Design, Synthesis, and Antiviral Evaluation of 2-Substituted 4,5-Dichloro- and 4,6-Dichloro-1-β-D-ribofuranosylbenzimidazoles as Potential Agents for Human Cytomegalovirus Infections[1]

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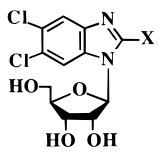
The syntheses of 2,4,6-trichlorobenzimidazole (**4a**) and 2-bromo-4,6-dichlorobenzimidazole (**4b**) were accomplished via the 2-amino intermediate (**3**) using a mild diazotization procedure. Ribosylation of **4a** and **4b** and subsequent deprotection afforded the corresponding 2,4,6-trichloro-1-β-D-ribofuranosylbenzimidazole (**7a**) and 2-bromo-4,6-dichloro-1-β-D-ribofuranosylbenzimidazole (**7b**). The 2-azido (**10**), 2-amino (**11**), 2-thione (**13**), 2-methylthio (**14a**), and 2-benzylthio (**14b**) derivatives were prepared via displacement reactions at the 2-position of the 2,3,5-tri-*O*-acetyl derivative of **7a**. 2,4,5-Trichlorobenzimidazole (**17a**) and 2-bromo-4,5-dichlorobenzimidazole (**17b**) were synthesized from the corresponding 1,2-phenylenediamines via successive cyclization with cyanogen bromide and diazotization in the presence of an appropriate cupric halide. Ribosylation of compounds **17a** and **17b** was followed by deprotection to afford 2,4,5-trichloro-1-β-D-ribofuranosylbenzimidazole (**20a**), and 2-bromo-4,5-dichloro-1-β-D-ribofuranosylbenzimidazole (**20b**). Heterocycles (**3**, **4a, 17a**) and nucleosides (**7a,b**, **8**, **10**, **11**, **13**, **14a,b**, **20a,b**) were evaluated for activity against human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1) and for cytotoxicity. The 2-chloro but not the 2-amino heterocycles were active against HCMV (IC_{50}'s = 5−8 μM) but not HSV-1; both also were somewhat cytotoxic to uninfected cells (IC_{50}'s = 32−100 μM). Among the nucleosides, the 2-chloro and 2-bromo analogs in both the 4,5- and 4,6-dichloro series (**20a,b**, **7a,b**, respectively) were active against HCMV (IC_{50}'s = 1−10 μM) and noncytotoxic in their antiviral dose ranges. The 2-bromo compounds were more active than the 2-chloro analogs; the 2-azido and 2-thiobenzyl analogs (**10**, **14b**) were weakly active against HCMV, but this activity was not well separated from cytotoxicity. None of the nucleosides were active against HSV-1. This pattern of activity and cytotoxicity is similar to that of the 2-chloro- and 2-bromo-5,6-dichloro analogs (TCRB, BDCRB) which we reported previously. Although these new 4,5- and 4,6-dichloro analogs are potent and selective inhibitors of HCMV, they are not as potent at TCRB and BDCRB.

Introduction

Human cytomegalovirus (HCMV) is the most common sight- and life-threatening opportunistic viral infection in immunocompromised individuals such as AIDS patients.[2,3] HCMV infection is also the primary cause of death in recipients of allogeneic bone marrow and renal transplantation.[4−6] The treatment of HCMV infection is difficult because few therapeutic options are available. Many well-known antiviral drugs, such as vidarabine, interferons, and acyclovir, have been tested and were not efficacious against HCMV.[7−10] Only ganciclovir (DHPG), foscarnet (PFA), and cidofovir (HPMPC) have been approved by the FDA for the treatment of HCMV diseases.[11−14] Although the treatment of HCMV infection with these drugs has produced clinical improvement in a large proportion of patients, the drugs suffer from poor oral bioavailability, low potency, and certain adverse effects.[12−14] In addition, drug-resistant HCMV has emerged in some patients.[15] Therefore, more active and less toxic HCMV agents which act by new mechanisms to circumvent resistance are still needed.

In our search for a more potent and nontoxic agent for the treatment of HCMV infection, we evaluated a number of benzimidazole nucleosides previously prepared in our laboratory.[16,17] We found that certain benzimidazole nucleoside analogs, e.g., 2,5,6-trichloro-1-β-D-ribofuranosylbenzimidazole (TCRB) and 2-bromo-5,6-dichloro1-β-D-ribofuranosylbenzimidazole (BDCRB), were highly active and selective against HCMV *in vitro* and were essentially noncytotoxic.[17,18] Both compounds act by a new mechanism involving inhibition of DNA processing.[19] As part of our comprehensive structure−activity relationship study of those benzimidazole ribonucleosides, we now have synthesized isomers of TCRB, 2,4,5- and 2,4,6-trichloro-1-β-D-ribofuranosylbenzimidazole, the corresponding isomers of BDCRB, and certain 2-substituted derivatives. The present work describes the synthesis and antiviral activity of these TCRB and BDCRB analogs.



X=Cl, TCRB
X=Br, BDCRB

Chemistry

2-Chlorobenzimidazole derivatives have been frequently synthesized via the chlorination of benzimid-

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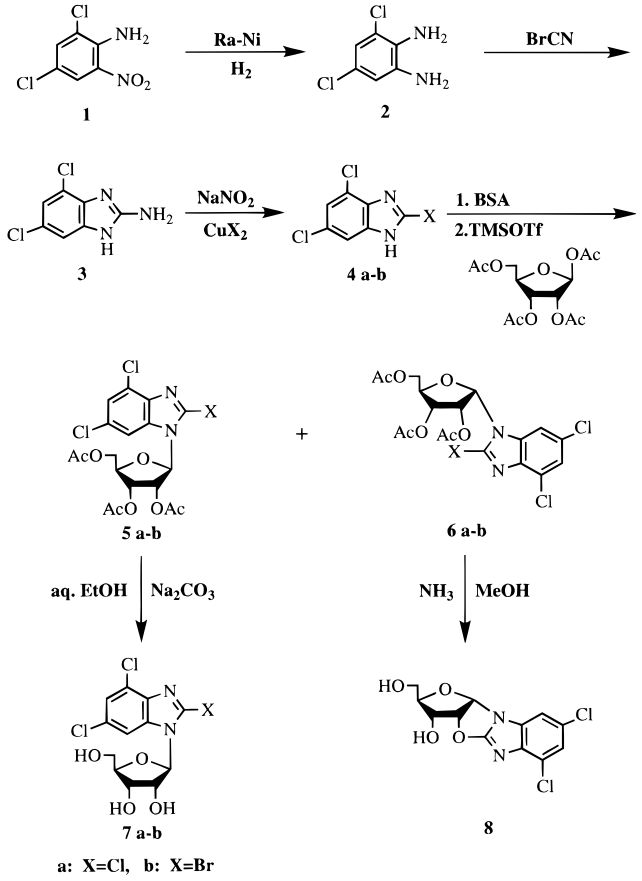
Scheme 1



azol-2-ones with phosphoryl chloride.[20,21] However, the application of this method to certain halogenated benzimidazol-2-ones has provided more capricious results.[22,23] Dimerization and other side reactions have resulted in diminished yields and difficulties in product purification. This prompted us to initiate an investigation designed to provide a generally applicable procedure for the synthesis of not only the 2-chlorobenzimidazoles but also the 2-bromobenzimidazoles.

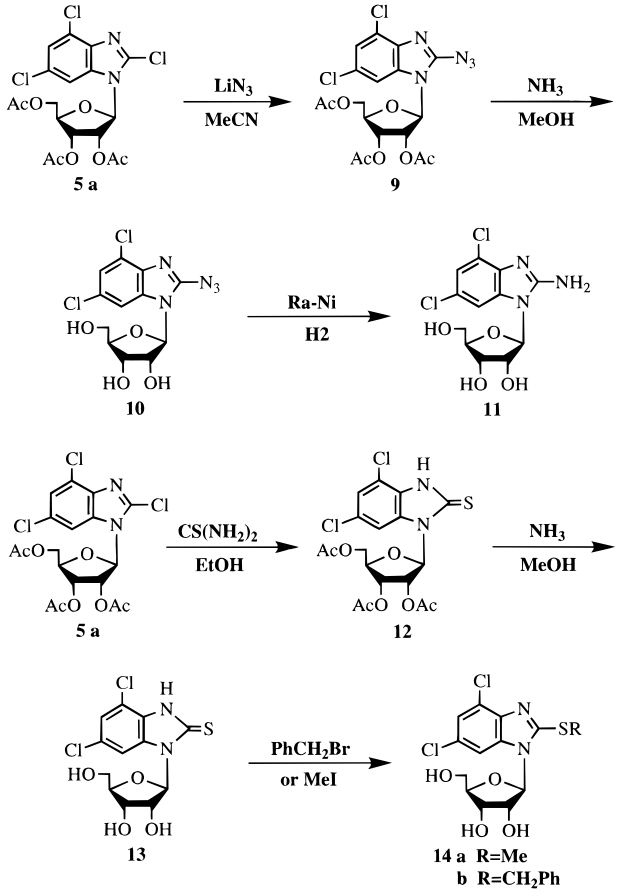
The route we envisaged for the 2-substituted 4,6-dichloro series of compounds used 2,4-dichloro-6-nitroaniline (**1**) as our starting material. Hydrogenation of **1** over Raney nickel has been reported[24] to give 3,5-dichloro-1,2-phenylenediamine (**2**) in 58% yield (Scheme 1). Subsequent cyclization of **2** with cyanogen bromide has also been reported[22] to afford 2-amino-4,6-dichlorobenzimidazole (**3**), but no yield was given. With modifications of the literature procedures, we were able to prepare **3** from **1** in 95% overall yield. Diazotization of **3** with sodium nitrite in aqueous cupric chloride solution afforded the desired 2,4,6-trichlorobenzimidazole (**4a**). This mild diazotization in aqueous cupric chloride medium alleviated the problem of forming a hydrolysis side product under the typical conditions of an aqueous diazotization reaction in which concentrated HCl solution was employed as the reaction medium. Similar diazotization of **3** in aqueous cupric bromide solution gave the corresponding 2-bromo-4,6-dichlorobenzimidazole (**4b**) in a good yield. Glycosylation of **4a** and **4b** with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose yielded what were subsequently established as the β nucleosides **5a** and **5b** as the major products along with a small amount of the α anomers (**6a** and **6b**). Deprotection of the β nucleosides **5a** and **5b** gave 2,4,6-trichloro-1-β-D-ribofuranosylbenzimidazole (an isomer of TCRB) and 2-bromo-4,6-dichloro-1-β-D-ribofuranosylbenzimidazole (an isomer of BDCRB), respectively. Similar deprotection of the α anomers **6a** and **6b** resulted in a removal of the acetyl protecting groups and a concomitant cyclization to give a 2,2′-*O*-cyclonucleoside (**8**).

