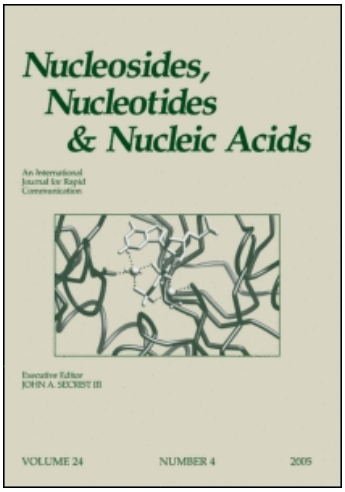

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 4′-THIO-L-XYLOFURANOSYL PURINE NUCLEOSIDES

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□ *A series of some new 4′-thio-L-xylofuranosyl nucleosides were prepared and evaluated as potential anticancer agents. A versatile sugar intermediate for direct coupling with the purine moiety is also synthesized by an efficient and high-yielding route. Proof of structure and configuration at all chiral centers of the nucleosides was obtained by proton NMR. All target compounds were evaluated in a series of human cancer cell lines in vitro. The details of the synthesis of the carbohydrate precursor 1-O-acetyl-2,3,5-tri-O-benzyl-4-thio-L-xylofuranose (***6***) and corresponding purine nucleosides are presented in the manuscript.*

Keywords Nucleosides; 4′-Thionucleosides; Purines; Antiviral activity

INTRODUCTION

In our ongoing initiative to develop novel anticancer agents, we have pursued the synthesis of many different types of nucleosides. These efforts have resulted in the development of fludarabine,[1,2] which received FDA approval over a decade ago, and clofarabine,[3–8] which received initial approval by the FDA at the end of 2004 for the treatment of pediatric acute lymphoblastic leukemia (ALL). Other nucleosides used regularly in cancer chemotherapy include gemcitabine, cladribine, pentostatin, capecitabine, and cytarabine.[9] For gemcitabine, the utility extends beyond hematologic malignancies to include some solid tumor indications. There continues to be a need for new agents, and nucleosides continue to show promise;[10]

Dedicated to the memory of John A. Montgomery.

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they also have the advantage of having the potential for multiple sites of action as well as activity against leukemias and lymphomas as well as solid tumors. For some years we have focused our attention on 4′-thionucleosides, which have structural similarities to natural nucleosides such that many of them function well as both substrates and inhibitors for enzymes on the nucleic acid biosynthetic pathways.[11] In particular, we focused initially on 4′-thio-ara-C,[12–17] which had been made previously[18] but had not been fully explored because of the synthetic challenge it presented. Suitable routes have been developed to prepare it efficiently, and it has been found to have significant activity and selectivity in a wide range of human tumor xenograft models.[12] In addition, we have studied its mechanism of action extensively in our laboratories.[19–23] On the basis of all of this research, 4′-thio-ara-C was moved into Phase I clinical trials.[18] Based upon the activity of this compound as well as 2′-deoxy[24] and other arabino 4′-thionucleosides,[25,26] we have continued to explore other types of 4′-thionucleosides. Herein we describe the preparation of a series of new purine 4′-thio-L-xylonucleosides as well as certain aspects of their biological activity. This series was prepared as a part of our program to explore the scope and limitations of activation of nucleosides by the enzyme deoxycytidine kinase as well as other kinases. Deoxycytidine kinase serves as the initial metabolic enzyme for many of the approved nucleoside anticancer agents, including both purine and pyrimidine nucleosides, as well as for 4′-thio-ara-C. Based upon our previous work in other 4′-thionucleoside series, we have developed an efficient route for the synthesis of the requisite sugar intermediate needed to make these kinds of nucleosides. All of the new compounds have been evaluated for their cytotoxicity in a panel of human tumor cell lines, and also against certain viruses.

CHEMISTRY

Our goal in this L-xylofuranose series, as with other 4′-thionucleoside analogs, was to prepare a series of compounds that included natural and modified purines chosen on the basis of biological evaluations within the series and biological information from our laboratories and others suggesting compounds with desirable properties. Thus, we had need of a versatile carbohydrate precursor for nucleoside synthesis in this series that would be readily accessible and that would give acceptable yields of both nucleoside anomers, because we often examine both the isomers if they are readily available in sufficient quantity. In this particular series examination of both anomers is of particular relevance if possible because of the change of configuration of both C-2′ and C-4′ from the standard nucleoside building blocks.

