gel with 25:70:30:0.2 MeOH/$CHCl_3$/H_2O/HOAc afforded 112 mg (40%) of the amino ester, judged to be 90% pure by TLC and NMR. Rechromatography of a portion of this material as before raised the purity to greater than 95%, and these samples were used for biological testing: R_f = 0.14 (silica gel, 25:70:3.0:0.1 MeOH/$CHCl_3$/H_2O/HOAc); IR (KBR pellet) 3420, 2920, 1735, 1685, 1570, 1420, 1205, 1185, 1130, 1060, 1010, 820, 765 cm^{-1}. ^1H NMR (500 MHz, D_2O, HOD = 4.80 ppm) 2.08 (ddd, 1 H, $J = J'$ = 13 Hz, J'' = 7 Hz, H-3ax), 2.19 (dd, 1 H, J = 13 Hz, J' = 5 Hz, H-3eq), 2.43 (s, 3 H, SCH_3), 2.46 (dd, 1 H, J = 13 Hz, J' = 8 Hz, H-8), 2.97 (dd, 1 H, J = 13 Hz, J' = 4 Hz, H-8), 3.49 (d, 1 H, J = 8 Hz, H-6), 3.78 (ddd, 1 H, J = 13 Hz, J' = 5 Hz J'' = 2.5 Hz, H-4), 3.90 (ddd, 1 H, $J = J'$ = 8 Hz, J'' = 4 Hz, H-7), 3.96 (d, 1 H, J = 2.5 Hz, H-5), 4.74 (d, 1 H, J = 7 Hz, H-2), 5.96, 6.04 (2 d, 2 H, J = 13 Hz, $ArCH_2$), 7.61, 7.615 (2 t, 2 H, $J = J'$ = 7.5 Hz, Ar-H-3 and Ar-H-6), 7.73, 8.07, 8.09, 8.17 (4 d, 4 H, J = 7.5 Hz); positive FAB MS (NBA), m/z 440 $(M + H)^+$; exact mass calcd for $C_{20}H_{26}NO_6S_2$ 440.1202, found 440.1208.

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Antitumor and Antiviral Activity of Synthetic α- and β-Ribonucleosides of Certain Substituted Pyrimido[5,4-*d*]pyrimidines: A New Synthetic Strategy for Exocyclic Aminonucleosides

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A novel and direct synthesis of the antiviral and antitumor agent 4-amino-8-(β-D-ribofuranosylamino)pyrimido-[5,4-*d*]pyrimidine (ARPP, **8**) and its α-anomer (**11**) has been developed. Treatment of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine (**1**) with 2,3-*O*-isopropylidene-D-ribofuranosylamine gave an anomeric mixture of 2,4,6-trichloro-8-(2,3-*O*-isopropylidene-β- and -α-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidines (**3** and **4**) in a ratio of 1.0:0.7. A nucleophilic displacement of the 4-chloro group of **3** and **4** with NH_3 furnished 4-amino-2,6-dichloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**6**) and its α-anomer (**9**), respectively. Catalytic hydrogenation of **6** and **9**, followed by deisopropylidenation gave ARPP (**8**) and the α-anomer **11**, respectively. Similarly, **3** and **4** have been transformed to 4-methoxy-8-(β-D-ribofuranosylamino)pyrimido-[5,4-*d*]pyrimidine (MRPP, **14**) and its α-anomer (**17**). Application of this procedure to **3** with NH_2Me or $NHMe_2$ resulted in the synthesis of 4-(methylamino)- and 4-(dimethylamino)-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]-pyrimidine (**24** and **27**, respectively). A synthesis of 8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one (**21**) has also been accomplished from **3** in three steps. Selective hydrogenation of **6** furnished 4-amino-6-chloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**36**), the structure of which was established by single-crystal X-ray diffraction analysis. Deisopropylidenation of **36** gave 6-chloro-ARPP (**37**). Extended treatment of **36** with NH_3 furnished 4,6-diamino-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**34**), which on deisopropylidenation gave 6-amino-ARPP (**35**). An unambiguous synthesis of **34** and **36** has also been accomplished by the reaction of 4,6,8-trichloropyrimido[5,4-*d*]pyrimidine (**28**) with **2**, followed by the treatment with NH_3. Nucleophilic displacement studies with **1**, **6**, and **28** indicated the reactivity of the halogens in these compounds is in the order of 8 > 4 > 6 > 2. The structures of **3** and **9** have been assigned on the basis of ^1H NMR data and further confirmed by single-crystal X-ray diffraction analysis. The exocyclic aminonucleosides synthesized during this study were tested for their activity against several RNA and DNA viruses in vitro and against L1210, WI-L2, and LoVo/L in cell culture. The effect of these compounds on the de novo nucleic acid biosynthesis has been studied. Compound **14** (MRPP) exhibited enhanced activity against L1210 in vivo, when compared to ARPP (**8**).

The recent molecular biology and biochemistry of purine and purine nucleoside analogues showing potent antiviral and antitumor activity has uncovered a number of new potential targets.[1-4] The pyrimido[5,4-*d*]pyrimidine ring system has attracted considerable attention in recent years as the deaza analogue of the naturally occurring antibiotics toxoflavin and fervenulin.[5] Dipyridamole, a pyrimido-[5,4-*d*]pyrimidine derivative, has shown coronary vasodilator properties.[6] The synthesis of the naturally occurring exocyclic aminonucleoside clitocine has recently been reported from our laboratory.[7] The synthesis[8] and the biological properties[9] of an unusual exocyclic aminonucleoside, 4-amino-8-(β-D-ribofuranosylamino)pyrimido-[5,4-*d*]pyrimidine (ARPP, **8**) has also been reported from our laboratory. ARPP has shown broad-spectrum antiviral activity against both DNA and RNA viruses in cell culture by inhibiting viral protein synthesis.[10] ARPP exhibited immunosuppressive activity and inhibited the growth of L1210 leukemia in mice.[2] Molecular mechanics calculations of ARPP and certain related nucleosides[11] showed that their conformational behavior is very similar even when groups like chloro or amino are introduced at pos-

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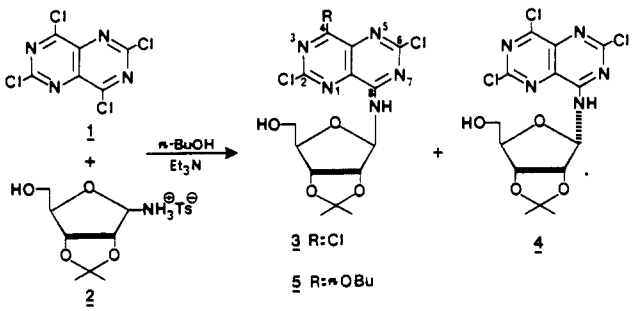
0022-2623/89/1832-0629$01.50/0

Scheme I



itions 2 and/or 6, suggesting chemical modifications at the aglycon portion of ARPP may prove useful. Moreover, our earlier work[12] on the modification of the glycon moiety of ARPP resulted in the loss of antiviral and antitumor activity. Therefore, we undertook the synthesis of certain heterocyclic ring substituted derivatives of ARPP. We now report a new and convenient synthesis of ARPP (8), its α-anomer (11), and certain substituted pyrimido[5,4-*d*]pyrimidine ribonucleosides. We have designed these ARPP derivatives with the view of increasing their specificity and separation of the antiviral and antitumor activities.

Chemistry

In the present study we considered the direct condensation of a halogen substituted pyrimido[5,4-*d*]pyrimidine with an aminoglycoside resulting in a one-step synthesis of the desired exocyclic aminonucleoside. Thus, treatment of free 2,3-*O*-isopropylidene-D-ribofuranosylamine[13] (generated in situ from its stable tosylate salt **2** by the addition of Et_3N) with dry 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine[14] (**1**) in 1-butanol at room temperature gave a mixture of two nucleoside products which were separated by silica gel column chromatography and identified as 2,4,6-trichloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**3**) and its α-anomer (**4**) (Scheme I). The anomeric ratio of **3**:**4** was 1.0:0.77. The assignment of the anomeric configuration of **3** and **4** was inconclusive from the ^1H NMR coupling constant values of the anomeric protons, since there was no significant difference in *J* values (10.8 and 10.0 Hz for **3** and **4**, respectively). Thus, ^1H–^1H 2D NMR of these two compounds were studied. The less polar compound **3** revealed an anomeric proton centered at δ 6.34 ppm (d, J = 10.8 Hz) coupled to another proton at δ 9.09 ppm (d, J = 10.8 Hz). Upon D_2O addition, the anomeric proton collapsed to a singlet and the downfield proton exchanged, indicating that the sugar moiety is located at the exocyclic amino group.[8] The appearance of the N*H* proton at low field is expected due to hydrogen bonding between the 5′OH and NH groups. This suggested a β-configuration for compound **3**. Imbach's empirical rule,[15] formulated for determining the anomeric configuration of azole nucleosides using the difference between the chemical shifts of the protons of the methyl groups of the dioxolane rings, did not prove helpful in this case, since the compound **3** exhibited $\Delta\delta$ = 0.22 ppm for the methyl groups, while compound **4** showed $\Delta\delta$ = 0.25 ppm. Furthermore, compound **4** revealed an anomeric proton at δ 6.19 ppm and an N*H* proton at δ 8.23 ppm. This high field placement of N*H* proton for **4** is due to the lack of hydrogen bonding between the 5′OH and NH groups, because of the α-configuration. Moreover, these compounds mutarotate in solution,[16] especially in Me_2SO-d_6. Mutarotation of similar aminoglycosides has been documented.[13] The anomeric configuration of **3** was established as β by X-ray diffraction analysis[17] (Figure 1). The anomeric configuration of **4** was then assigned as α.

