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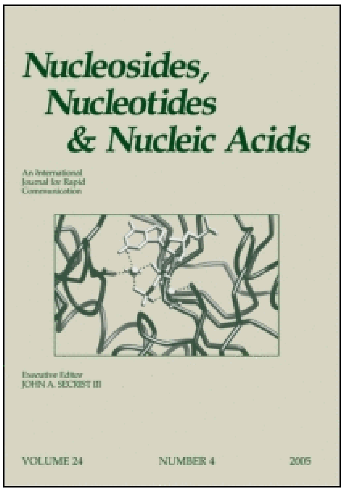

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Chemistry and Anti-HIV Activity of 2'-β-Fluoro-2',3'-dideoxyguanosine

Harry Ford Jr.[a]; John S. Driscoll[a]; Maqbool Siddiqui[a]; James A. Kelley[a]; Hioraki Mitsuya[b]; Takuma Shirasaka[b]; David G. Johns[a]; Victor E. Marquez[a]

[a] Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, Bethesda, MD [b] Experimental Retrovirology Section, Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD

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NUCLEOSIDES & NUCLEOTIDES, 13(1-3), 213-234 (1994)

CHEMISTRY AND ANTI-HIV ACTIVITY OF 2'-ß-FLUORO-2',3'-DIDEOXYGUANOSINE

Harry Ford, Jr.[†], John S. Driscoll,[†*] Maqbool Siddiqui[†], James A. Kelley[†], Hiroaki Mitsuya[‡], Takuma Shirasaka[‡], David G. Johns[†] and Victor E. Marquez[†]
[†]Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, and [‡]Experimental Retrovirology Section, Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, 9000 Rockville Pike, Building 37, Room 5C-02, Bethesda, MD 20892.

Abstract: The 2'-ß-fluoro analogue of 2',3'-dideoxyguanosine has been prepared by two synthetic routes. This compound and two analogues have anti-HIV activity in at least two of three host cell systems used (ATH8, CEM, PBL). These compounds, as well as their ddGuo parents, have been characterized with regard to their acid-stabilities, octanol-water partition coefficients, and enzyme substrate properties for adenosine deaminase and purine nucleoside phosphorylase. F-ddGuo analogues are less potent but more stable than their non-fluorinated parent compounds.

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INTRODUCTION

The 2',3'-dideoxy analogues of inosine (ddIno, didanosine, **1**) and adenosine (ddAdo, **2**) were among the earliest dideoxynucleoside analogues shown to have anti-HIV activity[1]. Clinical evaluation of these agents[2] quickly established ddIno as an effective anti-HIV drug[3,4]. The corresponding guanosine compound (ddGuo, **3a**), while showing an activity and a potency profile similar to ddAdo and ddIno[1], has not yet been evaluated clinically, although significant research has been conducted on the anti-viral activity[5], chemistry[6,7] and biochemistry[8-10] of **3a** and its analogues[11-13].

The simple purine 2',3'-dideoxyribofuranosides are unstable under acidic conditions[14,15] and this complicates the production of a convenient

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This article is dedicated to the memory of Dr. Roland K. Robins whose pioneering nucleoside research has been an inspiration for all workers in the field.

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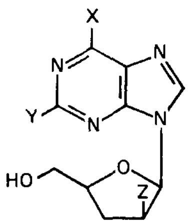
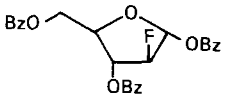


Compound	X	Y	Z	Name
1	OH	H	H	ddIno
2	NH_2	H	H	ddAdo
3a	OH	NH_2	H	ddGuo
3b	Cl	NH_2	H	2-NH_2-6-Cl-ddP
3c	NH_2	NH_2	H	2,6-diNH_2-ddP
4	OH	H	F	F-ddIno
5	NH_2	H	F	F-ddAdo
6	OH	NH_2	F	F-ddGuo
7	Cl	NH_2	F	2-NH_2-6-Cl-2'-F-ddP
8	NH_2	NH_2	F	2,6-diNH_2-2'-F-ddP



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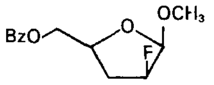

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oral dosage form[16]. The addition of a fluorine atom "up" in the 2'-position (F-ddI, **4** and F-ddA, **5**), however, makes these compounds acid-stable with retention of the activity and potency of the parent compounds[17,18]. In addition, fluorine-substitution increases the metabolic stability of the adenosine analogue, **5**, by making it more resistant to hydrolysis by adenosine deaminase (ADA), and of the inosine analogue, **4**, by making it resistant to degradation by purine nucleoside phosphorylase (PNP)[19,20]. Because of the improved properties observed with **4** and **5**, we undertook a study of the effects of 2'-β-F-substitution on ddGuo and two related compounds.

CHEMISTRY

Synthesis

The target compound, F-ddGuo (**6**), was prepared by two routes. The first method, shown in **Scheme** 1, utilizes the more traditional reduction of the 3'-hydroxyl group following the sugar-base condensation step. The starting, protected fluoro-sugar, **9**, was prepared by the method developed by the Bristol group[21]. Compound **9** was converted to the bromo-sugar, **11**,

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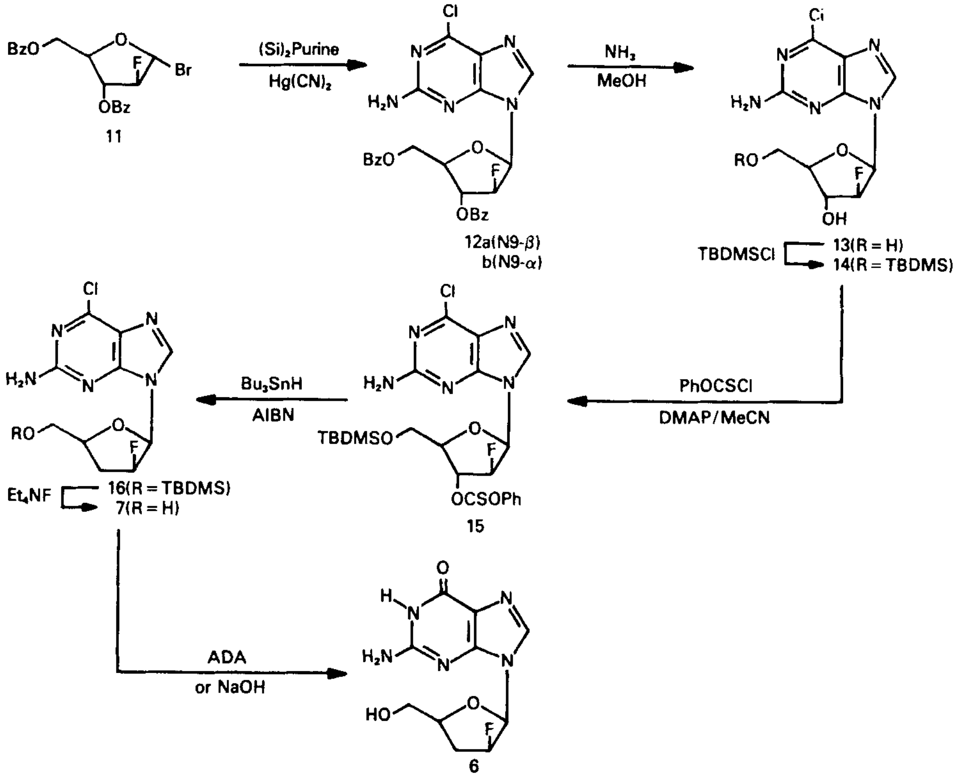

