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Benzimidazole 2'-Isonucleosides: Design, Synthesis, and Antiviral Activity of 2-Substituted-5,6-Dichlorobenzimidazole 2'-Isonucleosides

G. A. Freeman[a*], D. W. Selleseth,[b] J. L. Rideout[c] and R. J. Harvey[b]

Divisions of [a]Medicinal Chemistry and [b]Pharmacology, GlaxoWellcome Research, Five Moore Drive, RTP, North Carolina, USA, 27709

Dedicated to the memory of Gertrude B. Elion

ABSTRACT: 2,5,6-Trihalogenated benzimidazole-β-D-ribofuranosyl nucleosides and 2-substituted amino-5,6-dichlorobenzimidazole-β-L-ribofuranosyl nucleosides are potent and selective inhibitors of human cytomegalovirus (HCMV). The D-ribofuranosyl analogs are metabolized rapidly in vivo rendering them unsuitable as drug candidates. The primary source of instability is thought to be the anomeric bond. The synthesis of a series of chemically stable benzimidazole-2'-isonucleosides is presented. The synthetic schemes employed are based on nucleophilic displacements of a 2'-tosylate from carbohydrate intermediates with 2-bromo-5,6-dichlorobenzidazole. 2-Bromo and 2-isopropyl amino analogs with 3'- and 5'-oxo and deoxy substitutions were prepared. The benzimidazole-2'-isonucleosides presented here demonstrated reduced activity against HCMV when compared to other D-ribofuranosyl benzimidazole analogs. In addition, they were not found to be inhibitors of HIV.

Introduction

Potent inhibition of human cytomegalovirus (HCMV) by 2-chloro and 2-bromo-5,6-dichlorobenzimidazole-D-riboside (TCRB and BDCRB) was reported by Townsend and Drach[1].

The structure-activity-relationship (SAR) of the benzimidazole nucleosides has been examined extensively. Both the benzimidazole and carbohydrate moieties have been studied.[1,2] No analog in the D-ribofuranoside series is as active and selective as the 2-

Present address:
[c]Inspire Pharmaceuticals, Inc., 4222 Emperor Blvd., Suite 470, Durham, North Carolina, USA, 27703

chloro and 2-bromo-5,6-dichlorobenzimidazole. A novel mechanism of action was reported for these benzimidazoles nucleosides which does not involve anabolism to the triphosphate and subsequent inhibition of the viral polymerase but rather involves inhibition of processing and packaging of viral DNA.[3]

The potency, selectivity, and novel mechanism of action of these compounds make them promising candidates for development as a treatment for HCMV infections. Unfortunately, rapid in vivo metabolism to the inactive benzimidazole base renders them unsuitable for drug development.[4]

In related work, Chamberlain synthesized 5,6-dichloro-2-isopropylamino-benzimidazole-L-riboside, 1263W94, which also demonstrated potent in vitro inhibition of HCMV. Thus extending the SAR of the benzimidazoles to include certain 2-aminosubstituted L-ribose analogs.[5] In addition, the SAR of other benzimidazole L-ribofuranosyl analogs has been investigated. [6]

The metabolic instability of TCBR in rats and monkeys is thought to be caused by the chemical instability of the anomeric bond since trichlorobenzimidazole base was observed in plasma of the test animals. [7] Synthesis of potent HCMV inhibitors with increased stability of the anomeric bond has been approached by several molecular modifications including, carbocyclic analogs, C-nucleosides, and 2'-fluoro nucleoside analogs.[7]

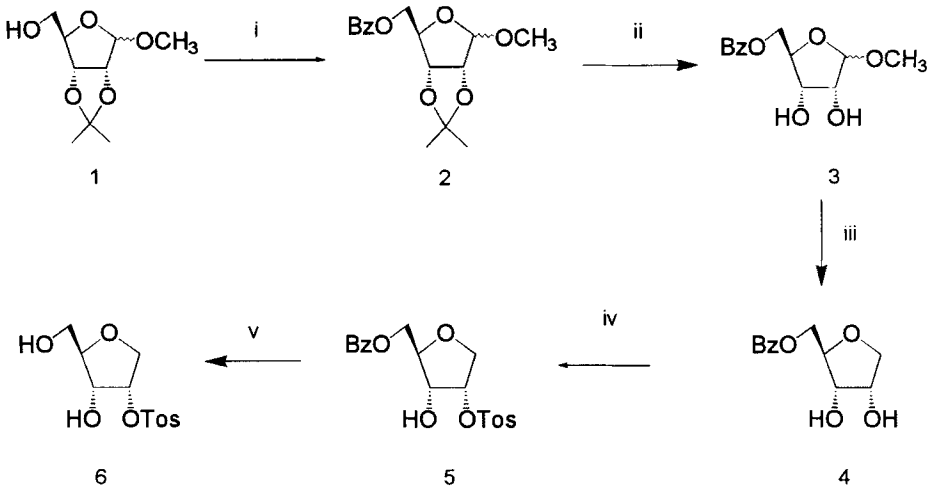
Presented here is an additional attempt to stabilize the anomeric bond, while maintaining the HCMV activity, by preparing benzimidazole 2'-isonucleosides. The concept of constructing nucleoside mimics by transposing the anomeric bond from C-1 to C-2, called 2'-isonucleosides, has been investigated over the last twenty years starting with John Montgomery.[8] A principle reason for transposing the anomeric bond to C-2 has been to gain chemical and metabolic stability. More recent work has focused on increasing the stability of the anomeric bond of ddA by preparing the 2'-isonucleoside analog (IsoddA).[9] In vivo experiments demonstrate that IsoddA is stable in rats when giving orally.[10] In addition to the normal D-ribofuranosyl stereochemistry, 2'-isonucleosides may also adopt an L-ribofuranosyl binding mode as demonstrated for IsoddA.[10]

In light of the above findings, the synthesis, antiviral evaluation, and selectivity index of a series of 2-bromo and 2-isopropylamino-5,6-dichloro-2'-isobenzimidazole analogs is presented.

Chemistry

The carbohydrate precursors used to generate the isomeric set of benzimidazole analogs were prepared using schemes I-III outlined below. The carbohydrate moieties were coupled to 2-bromo-5,6-dichlorobenzimidazole to give the 2-bromo analogs as outlined in Scheme IV. Each was converted to the 2-isopropylamino analog by heating with isopropyl amine.

