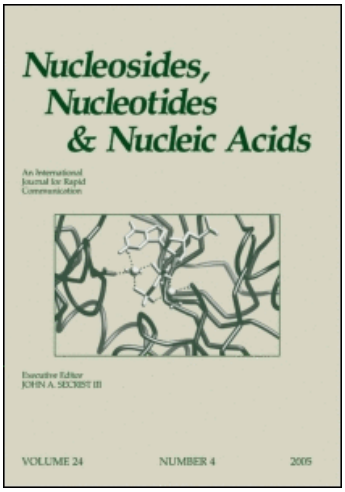

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Design, Synthesis, and Antiviral Evaluation of 2-Deoxy-D-Ribosides of Substituted Benzimidazoles as Potential Agents for Human Cytomegalovirus Infections

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DESIGN, SYNTHESIS, AND ANTIVIRAL EVALUATION OF 2-DEOXY-D-RIBOSIDES OF SUBSTITUTED BENZIMIDAZOLES AS POTENTIAL AGENTS FOR HUMAN CYTOMEGALOVIRUS INFECTIONS

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Dedicated to the memory of Dr. Gertrude B. Elion

ABSTRACT: Stereoselective glycosylation of 2,5,6-trichlorobenzimidazole (**1b**), 2-bromo-5,6-dichlorobenzimidazole (**1c**), 5,6-dichlorobenzimidazole (**1d**), 5,6-dichloro-benzimidazole-2-thione (**1e**), 5,6-dichloro-2-(methylthio)benzimidazole (**1f**), 2-(benzylthio)-5,6-dichlorobenzimidazole (**1g**), and 2-chloro-5,6-dimethylbenzimidazole (**1h**) with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride was achieved to give the desired β nucleosides **2b-h**. Subsequent deprotection afforded the corresponding free β-D-2-deoxyribosides **3b-h**. The 2-methoxy derivative **3i** was synthesized by the treatment of **2b** with methanolic sodium methoxide. Displacement of the 2-chloro group of **2b** with lithium azide followed by a removal of the protective groups gave the 2-azido-5,6-dichlorobenzimidazole derivative (**5**). The 2-amino derivative (**6**) was obtained by hydrogenolysis of **5** over Raney nickel. 5,6-Dichloro-2-isopropylamino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**10**) was prepared using 2'-deoxyuridine (**7**), N-deoxyribofuranosyl transferase and **1d** followed by functionalization of the C2 position. Antiviral evaluation of target compounds established that compounds **3b** and **3c** were active against human cytomegalovirus (HCMV) at non-cytotoxic concentrations. The activity of these 2-deoxy ribosides, however, was less than the activity of the parent riboside, 2,5,6-trichloro-1-β-D-ribofuranosylbenzimidazole (TCRB). Compared to TCRB, **3b** and **3c** were somewhat more cytotoxic and active against herpes simplex virus type 1. Compounds **3d-i** with other substituents in the 2-position were inactive against both viruses and non-cytotoxic. In contrast, compounds with amine substituents in the 2-position (**5**, **6**, **10**) were active against HCMV albeit less so than TCRB. These results establish that 2-deoxy-D-ribosyl benzimidazoles are less active against the DNA virus HCMV than are the corresponding D-ribosides.

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INTRODUCTION

Human cytomegalovirus (HCMV) is a common sight- and life-threatening opportunistic infection in immunocompromised individuals. These include AIDS patients, allogeneic bone marrow recipients, and renal transplant patients. HCMV also is among the leading cause of birth defects.[1] Currently only ganciclovir (GCV)[2], foscarnet[3], cidofovir[4] and the antisense oligonucleotide fomivirsen[5] have been approved in the United States for the treatment of HCMV diseases. Although these drugs are efficacious, they have poor oral bioavailability,[6] cause several adverse effects,[7,8] and the first three act by a similar mechanism. In addition, virus strains that are resistant to existing antiviral drugs are emerging.[9] Therefore the need exists for better drugs which act by a different mechanism to treat HCMV infections.

During the last several years, work in our laboratories has focused on benzimidazole nucleoside analogs as potential drugs for HCMV infections.[10,14,15] Interest in benzimidazole nucleosides began in the late 1940's when 5,6-dimethyl-1-(α-D-ribofuranosyl)benzimidazole was found as a constituent of vitamin B_{12}.[11] This led chemists to prepare a number of analogs including 5,6-dichloro-1-(ß-D-ribofuranosyl)benzimidazole (DRB, Fig. 2) as potential drugs.[12] Although initial data were interpreted to mean that DRB and analogs had antiviral activity especially against influenza, subsequent work established that the activity against viruses was not selective.[13]

In our search for more active, less toxic agents for the treatment of HCMV infection, we evaluated a number of benzimidazole nucleosides previously prepared in our laboratory.[14] We found that some of these substituted benzimidazole ribosides were highly active and selective against HCMV *in vitro* and were essentially non-cytotoxic. The most interesting of these compounds include 2-bromo-5,6-dichloro-1-(ß-D-ribofuranosyl)-benzimidazole (BDCRB) and TCRB (Fig. 2). Both of these compounds are active against HCMV at low or submicromolar concentrations and non-cytotoxic to growing cells at concentrations of 100 μM or more.[14]

Mode of action studies with TCRB and BDCRB in our laboratories established that these compounds did not inhibit DNA, RNA, or protein synthesis. Rather, the compounds

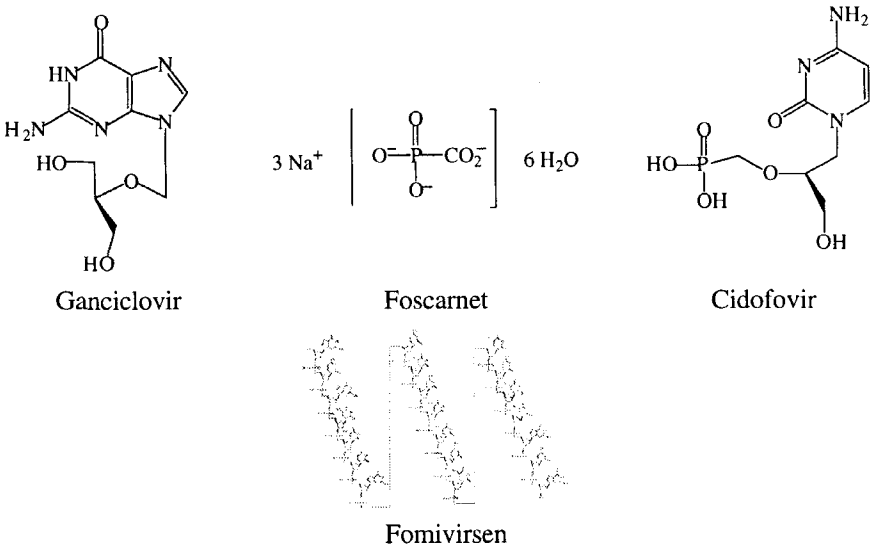


FIGURE 1. Structures of FDA-approved drugs for the treatment of human cytomegalovirus infections.

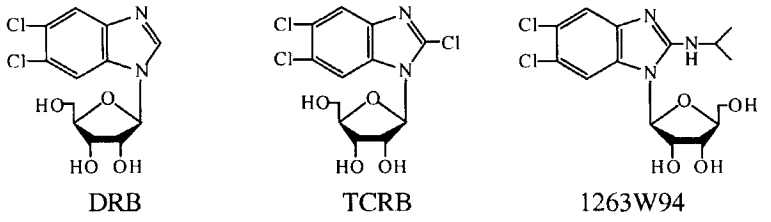


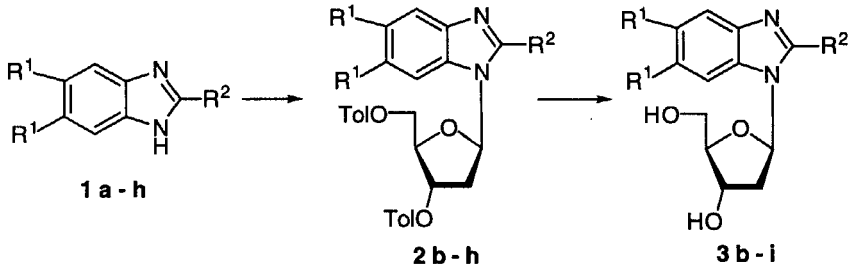
FIGURE 2. Structures of benzimidazole D- and L-ribosides .

act late in the viral replication cycle on viral assembly by inhibition of the processing of concatemeric DNA into monomeric genome length DNA.[15] Selection of drug-resistant virus and marker transfer studies established that resistance mapped to exon 2 of gene UL89 indicating that its gene product was the target for the compounds and that it is involved in HCMV DNA processing.[15] Studies with another TCRB-resistant HCMV strain has implicated another gene, UL56, in the action of these compounds and suggests that the gene products of UL89 and UL56 act as part of a complex that is inhibited by TCRB and BDCRB.[16]

In order to establish a structure-activity relationship and to better understand the mode of action of these compounds, we have synthesized a series of benzimidazole nucleoside analogs specifically modified on the heterocyclic and/or the sugar moieties. The present work describes a modification at the N-1 position and involves the synthesis of certain 2-deoxy-D-ribosyl benzimidazoles. The antiviral activity of these 2-deoxyribosides has been compared with that of their corresponding ribosides and found to be less than that of the parent ribosides.

