Notes

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

Ruiming Zou,† John C. Drach, and Leroy B. Townsend*

Department of Medicinal Chemistry, College of Pharmacy, Department of Chemistry, College of Literature, Sciences and Arts, Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, Michigan 48109-1065

Received June 27, 1996[⊗]

2-Chloro-5,6-difluorobenzimidazole (**8**) was prepared from 4,5-difluoro-2-nitroaniline (**5**) via successive reduction, cyclization, and diazotization reactions. 2-Chloro-5,6-dibromobenzimidazole (**10**) was obtained by a direct bromination of 2-chlorobenzimidazole (**9**) with bromine−water. 2-Chloro-5,6-diiodobenzimidazole (**15**) was synthesized by a stepwise transformation of the nitro functions of 2-chloro-5,6-dinitrobenzimidazole (**11**) into iodo groups via diazotization reactions. Ribosylation of **8**, **10**, and **15** gave the respective β nucleosides **16a**−**c** as the major products along with a small amount of the α anomers **17a**−**c**. Deprotection of **16a**−**c** afforded the corresponding free β nucleosides 2-chloro-5,6-difluoro-1-β-D-ribofuranosylbenzimidazole (**2**), 2-chloro-5,6-dibromo-1-β-D-ribofuranosylbenzimidazole (**3**), and 2-chloro-5,6-diiodo-1-β-D-ribofuranosylbenzimidazole (**4**). Similar deprotection of the α anomers (**17a**−**c**) resulted in a removal of the acetyl protecting groups and a concomitant cyclization to give the 2,2′-*O*-cyclonucleosides (**18a**−**c**). Most of the benzimidazole heterocycles, but not the difluoro analog, were active against human cytomegalovirus (HCMV) (IC_{50}'s = 3−40 μM) and herpes simplex virus type 1 (HSV-1) (IC_{50}'s = 50−90 μM). This activity, however, was not well separated from cytotoxicity, IC_{50}'s = 10−100 μM. The corresponding unsubstituted, the 5,6-dimethyl, and the 5,6-difluoro ribonucleosides (**19**, **20**, and **2**, respectively), were inactive against both viruses. Similar to the previously reported 2,5,6-trichloro analog (TCRB), the 5,6-dibromo ribonucleoside **3** was active against HCMV ($IC_{50} \approx 4$ μM) but more cytotoxic than TCRB. The 5,6-diiodo analog **4** also was active ($IC_{50} \approx 2$ μM) but more cytotoxic (IC_{50} = 10−20 μM) than either **3** or TCRB. The cyclonucleosides were inactive against both viruses and not cytotoxic, or slightly active with corresponding cytotoxicity. The order of activity against HCMV of the dihalobenzimidazole ribonucleosides was I ≃ Br ≃ Cl ≫ F > H = CH_3. The order of cytotoxicity among the most active compounds, however, was I > Br > Cl, thereby establishing that TCRB had the best antiviral properties.

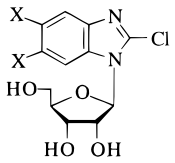
Introduction

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 transplantation and renal transplantation.[4,5] The treatment of HCMV infection is difficult because only a few therapeutic options are available. While many well-known antiviral drugs, such as vidarabine, interferons, and acyclovir, have been tested and proven not to be efficacious against HCMV,[6−9] only ganciclovir (DHPG),[10] foscarnet (PFA),[11] and cidofovir[12] have been approved by the FDA for the treatment of HCMV diseases. Although the treatment of HCMV infection with these drugs has produced clinical improvement in a large proportion of patients, the compounds suffer from poor oral bioavailability and produce adverse effects.[10−13] Therefore, there still is an urgent need for more active and nontoxic HCMV drugs with good oral availability. Moreover, as virus strains resistant to current drugs emerge,[14] drugs which act by new mechanisms will be needed to treat HCMV infections.

In our search for more potent and nontoxic agents for the treatment of HCMV infection, we evaluated a number of benzimidazole nucleosides previously prepared in our laboratory.[15] Unlike 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) which has antiviral and cytotoxic activities,[16−18] we discovered that other substituted benzimidazole ribosides, e.g., the 2-chloro analog of DRB (TCRB) and the 2-bromo analog (BDCRB), were highly active and selective against HCMV *in vitro* and were essentially noncytotoxic.[18,19] Both compounds act by a new mechanism involving inhibition of viral DNA processing.[20] As part of a comprehensive structure−activity relationship study related to the antiviral activity of TCRB, we have synthesized the ribosides of 2-chloro-5,6-difluoro-, 2-chloro-5,6-dibromo-, and 2-chloro-5,6-diiodobenzimidazole (**2**−**4**). The present work describes the synthesis and evaluation of these compounds for their activity against HCMV.

* Author to whom correspondence should be addressed.
† Present address: Gilead Sciences, 344 Lakeside Dr., Foster City, CA 94404.
⊗ Abstract published in *Advance ACS Abstracts,* January 1, 1997.

S0022-2623(96)00462-1 CCC: $14.00



1. X=Cl (TCRB)
2. X=F
3. X=Br
4. X=I

Results and Discussion

Chemistry. The reduction of 4,5-difluoro-2-nitroaniline (**5**) with iron powder in 1 N HCl gave 4,5-difluoro-1,2-phenylenediamine[21] (**6**), which was treated with cyanogen bromide to afford 2-amino-5,6-difluorobenzimidazole (**7**) in a good overall yield (Scheme 1). Diazotization of **7** with sodium nitrite in aqueous cupric chloride solution furnished 2-chloro-5,6-difluorobenzimidazole (**8**). The 5,6-dibromo derivative **10** was prepared via a direct bromination of 2-chlorobenzimidazole[22] (**9**) with bromine−water. The bromination reaction was controlled to give the desired 5,6-dibromo derivative as the major product by using mild reaction conditions and monitoring carefully the reaction process by TLC. The direct iodination of **9** with iodine monochloride was unsuccessful using a variety of reaction conditions. The introduction of iodine atoms into specific positions of the benzimidazole moiety was accomplished via stepwise diazotization and replacement reactions. Thus, the treatment of 2-chloro-5,6-dinitrobenzimidazole[23] (**11**) with iron powder in acetic acid effected a selective reduction of only one of the nitro groups and gave the 5(6)-amino-6(5)-nitro derivative **12**. Diazotization of the amino group and subsequent replacement with potassium iodide afforded the corresponding 5(6)-iodo-6(5)-nitro derivative **13**. Although the intermediate product **12** could be isolated in a pure form (41%) by successive column chromatography and recrystallization, it is experimentally preferable to carry out these reaction steps without purification of **12**. A selective reduction of the remaining nitro function of **13** to an amino group was achieved by hydrogenation over Raney nickel to yield the 5(6)-iodo-6(5)-amino derivative **14**. This reaction was closely monitored by TLC since prolonged hydrogenation also removed the iodine atom. Compound **14** was again subjected to the diazotization and replacement reactions and gave the desired 2-chloro-5,6-diiodobenzimidazole (**15**) in 87% yield. Glycosylation of **8**, **10**, and **15** with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose yielded the respective β nucleosides **16a**−**c** as the major products along with a small amount of the α anomers (**17a**−**c**) (Scheme 2). A removal of the protecting groups from **16a**−**c** gave the corresponding free β nucleosides 2-chloro-5,6-difluoro-1-β-D-ribofuranosylbenzimidazole (**2**), 2-chloro-5,6-dibromo-1-β-D-ribofuranosylbenzimidazole (**3**), and 2-chloro-5,6-diiodo-1-β-D-ribofuranosylbenzimidazole (**4**). Similar deprotection of the α anomers **17a**−**c** resulted in a removal of the acetyl protecting groups and a concomitant cyclization to give the 2,2′-*O*-cyclonucleosides (**18a**−**c**).

