Brief Articles

Synthesis of Fluorosugar Analogues of 2,5,6-Trichloro-1-(β-D-ribofuranosyl)benzimidazole as Antivirals with Potentially Increased Glycosidic Bond Stability

Kristjan S. Gudmundsson,† George A. Freeman,§ John C. Drach, and Leroy B. Townsend*

Department of Medicinal Chemistry, College of Pharmacy, Department of Chemistry, College of Literature, Sciences and Arts, and Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, Michigan 48109-1065, and Division of Medicinal Chemistry, Glaxo Wellcome Inc., Research Triangle Park, North Carolina 27709

Received May 3, 1999

The metabolic instability in vivo of the glycosidic bond of 2,5,6-trichloro-1-(β-D-ribofuranosyl)-benzimidazole (TCRB) prompted us to design and synthesize the hitherto unreported fluorinated benzimidazole nucleosides 2,5,6-trichloro-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)benzimidazole, 2,5,6-trichloro-1-(3-deoxy-3-fluoro-β-D-xylofuranosyl)benzimidazole, and 2-bromo-5,6-dichloro-1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)benzimidazole. TCRB was converted into the 2′,5′-dityrityl and 3′,5′-dityrityl derivatives, which were fluorinated with DAST and deprotected to yield 2,5,6-trichloro-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)benzimidazole and 2,5,6-trichloro-1-(3-deoxy-3-fluoro-β-D-xylofuranosyl)benzimidazole. The resulting low overall yield (5%) of 2,5,6-trichloro-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)benzimidazole encouraged us to develop an alternative route. The heterocycle 2,5,6-trichlorobenzimidazole was condensed with 1-bromo-3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose to give, after deprotection, 2,5,6-trichloro-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)benzimidazole in a 50% overall yield. The 2′-deoxy-2′-fluoro-β-D-ribofuranosyl compounds were prepared using 2′-deoxy-2′-fluorouridine, *N*-deoxyribofuranosyl transferase, and 5,6-dichlorobenzimidazole. Functionalization of the C2 position then gave the desired derivatives. Antiviral and cytotoxicity testing revealed that the deoxy fluoro arabinofuranosyl, xylofuranosyl, and ribofuranosyl derivatives were less active against human cytomegalovirus and more cytotoxic than TCRB.

Introduction

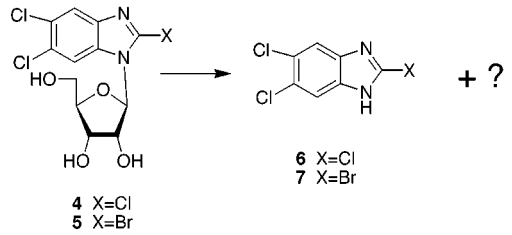
Human cytomegalovirus (HCMV) is one of eight human herpesviruses that belongs to the family Herpesviridae. It is estimated that, by adulthood, more than half of all Americans will have been infected with HCMV.[1] HCMV infections in immunocompetent individuals are usually asymptomatic. However, in immunocompromised patients, such as transplant recipients and individuals with acquired immune deficiency syndrome (AIDS), HCMV infections are often life-threatening. Currently, there are three FDA-approved drugs available for the treatment of HCMV infections: ganciclovir (**1**), foscarnet (**2**), and cidofovir (**3**).[2] Unfortunately, all three drugs can produce significant side effects and have limited oral bioavailability. Moreover virus strains resistant to each of these drugs are emerging.[3] Consequently, there is a need for a more potent and selective antiviral drug to treat HCMV infections.

As part of our search for new anticancer[4] and antiviral drugs,[5] a number of benzimidazole nucleosides have been synthesized. Certain compounds, including 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB, **4**) and 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BDCRB, **5**) are potent inhibitors of HCMV replication with low cellular toxicity at concentrations inhibiting viral growth.[5,6] Investigation of the disposition of **4** and **5** in rats and monkeys[7] has shown that the nucleosides are metabolized to a certain extent in vivo by glycosidic bond cleavage to yield plasma concentrations of the aglycons 2,5,6-trichlorobenzimidazole (**6**) and 2-bromo-5,6-dichlorobenzimidazole (**7**). The in vivo metabolic instability of **4** and **5** is detrimental to their use as antiviral agents because the half-lives of the parent compounds are less than an hour. The aglycons (**6** and **7**) also have lower antiviral activity than the parent nucleosides and are more cytotoxic.[5]Several structural modifications of TCRB and BDCRB have been explored as a way to reduce the metabolic instability and to improve pharmacokinetic properties. One successful approach involved the design and synthesis of carbocyclic benzimidazoles[8] and benzimidazole L-ribosides such as 1263W94.[8] A chemically more complicated approach involved the synthesis of C-nucleoside analogues of TCRB. Although more chemically stable than TCRB, the target compounds were inactive against HCMV.[8]

* To whom correspondence should be addressed. Tel: 734-764-7547. Fax: 734-763-5633. E-mail: ltownsen@umich.edu.
§ Glaxo Wellcome Inc.
† Present address: Glaxo Wellcome Inc., 5 Moore Dr., Research Triangle Park, NC 27709.

10.1021/jm990219s CCC: $19.00
Published on Web 05/16/2000

Chart 1



As another approach, we chose to investigate the synthesis of benzimidazole nucleosides with a 2′-fluorinated sugar. Replacement of a hydroxy group with a fluorine often increases chemical and metabolic stability of nucleosides. Fluorine is the most electronegative of the elements, and its powerful electron-withdrawing properties profoundly affect reactivity. Acid-catalyzed hydrolysis of benzimidazole nucleosides proceeds by an A1 mechanism in which the protonated nucleoside dissociates in the rate-controlling step to a glycosyl carbonium ion and the free heterocycle.[9] By replacing the hydroxy group at the C2′ position, adjacent to the reacting glycosidic bond, with the more electronegative fluorine, the carbonium ion should be destabilized and the rate of hydrolysis reduced. Replacement of the 2′-hydroxyl group with a fluorine atom has also been shown to inhibit enzyme catalyzed cleavage of the glycosidic bond, such as the cleavage of purines by purine nucleoside phosphorylase.[10]

