Purine 2'-Deoxy-2'-fluororibosides as Antiinfluenza Virus Agents

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Received August 10, 1992

Twenty purine 2'-deoxy-2'-fluororibosides were synthesized by enzymic pentosyl transfer from 2'-deoxy-2'-fluorouridine. Each nucleoside analogue was assayed for cytotoxicity in uninfected Madin-Darby canine kidney cells and for their ability to suppress influenza A virus infections in these cells. The most potent antivirial activity was observed with analogues having an amino group in the 2-position of the purine moiety. All 2-unsubstituted analogues were less potent than their 2-amino counterparts. Furthermore, 2-methyl, 2-methoxy, or 2-fluoro substitution obliterated antivirial activity. The most cytotoxic member of the series was the 2-fluoro-6-amino analogue (IC_{50} = 120 μM). 2'-Deoxy-2'-fluoroguanosine and those congeners readily converted to it by adenosine deaminase showed the most potent antivirial activity (IC_{50} = 15–23 μM). Little cytotoxicity was observed with this subgroup of analogues which renders them worthy of further investigation as potential antiinfluenza agents.

Introduction

Substitution of fluorine for hydrogen and/or hydroxyl in the pentosyl moiety of nucleosides has resulted in analogues with a wide variety of biological activities.[1-7] This highly electronegative substituent often increases the chemical[7-9] and metabolic[9-13] stability of the glycosidic bond and changes the pentosyl conformation (ring-pucker).[14-16] This latter property is an important difference between RNA and DNA.[17]

Pyrimidine nucleoside analogues with a 2'-deoxy-2'-fluoroarabinosyl moiety (fluoro-up) have shown activity against the following viruses: herpes simplex, simian varicella, varicella-zoster, human cytomegalo,[1,18-21] Epstein-Barr,[22] and hepatitis B.[1,23,24] Some of these analogues have also shown antibacterial[25] and antileukemic[26,27] activity. In contrast to their pyrimidine counterparts, purine nucleoside analogues of this type were reported to be inactive against herpes simplex virus, although they did inhibit the growth of leukemic cell lines[2,9,10] and protozoa.[28]

Of even more direct concern to this report are studies of nucleoside analogues with the 2'-deoxy-2'-fluororibosyl moiety (fluoro-down). Pyrimidine-containing members of this class have shown activity against herpes simplex,[29-31] pseudorabies, equine abortion,[29] influenza, and varicella-zoster[32] viruses as well as antibacterial activity.[25] Some also inhibit the growth of leukemic cells.[33,34] Only three purine-containing analogues have been reported. The purine moieties of these analogues were adenine,[35] hypoxanthine,[36] and guanine.[37] They and their corresponding nucleotides were used to study nucleic acid conformation.[14,17,36,38-42] Some of these nucleotides inhibit or inactivate certain enzymes.[43,44] Some of the polynucleotides induce interferon production in vitro.[17,45] The anabolism of 2'-deoxy-2'-fluoroadenosine in mammalian cell lines has been described.[12,46] Its deamination by adenosine deaminase and the slow phosphorolysis of the resulting inosine analogue by purine nucleoside phosphorylase has also been reported.[12] We recently reported, for the first time, on the antiinfluenza activity of some purine 2'-deoxy-2'-fluoronucleosides.[47] Here we describe further the synthesis of, and influenza virus inhibition by, a series of purine 2'-deoxy-2'-fluoronucleosides.

Results and Discussion

Chemistry. Synthetic data, physical properties, and ^{1}H-NMR spectral data of the purine 2'-deoxy-2'-fluororibosides are presented in Tables I, II, and III, respectively. The enzyme-catalyzed transfer of the pentosyl moiety from 2'-deoxy-2'-fluorouridine to a variety of purines is depicted in Scheme I. This method involves two coupled reactions with a pentosyl 1-phosphate intermediate and has been used to prepare a variety of nucleoside analogues.[48-51]

In general terms, pentosyl transfer from a pyrimidine pentosyl donor to a purine pentosyl acceptor is accomplished using either uridine phosphorylase (UPase) or thymidine phosphorylase (TPase) and purine nucleoside phosphorylase (PNPase) as catalysts. The pyrimidine enzyme that is used depends on the pentosyl donor. In the synthesis of purine 2'-deoxy-2'-fluororibosides, reactions containing UPase vs TPase were compared. With UPase, almost no product was detected by TLC, in contrast to the easily detectable product in the reactions containing TPase. This result is consistent with the finding that 2'-deoxy-2'-fluorouridine (0.5 mM) is not detectably phosphorolyzed by UPase (<0.0003% of the rate with uridine), whereas the reaction catalyzed by TPase was detectable but slow (0.0038% of the rate with 2'-deoxyuridine).[52] The procedure for the synthesis of purine 2'-deoxy-2'-fluororibosides using soluble TPase and PNPase at 37 °C (method A) was similar to the procedure previously described for the synthesis of 2'-deoxyribosides.[48] Compared to the synthesis of purine 2'-deoxyribosides, the synthesis of their fluorinated counterparts by this enzymatic procedure (method A) required large amounts of enzyme and long reaction times to obtain acceptable yields. The slow rate of phosphorolysis of 2'-deoxy-2'-fluorouridine by TPase mentioned above accounts for some of the inefficiency of this transfer. Further, it appears that the synthesis of the purine nucleoside products from 2'-deoxy-2'-fluororibose 1-phosphate and purines was also slow. The rate of phosphorolysis of 2'-deoxy-2'-fluoroguanosine (11) (1.0 mM) by PNPase was only 0.0031% of the rate with 2'-deoxyguanosine.[52] The marked decrease in the rate of phosphorolysis of substrates by the *Escherichia coli*

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0022-2623/93/1836-0119$04.00/0

Table I. Synthetic Data for Purine 2'-Deoxy-2'-fluororibosides

no.	purine substituent R_2	purine substituent R_6	synthesis method[a]	reaction time (days)	purification methods[b]	yield[c] (%)
1	H	OH	C	1	C	87
2	H	OCH_3	A	45	A	54
3	H	OCH_2CH_3	A	28	A, B1	40
4	H	SCH_3	B	8	A, D1	78
5	H	NH_2	A	17	A	82
6	H	$NHCH(CH_2)_2$[d]	A	20	A	67
7	F	NH_2	A	51	B1, B2	45
8	CH_3	NH_2	A	42	A	19
9	OCH_3	NH_2	A	22	A, B1, D2	26
10	NH_2	H	A	35	A	74
			B	2	A, D3	89
11	NH_2	OH	C	4	F	92
12	NH_2	OCH_3	A	24	A	61
			B	2	A	83
13	NH_2	OCH_2CH_3	A	14	A	65
14	NH_2	$OCH_2CH_2CH_3$	A	14	A	66
15	NH_2	SCH_3	A	38	A	23
16	NH_2	Cl	A	57	B1, C, E	34
17	NH_2	I	A	40	B1, C	26
18	NH_2	CH_3	A	20	A, C, F	55
19	NH_2	NH_2	A	12	A	78
			B	2	A	94
20	NH_2	$NHCH(CH_2)_2$[d]	A	20	A, D1	73

