Synthesis and Biological Activity of a Novel Adenosine Analogue, 3-β-D-Ribofuranosylthieno[2,3-*d*]pyrimidin-4-one

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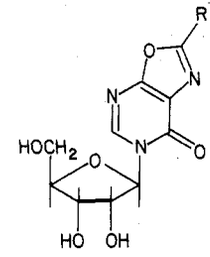
The title nucleoside **5** was prepared by a condensation of the silylated heterocycle thieno[2,3-*d*]pyrimidin-4-one (**1**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2a**) in the presence of a Lewis acid or with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide (**2b**) in the presence of mercuric oxide and mercuric bromide. The site of ribosylation and anomeric configuration of this nucleoside were established by ^{1}H NMR. The synthesis of 3-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidin-4-one (**8**), 1-phenyl-5-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-one (**9**), 5-methyl-3-β-D-ribofuranosylthieno[2,3-*d*]pyrimidin-4-one (**10**), and 2-methyl-6-β-D-ribofuranosyltriazolo[5,4-*d*]pyrimidin-7-one (**11**) is also described. The title compound inhibited the growth of murine L-1210 leukemic cells in vitro with an ID_{50} of 3×10^{-5} M. The growth inhibition could not be prevented by uridine, cytidine, thymidine, deoxycytidine, cytosine, hypoxanthine, or uridine and hypoxanthine together. On the other hand, inhibition of adenosine kinase by 10^{-7} M 5-iodotubercidin prevented the cytotoxic effect. Also a subline of L-1210 cells resistant to several cytotoxic adenosine analogues was also resistant to this nucleoside. Thus it appears that this compound **5** may act as an adenosine analogue.

We have pursued a strong interest in the chemical synthesis of bicyclic heterocyclic nucleosides which possess a pyrimidine ring fused to various five-membered heterocyclic systems with the glycosidic linkage positioned on a nitrogen atom in the pyrimidine ring. The rationale behind this program was based in part on the reported isolation of 7-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4,6-dione from patients being treated with allopurinol and on the ability of this nucleoside to inhibit pyrimidine biosynthesis de novo.[1] Additional interest in this area of research was generated by the reported in vitro and in vivo inhibition of various tumor cell lines by 3-β-D-ribofuranosyladenine.[2] During the course of this rather comprehensive study we have synthesized purine,[3-5] thiazolo[5,4-*d*]pyrimidine,[6] thieno[2,3-*d*]pyrimidine,[7] and oxazolo[5,4-*d*]pyrimidine[8] nucleosides which were similar to one another in that the carbohydrate moiety was bonded to the pyrimidine ring and as such were viewed as pyrimidine-type nucleosides. The evaluation of these compounds against L-1210 leukemic cells in vitro and in vivo showed that most of these nucleosides which were ribosylated at a position analogous to the N-3 in the purine ring system, i.e., N-6 in the thiazolo[5,4-*d*]pyrimidine ring system and N-1 of the thieno[2,3-*d*]pyrimidine, did not inhibit tumor growth.[3-7] However, it was subsequently discovered[8] that several 2-substituted 6-β-D-ribofuranosyloxazolo[5,4-*d*]-pyrimidin-7-ones (I) markedly inhibited the in vitro growth of both L-1210 leukemic cells and *Escherichia coli*, with inhibitory concentrations ranging from 5×10^{-7} to 8×10^{-4} M. These findings prompted us to investigate additional congeners of this type. We now report on the synthesis and antitumor evaluation of 3-β-D-ribofuranosylthieno-[2,3-*d*]pyrimidin-4-one (**5**) and several structurally similar



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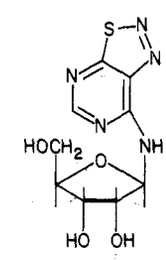

II

nucleosides. It is of considerable interest that compound **5** appears to be phosphorylated by adenosine kinase, suggesting that **5** acts as an analogue of the naturally occurring purine nucleoside adenosine, instead of a pyrimidine nucleoside.[9]

