# Synthesis and Cytotoxicity of Deoxyadenosine Analogues: Isomer Distribution in the Sodium Salt Glycosylation of 2,6-Disubstituted Purines

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Several 2-substituted deoxyadenosine derivatives were synthesized and screened for cytotoxicity toward hematopoietic cells in culture. To prepare intermediates for these syntheses, the sodium salts of 2,6-dibromopurine and 2,6-bis(methylthio)purine were reacted with 1-chloro-3,5-di-*p*-toluyl-α-D-*erythro*-pentofuranose in acetonitrile. Similar reactions using 6-chloropurines have been reported to give only 9-β and 7-β nucleosides as major and minor products, respectively. 2,6-Dibromopurine, however, gave 9-β and 9-α isomers as major and minor products, along with a lesser amount of the 7-β isomer. 2,6-Bis(methylthio)purine, in contrast, produced 9-β and 7-β isomers as major and minor products. These results are discussed in terms of sugar anomerization and possible steric and kinetic effects of base substituents in the sodium salt glycosylation reaction. Reactions of the 9-β nucleoside isomers with ammonia and alkylamines produced several 2-bromo, 2-methylthio, and 2-amino deoxyadenosines. All of the compounds showed weaker cytotoxic activity than 2-bromodeoxyadenosine against hematopoietic cells in culture, when [$^{14}$C]leucine incorporation into cellular proteins was measured.

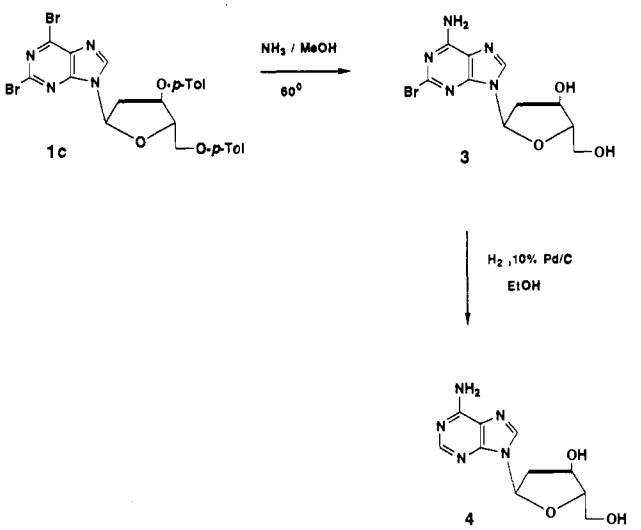
2-Bromodeoxyadenosine (BrdAdo, **5**) is one of a series of cytotoxic 2-haloadenine nucleosides that possess in vivo antitumor activity against T-cell leukemias and lymphomas.[1,2] The analogues owe their potency and selectivity, at least in part, to their activation by phosphorylation by deoxycytidine kinase and to their resistance to adenosine deaminase.[3] 2-Chlorodeoxyadenosine inhibited strongly the proliferation of T cells and non-B, non-T cells and other malignant hematopoietic cells in culture,[4] and the 2-halodeoxyadenosines were potent inhibitors of melanoma cell growth.[5] In comparative studies, **5** was highly effective in inhibiting the growth and DNA synthesis of the CCRF-CEM T-cell line, but the 2-SMe analogue **8** was nearly inactive.[6] In contrast, $N^6$-methyldeoxyadenosine was weak but $N^6$,$N^6$-dimethyldeoxyadenosine was a more potent inhibitor of the growth of the same cell line.[6]

We recently reported[7] a convenient synthesis of **5** via selective ammonolysis of the 6-bromo group of a protected 2,6-dibromo-9-(2-deoxy-β-D-ribofuranosyl)purine (**1a**). The latter compound was prepared by the efficient sodium salt glycosylation method in high yield from 2,6-dibromopurine.[7] The minor product of this reaction, however, was proposed to be the 9-α rather than the expected[8,9] 7-β isomer. In this paper we confirm the structure of this product as the 9-α nucleoside. In addition, in order to study the mechanistic basis for this result and to prepare intermediates for the synthesis of potentially cytotoxic deoxyadenosine derivatives, we have applied the sodium salt glycosylation procedure to 2,6-bis(methylthio)purine. We report the chemistry of these compounds and some preliminary results concerning the mechanism of isomer distribution during the sodium salt glycosylation. We also describe the synthesis of several 2-substituted deoxyadenosines and the results of their screening for cytotoxicity against hematopoietic cells in culture.

