g) was added, and the reaction mixt was heated at reflux temp for another 2 hr. The soln was cooled to 22°, concd to 5-8 ml, and chromatogd on a silica gel column with PhH-Me_2CO (1:1, v/v). The fraction contg IV (as shown by tlc) was evapd, and the residue was recrystd from EtOH-Et_2O.

1,2-Dihydro-1-(β-D-ribofuranosyl)-2-oxopyrazine (IX). A mixt of 1,2-dihydro-2-oxopyrazine (4.3 g, 0.045 mole), $(Me_3Si)_2NH$ (4 ml), and Me_3SiCl (3 ml) was heated at reflux temp with stirring for 1 hr. After cooling to 22°, PhMe (80 ml) was added, and the soln was concd to an oil, which was dissolved in dry $C_2H_4Cl_2$ (250 ml). 1,2,3,5-Tetra-*O*-acetyl-β-D-ribofuranose (9.55 g, 0.03 mole) and $TiCl_4$ (3 ml) were added to this soln, and the reaction mixt was heated for 5 hr at reflux temp with stirring and exclusion of atm moisture. The mixt was cooled to 22° and poured slowly with vigorous stirring into a satd soln of $NaHCO_3$ (1500 ml). It was filtered through a Celite pad, which was then washed with $CHCl_3$. The org layer was sepd, washed with H_2O, and dried (Na_2SO_4). The solvent was evapd, and the residue was dissolved in MeOH (100 ml). A catalytic amt of MeONa was added to this soln and after 0.5 hr at 22°, the soln was neutralized with HCl and evapd. The syrupy residue was dissolved in MeOH (5 ml) and applied to a dry column (2.5 × 8 cm) of silica gel. A Me_2CO-MeOH (4:1, v/v) mixt was applied to the column and the eluate was evapd. The syrupy residue was dissolved in Me_2CO-EtOH (1:2, v/v; 30 ml); and crystn occurred after standing of the soln overnight at 22°. Recrystn from EtOH gave pure IX: $[\alpha]^{25}D$ −21.2°, $\bar{\nu}_{max}$ 3400, 3280 (OH), 2945-2920 (CH), 1645 cm^{-1} (C=O).

1,2-Dihydro-1-(β-D-ribofuranosyl)-2-oxopyrazine 4-Oxide (XI). A mixt of IVa (1.12 g, 0.01 mole), $(Me_3Si)_2NH$ (3 ml), and Me_3SiCl (3 drops) was heated for 0.5 hr at 90-95° with stirring and exclusion of atm moisture. The resulting soln was cooled to 22°, dild with dry PhMe (40 ml), and evapd. The remaining solid was evapd once more from dry PhMe (40 ml), and dissolved in dry $C_2H_4Cl_2$ (100 ml). To this soln was added 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (3.20 g, 0.01 mole) and $TiCl_4$ (3 ml), and the reaction mixt was heated at reflux temp with stirring and exclusion of atm moisture for 4 hr. It was cooled to 22° and poured slowly, with vigorous stirring, into a satd soln of $NaHCO_3$ (500 ml). The mixt was filtered through a Celite pad, which was subsequently washed with $CHCl_3$. The org layer was sepd, washed with H_2O, and dried (Na_2SO_4). The syrup remaining after removal of the solvent was purified by column chromatog on silica gel with $CHCl_3$-Me_2CO (7:1, v/v) as the eluent. The syrupy 1,2-dihydro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-oxopyrazine 4-oxide (X) (480 mg) obtd by evapn of the solvent was dissolved in MeOH (50 ml). A catalytic amt of MeONa was added to this soln and after 0.5 hr at 22° the soln was neutralized with Dowex 50 (H^+) resin. The resin was filtered and washed with MeOH (20 ml). The syrup remaining after removal of the MeOH was dissolved in 96% EtOH and purified by column chromatog on silica gel with Me_2CO as the eluent. Me_2CO was removed by evapn and the remaining solid was recrystd from 96% EtOH: $[\alpha]^{25}D$ −204.2°, $\bar{\nu}_{max}$ 3400, 3300 (OH), 2960, 2925 (CH), 1660 (C=O), 1230, 840, 830 cm^{-1} (N→O).