Employing a synthetic strategy similar to those we have previously used for other 4-thiofuranose precursors,[25–27] we have developed a five step

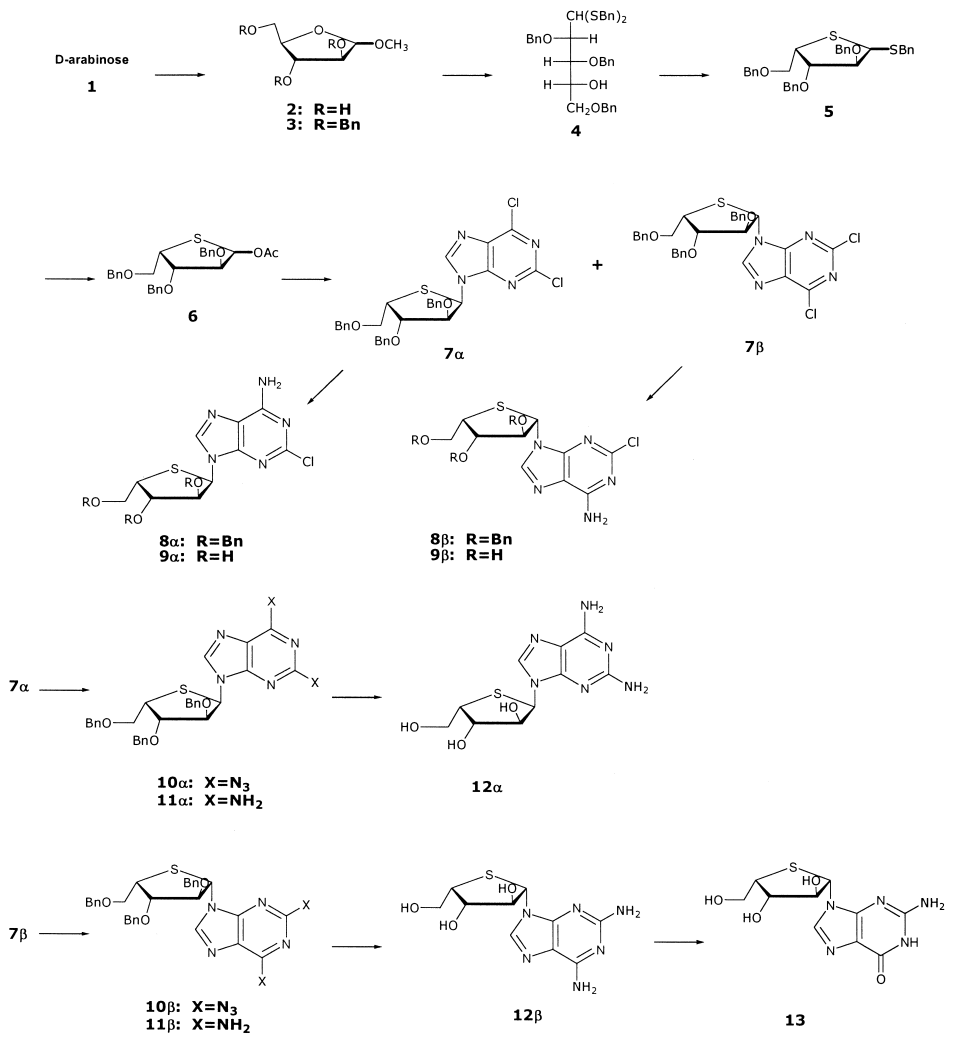

FIGURE 1

sequence to the key intermediate **6** (Figure 1). Conversion of D-arabinose to methyl 2,3,5-tri-*O*-benzyl-D-arabinofuranoside **(3)** was accomplished in two steps by the usual method. Conversion to dibenzyl dithioacetal **4** employing benzyl mercaptan and stannic chloride proceeded in 48% yield after chromatographic purification. Cyclization at C-4 involving a single inversion,[27] thus converting the D-*arabino* to the L-*xylo* configuration, was accomplished employing triphenylphosphine, iodine, and imidazole in 60% yield. The final step, replacement of the benzylthio group at C-1 by an acetoxy group, involved treatment of **5** with mercuric acetate in acetic acid

at room temperature. The overall yield of **6** from **1**, including four column purifications, was 25%, and afforded a ca. 1:1 mixture of α,β anomers.

