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# Preparation and Activity of the 4′-Thio Derivatives of Some 6-Substituted Purine Nucleosides[1]

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6-Chloro-9-(4-thio-β-D-ribofuranosyl)purine was prepared by condensation of 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride with the chloromercuri derivative of 6-chloropurine, followed by ammonolysis. Nucleophilic substitution was used to replace the 6-chloro with $NH_2$, $Me_2N$, or SH, and dehalogenation furnished the 4′-thio analog of 9-β-D-ribofuranosylpurine (nebularine). Replacement of the O in the carbohydrate skeleton with S led to marked changes in the potency of the compounds, as determined *in vitro* with *Streptococcus faecium*, *Escherichia coli*, Leukemia L-1210, and Ehrlich ascites cells. Depending on the test system used, the potency of the thioribosyl nucleosides was greater or smaller than that of the corresponding ribosyl analog. These differences in activity are likely related to differences in the metabolic disposition of the compounds. For example, unlike 6-mercapto-9-(β-D-ribofuranosyl)purine, the corresponding thio-D-ribosyl analog did not undergo enzymatic cleavage of its glycosyl bond. As a result, a mutant strain of *S. faecium* resistant to the inhibitory effect of 6-mercapto-9-(4-thio-β-D-ribofuranosyl)purine was still sensitive to the action of both 6-mercaptopurine and 6-mercapto-9-(β-D-ribofuranosyl)purine. When used in combination with the corresponding ribosyl analogs, the thioribosyl derivatives tested interfered with the growth of *S. faecium* in a synergistic manner. In view of this synergism and the observed activity against resistant strains, these compounds deserve evaluation *in vivo*.

In the past, numerous chemotherapeutically useful compounds of the nucleoside variety have been obtained through structural modification of either the base or the sugar moiety. Compounds of this nature include, for instance, 6-mercapto-9-(β-D-ribofuranosyl)-purine[2] and 1-(β-D-arabinofuranosyl)cytosine.[3] The simultaneous modification of both the base and the sugar has also been done, as, for example, 9-(β-D-arabinofuranosyl)-6-mercaptopurine.[4] Such double alterations offer the possibility for additional changes in the potency and selectivity of singly modified compounds, resulting, possibly, in their improved chemotherapeutic effectiveness. This paper reports the preparation and activity of a number of adenosine analogs, modified both at the 6 position of the heterocycle and in the 4 position of the carbohydrate (Figure 1).

From the available synthetic routes for the preparation of the desired purine nucleosides,[5-8] direct glycosylation of the chloromercuri derivative of 6-chloropurine was chosen. Treatment of 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride with the chloromercuri derivative of 6-chloropurine gave a 37% yield of the acetylated syrupy nucleoside (Ia). Deacetylation of Ia gave crystalline 6-chloro-9-(4-thio-β-D-ribofuranosyl)purine (Ib). Its structure was established by conversion into 9-(4-thio-β-D-ribofuranosyl)adenine through treatment in MeOH–$NH_3$ to produce compound III identical with that previously prepared[9] from chloromercuri 6-benzamidopurine and 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride. Treatment of Ib with thiourea in EtOH produced 6-mercapto-9-(4-thio-β-D-ribofuranosyl)purine (II). Nucleophilic displacement of the 6-Cl in Ib with $HNMe_2$ in MeOH gave 6-dimethylamino-(4-thio-β-D-ribofuranosyl)purine (IV). Compounds II and IV possessed uv spectra similar to 6-mercapto-9-(β-D-ribofuranosyl)purine[2,6] and 6-dimethylamino-9-(β-D-ribofuranosyl)purine, respectively.[10,11] Dehalogenation of Ib with $H_2$ and Pd catalyst gave 9-(4-thio-β-D-ribofuranosyl)purine (4′-thionebularine). An attempt was made to synthesize 4′-thionebularine by condensation[5] of 2,3,4-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride with the chloromercuri derivative of purine, but after the usual work-up and ammonolysis, tlc showed no evidence of compound V.

