7d. After liberation of 7d from the picrate, recrystallization from methanol initially yielded pure 7d. Later crops contained some of the α isomer of 7d, along with 7d. 7d: UV λ_{max} at pH 1, 264 (13.2), 286 nm (12.8); at pH 7, 261 (5.7), 314 nm (8.2); at pH 13, 261 (5.7), 313 nm (8.5). Anal. ($C_9H_{13}N_7O_4 \cdot 0.5H_2O$) C, H, N.

2-β-D-Ribofuranosyl-5-fluoro-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (7e). Solid 7d (220 mg, 0.78 mmol) was added to 8 mL of 48% HBF_4 at 10 °C. The resulting stirred solution was treated with solid KNO_2 (1.3 g, 15.6 mmol) over 3.7 h at 10 °C, at which time HPLC showed only a trace of starting material. The reaction was chilled to −20 °C and neutralized to pH 7 with 11.7 M KOH. After chilling, the precipitated salts were collected, and the filtrate was passed at 1 mL/mm through a 1.5 × 24 cm bed of Bio-Beads, SM-4, 20–50 mesh, which had been prepared in H_2O. The column was eluted with H_2O until the eluate was free of potassium ions. The product was isolated from a H_2O–CH_3OH (3:1) eluate, followed by chromatography on an Avicel F plate in CH_3CH_2OH–H_2O (3:2). The methanol extract of the plate band was evaporated and triturated with cold CH_3CN to give a white solid (32 mg, 14%), mp 190 °C dec. 7e: UV λ_{max} at pH 1, 247 (5.5), 292 nm (10.0); at pH 7, 247 (5.6), 292 nm (10.2); at pH 13, 243 (4.6), 297 nm (9.3). Anal. ($C_9H_{11}FN_6O_4 \cdot 0.25H_2O$) C, H, N.

Another nucleoside, 7f, was isolated from an aqueous column fraction by evaporation and trituration of the residue with CH_3OH: yield 70 mg, mp amorphous.

7f: UV λ_{max} at pH 1, 291 nm (9.7); at pH 7, 262 (10.4), 287 nm (11.0); at pH 13, 259 (5.1), 317 nm (8.1). Anal. ($C_9H_{12}N_6O_5 \cdot 0.7H_2O$) C, H, N.

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Registry No. 4, 61772-86-9; 5a, 40554-98-1; 5b, 13035-61-5; 6a, 86480-35-5; 6b, 86480-36-6; 6c, 86480-37-7; 6d, 70421-27-1; 6e, 86480-39-9; 6g, 86480-38-8; 7a, 86480-41-3; 7b, 86480-42-4; 7c, 86480-43-5; 7d, 86480-40-2; 7d picrate, 86480-44-6; 7e, 86480-45-7; 7f, 86480-46-8; 9a, 53458-40-5; 9b, 86480-47-9; 9c, 86480-48-0; 9d, 86480-49-1; 10, 86480-50-4.

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

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Ribonucleosides of 4-(alkylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidines have been shown to be useful anticoccidial agents [Krenitsky, T. A.; Rideout, J. L.; Koszalka, G. W.; Inmon, R. B.; Chao, E. Y.; Elion, G. B.; Latter, V. S.; Williams, R. B. *J. Med. Chem.* **1982**, *25*, 32. Rideout, J. L.; Krenitsky, T. A.; Elion, G. B. U.S. Patent 4 299 283, 1981]. In that study, the unsaturated 4-allylthio and 4-crotylthio derivatives (**19** and **20**) were shown to be more active in vivo against *Eimeria tenella* than their saturated congeners; therefore, some unsaturated (arylalkyl)thio derivatives were synthesized and investigated as anticoccidial agents. The novel compounds in this study (**2** to **18**) were prepared by the alkylation of 4-mercapto-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1**), which was prepared by an enzymatic method. The (*E*)-4-cinnamylthio derivative (**2**) and the 5′-monophosphate (**18**) were the most active compounds against *E. tenella* in vivo. None of the analogues with substituents in the aryl moiety (**3** to **13**) was more active than **2** in vivo. The geometry about the double bond was important, since the (*Z*)-4-cinnamylthio derivative (**14**) was inactive both in vitro and in vivo. The 4-(3-phenylpropynyl)thio and 4-(5-phenyl-2,4-pentadienyl)thio derivatives (**15** and **16**) were at least as active as **2** in vitro; however, they were less active than **2** in vivo. Compound **2** was effective in vivo against *E. tenella*, *E. necatrix*, *E. maxima*, and *E. brunetti*; these species of *Eimeria* were controlled when **2** was given in the diet at levels up to 100 ppm. Infections in vivo due to *E. acervulina* were controlled by **2** only at about 800 ppm. The broad spectrum of anticoccidial activity shown by **2** represents a significant improvement over the activities reported for related compounds [Krenitsky, T. A.; Rideout, J. L.; Koszalka, G. W.; Inmon, R. B.; Chao, E. Y.; Elion, G. B.; Latter, V. S.; Williams, R. B. *J. Med. Chem.* **1982**, *25*, 32].

