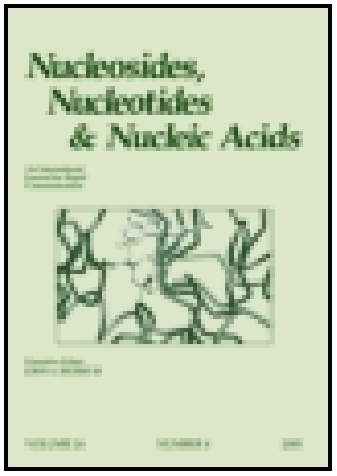

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Synthesis and Biological Activity of 4′-Thionucleosides of 2-Chloroadenine[1]

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 4'-THIONUCLEOSIDES OF 2-CHLOROADENINE[1]

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Abstract

1,2,3,5-Tetra-*O*-acetyl-4-thio-D-ribofuranose, prepared from 2,3,5-tri-*O*-benzyl-D-ribofuranose in four steps, was converted to the corresponding 2-chloroadenine nucleoside (**8**), which was deoxygenated to obtain 2-chloro-2'-deoxy-4'-thioadenosine (**12**). This is the first report of a 2'-deoxy-4'-thioribonucleoside of a purine rather than a pyrimidine. These novel nucleosides (**8** and **12**) were cytotoxic to several human tumor cell lines in culture.

Introduction

The synthesis and biological activity of nucleosides have been of interest for some time, particularly as antiviral and anticancer agents. Fludarabine phosphate,[2] which was developed in our laboratory, has shown activity in a number of human cancers and has been approved by the FDA for the treatment of refractory lymphocytic leukemia.[3] 2-Fluoro-, 2-chloro-, and 2-bromo-2'-deoxyadenosines have all shown very good activity in a murine leukemia model,[4] and 2-chloro-2'-deoxyadenosine has shown activity in clinical trials against human lymphomas and leukemia.[3] These 2-haloadenine nucleosides are resistant to deamination but are cleaved by *E. coli* PNP to the 2-haloadenines, which have no selective cytotoxicity.[5] 2-Fluoroadenine has been detected as a metabolite of fludarabine phosphate in animals and man.[2] The resistance of 4'-thioinosine to cleavage by mammalian purine nucleoside phosphorylase has been reported.[6] No 4'-thioribonucleosides or 2'-deoxy-4'-thionucleosides of the 2-haloadenines have been reported, but it is logical to assume that such nucleosides would be resistant to phosphorolytic cleavage. Since such metabolically stable nucleosides might have useful biological activity, we have undertaken their synthesis.

Reist *et al.*[7] and Whistler *et al.*[8] have converted L-lyxose to derivatives of 4-thio-D-ribofuranose. Thus, 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribose (**5**), used to prepare 4'-thioadenosine, was synthesized in six steps.[7] Recently, Bellon *et al.*[9] using the chemistry developed by Dyson *et al.*[10] prepared 1-*O*-acetyl-2,3,5-tri-*O*-benzyl-4-thio-D-ribose from L-lyxose, also in six steps. This latter sugar is less desirable than **5** for nucleoside syntheses because it does not have an acyloxy group at C-2 of the sugar to promote to the formation of trans (in this case, β)