Results and Discussion

Chemistry. The synthetic route we used for the preparation of 3-β-D-ribofuranosylthieno[2,3-*d*]pyrimidin-4-one (**5**) is outlined in Scheme I. The heterocycle thieno[2,3-*d*]pyrimidin-4-one was converted into the trimethylsilyl derivative **1** with use of hexamethyldisilazane and heating at reflux temperature. With use of the Lewis acid catalyzed silyl procedure,[10] the silyl derivative **1** was condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2a**) in the presence of stannic chloride in 1,2-dichloroethane to furnish a single blocked nucleoside as a syrup. This syrup (**3a**), without purification, was treated with methanolic ammonia to afford the deblocked nucleoside **5** in a 25% overall yield. Due to the low yield by this procedure, the nucleoside **5** was then prepared by condensing[11] the silyl derivative **1** with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide (**2b**) in benzene at reflux temperature in the presence of mercuric oxide and mercuric bromide followed by treatment with methanolic ammonia. However, the overall yield of **5**, after deacetylation of the intermediate blocked nucleoside **3b** with methanolic ammonia, was still only a modest 30%. To substantiate that the product of these condensations was indeed the desired N-3 ribosylated heterocycle rather than the N-1 ribosylated heterocycle, the nucleoside **5** was first converted into the triacetate

(1) Krenitsky, T. A.; Elion, G. B.; Strelity, R. A.; Hitchings, G. H. *J. Biol. Chem.* **1967**, *212*, 2675.
(2) Gerzon, K.; Johnson, I. S.; Boder, G. B.; Cline, J. C.; Simpson, P. J.; Speth, C.; Leonard, N. J.; Laursen, R. A. *Biochim. Biophys. Acta* **1966**, *119*, 445.
(3) Schmidt, C. L.; Townsend, L. B. *J. Org. Chem.* **1972**, *37*, 2300.
(4) Schmidt, C. L.; Townsend, L. B. *J. Heterocycl. Chem.* **1973**, *10*, 68.
(5) Schmidt, C. L.; Townsend, L. B. *J. Chem. Soc., Perkins Trans.* **1975**, 1257.
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(7) Patil, V. D.; Wise, D. S.; Townsend, L. B. *J. Chem. Soc.* **1980**, 1853.
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(9) A preliminary report of these results has been presented: Wotring, L. L.; Bloomer, L. C.; Townsend, L. B. *Proc. Am. Assoc. Cancer Res.* **1980**, *21*, 304.
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(11) Wittenberg, E. *Chem. Ber.* **1968**, *101*, 1095.

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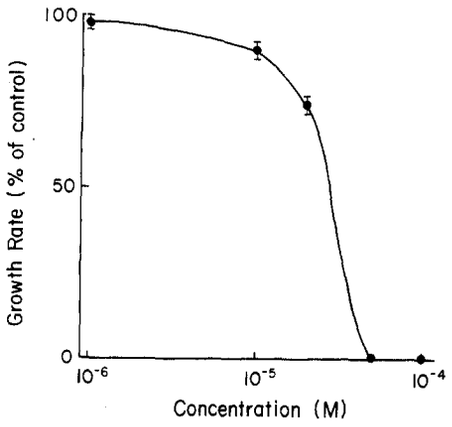
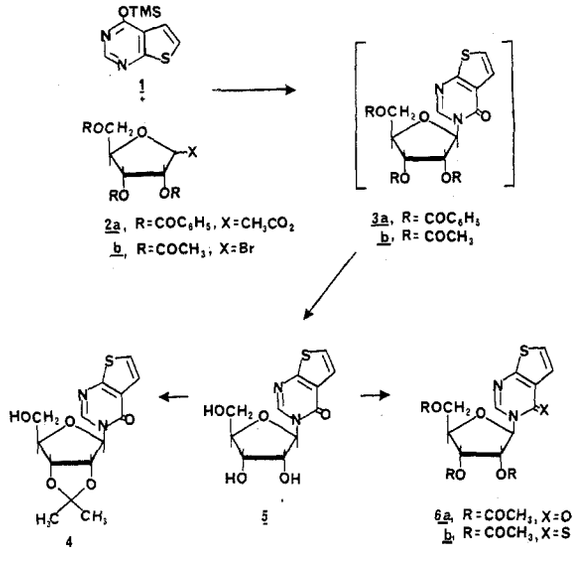


Figure 1. Dose–response for effect of **5** on growth rate of L-1210 cells in vitro. The values represent the means of four to seven determinations of growth rate (see Experimental Section), and the bars represent the standard error of the mean.