The major products (**5a,b**) of the glycosylation reactions were initially assigned as the β anomers since the preferential formation of the β anomer was expected with a protected sugar bearing a participating group at the 2-position.[25] This was also in agreement with the assignment of an α configuration to the minor products **6a,b** whose α configuration was established by a facile formation of a 2,2′-*O*-cyclonucleoside (**8**) upon deprotection. Additional support for the anomeric assignments were provided by comparing the 1′-H chemical shifts of the anomeric pairs.[26] The 1′-H signals of the major products **5a** (δ 6.26) and **5b** (δ 6.23) were found at higher (∼0.46 ppm) field than those of the corresponding anomers **6a** (δ 6.72) and **6b** (δ 6.69), and therefore it was consistent with the assignment of **5a,b** as β anomers. The unequivocal assignments were based on a nuclear Overhauser enhancement (NOE) experiment in which the 1′-H of **7a** was irradiated and the NOE at the 4′-H and 3′-H was measured. The observation of an NOE of the 4′-H signal and no NOE at the 3′-H proved its β configuration.[27,28] The assignment of the sugar attachment to the heterocycle as 1-ribosyl-2,4,6-trichloro (vs 1-ribosyl-2,5,7-trichloro) was based on an NOE experiment in which a 5.5% of enhancement of the 2′-H signal of **7a** was observed when 7-H was irradiated.

Successive treatment of **5a** with lithium azide at an elevated temperature and deprotection with methanolic ammonia gave 2-azido-4,6-dichloro-1-β-D-ribofuranosylbenzimidazole (**10**) in good yield. Subsequent hydrogenation of **10** over Raney nickel furnished 2-amino-4,6-dichloro-1-β-D-ribofuranosylbenzimidazole (**11**). Treatment of **5a** with thiourea followed by deprotection afforded the 2-thione derivative (**13**). Alkylation of **13** with methyl iodide and benzyl bromide gave excellent yields of the corresponding 2-methylthio (**14a**) and the 2-benzylthio (**14b**) analogs.

The route we envisaged for the 2-substituted 4,5-dichloro series of compounds used 3,4-dichloro-1,2-phenylenediamine (**15**) as our starting material. Compound **15** was cyclized with cyanogen bromide to give 2-amino-4,5-dichlorobenzimidazole (**16**) in an excellent overall yield (Scheme 3). Diazotization of **16** in the presence of cupric chloride afforded the desired 2,4,5-trichlorobenzimidazole (**17a**). Parallel diazotization of **16** in the presence of cupric bromide gave the corresponding 2-bromo-4,5-dichlorobenzimidazole (**17b**). Silylation of **17a** and **17b** with BSA and subsequent coupling with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose yielded the β nucleosides **19a** and **19b** as the major products along with a small amount of the α anomers (**18a** and **18b**). Deprotection of the β nucleosides **19a** and **19b** with sodium carbonate in an aqueous alcoholic medium gave 2,4,5-trichloro-1-β-D-ribofuranosylbenzimidazole (**20a**, an isomer of TCRB) and 2-bromo-4,5-dichloro-1-β-D-ribofuranosylbenzimidazole (**20b**, an isomer of BDCRB), respectively.

Scheme 2



The major products (**19a**,**b**) of the glycosylation reactions were assigned as the β anomers since the preferential formation of the β anomer was expected with a protected sugar bearing a participating group at the 2-position (Baker's trans rule).[25] Additional support for the anomeric assignments was provided by comparing the 1′-H chemical shifts of the anomeric pairs.[26] The 1′-H signals of the major products were found at a higher (~0.45 ppm) field than those of the corresponding anomers and therefore the major products are the β anomers. In the case of compounds **19a**,**b** and **20a**,**b**, the sugar attachment to the heterocycle was assigned as 1-ribosyl-2,4,5-trichloro (vs 1-ribosyl-2,6,7-trichloro) because the steric hindrance of the 2-chloro and the 4-chloro would be expected to prevent the attack of the sugar on the N-3. This assignment was also in agreement with our observation on the glycosylation products of other 2,4-disubstituted benzimidazoles, whose attachment was unequivocally established by the difference NOE experiments.[27,28]

Antiviral Studies

The benzimidazole heterocycles, cyclonucleoside, and ribosides were evaluated for activity against HCMV and HSV-1 and for cytotoxicity in two cell lines. The 2-chloro heterocycles **4a** and **17a** were active against HCMV at concentrations similar to that for ganciclovir (Table 1). In contrast to ganciclovir, both compound were inactive against HSV-1 and more cytotoxic (IC_{50} = 32 μM). Thus the activity against HCMV was less specific than that of ganciclovir. The 2-amino analog **3** was inactive against the viruses and was not cytotoxic (Table 1). Similarly, the cyclonucleoside **8** was inactive against HCMV and was not cytotoxic at concentrations up to 100 μM (data not shown).

Among the ribonucleosides, activity against HCMV was observed in both the 4,5- and 4,6-dichloro series but only with the 2-chloro and 2-bromo analogs **7a**,**b** and **20a**,**b** (Table 2). None of the compounds were active against HSV-1. Antiviral activity of the 2-thiomethyl and 2-thiobenzyl analogs **14a,b** was observed only at higher concentrations; consequently it was probably a consequence of cytotoxicity. These results are very similar to those obtained by us in a previous study[29] wherein the 2-thiobenzyl analog in the 5,6-dichloro series of benzimidazole nucleosides was somewhat active against HCMV but the activity was not well separated from cytotoxicity. In contrast, the activity of the four 2-halogen-substituted ribonucleosides (**7a,b** and **20a,b**) was well separated from cytotoxicity. Activity against HCMV in both plaque and yield reduction assays was similar to that of the 2,5,6-trichloro and 2-bromo-5,6-dichloro analogs TCRB and BDCRB, which we reported previously (see ref 17a and Table 2). Similar to the relative potencies of TCRB and BDCRB, the 2-bromo analogs **7b** and **20b** were the more active compounds in both HCMV assays. Also like TCRB and BDCRB, both compounds in both the 4,5- and 4,6-dichloro series effected multi-$\log_{10}$ reductions in HCMV titers at noncytotoxic concentrations. Specifically all four compounds reduced HCMV titers 10000-fold at 10−100 μM. These concentrations, however, are higher than those required for TCRB and BDCRB to produce a similar effect: due to dose−response curves with greater slopes, TCRB gave 10^4−10^5-fold titer reductions at 32 μM compared to 3−10 μM for BDCRB and 100 μM for ganciclovir.[17a]

Taken together, the data in Tables 1 and 2 confirm our prior observation[17a] that the addition of ribose to a polyhalogenated benzimidazole reduces cytotoxicity and retains or increases activity against HCMV. Furthermore, our results show that chlorine in the 4-position is nearly as effective as chlorine in the 5-position for producing activity against HCMV. We conclude, therefore, that the activity of the new 2-halo-4,6-dichloro and 2-halo-4,5-dichloro analogs **7a,b** and **20a,b** against HCMV is greater than that of ganciclovir but is slightly less than that of the 2-halo-5,6-dichloro analogs TCRB and BDCRB.

Experimental Section

General Method. Melting points (MP) were taken on a Thomas-Hoover Unimelt apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WM-360 spectrometer operating in FT mode. The chemical shift values were reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. Mass (MS) spectra were determined by the Mass Spectrometry Laboratory of the Chemistry Department, University of Michigan. High-resolution MS measurements were obtained on a VG 70-250-S MS spectrometer using a direct probe for sample introduction. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Chemical reactions and column chromatographic separations were followed by thin layer chromatography (TLC) on silica gel precoated glass plates (layer thickness 0.2 mm) purchased from Analtech, Inc. The TLC plates were observed under UV light (254 nm). Evaporations were effected using a Buchler flash-evaporator equipped with a Dewar "dry ice" condenser under water aspirator or mechanical oil pump vacuum at 40 °C or cooler unless otherwise specified.

