Synthesis and Antiviral Activity of 3-Formyl- and 3-Cyano-2,5,6-trichloroindole Nucleoside Derivatives

John D. Williams,[†] Jiong J. Chen,[‡] John C. Drach,[†,§] and Leroy B. Townsend*[,†,‡]

Department of Medicinal Chemistry, College of Pharmacy, Department of Chemistry, College of Literature, Sciences and the Arts, and Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, Michigan 48019

Received February 13, 2004

A series of trichlorinated 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 previously reported 3-formyl-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (FTCRI) and its 3-cyano homologue (CTCRI) were chemically modified at the 3-position. The formation of hydrazones and oximes of FTCRI was accomplished by a dehydrative addition of the appropriate hydrazine or hydroxylamine derivatives, respectively. A carboxamide oxime and imidate were synthesized from CTCRI by the addition of hydroxylamine or methanol, respectively, to the 3-nitrile substituent. Analogues synthesized from FTCRI generally had less antiviral activity than either FTCRI or CTCRI. However, the derivatives of CTCRI were potent and selective inhibitors of HCMV in vitro. The analogue 2,5,6-trichloro-1-(β-D-ribofuranosyl)indole-3-carboxamide oxime was especially selective (HCMV $IC_{50} = 0.30$ μM, $CC_{50} > 100$ μM). 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 (GCV),[4] valganciclovir,[5] cidofovir,[6] foscarnet,[7] and fomivirsen.[8] However, all of these compounds suffer limitations including poor oral 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 very likely.

Studies in our laboratories on new inhibitors of HCMV have led to the discovery that the benzimidazole nucleoside 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole[9] (TCRB, **1**, Figure 1) was a potent and selective inhibitor of HCMV replication in vitro but was rapidly degraded in vivo.[10] We have previously described a series of chlorinated indole nucleosides that are potent and selective inhibitors of HCMV replication[11] and should be resistant to the glycosidic bond cleavage that results in the inactivation of TCRB. This series of trichlorinated indole nucleoside analogues included 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). FTCRI and CTCRI were very amenable to further modifications at the 3-position, which broadened the structure−activity relationship (SAR) for this series. Derivatives of aromatic aldehydes (namely, hydrazones and oximes) are easily synthesized, and aromatic nitriles provide derivatives such as imidates and carboxamide oximes. All of these derivatives have potential hydrogen-bonding capabilities that we have previously shown[11] are required for antiviral activity in this polychlorinated indole nucleoside series. This prompted us to initiate studies into the modifications of FTCRI and CTCRI at the 3-position of the heterocycle.

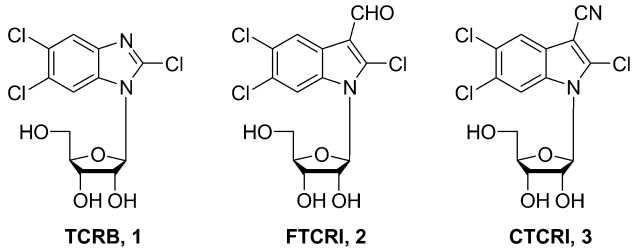


Figure 1. TCRB and analogous chlorinated indole nucleosides.

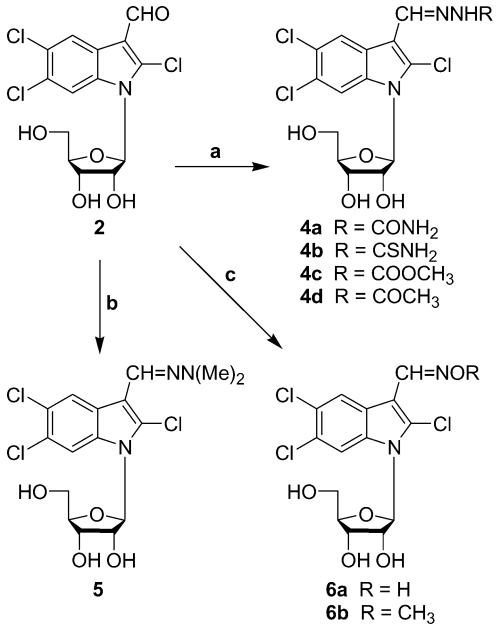
Results and Discussion

Chemistry. Dehydrative addition of substituted hydroxylamine and hydrazine derivatives to aldehydes is a very well-known process. Nitrophenyl hydrazones, for example, are commonly synthesized as derivatives that can be easily crystallized.[12] We did not require the steric bulk of this type of derivative and elected to prepare

* Corresponding author. Mailing address: Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, 428 Church St., Ann Arbor, MI 48109-1065. Fax: (734) 763-5633. E-mail: ltownsen@umich.edu.
[†] Department of Medicinal Chemistry.
[§] Department of Biologic and Materials Sciences.
[‡] Department of Chemistry.

10.1021/jm040032n CCC: $27.50
Published on Web 10/12/2004

Scheme 1[a]



[a] Reagents and conditions: (a) H_2NNHR, MeOH/H_2O, 20−60 °C, 16 h; (b) $H_2NN(Me)_2$, H_2O, 20 °C, 16 h; (c) $H_2NOR{\cdot}HCl$, K_2CO_3, MeOH/H_2O, 20 °C, 16 h.

