mixture was filtered through Celite, and the organic layer was separated. The aqueous layer was extracted again with EtOAc (2 × 300 mL), and the combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was chromatographed on a silica gel column (50 g), which was eluted first with $CHCl_3$ (250 mL) and then with 19:1 $CHCl_3$–EtOH. Evaporation of appropriately pooled 5–10 mL volumes of eluate yielded a straw-colored foam (1.8 g, 28%): R_f 0.60 (silica gel; 19:1 $CHCl_3$–EtOH); NMR ($CDCl_3$) δ 7.45 (d, 1, J = 6 Hz, H_6), 5.95 (d, 1, J = 2 Hz, $H_{1'}$), 5.53 (m, 2, $H_{2'}$ and $H_{3'}$), 4.6–3.8 (m, CH_2O), 2.15 and 2.10 (s, 6, CH_3CO). Anal. ($C_{12}H_{13}FN_2O_7 \cdot 0.6C_2H_5OH \cdot 0.2CHCl_3$) C, H, N.

1-β-D-**Erythrofuranosyl-5-fluorouracil** (8). The diacetate 13 (1.6 g, 5.1 mmol) was dissolved in MeOH (50 mL), and 1 M NaOMe (16 mL) was added. The resultant clear solution was stirred at room temperature for 2 h. Dowex 50W-X8 (H^+) was then added, stirring was continued for another 15 min, and the resin was removed by filtration. Evaporation of the filtrate under reduced pressure left a straw-colored residue, which was recrystallized from a mixture of MeOH and EtOAc to give a colorless solid (0.89 g, 76%): mp 192–194 °C; R_f 0.51 (silica gel; 7:2:1 i-PrOH–H_2O–concentrated NH_4OH), 0.84 (cellulose, H_2O); UV (EtOH) λ_{max} 269 nm; NMR (Me_2SO-d_6) δ 8.03 (d, 1, J = 7 Hz, H_6), 5.66 (d, 1, J = 3 Hz, $H_{1'}$), 5.3–5.0 (m, 2, $H_{2'}$ and $H_{3'}$), 4.3–3.9 (m, 2, CH_2O). Anal. ($C_8H_9FN_2O_5$) C, H, N.

Antitumor Assays. Standard NCI protocols were employed.[25] Groups of five male B6D2F$_1$J mice (Jackson Laboratories, Bar Harbor, ME) were inoculated ip with 10^6 P-388 leukemic cells on day 0, and drug treatment was begun on day 1 for 4 consecutive days (qd × 4). All compounds except 5 were administered in sterile saline; compound 5 was given in 10% Tween 80. The increase in survival (ILS) was calculated according to the formula % ILS = [(T/C) − 1]100, where T and C are the median survival times in days for the treated and control groups, respectively. All animals were weighed on days 1 and 7, and 7-day weight changes were calculated as a percentage. The results are given in Table I.

Analysis of Nucleoside Purity by HPLC. Compounds 1–3, 5, and 6 were analyzed by HPLC on a Waters 10μ C_8 reversed-phase column (RCM 8 radial compression cartridge, Waters Associates, Milford, MA) using 0.01 M sodium phosphate buffer, pH 5.7, as the mobile phase and a flow rate of 3 mL/min. Compound 8 was analyzed on a Waters μBondapak CN column with 15% EtOH in isooctane as the mobile phase and a flow rate of 1 mL/min. 5-Bromo-2′-deoxyuridine was used as an internal standard, with eluting peaks being monitored at 280 nm. Excellent base-line separation between FUR (2) and the other nucleosides was obtained, and it was determined by using standard mixtures that as little as 0.01% of 2 could be detected if it were present as a contaminant of 1, 3, or 5. Similarly, 8 and FU were readily separable and it was established that as little as 0.01% FU could be detected as a contaminant of the nucleoside 8. The following analyses of *maximum* contamination by 2 were obtained: 3, 0.02%; 5, 0.01%; other nucleosides, all <0.01%. The maximum possible content of FU in the sample of 8 was determined to be 0.7%.