Scheme 3

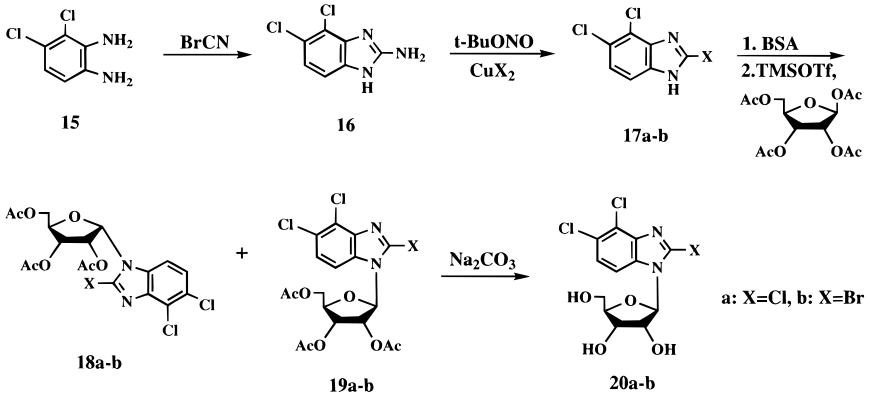
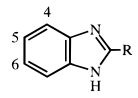


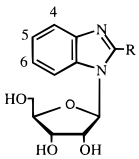
Table 1. Antiviral Activity and Cytotoxicity of 2-Substituted Dichlorobenzimidazoles



		50 or 90% inhibitory concentration (μM)				
		antiviral activity				
		HCMV[a]		HSV-1[b]	cytotoxicity[c]	
no.	R	plaque	yield	ELISA	visual	growth
4,6-dichloro benzimidazoles						
3	NH_2	>100[d]	–	>100[e]	100	–
4a	Cl	5.1[e]	–	>100[e]	32[e]	>100
4,5-dichloro benzimidazole						
17a	Cl	8		>100	32	80
foscarnet[f]		39±26	–	–	>100	–
ganciclovir (DHPG)[g]		7.4±6.5	1.6±1.2	3.5±2.1	>100	>100

[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] A plaque assay was used to determine the activity of DHPG against HSV-1; all other compounds were assayed by ELISA in quadruplicate wells. [c] Visual cytotoxicity was scored on HFF cells at time of HCMV plaque enumeration. Inhibition of KB cell growth was determined as described in the text in quadruplicate assays. Results are presented as IC_{50}'s. [d] >100 indicates IC_{50} or IC_{90} not reached at the noted (highest) concentration tested. [e] Average derived from two experiments. [f] Average ± standard deviation from 15 experiments. [g] Average ± standard deviation from 108, 33, and 3 experiments, respectively.

Table 2. Antiviral Activity and Cytotoxicity of 2-Substituted Dichlorobenzimidazole Ribonucleosides



		50 or 90% inhibitory concentration (μM)				
		antiviral activity				
		HCMV[a]		HSV-1[b]	cytotoxicity[c]	
no.	R	plaque	yield	ELISA	visual	growth
4,6-dichloro benzimidazole nucleosides						
7a	Cl	10	4.5	>300	77	230
7b	Br	1.6	2.1	>300	127	150
10	N_3	110	41[e]	–	>100	–
11	NH_2	>100	>100[e]	>300	>100	>300
13	SH	>100	–	>300	>100	>300[e]
14a	SCH_3	110	–	>300	150	270
14b	SBz	41	–	140	32	122
4,5-dichloro benzimidazole nucleosides						
20a	Cl	16	6.0	>100[e]	>100	>100[e]
20b	Br	2.4	1.0[e]	>100[e]	161	>100
5,6-dichloro benzimidazole nucleosides						
TCRB[f,g]	Cl	2.9	1.4	102	240	210
BDCRB[f,g]	Br	0.7	0.2	130	118	>100

[a–d] See Table 1 for footnotes *a*–*d*. [e] Average derived from a single experiment. All other data are averages of two to four experiments. [f] TCRB and BDCRB, the 2,5,6-trichloro and 2-bromo-5,6-dichloro analogs respectively, published previously as compounds **9** and **11** in ref 17a. [g] Averages derived from three to six experiments for each assay listed.

3,5-Dichloro-1,2-phenylenediamine (2) and 2-Amino-4,6-dichlorobenzimidazole (3). A mixture of **1** (purchased from MTM Research Chemicals, Lancaster Synthesis Inc., 8.28 g, 40 mmol) and 0.80 g (wet wt) of Raney nickel in 200 mL of EtOH was hydrogenated at 50 psi of H_2 at room temperature for 7 h. The reaction mixture was filtered, and the solid was washed with portions of MeOH. The filtrate and washings were combined, evaporated, and coevaporated with MeOH to give a brown foam (**2**). This product was directly used in the subsequent reaction without further purification. An analytical sample was obtained by recrystallization of the brown foam from CCl_4: mp 60−62 °C (lit.[21] mp 60−61 °C); HRMS (EI) *m*/*z* 175.9918 (100, M^+ = 175.9908); ^{1}H NMR (DMSO-d_6) δ 6.54, 6.50 (2 d, 2, 4-H and 6-H, J_{4-6} = 2.5 Hz), 5.15, 4.77 (2 br s, 4, 1-NH_2 and 2-NH_2).

This brown foam (**2**) was dissolved in 80 mL of MeOH, and this solution was added dropwise to a stirred solution of 5 M BrCN/MeCN (8.8 mL) in 80 mL of H_2O. After the addition had been completed, stirring was continued at room temperature for 4 h. The reaction mixture was concentrated to ∼80 mL and then was washed with EtOAc (100 mL). The EtOAc phase was extracted with H_2O (100 mL). The combined H_2O extracts were neutralized with a saturated $NaHCO_3$ solution to ∼pH 8, and the resulting suspension was extracted with EtOAc (400 mL). The EtOAc phase was washed successively with H_2O (200 mL) and a saturated NaCl solution (200 mL), dried (Na_2SO_4), and evaporated to give 7.698 g (95% from **1**) of **3** as a black foam. This product showed a single spot on TLC (R_f = 0.3, MeOH/$CHCl_3$, v/v) and was used directly in the subsequent reaction without further purification. An analytical sample was obtained by recrystallization from MeCN: mp 224−226 °C (lit.[22] mp 221−224 °C); HRMS: (EI) *m*/*z* 200.9866 (100, M^+ = 200.9861); ^{1}H NMR (DMSO-d_6) δ 11.42, 11.08 (2 br s*, 1, 1-NH), 7.10, 6.99 (d, s, 2, 5-H and 7-H, J_{5-7} = 2.0 Hz), 6.62 (br s, 2, 2-NH_2). Anal. ($C_7H_5Cl_2N_3$) C, H, N. [*The two broad singlets (combined integration as one proton) for 1-NH indicated the existence of two tautomers.]

2,4,6-Trichlorobenzimidazole (4a). To a solution of $CuCl_2/H_2O$ (67.23 g/250 mL), a solution of $NaNO_2/H_2O$ (10.35 g, 150 mmol/50 mL) was added dropwise and this was followed by a solution of compound **3**/MeOH (10.10 g, 50 mmol/50 mL). After the addition was complete, stirring was continued at room temperature for 1 h. The reaction mixture was then heated on a steam bath for 1 h. During this period of heating, an additional fresh $NaNO_2/H_2O$ solution (31.05 g, 450 mmol/150 mL) was added dropwise. The reaction mixture was cooled to room temperature, diluted with 500 mL of 1 *N* HCl solution, and extracted with EtOAc (500 mL). The EtOAc solution was filtered and the filtrate was washed with a 1 *N* HCl solution (300 mL), a sat. NaCl solution (300 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (4 × 20 cm, eluted with 1%, 2% MeOH/$CHCl_3$, v/v). Evaporation of fractions 15−75 (20 mL per fraction) and recrystallization from MeCN gave 5.156 g (47%) of **4a** as a crystalline compound. MP: 231−232 °C. HRMS: (EI) *m*/*z* 219.9357 (100%, M^+ = 219.9362). ^{1}H NMR (DMSO-d_6): δ 13.92 (br s, 1, 1-NH), 7.58, 7.44 (s, d, 2, 5-H and 7-H). Anal. ($C_7H_3Cl_3N_2$) C, H, N.

2-Bromo-4,6-dichlorobenzimidazole (4b). To a solution of $CuBr_2/H_2O$ (5.584 g, 25 mmol/10 mL) was added dropwise a solution of $NaNO_2/H_2O$ (0.518 g, 7.5 mmol/5 mL). The reaction mixture was stirred at room temperature for 10 min. Compound **3** (1.01 g, 5 mmol) was added portionwise over 5 min, and stirring was continued at room temperature for 2 h with the dropwise addition over a 2 h period of a fresh $NaNO_2/H_2O$ solution (1.36 g, 15 mmol/10 mL). The reaction mixture was then heated on a steam bath for 10 min, cooled to room temperature, and treated with 25 mL of a 1 N HBr solution and 100 mL of EtOAc for 5 min with vigorous stirring. The mixture was then transferred to a separatory funnel, and the two layers were separated. The EtOAc layer was washed successively with a 0.5 N HBr solution (100 mL) and a saturated $NaHCO_3$ solution (100 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (1.9 × 20 cm, eluted with 1:9 EtOAc/hexane). Evaporation of fractions 9−23 (15 mL per fraction) and recrystallization from MeCN gave 0.745 g (two crops, 56%) of **4b** as brownish crystals: mp 207−209 °C; HRMS (EI) *m/z* 263.8844 (63, $M^+ = 263.8857$); ^{1}H NMR (DMSO-d_6) δ 13.88 (br s, 1, 1-NH), 7.58, 7.41 (s, d, 2, 5-H and 7-H, $J_{5-7} = 1.5$ Hz). Anal. ($C_7H_3BrCl_2N_2$) C, H, N.