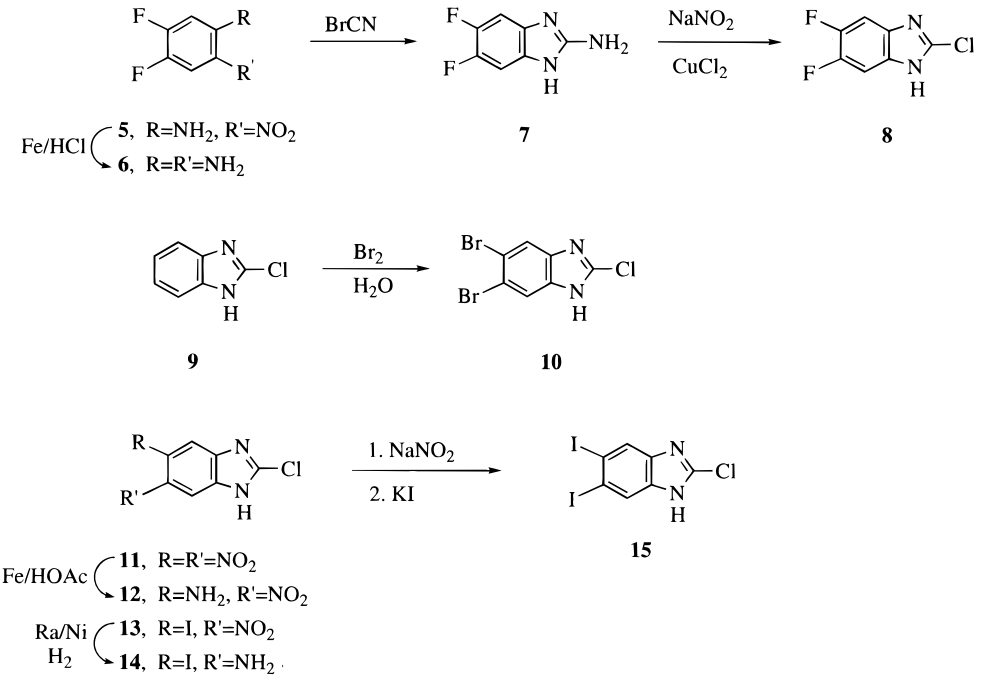
The major products (**16a**−**c**) of the glycosylation reactions were assigned as the β anomers since the preferential formation of the β anomer was expected with a protected sugar bearing a participating group at the 2-position.[24,25] This was also in agreement with the assignment of an α configuration to the minor products **17a**−**c** whose α configuration was established by the facile formation of 2,2′-*O*-cyclonucleosides **18a**−**c** upon deprotection. Additional support for the anomeric assignments was provided by comparing the H-1′ chemical shifts of the anomeric pairs.[19] The H-1′ signals of the major products **16a**−**c** were observed at a higher (∼0.45 ppm) field than those of the corresponding α anomers (**17a**−**c**), and therefore it was consistent with the assignment of **16a**−**c** as the β anomers.[25] An alternative synthesis of 2-chloro-5,6-dibromo-1-β-D-ribofuranosylbenzimidazole (**3**) was also achieved via a direct bromination of 2-chloro-1-β-D-ribofuranosylbenzimidazole[26] (**19**) with bromine−water.[27] This procedure not only provided a better synthesis of compound **3** but also gave further support for our assignments of anomeric configuration. Compounds **2**, **3**, **4**, **8**, **10**, and **15** were evaluated for their activities against human cytomegalovirus.

Antiviral Studies. The benzimidazole heterocycles, ribosides, and cyclonucleosides were evaluated for activity against HCMV and HSV-1 and for cytotoxicity in two cell lines. Three of the benzimidazole heterocycles (TCB,[18a] **10**, and **13)** were active against HCMV at concentrations similar to that found for ganciclovir (IC_{50}'s = 3−9 μM, Table 1). In contrast to ganciclovir, these compounds were less active against HSV-1 (IC_{50}'s = 25−65 μM) and much more cytotoxic (IC_{50}'s = 10−35 μM). Thus the activity against HCMV was much less specific than that of ganciclovir and the activity against HSV-1 was most likely a manifestation of cytotoxicity. The dinitro and diiodo compounds **11** and **15** were less active against HCMV with the antiviral activity not well separated from cytotoxicity (Table 1). The difluoro analog **8** and compounds **12** and **14** were essentially inactive against the viruses and not cytotoxic.

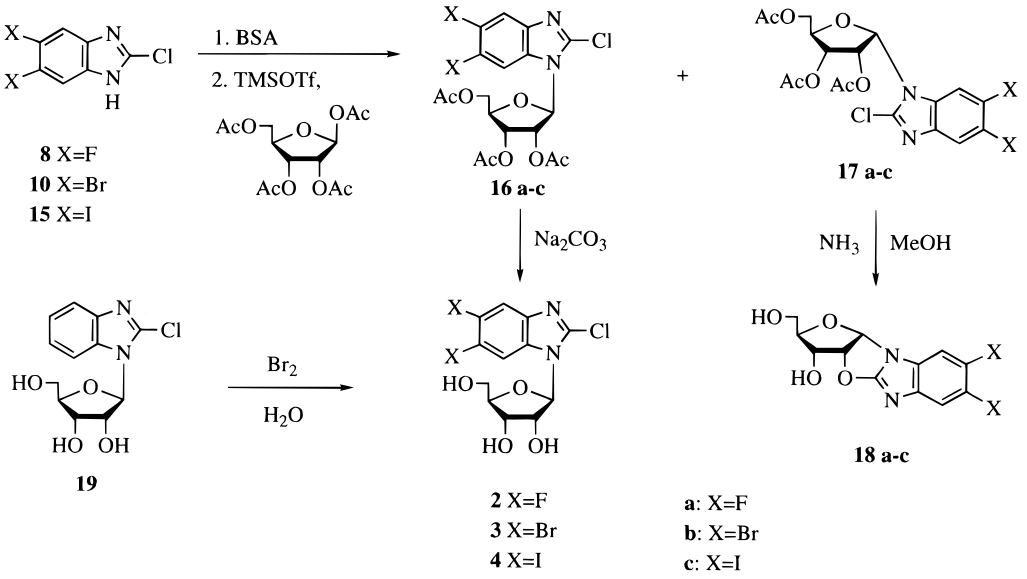
The activity of ribonucleosides **3** and **4** against HCMV was somewhat similar to that of the trichloro analog TCRB, which we have reported previously.[18a] Specifically, the dibromo and diiodo analogs were active against HCMV at low micromolar concentrations, but both analogs were more cytotoxic than TCRB (Table 2). The diiodo analog was most unlike TCRB; it was the most cytotoxic and exhibited some activity against HSV-1. In contrast, the 5,6-unsubstituted[26] analog **19**, the 5,6-dimethyl[28] analog **20**, and the 5,6-difluoro analog **2** were not cytotoxic and were inactive against HCMV and HSV-1 in the plaque assay and ELISA, respectively. The 5,6-difluoro analog was weakly active against HCMV in the yield assay (Table 2). The three cyclonucleosides **18a**−**c** were inactive against HSV-1 (IC_{50}'s >100 μM). They were inactive or weakly active against HCMV (IC_{50}'s = >100, 82, and 32 μM respectively for the 5,6-difluoro, 5,6-dibromo, and 5,6-diiodo analogs) and this activity closely paralleled their cytotoxicity in uninfected HFF cells. This would indicate that the activity of the cyclonucleosides was a manifestation of cytotoxicity.

Taken together, these data confirm our prior observation[18a] that the addition of ribose to a polyhalogenated benzimidazole reduces cytotoxicity and retains or increases activity against HCMV. The order for activity against HCMV among the 5,6-dihalo ribonucleosides was I ≃ Br ≃ Cl ≫ F > H compared to I > Br > Cl > F, H for cytotoxicity. Thus, our results show that the dichloro analog (TCRB) is the most potent and selective of these compounds and establish that the size

Scheme 1



Scheme 2



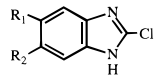
and electronegativity of substituents in the 5- and 6-positions are critical for activity against HCMV and for toxicity to uninfected cells.

Experimental Section

General Methods. 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 (HRMS) 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.

