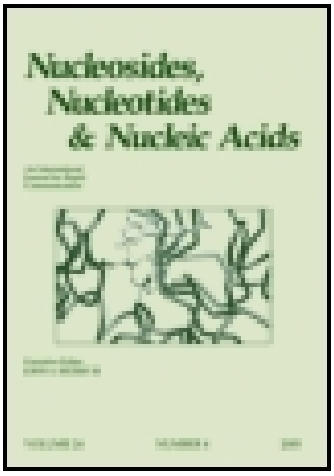

Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

Synthesis of 2′,3′-Dideoxy- and 3′-Azido-2′,3′-dideoxy-pyridazine Nucleosides as Potential Antiviral Agents

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Published online: 23 Sep 2006.

To cite this article: Biserka Kasnar, Dean S. Wise, Louis S. Kucera, John C. Drach & Leroy B. Townsend (1994) Synthesis of 2′,3′-Dideoxy- and 3′-Azido-2′,3′-dideoxy-pyridazine Nucleosides as Potential Antiviral Agents, Nucleosides and Nucleotides, 13:1-3, 459-479, DOI: 10.1080/15257779408013255

To link to this article: http://dx.doi.org/10.1080/15257779408013255

NUCLEOSIDES & NUCLEOTIDES, 13(1-3), 459-479 (1994)

SYNTHESIS OF 2',3'-DIDEOXY- AND 3'-AZIDO-2',3'-DIDEOXY-PYRIDAZINE NUCLEOSIDES AS POTENTIAL ANTIVIRAL AGENTS

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Abstract. The synthesis of 4-methoxy-, 4-amino-3-chloro-, and 4-amino-1-(2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)pyridazin-6-one nucleosides, **6**, **19** and **20** is described. The synthesis of 3,4-dichloropyridazin-6-one (**10**) was accomplished in 44% overall yield using bromomaleic anhydride (**17**) as the starting material. The condensation of the silylated base of **10** with the halogenose **12** in the presence of trimethylsilyl triflate as a catalyst afforded a mixture of 3,4-dichloro-1-(3,5-di-*O*-*p*-toluoyl-2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**13**) in 67% and its α-anomer **14** in 12% yield, respectively. A series of 3'-sulfonate esters were prepared to explore the synthesis of 3-chloro-4-hydroxy-1-(3-azido-2,3-dideoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**32**) via 6,3-anhydronucleoside analogues. Compounds **15**, **19** and **20** were evaluated against human immunodeficiency virus, human cytomegalovirus, and herpes simplex virus type 1 but were inactive.

INTRODUCTION

Analogues of naturally occurring nucleosides, modified in the heterocyclic base or carbohydrate moiety, have been studied extensively as antiviral and antitumor agents. Since the discovery of the human immunodeficiency virus (HIV) as the etiological agent of AIDS[1-4], increasing efforts have been devoted to the synthesis and biological evaluation of compounds with potential for anti-HIV activity.[5,6] The 2',3'-dideoxynucleosides have provided an active lead[7] to some of the more active anti-HIV agents, especially in the case of the pyrimidine nucleoside derivatives. 3'-Azido-3'-deoxythymidine (AZT),[8,9] 3'-azido-2',3'-dideoxyuridine and 2',3'-dideoxycytidine (DDC) have been shown to selectively suppress the replication of HIV through the inhibition of HIV-encoded reverse transcriptase in different cell models.[10] In previous studies, based upon the interesting biological and

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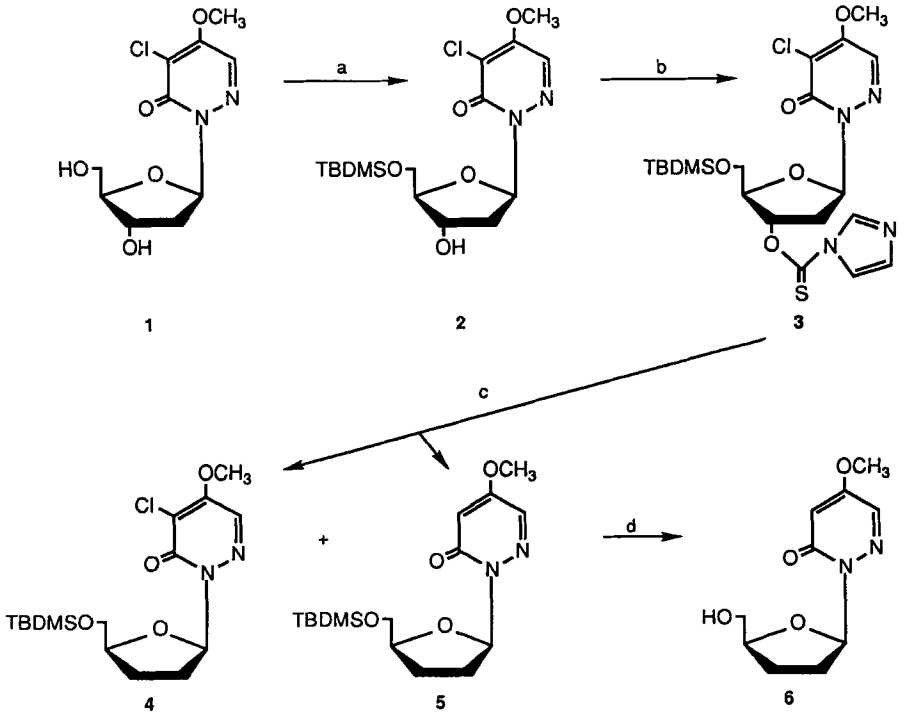
This manuscript is dedicated to the memory of Roland K. Robins

chemotherapeutic activity of 6-aza[11,12] and 3-deaza[13,14] analogues of pyrimidine nucleosides, we reported[15-17] the synthesis of several substituted pyridazine nucleosides which incorporate both of the above structural modifications. The present work describes the synthesis of 2',3'-dideoxy and 3'-azido-2',3'-dideoxy pyridazine nucleosides as potential antiviral agents.

RESULTS AND DISCUSSION

The synthesis of 2',3'-dideoxynucleosides have been approached by three general methods: synthesis from 2'-deoxynucleosides *via* a Barton-type deoxygenation reaction,[18] transformation of ribonucleosides to 2',3'-unsaturated dideoxynucleosides followed by a hydrogenation reaction,[19-21] and synthesis of a properly substituted 2',3'-dideoxy carbohydrate synthon followed by a condensation with an appropriate heterocycle.[22-24] The synthesis of 2',3'-dideoxynucleosides also has been accomplished *via* a ketonucleoside[25] and a photoreductive process.[26] We elected to use the Barton type approach and 5-chloro-4-methoxy-1-(2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**1**),[15] as our starting material. Compound **1**, was first protected at the 5'-hydroxyl group with a *tert*-butyldimethylsilyl group, since we wished to use mild deprotection conditions because of the well known acid lability of dideoxynucleosides.[26] Thus, treatment of **1** with *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in dimethylformamide (DMF) at room temperature for 16 h afforded 5-chloro-4-methoxy-1-(5-*O*-*tert*-butyldimethylsilyl-2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**2**) in 86% yield (Scheme I). Thiocarbonylation of **2** with 1,1-thiocarbonyldiimidazole, in DMF, at room temperature gave 5-chloro-4-methoxy-1-[5-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-(*N*-imidazothio-carbonyl)-ß-D-*erythro*-pentofuranosyl]pyridazin-6-one (**3**) in 80% yield. The homolytic cleavage of the C-O bond of nucleoside **3** with tri-n-butyltin hydride in the presence of 2,2'-azobis(isobutronitrile) (AIBN), in toluene at reflux, yielded the desired product 5-chloro-4-methoxy-1-(5-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)pyridazin-6-one (**4**) in 54% yield along with 4-methoxy-1-(5-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)pyridazin-6-one (**5**) in 36% yield. The aromatic region, of the ^{1}H NMR spectrum of **4**, showed a singlet resonance at δ 7.90 corresponding to the aromatic H-3. In contrast, the ^{1}H NMR spectrum of **5** showed two sets of doublets at δ 7.50 and δ 6.04, corresponding to the two aromatic protons H-3 and H-5, respectively. These signals exhibited a small through bond coupling (J = 2.87 Hz) which is characteristic for 4-substituted pyridazin-6-one nucleosides.[16,17] The sensitivity of the 5-chloro moiety to these reducing conditions was not expected since in general, aryl chlorides are inert to free radical halogen abstraction. However, the susceptibility of the 5-chloro group toward reduction in this case may be attributed to

