an incision was made in the fourth right intercostal space and the pericardium was incised. A Beckman polarographic PO_2 microelectrode (in a Riley needle) was placed in the coronary sinus via the superior vena cava. Compound, dissolved in isotonic saline, was administered as a bolus either intravenously (jugular) or intraduodenally through an indwelling catheter.

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Analogues of 8-Azaguanosine

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Two routes for the synthesis of 6-substituted 8-azaguanosine analogues are described. 2,5,6-Triamino-4(3*H*)-pyrimidinethione (1) was converted by methylation, nitrosation, and acetylation to *N*-acetyl-7-(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (**5**). The reaction of **5** with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride gave a mixture of the 7-, 8-, and 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-8-azapurines **4a–c** which was converted to 8-azaguanosine (**7c**) and the corresponding 7- and 8-substituted isomers **7a** and **7b**. **4a–c** were also converted with NaOMe to 6-*O*-methyl-8-azaguanosine (**8c**) and to the corresponding 7- and 8-substituted isomers **8a** and **8b**. The preferred route, however, to 6-substituted 8-azaguanosine analogues is an unambiguous synthesis through N^2-acetyl-6-(benzylthio)-N^4-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-nitro-2,4-pyrimidinediamine (**13**), prepared from the reaction of the chloropyrimidine **10** with the aminoribose **11**. Catalytic hydrogenation of **13** gave the aminopyrimidine **14**, which was converted with nitrous acid to the nucleoside β-**20**. Treatment of β-**20** with dilute acid gave 7-(benzylthio)-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (**19**). Replacement of the benzylthio group of **19** with various nucleophilic reagents gave 8-aza-6-thioguanosine **17** and analogues **8c**, **15**, and **16**. The thione **17** rearranges in aqueous solution to the thiadiazolopyrimidine **21**. The parent [1,2,3]thiadiazolo[5,4-*d*]pyrimidine-5,7-diamine (**24a**) was prepared by nitrosation of the triaminopyrimidine (**23a**). Rearrangement of **24a** in the presence of base gave a high yield of the thione **25a** which could be rearranged with heat to **24a**. Compounds **8a–c**, **15–19**, **24a**, and **25a** were evaluated in the H.Ep.-2 cell culture screen and compounds **8c**, **15–19**, **24a**, and **25a** in the L1210 mouse leukemia screen. Only one compound, **8c**, showed high cytotoxicity and borderline L1210 activity resulting from its enzymatic conversion to 8-azaguanosine.

The anticancer activity of 8-azaadenosine (3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine)[1] and 8-azainosine (3,6-dihydro-3-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one)[1,2] in experimental animals systems coupled with the clinical efficacy of the 6-thiopurines against human leukemias[3,4] led us to prepare alkoxy- and sulfur-containing congeners of these nucleosides. These compounds proved to be substrates for adenosine kinase, to be cytotoxic to cell lines possessing this enzyme but not to lines deficient in it, and to show some activity against murine leukemias.[1,5] These initially encouraging results and the knowledge of the anticancer activity of 6-thioguanine[3] and 8-azaguanine (5-amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one)[6] suggested the preparation and reevaluation of the known 8-aza-6-thioguanine (5-amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-7-thione) (**25a**)[7] and the synthesis of its ribonucleoside **17** since, although 6-thioguanosine is rapidly phosphorylyzed to 6-thioguanine,[3] 8-azapurine ribonucleosides such as 8-azainosine are not and show activity not possessed by the parent heterocycles.[1,2]

The successful synthesis of 7-(methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine[8] by the molecular sieve catalyzed reaction of 7-(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride and the reactivity of the methylthio group of this nucleoside to nucleophilic displacement reactions[8,9] suggested a similar sequence for the preparation of 8-aza-6-thioguanosine (5-amino-3,6-dihydro-3-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-7-thione, **17**).

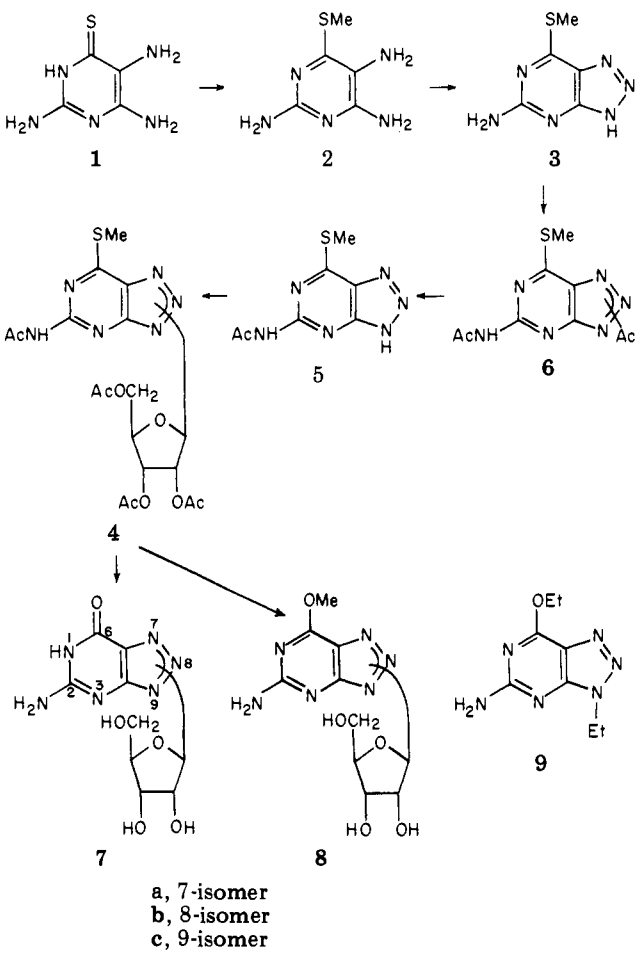
2,5,6-Triamino-4(3*H*)-pyrimidinethione[10] (**1**) was alkylated with methyl iodide to give a 72% yield of 6-(methylthio)-2,4,5-pyrimidinetriamine (**2**). This route to

