and dichloromethane to give on elution with 8–10% methanol/dichloromethane α-L-rhamnoside **15** (170 mg) recrystallized from methanol/water (81 mg, 18%), mp 243–246 °C. Anal. ($C_{27}H_{46}O_7 \cdot 0.5H_2O$) C, H.

(b) From 14. Compound **14** (22 mg, 0.046) was treated with LTBA as described for **3** to give **15** (7 mg, 32%), mp 238–244 °C from methanol/water.

20β-(*tert*-Butyldimethylsiloxy)-14-hydroxy-5β,14β-pregnane (23). Compound **22** (5.3 g, 10.8 mmol) prepared as described[1] was refluxed in 0.5 M KOH/MeOH (50 mL) under argon for 1 h and diluted with water, concentrated, neutralized, and extracted with ether to give **23** (4.0 g, 82%), mp 170–172 °C from dichloromethane/acetone (lit.[1] mp 171–173 °C).

Acknowledgment. We thank the Medical Research Council of Canada and the Manitoba Heart and Stroke Foundation for financial assistance. F.S.L. is a Career Investigator of the Medical Research Council of Canada. NMR spectra were recorded by T. Wolowiec, Department of Chemistry, University of Manitoba.

The Synthesis and Antiviral Activity of Some 4′-Thio-2′-deoxy Nucleoside Analogues

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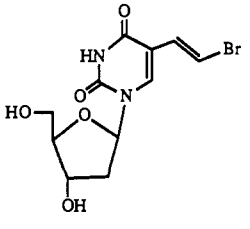
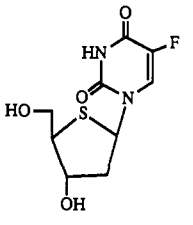
Starting from benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**4**), the following 2′-deoxy nucleoside analogues have been synthesized: 4′-thiothymidine (**8**), 3′-azido-4′-thio-deoxythymidine (**10**), and (*E*)-5-(2-bromovinyl)-4′-thio-2′-deoxyuridine (**22**). The first compound is toxic, the second is not toxic nor has detectable biological activity, and the third is not toxic and has significant activity against some herpesviruses.

Introduction

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU, **1**), a potent and selective inhibitor of HSV-1 and VZV, was first synthesized in this laboratory several years ago.[1] Its high therapeutic index (~10 000)[2] is due to a combination of its efficacy and its low toxicity because it is only phosphorylated to the 5′-mono- and 5′-diphosphates by the thymidine (and thymidylate) kinases of HSV-1 and VZV. Despite many subsequent attempts to synthesize other analogues,[3] no significant improvement in therapeutic index has been achieved. However, BVDU is a good substrate for pyrimidine phosphorylase[4] and is thus rapidly degraded in vivo into (*E*)-5-(2-bromovinyl)uracil (BVU) and 2-deoxyribose 1-phosphate. We have subsequently attempted to reduce this liability by making cytidine derivatives[5] and changing the halogen for iodo or chloro[6] but all were equally labile. An attempt to modify the sugar moiety by making the 3′-*O*-methyl ether in the hope of making the nucleoside resistant to nucleoside phosphorylase and also to be a chain terminator failed because the resultant nucleoside was no longer a substrate for the kinase.[7]

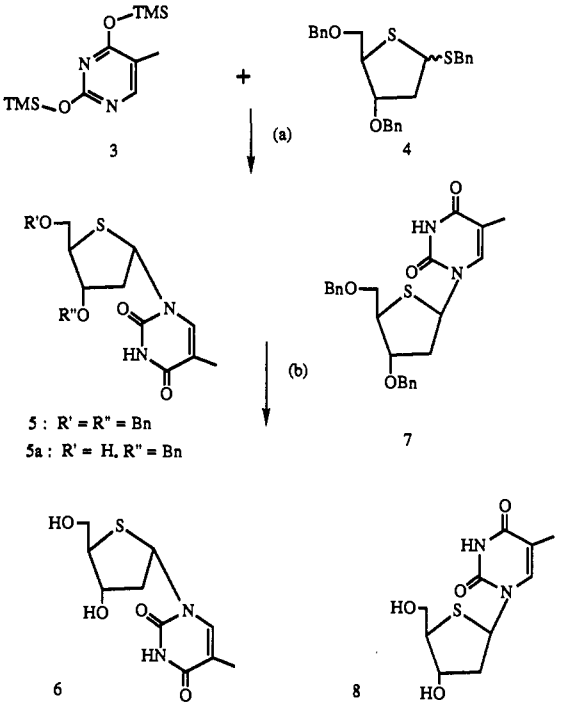


1



2

Scheme I[a]



[a] (a) $HgBr_2/CdCO_3$/toluene; (b) BCl_3/CH_2Cl_2.

We here describe the synthesis of some 4′-thio-2′-deoxy nucleosides in the hope that they might be more stable and thus bioavailable.

