Synthesis and Antiviral Evaluation of Certain Novel Pyrazinoic Acid *C*-Nucleosides

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Pyrazine (1,4-diazine) *C*-nucleosides constitute a rare class of nucleic acid analogues that has only recently been reported in the literature. As part of our ongoing investigation into the synthesis and reactivity of these compounds, we have developed an electrophilic esterification of a lithiated pyrazine *C*-nucleoside (**1**) to give, following deprotection, the versatile intermediate ethyl 3,5-dichloro-6-(β-D-ribofuranosyl)pyrazine-2-carboxylate (**4**). This intermediate was subjected to a variety of reaction conditions to generate a series of pyrazinoic acid *C*-nucleosides. These compounds, along with 3,5-dichloro-2-(β-D-ribofuranosyl)pyrazine (**2**) and **4**, were evaluated for antiviral activity and cytotoxicity. No significant activity was observed for compounds **2** and **5**−**9**, but **4** was active against two herpes viruses and cytotoxic in the micromolar range.

Introduction

Nucleosides possess a broad spectrum of biological functions, ranging from their primary roles as building blocks in the genetic code to other functions such as biosynthetic intermediates, energy donors, metabolic regulators, and cofactors in enzymatic processes. Because of this, nucleosides and their synthetic analogues have generated considerable scientific interest in their chemistry and biology.[1] Although *N*-nucleosides (in which the glycosidic bond is a C1′−N linkage) are the most abundant and therefore the most studied group of analogues, there is also a group of naturally occurring nucleosides in which the glycosidic bond is formed via a C1′−C linkage.[2] Within both classes of naturally occurring nucleosides are compounds which possess a carboxylic acid derivative (i.e., carboxylic acid, ester, amide, or nitrile) residing meta to the site of glycosylation. Many of these compounds, which have been isolated from various organisms, possess very interesting biological properties; e.g., compounds such as bredinin,[3] clitidine,[4] and the *C*-nucleoside pyrazofurin[5−7] are some examples of biologically active nucleoside carboxylic acid derivatives.

A number of synthetic analogues which are structurally related to these naturally occurring compounds have been synthesized and reported to possess significant biological properties, e.g., ribavirin,[8] tiazofurin,[9] and selenazofurin.[10,11] The *C*-nucleoside 3-(β-D-ribofuranosyl)benzamide has been prepared and shown to exhibit cytotoxicity in the nanomolar range to S49.1 lymphoma cells and leukemia K562 cells.[12]

Until very recently,[13,14] there had been no reports in the literature on the synthesis of pyrazine (1,4-diazine) *C*-nucleosides nor on their potential chemotherapeutic properties. We have published preliminary accounts of two convergent synthetic routes that lead to 2′-deoxypyrazine *C*-nucleosides[14a] and pyrazine *C*-ribosides[14b] efficiently and in good yields. On the basis of this work, we were interested in synthesizing some structurally related compounds for biological evaluation. One of the published ribosides, 3,5-dichloro-2-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrazine (**1**),[14b] was envisaged as a very versatile compound if a carboxylic acid derivative (i.e., acid, ester, amide, or nitrile) could be introduced at the unoccupied position on the pyrazine ring. This modification would provide a nucleoside that could lead to a variety of functionalized pyrazinoic acid *C*-nucleosides. We now report the synthesis of such pyrazinoic acid *C*-nucleosides and provide the preliminary evaluation for antiviral activity and cytotoxicity of these new target compounds.

Results and Discussion

Chemistry. Previous experience in our laboratories[15] and others[16] had established that trisubstituted pyrazines could be directly lithiated using nonnucleophilic lithium dialkylamides at low temperature. These lithiated pyrazines were reacted with various electrophilic reagents to introduce groups at the unoccupied position on the pyrazine ring. For our current investigation, these groups would ideally be a carboxylic acid derivative that could readily be converted to other functional groups at a subsequent stage of the synthetic route. We also planned to effect a nucleophilic aromatic substitution of the chloro groups at a later stage in the synthetic sequence.

Therefore, we needed a reagent that would efficiently provide a pyrazinoic acid derivative from a lithiopyrazine with a minimal amount of undesired side reactions. A search of the literature revealed only one instance where a direct formation of pyrazinoic acid derivatives had occurred via a substituted lithiopyrazine. Qué-