CHEMISTRY

The substituted benzimidazoles 2-amino-5,6-dichlorobenzimidazole[17,18] (**1a**), 2,5,6-trichlorobenzimidazole[18-20] (**1b**), 5,6-dichlorobenzimidazole[21] (**1d**), 5,6-dichlorobenzimidazole-2-thione[22] (**1e**), 5,6-dichloro-2-(methylthio)benzimidazole[22] (**1f**), 2-(benzylthio)-5,6-dichlorobenzimidazole[23] (**1g**), and 2-chloro-5,6-dimethylbenzimidazole[24] (**1h**) were synthesized according to the reported procedures. 2-Bromo-5,6-dichlorobenzimidazole (**1c**) was prepared from **1a** using a non-aqueous diazotization procedure, which gave a significantly higher yield of **1c** than the procedure previously developed in our laboratory[18]. Stereoselective glycosylation of **1b** with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride[25] was achieved via the sodium salt glycosylation procedure.[26,27] This gave predominantly the desired β anomer 2,5,6-trichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)benzimidazole (**2b**) in 89% yield (Scheme 1). Subsequent deprotection of **2b** furnished the free nucleoside

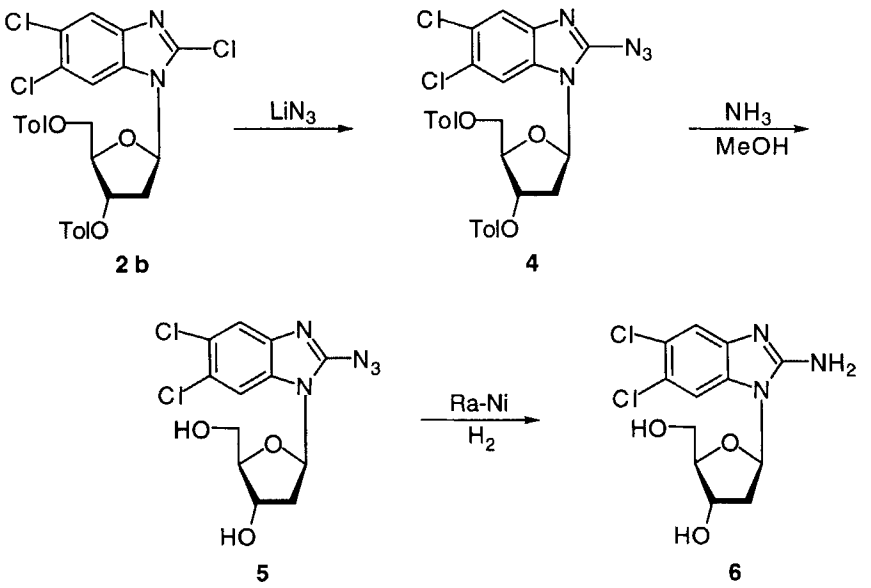


a. R^1=Cl, R^2=NH_2 b. R^1=Cl, R^2=Cl c. R^1=Cl, R^2=Br
d. R^1=Cl, R^2=H e. R^1=Cl, R^2=SH f. R^1=Cl, R^2=SMe
g. R^1=Cl, R^2=SBn h. R^1=Me, R^2=Cl i. R^1=Cl, R^2=OMe Tol = 

SCHEME 1.

2,5,6-trichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**3b**). The remaining β-D-2-deoxyribosides **3c-h** were prepared in a similar manner from the corresponding substituted benzimidazoles **1c-h**. The synthesis of 5,6-dichloro-2-methoxy-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**3i**) was accomplished by the treatment of **2b** with sodium methoxide in methanol. This resulted in a concomitant removal of the sugar protecting groups and a replacement of the 2-chloro group by a methoxy group. Treatment of **2b** with lithium azide followed by deprotection with methanolic ammonia furnished 2-azido-5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**5**) (Scheme 2). 2-Amino-5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**6**) was obtained by hydrogenolysis of **5** over Raney nickel.

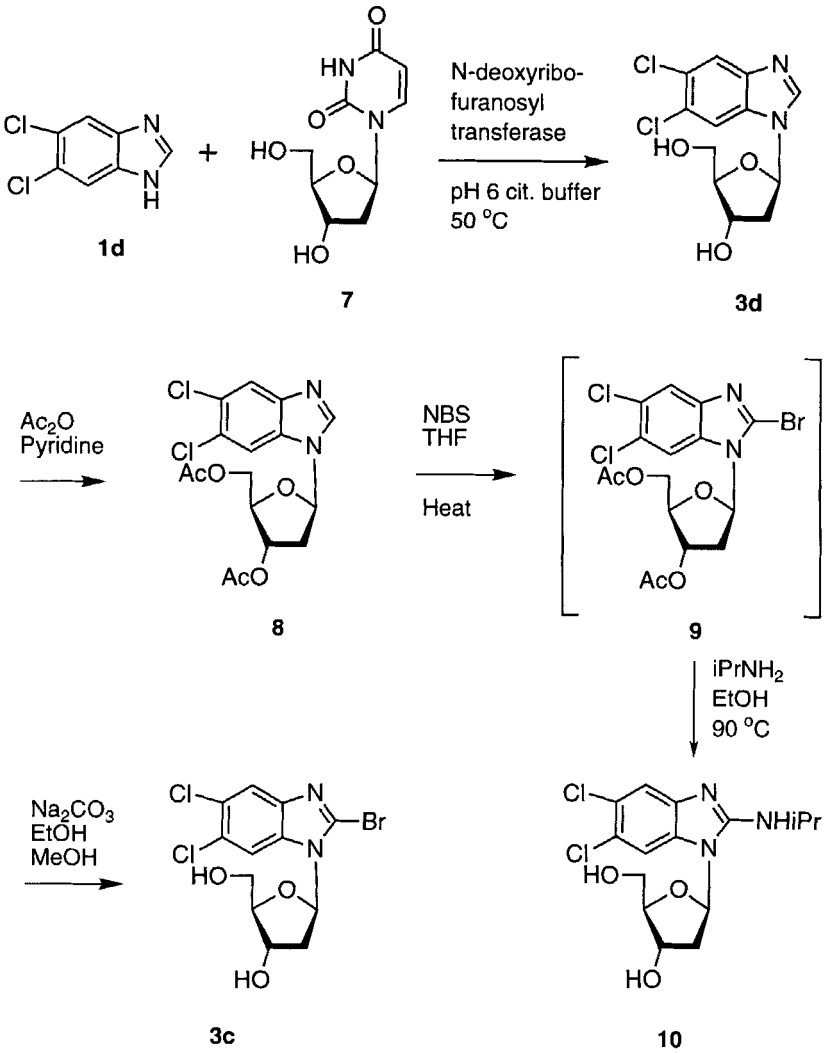
Assignment of the β configuration to the glycosylation products **2b-h** was based on the following facts. The sodium salt glycosylation procedure[26,27] using 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride as the sugar component has been reported to give predominantly the β anomers. The high stereoselectivity for the β anomer was attributed to the direct displacement of the 1-chloro group with an α configuration by the sodium salt of the heterocyclic component via a S_N2 type of reaction. A difference N.O.E.



SCHEME 2.

spectroscopic experiment was also conducted to allow an unambiguous assignment. Thus when the 1'-H of **3b** was irradiated, a 2% enhancement of the 4'-H signal was observed, while no enhancement was seen for the 3'-H signal. Also, a 2% enhancement of the 1'-H signal was observed when the 4'-H was irradiated, while no enhancement was observed when the 3'-H was irradiated. These data were in agreement with the assignment of a β configuration to **3b**.[28]

We also elected to explore the use of an enzymatic procedure for the synthesis of these compounds. We found that 5,6-dichlorobenzimidazole (*sans* a halogen at C-2) was



SCHEME 3.

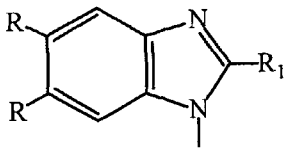
converted by **7** in the presence of N-deoxyribofuranosyl transferase[36] to 5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**3d**). Acetylation of **3d** provided a good yield of 5,6-dichloro-1-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)benzimidazole (**8**). Treatment of compound **8** with NBS furnished the 2-bromo analog (**9**) of **8**. The subsequent treatment of **9** with sodium carbonate furnished 2-bromo-5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**3c**) identical to **3c** as previously described (*vide supra*). A nucleophilic displacement of the 2-bromo group from **9** and a concomitant removal of the protecting groups with isopropylamine was accomplished to afford 5,6-dichloro-2-isopropylamino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**10**).

ANTIVIRAL STUDIES

Evaluation of target compounds against two herpesviruses established that the 2-halogen substituted analogs **3b** and **3c** were active against HCMV in plaque and yield reduction assays at non-cytotoxic concentrations (Table 1). The activity of these 2-deoxy ribosides, however, was less than the activity of the parent ribosides TCRB and BDCRB. In addition, the 2-deoxy analogs were somewhat more cytotoxic than TCRB and BDCRB but were weakly active against herpes simplex virus type 1 (HSV-1). Activity against both viruses was less than the activity of ganciclovir but activity against HCMV was similar to that observed with foscarnet (Table 1).

Compounds **3d-i** with other substituents in the 2-position were inactive against HCMV and/or HSV-1 and non-cytotoxic at the highest concentration tested, 100 μM. In contrast, compounds with amine substituents in the 2-position (**5**, **6**, **10**) were active against HCMV. Even though the 2-amino analog **6** was more cytotoxic, its more potent activity against HCMV (Table 1, yield assay) led us to synthesize the 2-isopropylamine analog **10** which has the same 2-substituent as the clinically-active compound 1263W94.[29] The activity of this compound was no better than **6** but it was less cytotoxic (Table 1). Compound **10** also was active against HCMV strain AD169

Table 1. Antiviral activity and cytotoxicity of 2-deoxy-D-ribosyl benzimidazoles.



			50 or 90% inhibitory concentration (μM)				
			antiviral activity				
			HCMV [a]		HSV-1 [b]	cytotoxicity [c]	
no.	R	R_1	plaque	yield	ELISA	visual	growth
3b	Cl	Cl	20 [d]	12	41	>100 [d]	100 [d]
3c	Cl	Br	35 [d]	6	50 [d]	50 [d]	100 [d]
3d	Cl	H	>100 [e]	-	>100	>100	>100
3e	Cl	SH	>100	-	>100	>100	>100
3f	Cl	SCH_3	>100	-	>100	>100	-
3g	Cl	$SCH_2C_6H_5$	>100 [d]	-	>100	>100[d]	-
3h	CH_3	Cl	>100	-	>100	>100	>100
3i	Cl	OCH_3	>100	-	-	>100	-
5	Cl	N_3	>100	38 [d]	-	100	-
6	Cl	NH_2	30	3.8 [d]	>100	32	30 [d]
10	Cl	NHC_3H_7	24	18	>100	>100	>100
TCRB [f]	Cl	Cl	2.9	1.4	102	238	210
BDCRB [f]	Cl	Br	0.7	0.2	130	118	>100
foscarnet [g]			39±26	-	-	>100	-
ganciclovir (GCV) [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 using the Towne strain of HCMV as described in the text. Results from plaque assays are reported as IC_{50}'s, those for yield reduction experiments as IC_{90}'s. [b] The plaque assay was used to determine the activity of GCV against HSV-1; all other compounds were assayed by ELISA in quadruplicate wells. [c] Visual cytotoxicity was scored on HFF cells at time of HCMV plaque enumeration. Inhibition of KB cell growth was determined as described in the text in quadruplicate assays. Results are presented as IC_{50}'s. [d] Average derived from two to four experiments. [e] >100 indicates IC_{50} or IC_{90} greater than the noted (highest) concentration tested. [f] Activity of TCRB and BDCRB as reported in reference 14. [g] Average ± standard deviation from 15 experiments. [h] Average ± standard deviation from 108, 33, and 3 experiments, respectively.

