Pyrazolo[3,4-*d*]pyrimidine Ribonucleosides as Anticoccidials. 1. Synthesis and Activity of Some Nucleosides of Purines and 4-(Alkylthio)pyrazolo[3,4-*d*]pyrimidines

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The finding that 6-(methylthio)-9-β-D-ribofuranosyl-9*H*-purine (**6**) was more toxic to the avian coccidium, *Eimeria tenella*, than to embryonic chick liver host cells in vitro prompted the synthesis and testing of analogues of this compound. It was revealed that the β-D-ribofuranosyl moiety was an important structural feature and that several types of 2-substituents in the purine ring decreased efficacy, as did 3-deaza and 8-aza ring modifications of **6**. In contrast, the pyrazolo[3,4-*d*]pyrimidine analogue of **6** (**24**) was an order of magnitude more active. Moreover, this analogue was 24-fold less toxic to the host cells than was **6**. A series of 4-(alkylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines was prepared from 4-mercapto-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**23**) and various alkyl halides. The most effective compound in this series in vivo, 4-(ethylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**25**), cleared chicks of the parasite at 50 ppm in the diet and was much less toxic than was **24**.

Avian coccidia, an economically important group of protozoan parasites, have been shown to readily incorporate various purines and purine nucleosides into nucleic acids. Moreover, all available data suggest that they are deficient in the synthesis of purines de novo.[1] This metabolic pattern should render these parasites particularly susceptible to chemotherapy by analogues of purines or their nucleosides. Indeed, some 9-benzylpurines have been shown to have anticoccidial activity.[2]

This report deals with the synthesis and anticoccidial activities of nucleosides of S-substituted 6-thiopurines and their analogues. The most potent and selective activity was found with a series of ribonucleosides of 4-(alkylthio)pyrazolo[3,4-*d*]pyrimidines.

Results and Discussion

Chemistry. The heterocycles (**1–4**) in Table I were synthesized as previously described.[3] Compound **9** was prepared by the method of Bell et al.[4] Procedures for the synthesis of the nucleosides listed in Table I, except for **15**, have been described.[5] However, for this study these nucleosides were synthesized from the appropriate heterocycles and nucleoside pentosyl donors by an enzymatic method.[6] Physical properties of these nucleosides were in agreement with those described in the literature. Representative examples (**15** and **16**) are provided under Experimental Section.

The pyrazolo[3,4-*d*]pyrimidines (**18–21**) in Table II were synthesized according to published procedures.[7] The syntheses of four of the 1-β-D-ribofuranosyl-1*H*-pyrazolo-[3,4-*d*]pyrimidines (**22–24** and **28**) have been described.[8] However, **22** and **23** were synthesized by the enzymatic method[6a] (method A in Table III) referred to above. The details of the synthesis of **23** are provided under Experi-

(1) (a) Wang, C. C. In "Avian Coccidiosis", Proceedings of the 13th Poultry Science Symposium, 1977; Long, P. L.; Boorman, K. N.; Freeman, B. M., Eds.; British Poultry Science, Ltd.: Edinburgh, 1978; pp 168–170. (b) Wang, C. C.; Simashkevich, P. M.; Stotish, R. L. *Biochem. Pharmacol.* **1979**, *28*, 2241.

(2) (a) Lire, E. P.; Barker, W. M.; McCrae, R. C. U.S. Patent 3 846 426, Nov 5, 1974. (b) U.S. Patent 3 953 597, Apr 27, 1976. (c) Miller, B. M.; McManus, E. C.; Olson, G.; Schleim, K. D.; Van Iderstine, A. A.; Graham, D. W.; Brown, J. E.; Rogers, E. F. *Poult. Sci.* **1977**, *56*, 2039. (d) Imai, K.; Seo, T. *Eur. J. Med. Chem.* **1980**, *15*, 207.