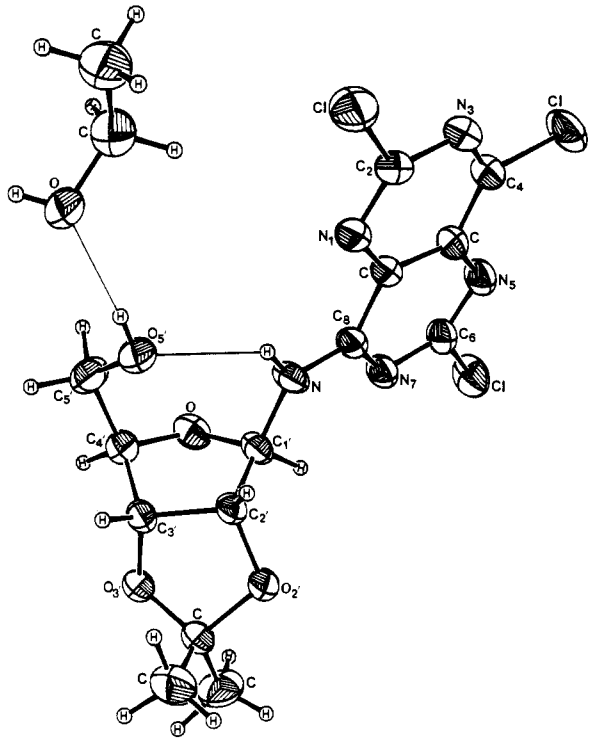


Figure 1. Compound **3**.

We have isolated 2,6-dichloro-4-(*n*-butyloxy)-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**5**) as a product of the solvent (1-butanol) participation when the reaction mixture of **1** and **2** was left for 16 h at ambient temperature, indicating the susceptibility of the 4-chloro group of **3** toward nucleophilic displacement reactions. Although the formation of the α-anomer of **5** was detected by TLC in the reaction mixture, no attempt was made to isolate it. In view of this observation, a brief (30 min at 0 °C) treatment of **3** and **4** with $EtOH/NH_3$ (saturated at 0 °C) resulted in the formation of 4-amino-2,6-dichloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**6**) and its α-anomer (**9**), respectively. Single-crystal X-ray study of **9** (Figure 2) established the α-configuration, and hence to its precursor **4**. Catalytic (Pd/C) hydrogenation of **6** and **9** at 50 psi for 24 h gave 4-amino-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-amino]pyrimido[5,4-*d*]pyrimidine (**7**) and the α-anomer

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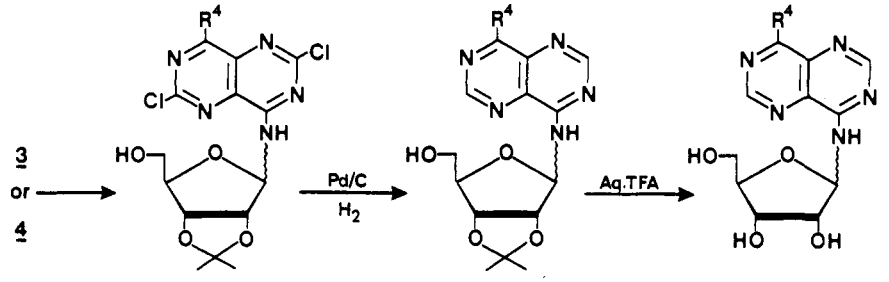
(16) At this point we assume that deisopropylidenation using aqueous TFA would result in the exclusive formation of the β-nucleoside due to the thermodynamic control. It is the NMR solvent (Me_2SO-d_6) in which most of the deblocked nucleosides mutarotate; therefore exhibiting two $C_{1'}H$ and two N*H* protons. However, no apparent mutarotation was observed when the ^1H NMR of **3** and **4** were run in $CDCl_3$. These compounds are also sensitive to heating in solution and start to decompose when in solution for an extended period of time.
(17) Larson, S. B.; Sanghvi, Y. S.; Revankar, G. R.; Robins, R. K. *Acta Crystallogr., Sect. C*, in press.

Scheme II[a]



compd	R^4	a.c.	compd	R^4	a.c.	compd	R^4	a.c.
6	NH_2	β	7	NH_2	β	8	NH_2	β (ARPP)
9	NH_2	α	10	NH_2	α	11	NH_2	α
12	OCH_3	β	13	OCH_3	β	14	OCH_3	β (MRPP)
15	OCH_3	α	16	OCH_3	α	17	OCH_3	α
18	OCH_2Ph	β						
19	OH*	β	20	OH*	β	21	OH*	β
22	NHMe	β	23	NHMe	β	24	NHMe	β
25	NMe_2	β	26	NMe_2	β	27	NMe_2	β

[a] a.c. = anomeric configuration; (*) present as the oxo tautomer.

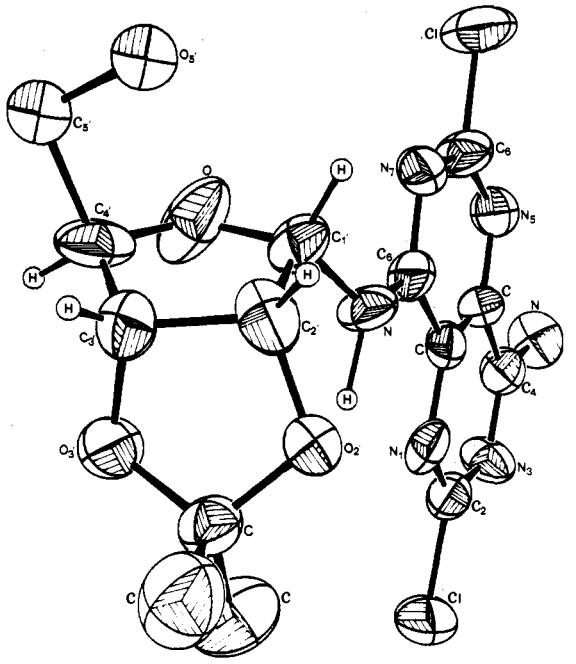


Figure 2. Compound **9**.

10, respectively (Scheme II). Careful treatment of **7** and **10** with aqueous trifluoroacetic acid (90% TFA) at room temperature cleaved the isopropylidene group to give ARPP (**8**) and its α-anomer **11** in >80% yield, respectively. The physical properties (melting point, TLC, HPLC, UV, and ^1H NMR) of **8** were identical[9] with those of a standard sample of ARPP, the structure of which had been confirmed previously by X-ray studies.[18] The anomeric protons of **8** and **11** appear at δ 5.88 ppm and δ 6.15 ppm, respectively. This observation is in agreement with the assignment of the anomeric protons for the α-anomer at lower field when compared to the β-anomer as in the case of similar exocyclic aminonucleosides recently reported.[19]

A nucleophilic displacement of the 4-chloro group from **3** and **4** with NaOMe in MeOH furnished 2,6-dichloro-4-methoxy-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-amino]pyrimido[5,4-*d*]pyrimidine (**12**) and the α-anomer **15**, respectively. Catalytic hydrogenation of **12** and **15** yielded **13** and **16**, which on independent deisopropylidenation gave 4-methoxy-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (MRPP, **14**) and the α-anomer **17**, respectively. We have assigned **14** as the β-anomer and **17** as the α-anomer on the basis of their ^1H NMR data. As expected the anomeric proton of **14** exhibited a quartet at δ 5.83 ppm, which on comparison with the anomeric proton of **17** was 0.33 ppm upfield (δ 6.16 ppm). Furthermore, the exocyclic N*H* proton of **14** revealed a doublet at δ 8.71 ppm, and the N*H* proton of **17** appeared at δ 8.39 ppm. This observation is consistent with the higher field N*H* proton of **11** (δ 8.19 ppm) and the lower field N*H* proton of **8** (δ 8.34 ppm).

In order to obtain the inosine analogue of ARPP we have developed a mild method for the preparation of **21**. When **3** was stirred with benzyl alcohol in the presence of Et_3N at room temperature for 24 h, 4-(benzyloxy)-2,6-dichloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**18**) was obtained in >60% yield. Debenzylation of **18** by hydrogenation in the presence of Pd/C at atmospheric pressure in dioxane gave 2,6-dichloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-amino]pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one (**19**). Deisopropylidenation of **19** with aqueous TFA furnished 2,6-dichloro-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one, which on dehalogenation furnished 8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidin-4-(3*H*)-one (**21**) in 66% yield. However, hydrogenation of **18** in MeOH in the presence of Pd/C resulted in solvent participation to furnish **13** and not the desired 8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]-pyrimidin-4(3*H*)-one (**20**).