Table I. Uv Spectra of Substituted 8-Azaguanine

8-Azaguanine[a]	λ_{max}, nm ($\epsilon \times 10^{-3}$)		
	0.1 N HCl	pH 7	0.1 N NaOH
1-Methyl-[b]	252 (5.65), 264 (sh) (4.16)		
3-Methyl-[c]	205 (25.7), 264 (7.39)	213 (7.56), 249 (9.85), 267 (11.2)	249 (9.76), 267 (11.2)
7-Methyl-[d]	210 (23.5), 270 (5.37)	210 (25.7), 240 (7.12), 296 (5.23)	219 (20.1), 245 (sh) (5.46), 297 (5.89)
8-Methyl-[d]	206 (26.8), 269 (8.84)	211 (28.7), 241 (6.69), 292 (6.32)	218 (22.7), 250 (4.97), 296 (7.98)
7a	210, 273	211, 240 (sh), 300	219, 245 (sh), 298
7b	208, 277	215, 240 (sh), 304	221, 257, 308
9-β-D-Ribofuranosyl-[e]	255 (13.4), 275 (sh) (9.34)	256 (12.9), 275 (sh) (9.00)	221 (23.3), 278 (11.7)

[a] Purine numbering. [b] See ref 15. [c] See ref 16. [d] See ref 17. [e] Sample prepared according to ref 14.

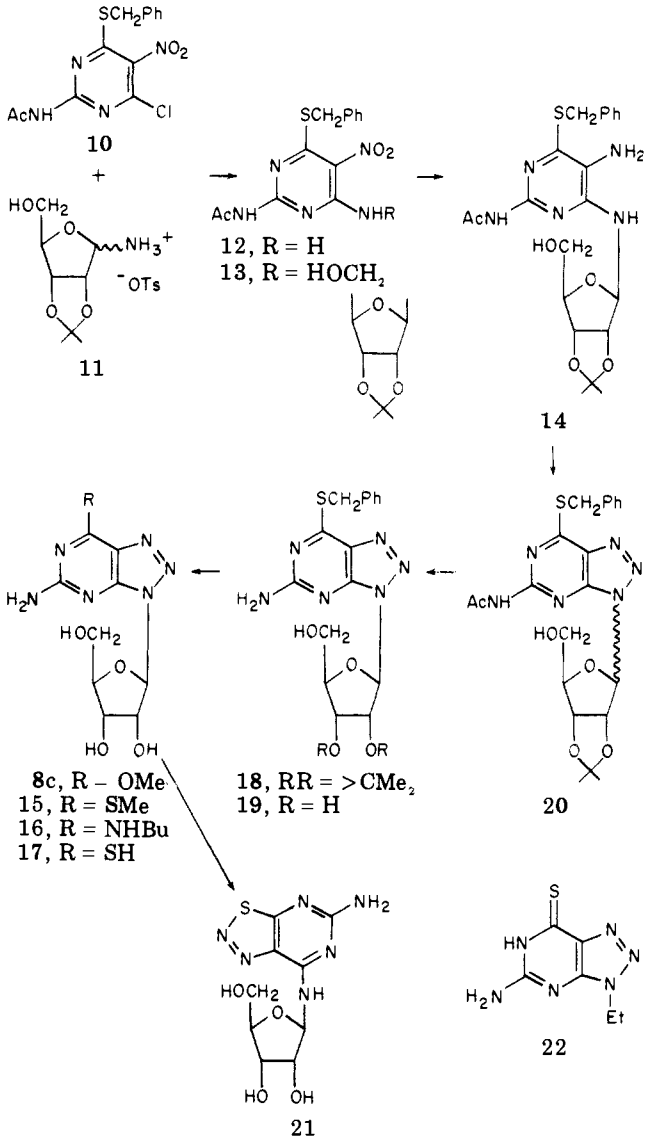
2 proved to be more convenient than that described by Daves et al.[11] Nitrosation of **2** gave a 90% yield of the known 7-(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine[12] (**3**). Attempts to convert **3** to the ribonucleoside by silylation with hexamethyldisilizane and treatment of the silylated derivative with triacetylribofuranosyl chloride or the corresponding bromide gave an impractically low yield of a mixture of nucleosides. The reaction of **3** with Ac_2O gave an 89% yield of a diacetyl derivative **6**, which was partially hydrolyzed in dilute base to give a 98% yield of *N*-acetyl-7-(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (**5**), the infrared spectrum of which contained an absorption band at 1660 cm^{-1}, confirming the position of the acetyl group. Treatment of **5** with triacetylribofuranosyl chloride in refluxing 1,2-dichloroethane containing Linde 4A molecular sieve[13] gave a low yield of a mixture of three nucleosides which was partially separated by column chromatography into one pure compound and a mixture of the other two. These nucleosides were identified by treatment with H_2O_2 in glacial HOAc followed by deblocking with methanolic NaOMe to give the corresponding crude 8-azaguanine ribonucleosides **7a–c**. The site of ribosylation was determined by comparison of the uv spectra of **7a–c** with the spectra of 8-azaguanosine[14] (**7c**) and the known *N*-methyl-8-azaguanines (see Table I). A comparison of the peak ratios and shapes of the uv curves left no doubt as to the identity of **7a–c** and, accordingly, **4a–c**, the nucleosides obtained in the sieve-catalyzed reaction. Treatment of **4a–c** with methanolic NaOMe gave the corresponding methoxy analogues **8a–c**. A uv spectrum of **8c** was similar to that reported for 7-ethoxy-3-ethyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine[18] (**9**).