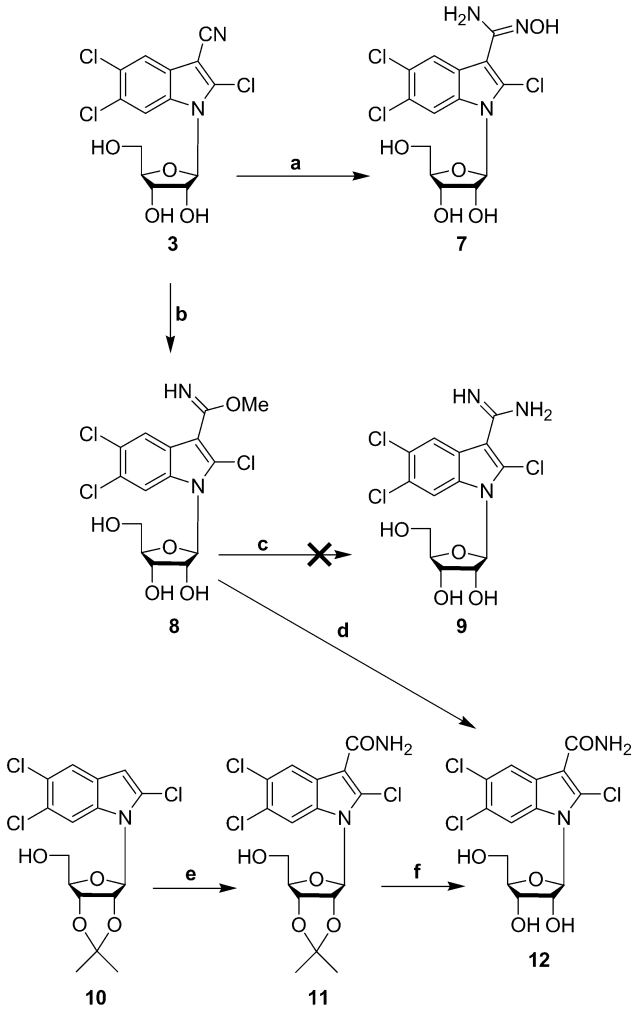
hydrazone and oxime analogues, which would be relatively small and should maintain the hydrogen-bonding capacity, which appears to be necessary for antiviral activity.

FTCRI (**2**, Scheme 1) was treated with semicarbazide (prepared in situ from the HCl salt) or thiosemicarbazide to provide the semicarbazone **4a** and thiosemicarbazone **4b**, respectively, in good yield. The methyl carbazate **4c** and acetylhydrazone **4d** were synthesized in a similar manner from **2** and methyl hydrazinocarboxylate and acethydrazide, respectively. In this case, heating was required to drive the reaction to completion. Simple hydrazine derivatives without electron-withdrawing substituents have been shown to cyclize upon reaction with **2** to form novel pyrazolo[3,4-*b*]indole nucleosides.[13] However, a condensation of FTCRI (**2**) and *asym*-dimethylhydrazine (in which one of the nitrogen atoms is fully substituted) did provide the hydrazone analogue 3-[*N*-(dimethylamino)iminomethylidene]-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (**5**).

Treatment of FTCRI (**2**) with hydroxylamine (again, generated in situ from the HCl salt) provided the oxime **6a** in good yield. In contrast to the case of the simple hydrazone derivatives, the oxime did not cyclize to the corresponding isoxazolo[5,4-*b*]indole nucleoside even under forcing conditions. The corresponding condensation of **2** with methoxylamine provided the oxime ester **6b**.

The addition of nucleophiles to aromatic nitriles has also been well demonstrated.[14] Powerful nucleophiles such as hydrazine and hydroxylamine can be used directly to provide the corresponding carboxamidrazones and carboxamide oximes,[15] respectively. The carboxamide oxime **7** was synthesized by a direct reaction of CTCRI (**3**) and hydroxylamine (Scheme 2). Surprisingly, methoxylamine did not react with **3**, even after prolonged heating. On the other hand, the reaction of **3** with hydrazine hydrate did occur but produced a

Scheme 2[a]

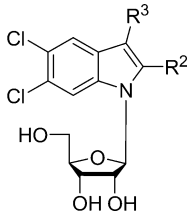


[a] Reagents and conditions: (a) H_2NOH, MeOH, 20 °C, 16 h; (b) HCl, MeOH, 20 °C, 24 h; (c) NH_3, MeOH, 0 °C, 60 min; (d) NH_4OAc, DMF, 85 °C, 4 h; (e) TFAA, CH_2Cl_2, 20 °C for 1 h, then CSI, CH_2Cl_2, 20 °C for 90 min, then H_2O for 15 min; (f) 90% TFA, 20 °C, 30 min.

tricyclic pyrazolo[3,4-*b*]indole nucleoside, in a manner similar to that cited above.[13]

Weak nucleophiles can also add to aromatic nitriles under acid catalysis. The imidate methyl 2,5,6-trichloro-1-(β-D-ribofuranosyl)indole-3-formimidate (**8**) was synthesized by the reaction of **3** with dry methanol using dry HCl gas to catalyze the addition. Our attempts to convert **8** into the amidine **9** were unsuccessful using either methanolic ammonia or ammonium acetate in DMF. The reaction of **8** with methanolic ammonia led to the decomposition of starting material. However, the carboxamide **12** was synthesized in low yield after a prolonged reaction of **8** with ammonium acetate in DMF. This material was found to be equivalent to a sample of carboxamide **12** synthesized via an alternative route beginning from the protected 2,5,6-trichloroindole nucleoside **10**.[16] Compound **10** was protected as the 5′-*O*-trifluoroacetate ester, then treated with chlorosulfonyl isocyanate followed by aqueous hydrolysis to provide **11** in moderate yield. The isopropylidene protecting group was removed with 90% aqueous trifluoroacetic acid to yield 2,5,6-trichloro-1-(β-D-ribofuranosyl)indole-3-carboxamide (**12**).