The fact that N^6-dimethyladenosine is a component of tRNA as well as the antibiotic puromycin encouraged us

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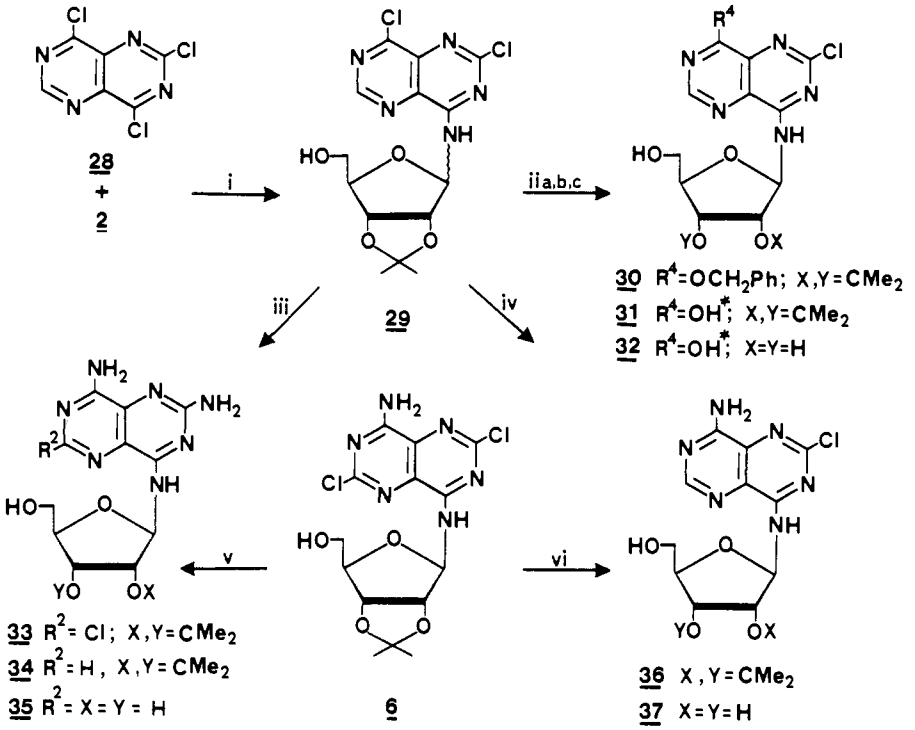
Scheme III[a]



[a] i, *n*-BuOH, Et_3N, room temperature, 6 h; iia, BnOH, Et_3N, room temperature; iib, Pd/C, H_2, atmospheric pressure; iic, aqueous TFA; iii, liquid NH_3, room temperature → iic; iv, MeOH/NH_3, 0 °C, 15 min → iic; v, iii → iib → iic; vi, iib → iic; * Present as the oxo tautomer.

to prepare the N^4-dimethyl derivative of ARPP. Hence, a nucleophilic displacement of the 4-chloro group of **3** with NH_2Me and $NHMe_2$ gave the corresponding 2,6-dichloro-4-(methylamino)- and 4-(dimethylamino)-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**22** and **25**, respectively). Hydrogenation of **22** and **25** provided **23** and **26**, respectively. Subsequent deisopropylidenation of **23** and **26** gave 4-(methylamino)-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (**24**) and 4-(dimethylamino)-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (**27**), respectively.

In view of our current interest in 8-chloroadenosine and 8-chloroadenosine 3′,5′-cyclic phosphate as growth inhibitors of cancer cells,[20,21] we have now prepared 6-chloro-ARPP (**37**). Controlled hydrogenation of **6** removed the 2-chloro group to furnish 4-amino-6-chloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**36**) in 65% yield. The proton NMR of **36** exhibited a singlet at δ 8.35 ppm for C_2H, thus confirming the removal of only one chloro group from **6**. The structure of this selective dehalogenation product was established by a single-crystal X-ray diffraction analysis[17] to be **36** (Figure 3). Subsequent deisopropylidenation of **36** gave the desired 4-amino-6-chloro-8-(β-D-ribofuranosylamino)-pyrimido[5,4-*d*]pyrimidine (**37**) in 67% yield (Scheme III).

Recently we[22] and others[23] have studied several amino group substituted nucleosides related to 8-aminoadenosine

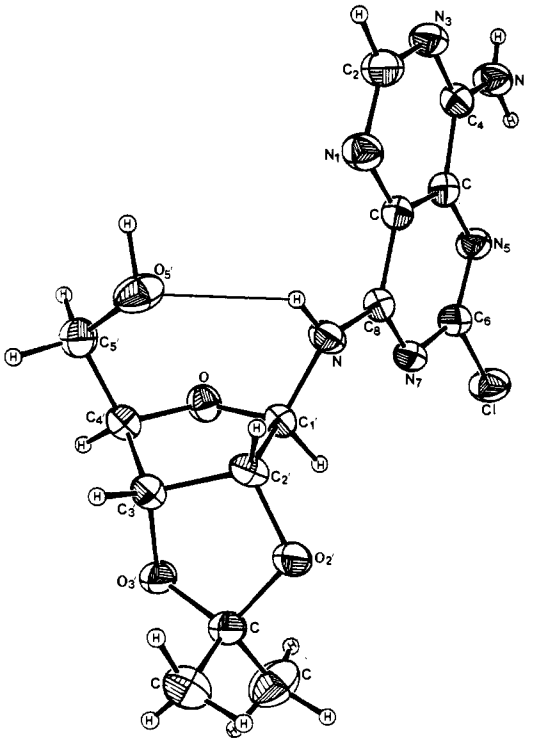


Figure 3. Compound **36**.

as potent inhibitors of purine nucleoside phosphorylase (PNPase). Introduction of an amino group at position 6 of ARPP would provide a compound of similar structure to 8-aminoadenosine. Thus, treatment of **6** with liquid NH_3 over a period of 6 days furnished a mixture of two nucleoside products. After separation by flash chromatography, the structure of the major product (59% yield) was assigned as the β-anomer **33** and the minor product (15% yield) as the α-anomer of **33**. Catalytic hydrogenation of **33** furnished **34** whose ^1H NMR spectrum exhibited

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Table I. In Vitro Antiviral and Antitumor Activity

compd	antiviral (VR)[a]			antitumor (ID_{50})[b]		
	HSV2[c]	VV[d]	PI3[e]	L1210[f]	WI-L2[g]	LoVo/L[h]
ARPP (8)	0.8	1.3	0.7	0.8	0.2	7.0
MRPP (14)	0.2	1.0	0.7	0.04	0.06	0.28
11	0.2	1.0	1.4	0.38	0.25	1.0
17	0.1	0.9	0.7	2.9	2.5	18.7

[a] (VR) virus ratings by convention ≥1.0 indicate marked antiviral activity; 0.5–0.9 indicate moderate activity, and <0.5 indicate weak or no activity. [b] (ID_{50}) inhibitory dose is the μM concentration of the compound that inhibits tumor cell growth by 50% as compared to the untreated controls. [c] Herpes simplex virus type 2 (MS strain) cell line in Vero cells. [d] Vaccinia virus (Elstree strain) cell line in HeLa cells. [e] Parainfluenza 3 (C243 strain) cell line in Vero cells. [f] Murine leukemia cell line. [g] Human B-lymphoblast cell line. [h] Human colon carcinoma cell line.

a C_2H proton at δ 8.05 ppm. In an effort to confirm the site of the ammonolysis in **6** we elected an unambiguous synthesis of **34** from 4,6,8-trichloropyrimido[5,4-*d*]pyrimidine[14] (**28**). Treatment of **28** with **2** as described earlier for the preparation of **3**, furnished an air-sensitive mixture of two compounds, presumably an anomeric mixture of 4,6-dichloro-8-[(2,3-*O*-isopropylidene-D-ribofuranosyl)-amino]pyrimido[5,4-*d*]pyrimidine (**29**). A brief EtOH/NH_3 treatment of **29** gave a mixture of two products, from which compound **36** was isolated as a major product. This compound was found to be identical with **36** prepared previously from **6**, thus confirming the structural assignment. Compound **29** upon ammonolysis (6 days) furnished an alternate route for the synthesis of **34**. This established the preferential site of the nucleophilic substitution at position 6 over position 2 in compound **6**. On the basis of the reactivity of various chloro groups in **1** and **28**, we suggest an order of nucleophilic substitution as positions 8 > 4 > 6 > 2. Deisopropylidenation of **34** gave the desired 4,6-diamino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]-pyrimidine (**35**), isolated as a TFA salt (Scheme III). Treatment of **29** with benzyl alcohol in a similar manner as described earlier for the preparation of **18** furnished **30**. Hydrogenation of **30** gave **31**, which on aqueous TFA treatment provided 6-chloro-8-(β-D-ribofuranosylamino)-pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one (**32**) in 58% yield.

Crystallographic Analysis

Colorless, transparent crystals of 2,4,6-trichloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**3**) and 4-amino-6-chloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]-pyrimidine (**36**) were obtained from EtOH solution. The trichloro nucleoside **3** crystallizes in space group *P*1 with cell a = 5.158 Å, b = 9.335 Å, c = 11.115 Å, α = 106.08°, β = 96.71°, and γ = 93.73°; compound **36** crystallizes in space group $P2_1$ with a = 5.043 Å, b = 9.994 Å, c = 15.713 Å, β = 99.13°. The proposed chemical structures and β-anomeric configurations of each were confirmed. Figures 1 and 3 illustrate the nearly identical conformations of **3** and **36** in the solid state. The sugar conformations are $^4T_0$ (C_4-endo) for **3** and $^4{}_3T$ (C_4-endo, C_3-exo) for **36** in contrast to the ^OE (O_1-endo) conformation of ARPP.[18] These conformations permit the bridging nitrogen in each to intramolecularly hydrogen bond to O5′ across the furan ring in contrast to the openness of the ARPP furan ring.[18] All other available hydroxyl- and amino-group hydrogens participate in intermolecular hydrogen bonding. Thus, in compound **3**, O5′ bonds to the oxygen of the EtOH solvate and the EtOH solvate hydrogen bonds to O2′ of the ribose; in compound **36**, O5′ and the 4-amino function are hydrogen bonded to N3 and the furan oxygen, respectively.

