NMR ($CDCl_3$) δ 2.42 (d, 8 H, 2- and 3-aziridinyl), 3.45 (s, 3 H, SO_3CH_3), 7.59 (d, 1 H, 6-H), 7.66 (m, 1 H, 7-H), 8.06 (d, 1 H, 8-H). Anal. ($C_{15}H_{14}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[(butylsulfonyl)oxy]-1,4-naphthoquinone (22): isolated as a glass; NMR ($CDCl_3$) δ 1.02 (t, 3 H, 4′-CH_3), 1.59 (m, 2 H, 3′-CH_3), 2.05 (m, 2 H, 2′-CH_2), 2.38–2.39 (2 s, 8 H, 2- and 3-aziridinyl), 3.59 (m, 2 H, 1′-CH_2), 7.57 (d, 1 H, 6-H), 7.62 (m, 1 H, 7-H), 8.02 (d, 1 H, 8-H). Anal. ($C_{18}H_{20}N_2O_5S{\cdot}0.5EtOAc$) C, H, N.

2,3-Diaziridinyl-5-[(hexadecylsulfonyl)oxy]-1,4-naphthoquinone (23): mp 71–73 °C; R_f 0.49 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 0.89 (t, 3 H, 16′-CH_3), 1.27–1.30 (m, 22 H, 5′- to 15′-CH_2), 1.40 (m, 2 H, 4′-CH_2), 1.54 (m, 2 H, 3′-CH_2), 2.07 (m, 2 H, 2′-CH_2), 2.40 (s, 4 H, 2-aziridinyl), 2.41 (s, 4 H, 3-aziridinyl), 3.60 (t, 2 H, 1′-CH_2), 7.60 (d, 1 H, 6-H), 7.64 (t, 1 H, 7-H), 8.04 (d, 1 H, 8-H). Anal. ($C_{30}H_{44}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[[(3-chloropropyl)sulfonyl]oxy]-1,4-naphthoquinone (24): mp 115–117 °C; R_f 0.22 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 2.41 (s, 8 H, 2- and 3-aziridinyl), 2.61 (m, 2 H, 2′-CH_2), 3.81 (m, 4 H, 3′- and 1′-CH_2), 7.60 (d, 1 H, 6-H), 7.65 (t, 1 H, 7-H), 8.06 (d, 1 H, 8-H). Anal. ($C_{17}H_{17}ClN_2O_5S{\cdot}0.5EtOAc$) C, H, N.

2,3-Diaziridinyl-5-[(β-styrylsulfonyl)oxy]-1,4-naphthoquinone (25): mp 171–173 °C dec; R_f 0.24 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 2.37 (2 s, 8 H, 2- and 3-aziridinyl), 7.21 (d, 1 H, styrene-H_α), 7.43–7.47 (m, 3 H, 6-, 3′-, and 5′-H), 7.25–7.56 (m, 3 H, 2′-, 6′-, and styrene-H_β), 7.62–7.65 (m, 3 H, 4′- and 7-H), 8.03 (q, 1 H, 8-H). Anal. ($C_{22}H_{18}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[(α-tolylsulfonyl)oxy]-1,4-naphthoquinone (26): isolated as a glass; R_f 0.24 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 2.42 (s, 4 H, 2-aziridinyl), 2.45 (s, 4 H, 3-aziridinyl), 4.91 (s, 2 H, α-CH_2), 7.43 (m, 3 H, 3′-, 4′-, and 5′-H), 7.51 (d, 1 H, 6-H), 7.60 (m, 2 H, 2′- and 6′-H), 7.61 (t, 1 H, 7-H), 8.02 (d, 1 H, 8-H). Anal. ($C_{21}H_{18}N_2O_5S$) C, H, N.

2,3-Diaziridinyl-5-[(d-10-camphorylsulfonyl)oxy]-1,4-naphthoquinone (27): mp 121–123 °C; R_f 0.33 (EtOAc/C_6H_{14}, 1:1, v/v); NMR ($CDCl_3$) δ 1.00 (s, 3 H, 9′-CH_3), 1.21 (s, 3 H, 8′-CH_3), 1.47 (m, 1 H, 4′-H, a or e), 1.77 (m, 1 H, 4′-H, a or e), 1.97 (m, 1 H, 5′-H, a or e), 2.11 (m, 1 H, 2′-H, a or e), 2.16 (m, 1 H, 3′-H), 2.41 (s, 8 H, 2- and 3-aziridinyl), 2.47 (m, 1 H, 5′-H, a or e), 2.55 (m, 1 H, 2′-H, a or e), 3.78 (d, 1 H, 10′-H_A or H_B), 4.05 (d, 1 H, 10′-H_A or H_B), 7.60 (d, 1 H, 6-H), 7.65 (m, 1 H, 7-H), 8.04 (d, 1 H, 8-H). Anal. ($C_{24}H_{24}N_2O_6S$) C, H, N.

