Synthesis and Antiviral Evaluation of Halogenated β-D- and -L-Erythrofuranosylbenzimidazoles

Kristjan S. Gudmundsson,[†] Jeffrey Tidwell,[§] Nicole Lippa, George W. Koszalka,[§] Nanine van Draanen,[§] Roger G. Ptak,[‡] John C. Drach, and Leroy B. Townsend*

Department of Medicinal Chemistry, College of Pharmacy, Department of Chemistry, College of Literature, Sciences and Arts, and Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, Michigan 48109-1065, and Division of Chemistry, Glaxo Wellcome Inc., 5 Moore Drive, Research Triangle Park, North Carolina 27709

A series of 2-substituted benzimidazole D- and L-erythrofuranosyl nucleosides were synthesized and tested for activity against herpesviruses and for cytotoxicity. The D-nucleosides 2,5,6-trichloro-1-(β-D-erythrofuranosyl)benzimidazole (**8a**) and 2-bromo-5,6-dichloro-1-(β-D-erythrofuranosyl)benzimidazole (**8b**) were prepared by coupling 1,2,3-tri-*O*-acetyl-β-D-erythrofuranose (D-**6**) with the appropriate benzimidazole, followed by removal of the acetyl protecting groups. The 2-isopropylamino (**9**), 2-cyclopropylamino (**10**), and 2-mercaptobenzyl (**11**) derivatives were synthesized by nucleophilic displacements of the C-2 chlorine in the benzimidazole moiety of **8a**. The D-nucleoside 4-bromo-5,6-dichloro-2-isopropylamino-1-(β-D-erythrofuranosyl)benzimidazole (**17**) was prepared by coupling D-**6** with the appropriate benzimidazole. The L-erythrofuranosyl derivatives, 5,6-dichloro-2-isopropylamino-1-(β-L-erythrofuranosyl)benzimidazole (**21a**), its 2-cyclopropylamino analogue (**21b**), and the 2-isopropylamino analogue (**25**), were prepared by coupling L-**6** with the appropriate benzimidazole. Several of these new derivatives had very good activity against HCMV in plaque and yield reduction assays (IC$_{50}$ = 0.05−19 μM against the Towne strain of HCMV) and DNA hybridization assays. Very little activity was observed against other herpesviruses. This pattern is similar to the antiviral activity profile observed for the corresponding ribofuranosides 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (**4a**), its 2-bromo analogue (**4b**), and the 2-cyclopropylamino analogue (**4c**). In comparison, **8a** was 15-fold more active against HCMV than **4a**, and **8b** was 4-fold more active against HCMV than **4b**. The 5,6-dichloro-2-isopropylamino-1-(β-L-erythrofuranosyl)-benzimidazole (**21a**) was less active than **4c**, which is now in clinical trials for HCMV infection. Both **8a,b** had comparable HCMV activity to **4c**. Mode of action studies with the D-erythrose analogues established that **8b** acted by inhibition of viral DNA processing whereas **9** and **10** may act via a different mechanism. The lack of a 5′-hydroxymethyl group in all members of this series established that antiviral activity occurred without 5′-phosphorylation, a feature required for the activity of most nucleoside analogues.

Introduction

Human cytomegalovirus (HCMV) is one of eight human herpesviruses. It is estimated that by adulthood, more than half of all Americans will have been infected with HCMV.[1] HCMV infections in immunocompetent individuals are usually asymptomatic. However, in immunocompromised patients, HCMV infections are often life-threatening. Transplant recipients[2] and individuals with acquired immune deficiency syndrome (AIDS)[3] are vulnerable to these infections. HCMV is also a leading cause of birth defects as a consequence of in utero fetal infection. Currently, there are four FDA-approved drugs available for the treatment of HCMV infections: ganciclovir (**1**),[5] foscarnet (**2**),[6] cidofovir (**3**),[7] and the 21-mer antisense oligonucleotide fomivirsen.[8] Unfortunately, these drugs can produce significant side

effects, they have limited oral bioavailability, or they must be injected intra-vitrally. Moreover, virus strains resistant to most of these drugs are emerging.[9] Consequently, there is a need for a more potent and selective antiviral drug to treat HCMV infections. As part of our search for new anticancer[10] and antiviral drugs,[11,12] a number of benzimidazole nucleosides have been synthesized. Certain compounds, including 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB, **4a**) and 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BDCRB, **4b**), have potent activity against HCMV with low cellular toxicity at concentrations inhibiting viral growth.[12] Biological evaluation of **4a,b** has established that their antiviral activity involves neither phosphorylation of the compound nor inhibition of DNA, RNA, or protein synthesis.[13] Rather, they act by a unique mechanism, which involves inhibition of viral DNA processing and virus assembly.[14] Related L-ribofuranosyl analogues, such as 2-isopropylamino-5,6-dichloro-1-(β-L-ribofuranosyl)benzimidazole (**4c**, 1263W94), also have been shown to have very good activity against HCMV. These L-ribofuranosyl derivatives inhibit HCMV DNA

* To whom correspondence should be addressed. Tel: 734-764-7547. Fax: 734-763-5633. E-mail: ltownsen@umich.edu.
§ Glaxo Wellcome Inc.
† Present address: Glaxo Wellcome Inc., 5 Moore Dr., Research Triangle Park, NC 27709.
‡ Present address: Southern Research Institute, 431 Aviation Way, Frederick, MD 21701.
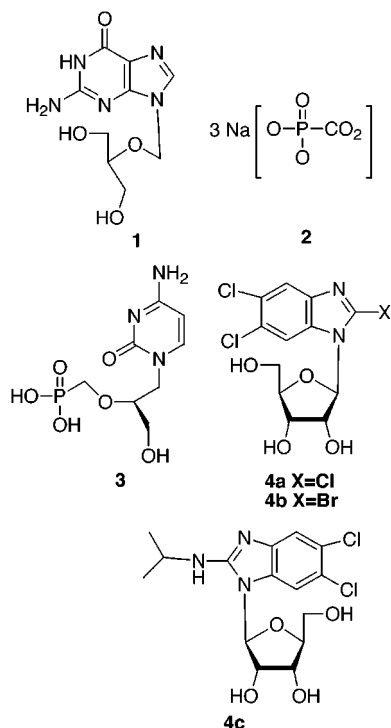
Figure 1. Structures of ganciclovir (**1**), foscarnet (**2**), cidofovir (**3**), and the benzimidazole nucleosides TCRB (**4a**), BDCRB (**4b**), and 1263W94 (**4c**).

synthesis by a mechanism that does not require phosphorylation and does not involve viral DNA polymerase.[15] Thus they have a different mode of action from the D-ribofuranose derivatives.[15] Compound **4c** is in clinical development for the treatment of HCMV infections.[15,16]

Numerous structure–activity relationship studies of derivatives of **4a**–**c**, modified in either the heterocycle or carbohydrate moiety, have been undertaken in our laboratories[10–12,17,18] In this article we describe studies on the effect of replacing a ribofuranosyl moiety with a D- or L-erythrofuranosyl moiety.