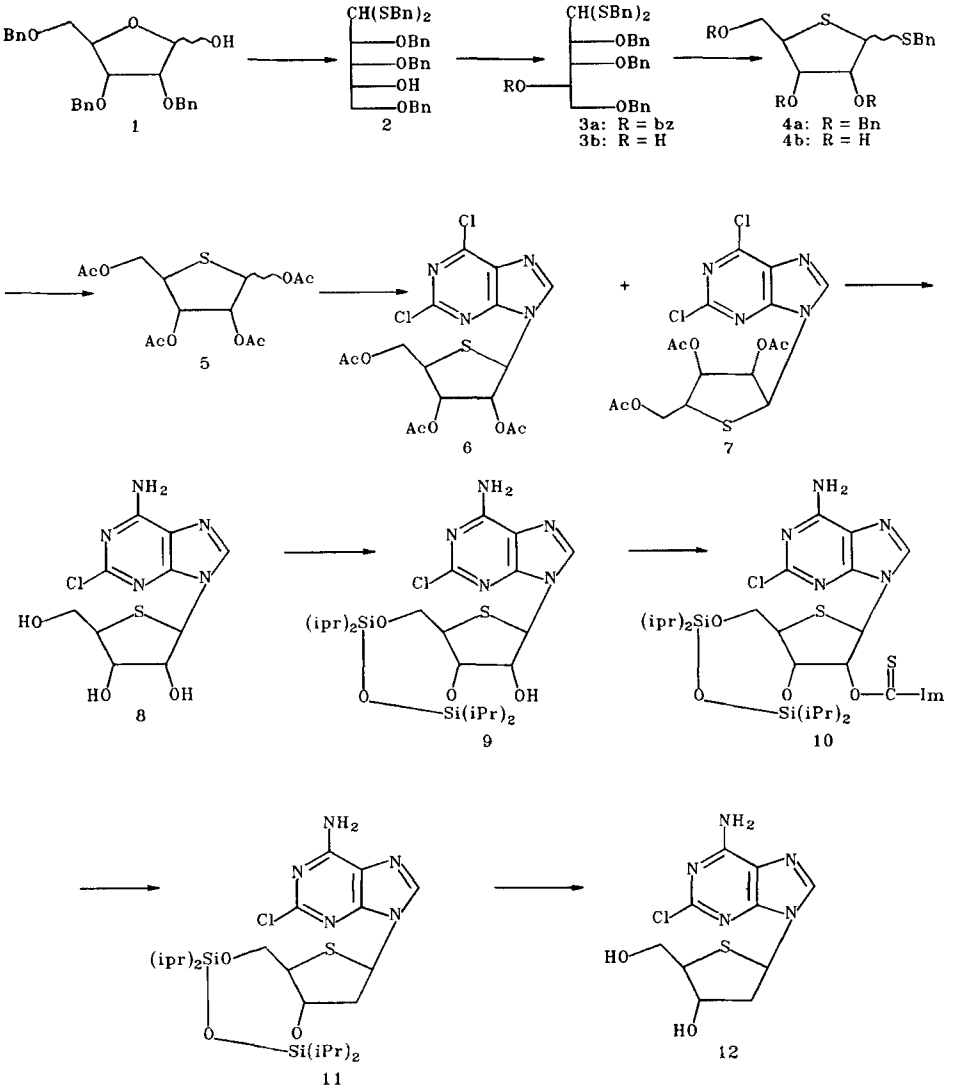
nucleosides. The starting material for these syntheses, L-lyxose, is an unnatural, expensive carbohydrate and therefore not suitable for large-scale nucleoside syntheses.

We have developed a synthesis of **5**, also based on the work of Dyson *et al.*[10] but starting with the readily-available, inexpensive D-ribose and have coupled this sugar to 2,6-dichloropurine from which the desired nucleosides were prepared.

Chemistry

2,3,5-Tri-*O*-benzyl-D-ribofuranose (**1**) was prepared from D-ribose by a literature procedure.[11] Treatment of **1** with benzyl mercaptan led to compound **2** in 69% yield. Reaction of **2** with benzoic acid, diethyl azodicarboxylate, and triphenylphosphine in THF at room temperature gave **3a** in 59% yield which after debenzoylation with sodium methoxide gave compound **3b**[9] in 82% yield. Conversion of compound **3b** to the 4-thioribose derivative **4a** was carried out in two ways. In the first procedure, compound **3b** was treated with mesyl chloride in pyridine to give the mesylate, which was allowed to react with tetrabutylammonium iodide and barium carbonate and provided compound **4a** in 72% yield after purification by column chromatography. In the second method, which is much cleaner and higher yielding, reaction of compound **3b** with iodine, triphenyl phosphine, and imidazole in toluene and acetonitrile provided sugar **4a** in 83% yield. After purification by silica gel chromatography, **4a** exhibited similar spectral properties to those reported earlier.[9] Removal of the benzyl groups followed by acetylation with acetic anhydride in pyridine and treatment of resulting product with mercuric acetate and acetic acid afforded 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose (**5**) in 81% yield. Compound **5** was obtained as a mixture of α and β anomers and has similar physical and spectral properties to those reported earlier.[7]