Table 1. Antiviral Activity and Cytotoxicity of 3-Substituted Indole Nucleosides

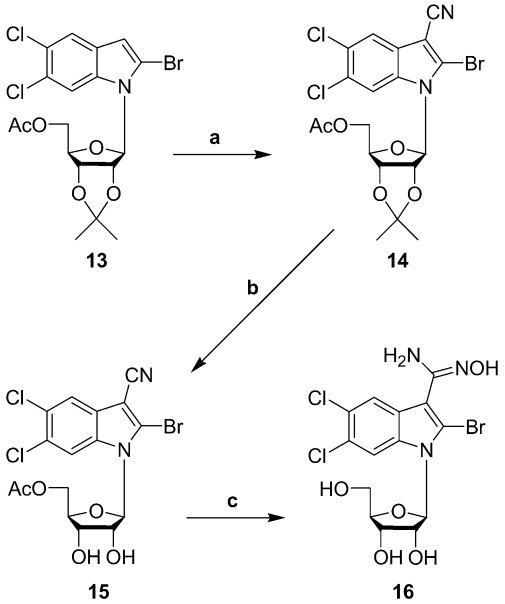


			50% inhibitory concentration (μM)			
			antiviral		cytotoxicity	
no.	R^2	R^3	HCMV plaque[a]	HSV-1 ELISA[b,d]	HFF visual[c,d]	KB growth[c,d]
2	−Cl	−CHO	0.23	40	45	45
3	−Cl	−CN	0.55	15	32	65
4a	−Cl	−CH=NNHCONH$_2$	3.0	>100	100	>100
4b	−Cl	−CH=NNHCSNH$_2$	2.2	>100	100	65
4c	−Cl	−CH=NNHCOOCH$_3$	1.7	30	32	20
4d	−Cl	−CH=NNHCOCH$_3$	1.7	30	32	9
5	−Cl	−CH=NN(Me)$_2$	0.27	15	32	70
6a	−Cl	−CH=NOH	3.9	5.0	20	25
6b	−Cl	−CH=NOCH$_3$	40	15	32	70
7	−Cl	−C(NH$_2$)=NOH	0.30	70	100	>100
8	−Cl	−C(OCH$_3$)=NH	0.36	70	100	50
12	−Cl	−CONH$_2$	2.0	40	45	45
16	−Br	−C(NH$_2$)=NOH	0.41	>100	>100	35
TCRB[e]			2.9	102	238	210
BDCRB[e]			0.70	130	118	>100
GCV[f]			7.4	3.5	>100	>100

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

It has been established that the 2-bromo analogue of TCRB (i.e., 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)-benzimidazole, BDCRB) is more active and selective against HCMV than TCRB itself.[9] The structural similarities of the indole nucleosides to the benzimidazole nucleosides prompted us to initiate studies on the synthesis of 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)-indole-3-carboxamide oxime (**16**). This compound was chosen because of the excellent activity and selectivity obtained for its 2-chloro congener, the carboxamide oxime **7**. The protected 2-bromo-5,6-dichloroindole nucleoside analogue **13**[16] was treated with chlorosulfonyl isocyanate (CSI) followed by DMF to provide the nitrile **14** (Scheme 3). Removal of the isopropylidene protecting group from **14**, followed by treatment of the intermediate **15** with hydroxylamine provided the carboxamide oxime **16** in good yield.

Scheme 3[a]



[a] Reagents and conditions: (a) CSI, CH_2Cl_2, 20 °C for 16 h, then DMF, 20 °C for 60 min; (b) 90% TFA, 20 °C, 2 min; (c) H_2NOH, MeOH, 20 °C, 4 d.

Biological Evaluation. The compounds synthesized above were tested for antiviral activity against HCMV and HSV-1 and for cytotoxicity. Most of the compounds demonstrated weak activity against HSV-1, but this generally occurred at drug concentrations approaching cytotoxic levels. This suggests that HSV-1 activity was a manifestation of cytotoxicity and not specific viral inhibition. The derivatives synthesized from FTCRI (**2**) generally had less anti-HCMV activity than **2**, and most were as cytotoxic as **2** (see compounds **4a**−**6b**, Table 1). The semicarbazide and thiosemicarbazide analogues **4a** and **4b** were not as toxic as **2**, but the activity against HCMV (and thereby the selectivity) was not as great as FTCRI. The dimethyl hydrazone **5** had HCMV activity and cytotoxicity that were very similar to compound **2**.

Better results were obtained from those derivatives synthesized from the 3-nitrile analogue **3**. Both the carboxamide oxime **7** and the methyl imidate **8** were more potent against HCMV and less toxic than the corresponding nitrile. However, carboxamide **12** was less potent and less selective than nitrile **3**. In contrast to the benzimidazole nucleoside series where the 2-bro-

mo homologue is more active then the 2-chloro lead compound (TCRB), the 2-bromo carboxamide oxime analogue **16** was no more active then the 2-chloro analogue **7**.

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. ^{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 was performed at the University of Michigan Department of Chemistry Mass Spectrometry facility. Elemental analysis was performed at the University of Michigan Chemistry Department Elemental Analysis facility.

3-[(4-Semicarbazono)methylidene]-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (4a). Compound **2**[11] (76 mg, 0.20 mmol) was dissolved in methanol (3 mL) to which was added a solution of semicarbazide hydrochloride (2.0 M, 0.20 mL, 0.40 mmol) and 2 drops of pyridine. The solution was warmed to 60 °C for 10 min, then stirred at room temperature for 16 h, during which time a fine white precipitate had developed. The suspension was cooled at 4 °C for 4 h; then the solids were collected by filtration and rinsed with cold water. The solids were dried under vacuum (0.5 mmHg, 65 °C) for 12 h to yield a white crystalline solid, which was recrystallized from MeOH to yield 49 mg (56%) of **4a** as a white powder: mp dec >250 °C; R_f 0.1 (10% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.19 (s, 1H, D_2O exch.), 8.50 (s, 1H), 8.48 (s, 1H), 8.39 (s, 1H), 8.07 (s, 1H), 6.40 (s, 2H, D_2O exch.), 5.91 (d, 1H), 5.38 (d, 1H, D_2O exch.), 5.35 (t, 1H, D_2O exch.), 5.23 (d, 1H, D_2O exch.), 4.41 (q, 1H), 4.13 (s, 1H), 3.97 (d, 1H), 3.70 (d, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.51, 133.73, 133.05, 128.96, 125.99, 124.92, 123.71, 122.27, 115.13, 108.53, 88.71, 85.99, 71.29, 69.64, 61.10. HRMS (EI) *m/z* calcd for $C_{15}H_{15}Cl_3N_4O_5$ 436.0108, found 436.0090. Anal. calcd for $C_{15}H_{15}Cl_3N_4O_5$: C, H, N.

