Synthesis and Antiviral Evaluation of Certain Disubstituted Benzimidazole Ribonucleosides[1]

Ruiming Zou,[†] Kevin R. Ayres, John C. Drach,[‡] and Leroy B. Townsend*

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

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Ribosylation of 2-chloro-5(6)-nitrobenzimidazole (**3**) gave 2-chloro-5-nitro-1-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)benzimidazole (**4a**) and 2-chloro-6-nitro-1-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)benzimidazole (**4b**) as a mixture of positional isomers. Subsequent hydrogenation of this mixture over Raney Nickel afforded the corresponding 5-amino and 6-amino derivatives **5** and **6**. At this stage the products were readily resolved via silica column chromatography into pure isomeric forms, and the pure isomers **5** and **6** were diazotized with *tert*-butyl nitrite and cupric chloride to furnish the isomerically pure 5-chloro derivative **2a** and 6-chloro derivative **2b**. Deprotection of **5**, **6**, **2a**, and **2b** with methanolic ammonia yielded the free nucleosides 5-amino-2-chloro-1-(*β*-D-ribofuranosyl)benzimidazole (**7**), 6-amino-2-chloro-1-(*β*-D-ribofuranosyl)-benzimidazole (**8**), 2,5-dichloro-1-(*β*-D-ribofuranosyl)benzimidazole (**9**), and 2,6-dichloro-1-(*β*-D-ribofuranosyl)benzimidazole (**10**), respectively. Treatment of **10** with thiourea afforded 6-chloro-1-(*β*-D-ribofuranosyl)benzimidazole-2-thione (**14**). Alkylation of **14** with methyl iodide and benzyl bromide gave good yields of the corresponding 2-methylthio (**12**) and the 2-benzylthio (**13**) analogs. The synthesis of 6-chloro-2-methoxy-1-(*β*-D-ribofuranosyl)benzimidazole (**11**) was accomplished by the treatment of **2b** with sodium methoxide in methanol. A difference NOE spectroscopic experiment was conducted to allow unequivocal assignment of regiochemistry to the positional isomers **5** and **6**. Evaluation of compounds for activity against human cytomegalovirus (HCMV) and herpes simplex virus type 1 revealed that the heterocycle **3** was active against both viruses but also was cytotoxic. Only the dichloro compounds **9** and **10** were weakly active against HCMV and noncytotoxic in their antiviral dose range. These data further substantiate the conclusion that activity against HCMV at noncytotoxic concentrations by benzimidazole ribonucleosides requires a halogen not only at the 2-position, but also more than one halogen on the benzene moiety.

Introduction

In 1954, Tamm and co-workers first reported the synthesis and antiviral activity of various halogenated benzimidazole nucleosides.[2] The most active compound, 5,6-dichloro-1-(*β*-D-ribofuranosyl)benzimidazole (DRB), was reported to be active against influenza virus[2,3] and other RNA viruses.[4] Subsequent studies expanded the list of viruses affected to include certain DNA viruses such as vaccinia virus.[4,5] The antiviral activity of DRB most likely is a consequence of specific and reversible inhibition of heterogeneous nuclear RNA synthesis,[6] probably as a consequence of inhibiting RNA polymerase II,[7] or by superinduction of interferon production.[8] Consequently its antiviral activity is not well separated from cytotoxicity resulting in little potential as an antiviral drug.[4,9]

These early studies by Tamm and co-workers prompted us to synthesize a series of 2-substituted benzimidazole ribonucleosides as potential anticancer agents.[10] More recently, we have examined the 2-chloro analog of DRB [2,5,6-trichloro-1-(*β*-D-ribofuranosyl)benzimidazole, (TCRB)] for activity against HCMV based in part on its low cytotoxicity and lack of activity in anticancer tests.[11] We found that TCRB is a potent and selective inhibitor of the replication of human cytomegalovirus (HCMV),[12] an opportunistic pathogen in neonates and immunocompromised patients.[13] In this search for therapeutic agents for the treatment of HCMV infections, we observed that DRB, in marked contrast to TCRB, had marginal activity and essentially no selectivity against HCMV.[12,14] As part of our structure−activity relationship studies related to DRB, we now have synthesized two DRB isomers 2,5-dichloro-1-(*β*-D-ribofuranosyl)benzimidazole (**9**) and 2,6-dichloro-1-(*β*-D-ribofuranosyl)-benzimidazole (**10**) and certain other 2-substituted analogs. The present work describes the synthesis and antiviral evaluation of these DRB isomers and analogs and establishes that the presence of chlorine in the 2-position of the heterocycle is essential for low cytotoxicity but is not sufficient for potent antiviral activity.

Chemistry

Silylation of 2,5(6)-dichlorobenzimidazole[15] (**1**) followed by coupling with 1,2,3,5-tetra-*O*-acetyl-*β*-D-ribofuranose in the presence of trimethylsilyl triflate (TMSOTf) gave an 82% yield of the desired *β* nucleosides as a mixture of positional isomers {2,5-dichloro-1-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)benzimidazole (**2a**) and 2,6-dichloro-1-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)benzimidazole (**2b**)}. Chromatographic separation of the positional isomers **2a**,**b** on silica was not accomplished in our hands because compounds **2a**,**b** comigrated in all solvent systems we used. Although repeated recrystallization (five times from methanol) afforded one pure

[†] Current address: Gilead Sciences, 346 Lakeside Drive, Foster City, CA 94404.
[‡] Department of Biological and Materials Sciences.
* To whom correspondence should be addressed at the College of Pharmacy.
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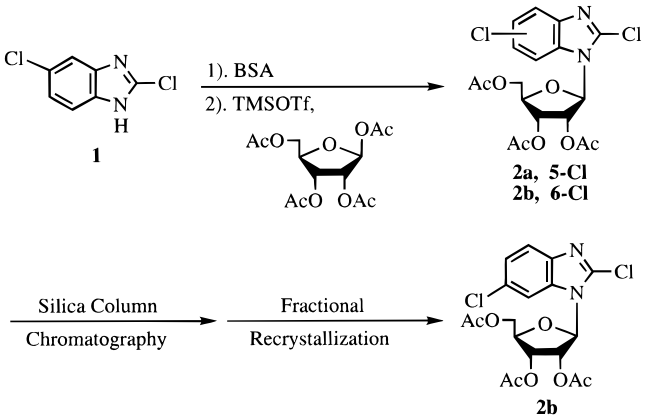
Scheme 1



positional isomer (the 2,6-dichloro derivative **2b**) in a low yield, the other isomer (**2a**) could not be obtained in a pure form. This prompted us to explore an alternative route to the synthesis of **2a** and **2b**.