Scheme 1

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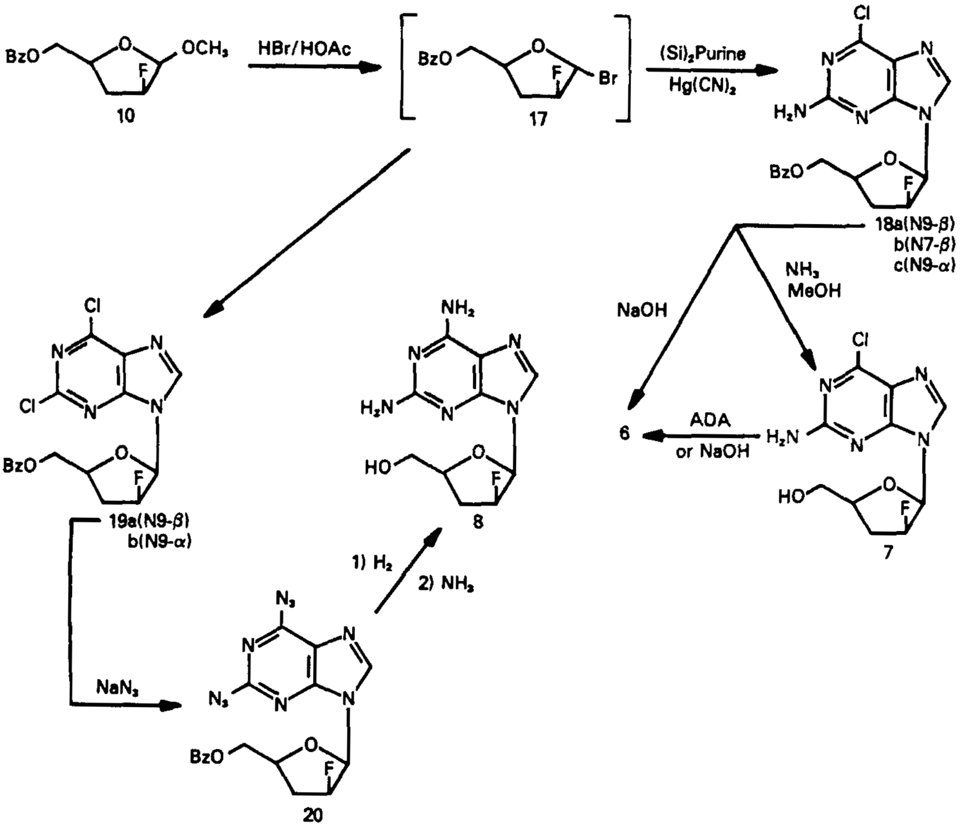
with HBr/HOAc and condensed with silylated 2-amino-6-chloropurine in the presence of $Hg(CN)_2$ to give the desired **12a** (28%), along with 6% of the N9-α anomer, **12b**. Deprotection of **12a** with ammonia, and 5'-reprotection with the TBDMS group set the stage for the preparation of the conventional 3'-phenoxythiocarbonyl derivative, **15**, which was reduced under Barton conditions to produce the 6-chloro analogue, **7**, after deprotection. The hydrolysis of **7** by either chemical (NaOH) or enzymatic (adenosine deaminase, ADA) means gave the target compound, 2'-fluoro-2',3'-dideoxy-ara-G (**6**, F-ddGuo) in roughly equivalent yields. ADA hydrolysis is the preferred method since it proved difficult to remove traces of inorganic materials after NaOH treatment of **7**.

The second synthetic method utilizes the fluoro-sugar, **10**, which allows the purine coupling to be performed after, rather than before the Barton reduction. This method[22] offers certain advantages for the



Scheme 2

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synthesis of 2'-fluoro analogues (**Scheme 2**). After formation of the bromo-sugar, **17**, and condensation with protected 2-amino-6-chloropurine, the N9-β isomer, **18a**, was obtained in 31% yield along with small amounts of the N7-β (**18b**, 7%) and N9-α (**18c**, 7%) isomers. Removal of the benzoyl protecting group from **18a** with methanolic ammonia gave **7**, which was identical with the material generated by **Scheme 1**. F-ddGuo (**6**) was produced from **7** either enzymatically or chemically as described above in **Scheme 1**, or by basic hydrolysis of **18a**. The 2,6-diamino analogue, **8**, was prepared from **10** in a similar manner. 2,6-Dichloropurine was condensed with **17** to give the desired N9-β anomer, **19a** (32%) along with some N9-α isomer (23%). Chlorine displacement with azide ion, reduction, and deprotection gave the desired 2,6-diamino analogue (**8**).

Table 1. Acid Stability[a]

Compound	$t_{1/2}$ (hr)	% Decomposition[b]		
		1 hr	3 hr	24 hr
3a (ddGuo)	0.014	100	100	100
3b (2-NH_2-6-Cl-ddP)[c]	0.016	100	100	100
8 (2,6-diNH_2-2'-F-ddP)	384	0	0	9
6 (F-ddGuo)	345	0	0.4	4
7 (2-NH_2-6-Cl-2'-F-ddP)	158	0	0.5	5

[a] Standard conditions: 50 μM substrate, pH 1.0 HCl/KCl, 37°C. [b] Actual measurement of product. [c] ddP = dideoxypurine

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Acid Stability

As mentioned above, the ability of an orally administered drug to survive acidic stomach conditions is an important practical consideration. A major advantage of 2'-fluoro-dideoxynucleosides[17,18,22] relative to their parent compounds[14,15] is their ability to survive this environment. For this reason, we extended this evaluation to the F-ddGuo analogues described here (**Table 1**). As expected, F-ddGuo is much more stable (>23,000 times) at pH 1, 37°C than ddGuo .