(3) (a) Elion, G. B.; Burgi, E.; Hitchings, G. H. *J. Am. Chem. Soc.* **1952**, *74*, 411. (b) Skinner, C. G.; Shive, W.; Ham, R. G.; Fitzgerald, D. C.; Eakin, R. E. *J. Am. Chem. Soc.* **1956**, *78*, 5097.

(4) Bell, J. P.; Faures, M. L.; LePage, G. A.; Kimball, A. P. *Cancer Res.* **1968**, *28*, 782.

(5) (a) Fox, J. J.; Wempen, I.; Hampton, A.; Doerr, I. L. *J. Am. Chem. Soc.* **1958**, *80*, 1669. (b) Hampton, A.; Biesele, J. J.; Moore, A. E.; Brown, G. B. *J. Am. Chem. Soc.* **1956**, *78*, 5695. (c) Honjo, M.; Furukawa, Y.; Yoshioka, Y.; Imada, A.; Fujii, S.; Ootsu, K.; Kimura, T.; Komeda, T.; Matsumoto, T. *Annu. Rep. Takeda Res. Lab.* **1968**, *27*, 1. (d) Reist, E. J.; Benitez, A.; Goodman, L.; Baker, B. R.; Lee, W. W. *J. Org. Chem.* **1962**, *27*, 3274. (e) Noell, C. W.; Robins, R. K. *J. Med. Pharm. Chem.* **1962**, *5*, 1074. (f) Yamazaki, A.; Furukawa, T.; Akiyama, M.; Okutsu, M.; Kumashiro, I.; Ikehara, M. *Chem. Pharm. Bull.* **1973**, *21*, 692. (g) Montgomery, J. A.; Johnson, T. P.; Gallagher, A.; Stringfellow, C. R.; Schabel, F. M., Jr. *J. Med. Pharm. Chem.* **1961**, *3*, 265. (h) Montgomery, J. A.; Hewson, K. *J. Med. Chem.* **1966**, *9*, 105. (i) Elliott, R. D.; Montgomery, J. A. *J. Med. Chem.* **1977**, *20*, 116.

(6) (a) Krenitsky, T. A.; Koszalka, G. W.; Tuttle, J. V. *Biochemistry* **1981**, *20*, 3615. (b) Krenitsky, T. A.; Koszalka, G. W.; Tuttle, J. V.; Rideout, J. L.; Elion, G. B. *Carbohydr. Res.* **1981**, *97*, 139.

(7) Koppel, H. C.; O'Brien, D. E.; Robins, R. K. *J. Org. Chem.* **1959**, *24*, 259.

(8) (a) Montero, J. G.; Bhat, G. A.; Panzica, R. P.; Townsend, L. B. *J. Heterocycl. Chem.* **1977**, *14*, 483. (b) Panzica, R. P.; Bhat, G. A.; Earl, R. A.; Montero, J.-L. G.; Roti Roti, L. W.; Townsend, L. B. In "Chemistry and Biology of Nucleosides and Nucleotides"; Harmon, R. E.; Robins, R. K.; Townsend, L. B., Eds.; Academic Press: New York, 1978; pp 121–134. (c) Korbukh, I. A.; Yakunina, N. G.; Preobrazhenskaya, M. N. *Bioorg. Khim.* **1980**, *6*, 1632.

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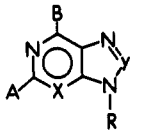
Table I. In Vitro Anticoccidial Activities of Some 6-Thiopurines and Corresponding Nucleoside Analogues