in a plaque assay (IC_{50} = 10 μM) but this activity was considerably less than the activity of its 2-deoxy-L-riboside homolog against strain AD169 - see accompanying paper.[30]

Overall these results establish that the 2-deoxy-D-ribosyl benzimidazoles are less active against HCMV than are the parent D-ribosides. This is noteworthy because

in this instance it is the ribosyl analogs, not the deoxyribosyl analogs, that are most active against a DNA virus which must reflect the unique antiviral mode of action of these compounds.[14-16]

EXPERIMENTAL

General Method: Melting points (MP) were taken on a Thomas-Hoover Unimelt apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WM-360 spectrometer operating in FT mode. The chemical shift values were reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. Mass (MS) spectra were determined by the Mass Spectrometry Laboratory of the Chemistry Department, University of Michigan. High resolution MS (HRMS) measurements were obtained on a VG 70-250-S MS spectrometer using a direct probe for sample introduction. Elemental analyses were performed by the Analytical Laboratory of the Chemistry Department, University of Michigan or by M-H-W Laboratories, Phoenix, AZ. Chemical reactions and column chromatographic separations were followed by thin layer chromatography (TLC) on silica gel pre-coated glass plates (layer thickness 0.2 mm) purchased from Analtech, Inc. The TLC plates were observed under UV light (254 nm). Evaporations were effected using a Buchler flash-evaporator equipped with a Dewar "dry ice" condenser under water aspirator or mechanical oil pump vacuum at 40 °C or cooler unless otherwise specified.

2,5,6-Trichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)benzimidazole (2b). To a suspension of 2,5,6-trichloro-benzimidazole[18-20] (**1b**, 4.43 g, 20 mmol) in 100 mL of dry MeCN, 1.2 g (30 mmol) of NaH (60% in oil) was added portionwise at room temperature. After the addition was complete, the reaction mixture was stirred at room temperature for 20 min to give a nearly clear yellowish solution. To this solution, 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride[25] (9.332 g, 24 mmol) was added portionwise over a period of 20 min and stirring was continued at room temperature for an additional 2 h. The reaction mixture was filtered and the solid was washed with portions of EtOAc (~300 mL). The filtrate was evaporated and the residue was dissolved in the EtOAc washings. This EtOAc

solution was washed with a NaCl solution (a sat. NaCl solution diluted with an equal volume of H_2O, 2 x 150 mL), dried (Na_2SO_4), and then evaporated. The residue was heated in 100 mL of MeOH at reflux temperature for 5 min and then allowed to cool to room temperature. Filtration of the resulting suspension and washing the solid with portions of MeOH gave 10.21 g (89%, 2 crops) of **2b** as white crystals. MP: 168-169 °C. HRMS: (EI) m/z 572.0664 (0.5%, M^+=572.0673). ^{1}H NMR (DMSO-d_6): δ 8.04, 7.94 (2 x s, 2, 7-H and 4-H), 7.97, 7.86, 7.37, 7.29 (4 x d, 8, 2 x p-MePhCO, J=8.0 Hz), 6.56 (dd, 1, 1'-H, $J_{1'-2'}$=8.5 Hz, $J_{1'-2''}$=6.0 Hz), 5.75 (m, 1, 3'-H, $J_{3'-2'}$=8.0 Hz, $J_{3'-2''}$=2.0 Hz, $J_{3'-4'}$=3.5 Hz), 4.72 (dd, 1, 5'-H, $J_{5'-4'}$=3.5 Hz, $J_{5'-5''}$=12.0 Hz), 4.69 (dd, 1, 5''-H, $J_{5''-4'}$=5.0 Hz), 4.61 (m, 1, 4'-H), 3.02 (m, 1, 2'-H, $J_{2'-2''}$=14.5 Hz), 2.72 (m, 1, 2''-H), 2.40, 2.36 (2 x s, 6, 2 x p-MePhCO). ^{13}C NMR (DMSO-d_6): δ 165.48, 165.34 (2 x p-MePhCO), 144.05, 143.81 (2 x p-MePhCO), 141.38 (C2), 140.81 (C3a), 132.43 (C7a), 129.48, 129.24 (2 x p-MePhCO), 126.47, 126.39, 126.23, 125.91 (2 x p-MePhCO, C6, and C5), 120.29 (C4), 113.43 (C7), 85.19 (C1'), 81.07 (C4'), 73.52 (C3'), 63.72 (C5'), 35.75 (C2'), 21.14, 21.09 (2 x p-MePhCO). *Anal.* Calcd. for $C_{28}H_{23}Cl_3N_2O_5$: C, 58.60; H, 4.04; N, 4.88. Found: C, 58.35; H, 4.09; N, 4.83.

2-Bromo-5,6-dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)benzimidazole (2c). To a suspension of 2-bromo-5,6-dichloro-benzimidazole[18] (**1c**, 1.55 g, 5.829 mmol) in 30 mL of dry MeCN, was added 0.35 g (8.750 mmol) of NaH (60% in oil) portionwise at room temperature. After the addition was complete, the reaction mixture was stirred at room temperature for 20 min to give a nearly clear yellowish solution. To this solution, 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride[25] (2.72 g, 6.995 mmol) was added portionwise over 20 min and stirring was continued at room temperature for an additional 2.5 h. The reaction mixture was diluted with EtOAc (100 mL), filtered and the solid was washed with portions of EtOAc (20 mL). This EtOAc solution was washed with a NaCl solution (a sat. NaCl solution diluted with an equal volume of H_2O, 2 x 100 mL), dried (Na_2SO_4), and then evaporated. The residue was chromatographed on a silica column (4 x 16 cm, eluted with pure chloroform). Evaporation of fractions 18-91 (15 mL per fraction) and recrystallization

of the solid from EtOH gave 2.927 g (81%, 2 crops) of **2c** as white crystals. MP: 157-159 °C. HRMS: (EI) m/z 616.0153 (0.2%, M^+=616.0167). ^{1}H NMR (DMSO-d_6): δ 8.02, 7.95 (2 x s, 2, 7-H and 4-H), 7.96, 7.86, 7.37, 7.29 (4 x d, 8, 2 x p-MePhCO, J=8.0 Hz), 6.52 (dd, 1, 1'-H, $J_{1'-2'}$=9.0 Hz, $J_{1'-2''}$=6.0 Hz), 5.76 (m, 1, 3'-H, $J_{3'-2'}$=8.0 Hz, $J_{3'-2''}$=2.0 Hz, $J_{3'-4'}$=3.5 Hz), 4.71 (m, 2, 5'-H and 5''-H, $J_{5'-4'}$=4.0 Hz, $J_{5''-4'}$=4.5 Hz, $J_{5'-5''}$=12.0 Hz), 4.63 (m, 1, 4'-H), 3.00 (m, 1, 2'-H, $J_{2'-2''}$=14.5 Hz), 2.70 (m, 1, 2''-H), 2.40, 2.36 (2 x s, 6, 2 x p-MePhCO). ^{13}C NMR (DMSO-d_6): δ 165.45, 165.27 (2 x p-MePhCO), 144.00, 143.75 (2 x p-MePhCO), 142.44 (C3a), 132.49 (C7a), 131.46 (C2), 129.40, 129.19 (2 x p-MePhCO), 126.43, 126.36, 126.03, 125.77 (2 x p-MePhCO, C6, and C5), 120.12 (C4), 113.29 (C7), 86.27 (C1'), 80.97 (C4'), 73.50 (C3'), 63.71 (C5'), 35.81 (C2'), 21.08, 21.03 (2 x p-MePhCO). *Anal.* Calcd. for $C_{28}H_{23}BrCl_2N_2O_5$: C, 54.39; H, 3.75; N, 4.53. Found: C, 54.54; H, 3.59; N, 4.44.

5,6-Dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-benzimidazole (2d). Compound **2d** was prepared from 5,6-dichlorobenzimidazole[21] (**1d**) following a reported procedure.[27] The final product was purified by recrystallization from CH_2Cl_2/hexane. MP: 109 °C. ^{1}H NMR (DMSO-d_6): δ 8.62 (s, 1, 2-H), 8.11 (s, 1, 7-H), 7.97 (s, 1, 4-H), 7.99, 7.81, 7.38, 7.29 (4 x d, 8, 2 x p-MePhCO, J=8.0 Hz), 6.60 (dd, 1, 1'-H, $J_{1'-2'}$=8.5 Hz, $J_{1'-2''}$=5.5 Hz), 5.71 (m, 1, 3'-H, $J_{3'-2'}$=6.5 Hz, $J_{3'-2''}$=2.0 Hz), 4.58 (m, 3, 4'-H and 5'-H), 3.05 (m, 1, 2'-H, $J_{2'-2''}$=14.5 Hz), 2.81 (m, 1, 2''-H), 2.41, 2.37 (2 x s, 6, 2 x p-MePhCO). ^{13}C NMR (DMSO-d_6): δ 165.38, 165.22 (2 x p-MePhCO), 144.14, 144.01, 143.74 (C2 and 2 x p-MePhCO), 143.16 (C3a), 132.36 (C7a), 129.47, 129.21, 129.14 126.47, 126.43 (2 x p-MePhCO), 125.49, 124.90 (C5 and C6), 120.79 (C4), 113.07 (C7), 84.94 (C1'), 81.66 (C4'), 74.63 (C3'), 63.98 (C5'), 36.41 (C2'), 21.12, 21.06 (2 x p-MePhCO). *Anal.* Calcd. for $C_{28}H_{24}Cl_2N_2O_5$: C, 62.35; H, 4.48; N, 5.19. Found: C, 62.45; H, 4.58; N, 5.36.