A series of purine nucleoside analogs were prepared through the coupling of **6** and 2,6-dichloropurine. A Lewis acid catalyzed reaction utilizing stannic chloride in acetonitrile was found to be an efficient method to achieve this coupling, and 30% and 25% yields of α and β anomers of **7** were obtained after chromatographic purification/separation. After treatment with ethanolic ammonia to produce the respective blocked 2-chloroadenine nucleosides **8α** and **8β**, removal of the *O*-benzyl groups was accomplished with boron trichloride in dichloromethane at −50°C to yield the final nucleoside targets **9α** (55%) and **9β** (45%). Treatment of **7α** and **7β** with sodium azide in 95% aqueous ethanol at reflux produced the corresponding 2,6-diazido intermediates **10α** and **10β**, which were subjected to reduction with stannous chloride in dichloromethane to afford the blocked diaminopurine nucleosides **11α** (80%) and **11β** (82%) respectively. Deblocking of **11α** and **11β** with boron trichloride in dichloromethane produced the target diamino nucleosides **12α** (71%) and **12β** (75%), respectively. The conversion of **12β** to the corresponding guanine nucleoside **13** (45%) was accomplished by treatment with adenosine deaminase under standard conditions. The deamination was slow, requiring 72 h at room temperature for completion, even with a high concentration of enzyme present. On the other hand, 9-(4-thio-α-L-xylofuranosyl)-9*H*-purine-2,6-diamine (**12α**) was resistant to deamination when treated with adenosine deaminase for several days.

BIOLOGICAL DATA

The cell culture cytotoxicity of all five target compounds (**9α**, **9β**, **12α**, **12β**, and **13**) was determined against our standard panel of human cancer cell lines, which includes CCRF-CEM (leukemia), CAKI-1 (renal), DLD-1 (colon), NCI-H23 (lung), SK-MEL-28 (melanoma), and SNB-7 (CNS) cells. No significant cytotoxicity was seen for any of the target compounds at concentrations up to 100 μM.

Compounds that do not show significant cytotoxicity in our panel of human cancer cell lines are typically evaluated in a series of antiviral assays. In this case, the same five compounds were examined in standard assays for herpes simplex type 1 (HSV-1), human cytomegalovirus AD169 (HCMV), respiratory syncytial virus (RSV), influenza A/PR/8, and human herpes virus type 8 (HHV8). Activity was seen in only two of these viruses, HCMV and HHV8. Data on active compounds only are presented in Table 1. It should be noted that **12α** has no detectable amount of **12β** in it, while **12β** has 1% of **12α** in it. Given that **12α** is only tenfold more potent than **12β**, the amount of **12α** present as a minor impurity does not account for all of the antiviral activity of **12β**.

TABLE 1 Antiviral Data

Compound	Virus[a,b]	IC50 (μM)	TC50 (μM)
Ganciclovir	HCMV	8.0	>10
9β	HCMV	0.17	>10
12α	HCMV	0.051	>10
12β	HCMV	0.49	>10
Cidofovir	HHV8	1.8	>25
13	HHV8	2.5	>10

[a]HCMV was grown in MRC-5 cells.
[b]HHV8 was grown in TPA-induced BCBL-1 cells.

EXPERIMENTAL SECTION

Melting points were determined on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Nicolet NT-300 NB spectrometer operating at 300.635 MHz (^{1}H). Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Decoupling methodology and NOE experiments were utilized to establish the carbohydrate configurations as well as the anomeric configuration. The NOE experiments were conducted on a degassed solution of DMSO-d_6. To minimize the effects of magnetic perturbations with the sample non-spinning, eight FIDs were acquired with the decoupler set to a desired frequency and eight FIDs were recorded with the decoupler off resonance. The process was repeated until 800 FIDs had been acquired. UV absorption spectra were determined on a Perkin-Elmer Lambda 9 spectrometer by dissolving each compound in methanol or water and diluting 10-fold with 0.1 N HCl, pH 7 buffer, or 0.1 N NaOH. Numbers in parentheses are extinction coefficients ($\times 10^{-3}$), sh = shoulder. Microanalyses were performed by Atlantic Microlab, Inc. (Atlanta, Georgia) or the Molecular Spectroscopy Section of Southern Research Institute. Analytical results indicated by elemental symbols were within $\pm 0.4\%$ of the theoretical values. Mass spectra were recorded on a Varian/MAT 311A double-focusing mass spectrometer in the fast atom bombardment (FAB) mode. Routine HPLC analyses were carried out on a Hewlett-Packard HP1084B liquid chromatograph with a Waters Associates μBondapak C_{18} column (3.9 mm × 30 cm) and UV monitoring (254 nm). HPLC purity determinations were carried out on a Sphereclone C_{18} 5 μ column using 0.01M $NH_4H_2PO_4$ (pH 5.1): CH_3OH in a 20 min linear gradient with a flow rate of 1.0 mL/min. All preparative chromatographic separations were carried out by flash chromatography using 230–400 mesh silica gel from E. Merck. TLC was carried out on Analtech precoated (250 μm) silica gel (GF) plates.