The only previous synthesis of a 4-thio-2-deoxy-D-*erythro*-pentose derivative (4-thio-2-deoxyribose) involved 14 steps,[8] and because of the difficulty and the low yield obtained, the synthesis of only one 4′-thio-2′-deoxy nucleoside has been reported in the literature.[9] Thus, Bobek

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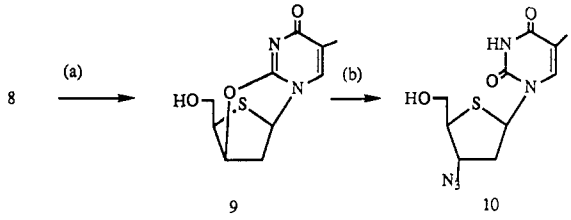
Scheme II[a]



[a] (a) $CHFClCF_2N(CH_3)_2$/DMF; (b) LiN_3/NH_4Cl/DMF.

and co-workers[9] published the synthesis of 5-fluoro-4'-thio-2'-deoxyuridine (2), but this was a rather unfortunate choice as the base, 5-fluorouracil, has an appreciable biological activity in its own right and thus it is difficult to distinguish between the activity of the intact compound and that of any degradation products, particularly the free base.

Using a recently published synthesis of a suitable sugar derivative,[10] we here report the synthesis and biological activity of several 5-substituted pyrimidine 4'-thio nucleosides and show that these are members of a class that is likely to contain other compounds with potentially useful and interesting biological activities.

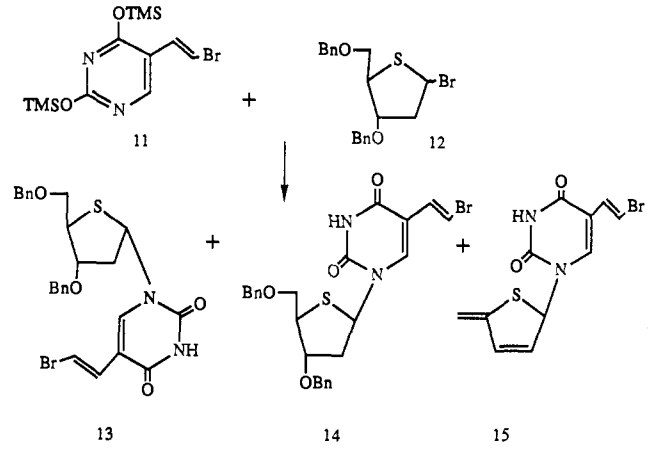
Chemistry

The synthesis of the necessary carbohydrate moiety, benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (4), will be described in detail elsewhere.[10] This synthesis is a dramatic improvement upon a previously published synthesis[8] both in terms of number of steps (7 vs 14) and overall yield (<10 vs >30%), which means that the synthesis of a large number of nucleoside analogues with the 4'-thio functionality is possible.

The initial synthesis reported here is that of the 4'-thio analogue of thymidine[8] and the method of Horton and Markovs was used (Scheme I).[11] In an attempt to minimize decomposition of the sugar moiety, initial experiments using the oxygen analogue of compound 4, benzyl 3,5-di-*O*-benzyl-2-deoxy-1-thio-D-*erythro*-pentofuranoside,[10] and 2,4-bis-*O*-(trimethylsilyl)-5-methyluracil (3) in dichloromethane instead of toluene were successfully achieved. However the thiosugar (4) did not react under those conditions, suggesting that the C-1 position is less susceptible to nucleophilic attack. Thus we reverted to the use of toluene, and although some decomposition occurred, it was possible to isolate a nucleoside product of α- (5) and β-anomers (7) (2.8:1) in 56% overall yield. The anomers could be separated chromatographically or by fractional crystallization from methanol in which the β-anomer was the least soluble.

Deprotection of the separated anomers was achieved using boron trichloride as described by Moffatt and coworkers[12] and Buchanan et al.[13] A large excess of boron trichloride is required, and with 20 equiv, a regioselective reaction is observed and reasonable yields of the 3'-*O*-benzyl nucleoside (5a) can be isolated. When 30 equiv are used, the fully deprotected nucleosides (6, 8) could be obtained in very high (93%) yield. Attempts to deprotect the nucleosides (5, 7) by catalytic hydrogenation under conditions that could be successfully employed on 3',5'-di-*O*-benzylthymidine (data not shown) failed, presumably because the presence of the sulfur heteroatom was causing the catalyst to be poisoned.

Scheme III



3'-Azido-3'-deoxy-4'-thiothymidine (10) was prepared from 4'-thiothymidine (8) by the method of Glinski et al. (Scheme II).[14] This proved not to be a very efficient synthesis but as at the time we had little of the 4'-thiothymidine, the method has the merit of not requiring a protection and deprotection step and, starting from the natural nucleoside thymidine, 3'-azido-3'-deoxythymidine (AZT) can be prepared in reasonable (25%) yield (data not shown). Unfortunately when preparing O^2,3'-anhydro-4'-thiothymidine (9) by use of the Yarovenko reagent[15] on 4'-thiothymidine, the specificity seen with the natural nucleoside was no longer present, and although some of the desired product was obtained, it had to be separated from other products, one of which was tentatively identified as the O^2,5'-anhydro-4'-thiothymidine, indicating that the sulfur heteroatom was possibly producing a different sugar ring conformation and hence modifying the reactivities of the hydroxyl groups. The ring opening of compound 9 with lithium azide occurred smoothly, but the overall yield was <5%.