5,6-Dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-benzimidazole-2-thione (2e). To a mixture of 5,6-dichlorobenzimidazole-2-thione[22] (**1e**, 1.1 g, 5.02 mmol) in 200 mL of dry dioxane was added 0.12 g (4.85 mmol) of 97%

NaH under N_2. The reaction mixture was stirred at room temperature for 30 min. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride[25] (2.137 g, 5.5 mmol) was added and stirring was continued at room temperature overnight. The reaction mixture was filtered through a Celite pad and the filtrate was evaporated. The residue was suspended in CH_2Cl_2 and the suspension was filtered to give 0.686 g of the starting material. The filtrate was evaporated and the residue was triturated with MeOH. The resulting suspension was filtered to give 0.938 g (33%) of **2e** as a white solid. An analytical sample was obtained by recrystallization from MeCN. MP: 258 °C. ^{1}H NMR (DMSO-d_6): δ 13.33 (s, 1, 3-NH), 7.94, 7.90, 7.36, 7.30 (4 x d, 8, 2 x p-MePhCO, J=8.0 Hz), 7.74 (s, 1, 7-H), 7.37 (s, 1, 4-H), 6.87 (dd, 1, 1'-H, $J_{1'-2'}$=9.5 Hz, $J_{1'-2''}$=5.5 Hz), 5.74 (m, 1, 3'-H, $J_{3'-2'}$=7.5 Hz, $J_{3'-2''}$=1.0 Hz, $J_{3'-4'}$=3.0 Hz), 4.72 (m, 2, 5'-H and 5''-H, $J_{5'-4'}$=4.5 Hz, $J_{5''-4'}$=3.5 Hz, $J_{5'-5''}$=12.0 Hz), 4.60 (m, 1, 4'-H), 2.86 (m, 1, 2'-H, $J_{2'-2''}$=14.0 Hz), 2.50 (m, 1, 2''-H), 2.40, 2.36 (2 x s, 6, 2 x p-MePhCO). ^{13}C NMR (DMSO-d_6): δ 169.93 (C2), 165.49, 165.24 (2 x p-MePhCO), 143.95, 143.74 (2 x p-MePhCO), 131.00, 129.53, 129.31, 129.20, 126.47, 126.39 (C3a, C7a, and 2 x p-MePhCO), 125.88, 124.78 (C5 and C6). 112.07, 110.97 (C4 and C7), 85.17 (C1'), 80.94 (C4'), 73.82 (C3'), 63.98 (C5'), 34.55 (C2'), 21.06, 21.02 (2 x p-MePhCO). *Anal.* Calcd. for $C_{28}H_{24}Cl_2N_2O_5S$: C, 58.85; H, 4.23; N, 4.90. Found: C, 58.91; H, 4.40; N, 4.80.

5,6-Dichloro-2-(methylthio)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)benzimidazole (2f). To a mixture of 5,6-dichloro-2-(methylthio)-benzimidazole[22] (**1f**, 0.217 g, 0.93 mmol) in 100 mL of dry MeCN was added 0.024 g (0.97 mmol) of 97% NaH under N_2. The reaction mixture was stirred at room temperature for 1 h. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride[25] (0.400 g, 1.03 mmol) was added and stirring was continued at room temperature overnight. The reaction mixture was filtered through a Celite pad and the filtrate was evaporated. The residue was triturated with MeCN. The resulting suspension was filtered to give 0.384 g (71%) of **2f** as a white solid. An analytical sample was obtained by recrystallization from MeCN. MP: 140-141.5 °C. ^{1}H NMR (DMSO-d_6): δ 7.97, 7.88, 7.39, 7.31 (4 x d, 8, 2 x

p-MePhCO, J=8.0 Hz), 7.93, 7.85 (2 x s, 2, 7-H and 4-H), 6.42 (dd, 1, 1'-H, $J_{1'-2'}$=8.5 Hz, $J_{1'-2''}$=6.0 Hz), 5.73 (m, 1, 3'-H, $J_{3'-2'}$=8.0 Hz, $J_{3'-2''}$=3.5 Hz, $J_{3'-4'}$=3.0 Hz), 4.75 (dd, 1, 5'-H, $J_{5'-4'}$=3.5 Hz, $J_{5'-5''}$=12.0 Hz), 4.65 (dd, 1, 5''-H, $J_{5''-4'}$=5.0 Hz), 4.57 (m, 1, 4'-H), 2.97 (m, 1, 2'-H, $J_{2'-2''}$=14.5 Hz), 2.68 (s, 3, 2-SMe), 2.63 (m, 1, 2''-H), 2.41, 2.38 (2 x s, 6, 2 x p-MePhCO). **2f**: *Anal.* Calcd. for $C_{29}H_{26}Cl_2N_2O_5S \cdot 0.5H_2O$: C, 58.59; H, 4.58; N, 4.71. Found: C, 58.48; H, 4.59; N, 4.73.

2-(Benzylthio)-5,6-dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)benzimidazole (2g). To a mixture of 2-(benzylthio)-5,6-dichloro-benzimidazole[23] (**1g**, 0.89 g, 2.88 mmol) and dry MeCN (90 mL) was added 0.072 g (2.91 mmol) of 97% NaH under N_2. The reaction mixture was stirred at room temperature for 1 h. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride[25] (1.23 g, 3.16 mmol) was then added and stirring was continued at room temperature for 18 h. Volatile materials were evaporated and the residue was purified on a silica column using 5% MeOH/CH_2Cl_2 (v/v) as the eluant. Evaporation of the appropriate fractions and recrystallization from MeOH gave 1.22 g (64%) of **2g** as white crystals. MP: 177-178.5 °C. ^{1}H NMR (DMSO-d_6): δ 7.96, 7.86, 7.43, 7.36 (4 x d, 8, 2 x p-MePhCO, J=8.0 Hz), 7.93, 7.89 (2 x s, 2, 7-H and 4-H), 7.27 (m, 5, 2-SCH_2Ph), 6.39 (dd, 1, 1'-H, $J_{1'-2'}$=9.0 Hz, $J_{1'-2''}$=6.0 Hz), 5.71 (m, 1, 3'-H, $J_{3'-2'}$=8.0 Hz, $J_{3'-2''}$=2.0 Hz, $J_{3'-4'}$=3.5 Hz), 4.70 (dd, 1, 5'-H, $J_{5'-4'}$=3.5 Hz, $J_{5'-5''}$=12.0 Hz), 4.64 (dd, 1, 5''-H, $J_{5''-4'}$=5.0 Hz), 4.58 (m, 2, 2-SCH_2Ph), 4.54 (m, 1, 4'-H), 2.92 (m, 1, 2'-H, $J_{2'-2''}$=14.5 Hz), 2.56 (m, 1, 2''-H), 2.40, 2.36 (2 x s, 6, 2 x p-MePhCO). ^{13}C NMR (DMSO-d_6): δ 165.44, 165.27 (2 x p-MePhCO), 153.73 (C2), 143.99, 143.73 (2 x p-MePhCO), 142.65 (C3a), 136.64 (2-SCH_2Ph), 133.66 (C7a), 129.44, 129.21, 128.93, 128.41, 127.44, 126.50, 126.40 (2-SCH_2Ph and 2 x p-MePhCO), 124.97, 124.56 (C5 and C6). 119.01 (C4), 112.53 (C7), 84.62 (C1'), 80.89 (C4'), 73.49 (C3'), 63.65 (C5'), 36.02, 35.57 (C2' and 2-SCH_2Ph), 21.11, 21.07 (2 x p-MePhCO). *Anal.* Calcd. for $C_{35}H_{30}Cl_2N_2O_5S$: C, 63.54; H, 4.57; N, 4.23. Found: C, 63.69; H, 4.45; N, 4.20.

2-Chloro-5,6-dimethyl-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pento-furanosyl)benzimidazole (2h). To a mixture of 2-chloro-5,6-dimethyl-

benzimidazole[24] (**1h**, 0.903 g, 5.00 mmol) and 97% NaH (0.13 g, 5.25 mmol) in 100 mL of dry MeCN, was added 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride[25] (1.90 g, 4.89 mmol). The reaction mixture was stirred at room temperature for 2 h and was then filtered through a Celite pad. The filtrate was evaporated and the residue was purified on a silica column using toluene as the eluant. Evaporation of the appropriate fractions gave 1.5 g (58%) of **2h** as a white solid. An analytical sample was obtained by recrystallization from EtOH. MP: 162-163 °C. ^{1}H NMR (DMSO-d_6): δ 7.97, 7.88, 7.38, 7.31 (4 x d, 8, 2 x p-MePhCO, J=8.0 Hz), 7.46, 7.39 (2 x s, 2, 7-H and 4-H), 6.50 (dd, 1, 1'-H, $J_{1'-2'}$=8.5 Hz, $J_{1'-2''}$=6.0 Hz), 5.79 (m, 1, 3'-H, $J_{3'-2'}$=8.5 Hz, $J_{3'-2''}$=2.5 Hz, $J_{3'-4'}$=3.5 Hz), 4.73 (dd, 1, 5'-H, $J_{5'-4'}$=3.5 Hz, $J_{5'-5''}$=12.0 Hz), 4.65 (dd, 1, 5''-H, $J_{5''-4'}$=5.0 Hz), 4.58 (m, 1, 4'-H), 3.06 (m, 1, 2'-H, $J_{2'-2''}$=14.0 Hz), 2.66 (m, 1, 2''-H), 2.41, 2.37 (2 x s, 6, 2 x p-MePhCO), 2.25, 2.05 (2 x s, 6, 5-Me and 6-Me). ^{13}C NMR (DMSO-d_6): δ 165.41, 165.29 (2 x p-MePhCO), 143.94, 143.76 (2 x p-MePhCO), 139.91 (C3a), 137.78 (C2), 132.12, 131.40, 131.28 (C7a, C5, and C6), 129.37, 129.17, 126.50, 126.39 (2 x p-MePhCO), 118.96 (C4), 111.73 (C7), 84.58 (C1'), 80.56 (C4'), 73.53 (C3'), 63.65 (C5'), 35.21 (C2'), 21.04, 20.99 (2 x p-MePhCO), 19.44 (5-Me and 6-Me). *Anal.* Calcd. for $C_{30}H_{29}ClN_2O_5$: C, 67.60; H, 5.48; N, 5.26. Found: C, 67.73; H, 5.61; N, 5.43.

2,5,6-Trichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (3b). A suspension of **2b** (7.30 g, 12.72 mmol) and KCN (8.284 g, 127.21 mmol) in 255 mL of 90% aq. EtOH was stirred at room temperature for 4 days. The reaction mixture was filtered and the filtrate was evaporated. The resulting solid was triturated successively with H_2O (3 x 50 mL), hexane (3 x 50 mL), $CHCl_3$ (50 mL), and then recrystallized from MeOH to give 3.027 g (70%, 2 crops) of **3b** as white crystals. MP: 178-180 °C. HRMS: (EI) m/z 335.9831 (12%, M^+=335.9835). ^{1}H NMR (DMSO-d_6): δ 8.44 (s, 1, 7-H), 7.94 (s, 1, 4-H), 6.35 (dd, 1, 1'-H, $J_{1'-2'}$=9.0 Hz, $J_{1'-2''}$=6.0 Hz), 5.42 (d, 1, 3'-OH, $J_{3'-3'OH}$=4.5 Hz), 5.24 (t, 1, 5'-OH, $J_{5'-5'OH}$=5.0 Hz), 4.43 (m, 1, 3'-H, $J_{3'-2'}$=7.0 Hz, $J_{3'-2''}$=2.0 Hz, $J_{3'-4'}$=2.5 Hz), 3.90 (m, 1, 4'-H, $J_{4'-5'}$=3.0 Hz), 3.70 (dd, 2, 5'-H), 2.51 (m, 1, 2'-H, $J_{2'-2''}$=13.5 Hz), 2.19 (m, 1, 2''-H). ^{13}C NMR (DMSO-d_6): δ

141.21 (C2), 140.95 (C3a), 132.27 (C7a), 125.91, 125.67 (C5 and C6), 120.02 (C4), 114.77 (C7), 87.68 (C4'), 85.70 (C1'), 69.99 (C3'), 60.86 (C5'), 38.96 (C2'). *Anal.* Calcd. for $C_{12}H_{11}Cl_3N_2O_3$: C, 42.69; H, 3.28; N, 8.30. Found: C, 42.40; H, 3.36; N, 8.07.