[a] Synthesis methods are described in more detail in Materials and Methods. In the synthesis of 9, the purine used was 2-fluoroadenine. After the enzymatic pentosyl transfer, the resulting product (7) was reacted with MeOH under basic conditions to form 9. A = pentosyl transfer from 2'-deoxy-2'-fluorouridine to a purine catalyzed by soluble TPase and PNPase at 37 °C. B = pentosyl transfer as in "A" except the enzymes were adsorbed to DEAE-cellulose and the reaction temperature increased to 50 °C. C = conversion of the 6-amino substituent of a purine 2'-deoxy-2'-fluororiboside to a 6-hydroxy substituent catalyzed by adenosine deaminase. [b] Purification methods are listed in the order in which they were performed. A = anion-exchange chromatography on AG1X2 (hydroxide) in H_2O. The product was eluted with MeOH/H_2O (9/1). B = gel filtration chromatography on Bio-Gel P-2 in solvent 1 (1-propanol/H_2O 3/7) or 2 (H_2O). C = gel filtration chromatography on Sephadex G-10 in 1-propanol/H_2O 3/7. D = adsorption chromatography on silica gel 60 in solvent 1 (MeCN/H_2O 98/2), 2 (MeCN/H_2O 95/5), or 3 ($CHCl_3$/MeOH/H_2O 75/25/4). E = reversed-phase chromatography on Prep-40-ODS in MeCN/H_2O 2/98. F = recrystallization from H_2O. [c] Yields were based on the amount of 2'-deoxy-2'-fluorouridine used except the yield for 20, which was based on the amount of purine used. [d] Cyclopropylamino.

enzymes which resulted from a 2'-fluoro substituent is consistent with data published for nucleoside phosphorylases from bacterial and mammalian sources.[9-13]

The long reaction times required in method A resulted in the accumulation of some 2,2'-anhydro-1-β-D-arabinofuranosyluracil and 1-β-D-arabinofuranosyluracil in the reactions. These compounds were identified based on their TLC R_f values in several different systems. In addition, a few mg of 1-β-D-arabinofuranosyluracil was isolated from a large-scale reaction for the synthesis of 19 (which contained 50 g of 2'-deoxy-2'-fluorouridine) and was unequivocally identified by ^{1}H-NMR spectra and elemental analysis. Cyclization of 2'-halo-2'-deoxyribosides to form 2,2'-anhydro arabinosides and the subsequent hydrolysis of these 2,2'-anhydro nucleosides to form arabinosides has been reported.[8,53] The formation of small amounts of uracil arabinoside in the reaction mixtures created the possibility that purine arabinosides could also be formed. Indeed, in some reaction mixtures, trace amounts of purine arabinoside products were detected.[54] The rate of phosphorolysis of uracil arabinoside (0.5 mM) by TPase is $^1/_3$ that of 2'-deoxy-2'-fluorouridine, whereas the rate of phosphorolysis of guanine arabinoside (1.0 mM) by PNPase is 4000 times faster than that of 2'-deoxy-2'-fluoroguanosine.[52] Apparently, the small concentration and slow rate of phosphorolysis of uracil arabinoside relative to the concentration and rate of phosphorolysis of 2'-deoxy-2'-fluorouridine was an important factor in limiting the formation of purine arabinoside byproducts. Although traces of purine arabinosides were detected by TLC in some reaction mixtures, no purine arabinosides were detectable in the purified 2'-deoxy-2'-fluororibosides listed in Table II.

Yields in individual reactions were mostly dependent on the aqueous solubility of the purine and its substrate efficiency for PNPase. In general, as solubility and substrate efficiency increased, yield increased and reaction time decreased. Because of the poor aqueous solubility of guanine, this purine was not directly used as a pentosyl acceptor. 2,6-Diaminopurine, which is approximately 500 times more soluble, was used and the resulting product (19) deaminated using adenosine deaminase (ADase) (method C) to form 2'-deoxy-2'-fluoroguanosine (11).

In order to overcome the problems of poor solubilities and long reaction times, the synthesis procedure using soluble enzymes (method A) was modified (method B). The enzymes were immobilized and stabilized by adsorption onto DEAE-cellulose before addition to reaction mixtures. Enzyme immobilization allowed elevation of the reaction temperature from 37 °C to 50 °C. Since it has been shown that the enzymes are also stabilized by pentose 1-phosphates,[48] the reactions were allowed to proceed at 37 °C for 24 h to allow pentose 1-phosphate accumulation before the temperature was increased to 50 °C. These modifications reduced the reaction times and thereby reduced the formation of arabinosides. For the sake of comparison, compounds 10, 12, and 19 were synthesized using both methods A and B. In each case, the synthesis by method B resulted in a higher yield and shorter reaction time (Table I).

The nucleoside analogue with 2-methoxy and 6-amino substituents (9) was synthesized inadvertently from the 2-fluoro-6-amino analogue (7). A reaction mixture containing 7 (method A) was chromatographed on AG1X2 (hydroxide) in water. The product was eluted with methanol/water (9/1). During chromatography, reaction of 7 with methanol under basic conditions displaced the 2-fluoro substituent to give 9. After further purification, as indicated in Table I, the resulting product (9) was identified by ^{1}H NMR, mass spectrometry, and elemental analysis.

Biology. The cytotoxicity and antiinfluenza A activity of compounds 1–20 are described in Table IV. None of these analogues were potent inhibitors of the growth of the Madin-Darby canine kidney (MDCK) cells. The most cytotoxic analogue was the 2-fluoroadenine-containing analogue (7, IC_{50} = 120 μM).

The antiinfluenza activity was generally strongest with those analogues containing a 2-amino substituent on the purine ring (10–20). In every case where appreciable activity was observed, the 2-amino-substituted analogue was more potent than its 2-unsubstituted counterpart. A comparison of the effect of different 2-substituents was made with the 6-amino-substituted analogue. The antiviral activity was best with the 2-substituent as amino (19, IC_{50} = 15 μM), weaker as hydrogen (5, IC_{50} = 52 μM), and not appreciable as fluoro (7), methyl (8), or methoxy (9). The activity of 2'-deoxy-2'-fluoroadenosine (5) was enhanced when tested in the presence of the adenosine