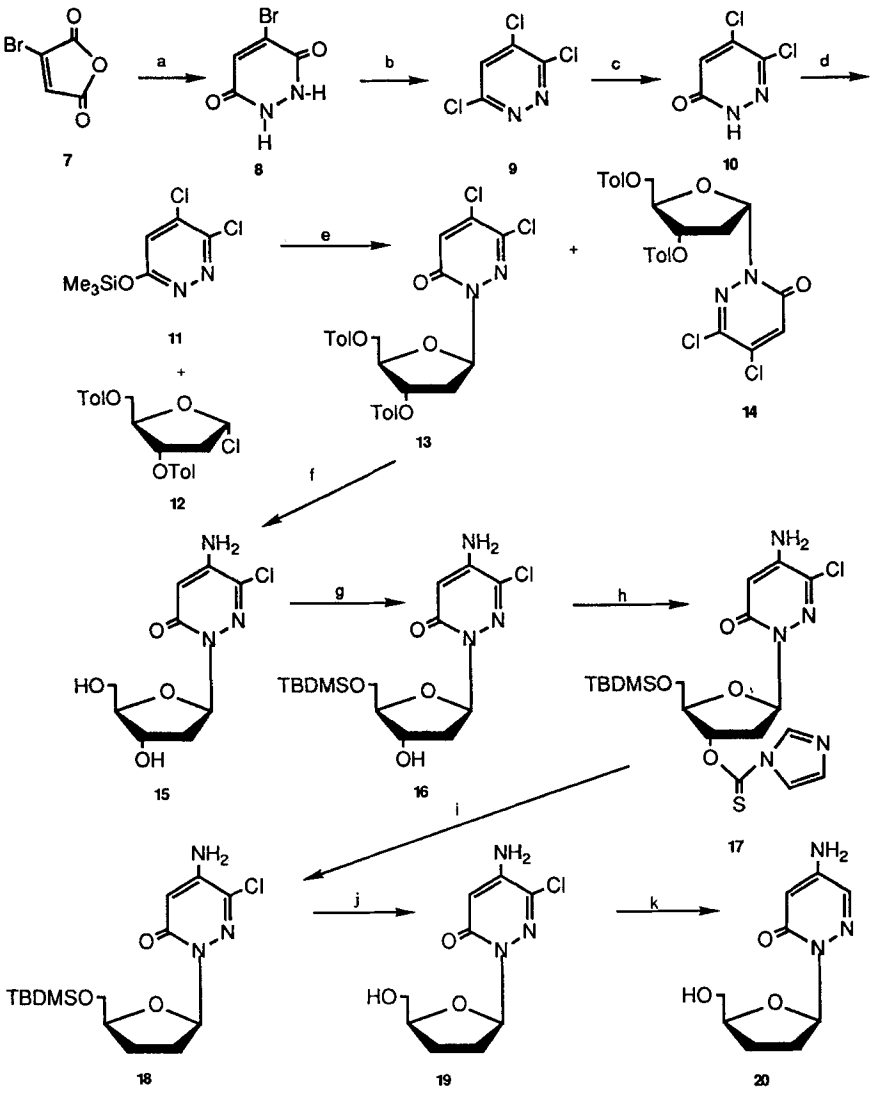


[a]Reagents: a) TBDMSCl, imidazole, DMF; b) (imid.)$_2$C=S, DMF; c) Bu_3SnH, AIBN, toluene; d) Bu_4NF, THF

SCHEME I[a]

activation by the adjacent carbonyl group.[27] Deprotection of **5** was effected with *tetra*-n-butylammonium fluoride to afford 4-methoxy-1-(2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)pyridazin-6-one (**6**) in 60% yield.

Amino-3-chloro-1-(2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**15**)[16] was used as our starting material in the synthesis of the 2',3'-dideoxycytidine analogues. To prepare **15** it was first necessary to synthesize 3,4-dichloropyridazin-6-one (**10**).[28,29] In previous studies, the synthesis of **10** started with chloromaleic anhydride. However, chloromaleic anhydride is no longer commercially available. Since bromomaleic anhydride is commercially available, we elected to use 4-bromo-3,6-dichloropyridazine as an alternate intermediate for the synthesis of **15**. Condensing bromomaleic anhydride (**7**) with hydrazine sulfate furnished 4-bromo-3,6-pyridazine-3,6-dione (**8**) in 71% yield (Scheme II). The ^{1}H NMR spectrum of **8** showed two broad singlets at δ 9.41 and 8.23



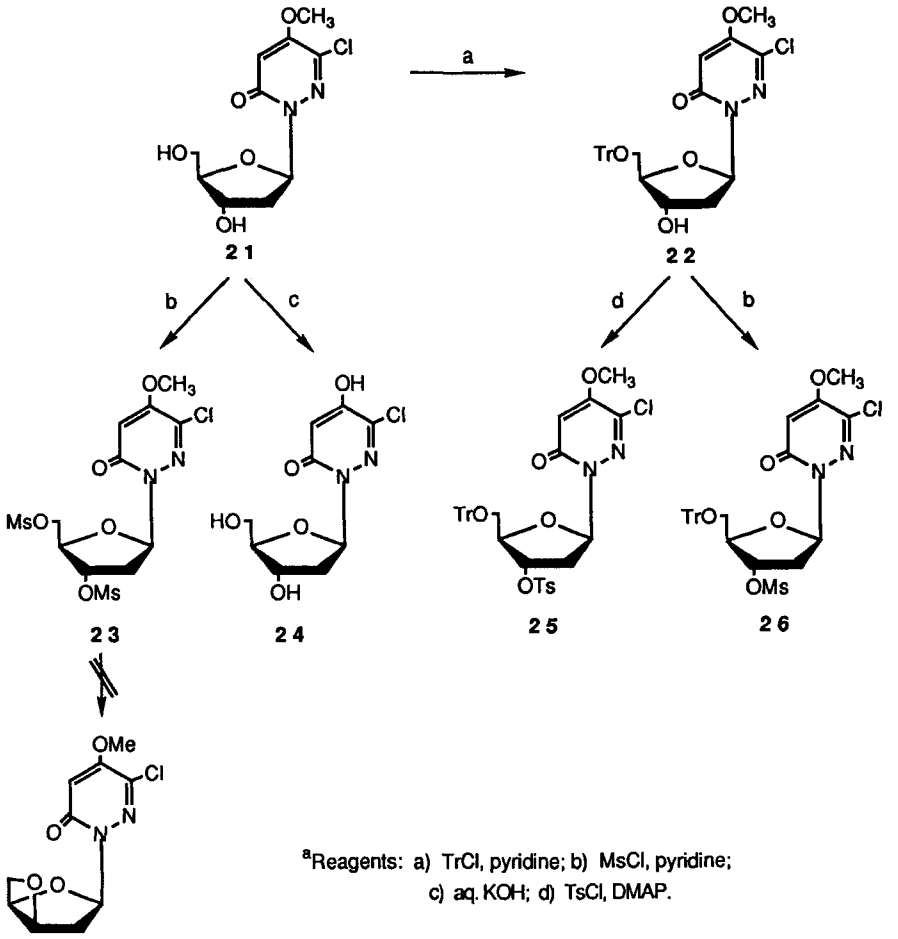
[a]Reagents: a) $NH_2NH_2 \cdot H_2SO_4$; b) $POCl_3$; c) AcOH; d) BSA; e) TMSOTf; f) NH_3/MeOH; g) TBDMSCl, pyridine; h) $(imid)_2C=S$, pyridine; i) Bu_3SnH, AIBN, Toluene; j) Bu_4NF, THF; k) Pd/C, H_2

SCHEME II[a]

corresponding to two amido protons and a singlet at δ 7.59 characteristic of the C-5 proton. The above structural assignment of **8** was confirmed by high resolution mass spectra (EI) which showed a molecular ion of m/z 189.9381 (M+ 189.9378). The reaction of **8** with boiling phosphorus oxychloride afforded 3,4,6-trichloropyridazine (**9**) as shown by elemental analysis and melting point, in an 84% yield rather than 4-bromo-3,6-dichloropyridazine. This reaction further supports the observed susceptibility of a 4-halo group to nucleophilic displacement.[16] Reaction of **9** with acetic acid at reflux yielded 3,4-dichloropyridazin-6-one (**10**)[28,29] in 70% yield.

In a previous study in our laboratory,[16] the condensation of silylated **10** with 3,5-*O*-p-toluoyl-2-deoxy-*erythro*-pentofuranosyl chloride (**12**)[30] was accomplished using stannic chloride as a catalyst to afford a 3/1 ß to α ratio of the nucleosides 3,4-dichloro-1-(3,5-di-*O*-p-toluoyl-2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**13**) and the α-anomer **14**. To maximize the yield of the ß-anomer, we modified our condensation conditions[16] of the base **10** with the glycosyl halide **12**.[30] Silylation of **10** was accomplished using *bis*(trimethylsilyl)acetamide in acetonitrile. The silyl derivative **11** was condensed with **12** in the presence of trimethylsilyl triflate (TMSOTf) to provide, after work-up, the anomers 3,4-dichloro-1-(3,5-di-*O*-p-toluoyl-2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**13**) and its α-anomer **14** (5.5/1, ß to α ratio). Nucleoside **13** was converted to the 4-amino-3-chloro derivative **15** using previous methodology.[16] Chemical transformation of the nucleoside **15** to the desired 2',3'-dideoxynucleosides **19** and **20** was accomplished in a manner similar to that described for the synthesis of **6**. The 5'-hydroxyl group of nucleoside **15** was selectively protected with TBDMSCl in the presence of imidazole to give 4-amino-3-chloro-1-(5-*O*-*tert*-butyldimethylsilyl-2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**16**) in 79% yield. Thiocarbonylation of **16** with 1,1'-thiocarbonyldiimidazole in dimethylformamide at room temperature afforded 4-amino-3-chloro-1-[5-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-(*N*-imidazothiocarbonyl)-ß-D-*erythro*-pentofuranosyl]pyridazin-6-one (**17**) in 88% yield. Homolytic reduction of nucleoside **17** with *tri*-n-butyltin hydride and AIBN in toluene gave 4-amino-3-chloro-1-(5-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-ß-D-*glycero*-4-pentofuranosyl)pyridazin-6-one (**18**) in 94% yield. The TBDMS-protecting group of **18** was removed with *tetra*-n-butylammonium fluoride to afford crystalline 4-amino-3-chloro-1-(2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)pyridazin-6-one (**19**) in 67% yield (44% overall yield from **15**). Treatment of **19** with hydrogen (53 psi) in the presence of 10% palladium on carbon catalyst afforded a 90% yield the of desired 4-amino-1-(2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)pyridazin-6-one (**20**).