no.	A	B	R	X	Y	min effective concn,[a] mg/L
1	H	SH	H	N	CH	>20
2	H	SCH_3	H	N	CH	>20
3	H	SCH_2CH_3	H	N	CH	>20
4	H	$S(CH_2)_4CH_3$	H	N	CH	20
5	H	SH	rib[b]	N	CH	>20
6	H	SCH_3	rib	N	CH	0.31
7	H	SCH_3	drib[c]	N	CH	50
8	H	SCH_3	arab[d]	N	CH	>20
9	H	SCH_3	$C_5H_7O_4$[e]	N	CH	>25
10	NH_2	SH	rib	N	CH	>50
11	NH_2	SCH_3	rib	N	CH	50
12	SCH_3	SCH_3	rib	N	CH	>50
13	CH_3	SCH_3	rib	N	CH	>25
14	H	SCH_2CH_3	rib	N	CH	1.25
15	H	$S(CH_2)_4CH_3$	rib	N	CH	50
16	H	SCH_3	rib	CH	CH	>20
17	H	SCH_3	rib	N	N	1.25

[a] Minimum effective concentration was the least amount of compound that completely inhibited the growth of *Eimeria tenella* in vitro. [b] rib = β-D-ribofuranosyl. [c] drib = β-D-2-deoxyribofuranosyl. [d] arab = β-D-arabinofuranosyl. [e] $C_5H_7O_4$ = periodate oxidation product (dialdehyde) of the β-D-ribofuranosyl moiety.

mental Section. Alkylation of the sulfur atom of **23** with the appropriate alkyl halide by several different procedures (methods B, C, and D in Table III) produced the remainder of the known (**24** and **28**) and novel nucleosides in Table II.

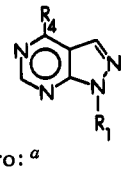
The yields obtained and some physical constants of the 1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines are given in Table III. The UV and proton NMR spectra are provided only for the compounds described in detail under Experimental Section.

Biology. Table I presents the activities of some 6-thiopurines and their nucleosides against developing schizonts of *Eimeria tenella* in vitro. The most active compound was the cytotoxic agent 6-(methylthio)-9-β-D-ribofuranosyl-9*H*-purine (**6**). Thiopurines without 9-substituents (**1–4**) or with 9-substituents other than the 9-β-D-ribofuranosyl moiety (**7–9**) were much less active than was **6**. Derivatives of **6** substituted in the 2-position with an amino (**11**), a methylthio (**12**), or a methyl (**13**) group were also less active. Changing the 6-substituent from methylthio (**6**) to ethylthio (**14**) decreased the activity 4-fold. The unalkylated (**5**) and pentyl (**15**) analogues of **6** were also less active.

Analogues of **6** with 3-deaza (**16**) or 8-aza (**17**) alterations in the purine ring system were less active. In contrast, the pyrazolo[3,4-*d*]pyrimidine analogue **24** (Table II) was about ten times more active than was **6**. Furthermore, **24** was much less cytotoxic to the host cells than was **6**. The lowest concentration of **24** at which toxicity was observed to the embryonic chick liver cells was 31 mg/L, whereas **6** was cytotoxic at 1.3 mg/L. Division of the minimum concentration required for toxicity to the host cells by that required to inhibit parasite growth completely gave an in vitro chemotherapeutic index of 4 for **6** and 1000 for **24**. This dramatic difference prompted the synthesis and evaluation of a variety of 1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines (Table II).

As with the purine series, the pyrazolo[3,4-*d*]pyrimidine ribonucleosides were more active than the corresponding aglycons (**23–25** and **33** vs. **18–21**). With the straight-chain alkyl-substituted ribonucleosides, only a 16-fold variation in activity in vitro was seen from the methylthio (**24**) through the heptylthio (**36**) analogues. The 4-thio analogue