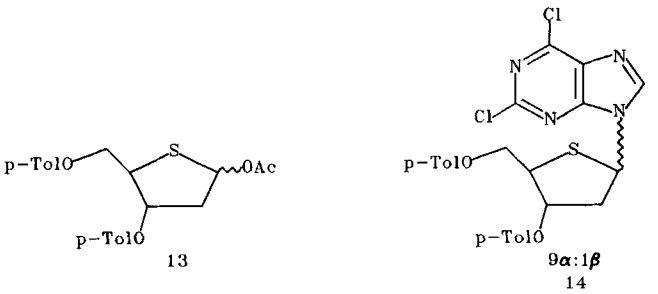
2,6-Dichloropurine was coupled with **5** in the presence of $SnCl_4$ to obtain a 3:1 mixture of 9β and 9α nucleosides (**6** and **7**) in 70% overall yield. Separation of isomers was carried out by fractional crystallization. The anomeric configuration was determined by ^1H and ^{13}C NMR spectra. Proton and anomeric assignments were made by NMR including decoupling experiments.[12] The assignments of the anomeric configuration of compounds **6** and **7** were made by NOE difference spectroscopy. The point of attachment of sugar and base were determined by ^{13}C-NMR and on the basis of coupling of H-1′ to C-4 and C-8. The reaction of **6** with ethanolic ammonia gave 2-chloro-4′-thioadenosine **8** in 81% yield. Treatment of **8** with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane produced **9** (85% yield), which upon reaction with 1,1′-thiocarbonyldiimidazole gave **10** in almost quantitative yield. Deoxygenation of compound **10** was carried out using tributyltin hydride and AIBN in toluene at reflux temperature to obtain 2′-deoxy derivative **11** in 24% yield. The other major product of the deoxygenation reaction appears from spectral data to be a result of dethiolation of the ring sulfur as well as the group at C-2′ to give an unsaturated acyclic derivative of 2-chloroadenine. The structure of this side product has not been established conclusively. Deprotection of **11** to give 2-chloro-2′-deoxy-4′-thioadenosine (**12**) was accomplished with tetraethylammonium



Scheme 1

fluoride in a mixture of tetrahydrofuran and acetonitrile. After purification by silica gel chromatography, compound **12** was isolated in 79% yield.

Efforts to prepare **12** more directly by the coupling of 1-*O*-acetyl-2-deoxy-4-thio-3,5-di-*O*-*p*-toluoyl-D-ribofurane (**13**) with 2,6-dichloropurine followed by removal of the *p*-toluoyl blocking groups were thwarted by the obtention of a very unfavorable ratio of anomers (**14**) (Scheme 2). Although a variety of conditions were tried, the best ratio obtained was 9 α-anomer to 1 β-anomer.[12] The cause of this unfavorable ratio is unclear since blocked ribofuranoses under



Scheme 2

Table. Cytotoxicity Data[a]

Compound	IC_{50} (μg/mL)[b,c]					
	H.Ep.-2	CCRF-CEM	ACHN (Renal)	CAKI-1 (Renal)	DLD-1 (Colon)	SK-MEL-28 (Melanoma)
8	2.0	4.0	20	40	30	20
12	<0.05	<0.5	2.0	0.09	20	3.0

[a]For details of the procedures used, see references 5 and 14. [b]The concentration required to inhibit cell proliferation to 50% untreated controls. [c]The cell lines are H.Ep.-2: human epidermoid carcinoma, CCRF-CEM human lymphoma, ACHN human renal cell carcinoma, CAKI-1 human renal cell carcinoma, DLD-1 human colon carcinoma, and SK-MEL-28 human melanoma.

similar coupling conditions give approximately 1α to 1β, and a similar ratio is obtained in the coupling of **13** with silylated pyrimidines.[13] This problem is under investigation.

Biological Evaluation

The target compounds **8** and **12** were evaluated for their cytotoxicity against a battery of human tumor cell lines selected from the National Cancer Institute primary screen (see Table). It is clear that the 2'-deoxyribonucleoside **12** is significantly more cytotoxic than the corresponding ribonucleoside **8**. Evaluation of **8** and **12** in an appropriate animal model is planned.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Nicolet NT-300 NB spectrometer operating