Biological Test Procedures. Transplantation of L1210 ascites cells was carried out by withdrawing peritoneal fluid from donor CDF_1 female mice bearing 7-day growths. The suspension was centrifuged for 2 min (1600 g), the supernatant peritoneal fluid was decanted, and a 10-fold dilution with isotonic saline was made. The cell number was determined with a Coulter particle counter and the cell population was adjusted to 10^6 cells/mL. One-tenth milliliter of the resulting cell suspension (containing approximately 10^5 cells) was injected intraperitoneally into each animal. The drug was administered by intraperitoneal injection beginning 24 h after tumor implantation, once daily for 6 consecutive days. Test compounds were injected intraperitoneally as fine suspensions in isotonic saline in a volume of 0.25 mL. For any one experiment, animals were distributed into groups of five mice of comparable weight and maintained throughout the course of the experiment on Laboratory Chow pellets and water ad libitum. Controls given injections of a comparable volume of vehicle (saline) were included in each experiment. Mice were weighed during the course of the experiments, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity.

The same methodology was used for the testing of compounds against B16 melanoma bearing mice.

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Nucleosides of Azathioprine and Thiamiprine as Antiarthritics

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Azathioprine [Imuran; 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purine] is a widely used immunosuppressive and antiarthritic drug. For the sake of comparison, the riboside, the 2′-deoxyriboside, and the arabinoside of azathioprine and its 2-amino congener, thiamiprine, were prepared by an enzymatic method. In vitro, the cytotoxicities of these aglycons and their nucleosides were similar (ED_{50} = 0.8–2 μM), except for the arabinosides, which were nontoxic (ED_{50} >100 μM). In vivo, their activities were compared in the rat adjuvant arthritis model. The ribosides and 2′-deoxyribosides were less potent than their corresponding aglycons. The safety indexes of these nucleosides were comparable to those of the corresponding aglycons except for the 2′-deoxyriboside of azathioprine, which had an appreciably lower safety index than did azathioprine. Both arabinosides were inactive and nontoxic. All of the aglycons tested (6-mercaptopurine, azathioprine, 6-thioguanine, and thiamiprine) were of similar potency. However, azathioprine had a more favorable therapeutic index than did 6-mercaptopurine. Similarly, thiamiprine was safer than was 6-thioguanine. In this model, the *S*-(1-methyl-4-nitro-1*H*-imidazol-5-yl) moiety imparted greater safety to these thiopurines by decreasing toxicity but not affecting potency.

6-Mercaptopurine (1) and 6-thioguanine (6) are antileukemic agents of long-standing clinical usefulness. These agents are also immunosuppressive. Not completely understood is the superiority of their *S*-(1-methyl-4-nitro-1*H*-imidazol-5-yl) derivatives as immunomodulators.[1] Early studies showed that azathioprine [2; 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purine; Imuran] was particularly effective in preventing rejection of kidney transplants in dogs.[2] Consequently, it was developed in the clinic as a chemotherapeutic adjunct to kidney transplant protocols. In time, the clinical use of this agent was extended to autoimmune disorders that are associated with chronic inflammation. Currently, the widest use of azathioprine is in the treatment of rheumatoid arthritis. The 2-amino congener of azathioprine, thiamiprine [7; 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purin-2-amine; Guaneran], was evaluated in man as an antitumor agent[3] but has not been clinically assessed as either an

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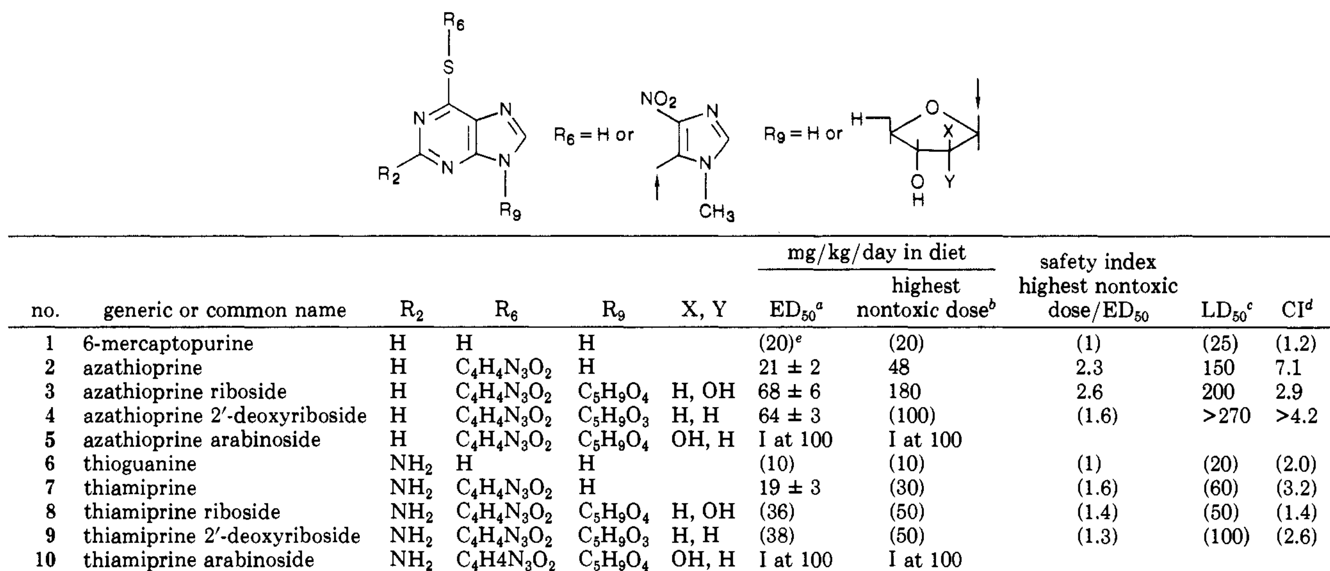
Table I. Evaluation of Some Thiopurines and Their Nucleosides against Adjuvant Arthritis in the Rat



