Novel 6-Alkoxypurine 2',3'-Dideoxynucleosides as Inhibitors of the Cytopathic Effect of the Human Immunodeficiency Virus

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Twenty-one 6-alkoxypurine 2',3'-dideoxynucleosides were enzymatically synthesized with nucleoside phosphorylases purified from *E. coli*. Eighteen analogs exhibited anti-HIV-1 activity in MT4 cells. Two analogs, 6-(hexyloxy)- (**17**) and 6-(heptyloxy)- (**18**) purine 2',3'-dideoxynucleoside, were as potent as 2',3'-dideoxyinosine (ddI, didanosine, Videx). Although the antiviral activities of **17** and **18** were equivalent, **18** was more cytotoxic. Analogs containing less than four carbons in the 6-alkoxypurine substituent exhibited weak anti-HIV-1 activity. Analogs containing more than seven carbons in the 6-alkoxypurine substituent were too cytotoxic to be effectively evaluated for antiviral activity. Several 6-alkoxypurine 2',3'-dideoxynucleosides were evaluated for substrate activity with calf intestinal adenosine deaminase (ADA). Increasing the carbon chain length of the 6-alkoxypurine substituent decreased the rate of dealkoxylation. The best substrate in this series was 6-methoxypurine 2',3'-dideoxynucleoside (**1**); however, the rate of dealkoxylation of 100 μM **1** was 0.17% of the rate of deamination of 100 μM 2',3'-dideoxyadenosine. Compound **17**, the most potent anti-HIV-1 analog, was not a substrate for ADA. EHNA (*erythro*-9-(2-hydroxy-3-nonyl)adenine), a potent inhibitor of ADA, had little effect on the antiviral activities of **17** and ddI. In contrast, coformycin, a potent inhibitor of both ADA and AMP deaminase, dramatically decreased the antiviral activity of **17**, but not the antiviral activity of ddI. Thus, AMP deaminase appeared to be involved in the anabolism of **17**. The pharmacokinetic profile of **17**, the most promising analog in this series, was determined in the rat. At least seventeen metabolites of **17**, including ddI, were detected in plasma samples. This analog also had poor oral bioavailability.

Introduction

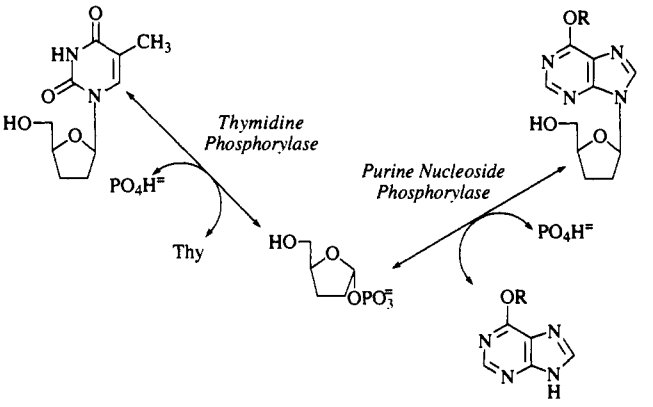
The 2',3'-dideoxynucleosides of adenine, hypoxanthine, guanine, and 2,6-diaminopurine protect ATH8 and MT4 cells against the cytopathology of the human immunodeficiency virus (HIV),[1,2] the causative agent of acquired immunodeficiency syndrome (AIDS).[3,4] Several 2-amino-6-halo- and 6-halopurine 2',3'-dideoxynucleosides have been synthesized and many of these analogs are also active against HIV-1.[5] In addition, 6-(alkylamino)- and 6-(alkylthio)purine 2',3'-dideoxynucleosides exhibit anti-HIV-1 activity in PBM (peripheral blood mononuclear) cells.[6] In contrast, little is known about the activity of 6-alkoxypurine 2',3'-dideoxynucleosides. In this report, we describe the synthesis and anti-HIV-1 activity of a series of novel 6-alkoxypurine 2',3'-dideoxynucleosides.

Chemistry

Commercially unavailable 6-alkoxypurines were prepared from either 6-chloropurine or purin-6-yltrimethylammonium chloride and an excess of the appropriate alkoxide ion in tetrahydrofuran. The typical yield of purified aglycone was 60%.

Twenty-one 6-alkoxypurine 2',3'-dideoxynucleosides were synthesized as shown in Scheme I using nucleoside phosphorylases isolated from *Eschrichia coli*.[7] α-D-2,3-Dideoxyribose-1-phosphate was generated in situ from 3'-deoxythymidine (**25**) and inorganic phosphate by thymidine phosphorylase. Purine nucleoside phosphorylase catalyzed the formation of the new glycosyl bond between the pentose-1-phosphate intermediate and the desired purine. The net result was the transfer of the 2,3-dideoxy sugar moiety from **25** to a purine with retention of the β-configuration in the nucleoside.[7] Although each phosphorylase reaction was reversible, the equilibrium favored formation of the purine nucleoside.[7] The phosphorylases were immobilized onto DE-52 cellulose to stabilize the enzymes in the presence of organic solvents and to facilitate the rapid removal of the catalysts from the reaction mixture.

Scheme I



Yields, melting points, optical rotations, UV spectral data, and hydrophobicity constants of the 6-alkoxypurine 2',3'-dideoxynucleosides are presented in Table I. Representative NMR data are included for **1** and **2**. NMR data for compounds **3**–**21** are included in the supplementary material.

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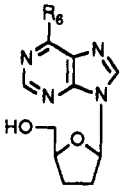
Table I. Synthetic Data and Physical Constants for Some 6-Alkoxypurine 2′,3′-Dideoxynucleosides