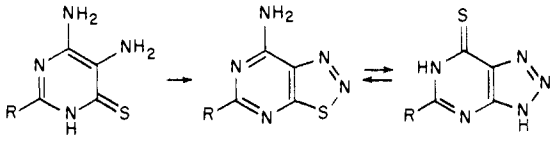
Unambiguous syntheses of 8-azapurine nucleosides have been effected by reaction of 4-chloro-5-nitropyrimidines with substituted glucopyranosylamines, followed by hydrogenation of the nitro group and cyclization with nitrous acid.[19,20] An attempt by Rokos and Pfleiderer[21] to apply this reaction to ribosylamine by treatment of 1-amino-2,3,5-tri-*O*-benzoyl-1-deoxy-D-ribofuranose with 4-chloro-5-nitropyrimidines was unsuccessful because of apparent instability of the aminoribose. The recent disclosure of a simple synthesis of the more stable 2,3-*O*-isopropylidene-D-ribofuranosylamine tosylate[22] (**11**) suggested its use in an unambiguous synthesis of 8-aza-6-thioguanosine (**17**).

The reaction of **11** with the chloropyrimidine[23] (**10**) in EtOH containing $NaHCO_3$ gave a 44% yield of anomerically pure N^2-acetyl-6-(benzylthio)-N^4-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-nitro-2,4-pyrimidinediamine (**13**). An initial attempt to carry out this reaction in the presence of Et_3N as the acid acceptor gave a 30% yield of **13** and a 12% yield of the aglycon **12**, probably resulting from base decomposition of **11** with liberation of NH_3. Hydrogenation of **13** in EtOH containing 5% Pd on charcoal gave an 82% yield of the anomerically pure ribosylaminopyrimidine (**14**). The use of Raney nickel in this reduction gave a lower (43%) yield of **14**. Addition of $NaNO_2$ to a cold solution of **14** in dilute AcOH gave a 4:1 mixture of *N*-acetyl-7-(benzylthio)-3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]-pyrimidin-5-amine (β-**20**) and the corresponding α-anomer (α-**20**). Although β-**20** was found to be stable in AcOH, the amine **14** racemized at an appreciable rate under these conditions. To minimize formation of the α-anomer, a dry mixture of **14** and $NaNO_2$ was added to a cold solution of dilute AcOH to give an 89% yield of anomerically pure β-**20**. An examination of the ^1H NMR spectra of α-**20** and β-**20** confirmed their identity. The observed difference in the chemical shifts ($\Delta\delta$) in Me_2SO-d_6 of the isopropylidene methyl groups is δ 0.10 for α-**20** and δ 0.19 for β-**20**, which is consistent with the values established for isopropylidene derivatives of D-ribonucleosides.[24] The coupling constant of β-**20** is <2.0 Hz, indicative of a β-ribonucleoside.[25] In addition, the H_1 chemical shift of β-**20** is upfield from the

same bands of the α-anomer α-**20** in keeping with published observations.[26,27] The acetyl and isopropylidene blocking groups of β-**20** were removed by hydrolysis in a solution of EtOH and 0.5 N H_2SO_4. Under optimum conditions, the hydrolysis gave a 48% yield of analytically pure deblocked nucleoside **19**, a 15% yield of crude isopropylidene nucleoside **18**, and a 10% yield of crude 8-azaguanosine (**7c**). The 8-azaguanosine was identified by comparison with an authentic sample,[14] and **18** was identified by a uv spectrum similar to **19**, a molecular ion of *m/e* 430, and a negative metaperiodate–Schiff test. Attempts to improve the yield of **19** by increasing or decreasing the reaction time gave higher yields of **7c** and **18**, respectively, with a resultant decrease in the yield of **19**.



The benzythio group of **19** could be easily displaced with a variety of nucleophiles at room temperature. Treatment of **19** with NaOMe in MeOH gave a 72% yield of 7-methoxy-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (**8c**). In a similar reaction with MeSH in MeOH containing NaOMe, **19** gave a 75% yield of 7-(methylthio)-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (**15**). The butylamino analogue **16** was prepared in 86% yield by treatment of **19** with excess *n*-butylamine. The reaction of **19** with NaSH in ethanol gave a precipitate of 8-aza-6-thioguanosine (**17**) as the sodium salt. An aqueous solution of this salt was acidified to pH 6 to give a precipitate of pure **17** in 66% yield. The structure of **17** was confirmed by comparison of its uv spectrum with that of 8-aza-9-ethyl-6-thioguanine (**22**).[18] A solution of **17** in water under N_2 rearranged slowly to give a 73% yield of N^7-β-D-ribofuranosyl[1,2,3]thiadiazolo[5,4-*d*]pyrimidine-5,7-diamine (**21**). A ^1H NMR spectrum of **21** showed only one anomer which is assumed to be β since anomerization would be expected to give a mixture. The peak for $H_{1'}$ is the predicted multiplet which forms a doublet upon exchange of the ribosyl NH with D_2O. Other evidence for this structure is its elemental analysis, a molecular ion of *m/e* 300 in its mass spectrum, and a uv spectrum similar to the parent [1,2,3]thiadiazolo[5,4-*d*]pyrimidine-5,7-diamine (**24a**), which was prepared by nitrosation of 2,5,6-triamino-4(3*H*)-pyrimidinethione[10] (**23a**) with $NaNO_2$ in 0.5 N HCl. This procedure has been used for the synthesis of [1,2,3]-thiadiazolo[5,4-*d*]pyrimidin-7-amine (**24b**) from 5,6-diamino-4(3*H*)-pyrimidinethione[28] (**23b**). Structural assignment of **24a** is based on elemental analysis, NMR data, insolubility in base, a molecular ion of *m/e* 168, absence of a molecular ion of *m/e* 334 for the disulfide of **23a** (field desorption mass spectrum), and negligible difference in the uv absorption at pH 7 and 13, indicative of the lack of an acidic proton on the chromophore.