2-Bromo-5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-benzimidazole (3c). METHOD A: A suspension of **2c** (0.618 g, 1 mmol) and KCN (0.330 g, 5 mmol) in 20 mL of 90% aq. EtOH was stirred at room temperature for 5 days. The reaction mixture was evaporated. The resulting solid was triturated successively with $CHCl_3$ (3 x 10 mL), H_2O (3 x 10 mL), and then recrystallized from EtOH to give 0.30 g (79%, 3 crops) of **3c** as white crystals. MP: 187-188 °C. HRMS: (EI) m/z 379.9332 (6%, M^+=379.9330). ^1H NMR (DMSO-d_6): δ 8.48 (s, 1, 7-H), 7.93 (s, 1, 4-H), 6.33 (dd, 1, 1'-H, $J_{1'-2'}$=9.0 Hz, $J_{1'-2''}$=5.5 Hz), 5.48 (d, 1, 3'-OH, $J_{3'-3'OH}$=4.0 Hz), 5.31 (t, 1, 5'-OH, $J_{5'-5'OH}$=4.5 Hz), 4.43 (m, 1, 3'-H, $J_{3'-2'}$=6.5 Hz, $J_{3'-2''}$=1.5 Hz), 3.91 (m, 1, 4'-H), 3.71 (m, 2, 5'-H), 2.50 (m, 1, 2'-H, $J_{2'-2''}$=13.5 Hz), 2.15 (m, 1, 2"-H). ^{13}C NMR (DMSO-d_6): δ 142.56 (C3a), 132.49 (C7a), 131.39 (C2), 125.75, 125.56 (C5 and C6), 119.83 (C4), 114.66 (C7), 87.68 (C4'), 86.94 (C1'), 70.02 (C3'), 60.86 (C5'), 39.00 (C2'). *Anal.* Calcd. for $C_{12}H_{11}BrCl_2N_2O_3$: C, 37.73; H, 2.90; N, 7.33. Found: C, 38.18; H, 2.80; N, 7.30.

METHOD B: 2-Bromo-1-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-5,6-dichlorobenzimidazole (**9**, 0.16 g, 0.34 mmoles) was dissolved in ethanol (8 mL) and methanol (8 mL). Sodium carbonate (0.080 g, 0.75 mmoles, 2.2 eq.) was dissolved in water (2 mL) and added to the starting material solution. The reaction was stirred at room temperature overnight. Water (40 mL) was added to the reaction and the product precipitated. The solid was collected by filtration to give 0.078 g of product in 60% yield. MS (FAB +) m/z, 383, M+1, 1-Br-2Cl pattern noted. ^1H NMR (DMSO- d_6) δ 8.44 (s, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 6.30 (t,1H, H-1', $J_{1',2'}$=5.8Hz), 5.4 (br s, 1H, OH), 5.2 (br s, 1H, OH), 4.4 (m, 1H, H-3'), 3.9 (m, 1H, H-4'), 3.7 (m, 2H, H-5'), 2.1 (ddd, 1H, H-2'). note: the other 2'-H obscured by DMSO.

5,6-Dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (3d).

METHOD A: Compound **3d** was prepared from **2d** following a reported procedure.[27] The final product was purified by recrystallization from EtOAc/hexane. MP: 169 °C. (Lit[27,31] MP: 168-169 °C). ^{1}H NMR (DMSO-d_6): δ 8.59 (s, 1, 2-H), 8.17 (s, 1, 7-H), 7.95 (s, 1, 4-H), 6.38 (dd, 1, 1'-H, $J_{1'-2'}$=7.0 Hz, $J_{1'-2''}$=6.0 Hz), 5.34 (d, 1, 3'-OH, $J_{3'-3'OH}$=4.0 Hz), 5.04 (t, 1, 5'-OH, $J_{5'-5'OH}$=5.0 Hz), 4.40 (m, 1, 3'-H, $J_{3'-2'}$=6.5 Hz, $J_{3'-2''}$=3.5 Hz, $J_{3'-4'}$=3.0 Hz), 3.88 (m, 1, 4'-H, $J_{4'-5'}$=$J_{4'-5''}$=4.0 Hz), 3.58 (m, 2, 5'-H and 5"-H, $J_{5'-5''}$=12.0 Hz), 2.56 (m, 1, 2'-H, $J_{2'-2''}$=13.5 Hz), 2.30 (m, 1, 2"-H). ^{13}C NMR (DMSO-d_6): δ 144.52 (C2), 143.25 (C3a), 132.27 (C7a), 125.14, 124.60 (C5 and C6), 120.60 (C4), 113.33 (C7), 87.66 (C4'), 84.90 (C1'), 70.24 (C3'), 61.21 (C5'), 39.69 (C2'). *Anal.* Calcd. for $C_{12}H_{12}Cl_2N_2O_3$: C, 47.55; H, 3.99; N, 9.24. Found: C, 47.65; H, 4.04; N, 9.09.

METHOD B: 2'-Deoxyuridine (1.83 g, 8 mmoles) was dissolved in 1600 mL of 50 mM, pH 6.0 citrate buffer. 5,6-Dichlorobenzimidazole (**1d**, 0.60 g, 3.2 mmoles) was added and the reaction was placed in a 50 °C water bath and gently shaken overnight. N-Deoxyribofuranosyl transferase[36], 400 units, was added and the reaction was gently shaken for 5 hr. 2'-Deoxyuridine (0.57 g, 2.5 mmoles) was added and the reaction continued another hour. The enzyme was precipitated by heating to 80 °C then cooling to 5 °C. Celite (50-60 g) was added and the reaction filtered. The product was eluted from the Celite cake with ethyl acetate (4 x 800 mL). The ethyl acetate was removed in vacuo to give the product in 85% yield, 2.05 g, based on 5,6-dichlorobenzimidazole. MS (FAB +) m/z, 303, M+1. ^{1}H NMR (DMSO-d_6) δ 8.56 (s, 1H, H-2), 8.14 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 6.34 (t,1H, H-1', $J_{1',2'}$=6.7Hz), 5.3 (br s, 1H, OH), 5.0(br s, 1H, OH), 4.36 (m, 1H, H-3'), 3.84 (m, 1H, H-4'), 3.54 (m, 2H, H-5'), 2.52 (ddd, 1H, H-2'), 2.27 (ddd, 1H, H-2'). *Anal.* Calcd. for $C_{12}H_{12}Cl_2N_2O_3$•1/4H_2O. C, 46.85; H, 4.10; N, 9.11. Found: C, 47.03; H, 4.13; N, 9.17.

5,6-Dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole-2-thione (3e). Compound **2e** (0.645 g, 1.13 mmol) was treated with 100 mL of NH_3/MeOH (sat. at 0 °C) in a pressure bottle at room temperature for 3 days. Volatile

materials were evaporated and the residue was purified on a silica column using 5% MeOH/CH_2Cl_2 (v/v) as the eluant. Evaporation of the appropriate fractions gave 0.31 g (82%) of **3e** as a white solid. An analytical sample was obtained by recrystallization from EtOH/H_2O. MP: 183 °C. ^{1}H NMR (DMSO-d_6): δ 13.14 (s, 1, 3-NH), 8.29 (s, 1, 7-H), 7.36 (s, 1, 4-H), 6.75 (dd, 1, 1'-H, $J_{1'-2'}$=9.5 Hz, $J_{1'-2''}$=5.5 Hz), 5.32 (d, 1, 3'-OH, $J_{3'-3'OH}$=3.0 Hz), 5.22 (t, 1, 5'-OH, $J_{5'-5'OH}$=4.5 Hz), 4.42 (m, 1, 3'-H, $J_{3'-2'}$=6.5 Hz, $J_{3'-2''}$=1.5 Hz, $J_{3'-4'}$=3.0 Hz), 3.86 (m, 1, 4'-H, $J_{4'-5'}$=2.5 Hz), 3.71 (m, 2, 5'-H), 2.32 (m, 1, 2'-H, $J_{2'-2''}$=13.0 Hz), 2.03 (m, 1, 2"-H). ^{13}C NMR (DMSO-d_6): δ 169.67 (C2), 130.93, 129.70, 125.60, 124.70 (C3a, C7a, C5, and C6), 113.55 (C7), 110.67 (C4), 87.33 (C4'), 85.41 (C1'), 70.04 (C3'), 60.79 (C5'), 37.78 (C2'). *Anal.* Calcd. for $C_{12}H_{12}Cl_2N_2O_3S$: C, 43.00; H, 3.61; N, 8.36. Found: C, 42.72; H, 3.86; N, 8.13.