Crystals of 4-amino-2,6-dichloro-8-[(2,3-*O*-isopropylidene-α-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**9**) were obtained from an EtOH/hexane (95:5) solution. Two crystalline forms were obtained, each pertaining to the space group $P2_1$. Form I with cell a = 17.058 Å, b = 20.176 Å, c = 5.902 Å, β = 90.19° contains two crystallographically independent molecules as well as unresolved disordered solvent (probably EtOH); form II with cell a = 15.724 Å, b = 24.577 Å, c = 10.352 Å, β = 97.76° contains four independent molecules and undetermined solvent. The preliminary results confirm the chemical structure and the α-anomeric configuration (Figure 2). All independent molecules have essentially the same conformation. The large thermal parameters and inconsistent bond lengths evident during refinement of both forms suggest that more than just the solvent is disordered. Similar characteristics for crystals of compounds **6** and **13** were observed in which the former appears to have four independent molecules and the latter appears to have three independent molecules. Details of the X-ray structural determinations of compounds **3** and **36** will be published elsewhere.[17]

Biological Evaluation

Antiviral Activity. Cell culture studies confirmed that ARPP was effective against both DNA (HSV-2 and VV) and RNA viruses (Para-3 and Rhino 1-A). Therefore, we tested (for experimental details, see ref 24) all of the new nucleosides described in the present study against Herpes 2 in Vero cells, Adeno 2 in HeLa cells, Rhino 1-A in HeLa cells, Para Flu 3 in Vero cells, Semliki forest in Vero cells, Visna virus in SCP cells, Influenza A in MDCK cells, and Vaccinia in HeLa cells. Among all compounds tested, only **11**, **14**, and **17** showed modest antiviral activity, and the results of a single experiment in parallel with ARPP (**8**) are summarized in Table I. Recently ARPP was evaluated by the National Cancer Institute for in vitro activity against the human immunodeficiency virus (HIV; cell line: CEM-V). At 9.51×10^{-8} M, ARPP exhibited a 50% reduction of viral cytopathic effect.

Antitumor Activity. In vitro cytotoxicity analysis was performed by using the following cell lines: L1210 (a murine leukemia), WI-L2 (a human B-lymphoblast), and LoVo/L (a human colon carcinoma) (for experimental details, see ref 25). The results for ARPP and the most active compounds are summarized in Table I. These data clearly demonstrate that MRPP (**14**) has greater antitumor activity but lesser antiviral activity than ARPP, thus achieving the goal of enhancing and separating the antitumor activity from antiviral activity. The best four compounds of this study **8**, **11**, **14**, and **17** were comparatively evaluated in vivo (for experimental details, see ref 26) for efficacy against L1210 in mice. ARPP (**8**) and the

(24) Smee, D. F.; McKernan, P. A.; Nord, L. D.; Willis, R. C.; Petrie, C. R.; Riley, T. M.; Revankar, G. R.; Robins, R. K.; Smith, R. A. *Antimicrob. Agents Chemother.* **1987**, *31*, 1535–1541.

(25) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.

α-anomer 11 differ both in their solubility in water and in their efficacy against L1210. Thus, the maximum water solubility of 8 was 17.3 mg/mL as compared to 2.2 mg/mL for 11, and when administered qd, day 1, the former produced a T/C of 162 as opposed to 138 for the latter. Neither drug was lethally toxic at its maximum soluble dosage, and scheduling trials to define the limits of dosing are in progress. In a similar manner, MRPP (14) and the α-anomer 17 were tested. Compound 17 was soluble in water to a maximum of 3.7 mg/mL and produced a treatment T/C of only 116. Conversely, MRPP (14) was soluble up to 17.3 mg/mL and was lethally toxic at that level for all treated mice. MRPP also caused death for 1/6 treated mice when made up at 10.4 mg/mL. The maximum nonlethal concentration of MRPP was 6.2 mg/mL, and when administered at 62 mg/kg, it produced a T/C of 182. Taken collectively, it appears from these results that the β-anomers tested have greater solubility than the α-anomers and when administered qd, day 1, were more effective in the treatment of L1210 leukemia.

Biochemical Studies

ARPP and other compounds were studied for their effect on de novo purine biosynthesis by observing inhibition of incorporation of [^{14}C]formate into the hot acid-soluble fraction of WI-L2 cells. Of the compounds examined (see Table II) ARPP (8) and MRPP (14) showed the greatest inhibitory activity and were active at concentrations as low as 0.25 μM. However, ARPP and MRPP (100 μM) were not cytotoxic to WI-L2 cells deficient in adenosine kinase activity (data not shown). Direct inhibition of WI-L2 adenosine kinase by MRPP was observed as a decrease in the rate of [^{14}C]AMP formation from [^{14}C]adenosine. MRPP demonstrated a K_i value of 8 μM at an apparent K_m value for adenosine of 0.9 μM. This suggests that phosphorylation of MRPP by adenosine kinase is required for inhibitory activity.

The formation of intracellular ARPP 5′-monophosphate but no higher phosphate analogues was previously shown by SAX-HPLC results from our laboratory;[7] however, two anomeric species were detected within the monophosphate region of the chromatogram. The similar appearance of two species was also seen in aqueous solution for ARPP analyzed by reverse-phase HPLC (data not shown). When ARPP was treated with adenosine deaminase as reagent, the preferential disappearance of the β-anomer (60% conversion in 5 min) was followed by a much slower decrease in the area of the α-anomer peak. This would be expected where the rate of anomerization is slow compared to the rate of deamination. The deamination product (21) similarly equilibrated from pure β-anomer into a mixture of α- and β-anomers. MRPP was less active as a substrate for adenosine deaminase and less than 10% was hydrolyzed in 18 h under the same conditions as described for ARPP. Prior addition of 1 μM coformycin to the assay completely prevented the deamination of ARPP.

The actual rate of anomerization was investigated in the absence of added protein by incubating a buffered sample of MRPP at 37 °C. After 9 h the solution analyzed by reverse-phase HPLC showed 1.35% of the α-anomer 17 and 0.42% of 21 as an anomeric mixture. Thus, the α-anomer 17 was inactive in the purine de novo assay over the short term (4 h; see Table II) but demonstrated the inhibitory activity in separate assays that employed longer incubation times. ARPP and MRPP appear to require activation to the 5′-monophosphate and inhibit an early step in de novo purine biosynthesis. The mechanism of inhibition is under investigation.

Table II. Comparative Effect of ARPP (8) and Related Compounds on de Novo Purine Biosynthesis

compd	concn, μM	% of control: cell	% of control: media	preincubation time, h
ARPP (8)	2.5	29.9	54.6	8
	0.25	79.5	71.9	8
MRPP (14)	25	4.3	*a*	4
	2.5	2.7	41.8	8
	0.25	41.9	63.0	8
21	50	98.2	105.9	8
27	50	50.7	85.8	8
37	50	16.4	43.4	8
35	50	91.2	89.8	8
17	25	98.2	*a*	4

[a] Not determined. De novo purine biosynthesis was measured by the incorporation of [^{14}C]formate into cellular and excreted purines after preincubation with compound.

In conclusion we have described the synthesis of several previously inaccessible ARPP derivatives. These compounds are capable of crossing the cell membrane and activated to the 5′-phosphate, thus causing the inhibition of the de novo purine biosynthesis.

Experimental Section

Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting point apparatus. Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ. Thin-layer chromatography (TLC) was conducted on plates of silica gel 60 F-254 (EM Reagents). Silica gel (E. Merck; 230–400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of nucleoside components in TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30 °C. Infrared (IR) spectra were recorded with a Beckman Acculab 2 spectrophotometer and ultraviolet (UV) spectra were recorded on a Beckman DU-50 spectrophotometer. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz with an IBM NR/300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The signals are described as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The presence of solvent as indicated by elemental analyses was verified by ^{1}H NMR spectroscopy.

2,4,6-Trichloro-8-[(2,3-*O*-isopropylidene-β- and -α-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (3 and 4). A suspension of dry 2,3-*O*-isopropylideneribofuranosylamine *p*-toluenesulfonate[13] (2; 3.61 g, 10 mmol) in 1-butanol (200 mL) was treated with Et_3N (4.18 mL, 30 mmol), and the mixture was stirred at ambient temperature under anhydrous conditions, furnishing a clear solution in 30 min. Finely powdered, dry 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine[14] (1; 4.04 g, 15 mmol) was added to the above solution and stirred for 6 h under similar conditions. The solution was evaporated and the residue dissolved in EtOAc (300 mL), filtered to remove some insoluble material, and washed with water (2 × 100 mL). After drying (Na_2SO_4), the solvent was evaporated and the residue was purified on a flash silica gel column (5 × 45 cm) by using hexanes/EtOAc (7:3, v/v), as the eluent, which provided two products in the following order. (i) 2,4,6-Trichloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (3; 1.89 g, 45%): R_f 0.65 (EtOAc/hexanes 3:7); mp 163 °C (EtOH); IR (KBr) ν_{max} 845 (C–Cl), 3300, 3455 (OH, NH) cm^{-1}; UV (MeOH) λ_{max} nm (ε × 10^{-3}) 256 (6.7), 298 (7.9), 346 (9.2), 362 (sh) (7.3); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3 CH_3), 1.59 (s, 3 CH_3), 2.61 (br s, 1, C$_5$′O*H*), 3.94 (s, 2, C$_5$′CH_2), 4.48 (s, 1, C$_4$′C*H*), 4.78 and 5.02 (2 m, 2, C$_{2'3'}$*H*), 6.34 (d, 1, *J* = 10.8 Hz, C$_1$′*H*), 9.09 (br d, 1, *J* = 10.8 Hz, N*H*). Anal. ($C_{14}H_{14}Cl_3N_5O_4$) C, H, N, Cl. (ii) 2,4,6-Trichloro-8-[(2,3-*O*-isopropylidene-α-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (4; 1.47 g, 35%): R_f 0.55 (EtOAc/hexanes, 3:7); mp 99 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 3, CH_3), 1.71 (s, 3, CH_3), 2.50 (br s, 1, C$_5$′O*H*), 3.77–3.91 (m, 2, C$_5$′CH_2), 4.27 (m, 1, C$_4$′*H*), 4.92 (m, 2, C$_{2'3'}$*H*), 6.19 (m, 1, C$_1$′*H*),

(26) Huang, M.; Avery, T. L.; Blakley, R. L.; Secrist, J. A., III; Montgomery, J. A. *J. Med. Chem.* 1984, *27*, 800–802.