Previous reports[1,2] have shown that ribonucleosides of 1*H*-pyrazolo[3,4-*d*]pyrimidines were able to inhibit the development of *Eimeria tenella* in chicks. It was shown that the alkylthio derivatives[1] were superior to the alkylamino derivatives.[2] In the study with the 4-alkylthio compounds,[1] it was apparent that unsaturation in the alkyl chain of the 4-substituent enhanced the activity in vivo relative to that found for the saturated congeners. The study has therefore been extended to include 4-(arylalkenyl)thio and 4-(arylalkynyl)thio analogues. The beneficial effects of these modifications and structure–activity relationships will be discussed.

Results and Discussion

Chemistry. The compounds (**2** to **16**, Table I) were prepared by the alkylation of 4-mercapto-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine[3] (**1**) with the ap-

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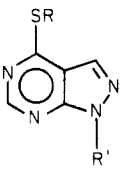
Table I. Anticoccidial Activities of Some 4-(Substituted-thio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines



no.	R	R′	in vitro[a] MIC, mg/L	in vivo: no. of chicks cleared of *E. tenella* lesions at the following levels in the diet[b] 400 ppm	300 ppm	200 ppm	100 ppm	50 ppm	25 ppm
1	H	rib[c]	0.31			2			
2	(*E*)-$CH_2CH{=}CHC_6H_5$	rib	0.078			5	5	5	5[d]
3	(*E*)-$CH_2CH{=}CHC_6H_4$-3-Br	rib	5			5	5	4	1
4	(*E*)-$CH_2CH{=}CHC_6H_4$-3-OH	rib	20	0					
5	(*E*)-$CH_2CH{=}CHC_6H_4$-3-CF_3	rib	5			1[e]			
6	(*E*)-$CH_2CH{=}CHC_6H_4$-4-Cl	rib	0.31			5	5	0	0
7	(*E*)-$CH_2CH{=}CHC_6H_4$-4-CH_3	rib	5			0[e]			
8	(*E*)-$CH_2CH{=}CHC_6H_4$-4-OCH_3	rib	1.25		2				
9	(*E*)-$CH_2CH{=}CHC_6H_4$-4-$OCH_2C_6H_5$	rib	1.25			5	2	0	1
10	(*E*)-$CH_2CH{=}CHC_6H_3$-2,4-Cl	rib	1.25			2			
11	(*E*)-$CH_2CH{=}CHC_6H_3$-3,4-Cl	rib	0.31			2			
12	(*E*)-$CH_2CH{=}CHC_6H_2$-3,4,5-OCH_3	rib	0.31	5		5	2		
13	(*E*)-$CH_2CH{=}CHC_6H_4$-2-OCH_3	rib	1.25		1				
14	(*Z*)-$CH_2CH{=}CHC_6H_5$	rib	>20				0	0	0[d]
15	$CH_2C{\equiv}CC_6H_5$	rib	0.019			5	2	0	1
16	(*E*)-$CH_2(CH{=}CH)_2C_6H_5$	rib	0.019			5		5	1
17	(*E*)-$CH_2CH{=}CHC_6H_5$	tri-*O*-Ac-rib	1.25			5		5	1
18	(*E*)-$CH_2CH{=}CHC_6H_5$	5-phos-rib	0.012			5	5	5	4 (5)[d,f]
19[g]	$CH_2CH{=}CH_2$	rib	0.019			5	5	2	1
20[g]	$CH_2CH{=}CHCH_3$ (1:5 *Z/E*)	rib	0.019			5	4	0	0

