Synthesis and Antiviral Activity of 2′-Deoxy-4′-thio Purine Nucleosides

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A series of 2′-deoxy-4′-thioribo purine nucleosides was prepared by *trans-N*-deoxyribosylase-catalyzed reaction of 2′-deoxy-4′-thiouridine with a variety of purine bases. This synthetic procedure is an improvement over methods previously used to prepare purine 4′-thio nucleosides. The compounds were tested against hepatitis B virus (HBV), human cytomegalovirus (HCMV), herpes simplex virus (HSV-1 and HSV-2), varicella zoster virus (VZV), and human immunodeficiency virus (HIV-1). Cytotoxicity was determined in a number of cell lines. Several compounds were extremely potent against HBV and HCMV and had moderate to severe cytotoxicity *in vitro*. The lead compound from the series, 2-amino-6-(cyclopropylamino)purine 2′-deoxy-4′-thioriboside, was the most potent and selective agent against HCMV and HBV replication *in vitro*; however, this analogue was nephrotoxic when tested *in vivo*.

Introduction

During the 1960s and 1970s, there was considerable interest in the preparation of nucleoside analogues with alternative heteroatoms such as nitrogen, selenium, and sulfur in the 4′-position. These compounds are structurally very similar to clinically useful 4′-oxo nucleosides. It was hoped that the slight change in the size and electronegativity of the ribose ring would lead to compounds that retained the activity of the 4′-oxo analogues but had decreased toxicity and increased metabolic stability. Of the heteroatom-substituted sugars studied, the 4′-thio class of nucleosides had the best biological profile.[1−9] Some of these early analogues had interesting antibiotic[5] and antineoplastic[6−9] activities, but these compounds were not developed further. Several 4′-thio sugar variations were made, including D-*ribo*,[1] D-*arabino*,[2] D-*xylo*,[2] D-*erythro*,[3] and D-*threo*[3] nucleosides. Owing to severe synthetic difficulties in preparing a 2-deoxy-4-thioribose derivative, the only 2′-deoxy-4′-thioribo nucleoside prepared was 2′-deoxy-4′-thio-5-fluorouridine.[6]

Recently, there has been resurging interest in 4′-thio nucleosides as potential antiviral and anticancer agents. Interest in this class of compounds stems from the demonstrated *in vitro* resistance of 4′-thioinosine toward phosphorylytic cleavage, a major metabolic pathway of nucleoside catabolism.[10] Thus, resistance toward degradation by phosphorylytic enzymes may provide 4′-thio analogues with a metabolic profile more favorable than found with traditional 4′-oxo nucleosides. Postulating that the glycosylic linkage of other 4′-thio nucleosides might also be resistant to phosphorylytic cleavage, Secrist and co-workers prepared a series of pyrimidine and purine 2′,3′-dideoxy-4′-thio D-nucleosides in search of a metabolically stable therapeutic agent for human immunodeficiency virus (HIV) disease.[11] From this series of compounds, 2′,3′-dideoxy-4′-thiocytidine was shown to be a selective anti-HIV agent *in vitro*.

Significant synthetic effort has also recently been focused on 2′-deoxy-4′-thio nucleosides. The original synthesis of 2′-deoxy-4′-thio-5-fluorouridine was inefficient, involving 14 steps and resulting in a very low overall yield.[6] Subsequently, several groups reported improved procedures for the synthesis of a 2-deoxy-4-thio sugar intermediate that was suitable for nucleoside synthesis (e.g., 2-deoxy-4-thio sugar **1**).[12,13] Coupling of **1** with a silylated pyrimidine base under Vorbrüggen conditions yields a mixture of α- and *β*-nucleosides (eq 1).[14−16] By this method, 2′-deoxy-4′-thio pyrimidine nucleosides such as **2a,b** have been prepared.

4′-Thiothymidine (**2a**, $R_5 = CH_3$) inhibited the replication of several herpes viruses (HSV) but also had significant cytotoxicity.[15,16] The 5-(bromovinyl)uridine analogue (4′-TBVDU; **2a**, $R_5 = CH{=}CHBr$) had potent activity against HSV-1 and varicella zoster virus (VZV) without toxicity *in vitro*.[14] No base formation was observed upon administration of 4′-TBVDU to mice, confirming the metabolic stability of the glycosylic linkage.

