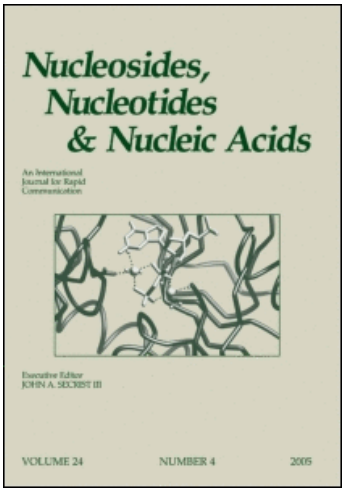

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Design, Synthesis, and Antiviral Evaluation of Some Polyhalogenated Indole *C*-nucleosides

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DESIGN, SYNTHESIS, AND ANTIVIRAL EVALUATION OF SOME POLYHALOGENATED INDOLE *C*-NUCLEOSIDES

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□ *2,5,6-Trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB), 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BDCRB) and 2-benzylthio-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BTDCRB) are benzimidazole nucleosides that exhibit strong and selective anti-HCMV activity. Polyhalogenated indole C-nucleosides were prepared as 1-deaza analogs of the benzimidazole nucleosides TCRB and BDCRB. A mild Knoevenagel coupling reaction between an indol-2-thione and a ribofuranose derivative was developed for the synthesis of 2-benzylthio-5,6-dichloro-3-(β-D-ribofuranosyl)indole (***12***). 3-(β-D-ribofuranosyl)-2,5,6-trichloroindole (***16***) was prepared from* ***12*** *in 4 steps. A Lewis acid-mediated glycosylation method was then developed to prepare the targeted 2-haloindole C-nucleoside* ***16*** *stereoselectively in four steps from the corresponding 2-haloindole aglycons. Only* ***12*** *was active against HCMV but it also was somewhat cytotoxic.*

Keywords Indole nucleoside; *C*-Nucleoside; Nucleoside analog; Antiviral; TCRB; Human cytomegalovirus (HCMV)

This manuscript is dedicated to the memory of Dr. John A. Montgomery.

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INTRODUCTION

A series of 2-substituted benzimidazole nucleosides have been synthesized in our group and displayed strong antiviral activities.[1,2] The lead compounds, including 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (**1**, TCRB, Figure 1), 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (**2**, BDCRB, Figure 1) and 2-benzylthio-5,6-dichloro-1-(β-D-ribofuranosyl) benzimidazole (**3**, BTDCRB, Figure 1), have shown potent activity against HCMV with low cellular toxicity at concentrations inhibiting viral growth (TCRB: $IC_{50} = 2.8$ μM, $CC_{50} = 238$ μM, BDCRB: $IC_{50} = 0.7$ μM, $CC_{50} = 118$ μM, BTDCRB: $IC_{50} = 22$ μM, $CC_{50} = 100$ μM in a plaque assay in human foreskin fibroblasts). However, the instability of the glycosyl bond of TCRB has been one of the major problems in its clinical development. It was found that TCRB disappears rapidly from the bloodstream of animals (rats or monkeys) following intravenous or oral dosage.[3] The disappearance of TCRB from the blood correlated with increased blood concentration of the aglycon, 2,5,6-trichlorobenzimidazole. This indicated that the lability of the glycosidic bond is at least partially responsible for the rapid disappearance of TCRB from the bloodstream.

Indole, a heterocycle that is structurally similar to benzimidazole, has the potential of bearing the same substitution patterns as that of TCRB, BDCRB and BTDCRB. Therefore, we proposed to synthesize some polyhalogenated indole nucleosides (Figure 2) as 1-deaza analogs of the antiviral benzimidazole nucleosides (Figure 1). Because the proposed indole *C*-nucleosides should resist enzymatic cleavage at the glycosyl bond, they may potentially solve the unstable glycosyl bond problem of TCRB. Several polyhalogenated indole *N*-nucleosides have been synthesized in our group as 3-deaza analogs of TCRB,[4–8] but the synthesis of indole *C*-nucleosides poses unique synthetic challenges and would help us further elucidate the structural and chemical requirements for activity in this series. In light of substantial structure activity relationship (SAR) studies of TCRB,[1,2,9–13] a limited number of 5,6-dichloroindole *C*-ribosides should be sufficient to evaluate the potential antiviral activity of this type of nucleosides as analogs of TCRB.

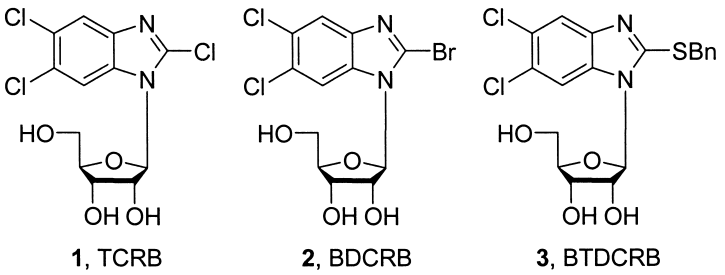


FIGURE 1 Some polyhalogenated benzimidazole nucleosides with activity against HCMV.

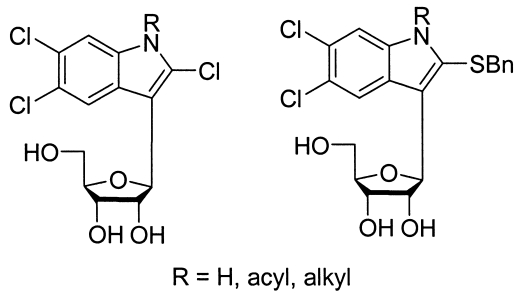


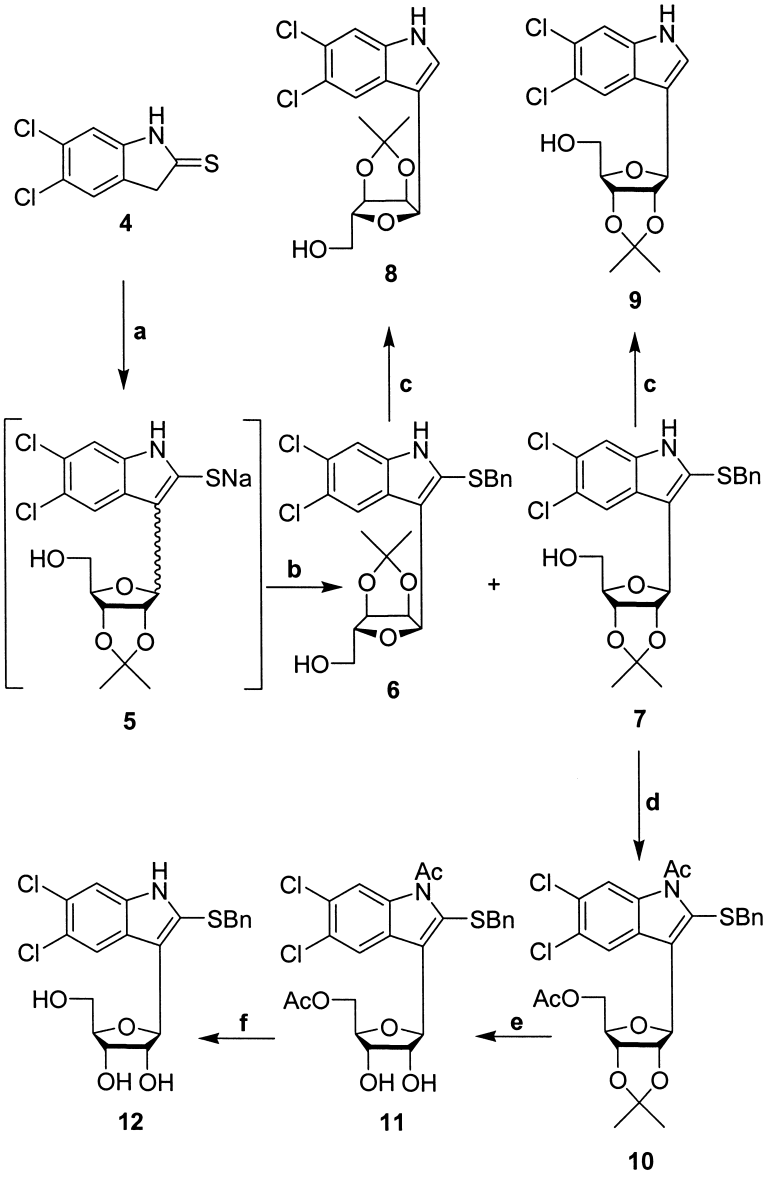
FIGURE 2 Proposed polyhalogenated indole *C*-nucleosides.