2,3,5-Tri-*O*-benzyl-D-arabinose Dibenzyl Dithioacetal (4). D-Arabinose (**1**, 25 g, 167 mmol) was stirred for 5 h in 0.5% hydrogen chloride in methanol (675 mL) at room temperature and then neutralized with

Amberlite IRA-400 OH anion exchange resin. The filtrate and washings were combined and evaporated to dryness and the crude product was purified by silica gel chromatography ($CHCl_3$/MeOH, 92:8) to afford 26.0 g of methyl D-arabinofuranoside (**2**, 94% yield) as α/β (1:1) mixture. MS 164 $(M)^+$.

To an ice-cold solution of **2** (20 g, 120.8 mmol) in dry tetrahydrofuran (500 mL) was added sodium hydride (60% dispersion in mineral oil, 29.6 g, 720 mmol) and the reaction mixture was stirred for 15 min under N_2. To this reaction mixture was added solid tetrabutylammonium iodide (0.72 g, 1.92 mmol) followed by a drop wise addition of benzyl bromide (73.2 g, 418 mmol). The reaction mixture was stirred for 3 days at room temperature. After the addition of methanol (50 mL) the solution was evaporated under reduced pressure, and the crude product was purified by silica gel chromatography (cyclohexane/EtOAc, 9:1) to afford pure methyl 2,3,5-tri-O-benzyl-D-arabinofuranoside (**3**, 44 g, 83% yield). MS 435 $(M+H)^+$. ^1H NMR ($CDCl_3$) 7.26–7.18 (m, 30H, aromatic *H*s), 4.92 (d, 1H, H-1, $J_{1,2}$ = 4.3 Hz), 4.82 (d, 1H, H-1, $J_{1,2} = 0.9$ Hz), 4.60–4.14 (m, 10H, PhC*H*$_2$s), 4.31 (m, 1H, H-4), 4.10 (dt, 1H, H-4, $J_{4,5a} = 3.7$ Hz, $J_{4,5b} = 6.5$ Hz, $J_{3,4} = 6.2$ Hz), 3.98 (t, 1H, H-3, $J_{3,4} = 6.9$ Hz, $J_{2,3} = 5.6$ Hz), 3.95 (dd, 1H, H-3, $J_{3,4}$ = 6.2 Hz, $J_{2,3} = 2.5$ Hz), 3.90 (dd, 1H, H-2, $J_{2,3} = 5.6$ Hz), 3.86 (t, 1H, H-2, $J_{2,3} = 2.5$ Hz), 3.63 (dd, 1H, H-5a, $J_{4,5a} = 4.5$ Hz, $J_{5a,5b} = 10.4$ Hz), 3.61 (dd, 1H, H-5a, $J_{4,5a} = 3.7$ Hz, $J_{5a,5b}$ =10.7 Hz), 3.54 (dd, 1H, H-5b, $J_{4,5b}$ = 7.5 Hz), 3.40 (dd, 1H, H-5b, $J_{4,5b} = 6.5$ Hz).

To a solution of **3** (42 g, 97 mmol) in dichloromethane (1000 mL) were added benzyl mercaptan (49.6 g, 400 mmol) and stannic chloride (4.93 g, 18.9 mmol), and the reaction mixture was stirred at room temperature overnight. After neutralization with 5% aqueous $NaHCO_3$ (750 mL), the organic layer was separated and the aqueous layer was extracted with dichloromethane (500 mL). The combined organic layers were evaporated, and crude **4** was purified by silica gel chromatography (cyclohexane/EtOAc, 99:1) to afford **4** (7.0 g, 48%) of sufficient purity to carry forward. MS 657 $(M+Li)^+$. ^1H NMR ($CDCl_3$) 7.28–7.25 (m, 19H, aromatic *H*s), 7.09–7.02 (m, 4H, aromatic *H*s), 6.99–6.96 (m, 2H aromatic *H*s), 4.78 (d, 1H, PhCH*H*, J = 11.1 Hz), 4.68 (two overlapping ds, 2H, PhC*H*H, PhCH*H*, $J = 11.1$ Hz, J = 11.2 Hz), 4.36 (d, 1H, PhC*H*H, 11.2 Hz), 4.31 (d, 1H, PhCH*H*, $J = 11.9$ Hz), 4.28 (d, 1H, PhC*H*H, $J = 11.9$ Hz), 4.02 (dd, 1H, H-2, $J_{1,2} = 3.0$ Hz, $J_{2,3} = 7.5$ Hz), 3.68–3.60 (m, 4H, two PhC*H*$_2$s), 3.56 (d, 1H, H-1, $J_{1,2} = 3.0$ Hz), 3.30–3.22 (m, 2H, H-4, H-5a), 3.12–3.08 (m, 1H, H-5b), 2.08 (d, 1H, 4-OH, $J = 6.2$ Hz).