Given the potent antiviral activity and metabolic stability of pyrimidine 2′-deoxy-4′-thio nucleosides, we were interested in preparing purine analogues in this series. This report describes the synthesis and *in vitro* antiviral and cytotoxic activities of 2′-deoxy-4′-thioribo purine nucleosides.

Chemistry

References to 2′-deoxy-4′-thio purine nucleosides are rare in the literature.[17] Secrist and co-workers have reported that the Lewis acid-catalyzed chemical coupling of a 2-deoxy-4-thio sugar with a purine base gave a 9:1 α:*β* mixture of purine nucleosides (eq 2).[18] This unfavorable anomeric ratio, coupled with the difficulty in preparing the requisite 4-thio sugar, accounts for the paucity of purine 4′-thio nucleosides prepared to date.

Because of the very poor anomeric ratio obtained from the chemical coupling of a 2-deoxy-4-thio sugar with a purine, an enzymatic route for the transfer of the 4′-thio sugar moiety from a pyrimidine nucleoside to a

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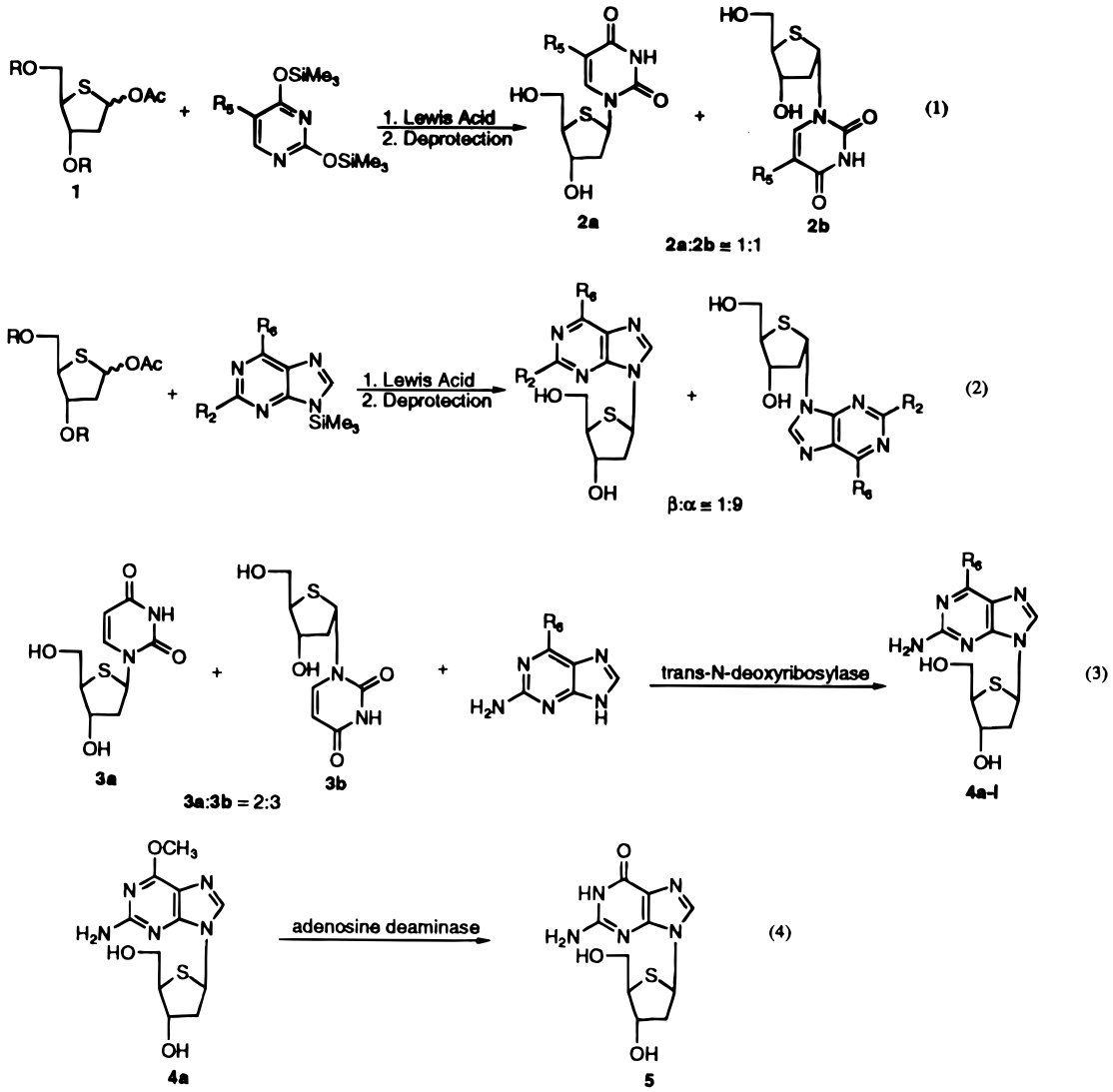
Chart 1