In our second approach, 2-chloro-5(6)-nitrobenzimidazole[15−17] (**3**) was similarly ribosylated to give the desired β nucleosides **4a**,**b** in 71% yield as a mixture of positional isomers {2-chloro-5-nitro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (**4a**) and 2-chloro-6-nitro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (**4b**)}. The positional isomers **4a**,**b**, which, like **2a**,**b**, could not be separated via silica chromatography and gave only one pure isomer in low yield upon repeated recrystallization, was directly converted to the corresponding amino derivatives **5** and **6** by hydrogenation over Raney nickel. At this stage the products could be readily resolved via silica column chromatography into pure isomeric forms {5-amino-2-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (**5**) and 6-amino-2-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (**6**)}. The pure positional isomers **5** and **6** served as two key intermediates for the introduction of a chlorine atom into the desired position. Thus diazotization of the 5-amino isomer **5** with *tert*-butyl nitrite and cupric chloride in acetonitrile furnished the pure 5-chloro derivative **2a** in 56% yield. Parallel diazotization of the 6-amino isomer **6** afforded the corresponding 6-chloro derivative **2b** in 59% yield. Deprotection of **5**, **6**, **2a**, and **2b** with methanolic ammonia gave the corresponding deprotected nucleosides 5-amino-2-chloro-1-(β-D-ribofuranosyl)benzimidazole (**7**), 6-amino-2-chloro-1-(β-D-ribofuranosyl)benzimidazole (**8**), 2,5-dichloro-1-(β-D-ribofuranosyl)benzimidazole (**9**), and 2,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (**10**), respectively. Treatment of **10** with thiourea afforded 6-chloro-1-(β-D-ribofuranosyl)benzimidazole-2-thione (**14**). This displacement of the chloro group with sulfur was confirmed as previously described in research from our laboratory, i.e.,[18] ^{1}H NMR.[19] Alkylation of **14** with methyl iodide and benzyl bromide gave good yields of the corresponding 2-methylthio (**12**) and the 2-benzylthio (**13**) analogs. The absence of a NH peak in the ^{1}H NMR spectrum of **14** at δ 13.01 and the appearance of a 3 proton signal at δ 2.71 in the spectrum of **12** confirms that alkylation occurred on sulfur rather than nitrogen. The synthesis of 6-chloro-2-methoxy-1-(β-D-ribofuranosyl)benzimidazole (**11**) was accomplished by the treatment of **2b** with sodium methoxide in methanol. This resulted in a concomitant removal of the sugar protecting groups and a replacement of the 2-chloro group by a methoxy group.

The assignment of β configuration to the glycosylation products was based on Baker's trans rule.[20] The preferential formation of the β anomers was attributed to the formation of an acyloxonium intermediate, which directed the attack of the base from the β face. The assignment of regiochemistry to the positional isomers **5** and **6** was based on their ^{13}C NMR data and was also supported by a series of difference NOE experiments. Thus, each aromatic proton of compound **5** was irradiated, and the NOE enhancement of the sugar protons was observed. The only aromatic proton, which resulted in an NOE enhancement of the sugar protons, was assigned as 7-H. The fact that this 7-H was a doublet with a coupling constant of 9 Hz (typical for ortho coupling) indicated that the amino group was attached to the C5. Similarly, a series of difference NOE experiments on compound **6** established its structure as a 6-amino derivative.

Antiviral Studies

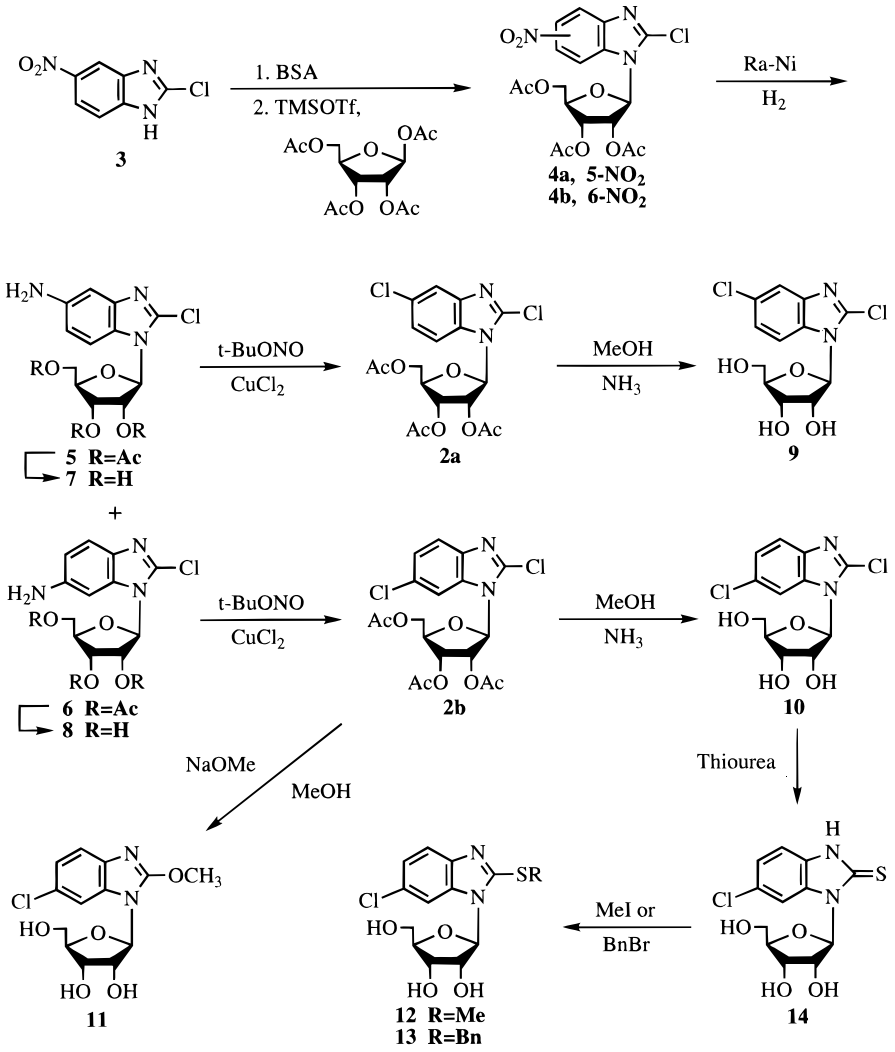
Compounds were evaluated for activity against HCMV and HSV-1 and for cytotoxicity in uninfected cells. The one heterocycle tested (**3**) was active against HCMV and to a lesser extent against HSV-1 but also was cytotoxic in nearly the same concentration range. This result is almost identical to that obtained previously for the heterocycle of TCRB.[12] In both cases, the benzimidazole heterocycle was both active and somewhat cytotoxic, in contrast TCRB was active but not cytotoxic (Table 1).