## Results

**Chemistry**. Glycosylation of the sodium salt of 6-chloropurines with 1-chloro-2-deoxy-3,5-di-*p*-toluyl-α-D-*erythro*-pentofuranose in acetonitrile solution has invariably produced only β anomers, with the 9-β isomer as major product and the 7-β isomer as minor product.[8-11] We recently suggested the formation of the 9-α isomer (**1c**)

Scheme I




as the second product from the analogous "sodium salt glycosylation" of 2,6-dibromopurine.[7] Although the appearance of the 1′-H resonance in the $^1$H NMR spectrum of **1c** in perdeuteriodimethyl sulfoxide was a pseudotriplet,

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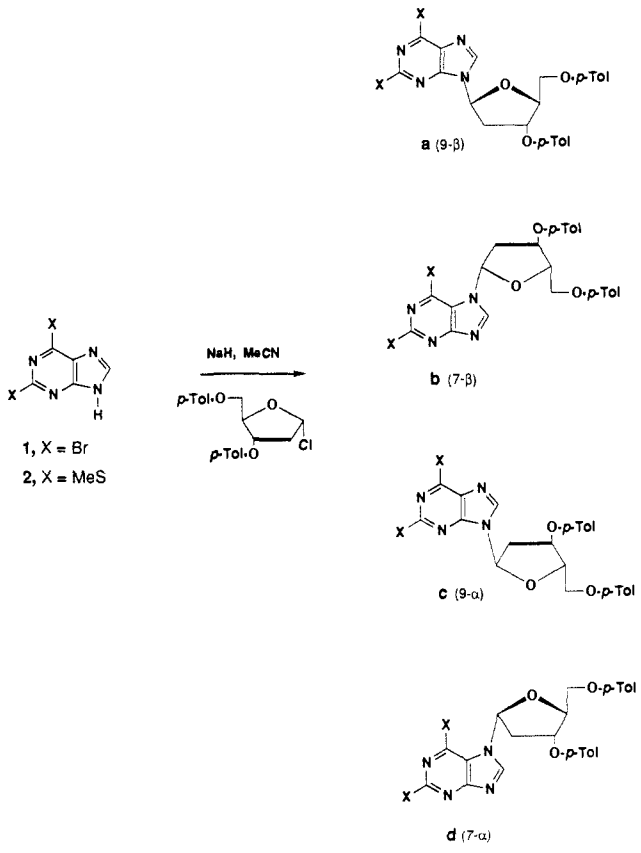
**Scheme II**




characteristic of β-deoxyribonucleosides,[12] the lack of overlap between the 4′ and 5′,5″ proton resonances suggested an α anomer.[13] We have now confirmed the structure of this product as **1c** by its selective ammonolysis to the 2-bromo-6-amino nucleoside **3** followed by hydrogenolysis to a compound with the proper ties of α-deoxyadenosine (**4**)[14] (Scheme I).

Confirmation of this structure prompted us to reexamine carefully the products from the sodium salt glycosylation of 2,6-dibromopurine and other substituted purines. After treatment of 2,6-dibromopurine under standard glycosylation conditions (see Experimental Section) and partial removal of the 9-β isomer **1a** by selective crystallization, preparative HPLC of the reaction mixture on silica gel revealed four components (Scheme II). An additional quantity of **1a** (giving a total yield of 48%) eluted first, followed by 12% of the product identified as the 9-α isomer **1c**. A third product was obtained in 6.6% yield and was identified as the 7-β isomer **1b** on the basis of both the pseudotriplet for the 1′-H and the overlap of 4′,5′,5″-Hs in its $^1$H NMR spectrum and on the similarity of the spectrum to that of the corresponding dichloro nucleoside.[15] Finally, 3.8% of a product identified as the 7-α isomer **1d** was obtained. Although the 1′-H of **1d** appeared as a doublet, the chemical shift pattern of the 4′-H and 5′,5″-Hs resembled that of the 9-α anomer (**1c**). In addition, chemical shifts of H-8 and H-1′ in **1d**, like those of the 7-β isomer **1b**, were downfield from those of the corresponding 9-isomers, **1a** and **1c**.

In separate experiments, we confirmed that the second product of the sodium salt glycosylation of 2,6-dichloropurine,[8] performed under conditions identical with those above, was, in fact, the 7-β isomer. However, HPLC of the reaction mixture showed the presence of 1.5% of a product with mp and $^1$H NMR identical with those of the 9-α deoxyribonucleoside isomer[15] (results not shown).

**Studies of Isomer Distribution.** The proposed mechanism of the sodium salt glycosylation of 6-chloropurines involves $S_N2$ attack of the base anion on the α-anomer of the 1-chloro sugar. Indeed, preparation of the protected 1-chlorodeoxyribose employed in these reactions gives the crystalline 1-α anomer, but isomerization of this sugar to give mixtures of 1-α and 1-β anomers occurs in solution and is strongly a function of solvent polarity.[16] Formation of only β anomers in reactions with 1-α-chloro sugars could only result if the rate of glycosylation was much faster than that of sugar anomerization or if the 1-β-chloro sugar was unreactive. The reported anomerization half-life of 1-chloro-2-deoxy-3,5-di-*p*-toluyl-α-D-*erythro*-pentofuranose at 21 °C in acetonitrile was about 90 min.[16] Analytical HPLC analysis of the glycosylation reactions reported in this paper have shown that the reactions run at 25 °C are substantially complete after 10 min (results to be published), but the data of Hubbard et al.[16] indicate that as much as 5–10% of 1-β sugar is formed after 10 min in acetonitrile at 21 °C. The β anomer of the 1-chloro sugar, in fact, reacted faster than the α anomer with pyrimidine bases.[16] The formation of a substantial amount of **1c** is, therefore, consistent with a steric effect of the 6-bromo group of the base preventing attack by N-7 on the 1-α-chloro sugar, allowing sufficient anomerization of sugar to occur and resulting in the 9-α nucleoside **1c** as the second most abundant product.