Scheme I



derivative **6a** with use of acetic anhydride and pyridine. The triacetate derivative **6a** was then treated with phosphorus pentasulfide in dioxane to furnish 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)thieno[2,3-*d*]pyrimidine-4-thione (**6b**). The chemical shift observed in the ^{1}H NMR spectra for the anomeric proton in **6b** appeared at δ 7.05, a significant downfield shift from the chemical shift (δ 6.13) of the peak attributed to the anomeric proton of **6a**. A downfield chemical shift of the anomeric proton resonance peak of a number of thionucleosides, attributed to the magnetic anisotropic effect of the thione group,[12] has been observed when the ribosyl moiety was on a ring nitrogen atom adjacent to or in very close proximity to the carbon atom bearing the thione group. This observation would seem to confirm that the site of glycosylation of **1** was indeed the N-3 atom. The assignment of anomeric configuration for **5** as β was based[13] on the observed difference of 0.23 Hz between the chemical shifts for the two methyl groups of the isopropylidene derivative **4**.

The stannic chloride procedure (described as method 1 in the synthesis of **5**) was also used to prepare the nucleosides **7–10**. However, the mercuric bromide/mercuric oxide method (described as method 2 in the synthesis of **5**) was used to prepare the nucleoside **11**.

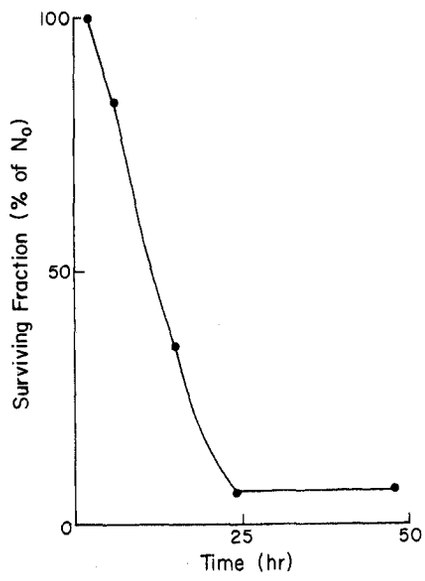
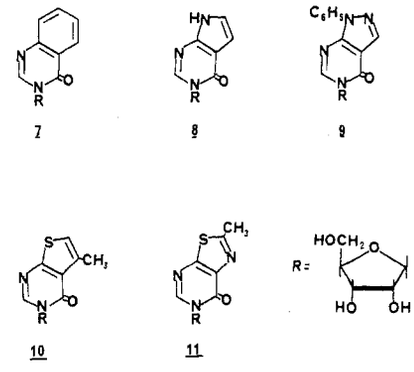


Figure 2. Effect of 10^{-4} M **5** on the viability of L-1210 cells after various treatment times. At the indicated times the cells were removed from the medium containing **5** and returned to normal medium, and their subsequent growth was monitored. The values represent the back extrapolation (see Experimental Section) of the growth curve consisting of the means of the six cell counts obtained in three experiments for the 24-h point and of duplicate cell counts in a single experiment for the remaining time points.

Scheme II



Antitumor and Biochemical Studies. In Vitro Cytotoxicity. 3-β-D-Ribofuranosylthieno[2,3-*d*]pyrimidin-4-one (**5**) inhibited growth of L-1210 cells in vitro (Figure 1). The ID_{50}, the concentration required to decrease the growth rate to 50% of the control rate, was 3×10^{-5} M, while 10^{-4} M caused total inhibition of cell growth. Furthermore, growth inhibition by **5** (10^{-4} M) was lethal to L-1210 cells. After treatment for various time intervals, the cells were returned to normal medium and their growth was monitored. The surviving fraction was estimated by back-extrapolation of the resulting growth curves, and the results are shown in Figure 2. Twenty-four-hour treatment of the cells with **5** (10^{-4} M) killed about 95% of the cells, and longer treatment, for a total of 48 h, did not increase the fraction killed. These results were confirmed by colony formation, an alternative method of determining cellular survival. After 24 or 48 h of treatment with **5** (10^{-4} M), ≥95% of the cells did not form colonies, as compared to control, untreated cells.

Nucleosides **7–11** (Scheme II) were also tested for their ability to inhibit growth of L-1210 cells in vitro. No effect on growth rate was observed for **7**, **9**, or **11** (10^{-4} M), while **8** and **10** at 10^{-4} M caused slight growth inhibition, with growth rates of 70% and 82% of control, respectively.

(12) Long, R. A.; Townsend, L. B. *J. Chem. Soc. D.* **1970**, 1087. Wise, D. S.; Townsend, L. B. *Tetrahedron Lett.* **1977**, 755.
(13) Imbach, J. L.; Barascut, J. L.; Kam, B.; Rayner, B.; Tapiero, C. *J. Heterocycl. Chem.* **1973**, *10*, 1069.