2,4,6-Trichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-benzimidazole (5a) and 2,4,6-Trichloro-1-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyl)benzimidazole (6a). To a suspension of **4a** (1.362 g, 6.15 mmol) in 31 mL of dry MeCN was added 1.52 mL (6.15 mmol) of BSA. The reaction mixture was stirred at 80 °C for 15 min to give a clear solution. This solution was treated with 2.153 g (6.675 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and 1.426 mL (7.380 mmol) of trimethylsilyl triflate (TMSOTf) at 80 °C for 1 h. The reaction mixture was cooled and diluted with EtOAc (150 mL). The EtOAc solution was washed with a saturated $NaHCO_3$ solution (150 mL × 2) and a saturated NaCl solution (150 mL), dried (Na_2SO_4), and evaporated. The residue was co-evaporated with MeOH and then suspended in 50 mL of hot MeOH. The suspension was cooled and filtered, and the solid product was washed with MeOH to give 2.103 g of **5a** as white crystals. (This product showed a single spot on TLC, $R_f = 0.75$, 2% MeOH/$CHCl_3$, v/v.) The mother liquor was evaporated to dryness, and the residue was chromatographed on a silica column (2.5 × 20 cm, eluted with $CHCl_3$ and 0.5% MeOH/$CHCl_3$, v/v). Evaporation of fractions 20−24 (20 mL per fraction) and recrystallization from MeOH gave an additional 0.258 g of **5a** as white crystals. The total yield of **5a** was 2.361 g (80%): mp 198−200 °C; HRMS (EI) *m/z* 480.0069 (4, for $C_{18}H_{17}{}^{35}Cl_2{}^{37}ClN_2O_7$: $M^+ = 480.0072$); ^{1}H NMR (DMSO-d_6) δ 7.89 (d, 1, 7-H, $J_{7-5} = 2.0$ Hz), 7.58 (d, 1, 5-H), 6.26 (d, 1, 1′-H, $J_{1'-2'} = 7.0$ Hz), 5.54 (t, 1, 2′-H, $J_{2'-3'} = 7.0$ Hz), 5.44 (dd, 1, 3′-H, $J_{3'-4'} = 4.5$ Hz), 4.49, 4.38 (2 m, 3, 4′-H and 5′-H), 2.139, 2.137, 2.02 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 169.8, 169.4, 169.1 (3 *C*OCH$_3$), 140.9 (C2), 137.4 (C3a), 134.1 (C7a), 128.5 (C6), 123.7 (C4), 123.3 (C5), 111.1 (C7), 86.9 (C1′), 79.6 (C4′), 70.5 (C2′), 68.6 (C3′), 62.5 (C5′), 20.5, 20.2, 19.9 (3 CO*C*H$_3$). Anal. ($C_{18}H_{17}Cl_3N_2O_7$) C, H, N.

Evaporation of fractions 29−39 (20 mL per fraction) gave 0.09 g (3%) of **6a** as a syrup: HRMS: (EI) *m/z* 480.0069 (6, for $C_{18}H_{17}{}^{35}Cl_2{}^{37}ClN_2O_7$: $M^+ = 480.0072$); ^{1}H NMR (DMSO-d_6) δ 7.74 (d, 1, 7-H, $J_{7-5} = 2.0$ Hz), 7.53 (d, 1, 5-H), 6.72 (d, 1, 1′-H, $J_{1'-2'} = 4.5$ Hz), 5.72 (t, 1, 2′-H, $J_{2'-3'} = 5.0$ Hz), 5.51 (dd, 1, 3′-H, $J_{3'-4'} = 7.0$ Hz), 4.84 (m, 1, 4′-H), 4.37 (dd, 1, 5′-H, $J_{5'-4'} = 3.5$ Hz, $J_{5'-5''} = 12.0$ Hz), 4.27 (dd, 1, 5″-H, $J_{5''-4'} = 5.5$ Hz), 2.09, 2.04, 1.58 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 170.0, 169.1, 168.3 (3 *C*OCH$_3$), 140.6 (C2), 137.2 (C3a), 134.9 (C7a), 127.9 (C6), 123.2 (C4), 122.7 (C5), 112.1 (C7), 86.5 (C1′), 78.4 (C4′), 70.9 (C2′), 70.4 (C3′), 62.7 (C5′), 20.4, 20.0, 19.4 (3 CO*C*H$_3$).

2-Bromo-4,6-dichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (5b) and 2-Bromo-4,6-dichloro-1-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyl)benzimidazole (6b). To a suspension of **4b** (0.532 g, 2 mmol) in 20 mL of dry MeCN was added 0.5 mL (2 mmol) of BSA. The reaction mixture was stirred at 75 °C for 10 min to give a clear solution. This solution was treated with 0.7 g (2.2 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and 0.58 mL (3 mmol) of TMSOTf at 75 °C for 30 min. The reaction mixture was cooled to room temperature and diluted with EtOAc (100 mL). The EtOAc solution was washed with a saturated $NaHCO_3$ solution (100 mL × 2), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (1.9 × 40 cm, eluted with $CHCl_3$). Evaporation of fractions 10−24 (20 mL per fraction) and recrystallization from MeCN gave 0.678 g of **5b** as white crystals. A second crop of 0.187 g was obtained by recrystallization of the mother liquor from MeOH. The total yield of **5b** was 0.865 g (83%): mp 194−197 °C (MeCN); HRMS (EI with a DCI probe) *m/z* 521.9589 (4, $M^+ = 521.9596$); ^{1}H NMR (DMSO-d_6) δ 7.88 (d, 1, 7-H, $J_{7-5} = 2.0$ Hz), 7.57 (d, 1, 5-H), 6.23 (d, 1, 1′-H, $J_{1'-2'} = 7.0$ Hz), 5.54 (t, 1, 2′-H, $J_{2'-3'} = 7.5$ Hz), 5.44 (dd, 1, 3′-H, $J_{3'-4'} = 4.5$ Hz), 4.50, 4.37 (2 m, 3, 4′-H and 5′-H), 2.15, 2.14, 2.01 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 170.0, 169.5, 169.2 (3 *C*OCH$_3$), 139.1 (C3a), 134.4 (C7a), 131.3 (C2), 128.4 (C6), 123.6 (C4), 123.2 (C5), 111.1 (C7), 87.9 (C1′), 79.7 (C4′), 70.4 (C2′), 68.6 (C3′), 62.6 (C5′), 20.7, 20.3, 20.0 (3 × CO*C*H$_3$). Anal. ($C_{18}H_{17}BrCl_2N_2O_7$) C, H, N.

Evaporation of fractions 29−39 (20 mL per fraction) gave 0.028 g (3%) of **6b** as a syrup: HRMS (EI with a DCI probe) *m/z* 521.9600 (6, $M^+ = 521.9596$); ^{1}H NMR (DMSO-d_6) δ 7.72 (d, 1, 7-H, $J_{7-5} = 1.5$ Hz), 7.53 (d, 1, 5-H), 6.69 (d, 1, 1′-H, $J_{1'-2'} = 4.5$ Hz), 5.70 (t, 1, 2′-H, $J_{2'-3'} = 5.0$ Hz), 5.53 (dd, 1, 3′-H, $J_{3'-4'} = 7.0$ Hz), 4.85 (m, 1, 4′-H), 4.36 (dd, 1, 5′-H, $J_{5'-4'} = 3.5$ Hz, $J_{5'-5''} = 12.5$ Hz), 4.28 (dd, 1, 5″-H, $J_{5''-4'} = 5.5$ Hz), 2.09, 2.04, 1.54 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 170.2, 169.4, 168.37 (3 *C*OCH$_3$), 138.8 (C3a), 135.4 (C7a), 130.7 (C2), 127.9 (C6), 123.1 (C4), 122.7 (C5), 112.2 (C7), 87.6 (C1′), 78.4 (C4′), 71.0 (C2′), 70.6 (C3′), 63.0 (C5′), 20.6, 20.2, 19.6 (3 CO*C*H$_3$).

