Synthesis and Antiviral Evaluation of Trisubstituted Indole *N*-Nucleosides as Analogues of 2,5,6-Trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB)

Jiong J. Chen,† Yuan Wei,‡ John C. Drach, and Leroy B. Townsend*

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

Received June 22, 1999

2,5,6-Trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB) and 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BDCRB) are nucleosides that exhibit strong and selective activity against human cytomegalovirus (HCMV). Selected polyhalogenated indole nucleosides have now been synthesized as 3-deaza analogues of the benzimidazole nucleosides using the sodium salt glycosylation method. 2-Benzylthio-1-[2-deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-5,6-dichloroindole (**8**) was prepared stereoselectively via the coupling of a 2-deoxyribofuranosyl α-chloride derivative with the sodium salt of 2-benzylthio-5,6-dichloroindole (**5**). Compound **8** was then elaborated into the targeted 2-benzylthio-1-(β-D-ribofuranosyl)-5,6-dichloroindole (**18**) in five steps. 2,5,6-Trichloro-(1-β-D-ribofuranosyl)indole (**19**) was prepared using the same synthetic route with 2,5,6-trichloroindole (**6**) as the starting material. We were subsequently able to prepare **19** in three steps using a modification of the sodium salt glycosylation method. 2-Bromo-5,6-dichloro-1-(β-D-ribofuranosyl)indole (**25**) was also prepared using the same procedures. Target compounds were tested for activity against HCMV, herpes simplex virus type 1 (HSV-1), and human herpes virus six (HHV-6) and for cytotoxicity. All of the compounds were less active against HCMV than TCRB and weakly active or inactive against HSV-1 and HHV-6.

Introduction

A series of 2-substituted benzimidazole nucleosides have been synthesized in our group and display strong antiviral activities.[1,2] The lead compounds 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB) and 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BDCRB) have potent activity against human cytomegalovirus (HCMV) with low cellular toxicity at concentrations that inhibit viral replication. 2-Benzylthio-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BTDCRB) (Figure 1) also is active against HCMV but is more cytotoxic.[2] In contrast to activity against HCMV, TCRB displays only weak antiviral activity against HSV-1, HSV-2, varicella zoster virus, Epstein Barr virus, human herpes virus six, and even murine cytomegalovirus.[3] TCRB was also found to be inactive against a selection of RNA viruses including the A and B strains of influenza, respiratory syncytia, measles virus, and HIV-1.[3] In contrast, TCRB was found to be active against three distinct clinical isolates of HCMV that had become resistant to ganciclovir. This is because both TCRB and BDCRB act by a unique mechanism which does not involve inhibition of DNA synthesis but does involve inhibition of viral DNA processing.[4] Genotypic characterization of HCMV resistant to TCRB and BDCRB has identified two viral genes, UL56 and UL89, which mutate to give drug-resistant virus.[4,5] Consequently, the proteins encoded by these two genes must be the target(s) for TCRB and related analogues.

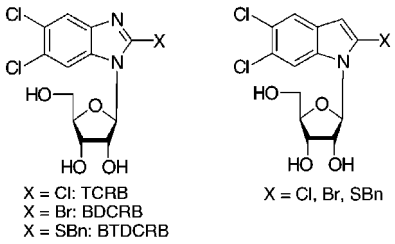


Figure 1. Polyhalogenated benzimidazole and indole nucleosides.

Indole, a heterocycle which is structurally similar to benzimidazole, has the potential of bearing the same substitution patterns as those of TCRB, BDCRB, and BTDCRB. Therefore, we elected to synthesize polyhalogenated indole nucleosides as 3-deaza analogues of the established antiviral benzimidazole nucleosides. In light of the substantial structure−activity relationship (SAR) studies of TCRB,[1,2,6−9] the synthesis and testing of a limited number of 5,6-dichloroindole ribosides (Figure 1) should be sufficient to evaluate the potential antiviral activity of these nucleosides as analogues of TCRB.

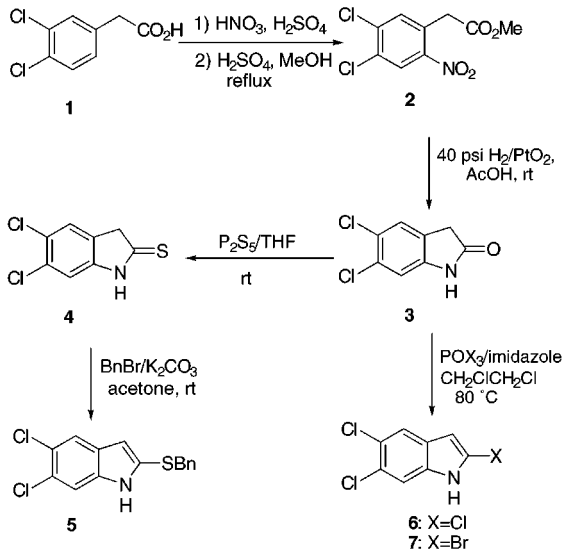
Results and Discussion

Chemistry. To date, the most successful preparation of indole nucleosides involved an indoline-indole strategy.[10] This strategy generally furnished indole nucleosides without a 2-substituent. Our targeted 2-substituted indole nucleosides made this strategy unattractive. Thus, our retrosynthetic approach involved the coupling[11] of a fully functionalized indole aglycon (sodium

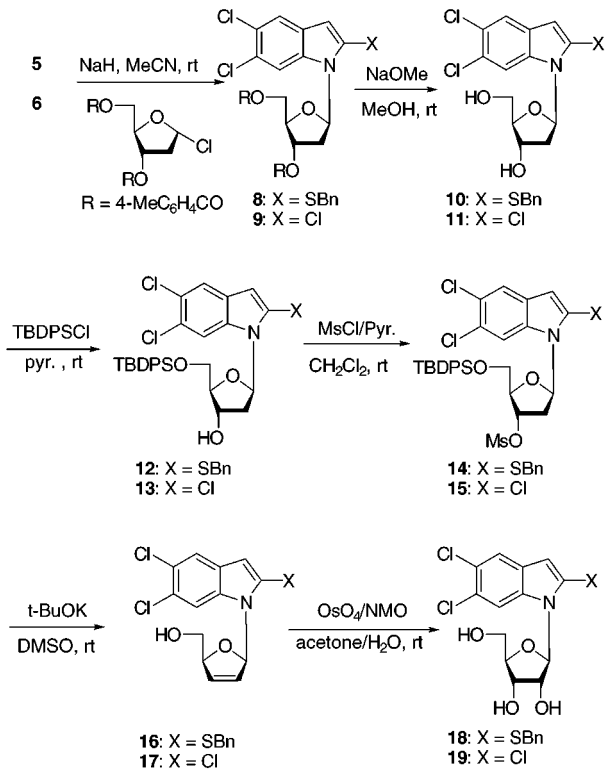
* To whom correspondence should be addressed. Tel: 734-764-7547. Fax: 734-763-5633. E-mail: ltownsen@umich.edu.
† Present address: Pharmacia & Upjohn, 7000 Portage Rd., Kalamazoo, MI 49001.
‡ Present address: Ganes Chemicals, Inc., 630 Broad St., Carlstadt, NJ 07072.

10.1021/jm990320x CCC: $19.00
Published on Web 05/17/2000

Scheme 1



Scheme 2

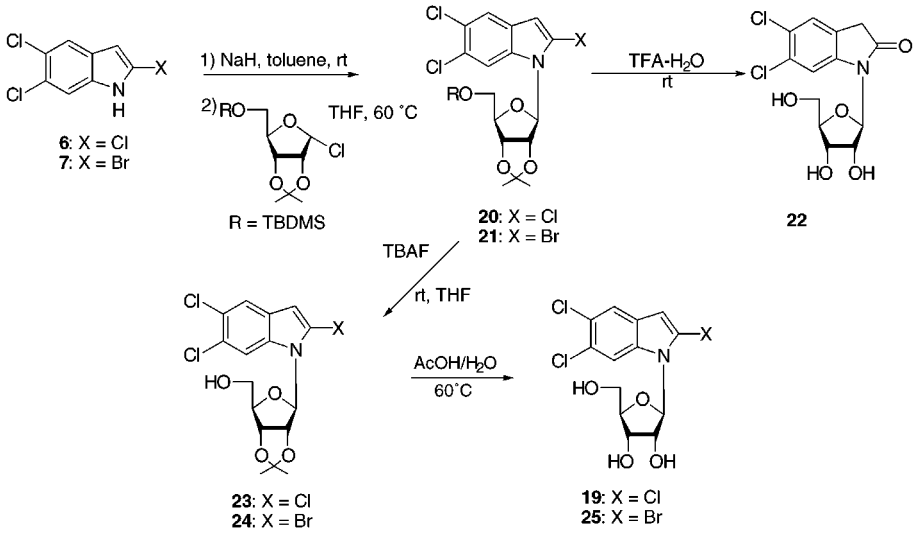


salt) with an appropriately protected ribofuranose donor, followed by deprotection to provide the appropriate 2,5,6-trisubstituted indole riboside.