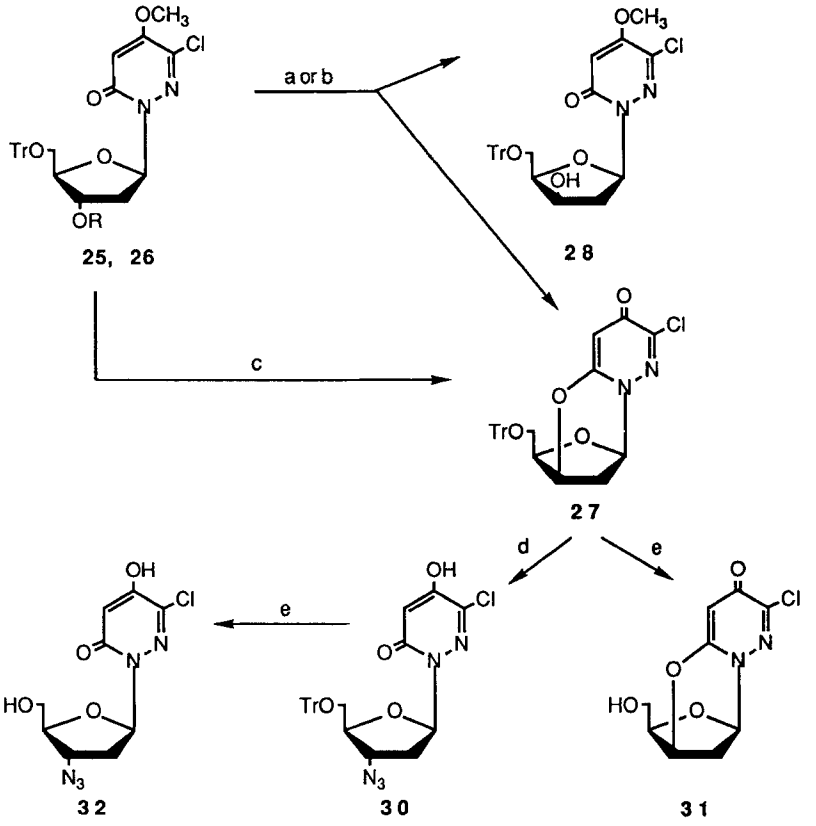
Since pyrimidine nucleosides with an azido group in the 3'-*erythro*-position have exhibited significant HIV antiviral activity[8,9] we investigated the synthesis of 3'-azido



SCHEME III[a]

pyridazine nucleoside analogues. Nucleoside **13** was converted to the key intermediate, 3-chloro-4-methoxy-1-(2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**21**) via a literature method.[16] In our initial study, we proposed to prepare the desired AZT analogue through a 3',5'-epoxide intermediate. Treatment of **21** with excess of methanesulfonyl chloride in dry pyridine at 0-10 °C afforded 3-chloro-4-methoxy-1-[2-deoxy-3,5-*bis*(methylsulfonyl)-ß-D-*erythro*-pentofuranosyl]pyridazin-6-one (**23**) in near quantitative yield (99%) (Scheme III). However, several attempts to obtain a 3',5'-epoxide nucleoside by the treatment of compound **23** with aqueous sodium hydroxide at reflux yielded only intractable mixtures. This is most likely due to the facile hydrolysis of the 4-

methoxy group on the pyridazine ring under the reaction conditions. This is supported by the facile conversion of nucleoside **21**, by treatment with potassium hydroxide, to the 5-chloro-2'-deoxyuridine analog, 3-chloro-4-hydroxy-1-(2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**24**). Thus, we abandoned this approach and adapted a more conventional method[31] which proceeded through an anhydronucleoside intermediate for the transformation of the 2'-deoxy-nucleoside **21** to the desired 3'-azido-2',3'-dideoxynucleosides. Treatment of **21** with trityl chloride in pyridine at 80 °C for 19 h afforded the 5'-hydroxyl blocked nucleoside 3-chloro-4-methoxy-1-(2-deoxy-5-*O*-triphenylmethyl-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**22**) in 82% yield. The tosylate and mesylate esters (**25** and **26**) were prepared in 94% and 96% yields, respectively, by treatment of **22** with p-toluenesulfonyl chloride and methanesulfonyl chloride in dry pyridine and in the presence of 4-(dimethylamino)pyridine. It is known that treatment of a 5'-protected 3'-mesyl or 3'-tosyl derivatives of 2'-deoxypyrimidine nucleosides with a base, i.e., ethanolic sodium hydroxide[32] or with 1,8-diazabicyclo-[5.4.0]unde-7-ene (DBU)[33] generally affords the corresponding anhydronucleoside derivatives. Interestingly, in this case, depending on the solvent used in the reaction, different reactions were observed in the treatment of the sulfonate esters **25** and **26** with base. When the tosylated or mesylated nucleoside **25** or **26** was treated with 1N aqueous sodium hydroxide in ethanol, only the desired 6,3'-anhydro-3-chloro-6-hydroxy-1-(2-deoxy-5-*O*-triphenylmethyl-ß-D-*threo*-pentofuranosyl)pyridazin-6-one (**27**) was isolated in a 15% yield (Scheme IV). Treatment of the mesylate nucleoside **26** with 1N aqueous sodium hydroxide in acetonitrile at reflux for 12 h gave the desired 6,3'-anhydronucleoside **27** in only a modestly improved yield of 27% along with 3-chloro-4-methoxy-1-(2-deoxy-5-*O*-triphenylmethyl-ß-D-*threo*-pentofuranosyl)pyridazin-6-one (**28**) in 30% yield. In this case, possibly due to the prolonged reaction conditions, two reactions can be envisioned. In one reaction displacement of the 4-methoxyl group by a hydroxide anion initiates the formation of the anhydronucleoside **27**, while in the second reaction the base effects an inversion of configuration at the 3'-position of the sugar moiety by SN_2 displacement of the mesylate group to afford the 2-deoxy-ß-D-*threo* derivative **28**. The structure of compound **28** was assigned by ^1H NMR spectroscopy and mass spectroscopy. Finally, the reaction of **26** with sodium iodide in 2-butanone effected a smooth conversion of **26** to **27** in 89% yield. This reaction most likely proceeds *via* demethylation of the 4-methoxyl group and subsequent nucleophilic displacement of the mesylate group by the 6-O moiety. Deprotection of **27** was accomplished by using a mixture of formic acid and ethyl acetate (2/3)[34] to give 6,3'-anhydro-3-chloro-1-(2-deoxy-ß-D-*threo*-pentofuranosyl)pyridazin-4-one (**31**) in 87% yield.



[a]Reagents: a) aq. NaOH, EtOH; b) aq. NaOH, CH_3CN; c) NaI, EtOAc; d) LiN_3, DMF; e) HCO_2H, EtOAc

SCHEME IV[a]

Nucleoside **27** was reacted with lithium azide in dimethylformamide to afford 3-chloro-4-hydroxy-1-(3-azido-2,3-dideoxy-5-*O*-triphenylmethyl-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**30**) in 96% yield. The structure of compound **30** was assigned by ^1H NMR spectroscopy, IR (2100 cm^{-1}), and elemental analysis. Deblocking the 3'-azido derivative **30** was also accomplished by using the above acidic conditions to give 3-chloro-4-hydroxy-1-(3-azido-2,3-dideoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (**32**) in 35% yield.

Three target compounds were evaluated against selected viruses. Neither the 2'-deoxy nucleoside **15** nor the 2',3'-dideoxy nucleosides **19** and **20** were active against HIV in a syncytial plaque assay. In addition, compounds **15** and **19** were inactive against

human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1) in plaque and ELISA assays, respectively. The compounds were not cytotoxic in CEM-SS cells nor in human foreskin fibroblasts (HFF cells) at 100 μM, the highest concentration used for antiviral and cytotoxicity testing.