2,4,6-Trichloro-1-β-D-ribofuranosylbenzimidazole (7a). To a solution of Na_2CO_3 (0.053 g, 0.5 mmol) in H_2O (1 mL) were added successively 4.5 mL of EtOH, 4.5 mL of MeOH, and 0.24 g (0.5 mmol) of **5a**. The reaction mixture was stirred at room temperature for 2 h. AcOH (0.06 mL) was added, and stirring was continued at room temperature for 15 min. Volatile materials were removed by evaporation. The residue was triturated with H_2O (10 mL × 2) and recrystallized from MeOH/H_2O to give 0.135 g (76%) of **7a** as white crystals: mp 176−177 °C; HRMS (EI) *m/z* 351.9795 (13, $M^+ = 351.9784$); ^{1}H NMR (DMSO-d_6) δ 8.36 (d, 1, 7-H, $J_{7-5} = 2.0$ Hz), 7.52 (d, 1, 5-H), 5.89 (d, 1, 1′-H, $J_{1'-2'} = 8.0$ Hz), 5.49 (d, 1, 2′-OH, $J_{2'-2'OH} = 6.5$ Hz), 5.38 (t, 1, 5′-OH, $J_{5'-5'OH} = 4.5$ Hz), 5.28 (d, 1, 3′-OH, $J_{3'-3'OH} = 4.5$ Hz), 4.41 (m, 1, 2′-H, $J_{2'-3'} = 5.5$ Hz), 4.14 (m, 1, 3′-H, $J_{3'-4'} = 2.0$ Hz), 4.02 (m, 1, 4′−H, $J_{4'-5'} = J_{4'-5''} = 2.5$ Hz), 3.71 (m, 2, 5′-H and 5″-H, $J_{5'-5''} = 12.0$ Hz); ^{13}C NMR (DMSO-d_6) δ 141.6 (C2), 137.5 (C3a), 134.2 (C7a), 128.0 (C6), 123.2 (C4), 122.8 (C5), 112.6 (C7), 89.4 (C1′), 86.5 (C4′), 71.7 (C2′), 69.7 (C3′), 61.0 (C5′). Anal. ($C_{12}H_{11}Cl_3N_2O_4$) C, H, N.

2-Bromo-4,6-dichloro-1-β-D-ribofuranosylbenzimidazole (7b). To a solution of Na_2CO_3 (0.106 g, 1 mmol) in H_2O (2 mL) were added successively 9 mL of EtOH, 9 mL of MeOH, and 0.524 g (1 mmol) of **5b**. The reaction mixture was stirred at room temperature for 2 h. AcOH (0.12 mL) was added, and stirring was continued at room temperature for 15 min. Volatile materials were removed by evaporation. The residue was triturated with H_2O (20 mL × 2) and recrystallized from MeCN to give 0.31 g (3 crops, 78%) of **7b** as white crystals: mp 175−176 °C; HRMS (EI) *m/z* 395.9290 (5, $M^+ = 395.9279$); ^{1}H NMR (DMSO-d_6) δ 8.37 (d, 1, 7-H, $J_{7-5} = 2.0$ Hz), 7.50 (d, 1, 5-H), 5.90 (d, 1, 1′-H, $J_{1'-2'} = 8.0$ Hz), 5.49 (d, 1, 2′-OH, $J_{2'-2'OH} = 6.5$ Hz), 5.40 (t, 1, 5′-OH, $J_{5'-5'OH} = 4.5$ Hz), 5.29 (d, 1, 3′-OH, $J_{3'-3'OH} = 4.0$ Hz), 4.42 (m, 1, 2′-H, $J_{2'-3'} = 5.5$ Hz), 4.14 (m, 1, 3′-H, $J_{3'-4'} = 2.0$ Hz), 4.02 (m, 1, 4′−H, $J_{4'-5'} = J_{4'-5''} = 2.5$ Hz), 3.71 (m, 2, 5′-H and 5″-H, $J_{5'-5''} = 12.0$ Hz); ^{13}C NMR (DMSO-d_6) δ 139.1 (C3a), 134.5 (C7a), 132.2 (C2), 127.9 (C6), 123.1 (C4), 122.7 (C5), 112.5 (C7), 90.4 (C1′), 86.5 (C4′), 71.6 (C2′), 69.7 (C3′), 61.1 (C5′). Anal. ($C_{12}H_{11}BrCl_2N_2O_4$) C, H, N.

4,6-Dichloro-1-α-D-ribofuranosylbenzimidazole 2,2′-*O*-cyclonucleoside (8). A mixture of 0.084 g (0.175 mmol) of **6a** in 10 mL of NH_3/MeOH (saturated at 0 °C) was stirred in a pressure bottle at room temperature for 5 h. Volatile materials were removed by evaporation and coevaporation

with MeOH (3×). The residue was recrystallized from MeOH/H_2O to give 38 mg (68%) of **8** as yellowish crystals: mp 204−206 °C; HRMS (EI) *m/z* 316.0005 (62, M^+ = 316.0018); ^{1}H NMR (DMSO-d_6) δ 7.57 (d, 1, 7-H, J_{7-5} = 1.5 Hz), 7.35 (d, 1, 5-H), 6.55 (d, 1, 1′-H, $J_{1'-2'}$ = 5.0 Hz), 5.79 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 7.0 Hz), 5.76 (t, 1, 2′-H, $J_{2'-3'}$ = 5.5 Hz), 4.87 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.0 Hz), 4.08 (m, 1, 3′-H, $J_{3'-4'}$ = 9.0 Hz), 3.67 (dd, 1, 5′-H, $J_{4'-5'}$ = 0 Hz, $J_{5'-5''}$ = 12.0 Hz), 3.55 (m, 1, 4′-H, $J_{4'-5''}$ = 5.0 Hz), 3.48 (m, 1, 5″-H); ^{13}C NMR (DMSO-d_6) δ 164.3 (C2), 142.2 (C3a), 130.5 (C7a), 125.1 (C6), 121.6 (C5 and C4), 109.1 (C7), 91.4 (C2′), 84.8 (C1′), 81.1 (C4′), 69.5 (C3′), 59.44 (C5′). Anal. ($C_{12}H_{10}Cl_2N_2O_4$) C, H, N.

2-Azido-4,6-dichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (9). To a mixture of 0.198 g (0.413 mmol) of **5a** in 5 mL of MeCN was added 0.202 g (4.13 mmol) of LiN_3. The reaction mixture was stirred at 80°C for 2 days. The mixture was then cooled to room temperature and partitioned between EtOAc/H_2O (50 mL/50 mL). The EtOAc layer was washed with a saturated NaCl solution (50 mL × 2), dried (Na_2SO_4), and evaporated. The residue was recrystallized from MeOH to give 0.161 g (2 crops, 80%) of **9** as a slightly yellowish crystalline compound: mp 140−142 °C; HRMS (EI) *m/z* 485.0494 (7, M^+ = 485.0505); ^{1}H NMR (DMSO-d_6) δ 7.81 (d, 1, 7-H, J_{7-5} = 2.0 Hz), 7.49 (d, 1, 5-H), 6.04 (d, 1, 1′-H, $J_{1'-2'}$ = 6.5 Hz), 5.59 (t, 1, 2′-H, $J_{2'-3'}$ = 6.5 Hz), 5.43 (dd, 1, 3′-H, $J_{3'-4'}$ = 4.5 Hz), 4.38 (m, 3, 4′-H and 5′-H), 2.13, 2.11, 2.03 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 169.9, 169.4, 169.2 (3 *C*OCH$_3$), 148.7 (C2), 137.1 (C3a), 134.1 (C7a), 126.9 (C6), 122.8 (C5), 122.4 (C4), 110.5 (C7), 85.6 (C1′), 79.3 (C4′), 70.6 (C2′). 69.0 (C3′), 62.7 (C5′), 20.5, 20.2, 20.1 (3 CO*C*H$_3$). Anal. ($C_{18}H_{17}Cl_2N_5O_7$) C, H, N.

2-Azido-4,6-dichloro-1-β-D-ribofuranosylbenzimidazole (10). A mixture of compound **9** (0.875 g, 1.8 mmol) and NH_3/MeOH (saturated at 0 °C, 18 mL) was stirred in a pressure bottle at room temperature for 4.5 h. Volatile materials were removed by evaporation and coevaporation with MeOH (3×). The residue was recrystallized from MeOH to give 0.605 g (2 crops, 93%) of **10** as a white crystalline compound: mp ∼154 °C dec; HRMS (EI with a DCI probe) *m/z* 359.0197 (50, M^+ = 359.0188); ^{1}H NMR (DMSO-d_6) δ 8.15 (d, 1, 7-H, J_{7-5} = 1.5 Hz), 7.43 (d, 1, 5-H), 5.63 (d, 1, 1′-H, $J_{1'-2'}$ = 7.5 Hz), 5.41 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.0 Hz), 5.27 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.0 Hz), 5.19 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 4.5 Hz), 4.39 (m, 1, 2′-H, $J_{2'-3'}$ = 5.5 Hz), 4.11 (m, 1, 3′-H, $J_{3'-4'}$ = 2.0 Hz), 3.95 (m, 1, 4′−H, $J_{4'-5'}$ = $J_{4'-5''}$ = 3.0 Hz), 3.67 (m, 2, 5′-H and 5″-H, $J_{5'-5''}$ = 12.0 Hz); ^{13}C NMR (DMSO-d_6) δ 149.1 (C2), 137.3 (C3a), 133.9 (C7a), 126.5 (C6), 122.3 (C5), 122.0 (C4), 112.1 (C7), 87.9 (C1′), 86.1 (C4′), 71.4 (C2′), 69.8 (C3′), 61.2 (C5′). Anal. ($C_{12}H_{11}Cl_2N_5O_4$) C, H, N.