RESULTS AND DISCUSSION

Chemistry

The synthesis of indole *C*-nucleosides has been of interest for some time.[14,15] The previous synthesis generally involved a coupling of indole aglycons and sugar donors to directly provide indole *C*-nucleosides. In order to use this synthetic approach, some 2-substituted (benzylthio and chloro) 5,6-dichloroindoles were required as aglycons for our targeted nucleosides. Although 2-benzylthio-5,6-dichloroindole was readily available,[4] the 2-chloro analog had not been reported in the literature at the beginning of our study. Thus, we initially decided to prepare a *C*-nucleoside of 2-benzylthio-5,6-dichloroindole, and then transform this nucleoside to the *C*-nucleosides of the 2-chloro analog.

In previous research from our laboratory,[4] we had established that 2-benzylthio-5,6-dichloroindole did not undergo a coupling with certain ribofuranosyl donors to yield an indole *N*-nucleoside due to the bulky 2-benzylthio group. Therefore, we assumed that the direct coupling procedures of Sokolova and Cornia could encounter a similar problem. However, an indole-2-thione has been known to react with some ketones at the 3-position in a Knoevenagel type fashion.[16] If the ketones were replaced with a ribofuranose derivative, a condensation of 5,6-dichloroindole-2-thione (**1**) and the ribofuranose derivative, followed by a benzylation, could lead directly to a 2-benzylthio-5,6-dichloroindole *C*-nucleoside. Indeed, when a mixture of compound **1** and 2,3-*O*-isopropylidene-D-ribofuranose in MeOH was treated with sodium carbonate, a precipitate was formed. This precipitate was likely the salt intermediate **5** (Scheme 1). Without isolation, this salt was treated with benzyl bromide to afford 2-benzylthio-5,6-dichloro-3-(2,3-*O*-isopropylidene-α-D-ribofuranosyl)indole (**6**) and 2-benzylthio-5,6-dichloro-3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (**7**) in a 2:1 ratio. It should be noted that the ring closure to form possible pyranose isomers was not observed, since the presence of a five-membered isopropylidene ring



SCHEME 1 Reagents and conditions: (**a**) Na_2CO_3, MeOH, 20°C, 20 h; (**b**) BnBr, acetone, 20°C, 30 min; (**c**) Raney Ni, EtOH, 20°C, 1 h; (**d**) Ac_2O, Et_3N, CH_3CN, 20°C, 24 h; (**e**) 90% TFA, 20°C, 10 min; (**f**) Na_2CO_3, MeOH, 20°C, 2 h.

strongly favors the formation of a second fused, five-membered ring.[17] The Knoevenagel reaction has been applied in the *C*-nucleoside synthesis area with limited successes.[18,19] Ketones and carboxamides were generally used to couple with sugars, and harsh reaction conditions were usually required, which resulted in low yields and some epimerizations.[20] To the best of our knowledge, this is the first example of a thioamide Knoevenagel reaction being used in a *C*-nucleoside synthesis. The mild reaction conditions may find some use in the synthesis of other *C*-nucleosides.

Some difficulties were encountered in our efforts to determine the anomeric configurations of compounds **6** and **7**. The chemical shift difference for the H-1 protons suggested that compound **7** was the α-anomer, since the peak for an α-anomer's H-1′ is generally at a lower field than that of a β-anomer (Table 1).[21] However, the $\Delta\delta$ value observed for the isopropylidene group of **7** was larger than the $\Delta\delta$ value for **6**, which suggested that **7** was the β-anomer.[21] This β-anomeric assignment was further supported by the ^{13}C NMR data, since δ values of a β-anomer's two methyls on the isopropylidene group are generally larger than those of the α-anomer's methyls. The bulky benzylthio group may have exhibited some substituent effects on the H-1s of **6** and **7**. Thus, 5,6-dichloro-3-(2,3-*O*-isopropylidene-α-D-ribofuranosyl)indole and 5,6-dichloro-3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (**8** and **9**) were prepared from **6** and **7**, respectively, to eliminate this substituent effect. This time, all three NMR spectrum criteria of **8** and **9** agreed (Table 1) with the general trends and we were confident in assigning compound **7** as the β-anomer and compound **6** as the α-anomer.

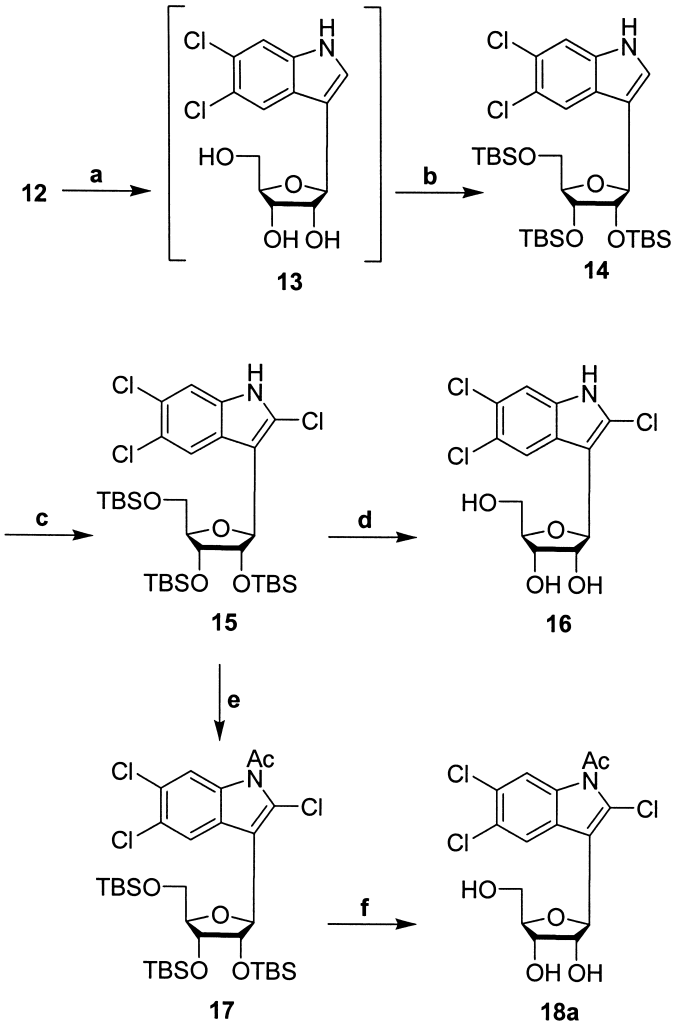
In order to reduce the likelihood of a possible opening of the ribofuranose ring and a subsequent cyclization to form the thermodynamically more stable pyranose isomers under acidic conditions, the 1- and 5′-positions of compound **7** were protected with acetyl groups to give 1-acetyl-3-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-2-benzylthio-5,6-dichloroindole (**10**). The treatment of **10** with aqueous TFA provided 1-acetyl-3-(5-*O*-acetyl-β-D-ribofuranosyl)-2-benzylthio-5,6-dichloroindole (**7**) with a small amount of the α-anomer (β:α = 3.5:1). Due to the π-electron rich property of an indole, the protonation of the ribofuranose ring oxygen may be followed with an anomerization. This type of anomerization has been reported

TABLE 1 NMR Spectral Data Used to Determine Anomeric Configuration

Compound #	H-1′ (ppm)	$\Delta\delta$ of CMe_2 (ppm) in ^{1}H NMR	CMe_2 (ppm) in ^{13}C NMR	Anomeric configuration
6	5.04	0.29	24.0, 26.1	α
7	5.24	0.31	25.6, 27.6	β
8	5.26	0.23	24.5, 26.2	α
9	5.08	0.25	25.4, 27.4	β

previously.[14] The attempted separation of **11** and the α-anomer by flash column chromatography proved fruitless. However, pure **11** was obtained by crystallization from a mixture of EtOAc and hexane. Removal of the acetyl group of **11** was performed with Na_2CO_3 in MeOH to afford the target compound, 2-benzylthio-5,6-dichloro-3-(β-D-ribofuranosyl)indole (**12**).