Biological Assays. The techniques used for these detns have been published previously.[25]

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Synthesis and Biological Activity of 4′-Thio Analogs of the Antibiotic Toyocamycin

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The 4′-thio analog of the antibiotic toyocamycin was prepared by condensation of 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride with the chloromercuri derivative of 4-acetamino-6-bromo-5-cyanopyrrolo-[2,3-*d*]pyrimidine, followed by removal of the protecting groups with MeOH–NH_3 and removal of Br with H_2/Pd catalyst. Condensation with the chloromercuri derivative of 4-chloro-6-bromo-5-cyanopyrrolo-[2,3-*d*]pyrimidine, followed by treatment with MeOH–NH_3 at 5°, effected removal of the protecting groups and nucleophilic substitution of the Br group to furnish 4-chloro-6-amino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. When treatment with MeOH–NH_3 was carried out at 120°, 4,6-diamino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine was formed. The 4′-thio derivatives proved to be effective inhibitors of the growth of leukemia L-1210 cells *in vitro*, their concn for 50% reduction of growth ranging from 4×10^{-7} to 5×10^{-6} *M*. 4′-Thiotoyocamycin retained full inhibitory activity against a strain of *Streptococcus faecium* resistant to 10^{-3} *M* toyocamycin.

The antibiotic toyocamycin[1] is an analog of adenosine in which N-7 of the imidazole ring is replaced by C, to which is attached a CN group.[2] In exptl systems, this antibiotic showed marked antitumor activity,[3] but the severe local toxocity in man[4] which it produced limited its clinical usefulness.

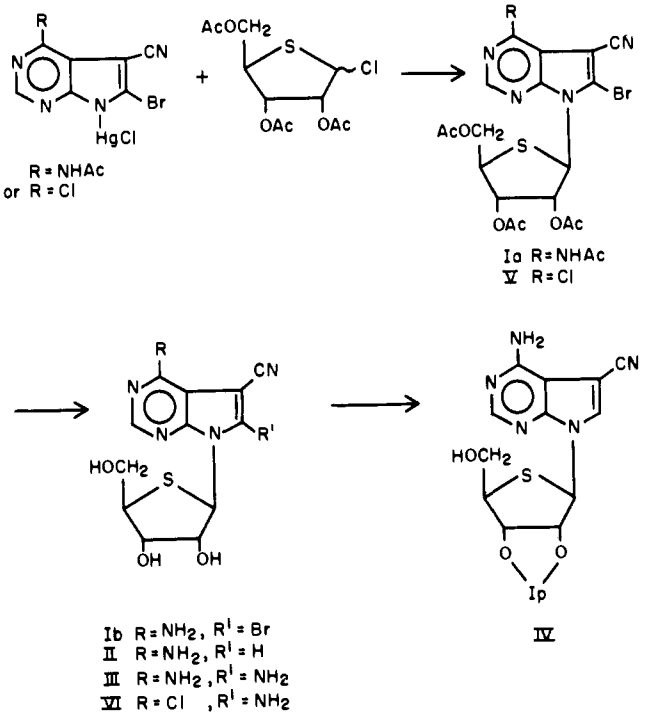
In an attempt at decreasing this toxicity, two structural modifications of the toyocamycin molecule were made. In one, the ring O of the carbohydrate moiety was replaced by S; the other involved, in addition to this replacement, the substitution of the 4 and 6 position of the heterocycle with Cl and amino groups, resp. The results obtained in *in vitro*

systems showed that these deriv had biol properties different from those of the parent compd. These properties and the procedures used for the synthesis of the compds are described in this paper.