Partition Coefficients

Generally the lipophilicity of a drug plays an important role in its *in vivo* transport properties[23], especially as related to central nervous system (CNS) penetration.[24,25] This also appears to be the case with anti-HIV drugs since AZT, the most lipophilic of the drugs currently available in the U.S., produces a higher CSF to plasma ratio than either ddI or ddC[26-28]. Using a microscale partition coefficient method devised earlier in this laboratory,[29] octanol-water log P values were determined for ddGuo and its analogues (**Table 2**). While the addition of fluorine does not appreciably alter the partition coefficient, a 6-chloro substituent (**7** and **3b**) increases the lipophilicity over 20 times relative to their parent compounds, **6** and **3a**.

Table 2. Octanol-Water Partition Coefficients and Chromatographic Properties of ddGuo Analogues

Compound	Log P[a]	HPLC[b]	k'[c]	UV[d] (nm)
6 (F-ddGuo)	-1.178 ± 0.012	A	1.16	252
3a (ddGuo)	-1.091 ± 0.006	A	1.17	253
8 (2,6-diNH_2-2'-F-ddP)	-0.495 ± 0.008	B	1.37	255, 279
3c (2,6-diNH_2-ddP)	-0.493 ± 0.004	B	1.38	215, 255, 280
3b (2-NH_2-6-Cl-ddP)	0.211 ± 0.011	B	4.12	220, 245, 309
7 (2-NH_2-6-Cl-2'-F-ddP)	0.276 ± 0.010	B	5.40	220, 246, 306

[a] Mean ± standard deviation of three independent partition coefficient (P) determinations.

[b] The following mobile phases were used at 1.0 ml/min with a 4.6 x 250 mm 5-μm Ultrasphere ODS column which was protected with a 4.6 x 30 mm Brownlee C_{18} precolumn: A) 8.5% or B) 10% CH_3CN in pH 7.0, 0.01M phosphate buffer.

[c] Mean capacity factor ($k' = (t_r - t_o)/t_o$) for the indicated mobile phase. The standard deviation of three determinations was less than 0.02 in all cases.

[d] Wavelengths determined on-the-fly with a diode-array detector in HPLC mobile phases.

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BIOLOGY

Anti-HIV Activity

Because nucleoside antiviral activity is critically dependent upon specific cellular activating enzymes, the host cell line is a crucial variable in determining whether a compound has activity[30,31]. For this reason, anti-HIV testing was carried out using the ATH8[1], CEM[32] and PHA/PBL[33] cell lines infected with HIV/LAI.

In the ATH8 cell line (**Table 3**), ddGuo (**3a**) proved to be active, potent and non-cytotoxic over a large range of concentrations (2-100 μM). These results are similar to those shown earlier[1]. While active, F-ddGuo (**6**) was neither as active nor as potent as **3a** and was more toxic at 100 μM concentration. The 6-chloro (**7**) and 6-amino (**8**) analogues of 2-NH_2-2'-F-

Table 3. Anti-HIV Activity in ATH-8 Cells

Compound	Concentration (μM)	% Protection	% Cytotoxicity
3a	2[a],10,20,50,100	75,88,100,91,91	0,0,3,6,0
6	2,10,20,50,100	25,44,47,50,66	3,6,0,0,26
7	10,20,50,100,200	33,38,61,51,30	0,0,0,43,56
8	1,10,100	23,36,40	9,0,24

[a] Concentration of **3a** giving 75% protection with 0% cytotoxicity

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ddP are both active but not as potent or active as ddGuo. It should be noted that **8** had been reported to be inactive against HIV in MT-4 cells[34], and that the non-fluorinated analogues, 2-NH_2-6-Cl-ddP (**3b**) and 2,6-diamino-ddP (**3c**), earlier had shown impressive anti-HIV activity (ATH8 and MT-2 cells)[35].

CEM cellular experiments (**Table 4**) produced an activity profile somewhat similar to that observed in ATH8 cells. In this cell line, **7** was the only compound completely devoid of activity. ddGuo (**3a**) was again the most potent compound among those tested.

All compounds tested using phytohemagglutinin-stimulated peripheral blood lymphocytes (PHA/PBL) as the host cell line showed anti-HIV activity (**Table 5**). Again, ddGuo (**3a**) was the most potent compound in this group.

Enzyme-Substrate Activity

Adenosine Deaminase (ADA)

ADA is a ubiquitous catabolic enzyme present in many human tissues.[36] There is a large amount of experimental work which shows that many 6-

Table 4. Anti-HIV Activity in CEM Cells[a]

Compound	EC_{50} (μM)	IC_{50} (μM)	TI_{50}
3a	2	> 10	> 5
6	20	> 53	> 2.5
7	> 50	> 50	--
8	37	> 93	> 2.5

[a] EC_{50} = concentrations effective in protecting 50% of the CEM cells from the cytopathogenic effects of HIV. IC_{50} = concentration which inhibits CEM cell proliferation by 50%. TI_{50} = IC_{50} divided by EC_{50}.

Table 5. Anti-HIV Activity in PHA-Stimulated PBL Cells

Compound	IC_{90}[a] (μM)	CN_{50}[b] (μM)
3a	<2.5	<2.5
6	7.1	10
7	3.8	10
8	6.4	50

[a] Compound concentration which inhibits HIV p24 Gag protein expression by 90% relative to controls. [b] Compound concentration which yielded 50% p24 Gag protein negative wells.

substituted purine ribonucleosides are substrates for ADA hydrolysis, producing inosine and guanosine.[37] Since these 6-substituted compounds are normally more lipophilic than their parents, it is possible that they might function as prodrugs with favorably altered biotransport properties which could be converted to active ddGuo analogues in a target tissue.[38] For this reason, we evaluated the rates of the ADA-catalyzed hydrolysis of **7** and **8** to F-ddGuo (**6**). As shown in **Table 6**, the addition of a 2'-fluoro group produces a reduction in the ADA-catalyzed hydrolysis rate of ddAdo

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Table 6. Relative Rates of Enzyme Catalysis

Compound	ADA[a]	PNP[b]
2 (ddAdo)	2080	ND[c]
3a (ddGuo)	ND	100
3b ($2-NH_2-6-Cl-ddP$)	90	< 0.14
3c ($2,6-diNH_2-ddP$)	156	ND
5 (F-ddAdo)	100	ND
6 (F-ddGuo)	ND	NR[d]
7 ($2-NH_2-6-Cl-2'-F-ddP$)	6	< 0.03
8 ($2,6-diNH_2-2'-F-ddP$)	11	< 0.03

[a] Standard adenosine deaminase (ADA) conditions: 50 μM substrate; 0.01 M phosphate buffer, pH 7.4; 37°C. Disappearance rate constant per unit ADA normalized to that of ß-F-ddA.