The above steric hypothesis was tested by the use of 2,6-bis(methylthio)purine as base in the glycosylation reaction. The methylthio group is larger than bromo (molar refraction (MR) values 13.82 and 8.88, respectively[17]), and it would be expected to hinder glycosylation at N-7 even more than bromo. Glycosylation of 2,6-bis(methylthio)-purine under conditions identical with those for the dibromo compound (see Scheme II) gave the 9-β isomer **2a** in 62% yield, but 18% of a second compound with NMR characteristics of the 7-β isomer **2b**. Indeed, only 0.3% of the 9-α isomer (**2c**) and 0.2% of the 7-α isomer (**2d**) could be isolated by preparative HPLC. Failure of this reaction to produce substantial 9-α isomer clearly eliminates a simple steric effect of the 6-substituent in directing formation of 9-α isomers during sodium salt glycosylations. Considering the rapidity with which these reactions proceed, clearly faster than substantial anomerization of the starting sugar, substituent-induced differences in the rates of attack of the base ambident anions on the starting sugar may explain the anomalous product distribution in the case of the 2,6-dibromo compound. Kinetic experiments and glycosylation reactions of simple 6-substituted purines, the results of which may resolve this problem and which may allow control of isomer distribution, are in progress.

**Deoxyadenosine Derivatives.** As illustrated in Scheme III, deblocking and selective displacement of the 6-bromo group of **1a** with amines was achieved at 25 °C to give the $N^6$-methyl analogue **6** and the $N^6$,$N^6$-dimethyl analogue **7** in 78% and 72% yields, respectively. Relative

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**Table I.** Toxicity of Deoxyadenosine Analogues against Malignant and Normal Hematopoietic Cells in Vitro

| | $ID_{50}$[a] (μM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| compd | MOLT-4[b] | Raji | U-937 | NALL-1 | HL-60 | K-562 | PHA-LY | B-95-8 |
| **5** | 3.2 | 0.7 | 0.8 | 12 | 41 | 83 | 1.6 | 6 |
| **6** | 177 | 80 | 140 | 230 | 143 | 110 | 30 | 253 |
| **7** | >300 | >300 | 164 | 290 | >300 | >300 | 88 | >300 |
| **8** | >300 | 107 | >300 | 96 | >300 | >300 | 82 | >300 |
| **9** | >300 | >300 | >300 | 186 | >300 | >300 | 81 | >300 |
| **10** | 172 | 233 | 126 | 188 | >300 | >300 | 260 | 116 |
| **11** | >300 | 125 | >300 | >300 | >300 | >300 | >300 | 182 |
| **12**[c] | – | – | – | – | – | – | – | – |
| **13** | 141 | 170 | >300 | 173 | >300 | >300 | 167 | 225 |

[a] $ID_{50}$ is the concentration (μM) of compound which caused a 50% decrease in [$^{14}$C]leucine incorporation by the cells in culture. Values were calculated from dose–response curves done in triplicate for each analogue. [b] Abbreviations are as follows: MOLT-4, acute T cell leukemia; Raji, Burkitt's lymphoma (B cell); U-937, histiocytic lymphoma; NALL-1, non-B, non-T acute lymphatic leukemia; HL-60, acute promyelocytic leukemia; K-562, chronic myelogenous leukemia; PHA-LY, phytohemagglutinin-stimulated peripheral blood lymphocytes; B-95-8, EBV-transformed monkey lymphoblastoid cell. [c] Inactive at 40 μM (limit of solubility) in all cases.

resistance to displacement of the second bromo group is demonstrated by the prolonged heating of **5** with dimethylamine at 120 °C necessary to produce 2-(dimethylamino)deoxyadenosine (**11**) in 73% yield, and by similar treatment of **1a** with dimethylamine to produce the 2,6-bis(dimethylamino) analogue **12** in 71% yield.