The preparation of some key indole aglycons was initiated with the commercially available 3,4-dichlorophenylacetic acid (**1**) (Scheme 1). Compound **1** was nitrated[12] and methylated to afford methyl 4,5-dichloro-2-nitrophenyl acetate (**2**) in a 65% yield over two steps. 5,6-Dichloroindol-2-one (**3**) was then prepared in a 76% yield under catalytic hydrogenation conditions. A conversion of compound **3** to 5,6-dichloroindole-2-thione (**4**) was effected with phosphorus pentasulfide. Compound **4** was then benzylated to give 2-benzylthio-5,6-dichloroindole (**5**) in a high yield under weakly basic conditions. The preparation of the requisite 2-haloindoles was not as straightforward. Using the reaction conditions described by Powers and Brennan,[13,14] i.e., phosphorus oxychloride in chloroform or phosphorus oxybromide in *N*,*N*-dimethylaniline, the corresponding 2-chloroindole and 2-bromoindole derivatives (**6**, **7**) were obtained in only 10−15% yields. The formation of **6** and **7** was accompanied by considerable amounts of highly insoluble solids. We found that both compounds **6** and **7** deteriorated in solution, especially in a weakly acidic solvent such as chloroform. The low yields and decomposition confirmed the reported acid lability of 2-haloindoles.[13,14] Strong acids, such as HCl or HBr, which were generated in the above halogenation reactions, may have hydrolyzed or polymerized the 2-haloindole products and resulted in the highly insoluble solids. We reasoned that the use of a weak base should prevent or inhibit such side reactions. We subsequently found that by using imidazole in the reaction the yields increased to 70% for 2,5,6-trichloroindole (**6**) and 80% for 2-bromo-5,6-dichloroindole (**7**).

Indole 2′-deoxyribosides have been prepared via the sodium salt glycosylation method.[16,17] However, the preparation of indole ribosides via this method was unsuccessful according to the literature.[18] Our initial attempts to couple 2-benzylthio-5,6-dichloroindole (**5**) with various ribofuranosyl halides confirmed the unsuccessful attempts reported in the literature.[18] For example, with 5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-α-D-ribofuranosyl chloride, the glycosylation reaction gave only a trace amount of the desired product. The glycosylation reaction with 2,3,5-tri-*O*-benzoyl-α,*β*-D-ribofuranosyl bromide afforded a 1,2-*O*-alkylidene nucleoside (orthoamide), which was formed via the participation of a neighboring 2-alkyloxy group of the ribofuranosyl donor. These unsuccessful attempts prompted us to prepare the 2′-deoxy analogues according to the literature procedures[16,17] and then elaborate the resulting indole 2′-deoxyribosides into the targeted indole ribosides (Scheme 2).[17] The glycosylation of compound **5** with 2-deoxy-3,5-bis-*O*-(4-methylbenzoyl)-α-D-ribofuranosyl chloride resulted in a very good yield of the *O*-protected 2′-deoxyriboside **8**. A removal of the 4-methylbenzoyl groups from the carbohydrate moiety provided the indole 2′-deoxyriboside **10**. The 5′-position of **10** was selectively "masked" with a *tert*-butyldiphenylsilyl (TBDMS) group to afford 2-benzylthio-1-(2-deoxy-5-*O*-*tert*-butyldiphenylsilyl-*β*-D-*erythro*-pentofuranosyl)-5,6-dichloroindole (**12**). A subsequent mesylation of the 3′-hydroxyl group of **12** afforded 2-benzylthio-1-(2-deoxy-5-*O*-*tert*-butyldiphenylsilyl-3-*O*-mesyl-*β*-D-*erythro*-pentofuranosyl)-5,6-dichloroindole (**14**). The elimination procedure that was employed by Seela[17] did not yield the desired nucleoside. However, we found that 2-benzylthio-5,6-dichloro-1-(2,3-dideoxy-*β*-D-*glycero*-pent-2-enofuranosyl)indole (**16**) can be isolated in a moderate yield by treating **14** with potassium *tert*-butoxide in DMSO at room temperature. The dihydroxylation procedure using osmium tetraoxide-NMO conditions occurred on the less hindered α-face of the 2′,3′-didehydronucleoside **16** and provided the targeted 2-benzylthio-1-(*β*-D-ribofuranosyl)-5,6-dichloroindole (**18**) stereoselectively. The 2-chloroindole analogue **19** was prepared using the same procedures as those described for the preparation of **18**. Instead of preparing the BDCRB analogue using this rather lengthy synthetic route, we

Scheme 3



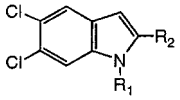
elected to reinvestigate the conditions necessary for a successful coupling of an indole sodium salt and a ribofuranosyl chloride. We selected 5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-α-D-ribofuranosyl chloride as the ribofuranosyl donor.[19] This ribofuranosyl chloride has been used in the sodium salt glycosylation reactions for the preparation of various other nucleosides. However, due to the increased steric hindrance at the coupling site as compared to 2-deoxy ribofuranosyl chloride, the glycosylation reaction of this ribofuranosyl chloride proceeded much slower and yields were variable.[11] In our case, the initial attempts were made with the indole aglycon **5**, which had a benzylthio substituent near the coupling site, and further increased the steric hindrance. This prompted us to change the heterocycle to 2,5,6-trichloroindole (**6**); however, once again very little glycosylation occurred even after the resulting mixture was stirred at room temperature for a prolonged period of time. Normally, the sodium salt glycosylation reaction is conducted at room temperature, because the ribofuranosyl α-chloride epimerizes at a high temperature.[11,20] However, due to the above results we felt that some forcing conditions might be necessary to effect the desired glycosylation. When the reaction mixture was heated at reflux temperature for 2 h (ca. 80 °C) (Scheme 3), 1-(5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)-2,5,6-trichloroindole (**20**) and its α-anomer were isolated in a 15% combined yield and a 4:1 ratio. It was most likely that the ribofuranosyl α-chloride epimerized to its thermodynamically more stable *β*-anomer at the higher temperature and led to the formation of the indole α-nucleoside. To minimize the epimerization of the ribofuranosyl chloride, the reaction temperature was lowered to 60 °C. At this temperature, the combined yield was increased to 60% yield. However, the stereoselectivity was still in a 4:1 ratio for *β*- vs α-anomers. A second possible pathway for the formation of an α-nucleoside was through a ribofuranosyl oxonium ion. If the formation of this oxonium ion could be inhibited, the stereoselectivity of the glycosylation reaction would be improved. Indeed, when the nonpolar solvent toluene was used in combination with THF in the glycosylation reaction, only a trace amount of the α-anomer of **20** was observed. Moreover, the yield was increased to 73% for the chloro analogue **20**. We then used 2-bromo-5,6-dichloroindole (**7**) as the aglycon and obtained a 92% yield for the bromo analogue **21**. This simple modification has expanded the scope of the sodium salt glycosylation method to some of the less reactive heterocycles.