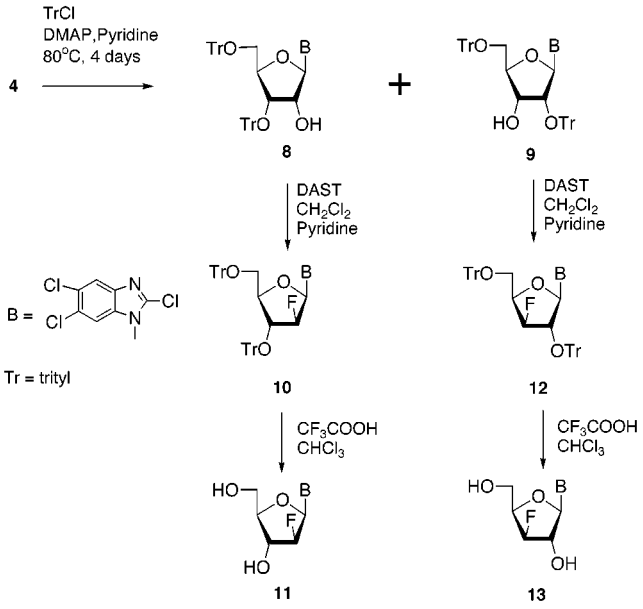
Therefore, we elected to synthesize the 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl, 3′-deoxy-3′-fluoro-β-D-xylofuranosyl, and the 2′-deoxy-2′-ribofuranosyl derivatives to determine what effect a substitution of the corresponding hydroxyl group with a fluorine would have on the antiviral activity and thus the feasibility of stabilizing the glycosidic bond.

Results and Discussion

Chemistry. Fluorinated nucleosides have commonly been synthesized by two different routes. One route introduces the fluorine into a suitably protected nucleoside, while the other route condenses (synthetic or enzymatic) a heterocycle with a fluorinated sugar derivative. Toward our goal of synthesizing a TCRB analogue with an increase in the stability of the glycosidic bond, we have investigated both of these strategies.

Fluorination of TCRB. As the glycosylation of several purines with 2′-deoxy-2′-fluoroarabinofuranosyl derivatives has been reported to be difficult,[11] we initially investigated the fluorination of a suitably protected derivative of **4** with dialkylaminosulfur trifluoride (DAST). While DAST has been reported to be an efficient fluorinating reagent for several nucleosides, it is well-known that the conformation of the protected furanose moiety is crucial for obtaining the desired attack of the weakly nucleophilic fluorine from the β-side.[12] For a displacement of a leaving group at the 2′-position it is important that the furanose ring assumes a conformation unfavorable for trans-elimination. Such a conformation can be induced by using bulky protecting groups at C5′ and C3′. We elected to synthesize 2,5,6-trichloro-1-(3,5-di-*O*-trityl-β-D-ribofuranosyl)-benzimidazole (**8**) (Scheme 1). Tritylation of **4** using TrCl and DMAP in pyridine at 80 °C for 4 days gave a

Scheme 1

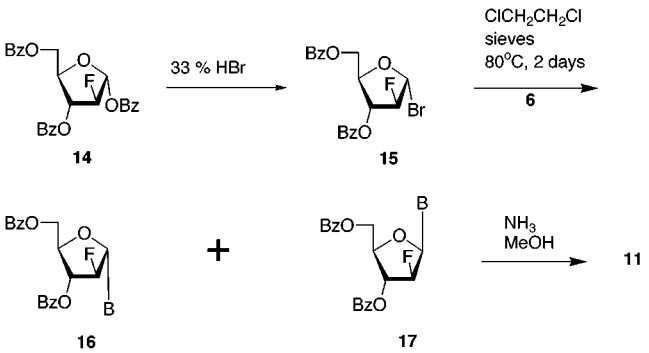


mixture of the ditrityl derivatives **8** and **9** in a combined yield of 38%. All attempts to improve the yield by increasing the reaction time, increasing the reaction temperature, or using bases other than DMAP to catalyze the reaction failed to improve the yield. The di-*O*-trityl derivatives **8** and **9** could not be separated by flash column chromatography but were separable by thin-layer chromatography (EtOAc/hexane: 1:2, three to four submersions). They were more conveniently separated by fractional crystallization from diethyl ether to give a 1:3 ratio of **9** and **8**. Substantiation for the assignment of **9** as the 2′,5′-di-*O*-trityl derivative and **8** as the 3′,5′-di-*O*-trityl derivative was based on homonuclear decoupling experiments.

While the desired 3′,5′-di-*O*-trityl derivative **8** was only obtained in 10% yield from **4**, the isomeric 2′,5′-ditrityl derivative **9** was obtained in a 30% yield. This prompted us to use **9** for the synthesis of fluorinated (xylofuranosyl)trichlorobenzimidazole derivatives. The fluorination of **9** using DAST and pyridine in CH_2Cl_2 gave compound **12** in a 71% yield. The fluorination of **8** using the same conditions gave compound **10** in a 63% yield. Both **10** and **12** were deprotected using 10% CF_3-COOH in $CHCl_3$ to give the target compounds 2,5,6-trichloro-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)benzimidazole (**11**) and 2,5,6-trichloro-1-(3-deoxy-3-fluoro-β-D-xylofuranosyl)benzimidazole (**13**), respectively.

The fluoro derivatives **11** and **13** are the β-arabinofuranosyl and β-xylofuranosyl derivatives, respectively, as they were synthesized from the preformed β-ribofuranosyl nucleoside **4** using DAST. These DAST fluorinations are known to replace a hydroxyl group with fluorine with an inversion of configuration.[13] Further support for the assignments of **11** and **13** as the β-arabinofuranosyl and β-xylofuranosyl derivatives is supported by the fact that both show long-range coupling[14,16] between the C_7-H and F, and this coupling is slightly larger for **11** (J = 1.9 Hz) than for **13** (J = 1.7 Hz). This indicates that the fluorine and the heterocycle are on the same face of the furanose ring. Finally for the arabinose derivative **11**, the coupling between the

Scheme 2



1′-H and the F is 17.7 Hz. This coupling is indicative of a vicinal trans relation between the 1′-H and F.[14]

Synthetic Coupling of a Fluorinated Sugar with Trichlorobenzimidazole. The arabinofuranosyl derivative **11** was only obtained in 5% yield from **4**. This prompted us to initiate an investigation into the synthesis of **11** by the coupling of a fluorinated arabinofuranose compound (such as **14** or **15**) to 2,5,6-trichlorobenzimidazole (**6**). Initial attempts to use Vorbruggen conditions and attempts to glycosylate an alkali salt of **6** were unsuccessful.