The reversible rearrangement of **24b** to the thione **25b** reported by Albert[28] also occurs with **24a**. Treatment of **24a** with hot base gave an 87% yield of the thione **25a**. Evidence for the structure of **25a** is based on elemental analysis, solubility in base, a uv spectrum similar to **17** and **22** at pH 1, a molecular ion of *m/e* 168, and the expected ^1H NMR absorption. This compound has essentially the same physical properties as those described for a sample of **25a** prepared from 8-azaguanine.[7] The reverse rearrangement was demonstrated by heating dry **25a** at 165° to give a low yield of **24a**.

Both ^1H and ^{13}C NMR spectra support the structures assigned to **24a** and **25a**. Assignments for the ^{13}C NMR spectra were made by comparison with 2,6-diaminopurine, 6-mercaptopurine, and related compounds and by intensity changes observed on addition of D_2O.[29] The ^1H NMR spectrum of **24a** shows two absorption peaks of about the same intensity and shape, attributed to the two NH_2 groups. In contrast, the spectrum of **25a** shows a sharp absorption peak assigned to the NH_2 group and very broad absorption assigned to the two NH groups.

Biologic Evaluation. Compounds **8a–c**, **15–19**, **24a**, and **25a** were evaluated in the H.Ep.-2 cell culture screen[31] and compounds **8c**, **15–19**, **24a**, and **25a** in the L1210 mouse leukemia screen[30] (see Table II). Only compounds **8c** and **25a** were highly cytotoxic. Compound **8c** proved to be a good substrate for adenosine deaminase indicating that its cytotoxicity is a result of its conversion to 8-azaguanosine (**7c**). In contrast to **8c**, the positional isomers **8a** and **8b** show a very low order of toxicity. The low toxicity of the remaining 8-azaguanosine analogues in Table II is also contrasted with the high toxicity of the corresponding 8-azainosine analogues.[1] The lack of L1210 activity and low toxicity of the thio and alkylthio analogues **15**, **17**, and **19** indicate that simple hydrolysis to **7c** or **25a**

Table II. Cytotoxicity and L1210 Screening Data

Compd no.	Cytotoxicity to H.Ep.-2 cells in culture, ED_{50}, μM[a]	L1210[b] Dose, mg/kg	L1210[b] % ILS[c]
7c	2	25	32[d]
8a	>70		
8b	>70		
8c	2	100	29
15	>60	400	12
16	>60	400	7
17	>100	200	4
18	>50	400	1
19	>50	400	11
24a	>100	400	0
25a	4	100	0

[a] The concentration required to inhibit the growth of cells to 50% of controls. See ref 31. [b] L1210 leukemic cells (10^5) were implanted intraperitoneally in mice and treated with a single dose of compound on day 1. See ref 30. [c] Increase in life span at optimum dose. [d] See ref 32.

does not occur in cell culture or in vivo and that the presence of the amino group in **15** prevents it from functioning as a substrate for adenosine kinase, the enzyme that activates 8-aza-6-(methylthio)purine ribonucleoside.[1] In contrast, the presence of the amino group of **8c** does not interfere with its ability to function as a substrate for adenosine deaminase (see above).

Of the compounds tested against L1210 leukemia in mice, only **8c** showed borderline activity.

Experimental Section

Melting points were determined on a Kofler Heizbank or, when indicated, on a Mel-Temp apparatus. Absence of melting point data indicates an indefinite melting point. The ultraviolet absorption spectra were determined with a Cary Model 17 spectrophotometer. Each compound was dissolved in the solvent indicated in parentheses and diluted tenfold with 0.1 N HCl, pH 7 buffer, and 0.1 N NaOH. The ^1H NMR spectra were determined in 3–7% w/v solutions in Me_2SO-d_6 with a Varian XL-100-15 spectrometer operating at 100 MHz or T-60A operating at 60 MHz (internal Me_4Si). The relative peak areas are given to the nearest whole number, and chemical shifts quoted in the case of multiples are measured from the approximate center. The ^{13}C NMR spectra were determined in 12% w/v solutions in Me_2SO-d_6 with a Varian XL-100-15 spectrophotometer operating at 25.160 MHz and equipped with a Digilab FTS NMR-3 pulser and data system. Mass spectral data were taken with a Varian MAT 311A instrument equipped with a combination E1/F1/FD ion source. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

6-(Methylthio)-2,4,5-pyrimidinetriamine (2). A mixture of 2,5,6-triamino-4(3*H*)-pyrimidinethione[10] (1, 8.40 g, 36.5 mmol), 2 N NaOH (56.5 ml), and methyl iodide (2.39 ml, 38.4 mmol) was stirred vigorously for 15 min at 0° and 45 min at 25°. The reaction mixture was adjusted to pH 8.6 with 6 N HCl and cooled in an ice bath to give a red precipitate which was collected, washed with cold H_2O, and dried in vacuo (P_2O_5): yield 4.52 g (72%); mp 190° (lit.[11] mp 191–192°).