Cytotoxicity Assays. Cell growth inhibition by the compounds described in this paper was measured as described previously,[30] using L1210 murine leukemia cells in Eagle's minimal essential medium supplemented with 15% fetal calf serum and containing streptomycin (100 μg/mL), penicillin (100 units/mL), and 0.05 mM 2-mercaptoethanol. Cells were counted after 48 h with the aid of a Coulter hemocytometer (Model F). Assays were performed in triplicate and have a standard deviation of ±10%. The results are given in Table II.

Acknowledgment. This work was supported in part by Grants CA 23151 and CA 19589 from the National Cancer Institute (NIH). The authors are indebted to Linda Anderson and Gerda Swedowsky for their help in obtaining in vivo antitumor data and in vitro cytotoxicity data, respectively.

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Pyrazolo[3,4-*d*]pyrimidine Ribonucleosides as Anticoccidials. 2. Synthesis and Activity of Some Nucleosides of 4-(Alkylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidines

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A series of 4-(alkylamino)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines was synthesized by enzymatic and chemical methods. On the basis of the previous finding that 4-(alkylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines were effective anticoccidial agents, this series was examined for efficacy against *Eimera tenella* in chicks. The most active anticoccidial agent in the present study was the 4-cyclopentylamino derivative (8), which cleared chicks of the parasite at 200 ppm in the diet. Some members of this series were toxic to embryonic chick liver cells, mouse cells, and human cells in vitro. The 4-diethylamino derivative (16), which was not toxic in vitro, appeared to be toxic in chicks.

In the first paper of this series,[1] it was shown that ribonucleosides of 4-(alkylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidines inhibited the growth of avian coccidia both in vitro and in vivo. The most active compound of that series was the 4-ethylthio derivative. As part of this continuing investigation, a series of 4-(alkylamino)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines was synthesized, and the structure–activity relationships were investigated.

Results and Discussion

Chemistry. Scheme I shows the methods (A–D) used to prepare the compounds. Methods are also indicated in Table II, and examples are provided under Experimental

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(1) Krenitsky, T. A.; Rideout, J. L.; Koszalka, G. W.; Inmon, R. B.; Chao, E. Y.; Elion, G. B.; Latter, V. S.; Williams, R. B. *J. Med. Chem.* **1982**, *25*, 32.

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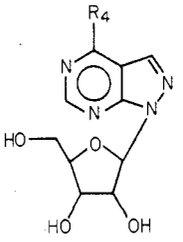
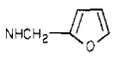
Table I. Anticoccidial Activities of Some Pyrazolo[3,4-*d*]pyrimidine Ribonucleosides



no.	R_3	in vitro:[a] MIC, mg/L	in vivo:[b] ppm in diet 400	200	150	100	50	25
1	OH	20	0					
2	NH_2	20 (T)		0				
3	$NHC(=O)C_6H_5$	5 (T)		0				
4	$NHNH_2$	5	0[c]					
5	$NHCH_3$	0.31		0				
6	NHC_2H_5	5	1					
7	NHC_4H_9	1.25		0				
8	NH-cyclopentyl	0.31		5		0	2	0
9	$NHCH_2CH=C(CH_3)_2$	5						
10	NHC_6H_{13}	1.25		0				
11	NHC_8H_{17}	5			0[c]			
		0.31		0				
13	$NHCH_2C_6H_5$	0.31		0[c]				
14	$NH(CH_2)_2C_6H_5$	5			2			
15	$N(CH_3)_2$	5		2				
16	$N(C_2H_5)_2$	5	1[d]					
17	$N(C_2H_5)C_4H_9$	5		0				
18	$N(C_6H_{13})_2$	>20		1				
19	$c\text{-}N(CH_2CH_2)_2O$	20 (ST)	0					

[a] Minimum inhibitory concentration (MIC) was the least amount of compound that completely inhibited the growth of *Eimera tenella* in vitro. (T = toxic; ST = slightly toxic). [b] Number of chicks cleared of *Eimeria tenella* lesions out of a group of five. [c] One chick out of a group of five died and contained lesions due to the parasite. [d] Chicks showed decreased weight gains and abnormal feces, suggesting that the compound was itself toxic.

