Synthesis, Antiviral Activity, and Mode of Action of Some 3-Substituted 2,5,6-Trichloroindole 2′- and 5′-Deoxyribonucleosides

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A series of chlorinated indole nucleosides has been synthesized and tested for activity against human cytomegalovirus (HCMV) and herpes simplex virus type-1 (HSV-1) and for cytotoxicity. The 2′- and 5′-deoxy derivatives of the reported 3-formyl-2,5,6-trichloro-1-(β-D-ribofuranosyl)-indole (FTCRI) and 3-cyano-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (CTCRI) were synthesized by either a modification of the appropriate 3-unsubstituted sugar-modified nucleoside analogues or by a glycosylation of 3-substituted heterocycles with a protected α-chlorosugar. The modifications were guided in part by structural similarity to the corresponding series of chlorinated benzimidazole ribonucleosides and the fact that 5′-deoxy analogues of 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB) are very active against HCMV. The 5′-deoxy analogues of FTCRI and CTCRI were nearly as active as FTCRI and CTCRI, suggesting that the chlorinated benzimidazole nucleosides and the chlorinated indole nucleosides act in a similar manner. This hypothesis was supported by time-of-addition studies using FTCRI and by the resistance of TCRB-resistant strains of HCMV to four different 3-substituted indole ribonucleosides. The 2′-deoxy analogues of the trichlorinated indole nucleosides also had potent antiviral activity, in contrast to decreased activity and selectivity observed for 2′-deoxy TCRB compared to TCRB. In addition, 3-acetyl-2,5,6-trichloro-1-(2-deoxy-β-D-ribofuranosyl)indole was also active and much less cytotoxic (HCMV $IC_{50} = 0.30\ \mu M$, HFF $CC_{50} > 100\ \mu M$) than previous analogues. None of the analogues had significant activity against HSV-1.

Introduction

Human cytomegalovirus (HCMV) is an opportunistic pathogen that is endemic in both industrialized and developing nations.[1] It is estimated that 50% of the American public is seropositive for HCMV.[2] Although HCMV poses little risk to healthy individuals, a variety of immunocompromised populations are susceptible to HCMV-related pathologies. AIDS patients, for example, are susceptible to retinitis and gastritis, transplant recipients are susceptible to organ rejection, and neonates are at risk for a host of birth defects and developmental disorders.[1,3]

There are currently five FDA-approved drugs available for the treatment of HCMV, namely, ganciclovir (CGV),[4] valganciclovir,[5] cidofovir,[6] foscarnet,[7] and fomivirsen.[8] All of these compounds suffer limitations, however, including poor bioavailability and toxicity. Furthermore, all of the licensed compounds (with the exception of fomivirsen) act upon the viral DNA polymerase, making the emergence of new drug-resistant viral strains more likely.

The search for new compounds with fewer or less severe limitations has led our laboratory to synthesize a wide range of nucleoside analogues, including 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB, **1**, Figure 1).[9] Although this compound demonstrated excellent antiviral activity and selectivity in vitro, it is degraded (via glycosidic bond cleavage) too rapidly in vivo to be of interest as a clinical candidate.[10] Further investigations have led to the syntheses of numerous TCRB analogues with stabilized glycosidic bonds,[11−13] including the clinical candidate maribavir.[14]

We recently reported that certain indole nucleosides including 3-formyl-2,5,6-trichloro-1-(β-D-ribofuranosyl)-indole (FTCRI, **2**, Figure 1) and 3-cyano-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (CTCRI, **3**, Figure 1) are potent and selective inhibitors of HCMV replication in vitro.[15] On the basis of the structural similarity between TCRB and FTCRI, we wanted to determine whether FTCRI shares the unique mode of action demonstrated by TCRB.[16]

Two virological lines of evidence can be used to support our hypothesis that TCRB, FTCRI, and CTCRI share a common mode of action. First, because TCRB exerts its inhibitory effect late in the viral lifecycle,[16] a time-of-addition study would be useful in determining whether this feature is shared with FCTRI and CTCRI. Second, several viral strains that are resistant to TCRB have been isolated and characterized in our laboratories.[17] If the analogues of FTCRI and CTCRI show reduced efficacy against these mutant virus strains, then the specific mutations found in the viruses would be implicated in the mode of action for the indole nucleosides as well.

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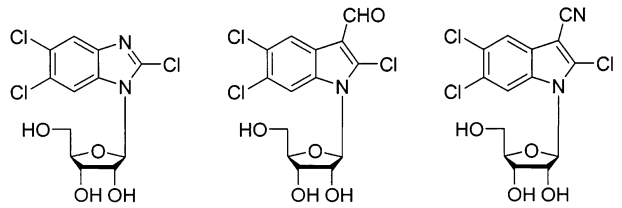


Figure 1. TCRB and analogous indole nucleosides.

We would also like to establish a structural line of evidence that the benzimidazole and indole nucleosides share a common pathway. It has been established that phosphorylation of TCRB at the 5′-position is not required for antiviral activity because the 5′-deoxy, 5′-deoxy-5′-fluoro, and 5′-deoxy-5′-azido analogues of TCRB are all as active as TCRB itself.[18−20] These hypothesized similarities between the benzimidazole and indole nucleosides prompted us to synthesize a series of 5′-deoxyindole nucleosides. If these 5′-deoxy FTCRI and CTCRI analogues retained their antiviral activity, this would strongly suggest that (as is the case for TCRB[21]) 5′-phosphorylation is not required for antiviral activity and would further strengthen our hypothesis that both chlorinated indole and benzimidazole nucleosides share a common mechanism of action.

In contrast to the 5′-deoxy analogue of TCRB, its 2′-deoxy analogue was not as active as the ribofuranosyl analogues.[22] This difference in antiviral activity prompted us to also synthesize the 2′-deoxy analogues of FTCRI and CTCRI in order to determine whether the same trend observed for 2′-deoxy TCRB would hold for these 2′-deoxyindole nucleosides.

Results and Discussion

Chemistry. For the desired series of 5′-deoxyindole nucleosides, two methodologies could be pursued: (1) modification of an appropriate sugar precursor followed by glycosylation and (2) modification of existing nucleoside precursors (with the glycosidic bond already established). The latter strategy was chosen for the synthesis of the target nucleoside analogues because it had the advantage of requiring fewer overall synthetic transformations.

To synthesize the desired 5′-deoxy analogues, the partially protected intermediate 2,5,6-trichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole[13] (**4**, Scheme 1) was tosylated at the 5′-position and this compound (**5**) was then reduced with sodium borohydride in DMSO[23] to provide the deoxygenated intermediate **6**. This nucleoside was either formylated under Vilesmeier−Haack[24] conditions using phosphorus oxychloride in DMF or cyanated with chlorosulfonyl isocyanate (CSI) followed by DMF[25] to provide the 3-substituted analogues **7a** and **7b**, respectively. These compounds were then deprotected with 90% aqueous trifluoroacetic acid to yield the desired 5′-deoxyindole nucleosides 3-formyl-2,5,6-trichloro-1-(5-deoxy-β-D-ribofuranosyl)indole (**8a**) and 3-cyano-2,5,6-trichloro-1-(5-deoxy-β-D-ribofuranosyl)indole (**8b**).

The 5′-deoxy-5′-azido analogues of FTCRI and CTCRI were also synthesized from the 5′-tosyloxy intermediate **5** (Scheme 1). The nucleoside **5** was treated with sodium azide in DMSO to provide compound **9** (Scheme 2). Formylation and cyanation of **9** were performed as above, and the intermediates **10a** and **10b** were deprotected with 90% aqueous trifluoroacetic acid to provide the 5′-deoxy-5′-azido analogues 3-formyl-2,5,6-trichloro-1-(5-deoxy-5-azido-β-D-ribofuranosyl)indole (**11a**) and 3-cyano-2,5,6-trichloro-1-(5-deoxy-5-azido-β-D-ribofuranosyl)indole (**11b**).

