Benzimidazole Ribonucleosides: Design, Synthesis, and Antiviral Activity of Certain 2-(Alkylthio)- and 2-(Benzylthio)-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazoles[1]

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Several 2-alkylthio- and 2-benzylthio derivatives of 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB) have been designed and synthesized from 5,6-dichloro-1-(β-D-ribofuranosyl)-benzimidazole-2-thione. All compounds were evaluated for activity against human cytomegalovirus (HCMV) and/or herpes simplex virus type-1 (HSV-1). Three different cytotoxicity assays were used to determine if the compounds were toxic to uninfected cells. Most of the 2-alkylthio compounds were either inactive against HCMV and HSV-1 or were active only at concentrations at or near those which produced toxicity in uninfected cells. The best separation between activity against HCMV and cytotoxicity was observed with the 2-benzylthio analog **7**. This prompted us to synthesize the substituted 2-benzylthio analogs **11-23** using a Topliss Tree approach. None of these compounds were more active than compound **7**; most of the analogs were weakly active against both HCMV and HSV-1, but the activity was not separated from cytotoxicity. On the basis of both antiviral and cytotoxicity data, compound **7** was the best compound in the series. It was more active against HCMV than DRB (the 2-unsubstituted analog), acyclovir, and foscarnet, but it was less active than ganciclovir.

Introduction

Human cytomegalovirus (HCMV) is a leading cause of opportunistic infections among immunosuppressed individuals including AIDS patients, neonates, and bone marrow and organ transplant recipients.[2] Despite extensive research aimed at developing new compounds to treat HCMV infections, ganciclovir[3] and foscarnet[4] remain as two of the most promising agents for treating HCMV. Since the use of ganciclovir can lead to granulocytopenia[3] and foscarnet can cause severe renal dysfunction,[5] it is important to search for new agents that show promise in controlling HCMV infections.

Several 5,6-dihalogenated benzimidazole nucleosides have been synthesized as potential antiviral agents,[6,7] but until our recent work with 2,5,6-trichloro-1-β-D-ribofuranosylbenzimidazole and its 2-bromo analog,[8] we know of no reports on the activity of benzimidazole ribonucleosides against HCMV. In contrast, the 2-unsubstituted analog (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, DRB[6]) has been studied extensively for other biological activities. DRB is active against RNA[7] and DNA[9,10] viruses, but its antiviral activity is poorly separated from cytotoxicity; consequently, it has no utility as an antiviral drug. DRB inhibits viral[11] and cellular[12] RNA synthesis, most likely as a consequence of inhibiting cellular RNA polymerase II.[13] DRB also is an inhibitor of casein kinase,[14] DNA topoisomerase II,[15] and is an interferon inducer.[16] Together these findings have established that DRB is a nucleoside that affects multiple cellular processes.

In order to improve the antiviral profile of DRB, various synthetic modifications have been performed on the parent structure. With one exception,[17] DRB derivatives modified in the benzene moiety with different halogens[18] or halogens at different positions have shown weaker antiviral activity than DRB itself. Most sugar-modified analogs also are less active than DRB as antiviral agents[6,19] except for the xylo and lyxo analogs which are active against herpes simplex virus types 1 and 2 (HSV-1, HSV-2) at concentrations below their cytotoxic concentrations.[20] The xylo and lyxo analogs also demonstrated significant activity against parainfluenza virus type-3 but only marginal activity against HIV.

Apart from our own work,[8,21,38] there have been few reports on 2-substituted DRB analogs, although non-nucleoside 2-substituted benzimidazoles are known to have a broad range of biological activity including inhibition of nucleic acid synthesis[22] and potent antiviral activity against enteroviruses (e.g., LY122771-72)[23] and human immunodeficiency virus (e.g., TIBO analogs).[24] The paucity of information on nucleosides of 2-substituted benzimidazoles prompted us to initiate a study involving certain modifications of DRB at the 2-position in an effort to alter its antiviral profile. In view of our recent results that certain 2-substituted DRB analogs were active against HCMV,[21] we expanded our studies on the substitution pattern at this position. Because the high inhibitory activity of N-glycosides of halogenated benzimidazoles depends upon both the halogen and carbohydrate substituents, with the highest inhibitory activity among the β-ribofuranosides,[8,25] we elected to prepare and evaluate selected 2-thio-5,6-dichloro-1-β-D-ribofuranosylbenzimidazoles as potential inhibitors of HCMV.

Results and Discussion

Chemistry. The commercially available 4,5-dichloro-1,2-phenylenediamine (**1**) was cyclized[26] with carbon disulfide in the presence of KOH in ethanol to afford

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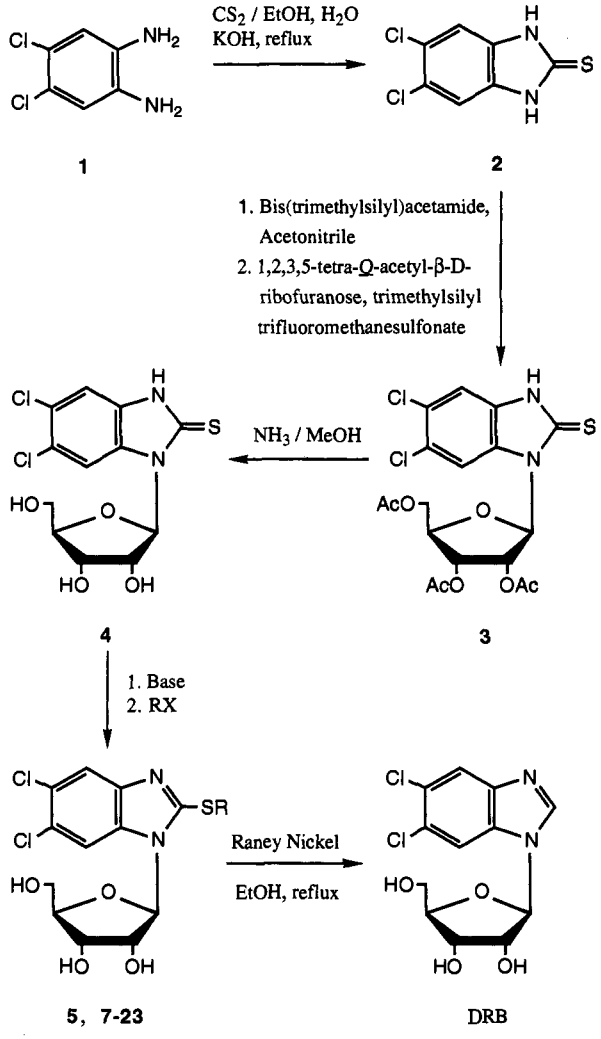
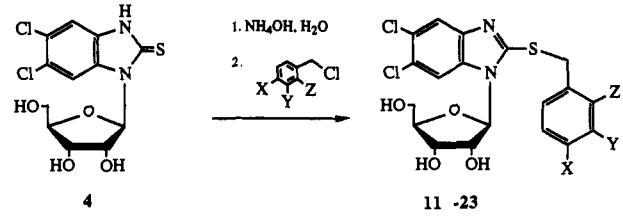
Scheme 1



