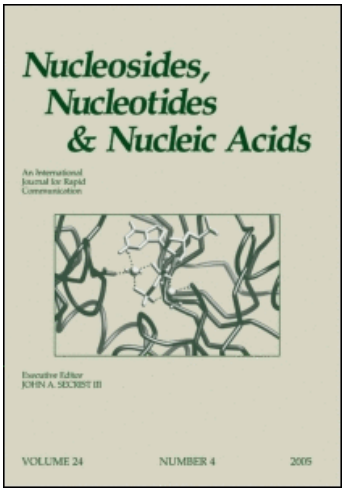

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SYNTHESIS AND BIOLOGIC EVALUATION OF 8-SUBSTITUTED DERIVATIVES OF NEBULARINE (9-β-D-RIBOFURANOSYLPURINE)

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Abstract

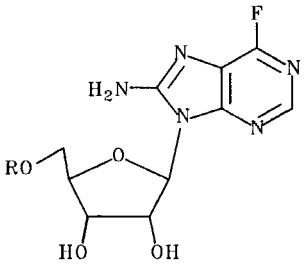
A series of 8-substituted purine ribonucleosides were prepared from 2',3',5'-tri-*O*-acetyl-8-bromoadenosine and evaluated for cytotoxicity and antiviral activity. Four of these nucleosides (**6b**-**9b**) were significantly toxic to both HEp-2 and L1210 cells in culture but the most cytotoxic one (**9b**) was inactive against the P388 leukemia in mice. None of these nucleosides showed significant antiviral activity against Herpes Simplex 1 or 2, vaccinia, or influenza A.

Introduction

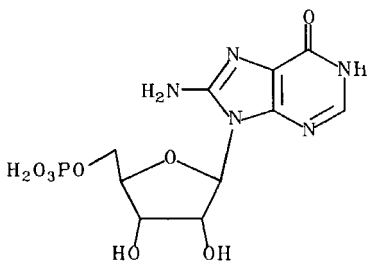
8-Amino-6-fluoropurine ribonucleoside (**1**), prepared by the diazotization of 8-aminoadenosine,[1] is cytotoxic to HEp-2 and L1210 cells in culture with an IC_{50} of about 2 μM. The major metabolite (>90%) of **1** in these cells is 8-aminoinosinic acid (**3**) formed by the hydrodefluorination of the 5'-monophosphate **2** by adenylate deaminase. The 5'-monophosphate **2** is formed by the phosphorylation of **1** by adenosine kinase. The nucleotide **3** is not detectably metabolized further. Since 8-aminoinosine is not phosphorylated in these cells, its cytotoxicity cannot readily be determined, but coformycin increases the cytotoxicity of **1** by almost an order of magnitude, indicating that 8-aminoinosine is at best only weakly cytotoxic.[1] These results led us to prepare a number of 8-substituted purine ribonucleosides including 8-aminopurine ribonucleoside (**9b**) which would be unaffected by adenosine deaminase (although it could be oxidized to 8-aminoinosine[2]). The 5'-monophosphate of **9b** would likewise be unaffected by adenylate deaminase. Purine ribonucleoside itself (nebularine) behaves biologically as an adenosine analog, is cytotoxic,[3-5] and is rapidly phosphorylated by adenosine kinase[5] and on to the triphosphate, its toxic metabolite.[6] When given to L1210 leukemic mice along with 6-(4-nitrobenzylthio)purine ribonucleoside, a nucleoside transport inhibitor, it is curative.[7]

Chemistry

Our synthesis of this series began with the preparation of **4a** from commercially-available 8-bromoadenosine.[8] A clean conversion of **4a** to **6a** was achieved in an anhydrous diazotization/deamination reaction with n-pentyl nitrite in refluxing THF.[9] This crystalline 8-bromo intermediate (**6a**) was used in subsequent displacement reactions to produce structures **8a**, **10a**, **11a**, **12b**, **13b**, and **15a**. The most rapid of these reactions occurred when **6a** was treated



1: R = H
2: R = PO_3H_2



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with NaN_3 in DMSO to yield **8a**. Compound **11a** was also prepared in DMSO from **6a** using $NaSCH_3$. The remaining bromo displacements that yielded **10a**, **12b**, **13b**, and **15a** were carried out in an alcohol with either dimethylamine, sodium methoxide, sodium benzoxide, or thiourea.[10,11] Catalytic hydrogenolysis at atmospheric pressure of the azido group of **8a** or benzyloxy group of **13b** gave the 8-amino (**9a**) or 8-oxo (**14b**) nucleoside. The one (**14b**) and thione (**15a** and **b**) structural assignments were made on the basis of the literature[11-13] and were confirmed by our spectral data, particularly the ^{13}C nmr. For those compounds requiring deacylation, NaOH (~3 eq) in 1:1 MeCN:H_2O caused rapid acetyl removal without loss of the 8-substituent except in the case of the 8-halonucleosides **6a** and **7a**.

All of the conventional deprotection methods used for **6a** (8-Br) and **7a** (8-Cl) resulted in halogen displacement or partial deacetylation. By treating a buffered suspension of **6a** or **7a** with pig liver esterase,[14] we were able to obtain the desired targets **6b** and **7b** that were free of any side-products. To our knowledge, deacetylation of a nucleoside with this particular enzyme has not been reported. Use of goat intestine esterase for this purpose was described earlier.[15]

During the course of this work, a variety of reaction conditions were tried to introduce a chlorine into the 8-position of purine riboside. Attempts at halogen exchange failed to produce the 8-chloro (**7a**) cleanly from the 8-bromo (**6a**) nucleoside. A deamination/halogenation reaction of 8-amino (**9a**) using n-pentyl nitrite in refluxing CCl_4 as a chlorine atom donating solvent gave purine ribonucleoside triacetate as the major product.[9] Substituting *t*-butyl nitrite reduced this by-product, but due to the low solubility of **9a** in CCl_4, the yield of **7a** remained low even with the use of co-solvents. Consequently, we elected to prepare 8-chloroadenosine (**5b**)[16,17] which was acetylated (**5a**), deaminated to **7a**, and deprotected to **7b** by the route used for **6b** with one exception. The deamination was done in THF using *t*-butyl nitrite which reduced the reaction time from 90 h to 1 h. This time reduction proved important since prolonged heating produced highly-colored side-products from the less stable 8-Cl nucleoside (**7a**).