Compound **12** was treated with Raney Ni (Scheme 2) to afford 5,6-dichloro-3-(β-D-ribofuranosyl)indole (**13**), which was then silylated to give 5,6-dichloro-3-[2,3,5-tri-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl]indole (**14**). Treatment of **14** with NCS in CCl_4 at room temperature yielded



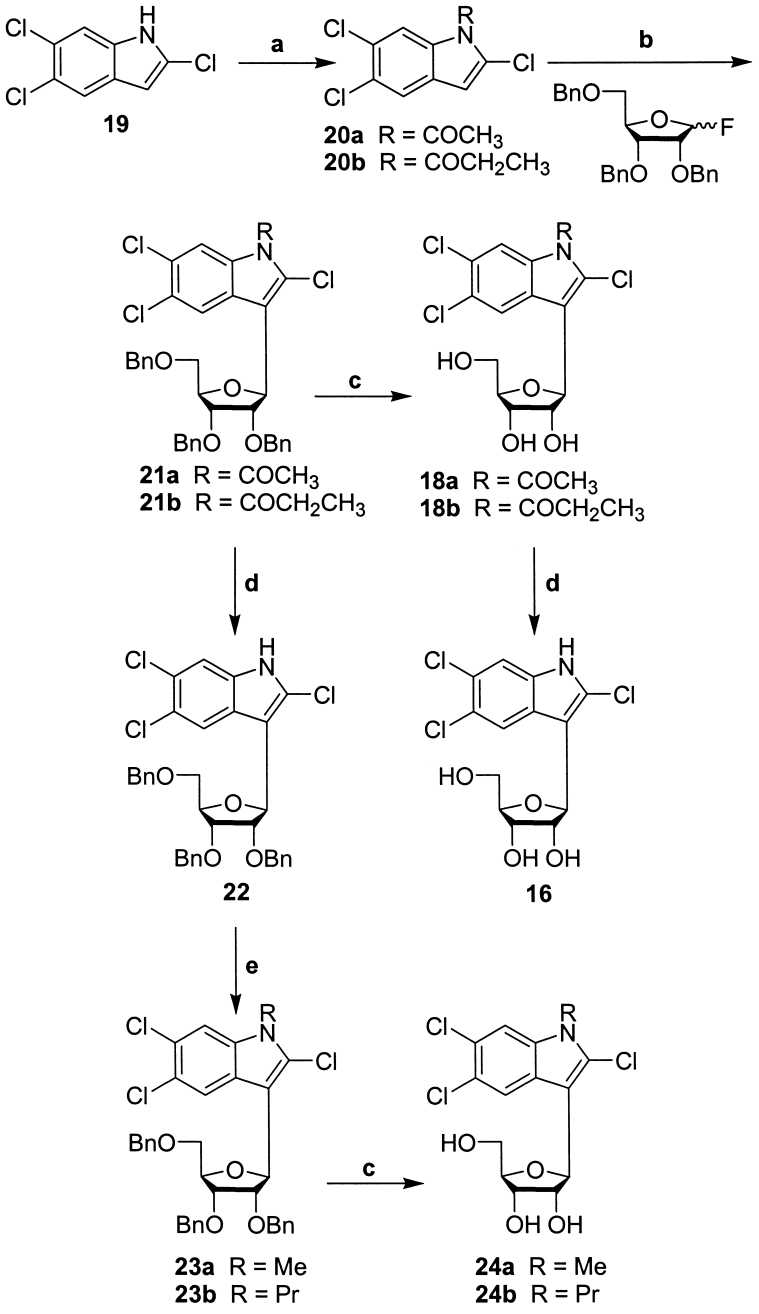
SCHEME 2 Reagents and conditions: (**a**) Raney Ni, EtOH, 20°C, 30 min; (**b**) TBSCl, imidazole, DMF, 20°C, 26 h; (**c**) NCS, CCl_4, 20°C, 24 h; (**d**) TBAF, THF, 20°C, 30 min; (**e**) Ac_2O, Et_3N, CH_3CN, 20°C, 30 min; (**f**) KF, 80% TFA, 20°C, 1 h.

3-[2,3,5-tri-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl]-2,5,6-trichloroindole (**15**) and the α-anomer in a 6.5:1 ratio based on ^{1}H NMR spectral data. Pure **15** was obtained in a 51% yield by crystallization from acetonitrile. The cleavage of TBDMS groups was effected by treating compound **15** with TBAF and resulted in the targeted nucleoside, 3-(β-D-ribofuranosyl)-2,5,6-trichloroindole (**16**). The *N*-acetyl derivative **18a** was synthesized through an acylation of **15** with Ac_2O and followed by deprotection of the silyl intermediate **17** under KF-TFA-H_2O conditions. Deprotection of **17** with TBAF caused a substantial cleavage of the labile acetyl group.

Although the above strategy yielded the target compounds **16** and **18a**, it had two major drawbacks: 1) the β-anomer **7** was the minor product of the coupling reaction; and 2) the synthetic route for the 2-chloroindole nucleoside **16** was tedious. At the same time, our studies in the indole *N*-nucleoside area had resulted in an optimized procedure for the synthesis of 2,5,6-trichloroindole (**19**, Scheme 3).[4] Thus, we elected to investigate a direct coupling method for the synthesis of the 2-haloindole *C*-nucleosides.

Recently, Yokoyama and coworkers reported a successful preparation of some indole *C*-nucleosides under Lewis acid–mediated glycosylation conditions.[22] Thus, we elected to apply this method to our 2,5,6-trichloroindole substrates. We found that the acyl-protected indoles were stable under Lewis acid conditions and were reactive toward the desired glycosylation. The use of an acetyl group is preferred to Yokoyama's use of a phenylsulfonyl group, because the removal of an acetyl group is usually more facile than that of a phenylsulfonyl group. The coupling between 1-acetyl-2,5,6-trichloroindole (**20a**) and 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl fluoride[23] proceeded smoothly at 0°C. The glycosylation reaction yielded 1-acetyl-2,5,6-trichloro-3-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)indole (**21b**) and its α anomer in a ratio of approximately 4:1 (as determined by integration of the appropriate 1′ protons in the crude mixture). Similar results were obtained from the coupling of 1-propionyl-2,5,6-trichloroindole (**20b**) and the fluorosugar (Scheme 3). The assignment of anomeric configurations were based on comparisons with Yokoyama's results,[22] in which the coupling constant of the β-anomer ($J > 6.3$ Hz) was always larger than that of the α-anomer ($J < 3.3$ Hz). The coupling constants of the β-anomers ($J > 8$ Hz) in the current series were much higher than the corresponding α-anomers ($J <$ 3.5 Hz).

Initially, a removal of the benzyl groups from the carbohydrate moiety under catalytic hydrogenation conditions, i.e., Pearlman's catalyst (20% palladium hydroxide on carbon, moisturized) in ethanol under 1 atm hydrogen, resulted in multiple products. The ^{1}H NMR spectra of all the products revealed that all of the benzyl groups had been removed but none of the spectra displayed the characteristic peaks observed for the previously prepared compound **18a**, probably due to overreduction



SCHEME 3 Reagents and conditions: (**a**) Ac_2O, pyridine, CH_2Cl_2, 20°C, 24 h or CH_3CH_2COCl, pyridine, 20°C, 16 h; (**b**) BF_3OEt_2, CH_2Cl_2, 0°C, 10 min, (**c**) H_2 (1 atm), 5% $Pd/BaSO_4$, AcOH, 20°C, 45 min; (**d**) $MeNH_2$, MeOH, 20°C, 30 min; (**e**) NaH, THF, 0°C, 10 min then MeI or 1-PrI, 20°C, 60 min.