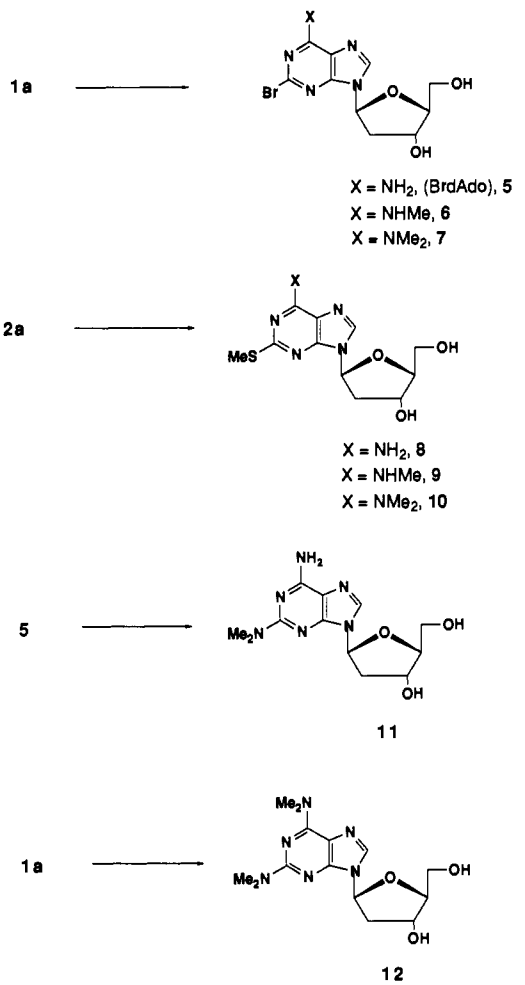
Displacement of the 6-SMe group of **2a** by ammonia or amines and concomitant deblocking required more strenuous conditions than the analogous reactions with **1a**. Treatment of **2a** at high temperatures (110–140 °C) was necessary to give the deoxyadenosine derivative **8** in 32% yield and the $N^6$-methyl and $N^6$,$N^6$-dimethyl analogues (**9** and **10**, respectively) in 66% and 52% yields, respectively.

**Cytotoxicity Screening.** The toxicities of the deoxyadenosine derivatives **5–12** and of the 9-β deoxyribonucleoside of 2,6-bis(methylthio)purine **13** were tested against eight different hematopoietic cell populations in vitro. The cells represented six established human leukemia/lymphoma lines, mitogen-stimulated human peripheral blood lymphocytes, and an Epstein-Barr virus transformed monkey lymphoblastoid cell line. The cells were cultured for 3 days with the test compounds, and the extent of [$^{14}$C]leucine incorporation into cellular proteins during the final 24 h of culture was used as the end point.[18] This system is well-suited for the assessment of nucleoside toxicity, and a good correlation has been noted between cell number and [$^{14}$C]leucine incorporation per culture.[19]

The results of the cytotoxicity assays are presented in Table I. BrdAdo (**5**) was strongly cytotoxic to most of the cell lines tested, showing highest activity against the MOLT-4 T cell line, the Burkitt's lymphoma-derived Raji B line, the histiocytic lymphoma line U-937, and normal human lymphocytes. These results contrast with those previously reported for 2-chlorodeoxyadenosine in which, for example, HL-60 and NALL-1 cells were highly sensitive to the compound, and an EBV-transformed B cell line was resistant.[4] All of the analogues tested had considerably weaker cytotoxic activity than **5**. Only the $N^6$-methyl derivative **6** showed consistent activity, with highest activity against normal lymphocyte cells; the $N^6$,$N^6$-dimethyl analogue **7** was inactive.

The 2-methylthio derivatives weakly inhibited growth of the NALL-1 and normal lymphocyte lines, and, in addition, the $N^6$,$N^6$-dimethyl analogue **10** weakly inhibited all but the myelocytic (HL-60) and myelogenous (K-562) leukemia cell lines (Table I). These results are consistent with those of Huang et al.[6] with the CCRF-CEM T-cell line: $ID_{50}$ for growth inhibition by $N^6$,$N^6$-dimethyl-

**Scheme III**




deoxyadenosine (5 μM) was much lower than that of $N^6$-methyldeoxyadenosine (87 μM) in that study.

It appears likely that the 5′-triphosphates of 2-halodeoxyadenosines are the active forms of the compounds in cells[3] and that inhibition of DNA synthesis and DNA fragmentation result from the active form.[5] Indeed, the triphosphates have recently been shown to be incorporated into DNA by the mammalian DNA polymerases in vitro.[20] Weak cytotoxic activity of the analogues reported in this paper may be attributed to their inefficient phosphorylation in cells or to the lack of effect of phosphorylated forms on DNA synthesis in cells.

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## Experimental Section

Proton nuclear magnetic resonance spectra were obtained at 250 MHz with a Bruker WM250 instrument or at 200 MHz with a Bruker AC200 instrument; chemical shifts are reported in parts per million ($\delta$) relative to internal tetramethylsilane. Only characteristic chemical shifts and coupling constants of sugar resonances in base modified nucleosides are given. UV spectra were obtained with a Gilford Response spectrophotometer. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Purifications by HPLC were achieved with a Waters model 6000 pump and model 401 Differential Refractometer detector. Elemental analyses were performed by the Microanalysis Laboratory, University of Massachusetts, Amherst, MA; analytical results were within ±0.4% of calculated values. 2-Bromo-2′-deoxyadenosine (**5**) was prepared as described by Wright et al.,[7] and 2,6-bis(methylthio)purine was prepared by the method of Montgomery et al.[21] 1-Chloro-2-deoxy-3,5-di-*p*-toluyl-α-D-*erythro*-pentofuranose was prepared by the method of Hoffer.[22]