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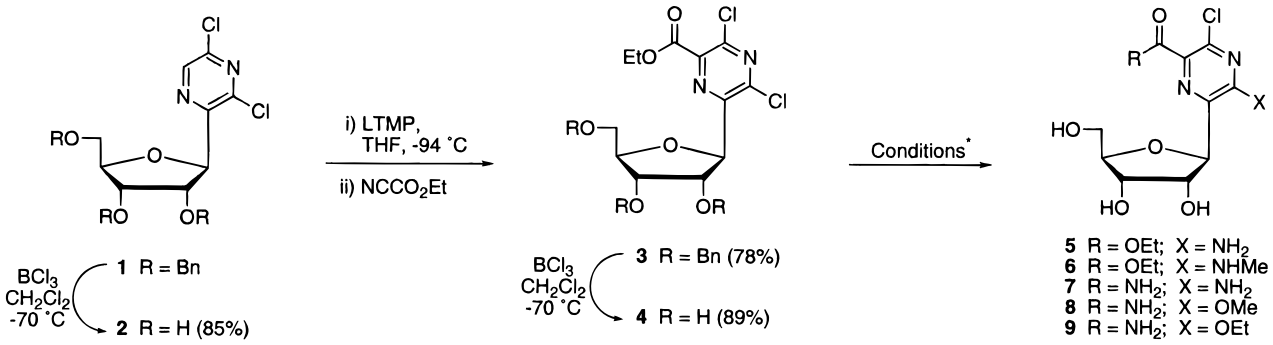
Scheme 1[a]



[a] Conditions: (A) 2 equiv of NH_3/1,4-dioxane, 40 °C, 42 h; (B) 2 equiv of $MeNH_2$/THF, rt, 30 min; (C) satd NH_3/MeOH, 50 °C, 20 h; (D) satd NH_3/MeOH, MeOH, rt, 48 h; (E) satd NH_3/EtOH, 4 °C, 48 h; (F) satd NH_3/EtOH, 20 °C, 70 h.

guiner and co-workers had been successful in reacting 3-chloro-2-lithiopyrazine with carbon dioxide to obtain 3-chloropyrazinoic acid (30% yield).[16] Numerous reagents are known to react with organolithium species to give carboxylic acid derivatives, and we initially felt that this would be a straightforward process to obtain our target compounds. However, lithiation of **1** followed by treatment with carbon dioxide[17] (solid or gaseous) unfortunately resulted in a rapid decomposition of the starting material. We then investigated the synthesis of pyrazine esters using alkyl chloroformates or alkyl carbonates. In all instances, the organolithium intermediate was destroyed rapidly upon the addition of an electrophile and only **1** was isolated from the reaction mixture. Model studies in our laboratories with alkyl isocyanates had indicated that these reagents would react with the lithiopyrazines, but all attempts to cleave the amide/alkyl bond via acidic hydrolysis ultimately resulted in complete destruction of the compound.[18] Parker reported that the reaction of trimethylsilyl isocyanate with organomagnesium compounds can lead to the isolation of a free carboxamide upon aqueous workup and hydrolysis of the silylated amide product.[19] Our use of this reagent at −94 °C resulted only in the isolation of a product that was silylated on the heterocycle. This finding was not contrary to literature reports since it has been noted that mixtures of amides and silyl products are produced unless the temperature of the reaction is maintained between 85 and 95 °C.[19] Due to the instability of the lithiopyrazine intermediate, we could not raise the temperature of our reaction this high. Even at −78 °C, a large amount of highly colored, uncharacterizable material was formed and yields of the product were low. The use of chloroacetyl isocyanate to give free amides has been reported,[19] but in our hands only decomposition products were observed when this reagent was used. Other electrophiles, e.g., chlorosulfonyl isocyanate (CSI)[20] and urethane (ethyl carbamate),[21] also did not provide the desired products under these conditions.

Mander and co-workers have demonstrated that alkyl cyanoformates are useful reagents to effect *C*-alkylation of lithium enolates preferential to *O*-alkylation.[22] Others have successfully used these reagents to form heterocyclic alkyl esters.[23] We found that the treatment of **1** with lithium 2,2,6,6-tetramethylpiperidide (LTMP) at low temperature followed by quenching with 5 equiv of ethyl cyanoformate gave, after aqueous workup and chromatographic purification, the protected 6-(β-D-ribofuranosyl)pyrazine-2-carboxylate **3** in a 78% yield (Scheme 1). Debenzylation of **3** using boron trichloride yielded ethyl 3,5-dichloro-6-(β-D-ribofuranosyl)pyrazine-2-carboxylate (**4**) in an 89% yield. The preparation of **4** was achieved in a 50% overall yield from the commercially available 2,6-dichloropyrazine.[14b] This method compares very well to any nucleoside preparation in the literature, emphasizes the utility of a convergent approach to *C*-nucleosides, and provides a versatile intermediate from which a variety of analogues could be prepared.

Table 1. Reactivity of Nucleoside **4** toward Nucleophiles

product	conditions[a]	yield[b]
5	A	59
	E	38
	F	30
6	B	57
7	C	23
	E	35
	F	17
8	D	30
9	F	12

[a] See Scheme 1 for reaction conditions. [b] Percent (%) isolated product.