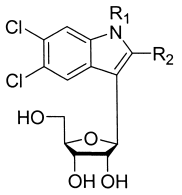
(i.e., dechlorination of the heterocycle). To avoid the over-reduction, we elected to use the debenzylation reagent boron trichloride. This reagent has been used previously for the debenzylation of *C*-nucleosides,[22,24] although it generally offers moderate to low yields. Our use of this reagent resulted in the production of some of the desired product, but in a low yield. The low yield was partially due to the loss of the labile acetyl group. In addition, epimerization was also observed. These disappointing results forced us to reexamine the catalytic hydrogenation conditions. In an effort to stop or reduce the overreduction, ethyl acetate was tested as the solvent. Encouragingly, some of the desired product **18a** was isolated in a low yield along with some overreduced products and some starting material. This prompted us to initiate a thorough study on the effect of solvents on the reaction. We found that acetic acid worked best as the solvent in this reaction and yielded **18a** as the major product. To further optimize the reaction conditions, we tested a variety of different catalysts: e.g., 5% Pd/C, 10% Pd/C, 20% Pd/C, 5% Pd/$CaSO_4$, 5% Rh/C, PtO_2, and 5% Pd/$BaSO_4$. The highest ratio of the desired product in comparison to the side products was obtained with 5% Pd/BaSO4. Deprotection of the labile acetyl group of **18a** was then effected with methylamine in EtOH at room temperature to afford the target compound **16** in a quantitative yield.

In order to determine whether different substituents would be tolerated at the 1-position of the indole, we decided to alkylate this position with some simple electrophiles. The protected intermediate **21a** was deacetylated using the methylamine procedure, and the indole nitrogen was then deprotonated using sodium hydride. The resulting sodium anion was treated with methyl iodide or 1-iodopropane in situ to provide the desired 1-methyl analog **23a** or the 1-propyl analog **23b**. These compounds (**23a** and **23b**) were debenzylated under the conditions optimized above using 1 atm H_2 and 5% Pd/$BaSO_4$ in acetic acid to provide 1-methyl-2,5,6-trichloro-3-(β-D-ribofuranosyl)indole (**24a**) and 1-(1-propyl)-2,5,6-trichloro-3-(β-D-ribofuranosyl)indole (**24b**), respectively, in good yield.

Biological Evaluation

The compounds synthesized as described above were tested for antiviral activity against HCMV and HSV-1 and for cytotoxicity. Although the compounds are structurally similar to the benzimidazole nucleosides, only **12** appeared to have antiviral activity, but this was most likely due to cytotoxicity (Table 2). Thus, none of these indole *C*-nucleosides demonstrated the potent antiviral activity or lack of cytotoxicity possessed by the benzimidazole nucleosides, or by a number of the analogous indole *N*-nucleosides.[5–8] Because these representative chlorinated indole *C*-nucleosides were not active, further analogs were not pursued.

TABLE 2 Antiviral Activity and Cytotoxicity of 3-Substituted Indole Nucleosides



			50% Inhibitory concentration (μM)			
			Antiviral		Cytotoxicity	
No.	R_1	R_2	HCMV plaque[a]	HSV-1 ELISA[b]	HFF visual[c]	KB growth[c]
12	-H	-SBn	32	>100	32	50
16	-H	-Cl	>100	>100	>100	65
18a	-$COCH_3$	-Cl	>100	>100	>100	50
24a	-Me	-Cl	>100	>100	>100	>100
TCRB[e]			2.9	102	238	210
BDCRB[e]			0.70	130	118	>100
GCV[f]			7.4	3.5	>100	>100

[a]Plaque reduction assays were performed in duplicate wells as described in the text.
[b]Compounds were assayed by ELISA in quadruplicate wells.
[c]Visual cytotoxicity was scored on HFF cells at the time of HCMV plaque enumeration in duplicate wells; inhibition of KB cell growth was determined in triplicate wells as described in the text.
[d]>100 indicates an IC_{50} greater than the highest concentration tested.
[e]Data for TCRB and BDCRB published previously as compounds **9** and **11**, respectively, in reference 1.
[f]Averages from 108, 33, and 3 experiments, respectively, using ganciclovir (GCV).

EXPERIMENTAL

General Procedures

All solvents were dried prior to use according to known procedures; all reagents were obtained from commercial sources or were synthesized from literature procedures, and were used without further purification unless otherwise noted. Air-sensitive reactions were performed under a slight positive pressure of argon. Room temperature is assumed to be between 20 and 25°C. Evaporation of solvents was accomplished under reduced pressure (water aspirator, 12 mmHg), at less than 40°C, unless otherwise noted. Chromatography solvent systems are expressed in v:v ratios or as %v. Melting points were taken on a Mel-Temp apparatus, and are uncorrected. Thin-layer chromatography was performed on silica gel GHLF plates from Analtech (Newark, Delaware). Chromatograms were visualized under UV light at 254 nm. ^{1}H-NMR spectra were obtained at 500 MHz on a Bruker DRX500 spectrometer. ^{13}C-NMR spectra were obtained at 125 MHz on a Bruker DRX500 spectrometer. Chemical shift values for ^{1}H were determined relative to an internal tetramethylsilane standard (0.00 ppm); chemical shift values for ^{13}C were determined relative to the solvent used (39.52 ppm for DMSO-d_6 and 77.23 ppm for $CDCl_3$). Mass spectrometry and elemental

analysis for selected compounds (listed in supplemental information) were performed at the University of Michigan Department of Chemistry Mass Spectrometry facility and Elemental Analysis facility, respectively.

2-Benzylthio-5,6-dichloro-3-(2,3-*O*-isopropylidene-α-D-ribofuranosyl) indole (6) and 2-Benzylthio-5,6-dichloro-3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (7). A mixture of 5,6-dichloroindol-2-thione (**4**) (3.00 g, 13.7 mmol), 2,3-*O*-isopropylidene-D-ribofuranose (3.84 g, 20.5 mmol) and Na_2CO_3 (5 g, 47 mmol) in 45 mL of MeOH was stirred at room temperature for 20 h, and then concentrated to dryness. The residue was dried in vacuo, treated with 60 mL of acetone and then with benzyl bromide (1.79 mL, 15.1 mmol). The mixture was stirred at room temperature for 30 min. The solids were removed by filtration. The filtrate was concentrated to dryness. The residue was purified by flash column chromatography (25% EtOAc/Hex, 4 cm × 10 cm) to give 3.46 g (52%) of **6** as a light yellow foam and 1.70 g (26%) of **7** as a light yellow foam. **6**: R_f 0.30 (30% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ 1.25 (s, 3H), 1.54 (s, 3H), 1.74 (t, 1H, $J = 5.9$), 3.61 (t, 2H, $J = 5.9$), 3.86 (s, 2H), 4.34 (m, 2H), 4.63 (m, 1H), 5.04 (d, 1H, $J = 3.7$), 7.04 (m, 2H), 7.21 (s, 1H), 7.25 (m, 3H), 8.04 (broad s, 1H), 8.06 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 24.0, 26.1, 41.5, 61.7, 77.9, 82.2, 83.2, 84.2, 111.8, 112.6, 117.2, 123.9, 124.1, 127.0, 127.1, 127.2, 127.4, 128.7, 128.8, 135.5, 138.1. **7**: R_f 0.60 (30% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 3H), 1.66 (s, 3H), 2.10 (broad s, 1H), 3.92–4.03 (m, 4H), 4.15 (m, 1H), 4.77 (m, 1H), 4.89 (m, 1H), 5.24 (d, 1H, $J = 6.1$), 7.10 (m, 2H), 7.23 (m, 4H), 7.73 (s, 1H), 7.78 (broad s, 1H). ^{13}C NMR ($CDCl_3$): δ 25.6, 27.6, 42.4, 62.8, 80.4, 81.4, 84.1, 85.0, 112.3, 115.3, 118.1, 120.7, 124.4, 125.6, 127.4, 127.6, 128.69, 128.72, 129.2, 135.1, 137.9. HRMS for $C_{23}H_{23}Cl_2NO_4S$: Calcd, 479.0725; Found, 479.0713.