Table II. Physical Properties of 2'-Deoxy-2'-fluororibosides

no.	formula[a]	mp (°C)	UV λ_{max} ($\epsilon \times 10^{-3}$)[b] 0.1 N HCl	pH 7	0.1 N NaOH	TLC R_f (system)[c]
1	$C_{10}H_{11}FN_4O \cdot H_2O$	175[d]	249 (11.8)	248.5 (12.0)	253 (13.0)	0.78 (C)
2	$C_{11}H_{13}FN_4O_4 \cdot 0.3H_2O$	182	250 (8.72)	248 (8.95)	250 (9.14)	0.65 (S1)
			259 (sh)	259 (sh)	259 (sh)	
3	$C_{12}H_{15}FN_4O_4 \cdot 0.5H_2O$	86	249 (11.4)	248 (11.7)	249 (12.0)	0.59 (S3)
4	$C_{11}H_{13}FN_4O_3 \cdot 0.3H_2O$	95[e]	292.5 (17.7)	287–291 (19.1)	287–291 (19.3)	0.68 (S4)
5	$C_{10}H_{12}FN_5O_3 \cdot 0.6H_2O$	226[f]	257 (14.6)	258.5 (14.5)	260 (14.9)	0.56 (C)
6	$C_{13}H_{16}FN_5O_3 \cdot 0.4H_2O$	208	264 (19.1)	268 (18.2)	268 (18.6)	0.47 (S1)
7	$C_{10}H_{11}F_2N_5O_3 \cdot 0.3H_2O$	228	261.5 (13.4)	261 (14.1)	261.5 (14.4)	0.67 (C)
8	$C_{11}H_{14}FN_5O_3 \cdot H_2O$	121	256.5 (9.99)	262 (10.6)	262.5 (10.8)	0.59 (C)
9	$C_{11}H_{14}FN_5O_4 \cdot 0.8H_2O$	163	275 (13.1)	267 (16.2)	268 (13.5)	0.53 (C)
			246 (9.43)			
10	$C_{10}H_{12}FN_5O_3 \cdot 0.6H_2O$	152	313 (4.00)	304 (7.00)	304 (7.30)	0.62 (C)
			240–245 (4.90)	243 (6.20)	243 (5.90)	
11	$C_{10}H_{12}FN_5O_4 \cdot 1.3H_2O$	>250	257 (12.2)	252.5 (13.9)	257–264 (10.9)	0.60 (S2)
			280 (sh)	271 (sh)		
12	$C_{11}H_{14}FN_5O_4 \cdot 0.5H_2O$	201	288 (7.85)	279.5 (8.09)	280 (8.40)	0.65 (S1)
			244.5 (6.43)	248 (8.85)	248.5 (8.59)	
13	$C_{12}H_{16}FN_5O_4 \cdot 0.7H_2O$	85	288 (8.78)	280 (8.96)	280 (9.25)	0.63 (C)
			244.5 (7.19)	247.5 (9.55)	249 (9.15)	
14	$C_{13}H_{16}FN_5O_4 \cdot 0.8H_2O$	93	289 (9.05)	280 (9.28)	280.5 (9.67)	0.62 (S1)
			245 (7.37)	248 (9.82)	249.5 (9.55)	
15	$C_{11}H_{14}FN_5O_3S \cdot 0.5H_2O$	86	327 (11.6)	311 (12.8)	311 (13.3)	0.62 (C)
			250 (11.2)	246 (15.1)	246 (14.8)	
			263 (sh)	257 (sh)	257 (sh)	
16	$C_{10}H_{11}ClFN_5O_3$	212[g]	309 (6.00)	307.5 (6.30)	307 (6.40)	0.60 (C)
			247 (5.60)	247 (5.80)	247 (5.30)	
17	$C_{10}H_{11}FIN_5O_3 \cdot 0.6H_2O$	135[h]	318 (7.94)	315 (8.30)	315 (8.36)	0.57 (C)
18	$C_{11}H_{14}FN_5O_3 \cdot 0.4H_2O$	215	311 (5.00)	300 (6.60)	300 (6.90)	0.70 (C)
			247 (4.80)	245.5 (6.10)	245 (5.70)	
19	$C_{10}H_{13}FN_6O_3 \cdot 1.2H_2O$	124	291 (10.2)	278.5 (10.3)	279 (10.6)	0.48 (S2)
			252 (11.8)	255 (9.69)	255 (9.69)	
20	$C_{13}H_{17}FN_6O_3 \cdot 0.6H_2O$	120	295.5 (14.3)	282 (14.8)	282 (15.2)	0.51 (S1)
			254 (12.7)	262 (sh)	262 (sh)	

[a] Satisfactory elemental analyses (±0.4%) were obtained for all compounds. [b] sh = shoulder. [c] All compounds were one spot by TLC except 17, which had one trace spot (R_f = 0.25). C = cellulose sheets developed in H_2O, S = silica gel plates developed in $CH_3CN/H_2O/NH_4OH$ 85/10/5 (1) or CH_3CN/H_2O 85/15 (2), 95/5 (3), or 98/2 (4). [d] Partial melt at 125 °C. [e] Partial melt at 75 °C. [f] Decomposes. [g] Partial melt at 205 °C. [h] Partial melt at 109 °C.

deaminase inhibitor EHNA (*erythro*-9-(2-hydroxy-3-nonyl)adenine). The weak activity shown in Table IV for this intrinsically active compound was, therefore, probably due to its incomplete deamination to the inactive inosine analogue (1).

Within the 2-amino-substituted series, the 2'-fluoro analogue of 2'-deoxyguanosine (11) and those congeners (12, 16, 17, 19) that were readily converted to it by adenosine deaminase[55] had the most potent antiviral activity. Because compounds 12 and 19 were inactive when tested in the presence of EHNA, it appears that 11 was the only intrinsically active 2-amino-substituted compound and that the other active 2-amino-substituted compounds were prodrugs of 11.

2'-Deoxy-2'-fluoroguanosine (11) was a substrate for calf thymus deoxycytidine kinase.[52] It had a K_m value of 0.89 mM, whereas this value for 2'-deoxyguanosine was 0.18 mM. The V_{max} value was 23% of that of 2'-deoxyguanosine. It, therefore, appears probable that this enzyme was involved in the anabolism of the active 2-amino-substituted compounds.

The in vitro selectivity of the more potent 2-amino-substituted analogues (11, 12, 16, 17, 19) rendered these compounds sufficiently interesting for further biological studies that will be published elsewhere. The 2-amino-6-substituted congeners that did not show appreciable antiviral activity in the in vitro assay should not necessarily be dismissed. The metabolic pathways that can convert some of these compounds to 11 or indirectly to its nucleotides might be lacking or low in cultured cells. One known example of this is the 2-amino-6-unsubstituted analogue (10), which is converted by xanthine oxidase to 11 in vivo.

Experimental Section

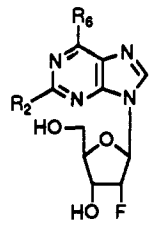
Materials. Adenine was purchased from United States Biochemical Corp., Cleveland, OH. 2-Aminopurine and 2,6-diaminopurine were purchased from Pacific Chemical Laboratories, Albany, OR. 2-Amino-6-methylpurine was purchased from Cyclo Chemical Co., Los Angeles, CA. 6-Methylthiopurine was purchased from Aldrich Chemical Co., Milwaukee, WI. 2'-Deoxyguanosine, 2'-deoxyuridine, uridine, 9-β-D-arabinofuranosylguanine, and 1-β-D-arabinofuranosyluracil were purchased from Calbiochem Corp., San Diego, CA. 6-Methoxypurine, 6-ethoxypurine, 2-methyladenine (hemisulfate), 2-amino-6-chloropurine, 2-amino-6-methylthiopurine, and 2,2'-anhydro-1-β-D-arabinofuranosyluracil were purchased from Sigma Chemical Co., St. Louis, MO. 2-Amino-6-methoxypurine,[56] 2-amino-6-ethoxypurine,[56] 2-amino-6-propoxypurine,[56] 2-amino-6-iodopurine,[57] 2-fluoroadenine,[58] 6-(cyclopropylamino)purine,[59] 2-amino-6-(cyclopropylamino)purine hydrochloride,[60] and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil[8] were synthesized at the Wellcome Research Laboratories. AG1X2-hydroxide form (200–400 mesh) anion exchange resin and Bio-Gel P-2 (200–400 mesh) gel filtration medium were purchased from Bio-Rad Laboratories, Richmond, CA. Sephadex G-10 (40–120 μm) gel filtration medium was purchased from Pharmacia LKB Biotechnology Inc., Piscataway, NJ. Silica gel 60 (230–400 mesh) for column chromatography and thin-layer chromatography plates of silica gel 60 F-254 were purchased from EM Science, Cherry Hill, NJ. Hi-chrom Prep-40-ODS, a C_{18}-derivatized silica gel (32–74 μm) for reversed-phase chromatography, was purchased from Regis Chemical Co., Morton Grove, IL. DE-52, a microgranular DEAE-cellulose, was purchased from Whatman, Clifton, NJ. Cellulose sheets with