5,6-Dichloro-2-(methylthio)-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-benzimidazole (3f). Compound **2f** (0.228 g, 0.392 mmol) was treated with 50 mL of NH_3/MeOH (sat. at 0°C) in a pressure bottle at room temperature for 3 days. Volatile materials were evaporated and the residue was purified on a silica column using 5% MeOH/CH_2Cl_2 (v/v) as the eluant. Evaporation of the appropriate fractions gave 0.092 g (68%) of **3f** as a white solid. An analytical sample was obtained by recrystallization from MeOH. MP: 197.5 °C. ^{1}H NMR (DMSO-d_6): δ 8.25 (s, 1, 7-H), 7.82 (s, 1, 4-H), 6.16 (dd, 1, 1'-H, $J_{1'-2'}$=9.0 Hz, $J_{1'-2''}$=6.0 Hz), 5.40 (d, 1, 3'-OH, $J_{3'-3'OH}$=4.5 Hz), 5.19 (t, 1, 5'-OH, $J_{5'-5'OH}$=5.0 Hz), 4.40 (m, 1, 3'-H, $J_{3'-2'}$=6.5 Hz, $J_{3'-2''}$=2.0 Hz, $J_{3'-4'}$=3.0 Hz), 3.88 (m, 1, 4'-H, $J_{4'-5'}$=3.5 Hz), 3.69 (m, 2, 5'-H), 2.73 (s, 3, 2-SMe), 2.47 (m, 1, 2'-H, $J_{2'-2''}$=13.0 Hz), 2.09 (m, 1, 2"-H). ^{13}C NMR (DMSO-d_6): δ 154.85 (C2), 143.05 (C3a), 133.58 (C7a), 124.50, 124.00 (C5 and C6), 118.56 (C4), 113.67 (C7), 87.50 (C4'), 85.03 (C1'), 70.10 (C3'), 60.98 (C5'), 38.69 (C2'), 14.39 (2-SMe). *Anal.* Calcd. for $C_{13}H_{14}Cl_2N_2O_3S$: C, 44.71; H, 4.04; N, 8.02. Found: C, 44.92; H, 4.12; N, 8.13.

2-(Benzylthio)-5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-benzimidazole (3g). Compound **2g** (0.700 g, 1.058 mmol) was treated with 40 mL of NH_3/MeOH (sat. at 0 °C) in a pressure bottle at room temperature for 4 days. Volatile

materials were evaporated *in vacuo* and the residue was purified on a silica column using 5% MeOH/CH_2Cl_2 (v/v) as the eluant. Evaporation of the appropriate fractions gave 0.180 g (40%) of **3g** as a white solid. An analytical sample was obtained by recrystallization from MeOH/CH_2Cl_2. MP: 187 °C. ^{1}H NMR (DMSO-d_6): δ 8.26 (s, 1, 7-H), 7.86 (s, 1, 4-H), 7.50-7.20 (m, 5, 2-SCH_2Ph), 6.16 (dd, 1, 1'-H, $J_{1'-2'}$=9.0 Hz, $J_{1'-2''}$=6.0 Hz), 5.36 (d, 1, 3'-OH, $J_{3'-3'OH}$=4.5 Hz), 5.17 (t, 1, 5'-OH, $J_{5'-5'OH}$=4.5 Hz), 4.63 (m, 2, 2-SCH_2Ph), 4.38 (m, 1, 3'-H, $J_{3'-2'}$=7.0 Hz, $J_{3'-2''}$=2.0 Hz, $J_{3'-4'}$=3.0 Hz), 3.85 (m, 1, 4'-H, $J_{4'-5'}$=3.0 Hz), 3.68 (m, 2, 5'-H), 2.44 (m, 1, 2'-H, $J_{2'-2''}$=13.5 Hz), 2.05 (m, 1, 2''-H). ^{13}C NMR (DMSO-d_6): δ 153.46 (C2), 142.96 (C3a), 136.71 (2-SCH_2Ph), 133.37 (C7a), 128.93, 128.44, 127.47 (2-SCH_2Ph), 124.71, 124.25 (C5 and C6), 118.76 (C4), 113.85 (C7), 87.50 (C4'), 85.07 (C1'), 70.08 (C3'), 60.96 (C5'), 38.74 (C2'), 35.83 (2-SCH_2Ph). *Anal.* Calcd. for $C_{19}H_{18}Cl_2N_2O_3S$: C, 53.65; H, 4.27; N, 6.59. Found: C, 53.90; H, 4.35; N, 6.86.

2-Chloro-5,6-dimethyl-1-(deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (3h). Compound **2h** (1.0 g, 1.88 mmol) was treated with 50 mL of NH_3/MeOH (sat. at 0°C) in a pressure bottle at room temperature for 2 days. Volatile materials were evaporated and the residue was purified on a silica column using 5% MeOH/CH_2Cl_2 (v/v) as the eluant. Evaporation of the appropriate fractions gave 0.40 g (72%) of **3h** as a white solid. An analytical sample was obtained by recrystallization from EtOAc/CH_2Cl_2. MP: 168 °C. ^{1}H NMR (DMSO-d_6): δ 7.70 (s, 1, 7-H), 7.37 (s, 1, 4-H), 6.32 (dd, 1, 1'-H, $J_{1'-2'}$=9.0 Hz, $J_{1'-2''}$=6.0 Hz), 5.41 (d, 1, 3'-OH, $J_{3'-3'OH}$=4.5 Hz), 5.08 (t, 1, 5'-OH, $J_{5'-5'OH}$=5.0 Hz), 4.41 (m, 1, 3'-H, $J_{3'-2'}$=7.0 Hz, $J_{3'-2''}$=2.5 Hz, $J_{3'-4'}$=3.0 Hz), 3.85 (m, 1, 4'-H, $J_{4'-5'}$=4.0 Hz, $J_{4'-5''}$=4.5 Hz), 3.67 (m, 2, 5'-H and 5''-H, $J_{5'-5''}$=11.5 Hz), 2.62 (m, 1, 2'-H, $J_{2'-2''}$=13.5 Hz), 2.30, 2.28 (2 x s, 6, 5-Me and 6-Me), 2.13 (m, 1, 2''-H). ^{13}C NMR (DMSO-d_6): δ 139.99 (C3a), 137.66 (C2), 131.80, 131.40, 131.16 (C7a, C5 and C6), 118.71 (C4), 112.81 (C7), 87.21 (C4'), 84.87 (C1'), 70.14 (C3'), 61.22 (C5'), 38.16 (C2'), 20.01, 19.58 (5-Me and 6-Me). *Anal.* Calcd. for $C_{14}H_{17}ClN_2O_3$: C, 56.66; H, 5.77; N, 9.44. Found: C, 56.68; H, 5.73; N, 9.18.

5,6-Dichloro-2-methoxy-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (3i). Compound **2b** (0.230 g, 0.40 mmol) was treated with 10 mL of 1 N NaOMe/MeOH at 65-70°C for 10 min. Volatile materials were evaporated and the residue was purified on a silica column using 5% MeOH/CH_2Cl_2 (v/v) as the eluant. Evaporation of the appropriate fractions gave 0.126 g (95%) of **3i** as a white solid. An analytical sample was obtained by recrystallization from CH_2Cl_2. MP: 168-169 °C. ^{1}H NMR (DMSO-d_6): δ 8.05 (s, 1, 7-H), 7.66 (s, 1, 4-H), 6.17 (dd, 1, 1'-H, $J_{1'-2'}$=8.5 Hz, $J_{1'-2''}$=6.0 Hz), 5.32 (d, 1, 3'-OH, $J_{3'-3'OH}$=4.5 Hz), 5.07 (t, 1, 5'-OH, $J_{5'-5'OH}$=5.0 Hz), 4.35 (m, 1, 3'-H, $J_{3'-2'}$=6.5 Hz, $J_{3'-2''}$=2.5 Hz, $J_{3'-4'}$=2.5 Hz), 4.12 (s, 3, 2-OMe), 3.80 (m, 1, 4'-H, $J_{4'-5'}$=3.5 Hz, $J_{4'-5''}$=4.0 Hz), 3.60 (m, 2, 5'-H and 5''-H, $J_{5'-5''}$=12.0 Hz), 2.52 (m, 1, 2'-H, $J_{2'-2''}$=13.0 Hz), 2.07 (m, 1, 2''-H). ^{13}C NMR (DMSO-d_6): δ 158.15 (C2), 139.78 (C3a), 131.56 (C7a), 123.89, 122.93 (C5 and C6), 118.27 (C4), 112.97 (C7), 87.18 (C4'), 83.00 (C1'), 70.26 (C3'), 61.26 (C5'), 57.67 (2-OMe), 37.91 (C2'). *Anal.* Calcd. for $C_{13}H_{14}Cl_2N_2O_4$: C, 46.86; H, 4.24; N, 8.41. Found: C, 46.66; H, 4.08; N, 8.18.

2-Azido-5,6-dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)benzimidazole (4). Compound **2b** (5.74 g, 10 mmol) was stirred with LiN_3 (4.90 g, 100 mmol) in 50 mL of dry DMF at 70 °C for 16 h. The reaction mixture was evaporated and co-evaporated with toluene. The residue was dissolved in 250 mL of EtOAc and the EtOAc solution was washed with a NaCl solution (a sat. NaCl solution diluted with an equal volume of H_2O, 2 x 200 mL), dried (Na_2SO_4), and then evaporated. The residue was dried at oil-pump vacuum at room temperature for 2 h to give **4** as a yellowish foam (6.0 g). This sample contained a small amount of solvents, but was pure by TLC and used directly in the subsequent reactions without further purification. An analytical sample was obtained by recrystallization from EtOH. MP: 82-85 °C. HRMS: (EI) m/z 579.1065 (2.5%, M^+=579.1076). ^{1}H NMR (DMSO-d_6): δ 7.97, 7.81 (2 x s, 2, 7-H and 4-H), 7.96, 7.88, 7.36, 7.30 (4 x d, 8, 2 x p-MePhCO, J=8.0 Hz), 6.36 (dd, 1, 1'-H, $J_{1'-2'}$=8.5 Hz, $J_{1'-2''}$=6.0 Hz), 5.72 (m, 1, 3'-H, $J_{3'-2'}$=7.0 Hz, $J_{3'-2''}$=2.0 Hz, $J_{3'-}$

$_{4'}$=3.5 Hz), 4.71 (dd, 1, 5'-H, $J_{5'-4'}$=3.5 Hz, $J_{5'-5''}$=12.0 Hz), 4.63 (dd, 1, 5"-H, $J_{5''-4'}$=5.0 Hz), 4.53 (m, 1, 4'-H), 3.05 (m, 1, 2'-H, $J_{2'-2''}$=15.0 Hz), 2.60 (m, 1, 2"-H), 2.40, 2.36 (2 x s, 6, 2 x p-MePhCO). ^{13}C NMR (DMSO-d_6): δ 165.46, 165.31 (2 x p-MePhCO), 149.07 (C2), 144.04, 143.78 (2 x p-MePhCO), 140.52 (C3a), 132.63 (C7a), 129.49, 129.24 129.20 (2 x p-MePhCO), 126.55, 126.43, 125.35, 124.57 (2 x p-MePhCO, C6, and C5), 118.98 (C4), 112.78 (C7), 83.78 (C1'), 81.19 (C4'), 73.90 (C3'), 63.72 (C5'), 35.31 (C2'), 21.15, 21.10 (2 x p-MePhCO). *Anal.* Calcd. for $C_{28}H_{23}Cl_2N_5O_5$: C, 57.94; H, 3.99; N, 12.07. Found: C, 58.07; H, 4.12; N, 11.94.