Evaluation of the disubstituted ribonucleosides **7**−**14** revealed a different pattern of activity. These compounds were either inactive or weakly active against HCMV, inactive against HSV-1, and not cytotoxic (Table 1). The two dichloro substituted benzimidazole nucleosides **9** and **10** were weakly active against HCMV with the 2,6 dichloro isomer **10** somewhat more active than the 2,5-dichloro isomer **9**. The activity of these two dichloro isomers and their lack of cytotoxicity is in marked contrast to the activity of DRB, the 5,6-dichloro isomer. The fact that DRB inhibited HCMV, HSV-1, HFF cells, and KB cells to nearly the same extent (Table 1) strongly suggests that its apparent activity against the viruses is not antiviral activity but is a manifestation of cytotoxicity. In contrast, the weak activity of the 2-substituted analogs **9** and **10** against HCMV most likely is specific antiviral activity because it was observed at noncytotoxic concentrations. This is consistent with the known potent and specific antiviral activity of the 2,5,6-trichloro analog, TCRB, against HCMV[12,14] and its low cytotoxicity (Table 1). We conclude, therefore, that substitution of the 2-position of a benzimidazole ribonucleoside with halogen is critical in enduing the molecule with low cytotoxicity. For effective activity against HCMV, however, the molecule must have a halogen not only at the 2-position, but it must also have more than one chlorine in the benzene moiety.

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

Scheme 2

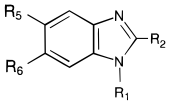


Laboratory in the Chemistry Department, University of Michigan. High resolution MS (HRMS) measurements were obtained on a VG 70-250-S MS spectrometer using a direct probe for sample introduction. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Chemical reactions and column chromatographic 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 Buchi rotavapor under water aspirator or mechanical oil pump vacuum at 40 °C or cooler unless otherwise specified.

2,5-Dichloro-1-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)-benzimidazole (2a) and 2,6-Dichloro-1-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)benzimidazole (2b). A stirred suspension of 2,5(6)-dichlorobenzimidazole[15] (**1**, 0.75 g, 4.01 mmol) in dry MeCN (20 mL) was treated with bis(trimethylsilyl)-trifluoroacetamide (BSTFA, 1.1 mL, 4.14 mmol) at 80 °C for 15 min to give a clear solution. To this solution were added 1,2,3,5-tetra-*O*-acetyl-*β*-D-ribofuranose (1.27 g, 3.99 mmol) and TMSOTf (0.856 mL, 4.43 mmol), and stirring was continued at 80 °C for 1 h. The reaction mixture was cooled to room temperature and diluted with EtOAc (100 mL). The EtOAc solution was extracted with a saturated $NaHCO_3$ solution (100 mL × 2) and a saturated NaCl solution (100 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (3 × 8 cm, eluted with $CHCl_3$). The fractions containing the first eluted spot were collected and evaporated. The residue was coevaporated with MeOH and then recrystallized from MeOH to afford 1.451 g (two crops, 82%) of **2a**,**b** as white crystals (as a mixture of the 2,5-dichloro and the 2,6-dichloro positional isomers). Repeated recrystallization (five times) of this material yielded pure isomer **2b** as white crystals. Mp 154−155 °C. HRMS (EI) *m*/*z* 444.0492 (6%, M^+ = 444.0491). ^{1}H NMR (DMSO-d_6) δ 7.89 (d, 1, 7-H, J_{7-5} = 2.0 Hz), 7.68 (d, 1, 4-H, J_{4-5} = 8.5 Hz), 7.37 (dd, 1, 5-H), 6.25 (d, 1, 1′-H, $J_{1'-2'}$ = 7.0 Hz), 5.57 (t, 1, 2′-H, $J_{2'-3'}$ = 7.0 Hz), 5.44 (dd, 1, 3′-H, $J_{3'-4'}$ = 4.5 Hz), 4.47, 4.39 (2 × 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 169.94, 169.48, 169.15 (3 × $OCOCH_3$), 140.06 (C3a and C2), 133.48 (C7a), 128.34 (C6), 123.78 (C5), 120.52 (C4), 111.71 (C7), 86.65 (C1′), 79.40 (C4′), 70.35 (C2′), 68.64 (C3′), 62.58 (C5′), 20.55, 20.26, 19.97 (3 × $OCOCH_3$). Anal. ($C_{18}H_{18}Cl_2N_2O_7$) C, H, N.