Table III. ^{1}H-NMR Parameters for Purine 2′-Deoxy-2′-fluororibosides[a]

	chemical shifts, ∂												coupling constants, Hz					
no.	H-8[b]	H-2[b]	2-NH_2	6-NH_2	H-1′	3′-OH	H-2′	5′-OH	H-3′	H-4′	H-5′a	H-5′b	other	$J_{1',F}$	$J_{1',2'}$	$J_{2',F}$	$J_{2',3'}$	$J_{3'\text{-OH},3'}$	$J_{5'\text{-OH},5'}$
1	8.33 s	8.09 s			6.21 dd	5.75 bs	5.36 ddd	5.20 bs	4.43 m	3.97 m	3.75 m	3.59 m	12.41 (bs, 1H, H-N1)	16.8	2.4	52.7	4.4	c	c
2	8.63 s	8.58 s			6.34 dd	5.76 d	5.45 ddd	5.17 t	4.50 m	4.00 m	3.75 m	3.62 m	4.11 (s, 3H, 6-OCH_3)	17.1	2.4	52.7	4.3	6.1	4.1
3	8.59 s	8.53 s			6.31 dd	5.70 d	5.43 ddd	5.12 t	4.51 m	3.98 m	3.73 m	3.61 m	4.59 (q, 2H, 6-OCH_2, J = 7.0 Hz), 1.40 (t, 3H, CH_3, J = 7.0 Hz)	17.0	2.5	52.9	4.4	6.3	5.4
4	8.75 s	8.67 s			6.32 dd	5.72 d	5.44 ddd	5.12 t	4.50 m	3.98 m	3.77 m	3.58 m	2.66 (s, 3H, 6-SCH_3)	17.1	2.3	52.8	4.0	6.3	5.4
5	8.35 s	8.14 s		7.36 s	6.21 dd	5.71 d	5.42 ddd	5.25 t	4.47 m	3.97 m	3.74 m	3.56 m		16.8	3.0	53.0	4.5	6.0	5.6
6	8.35 s	8.24 s			6.23 dd	5.68 d	5.42 ddd	5.20 t	4.46 m	3.97 m	3.73 m	3.55 m	7.98 (bd, 1H, 6-NH, J = 4.10), 3.04 (m, 1H, NCH), 0.66 (m, 4H, CH_2CH_2)	16.8	2.9	53.0	4.6	5.9	5.6
7	8.35 s			7.92 s	6.14 dd	5.73 d	5.38 ddd	5.11 t	4.47 m	3.96 m	3.75 m	3.59 m		17.1	2.4	52.7	4.4	5.9	5.6
8	8.28 s			7.29 s	6.21 dd	5.74 d	5.43 adt	5.49 t	4.46 m	4.01 m	3.74 m	3.59 m	2.39 (s, 3H, 2-CH_3)	16.1	3.7	52.7	c	5.9	5.8
9	8.16 s			7.37 s	6.13 dd	5.71 d	5.45 ddd	5.11 t	4.53 m	3.95 m	3.73 m	3.49 m	3.82 (s, 3H, 2-OCH_3)	17.9	2.9	53.3	4.4	6.1	5.6
10	8.31 s		6.64 s		6.15 dd	5.68 d	5.37 ddd	5.14 t	4.44 m	3.96 m	3.75 m	3.59 m	8.61 (s, 1H, H-6)	16.6	2.4	52.9	4.2	6.2	5.3
11	7.95 s		6.56 s		6.01 dd	5.67 d	5.25 ddd	5.15 t	4.37 m	3.93 m	3.72 m	3.58 m	10.63 (bs, 1H, H-N1)	16.6	2.7	52.7	4.4	6.1	5.6
12	8.12 s		6.57 s		6.11 dd	5.69 d	5.31 ddd	5.17 t	4.40 m	c	3.74 m	3.58 m	3.95 (m, 4H, H-4′ and 6-OCH_3)	16.6	2.7	53.0	4.4	6.1	4.2
13	8.11 s		6.51 s		6.10 dd	5.68 d	5.31 ddd	5.17 t	4.41 m	3.94 m	3.75 m	3.59 m	4.46 (q, 2H, 6-OCH_2, J = 7.1 Hz), 1.36 (t, 3H, CH_3, J = 7.1 Hz)	16.5	2.9	52.9	4.4	6.0	5.4
14	8.11 s		6.51 s		6.10 dd	5.68 d	5.31 ddd	5.17 t	4.40 m	3.95 m	3.75 m	3.59 m	4.36 (t, 2H, 6-OCH_2, J = 6.7 Hz), 1.77 (a sex, 2H, CH_2, J = 7.1 Hz), 0.97 (t, 3H, CH_3, J = 7.5 Hz)	16.6	2.9	53.0	4.3	6.0	5.4
15	8.19 s		6.63 s		6.11 dd	5.69 d	5.33 ddd	5.16 t	4.42 m	3.95 m	3.75 m	3.59 m	2.58 (s, 3H, 6-SCH_3)	16.7	2.7	53.0	4.3	5.8	5.4
16	8.37 s		7.07 s		6.12 dd	5.72 d	5.34 ddd	5.19 t	4.42 m	3.96 m	3.76 m	3.61 m		16.6	2.0	52.9	4.2	6.4	5.3
17	8.33 s		6.96 s		6.09 dd	5.69 d	5.33 ddd	5.16 t	4.42 m	3.95 m	3.77 m	3.60 m		16.7	2.4	52.8	4.2	6.3	5.3
18	8.23 s		5.52 s		6.12 dd	5.69 d	5.35 ddd	5.15 t	4.43 m	3.95 m	3.75 m	3.59 m	2.48 (s, 3H, 6-CH_3)	16.7	2.8	53.0	4.4	6.1	5.4
19	7.93 s		5.82 s	6.76 s	6.03 dd	5.64 d	5.30 ddd	5.27 t	4.38 m	3.92 m	3.71 m	3.57 m		16.4	3.3	53.1	4.4	6.0	5.5
20	7.93 s		5.88 s		6.04 dd	5.62 d	5.30 adt	5.23 t	4.38 m	3.92 m	3.69 m	3.59 m	7.38 (bd, 1H, 6-NH, J = 4.5 Hz), 3.10 (m, 1H, NCH), 0.62 (m, 4H, CH_2CH_2)	16.4	3.3	≈53.7	c	5.9	5.4

[a] Spectra were recorded in Me_2SO-d_6. Signals are quoted as s (singlet), bs (broad singlet), d (doublet), bd (broad doublet), dd (double doublet), ddd (double double-doublet), t (triplet), a dt (apparent double triplet), q (quartet), ab q (ab quartet), a sex (apparent sextet), and m (multiplet). [b] Assignments for H-8 and H-2 are not unequivocal and may be reversed. [c] Values not established due to signal overlap.