[b] Standard purine nucleoside phosphorylase (PNP) conditions: 50 μM substrate; 0.1M phosphate buffer, pH 7.4; 37°C. Relative rates calculated on the basis of t_{90} (the time required for 10% conversion).

[c] Not determined.

[d] No conversion observed in 24 hr.

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by about 20 times, and causes a ca. 15-fold decrease in the $2-NH_2$-6-chloro conversion.

Purine Nucleoside Phosphorylase

Purine nucleoside phosphorylase (PNP) is an enzyme which catalyzes the reversible phosphorolysis of ribonucleoside analogues of guanine and hypoxanthine. In its catabolic mode, PNP cleaves the glycosidic bond to produce a sugar and an aglycon[39]. This reaction could reduce the anti-HIV potency of an active guanosine analogue *in vivo* by converting the drug to inactive products. A comparison of the decomposition rates of ddGuo (**3a**) and F-ddGuo (**6**) in the presence of PNP (**Table 6**) shows that the addition of fluorine substitution completely abolishes this glycosidic cleavage reaction. Also seen, in contrast to the situation described above with ADA-catalyzed hydrolysis, a 6-chloro substituent in the 2-aminopurine ring (**3b**) virtually abolishes PNP substrate activity.

EXPERIMENTAL

HPLC Analysis of Guanine Dideoxynucleosides Solutions and diluted enzymatic reaction mixtures of dideoxynucleosides were analyzed on an liquid chromatography system consisting of a Rheodyne 7125 syringe-loading sample injection valve, a Waters Model 6000A pump and a Gilson Model 116 variable wavelength UV detector. On occasion, a Spectra-Physics AS3000 automatic sample injector or a Perkin-Elmer LC-235 UV diode array detector were substituted for the corresponding system components, depending on the number of samples and the analytical requirements of the experiment. Dideoxynucleosides were separated on a 4.6 X 250 mm 5-μm Altex/Beckman Ultrasphere C18 column that was protected by a 4.6 X 30 mm Brownlee Spheri-5 RP-18 precolumn. This column system was eluted isocratically at 1.0 mL/min with a mobile phase of either 8.5 or 10% CH_3CN in pH 7.0, 0.01M phosphate buffer so that all dideoxynucleosides had retention times in the range of 6-18 min. Enzymatic reaction products were identified by coincidence of retention time with standards and by comparison of full-scan (195-360 nm) on-the-fly UV spectra obtained with the diode array detector. Peak areas were measured on a Spectra-Physics SP4400 Chromjet computing integrator. For kinetic studies, this data was plotted as a function of time and fitted to a first order decomposition ($y = Ae^{-kt}$) using GraphPad (ISI Software, Philadelphia, PA), a PC-based curve-fitting program.

Mass Spectrometry Positive-ion fast atom bombardment mass spectra were obtained on a VG 7070E mass spectrometer operated at an accelerating voltage of 6 kV and a resolution of 1500. Glycerol and 3-nitrobenzyl alcohol were used as sample matrices, and ionization was effected by a beam of xenon atoms derived by charge-exchange neutralization of a 1.0 - 1.2 mA beam of xenon ions accelerated through 8.4-8.9 kV. Spectra were acquired under the control of a VG 11/250 J^+ data system at a scan speed of 10 s/decade, and the matrix background was automatically subtracted. Accurate mass measurement of the protonated molecular ion (MH^+) peak was carried out in a separate experiment at a dynamic resolution of 2500 using a voltage scan over a limited mass range and signal acquisition in a multi-channel analysis mode. Software-based peak matching was then employed using selected glycerol peaks within the mass range as internal references.

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Measurement of Octanol-Water Partition Coefficients *n*-Octanol-water partition coefficients (P) were determined for individual compounds using a microscale shake-flask method employing HPLC analysis of both the buffer and *n*-octanol phases[29]. This determination was conducted in triplicate.

Acid Stability of Guanine Dideoxynucleosides Fifty microliters of a 1.0 mM aqueous solution of dideoxynucleoside was added to 0.95 mL pH 1.0 HCl/KCl in a 1.5-mL polypropylene microcentrifuge tube (Eppendorf) that had been prewarmed to 37°C. A 50-μL aliquot was immediately removed for analysis and the tube incubated at 37°C in an Eppendorf Model 5320 thermostated heater. This initial sample aliquot was diluted with 0.45 mL pH 7.4, 0.1M phosphate buffer. Subsequent 50-μL samples were taken at predetermined times and treated in the same manner as the first. The concentration of dideoxynucleoside in each sample was determined by HPLC analysis of a 50-μL aliquot as described above.

Enzymatic Hydrolysis by Adenosine Deaminase Adenosine deaminase (ADA, adenosine aminohydrolase, EC 3.5.4.4, Boehringer Mannheim) from calf intestinal mucosa was prepared as previously described[38] to give a stock solution of 1 mg/mL (250-280 units/mL) in pH 7.4, 0.01 M phosphate buffer. The adenosine deaminase inhibitor 2'-deoxycoformycin (dCF) was obtained from the Pharmaceutical Resources Branch, NCI. Relative rates of hydrolysis of selected nucleosides by ADA and characterization of products were carried out as previously described.[38] Briefly, 0.02-1.0 units of ADA were added to 1.0 mL of a 50 μM solution of a given compound in pH 7.4, 0.01 M phosphate buffer and the solution incubated at 37°C. Fifty-microliter aliquots were withdrawn at timed intervals and hydrolysis was terminated by mixing with 0.450 mL 4 μM dCF. The diluted sample was heated at 95°C for 1 min to insure enzyme deactivation, and the decrease in substrate concentration and the formation of product was followed as a function of time by HPLC analysis.

