxazinomycin[2] (minimycin,[3] **1**) is a C-nucleoside antibiotic and is structurally



related to uridine and pseudouridine. However, due to the oxazinedione ring system, oxazinomycin is quite susceptible to hydrolytic cleavage and is consequently less stable

(1) R. F. Struck, *Annu. Rep. Med. Chem.*, 15, 130 (1980).
(2) T. Haneishi, T. Okazaki, T. Hata, C. Tamura, M. Nomura, A. Naito, I, Seki, and M. Arai, *J. Antibiot.*, 24, 797 (1971).
(3) K. Sasaki, Y, Kusakabe, and S. Esumi, *J. Antibiot.*, **25**, 151 (1972).

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