Biological Results

The target compounds were evaluated for cytotoxicity to the HEp-2 and L1210 cell lines in culture.[1] Some interesting structure-activity relationships were observed (Table). Compounds

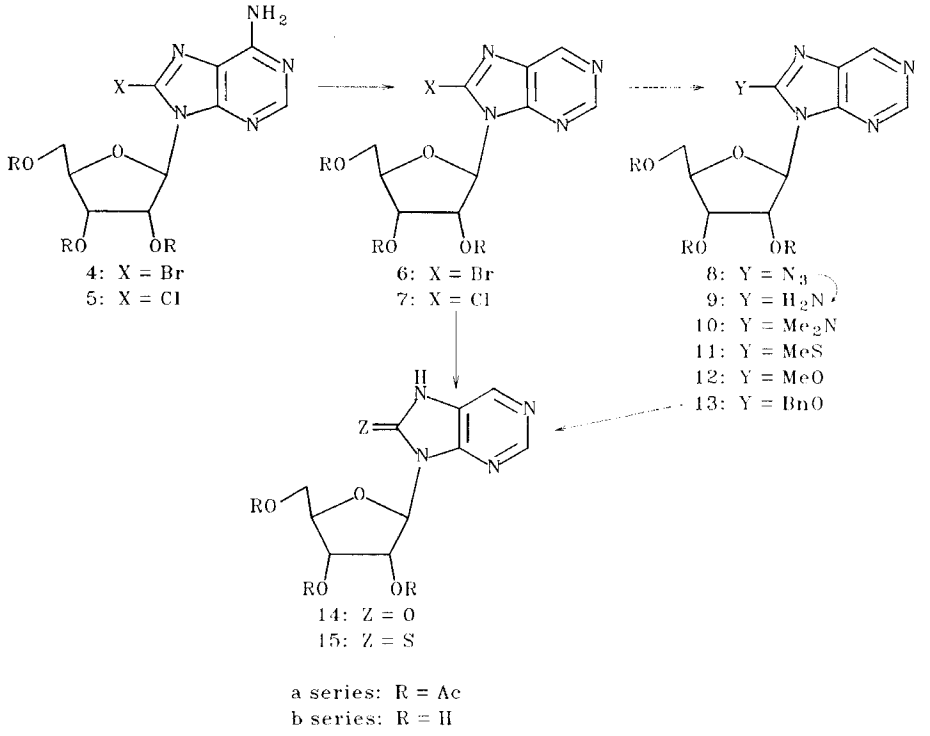
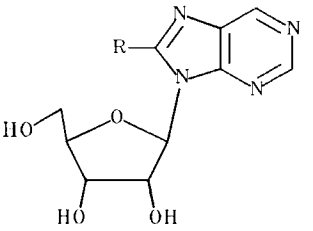


Table. Cytotoxicity Data



Compound Number	R	IC_{50} (μM)	
		HEp-2	L1210
Nebularine	H	0.08	-
9b	NH_2	0.3	3-4
8b	N_3	0.5	10
6b	Br	2	2
7b	Cl	<3	<3
11b	MeS	~7	I[a]
15b	HS (=S)	~60	I
10b	Me_2N	I	I
12b	MeO	I	I
14b	HO (=O)	I	I

[a]IC_{50} >130, the highest level tested.

10b, **12b**, **14b**, and **15b** showed little toxicity to either cell line. Compound **11b** was toxic to HEp-2 cells only. The halo (**6b** and **7b**), azido (**8b**), and amino (**9b**) compounds were significantly toxic to both cell lines, but none of them was as potent as purine ribonucleoside itself. These compounds were all tested as substrates for adenosine kinase[1] and all of the cytotoxic compounds were substrates, whereas the inactive compounds were not. 8-Aminopurine ribonucleoside (**9b**) was selected for further study. It (**9b**) was facilely converted to the triphosphate in L1210 cells, but was not cytotoxic (>150 μM) to mutant L1210 cell lines deficient in adenosine kinase activity compared to 3-4 μM in the parent line.[1] Further testing of **9b** against the P388 leukemia in mice produced no increase in lifespan up to a toxic dose of 400 mg/kg on a qd 1-5 schedule.[18] Antiviral evaluation of **9b**, **10b**, and **11b** against Herpes Simplex Types I (E-377) and II (MS) in Vero cell monolayers showed no effect.[19] Additional tests against influenza A in MDCK cells and against vaccinia were essentially negative.[20]

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. Microanalyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and by the Molecular Spectroscopy Section of Southern Research Institute. Analytical results indicated by elemental symbols were within $\pm$0.4% of the theoretical values. Where solvents were noted as part of the elemental analysis, they were seen in the ^{1}H-NMR spectrum in the proper amounts. Mass spectra were recorded on a Varian/MAT 311A double-focusing mass spectrometer in the fast atom bombardment (FAB) mode. 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 0.1N HCl, pH 7 buffer, and 0.1N NaOH. Maxima are expressed in nanometers with the extinction coefficient given in parentheses (ϵ x 10^{-3}); sh=shoulder. Infrared spectra were recorded on a Nicolet FT IR spectrometer, Model 10 DX. HPLC analyses were carried out on a Hewlett Packard 1084B liquid chromatograph equipped with a Waters Associates μBondapak C_{18} column (3.9 mm x 30 cm) and a UV detector monitoring at 254 nm. All flash column chromatography used 230-400 mesh silica gel from E. Merck. TLC examinations were done on Analtech precoated (250 μm) silica gel (GF) plates. The Bio-Beads SM-4 macroporous adsorbent (20-50 mesh) were obtained from Bio-Rad. Esterase from porcine liver was purchased from Sigma as a suspension in aqueous $(NH_4)_2SO_4$. 8-Bromoadenosine was also purchased from Sigma.