purine base was investigated. Enzymatic transfer reactions have been used successfully in the preparation of a wide variety of sugar-modified purine nucleosides.[19−21] For the 4′-thio nucleoside series, this procedure offered two advantages over Vorbrüggen-type coupling. Firstly, the pyrimidine 'donor' was synthetically accessible as a 3:2 α:β mixture.[14,15] By using a standard pyrimidine for all transfer reactions, more 4-thio sugar could be converted to the desired β-purine nucleoside (40% available as β-anomer from the pyrimidine nucleoside vs 10% from direct chemical coupling of the thio sugar). Secondly, on the basis of the specificity of enzyme−substrate interactions, only the β-anomer should serve as a substrate, yielding the biologically relevant β-purine nucleoside. The product purine nucleosides can then be readily separated from the other reaction mixture components by simple anion-exchange chromatography. The selectivity of the enzyme-catalyzed reaction in conjunction with a simple purification procedure would provide an efficient method to prepare a variety of β-D-purine nucleosides.

The requisite pyrimidine starting material 2′-deoxy-4′-thiouridine (**3**) was prepared as a 3:2 α:β mixture according to the method of Dyson.[12,16] The spectroscopic characteristics of **3** matched those previously reported.[15]

Two enzymatic systems were investigated. The combination of thymidine phosphorylase (TPase) and purine nucleoside phosphorylase (PNP) has proven to be extremely useful for purine synthesis in a variety of sugar series.[19−21] Compound **3** and 2-aminopurine were combined with *Escherichia coli* TPase and PNP in phosphate buffer to determine if transfer of the thio sugar subunit to a purine base would occur. Evidence of purine nucleoside formation was found, but the reaction was too slow to be synthetically useful. This was not a surprising result in light of the resistance of 4′-thio nucleosides to phosphorylytic enzymes. The second enzyme studied, *trans*-*N*-deoxyribosylase (E.C. 2.4.2.6), has a reaction mechanism different from that of the phosphorylases and served as an efficient biocatalyst for 4′-thio purine nucleoside synthesis. On a preparative scale, this enzyme catalyzed the complete transfer of the 4′-thio sugar from the β-anomer of **3** to a variety of purine bases, yielding purine nucleosides **4a**−**l** (eq 3). The guanosine analogue **5** was readily prepared by treatment of the 2-amino-6-methoxy analogue **4b** with adenosine deaminase (ADA) (eq 4).

Biological Results

Compounds **4a**−**l** and **5** were tested in a number of antiviral assays, including hepatitis B virus (HBV),[22] human cytomegalovirus (HCMV),[23] HSV-1 and HSV-

Table 1. Antiviral Activity and Cytotoxicity Evaluation for a Series of 2-Amino-6-substituted-purine 2′-Deoxy-4′-thio Nucleosides[a]

compd no.	R_6	HBV[b] efficacy IC_{50} (μM)	HBV[b] toxicity CC_{50} (μM)	HCMV efficacy IC_{50} (μM)	HCMV toxicity CC_{50} (μM)	cytotoxicity[c] (% of control at 100 μM or (CC_{50}, μM)) IM-9	CEM	Molt 4
4a	Cl	0.001	>0.2	0.1	2	(42)	(10)	(3.8)
4b	OCH_3	0.0025	14	0.062	>5	(5)	(8)	(2)
4c	OCH_2cPr	0.035	>0.2	0.6	>20	(61)	(41)	(13)
4d	SCH_3	0.45	>200	2	>20	88	78	73
4e	NHPr	0.061	>0.2	2	>50	92	68	52
4f	NHallyl	0.058	>0.2	1.5	>200	67	(60)	(40)
4g	NHiPr	0.3	>200	4	>100	90	80	77
4h	NHcPr	0.0072	77	0.2	15	78	68	11
4i	$N(Et)CH_3$	0.19	>200	2	>20	85	69	77
4j	$N(CH_3)$cPr	0.3	101	2	>20	72	58	(100)
4k	piperidino	4	>200	6	>200	88	80	77
4l	pyrrolidino	0.85	>200	10	>200	99	71	112
5	OH	0.002	13	0.06	2	(4)	(9)	(14)