Table II. Anticoccidial Activities of Some Pyrazolo[3,4-*d*]pyrimidines and Corresponding Ribonucleosides



no.	R_4	R_1	in vitro:[a] min effective concn, mg/L	in vivo: no. of chicks[b] ppm in diet: 400	200	100	50	25
18	SH	H	>20	0				
19	SCH_3	H	20					
20	SCH_2CH_3	H		0				
21	$S(CH_2)_4CH_3$	H	5					
22	H	rib[c]	1.3		1			
23	SH	rib	0.31		2			
24	SCH_3	rib	0.03	1 (4)[d]	3 (2)[d]	2 (1)[d]	2	0
25	SCH_2CH_3	rib	0.31		5	5	5	2
26	$S(CH_2)_2CH_3$	rib	0.019		4	1	0	0
27	$SCH(CH_3)_2$	rib	0.019	0				
28	$SCH_2CH{=}CH_2$	rib	0.019		5	5	2	1
29	SCH_2COCH_3	rib	>20		0			
30	$SCH_2CO_2CH_3$	rib	20		0			
31	$S(CH_2)_3CH_3$	rib	0.019		5	1	0	0
32	$SCH_2CH{=}CHCH_3$	rib	0.019		5	4	0	0
33	$S(CH_2)_4CH_3$	rib	0.31		5	3	0	0
34	*S*-cyclopentyl	rib	5.0		1			
35	$S(CH_2)_5CH_3$	rib	0.019		5	2	0	0
36	$S(CH_2)_6CH_3$	rib	0.31		1			
37	$S(CH_2)_2PO(OCH_2CH_3)_2$	rib	5.0		1			

[a] See Table I. [b] Number of chicks cleared of *Eimeria tenella* lesions out of a group of 5. [c] rib = β-D-ribofuranosyl. [d] Parenthetical value is the number of chicks out of a group of five that died due to toxicity of the compound.

Table III. Synthetic Data and Physical Constants for Some 1-β-D-Ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines

no.	formula	synthetic method[a]	yield, %	mp,[b] °C	$[\alpha]^{20}_D$ (*c* 1, DMF), deg
22	$C_{10}H_{12}N_4O_4$	A	32[c]	217-218	-92.1
23	$C_{10}H_{12}N_4O_4S$	A	63[c]	215-217	-73.7
24	$C_{11}H_{14}N_4O_4S$	B (I)	92	180-181	-76.2
25	$C_{12}H_{16}N_4O_4S$	B (Br)	83	157-159	-81.6
26	$C_{13}H_{18}N_4O_4S$	B (I)	86	93-95	-62.8
27	$C_{13}H_{18}N_4O_4S$	B (Br)	64	101-102	-52.0
28	$C_{13}H_{16}N_4O_4S$	B (Br)	88	114-115	-87.9
29	$C_{13}H_{16}N_4O_5S \cdot 0.25H_2O$	C (Cl)	24	95-97	-58.5
30	$C_{13}H_{16}N_4O_6S$	B (Cl)	29	99-101	-70.4
31	$C_{14}H_{20}N_4O_4S$	B (Br)	42	88-89	-66.2
32	$C_{14}H_{18}N_4O_4S$	D (Br)	30	139-141	-65.2
33	$C_{15}H_{22}N_4O_4S$	B (Br)	63	81-82	-77.0
34	$C_{15}H_{20}N_4O_4S$	B (Br)	9	70-72	-67.9
35	$C_{16}H_{24}N_4O_4S$	B (Br)	85	71-73	-70.4
36	$C_{17}H_{27}N_4O_4S$	B (I)	90	70-72	-63.7
37	$C_{16}H_{25}N_4O_7PS \cdot H_2O$	D (Br)	28	*d*	-46.6

[a] See Experimental Section; parenthetical notation pertains to the type of alkyl halide used. [b] Literature values[8a] for 23, 209-210; 24, 163-164; 28, 125-126. [c] Yield was calculated on the basis of the pyrazolo[3,4-*d*]pyrimidine rather than the pentosyl donor (uridine). [d] Amorphous solid.

23 had activity comparable to the less active members of the simple alkylthio series (25, 33, and 36). Even the 4-unsubstituted derivative 22 had some activity. This tolerance for bulky thio substituents or lack thereof contrasted with the results with the purine series (5, 14, and 15 vs. 6 in Table I).

