Nucleic Acid Related Compounds. 114. Synthesis of 2,6-(Disubstituted)purine 2',3'-Dideoxynucleosides and Selected Cytotoxic, Anti-Hepatitis B, and Adenosine Deaminase Substrate Activities

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Dedicated to Professor Jerald S. Bradshaw on the occasion of his retirement from Brigham Young University

Selected 2,6-(disubstituted)purine 2',3'-didehydro-2',3'-dideoxynucleosides and 2',3'-dideoxynucleosides were prepared and evaluated. Treatment of 5'-protected ribonucleosides with phenoxythiocarbonyl chloride and 4-(dimethylamino)pyridine, or under Schotten-Baumann conditions, gave high yields of 2',3'-*O*-thionocarbonates that underwent Corey-Winter elimination. Treatment of unprotected ribonucleosides with α-acetoxyisobutyryl bromide in "moist" acetonitrile gave *trans* 2',3'-bromohydrin acetate mixtures that underwent reductive elimination with zinc-copper couple or zinc/acetic acid. Catalytic hydrogenation of the resulting 2',3'-enes gave 2',3'-dideoxynucleosides. Treatment of the 2-amino-6-chloropurine and 6-amino-2-fluoropurine derivatives with nucleophiles gave 2,6-(disubstituted)purine 2',3'-dideoxynucleosides. 2',3'-Dideoxyguanosine and the 2-amino-6-[amino (**16d**), methoxy (**16b**), ethoxy (**16c**), and methylamino (**16j**)]purine 2',3'-dideoxynucleosides showed good anti-hepatitis B activity with infected primary duck hepatocytes. Cytotoxic effects with selected analogues were evaluated in human T-lymphoblastic and promyelocytic leukemia cell lines. The 2-amino-6-fluoro derivative **16m** was the most cytotoxic of the 2-amino-6-(substituted)purine 2',3'-dideoxynucleosides, and 2-fluoro-2',3'-dideoxyadenosine (**21a**) was the most cytotoxic compound. The order of efficiency of hydrolysis of the 6-substituent from 2-amino-6-(substituted)purine 2',3'-dideoxynucleosides (V_{max}/K_m) with adenosine deaminase from calf intestine was: 2-amino-6-[amino (**16d**) > methoxy (**16b**) > ethoxy (**16c**)], all of which were ≤3% of the efficiency with adenosine. The 6-methylamino derivative **16j**, as well as **16b**, **16c**, and **16d** were readily converted into 2',3'-dideoxyguanosine by duck cell supernatants.

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Introduction.

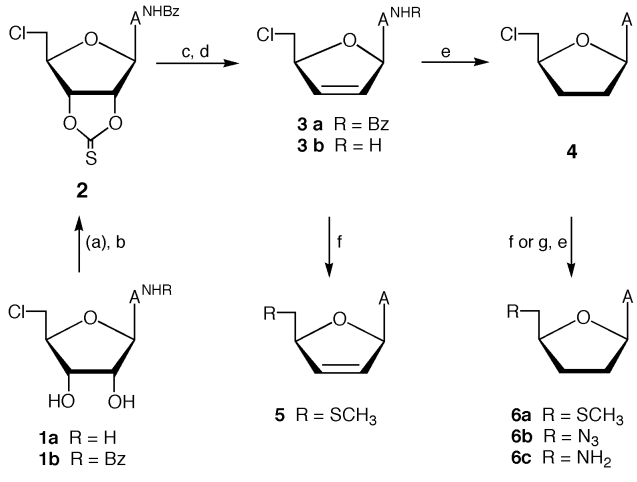
The first 2',3'-dideoxynucleoside, 3'-deoxythymidine, was reported by Michelson and Todd in 1955 [1]. The first purine dideoxynucleoside, 2',3'-dideoxyadenosine, was reported by Robins and Robins in 1964, who noted that such analogues might function as terminators of nucleic acid biosynthesis if converted into 5'-phosphates and incorporated onto growing DNA chains [2]. This concept, demonstrated independently by Cohen [3] and Kornberg [4] and their coworkers, was developed by Sanger into the standard methodology for DNA sequencing [5]. β-Elimination reactions were then employed for synthesis of 2',3'-didehydro-2',3'-dideoxynucleosides, which could be hydrogenated to give 2',3'-dideoxynucleosides [6,7]. The discovery that such dideoxynucleosides had activity against human immunodeficiency viruses (HIV) [8] ignited major interest in their synthesis and biological evaluation. Chemistry associated with AIDS-related dideoxynucleosides, their derivatives, and analogues have been reviewed [9]. A number of 6-halo, 2-amino-6-halo [10], and 6-amino-2-halo [11] purine 2',3'-dideoxynucleosides with anti-HIV activity have been reported. Biological activities of 6-(alkyl)thio, 6-(alkyl)amino [12], 6-alkoxy [13], and related 2,6-(disubstituted)purine 2',3'-dideoxynucleosides have been evaluated [14-18]. Chemistry utilized for the synthesis of these purine-modified 2',3'-dideoxynucleosides has included: (i) dideoxygenation of the vicinal diol moiety of base-modified ribonucleosides [14,18], (ii) coupling of deoxygenated sugar and/or precursors with substituted purines [12,15], (iii) modification of the purine base of dideoxynucleosides [10,12,14], and (iv) enzymatic transfer of deoxygenated sugars from modified nucleosides to substituted purine bases [10,11,13,17]. During the course of this work [18a], several of the present dideoxynucleosides have been prepared by enzymatic glycosyl transfer and other procedures [9].

We reported the discovery that certain 2',3'-dideoxynucleosides are potent inhibitors of duck hepatitis B virus (DHBV) [19]. It has been shown that dGTP binds to tyrosine-96 in the *in vitro* translated DHBV polymerase [20], and we have demonstrated that ddGTP binds to the *in vitro* translated DHBV polymerase [21], possibly *via* Tyr96 in the primer domain of the enzyme. This may rationalize the potent inhibition of DHBV by ddGuo and ddGuo prodrugs. The current status of anti-HBV chemotherapy and the pronounced activity of a number of dideoxynucleosides have been reviewed [22].

Chemistry.

We have utilized 5'-chloro-5'-deoxyadenosine (**1a**) [23] to prepare selected 5'-modified 2',3'-dideoxynucleosides. Treatment of 6-*N*-benzoyl-5'-chloro-5'-deoxyadenosine (**1b**) (obtained from **1a** *via* the Jones transient protection protocol [24]) with phenoxythiocarbonyl chloride (PTCCl)/4-(dimethylamino)pyridine (DMAP) gave the cyclic 2',3'-*O*-thionocarbonate **2** (90%) [25a] (Scheme 1). A subsequent communication reported a similar conversion with PTCCl, tributyltin oxide, and a quaternary ammonium salt [25b], and other nucleoside thionocarbonates have been prepared with 1,1'-thiocarbonyldiimidazole [26]. Corey-Winter treatment of **2** with triethyl phosphite at 120 °C for 4 hours gave 6-*N*-benzoyl-9-(5-chloro-2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl)adenine (**3a**) (95%) which was debenzoylated to give **3b** (quantitative). Catalytic hydrogenation of **3b** gave 9-(5-chloro-2,3,5-trideoxy-β-D-*glycero*-pentofuranosyl)adenine (**4**) (80%). Some glycosyl cleavage occurred at atmospheric pressure with **3b**, and this became predominant at higher pressures. Extensive glycosyl cleavage occurred upon attempted hydrogenation of **3a**. Treatment of **3b** (or **4**) with sodium thiomethoxide gave **5** (or **6a**) quantitatively. Treatment of **4** with sodium azide in DMF (90 °C) gave the 5'-azido compound **6b** which was hydrogenolyzed to give 5'-amino-2',3',5'-trideoxyadenosine (**6c**).

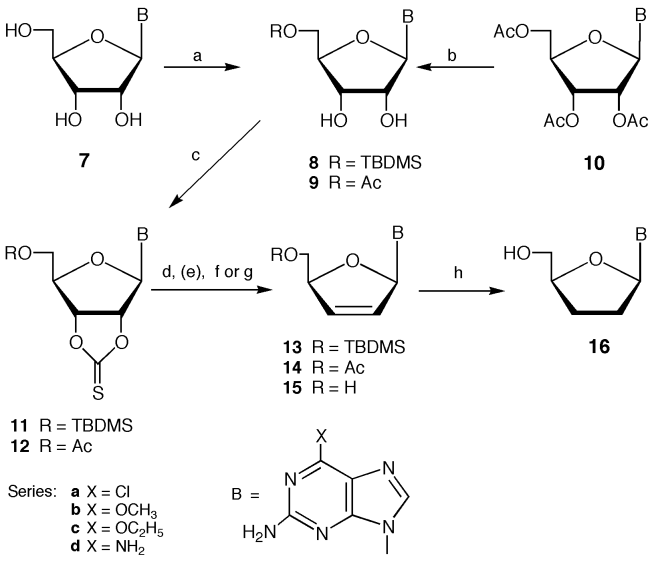
Scheme 1



(a) (i) TMSCl/pyridine; (ii) BzCl; (iii) NH_3/H_2O. (b) PTCCl/DMAP/CH_3CN. (c) $(EtO)_3P$/120 °C/5 h. (d) NH_3/MeOH. (e) H_2/Pd•C/MeOH. (f) NaSMe/DMF. (g) NaN_3/DMF.