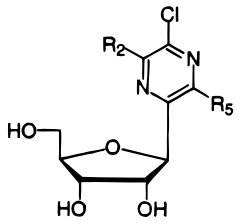
In addition to the reactive carbonyl center of the ethyl ester, **4** also possesses two chloro groups that could be activated toward nucleophilic aromatic displacement due to the ester functionality ortho and para to these halogens. In order to determine the order and reactivity of these sites to nucleophilic addition, we chose to treat **4** with 2 equiv of ammonia in the aprotic solvent 1,4-dioxane (condition A). After 42 h at 40 °C, TLC analysis indicated a complete consumption of the starting material and the formation of only one product. This compound was isolated, purified by silica gel chromatography, and identified as ethyl 5-amino-3-chloro-6-(β-D-ribofuranosyl)pyrazine-2-carboxylate (**5**) (Table 1). The ^1H NMR spectrum of **5** showed the presence of an aromatic amino functionality (as a D_2O exchangeable broad singlet centered at δ 7.65) as well as showed that the ethyl ester was still intact. The site of nucleophilic aromatic displacement was unequivocally proven to occur at the 5-position (para to the carboxylate) by a single-crystal X-ray structural determination of compound **5**.[24] It is interesting to note that under these conditions (where the amount of nucleophile may be controlled), the order of reactivity is very selective. For example, treatment of **4** with 2 equiv of the more nucleophilic reagent methylamine in tetrahydrofuran

at room temperature (condition B) led exclusively to the formation of ethyl 3-chloro-5-(methylamino)-6-(β-D-ribofuranosyl)pyrazine-2-carboxylate (**6**) in which the 5-chloro group had also been preferentially displaced by the methylamino group. In an attempt to synthesize substituted pyrazinamide ribosides from **4**, we treated **4** with a saturated solution of ammonia in methanol (condition C). At room temperature, TLC analysis of the reaction after 1 h revealed the formation of several products as well as a sizable amount of unreacted starting material. Upon heating the reaction for 20 h at 50 °C, TLC analysis showed only one product along with some highly colored, unidentifiable baseline material. Upon isolation of the product, it was determined that ammonolysis of the ethyl ester had been achieved as well as a nucleophilic substitution of the 5-chloro substituent by ammonia to give 5-amino-3-chloro-6-(β-D-ribofuranosyl)pyrazine-2-carboxamide (**7**). Compound **1** was isolated in only a 23% yield via this route.

As stated above, TLC analysis of the reaction after 1 h had shown the presence of more than one product (or intermediate) being formed. To isolate and characterize these other products, **4** was dissolved in methanol and treated with saturated methanolic ammonia (condition D) at room temperature. After 24 h, TLC analysis showed that all of **4** had been consumed and that only one major product had been formed. Isolation and preliminary identification of this compound showed that transesterification had occurred to give the methyl carboxylate and that a nucleophilic substitution of the 5-chloro substituent by a methoxy group had occurred. The yield of 3-chloro-5-methoxy-6-(β-D-ribofuranosyl)-pyrazine-2-carboxamide (**8**) was 30% overall. Under these conditions, the formation of **7** was not observed. The reaction of **4** in saturated methanolic ammonia for 2 days at room temperature resulted in the formation and isolation of both **7** and **8**. No evidence of the amino ester compound **5** was observed. It is assumed that an amino ester compound must be an intermediate in the formation of **7** via a nucleophilic displacement of the 5-chloro group by ammonia followed by ammonolysis of the ester. The reaction of **4** in ethanolic ammonia at 4 °C for 2 days (condition E) resulted in the formation of **5** and **7**. There was no evidence that a displacement of the 5-chloro group by ethanol to give **9** had occurred. Raising the temperature of this reaction to 20 °C (condition F) resulted in the isolation of not only **5** and **7** but also 3-chloro-5-ethoxy-6-(β-D-ribofuranosyl)pyrazine-2-carboxamide (**9**) in a 12% yield. These results suggest that under more kinetic conditions ammonia is the only viable nucleophile, while raising the temperature results in a competition between ammonia and the alcohol (or its conjugate base). Thus, from the versatile intermediate **4**, a series of novel substituted pyrazinoic acid *C*-ribosides was prepared quite readily.

Biological Evaluation. The capacity of the compounds to inhibit the replication of human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1) was examined in an initial study of their antiviral effects. To determine if the compounds had specific antiviral activity, the cytotoxicities of compounds were investigated in uninfected stationary human foreskin fibroblasts (HFF cells) and growing KB cells (Table 2). The two dichloro-substituted compounds (**2**, **4**) were active against HCMV, but the activity of compound **4** against both viruses occurred at cytotoxic concentrations. Although the activity of **2** was weak, it did occur at noncytotoxic concentrations suggesting some possibilities for additional synthesis. None of the compounds with other substituents in the 5-position (**5**−**9**) were active against either virus.