It is known that the presence of the bromovinyl side chain substituted at C-5 of uracil causes a decrease in nucleophilicity of the corresponding bis(trimethylsilyl) derivative in nucleoside condensation reactions.[16] Thus when an attempt was made to synthesize compound 14 using the method of Horton and Markovs,[11] the overall yield of nucleoside was low and the product 15 was tentatively identified along with a mixture of the α- and β-anomers 13 and 14. It can be assumed that the α-anomer corresponding to compound 15 is also formed, but its presence was not confirmed. It is known that trimethylsilyl bromide formed as a byproduct can act as a debenzlating agent[17] and causes elimination. The reason why the corresponding analogue is not seen in the case of reaction with thymine is probably due to the increased nucleophilicity of thymine and the fact that this reaction thus proceeds much faster.

Attempts to increase the reaction rate so that the condensation conditions could be made less drastic was attempted by producing the α,β-bromo sugar (12) in situ. This gave a much cleaner reaction (Scheme III) but now

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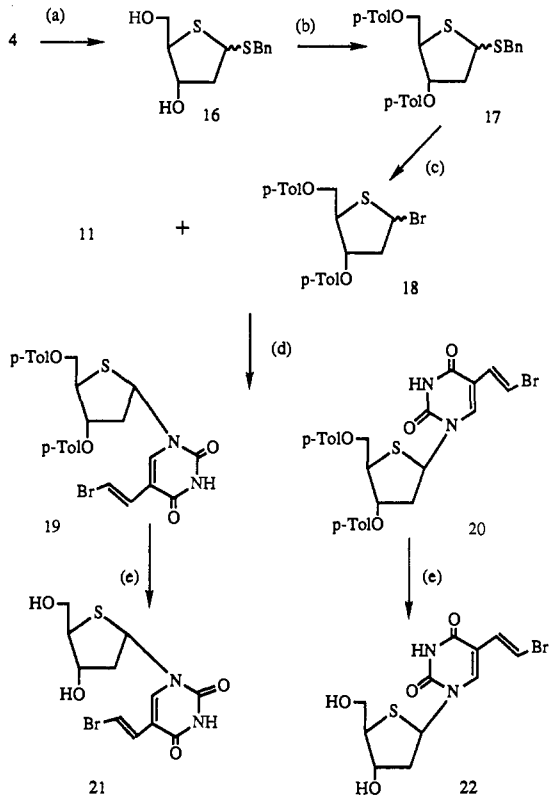
(17) Jung, M. E.; Lyster, M. A. *J. Org. Chem.* **1977**, *42*, 2953.

Table I. Antiviral Activities of 4′-Thio Nucleosides

compd	MIC_{50}[a] (μM)					ID_{50}[b]	
	HIV-1	HSV-1	HSV-2	VZV	CMV	VERO	MT-4
6	d	>100	>100	>100	ND[c]	>100	>100
8	d	0.37	2.3	10	0.98	7.1	1
10	d	>100	>100	ND	ND	>100	>100
21	ND	>500	>500	ND	ND	ND	>500
22	d	0.6	~10	0.08	ND	>500	>500
BVDU	-	0.03	12	0.02	>100	>500	-
acyclovir	-	1.3	1.8	14.5	60–100	>500	-

[a] Minimum inhibitory concentration, required to reduce virus-induced cytopathogenicity by 50%. [b] Inhibitory dose required to reduce the viability of cells by 50%. [c] ND denotes an antiviral assay was not performed. [d] No activity below ID_{50} observed. HSV-2 assays were done in VERO cells, other herpesvirus assays were done in MRC-5 cells. No significant difference in toxicity of the compounds between VERO and MRC-5 cells was seen.

Scheme IV[a]



[a] (a) BCl_3/CH_2Cl_2; (b) p-toluoyl chloride/pyridine; (c) Br_2/CBr_4; (d) heat, 90 C; (e) NaOMe/MeOH.

compound 15 predominated although it was possible to isolate and identify the two desired protected α- and β-nucleosides (13, 14).

In view of the problems caused by the presence of the benzyl protecting groups on the sugar, it was decided to replace those with acyl groups and in particular the p-toluoyl group which is often used (Scheme IV). Debenzylation of 4 was achieved using boron trichloride, and this resulted in the selective removal of the O-benzyl (16), leaving the benzyl glycoside unaffected. The 3′- and 5′-hydroxyl group could then be acylated in the usual way, and before condensing this sugar analogue (17) with a suitably protected pyrimidine, the 1-bromo derivative (18) of the former was produced in situ in the usual way. Reaction with the pyrimidine 11 now could be achieved in a smooth reaction with only starting material and the α- and β-nucleosides (19, 20) being present. The nucleosides could be separated, crystallized, deprotected, and finally isolated as crystalline compounds, although the yield of isolated β-isomer (24) was only around 10% due mainly to the predominant isomer formed in the condensation being the α-anomer (21). Whether this depends upon the anomeric configuration of the starting sugar (18) and/or the nucleoside-forming conditions is as yet unclear and will be the subject of further investigations.