Results and Discussion

Chemistry. As part of our studies on the structure–activity relationships for the benzimidazole nucleosides, 2,5,6-trichloro-1-(5-deoxy-*β*-D-ribofuranosyl)benzimidazole[18] and various 5′-modified analogues have been prepared.[19] Several of these compounds had better antiviral activity than TCRB. This prompted the synthesis and antiviral evaluation of the erythrofuranosyl analogues described herein.

D-Erythrose[20] was acetylated with acetic anhydride in pyridine to give 1,2,3-tri-*O*-acetyl-D-erythrofuranose (D-**6**).[21] Silylation of 2,5,6-trichlorobenzimidazole (**5a**)[22] with bis(trimethylsilyl)acetamide (BSA) was followed by the addition of D-**6** and trimethylsilyl trifluoromethanesulfonate (TMSOTf) to the reaction mixture to give exclusively the desired 2,5,6-trichloro-1-(2,3-di-*O*-acetyl-*β*-D-erythrofuranosyl)benzimidazole (**7a**) in 75% yield. The desired *β*-anomer (**7a**) was the only isolated product since a condensation of the acetylated carbohydrate D-**6** and 2,5,6-trichlorobenzimidazole (**5a**), under Vorbruggen conditions, follows Bakers rule,[23] due to the 2′-*O*-acetyl participation during the condensation. Comparison of the chemical shifts and coupling constants of the ano-

meric proton of **7a** with the chemical shift and coupling constants of related 2,3-di-*O*-acetyl-*β*-D-benzimidazole nucleosides[12,18,19] supported the *β*-assignment. Compound **7a** was deprotected with sodium carbonate in aqueous ethanol to give 2,5,6-trichloro-1-(*β*-D-erythrofuranosyl)benzimidazole (**8a**) in 81% yield. Similarly, 2-bromo-5,6-dichloro-1-(*β*-D-erythrofuranosyl)benzimidazole (**8b**) was synthesized by a condensation of 2-bromo-5,6-dichlorobenzimidazole (**5b**)[12] with D-**6**, followed by deprotection with sodium carbonate in aqueous ethanol. The chlorine at C-2 of compound **7a** can be readily displaced with a variety of nucleophiles. A series of C-2 substituted D-erythrofuranosides **9**–**11** were formed by the treatment of **7a** with the appropriate nucleophiles, under mild conditions, resulting in substitution of the 2-chloro group and concomitant deacetylation of the carbohydrate moiety. The choice of nucleophiles was based on the previously established activity of C-2 substituted benzimidazole ribonucleosides. Thus, compound **7a** was treated with cyclopropylamine, isopropylamine, and sodium mercaptobenzylate to afford 2-cyclopropylamino-5,6-dichloro-1-(*β*-D-erythrofuranosyl)-benzimidazole (**9**), 2-isopropylamino-5,6-dichloro-1-(*β*-D-erythrofuranosyl)benzimidazole (**10**), and 5,6-dichloro-2-mercaptobenzyl-1-(*β*-D-erythrofuranosyl)benzimidazole (**11**), respectively.

In the synthesis of 4-bromo-5,6-dichloro-2-isopropylamino-1-(*β*-D-erythrofuranosyl)benzimidazole (**17**), 4-bromo-5,6-dichloro-2-isopropylaminobenzimidazole (**15**) was synthesized first and then coupled with D-**6**. Thus, 2-nitro-4,5-dichloroaniline (**12**) was brominated using NBS to give **13** and then reduced (Fe, HCl) to 3-bromo-4,5-dichloro-1,2-phenylenediamine (**14**). Phenylenediamine **14** was condensed with isopropyl isothiocyanate in pyridine in the presence of the desulfurizing agent, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate,[24] to give **15**. Condensation of **15** with D-**6** under Vorbruggen conditions, followed by deprotection, gave **17**. The L-isomers 5,6-dichloro-2-isopropylamino-1-(*β*-L-erythrofuranosyl)benzimidazole (**21a**), 5,6-dichloro-2-cyclopropylamino-1-(*β*-L-erythrofuranosyl)-benzimidazole (**21b**), and 4,5,6-trichloro-2-isopropylamino-1-(*β*-L-erythrofuranosyl)benzimidazole (**25**) were synthesized in a similar fashion by condensing 1,2,3-tri-*O*-acetyl-*β*-L-erythrofuranose (L-**6**)[25] with the appropriate benzimidazole. The cyclopropylamino and isopropylamino groups were chosen as C-2 substituents based on structure–activity relationships from the L-ribofuranosyl benzimidazole series.

Biology. This new series of compounds was evaluated for activity against HCMV and HSV-1 as well as for cytotoxicity in a number of cell lines (Table 1). Like the lead compounds (**4a**–**c**), none of the compounds in this study had significant activity against HSV-1. Several of the tested compounds were more cytotoxic than **4a**–**c**, but for the most active compounds (e.g. **8a,b**) cytotoxicity was well-separated from antiviral activity.

Evaluation of the compounds against HCMV revealed that the erythro compounds **8a,b** were approximately 10 times more active than the ribo-**4a** and 4 times more active than the ribo-**4b**. Compounds **8a,b** were about 40 times more active than ganciclovir (Table 1).

Interestingly, the antiviral activity observed for the di-*O*-acetates (**7a,b**) was nearly the same as the activity