***N*-Acetyl-7-(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (5).** A solution of **3**[12] (2.50 g, 13.8 mmol), prepared in 90% yield from **2**, was heated in refluxing acetic anhydride (28 ml) for 2 h, cooled to 25°, and diluted with Et_2O (60 ml). The crystalline diacetyl derivative **6** was collected, washed with Et_2O, and dried in vacuo; yield 3.26 g (89%); mp ~240° dec. Anal. ($C_9H_{10}N_6O_2S$) C, H, N. The diacetyl derivative (3.26 g, 12.3 mmol) was added in small portions to a stirred 1 N NaOH solution (24.5 ml) and the resulting mixture stirred for 20 h, filtered, treated with 1 N HCl (24.5 ml), and cooled in an ice bath. The crystalline product was collected, washed with H_2O, and dried in vacuo (P_2O_5): yield 2.69 g (98%); mp 251° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$) (EtOH), (0.1 N HCl) 227 (13.4), 252 (16.2), 277 (10.8), 302 (13.6); λ_{max} (pH 7) 238 (21.4), 307 (12.3); λ_{max} (0.1 N NaOH) 237 (18.1), 308 (11.3). Anal. ($C_7H_8N_6OS$) C, H, N.

Nucleosides from the Reaction of 5 with 2,3,5-Tri-*O*-acetyl-D-ribofuranosyl Chloride. A mixture of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride[33] (8.6 mmol), **5** (1.81 g, 8.08 mmol), Linde 4A molecular sieve[13] (40 g), and 1,2-dichloroethane (450 ml) was stirred at 65° for 5 days. The mixture was cooled at 25°, filtered, and evaporated to dryness in vacuo. A solution of the residue in benzene (50 ml) was filtered and evaporated to give 3.25 g of mixed nucleosides. A solution of the mixture in a minimum of $CHCl_3$ was absorbed on a 28-mm diameter column of E. Merck silica gel 60 (230–400 mesh, 150 g) equilibrated with $CHCl_3$ and developed with $CHCl_3$–MeOH (99:1). The faster moving fraction was evaporated to a foam (3.25 g) consisting of a mixture of *N*-acetyl-7-(methylthio)-2-(2,3,5-tri-*O*-acetyl)-β-D-ribofuranosyl-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (**4b**) and the corresponding 3-substituted isomer **4c**. A slower moving fraction was evaporated to a foam (470 mg) which was identified as the crude 1-substituted isomer **4a**: λ_{max}, nm (EtOH), (0.1 N HCl and pH 7) 248, 323.

5-Amino-1,6-dihydro-1-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (7a). A solution of crude **4a** (100 mg) in AcOH (1 ml) was stirred with 30% H_2O_2 (0.12 ml) for 18 h and evaporated to a gum in vacuo. A solution of the residue in $CHCl_3$ was washed with H_2O and evaporated to dryness, and the residue was stirred with NaOMe (78 mg) in MeOH (4 ml) for 18 h. The solution was neutralized with Amberlite IR 120 cation-exchange resin and evaporated to give 27 mg of crude **7a**: λ_{max}, see Table I.

8-Azaguanosine (7c) and 5-Amino-2,6-dihydro-2-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (7b). The mixture of **4b** and **4c** (100 mg) was oxidized with H_2O_2 and hydrolyzed as above to give a mixture of **7b** and **7c** (55 mg). Extraction of the mixture with H_2O (2 ml) left crude **7b** as an insoluble white powder (13 mg): λ_{max}, see Table I. The extract was evaporated and the residue again extracted with water (1 ml) and evaporated in vacuo to give 24 mg of crude **7c**. A TLC and uv spectrum of this material was identical with that of previously prepared **7c**.[14]

7-Methoxy-1-β-D-ribofuranosyl-1*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (8a). A solution of crude **4a** (200 mg) in MeOH (6 ml) containing NaOMe (78.3 mg, 1.45 mmol) was stirred for 18 h and a white precipitate of **8a** was collected by filtration, washed with MeOH, and dried in vacuo (P_2O_5): yield 51 mg; mp ~173°. Additional product, 24 mg, mp ~173°, was obtained from the filtrate by neutralization with Amberlite IR-120 cation-exchange resin, evaporation, and crystallization from EtOH: λ_{max}, nm ($\epsilon \times 10^{-3}$) (EtOH), (0.1 N HCl) 215 (sh) (18.9), 245 (sh) (7.23), 294 (6.65); λ_{max} (pH 7) 211 (24.7), 313 (4.53); λ_{max} (0.1 N NaOH) 310 (4.76). Anal. ($C_{10}H_{14}N_6O_5 \cdot 0.5H_2O$) C, H, N.

7-Methoxy-2-β-D-ribofuranosyl-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (8b). A solution of the mixture of **4b** and **4c** (338 mg) in MeOH (10 ml) containing NaOMe (132 mg, 2.45 mmol) was stirred for 18 h, neutralized with IR-120 cation-exchange resin, and evaporated to dryness. The product mixture was separated by chromatography on a 2-mm Merck silica gel 60 F-254 preparative TLC plate using $CHCl_3$–MeOH (5:1) as the developing solvent. The faster moving band was extracted with MeOH to give 29 mg of crude **8c**; this compound was found to be identical (TLC, uv) with the analytical sample described below. The slower moving band was extracted with MeOH to give 74 mg of crude **8b** which was recrystallized from EtOH to give 18 mg of pure product: mp 190°; λ_{max}, nm ($\epsilon \times 10^{-3}$) (H_2O), (0.1 N HCl) 204 (22.9), 243 (6.30), 292 (13.3); λ_{max} (pH 7) 215 (3.13), 311 (8.33); λ_{max} (0.1 N NaOH) 216 (2.67), 255 (sh) (4.41), 307 (8.57). Anal. ($C_{10}H_{14}N_6O_5$) C, H, N.