8-Chloro-2',3',5'-tri-*O*-acetyladenosine (5a). A solution of 8-chloroadenosine (**5b**)[17] (2.11 g, 7 mmol) in 60 mL of anhydrous pyridine was chilled to 5 °C and treated dropwise with acetic anhydride (6 mL). The reaction was stirred at room temperature for 16-h and poured into ice water (250 mL). After 15 min of stirring, $CHCl_3$ (150 mL) was added, and the layers were separated. The aqueous layer was extracted with additional $CHCl_3$ (2 x 100 mL), and the

combined organic extracts were washed with 5% aqueous $NaHCO_3$ (90 mL) and water (3 x 100 mL), dried ($MgSO_4$), and evaporated. The residue was evaporated in vacuo with toluene and then dissolved in hot EtOH. The solid that formed at room temperature was chilled, collected, washed with cold EtOH, and dried in vacuo at room temperature to give pure **5a**: yield 2.63 g (88%); mp 154-156 °C; TLC 95:5 $CHCl_3$-MeOH, R_f 0.55; MS, *m/z* 428 $(M + 1)^+$.

8-Bromo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (6a). A solution of **4a**[8] (6.0 g, 12.7 mmol) in 250 mL dry THF under a nitrogen atmosphere was added portionwise over 1.5 h to a stirred refluxing solution of *n*-pentyl nitrite (34 mL, 256 mmol) in 250 mL dry THF. A 24-h TLC aliquot indicated incomplete reaction. More *n*-pentyl nitrite (4 mL, 30 mmol) was added, and the reflux was continued for a total reaction time of 90 h. The reaction was evaporated to give a yellow-orange residue that was solidified by EtOH trituration to give essentially pure **6a**: yield 2.5 g (43%). The filtrate was evaporated, and the residue dissolved in $CHCl_3$ was applied to a flash column containing 150 g of silica gel. The column was eluted with $CHCl_3$, and the pure fractions were combined and crystallized from EtOH to give more material: yield 1.6 g (27%). An analytical sample was obtained by a second recrystallization from EtOH; mp 115 °C; TLC 95:5 $CHCl_3$-MeOH, R_f 0.65; HPLC 99%, linear gradient, 50→95% acetonitrile in water, 20 min; MS, *m/z* 457 $(M + 1)^+$; ^1H NMR ($CDCl_3$) δ 9.06 (s, 1, H_6), 8.97 (s, 1, H_2), 6.38 (dd, 1, $H_{2'}$, $J_{1',2'}$ = 4.4, $J_{2',3'}$ = 6.0 Hz), 6.18 (d, 1, $H_{1'}$), 5.93 (t, 1, $H_{3'}$, $J_{3',4'}$ = 5.9 Hz), 4.52 (dd, 1, $H_{5'a}$, $J_{4',5'a}$ = 3.4, $J_{5'a,5'b}$ = 11.9 Hz), 4.42 (m, 1, $H_{4'}$, $J_{4',5'b}$ = 5.5 Hz), 2.17 (s, 3, CH_3), 2.12 (s, 3, CH_3), 2.03 (s, 3, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.4, 169.4, 169.3 (3 C=O's), 152.6 (C_2, J_{C_2,H_2} = 207.5, J_{C_2,H_6} = 10.9 Hz), 151.7 (C_4), 147.7 (C_6, J_{C_6,H_6} = 186.2, J_{C_6,H_2} = 10.4 Hz), 134.7 (C_8, J_{C_8,H_2} = 5.0 Hz), 133.8 (C_5, J_{C_5,H_2} = 4.6 Hz), 88.7 ($C_{1'}$, $J_{C_{1'},H_{1'}}$ = 166.3 Hz), 80.2 ($C_{4'}$), 71.9 ($C_{2'}$), 70.3 ($C_{3'}$), 62.8 ($C_{5'}$), 20.6, 20.5, 20.4 (3 CH_3's). Anal. calcd for $C_{16}H_{17}BrN_4O_7$: C, 42.03; H, 3.75; N, 12.25. Found: C, 41.82; H, 3.81; N, 12.18.

8-Bromo-9-(β-D-ribofuranosyl)purine (6b). A suspension of **6a** (150 mg, 0.33 mmol) in 15 mL of 1% NH_4HCO_3 was treated with porcine liver esterase (858 units, 300 μL, Sigma) and stirred at room temperature. The mixture became a clear solution after 3 h. A 22-h TLC aliquot showed the absence of starting material and the presence of partially deblocked products. More esterase (143 units, 50 μL) was added on day 4 and on day 5. On day 6, the white solid that had precipitated during the course of the reaction was collected by filtration, washed with water, and dried in vacuo to give crude **6b**, 73 mg. This solid was recrystallized from 7 mL of 1:1 DMF-EtOH with Celite filtration to remove insoluble material. The crystals that formed at room temperature were collected after 16 h at -20 °C, washed with cold EtOH, and dried in vacuo for 16 h at 56 °C to give pure **6b**: yield 42 mg (39%); mp 155-160 °C dec; TLC 7:1 $CHCl_3$-MeOH, R_f 0.55; HPLC 99%, 85:15 H_2O-MeCN; MS, *m/z* 331 $(M + 1)^+$; ^1H NMR (Me_2SO-d_6) δ 9.18 (s, 1, H_6), 8.96 (s, 1, H_2), 5.94 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.2 Hz), 5.53 (d, 1, 2'-OH, $J_{2',2'\text{-OH}}$ = 6.0 Hz), 5.31 (d, 1, 3'-OH, $J_{3',3'\text{-OH}}$ = 5.0 Hz), 5.25 (m, 1, $H_{2'}$, $J_{2',3'}$ = 5.2 Hz), 4.92 (dd, 1, 5'-OH, $J_{5'a,5'\text{-OH}}$ = 5.3 and $J_{5'b,5'\text{-OH}}$ = 6.7 Hz), 4.30 (m, 1, $H_{3'}$, $J_{3',4'}$ = 3.2 Hz), 3.96 (m, 1, $H_{4'}$), 3.70 (m, 1, $H_{5'a}$, $J_{5'a,5'b}$ = 11.8, $J_{4',5'a}$ = 4.9 Hz), 3.53 (m, 1, $H_{5'b}$, $J_{4',5'b}$ = 5.4 Hz);