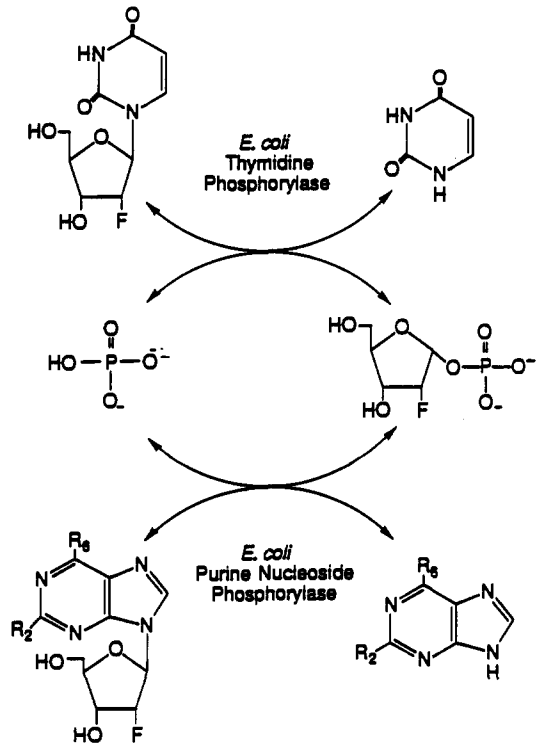
Table IV. Antiinfluenza Activity and Cytotoxicity of Purine 2'-Deoxy-2'-fluororibosides in Dog Kidney (MDCK) Cells



no.	R_2	R_6	IC_{50} (μM) antiviral	IC_{50} (μM) cytotoxicity
1	H	OH	>100	>500
2	H	OCH_3	>100	>500
3	H	OCH_2CH_3	>100	>500
4	H	SCH_3	>100	>500
5	H	NH_2	52	320
6	H	$NHCH(CH_2)_2$[a]	>100	>500
7	F	NH_2	>100	120
8	CH_3	NH_2	>100	>500
9	OCH_3	NH_2	>100	>500
10	NH_2	H	>100	>500
11	NH_2	OH	19	440
12	NH_2	OCH_3	16	>500
13	NH_2	OCH_2CH_3	29	>500
14	NH_2	$OCH_2CH_2CH_3$	36	>500
15	NH_2	SCH_3	>100	>500
16	NH_2	Cl	17	>500
17	NH_2	I	23	300
18	NH_2	CH_3	>100	>500
19	NH_2	NH_2	15	>500
20	NH_2	$NHCH(CH_2)_2$[a]	72	>500

[a] Cyclopropylamino.

Scheme I



fluorescent indicator for thin-layer chromatography were purchased from Eastman Kodak Co., Rochester, NY. Coomassie Blue G-250 protein assay reagent was purchased from Pierce Chemical Co., Rockford, IL.

Enzyme Catalysis. TPase (EC 2.4.2.4), PNPase (EC 2.4.2.1), and UPase (EC 2.4.2.3) were purified from *E. coli* as previously described.[48] Solutions of each of these enzymes in 5 mM potassium phosphate buffer pH 7.0 were stored at −70 °C. ADase (EC 3.5.4.4) from calf intestine was purchased from Boehringer Mannheim Biochemicals, Indianopolis, IN. Suspensions of this enzyme in 3.2 M $(NH_4)_2SO_4$ were stored at 4 °C. TPase,[61] UPase,[61] and ADase[62] were assayed spectrophotometrically as previously described. PNPase was assayed using the xanthine oxidase coupled spectrophotometric assay with 1.0 mM inosine as substrate. Assay conditions were as previously described[63] except the concentration of potassium phosphate was 0.1 M. Enzyme activities are expressed as international units (IU). One international unit of activity will convert 1 μmol of substrate to product in 1 min at 25 °C under the defined assay conditions. In some reactions, enzymes which had been adsorbed onto DEAE-cellulose were used. DE-52 was equilibrated with 5 mM potassium phosphate buffer which contained 0.04% (w/v) KN_3 as an antimicrobial agent. After the excess buffer was removed by filtration through a sintered glass funnel, portions of this moist DE-52 were added batchwise, with gentle stirring, to a mixture of TPase and PNPase (2 IU of PNPase/IU of TPase) in the above buffer until >99% of the protein had been adsorbed. Each milliliter of the final enzyme–DEAE-cellulose suspension contained 0.45 g of DE-52, 1660 IU of TPase, and 3330 IU of PNPase (IU of enzyme based on the IU of soluble enzyme added). This suspension was stored at 4 °C. Protein was assayed using the method of Bradford.[64]

Enzymatic Syntheses. Conversion of reactants to products was followed by thin-layer chromatography (TLC) using the systems listed in Table II. Detection of purines, pyrimidines, and nucleosides on TLC was by UV light. Solvents were evaporated under reduced pressure with a rotary evaporator at temperatures not above 50 °C. The synthesis methods, reaction times, purification methods, and yields for the purine 2'-deoxy-2'-fluororibosides are listed in Table I. The synthesis methods are described below.

Method A. These reactions were catalyzed by soluble enzymes. Reaction mixtures contained 5 mM of potassium phosphate buffer, pH 7, and 0.04% (w/v) KN_3 as an antimicrobial agent. Mixtures were stirred at 37 °C, and the typical reaction contained 1.6 mmol of pentosyl donor, 4.1 mmol of purine, 6000 IU of TPase, and 11 800 IU of PNPase. Except for the synthesis of **20**, the reactions typically contained 2.5 times as much purine as pentosyl donor. The reactions typically contained twice as much PNPase as TPase. Method A is exemplified by the synthesis of **5**.

6-Amino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9*H*-purine (5). Adenine (0.80 g, 5.9 mmol) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.40 g, 1.6 mmol) were suspended in 20 mL of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) KN_3. TPase (2400 IU) and PNPase (3900 IU) were added, and the suspension was stirred at 37 °C. On day 6, the reaction was diluted to 100 mL with the above buffer. On day 17, the suspension was filtered and the filtrate was evaporated. The residue was suspended in warm water. The suspension was filtered and the filtrate applied to a 1.5 × 12 cm column of AG1X2 (hydroxide). The product was eluted with water. Product containing fractions were combined, and the solvent was evaporated. The residue was dissolved in water and lyophilization yielded 0.363 g (82%) of **5** that analyzed as a 0.6 hydrate.

Method B. These reactions were catalyzed by enzymes adsorbed to DEAE-cellulose. Reaction mixtures contained 5 mM potassium phosphate buffer, pH 7, and 0.04% (w/v) KN_3 as an antimicrobial agent. Mixtures were shaken at 37 °C for 24 h and then the temperature increased to 50 °C. Typical reactions contained 2.7 mmol of pentosyl donor, 23 mmol of purine, 42 000 IU of TPase, and 83 000 IU of PNPase. The reactions typically contained 9.4 times as much purine as pentosyl donor. Method B is exemplified by the synthesis of **19**.

2,6-Diamino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9*H*-purine (19). 2,6-Diaminopurine (2.0 g, 12.7 mmol) and 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)uracil (0.8 g, 3.3 mmol) were suspended in 500 mL of 5 mM potassium phosphate buffer, pH 7.0, which contained 0.04% (w/v) KN_3. TPase (42 000 IU) and PNPase (84 000 IU) which had been absorbed onto 10.5 g of DEAE-cellulose (25 mL) were added, and the suspension was shaken at 37 °C. After 24 h, 2.0 g of 2,6-diaminopurine was added and the temperature increased to 50 °C. After another 24 h, the reaction was filtered. The filter cake was washed with water, the filtrates were combined, and the solvent was removed under vacuum. The residue was dissolved in hot water and the

pH adjusted to pH 9.4 with NH_4OH. The solution was applied to a 2.5 × 13 cm column of AG1X2 (hydroxide). After the column was washed with water, the product was eluted with methanol/water (9/1). Product-containing fractions were combined, and the solvent was removed under vacuum. The residue was dissolved in water and applied to another column of AG1X2, and the product was eluted as above. Product-containing fractions were combined, and the solvent was removed under vacuum. The residue was dissolved in water and lyophilization yielded 0.89 g (94%) of 19 that analyzed as a 0.5 hydrate.

Method C. In this method, ADase catalyzed the conversion of the 6-amino substituent of a purine 2'-deoxy-2'-fluororiboside to the corresponding 6-hydroxy substituent. The purine 2'-deoxy-2'-fluororibonucleoside (0.64–1.0 mmol), prepared by method A or B, was dissolved in water and 4 IU of adenosine deaminase was added. The reaction was incubated at 37 °C. Method C is exemplified by the synthesis of 11 from 19.