When compound **20** was deprotected with a strong acid such as trifluoroacetic acid (TFA) to remove both the 5′-silyl and the 2′,3′-isopropylidene groups in one step, 5,6-dichloro-1-(*β*-D-ribofuranosyl)indol-2-one (**22**) was isolated as the major product rather than the desired TCRB analogue (**19**). This prompted us to use a two-step deprotecting sequence, i.e., TBAF at room temperature then aqueous acetic acid at 60 °C, which afforded our desired target compound **19** in a good yield. Although using acetic acid alone may cleave both protecting groups in one step, we found that a separate TBAF step enabled us to purify the indole nucleosides more extensively and led to the isolation of a cleaner product. The BDCRB analogue 2-bromo-5,6-dichloro-1-(*β*-D-ribofuranosyl)indole (**25**) was prepared using the same procedure as that described for compound **19**. In general, the 2-bromoindole compounds behaved similarly to their 2-chloroindole analogues, although they were found to be relatively more stable. The anomeric configurations of compounds **20**, **21**, **23**, and **24** were established as *β* by their ^{1}H NMR spectral data.[21] The $\Delta\delta$ values of **23** and **24** agree with the Imbach rule[21] ($\Delta\delta$ for *β*-anomer > 0.15 ppm and the α-anomers' $\Delta\delta$ < 0.15 ppm). The successful synthesis of the target compound **19** via this direct method also proved the stereochemistry of the indole nucleosides **18** and **19** prepared by the previous route described in this manuscript.

Biology. Selected compounds were evaluated for activity against two or three herpes viruses and for cytotoxicity. The unsubstituted heterocycle **7** was active against HCMV and HSV-1 but at concentrations similar to those that produced cytotoxicity in uninfected cells (Table 1). A similar pattern of antiviral activity poorly separated from cytotoxicity also was noted with the 2′-deoxy analogue **10**. This same pattern also had been observed with the 2-benzylthio-5,6-dichlorobenzimidazole ribonucleoside.[2]

In contrast, the two 2-halogen-substituted indole D-ribonucleosides **19** and **25** were as active against

Table 1. Antiviral Activity and Cytotoxicity of 2-Substituted-5,6-dichloro Indole *N*-Nucleosides



			50 or 90% inhibitory concentration (μM)				
			antiviral activity				
			HCMV[a]		HSV-1[b]	cytotoxicity[c]	
no.	R_1	R_2	plaque	yield	ELISA	visual	growth
7	H	Br	14		70	32	70
10	2-D-ribose	SBn	32		40	32	60
19	ribose	Cl	42	50	>100[d]	>100	>100
25	ribose	Br	18	42	>100	100	>100
TCRB[e]	ribose	Cl	2.9	1.4	102	238	210
foscarnet[f]			39 ± 26			>100	
ganciclovir (DHPG)[g]			7.4 ± 6.5	1.6 ± 1.2	3.5 ± 2.1	>100	>100

[a] Plaque and yield reduction assays were performed in duplicate as described in the text. Results from plaque assays are reported as IC_{50}'s, those for yield reduction experiments as IC_{90}'s. [b] A plaque assay was used to determine the activity of DHPG against HSV-1; all other compounds were assayed by ELISA in quadruplicate wells. [c] Visual cytotoxicity was scored on HFF cells at time of HCMV plaque enumeration. Inhibition of KB cell growth was determined as described in the text in quadruplicate assays. Results are presented as IC_{50}'s. [d] >100 indicates IC_{50} or IC_{90} greater than the noted (highest) concentration tested. [e] Data for TCRB published previously as compound **9** in ref 1. [f] Average ± standard deviation from 15 experiments. [g] Average ± standard deviation from 108, 33, and 3 experiments, respectively.

HCMV in both plaque and yield assays as compounds **7** and **10** but they were not cytotoxic at 100 μM, the highest concentration tested (Table 1). Both were inactive against HSV-1, and compound **19** was inactive against human herpes virus six (HHV-6) as well.

The activity of the latter two compounds is similar to what we have observed with the corresponding benzimidazole D-ribonucleosides, TCRB and BDCRB,[1,5] albeit at less efficacious concentrations. The observation that the addition of ribose to the indole heterocycle **7** to give a less cytotoxic nucleoside also is similar to what we observed with the benzimidazoles. In contrast to the benzimidazoles, the addition of ribose did not increase activity against HCMV.[1] We conclude that although the indole *N*-ribonucleosides are as active against HCMV as foscarnet, their lower activity compared to TCRB, BDCRB, and ganciclovir (Table 1) most likely precludes their further development as antiviral drugs.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and were used as provided. Acetonitrile (calcium hydride), dichloromethane (phosphorus pentoxide), toluene (phosphorus pentoxide), dimethylformamide (calcium oxide), and tetrahydrofuran (sodium/benzophenone) were distilled from the indicated drying agent and stored under a positive pressure of argon prior to use (if not used immediately). The phrases "evaporated in vacuo" and "concentrated" were meant to imply the use of a rotary evaporator with a bath temperature not exceeding 40 °C using a water aspirator. Thin-layer chromatography (TLC) was carried out on Analtech 60F-254 silica gel plates, and detection of components on TLC was made by UV light absorption at 254 nm and 365 nm, staining with iodine vapor, or heating to a char following treatment with 10% sulfuric acid in methanol. Solvent systems are expressed as a percentage of the more polar component with respect to total volume (v/v%). Flash column chromatography refers to the chromatography technique described by Still (*J. Org. Chem.* **1978**, *43*, 2923−2925). The notation "X% EtOAc/Hex, Y × Z cm" indicates the solvent system that was used in the column, the diameter of the column size, and the height of silica gel. Mallinckrodt SilicAR 230−400 mesh (40−63 μm) was used for chromatography. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. The ^{1}H (300, 360, or 500 MHz) and ^{13}C (67.5, 90, or 125 MHz) NMR spectra were recorded on Bruker instruments. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as an internal standard or the standard chemical shift of the solvents for ^{1}H NMR, and they are expressed (ppm) relative to the standard chemical shift of the solvent for ^{13}C NMR. The coupling constant values are expressed in hertz. Mass spectroscopy and elemental analyses were performed by the University of Michigan Chemistry Department.

4,5-Dichloro-2-nitrophenylacetic Acid, Methyl Ester (2). To a solution of 4,5-dichloro-2-nitrophenylacetic acid[12] (5.3 g, 21 mmol) in 70 mL of MeOH at room temperature, was added dropwise concentrated H_2SO_4 (4.2 mL) (exothermic!). The resulting yellow solution was heated at reflux temperature for 7 h. MeOH was evaporated in vacuo, and the resulting yellow oil was poured into ice-H_2O (ca. 70 mL). The mixture was extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with 70 mL of H_2O, 70 mL of saturated $NaHCO_3$, and 70 mL of brine. The organic layers were dried over $MgSO_4$ and concentrated to give a yellow syrup. This syrup was crystallized from EtOAc/hexane to give 4.6 g (82%) of compound **2** as a pale-yellow solid: mp 67−68 °C; R_f (20% EtOAc/Hex) = 0.40. ^{1}H NMR ($CDCl_3$): δ 3.74 (s, 3H), 4.01 (s, 2H), 7.49 (s, 1H), 8.28 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 39.2, 52.8, 127.5, 129.8, 133.1, 134.9, 138.6, 147.2, 169.8. Anal. ($C_9H_7Cl_2NO_4$) C, H, N.

5,6-Dichloroindol-2-one (3). A mixture of compound **2** (19.3 g, 73.1 mmol) and 1.0 g of PtO_2 in 150 mL of AcOH was shaken on a Parr apparatus under 40 psi H_2 for 20 h. The resulting black suspension was filtered through Celite, and the Celite was washed with 100 mL of AcOH. The filtrate was evaporated in vacuo to give an orange solid. This solid was recrystallized from EtOAc/MeOH to give 11.3 g (76%) of compound **3** as a pale-pink solid: mp 209−210 °C; R_f (40% EtOAc/Hex) = 0.30. ^{1}H NMR (DMSO-d_6): δ 3.48 (s, 2H), 6.93 (s, 1H), 7.41 (s, 1H), 10.6 (broad s, 1H, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 35.5, 110.5, 122.9, 126.2, 127.0, 129.6, 143.9, 176.0. Anal. ($C_8H_5Cl_2NO$) C, H, N.