EXPERIMENTAL SECTION

Chemistry

Proton magnetic resonance (^{1}H NMR) spectra were obtained with a Bruker WP-270SY, IBM AM-300 or an IBM WM-360 Spectrophotometer [solutions in dimethylsulfoxide-d_6 (DMSO-d_6) or deuteriochloroform ($CDCl_3$)] with chemical shift values reported in δ, parts per million, relative to the internal standard. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. E. Merck silica gel (230-400 mesh) was used for column chromatography. Thin layer chromatography was performed on silica gel GHLF-254 plates. R_f's were determined using the solvent systems recorded. Solvent systems are reported in volume/volume ratios. Compounds of interest were detected by either ultraviolet lamp (254 nm) or treatment with 10% H_2SO_4 in MeOH followed by heating (charring). Evaporations were performed with a rotary evaporator under reduced pressure with a bath temperature <50 °C. UV spectra were recorded on a Hewlet-Packard UV 8450 spectrometer. High resolution MS measurements were obtained on a VG 70-250-S MS spectrometer using a direct probe for sample introduction. Low resolution MS spectra were obtained on a Finnigan 4023 (GC/MS) instrument, using electron ionization or chemical ionization. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

5-Chloro-4-methoxy-1-(5-*O*-*tert*-butyldimethylsilyl-2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (2). Nucleoside **1** (800 mg, 2.89 mmol) was dissolved in dimethylformamide (7 mL) and treated with imidazole (471 mg, 6.9 mmol) and *tert*-butyldimethylchlorosilane (520 mg, 3.5 mmol) with the exclusion of moisture. The reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated and the residual oil was dissolved in dichloromethane (4 mL) and applied to an open-bed silica gel column (40 g SiO_2). The product was eluted from the column using dichloromethane/methanol (40/1), collecting 15 mL fractions. Concentration of the fractions containing the product, as determined by tlc, gave **2** as an oil: Yield 972 mg (86%); R_f 0.39 (CH_2Cl_2/MeOH, 20/1); UV λ max nm (ε x 10^{-3}): (MeOH) 262 (4.6), 295 (5.2); (pH 1) 262 (5.7), 291 (5.9); (pH 11) 263 (6.0), 292 (6.4); ^{1}H NMR (DMSO-d_6): δ 8.30 (s, 1 H, H-3), 6.61 (dd, 1 H, J = 4.9 and 7 Hz, H-1'), 5.24 (d, 1 H, J = 4.9 Hz, OH-3'), 4.30 (m, 1 H, H-3'), 4.07 (s, 3 H, OCH_3), 3.76-3.51 (m, 3 H, H-4' and 2 H-5'), 2.45 (1 H, H-2'a), 2.14 (m, 1 H, H-2'b), 0.83 (s, 9 H, CH_3-C), -0.02 and -0.03 (2 s, 6 H, CH_3Si). *Anal.* Calcd for $C_{16}H_{27}N_2ClO_5Si$ (390.95): C, 49.15; H, 6.96; N, 7.17. Found: C, 49.38; H, 7.14; N, 7.23.

5-Chloro-4-methoxy-1-[5-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-(*N*-imidazo-thiocarbonyl)-ß-D-*erythro*-pentofuranosyl]pyridazin-6-one (3). 1,1'-Thiocarbonyldiimidazole (453 mg, 3.35 mmol) was added to a solution of **2** (900 mg, 2.3 mmol) in dimethylformamide (7 mL). The reaction mixture was stirred at room temperature for 48 h. The solvent was evaporated and the residue was taken up in chloroform and washed twice with water. The organic layer was dried over anhydrous sodium sulfate and evaporated. The product was purified on a silica gel column (60 g SiO_2) using dichloromethane/methanol (20/1) as eluent. Crystallization from ether gave **3** as a white powder: Yield 922 mg (80%), mp 135-137 °C; R_f 0.39 (CH_2Cl_2/MeOH, 20/1); UV λ max nm (ε x 10^{-3}): (MeOH) 288 (13.8); (pH 1) 260 (6.8); (pH 11) 290 (4.4); ^1H NMR (DMSO-d_6): δ 8.55 (s, 1 H, H-2"), 8.37 (s, 1 H, H-3), 7.86 (br s, 1 H, H-4"), 7.09 (br s, 1 H, H-5"), 6.76 (t, 1 H, J = 6.5 Hz, H-1'), 6.03 (m, 1 H, H-3'), 4.43 (m, 1 H, H-4'), 4.10 (s, 3 H, OCH_3), 3.73 (m, 2 H, 2 H-5'), 2.97 (m, 1 H, H-2'a), 2.64 (m, 1 H, H-2'b), 0.80 (s, 9 H, CH_3-C), -0.01 (br, s, 6 H, CH_3-Si). *Anal.* Calcd for $C_{20}H_{29}N_4O_5SiSCl$ (501.08): C, 47.94; H, 5.83; N, 11.18. Found: C, 48.03; H, 6.00; N, 11.11.

5-Chloro-4-methoxy-1-(5-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-ß-D-glycero-pentofuranosyl)pyridazin-6-one (4) and 4-methoxy-1-(5-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)pyridazin-6-one (5). A solution of compound **3** (279 mg, 0.17 mmol) in boiling toluene (8 mL) was treated with 2,2'-azobis-2-methylpropionitrile (57 mg, 0.35 mmol). The *tri*-n-butyltin hydride (0.57 mL, 2.18 mmol) in toluene (5 mL) was then added dropwise during 45 min. The mixture was heated at reflux for a total period of 3 h (TLC showed two products). Toluene was evaporated and the residual oil was purified by flash chromatography (50 g, SiO_2). The column was eluted with dichloromethane/methanol (90/1) to give 171 mg (54%) of **4** as an oil: R_f 0.89 (CH_2Cl_2/MeOH, 30/1); ^1H NMR ($CDCl_3$): δ 7.90 (s, 1 H, H-3), 6.86 (t, 1 H, H-1'), 4.18 (m, 1 H, H-4'), 4.07 (s, 3 H, OCH_3), 3.99 (m, 1 H, H-5'a), 3.86 (m, 1 H, H-5'b), 2.44 (m, 2 H, H-2'a and H-3'a), 2.23 (m, 2 H, H-2'b and H-3'b), 0.89 (s, 9 H, CH_3-C), -0.03 and -0.02 (2 s, 3 H, CH_3-Si).

The compound **5** (105 mg, 36%) was also isolated as an oil: R_f 0.38 (CH_2Cl_2/MeOH, 30/1), ^1H NMR ($CDCl_3$): δ 7.53 (d, 1 H, $J_{3,5}$ = 2.78 Hz, H-3), 6.62 (t, 1 H, H-1'), 6.04 (d, 1 H, $J_{5,3}$ = 2.78 Hz, H-5), 4.13 (m, 1 H, H-4'), 3.78 (m, 5 H, 2 H-5'+OCH_3), 2.24 (m, 2 H, H-2'a and H-3'a), 2.03 (m, 2 H, H-2'b and H-3'b), 0.89 (s, 9 H, CH_3-C), -0.03 and -0.02 (2xs, 6 H, CH_3-Si). Although the ^1H NMR spectrum showed little impurities, the purification of both nucleosides was difficult and we were unable to obtain a pure sample for analysis. Therefore, compounds **4** and **5** were used in subsequent reactions without further purification.

4-Methoxy-1-(2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)pyridazine-6-one (6). Method A: A solution of compound **5** (168 mg, 0.45 mmol) in 1M *tetra*-n-butylammonium fluoride in tetrahydrofuran (2 mL) was stirred at room temperature for 25 min. TLC showed two products. After evaporation of the solvent, the mixture was purified on a silica gel column (40 g, SiO_2) using chloroform/ethanol (92/8) as the eluent. Concentration of the fractions containing the product gave **6** as oil: Yield 61 mg (61%); R_f 0.27 (CH_2Cl_2/MeOH, 20/1); ^1H NMR (DMSO-d_6): δ 7.79 (d, 1 H, J = 2.86 and 7.3 Hz, H-1'), 4.65 (t, 1 H, J = 5.73 Hz, OH-5'), 3.99 (m, 1 H, H-4'), 3.78 (s, 3 H, OCH_3), 3.35 (m, 2 H, 2 H-5'), 2.15 (m, 2 H, H-2'a and H-3'a), 1.92 (m, 2 H, H-2'b and H-3'b). *Anal.* Calcd for $C_{10}H_{14}N_2O_4$ (226.23): C, 53.09; H, 6.24; N, 12.39. Found: C, 52.92; H, 6.05; N, 12.22.

Method B: Deprotection of **4** was performed in a manner similar to that used to afford **6** (56% yield).

4-Bromo-3,6-pyridazinediol (8). Hydrazine sulfate (28 g) was dissolved in boiling water (125 mL) and bromomaleic anhydride (**7**) (50 g, 0.283 mol) was added. The reaction mixture was heated at reflux for 6 h and then cooled to room temperature. The solid which had separated was collected by filtration, washed with acetone, and dried in an oven to yield 38.6 g (71%) of **8** as a white powder: mp 260-263 °C dec.; R_f 0.10 (CH_2Cl_2/MeOH, 4/1); ^1H NMR (DMSO-d_6): δ 9.41 (br s, 1 H, NH), 8.23 (br s, 1 H, NH), 7.59 (s, 1 H, H-5); MS (EI) exact mass calcd for $C_4H_3N_2O_2Br$ m/e 189.9381, found 189.9378.