9-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)guanine (11). 2,6-Diamino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-9*H*-purine (19; 0.20 g, 0.64 mmol), prepared as described above, was dissolved in 15 mL of water. Adenosine deaminase (4 IU) was added and the solution incubated at 37 °C for 4 days. The solution was cooled to 4 °C. After 3 h, the suspension was filtered to remove the first batch of product crystals. The volume of the filtrate was reduced under vacuum and the suspension cooled to 4 °C. The suspension was filtered to remove the second batch of product crystals. The batches of product crystals were combined and suspended in water, and lyophilization yielded 0.182 g (92%) of 11 that analyzed as a 1.3 hydrate.

Physical Characterization of Compounds. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses for fluorine were performed by Galbraith Laboratories, Knoxville, TN. Elemental analyses for elements other than fluorine were performed by Atlantic Microlabs, Atlanta, GA. Elemental analyses were within 0.4% of the theoretical values. Proton nuclear magnetic resonance spectra (^1H NMR) were recorded at ambient temperature on a Varian XL-200, XL-250, or XL-300 spectrophotometer in Me_2SO-d_6 with Me_4Si as the internal standard. The presence of H_2O as indicated by elemental analysis was verified by ^1H NMR. Ultraviolet spectra (UV) were recorded on a Gilford 250, a Perkin-Elmer Lambda 7, or a Kontron Uvikon 870 spectrophotometer.

Antiinfluenza Activity. Compounds were evaluated for antiinfluenza [A/Sweden/3/50 (H1N1)] activity in Madin-Darby canine kidney (MDCK) cells by plaque reduction as described previously.[65] Confluent monolayers of MDCK cells in 35 mm diameter plastic petri dishes were infected, in the presence of test compound, with 50–150 plaque forming units of virus suspension. The cell monolayer was then overlaid with nutrient agarose containing 0.1 μg/mL trypsin, 100 μg/mL DEAE-dextran, and the test compound at an appropriate concentration. After incubation at 36 °C for 3–4 days, the cell monolayer was fixed with formalin and stained with methyl violet. The plaques of dead cells resulting from viral growth were counted. Plaque counts, expressed as a percentage of the infected control (no compound) value, were determined in triplicate at each compound concentration. The mean was plotted against the logarithm of the compound concentration to yield a dose–response curve from which the 50% inhibitory concentration (IC_{50}) was determined.

Cytotoxicity Evaluation. The effect of compounds on dividing MDCK cells was determined in 24-well microtitre plates seeded at 2 × 10^4 cells/well. Compound was included in the growth medium at various concentrations and cell growth compared with untreated controls over 4 days incubation (3–4 cell doublings). Cell viability was quantified spectrophotometrically after adding the tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which was metabolized by viable cells to the blue Formazan derivative, and solubilizing cells using acidified 2-propanol. Results are expressed as IC_{50} values.

Acknowledgment. We are indebted to Susan Daluge, Mike English, Bill Patterson, and Lucinda Jones of these laboratories for their contributions to this study.

References

(1) Fox, J. J.; Watanabe, K. A.; Chou, T. C.; Schinazi, R. F.; Soike, K. F.; Fourel, I.; Hantz, O.; Trepo, C. Recent studies on the antiviral activities of certain 2'-fluorinated arabinsoyl-pyrimidine nucleosides. In *Fluorinated carbohydrates: chemical and biochemical aspects*; Taylor, N. F., Ed.; ACS Symposium Series 374; American Chemical Society: Washington, D.C., 1988, pp 176–190.
(2) Chu, C. K.; Matulic-Adamic, J.; Huang, J.-T.; Chou, T.-C.; Burchenal, J. H.; Fox, J. J.; Watanabe, K. A. Nucleosides. CXXXV. Synthesis of some 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9*H*-purines and their biological activities. *Chem. Pharm. Bull.* **1989**, *37*, 336–339.
(3) Koshida, R.; Cox, S.; Harmenberg, J.; Gilljam, G.; Wahren, B. Structure activity relationships of fluorinated nucleoside analogs and their synergistic effect in combination with phosphonoformate against human immunodeficiency virus type I. *Antimicrob. Agents Chemother.* **1989**, *33*, 2083–2088.
(4) De Clercq, E.; Van Aerschot, A.; Herdewijn, P.; Baba, M.; Pauwels, R.; Balzarini, J. Anti-HIV-1 activity of 2',3'-dideoxynucleoside analogs—structure–activity relationship. *Nucleosides Nucleotides* **1989**, *8*, 659–671.
(5) Hertel, L. W.; Grossman, C. S.; Kroin, J. S.; Mineishib, S.; Chubb, S.; Nowak, B.; Plunkett, W. Synthesis, cytotoxicity and metabolism of the 2',2'-difluoro-analogs of deoxyadenosine (DFDA) and deoxyguanosine (DFDG). *Nucleosides Nucleotides* **1989**, *8*, 951–955.
(6) Borthwick, A. D.; Kirk, B. E.; Biggadike, K.; Exall, A. M.; Butt, S.; Roberts, S. M.; Knight, D. J.; Coates, J. A. V.; Ryan, D. M. *Fluorocarbocyclic* nucleosides: Synthesis and antiviral activity of 2'- and 6'-fluorocarbocyclic 2'-deoxy guanosines. *J. Med. Chem.* **1991**, *34*, 907–914.
(7) Marquez, V. E.; Tseng, C. K.-H.; Mitsuya, H.; Aoki, S.; Kelly, J. A.; Ford, H., Jr.; Roth, J. S.; Broder, S.; Johns, D. G.; Driscoll, J. S. Acid-stable 2'-fluoro purine dideoxynucleosides as active agents against HIV. *J. Med. Chem.* **1990**, *33*, 978–985.
(8) Codington, J. F.; Doerr, I. L.; Fox, J. J. Nucleosides. XVIII. Synthesis of 2'-fluorothymidine, 2'-fluorodeoxyuridine, and other 2'-halogeno-2'-deoxy nucleosides. *J. Org. Chem.* **1964**, *29*, 558–564.
(9) Carson, D. A.; Wasson, D. B.; Esparza, L. M.; Carrera, C. J.; Kipps, T. J.; Cottam, H. B. Oral antilymphocyte activity and induction of apoptosis by 2-chloro-2'-*arabino*-fluoro-2'-deoxyadenosine. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2970–2974.
(10) Montgomery, J. A.; Shortnacy, A. T.; Carson, D. A.; Secrist, J. A., III 9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)guanine: A metabolically stable cytotoxic analogue of 2'-deoxyguanosine. *J. Med. Chem.* **1986**, *29*, 2389–2392.
(11) Coderre, J. A.; Santi, D. V.; Matsuda, A.; Watanabe, K. A.; Fox, J. J. Mechanism of action of 2',5-difluoro-1-arabinosyluracil. *J. Med. Chem.* **1983**, *26*, 1149–1152.
(12) Stoeckler, J. D.; Bell, C. A.; Parks, R. E., Jr.; Chu, C. K.; Fox, J. J.; Ikehara, M. C(2')-Substituted purine nucleoside analogs: Interactions with adenosine deaminase and purine nucleoside phosphorylase and formation of analog nucleotides. *Biochem. Pharmacol.* **1982**, *31*, 1723–1728.
(13) Heidelberger, C.; Boohar, J. Fluorinated pyrimidines. XXIII. Further studies on nucleoside phosphorylase. *Biochim. Biophys. Acta* **1964**, *91*, 639–641.
(14) Ikehara, M. 2'-Substituted 2'-deoxypurine nucleotides: Their conformation and properties. *Heterocycles* **1984**, *21*, 75–90.
(15) Guschlbauer, W.; Jankowski, K. Nucleoside conformation is determined by the electronegativity of the sugar substituent. *Nucleic Acids Res.* **1980**, *8*, 1421–1433.
(16) Uesugki, S.; Miki, H.; Ikehara, M.; Iwahashi, H.; Kyogoku, Y. A linear relationship between electronegativity of 2'-substituents and conformation of adenine nucleosides. *Tetrahedron Lett.* **1979**, *42*, 4073–4076.
(17) Kakiuchi, N.; Marck, C.; Rousseau, N.; Leng, M.; De Clercq, E.; Guschlbauer, W. Polynucleotide helix geometry and stability: Spectroscopic, antigenic, and interferon-inducing properties of deoxyribose-, ribose-, or 2'-deoxy-2'-fluororibose-containing duplexes of poly(inosinic acid)·poly(cytidylic acid). *J. Biol. Chem.* **1982**, *257*, 1924–1928.
(18) Fox, J. J.; Watanabe, K. A.; Schinazi, R. F.; Lopez, C. Antiviral activities of some newer 2'-fluoro-5-substituted arabinosylpyrimidine nucleosides. In *Herpes Viruses and Virus Chemotherapy. Pharmalogical and Clinical Approaches*; Kono, R., Nakajima, A., Eds.; Excerpta Medica 667; Amsterdam, 1985, pp 53–56.
(19) Harada, K.; Matulic-Adamic, J.; Price, R. W.; Schinazi, R. F.; Watanabe, K. A.; Fox, J. J. Nucleosides. 139. Synthesis and anticytomegalovirus and antiherpes simplex virus activity of 5'-modified analogs of 2'-fluoroarabinosylpyrimidine nucleosides. *J. Med. Chem.* **1987**, *31*, 226–229.
(20) Mar, E.-C.; Patel, P. C.; Cheng, Y.-C.; Fox, J. J.; Watanabe, K. A.; Huang, E.-S. Effects of certain nucleoside analogues on human cytomegalovirus replication in vitro. *J. Gen. Virol.* **1984**, *65*, 47–53.