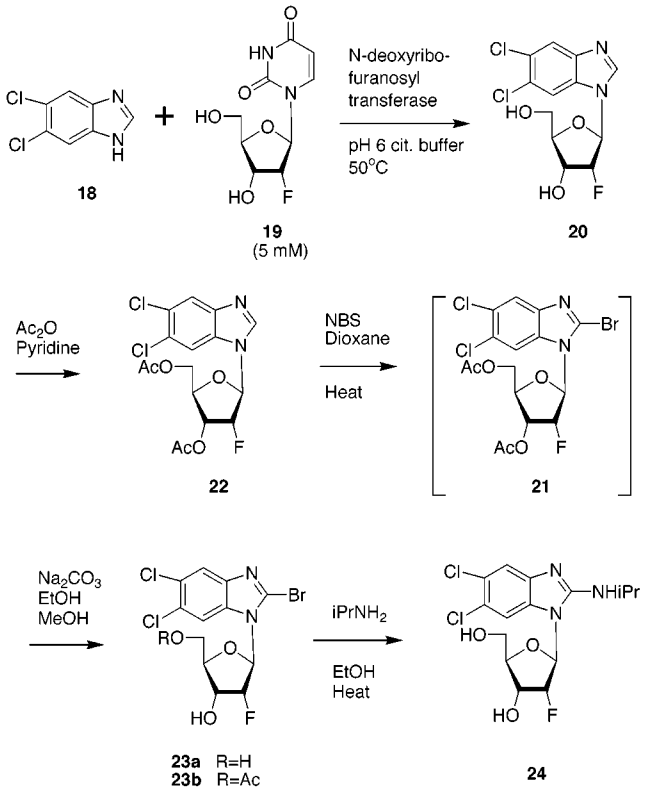
We were finally able to condense **6** with 1-bromo-3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose **(15)**[15] using conditions similar to those described by Secrist et al.[16] Thus, condensation of **6** with **15** at 80 °C in dichloroethane in the presence of 4 A molecular sieves gave compound **17** and its α-isomer **16** (Scheme 2). The assignment of compound **17** as the *β*-anomer and **16** as the α-anomer is based partially on their proton spectra. The coupling constant between the fluorine and the 1′-H is $J_{1',F} = 23.1$ Hz for **17** indicating a vicinal trans relation of the anomeric proton to the fluorine. For **16** this coupling constant is $J_{1',F} = 17.4$ Hz indicating a vicinal cis relation of the anomeric proton to the fluorine.[14,15,17] Further substantiation for the anomeric assignment stems from the fact that the C_7-H of **17** at 7.91 ppm is split by long-range coupling to fluorine (the signal is a doublet, $J = 3.2$ Hz, due to coupling between C_7-H and F) which is not observed at C_7-H for **16** at 7.79 ppm (in this case the signal is a singlet).

Further investigation of the condensation conditions showed that the anomeric ratio was highly dependent on condensation conditions. The condensation conditions described above gave mostly the *β*-anomer **17** (α/*β* ratio: 1/5−1/10) in nonpolar solvents such as dichloroethane and benzene. However, the α-anomer **16** (α/*β* ratio: 5/1−10/1) was the major product in more polar solvents such as acetonitrile and nitromethane. Yields were significantly better in nonpolar solvents such as dichloroethane (80%, 8/1: *β*/α) than in polar solvents such as acetonitrile (9%, 1/7: *β*/α).

Deprotection of **17** in methanolic ammonia gave a compound identical to the previously characterized **11**. Thus, this alternative route for the synthesis of **11** gave **11** in approximately 50% yield from the heterocycle **6** and bromo sugar **15** (Scheme 2).

Synthesis of 2′-Deoxy-2′-fluoro-*β*-D-ribofuranosylbenzimidazoles by an Enzymatic Method. We

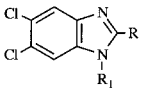
Scheme 3. Preparation of 5,6-Dichloro-2-substituted-1-(2-deoxy-2-fluoro-*β*-D-ribofuranosyl)benzimidazole Analogues



elected to explore the use of an enzymatic procedure for the synthesis of the 2′-deoxy-2′-fluororibofuranosyl derivative. Initial attempts to effect a enzymatic transfer of the carbohydrate moiety of 2′-deoxy-2′-fluorouridine (**19**) to the halogenated benzimidazoles **6** and **7** were unsuccessful. However, we found that 5,6-dichlorobenzimidazole (sans a halogen at C2) was ribosylated by **19** in the presence of *N*-deoxyribofuranosyl transferase to furnish the DRB analogue **20** (Scheme 3). Acetylation of **20** provided a good yield of compound **22**. Treatment of compound **22** with NBS furnished the 2-bromo analogue **21**, and subsequent treatment of **21** with sodium carbonate gave 2-bromo-5,6-dichloro-1-(2-deoxy-2-fluoro-*β*-D-ribofuranosyl)benzimidazole (**23a**) accompanied by a significant amount of the 5′-acetyl derivative **23b**. We chose to use this 5′-acetyl derivative **23b** for the synthesis of 5,6-dichloro-2-isopropylamino-1-(2-deoxy-2-fluoro-*β*-D-ribofuranosyl)benzimidazole (**24**), a 2′-F analogue of the highly active 5,6-dichloro-2-isopropylamino-1-(*β*-L-ribofuranosyl)benzimidazole.[8] Thus, a nucleophilic displacement of the 2-bromo group and concomitant removal of the acetyl group from **23b** with isopropylamine occurred at 90 °C to afford **24.**

Biology. Five target fluorinated benzimidazole nucleosides (**11, 13, 20, 23a, 24**) and three protected intermediates (**8, 9, 17**) were evaluated in vitro for their activity against herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV) and for cytotoxicity in human foreskin fibroblasts (HFF) and a human oral carcinoma cell line (KB cells). Solubility limited the testing of **8** and **9,** but **17** was found to be weakly active against HCMV but not HSV-1 (Table 1). Likewise, the target compounds were not active against HSV-1 but the 2′-fluoroarabinose (**11**) and 3′-fluoroxylose (**13**)