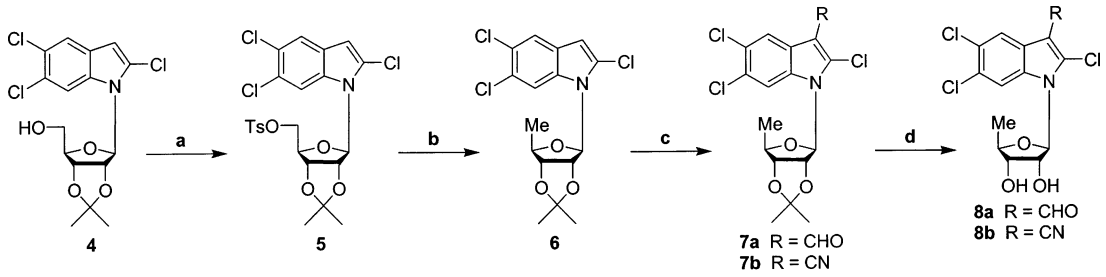
Synthesis of the 5′-deoxy-5′-fluoro derivatives proceeded once again from the partially protected indole nucleoside analogue **4**. The unprotected hydroxyl group at the 5′-position of **4** was fluorinated using Deoxofluor reagent[26] to provide 2,5,6-trichloro-1-(5-deoxy-5-fluoro-β-D-ribofuranosyl)indole (**12**, Scheme 3) in good yield. As above, formylation and cyanation of intermediate **12** was followed by deprotection with 90% aqueous trifluoroacetic acid to provide 3-formyl-2,5,6-trichloro-1-(5-deoxy-5-fluoro-β-D-ribofuranosyl)indole (**14a**) and 3-cyano-2,5,6-trichloro-1-(5-deoxy-5-fluoro-β-D-ribofuranosyl)indole (**14b**).

We initially elected to use a similar strategy for the synthesis of our target 2′-deoxy nucleoside analogues (i.e., modification of preformed 2′-deoxy nucleosides). Unfortunately, modification of the intermediate **15**[13] proved to be difficult in the case of formylation and acetylation. Formylation of **15** under the Vilsmeier−Haack conditions used previously did not produce the desired nucleoside analogue **16**. Instead, the formylated heterocycle was the only material isolated in any significant quantity. Acetylation of **15** also resulted in a glycosidic bond cleavage of the nucleoside instead of providing the intermediate **17** (Scheme 4).

Although the strategy is less efficient, we then elected to synthesize the target nucleosides by glycosylation of the preformed heterocycles with the 2-deoxy α-chlorosugar **20**.[27] The formylated heterocycle **19** and acetylated heterocycle **23** could be easily synthesized by a Vilsmeier−Haack formylation and a modified Friedel−Crafts acylation,[28] respectively (Scheme 5). Compounds **19** and **23** were glycosylated with the α-chlorosugar **20** to provide the protected nucleoside analogues **21** and **24**, respectively. However, deprotection of these intermediates produced two very different outcomes. The 3-acetyl derivative **24** was deprotected and provided the expected nucleoside 3-acetyl-2,5,6-trichloro-1-(2-deoxy-β-D-ribofuranosyl)indole (**25**). When identical conditions were applied to the 3-formyl analogue **21**, they produced only the 2-methoxy derivative **22**. We were unable to find conditions that would afford **21** without a concomitant displacement of the 2-chloro substituent. Interestingly, the protected 3-nitrile derivative **26**, synthesized from **15** without glycosidic bond cleavage, was deprotected with methanolic sodium methoxide to provide both the 2-chloro analogue 3-cyano-2,5,6-trichloro-1-(2-deoxy-β-D-ribofuranosyl)indole (**27a**) and the 2-methoxy analogue 3-cyano-2-methoxy-5,6-dichloro-1-(2-deoxy-β-D-ribofuranosyl)indole (**27b**) in a 2.5:1 ratio.

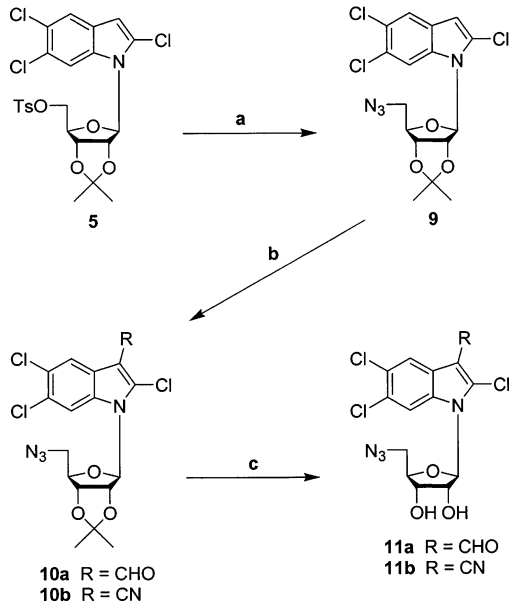
We also pursued the synthesis of some other selected 2′-deoxyindole nucleosides. The 3-iodinated nucleoside analogue **28** was available from a previous investigation in our lab.[15] This prompted us to use **28** in the synthesis of some selected 3-heteroaryl derivatives via palladium-catalyzed coupling reactions. Compound **28** was subjected to a Suzuki-type coupling with either 2-furylboronic acid or 3-thienylboronic acid according to the

Scheme 1[a]



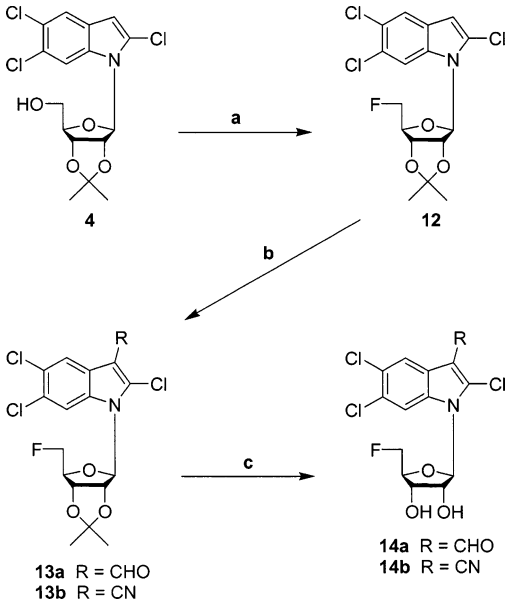
[a] Reagents and conditions: (a) TsCl, pyridine, 20 °C, 16 h; (b) $NaBH_4$, DMSO, 75 °C, 16 h; (c) $POCl_3$, DMF, 70 °C, 16 h or CSI, CH_2Cl_2, 20 °C, 16 h, then DMF, 20 °C, 1 h; (d) 90% TFA, 20 °C, 2 min.

Scheme 2[a]



[a] Reagents and conditions: (a) NaN_3, DMF, 75 °C, 16 h; (b) $POCl_3$, DMF, 70 °C, 16 h or CSI, CH_2Cl_2, 20 °C, 16 h, then DMF, 20 °C, 1 h; (c) 90% TFA, 20 °C, 2 min.

Scheme 3[a]



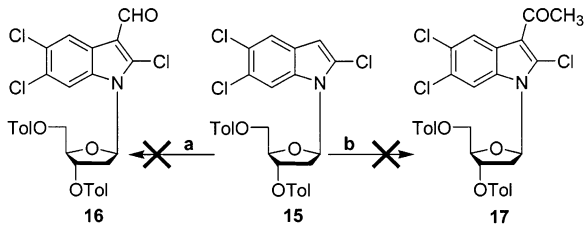
[a] Reagents and conditions: (a) Deoxofluor, CH_2Cl_2, 20 °C, 16 h; (b) $POCl_3$, DMF, 70 °C, 16 h or CSI, CH_2Cl_2, 20 °C, 16 h, then DMF, 20 °C, 1 h; (c) 90% TFA, 20 °C, 2 min.

procedure of Huff[29] to provide **29a** and **29b**, respectively (Scheme 6). These 3-heteroaryl analogues were then deprotected with methanolic sodium methoxide to provide 3-(2-furyl)-2,5,6-trichloro-1-(2-deoxy-β-D-ribofuranosyl)indole (**30a**) and 3-(3-thienyl)-2,5,6-trichloro-1-(2-deoxy-β-D-ribofuranosyl)indole (**30b**) in good yields.