no.	R_6 substituent	yield,[a] %	mp, °C	formula	$[\alpha]^{20}_D$ in DMF,[b] deg	UV data at pH 7		log k'_w
						λ_{max}[c]	ϵ[d]	
1	OCH_3	27	122–123	$C_{11}H_{14}N_4O_3$	−22.4	251	11.8	0.16
2	OCH_2CH_3	28	93–95	$C_{12}H_{16}N_4O_3 \cdot 0.15H_2O$	−23.8	247	11.9	–
3	OCH_2CH_2F	34	100	$C_{12}H_{15}FN_4O_3$[e]	−20.2	251	11.6	0.25
4	OCH_2CF_3	16	88	$C_{12}H_{13}F_3N_4O_3$[e]	−14.6	250	10.9	1.34
5	$O(CH_2)_2CH_3$	69	*f*	$C_{13}H_{18}N_4O_3 \cdot 0.3H_2O$	−19.2[g]	253	13.2	1.25
6	$OCH(CH_3)_2$	38	75	$C_{13}H_{18}N_4O_3$	−23.8	253	12.8	1.16
7	$OCH_2CH{=}CH_2$	61	53	$C_{13}H_{16}N_4O_3 \cdot 0.4H_2O$	−21.6	252	11.3	1.05
8	$O(CH_2)_3CH_3$	36	55	$C_{14}H_{20}N_4O_3$	−20.8[h]	252.5	13.7	1.82
9	(*RS*)-$OCH(CH_3)CH_2CH_3$	60	*f*	$C_{14}H_{20}N_4O_3$	−20.4	253	15.8	–
10	(*R*)-$OCH(CH_3)CH_2CH_3$	68	*f*	$C_{14}H_{20}N_4O_3 \cdot 0.25H_2O$	−49.2	252.5	12.9	1.64
11	(*S*)-$OCH(CH_3)CH_2CH_3$	66	*f*	$C_{14}H_{20}N_4O_3 \cdot 0.25H_2O$	7.1[h]	252.5	12.6	1.66
12	$OCH_2CH(Me)_2$	59	*f*	$C_{14}H_{20}N_4O_3 \cdot 0.15H_2O$	−19.0	252.5	14.0	1.74
13	$OCH_2CH{=}CHCH_3$	56	*f*	$C_{14}H_{18}N_4O_3 \cdot 0.25H_2O$	−15.0	252	15.4	1.60
14	$O(CH_2)_4CH_3$	57	51	$C_{15}H_{22}N_4O_3$	−19.4	252	12.4	2.73
15	(*RS*)-$OCH(CH_3)(CH_2)_2CH_3$	61	*f*	$C_{15}H_{22}N_4O_3$	−18.6	252	15.3	2.58
16	$OCH(CH_2CH_3)_2$	61	*f*	$C_{15}H_{22}N_4O_3 \cdot 0.3H_2O$	−21.4	252.5	16.8	2.31
17	$O(CH_2)_5CH_3$	34	50	$C_{16}H_{24}N_4O_3$	−16.0	252	13.0	3.33
18	$O(CH_2)_6CH_3$	36	*f*	$C_{17}H_{26}N_4O_3 \cdot 0.3H_2O$	−8.2[h]	252	12.5	3.98
19	$O(CH_2)_7CH_3$	11	50–52	$C_{16}H_{28}N_4O_3$	−8.1[i]	252	13.2	4.55
20	$O(CH_2)_8CH_3$	14	55–57	$C_{19}H_{30}N_4O_3$	−7.9[j]	252	15.4	5.13
21	$O(CH_2)_9CH_3$	19	44–46	$C_{20}H_{32}N_4O_3$	−15.6	252	10.9	5.69

[a] Based on amount of aglycone in the enzymatic synthesis. [b] Concentration was 0.5 g/100 mL unless otherwise specified. [c] In nanometers. [d] mM^{-1} cm^{-1}. [e] These compounds were analyzed for fluorine. [f] These compounds were not solids at room temperature. [g] 0.625 g/100 mL. [h] 0.49 g/100 mL. [i] 0.54 g/100 mL. [j] 0.52 g/100 mL.

Biology

Cytotoxicity. Cytotoxicity of the 2′,3′-dideoxynucleosides was assessed in MT4 cells, human D-98 cells, and mouse L cells (Table II). In MT4 cells, the 6-(heptyloxy)- (**18**) and 6-(octyloxy)- (**19**) purine 2′,3′-dideoxynucleosides inhibited cell growth by about 40% at 32 μM, whereas the 6-(nonyloxy)- (**20**) and 6-(decyloxy)- (**21**) analogs inhibited cell growth by about 80% at 32 μM. In D-98 and L cells, the 2′,3′-dideoxynucleosides of 6-(2-butenyloxy)- (**13**), 6-(pentyloxy)- (**14**), 6-[(1-ethylpropyl)oxy]- (**16**), and 6-(hexyloxy)- (**17**) purine, as well as **18–21** inhibited cell growth by more than 30% at 100 μM (Table II). Of the 21 analogs tested, only the long-chain substituted analogs (**18–21**) showed significant cytotoxicity in all three cell lines. Compounds **20** and **21** were the most cytotoxic analogs in this series.

Anti-HIV-1 Activity in MT4 Cells. Antiviral activity was assessed in MT4 cells and the results are presented in Table II. All but three of the nucleosides had detectable anti-HIV-1 activity. The 6-methoxy- (**1**), 6-ethoxy- (**2**), and 6-propoxy- (**5**) purine 2′,3′-dideoxynucleosides had weak anti-HIV-1 activity (IC_{50} > 100 μM) compared to ddI (IC_{50} = 22 μM). However, the antiviral activity increased as the length of the alkoxy carbon chain increased. For example, the 6-butoxy- (**8**) and 6-(pentyloxy)- (**14**) analogs were more active than **1**, **2**, or **3** (IC_{50} = 68 and 58 μM vs >100 μM, respectively). Surprisingly, the 6-(hexyloxy)- (**17**) and 6-(heptyloxy)- (**18**) analogs were the most active nucleosides in this series (IC_{50} = 18 and 25 μM, respectively). The anti-HIV-1 activities of **17**, **18**, and ddI were identical in this assay system. The 6-(octyloxy)- (**19**), 6-(nonyloxy)- (**20**), and 6-(decyloxy)- (**21**) analogs had no anti-HIV-1 activity at concentrations below 32 μM and were cytotoxic at concentrations greater than 32 μM.

In general, several 6-iso- and 6-*sec*-alkoxy-substituted purine 2′,3′-dideoxynucleosides had IC_{50} values similar to those of the unbranched parent compounds. However, in the isomeric group of 6-(pentyloxy)- analogs, the IC_{50} increased as the substituent was varied from *n*-(pentyloxy)- (**14**, IC_{50} = 50 μM), to 2-(pentyloxy)- (**15**, IC_{50} = 80 μM), to 3-(pentyloxy)- (**16**, IC_{50} > 100 μM).

Introducing unsaturation into the side chain of two 6-alkoxypurine 2′,3′-dideoxynucleosides did not significantly increase the antiviral activity. The 6-(allyloxy)- (**7**) and 6-(2-butenyloxy)- (**13**) analogs had IC_{50} values comparable to the saturated parent analogs (**5** and **8**, respectively).

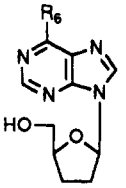
Substitution of either one (**3**) or three (**4**) fluorine atoms onto the β carbon of the ethoxy side chain of **2** did not increase the antiviral activity.

Hydrophobicity Constants. Hydrophobicity constants for the compounds in this series were estimated by HPLC and the results (log k'_w values) are presented in Table I. All of the 6-alkoxypurine 2′,3′-dideoxynucleosides were more hydrophobic than ddI (log k'_w = −0.64). However, there was no correlation between the log k'_w values and anti-HIV-1 activities for the entire set of compounds.

Anti-HIV-1 Activity in PBM Cells and Macrophages. Because **17** was as potent as ddI in MT4 cells and was not cytotoxic, **17** and ddI were evaluated for anti-HIV-1 activity in human PBM cells and macrophages. Compound **17** and ddI were equivalent in the PBM cell assay (IC_{50} values of 16 and 15 μM, respectively). However, ddI was more active than **17** in macrophages (IC_{50} values of 5 vs 20 μM, respectively).