5,6-dichlorobenzimidazole-2-thione (**2**) in 95% yield (Scheme 1). The IR absorption bands at 1460 and 1311 cm^{-1} and the lack of any absorption at 2550–2600 cm^{-1} suggested that **2** existed in the thione form. The chemical shift and integration of the peak for the D_2O-exchangeable proton also supported the structure of **2**. Compound **2** was silylated[27] with bis(trimethylsilyl)-acetamide (BSA) in acetonitrile and subsequently ribosylated by the addition of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of trimethylsilyl trifluoromethanesulfonate[28] (TMSOTf) to give 5,6-dichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole-2-thione (**3**) in 90% yield. The site of ribosylation and anomeric configuration for **3** was subsequently determined by a conversion of **3** into DRB. Deprotection of **3** was achieved with methanolic ammonia to obtain 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole-2-thione (**4**) and methylation of **4** with iodomethane[29] gave 5,6-dichloro-2-(methylthio)-1-β-D-ribofuranosylbenzimidazole (**5**). The site of ribosylation for compound **4** was determined by observing seven unequivalent carbon resonances in the aromatic region of the ^{13}C spectrum, an IR absorbance at 1463 cm^{-1} (C=S), and a exchangeable proton resonance at 13.14 ppm (N-*H*). Furthermore, Raney nickel mediated dethiation of **5** furnished a compound with the same properties as those reported for DRB.[19] Previous reports of 1-β-D-ribofuranosylbenzimidazole nucleosides[29-31] indicate that the $J_{1'-2'}$ coupling constants tend to be >5 Hz for such compounds

Scheme 2



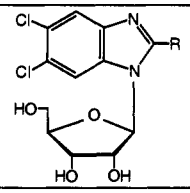
and therefore cannot be used to establish anomeric configuration. We found that all nucleosides (**4–23**) prepared in this study possessed a $J_{1'-2'}$ >5 Hz. However, the dethiation of **5** to give DRB (with a $J_{1'-2'}$ of 6.3 Hz) established the β-anomeric configuration for all nucleosides (**4–23**). Compounds **5–23** were obtained by the treatment of **4** with a base followed by the addition of an appropriate alkenyl, alkynyl, or benzyl halide (Schemes 1 and 2). The 2-methylsulfonyl compound (**6**) was obtained from the corresponding 2-methylthio compound (**5**) by oxidation with the magnesium hexahydrate salt of monoperoxyphthalic acid (MMPP) in acetonitrile at 0 °C. A downfield shift of the methyl protons coupled with an upfield shift of the methylsulfonyl carbon in the ^{13}C NMR spectra and a molecular ion at 397 ($[M + H]^+$) established **6** as the 2-methylsulfonyl analog of DRB.

Biology. The 2-(alkylthio)-DRB derivatives (**4–6, 8–10**) were evaluated for activity against HCMV in the plaque reduction assay and for cytotoxicity in uninfected human foreskin fibroblasts (HFF cells) (Table 1). Although compounds **4**, **5**, and **9** were inactive, the 2-methylsulfonyl analog (**6**) was active but cytotoxic, indicating activity against the virus was most likely a consequence of cytotoxicity. The 2-(propargylthio)- (**8**), and the 2-(cyanomethylthio)-DRB (**10**) derivatives were slightly more active against HCMV and about 4-fold less cytotoxic against HFF cells than DRB based upon visual inspection of stationary cells. Additional experiments showed that compound **10** also was active against HCMV in a yield reduction assay but was not active against HSV-1. The lack of activity against HSV-1 in an ELISA and low cytotoxicity in KB cells established that **10** was active against HCMV with better separation from cytotoxicity than compound **8**.

The 2-benzylthio analog **7** was the most promising member of the group. It was active against HCMV in both plaque- and yield-reduction assays, and this activity was separated from cytotoxicity based upon visual inspection of HFF cells and growth of KB cells (Table 1). It also had some activity against HSV-1. The cytotoxicity of compound **7** was evaluated further to better ascertain the separation between antiviral activity and cytotoxicity. Labeled precursor uptake studies were employed as a measure of effects on DNA, RNA, and protein synthesis. Compound **7** inhibited the uptake of tritiated thymidine, uridine, and leucine in uninfected KB cells with IC_{50}'s of 23, 15, and 22 μM, respectively, thereby raising the question if the antiviral activity could be a consequence of inhibition of cellular functions.

In an effort to improve the selectivity of compound **7** and increase the chances of synthesizing the most potent compound in the benzylthio series, we adopted the Topliss Tree approach[32] for aromatic substitution.

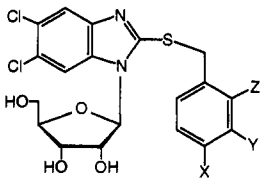
Table 1. Antiviral Activity and Cytotoxicity of 2-(Alkylthio)-5,6-dichloro-1-β-ribofuranosylbenzimidazoles



no.	R	50 or 90% inhibitory concentration (μM)				
		antiviral activity[a]				
		HCMV		HSV-1[b]	cytotoxicity[c]	
		plaque	yield	ELISA	visual	growth
4	SH	>100[d]	–	–	100	–
5	SCH_3	>100	–	–	>100	–
6	SO_2CH_3	10	–	–	10	–
7	$SCH_2C_6H_5$	22[e]	7	25	100[e]	>100
8	$SCH_2C{\equiv}CH$	25	–	100	100	60
9	$SCH_2CH{=}CH_2$	>100	–	>100	24	68[e]
10	$SCH_2C{\equiv}N$	30	10	>100	100	>100
DRB	H	42[e]	19[e]	30[e]	24[e]	100
foscarnet[f]		39 ± 26	–	–	>100	–
acyclovir[g]		86 ± 28	85 ± 82	1.7 ± 2.1	>100	>100
ganciclovir (DHPG)[h]		7.4 ± 6.5	1.6 ± 1.2	3.5 ± 2.1	>100	–

[a] Plaque and yield reduction assays were performed 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 activity of compound **7** and DHPG against HSV-1; all other compounds were assayed by ELISA. [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. Results are presented as IC_{50}'s. [d] >100 indicates IC_{50} or IC_{90} not reached at the noted (highest) concentration tested. [e] Average derived from two experiments. [f] Average ± standard deviation from 15 experiments. [g] Average ± standard deviation from 5, 5, and 26 experiments, respectively. [h] Average ± standard deviation from 108, 33, and 3 experiments, respectively.

Table 2. Antiviral Activity and Cytotoxicity of 2-(Benzylthio)-5,6-dichloro-1-β-ribofuranosylbenzimidazoles



no.	X	Y	Z	50% inhibitory concentration (μM)			
				antiviral activity[a]			
				HCMV plaque	HSV-1 ELISA	cytotoxicity[b] visual	growth
11	Cl	H	H	>100[c,d]	>30	100[d]	–
17	H	Cl	H	25[d]	70	21[d]	19
18	H	H	Cl	40	60	32	30
16	Cl	Cl	H	28[d]	>30	32[d]	–
12	Br	H	H	30	30	32	20
13	F	H	H	30[d]	>30[d]	21[d]	–
14	CH_3	H	H	30[d]	>30	32[d]	–
19	H	CH_3	H	40	38	32	30
15	OCH_3	H	H	40[d]	>100	66[d]	41
20	NO_2	H	H	40	40	32	30
21	H	NO_2	H	40	50	32	25
22	H	CF_3	H	40	40	32	15
23	*t*-Bu	H	H	20	20	32	50

[a] A plaque assay was used to quantitate HCMV, an ELISA was used for HSV-1. [b] 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. [c] >100 indicates IC_{50} not reached at the noted (highest) concentration tested. [d] Average derived from two experiments.