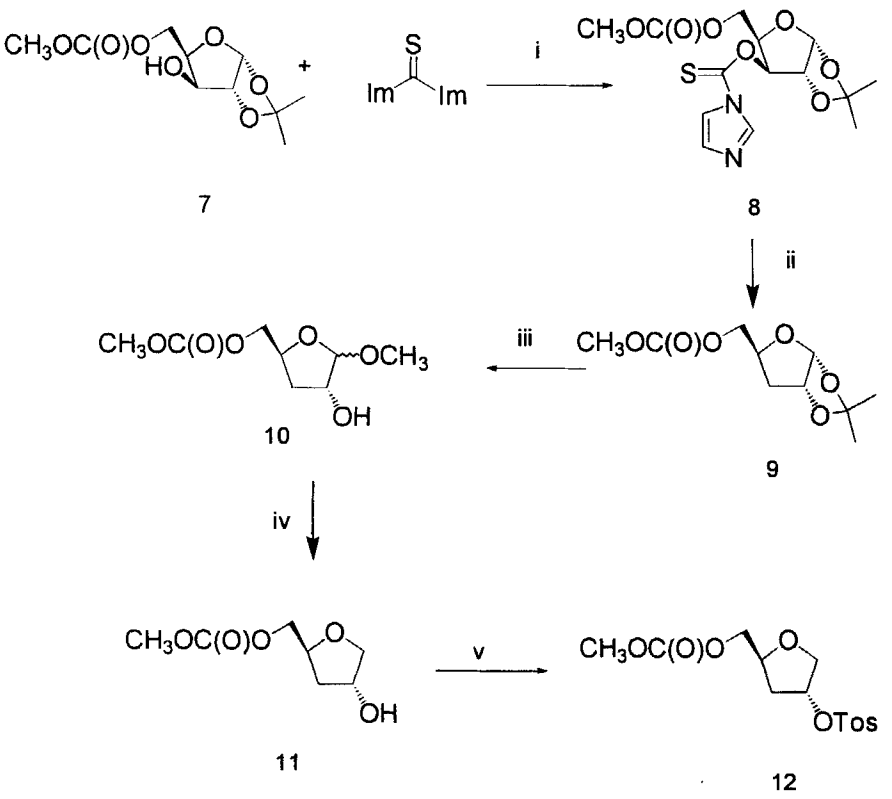
Scheme I



i) Benzoyl chloride/pyridine, ii) Dowex 50 H^+/ MeOH, iii) 1) HMDS 2) $Et_3SiH/TMSTfl/CH_3CN$, iv) $Bu_2SnO/Bu_4NBr/TosCl/CH_2Cl_2$; 4/1 ratio of 2'/3' tosylates, v) $EtOH/MeOH/H_2O/Na_2CO_3$.

The carbohydrate precusor to the 3',5'-dihydroxy-2'-isosteres, 19 and 23, was prepared from 2,3-isopropylidene-1-methyl-D-ribofuranoside, 1 (Scheme I) by protecting the 5'-hydroxyl with a benzoyl group to give 2. The isopropylidene group was removed by stirring in Methanol with Dowex 50 H^+ to give 3 followed by reductive removal of the 1-

Scheme II

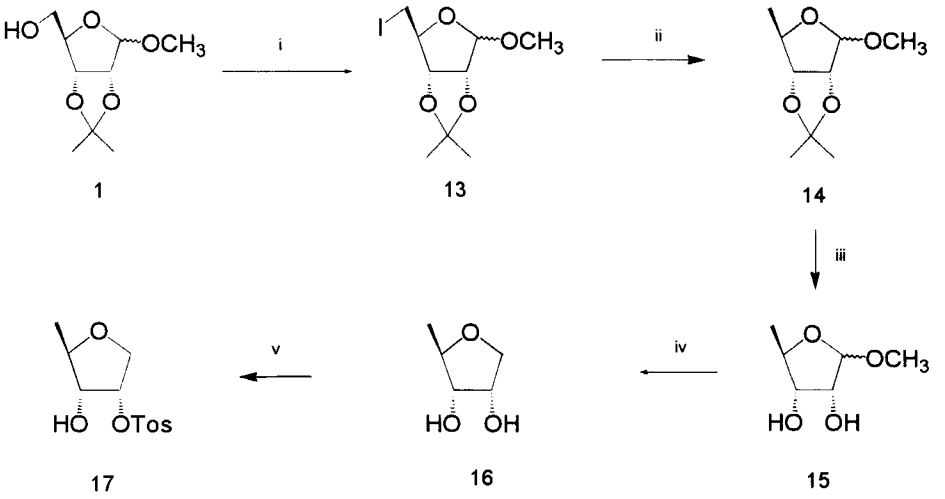


i) $C_2H_2Cl_2$, ii) Bu_3SnH/AIBN/Toluene, iii) HCl/MeOH, iv) 1) HMDS, 2) Et3SiH/TMSTfl/CH3CN, v) TosCl/pyridine.

methoxide with trimethylsilyl triflate and triethyl silane giving 4.[9] Tosylation was accomplished using dibutyltin oxide, tosyl chloride and tetrabutyl ammonium bromide in dichloromethane to give a mixture of 5 and the 3'-tosylate in a ratio of 4/1 respectively as demonstrated by the chemical shifts of the attached 2'/3' protons in the NMR spectra.[12] The 5'-benzoyl protecting group was removed with potassium carbonate in an aqueous alcohol solution to give 6. The coupling reaction for the final products is outlined in scheme IV.

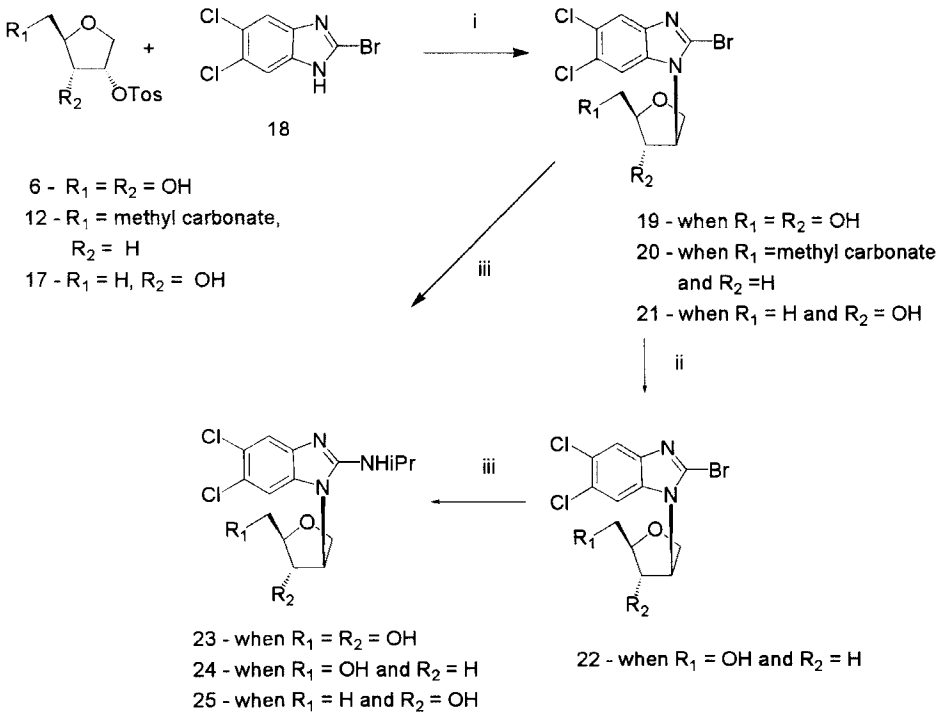
The 3'-deoxy-5'-hydroxy precursor was prepared as outlined in Scheme II. Deoxygenation of 6 was accomplished by preparation of the imidazole thiocarbonyl analog 7 followed by AIBN catalyzed reduction with tributyl tin hydride to give 8. Removal of