It has been established that the 2-bromo analogue of TCRB [i.e., 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)-benzimidazole, BDCRB] has greater antiviral activity and selectivity than TCRB itself.[9] Our preliminary evaluation of the chlorinated 2′-deoxy nucleosides (vide supra) revealed that 3-acetyl-2,5,6-trichloro-1-(2-deoxy-β-D-ribofuranosyl)indole (**25**) was a particularly selective compound. This prompted us to synthesize the 2-bromo analogue of **25** to determine whether this modification at the 2-position of the heterocycle would increase the activity or selectivity as was the case in the benzimidazole series.

However, our initial attempts to synthesize the requisite 3-acetyl-2-bromo-5,6-dichloroindole (**32**, Scheme 7) were complicated by a halogen exchange at the 2-position. Acetylation of **31**[13] using the same procedure[28] as that used for the 2-chloro congener **18** provided a mixture of the desired 2-bromo heterocycle **32** along with a substantial amount of the 2-chloro analogue **23**. Replacing the tin(IV) chloride with tin(IV) bromide did not improve the outcome but instead resulted in the exclusive production of the undesired 2-chloro analogue **23**. When tin(IV) chloride and acetyl chloride were replaced by tin(IV) bromide and acetyl bromide, the desired product **32** was produced in good yield without the concomitant halogen exchange. The requisite heterocycle was then glycosylated as above with α-chlorosugar **20** and the intermediate (**33**) was deprotected with sodium methoxide to yield the desired nucleoside 3-acetyl-2-bromo-5,6-dichloro-1-(2-deoxy-β-D-ribofuranosyl)indole (**34**).

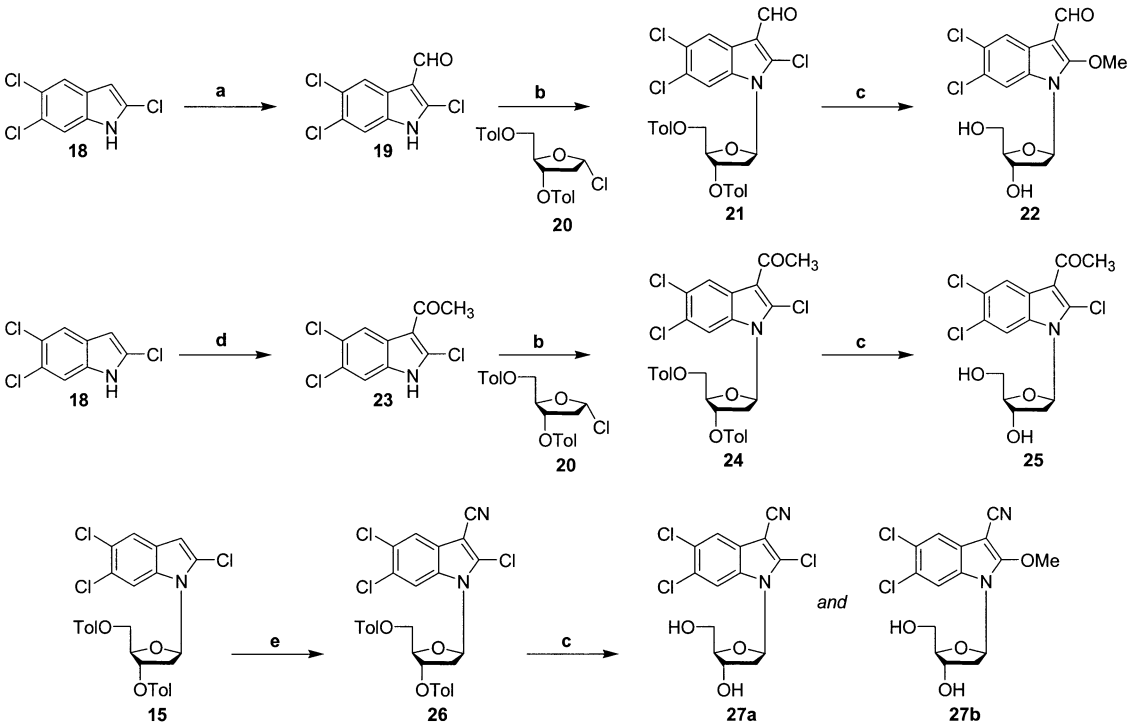
Scheme 4[a]



[a] Reagents and conditions: (a) $POCl_3$, DMF, 60 °C, 16 h; (b) AcCl, $AlCl_4$, CH_2Cl_2, 20 °C, 15 min.

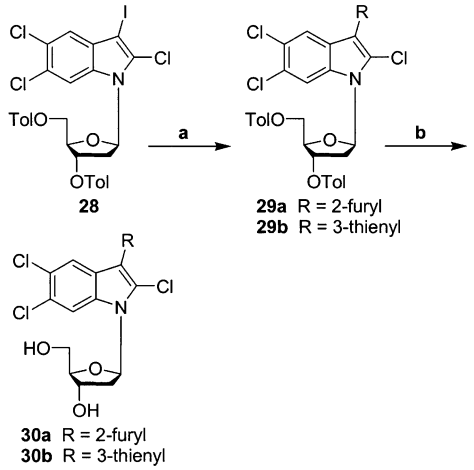
Biological Evaluation. The compounds were tested for antiviral activity against HCMV and HSV-1 and for cytotoxicity in HFF and KB cell lines. Among the series

Scheme 5[a]



[a] Reagents and conditions: (a) $POCl_3$, DMF, 60 °C, 16 h; (b) NaH, THF, 0 °C, 10 min, then **20**, THF/toluene, 20 °C, 16 h; (c) NaOMe, MeOH, 20 °C, 16 h; (d) AcCl, $SnCl_4$, CH_2Cl_2, $MeNO_2$, 0−20 °C, 45 min; (e) CSI, CH_2Cl_2, 20 °C, 16 h, then DMF, 20 °C, 1 h.

Scheme 6[a]



[a] Reagents and conditions: (a) Ar−$B(OH)_2$, $Pd(OAc)_2$, (*o*-tol)$_3$P, Na_2CO_3, DMF/*n*-PrOH/H_2O, 120 °C, 5−15 min; (b) NaOMe, MeOH, 20 °C, 45−90 min.

of 5′-deoxy nucleoside analogues, most were as potent or more potent than their ribofuranosyl counterparts (Table 1). Both 5′-deoxy analogues **8a** and **8b** were as potent as their congeners **2** and **3**, respectively. This strongly suggests that, as is the case for TCRB and its 5′-deoxy analogue, no phosphorylation is required for antiviral activity in these chlorinated indole nucleosides. The 5′-deoxy-5′-fluoro and 5′-deoxy-5′-azido analogues of FTCRI (i.e., **11a** and **14a**) were also nearly as active as FTCRI itself, but the corresponding analogues of CTCRI (i.e., **11b** and **14b**) were substantially less active. The reason for this difference in activity among the different 3-substituted indole nucleosides is unknown.