Selective O5' silylation of 2-amino-6-chloropurine riboside (**7a**) [27] gave **8a** (90%) which was converted into its 2',3'-*O*-thionocarbonate **11a** (Scheme 2). Heating **11a** in triethyl phosphite effected quantitative dideoxygenation to give the 2',3'-didehydro-2',3'-dideoxy analogue **13a**, which was deprotected (tetrabutylammonium fluoride, TBAF) to

Scheme 2



(a) TBDMSCl/pyridine or imidazole/DMF. (b) MeOH/TEA/$CHCl_3$. (c) PTCCl/DMAP or KOH/CH_3CN. (d) $(EtO)_3P$ or $(EtO)_3P$/dioxane. (e) TBAF/THF or NH_4F/MeOH/TEA. (f) NaOMe/MeOH. (g) NH_3/MeOH. (h) H_2/Pd•C.

give 2-amino-6-chloro-9-(2,3-dideoxy-β-D-*erythro*-pent-2-enofuranosyl)purine (**15a**). Extensive glycosyl hydrogenolysis occurred upon attempted catalytic hydrogenation of **13a** and **15a**. Nucleophilic displacement of chloride from **15a** by methoxide gave the 2-amino-6-methoxy analogue **15b** (quantitative conversion) which underwent clean hydrogenation to give 2-amino-9-(2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-6-methoxypurine (**16b**). Treatment of **15a** with methanolic ammonia (85 °C for 36 hours) gave the 2,6-diaminopurine analogue **15d** (47%) plus the 2-amino-6-methoxy byproduct **15b** and unreacted **15a**. Hydrogenation of **15d** proceeded smoothly to give 2,6-diamino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)purine (**16d**) (99%) [18b].

A suspension of 2-amino-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**10a**) [27a] in methanol/triethylamine/chloroform was heated at reflux, and the residual solid was mainly the 5'-*O*-acetyl derivative **9a** (85%). Treatment of **9a** with PTCCl/DMAP gave the 2',3'-*O*-thionocarbonate derivative **12a** which underwent dideoxygenation very slowly [$(EtO)_3P$/115 °C] with accompanying formation of fluorescent baseline spots (TLC). Addition of 1,4-dioxane markedly accelerated the elimination to give 2-amino-9-(5-*O*-acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-6-chloropurine (**14a**). Treatment of **14a** with methanolic sodium methoxide effected nucleophilic substitution of chloride and deacetylation to give **15b** which was hydrogenated to give **16b** (43% overall from **9a**).

The 2-amino-6-methoxy, **16b**, and 2-amino-6-ethoxy, **16c**, compounds were active against duck hepatitis B virus

(DHBV) and had low toxicity in cell cultures. Therefore, more efficient routes to these compounds were pursued. Treatment of 2-amino-6-methoxypurine riboside (**7b**) [27b] with α-acetoxyisobutyryl bromide (AIB-Br) [18] failed to give the bromohydrin acetates. Selective silylation of the 5'-hydroxyl group of **7b** (*tert*-butyldimethylsilyl chloride/pyridine) gave **8b** which was treated with $PTCCl/CH_3CN/Et_2O//KOH/H_2O$ to give the 2',3'-*O*-thionocarbonate **11b** in high yield. Application of these modified Schotten-Baumann conditions circumvented the separation problems and avoided the use of amines that can interact with triethyl phosphite. Corey-Winter treatment of **11b** [$(EtO)_3P$/115 °C)] gave **13b** smoothly, but purification of the deprotected **15b** from TBAF was difficult. Desilylation of **13b** with ammonium fluoride in methanol [28] in the presence of triethylamine allowed straightforward isolation of **15b**. Treatment of 2-amino-6-ethoxy analogue **7c** [29] by this sequence gave **15c** which was hydrogenated to give 2-amino-9-(2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-6-ethoxypurine (**16c**).

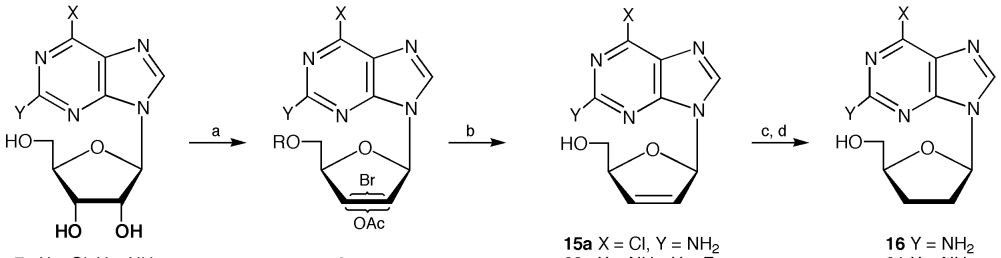
Our efficient three-step sequence [18]: (1) conversion of purine ribonucleosides into *trans* bromohydrin acetates with AIB-Br, (2) reductive elimination with zinc-copper couple or zinc/acetic acid, (3) hydrogenation ($Rh•Al_2O_3$) was used to prepare the 2-amino-6-chloro precursor, **16a**, for the synthesis of biological test quantities of 2-amino-6-(substituted)purine 2',3'-dideoxynucleosides. Treatment of 2-amino-6-chloropurine riboside (**7a**) with AIB-Br caused some replacement of chloride by bromide at C6 [18b]. However, since the 6-(bromo and chloro) compounds underwent equivalent displacement reactions with nucleophiles, these mixtures were treated as 2-amino-6-chloropurine compounds **15a**, **16a**, **17a** in the reaction sequences of Scheme 3. Treatment of **16a** with sodium alkoxides (or thioalkoxides) gave 2-amino-6-alkoxy **16b**,**c**,**e**,**f** (or 2-amino-6-alkylthio **16g-i**) derivatives (Scheme 3). Treatment of **16a** with an excess of the amines (80 °C, steel bomb) gave the alkylamino analogues **16 j-l**. The 2-amino-6-fluoro derivative **16m** was obtained by displacement of chloride from **16a** with fluoride. Our three-step sequence [18] (hydrogenation with Pd•C) was applied to 2-fluoroadenosine (**18**) [30] to give the 2-fluoroadenine 2',3'-dideoxynucleoside **21a** (72% from **18**, *via* **19** and **20**) [18b]. Treatment of **21a** with nucleophiles gave the 2-substituted-2',3'-dideoxyadenosine derivatives **21b-e**.

It was observed that several of the purine 2',3'-didehydro-2',3'-dideoxynucleosides underwent decomposition with release of the purine base during chromatographic purification and/or upon incubation with adenosine deaminase in aqueous buffer at 37 °C. Preliminary tests indicated enhanced cellular toxicity with certain of these compounds relative to their saturated 2',3'-dideoxy derivatives. Therefore, these somewhat unstable 2',3'-didehydro intermediates were not subjected to either anti-DHBV testing in duck hepatocyte cultures for the required extended periods at 37 °C or controlled cytotoxicity screening.

Cytotoxic Effects.