5,6-Dichloroindole-2-thione (4). To a solution of compound **3** (2.0 g, 9.9 mmol) in 22 mL of THF was added P_2S_5 (2.2 g, 9.9 mmol). The mixture was stirred at room temperature for 30 min, and $NaHCO_3$ (2.8 g, 33 mmol) was then added in three portions. The resulting mixture was stirred at room temperature for 3 h. The solid in the mixture was removed by filtration, and the filtrate was concentrated to dryness. The residue was treated with 100 mL of ice-water and extracted

with $CHCl_3$ (3 × 60 mL). The combined $CHCl_3$ extracts were washed with 70 mL of brine. The organic layer was dried over Na_2SO_4 and concentrated to give a yellow solid. This solid was recrystallized from MeOH to give 1.5 g (69%) of compound **4** as a yellow solid. mp 165−166 °C; R_f (40% EtOAc/Hex) = 0.40. ^{1}H NMR ($CDCl_3$): δ 4.04 (s, 2H), 7.07 (s, 1H), 7.35 (s, 1H), 10.1 (broad s, 1H, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 48.3, 111.3, 126.0, 128.0, 130.0, 132.2, 143.5, 203.9. HRMS for $C_8H_5Cl_2NS$: Calcd, 216.9520; Found, 216.9521.

2-Benzylthio-5,6-dichloroindole (5). A mixture of compound **4** (640 mg, 2.94 mmol), benzyl bromide (420 mL, 3.50 mmol), and K_2CO_3 (480 mg, 3.50 mmol) in 10 mL of acetone was stirred at room temperature for 3.5 h. The solid in the mixture was removed by filtration, and the filtrate was concentrated to dryness. The residue was purified by flash column chromatography (30% EtOAc/Hex, 3 × 10 cm) to give 885 mg (98%) of compound **5** as a white solid: mp 141−142 °C. R_f (15% EtOAc/Hex) = 0.80. ^{1}H NMR ($CDCl_3$): δ 3.99 (s, 2H), 6.47 (m, 1H), 7.14−7.29 (m, 5H), 7.30 (s, 1H), 7.69 (broad s, 1H, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 41.5, 108.9, 111.9, 121.1, 124.2, 126.5, 127.6, 128.68, 128.72, 128.7, 130.9, 135.5, 137.8. HRMS for $C_{15}H_{11}Cl_2NS$: Calcd, 306.9989; Found, 306.9980.

2,5,6-Trichloroindole (6). To a suspension of compound **3** (10.4 g, 51.7 mmol) in 200 mL of 1,2-dichloroethane, was added $POCl_3$ (9.6 mL, 103 mmol) at room temperature. The resulting mixture was heated at reflux temperature in a 90 °C oil bath for 0.5 h (the reaction formed a copious amount of precipitate and an oil bath was preferred to a heating mantle, since it provided a gentle heating and avoided a darkening of the precipitate). The reaction was cooled just below reflux temperature, and imidazole (4.22 g, 62 mmol) was added in one portion. The resulting gummy suspension was heated at reflux temperature in an oil bath for another 2 h. The reaction was cooled to room temperature and 100 mL of ice-water was added. Solid $NaHCO_3$ (ca. 50 g) was added to the mixture until no further gas was evolved. The suspension was extracted with dichloromethane (4 × 100 mL), and the combined dichloromethane extracts were washed with 300 mL of brine. The dichloromethane extracts were filtered through a band of silica gel and concentrated to dryness to afford a crude product. The crude product was recrystallized from chloroform to give 5.41 g (47%) of compound **6** as a white solid. The filtrate was concentrated to dryness, and the residue was purified by flash column chromatography (30% EtOAc/Hex, 2 × 15 cm) to give an additional 2.6 g of desired product (23%, total: 70%): mp 129 °C (dec): R_f (20% EtOAc/Hex) = 0.55. ^{1}H NMR ($CDCl_3$): δ 6.38 (s, 1H), 7.42 (s, 1H), 7.61 (s, 1H), 8.12 (broad s, 1H, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 100.5, 111.9, 120.8, 124.9, 125.4, 126.2, 127.8, 133.6. HRMS for $C_8H_4Cl_3N$: Calcd, 218.9409; Found, 218.9409. Anal. ($C_8H_4Cl_3N$) C, H, N.

2-Bromo-5,6-dichloroindole (7). The procedure is the same as that described for **6**, except that $POBr_3$ (14.1 g, 49 mmol) was used instead of $POCl_3$ and more $NaHCO_3$ (×2) was needed to quench the reaction. An 80% yield (5.3 g) of **7** was obtained from **3** (5.0 g, 24.8 mmol) as an off-white solid: mp 115 °C (dec); R_f (20% EtOAc/Hex) = 0.55. ^{1}H NMR ($CDCl_3$): δ 6.49 (d, 1H, J = 0.8), 7.43 (s, 1H), 7.62 (s, 1H), 8.17 (broad s, 1H, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 105.0, 111.2, 112.2, 121.0, 125.2, 126.6, 128.7, 135.5. Anal. ($C_8H_4BrCl_2N$) C, H, N.

2-Benzylthio-1-[2-deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-5,6-dichloroindole (8). To a solution of compound **5** (100 mg, 0.32 mmol) in 10 mL of CH_3CN was added NaH (15 mg, 0.50 mmol, 80% in mineral oil) at room temperature. The resulting solution was stirred at room temperature for 10 min. 3,5-Bis-*O*-(4-methylbenzoyl)-D-*erythro*-pentofuranosyl α-chloride (150 mg, 0.39 mmol) was then added, and stirring was continued for 20 min. The reaction was quenched by adding several drops of acetic acid, and the resulting mixture was concentrated to dryness. The residue was purified by flash column chromatography (15% EtOAc/Hex, 2 × 10 cm) to give 194 mg (92%) of **8** as a white solid: mp 143−144 °C; R_f (20% EtOAc/Hex) = 0.60. ^{1}H NMR ($CDCl_3$): δ 1.85 (m, 1H), 2.40 (s, 3H), 2.44 (s, 3H), 2.81 (m, 1H), 3.96 (s, 2H), 4.38 (m, 1H), 4.73 (m, 2H), 5.63 (m, 1H), 6.50 (dd, 1H, J = 9.6, 5.5), 6.57 (s, 1H), 7.07−7.32 (m, 9H), 7.57 (s, 1H), 7.72 (s, 1H), 7.96 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 21.6, 21.7, 36.0, 42.6, 64.0, 73.8, 80.8, 85.2, 111.0, 113.5, 121.4, 124.9, 126.6, 126.8, 127.6, 128.1, 128.7, 129.3, 129.76. 129/78, 132.8, 134.6, 137.3, 144.0, 144.4, 165.9, 166.4. Anal. ($C_{36}H_{31}Cl_2NO_5S$) C, H, N.

1-[2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-D-*erythro*-pentofuranosyl]-2,5,6-trichloroindole (9). The procedure is the same as that described for **8**, except that **6** (1.8 g, 8.2 mmol) was used instead of **5**. A 92% yield (4.3 g, 7.5 mmol) of **9** was obtained as a white solid: mp 156−157 °C; R_f (20% EtOAc/Hex) = 0.60. ^{1}H NMR ($CDCl_3$): δ 2.41 (s, 3H), 2.44 (s, 3H), 2.50 (m, 1H), 3.00 (m, 1H), 4.53 (m, 1H), 4.79 (m, 2H), 5.72 (m, 1H), 6.43 (s, 1H), 6.47 (dd, 1H, J = 9.4, 5.7), 7.25 (m, 4H), 7.56 (s, 1H), 7.76 (s, 1H), 7.97 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 21.70, 21.73, 36.7, 63.9, 73.8, 81.4, 85.1, 102.0, 113.2, 121.0, 125.4, 126.4, 126.8, 127.4, 129.26, 129.30, 129.8, 133.0, 144.1, 144.5, 166.0, 166.4. Anal. ($C_{29}H_{24}Cl_3NO_5$) C, H, N.