The Topliss Tree approach incorporates a substituent's lipophilic, electronic, and steric parameters. Using this approach, we synthesized and evaluated several new analogs. Although most of the compounds (**11**–**23**) demonstrated some activity against HCMV and HSV-1, there was little or no separation from cytotoxicity in either HFF or KB cells (Table 2). Thus the parent compound, **7**, was as or more active against HCMV and less cytotoxic than the substituted analogs.

Our present study has confirmed[8] that certain substituents at the 2-position can favorably influence the selectivity of DRB against HCMV. In our *in vitro* assays, compound **7** was 2–3 times more active against HCMV and 2–4 times less cytotoxic than the unsubstituted analog DRB (Table 1). Compounds **8** and **10** also appeared to be more active against HCMV than DRB, but the separation from cytotoxicity was not as good as that of compound **7**. All three compounds were more active against HCMV than foscarnet or acyclovir but were less active than ganciclovir. All three compounds, however, were more cytotoxic than these known drugs. These results suggest that other types of substitutions at the 2-position of DRB could lead to compounds with useful antiviral activity.

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Silica gel 60 230–400 mesh (E. Merck, Darmstadt, West Germany) was used for chromatography. Thin-layer chromatography (TLC) was performed on prescored SilicAR 7GF plates (Analtech, Newark, DE). TLC plates were developed in the following solvent systems: system 1 (2% MeOH/$CHCl_3$, v/v), system 2 (10% MeOH/$CHCl_3$, v/v), system 3 (50% EtOAc/hexane). Compounds were visualized by illumination with UV light (254 nm) or by spraying with 20% methanolic sulfuric acid followed by charring on a hot plate. Evaporations were carried out under reduced pressure (water aspirator) with the bath temperature below 40 °C, unless specified otherwise. IR spectra were obtained on a Nicolet 5DXB FT-IR spectrophotometer. UV spectra were performed on a Hewlett-Packard 8450-A UV/vis spectrophotometer. Nuclear magnetic resonance (NMR) spectra were determined at 360 MHz with a Bruker WP 360 SY. The chemical shift values are expressed in δ values (parts per million) relative to the standard chemical shift of the solvent DMSO-d_6. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

5,6-Dichlorobenzimidazole-2-thione (2). 4,5-Dichloro-1,2-phenylenediamine (1, 17.7 g, 0.1 mol) was suspended in a mixture of EtOH (100 mL) and H_2O (15 mL). Carbon disulfide (9 g, 0.11 mol) and potassium hydroxide (6.3 g, 0.11 mol) were added, and the mixture was heated at reflux for 3 h. Norit (4.0 g) was added, and the mixture was heated at reflux for an additional 10 min. The hot solution was gravity filtered. The filtrate was diluted with H_2O (100 mL), and a solid was precipitated with 8 mL of glacial acetic acid. The mixture was then allowed to stand at 5 °C for 18 h. The precipitate was collected by filtration, and the brown material was dried in a dessicating oven for 18 h at 40 °C to give 20.7 g (95%) of

product. Mp: >360 °C (lit.[33] mp >320 °C). R_f = 0.62 (solvent system 2), R_f = 0.70 (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 12.73 (s, 2 H, N-*H*, D_2O exchangeable), 7.30 (s, 2 H, C-*H*). ^{13}C NMR (DMSO-d_6): δ 170.46, 132.38, 124.44, 110.45. UV [λ_{max}, nm (ε)]: (pH 7) 253 (21 211), 323 (27 510); (pH 1) 248 (20 133), 316 (24 247); (pH 11) 236 (23 064), 260 (6875), 268 (6161), 315 (19 102).

5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole-2-thione (4). 5,6-Dichlorobenzimidazole-2-thione (**2**, 17.84 g, 0.081 mol) was suspended in dry acetonitrile (300 mL), and BSA (25.22 mL, 0.102 mol) was added to effect a clear solution. After 15 min, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (25.92 g, 0.081 mol) and TMSOTf (19.68 mL, 0.102 mol) were added. The reaction was heated to 50 °C in an oil bath and allowed to stir for 18 h. TLC analysis (solvent system 1) indicated that only one product had been formed with an R_f = 0.42. The mixture was concentrated under reduced pressure and the residue purified on a silica gel column (9.5 × 20 cm) using chloroform for elution. The fractions containing UV-absorbing material were pooled and concentrated under reduced pressure to give 34.71 g (90%) of the desired 5,6-dichloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)benzimidazole-2-thione (**3**) as a foam. R_f = 0.44 (solvent system 1), R_f = 0.13 (solvent system 3). ^{1}H NMR ($CDCl_3$): δ 11.05 (s, 1 H, N_3-*H*), 7.64 (s, 1 H, C_7-*H*), 7.25 (s, 1 H, C_4-*H*), 6.76 (d, 1 H, 1′-*H*, 7.4 Hz), 5.59 (t, 1 H, 2′-*H*, 6.9 Hz), 5.44 (m, 1 H, 3′-*H*), 4.46 (dm, 2 H, 5′-*H*), 4.39 (m, 1 H, 4′-*H*), 2.29 (s, 3 H), 2.13 (s, 3 H), 2.06 (s, 3 H). ^{13}C NMR ($CDCl_3$): δ 170.6, 169.9, 130.1, 129.6, 128.2, 127.1, 112.3, 111.4, 86.9, 79.9, 70.3, 69.3, 62.8, 21.0, 20.5, 20.4. This protected nucleoside (**3**, 28.34 g, 0.06 mol) was treated with methanolic ammonia (methanol saturated with ammonia at 0 °C) at room temperature for 18 h. The mixture was concentrated to dryness under reduced pressure and triturated with chloroform. The precipitate that formed was collected by vacuum filtration to give 15.76 g (76%) of unprotected 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole-2-thione (**4**) which was recrystallized from EtOH/H_2O. Mp: 241–242 °C. R_f = 0.28 (solvent system 2), R_f = 0.11 (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 13.14 (s, 1 H, N_3-*H*), 8.30 (s, 1 H, C_7-*H*), 7.36 (s, 1 H, C_4-H), 6.43 (d, 1 H, 1′-*H*, 7.6 Hz), 5.34 (t, 1 H, 5′-O*H*, 4.3 Hz), 5.19 (d, 1 H, 2′-O*H*, 6.5 Hz), 5.14 (d, 1 H, 3′-O*H*, 4.1 Hz), 4.42 (q, 1 H, 2′-*H*, 6.2 Hz), 4.12 (m, 1 H, 3′-*H*), 3.93 (m, 1 H, 4′-*H*), 3.67 (m, 2 H, 5′-H). ^{13}C NMR (DMSO-d_6): δ 171.4, 131.0, 130.2, 125.7, 124.8, 113.5, 110.7, 88.4, 85.6, 70.7, 69.9, 61.1. MS (DCI): *m*/*z* 351. UV [λ_{max}, nm (ε)]: (pH 7) 238 (8508), 255 (15 451), 324 (26 637); (pH 1) 233 (12 446), 252 (15 209), 319 (25 398); (pH 11) 236 (21 165), 274 (8143), 318 (18 431). Anal. ($C_{12}H_{12}Cl_2N_2O_4S \cdot {}^1/_2H_2O$) C, H, N.