[a] IC_{50} values and 50% cytotoxic concentrations (CC_{50}) were determined on the basis of five compound concentrations encompassing the IC_{50} and CC_{50}, respectively. Calculations were performed as described previously.[31] [b] Data are means of four replicate samples. Antiviral activity and cytotoxicity were determined in growing cultures of 2.2.15 cells, as described elsewhere.[23] [c] Data are means of three replicate samples. Cytotoxicity was determined in growing cell culture, as described previously.[28]

2,[24] HIV-1,[25] and VZV.[26] Growth inhibition of each cell line used in the antiviral assays was also determined. Additional cellular toxicities produced by these compounds were determined in growing cultures of human leukemic B-cell line IM-9 and two human leukemic T-cell lines, CEM and Molt 4.[27] The results for antiviral activity against HBV and HCMV are shown in Table 1 along with the cytotoxicities observed with various cell lines. Except where noted below, none of the analogues prepared had significant activity against HSV-1, HSV-2, HIV-1, or VZV.

The guanine analogue **5** showed potent activity against both HBV and HCMV (IC_{50} = 0.002 and 0.006 μM, respectively). Moderate activity against HSV-1 and VZV was also observed for **5** (IC_{50} = 7.6 and 6 μM, respectively), but no anti-HIV activity was found. The 2-amino-6-chloro and 2-amino-6-methoxy analogues **4a,b** showed activity profiles similar to that of **5**. This similarity most probably results from reaction of the 6-chloro and 6-methoxy groups of these purine thio nucleosides with ADA to yield the guanosine analogue.[21] In cell culture, compounds **4a,b** would thus serve as prodrugs of **5**.

Significant leukemic cell cytotoxicity was observed with compounds **5** and **4a,b**. While the differential between antiviral activity and cytotoxicity for **5** and **4a,b** was significant, we were interested in ameliorating the toxicity while maintaining the antiviral activity. We therefore prepared a series of compounds with a variety of substitutions at R_6 in an attempt to reduce the cytotoxicity of these thio nucleosides.

In addition to the 6-methoxy compound **4b**, we prepared the cyclopropylmethoxy analogue **4c**. While this compound was 10-fold less toxic to leukemic cells than was **5**, it was also at least 10-fold less active against HBV and HCMV, yielding no therapeutic advantage. The 6-methylthio analogue **4d** showed an even more dramatic drop in efficacy. However, analogues with substituted amine functionalities at R_6 were more promising. Simple monoalkylamines such as the *N*-propyl and *N*-allyl analogues **4e,f** were 10−50-fold less active than **5** against HBV and HCMV but showed an even greater decrease in cytotoxicity. The bulkier isopropylamine analogue **4g** was inactive. Simple steric hindrance cannot account for the lack of activity because the cyclopropylamine compound **4h** was very potent against HBV and HCMV (IC_{50} = 0.007 and 0.2 μM, respectively) and only moderately toxic to leukemic cells. Disubstituted amines **4i**−**l** showed little activity.

The two most potent and selective compounds in this series, **4b,h**, were evaluated for their ability to inhibit bone marrow progenitor stem cell growth.[28] Compound **4b** was a potent inhibitor of colony-forming unit granulocyte macrophage (CFU-GM) and burst-forming unit erythroid (BFU-E) colonies (CC_{50} = 0.16 and 0.8 μM, respectively). Compound **4h** was significantly less toxic than **4b** in this assay (CC_{50} = 1.0 and 10 for CFU-GM and BFU-E colonies, respectively).

Compounds **4b,h** are novel compounds active against two therapeutically important targets, HCMV and HBV. The high level of potency and selectivity observed with **4h** *in vitro* prompted us to evaluate the *in vivo* toxicity of this analogue. Compound **4h** was given orally once daily at a dose of 10 mg/kg to a beagle dog in a 30-day toxicity model. The study had to be terminated on day 18 and the dog euthanized due to the toxicity of **4h**. Clinical signs of toxicity first appeared on day 14 and included dry mucous membranes inside the mouth, soft stool, ataxia, decreased activity, slight dehydration, pale gingiva, and ulcers inside the mouth. Serum biochemical tests indicated severe kidney and liver toxicity beginning on dose day 11. The kidney and liver toxicity was confirmed histopathologically at the termination of the study. In addition, there was cell maturation arrest in many organs. Except for signs of hemoconcentration, there was no indication of hematological toxicity. The toxicities noted with **4h** limit its utility as an antiviral agent.