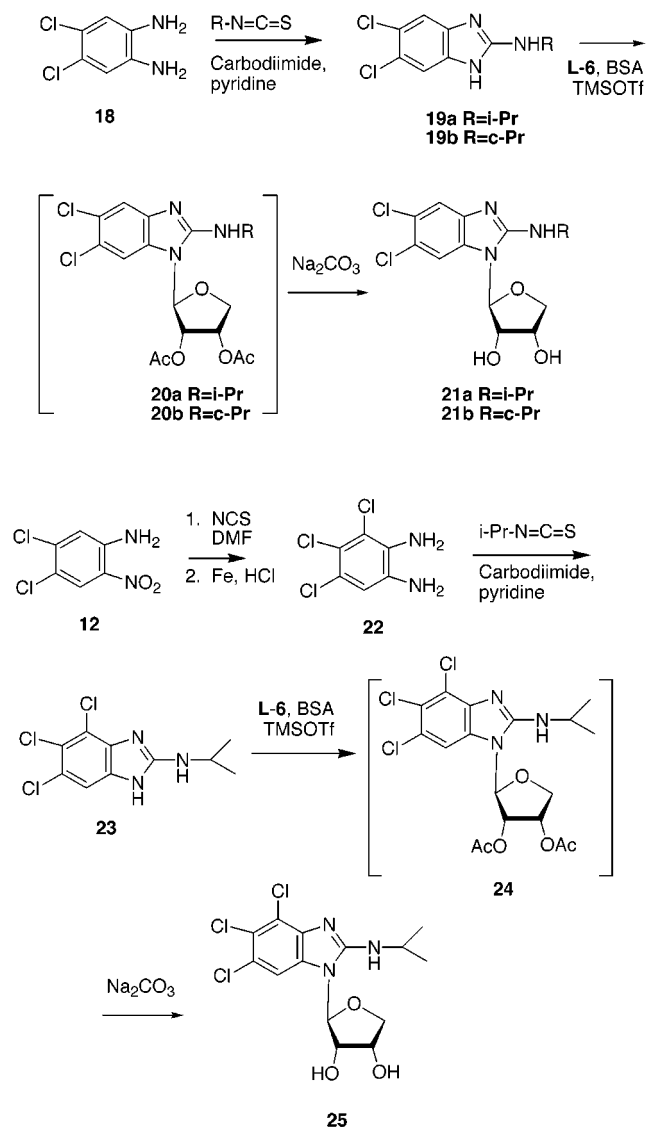**Scheme 1.** Synthesis of D-Erythrofuranosylbenzimidazole Derivatives
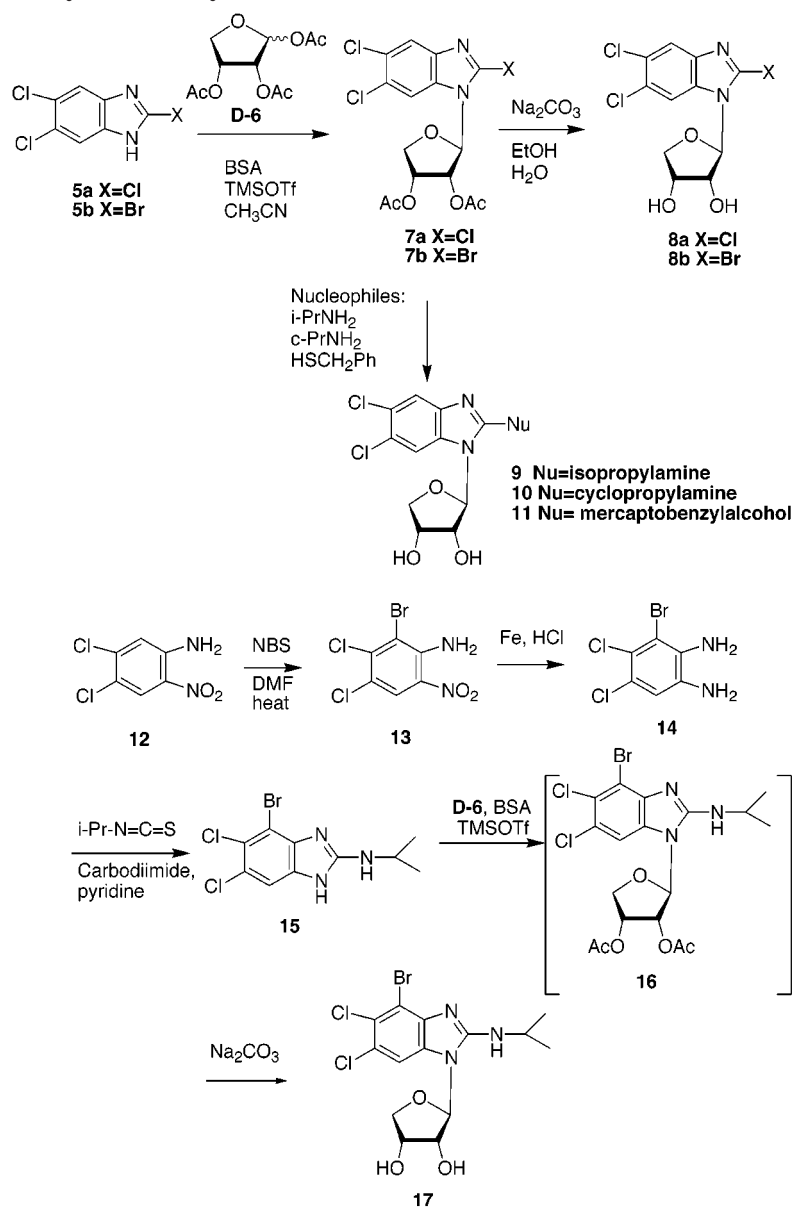
observed for the deprotected erythrosides (**8a,b**). We speculate that this may be due to deacetylation of **7a**,**b** by serum esterases in the cell culture medium. Results from yield reduction assays established more firmly the greater activity of **7a,b** and **8a,b** against HCMV than the activity of **4a**,**b** and ganciclovir. The 2-isopropyl-amino derivative **9** and the 2-cyclopropylamino deriva-tive **10** were considerably less active than the 2-halo analogues and also less active than ganciclovir against HCMV with less separation between antiviral activity and cytotoxicity (Table 1). The same was true for the mercaptobenzyl derivative **11**.

The nucleosides **7b**, **8b**, and **9** were also tested against the AD169 strain of HCMV in a plaque assay. All three compounds were 4−5-fold more active against AD169 than against the Towne strain. The 2-isopropylamino analogue **9** was less active than the 2-halo analogues against both strains of HCMV in the plaque assays but had equivalent activity in viral cytopathic effect (CPE) and DNA hybridization assays.

The L-erythrofuranosyl derivatives **21a,b** and **25** were less active than their D-analogues (**9**, **10**, and **17**,
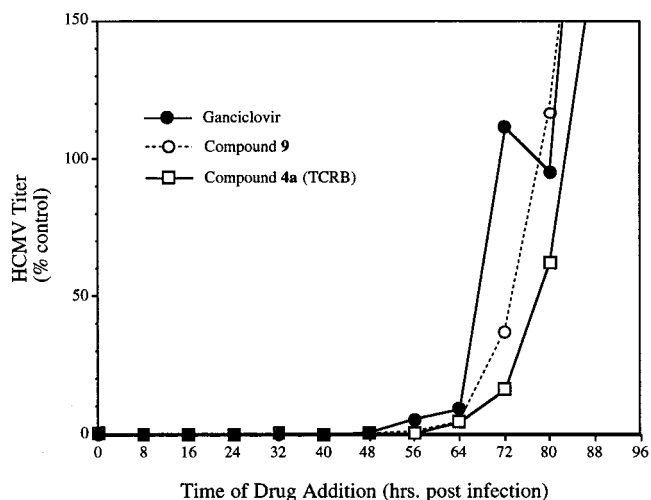
respectively). Furthermore, whereas the D-erythrofur-anosyl derivatives were more active than the corre-sponding D-ribose derivatives (compare **8a,b** to **4a,b**), this was not the case for the L-erythrofuranosyl deriva-tives, which were less active than the corresponding L-ribose derivatives. These differences in structure−activity trends are not surprising in view of the fact that L-benzimidazole nucleosides have a different mode of action (inhibit viral DNA synthesis)[15] than the D-benzimidazole nucleosides (inhibit viral DNA process-ing).[14]