Table II. Mouse Bioavailability Experiments[a]

compd	max. plasma level (μM)	$T_{1/2}$ (h)	toxicity (mg/kg)
8	>35	1–2	>40
22	>50	2–3	>40

[a] Mice were administered the test compound intraperitoneally at a dose of 40 mg of test compound/kilogram of mouse weight.

Antiviral Testing and Biological Results

The antiviral activity of the deprotected nucleosides tested are given in Table I. α-4′-Thiothymidine appeared to have no significant activity whereas β-4′-thiothymidine was surprisingly active, particularly against human cytomegalovirus but it was also very toxic. This activity and concomitant toxicity will be the subject of further investigations. Compound 10, the 4′-analogue of AZT, showed no toxicity nor any antiviral activity whereas the BVDU analogue 22 was almost as active as the normal compound. As BVDU suffers in vivo from the fact that it is particularly susceptible to nucleoside phosphorylases (which do not detract from its in vitro activity), it was of particular interest to see whether the 4′-thio nucleosides were more stable. Preliminary mouse bioavailability studies are given in Table II. These show a significant half-life for the two compounds tested and also high plasma levels. Indeed no (E)-5-(2-bromovinyl)uracil could be detected, which indicates that these analogues may not be good substrates for pyrimidine nucleoside phosphorylases.

The biological results can be explained if it is assumed that 4-′thiothymidine is a substrate for cellular and viral kinases and is therefore active and toxic and that 3′-azido-3′-deoxy-4-thiothymidine is not a substrate for cellular or viral (as it has no antiherpesvirus activity) kinases and is therefoe not active nor toxic. (E)-5-(2-Bromovinyl)-4′-thio-2′-deoxyuridine however is probably a substrate for some herpesvirus kinases (as is BVDU) but not the cellular enzyme and is therefore active and not toxic. Its stability toward phosphorylases means that one might expect to see an even greater activity in vivo than one sees for BVDU itself.

Thus this new series of nucleoside analogues has already produced some compounds with significant biological activity and it is confidently expected that other members of this series will be found with equally interesting and potentially useful properties.

Experimental Section

General Procedures. Melting points were obtained using a Gallenkamp apparatus. ^1H NMR spectra were recorded with a JEOL FX90Q (90 MHz) or a JEOL GX270 (270 MHz) spec-

trometer in d_6-DMSO solution relative to an internal tetramethylsilane reference. ^{19}F NMR spectra were recorded in the same solvent with the 90-MHz machine with trichlorofluoromethane as internal standard. FAB mass spectra were obtained on a Kratos MS80 spectrometer from samples dissolved in DMSO with 3-nitrobenzyl alcohol as matrix; sodium ion doping to give enhanced peaks was used as necessary. Samples for UV spectrophotometry were dissolved in spectroscopic grade ethanol, and spectra were recorded on a Perkin-Elmer 552 spectrophotometer. Precoated, aluminum-backed, silica gel TLC plates (silica gel 60 F_{254}, 0.2-mm thickness) were supplied by E. Merck, A. G. Detection was achieved under UV light (254 nm) or by spraying with 30% H_2SO_4 in ethanol and heating. Column chromatography was performed on silica gel 60, 230–400 mesh (Merck).

The following solvents were distilled before use: carbon tetrachloride, dichloromethane (from P_2O_5), 1,2-dimethoxyethane and pyridine (from CaH_2), and methanol (from Mg/I_2). Calcium chloride dried toluene was further dried over sodium wire.