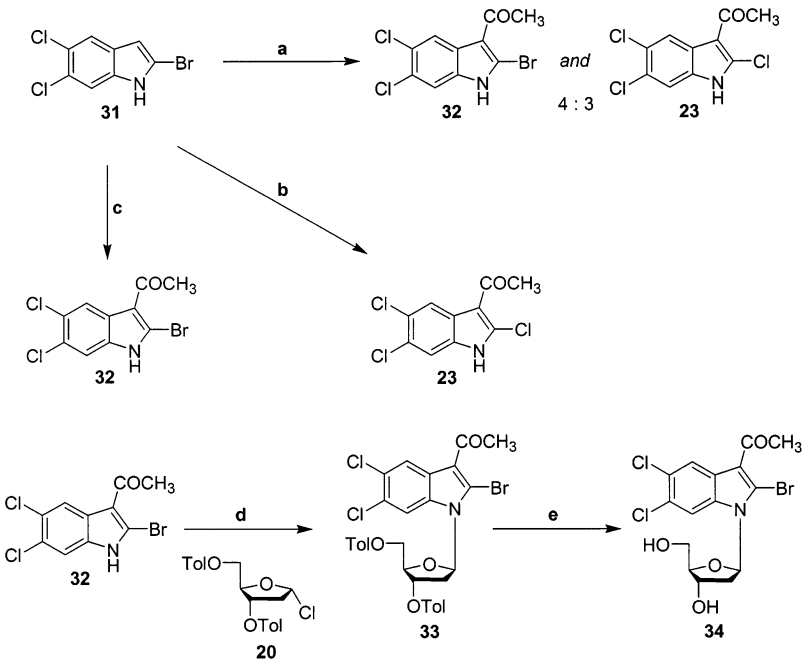
As expected from our experiences with 2-substituted indole nucleosides in a previous study,[30] the 2′-deoxy analogues with 2-methoxy substituents, **22** and **27b**, were substantially less active than their 2-chloro counterparts. However, we were surprised by the potent anti-HCMV activity of the corresponding 2-chloro analogues **25** and **27a**. Neither **25** nor **27a** were substantially less active than FTCRI and CTCRI. In fact, the 3-acetyl analogue **25** was also much less toxic than any previous chlorinated indole nucleosides in this series. The selectivity of **25** is quite remarkable, with the concentration required for toxicity approximately 1000-fold more than that needed for antiviral activity.

The 3-heteroaryl analogues of 2′-deoxyindole nucleosides (**30a** and **30b**) were again less active than the either 2′-deoxy nucleoside analogues **25** and **27a** or ribofuranosyl analogues FTCRI (**2**) or CTCRI (**3**). In contrast, the 2-bromo analogue of the very selective compound **25** (i.e., **34**) was as active and no more cytotoxic than **25** (see Table 1), although we had expected an increase in activity and selectivity.

Mechanism of Action. In our studies to determine the mechanism of action for the chlorinated indole nucleosides, we have established several lines of evidence. Because TCRB has a unique mechanism of action and acts very late in the viral replication cycle,[16] the time-of-addition study is especially diagnostic. Data in Figure 2 demonstrate that as expected, TCRB acted late in the viral replication cycle, at a time later than the DNA polymerase inhibitor ganciclovir (GCV). The indole nucleoside FTCRI (**2**) also acted very late in the replication cycle, losing its effectiveness later than GCV, and at a time that was virtually indistinguishable from TCRB. Thus, FTCRI acted very late in the viral replication cycle at a time when DNA synthesis had been mostly concluded.

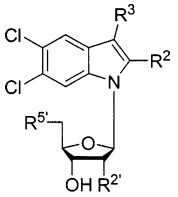
As a second line of evidence, the activity of four chlorinated indole nucleosides was determined in viral

Scheme 7[a]



[a] Reagents and conditions: (a) AcCl, $SnCl_4$, CH_2Cl_2, $MeNO_2$, 0−20 °C, 45 min; (b) AcCl, $SnBr_4$, CH_2Cl_2, $MeNO_2$, 0−20 °C, 45 min; (c) AcBr, $SnBr_4$, CH_2Cl_2, $MeNO_2$, 0−20 °C, 45 min; (d) NaH, THF, 0 °C, 10 min, then **20**, THF/toluene, 20 °C, 16 h; (e) NaOMe, MeOH, 20 °C, 16 h.

Table 1. Antiviral Activity and Cytotoxicity of 2′- and 5′-Deoxyribosylindole Nucleosides



					50% inhibitory concentration (μM)			
					antiviral		cytotoxicity	
compd	R^2	R^3	$R^{2'}$	$R^{5'}$	HCMV plaque[a]	HSV-1 ELISA[b]	HFF visual[c]	KB growth[c]
8a	−Cl	−CHO	−OH	−H	0.30	20	32	25
8b	−Cl	−CN	−OH	−H	0.19	32	32	40
11a	−Cl	−CHO	−OH	$-N_3$	0.32	10	32	25
11b	−Cl	−CN	−OH	$-N_3$	3.0	15	>100[d]	60
14a	−Cl	−CHO	−OH	−F	0.60	20	32	20
14b	−Cl	−CN	−OH	−F	2.7	40	32	50
22	−OMe	−CHO	−H	−OH	>100	>100	100	20
25	−Cl	$-COCH_3$	−H	−OH	0.30	8.0	>100	400
27a	−Cl	−CN	−H	−OH	0.30	35	32	60
27b	−OMe	−CN	−H	−OH	68	90	>100	>100
30a	−Cl	−(2-furyl)	−H	−OH	3.6	20	3.2	50
30b	−Cl	−(3-thienyl)	−H	−OH	38	20	32	50
34	−Br	$-COCH_3$	−H	−OH	0.34	>100	>100	>100
2[e]	−Cl	−CHO	−OH	−OH	0.23	40	45	45
3[e]	−Cl	−CN	−OH	−OH	0.55	>100	32	35
TCRB[f]					2.9	102	238	210
5′-deoxy TCRB[g]					0.36		100	
2′-deoxy TCRB[h]					20	41	>100	100
BDCRB[f]					0.7	130	118	>100
GCV[i]					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 compounds **2** and **3** published previously as compounds **9a** and **9b**, respectively, in ref 15. [f] Data for TCRB and BDCRB published previously as compounds **9** and **11**, respectively, in ref 9. [g] Data for 5′-deoxy TCRB published previously as compound **210** in ref 19. [h] Data for 2′-deoxy TCRB published previously as compound **3b** in ref 22. [i] Averages from 108, 33, and 3 experiments, respectively, using GCV.

strains that are resistant to TCRB. Data in Table 2 compare the activity of these compounds to that of BDCRB and GCV in wild-type HCMV and three viral strains that are resistant to TCRB and BDCRB. The data readily establish that all of the indole nucleosides were less active against the TCRB-resistant viruses

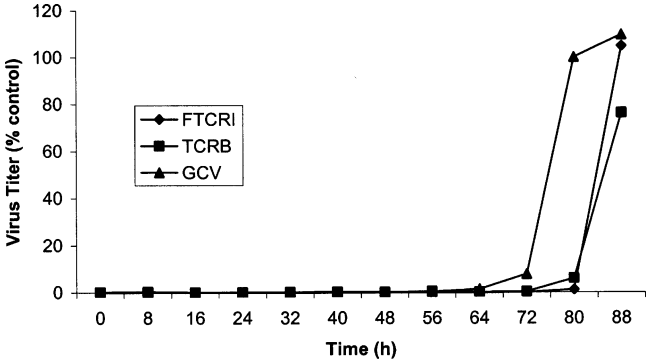
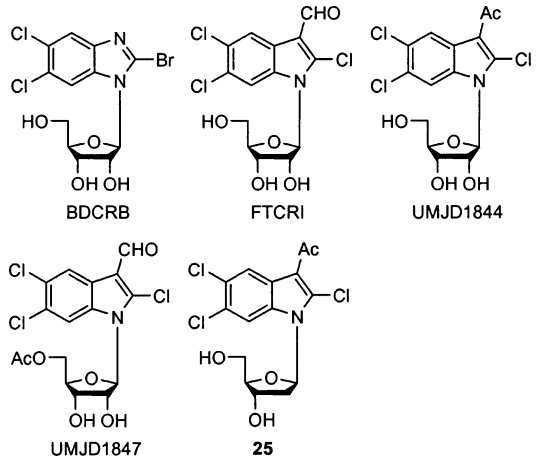


Figure 2. HCMV time-of-addition study comparing the activity of the indole nucleoside FTCRI (**2**) to that of the benzimidazole nucleoside TCRB (**1**) and the DNA synthesis inhibitor GCV. Drugs were added to cells infected with HCMV at time zero and at the indicated time points. Inhibition of viral replication was measured by yield reduction assays on samples taken at 96 h at the end of one viral replication cycle. Data are presented as the mean of duplicate assays.