Table 2. Antiviral Activity and Cytotoxicity of Pyrazine *C*-Nucleosides



			50% inhibitory concentration (μM)			
			antiviral activity		cytotoxicity[c]	
	substituent		HCMV,[a] plaque	HSV-1,[b] ELISA	HFF cells, visual	KB cells, growth
compd	R_2	R_5				
2	H	Cl	70		>100	
4	CO_2Et	Cl	3.2	20	3.2	20
5	CO_2Et	NH_2	>100	>100	>100	>100
6	CO_2Et	NHMe	>100	>100	>100	>100
7	$CONH_2$	NH_2	>100	>100	>100	>100
8	$CONH_2$	OMe	>100	>100	>100	>100
9	$CONH_2$	OEt	>100	>100	>100	>100
ganciclovir (DHPG)			7.7[d]	1.8[e]	>100[d]	>100[e]

[a] Plaque reduction assays were performed in duplicate wells as described in the text. [b] Assayed by ELISA in quadruplicate wells. [c] Visual cytotoxicity scored on HFF cells at time of HCMV plaque enumeration. Inhibition of KB cell growth was determined as described in the text in quadruplicate wells. [d] Average of 88 experiments in which DHPG was used as a positive control. [e] Average from 2−4 separate experiments.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and used as provided. Dichloromethane (phosphorous pentoxide) and tetrahydrofuran (sodium/benzophenone) were distilled from the indicated drying agent and stored over activated 4-Å molecular sieves under a positive pressure of argon prior to use (if not used immediately). The phrase "evaporated in vacuo" is meant to imply the use of a rotary evaporator with a bath temperature not exceeding 40 °C using a water aspirator. Thin-layer chromatography (TLC) was carried out on Analtech 60F-254 silica gel plates, and detection of components on TLC was made by UV light absorption at 254 or 365 nm, staining with iodine vapor, or heating to a char following treatment with 10% sulfuric acid in methanol. Solvent systems are expressed as a percentage of the more polar component with respect to total volume (v/v %). Mallinckrodt SilicAR 230−400 mesh (40−63 μm) was used for chromatography, which was carried out utilizing Ace Glass Michel-Miller columns. A Rainin Rabbit HPX pump was used for solvent delivery. UV light-active product-containing fractions were detected on an Isco V^4 UV detector and collected by an Isco Foxy fraction collector. Flow rates, sample loading, and fraction size were determined using the guidelines outlined by Still and co-workers.[25] Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. The ^{1}H (300 or 360 MHz) and ^{13}C (90 or 125 MHz) NMR spectra were recorded on Bruker instruments. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard for ^{1}H NMR and relative to the standard chemical shift of the solvent for ^{13}C NMR. Mass spectroscopy was performed by The

University of Michigan Chemistry Department. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ, or by The University of Michigan Chemistry Department. The presence of solvent as indicated by analysis was always confirmed by ^{1}H NMR spectroscopy.

3,5-Dichloro-2-(β-D-ribofuranosyl)pyrazine (2). A flame-dried, evacuated, 100-mL round-bottom flask was charged, under argon, with **1**[14b] (550 mg, 1.0 mmol). This was dissolved in dry dichloromethane (30 mL) and cooled to −70 °C. To this solution was added boron trichloride (1.0 M solution in dichloromethane, 10.0 mL, 10.0 mmol) dropwise, and stirring was continued at −70 °C for 120 min. The reaction was then warmed to room temperature and stirred for an additional 60 min. The excess boron trichloride was then quenched with methanol/dichloromethane (1:1, 20 mL), and the solution was adjusted to pH 7 with 2 N aqueous ammonium hydroxide. A minimal amount of silica gel was added and the solvent evaporated. The compound (absorbed on silica gel) was subjected to column chromatography (SiO_2, 40 × 150 mm, 5% methanol/chloroform, R_f = 0.25) to give, following solvent evaporation, **2** (240 mg, 85%) as a white solid: mp 132−132.5 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.87 (s, 1), 5.19 (d, 1, J = 5.7 Hz), 5.10 (d, 1, J = 5.1 Hz, D_2O exchangeable), 5.03 (d, 1, J = 5.6 Hz, D_2O exchangeable), 4.65 (pt, 1, J = 5.3, 6.1 Hz, D_2O exchangeable), 4.30 (dd, 1, J = 5.3, 10.6 Hz), 3.99 (dd, 1, J = 5.3, 10.5 Hz), 3.88 (dd, 1, J = 4.9, 9.6 Hz), 3.53 (m, 1), 3.43 (m, 1); ^{13}C NMR (90 MHz, DMSO-d_6) δ 150.8, 146.2, 145.5, 142.8, 85.3, 80.2, 74.3, 71.4, 61.6. Anal. ($C_9H_{10}Cl_2N_2O_4$) C,H,N.