8.23 (br d, 1, J = 10.0 Hz, N*H*). Anal. ($C_{14}H_{14}Cl_3N_5O_4$·0.5EtOH) C, H, N, Cl.

2,6-Dichloro-4-(*n*-butyloxy)-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (5). The title compound was obtained in 30% yield when the above reaction mixture was left for 16 h or longer at ambient temperature and when a reverse addition of free glycosylamine **2** was made to a solution of **1** in 1-butanol: R_f 0.68 (EtOAc/hexanes, 3:7); mp 175 °C; ^{1}H NMR ($CDCl_3$) δ 1.00 (t, 3, CH_2CH_3), 1.39 (s, 3, CH_3), 1.61 (s, 3, CH_3), 1.52 (m, 2, CH_2CH_3), 1.92 (m, 2, CH_2CH_2), 2.79 (br s, 1, $C_{5'}OH$), 3.94 (m, 2, $C_{5'}CH_2$), 4.46 (br s, 1, $C_{4'}H$), 4.63 (m, 2, OCH_2), 4.84 and 5.02 (2 m, 2, $C_{2'3'}H$), 6.27 (d, 1, J = 10.5 Hz, $C_{1'}H$), 8.69 (d, 1, J = 10.5 Hz, N*H*). Anal. ($C_{18}H_{23}Cl_2N_5O_5$·0.25H_2O) C, H, N, Cl.

2,6-Dichloro-4-amino-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (6). A solution of **3** (0.85 g, 2 mmol) in EtOH/NH_3 (20 mL, saturated at 0 °C) was stirred at 0 °C for 30 min. The solvent was removed, and the residue was purified by chromatography on a silica gel column (5 × 45 cm) using hexanes/EtOAc (1:3, v/v) as the eluent to provide 0.59 g (74%) of **6**: mp 148 °C (EtOH/hexanes); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3, CH_3), 1.59 (s, 3, CH_3), 2.86 (t, 1, $C_{5'}OH$), 3.85–3.98 (m, 2, $C_{5'}CH_2$), 4.44 (br s, 1, $C_{4'}H$), 4.83 and 5.00 (m, 2, $C_{2'3'}H$), 6.15 and 6.79 (2 br s, 2, NH_2), 6.22 (d of d, 1, $C_{1'}H$), 8.47 (d, 1, J = 10.5 Hz, N*H*). Anal. ($C_{14}H_{16}Cl_2N_6O_4$) C, H, N, Cl.

2,6-Dichloro-4-amino-8-[(2,3-*O*-isopropylidene-α-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (9). The title compound was prepared in a similar manner as described for **6**, by using **4** (0.85 g, 2 mmol) and EtOH/NH_3 (20 mL). The product was isolated as a crystalline solid and recrystallized from EtOH to yield 0.56 g (70%): mp 208 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 3, CH_3), 1.74 (s, 3, CH_3), 2.78 (t, 1, $C_{5'}OH$), 3.76–3.92 (2 m, 2, $C_{5'}CH_2$), 4.27 (t, 1, $C_{4'}H$), 4.92 (m, 2, $C_{2'3'}H$), 6.18 (m, 1, $C_{1'}H$), 6.54 and 6.69 (2 br s, 2, NH_2), 8.00 (d, 1, J = 10.5 Hz, N*H*). Anal. ($C_{14}H_{16}Cl_2N_6O_4$·0.25EtOH) C, H, N, Cl.

General Procedure for Hydrogenation. A mixture of the appropriate nucleoside (1 mmol), Pd/C (10%; 0.1 g), and anhydrous Et_3N (3 mmol) in absolute EtOH (100 mL) was shaken in a pressure bottle on a Parr hydrogenator at 50 psi for 24 h at ambient temperature. The catalyst was removed by filtration through a Celite pad and washed with EtOH (2 × 25 mL). The combined filtrates were evaporated, and the residue was purified by either column chromatography or direct crystallization.

4-Amino-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (7). Hydrogenation of **6** by the general procedure gave **7** in 75% yield: mp 178 °C (EtOH); ^{1}H NMR (Me_2SO-d_6) δ 1.28 (s, 3, CH_3), 1.47 (s, 3, CH_3), 3.51 (m, 2, $C_{5'}CH_2$), 4.18 (m, 1, $C_{4'}H$), 4.81 (m, 2, $C_{2'3'}H$), 5.54 (t, 1, $C_{5'}OH$), 6.10 (d, 1, J = 10.6 Hz, $C_{1'}H$), 7.80 and 7.99 (2 br s, 2, NH_2), 8.36 and 8.49 (2 s, 2, C_2H and C_6H), 8.83 (d, 1, J = 10.6 Hz, N*H*). Anal. ($C_{14}H_{18}N_6O_4$) C, H, N.

4-Amino-8-[(2,3-*O*-isopropylidene-α-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (10). Hydrogenation of **9** by the general procedure gave **10** in 71% yield; isolated as homogeneous foam: ^{1}H NMR (Me_2SO-d_6) δ 1.36 (s, 3, CH_3), 1.55 (s, 3, CH_3), 3.52 (m, 2, $C_{5'}CH_2$), 4.00 (m, 1, $C_{4'}H$), 4.83 (m, 2, $C_{2'3'}H$), 5.07 (t, 1, $C_{5'}OH$), 6.15 (d of d, 1, $C_{1'}H$), 7.68 (d, 1, J = 10.4 Hz, N*H*), 7.89 and 8.08 (2 br s, 2, NH_2), 8.40 and 8.55 (2 s, 2, C_2H and C_6H). Anal. ($C_{14}H_{18}N_6O_4$) C, H, N.

General Procedure for Deisopropylidenation Reaction. A suspension of the corresponding 2′,3′-*O*-isopropylidene nucleoside (0.5 mmol) in a mixture of TFA/H_2O (2 mL; 9:1, v/v) was stirred at room temperature for 30 min. The solvent was evaporated under a stream of argon, and the residue was coevaporated with EtOH (3 × 20 mL), redissolved in EtOH (2 mL), and precipitated by slow addition of dry diethyl ether (100 mL). Compounds were further purified by flash silica gel column chromatography using EtOAc/H_2O/MeOH/acetone (3:1:1:1, v/v) as the eluent or by HPLC followed by crystallization from a suitable solvent.

4-Amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (ARPP, 8). Deisopropylidenation of **7** with aqueous TFA gave **8** in 85% yield: mp 212 °C (H_2O) (lit.[9] mp 214–216 °C); UV and ^{1}H NMR spectra are identical with those of an authentic sample of **8**.

4-Amino-8-(α-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (11). Deisopropylidenation of **10** with aqueous TFA gave **11** in 82% yield: mp 165 °C (EtOH); ^{1}H NMR (Me_2SO-d_6)[16] δ 3.50 (m, 2, $C_{5'}CH_2$), 3.77, 3.96, and 4.11 (3 m, 3, $C_{2'3'4'}H$), 4.91 (t, 1, $C_{5'}OH$), 5.31 and 5.64 (2 d, 2, J = 5.6 Hz, $C_{2'3'}OH$), 5.78 (q, 1, $C_{1'}H$, collapsed to a d, $J_{1'2'}$ = 5.80 Hz after deuteration), 5.97 (q, 1, $C_{1'}H$, collapsed to a d, $J_{1'2'}$ = 5.43 Hz after deuteration), 7.78 and 8.0 (2 br s, 2, NH_2), 8.19 (d, 1, $J_{1',NH}$ = 10.5 Hz, N*H*), 8.39 (d, 1, partially overlapped with C_2H proton, N*H*), 8.38 and 8.49 (2 s, 2, C_2H and C_6H). Anal. ($C_{11}H_{14}N_6O_4$) C, H, N.

2,6-Dichloro-4-methoxy-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (12). To a stirred solution of **3** (0.42 g, 1 mmol) in dry MeOH (20 mL) was added NaOMe (0.108 g, 2 mmol). When TLC (solvent: EtOAc/hexanes, 3:7) showed the reaction was complete (in 30 min), the solution was neutralized by the addition of Dowex-50 (H^+) resin and filtered. The resin was washed with MeOH (2 × 20 mL), and the combined filtrates were evaporated to dryness. The residue was purified by flash chromatography using hexanes/EtOAc (3:1, v/v) as the eluent to furnish 0.27 g (65%) of **12**: mp 211 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3, CH_3), 1.48 (s, 3, CH_3), 2.49 (br s, 1, $C_{5'}OH$), 3.62 (m, 2, $C_{5'}CH_2$), 4.11 (s, 3, OCH_3), 4.34 (s, 1, $C_{4'}H$), 4.69 and 4.89 (2 m, 2, $C_{2'3'}H$), 6.15 (d of d, 1, $C_{1'}H$), 8.57 (d, 1, J = 10.8 Hz, N*H*). Anal. ($C_{15}H_{17}Cl_2N_5O_5$) C, H, N, Cl.

4-Methoxy-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (13). The title compound was prepared by hydrogenation of **12** following the general procedure, in 84% yield: mp 218 °C (EtOH); ^{1}H NMR (Me_2SO-d_6) δ 1.28 (s, 3, CH_3), 1.47 (s, 3, CH_3), 3.55 (m, 2, $C_{5'}CH_2$), 4.11 (s, 3, OCH_3), 4.20 (s, 1, $C_{4'}H$), 4.82 (m, 2, $C_{2'3'}H$), 5.63 (br s, 1, $C_{5'}OH$), 6.13 (br s, 1, $C_{1'}H$), 8.60 and 8.79 (2 s, 2, C_2H and C_6H), 9.11 (br s, 1, N*H*). Anal. ($C_{15}H_{19}N_5O_5$) C, H, N.