Table II. Synthetic Data and Physical Constants for Pyrazolo[3,4-*d*]pyrimidine Ribonucleosides

no.	formula	synth method[a]	yield, %	mp,[b] °C	$[\alpha]^{20}_D$ (*c* 0.5, DMF), deg
1	$C_{10}H_{12}N_4O_5 \cdot 0.5H_2O$	A	24	172-175	-70.1
2	$C_{10}H_{13}N_5O_4 \cdot H_2O$	A	30	251-254	-81.2[c]
3	$C_{17}H_{17}N_5O_5 \cdot 0.5H_2O$	A	50	105-107 (softens 71)	-61.5[c]
4	$C_{10}H_{14}N_6O_4$	D	70	188-189	-78.5[c]
5	$C_{11}H_{15}N_5O_4$	B	50	224-225	-75.5
6	$C_{12}H_{17}N_5O_4 \cdot 0.2H_2O$	D	55	188-189	-72.2[c]
7	$C_{14}H_{21}N_5O_4 \cdot 0.65H_2O$	B	37	118-119	-67.9
7	$C_{14}H_{21}N_5O_4 \cdot 0.60H_2O$[d]	C	38	120-124	-62.3[c]
8	$C_{15}H_{21}N_5O_4 \cdot 0.25H_2O$	D	50	179-180	-65.6
9	$C_{15}H_{21}N_5O_4 \cdot 0.4H_2O$	C	12	96-100	-67.0[e]
10	$C_{16}H_{25}N_5O_4 \cdot H_2O$	D	20	68-70	-63.3
11	$C_{18}H_{29}N_5O_4 \cdot 0.7H_2O$	D	20	114-116	-55.7
12	$C_{15}H_{17}N_5O_5 \cdot 0.75H_2O$	D	30	104-106	-64.2
13	$C_{17}H_{19}N_5O_4 \cdot 0.6H_2O$	D	15	130-131	-65.2
14	$C_{18}H_{21}N_5O_4 \cdot 0.3H_2O$	D	20	104-106	-60.6
15	$C_{12}H_{17}N_5O_4 \cdot 0.5H_2O$	A	57	187-189	-70.4[c]
16	$C_{14}H_{21}N_5O_4$	D	59	123-124	-68.7[c]
17	$C_{16}H_{25}N_5O_4 \cdot 0.5CH_3OH$[f]	B	20	40-51[g]	-66.2
18	$C_{22}H_{37}N_5O_4$	B	25	*h*	-52.8
19	$C_{14}H_{19}N_5O_5 \cdot 0.2H_2O$	D	77	161-166	-56.6[d]

[a] See Experimental Section. [b] Literature values: 1, 172-174 (see ref 4f); 2, 258-259,[5a] 253,[5b] 246;[2] 4, 220-222;[6a] 5, 232-233;[6a] 12, 124-125;[6c] 13, 185-186;[6c] 15, 192.5-193.[6a] [c] *c* 1, DMF. [d] H: calcd, 6.70; found, 6.25. [e] *c* 0.4, DMF. [f] C: calcd, 53.93; found, 54.35. NMR indicated the presence of methanol [CH_3, δ 3.16 (d, J = 4.5 Hz)]. [g] Amorphous. [h] Yellow glass.

Section with further details under Syntheses Section for those not exemplified in full. The yields obtained and some physical constants of the 1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines are given in Table II. The UV and NMR spectral data are provided only for compounds described in detail under Experimental Section.