no.	generic or common name	R_2	R_6	R_9	X, Y	mg/kg/day in diet: ED_{50}[a]	mg/kg/day in diet: highest nontoxic dose[b]	safety index highest nontoxic dose/ED_{50}	LD_{50}[c]	CI[d]
1	6-mercaptopurine	H	H	H		(20)[e]	(20)	(1)	(25)	(1.2)
2	azathioprine	H	$C_4H_4N_3O_2$	H		21 ± 2	48	2.3	150	7.1
3	azathioprine riboside	H	$C_4H_4N_3O_2$	$C_5H_9O_4$	H, OH	68 ± 6	180	2.6	200	2.9
4	azathioprine 2′-deoxyriboside	H	$C_4H_4N_3O_2$	$C_5H_9O_3$	H, H	64 ± 3	(100)	(1.6)	>270	>4.2
5	azathioprine arabinoside	H	$C_4H_4N_3O_2$	$C_5H_9O_4$	OH, H	I at 100	I at 100			
6	thioguanine	NH_2	H	H		(10)	(10)	(1)	(20)	(2.0)
7	thiamiprine	NH_2	$C_4H_4N_3O_2$	H		19 ± 3	(30)	(1.6)	(60)	(3.2)
8	thiamiprine riboside	NH_2	$C_4H_4N_3O_2$	$C_5H_9O_4$	H, OH	(36)	(50)	(1.4)	(50)	(1.4)
9	thiamiprine 2′-deoxyriboside	NH_2	$C_4H_4N_3O_2$	$C_5H_9O_3$	H, H	(38)	(50)	(1.3)	(100)	(2.6)
10	thiamiprine arabinoside	NH_2	$C_4H4N_3O_2$	$C_5H_9O_4$	OH, H	I at 100	I at 100			

[a] Dose at which there was a 50% reduction in arthritic score relative to untreated animals. [b] Highest dose at which there was no significant weight loss at day 16. [c] LD_{50} = dose (mg/kg/day in diet) producing 50% lethality at day 16. [d] Chemotherapeutic index = ratio of LD_{50} to ED_{50}. [e] Values in parentheses are approximate; two dose levels.

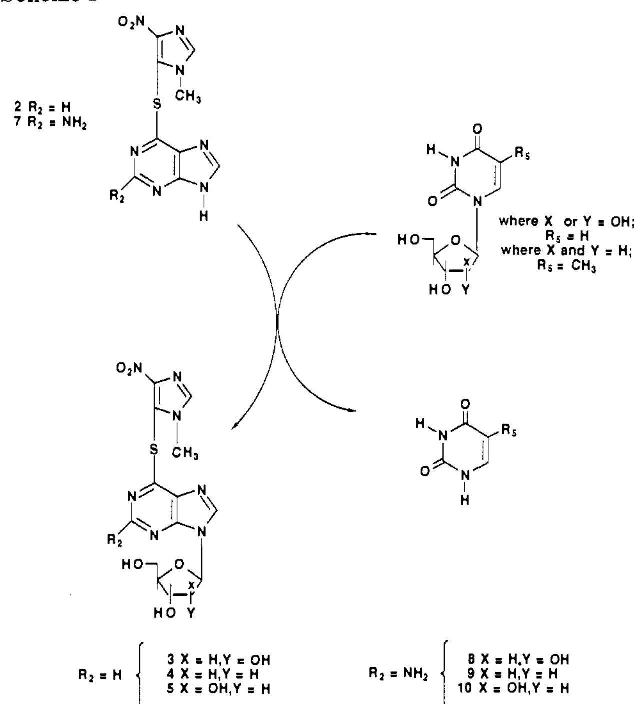
immunosuppressive or antiarthritic agent. It is, however, immunosuppressive in animal models.[1,4]

In this study, the riboside, the 2′-deoxyriboside, and the arabinoside of azathioprine and thiamiprine were synthesized and evaluated both in vitro and in vivo. In the rat adjuvant arthritis model,[5] these nucleosides were compared to the aglycons 6-mercaptopurine, azathioprine, 6-thioguanine, and thiamiprine.

Results and Discussion

Chemistry. Some of the nucleosides of azathioprine and thiamiprine have been previously synthesized (**3**,[6] **8**,[6,7] **9**[8]) by conventional procedures. In this study, all six nucleosides listed were prepared by an adaptation of an enzymatic method previously applied to purines,[9] pyrazolo[3,4-*d*]pyrimidines,[10] pyrimidines,[11] and imidazo[4,5-*c*]pyridines.[12] The synthesis is outlined in Scheme I and involves the coupling of two enzyme-catalyzed phospho-

Scheme I



rolytic reactions via an α-D-pentose 1-phosphate intermediate (not shown in Scheme I). The net effect is the transfer of the pentosyl moiety stereospecifically from a pyrimidine nucleoside to the 9-position of a purine. In all cases, the reaction of α-D-pentose 1-phosphate with the purine was catalyzed by purine nucleoside phosphorylase (EC 2.4.2.1). The phosphorolysis of the donor pyrimidine nucleoside was catalyzed by uridine phosphorylase (EC 2.4.2.3) for the synthesis of ribosides (**3**, **8**) and arabinosides (**5**, **10**) and by thymidine phosphorylase (EC 2.4.2.4) for 2′-deoxyribosides (**4**, **9**). The respective pentosyl donors were uridine, uracil arabinoside, and thymidine.