Unsaturation in the alkyl chains (28 and 32) did not alter the activity in vitro. The propyl analogue 26 was as active in vitro as was the isopropyl derivative 27. However, the cyclopentyl derivative 34 was much less active than was the *n*-pentyl analogue 33. Carbonyl (29), carboxylic acid ester (30), or phosphonate ester (37) groups introduced into the chain decreased activity.

The structure–activity relationships with tests performed in vivo differed from those derived from the in vitro data. The most active compound in vivo was 4-(ethylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (25). It cleared all chicks of *Eimeria tenella* lesions when given in the diet at 50 ppm. Next in potency was the allylthio (28) compound, which cleared all lesions at 100 ppm. The crotylthio (32), *n*-pentylthio (33), *n*-hexylthio (35), and methylthio (24) analogues approached the potency of 28, but the last of these (24) was toxic, which made its potency difficult to assess. In contrast to the in vitro results, unsaturation of the alkylthio substituent (28 vs. 26 and 32 vs. 31) increased activity in vivo.

As well as being the most active compound in vivo, the ethylthio derivative 25 was much less toxic than the methylthio compound 24. In vitro, 24 was cytotoxic to embryonic chick liver cells at concentrations as low as 31 mg/L, whereas 25 showed no signs of cytotoxicity at 125 mg/L. This trend was also observed in vivo. Four out of five chicks died, apparently due to toxicity, when 24 was administered in the diet at 400 ppm, and at 100 ppm one death was observed. In repeated experiments with 25, no deaths attributable to toxicity were seen at 400 ppm in the diet.

In the test in vivo, *E. acervulina* was administered to the chicks in a mixture with *E. tenella*. Using oocyst production as the criterion, no activity against *E. acervulina* was observed with most of the compounds listed in Table II. Only 24–26 were active at 200 ppm in the diet.

The findings presented here represent the discovery of a selective and effective series of anticoccidial agents, the ribonucleosides of 4-substituted pyrazolo[3,4-*d*]pyrimidines. Subsequent reports will deal with further exploration of the structure–activity relationships and further biological properties of this series.

Experimental Section

Anticoccidial Evaluation. Activity against *E. tenella* (Weybridge strain) growing in embryonic chick liver cells in vitro was determined as previously described.[9] Activity against this organism in vivo with a simultaneous infection of *E. acervulina* (Ongar strain) was evaluated in 1-week-old chicks. The chicks were infected 1 day after the beginning of medication. The test compound was mixed in the diet and fed ad libitum until the end of the test. The details of this in vivo test will be described elsewhere (R.B.W.).

Enzyme Catalysts. Uridine phosphorylase (EC 2.4.2.3), thymidine phosphorylase (EC 2.4.2.4), and purine nucleoside phosphorylase (EC 2.4.2.1) were purified from *Escherichia coli* as previously described.[6a] One unit of enzyme activity was that amount which catalyzed the formation of 1 μmol of product per minute under the defined assay conditions.

Physical Characterization of Compounds. All compounds listed in Tables I and II gave elemental analyses within ±0.4% of calculated values. Analyses were performed by Integral Microanalytical Laboratories, Raleigh, NC, or Atlantic Microlabs, Atlanta, GA. Melting points were obtained on a Thomas-Hoover capillary apparatus and are uncorrected. UV spectra were recorded using a Varian Super-Scan 3 or Cary 118. A Varian XL-100 or CFT-20 provided the NMR spectra in Me_2SO-d_6. Optical rotations were obtained using a Perkin-Elmer Model 141 polarimeter. Table III lists some physical constants for the pyrazolo[3,4-*d*]pyrimidine ribonucleosides.