^{13}C NMR (Me_2SO-d_6) δ 151.9 (C_2, J_{C_2,H_2} = 207.0, J_{C_2,H_6} = 10.9 Hz), 151.3 (C_4, J_{C_4,H_2} = 11.6, $J_{C_4,H_{1'}}$ = 5.5, J_{C_4,H_6} = 5.5 Hz), 147.3 (C_6, J_{C_6,H_6} = 186.3, J_{C_6,H_2} = 10.3 Hz), 135.4 (C_8, $J_{C_8,H_{1'}}$ = 4.0 Hz), 134.3 (C_5, J_{C_5,H_6} = 6.6 Hz), 90.4 ($C_{1'}$, $J_{C_{1'},H_{1'}}$ = 164.3 Hz), 86.1 ($C_{4'}$), 70.6 ($C_{2'}$), 70.3 ($C_{3'}$), 61.6 ($C_{5'}$). Anal. calcd for $C_{10}H_{11}BrN_4O_4 \cdot 0.04$ DMF $\cdot 0.04$ EtOH $\cdot 0.20$ H_2O: C, 36.09; H, 3.54; N, 16.67. Found: C, 36.35; H, 3.40; N, 16.87.

8-Chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (7a). A solution of **5a** (1.0 g, 2.34 mmol) in 40 mL anhydrous THF under a nitrogen atmosphere was treated in one portion with *t*-butyl nitrite (3.6 g, 35 mmol). The reaction was placed in a 95 °C bath and allowed to reflux gently for 1 h. A TLC aliquot indicated complete reaction, and the solution was evaporated to dryness. This residue in $CHCl_3$ was applied to a flash column containing 135 g of silica gel with $CHCl_3$ as eluant followed by 97:3 $CHCl_3$-MeOH. The crude material obtained was further purified on silica gel thick plates (Analtech, GF, 2000 μm) in 97:3 $CHCl_3$-MeOH and then 3:1 ethyl acetate-cyclohexane to give pure **7a**: yield 380 mg (39%); TLC 95:5 $CHCl_3$-MeOH, R_f 0.65; MS, *m/z* 413 $(M + 1)^+$; ^1H NMR ($CDCl_3$) δ 9.05 (s, 1, H_6), 8.98 (s, 1, H_2), 6.35 (dd, 1, $H_{2'}$, $J_{1',2'}$ = 4.5, $J_{2',3'}$ = 6.0 Hz), 6.19 (d, 1, $H_{1'}$), 5.91 (t, 1, $H_{3'}$, $J_{3',4'}$ = 5.8 Hz), 4.52 (A part of an ABC spin system, 1, $H_{5'a}$, $J_{5'a,5'b}$ = 11.8, $J_{4',5'a}$ = 3.5 Hz), 4.43 (C part of an ABC spin system, 1, $H_{4'}$, $J_{4',5'b}$ = 5.6 Hz), 4.32 (B part of an ABC spin system, 1, $H_{5'b}$), 2.18 (s, 3, CH_3), 2.11 (s, 3, CH_3), 2.04 (s, 3, CH_3).

8-Chloro-9-(β-D-ribofuranosyl)purine (7b). A suspension of **7a** (379 mg, 0.92 mmol) in 25 mL of 2% NH_4HCO_3 was treated with porcine liver esterase (450 μL, 1287 units, Sigma), stirred at room temperature, and examined periodically by TLC. On day 2, more enzyme was added (100 μL, 286 units), and the stirring was continued. On day 5, the essentially complete reaction was filtered and passed through a 1.5 x 30 cm column of Bio-Beads SM-4, 20-50 mesh, equilibrated with water, at a flow of ~1 mL/min. The column was eluted with water followed by a stepwise gradient from 9:1 to 3:1 water-EtOH. The pure fractions were combined, evaporated, and recrystallized from 95% EtOH. After 16 h at 0 °C, the solid was collected, washed with cold EtOH, and dried in vacuo at 56 °C for 16 h to give pure **7b**: yield 97 mg (37%); mp 157 °C dec; TLC 7:1 $CHCl_3$-MeOH, R_f 0.55; HPLC 99%, 85:15 H_2O-MeCN; MS, *m/z* 287 $(M + 1)^+$; UV λ_{max} pH 1 267 (7.79), pH 7 245 (sh), 266 (9.41), pH 13 266 (9.00); ^1H NMR (Me_2SO-d_6) δ 9.19 (s, 1, H_6), 9.00 (s, 1, H_2), 5.96 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.0 Hz), 5.55 (d, 1, 2'-OH, $J_{2',2'\text{-OH}}$ = 5.8 Hz), 5.33 (d, 1, 3'-OH, $J_{3',3'\text{-OH}}$ = 4.9 Hz), 5.19 (m, 1, $H_{2'}$, $J_{2',3'}$ = 5.3 Hz), 4.92 (t, 1, 5'-OH, $J_{5',5'\text{-OH}}$ = 5.9 Hz), 4.30 (m, 1, $H_{3'}$, $J_{3',4'}$ = 3.3 Hz), 3.97 (m, 1, $H_{4'}$), 3.69 (m, 1, $H_{5'a}$, $J_{4',5'a}$ = 3.9, $J_{5'a,5'b}$ = 12.0 Hz), 3.54 (m, 1, $H_{5'b}$, $J_{4',5'b}$ = 5.9 Hz); ^{13}C NMR (Me_2SO-d_6) δ 152.1 (C_2, J_{C_2,H_2} = 207.2, J_{C_2,H_6} = 10.9 Hz), 151.2 (C_4, J_{C_4,H_2} = 11.6, $J_{C_4,H_{1'}}$ = 5.4, J_{C_4,H_6} = 5.6 Hz), 147.4 (C_6, J_{C_6,H_6} = 186.5, J_{C_6,H_2} = 10.4 Hz), 144.2 (C_8, $J_{C_8,H_{1'}}$ = 4.5 Hz), 132.7 (C_5, J_{C_5,H_6} = 5.8 Hz), 89.2 ($C_{1'}$, $J_{C_{1'},H_{1'}}$ = 164.6 Hz), 86.1 ($C_{4'}$), 70.7 ($C_{2'}$), 70.2 ($C_{3'}$), 61.5 ($C_{5'}$). Anal. calcd for $C_{10}H_{11}ClN_4O_4$: C, 41.90; H, 3.87; N, 19.54. Found: C, 42.15; H, 3.73; N, 19.69.