In previously described applications of this enzymatic method for nucleoside synthesis,[9-12] most of the reactions were in an aqueous buffered milieu. In cases where the pentosyl acceptor had poor water solubility, some ethanol

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or 1-propanol was added to the reaction mixtures. With azathioprine or thiamiprine, alcohol addition did not improve unsatisfactory yields that were attributed to low solubility. Many other cosolvents were tried. With azathioprine (2), the most favorable procedure was to add a solution of 2 in equal parts of *N,N*-dimethylformamide and *N,N*-dimethylacetamide to the aqueous mixture containing enzymes and donor. A similar procedure was adopted with thiamiprine (6) except that the solvent mixture was dimethyl sulfoxide and either glyme or diglyme. The ratio of organic cosolvent to water was approximately 1:10 (v/v). Nucleoside products were isolated from these reactions in yields of 40–69% calculated on the basis of aglycon used. The addition of these organic solvents to the reaction mixtures made practical the synthesis of the nucleosides in the quantities necessary for in vivo evaluation in the rat adjuvant arthritis model.

Biology. In vitro cytotoxicity in human epitheloid (detroit 98) cells[10b] gave ED_{50} values of 0.8–2.0 μM for azathioprine and thiamiprine and their ribosides and 2′-deoxyribosides (2–4, 6–9). The corresponding arabinosides (5, 10) were relatively nontoxic, with ED_{50} values greater than 100 μM.

Table I presents the activities of azathioprine (2) and thiamiprine (7), their nucleosides (3–5, 8–10), and the parent S-unsubstituted purines, 6-mercaptopurine (1) and 6-thioguanine (6), in the rat adjuvant arthritis model. These compounds were administered in the diet, because it was previously found that with azathioprine this route gave a higher chemotherapeutic index than did dosing by gavage.[13] The ED_{50} and LD_{50} values for azathioprine, although previously reported,[13] were redetermined for direct comparison to the data presented in Table I. The more recent values (Table I) are similar to the earlier data.[13] Azathioprine (2) and 6-mercaptopurine (1) were of nearly equal efficacy. Azathioprine (2) was approximately three times more potent than its riboside (3) and 2′-deoxyriboside (4). Its arabinoside (5) was inactive and nontoxic at the highest dose tested (100 mg/kg). The ratio of the highest dose at which there was no significant weight loss to the ED_{50} was used as the safety index. Thusly evaluated, the safety index was highest and approximately equal for azathioprine (2) and its riboside (3), somewhat lower for its 2′-deoxyriboside (4), and lowest for the parent compound 6-mercaptopurine (1). These results indicate that S-substitution of 6-mercaptopurine with the 1-methyl-4-nitro-1*H*-imidazol-5-yl moiety does not affect potency but increases safety by decreasing toxicity. 9-Substitution of azathioprine with 9-(β-D-ribofuranosyl) or 9-(β-D-2′-deoxyribofuranosyl) decreased potency but did not increase safety. 9-Substitution with β-D-arabinofuranosyl resulted in a virtually complete loss of both activity and toxicity.

Thioguanine (6) was twice as potent as 6-mercaptopurine (1), but neither showed separation between an efficacious and a toxic dose. S-Substitution of thioguanine with the 1-methyl-4-nitro-1*H*-imidazol-5-yl moiety resulted in a small decrease in potency and a somewhat larger decrease in toxicity, thereby increasing its chemotherapeutic index but not by as much as was seen with azathioprine (2) versus 6-mercaptopurine (1). 9-Substitution with β-D-ribofuranosyl or β-D-2′-deoxyribofuranosyl resulted in further loss of potency but less than that seen with the corresponding azathioprine nucleosides (3, 4). These substitutions did not increase safety. Again, 9-substitution with β-D-arabinofuranosyl obliterated both activity and toxicity. It has been reported that thiamiprine had a much better therapeutic index than did azathioprine in suppressing the immune response to bacterial and mammalian antigenic stimuli.[4] No such superiority of thiamiprine over azathioprine was observed with the model employed in this study (Table I).