3',5'-Di-*O*-benzyl-4'-thiothymidine (7) and Its α-Anomer (5). A suspension of benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (4) (22.5 g, 51.6 mmol), 2,4-bis-*O*-(trimethylsilyl)-5-methyluracil (3) (46 g, 170 mmol), mercuric bromide (20.5 g, 56.7 mmol), cadmium carbonate (29.3 g, 170 mmol), and dry toluene (1 L) was boiled under reflux with stirring for 24 h. The hot suspension was then filtered, the residual solids were washed with dichloromethane, and the combined filtrate and washings were successively washed with potassium iodide solution (30% aqueous) and water and concentrated to dryness. The residue was added to methanol–water, 4:1 (250 mL), stirred for 30 min, and filtered, and the filtrate was concentrated to dryness. The residue was applied to a silica column, and chromatography using hexane–ethyl acetate (1:1) as solvent gave a mixed product of **5** and **7** as a clear colorless (12.7 g, 56%). ^1H NMR indicated the ratio of α:β-anomers to be 2.8:1. Further column chromatography on silica gel using hexane–ethyl acetate (1:1) as solvent gate two separate fractions, the faster running of which was recrystallized from methanol to give **3',5'-di-*O*-benzyl-4'-thiothymidine (7)** as colorless crystals (1.7 g, 7.5%): mp 140–142 °C; UV λ_{max} 269 nm (ε 14 300); ^1H NMR δ 11.36 (1 H, s NH), 7.69 (1 H, s, H6), 7.22–7.48 (10 H, m, aromatic), 6.27–6.33 (1 H, t, H1'), 4.51–4.61 (4 H, M, PhCH_2O), 4.30 (1 H, s, H3'), 3.66–3.76 (3 H, m, H4', H5'), 2.32–2.42 (2 H, m, H2'), 1.66 (3 H, s, CH_3); MS *m/z* 439 $(M + H)^+$. Anal. ($C_{24}H_{26}N_2O_4S$) C, H, N, S. The slower running component was eluted from the column as a colorless syrup to give **5** (6.3 g, 27.8%): UV λ_{max} 268 nm (ε 10 900); ^1H NMR δ 11.28 (1 H, s, NH), 7.95 (1 H, s, H6), 7.20–7.43 (10 H, m, aromatic), 6.21–6.25 (1 H, d, H1'), 4.47–4.60 (4 H, m, PhCH_2O), 4.25 (1 H, s, H3'), 4.06–4.10 (1 H, m, H4'), 3.42–3.57 (2 H, m, H5'), 2.26–2.68 (2 H, m, H2'), 1.55 (3 H, s, CH_3); MS *m/z* 439 $(M - H)^+$. Anal. ($C_{24}H_{26}N_2O_4S$) C, H, N, S.

4'-Thiothymidine (8) and Its α-Anomer (6). To a 2 M boron trichloride solution in dry dichloromethane (55 mL) cooled to −78 °C was added a solution of **7** (1.6 g, 3.7 mmol) in dry dichloromethane (30 mL). The solution was stirred for 5 h at −78 °C, and then methanol–dichloromethane (1:1) (200 mL) was added dropwise over a period of 40 min. The reaction mixture was allowed to warm up to room temperature over a period of 1 h, the solvent was removed in vacuo, and the resulting residue was coevaporated with dry methanol (3 × 30 mL). The residue was chromatographed on a silica column with chloroform–methanol (85:15) to give **8** as colorless crystals (0.92 g, 93%): mp 208–209 °C; UV λ_{max} 270.5 nm (ε 10 300); ^1H NMR δ 11.34 (1 H, s, NH), 7.81 (1 H, s, H6), 6.26–6.32 (1 H, t, H1'), 5.25–5.26 (1 H, d, OH-3'), 5.16–5.20 (1 H, t, OH-5'), 4.35–4.40 (1 H, m, H3'), 3.16–3.68 (3 H, m, H4', H5'), 2.13–2.25 (1 H, m, H2'), 1.8 (3 H, s, CH_3); MS *m/z* 259 $(M + H)^+$. Anal. ($C_{10}H_{14}N_2O_4S$) C, H, N, S.

α-4'-Thiothymidine (**6**) was prepared from **5** (2.3 g, 5.2 mmol) in an exactly analogous manner although under these conditions partial debenzylation of the α-anomer occurred and on chromatography on silica gel, two compounds could be recovered. The first component obtained as colorless crystals (0.42 g, 23%) was **α-3'-*O*-benzyl-4'-thiothymidine (5a)**: mp 139–141 °C; UV λ_{max} 269.5 nm (ε 12 200); ^1H NMR δ 11.26 (1 H, s, NH), 7.95 (1 H, s, H-6), 7.33 (5 H, s, aromatic), 6.11–6.30 (1 H, q, H1'), 5.05–5.24 (1 H, t, OH-5'), 4.39–4.74 (2 H, m, PhCH_2O), 4.19–4.39 (1 H, m, H3'), 3.70–4.06 (1 H, m, H4'), 3.30–3.52 (2 H, m, H5'), 2.10–2.65 (2 H, m, H2'), 1.50 (3 H, s, CH_3); MS *m/z* 349 $(M + H)^+$. Anal. ($C_{17}H_{20}N_2O_4S$) C, H, N, S.

The second compound to be eluted from the column was recrystallized from methanol to give **α-4'-thiothymidine (6)** (0.77 g, 57%) as colorless crystals: mp 194–196 °C; UV λ_{max} 271 (ε 13 300); ^1H NMR δ 11.27 (1 H, s, NH), 8.11 (1 H, s, H6), 6.14–6.18 (1 H, q, H1'), 5.48–5.50 (1 H, d, OH-3'), 4.98–5.03 (1 H, 5, OH5'), 4.23–4.29 (1 H, m, H3'), 3.26–3.58 (3 H, m, H4', H5'), 1.98–2.11 (2 H, m, H2'), 1.78 (3 H, s, CH_3); MS *m/z* 259 $(M + H)^+$. Anal. ($C_{10}H_{14}N_2O_4S$) C, H, N, S.