at 300.635 MHz (^{1}H) or 75.6 MHz (^{13}C). Chemical shifts are expressed in parts per million downfield from tetramethylsilane. The hydrogen-decoupled ^{13}C NMR were assigned by comparison of the $J^{C,H}$ values obtained from the hydrogen-coupled ^{13}C NMR spectra, and when necessary, selective hydrogen decoupling was performed in order to confirm the assignments. The NOE experiments were conducted on a degassed solution of $CDCl_3$. To minimize the effects of magnetic perturbations with the sample non-spinning, eight FID's were acquired with the decoupler set to a desired frequency and eight FID's were recorded with the decoupler off resonance. The process was repeated until 800 FID's had been acquired. Ultraviolet 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, and 0.1 N NaOH. Numbers in parentheses are extinction coefficients (ϵ x 10^{-3}), sh=shoulder. Microanalyses were performed by Atlantic Microlab, Inc. (Atlanta, GA) 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. HPLC analyses were carried out on a Hewlett-Packard HP1084B liquid chromatograph with a Waters Associates μBondapak C_{18} column (3.9 mm x 30 cm) and UV monitoring (254 nm). 230-400 Mesh silica gel from E. Merck was used for all flash-column chromatography. TLC was carried out on Analtech precoated (250 μM) silica gel (GF) plates.

2,3,5-Tri-*O*-benzyl-D-ribose Dibenzyldithioacetal (2). Compound **1** (4.20 g, 10 mmol) was stirred at 0 °C with concentrated hydrochloric acid (3.0 mL) and benzyl mercaptan (4.96 g, 40 mmol) for 2 h. The reaction mixture was then stirred for 3 h at room temperature at which time starting material was almost consumed. The reaction mixture was neutralized with 10% aqueous $NaHCO_3$ solution (150 mL) and extracted with dichloromethane (150 mL). The organic layer was dried ($MgSO_4$) and then evaporated to dryness *in vacuo*. The crude product was applied to a column of silica gel and eluted with 6:1 cyclohexane:ethyl acetate to give pure **2** (4.48 g, 69%) as a syrup; MS *z/e* 651 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 3.61 (m, 3H, H-4, H-5, H-5'), 3.70 (m, 6H, O-C*H*$_2$-C$_6$H$_5$), 4.05 (m, 3H, H-1, H-2, H-3), 4.40-4.90 (m, 4H, -SC*H*$_2$-C$_6$H$_5$), 5.19 (d, 1H, OH), 7.11 (m, 25H, -O-CH$_2$-C$_6$*H*$_5$ and S-CH$_2$-C$_6$H$_5$).

2,3,5-Tri-*O*-benzyl-L-lyxose Dibenzyldithioacetal[9] (3b). To a solution of **2** (4.0 g, 6.15 mmol), triphenylphosphine (2.62 g, 10 mmol), and benzoic acid (1.22 g, 10 mmol) in dry tetrahydrofuran (100 mL) was added a solution of diethyl azodicarboxylate (1.74 g, 10 mmol) in dry tetrahydrofuran (25 mL) dropwise at room temperature. The mixture was stirred at room temperature for 24 h and then concentrated *in vacuo*. The crude product was applied to a short column of silica gel and eluted with 7:1 cyclohexane:ethyl acetate to afford 2.73 g (59%) of **3a** as a light yellow syrup: MS *z/e* 755 $(M + H)^+$. This syrup was dissolved in 50 mL of anhydrous methanol, sodium methoxide (0.32 g) added, and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was poured into aqueous 5% NaH_2PO_4 and the solution extracted with dichloromethane. The organic layer was washed with aqueous 5% sodium bicarbonate solution and water, dried ($MgSO_4$) and concentrated. Column

chromatography (5:1 cyclohexane:ethyl acetate) of the residue gave compound **3b** (1.92 g, 82%), MS *z/e* 651 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 3.61 (m, 3H, H-4, H-5, H-5'), 3.80 (m, 4H, -S-CH_2-C_6H_5), 4.06-4.30 (m, 3H, H-1, H-2, H-3), 4.40-4.90 (m, 6H, -O-CH_2-C_6H_5), 4.80 (d, 1H, OH), 7.30 (m, 25H, -O-CH_2-C_6H_5 and -S-CH_2-C_6H_5).