Azathioprine (2) and thiamiprine (7) clearly had more favorable chemotherapeutic indexes than did 6-mercaptopurine (1) and 6-thioguanine (6) (Table I). The findings suggest that endogenous sulfhydryl modification[14] by reaction with the 1-methyl-4-nitro-1*H*-imidazol-5-yl moiety might contribute to the increased safety and reduced toxicity of 2 and 7. The possibility that there might be a difference in the nonenzymatic rates of reaction of the imidazolyl moieties of the compounds in Table I with glutathione was explored. The conditions of temperature (37 °C) and pH (6.8) used were chosen to approximate in vivo conditions. The pseudo-first-order rate constant for the reaction of azathioprine with 5 mM reduced glutathione was 0.030 ± 0.002 min^{-1} ($t_{1/2}$ = 23 min) and was independent of azathioprine concentration below 100 μM. The reaction of the nucleosides of azathioprine (3–5) and thiamiprine (7) and its nucleosides (8–10) with 5 mM glutathione was characterized by a similar, slightly higher, rate constant, 0.038 ± 0.002 min^{-1} ($t_{1/2}$ = 18 min). The bimolecular rate constant for the reaction of azathioprine and reduced glutathione was 5.8×10^3 $min{\cdot}mM^{-1}$. The similarity of the rates of reaction of the S-substituted compounds in Table I with reduced glutathione suggests that this reaction is not the basis for the differences in biological activities among these compounds. Further, the similar reactivity of the arabinosides 5 and 10, together with their lack of activity in vitro and in vivo, suggests that sulfhydryl modification is, in and of itself, not sufficient for activity in these systems. In vitro studies with human lymphoblasts have indicated the presence of some phosphoribosyltransferase-independent toxicity with azathioprine.[15] However, there is considerable evidence[16] to suggest that in the latter system the generation of 6-mercaptopurine rather than the reaction of cell components with the 1-methyl-4-nitro-1*H*-imidazol-5-yl moiety causes the cell toxicity.

In conclusion, it is clear that, in the rat adjuvant arthritis model, none of the congeners studied was superior to azathioprine itself. The lack of activity of the arabinosides (5, 10) despite their undiminished ability to react with glutathione puts into question the importance of sulfhydryl modification or the formation of imidazole metabolites per se in the mechanism of action of this class of compounds in vivo. It is also clear that, in the rat adjuvant arthritis model, the 1-methyl-4-nitro-imidazol-5-yl moiety increases the safety index of thiopurines by decreasing their toxicity without changing their potency. At present, it is uncertain whether sulfhydryl modification directly ameliorates toxicity or whether the 1-methyl-4-nitro-imidazol-5-yl moiety

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merely affects pharmacokinetic parameters critical to in vivo toxicity.

Experimental Section

Biology. The in vitro cytotoxicity tests were performed as previously described.[10b] The adjuvant-induced arthritis assay in the rat has been described in detail elsewhere.[5] The rats used in this study were purchased from Charles River, Wilmington, MA. They were female Lewis rats whose starting weight was 190 ± 10 g. All compounds were administered in the diet, which was prepared by milling with Wayne Rodent Blox diet (Continental Grain Co., Chicago, IL). Drug-containing diet was initiated on the same day that adjuvant was injected and continued for 14 days. Animals were evaluated for weight gain or loss and arthritic score on day 16. There were six animals in each group. The standard deviations for the arthritic scores were lowest (±0.7%) for the highest scores (little or no joint abnormalities) and greatest (±20%) for the lowest scores. At least three dose levels were used for determining each ED_{50} value unless otherwise noted.

Syntheses. Azathioprine (2) and thiamiprine (7) were prepared by the Wellcome Chemical Development Laboratories.[17] 1-β-D-Arabinofuranosyluracil was synthesized from 2,2′-anhydro-1-β-D-arabinofuranosylcytosine hydrochloride as previously described.[18] This precursor as well as uridine and thymidine was purchased from Sigma Chemical Co., St. Louis, MO. Q-Sepharose, an anion-exchange resin, was purchased from Pharmacia, Uppsala, Sweden. Reduced glutathione was purchased from Calbiochem, San Diego, CA.

Filtrations were performed by using Büchner funnels and Whatman 1 filter paper under reduced pressure. Solvents were removed in vacuo with a rotary evaporator at temperatures not above 45 °C. Solids were dried in vacuo at temperatures not above 50 °C. Yields were calculated on the basis of aglycon used.

Enzyme catalysts were purified from *Escherichia coli* and assayed, with units defined as previously described.[9] Thin-layer chromatography was on cellulose plates (Eastman, Rochester, NY; No. 13254 with fluorescent indicator) with water as the liquid phase or on silica gel plates (Merck, Darmstadt, West Germany, No. 5735) with chloroform/methanol/water (80:20:2). The cellulose system was more useful for monitoring the progress of the reactions and the silica gel system for the determination of purity during isolation of the products. Solid samples were dissolved in *N,N*-dimethylformamide for application to the plates.

A Varian XL-200 was used for the proton NMR spectra in Me_2SO-d_6. The exchangeability of protons with D_2O was used to assist in the assignments. Two-dimensional NMR was also performed on some of the products (**9** and **10**) to assist in assignments. Assignments of H_2 and H_8 are equivocal and may be reversed. All compounds listed in Table I gave elemental analyses within ±0.4% of calculated values. Analyses were performed by Atlantic Microlabs, Atlanta, GA. Melting points were obtained on a Thomas-Hoover capillary apparatus and are uncorrected. UV spectra were obtained by using a scanning Gilford spectrophotometer.