A. Chemical. Various methods have, in the past, been used for the prepn of pyrrolopyrimidine nucleosides. Ribosidation of the chloromercuri salt of 4-amino-5-cyanopyrrolo[2,3-*d*]pyrimidine, for example, produced a nucleoside material which, because of the low yield obtained (less than 1%), could not be completely characterized.[5] The heavy metal salt procedure was also used in an attempt to synthesize tubercidin, but the desired nucleoside was not obtained.[6] Recently, the fusion procedure has been employed for the prepn of a number of pyrrolopyrimidine nucleosides in yields varying from 3 to 20% (based on the sugar component).[7] Application of the procedure employing trimethylsilyl derivs to pyrrolo[2,3-*d*]pyrimidines has furnished mixts of isomeric nucleosides in high yield.[8]

Since the chloromercuri procedure had been used by us successfully for the prepn of 4'-thio purine nucleosides,[9] we attempted to apply this method to the synthesis of 4'-thiopyrrolo[2,3-*d*]pyrimidine nucleosides (Scheme I).

Scheme I



Treatment of 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride with the chloromercuri deriv of 4-acetamino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine furnished a 20% yield of the acetylated syrupy nucleoside (Ia). The structure of this product was established as 4-acetamino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine on the basis of its uv, pmr, and ir spectra. On the basis of the trans rule, the anomeric configuration was presumed to be β. Deacetylation of Ia with methanolic NH_3 at 5° gave crystalline 4-amino-6-bromo-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (Ib). Dehalogenation of Ib with H_2/Pd gave 4-amino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (II, 4'-thiotoyocamycin). Treatment of Ib with MeOH-NH_3 at 115–120° produced 4,6-diamino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (III). The site of attachment of 4-thio-D-ribose was assigned to N-7, on the basis of uv absorption. As a result of substitution of S for O in the ribofuranosyl moiety, the uv absorption maxima for II in EtOH, pH 1 and pH 12, exhibit small bathochromic shifts relative to those obsd for toyocamycin.[7] Similar bathochromic shifts were exhibited by the 9,4'-thio-β-D-ribofuranosyladenine ring.[9,10]

Treatment of II with Me_2CO, 2,2-dimethoxypropane, and TsOH produced 4-amino-5-cyano-7-(2,3-*O*-isopropylidene-4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (IV), indicating the presence of a cis diol.

To evaluate the influence of certain substituents in the pyrrolopyrimidine ring structure on the yield, and the site of ribosidation, 4-chloro-6-bromo-5-cyanopyrrolopyrimidine was used in the condensation reaction. The chloromercuri salt of 4-chloro-6-bromo-5-cyanopyrrolo[2,3-*d*]-pyrimidine, prepd in 85% yield, was condensed with 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride in dry PhMe to give 4-chloro-6-bromo-5-cyano-7-(2,3,5-tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (V), which was isolated as a cryst substance by column chromatog. The yield of V was 25.4%. The site of glycosidation of V was assigned at N-7 on the basis of the uv spectra. Removal of the protecting groups from V was accomplished with MeOH-NH_3 at 5°. Elemental analysis and spectral examn of the product isolated indicated that nucleophilic displacement of the 6-Br by NH_2 had occurred, to furnish the 4-chloro-6-amino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (VI). This finding was rather unexpected, since treatment of 6-bromo-4-chloro-5-cyano-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine with MeOH-NH_3 at 110° affected removal of the blocking groups with concomitant displacement of the 4-Cl to give 4-amino-6-bromo-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine.[7] The susceptibility of the 6-Br of V to nucleophilic substitution is presumably due to the ability of the S atom to accommodate both positive and negative charges.

Treatment of V with MeOH-NH_3 at 115° produced 4,6-diamino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (III), which was identical in every respect with the compd prepd from Ib, thus proving that the site of ribosidation of the chloromercury deriv of both 4-chloro-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine and 4-acetamino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine is the same.