2-Azido-5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-benzimidazole (5). Compound **4** (6.0 g, prepared from 10 mmol of **2b**) was treated with 150 mL of NH_3/MeOH (sat. at 0 °C) in a pressure bottle at room temperature for 2 days. The reaction mixture was evaporated and co-evaporated with MeOH. The resulting solid was suspended in $CHCl_3$ (50 mL). The $CHCl_3$ suspension was kept at 0 °C for 1 h and then filtered. The solid was washed with portions of $CHCl_3$ and then recrystallized from MeOH to give 2.462 g (2 crops) of **5** as white needles. The $CHCl_3$ filtrate was concentrated to 20 mL and kept in a freezer (~ -15 °C) overnight. The precipitate was collected and recrystallized from MeOH to give an additional 0.569 g (2 crops) of **5**. The total yield was 3.031 g (88% from **2b**). MP: 159-161 °C (dec). HRMS: (EI) m/z 343.0232 (30%, M^+=343.0239). ^{1}H NMR (DMSO-d_6): δ 8.26 (s, 1, 7-H), 7.81 (s, 1, 4-H), 6.09 (dd, 1, 1'-H, $J_{1'-2'}$=9.0 Hz, $J_{1'-2''}$=6.0 Hz), 5.36 (d, 1, 3'-OH, $J_{3'-3'OH}$=4.5 Hz), 5.15 (t, 1, 5'-OH, $J_{5'-5'OH}$=5.0 Hz), 4.39 (m, 1, 3'-H, $J_{3'-2'}$=6.5 Hz, $J_{3'-2''}$=2.5 Hz, $J_{3'-4'}$=3.0 Hz), 3.84 (m, 1, 4'-H, $J_{4'-5'}$=3.5 Hz), 3.65 (m, 2, 5'-H), 2.50 (m, 1, 2'-H, $J_{2'-2''}$=13.0 Hz), 2.09 (m, 1, 2"-H). ^{13}C NMR (DMSO-d_6): δ 149.00 (C2), 140.90 (C3a), 132.11 (C7a), 125.13, 124.25 (C5 and C6), 118.79 (C4), 114.11 (C7), 87.52 (C4'), 84.08 (C1'), 70.15 (C3'), 60.10 (C5'), 38.54 (C2'). *Anal.* Calcd. for $C_{12}H_{11}Cl_2N_5O_3$: C, 41.88; H, 3.22; N, 20.35. Found: C, 42.01; H, 3.13; N, 19.98.

2-Amino-5,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-benzimidazole (6). A mixture of **5** (1.032 g, 3 mmol) and Raney nickel (0.24 g, wet

wt.) in 30 mL of EtOH was hydrogenated (50 psi of H_2) at room temperature for 6 h. The reaction mixture was filtered and the filtrate was evaporated. The residue was suspended in 20 mL of MeCN and the suspension was filtered to give 0.856 g (90%, 2 crops) of **6** as a white solid. An analytical sample (as white needles) was obtained by recrystallization from MeOH/MeCN. MP: 216-221 °C (dec.). HRMS: (EI) m/z 317.0340 (37%, M^+=317.0334). ^{1}H NMR (DMSO-d_6): δ 7.71 (s, 1, 7-H), 7.29 (s, 1, 4-H), 6.93 (br s, 2, 2-NH_2), 6.23 (dd, 1, 1'-H, $J_{1'-2'}$=8.5 Hz, $J_{1'-2''}$=6.0 Hz), 5.40 (t, 1, 5'-OH, $J_{5'-5'OH}$=4.5 Hz), 5.34 (d, 1, 3'-OH, $J_{3'-3'OH}$=4.0 Hz), 4.40 (m, 1, 3'-H, $J_{3'-2'}$=7.0 Hz, $J_{3'-2''}$=2.0 Hz, $J_{3'-4'}$=2.5 Hz), 3.82 (m, 1, 4'-H, $J_{4'-5'}$=2.5 Hz), 3.68 (m, 2, 5'-H), 2.40 (m, 1, 2'-H, $J_{2'-2''}$=13.0 Hz), 2.05 (m, 1, 2"-H). ^{13}C NMR (DMSO-d_6): δ 155.85 (C2), 143.16 (C3a), 132.40 (C7a), 122.94, 119.77 (C5 and C6), 115.35 (C4), 110.69 (C7), 86.91 (C4'), 83.87 (C1'), 70.20 (C3'), 60.74 (C5'), 38.05 (C2'). *Anal.* Calcd. for $C_{12}H_{13}Cl_2N_3O_3$: C, 45.30; H, 4.12; N, 13.21. Found: C, 45.44; H, 4.10; N, 13.28.

1-(2-Deoxy-3,5-di-O-acetyl-β-D-*erythro*-pentofuranosyl)-5,6-dichlorobenzimidazole (8). 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5,6-dichlorobenzimidazole (**3d**, 0.85 g, 2.8 mmoles) was dissolved in pyridine (20 mL) and boiled to remove water. The solution was chilled to 0 °C in an ice bath. Acetic anhydride (260 μL, 2.9 mmoles, 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 x 20 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 was used without further purification. The yield was 1.1 g, 95%. MS (FAB +) m/z, 388, M+1. ^{1}H NMR (DMSO-d_6) δ 8.56 (s, 1H, H-2), 8.08 (s, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 6.45 (t,1H, H-1', $J_{1',2'}$=5.9Hz), 5.27 (m, 1H, H-3'), 4.2 (m, 3H, H-4',5'), 2.86 (m, 1H, H-2'), 2.07 (s, 3H, acetyl-CH_3), 2.00 (s, 3H, acetyl-CH_3), note: the other 2'-H was obscured by DMSO.

2-Bromo-1-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-5,6-dichlorobenzimidazole (9). 1-(2-Deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pento-

furanosyl)-5,6-dichlorobenzimidazole (**8**, 1.0 g, 2.6 mmoles) was dissolved in toluene (50 mL) and boiled to remove water. The excess solvent was removed in vacuo. The residue was dissolved in THF (50 mL) and heated to reflux in an 85 °C oil bath. NBS (1.02 g, 5.7 mmoles, 2.2 eq.) was added and the reaction refluxed for 9 min. The reaction was removed from the heat and poured into cold sat. sodium bicarbonate. The product was extracted with chloroform (2 x 50 mL). The solvent was removed in vacuo and the residue purified by filtration through 125 g of silica gel eluted with ethyl acetate/ hexane (1:2, v/v). The product containing fractions were combined and the solvents removed in vacuo to give one pure fraction, 0.35 g, plus a second fraction that was one major spot on tlc for the expected product plus one minor spot. MS (FAB +) m/z, 465, M+1, 1-Br-2Cl pattern noted. ^{1}H NMR (DMSO-*d6*) δ 8.04 (s, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 6.32 (t,1H, H-1', $J_{1',2'}$=5.9Hz), 5.3 (m, 1H, H-3'), 4.43 (m, 1H, H4'), 4.27 (m, 2H, H-5'), 2.76 (m, 1H, H-2'), 2.10 (s, 3H, acetyl-CH_3), 2.07 (s, 3H, acetyl-CH_3). note: the other 2'-H was obscured by DMSO.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5,6-dichloro-2-isopropylamino-benzimidazole (10). 2-Bromo-1-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pento-furanosyl)5,6-dichlorobenzimidazole (**9**) (0.06 g, 0.14 mmoles) was dissolved in ethanol (4 mL) and isopropylamine (1.3 mL) was added. The reaction was heated in a sealed tube in a 90 °C oil bath overnight. The solvents were removed in vacuo and the residue purified by filtration through 15 g of silica gel, eluted with chloroform/methanol (98:2). The product containing fractions were combined and the solvents removed in vacuo to give a 42 % yield (0.052 g) of **10**. MS (APCH +) m/z, 360, M+1. ^{1}H NMR (DMSO-*d6*) δ 7.63 (s, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 6.94 (d, 1H, NH, J=7.7Hz), 6.17 (dd,1H, H-1', $J_{1',2'}$=6.0Hz and 9.0 Hz), 5.45 (t, 1H, OH-5', J=4.5Hz), 5.30 (d, 1H, OH-3', J=4.0Hz), 4.37 (m, 1H, H-3'), 4.02 (m, 1H, H-4'), 3.8 (m, 1H, H-5'), 3.66 (m, 1H, H-5'), 2.33 (m, 1H, H-2'), 2.0 (m, 1H, H-2').

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.[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 (m.o.i.) of <0.01 plaque-forming units (p.f.u.) per cell as detailed previously.[32] High titer HSV-1 stocks were prepared by infecting KB cells at an m.o.i. of <0.1 also as detailed previously.[32] 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.[33]

HCMV plaque reduction assay. HFF cells in 24-well cluster dishes were infected with approximately 100 p.f.u. of HCMV per cm^2 cell sheet using the procedures detailed above.[32] Following virus adsorption, compounds dissolved in growth medium were added to duplicate wells in four to eight selected concentrations. After incubation at 37 °C for 7 days, cell sheets were fixed, stained with crystal violet and microscopic plaques enumerated as described above. Drug effects were calculated as a percentage of reduction in number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug.

HCMV yield assay. HFF cells were planted as described above in 96-well cluster dishes, incubated overnight, medium removed and the cultures were inoculated with HCMV at a m.o.i. of 0.5 to 1 p.f.u. per cell as reported elsewhere.[33] After virus adsorption, inoculum was replaced with 0.2 mL of fresh medium containing test compounds. The first row of 12 wells was left undisturbed and served as virus controls. Each well in the second row received an additional 0.1 mL of medium with test compound

at three times the desired final concentration. The contents of the 12 wells were mixed by repeated pipetting and then serially diluted 1:3 along the remaining wells. In this manner, six compounds could be tested in duplicate on a single plate with concentrations from 100 μM to 0.14 μM. Plates were incubated at 37° C for seven days, subjected to one cycle of freezing and thawing; aliquots from each of the eight wells of a given column were transferred to the first column of a fresh 96-well monolayer culture of HFF cells. Contents were mixed and serially diluted 1:3 across the remaining eleven columns of the secondary plate. Each column of the original primary plate was diluted across a separate plate in this manner. Cultures were incubated, plaques were enumerated, and titers calculated as described above.