4-Methoxy-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (MRPP, 14). Deisopropylidenation of **13** by the general procedure gave **14** in 82% yield: mp 183 °C (aqueous EtOH); UV λ_{max} nm (ε × 10^{-3}) (pH 1) 299 (13.9), 310 (16.2), 324 (11.5), (pH 7) 210 (10.5), 283 (11.6), 297 (sh) (10.3), 310 (10.1), 326 (sh) (7.2), (pH 11) 283 (11.4), 297 (sh) (10.3), 309 (10.0), 326 (sh) (7.2); ^{1}H NMR (Me_2SO-d_6) δ 3.44 (m, 2, $C_{5'}CH_2$), 3.78 (m, 1, $C_{4'}H$), 4.02 and 4.15 (2 m, 2, $C_{2'3'}H$), 4.11 (s, 3, OCH_3), 5.83 (q, 1, $C_{1'}H$, collapsed to a d, $J_{1'2'}$ = 5.86 Hz, after deuteration), 8.59 and 8.82 (2 s, 2, C_2H and C_6H), 8.71 (d, 1, $J_{1',NH}$ = 10.0 Hz, N*H*). Anal. ($C_{12}H_{15}N_5O_5$·1.5H_2O) C, H, N.

4-Methoxy-8-(α-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (17). Compound **4** was transformed to **16** via the intermediate **15** in a similar way as described above for **13**. The overall yield of **16** was 65%: mp 105 °C (EtOH); ^{1}H NMR (Me_2SO-d_6) δ 1.37 (s, 3, CH_3), 1.56 (s, 3, CH_3), 3.54 (m, 2, $C_{5'}CH_2$), 4.02 (m, 1, $C_{4'}H$), 4.12 (s, 3, OCH_3), 4.85 (m, 2, $C_{2'3'}H$), 5.09 (t, 1, $C_{5'}OH$), 6.16 (q, 1, $C_{1'}H$, collapsed to a d after deuteration, $J_{1'2'}$ = 4.6 Hz), 7.83 (d, 1, $J_{1',NH}$ = 10.4 Hz, N*H*), 8.67 and 8.86 (2 s, 2, C_2H and C_6H). Anal. ($C_{15}H_{19}N_5O_5$) C, H, N. Deisopropylidenation of **16** with aqueous TFA by the general procedure furnished 71% yield of **17**: mp 213 °C (H_2O); ^{1}H NMR (Me_2SO-d_6) δ 3.44 (m, 2, $C_{5'}CH_2$), 3.84, 3.99, and 4.18 (3 m, 3, $C_{4'2'3'}H$), 4.11 (s, 3, OCH_3), 6.00 (q, 1, $C_{1'}H$, collapsed to a d after deuteration, $J_{1'2'}$ = 5.5 Hz), 8.39 (d, 1, $J_{1',NH}$ = 10.3 Hz, N*H*), 8.60 and 8.83 (2 s, 2, C_2H and C_6H). Anal. ($C_{12}H_{15}N_5O_5$) C, H, N.

2,6-Dichloro-4-(benzyloxy)-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (18). To a stirred solution of **3** (0.84 g, 2 mmol) in dry BnOH (3 mL) was added Et_3N (0.41 mL, 3 mmol), and the mixture was stirred at ambient temperature for 24 h with the exclusion of moisture. The dark colored reaction mixture was washed with water (2 × 20 mL) and hexanes (3 × 20 mL) to furnish a gummy residue, which on purification by flash column chromatography using hexanes/EtOAc (7:3, v/v) as the eluent afforded 0.84 g (85%) of **18**: mp 178 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 3, CH_3), 1.59 (s, 3, CH_3), 2.65 (br s, 1, $C_{5'}OH$), 3.91 (m, 2, $C_{5'}CH_2$), 4.43 (m, 1, $C_{4'}H$), 4.80 and 4.99 (2 m, 2, $C_{2'3'}H$), 5.66 (s, 2, CH_2Ph), 6.22 (d of d, 1, $C_{1'}H$, collapsed to a br s after deuteration), 7.33–7.55 (m, 5, CH_2Ph), 8.68 (d, 1, J = 10.4 Hz, N*H*). Anal. ($C_{21}H_{21}Cl_2N_5O_5$) C, H, N, Cl.

2,6-Dichloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one (19).

A mixture of **18** (2.47 g, 5 mmol) and Pd/C (10%, 1 g) in dry dioxane (100 mL) was hydrogenated at atmospheric pressure for 4 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was purified by flash chromatography to furnish 1.0 g (50%) of **19**: mp 248 °C (dec); ^{1}H NMR (Me_2SO-d_6) δ 1.27 (s, 3, CH_3), 1.46 (s, 3, CH_3), 3.51 (m, 2, $C_{5'}CH_2$), 4.14 (m, 1, $C_{4'}H$), 4.79 (m, 2, $C_{2'3'}H$), 5.44 (t, 1, $C_{5'}OH$), 5.90 (d of d, 1, $C_{1'}H$, collapsed to a d on deuteration, $J_{1',2'}$ = 1.4 Hz), 8.56 (d, 1, $J_{1',NH}$ = 10.6 Hz, NH). Anal. ($C_{14}H_{15}Cl_2N_5O_5$) C, H, N, Cl.

8-(β-D-Ribofuranosylamino)pyrimido[5,4-*d*]pyrimidin-4-(3*H*)-one (21). Compound **19** on deisopropylidenation with aqueous TFA by the general procedure gave 71% yield of 2,6-dichloro-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidin-4-(3*H*)-one: mp 200 °C (dec); UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1) 229 (8.4), 286 (17.9), 324 (9.7), 339 (6.9), (pH 7) 291 (18.3), 323 (11.2), 338 (8.3), (pH 11) 291 (18.2), 323 (11.2), 338 (8.3); ^{1}H NMR (Me_2SO-d_6) δ 3.84 (m, 1, $C_{4'}H$), 3.96 and 4.14 (2 t, 2, $C_{2'3'}H$), 5.82 (q, 1, $C_{1'}H$, collapsed to a d of $J_{1',2'}$ = 5.6 Hz after deuteration), 8.10 (d, 1, $J_{1',NH}$ = 10.0 Hz, NH). Anal. Calcd for $C_{11}H_{11}Cl_2N_5O_5 \cdot 0.5H_2O$: C, 35.40; H, 3.24; N, 18.76. Found: C, 35.27; H, 3.03; N, 18.38.

Catalytic hydrogenation of the above dichloro compound by the general procedure gave the title compound in a 66% yield: mp 220 °C (dec); UV λ_{max} ($\epsilon \times 10^{-3}$) (pH 1) 294 (18.2), 309 (16.5), 323 (8.9), (pH 7) 280 (14.1), 315 (7.9), 328 (5.7), (pH 11) 287 (14.6), 313 (10.5), 328 (7.3); ^{1}H NMR (Me_2SO-d_6) δ 3.80 (m, 1, $C_{4'}H$), 3.96 and 4.11 (2 m, 2, $C_{2'3'}H$), 4.77 (t, 1, $C_{5'}OH$), 5.31 and 5.64 (2 m, 2, $C_{2'3'}OH$), 5.95 (q, 1, $C_{1'}$H, collapsed to a d after deuteration, $J_{1',2'}$ = 5.4 Hz), 8.05 (d, 1, $J_{1',NH}$ =10.0 Hz, NH), 8.18 and 8.49 (2 s, 2, C_2H and C_6H), 12.5 (br s, 1, NH). Anal. ($C_{11}H_{13}N_5O_5 \cdot 0.5H_2O$) C, H, N.

2,6-Dichloro-4-(methylamino)-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (22). To a solution of **3** (0.84 g, 2 mmol) in dry CH_3CN (80 mL) was added an ethanolic solution of CH_3NH_2 (0.52 mL, 5 mmol, 30%) at 0 °C and the mixture was stirred for 1 h. The solvent was evaporated and the residue was purified by crystallization from EtOH/hexanes to furnish 0.66 g (79%) of **22**: mp 192 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.28 (s, 3, CH_3), 1.46 (s, 3, CH_3), 2.92 (d, 3, J = 5.3 Hz, $NHCH_3$, collapsed to a s after deuteration), 3.54 (m, 2, $C_{5'}CH_2$), 4.19 (m, 1, $C_{4'}H$), 4.83 (m, 2, $C_{2'3'}H$), 5.61 (t, 1, $C_{5'}OH$), 5.95 (d, 1, J = 6.3 Hz, collapsed to a br s after deuteration), 8.82 (d, 1, J = 5.3 Hz, $NHCH_3$), 9.13 (d, 1, J = 6.3 Hz, NH). Anal. ($C_{15}H_{18}Cl_2N_6O_4$) C, H, N, Cl.

4-(Methylamino)-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (23). Catalytic hydrogenation of **22** by the general procedure gave the title compound in 65% yield: mp 130 °C (EtOH); ^{1}H NMR (Me_2SO-d_6) δ 1.28 (s, 3, CH_3), 1.46 (s, 3, CH_3), 2.96 (d, 3, J = 5.40 Hz, $NHCH_3$, collapsed to a s after deuteration), 3.54 (m, 2, $C_{5'}CH_2$), 4.18 (br s, 1, $C_{4'}H$), 4.80 (m, 2, $C_{2'3'}H$), 5.56 (t, 1, $C_{5'}OH$), 6.10 (d, 1, J = 10.5 Hz, $C_{1'}H$, collapsed to a br s after deuteration), 8.39 (d, 1, J = 5.4 Hz, $NHCH_3$), 8.43 and 8.50 (2 s, 2, C_2H and C_6H), 8.83 (d, 1, J = 10.5 Hz, NH). Anal. ($C_{15}H_{20}N_6O_4$) C, H, N.