Syntheses. Reagents were used as received. DMF was stored over 4Å sieves. Dowex 1-X8 (Cl^- form) was converted to the hydroxide form by treatment with 2 N NaOH and then to the bicarbonate form by treatment with 2 N $NaHCO_3$, washed with MeOH, filtered, and stored at room temperature until used. Evaporations in vacuo were performed at or below 40 °C. Table III provides synthetic procedures used and yields for the pyrazolo[3,4-*d*]pyrimidine ribonucleosides. Methods A–D are exemplified below. The trituration solvent used in method B was petroleum ether, except in the sample described below (26) where methanol was used. Compound 31 was purified by column chromatography on polyacrylamide gel (P-2, Bio-Rad) in 1-propanol–H_2O (3:7, v/v) and then crystallized from ethyl acetate. Compound 34 was purified by extraction of a $CHCl_3$ solution with water, evaporation of the $CHCl_3$, dissolution in ether, evaporation of the solvent, then dissolution in ethanol, and evaporation of the solvent to give the solid. Compound 30 was treated as for 34, except that after the evaporation of the $CHCl_3$ the oil was triturated with warm ether. With 37, the reaction mixture was refluxed in ethanol for 11 h. The product was crystallized from

(9) Latter, V. S.; Wilson, R. G. *Parasitology* **1979**, *79*, 169.

methanol and washed with ether.

6-(Pentylthio)-9-β-D-ribofuranosyl-9*H*-purine (15; Method A). A solution containing 6-(pentylthio)purine[3b] (7.6 g, 0.034 mol), inosine (20 g, 0.075 mol), KH_2PO_4 (1.4 g, 0.01 mol), 1.0 L of water, and 0.43 L of 1-propanol was adjusted to pH 7.4 with KOH. Purine nucleoside phosphorylase (43 units) was added, and this mixture was maintained at 34 °C for 22 days. More enzyme (14 units) was added, and the incubation was continued for 12 more days. Twenty milliliters of 15 N NH_4OH was then added to the reaction mixture, and the solution was applied to a Dowex 1-X8 (formate form) column, 9.5 × 20 cm, that had been washed with a 1-propanol–water mixture (30:70; v/v). After the sample was applied, the column was washed with 2.0 L of this mixture. Fractions that contained the bulk of the product were combined and evaporated under reduced pressure to 200 mL. To dissolve the product, 100 mL of 1-propanol was added, and the resulting solution was applied to a column packed with polyacrylamide gel (7.5 × 90 cm; P-2, Bio-Rad) that had been equilibrated with the 1-propanol–water mixture. The product was eluted with this mixture. The 1-propanol was removed by evaporation in vacuo, and the residue was then lyophilized. Product (7.2 g) was obtained in 59% yield with respect to the amount of 6-(pentylthio)purine used: mp 62–65 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 296 nm (14.7); at pH 13, 294 nm (15.7); NMR δ 8.73 and 8.69 (2 s, 2 H, H_2 and H_8), 6.00 (d, 1 H, J = 5.6 Hz, $H_{1'}$), 3.30 (m, 2 H, 5′-CH_2), 1.40 (mm, 6 H, 3 CH_2), 0.85 (t, 3 H, CH_3); $[\alpha]^{20}_D$ −44.2° (*c* 1, DMF). Anal. ($C_{15}H_{22}N_4O_4S$) C, H, N, S.

4-(Methylthio)-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine (16; Method A).[5h] Uridine (1.35 g, 5.5 mmol), 4-(methylthio)-1*H*-imidazo[4,5-*c*]pyridine[10] (0.68 g, 4.1 mmol), KH_2PO_4 (0.14 g, 1 mmol), and KN_3 (0.014 g, 0.17 mmol) were suspended in 10 mL of water. The pH was adjusted to 7.4 with KOH. Purine nucleoside phosphorylase (320 units) and uridine phosphorylase (58 units) were added to the mixture, which was then stored at 37 °C. Nineteen days later, the warm reaction mixture was filtered. The filtrate on cooling formed white crystals, which were washed with water and dried to give 0.61 g of **16**. The yield on the basis of the amount of 4-(methylthio)-1*H*-imidazo-[4,5-*c*]pyridine used was 50%: mp 194–196 °C (lit.[5h] mp 195 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 307 (17.2), 238 (sh) (10.1), 223 nm (sh) (15.1); at pH 13, 285 nm (13.8) [lit.:[5h] at pH 1, 307 nm (16.9); at pH 13, 284 nm (13.7)]; NMR δ 8.52 (s, 1 H, H_2), 8.30 (d, 1 H, J = 5.7 Hz, H_6), 7.57 (d, 1 H, J = 5.8 Hz, H_7), 5.88 (d, 1 H, J = 6.1 Hz, $H_{1'}$), 3.62 (s, 3 H, CH_3); $[\alpha]^{20}_D$ −55.8° (*c* 1.1, DMF). Anal. ($C_{12}H_{15}N_3O_4S$) C, H, N, S.