3,4,6-Trichloropyridazine (9).[28,29] Compound **8** (37 g, 0.194 mol) was heated with phosphorous oxychloride (250 mL) at reflux for 4 h. Excess reagent was removed in vacuo and the cooled residue was poured onto ice (350 mL). The solid was collected by filtration. An additional crop was obtained by the addition of ammonium hydroxide (28%) to the filtrate (slightly alkaline) and extraction with chloroform. The organic layer was dried over anhydrous sodium sulfate and evaporated. The combined material was recrystallized from dichloromethane-hexane to give **9** as white crystals: yield 30.2 g (84%); mp 50-52 °C (lit.[24,25] 50 °C); R_f 0.89 (CH_2Cl_2/MeOH, 20/1); ^1H NMR (DMSO-d_6): δ 8.58 (s, 1 H, H-5). Caution: Compound **9** is a strong vesicant. Several layers of gloves should be worn throughout the work-up and the handling of **9** to avoid painful blisters.

3,4-Dichloro-1-(3,5-di-*O*-toluoyl-2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (13) and 3,4-dichloro-1-(3,5-di-O-toluoyl-2-deoxy-α-D-*erythro*-pentofuranosyl)pyridazin-6-one (14).[16] 3,4-Dichloropyridazin-6-one (**10**)[28,29] (5 g, 30.3 mmol) and *bis*(trimethylsilyl)acetamide (5.9 mL, 23.9 mmol) in dry acetonitrile (30 mL) were heated at 70 °C for 1 h. The resulting

solution was cooled to 0 °C and 3,5-di-*O*-p-toluoyl-2-deoxy-*erythro*-pentofuranosyl chloride[30] (**12**) (11.14 g, 28.6 mmol) in acetonitrile was added. This suspension was treated with trimethylsilyl trifluoromethanesulfonate (6 mL, 31.03 mmol) dropwise over 30 min and then stirred at room temperature for 2 h. The reaction mixture was poured into an ice cold mixture of ethyl acetate (90 mL) and a saturated solution of sodium bicarbonate in water (40 mL). The organic layer was separated and the residue was extracted with dichloromethane (2 x 50 mL). The combined EtOAc and CH_2Cl_2 solutions were dried over anhydrous sodium sulfate, concentrated and filtered through a column of silica gel (10 x 7 cm). The column was eluted with hexane-ethyl acetate (4/1) and dichloromethane/ethyl acetate (20/1). The combined eluants were evaporated to give both anomers **13** and **14** as crystals, yield 12.5 g (84%; α/β=1/6). Boiling methanol was added to the anomeric mixture and the solution was then allowed to stand at room temperature for 48 h. Crystals of **13** were collected by filtration: yield 10 g (67%); mp 144-145 °C (lit.[12] mp 143-145 °C); R_f 0.49 (CH_2Cl_2/EtOAc, 20/1). The filtrate was evaporated and the residue was dissolved in dichloromethane and treated with hexane to give 1.8 g of **14** (12%) as white crystals, mp 106-108 °C (lit.[16] mp 105-106 °C); R_f 0.34 (CH_2Cl_2/EtOAc, 20/1).

4-Amino-3-chloro-1-(5-*O*-*tert*-butyldimethylsilyl-2-deoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (16). Nucleoside **15**[16] (338 mg, 1.3 mmol) was dissolved in dimethylformamide (2.5 mL) and treated with imidazole (211 mg, 3.1 mmol) and *tert*-butyldimethylchlorosilane (273 mg, 1.8 mmol) with the exclusion of moisture. The reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated, the residual oil was dissolved in chloroform (4 mL) and applied to an open-bed silica gel column (SiO_2, 40 g). The fractions containing product were eluted with chloroform/methanol (20/1), collecting 15 mL fractions. The product was evaporated to dryness and the resulting solid was crystallized from chloroform/n-hexane (1/1) to give **16** as white crystals, yield 383 mg (79%); mp 109-110 °C; R_f 0.34 (CH_2Cl_2/MeOH, 20/1): UV λ max nm ($\varepsilon x10^{-3}$): (MeOH) 227 (11.2), 301 (9.1); (pH 1) 227 (13.0), 300 (9.9); (pH 11) 300 (9.9); ^1H NMR ($CDCl_3$): δ 6.78 (dd, 1 H, J = 3.5 and 7.4 H, H-1'), 6.17 (s, 1 H, H-5), 5.12 (br s, 2 H, NH_2), 4.69 (m, 1 H, H-3'), 3.92-3.67 (m, 3 H, H-4' and 2 H-5'), 2.58 (m, 1 H, H-2b) 2.03 (br s, 1 H, OH-3'), 0.88 (s, 9 H, CH_3-C), 0.06 and 0.05 (2 x s, 6 H, CH_3Si). *Anal.* Calcd for $C_{15}H_{26}N_3ClO_4Si$ (375.93): C, 47. 92; H, 6.97; N, 11.18. Found: C, 47.90; H, 7.00; N, 11.20.

4-Amino-3-chloro-1-5-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-(*N*-imidazothiocarbonyl)-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (17). 1,1'-Thiocarbonyldiimidazole (131 mg, 0.97 mmol) was added to a solution of nucleoside **16** (250 mg, 0.67 mmol) in dimethylformamide (2 mL). The reaction mixture was stirred at room temperature for 48 h. The solvent was evaporated and the residue was taken up in

chloroform and washed twice with water. The organic layer was dried over anhydrous sodium sulfate and evaporated. The resulting oil was crystallized from ethanol/n-hexane to give **17** as a white solid, yield 283 mg (88%); mp 146-147 °C; R_f 0.37 (CH_2Cl_2/MeOH, 20/1); UV λ max nm (εx10^{-3}): (MeOH) 221 (14.6), 227 (14.4), 278 (12.5); (pH 1) 228 (15.5), 273 (10.1), 291 (10.2), 300 (10.2); (pH 11) 300 (10.1); ^{1}H NMR ($CDCl_3$): δ 8.35 (s, 1 H, H-2"), 7.62 (d, 1 H, $J_{4",5"}$=1.3 Hz, H-4"), 7.04 (d, 1 H, $J_{5",4"}$=1.1 Hz, H-5"), 6.86 (t, 1 H, J=6.5 Hz, H-1'), 6.20 (s, 1 H, H-5), 6.11 (m, 1 H, H-3'), 5.19 (br s, 2 H, NH_2), 4.37 (m, 1 H, H-4'), 3.82 (m, 2 H, 2 H-5'), 3.13 (m, 1 H, H-2'a), 2.46 (m, 1 H, H-2'b), 0.86 (s, 9 H, CH_3-C), 0.05 and 0.04 (2 x s, 6 H, CH_3-Si). *Anal.* Calcd for $C_{19}H_{28}N_5ClO_4SSi$ (486.07): C, 46.95; H, 5.81; N, 14.41. Found: C, 46.83; H, 5.93; N, 14.30.

4-Amino-3-chloro-1-(5-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)pyridazin-6-one (18). A solution of compound **17** (287 mg, 0.59 mmol) in boiling toluene (9 mL) was treated with 2,2'-azobis-2-methylpropionitrile (61 mg, 0.37 mmol). The *tri*-n-butyltin hydride (0.61 mL, 2.3 mmol) in toluene (5 mL) was then added dropwise during 45 min. The mixture was boiled under reflux for a total period of 3 h. Toluene was evaporated and the residual oil was dissolved in dichloromethane (2 mL) and applied to an open-based silica gel column (SiO_2, 40 g). The column was eluted with dichloromethane/methanol (40/1). Concentration of the fractions containing the product gave **18** as an oil, yield 199 mg (94%), R_f 0.66 (CH_2Cl_2/MeOH, 20/1), UV λ max nm (εx10^{-3}): (MeOH) 227 (11.6) 301, (9.1); (pH 1) 227 (14.3), 299 (10.4); (pH 11) 300 (9.6); ^{1}H NMR ($CDCl_3$): δ 6.66 (dd, 1 H, J=3.1 and 6.3 Hz, H-1'), 6.17 (s, 1 H, H-5), 5.09 (br s, 2 H, NH_2), 4.18 (m, 1 H, H-4'), 3.76 (m, 2 H, 2 H-5'), 2.30-2.00 (m, 4 H, 2H, H-2' and 2 H-3'), 0.89 (s, 9 H, CH_3-C), 0.03 and 0.02 (2 x s 6 H, CH_3-Si). *Anal.* Calcd for $C_{15}H_{26}N_3ClO_3Si$ (359.93): C, 50.05; H, 7.28; N, 11.67. Found: C, 50.23; H, 7.38; N, 11.60.