Scheme III



i) $(PhO)_3PCH_3I/DMF$, ii) 10% Pd-C/TEA/EtOH, iii) Dowex $50H^+$/MeOH, iv) 1) HMDS 2) $Et_3SiH/TMSTfl/CH_3CN$, v) $Bu_2SnO/Bu_4NBr/TosCl/CH_2Cl_2$;
85/15 ratio of 2'/3' tosylates.

Scheme IV



i) K_2CO_3/18-Crown-6/Toluene, ii) $EtOH/MeOH/H_2O/K_2CO_3$, iii) $iPrNH_2$/EtOH.

the 1,2-isopropylidene and concomitant formation of the methyl glycoside was achieved by reaction of 8 in methanolic HCl to give 9. [9] Reductive removal of the 1-methoxide group was accomplished as for 3 in Scheme I. Coupling to give the final products was accomplished as outlined in Scheme IV.

The 5'-deoxy-3'-hydroxy precursor was prepared as outlined in Scheme III. The 5-hydroxyl of 1 reacted with triphenoxymethyl phosphonium iodide to give the 5'-iodo analog 12. 5'-Dehalogenation was accomplished by catalytic reduction with palladium in ethanol. Removal of the isopropylidene and reduction of the 1-methoxide was accomplished as in Scheme I.. Tosylation was performed as in Scheme I to give a mixture of 17 and the 3'-tosylate in a ratio of 85/15 respectively as demonstrated by the chemical shifts of the attached 2'/3' protons in the NMR spectra.

Coupling to give the final product is outlined in Scheme IV. Intermediates 5, 11, and 14 were heated with 2-bromo-5,6-dichlorobenzimidazole in toluene to give the products 18, 19, and 20 respectively. The 5'-hydroxyl of 19 was deprotected by treatment with potassium carbonate in an alcoholic solution of water to give 21. Conversion of the 2-bromo analogs to the 2-isopropyl amino analogs was accomplished by heating with isopropyl amine in ethanol to give 22, 23, and 24.

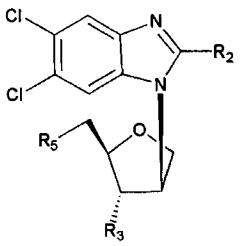
Antiviral Testing and Results

The 2-bromo and 2-isopropylamino benzimidazole analogs 18, 20, 21, 22, 23, and 24 were screened against HCMV in MRC5 cells using the method of outlined below. The results are presented in Table I.

Discussion

A series of 3',5'-dihydroxy, 3'-deoxy-5'-hydroxy and 5'-deoxy-3'-hydroxy-2-bromo and 2-isopropylamino 5,6-dichlorobenzimidazole-2'-isonucleosides was prepared and tested against HCMV. As a set they gave IC_{50}s in the range of 6-25 μM and therefore demonstrated little change in IC_{50} with the carbohydrate modifications. Little difference

Table 1. Antiviral Activity and Selectivity of 2-Substituted-5,6-dichlorobenzimidazole-2'-isonucleosides against HCMV



Compound	R_2	R_3	R_5	HCMV IC_{50}	TC_{50}[1] - MRC5 cells	SI[2]
18	Br	OH	OH	25 μM	100 μM	4
20	Br	H	OH	14 μM	100 μM	7
21	Br	OH	H	11 μM	100 μM	9
22	NHiPr	OH	OH	10 μM	32 μM	3.2
23	NHiPr	H	OH	6 μM	65 μM	11
24	NHiPr	OH	H	10 μM	32 μM	3.2
BDCRB				0.5 μM	100 μM	200
1263W94				0.05 μM	100 μM	2000

[1] TC_{50} is the concentration causing a 50% reduction in the number of host cells.

[2] SI is the selectivity index obtained by dividing the EC_{50} by the TC_{50}.

was noted between the bromo and isopropyl amino analogs. The cytotoxicities to the host MRC cells were in the range of 32-100 mM with selectivity indices in the range of 3-11. In comparison to BDCBR and 1263W93 they were 10 to 400 fold less active and less selective. Evaluation of these compounds in vivo for increased metabolic stability was not pursued. In comparison to other modifications aimed at increasing the metabolic stability of the benzimidazole nucleosides, the 2'-deoxy-2'-fluoro-ß-D-ribofuranosyl, 2'-deoxy-2'-fluoro-ß-D-arabino, and 3'-deoxy-3'-fluoro-ß-D-xylofuranosyl analogs all demonstrated

significantly reduced antiviral activity as was also noted for the C-nucleoside analogs. In contrast, some of the carbocyclic analogs, in particular the L-ribofuranosyl types, demonstrated potent inhibition of HCMV.[7]

Further evaluation of analogs 18, 21, and 23 against HIV demonstrated no antiviral inhibition.

In conclusion, while transposing the anomeric bond to the 2'-position of the benzimidazole nucleosides should increase the chemical and metabolic stability of the resulting molecule it also resulted in decreased antiviral activity and selectivity.

Experimental Section

General Chemical Procedures

^{1}H NMR spectra were recorded on Varian Unity Plus 400 MHz and 300 MHz NMR spectrometers. Mass spectral data were obtained using a Micromass Platform Mass Spectrometer utilizing either an electro-spray (ES) technique or atmospheric pressure chemical ionization (APCI). Some mass spectra data were obtained by fast atom bombardment (FAB). 2-Bromo-5,6-dichlorobenzimidazole was prepared as described in reference 13. Commercial reagents were used without further purification. Flash column chromatography was performed according to reference 14. The reported experimental procedures were sometimes representative of many runs.