4-(Methylamino)-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (24). Deisopropylidenation of **23** following the general procedure gave **24** in 60% yield: mp 230 °C (EtOH); UV λ_{max} ($\epsilon \times 10^{-3}$) (pH 1) 292 (11.3), 306 (sh) (10.3), 322 (10.0), 336 (7.4), (pH 7) 297 (11.5), 303 (sh) (11.4), 318 (11.2), 334 (7.7), (pH 11) 296 (12.0), 302 (12.0), 317 (11.8), 335 (7.9); ^{1}H NMR (Me_2SO-d_6)[16] δ 2.98 and 3.00 (2 s, 3, $NHCH_3$), 5.72 (dd, 1, $C_{1'}H$), 6.0 (dd, 1, $C_{1'}H$), 8.21 (d, 1, J = 10.0 Hz, NH), 8.95 (d, 1, J = 10.0 Hz, NH), 8.47 and 8.51 (2 s, 2, C_2H and C_6H), and other sugar protons. Anal. ($C_{12}H_{16}N_6O_4 \cdot 0.5H_2O$) C, H, N.

2,6-Dichloro-4-(dimethylamino)-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (25). To a stirred solution of **3** (0.42 g, 1 mmol) in dry CH_3CN (25 mL) was added cold $HN(CH_3)_2$ (0.20 mL, 3 mmol) at 0 °C. TLC (EtOAc/hexanes, 3:7) indicated formation of a high-R_f compound (in 5 min). The reaction mixture was evaporated to dryness, and the residue was adsorbed on silica gel and purified by flash column chromatography to furnish 0.36 g (84%) of **25**: mp 208 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3, CH_3), 1.61 (s, 3, CH_3), 2.82 (t, 1, $C_{5'}OH$), 3.18 [s, 6, $N(CH_3)_2$], 3.85 (m, 2, $C_{5'}CH_2$), 4.32 (m, 1, $C_{4'}H$), 4.82 and 4.97 (2 m, 2, $C_{2'3'}H$), 6.20 (dd, 1, $C_{1'}H$), 7.73 (d, 1, J = 10.8 Hz, NH). Anal. ($C_{16}H_{20}Cl_2N_6O_4$) C, H, N, Cl.

4-(Dimethylamino)-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (26). Catalytic hydrogenation of **25** by the general procedure furnished **26** in 70% yield: mp 180 °C (EtOH); ^{1}H NMR (Me_2SO-d_6) δ 1.28 (s, 3, CH_3), 1.46 (s, 3, CH_3), 3.40–3.60 [br s, 6, $N(CH_3)_2$], 3.53 (m, 2, $C_{5'}CH_2$), 4.18 (m, 1, $C_{4'}H$), 4.76 and 4.82 (2 m, 2, $C_{2'3'}H$), 5.54 (t, 1, $C_{5'}OH$), 6.10 (q, 1, $J_{1',NH}$ = 10.6 Hz, $C_{1'}H$, collapsed to a d on deuteration, $J_{1',2'}$ = 1.7 Hz), 8.43 and 8.46 (2 s, 2, C_2H and C_6H), 8.82 (d, 1, $J_{1',NH}$ = 10.6 Hz, NH). Anal. ($C_{16}H_{22}N_6O_4 \cdot 0.5H_2O$) C, H, N.

4-(Dimethylamino)-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (27). Deisopropylidenation of **26** by the general procedure furnished **27** in 75% yield: mp 231 °C (EtOH); UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1) 300 (16.4), 320 (15.0), 336 (17.0), 351 (12.8), (pH 7) 210 (19.9), 307 (19.6), 320 (18.5), 334 (17.5), 351 (12.8), (pH 11) 306 (18.8), 320 (17.9), 334 (17.0), 351 (12.5); ^{1}H NMR (Me_2SO-d_6)[16] δ 3.31–4.13 [m, 11, $N(CH_3)_2$, $C_{5'}CH_2$, $C_{2'3'4'}H$], 5.58 (q, 1, $C_{1'}H$, collapsed to a d after deuteration, $J_{1',2'}$ = 4.4 Hz), 5.97 (q, 1, $C_{1'}H$, collapsed to d after deuteration, $J_{1',2'}$ = 5.3 Hz), 8.29 (d, 1, $J_{1',NH}$ = 10.5 Hz, NH), 8.97 (d, 1, $J_{1',NH}$ = 10.5 Hz, NH), 8.46 and 8.47 (2 s, 2, C_2H and C_6H). Anal. ($C_{13}H_{18}N_6O_4$) C, H, N.

2-Chloro-4,6-diamino-8-[(2,3-*O*-isopropylidene-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (33). A mixture of **6** (1.41 g, 3.5 mmol) and liquid NH_3 (50 mL) was stirred in a sealed reaction vessel at room temperature for 6 days. After removal of NH_3, the residue was purified by flash chromatography using EtOAc/hexanes (3:7) as eluent to give two nucleoside products in the order described. (i) Compound **33**: crystallized from EtOH to give 0.81 g (59%): mp 291 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.27 (s, 3, CH_3), 1.45 (s, 3, CH_3), 3.53 (m, 2, $C_{5'}CH_2$), 4.11 (m, 1, $C_{4'}H$), 4.79 (m, 2, $C_{2'3'}H$), 5.44 (t, 1, $C_{5'}OH$), 6.04 (d, 1, J = 10.8 Hz, $C_{1'}H$), 6.45 (br s, 2, NH_2), 7.21 and 8.13 (2 br s, 2, NH_2), 8.40 (d, 1, J = 10.8 Hz, NH). Anal. ($C_{14}H_{18}ClN_7O_4 \cdot 0.5H_2O$) C, H, N, Cl. (ii) The α-anomer of **33**: 0.20 g (15% yield as foam); ^{1}H NMR (Me_2SO-d_6) δ 1.28 (s, 3, CH_3), 1.46 (s, 3, CH_3), 3.61 (m, 2, $C_{5'}CH_2$), 4.21 (br s, 1, $C_{4'}H$), 4.70 and 4.84 (2 d, 2, $C_{2'3'}H$), 5.55 (t, 1, $C_{5'}OH$), 5.89 (d, 1, J = 10.7 Hz, $C_{1'}H$), 6.19 (br s, 2, NH_2), 7.28 and 7.47 (2 br s, 2, NH_2), 8.78 (d, 1, J = 10.7 Hz, NH). Anal. Calcd for $C_{14}H_{18}ClN_7O_4$: C, 43.81; H, 4.72; N, 25.54; Cl, 9.23. Found: C, 43.87; H, 4.75; N, 25.65; Cl, 9.09.

4,6-Diamino-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (34). Catalytic hydrogenation of **33** by the general procedure furnished **34** in 72% yield: mp 150 °C (foam); ^{1}H NMR (Me_2SO-d_6) δ 1.27 (s, 3, CH_3), 1.45 (s, 3, CH_3), 3.61 (m, 2, $C_{5'}CH_2$), 4.13 (br s, 1, $C_{4'}H$), 4.80 (m, 2, $C_{2'3'}H$), 5.47 (t, 1, $C_{5'}OH$), 6.05 (dd, 1, $C_{1'}H$, collapsed to a s on deuteration), 6.37 (br s, 2, NH_2), 6.71 and 7.50 (2 br s, 2, NH_2), 8.05 (s, 1, C_2H), 8.54 (d, 1, J = 10.5 Hz, NH). Anal. ($C_{14}H_{19}N_7O_4 \cdot 0.25H_2O$) C, H, N.

4,6-Diamino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (6-Amino-ARPP, 35). Deisopropylidenation of **34** by the general procedure gave the title compound, which was isolated as its TFA salt in 66% yield: mp 170 °C (dec); UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1) 244 (13.7), 269 (15.3), 334 (7.1), 350 (6.6), (pH 7) 242 (12.4), 280 (14.6), 343 (8.0), (pH 11) 210 (21.1), 242 (12.4), 281 (14.4), 344 (8.0); ^{1}H NMR (Me_2SO-d_6)[16] δ 3.20–4.21 (m, 5, $C_{2'3'4'}H$ and $C_{5'}H_2$), 5.90–5.98 (q, 1, $C_{1'}H$, collapsed to a d on deuteration, $J_{1',2'}$ = 5.61 Hz), 7.81 (br s, 4, $2NH_2$), 8.30 (s, 1, C_2H), 8.73 and 9.20 (2 br s, 1, NH). Anal. ($C_{11}H_{15}N_7O_4 \cdot$TFA) C, H, N.

4-Amino-6-chloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (36). Controlled hydrogenation of **6** at atmospheric pressure for 16 h gave **36** in 65% yield following the general workup procedure: mp 206 °C (EtOH); ^{1}H NMR (Me_2SO-d_6) δ 1.28 (s, 3, CH_3), 1.47 (s, 3, CH_3), 3.57 (m, 2, $C_{5'}CH_2$), 4.21 (br s, 1, $C_{4'}H$), 4.82 (m, 2, $C_{2'3'}H$), 5.64 (br s, 1, $C_{5'}OH$), 5.97 (d, 1, J = 10.0 Hz, $C_{1'}H$), 7.79 and 8.06 (2 br s, 2, NH_2), 8.35 (s, 1, C_2H), 9.24 (d, 1, J = 10.0 Hz, NH). Anal. ($C_{14}H_{17}ClN_6O_4$) C, H, N, Cl.