5,6-Dichloro-3-(2,3-*O*-isopropylidene-α-D-ribofuranosyl)indole (8). A mixture of compound 6 (500 mg, 1.04 mmol) and Raney Ni (4 g, wet, W-4) in 15 mL of EtOH was stirred at room temperature for 1 h. The Raney Ni was removed by filtration. The filtrate was concentrated to dryness, and the residue was purified by flash column chromatography (90% EtOAc/Hex, 2 cm × 10 cm) to give 250 mg (67%) of **8** as a light white foam. R_f 0.30 (90% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ 1.33 (s, 3H), 1.55 (s, 3H), 1.96 (broad s, 1H), 3.75 (m, 2H), 4.35 (m, 1H), 4.86–5.08 (m, 2H), 5.26 (d, 1H, $J = 3.6$), 7.30 (d, 1H, $J = 2.4$), 7.41 (s, 1H), 7.86 (s, 1H), 8.25 (broad s, 1H). ^{13}C NMR ($CDCl_3$): δ 24.5, 26.2, 62.0, 77.4, 82.5, 82.7, 84.0, 110.7, 112.6, 112.8, 121.5, 123.7, 125.6, 125.9, 126.4, 134.9.

5,6-Dichloro-3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (9). The procedure is the same as that described for **8**, except that **7** (500 mg, 1.04 mmol) was used instead of **6**. A 72% yield (268 mg, 0.75 mmol) of **9** was

obtained as a white foam. R_f 0.50 (90% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ 1.39 (s, 3H), 1.64 (s, 3H), 3.80 (m, 1H), 3.98 (m, 1H), 4.19 (m, 1H), 4.80 (m, 2H), 5.08 (dd, 1H, J = 5.4, 1.4), 7.24 (d, 1H, J = 2.5), 7.46 (s, 1H), 7.80 (s, 1H), 8.23 (broad s, 1H). ^{13}C NMR ($CDCl_3$): δ 25.4, 27.4, 62.7, 80.7, 81.5, 84.3, 85.0, 112.8, 114.2, 115.2, 120.8, 123.7, 124.1, 125.4, 126.5, 135.4. HRMS for $C_{16}H_{17}Cl_2NO_4$: Calcd, 357.0535; Found, 357.0519.

1-Acetyl-3-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-2-benzylthio-5,6-dichloroindole (10). To a solution of compound **7** (1.00 g, 2.08 mmol) in 12 mL of CH_3CN, Ac_2O (0.82 mL, 8.7 mmol) and Et_3N (1.21 mL, 8.7 mmol) were added at room temperature. The mixture was stirred for 24 h, and then filtered to give 0.92 g (79%) of **10** as a white solid. The filtrate was concentrated to dryness. The residue was purified by flash column chromatography (30% EtOAc/Hex, 1 cm × 10 cm) to give 102 mg (8.7%, total 88%) of additional **10**: mp. 178–179°C. R_f 0.70 (30% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ 1.37 (s, 3H), 1.68 (s, 3H), 2.29 (s, 3H), 2.66 (s, 3H), 4.01 (m, 2H), 4.28 (m, 1H), 4.40–4.52 (m, 2H), 4.81–4.91 (m, 2H), 5.39 (d, 1H, J = 6.1), 7.02 (m, 2H), 7.22 (m, 3H), 7.83 (s, 1H), 8.42 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 21.1, 25.6, 27.5, 28.3, 44.9, 63.5, 80.7, 81.2, 81.7, 84.4, 116.0, 118.2, 120.9, 125.8, 126.9, 127.3, 127.9, 128.5, 128.8, 130.3, 130.5, 136.4, 136.5, 170.8, 170.9. Anal calcd for $C_{27}H_{27}Cl_2NO_6S$: C, 57.45; H, 4.82; N, 2.48. Found: C, 57.58; H, 5.00; N, 2.50.

1-Acetyl-3-(5-*O*-acetyl-β-D-ribofuranosyl)-2-benzylthio-5,6-dichloroindole (11). Compound **10** (240 mg, 0.43 mmol) was dissolved in 7 mL of TFA-water (9:1) at room temperature. The mixture was stirred for 10 min, and then concentrated to dryness. The residue was recrystallized from EtOAc/Hex to give 114 mg (51%) of **11**: mp. 135–136°C. R_f 0.20 (30% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ 2.25 (s, 3H), 2.50 (broad s, 1H), 2.60 (broad s, 1H), 2.72 (s, 3H), 4.00 (s, 2H), 4.04 (m, 1H), 4.20–4.28 (m, 2H), 4.37–4.55 (m, 2H), 5.18 (d, 1H, J = 7.2), 7.01 (m, 2H), 7.23 (m, 3H), 7.85 (s, 1H), 8.41 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 21.1, 28.1, 44.3, 63.6, 70.9, 74.5, 80.0, 82.6, 118.0, 121.0, 126.0, 127.1, 127.3, 127.9, 128.6, 128.9, 130.1, 130.5, 136.3, 136.5, 170.8, 171.1. Anal calcd for $C_{24}H_{23}Cl_2NO_6S$: C, 54.97; H, 4.42; N, 2.67. Found: C, 55.09; H, 4.56; N, 2.65.

2-Benzylthio-5,6-dichloro-3-(β-D-ribofuranosyl)indole (12). Compound **11** (2.80 g, 5.34 mmol) and Na_2CO_3 (6.0 g, 57 mmol) in 80 mL of MeOH were stirred at room temperature for 2 h, and then treated with 200 mL of water. The resulting mixture was concentrated first to remove most of the organic solvents, and then extracted with EtOAc (3 × 100 mL). The combined EtOAc extracts were washed with 200 mL of brine and dried over $MgSO_4$. The organic layer was concentrated to dryness. The residue was

purified by flash column chromatography (60% EtOAc/Hex, 4 cm × 10 cm) to give 2.4 g of the crude product, which was crystallized from EtOAc/Hex to give 1.85 g (79%) of **12** as a white solid. mp 164–165°C. R_f 0.40 (60% EtOAc/Hex). ^{1}H NMR (DMSO-d_6): δ 3.63 (m, 2H), 3.78 (m, 1H), 4.03 (m, 2H), 4.19 (m, 2H), 4.83 (d, 1H, $J = 6.3$), 4.89 (m, 2H), 4.96 (t, 1H, $J =$ 5.0), 7.23 (m, 5H), 7.46 (s, 1H), 8.09 (s, 1H), 11.7 (broad s, 1H). ^{13}C NMR (DMSO-d_6): δ 40.7, 61.8, 71.3, 74.7, 77.6, 85.6, 112.2, 117.3, 121.6, 124.3, 125.6, 127.2, 128.4, 128.7, 130.6, 135.7, 137.6. HRMS for $C_{20}H_{19}Cl_2NO_4S$: Calcd, 439.0412; Found, 439.0399. Anal calcd for $C_{20}H_{19}Cl_2NO_4S$: C, 54.55; H, 4.35; N, 3.18. Found: C, 54.57; H, 4.51; N, 3.27.

5,6-Dichloro-3-(β-D-ribofuranosyl)indole (13). A mixture of compound **12** (5.0 g, 11 mmol) and Raney Ni (30 g, wet, W-4) in 100 mL of EtOH was stirred at room temperature for 0.5 h. The Raney Ni was removed by filtration. The filtrate was concentrated to dryness to give compound **13** as a white solid. Compound **13** was used in the next reaction without further purification: R_f 0.15 (EtOAc). ^{1}H NMR (DMSO-d_6): δ 3.56 (m, 2H), 3.79 (m, 1H), 3.94 (m, 2H), 4.77 (d, 1H, $J = 6.1$), 4.85 (m, 2H), 4.93 (d, 1H, $J =$ 5.7), 7.43 (s, 1H), 7.60 (s, 1H), 7.94 (s, 1H), 11.2 (broad s, 1H). ^{13}C NMR (DMSO-d_6): δ 62.0, 71.2, 75.4, 78.0, 84.9, 112.9, 114.5, 120.9, 121.0, 123.3, 125.8, 126.1, 135.5.