Adenosine Deaminase Assay. 6-Alkoxypurine 2′,3′-dideoxynucleoside analogs that were dealkoxylated in the 6-position of the purine ring could serve as prodrugs for

Table II. Anti-Human Immunodeficiency Virus Activity in MT-4 Cells and in Vitro Cytotoxicity of Some 6-Alkoxypurine 2′,3′-Dideoxynucleosides



no.	substituent	anti-HIV effect in MT4 Cells[a] (IC_{50}, μM)	% of control cell growth at 100 μM nucleosides: MT4 cells[b]	D-98 cells[b]	L cells[b]
ddI	(OH)	23 ± 3[c]	100	97	100
1	OCH_3	>100	100	97	96
2	OCH_2CH_3	>100	100	82	90
3	OCH_2CH_2F	>100	100	76	100
4	OCH_2CF_3	>100	100	82	82
5	$O(CH_2)_2CH_3$	>100	100	87	87
6	$OCH(CH_3)_2$	100 ± 9	100	74	72
7	$OCH_2CH{=}CH_2$	97 ± 7	100	68	70
8	$O(CH_2)_3CH_3$	70 ± 20	100	85	68
9	(*RS*)-$OCH(CH_3)CH_2CH_3$	>100	100	77	77
10	(*R*)-$OCH(CH_3)CH_2CH_3$	>100	100	81	81
11	(*S*)-$OCH(CH_3)CH_2CH_3$	80 ± 10	100	81	85
12	$OCH_2CH(CH_3)_2$	52 ± 9	100	98	98
13	$OCH_2CH{=}CHCH_3$	59 ± 9	100	35	58
14	$O(CH_2)_4CH_3$	50 ± 10	100	60	48
15	(*RS*)-$OCH(CH_3)CH_2CH_2CH_3$	80 ± 20	100	81	67
16	$OCH(CH_2CH_3)CH_2CH_3$	>100	100	60	68
17	$O(CH_2)_5CH_3$	18 ± 5	100	54	42
18	$O(CH_2)_6CH_3$	25 ± 6	55[d]	47	34
19	$O(CH_2)_7CH_3$	>32[e]	62[d]	29	28
20	$O(CH_2)_8CH_3$	>5[e]	23[d]	27	23
21	$O(CH_2)_9CH_3$	>5[e]	20	<1	6

[a] MT4 cells were infected with 100-fold the concentration of HTLV-III_B virus stock necessary to inhibit cell growth by 50%. [b] Nucleoside was in contact with MT4 cells for 5 days and with D-98 and L cells for 4 days. Untreated cells underwent at least three doublings during the course of the assay. [c] Mean ± standard error (n = 15). [d] % of control cell growth at 32 μM nucleoside. [e] Could not be effectively evaluated due to cytotoxicity.

Table III. 6-Alkoxypurine 2′,3′-Dideoxynucleosides as Substrates of Calf Intestinal Adenosine Deaminase

no.	R_6 substituent	relative velocity[a]	K_m (μM)
adenosine		100	30
ddA	NH_2	47	45
1	OCH_3	0.08	150
2	OCH_2CH_3	0.002	650
3	OCH_2CH_2F	0.0006	ND[b]
5	$O(CH_2)_2CH_3$	0.0005	ND
10	(*R*)-$OCH(CH_3)CH_2CH_3$	<0.0004	ND
11	(*S*)-$OCH(CH_3)CH_2CH_3$	0.0004	ND
17	$O(CH_2)_5CH_3$	<0.0004	ND

[a] Measured at 100 μM nucleoside. [b] ND: These K_m values could not be determined due to their high UV absorbances.

ddI. Therefore, several analogs were tested as substrates of purified adenosine deaminase. The rates of dealkoxylation of these analogs were compared to the rate of deamination of adenosine in Table III. 2′,3′-Dideoxyadenosine (ddA), the most efficient dideoxynucleoside substrate, was deaminated at one-half the rate of adenosine. In the 6-alkoxypurine 2′,3′-dideoxynucleoside series, the 6-methoxy analog (1) was the best substrate; however, the relative rate of dealkoxylation was 0.17% the rate of deamination of ddA. Increasing the carbon chain length of the 6-alkoxypurine substituent decreased the rate of dealkoxylation. No substrate activity was detected with 17.

Reversal of Antiviral Activity. Since some of the 6-alkoxypurine 2′,3′-dideoxynucleosides were poor substrates for adenosine deaminase, small quantities of ddI could be generated by ADA during the 5-day anti-HIV-1 assay. The observed antiviral activity may have been influenced by these small quantities of ddI; therefore, several compounds were assayed for anti-HIV-1 activity in the presence of EHNA, a potent inhibitor of ADA,[8] and coformycin, a potent inhibitor of both ADA and AMP deaminase.[9] The results are presented in Table IV. The antiviral activities of 8, 13, 17 and ddI were not significantly different from control values when the assays were performed in the presence of EHNA. These data indicate that dealkoxylation of the 2′,3′-dideoxynucleosides by ADA is not mandatory for antiviral activity. In contrast, because the antiviral activities of 8, 13, and 17 were significantly decreased in the presence of coformycin, AMP deaminase might be involved in the metabolism of these compounds. Coformycin had no effect on the antiviral activity of ddI.

Table IV. Effect of *erythro*-9-(2-Hydroxy-3-nonyl)adenine (EHNA) and Coformycin on the Anti-Human Immunodeficiency Virus Activity in MT4 Cells of Some 6-Alkoxypurine 2′,3′-Dideoxynucleosides

			anti-HIV IC_{50} values (μM) measured in the presence of: EHNA at		coformycin at	
no.	R_6 substituent	PBS[a]	0.10 μM	1.0 μM	0.10 μM	1.0 μM
ddI	OH	30 ± 6	30 ± 10	31 ± 7	28 ± 4	39 ± 6
8	$O(CH_2)_3CH_3$	38 ± 4	26 ± 6	70 ± 20	>200	>200
13	$OCH_2CH{=}CHCH_3$	70 ± 10	50 ± 10	80 ± 20	>200	>200
17	$O(CH_2)_5CH_3$	24 ± 4	25 ± 3	60 ± 30	>200	>200

[a] Phosphate buffered saline.

In Vivo Metabolism Studies. As compound 17 emerged as the lead compound in this series because of potent anti-HIV-1 activity and low cytotoxicity, the pharmacokinetics of 17 were studied in the rat. The compound was administered intravenously and orally at 10 mg/kg. In each case, at least 17 metabolites, including ddI, were observed in the plasma. The plasma protein binding of 17 was 85%. After intravenous administration, areas under the plasma concentration–time curves (AUC) for 17 and ddI were 500 and 32 μM·min, respectively. The elimination of 17 followed biphasic kinetics with $t_{1/2}$ α and $t_{1/2}$ β of 4 ± 2 and 28 ± 2 min, respectively. The volume of distribution at steady state of 17 was 1.8 ± 0.2 L/kg, whereas the total body clearance rate was 60 ± 10 mL/min per kg. After oral administration, 17 and ddI derived from 17 were barely detectable (<0.5 μM) in the plasma.