Table 1. Antiviral Activity and Cytotoxicity of 2-Substituted-5,6-dichloro-1-(fluorofuranosyl)benzimidazoles



			50 or 90% inhibitory concentration (μM)				
			antiviral activity				
			HCMV[a]		HSV-1[b]	cytotoxicity[c]	
Cmpd no.	R	R_1	plaque	yield	ELISA	visual	growth
8	Cl	3,5-di-Tr-ribose	>10[d,e]		>100	>100[d]	>100
9	Cl	2,3,5-tri-Tr-ribose	>10[d,e]		>100	>100[d]	>100
11	Cl	2-F-arabinose	8[d]	8	50	32[d]	60
13	Cl	3-F-xylose	9.4[d]	13	>100	40[d]	>100
17	Cl	2-F-3,5-di-Bz-arabinose	23		>100	>100[d]	100
20	H	2-F-ribose	>100[e]		>100	>100	>100
23a	Br	2-F-ribose	17		>100	100	80
24	*i*Pr-NH	2-F-ribose	>100[e]		>100	>100	>100
DRB[f,g]	H	ribose	42	19	30	24	36
4 (TCRB)[g]	Cl	ribose	2.9	1.4	102	238	210
5 (BDCRB)[g]	Br	ribose	0.7	0.2	130	118	>100
ganciclovir (DHPG)[h]			7.4±6.5	1.6±1.2	3.5±2.1	>100	>100

[a] Plaque and yield reduction assays were performed in duplicate as described in ref 20. Results from plaque assays are reported as IC_{50}'s, those for yield reduction experiments as IC_{90}'s. [b] The plaque assay was used to determine the activity of DHPG against HSV-1; all other compounds were assayed by ELISA in quadruplicate wells. [c] Visual cytotoxicity was scored on HFF cells at time of HCMV plaque enumeration. Results of duplicate experiments presented. Inhibition of KB cell growth was determined as described in ref 20 in quadruplicate assays. Results are presented as IC_{50}'s. [d] Average derived from two experiments. [e] >10 or 100 indicates IC_{50} or IC_{90} greater than the noted (highest) concentration tested. [f] Referred to as DRB by Tamm et al. [g] Data previously reported as compounds DRB, **9**, and **11**, respectively, in refs 5 and 20. [h] Average ± standard deviation from 108, 33, and 3 experiments, respectively.

analogues of TCRB and the 2′-fluororibose analogue of BDCRB (**23a**) were active against HCMV (Table 1). Although active in both plaque and yield reduction assays, the activity was less than that observed with TCRB and BDCRB. Furthermore the compounds were more cytotoxic than their ribosyl analogues leading to the decision that further studies of these fluoro nucleosides were not warranted.

The other two target compounds (**20, 24**) were inactive against HCMV. This lack of activity was surprising inasmuch as **20** is the direct 2′-fluororibofuranosyl analogue of DRB[18] which is both active and cytotoxic (Table 1). In a similar manner, the lack of activity of **24** is unexpected because the L-ribosyl analogue (1263W94) is highly active against HCMV[8] and was active and nontoxic in early clinical trials.[19]

Experimental Section

General Chemical Procedures.[20]

2,5,6-Trichloro-1-(2,5-di-*O*-trityl-*β*-D-ribofuranosyl)benzimidazole (9) and 2,5,6-Trichloro-1-(3,5-di-*O*-trityl-*β*-D-ribofuranosyl)benzimidazole (8). A mixture of **4** (5.0 g, 0.014 mol), DMAP (1.25 g, 0.014 mol), and TrCl (12.0 g, 0.043 mol) in dry pyridine (100 mL) was heated at 80 °C for 3 days. Additional amounts of TrCl (12.0 g, 0.043 mol) were added on the second and third day. After 3 days the reaction was quenched with MeOH (60 mL). The reaction mixture was concentrated under reduced pressure and coevaporated with toluene (3 × 100 mL). The residue was stirred in toluene (150 mL), filtered and the filtrate evaporated to dryness. The resulting yellow foam was purified by flash chromatography (toluene 0.5 L, then toluene/EtOAc 20:1, 5 cm × 20 cm) to give, after combining fractions containing ditrityl compounds and removal of the solvent under reduced pressure a mixture of **8** and **9** (4.5 g, 38%). Recrystallization from EtOEt gave mostly **9** while the filtrate was enriched in **8**. Compound **9** could be purified to homogeneity by two further recrystallizations from EtOEt to give 3.0 g (25%) of **9** as white crystals. The filtrate, which contained mostly **8**, was evaporated to dryness and subsequently purified by recrystallization from EtOAc/hexane to give 1.0 g (8.4%) of pure **8** as white crystals. **9**: mp 193−195 °C; R_f 0.19 (toluene/EtOAc 20:1); R_f 0.62 (EtOAc/hexane 1:2). Anal. Calcd for $C_{50}H_{39}Cl_3N_2O_4 \cdot 1/2H_2O$: C, H, N. **8**: mp 174−175 °C; R_f 0.19 (toluene/EtOAc 20:1); R_f 0.62 (EtOAc/hexane 1:2). Anal. Calcd for $C_{50}H_{39}Cl_3N_2O_4$: C, H, N.

2,5,6-Trichloro-1-(2,5-di-*O*-trityl-3-deoxy-3-fluoro-*β*-D-xylofuranosyl)benzimidazole (12). The 2′,5′-ditrityl compound **9** (1.2 g, 1.44 mmol) was dissolved in dry CH_2Cl_2 (30 mL). To this solution was added pyridine (1.1 mL, 14.4 mmol) and DAST (1.0 mL, 7.2 mmol) and the reaction mixture was stirred at room temperature for 24 h. Additional CH_2Cl_2 (200 mL) was added to the reaction mixture and the mixture extracted with saturated $NaHCO_3$ (100 mL) and washed with water (100 mL). The organic phase was then dried over magnesium sulfate, filtered and the solvent removed in vacuo. The resulting syrup was purified by flash chromatography (EtOAc/hexane 1:2, 4 cm × 15 cm), fractions containing product were combined and the solvent removed under reduced pressure to give, after recrystallization from EtOH, 0.85 g (70%) of **12** as a white solid: mp >240 °C dec; R_f 0.6 (EtOAc/hexane 1:2); HRMS *m/z* calcd for $C_{50}H_{38}Cl_3FN_2O_3$ 838.1924, found 838.1957. Anal. Calcd for $C_{50}H_{38}Cl_3FN_2O_3 \cdot 1/2H_2O$: C, H, N.