4-Mercapto-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (23; Method A).[8a] Uridine (230 g, 0.94 mol), compound **18** (95 g, 0.625 mol), K_2HPO_4 (3.3 g, 0.019 mol) and NaN_3 (0.4 g, 0.006 mol) were suspended in 13.3 L of deionized water. Purine nucleoside phosphorylase (12000 units) and uridine phosphorylase (735 units) were added. The suspension was gently stirred at 35 °C. The initial pH value of 6.9 slowly decreased. The pH was monitored and kept near neutrality by the addition of 0.5 N KOH. After 14 days, the initial quantities of enzyme and NaN_3 were added again. After another 14 days, the enzyme additions were again repeated. Nine days later, the precipitate in the reaction mixture was collected by filtration. Uracil and other minor components were removed by successive extractions with 1.0-L portions of water at 3 °C. After five extractions, only **23** was detectable by TLC.[11] Upon drying, the first crop of **23**, 42.4 g, was obtained.

The filtrate of the reaction mixture was stored at 3 °C for 20 h. A precipitate formed, which was collected and washed with five 1.0-L portions of water at 3 °C as described above. The residue was suspended in 2.0 L of water, heated to 75 °C, and filtered while hot. The filtrate was stored at 24 °C for 22 h. The precipitate was collected by filtration, washed with water, and dried. This material provided the second crop of **23**, 39.2 g.

The filter cake from the first 75 °C water extraction was reextracted at 75 °C with 2.0 L of water and filtered while hot. The filter cake was again extracted in the same manner with 1.0 L of water. The precipitates that formed on cooling both filtrates were combined and recrystallized from 600 mL of water. The precipitate was washed with water and dried to give the third crop of **23**, 30.5 g. The overall yield on the basis of the amount of **18** used was 63%: mp 215–217 °C (lit.[8a] 209–210 °C); UV at pH 1, λ_{max} ($\epsilon \times 10^{-3}$) 319 (25.7), 235 (9.6), λ_{min} 270 nm (2.1); at pH 13, λ_{max} 318 (20.4), λ_{min} 265 nm (2.3) [lit.:[8a] at pH 1, 321 (23.6), 236.5 (9.1), 271.5 nm (2.4)]; NMR δ 13.6 (br s, 1 H, thione NH), 8.25 (d, 1 H, J = 0.5 Hz, H_3), 8.19 (s, 1 H, H_6), 6.04 (d, 1 H, J = 4.4 Hz, 1′-H), 4.55 (dd, 1 H, 2′-H), 4.20 (dd, 1 H, 3′-H), 3.90 (dt, 1 H, 4′-H), 3.45 (AB q, 2 H, 5′-CH_2). The ribofuranosyl protons were assigned after D_2O exchange: pK_a = 7.6 ± 0.1. Anal. ($C_{10}H_{12}N_4O_4S$) C, H, N, S.