Discussion

Eighteen of twenty-one 6-alkoxypurine 2′,3′-deoxynucleosides synthesized for this study had anti-HIV-1 activity in the MT4 cell assay. This indicates that a wide variety of alkoxy substituents can be tolerated in the 6-position of the purine ring. The length of the substituent had a dramatic influence on the antiviral activity. Analogs substituted with short alkyl chains (C_{1-3}) had weak anti-HIV-1 activity, whereas 17 (C_6) and 18 (C_7) were as active as ddI. Analogs substituted with long alkyl chains (C_{8-10}) could not be effectively evaluated for antiviral activity because of cytotoxicity. Neither 17 nor ddI had significant toxicity against MT4 cells at 100 μM, the highest concentration tested. However, 17 was more cytotoxic than ddI to D98 and L cells.

6-(*n*-Hexyloxy)purine 2′,3′-dideoxynucleoside (17) was not detectably dealkoxylated by purified ADA (adenosine

deaminase). As expected, the antiviral activity of 17 was not affected by EHNA, a potent ADA inhibitor.[8] In contrast, the antiviral activity of 17 was significantly decreased when the compound was assayed in the presence of coformycin, an inhibitor of ADA and AMP deaminase.[9] Thus, AMP deaminase appears to be involved in the metabolism of 17. The antiviral activity of ddI was not affected by the presence of either EHNA or coformycin.

Previously, our laboratories have shown that 6-methoxypurine arabinoside (*ara*-M), a potent antiviral agent against varicella-zoster virus (VZV), is selectively phosphorylated to *ara*-M-MP by a VZV-induced thymidine kinase.[10] We have also shown that *ara*-M-MP is dealkoxylated by AMP deaminase to *ara*-IMP and subsequently metabolized to *ara*-ATP.[11] Although *ara*-M fully retains anti-VZV activity when assayed in the presence of EHNA, the IC_{50} is increased 60-fold when assayed in the presence of coformycin.[10] The reversal of *ara*-M's anti-VZV activity by coformycin was linked to increased levels of *ara*-M-MP and decreased levels of *ara*-ATP, the anabolic end product of *ara*-M in VZV-infected cells.[11] These results are consistent with the inhibition of AMP deaminase by coformycin. The studies with *ara*-M coupled with the results from the EHNA and coformycin experiments with the purine 2',3'-dideoxynucleosides suggest that dealkoxylation of 17 is catalyzed by AMP deaminase; therefore, the anabolic pathways of 17 and ddI may converge with the formation of ddIMP. ddIMP is aminated to ddAMP via adenylosuccinate synthetase/lyase and further phosphorylated to ddATP in human lymphoid cells.[12] Thus, the active metabolite of 17 is probably ddATP.

The potency of 17 against HIV-1 and the lack of cytotoxicity warranted an investigation of its pharmacokinetics in the rat. Many metabolites were observed in the plasma after either oral or intravenous administration of 17. In addition, 17 had poor oral bioavailability, which resulted in low plasma concentrations of the drug. A prodrug of 17 might improve the oral bioavailability.

Experimental Section

Anti-HIV-1 Activity Evaluation. Antiviral activity was assessed in MT4 cells,[13] PBM cells, and macrophages.[14] Cells were infected with 100-fold $TCID_{50}$ of $HTLV\text{-}III_B$. Each compound was assayed in triplicate at least five times. Antiviral activity reversal studies were performed in the presence of either 0.1 or 1.0 μM EHNA or coformycin. Control assays in the reversal studies contained PBS instead of EHNA or coformycin.

Cytotoxicity Evaluation. Cytotoxicity was measured in cultured human sternal marrow (D-98 cells),[15] mouse connective tissue (L cells),[15] and MT4 cells.[13]

Enzymes. Thymidine phosphorylase (EC 2.4.2.4, TPase) and purine nucleoside phosphorylase (EC 2.4.2.1, PNP) were purified from *E. coli*.[7] One unit of enzyme catalyzed the formation of 1 μmol of product per minute under the defined assay conditions. The phosphorylases were immobilized at room temperature. Aliquots of preswollen Whatman DE-52 resin (10–20 g) were added to a mixture of PNP (1.1×10^6 units) and TPase (5.5×10^5 units) at a protein concentration of about 50 mg/mL in 10 mM potassium phosphate buffer, pH 7.0 until the amount of protein in the supernatant was <0.05 mg/mL. During resin addition, the pH was maintained at 7.0 with either 1 M acetic acid or 1 M potassium hydroxide. Protein concentrations were determined by the Coomassie blue method[16] with bovine γ globulin as the standard. The immobilized enzyme was stored at 4 °C.

Calf intestinal adenosine deaminase (ADA, EC 3.5.4.4) purchased from Boehringer Mannheim was dialyzed against deionized water to remove the ammonium sulfate. Activities were measured spectrophotometrically at 25 °C, pH 6.8. Kinetic constants were determined from initial velocity analysis.[17] Relative velocities were calculated by comparing velocities of 100 μM 2',3'-dideoxynucleoside versus 100 μM adenosine. Change in extinction coefficients ($mM^{-1}\cdot cm^{-1}$) and wavelengths (nm) used in the assay are as follows: for adenosine, −5.85 at 270; for 2',3'-dideoxyadenosine, −8.2 at 265; for 1, 3.6 at 269; for 2, 3.6 at 269; for 3, 2.4 at 268; for 5, 2.9 at 253; for 10, 2.3 at 252.5; for 11, 2.5 at 252.5; and for 17, 2.0 at 252. All enzymatically formed products had UV spectra identical to that of ddI.

In Vivo Metabolism Studies. Male Sprague-Dawley rats (200–350 g) were fitted with a jugular cannula and fasted overnight. Compound 17 was dissolved in saline containing 10% ethanol (2 mg/mL). Three rats were dosed orally and three rats were dosed intravenously through the cannula at 10 mg/kg. Blood samples (0.5 mL) were collected into syringes containing 30 μL of 5% disodium EDTA and 5 μL of 1 mM 2'-deoxycoformycin. After centrifugation, plasmas were ultrafiltered through a Centricon ultrafiltration apparatus and were either analyzed immediately or stored at −15 °C.

Ultrafiltrates were chromatographed on a Rainin Microsorb C_{18} reverse-phase column (4.3 × 250 mm, 5-μm particle size) at a flow rate of 1 mL/min. Eluting buffers were 50 mM ammonium acetate, pH 5.5, containing either 0.5% acetonitrile (mobile phase 1) or 60% acetonitrile (mobile phase 2). The column was eluted with a two-step gradient. Step I was a linear gradient from 5 to 10% mobile phase 2 over 1000 s and step II was a concave gradient from 10 to 100% mobile phase 2 over the next 3400 s. Column effluent was monitored at 280 and 254 nm.