Depurination by Purine Nucleoside Phosphorylase A 0.50-mL aliquot of a 10 mg/mL suspension of purine nucleoside phosphorylase (PNP, EC 2.4.2.1, Boehringer Mannheim) from calf spleen was centrifuged at 12,000 X g for 3 min. The pellet remaining after removal of the $(NH_4)_2SO_4$ supernatant was dissolved in sufficient pH 7.4, 0.01M phosphate buffer to make a final volume of 0.100 mL and a PNP concentration of 100 units/mL. Serial dilution was employed to make enzyme solutions of 4.0 and 0.08 units/mL, respectively. Depurination of selected substrates was assessed by mixing 2.5-10 μL of the appropriate enzyme solution with 50 μL of 1 mM nucleoside solution, 500 μL pH 7.4, 0.2 M phosphate buffer, and sufficient distilled H_2O to give a final volume of 1.0 mL. This reaction mixture containing 50 μM substrate and varying PNP concentration (0.0002-1.0 units/mL) was incubated at 37°C in a 1.5-ml polypropylene microcentrifuge tube. At predetermined times, 50-μL aliquots were withdrawn, diluted with 450 μL distilled water, and heated at 95°C for 1.5 min to inactivate the enzyme. The decrease, if any, in substrate concentration over time was determined by HPLC analysis (*vide supra*).

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HIV Cytopathic Effect Inhibition Assays The anti-HIV assays in ATH8[1,22b], CEM[22b,32] and PHA-stimulated PBL[33] cells were performed as previously described. CEM assays were carried out by PRI Inc., FCRDC, Frederick, MD, under contract to the Developmental Therapeutics Program, NCI.

Reagents Compounds **1**, **2**, and **3a** were provided by the Drug Synthesis and Chemistry Branch (Dr. Narayanan) and the Pharmaceutical Resources Branch (Dr. Vishnuvajjala), Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. Compounds **3b** and **3c** were synthesized by the Sanyo-Kokusaku Pulp Co., Iwakuni, Japan.[35a] All other reagents were commercially available and used without further purification.

3,5-Di-O-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**11**)
A solution of 1,3,5-tri-O-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose (**9**, 3.0 g, 6.45 mmol) in dry CH_2Cl_2 (10 mL) was treated with a solution of 33% HBr in AcOH (3.2 mL, 13.0 mmol) and stirred at rt under nitrogen for 20 hr. The reaction mixture was evaporated and then coevaporated twice with toluene (2 x 25 mL). The residue was dissolved in CH_2Cl_2 (100 mL) and washed successively with water (2 x 50 mL) and saturated $NaHCO_3$ (2 x 50 mL). The organic layer was dried (Na_2SO_4) and concentrated to a viscous syrup which was further dried under vacuum for 16 hr to give 2.70 g (99%) of **11**. This material was used in the following step without further purification.

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2-Amino-6-chloro-9-(3,5-di-O-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9*H*-purine (12a) A suspension of 2-amino-6-chloropurine (1.07 g, 6.30 mmol) in dry CH_3CN (20 mL) was treated with bis(trimethylsilyltrifluoro)acetamide (8 mL, 29.8 mmol) and the resulting mixture was stirred at rt until it became homogeneous (30 min). The solvent was removed and the residue was protected from moisture. Immediately after, the residue was dissolved in dry benzene (30 mL) and after the addition of $Hg(CN)_2$ (1.62 g, 6.41 mmol) the solution was refluxed for 30 min under nitrogen with the aid of a Dean-Stark trap to insure anhydrous conditions. A solution of bromosugar **11** (2.70 g, 6.30 mmol) in dry benzene (25 mL) was gradually added and refluxing continued for 3 hr. The mixture was allowed to cool to rt and after the addition of 200 mL of $CHCl_3$ the organic solution was washed successively with aqueous $NaHCO_3$ (2 x 80 mL) and 1M KI solution (2 x 80 mL). The organic layer was dried ($NaSO_4$) and reduced to dryness. The residue was purified by flash column chromatography (silica gel, hexane:EtOAc, 2:1) and two isomeric compounds were isolated. The desired N9-β-isomer **12a** (0.894 g, 27.7%) was isolated as the major component (R_f = 0.22, hexane:EtOAc, 3:2). This compound was recrystallized to give a solid, mp 98-103 °C (EtOH); ^1H NMR ($CDCl_3$) δ 4.55 (m, 1 H, H-4'), 4.70 (d, J = 4 Hz, 2 H, H-5'$_{a,b}$), 5.20 (brs, 2 H, NH_2), 5.30 (dd, J = 50, 2.7 Hz, 1 H, H-2'), 5.75 (dd, J = 16, 3 Hz, 1 H, H-3'), 6.44 (dd, J = 22, 2.7 Hz, 1 H, H-1'), 7.40-7.70 (m, 6 H, Ph), 8.20 (m, 5 H, H-8, Ph). Anal. Calcd for $C_{24}H_{19}ClFN_5O_5 \cdot 0.75$ EtOH: C, 56.04; H, 4.33; N, 12.82. Found: C, 56.00; H, 4.32; N, 12.98. The minor N9-α isomer **12b** (0.21 g, 6.3%, R_f = 0.34) was obtained as an oil; ^1H NMR ($CDCl_3$) δ 4.70 (d, J = 5 Hz, 2 H, H-5'$_{a,b}$), 4.92 (m, 1 H, H-4'), 5.30 (br s, 1 H, NH_2), 5.75 (dm, J = 17 Hz, 1 H, H-3'), 6.09 (d, J = 49 Hz, 1 H, H-2'), 6.40 (d, J = 14.0 Hz, 1 H, H-1'), 7.30-7.75 (m, 6 H, Ph), 8.10 (m, 5 H, H-8, Ph).

2-Amino-6-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9*H*-purine(13)

In a sealed pressure bottle, protected compound **12a** (0.30 g, 0.58 mmol) was stirred at rt with saturated methanolic ammonia (15 mL) for 4 hr. The solvent was evaporated and the residue was purified by preparative tlc (silica gel, Analtech 2000 μ) developed with CH_2Cl_2/MeOH (9:1). Compound **13** (0.13 g, 75%) was isolated as a solid, mp 173-74 °C dec; ^1H NMR (Me_2SO-d_6) δ 3.60 (m, 2 H, H-5'$_{a,b}$), 3.83 (br q, 1 H, H-4'), 4.40 (dm, J = 14.5

Hz, 1 H, H-3'), 5.05 (m, 1 H, 5'-OH), 5.20 (dm, *J* = 52 Hz, 1 H, H-2'), 5.97 (d, *J* = 4.5 Hz, 1 H, 3'-OH), 6.25 (dd, *J* = 14, 4.4 Hz, 1 H, H-1'), 7.01 (br s, 2 H, NH_2), 8.23 (d, *J* = 1.9 Hz, 1 H, H-8). Anal. Calcd for $C_{10}H_{11}ClFN_5O_3$: C, 39.55; H, 3.65; N, 23.06. Found: C, 39.48; H, 3.69; N, 22.97.