Cytotoxic potencies were determined by measuring effects of the purine 2',3'-dideoxynucleosides on the viability of human T-lymphoblastoid CCRF-CEM cells and human promyelocytic leukemia HL-60 cells (Table 1). In general, the orders of cytotoxic potencies of the agents were similar in the two cell lines. However, **16b**, **16d**, and **16m** were less toxic in HL-60 cells than in CCRF-CEM cells, and **21e** was more toxic in HL-60 cells. IC_{50} values for most of the **16**-series compounds [2-amino-6-(substituted)purine 2',3'-dideoxynucleosides] exceeded 900 μ*M*, indicating the low cytotoxicity of these agents. Exceptions included the 6-fluoro derivative, **16m**, which was the most cytotoxic, followed by the 6-methylamino, **16j**, and 6-chloro, **16a**, derivatives. IC_{50} values for **16b**, **16c**, and **16d** all exceeded 2.5 mM in primary duck hepatocyte cultures (data not shown). The 2-fluoro derivative **21a** was the most cytotoxic compound tested (IC_{50}, 5 μ*M*), whereas the cytotoxicities of the other 2-substituted-2',3'-

Scheme 3



7a X = Cl, Y = NH_2
18 X = NH_2, Y = F

17 X = Cl, Y = NH_2
19 X = NH_2, Y = F

15a X = Cl, Y = NH_2
20 X = NH_2, Y = F

16 Y = NH_2
21 X = NH_2

16: **a** X = Cl; **b** X = OMe; **c** X = OEt; **d** X = NH_2; **e** X = OPr; **f** X = O*i*-Pr; **g** X = SH; **h** X = SMe; **i** X = SEt; **j** X = NHMe; **k** X = NHEt; **l** X = NMe_2; **m** X = F

21: **a** Y = F; **b** Y = OMe; **c** Y = NHMe; **d** Y = NHEt; **e** Y = SMe

(a) $(CH_3)_2C(OAc)COBr/CH_3CN$. (b) Zn-Cu/DMF or Zn/HOAc/DMF. (c) H_2/Pd•C or $Rh•Al_2O_3$. (d) Nucleophiles.

Table 1

Cytotoxic Activities of 2,6-(Disubstituted)purine 2',3'-Dideoxynucleosides against Human Leukemia Cell Lines *In Vitro*

Compound	IC_{50} (μM) [a]	
	CCRF-CEM	HL-60
16a	640 ± 40	620 ± 45
16b	1300 ± 360	3700 ± 500
16c	2000 ± 530	2100 ± 48
16d	950 ± 190	1700 ± 320
16h	1700 [b]	2300 ± 450
16j	160 ± 38	240 ± 120
16l	1300 ± 200	1900 [b]
16m	72 ± 10	300 ± 75
21a	4.8 ± 1.2	5.0 ± 1.5
21b	1700 ± 280	1900 ± 200
21c	3400 ± 150	3200 [b]
21e	1700 [b]	900 [b]

[a] Concentration of compound that reduced the number of viable cells by 50%. Results are the means (±S.D.) of 2-6 experiments, each with 6 replicate measurements. [b] Single experiment.

dideoxyadenosine derivatives tested were very low (IC_{50} values ≥900 μ*M*, Table 1).

Adenosine Deaminase Substrate Activity.

The 2-amino-6-[methoxy (**16b**), ethoxy (**16c**), and amino (**16d**)]purine 2',3'-dideoxynucleosides which showed potent antiviral activity (≤10 μ*M*) and low cytotoxicity (IC_{50} ≥100 μ*M*) were tested as substrates of adenosine deaminase from calf intestine (Table 2). The K_m values were similar to that of the natural substrate, adenosine. In contrast, V_{max} values for the deaminase-mediated hydrolysis of 6-substituents from **16b** and **16d** were about two orders of magnitude less than that of adenosine, and the rate for **16c** was less by over four orders of magnitude. The relative efficiencies (V_{max}/K_m, Table 2) of adenosine deaminase-mediated hydrolysis at C6 of these compounds were adenosine > **16d** > **16b** > **16c**, with the efficiency of **16d** ~3% of that of adenosine. Thus, the increased size of the 6-substituent correlated with decreased V_{max} and efficiencies of deaminase-mediated hydrolysis at C6 in harmony with the report of Burns *et al.*, which showed a carbon chain length-associated decrease in the rate of deaminase-mediated dealkoxylation of 6-alkoxypurine 2',3'-dideoxynucleosides [13a]. In the present study, the rate for the 6-methylamino derivative, **16j**, was more than an order of magnitude lower than that of **16c**, and the 6-propoxy, **16e**, and 6-isopropoxy, **16f**, derivatives were not measurably cleaved under these assay conditions.

Table 2

Kinetic Constants for the Hydrolysis of the C6 Substituent from 2-Amino-6-(substituted)purine 2',3'-Dideoxynucleosides with Adenosine Deaminase from Calf Intestine

Substrate	K_m (μM)	V_{max} [a]	V_{max}/K_m
Ado	34.5	561	16.3
16b	55.5	9.28	0.167
16c	33.8	0.0298	0.000882
16d	12.9	6.35	0.492

[a] Units are: μmol min^{-1} (mg protein)$^{-1}$.

Selected dideoxynucleosides were tested as substrates for duck adenosine deaminase by measuring rates of hydrolysis of 6-substituents during incubation with supernatant preparations from broken erythrocytes and liver cells from Pekin ducks (Table 3). Although specific activities (based on adenosine) of adenosine deaminase in these crude preparations from erythrocytes and liver differed about 20-fold, the orders of initial hydrolysis rates for the substrates (50 μ*M*) were similar. The relative hydrolysis rates for compounds **16c** and **16j** were lower than those of **16b** and **16d**, which is in harmony with the observed size effects of the 6-substituent in the experiments with adenosine deminase from calf intestine.

Table 3

Hydrolysis of the C6 Substituent from 2-Amino-6-(substituted)purine 2',3'-Dideoxynucleosides with Supernatants from Duck Erythrocytes and Duck Liver Cells

Substrate [a]	Initial Rate of Hydrolysis [b]	
	Erythrocyte	Liver
Ado	29.7 ± 9.2	1.46 [c]
16b	7.81 [c]	0.855 ± 0.045
16c	1.31 ± 0.77	0.310 ± 0.194
16d	9.43 ± 1.24	0.496 ± 0.068
16j	0.611 ± 0.199	[d]

[a] Substrate concentrations were 50 μ*M*. [b] Units are: nmol min^{-1} (mg protein)$^{-1}$. Each value is the mean (±S.E.M.) of 2-5 experiments. [c] Single experiment. [d] Not determined.

Hepatitis B Antiviral Activity.

In vitro anti-hepatitis B activities (IC_{50}) of 2',3'-dideoxy(adenosine, guanosine, and inosine) in primary duck hepatocytes cultured for 18 days were in the range of 0.1–1 μg/mL [19]. The 2',3'-dideoxynucleosides of 2-amino-6-methoxypurine (**16b**) and 2,6-diaminopurine (**16d**) also had similar potencies in this system. Conversion of **16b** and **16d** into 2',3'-dideoxyguanosine occurred rapidly in duck blood, and they presumably function as prodrugs. The analogous 9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl) nucleosides of 2-amino-6-chloropurine (**16a**), 2-amino-6-ethoxypurine (**16c**), 2-amino-6-methylaminopurine (**16j**), 2-amino-6-ethylaminopurine (**16k**), and 2-amino-6-fluoropurine (**16m**) were approximately two orders of magnitude less active, and 2-amino-6-dimethylaminopurine 2',3'-dideoxynucleoside (**16l**) was

less active by about another order of magnitude. Compounds with significant toxicity in uninfected duck hepatocytes or which did not reduce DHBV proliferation by ≥50% (qualitative estimation) at these concentrations were not examined further. Compounds **16b** and **16d**, as well as 2',3'-dideoxyguanosine, were highly effective *in vivo* and rapidly eliminated DHBV from duck serum [19].

EXPERIMENTAL

Reagent grade solvents were distilled before use. "Dried" solvents were freshly distilled from calcium hydride under a nitrogen atmosphere. Uncorrected melting points were determined with a capillary apparatus. ^{1}H NMR spectra (solutions in deuterated dimethylsulfoxide unless otherwise noted) were recorded on a Varian Gemini-200 spectrometer. Ultraviolet (uv) spectra (solutions in methanol) were recorded on a Hewlett-Packard 8451A diode array spectrophotometer. Mass Spectra (ms) were determined on a Finnigan MAT 8430 mass spectrometer. Elemental analyses were determined by MHW Laboratories, Phoenix, AZ or the Microanalytical Laboratory at the University of Alberta. Evaporations *in vacuo* were effected with a rotary evaporator equipped with a Dewar "Dry-Ice" condenser under mechanical oil pump vacuum. Column chromatography was performed with EM Kieselgel 60 (200-400 mesh). "Diffusion crystallization" was effected as described [31]. Treatment of 2-amino-6-chloropurine riboside (**7a**) with α-acetoxyisobutyryl bromide (AIB-Br) caused some replacement of chloride by bromide at C6 [18b]. However, since the 6-(bromo and chloro) compounds underwent equivalent displacement reactions with nucleophiles, these mixtures were treated as the 2-amino-6-chloropurine compounds **15a**, **16a**, **17a** in the reaction sequences of Scheme 3.

6-*N*-Benzoyl-5'-chloro-5'-deoxyadenosine (**1b**).