Compound 17 and ddI in the plasma samples were identified by retention times and by comparisons of the ratio of peak heights at 280/254 nm to the peak height ratio of authentic standards. The plasma concentrations of 17 versus time data were fitted to a two-compartment model using a nonlinear least squares program (NONLIN). The AUC for ddI was calculated using the linear trapezoid method.

Physical Characterization of Compounds. Solvents were removed in vacuo with a rotary evaporator at temperatures not exceeding 45 °C. Elemental analyses were performed either by Atlantic Microlabs, Atlanta, GA or by Oneida Research Services, Inc., Whitesboro, NY. Results for all of the compounds in Table I were within ±0.4% of calculated values. ^{1}H-NMR spectra were recorded on either a Varian XL-200 or a Varian XL-300 in DMSO-d_6. Assignments of H_8 and H_2 were equivocal and could be reversed. Mass spectra were obtained from Oneida Research Services, Inc., Whitesboro, NY on a Finnegan 45 TFQ mass spectrometer. Melting points were obtained on a Thomas-Hoover capillary apparatus and are uncorrected. Ultraviolet spectra were recorded using either a Gilford 250 scanning spectrophotometer or a Kontron Uvikon 860. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. All rotations were measured at 20 °C in DMF at a concentration of 5 mg/mL unless otherwise specified. The hydrophobicity constants ($\log k'_w$) were estimated by a modified reverse-phase HPLC method designed to model octanol/water partitioning.[18]

Materials. Amberlite XAD-2 resin (Chemical Dynamics Corporation, South Plainfield, NJ), Sephadex G-10, 40–120 μm (Pharmacia, Piscataway, NJ), Polygosil C_{18} reverse-phase resin (Macherey-Nagel and Company, D-516, Düren, Germany), and AG1-X2 (OH^- form) and polyacrylamide gel P-2 (Bio-Rad Laboratories, Richmond, CA), were purchased. Thin-layer and preparative flash chromatography were performed on Merck silica gel 60. Centricon ultrafiltration units were purchased from Amicon.

Syntheses. Yields, melting points, chemical formulas, optical rotations, UV absorbance data, and hydrophobicity constants for the purine 2',3'-dideoxynucleosides synthesized for this study are summarized in Table I. Representative NMR data are included for 1 and 2. NMR data for compounds 3–21 are included in the supplementary material.

Commercially unavailable purine bases were prepared by one of two methods, A or B. In method A, 6-chloropurine (Aldrich Chemical Co., Milwaukee, WI) and at least 4 equiv of the appropriate alkoxy anion were refluxed in tetrahydrofuran.[19] After completion, the reaction mixture was neutralized with 6 N HCl and dried in vacuo, and the product was recrystallized. In method B, purin-6-yltrimethylammonium chloride (Aldrich Chemical Co.,

Milwaukee, WI), the appropriate alcohol, and 2 equiv of sodium hydroxide pellets were stirred at ambient temperature in tetrahydrofuran.[20] After completion, the product was isolated as described in method A.

Three general methods were used to synthesize the majority of the nucleosides. In method 1, the purine base was dissolved in 10 mL of a hot 1:1 (v/v) mixture of *N,N*-dimethylformamide and dimethyl sulfoxide. After cooling to room temperature, 3′-deoxythymidine (**25**) and 30 mL of 10 mM potassium phosphate buffer, pH 6.8, containing 0.04% potassium azide (buffer A) were added. The apparent pH was adjusted to 6.8 with either dilute acetic acid or potassium hydroxide. PNP (20 000 units) and TPase (10 000 units) immobilized on DE-52 resin were added to the mixture and stirred at 35 °C. After 7.5 h, the immobilized protein was removed by filtration and washed with methanol until the filtrate was free of UV absorbing material. The filtrate was further diluted with water until the organic solvent content was <10%. This solution was applied to two columns in series. The first column contained AG1-X2 (OH^-) and the second column contained XAD-2. The columns were flushed with at least 500 mL of water before the product was eluted with MeOH.

Method 2 is identical to method 1, except that less immobilized PNP (16 000 units) and TPase (8 000 units) were used.

In method 3, the purine base, organic solvent, 3′-deoxythymidine, and buffer were combined as in method 1. PNP (65 000 units) and TPase (33 000 units) immobilized on DE-52 resin were added to the mixture which was then stirred at 40 °C. Non-immobilized PNP (16 000 units) and TPase (13 000 units) were added to the reaction mixture at 12 h and again at 24 h after initialization. The product was harvested at 30 h after initialization and workup proceeded as in method 1.

Column dimensions for the chromatographic separations were as follows: AG1-X2, 2.5 × 10 cm; XAD-2, 2.5 × 20 cm; P-2, 5 × 90 cm; and G-10, 5 × 90 cm. The following chromatographic solvent systems (v/v) were used: A (MeOH–H_2O, 9/1); B (propanol–H_2O, 3/7); C (CH_2Cl_2–MeOH, 95/5); D (EtOAc–MeOH, 95/5); E ($CHCl_3$–MeOH, 9/1); F ($CHCl_3$–MeOH, 98/2); G (MeOH–H_2O, 8/2); H ($CHCl_3$–MeOH, 95/5); I (CH_2Cl_2–acetone, 1/1); and J (CH_2Cl_2–MeOH, 9/1).

1-[(2*R*,5*S*)-2,5-Dihydro-5-[(trityloxy)methyl]-2-furyl]-thymine (23). 1-(2-Deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[21-23] (**22**, 700 g, 1.24 mol) was suspended in a mixture of NaOH (149.1 g, 3.73 mol), deionized water (1070 mL), and ethanol (4200 mL). The reaction mixture was refluxed for 1 h, cooled to 40 °C, poured into a slurry of 8 L of ice and 16 L of water (v:v), and neutralized with 1 N HCl. Solids were collected by filtration and washed with 3 L of water. Drying under vacuum at 45 °C afforded 551.4 g of **23** (95%): mp 104–106 °C (lit.[24] mp 92–109 °C).

1-[(2*R*,5*S*)-2,5-Dihydro-5-(hydroxymethyl)-2-furyl]thymine (24). After **23** (580.0 g, 1.24 mol) was dissolved in 8 L of MeOH, a solution of concentrated HCl in MeOH (140 mL in 3.2 L) was added. The reaction mixture was stirred for 2 h at 25 °C, neutralized with 1 N NaOH, and concentrated in vacuo to 2 L. The triphenylcarbinol precipitated as a white solid and was separated by filtration. The resulting filtrate was concentrated in vacuo to dryness and recrystallization from ethanol to give 208.9 g of **24** (74.9%): mp 162.5–163.5 °C (lit.[24] mp 165–166 °C).

3′-Deoxythymidine (25). A mixture of **24** (185.5 g, 830 mmol) and 5% Pd/C (19.0 g) in 1,4-dioxane (6 L) was stirred under 1 atm of H_2. After the reduction was complete, the catalyst was removed by filtration and the solvent was removed in vacuo. Recrystallization of the residue from ethanol gave 173.7 g of **25** (92.8%): mp 148.5–150.0 °C (lit.[24] mp 149–150 °C).