2-Chloro-5-nitro-1-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)-benzimidazole (4a) and 2-Chloro-6-nitro-1-(2,3,5-tri-*O*-acetyl-*β*-D-ribofuranosyl)benzimidazole (4b). A stirred suspension of 2-chloro-5(6)-nitrobenzimidazole[15−17] (**3**, 3.952 g, 20.22 mmol) in $ClCH_2CH_2Cl$ (100 mL) was treated with bis-(trimethylsilyl)acetamide (BSA, 5 mL, 20 mmol) at 75 °C for 15 min to give a clear solution. This solution was cooled to room temperature and treated with 1,2,3,5-tetra-*O*-acetyl-*β*-D-ribofuranose (7.0 g, 22 mmol) and TMSOTf (4.638 mL, 24 mmol) at room temperature for 2 h. The reaction mixture was diluted with 200 mL of $CHCl_3$. The $CHCl_3$ solution was washed with a saturated $NaHCO_3$ solution (200 mL × 2) and a saturated NaCl solution (200 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (4 × 35 cm, eluted with $CHCl_3$ and 0.5% MeOH/$CHCl_3$, v/v). The fractions containing the first eluted spot were collected and evaporated to give 6.50 g (71%) of **4a**,**b** as a white foam (as a mixture of the 5-nitro and the 6-nitro positional isomers). Fractional recrystallization of this foam (five times from MeOH) gave 1.59 g (17%) of pure 6-nitro isomer **4b**. Mp

Table 1. Antiviral Activity and Cytotoxicity of Disubstituted Benzimidazoles and Benzimidazole Ribonucleosides



compound no.	R_1	R_2	R_5	R_6	50 or 90% inhibitory concentration (μM) antiviral activity HCMV[a] plaque	HCMV[a] yield	HSV-1[b] ELISA	cytotoxicity[c] visual	cytotoxicity[c] growth
3	H	Cl	NO_2	H	6[d]	–	21[d]	20[d]	–
7	ribose	Cl	NH_2	H	>300[d,e]	–	>100	>300[d]	–
8	ribose	Cl	H	NH_2	>100[d]	–	>100	>300[d]	–
9	ribose	Cl	Cl	H	150[d]	100[d]	>100	>100[d]	–
10	ribose	Cl	H	Cl	58[d]	52[d]	>100	>100[d]	–
DRB[f,g]	ribose	H	Cl	Cl	42[d]	19[d]	30[d]	24[d]	36[d]
14	ribose	SH	H	Cl	>100	–	>100[d]	>100	>100
12	ribose	SCH_3	H	Cl	>100	–	<100[d]	>100	–
13	ribose	$SCH_2C_6H_5$	H	Cl	>100	–	>100[d]	>100	–
11	ribose	OCH_3	H	Cl	>100	–	>100[d]	>100	>100
TCRB[g]	ribose	Cl	Cl	Cl	2.8 ± 0.8	1.3 ± 0.8	102[d]	238	210
ganciclovir[h]					7.4 ± 6.5	1.6 ± 1.2	3.5 ± 2.1	>100	>100

[a] Plaque and yield reduction assays were performed as described in the text. Results from plaque assays are reported as IC_{50}'s and those for yield reduction experiments as IC_{90}'s. [b] All 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] Average of duplicate or triplicate experiments. [e] > indicates IC_{50} or IC_{90} not reached at the noted (highest) concentration tested. [f] Compound referred to as DRB by Tamm and co-workers.[2] [g] Data also reported in ref 12; for TCRB averages ±sd derived from 5 and 15 experiments. [h] Averages ±sd derived from 108, 33, and 3 experiments, respectively, in which ganciclovir was used as a positive control.

127−129 °C. HRMS (EI) *m/z* 455.0750 (2%, M^+ = 455.0732). ^{1}H NMR (DMSO-d_6) δ 8.68 (d, 1, 7-H, J_{7-5} = 2.0 Hz), 8.21 (dd, 1, 5-H, J_{5-4} = 9.0 Hz), 7.88 (d, 1, 4-H), 6.41 (d, 1, 1′-H, $J_{1'-2'}$ = 7.0 Hz), 5.58 (t, 1, 2′-H, $J_{2'-3'}$ = 7.0 Hz), 5.45 (dd, 1, 3′-H, $J_{3'-4'}$ = 4.0 Hz), 4.50, 4.40 (2 × m, 3, 4′-H and 5′-H), 2.15, 2.12, 2.03 (3 × s, 9, 3 × Ac). ^{13}C NMR (DMSO-d_6) δ 170.12, 169.49, 169.25 (3 × O*C*OCH$_3$), 145.60 (C3a), 143.96 (C2), 143.65 (C6), 132.46 (C7a), 119.65 (C4), 118.92 (C5), 108.47 (C7), 86.96 (C1′), 79.67 (C4′), 71.12 (C2′), 68.75 (C3′), 62.56 (C5′), 20.39, 20.31, 20.03 (3 × OCO*C*H$_3$). Anal. ($C_{18}H_{18}ClN_3O_9$) C, H, N.