Table 2. Cross-Resistance between BDCRB and Selected Indole Nucleosides in BDCRB-Resistant Viruses



	50% inhibitory concentration (μM)[a] viral strain (mutant gene)			
compd	wild type	r56 (UL56)	D10 (UL89)	C4 (UL56 + UL89)
BDCRB[b]	0.99 ± 0.12	6.7 ± 0	12 ± 5	29 ± 0
GCV	1.2	1.2	1.2	2.5
FTCRI[b]	0.46 ± 0.24	1.8 ± 0	0.88 ± 0.08	4.8 ± 1.7
UMJD1844[b,c]	0.26 ± 0.04	1.2 ± 0.1	0.72 ± 0.09	6.2 ± 0.3
UMJD1847[b,c]	0.22 ± 0	1.6 ± 0.7	0.88 ± 0.03	3.3 ± 1.7
25[b]	0.22 ± 0	1.8 ± 0.6	0.76 ± 0.07	2.1 ± 0.5

[a] Plaque reduction assays were performed in duplicate wells as described in the text. Wild-type virus is Towne strain; UL56, UL89, and UL56 + UL89 viral strains contain single mutations in the specified ORF and have been characterized as found in ref 17.[b] IC_{50} values were calculated from the average of two experiments. [c] Compounds UMJD1844 and UMJD1847 published previously as compounds **9c** and **7a**, respectively, in ref 15.

than wild-type HCMV. Resistance to TCRB and BDCRB has been traced to point mutations in both UL56 and UL89 genes of HCMV.[17] Although the exact nature of inhibition of these gene products has not been definitively proven,[31] both genes are involved in the cleavage of concatemeric viral DNA into genome-sized pieces before packaging. Because mutations causing resistance to TCRB and BDCRB also caused resistance to several chlorinated indole nucleosides, this is further evidence that the chlorinated benzimidazole nucleosides (i.e., TCRB and BDCRB) share a common viral target with the chlorinated indole nucleosides (i.e., FTCRI and its analogues).

A third line of evidence for a common mechanism between chlorinated indole and benzimidazole nucleosides is our observation that both 5′-deoxy TCRB analogues and 5′-deoxy FTCRI analogues are potent inhibitors of HCMV replication (Table 1). These data strongly suggest that as in the chlorinated benzimidazole series[21] 5′-phosphorylation is not required for activity in the chlorinated indole series. This parallel further strengthens the similarities between chlorinated indole nucleosides and chlorinated benzimidazole nucleosides.

Experimental Section

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 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 % vol. 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, DE). Chromatograms were visualized under UV light at 254 nm. Spectra for all compounds are presented in Supporting Information. ^{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. ^{19}F NMR spectra were obtained at 300 MHz on a Bruker DPX300 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 $CDCl_3$). Chemical shift values for ^{19}F were determined relative to an external TFA standard (−76.50 ppm). Mass spectrometry and elemental analysis for selected compounds (listed in Supporting Information) were performed at the University of Michigan Department of Chemistry mass spectrometry facility and elemental analysis facility, respectively.

2,5,6-Trichloro-1-(2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-*β*-D-ribofuranosyl)indole (5). 2,5,6-Trichloro-1-(2,3-*O*-isopropylidene-*β*-D-ribofuranosyl)indole[13] (**4**, 0.79 g, 2.0 mmol) was dissolved in dry pyridine (20 mL) to which was added *p*-toluenesulfonyl chloride (0.77 g, 4.0 mmol). The resulting solution was stirred at room temperature for 16 h, then the solvent was evaporated and the residual oil diluted with 5% aqueous Na_2CO_3 (50 mL) and brine (50 mL). This mixture was extracted with EtOAc (2 × 50 mL) and the combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield a pale-yellow oil. The oil was dissolved in $CHCl_3$ (2 mL) and subjected to column chromatography (50 mm × 450 mm) on silica gel with 3:1 hexane/EtOAc. Fractions containing product were pooled and evaporated to yield 0.83 g (75%) of **5** as a colorless foam: mp 65−70 °C; R_f = 0.4 (3:1 hexane/EtOAc).

2,5,6-Trichloro-1-(2,3-*O*-isopropylidene-5-deoxy-*β*-D-ribofuranosyl)indole (6). Compound **5** (693 mg, 1.3 mmol) was dissolved in dry DMSO (15 mL) to which was added sodium borohydride (0.24 g, 6.3 mmol). The resulting solution was heated on a 45 °C oil bath for 16 h, then cooled to room temperature and poured into brine (200 mL). The suspension was extracted with EtOAc (2 × 200 mL). The organic extracts were washed successively with water (200 mL) and brine (200 mL), then combined and dried over $MgSO_4$, filtered, and

evaporated to yield a clear residue. The residue was dissolved in $CHCl_3$ (2 mL) and subjected to column chromatography (50 mm × 450 mm) on silica gel with 5:1 hexane/EtOAc. Fractions containing product were pooled and evaporated to yield 355 mg (74%) of **6** as a colorless oil that solidifies upon standing: mp 69−71 °C; R_f = 0.6 (5:1 hexane/EtOAc).

3-Formyl-2,5,6-trichloro-1-(2,3-*O*-isopropylidene-5-deoxy-β-D-ribofuranosyl)indole (7a). Compound **6** (192 mg, 0.51 mmol) was dissolved in dry DMF (5 mL) to which was added phosphorus oxychloride (0.25 mL, 0.41 g, 2.7 mmol). The resulting yellow solution was heated on a 60 °C oil bath for 16 h, then cooled to room temperature and poured into 10% $NaHCO_3$ (200 mL). The aqueous suspension was extracted with EtOAc (2 × 50 mL), and the combined organic extracts were washed with brine (50 mL), dried over $MgSO_4$, filtered, and evaporated to yield a yellow oil. The oil was dissolved in $CHCl_3$ (1 mL) and subjected to column chromatography (40 mm × 350 mm) on silica gel with 3:1 hexane/EtOAc. Fractions containing product were pooled and evaporated to yield 180 mg (87%) of **7a** as a white solid. A sample was recrystallized from $CHCl_3$/hexane to yield a white crystalline solid: mp 153−154 °C; R_f = 0.3 (5:1 hexane/EtOAc).

3-Cyano-2,5,6-trichloro-1-(2,3-*O*-isopropylidene-5-deoxy-β-D-ribofuranosyl)indole (7b). Compound **6** (450 mg, 1.2 mmol) was dissolved in dry CH_2Cl_2 (10 mL) to which was added chlorosulfonyl isocyanate (0.20 mL, 0.32 g, 2.2 mmol). The resulting solution was stirred at room temperature for 16 h, then dry DMF (1 mL) was added, and the solution was stirred an additional 1 h at room temperature. To the resulting solution were added 5% Na_2CO_3 (20 mL) and brine (30 mL). This aqueous suspension was extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield a yellow-orange oil. The oil was dissolved in $CHCl_3$ (1 mL) and subjected to column chromatography (40 mm × 350 mm) on silica gel with 5:1 hexane/EtOAc. Fractions containing product were pooled and evaporated to yield 153 mg (32%) of **7b** as a white crystalline solid: mp 165−166 °C; R_f = 0.4 (5:1 hexane/EtOAc).