4-Amino-3-chloro-1-(2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)-pyridazin-6-one (19). A solution of compound **18** (199 mg, 0.55 mmol) in 1M *tetra*-n-butylammonium fluoride in tetrahydrofuran (2 mL) was stirred at room temperature for 25 min. After evaporation of solvent, the product was purified on a silica gel column (SiO_2, 60 g), using dichloromethane/methanol (20/1) as eluent. Crystallization from ethanol gave **19** as white needles, yield 91 mg (67%); mp 101-102 °C; R_f 0.30 (CH_2Cl_2/MeOH, 20/1); UV λ max nm (εx10^{-3}): (MeOH) 301, (13.9); (pH 1) 227 (206.6), 299 (15.9); (pH 11) 299 (14.3); ^{1}H NMR (DMSO-d_6): δ 6.94 (br s, 2 H, NH_2), 6.49 (dd, 1 H, J=3.2 and 6.9 Hz, H-1'), 6.16 (s, 1 H, H-5), 4.66 (t, 1 H, J=5.7 Hz, OH-5'), 4.01 (m, 1 H, H-4'), 3.38 (m, 2 H, 2 H-5'), 2.30-2.00 (m, 2 H, H-2'a and H-3'a), 1.97-1.85 (m, 2 H, H-2'b and H-3'b). *Anal.* Calcd for $C_9H_{12}N_3O_3Cl$ (245.67): C, 44.00; H, 4.93; N, 17.10. Found: C, 44.18; H, 5.20; N, 16.87.

4-Amino-1-(2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)pyridazin-6-one (20). Compound **19** (90 mg, 0.37 mmol) was dissolved in methanol (43 mL) and 0.1N aqueous sodium hydroxide (3.5 mL) was added. The solution was cooled to 5 °C and purged with nitrogen. Palladium on carbon catalyst (10%, 86 mg) was added and the reaction mixture was treated with hydrogen gas at 53 psi in a Parr hydrogenation apparatus for 3 h. The mixture was filtered through a Celite pad and the pad was washed with boiling methanol (50 mL). The combined methanol filtrates were concentrated under reduced pressure and the resulting residue was triturated with ethanol (50 mL), filtered and the filtrate evaporated to give a yellow oil. Crystallization from ethanol gave **20**, as white needles, yield 70 mg (90%), mp 136-137 °C, R_f 0.2 (CH_2Cl_2/MeOH, 9/1); UV λ max nm (εx10^{-3}): (MeOH) 298 (11.4); (pH 1) 295 (12.8); (pH 11) 297 (12.5); ^{1}H-NMR (DMSO-d_6): δ 7.52 (d, 1 H, $J_{3,5}$=2.8 Hz, H-6), 6.56 (t, 1 H, J=5.6 Hz, H-1'), 6.44 (br s, 2 H, NH_2), 6.14 (d, 1 H, $J_{5,3}$=2.8 Hz, H-5), 4.69 (t, 1 H, J=5.7 Hz, OH-5'), 4.02 (m, 1 H, H-4'), 3.38 (m, 2 H, 2 H-5'), 2.22-2.09 (m, 2 H, H-2'a and H-3'a), 1.98-1.90 (m, 2 H, H-2'b, and H-3'b). *Anal.* Calcd. for $C_9H_{13}N_3O_3$ (211.21): C, 51.18; H, 6.20; N, 19.89. Found: C, 51.39; H, 6.18; N, 19.88.

3-Chloro-4-methoxy-1-(2-deoxy-5-*O*-triphenylmethyl-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (22). To a solution of **21**[16] (950 mg, 3.4 mmol) in dry pyridine (9.5 mL) was added triphenylmethyl chloride (1.31 g, 4.7 mmol). The mixture was stirred at room temperature for 19 h and then 20 min at 80 °C. After cooling, the mixture was poured into an ice water mixture (150 mL) and the resulting white precipitate was filtered and washed with cold water. The precipitate was dissolved in dichloromethane, washed with water, and dried over anhydrous sodium sulfate. The solvent was evaporated to give a foam 1.46 g (82% yield). An analytical sample was obtained by crystallization from acetone/hexane to give white crystals of **22**, mp 162-163 °C, R_f 0.39 (CH_2Cl_2/MeOH, 20/1); ^{1}H NMR ($CDCl_3$): δ 7.44-7.20 (m, 15H, arom. H's), 6.73 (dd, 1 H, J=4 and 7.2 Hz, H-1'), 6.10 (s, 1 H, H-5), 4.62 (m, 1 H, H-3'), 4.01 (m, 1 H, H-4'), 3.85 (s, 3 H, OCH_3), 3.39 (m, 1 H, H-5'a), 3.26 (m, 1 H, H-5'b), 2.55 (m, 1 H, H-2'a), 2.24 (m, 1 H, H-2'b), 1.97 (d, 1 H, J=3.5 Hz, OH-3'). *Anal.* Calcd. for $C_{29}H_{27}N_2O_5Cl$ (518.99): C, 67.11; H, 5.24; N, 5.40. Found: C, 67.11; H, 5.50; N, 5.41.

3-Chloro-4-methoxy-1-(2-deoxy-3,5-*bis*(methylsulfonyl)-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (23). Nucleoside **21**[16] (1.06 g, 3.8 mmol) was dissolved in dry pyridine (2.6 mL) and the solution was cooled to 0 °C. The methanesulfonyl chloride (1.06 mL) was added dropwise over a period of 15 min and the reaction mixture was stirred for an additional 30 min at 10 °C. The resulting mixture was then poured into water extracted with dichloromethane and dried over anhydrous sodium

sulfate. The solvent was evaporated to give 1.65 g (99%) of **23** as a foam. The product recrystallized from hot acetone to yield an analytical sample: mp 106-107 °C; R_f 0.60 (CH_2Cl_2/MeOH, 20/1); ^1H NMR ($CDCl_3$): δ 6.80 (dd, 1 H, H-1'), 6.13 (s, 1 H, H-5), 5.45 (m, 1 H, H-3'), 4.42 (m, 3 H, H-4' and 2 H-5'); 3.90 (s, 3 H, OCH_3), 3.11 and 3.06 (2xs, 6 H, SCH_3), 3.01 (m, 1 H, H-2'a), 2.26 (dd, 1 H, H-2'b). *Anal.* Calcd. for $C_{12}H_{17}N_2ClO_9S_2$ (432.87). C, 33.29; H, 3.96; N, 6.47. Found: C, 33.47; H, 4.10; N, 6.35.

3-Chloro-4-hydroxy-1-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-pyridazin-6-one (24). Nucleoside **21** (680 mg, 2.46 mmol) was added to a solution of potassium hydroxide (2.6 g) in distilled water (13 mL) and the solution was heated at reflux for 30 min. The reaction mixture was then cooled in an ice bath, and the solution was carefully acidified to pH~5 with conc. hydrochloric acid. The resulting solution was evaporated to dryness at 30 °C *in vacuo* and the residue was triturated at room temperature with ethanol (100 mL) for 15 h. The mixture was filtered and the filtrate evaporated to dryness. Crystallization of the residue from an ethanol-ethyl acetate mixture afforded 493 mg (81%) of **24** mp >210 ° dec. R_f 0.25 (CH_2Cl_2/MeOH, 2/1); MS (CI, NH_3) m/z 263 $(m + 1)^+$; ^1H NMR (DMSO-d_6): δ 6.98 (t, 1 H, H-1'), 5.57 (br s, 1 H, OH-2'), 5.50 (br s, 1 H, H-5), 5.17 (t, 1 H, OH-5'), 4.52 (br s, 1 H, H-3'), 4.15 (m, 1 H, H-4'), 3.84 (m, 2 H, H-5'), 2.83 (m, 1 H, H-2'a), 2.40 (m, 1 H, H-2'b). Anal. Calcd. for $C_9H_{11}N_2O_5Cl \cdot HCl \cdot 1/2H_2O$ (308.092): C, 35.08; H, 3.89; N, 09.09. Found: C, 35.13; H, 3.89; N, 8.65.

3-Chloro-4-methoxy-1-(2-deoxy-3-*O*-p-toluenesulfonyl-5-*O*-triphenylmethyl-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (25). A mixture of nucleoside **22** (304 mg, 0.58 mmol), p-toluenesulfonyl chloride (334 mg, 1.75 mmol), and 4-(dimethylamino)pyridine (DMAP) (136 mg, 1.11 mmol) in dry pyridine (2 mL) was stirred at room temperature for 65 h. The reaction was quenched with ice water and the precipitate was collected by filtration and washed with cold water. The precipitate was dissolved in dichloromethane, washed with water and dried over anhydrous sodium sulfate. After evaporation of solvents, the product was purified on a silica gel column (SiO_2, 45 g), using dichloromethane/ethyl acetate (3/1) as eluent, to give **25** (371 mg, 94%) as a foam, R_f 0.7 (CH_2Cl_2/EtOAc, 1/1), ^1H NMR ($CDCl_3$): δ 7.70 (d, 2 H, arom. H Tol H's), 7.34-7.17 (m, arom. H), 6.70 (t, 1 H, J=6.3 Hz, H-1'), 6.08 (s, 1 H, H-5), 5.14 (m, 1 H, H-3'), 4.11 (m, 1 H, H-4'), 3.83 (s, 3 H, OCH_3), 3.16 (m, 1 H, H-5'a), 3.00 (m, 1 H, H-5'b), 2.70 (m, 1 H, H-2'a), 2.36 (m, 4 H, CH_3-C_6H_4 and H-2'a). *Anal.* Calcd. for $C_{36}H_{33}N_2O_7ClS$ (673.17): C, 64.23; H, 4.94; N, 4.16. Found: C, 64.11; H, 5.10; N, 4.11.