B. Biological. The effect of the 4'-thionucleosides on the growth of *Streptococcus faecium* and leukemia L-1210 cells is summarized in Table I. In the bacterial test system, 4'-thiotoyocamycin was approximately 10 times more effective an inhibitor than was toyocamycin, while the 6-amino deriv was 3 times more potent. The 6-Br deriv was inactive at $10^{-3}M$. This variance in activity constitutes a parallel to the marked growth-inhibitory activity of 8-aminoadenosine and the inactivity of 8-bromoadenosine in the *S. faecium* system.[11]

The inhibitory activity of the analogs in the mammalian cell system was found to differ from their effect on the bacteria primarily by the fact that toyocamycin was approx 10 times more potent an inhibitor of L-1210 growth than was the 4'-thio analog or its 6-amino deriv. Furthermore, unlike its inactivity in the bacterial system, 4'-thio-6-bromotoyocamycin was markedly inhibitory of the tumor cell growth.

Of importance for the potential chemotherapeutic use of these analogs is the finding that the active 4'-thio derivatives retained their full activity against a strain of *S. faecium* resistant to $10^{-3}M$ toyocamycin or to the related analog

Table I. Inhibition of Cell Growth by 4'-Thiotoyocamycin and Derivatives

Compound	Concentration (M) for 50% growth inhibition of			
	S. faecium	*S. faecium* resistant to Toyocamycin	*S. faecium* resistant to Tubercidin	Leukemia L-1210
Toyocamycin	9×10^{-5}	$>10^{-3}$	$>10^{-3}$	4×10^{-8}
4'-Thiotoyocamycin	7×10^{-6}	7×10^{-6}	7×10^{-6}	4×10^{-7}
4'-Thio-6-aminotoyocamycin	4×10^{-5}	4×10^{-5}	4×10^{-5}	6×10^{-7}
4'-Thio-6-bromotoyocamycin	$>10^{-3}$	$>10^{-3}$	$>10^{-3}$	5×10^{-6}

tubercidin (7-deazaadenosine). This finding parallels the observation reported by us previously,[9] showing that the 4'-thio derivative of 6-mercapto-9-(β-D-ribofuranosyl)purine retained its activity against a strain of *S. faecium* resistant to the corresponding 6-mercapto-9-(β-D-ribofuranosyl)-purine.

Whether these observed biol differences between toyocamycin and the 4'-thio derivs result in an improvement of their toxic properties remains to be detd.

Experimental Section†

4-Acetamino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (Ia). 4-Acetamino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine[7] (5.6 g; 0.02 mole) was added to 300 ml of H_2O, and to this mixt 10% NaOH soln was added dropwise with stirring until a clear soln was achieved (approx 8 ml). A soln of 5.42 g (0.02 mole) of $HgCl_2$ in 100 ml of EtOH, and 6 g of Celite was added. The pH of the mixt was adjusted to 7.5-8 with 10% NaOH soln, and the mixt was evapd to dryness. The residue was dried by azeotropic distn with xylene. A soln of 6.30 g (0.02 mole) of 2,3,5-tri-*O*-acetyl-4-thio-α,β-D-ribofuranosyl chloride[12] prepd from 1,2,3,5-tetra-*O*-acetyl-4-thio-β-D-ribofuranose) in 350 ml of dry PhMe was added, and the reaction mixt was stirred under exclusion of moisture at 90-95° for 50 hr. The solids were removed by filtration and washed with 200 ml of EtOAc. This soln was washed with 30% KI (2 × 80 ml) and once with H_2O (100 ml) and dried (Na_2SO_4). The syrup remaining after removal of the solvent was purified by column chromatog on silica gel with PhH-Me_2CO (8:2, v/v) as the eluent. The syrup was dissolved in 50 ml of MeOH and evapd to yield 2.2 g (19.8% of Ia: uv_{max} (EtOH) 288 (ε 12,580), (pH 1) 284 (12,790), (pH 12) 289 (14,070), 298 mμ (11,510); ir (CCl_4) 2235 (C≡N), 1765 (OAc), 1735 cm^{-1} (NHAc); pmr ($CDCl_3$) 1.95, 2.06, 2.15 (CCH_3), 6.28 d (J_1J_2, = 7.5 Hz), 8.83 (C_2H).