3-Formyl-2,5,6-trichloro-1-(5-deoxy-β-D-ribofuranosyl)-indole (8a). Compound **7a** (139 mg, 0.34 mmol) was dissolved in 90% aqueous trifluoroacetic acid (5 mL). The solution was stirred at room temperature for 5 min, then evaporated to approximately 1 mL. The remainder was poured into 5% aqueous Na_2CO_3 (10 mL) and brine (40 mL). The aqueous suspension was extracted with EtOAc (2 × 100 mL), and the combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield a pale-yellow solid. The solid was dissolved in DMF (0.5 mL) and subjected to column chromatography (40 mm × 350 mm) on silica gel with 10% MeOH/$CHCl_3$. Fractions containing product were pooled and evaporated to yield a white solid. This solid was recrystallized from boiling hexane to yield 86 mg (70%) of **8a** as a white crystalline solid: mp 217−218 °C; R_f = 0.6 (10% MeOH/$CHCl_3$). Anal. ($C_{14}H_{12}Cl_3NO_4$) C, H, N.

3-Cyano-2,5,6-trichloro-1-(5-deoxy-β-D-ribofuranosyl)-indole (8b). Compound **7b** (207 mg, 0.52 mmol) was treated as per **8a** above with 90% aqueous TFA (5 mL) and recrystallized from 10% MeOH/$CHCl_3$ and hexane to yield 152 mg (82%) of **8b** as a white crystalline solid: mp 227−228 °C; R_f = 0.6 (10% MeOH/$CHCl_3$). Anal. ($C_{14}H_{11}Cl_3N_2O_3 \cdot {}^1/_5H_2O$) C, H, N.

2,5,6-Trichloro-1-(2,3-*O*-isopropylidene-5-deoxy-5-azido-β-D-ribofuranosyl)indole (9). Compound **5** (0.77 g, 1.4 mmol) was dissolved in dry DMF (20 mL) to which was added sodium azide (1.5 g, 23.0 mmol), and the mixture was heated on a 75 °C oil bath for 16 h. The resulting suspension was cooled to room temperature and evaporated under high vacuum (0.5 mmHg, 35 °C), and the residue was dissolved in EtOAc (150 mL). The organic solution was washed with brine (50 mL), then dried over $MgSO_4$, filtered, and evaporated to yield a clear oil. This oil was dissolved in $CHCl_3$ (2 mL) and subjected to column chromatography (50 mm × 450 mm) on silica gel with 3:1 hexane/EtOAc. Product-containing fractions were pooled and evaporated to yield 0.56 mg (95%) of **9** as a clear oil: R_f = 0.6 (3:1 hexane/EtOAc).

3-Formyl-2,5,6-trichloro-1-(2,3-*O*-isopropylidene-5-deoxy-5-azido-β-D-ribofuranosyl)indole (10a). Compound **9** (283 mg, 0.68 mmol) was treated as per **7a** above with dry DMF (10 mL) and phosphorus oxychloride (1.5 mL, 2.5 g, 16 mmol), then recrystallized from boiling $CHCl_3$/hexane to yield 187 mg (62%) of **10a** as a pale-yellow crystalline solid: mp 142−143 °C; R_f = 0.4 (2:1 hexane/EtOAc).

3-Cyano-2,5,6-trichloro-1-(2,3-*O*-isopropylidene-5-deoxy-5-azido-β-D-ribofuranosyl)indole (10b). Compound **9** (426 mg, 1.0 mmol) was treated as per **7b** above with chlorosulfonyl isocyanate (135 μL, 220 mg, 1.5 mmol) and recrystallized from boiling hexane to yield 240 mg (53%) of **10b** as a white crystalline solid: mp 144−145 °C; R_f = 0.5 (2:1 hexane/EtOAc).

3-Formyl-2,5,6-trichloro-1-(5-deoxy-5-azido-β-D-ribofuranosyl)indole (11a). Compound **10a** (168 mg, 0.38 mmol) was treated as per **8a** above with 90% aqueous TFA (10 mL) and recrystallized from boiling $CHCl_3$ to yield 117 mg (76%) of **11a** as a pale-yellow crystalline solid: mp 160−161 °C; R_f = 0.5 (10% MeOH/$CHCl_3$). Anal. ($C_{14}H_{11}Cl_3N_4O_4 \cdot {}^1/_{20}CHCl_3$) C, H, N.

3-Cyano-2,5,6-trichloro-1-(5-deoxy-5-azido-β-D-ribofuranosyl)indole (11b). Compound **10b** (216 mg, 0.49 mmol) was treated as per **8a** above with 90% aqueous TFA (10 mL) and recrystallized from boiling $CHCl_3$ to yield 165 mg (84%) of **11b** as a white powder: mp 161−162 °C; R_f = 0.5 (10% MeOH/$CHCl_3$). Anal. ($C_{14}H_{10}Cl_3N_5O_3 \cdot {}^1/_{20}CHCl_3$) C, H, N.

2,5,6-Trichloro-1-(2,3-*O*-isopropylidene-5-deoxy-5-fluoro-β-D-ribofuranosyl)indole (12). Compound **4**[13] (0.98 g, 2.5 mmol) was dissolved in dry CH_2Cl_2 (5 mL) to which was added bis(2-methoxyethyl)aminosulfur trifluoride (Deoxofluor,[26] 0.70 mL, 0.84 g, 3.8 mmol). The resulting solution was stirred at room temperature for 16 h, and then the reaction was quenched with 5% Na_2CO_3 (10 mL). The biphasic mixture was poured into brine (40 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield a yellow oil. This oil was dissolved in $CHCl_3$ (1 mL) and subjected to column chromatography (40 mm × 350 mm) on silica gel with 5:1 hexane/EtOAc. Fractions containing product were pooled and evaporated to yield 0.46 mg (47%) of **12** as a pale-yellow oil. R_f = 0.4 (5:1 hexane/EtOAc).

3-Formyl-2,5,6-trichloro-1-(2,3-*O*-isopropylidene-5-deoxy-5-fluoro-β-D-ribofuranosyl)indole (13a). Compound **12** (232 mg, 0.59 mmol) was treated as per **7a** above with dry DMF (5 mL) and phosphorus oxychloride (0.30 mL, 0.49 g, 3.2 mmol) to yield 126 mg (51%) of **13a** as a white crystalline solid: mp 147−148 °C; R_f = 0.5 (2:1 hexane/EtOAc).

3-Cyano-2,5,6-trichloro-1-(2,3-*O*-isopropylidene-5-deoxy-5-fluoro-β-D-ribofuranosyl)indole (13b). Compound **12** (412 mg, 1.0 mmol) was treated as per **7b** above with chlorosulfonyl isocyanate (0.14 mL, 0.23 g, 1.6 mmol) to yield 198 mg (45%) of **13b** as a white crystalline solid: mp 162−163 °C; R_f = 0.5 (2:1 hexane/EtOAc).

3-Formyl-2,5,6-trichloro-1-(5-deoxy-5-fluoro-β-D-ribofuranosyl)indole (14a). Compound **13a** (133 mg, 0.31 mmol) was treated as per **8a** above with 90% aqueous TFA (5 mL) and recrystallized from 10% MeOH/$CHCl_3$ and hexane to yield 82 mg (68%) of **14a** as a white crystalline solid: mp 207−208 °C; R_f = 0.5 (10% MeOH/$CHCl_3$). Anal. ($C_{14}H_{11}Cl_3FNO_4$) C, H, N.

3-Cyano-2,5,6-trichloro-1-(5-deoxy-5-fluoro-β-D-ribofuranosyl)indole (14b). Compound **13b** (133 mg, 0.31 mmol) was treated as per **8a** above with 90% aqueous TFA (5 mL) and recrystallized from EtOAc/hexane to yield 150 mg (88%) of **14b** as a white crystalline solid: mp 246−247 °C; R_f = 0.6 (10% MeOH/$CHCl_3$). Anal. ($C_{14}H_{10}Cl_3FN_2O_3$) C, H, N.