5-Amino-2-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (5) and 6-Amino-2-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole (6). Compound **4a**,**b** (3.96 g, 8.688 mmol, as a mixture of the 5-nitro and the 6-nitro positional isomers) was dissolved in EtOH (90 mL) and hydrogenated at room temperature, 50 psi of H_2 for 1 day using 0.30 g of Raney nickel (wet weight) as catalyst. The reaction mixture was then filtered, and the filtrate was evaporated. The residue was chromatographed on a silica column (4 × 45 cm, eluted with $CHCl_3$). Evaporation of the appropriate fractions gave 1.54 g (41% as a hemihydrate) of **6** as a white foam. HRMS (EI) *m/z* 425.0987 (14%, M^+ = 425.0990). ^{1}H NMR (DMSO-d_6) δ 7.29 (d, 1, 4-H, J_{4-5} = 8.5 Hz), 6.75 (d, 1, 7-H, J_{7-5} = 1.5 Hz), 6.61 (dd, 1, 5-H), 6.09 (d, 1, 1′-H, $J_{1'-2'}$ = 6.5 Hz), 5.57 (t, 1, 2′-H, $J_{2'-3'}$ = 7.5 Hz), 5.37 (dd, 1, 3′-H, $J_{3'-4'}$ = 5.5 Hz), 5.19 (s, 2, 6-NH$_2$), 4.43 (dd, 1, 5′-H, $J_{5'-4'}$ = 2.5 Hz, $J_{5'-5''}$ = 11.0 Hz), 4.40 (m, 1, 4′-H), 4.36 (dd, 1, 5″-H, $J_{5''-4'}$ = 6.0 Hz), 2.13, 2.09, 2.04 (3 × s, 9, 3 × Ac). ^{13}C NMR (DMSO-d_6) δ 170.10, 169.45, 169.19 (3 × O*C*OCH$_3$), 146.03 (C6), 134.83 (C2), 134.06 (C7a), 132.92 (C3a), 119.35 (C4), 112.17 (C5), 94.72 (C7), 86.67 (C1′), 78.46 (C4′), 69.75 (C2′), 68.58 (C3′), 62.51 (C5′), 20.50, 20.21, 19.99 (3 × OCO*C*H$_3$). Anal. ($C_{18}H_{20}ClN_3O_7$·0.5H_2O) C, H, N. Further elution and evaporation of the appropriate fractions gave 1.86 g (49% as a hemihydrate) of **5** as a white foam. HRMS (EI) *m/z* 425.0976 (9%, M^+ = 425.0990). ^{1}H NMR (DMSO-d_6) δ 7.40 (d, 1, 7-H, J_{7-6} = 8.5 Hz), 6.76 (d, 1, 4-H, J_{4-6} = 2.0 Hz), 6.66 (dd, 1, 6-H), 6.10 (d, 1, 1′-H, $J_{1'-2'}$ = 6.5 Hz), 5.56 (t, 1, 2′-H, $J_{2'-3'}$ = 7.0 Hz), 5.39 (dd, 1, 3′-H, $J_{3'-4'}$ = 5.0 Hz), 5.10 (br s, 2, 5-NH$_2$), 4.40 (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 169.91, 169.52, 169.11 (3 × O*C*OCH$_3$), 145.31 (C5), 142.74 (C3a), 137.80 (C2), 124.66 (C7a), 112.60 (C6), 111.62 (C7), 102.23 (C4), 86.57 (C1′), 78.89 (C4′), 70.05 (C2′), 68.78 (C3′), 62.69 (C5′), 20.45, 20.26, 19.98 (3 × OCO*C*H$_3$). Anal. ($C_{18}H_{20}ClN_3O_7$·0.5H_2O) C, H, N.

5-Amino-2-chloro-1-(β-D-ribofuranosyl)benzimidazole (7). A solution of **5** (0.363 g, 0.835 mmol, based on the hemihydrate of **5**) in NH$_3$/MeOH (saturated at 0 °C, 10 mL) 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/Et_2O (Et_2O diffusion) to give 0.203 g (two crops, 81%) of **7** as beige crystals. Mp ~ 154 °C dec. HRMS (EI) *m/z* 299.0675 (10%, M^+ = 299.0673). ^{1}H NMR (DMSO-d_6) δ 7.56 (d, 1, 7-H, J_{7-6} = 8.5 Hz), 6.73 (d, 1, 4-H, J_{4-6} = 2.0 Hz), 6.57 (dd, 1, 6-H), 5.77 (d, 1, 1′-H, $J_{1'-2'}$ = 7.5 Hz), 5.39 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.5 Hz), 5.18 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 4.5 Hz), 5.11 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.0 Hz), 4.90 (s, 2, 5-NH$_2$), 4.44 (m, 1, 2′-H, $J_{2'-3'}$ = 6.0 Hz), 4.09 (m, 1, 3′-H, $J_{3'-4'}$ = 2.5 Hz), 3.91 (m, 1, 4′-H, $J_{4'-5'}$ = 3.5 Hz), 3.65 (t, 2, 5′-H). ^{13}C NMR (DMSO-d_6) δ 144.82 (C5), 142.77 (C3a), 138.25 (C2), 125.13 (C7a), 112.67 (C7), 112.22 (C6), 101.83 (C4), 88.81 (C1′), 85.48 (C4′), 70.89 (C2′), 69.61 (C3′), 61.31 (C5′). Anal. ($C_{12}H_{14}ClN_3O_4$) C, H, N.

6-Amino-2-chloro-1-(β-D-ribofuranosyl)benzimidazole (8). A solution of **6** (0.283 g, 0.651 mmol, based on the hemihydrate of **6**) in NH$_3$/MeOH (10 mL) 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 to give 0.170 g (two crops, 87%) of **8** as beige crystals. Mp ~170 °C dec. HRMS (EI) *m/z* 299.0862 (16%, M^+ = 299.0673). ^{1}H NMR (DMSO-d_6) δ 7.25 (d, 1, 4-H, J_{4-5} = 8.5 Hz), 6.87 (d, 1, 7-H, J_{7-5} = 2.0 Hz), 6.57 (dd, 1, 5-H), 5.75 (d, 1, 1′-H, $J_{1'-2'}$ = 7.5 Hz), 5.43 (d, 1, 2′-OH, $J_{2'-2'OH}$ = 6.5 Hz), 5.18 (d, 1, 3′-OH, $J_{3'-3'OH}$ = 5.0 Hz), 5.04 (s, 2, 6-NH$_2$), 5.03 (t, 1, 5′-OH, $J_{5'-5'OH}$ = 5.5 Hz), 4.48 (m, 1, 2′-H, $J_{2'-3'}$ = 6.0 Hz), 4.07 (m, 1, 3′-H, $J_{3'-4'}$ = 3.0 Hz), 3.90 (m, 1, 4′-H, $J_{4'-5'}$ = 4.0 Hz, $J_{4'-5''}$ = 5.0 Hz), 3.66 (m, 2, 5′-H and 5″-H, $J_{5'-5''}$ = 12.0 Hz). ^{13}C NMR (DMSO-d_6) δ 145.34 (C6), 135.63 (C2), 134.34 (C7a), 133.18 (C3a), 118.83 (C4), 111.71 (C5), 95.79 (C7), 88.79 (C1′), 85.24 (C4′), 70.17 (C2′), 69.70 (C3′), 61.55 (C5′). Anal. ($C_{12}H_{14}ClN_3O_4$) C, H, N.