8-Azido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (8a). A solution of **6a** (300 mg, 0.66 mmol) in 6 mL sieve-dried DMSO was treated in one portion with solid NaN_3 (120 mg, 1.8

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dryness and the residue crystallized from 75 mL boiling H_2O with charcoal treatment to give pure **9b**, that was dried in vacuo at 56 °C for 16 h: yield from 8-Br (**6a**) 1.4 g (61%); mp 219-221 °C dec; TLC 3:1 $CHCl_3$-MeOH + 1% HOAc, R_f 0.45; HPLC 100%, 90:10 H_2O-MeCN; MS, *m/z* 268 $(M + 1)^+$; UV λ_{max} pH 1 276 (9.58), 305 (sh), pH 7 252 (6.70), 285 (10.9), pH 13 252 (5.94), 286 (10.8); ^1H NMR (Me_2SO-d_6) δ 8.50 (s, 1, H_2), 8.44 (s, 1, H_6), 7.38 (bs, 2, 8-NH_2), 6.02 (d, 1, $H_{1'}$, $J_{1',2'}$ = 7.4 Hz), 5.65 (t, 1, 5'-OH, $J_{5',5'\text{-OH}}$ = 5.7 Hz), 5.36 (d, 1, 2'-OH, $J_{2',2'\text{-OH}}$ = 6.4 Hz), 5.20 (d, 1, 3'-OH, $J_{3',3'\text{-OH}}$ = 4.2 Hz), 4.70 (m, 1, $H_{2'}$, $J_{2',3'}$ = 5.3 Hz), 4.16 (m, 1, $H_{3'}$, $J_{3',4}$ = 1.9 Hz), 4.00 (m, 1, $H_{4'}$, $J_{4',5'}$ = 2.4 Hz), 3.63 (m, 1, $H_{5'}$); ^{13}C NMR (Me_2SO-d_6) δ 155.2 (C_8, $^3J_{C_8,H_{1'}}$ = 4.9 Hz), 152.5 (C_4, $^3J_{C_4,H_{1'}}$ = 4.0, J_{C_4,H_2} = 10.2, J_{C_4,H_6} = 5.5 Hz), 147.5 (C_2, J_{C_2,H_2} = 203.5, J_{C_2,H_6} = 10.5 Hz), 139.0 (C_6, J_{C_6,H_6} = 181.2, J_{C_6,H_2} = 10.7 Hz), 134.4 (C_5, J_{C_5,H_6} = 6.8 Hz), 86.2 ($C_{1'}$, $J_{C_{1'},H_{1'}}$ = 161.6 Hz), 85.6 ($C_{4'}$), 70.8 ($C_{3'}$), 70.4 ($C_{2'}$), 61.4 ($C_{5'}$). Anal. calcd. for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.90; N, 26.21. Found: C, 44.74; H, 5.06; N, 25.94.

8-*N,N*-Dimethylamino-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (10a). A solution of **6a** (200 mg, 0.44 mmol) in 20 mL anhydrous EtOH at 5 °C was treated with ethanolic 3.2 M $(CH_3)_2NH$ (0.68 mL, 2.18 mmol). The reaction protected from moisture was stirred at 5 °C for 2 h. A TLC aliquot showed the absence of **6a**. The reaction was evaporated to dryness, and the residue was dried in vacuo at room temperature for 16 h to give crude **10a**, 233 mg. TLC 95:5 $CHCl_3$-MeOH, R_f 0.45; MS, *m/z* 422 $(M + 1)^+$. This material was used in the next step without further purification.