5,6-Dichloro-3-[2,3,5-tri-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl]indole (14). To a solution of compound **13** (used directly from the previous reaction without purification) in 60 mL of DMF, imidazole (5.0 g, 73 mmol) and TBDMSCl (6.5 g, 43 mmol) were added at room temperature. The resulting mixture was stirred at room temperature for 26 h, and then concentrated to dryness. The residue was treated with 100 mL of EtOAc/Hex (1:1). The solid was removed by filtration and the filtrate was concentrated to dryness. The residue was purified by flash column chromatography (10% EtOAc/Hex, 4 cm × 10 cm) to give 5.32 g (73% over 2 steps) of **14** as a white solid: mp. 103–107°C. R_f 0.60 (15% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ −0.45 (s, 3H), −0.17 (s, 3H), 0.10 (s, 3H), 0.11 (s, 3H), 0.16 (s, 6H), 0.74 (s, 9H), 0.95 (s, 9H), 0.96 (s, 9H), 3.86 (m, 2H), 4.04 (m, 1H), 4.10 (m, 1H), 4.20 (m, 1H), 4.97 (d, 1H, $J = 8.4$), 7.22 (d, 1H, $J = 2.4$), 7.43 (s, 1H), 7.94 (s, 1H), 8.10 (broad s, 1H). ^{13}C NMR ($CDCl_3$): δ −5.4, −5.3, −4.5, −4.4, −4.3, 18.0, 18.1, 18.6, 25.8, 25.9, 26.2, 63.9, 73.7, 77.2, 86.3, 112.5, 115.0, 121.8, 123.7, 125.0, 126.0, 135.4. HRMS for $C_{31}H_{55}Cl_2NO_4Si_3$ [M + H]: Calcd, 660.2894; Found, 660.2886.

3-[2,3,5-Tri-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl]-2,5,6-trichloroindole (15). A mixture of compound **14** (1.80 g, 2.72 mmol) and NCS (378 mg, 2.83 mmol) in 20 mL of CCl_4 was stirred at room temperature

for 24 h, and then concentrated to dryness. The residue was purified by flash column chromatography (5% EtOAc/Hex, 3 cm × 10 cm), followed by crystallization from MeCN to give 0.97 g (51%) of **15** as a white solid. mp. 158–164°C. R_f 0.30 (50% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ −0.61 (s, 3H), −0.19 (s, 3H), 0.11 (s, 3H), 0.16 (s, 3H), 0.19 (s, 6H), 0.74 (s, 9H), 0.96 (s, 9H), 0.99 (s, 9H), 3.89 (m, 2H), 4.05 (m, 1H), 4.22 (m, 2H), 5.05 (d, 1H, $J = 8.4$), 7.29 (s, 1H), 7.94 (s, 1H), 8.20 (broad s, 1H). ^{13}C NMR ($CDCl_3$): δ −5.5, −5.3, −5.2, −4.7, −4.5, −4.3, 17.8, 18.1, 18.7, 25.8, 25.9, 26.3, 63.9, 73.5, 75.4, 75.8, 87.1, 109.5, 111.9, 121.3, 124.6, 125.1, 126.0, 126.4, 133.2. HRMS for $C_{31}H_{54}Cl_3NO_4Si_3$ [M + NH_4]: Calcd, 711.2770; Found, 711.2753.

3-(β-D-Ribofuranosyl)-2,5,6-trichloroindole (16). Method 1: Compound **15** (600 mg, 0.86 mmol) was dissolved in 15 mL of a THF solution of TBAF (1.0 M). The mixture was stirred at room temperature for 0.5 h. Water (80 mL) was added, and the mixture was extracted with EtOAc (3 × 50 mL). The combined extracts were washed with brine (70 mL), dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash column chromatography (90% EtOAc/Hex, 2 cm × 10 cm) to give 262 mg (86%) of **16** as a white solid.

Method 2: Compound **18a** (38 mg) was dissolved in 33% methylamine in EtOH (1 mL) at room temperature. The mixture was stirred at room temperature for 30 min, and then concentrated to dryness. The residue was subjected to flash column chromatography (from 5% MeOH/CH_2Cl_2 to 8% MeOH/CH_2Cl_2, 1 cm × 10 cm) to give 18 mg, (100%) of **16** as a white solid, which has the same ^{1}H NMR spectrum as that of the solid prepared in method 1: mp dec >179°C. R_f 0.35 (EtOAc). ^{1}H NMR (DMSO-d_6): δ 3.61 (m, 2H), 3.79 (m, 1H), 4.03 (m, 2H), 4.72 (d, 1H, $J = 8.2$), 4.88 (m, 2H, D_2O exchangeable), 4.98 (t, 1H, D_2O exchangeable, $J = 5.1$), 7.51 (s, 1H), 8.07 (s, 1H), 12.2 (broad s, 1H, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 61.8, 71.2, 74.3, 76.4, 85.7, 109.3, 112.4, 121.1, 122.2, 124.1, 124.8, 125.4, 133.7. Anal calcd for $C_{13}H_{12}Cl_3NO_4$: C, 44.28; H, 3.43; N, 3.97. Found: C, 43.95; H, 3.28; N, 3.82.

1-Acetyl-3-[2,3,5-tri-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl]-2,5,6-trichloroindole (17). To a solution of compound **15** (440 mg, 0.63 mmol) in 15 mL of MeCN, Ac_2O (0.8 mL) and Et_3N (0.5 mL) were added at room temperature. The mixture was stirred for 0.5 h, and then filtered to give 360 mg (77%) of **17** as a white solid. The filtrate was concentrated to dryness. The residue was purified by flash column chromatography (5% EtOAc/Hex, 1 cm × 10 cm) to give an additional 64 mg (14%, total 91%) of **17**. mp. 120–121°C. R_f 0.40 (50% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ −0.56 (s, 3H), −0.15 (s, 3H), 0.11 (s, 3H), 0.12 (s, 3H), 0.19 (s, 3H), 0.20 (s, 3H), 0.76 (s, 9H), 0.96 (s, 9H), 0.98 (s, 9H), 2.79 (s, 3H), 3.91 (m, 2H), 4.06 (m, 1H), 4.20 (m,

2H), 5.13 (m, 1H), 8.00 (s, 1H), 8.54 (s, 1H). ^{13}C NMR ($CDCl_3$): δ −5.5, −5.3, −5.2, −4.6, −4.5, −4.2, 17.8, 18.1, 18.7, 25.7, 25.9, 26.3, 27.7, 63.8, 73.3, 75.3, 76.0, 87.7, 117.1, 118.0, 121.1, 123.7, 126.2, 127.9, 129.4, 134.5, 169.3. Anal calcd for $C_{33}H_{56}Cl_3NO_5Si_3$: C, 44.28; H, 3.43; N, 3.97. Found: C, 43.95; H, 3.28; N, 3.82.

1-Acetyl-3-(β-D-ribofuranosyl)-2,5,6-trichloroindole (18a). Method 1: A mixture of compound **17** (300 mg, 0.41 mmol) and KF (700 mg, 12 mmol) in 7 mL of 80% aqueous TFA was stirred at room temperature for 1 h. The resulting mixture was concentrated to dryness, and then treated with 50 mL of brine and extracted with EtOAc (2 × 50 mL). The combined extracts were dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash column chromatography (95% EtOAc/Hex, 2 cm × 10 cm) to give 108 mg (67%) of **18a** as a white solid. **Method 2:** Compound **21a** (324 mg, 0.456 mmol) was suspended in 12 mL of AcOH, and 5% $Pd/BaSO_4$ (300 mg) was added. The mixture was stirred under 1 atm of H_2 for 45 min. The black suspension was filtered through Celite. The filtrate was concentrated to dryness and the residue was subjected to flash column chromatography (from 5% MeOH/CH_2Cl_2 to 8% MeOH/CH_2Cl_2, 2 cm × 10 cm) to give 180 mg (90%) of compound **18a** as a white solid: mp. 165–166°C (EtOAc/Hex). R_f 0.40 (10% MeOH/CH_2Cl_2). ^{1}H NMR (DMSO-d_6): δ 2.80 (s, 3H), 3.66 (m, 2H), 3.87 (m, 1H), 4.08 (m, 2H), 4.85 (d, 1H, J = 7.7), 5.00 (m, 2H, D_2O exchangeable), 5.11 (t, 1H, D_2O exchangeable, J = 4.3), 8.37 (s, 1H), 8.43 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 27.5, 61.5, 71.1, 74.7, 76.5, 86.3, 117.2, 117.3, 121.8, 123.8, 126.10, 126.14, 127.3, 134.1, 169.9. HRMS for $C_{15}H_{14}Cl_3NO_5$: Calcd, 392.9938; Found, 392.9919. Anal calcd for $C_{15}H_{14}Cl_3NO_5$: C, 53.75; H, 7.65; N, 1.90. Found: C, 53.51; H, 7.60; N, 1.89.