3-[(4-Thiosemicarbazono)methylidene]-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (4b). Compound **2**[11] (94 mg, 0.25 mmol) was dissolved in methanol (10 mL) to which was added thiosemicarbazide (24 mg, 0.26 mmol). The solution was stirred at room temperature for 16 h, during which time a fine white precipitate had developed. The suspension was cooled at 4 °C for 4 h; then the solids were collected by filtration and rinsed with cold water. The solids were dried under vacuum (0.5 mmHg, 65 °C) for 12 h to yield 72 mg (65%) of **4b** as a white powder: mp dec >225 °C; R_f 0.7 (20% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.36 (s, 1H, D_2O exch.), 8.53 (s, 1H), 8.48 (s, 1H), 8.35 (s, 1H), 8.19 (s, 1H, D_2O exch.), 7.84 (s, 1H, D_2O exch.), 5.94 (d, 1H), 5.41 (d, 1H, D_2O exch.), 5.37 (t, 1H, D_2O exch.), 5.25 (d, 1H, D_2O exch.), 4.42 (q, 1H), 4.15 (m, 1H), 3.99 (m, 1H), 3.72 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 177.23, 137.32, 133.09, 126.22, 125.28, 123.50, 122.63, 115.06, 108.11, 88.84, 86.11, 71.35, 69.63, 61.08. HRMS (ES) *m/z* calcd for $C_{15}H_{15}Cl_3N_4O_4S$·Na 472.9777, found 474.9789. Anal. calcd for $C_{15}H_{15}Cl_3N_4O_4S$·1/4 MeOH: C, H, N.

3-[*N*-(Methoxycarbonylamino)iminomethylidene]-2,5,6-trichloro-1-(β-d-ribofuranosyl)indole (4c). Compound **2**[11] (122 mg, 0.32 mmol) was dissolved in MeOH (10 mL) to which was added methyl hydrazinocarboxylate (115 mg, 1.3 mmol). The resulting solution was heated on a 60 °C oil bath for 16 h, then cooled to room temperature, and poured into 100 mL of water, and the solvent was evaporated to approx 50 mL. The resulting suspension was cooled to 4 °C, then filtered, and the solids were rinsed with cold water (25 mL). The solids were recrystallized from boiling EtOAc/hexane to yield 123 mg (85%) of **4c** as a pale pink crystalline solid: mp 217−219 °C; R_f 0.2 (10% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.17 (b, 1H, D_2O exch.), 8.54 (s, 1H), 8.42 (s, 1H), 8.22 (b, 1H), 5.92 (d, 1H), 5.42−5.37 (m, 2H, D_2O exch.), 5.25 (d, 1H, D_2O exch.), 4.43 (q, 1H), 4.15 (d, 1H), 3.98 (d, 1H), 3.72 (b, 5H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 153.88, 133.17, 129.33, 126.08, 124.96, 124.06, 122.17, 115.34, 108.59, 88.76, 86.03, 71.30, 69.73, 69.62, 61.07, 52.04. HRMS (ES) *m/z* calcd for $C_{16}H_{16}Cl_3N_3O_6$·Na 474.0002, found 473.9999. Anal. calcd for $C_{16}H_{16}Cl_3N_3O_6$·$^1/_4H_2O$: C, H, N.

3-[*N*-(Acetylamino)iminomethylidene]-2,5,6-trichloro-1-(β-d-ribofuranosyl)indole (4d). Compound **2**[11] (109 mg, 0.29 mmol) was dissolved in MeOH (5 mL) to which was added acethydrazine (85 mg, 1.1 mmol). The resulting solution was heated on a 45 °C oil bath for 16 h, then cooled to room temperature, and poured into 15 mL of water. The resulting suspension was cooled to 4 °C, then filtered, and the solids were rinsed with cold water (25 mL). The solids were 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 product were pooled and evaporated to yield a white crystalline solid, which was recrystallized from acetone/MeOH to yield 70 mg (56%) of **4d** as a white microcrystalline solid, which is an inseparable mixture of isomers in a ratio of 60:40: mp 270−271 °C; R_f 0.2 (10% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6): (major isomer) δ 11.25 (s, 1H, D_2O exch.), 8.56 (s, 1H), 8.27 (s, 1H), 8.18 (s, 1H), 5.94 (d, 1H), 5.40−5.36 (m, 2H, D_2O exch.), 5.25 (d, 1H, D_2O exch.), 4.42 (q, 1H), 4.13 (s, 1H), 3.98 (s, 1H), 3.70-(s, 2H), 2.24 (s, 3H); (minor isomer) δ 11.41 (s, 1H, D_2O exch.), 8.56 (s, 1H), 8.44 (s, 1H), 8.32 (s, 1H), 5.94 (d, 1H), 5.40−5.36 (m, 2H, D_2O exch.), 5.25 (d, 1H, D_2O exch.), 4.42 (q, 1H), 4.13 (s, 1H), 3.98 (s, 1H), 3.70(s, 2H), 1.95 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): (major isomer) δ 171.39, 135.98, 133.15, 129.73, 126.01, 124.96, 123.86, 121.69, 115.41, 108.31, 88.78, 86.09, 71.41, 69.66, 61.10, 20.36; (minor isomer) δ 165.24, 138.70, 133.12, 129.82, 126.06, 124.96, 123.99, 122.17, 115.33, 108.51, 88.78, 86.09, 71.41, 69.66, 61.10, 21.67. HRMS (ES) *m/z* calcd for $C_{16}H_{16}Cl_3N_3O_5$·Na 458.0053, found 458.0043. Anal. calcd for $C_{16}H_{16}Cl_3N_3O_5$: C, H, N.