General Procedure for the Synthesis of Compounds 5 and 7–23. 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole-2-thione (**4**, 351 mg, 1 mmol) was suspended in H_2O (25 mL), and CH_3CN (15 mL) was added for solubility. Twelve drops of concentrated NH_4OH were added, and the mixture was stirred at room temperature for 15 min. The appropriate alkyl, alkenyl, alkynyl, or benzyl halide (1 mmol) was added, and the mixture was stirred at room temperature for 18 h. Excess acetonitrile was removed under reduced pressure, and the aqueous layer was extracted with ethyl acetate (2 × 40 mL). The organic extracts were dried over anhydrous Na_2SO_4, concentrated to a thick syrup, dissolved in MeOH (100 mL), treated with charcoal, and filtered, and the filtrate was concentrated to dryness to give the appropriate 2-(substituted thio)-DRB analogs which were then recrystallized from MeOH/H_2O.

5,6-Dichloro-2-(methylthio)-1-β-D-ribofuranosylbenzimidazole (5). Yield: 285 mg (78%). Mp: 175–176 °C. R_f = 0.24 (solvent system 2), R_f = 0.10 (solvent system 3). ^{1}H NMR (DMSOd_6): δ 8.31 (s, 1 H, C_7-*H*), 7.82 (s, 1 H, C_4-*H*), 5.67 (d, 1 H, 1′-*H*, 7.6 Hz), 5.40 (d, 1 H, 2′-O*H*, 6.6 Hz), 5.32 (t, 1 H, 5′-O*H*, 4.3 Hz), 5.22 (d, 1 H, 3′-O*H*, 4.1 Hz), 4.39 (q, 1 H, 2′-*H*, 5.8 Hz), 4.10 (m, 1 H, 3′-*H*), 3.97 (m, 1 H, 4′-*H*), 3.67 (m, 2 H, 5′-*H*), 2.71 (s, 3 H, SC*H*$_3$). ^{13}C NMR (DMSO-d_6): δ 158.9, 140.0, 131.7, 124.1, 123.1, 118.4, 113.4, 86.8, 85.6, 70.9, 70.0, 61.4, 57.8. MS (DCI): *m*/*z* 365. UV [λ_{max}, nm (ε)]: (pH 7) 223 (15 160), 260 (3853), 267 (3727), 298 (5067), 308 (5724); (pH 1) 242 (6066), 298 (6528), 308 (7355); (pH 11) 218 (25 700), 259 (4209), 266 (3990), 298 (5207), 307 (5625). Anal. ($C_{13}H_{14}Cl_2N_2O_4S$) C, H, N.

Dethiation of 5 with Raney Nickel. 5,6-Dichloro-2-(methylthio)-1-β-D-ribofuranosylbenzimidazole (**5**, 1 g, 3.1 mmol) was dissolved in absolute ethanol (50 mL) and heated at reflux in the presence of Raney nickel for 8 h. The mixture was gravity filtered, and the filtrate was concentrated under reduced pressure. Purification on a silica gel column (3.5 × 7 cm) using 10% MeOH/$CHCl_3$(v/v) gave 740 mg (85%) of DRB. Mp: 215–216 °C (lit.[19] mp 215–216 °C). R_f = 0.33 (solvent system 2), R_f = 0.08 (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.57 (s, 1 H, C_7-*H*), 8.22 (s, 1 H, C_2-*H*), 7.97 (s, C_4-*H*), 5.87 (d, 1 H, 1′-*H*, 6.3 Hz), 5.49 (d, 1 H, 2′-O*H*, 6.4 Hz), 5.21 (m, 2 H, 3′-O*H*, 5′-O*H*), 4.30 (q, 1 H, 2′-*H*, 5.7 Hz), 4.10 (m, 1 H, 3′-*H*), 3.99 (m, 1 H, 4′-*H*), 3.64 (m, 2 H, 5′-0*H*). ^{13}C NMR (DMSO-d_6): δ 144.8, 143.4, 132.2, 125.2, 124.6, 120.6, 113.6, 89.0, 85.8, 73.7, 70.0, 61.0. MS (FAB): *m*/*z* 319. UV [λ_{max}, nm (ε)]: (pH 7) 256 (5145), 287 (4393), 296 (4147); (pH 1) 253 (3869), 285 (6166), 294 (5659); (pH 11) 255 (4386), 287 (3388), 296 (3267).

5,6-Dichloro-2-(methylsulfonyl)-1-β-D-ribofuranosylbenzimidazole (6). 5,6-Dichloro-2-(methylthio)-1-β-D-ribofuranosylbenzimidazole (**5**, 2.22 g, 6.08 mmol) was suspended in acetonitrile (200 mL) and cooled in an ice bath to 0 °C. Monoperoxyphthalic acid, $Mg^{2+}-6H_2O$ (3.02 g, 6.1 mmol), was added and the reaction was allowed to stand at 0 °C for an additional h. The mixture was then allowed to warm to room temperature, and stirring was continued for 18 h. TLC analysis (10% MeOH/$CHCl_3$) indicated that the reaction had not gone to completion; therefore, the reaction was heated to 50 °C for 1 h and then allowed to stir at room temperature for an additional 18 h. The mixture was concentrated under reduced pressure, suspended in H_2O (100 mL), and filtered, and the filtrate was extracted with ethyl acetate (5 × 50 mL). The EtOAc extracts were dried, concentrated, applied to a silica gel column (3.5 × 9 cm), and eluted with 2% MeOH/$CHCl_3$. The collected material was dissolved in MeOH (100 mL), treated with charcoal, filtered, and concentrated to dryness to give 1.23 g (51%) of 5,6-dichloro-2-(methylsulfonyl)-1-β-D-ribofuranosylbenzimidazole (**6**). Mp: 187–188 °C. R_f = 0.24 (solvent system 2), R_f = 0.08 (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.79 (s, 1 H, C_7-*H*), 8.21 (s, 1 H, C_4-*H*), 6.45 (d, 1 H, 1′-*H*, 7.7 Hz), 5.52 (m, 2 H, 2′-O*H*, 5′-O*H*), 5.33 (m, 1 H, 3′-O*H*), 4.41 (q, 1 H, 2′-*H*, 6.4 Hz), 4.17 (m, 1 H, 3′-*H*), 4.03 (m, 1 H, 4′-*H*), 3.73 (m, 2 H, 5′-*H*), 3.36 (s, 3 H, SO_2CH_3). ^{13}C NMR (DMSO-d_6): δ 151.0, 140.1, 132.6, 128.7, 127.2, 122.4, 117.2, 88.9, 86.7, 72.4, 69.7, 61.0, 43.8. MS (FAB): *m*/*z* [M + H]$^+$ 397. UV [λ_{max}, nm (ε)]: (pH 7) 230 (12 942), 271 (8373), 296 (8055); (pH 1) 227 (18 262), 274 (8532), 298 (8674); (pH 11) 229 (13 101), 297 (6936). Anal. ($C_{13}H_{14}Cl_2N_2O_6S$) C, H, N.