6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9-(β-D-ribofuranosyl)purine (3) (Azathioprine Riboside).[6] Azathioprine (**2**; 10 g, 36 mmol) was suspended in 60 mL each of *N,N*-dimethylacetamide (DMA) and *N,N*-dimethylformamide (DMF) at 40 °C. The suspension was added to 1080 mL of deionized water containing KH_2PO_4 (2 mmol), K_2HPO_4 (8 mmol), 26 g of uridine (106 mmol), 5300 units of uridine phosphorylase (EC 2.4.2.3), and 279 000 units of purine nucleoside phosphorylase (EC 2.4.2.1). After 72 h at 35 °C, additional uridine (5 g, 20 mmol), uridine phosphorylase (1300 units), and purine nucleoside phosphorylase (70 000 units) were mixed into the reaction. Forty-eight hours later, the pale yellow precipitate was collected by filtration. The filtrate was stored overnight at 3 °C, and the precipitate that formed was collected by filtration and combined with the solid from the first filtration. Contaminating uracil and azathioprine were eliminated by three recrystallizations from DMA/H_2O (1:5). The yield of product (8.73 g as the hemihydrate) was 58%: mp 180 °C (lit.[6] 169–171 °C); $[\alpha]^{20}_D$ −41.2° (*c* 0.5, DMF); UV λ_{max} (pH 7) 279 nm (ϵ 18 400) (lit.[5] pH 1, 279); NMR δ 8.79 (s, 1 H, H_8), 8.63 (s, 1 H, H_2), 8.23 (s, 1 H, imidazole H_2), 5.99 (d, 1 H, J = 5.4 Hz, $H_{1'}$), 5.48 (d, 1 H, J = 5.8 Hz, $OH_{2'}$), 5.18 (d, 1 H, J = 5.2 Hz, $OH_{3'}$), 5.03 (t, 1 H, J = 5.6 Hz, $OH_{5'}$), 4.58 (m, 1 H, $H_{2'}$), 4.17 (m, 1 H, $H_{3'}$), 3.97 (m, 1 H, $H_{4'}$), 3.69 (s, 3 H, CH_3), 3.58 (m, 2 H, $H_{5'}$). Anal. ($C_{14}H_{15}N_7SO_6{\cdot}0.5H_2O$) C, H, N, S.

6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9-(β-D-2′-deoxyribofuranosyl)purine (4) (Azathioprine 2′-Deoxyriboside). Thymidine (30 g, 124 mmol), KH_2PO_4 (5.5 mmol), K_2HPO_4 (5.5 mmol), and 4000 units of thymidine phosphorylase were dissolved in 965 mL of deionized water at 40 °C. The pH of the solution was 6.8. After 0.5 h, purine nucleoside phosphorylase (29 000 units) was added. A suspension of **2** (10 grams, 36 mmol) in 60 mL each of DMA and DMF was prepared separately at 45 °C. This suspension was added to the reaction mixture in 10-mL aliquots at 10-min intervals starting 5 min after the addition of purine nucleoside phosphorylase. The reaction mixture clarified before each subsequent addition. After 80% of the azathioprine suspension had been added, more thymidine (5 g, 21 mmol) was added. Twenty-five minutes after the final azathioprine addition, the solution was quickly chilled to 0 °C in a dry ice/acetone bath. It was stored on ice for 4 h, and then at −20 °C overnight. After thawing, the yellowish white precipitate was collected by filtration. The solids were suspended in 150 mL of deionized water, stirred for 1 h at 40 °C, and then stored overnight at 3 °C. The precipitate collected on the following day was extracted five more times in this manner with 100-mL aliquots of water. The final product was dried in a vacuum oven overnight at 40 °C. The yield (7.19 g as the monohydrate) was 49%; mp 190 °C; $[\alpha]^{20}_D$ −17.8° (*c* 0.5, DMF); UV λ_{max} (pH 7) 279 nm (ϵ 19 300); NMR δ 8.74 (s, 1 H, H_8), 8.62 (s, 1 H, H_2), 8.23 (s, 1 H, imidazole H_2), 6.42 (t, 1 H, J = 6.6 Hz, $H_{1'}$), 5.30 (d, 1 H, J = 4.2 Hz, $OH_{3'}$), 4.91 (t, 1 H, J = 5.6 Hz, $OH_{5'}$), 4.42 (m, 1 H, $H_{3'}$), 3.87 (m, 1 H, $H_{4'}$), 3.68 (s, 3 H, CH_3), 3.57 (m, 2 H, $H_{5'}$), 3.11 (m, 1 H, $H_{2'\alpha}$), 2.34 (m, 1 H, $H_{2'\beta}$). Anal. ($C_{14}H_{15}N_7SO_5{\cdot}H_2O$) C, H, N, S.