HCMV assay system

Cells, viruses, and viral infection. Human diploid fibroblasts (MRC-5) were from BioWhittaker, (Watersville, MD) and human cytomegalovirus (HCMV) strain AD169 was from American Type Culture Collection (Rockville, MD). Monolayer cultures were grown at 37°C in Eagle's minimal essential medium (MEM, GIBCO) supplemented with Earle's salts, L-glutamine, antibiotics, and 8% fetal calf serum (Hyclone Laboratories, Logan, UT) as described [MRU]. HCMV (strain AD169) was plaque purified and mycoplasma free (Gen Probe Mycoplasma Rapid Detection System, Fisher Scientific, Pittsburgh, PA).

For viral infection, monolayers were grown to confluence. The medium was removed; virus was added and suspended in the minimum volume of MEM with the serum concentration reduced to 2%. Plates were centrifuged at 1500 rpm for 10 min at 25°C and incubated at 37°C for 90 min to allow virus adsorption. MEM containing 2% fetal calf serum ± test compounds was added to the wells.

DNA hybridization assay. MRC-5 cells were seeded (1 x 10^4 cells/well), grown to confluence (~2 x 10^4 cells/well), and infected at a multiplicity of infection (MOI) of 0.01. For drug studies, a range of eight concentrations of compound were added to define the dose-response curves for each inhibitor. After six days, 50 µL of lysis buffer (100 mM Tris-HCl, pH 8, 50 mM EDTA, 0.2% SDS, 0.1 mg/mL proteinase K) was added per well. Plates were incubated for 1 h at 55°C after which the DNA was extracted by the addition and mixing of 65 µL of TE (10 mM Tris-HCl, 1 mM EDTA [pH 7.0])-saturated phenol-chloroform and 150 µL water. After centrifugation (2200 rpm for 15 min), a 50 µL sample of the aqueous phase was mixed with an equal volume of 0.6 M NaOH, denatured at 95°C for 15 min and made to 1.5 M ammonium acetate, 1.5 M ammonium dihydrogen phosphate, 5 mM EDTA (pH 6.5). Samples were blotted onto GIBCO supported nitrocellulose filters (catalog no. 1465MH) on a 96-well BRL Convertible Filtration Manifold. Wells were rinsed with 200 µL of the above buffer. The DNA was cross-linked to the filter with a Stratlinker 1800 UV oven (Stratagene, La Jolla, CA) on the Autolink setting. Filters were prehybridized overnight at 45°C in 10 mL of 6X SSPE (1X SSPE is 0.18 M NaCl and 10 mM sodium phosphate, pH 7.7, and 1 mM EDTA), 0.5% SDS, 50 µg/mL salmon sperm DNA, and 10X Denhardt's solution (0.2% each of Ficoll, polyvinylpyrrolidone and bovine serum albumin). Nick-translated DNA probe was prepared from two plasmids carrying 35 kb each of HCMV sequence from UL region (pC7S31 and 37, 0.22- 0.6 map units). The probes are labeled with [^{32}P- or ^{33}P] -dCTP using random primers and T7 DNA polymerase (Pharmacia) after cutting with XbaI. After being denatured at 95°C for 15 min, the probes were diluted into hybridization solution (6X SSPE and 0.5% SDS) containing 50 µg/mL salmon sperm DNA. The prehybridization solution was replaced with 5 mL of hybridization solution containing 1 x 10^6 cpm of each ^{32}P- or ^{33}P-labelled probe per mL and incubated at 65°C for 16 h. The membranes were then washed as follows: twice with 6X SSPE with 0.5% SDS for 15 min

at room temp, twice with 1X SSPE with 0.5% SDS for 15 min at 42°C, and once with 0.1X SSPE with 0.5% SDS for 30 min at 65°C. Filters were dried and their associated radioactivity was measured with a PhosphorImager with its associated ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Test compound IC_{50} and IC_{90} values were determined using the probit analysis method. Alternatively, an IC_{50} program based on the Hill equation was used to generate IC_{50} and IC_{90} values.

For cell-associated virus, 50 to 150 infected cells were added per well of confluent MRC-5 cells and the plates centrifuged at 750 x g for 10 min to assure optimal cell-to-cell contact. Following incubation of the cells for 90 min at 37°C, test compound (in MEM containing 2% serum) was added as a serial three-fold dilution. Six concentrations of each compound were tested in triplicate. After incubation for six days at 37°C, (CPE 25 - 100%), lysis buffer was added and the DNA was extracted, immobilized, and quantitated as described above.

5-(O-Benzoyl-2,3-isopropylidene-1-Methoxy-D-ribofuranoside (2). 2,3-Isopropylidene-1-methoxy-D-ribofuranoside (1, 25g, 0.12 mol) was dissolved in pyridine (125 mL) and chilled to 0° C in an ice bath. Benzoyl chloride (13.7 mL, 0.15 mol, 1.25eq.) was added and the reaction was allowed to warm to room temperature. After four hours tlc (hexane/ ethyl acetate, 4/1, v/v) indicated a complete reaction. Methanol was added to the reaction and stirred several hours. The solvents were removed in vacuo. Toluene (100 mL, 2X) was added and removed in vacuo. The residue was partitioned between water and ethyl acetate. The ethyl acetate layer was removed and washed with brine. The organic fraction was dried with $MgSO_4$ and filtered. The solvent was removed in vacuo and the residue was purified by chromatography on silica gel (500g) eluted with hexane/ethyl acetate (9:1, v/v). The product containing fractions were combined and the solvents removed in vacuo to give a 91% yield. MS (FAB+); m/z, 309, M+1: ^{1}H NMR (DMSO-d6) δ 7.96 (d, 2H, H-o-Ph, J=7.1Hz), 7.65 (t, 1H, H-p-Ph, J= 7.5Hz), 7.53, (d, H2, H-m-Ph, J=7.7Hz), 4.94 (s, 1H, H-1), 4.80 (d, 1H, H-2, J=5.8Hz), 4.60 (d, 1H, H-3, J=6.0Hz), 4.39 (m, 1H, H-4), 4.3-4.17 (m, 2H, H-5), 3.19 (s, 3H, H-OCH_3), 1.37 (s, 3H, H-CH_3), 1.24 (s, 3H, H-CH_3).