Benzyl 2,3,5-Tri-*O*-benzyl-1,4-dithio-D-ribofuranoside[9] (4a). Method 1: Mesyl chloride (0.19 g, 1.67 mmol) was added to a solution of compound **3b** (650 mg, 1 mmol) in anhydrous pyridine (15 mL). The solution was stirred for 4 h and TLC showed complete comsumption of starting material. Barium carbonate (0.253 g, 1.28 mmol) and tetrabutylammonium iodide (0.475 g, 1.28 mmol) was added to the reaction mixture and the dark yellow heterogenous suspension heated under reflux for 1 h to give a new product on TLC. The reaction mixture was evaporated under reduced pressure, diluted with dichloromethane, washed with a 5% aqueous sodium bicarbonate solution, and the organic layer dried over anhydrous $MgSO_4$ and concentrated. The crude product was purified on a silica gel column eluted with 4:1 cyclohexane:ethyl acetate to afford compound **4a** as a syrup (375 mg, 72%); MS *z/e* 543 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 3.51 (dd, 1H, H-5, $J_{5,5'}$ = 9.5; $J_{5,4}$ = 6.7 Hz), 3.64 (dd, 1H, H-5', $J_{5',5}$ = 9.5; $J_{5',4}$ = 6.8 Hz), 3.75 (m, 3H, H-4, -S-CH_2-C_6H_5), 3.84 (t, 1H, H-2, $J_{2,1}$ = 3.7; $J_{2,3}$ = 3.7 Hz), 4.06 (dd, 1H, H-3, $J_{3,2}$ = 3.4, $J_{3,4}$ = 6.4 Hz), 4.30 (d, 1H, H-1, $J_{1,2}$ = 4.0 Hz), 4.4-4.5 (m, 6H, -O-CH_2-C_6H_5), 7.30 (m, 20H, -O-CH_2-C_6H_5, -S-CH_2-C_6H_5).

Method 2: To a solution of compound **3b** (1.30 g, 2 mmol) in anhydrous tetrahydrofuran (20 mL) was added triphenylphosphine (1.57 g, 6 mmol), iodine (1.27 g, 5 mmol) and imidazole (544 mg, 8 mmol). The reaction mixture was stirred at 90 °C for 24 h before the solution was evaporated to dryness. The product was purified by silica gel chromatography (4:1 cyclohexane:ethyl acetate) to obtain compound **4a** (900 mg, 83%), which was identical to the compound obtained by Method 1: the same R_f values, M.S., and ^1H NMR spectrum.

1,2,3,5-Tetra-*O*-acetyl-4-thio-D-ribofuranose[7] (5). A solution of **4a** (1.08 g, 2 mmol) in dichloromethane (30 mL) was treated at -78 °C under argon with a solution of 1M boron tribromide in dichloromethane (15 mL). After stirring for 0.5 h at -78 °C, methanol (10 mL) was added followed by pyridine (10 mL) and the solution was evaporated to dryness. Dry pyridine (3 x 20 mL) was added to the residue and the resulting solutions evaporated to dryness (three times). The crude brown **4b** was dissolved in pyridine (30 mL) and to this solution was added acetic anhydride (10 mL). After 12 h the pyridine was removed *in vacuo* and the residue was dissolved in chloroform, washed with HCl (2 M), sodium carbonate (1 M) and water, dried ($MgSO_4$) and concentrated to dryness. The brown syrup was passed through a short bed of silica gel on a filter funnel and washed first with cyclohexane and then with 6:1 cyclohexane:ethyl acetate to obtain a light yellow syrup (600 mg) that was dissolved in a mixture of acetic anhydride (5 mL) and acetic acid (5 mL) containing mercuric acetate (2 g). The solution was kept at 45-50 °C for 30 min before adding water (50 mL) and $CHCl_3$ (50 mL). The organic layer was dried, evaporated to dryness, and the residue purified by silica gel chromatography (6:1 cyclohexane:ethyl acetate) to give **5** (540 mg, 81%) as a

mixture of α and β anomers. A small amount of β-anomer was obtained by fractional crystallization (methanol) mp 65-67 °C; MS *z/e* 335 $(M + H)^+$; ^1H NMR β-anomer ($CDCl_3$) δ 2.05, 2.10, 2.12, 2.14 (s, 12H, OAc), 3.81 (m, 1H, H-4), 4.15 (m, 1H, H-5), 4.39 (m, 1H, H-5), 5.37 (m, 1H, H-3), 5.07 (dd, 1H, H-2, J = 2 and 4 Hz), 5.32 (d, 1H, H-1, J = 2 Hz).