2,5-Dichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-benzimidazole (2a). To a stirred mixture of $CuCl_2$ (0.292 g, 2.172 mmol) and 90% t-BuONO (0.259 mL, 1.960 mmol) in MeCN (4 mL) was added dropwise a solution of **5** (0.463 g,

1.065 mmol, based on the hemihydrate of **5**) in MeCN (2 mL). After the addition was complete, stirring was continued at room temperature for 2 h. The reaction mixture was diluted with EtOAc (50 mL). The EtOAc solution was washed with H_2O (50 mL), a saturated $NaHCO_3$ solution (50 mL × 2), and a saturated NaCl solution (50 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (2 × 15 cm, eluted with $CHCl_3$). Evaporation of the appropriate fractions and recrystallization from MeOH gave 0.265 g (56%) of **2a** as a white crystalline compound. Mp 98−100 °C. HRMS (EI) *m/z* 444.0487 (8%, $M^+ = 444.0491$). ^{1}H NMR (DMSO-d_6) δ 7.81 (d, 1, 7-H, $J_{7-6} = 9.0$ Hz), 7.77 (d, 1, 4-H, $J_{4-6} = 2.0$ Hz), 7.42 (dd, 1, 6-H), 6.25 (d, 1, 1′-H, $J_{1'-2'} = 7.0$ Hz), 5.56 (t, 1, 2′-H, $J_{2'-3'} = 7.0$ Hz), 5.42 (dd, 1, 3′-H, $J_{3'-4'} = 5.0$ Hz), 4.43 (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 170.01, 169.52, 169.20 (3 × O*C*OCH$_3$), 142.17 (C3a), 140.66 (C2), 131.73 (C7a), 127.94 (C5), 123.91 (C6), 118.80 (C4), 113.19 (C7), 86.88 (C1′), 79.26 (C4′), 70.46 (C2′), 68.73 (C3′), 62.65 (C5′), 20.52, 20.29, 20.01 (3 × OCO*C*H$_3$). Anal. ($C_{18}H_{18}Cl_2N_2O_7$) C, H, N.

2,6-Dichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-benzimidazole (2b). To a stirred mixture of $CuCl_2$ (0.419 g, 3.116 mmol) and 90% t-BuONO (0.371 mL, 2.807 mmol) in MeCN (5 mL) was added dropwise a solution of **6** (0.633 g, 1.456 mmol, based on the hemihydrate of **6**) in MeCN (3 mL). After the addition was complete, stirring was continued at room temperature for 2 h. The reaction mixture was diluted with EtOAc (60 mL). The EtOAc solution was washed with H_2O (50 mL), a saturated $NaHCO_3$ solution (50 mL × 2), and a saturated NaCl solution (50 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica column (1.9 × 35 cm, eluted with $CHCl_3$). Evaporation of the appropriate fractions and recrystallization from MeOH gave 0.380 g (59%) of **2b** as a white crystalline compound. Mp 152−154 °C. HRMS (EI) *m/z* 444.0487 (7%, $M^+ = 444.0491$). ^{1}H and ^{13}C NMR spectra were identical to those of **2b** prepared via ribosylation of 2,5-dichlorobenzimidazole (**1**) followed by fractional recrystallization. Anal. ($C_{18}H_{18}Cl_2N_2O_7$) C, H, N.

2,5-Dichloro-1-(β-D-ribofuranosyl)benzimidazole (9). A solution of **2a** (0.226 g, 0.508 mmol) in NH_3/MeOH (saturated at 0 °C, 10 mL) 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 to give 0.136 g (76%, based on $C_{12}H_{12}Cl_2N_2O_4$·MeOH) of **9** as white crystals. Mp 102−150 °C (melted slowly over a wide range of temperature). HRMS (CI) *m/z* 319.0242 (37%, $MH^+ = 319.0252$). ^{1}H NMR (DMSO-d_6) δ 8.06 (d, 1, 7-H, $J_{7-6} = 9.0$ Hz), 7.73 (d, 1, 4-H, $J_{4-6} = 2.0$ Hz), 7.30 (dd, 1, 6-H), 5.89 (d, 1, 1′-H, $J_{1'-2'} = 8.0$ Hz), 5.51 (d, 1, 2′-OH, $J_{2'-2'OH} = 6.5$ Hz), 5.29 (d, 1, 3′-OH, $J_{3'-3'OH} = 4.5$ Hz), 5.26 (t, 1, 5′-OH, $J_{5'-5'OH} = 5.0$ Hz), 4.45 (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, $J_{4'-5'} = J_{4'-5''} = 3.5$ Hz), 3.69 (m, 2, 5′-H and 5″-H, $J_{5'-5''} = 12.0$ Hz). ^{13}C NMR (DMSO-d_6) δ 142.36 (C3a), 141.40 (C2), 131.88 (C7a), 127.49 (C5), 123.32 (C6), 118.40 (C4), 114.51 (C7), 89.07 (C1′), 86.23 (C4′), 71.49 (C2′), 69.75 (C3′), 61.23 (C5′). Anal. ($C_{12}H_{12}Cl_2N_2O_4$·MeOH) C, H, N.