3-[*N*-(Dimethylamino)iminomethylidene]-2,5,6-trichloro-1-(β-d-ribofuranosyl)indole (5). Compound **2**[11] (100 mg, 0.26 mmol) was dissolved in MeOH (5 mL) to which was added *asym*-dimethylhydrazine (0.5 mL). The resulting solution was stirred at room temperature for 16 h, then evaporated under vacuum to yield a yellow 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 87 mg (78%) of **5** as a white powder: mp dec >170 °C; R_f 0.4 (10% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.44 (s, 1H), 8.36 (s, 1H), 7.35 (s, 1H), 5.91 (d, 1H), 5.36−5.31 (m, 2H, D_2O exch.), 5.21 (d, 1H, D_2O exch.), 4.42 (q, 1H), 4.13 (s, 1H), 3.96 (d, 1H), 3.70 (b, 2H), 2.93 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 133.01, 125.64, 125.62, 125.37, 124.26, 124.04, 121.97, 114.85, 110.14, 88.58, 85.75, 71.18, 69.66, 61.14, 42.58. HRMS (ES) *m/z* calcd for $C_{16}H_{18}Cl_3N_3O_4$·Na 444.0261, found 444.0269. Anal.calcd for $C_{16}H_{18}Cl_3N_3O_4$: C, H, N.

3-(*N*-Hydroxyiminomethylidene)-2,5,6-trichloro-1-(β-d-ribofuranosyl)indole (6a). To a solution of compound **2**[11] (170 mg, 0.45 mmol) in MeOH (10 mL) was added a solution of methoxylamine hydrochloride (39 mg, 0.56 mmol) and

sodium bicarbonate (49 mg, 0.48 mmol) in water (2.0 mL). The resulting mixture was stirred at room temperature for 16 h; then the solvent was evaporated to provide a pale yellow residue. The residue was suspended in 20 mL of 5% aqueous sodium thiosulfate and extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine (25 mL), dried over $MgSO_4$, filtered, and evaporated to yield a pink residue. The residue was subjected to column chromatography (40 mm × 350 mm) on C18 reverse-phase silica gel with 75% MeOH/H_2O. The appropriate UV-active fractions were pooled and evaporated to yield 124 mg (70%) of **6a** as a white powder. A portion was recrystallized from Et_2O/hexane to yield a white crystalline solid: R_f 0.2 (10% MeOH/$CHCl_3$); mp 208−209 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (s, 1H), 8.54 (s, 1H), 8.22 (s, 1H), 8.19 (s, 1H), 5.93 (d, 1H), 5.37 (m, 2H, D_2O exch.), 5.25 (m, 1H, D_2O exch.), 4.43 (m, 1H), 4.16 (m, 1H), 3.99 (m, 1H), 3.73 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 141.30, 133.03, 128.48, 125.85, 124.73, 124.05, 121.76, 115.31, 106.70, 88.81, 86.02, 71.39, 69.65, 61.11. HRMS (EI) *m/z* calcd for $C_{14}H_{13}Cl_3N_2O_5$ 393.9890, found 393.9892. Anal. calcd for $C_{14}H_{13}Cl_3N_2O_5 \cdot 1/2\ Et_2O$: C, H, N.

3-(*N*-Methoxyiminomethylidene)-2,5,6-trichloro-1-(β-d-ribofuranosyl)indole (6b). To a solution of compound **2**[11] (103 mg, 0.27 mmol) in MeOH (5 mL) was added a solution of methoxylamine hydrochloride (27 mg, 0.32 mmol) and sodium bicarbonate (22 mg, 0.26 mmol) in water (1.0 mL). The resulting mixture was stirred at room temperature for 16 h; then the solvent was evaporated to provide a pale yellow residue. The residue was suspended in 10 mL of 5% aqueous sodium thiosulfate and extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed with brine (10 mL), dried over $MgSO_4$, filtered, and evaporated to yield a yellow residue. The residue was subjected to column chromatography (40 mm × 350 mm) on C18 reverse-phase silica gel with 80% MeOH/H_2O. The appropriate UV-active fractions were pooled and evaporated to yield a white powder. The powder was recrystallized from Et_2O to yield 45 mg (41%) of **6b** as a white crystalline solid: R_f 0.4 (10% MeOH/$CHCl_3$); mp 197−198 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.57 (s, 1H), 8.27 (s, 1H), 8.17 (s, 1H), 5.93 (d, 1H), 5.42 (d, 1H, D_2O exch.), 5.40 (t, 1H, D_2O exch.), 5.27 (d, 1H, D_2O exch.), 4.42 (q, 1H), 4.14 (m, 1H), 4.00 (d, 1H), 3.96 (s, 3H), 3.71 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 141.84, 133.04, 129.74, 126.09, 125.03, 123.82, 121.70, 115.46, 105.59, 88.85, 86.13, 71.43, 69.65, 61.84, 61.09. HRMS (EI) *m/z* calcd for $C_{15}H_{15}Cl_3N_2O_5$ 408.0047, found 408.0047. Anal. calcd for $C_{15}H_{15}Cl_3N_2O_5$: C, H, N.

2,5,6-Trichloro-1-(β-d-ribofuranosyl)indole-3-carboxamide Oxime (7). Compound **3**[11] (107 mg, 0.28 mmol) was dissolved in dry MeOH (5 mL) and dry DMF (1 mL) to which were added hydroxylamine hydrochloride (0.50 g, 7.2 mmol) and potassium hydroxide (0.39 g, 7.0 mmol). The resulting suspension was stirred at room temperature for 16 h, then poured into brine (25 mL) and water (25 mL), and the resulting aqueous suspension was extracted with EtOAc (2 × 100 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield a clear oil. The oil was dissolved in MeOH (1 mL) and subjected to column chromatography (40 mm × 350 mm) on C18 reverse-phase silica gel with 75% MeOH/H_2O. Fractions containing product were pooled and evaporated to yield a light tan solid, which was recrystallized from MeOH/H_2O to yield 81 mg (70%) of **7** as a light tan solid: mp dec >150 °C; R_f 0.4 (20% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.71 (s, 1H, D_2O exch.), 8.47 (s, 1H), 7.97 (s, 1H), 5.95 (d, 1H), 5.78 (s, 2H, D_2O exch.), 5.36−5.33 (m, 2H, D_2O exch.), 5.23 (d, 1H, D_2O exch.), 4.44 (q, 1H), 4.14 (m, 1H), 3.96 (d, 1H), 3.71 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 145.92, 132.24, 125.86, 125.78, 125.10, 123.92, 121.27, 114.85, 107.15, 88.61, 85.77, 71.24, 69.59, 61.09. HRMS (EI) *m/z* calcd for $C_{14}H_{14}Cl_3N_3O_5$ 408.9999, found 408.9999. Anal. calcd for $C_{14}H_{14}Cl_3N_3O_5$: C, H, N.