**2-Bromo-6-amino-9-(2-deoxy-α-D-ribofuranosyl)purine (3).** A mixture of 2,6-dibromo-9-(2-deoxy-3,5-di-*p*-toluyl-α-D-ribofuranosyl)purine (**1c**) (468 mg, 0.743 mmol) and methanolic ammonia saturated at 0 °C (15 mL) was heated in a steel bomb at 65 °C for 32 h. The solvent was evaporated, and the residue was adsorbed onto silica gel. The impregnated silica gel was placed on top of a silica gel column (6 g, 70–230 mesh) and the product was eluted with chloroform/methanol (4:1, 500 mL). The nucleoside-containing fractions were evaporated, and the product was crystallized from methanol to give 111 mg (45%) of **3**: mp 205 °C dec; UV (EtOH) $\lambda_{max}$ 265.5 nm ($\epsilon$ 15300); $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 8.35 (s, 1 H, C8-H), 7.68 (bd s, 2 H, $NH_2$), 6.25 (pseudo q, 1 H, C1′-H, $J$ = 7.8, 2.8 Hz), all other resonances as expected. Anal. ($C_{10}H_{12}N_5O_3Br$) C, H, N, Br.

**6-Amino-9-(2-deoxy-α-D-ribofuranosyl)purine (α-Deoxyadenosine) (4).** A solution of **3** (75 mg, 0.227 mmol), potassium carbonate (55 mg 0.398 mmol), and 10% palladium on carbon (55 mg) in EtOH (11 mL) was sealed in a hydrogenation vessel. The vessel was evacuated, filled with hydrogen gas to 50 psi, and shaken for 20 h. The reaction mixture was filtered through a 0.45 μm filter and the solvent was evaporated. The residue, as a slurry in chloroform, was layered onto a short column of silica gel (1 g, 70–230 mesh), and the product was eluted in 20% methanol in chloroform (100 mL). The solvents were evaporated to give 52.5 mg (92%) of α-deoxyadenosine: mp 200 °C dec; UV ($H_2O$) $\lambda_{max}$ 260 nm ($\epsilon$ 13500) (lit.[14] mp 210–211 °C; UV ($H_2O$) $\lambda_{max}$ 262 nm ($\epsilon$ 14100)); $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 8.37 (s, 1 H, C8-H), 8.14 (s, 1 H, C2-H), 7.28 (bd s, 2 H, $NH_2$), 6.31 (pseudo q, 1 H, C1′-H, $J$ = 8.0, 3.0 Hz), 4.28 (m, 1 H, C3′-H), 4.11 (m, 1 H, C4′-H), 3.44 (m, 2 H, 5′,5″-H), 2.74 (m, 1 H, C2′-H, $J$ = 11.5, 8.0, 5.7 Hz), 2.32 (m, 1 H, C2″-H, $J$ = 11.5, 5.7, 3.0 Hz).

**2,6-Dibromo-7-(2-deoxy-3,5-di-*p*-toluyl-β-D-ribofuranosyl)purine (1b) and Its Isomers.** The sodium salt glycosylation of 2,6-dibromopurine (1 g, 3.6 mmol) was carried out as described by Wright et al.[7] The products were purified by HPLC (silica gel, 50 cm × 22.5 mm) following filtration of the 9-β isomer **1a** (481 mg, 20%) from toluene. Elution with 4% acetone in toluene (567 mL) gave an additional 644 mg (28%) of **1a**: $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 8.85 (s, 1 H, C8-H), 6.57 (pseudo t, 1 H, C1′-H, $J_{av}$ = 6.6 Hz), 5.81 (m, 1 H, C3′-H), ca. 4.62 (m, 3 H, 4′,5′,5″-H), 3.24 (m, 1 H, C2′-H), 2.84 (m, 1 H, C2″-H). Continued elution (425 mL) gave 274 mg (12%) of the 9-α isomer **1c**: $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 8.88 (s, 1 H, C8-H), 6.65 (pseudo t, 1 H, C1′-H, $J_{av}$ = 4.3 Hz), 5.63 (m, 1 H, C3′-H), 5.05 (m, 1 H, C4′-H), 4.54 (m, 2 H, 5′,5″-H), 3.21 (m, 1 H, C2′-H), 3.07 (m, 1 H, C2″-H). Further elution (851 mL) gave 151 mg (6.6%) of the 7-β isomer **1b**: mp 139–140 °C (from EtOH); UV (EtOH) $\lambda_{max}$ 283 nm ($\epsilon$ 9500); $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 9.16 (s, 1 H, C8-H), 6.91 (pseudo t, 1 H, C1′-H, $J_{av}$ = 6.3 Hz), 5.71 (m, 1 H, C3′-H), ca. 4.65 (m, 3 H, 4′,5′,5″-H), 3.15 (m, 1 H, C2′-H), 2.98 (m, 1 H, C2″-H). Anal. ($C_{26}H_{22}N_4O_5Br_2$) C, H, N, Br. Finally, elution with 536 mL gave 86 mg (3.8%) of the 7-α isomer **1d**: mp 125–128 °C (from MeOH); UV (EtOH) $\lambda_{max}$ 282 nm ($\epsilon$ 10100); $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 9.15 (s, 1 H, C8-H), 6.98 (bd d, 1 H, C1′-H, $J \simeq$ 6.3 Hz), 5.66 (m, 1 H, C3′-H), 5.23 (m, 1 H, C4′-H), 4.54 (m, 2 H, 5′,5″-H), 3.15 (m, 1 H, C2′-H), 2.92 (m, 1 H, C2″-H). Anal. ($C_{26}H_{22}N_4O_5Br_2$) C, H, N, Br.