8-*N,N*-Dimethylamino-9-(β-D-ribofuranosyl)purine (10b). A solution of **10a** (~0.44 mmol) in 35 mL anhydrous EtOH saturated with NH_3 at 5 °C was allowed to stand in a bomb at room temperature for 3 days before it was evaporated to dryness. This residue in MeOH was applied to one silica gel thick plate (Analtech, GF, 2000 μm) that was developed three times with 5:1 $CHCl_3$-MeOH. The product was extracted with MeOH, crystallized twice from boiling MeCN, and dried in vacuo at 56 °C for 16 h to give pure **10b**: yield from 8-Br (**6a**) 56 mg (43%); mp 145-146 °C; TLC 5:1 $CHCl_3$-MeOH, R_f 0.60; HPLC 99%, 85:15 H_2O-MeCN; MS, *m/z* 296 $(M + 1)^+$; UV λ_{max} pH 1 306 (19.0), pH 7 262 (7.52), 293 (12.0), pH 13 263 (6.91), 293 (12.0); ^1H NMR (Me_2SO-d_6) δ 8.75 (s, 1, H_6), 8.67 (s, 1, H_2), 5.71 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.6 Hz), 5.42 (d, 1, 2'-OH, $J_{2',2'\text{-OH}}$ = 6.2 Hz), 5.25 (m, 1, $H_{2'}$, $J_{2',3'}$ = 6.4 Hz), 5.21 (d, 1, 3'-OH, $J_{3',3'\text{-OH}}$ = 4.9 Hz), 5.03 (dd, 1, 5'-OH, $J_{5'a,5'\text{-OH}}$ = 7.1, $J_{5'b,5'\text{-OH}}$ = 4.9 Hz), 4.23 (m, 1, $H_{3'}$, $J_{3',4'}$ = 2.9 Hz), 3.92 (m, 1, $H_{4'}$, $J_{4',5'a}$ = 4.9, $J_{4',5'b}$ = 5.2 Hz), 3.69 (m, 1, $H_{5'a}$, $J_{5'a,5'b}$ = 11.8 Hz), 3.55 (m, 1, $H_{5'b}$), 3.03 (s, 6, N-CH_3); ^{13}C NMR (Me_2SO-d_6) δ 160.1 (C_8), 152.5 (C_4, J_{C_4,H_2} = 11.3, $J_{C_4,H_{1'}}$ = J_{C_4,H_6} = 5.6 Hz), 149.1 (C_2, J_{C_2,H_2} = 204.7, J_{C_2,H_6} = 10.6 Hz), 142.7 (C_6, J_{C_6,H_6} = 183.0, J_{C_6,H_2} = 10.7 Hz), 133.6 (C_5, J_{C_5,H_6} = 6.5, J_{C_5,H_2} = 1.4 Hz), 88.7 ($C_{1'}$, $J_{C_{1'},H_{1'}}$ = 161.5 Hz), 85.7 ($C_{4'}$), 70.5 ($C_{3'}$), 69.9 ($C_{2'}$), 61.9 ($C_{5'}$), 41.8 (CH_3). Anal. calcd for $C_{12}H_{17}N_5O_4$: C, 48.81; H, 5.80; N, 23.72. Found: C, 49.05; H, 6.03; N, 23.84.

8-(Methylthio)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (11a). A solution of **6a** (300 mg, 0.66 mmol) in 6 mL sieve-dried DMSO was treated in one portion with solid $NaSCH_3$ (138 mg, 1.97 mmol). The reaction was stirred at room temperature for 2 h, then evaporated to

dryness. The residue was partitioned between $CHCl_3$ and water. The aqueous layer was extracted twice with $CHCl_3$. The combined organic layer was washed twice with water, dried ($MgSO_4$), and evaporated to give crude **11a**: yield 121 mg (44%). This material was used without further purification in the next step.

8-(Methylthio)-9-(β-D-ribofuranosyl)purine (11b). A solution of **11a** (121 mg, 0.29 mmol) in 1:1 MeCN-H_2O (12 mL) was treated in one portion with 1 N NaOH (0.8 mL). After being stirred at room temperature for 1 h, the reaction was complete as indicated by TLC. At 1.5 h, the solution was neutralized with glacial acetic acid and evaporated. The resulting residue was dissolved in MeOH and applied to one silica gel thick plate (Analtech, GF, 2000 μm) that was developed twice with 5:1 $CHCl_3$-MeOH. The major band was extracted with MeOH to give essentially pure **11b**, 66 mg.

Some partially blocked material was recovered from the aqueous layer of **11a**. Treatment with 1 N NaOH and chromatography as above gave more product, 37 mg.

The two residues were combined in hot water and allowed to crystallize at room temperature. The white solid was collected after being chilled, washed with cold water, and dried in vacuo at 56 °C for 16 h to give pure **11b**: yield from 8-Br (**6a**) 69 mg (35%); mp 192-193 °C; TLC 5:1 $CHCl_3$-MeOH, R_f 0.50; HPLC 99%, linear gradient, 10→90% acetonitrile in water, 20 min; MS, *m/z* 299 $(M + 1)^+$; UV λ_{max} pH 1 238 (12.3), 300 (13.7), pH 7 254 (5.48), 288 (17.3), pH 13 254 (5.11), 289 (17.2). ^1H NMR (Me_2SO-d_6) δ 9.02 (s, 1, H_6), 8.82 (s, 1, H_2), 5.79 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.4 Hz), 5.50 (bs, 1, 2'-OH), 5.28 (bs, 1, 3'-OH), 5.12 (m, 1, $H_{2'}$), 5.00 (bs, 1, 5'-OH), 4.23 (m, 1, $H_{3'}$, $J_{2',3'}$ = 5.3, $J_{3',4'}$ = 3.1 Hz), 3.96 (m, 1, $H_{4'}$, $J_{4',5'a}$ = 5.0, $J_{4',5'b}$ = 4.9 Hz), 3.69 (m, 1, $H_{5'a}$, $J_{5'a,5'b}$ = 11.8 Hz), 3.55 (m, 1, $H_{5'b}$), 2.79 (s, 3, S-CH_3); ^{13}C NMR (Me_2SO-d_6) δ 157.8 (C_8, J_{C_8,SCH_3} = 4.8, $J_{C_8,H_{1'}}$ = 4.1 Hz), 152.9 (C_4, J_{C_4,H_6} = 5.1, J_{C_4,H_2} = 11.1, $J_{C_4,H_{1'}}$ = 3.7 Hz), 150.5 (C_2, J_{C_2,H_2} = 205.7, J_{C_2,H_6} = 10.8 Hz), 144.8 (C_6, J_{C_6,H_6} = 184.6, J_{C_6,H_2} = 10.5 Hz), 134.3 (C_5, J_{C_5,H_6} = 7.9 Hz), 88.5 ($C_{1'}$, $J_{C_{1'},H_{1'}}$ = 161.9 Hz), 86.1 ($C_{4'}$), 70.7 ($C_{2'}$), 70.4 ($C_{3'}$), 61.7 ($C_{5'}$), 14.2 (SCH_3). Anal. calcd for $C_{11}H_{14}N_4O_4S \cdot 0.50\ H_2O$: C, 42.99; H, 4.92; N, 18.23. Found: C, 43.03; H, 5.31; N, 18.22.