Ethyl 3,5-Dichloro-6-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrazine-2-carboxylate (3). A flame-dried, evacuated, 3-neck, 250-mL round-bottom flask equipped with an addition funnel was charged, under argon, with dry tetrahydrofuran (60 mL) and cooled to −70 °C. To this flask was added *n*-butyllithium (2.5 M solution in hexane, 3.2 mL, 8.0 mmol) followed by 2,2,6,6-tetramethylpiperidine (1.52 mL, 9.0 mmol), and the solution was warmed and allowed to stir at 0 °C for 60 min. After cooling to −94 °C, a solution of **1**[14b] (4.03 g, 7.3 mmol in 40 mL of dry tetrahydrofuran) was added dropwise (ca. 20 min). The lithiation was allowed to take place at −94 °C for 120 min, at which time ethyl cyanoformate (3.61 mL, 37.0 mmol) was added neat in one portion. The condensation was complete in 60 min (as determined by TLC), at which time the reaction was quenched with 25 mL of water, warmed to room temperature, and diluted with ethyl acetate to a total volume of 200 mL. The organic layer was isolated, dried over magnesium sulfate, and filtered, and the solvent was evaporated in vacuo. The resultant orange oil was subjected to column chromatography (SiO_2, 50 × 220 mm, 15% ethyl acetate/hexanes, R_f = 0.38) to give, following solvent evaporation, **3** (3.56 g, 78%) as a colorless oil: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.35−7.23 (m, 15), 5.47 (d, 1, J = 5.7 Hz), 4.67−4.37 (m, 10), 4.12 (pt, 1, J = 4.8, 4.9 Hz), 3.59 (dd, 2, J = 3.5, 4.6 Hz), 1.34 (t, 3, J = 7.1 Hz); ^{13}C NMR (90 MHz, $CDCl_3$) δ 162.7, 149.6, 148.7, 145.0, 141.7, 138.3, 137.9, 137.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.7, 82.8, 80.5, 79.1, 78.1, 73.5, 72.9, 72.5, 70.5, 62.7, 14.2; HRMS calcd for $C_{33}H_{36}Cl_2N_2O_6$ 622.1637, found 622.1617. Anal. ($C_{33}H_{36}Cl_2N_2O_6$) H,N; C: calcd, 63.57; found, 62.90.

Ethyl 3,5-Dichloro-6-(β-D-ribofuranosyl)pyrazine-2-carboxylate (4). A flame-dried, evacuated, 100-mL round-bottom flask was charged with **3** (680 mg, 1.1 mmol) under argon. This was dissolved in dry dichloromethane (25 mL) and cooled to −70 °C. To this solution was added boron trichloride (1.0 M solution in heptane, 6.5 mL, 6.5 mmol) dropwise, and the bright yellow mixture was allowed to stir at −70 °C for 150 min. The excess boron trichloride was then quenched at −70 °C with ethanol/dichloromethane (1:1, 10 mL) and warmed to room temperature, and the solution was neutralized with 3 N aqueous ammonium hydroxide. The solvent was evaporated to give a yellowish solid. This solid was then taken up in hot ethyl acetate, and the insoluble ammonium chloride was filtered off through Celite. A minimal amount of silica gel was added to the filtrate, and the solvent was evaporated in vacuo. The compound (absorbed on silica gel) was subjected to column chromatography (SiO_2, 40 × 220 mm, 7% methanol/chloroform, R_f = 0.38) to give, following solvent evaporation, **4** (139 mg, 89%) as a white solid. An analytical sample was crystallized from acetone/petroleum ether to afford a flocculent white solid: mp 122.5−123 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 5.32 (d, 1, J = 5.3 Hz, D_2O exchangeable), 5.14 (d, 1, J = 3.9 Hz), 5.03 (d, 1, J = 6.0 Hz, D_2O exchangeable), 4.49 (dd, 1, J = 4.5, 7.0 Hz, D_2O exchangeable), 4.41 (q, 2, J = 7.1 Hz), 4.23 (dd, 1, J = 4.8, 9.1 Hz), 3.98 (dd, 1, J = 5.9, 10.9 Hz), 3.91 (dd, 1, J = 4.5, 9.4 Hz), 3.61 (m, 1), 3.51 (m, 1), 1.34 (t, 3, J = 7.1 Hz); ^{13}C NMR (90 MHz, DMSO-d_6) δ 162.3, 151.1, 147.2, 143.3, 140.8, 84.8, 80.7, 74.6, 70.9, 62.5, 61.8, 13.8. Anal. ($C_{12}H_{14}Cl_2N_2O_6$) C,H,N.