5,6-Dichloro-2-(benzylthio)-1-β-D-ribofuranosylbenzimidazole (7). Yield: 350 mg (79%). Mp: 155–156 °C. R_f = 0.93 (solvent system 2), R_f = 0.82 (solvent system 3). ^{1}H NMR (DMSOd_6): δ 8.34 (s, 1 H, C_7-*H*), 7.87 (s, 1 H, C_4-*H*), 7.46 (m, 2 H, Ar-*H*), 7.28 (m, 3 H, Ar-*H*), 5.67 (d, 1 H, 1′-*H*, 7.7 Hz), 5.42 (d, 1 H, 2′-O*H*, 6.6 Hz), 5.32 (t, 1 H, 5′-O*H*, 4.5 Hz), 5.23 (d, 1 H, 3′-O*H*, 4.2 Hz), 4.61 (q, 2 H, SCH_2Ar, 13.1 Hz), 4.38 (q, 1 H, 2′-*H*, 6.2 Hz), 4.09 (m, 1 H, 3′-*H*), 3.95 (m, 1 H, 4′-*H*), 3.67 (m, 2 H, 5′-*H*). ^{13}C NMR (DMSO-d_6): δ 154.5, 142.9, 136.7, 133.5, 128.8, 128.3, 127.3, 124.7, 124.2, 118.6, 113.9, 88.9, 86.0, 71.6, 69.7, 61.1, 35.9. MS (EI 70 eV (with DCI probe)): *m*/*z* 440. UV [λ_{max}, nm (ε)]: (pH 7) 261 (2740), 268 (2613), 301 (3910), 309 (4226); (pH 1) 309 (4305); (pH 11) 216 (25 900), 260 (2695), 267 (2604), 300 (3671), 308 (3782). Anal. ($C_{19}H_{18}Cl_2N_2O_4S$) C, H, N.

5,6-Dichloro-2-(2-propynylthio)-1-β-D-ribofuranosylbenzimidazole (8). Reaction performed at 0.57-mmol scale. Yield: 155 mg (70%). Mp: 182–183 °C. R_f = 0.34 (solvent system 2), R_f = 0.07 (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.36 (s, 1 H, C_7-*H*), 7.88 (s, 1 H, C_4-*H*), 5.66 (d, 1 H, 1′-*H*, 7.7 Hz), 5.45 (d, 1 H, 2′-O*H*, 6.7 Hz), 5.33 (t, 1 H, 5′-O*H*, 4.6 Hz), 5.26 (d, 1 H, 3′-O*H*, 4.4 Hz), 4.37 (q, 1 H, 2′-*H*, 6.0 Hz), 4.18 (2 H, SCH_2C), 4.11 (m, 1 H, 3′-*H*), 3.98 (m, 1 H, 4′-*H*), 3.70 (m, 2 H, 5′-*H*), 3.23 (1 H, CC*H*). ^{13}C NMR (DMSO-d_6): δ 153.3, 142.9, 133.6, 124.9, 124.5, 118.9, 114.1, 88.9, 86.3, 79.5, 74.2,

71.7, 69.8, 61.2, 20.6. MS (EI 70 eV (with DCI probe)): m/z 388. Anal. ($C_{15}H_{14}Cl_2N_2O_4S$) C, H, N.

5,6-Dichloro-2-(2-propenylthio)-1-β-D-ribofuranosylbenzimidazole (9). Yield: 370 mg (95%). Mp: 145–146 °C. TLC: $R_f = 0.44$ (solvent system 2), $R_f = 0.19$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.35 (s, 1 H, C_7-H), 7.84 (s, 1 H, C_4-H), 6.01 (m, 1 H, SCH_2CH=), 5.70 (d, 1 H, 1′-H, 7.7 Hz), 5.43 (d, 1 H, 2′-OH, 6.5 Hz), 5.34 (m, 3 H, =CH_2 (trans), 5′-OH), 5.25 (d, 1 H, 3′-OH, 4.1 Hz), 5.14 (m, 1 H, =CH_2(cis)), 4.39 (q, 1 H, 2′-H, 6.3 Hz), 4.10 (m, 1 H, 3′-H), 4.02 (m, 2 H, SCH_2), 3.97 (m, 1 H, 4′-H), 3.71 (m, 2 H, 5′-H). ^{13}C NMR (DMSO-d_6): δ 154.4, 143.1, 133.5, 133.0, 124.7, 124.3, 118.8, 118.7, 114.0, 88.8, 86.2, 71.6, 69.8, 61.2, 34.6. MS (EI 70 eV (with DCI probe)): m/z 390. Anal. ($C_{15}H_{16}Cl_2N_2O_4S$) C, H, N.

5,6-Dichloro-2-[(cyanomethyl)thio]-1-β-D-ribofuranosylbenzimidazole (10). Yield: 200 mg (51%). Mp: 210–211 °C. TLC: $R_f = 0.38$ (solvent system 2), $R_f = 0.08$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.38 (s, 1 H, C_7-H), 7.93 (s, 1 H, C_4-H), 5.65 (d, 1 H, 1′-H, 7.6 Hz), 5.52 (d, 1 H, 2′-OH, 6.7 Hz), 5.34 (t, 1 H, 5′-OH, 4.7 Hz), 5.30 (d, 1 H, 3′-OH, 4.4 Hz), 4.46 (q, 2 H, SCH_2CN, 16.8 Hz), 4.36 (q, 1 H, 2′-H, 7.2 Hz), 4.11 (m, 1 H, 3′-H), 4.01 (m, 1 H, 4′-H), 3.71 (m, 2 H, 5′-H). ^{13}C NMR (DMSO-d_6): δ 151.7, 142.7, 133.9, 125.1, 124.9, 119.1, 117.2, 114.1, 89.2, 86.5, 72.1, 69.7, 61.2, 17.7. MS (EI 70 eV (with DCI probe)): m/z 389. Anal. ($C_{14}H_{13}Cl_2N_3O_4S$) C, H, N.

5,6-Dichloro-2-[(4-chlorobenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (11). Yield: 360 mg (76%). Mp: 160–161 °C. $R_f = 0.40$ (solvent system 2), $R_f = 0.18$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.35 (s, 1 H, C_7-H), 7.86 (s, 1 H, C_4-H), 7.49 (d, 2 H, Ar-H), 7.36 (d, 2 H, Ar-H), 5.67 (d, 1 H, 1′-H, 7.7 Hz), 5.42 (d, 1 H, 2′-OH, 6.6 Hz), 5.33 (t, 1 H, 5′-OH, 4.3 Hz), 5.24 (d, 1 H, 3′-OH, 3.3 Hz), 4.61 (q, 2 H, SCH_2Ar, 13.4 Hz), 4.37 (q, 1 H, 2′-H, 6.3 Hz), 4.09 (m, 1 H, 3′-H), 3.95 (m, 1 H, 4′-H), 3.71 (m, 2 H, 5′-H). ^{13}C NMR (DMSO-d_6): δ 154.3, 143.0, 136.2, 133.6, 132.1, 130.8, 128.4, 124.8, 124.3, 118.8, 114.1, 88.8, 86.2, 71.6, 69.8, 61.1, 35.0. MS (EI 70 eV (with DCI probe)): m/z 476. Anal. ($C_{19}H_{17}Cl_3N_2O_4S$) C, H, N.