6-Methoxy-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (1). 6-Methoxypurine (1.0 g, 6.7 mmol) and **25** (1.0 g, 4.4 mmol) were suspended in 50 mL of buffer A. PNP (2400 units) and TPase (2000 units) were added and the mixture was stirred at 35 °C. After 3 days, the solvent was removed and the residue was dissolved in solvent A. The solution was applied to a column of AG1-X2 and eluted with solvent A. The product was then chromatographed on two consecutive G-10 columns; the first column was developed with solvent B and the second column was developed with water. Lyophilization yielded 0.303 g of **1**: ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.59 and 8.52 (2 s, 2 H, H_2 and H_8), 6.31 (dd, 1 H, H_1', J = 6.0 Hz, J = 4.2 Hz), 4.95 (t, 1 H, OH_5', J = 5.5 Hz), 4.08–4.18 (m, 1 H, H_4'), 4.08 (s, 3 H, OCH_3), 3.43–3.67 (m, 2 H, H_5' and H_5''), 2.39–2.44 (m, 2 H, H_2' and H_2''), and 1.98–2.11 (m, 2 H, H_3' and H_3'').

6-Ethoxy-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (2). 6-Ethoxypurine (0.5 g, 3.0 mmol) and **25** (0.75 g, 3.3 mmol) were suspended in 25 mL of buffer A. PNP (800 units) and TPase (1200 units) were added and the mixture was stirred at 37 °C. After 24 h, the mixture was diluted to 110 mL with buffer A. After an additional 120 h, the reaction mixture was filtered and the filtrate was applied to a column of AG1-X2. The product was eluted with solvent A. Solvent was removed from product fractions and the residue dissolved in solvent B. This sample was applied to a P-2 column and eluted with solvent B. Lyophilization yielded 0.225 g of **2**.

^{1}H NMR (200 MHz, DMSO-d_6) δ 8.60 and 8.50 (2 s, 2 H, H_2 and H_8), 6.31 (dd, 1 H, H_1', J = 5.8 Hz, J = 4.2 Hz), 4.98 (t, 1 H, OH_5', J = 5.5 Hz), 4.58 (q, 2 H, OCH_2, J = 7.0 Hz), 4.11–4.14 (m, 1 H, H_4'), 3.50–3.62 (m, 2 H, H_5' and H_5''), 2.12–2.44 (m, 2 H, H_2' and H_2''), 1.99–2.09 (m, 2 H, H_3' and H_3''), and 1.40 (t, 3 H, CH_3, J = 7.0 Hz).

6-(2-Fluoroethoxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (3). Purin-6-yltrimethylammonium chloride (5.1 g, 23.4 mmol), NaOH (1.98 g, 49.4 mmol), and 2-fluoroethanol (25 g, 390 mmol) were reacted as described in method B. Recrystallization from acetonitrile yielded 1.07 g of 6-(2-fluoroethoxy)-9*H*-purine: mp 199 °C; UV λ_{max} at pH 7, 252 nm (10.0 $mM^{-1}\cdot cm^{-1}$); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.48 and 8.38 (2 s, 2 H, H_2 and H_8), 4.83–4.96 (m, 2 H, OCH_2), and 4.70 (m, 2 H, CH_2F). This purine (0.50 g, 2.77 mmol) and **25** (0.76 g, 3.4 mmol) were reacted as described in method 2. Solvent was removed from the product fractions and the residue was flash chromatographed on a 2.5 × 40 cm silica gel column with solvent C. Lyophilization afforded 0.266 g of **3**.

6-(2,2,2-Trifluoroethoxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (4). Purin-6-yltrimethylammonium chloride (5.0 g, 23.6 mmol), NaOH (2.02 g, 50.6 mmol), and 2,2,2-trifluoroethanol (25 g, 250 mmol) were reacted as described in method B. Recrystallization from water yielded 4.09 g of 6-(2,2,2-trifluoroethoxy)-9*H*-purine: mp 218 °C; UV λ_{max} at pH 7, 252.5 nm (9.9 $mM^{-1}\cdot cm^{-1}$); ^{1}H NMR (200 MHz, DMSO-d_6) δ 13.6 (b, 1 H, H_9), 8.54 and 8.46 (2 s, 2 H, H_2 and H_8), 5.26 (q, 2 H, J = 9.0 Hz, OCH_2). This purine (0.51 g, 2.33 mmol) and **25** (0.64 g, 2.83 mmol) were reacted as described in method 2. Solvent was removed from the product fractions and the residue was flash chromatographed on a 2.5 × 50 cm silica gel column with solvent D. Lyophilization afforded 0.266 g of **4**.

6-Propoxy-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (5). 6-Propoxypurine (0.5 g, 2.8 mmol) and **25** (0.96 g, 4.2 mmol) were reacted as described in method 1. Product containing fractions were pooled and flash chromatographed on a 2.5 × 50 cm silica gel column with solvent E. Lyophilization afforded 0.554 g of **5**.

6-Isopropoxy-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (6). 6-Isopropoxypurine (0.5 g, 2.8 mmol) and **25** (0.95 g, 4.2 mmol) were reacted as described in method 1. Solvent was removed from product fractions and the residue was dissolved in solvent B. The product was applied to a G-10 column and eluted with solvent B. Lyophilization yielded 0.313 g of **6**.

6-(Allyloxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (7). Purin-6-yltrimethylammonium chloride (5.0 g, 23.4 mmol), NaOH (1.9 g, 47.5 mmol), and allyl alcohol (100 mL, 1500 mmol) were reacted as described in method B. Recrystallization from acetonitrile yielded 3.41 g of 6-(allyloxy)-9*H*-purine: mp 182 °C; UV λ_{max} at pH 7, 252 nm (11.2 $mM^{-1}\cdot cm^{-1}$); ^{1}H NMR (200 MHz, DMSO-d_6) δ 13.4 (b, 1 H, H_9), 8.47 and 8.35 (2 s, 2 H, H_2 and H_8), 6.04–6.23 (m, 1 H), 5.24–5.48 (m, 2 H), and 5.06 (d, 2 H, J = 5.5 Hz). This purine (0.50 g, 2.85 mmol) and **25** (0.79 g, 3.51 mmol) were reacted as described in method 2. Solvent was removed from the product fractions and the residue was chromatographed on a Chromatotron on a 2-mm silica gel plate with solvent D. Lyophilization afforded 0.492 g of **7**.

6-Butoxy-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (8). 6-Butoxypurine (0.5 g, 2.6 mmol) and **25** (0.70 g, 3.1 mmol) were suspended in 100 mL of buffer A. PNP

(3,500 units) and TPase (800 units) were added and the solution was stirred at 32 °C. After 7 days, the mixture was filtered and the filtrate applied to a column containing AG1-X2. Product was eluted with solvent A. Solvent was removed and the residue was flash chromatographed on a 2.5 × 80 cm silica gel column with solvent F. The product was dissolved in water and applied to a column containing XAD-2. The column was washed with 500 mL of water and then developed with methanol. Lyophilization of the product yielded 0.276 g of **8**.