O^2,3'-Anhydro-4'-thiothymidine (9). To a solution of 4'-thiothymidine (**8**) (500 mg, 1.9 mmol) in dry DMF (1 mL) was added 2-chloro-1,1,2-trifluorotriethylamine (640 mg, 3.4 mmol), and the mixture heated at 70 °C for 30 min and poured into dry acetone (2 mL). The solvent was removed in vacuo, and the residue was chromatographed on silica gel with methanol–chloroform (1:4) as solvent. Combination of the apprropriate fractions gave **9** as a white solid (40 mg, 9%): UV λ_{max} 254 nm (ε 9200); ^1H NMR δ 7.50 (1 H, s, H6), 5.66–5.90 (1 H, m, H1'), 5.20–5.58 (2 H, m, H3', OH-5'), 4.84–5.10 (1 H, t, H4'), 4.00–4.60 (2 H, m, H5'), 2.60–3.00 (2 H, m, H2'), 1.75 (3 H, s, CH_3); MS *m/z* 241 $(M + H)^+$. Anal. ($C_{10}H_{12}N_2O_3S$) C, H, N, S.

3'-Azido-3'-deoxy-4'-thiothymidine (10). Compound **9** (30 mg, 0.12 mmol), lithium azide (16.4 mg, 0.38 mmol), and ammonium chloride (1.6 mg) were heated in DMF (1 mL) at 110 °C for 18 h. The mixture was then poured into water (30 mL), and the product was isolated by repeated extraction of the aqueous solution with ethyl acetate (12 × 20 mL). The ethyl acetate solution was concentrated, and the residue was chromatographed on silica gel with methanol–chloroform (7:93) as solvent to give **10** (15 mg, 42%) as a yellow oil: UV λ_{max} 265 nm (ε 10 500); ^1H NMR δ 11.35 (1 H, s, NH), 7.67 (1 H, s, H6), 6.18–6.27 (1 H, t, H1'), 4.43–4.90 (4 H, H4', H5', OH-5'), 3.58–3.77 (1 H, m, H3'), 2.34–2.62 (2 H, m, H2'), 1.81 (3 H, s, CH_3); MS *m/z* 284 $(M + H)^+$. Anal. ($C_{10}H_{13}N_5O_3S$) C, H, N.

(*E*)-5-(2-Bromovinyl)-3',5'-di-*O*-benzyl-2'-deoxy-4'-thiothymidine (14) and Its α-Anomer (13). To a solution of **4** (0.5 g, 1.19 mmol) in carbon tetrachloride (5 mL) was added a solution of bromine (0.21 g, 1.34 mmol) in carbon tetrachloride with stirring at 25 °C until no starting material remained (TLC, 5 min). The mixture was then concentrated under reduced pressure, and the residue coevaporated with carbon tetrachloride (5×). The resulting syrupy residue (**12**) was dissolved in carbon tetrachloride (10 mL) to which was added 2,4-bis-*O*-(trimethylsilyl)-(*E*)-5-(2-bromovinyl)uracil (**11**) (1.0 g, 2.77 mmol). The resulting mixture was stirred at room temperature for 1 h and concentrated in vacuo, and the residue was heated for 1 h at 90–100 °C. The cooled residue was dissolved in methanol–water (4:1) (10 mL), the solution was boiled under reflux for 15 min and concentrated, the residue was triturated with chloroform (12.5 mL), and the unreacted (*E*)-5-(2-bromovinyl)uracil was removed by filtration. The organic layer was washed with aqueous sodium bicarbonate solution (5%) and water, dried (sodium sulfate), and concentrated. The residue was chromatographed on silica gel using ethyl acetate–hexane (2:3) as solvent. Combination of the relevant fractions gave three nucleosides, isolated as clear syrups. The first to emerge was the title compound (**14**) (40 mg, 6%): UV λ_{max} 292 nm (ε 10 100); ^1H NMR δ 11.26 (1 H, s, NH), 8.15 (1 H, s, H6), 7.20–7.51 (11 H, m, aromatic + vinylic), 6.80–6.85 (1 H, d, J = 13.8 Hz, vinylic), 6.06–6.02 (1 H, t, H1'), 4.48–4.59 (4 H, m, PhCH_2O), 4.28 (1 H, s, H3'), 3.34–3.73 (3 H, m, H4', H5'), 2.30–2.41 (2 H, m, H2'); MS *m/z* 529, 531 $(M + H)^+$. Anal. ($C_{25}H_{25}N_2O_4SBr$) C, H, N.

The second compound was tentatively identified as **15** (0.18 g, 30%): UV λ_{max} 294 nm (ε 11 100); ^1H NMR δ 11.67 (1 H, s, NH), 8.08 (1 H, s, H6), 6.76–7.50 (7 H, m, H5', H4', H2', H1'', H1'', H2''); MS *m/z* 312, 314 $(M + H)^+$. Anal. ($C_{11}H_9N_2O_2SBr$), C, H, N.