3-Formyl-2,5,6-trichloroindole (19). 2,5,6-Trichloroindole[13] (**18**, 2.19 g, 9.9 mmol) was dissolved in dry DMF (25 mL) to which was added phosphorus oxychloride (2.8 mL, 4.6 g, 30 mmol). The solution was heated on a 60 °C oil bath for 16 h, and then the solvent was removed under vacuum (0.5 mmHg, 40 °C). The residual orange oil was poured into cold water (400 mL), and the pale-yellow suspension was stirred at 4 °C for 2 h. The suspension was filtered, and the solids

were rinsed with cold water (100 mL). The solids were suspended in boiling MeOH (250 mL), and the suspension was evaporated to approximately 150 mL and then allowed to stand at 4 °C for 8 h. The resulting suspension was filtered, and the solids were rinsed with cold MeOH (25 mL). The solids were dried in a vacuum oven (60 °C, 12 mmHg) for 12 h to yield 1.80 g (73%) of **19** as a pale-yellow powder: mp >280 °C (dec); R_f = 0.5 (5% MeOH/$CHCl_3$).

3-Formyl-2,5,6-trichloro-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-*β*-D-ribofuranosyl]indole (21). Compound **19** (0.63 g, 2.5 mmol) was suspended in dry THF (75 mL) to which was added 60% sodium hydride in mineral oil (0.20 g, 5.0 mmol). The suspension was stirred at room temperature for 15 min until gas evolution had ceased, then filtered into a suspension of 3,5-di-(*O*-*p*-toluoyl)-2-deoxy-α-D-ribofuranosyl chloride[27] (**20**, 1.00 g, 2.6 mmol) in toluene (25 mL). The resulting solution was stirred at room temperature for 16 h, then evaporated to dryness. The residue was suspended in brine (20 mL) and extracted with EtOAc (2 × 40 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield a pale-yellow solid. The solid was suspended in $CHCl_3$ (10 mL), and the residual solids were filtered off. The filtrate was evaporated, then dissolved in $CHCl_3$ (2 mL) and subjected to column chromatography (50 mm × 450 mm) on silica gel with 3:1 hexane/EtOAc. Fractions containing product were pooled and evaporated to yield 0.75 g (49%) of **21** as a white solid. An analytical sample was prepared by recrystallization from MeOH/$CHCl_3$: mp 153−154 °C; R_f = 0.3 (3:1 hexane/EtOAc).

3-Formyl-2-methoxy-5,6-dichloro-1-(2-deoxy-*β*-D-ribofuranosyl)indole (22). Compound **21** (198 mg, 0.33 mmol) was suspended in dry MeOH (10 mL) to which was added sodium methoxide (75 mg, 1.4 mmol). The suspension was stirred at room temperature for 16 h, after which time the solution clarified. The solvent was then removed under vacuum, and the residue was suspended in water (50 mL) and extracted with EtOAc (2 × 100 mL). The combined organic extracts were washed with brine (25 mL), dried over $MgSO_4$, filtered, and evaporated to yield a damp solid. The crude material was dissolved in MeOH (1 mL) and subjected to column chromatography (40 mm × 350 mm) on silica gel with 10% MeOH/$CHCl_3$. Fractions containing product were pooled and evaporated to yield a white solid. The solid was recrystallized from warm MeOH to yield 82 mg (68%) of **22** as a white crystalline solid: mp 186−187 °C; R_f = 0.3 (10% MeOH/$CHCl_3$). Anal. ($C_{15}H_{15}Cl_2NO_5 \cdot {}^1/_2H_2O$) C, H, N.

3-Acetyl-2,5,6-trichloroindole (23). Compound **18**[13] (2.21 g, 10.0 mmol) was dissolved in CH_2Cl_2 (50 mL) and cooled to 0 °C. Tin tetrachloride (1.4 mL, 12 mmol) was added over 5 min, then the resulting suspension was warmed to room temperature and stirred for 30 min at that temperature. Acetyl chloride (0.7 mL, 0.77 g, 10 mmol) was added, followed by nitromethane (50 mL), and the resulting suspension was stirred for 15 min at room temperature. The reaction was quenched with 50 mL of cold water, and the biphasic suspension was stirred vigorously for 30 min. The organic phase was separated, and the organic phase was extracted with THF (2 × 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to dryness. The red solid was recrystallized from boiling THF to yield 0.98 g (37%) of **23** as a pale-pink powder: mp >320 °C (dec); R_f = 0.6 (5% MeOH/$CHCl_3$).

3-Acetyl-2,5,6-trichloro-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-*β*-D-ribofuranosyl]indole (24). Compound **23** (0.63 g, 2.5 mmol) was treated as per **21** above with 60% sodium hydride in mineral oil (0.25 g, 6.3 mmol) and 3,5-di-(*O*-*p*-toluoyl)-2-deoxy-α-D-ribofuranosyl chloride[27] (**20**, 1.00 g, 2.6 mmol) to yield 1.12 g (61%) of **24** as a white solid. An analytical sample was prepared by recrystallization from MeOH/$CHCl_3$: mp 100−103 °C; R_f = 0.4 (3:1 hexane/EtOAc).

3-Acetyl-2,5,6-trichloro-1-(2-deoxy-*β*-D-ribofuranosyl)-indole (25). Compound **24** (0.62 g, 1.0 mmol) was suspended in dry MeOH (50 mL) to which was added sodium methoxide (220 mg, 4.1 mmol). The suspension was stirred at room temperature for 16 h, after which time the solution first clarified and a precipitate then formed. The suspension was allowed to stand at 4 °C for 4 h and was then filtered, and the solids were rinsed with cold MeOH (10 mL). The solid was recrystallized from warm MeOH to yield 0.28 g (74%) of **25** as a white crystalline solid: mp >200 °C (dec); R_f = 0.4 (10% MeOH/$CHCl_3$). Anal. ($C_{15}H_{14}Cl_3NO_4$) C, H, N.

3-Cyano-2,5,6-trichloro-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-*β*-D-ribofuranosyl]indole (26). 2,5,6-Trichloro-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-*β*-D-ribofuranosyl]indole[13] (**15**, 1.15 g, 2.0 mmol) was treated as per **7b** above with chlorosulfonyl isocyanate (0.35 mL, 0.57 g, 4.0 mmol) and recrystallized from $CHCl_3$/MeOH to yield 0.64 g (55%) of **26** as a white crystalline solid: mp 192−193 °C; R_f = 0.5 (3:1 hexane/EtOAc).

3-Cyano-2,5,6-trichloro-1-(2-deoxy-*β*-D-ribofuranosyl)-indole (27a) and 3-Cyano-2-methoxy-5,6-dichloro-1-(2-deoxy-*β*-D-ribofuranosyl)indole (27b). Compound **26** (366 mg, 0.63 mmol) was suspended in dry MeOH (30 mL) to which was added sodium methoxide (82 mg, 1.5 mmol). The suspension was stirred at room temperature for 16 h, then the solvent was removed under vacuum, and the residue was suspended in brine (100 mL) and water (10 mL). The aqueous mixture was extracted with EtOAc (2 × 100 mL), and the combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield a viscous residue. The residue was dissolved in DMF (0.5 mL) and subjected to column chromatography (40 mm × 350 mm) on silica gel with 10% MeOH/$CHCl_3$. Fractions containing product were pooled and evaporated to yield a white solid. The solid was dissolved in DMF (0.5 mL) and subjected to column chromatography (40 mm × 350 mm) on C18 reverse-phase silica gel with 75% MeOH/H_2O. Fractions containing the more rapidly eluting material were pooled and evaporated to yield 47 mg (21%) of **27b** as a white powder. Fractions containing the more slowly eluting material were pooled and evaporated to yield 120 mg (53%) of **27a** as a white powder. **27b**: mp 222−223 °C; R_f = 0.4 (10% MeOH/$CHCl_3$). Anal. ($C_{15}H_{14}Cl_2N_2O_4 \cdot {}^1/_5H_2O$) C, H, N. **27a**: mp 187−188 °C; R_f = 0.4 (10% MeOH/$CHCl_3$). Anal. ($C_{14}H_{11}Cl_3N_2O_3 \cdot {}^1/_5H_2O$) C, H, N.