Because two separate modes of action have been discovered with the ribosylbenzimidazoles, initial mode of action studies were performed with the erythro-furanosylbenzimidazoles. Compounds **8b**, **9**, and **10** were selected for study based upon the different sub-stituents in the 2-position. Their activity was compared in strains of HCMV resistant to one or the other mechanism of benzimidazole action. Strain C4 was derived by selection with **4a** and acquired resistance to **4a,b** by mutation of genes *UL56* and *UL89*.[26] Strain 2916[r] was derived by selection with an analogue of **4c**

Scheme 2. Synthesis of
L-Erythrofuranosylbenzimidazoles

Figure 2. Time of drug action in the HCMV replication cycle. Each compound was added at each of the indicated times to duplicate cultures of infected cells to give a final concentration of 100 μM. An identical aliquot of control medium also was added to separate cultures in duplicate. Incubation was continued for one replication cycle (96 h), and all the cultures were frozen. HCMV titers were determined subsequently in separate assays and are presented as percent of control titer at each time point. Ganciclovir is a known inhibitor of HCMV DNA synthesis;[5] TCRB is an inhibitor of DNA processing.[14]

and acquired resistance by mutation of gene *UL97*.[27] In plaque assays, compound **8b** was very active against wild-type Towne and AD169 viruses as well as against strain 2916ʳ (Table 2). In contrast, strain C4 was as or more resistant to **8b** as it was to **4b** (Table 2). As expected, all four strains were equally sensitive to ganciclovir. Together these data strongly suggest that **8b** acts by the same mechanism as **4b**: namely, inhibition of viral DNA processing via the proteins specified by genes *UL56* and *UL89*.

A more limited examination of the activity of compounds **9** and **10** by the more sensitive yield reduction assay revealed that in contrast to **8b**, both wild-type and C4 HCMV strains had about the same sensitivity to **9** and **10** (Table 2). The observation that C4 was resistant to **4b** in this assay but not to **4c** suggests either **9** and **10** interact with a UL56/UL89 protein complex very differently than does **4b** or **9** and **10** may act in a manner similar to **4c** and not inhibit viral DNA processing. To explore this possibility further, a drug time of addition study was accomplished with compound **9** along with control compounds that act by related mechanisms. Figure 2 shows that ganciclovir, a known

viral DNA synthesis inhibitor, could be added to HCMV-infected cultures as late as 56−64 h without a significant loss of activity. Activity of compound **4a** was lost even later, consistent with ganciclovir inhibition of viral DNA synthesis and **4a** inhibition of viral DNA processing once DNA was synthesized. Activity of compound **9** was lost at approximately the same time as observed with ganciclovir and **4a** thereby establishing that the compound acted late in the viral replication cycle. Even though the results for **9** do not unambiguously differentiate between the two mechanisms, the results, together with data in Table 2, show that compound **9** affects either DNA synthesis, DNA processing, or both. Whatever the reason, the change from a Cl or Br to an iso- or cyclopropylamine in the 2-position not only affected the potency of the compounds but also may affected the mechanism by which the compounds act. Regardless of mechanism, it is clear that the compounds cannot be phosphorylated at the 5′-position as a consequence of their design, thereby establishing that they act differently than many nucleoside analogues.

Experimental Section

General Chemical Procedures. Melting points were taken on a Thomas-Hoover Unimelt apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained at 300, 360, or 500 MHz. Flash column chromatography was performed using silica gel 60 230−400 mesh (ICN) and the technique described by Still et al.[28] Thin-layer chromatography (TLC) was performed on prescored silica gel GHLF plates (Analtech, Newark, DE). Compounds were visualized by illumination under UV light (254 nm) or by spraying with 20% methanolic sulfuric acid followed by charring on a hot plate.

2,5,6-Trichloro-1-(2,3-di-*O*-acetyl-*β*-D-erythrofuranosyl)benzimidazole[22] (7a). 2,5,6-Trichlorobenzimidazole[22] (**5a**; 350 mg, 1.6 mmol) was suspended in dry CH₃CN (5 mL). To this suspension was added BSA (0.6 mL, 2.4 mmol) and the resulting solution stirred at room temperature for 20 min. 1,2,3-Tri-*O*-acetyl-D-erythrofuranose (D-**6**; 390 mg, 1.6 mmol)

Table 1. Antiviral Activity and Cytotoxicity of Erythrofuranosylbenzimidazole Nucleosides

	IC$_{50}$ or IC$_{90}$ (μM)								
	antiviral activity						cytotoxicity[c]		
	HCMV[a]					HSV-1[b]			
compd	plaque (Towne)	plaque (AD169)	yield (Towne)	CPE	DNA hybrid	ELISA	visual	growth	MOLT 4
7a	0.25		0.10	0.1	0.33	>100[e]	41	45	
8a	0.24		0.12	0.1	0.1	>100	32	60	
7b	0.22	0.05[d]	0.05	0.05	0.21	>100	59	60	
8b	0.24	0.04[d]	0.06	0.05	0.23	50	32	50	47
9	7.9	1.8	5.0[d]	0.8	0.42	>100	>100	>100	84
10	19		6.8[d]	0.6	0.26	>100	>100	>100	132
11	32			>10	>10	65	32	60	
17				1.0	0.16				13
21a				2.3	0.8				15
21b				2.3	2.0				43
25				0.8	0.15				
4a (TCRB)[f]	2.8		1.3 ± 0.8			102	238	210	
4b (BDCRB)[f]	0.70		0.20	0.3	0.52	130	118	>100	
4c (1263W94)		0.45	0.60[d]		0.1	>100	>100	65[d]	
1 (GCV)[g]	7.4 ± 6.5		1.6 ± 1.2	2	0.88	3.5 ± 2.1	>100	>100	

[a] Plaque and yield reduction assays were performed in duplicate wells as described in the Experimental Section. Results from plaque assays are reported as IC$_{50}$'s, those for yield reduction experiments as IC$_{90}$'s. CPE is cytophathic effect and measures microscopically visible effects of virus on cells. The DNA hybridizaton assay was performed as described.[33] [b] HSV-1 was assayed by ELISA in quadruplicate wells. [c] Visual cytotoxicity was scored on HFF cells unaffected by HCMV at the time of plaque enumeration. Inhibition of KB cell growth was determined in quadruplicate assays. Inhibition of MOLT 4 cells was determined as described in the literature.[36] Results are presented as IC$_{50}$'s. [d] Average of duplicate or triplicate experiments. [e] >100 μM indicates that IC$_{50}$ not reached at the noted (highest) concentration tested. [f] Results for compounds **4a,b**, also reported in ref 12 as compounds **9** and **11**; averages ± SD from 5 and 15 experiments. [g] Averages ± SD derived from 108, 33, and 3 experiments, respectively, in which ganciclovir was used as a positive control.