The 4-amino- and 4-hydroxy-1*H*-pyrazolo[3,4-*d*]pyrimidines were supplied by Burroughs Wellcome Co. The 4-benzamido- and 4-(dimethylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidines were prepared according to literature procedures.[2,3]

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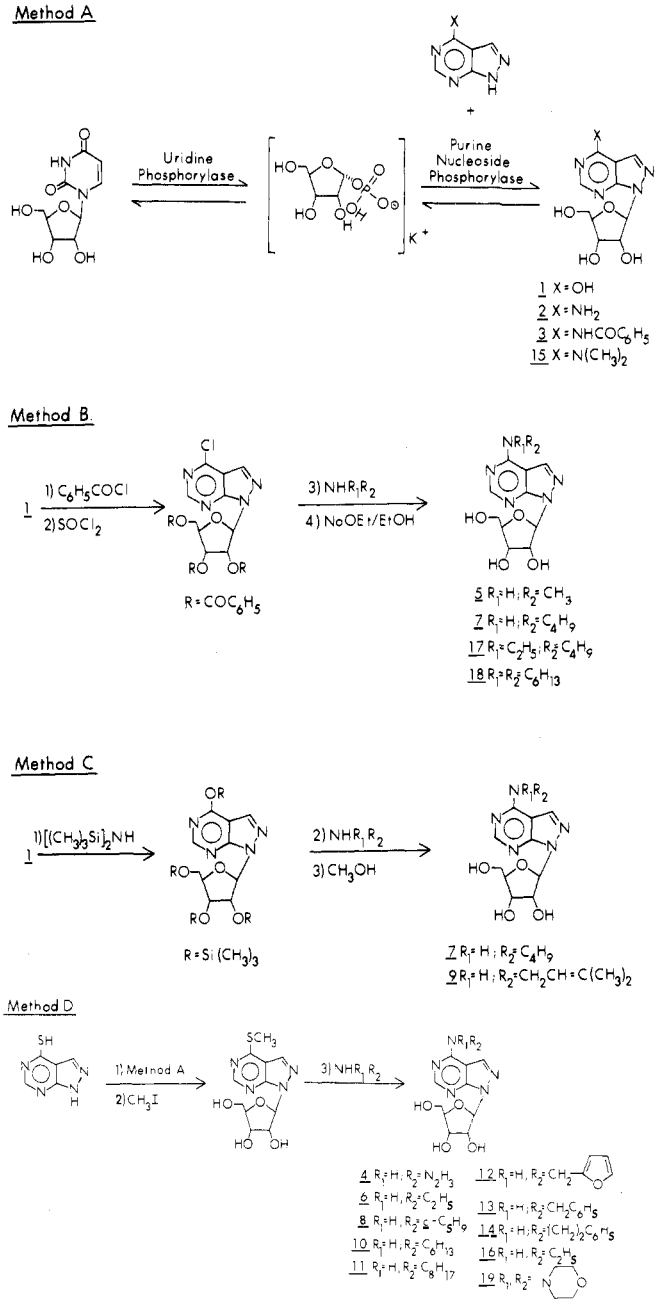
Scheme I



In this study, **1**,[4] **2**,[2,4g-i,5] **3** and **15**[6] were prepared from the corresponding heterocycles by enzymatic ribosylation[1,7] (Scheme I, method A). The details of the enzyme-catalyzed synthesis of **1** and **3** are provided under Experimental Section. Preparations of **7** (Scheme I, methods B and C) are also described in detail. A procedure similar to that outlined in method B (Scheme I) has been reported.[6] Method C in Scheme I was based on the work of Vorbrüggen and Kroliewiez[8] with purine ribonucleosides. Compound **7** was prepared by methods B and C. Two chromatographic purifications were necessary in the first; the second was a "one-pot" procedure. Equal yields of **7** resulted. The simplicity of the silylation reaction makes it an attractive method for the synthesis of ribonucleosides of 4-(alkylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidines. The 4-[(3-methyl-2-butenyl)amino] derivative (**9**) was prepared using method C. The enzymatic preparation and methylation of 4-(methylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine that were used in method D (Scheme I) have been reported.[1] The displacement of the 4-methylthio group by the appropriate amine was carried out as described in the literature,[6a] as for **4**, or in a slightly modified manner (Syntheses and Experimental Sections) to give the remainder of the known (**5**,[6] **12**,[6c] **13**[6c]) and novel (**6**, **8**, **10–12**, **14**, **16–19**) compounds, with **6** being described in detail.