The effects of the thionucleosides on the *in vitro* growth of 4 cell systems is shown in Table I. To evaluate the contribution which the ring S makes to the potency of the compounds, the corresponding nucleosides were examined in parallel, with the exception of 6-dimethylamino-9-(β-D-ribofuranosyl)purine. The introduction of S as the sugar ring heteroatom led to marked changes both in potency and in selectivity of the compounds. Thus, the 6-chloro-9-(4-thio-β-D-ribofuranosyl)purine was approximately 300 times more active against *S. faecium* than was the 6-chloro-9-(β-D-ribofuranosyl)purine. Similarly, the 9-(4-thio-β-D-ribofuranosyl)purine was an effective inhibitor of the growth of this organism, whereas 9-(β-D-ribofuranosyl)-purine was inactive. This change in potency occurred also in the reverse direction, as shown by the fact that 6-mercapto-9-(β-D-ribofuranosyl)purine was approxi-

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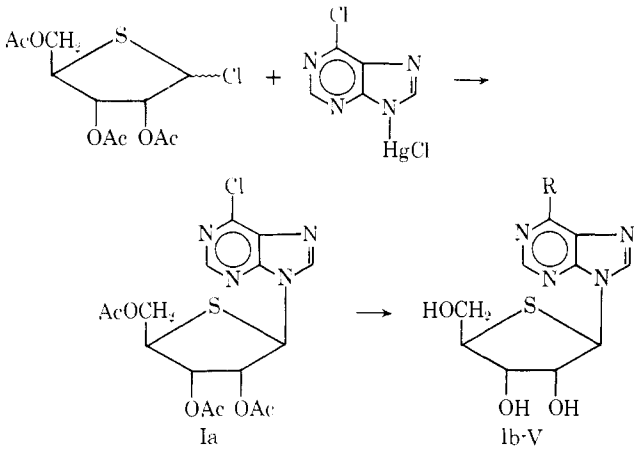
TABLE I

*In Vitro* GROWTH INHIBITION BY 4'-THIONUCLEOSIDES

| Derivative of Purine | *M* concentration required for 50% growth inhibition of | | | |
|---|---|---|---|---|
| | *S. faecium* | *E. coli* | Leukemia L-1210 | Ehrlich ascites |
| 6-Chloro-9-(4-thio-β-D-ribofuranosyl) (Ib) | $6 \times 10^{-7}$ | $>10^{-3}$ | $2 \times 10^{-5}$ | $1 \times 10^{-5}$ |
| 6-Chloro-9-(β-D-ribofuranosyl) | $2 \times 10^{-4}$ | $9 \times 10^{-5}$ | $3 \times 10^{-5}$ | $5 \times 10^{-5}$ |
| 6-Mercapto-9-(4-thio-β-D-ribofuranosyl) (II) | $2 \times 10^{-6}$ | $>10^{-3}$ | $5 \times 10^{-5}$ | $1 \times 10^{-4}$ |
| 6-Mercapto-9-(β-D-ribofuranosyl) | $9 \times 10^{-5}$ | $3 \times 10^{-5}$ | $7 \times 10^{-7}$ | $8 \times 10^{-6}$ |
| 9-(4-Thio-β-D-ribofuranosyl) (V) | $8 \times 10^{-5}$ | $>10^{-3}$ | $6 \times 10^{-4}$ | $4 \times 10^{-4}$ |
| 9-(β-D-Ribofuranosyl)purine (Nebularine) | $>10^{-3}$ | $>10^{-4}$ | $2 \times 10^{-5}$ | $3 \times 10^{-5}$ |
| 6-Dimethylamine-9-(4-thio-β-D-ribofuranosyl) (IV) | $7 \times 10^{-5}$ | $>10^{-3}$ | $4 \times 10^{-4}$ | $5 \times 10^{-4}$ |




Ib, R = Cl
II, R = SH
III, R = $NH_2$
IV, R = $N(CH_3)_2$
V, R = H

Figure 1.