Ethyl 5-Amino-3-chloro-6-(β-D-ribofuranosyl)pyrazine-2-carboxylate (5). Condition A: A 10-mL pressure tube was charged with **4** (176 mg, 0.5 mmol). To this tube was added a 0.5 M solution of ammonia in 1,4-dioxane (Aldrich; 2.2 mL, 1.1 mmol), the tube was sealed, and the reaction continued at 40 °C for 42 h. At this point, TLC indicated that all starting material had been consumed. A minimal amount of silica gel was added, and the solvent was evaporated in vacuo. The compound (absorbed on silica gel) was subjected to column chromatography (SiO_2, 20 × 180 mm, 10% methanol/chloroform, R_f = 0.38) to give, following solvent evaporation, **5** (98 mg, 59%) as a white solid: mp 164−166 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 7.65 (bs, 2, D_2O exchangeable), 5.36 (pt, 1, J = 4.6 Hz, D_2O exchangeable), 5.12 (d, 1, J = 6.5 Hz, D_2O exchangeable), 4.99 (d, 1, J = 4.8 Hz, D_2O exchangeable), 4.72 (d, 1, J = 7.6 Hz), 4.26 (m, 3), 4.01 (dd, 1, J = 4.7, 8.4 Hz), 3.92 (m, 1), 3.61 (m, 2), 1.30 (t, 3, J = 7.1 Hz); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.2, 153.6, 145.6, 136.3, 126.4, 86.0, 83.7, 71.8, 71.4, 61.0, 60.8, 14.1; HRMS calcd for $C_{12}H_{16}ClN_3O_6$ 333.0728, found 333.0733. Anal. ($C_{12}H_{16}ClN_3O_6$) C,H,N.

Condition E: Alternately, a 25-mL pressure tube was charged with **4** (200 mg, 0.57 mmol). To this tube was added ethanolic ammonia (saturated at 0 °C, 7.5 mL), the tube was sealed, and the reaction continued at 4 °C for 48 h. At this point, TLC indicated that all starting material had been consumed. A minimal amount of silica gel was added, and the solvent was evaporated in vacuo. The compound (absorbed on silica gel) was subjected to column chromatography (SiO_2, 40 × 150 mm, 10% methanol/chloroform, R_f = 0.38) to give, following solvent evaporation, **5** (73 mg, 38%) as a white solid: mp, ^{1}H NMR, and ^{13}C NMR all correspond to the compound prepared above.

Also isolated was 61 mg (35%) of **7** as a white solid: R_f, mp, ^{1}H NMR, and ^{13}C NMR all correspond to the compound prepared below.

Ethyl 3-Chloro-5-(methylamino)-6-(β-D-ribofuranosyl)-pyrazine-2-carboxylate (6). Condition B: A flame-dried, evacuated 50-mL pressure tube was charged with **4** (353 mg, 1.0 mmol) under argon, and this was dissolved in dry tetrahydrofuran (10 mL). To this solution was added a solution of methylamine (2.0 M in tetrahydrofuran, 1.1 mL, 2.2 mmol) in one portion, and the tube was sealed. A precipitate formed immediately and, after 60 min at room temperature, TLC indicated that all starting material had been consumed. A minimal amount of silica gel was added, and the solvent was evaporated in vacuo. The compound (absorbed on silica gel) was subjected to column chromatography (SiO_2, 20 × 180 mm, 10% methanol/chloroform, R_f = 0.37) to give, following solvent evaporation, **6** (198 mg, 57%) as white needles: mp 174−175 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.01 (bd, 1, J = 4.6 Hz, D_2O exchangeable), 5.54 (pt, 1, J = 4.2 Hz, D_2O exchangeable), 5.12 (d, 1, J = 6.5 Hz, D_2O exchangeable), 5.01 (d, 1, J = 4.8 Hz, D_2O exchangeable), 4.74 (d, 1, J = 7.7 Hz), 4.26 (m, 3), 4.01 (dd, 1, J = 5.0, 8.2 Hz), 3.95 (m, 1), 3.63 (m, 2), 2.85 (d, 3, J = 4.6 Hz), 1.30 (t, 1, J = 7.1 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.3, 153.5, 146.8, 138.2, 126.1, 86.9, 84.8, 72.8, 72.4, 61.7, 61.6, 28.7, 15.0. Anal. ($C_{13}H_{18}ClN_3O_6$) C,H,N.

5-Amino-3-chloro-6-(β-D-ribofuranosyl)pyrazine-2-carboxamide (7). Condition C: A 25-mL pressure tube was charged with **4** (177 mg, 0.5 mmol). To this tube was added