7-Methoxy-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (8c). A solution of **19** (1.00 g, 2.56 mmol) and NaOMe (415 mg, 7.68 mmol) in anhydrous MeOH (200 ml) was stirred for 4 days exposed to air through a drying tube containing soda lime and Drierite. The solution was neutralized with Amberlite IR-120 cation-exchange resin and filtered, and the resin was washed thoroughly with MeOH and H_2O. The combined filtrate and wash was lyophilized and the residue washed

with Et_2O to remove benzyl disulfide. A solution of the dried residue in water (200 ml) was washed with Et_2O and lyophilized. The residue (650 mg) was crystallized from hot H_2O (charcoal, Celite) to give white needles which were dried at 100° in vacuo: yield 546 mg (72%); mp 209°; λ_{max}, nm ($\epsilon \times 10^{-3}$) (H_2O), (0.1 N HCl) 213 (22.0), 244 (5.10), 283 (10.7); λ_{max} (pH 7) 214 (22.2), 246 (5.36), 287 (10.6); λ_{max} (0.1 N NaOH) 244 (5.40), 287 (10.7). Anal. ($C_{10}H_{14}N_6O_5$) C, H, N.

N^2-Acetyl-6-(benzylthio)-N^4-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-nitro-2,4-pyrimidinediamine (13). A suspension of *N*-acetyl-4-(benzylthio)-6-chloro-5-nitro-2-pyrimidineamine[23] (**10**, 5.00 g, 14.8 mmol), 2,3-*O*-isopropylidene-D-ribofuranosylamine *p*-toluenesulfonate[22] (11, 10.6 g, 29.5 mmol), and $NaHCO_3$ (2.48 g, 29.5 mmol) in EtOH (75 ml) was stirred in an oil bath at 59° for 17 h, cooled to 25°, and stirred for 1 h. The white precipitate was collected by filtration, washed with a small quantity of EtOH, dried in vacuo, stirred with H_2O (50 ml) for 1 h, collected, and dried in vacuo (P_2O_5): yield 3.20 g (44%); mp 192°; λ_{max}, nm ($\epsilon \times 10^{-3}$) (EtOH), (0.1 N HCl) 219 (23.4), 261 (27.2), 352 (13.5); λ_{max} (0.1 N NaOH) 260 (15.7), 357 (17.4). Anal. ($C_{21}H_{25}N_5O_7S$) C, H, N. Substitution of Et_3N for $NaHCO_3$ in this reaction gave, after fractional crystallization from EtOH, a 30% yield of **13** and a 12% yield of N^2-acetyl-6-(benzylthio)-5-nitro-2,4-pyrimidinediamine (**12**): λ_{max}, nm ($\epsilon \times 10^{-3}$) (EtOH), (0.1 N HCl) 259 (20.3), 345 (12.9); λ_{max} (pH 7) 215 (20.6), 256 (20.6), 348 (13.4); λ_{max} (0.1 N NaOH) 256 (12.6), 351 (17.2). Anal. ($C_{13}H_{13}N_5O_3S$) C, H, N.

N^2-Acetyl-6-(benzylthio)-N^4-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-2,4,5-pyrimidinetriamine (14). A solution of **13** (3.65 g, 7.42 mmol) in EtOH (600 ml) was hydrogenated at atmospheric pressure in the presence of 5% Pd on charcoal (5.5 g) for 6 h. The solution, which took up 700 ml (28.8 mmol) of H_2, was warmed to 80° on a water bath and filtered and the catalyst extracted with boiling EtOH (500 ml). The combined filtrate and extract was evaporated in vacuo leaving a residue of white solid which was used for the preparation of β-**20**: yield 2.81 g (82%); mp 171°. Recrystallization of a portion from EtOH gave an analytical sample: mp 175°; λ_{max}, nm ($\epsilon \times 10^{-3}$) (EtOH), (0.1 N HCl) 218 (18.6), 243 (21.1), 290 (8.00), 330 (9.00); λ_{max} (pH 7) 213 (26.5), 273 (br) (9.57), 310 (11.2); λ_{max} (0.1 N NaOH) 310 (10.7). Anal. ($C_{21}H_{27}N_5O_5S$) C, H, N.

7-(Methylthio)-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (15). A solution of NaOMe (138 mg, 2.56 mmol) in MeOH (100 ml) was saturated with MeSH, treated with **19** (500 mg, 1.28 mmol), allowed to stand in a stoppered flask for 5 days, and diluted dropwise with H_2O (100 ml). The solution was neutralized with Amberlite IR-120 cation-exchange resin, filtered, and lyophilized. The residual powder was triturated with Et_2O (3 × 25 ml), dried in vacuo, and recrystallized from hot H_2O (5 ml) to give white crystals, which were dried at 56° in vacuo (P_2O_5): yield 302 mg (75%); mp 209°; λ_{max}, nm ($\epsilon \times 10^{-3}$) (H_2O), (0.1 N HCl) 221 (14.2), 247 (9.72), 279 (9.19), 315 (12.5); λ_{max} (pH 7) 221 (14.6), 248 (10.6), 277 (8.64), 316 (12.6); λ_{max} (0.1 N NaOH) 221 (13.2), 244 (10.6), 277 (8.64), 316 (12.6). Anal. ($C_{10}H_{14}N_6O_4S$) C, H, N.

N^7-Butyl-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-5,7-diamine (16). A solution of **19** (100 mg, 0.256 mmol) and *n*-butylamine (0.5 ml) in MeOH (0.5 ml) was allowed to stand at 25° for 4 days and evaporated in vacuo. The residue was triturated with Et_2O (2 × 2 ml), dried, and triturated with $CHCl_3$ (1 ml). The resulting white crystalline precipitate was collected, washed with $CHCl_3$, and dried at 56° in vacuo (P_2O_5): yield 75 mg (86%); mp 135°; λ_{max}, nm ($\epsilon \times 10^{-3}$) (H_2O), (0.1 N HCl) 213 (21.7), 261 (13.2), 285 (10.7); λ_{max} (pH 7) 233 (20.4), 291 (14.1); λ_{max} (0.1 N NaOH) 232 (21.2), 291 (14.3). Anal. ($C_{13}H_{21}N_7O_4$) C, H, N.