2,3,5-Tri-*O*-benzyl-1-*O*-acetyl-4-thio-L-xylofuranose (6). To a solution of **4** (6.5 g, 10 mmol) in dry 2:1 toluene/acetonitrile (150 mL) were added triphenylphosphine (7.85 g, 30 mmol), iodine (6.35 g, 25 mmol) and

imidazole (2.72 g, 40 mmol). The reaction mixture was stirred at 90°C for 24 h, after which time the solution was evaporated to dryness. The crude product was purified by silica gel chromatography (cyclohexane/EtOAc, 4:1) to afford benzyl 2,3,5-tri-O-benzyl-1,4-dithio-L-xylofuranoside as a syrup (**5**, 3.75 g, 60%). MS 543 $(M+H)^+$. ^1H NMR ($CDCl_3$) 7.32–7.14 (m, 20H, aromatic *H*s), 4.62–4.39 (m, 6H, three PhC*H*$_2$Os), 4.32 (m, 1H, H-1), 4.14 (m, 2H, H-2, H-3), 3.81 (s, 2H, PhC*H*$_2$S-), 3.73 (dd, 1H, H-5a, $J_{4,5a} = 7.4$ Hz, $J_{5a,5b} = 9.3$ Hz), 3.51 (dd, 1H, H-5b, $J_{4,5b} = 7.1$ Hz), 3.40 (m, 1H, H-4).

To a suspension of mercuric acetate (4.64 g, 11.45 mmol) in acetic acid (50 g) was added **5** (2.71 g, 5 mmol), and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with dichloromethane (150 mL) and washed successively with water, saturated aqueous $NaHCO_3$ and 5% aqueous KCN solution. The organic layer was dried over Na_2SO_4 and concentrated. Chromatography of the crude product using cyclohexane:ethyl acetate (98:2) as eluent gave a mixture of and (1:1) anomers of **6** (1.85 g, 77%) as a colorless syrup. MS 479 $(M+H)^+$. ^1H NMR ($CDCl_3$) 7.31–7.20 (m, 15H, aromatic *H*s), 6.03 (d, 0.25H, H-1, $J_{1,2} = 4.0$ Hz), 5.95 (d, 0.75H, H-1, $J_{1,2} = 2.8$ Hz), 4.78–4.40 (m, 6H, PhC*H*$_2$s), 4.21 (dd, 0.75H, H-2, $J_{2,3} = 5.4$ Hz), 4.10–4.06 (m, 0.5H, H-2, H-3), 4.00 (t, 0.75H, H-3, $J_{3,4} = 6$ Hz), 3.72–3.65 (m, 1.25H, H-4, H-5a, H-5a), 3.49–3.36 (m, 1.75H, H-5b, H-4, H-5b), 2.05 (s, 3H, CH3- and CH3-).

9-(2,3,5-Tri-*O*-benzyl-4-thio-α and -β-L-Xylofuranosyl)-2,6-dichloropurine (7α and 7β). To a stirred mixture of **6** (0.956 g, 2 mmol) and 2,6-dichloropurine (0.568 g, 3 mmol) in acetonitrile (50 mL) at room temperature was added a solution of stannic chloride in dichloromethane (3 mL of 1.0 M) over 1 min and stirring was continued for 2 h. The reaction mixture was quenched by pouring it into a mixture of 50 mL of dichloromethane and 25 mL of saturated $NaHCO_3$. The organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by silica gel chromatography (cyclohexane/EtOAc, 9:1) to afford **7α** (364 mg, 30%) eluting first followed by **7β** (291 mg, 25%).

Compound **7α**: MS 608 $(M+H)^{+,1}H$ NMR ($CDCl_3$) 8.88 (s, 1H, H-8) 7.30–7.14 (m, 13H, aromatic *H*s), 6.92 (m, 2H, aromatic *H*s), 6.18 (d, 1H, H-1′, $J_{1,2} = 4.9$ Hz), 4.65 (d, 1H, PhC*H*H, $J = 11.6$ Hz), 4.52 (s, 2H, PhC*H*$_{2-}$), 4.48 (s, 2H, PhC*H*$_{2-}$), 4.44 (d, 1H, PhCH*H*-, $J = 11.6$ Hz), 4.32–4.23 (m, 2H, H-2′, H-3′), 3.65 (dd, 1H, H-5′ a, $J_{4,5a} = 4.6$ Hz, $J_{5a,5b} = 10.0$ Hz), 3.61 (dd, 1H, H-5′ b, $J_{4,5b} = 4.8$ Hz), 3.49 (m, 1H, H-4′, $J_{3,4} = 5.0$ Hz).