4-Amino-6-chloro-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (6-Chloro-ARPP, 37). Deisopropylidenation of **36** by the general procedure gave 6-chloro-ARPP in 67% yield: mp 205 °C (dec); UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1) 290 (16.8), 328 (12.2), 342 (9.5), (pH 7) 291 (16.5), 308 (sh) (13.0), 322 (13.0), 337

(9.7), (pH 11) 291 (16.4), 308 (sh) (13.0), 323 (12.9), 337 (9.7); ^{1}H NMR (Me_2SO-d_6)[16] δ 3.41–4.12 (m, 5, $C_{2'3'4'}H$ and $C_{5'}CH_2$), 5.70 (q, 1, $C_{1'}H$, collapsed to d on deuteration, $J_{1',2'}$ = 6.0 Hz), 5.86 (q, 1, $C_{1'}H$, collapsed to d on deuteration, $J_{1',2'}$ = 5.63 Hz), 7.79 and 8.07 (2 br s, 2, NH_2), 8.38 (s, 1, C_2H), 8.45 (d, 1, $J_{1',NH}$ = 10.9 Hz), 8.90 (d, 1, $J_{1',NH}$ = 10.9 Hz). Anal. Calcd for $C_{11}H_{13}ClN_6O_4 \cdot 0.75H_2O$: C, 38.60; H, 4.26; N, 24.55; Cl, 10.37. Found: C, 38.51; H, 4.07; N, 24.45; Cl, 11.02.

4,6-Dichloro-8-[(2,3-*O*-isopropylidene-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (29). Condensation of dry 4,6,8-trichloropyrimido[5,4-*d*]pyrimidine[14] (**28**, 3.52 g, 15 mmol) with **2** (3.61 g, 10 mmol) was carried out in a similar way as described for the preparation of **3**, and a 3:7 mixture of α- and β-anomers of **29** was obtained as a foam in 45% yield. Attempted crystallization and extended solvent contact decomposed **29** into unidentified compounds: UV (MeOH) λ_{max} nm ($\epsilon \times 10^{-3}$) 244 (5.8), 287 (8.0), 310 (sh) (8.3), 329 (11.1), 348 (sh) (7.6); ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 3, CH_3), β), 1.62 (s, 3, CH_3, β), 1.48 (s, 3, CH_3, α), 1.72 (s, 3, CH_3, α), 2.71 (br s, 1, $C_{5'}OH$, β), 2.78 (t, 1, $C_{5'}OH$, α), 6.25 (dd, 1, $C_{1'}H$, α), 6.36 (d, 1, $C_{1'}H$, β), 8.40 (d, 1, $J_{1',NH}$ = 10.6 Hz, NH, α), 8.93 (s, 1, C_2H, β), 9.03 (s, 1, C_2H, α), 9.08 (d, 1, $J_{1',NH}$ = 10.5 Hz, NH, β), and other sugar protons. Anal. ($C_{14}H_{15}Cl_2N_5O_4 \cdot 0.5n$-BuOH) C, H, N, Cl.

4-(Benzyloxy)-6-chloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (30). The title compound was prepared from **29** in 65% yield, following the procedure as described for the preparation of **18**. Pure β-anomer, being the less soluble compound, crystallized from EtOH and was found to be quite stable in solution compared to its precursor **29**: mp 188–190 °C; ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 3, CH_3), 1.55 (s, 3, CH_3), 2.68 (t, 1, $C_{5'}OH$), 3.88 (m, 2, $C_{5'}CH_2$), 4.40 (br s, 1, $C_{4'}H$), 4.76 and 4.96 (2 m, 2, $C_{2'3'}H$), 5.63 (s, 2, CH_2Ph), 6.22 (dd, 1, $C_{1'}H$), 7.31–7.52 (m, 5, CH_2Ph), 8.64 (d, 1, J = 10.0 Hz, NH). Anal. ($C_{21}H_{22}ClN_5O_5$) C, H, N, Cl.

6-Chloro-8-[(2,3-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one (31). Following the procedure as described for the preparation of **19**, compound **30** was hydrogenated to give **31** in 69% yield: mp >220 °C (dec); ^{1}H NMR (Me_2SO-d_6) δ 1.25 (s, 3, CH_3), 1.43 (s, 3, CH_3), 3.50 (m, 2, $C_{5'}CH_2$), 4.19 (m, 1, $C_{4'}H$), 4.71–4.80 (m, 2, $C_{2'3'}H$), 5.94 (d, 1, J = 10.0 Hz, $C_{1'}H$), 8.14 (s, 1, C_2H), 9.07 (d, 1, J = 10.0 Hz, NH), 12.9 (br s, 1, N_3H). Anal. Calcd for $C_{14}H_{16}ClN_5O_5 \cdot H_2O$: C, 43.35; H, 4.67; N, 18.06. Found: C, 43.02; H, 4.16; N, 17.24.

6-Chloro-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one (32). Deisopropylidenation of **31** by aqueous TFA following the general procedure furnished the title compound in 58% yield: mp 180–182 °C (dec); UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1) 230 (6.1), 277 (sh) (15.3), 285 (16.2), 310 (sh) (8.8), 320 (9.4), 334 (7.0), (pH 7) 228 (6.1), 277 (sh) (15.3), 285 (15.8), 308 (sh) (8.8), 320 (9.4), 334 (6.9), (pH 11) 290 (15.3), 308 (sh) (12.9), 319 (10.8), 334 (7.8); ^{1}H NMR (Me_2SO-d_6)[16] δ 3.48 (m, 2, $C_{5'}CH_2$), 3.83 (m, 1, $C_{4'}H$), 3.98–4.06 (m, 2, $C_{2'3'}H$), 5.54 (q, 1, collapsed to d on deuteration, $J_{1',2'}$ = 5.5 Hz), 5.84 (q, 1, collapsed to d on deuteration, $J_{1',2'}$ = 5.1 Hz), 8.20 (s, 1, C_2H), 8.30 (d, 1, $J_{1',NH}$ = 10.0 Hz, NH), 9.0 (d, 1, $J_{1',NH}$ = 10.0 Hz, NH), 12.95 (br s, 1, N_3H), and other sugar protons. Anal. ($C_{11}H_{12}ClN_5O_5 \cdot 0.25$EtOH) C, H, N, Cl.

Biological Methods: Assays on Cell Culture. Materials. [^{14}C]Formate (57 Ci/mol) and [8-^{14}C]adenosine (51 Ci/mol) were purchased from ICN Biomedicals. Adenosine deaminase was from Sigma Chemical Co.

Cell Lines. The B cell line, WI-L2, and derivatives have been previously described.[27] WI-L2 is the normal lymphoblast phenotype. The enzyme-deficient cell lines selected in vitro are as follows: HPRT$^-$, a hypoxanthine-guanine phosphoribosyl transferase (EC 2.4.2.8) deficient line and AK$^-$, an adenosine kinase (EC 2.7.1.20) deficient line. Cells were cultured in RPMI 1640 medium containing 5% dialyzed fetal bovine serum, 20 mM NaHEPES, pH 7.5, and 2 mM glutamine and maintained in log phase growth between 0.5 and 12 × 10^5 cells/mL. The mutant cell lines were periodically reselected by treatment with 6-thioguanine (HPRT$^-$) or tubercidin (AK$^-$).

HPLC Analysis. Sample components were separated with an LKB Model 2150 gradient HPLC system at ambient temperature on an Altex Ultrasphere-ODS reverse-phase column (Beckman) developed with a linear gradient of buffer A (10 mM KPO_4, pH 3.83) to 20% component B (60% aqueous CH_3CN) at a combined flow rate of 1.0 mL/min over 15 min. Ultraviolet (UV) absorbance was monitored with an LKB Model 2140 diode array detector.

Inhibition of de Novo Purine Biosynthesis. WI-L2 or adenosine kinase deficient cells were preincubated at 37 °C with drug for various time periods prior to labeling with [^{14}C]formate as previously described.[28]

Enzyme Assays. Adenosine kinase inhibition studies were accomplished by the filter binding assay method.[29] Inhibition studies were done at 37 °C with an assay mixture containing 4 mM ATP, 1.5 mM $MgCl_2$, 5 or 10 μM [8-^{14}C]adenosine (50 mCi/mmol), and 100 mM Tris–maleate, pH 5.5. To prevent the enzymatic breakdown of adenosine, *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) was added to the cell-free WI-L2 lysate to give 5 μM final assay concentration. The assay was started by the addition of protein. The amount of enzyme, time of assay, and sampling volume were adjusted to give acceptable conversion of [8-^{14}C]adenosine to [8-^{14}C]AMP. Each DE-81 filter was spotted with a constant sampling volume and immersed in H_2O (4 L) to terminate the reaction. Filters were washed three times with H_2O (4 L) and once with EtOH (100 mL). Dry filters were placed in scintillation vials, and radioactivity was counted with 10 mL of toluene-based scintillation cocktail.

Adenosine deaminase substrate activity was accomplished by incubating each compound (1.0 mM) in potassium phosphate buffer (100 mM, pH 7.5) with 0.25 unit/mL of adenosine deaminase. Samples were analyzed directly on reverse-phase HPLC. Enzymatic hydrolysis was distinguished from nonenzymatic by treating a parallel sample with EHNA.

Cell Culture Toxicity Studies. The cell lines used were L1210 (a murine leukemia), WI-L2 (a human B-lymphoblast), and LoVo/L (a human colon carcinoma). Cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 20 mM HEPES, pH 7.4, and 2 mM glutamine. The cytotoxicity determinations were carried out in 96-well microtiter dishes containing a starting number of (5–10) × 10^3 cells per well and 0.1–100 μM concentrations of the compounds in triplicate wells. L1210 and WI-L2 were incubated with the compounds at 37 °C for 3 days, while LoVo/L was incubated for 5 days. After this time period, 25 μL of 4 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to each well and incubation was continued for 2 to 5 h.[25,30] The formazan product was dissolved in 2-propanol containing 0.04 M HCl, and the absorbance was determined with a microtiter plate reader. The absorbance was proportional to the number of cells. The absorbance values were used to calculate the ID_{50} value for each compound, the concentration that inhibited cell growth to 50% of the value for untreated, control cells.

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