Trimethylsilyl chloride (10 mL, 8.56 g, 79 mmol) was added dropwise over a 10 minute period to a cooled (ice bath) suspension of **1a** (5.0 g, 16 mmol) [23] in pyridine (50 mL) and stirring was continued for 30 minutes. Benzoyl chloride (10 mL, 12.1 g, 86 mmol) was added, the reaction mixture was allowed to warm to ambient temperature with stirring (2 hours), and then cooled (ice-water bath). H_2O (10 mL) was added slowly, the mixture was stirred (30 minutes), and NH_3/H_2O (29%, 10 mL) was added dropwise with stirring at ambient temperature. The mixture was adjusted to pH ~9 (NH_3/H_2O, 29%; ~1 mL), cooled (-10 °C) overnight, and evaporated. EtOAc and heptane were added and evaporated. The off-white material was stirred with H_2O (100 mL) and filtered, and stirred with Et_2O (100 mL) and filtered to give an off-white solid of sufficient purity for subsequent reactions. Recrystallization (*i*-PrOH/H_2O, ~2:3; 60 mL) gave first (5.5 g, 88%) and second crops (0.60 g) of **1b**•0.5 H_2O (6.1 g, 98%): mp 145-146 °C (lit [32] mp 166.5-167.5 °C); uv: λ max 280 nm (ε 19 400), λ min 247 nm (ε 11 900); ^{1}H nmr: δ 3.91 (m, 2, H5',5''), 4.16 (m, 1, H3'), 4.26 (m, 1, H2'), 4.85 (m, 1, H4'), 5.53 (d, *J* = 5.1 Hz, 1, OH3'), 5.72 (d, *J* = 5.9 Hz, 1, OH2'), 6.08 (d, *J* = 5.8 Hz, 1, H1'), 7.50-8.07 (m, 5, Bz), 8.70 (s, 1, H2), 8.77 (s, 1, H8), 11.20 (br s, 1, NH); ms: m/z 389 (M^+ [^{35}Cl]).

Anal. Calcd. for $C_{17}H_{16}ClN_5O_4$•0.5 H_2O (398.8): C, 51.20; H, 4.30; Cl, 8.89; N, 17.56. Found: C, 51.53; H, 4.08; Cl, 8.89; N, 17.36.

6-*N*-Benzoyl-5'-chloro-5'-deoxy-2',3'-*O*-thiocarbonyladenosine (**2**).

Typical Procedure A.

PTCCl (1.80 mL, 2.25 g, 13.0 mmol) was added dropwise to a suspension of **1b** (1.95 g, 4.89 mmol) and DMAP (1.79 g, 14.7 mmol) in dry CH_3CN (20 mL) and stirring of the resulting solution was continued at ambient temperature for 1 hour. Volatiles were evaporated and the thick slurry was stirred with Et_2O (100 mL) and H_2O (50 mL). The suspension was filtered and the filter cake was washed with Et_2O (50 mL) to give **2** (2.16 g; quantitative, after drying *in vacuo* over P_4O_{10}) as a tan solid. A sample of this crude material was stirred with MeOH/$CHCl_3$ (1:9) and the resulting solution was filtered through charcoal/Celite to give **2** (1.62 g, 75%) as a white granular solid: mp 194.5-195.5 °C; uv: λ max 280, 236 nm (ε 21 200, 23 700), λ min 259, 219 nm (ε 13 200, 15 400); ^{1}H nmr: δ 3.90 (m, 2, H5',5''), 4.78 (m, 1, H4'), 5.94 (dd, *J* = 7.7, 7.4 Hz, 1, H3'), 6.44 (dd, *J* = 7.6, 1.9 Hz, 1, H2'), 6.79 (d, *J* = 1.9 Hz, 1, H1'), 7.53-8.08 (m, 5, Bz), 8.67 (s, 1, H2), 8.81 (s, 1, H8), 11.34 (br s, 1, NH); ms: m/z 431 (M^+ [^{35}Cl]).

Anal. Calcd. for $C_{18}H_{14}ClN_5O_4S$ (431.9): C, 50.06; H, 3.27; N, 16.22; S, 7.42. Found: C, 49.66; H, 3.19; N, 15.89; S, 7.23.

9-(5-Chloro-2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl)-adenine (**3b**).

Typical Procedure B (Elimination).

A suspension of **2** (1.50 g, 3.47 mmol) in freshly distilled $(EtO)_3P$ (100 mL) was deoxygenated (Ar, 30 minutes) and heated in an oil bath at 120 °C for 5 hours under Ar. Volatiles were removed *in vacuo* and the syrup was stirred with Et_2O/hexanes (~1:1, 150 mL) to give a precipitate that was filtered and washed with hexanes to give **3a** (1.24 g, quantitative) as a white solid of sufficient purity for subsequent reactions. Column chromatography (EtOH/$CHCl_3$, 5:95) gave **3a** (1.11 g, 90%) as a white granular solid: mp 85-86 °C; ^{1}H nmr ($CDCl_3$): δ 3.74 (m, 2, H5',5''), 5.24 (m, 1, H4'), 6.11 (m, 1, H2'), 6.34 (m, 1, H3'), 7.06 (m, 1, H1'), 7.32-7.94 (m, 5, Bz), 8.14 (s, 1, H2), 8.64 (s, 1, H8), 9.55 (br s, 1, NH); ms: m/z 355 (M^+ [^{35}Cl]).

A solution of **3a** (860 mg, 2.42 mmol) in saturated NH_3/MeOH (100 mL) was stirred at ambient temperature overnight and evaporated. The white solid was washed with H_2O (20 mL), filtered, and dried *in vacuo* to give **3b** (608 mg, quantitative). Chromatography (EtOH/$CHCl_3$, 1:9) gave **3b** (500 mg, 82%): mp 127-128 °C; uv: λ max 260 nm (ε 14 000), λ min 226 nm (ε 1000); ^{1}H nmr: δ 3.87 (d, *J* = 4.8 Hz, 2, H5',5''), 5.13 (m, 1, H4'), 6.27 (m, 1, H2'), 6.48 (m, 1, H3'), 6.95 (m, 1, H1'), 7.30 (br s, 2, NH_2), 8.10 (s, 1, H2), 8.16 (s, 1, H8); ms: m/z 251 (M^+ [^{35}Cl]).

Anal. Calcd. for $C_{10}H_{10}ClN_5O$ (251.7): C, 47.72; H, 4.01; N, 27.83. Found: C, 47.57; H, 4.25; N, 27.70.

9-(5-Chloro-2,3,5-trideoxy-β-D-*glycero*-pentofuranosyl)adenine (**4**).

Typical Procedure C.

A suspension of **3b** (175 mg, 0.69 mmol) and 10% Pd•C (18 mg) in MeOH (20 mL) was stirred overnight with H_2 (atmospheric pressure), filtered (glass fiber filter), and the filtrate was evaporated to give **4** (140 mg, 80%) as a white solid. Chromatography (EtOH/$CHCl_3$, 1:9) and crystallization (Et_2O/MeOH) gave **4**: mp 149-150 °C; uv: λ max 260 nm (ε 13 500), λ min 226 nm (ε 1000); ^{1}H nmr: δ 2.20 (m, 2, H3',3''), 2.50 (m, 2, H2',2''), 3.82 (m, 2,

H5',5''), 4.29 (m, 1, H4'), 6.25 (t, *J* = 6.7 Hz, 1, H1'), 8.14 (s, 1, H2), 8.30 (s, 1, H8); ms: m/z 253 (M^+ [^{35}Cl]).

Anal. Calcd. for $C_{10}H_{12}ClN_5O$ (253.7): C, 47.34; H, 4.77; N, 27.61. Found: C, 47.25; H, 4.85; N, 27.43.

9-(2,3-Dideoxy-5-*S*-methyl-5-thio-β-D-*glycero*-pent-2-enofuranosyl)adenine (**5**).

A solution of **3b** (50 mg, 0.20 mmol) and NaSMe (20 mg, 29 mmol) in DMF (1 mL) was stirred at ambient temperature for 30 minutes and evaporated. Chromatography (EtOH/$CHCl_3$, 1:9) gave **5** (52 mg, 100%) as a white powder: mp >130 °C dec; uv: λ max 260 nm (ε 14 700), λ min 227 (ε 2900); ^{1}H nmr: δ 1.99 (s, 3, SMe), 2.78 (d, 2, *J* = 6 Hz, H5',5''), 5.05 (m, 1, H4'), 6.21 (m, 1, H2'), 6.54 (m, 1, H3'), 6.93 (m, 1, H1'), 7.28 (br s, 2, NH_2), 8.16 (s, 1, H2), 8.16 (s, 1, H8); ms: m/z 263 (M^+).