8-(Methoxy)-9-(β-D-ribofuranosyl)purine (12b). A solution of **6a** (100 mg, 0.22 mmol) in 10 mL of sieve-dried MeOH was chilled to 5 °C and treated in one portion with methanolic 1 N $NaOCH_3$ (0.36 mL). After refrigeration for 16 h, the reaction was complete as indicated by TLC (9:1 $CHCl_3$-MeOH, R_f 0.30). The reaction was neutralized with glacial acetic acid and evaporated to dryness. The residue was crystallized from 3 mL of boiling MeOH to give pure **12b**: yield 44 mg (71%); mp 179-180 °C dec; TLC 7:1 $CHCl_3$-MeOH, R_f 0.50; HPLC 98%, linear gradient, 10→90% acetonitrile in water, 20 min; MS, *m/z* 283 $(M + 1)^+$; UV λ_{max} pH 1 274 (6.74), pH 7 239 (4.63), 270 (8.37), pH 13 239 (3.99), 271 (8.16); ^1H NMR (Me_2SO-d_6) δ 8.85 (s, 1, H_6), 8.76 (s, 1, H_2), 5.82 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.1 Hz), 5.44 (d, 1, 2'-OH, $J_{2',2'\text{-OH}}$ = 5.5 Hz), 5.21 (d, 1, 3'-OH, $J_{3',3'\text{-OH}}$ = 4.9 Hz), 4.99 (m, 1, $H_{2'}$, $J_{2',3'}$ = 4.6 Hz), 4.93 (t, 1, 5'-OH, $J_{5'a,5'\text{-OH}}$ = 5.1, $J_{5'b,5'\text{-OH}}$ = 5.9 Hz), 4.20 (m, 4, $H_{3'}$ and OCH_3), 3.90 (m, 1, $H_{4'}$, $J_{3',4'}$ = 2.3 Hz), 3.63 (m, 1, $H_{5'a}$, $J_{5'a,5'b}$ = 11.8, $J_{4',5'a}$ = 5.1 Hz), 3.50 (m, 1, $H_{5'b}$, $J_{4',5'b}$ = 5.8 Hz); ^{13}C NMR

(Me_2SO-d_6) δ 158.3 (C_8, J_{C_8,OCH_3} = 3.4), 151.3 (C_4, $J_{C_4,H_{1'}}$ = 5.0, J_{C_4,H_6} = 5.5, J_{C_4,H_2} = 10.6 Hz), 150.1 (C_2, J_{C_2,H_2} = 205.5, J_{C_2,H_6} = 10.7 Hz), 143.5 (C_6, J_{C_6,H_6} = 183.9, J_{C_6,H_2} = 10.6 Hz), 131.6 (C_5, J_{C_5,H_2} = 6.3, J_{C_5,H_2} = 1.2 Hz), 86.5 ($C_{1'}$, $J_{C_{1'},H_{1'}}$ = 165.4 Hz), 85.5 ($C_{4'}$), 70.5 ($C_{2'}$), 70.3 ($C_{3'}$), 61.7 ($C_{5'}$), 57.8 (OCH_3). Anal. calcd for $C_{11}H_{14}N_4O_5 \cdot 0.05$ MeOH: C, 46.75; H, 5.04; N, 19.74. Found: C, 46.62; H, 5.23; N, 19.34.

8-Benzyloxy-9-(β-D-ribofuranosyl)purine (13b). Sodium (22 mg, 0.96 mmol) was dissolved in 5 mL benzyl alcohol at room temperature. The solution was chilled to 5 °C, treated in one portion with solid **6a** (200 mg, 0.44 mmol), and refrigerated. A 17-h TLC aliquot showed complete reaction. The reaction pH was adjusted to 5 with glacial acetic acid, and the solution was evaporated in vacuo. The liquid obtained was diluted with $CHCl_3$ and applied to one silica gel thick plate (Analtech, GF, 2000 μm) that was developed with 9:1 $CHCl_3$-MeOH. The product was extracted with hot MeOH, evaporated, and crystallized from 5 mL hot water. After being chilled, the white solid was collected and dried in vacuo at 56 °C for 5 h to give pure **13b**: yield 84 mg (53%); mp 154-155 °C; TLC 85:15 $CHCl_3$-MeOH, R_f 0.65; HPLC 97%, 75:25 H_2O-MeCN; MS; *m/z* 359 $(M + 1)^+$.