Compound **7β**: MS 608 $(M+H)^{+1}H$ NMR ($CDCl_3$) 8.46 (s, 1H, H-8), 7.32–7.19 (m, 13H, aromatic *H*s), 7.00 (m, 2H, aromatic *H*s), 6.05 (d, 1H, H-1′, $J_{1,2} = 2.1$ Hz), 4.67 (s, 2H, PhC*H*$_{2-}$), 4.56 (d, 1H, PhC*H*H-, $J = 12.0$ Hz), 4.48 (d, 1H, PhC*H*H-, $J = 12.0$ Hz), 4.46 (d, 1H, PhCH*H*-, $J = 11.9$ Hz), 4.34 (d, 1H, PhCH*H*-, $J = 11.9$ Hz), 4.24 (t, 1H, H-2′, $J_{1,2} = 2.1$ Hz,

$J_{2,3}$ = 2.5 Hz), 4.20 (t, 1H, H-3′, $J_{3,4}$ = 2.5 Hz), 4.05 (m, 1H, H-4′), 3.80 (dd, 1H, H-5′ a, $J_{3,4}$ = 8.2 Hz, $J_{5a,5b}$ = 9.5 Hz), 3.54 (dd, 1H, H-5′ b, $J_{4,5b}$ = 6.7 Hz.

2-Chloro-9-(4-thio-α-L-xylofuranosyl)adenine (9α). A mixture of compound **7α** (608 mg, 1 mmol) and saturated ethanolic ammonia (100 mL) was heated at 50°C in a glass-lined stainless steel pressure vessel for 48 h. The reaction mixture was evaporated to dryness to afford a solid (**8α**) that was dissolved in dichloromethane (50 mL) and was added dropwise to a solution of 1 M BCl_3 in CH_2Cl_2 (100 mL) at −50°C. Solid precipitated from the solution near the end of addition. The reaction in a tightly sealed flask was stored at −20°C for 16 h. The resulting clear solution was evaporated to dryness at −20°C to give a dark residue. A solution of this material in ice-cold CH_2Cl_2 (25 mL) was evaporated to dryness four times to provide a foam. Ice-cold saturated aqueous $NaHCO_3$ (20 mL) was added to the foam, and the mixture was stirred vigorously until the pH remained stable (pH 7–8). Water (150 mL) was added to form a clear solution that was extracted with two portions of CH_2Cl_2 (50 mL, 25 mL) to remove color and impurities. The colorless aqueous layer was held briefly under vacuum to remove residual CH_2Cl_2 before being applied to a column (13 × 190 mm) of Bio Beads SM-4 (100–200 mesh) equilibrated in water. Water elution with fractions monitored at 254 nm provided pure **9α** (175 mg, 55%);TLC, 3:1:0.1 $CHCl_3$-MeOH-NH_4OH, R_f 0.55; m.p. 140°C; MS 318 $(M+H)^+$, ^1H NMR (DMSO-d_6) 8.24 (s, 1H, H-8), 7.76 (br s, 2H, NH_2), 5.90 (d, 1H, H-1′, $J_{1,2}$ = 5.2 Hz), 5.72 (d, 1H, 2′ -OH, J = 4.3 Hz), 5.49 (d, 1H, 3′ -OH, J = 4.6 Hz), 5.15 (t, 1H, 5′ -OH, J = 5.1 Hz), 4.09-4.07 (m, 2H, H-3′, H-2′), 3.77 (dd, 1H, H-5′ a, $J_{4,5a}$ = 4.3 Hz, $J_{5a,5b}$ = 11.2 Hz), 3.73 (dd, 1H, H-5′ b, $J_{4,5b}$ = 6.5 Hz), 3.19 (mm, 1H, H-4′, $J_{3,4}$ = 6.4 Hz). HPLC purity 96.4%. HRMS calcd for $C_{10}H_{12}N_5O_3ClS$ $(M+Na)^+$: 340.0242. Found: 340.0246.