(21) Fox, J. J.; Lopez, C.; Watanabe, K. A. 2'-Fluoroarabinosylpyrimidine nucleosides: Chemistry, antiviral and potential antitumor activities. In *Medicinal Chemistry Advances*; de las Hevas, F. G., Vega, S., Eds.; Pergamon: Oxford, 1981; pp 27–40.
(22) Lin, J. C.; Nelson, D. J.; Lambe, C. U.; Choi, E. I.; Pagano, J. S. Effects of nucleoside analogs in inhibition of Epstein-Barr virus. In *Herpes Viruses and Virus Chemotherapy: Pharmalogical and Clinical Approaches*; Kono, R., Nakajima, A., Eds.; Exerpta Medica: Amsterdam, 1985; pp 225–227.
(23) Fourel, I.; Hantz, O.; Watanabe, K. A.; Jacquet, C.; Chomel, B.; Fox, J. J.; Trepo, C. Inhibitory effects of 2'-fluorinated arabinosylpyrimidine nucleosides on woodchuck hepatitis virus replication in chronically infected woodchucks. *Antimicrob. Agents Chemother.* **1990**, *34*, 473–475.
(24) Fourel, I.; Gripon, P.; Hantz, O.; Cova, L.; Lambert, V.; Jacquet, C.; Watanabe, K. A.; Fox, J. J.; Guillouzo, C.; Trepo, C. Prolonged duck hepatitis B virus replication in duck hepatocytes cocultivated with rat epithelial cells: A useful system for antiviral testing. *Hepatology* **1989**, *10*, 186–191.
(25) Metha, B. M.; Hutchison, D. J. Inhibition of microorganisms by pyrimidine nucleosides. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 559–563.
(26) Kong, X.-B.; Andreeff, M.; Fanucchi, M. P.; Fox, J. J.; Watanabe, K. A.; Vidal, P. M.; Chou, T.-C. Cell-differentiation effects of 2'-fluoro-1-beta-D-arabinofuranosyl pyrimidines in HL-60 cells. *Leuk. Res.* **1987**, *11*, 1031–1039.
(27) Burchenal, J. H.; Chou, T.-C.; Lokys, L.; Smith, R. S.; Watanabe, K. A.; Su, T.-L.; Fox, J. J. Activity of 2-fluoro-5-methylarabinofuranosyluracil against mouse leukemias sensitive to and resistant to 1-β-D-arabinofuranosylcytosine. *Cancer Res.* **1982**, *42*, 2598–2600.
(28) Rainey, P.; Nolan, P. A.; Townsend, L. B.; Robins, R. K.; Fox, J. J.; Secrist, J. A., III; Santi, D. V. Inosine analogs as anti-leishmanial agents. *Pharm. Res.* **1985**, *5*, 217–220.
(29) Wohlrab, F.; Jamieson, A. T.; Hay, J.; Mengel, R.; Guschlbauer, W. The effect of 2'-fluoro-2'-deoxycytidine on herpes virus growth. *Biochim. Biophys. Acta* **1985**, *824*, 233–242.
(30) Watanabe, K. A.; Su, T.-L.; Klein, R. S.; Chu, C. K.; Matsuda, A.; Chun, M. W.; Lopez, C.; Fox, J. J. Nucleosides. 123. Synthesis of antiviral nucleosides: 5-Substituted 1-(2-deoxy-2-halogeno-β-D-arabinofuranosyl)cytosines and -uracils. Some structure–activity relationships. *J. Med. Chem.* **1983**, *26*, 152–156.
(31) Kumar, R.; Xu, L.; Knaus, E. E.; Wiebe, L. I.; Tovell, D. R.; Tyrrell, D. L.; Allen, T. M. Synthesis and antiviral and cytotoxic activity of iodohydrin and iodomethoxy derivatives of 5-vinyl-2'-deoxyuridines, 2'-fluoro-2'-deoxyuridine, and uridine. *J. Med. Chem.* **1990**, *33*, 717–723.
(32) Slater, M. J.; Tisdale, S. M.; Gowrie, C. 2'-Deoxy-2'-fluoropyrimidine nucleosides with antiviral activity. *Antiviral Res.* **1991**, *15* (Suppl. 1), 52.
(33) Burchenal, J. H.; Currie, V. E.; Dowling, M. D.; Fox, J. J.; Krakoff, I. H. Experimental and clinical studies on nucleoside analogs as antitumor agents. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 202–212.
(34) Brox, L. W.; LePage, G. A.; Hendler, S. S.; Shannahoff, D. H. Studies on the growth inhibition and metabolism of 2'-deoxy-2'-fluorocytidine in cultured human lymphoblasts. *Cancer Res.* **1974**, *34*, 1838–1842.
(35) Uesugi, S.; Kaneyasu, T.; Matsugi, J.; Ikehara, M. Improved synthesis of 2'-fluoro-2'-deoxyadenosine and synthesis and carbon-13 NMR spectrum of its 3',5'-cyclic phosphate derivative. *Nucleosides Nucleotides* **1983**, *2*, 373–385.
(36) Hakoshima, T.; Omori, H.; Tomita, K.; Miki, H.; Ikehara, M. The crystal and molecular structure of 2'-deoxy-2'-fluoroinosine monohydrate. *Nucleic Acids Res.* **1981**, *9*, 711–729.
(37) Ikehara, M.; Imura, J. Studies on nucleosides and nucleotides. LXXXIX. Purine cyclonucleosides. (43). Synthesis and properties of 2'-halogeno-2'-deoxyguanosines. *Chem. Pharm. Bull.* **1981**, *29*, 3281–3285.
(38) Morishita, K.; Hakoshima, T.; Fujiwara, T.; Tomita, K.-I.; Kaneyasu, T.; Uesugi, S.; Ikehara, M. Structure of 2'-deoxy-2'-fluoroadenosine (DAFL)-C10H12N503F. *Acta Cryst.* **1984**, *C40*, 434–436.
(39) Tomita, K.-I.; Hakoshima, T.; Fukui, T.; Ikehara, M. X-ray diffraction studies of poly (I)·poly (C) analogues with substitution of the 2'-hydroxyl group by a fluorine atom. *Nucleic Acids Symp. Ser.* **1982**, *11*, 177–180.
(40) Uesugi, S.; Kaneyasu, T.; Ikehara, M. Synthesis and properties of ApU analogues containing 2'-halo-2'-deoxyadenosines: Effects of 2' substituents on oligonucleotide conformation. *Biochemistry* **1982**, *21*, 5870–5877.