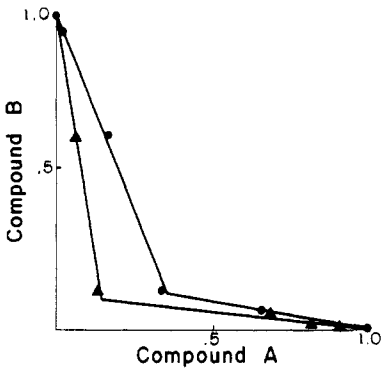



Figure 2.—Effect of combinations of D-ribofuranosides and 4'-thio-D-ribofuranosides on the growth of *S. faecium* as expressed in terms of fractional inhibitory concentrations: (▲) compound A, 6-mercaptopurine D-riboside; B, 6-mercaptopurine 4-thio-D-riboside; (●) compound A, 6-chloropurine D-riboside; B, 6-chloropurine 4-thio-D-riboside.

mately 400 times more inhibitory to *S. faecium* than was the corresponding 4-thio-D-ribofuranosyl analog. The inhibition of growth of this organism by both the β-D-ribofuranosyl analog and the 4'-thio-β-D-ribofuranosyl analogs was readily reversed by the natural purine nucleosides, indicating their antimetabolite nature.

With the exception of the 6-chloro- and 6-mercapto-9-(β-D-ribofuranosyl)purine, none of the analogs was active against *E. coli*, demonstrating a selective effect which, in turn, reflects differences in the metabolic disposition of the compounds by the cells. For example, in cell-free extracts of *E. coli* 6-mercapto-9-(β-D-ribofuranosyl)purine underwent cleavage, whereas the 4-thio-β-D-ribofuranosyl analog did not. Since 6-mercaptopurine itself was somewhat more active than the nucleoside ($9 \times 10^{-7}$ *M*) it appears that, in this organism, activation of the nucleoside proceeds *via* a pyrophosphorylase rather than *via* a kinase. The lack of cleavage of the 9-(4-thio-β-D-ribofuranosyl)purine could then be responsible for the lack of its metabolic activation.

In *S. faecium* the 6-mercapto-9-(4-thio-β-D-ribofuranosyl)purine did not undergo cleavage either, and its biological activity may result from its activation *via* a kinase. This deduction receives support from cross-resistance studies. As summarized in Table II, the strain of *S. faecium* resistant to 6-mercapto-9-(β-D-ribofuranosyl)purine was completely cross-resistant to the effects of the corresponding 4-thio-β-D-ribofuranosyl analog, while the mutant resistant to the action of the 6-mercapto-9-(4-thio-β-D-ribofuranosyl)purine was still sensitive to inhibition by the corresponding D-ribosylpurine and by the base. In this organism the 6-mercapto-9-(β-D-ribofuranosyl)purine resistance involves the deletion of both a phosphorylase and a kinase, whereas resistance to the 6-mercapto-9-(4-thio-β-D-ribofuranosyl)purine may require deletion of only a kinase. Biochemical differences of this nature may well contribute to the synergistic effects seen when corresponding pairs of β-D-ribofuranosyl and 4-thio-β-D-ribofuranosyl nucleosides were tested in combination (Figure 2). Differences in potency between the ribosyl and thioribosyl analogs were also seen in the two tumor lines, leukemia L-1210 and Ehrlich ascites carcinoma (Table I). With the exception of the 6-Cl derivative, the 4-thio-β-D-ribofuranosyl analogs were less effective inhibitors of these cell lines than were the corresponding β-D-ribofuranosyl nucleosides. Nevertheless, in view of the synergistic effects produced by combinations of corresponding D-ribosyl and 4-thio-D-ribosyl nucleosides, and the continued sensitivity of the 6-mercapto-9-(4-thio-β-D-ribofuranosyl)purine-resistant mutant to inhibition by 6-mercapto-9-(β-D-ribofuranosyl)purine, further evaluation of the compounds *in vivo* is indicated.

## Experimental Section[12]

**6-Chloro-9-(2,3,5-tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)purine (Ia).**—To a suspension of an azeotropically dried mixture (7 g)

(12) Where analyses are indicated by the symbols of the elements analytical results for those elements were within ±0.4% of the theoretical values. Melting points were taken on a Fisher–Johns apparatus and are corrected. Optical rotations are equilibrium values and were determined on a Perkin-Elmer, Model 141 polarimeter at 0.5 concentration in pyridine–$H_2O$ (1:1). Solvent concentration was conducted under reduced pressure in a rotary evaporator.