The third compound to be eluted was the α-anomer **13** (20 mg, 3%): UV λ_{max} 291 nm (ε 9200); ^1H NMR δ 11.58 (1 H, s, NH), 8.35 (1 H, s, H6), 7.13–7.40 (11 H, m, aromatic + vinylic), 6.48–6.53 (1 H, d, J = 13.6 Hz, vinylic), 6.18–6.20 (1 H, d, H1'), 4.13–4.60 (5 H, m, PhCH_2O and H3'), 3.32–3.61 (3 H, m, H4', H5'), 2.30–2.32 (2 H, m, H2'); MS *m/z* 529, 531 $(M + H)^+$. Anal. $C_{25}H_{25}N_2O_4SBr$) C, H, N.

Compounds **13** and **14** could be deprotected using boron trichloride in dry dichloromethane as described for compounds **5** and **7**, and the resulting compounds were identical in all respects

with the compounds isolated by deprotection of the relevant 3',5'-di-*O*-*p*-toluoyl esters (**19**, **20**), the preparation of which is described below. This following method is much to be preferred as the yields are much higher and, due to the absence of compound **15**, the separation of the anomers is much simplified.

Benzyl 2-Deoxy-3,5-di-*O*-*p*-toluoyl-1,4-dithio-D-*erythro*-pentofuranoside (17). To a solution of boron trichloride (2 M) in dry dichloromethane (150 mL) cooled to −78 °C was added dropwise over a period of 30 min a solution of compound **4** (4.2 g, 10 mmol) in dry dichloromethane (100 mL). Stirring was continued for 5 h at −78 °C, and the reaction mixture was then allowed to warm up to room temperature over a period of 1 h, the solvent was removed in vacuo and the residue (**16**) was coevaporated with dry methanol (3 × 30 mL). The residue was then dissolved in dry pyridine (25 mL) and cooled to 0 °C, and a solution of *p*-toluoyl chloride (4.6 g, 30 mmol) in dry pyridine (25 mL) was added dropwise with stirring. The pyridine was removed in vacuo, and the residue was extracted with chloroform, washed with hydrochloric acid (2 M), sodium carbonate (M), and water, dried (magnesium sulfate), and concentrated to dryness. The residue was chromatographed on a silica gel column with hexane–ethyl acetate (9:1) as solvent to give compound **17** as a clear slightly yellow syrup (2.5 g, 53%): ^{1}H NMR δ 7.25–7.94 (13 H, m, aromatic), 5.62–5.68 (1 H, m, H1), 4.66–4.74 (1 H, m, H3), 3.83–4.39 (5 H, m, H4, H5, and PhCH_2S), 2.25–2.51 (2 H, m, H2), 2.39 (6 H, s, OCH_3); MS *m/z* 515 $(M + Na)^+$. Anal. ($C_{28}H_{28}O_4S_2$) C, H, S.

(*E*)-5-(2-Bromovinyl)-3',5'-di-*O*-*p*-toluoyl-2'-deoxy-4'-thiothymidine (20) and Its α-Anomer (19). To a solution of **17** (1.4 g, 2.8 mmol) in carbon tetrachloride (15 mL) was added with stirring at room temperature a solution of bromine (0.05 g, 3.1 mmol) in carbon tetrachloride (15 mL). After 5 min, the solution was concentrated in vacuo, carbon tetrachloride (4 × 5 mL) was added and the solution was concentrated to remove the excess of bromine. The resulting syrup (**18**) was unstable and was used directly in the next step.

To a solution of **18** in carbon tetrachloride (10 mL) was added 2,4-bis-*O*-(trimethylsilyl)-(*E*)-5-(2-bromovinyl)uracil (**11**) (1.7 g, 4.7 mmol) in carbon tetrachloride (10 mL). The mixture was stirred until homogeneous and concentrated, and the residue was heated at 90–100 °C for 1 h. The cooled dark residue was dissolved in methanol–water (4:1) (30 mL), boiled under reflux for 15 min, and concentrated to dryness. The residue was triturated with chloroform (40 mL), and the unreacted (*E*)-5-(2-bromovinyl)uracil was removed by filtration. The filtrate was washed with aqueous sodium hydrogen carbonate and water, dried (sodium sulfate), and concentrated to dryness. The residue was chromatographed on silica gel with hexane–ethyl acetate (3:2) as solvent to give the product as a mixture of the α- (**19**) and β-anomers (**20**) (820 mg, 50%), in the ratio of 1.8:1. Further column chromatography with chloroform–propan-2-ol (99:1) gave the separated isomers. The first to be eluted was compound **20**, which could be crystallized from methanol to give colorless crystals (230 mg, 14%): mp 182–184 °C; UV λ_{max} 297 nm (ε 10 100); ^{1}H NMR δ 11.73 (1 H, s, NH), 8.10 (1 H, s, H6), 7.86–7.94 (4 H, m, aromatic), 7.19–7.39 (5 H, m, aromatic and H2''), 6.89 (1 H, d, vinylic H1'', *J* = 14 Hz), 6.40–6.45 (1 H, t, H1'), 5.80–5.85 (1 H, m, H-3'), 4.53–4.71 (2 H, m, H5'), 3.92–4.00 (1 H, m, H4'), 2.50–2.83 (2 H, m, H2'), 2.39 (6 H, s, OCH_3); MS *m/z* 586 $(M + H)^+$. Anal. ($C_{27}H_{25}BrN_2O_6S$) C, H, N.