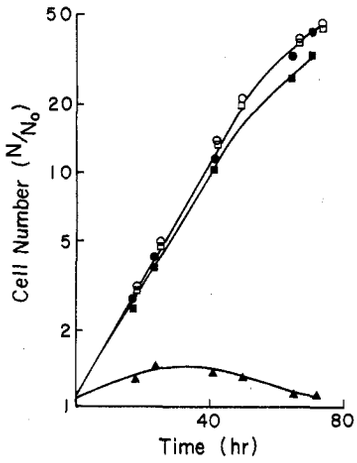


Figure 3. The effect of adenosine kinase deficiency on inhibition by 10^{-4} M **5** of L-1210 cell growth in vitro. The cell numbers for each experiment were normalized by calculating the ratio of the cell number at any time point, N, to the cell number at zero time, N_0, which was approximately 10^4 cells/mL. The solid points represent the parent, sensitive line of L-1210: (●) control, no **5**, with or without 10^{-7}M 5-iodotubercidin (ITu); (▲) 10^{-4} M **5**, no ITu; (■) 10^{-4} M **5**, 10^{-7} M ITu. The open points represent the resistant subline L-1210/T: (○) control, no additions; (□) 10^{-4} M **5**, no ITu. The values are the means of the four normalized cell counts obtained in two experiments, and the standard deviations were all less than 10% of the means.

Thus, the ID_{50} for both **8** and **10** was $>10^{-4}$ M.

Cellular and Biochemical Studies on the Metabolic Activation of 5. Biological activity of nucleosides generally requires metabolic activation to the 5′-monophosphate derivative.[14,15] The resemblance of **5** to a 3,4-disubstituted 5-azauridine initially suggested that it might be a substrate for uridine-cytidine kinase, which has previously been shown to activate a variety of pyrimidine-type nucleosides[16] including 5-azacytidine.[17] However, preliminary testing indicated that **5** did not appear to be an alternative substrate for uridine-cytidine kinase. Specifically, the phosphorylation of uridine (40 μM) by this enzyme, partially purified from murine kidney as previously described (K_m for uridine = 82 μM),[18] was not inhibited by the addition of 1000 μM **5**; i.e., a 25-fold excess of **5** did not effectively compete with the natural substrate, which was present at $^1/_2$ its K_m concentration. This result indicated that **5** did not interact with uridine-cytidine kinase to a significant degree and therefore could not be considered a possible alternative substrate.

Adenosine kinase was then investigated as a possible activating enzyme for **5**. For these studies, L-1210 cells were made deficient in adenosine kinase, first by selection of a subline resistant to cytotoxic analogues of adenosine[19] and second by treatment of cells from the parent, sensitive line with 5-iodotubercidin, an inhibitor of adenosine kinase.[20] As shown in Figure 3, at 10^{-4} M (the highest concentration tested), **5** did not inhibit the growth of L-1210 cells when the activity of adenosine kinase was decreased with use of either of these two approaches. Thus, it is clear that adenosine kinase is required for the activation of **5** to its cytotoxic form, presumably a phosphorylated derivative. While **5** is somewhat unusual as a substrate for adenosine kinase, there is precedent for this enzyme phosphorylating compounds that in some ways resemble **5**, for example, a bicyclic nucleoside with the glycosidic bond to a pyrimidine ring, namely, 3-β-D-ribofuranosyladenine (3-isoadenosine),[21] and a ribosyl amine in which a sulfur-containing five-membered ring is fused to the pyrimidine ring in a position analogous to the thieno ring of **5**, namely, 7-(1-β-D-ribofuranosylamino)[1,2,3]-thiadiazolo[5,4-*d*]pyrimidine (II).[22]

A preliminary indication of the biochemical effect of the cytotoxic metabolite of **5** was sought by testing for prevention of the L-1210 cell growth inhibition by various naturally occurring purine and pyrimidine bases and nucleosides (Table I). None of the compounds listed had any effect on the inhibition of L-1210 cell growth inhibition by **5**. Thus, it was clear that inhibition of purine or pyrimidine de novo synthesis or of pyrimidine interconversions was not the mechanism of the cytotoxicity of **5**. On the other hand, adenine and adenosine both slightly decreased the effect of **5** on the initial growth rate of L-1210 cells (Table II). The cell number in these cultures reached