2-Amino-4,6-dichloro-1-β-D-ribofuranosylbenzimidazole (11). A mixture of compound **10** (0.227 g, 0.63 mmol) and Raney nickel (0.05 g, wet wt) in EtOH (10 mL) was hydrogenated at 50 psi of H_2 at room temperature for 6 h. The reaction mixture was filtered, and the filtrate was evaporated. The residue was recrystallized from H_2O to give 0.143 g (2 crops, 68%) of **11** as a white powder: mp 135−140 °C dec; HRMS (EI with a DCI probe) *m/z* 333.0301 (24, M^+ = 333.0283); ^{1}H NMR (DMSO-d_6) δ 7.50 (d, 1, 7-H, J_{7-5} = 2.0 Hz), 7.10 (d, 1, 5-H), 7.06 (s, 2, 2-NH$_2$), 5.73 (d, 1, 1′-H, $J_{1'-2'}$ = 7.5 Hz), 5.57 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 4.5 Hz), 5.29 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 7.5 Hz), 5.25 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 4.0 Hz), 4.32 (m, 1, 2′-H, $J_{2'-3'}$ = 5.5 Hz), 4.09 (m, 1, 3′-H, $J_{3'-4'}$ = 2.0 Hz), 3.97 (m, 1, 4′−H, $J_{4'-5'}$ = 2.0 Hz, $J_{4'-5''}$ = 2.5 Hz), 3.67 (m, 2, 5′-H and 5″-H, $J_{5'-5''}$ = 12.0 Hz); ^{13}C NMR (DMSO-d_6) δ 155.6 (C2), 139.0 (C3a), 134.8 (C7a), 122.2 (C6), 120.1 (C5), 118.5 (C4), 108.5 (C7), 87.7 (C1′), 85.8 (C4′), 71.3 (C2′), 70.1 (C3′), 61.0 (C5′). Anal. ($C_{12}H_{13}Cl_2N_3O_4$) C, H, N.

4,6-Dichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-benzimidazole-2-thione (12) and 4,6-Dichloro-1-β-D-ribofuranosylbenzimidazole-2-thione (13). A mixture of **5a** (2.399 g, 5 mmol) and thiourea (0.76 g, 10 mmol) in EtOH (50 mL) was heated at 80 °C for 1 h (it became a clear solution). Volatile materials were then remove by evaporation. The residue was partitioned between H_2O/$CHCl_3$ (100 mL/100 mL). The H_2O layer was again extracted with $CHCl_3$ (50 mL). The combined $CHCl_3$ extracts were washed with a saturated NaCl solution (100 mL), dried (Na_2SO_4), evaporated, and coevaporated with MeOH to give **12** as a white foam. This material was directly used in the subsequent reaction without further purification. An analytical sample of **12** (as white crystals) was obtained by a combination of silica column chromatography (using $CHCl_3$ as eluant) and recrystallization (from MeOH): mp 195−197 °C; HRMS (EI with a DCI probe) *m/z* 476.0200 (12, M^+ = 476.0212); ^{1}H NMR (DMSO-d_6) δ 13.79 (s, 1, 3-NH), 7.65 (d, 1, 7-H, J_{7-5} = 1.5 Hz), 7.49 (d, 1, 5-H), 6.67 (d, 1, 1′-H, $J_{1'-2'}$ = 7.5 Hz), 5.67 (t, 1, 2′-H, $J_{2'-3'}$ = 7.0 Hz), 5.46 (dd, 1, 3′-H, $J_{3'-4'}$ = 4.5 Hz), 4.49 (dd, 1, 5′-H, $J_{5'-4'}$ = 4.5 Hz, $J_{5'-5''}$ = 12.0 Hz), 4.43 (m, 1, 4′-H), 4.32 (dd, 1, 5″-H, $J_{5''-4'}$ = 2.5 Hz), 2.15, 2.14, 1.98 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 171.0 (C2), 170.1, 169.6, 169.3 (3 *C*OCH$_3$), 131.9, 128.3, 127.5 (C3a, C7a, and C6), 123.1 (C5), 114.7 (C4), 109.5 (C7), 86.2 (C1′), 79.5 (C4′), 69.3 (C2′), 69.0 (C3′), 62.8 (C5′), 20.7, 20.4, 20.2 (3 CO*C*H$_3$). Anal. ($C_{18}H_{18}Cl_2N_2O_7S$) C, H, N.

The foam of crude **12** was treated with MeOH/NH_3 (saturated at 0 °C, 50 mL) in a pressure bottle at room temperature for 1 day. The reaction mixture was evaporated and coevaporated with MeOH (3×). The residue was recrystallized from MeOH/$CHCl_3$ (dissolving the residue in 10 mL of hot MeOH, diluting it with 90 mL of $CHCl_3$, and allowing it to stand) to give 1.509 g (2 crops, 86%) of **13** as white crystals: mp 213−215 °C; HRMS (EI with a DCI probe) *m/z* 349.9892 (17, M^+ = 349.9895). ^{1}H NMR (DMSO-d_6) δ 13.61 (s, 1, 3-NH), 8.13 (d, 1, 7-H, J_{7-5} = 1.5 Hz), 7.41 (d, 1, 5-H), 6.48 (d, 1, 1′-H, $J_{1'-2'}$ = 8.0 Hz), 5.35 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 4.5 Hz), 5.22 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.5 Hz), 5.16 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 4.0 Hz), 4.42 (m, 1, 2′-H, $J_{2'-3'}$ = 6.0 Hz), 4.13 (m, 1, 3′-H, $J_{3'-4'}$ = 2.0 Hz), 3.93 (m, 1, 4′-H), 3.67 (m, 2, 5′-H, and 5″-H, $J_{5'-4'}$ = $J_{5''-4'}$ = 2.5 Hz, $J_{5'-5''}$ = 12.0 Hz); ^{13}C NMR (DMSO-d_6) δ 171.6 (C2), 132.2, 128.2, 127.3 (C3a, C7a, and C6), 122.5 (C5), 114.1 (C4), 111.0 (C7), 88.4 (C1′), 85.6 (C4′), 70.6 (C2′), 69.8 C3′), 61.1 (C5′). Anal. ($C_{12}H_{12}Cl_2N_2O_4S$) C, H, N.

4,6-Dichloro-2-methylthio-1-β-D-ribofuranosylbenzimidazole (14a). To a solution of **13** (0.351 g, 1 mmol) in 10 mL of H_2O and 1 mL of concentrated NH_4OH was added 0.125 mL (2 mmol) of methyl iodide. The reaction mixture was stirred at room temperature for 2 h. The resulting suspension was filtered. The white solid was air dried and recrystallized from MeOH/H_2O to give 0.337 g (2 crops, 92%) of **14a** as white crystals: mp 202−205 °C; HRMS (EI with a DCI probe) *m/z* 364.0030 (39, M^+ = 364.0051); ^{1}H NMR (DMSO-d_6) δ 8.15 (d, 1, 7-H, J_{7-5} = 2.0 Hz), 7.38 (d, 1, 5-H), 5.69 (d, 1, 1′-H, $J_{1'-2'}$ = 7.5 Hz), 5.44 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.5 Hz), 5.32 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.0 Hz), 5.25 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 4.5 Hz), 4.40 (m, 1, 2′-H, $J_{2'-3'}$ = 5.5 Hz), 4.12 (m, 1, 3′-H, $J_{3'-4'}$ = 2.0 Hz), 3.98 (m, 1, 4′-H, $J_{4'-5'}$ = $J_{4'-5''}$ = 3.0 Hz), 3.69 (m, 2, 5′-H and 5″-H, $J_{5'-5''}$ = 12.0 Hz), 2.75 (s, 3, 2-SMe); ^{13}C NMR (DMSO-d_6) δ 155.5 (C2), 139.5 (C3a), 135.7 (C7a), 126.3 (C6), 121.9 (C4), 121.7 (C5), 111.8 (C7), 88.9 (C1′), 86.3 (C4′), 71.5 (C2′), 69.9 (C3′), 61.2 (C5′), 14.7 (2-SMe). Anal. ($C_{13}H_{14}Cl_2N_2O_4S$) C, H, N.

2-(Benzylthio)-4,6-dichloro-1-β-D-ribofuranosylbenzimidazole (14b). To a solution of **13** (0.351 g, 1 mmol) in 10 mL of H_2O and 1 mL of concentrated NH_4OH was added 0.238 mL (2 mmol) of benzyl bromide. The reaction mixture was stirred at room temperature for 2 h. The resulting suspension was filtered. The white solid was washed with portions of H_2O and dissolved in 50 mL of EtOAc. The EtOAc solution was washed with H_2O, dried (Na_2SO_4), and evaporated. The residue was triturated with hexane (10 mL × 2) and then recrystallized from EtOAc/hexane to give 0.418 g (95%) of **14b** as white crystals: mp 104−107 °C; HRMS (EI with a DCI probe) *m/z* 440.0364 (4, M^+ = 440.0364); ^{1}H NMR (DMSO-d_6) δ 8.16 (d, 1, 7-H, J_{7-5} = 2.0 Hz), 7.52 (m, 2, Ph), 7.40 (d, 1, 5-H), 7.31 (m, 3, Ph), 5.68 (d, 1, 1′-H, $J_{1'-2'}$ = 7.5 Hz), 5.43 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.5 Hz), 5.32 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.0 Hz), 5.24 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 4.5 Hz), 4.64 (m, 2, CH_2Ph), 4.37 (m, 1, 2′-H, $J_{2'-3'}$ = 5.5 Hz), 4.10 (m, 1, 3′-H, $J_{3'-4'}$ = 2.0 Hz), 3.96 (m, 1, 4′-H, $J_{4'-5'}$ = $J_{4'-5''}$ = 3.0 Hz), 3.67 (m, 2, 5′-H and 5″-H, $J_{5'-5''}$ = 12.0 Hz); ^{13}C NMR (DMSO-d_6) δ 154.1 (C2), 139.5 (C3a), 136.8 (Ph), 135.5 (C7a), 129.2, 128.5, 127.5 (Ph), 126.5 (C6), 122.1 (C4), 121.8 (C5), 111.9 (C7),

88.9 (C1′), 86.3 (C4′), 71.6 (C2′), 69.8 (C3′), 61.2 (C5′), 35.9 (2-SCH_2Ph). Anal. ($C_{19}H_{18}Cl_2N_2O_4S$) C, H, N.