Experimental Section

General. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points (Pyrex capillary) are uncorrected. Concentrations for rotation data are given as g/100 mL of solvent, and all optical rotations were run at c = 0.5, DMF. ^{1}H NMR spectra were recorded on a Varian XL-300 spectrometer in DMSO-d_6. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane; coupling constants are expressed in hertz. UV data are reported as nm (ϵ (M^{-1} cm^{-1})). Modified purine bases which were not commercially available were prepared by displacement of the chlorine in 2-amino-6-chloropurine with an appropriate nucleophile according to literature procedures.[21] *trans*-*N*-Deoxyribosylase (E.C. 2.4.2.6)

was purified from *E. coli*.[29,30] Calf intestinal adenosine deaminase (E.C. 3.5.4.4; ADA) was purchased from Boehringer Mannheim. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, GA.

General Procedure for the Enzymatic Preparation of 2′-Deoxy-4′-thio Purine Nucleosides. Citrate buffer was prepared by adding citric acid (9.46 g, 45 mmol) to deionized H_2O (900 mL) and adjusting the pH to 6.0 with NaOH. An appropriately substituted purine base (0.9 mmol) was added to the citrate buffer solution. A 3:2 α:β mixture of 2′-deoxy-4′-thiouridine (**3**) was added to give a concentration of 5 mM in β-anomer. Homogeneity was achieved by sonicating the mixture in a 50 °C water bath. *trans*-*N*-Deoxyribosylase (2050 units/mL) was added to a final concentration of 5 units of enzyme/mL of reaction mixture. The reaction mixture was maintained at 50 °C. Every day for 4 days, an equivalent portion of purine base was added to the reaction mixture. After 5 days, the enzyme was removed by ultrafiltration. The water was removed by lyophilization. The resulting white powdery residue was slurried with methanol (50 mL) and filtered. The solid was rinsed thoroughly with CH_3OH (3 × 100 mL, or until no substantial UV absorbance was present in the filtrate). The combined filtrates were slurried with Dowex AG-1 (HO^- form) resin (200 mL) and filtered. The resin was rinsed with CH_3OH until no UV activity was present in the filtrate. The solvent was removed with a rotary evaporator. The sticky residue was dissolved in CH_3OH (100 mL), and silica gel (20 mL) was added. The solvent was removed with a rotary evaporator, and the residue was applied to the top of a 5 × 20 cm flash chromatography column packed with 95:5 CH_2Cl_2:CH_3OH. Elution with the same solvent followed by concentration with a rotary evaporator and lyophilization from H_2O gave the target nucleosides as white powders.

2-Amino-6-chloro-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-9*H*-purine (4a): yield 0.65 g (48%); mp 145 °C dec; $[\alpha]^{20}_D = -65.6°$. ^{1}H NMR: δ 2.33−2.41 (m, 1), 2.57−2.65 (m, 1), 3.34−3.37 (m, 1), 3.55−3.61 (m, 1), 3.69−3.77 (m, 1), 4.44−4.49 (m, 1), 5.13 (t, 1, J = 5.5), 5.32 (d, 1, J = 3.9), 6.12 (dd, 1, J = 6.6, 7.5), 6.99 (s, 2), 8.46 (s, 1). UV: pH = 7 λ_{max} 309 (7400), λ_{min} 268 (950); pH = 13 λ_{max} 308 (7800), λ_{min} 268 (1200). Anal. ($C_{10}H_{12}N_5O_2ClS$) C, H, N, Cl, S.

2-Amino-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-6-methoxy-9*H*-purine (4b): yield 0.47 g (34%); mp 114−116 °C. ^{1}H NMR: δ 2.31−2.38 (m, 1), 2.51−2.58 (m, 1), 3.28−3.36 (m, 1), 3.54−3.60 (m, 1), 3.67−3.73 (m, 1), 3.95 (s, 3), 4.46 (t, 1, J = 3.1), 5.13 (t, 1, J = 5.5), 5.30 (d, 1, J = 3.9), 6.13 (t, 1, J = 7.2), 6.50 (s, 2), 8.19 (s, 1). UV: pH = 7 λ_{max} 281 (9800), 251 (8500), λ_{min} 262 (5800); pH = 13 λ_{max} 281 (10 000), 251 (8400), λ_{min} 262 (5800). Anal. ($C_{11}H_{15}O_3N_5S\cdot0.5H_2O$) C, H, N, S.