methanolic ammonia (saturated at 0 °C, 5 mL), the tube was sealed, and the reaction continued at room temperature for 1 h. TLC indicated that the reaction was sluggish and producing multiple products. An additional quantity of methanolic ammonia (5 mL) was added, the tube sealed, and stirring continued at 50 °C for 20 h. TLC indicated that there was only one major mobile product. A minimal amount of silica gel was added, and the solvent was evaporated in vacuo. The compound (absorbed on silica gel) was subjected to column chromatography (SiO_2, 40 × 200 mm, 15% methanol/chloroform, $R_f = 0.27$) to give, following solvent evaporation, **7** (35 mg, 23%) as a white, hygroscopic solid: mp 84.5−85 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 7.66 (bs, 1, D_2O exchangeable), 7.40 (bs, 2, D_2O exchangeable), 7.36 (bs, 1, D_2O exchangeable), 5.30 (pt, 1, $J = 4.6$ Hz, D_2O exchangeable), 5.09 (d, 1, $J = 6.7$ Hz, D_2O exchangeable), 4.96 (d, 1, $J = 4.2$ Hz, D_2O exchangeable), 4.72 (d, 1, $J = 7.8$ Hz), 4.27 (dd, 1, $J = 6.5, 12.9$ Hz), 4.01 (m, 1), 3.90 (m, 1), 3.58 (m, 2); ^{13}C NMR (90 MHz, DMSO-d_6) δ 165.0, 153.6, 143.9, 135.1, 128.7, 86.0, 83.2, 71.8, 71.4, 61.1; HRMS calcd for $C_{10}H_{13}ClN_4O_5$ 304.0574, found 304.0563. Anal. ($C_{10}H_{13}ClN_4O_5 \cdot {}^1/_2H_2O$) C,H,N.

3-Chloro-5-methoxy-6-(β-D-ribofuranosyl)pyrazine-2-carboxamide (8). Condition D: A 25-mL pressure tube was charged with **4** (150 mg, 0.42 mmol), and this was dissolved in 10 mL of methanol. To this solution was added methanolic ammonia (saturated at 0 °C, 250 μL), the tube was sealed, and the solution stirred at room temperature for 24 h. TLC indicated that all starting material had been consumed. The solvent was evaporated in vacuo, and the residue was redissolved in 5 mL of methanolic ammonia (saturated at 0 °C) and allowed to react at room temperature for 24 h. A minimal amount of silica gel was added to the solution, and the solvent was evaporated in vacuo. The compound (absorbed on silica gel) was subjected to column chromatography (SiO_2, 20 × 150 mm, 10% methanol/chloroform, $R_f = 0.15$) to give, following solvent evaporation, **8** (40 mg, 30%) as a white solid: mp 169−170.5 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 7.95 (bs, 1, D_2O exchangeable), 7.74 (bs, 1, D_2O exchangeable), 5.10 (d, 1, $J =$ 5.6 Hz, D_2O exchangeable), 4.96 (d, 1, $J = 4.9$ Hz), 4.91 (d, 1, $J = 5.7$ Hz, D_2O exchangeable), 4.67 (pt, 1, $J = 5.5, 5.8$ Hz, D_2O exchangeable), 4.32 (dd, 1, $J = 5.1, 10.0$ Hz), 4.02 (dd, 1, $J = 5.5, 10.6$ Hz), 3.98 (s, 3), 3.82 (dd, 1, $J = 4.9, 9.6$ Hz), 3.56 (m, 1), 3.45 (m, 1); ^{13}C NMR (90 MHz, DMSO-d_6) δ 164.8, 157.3, 141.5, 141.0, 136.4, 84.7, 78.9, 73.4, 71.1, 61.6, 55.0; HRMS calcd for $C_{11}H_{14}ClN_3O_6$ 319.0571, found 319.0566. Anal. ($C_{11}H_{14}ClN_3O_6$) C,H,N.

3-Chloro-5-ethoxy-6-(β-D-ribofuranosyl)pyrazine-2-carboxamide (9). Condition F: A 25-mL pressure tube was charged with **4** (353 mg, 1.0 mmol). To this tube was added ethanolic ammonia (saturated at 0 °C, 10 mL), the tube was sealed, and the reaction continued at 20 °C for 70 h. TLC indicated that all starting material had been consumed, a minimal amount of silica gel was added, and the solvent was evaporated in vacuo. The compound (absorbed on silica gel) was subjected to column chromatography (SiO_2, 40 × 150 mm, 10% methanol/chloroform, $R_f = 0.24$) to give, following solvent evaporation, **9** (40 mg, 12%) as a clear glass which could be triturated with methanol to give a white solid: mp 153.0−154.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (bs, 1, D_2O exchangeable), 7.72 (bs, 1, D_2O exchangeable), 5.09 (d, 1, $J =$ 5.3 Hz, D_2O exchangeable), 4.96 (d, 1, $J = 4.9$ Hz), 4.92 (d, 1, $J = 5.6$ Hz, D_2O exchangeable), 4.66 (pt, 1, $J = 5.5$ Hz, D_2O exchangeable), 4.41 (q, 2, $J = 6.9$ Hz), 4.33 (dd, 1, $J = 5.0, 10.0$ Hz), 4.03 (dd, 1, $J = 5.4, 10.6$ Hz), 3.82 (m, 1), 3.41 (m, 1), 1.37 (t, 3, $J = 6.9$ Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.8, 156.8, 141.4, 141.0, 136.2, 84.7, 79.2, 73.4, 71.9, 63.8, 61.7, 14.0. Anal. ($C_{12}H_{16}ClN_3O_6 \cdot$MeOH) C,H,N.