9-[2,3,5-Tetra-*O*-acetyl-β- and α-D-ribofuranosyl]-2,6-dichloropurine (6 and 7). A mixture of sugar **5** (334 mg, 1 mmol) and 2,6-dichloropurine (236 mg, 1.25 mmol) in 30 mL of acetonitrile was cooled to 0 °C and a 1.0 M solution of tin (IV) chloride in dichloromethane (1.25 mL, 1.25 mmol) was added. Stirring was continued for 12 h at room temperature. The reaction mixture was quenched by pouring into a mixture of 30 mL of dichloromethane and 15 mL of saturated $NaHCO_3$. The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was subjected to flash chromatography with 3:1 cyclohexane:ethyl acetate. Removal of the solvent gave a 3:1 β:α-anomeric mixture (323 mg, 70%). Fractional crystallization of the mixture from diethyl ether gave 225 mg of pure **6** and crystallization of the mother liquor from ethyl alcohol provided 75 mg of pure **7**. Compound **6** (β-anomer) mp 171-173 °C, MS *z/e* 463 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 2.02 (s, 3H, OAc), 2.20 (s, 6H, OAc), 3.82 (m, 1H, H-4'), 4.24 (m, 2H, 2H-5'), 5.62 (t, 1H, H-3', J = 4 Hz), 5.98 (dd, 1H, H-2', J = 4 and 6 Hz), 6.28 (d, 1H, H-1', J = 6 Hz), 8.5 (s, 1H, H-8). ^{13}C NMR ($CDCl_3$) δ 20.44 and 20.78 (CH_3 of OAc), 47.83 (C-4'), 60.81 (C-1'), 63.85 (C-5'), 73.47 (C-3'), 76.03 (C-2'), 131.36 (C-5), 144.21 (C-8), 152.36 (C-6 or C-2), 152.83 (C-4), 153.31 (C-2 or C-6), 169.33, 169.59, 170.23 (carbonyl of acetates). Anal. calcd for $C_{16}H_{16}Cl_2N_4O_6S$: C, 41.48; H, 3.48; N, 12.09. Found: C, 41.56; H, 3.29; N, 11.97.

Compound **7** (α-anomer) mp 139-141 °C; MS *z/e* 463 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 1.90, 2.0, 2.18 (3s, 9H, 3OAc), 4.08 (m, 1H, H-4'), 4.32 (m, 2H, 2H-5'), 5.52 (t, 1H, H-3', J = 4 Hz), 5.80 (dd, 1H, H-2', J = 4 and 2 Hz), 6.43 (d, 1H, H-1', J = 4 Hz), 8.62 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$) δ 20.33, 20.66, 20.70 (CH_3 of acetyls), 48.29 (C-4'), 58.19 (C-1'), 63.87 (C-5'), 72.85 (C-2'), 74.41 (C-3'), 130.79 (C-5), 146.10 (C-8), 151.81, 153.13 (C-6, C-2), 153.41 (C-4), 168.76, 169.02, 170.30 (carbonyl of acetyls).

2-Chloro-4'-thioadenosine (8). A mixture of compound **6** (140 mg, 0.3 mmol) and saturated ethanolic ammonia (50 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 that was purified on a silica gel column (5:1 chloroform:methanol), and the resulting product was crystallized from ethyl alcohol to provide **8** (77 mg, 81%), mp 222-223 °C; MS *z/e* 318 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 3.25 (m, 1H, H-4'), 3.62 (m, 1H, H-5'), 3.78 (m, 1H, H-5'), 4.20 (m, 1H, H-3'), 4.62 (m, 1H, H-2'), 5.18 (t, 1H, 5'-OH, J = 4 Hz), 5.36 (d, 1H, 3'-OH, J = 4 Hz), 5.59 (d, 1H, 2'-OH, J = 6 Hz), 5.78 (d, 1H, H-1', J = 7 Hz), 7.82 (s, 2H, NH_2), 8.50 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6), δ 53.25 (C-4'), 61.15 (C-1'), 63.04 (C-5'), 72.99 (C-3'), 76.82 (C-2'), 117.91 (C-5), 140.27 (C-8), 150.55 (C-4), 152.76 (C-2), 156.60 (C-6). Anal. calcd for $C_{10}H_{12}ClN_5O_3S$: C, 37.79; H, 3.80; N, 22.04. Found: C, 37.58; H, 3.70; N, 21.69.