The second component to be eluted was the α-anomer (**19**), which was crystallized from methanol to give colorless crystals (450 mg, 27%): mp 170–172 °C; UV λ_{max} 296 nm (ε 11 500); ^{1}H NMR δ 11.64 (1 H, s, NH), 8.36 (1 H, s, H6), 7.77–7.91 (4 H, m, aromatic), 7.22–7.36 (5 H, m, aromatic and H2''), 6.80 (1 H, d, vinylic H1'', *J* = 14 Hz), 6.27–6.29 (1 H, d, H1'), 5.62–5.74 (1 H, m, H3'), 4.39–4.48 (3 H, m, H4', H5'), 2.85–2.94 (2 H, m, H2'), 2.37 (6 H, s, OCH_3); MS *m/z* 586 $(M + H)^+$. Anal. ($C_{27}H_{25}BrN_2O_6S$) C, H, N.

(*E*)-5-(2-Bromovinyl)-2'-deoxy-4'-thiouridine (22) and Its α-Anomer (21). Compound **20** (200 mg, 0.34 mmol) was dissolved in a solution of sodium methoxide (0.1 M) in methanol (7.5 mL) and allowed to stand at 22 °C for 24 h. The solution was neutralized by careful addition of Dowex 50 ion exchange resin (H^+ form) to pH 6. The resin was removed by filtration and washed with methanol, and the filtrate and washings were concentrated to dryness to yield a white solid that was chromatographed on silica gel with chloroform–methanol (9:1). Compound **22** was recovered and could be crystallized from methanol–water (1:1) to give colorless crystals (90 mg, 75%): mp 190–191 °C; UV λ_{max} 297 nm (ε 14 300); ^{1}H NMR δ 11.63 (1 H, s, NH), 8.20 (1 H, s, H6), 7.30 (1 H, d, H2'', *J* = 14 Hz), 6.97 (1 H, d, H1'', *J* = 14 Hz), 6.22–6.27 (1 H, t, H1'), 5.28–5.29 (1 H, d, OH-3'), 5.20–5.24 (1 H, t, OH-5'), 4.32–4.40 (1 H, m, H3'), 3.16–3.69 (3 H, m, H4', H5'), 2.15–2.30 (2 H, m, H2'); MS *m/z* 348 $(M + H)^+$. Anal. ($C_{11}H_{13}BrN_2O_4S$) C, H, N, S.

Compound **19** was deprotected in a similar manner to give the α-anomer (**21**), which could be crystallized from methanol: mp 186–187 °C; UV λ_{max} 296 nm (ε 14 300); ^{1}H NMR δ 11.56 (1 H, s, NH), 8.44 (1 H, s, H6), 7.24 (1 H, d, H2'', *J* = 14 Hz), 6.86 (1 H, d, H1'', *J* = 14 Hz), 6.09–6.13 (1 H, q, H1'), 5.45–5.46 (1 H, d, OH-3'), 5.02–5.06 (1 H, t, OH-5'), 4.24–4.30 (1 H, m, H3'), 3.16–3.63 (3 H, m, H4', H5'), 2.09–2.17 (2 H, m, H2'); MS *m/z* 348, 350 $(M + H)^+$. Anal. ($C_{11}H_{13}BrN_2O_4S$) C, H, N, S.

Antiviral Assay Procedures. The human immunodeficiency virus (HIV) assay was based on the ability of a compound to reverse HIV-mediated growth inhibition in MT-4 cells infected with the HTLV-III_B strain grown in T-cell line H9. The test involved infection of cells (1 h at 37 °C with 10 $TCID_{50}$ HIV) followed by immediate exposure to the candidate drug at concentrations of 100, 10, 1, and 0.1 μM. Mock infected cells were used as controls for all drug concentrations on the same 96-well dish, allowing a simultaneous assessment of toxicity (by growth inhibition). Triplicate wells were used for infected or uninfected cells at each drug concentration. After 5 days cell number was assessed by the uptake of a tetrazolium dye MTT into viable cells, extraction with acidified propan-2-ol, and spectrophotometric determination.

For the cytomegalovirus assay, monolayers of MRC-5 cells were formed in 24-well tissue culture panels. After 24 h the wells were infected and overlaid with 0.5% indubiose A37 medium. The candidate drug was dissolved in a suitable solvent and incorporated into the overlay medium at 10 and 100 μM. After 5 days giant cells (plaques) were visualized by methylene blue stain and examined by microscope.

The other antiviral assays were based on plaque reduction. Confluent monolayers of the appropriate cells in 50-mm diameter plastic petri dishes were infected with a suspension of the virus and overlaid with nutrient agarose in which the candidate drug was dissolved in doubling dilutions. After 5 days plaques were counted and estimated as a percentage of the control and plotted against the logarithm of the compound concentration. From this the IC_{50} was determined.

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