**Glycosylation of 2,6-Bis(methylthio)purine.** A mixture of 2,6-bis(methylthio)purine (1.76 g, 8.26 mmol) and sodium hydride (60% suspension in mineral oil, 393 mg, 9.1 mmol) in acetonitrile (35 mL) was stirred at room temperature under nitrogen atmosphere for 30 min. 1-Chloro-2-deoxy-3,5-di-*p*-toluyl-α-D-*erythro*-pentofuranose (3.2 g, 8.26 mmol) was added in one portion and stirring was continued for 1 h. The mixture was diluted with an equal volume of chloroform (35 mL), filtered through Celite, and washed with chloroform (100 mL). The filtrate was concentrated to a minimum volume and layered onto a column of silica gel (80 g, 230–400 mesh). The combined products were eluted with chloroform (1.5 L), and after removal of solvent, the residue was mixed with 6% acetone in toluene. Following filtration of 1.89 g (41%) of **2a**, the solutes were purified by HPLC (silica gel, 50 cm × 22.5 mm). Elution with 6% acetone in toluene (394 mL) gave an additional 1.0 g (21%) of **2a**: mp 159–161 °C (from MeOH); UV (MeOH) $\lambda_{max}$ 238.5 nm ($\epsilon$ 38900), 256.5 nm (sh); $^1$H NMR ($CDCl_3$/$Me_2SO$-$d_6$) $\delta$ 8.26 (s, 1 H, C8-H), 6.55 (pseudo t, 1 H, C1′-H, $J_{av}$ = 6.8 Hz), 5.88 (m, 1 H, C3′-H), 4.62 (m, 3 H, 4′,5′,5″-H), 3.35 (m, 1 H, C2′-H), 2.79 (m, 1 H, C2″-H), 2.66 and 2.63 (s, 6 H, 2 × $SCH_3$). Anal. ($C_{28}H_{28}N_4O_5S_2$) C, H, N.

Continued elution (740 mL) gave 900 mg (18%) of the 7-β isomer **2b**: mp 92–95 °C (from MeOH); UV (MeOH) $\lambda_{max}$ 240.5 ($\epsilon$ 53900), 265 nm (sh); $^1$H NMR ($CDCl_3$) $\delta$ 8.48 (s, 1 H, C8-H), 6.75 (pseudo q, 1 H, C1′-H, $J$ = 7.5, 5.6 Hz), 5.69 (m, 1 H, C3′-H), 4.71 (m, 3 H, 4′,5′,5″-H), 2.97 (m, 1 H, C2′-H), 2.67 (m, 1 H, C2″-H), 2.73 and 2.64 (s, 6 H, 2 × $SCH_3$). Anal. ($C_{28}H_{28}N_4O_5S_2$) C, H, N.

Further elution (331 mL) gave 14 mg (0.3%) of a product tentatively identified as the 9-α isomer **2c**: mp 88–91 °C (from MeOH); UV (MeOH) $\lambda_{max}$ 236 ($\epsilon$ 40700), 245 and 256 nm (sh); $^1$H NMR ($CDCl_3$) $\delta$ 8.39 (s, 1 H, C8-H), 6.57 (pseudo q, 1 H, C1′-H, $J$ = 6.6, 1.4 Hz), 5.67 (m, 1 H, C3′-H), 4.91 (m, 1 H, C4′-H), 4.61 (m, 2 H, 5′,5″-H), 3.26 (m, 1 H, C2′-H), 3.0 (m, 1 H, C2″-H), 2.67 and 2.59 (s, 6 H, 2 × $SCH_3$). Finally, elution in 580 mL gave 11 mg (0.2%) of a fourth nucleoside tentatively identified as the 7-α isomer **2d**: $^1$H NMR ($CDCl_3$) $\delta$ 8.36 (s, 1 H, C8-H), 6.82 (bd d, 1 H, C1′-H, $J \simeq$ 5.7 Hz), 5.70 (m, 1 H, C3′-H), 4.95 (m, 1 H, C4′-H), 4.61 (m, 2 H, 5′,5″-H), 3.13 (m, 1 H, C2′-H), ca. 2.59 (m, 1 H, C2″-H), 2.72 and 2.65 (s, 6 H, 2 × $SCH_3$).