2-Amino-6-chloro-9-[5-O-(*tert*-butyldimethylsilyl)-2-deoxy-2-fluoro-ß-D-arabinofuranosyl]-9*H*-purine (14) A solution of **13** (0.23 g, 0.75 mmol) in dry DMF (4 mL) was treated with a mixture of *t*-butyldimethylsilyl chloride (3 mmol) and imidazole (7.5 mmol) in DMF. After stirring at rt for 30 min, water (100 mL) was added and the resulting mixture was extracted with EtOAc (3 x 100 mL). The organic layer was washed with brine (3 x 75 mL) and dried ($NaSO_4$). The solvent was evaporated and the residue was purified by preparative tlc (silica gel, Analtech 2000 μ, EtOAc:hexane, 1:1) to give compound **14** (0.270 g, 86%), mp 174-75 °C (EtOAc/hexane); ^1H NMR (Me_2SO-d_6) δ 0.05 (s, 6 H, Me_2Si), 0.85 (s, 9 H, Me_3C), 3.82 (m, 3 H, H-4', H-5'$_{a,b}$), 4.40 (dm, *J* = 14.5 Hz, 1 H, H-3'), 5.25 (dm, *J* = 52 Hz, 1 H, H-2'), 6.01 (br d, 1 H, 3'-OH), 6.35 (dd, *J* = 14.0, 4.4 Hz, 1 H, H-1'), 7.01 (br s, 2 H, NH_2), 8.23 (d, *J* = 2 Hz, 1 H, H-8). Anal. Calcd for $C_{16}H_{25}ClFN_5O_3Si$: C, 45.98; H, 6.03; N, 16.76. Found: C, 45.96; H, 6.05; N, 16.65. A second, less polar band afforded 0.05 g (13%) of the bis-silylated analogue.

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2-Amino-6-chloro-9-[3-O-[phenoxy(thiocarbonyl)]-5-O-(*tert*-butyldimethylsilyl)-2-deoxy-2-fluoro-ß-D-arabinofuranosyl]-9*H*-purine (15) A solution of **14** (0.052 g, 0.124 mmol) in dry CH_3CN (2 mL) was treated with phenyl chlorothionoformate (35 μL, 0.253 mmol) in the presence of dimethylaminopyridine (DMAP, 0.055 g, 0.450 mmol) and stirred at rt for 24 hr. The solvent was removed under vacuum and the crude product was purified by preparative tlc (silica gel, Analtech 2000 μ, hexane:EtOAc, 2:1) to give 0.039 g (57%) of **15** as an oil; ^1H NMR ($CDCl_3$) δ 0.06 (s, 6 H, Me_2Si), 0.90 (s, 9 H, Me_3C), 4.00 (m, 2 H, H-5'$_{a,b}$), 4.45 (m, 1 H, H-4'), 5.20 (br s, 2 H, NH_2), 5.30 (dd, *J* = 50, 3 Hz, 1 H, H-2'), 6.00 (dd, *J* = 16, 2.5 Hz, 1 H, H-3'), 6.40 (dd, *J* = 22, 3 Hz, 1 H, H-1'), 6.90-7.50 (m, 5 H, Ph), 8.08 (d, *J* = 2.9 Hz, 1 H, H-8). This material was used in the following step without further purification.

2-Amino-6-chloro-9-[5-O-(*tert*-butyldimethylsilyl)-2,3-dideoxy-2-fluoro-ß-D-*threo*-pentofuranosyl]-9*H*-purine (16) A solution of **15** (0.142 g, 0.256 mmol) in dry toluene (10 mL) was treated with Bu_3SnH (0.41 mL, 1.54 mmol) and 2,2′-azobis(2-methylpropionitrile) (AIBN, 0.044 g, 0.27 mmol). The reaction mixture was heated at reflux for 3 hr and cooled to rt. The solvent was removed under vacuum and the crude product was purified by preparative tlc (silica gel, Analtech 2000 μ, CH_2Cl_2:MeOH, 19:1) to give 0.071 g (69%) of **16** as a foam; ^1H NMR ($CDCl_3$) δ 0.06 (s, 6 H, Me_2Si), 0.92 (s, 9 H, Me_3C), 2.38 (m, 1 H, H-3′$_a$), 2.52 (m, 1 H, H-3′$_b$), 3.81 (d, J = 5 Hz, 2 H, H-5′$_{a,b}$), 4.25 (m, 1 H, H-4′), 5.15 (br s, 2 H, NH_2), 5.23 (dm, J = 50 Hz, 1 H, H-2′), 6.14 (dd, J = 16.5, 3.6 Hz, 1 H, H-1′), 8.08 (d, J = 2.7 Hz, 1 H, H-8). This material was used in the following step without further purification.

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2-Amino-6-chloro-9-(5-O-benzoyl-2,3-dideoxy-2-fluoro-ß-D-*threo*-pentofuranosyl]-9*H*-purine (18a) A suspension of 2-amino-6-chloropurine (0.184 g, 1.08 mmol) in dry CH_3CN (15 mL) was treated with bis(trimethylsilyltrifluoro)acetamide (1.5 mL, 5.4 mmol) and the resulting mixture was stirred at rt until it became homogeneous (30 min). The solvent was removed and the residue was protected from moisture. Immediately after, the residue was dissolved in acetonitrile (15 mL) and after the addition of $Hg(CN)_2$ (0.28 g, 1.08 mmol) the solution was refluxed for 30 min under nitrogen with the aid of a Dean-Stark trap to insure anhydrous conditions. A solution of bromosugar **17** in dry benzene (10 mL), prepared from 5-O-benzoyl-2,3-dideoxy-2-fluoro-1-O-methyl-ß-D-*threo*-pentofuranose (**10**, 0.092 g, 0.361 mmol),[22] was gradually added and refluxing continued for 3 hr. The mixture was allowed to cool to rt and after the addition of 100 mL of $CHCl_3$ the organic solution was washed successively with aqueous $NaHCO_3$ (2 x 50 mL) and 1M KI solution (2 x 50 mL). The organic layer was dried ($NaSO_4$) and reduced to dryness. The residue was purified by flash column chromatography (silica gel, EtOAc:hexane, gradient from 0% to 30%) to give the desired N9-ß isomer **18a** (0.044 g, 31%) as a foam (R_f = 0.37, silica gel, EtOAc:hexane, 20:1); ^1H NMR ($CDCl_3$) δ 2.60-2.80 (m, 2 H, H-3′$_{a,b}$), 4.60 (m, 3 H, H-4′, H-5′$_{a,b}$), 5.05 (br s, 2 H, NH_2), 5.25 (dm, J = 50 Hz, 1 H, H-2′), 6.20 (dd, J = 20, 2.9 Hz, 1 H, H-1′), 7.40-7.70 (m, 3 H, Ph), 8.02 (d, J = 1.5 Hz, 1 H, H-8), 8.10 (m, 2 H, Ph). This product was used in the following step without further purification.