4,5-Difluoro-1,2-phenylenediamine (6). To a solution of 4,5-difluoro-2-nitroaniline (**5**, 1.106 g, 6.352 mmol, purchased from Aldrich Chemical Co.) in MeOH (20 mL) were added 50 mL of 1 N HCl and 1.774 g (31.764 mmol) of iron powder. The reaction mixture was stirred at room temperature for 3 h and then filtered. The filtrate was neutralized with concentrated NH_4OH to about pH 8. The resulting suspension was filtered again, and the filter cake was washed thoroughly with cold MeOH. The filtrate and washings were combined, concentrated to about 50 mL, and extracted with $CHCl_3$ (50 mL × 3). The $CHCl_3$ extracts were combined, washed with a saturated NaCl solution (100 mL × 2), dried (Na_2SO_4), and evaporated. The residue was coevaporated with $CHCl_3$ to give 0.78 g (85%) of **6** as a red solid. This material was used in the subsequent reaction without further purification. A brown crystalline sample of **6** was obtained by recrystallization from CCl_4: mp 126−129 °C [lit.[21] mp 130−131 °C (EtOAc/hexane)];

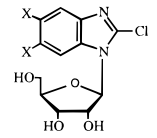
Table 1. Antiviral Activity and Cytotoxicity of 2-Chloro-5,6-disubstituted Benzimidazoles



			50 or 90% inhibitory concentration (μM)				
			antiviral activity				
			HCMV[a]		HSV-1[b]	cytotoxicity[c]	
no.	R_1	R_2	plaque	yield	ELISA	visual	growth
8	F	F	>100[d]	–	–	>100	>100
TCB[e,f]	Cl	Cl	6.4	–	51	24	19
10	Br	Br	8.5	–	65	32	35
11	NO_2	NO_2	38	–	>100	>100	–
12	NH_2	NO_2	>100	–	90[f]	>100	–
13	I	NO_2	2.8	7	25	10	–
14	I	NH_2	>100	–	>100	>100	–
15	I	I	20	–	–	32	–
foscarnet[g]			39±26	–	–	>100	–
ganciclovir (DHPG)[h]			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] The 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] 2,5,6-Trichlorobenzimidazole, published previously as compound **4** in ref 18a. [f] Average derived from two to four experiments for each parameter studied. [g] Average ± standard deviation from 15 experiments. [h] Average ± standard deviation from 108, 33, and three experiments, respectively.

Table 2. Antiviral Activity and Cytotoxicity of 2-Chloro-5,6-disubstituted-benzimidazole Ribonucleosides



		50 or 90% inhibitory concentration (μM)				
		antiviral activity				
		HCMV[a]		HSV-1[b]	cytotoxicity[c]	
no.	X	plaque	yield	ELISA	visual	growth
19[i]	H	>100[d,e]	>100	–	>100[e]	>100
2	F	>100[e]	75[e]	>100	>100[e]	>100
TCRB[f,g]	Cl	2.9	1.4	102	240	210
3	Br	3.8[e]	1.3	>100[e]	44[e]	88[e]
4	I	2.5[e]	2.2[e]	57[e]	13[e]	27[e]
20[h]	CH_3	>100	–	>100	>100	>100

[a–d] See Table 1 for footnotes *a*–*d*. [e] Average derived from two or three experiments. [f] The trichloro analog, published previously as compound **9** in ref 18a. [g] Averages derived from three to six experiments for each assay listed. [h] The dimethyl analog **20**, synthesized as reported in ref 28. [i] Compound **19** was synthesized and reported in ref 26.

HRMS (EI) *m/z* 144.0490 (100, M^+ = 144.0499); ^{1}H NMR (DMSO-d_6) δ 6.44 (t, 2, 3-H and 6-H, J_{F-H} = 10.5 Hz), 4.59 (br s, 4, 2 NH_2). Anal. ($C_6H_6F_2N_2$) C, H, N.

2-Amino-5,6-difluorobenzimidazole (7). To a stirred solution of 5 M BrCN/MeCN (4.9 mL, 24.5 mmol) and H_2O (50 mL) was added dropwise a solution of **6** (3.515 g, 24.389 mmol) in MeOH (50 mL) over 20 min. After the addition was complete, stirring was continued at room temperature for 2 h. The reaction mixture was concentrated to about 50 mL and then washed with EtOAc (50 mL × 3). The combined EtOAc solution was extracted with 100 mL of H_2O and then discarded. The combined H_2O phase was made basic with a saturated $NaHCO_3$ solution (precipitation occurred) and was extracted again with EtOAc (70 mL × 3). The EtOAc solution was dried (Na_2SO_4) and evaporated to dryness. The residue was suspended in 50 mL of $CHCl_3$ and filtered. The filter cake was washed with portions of $CHCl_3$ to give 3.40 g of **7** as a yellowish solid. The filtrate and washings were evaporated to dryness. The residue was again suspended in a small amount of $CHCl_3$ and filtered to give an additional 0.355 g of **7**. The total yield of **7** was 3.755 g (91%): mp 152–153 °C; HRMS: (EI) *m/z* 169.0455 (100, M^+ = 169.0452); ^{1}H NMR (DMSO-d_6) δ 10.79 (br s, 1, 1-NH), 7.06 (dd, 2, 4-H and 7-H, $^3J_{F-H}$ = 11.0 Hz, $^4J_{F-H}$ = 7.5 Hz), 6.30 (br s, 2, 2-NH_2). Anal. ($C_7H_5F_2N_3$) C, H, N.

2-Chloro-5,6-difluorobenzimidazole (8). To a stirred mixture of an aqueous $CuCl_2$ solution (40 mL, 60% by weight) and an aqueous $NaNO_2$ solution (2.08 g, 30 mmol/10 mL) in H_2O (20 mL) was added portionwise compound **7** (1.69 g, 10 mmol) over a period of 5 min. The reaction mixture was stirred at room temperature for 1 h. A fresh $CuCl_2$ solution (30 mL, 60% by weight) was added, and stirring was continued at 100 °C for 10 min (a small amount of MeOH was added to suppress the formation of foam). The mixture was cooled to room temperature and extracted with EtOAc (150 mL × 2). The EtOAc solution was washed with a saturated NaCl solution (100 mL × 2), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (3 × 15 cm) using 2% and 3% MeOH/$CHCl_3$ as eluants. Evaporation of the appropriate fractions gave a brownish solid. This solid was washed with Et_2O and dried to give 0.962 g of **8**. The Et_2O washings were evaporated, and the residue was repurified on a silica column (2 × 10 cm, eluted successively with 1%, 2% MeOH/$CHCl_3$, v/v). Evaporation of the appropriate fractions and washing of the residue with Et_2O gave an additional 0.152 g of **8**. The total yield of **8** was 1.114 g (59%): mp ~274 °C dec; HRMS: *m/z* 187.9954 (100, M^+ = 187.9953); ^{1}H NMR (DMSO-d_6) δ 13.5 (br s, 1, 1-NH), 7.62 (t, 2, 4-H and 7-H, J_{F-H} = 9.0 Hz). Anal. ($C_7H_3ClF_2N_2$) C, H, N.

2-Chloro-5,6-dibromobenzimidazole (10). To a mixture of 2-chlorobenzimidazole[22] (**9**, 0.763 g, 5 mmol) in MeOH (25 mL) was added dropwise a solution of Br_2/MeOH (1 mL/10 mL). The reaction mixture was stirred at room temperature for 5 h. H_2O (25 mL) was added, and stirring was continued at room temperature for 18 h. The resulting suspension was filtered, and the filter cake was washed thoroughly with portions of cold H_2O until the washings were neutral. The solid was air-dried and recrystallized from MeOH to give 1.115 g (3 crops, 72%) of **10** as a crystalline compound: mp 228–229 °C; HRMS (EI) *m/z* 307.8348 (46, M^+ = 307.8351); ^{1}H NMR (DMSO-d_6) δ 13.70 (br s, 1, 1-NH), 7.93 (s, 2, 4-H and 7-H). Anal. ($C_7H_3Br_2ClN_2$) C, H, N.

5(6)-Amino-2-chloro-6(5)-nitrobenzimidazole (12). A mixture of 2-chloro-5,6-dinitrobenzimidazole[23] (**11**, 1.213 g, 5.0 mmol) and iron powder (1.398 g, 25 mmol) in AcOH (50 mL) was stirred at room temperature for 4 h. The reaction mixture was diluted with 100 mL of EtOAc and filtered, and the solid was washed with portions of EtOAc (~100 mL). The filtrate and washings were combined and washed with H_2O (100 mL × 3). The H_2O layer was extracted with 100 mL of EtOAc. The EtOAc solutions were combined, evaporated, and coevaporated with toluene (10 mL × 3) and MeOH (10 mL × 2) to give a brown solid. The brown solid was purified on a silica column (3 × 25 cm, eluted successively with 2%, 4%, 8% MeOH/$CHCl_3$, v/v). Evaporation of fractions 30–58 (20 mL per fraction) and recrystallization from MeOH gave 0.431 g (two crops, 41%) of **12** as red crystals: mp >250 °C dec; HRMS: (EI) *m/z* 212.0096 (100, M^+ = 212.0101); ^{1}H NMR (DMSO-d_6) δ 13.07 (br s, 1, 1-NH), 8.15 (s, 1, 7-H), 7.06 (s, 2, 5-NH_2), 6.91 (s, 1, 4-H). Anal. ($C_7H_5ClN_4O_2$) C, H, N.