[(2R,3S,4S)-3,4-dihydroxytetrahydro-2-furanyl]methyl benzoate (4). 5-(O-Benzoyl)-2,3-isopropylidene-1-methoxy-D-ribofuranoside (2, 13g, 0.042 mol) was dissolved in

methanol (150 mL). Dowex 50 H^+ resin (1g), freshly washed with methanol, was added and the reaction stirred at room temperature. Reaction progress was monitored by tlc (ethyl acetate/hexane, 2/1, v/v). After I hr, Dowex 50 H^+ resin was added again and the reaction stirred overnight. Dowex 50 H^+ resin was added again and the reaction stirred a second night. The reaction was filtered and the methanol was removed in vacuo. The product was used in the next step without purification. 5-Benzoyl methyl-D-ribofuranoside (3) (1.5g, 0.0056 mol) was combined with HMDS (15 mL) and ammomium sulfate (0.002g) and heated to reflux overnight. The HMDS was removed in vacuo. The residue was dissolved in acetonitrile (25 mL) and triethyl silane (2.7 mL, 0.017 mol, 3 eq.) was added. TMS triflate (3.2 mL, 0.017 mol, 3 eq.) was added over fifteen minutes. The reaction was stirred at room temperature for 75 minutes until tlc (hexane/ethyl acetate, ½, v/v) indicated no further reaction. The reaction was chilled in an ice bath and water (25 mL) was added. The pH was adjusted to 7 with sodium hydroxide (5N) solution. The product was extracted with ethyl acetate (3X). The organic fractions were combined, dried with $MgSO_4$, and filtered. The product was isolated by chromatography on a 5 by 13 cm column of silica gel eluted with chloroform/methanol (99/1, v/v) in a 44% yield. MS (APCI+); m/z, 239, M+1: ^{1}H NMR (DMSO-d_6) δ 7.93 (d, 2H, H-o-Ph, J=7.7Hz), 7.64 (t, 1H, H-p-Ph, J= 7.5Hz), 7.51, (m, H2, H-m-Ph, J=7.7Hz), 4.99 (d, 1H, H-OH, J=5.5Hz), 4.85 (d, 1H, H-OH, J=4.4Hz), 4.4 (m, 1H, H-4), 4.2 (m, 1H, H-3), 4.0 (m, 1H, H-2), 3.9 (m, 3H, H-5,CH_2-benzoate), 3.5(m, 1H, H-5).

((2R,3R,4S)-3-hydroxy-4-{[(4-methylphenyl)sulfonyl]oxy}tetrahydro-2-furanyl)methyl benzoate (5). [(2R,3S,4S)-3,4-dihydroxytetrahydro-2-furanyl]methyl benzoate and tetrabutylammonium bromide (0.47g, 0.0015 mol) were slurrried in toluene (30 mL) and heated to boiling to remove residual water. The toluene was removed in vacuo. Dichloromethane (15 mL) was added to the residue followed by dibutyl tin oxide (0.37g, 0.0015 mol, 1 eq.) The reaction was stirred for 10 minutes at room temperature and tosyl chloride (0.31g, 0.0016 mol, 1.1 eq.) was added. The reaction was stirred at room temperature for 4.5h. The reaction was followed by tlc on silica gel eluted with chloroform/methanol (95/5, v/v). The solvent was removed in vacuo and the products isolated by chromatography on silica gel (15g) eluted with chloroform (200 mL) followed by chloroform/methanol (99/1, 200 mL). The products were collected as a mixture of 2- and 3-tosylates (4:1 ratio) in a total yield of 83%. Structural assignment was based on

NMR chemical shifts of the 2 and 3 protons. MS (APCI+); m/z, 393, M+1; ^{1}H NMR (DMSO-d_6) δ 8-7.4 (m, 9H, aryls), 5.62 (d, 1H, OH of 4-tosylate, J=6.8 Hz), 5.51 (d, OH-3-tosylate, J=5.7 Hz), 4.82 (m, 1H, H-4 4-tosylate), 4.7 (m, H-3 of 3-tosylate), 4.42 (d, 1H, H-CH_2-benzoate$_a$, J= 12 Hz), 4.2 (dd, 1H, H-CH_2-benzoate, J=5.3, 12 Hz), 4.1 (m, 1H, H-3) 3.95-3.86 (m, 2H, H-2, H-5_a), 3.66 (d, 1H, H-5_b, J=11Hz), 2.4 (s, 3H, CH_3-tosylate).

(3S,4R,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydro-3-furanyl 4-methyl-benzenesulfonate (6). ((2R,3R,4S)-3-hydroxy-4-{[(4-methylphenyl)sulfonyl]-oxy}tetrahydro-2-furanyl)methyl benzoate, 5, (1.0g, 0.0025 mol) was dissolved in a solution of ethanol and methanol (80 mL, 1/1, v/v). Sodium carbonate (0.54g, 0.005 mol, 2 eq.), dissolved in water (10 mL), was added. The reaction was stirred at room temperature for 24 h. Brine was added (100 mL) and the solution was extracted with ethyl acetate (150 mL, 2X). The organic fraction was dried with $MgSO_4$ and the solvents removed in vacuo. The product was isolated by chromatography on silica gel (50g) eluted with chloroform/methanol (95/5, v/v) giving compound 6, 0.55g, 75% yield. MS (ES-); m/z, 323, M+Cl^-; ^{1}H NMR (DMSO-d_6) δ 7.83 (d, 2H, aryl, J=8.3Hz), 7.49 (d, 2H, aryl, J=8.3Hz), 5.34(d, 1H, 4-OH, J=6.5 Hz), 4.77 (m, 1H, H-3), 4.67 (t, 1H, CH_2OH, J= 5.6Hz), 3.95 (m, 1H), 3.85-3.75 (m, 2H), 3.6-3.4 (m, 3H), 2.4 (s, 3H, CH_3-tosylate).

(2R,3S,4R)-4-(2-bromo-5,6-dichloro-1H-benzimidazol-1-yl)-2-(hydroxymethyl)-tetrahydro-3-furanol (19). (3S,4R,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydro-3-furanyl 4-methylbenzenesulfonate, 6, (0.55g, 0.0019 mol), 2-bromo-5,6-dichlorobenzimidazole (0.5g, 0.0019 mol, 1 eq.), potassium carbonate (0.315g, 0.0023mol, 1.2 eq.), and 18-Crown-6 (0.5g, 0.0019mol, 1 eq.) were combined in toluene (25 mL) and heated in a 95°C oil bath for 6.5 hrs. The reaction was partitioned between water and ethyl acetate. The product containing ethyl acetate phase was collected and the solvent removed in vacuo. The dark residue was purified by chromatography on a 2.5 by 14 cM column of silica gel eluted with chloroform/methanol (98/2, 500 mL; followed by 95/5, 500 mL). The product containing fractions were combined and the solvents removed in vacuo. Final purification was accomplished by treating the product with ethyl ether. A 10% yield was obtained. MS (FAB+); m/z, 346, ($M+Cl^+$), ^{1}H NMR (DMSO-d_6) δ 8.08

(s, 1H, aryl), 7.93 (s, 1H, aryl), 5.72 (d, 1H, 3'-OH, J=5.8Hz), 5.08 (m, 2H, H-4' and 5'-OH), 4.4 (m, 1H, H-3'), 4.23 (m, 1H, H-5_a'), 4.1 (m, 1H, H-5_b'), 3.7 (m, 1H, H-CH_2OH), 3.6 (m, 1H, H-2'), 3.55 (m, 1H, CH_2OH).