2-Chloro-4'-thio-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (9). A solution of 2-chloro 4'-thioadenosine (**8**, 200 mg, 0.66 mmol) in 10 mL of pyridine was treated at room temperature with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (230 mg, 0.73 mmol). After stirring for 1 h, the reaction was essentially complete as indicated by TLC (9:1 $CHCl_3$:MeOH). After 3 h, the reaction mixture was poured into ice water, stirred for 15 min, and then diluted with 20 mL of $CHCl_3$ to give a total volume of 80 mL. The aqueous layer was extracted with $CHCl_3$ (2 x 25 mL). The combined $CHCl_3$ extracts were washed with water and dried over $MgSO_4$ before evaporation to dryness. The residue was co-evaporated with toluene to remove pyridine and the crude product was purified by silica gel column chromatography (97:3 $CHCl_3$:MeOH) to provide **9** (313 mg, 85%); MS *z/e* 560 $(M + 1)^+$; ^1H NMR ($CDCl_3$) δ 1.09 (m, 28 H, 4 $CH(CH_3)_2$), 3.02 (d, 1H, 2'-OH, J = 1 Hz), 3.68 (m, 1H, H-4'), 4.10 (m, 2H, H-5',5''), 4.18 (m, 1H, H-2'), 4.66 (dd, 1H, H-3', J = 4 and 6 Hz), 5.81 (brs, 2H, NH_2), 5.84 (s, 1H, H-1'), 8.21 (s, 1H, H-8).

2-Chloro-4'-thio-2'-*O*-[1-imidazolyl(thiocarbonyl)]-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (10): Thiocarbonyldiimidazole (178 mg, 1.0 mmol) was added to a solution of **9** (279 mg, 0.5 mmol) in 20 mL of molecular sieve-dried dichloromethane, and the solution was refluxed for 2 h during which time starting material was completely consumed (monitored by TLC - 95:5 $CHCl_3$:MeOH). The reaction mixture was evaporated to dryness and the crude product was purified on a column of silica gel (99:1 $CHCl_3$:MeOH) to afford 302 mg (90%) of compound **10** as a light yellow solid; MS *z/e* 670 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 1.15 (m, 28 H, 4 $CH(CH_3)_2$), 3.68 (m, 1H, H-4'), 4.18 (m, 2H, H-5',5''), 5.07 (dd, 1H, H-3', J = 4 and 6 Hz), 5.98 (brs, 2H, NH_2), 6.06 (s, 1H, H-1'), 6.30 (d, 1H, H-2', J = 4 Hz), 7.18 (s, 1H, H of imidazole), 7.54 (s, 1H, H-5 of imidazole), 8.22 (s, 1H, H-8), 8.45 (s, 1H, H-2 of imidazole).

2-Chloro-2'-deoxy-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (11). Tributyltin hydride (175 mg, 0.6 mmol) and 2,2'-azobis-(2-methylpropionitrile) (AIBN, 6.6 mg, 0.4 mmol) were added to a solution of **10** (275 mg, 0.41 mmol) in dry toluene (20 mL). The stirred reactants were refluxed for 3 min before the solvent was removed *in vacuo* and the residue applied on a column of silica gel. Elution of column with 99:1 $CHCl_3$:MeOH afforded the product **11** as a colorless foam (53.5 mg, 24%); MS *z/e* 544 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 1.10 (m, 28H, 4 $CH(CH_3)_2$), 2.50 (m, 1H, H-2'), 2.60 (m, 1H, H-2'), 3.43 (m, 1H, H-4'), 4.0 (m, 1H, H-5'), 4.18 (m, 1H, H-5''), 4.60 (m, 1H, H-3'), 6.0 (d, 1H, H-1', J = 6 Hz), 8.48 (s, 1H, H-8).

2-Chloro-2'-deoxy-4'-thioadenosine (12). A solution of **11** (50 mg, 0.09 mmol) in 5 mL of dry tetrahydrofuran and 5 mL of acetonitrile (Aldrich Sureseal) was treated for 1 h at room temperature with tetraethylammonium fluoride (31 mg, 0.20 mmol). The solution was evaporated to dryness, the residue dissolved in MeOH, and the solution applied to silica gel thick plates (Analtech, GF, 2000 μM) that were developed in 5:1 $CHCl_3$:MeOH. The product was extracted from the silica gel with warm EtOH and the solution evaporated to obtain compound **12** (22 mg, 79%) as a white powder. MS *z/e* 302 $(M + H)^+$; UV λ_{max} pH 1 267