4-Amino-6-bromo-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (Ib). Ia (1.11 g; 0.002 mole) was covered with 50 ml of MeOH-NH_3 (satd at 0°), allowed to stand at 5° for 24 hr, and was concd to dryness at 40-45°. The remaining solid material was triturated with EtOH, and crystals were collected by filtration: yield 713 mg; mp 195-197° dec. Recrystn of this solid from EtOH-H_2O mixt afforded the product in 87.5% yield (675 mg): mp 199-201° dec; $[\alpha]^{25}D$ −103.1° (solvent a); uv max (EtOH) 297 (sh) (ε 10,420), 288 (13,510), 218 (16,800), 205 (17,180), (pH 1) 285 (12,510), 234 (14,670), 210 mμ (18,530); ir (Nujol) 2235 cm^{-1} (C≡N). *Anal.* ($C_{12}H_{12}BrN_5O_3S \cdot 0.5H_2O$) C, H, N, S.

4-Amino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (4'-Thiotoyocamycin) (II). Ib (505 mg) was added to a mixt of MeOH (100 ml) and Pd black (100 mg). MeOH-NH_3 (0.5 ml, satd at 0°) was added, and the mixt was hydrogenated at 25° at atm pressure for 4 hr. After removal of the catalyst by filtration and concn of the filtrate to near dryness, EtOH (8 ml) was added, and the mixt was allowed to stand at 5° for 4 hr. Removal of the crystals by filtration gave II: yield, 350 mg (87.2%). Recrystn from an EtOH-H_2O mixt afforded an analytical sample: mp 202-205°; $[\alpha]^{25}D$ −88.4° (solvent a); uv (EtOH) 293 (ε 9920), 283 (14,650), 276 (sh) (14,060), 235 (9690), 208 (22,810), (pH 1) 277 (11,810), 237 (17,960), (pH 12) 292 (sh) (9980), 281 mμ (14,860); ir (Nujol) 2235 cm^{-1} (C≡N). *Anal.* ($C_{12}H_{13}N_5O_3S \cdot 0.5H_2O$) C, H, N, S.

†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 detd on a Jasco Model ORD/uv-5 at 0.1 concn in (a) DMF-H_2O (1:4, v/v) and (b) EtOH. Ir spectra were detd on a Perkin-Elmer, Model 337 spectrophotometer. Pmr spectra were obtd on a Varian A-60 instrument (Me_4Si). Uv spectra were taken on a Cary Model 14 spectrophotometer. Solvent concn was carried out at reduced pressure in a rotary evaporator.

4,6-Diamino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (III). Method 1. V (2.126 g; 0.004 mole) was covered with 40 ml of methanolic NH_3 (satd at 0°) and the reaction mixt was heated in a sealed tube for 14 hr at 115-120°. The cooled soln was evapd to dryness, and MeOH was added to the residue and then removed until the odor of NH_3 was no longer evident. Dry column chromatog utilizing silica gel and $CHCl_3$-MeOH (4:1, v/v) as the developing solvent resolved the nucleoside and decompn products. The solvent was evapd and the residue was recrystd from H_2O: yield, 0.46 g (34.7%); mp 221-222°; $[\alpha]^{25}D$ −15.2° (solvent a); uv max (EtOH) 294 (ε 19,980), (pH 1) 298 (15,200), 237 (19,690), 224 mμ (21,080). *Anal.* ($C_{12}H_{14}N_6O_3S \cdot 0.5H_2O$) C, H, N, S.

Method 2. Ib (60 mg) was covered with 5 ml of MeOH-NH_3 (satd at 0°) and the reaction mixt heated in a sealed tube for 14 hr at 115-120°. The cooled soln was evapd to dryness and the residue was recrystd from H_2O: yield, 36 mg. The uv spectra and the chromatographic mobilities were identical with those of the product prepared by method 1. A mmp showed no depression.