Table I. Naturally Occurring Purine and Pyrimidine Bases and Nucleosides Which Had No Effect on L-1210 Cell Growth Inhibition by **5**[a]

compd	concn range, M	concn range of 5 tested, M
uridine	10^{-4} to 10^{-3}	10^{-4}
thymidine	10^{-5} to 10^{-4}	2×10^{-5} to 10^{-4}
cytidine	10^{-4} to 10^{-3}	10^{-4}
2′-deoxycytidine	10^{-4} to 5×10^{-4}	10^{-4}
cytosine	10^{-4}	10^{-4}
cytidine	10^{-4}	10^{-4}
plus 2′-deoxycytidine	10^{-4}	
hypoxanthine	5×10^{-5}	10^{-5} to 10^{-4}
hypoxanthine	5×10^{-5}	2×10^{-5} to 10^{-4}
plus uridine	5×10^{-5}	

[a] The growth rate of L-1210 cells was determined in cultures containing **5** alone and with the naturally occurring compounds listed. The latter were used singly and in combinations as indicated. The concentration, or range of concentrations, of each naturally occurring compound is listed in the second column and that of **5** in the third column. The naturally occurring compounds alone had no effect on the growth rate of L-1210 cells. Each experiment was performed one to four times.

Table II. Effect of Adenine and Adenosine on Inhibition of L-1210 Cell Growth by **5**[a]

compd added to culture	initial growth rate, % of control	
	5×10^{-5} M **5**	10^{-4} M **5**
5 alone	32 (±3.8)[b]	16 (±3.0)[b]
5 + 10^{-5} M adenine	52[c]	25
5 + 10^{-5} M adenosine	57[c]	50

[a] Initial growth rate refers to the rate of increase in the cell number during the first 24 h of incubation with the indicated compounds. [b] Mean (± SD), $n = 5$. [c] Mean, $n = 2$.

(14) Hakala, M. T. In "Drug Resistance and Selectivity, Biochemical and Cellular Basis"; Mihich, E., Ed.; Academic Press: New York, 1973; p 263.
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a plateau after about 48 h, as compared with about 24 h when the cells were treated with **5** alone. Also, the total cell number reached was significantly higher when adenine or adenosine was added. This decrease in the cytotoxicity of **5** in the presence of either adenine or adenosine suggests that a metabolite of **5**, presumably a nucleotide, competitively inhibits a reaction for which an adenine nucleotide is the substrate, to produce the cytotoxic action of **5**. Possible sites of action would include various steps in glycolysis and synthesis of nucleic acids. At present, no data is available on the validity of these or other possible mechanisms of action of **5**.

In Vivo Antitumor Testing. Compound **5** did not significantly lengthen the lifespans of mice bearing L1210 leukemia, when **5** was administered once daily for 9 days in doses of 12.5–400 mg/kg. At doses ≥ 100 mg/kg, the lifespan of the mice was shortened by the toxic effects of **5**.

Experimental Section

Chemistry. Proton magnetic resonance (^{1}H NMR) spectra were obtained with a Varian A56/60 or EM-390 spectrometer (solutions in dimethyl-d_6 sulfoxide or deuteriochloroform with sodium 2,2-dimethyl-2-disilapentane-5-sulfonate or tetramethylsilane, respectively, as internal standard), with chemical shift values reported in δ, parts per million, relative to the internal standard. Ultraviolet spectra were recorded on a Beckman Acta CIII spectrophotometer. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Thin-layer chromatography was run on glass plates coated (0.25 mm) with silica gel (SilicAR 7GF, Mallinckrodt). Compounds of interest were detected by either ultraviolet lamp (254 nm), iodine vapors, or treatment with sulfuric acid followed by heating. Evaporations were performed under reduced pressure at 40 °C with a rotary evaporator unless otherwise stated. Elemental analysis were performed by M-H-W Laboratories, Phoenix, AZ. Analytical results indicated by elemental symbols were within ±0.4% of the theoretical values. Water of hydration was substantiated by ^{1}H NMR.