2-Amino-6-(cyclopropylmethoxy)-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-9*H*-purine (4c): yield 0.080 g (5.2%); mp 183−184 °C. ^{1}H NMR: δ 0.32−0.37 (m, 2), 0.54−0.60 (m, 2), 1.26−1.31 (m, 1), 2.31−2.37 (m, 1), 2.51−2.61 (m, 1), 3.28−3.36 (m, 1), 3.52−3.59 (m, 1), 3.67−3.73 (m, 1), 4.23 (d, 2, J = 7.2), 4.46 (t, 1, J = 3.3), 5.3 (t, 1, J = 5.5), 5.29 (br s, 1), 6.12 (dd, 1, J = 6.5, 7.8), 6.44 (br s, 2), 8.19 (s, 1). UV: pH = 13 λ_{max} 282 (9400), 252 (7700), λ_{min} 262 (5300), 231 (4600). Anal. ($C_{14}H_{19}N_5O_3S$) C, H, N, S.

2-Amino-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-6-(methylthio)-9*H*-purine (4d): yield 0.16 g (11%); mp 197−199 °C; $[\alpha]^{20}_D = -94.4°$. ^{1}H NMR: δ 2.31−2.39 (m, 1), 2.57−2.64 (m, 1), 2.60 (s, 3), 3.30−3.37 (m, 1), 3.53−3.60 (m, 1), 3.69−3.75 (m, 1), 4.45−4.48 (m, 1), 5.13 (t, 1, J = 5.5), 5.31 (d, 1, J = 3.7), 6.13 (t, 1, J = 7.1), 6.58 (s, 2), 8.26 (s, 1). UV: pH = 7 λ_{max} 311 (13 500), λ_{min} 277 (2600); pH = 13 λ_{max} 311 (12 900), λ_{min} 277 (2100). Anal. ($C_{11}H_{15}N_5S_2O_2\cdot0.5H_2O$) C, H, N, S.

2-Amino-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-6-(propylamino)-9*H*-purine (4e): yield 0.53 g (35%); mp 74−78 °C; $[\alpha]^{20}_D = -74.0°$. ^{1}H NMR: δ 0.87 (t, 3, J = 7.3), 1.50−1.61 (m, 2), 2.28−2.34 (m, 1), 2.52−2.55 (m, 1), 3.30−3.38 (m, 1), 3.51−3.59 (m, 1), 3.66−3.72 (m, 1), 4.44−4.46 (m, 1), 5.14 (t, 1, J = 5.5), 5.26 (d, 1, J = 3.7), 5.85 (br s, 2), 6.09 (dd, 1, J = 6.3, 8.0), 7.20−7.30 (br s, 1), 8.00 (s, 1). UV: pH = 7 λ_{max} 282 (14 200), λ_{min} 243 (5300); pH = 13 λ_{max} 282 (15 000), λ_{min} 243 (5800). Anal. ($C_{13}H_{20}N_6O_2S\cdot0.4H_2O\cdot0.1EtOH$) C, H, N, S.

6-(Allylamino)-2-amino-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-9*H*-purine (4f): yield 0.073 g (6.6%); mp 171−172 °C. ^{1}H NMR: δ 2.31−2.33 (m, 1), 2.51−2.56 (m, 1), 3.30−3.34 (m, 1), 3.54−3.59 (m, 1), 3.67−3.72 (m, 1), 4.04−4.08 (br s, 2), 4.46 (t, 1, J = 3.4), 5.02 (dd, 1, J = 10.2, 1.8), 5.11 (dd, 1, J = 8.3, 1.8), 5.15 (d, 1, J = 5.2), 5.27 (d, 1, J = 3.7), 5.86−5.97 (m, 1), 5.89 (s, 2), 6.10 (dd, 1, J = 6.3, 8.0), 7.30−7.50 (br s, 1), 8.02 (s, 1). UV: pH = 7 λ_{max} 283 (14 200), λ_{min} 243 (5400); pH = 13 λ_{max} 283 (13 900), λ_{min} 243 (5500). Anal. ($C_{13}H_{18}N_6O_2S$) C, H, N, S.