Methyl 2,5,6-trichloro-1-(β-d-ribofuranosyl)indole-3-formimidate (8). Compound **3**[11] (107 mg, 0.28 mmol) was dissolved in dry MeOH (10 mL), which was then cooled to 0 °C in an ice bath. Hydrogen chloride gas was slowly bubbled through the solution for 2 h, the reaction vessel was tightly capped, and the resulting solution was stirred at room temperature for 24 h. The acidic solution was diluted with Et_2O (20 mL) and evaporated to dryness. The residual solid was suspended in 10% aqueous $NaHCO_3$ (100 mL) and extracted with EtOAc (3 × 100 mL with vigorous shaking). The combined organic extracts were dried over $MgSO_4$, filtered, 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 product were pooled and evaporated to yield 64 mg (58%) of **8** as a white crystalline solid: mp 248−249 °C; R_f 0.6 (20% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.55 (s, 1H), 8.43 (s, 1H, D_2O exch.), 7.96 (s, 1H), 5.98 (d, 1H), 5.39 (d, 1H, D_2O exch.), 5.37 (t, 1H, D_2O exch.), 5.24 (d, 1H, D_2O exch.), 4.41 (q, 1H), 4.14 (s, 1H), 3.98 (d, 1H), 3.85 (s, 3H), 3.71 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.40, 132.01, 128.23, 125.67, 124.82, 124.50, 121.32, 115.19, 106.29, 88.73, 86.08, 71.40, 69.50, 60.99, 52.45. HRMS (EI) *m/z* calcd for $C_{15}H_{15}Cl_3N_2O_5$ 408.0047, found 408.0043. Anal. calcd for $C_{15}H_{15}Cl_3N_2O_5$: C, H, N.

2,5,6-Trichloro-1-(2,3-*O*-isopropylidene-β-d-ribofuranosyl)indole-3-carboxamide (11). Compound **10**[16] (172 mg, 0.40 mmol) was dissolved in CH_2Cl_2 (3 mL) to which was added trifluoroacetic anhydride (0.17 mL, 1.2 mmol). The solution was stirred at room temperature for 1 h, then evaporated to dryness. The residue was dried in vacuo (0.5 mmHg, 30 °C) for 1 h, then the residue was dissolved in CH_2Cl_2, and chlorosulfonyl isocyanate (0.052 mL, 0.60 mmol) was added in one portion. The resulting yellow solution was stirred at room temperature for 90 min. H_2O (2 mL) was then added, and the mixture was stirred vigorously for an additional 15 min. The biphasic mixture was extracted with EtOAc (3 × 5 mL), the combined extracts were washed with brine (10 mL), dried over $MgSO_4$, filtered, and evaporated to yield a yellow residue. The residue was subjected to column chromatography (20 mm × 100 mm) on silica gel with 2:3 hex/EtOAc. Fractions containing product were pooled and evaporated to yield 102 mg (59%) of **11** as a white solid: mp 193−194 °C; R_f 0.2 (3:2 hex/EtOAc). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.27 (s, 1H), 8.08 (s, 1H), 7.60 (b, 1H, D_2O exch.), 7.44 (b, 1H, D_2O exch.), 6.13 (d, 1H), 5.38 (t, 1H, D_2O exch.), 5.01 (m, 2H, 4.14 (m, 1H), 3.37 (m, 2H), 1.56 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.33, 132.04, 126.89, 125.96, 125.76, 125.01, 121.47, 114.75, 114.61, 109.51, 89.89, 83.98, 81.73, 79.39, 60.39, 27.16, 25.31. HRMS (ES) *m/z* calcd for $C_{17}H_{17}Cl_3N_2O_5 \cdot H$ 435.0281, found 435.0273.

2,5,6-Trichloro-1-(β-d-ribofuranosyl)indole-3-carboxamide (12). Method A. Compound **11** (60 mg, 0.14 mmol) was dissolved in 90% aqueous trifluoroacetic acid (2 mL) and stirred at room temperature for 30 min. The solvent was then removed under vacuum, and the residue subjected to column chromatography (10 mm × 100 mm) on silica gel with 10% MeOH/CH_2Cl_2. Fractions containing product were pooled and evaporated to yield 44 mg (81%) of **12** as a white powder: mp 256−257 °C; R_f 0.2 (10% MeOH/CH_2Cl_2). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.53 (s, 1H), 8.10 (s, 1H), 7.58 (b, 1H, D_2O exch.), 7.41 (b, 1H, D_2O exch.), 5.96 (d, 1H), 5.38 (m, 2H, D_2O exch.), 5.25 (d, 1H, D_2O exch.), 4.42 (m, 1H), 4.12 (m, 1H), 3.97 (m, 1H), 3.70 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.51, 132.19, 128.09, 126.00, 125.57, 124.76, 121.44, 115.21, 108.80, 88.66, 86.07, 71.33, 69.56, 61.00. Anal. calcd for $C_{14}H_{13}Cl_3N_2O_5$: C, H, N.

Method B. Compound **8** (103 mg, 0.25 mmol) was dissolved in DMF (10 mL) to which was added ammonium acetate (1.0 g, 13 mmol). The resulting suspension was heated in a sealed tube (25 mL) on an 85 °C oil bath for 4 h. The reaction was then cooled to room temperature, and the solvent was removed under vacuum (0.5 mmHg, 40 °C). The residual oil was poured into brine (50 mL) and extracted with EtOAc (3 × 25 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield an orange oil. The oil was dissolved in MeOH (1 mL) and subjected to column chromatography (40 mm × 350 mm) on C18 reverse-phase silica gel with 75%

MeOH/H_2O. Fractions containing product were pooled and evaporated to yield 13 mg (13%) of the product as a white powder: mp 255−257 °C; R_f 0.2 (10% MeOH/CH_2Cl_2). ^{1}H NMR and ^{13}C NMR match those obtained for method A above.