Table III. Inhibitory Concentrations of Some Pyrazolo[3,4-*d*]pyrimidine Ribonucleosides for Cultured Human Detroit 98 and Mouse L Cells

no.	ED_{50}, μM	
	Detroit 98	L
2	0.08	0.3
3	>100	>100
4	20.0	100
9	1.5	>100
12	0.3	3.0
18	50	50

[a] Concentration that inhibited growth by 50%.

Biology. Table I presents the activities of some 4-(alkylamino)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines against developing schizonts of *Eimeria tenella* in vitro. In contrast to the predominantly nontoxic 4-alkylthio series,[1] several of the compounds in Table I showed toxicity to the host cells in the in vitro system. Compounds **2–5**, **8**, **10–13**, and **19** were toxic to the host cells at 20 mg/L, and for **2**, **3**, and **19**, toxicity was associated with the minimum inhibitory concentration (MIC[9]).

In view of the toxicity toward the chick host cells, these compounds were examined for their ability to inhibit the multiplication of cultured mammalian cells. The objective was to determine if any members of the series showed potential utility as antitumor agents. Human (Detroit 98) and mouse (L) cells were exposed to the compounds during a 3-day period. Data for the more toxic compounds are given in Table III. The cytotoxicity of 4-amino-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**2**) toward

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(9) See footnote *a* of Table I.

various cell lines has been reported.[6b,c,10-13] In the present study, **2** and **12** were the most cytotoxic compounds, with ED_{50} values of 0.08 and 0.3 M (D-98) and 0.3 and 3.0 M (L), respectively. Neither **2** nor **12** was effective as an anticoccidial agent; however, no toxicity due to either compound was noted when administered to chicks at 200 ppm in the diet. Unexpectedly, the 4-diethylamino derivative (**16**) appeared somewhat toxic in vivo; the treated group of chicks showed much reduced weight gains relative to controls, and the feces were normal in appearance.

Examination of the data in Table I for efficacy of the compounds as anticoccidial agents in vivo shows that the majority were unable to clear any chicks of *Eimeria tenella*. The least effective were the hydrazino (**4**), octylamino (**11**), and benzylamino (**13**) derivatives, where in a group of five chicks, one died, and autopsy revealed parasitic lesions. No deaths similarly due to coccidiosis occurred in chicks medicated with any of the other compounds. Additionally, there appears to be little correlation between activity in vitro and in vivo for the members of this series (Table I). Some lack of correlation was also observed in the 4-alkylthio series.[1] Of the compounds with the lowest MIC in vitro (**5**, **8**, **12**, and **13**), only **8** was highly active in vivo. The dialkylamino compounds (**15**, **16**, and **18**) were slightly active, but the unsymmetrically disubstituted compound (**17**) and the morpholinyl derivative (**19**) were inactive. Among the monosubstituted straight-chain analogues (**5–7**, **10**, **11**, **13**, and **14**), only the ethylamino (**6**) and phenethylamino (**14**) derivatives showed slight activity. In this series, branching adjacent to the nitrogen as in the cyclopentylamino derivative (**8**) or disubstitution on nitrogen (**15**, **16**, and **18**) was tolerated, and these derivatives were among the most active in vivo. This is in contrast to the 4-alkylthio series, where branching in the alkyl chain was not tolerated.

In the in vivo screen, the parasitic infection was established by the simultaneous administration of *E. acervulina* and *E. tenella*. Based upon oocyst production at day 5, none of the compounds was effective against the infection due to *E. acervulina*.

It can be concluded that the ribonucleoside derivatives containing a 4-alkylthio substituent[1] are more promising as anticoccidials than the 4-alkylamino derivatives described in this report.

Experimental Section

Anticoccidial Evaluation. Activity against *E. tenella* (Weybridge strain) growing in embryonic chick liver cells in vitro was determined as previously described.[14] Activity against this organism in vivo with a simultaneous infection of *E. acervulina* (Ongar strain) was evaluated in week-old chicks as previously described.[1]

Enzyme Catalysts. Uridine phosphorylase (EC 2.4.2.3), thymidine phosphorylase (EC 2.4.2.4), and purine nucleoside phosphorylase (EC 2.4.2.1) were purified from *Escherichia coli* as previously described.[7] One unit of enzyme activity was that amount which catalyzed the formation of 1 μmol of product per minute under the defined assay conditions.