(2R,3S,4R)-4-[5,6-dichloro-2-(isopropylamino)-1H-benzimidazol-1-yl]-2-(hydroxymethyl)tetrahydro-3-furanol (23). (2R,3S,4R)-4-(2-bromo-5,6-dichloro-1H-benzimidazol-1-yl)-2-(hydroxymethyl)tetrahydro-3-furanol,19, (0.05g, 0.00013 mol) was heated with ethanol (5 mL) and isopropylamine (2 mL) in a 90 ^{o}C oil bath overnight. The solvents were removed in vacuo. The product was purified by chromatography on a 2.5 by 8 cm column of silica gel eluted with ethyl acetate/hexane (1/2, v/v) followed by chromatography on a 2.5 by 8 cm column of silica gel eluted with chloroform. The product containing fractions were combined and the solvents removed in vacuo to give a 45% yield. MS (ES+); m/z, 360, $M+H^+$; ^{1}H NMR (DMSO-d_6) δ 7.54 (s, 1H, aryl), 7.35 (s, 1H, aryl), 6.57 (d, 1H, NH, J=7.4 Hz), 5.66 (d, 1H, 3'-OH, J=5.1Hz), 5.10 (t, 1H, OH of CH_2OH, J=5.3 Hz), 4.93 (m, 1H, H-4'), 4.42 (m, 1H, H-3'), 4.10 (m, 1H, H-5_a'), 4.06-3.96 (m, 2H, H-CH, 5_b'), 3.74-3.71 (m, 1H, CH_2OH'), 3.60-3.55 (m, 2H, H-CH_2OH, 2'), 1.20-1.17 (m, 6H, CH_3).

3-O-(1-(1H-Imidazolyl)thiocarbonyl)-1,2-O-isopropylidene-5-O-(methoxycarbonyl)-α-D-xylose (7). 1,2-O-Isopropylidene-5-O-(methoxycarbonyl)-α-D-xylose (18.5g, 0.0746 mol, Pfanstiehl) was dissolved in 240 mL of 1,2-dichloroethane. 1,1-Thiocarbonyldiimidazole (20 g, 0.112 mol, 1.5 equivalents) was added. The reaction was refluxed for three hours under nitrogen then cooled and washed with water (3 X). The organic fraction was dried with Na_2SO_4, filtered and the solvent removed in vacuo. The residue was crystallized from ethyl acetate/ hexane to give the product in 69% yield. MS, (CI+); m/z, 359, M+1; ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1H, aryl), 7.63 (s, 1H, aryl), 7.17 (s, 1H, aryl), 6.0 (d, 1H), 5.9 (d,1H), 4.89 (d, 1H), 4.70-4.67 (m, 1H), 4.5-4.3 (m, 2H), 3.80 (s, 3H, OCH_3), 1.55, 1.3 (2s, 3H each, $2CH_3$).

3-Deoxy-1,2-O-isopropylidene-5-O-(methoxycarbonyl)-α-D-erythro-pentofuranose (8). 3-O-(1-(1H-Imidazolyl)thiocarbonyl)-1,2-O-isopropylidene-5-O-(methoxycarbonyl)-

α-D-xylose (5g, 14 mmol) was dissolved in toluene (300 mL) and heated to reflux under nitrogen. AIBN (2g, 12 mmol) was dissolved in toluene (50 mL). Tributyltin hydride (5.66 mL, 21 mmol, 1.5 eq.) was added to the AIBN solution. The tributyltin hydride/AIBN solution was added to the refluxing reaction over thirty minutes. The reaction was continued for 2 h. and the solvent removed in vacuo. The residue was partitioned between acetonitrile (400 mL) and hexane (400 mL). The acetonitrile solution was extracted with hexane (3X). The acetonitrile was removed in vacuo and the residue purified by chromatography on 200g of silica gel eluted with 2 L hexane followed by 2.5 L hexane/ethyl acetate (4:1). The product containing fractions were combined and the solvents removed in vacuo to give a 58% yield. MS, (CI+); m/z, 255, M+Na; ^{1}H NMR ($CDCl_3$) δ 5.85 (d, 1H), 4.88 (m,1H), 4.50-4.28 (m, 2H), 4.22-4.15 (dd, 1H), 3.80 (s, 3H, OCH_3), 2.20-2.02 (m,1H), 1.80-1.60 (m, 1H), 1.50, 1.30 (2s, 3H each, $2CH_3$).

1,3-Dideoxy-2-hydroxy-5-O-(methoxycarbonyl)-D-pentofuranose (10). 3-Deoxy-1,2-O-isopropylidene-5-O-(methoxycarbonyl)-α-D-erythro-pentofuranose (1.9g, 8.2 mmol) was dissolved in methanol (75 mL). HCl(g) was bubbled into the solution for several minutes giving a pH of approximately 1. The reaction was stirred at room temperature for ten hours. The reaction was neutralized with AG-1 hydroxide resin. The mixture was filtered and the methanol removed in vacuo. The product (10) was purified by chromatography on 50g of silica gel eluted with hexane/ethyl acetate (1:1). The product containing fractions were combined and the solvents removed in vacuo. The yield of the 2-hydroxy-1-methoxy analog was 71%. 3-Deoxy-2-hydroxy-1-methoxy-5-O-(methoxy-carbonyl)-D-pentofuranose, 9, (1.2g, 5.8 mmol), hexamethyldisilazane (20 mL) and a catalytic amount of ammonium chloride were refluxed overnight. The hexamethyldisilazane was removed in vacuo. The residue was evaporated with toluene (20 mL, 2 X) in vacuo. The residue was dissolved in acetonitrile (30 mL). Triethylsilane (2.88 mL, 17.4 mmol, 3eq.) was added. Addition of trimethylsilyl triflate (3.5 mL, 17.4 mmol, 3 eq.) was added over ten minutes. The reaction was stirred at room temperature for 2 h. Water (10 mL) was added and the pH adjusted to 7 with 5N NaOH. The product was extracted with ethyl acetate (2X) and dried with $MgSO_4$. The mixture was filtered and the solvent removed in vacuo. The residue was purified by chromatography on 50g of silica gel eluted with hexane/ethyl acetate (1:2). The product containing fractions were combined and the solvents removed in vacuo to give a 58% yield. MS, (APCI+); m/z, 199

M+Na; ^{1}H NMR (DMSO-d_6) δ 4.92 (d, 1H, OH, J=3.7Hz), 4.26 (m,1H, H-4), 4.18-4.13 (m, 1H, H-5_a), 4.12-3.95 (m, 1H, H-5_b), 3.76-3.73 (dd, 1H, H-1_a), 3.49-3.41 (m,1H,H-1_b), 1.80-1.75 (dd, 1H, H-3_a), 1.66-1.60 (m, 1H H-3_b).