3-Cyano-2-bromo-5,6-dichloro-1-(2,3-*O*-isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl)indole (14). Compound **13**[16] (0.50 g, 1.0 mmol) was dissolved in dry CH_2Cl_2 (15 mL) to which was added chlorosulfonyl isocyanate (135 μL, 220 mg, 1.5 mmol). The resulting solution was stirred at room temperature for 16 h, then dry DMF (2 mL) was added, and the solution was stirred for an additional 1 h. Water (10 mL) was then added, and the biphasic suspension was stirred vigorously for 10 min. The mixture was poured into 10% $NaHCO_3$ (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine (20 mL), dried over $MgSO_4$, filtered, and evaporated to yield an orange oil. The oil was dissolved in $CHCl_3$ (1 mL) and subjected to column chromatography (40 mm × 350 mm) on silica gel with 2:1 hex/EtOAc. Fractions containing product were pooled and evaporated to yield 204 mg (41%) of **14** as a white crystalline solid: mp 151−152 °C; R_f 0.5 (2:1 hex/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.74 (s, 1H), 6.11 (d, 1H), 5.00 (dd, 1H), 4.94 (dd, 1H), 4.52 (dd, 1H), 4.44 (dd, 1H), 4.36 (q, 1H), 2.28 (s, 3H), 1.66 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (125 MHz,$CDCl_3$): δ 170.76, 133.02, 128.99, 128.50, 127.38, 122.69, 120.50, 116.84, 114.07, 113.32, 92.29, 92.14, 82.40, 81.67, 79.28, 62.91, 27.45, 25.60, 21.24.

3-Cyano-2-bromo-5,6-dichloro-1-(5-*O*-acetyl-β-D-ribofuranosyl)indole (15). Compound **14** (184 mg, 0.36 mmol) was dissolved in 90% aqueous trifluoroacetic acid (5 mL). The resulting solution was stirred at room temperature for 2 min, then evaporated to approx 1 mL. The remaining solution was poured into 10% aqueous $NaHCO_3$ (50 mL). The aqueous suspension was extracted with EtOAc (2 × 50 mL), and the combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield a white solid. The solid was recrystallized from boiling EtOAc and hexane to yield 149 mg (88%) of **15** as a white crystalline solid: mp 103−105 °C; R_f 0.5 (10% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.04 (s, 1H), 7.97 (s, 1H), 5.97 (d, 1H), 5.58 (d, 1H, D_2O exch.), 5.48 (d, 1H, D_2O exch.), 4.48 (dd, 1H), 4.39 (q, 1H), 4.27 (dd, 1H), 4.18 (q, 1H), 4.14 (m, 1H), 2.16 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.24, 132.95, 126.92, 126.81, 126.54, 125.65, 119.79, 114.75, 113.63, 91.19, 90.20, 82.81, 71.62, 68.85, 63.40, 20.84.

2-Bromo-5,6-dichloro-1-(β-D-ribofuranosyl)indole-3-carboxamide Oxime (16). A solution of hydroxylamine in absolute MeOH was prepared by adding potassium hydroxide (1.83 g, 33 mmol) to hydroxylamine hydrochloride (2.50 g, 36 mmol) dissolved in absolute MeOH (20 mL) with stirring for 10 min. The solids that had developed were collected by filtration and rinsed with cold absolute MeOH (10 mL), and the filtrate was used without further purification. Compound **15** (133 mg, 0.29 mmol) was dissolved in the crude solution of hydroxylamine in MeOH and stirred at room temperature for 96 h. The solvent was then removed under vacuum, and the residual solid was dissolved in brine (40 mL). The aqueous suspension 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 solid. The solid was dissolved in MeOH (1 mL) and subjected to column chromatography (40 mm × 350 mm) on silica gel with 20% MeOH/$CHCl_3$. Fractions containing product were pooled and evaporated to yield a pale yellow solid. The solid was dissolved in MeOH (1 mL) and subjected to column chromatography (40 mm × 350 mm) on C18 reverse-phase silica gel with 75% MeOH/H_2O. Fractions containing product were pooled and evaporated to yield a pale yellow solid, which was recrystallized from MeOH/H_2O to yield 54 mg (41%) of **16** as a pale yellow powder: mp slow dec >170 °C; R_f 0.5 (20% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.66 (s, 1H, D_2O exch.), 8.48 (s, 1H), 7.89 (s, 1H), 5.99 (d, 1H), 5.76 (s, 2H, D_2O exch.), 5.35 (t, 1H, D_2O exch.), 5.31 (d, 1H, D_2O exch.), 5.22 (d, 1H, D_2O exch.), 4.45 (q, 1H), 4.15 (t, 1H), 3.96 (d, 1H), 3.72 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 146.18, 133.16, 127.04, 124.88, 123.77, 120.74, 115.72, 114.86, 110.61, 90.17, 85.68, 71.21, 69.55, 61.07. HRMS (ES) *m/z* calcd. for $C_{14}H_{14}BrCl_2N_3O_5$·H 453.9572, found 453.9570. Anal. calcd for $C_{14}H_{14}BrCl_2N_3O_5$·1/4 MeOH: 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.[17]