5,6-Dichloro-2-[(4-bromobenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (12). Yield: 460 mg (88%). Mp: 175–176 °C. TLC: $R_f = 0.49$ (solvent system 2), $R_f = 0.19$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.34 (s, 1 H, C_7-H), 7.85 (s, 1 H, C_4-H), 7.46 (dd, 4 H, Ar-H), 5.66 (d, 1 H, 1′-H, 7.7 Hz), 5.42 (d, 1 H, 2′-OH, 6.4 Hz), 5.33 (t, 1 H, 5′-OH, 4.1 Hz), 5.24 (d, 1 H, 3′-OH, 3.8 Hz), 4.58 (q, 2 H, SCH_2Ar, 13.4 Hz), 4.37 (q, 1 H, 2′-H, 6.5 Hz), 4.09 (m, 1 H, 3′-H), 3.95 (m, 1 H, 4′-H), 3.71 (m, 2 H, 5′-H). ^{13}C NMR (DMSO-d_6): δ 154.3, 143.0, 136.6, 133.6, 131.3, 131.2, 124.8, 124.3, 120.6, 118.8, 114.1, 88.8, 86.2, 71.6, 69.8, 61.1, 35.1. MS (EI 70 eV (with DCI probe)): m/z 520. Anal. ($C_{19}H_{17}BrCl_2N_2O_4S$) C, H, N.

5,6-Dichloro-2-[(4-fluorobenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (13). Yield: 430 mg (94%). Mp: 91–92 °C. $R_f = 0.36$ (solvent system 2), $R_f = 0.12$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.35 (s, 1 H, C_7-H), 7.87 (s, 1 H, C_4-H), 7.52 (dd, 2 H, Ar-H), 7.14 (t, 2 H, Ar-H), 5.66 (d, 1 H, 1′-H, 7.7 Hz), 5.43 (d, 1 H, 2′-OH, 6.5 Hz), 5.34 (t, 1 H, 5′-OH, 4.3 Hz), 5.25 (d, 1 H, 3′-OH, 4.0 Hz), 4.60 (q, 2 H, SCH_2Ar, 13.3 Hz), 4.37 (q, 1 H, 2′-H, 6.6 Hz), 4.08 (m, 1 H, 3′-H), 3.95 (m, 1 H, 4′-H), 3.66 (m, 2 H, 5′-H). ^{13}C NMR (DMSO-d_6): δ 162.8, 154.4, 143.0, 133.5, 133.1, 131.0, 130.9, 124.7, 124.3, 118.7, 115.3, 115.0, 114.0, 88.8, 86.2, 71.5, 69.8, 61.1, 35.0. MS (EI 70 eV (with DCI probe)): m/z 458. Anal. ($C_{19}H_{17}Cl_2FN_2O_4S$) C, H, N.

5,6-Dichloro-2-[(4-methylbenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (14). Yield: 440 mg (97%). Mp: 154–155 °C. $R_f = 0.40$ (solvent system 2), $R_f = 0.17$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.34 (s, 1 H, C_7-H), 7.86 (s, 1 H, C_4-H), 7.34 (d, 2 H, Ar-H), 7.11 (d, 2 H, Ar-H), 5.67 (d, 1 H, 1′-H, 7.7 Hz), 5.41 (d, 1 H, 2′-OH, 6.6 Hz), 5.33 (t, 1 H, 5′-OH, 4.5 Hz), 5.23 (d, 1 H, 3′-OH, 4.3 Hz), 4.57 (q, 2 H, SCH_2Ar, 13.0 Hz), 4.37 (q, 1 H, 2′-H, 6.7 Hz), 4.09 (m, 1 H, 3′-H), 3.95 (m, 1 H, 4′-H), 3.67 (m, 2 H, 5′-H), 2.25 (s, 3 H, Ar-CH_3). ^{13}C NMR (DMSO-d_6): δ 154.7, 143.1, 136.7, 133.6, 129.0, 128.9, 124.7, 124.2, 118.7, 114.1, 88.8, 86.2, 71.6, 69.8, 61.2, 35.8, 20.6. MS (EI 70 eV (with DCI probe)): m/z 454. Anal. ($C_{20}H_{20}Cl_2N_2O_4S$) C, H, N.

5,6-Dichloro-2-[(4-methoxybenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (15). Yield: 290 mg (62%). Mp: 93–94 °C. $R_f = 0.38$ (solvent system 2), $R_f = 0.10$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.34 (s, 1 H, C_7-H), 7.86 (s, 1 H, C_4-H), 7.39 (d, 2 H, Ar-H), 6.86 (d, 2 H, Ar-H), 5.66 (d, 1 H, 1′-H, 7.7 Hz), 5.43 (d, 1 H, 2′-OH, 6.5 Hz), 5.34 (t, 1 H, 5′-OH, 4.5 Hz), 5.24 (d, 1 H, 3′-OH, 3.8 Hz), 4.58 (q, 2 H, SCH_2Ar, 7.9 Hz), 4.37 (q, 1 H, 2′-H, 6.4 Hz), 4.09 (m, 1 H, 3′-H), 3.95 (m, 1 H, 4′-H), 3.71 (s, 3 H, OCH_3), 3.66 (m, 2 H, 5′-H). ^{13}C NMR (DMSO-d_6): δ 158.7, 154.8, 143.1, 133.5, 130.3, 128.5, 124.7, 124.3, 118.7, 114.1, 113.9, 88.8, 86.2, 72.2, 69.8, 61.2, 55.0, 35.6. MS (EI 70 eV (with DCI probe)): m/z 470. Anal. ($C_{20}H_{20}Cl_2N_2O_5S$) C, H, N.

5,6-Dichloro-2-[(3,4-dichlorobenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (16). Yield: 450 mg (88%). Mp: 94–95 °C. $R_f = 0.39$ (solvent system 2), $R_f = 0.14$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.36 (s, 1 H, C_7-H), 7.85 (s, 1 H, C_4-H), 7.75 (bs, 1 H, Ar-H), 7.50 (dd, 2 H, Ar-H), 5.66 (d, 1 H, 1′-H, 7.7 Hz), 5.44 (d, 1 H, 2′-OH, 6.5 Hz), 5.35 (t, 1 H, 5′-OH, 4.3 Hz), 5.26 (d, 1 H, 3′-OH, 3.9 Hz), 4.60 (q, 2 H, SCH_2Ar, 13.6 Hz), 4.37 (q, 1 H, 2′-H, 6.2 Hz), 4.10 (m, 1 H, 3′-H), 3.97 (m, 1 H, 4′-H), 3.67 (m, 2 H, 5′-H). ^{13}C NMR (DMSO-d_6): δ 154.2, 143.0, 138.6, 133.7, 131.0, 130.9, 130.6, 130.1, 129.4, 124.9, 124.5, 118.9, 114.2, 88.8, 86.46, 71.7, 69.9, 61.2, 34.5. MS (EI 70 eV (with DCI probe)): m/z 510. Anal. ($C_{19}H_{16}Cl_4N_2O_4S$) C, H, N.