9-(β-D-Ribofuranosyl)purine-8(7H)-one (14b). To a solution of **13b** (83 mg, 0.23 mmol) in 10 mL of EtOH was added 20 mg of 5% palladium-on-carbon powder (Engelhard), and the mixture was stirred under hydrogen at atmospheric pressure and room temperature. A 17-h TLC aliquot showed complete reaction. The catalyst was filtered off and washed with EtOH, and the filtrate was evaporated to dryness. This residue in MeOH was applied to one silica gel thick plate (Analtech, GF, 1000 μm) that was developed once with 5:1 $CHCl_3$-MeOH followed by a second development with 3:1 $CHCl_3$-MeOH. The product was extracted with MeOH, evaporated, and solidified by trituration with MeCN. The white solid was chilled, collected, and then dried in vacuo at room temperature for 16 h to give pure **14b**: yield 44 mg (71%); mp 140-190 °C dec; TLC 3:1 $CHCl_3$-MeOH, R_f 0.50; HPLC 99%, 85:15 0.01 M $NH_4H_2PO_4$ (pH 5.1)-MeOH; MS, *m/z* 269 $(M + 1)^+$; IR 1732 cm^{-1} (C=O); UV λ_{max} pH 1 281 (10.8), pH 7 236 (3.92), 276 (10.4), pH 13 256 (6.35), 291 (9.70); ^1H NMR (Me_2SO-d_6) δ 8.57 (s, 1, H_2), 8.29 (s, 1, H_6), 6.1-4.2 (2'-OH, 3'-OH, 5'-OH, 7-NH, and H_2O), 5.74 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.3 Hz), 4.93 (t, 1, $H_{2'}$, $J_{2',3'}$ = 5.1 Hz), 4.18 (dd, 1, $H_{3'}$, $J_{3',4'}$ = 3.5 Hz), 3.86 (m, 1, $H_{4'}$, $J_{4',5'a}$ = 4.6, $J_{4',5'b}$ = 5.2 Hz), 3.62 (A part of an ABX spin system, 1, $H_{5'a}$ = $J_{5'a,5'b}$ = 11.8 Hz), 3.47 (B part of an ABX spin system, 1, $H_{5'b}$); ^{13}C NMR (Me_2SO-d_6) δ 152.8 (C_8, $J_{C_8,H_{1'}}$ = 4.0 Hz), 149.8 (C_2, J_{C_2,H_6} = 10.2, J_{C_2,H_2} = 205.7 Hz), 149.2 (C_4, J_{C_4,H_2} = 10.9, $J_{C_4,H_{1'}}$ = J_{C_4,H_6} = 4.4 Hz), 133.5 (C_6, J_{C_6,H_2} = 10.8, J_{C_6,H_6} = 186.5 Hz), 122.6 (C_5, J_{C_5,H_6} = 5.7 Hz), 85.6 ($C_{1'}$, $J_{C_{1'},H_{1'}}$ = 163.9 Hz), 85.2 ($C_{4'}$), 70.6 ($C_{3'}$), 69.6 ($C_{2'}$), 62.1 ($C_{5'}$). Anal. calcd for $C_{10}H_{12}N_4O_5 \cdot 0.1CH_3CN \cdot 0.8\ H_2O$: C, 42.73; H, 4.89; N, 20.03. Found: C, 42.76; H, 4.75; N, 19.72.

9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)purine-8(7H)-thione (15a). A solution of **6a** (200 mg, 0.44 mmol) in 20 mL of EtOH was treated with thiourea (33 mg, 0.44 mmol) and stirred at room temperature. A 5-h TLC aliquot showed complete reaction. The solution was evaporated, dissolved in $CHCl_3$, and applied to one silica gel thick plate (Analtech, GF, 2000 μm) that was

developed three times with 95:5 $CHCl_3$-MeOH. The product was extracted with 5:1 $CHCl_3$-EtOH and evaporated to give essentially pure **15a**: yield 149 mg (83%); TLC 95:5 $CHCl_3$-MeOH, R_f 0.40; MS, *m/z* 411 $(M + 1)^+$. This material was used directly in the next step.

9-(β-D-Ribofuranosyl)purine-8(7H)-thione (15b). A solution of **15a** (149 mg, 0.36 mmol) in 1:1 MeCN-H_2O (10 mL) was treated with 1 N NaOH (1 mL) and stirred at room temperature. A 16-h TLC aliquot indicated incomplete reaction. More 1 N NaOH (0.4 mL) was added, and stirring was continued for 48 h. The reaction was neutralized with glacial acetic acid and evaporated. This residue in water was applied to one silica gel thick plate (Analtech, GF, 1000 μm) that was developed twice with 5:1 $CHCl_3$-MeOH. A broad, streaking band was extracted with warm EtOH and crystallized from hot water (5 mL). The white, crystalline solid was collected after chilling, washed with cold water, and dried in vacuo at 56 °C for 16 h to give pure **15b**: yield from **6a** 54 mg (43%); mp 227-228 °C dec; TLC 5:1 $CHCl_3$-MeOH, R_f 0.40; HPLC 99%, 90:10 H_2O-MeCN; MS, *m/z* 285 $(M + 1)^+$; UV λ_{max} pH 1 241 (9.63), 317 (17.3), pH 7 235 (9.60), 268 (sh), 313 (21.4), pH 13 235 (10.3), 272 (sh), 314 (19.2); ^1H NMR (Me_2SO-d_6) δ 8.78 (s, 1, H_2), 8.56 (s, 1, H_6), 6.40 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.9 Hz), 5.30 (bs, 1, 2'-OH), 5.15 (bs, 1, 3'-OH), 5.09 (m, 1, $H_{2'}$, $J_{2',3'}$ = 5.2 Hz), 4.83 (bs, 1, 5'-OH), 4.31 (m, 1, $H_{3'}$, $J_{3',4'}$ = 4.1 Hz), 3.90 (m, 1, $H_{4'}$, $J_{4',5'a}$ = 5.5, $J_{4',5'b}$ = 4.5 Hz), 3.69 (m, 1, $H_{5'a}$, $J_{5'a,5'b}$ = 11.9 Hz), 3.52 (m, 1, $H_{5'b}$); ^{13}C NMR (Me_2SO-d_6) δ 172.3 (C_8, $J_{C_8,H_{1'}}$ = 3.4 Hz), 151.1 (C_2, J_{C_2,H_2} = 207.6, J_{C_2,H_6} = 10.4 Hz), 150.0 (C_4, J_{C_4,H_2} = 11.2, $J_{C_4,H_{1'}}$ = 5.5, J_{C_4,H_6} = 5.7 Hz), 135.8 (C_6, J_{C_6,H_6} = 189.1, J_{C_6,H_5} = 10.8 Hz), 123.7 (C_5, J_{C_5,H_6} = 6.4 Hz), 88.6 ($C_{1'}$, $J_{C_{1'},H_{1'}}$ = 165.2 Hz), 85.2 ($C_{4'}$), 70.4 ($C_{3'}$), 70.2 ($C_{2'}$), 61.8 ($C_{5'}$). Anal. calcd for $C_{10}H_{12}N_4O_4S$: C, 42.25; H, 4.25; N, 19.71. Found: C, 42.49; H, 4.54; N, 19.84.

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