2-Chloro-5(6)-iodo-6(5)-nitrobenzimidazole (13). The procedure for the preparation of **12** was followed to give a brown solid. This solid was dissolved in a mixture of concentrated H_2SO_4/ice–H_2O (14 mL/20 mL), and a solution of $NaNO_2$/H_2O (0.994 g, 13.688 mmol/5 mL) was added dropwise at 0 °C. After the addition was complete, stirring was continued at 0 °C for 1 h. The excess $NaNO_2$ was destroyed by the addition of an aqueous urea solution (0.411 g/3 mL), and the reaction mixture was treated with an aqueous KI solution (2.272 g/5 mL) at room temperature for 18 h. The contents were transferred to a separatory funnel and extracted with EtOAc (100 mL × 2). The EtOAc solution was washed with H_2O (100 mL), a saturated $NaHCO_3$ solution (100 mL) and a saturated NaCl solution (100 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (2.2 × 25 cm, eluted successively with 1%, 2% MeOH/$CHCl_3$, v/v). Fractions 20–41 (20 mL per fraction) were collected, washed with an aqueous $Na_2S_2O_3$ solution (1 g/100 mL), dried (Na_2SO_4), and evaporated. The residue was

recrystallized from MeOH to give 0.593 g (three crops, 37%) of **13** as yellowish crystals: mp ~213 °C dec; HRMS: (EI) *m/z* 322.8968 (100, M^+ = 322.8957); ^{1}H NMR (DMSO-d_6) δ 13.98 (br s, 1, 1-NH), 8.24, 8.16 (2 s, 2, 4-H and 7-H). Anal. ($C_7H_3ClIN_3O_2$) C, H, N.

6(5)-Amino-2-chloro-5(6)-iodobenzimidazole (14). A mixture of **13** (1.04 g, 3.215 mmol) and Raney nickel (0.322 g, wet weight) in 32 mL of EtOH was hydrogenated (50 psi of H_2) at room temperature for 6 h. The reaction mixture was filtered, and the filtrate was evaporated. The residue was chromatographed on a silica column (2 × 30 cm, eluted successively with $CHCl_3$, 1%, 2% MeOH/$CHCl_3$, v/v). Evaporation of fractions 24−37 (20 mL per fraction) gave 0.72 g (76%) of **14** as a solid. An analytical sample was obtained by recrystallization from MeCN: mp >200 °C dec; HRMS: (EI) *m/z* 292.9202 (100, M^+ = 292.9217). ^{1}H NMR (DMSO-d_6) δ 12.80 (br s, 1, 1-NH), 7.75 (s, 1, 4-H), 6.87 (s, 1, 7-H), 5.03 (s, 2, 6-NH_2). Anal. ($C_7H_5ClIN_3$) C, H, N.

2-Chloro-5,6-diiodobenzimidazole (15). Compound **14** (0.72 g, 2.453 mmol) was dissolved in a mixture of concentrated H_2SO_4/ice−H_2O (6.6 mL/10 mL), and a solution of $NaNO_2/H_2O$ (0.508 g, 7.362 mmol/25 mL) was added dropwise at 0 °C. After the addition was complete, the reaction mixture was stirred at room temperature for 1 h. An aqueous KI solution (2.036 g, 12.265 mmol/15 mL) was added, and stirring was continued at room temperature for 3 h and then at 100 °C for 15 min. The reaction mixture was cooled to room temperature and extracted with EtOAc (100 mL). The EtOAc solution was washed with an aqueous $Na_2S_2O_3$ solution (3 g/100 mL) and a saturated NaCl solution (100 mL), dried (Na_2SO_4), and evaporated. The residue was recrystallized from MeCN to give 0.501 g of **15** as a crystalline compound. The mother liquor was evaporated, and the residue was chromatographed on a silica column (2 × 20 cm, eluted successively with $CHCl_3$, 1%, MeOH/$CHCl_3$, v/v). Evaporation of fractions 13−20 (15 mL per fraction) gave an additional 0.363 g of **15**. The total yield of **15** was 0.864 g (87%): mp 228−229 °C dec; HRMS (EI) *m/z* 403.8064 (100, M^+ = 403.8072); ^{1}H NMR (DMSO-d_6) δ 13.50 (br s, 1, 1-NH), 8.11 (s, 2, 4-H and 7-H). Anal. ($C_7H_3ClI_2N_2$) C, H, N.

2-Chloro-5,6-difluoro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (16a) and 2-chloro-5,6-difluoro-1-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyl)benzimidazole (17a). To a suspension of **8** (0.943 g, 5 mmol) in dry $ClCH_2CH_2Cl$ (25 mL) was added 1.25 mL (5 mmol) of BSA. The reaction mixture was stirred at 75 °C for 30 min to give a clear solution. To this solution were added 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (1.75 g, 5.5 mmol) and TMSOTf (1.07 mL, 5.5 mmol), and stirring was continued at 75 °C for 30 min. The reaction mixture was cooled to room temperature, diluted with 100 mL of $CHCl_3$, and washed with a saturated $NaHCO_3$ solution (100 mL × 2) and a saturated NaCl solution (100 mL). The $CHCl_3$ phase was dried (Na_2SO_4) and evaporated. The residue was chromatographed on a silica column (3 × 25 cm, eluted with $CHCl_3$). Evaporation of the appropriate fractions and recrystallization of the residue from MeOH gave 1.257 g (3 crops, 56%) of **16a** as crystalline needles: mp 90−91 °C; HRMS (EI) *m/z* 446.0694 (5, M^+ = 446.0692); ^{1}H NMR (DMSO-d_6) δ 7.93, 7.82 (2 dd, 2, 7-H and 4-H, $^3J_{F-H}$ = 10.5 Hz, $^4J_{F-H}$ = 7.5 Hz), 6.23 (d, 1, 1′-H, $J_{1'-2'}$ = 7.0 Hz), 5.55 (t, 1, 2′-H, $J_{2'-3'}$ = 7.0 Hz), 5.44 (m, 1, 3′-H, $J_{3'-4'}$ = 4.5 Hz), 4.45 (m, 3, 4′-H and 5′-H), 2.13, 2.10, 2.02 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 169.95, 169.50, 169.18 (3 *C*OCH$_3$), 148.76, 148.62, 146.10, 145.93 (C5 and C6, $^1J_{F-C}$ = 242 Hz, $^2J_{F-C}$ = 15 Hz), 140.16 (C2), 137.01, 136.88 (C3a, $^3J_{F-C}$ = 12 Hz), 128.54, 128.40 (C7a, $^3J_{F-C}$ = 12 Hz), 107.31, 107.09 (C4, $^2J_{F-C}$ = 20 Hz), 100.93, 100.66 (C7, $^2J_{F-C}$ = 24 Hz), 86.89 (C1′), 79.41 (C4′), 70.40 (C2′), 68.62 (C3′), 62.67 (C5′), 20.43, 20.28, 20.00 (3 CO*C*H$_3$). Anal. ($C_{18}H_{17}ClF_2N_2O_7$) C, H, N.

Further elution and evaporation of the appropriate fractions gave 0.456 g (20%) of **17a** as a syrup: HRMS (EI) *m/z* 446.0680 (12, M^+ = 446.0692); ^{1}H NMR (DMSO-d_6) δ 7.75 (m, 2, 7-H and 4-H), 6.69 (d, 1, 1′-H, $J_{1'-2'}$ = 4.0 Hz), 5.69 (t, 1, 2′-H, $J_{2'-3'}$ = 5.0 Hz), 5.49 (dd, 1, 3′-H, $J_{3'-4'}$ = 7.0 Hz), 4.90 (m, 1, 4′-H), 4.37 (dd, 1, 5′-H, $J_{5'-4'}$ = 3.5 Hz, $J_{5'-5''}$ = 12.0 Hz), 4.26 (dd, 1, 5″-H, $J_{5''-4'}$ = 5.5 Hz), 2.09, 2.03, 1.54 (3 s, 9, 3 Ac). ^{13}C NMR (DMSO-d_6) δ 170.05, 169.27, 168.34 (3 *C*OCH$_3$), 148.54, 148.37, 148.19, 145.88, 145.71, 145.54 (C5 and C6, $^1J_{F-C}$ = 241 Hz, $^2J_{F-C}$ = 16 Hz), 139.68 (C2), 136.71, 136.58 (C3a, $^3J_{F-C}$ = 11 Hz), 129.45, 129.32 (C7a, $^3J_{F-C}$ = 12 Hz), 106.66, 106.44 (C4, $^2J_{F-C}$ = 20 Hz), 101.79, 101.52 (C7, $^2J_{F-C}$ = 24 Hz), 86.43 (C1′), 78.16 (C4′), 70.97 (C2′), 70.39 (C3′), 62.69 (C5′), 20.49, 20.09, 19.48 (3 CO*C*H$_3$).