3-(2-Furyl)-2,5,6-trichloro-1-(3,5-di-*O*-toluoyl-2-deoxy-*β*-D-ribofuranosyl)indole (29a). 3-Iodo-2,5,6-trichloro-1-(3,5-di-*O*-*p*-toluoyl-2-deoxy-*β*-D-ribofuranosyl)indole[15] (**28**, 300 mg, 0.43 mmol) and 2-furanboronic acid (55 mg, 0.49 mmol) were suspended in *n*-propanol (2 mL) and dry DMF (0.5 mL). The suspension was stirred at room temperature for 10 min, then palladium(II) acetate (10 mg, 0.045 mmol) and tri-*o*-tolylphosphine (42 mg, 0.14 mmol) were added, followed by 2.0 M aqueous Na_2CO_3 (0.25 mL, 0.52 mmol) and H_2O (0.5 mL). The resulting suspension was heated on a 120 °C oil bath for 20 min. The resulting dark solution was cooled and poured into EtOAc (50 mL). The organic solution was washed with brine (50 mL), dried over $MgSO_4$, filtered, and evaporated to yield an orange solid. The solid was dissolved in $CHCl_3$ (1 mL) and subjected to column chromatography (40 mm × 350 mm) on silica gel with 5:1 hexane/EtOAc. Fractions containing product were pooled and evaporated to yield 158 mg (58%) of **29a** as a white crystalline solid: mp 85−87 °C; R_f = 0.5 (3:1 hexane/EtOAc).

3-(3-Thienyl)-2,5,6-trichloro-1-(3,5-di-*O*-toluoyl-2-deoxy-*β*-D-ribofuranosyl)indole (29b). Compound **28**[15] (280 mg, 0.40 mmol) was treated as per **29a** above with 3-thiopheneboronic acid (58 mg, 0.45 mmol), palladium(II) acetate (10 mg, 0.045 mmol), tri-*o*-tolylphosphine (42 mg, 0.14 mmol), 2.0 M aqueous Na_2CO_3 (0.25 mL, 0.50 mmol), and H_2O (0.5 mL) to yield 184 mg (70%) of **29b** as a white crystalline solid: mp 94−96 °C; R_f = 0.3 (5:1 hexane/acetone).

3-(2-Furyl)-2,5,6-trichloro-1-(2-deoxy-*β*-D-ribofuranosyl)-indole (30a). Compound **29a** (142 mg, 0.22 mmol) was treated as per **22** above with sodium methoxide (30 mg, 0.56 mmol) and recrystallized from 10% MeOH/$CHCl_3$ and hexane to yield 67 mg (75%) of **30a** as a white crystalline solid: mp 140−141 °C; R_f = 0.5 (10% MeOH/$CHCl_3$). Anal. ($C_{17}H_{14}Cl_3NO_4 \cdot {}^1/_4H_2O$) C, H, N.

3-(3-Thienyl)-2,5,6-trichloro-1-(2-deoxy-β-D-ribofuranosyl)indole (30b). Compound **29b** (159 mg, 0.24 mmol) was treated as per **22** above with sodium methoxide (30 mg, 0.56 mmol) and recrystallized from 10% MeOH/$CHCl_3$ and hexane to yield 84 mg (82%) of **30b** as a white crystalline solid: mp 144−145 °C; R_f = 0.5 (10% MeOH/$CHCl_3$). Anal. ($C_{17}H_{14}Cl_3NO_3S\cdot{}^1/_4H_2O$) C, H, N.

3-Acetyl-2-bromo-5,6-dichloroindole (32). 2-Bromo-5,6-dichloroindole[13] (**31**, 1.25 g, 4.7 mol) was dissolved in dry CH_2Cl_2 (25 mL) to which was added tin tetrabromide (2.50 g, 5.7 mmol). The suspension was stirred for 10 min at room temperature until the solids had completely dissolved, then acetyl bromide (0.37 mL, 0.62 g, 5.0 mmol) was added in one portion, followed by nitromethane (25 mL). The resulting dark solution was stirred at room temperature for 20 min. Water (25 mL) was then added, and the biphasic mixture was stirred vigorously for 10 min. The mixture was poured into brine (200 mL) and EtOAc (550 mL), and the mixture was heated on a steam bath until the solids had mostly dissolved. The aqueous layer was separated and discarded, and the organic layer was heated to reflux on a steam bath. The organic suspension was filtered, and the filtrate was evaporated to approximately 50 mL. The resulting solids were filtered and rinsed with cold EtOAc to yield 1.05 g (72%) of **32** as a pale-pink powder: mp >285 °C (dec); R_f = 0.6 (1:1 hexane/EtOAc).

3-Acetyl-2-bromo-5,6-dichloro-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-β-D-ribofuranosyl]indole (33). Compound **32** (0.92 g, 3.0 mmol) was suspended in dry THF (150 mL) to which was added sodium hydride (60% in mineral oil, 200 mg, 5.0 mmol). The suspension was stirred at room temperature for 15 min until gas evolution had ceased, then filtered into a suspension of 3,5-di-(*O*-*p*-toluoyl)-2-deoxy-α-D-ribofuranosyl chloride[27] (**20**, 1.22 g, 3.1 mmol) in dry toluene (150 mL). The resulting solution was stirred at room temperature for 16 h, then evaporated to dryness. The residue was suspended in brine (90 mL) and water (10 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield an orange oil. The oil was dissolved in $CHCl_3$ (3 mL) and subjected to column chromatography (50 mm × 450 mm) on silica gel with 2:1 hexane/EtOAc. Fractions containing product were pooled and evaporated to yield a pale-yellow solid. This solid was recrystallized from EtOAc/hexane to yield 1.13 g (57%) of **33** as a white crystalline solid: mp 100−101 °C; R_f = 0.4 (2:1 hexane/EtOAc).

3-Acetyl-2-bromo-5,6-dichloro-1-(2-deoxy-β-D-ribofuranosyl)indole (34). Compound **33** (1.24 g, 1.7 mmol) was suspended in absolute MeOH (75 mL) to which was added sodium methoxide (250 mg, 4.6 mmol). The suspension was stirred at room temperature for 2 h, after which time the solution first clarified and then a precipitate formed. Water (100 mL) was added, and the suspension was evaporated until no more MeOH remained. The remaining solids were filtered and recrystallized twice from MeOH/H_2O to yield 475 mg (68%) of **34** as a white crystalline solid: mp slow >235 °C (dec); R_f = 0.5 (10% MeOH/$CHCl_3$). Anal. ($C_{15}H_{14}BrCl_2NO_4$) C, H, N.

Biological Evaluation. Cell Culture Procedures. The routine growth and passage of KB, BSC-1, and HFF cells were 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.[32]

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.[33] High-titer HSV-1 stocks were prepared by infecting KB cells at a moi of <0.1 also as detailed previously.[33] 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.[34] 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 for 2 or 3 days for HSV-1. Plaques were enumerated under 20-fold magnification in wells having the dilution that 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, 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 and stained with crystal violet and microscopic plaques were 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[35] 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, the 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 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. (i) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[33] (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.[36] 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 linear regression of the percent inhibition of parameters derived in the preceding assays against $\log_{10}$ drug concentrations. Fifty percent inhibitory concentrations (IC_{50} values) 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.

HCMV Time-of-Addition Study. As described above, HFF cells at 10000 cells per well in 96-well cluster plates were infected with HCMV (Towne strain) at 0.5 pfu/cell. At infection and the indicated times postinfection, media were replaced with either fresh media or media containing selected

virus inhibitory but nontoxic drug concentrations plus 0.5% methylcellulose and 5% FBS. After incubation at 37 °C for 96 h in an atmosphere of 5% CO_2, plates were frozen at −80 °C. They were subsequently thawed, and the titer in each well was determined by plaque assay in 96-well plates as we have described previously.[34] Drug effects on HCMV titers were calculated as a percentage of reduction in titer in the presence of each drug compared to the titer observed in the absence of drug at each time point. Data are presented as the mean duplicate results for each drug at each time point.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR for all new compounds and HRMS and elemental analysis results for all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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