5,6-Dichloro-2-[(3-chlorobenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (17). Yield: 370 mg (78%). Mp: 130–131 °C. $R_f = 0.42$ (solvent system 2), $R_f = 0.24$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.36 (s, 1 H, C_7-H), 7.86 (s, 1 H, C_4-H), 7.55 (s, 1 H, Ar-H), 7.45 (m, 1 H, Ar-H), 7.32 (m, 2 H, Ar-H), 5.67 (d, 1 H, 1′-H, 7.3 Hz), 5.43 (d, 1 H, 2′-OH, 6.3 Hz), 5.33 (m, 1 H, 5′-OH), 5.24 (d, 1 H, 3′-OH, 3.4 Hz), 4.62 (q, 2 H, SCH_2Ar, 13.5 Hz), 4.38 (q, 1 H, 2′-H, 6.5 Hz), 4.10 (m, 1 H, 3′-H), 3.96 (m, 1 H, 4′-H), 3.68 (m, 2 H, 5′-H). ^{13}C NMR (DMSO-d_6): δ 154.4, 143.0, 139.7, 133.6, 133.0, 130.3, 128.8, 127.3, 127.4, 124.9, 124.4, 118.8, 114.2, 88.8, 86.3, 71.7, 69.9, 61.2, 35.1. MS (EI 70 eV (with DCI probe)): m/z 476. Anal. ($C_{19}H_{17}Cl_3N_2O_4S$) C, H, N.

5,6-Dichloro-2-[(2-chlorobenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (18). Yield: 360 mg (76%). Mp: 102–103 °C. TLC: $R_f = 0.49$ (solvent system 2), $R_f = 0.22$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.36 (s, 1 H, C_7-H), 7.89 (s, 1 H, C_4-H), 7.65 (dd, 1 H, Ar-H), 7.48 (dd, 1 H, Ar-H), 7.31 (dq, 2 H, Ar-H), 5.67 (d, 1 H, 1′-H, 7.7 Hz), 5.42 (d, 1 H, 2′-OH, 6.7 Hz), 5.33 (t, 1 H, 5′-OH, 4.6 Hz), 5.23 (d, 1 H, 3′-OH, 4.4 Hz), 4.71 (q, 2 H, SCH_2Ar, 13.2 Hz), 4.38 (q, 1 H, 2′-H, 3.6 Hz), 4.11 (m, 1 H, 3′-H), 3.97 (m, 1 H, 4′-H), 3.68 (m, 2 H, 5′-H). ^{13}C NMR (DMSO-d_6): δ 154.2, 143.0, 134.2, 133.7, 133.4, 131.5, 129.6, 129.5, 127.4, 124.9, 124.5, 118.9, 114.2, 88.9, 86.3, 71.7, 69.9, 61.2, 34.1. MS (EI 70 eV (with DCI probe)): m/z 474. Anal. ($C_{19}H_{17}Cl_3N_2O_4S$) C, H, N.

5,6-Dichloro-2-[(3-methylbenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (19). Yield: 370 mg (81%). Mp: 166–167 °C. TLC: $R_f = 0.57$ (solvent system 2), $R_f = 0.29$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.35 (s, 1 H, C_7-H), 7.86 (s, 1 H, C_4-H), 7.21 (m, 3 H, Ar-H), 7.07 (d, 1 H, Ar-H), 5.69 (d, 1 H, 1′-H, 7.6 Hz), 5.41 (d, 1 H, 2′-OH, 6.6 Hz), 5.32 (t, 1 H, 5′-OH, 4.5 Hz), 5.23 (d, 1 H, 3′-OH, 4.2 Hz), 4.58 (q, 2 H, SCH_2-Ar, 12.9 Hz), 4.39 (q, 1 H, 2′-H, 7.0 Hz), 4.11 (m, 1 H, 3′-H), 3.96 (m, 1 H, 4′-H), 3.72 (m, 2 H, 5′-H), 2.27 (s, 3 H, Ar-CH_3). ^{13}C NMR (DMSO-d_6): δ 154.8, 143.1, 137.7, 136.5, 133.6, 129.6, 128.4, 128.2, 126.1, 124.8, 124.3, 118.8, 114.1, 88.8, 86.3, 71.6, 69.9, 61.2, 36.0, 20.9. MS (EI 70 eV (with DCI probe)): m/z 454. Anal. ($C_{20}H_{20}Cl_2N_2O_4S$) C, H, N.

5,6-Dichloro-2-[(4-nitrobenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (20). Yield: 342 mg (70%). Mp: 176–177 °C. TLC: $R_f = 0.49$ (solvent system 2), $R_f = 0.11$ (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.35 (s, 1 H, C_7-H), 8.16 (d, 2 H, Ar-H), 7.84 (s, 1 H, C_4-H), 7.75 (d, 2 H, Ar-H), 5.67 (d,

1 H, 1′-*H*, 7.7 Hz), 5.42 (d, 1 H, 2′-O*H*, 6.5 Hz), 5.31 (t, 1 H, 5′-O*H*, 4.4 Hz), 5.23 (d, 1 H, 3′-O*H*, 4.1 Hz), 4.73 (q, 2 H, SC*H*$_2$-Ar, 13.7 Hz), 4.36 (q, 1 H, 2′-*H*, 5.8 Hz), 4.08 (m, 1 H, 3′-*H*), 3.95 (m, 1 H, 4′-*H*), 3.66 (m, 2 H, 5′-*H*). ^{13}C NMR (DMSO-d_6): δ 153.9, 146.7, 145.4, 142.9, 133.6, 130.2, 124.9, 124.5, 123.4, 118.8, 114.1, 88.9, 86.3, 71.7, 69.8, 61.2, 34.9. MS (EI 70 eV (with DCI probe)): *m*/*z* 485. Anal. ($C_{19}H_{17}Cl_2N_3O_6S$) C, H, N.

5,6-Dichloro-2-[(3-nitrobenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (21). Yield: 332 mg (68%). Mp: 195–196 °C. TLC: R_f = 0.48 (solvent system 2), R_f = 0.08 (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.39 (s, 1 H, Ar-*H*), 8.35 (s, 1 H, C$_7$-*H*), 8.10 (d, 1 H, Ar-*H*), 7.94 (d, 1 H, Ar-*H*), 7.85 (s, 1 H, C$_4$-*H*), 7.60 (t, 1 H, Ar-*H*), 5.67 (d, 1 H, 1′-*H*, 7.6 Hz), 5.42 (d, 1 H, 2′-O*H*, 6.5 Hz), 5.33 (m, 1 H, 5′-O*H*), 5.24 (d, 1 H, 3′-O*H*, 3.9 Hz), 4.74 (q, 2 H, SC*H*$_2$Ar, 13.7 Hz), 4.36 (q, 1 H, 2′-*H*, 6.6 Hz), 4.09 (m, 1 H, 3′-*H*), 3.95 (m, 1 H, 4′-*H*), 3.67 (m, 2 H, 5′-*H*). ^{13}C NMR (DMSO-d_6): δ 154.1, 147.8, 143.0, 139.9, 135.7, 133.7, 129.9, 125.0, 124.5, 123.7, 122.3, 118.9, 114.2, 89.0, 86.3, 71.8, 69.9, 61.2, 34.9. MS (EI 70 eV (with DCI probe)): *m*/*z* 485. Anal. ($C_{19}H_{17}Cl_2N_3O_6S$) C, H, N.