3-β-D-Ribofuranosylthieno[2,3-*d*]pyrimidin-4-one (5). Method 1. Thieno[2,3-*d*]pyrimidin-4-one[23] (3.04 g, 20 mmol) was silylated with hexamethyldisilazane (HMDS, 80 mL), by heating the solution at reflux temperature for 4 h. The excess HMDS was removed by vacuum distillation and the silylated base, without further purification, was dissolved in 1,2-dichloroethane (60 mL). To this solution was added 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (10.12 g, 20 mmol) followed by stannic chloride (4 mL), and the mixture was stirred for 18 h at room temperature. Pyridine (4 mL) was then added, and the precipitate which formed was collected by filtration and washed with chloroform (2 × 30 mL). The filtrate and wash were combined and extracted with saturated aqueous sodium bicarbonate (2 × 120 mL) and water (3 × 80 mL). The organic layer was dried over sodium sulfate and filtered, and the sodium sulfate was washed with chloroform (50 mL). The combined filtrate and washings were evaporated to dryness, and the resulting residue was treated with methanolic ammonia (225 mL, saturated at −5 °C) in a sealed pressure bottle at room temperature for 24 h. The solution was then evaporated to dryness and the residue extracted with ethyl acetate (20 mL). The resulting solid was recrystallized from water to yield 1.4 g (25%) of **5**: mp 184–185 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.1 (d, 1, H-1′, $J_{1',2'}$ = 4.0 Hz), 7.38 (d, 1, H-5, $J_{5,6}$ = 6 Hz), 7.63 (d, 1, H-6, $J_{6,5}$ = 6 Hz), 8.70 (s, 1, H-2); UV λ_{max} nm ($\epsilon \times 10^{-3}$) pH 1, 293 (7.1), 264 (5.4); MeOH, 294 (7.2), 263 (4.5); pH 11, 293 (7.1), 264 (5.1), 227 (15.3). Anal. ($C_{11}H_{12}N_2O_5S \cdot H_2O$) C, H, N.

Method 2. The silylated heterocycle 1 [prepared as in method 1, from 3.04 g (20 mmol) of thieno[2,3-*d*]pyrimidin-4-one] was dissolved in benzene (50 mL) and then added to a suspension of mercuric bromide (5 g) and mercuric oxide (5 g) in 250 mL of azeotropically dried benzene. 2,3,5-Tri-*O*-acetyl-D-ribofuranosyl bromide (**2b**)[24] (prepared from 6.36 g of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose) was dissolved in 50 mL of anhydrous benzene and this solution was added to the above reaction mixture. The suspension protected from moisture was heated at reflux temperature for 20 h. The reaction mixture was allowed to cool, and the mercury salts were removed by filtration through a Celite pad, and the Celite pad was washed with chloroform (3 × 50 mL). The filtrate and washings were combined and evaporated in vacuo to afford a syrup. This syrup was dissolved in chloroform (350 mL) and the solution was washed successively with a 30% aqueous solution of potassium iodide (3 × 50 mL), a cold saturated aqueous sodium bicarbonate solution (4 × 50 mL), and distilled water (4 × 50 mL) before being dried over anhydrous sodium sulfate. The drying agent was removed by filtration. The chloroform solution was then evaporated under reduced pressure to afford a syrup (7 g). The syrup was treated with methanol (400 mL), which had been previously saturated with ammonia at 0 °C. The solution was allowed to stand at room temperature for 20 h with occasional shaking. The solution was filtered, and the filtrate was evaporated in vacuo to yield a solid material which was crystallized from water to yield 1.8 g (30%) of **5** identical in all respects with **5** prepared by method 1.