Also isolated was 101 mg (30%) of **5** as a white solid: R_f, mp, ^{1}H NMR, and ^{13}C NMR all correspond to the compound prepared above.

Also isolated was 53 mg (17%) of **7** as a white solid ($R_f =$ 0.10 in 10% methanol/chloroform): mp, ^{1}H NMR, and ^{13}C NMR all correspond to the compound prepared above.

Cell Culture Procedures. The routine growth and passage of KB, BSC-1, and HFF cells was performed in monolayer cultures using minimal essential medium (MEM) with either Hanks salts [MEM(H)] or Earle salts [MEM(E)] supplemented with 10% calf serum or 10% fetal bovine serum (HFF cells). The sodium bicarbonate concentration was varied to meet the buffering capacity required. Cells were passaged at 1:2 to 1:10 dilutions according to conventional procedures using 0.05% trypsin plus 0.02% EDTA in a HEPES-buffered salt solution.

Virological Procedures. The Towne strain, plaque-purlfied isolate P_O, of HCMV was kindly provided by Dr. Mark Stinski, University of Iowa. The KOS strain of HSV- 1 was used in most experiments and was provided by Dr. Sandra K. Weller, University of Connecticut. Stock HCMV was prepared by infecting HFF cells at a multiplicity of infection (moi) of <0.01 plaque-forming units (pfu)/cell as detailed previously.[26] High-titer HSV-1 stocks were prepared by infecting KB cells at an moi of <0.1 also as detailed previously.[26] Virus titers were determined using monolayer cultures of HFF cells for HCMV and monolayer cultures of BSC-1 cells for HSV-1 as described earlier.[27] Briefly, HFF or BSC-1 cells were planted as described above in 96-well cluster dishes and incubated overnight at 37 °C. The next day cultures were inoculated with HCMV or HSV-1 and serially diluted 1:3 across the remaining 11 columns of the 96-well plate. After virus adsorption the inoculum was replaced with fresh medium, and cultures were incubated for 7 days for HCMV and for 2 or 3 days for HSV-1. Plaques were enumerated under 20-fold magnification in wells having the dilution which gave 5−20 plaques/well. Virus titers were calculated according to the following formula: titer (pfu/mL) = number of plaques × 5 × 3^n, where n represents the nth dilution of the virus used to infect the well in which plaques were enumerated.

HCMV Plaque Reduction Assay. HFF cells in 24-well cluster dishes were infected with approximately 100 pfu of HCMV/cm^2 cell sheet using the procedures detailed above. Following virus adsorption, compounds dissolved in growth medium were added to duplicate wells in 4−8 selected concentrations. After incubation at 37 °C for 10−12 days, cell sheets were fixed and stained with crystal violet and microscopic plaques enumerated as described above. Drug effects were calculated as a percentage of reduction in number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug.

HSV-1 ELISA. An ELISA was employed to detect HSV-1;[28] 96-well cluster dishes were planted with 10 000 BSC-1 cells/well in 200 μL/well of MEM(E) plus 10% calf serum. After overnight incubation at 37 °C, selected drug concentrations in quadruplicate and HSV-1 at a concentration of 100 pfu/well were added. Following a 3-day incubation at 37 °C, medium was removed, plates were blocked and rinsed, and horseradish peroxidase-conjugated rabbit anti-HSV-1 antibody was added. Following removal of the antibody-containing solution, plates were rinsed and then developed by adding 150 μL/well of a solution of tetramethylbenzidine as substrate. The reaction was stopped with H_2SO_4, and absorbance was read at 450 and 570 nm. Drug effects were calculated as a percentage of the reduction in absorbance in the presence of each drug concentration compared to absorbance obtained with virus in the absence of drug.

Cytotoxicity Assays. Two different assays were used to explore cytotoxicity of selected compounds using methods we have detailed previously. (i) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[26] (ii) The effect of compounds during two population doublings of KB cells was determined by crystal violet staining and spectrophotometric quantitation of dye eluted from stained cells as described earlier.[29] Briefly, 96-well cluster dishes were planted with KB cells at 3000−5000 cells/well. After overnight incubation at 37 °C, test compound was added in quadruplicate at 6−8 concentrations. Plates were incubated at 37 °C for 48 h in a CO_2 incubator, rinsed, fixed with 95% ethanol, and stained with 0.1% crystal violet. After drying, 0.1 N HCl in ethanol

was added, and the plates were read at 570 nm in a spectrophotometer designed to read 96-well ELISA assay plates.

Data Analysis. Dose−response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations; 50% inhibitory concentrations (IC_{50}) or IC_{90}'s were calculated from the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

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Supporting Information Available: X-ray structures and relevant data for **5** (13 pages). Ordering information is given on any current masthead page.

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