6-((*RS*)-2-Butoxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (9). Purin-6-yltrimethylammonium chloride (2.48 g, 11.6 mmol), NaOH (1.08 g, 27.1 mmol), and (*RS*)-2-butanol (80 mL, 870 mmol) were reacted as described in method B. Recrystallization from water yielded 1.79 g of 6-((*RS*)-2-butoxy)-9*H*-purine: mp 165–167 °C; $[\alpha]^{20}_D$ 1.2° (*c* = 0.50, DMF); UV λ_{max} at pH 7, 253.5 nm (11.5 $mM^{-1}\cdot cm^{-1}$); ^1H NMR (200 MHz, DMSO-d_6) δ 13.3 (b, 1 H, H_9), 8.44 and 8.32 (2 s, 2 H, H_2 and H_8), 5.42 (m, 1 H, OCH), 1.61–1.85 (m, 2 H, CH_2), 1.34 (d, 3 H, *J* = 6.2 Hz, CH_3), and 0.92 (t, 3 H, *J* = 7.4 Hz, CH_3). This purine (0.501 g, 2.61 mmol) and **25** (0.889 g, 3.92 mmol) were reacted as described in method 1. Solvent was removed from the product fractions and the residue was flash chromatographed on a 5 × 23 cm silica gel column with solvent E. Appropriate fractions were pooled, solvent was removed, and the residue was dissolved in a minimum amount of solvent G. Product was eluted from a 2.5 × 25 cm C_{18} reverse-phase column with solvent G. Lyophilization yielded 0.457 g of **9**.

6-((*R*)-2-Butoxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (10). NaH (8.4 g), (*R*)-2-butanol (9 mL, 150 mmol), and 6-chloropurine (5.0 g, 32.4 mmol) were reacted as described in method A. Recrystallization from water afforded 4.25 g of 6-((*R*)-2-butoxy)-9*H*-purine: mp 158 °C; $[\alpha]^{20}_D$ −45.4° (*c* = 0.50, DMF). This purine (0.50 g, 2.61 mmol) and **25** (0.74 g, 3.3 mmol) were reacted as described in method 2. Lyophilization yielded 0.526 g of **10**.

6-((*S*)-2-Butoxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (11). NaH (6.1 g), (*S*)-2-butanol (10 g, 135 mmol), and 6-chloropurine (5.0 g, 32.3 mmol) were reacted as described in method A. Recrystallization from water afforded 3.10 g of 6-((*S*)-2-butoxy)-9*H*-purine: mp 160 °C; $[\alpha]^{20}_D$ 46.0° (*c* = 0.50, DMF). This purine (0.50 g, 2.6 mmol) and **25** (0.72 g, 3.2 mmol) were reacted as described in method 2. Lyophilization yielded 0.515 g of **11**.

6-(2-Methyl-1-propoxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (12). NaH (8.1 g), 2-methyl-1-propanol (30 mL, 325 mmol), and 6-chloropurine (5.1 g, 33.0 mmol) were reacted as described in method A. Recrystallization from ethyl acetate afforded 4.14 g of 6-(2-methyl-1-propoxy)-9*H*-purine: mp 188–189 °C; UV λ_{max} at pH 7, 253 nm (8.9 $mM^{-1}\cdot cm^{-1}$); ^1H NMR (200 MHz, DMSO-d_6) δ 13.4 (b, 1 H, H_9), 8.45 and 8.34 (2 s, 2 H, H_2 and H_8), 4.30 (d, 2 H, *J* = 6.6 Hz, OCH_2), 2.12 (m, 1 H, CH), and 0.99 (d, 6 H, *J* = 6.8 Hz, CH_3). This purine (0.501 g, 2.61 mmol) and **25** (0.907 g, 4.00 mmol) were reacted as described in method 1. Solvent was removed from the product and the residue was flash chromatographed on a 5 × 23 cm silica gel column with solvent H. Appropriate fractions were pooled, solvent was removed, and the residue was dissolved in a minimum amount of solvent G. Product was eluted from a 2.5 × 25 cm C_{18} reverse-phase column with solvent G. Lyophilization yielded 0.453 g of **12**.

6-(2-Butenyloxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (13). Purin-6-yltrimethylammonium chloride (5.0 g, 23.4 mmol), NaOH (1.90 g, 47.5 mmol), and 2-buten-1-ol (25 g, 350 mmol) were reacted as described in method B. Recrystallization from acetonitrile yielded 2.38 g of 6-(2-butenyloxy)-9*H*-purine (70% trans isomer by NMR): mp 171 °C; UV λ_{max} at pH 7, 253.5 nm (10.4 $mM^{-1}\cdot cm^{-1}$); ^1H NMR (200 MHz, DMSO-d_6) δ 13.3 (b, 1 H, H_9), 8.45 and 8.34 (2 s, 2 H, H_2 and H_8), 5.71–5.98 (m, 2 H), 4.95–5.12 (m, 1 H), and 1.68–1.75 (m, 3 H, CH_3). This purine (0.50 g, 2.66 mmol) and **25** (0.732 g, 3.24 mmol) were reacted as described in method 2. Solvent was removed from the product and the residue was flash chromatographed on a 2.5 × 20 cm silica gel column with solvent C. Lyophilization yielded 0.441 g of **13** (75% trans isomer by NMR).

6-(Pentyloxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (14). Purin-6-yltrimethylammonium chloride (5.0 g, 23.4 mmol), NaOH (1.90 g, 47.5 mmol), and 1-pentanol (100 mL, 920 mmol) were reacted as described in method B. Recrystallization from acetonitrile yielded 3.89 g of 6-(pentyloxy)-9*H*-purine: mp 160–161 °C (lit.[25] mp 158 °C). This purine (0.50 g, 2.66 mmol) and **25** (0.83 g, 3.7 mmol) were reacted as described in method 1. Solvent was removed from product fractions and the residue was flash chromatographed on a 2.5 × 48 cm silica gel column with solvent I. Lyophilization yielded 0.425 g of **14**.

6-[(*RS*)-2-Pentyloxy]-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (15). Purin-6-yltrimethylammonium chloride (5.0 g, 23.4 mmol), NaOH (1.90 g, 47.5 mmol), and (*RS*)-2-pentanol (100 mL, 920 mmol) were reacted as described in method B. The residue was flash chromatographed on a 5 × 20 cm silica gel column with solvent J. Recrystallization of the product from acetonitrile yielded 2.17 g of 6-((*RS*)-2-pentyloxy)-9*H*-purine: mp 128 °C; UV λ_{max} at pH 7, 253.5 nm (10.6 $mM^{-1}\cdot cm^{-1}$); ^1H NMR (200 MHz, DMSO-d_6) δ 13.3 (b, 1 H, H_9), 8.43 and 8.28 (2 s, 2 H, H_2 and H_8), 5.50 (q, 1 H, *J* = 6.3 Hz, OCH), 1.60–1.78 (m, 2 H, CH_2), 1.32–1.44 (m, 5 H), and 0.88 (t, 3 H, *J* = 7.2 Hz, CH_3). This purine (0.50 g, 2.6 mmol) and **25** (0.72 g, 3.2 mmol) were reacted as described in method 1. Solvent was removed from product fractions and the residue was flash chromatographed on a 2.5 × 35 cm silica gel column with solvent C. Lyophilization yielded 0.496 g of **15**.