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.[18] High-titer HSV-1 stocks were prepared by infecting KB cells at an moi of <0.1 pfu/cell also as detailed previously.[18] 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.[19] Briefly, HFF or BSC-1 cells were planted as described above in 96-well cluster dishes and incubated overnight at 37 °C. The next day cultures were inoculated with HCMV or HSV-1 and serially diluted 1:3 across the remaining eleven columns of the 96-well plate. After virus adsorption, the inoculum was replaced with fresh medium, and cultures were incubated for 7 days for HCMV or 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[20] to detect HSV-1. Ninety-six-well cluster dishes were planted with 10 000 BSC-1 cells per well in 200 μL per well of MEM(E) plus 10% calf serum. After overnight incubation at 37 °C, selected drug concentrations in quadruplicate and HSV-1 at a concentration of 100 pfu/well were added. Following a 3-day incubation at 37 °C, medium was removed, plates were blocked 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.[18] (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.[21] Briefly, 96-well cluster dishes were

planted with KB cells at 3000−5000 cells per well. After overnight incubation at 37 °C, test compound was added in quadruplicate at six to eight concentrations. Plates were incubated at 37 °C for 48 h in a CO_2 incubator, rinsed, fixed with 95% ethanol, and stained with 0.1% crystal violet. Acidified ethanol was added and plates read at 570 nm in a spectrophotometer designed to read 96-well ELISA assay plates.

Data Analysis. Dose response relationships were used to quantitate drug effects by linear regression of the percent inhibition of parameters derived in the preceding assays against $\log_{10}$ drug concentrations. Fifty percent inhibitory concentrations (IC_{50}'s) were calculated from the linear portions of the regression lines. Samples containing positive controls (acyclovir for HSV-1, GCV for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

Acknowledgment. We thank Julie M. Breitenbach and Kathy Z. Borysko for expert performance of antiviral and cytotoxicity assays. These studies were supported by Training Grant T32-GM07767 and Research Grant P01-AI46390 from the National Institutes of Health.

Supporting Information Available: CHN for all target compounds. This material is available free of charge via the Internet at http://www.acs.org.

References

(1) Pass, R. F. Cytomegalovirus. In *Fields Virology*, 4th. ed.; Knipe, D. M., Howley, P. M., Eds.; Lippincott, Williams & Wilkins: Philadephia, 2001; Chapter 77, pp 2675−2705.
(2) Alford, C. A.; Britt, W. J. Cytomegalovirus. In *The Human Herpesviruses*; Roizman, B., Whitley, R. J., Lopez, C., Eds.; Raven Press: New York, 1993; pp 227−256.
(3) Britt, W. J. Infections Associated with Human Cytomegalovirus. In *Herpesvirus Infections*; Glaser, R., Jones, J. F., Eds.; Marcel Dekker: New York, 1994; pp 59−116.
(4) Faulds, D.; Heel, R. C. Ganciclovir: A review of its Antiviral Activity, Pharmacokinetic Properties and Therapeutic Efficacy in Cytomegalovirus Infections. *Drugs* **1990**, *39*, 597−638.
(5) Curran, M.; Noble, S. Valganciclovir. *Drugs* **2001**, *61*, 1145−1150.
(6) Lea, A. P.; Bryson, H. M. Cidofovir. *Drugs* **1996**, *52*, 225−230.
(7) Wagstaff, A. J.; Bryson, H. M. Foscarnet: A Reappraisal of its Antiviral Activity, Pharmacokinetic Properties, and Therapeutic Use in Immunocompromised Patients with Viral Infections. *Drugs* **1994**, *48*, 199−226.
(8) Perry, C. M.; Barman-Balfour, J. A. Fomivirsen. *Drugs* **1999**, *57*, 375−380.
(9) 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.
(10) Good, S. S.; Owens, B. S.; Townsend, L. B.; Drach, J. C. The Disposition in Rats and Monkeys of 2-Bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BDCRB) and its 2,5,6-Trichloro Congener. *Antiviral Res.* **1994**, *23* (S), 103.
(11) Williams, J. D.; Chen, J. J.; Drach, J. C.; Townsend, L. B. Design, Synthesis, and Antiviral Activity of Certain 3-Substituted 2,5,6-Trichloroindole Nucleosides. *J. Med. Chem.* **2004**, *47*, xxxx−xxxx.
(12) Allen, C. F. H. The Identification of Carbonyl Compounds by Use of 2,4-Dinitrophenylhydrazine. *J. Am. Chem. Soc.* **1930**, *52*, 2955−2959.
(13) Williams, J. D.; Drach, J. C.; Townsend, L. B. Synthesis and Antiviral Evaluation of Some Novel Tricyclic Pyrazolo[3,4-*b*]-indole Nucleosides. *Nucleosides Nucleotides Nucleic Acids*, in press.
(14) Schaefer, F. C. Nitrile Reactivity. In *The Chemistry of the Cyano Group*; Rappoport, Z., Ed.; Interscience: New York, 1970; pp 239−305.
(15) Eloy, F.; Lenaers, R. The Chemistry of Amidoximes and Related Compounds. *Chem. Rev.* **1962**, *62*, 155−183.
(16) Chen, J. J.; Wei, Y.; Drach, J. C.; Townsend, L. B. Synthesis and Antiviral Evaluation of Trisubstituted Indole *N*-Nucleosides as Analogues of 2,5,6-Trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB). *J. Med. Chem.* **2000**, *43*, 2449−2456.
(17) Shipman, C., Jr.; Smith, S. H.; Carlson, R. H.; Drach, J. C. Antiviral Activity of Arabinofuranosyladenosine and Arabinofuranosylhypoxanthine in Herpes Simplex Virus-Infected KB Cells. *Antimicrob. Agents Chemother.* **1976**, *9*, 120−127.
(18) Turk, S. R.; Shipman, C., Jr.; Nassiri, M. R.; Genzlinger, 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.
(19) 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. *J. Virol. Methods* **1990**, *28*, 101−106.
(20) Prichard, M. N.; Shipman, C., Jr. A Three-Dimensional Model to Analyze Drug-Drug Interactions. *Antiviral Res.* **1990**, *14*, 181−206.
(21) Prichard, M. N.; Prichard, L. E.; Baguley, W. A.; Nassiri, M., R.; Shipman, C., Jr. Three-Dimensional Analysis of Synergistic Cytotoxicity of Ganciclovir and Zidovudine. *Antiviral Res.* **1991**, *35*, 1060−1065.

JM040032N