(41) Uesugi, S.; Takatsuka, Y.; Ikehara, M.; Cheng, D. M.; Kan, L. S.; Ts'o, P. O. P. Synthesis and characterization of the dinucleoside monophosphates containing 2'-fluoro-2'-deoxyadenosine. *Biochemistry* **1981**, *20*, 3056–3062.
(42) Cheng, D. M.; Kan, L.-S.; Tsa'o, P. O. P.; Uesugi, S.; Takatsuka, Y.; Ikehara, M. Multinuclear magnetic resonance studies of monomers and dimers containing 2'-fluoro-2'-deoxyadenosine. *Biopolymers* **1983**, *22*, 1427–1444.
(43) Ishihama, A.; Enami, M.; Nishijima, Y.; Fukui, T.; Ohtsuka, E.; Ikehara, M. 2'-Deoxy-2'-azidoadenosine triphosphate and 2'-deoxy-2'-fluoroadenosine triphosphate as substrates and inhibitors for *Escherichia coli* DNA-dependent RNA polymerase. *J. Biochem.* **1980**, *87*, 825–830.
(44) Stubbe, J.; Kozarich, J. W. Fluoride, pyrophosphate, and base release from 2'-deoxy-2'-fluoronucleoside 5'-diphosphates by ribonucleoside-diphosphate reductase. *J. Biol. Chem.* **1980**, *255*, 5511–5513.
(45) DeClercq, E.; Stollar, B. D.; Hobbs, J.; Fukui, T.; Kakiuchi, N.; Ikehara, M. Interferon induction by two 2'-modified double-helical RNAs, poly(2'-fluoro-2'-deoxyinosinic acid)·poly (cytidylic acid) and poly(2'-chloro-2'-deoxyinosinic acid)·polycytidylic acid). *Eur. J. Biochem.* **1980**, *107*, 279–288.
(46) Burgess, F. W.; Stoeckler, J. D.; Parks, R. E., Jr. Differential incorporation of 2'-deoxyadenosine into human peripheral lymphocytes. *Biochem. Pharmacol.* **1985**, *34*, 3353–3360.
(47) Tisdale, M.; Tuttle, J.; Appleyard, G.; Ellis, M.; Daluge, S.; Nelson, D.; Miller, W.; Krenitsky, T. Inhibition of influenza A and B viruses by 2'-fluoronucleosides, mechanism of action on virus replication. *Antiviral Res.* **1992**, *17* (Suppl. 1), 90.
(48) Krenitsky, T. A.; Koszalka, G. W.; Tuttle, J. V. Purine nucleoside synthesis: An efficient method employing nucleoside phosphorylases. *Biochemistry* **1981**, *20*, 3615–3621.
(49) Krenitsky, T. A.; Koszalka, G. W.; Tuttle, J. V.; Rideout, J. L.; Elion, G. B. An enzymatic synthesis of purine D-arabinonucleosides. *Carbohydr. Res.* **1981**, *97*, 139–146.
(50) Krenitsky, T. A.; Freeman, G. A.; Shaver, S. R; Beacham, L. M. III; Hurlbert, S.; Cohn, N. K.; Elwell, L. P.; Selway, J. W. T. 3'-Amino-2',3'-dideoxyribonucleosides of some pyrimidines: Synthesis and biological activities. *J. Med. Chem.* **1983**, *26*, 891–895.
(51) Krenitsky, T. A.; Rideout, J. L.; Chao, E. Y.; Koszalka, G. W.; Gurney, F.; Crouch, R. C.; Cohn, N. K.; Wolberg, G.; Vinegar, R. Imidazo (4,5-C) pyridines (3-deazapurines) and their nucleosides as immunosuppressive and antiinflammatory agents. *J. Med. Chem.* **1986**, *29*, 138–143.
(52) Tuttle, J. V. Unpublished results.
(53) Doerr, I. L.; Fox, J. J. Nucleosides. XXXIX. 2'-Deoxy-2'-fluorocytidine, 1-β-D-arabinofuranosyl-2-amino-1,4(2H)-4-iminopyrimidine, and related derivatives. *J. Org. Chem.* **1967**, *32*, 1462–1471.
(54) In general, the R_f value of the purine arabinoside was less than that of the purine 2'-deoxy-2'-fluororiboside when the TLC was performed on silica gel plates using the solvents listed in Table II.
(55) Resetar, A. M.; Yale, J. L.; Spector, T. Unpublished results.
(56) Balsiger, R. W.; Montgomery, J. A. Synthesis of potential anticancer agents. XXV. Preparation of 6-alkoxy-2-aminopurines. *J. Org. Chem.* **1960**, *25*, 1573–1575.
(57) Koda, R. T.; Biles, J. A.; Wolf, W. Synthesis of some iodopurine derivatives. *J. Pharm. Sci.* **1968**, *57*, 2056–2061.
(58) Eaton, C. N.; Denny, G. H., Jr. Convenient synthesis of 2-fluoroadenine. *J. Org. Chem.* **1969**, *34*, 747–748.
(59) Girgis, N. S.; Pedersen, E. B. Phosphorus pentoxide in organic synthesis; II. A new, one-step conversion of hypoxanthine into N^6-substituted adenines. *Synthesis* **1982**, 480–482.
(60) Tuttle, J. V.; Krenitsky, T. A. Antiviral nucleoside derivatives and pharmaceutical compositions containing them. Eur. Patent 285,432A, 1988; *Chem. Abstr.* **1988**, *111*, 55835.
(61) Krenitsky, T. A.; Tuttle, J. V.; Koszalka, G. W.; Chen, I. S.; Beacham, L. M., III; Rideout, J. L.; Elion, G. B. Deoxycytidine kinase from calf thymus: Substrate and inhibitor specificity. *J. Biol. Chem.* **1976**, *251*, 4055–4061.
(62) Bergmeyer, H. U.; Grassl, M.; Walter, H.-E. Enzymes: Adenosine deaminase from calf intestine. In *Methods of enzymatic analysis*, 3rd ed.; Bergmeyer, H. U., Ed.; VCH: Deerfield Beach, FL, 1983; pp 135–136.
(63) Tuttle, J. V.; Krenitsky, T. A. Effects of acyclovir and its metabolites on purine nucleoside phosphorylase. *J. Biol. Chem.* **1984**, *259*, 4065–4069.
(64) Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
(65) Hayden, F. G.; Cote, K. M.; Douglas, R. G., Jr. Plaque inhibition assay for drug susceptibility testing of influenza viruses. *Antimicrob. Agents Chemother.* **1980**, *17*, 865–870.