2,5,6-Trichloro-1-(3,5-di-*O*-trityl-2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)benzimidazole (10). The 3′,5′-di-*O*-trityl compound **8** (0.3 g, 0.36 mmol) was dissolved in dry CH_2Cl_2 (10 mL). To this solution was added pyridine (0.3 mL, 3.6 mmol) and DAST (0.24 mL, 1.8 mmol) and the reaction stirred at room temperature for 24 h. Additional CH_2Cl_2 (200 mL) was added to the reaction mixture and the mixture extracted with saturated $NaHCO_3$ (100 mL) and washed with water (100 mL). The organic phase was dried over magnesium sulfate, filtered and the solvent removed in vacuo. The resulting syrup was purified by flash chromatography (EtOAc/hexane 1:2, 2 cm × 15 cm), fractions containing product were pooled and solvent removed in vacuo to give, after recrystallization from EtOH, 0.20 g (67%) of **10** as a white solid: mp 145 °C; R_f 0.57 (EtOAc/hexane 1:2); HRMS *m/z* calcd for $C_{50}H_{38}Cl_3FN_2O_3$ 838.1932, found 838.1949. Anal. Calcd for $C_{50}H_{38}Cl_3FN_2O_3$: C, H, N.

2,5,6-Trichloro-1-(3-deoxy-3-fluoro-*β*-D-xylofuranosyl)-benzimidazole (13). Compound **12** (0.28 g, 0.32 mmol) was dissolved in 10% CF_3COOH in $CHCl_3$ (20 mL) and stirred at room temperature in a stoppered flask for 45 min. The reaction mixture was evaporated to dryness in vacuo, the oily residue was purified by flash chromatography (EtOAc/hexane 5:1, 2 cm × 15 cm), appropriate fractions pooled, evaporated to dryness and crystallized from MeOH/H_2O to give 85 mg (74%) of **13** as white crystals: mp 238 °C; R_f 0.42 (EtOAc/hexane 5:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.01 (s, 1H, C_4−H), 7.93 (d, 1H, C_7−H, J = 1.7 Hz, long-range coupled to F), 6.29 (d, 1H, 2′-OH, D_2O exchangeable), 5.91 (d, 1H, 1′−H, $J_{1',2'}$ = 4.6 Hz), 5.17 (dd, 1H, 3′−H, $J_{3',F}$ = 52.8 Hz), 5.14 (t, 1H, 5′-OH, D_2O exchangeable), 4.63 (dm, 1H, 2′−H, $J_{2',F}$ = 22.6 Hz, becomes dd on D_2O wash with $J_{2',F}$ = 22.6 Hz and $J_{1',2'}$ = 4.6 Hz), 4.30 (dm, 1H, 4′−H, $J_{4',F}$ = 28.5 Hz), 3.69−3.84 (m, 2H, 5′-H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 141.80, 140.94, 132.19, 125.967, 120.40, 113.61, 113.53, 96.90 (3′C, $J_{3'C,F}$ = 184.1 Hz), 91.14 (1′C, $J_{1'C,F}$ = 4.6 Hz), 80.46 (4′C, $J_{4'C,F}$ = 19.8 Hz), 77.81 (2′C, $J_{2'C,F}$ = 26.89 Hz), 57.71 (5′C, $J_{5'C,F}$ = 9.96 Hz); HRMS *m/z* calcd for $C_{12}H_{10}Cl_3FN_2O_3$ 353.9741, found 353.9747. Anal. Calcd for $C_{12}H_{10}Cl_3FN_2O_3$: C, H, N.

2,5,6-Trichloro-1-(2-deoxy-2-fluoro-*β*-D-arabinofuranosyl)benzimidazole (11). Compound **10** (0.10 g, 0.12 mmol) was dissolved in 10% CF_3COOH in $CHCl_3$ (10 mL) and stirred at room temperature in a stoppered flask for 60 min. The reaction mixture was evaporated to dryness in vacuo, the oily residue was purified by flash chromatography (EtOAc/hexane 5:1, 2 cm × 15 cm), appropriate fractions pooled, solvent removed in vacuo and the white residue crystallized from MeOH/H_2O to give 25 mg (60%) of **11** as white crystals: mp 223 °C; R_f 0.43 (EtOAc/hexane 5:1); ^{1}H NMR (360 MHz,

DMSO-d_6) δ 8.29 (d, 1H, C_7−H, J = 1.9 Hz, long-range coupled to F), 7.93 (s, 1H, C_4−H), 6.44 (dd, 1H, 1′−H, $J_{1',F}$ = 17.7 Hz, $J_{1',2'}$ = 4.5 Hz), 6.02 (d, 1H, 3′-OH, D_2O exchangeable), 5.31−5.35 (m, 1.5 H, 5′-OH and 0.5 of 2′−H, 5′-OH D_2O exchangeable), 5.25 (dm, 0.5 H, 2′−H, $J_{2',F}$ = 53.2 Hz, $J_{2',3'}$ = 2.7 Hz), 4.42 (dm, 1H, 3′−H, becomes ddd on D_2O wash with $J_{3',F}$ = 24.2 Hz, $J_{2',3'}$ = 2.7 Hz and $J_{3',4'}$ = 5.9 Hz), 3.71−3.86 (m, 3H, 4′−H and 5′−H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 140.81, 140.60, 134.07, 126.07, 125.68, 119.88, 115.61, 97.38 (2′C, $J_{2'C,F}$ = 192.5 Hz), 84.98 (1′C, $J_{1'C,F}$ = 17.4 Hz), 82.89 (4′C), 73.25 (3′C, $J_{2'C,F}$ = 24.4 Hz), 59.05 (5′C, $J_{5'C,F}$ = 9.9 Hz); HRMS *m/z* calcd for $C_{12}H_{10}Cl_3FN_2O_3$ 353.9741, found 353.9735. Anal. Calcd for $C_{12}H_{10}Cl_3FN_2O_3$: C, H, N.