(11.7), pH 7 266 (12.7), pH 13 265 (12.7); ^{1}H NMR (DMSO-d_6) δ 2.40 (m, 1H, H-2′), 2.65 (m, 1H, H-2′), 3.30 (m, 1H, H-4′), 3.60 (m, 1H, H-5′), 3.78 (m, 1H, H-5″), 4.50 (m, 1H, H-3′), 5.14 (t, 1H, 5′-OH, J = 6 Hz), 5.34 (d, 1H, 3′-OH, J = 4 Hz), 6.17 (t, 1H, H-1′, J = 6 Hz), 7.82 (brs, 2H, NH_2), 8.45 (s, 1H, H-8). Anal. calcd for $C_{10}H_{12}ClN_5O_2S$: C, 39.80; H, 4.00; N, 23.20. Found: C, 39.79; H, 4.04; N, 23.12.

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References

1. Presented at the Tenth International Roundtable: Nucleosides and Nucleosides, Park City, Utah, U.S.A., September 16-20, 1992, Abstract No. 21.
2. Montgomery, J. A.; Hewson, K. Nucleosides of 2-Fluoroadenine. *J. Med. Chem.* **1969**, *12*, 498-504.
3. Cheson, B. D. New Antimetabolites in the Treatment of Human Malignancies. *Semin. Oncol.* **1992**, *19*, 695-706.
4. Montgomery, J. A. Has the Well Gone Dry? The First Cain Memorial Award Lecture. *Cancer Res.* **1982**, *42*, 3911-3917.
5. Secrist III, J. A.; Shortnacy, A. T.; Montgomery, J. A. Synthesis and Biological Evaluations of Certain 2-Halo-2′-substituted Derivatives of 9-β-D-Arabinofuranosyladenine. *J. Med. Chem.* **1988**, *31*, 405-410.
6. Parks, Jr., R. E.; Stoeckler, J. D.; Cambor, C.; Savarese, T. M.; Crabtree, G. W.; Chu, S.-H. Purine Nucleoside Phosphorylase and 5′-Methylthioadenosine Phosphorylase: Targets of Chemotherapy. In: *Molecular Actions and Targets for Cancer Chemotherapeutic Agents*; Sartorelli, A. C.; Lazo, J. S.; Bertino, J. R., Eds.; Academic Press: New York, 1981; pp 229-252.
7. Reist, E. J.; Gueffroy, D. E.; Goodman, L. Synthesis of 4-Thio-D- and -L-ribofuranose and the Corresponding Adenine Nucleosides. *J. Am. Chem. Soc.* **1964**, *86*, 5658-5663.
8. Whistler, R. L.; Dick, W. E.; Ingle, T. R.; Rowell, R. M.; Urbas, B. Methyl 4-Deoxy-4-mercapto-D-ribofuranoside. *J. Org. Chem.* **1964**, *29*, 3723-3725.
9. Bellon, L.; Barascut, J.-L.; Imbach, J.-L. Efficient Synthesis of 4-Thio-D-ribofuranose and Some 4′-Thioribonucleosides. *Nucleosides, Nucleotides* **1992**, *11*, 1467-1479.
10. Dyson, M. R.; Coe, P. L.; Walker, R. T. An Improved Synthesis of Benzyl 3,5-di-*O*-Benzyl-2-deoxy-1,4-dithio-D-erythro-pentofuranoside, An Intermediate in the Synthesis of 4′-Thionucleosides. *Carbohydr. Res.* **1991**, *216*, 237-248.

11. Barker, R.; Fletcher, Jr., H. G. 2,3,5-Tri-*O*-benzyl-D-ribosyl and L-Arabinosyl Bromides *J. Org. Chem.* **1961**, *26*, 4605-4609.

12. Manuscript in preparation.

13. Secrist III, J. A.; Tiwari, K. N.; Riordan, J. M.; Montgomery, J. A. Synthesis and Biological Activity of 2'-Deoxy-4'-thio Pyrimidine Nucleosides. *J. Med. Chem.* **1991**, *34*, 2361-2366.

14. Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. Evaluation of a Soluble Tetrazolium/Formazan Assay for Cell Growth and Drug Sensitivity in Culture Using Human and Other Tumor Cell Lines. *Cancer Res.* **1988**, *48*, 4827-4833.

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