2-Chloro-5,6-dibromo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (16b) and 2-Chloro-5,6-dibromo-1-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyl)benzimidazole (17b). To a suspension of **10** (2.483 g, 8 mmol) in dry MeCN (40 mL) was added 2 mL (8 mmol) of BSA. The reaction mixture was stirred at 70 °C for 15 min to give a clear solution. This solution was cooled to room temperature and then treated with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (2.8 g, 8.8 mmol) and TMSOTf (1.855 mL, 9.6 mmol) at room temperature for 30 min. The reaction mixture was diluted with 160 mL of EtOAc. The EtOAc solution was washed with a saturated $NaHCO_3$ solution (160 mL × 2) and a saturated NaCl solution (160 mL), dried (Na_2SO_4), and evaporated. The residue was recrystallized from MeOH to give 3.51 g of **16b** as white crystals. The mother liquor was evaporated, and the residue was chromatographed on a silica column (2 × 10 cm, eluted with $CHCl_3$). Evaporation of the fractions 10−17 (5 mL per fraction) and recrystallization from MeOH gave an additional 0.293 g of **16b** as white crystals. The total yield of **16b** was 3.803 g (84%): mp 142−143 °C; HRMS (EI) *m/z* 565.9082 (1, M^+ = 565.9091); ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1, 7-H), 8.12 (s, 1, 4-H), 6.25 (d, 1, 1′-H, $J_{1'-2'}$ = 7.0 Hz), 5.55 (t, 1, 2′-H, $J_{2'-3'}$ = 7.0 Hz), 5.43 (m, 1, 3′-H, $J_{3'-4'}$ = 4.5 Hz), 4.52−4.34 (m, 3, 4′-H and 5′-H), 2.15, 2.14, 2.02 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 169.96, 169.47, 169.17 (3 *C*OCH$_3$), 141.65, 141.31 (C3a and C2), 133.16 (C7a), 123.53 (C4), 118.44, 118.08 (C5 and C6), 116.38 (C7), 86.67 (C1′), 79.59 (C4′), 70.56 (C2′), 68.68 (C3′), 62.59 (C5′), 20.69, 20.29, 20.00 (3 CO*C*H$_3$). Anal. ($C_{18}H_{17}Br_2ClN_2O_7$) C, H, N.

Evaporation of fractions 21−33 (5 mL per fraction) gave 0.37 g (8%) of **17b** as a syrup. HRMS (EI) *m/z* 565.9116 (5, M^+ = 565.9091). ^{1}H NMR (DMSO-d_6) δ 8.07, 8.06 (2 s, 2, 7-H and 4-H), 6.70 (d, 1, 1′-H, $J_{1'-2'}$ = 4.5 Hz), 5.71 (t, 1, 2′-H, $J_{2'-3'}$ = 5.0 Hz), 5.50 (dd, 1, 3′-H, $J_{3'-4'}$ = 6.5 Hz), 4.81 (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), 4.27 (dd, 1, 5″-H), 2.09, 2.05, 1.57 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 170.08, 169.27, 168.33 (3 *C*OCH$_3$), 141.45 (C3a), 141.06 (C2), 133.95 (C7a), 123.22 (C4), 117.86, 117.39 (C5 and C6), 117.39 (C7), 86.33 (C1′), 78.42 (C4′), 70.95 (C2′), 70.46 (C3′), 62.82 (C5′), 20.55, 20.17, 19.56 (3 CO*C*H$_3$).

2-Chloro-5,6-diiodo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (16c) and 2-chloro-5,6-diiodo-1-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyl)benzimidazole (17c). To a suspension of **15** (0.809 g, 2 mmol) in dry MeCN (20 mL) was added 1 mL (4 mmol) of BSA. The reaction mixture was stirred at 80 °C for 15 min to give a clear solution. This solution was cooled to room temperature and then treated with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (0.70 g, 2.2 mmol) and TMSOTf (0.964 mL, 5 mmol) at room temperature for 1 h. The reaction mixture was diluted with EtOAc (100 mL). The EtOAc solution was washed with a 1:1 saturated $NaHCO_3$/saturated NaCl solution (100 mL × 2), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (2 × 35 cm, eluted with $CHCl_3$, 0.5% MeOH/$CHCl_3$, v/v). Evaporation of fractions 11−30 (15 mL per fraction) and recrystallization from MeOH gave 1.023 g (2 crops, 77%) of **16c** as white crystals: mp 123−125 °C; HRMS (EI) *m/z* 661.8823 (14, M^+ = 661.8811); ^{1}H NMR (DMSO-d_6) δ 8.34 (s, 1, 7-H), 8.25 (s, 1, 4-H), 6.22 (d, 1, 1′-H, $J_{1'-2'}$ = 7.0 Hz), 5.54 (t, 1, 2′-H, $J_{2'-3'}$ = 7.0 Hz), 5.41 (m, 1, 3′-H, $J_{3'-4'}$ = 4.5 Hz), 4.46, 4.36 (2 m, 3, 4′-H and 5′-H), 2.17, 2.14, 2.01 (3 s, 9, 3 Ac). ^{13}C NMR (DMSO-d_6) δ 170.05, 169.56, 169.26 (3 *C*OCH$_3$), 142.58 (C3a), 140.73 (C2), 134.10 (C7a), 128.93 (C4), 121.54 (C7), 102.17, 101.51 (C5 and C6), 86.53 (C1′), 79.53 (C4′), 70.54 (C2′), 68.74 (C3′), 62.67 (C5′), 21.01, 20.38, 20.09 (3 CO*C*H$_3$). Anal. ($C_{18}H_{17}ClI_2N_2O_7$) C, H, N.

Evaporation of fractions 40−44 (20 mL per fraction) gave 0.108 g (8%) of **17c** as a syrup: HRMS (EI) *m/z* 661.8798 (21, M^+ = 661.8811); ^{1}H NMR (DMSO-d_6) δ 8.24 (s, 1, 7-H), 8.21

(s, 1, 4-H), 6.67 (d, 1, 1′-H, $J_{1'-2'}$ = 4.5 Hz), 5.69 (t, 1, 2′-H, $J_{2'-3'}$ = 5.0 Hz), 5.49 (dd, 1, 3′-H, $J_{3'-4'}$ = 7.0 Hz), 4.74 (m, 1, 4′-H, $J_{4'-5'}$ = 3.5 Hz, $J_{4'-5''}$ = 5.5 Hz), 4.35 (dd, 1, 5′-H, $J_{5'-5''}$ = 12.0 Hz), 4.26 (dd, 1, 5″-H), 2.09, 2.06, 1.58 (3 s, 9, 3 Ac); ^{13}C NMR (DMSO-d_6) δ 170.06, 169.28, 168.30 (3 *C*OCH$_3$), 142.37 (C3a), 140.43 (C2), 134.83 (C7a), 128.60 (C4), 122.70 (C7), 101.26, 100.46 (C5 and C6), 86.23 (C1′), 78.36 (C4′), 70.89 (C2′), 70.45 (C3′), 62.88 (C5′), 20.55, 20.24, 19.58 (3 CO*C*H$_3$).

2-Chloro-5,6-difluoro-1-(β-D-ribofuranosyl)benzimidazole (2). Compound **16a** (0.894 g, 2 mmol) was treated with 20 mL of NH$_3$/MeOH (saturated at 0 °C) in a pressure bottle at room temperature for 3 h. The reaction mixture was evaporated and coevaporated with MeOH to give a solid. This was recrystallized from MeOH to give 0.573 g (3 crops, 89%) of **2** as a crystalline compound: mp ∼210 °C dec; HRMS (EI) *m*/*z* 320.0385 (20, M$^+$ = 320.0375); ^{1}H NMR (DMSO-d_6) δ 8.34 (dd, 1, 7-H, $^3J_{F-H}$ = 11.5 Hz, $^4J_{F-H}$ = 7.5 Hz), 7.77 (dd, 1, 4-H, $^3J_{F-H}$ = 11.0 Hz, $^4J_{F-H}$ = 7.5 Hz), 5.88 (d, 1, 1′-H, $J_{1'-2'}$ = 8.0 Hz), 5.50 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.5 Hz), 5.44 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 4.5 Hz), 5.29 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 4.5 Hz), 4.40 (m, 1, 2′-H, $J_{2'-3'}$ = 5.5 Hz), 4.14 (m, 1, 3′-H, $J_{3'-4'}$ = 1.5 Hz), 4.01 (m, 1, 4′-H), 3.72 (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 148.53, 148.42, 148.37, 148.26, 145.88, 145.76, 145.72, 145.60 (C5 and C6, $^1J_{F-C}$ = 241 Hz, $^2J_{F-C}$ = 16 Hz), 140.84 (C2), 137.16, 137.04 (C3a, $^3J_{F-C}$ = 11 Hz), 128.55, 128.42 (C7a, $^3J_{F-C}$ = 12 Hz), 106.77, 106.54 (C4, $^2J_{F-C}$ = 20 Hz), 102.12, 101.84 (C7, $^2J_{F-C}$ = 25 Hz), 89.10 (C1′), 86.39 (C4′), 71.55 (C2′), 69.81 (C3′), 61.12 (C5′). Anal. ($C_{12}H_{11}ClF_2N_2O_4$) C, H, N.