HSV-1 ELISA. An ELISA was employed[34] 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 p.f.u./well were added. Following a 3-day incubation at 37 °C, medium was removed, plates were blocked, rinsed, and horse radish 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: (*i*) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[32] (*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.[35] 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 hours 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 quantify drug effects by linearly regressing the percent inhibition of parameters derived in the preceding assays (except for yield experiments) against log drug concentrations. For yield experiments, the log of viral titer was plotted against the log drug concentration. Fifty percent inhibitory concentrations (IC_{50}'s) and ninety percent inhibitory concentrations (IC_{90}'s, yield experiments) were calculated from the linear portions of the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

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REFERENCES

1. Britt, W. J.; Alford, C. A. Cytomegalovirus, p 2493-2523. *In* B.N. Fields, D.M. Knipe, and P.M. Howley (ed.), Fields Virology, 3rd ed., 1996. Lippincott - Raven Publishers, Philadelphia, PA.
2. Crumpacker, C. S. Ganciclovir. *New England Journal of Medicine* **1996**; *335*:721-729.
3. Chrisp, P.; Clissold, S. P. Foscarnet. A review of its antiviral activity,

pharmacokinetic properties and therapeutic use in immunocompromised patients with cytomegalovirus retinitis. *Drugs* **1991**; *41*, 104-129.

4. Hitchcock, M. J.; Jaffe, H. S.; Martin, J. C.; Stagg, R. J. Cidofovir, a new agent with potent anti-herpesvirus activity. *Antiviral Chemistry & Chemotherapy* **1996**; *7:*115-127.

5. Marwick, C. First "antisense" drug will treat CMV retinitis. *J. Amer. Med. Assn.* **1998** *280*:871.

6. Skolnik, P. R.; Hirsch, M. S. Therapy and Prevention of Cytomegalovirus Infections. In "*Clinical Use of Antiviral Drugs*", DeClercq, E. Ed., Martinus Nijhoff Publishing, Norwell, **1988**, pp. 159-194.

7. Deray, G.; LeHoang, P.; Soubrie, C.; Morer, I.; Cacoub, P.; Baumelou, A.; Rousselie, F.; Jacobs, C. Foscarnet-Induced Acute Renal Failure and Effectiveness of Haemodialysis. *Lancet* **1987**, *2*, 216.

8. Bowden, R. A.; Digel, J.; Reed, E. C.; Meyers, J. D. Immunosuppressive Effects of Ganciclovir on in vitro Lymphocyte Responses. *J. Infect. Dis.* **1987**, *156*, 899-903.

9. Field, A. K.; Biron, K. K. "The end of innocence" revisited: Resistance of herpesviruses to antiviral drugs. *Clinical Microbiology Reviews* **1994**; *7*:1-13.

10. (a) Townsend, L. B.; Revankar, G. R. Benzimidazole Nucleosides, Nucleotides, and Related Derivatives. *Chem. Rev.* **1970**, 70, 389-438. (b) Revankar, G. R., Townsend, L. B. The Synthesis of 2-Chloro-1-(β-D-ribofuranosyl)benzimidazole and Certain Related Derivatives. *J. Heterocycl. Chem.* **1968**, *5*, 477-483.

11. Beavan, G. R.; Holiday, E. R.; Johnson, E. A.; Ellis, B.; Mamalis, P.; Petrow, V.; Sturgeon, B. The chemistry of anti-pernicious anaemia factors Part III. 5:6-Disubstituted benzimidazoles as products of acid hydrolysis of vitamin B_{12}. *Journal of Pharmacy and Pharmacology* **1949**; *1*:957-970.

12. Tamm, I.; Folkers, K.; Shunk, C. H.; Horsfall, F. L. Jr. Inhibition of Influenza Virus Multiplication by N-Glycosides of Benzimidazoles. *J. Exp. Med.* **1954**, *99*, 227-248.

13. Tamm, I.; Sehgal, P. B. Halobenzimidazole ribosides and RNA synthesis of cells and viruses. *Advances in Virus Research* **1978**; *22*:187-258.

14. (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 analogs of 2,5,6,-trichloro-1-(ß-D-ribofuranosyl)benzimidazole (TCRB). *J. Med. Chem.* **1997**, *40*:785-793. (c) Zou, R.; Drach, J. C.; Townsend, L.B. Design, synthesis and antiviral evaluation of 2-chloro-5,6-dihalo-1-(ß-D-ribofuranosyl)benzimidazoles as potential agents for human cytomegalovirus infections. *J. Med. Chem* **1997**, *40*, 811-818.

15. Underwood, M. R.; Harvey, R. J.; Stanat, S. C.; Hemphill, M. L.; Miller, T.; Drach, J. C.; Townsend, L. B.; Biron, K. K. Inhibition of human cytomegalovirus DNA maturation by a benzimidazole ribonucleoside is mediated through the UL89 gene product. J. Virol. **1998**, *72*:717-725.

16. Krosky, P. M.; Underwood, M. R.; Turk, S. R.; Feng, K. W.-H.; Jain, R. K.; Ptak, R. G.; Westerman, A. C.; Biron, K. K.; Townsend, L. B.; Drach, J. C. Resistance of human cytomegalovirus to benzimidazole nucleoside analogs maps to two open reading frames: UL89 and UL56. J. Virol. **1998**, *72*:4721-4728.

17. Horner, J. K.; Henry, D. W. Analogs of 3-Amino-7-chloro-1,2,4-benzotriazine 1-Oxide as Antimalarial Agents. *J. Med. Chem.* **1968**, *11*, 946-949.

18. Devivar, R. V. Design and Synthesis of 2-Substituted Analogs of 2,5,6-Trichloro-1-(β-D-ribofuranosyl)benzimidazole and Related Imidazo[4,5-b]pyridine Nucleosides as Potential Antiviral Agents for the Treatment of Human Cytomegalovirus Infections. Ph. D. Dissertation, University of Michigan, Ann Arbor, Michigan. **1992.**

19. Mandel, L. R.; Porter, C. C.; Kuehl, F. A. Jr.; Jensen, N. P.; Schmitt, S. M.; Windholz, T. B.; Beattie, T. R.; Garty, J. A.; Christensen, B. G.; Shen, T. Y. Inhibition of Adrenal Phenethanolamine N-Methyltransferase by Substituted Benzimidazoles. *J. Med. Chem.* **1970**, *13*, 1043-1047.

20. Kawashima, E.; Gupta, P. K.; Devivar, R. V.; Townsend, L. B. Synthesis of a Precursor for 2-Substituted 5,6-Dichlorobenzimidazoles. In "*Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods and Techniques*", Vol. 4, Townsend, L. B.; Tipson, R. S. Eds., Wiley-Interscience, New York, **1991**, pp. 24-26.

21. Weygand, F.; Wacker, A.; Wirth, F. Vitamin B_{12}, Purine, and Pyrimidine Requirement of Lactobacillus Leichmannii 313. *Z. Naturforsch.* **1951**, *6b*, 25-34.

22. Buchel, K. H. Inhibitors of Photosynthesis VI, Synthesis of Electronegatively Substituted Benzimidazoles. *Z. Naturforsch.* **1970**, *25b*, 945-953.

23. Pande, A. V.; Kirke, P. A.; Khadse, B. G. Synthesis and Study of 2-Benzylthio benzothiazoles and 2-Benzylthiobenzimidazoles as Antituberculosis Agents. *Bull. Haffkine Inst.* **1983**, *11*, 51-55.

24. Bednyagina, N. P.; Tyurenkova, G. N.; Panov, I. V. Benz- and Naphthazole Series. V. 5,6-Dimethyl-2-hydrazinobenzimidazole and its N-Alkylated Derivatives. *Zh. Obshch. Khim.* **1964**, *34*. 1575-1577. CA. **61**: 8297a.

25. Bhat, C. C. Preparation of a Crystalline O-Acyl-2-deoxypentofuranosyl Halide. In "*Synthetic Procedures in Nucleic Acid Chemistry*", Vol. 1, Zorbach, W. W.; Tipson, R. S. Eds. Interscience Publishers, New York, **1968**, pp. 521-522.

26. Kazimierczuk, Z.; Cottam, H. B.; Revankar, G. R.; Robins, R. K. Synthesis of 2'-Deoxytubercidin, 2'-Deoxyadenosine, and Related 2'-Deoxynucleosides via a Novel Direct Stereospecific Sodium Salt Glycosylation Procedure. *J. Am. Chem. Soc.* **1984**, *106*, 6379-6382.

27. Kazimierczuk, Z.; Stolarski, R.; Shugar, D. Stereospecific Synthesis by the Sodium Salt Glycosylation Method of Halogeno Benzimidazole 2'-Deoxyribose Analogues of the Inhibitor of hnRNA Synthesis, 5,6-Dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB). *Z. Naturforsch.* **1985**, *40c*, 715-720.

28. Rosemeyer, H.; Toth, G.; Seala, F. Assignment of Anomeric Configuration of D-ribo-, Arabino-, 2'-Deoxyribo-, and 2',3'-Dideoxyribonucleosides by NOE Difference Spectroscopy. *Nucleosides & Nucleotides*, **1989**, *8*, 587-597.

29. Koszalka, G. W.; Chamberlain, S. D.; Daluge, S. M.; Boyd, F. L.; Tidwell, J. H.; Martin, M. T.; Harvey, R. J.; Frick, L. W.; Perkins, D. G.; Wang, L. H.; Drach, J. C.; Townsend, L. B.; Biron, K. K. Benzimidazoles for the treatment of human cytomegalovirus infection. XII International Roundtable: Nucleosides, Nucleotides and their Biological Applications, La Jolla, CA, September 1996.

30. Chan, J. H.; Chamberlain, S. D.; Davis, M. G.; Biron, K. K.; Harvey, R. J.; Selleseth, D. W.; Dornsife, R. E.; Dark, E. H.; Frick, L. W.; Koszalka, G. W.; Townsend, L. B.; Drach, J. C. Synthesis and evaluation of a series of 2'-Deoxy-(b-L-ribofuranosyl)benzimidazole analogues of the anti-HCMV agent 5,6-dichloro-2-iospropylamino-1-(β-L-ribofuranosyl) 1H benzimidazole (1263W94). *Nucleosides & Nucleotides,* **2000**, submitted (this issue).

31. Whittle, C. P.; Robins, R. K. The Synthesis of 1-(2-Deoxy-α- and -β-D-ribofuranosyl)benzimidazoles Related to the Naturally Occurring Nucleosides of Vitamin B_{12}. *J. Am. Chem. Soc.* **1965**, *87*, 4940-4944.

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

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

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

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

36. Cook, W. J.; Short, S. A.; Ealick, S. E. Crystallization and Preliminary X-ray Investigation of Recombinant Lactobacillus Leichmannii Nucleoside Deoxyribosyltransferase. *Journal of Biological Chemistry* **1990**, *265*, 2682-2683.