Two other minor isomers were isolated and their structures were tentatively assigned by characteristic features in the ^{1}H NMR spectra. The N7-ß isomer **18b** (0.009 g, 7%, R_f = 0.58): δ 6.53 (dd, *J* = 17, 3 Hz, 1 H, H-1′) and 8.40 (d, *J* = 1.8 Hz, 1 H, H-8). The more polar N9-α isomer **18c** (0.009 g, 7%, R_f = 0.18): δ 6.20 (d, *J* = 19 Hz, 1 H, H-1′) and 7.80 (s, 1 H, H-8).

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2-Amino-6-chloro-9-(2,3-dideoxy-2-fluoro-ß-D-*threo*-pentofuranosyl]-9*H*-purine (7)

Method A A solution of **16** (0.071 g, 0.176 mmol) in THF (5 mL) was treated with tetraethylammonium fluoride dihydrate (0.136 g, 0.734 mmol) and stirred at rt for 6 hr. The solvent was removed under reduced pressure and the residue was purified by preparative tlc (silica gel, Analtech 2000 μ, CH_2Cl_2:MeOH, 9:1) to give **6** (0.043 g, 86%) as a semisolid. This material was dissolved in water and lyophilized to give **7** as a white fluffy solid, mp 94-97 °C; ^{1}H NMR (D_2O) δ 2.15 (m, 1 H, H-3′$_a$), 2.52 (m, 1 H, H-3′$_b$), 3.65 (m, 2 H, H-5′$_{a,b}$), 4.25 (m, 1 H, H-4′), 5.22 (dm, *J* = 50 Hz, 1 H, H-2′), 6.01 (dd, *J* = 18.8, 3.1 Hz, 1 H, H-1′), 8.12 (d, *J* = 2.5 Hz, 1 H, H-8); FAB mass spectrum, *m/z* (relative intensity) 288 (^{35}Cl MH$^+$, 100), 254 (39, MH-Cl+H), 170 (50, ^{35}Cl, bH$_2^+$), 136 (13, bH$_2$-Cl+H), Calcd: 288.0667. Found: 288.0664 (peak matching). Anal. Calcd for $C_{10}H_{11}ClFN_5O_2$: C, 39.88; H, 4.18; N, 23.25. Found: C, 39.70; H, 3.98; N, 23.16.

Method B A solution of **18a** (0.257 g, 0.655 mmol) in saturated methanolic ammonia (30 mL) was stirred in a pressure bottle at rt for 36 hr. The solvent was removed under reduced pressure and the product was purified by flash column chromatography (silica gel, MeOH:CH_2Cl_2, gradient from 0% to 10%) to afford 0.150 g (66%) of 7, mp 94-97 °C. This compound was identical in every respect to the same material synthesized from **16**. A small amount (0.019 g, 9%) of the diamino compound **8** was also obtained.

9-(2,3-Dideoxy-2-fluoro-ß-D-*threo*-pentofuranosyl)guanine (6).

(From Compound 7)

Method A Sodium hydroxide (0.063 g, 1.57 mmol) was added to a solution of **7** (0.042 g, 0.146 mmol) in water (10 mL). The solution was heated to 80-90 °C for 4 hr and then cooled to rt and neutralized with 2 N acetic acid. After lyophilization, the white, salt-contaminated solid was purified by reverse phase chromatography (Baker octadecyl C-18, 40 μm average particle diameter) using a gradient from 0 to 5% MeOH in water to give 0.022 g (57%) of **6** as a fluffy white solid; mp > 230 °C (dec).

Method B Adenosine deaminase (1 mL calf intestine enzyme suspension, 2000 U/mL, from Boehringer Mannheim) was added to a solution of **7** (0.080 g, 0.27 mmol) in 0.025 *M* aqueous phosphate buffer (50 mL, pH 7.5) and the mixture was stirred overnight at rt. The reaction mixture was then divided into six portions and each portion was ultrafiltered by centrifugation (3,000 rpm) for 30 min through an Amicon Centripep-10 concentrator unit to remove enzyme. The filtrate from each vessel was decanted and combined. Each unit was rinsed with pure water and the operation repeated. The combined filtrates from both operations were lyophilized and the product purified as in Method A by reverse phase chromatography to give pure **6**, 0.051 g (68%) as before; ^{1}H NMR (D_2O) δ 2.20 (m, 1 H, H-3'$_a$), 2.60 (m, 1 H, H-3'$_b$), 3.70 (m, 2 H, H-5'$_{a,b}$), 4.30 (m, 1 H, H-4'), 5.30 (dm, *J* = 50 Hz, 1 H, H-2'), 6.10 (dd, *J* = 19.0, 3.4 Hz, 1 H, H-1'), 7.95 (d, *J* = 2.7 Hz, 1 H, H-8); FAB mass spectrum, *m/z* (relative intensity) 270 (MH^+, 100), 152 (53, bH_2^+). Calcd: 270.1002. Found: 270.1005. Anal. Calcd for $C_{10}H_{12}FN_5O_3$: C, 41.82; H, 4.91; N, 24.38. Found: C, 41.74; H, 4.84; N, 24.22.

(From Compound 18a)

A mixture of **18a** (0.190 g, 0.484 mmol) and 1.6 N NaOH (15 mL) was heated to reflux for 4 hr. The solution was neutralized with 2 N HOAc, concentrated and lyophilized. The crude product was purified by reverse phase column chromatography as in Method A above to afford **6** (0.062 g, 47%) as a white, fluffy solid, mp > 230° C dec, identical in every respect to the product isolated from **7**.