Anal. Calcd. for $C_{11}H_{13}N_5OS$ (263.3): C, 50.17; H, 4.98; N, 26.60. Found: C, 50.39; H, 5.11; N, 26.35.

9-(2,3-Dideoxy-5-*S*-methyl-5-thio-β-D-*glycero*-pentofuranosyl)adenine (**6a**).

Treatment of **4** (50 mg, 0.20 mmol) with NaSMe and purification (as described for **5**) gave **6a** (52 mg, 100%) as a white powder: mp 172-173 °C; uv: λ max 260 nm (ε 14 400), λ min 226 nm (ε 3400); ^{1}H nmr: δ 2.03 (s, 3, SMe), 2.15 (m, 2, H3',3''), 2.48 (m, 2, H2',2''), 2.76 (d, *J* = 6 Hz, 2, H5',5''), 4.22 (m, 1, H4'), 6.21 (t, 1, H1'), 7.25 (br s, 2, NH_2), 8.14 (s, 1, H2), 8.29 (s, 1, H8); ms: m/z 265 (M^+).

Anal. Calcd. for $C_{11}H_{15}N_5OS$ (265.3): C, 49.79; H, 5.70; N, 26.39. Found: C, 49.76; H, 5.60; N, 26.18.

9-(5-Azido-2,3,5-trideoxy-β-D-*glycero*-pentofuranosyl)adenine (**6b**).

A suspension of **4** (75 mg, 0.30 mmol) and NaN_3 (0.20 g, 3.1 mmol) in DMF (1 mL) was heated at 90 °C for 12 hours under N_2 and evaporated. The syrup was chromatographed (EtOH/$CHCl_3$, 1:9) to give **6b** (50 mg, 65%) as a white powder. "Diffusion crystallization" (MeOH/Et_2O) gave **6b** (38 mg, 50%) as colorless crystals: mp 152.5-154 °C; uv: λ max 260 nm (ε 14 000), λ min 227 nm (ε 2500); ^{1}H nmr: δ 2.03 (m, 2, H3',3''), 2.59 (m, 2, H2',2''), 3.53 (m, 2, H5',5''), 4.26 (m, 1, H4'), 6.25 (t, *J* = 5.7 Hz, 1, H1'), 7.25 (br s, 2, NH_2), 8.14 (s, 1, H2), 8.31 (s, 1, H8); ms: m/z 260 (M^+).

Anal. Calcd. for $C_{10}H_{12}N_8O$ (260.3): C, 46.15; H, 4.65; N, 43.06. Found: C, 46.40; H, 4.73; N, 42.91.

9-(5-Amino-2,3,5-trideoxy-δ-D-*glycero*-pentofuranosyl)adenine (**6c**).

Hydrogenation of **6b** (45 mg, 0.17 mmol) by typical procedure C gave a white solid (40 mg, quantitative) that was "diffusion crystallized" (*i*-PrOH/Et_2O) to give **6c** (31 mg, 77%): mp 174-174.5 °C; uv: λ max 260 nm (ε 12 900), λ min 227 nm (ε 2100); ^{1}H nmr: δ 2.02 (m, 2, H3',3''), 2.40 (m, 2, H2',2''), 2.71 (d, *J* = 5.1 Hz, 2, H5',5''), 3.32 (br s, 2, 5'-NH_2), 4.00 (m, 1, H4'), 6.19 (dd, *J* = 6.1, 5.8 Hz, 1, H1'), 7.24 (br s, 2, 6-NH_2), 8.13 (s, 1, H2), 8.30 (s, 1, H8); ms: m/z 234 (M^+).

Anal. Calcd. for $C_{10}H_{14}N_6O$ (234.3): C, 51.27; H, 6.02; N, 35.88. Found: C, 51.40; H, 5.97; N, 35.76.

2-Amino-9-(5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-6-chloropurine (**8a**).

TBDMSCl (523 mg, 3.47 mmol) was added to a solution of **7a** (620 mg, 2.06 mmol) [27b] and imidazole (500 mg, 7.34 mmol) in dried DMF (5 mL) and stirring was continued for 18 hours at ambient temperature. Volatiles were evaporated and the residue was stirred with EtOAc, filtered, and the filter cake was washed (EtOAc). The combined filtrate was chromatographed [EtOAc/hexanes (1:4) → EtOAc] to give **8a** (763 mg, 90%) as a white solid foam of sufficient purity for further reactions. Crystallization (EtOAc/hexanes, 2:3) gave **8a** as a white powder: mp 137-139 °C; uv: λ max 310, 248 nm (ε 8000, 6900), λ min 266, 236 nm (ε 1000, 5500); ^{1}H nmr ($CDCl_3$/Me_2SO-d_6): δ -0.04, 0.05 (s, s; 3, 3; Me's), 0.81 (s, 9, *t*-Bu), 3.80 (m, 2, H5',5''), 4.06 (d, *J* = 4 Hz, 1, OH3'), 4.11 (m, 1, H4'), 4.27 (m, 1, H3'), 4.39 (m, 1, H2'), 5.02 (d, *J* = 5 Hz, 1, OH2'), 5.45 (br s, 2, NH_2), 5.90 (d, *J* = 5 Hz, 1, H1'), 8.05 (s, 1, H8); ms: m/z 415 (M^+ [^{35}Cl]).

Anal. Calcd. for $C_{16}H_{26}ClN_5O_4Si$ (415.9): C, 46.20; H, 6.30; N, 16.84. Found: C, 46.09; H, 6.30; N, 16.76.

2-Amino-9-(5-*O*-acetyl-β-D-ribofuranosyl)-6-chloropurine (**9a**).

A suspension of **10a** (46 g, 108 mmol) [27a] in MeOH (40 mL), triethylamine (345 mL), and $CHCl_3$ (115 mL) was refluxed for 5 hours. The supernatant solution was decanted from a dark-colored, oily residue and reflux was continued overnight. The resulting thick suspension was cooled and the precipitate was filtered to give **9a** (31.5 g, 85%): mp 190-191 °C; uv: λ max 309, 248 nm (ε 7900, 7300); ^{1}H nmr: δ 2.00 (s, 3, Ac), 4.05 (m, 1, H4'), 4.17 (dd, *J* = 4, 11.5 Hz, 1, H5''), 4.28 (dd, *J* = 4, 11.5 Hz, 1, H5'), 4.18 (m, 1, H3'), 4.55 (m, 1, H2'), 5.34 (d, *J* = 5 Hz, 1, OH3'), 5.60 (d, *J* = 5 Hz, 1, OH2'), 5.82 (d, *J* = 5 Hz, 1, H1'), 7.00 (br s, 2, NH_2), 8.30 (s, 1, H8); hrms: m/z 343.0678 (M^+ [$C_{12}H_{14}{}^{35}ClN_5O_5$] = 343.0683).

Anal. Calcd. for $C_{12}H_{14}ClN_5O_5$ (343.8): C, 41.93; H, 4.11; N, 20.38. Found: C, 41.66; H, 4.12; N, 20.12.

2-Amino-9-(5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-thiocarbonyl-β-D-ribofuranosyl)-6-chloropurine (**11a**).

Treatment of **8a** (456 mg, 1.1 mmol) by typical procedure A [PTCCl (2.2 mmol); cold (ice-water bath), 1 hour] and evaporation gave a residue that was chromatographed [hexanes/EtOAc (1:4) → EtOAc] to give **11a** (483 mg, 96%) as a white foam: uv: λ max 310, 238 nm (ε 8000, 20 900), λ min 266, 234 nm (ε 1100, 10 100); ^{1}H nmr ($CDCl_3$): δ -0.05, -0.02 (s, s; 3, 3; Me's), 0.79 (s, 9, *t*-Bu), 3.76 (m, 2, H5',5''), 4.62 (m, 1, H4'), 5.18 (br s, 2, NH_2), 5.60 (dd, *J* = 2.1, 2.6 Hz, 1, H3'), 6.14 (dd, *J* = 1.9, 2.1 Hz, 1, H2'), 6.20 (d, *J* = 1.9 Hz, 1, H1'), 7.90 (s, 1, H8); ms: m/z 457 (M^+ [^{35}Cl]).

Anal. Calcd. for $C_{17}H_{24}ClN_5O_4SSi$ (458.0): C, 44.58; H, 5.28; N, 15.29; S, 7.00. Found: C, 44.65; H, 5.14; N, 15.05; S, 6.98.

2-Amino-6-chloro-9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)purine (**15a**).