2-Chloro-5,6-dibromo-1-β-D-ribofuranosylbenzimidazole (3). To a solution of Na_2CO_3 (0.212 g, 2 mmol) in H_2O (4 mL) were added successively 18 mL of EtOH, 18 mL of MeOH, and 1.137 g (2 mmol) of **16b**. The reaction mixture was stirred at room temperature for 2 h. AcOH (0.24 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 (40 mL × 2) and recrystallized from MeOH to give 0.719 g (2 crops, 76% based on $C_{12}H_{11}Br_2ClN_2O_4$·MeOH) of **3** as white crystals: mp 128−137 °C; HRMS: (FAB) *m*/*z* 440.8862 (3, MH$^+$ = 440.8852); ^{1}H NMR (DMSO-d_6) δ 8.68 (s, 1, 7-H), 8.09 (s, 1, 4-H), 5.88 (d, 1, 1′-H, $J_{1'-2'}$ = 8.0 Hz), 5.51 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.5 Hz), 5.40 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 4.5 Hz), 5.30 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 4.5 Hz), 4.42 (m, 1, 2′-H, $J_{2'-3'}$ = 5.5 Hz), 4.13 (m, 1, 3′-H, $J_{3'-4'}$ = 2.0 Hz), 4.01 (m, 1, 4′-H), 3.69 (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 142.08 (C2), 141.84 (C3a), 133.14 (C7a), 123.14 (C4), 117.94 [C7 and C5 (or C6)], 117.58 {C6 (or C5)}, 89.14 (C1′), 86.48 (C4′), 71.68 (C2′), 69.83 (C3′), 61.09 (C5′). Anal. ($C_{12}H_{11}Br_2ClN_2O_4$) C, H, N.

2-Chloro-5,6-diiodo-1-β-D-ribofuranosylbenzimidazole (4). 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 0.331 g (0.5 mmol) of **16c**. 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 0.237 g (3 crops, 88%) of **4** as white crystals; mp 147−149 °C; HRMS (CI) *m*/*z* 536.8571 (5, MH$^+$ = 536.8573); ^{1}H NMR (DMSO-d_6) δ 8.78 (s, 1, 7-H), 8.23 (s, 1, 4-H), 5.85 (d, 1, 1′-H, $J_{1'-2'}$ = 8.0 Hz), 5.49 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.5 Hz), 5.33 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.0 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), 4.12 (m, 1, 3′-H, $J_{3'-4'}$ = 2.0 Hz), 3.99 (m, 1, 4′-H), 3.68 (m, 2, 5′-H and 5″-H, $J_{5'-4'}$ = $J_{5''-4'}$ = 3.0 Hz, $J_{5'-5''}$ = 12.0 Hz); ^{13}C NMR (DMSO-d_6) δ 142.79 (C3a), 141.52 (C2), 133.04 (C7a), 128.58 (C4), 123.19 (C7), 101.34, 100.87 (C5 and C6), 89.02 (C1′), 86.42 (C4′), 71.53 (C2′), 69.87 (C3′), 61.13 (C5′). Anal. ($C_{12}H_{11}ClI_2N_2O_4$) C, H, N.

5,6-Difluoro-1-α-D-ribofuranosylbenzimidazole 2,2′-*O*-Cyclonucleoside (18a). A solution of compound **17a** (0.376 g, 0.842 mmol) in 15 mL of NH$_3$/MeOH (saturated at 0 °C) was stirred in a pressure bottle at room temperature for 20 h. Volatile materials were removed by evaporation and coevaporation with MeOH. The residue was dissolved in 15 mL of MeOH, decolorized with activated carbon, and filtered. The filtrate was evaporated, and the residue was recrystallized twice from MeOH/H_2O to give 0.132 g (55%) of **18a** as white crystalline needles: mp 110−115 °C; HRMS (EI) *m*/*z* 284.0610 (25, M$^+$ = 284.0609); ^{1}H NMR (DMSO-d_6) δ 7.59 (dd, 1, 7-H, $^3J_{F-H}$ = 10.5 Hz, $^4J_{F-H}$ = 7.5 Hz), 7.50 (dd, 1, 4-H, $^3J_{F-H}$ = 11.5 Hz, $^4J_{F-H}$ = 7.5 Hz), 6.49 (d, 1, 1′-H, $J_{1'-2'}$ = 5.0 Hz), 5.76 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 7.0 Hz), 5.72 (t, 1, 2′-H, $J_{2'-3'}$ = 5.5 Hz), 4.84 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.5 Hz), 4.08 (m, 1, 3′-H, $J_{3'-4'}$ = 9.0 Hz), 3.69 (m, 1, 5′-H, $J_{5'-4'}$ = 1.5 Hz, $J_{5'-5''}$ = 12.0 Hz), 3.54 (m, 1, 4′-H), 3.49 (m, 1, 5″-H, $J_{4'-5''}$ = 5.0 Hz); ^{13}C NMR (DMSO-d_6) δ 164.66 (C2), 147.81, 147.57, 145.12, 144.88 (C5 or C6, $^1J_{F-C}$ = 244 Hz, $^2J_{F-C}$ = 22 Hz), 146.71, 146.58, 144.13, 144.00 (C6 or C5, $^1J_{F-C}$ = 234 Hz, $^2J_{F-C}$ = 11 Hz), 142.00, 141.87 (C3a, $^3J_{F-C}$ = 11 Hz), 124.84, 124.71 (C7a, $^3J_{F-C}$ = 12 Hz), 106.30, 106.08 (C4, $^2J_{F-C}$ = 21 Hz), 98.89, 98.63 (C7, $^2J_{F-C}$ = 24 Hz), 91.11 (C2′), 84.64 (C1′), 80.93 (C4′), 69.61 (C3′), 59.50 (C5′). Anal. ($C_{12}H_{10}F_2N_2O_4$) C, H, N.

5,6-Dibromo-1-α-D-ribofuranosylbenzimidazole 2,2′-*O*-Cyclonucleoside (18b). A solution of 0.37 g (0.65 mmol) of **17b** in 15 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 the resulting solid was recrystallized from MeOH to give 0.140 g of **18b** as white crystals. The mother liquor was evaporated to dryness and the residue was triturated with H_2O (10 mL × 3) and then recrystallized from MeOH/Et_2O (diffusion) to give an additional 0.084 g of **18b** as white crystals. The total yield of **18b** was 0.224 g (85%): mp 140−160 °C (melted slowly over a large range of temperature); HRMS (EI) *m*/*z* 403.8995 (26, M$^+$ = 403.9007); ^{1}H NMR (DMSO-d_6) δ 7.88 (s, 1, 7-H), 7.80 (s, 1, 4-H), 6.52 (d, 1, 1′-H, $J_{1'-2'}$ = 5.0 Hz), 5.78 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 7.0 Hz), 5.74 (t, 1, 2′-H, $J_{2'-3'}$ = 5.5 Hz), 4.84 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.0 Hz), 4.08 (m, 1, 3′-H, $J_{3'-4'}$ = 9.0 Hz), 3.69 (m, 1, 5′−H, $J_{4'-5'}$ = 0.5 Hz, $J_{5'-5''}$ = 11.5 Hz), 3.49 (m, 2, 4′-H and 5″-H, $J_{4'-5''}$ = 5.0 Hz); ^{13}H NMR (DMSO-d_6) δ 164.87 (C2), 147.18 (C3a), 129.70 (C7a), 122.19 (C4), 116.15, 114.34 (C5 and C6), 114.49 (C7), 91.41 (C2′), 84.64 (C1′), 80.91 (C4′), 69.60 (C3′), 59.50 (C5′). Anal. ($C_{12}H_{10}Br_2N_2O_4$) C, H, N.