2-Benzylthio-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6-dichloroindole (10). A mixture of compound **8** (194 mg, 0.29 mmol) and NaOMe (100 mg, 1.85 mmol) in 10 mL of MeOH was stirred at room temperature for 2 h. The reaction was quenched by adding several drops of acetic acid, and the resulting mixture was concentrated to dryness. The residue was purified by flash column chromatography (50% EtOAc/Hex, 2 × 10 cm) to give 112 mg (91%) of **10** as a white solid: mp 106−107 °C; R_f (50% EtOAc/Hex) = 0.30. ^{1}H NMR (DMSO-d_6): δ 1.85 (m, 1H), 2.45 (m, 1H), 3.70 (m, 2H), 3.82 (m, 1H), 4.15 (s, 2H), 4.41 (m, 1H), 5.16 (t, 1H, J = 4.8, D_2O exchangeable), 5.34 (d, 1H, J = 4.3, D_2O exchangeable), 6.47 (dd, 1H, J = 8.9, 5.8), 6.57 (s, 1H), 7.25 (m, 5H), 7.73 (s, 1H), 8.26 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 61.0, 70.1, 84.9, 86.9, 107.8, 114.5, 120.7, 122.9, 124.4, 127.4, 128.0, 128.5, 128.8, 133.6, 134.3, 136.9. Anal. ($C_{20}H_{19}Cl_2NO_3S$) C, H, N.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2,5,6-trichloroindole (11). The procedure is the same as that described for **10**, except that **9** (4.2 g, 7.3 mmol) was used instead of **8**. A 98% yield (2.4 g, 7.1 mmol) of **11** was obtained as a colorless foam: R_f (70% EtOAc/Hex) = 0.40. ^{1}H NMR (DMSO-d_6): δ 2.10 (m, 1H), 2.52 (m, 1H), 3.70 (m, 2H), 3.85 (m, 1H), 4.43 (m, 1H), 5.19 (t, 1H, J = 4.9, D_2O exchangeable), 5.39 (d, 1H, J = 4.4, D_2O exchangeable), 6.37 (dd, 1H, J = 8.9, 5.5), 6.67 (s, 1H), 7.78 (s, 1H), 8.34 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 61.0, 70.0, 84.7, 87.1, 101.3, 114.6, 120.8, 123.6, 124.5, 126.2, 127.2, 132.7. HRMS for $C_{13}H_{12}Cl_3NO_3$: Calcd, 334.9883; Found, 334.9874.

2-Benzylthio-1-(2-deoxy-5-*O*-*tert*-butyldiphenylsilyl-β-D-*erythro*-pentofuranosyl)-5,6-dichloroindole (12). To a solution of compound **10** (1.80 g, 4.24 mmol) in 25 mL of pyridine at 0 °C was added dropwise *tert*-butylchlorodiphenylsilane (1.26 mL, 4.84 mmol) in 30 min. The mixture was stirred at room temperature for 24 h, concentrated to dryness, and then coevaporated with toluene (2 × 20 mL). The residue was purified by flash column chromatography (20% EtOAc/Hex, 4 × 10 cm) to give 2.46 g (88%) of **12** as a colorless foam: R_f (50% EtOAc/Hex) = 0.60. ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 9H), 1.90 (m, 1H), 2.60 (m, 1H), 3.92−4.05 (m, 5H), 4.58 (m, 1H), 6.45 (s, 1H), 6.48 (d, 1H, J = 9.0), 7.07−7.43 (m, 11H), 7.47 (s, 1H), 7.55 (s, 1H),7.70 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 19.3, 27.0, 37.9, 42.0, 64.1, 72.5, 84.4, 84.7, 110.4, 113.1, 121.3, 124.6, 126.4, 127.5, 127.9, 128.1, 128.6, 128.8, 130.0, 132.8, 132.9, 133.1, 134.5, 135.6, 137.1. HRMS for $C_{36}H_{37}Cl_2NO_3SSi$: Calcd, 661.1640; Found, 661.1665.

1-(2-Deoxy-5-*O*-*tert*-butyldiphenylsilyl-β-D-*erythro*-pentofuranosyl)-2,5,6-trichloroindole (13). The procedure is the same as that described for **12**, except that **11** (2.2 g, 6.5 mmol) was used instead of **10**. A 90% yield (3.4 g, 5.9 mmol) of **13** was obtained as a colorless foam: R_f (25% EtOAc/Hex) = 0.40. ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 9H), 2.23 (m, 1H), 2.65 (m, 1H), 3.90−4.01 (m, 3H), 4.64 (m, 1H), 6.36 (d, 1H, J =

8.9), 6.39 (s, 1H), 7.44 (m, 6H), 7.54 (s, 1H), 7.55 (s, 1H), 7.70 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 19.3, 27.0, 38.4, 64.0, 72.3, 84.4, 85.2, 101.7, 113.0, 120.9, 125.2, 126.0, 126.7, 127.4, 127.9, 130.0, 132.77, 132.83, 133.0, 135.6. HRMS for $C_{29}H_{30}Cl_3NO_3Si$: $[M + NH_4]^+$ Calcd, 591.1404; Found, 591.1389.

2-Benzylthio-1-(2-deoxy-5-*O*-*tert*-butyldiphenylsilyl-3-*O*-mesyl-β-D-*erythro*-pentofuranosyl)-5,6-dichloroindole (14). To a solution of compound **12** (2.46 g, 3.71 mmol) in 60 mL of CH_2Cl_2 and 14 mL of pyridine at 0 °C was added MsCl (1.4 mL, 18 mmol). The mixture was stirred at room temperature for 4.5 h. After the addition of MeOH (12.4 mL) and further stirring for 20 min, the mixture was concentrated to dryness and coevaporated with toluene (2 × 15 mL). The residue was dissolved in 200 mL of CH_2Cl_2. The CH_2Cl_2 solution was washed with brine (50 mL), dried over Na_2SO_4, and concentrated to dryness. The residue was purified by flash column chromatography (20% EtOAc/Hex, 4 × 10 cm) to give 2.52 g (92%) of **14** as a colorless foam: R_f (40% EtOAc/Hex) = 0.40. ^{1}H NMR ($CDCl_3$): δ 1.12 (s, 9H), 1.99 (m, 1H), 2.70 (m, 1H), 2.99 (s, 3H), 3.92 (s, 2H), 3.97 (m, 2H), 4.14 (m, 1H), 5.39 (m, 1H), 6.42 (dd, 1H, J = 9.7, 5.6), 6.51 (s, 1H), 7.10−7.56 (m, 11H), 7.54 (s, 1H), 7.57 (s, 1H), 7.70 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 19.3, 27.1, 36.0, 38.4, 42.3, 62.8, 79.1, 82.9, 84.7, 111.0, 113.3, 121.4, 124.9, 126.8, 127.6, 127.89, 127.91, 128.0, 128.1, 128.71, 128.74, 130.00, 130.04, 132.4, 132.6, 133.0, 134.6, 135.6, 135.7, 137.0. HRMS for $C_{37}H_{39}Cl_2NO_5S_2Si$: Calcd, 739.1416; Found, 739.1425.

1-(2-Deoxy-5-*O*-*tert*-butyldiphenylsilyl-3-*O*-mesyl-β-D-*erythro*-pentofuranosyl)-2,5,6-trichloroindole (15). The procedure is the same as that described for **14**, except that **13** (3.3 g, 5.7 mmol) was used instead of **12**. A 91% yield (3.4 g, 5.2 mmol) of **15** was obtained as a colorless foam: R_f (25% EtOAc/Hex) = 0.30. ^{1}H NMR ($CDCl_3$): δ 1.12 (s, 9H), 2.50 (m, 1H), 2.80 (m, 1H), 3.01 (s, 3H), 4.00 (m, 2H), 4.25 (m, 1H), 5.46 (m, 1H), 6.34 (dd, 1H, J = 9.7, 5.4), 6.43 (d, 1H, J = 0.7), 7.44 (m, 6H), 7.57 (s, 1H), 7.60 (s, 1H), 7.70 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 19.3, 27.0, 36.7, 38.4, 62.7, 78.9, 83.4, 84.7, 102.1, 113.1, 121.0, 125.5, 126.4, 126.5, 127.4, 127.7, 127.89, 127.93, 130.0, 130.1, 132.3, 132.5, 133.0, 134.8, 135.6, 135.7. HRMS for $C_{30}H_{32}Cl_3NO_5SSi$: $[M + NH_4]^+$ Calcd, 669.1180; Found, 669.1165.

2-Benzylthio-5,6-dichloro-1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)indole (16). To a solution of compound **14** (1.00 g, 1.35 mmol) in 14 mL of DMSO was added *t*-BuOK (0.59 g, 5.25 mmol). The mixture was stirred at room temperature for 10 min, then treated with 100 mL of cold water, and extracted with EtOAc (2 × 100 mL). The combined extracts were washed with water (150 mL) and brine (150 mL), dried over Na_2SO_4, and concentrated to dryness. The residue was purified by flash column chromatography ($CHCl_3$, 2 × 10 cm) to give 370 mg (67%) of **16** as a pale-yellow foam: R_f (50% EtOAc/Hex) = 0.30. ^{1}H NMR (DMSO-d_6): δ 3.61 (m, 2H), 4.18 (s, 2H), 4.80 (m, 1H), 4.99 (t, 1H, J = 4.8, D_2O exchangeable), 6.01 (m, 1H), 6.51 (m, 1H), 6.64 (s, 1H), 7.02 (m, 1H), 7.25 (m, 5H), 7.75 (s, 1H), 8.04 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 42.0, 64.1, 86.2, 90.5, 111.1, 113.8, 121.2, 124.8, 126.4, 127.5, 128.3, 128.5, 128.8, 133.0, 133.9, 135.8, 137.1. HRMS for $C_{20}H_{17}Cl_2NO_2S$: Calcd, 405.0357; Found, 405.0338.