Treatment of **11a** (229 mg, 0.5 mmol) by typical procedure B (100 → 115 °C, 8 hours) and chromatography [hexanes/EtOAc (1:4) → EtOAc] gave **13a** (189 mg, 99%) as a white foam: ^{1}H nmr ($CDCl_3$): δ -0.02 (s, 6, Me's), 0.85 (s, 9, *t*-Bu), 3.77 (m, 2, H5',5''), 4.95 (m, 1, H4'), 5.18 (br s, 2, NH_2), 5.99 (m, 1, H2'), 6.39 (m, 1, H3'), 6.92 (m, 1, H1'), 8.00 (s, 1, H8); ms: m/z 381 (M^+ [^{35}Cl]).

TBAF/THF (1 *M*, 1 mL) was added to a solution of **13a** (330 mg, 0.86 mmol) in THF (10 mL) and stirring was continued with cooling (ice-water bath) for 10 minutes. Evaporation of volatiles and chromatography of the residue (EtOH/$CHCl_3$, 5:95) gave

15a (186 mg, 81%) as a white powder: mp 164-165 °C (lit [15] mp 167 °C); uv: λ max 308, 248 nm (ε 7900, 7300), λ min 266, 234 nm (ε 5100, 1000); ^{1}H nmr: δ 3.55 (m, 2, H5',5''), 4.90 (m, 2, H4', OH5'), 6.11 (m, 1, H2'), 6.48 (m, 1, H3'), 6.81 (m, 1, H1'), 6.98 (br s, 2, NH_2), 8.13 (s, 1, H8); ms: m/z 267 (M^+ [^{35}Cl]).

Anal. Calcd. for $C_{10}H_{10}ClN_5O_2$ (267.7): C, 44.87; H, 3.77; N, 26.16. Found: C, 45.18; H, 4.04; N, 26.03.

2-Amino-9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-6-methoxypurine (**15b**).

Method A.

A suspension of **15a** (75 mg, 0.28 mmol) and NaOMe (0.10 g, 1.9 mmol) in MeOH (1 mL) was refluxed for 5 hours, evaporated, and the residue was chromatographed (MeOH/$CHCl_3$, 1:9) to give **15b** (65 mg, 88%) as a white powder: mp 108-110 °C; uv: λ max 282, 248 nm (ε 9400, 10 200), λ min 262, 236 nm (ε 5100, 4600); ^{1}H nmr: δ 3.95 (s, 3, MeO), 3.54 (m, 2, H5',5''), 4.84 (m, 1, H4'), 4.94 (t, 1, OH5'), 6.01 (m, 1, H2'), 6.46 (m, 3, H3', NH_2), 6.78 (m, 1, H1'), 7.88 (s, 1, H8); ms: *m/z* 263 (M^+).

Anal. Calcd. for $C_{11}H_{13}N_5O_3$ (263.3): C, 50.19; H, 4.98; N, 26.60. Found: C, 49.97; H, 4.92; N, 26.80.

Method B.

A suspension of **7b** (8 g, 27 mmol) [27b] in dried pyridine (50 mL) was heated, the resulting solution was cooled to ~4 °C (ice-water bath), TBDMSCl (4.4 g, 30 mmol) was added, and stirring (with warming to ambient temperature) was continued for 5 hours. H_2O (1 mL) was added, stirring was continued for 15 minutes, and volatiles were evaporated *in vacuo*. The residue was dissolved in a minimum volume of MeOH and precipitated into H_2O (500 mL). The precipitate was filtered and washed well with H_2O and then petroleum ether to give **8b** (11.2 g, 89%) as a white powder. Crystallization (*i*-PrOH) gave **8b**: mp 185-187 °C.

KOH/H_2O (2 *M*, 30 mL) and PTCCl (4 x 1 mL aliquots) were added to a cold (~4 °C), rapidly stirred suspension of finely powdered **8b** (13 g, 31 mmol) in Et_2O/MeCN (1:1, 250 mL). A second portion of KOH/H_2O (2 *M*, 30 mL) and PTCCl (2.5 mL; total 8.0 g, 46.5 mmol) was added and stirring was continued for 15 minutes. The layers were separated and the organic phase was diluted (Et_2O, 100 mL), washed [H_2O (100 mL), $NaHCO_3$/H_2O (100 mL), and brine], concentrated *in vacuo* to ~50 mL, and slowly added to stirred petroleum ether (500 mL). The off-white precipitated **11b** (12 g, 86%) had mp 93-95 °C.

Powdered **11b** (12 g, 27 mmol) was treated by typical procedure B (115 °C, 15 hours), volatiles were evaporated *in vacuo*, and the residue was refluxed for 3 hours with NH_4F (3.9 g, 108 mmol)/MeOH (200 mL)/TEA (100 mL). Volatiles were evaporated and the residue was chromatographed [Dowex 1 x 2 (OH^-) resin, H_2O] to give **15b** (6.1 g, 85%) as a gelatinous white solid. Crystallization (THF) gave **15b** with mp 117-120 °C and spectral data as in method A.

2-Amino-9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-6-ethoxypurine (**15c**).

Treatment of **7c** (4.4 g, 14.4 mmol; prepared in 87% yield by treatment of **7a** [27b] with NaOEt; spectral data as reported [29]) with TBDMSCl (2.38 g, 22 mmol) [as described for **15b** (method B; 15 hours, ~4 °C); the residue was extracted ($CHCl_3$, 700 mL), washed ($NaHCO_3$/H_2O and brine), dried (Na_2SO_4), and chromatographed (MeOH/$CHCl_3$, 1:9)] gave **8c** (5.2 g, 85%). Crystallization (*i*-PrOH) gave **8c** with mp 140-143 °C. Amorphous **8c** (5.2 g, 12.2 mmol) was treated by typical procedure A [PTCCl (24 mmol) and chromatography (MeOH/$CHCl_3$, 5:95) to give **11c** (4.25, 81%)] and **11c** was treated by typical procedure B (115 °C, 8 hours). The reaction mixture was concentrated *in vacuo* and the residue was stirred with TBAF/THF (1 *M*; 10 mL, 10 mmol) at ambient temperature for 15 hours. Volatiles were evaporated *in vacuo*, the residue was dissolved (H_2O) and chromatographed [Dowex 1 x 2 (OH^-), H_2O] to give **15c** (2.4 g, 89%): mp 105-106 °C; uv: λ max 281, 247 nm (ε 8000, 8300); ^{1}H nmr: δ 1.35 (t, *J* = 7.0 Hz, 3, Et), 3.55 (m, 2, H5',5''), 4.45 (q, *J* = 7.0 Hz, 2, Et), 4.85 (m, 1, H4'), 4.95 (t, *J* = 5 Hz, 1, OH5'), 6.10 (dt, *J* = 1.5, 6.0 Hz, 1, H2'), 6.42 (m, 1, H3'), 6.42 (br s, 2, NH_2), 6.78 (m, 1, H1'), 7.90 (s, 1, H8); hrms: m/z 277.1175 (M^+ [$C_{12}H_{15}N_5O_3$] = 277.1175).

2,6-Diamino-9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-purine (**15d**).

A solution of **15a** (85 mg, 0.31 mmol) in saturated NH_3/MeOH (8 mL) was heated at 90 °C (oil bath) for 36 hours in a steel pressure vessel. The mixture was cooled, evaporated, and the residue was chromatographed (EtOH/$CHCl_3$, 5:95) to give **15d** (37 mg, 47%) with nmr spectral data as reported [18b]: mp softening from 135 °C, dec (lit [18b] mp 168-169 °C); uv: λ max 282, 256 nm (ε 9500, 8600), λ min 266, 237 nm (ε 6900, 5300); ms: m/z 248 (M^+).

Anal. Calcd. for $C_{10}H_{12}N_6O_2$ (248.2): C, 48.38; H, 4.87; N, 33.85. Found: C, 48.38; H, 4.54; N, 33.87.

2-Amino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-6-methoxypurine (**16b**).

Method A.

Hydrogenation of **15b** (100 mg, 0.380 mmol) by typical procedure C (6 hours) gave a syrup that was refrigerated (~5 °C). The resulting crystals were washed with cold H_2O, filtered, and dried (P_4O_{10}) *in vacuo* at 35 °C for 3 days to give **16b** (53 mg, 53%) as its hemihydrate with nmr spectral data as reported [18b]: mp softening from 65 °C, dec (lit [18b mp 76-78 °C; uv: λ max 282, 248 nm (ε 9200, 9500), λ min 261, 226 nm (ε 4600, 4000); ms: m/z 265 (M+).

Anal. Calcd. for $C_{11}H_{15}N_5O_3$•0.5 H_2O (274.3): C, 48.17; H, 5.88; N, 25.53. Found: C, 48.48; H, 5.95; N, 25.76.