5,6-Diiodo-1-α-D-ribofuranosylbenzimidazole 2,2′-*O*-Cyclonucleoside (18c). A solution of 0.10 g (0.151 mmol) of **17c** 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 resulting solid was recrystallized from MeOH to give 0.054 g (2 crops, 72%) of **18c** as white crystals: mp ∼155 °C (melted over a large range of temperature); HRMS (EI) *m*/*z* 499.8719 (100, M$^+$ = 499.8728); ^{1}H NMR (DMSO-d_6) δ 8.01, 7.97 (2 s, 2, 7-H and 4-H), 6.51 (d, 1, 1′-H, $J_{1'-2'}$ = 5.0 Hz), 5.73 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 7.0 Hz), 5.725 (t, 1, 2′-H, $J_{2'-3'}$ = 5.0 Hz), 4.79 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.0 Hz), 4.06 (m, 1, 3′-H, $J_{3'-4'}$ = 9.0 Hz), 3.69 (m, 1, 5′−H, $J_{4'-5'}$ = 1.0 Hz, $J_{5'-5''}$ = 11.0 Hz), 3.50 (m, 2, 4′-H and 5″-H, $J_{4'-5''}$ = 5.0 Hz); ^{13}H NMR (DMSO-d_6) δ 164.30 (C2), 148.16 (C3a), 130.55 (C7a), 127.73 (C4), 119.63 (C7), 98.92, 96.97 (C5 and C6), 91.26 (C2′), 84.53 (C1′), 80.83 (C4′), 69.61 (C3′), 59.50 (C5′). Anal. ($C_{12}H_{10}I_2N_2O_4$) C, H, N.

2-Chloro-5,6-dibromo-1-β-D-ribofuranosylbenzimidazole (3) from 19. To a stirred suspension of 2-chloro-1-β-D-ribofuranosylbenzimidazole[23] (**19**, 0.57 g, 2 mmol) in H_2O (50 mL) was added dropwise 50 mL of Br_2/H_2O (saturated at 20 °C) over a period of 30 min. After the addition was complete, stirring was continued at room temperature for 3 h. The reaction mixture was allowed to stand in an ice−H_2O bath for 30 min and then filtered. The filter cake was washed with portions of cold H_2O until the washings were neutral. The resulting white solid was air-dried and recrystallized from MeOH to give 0.757 g (3 crops, 80% as $C_{12}H_{11}Br_2ClN_2O_4$·MeOH) of **3** as white crystals. This material was identical to the product obtained from the deprotection of **16b**.

Cell Culture Procedures. The routine growth and passage of KB, BSC-1, and HFF cells was performed in monolayer cultures using minimal essential medium (MEM) with either Hanks salts [MEM(H)] or Earle salts [MEM(E)] supplemented with 10% calf serum or 10% fetal bovine serum (HFF cells). The sodium bicarbonate concentration was varied to meet the

buffering capacity required. Cells were passaged at 1:2 to 1:10 dilutions according to conventional procedures 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.[29] High titer HSV-1 stocks were prepared by infecting KB cells at an moi of <0.1 also as detailed previously.[29] 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.[30] Briefly, HFF or BSC-1 cells were planted as described above in 96-well cluster dishes and incubated overnight at 37 °C. The next day cultures were inoculated with HCMV or HSV-1 and serially diluted 1:3 across the remaining 11 columns of the 96-well plate. After virus adsorption, the inoculum was replaced with fresh medium and cultures were incubated for 7 days for HCMV, 2 or 3 days for HSV-1. Plaques were enumerated under 20-fold magnification in wells having the dilution 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 nth dilution of the virus used to infect the well in which plaques were enumerated.

HCMV Plaque Reduction Assay. HFF cells in 24-well cluster dishes were infected with approximately 100 pfu of HCMV per 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 concentrations. After incubation at 37 °C, for 7 days, cell sheets were fixed and stained with crystal violet and microscopic plaques enumerated as described above. Drug effects were calculated as a percentage of reduction in number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug.

HCMV Yield Assay. HFF cells were planted as described above in 96-well cluster dishes and incubated overnight, the medium was removed, and the cultures were inoculated with HCMV at a moi of 0.5 to 1 pfu per cell as reported elsewhere.[30] After virus adsorption, inoculum was replaced with 0.2 mL of fresh medium containing test compounds. The first row of 12 wells was left undisturbed and served as virus controls. Each well in the second row received an additional 0.1 mL of medium with test compound at three times the desired final concentration. The contents of the 12 wells were mixed by repeated pipetting and then serially diluted 1:3 along the remaining wells. In this manner, six compounds could be tested in duplicate on a single plate with concentrations from 100 to 0.14 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 11 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[31] 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 absorbence was read at 450 and 570 nm. Drug effects were calculated as a percentage of the reduction in absorbence in the presence of each drug concentration compared to absorbence obtained with virus in the absence of drug.

Cytotoxicity Assays. Two different assays were used to explore cytotoxicity of selected compounds using methods we have detailed previously. (i) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[29] (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.[32] 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}) 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.

Acknowledgment. This research work was supported by federal funds from the Department of Health and Human Services research contracts NOl-AI42554 and NOl-AI7264, Research Grant UOl-AI31718 from the National Institute of Allergy and Infectious Diseases, and Research Agreement DRDA-942921 with Glaxo-Wellcome. The authors thank members of Dr. Drach's research group, especially Roger G. Ptak and Drs. Edward D. Kreske, Mary S. Ludwig, and Allison C. Westerman, for the antiviral evaluations. We also appreciate the contributions of J. Hinkley for the preparation of chemical intermediates and Ms. Marina Savic for preparation of the manuscript.

References

(1) Zou, R.; Kreske, E. D.; Drach, J. C.; Townsend, L. B. Benzimidazole Ribonucleosides: Design, Synthesis, and Evaluation of 2-Chloro-5,6-diiodo-1-(β-D-ribofuranosyl) benzimidazole, an analog of TCRB. A portion of this work was presented at the Fifth International Conference on Antiviral Research, Vancouver, B.C., Canada, March 8, 1992.

(2) Jacobson, M. A.; Mills, J. Serious Cytomegalovirus Disease in the Acquired Immunodeficiency Syndrome (AIDS), Clinical Findings, Diagnosis, and Treatment. *Ann. Intern. Med.* **1988**, *108*, 585−594.

(3) Alford, C. A.; Britt, W. J. Cytomegalovirus. In *The Human Herpesviruses;* Roizman, B., Whitley, R. J., Lopez, C., Eds.; Raven Press: New York, 1993; pp 227−255.

(4) Meyers, J. D.; Flournoy, N.; Thomas, E. D. Risk Factors for Cytomegalovirus Infection after Human Marrow Transplantation. *J. Infect. Dis.* **1986**, *153*, 478−488.

(5) Glenn, J. Cytomegalovirus Infections Following Renal Transplantation. *Rev. Infect. Dis.* **1981**, *3*, 1151−1178.

(6) Rytel, M. W.; Kauffman, H. M. Clinical Efficacy of Adenine Arabinoside in Therapy of Cytomegalovirus Infections in Renal Allograft Recipients. *J. Infect. Dis.* **1976**, *133*, 202−205.

(7) Meyers, J. D.; McGuffin, R. W.; Neiman, P. E.; Singer, J. W.; Thomas, E. D. Toxicity and Efficacy of Human Leukocyte Interferon for Treatment of Cytomegalovirus Pneumonia after Marrow Transplantation. *J. Infect. Dis.* **1980**, *141*, 555−562.

(8) Meyers, J. D.; McGuffin, R. W.; Bryson, Y. J.; Cantell, K.; Thomas, E. D. Treatment of Cytomegalovirus Pneumonia after Marrow Transplant with Combined Vidarabine and Human Leukocyte Interferon. *J. Infect. Dis.* **1982**, *146*, 80−84.

(9) Wade, J. C.; McGuffin, R. W.; Springmeyer, S. C.; Newton, B.; Singer, J. W.; Meyers, J. D. Treatment of Cytomegaloviral Pneumonia with High-Dose Acyclovir and Human Leukocyte Interferon. *J. Infect. Dis.* **1983,** *148*, 557−562.

(10) Faulds, D.; Heel, R. C. Ganciclovir. A Review of its Antiviral Activity, Pharmacokinetic Properties and Therapeutic Efficacy in Cytomegalovirus Infections. *Drugs* **1990**, *39*, 597−638.

(11) Chrisp, P.; Clissold, S. P. Foscarnet. A Review of its Antiviral Activity, Pharmacokinetic Properties and Therapeutic use in Immunocompromised Patients with Cytomegalovirus Retinitis. *Drugs* **1991**, *41*, 104−129.