5,6-Dichloro-2-[[3-(trifluoromethyl)benzyl]thio]-1-β-D-ribofuranosylbenzimidazole (22). Yield: 410 mg (81%). Mp: 163–164 °C. TLC: R_f = 0.45 (solvent system 2), R_f = 0.17 (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.35 (s, 1 H, C$_7$-*H*), 7.81 (s, 1 H, C$_4$-*H*), 7.55 (m, 2 H, Ar-*H*), 5.67 (d, 1 H, 1′-*H*, 7.3 Hz), 5.42 (d, 1 H, 2′-O*H*, 6.3 Hz), 5.33 (m, 1 H, 5′-O*H*), 5.24 (d, 1 H, 3′-O*H*, 3.3 Hz), 4.70 (q, 2 H, SC*H*$_2$Ar, 13.4 Hz), 4.37 (m, 1 H, 2′-*H*), 4.10 (m, 1 H, 3′-*H*), 3.96 (m, 1 H, 4′-*H*), 3.67 (m, 2 H, 5′-*H*). ^{13}C NMR (DMSO-d_6): δ 154.2, 143.0, 138.8, 133.7, 133.1, 129.8, 129.5, 125.7, 124.9, 124.5, 124.1, 118.8, 114.2, 89.0, 86.3, 71.8, 69.9, 61.2, 35.3. MS (EI 70 eV (with DCI probe)): *m*/*z* 508. Anal. ($C_{20}H_{17}Cl_2F_3N_2O_4S$) C, H, N.

5,6-Dichloro-2-[(4-*tert*-butylbenzyl)thio]-1-β-D-ribofuranosylbenzimidazole (23). Yield: 440 mg (89%). Mp: 115–116 °C. TLC: R_f = 0.57 (solvent system 2), R_f = 0.28 (solvent system 3). ^{1}H NMR (DMSO-d_6): δ 8.34 (s, 1 H, C$_7$-*H*), 7.86 (s, 1 H, C$_4$-*H*), 7.36 (dd, 4 H, Ar-*H*), 5.66 (d, 1 H, 1′-*H*, 7.7 Hz), 5.41 (d, 1 H, 2′-O*H*, 6.5 Hz), 5.31 (t, 1 H, 5′-O*H*, 4.3 Hz), 5.23 (d, 1 H, 3′-O*H*, 3.9 Hz), 4.57 (q, 2 H, SC*H*$_2$Ar, 13.1 Hz), 4.36 (q, 1 H, 2′-*H*, 6.0 Hz), 4.09 (m, 1 H, 3′-*H*), 3.94 (m, 1 H, 4′-*H*), 3.66 (m, 2 H, 5′-*H*), 1.24 (s, 9 H, t-Bu). ^{13}C NMR (DMSO-d_6): δ 154.8, 150.0, 143.1, 133.6, 128.7, 125.2, 124.8, 124.3, 118.7, 114.1, 88.8, 86.2, 71.6, 69.8, 61.2, 48.6, 35.6, 34.2, 31.0. MS (EI 70 eV (with DCI probe)): *m*/*z* 496. Anal. ($C_{23}H_{26}Cl_2N_2O_4S$) C, H, N.

Cells Culture Procedures. The routine growth and passage of KB and BSC-1 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. The sodium bicarbonate concentration was varied to meet the buffering capacity required. Cultures of diploid human foreskin fibroblasts (HFF) or MRC-5 cells were grown in medium consisting of MEM(E) with 10% fetal bovine serum. 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 as described previously.[34] HFF and MRC-5 cells were passaged only at 1:2 dilutions.

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. Cell growth medium was changed every 4 days until cytopathology was evident in all cells (approximately 21 days). Supernatant fluids were retained as the virus stock. High titer HSV-1 stocks were prepared by infecting KB cells at an moi of <0.1 as detailed previously.[34] 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.[35] Briefly, HFF or BSC-1 cells were planted as described above in 96-well cluster dishes and incubated overnight at 37 °C in a humidified 3% CO_2–97% air atmosphere. 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. Cultures were incubated at 37 °C for 2 h to permit virus adsorption, and then virus innoculum was replaced with 0.2 mL of fresh medium. Cultures were incubated for 7 days for HCMV, 2 or 3 days for HSV-1, medium was removed, and the cell sheets were stained with 0.1% crystal violet in 20% methanol. 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 *n*th dilution of the virus used to infect the well in which plaques were enumerated.

Assays for Antiviral Activity. The effect of compounds on the replication of HCMV has been measured using both a plaque reduction assay and a titer (yield) reduction assay. For the former, 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 three to six selected concentrations. Following incubation at 37 °C for 7–10 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. A procedure devised by us[35] was used for yield reduction assays. HFF or MRC-5 cells were planted as described above in 96-well cluster dishes at a concentration of 12 500 cells per well and incubated overnight. The next day, the medium was shaken out and the cultures were inoculated with HCMV at a moi of 0.5–1 pfu/cell. After virus adsorption, virus 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 MEM(E) containing 5% serum, antibiotics, and test compound at 3 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 ranging nearly 1000-fold between the highest and lowest dilutions (100–0.14 μM, for example). Plates were incubated at 37 °C for 7 days and then subjected to one cycle of freezing at −76 °C and thawing at 37 °C to disrupt the cells. 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.

Plaque reduction experiments with HSV-1 were performed using monolayer cultures of BSC-1 cells. The assay was performed exactly as described above except that the 0.2-μL virus suspension contained approximately 100 pfu of HSV-1. Compounds were tested in duplicate by dissolving in overlay medium at concentrations usually ranging from 0.1 to 100 μM in half- or one-logarithm dilutions. An ELISA also was employed 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 3–5-day incubation at 37 °C, medium was removed and plates were blocked with 200 μL per well of 10% calf serum and 0.05% Tween in hepes-buffered saline[36] (HBS-T). After 30 min, the blocking agent was removed and the wells rinsed with HBS-T. Horse radish peroxidase conjugated rabbit anti-HSV-1 antibody in HBS was added and incubated on a rocker for 1 h at room temperature. Following removal of the antibody-containing solution, plates were rinsed four times with HBS-T and then developed by adding 150 μL per well of a solution of tetramethylbenzidine as substrate. The reaction

was stopped with H_2SO_4 and absorbance was read at 450 and 570 nm. Drug effects were calculated as a percentage of the reduction in absorbance in the presence of each drug concentration compared to absorbance obtained with virus in the absence of drug.

Cytotoxicity Assays. Three different assays were used to explore cytotoxicity of selected compounds using methods we have detailed previously. (*i*) Cytotoxicity produced in stationary HFF, BSC-1, and MRC-5 cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[34] (*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.[37] (*iii*) Effects of compound **7** on the incorporation of radio-labeled precursors into logarithmically growing KB cells also were determined using an established procedure.[34]

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 (IC_{50}) concentrations 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. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by > ±1.5 standard deviations.

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