**2-Bromo-6-(methylamino)-9-(2-deoxy-β-D-ribofuranosyl)-purine (6).** A solution of **1a** (50 mg, 0.08 mmol) in toluene (1 mL) was treated with 15% methylamine in ether (0.16 mL, 0.256 mmol) and placed in a sealed container at 25 °C for 15 h. The solvent was evaporated, and the residue was treated with methanolic ammonia saturated at 0 °C (2 mL). The mixture was kept in a sealed container at 25 °C for 7 days. The solvent was evaporated, and the residue, as a slurry in chloroform, was layered onto a short column of silica gel (1 g, 70–230 mesh). The products were eluted in 5% methanol in chloroform (200 mL), and evaporation of solvents gave 21.5 mg (78%) of pure **6**: mp 106–109 °C; UV (pH 1) $\lambda_{max}$ 271.5 ($\epsilon$ 18800); UV (pH 7–12) $\lambda_{max}$ 277.5 nm ($\epsilon$ 14100); $^1$H NMR ($Me_2SO$-$d_6$, $D_2O$) $\delta$ 8.32 (s, 1 H, C8-H), 6.28 (pseudo t, 1 H, C1′-H, $J_{av}$ = 6.7 Hz), 2.91 (s, 3 H, N-$CH_3$), all other resonances as expected. Anal. ($C_{11}H_{14}N_5O_3Br$) C, H, N.

**2-Bromo-6-(dimethylamino)-9-(2-deoxy-β-D-ribofuranosyl)purine (7).** A solution of **1a** (500 mg, 0.8 mmol) in toluene (5 mL) was treated with 22% dimethylamine in ether (0.75 mL, 2.56 mmol) and placed in a sealed container at 25 °C for 52 h. The solvent was evaporated, and the residue was treated with methanolic ammonia saturated at 0 °C (10 mL). The mixture was heated in a steel bomb at 50 °C for 4 days. The solvent was evaporated, and the residue was adsorbed onto silica gel. The impregnated silica gel was placed on top of a column of silica gel (10 g, 70–230 mesh), and the products were eluted in 5% methanol in chloroform (350 mL). The solvents were evaporated to give 206 mg (72%) of pure **7**: mp 115–118 °C; UV (pH 1) $\lambda_{max}$ 295 ($\epsilon$ 10200); UV (pH 7–12) $\lambda_{max}$ 259 nm ($\epsilon$ 12500); $^1$H NMR ($Me_2SO$-$d_6$, $D_2O$) $\delta$ 8.35 (s, 1 H, C8-H), 6.28 (pseudo t, 1 H, C1′-H, $J_{av}$ = 6.7 Hz), 3.40 (s, 6 H, N-$(CH_3)_2$), all other resonances as

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expected. Anal. ($C_{12}H_{16}N_5O_3Br$) C, H, N.

**2-(Methylthio)-6-amino-9-(2-deoxy-β-D-ribofuranosyl)purine (8).** A mixture of **2a** (615 mg, 1.15 mmol) and methanolic ammonia saturated at 0 °C (20 mL) was heated in a steel bomb at 140–145 °C for 20 h. The solvent was evaporated, and the residue was dissolved in methanol. The solution was chromatographed on a silica gel plate (20 × 20 cm) in chloroform/methanol (9:1) as developing solvent. The product ($R_f$ 0.15) was crystallized from acetone to give 110 mg (32%) of **8**, mp 195–196 °C (lit.[21] mp 194–196 °C).

**2-(Methylthio)-6-(methylamino)-9-(2-deoxy-β-D-ribofuranosyl)purine (9).** A mixture of **2a** (1.08 g, 2 mmol) and 30% methanolic methylamine (30 mL) was heated in a steel bomb at 110 °C for 20 h. The solvent was evaporated, and the residue was adsorbed onto silica gel. The impregnated silica gel was placed on top of a silica gel column (2.5 × 15 cm), and the product was eluted with chloroform/methanol (9:1, 0.8 L). The nucleoside-containing fractions were evaporated, and the product was crystallized from ethyl acetate to give 410 mg (66%) of **9**: mp 107–109 °C; UV (pH 1) $\lambda_{max}$ 272 (ε 13900), 290 nm (sh); UV (pH 7–12) $\lambda_{max}$ 241 (ε 19900), 280 nm (ε 15400); $^1$H NMR ($Me_2SO$-$d_6$, $D_2O$) δ 8.18 (s, 1 H, C8-H), 6.28 (pseudo t, 1 H, C1′-H, $J_{av}$ = 6.5 Hz), 2.92 (s, 3 H, N-$CH_3$), 2.51 (s, 3 H, $SCH_3$), all other resonances as expected. Anal. ($C_{12}H_{17}N_5O_3S$) C, H, N.

**2-(Methylthio)-6-(dimethylamino)-9-(2-deoxy-β-D-ribofuranosyl)purine (10).** A mixture of **2a** (1.89 g, 3.5 mmol) and 30% methanolic dimethylamine (40 mL) was heated at 110 °C for 20 h. The solvent was evaporated, and the residue was adsorbed onto silica gel. The impregnated silica gel was placed on top of a silica gel column (3 × 15 cm), and the product was eluted with chloroform/methanol (9:1, 1 L). The nucleoside-containing fractions were evaporated, and the product was crystallized from ethyl acetate to give 590 mg (52%) of **10**, mp 183–185 °C; UV (pH 1) $\lambda_{max}$ 275 (ε 14700), 255 and 290 nm (sh); UV (pH 7–12) $\lambda_{max}$ 247 (ε 23000), 287 nm (ε 17100); $^1$H NMR ($Me_2SO$-$d_6$, $D_2O$) δ 7.94 (s, 1 H, C8-H), 6.24 (pseudo t, 1 H, C1′-H, $J_{av}$ = 6.5 Hz), 3.08 (s, 6 H, $N(CH_3)_2$), 2.50 (s, 3 H, $SCH_3$), all other resonances as expected. Anal. ($C_{13}H_{19}N_5O_3S$) C, H, N.