1-Propionyl-3-(β-D-ribofuranosyl)-2,5,6-trichloroindole (18b). The procedure is the same as that described for **18a** (Method 2), except that **21b** (380 mg, 0.56 mmol) was used instead of **21a**. A 70% yield (160 mg, 0.39 mmol) of **18b** was obtained as a white foam: mp 182–184°C. R_f 0.28 (10% MeOH/$CHCl_3$). ^{1}H NMR (DMSO-d_6): δ 1.20 (t, 3H), 3.20 (m, 2H) 3.67 (m, 2H), 3.87 (d, 1H), 4.06–4.10 (m, 2H), 4.85 (d, 1H), 4.99–5.01 (m, 2H, D_2O exchangeable), 5.11 (t, 1H, D_2O exchangeable), 8.36 (s, 1H), 8.44 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 8.9, 32.2, 61.5, 71.1, 74.7, 76.5, 86.3, 117.1, 117.2, 121.8, 123.6, 126.0, 126.1, 127.2, 134.1, 173.7. HRMS for: $C_{16}H_{16}Cl_3NO_5$ [M + Na]: Calcd, 429.9992; Found, 430.0006. Anal calcd for $C_{16}H_{16}Cl_3NO_5$: C, 47.02; H, 3.95; N 3.43. Found: C, 47.17; H, 4.15; N, 3.25.

1-Acetyl-2,5,6-trichloroindole (20a). To a solution of compound **19**[4] (4.40 g, 20.0 mmol) in CH_2Cl_2 (50 mL) and dry pyridine (20 mL) was

added Ac_2O (10.0 g, 100 mmol) at room temperature. The mixture was stirred at room temperature for 24 h, and then concentrated to dryness. The residue was dissolved in EtOAc (100 mL), washed with 1.0 M HCl (50 mL), 10% $NaHCO_3$ (50 mL), brine (20 mL), dried over $MgSO_4$, filtered, and the filtrate evaporated to dryness. This furnished an orange solid which was recrystallized from EtOAc/Hex to give 2.77 g (53%) of **20a** as a pale-pink solid: mp. 135–137°C. R_f 0.30 (10% EtOAc/Hex). ^1H NMR (DMSO-d_6): δ 2.76 (s, 3H), 6.89 (s, 1H), 7.80 (s, 1H), 8.36 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 27.2, 108.4, 117.4, 120.9, 125.4, 126.2, 127.0, 127.7, 134.3, 169.8. Anal calcd for $C_{10}H_6Cl_3NO$: C, 45.75; H, 2.30; N, 5.34. Found: C, 45.43; H, 2.63; N, 5.39.

1-Propionyl-2,5,6-trichloroindole (20b). To a solution of compound **19** (1.00 g, 4.5 mmol) in dry pyridine (20 mL) was added propionyl chloride (2.0 mL, 2.1 g, 23 mmol) at room temperature. The mixture was stirred at room temperature for 24 h, and then concentrated to dryness. The residue was dissolved in EtOAc (75 mL), washed with 1.0 M HCl (50 mL), 10% $NaHCO_3$ (50 mL), brine (20 mL), then dried over $MgSO_4$, filtered, and the filtrate was evaporated to yield an orange solid. The solid was subjected to flash column chromatography (3:1 hex:EtOAc, 5 cm × 45 cm) to give 0.64 g (51%) of compound **20b** as a white solid: mp. 124–125°C. R_f 0.65 (25% EtOAc/Hex). ^1H NMR ($CDCl_3$): δ 1.32 (t, 3H), 3.13 (q, 2H), 6.52 (s, 1H), 7.48 (s, 1H), 8.50 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 9.2, 32.7, 108.8, 118.5, 120.5, 124.8, 127.5, 128.1, 129.2, 135.1, 173.6.

1-Acetyl-3-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-2,5,6-trichloroindole (21a). To a solution of compound **20a** (802 mg, 3.06 mmol) and 2,3,5-tri-*O*-benzyl-α,β-D-ribofuranosyl fluoride[23] (800 mg, 1.89 mmol) in 15 mL of CH_2Cl_2, boron trifluoride etherate (0.23 mL, 1.89 mmol) was added dropwise at 0°C. The resulting mixture was stirred at that temperature for 10 minutes. The reaction was quenched with sat. $NaHCO_3$ (10 mL). The mixture was extracted with CH_2Cl_2 (3 × 5 mL). The organic layers were washed with brine (10 mL), dried over $MgSO_4$ and concentrated to dryness. The resulting yellow oil was subjected to flash column chromatography (from 10% EtOAc/Hex to 20% EtOAc/Hex) to give 0.94 g of a crude product. Recrystallization from EtOAc/Hex provided 590 mg (45%) of compound **21a** as a white solid: mp. 115–116°C (EtOAc/Hex). R_f 0.70 (30% EtOAc/Hex). ^1H NMR (DMSO-d_6): δ 2.78 (s, 3H), 3.59 (m, 1H), 3.71 (m, 1H), 4.19 (m, 2H), 4.23 (m, 1H), 4.33 (d, 1H, J = 12.1), 4.49 (d, 1H, J = 12.1), 4.57 (d, 1H, J = 12.3), 4.64 (q, 2H, J = 9.6), 4.72 (d, 1H, J = 12.3), 5.04 (d, 1H, J = 8.3), 7.07 (m, 5H), 7.32 (m, 10H), 8.02 (s, 1H), 8.37 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 27.6, 69.9, 71.3, 71.4, 72.4, 75.1, 76.8, 80.6, 82.9, 115.9, 117.2, 121.2, 124.2, 125.6, 126.0, 127.3, 127.5, 127.6, 127.7, 127.9,

128.2, 128.3, 134.0, 137.7, 138.0, 138.3, 169.8. Anal calcd for $C_{36}H_{32}Cl_3NO_5$: C, 65.02; H, 4.85; N, 2.11. Found: C, 65.08; H, 5.05; N, 2.10.

1-Propionyl-3-(2,3,5-tri-*O*-benzyl-*β*-D-ribofuranosyl)-2,5,6-trichloroindole (21b). The procedure is the same as that described for **21a** (Method 2), except that **20b** (540 mg, 2.0 mmol) was used instead of **20a**. A 31% yield (400 mg, 0.75 mmol) of **21b** was obtained as a white crystalline solid: mp. 94–95°C. R_f 0.45 (25% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ 1.32 (t, 3H), 3.13 (m, 2H), 3.59 (dd, 1H), 3.68 (dd, 1H), 4.11 (dd, 1H), 4.19 (dd, 1H), 4.24 (d, 1H), 4.33 (d, 1H), 4.51 (d, 2H), 4.60 (d, 1H), 4.73 (d, 1H), 4.81 (d, 1H), 5.20 (d, 1H), 7.03–7.13 (m, 5H), 7.28–7.37 (m, 10H), 7.99 (s, 1H), 8.50 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 9.3, 33.0, 34.9, 70.0, 72.6, 73.0, 73.6, 76.1, 81.1, 83.6, 116.9, 118.1, 121.4, 123.5, 125.9, 127.8, 127.9, 128.0, 128.1, 128.2, 128.4, 128.7, 129.4, 134.9, 137.6, 138.0, 138.1, 173.6.

3-(2,3,5-Tri-*O*-benzyl-*β*-D-ribofuranosyl)-2,5,6-trichloroindole (22). The procedure is the same as that described for **16** (Method 2), except that **21a** (2.46 g, 3.7 mmol) was used instead of **18a**. A 99% yield (2.27 g, 3.6 mmol) of **22** was obtained as a clear foam: mp. 94–95°C. R_f 0.44 (1% MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 3.53 (dd, 1H), 3.68 (dd, 1H), 4.11 (dd, 1H), 4.23–4.27 (m, 2H), 4.41 (s, 2H), 4.51 (d, 1H), 4.59 (d, 1H), 4.71 (d, 1H), 4.76 (d, 1H), 5.20 (d, 1H), 7.06–7.14 (m, 5H), 7.25–7.33 (m, 11H), 7.86 (s, 1H), 9.45 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 70.1, 72.5, 73.6, 76.4, 77.4, 82.7, 109.1, 112.4, 121.2, 124.4, 125.2, 125.6, 126.2, 127.8, 127.9, 128.0, 128.3, 128.4, 128.6, 133.8, 137.7, 138.0, 138.1.