1-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-2,5,6-trichloroindole (17). The procedure is the same as that described for **16**, except that **15** (1.8 g, 2.7 mmol) was used instead of **14**. A 76% yield (0.66 g, 2.1 mmol) of **17** was obtained as a pale-yellow oil: R_f (2% MeOH/$CHCl_3$) = 0.25. ^{1}H NMR ($CDCl_3$): δ 3.87 (m, 2H), 4.96 (m, 1H), 6.24 (m, 1H), 6.44 (m, 2H), 7.01 (m, 1H), 7.56 (s, 1H), 7.88 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 64.0, 86.7, 90.3, 101.9, 113.7, 120.8, 125.4, 125.9, 127.4, 127.8, 128.0, 133.8, 134.0. HRMS for $C_{13}H_{10}Cl_3NO_2$: Calcd, 316.9777; Found, 316.9771.

2-Benzylthio-1-(β-D-ribofuranosyl)-5,6-dichloroindole (18). Compound **16** (1.27 g, 3.13 mmol) and *N*-methylmorpholine oxide (1.00 g, 8.51 mmol) were dissolved in 30 mL of acetone−water (8:1), and OsO_4 (3 mL, 0.30 mmol, 2.5 wt % in 2-methyl-2-propanol) was added. The mixture was stirred at room temperature for 20 h, 10% $Na_2S_2O_4$ (10 mL) was then added, and the stirring was continued for 15 min. The mixture was treated with 100 mL of water and extracted with EtOAc (2 × 150 mL). The combined extracts were washed with brine (100 mL), dried over Na_2SO_4, and concentrated to dryness. The residue was purified by flash column chromatography (60% EtOAc/Hex, 3 × 10 cm) to give 845 mg (61%) of **18** as a white solid: mp 139−140 °C; R_f (60% EtOAc/Hex) = 0.30. ^{1}H NMR (DMSO-d_6): δ 3.71 (m, 2H), 3.94 (m, 1H), 4.16 (m, 3H), 4.48 (m, 1H), 5.22 (d, 1H, J = 4.2, D_2O exchangeable), 5.30 (t, 1H, J = 4.5, D_2O exchangeable), 5.38 (d, 1H, J = 6.6, D_2O exchangeable), 6.15 (d, 1H, J = 7.8), 6.46 (s, 1H), 7.25 (m, 5H), 7.73 (s, 1H), 8.38 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 61.2, 69.7, 71.0, 85.3, 88.7, 109.1, 114.9, 120.8, 122.9, 124.6, 127.2, 127.7, 128.3, 129.0, 133.9, 134.6, 137.0. HRMS for $C_{20}H_{19}Cl_2NO_4S$: Calcd, 439.0412; Found, 439.0408. Anal. ($C_{20}H_{19}Cl_2NO_4S$) C, H, N.

2,5,6-Trichloro-(1-β-D-ribofuranosyl)indole (19). Method I. The procedure is the same as that described for **18**, except that **17** (0.65 g, 2.0 mmol) was used instead of **16**. A 84% yield (0.59 g, 1.7 mmol) of **19** was obtained as a white solid: mp 135 °C (dec); R_f (60% EtOAc/Hex) = 0.30. ^{1}H NMR (DMSO-d_6): δ 3.70 (m, 2H), 3.94 (m, 1H), 4.13 (m, 1H), 4.43 (m, 1H), 5.23 (d, 1H, J = 4.5, D_2O exchangeable), 5.32 (m, 2H, D_2O exchangeable), 5.88 (d, 1H, J = 7.7), 6.68 (s, 1H), 7.80 (s, 1H), 8.42 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 61.2, 69.6, 71.2, 85.7, 88.5, 101.3, 114.8, 120.8, 123.6, 124.5, 127.3, 132.9. Anal. ($C_{13}H_{12}Cl_3NO_4$) C, H, N.

Method II. Compound **19** may also be prepared from compound **23**. The procedure is the same as that described for **25**, except that **23** (5 mg, 0.013 mmol) was used instead of **24**. A 54% yield (2.4 mg, 0.007 mmol) of **19** was obtained as a white solid: mp 135 °C (dec).

1-(5-*O*-*tert*-Butyldimethylsilyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-2,5,6-trichloroindole (20). The procedure is the same as that described for **21**, except that **6** (1.6 g, 7.3 mmol) was used instead of **7**. A 73% yield (2.7 g, 5.3 mmol) of **20** was obtained as a white foam: R_f (10% EtOAc/Hex) = 0.70. ^{1}H NMR ($CDCl_3$): δ 0.16 (2s, 6H), 0.96 (s, 9H), 1.38 (s, 3H), 1.65 (s, 3H), 3.98 (m, 2H), 4.11 (m, 1H), 5.00 (m, 2H), 6.07 (d, 1H, J = 4.1), 6.45 (s, 1H), 7.60 (s, 1H), 7.61 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 19.0, 25.8, 26.4, 27.7, 62.8, 79.4, 82.5, 84.1, 90.0, 102.2, 113.1, 116.0, 121.3, 125.8, 126.7, 127.1, 127.7, 133.5. HRMS for $C_{22}H_{30}Cl_3NO_4Si$: Calcd, 505.1010; Found, 505.1009.

2-Bromo-1-(5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5,6-dichloroindole (21). Compound **7** (625 mg, 2.36 mmol) was dissolved in toluene (25 mL), and NaH (118 mg, 2.95 mmol, 60% in mineral oil) was then added at room temperature. The resulting brown suspension was stirred at room temperature for 1 h, and the preprepared 5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-D-ribofuranosyl α-chloride (4.9 mmol, in 16 mL of THF, not purified)[19] was then added at room temperature to give a brown solution. This brown solution was stirred at 70 °C for 16 h. The reaction mixture was cooled to room temperature and quenched with 10 mL of H_2O dropwise. The resulting mixture was concentrated first to remove most of the organic solvents and then extracted with EtOAc (3 × 20 mL). The combined EtOAc extracts were washed with 30 mL of brine and dried over $MgSO_4$. The organic layer was concentrated to dryness. The residue was purified by flash column chromatography (5% EtOAc/Hex, 3 × 10 cm) to give 133 mg (21%) of compound **7** and 950 mg (92%, based on recovered starting material **7**) of compound **21** as a white solid: mp 88−89 °C; R_f (10% EtOAc/Hex) = 0.70. ^{1}H NMR ($CDCl_3$): δ 0.16 (2s, 6H), 0.96 (s, 9H), 1.36 (s, 3H), 1.65 (s, 3H), 3.96 (m, 2H), 4.12 (m, 1H), 4.98 (m, 2H), 6.08 (d, 1H, J = 4.4), 6.55 (s, 1H), 7.59 (s, 1H), 7.62 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 18.3, 25.2, 25.8, 27.0, 62.2, 78.8, 81.7, 83.3, 90.8, 105.7, 112.5, 113.8, 115.4, 120.5, 125.1, 125.9, 128.2, 133.5. Anal. ($C_{22}H_{30}BrCl_2NO_4Si$) C, H, N.

5,6-Dichloro-1-(β-D-ribofuranosyl)indol-2-one (22). Compound **20** (130 mg, 0.33 mmol) was dissolved in 2 mL of TFA−water (9:1). The resulting solution was stirred at room temperature for 30 min, and then concentrated to dryness to give a colorless oil. This oil was purified by flash column

chromatography (from 30% EtOAc/Hex to 6% MeOH/EtOAc, 2 × 10 cm) to give 61 mg (55%) of compound **22** as a white solid: mp 132−133 °C; R_f (10% MeOH/EtOAc) = 0.50. ^{1}H NMR (DMSO-d_6): δ 3.60 (s, 2H), 3.68 (m, 2H), 3.84 (m, 1H), 4.06 (m, 1H), 4.36 (m, 1H), 5.09 (broad s, 1H, D_2O exchangeable), 5.19 (broad s, 2H, D_2O exchangeable), 5.67 (dd, 1H, J = 7.6, 1.9), 7.54 (s, 1H), 7.88 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 35.9, 62.0, 69.2, 70.6, 85.6, 85.8, 114.3, 124.9, 126.6, 126.8, 130.6, 142.9, 175.3. Anal. ($C_{13}H_{13}Cl_2NO_5$) C, H, N.