**2-(Dimethylamino)-6-amino-9-(2-deoxy-β-D-ribofuranosyl)purine (11).** A solution of **5** (220 mg, 0.67 mmol) in 30% methanolic dimethylamine (10 mL) was heated in a steel bomb at 120 °C for 20 h. The solvent was evaporated to dryness, and the residue was crystallized from ethanol to give 145 mg (73%) of **11**, mp 204–206 °C; UV (pH 1) $\lambda_{max}$ 260 (ε 14600), 305 nm (ε 7800); UV (pH 7–12) $\lambda_{max}$ 261.5 nm (ε 13000), 295.5 nm (ε 8100); $^1$H NMR ($Me_2SO$-$d_6$, $D_2O$) δ 7.91 (s, 1 H, C8-H), 6.20 (pseudo t, 1 H, C1′-H, $J_{av}$ = 6.5 Hz), 3.09 (bd s, 6 H, $N(CH_3)_2$), all other resonances as expected. Anal. ($C_{12}H_{18}O_3N_6$) C, H, N.

**2,6-Bis(dimethylamino)-9-(2-deoxy-β-D-ribofuranosyl)purine (12).** A solution of **1a** (0.89 g, 1.4 mmol) in 30% methanolic dimethylamine (30 mL) was heated at 130 °C for 16 h. The solvent was evaporated, and the residue was crystallized from ethyl acetate and then from water to give 320 mg (71%) of **12**; mp 207–208 °C; UV (pH 1) $\lambda_{max}$ 244 (ε 12000), 263 nm (ε 13600), 285 nm (sh); UV (pH 7–12) $\lambda_{max}$ 247 (ε 18700), 295 nm (ε 8700); $^1$H NMR ($Me_2SO$-$d_6$, $D_2O$) δ 7.93 (s, 1 H, C8-H), 6.24 (pseudo t, 1 H, C1′-H, $J_{av}$ = 6.5 Hz), 3.44 (s, 6 H, 6-$N(CH_3)_2$), 3.09 (s, 6 H, 2-$N(CH_3)_2$), all other resonances as expected. Anal. ($C_{14}H_{22}N_6O_3$) C, H, N.

**2,6-Bis(methylthio)-9-(2-deoxy-β-D-ribofuranosyl)purine (13).** A mixture of **2a** (1.2 g, 2.2 mmol) and methanolic ammonia saturated at 0 °C (30 mL) was heated in a steel bomb at 80 °C for 15 h. The solvent was evaporated and the residue was adsorbed onto silica gel. The impregnated silica gel was placed on top of a silica gel column (3 × 15 cm), and the product was eluted with chloroform/methanol (9:1, 1 L). The combined nucleoside-containing fractions were evaporated, and the product was crystallized from water to give 510 mg (70%) of **13**, mp 143–145 °C; UV (pH 1) $\lambda_{max}$ 264 (ε 21200), 312.5 nm (ε 11600); UV (pH 7–12) $\lambda_{max}$ 261 (ε 25000), 309 nm (ε 14500); $^1$H NMR ($Me_2SO$-$d_6$, $D_2O$) δ 8.45 (s, 1 H, C8-H), 6.32 (pseudo t, 1 H, C1′-H, $J_{av}$ = 6.5 Hz), 2.61 and 2.56 (s, 6 H, 2 × $SCH_3$), all other resonances as expected. Anal. ($C_{12}H_{16}N_4O_3S_2$) C, H, N.

**Cytotoxicity Studies.** Toxicity of the compounds was determined by their effects on protein synthesis in cells in culture. The cell lines (Table I) were generous gifts from Dr. Leif Andersson, Department of Pathology, University of Helsinki. Test compounds were added to cultures in 96-well microplates containing $10^4$ cells per 200-μL well. Cells were cultured in RPMI 1640 medium containing glutamine (2 mM), penicillin (100 units/mL), streptomycin (100 μg/mL), and fetal calf serum (10%, v/v), in humidified atmosphere containing 5% $CO_2$ at 37 °C. [U-$^{14}$C]-L-Leucine (specific activity, 1.3 mCi/mmol and 0.5 μCi/mL) was added to the cultures for the final 24 h of the 3-day culture period. After incubation the proteins were precipitated with 0.2 N perchloric acid and collected on glass fiber filters with use of a multiple cell harvester (Cell Harvester D-001, Flow Laboratories). The radioactivity incorporated into proteins was measured in a scintillation spectrophotometer (LKB-Wallac, 81000). The incorporation of [$^{14}$C]leucine per cell remained constant during the final 24 h of culture, and there was a good correlation between cell number and [$^{14}$C]leucine incorporation per cell.[18,19]

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