Table II

Cross-Resistance of *Streptococcus faecium* Cells Resistant to 6-Mercaptopurines

| | Resistant to inhibition[a] by | | |
|---|---|---|---|
| | 6-Mercaptopurine | 6-Mercapto-9-(β-D-ribofuranosyl)purine | 6-Mercapto-9-(4-thio-β-D-ribofuranosyl)purine |
| *S. faecium*/6-mercaptopurine | + | + | + |
| *S. faecium*/6-mercapto-9-(β-D-ribofuranosyl)purine | + | + | + |
| *S. faecium*/6-mercapto-9-(4-thio-β-D-ribofuranosyl)purine | − | − | + |

[a] (+) Resistant, (−) not resistant.

of the Hg derivative[13] of 6-chloropurine and Celite and 5 g of molecular sieve (Fisher Scientific Co., 4 A) in 50 ml of dry PhMe was added 3.15 g (0.01 mol) of 2,3,5-tri-*O*-acetyl-4-thio-α,β-D-ribofuranosyl chloride[14] (prepared from 1,2,3,5-tri-*O*-acetyl-4-thio-β-ribofuranose in 50 ml of dry PhMe). The reaction mixture was stirred with exclusion of moisture at 80–85° for 38 hr. The solids were removed by filtration and washed with 80 ml of EtOAc. This solution was washed with 30% KI (2 × 80 ml), once with $H_2O$ (100 ml), and dried over anhyd $Na_2SO_4$. The syrup remaining after removal of the EtOAc was purified by column chromatography on silica gel with hexane–EtOAc (3:2, v/v) as the eluent. After removal of the solvent, the syrup was dissolved in 50 ml of MeOH and evaporated to yield 1.62 g (37.3%); uv max (MeOH) 264 mμ.

**6-Chloro-9-(4-thio-β-D-ribofuranosyl)purine (Ib).**—Ia (1.27 g, 0.003 mol) was dissolved in 50 ml of methanolic $NH_3$ (saturated at 0°), allowed to stand at 5° overnight, and was concentrated to dryness at 40–45°. The product was dissolved in 20 ml of hot MeOH from which it crystallized on standing overnight. The colorless needles were removed by filtration, washed with EtOH, and dried; yield 680 mg; mp 190–192° dec. Partial evaporation of the filtrate yielded an additional 65 mg; total yield 82%. Recrystallized from MeOH, mp 190–192° dec; $[\alpha]^{25}D$ −41.8°; uv max ($H_2O$) 264 mμ (ε 10,200). *Anal.* ($C_{10}H_{11}ClN_4O_3S$) C, H, Cl, N, S.

**6-Mercapto-9-(4-thio-β-D-ribofuranosyl)purine (II).**—A mixture of 0.605 g (0.002 mol) of Ib, 0.2 g of thiourea, and 60 ml of 95.6% EtOH was refluxed 1.6 hr on a steam bath. Charcoal was added to the warm solution and the mixture filtered. The residue remaining after concentration of the filtrate was dissolved in 5 ml of hot $H_2O$ and 10 ml of hot EtOH was added. Crystallization occurred after standing of the solution overnight at 25°; yield 300 mg; mp 233–235° dec. Partial concentration of the filtrate yielded an additional 200 mg. Recrystallization from $H_2O$ gave 390 mg (65%); mp 234–235° dec; $[\alpha]^{25}D$ −55.6°; uv max ($H_2O$) 322 mμ (ε 24,900). *Anal.* ($C_{10}H_{12}N_4O_3S_2$) C, H, N, S.

**9-(4-Thio-β-D-ribofuranosyl)adenine (III).**—Ib (100 mg, 0.0005 mol) in 5 ml of methanolic $NH_3$ (saturated at 0°) was heated in a sealed tube at 100° for 7 hr. The residue after evaporation of the solvent was recrystallized twice from MeOH; yield 74 mg; mp and mixture mp 247–248°; $[\alpha]^{25}D$ −41.5°, uv max ($H_2O$) 261 mμ (ε 14,600); lit.[9] mp 248–249°; $[\alpha]^{25}D$ −42°; uv max (pH 7, 12) 261 mμ (ε 14,870). *Anal.* ($C_{10}H_{13}N_5O_3S \cdot 0.5H_2O$) C, H, N, S.