1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-2,5,6-trichloroindole (23). The procedure is the same as that described for **24**, except that **20** (1.4 g, 2.8 mmol) was used instead of **21**. A 73% yield (0.80 g, 2.0 mmol) of **23** was obtained as a white solid: mp 57−60 °C; R_f (20% EtOAc/Hex) = 0.40. ^{1}H NMR ($CDCl_3$): δ 1.39 (s, 3H), 1.66 (s, 3H), 4.00 (m, 2H), 4.71 (m, 1H), 5.12 (m, 2H), 6.13 (d, 1H, J = 4.0), 6.46 (s, 1H), 7.60 (s, 1H), 7.73 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 25.8, 27.7, 62.4, 79.6, 83.0, 84.2, 90.4, 102.6, 113.1, 116.2, 121.5, 125.9, 126.9, 127.1, 127.7, 133.8. Anal. ($C_{16}H_{16}Cl_3NO_4$) C, H, N.

2-Bromo-5,6-dichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (24). To a solution of compound **21** (0.95 g, 1.7 mmol) in 5 mL of THF was added TBAF (1.7 mL, 1.0 M solution in THF) at room temperature. The resulting mixture was stirred at room temperature for 10 min, and then concentrated to dryness. The residue was subjected to flash column chromatography (from 20% EtOAc/Hex to 30% EtOAc/Hex, 3 × 10 cm) to give 615 mg (81%) of compound **24** as a white solid: mp 55−57 °C; R_f (20% EtOAc/Hex) = 0.40. ^{1}H NMR ($CDCl_3$): δ 1.38 (s, 3H), 1.66 (s, 3H), 4.00 (m, 2H), 4.18 (m, 1H), 5.10 (m, 2H), 6.14 (d, 1H, J = 4.1), 6.57 (s, 1H), 7.61 (s, 1H), 7.78 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 25.0, 26.9, 61.6, 78.8, 82.1, 83.3, 91.0, 105.9, 112.4, 113.5, 115.4, 120.5, 125.1, 126.0, 128.1, 133.6. Anal. ($C_{16}H_{16}BrCl_2NO_4$) C, H, N.

2-Bromo-5,6-dichloro-1-(β-D-ribofuranosyl)indole (25). Compound **24** (440 mg, 1.01 mmol) was dissolved in 5 mL of AcOH/H_2O (4:1). The resulting solution was heated at 60 °C for 36 h and then concentrated to dryness. The residue was subjected to flash column chromatography (from 60% EtOAc/Hex to 80% EtOAc/Hex, 2 cm × 10 cm) to give 230 mg (58%) of compound **25** as a white solid: mp 88−90 °C (dec); R_f (60% EtOAc/Hex) = 0.30. ^{1}H NMR (DMSO-d_6): δ 3.70 (m, 2H), 3.94 (m, 1H), 4.13 (m, 1H), 4.44 (m, 1H), 5.22 (d, 1H, J = 4.4, D_2O exchangeable), 5.33 (m, 2H, D_2O exchangeable), 5.89 (d, 1H, J = 7.7), 6.75 (s, 1H), 7.80 (s, 1H), 8.44 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 61.2, 69.7, 71.2, 85.7, 90.0, 105.3, 114.8, 115.9, 120.6, 123.6, 124.4, 128.5, 133.6. Anal. ($C_{13}H_{12}BrCl_2NO_4 \cdot 0.25C_6H_6$) C, H, N.

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.[22]

Virological Procedures. The Towne strain, plaque-purified isolate P_o, of HCMV was kindly provided by Dr. Mark Stinski, University of Iowa. The KOS strain of HSV-1 was used in most experiments and was provided by Dr. Sandra K. Weller, University of Connecticut. Stock HCMV was prepared by infecting HFF cells at a multiplicity of infection (moi) of <0.01 plaque-forming units (pfu) per cell as detailed previously.[23] High titer HSV-1 stocks were prepared by infecting KB cells at an moi of <0.1 also as detailed previously.[23] 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.[24] Briefly, HFF or BSC-1 cells were planted as described above in 96-well cluster dishes and incubated overnight at 37 °C. The next day cultures were inoculated with HCMV or HSV-1 and serially diluted 1:3 across the remaining 11 columns of the 96-well plate. After virus adsorption, the inoculum was replaced with fresh medium, and cultures were incubated for 7 days for HCMV and 2 or 3 days for HSV-1. Plaques were enumerated under 20-fold magnification in wells having the dilution which gave 5−20 plaques per well. Virus titers were calculated according to the following formula: Titer (pfu/mL) = number of plaques × 5 × 3^n; where n represents the nth dilution of the virus used to infect the well in which plaques were enumerated.

HCMV Plaque Reduction Assay. HFF cells in 24-well cluster dishes were infected with approximately 100 pfu of HCMV per cm^2 cell sheet using the procedures detailed above. Following virus adsorption, compounds dissolved in growth medium were added to duplicate wells in four to eight selected concentrations. After incubation at 37 °C for 7 days, cell sheets were fixed and stained with crystal violet, and microscopic plaques were enumerated as described above. Drug effects were calculated as a percentage of reduction in the number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug.

HCMV Yield Assay. HFF cells were planted as described above in 96-well cluster dishes and incubated overnight, medium was removed, and the cultures were inoculated with HCMV at a moi of 0.5−1 pfu per cell as reported elsewhere.[24] After virus adsorption, inoculum was replaced with 0.2 mL of fresh medium containing test compounds. The first row of 12 wells was left undisturbed and served as virus controls. Each well in the second row received an additional 0.1 mL of medium with test compound at three times the desired final concentration. The contents of the 12 wells were mixed by repeated pipetting and then serially diluted 1:3 along the remaining wells. In this manner, six compounds could be tested in duplicate on a single plate with concentrations from 100 μM to 0.14 μM. Plates were incubated at 37 °C for 7 days and subjected to one cycle of freezing and thawing; aliquots from each of the eight wells of a given column were transferred to the first column of a fresh 96-well monolayer culture of HFF cells. Contents were mixed and serially diluted 1:3 across the remaining 11 columns of the secondary plate. Each column of the original primary plate was diluted across a separate plate in this manner. Cultures were incubated, plaques were enumerated, and titers calculated as described above.

HSV-1 ELISA. An ELISA was employed[25] to detect HSV-1. Ninety-six-well cluster dishes were planted with 10 000 BSC-1 cells per well in 200 mL per well of MEM(E) plus 10% calf serum. After overnight incubation at 37 °C, selected drug concentrations in quadruplicate and HSV-1 at a concentration of 100 pfu/well were added. Following a 3 day incubation at 37 °C, medium was removed, plates were blocked and rinsed, and 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 mL 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.

HHV-6. An ELISA was performed using covalent amine plates (Costar, Cambridge, MA). The plates were activated by the addition of a homobifunctional cross-linking agent, bis-(sulfosuccinimidyl) suberate, and then washed with PBS. Samples consisting of 150 μL of suspended HSB_2 cells infected with HHV-6 strain GS (obtained through the courtesy of Dr. Robert C. Gallo, NIH) and previously incubated with selected concentrations of drug on a separate plate were solubilized in Triton X-100 in coating buffer. The plate was covered and incubated for 1 h at 37 °C in a 5% CO_2 atmosphere. These binding conditions facilitated covalent attachment of the antigen to the free end of the cross-linker.