2,5,6-Trichloro-1-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)benzimidazole (17) and Its α-Isomer 16. The 2-fluoro sugar **14**[14] (1.20 g, 2.6 mmol) was dissolved in CH_2Cl_2 (10 mL) and then a 33% HBr in CH_3COOH solution was added (2.64 mL, 10.4 mmol). The reaction mixture was stirred in a stoppered flask for 6 h. Additional CH_2Cl_2 (100 mL) was added to the reaction mixture and the organic phase was washed sequentially with ice cold saturated $NaHCO_3$ (100 mL) and ice cold water (100 mL). The CH_2Cl_2 solution was dried over magnesium sulfate, filtered and the solvent was removed under reduced pressure to give a colorless syrup of **15**. This syrup was dissolved in dry $ClCH_2CH_2Cl$ (10 mL) and added to a previously prepared solution containing **6** (0.6 g, 2.6 mmol) in $ClCH_2CH_2Cl$ (10 mL) and activated 4A sieves. The resulting mixture was heated at 80 °C under an inert atmosphere for 2 days. Then CH_2Cl_2 (100 mL) and a saturated $NaHCO_3$ solution (100 mL) were added to the reaction mixture. The organic phase was separated and washed with water (100 mL), then dried over magnesium sulfate, filtered and the solvent was removed in vacuo. The resulting solid was purified by flash chromatography (EtOAc/hexane: 1:2, 4 cm × 15 cm) with the fractions containing the faster moving nucleoside being pooled, concentrated to dryness and recrystallized from MeOH/H_2O and then from EtOH to give 0.12 g (8%) of **16** as white crystals. The fractions containing the slower moving nucleoside were contaminated with a small amount of **6**. These contaminated fractions were pooled, concentrated to dryness and rechromatographed on a second column (5% MeOH in $CHCl_3$, 4 cm × 15 cm) to give after pooling appropriate fractions and removing solvent under reduced pressure a white solid which after recrystallizations from MeOH/H_2O gave 1.0 g (72%) of **17**. **17:** mp 88−90 °C; R_f 0.36 (EtOAc/hexane 1:2); R_f 0.67 (5% MeOH in $CHCl_3$); HRMS *m/z* calcd for $C_{26}H_{18}Cl_3FN_2O_5$ 562.0265, found 562.0254. Anal. Calcd for $C_{26}H_{18}Cl_3FN_2O_5$: C, H, N. **16**: mp 78−80 °C; R_f 0.60 (EtOAc/hexane 1:2); R_f 0.9 (5% MeOH in $CHCl_3$); HRMS *m/z* calcd for $C_{26}H_{18}Cl_3FN_2O_5$ 562.0265, found 562.0276. Anal. Calcd for $C_{26}H_{18}Cl_3FN_2O_5$: C, H, N.

2,5,6-Trichloro-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)benzimidazole (11). Compound **17** (0.5 g, 0.9 mmol) was dissolved in methanolic ammonia and the solution was stirred at room temperature for 6 h. The solvent was removed in vacuo and the residue purified by flash chromatography (EtOAc/hexane 5:1, 2 cm × 15 cm), appropriate fractions pooled and evaporated to dryness to give after crystallization from MeOH, 0.23 g (74%) of a white solid identical to the previously characterized **11**.

5,6-Dichloro-1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)benzimidazole (20). 2′-Deoxy-2′-fluorouridine[21] (**19**; 0.99 g, 4 mmol) was dissolved in 800 mL of 50 mM pH 6.0 citrate buffer. 5,6-Dichlorobenzimidazole[4] (**18**; 0.30 g, 1.6 mmol) was added and the reaction was placed in a 50 °C water bath. *N*-Deoxyribofuranosyl transferase, 60 000 units,[22] was added and the reaction was gently shaken overnight. 5,6-Dichlorobenzimidazole (0.30 g, 1.6 mmol) was again added and the reaction continued for 2 days. The enzyme was precipitated by heating to 80 °C then cooling to room temperature. Celite (50−60 g) was added and the reaction filtered. The product was extracted with ethyl acetate (3 × 100 mL). The ethyl acetate was removed in vacuo and the residue purified by chromatography on 75 g of basic alumina. The column was eluted with $CHCl_3$/MeOH (95:5 followed by 9:1 then 2:1 and finally 1:1; v/v). The product containing fractions were combined and the solvents removed in vacuo to give 0.54 g (67%) of a white solid **20**: MS (FAB+) *m/z* 321, M + 1. Anal. Calcd for $C_{12}H_{11}Cl_2FN_2O_3 \cdot 0.05CHCl_3 \cdot 0.1CH_3OH$: C, H, N.

5,6-Dichloro-1-(2-deoxy-3,5-di-*O*-acetyl-2-fluoro-β-D-ribofuranosyl)benzimidazole (22). Compound **20** (0.45 g, 1.4 mmol) was dissolved in pyridine (20 mL) and concentrated in vacuo to remove water. The solution was chilled to 0 °C in an ice bath. Acetic anhydride (260 μL, 2.9 mmol, 2 eq.) was added and the reaction was allowed to warm to room temperature while stirring overnight. Methanol (3 mL) was added and the solvents removed in vacuo. Residual pyridine was removed by coevaporation with toluene (3 × 10 mL). The residue was partitioned between water and ethyl acetate. The ethyl acetate solution was dried with $MgSO_4$, filtered, and the solvent removed in vacuo. The product **22** was used without further purification: yield 0.56 g, 98%; MS (FAB+) *m/z* 405, M + 1.