8-Aza-6-thioguanosine [5-Amino-3,6-dihydro-3-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-7-thione Hydrate (17)]. A suspension of powdered Fisher purified sodium sulfhydrate (1.00 g) and **19** (500 mg, 1.28 mmol) in EtOH (20 ml) was stirred in a stoppered flask for 2 days. The resulting sodium salt of **17** was collected by filtration under N_2, washed with a small quantity of cold EtOH, and dried in vacuo: yield 572 mg. A portion of the salt (100 mg) in 2 ml of H_2O was filtered, acidified with 1 N HCl to pH 6, and cooled in an ice bath. The white precipitate of **17** was collected by filtration, washed with cold H_2O, and dried in vacuo (P_2O_5): yield 47 mg (66%); mp 139–140° (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$) (CH_3OH), (0.1 N HCl) 211 (21.5), 230 (sh) (11.8), 263 (8.90), 338 (20.3); λ_{max} (pH 7) 212 (18.0), 258 (10.4), 334 (17.6); λ_{max} (0.1 N NaOH) 257 (10.9), 292 (7.82), 328 (17.1). Anal. ($C_9H_{12}N_6O_4S \cdot H_2O$) C, H, N.

7-(Benzylthio)-3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (18) and 7-(Benzylthio)-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (19). A solution of β-**20** (500 mg, 1.06 mmol) in EtOH (20 ml) and 0.5 N H_2SO_4 (5 ml) was stirred in an oil bath at 44° for 41 h, cooled to 25°, neutralized with 4 N NaOH to pH 7, filtered, and evaporated to dryness in vacuo. The residue was washed with benzene (2 × 5 ml), dried, and extracted with EtOH. This extract was filtered, concentrated in vacuo, and chromatographed on four 2-mm Merck silica gel 60F-254 preparative TLC plates using 9:1 $CHCl_3$–MeOH as the developer. A faster moving minor band was extracted with MeOH to give 69 mg of crude **18**: λ_{max}, nm (EtOH), (0.1 *N* HCl) 249, 280, 318; λ_{max} (pH 7) 250, 279, 318. A nonmoving minor band was extracted with MeOH to give 30 mg of crude 8-azaguanosine (**7c**), which was identical (TLC and uv) with a previously prepared sample.[14] The major band was extracted with MeOH to give 291 mg of crude **19**, which was recrystallized from hot 5:1 H_2O–EtOH (30 ml): yield 199 mg (48%); mp 162°; λ_{max}, nm ($\epsilon \times 10^{-3}$) (EtOH), (0.1 N HCl) 216 (19.0), 249 (9.36), 280 (9.14), 317 (13.6); λ_{max} (pH 7) 216 (19.3), 249 (9.82), 278 (9.00), 317 (13.6); ^1H NMR δ 3.54 (m, $H_{5'}$), 3.97 (m, 1, $H_{4'}$), 4.27 (m, 1, $H_{3'}$), 4.63 (s, SCH_2), 4.75 (m, $H_{2'}$), 5.17 (d, 1, $O_{3'}H$), 5.52 (d, 1, $O_{2'}H$), 6.00 (d, 1, $J_{1',2'} \simeq 5.0$ Hz, $H_{1'}$), 7.4 (m, 7, NH_2, C_6H_5). Anal. ($C_{16}H_{18}N_6O_4S$) C, H, N.

***N*-Acetyl-7-(benzylthio)-3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (β-20).** A mixture of **14** (2.81 g, 6.09 mmol) and $NaNO_2$ (740 mg, 10.7 mmol) in a 50-ml flask was cooled in an ice bath and treated rapidly with a cold (0°) solution of AcOH (28 ml) and H_2O (2.8 ml). The resulting mixture, after stirring for 30 min at 0° and 30 min at 25°, was poured into ice water (280 ml) and stirred for 5 min. The white precipitate was collected, washed with cold H_2O, and dried in vacuo (P_2O_5): yield 2.57 g (89%); melting point indefinite; λ_{max}, nm ($\epsilon \times 10^{-3}$) (EtOH), (0.1 N HCl) 231 (14.5), 257 (16.7), 283 (sh) (11.3), 307 (16.6); λ_{max} (pH 7) 231 (14.6), 257 (16.9), 283 (sh) (11.3), 307 (16.7); ^1H NMR δ 1.36, 1.55 (d, 6, CMe_2), 2.31 (s, 3, $COCH_3$), 4.24 (m, 1, $H_{4'}$), 5.17 (m, 1, $H_{3'}$), 5.62 (m, 1, $H_{2'}$), 6.41 (d, 1, $J_{1',2'} = <2.0$ Hz, $H_{1'}$), 7.42 (m, 5, C_6H_5). Anal. ($C_{21}H_{24}N_6O_5S$) C, H, N.

In a preliminary experiment (see discussion) a small quantity of the α-anomer (α-**20**) was isolated by separation of a 4:1 mixture of β-**20** and α-**20** on a 2-mm Merck silica gel 60 F-254 preparative TLC plate using $CHCl_3$–MeOH (97:3) as developing solvent: λ_{max}, nm (EtOH), (0.1 N HCl and pH 7) 233, 256, 283 (sh), 306; ^1H NMR δ 1.11, 1.21 (d, 6, CMe_2), 2.32 (s, 3, $COCH_3$), 3.66 (d, 2, $H_{5'}$), 6.71 (d, 1, $J_{1',2'} = 5.0$ Hz, $H_{1'}$), 7.42 (m, 5, C_6H_5).