Table 2. Activity of D-Erythrofuranosylbenzimidazole Nucleosides in Drug-Resistant Strains of HCMV

				IC$_{50}$ or IC$_{90}$ (μM)			
				HCMV strain[a]			
				Towne		AD169	
compound		substituents		wild-type	C-4[b]	wild-type	2916r[c]
no.	name	R$_1$	R$_2$				
		Plaque Reduction Assay					
4b	BDCRB	β-D-ribose	Br	1.7[d]	31[d]	0.34[d]	0.41[d]
4c	1263W94	β-L-ribose	NHC$_3$H$_7$			0.33[d]	>100[d]
8b		β-D-erythrose	Br	0.23[d]	12[d]	0.03	0.03
1	ganciclovir			4.5	4.1	3.3	3.6
		Yield Reduction Assay					
4b	BDCRB	β-D-ribose	Br	0.3	8.0		
4c	1263W94	β-L-ribose	NHC$_3$H$_7$	0.59	0.70		
9		β-D-erythrose	NHC$_3$H$_7$	6.9	8.5		
10		β-D-erythrose	NHC$_3$H$_5$	10	5.0		

[a] Plaque and yield reduction assays were performed in duplicate as described in the text. Results from plaque assays are reported as IC$_{50}$'s, those for yield reduction experiments as IC$_{90}$'s. [b] HCMV resistant to **4a,b** – see ref 26. [c] HCMV resistant to **4c** – see ref 27. [d] Average derived from two or three experiments.

in CH$_3$CN (4 mL) was added to the solution and this was then followed by TMSOTf (0.3 mL, 1.6 mmol). The reaction mixture was stirred at ambient temperature for 2 h. Saturated NaHCO$_3$(aq) solution was then added and the resulting mixture extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered and evaporated under reduced pressure to give the crude product as a foam. This foam was purified using flash column chromatography (SiO$_2$, CHCl$_3$). The appropriate fractions were combined and the solvent was removed in vacuo to yield, after recrystallization from MeOH, 400 mg (61%) of **7a** as white crystals: mp 166–168 °C; R_f 0.52 (2% MeOH in CHCl$_3$); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.13 (s, 1H), 8.00 (s, 1H), 6.19 (d, 1H, 1'-H, J = 7.8 Hz), 5.81 (m, 1H), 5.60 (t, 1H), 4.79 (dd, 1H), 4.12 (d, 1H), 2.16 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (90 MHz,

DMSO-d_6) δ 169.64, 169.27, 141.54, 140.85, 132.18, 126.55, 126.20, 120.41, 118.49, 85.81, 71.98, 71.94, 70.75, 20.56, 20.11. Anal. Calcd for C$_{15}$H$_{13}$Cl$_3$N$_2$O$_5$: C, H, N.

2,5,6-Trichloro-1-(β-D-erythrofuranosyl)benzimidazole (8a). Compound **7a** (0.35 g, 0.85 mmol) was dissolved in EtOH (40 mL). To this solution was added Na$_2$CO$_3$ (0.62 g, 5.84 mmol) in water (40 mL) and the resulting mixture stirred for 4 h. Acetic acid was then added until a pH of 7 was obtained and the resulting suspension was concentrated in vacuo. The suspension was triturated with water and the solid collected by filtration and recrystallized from MeOH to give 225 mg (81%) of **8a** as a white crystalline solid: mp 115–117 °C; R_f 0.19 (EtOAc/hexane 1:1); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.00 (s, 1H), 7.90 (s, 1H), 5.93 (d, 1H, 1'-H, J = 7.8 Hz), 5.59 (d, 1H, D$_2$O exchangeable), 5.37 (d, 1H, D$_2$O exchangeable), 4.55 (m, 2H), 4.24 (d, 1H), 3.90 (d, 1H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 142.14, 141.01, 132.41, 126.16, 125.85, 120.36, 113.45, 89.31, 74.42, 73.31, 70.13. Anal. Calcd for C$_{11}$H$_9$Cl$_3$N$_2$O$_3$: C, H, N.

2-Bromo-5,6-dichloro-1-(2,3-di-O-acetyl-β-D-erythrofuranosyl)benzimidazole (7b). 2-Bromo-5,6-dichlorobenzimidazole[12] (**5b**; 1.0 g, 3.8 mmol) was suspended in dry CH$_3$CN (20 mL). BSA (1.3 mL, 5.2 mmol) was added to this suspension and the resulting solution stirred at room temperature for 20 min. 1,2,3-Tri-O-acetyl-D-erythrofuranose (D-**6**; 1.0 g, 4.1 mmol) in CH$_3$CN (15 mL) was added and this was followed by TMSOTf (1.3 mL, 7.0 mmol). The reaction mixture was stirred at ambient temperature for 2 h. Saturated NaHCO$_3$(aq) solution (100 mL) was added and the resulting mixture extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered and evaporated in vacuo to give the crude product as a foam, which was purified using flash column chromatography (SiO$_2$, EtOAc/hexane 1:2). The appropriate fractions were combined and the solvent removed in vacuo to yield, after recrystallization from MeOH, 1.3 g (75%) of **7b** as white crystals: mp 168–170 °C; R_f 0.2 (EtOAc/hexane 1:2); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.99 (s, 1H), 6.17 (d, 1H, J = 7.9 Hz, 1'-H), 5.81 (m, 1H), 5.61 (t, 1H), 4.79 (dd, 1H), 4.12 (d, 1H), 2.17 (s, 3H), 1.98 (s, 3H). Anal. Calcd for C$_{15}$H$_{13}$BrCl$_2$N$_2$O$_5$: C, H, N.

2-Bromo-5,6-dichloro-1-(β-D-erythrofuranosyl)benzimidazole (8b). Compound **7b** (0.4 g, 0.88 mmol) was dissolved in EtOH (40 mL). To this solution was added Na$_2$CO$_3$ (0.62 g, 5.84 mmol) in water (40 mL) and the resulting mixture

stirred for 4 h, then neutralized with acetic acid and concentrated under reduced pressure. The suspension was triturated with water and the solid collected by filtration. This solid was recrystallized from MeOH to give 275 mg (85%) of **8b** as a white crystalline solid: mp 138–139 °C; R_f 0.5 (EtOAc/hexane 2:1); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.00 (s, 1H), 7.88 (s, 1H), 5.93 (d, 1H, 1′-H, $J = 7.9$ Hz), 5.56 (d, 1H, D$_2$O exchangeable), 5.35 (d, 1H, D$_2$O exchangeable), 4.55 (m, 2H), 4.24 (m, 1H), 3.88 (d, 1H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 142.60, 132.58, 132.43, 125.93, 125.68, 120.17, 113.23, 90.19, 74.31, 73.10, 70.10. Anal. Calcd for $C_{11}H_9BrCl_2N_2O_3$: C, H, N.