3-Chloro-4-methyl-1-(2-deoxy-3-*O*-methanesulfonyl-5-*O*-triphenylmethyl-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (26). To a solution of nucleoside **23** (332 mg, 0.64 mmol) in dry pyridine (2 mL) was added methanesulfonyl chloride (0.21 mL, 1.55 mmol) with cooling in an ice water bath. The mixture was stirred for 2 h at 0 °C and then poured into an ice water mixture (30 mL). The mixture was stirred for an additional 1 h and the resulting precipitate was collected by filtration, washed with water, dissolved in dichloromethane and dried over anhydrous sodium sulfate. The solvent was then evaporated to give **26** (838 mg, 96%); as a foam R_f 0.55 (CH_2Cl_2/EtOAc, 1/1); ^1H NMR ($CDCl_3$): δ 7.45-7.20 (m, 15 H, arom. H's), 6.79 (t, 1 H, H-4'), 3.87 (s, 3 H, OCH_3), 3.32 (m, 2 H, 2 H-5'), 2.98 (s, 3 H, SCH_3), 2.28 (m, 1 H, H-2'a), 2.50 (m, 1 H, H-2'b). *Anal.* Calcd. for $C_{30}H_{29}N_2O_7ClS$ (597.08): C, 60.34; H, 4.90; N, 4.69. Found: C, 60.27; H, 5.00; N, 4.46.

6,3'-Anhydro-3-chloro-1-(2-deoxy-5-*O*-triphenylmethyl-ß-D-*threo*-pentofuranosyl)pyridazin-4-one (27). Method A: To a refluxing solution of **25** (100 mg, 0.15 mmol) in ethanol (5 mL) was added dropwise aqueous 1 N sodium hydroxide (0.3 mL). The mixture was refluxed for 15 min, cooled, and neutralized with Dowex 50X. The solution was evaporated and purified by column chromatography (SiO_2, 30 g) using dichloroethane/ethyl acetate (3/1) as an eluent to afford 11.4 mg (15%) as white crystals, mp 223-224 °C; R_f 0.42 (CH_2Cl_2/MeOH, 20/1). ^1H NMR ($CDCl_3$): δ 7.4-7.24 (m, 15 H, arom. H's), 5.81 (d, 1 H, J=3.8 Hz, H-1'), 5.76 (s, 1 H, H-5), 5.27 (br s, 1 H, H-3'), 4.37 (m, 1 H, H-4'), 3.40 (dd, 1 H, J=6.1 and 9.4 Hz, H-5'a), 5.17 (t, 1 H, H-5'b), 2.61 (d, 1 H, J=13.4, Hz, H-2'a), 2.52 (d, 1 H, J=13.8 Hz, H-2'b). *Anal.* Calcd. for $C_{28}H_{23}N_2O_4Cl$ (486.94). C, 69.06; H, 4.76; N, 5.75. Found: C, 69.10; H, 4.84; N, 5.55.

Method B: Nucleoside **26** (200 mg, 0.33 mmol) was dissolved in acetonitrile (5 mL) and treated with a 1 N aqueous solution of sodium hydroxide (0.4 mL). The reaction mixture was stirred at reflux for 12 h and then evaporated. The residue was dissolved in dichloromethane and filtered. The filtrate was concentrated and applied to a preparative thick-layer chromatographic plate (20 x 40 cm), and the plate was developed with dichloromethane/methanol (20/1). Two major products were isolated: 3-chloro-4-methoxy-1-(2-deoxy-5-*O*-triphenylmethyl-ß-D-*threo*-pentofuranosyl)pyridazin-6-one (**28**), 53 mg (30% yield) and **27**, 44 mg (27%), identical in all respects with **27** produced in the reaction from **25**. Crystallization of **28** from dichloromethane-hexane gave white crystals, mp 157-159 °C, R_f 0.58 (CH_2Cl_2/MeOH, 2/1). MS (CI, NH_3) m/z 519 (M^+), 536 $(M+NH4)^+$, 487, 277, 243; ^1H NMR (DMSO-d_6): δ 7.2 g (m, 15H, arom. H's), 6.47 (s, 1 H, H-5), 6.40 (t, 1 H, H-1'), 4.79 (d, 1 H, J=6.7 Hz, OH-3'), 4.29 (m, 1 H, H-3'), 4.13 (m, 1 H, H-4'), 3.91 (s, 3 H, OCH_3), 3.22 (m, 2 H, 2 H-5'), 2.46 (m, 1 H, H-2'a), 2.12 (m. 1 H, H-2'b). *Anal.* Calcd. for $C_{29}H_{27}N_2O_5Cl \cdot 3/4\ H_2O$ (532.49). C, 65.40; H, 5.39; N, 5.28. Found: C, 65.33; H, 5.42; N, 5.00.

Method C: Nucleoside **26** (590 mg, 0.99 mmol) was added to a solution of sodium iodide (1.48 g, 9.9 mmol) in 2-butanone. The resulting solution was heated at reflux for 22 h. The precipitate formed during the reaction was removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in dichloromethane, and washed with 5% aqueous sodium thiosulfate. The solvent was evaporated and chromatographed on a short silica gel column (15 g, SiO_2) using dichloromethane/methanol (20/1) as the eluent. Evaporation of the solvent gave **27** (429 mg, 89%) as a foam. Crystallization from dichloromethane/hexane afforded **27** (mp. 223-224 °C) which was identical with **27** produced in the previous two methods.

3-Chloro-4-hydroxy-1-(3-azido-2,3-dideoxy-5-*O*-triphenylmethyl-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (30). Nucleoside **27** (200 mg, 0.41 mmol) and lithium azide (80 mg, 1.64 mmol) in dimethylformamide (3 mL) were heated to 100 °C for 5 h under argon. The solvent was removed under high vacuum and the resulting residue was dissolved in dichloromethane and extracted with water, washed with brine and dried over anhydrous sodium sulfate. After evaporation of the solvent, the product was crystallized from dichloromethane/hexane to afford **30**, 209 mg (96%); mp. 194-195 °C dec.; R_f 0.52 (CH_2Cl_2/MeOH, 2/1), IR (KBr) 2109 cm^{-1} (N_3); ^1H NMR ($CDCl_3$): δ 7.29 (m, 15H, arom. H's), 6.52 (m, 1 H, H-1'), 5.97 (br s, 1 H, H-5), 4.37 (m, 1 H, H-3'), 3.94 (m, 1 H, H-4'), 3.32 (br s, 1 H, OH-4' + H_2O peak), 3.11 (m, 2 H, 2 H-5'), 2.60-2.28 (m, 2 H, H-2'). *Anal.* Calcd. for $C_{28}H_{24}N_5O_4Cl$ (529.97): C, 63.45; H, 4.56; N, 13.22. Found: C, 63.21; H, 4.65; N, 13.20.

6,3'-Anhydro-3-chloro-6-hydroxy-1-(2-deoxy-ß-D-*threo*-pentofuranosylpyridazin-4-one (31). Nucleoside **27** (93.5 mg, 0.19 mmol) was treated with a mixture of formic acid and ethyl acetate (1.5 mL, HCO_2H/EtOAc, 2/3). The solution was stirred at room temperature for 3.5 h. The solvent was evaporated and the residue dissolved in methanol and applied to a preparative thick-layer chromatographic plate (20 x 40 cm). The plate was developed with dichloromethane/methanol (9/1). The UV-absorbing band (R_f 0.24) was removed and the silica gel was extracted with boiling ethanol. The filtrate was evaporated to give **31** (41 mg, 87%) as a foam; R_f 0.24 (CH_2Cl_2/MeOH, 9/1), ^1H NMR (DMSO-d_6): δ 5.98 (d, 1 H, J=4.2, H-1'), 5.95 (s, 1 H, H-5), 5.34 (br s, 1 H, H-3'), 5.05 (t, 1 H, J=5.5 Hz, OH-5'), 4.22 (t, 1 H, H-4'), 3.50 (t, 2 H, 2 H-5'), 2.69 (d, 1 H, J=6.5 Hz, H-2'a), 2.51 (m, 1 H, H-2'b). MS, EI m/z 244 (M^+), 213, 185, 152; exact mass (FAB) m/z calculated for $C_9H_9N_2O_4Cl$. (m+) 244.0251, found: 244.0240.

3-Chloro-4-hydroxy-1-(3-azido-2,3-dideoxy-ß-D-*erythro*-pentofuranosyl)pyridazin-6-one (32). Nucleoside **30**, (174 mg, 0.33 mmol) was treated with a mixture of formic acid and ethyl acetate (1.5 mL, HCOOH/EtOAc, 2/3). The solution was

stirred at room temperature for 1.5 h. The solution was evaporated to dryness and purified by flash chromatography (15 g, SiO_2) using dichloromethane/methanol (5/1) as the eluent. Evaporation of the solvent and crystallization from methanol/ethyl acetate afforded **32**, 33 mg (35%) as white crystals, mp >210 °C dec.; R_f 0.37 (CH_2Cl_2/MeOH, 9/1); IR (KBr) 2090 cm^{-1} (N_3); ^1H NMR (DMSO-d_6): δ 6.45 (t, 1 H, J=6.2 Hz, H-1'), 4.93 (t, 1 H, J=5.7 Hz, OH-5'), 4.80 (s, 1 H, H-5), 4.31 (m, 1 H, H-3'), 3.72 (m, 1 H, H-4'), 3.46-3.15 (m, 2 H, H-5' + H_2O peak), 2.14-2.07 (m, 2 H, 2 H-2'). Exact mass spectrum $C_9H_{10}N_5O_4Cl$ (287.68): Calcd[M+Na]: 310.0319, found 310.0324.