2,6-Dichloro-9-(5-O-benzoyl-2,3-dideoxy-2-fluoro-ß-D-*threo*-pentofuranosyl]-9*H*-purine (**19a**) A suspension of 2,6-dichloropurine (0.354 g, 1.87 mmol) in dry CH_3CN (15 mL) was treated with bis(trimethylsilyltrifluoro)acetamide (3 mL, 10.8 mmol) and the resulting mixture was stirred at rt until it became homogeneous (30 min). The solvent was removed and the white solid residue was redissolved in dry acetonitrile (30 mL) in the presence of molecular sieves (4Å). A solution of bromosugar **17** in dry acetonitrile (20 mL) was prepared separately from 5-O-benzoyl-2,3-dideoxy-2-fluoro-1-O-methyl-ß-D-*threo*-pentofuranose (10,

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0.159 g, 0.625 mmol),[22] and it was gradually added to the solution of the silylated base. The resulting reaction mixture was refluxed for 20 hr, cooled and quenched with methanol (10 mL). The mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The crude product obtained was purified by preparative tlc (silica gel, Analtech® 2000 μ, CH_2Cl_2:MeOH, 40:1) and two major bands were isolated. The top band (R_f = 0.5, silica gel, EtOAc:hexane, 1:1) afforded 0.081 g (32%) of the desired N9-ß isomer **19a**, which was obtained as a solid, mp 169-170 °C (EtOH/ether); ^{1}H NMR ($CDCl_3$) δ 2.40-2.90 (m, 2 H, H-3'$_{a,b}$), 4.65 (m, 3 H, H-4', H-5'$_{a,b}$), 5.32 (dm, J = 50 Hz, 1 H, H-2'), 6.35 (dd, J = 19.5, 2.8 Hz, 1 H, H-1'), 7.40-7.70 (m, 3 H, Ph), 8.10 (m, 2 H, Ph), 8.42 (d, J = 2.7 Hz, 1 H, H-8). Anal. Calcd for $C_{17}H_{13}Cl_2FN_4O_3 \cdot 0.75 H_2O$: C, 48.07; H, 3.44; N, 13.19. Found: C, 48.11; H, 3.08; N, 13.13. The lower band (R_f = 0.40, silica gel, EtOAc:hexane, 1:1) afforded 0.061 g (23%) of the N9-α **19b** as a thick oil; ^{1}H NMR ($CDCl_3$) δ 2.20-2.50 (m, 1 H, H-3'$_a$), 2.80-3.10 (m, 1 H, H-3'$_b$), 4.50 (AB doublet, 2 H, H-5'$_{a,b}$), 5.00 (m, 1H, H-4'), (dd, J = 53, 6 Hz, 1 H, H-2'), 6.35 (d, J = 14.5 Hz, 1 H, H-1'), 7.40-7.60 (m, 3 H, Ph), 8.10 (m, 2 H, Ph), 8.20 (s, 1 H, H-8). No futher purification of this compound was attempted.

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2,6-Diazido-9-(5-O-benzoyl-2,3-dideoxy-2-fluoro-ß-D-*threo*-pentofuranosyl]-9*H*-purine (20) A suspension of **19a** (0.238 g, 0.578 mmol) in EtOH (20 mL) was treated with an aqueous solution (10 mL) of NaN_3 (0.090 g, 1.38 mmol). The resulting mixture was heated at reflux for 3 hr and cooled. The solvent was removed under reduced pressure and crystallization of the solid residue from EtOH/ether afforded 0.152 g (62%) of pure **20**, mp 177-180° C dec. Purification of the mother liquor by preparative tlc (silica gel, Analtech 2000 μ, CH_2Cl_2:MeOH, 40:1) afforded an additional amount of compound (0.045 g). The total yield of pure **20** was 81%; ^{1}H NMR ($CDCl_3$) δ 2.40-2.90 (m, 2 H, H-3'$_{a,b}$), 4.65 (m, 3 H, H-4', H-5'$_{a,b}$), 5.30 (dm, J = 50 Hz, 1 H, H-2'), 6.35 (dd, J = 20, 2.8 Hz, 1 H, H-1'), 7.40-7.60 (m, 3 H, Ph), 8.10 (m, 2 H, Ph), 8.21 (d, J = 2.8 Hz, 1 H, H-8). Anal. Calcd for $C_{17}H_{13}FN_{10}O_3$: C, 48.11; H, 3.09; N, 33.01. Found: C, 48.06; H, 3.04; N, 33.11.

2,6-Diamino-9-(2,3-dideoxy-2-fluoro-ß-D-*threo*-pentofuranosyl]-9*H*-purine (8) A solution of the diazide **20** (0.130 g, 0.306 mmol) prepared by first

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dissolving the solid in EtOAc (2 mL) and diluted with EtOH (10 mL) was hydrogenated at 45 psi on a Parr hydrogenator for 72 hr in the presence of 10% Pd/C. The solution was then treated with a few drops of glacial AcOH and filtered through a pad of celite. The filtrate was concentrated under reduced pressure and the residue was purified by preparative tlc (silica gel, Analtech, 2000 μ, CH_2Cl_2:MeOH, 9:1). The resulting product (0.081 g) was immediately treated with 15 mL of saturated methanolic ammonia and stirred at rt for 36 hr in a pressure vessel. After the solvent was removed under reduced pressure, the solid obtained was dissolved in water (50 mL) and extracted with $CHCl_3$ (3 x 20 mL) and ether (3 x 20 mL). The aqueous layer was concentrated and lyophilized to give 0.032 g (39%) of **8** as a white fluffy solid, mp 239-241 °C; ^1H NMR (D_2O) δ 2.10-2.30 (m, 1 H, H-3′$_a$), 2.40-2.70 (m, 1 H, H-3′$_b$), 3.70 (m, 2 H, H-5′$_{a,b}$), 4.25 (m, 1 H, H-4′), 5.25 (dm, J = 53 Hz, 1 H, H-2′), 6.05 (dd, J = 19, 3.2 Hz, 1 H, H-1′), 7.92 (d, J = 2.6 Hz, 1 H, H-8); FAB mass spectrum, *m/z* (relative intensity) 269 (100, MH^+), 151 (36, bH_2^+). Calcd: 269.116. Found: 269.115. Anal. Calcd for $C_{10}H_{13}FN_6O_2 \cdot 0.75\ H_2O$: C, 42.62; H, 5.19; N, 29.83. Found: C, 42.62; H, 5.19; N, 29.61.

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