4-Amino-5-cyano-7-(2,3-*O*-isopropylidine-4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (IV). II (133 mg) was dissolved in a mixt of Me_2CO (10 ml), 2,2-dimethoxypropane (0.5 ml), and TsOH · H_2O (150 mg). The reaction mixt was allowed to stand at 22° for 12 hr, then 450 mg of $KHCO_3$ was added, and the mixt was stirred for 1 hr. The solids were removed by filtration and washed with 30 ml of Me_2CO. The soln was evapd to dryness and the residue was extd with 5 portions (20 ml each) of $CHCl_3$. The solvent was evapd to dryness and the residue was recrystd from a H_2O-EtOH mixt: yield, 120 mg (80%); mp 112-113°; $[\alpha]^{25}D$ −119.5° (solvent b); uv max (EtOH) 292 (ε 12,670), 282 (18,680), 275 (sh) (18,340), 234 mμ (12,320). *Anal.* ($C_{15}H_{17}N_5O_3S \cdot 0.5H_2O$) C, H, N, S.

4-Chloro-6-bromo-5-cyano-7-(2,3,5-tri-*O*-acetyl-4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (V). To a mixt of 4-chloro-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine[7] (2.58 g; 0.01 mole) and H_2O (150 ml), a 10% NaOH soln was added dropwise with stirring until a clear soln was achieved. Then, 2.6 g of Celite and a soln of $HgCl_2$ (2.72 g; 0.01 mole) in 40 ml of 20% EtOH were added, and the mixt was stirred for an additional 30 min. The mixt of the HgCl deriv of 4-chloro-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine and Celite was then collected by filtration, washed with H_2O and dried in a desiccator under reduced pressure, yield 6.8 g (85.3%). To 7.7 g of this mixt, dried by azeotropic distn with xylene, was added a soln of 2,3,5-tri-*O*-acetyl-4-thio-α,β-D-ribofuranosyl chloride (3.15 g; 0.01 mole) in 100 ml of dry PhMe, and the reaction mixt was stirred with exclusion of moisture at 85-90° for 30 hr. It was worked up as described for the prepn of Ia, giving a pale yellow syrup. Column chromatog on silica gel with hexane-EtOAc (6:4, v/v) as the developing solvent sepd the nucleoside from the decompn products. The syrup remaining after removal of the solvent was dissolved in 10 ml of MeOH. After 5 hr at 5°, crystals sepd which were collected by filtration: yield, 1.35 g (25.4%); mp 134-136°. Recrystn from MeOH afforded an analytical sample: mp 136-137.5°; uv max (EtOH) 293 (sh) (ε 13,550), 285 (14,090), 228 (34,000), (pH 1) 292 (sh) (14,090), 284 (14,880), 228 mμ (38,280); ir (CCl_4) 2235 (C≡N), 1755 cm^{-1} (OAc). *Anal.* ($C_{18}H_{16}BrClN_4O_6S$) C, H, N, S.

4-Chloro-6-amino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (VI). IV (1.064 g; 0.002 mole) was dissolved in 50 ml of MeOH-NH_3 (satd at 0°) and allowed to stand at 5° for 15 hr. The soln was then evapd to dryness, EtOH (50 ml) was added to the residue and evapd to dryness. H_2O (8 ml) was added to the residue, and after 4 hr the cryst compd was collected by filtration. Recrystn from H_2O afforded the product in 43.1% yield (0.31 g): mp 208-210° (H_2O); $[\alpha]^{25}D$ −38.3° (solvent b); uv max (EtOH) 316 (ε 11,080), 240 (19,780), 213 (21,080), (pH 1) 314 (10,790), 239 (20,140), 213 mμ (23,490); ir (Nujol) 2220 cm^{-1} (C≡N). *Anal.* ($C_{12}H_{12}ClN_5O_3S \cdot H_2O$) C, H, N, Cl, S.