Method B.

PTCCl (14 mL) was added to **9a** (35 g, 102 mmol) and DMAP (17 g, 142.8 mmol) in MeCN (250 mL) and the mixture was stirred at ~4 °C for 1 hour. TEA (25 mL, 102 mmol) and PTCCl (7 mL, 51 mmol) were added and stirring was continued for 30 minutes. EtOAc (250 mL) was added and the solution was washed [0.5 *M* HCl/H_2O (3 x 100 mL), $NaHCO_3$/H_2O, and brine], dried (Na_2SO_4), and concentrated to ~50 mL. Slow addition of this solution to vigorously stirred hexane (500 mL) caused separation of a precipitate that was filtered to give **12a** (33.5 g, 85%) as a white powder: mp 207-208 °C.

Treatment of **12a** (29 g, 75 mmol) by typical procedure B (115 °C, 5 hours) gave **14a** that was stirred overnight with NaOMe/MeOH (1 *M*, 200 mL). The solution was evaporated and the residue was dissolved (H_2O), washed with hexane (3 x 100 mL), and chromatographed [Dowex 1 x 2 (OH^-), H_2O] to give a gelatinous solid that was subjected to repeated additions and evaporations of MeOH/*i*-PrOH/$PhCH_3$ until a clear distillate was produced. The solid was then hydrogenated by procedure C [10% Pd•C (700 mg), 3 hours] and the product was crystallized (H_2O) to

give **16b** (10 g, 51%) as a white powder: mp 76-78 °C; hrms: m/z 265.1171 (M^+ [$C_{11}H_{15}N_5O_3$] = 265.1172.

Anal. Calcd. for $C_{11}H_{15}N_5O_3 \cdot H_2O$ (283.3): C, 46.64; H, 6.05; N, 24.75. Found: C, 46.86; H, 5.86; N, 24.49.

2-Amino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-6-ethoxypurine (**16c**).

Hydrogenation of **15c** (2.4 g, 8.6 mmol) by typical procedure C (EtOH, 1 hour) and chromatography [Dowex 1 x 2 (OH^-), H_2O] gave **16c** (1.4 g, 58%). Crystallization (H_2O) gave **16c**: mp 108-110 °C (lit [13b] mp 150 °C); hrms: m/z 279.1332 (M^+ [$C_{12}H_{17}N_5O_3$] = 279.1332).

Anal. Calcd. for $C_{12}H_{17}N_5O_3$ (279.3): C, 51.60; H, 6.09; N, 25.08. Found: C, 51.10; H, 5.78; N, 24.72.

2,6-Diamino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)purine (**16d**).

Hydrogenation of **15d** (25 mg, 0.097 mmol) by typical procedure C (24 hours) gave **16d** (25 mg, 99%) as a white powder with nmr spectral data as reported [18b]: mp 173-174 °C (lit [18b] mp 194-195 °C); uv: λ max 282, 256 nm (ε 9500, 8400), λ min 266, 237 nm (ε 6400, 5400); ms: m/z 250 (M^+).

Anal. Calcd. for $C_{10}H_{14}N_6O_2 \cdot 0.5\ H_2O$ (259.3): C, 46.33; H, 5.83; N, 32.41. Found: C, 46.70; H, 5.50; N, 32.40.

2-Amino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-6-isopropoxypurine (**16f**).

Typical Procedure D.

A solution of **16a** (100 mg, 0.37 mmol) [18b] in NaO*i*-Pr/*i*-PrOH [prepared from sodium (0.5 g) and *i*-PrOH (20 mL)] was stirred overnight and tlc indicated complete conversion to **16f**. Volatiles were evaporated, the residue was extracted (EtOAc), and the combined extract was evaporated *in vacuo* to give **16f** (100 mg, 92%) as a syrup which resisted crystallization: ^{1}H nmr: δ 1.32 (d, *J* = 6.0 Hz, 6, 2 x CH_3), 2.00 (m, 2, H3',3"), 2.37 (m, 2, H2',2"), 3.50 (m, 1, H5"), 3.62 (m, 1, H5'), 4.09 (m, 1, H4'), 4.95 (t, *J* = 5.5 Hz, 1, OH5'), 5.48 (m, 1, CH), 6.07 (dd, *J* = 3.5, 6.5 Hz, 1, H1'), 6.39 (br s, 2, NH_2), 8.10 (s, 1, H8); hrms: m/z 293.1487 (M^+ [$C_{13}H_{19}N_5O_3$] = 293.1492).

Analogous treatment of **16a** [18b] with NaOMe/MeOH, NaOEt/EtOH, and NaOPr/PrOH gave **16b** (59%) [18b], **16c** (80%) [13b], and **16e** (88%) [13b], respectively (quantitative conversions, yields after recrystallizations).

2-Amino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)purine-6-thione (**16g**).

A solution of NaOMe [prepared from sodium (50 mg) and anhydrous MeOH (50 mL)] was saturated with H_2S (15 minutes). Compound **16a** (268 mg, 1 mmol) [18b] was added and H_2S was bubbled through the solution. After 7 hours of reflux, the flow of H_2S was stopped, reflux was continued overnight, and volatiles were evaporated. The residue was partitioned (EtOAc/H_2O) and the aqueous layer was washed with EtOAc (2 x 50 mL) and carefully neutralized (dilute AcOH/H_2O). Separation of **16g** (160 mg, 60%) as yellow powder with mp 200 °C, dec (lit [10a] mp 201-203 °C) and spectral data as reported [10a] occurred upon concentration of the aqueous phase.

2-Amino-6-methylthio-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)purine (**16h**).

Typical Procedure E.

A solution of dimethyl disulfide (1 mL, 11 mmol) in THF (25 mL) and CH_3CN (10 mL) was treated with sodium (130 mg) and heated to reflux until all of the sodium had reacted. The suspension was cooled to ambient temperature, **16a** (200 mg, 0.66 mmol) [18b] was added, and the suspension was stirred for 2 hours. H_2O (50 mL) was added and the solution was carefully neutralized (dilute AcOH/H_2O) and extracted with EtOAc (3 x 50 mL). The combined organic phase was washed ($NaHCO_3$/H_2O, brine), dried (Na_2SO_4), and evaporated, and the residue was column chromatographed (10% MeOH/$CHCl_3$) and crystallized (THF/Et_2O) to give off-white needle clusters of **16h** (150 mg, 69%) [17]: mp 93-94 °C; hrms: m/z 281.0946 (M^+ [$C_{11}H_{15}N_5O_2S$] = 281.0952).

Analogous treatment of **16a** [18b] with diethyl disulfide, and crystallization of the product (THF/toluene) gave **16i** (63%): mp 74-76 °C; ^{1}H nmr: δ 1.32 (t, *J* = 7.5 Hz, 3, CH_3), 2.02 (m, 2, H3',3"), 2.35 (m, 2, H2',2"), 3.25 (q, *J* = 7.5 Hz, 2, CH_2), 3.50 (m, 1, H5"), 3.60 (m, 1, H5'), 3.60 (m, 1, H5'), 4.18 (m, 1, H4'), 4.95 (t, *J* = 5.5 Hz, 1, OH5'), 6.19 (dd, *J* = 4.0, 6.5 Hz, 1, H1'), 6.5 (br s, 2, NH_2), 8.18 (s, 1, H8); hrms: m/z 295.1099 (M^+ [$C_{12}H_{17}N_5O_2S$] = 295.1112).

2-Amino-6-*N*-methylamino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)purine (**16j**).

Typical Procedure F.

Compound **16a** (100 mg, 0.37 mmol) [18b] was treated with 40% $MeNH_2$/H_2O (10 mL) in a stirred pressure vessel at 80 °C for 12 hours. Volatiles were evaporated and the residue was purified (preparative tlc) and crystallized (THF/toluene) to give **16j** (65 mg, 64%): mp 163-165 °C {lit [13b] mp 95 °C (hydrate)}.

Anal. Calcd. for $C_{11}H_{16}N_6O_2$ (264.3): C, 50.00; H, 6.10; N, 31.80. Found: C, 49.90, H, 5.88; N, 31.40.

Analogous treatment of **16a** [18b] with 30% $EtNH_2$/MeOH and crystallization (H_2O) gave **16k** (49%) (mp 117-119 °C).

Anal. Calcd. for $C_{12}H_{18}N_6O_2$ (278.3): C, 51.77; H, 5.62, N, 30.21. Found: C, 52.03; H, 5.66; N, 29.70.