2-Amino-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-6-(isopropylamino)-9*H*-purine (4g): yield 0.56 g (35%); mp 81−85 °C; $[\alpha]^{20}_D = -21.8°$. ^{1}H NMR: δ 1.16 (d, 6, J = 6.5), 2.26−2.34 (m, 1), 2.52−2.58 (m, 1), 3.34−3.35 (m, 2), 3.51−3.59 (m, 1), 3.67−3.73 (m, 1), 4.43−4.48 (m, 1), 5.14 (t, 1, J = 5.6), 5.26 (d, 1, J = 3.7), 5.84 (br s, 2), 6.10 (dd, 1, J = 6.3, 6.4), 6.94−6.98 (br s, 1), 8.00 (s, 1). UV: pH = 7 λ_{max} 283 (15 600), λ_{min} 244 (6400); pH = 13 λ_{max} 283 (16 000), λ_{min} 244 (6300). Anal. ($C_{13}H_{20}N_6O_2S\cdot0.5H_2O\cdot0.5EtOH$) C, H, N, S.

2-Amino-6-(cyclopropylamino)-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-9*H*-purine (4h): yield 0.35 g (23%); mp 98−126 °C; $[\alpha]^{20}_D = -73.8°$. ^{1}H NMR: δ 0.54−0.68 (m, 4), 2.27−2.35 (m, 1), 2.50−2.60 (m, 1), 3.01 (br s, 1), 3.31 (m, 1), 3.51−3.59 (m, 1), 3.67−3.73 (m, 1), 4.46 (t, 1, J = 3.3), 5.15 (t, 1, J = 5.5), 5.27 (d, 1, J = 3.8), 5.90 (s, 2), 6.10 (dd, 1, J = 6.5, 7.9), 7.36 (d, 1, J = 2.3), 8.01 (s, 1). UV: pH = 7 λ_{max} 285 (15 200), λ_{min} 246 (6400); pH = 13 λ_{max} 297 (14 200), λ_{min} 274 (7200). Anal. ($C_{13}H_{18}N_6O_2S\cdot0.5H_2O$) C, H, N, S.

2-Amino-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-6-(ethylmethylamino)-9*H*-purine (4i): yield 0.59 g (39%); mp 78−82 °C; $[\alpha]^{20}_D = -84.0°$. ^{1}H NMR: δ 1.12 (t, 3, J = 7.0), 2.31−2.35 (m, 1), 2.50−2.53 (m, 1), 3.25−3.31 (br s, 1), 3.33 (s, 3), 3.53−3.60 (m, 11), 3.66−3.71 (m, 1), 3.94−3.98 (m, 1), 4.44−4.47 (m, 1), 5.14 (t, 1, J = 5.5), 5.27 (d, 1, J = 3.8), 5.86 (s, 2), 6.11 (dd, 1, J = 6.3, 8.0), 8.03 (s, 1). UV: pH = 7 λ_{max} 285 (16 100), λ_{min} 249 (6900); pH = 13 λ_{max} 286 (15 900), λ_{min} 250 (7000). Anal. ($C_{13}H_{20}N_6O_2S\cdot0.5H_2O\cdot0.1EtOH$) C, H, N, S.

2-Amino-6-(cyclopropylmethylamino)-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-9*H*-purine (4j): yield 0.24 g (15%); mp 72−76 °C; $[\alpha]^{20}_D = -78.0°$. ^{1}H NMR: δ 0.65−0.70 (m, 2), 0.78−0.84 (m, 2), 2.27−2.35 (m, 1), 2.51−2.58 (m, 1), 3.18−3.31 (m, 1), 3.33 (s, 3), 3.34−3.36 (m, 1), 3.51−3.59 (m, 1), 3.66−3.74 (m, 1), 4.44−4.47 (m, 1), 5.14 (t, 1, J = 5.5), 5.27 (d, 1, J = 3.7), 5.89 (s, 2), 6.13 (dd, 1, J = 6.5, 7.9), 8.04 (s, 1). UV: pH = 7 λ_{max} 288 (16 500), λ_{min} 251 (8200); pH = 13 λ_{max} 288 (16 100), λ_{min} 251 (7900). Anal. ($C_{14}H_{20}N_6O_2S\cdot0.9H_2O\cdot0.1EtOH$) C, H, N, S.