2-Bromo-5,6-dichloro-1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)benzimidazole (23a) and 2-Bromo-5,6-dichloro-1-(5-*O*-acetyl-2-deoxy-2-fluoro-β-D-ribofuranosyl)benzimidazole (23b). Compound **22** (0.55 g, 1.4 mmol) was dissolved in dioxane (25 mL) and boiled to remove water. The solution was heated to reflux in a 120 °C oil bath. NBS (0.48 g, 2.8 mmol, 2 equiv) was added and the reaction mixture heated at reflux for 4 min. A second portion (0.48 g, 2.89 mmol, 2 eq.) was then added and heating at reflux was continued for an additional 6 min. The reaction was removed from the heat source, diluted with chloroform (40 mL) and cooled to room temperature. The solution was washed with saturated sodium bicarbonate (2 × 75 mL), dried ($MgSO_4$) and filtered. The solvents were removed in vacuo and the residue purified by flash chromatography (ethyl acetate/hexane 1:1, v/v). The product containing fractions were combined and the solvents removed in vacuo to give 2-bromo-5,6-dichloro-1-(2-deoxy-3,5-di-*O*-acetyl-2-fluoro-β-D-ribofuranosyl)benzimidazole (**21**). A removal of the acetyl groups was accomplished by treatment of **21** in methanol and ethanol (17 mL each) with sodium carbonate (0.22 g, 2.1 mmol, 2 equiv) dissolved in 4.2 mL water. The reaction was stirred at room temperature for 18 h. The solution was diluted with water (40 mL) and the product was extracted with ethyl acetate (2 × 75 mL). The ethyl acetate solution was dried with $MgSO_4$, filtered, and the solvent removed in vacuo. The residue was purified by chromatography on 75 g of silica gel. The column was eluted with ethyl acetate/hexane (1:4, v/v) followed by ethyl acetate/hexane (1:2, v/v). The faster eluting product was the 5′-acetyl compound **23b** (0.17 g). The second product to elute was 2-bromo-5,6-dichloro-1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)benzimidazole (**23a**; 0.03 g): MS (FAB+) *m/z* 399, M + 1; ^{1}H NMR (DMSO-d_6) δ 8.46 (s, 1H, Ar−H), 7.95 (s, 1H, Ar−H), 6.22 (dd, 1H, H-1′, $J_{1',2'}$ = 5.4 Hz, $J_{1',F}$ = 14.5 Hz), 5.82 (d, 1H, OH-3′, J = 5.6 Hz), 5.45 (t, 1H, OH-5′, J = 4.5 Hz), 5.29 (dt, 1H, H-2′, J = 5.2 Hz, $J_{2',F}$ = 53 Hz), 4.3 (m, 1H, H-3′), 4.0 (m, 1H, H-4′), 3.7 (m, 2H, H-5′).

2-Bromo-5,6-dichloro-1-(5-*O*-acetyl-2-deoxy-2-fluoro-β-D-ribofuranosyl)benzimidazole (23b): ^{1}H NMR (DMSO-d_6) δ 8.00 (s, 1H, Ar−H), 7.90 (s, 1H, Ar−H), 6.25 (dd, 1H, H-1′, $J_{1',2'}$ = 5.1 Hz, $J_{1',F}$ = 16.5 Hz), 5.95 (d, 1H, OH-3′, J = 6 Hz), 5.40 (dt, 1H, H-2′, J = 5.3 Hz, $J_{2',F}$ = 53 Hz), 4.4−4.1 (m, 4H, H-3′,4′,5′), 2.13 (s, 3H, acetyl-CH_3).

5,6-Dichloro-2-isopropylamino-1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)benzimidazole (24). Compound **23b** (0.06 g, 0.14 mmol) was dissolved in ethanol (4 mL) and isopropylamine (Fluka; 1.3 mL) was added to this solution. The reaction was heated in a sealed tube in a 90 °C oil bath for 17 h. The solvents were removed in vacuo and the residue purified by chromatography on silica gel (6 g). The column was eluted with chloroform/methanol (95:5). The product containing fractions were combined and the solvents removed in vacuo to afford 0.03 g (57%) of **24**: MS (APCH+) *m/z* 378, M + 1; ^{1}H NMR (DMSO-d_6) 7.66 (s, 1H, Ar−H), 7.37 (s, 1H, Ar−H), 6.92 (d, 1H, NH, J = 7.7 Hz), 6.17 (dd, 1H, H-1′, $J_{1',2'}$ = 5.3 Hz, $J_{1',F}$ = 15.4 Hz), 5.73 (d, 1H, OH-3′, J = 5.7 Hz), 5.63 (t, 1H, OH-5′,

$J = 4.3$ Hz), 5.12 (dt, 1H, H-2′, $J = 5.3$ Hz, $J_{2',F} = 53$ Hz), 4.29 (m, 1H, H-3′), 4.02 (m, 1H, CH), 3.94 (m, 1H, H-4′), 3.68 (m, 2H, H-5′), 1.16 (d, 6H, CH_3, $J = 6.5$ Hz). Anal. Calcd for $C_{15}H_{18}Cl_2FN_3O_3 \cdot 0.25C_4H_8O_2$: C, H, N.

Biological Procedures and Assays. Cell culture and virological procedures, HCMV plaque and yield reduction assays, an HSV-1 ELISA, cytotoxicity assays, and data analyses were performed as described in a preceding paper.[20]

Acknowledgment. We thank Julie M. Breitenbach and Roger G. Ptak for expert performance of antiviral and cytotoxicity assays and Kimberly Barrett for assistance in manuscript preparation. These studies were supported by Research Grant UOI-AI31718 from the National Institute of Allergy and Infectious Diseases and Research Agreement DRDA-942921 with Glaxo Wellcome.

References

(1) Alford, C. A.; Britt, W. J. Cytomegalovirus. In *The Human Cytomegalovirus*; Roizman, B., Whitley, R. J., Lopez, C., Eds.; Raven Press: New York, 1993; pp 227−255.

(2) Lalezari, J. P.; Drew, W. L.; Glutzer, E.; James, C.; Miner, D.; Flaherty, J.; Fisher, P. E.; Cundy, K.; Hannigan, J.; Martin, J. C.; Jaffe, H. S. (S)-1-[3-Hydroxy-2-(phosphonylmethoxy)propyl]-cytosine (Cidofovir): Results of a Phase I/II Study of a Novel Antiviral Nucleotide Analogue. *J. Infect. Dis*. **1995**, *171*, 788−796 and references therein.

(3) Field, A. K.; Biron, K. K. "The end of Innocence" Revisited: Resistance to Antiviral Drugs. *Clin. Microbiol. Rev*. **1994**, *7*, 1−13.