2-Chloro-9-(4-thio-β-L-xylofuranosyl)adenine (9β). This compound was prepared from **7** by the same procedure as reported for **9α** in 45% yield; TLC, 3:1:0.1 $CHCl_3$ -MeOH-NH_4OH, R_f 0.45; m.p. 235: MS 318 $(M+H)^{+1}H$ NMR (DMSO-d_6) 8.46 (s, 1H, H-8), 7.80 (br s, 2H, NH_2), 5.79 (d, 1H, 2′ -OH, J = 5.5 Hz), 5.65 (d, 1H, 3′ -OH, J = 5.0 Hz), 5.62 (d, 1H, H-1′, $J_{1,2}$ = 7.1 Hz), 4.95 (dd, 1H, 5′ -OH, $J_{5a,OH}$ = 4.6 Hz, $J_{5b,OH}$ = 6.0 Hz), 4.43 (m, 1H, H-2′, $J_{2,3}$ = 7.7 Hz), 3.87 (m, 1H, H-5′ a, $J_{4,5a}$ = 3.8 Hz, $J_{5a,5b}$ = 10.9 Hz), 3.74 (m, 1H, H-3′, $J_{3,4}$ = 8.2 Hz), 3.62 (m, 1H, H-4′), 3.42 (m, 1H, H-5′ b, $J_{4,5b}$ = 8.3 Hz). HPLC purity 97.8%. HRMS calcd for $C_{10}H_{12}N_5O_3ClS$ $(M+Na)^+$: 340.0242. Found: 340.0239.

9-(2,3,5-Tri-*O*-benzyl-4-thio-α-L-xylofuranosyl)-9H-purine-2,6-diamine (11α). A solution of **7α** (303 mg, 0.5 mmol) and sodium azide (162.5 mg,

2.5 mmol) in 20 mL of 95% ethanol was heated at reflux for 2 h. The solvent was removed in vacuo, and the residue was partitioned between dichloromethane and water. The organic phase was dried ($MgSO_4$) and concentrated in vacuo to yield 290 mg of a yellowish solid (**10α**) [TLC: $CHCl_3$/MeOH, 97:3; Rf 0.45; mass spectrum, m/z 621 $(M+H)^+$], which was redissolved in 20 mL of dichloromethane and 2 mL of methanol. This solution was treated with stannous chloride (190 mg, 1 mmol) and the resulting suspension was stirred for 30 min. After evaporation of solvent, purification was accomplished by silica gel chromatography ($CHCl_3$/MeOH, 97:3) to afford **11α** (214 mg, 75%), which was suitable for deblocking. MS 569 $(M+H)^+$. ^{1}H NMR ($CDCl_3$) 8.04 (s, 1H, H8), 7.30–7.50 (m, 13H, aromatic *H*s), 7.01 (m, 2H, aromatic *H*s), 6.44 (d, 1H, H-1′, $J_{1,2} = 5.3$ Hz), 5.36 (bs, 2H, NH_2), 4.68 (d, 1H, PhCH*H*, $J = 11.7$ Hz), 4.67 (bs, 2H, NH_2), 4.58–4.50 (m, 4H, two PhCH_2s), 4.32(dd, 1H, H-3′, $J_{3,4} = 6.3$ Hz), 4.22 (dd, 1H, H-2′, $J_{2,3} = 7.0$ Hz), 3.72–3.62 (m, 2H, H-5′ a, H-5′ b), 3.53 (m, 1H, H-4′).

9-(2,3,5-Tri-*O*-benzyl-4-thio-β-L-xylofuranosyl)-9H-purine-2,6-diamine (11β). This compound was prepared in 78% yield by the same procedure as reported above for **11α** but starting from **7β**, affording material suitable for deblocking. MS 569 $(M+H)^+$. ^{1}H NMR ($CDCl_3$) 7.89 (s, 1H, H-8), 7.35–7.24 (m, 13H, aromatic CHs), 7.13–718 (m, 2H, aromatic CHs), 6.00 (d, 1H, H-1′, $J = 4.0$ Hz), 5.44 (bs, 2H, NH_2), 4.74 (bs, 2H, NH_2), 4.64 (d, 1H, PhC*H*H-, $J = 12.1$ Hz), 4.52–4.49 (m, 4H, $PhCH_2$s), 4.41 (t, 1H, H-2′, $J = 3.2$ Hz), 4.19 (t, 1H, H-3′, $J_{3,4} = 4.0$ Hz), 4.01 (ddd, 1H, H-4′), 3.76 (dd, 1H, H-5′ b, $J_{4,5b} = 7.4$ Hz, $J_{5a,5b} = 9.6$ Hz), 3.54 (dd, 1H, H-5′ a, $J_{4,5a} = 6.7$ Hz).