Cell Culture Materials and Methods. Detroit 98 cells were a cloned derivative from American Type Culture Collection CCL 18.1 (human sternal marrow). L cells were a cloned derivative from ATCC CCL (C3H/AN mouse connective tissue). These cultures were grown in a modification[15] of Eagle's medium and incubated in a water-jacketed CO_2 incubator at 36 °C and 100% humidity.

Total cell counts (made with an electronic cell counter) were compared between replicate test and control monolayer cultures. Controls showed at least two population doublings in the test period of 70–76 h. Percentages of control were plotted against molar concentration of test compound. ED_{50} is the concentration giving 50% of controls.

Physical Characterization of Compounds. All compounds listed in Table III gave elemental analyses within ±0.4% of calculated values, except as noted for **7** and **17**. Analyses were performed by Integral Microanalytical Laboratories, Raleigh, NC, or Atlantic Microlabs, Atlanta, GA. Melting points were obtained on a Thomas-Hoover capillary apparatus and are uncorrected. UV spectra were recorded using a Varian Super-Scan 3 or Pye-Unicam SP1800 spectrophotometer. A Varian XL-100 or CFT-20 provided the NMR spectra in Me_2SO-d_6. Optical rotations were obtained using a Perkin-Elmer Model 141 polarimeter. Table II lists some physical constants for the pyrazolo[3,4-*d*]pyrimidine ribonucleosides.

Syntheses. Reagents were used as received. Chromatography was performed on Merck silica gel, 0.02–0.6 μm. Preparative reverse-phase chromatography was performed at 80–100 psi on Merck RP-18 silica gel, 25–40 μm, packed in a glass column, 1.5 × 50 cm (Laboratory Data Control), using water or aqueous methanol as the eluent. The styrene–divinylbenzene copolymer, Amberlite XAD-2, was from Rohm and Haas. The samples were applied in water and eluted with mixtures of water and methanol. Analytical HPLC was conducted on a Whatman ODS-2 column, 4.2 × 25 cm, at 1200 to 1500 psi. Compounds were eluted with aqueous methanol. Polyacrylamide gel, P-2, was supplied by Bio-Rad Laboratories. Column dimensions are given as width × length. Evaporations in vacuo were performed at or below 40 °C.

Table II provides synthetic procedures used. Methods A–D are exemplified below. Compound **5** was deblocked using alcoholic aqueous methylamine, chromatographed on silica gel, and crystallized from methanol. Compound **17** was deblocked with hot 40% aqueous methylamine, followed by chromatography on silica gel and XAD-2; removal of the solvent gave the product. We obtained compound **18** in the deblocked form by conducting the reaction in refluxing ethanol. Chromatography on silica gel and XAD-2 gave the analytically pure compound. Compound **9** was purified by reverse-phase chromatography and crystallized from acetonitrile and ethanol. For compounds **8**, **11–14**, and **19**, the neat amine was reacted with the substrate at steam-bath temperature. Chromatography on silica gel and crystallization from chloroform gave **14**; chromatography on silica gel and removal of the solvent gave **12**. Compounds **8**, **10**, **11**, and **13** were precipitated from a solution in chloroform by the addition of water and crystallized as follows: **8** and **11** from ethanol; **14** from chloroform; **10** and **13** from water. Compound **19** crystallized from dichloromethane–ether. Chromatography on silica gel and crystallization from dichloromethane–methanol gave **16**. Compound **4** was prepared according to the literature method.[6a]