Antiviral Evaluation.

Cells and Viruses. The continuous human T-cell line (CEM-SS) was maintained in RPMI-1640 medium supplemented with 20% (v/v) fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL) and 2 mM L-glutamine. Cells were seeded at a density of 5 x 10^5 cells/mL and subcultured 1:3 three times a week. Cells were subcultured 1:2 18 to 24 h before use in experiments to maintain the cells in an exponential phase. Diploid HFF cells were grown in minimal essential medium with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[35]

HIV-1 (strain HTLV-III_B) was propagated in CEM-SS cells or H9III_B cells as previously described by us.[36] Infectious HIV-1 recovered from cell supernatants was clarified by centrifugation, filtered through a 0.45 μ Millipore filter, stored at -80 °C and assayed by syncytial plaque count. HCMV and HSV-1 were grown and titers determined as described elsewhere.[35] A plaque-purified isolate, P_O, of the Towne strain of HCMV was used and was a gift of Dr. M.F. Stinski, University of Iowa. The S-148 strain of HSV-1 was provided by Dr. T.W. Schafer of Schering Corp.

Assays for Antiviral Activity. *HIV:* The syncytial plaque assay performed as previously described by us[36] was used to measure the effect of compounds on HIV-1.

HCMV: The activity of compounds against HCMV replication was measured using a modification of the plaque reduction assay described previously.[35] In brief, HFF cells were planted in 24-well cluster dishes at a concentration of 50,000 cells per well. When cells were ~80% confluent, monolayers were inoculated with 0.2 mL of a suspension containing 100 PFU of HCMV and incubated for 1 h. Following adsorption, the virus suspension was aspirated and 1.0 mL of overlay medium was added which contained drug. Plates were incubated for 6 to 9 days, cells were fixed and stained with 0.1% crystal violet in 20% methanol, and microscopic plaques were enumerated. All assays were performed in duplicate.

HSV-1: HSV-1 was assayed using an enzyme immunoassay described by Prichard and Shipman.[37]

Cytotoxicity Assays. Two basic tests for cellular toxicity were routinely employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF cells was estimated by visual scoring of cells not affected by virus infection in the HCMV plaque reduction assays described above. Drug-induced cytopathology was estimated at 30-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration.[35] Cytotoxicity in CEM-SS cells was determined by seeding the cells at a density of 1×10^4 cells per well in growth medium using a 96-well flat bottom plate. Serial 5-fold dilutions of compounds were prepared in growth medium and added to the wells as a second overlay. After 48-h incubation at 37 °C, cells were pulse-labeled with [^{3}H]dThd (1 μCi per well, 20 Ci/mmol) for 6 h and the cells were harvested to measure total incorporation of label into DNA.

Data Analysis. Dose-response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against $\log_{10}$ of drug concentration. Fifty-percent inhibitory concentrations were not calculated because little or no inhibition was obtained with the target compounds. Samples containing positive controls (AZT, acyclovir, ganciclovir for HIV, HSV-1 and HCMV, respectively) were used in all assays.

Acknowledgment. This investigation was supported by grant U01-AI25739 from the Division of AIDS, National Institute of Allergy and Infectious Diseases, for a National Cooperative Drug Discovery Group (NCDDG), and contract N01-AI72641 from the National Institute of Allergy and Infectious Diseases. The authors thank Nathan Iyer, Shelby Puckett, Lisa Coleman and Sandra Engelhardt for technical support. The authors also thank Rae L. Herrst and Patricia J. Whittler for their expert preparation of the manuscript.

REFERENCES

(1) Barre-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier L. *Science* **1983**, *220*, 868.

(2) Gallo, R. C.; Salahuddin, S. Z.; Popovic, M.; Shearer, G. M.; Kaplan, M.; Haynes, B. F.; Palker, T. J.; Redfield, R.; Oleske, J.; Safai, B.; et. al. *Science* **1984**, 500.

(3) Fauci, A. S. *Science* **1988**, *239*, 617.

(4) Smith, A. J.; Walker, D. M.; *J. Oral. Pathol. Med.* **1992**, *21*, 145.

(5) De Clercq, E. *J. Med. Chem.* **1986**, *29*, 1561.

(6) De Clercq, E. *Antiviral Res.* **1989**, *12*, 1.

(7) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 1911.
(8) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S.; St. Clair, M. H.; *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7096.
(9) Lin, T. S.; Chen, M. S.; McLaren, C.; Gao, Y. S.; Ghazzouli, I.; Prusoff, W. H. *J. Med. Chem.* **1987**, *30*, 440.
(10) Eriksson, B. F.; Chu, C. K.; Schinazi, R. F.; *Antimicrob. Agents Chemother.* **1989**, *33*, 1729.
(11) Skoda, J. *Progr. Nucl. Acid Res. Mol. Biol.* **1963**, *2*, 197.
(12) Cihak, A. *Collect. Czech. Chem. Commun.* **1974**, *39*, 673.
(13) Bloch, A.; Dutschman, G.; Currie, B. L.; Robins, R. K.; Robins, M. J. *J. Med. Chem.* **1973**, *16*, 294.
(14) Shannon, W. M.; Arnett, G.; Schabel, F. M., Jr. *Antimicrob. Agents Chemother.* **1972**, *2*, 159.
(15) Katz, D. J.; Wise, D. S.; Townsend, L. B. *J. Med. Chem.* **1982**, *25*, 813.
(16) Katz, D. J.; Wise, D. S.; Townsend, L. B. *J. Heterocyclic Chem.* **1983**, *20*, 369.
(17) Katz, D. J.; Wise, D. S.; Townsend, L. B. *J. Org. Chem.* **1983**, *48*, 3765.
(18) Webb, R. R.; Wos, J. A.; Martin, J. C.; Brodfuehrer, P. R. *Nucleosides Nucleosides* **1988**, *7*, 147.
(19) Russell, A. F.; Greenberg, S.; Moffatt, J. G. *J. Am. Chem. Soc.* **1973**, *95*, 4025.
(20) Chu, C. K.; Bhadti, V. S.; Doboszewski, B.; Gu, Z. P.; Kosugi, Y.; Pullaiah, K. C.; Van Roey, P. *J. Org. Chem.* **1989**, *54*, 2217.
(21) Robins, M. J.; Hansske, F.; Low, N. H.; Park, J. I. *Tetrahedron Lett.*, **1984**, *25*, 367.
(22) Okabe, M.; Sun, R.-C.; Tam, S. Y.-K.; Todaro, L. J.; Coffen, D. L. *J. Org. Chem.* **1988**, *53*, 4780.
(23) Motawia, M. S.; Pedersen, E. B. *Liebigs Ann. Chem.* **1990**, 599.
(24) Chu, C. K.; Babu, J. R.; Beach, J. W.; Ahn, S. K.; Huang, H.; Jeong, L. S.; Lee, S. L. *J. Org. Chem.* **1990**, *55*, 1418.
(25) Thiem, J.; Rasch, D. *Nucleosides Nucleotides* **1985**, *4*, 487.
(26) York, J. L. *J. Org. Chem.* **1981**, *46*, 2171.
(27) Takano, S.; Nishizawa, S.; Akiyama, M.; Ogasawara, K. *Synthesis* **1984**, 949.
(28) Kuraishi, T. *Chem. Pharm. Bull.* **1957**, *5*, 376.
(29) Schonbeck, R.; Kloimstein, E. *Monatsh. Chem.* **1968**, *99*, 15.
(30) Bhat, C. C. "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, Zorbach, W. W.; Tipson, R. S., eds. John Wiley, New York, **1968**, p. 521.

(31) Chu, C. K.; Schinazi, R. F.; Ahn, M. K.; Ullas, G. V.; Gu, Z. P. *J. Med. Chem.* **1989**, *32*, 612 and references cited therein.

(32) Doboszewski, B.; Chu, C. K.; Van Halbeek, H. *J. Org. Chem.* **1988**, *53*, 2777.

(33) Hrebabecky, H.; Holy, A.; de Clercq, E. *Collect. Czech. Chem. Commun.* **1990**, *55*, 1801.

(34) Bessodes, M.; Komotis, D.; Antonakis, K. *Tetrahedron Lett.* **1986**, *27*, 579.

(35) Turk, S. R.; Shipman, Jr., C.; Nassiri, R.; Genzlinger, G.; Krawczyk, S. H.; Townsend, L. B.; Drach, J. C. *Antimicrob. Agents Chemother.* **1987**, *31*, 544.

(36) Kucera, L. S.; Iyer, N.; Leake, E.; Raben, A.; Modest, E. J.; Daniel, L. W.; Piantadosi, C. *AIDS Res. Hum. Retroviruses* **1990**, *6*, 491.

(37) Prichard, M. N.; Shipman, C., Jr. *Antiviral Res.* **1990**, *14*, 181.

Received 8/2/93
Accepted 10/12/93