1-Methyl-3-(2,3,5-tri-*O*-benzyl-*β*-D-ribofuranosyl)-2,5,6-trichloroindole (23a). A solution of compound **22** (0.47 g, 0.75 mmol) in dry THF (10 mL) was cooled to 0°C in an ice bath and sodium hydride (60% in mineral oil, 100 mg, 2.5 mmol) was added in one portion. The suspension was stirred at 0°C for 15 min, then methyl iodide (1.0 mL, 2.3 g, 16 mmol) was added and the resulting suspension was allowed to warm to room temperature over 20 min. The solvent was removed under vacuum, and the residue dissolved in EtOAc (20 mL). The organic suspension was washed with 5% aqueous sodium thiosulfate (25 mL), brine (20 mL), dried over $MgSO_4$, filtered, and the filtrate was evaporated to yield a yellow residue. The residue was subjected to flash column chromatography (2:1 hex:EtOAc, 5 cm × 45 cm) to give 0.45 g (94%) of compound **23a** as a clear oil: R_f 0.60 (33% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ 3.59 (dd, 1H), 3.66 (s, 3H), 3.74 (dd, 1H), 4.18 (dd, 1H), 4.29–4.33 (m, 2H), 4.45 (d, 1H), 4.50 (d, 1H), 4.58 (d, 1H), 4.67 (d, 1H), 4.78 (d, 1H), 4.83 (d, 1H), 5.30 (d, 1H), 7.12–7.21 (m, 5H), 7.35–7.41 (m, 11H), 8.00 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 30.2, 70.1, 72.3, 72.6, 73.5, 76.6, 77.3, 82.7, 108.7, 110.8, 121.4, 124.3, 124.7, 126.1, 127.5, 127.6, 127.8, 127.9, 128.1, 128.3, 128.5, 128.6, 135.0, 137.8, 138.1 138.2.

1-(1-Propyl)-3-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-2,5,6-trichloroindole (23b). The procedure is the same as that described for **23a**, except that **22** (0.49 g, 0.79 mmol) was treated with 1-iodopropane instead of methyl iodide. A 100% yield (0.52 g, 0.79 mmol) of **23b** was obtained as a clear oil: Rf 0.71 (33% EtOAc/Hex). ^{1}H NMR ($CDCl_3$): δ 0.90 (t, 3H), 1.75 (q, 2H), 3.53 (dd, 1H), 3.67 (dd, 1H), 4.04 (dt, 2H), 4.11 (q, 2H), 4.21–4.25 (m, 2H), 4.40 (dd, 2H), 4.51 (d, 1H), 4.60 (d, 1H), 4.72 (d, 1H), 4.76 (d, 1H), 5.24 (d, 1H), 7.04–71.4 (m, 5H), 7.26–7.37 (m, 11H), 7.93 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 11.4, 23.1, 45.5, 70.1, 72.4, 72.5, 73.5, 76.7, 77.3, 80.9, 82.7, 108.7, 111.0, 124.3, 124.8, 126.0, 127.2, 127.6, 127.8, 127.9, 128.2, 128.3, 128.5, 128.6, 134.5, 137.8, 138.1, 138.2.

1-Methyl-3-(β-D-ribofuranosyl)-2,5,6-trichloroindole (24a). The procedure is the same as that described for **18a** (Method 2), except that **23a** (442 mg, 0.69 mmol) was used instead of **21a**. A 70% yield (175 mg, 0.48 mmol) of **24a** was obtained as a white powder: mp dec >112°C. R_f 0.37 (10% MeOH/$CHCl_3$). ^{1}H NMR (DMSO-d_6): δ 3.49–3.55 (m, 2H), 3.72 (s, 3H), 3.77–3.82 (m, 2H), 3.98 (m, 1H), 4.51 (b, 1H, D_2O exchangeable), 4.55 (d, 1H), 4.71 (b, 1H, D_2O exchangeable), 4.90 (b, 1H, D_2O exchangeable), 7.79 (s, 1H), 7.88 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 30.3, 65.7, 69.6, 70.6, 71.1, 109.8, 112.0, 120.6, 122.2, 124.1, 124.6, 127.3, 134.6. HRMS for $C_{14}H_{14}Cl_3NO_4$ [M + Na]: Calcd, 387.9886; Found, 387.9877. Anal calcd for $C_{14}H_{14}Cl_3NO_4 \cdot 1/4$ EtOAc: C, 46.36; H, 4.15; N, 3.60. Found: C, 46.58; H, 4.20; N, 3.65.

1-(1-Propyl)-3-(β-D-ribofuranosyl)-2,5,6-trichloroindole (24b). The procedure is the same as that described for **18a** (Method 2), except that **23b** (442 mg, 0.69 mmol) was used instead of **21a**. A 61% yield (167 mg, 0.42 mmol) of **24b** was obtained as a white powder: mp dec >135°C. R_f 0.34 (10% MeOH/$CHCl_3$). ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H), 1.68 (m, 2H), 3.50–3.55 (m, 2H), 3.78–3.18 (m, 2H), 3.97 (s,1H), 4.17 (m, 2H), 4.50 (b, 1H, D_2O exchangeable), 4.55 (d, 1H), 4.69 (b, 1H, D_2O exchangeable), 4.78 (b, 1H, D_2O exchangeable), 7.79 (s, 1H), 7.93 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 10.9, 22.7, 44.8, 65.7, 67.1, 69.5, 70.6, 71.1, 109.9, 112.0, 120.7, 122.2, 124.2, 124.7, 126.8, 134.1. HRMS for $C_{16}H_{18}Cl_3NO_4$ [M + Na]: Calcd, 416.0199; Found, 416.0200. Anal calcd for $C_{16}H_{18}Cl_3NO_4 \cdot 1/4$ EtOAc: C, 49.00; H, 4.84; N, 3.36. Found: C, 49.21; H, 4.90; N, 3.31.

Biological Evaluation

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.[25]

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 (m.o.i.) of <0.01 plaque-forming units (p.f.u.) per cell as detailed previously.[26] High titer HSV-1 stocks were prepared by infecting KB cells at an m.o.i. of <0.1 also as detailed previously.[26] 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.[27] Briefly, HFF or BSC-1 cells were planted as described above in 96-well cluster dishes and incubated overnight at 37°C. The next day cultures were inoculated with HCMV or HSV-1 and serially diluted 1:3 across the remaining eleven columns of the 96-well plate. After virus adsorption the inoculum was replaced with fresh medium and cultures were incubated for seven days for HCMV, two or three days for HSV-1. Plaques were enumerated under 20-fold magnification in wells having the dilution which gave 5 to 20 plaques per well. Virus titers were calculated according to the following formula: Titer (p.f.u./mL) = number of plaques $\times$ 5 $\times$ 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, the compounds, prepared as 10 mg/mL stock solutions in DMSO were diluted with growth medium and were added to duplicate wells in four to eight selected concentrations. After incubation at 37°C for 7–10 days, cell sheets were fixed, 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.

HSV-1 ELISA. An ELISA was employed[28] 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, rinsed, and horseradish peroxidase conjugated rabbit anti-HSV-1 antibody was added. Following

removal of the antibody containing solution, plates were rinsed, and then developed by adding 150 μL per well of a solution of tetramethylbenzidine as substrate. The reaction was stopped with H_2SO_4 and absorbance was read at 450 and 570 nm. Drug effects were calculated as a percentage of the reduction in absorbance in the presence of each drug concentration compared to absorbance obtained with virus in the absence of drug.

Cytotoxicity Assays. Two different assays were used for routine cytotoxicity testing. (a) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[26] (b) 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.[29] 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 used to quantitate drug effects by linear regression of the percent inhibition of parameters derived in the preceding assays against $\log_{10}$ drug concentrations. Fifty percent inhibitory concentrations (IC_{50}s) were calculated from the linear portions of the regression lines. Samples containing positive controls (acyclovir for HSV-1, GCV for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

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