1-β-D-Ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (Allopurinol Riboside) (**1**; **Method A**). Uridine (40 g, 0.164 mol) and pyrazolo[3,4-*d*]pyrimidin-4-one (10 g, 0.073 mol) were suspended in a solution of K_2HPO_4 (3.5 g, 0.02 mmol) in 2 L of deionized water. The pH of the medium was 7.2. After the addition of purine nucleoside phosphorylase (254 units) and uridine phosphorylase (90 units), the suspension was gently stirred at 36 °C. The solution was readjusted occasionally by the addition of 1 N KOH to give a pH value of 7.2. After 6 days, both enzymes were added in the amounts indicated above. Twenty-five days later, the reaction mixture was filtered, and the filtrate was brought to pH 9.2 using 15 N NH_4OH. The solution was applied to a column of Dowex 1-X8 (formate), 5 × 6.5 cm, packed in water. The column was washed with water, 0.12 N NH_4HCO_3 (2 L, pH 5.6), 2 L of 0.01 N HCO_2H, and then 0.5 N HCO_2H. Fractions

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containing product by TLC[16] were combined, neutralized with 15 N NH_4OH, and evaporated in vacuo. The residue was suspended in 0.2 L of methanol, heated at 70 °C, and filtered while hot, and the solution was evaporated in vacuo. The residue was dissolved in 1-propanol/water (30:70, v/v, 0.05 L), and the solution was chromatographed on a column of polyacrylamide P-2, 5 × 90 cm, which had been equilibrated in the same solvent. Fractions containing product were combined and taken to dryness. The residue was suspended in water, heated to 55 °C, and filtered. The filtrate was applied to a column of ion-exchange resin [Bio-Rad, AG-50-X8 (hydrogen), 2.5 × 5 cm]. Elution with water gave fractions containing product. These were combined, neutralized with NH_4OH, and evaporated. The residue was dissolved in 0.05 L of water and chromatographed on a column of polyacrylamide gel P-2, 5 × 90 cm. Elution with water and lyophilization gave the product as the hemihydrate (4.8 g, 24%); yield based on the heterocycle: mp 172–175 °C (lit. mp 172–174 °C and various values reported between 164 and 271 °C; see ref 4f for a full discussion of this); $[\alpha]^{20}_D$ −70.1° (c 0.5, DMF) [lit.[4f] $[\alpha]^{20}_D$ −70.2° (c 0.53, CH_3OH)]; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 253 nm (8.5); at pH 13, 272 (11.6), 255 nm (sh) (8.2) [lit.[4f] at pH 7, 252 nm (3.97); at pH 11, 271 (4.08), 254 nm (sh)]; NMR δ 8.17 and 8.13 (2 s, 2 H, H_3 and H_6), 6.07 (d, 1 H, J = 4.4 Hz, $H_{1'}$); [lit.[4f] δ 8.23 and 8.18 (H_3 and H_6), 6.13 (d, J = 4.5 Hz, $H_{1'}$)]. Anal. ($C_{10}H_{12}N_4O_5 \cdot 0.5H_2O$) C, H, N.

4-Benzamido-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (3; Method A). A mixture of 4-benzamido-1*H*-pyrazolo[3,4-*d*]pyrimidine[2] (2.0 g, 8.4 mmol), uridine (2.4 g, 10 mmol), purine nucleoside phosphorylase (4900 units), uridine phosphorylase (880 units), and 1-propanol (15 mL) in 313 mL of potassium phosphate buffer (100 mM, pH 6.2) was stirred at 53 °C for 5 days. The suspension was filtered hot, and the filtrate was cooled to 23 °C to give a precipitate. The solid was collected and washed with water. It was suspended in 50% aqueous ethanol, heated to 68 °C, and filtered. Cooling gave the product, which was collected, washed with water, and dried: yield 1.6 g (50%); mp (softens 71 °C) 105–107 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 237 (sh) (18.2), 284 nm (21.9); at pH 13, 320 nm (18.1); NMR δ 11.9 (br s, 1 H, NH), 8.78 (s, 1 H, H_6), 8.51 (d, 1 H, J = 0.5 Hz, H_3), 8.11 and 7.59 (2 multiplets, 2 H and 3 H, C_6H_5), 6.30 (d, 1 H, J = 4.4 Hz, $H_{1'}$, 4.72 (m, 1 H, H_2), 4.22 (m 1 H, H_3), 3.95 (m, 1 H, H_4), 3.55 (m, 2 H, 5′-CH_2). Anal. ($C_{17}H_{17}N_5O_5 \cdot 0.5H_2O$) C, H, N.