Biological Assays. The techniques used for the microbial assays have been published previously.[9] *S. faecium* was grown in a defined medium free of purines but containing 1 mg/ml of folate. The resistant strains were selected by serial transfer in increasing

concns of inhibitor. The *in vitro* antitumor assays were carried out by our microassay technique which involves the introduction of 0.5 ml aliquots of the medium (RPMI 1630 + 10% calf serum) contg the various concns of the analog into 16 × 125 mm screw cap culture tubes, followed by 0.5 ml portions of medium contg 3×10^5 L-1210 cells. The cultures are incubated at 37° for 40 hr, after which the viable cells are counted by trypan blue exclusion. During this time the cell number in the controls increases approximately eight- to ninefold, with an average viability of 99%.

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Synthesis and Antimicrobial Activity of a Carbocyclic Puromycin Analog. 6-Dimethylamino-9-{*R*-[2*R*-hydroxy-3*R*-(*p*-methoxyphenyl-L-alanylamino)]-cyclopentyl}purine†

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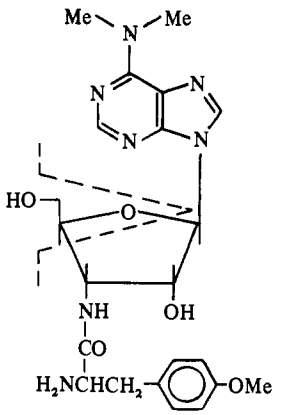
An assessment of the requirement for the furanosyl O and the CH_2OH moiety in the puromycin molecule was undertaken by the synthesis of a novel puromycin analog. A carbocyclic analog, 6-dimethylamino-9-{*R*-[2*R*-hydroxy-3*R*-(*p*-methoxyphenyl-L-alanylamino)]cyclopentyl}purine (**2**), was synthesized and evaluated for antimicrobial activity. The carbocyclic analog exhibited antimicrobial activity comparable to puromycin, and also circumvented the nephrotic syndrome associated with puromycin by releasing a nontoxic aminonucleoside upon hydrolysis. The diastereoisomer (**19**) of **2** was also isolated and found to be devoid of antimicrobial activity.

Puromycin (**1**), an antibiotic with antitumor activity,[1] has been found to inhibit protein synthesis in a wide variety of organisms. Its structure has a striking resemblance to that of the aminoacyl-adenyl terminus of aminoacyl-tRNA, and it has been demonstrated that the antibiotic causes premature release of the polypeptide chains from the ribosome.[2] For this reason, puromycin has been used extensively as a tool in the investigation of protein biosynthesis.



1

A variety of analogs and isomers of puromycin have been prepared to define the structural requirements for inhibition in an attempt to further understand its mode of action.[3,4] However, all of these structures have been of the classical nucleoside type in which an N-substituted amino sugar is attached to a purine or pyrimidine ring through a glycosidic linkage.[3-6] The difficulties encountered in preparing 3-aminoribosyl nucleosides have severely limited the availability of these compounds. Also, the classical nucleoside compounds introduce two undesirable structural features into the puromycin analogs which have not been demonstrated as essential for biological activity; *i.e.*, the furanosyl O and the 5′-OH group. Thus, it may be possible to modify **1** within the region outlined by the dotted line and still retain the activity of the antibiotic.

Since ribonucleosides are easily cleaved hydrolytically or enzymatically, many nucleosides which may be effective chemotherapeutic agents become ineffective *in vivo* because they are rapidly destroyed by cleavage into a purine or pyrimidine and a carbohydrate moiety.[7,8] This difficulty could be circumvented by replacing the furanosyl ring with a cyclopentyl system which sterically simulates the sugar moiety and provides a hydrolytically stable C-N bond. The removal of the 5′-OH group from puromycin and its analogs would be desirable from a toxicity standpoint. Toxic manifestations, including renal lesions, have precluded the use of puromycin in the treatment of human or animal infectious diseases or neoplasms.[9] The nephrotic syndrome results from small amounts of aminonucleoside produced by the hydrolytic removal of the amino acid moiety from administered puromycin.[9] Recent studies demonstrate that the aminonucleoside is first monodemethylated[10] and subsequently converted to the 5′-nucleotide.[11] It has been sug-

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