2-Isopropylamino-5,6-dichloro-1-(β-D-erythrofuranosyl)benzimidazole (9). Compound **7a** (0.66 g, 1.6 mmol) was treated with isopropylamine (5 mL) and the resulting solution stirred at room temperature for 7 days. The solution was concentrated under reduced pressure and purified by flash column chromatography (SiO$_2$, EtOAc/hexane 2:1). Fractions containing the product were pooled, solvent was removed in vacuo and the resulting solid was recrystallized from aqueous ethanol to give 450 mg (80%) of **9** as a white solid: mp 185–187 °C dec; R_f 0.33 (EtOAc/hexane 1:1); ^{1}H NMR (360 MHz, DMSO-d_6) δ 7.40 (s, 1H), 7.30 (s, 1H), 6.60 (d, 1H, D$_2$O exchangeable), 5.76 (d, 1H, 1′-H, $J = 7.8$ Hz), 5.33 (d, 1H, D$_2$O exchangeable), 5.23 (d, 1H, D$_2$O exchangeable), 4.42 (m, 2H), 4.20 (m, 1H), 4.01 (m, 1H), 3.82 (d, 1H), 1.21 (s, 3H), 1.19 (s, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 155.78, 143.66, 131.88, 123.10, 119.95, 115.92, 110.96, 86.49, 73.64, 72.36, 69.72, 44.58, 22.50, 22.40. Anal. Calcd for $C_{14}H_{17}Cl_2N_3O_3$: C, H, N.

2-Cyclopropylamino-5,6-dichloro-1-(β-D-erythrofuranosyl)benzimidazole (10). Compound **7a** (0.7 g, 1.7 mmol) was added to cyclopropylamine (5 mL) and treated as described for **9** above to give 480 mg (87%) of **10** as a white crystalline solid: mp 223–225 °C; R_f 0.23 (EtOAc/hexane 2:1); ^{1}H NMR (360 MHz, DMSO-d_6) δ 7.47 (s, 1H), 7.33 (s, 1H), 7.08 (d, 1H, D$_2$O exchangeable), 5.69 (d, 1H, 1′-H, $J = 7.8$ Hz), 5.29 (d, 1H, D$_2$O exchangeable), 5.20 (d, 1H, D$_2$O exchangeable), 4.42 (m, 2H), 4.19 (m, 1H), 3.81 (d, 1H), 2.75 (m, 1H), 0.70 (m, 2H), 0.51 (m, 2H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 156.84, 143.51, 132.00, 123.19, 120.30, 116.33, 111.07, 86.60, 73.62, 72.33, 69.68, 25.22, 6.55, 6.20. Anal. Calcd for $C_{14}H_{15}Cl_2N_3O_3$: C, H, N.

5,6-Dichloro-2-mercaptobenzyl-1-(β-D-erythrofuranosyl)benzimidazole (11). Compound **7a** (0.57 g, 1.4 mmol) was dissolved in THF (5 mL) and to this solution were added mercaptobenzyl alcohol (2 mL) and sodium hydride (80% sodium hydride, 220 mg, 5.6 mmol). The resulting solution was stirred at ambient temperature for 8 h. Water was added to the reaction mixture and extracted with EtOAc (3 × 50 mL). The organic phase was extracted several times with 10% NaOH(aq), then dried over magnesium sulfate and concentrated in vacuo to give a solid. This solid was purified by flash chromatography (SiO$_2$, EtOAc/hexane 1:2), fractions containing product were combined and evaporated to a solid and the solid was recrystallized from aqueous ethanol to give 500 mg (87%) of **11** as a white solid: mp 136–138 °C dec; R_f 0.2 (EtOAc/hexane 1:2); ^{1}H NMR (360 MHz, DMSO-d_6) δ 7.89 (s, 1H), 7.77 (s, 1H), 7.47 (m, 2H), 7.31 (m, 3H), 5.78 (d, 1H, 1′-H, $J = 7.7$ Hz), 5.50 (d, 1H, D$_2$O exchangeable), 5.33 (m, 1H, D$_2$O exchangeable), 4.61 (dd, 2H), 4.45 (m, 2H), 4.20 (m, 1H), 3.85 (d, 1H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 154.35, 142.87, 136.82, 134.00, 129.10, 128.55, 127.54, 124.73, 124.34, 118.94, 112.43, 88.76, 74.09, 73.47, 70.08, 35.76. Anal. Calcd for $C_{18}H_{16}Cl_2N_2O_3S·C_2H_5OH$: C, H, N.

2-Bromo-3,4-dichloro-6-nitroaniline (13). 4,5-Dichloro-2-nitroaniline (**12**; 41.0 g, 198 mmol) was dissolved in DMF (200 mL) and to this solution was added *N*-bromosuccinimide (42.9 g, 241 mmol). The resulting solution was stirred at 100 °C for 1 h. The mixture was allowed to cool to room temperature and then poured into a flask containing ice–water (1 L). A yellow solid formed and was collected by filtration. This solid was dissolved in CH$_2$Cl$_2$ (2 L) and the organic phase washed with water (3 × 500 mL). The organic phase was dried over magnesium sulfate, filtered and concentrated in vacuo to give 48.6 g (86%) of **13** as a solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.3 (s, 1H), 7.5 (br s, 2H). Anal. Calcd for $C_6H_3BrCl_2N_2O_2$: C, H, N.

3-Bromo-4,5-dichloro-1,2-phenylenediamine (14). Compound **13** (48.3 g, 169 mmol) was suspended in ethanol (1 L) and the resulting suspension cooled to 0 °C. Concentrated HCl (37%, 193 mL, 2.36 mol) was added dropwise to this suspension over a period of 15 min. The resulting reaction mixture was heated at reflux temperature for 1 h and subsequently cooled to room temperature. The mixture was diluted with water (1.5 L) and the pH of this mixture was adjusted to 8 by the addition of solid NaHCO$_3$. The mixture was extracted with EtOAc (3 × 300 mL), the extracts were dried over magnesium sulfate and filtered, and the filtrate was concentrated in vacuo to give a solid. Recrystallization of the solid from aqueous methanol gave 34.2 g (79%) of **14** as a solid: mp 128–129 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.69 (s, 1H), 5.24 (broad s, 2H), 5.06 (broad s, 2H). Anal. Calcd for $C_6H_5BrCl_2N_2$: C, H, N.

4-Bromo-5,6-dichloro-2-isopropylaminobenzimidazole (15). Compound **14** (25.0 g, 97.7 mmol) and isopropyl isothiocyanate (11.2 g, 110.5 mmol) were added to anhydrous pyridine (0.5 L). The resulting mixture was heated to 80 °C for 30 min, then 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (56.8 g, 134.1 mmol) was added as a solid in one portion. The resulting mixture was allowed to stir at 80–90 °C for 1 h. The reaction was subsequently allowed to cool to ambient temperature. Solids were removed from the mixture by filtration and the filtrate was concentrated under reduced pressure to afford a solid. This solid was dissolved in EtOAc (200 mL) and the organic phase washed with water (100 mL) and saturated NaCl(aq) (100 mL), dried over magnesium sulfate, filtered and concentrated to a solid. This solid was purified using flash column chromatography (SiO$_2$, EtOAc/hexane 7:3). The appropriate fractions were combined and evaporated to give 19.2 g (62%) of **15** as a tan solid: mp 247–249 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.1 (broad s, 1H), 7.28 (s, 1H), 7.17 (s, 1H), 3.87 (m, 1H), 1.17 (d, 6H). Anal. Calcd for $C_{10}H_{10}BrCl_2N_3·0.08C_6H_{14}$: C, H, N.