4-(Ethylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (26; Method B). Dowex 1-X8 (HCO_3 form) (4.0 g, 12.8 mequiv) and **23** (2.0 g, 7 mmol) were added to 0.1 L of MeOH, and the mixture was warmed and stirred until there was no UV-absorbing material remaining in solution. EtBr (0.76 g, 7 mmol) was added, and the mixture was stirred at 24 °C for 20 h. The resin was removed by filtration and washed with MeOH. Evaporation of the filtrate and washes gave a light-tan solid, which was dried in vacuo: yield 1.18 g (83%); mp 157–159 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 294 nm (16); at pH 13, 292 nm (16); NMR δ 8.75 (s, 1 H, H_6), 8.35 (s, 1 H, H_3), 6.17 (d, 1 H, J = 5 Hz, $H_{1'}$), 1.35 (t, 3 H, CH_3), 3.45 (mm, 4 H, CH_2 and 5′-CH_2). Anal. ($C_{12}H_{16}N_4O_4S$) C, H, N, S.

4-(Acetonylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (29; Method C). Compound **23** (2.0 g, 7 mmol) was suspended with stirring in 0.075 L of EtOH, and a solution of $NaHCO_3$ (0.6 g, 7.1 mequiv) in a minimum amount of H_2O was added. After the mixture had been stirred for 2 h, a solution of chloroacetone (0.65 g, 7 mmol) in 0.025 L of EtOH was added slowly over 30 min. The mixture was heated on the steam bath for 30 min and was allowed to stand at ambient temperature for 1.5 h. It was then heated briefly and evaporated in vacuo to a syrup, which was dissolved in H_2O and extracted with ethyl acetate. Solid which formed in the aqueous layer was isolated, washed with H_2O, and dried: yield 0.57 g (24%). A second crop was isolated from the ethyl acetate layer by evaporation of the solvent. This solid was washed with H_2O and dried: yield 0.84 g; total yield 58%; mp 95–97.5 °C; UV (MeOH) λ_{max} ($\epsilon \times 10^{-3}$) 282 nm (15.3); NMR δ 8.75 (s, 1 H, H_6), 8.48 (d, 1 H, J = 0.4 Hz, H_3), 6.21 (d, 1 H, J = 4.5 Hz, 1′-H), 2.30 (s, 3 H, CH_3), 4.39 (s, 3 H, CH_2 and ribose $H_{3'}$). Anal. ($C_{13}H_{16}N_4O_5S \cdot 0.25H_2O$) C, H, N, S.

4-(2-Butenylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (32; Method D). A mixture of **23** (2.0 g, 7 mmol) and K_2CO_3 (0.97 g, 7 mequiv) in 0.02 L of DMF was heated at 40 °C. Technical grade crotyl bromide (1.14 g, 8.45 mmol, 21% 3-bromo-1-butene, 36% *cis*- and 43% *trans*-crotyl bromide by NMR) was added. The reaction was stirred and heated until the UV spectrum no longer increased at 296 nm or decreased at 323 nm, indicating that the reaction was complete. The solution was poured into ice–water. The mixture was extracted with ethyl acetate and dried (Na_2SO_4), and the solvent was evaporated. Recrystallization from MeOH gave the product as a mixture of cis and trans isomers in a ratio of 1:5: yield 0.7 g (30%); mp 139–141 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 295 nm (15); at pH 13, 293 nm (11.5); NMR δ 8.82 (s, 1 H, H_6), 8.41 (s, 1 H, H_3), 6.23 (d, 1 H, J = 4.63 Hz, $H_{1'}$), 5.70 (m, 2 H, trans and cis CH=), 4.40 (d, 2 H, CH_2). Anal. ($C_{14}H_{18}N_4O_4S$) C, H, N, S.

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(10) De Roos, K. B.; Salemink, C. A. *Recl. Trav. Chim. Pays-Bas* **1971**, *90*, 1166.

(11) TLC on Eastman cellulose sheets with fluorescent indicator (no. 6063) in acetonitrile–H_2O–15 N NH_4OH (85:10:5, v/v) was used to monitor the isolation of **23**. R_f values were 0.36, 0.24, 0.29, and 0.14 for **18**, **23**, uracil, and uridine, respectively.