2-Amino-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-6-piperidino-9*H*-purine (4k): yield 0.40 g (24%); mp 85−90 °C; $[\alpha]^{20}_D = -75.0°$. ^{1}H NMR: δ 1.52−1.53 (br s, 4), 1.63−1.65 (br s, 2), 2.27−2.35 (m, 1), 2.53−2.57 (m, 1), 3.28−3.30 (m, 1), 4.02−4.15 (br s, 2), 4.42−4.46 (m, 1), 5.14 (t, 1, J = 5.5), 5.27 (d, 1, J = 3.7), 5.89 (s, 2), 6.11 (dd, 1, J = 6.5, 7.8), 8.03 (s, 1). UV: pH = 7 λ_{max} 289 (21 800), λ_{min} 252 (9300); pH = 13 λ_{max} 289 (22 100), λ_{min} 252 (9400). Anal. ($C_{15}H_{22}N_6O_2S\cdot0.5H_2O\cdot0.3EtOH$) C, H, N, S.

2-Amino-9-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-6-(1-pyrrolidinyl)-9*H*-purine (4l): yield 0.47 g (30%); mp 113−117 °C; $[\alpha]^{20}_D = -79.0°$. ^{1}H NMR: δ 1.90 (br s, 4), 2.27−2.35 (m, 1), 2.52−2.54 (m, 1), 3.31−3.32 (m, 1), 3.52−3.60 (m, 1), 3.67−3.69 (m, 1), 3.71−4.02 (br s, 4), 4.46 (t, 1, J = 3.4), 5.15 (t, 1, J = 5.5), 5.28 (d, 1, J = 3.7), 5.86 (s, 2), 6.12 (dd, 1, J = 6.5, 7.9), 8.01 (s, 1). UV: pH = 7 λ_{max} 285 (16 700), λ_{min} 249 (6700); pH = 13 λ_{max} 285 (16 500), λ_{min} 249 (6700). Anal. ($C_{14}H_{20}N_6O_2S\cdot1.0H_2O$) C, H, N.

2′-Deoxy-4′-thioguanosine (5). To a solution of **4b** (0.40 g, 1.3 mmol) in 1 M pH 7.4 potassium phosphate buffer (10 mL) in a 250-mL Erlenmeyer flask was added H_2O (180 mL) and adenosine deaminase (100 μL of 10 mg/mL suspension). The flask was covered and the mixture allowed to stir slowly at 37 °C overnight. The H_2O was removed with a rotary evaporator. The product was dissolved in CH_3OH and silica

gel (10 mL) added. The solvent was removed with a rotary evaporator, and the residue was applied to a 2.5 × 20 cm flash chromatography column packed with 85:15 CH_2Cl_2:CH_3OH. Gradient elution with 85:15−80:20 CH_2Cl_2:CH_3OH followed by concentration with a rotary evaporator gave 2′-deoxy-4′-thioguanosine (**5**; 0.33 g, 87%): mp 235−245 °C dec; $[\alpha]^{20}_D$ = −76.4°. ^{1}H NMR: δ 2.26−2.33 (m, 1), 2.45−2.53 (m, 1), 3.52−3.56 (m, 1), 3.63−3.71 (m, 1), 4.42 (t, 1, J = 3.6), 5.10 (t, 1, J = 5.5), 5.27 (d, 1, J = 3.9), 6.00 (dd, 1, J = 6.6, 7.9), 6.48 (br s, 2), 8.00 (s, 1), 10.59 (br s, 1). UV: pH = 7 λ_{max} 256 (14 200), λ_{min} 228 (4500); pH = 13 λ_{max} 268 (12 000), λ_{min} 232 (3800). Anal. ($C_{10}H_{13}N_5O_3S$) C, H, N, S.

Determination of Anti-HCMV Activity. HCMV strain AD169 was grown on monolayers of human embryonic lung cells (MRC5 cells) in 96-well plates. After infection of the cells at a ratio of ca. 0.01 infectious virus particles/cell, the compounds to be tested were added to selected wells at six different concentrations, each in triplicate. The same concentrations of compound were also applied to wells containing monolayers of uninfected cells in order to assess compound cytotoxicity. The plates were incubated for 5 days, and the minimum cytotoxic dose was estimated from microscopic examination. The IC_{50} for antiviral effect was estimated from measurements of HCMV DNA in each well by blotting and quantitative specific DNA hybridization, similar to the method of Gadlen.[23] The values for IC_{50} reported are the mean of at least two such determinations carried out at different times.

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