**6-Dimethylamino-9-(4-thio-β-D-ribofuranosyl)purine (IV).**—Ib (303 mg, 0.001 mol) dissolved with stirring in 10 ml of MeOH containing 0.5 ml of $Me_2NH$ was allowed to stand at 25° for 2 hr. Ib went into solution in 15 min and a white solid began to precipitate. This solid was removed by filtration; yield 257 mg (82.6%); mp 225.5–227°. The residue after concentration of the filtrate was dissolved in 8 ml of EtOH after 1 hr at 25 °the crystals were removed by filtration to yield an additional 21 mg of IV; total yield 278 mg (88.7%); $[\alpha]^{25}D$ −59.2°; uv max ($H_2O$) 271 mμ (ε 14,700). *Anal.* ($C_{12}H_{17}N_5O_3S$) C, H, N, S.

**9-(4-Thio-β-D-ribofuranosyl)purine, 4'-Thionebularine (V).**—Ib (454 mg, 1.5 mmol) was dissolved in a mixture of 50 ml of MeOH, 10 ml of $H_2O$, and 0.2 ml of methanolic $NH_3$ (saturated at 0°). To this solution Pd black (100 mg) was added and the mixture hydrogenated at 25° at atmospheric pressure for 8 hr. After removal of the catalyst by filtration and concentration of the filtrate to dryness, the residue was dissolved in 6 ml of EtOH and allowed to crystallize at 25°. Removal of the crystals by filtration gave V, yield 302 mg (75%). Recrystallization from $H_2O$–EtOH gave pure V; mp 214–215°; $[\alpha]^{25}D$ − 17.6°; uv max ($H_2O$) 262 mμ (ε 7,500). *Anal.* ($C_{10}H_{12}N_4O_3S$) C, H, N, S.

**Biological and Biochemical Assays.**—6-Mercaptopurine was purchased from Sigma Chemical Co; 6-mercapto-9-(β-D-ribofuranosyl)purine from Cyclo Chemical Corp.; 6-chloro-9-(β-D-ribofuranosyl)purine from K & K Labs, Inc., and nebularine was kindly provided by the Sloan-Kettering Institute for Cancer Research.

The techniques used for the microbial assays have been published previously.[15] *E. coli* K-12 was grown in the synthetic medium of Gray and Tatum,[16] *S. faecium* in the medium of Flynn, *et al.*,[17] from which the purines and pyrimidines were deleted, and to which 1 mμ/ml of folate was added. The inhibition analyses were performed by adding the natural nucleosides at concentrations ranging from $10^{-3}$ to $10^{-6}$ *M* to the growth medium containing the inhibitors. The resistant strains were selected by serial transfer in increasing concentrations of inhibitor. The *in vitro* antitumor assays were made by a microassay technique developed recently.[18]

Aliquots (0.5 ml) of medium[19] containing the various concentrations of the analog were added to 0.5-ml portions of medium containing 3 × $10^5$ L-1210 or 4 × $10^5$ Ehrlich ascites cells. Incubation at 37° proceeded for 40 hr, after which the viable cells were counted with trypan blue. During this time the cell number in the controls increased five to ninefold, with a viability of 99%.

The susceptibility of 6-mercapto-9-(β-D-ribofuranosyl)purine and the 4-thio-β-D-ribofuranosyl analog to enzymatic cleavage was examined in cell-free extracts of *S. faecium* and *E. coli*. Both nucleosides were rechromatographed before use with $H_2O$, pH 10, as the solvent. Preparation of the extracts was conducted as described previously[20] and the 40% $(NH_4)_2SO_4$ fraction of *E. coli* and the 60% fraction of *S. faecium* were used for the assays. The latter fraction was further purified by chromatography on Sephadex G-25, with 0.05 *M* Tris, pH 7.0, as the eluent. The incubation mixture included 0.08 ml of the nucleoside (2 × $10^{-3}$ *M*), 0.03 ml of 0.1 *M* $MgCl_2$, 0.04 ml of 0.1 *M* phosphate or Tris buffer, pH 7.5, and 0.2 ml of enzyme in a total volume of 0.35 ml. Incubation proceeded at 37° for 60 and 120 min. The reaction products were identified by comparison with authentic samples according to their $R_f$ and their spectral properties after chromatography of the mixtures in *n*-BuOH–glacial AcOH–$H_2O$ (2:1:1 v/v); *i*-AmOH–5% $Na_3PO_4$ (1:1); and $H_2O$ pH 10.

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