3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)thieno[2,3-*d*]pyrimidine-4-thione (6b). Compound **5** (300 mg, 1.1 mmol) was dissolved in pyridine (4 mL) and acetic anhydride (3 mL) was added dropwise over a period of 30 min. The reaction mixture was stirred at room temperature for 18 h. The solvent was removed in vacuo (bath temperature 50 °C), and the resulting residue was coevaporated with ethanol (3 × 5 mL). The residue was dissolved in chloroform (20 mL) and extracted successively with 5% aqueous hydrochloric acid (1 × 10 mL), a cold saturated aqueous sodium bicarbonate solution (2 × 5 mL), and then cold water (2 × 5 mL). The organic layer was dried over magnesium sulfate, and the drying agent was then removed by filtration, and the chloroform mixture was evaporated under reduced pressure to afford 420 mg of **6a** as a syrup: ^{1}H NMR (Me_2SO-d_6) δ 2.06 (s, 3, CH_3), 2.11 (s, 3, CH_3), 6.13 (d, 1, H-1′, $J_{1',2'}$ = 4 Hz), 7.41 (d, 1, H-5, $J_{5,6}$ = 6 Hz), 7.65 (d, 1, H-6, $J_{6,5}$ = 6 Hz), 8.48 (s, 1, H-2); UV λ_{max} nm ($\epsilon \times 10^{-3}$) pH 1, 294 (6.9), 263 (4.5); MeOH, 294 (7.1), 263 (5.1); pH 11, 294 (7.0), 263 (5.0). The blocked intermediate **6a** was used directly without further purification in the thiation reaction. The blocked nucleoside **6a** (400 mg) and phosphorus pentasulfide (0.22 g) were added to dioxane (20 mL), and the mixture was heated at reflux temperature for 40 min. A further charge of phosphorus pentasulfide (0.22 g) was added and the heating continued for an additional 8 h. The resulting solution was evaporated to dryness, water (30 mL) was added, and the suspension was heated on a stream bath with stirring for 1 h. The mixture was then extracted with chloroform (2 × 25 mL). An aqueous saturated sodium chloride solution (15 mL) was added to break up the observed emulsion. The organic layer was washed with saturated aqueous sodium chloride (3 × 15 mL) and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the filtrate was evaporated to dryness. The resulting syrup was dissolved in a minimum amount of chloroform (2 mL), and this solution was applied to the top of a dry packed SilicAR CC-7 chromatography column (3.5 × 20 cm) and then eluted with a chloroform:acetone mixture (95:5, v:v). The fractions containing **6b** were combined and evaporated to dryness. The residue was crystallized from ethanol to yield 80 mg (20%) of **6b**: mp 184 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.11 (s, 3, CH_3), 2.17 (s, 3, CH_3), 7.05 (d, 1, H-1′, $J_{1',2'}$ = 3 Hz), 7.58 (d, 1, H-5, $J_{5,6}$ = 5 Hz), 7.79 (d, 1, H-6, $J_{6,5}$ = 5 Hz), 8.8 (s, 1, H-2); UV λ_{max} nm ($\epsilon \times 10^{-3}$) pH 1, 327 (10.2), 275 (9.8), 228 (23.0); MeOH, 317 (10.7), 273 (8.3), 225 (20.9); pH 11, 327 (10.2), 274 (9.0), 231 (17.5). Anal. ($C_{17}H_{18}N_2O_7S_2 \cdot 0.5H_2O$) C, H, N.

3-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)thieno[2,3-*d*]pyrimidin-4-one (4). Compound **5** (284 mg, 1 mmol) was suspended in anhydrous acetone (200 mL) and the reaction mixture cooled to −5 °C. Perchloric acid (2 drops) was added and the mixture was allowed to warm to room temperature and was then stirred for 2 h. Sodium bicarbonate (2 g) was added and

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the stirring continued for an additional 0.5 h. The mixture was filtered, and the resulting residue was washed with acetone (20 mL). The combined filtrate and washings were evaporated to dryness, and the residue was triturated with chloroform (30 mL) and subsequently crystallized from water to yield 160 mg (49%) of compound **4**: mp 165 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.46 (s, 3, CH_3), 1.69 (s, 3, CH_3), 6.28 (d, 1, H-1′, $J_{1',2'}$ = 1.5 Hz), 7.50 (d, 1, H-5, $J_{5,6}$ = 6 Hz), 7.73 (d, 1, H-6, $J_{6,5}$ = 6 Hz), 8.68 (s, 1, H-2). Anal. ($C_{14}H_{16}N_2O_5S$) C, H, N.

3-β-D-Ribofuranosylquinazolin-4-one (7). This nucleoside was prepared by a procedure similar to the one used to prepare **5** (method 1). The yield of **7**, crystallized from methanol, was 27%; mp 164 °C (lit.[25] mp 164 °C).

3-β-D-Ribofuranosylpyrrolo[2,3-*d*]pyrimidin-4-one (8). This nucleoside was prepared by a procedure similar to the one used to prepare **5** (method 1). The yield of **8**, crystallized from methanol, was 25%: mp 181–182 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.24 (d, 1, H-1′, $J_{1',2'}$ = 2 Hz), 6.59 (d, 1, H-5, $J_{5,6}$ = 4 Hz), 7.16 (d, 1, H-6, $J_{6,5}$ = 4 Hz), 8.53 (s, 1, H-2); UV λ_{max} nm (ε × 10^{-3}) pH 1, 264 (8.8); MeOH, 261 (8.5); pH 11, 264 (8.7). Anal. ($C_{11}H_{13}N_3O_5 \cdot H_2O$) C, H, N.

1-Phenyl-5-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-one (9). This nucleoside was prepared by a procedure similar to the one used to prepare **5** (method 1). The yield of **9**, crystallized from methanol, was 30%: mp 182–183 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.28 (d, 1, H-1′, $J_{1',2'}$ = 3 Hz), 7.9 (m, 5, C_6H_5), 8.5 (s, 1, H-6), 9.03 (s, 1, H-3); UV λ_{max} nm (ε × 10^{-3}) pH 1, 277 (11.4); MeOH, 272 (10.0); pH 11, 272 (9.6). Anal. ($C_{16}H_{16}N_4O_5 \cdot 2H_2O$) C, H, N.