6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9-(β-D-arabinofuranosyl)purine (5) (Azathioprine Arabinoside). 1-(β-D-Arabinofuranosyl)uracil (8.6 g, 35 mmol) was dissolved in 1350 mL of deionized water containing K_2HPO_4 (28 mmol), KH_2PO_4 (7 mmol), 30 000 units of uridine phosphorylase, and 320 000 units of purine nucleoside phosphorylase. The pH was 7.4. After 6 h at 40 °C, 4 g (14 mmol) of **2** dissolved in 100 mL of a mixture of DMA and DMF (50/50) at 45 °C was added slowly over a 45-min period. The reaction mixture was stirred at 35 °C. At 24 h and again at 48 h, 1 g (4.1 mmol) of 1-(β-D-arabinosyl)uracil, 1000 units of uridine phosphorylase, and 6000 units of purine nucleoside phosphorylase were added to the reaction mixture. After 72 h, the reaction mixture was cooled to 25 °C and acetone was added to 60% by volume. Celite filter aid (28 g) was added and the suspension stirred for 15 min, followed by filtration. The solids, consisting mostly of denatured protein, were discarded. The acetone and water were evaporated from the filtrate, leaving a thick suspension which was thoroughly mixed with 250 mL of methanol at 40 °C. After this suspension remained at room temperature for 18 h and following subsequent filtration, methanol was evaporated from the filtrate and 500 mL of 5 mM Tris, pH 9.2, aqueous buffer (buffer A) was added to the suspension in the residual DMA and DMF. This solution was applied to a 7 × 14 cm Q-Sepharose column equilibrated with buffer A. The resin was washed with buffer A, and eluate that contained product was evaporated to dryness. The pale yellow solid was triturated four times in water, maintaining the pH at 9.2 by addition of 1% ammonium hydroxide. This trituration removed most of the remaining uracil from the product. A final crystallization from methanol yielded 2.65 g of **5** as the 0.1 hydrate (44%): mp 221–223 °C; $[\alpha]^{20}_D$ −13.4° (*c* 0.5, DMF); UV λ_{max} (pH 7) 279 nm (ϵ 19 100); NMR δ 8.61 (s, 1 H, H_8 or H_2), 8.60 (s, 1 H, H_8 or H_2), 8.23 (s, 1 H, imidazole H_2), 6.35 (d, 1 H, J = 4.8 Hz, $H_{1'}$), 5.61 (d, 1 H, J = 5.2 Hz, $OH_{2'}$), 5.52 (d, 1 H, J = 4.6 Hz, $OH_{3'}$), 5.05 (t, 1 H, J = 5.4 Hz, $OH_{5'}$), 4.17 (m, 2 H, $H_{2',3'}$), 3.81 (m, 1 H, $H_{4'}$), 3.69 (s, 3 H, CH_3), 3.66 (m, 2 H, $H_{5'}$). Anal. ($C_{14}H_{15}N_7SO_6{\cdot}0.1H_2O$) C, H, N, S.

2-Amino-6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9-(β-D-ribofuranosyl)purine (8) (Thiamiprine Riboside).[6,7]

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(18) Krenitsky, T. A.; Koszalka, G. W.; Tuttle, J. V.; Rideout, J. L.; Elion, G. B. *Carbohydr. Res.* 1981, 97, 139.

Thiamiprine (7; 10 g, 34 mmol) was suspended in 70 mL of dimethyl sulfoxide and 209 mL of bis(2-methoxyethyl) ether. This suspension was mixed with 600 mL of deionized water containing K_2HPO_4 (6 mmol), KH_2PO_4 (6 mmol), and uridine (29 g, 120 mmol). The pH of this suspension was 7.6. Uridine phosphorylase (8000 units) was added. After 2 h at 24 °C, purine nucleoside phosphorylase (67 000 units) was added and the suspension placed in an incubator at 39 °C. After 24 h, the suspension was removed from the incubator, 800 mL of ethyl acetate was added, and the two-phase mixture was mechanically stirred for 1 h. After 17 h at 24 °C, the mixture was filtered and the solids were extracted with two 500-mL portions of ethyl acetate. The remaining solids were suspended in 1800 mL of methanol, stirred, and brought to a boil. The hot suspension was rapidly filtered, and the filtrate was evaporated to dryness. The residue was dissolved in 55 mL of DMF by warming. Water (250 mL) was slowly added to this solution. After 1.5 h at 3 °C, the product was collected by filtration and dried. The yield (8.7 g as the 0.75 hydrate) was 58%: mp 235–237 °C (lit.[6,7] 234–236 °C dec; 208–209 °C); $[\alpha]^{20}_D$ −24.0° (*c* 0.5, DMF); UV λ_{max} (pH 7) 317 nm (ϵ 14 700) [lit.[6,7] pH 1, 315; MeOH 311 (14 600)]; NMR δ 8.21 (s, 1 H, H_8), 8.18 (s, 1 H, imidazole H_2), 6.61 (s, 2 H, NH_2), 5.76 (d, 1 H, J = 6.0 Hz, $H_{1'}$), 5.41 (d, 1 H, J = 6.0 Hz, $OH_{2'}$), 5.13 (d, 1 H, J = 6.8 Hz, $OH_{3'}$), 5.00 (t, 1 H, J = 5.4 Hz, $OH_{5'}$), 4.45 (m, 1 H, $H_{2'}$), 4.09 (m, 1 H, $H_{3'}$), 3.87 (m, 1 H, $H_{4'}$), 3.67 (s, 3 H, CH_3), 3.56 (m, 2 H, $H_{5'}$). Anal. ($C_{14}H_{16}N_8SO_6 \cdot 0.75H_2O$) C, H, N, S.