5-(O-Methoxycarbonyl)-(2S,4R)-tetrahydro-4-(tosyloxy)furan (12).

1,3-Dideoxy-2-hydroxy-5-O-(methoxycarbonyl)-D-pentofuranose (0.6g, 3.4 mmol) was dissolved in pyridine (5mL) and cooled to 5°C. Toluenesulfonyl chloride (0.975g, 5.1 mmol, 1.5 eq.) was added and the reaction stirred at 5°C overnight. The reaction was poured into water (10 mL) and the product extracted with ethyl ether (2X). The ether solution was extracted with 1N HCL followed by water. The ether solution was dried with $MgSO_4$ and filtered. The solvent was removed in vacuo and the residue purified by chromatography on 50g of silica gel eluted with hexane/ethyl acetate (1:1). The product containing fractions were combined and the solvents removed in vacuo to give a 38% yield. MS, ES +; m/z, 353, M+Na; ^{1}H NMR (DMSO-d_6) δ 7.8 (d, 2H, aryls), 7.45 (d, 2H, aryls), 5.1 (m, 1H, H-2), 4.2 (m, 1H, H-4), 4.1-3.95 (m, 2H, H-5), 3.85-3.6 (m, 2H, H-1), 3.65 (s, 3H, H-OCH_3), 2.4 (s, 3H, tosyl-CH_3) 2.1-1.8 (m, 2H, H-3).

1,4-Anhydro-2-(2-bromo-5,6-dichloro-1H-benzimidazole-1-yl)-2,3-dideoxy-D-threo-pentitol (22). 5-(O-Methoxycarbonyl)-(2S,4R)-tetrahydro-4-(tosyloxy)furan (0.4g, 1.2 mmol), 2-Bromo-5,6-dichlorobenzimidazole (0.36g, 1.3 mmol, 1.1 eq.), and K_2CO_3 (0.17g, 1.26mmol, 2.1 eq.) were combined in DMF (10 mL). The reaction was heated in an 80°C oil bath for four days. The reaction was poured into 100 mL of ice water and extracted with toluene (3X). The toluene solution was dried with $MgSO_4$ and filtered. The solvent was removed in vacuo and the residue purified by chromatography on a 2.5 by 10 cm column of silica gel eluted with chloroform followed by 1% methanol in chloroform. The solvent was removed from the product containing fractions. The product was purified by chromatography a second time on a 2.5 by 10 cm column of silica gel eluted with hexane/ethyl acetate 300 mL (2:1), 400 mL(1:1), and 600 mL (1:2). The solvent was removed to give 5-(O-Methoxycarbonyl)-(2S,4R)-tetrahydro-4-(2-bromo-5,6-dichlorobenzimidazole)furan (19) in 20% yield. The 19 was treated in EtOH/MeOH/H_2O (4/4/1), 9 mL, with K_2CO_3 (0.053g, 2eq.). The reaction was stirred at rt overnight. The reaction was poured into water and extracted with ethyl acetate. The ethyl acetate solution

was dried with $MgSO_4$, filtered and the solvent removed in vacuo. The residue purified by chromatography on 10g of silica gel eluted with hexane/ethyl acetate (1:2). The product containing fractions were combined and the solvents removed in vacuo. The yield was 38%. MS (FAB): m/z, 365, M+1. ^{1}H NMR (DMSO-d_6) δ 8.20 (s, 1H, aryl), 7.90 (s, 1H, aryl), 5.4 (m, 1H, H-2'), 5.0 (t, 1H, OH), 4.15 (dd, 1H, H-1_a'), 4.0 (m, 1H, H-1_b'), 3.9 (m, 1H, H-4'), 3.7 (m, 1H, H-5_a'), 3.55 (m, 1H, H-5_b'), 2.4 (m, 1H, H-3_a'), 2.0 (m, 1H, H-3_b').

1,4-Anhydro-2-(5,6-dichloro-2-isopropylamino-1H-benzimidazole-1-yl)-2,3-dideoxy-D-threo-pentitol (24). 1,4-Anhydro-2-(2-bromo-5,6-dichloro-1H-benzimidazole-1-yl)-2,3-dideoxy-D-threo-pentitol was converted to the title compound by the method used for compound 23. MS (APCI+); m/z, 344, M+1. ^{1}H NMR (DMSO-d_6) δ 7.6 (s, 1H, aryl), 7.3 (s, 1H, aryl), 6.6 (d,1H,NH, J=7.2Hz), 5.2 (m,1H, H-2'), 5.05 (t, 1H, OH), 4.05 (dd, 1H, H-1_a'), 4.0 (m, 1H, CH), 3.85 (m, 2H, H-1_b' and 4'), 3.7 (m, 1H, H-5_a'), 3.55 (m, 1H, H-5_b'), 2.3 (m, 1H, H-3_a'), 1.9 (m, 1H, H-3_b'), 1.2 (m, 6H, 2-CH_3s).