4-Bromo-5,6-dichloro-2-isopropylamino-1-(β-D-erythrofuranosyl)benzimidazole (17). A mixture of 4-bromo-5,6-dichloro-2-isopropylaminobenzimidazole (**15**; 1.19 g, 3.69 mmol), ClCH$_2$CH$_2$Cl (30 mL) and BSA (0.96 mL, 3.87 mmol) was heated to 80 °C for 30 min. TMSOTf (0.46 mL, 2.39 mmol) was added and the mixture stirred for 15 min, then 1,2,3-tri-*O*-acetyl-D-erythrofuranose (D-**6**; 1.0 g, 4.1 mmol) was added and the reaction stirred for 12 h at 80 °C. The reaction mixture was allowed to cool to room temperature and was then diluted with 5% NaHCO$_3$ (35 mL) and CH$_2$Cl$_2$ (20 mL). This mixture was stirred for 30 min, the phases were separated and the aqueous phase was extracted with additional CH$_2$Cl$_2$ (2 × 20 mL). The combined organic phases were dried over magnesium sulfate, filtered and concentrated in vacuo. The resulting residue was purified using flash column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 60:1). The appropriate fractions were combined and evaporated to give 0.76 g of 4-bromo-5,6-dichloro-2-isopropylamino-1-(2,3-di-*O*-acetyl-β-D-erythrofuranosyl)benzimidazole, **16**. Compound **16** was deprotected using Na$_2$CO$_3$ (0.24 g, 2.29 mmol) in aqueous ethanol in a similar fashion as described above. This gave, after purification using flash column chromatography (SiO$_2$, EtOAc/hexane 7:3), 0.13 g (25%) of **17** as a white solid: mp 200–205 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.41 (s, 1H), 6.89 (d, 1H), 5.83 (d, 1H), 5.39 (d, 1H), 5.32 (d, 1H), 4.51 (m, 2H), 4.23 (m, 2H), 3.88 (d, 1H), 1.28 (d, 6H). Anal. Calcd for $C_{14}H_{16}BrCl_2N_3O_3·0.35C_6H_{14}$: C, H, N.

1,2,3-Tri-*O*-acetyl-L-erythrofuranose (L-6). L-Erythrofuranose[29] (5.0 g, 41.6 mmol), DMAP (0.2 g, 1.67 mmol) and acetic anhydride (23.61 mL, 249.5 mmol) were added to pyridine (150 mL) and the reaction stirred for 3 h at room temperature. The reaction mixture was then poured into a flask containing saturated NaHCO$_3$ (100 mL). The product was extracted with CH$_2$Cl$_2$ (500 mL) and concentrated. The residual syrup was dissolved in EtOAc (2 × 250 mL) and washed with 0.1 N HCl (200 mL), and the organic phase was dried over MgSO$_4$, filtered and concentrated. The product was

purified using flash column chromatography (SiO$_2$, hexane/EtOAc 3:1). Appropriate fractions were obtained to give 4.35 g (42%) of L-**6** as a yellow syrup: Spectral data was identical with that for the referenced D-enantiomer. Anal. Calcd for C$_{10}$H$_{14}$O$_7$: C, H.

5,6-Dichloro-2-isopropylaminobenzimidazole[30] (19a). 4,5-Dichloro-1,2-phenylenediamine (**18**; 80.02 g, 452 mmol), isopropyl isothiocyanate (48.86 g, 483 mmol), 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (249 g, 587 mmol) and pyridine (1.15 L) were used as previously described for the synthesis of **15**. The product was recrystallized from dioxane to afford 79 g (72%) of **19a** as a tan solid: mp 218−220 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.7 (broad s, 1H), 7.25 (s, 2H), 6.80 (d, 1H), 3.87 (m, 1H), 1.16 (d, 6H). Anal. Calcd for C$_{10}$H$_{11}$Cl$_2$N$_3$: C, H, N.

5,6-Dichloro-2-isopropylamino-1-(β-L-erythrofuranosyl)benzimidazole (21a). 5,6-Dichloro-2-isopropylaminobenzimidazole (**19a**; 1.0 g, 4.09 mmol), BSA (1.06 mL, 4.29 mmol), TMSOTf (0.51 mL, 2.66 mmol), 1,2,3-tri-*O*-acetyl-L-erythrofuranose (L-**6**; 1.10 g, 4.49 mmol) and ClCH$_2$CH$_2$Cl (25 mL) were used as described above for **17** to give, after flash column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 30:1), 0.45 g (26%) of **20a** as a brown solid. This solid (0.34 g, 0.79 mmol) was deprotected using Na$_2$CO$_3$ (0.13 g, 1.23 mmol) in an aqueous mixture of ethanol and methanol in a similar fashion as described above for **17**. This gave 0.17 g (63%) of **21a** as a white solid: mp 180−181 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.38 (s, 1H), 7.28 (s, 1H), 6.58 (d, 1H), 5.75 (d, 1H), 5.30 (d, 1H), 5.21 (d, 1H), 4.41 (m, 2H), 4.19 (m, 1H), 4.02 (m, 1H), 3.80 (d, 1H), 1.19 (d, 6H). Anal. Calcd for C$_{14}$H$_{17}$Cl$_2$N$_3$O$_3$: C, H, N.

2-Cyclopropylamino-5,6-dichlorobenzimidazole (19b). 4,5-Dichloro-1,2-phenylenediamine (**18**; 6.04 g, 34.1 mmol), cyclopropyl isothiocyanate (3.69 g, 37.2 mmol), 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (20.1 g, 47.4 mmol) and pyridine (135 mL) were used as previously described for **19a**. The product was recrystallized from acetonitrile to afford 5.82 g (70%) of **19b** as a yellow solid: mp 223−225 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.2 (s, 1H), 7.43 (s, 1H), 7.27 (s, 2H), 2.49 (m, 1H), 0.70 (m, 2H), 0.49 (m, 2H). Anal. Calcd for C$_{10}$H$_9$Cl$_2$N$_3$: C, H, N.