2,6-Dichloro-1-(β-D-ribofuranosyl)benzimidazole (10). A solution of **2b** (0.362 g, 0.813 mmol) in NH_3/MeOH (10 mL) 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 to give 0.183 g (two crops, 71%) of **10** as white crystalline needles. Mp 160−161 °C. HRMS (EI) *m/z* 318.0169 (9%, $M^+ = 318.0174$). ^{1}H NMR (DMSO-d_6) δ 8.30 (d, 1, 7-H, $J_{7-5} = 2.0$ Hz), 7.64 (d, 1, 4-H, $J_{4-5} = 8.5$ Hz), 7.31 (dd, 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), 5.35 (t, 1, 5′-OH, $J_{5'-5'OH} = 4.5$ Hz), 5.28 (d, 1, 3′-OH, $J_{3'-3'OH} = 4.5$ Hz), 4.46 (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, $J_{4'-5'} = J_{4'-5''} = 3.0$ Hz), 3.70 (m, 2, 5′-H and 5″-H, $J_{5'-5''} = 12.0$ Hz). ^{13}C NMR (DMSO-d_6) δ 140.79 (C2), 140.21 (C3a), 133.58 (C7a), 127.90 (C6), 123.29 (C5), 120.08 (C4), 113.22 (C7), 89.00 (C1′), 86.32 (C4′), 71.39 (C2′), 69.79 (C3′), 61.16 (C5′). Anal. ($C_{12}H_{12}Cl_2N_2O_4$) C, H, N.

6-Chloro-2-methoxy-1-(β-D-ribofuranosyl)benzimidazole (11). A mixture of **2b** (0.445 g, 1 mmol) and NaOMe (0.108 g, 2 mmol) in MeOH (20 mL) was stirred at reflux temperature for 18 h. The resulting solution was cooled to room temperature, neutralized with NH_4Cl (0.107 g, 2 mmol), and then evaporated. The resulting solid was triturated with cold H_2O and recrystallized from MeOH/H_2O to give 0.314 g (91%, two crops, the 2nd crop was recrystallized from EtOAc/hexane) of **11** as a white powder. Mp 206−209 °C (MeOH). HRMS (EI) *m/z* 314.0681 (21%, $M^+ = 314.0669$). ^{1}H NMR (DMSO-d_6) δ 7.89 (d, 1, 7-H, $J_{7-5} = 2.0$ Hz), 7.42 (d, 1, 4-H, $J_{4-5} = 8.5$ Hz), 7.14 (dd, 1, 5-H), 5.71 (d, 1, 1′-H, $J_{1'-2'} = 7.5$ Hz), 5.34 (d, 1, 2′-OH, $J_{2'-2'OH} = 6.5$ Hz), 5.16 (m, 2, 3′-OH and 5′-OH, $J_{3'-3'OH} = 4.5$ Hz, $J_{5'-5'OH} = 5.5$ Hz), 4.46 (m, 1, 2′-H, $J_{2'-3'} = 5.5$ Hz), 4.11 (s, 3, 2-OMe), 4.09 (m, 1, 3′-H, $J_{3'-4'} = 2.5$ Hz), 3.90 (m, 1, 4′-H, $J_{4'-5'} = 3.0$ Hz), 3.63 (t, 2, 5′-H). ^{13}C NMR (DMSO-d_6) δ 157.95 (C2), 138.76 (C3a), 132.76 (C7a), 125.14 (C6), 121.66 (C5), 118.34 (C4), 111.77 (C7), 86.53 (C1′), 85.48 (C4′), 70.65 (C2′), 70.00 (C3′), 61.41 (C5′), 57.48 (2-OMe). Anal. ($C_{13}H_{15}ClN_2O_5$) C, H, N.

6-Chloro-2-(methylthio)-1-(β-D-ribofuranosyl)benzimidazole (12). A solution of compound **14** (0.317 g, 1 mmol) in 10 mL of H_2O and 1.13 mL of concd NH_4OH was treated with 0.14 mL (2.25 mmol) of MeI at room temperature for 2 h and then was allowed to stand at 5 °C overnight. The white precipitate was collected by filtration and air-dried. The filtrate was extracted with EtOAc (30 mL), and the EtOAc solution was evaporated to give a white solid. This was combined with the precipitate and recrystallized from MeOH/H_2O to afford 0.256 g (two crops, 77%) of **12** as white crystals. Mp 106−109 °C. HRMS (EI) *m/z* 330.0442 (27%, $M^+ = 330.0441$). ^{1}H NMR (DMSO-d_6) δ 8.09 (d, 1, 7-H, $J_{7-5} = 2.0$ Hz), 7.55 (d, 1, 4-H, $J_{4-5} = 8.5$ Hz), 7.19 (dd, 1, 5-H), 5.69 (d, 1, 1′-H, $J_{1'-2'} = 7.5$ Hz), 5.24 (d, 1, 2′-OH, $J_{2'-2'OH} = 6.5$ Hz), 5.28 (t, 1, 5′-OH, $J_{5'-5'OH} = 5.0$ Hz), 5.42 (d, 1, 3′-OH, $J_{3'-3'OH} = 4.5$ Hz), 4.44 (m, 1, 2′-H, $J_{2'-3'} = 5.5$ Hz), 4.11 (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), 3.69 (m, 2, 5′-H and 5″-H, $J_{5'-5''} = 12.0$ Hz), 2.71 (s, 3, 2-SMe). ^{13}C NMR (DMSO-d_6) δ 154.19 (C2), 142.31 (C3a), 134.98 (C7a), 126.17 (C6), 122.06 (C5), 118.66 (C4), 112.35 (C7), 88.66 (C1′), 85.94 (C4′), 71.21 (C2′), 69.79 (C3′), 61.22 (C5′), 14.60 (2-SMe). Anal. ($C_{13}H_{15}ClN_2O_4S$) C, H, N.