2-Amino-6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9-(β-D-2′-deoxyribofuranosyl)purine (9) (Thiamiprine 2′-Deoxyriboside).[8] Thiamiprine (7; 2 g, 6.8 mmol) was suspended in 10 mL of dimethyl sulfoxide and 60 mL of glyme (1,2-dimethoxyethane). This suspension was mixed with 120 mL of deionized water containing K_2HPO_4 (1.2 mmol), KH_2PO_4 (1.2 mmol), potassium acetate (1.2 g), and thymidine (8 g, 33 mmol). The pH of the mixture was 7.8. Thymidine phosphorylase (800 units) and purine nucleoside phosphorylase (27 000 units) were added. The suspension was stirred for 1 h at 24 °C and then at 35 °C for 17 h. The mixture was filtered and the filtrate was evaporated to a thick suspension. Water (250 mL) was added to this suspension with stirring and then placed at 3 °C for 7 h. The solids were collected by filtration, washed with water, and after drying suspended in 7 mL of DMF and 7 mL of glyme. After warming, the suspension was filtered and the solids were washed with two 5-mL portions of glyme. The filtrate and washes were evaporated to a thick clear syrup to which was added 70 mL of water. After 3 h at 24 °C and 18 h at 3 °C, the precipitated product was collected by filtration, washed with water, and dried. The yield (2 g as the monohydrate) was 69%: mp 143–145 °C (lit.[8] 150–151 °C); $[\alpha]^{20}_D$ −21.6° (*c* 0.5, DMF) [lit.[8] −22.5° (*c* 0.25, DMF)]; UV λ_{max} (pH 7) 317.5 nm (ϵ 14 400); NMR δ 8.18 (s, 2 H, H_8 and imidazole H_2), 6.59 (s, 2 H, NH_2), 6.18 (dd, 1 H, J = 6.3 and 7.4 Hz, $H_{1'}$), 5.26 (d, 1 H, J = 4.0 Hz, $OH_{3'}$), 4.91 (t, 1 H, J = 5.6 Hz, $OH_{5'}$), 4.33 (m, 1 H, $H_{3'}$), 3.80 (m, 1 H, $H_{4'}$), 3.66 (s, 3 H, CH_3), 3.50 (m, 2 H, $H_{5'}$), 2.55 (m, 1 H, $H_{2'\alpha}$), 2.20 (m, 1 H, $H_{2'\beta}$). Anal. ($C_{14}H_{16}N_8SO_5 \cdot H_2O$) C, H, N, S.

2-Amino-6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9-(β-D-arabinofuranosyl)purine (10) (Thiamiprine Arabinoside). The reaction mixture was the same as that described for 8 except that 1-(β-D-arabinofuranosyl)uracil (29.3 g, 120 mmol) was the pentosyl donor instead of uridine and greater quantities of enzyme catalysts were used (190 000 units of purine nucleoside phosphorylase and 20 000 units of uridine phosphorylase). After 24 h at 39 °C, ethyl acetate was added to the reaction mixture and treated subsequently as described for **5** except that the solids were extracted with ethyl acetate only once. The remaining solids were extracted twice with 1300 mL and five times with 300-mL portions of boiling methanol. All of the methanol extracts were combined and evaporated to dryness. The residue was dissolved in 55 mL of warm DMF. Water (750 mL) was then added to this clear solution, and after 1.5 h at 3 °C, the solids were collected by filtration. After extraction with a mixture of 50 mL of 1-propanol and 100 mL of water, the solids were dried and dissolved in 30 mL of DMA and reprecipitated by adding 55 mL of water. After collecting the precipitate by filtration and drying, the product was crystallized from a DMA solution with ethanol and then recrystallized twice from DMF solutions with methanol to remove slight impurities that had lower R_f values in the silica gel TLC system described above. The final product (5.75 g) was obtained in 40% yield: mp 250 °C dec; $[\alpha]^{20}_D$ 18.4° (*c* 0.5, DMF); UV λ_{max} (MeOH) 310 nm (ϵ 11 300); NMR δ 8.18 (s, 1 H, imidazole H_2), 8.01 (s, 1 H, H_8), 6.56 (s, 2 H, NH_2), 6.09 (d, 1 H, J = 4.0 Hz, $H_{1'}$), 5.59 (d, 1 H, J = 5.0 Hz, $OH_{2'}$), 5.48 (d, 1 H, J = 3.8 Hz, $OH_{3'}$), 5.01 (t, 1 H, J = 5.2 Hz, $OH_{5'}$), 4.06 (m, 2 H, $H_{2',3'}$), 3.74 (m, 1 H, $H_{4'}$), 3.67 (s, 3 H, CH_3), 3.60 (m, 2 H, $H_{5'}$). Anal. ($C_{14}H_{16}N_8SO_6$) C, H, N, S.

Rate Constants. The reaction of excess reduced glutathione (GSH) with limiting azathioprine (50 μM) was a first-order process. Pseudo-first-order rate constants for the reaction at varying GSH concentrations (1.0–10 mM) in 50 mM potassium phosphate buffer at pH 6.8 and 37 °C were estimated from time-dependent absorbance changes at 323 nm ($\Delta\epsilon$ = +11 mM^{-1} cm^{-1}). These absorbance data were fit by an iterative nonlinear least-squares routine to the function $a + be^{-ct}$.[19] The plot of these pseudo-first-order rate constants versus GSH concentration was linear. From the slope of this line, the bimolecular rate constant for the reaction of azathioprine with GSH was calculated. For the other compounds, the pseudo-first-order rate constants were determined at 5 mM GSH, otherwise under the same conditions as for azathioprine except that 8–10 were monitored at 343 nM ($\Delta\epsilon$ = +11 mM^{-1} cm^{-1}).

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Registry No. **1**, 50-44-2; **2**, 446-86-6; **3**, 3052-02-6; **4**, 116019-33-1; **5**, 120416-99-1; **6**, 154-42-7; **7**, 5581-52-2; **8**, 3384-61-0; **9**, 61552-42-9; **10**, 120417-00-7; uridine, 58-96-8; thymidine, 50-89-5; 1-(β-D-arabinofuranosyl)uracil, 3083-77-0.

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