Analogous treatment of **16a** [18b] with 40% Me_2NH/H_2O and crystallization (THF/Et_2O) gave **16l** (44%) [10b]: mp 64-67 °C; hrms: m/z: 278.1490 (M^+ [$C_{12}H_{18}N_6O_2$] = 278.1491).

2-Amino-6-fluoro-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-purine (**16m**).

A suspension of **16a** (200 mg, 0.7 mmol) [18b], anhydrous KF (1.2 g), and Me_3N (0.5 mL) in DMF (20 mL) was stirred at ambient temperature for 18 hours. Volatiles were evaporated and the residue was extracted (EtOAc). The extract was concentrated and chromatographed on silica (4% MeOH/$CHCl_3$) and the residue was crystallized (H_2O) to give **16m** (85 mg, 45%): mp 109 °C, dec (lit [10a] mp 138-140 °C).

Anal. Calcd. for $C_{10}H_{12}FN_5O_2 \cdot H_2O$ (271.3): C, 44.30; H, 5.20; N, 25.83. Found: C, 44.33; H, 4.70; N, 25.66.

6-Amino-2-methoxy-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)purine (**21b**).

Treatment of **21a** (150 mg, 0.59 mmol) [18b] with NaOMe/MeOH (1 *M*, 15 mL) at ambient temperature for 24 hours, evaporation of volatiles, and purification of the residue [Dowex 1 x 2 (OH^-), MeOH/H_2O] gave **21b** (55 mg, 35%): mp 164-166 °C; hrms: m/z 265.1162 (M^+ [$C_{11}H_{15}N_5O_3$] = 265.1172).

Anal. Calcd. for $C_{11}H_{15}N_5O_3$ (265.3): C, 49.81; H, 5.66; N, 26.41. Found: C, 49.34; H, 5.46; N, 26.14.

6-Amino-2-*N*-methylamino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)purine (**21c**).

Typical Procedure G.

A solution of **21a** (78 mg, 0.31 mmol) [18b] in 40% $MeNH_2/H_2O$ was stirred at ambient temperature for 24 hours. Evaporation of volatiles and purification of the residue (preparative tlc) gave **21c** (52 mg, 81%): mp 165-167 °C; hrms: m/z 264.1335 (M^+ [$C_{11}H_{16}N_6O_2$] = 264.1335).

Anal. Calcd. for $C_{11}H_{16}N_6O_2$ (264.3): C, 50.00; H, 6.10; N, 31.80. Found: C, 49.90; H, 5.88; N, 31.40.

Analogous treatment of **21a** [18b] with 30% $EtNH_2$/MeOH gave **21d** (48%): mp 213-215 °C; hrms: m/z 278.1490 (M^+ [$C_{12}H_{18}N_6O$] = 278.1491).

Analogous treatment of **21a** [18b] with NaSMe/MeOH [prepared from dimethyl disulfide and NaOMe/MeOH (pH ~14); see typical procedure E (ambient temperature, 30 minutes)] gave **21e** [14] (10%, isolated from a mixture of **21b** and **21e** by preparative tlc).

Cytotoxicity Assays.

Cytotoxic potencies of the dideoxynucleosides were determined with the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay of Mosmann [33], as described by Alley *et al*. [34]. Briefly, microcultures of exponentially proliferating CCRF-CEM or HL-60 cells (plated at 8 x 10^3 cells/well or 1.6 x 10^4 cells/well, respectively) in RPMI 1640 medium containing 2 m*M* HEPES, pH 7.4, and 10% (CCRF-CEM cells) or 15% (HL-60 cells) fetal bovine serum were treated with graded concentrations of the test compounds in 96-well microtiter plates. The microcultures were incubated for 48 hours at 37 °C in a humidified atmosphere of 5% CO_2 in air. MTT solution (30 μg in 30 μL) was added to each microwell and the cultures were incubated for a further 2 hours. The plates were centrifuged and supernatants were removed by aspiration. The MTT formazan crystals were dissolved in dimethylsulfoxide (spectrophotometric grade, Aldrich) and absorbances of the microwell contents were measured at 540 nm in a microplate reader. Cell viability (proportional to A_{540} values) in the test microwells was expressed as a percentage of the A_{540} values in control wells to which no test compounds were added, and plotted against the log of the concentration of test compound to yield concentration-effect curves, from which IC_{50} values were obtained.

Substrate Activity with Adenosine Deaminase.

Adenosine deaminase-mediated hydrolysis of 2-amino-6-(substituted)purine 2',3'-dideoxynucleosides was determined by spectrophotometric assays in which time courses of formation of the product, 2',3'-dideoxyguanosine, were measured as the increase in A_{255} of assay solutions. Kinetic constants for the interaction of test compounds with adenosine deaminase from calf intestine (Sigma A-1155, 160-194 U/mg protein) were determined at 22 °C in solutions (1.0 ml) containing graded concentrations (10 μ*M* - 200 μ*M*) of test compounds in 50 m*M* sodium phosphate buffer, pH 7.5, to which the enzyme (final concentration, 0.06-0.9 U/mL) was added to begin the reactions. Initial rates of hydrolysis of the 6-substituents were determined from the initial, linear portions of progress curves. Kinetic constants were determined by fitting the Michaelis-Menten equation to initial rate data with the computer program "Enzfitter." Deamination of adenosine was measured for comparison.

Compounds also were tested as substrates for adenosine deaminase in supernatant fractions from adult Pekin duck erythrocytes and liver cells. Duck blood was collected into heparin and centrifuged to recover the erythrocyte fraction, which was washed in 0.9% $NaCl/H_2O$ solution. The packed, washed cells were lysed in distilled water at 4 °C and centrifuged (39,000 x *g*, 45 min, 4 °C). This supernatant was stored in small portions at -70 °C until use. A duck hepatocyte suspension was prepared from the collagenase-perfused liver of an adult Pekin duck. The dissociated cell preparation was washed (3 x) with 0.9% $NaCl/H_2O$ solution, and the cell pellet was lysed by additon of 4 volumes of distilled water and sonication at 4 °C. The broken cell preparation was centrifuged (39,000 x *g*, 45 min, 4 °C), and the supernatant was stored at -70 °C until use. Initial rates of 6-substituent hydrolysis were determined by measuring time courses of the increase in A_{255} (**16**-series compounds) or the increase in A_{250} (adenosine) at 22 °C in assay solutions (1.0 mL) that contained 50 μ*M* test compound, 20 μL (erythrocyte supernatant, 980 μg protein) or 50 μL (liver supernatant, 190 μg protein) of cell supernatant, in 50 m*M* sodium phosphate buffer (pH 7.5). Protein was determined by the Peterson modification [35] of the method of Lowry with bovine serum albumin as standard.

Duck Hepatitis B Activity Assays.

The 2',3'-dideoxynucleosides were evaluated for inhibition of DHBV in primary Pekin duck hepatocytes. Pekin ducks congenitally infected with DHBV were raised at the University of Alberta. Primary cultures of duck hepatocytes were prepared as previously described [36]. Cells were cultured in 60-mm cell culture dishes in L-15 medium, 5% fetal calf serum, 50 IU/mL penicillin G, 10 μg/mL streptomycin, and 25 units/mL of nystatin at 37 °C. Nucleosides were added at 0.1, 1, 10, and 100 μ*M* on day 2 and replenished in fresh medium every second day until the cultures were harvested on day 16.

Cellular and viral DNA preparations were performed as previously described [21]. Cells were lysed with a buffer containing 0.2% sodium dodecyl sulfate, 100 m*M* Tris hydrochloride (pH 8.0), 10 m*M* EDTA, 5 m*M* EGTA, and 150 m*M* NaCl. The cell lysate was digested with 0.5 mg of pronase E (Sigma Chemical Co.) per mL at 37 °C for 1.5 hours. The contents were extracted with an equal volume of phenol saturated with 20 m*M* Tris hydrochloride (pH 7.5), 0.5 m*M* EDTA and 0.1% 8-hydroxyquinoline. Concentrated ammonium acetate (2 *M*, pH 7.0) was added to the aqueous phase to yield a final concentration of 0.2 *M* ammonium acetate. The nucleic acids were precipitated with 2 volumes of ethanol, pelleted, washed with ethanol, and dried. The DNA was dissolved in 10 m*M* Tris hydrochloride (pH 7.5), EDTA (1 m*M*).

DNA samples were spotted on nylon (Hybond-N: Amersham Co.) filtered with a Bio-Dot micro filtration apparatus. The dot-blot assay was performed as described previously [19b] using a ^{32}P-labeled DHBV probe prepared by nick translation of the *Escherichia coli* plasmid containing DHBV (pDH010-DHBV) which was a generous gift from Dr. J. W. Summers.

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