2-Benzylthio-6-chloro-1-(β-D-ribofuranosyl)benzimidazole (13). A solution of compound **14** (0.317 g, 1 mmol) in 10 mL of H_2O and 1.0 mL of conc. NH_4OH was treated with 0.240 mL (2.0 mmol) of benzyl bromide at room temperature for 2 h. The white precipitate was filtered, air-dried, and recrystallized from EtOAc/hexane to give 0.339 g (83%) of **13** as white crystals. Mp 152−155 °C. HRMS (EI) *m/z* 406.0753 (5%, $M^+ = 406.0754$). ^{1}H NMR (DMSO-d_6) δ 8.11 (d, 1, 7-H, $J_{7-5} = 2.0$ Hz), 7.59 (d, 1, 4-H, $J_{4-5} = 8.5$ Hz), 7.48, 7.31 (2 × m, 5, Ph), 7.21 (dd, 1, 5-H), 5.70 (d, 1, 1′-H, $J_{1'-2'} = 7.5$ Hz), 5.41 (d, 1, 2′-OH, $J_{2'-2'OH} = 6.5$ Hz), 5.28 (t, 1, 5′-OH, $J_{5'-5'OH} = 5.0$ Hz), 5.22 (d, 1, 3′-OH, $J_{3'-3'} = 4.5$ Hz), 4.61 (m, 2, C*H*$_2$Ph), 4.42 (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 152.78 (C2), 142.19 (C3a), 136.90 (Ph), 134.78 (C7a), 128.90, 128.39,127.36 (Ph), 126.35(C6), 122.20 (C5), 118.82 (C4), 112.46 (C7), 88.66 (C1′), 85.95 (C4′), 71.27 (C2′), 69.74 (C3′), 61.18 (C5′), 35.90 (*C*H$_2$Ph). Anal. ($C_{12}H_{13}N_2ClSO_4$) C, H, N.

6-Chloro-1-(β-D-ribofuranosyl)benzimidazole-2-thione (14). A mixture of **10** (0.82 g, 2.57 mmol) and thiourea (0.391 g (5.14 mmol) in anhydrous EtOH (50 mL) was stirred at reflux temperature for 2.5 h and then allowed to stand at room temperature for 12 h. A small amount of solid material was filtered off and discarded. The filtrate was evaporated, and the residue was triturated with cold H_2O. The resulting white precipitate was collected by filtration and washed with portions of cold water to give 0.694 g (85%) of **14** as a white powder. This material was used directly in the subsequent alkylation reactions without further purification. An analytically pure sample was obtained by recrystallization from EtOH/H_2O. Mp 175−177 °C. HRMS (EI) *m/z* 316.0286 (6%, $M^+ = 316.0285$). ^{1}H NMR (DMSO-d_6) δ 13.01 (br s, 1, 3-NH), 8.06 (d, 1, 7-H, $J_{7-5} = 2.0$ Hz), 7.23 (dd, 1, 5-H, $J_{5-4} = 8.5$ Hz),

7.17 (d, 1, 4-H), 6.46 (d, 1, 1′-H, $J_{1'-2'}$ = 8.0 Hz), 5.25, 5.15, 5.11 (3 × s, 3, 2′-OH, 3′-OH, and 5′-OH), 4.47 (m, 1, 2′-H, $J_{2'-3'}$ = 6.0 Hz), 4.15 (m, 1, 3′-H, $J_{3'-4'}$ = 2.5 Hz), 3.92 (m, 1, 4′-H, $J_{4'-5'}$ = $J_{4'-5''}$ = 2.5 Hz), 3.68 (m, 2, 5′-H and 5″-H, $J_{5'-5''}$ = 12.0 Hz). ^{13}C NMR (DMSO-d_6) δ 170.52 (C2), 131.20, 129.84 (C3a and C7a), 126.70 (C6), 122.93 (C5), 111.86 (C7), 110.53 (C4), 88.31 (C1′), 85.29 (C4′), 70.42 (C2′), 69.63 (C3′), 61.01 (C5′). Anal. ($C_{12}H_{13}ClN_2O_4S$) C, H, N.

Antiviral Evaluation. **(a) Cells and Viruses.** Human foreskin fibroblasts (HFF cells) and MRC-5 cells, a human embryonic lung cell line, were grown in minimal essential medium (MEM) with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum (FBS). KB cells, an established human cell line derived from an epidermoid oral carcinoma, were grown in MEM with Hank's salts [MEM(H)] supplemented with 10% calf serum (CS). These cell lines were subcultured according to conventional procedures as described previously.[21] All cell lines were screened periodically for mycoplasma contamination and were negative. A plaque purified isolate, P_o, of the Towne strain of HCMV was used in all experiments and was a gift of 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 preparations of HCMV and HSV-1 were prepared and titer determined as described elsewhere.[21,22]

(b) Assays for Antiviral Activity. HCMV plaque-reduction assays were performed using monolayer cultures of HFF cells by a procedure similar to that referenced above for HCMV,[21] with the exception that the virus inoculum (0.2 mL) contained approximately 100 plaque forming units (PFU) of HCMV and the compounds to be assayed were dissolved in the overlay medium. HCMV yield reduction assays were performed as described previously.[21,22] HSV-1 ELISA assays were performed using a procedure described by Prichard and Shipman.[23]

(c) Cytotoxicity Assays. Two basic tests for cellular cytotoxicity were employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF cells was estimated by visual scoring of cells not affected by virus infection in the plaque-reduction assay described above. Drug-induced cytopathology was estimated at 30-fold magnification and was scored on a zero to four basis on the day of staining for plaque counting. Cytotoxicity in logarithmically growing KB cells was determined by a staining method previously described.[24]

(d) Data Analysis. Dose−response relationships were used to quantify drug effects. These were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. The 50% inhibitory (IC_{50}) concentrations were calculated from the regression lines. Ganciclovir (GCV), acyclovir (ACV), and 2-acetylpyridine thiosemicarbazone were used as positive controls in HCMV and HSV-1 assays, respectively.

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