5-Deoxy-1-methoxy-D-ribofuranoside (15). 2,3-Isopropylidene-1-methoxy-D-ribofuranoside (17g, 0.083 mol) was dissolved in toluene and boiled to remove residual water. The excess toluene was removed in vacuo. The residue was dissolved in DMF (anhydrous, 40 mL). Methyl triphenoxyphosphonium iodide (39.4g, 0.087 mol, 1.05 eq.) was weight out and dissolved in DMF (anhydrous, 60 mL) in a glove bag and sealed with a septum. The solution of the carbohydrate in DMF was added to the reagent by canula. The reaction was stirred at room temperature for 1 hr. Thin layer chromatography, tlc, on silica gel eluted with hexane/ethyl acetate (4/1,v/v) indicated a complete reaction. The solution was poured onto 1L of ice. The product was extracted with hexane (300 mL, 3X). The fractions were combined and the hexane removed in vacuo to give the crude product, 13. Removal of the 5'-iodo group was accomplished by catalytic reduction in ethanol (150 mL) and 10% Pd/C (0.8g) and ammonium hydroxide (5.6 mL) under 50 psi of hydrogen for 4.5 h. The reaction was followed by tlc, silica gel eluted with hexane/ethyl acetate (4/1,v/v). The reaction was filtered and the solvent removed in vacuo. The residue was partitioned between water and ethyl acetate. The organic layer was dried with $MgSO_4$, filtered and the solvent removed in vacuo. The residue was purified by filtration through a silica gel pad (125 mL in a 250 mL glass filtration funnel) eluted with

hexane/ethyl acetate (4/1,v/v). The product containing fractions were combined and the solvents removed in vacuo. The isopropylidene group was removed by stirring in methanol with Dowex 50 H^+ as described for 3. The reaction was followed by tlc, silica gel eluted with ethyl acetate/hexane (2/1, v/v). After completion of the reaction, the resin was removed by filtration and the solvent was removed in vacuo. The product was purified by filtration through a silica gel pad, 150g, in a glass funnel, 300 mL, eluted with ethyl acetate/hexane (1/1, v/v). The product containing fractions were combined and the solvents removed in vacuo to give a 20% yield over three steps. ^{1}H NMR (DMSO-d_6) δ 4.55 (s,1H, H-1), 3.78 (m, 1H, H-4, J=6.5Hz), 3.68 (d, 1H, H-2, J=4.7Hz), 3.59 (m, 1H, H-3), 3.18 (s, 3H, OCH_3), 1.15 (d, 3H, CH_3).

(2R,3S,4S)-2-methyltetrahydro-3,4-furandiol (16). 5-Deoxy-1-methoxy-D-ribofuranoside was converted to 16 by the method used for 4.The reaction was placed in an ice bath and water was added. The pH was adjusted to 8 with sodium hydroxide solution (5N). The product was extracted with ethyl acetate (6X). The fractions were combined, dried with $MgSO_4$ and filtered. The solvent was removed in vacuo and the residue purified by filtration on a pad of silica gel, 20g in a 100mL glass filter, eluted with neat chloroform followed by chloroform/methanol (98/2, then 95/5, v/v). The solvents were removed in vacuo to give the product in 47% yield. MS (FAB+); m/z, 119, M+H; ^{1}H NMR (DMSO-d_6) δ 4.7-4.68 (m, 2H, H-OH), 4.0-3.9 (m, 1H, H-2), 3.9-3.85 (m, 1H, H-1_a), 3.6-3.5 (m, 1H, H-4), 3.45-3.35(m, 2H,H-3,1_b), 1.10 (d, 3H, CH_3).

(3S,4R,5R)-4-hydroxy-5-methyltetrahydro-3-furanyl 4-methylbenzenesulfonate (17). (2R,3S,4S)-2-methyltetrahydro-3,4-furandiol (16) was converted to 17 by the method used for 5. The product was purified by filtration through a silica gel pad, 100g in a 300 mL glass funnel, eluted with chloroform followed by chloroform/methanol (98/2, v/v). The solvents were removed in vacuo to give the product in 86% yield as a mixture of 2',3'-tosylates in a ratio of 85/15 as determined by comparison of the 2, 3 proton NMR chemical shifts. MS (APCI+); m/z, 295, $M+Na^+$; 2-Tosylate: ^{1}H NMR (DMSO-d_6) δ 7.77 (d, 2H, aryl, J=8.3Hz), 7.43 (d, 2H, aryl, J=8.3Hz), 5.38 (d, 1H, OH, J=7.4Hz), 4.8 (m, 1H, H-2), 3.9 (m, 1H, H-1_a), 3.5 (m, 3H, H-1_b, 3, and 4), 2.4 (s, 3H, CH_3-tosylate), 1.08 (d, 3H, CH_3-5, J=5.5 Hz).

(2R,3S,4R)-4-(2-bromo-5,6-dichloro-1H-benzimidazol-1-yl)-2-methyltetrahydro-3-furanol (21). (3S,4R,5R)-4-hydroxy-5-methyltetrahydro-3-furanyl 4-methylbenzene-sulfonate was converted to 21 by the method used for 19. The reaction was partitioned between water and ethyl acetate. The organic phase was dried with $MgSO_4$, filtered and the solvent removed in vacuo. The residue was purified by chromatography on a 2.5 by 14 cm column of silica gel eluted ethyl acetate/hexane (1/2; v/v). The product containing fractions were combined and the solvents removed in vacuo. Final purification of the product was achieved by slurrying in methanol with Dowex hydroxide resin to remove the 2-bromo-5,6-dichlorobenzimidazole starting material. A 25% yield was obtained. MS (APCI+); m/z, 366, $M+H^+$; ^1H NMR (DMSO-d_6) δ 7.95 (s, 1H, aryl), 7.86 (s, 1H, aryl), 5.77 (d, 1H, OH, J=5.8Hz), 5.0 (m, 1H, H-4'), 4.2-4.1 (m, 2H, H-5'), 3.9-3.85 (m, 1H, 3'), 3.7-3.6 (m, 1H, H-2'), 1.31 (d, 3H, CH_3, J=6.2 Hz).

(2R,3S,4R)-4-[5,6-dichloro-2-(isopropylamino)-1H-benzimidazol-1-yl]-2-methyl-tetrahydro-3-furanol (25). (2R,3S,4R)-4-(2-bromo-5,6-dichloro-1H-benzimidazol-1-yl)-2-methyltetrahydro-3-furanol (21) was converted to 25 by the method used for 23. The product was partially purified by chromatography on a 2.5 by 8 cm column of silica gel eluted with ethyl acetate/ hexane (1/2, v/v) followed by ethyl acetate/hexane (1/1, v/v). Final purification was achieved by chromatography on silica gel eluted with chloroform. A 15% yield was obtained. MS (APCI+): m/z, 344, $M+H^+$, ^1H NMR (DMSO-d_6) δ 7.38 (s, 2H, aryl), 6.6 (br s, 1H, NH), 5.72 (d, 1H, OH, J=5.1Hz), 4.9 (m, 1H, H-4'), 4.1-3.95 (m, 2H, H-5'), 3.95-3.8 (m, 1H, 3'), 3.65-3.55 (m, 1H, H-2'), 1.20 (d, 3H, CH_3, J=6.9 Hz).

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