4-(Butylamino)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (7; Method B). 2′,3′,5′-Tri-*O*-benzoyl-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one[4c,d,f] was prepared from 1 in 80% yield according to the method of Fox et al.,[17] for the benzoylation of inosine. The compound was isolated by chromatography on silica gel [mp 176–177 °C (lit.[4c,d,f] mp 180–181 °C)] and converted quantitatively to the 4-chloro derivative using thionyl chloride and DMF in dry chloroform according to the method of Ikehara and Uno.[18]

A solution of the crude 4-chloro-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (2.1 g, 3.5 mmol) and butylamine (2 mL) in ethanol was refluxed 4 h. The solvent and reagent were removed in vacuo to give a gummy residue, which was extracted in to ether. After removal of the ether under reduced pressure, the compound was deblocked using NaOEt in ethanol (from 0.3 g of Na metal). After 2 h at ambient temperature, the solution was neutralized with glacial AcOH and extracted with chloroform. Removal of the chloroform gave the crude product. Chromatography on silica gel and crystallization from chloroform gave 0.42 g of 7 (37% yield): mp 118–119 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 263 nm (13.3); at pH 7, 263 (sh) (8.8), 281 nm (13.5); at pH 13, 263 (sh) (8.8), 281 nm (13.3); NMR δ 8.30 (t, 1 H, NH), 8.26 and 8.20 (2 s, 2 H, H_6 and H_3), 6.09 (d, 1 H, J = 4.5 Hz, $H_{1'}$), 3.50 (m, 2 H, NCH_2), 1.53 (m, 4 H, $2CH_2$), 0.92 (t, 3 H, CH_3). Anal. ($C_{14}H_{21}N_5O_4 \cdot 0.65H_2O$) C, H, N.

Method C. A suspension of 1 (0.54 g, 2.0 mmol) was refluxed, under a nitrogen atmosphere, in 5 mL of hexamethyldisilazane with $(NH_4)_2SO_4$ (29 mg, 0.22 mequiv) as catalyst according to methods in the literature.[8,4d,f] The excess reagent was removed by vacuum distillation. The oily residue was dissolved in 2 mL of dry pyridine, and 2 mL of butylamine was added. The reaction mixture was refluxed for 20 h, at which time HPLC analysis showed the reaction was approximately 70% complete. The addition of more amine did not increase product formation. Methanol (50 mL) was added, and the reaction was refluxed for 3 h. Volatile components were removed in vacuo, and the residue was dissolved in water. After the aqueous solution was washed with chloroform, a precipitate formed. This was collected, washed with water, and dried in vacuo at 70 °C. Compound 7, 0.25 g, was obtained in 38% yield, mp 120–124 °C. All other physical properties were in agreement with those above. Anal. ($C_{14}H_{21}N_5O_4 \cdot 0.6H_2O$) C, H, N.

4-(Ethylamino)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (6; Method D). 4-(Methylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine[1,6,4j] (2.0 g, 6.8 mmol) was dissolved in 10 mL of 30% aqueous ethylamine and heated with stirring at 80 °C for 3 h. The cooled reaction mixture was taken to dryness in vacuo. The product, 6, crystallized from methanol in 55% yield (1.12 g): mp 188–189 °C; $[\alpha]^{20}_D$ −72.2° (c 1, DMF); UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 263 nm (13.8); at pH 13, 263 (10.4), 283 nm (15.5); NMR δ 8.30 (m, 1 H, NH), 8.26 and 8.19 (2 s, 2 H, H_6 and H_3), 6.09 (d, 1 H, J = 4.6 Hz, $H_{1'}$), 3.50 (m, 2 H, NCH_2), 1.21 (t, 3 H, NCH_3). Anal. ($C_{12}H_{17}N_5O_4 \cdot 0.2H_2O$) C, H, N.

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(16) TLC on silica gel glass plates in CH_3CN/15 N NH_4OH/H_2O (85:5:10, v/v). R_f values were 0.15 for uridine, 0.37 for pyrazolo[3,4-*d*]pyrimidin-4-one and uracil, and 0.25 for 1.

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