2-Amino-4,5-dichlorobenzimidazole (16). To a stirred solution of 13.2 mL of 5 M BrCN/MeCN in 120 mL of H_2O was added dropwise a solution of 3,4-dichloro-1,2-phenylenediamine (10.60 g, 59.88 mmol) in 120 mL of MeOH at room temperature over 1 h. After the addition was complete, stirring was continued at room temperature for 2 h. The reaction mixture was concentrated to ∼120 mL and was washed with EtOAc (100 mL × 2). The combined EtOAc phase was extracted with H_2O (100 mL). The combined H_2O phase was neutralized with sat. $NaHCO_3$ solution to pH ∼8 and was extracted with EtOAc (800 mL). The EtOAc phase was washed with half-saturated NaCl solution (400 mL), dried (Na_2SO_4), and evaporated. The residue was recrystallized from MeCN to give 10.06 g of **16** as beige crystals. The mother liquor was evaporated, and the residue was suspended in a small amount of $CHCl_3$. The $CHCl_3$ suspension was filtered to give an additional 1.18 g of **16** as a solid (pure by TLC). The total yield of **16** was 11.24 g (93%): mp 239−241 °C; HRMS (EI) *m/z* 200.9872 (100, M^+ = 200.9861); ^{1}H NMR (DMSO-d_6) δ 11.44, 11.06 (2 br s, 1, 1-NH), 7.05, 699 (2 d, 2, 6-H and 7-H), 6.62, 6.28 (2 br s, 2, 2-NH_2). Anal. ($C_7H_5Cl_2N_3$) C, H, N.

2,4,5-Trichlorobenzimidazole (17a). To a mixture of $CuCl_2$ (1.345 g, 10 mmol) in 25 mL of acetone was added 0.99 mL (7.5 mmol) of 90% t-BuONO. The reaction mixture was stirred at room temperature for 20 min. Compound **16** (1.01 g, 5 mmol) was added portionwise over 15 min, and the reaction mixture was heated under gentle reflux for 2 h (with addition of 0.99 mL of fresh 90% t-BuONO every 30 min). The reaction mixture was then cooled to room temperature, treated with 50 mL of 2 N HCl solution, and extracted with 100 mL of EtOAc. The EtOAc layer was washed with 2 N HCl solution (50 mL × 2) and a saturated NaCl solution (50 mL), dried (Na_2SO_4), and evaporated. The residue was recrystallized from MeCN to give 0.498 (45%) of **17a** as a brownish solid. Analytical sample was obtained via silica column chromatography followed by recrystallization from MeCN: mp 244−247 °C; HRMS (EI with DCI probe) *m/z* 219.9372 (100, M^+ = 219.9362); ^{1}H NMR (DMSO-d_6) δ 13.89 (br s, 1, 1-NH), 7.46 (m, 2, 6-H and 7-H). Anal. ($C_7H_3Cl_3N_2$) C, H, N.

2-Bromo-4,5-dichlorobenzimidazole (17b). To a solution of $CuBr_2/H_2O$ (5.584 g, 25 mmol/10 mL) was added a solution of $NaNO_2/H_2O$ (0.518 g, 7.5 mmol/5 mL). The reaction mixture was stirred at room temperature for 10 min. Compound **16** (1.01 g, 5 mmol) was added portionwise over 5 min. Stirring was continued at room temperature for 2 h [with dropwise addition of a fresh solution of $NaNO_2/H_2O$ (1.36 g, 15 mmol/10 mL)] and then at 100 °C for 10 min. The reaction mixture was cooled to room temperature and treated with 100 mL of EtOAc and 25 mL of 1 N HBr solution for 5 min with vigorous stirring. The content was transferred to a separatory funnel, and the two layers were separated. The EtOAc layer was washed with a 0.5 N HBr solution (100 mL) and a saturated Na_2CO_3 solution (100 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (1.9 × 20 cm, eluted successively with 10%, 20%, 40% EtOAc/hexane). Evaporation of fractions 11−17 (15 mL/fraction) and recrystallization from MeCN gave 0.75 g (2 crops, 56%) of **17b** as yellowish crystals: mp 221−223 °C; HRMS (EI with DCI probe) *m/z* 263.8849 (65, M^+ = 263.8857); ^{1}H NMR (DMSO-d_6) δ 13.84 (br s, 1, 1-NH), 7.45 (m, 2, 6-H and 7-H). Anal. ($C_7H_3BrCl_2N_2$) C, H, N.

2,4,5-Trichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-benzimidazole (19a) and 2,4,5-Trichloro-1-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyl)benzimidazole (18a). To a suspension of **17a** (1.772 g, 8 mmol) in dry MeCN (40 mL) was added 3 mL (12 mmol) of BSA. The reaction mixture was stirred at ambient temperature for 15 min. This was treated with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (3.055 g, 9.6 mmol) and TMSOTf (3.092 mL, 16 mmol) at ambient temperature for 30 min. (It became a clear solution and then turned into a suspension again.) The reaction mixture was diluted with EtOAc (120 mL) and filtered. The solid was pure product **19a**. The filtrate was washed with 1:1 saturated $NaHCO_3$ solution/saturated NaCl solution (100 mL × 2), dried (Na_2SO_4), and evaporated. The residue was heated in 50 mL of MeOH and cooled, and the resultant suspension was filtered. The solid products were combined and recrystallized from MeCN to give 1.898 g of **19a** as white crystals. The mother liquor and the filtrate were combined and evaporated. The residue was chromatographed on a silica column (2 × 27 cm, eluted with $CHCl_3$). Evaporation of fractions 4−11 (15 mL per fraction) and recrystallization from MeCN gave an additional 0.905 g of **19a** as white crystals. The total yield of **19a** was 2.803 g (73%): mp 194−197 °C; HRMS (EI with DCI probe) *m/z* 480.0073 (6, M^+ = 480.0072 for $C_{18}H_{17}{}^{35}Cl_2{}^{37}ClN_2O_7$); ^{1}H NMR (DMSO-$d_6$) δ 7.80 (d, 1, 7-H, J_{7-6} = 9.0 Hz), 7.61 (d, 1, 6-H), 6.27 (d, 1, 1′-H, $J_{1'-2'}$ = 6.5 Hz), 5.53 (t, 1, 2′-H, $J_{2'-3'}$ = 7.0 Hz), 5.42 (m, 1, 3′-H, $J_{3'-4'}$ = 5.0 Hz), 4.42 (m, 3, 4′-H and 5′-H), 2.14, 2.11, 2.02 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 170.1, 169.6, 169.3 (3 *C*OCH_3), 141.3 (C2), 139.7 (C3a), 132.5 (C7a), 126.3 (C5), 125.2 (C6), 121.3 (C4), 111.9 (C7), 87.1 (C1′), 79.5 (C4′), 70.7 (C2′), 68.7 (C3′), 62.7 (C5′), 20.6, 20.3, 20.1 (3 × CO*C*H_3). Anal. ($C_{18}H_{17}Cl_3N_2O_7$) C, H, N.

Evaporation of fractions 13−17 (15 mL per fraction) gave 0.089 g (2%) of **18a** as a yellowish syrup: HRMS (EI with DCI probe) *m/z* 478.0089 (8, M^+ = 478.0101); ^{1}H NMR (DMSO-d_6) δ 7.70 (d, 1, 7-H, J_{7-6} = 9.0 Hz), 7.54 (d, 1, 6-H), 6.74 (d, 1, 1′-H, $J_{1'-2'}$ = 4.0 Hz), 5.68 (t, 1, 2′-H, $J_{2'-3'}$ = 5.0 Hz), 5.50 (dd, 1, 3′-H, $J_{3'-4'}$ = 7.5 Hz), 4.82 (m, 1, 4′-H, $J_{4'-5'}$ = 3.0 Hz, $J_{4'-5''}$ = 5.5 Hz), 4.37 (dd, 1, 5′-H, $J_{5'-5''}$ = 12.0 Hz), 4.26 (dd, 1, 5″-H), 2.08, 2.02, 1.54 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 170.2, 169.4, 168.5 (3 × *C*OCH_3), 140.8 (C2), 139.43 (C3a), 133.3 (C7a), 125.6 (C5), 124.7 (C6), 120.7 (C4), 113.3 (C7), 86.7 (C1′), 78.2 (C4′), 71.0 (C2′), 70.4 (C3′), 62.8 (C5′), 20.6, 20.2, 19.7 (3 CO*C*H_3).