(12) (a) Kirsch, L. S.; Arevalo, J. F.; De Clercq, E.; Chavez, de la Paz. E.; Munguia, D.; Garcia, R.; Freeman, W. R. Phase I/II study of intravitreal cidofovir for the treatment of cytomegalovirus retinitis in patients with the acquired immunodeficiency syndrome. *Am. J. Ophthalmol.* **1995**, *119*, 466−476. (b) Polis, M. A.; Spooner, K. M.; Baird, B. F.; Manischewitz, J. F.; Jaffe, H. S.; Fisher, P. E.; Falloon, J.; Davey, R. T., Jr.; Kovacs, J. A.; Walker, R. E.; et al. Anticytomegaloviral activity and safety of cidofovir in patients with human immunodeficiency virus infection and cytomegalovirus viruria. *Antimicrob. Agents Chemother.* **1995**, *39*, 882−886.

(13) Morris, D. J. Antiviral Chemotherapy for Cytomegalovirus Disease. *J. Antimicrob. Chemother.* **1988**, *21*, 519−522.

(14) Field, A. K.; Biron, K. K. "The End of Innocence" Revisited: Resistance of Herpesviruses to Antiviral Drugs. *Clin. Microbiol. Rev.* **1994**, *7*, 1−13.

(15) Townsend, L. B.; Revankar, G. R. Benzimidazole Nucleosides, Nucleotides, and Related Derivatives. *Chem. Rev.* **1970**, *70*, 389−438.

(16) Tamm, I; Folkers, K.; Shunk, C. H.; Horsfall, F. L. Inhibition of Influenza Virus Multiplication by N-Glycosides of Benzimidazoles. *J. Exp. Med.* **1954**, *99*, 227−250.

(17) Tamm, I.; Sehgal, P. B. Halobenzimidazole Ribosides and RNA Synthesis of Cells and Viruses. *Adv. Virus Res.* **1978**, *22*, 187−258.

(18) (a) Townsend, L. B.; Devivar, R. V.; Turk, S. R.; Nassiri, M. R.; Drach, J. C. Design, Synthesis and Antiviral Activity of Certain 2,5,6-trihalo-1-(β-D-ribofuranosyl)benzimidazoles. *J. Med. Chem.* **1995**, *38*, 4098−4105. (b) Townsend, L. B. Benzimidazole Ribonucleosides: Design, Synthesis, and Evaluation of 2,5,6-Trichloro-1-(β-D-ribofuranosyl) benzimidazole (TCRB), 2-Bromo-5,6-dichloro-1-(β-D-ribofuranosyl) benzimidazole (BDCRB), and Some Structurally Related Analogs as Agents for Human Cytomegalovirus Infections. Fifth International Conference on Antiviral Research, Vancouver, B.C., Canada, 8−13 March, 1992. (c) Drach J. C.; Townsend, L. B.; Nassiri, M. R.; Turk, S. R.; Coleman, L. A.; Devivar, R. V.; Genzlinger, G; Kreske, E. D.; Renau, T. E.; Westerman, A. C.; Shipman, C. Jr.; Biron, K. K.; Dornsife, R.; Kern, E. R. Benzimidazole Ribonucleosides: A New Class of Antivirals with Potent and Selective Activity Against Human Cytomegalovirus. Fifth International Conference on Antiviral Research, Vancouver, B.C., Canada, 8−13 March, 1992.

(19) Nassiri, M. R.;. Emerson, S. G.; Townsend, L. B.; Drach, J. C.; Taichman, R. S. Comparison of benzimidazole nucleosides and ganciclovir on the *in vitro* proliferation and colony formation of human bone marrow progenitor cells. *Br. J. Haematol.* **1996**, *93*, 273−279.

(20) (a) Underwood, M. R.; Biron, K. K.; Hemphill, M. L.; Miller, T. J.; Stanat, S. C.; Dornsife, R. E.; Drach, J. C.; Townsend, L. B.; Edwards, C. A.; Harvey, R. J. High Molecular Weight HCMV DNA Does Not Properly Mature in the Presence of 2-Bromo-5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (BDCRB). Herpesvirus Workshop, Pittsburgh, PA, July 1993. (b) Underwood, M. R.; Stanat, S. C.; Drach, J. C.;Harvey R. J.; Biron, K. K. Inhibition of HCMV DNA Processing by a New Class of Anti-HCMV Compounds is Mediated Through the UL89 Gene Product. Herpesvirus Workshop, Vancouver, B.C., August 1994.

(21) Uchida, M.; Morita, S.; Chihiro, M.; Kanbe, T.; Yamasaki, K.; Yabuuchi, Y.; Nakagawa, K. Studies on Proton Pump Inhibitors. I. Synthesis of 8-[(2-Benzimidazolyl)sulfinyl]-5,6,7,8-tetrahydroquinolines and Related Compounds. *Chem. Pharm. Bull.* **1989**, *37*, 1517−1523.

(22) Harrison, D.; Ralph, J. T.; Smith, A. B. C. Some 1- and 2-Halogenobenzimidazoles. *J. Chem. Soc.* **1963**, 2930−2937.

(23) Chernova, E. Y.; Mokrushina, G. A.; Chupakhin, O. N.; Kotovskaya, S. K.; Il'enko, V. I.; Andreeva, O. T.; Boreko, E. I.; Vladyko, G. V.; Korobchenko, L. V.; et al. Synthesis and Biological Activity of 5,6-Dinitro Derivatives of Benzimidazole. *Khim.-Farm. Zh.* **1991**, *25*, 50−52; *Chem. Abstr.* 1991, *115*, 29203x.

(24) Baker, B. R.; Joseph, J. P.; Schaub, R. E.; Williams, J. H. Puromycin Synthetic Studies. V. 6-Dimethylamino-9-(2′-acetamino-β-D-glucopyranosyl)purine. *J. Org. Chem.* **1954**, *19*, 1786−1792.

(25) Townsend, L. B. Nuclear Magnetic Resonance Spectroscopy in the Study of Nucleic Acid Components and Certain Related Derivatives. In *Synthetic Procedures in Nucleic Acid Chemistry Vol. 2, Physical and Physicochemical Aids in Characterization and in Determination of Structure*; Zorbach, W. W., Tipson, R. S., Eds.; Wiley-Interscience: New York, 1973; pp 267−398.

(26) Revankar, G. R.; Townsend, L. B. The Synthesis of 2-Chloro-1-(β-D-ribofuranosyl)-benzimidazole and Certain Related Derivatives. *J. Heterocycl. Chem.* **1968**, *5*, 477−483.

(27) Kazimierczuk, Z.; Dudycz, L.; Stolarski, R.; Shugar, D. Preparation of 1-α-D-Arabinofuranosylbenzimidazole and its 5,6-Dichloro Derivative, and the Direct Bromination of Benzimidazole Nucleosides. *Z. Naturforsch.* **1980**, *35c*, 30−35.

(28) Revankar, G. R.; Townsend, L. B. The Synthesis of 2-Chloro-1-(β-D-ribofuranosyl)-5,6-dimethylbenzimidazole and Certain Related Derivatives. *J. Heterocycl. Chem.* **1968**, *5*, 615−620.

(29) Turk, S. R.; Shipman, C., Jr.; Nassiri, M. R.; Genzingler, G.; Krawczyk, S. H.; Townsend, L. B.; Drach, J. C. Pyrrolo[2,3-*d*]-pyrimidine Nucleosides as Inhibitors of Human Cytomegalovirus, *Antimicrob. Agents Chemother.* **1987**, *31*, 544−550.

(30) Prichard, M. N.; Turk, S. R.; Coleman, L. A.; Engelhardt, S. L.; Shipman, C., Jr.; Drach, J. C. A Microtiter Virus Yield Reduction Assay for The Evaluation of Antiviral Compounds Against Human Cytomegalovirus and Herpes Simplex Virus. *J. Virol. Methods* **1990**, *28*, 101−106.

(31) Prichard, M. N.; Shipman, C., Jr. A Three-Dimensional Model To Analyze Drug-Drug Interactions. *Antiviral Res.* **1990**, *14*, 181−206.

(32) Prichard, M. N.; Prichard, L. E.; Baguley, W. A.; Nassiri, M. R.; Shipman, C., Jr. Three-Dimensional Analysis of the Synergistic Cytotoxicity of Ganciclovir and Zidovudine. *Antimicrob. Agents Chemother.* **1991**, *35*, 1060−1065.

JM960462G