9-(4-Thio-α-L-xylofuranosyl)-9*H*-purine-2,6-diamine (12α). An ice-cold solution of **11α** (218 mg, 0.38 mmol) in CH_2Cl_2 (2.6 mL) was added dropwise to a solution of 1 M BCl_3 in CH_2Cl_2 (30 mL) at −50°C. Solid precipitated from the solution near the end of addition. The reaction in a tightly sealed flask was stored at −20°C for 16 h. The resulting clear solution was evaporated to dryness at −20°C to give a dark residue. A solution of this material in ice-cold CH_2Cl_2 (25 mL) was evaporated to dryness four times to provide a foam. Ice-cold saturated aqueous $NaHCO_3$ (20 mL) was added to the foam, and the mixture was stirred vigorously until the H remained stable (pH 7–8). Water (150 mL) was added to form a clear solution that was extracted with two portions of CH_2Cl_2 (50 mL, 25 mL) to remove color and impurities. The colorless aqueous layer was held briefly under vacuum to remove residual CH_2Cl_2 before being applied to a column (13 × 190 mm) of Bio Beads SM-4 (100–200 mesh) equilibrated in water. Water elution with fractions monitored at 254 nm provided pure **12α** that was crystallized from hot MeOH (81 mg, 71%). TLC, 3:1:0.1 $CHCl_3$-MeOH-NH_4OH, R_f 0.48; m.p.

140°C; MS 299 $(M+H)^+$) ^1H NMR (DMSO-d_6) 7.88 (s, 1H, H8), 6.66 (s, 2H, NH_2), 6.40 (d, 1H, H-1′, $J_{1,2} = 4$ Hz), 5.82 (bs, 2H, NH_2), 4.20(dd, 1H, H-3′, $J_{3,4} = 6.3$ Hz), 4.06 (bdd, 2H, H-2′ H-4′), 3.72–3.82 (m, 1H, H-5′), 3.48–3.54 (m, 1H, H-5′). HPLC purity, 99.3%. HRMS calcd for $C_{10}H_{14}N_6O_3S$ $(M+H)^+$: 299.0921. Found: 299.0923.

9-(4-Thio-β-L-xylofuranosyl)-9*H*-purine-2,6-diamine (12β). This compound was prepared in 75% yield (crystallized from water) by the same procedure as reported above for **12α** but starting from **11β**. TLC, 3:1:0.1 $CHCl_3$-MeOH-NH_4OH, R_f 0.43 m.p. 130°C; MS 299 $(M+H)^+$, ^1H NMR (DMSO-d_6) 8.08 (s, 1H, H8), 6.68 (s, 2H, NH_2), 5.80 (bs, 2H, NH_2), 5.60 (d, 1H, H-1′, $J_{1,2} = 4$ Hz), 4.41 (t, 1H, H-2′, $J = 3.2$ Hz), 4.16 (t, 1H, H-3′, $J = 4.0$ Hz), 3.60 9m. 1H. H-4′), 3.78 (d, 1H, H-5′), 3.70 (d, 1H, H-5′). HPLC purity, 94.7%. HRMS calcd for $C_{10}H_{14}N_6O_3S$ $(M+H)^+$: 299.0921. Found: 299.0923. Anal. calcd. for $C_{10}H_{14}N_6O_3S$: H_2O: C 37.96; H 5.10; N 26.58. Found: C 38.14; H 4.94; N 26.56.

9-(4-Thio-β-L-xylofuranosyl)guanine (13). To a solution of **12β** (50 mg, 0.17 mmol) in 20 mL of water was added 100 u of adenosine deaminase type VIII (40 μL). The reaction was stirred for 72 h, the solution was boiled for 3 min to deactivate the enzyme, and the suspension was treated with charcoal and filtered through Celite. The filtrate was concentrated to give gelatinous **13** which was dissolved in hot water (4 mL) and filtered through 0.45-μm filter (25 mm, Gelman Acrodisc GHP-GF). The clear filtrate was lyophilized to provide **13** as a fluffy white solid which was crystallized from water (20 mg, 40%): TLC, 3:1:0.1 $CHCl_3$-MeOH-NH_4OH, R_f 0.40; m.p. 260°C; MS 300 $(M+H)^+$, ^1H NMR (DMSO-d_6) 10.5 (bs, 1H, N-3H) 8.02 (s, 1H, H-8), 6.48 9s, 2H, NH2), 6.00 (d, 1H, 2′-OH, $J = 4.0$ Hz), 5.58 (d, 1H, 3′-OH), 4.90 (t, 1H, 5′-OH, $J = 3.0$ Hz), 4.35 (t, 1H, H-2′, $J = 3.2$ Hz), 4.18 (t, 1H, H-3′, $J_{3,4} = 4.0$ Hz), 3.80 (dd, 1H, H-5′ b, $J_{4,5b} = 7.4$ Hz, $J_{5a,5b} = 9.6$ Hz), 3.68 (dd, 1H, H-5′ a, $J_{4,5a} = 6.7$ Hz) 3.60 (ddd, 1H, H-4′). HPLC purity, 95.6%. HRMS calcd for $C_{10}H_{13}N_5O_4S$: 322.0581. Found: 322.0581.

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