2-Bromo-4,5-dichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (19b) and 2-Bromo-4,5-dichloro-1-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyl)benzimidazole (18b). A mixture of **17b** (0.532 g, 2 mmol) and BSA (0.5 mL) in dry MeCN (20 mL) was stirred at 75 °C for 10 min to give a clear solution. To this solution were added 0.70 g (2.2 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and 0.58 mL (3 mmol) of TMSOTf. Stirring was continued at 75 °C for 30 min. The reaction mixture was cooled to room temperature and diluted with EtOAc (100 mL). The EtOAc solution was washed with a saturated $NaHCO_3$ solution (100 mL × 2), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (2 × 40 cm, eluted with $CHCl_3$). Evaporation of fractions 9−23 (20 mL per fraction) and recrystallization from MeCN gave 0.918 g (2 crops, 88%) of **19b** as white crystals: mp 194−197 °C; HRMS (EI with DCI probe) *m/z* 521.9590 (7, M^+ = 521.9596); ^{1}H NMR (DMSO-d_6) δ 7.81 (d, 1, 7-H, J_{7-6} = 9.0 Hz), 7.59 (d, 1, 6-H), 6.24 (d, 1, 1′-H, $J_{1'-2'}$ = 7.0 Hz), 5.54 (t, 1, 2′-H, $J_{2'-3'}$ = 7.0 Hz), 5.42 (dd, 1, 3′-H, $J_{3'-4'}$ = 4.5 Hz), 4.45 (m, 3, 4′-H and 5′-H), 2.14, 2.12, 2.01 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 170.0, 169.5, 169.1 (3 *C*OCH_3), 141.3 (C3a), 132.8 (C7a), 131.6 (C2), 126.2 (C5), 125.0 (C6), 121.2 (C4), 111.7 (C7), 88.1 (C1′), 79.5 (C4′), 70.5 (C2′), 68.7 (C3′), 62.7 (C5′), 20.6, 20.3, 20.0 (3 CO*C*H_3). Anal. ($C_{18}H_{17}BrCl_2N_2O_7$) C, H, N.

Evaporation of fractions 27−33 (20 mL per fraction) gave 0.032 g (3%) of **18b** as a yellowish syrup: HRMS (EI with DCI probe) *m/z* 521.9617 (4, M^+ = 521.9596); ^{1}H NMR (DMSO-d_6) δ 7.70 (d, 1, 7-H, J_{7-6} = 9.0 Hz), 7.52 (d, 1, 6-H), 6.71 (d, 1, 1′-H, $J_{1'-2'}$ = 4.0 Hz), 5.67 (t, 1, 2′-H, $J_{2'-3'}$ = 5.0 Hz), 5.53 (dd, 1, 3′-H, $J_{3'-4'}$ = 7.5 Hz), 4.82 (m, 1, 4′-H, $J_{4'-5'}$ = 3.5 Hz, $J_{4'-5''}$ = 5.5 Hz), 4.36 (dd, 1, 5′-H, $J_{5'-5''}$ = 12.0 Hz), 4.28 (dd, 1, 5″-H), 2.08, 2.02, 1.51 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 170.2, 169.4, 168.5 (3 *C*OCH_3), 140.9 (C3a), 133.8 (C7a), 130.8 (C2), 125.5 (C5), 124.5 (C6), 120.6 (C4), 113.3 (C7), 87.8 (C1′), 78.2 (C4′), 70.9 (C2′), 70.5 (C3′), 63.0 (C5′), 20.6, 20.2, 19.7 (3 CO*C*H_3).

2,4,5-Trichloro-1-β-D-ribofuranosylbenzimidazole (20a). To a solution of Na_2CO_3 (0.106 g, 1 mmol) in H_2O (2 mL) were added successively 9 mL of EtOH, 9 mL of MeOH, and 0.480 g (1 mmol) of **19a**. The reaction mixture was stirred at room temperature for 2 h. AcOH (0.12 mL) was added, and stirring was continued at room temperature for 15 min. Volatile materials were removed by evaporation. The residue was

triturated with H_2O (20 mL) and recrystallized from MeCN to give 0.308 g (2 crops, 87%) of **20a** as white crystals: mp 178−180 °C; HRMS (EI with DCI probe) *m/z* 351.9774 (10, M^+ = 351.9784); ^{1}H NMR (DMSO-d_6): δ 8.09 (d, 1, 7-H, J_{7-6} = 9.0 Hz), 7.48 (d, 1, 6-H), 5.90 (d, 1, 1′-H, $J_{1'-2'}$ = 7.5 Hz), 5.53 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.5 Hz), 5.30 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 4.5 Hz), 5.29 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.0 Hz), 4.40 (m, 1, 2′-H, $J_{2'-3'}$ = 6.0 Hz), 4.13 (m, 1, 3′-H, $J_{3'-4'}$ = 2.0 Hz), 4.00 (m, 1, 4′-H, $J_{4'-5'}$ = 3.5 Hz, $J_{4'-5''}$ = 3.0 Hz), 3.70 (m, 2, 5′-H and 5″-H, $J_{5'-5''}$ = 12.0 Hz); ^{13}C NMR (DMSO-d_6) δ 142.0 (C2), 139.8 (C3a), 132.6 (C7a), 125.8 (C5), 124.6 (C6), 120.9 (C4), 113.2 (C7), 89.4 (C1′), 86.5 (C4′), 71.8 (C2′), 69.7 (C3′), 61.2 (C5′). Anal. ($C_{12}H_{11}Cl_3N_2O_4$) C, H, N.

2-Bromo-4,5-dichloro-1-β-D-ribofuranosylbenzimidazole (20b). To a solution of Na_2CO_3 (0.106 g, 1 mmol) in H_2O (2 mL) were added successively 9 mL of EtOH, 9 mL of MeOH, and 0.524 g (1 mmol) of **19b**. The reaction mixture was stirred at room temperature for 2 h. AcOH (0.12 mL) was added, and stirring was continued at room temperature for 15 min. Volatile materials were removed by evaporation. The residue was triturated with H_2O (20 mL × 2) and recrystallized from MeCN to give 0.338 g (2 crops, 85%) of **20b** as white crystals: mp 174−175 °C; HRMS (EI) *m/z* 395.9276 (11, M^+ = 395.9279); ^{1}H NMR (DMSO-d_6) δ 8.09 (d, 1, 7-H, J_{7-6} = 9.0 Hz), 7.46 (d, 1, 6-H), 5.90 (d, 1, 1′-H, $J_{1'-2'}$ = 8.0 Hz), 5.50 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.5 Hz), 5.30 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 4.5 Hz), 5.29 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.0 Hz), 4.43 (m, 1, 2′-H, $J_{2'-3'}$ = 5.5 Hz), 4.13 (m, 1, 3′-H, $J_{3'-4'}$ = 2.0 Hz), 4.00 (m, 1, 4′-H, $J_{4'-5'}$ = $J_{4'-5''}$ = 3.0 Hz), 3.70 (m, 2, 5′-H and 5″-H, $J_{5'-5''}$ = 12.0 Hz); ^{13}C NMR (DMSO-d_6) δ 141.4 (C3a), 132.9 (C7a), 132.5 (C2), 125.7 (C5), 124.4 (C6), 120.8 (C4), 112.9 (C7), 90.4 (C1′), 86.4 (C4′), 71.7 (C2′), 69.7 (C3′), 61.1 (C5′). Anal. ($C_{12}H_{11}BrCl_2N_2O_4$) C, H, N.

Cell Culture Procedures. The routine growth and passage of KB cells (human oral epidermoid carcinoma), BSC-1 cells (African green monkey kidney cells), and human foreskin fibroblasts (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 by using 0.05% trypsin plus 0.02% EDTA in a HEPES buffered salt solution.

Virological Procedures. The Towne strain, plaque-purified 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) per cell as detailed previously.[30] High titer HSV-1 stocks were prepared by infecting KB cells at an m.o.i. of <0.1 also as detailed previously.[30] 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.[31] 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 eleven 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, 2 or 3 days for HSV-1. Plaques were enumerated under 20-fold magnification in wells having the diludon which gave 5−20 plaques per well. Virus titers were calculated according to the following formula: Titer (pfu/mL) = number of plaques × 5 × 3^n; where *n* represents the *n*th 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 four to eight selected concentradons. After incubation at 37 °C for 7−10 days, cell sheets were fixed and stained with crystal violet and microscopic plaques were 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.

HCMV Yield Reduction Assay. The effect of compounds on the replication of HCMV also was measured using a yield reduction assay. HFF cells were planted as described above in 96-well cluster dishes and inoculated overnight, medium was removed, and the cultures were inoculated with HCMV in 0.1 mL at a moi of 0.5−1 pfu/cell as reported elsewhere.[31] After virus adsorption, 0.1 mL of fresh medium containing test compounds in twice the desired final concentration (2×) was added. This was accomplished by preparing a 2× solution of the highest desired concentrations and then serially diluting 1:3 along the eight wells. In this manner, six compounds could be tested in duplicate on a single plate with concentrations from 100 to 0.05 μM. Plates were incubated at 37 °C for 7 days and subjected to one cycle of freezing and thawing; aliquots from each of the eight wells of a given column were transferred to the first column of a fresh 96-well monolayer culture of HFF cells. Contents were mixed and serially diluted 1:3 across the remaining eleven columns of the secondary plate. Each column of the original primary plate was diluted across a separate plate in this manner. Cultures were incubated, plaques were enumerated, and titers were calculated as described above.

HSV-1 ELISA. An ELISA was employed[32] to detect HSV-1. Ninety-six-well cluster dishes were planted with 10 000 BSC-1 cells per well in 200 μL per 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 horse radish 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 per 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 infected by HCMV used in plaque assays.[30] (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.[33] Briefly, 96-well cluster dishes were planted with KB cells at 3000−5000 cells per well. After overnight incubation at 37 °C, test compound was added in quadruplicate at six to eight 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. Acidified ethanol was added and plates 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. Fifty-percent inhibitory concentrations (IC_{50}'s) 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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