2-Cyclopropylamino-5,6-dichloro-1-(β-L-erythrofuranosyl)benzimidazole (21b). 2-Cyclopropylamino-5,6-dichlorobenzimidazole (**19b**; 1.0 g, 4.13 mmol), BSA (1.00 mL, 4.30 mmol), TMSOTf (0.52 mL, 2.68 mmol), 1,2,3-tri-*O*-acetyl-L-erythrofuranose (L-**6**; 1.12 g, 4.54 mmol) and ClCH$_2$CH$_2$Cl (25 mL) were used as described above for **21a** to give, after flash column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 98:2), 1.81 g of **20b** as a solid. Compound **20b** (1.81 g) was deprotected using Na$_2$CO$_3$ (0.67 g, 6.34 mmol) in an aqueous mixture of methanol and ethanol in a similar fashion as described above for **21a**. This gave, after flash column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 99:1), 0.18 g (13%) of **21b** as a white solid: mp 201−202 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.54 (s, 1H), 7.39 (s, 1H), 7.15 (s, 1H), 5.74 (d, 1H), 5.36 (d, 1H), 5.26 (d, 1H), 4.47 (m, 2H), 4.24 (br s, 1H), 3.86 (d, 1H), 2.81 (br s, 1H), 0.74 (m, 2H), 0.55 (m, 2H). Anal. Calcd for C$_{14}$H$_{15}$Cl$_2$N$_3$O$_3$: C, H, N.

3,4,5-Trichloro-1,2-phenylenediamine (22). 4,5-Dichloro-2-nitroaniline (**12**; 15.2 g, 73.2 mmol) and *N*-chlorosuccinimide (12.2 g, 91.6 mmol) were dissolved in DMF (150 mL). The resulting orange solution was heated to 100 °C for 1 h. After cooling to room temperature this solution was poured into ice water (1.2 L). A yellow solid formed which was collected by filtration and dissolved in CH$_2$Cl$_2$, and the organic phase was washed with water, dried (MgSO$_4$), filtered and concentrated to give 17.6 g (98%) of 2,3,4-trichloro-6-nitroaniline as a yellow solid. The solid (17.6 g, 72.8 mmol) was dissolved in EtOH (400 mL) and iron powder (14.0 g, 250 mmol) was added and the resulting suspension was cooled to 0 °C. Concentrated HCl (93 mL, 1.14 mol) was added dropwise over a period of 15 min. When the addition was complete, the suspension was heated at reflux for 3.5 h, then allowed to cool to room temperature. The resulting suspension was diluted with water (2 L) and the pH of the mixture adjusted to 8 by the slow addition of solid Na$_2$CO$_3$. The aqueous phase was extracted with EtOAc (3 × 700 mL) and the organic phase dried (MgSO$_4$), filtered and concentrated under reduced pressure. Purification using flash column chromatography (SiO$_2$, EtOAc/hexane 35:65) gave 10.9 g (72%) of **22** as a tan solid: mp 113−115 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.66 (s, 1H), 5.24 (broad s, 2H), 5.10 (broad s, 2H). Anal. Calcd for C$_6$H$_5$Cl$_3$N$_2$: C, H, N.

2-Isopropylamino-4,5,6-trichlorobenzimidazole (23). 3,4,5-Trichloro-1,2-phenylenediamine (**22**; 3.12 g, 14.8 mmol), isopropyl isothiocyanate (1.62 g, 16.0 mmol) and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (8.4 g, 19.9 mmol) in anhydrous pyridine (50 mL) were used as described for **19a,b** to give, after recrystallization from dioxane, 2.9 g (72%) of **23** as a tan solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.2 (s, 1H), 7.34 (s, 1H), 7.20 (d, 1H), 3.93 (m, 1H), 1.25 (d, 6H). Anal. Calcd for C$_{10}$H$_{10}$Cl$_3$N$_3$·0.10C$_4$H$_8$O$_2$: C, H, N.

2-Isopropylamino-4,5,6-trichloro-1-(β-L-erythrofuranosyl)benzimidazole (25). 2-Isopropylamino-4,5,6-trichlorobenzimidazole (**23**; 0.86 g, 3.10 mmol), BSA (0.80 mL, 3.26 mmol), TMSOTf (0.39 mL, 2.01 mmol), 1,2,3-tri-*O*-acetyl-L-erythrofuranose (L-**6**; 0.84 g, 3.41 mmol) and ClCH$_2$CH$_2$Cl (25 mL) were used as described above for **21a,b** to give, after flash column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 60:1), 0.28 g (20%) of **24** as a white foam. This foam (0.05 g, 0.12 mmol) was deprotected using Na$_2$CO$_3$ (0.02 g, 0.17 mmol) in an aqueous mixture of methanol and ethanol in a similar fashion as described above for **21a,b**. This gave, after flash column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 98:2), 0.04 g (91%) of **25** as a white solid: mp 199−200 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.30 (s, 1H), 6.80 (d, 1H), 5.76 (d, 1H), 5.31 (d, 1H), 5.23 (d, 1H), 4.44 (m, 2H), 4.19 (m, 1H), 4.09 (m, 1H), 3.80 (d, 1H), 1.2 (d, 6H). Anal. Calcd for C$_{14}$H$_{16}$Cl$_3$N$_3$O$_3$: C, H, N.

In Vitro Antiviral Evaluation. Cell lines were subcultured according to conventional procedures previously described.[31] Stock preparations of HCMV and HSV-1 were prepared as described elsewhere.[26,31] The effect of compounds on the replication of HCMV was measured using plaque reduction assays,[31] a yield reduction assay using a multiplicity of infection = 0.5 PFU/mL,[32] and a DNA hybridization assay.[33] Effects on HSV-1 replication were measured in an ELISA assay.[34] Three 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 the plaque assays;[17] (ii) effect of compounds on proliferation of KB cells during two population doublings of the controls was determined by crystal violet staining and spectrophotometric quantification of dye eluted from stained cells as described earlier;[35] (iii) cytotoxicity in MOLT 4 cells (human T-cell lymphocytic leukemia cell line) was performed as described elsewhere.[36]

Dose−response relationships for most assays were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. For yield reduction experiments, the log of virus titer was regressed against log drug concentration. Fifty-percent inhibitory concentrations (IC$_{50}$'s) or IC$_{90}$'s (yield experiments) were calculated from the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV) were used in all assays.

Time of Drug Addition Protocol. Ninety-six-well culture plates were seeded at 12 500 HFF cells/well in MEM(E) with 10% fetal bovine serum (FBS) and penicillin plus streptomycin (pen/strep) the evening before infection. On the day of infection, medium was removed from the plates and replaced with 180 μL of virus inoculum. The inoculum was prepared by diluting previously titered stock virus (Towne strain) in MEM(E) with 5% FBS + pen/strep to obtain a multiplicity of infection of 0.5 PFU/cell. At each time point, 20 μL of 10× drug prepared in MEM(E), 5% FBS + pen/strep was added to a subset of wells on the plates. Each drug was tested in duplicate at each time point. The drugs were prepared prior to infection and were

stored at −80 °C between time points. In addition, duplicate virus control cultures received medium without drug at each time point. At 96 h post-infection, all plates were frozen at −80 °C. Subsequently the plates were thawed and virus titer in each well was determined by end-point dilution and plaque enumeration as detailed previously.[32]

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