N^7-β-D-Ribofuranosyl[1,2,3]thiadiazolo[5,4-*d*]pyrimidine-5,7-diamine (21). A solution of **17** (15 mg) in H_2O (2 ml) was stirred in a stoppered vial under N_2 at ~45° for 5 days. The mixture was cooled in an ice bath and the white precipitate collected and dried in vacuo (P_2O_5): yield 11 mg (73%); mp 247° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$) (Me_2SO–H_2O, 2:23), (0.1 N HCl) 248 (21.8), 275 (11.1), 285 (sh) (10.6); λ_{max} (pH 7) 247 (14.9), 311 (11.6); λ_{max} (0.1 N NaOH) 248 (14.3), 313 (11.2); ^1H NMR δ 3.47 (m, 2, $H_{5'}$), 3.75 (m, 1, $H_{4'}$), 4.07 (m, 2, $H_{2',3'}$), 4.73 (m, 1, $O_{5'}H$), 4.88 (d, 1, $O_{2'}$ or $_{3'}H$), 5.07 (d, 1, $O_{2'}$ or $_{3'}H$), 5.89 (m, 1, H_1), 7.22 (s, 2, NH_2), 9.06 (d, 1, NH). Anal. ($C_9H_{12}N_6O_4S$) C, H, N.

[1,2,3]Thiadiazolo[5,4-*d*]pyrimidine-5,7-diamine (24a). (a) A solution of **23a**[10] (10.0 g, 43.4 mmol) in hot 0.5 N HCl (700 ml) was cooled to 15–20° and treated dropwise with a solution of $NaNO_2$ (4.49 g, 65.2 mmol) in H_2O (20 ml). The solution was stirred at 25° for 5 h, filtered, neutralized with 50% NaOH, and cooled in an ice bath. The precipitate was collected, washed with H_2O, and stirred with 1 N NaOH (100 ml) for 30 min. The crude product was collected, washed with H_2O, dried, triturated with 1 N NaOH (20 ml), collected, washed with H_2O, and dried in vacuo (P_2O_5): yield 2.77 g (38%); mp 256° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$) (Me_2SO–MeOH, 2:23), (0.1 N HCl) 235 (17.0), 274 (sh) (8.44), 283 (8.62); λ_{max} (pH 7) 243 (12.4), 310 (10.1); λ_{max} (0.1 N NaOH) 243 (12.5), 310 (10.1); ^1H NMR δ 6.98 (s, 2, NH_2), 8.03 (s, 2, NH_2); ^{13}C NMR δ (±0.02) 162.54 (C_5), 172.48 (C_{3a}), 133.21 (C_{7a}), 158.00

(C_7). Anal. ($C_4H_4N_6S$) C, H, N.

(b) Powdered **25a** (20 mg) in a stoppered vial under N_2 was heated in an oil bath at 165° for 3 h. The resulting solid was triturated with 1 N NaOH (1 ml), collected, washed with H_2O, and dried in vacuo (P_2O_5): yield 6.1 mg (31%). This product was the same (TLC, uv) as that described in (a).

5-Amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-7-thione (25a). A suspension of **24a** (500 mg, 2.98 mmol) in 1 N NaOH (30 ml) was heated under N_2 at ~98° until complete solution occurred (20 min). The solution was heated an additional 10 min, cooled to 25°, filtered under N_2, and acidified with 6 N HCl to pH 6. The white precipitate was collected, washed with cold H_2O, and dried in vacuo (P_2O_5): yield 434 mg (87%); mp 258° dec (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$) (Me_2SO–MeOH, 2:23), (0.1 N HCl) 224 (14.1), 258 (7.41), 338 (17.3); λ_{max} (pH 7) 231 (19.5), 257 (5.04), 282 (sh) (34.7), 343 (16.8); λ_{max} (0.1 N NaOH) 224 (19.6), 279 (8.40), 328 (14.9); ^1H NMR δ 6.95 (s, 2, NH_2), 10–16 (2, NH); ^{13}C NMR δ (±0.02) 154.02 (C_5), 148.90 (C_{3a}), 132.82 (C_{7a}), 177.62 (C_7). Anal. ($C_4H_4N_6S$) C, H, N.

Reaction of 8c with Adenosine Deaminase. A solution of 22 units of a crystalline suspension of adenosine deaminase from calf intestinal mucosa in 0.01 ml of 3.2 M $(NH_4)_2SO_4$ (Sigma Chemical Co.) was added to a solution of **8c** (4.1 mg) in 50 ml of 0.05 M phosphate buffer at pH 7.5. A uv spectrum of this solution after 16 min showed complete conversion to **7c**.

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Inhibitors of tRNA Methyltransferases. *S*-Adenosylsulfonium Salts

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Three new compounds have been synthesized and tested as in vitro inhibitors of normal and tumor tRNA methyltransferases. These compounds are 5′-methylethyl(5′-adenosyl)sulfonium chloride (MEAS), 5′-methylpropyl-(5′-adenosyl)sulfonium chloride (MPAS), and 5′-ethylpropyl(5′-adenosyl)sulfonium chloride (EPAS). They were prepared by reacting an alkyl iodide with the appropriate alkyladenosyl thioether. Inhibition assays revealed all three compounds to be inhibitors of normal and tumor tRNA methyltransferases. The propyl compounds were slightly better inhibitors of the tumor tRNA methyl transferases. MPAS, EPAS, and MEAS had K_I's of 58.5, 61.7, and 24.5, respectively, for the normal tRNA methyltransferases and 15.3, 13.8, and 44.3, respectively, for the tumor tRNA methyltransferases.

The discovery by Magee and Farber[1] that the chemical carcinogen dimethylnitrosamine would methylate tRNA to a greater extent than DNA led Borek[2] to postulate that chemical alkylation might result in aberrant methylation in tumor cells. In a wide variety of tumor and oncogenic systems the tRNA methyltransferase activity is increased