After covalent binding, the antigen solution was decanted, and the plate was washed four times in HEPES buffered saline with 0.05% Tween 20 (HBS-T), soaking for 3 min for each wash. Unbound sites on the plate were blocked, the blocker was decanted, and diluted primary monoclonal antibody, specific for HHV-6 (GS) glycoprotein gp116, was added. The

plate was covered and incubated for 1 h at 37 °C. The plate was washed again, blocker again added, and horseradish peroxidase-labeled rabbit anti-mouse antibody added to each well. The plate was incubated for 1 h at 37 °C, washed again as described above, and developed using TMB-Turbo (Pierce, Rockford, IL) for 30 min at room temperature. The reaction was stopped with 2 M H_2SO_4. Absorbance in each well was determined at 450/570 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. (*i*) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[23] (*ii*) The effect of compounds during two population doublings of KB cells was determined by crystal violet staining and spectrophotometric quantitation of dye eluted from stained cells as described earlier.[26] 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 were 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 linearly regressing the percent inhibition of parameters derived in the preceding assays (except for yield experiments) against log drug concentrations. For yield experiments, the log of viral titer was plotted against the log drug concentration. Fifty percent inhibitory concentrations (IC_{50}'s) and 90% inhibitory concentrations (IC_{90}'s, yield experiments) were calculated from the linear portions of the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, foscarnet for HHV-6, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

Acknowledgment. We thank Ms. Julie M. Breitenbach and Mr. Roger G. Ptak for their expert performance of antiviral and cytotoxicity assays and Ms. Kimberly J. Barrett for her assistance with the preparation of this manuscript. These studies were supported by Research Grants U19-AI31718 and PO1-AI46390 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

References

(1) Townsend, L. B.; Devivar, R. V.; Turk, S. R.; Nassiri, M. R.; Drach, J. C. Design, Synthesis, and Antiviral Activity of Certain 2,5,6-Trihalo-1-(β-D-ribofuranosyl)benzimidazoles. *J. Med. Chem.* **1995**, *38*, 4098−4105.

(2) Devivar, R. V.; Kawashima, E.; Revankar, G. R.; Breitenbach, J. M.; Kreske, E. D.; Drach, J. C.; Townsend, L. B. Benzimidazole Ribonucleosides: Design, Synthesis, and Antiviral Activity of Certain 2-(Alkylthio)- and 2-(Benzylthio)-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazoles. *J. Med. Chem.* **1994**, *37*, 2942−2949.

(3) Drach, J. C.; Townsend, L. B.; Biron, K. K.; Kern, E. R. Selective Inhibition of Human Cytomegalovirus by Polyhalogenated Benzimidazole Ribonucleosides, a New Class of Antivirals. 32nd Interscience Conf. Antimicrob. Agents Chemother., Session #6, Symposium: New Antiviral Agents. Anaheim, CA. October, 1992.

(4) Underwood, M. R.; Harvey, R. J.; Stanat, S. C.; Hemphill, M. L.; Miller, T.; Drach, J. C.; Townsend, L. B.; Biron, K. K. Inhibition of Human Cytomegalovirus DNA Maturation by a Benzimidazole Ribonucleoside is Mediated Through the UL89 Gene Product. *J. Virol.* **1998**, *72*, 717−725.

(5) Krosky, P. M.; Underwood, M. R.; Turk, S. R.; Feng, K. W.-H.; Jain, R. K.; Ptak, R. G.; Westerman, A. C.; Biron, K. K.; Townsend, L. B.; Drach, J. C. Resistance of Human Cytomegalovirus to Benzimidazole Nucleoside Analogues Maps to Two Open Reading Frames: UL89 and UL56. *J. Virol.* **1998**, *72*, 4721−4728.

(6) Zou, R.; Ayres, K. R.; Drach, J. C.; Townsend, L. B. Synthesis and Antiviral Evaluation of Certain Disubstituted Benzimidazole Ribonucleosides. *J. Med. Chem.* **1996**, *39*, 3477−3482.

(7) Zou, R.; Drach, J. C.; Townsend, L. B. Design, Synthesis, and Antiviral Evaluation of 2-Chloro-5,6-dihalo-1-β-D-ribofuranosylbenzimidazoles as Potential Agents for Human Cytomegalovirus Infections. *J. Med. Chem.* **1997**, *40*, 811−818.

(8) Zou, R.; Drach, J. C.; Townsend, L. B. Design, Synthesis, and Antiviral Evaluation of 2-Substituted 4,5-Dichloro- and 4,6-Dichloro-1-β-D-ribofuranosylbenzimidazoles as Potential Agents for Human Cytomegalovirus Infections. *J. Med. Chem.* **1997**, *40*, 802−810.

(9) Gudmundsson, K. S.; Drach, J. C.; Wotring, L. L.; Townsend, L. B. Synthesis and Antiviral Activity of Certain 5′-Modified Analogues of 2,5,6-Trichloro-1-(β-D-ribofuranosyl)benzimidazole. *J. Med. Chem.* **1997**, *40*, 785−793.

(10) Preobrazhenskaya, M. N.; Vigdorchik, M. M.; Suvorov, N. N. Glycosylindoles-VII: Synthesis of 1-(D-β-ribofuranosyl)indole. *Tetrahedron* **1967**, *23*, 4653−4660.

(11) Revankar, G. R.; Robins, R. K. Use of the Sodium Salt Glycosylation Method in Nucleoside Synthesis. *Nucleosides Nucleotides* **1989**, *8*, 709−724.

(12) Nodiff, E. A.; Saggiomo, A. J.; Shinbo, M.; Chen, E. H.; Otomasu, H.; Kondo, Y.; Kikuchi, T.; Verma, B. L.; Matsuura, S.; Tanabe, K.; Tyagi, M. P.; Morosawa, S. Antimalarial Phenanthrene Amino Alcohols. 2. Trifluromethyl-Containing 9-Phenanthrenemethanols. *J. Med. Chem.* **1972**, *15*, 775−780.

(13) Powers, J. C. Chloroindoles. *J. Org. Chem.* **1966**, *31*, 2627−2631.

(14) Brennan, M. R.; Erickson, K. L.; Szmalc, F. S.; Tansey, M. J.; Thornton, J. M. The Preparation and Spectral Characterization of 2-Haloindoles, 3-Haloindoles, and 2,3-Dihaloindoles. *Heterocycles* **1986**, *24*, 2879−2885.

(15) Bergman, J.; Venemalm, L. Efficent Synthesis of 2-Chloro-, 2-Bromo-, and 2-Iodoindole. *J. Org. Chem.* **1992**, *57*, 2495−2497.

(16) Girgis, N. S.; Cottam, H. B.; Robins, R. K. Synthesis of 2′-Deoxyribofuranosyl Indole Nucleosides Related to the Antibiotics SF-2140 and Neosidomycin. *J. Heterocycl. Chem.* **1988**, *25*, 361−366.

(17) Seela, F.; Bourgeois, W. Synthesis of Indole 2′,3′-Dideoxyribonucleosides. *Synthesis* **1990**, 945−950.

(18) Preobrazhenskaya, M. N.; Korbukh, I. A. The Synthesis and Reactions of Pyrrole, Pyrazole, Triazole, Indole, Indazole, and Benzotriazole Nucleosides and Nucleotides In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1994; Vol. 3, p 1−105.

(19) Rosemeyer, H.; Seela, F. Stereoselective Synthesis of Pyrrolo-[2,3-*d*]pyrimidine α- and β-D-Ribonucleosides from Anomerically Pure D-Ribofuranosyl Chlorides: Solid−Liquid Phase-Transfer Glycosylation and ^{15}N NMR Spectra. *Helv. Chim. Acta* **1988**, *71*, 1573−1585.

(20) Wilcox, C. S.; Otoski, R. M. Seteroselective Preparations of Ribofuranosyl Chlorides and Ribofuranosyl Acetates. Solvent Effects and Stereoselectivity in the Reaction of Ribofuranosyl Acetates with Trimethylallylsilane. *Tetrahedron Lett.* **1986**, *27*, 1011−1014.

(21) Chu, C. K.; El-Kabbani, F. M.; Thompson, B. B. Determination of the Anomeric Configuration of *C*-Nucleosides by ^1H and ^{13}C NMR Spectroscopy. *Nucleosides Nucleotides* **1984**, *3*, 1−31 and references therein.

(22) Shipman, C., Jr.; Smith, S. H.; Carlson, R. H.; Drach, J. C. Antiviral Activity of Arabinofuranosyladenine and Arabinofuranosylhypoxanthine in Herpes Simplex Virus-Infected KB Cells. I. Selective Inhibition of Viral DNA Synthesis in Synchronized Suspension Cultures. *Antimicrob. Agents Chemother.* **1976**, *9*, 120−127.

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

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

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

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

JM990320X