6-[(1-Ethylpropyl)oxy]-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (16). Purin-6-yltrimethylammonium chloride (5.0 g, 23.4 mmol), NaOH (1.90 g, 47.5 mmol), and 3-pentanol (25 g, 280 mmol) were reacted as described in method B. The residue was flash chromatographed on a 5 × 45 cm silica gel column with solvent J. Recrystallization of the product from acetonitrile yielded 1.59 g of 6-[(1-ethylpropyl)oxy]-9*H*-purine: mp 166 °C; UV λ_{max} at pH 7, 253 nm (11.2 $mM^{-1}\cdot cm^{-1}$); ^1H NMR (200 MHz, DMSO-d_6) δ 13.3 (b, 1 H, H_9), 8.44 and 8.33 (2 s, 2 H, H_2 and H_8), 5.37 (m, 1 H, OCH), 1.66–1.80 (m, 4 H, CH_2), and 0.90 (t, 6 H, *J* = 7.4 Hz, CH_3). This purine (0.50 g, 2.4 mmol) and **25** (0.82 g, 3.6 mmol) were reacted as described in method 1. Solvent was removed from the product and the residue was flash chromatographed on a 5 × 30 cm silica gel column with solvent E. Lyophilization yielded 0.461 g of **16**.

6-(Hexyloxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (17). Purin-6-yltrimethylammonium chloride (5.0 g, 23.4 mmol), NaOH (1.90 g, 47.5 mmol), and 1-hexanol (100 mL, 800 mmol) were reacted as described in method B. Recrystallization from acetonitrile yielded 3.54 g of 6-(hexyloxy)-9*H*-purine: mp 148 °C (lit.[25] mp 149–150 °C). This purine (0.50 g, 2.3 mmol) and **25** (0.63 g, 2.8 mmol) were reacted as described in method 2. Solvent was removed from the product and the residue was flash chromatographed on a 2.5 × 40 cm silica gel column with solvent C. Lyophilization yielded 0.251 g of **17**.

6-(Heptyloxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (18). NaH (4.4 g), heptanol (21 mL, 150 mmol), and 6-chloropurine (5.0 g, 32.4 mmol) were reacted as described in method A. The residue was chromatographed on a 4.8 × 20 cm silica gel column with solvent C. Recrystallization from acetonitrile afforded 6.45 g of 6-(heptyloxy)-9*H*-purine: mp 128–129 °C (lit.[25] mp 128 °C). This purine (0.50 g, 2.1 mmol) and **25** (0.71 g, 3.2 mmol) were reacted as described in method 3. Solvent was removed from the product and the residue was flash chromatographed on a 5 × 30 cm silica gel column with chloroform. Lyophilization yielded 0.257 g of **18**.

6-(Octyloxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (19). NaH (4.3 g), octanol (26 mL, 165 mmol), and 6-chloropurine (5.0 g, 32.6 mmol) were reacted as described in method A. The residue was flash chromatographed on a 5 × 20 cm silica gel column with solvent C. Recrystallization from acetonitrile afforded 6.66 g of 6-(octyloxy)-9*H*-purine: mp 119–120 °C; UV λ_{max} at pH 7, 252.5 nm (10.9 $mM^{-1}\cdot cm^{-1}$); ^1H NMR (300 MHz, DMSO-d_6) δ 13.4 (b, 1 H, H_9), 8.47 and 8.36 (2 s, 2 H, H_2 and H_8), 4.52 (t, 2 H, *J* = 6.6 Hz, OCH_2), 1.78–1.82 (m, 2 H), 1.26–1.46 (b, 10 H), and 0.86 (t, 3 H, *J* = 6.7 Hz, CH_3). This purine (0.50 g, 2.0 mmol) and **25** (0.68 g, 3.0 mmol) were reacted as described in method 3. Solvent was removed from the product

and the residue was flash chromatographed on a 5 × 30 cm silica gel column with chloroform. Lyophilization yielded 0.077 g of **19**.

6-(Nonyloxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (20). NaH (4.4 g), nonanol (30 mL, 170 mmol), and 6-chloropurine (5.0 g, 32.4 mmol) were reacted as described in method A. The residue was chromatographed on a 5 × 20 cm silica gel column with solvent J. Recrystallization from acetonitrile afforded 4.61 g of 6-(nonyloxy)-9*H*-purine: mp 123–125 °C; UV λ_{max} at pH 7, 253 nm (9.3 $mM^{-1}\cdot cm^{-1}$); ^1H NMR (300 MHz, DMSO-d_6) δ 13.4 (b, 1 H, H_9), 8.47 and 8.36 (2 s, 2 H, H_2 and H_8), 4.52 (t, 2 H, J = 6.6 Hz, OCH_2), 1.77–1.82 (m, 2 H), 1.25–1.43 (b, 12 H), and 0.85 (t, 3 H, J = 6.7 Hz, CH_3). This purine (0.50 g, 1.9 mmol) and **25** (0.64 g, 2.8 mmol) were reacted as described in method 3. Solvent was removed from the product and the residue was flash chromatographed on a 5 × 30 cm silica gel column with solvent E. Lyophilization yielded 0.100 g of **20**.

6-(Decyloxy)-9-[(2*R*,5*S*)-tetrahydro-5-(hydroxymethyl)-2-furyl]-9*H*-purine (21). NaH (6.1 g), decyl alcohol (31 mL, 162 mmol), and 6-chloropurine (5.0 g, 32.6 mmol) were reacted as described in method A. Recrystallization from acetonitrile afforded 5.25 g of 6-(decyloxy)-9*H*-purine: mp 116 °C; UV λ_{max} at pH 7, 253 nm (7.3 $mM^{-1}\cdot cm^{-1}$); ^1H NMR (200 MHz, DMSO-d_6) δ 13.4 (b, 1 H, H_9), 8.43 and 8.32 (2 s, 2 H, H_2 and H_8), 4.49 (t, 2 H, J = 6.6 Hz, OCH_2), 1.70–1.80 (m, 2 H), 1.21–1.43 (b, 14 H), and 0.82 (t, 3 H, J = 6.4 Hz, CH_3). This purine (0.50 g, 1.8 mmol) and **25** (0.53 g, 2.4 mmol) were reacted as described in method 2, except that the reaction was allowed to proceed for 20.5 h at 37 °C. Lyophilization yielded 0.129 g of **17**.

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Supplementary Material Available: A table of ^1H NMR data for compounds **3–21** (2 pages). Ordering information is given on any current masthead page.

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