5-Methyl-3-β-D-ribofuranosylthieno[2,3-*d*]pyrimidin-4-one (10). This nucleoside was prepared by a procedure similar to the one used to prepare **5** (method 2). The yield of **10**, crystallized from water, was 40%: mp 190–191 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.55 (s, 3, CH_3), 6.16 (d, 1, H-1′, $J_{1',2'}$ = 2 Hz), 7.17 (s, 1, H-6), 8.73 (s, 1, H-2); UV λ_{max} nm (ε × 10^{-3}) pH 1, 301 (7.5); MeOH, 301 (7.8); pH 11, 301 (7.5). Anal. ($C_{12}H_{14}N_2O_5S$) C, H, N.

2-Methyl-6-β-D-ribofuranosylthiazolo[5,4-*d*]pyrimidin-7-one (11). This nucleoside was prepared by a procedure similar to the one used to prepare **5** (method 1). The yield of **11**, crystallized from acetone, was (25%): mp 190–191 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.83 (s, 3, CH_3), 6.15 (d, 1, H-1′, $J_{1',2'}$ = 3 Hz), 8.87 (s, 1, H-5); UV λ_{max} nm (ε × 10^{-3}) pH 1, 286 (7.5), 270 (7.5), 262 (7.2); MeOH, 292 (7.2), 269 (6.8), 262 (6.8); pH 11, 287 (7.2), 270 (6.9), 262 (6.6). Anal. ($C_{11}H_{13}N_3O_5S$) C, H, N.

Antitumor and Biochemical Evaluation. L-1210 Cells. Murine leukemic cells L-1210 and the adenosine kinase deficient subline L-1210/T[19] were grown in static suspension cultures in Fischer's medium for leukemic cells of mice as described previously.[26] The growth rate relative to control, untreated cells, and the ID_{50}, the concentration required to reduce the growth rate to 50% of control, were determined from the data obtained by counting the cell number six times during a 3-day period after adding the test compound.[26] The viability of L-1210 cells was determined by using two methods: (1) *Growth curve back-extrapolation.*[26] After treatment for the designated time with the test compound, the cells were removed from the medium and returned to normal medium without compound. Their subsequent growth was monitored and the exponential portion of the resulting curve was extrapolated back to zero time to obtain an estimate of the minimal number of cells surviving the treatment. (2) *Colony formation in soft agar.*[26] After the cells were returned to normal medium as described above, they were diluted in soft agar to provide 20–200 colonies/tube.

Uridine Kinase. Murine kidney was the source of enzyme partially purified as described previously, by ammonium sulfate fractionation and gel filtration on Sephacryl S-200 Superfine.[18] The activity of uridine kinase was assayed by using [2-^{14}C]uridine as the substrate, collecting the phosphorylated products on DEAE-cellulose filter disks, and counting the radioactive products by liquid scintillation counting.[18]

In Vivo Antitumor Testing. These results were provided though the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, by the screening contractors using standard protocols.[27] The mice were inoculated on day 0 with 10^5 tumor cells, and drug administration was initiated on day 1.

Other Compounds. 5-Iodotubercidin was prepared according to the procedure published previously.[28] The naturally occurring heterocyclic bases and nucleosides were obtained from commercial sources.

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Registry No. **1**, 94620-72-1; **2a** (β-isomer), 6974-32-9; **2b**, 39110-68-4; **3a**, 94620-73-2; **3b**, 94620-74-3; **4**, 94620-75-4; **5**, 94644-73-2; **6a**, 94620-74-3; **6b**, 94620-76-5; **7**, 23701-75-9; **8**, 30066-71-8; **9**, 94620-77-6; **10**, 94620-78-7; **11**, 94620-79-8; thieno[2,3-*d*]pyrimidin-4-one, 94620-80-1; quinazolin-4-one, 491-36-1; 3*H*-pyrrolo[2,3-*d*]pyrimidin-4-one, 3680-71-5; 1-phenyl-5*H*-pyrazolo[3,4-*d*]pyrimidin-4-one, 21314-17-0; 3*H*-5-methylthieno[2,3-*d*]pyrimidin-4-one, 43088-64-8; 2-methyl-6*H*-thiazolo[5,4-*d*]pyrimidin-7-one, 5021-51-2; adenosine kinase, 9027-72-9.

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