(4) Townsend, L. B.; Revankar, G. R. Benzimidazole Nucleosides, Nucleotides, and Related Derivatives. *Chem. Rev*. **1970**, *70*, 389−438.

(5) (a) 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. (b) Gudmundsson, K. S.; Drach, J. C.; Wotring, L. L.; Townsend, L. B. Synthesis and antiviral activity of certain 5′-modified analogues of 2,5,6,-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB). *J. Med. Chem*. **1997**, *40*, 785−793 and references cited therein.

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

(7) 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 (TCRB). 7th International Conference on Antiviral Research, Charleston, SC, March 1994.

(8) Townsend, L. B.; Gudmundsson, K. S.; Daluge, S. M.; Chen, J. J.; Zhu, Z.; Koszalka, G. W.; Boyd, F. L.; Chamberlain, S. D.; Freeman, G. A.; Biron, K. K.; Drach, J. C. Studies Designed to Increase the Stability and Antiviral Activity (HCMV) of the Active Benzimidazole Nucleoside, TCRB. *Nucleosides Nucleotides* **1999**, *18*, 509−519 and references cited therein.

(9) (a) York, J. L. Effect of the Structure of the Glycon on the Acid-Catalyzed Hydrolysis of Adenine Nucleosides. *J. Org. Chem*. **1981**, *46*, 2171−2173. (b) Oivanen, M.; Lonnberg, H.; Kazimierczuk, Z.; Shugar, D. Mechanism for Acid-Catalyzed Hydrolysis of Nucleoside and Acyclonucleoside Analogues Benzimidazole. *Nucleosides Nucleotides* **1989**, *8*, 133−144.

(10) Stoeckler, J. D.; Bell, C. A.; Parks, R. E.; Chu, C. K.; Fox, J. J.; Ikehara, M. C(2′)-Substituted Purine Nucleoside Analogues, Interaction with Adenosine Deaminase and Purine Nucleoside Phosphorylase and Formation of Analogue Nucleotides. *Biochem. Pharmacol*. **1982**, *31*, 1723−1728.

(11) Pankiewicz, K. W.; Krzeminski, J.; Ciszewski, L. A.; Ren, W. Y.; Watanabe, K. A. A Synthesis of 9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)adenine and Hypoxanthine. An Effect of C3′-Endo to C2′-Endo Conformational Shift on the Reaction Course of 2′-Hydroxyl Group with DAST. *J. Org. Chem*. **1992**, *57*, 553−559 and references cited therein.

(12) Krezeminski, J.; Nawrot, B.; Pankiewicz, K. W.; Watanabe, K. A. Synthesis of 9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)hypoxanthine. The First Direct Introduction of a 2′-β-Fluoro Substituent in Preformed Purine Nucleosides. Studies Directed Toward The Synthesis of 2′-Deoxy-2′-Substituted Arabinonucleosides. *Nucleosides Nucleotides* **1991**, *19*, 781−798.

(13) Herdewijn, P.; Aerchot, A.; Kerremans, L. Synthesis of Nucleosides Fluorinated in the Sugar Moiety. The Application of Diethylaminosulfur Trifluoride to the Synthesis of Fluorinated Nucleosides. *Nucleosides Nucleotides* **1989**, *8*, 65−96 and references therein.

(14) Wright, J. A.; Taylor, N. F.; Fox, J. J. Nucleosides. LX. Fluorocarbohydrates. XXII. Synthesis of 2-deoxy-2-fluoro-D-arabinose and 9-(2-deoxy-2-fluoro-α- and β-arabinofuranosyl)adenines. *J. Org. Chem*. **1969**, *34*, 2632−2636.

(15) Howell, H. G.; Brodfuehrer, P. R.; Brundige, S. P.; Benigne, D. A.; Sapino, C. Antiviral Nucleosides. A Stereospecific, Total Synthesis of 2′-Fluoro-2′-deoxy-β-D-arabinofuranosyl Nucleosides. *J. Org. Chem*. **1988**, *53*, 85−88 and references therein.

(16) Montgomery, J. A.; Shortnacy, A. T.; Carson, D. A.; Secrist III, J. A. 9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)guanine: A Metabolically Stable Cytotoxic Analogue of 2′-Deoxyguanosine. *J. Med. Chem*. **1986**, *29*, 2389−2392.

(17) Reichman, U.; Watanabe, K. A.; Fox, J. J. A Practical Synthesis of 2-Deoxy-2-Fluoro-D-Arabinofuranose Derivatives. *Carbohydr. Res*. **1975**, *42*, 233−240.

(18) Tamm, I.; Folkers, K.; Shunk, C. H.; Horsfall, F. L. Inhibition of Influenza Virus Multiplication by N-Glycosides of Benzimidazoles. *J. Exp. Med*. **1954**, *99*, 227−250.

(19) Drew, W. L.; Lalezari, J. P.; Wang, L. H.; Miner, R. C.; Aberg, J. A.; Wire, M. B.; Jacobson, M. A. In Vivo Anti-CMV Activity and Safety of Oral 1263W94 in HIV-Infected Subjects with Asympotomatic CMV Shedding. *Antiviral Res.* **1998**, *37*, A70.

(20) Gudmundsson, K. S.; Tidwell, J.; Lippa, N.; Koszalka, G. W.; van Draanen, N.; Ptak, R. G.; Drach, J. C.; Townsend, L. B. Synthesis and Antiviral Evaluation of Halogenated β-D- and L-Erythrofuranosylbenzimidazoles. *J. Med Chem*. **2000**, *43*, 2464−2472.

(21) Codington, J. F.; Doerr, I. L.; and Fox, J. J. Nucleosides. XVIII. Synthesis of 2′-Fluorothymidine, 2′-Fluorodeoxyuridine, and Other 2′-Halogeno-2′-Deoxy Nucleosides. *J. Org. Chem*. **1964**, *29*, 558−564.

(22) Cook, W. J.; Short, S. A.; Ealick, S. E.; Crystallization and Preliminary X-ray Investigation of Recombinant Lactobacillus Leichmannii Nucleoside Deoxyribosyltransferase. *J. Biol. Chem*. **1990**, *265*, 2682−2683.

JM990219S