[a] Minimum inhibitory concentration (MIC) was the least amount of compound that completely inhibited the growth of *Eimeria tenella* in vitro. [b] Number of chicks cleared of *Eimeria tenella* lesions out of a group of five. [c] rib = 1-β-D-ribofuranosyl; tri-*O*-Ac-rib = 2′,3′,5′-tri-*O*-acetyl-1-β-D-ribofuranosyl; 5-phos-rib = 1-β-D-ribofuranosyl 5′-monophosphate disodium salt. [d] At 12.5 ppm, no chicks were cleared. [e] One chick out of a group of five died and contained lesions due to the parasite. [f] Values of duplicate tests. [g] Reference 1.

propriate halide. Compound 1 was prepared by an enzymatic method as described previously.[1] Four methods (A–D) were used to prepare the requisite halides. The halides were used as the crude compounds, except as shown for the preparation of 4 in the Experimental Section. Method A involved the reduction of the acyl chloride to the alcohol[4] and chlorination with thionyl chloride in benzene containing pyridine,[5] as described for the preparation of 3 in the Experimental Section. Method B involved the preparation of the methyl ester,[6] rapid reduction with lithium aluminum hydride at low temperature to avoid reduction of the double bond, and finally halide formation using thionyl chloride in petroleum ether–ether[7] at −10 °C, as shown for the preparation of 4 in the Experimental Section. In the preparation of 4-[(2-methoxycinnamyl)thio]-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (13), this procedure was used to reduce methyl 2-methoxycinnamate at 0 °C, and some overreduction to the saturated alcohol occurred. The desired product was contaminated with 12% of the saturated analogue, based on NMR analysis of the mixture. Results reported for 13 are for the mixture. For the remainder of the compounds prepared by this method, the reduction step was conducted at −10 to −50 °C, and saturation of the double bond was not observed. Method C involved borohydride reduction of phenylpropargyl aldehyde to the alcohol, catalytic reduction to the (*Z*)-cinnamyl alcohol using Lindlar catalyst in the presence of quinoline,[8] and chlorination to the halide by the method of Collington and Meyers,[9] as shown in the Experimental Section for the preparation of 14. In Method D, phenylpropargyl chloride was prepared from the alcohol by using triphenylphosphine in carbon tetrachloride according to the method of Snyder.[10] This was used for the preparation of 15. The method (A, B, C, or D) used to obtain each halide is indicated in Table II. The 5′-phosphate (18) was prepared by the method of Yoshikawa,[11] as shown in the Experimental Section. Alkylation of 4-mercapto-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine gave 17, as shown in the Experimental Section. The preparation of the acetylated intermediate by the thiation of 4-hydroxy-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine has been published.[3a] In the present study, this intermediate was prepared by the direct acetylation of the 4-mercapto compound (1) using acetic anhydride in the presence of potassium carbonate. There

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Table II. Synthetic Data and Physical Constants for Some 4-(Substituted-thio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidines

no.	formula	synth method[a]	yield, %	mp, °C	$[\alpha]_D^{20}$ (*c* 1, DMF), deg
2	$C_{19}H_{20}N_4O_4S$	*b*	48	165-167	−66.8
3	$C_{19}H_{19}BrN_4O_4S \cdot 0.5H_2O$	A	36	92	−56.1
4	$C_{19}H_{20}N_4O_5S \cdot 0.1EtOH$[c]	B	30	176-178	−62.9
5	$C_{20}H_{19}F_3N_4O_4S$	A	51	105-110	−55.4
6	$C_{19}H_{19}ClN_4O_4S$	A	30	113-116	−61.7
7	$C_{20}H_{22}N_4O_4S$	A	14	134-138	−59.4
8	$C_{20}H_{22}N_4O_5S$	B	11	154-155	−61.5[d]
9	$C_{26}H_{26}N_4O_5S$	B	21	160-166	−49.7
10	$C_{19}H_{18}Cl_2N_4O_4S$	A	36	(softens 90) 96	−56.2
11	$C_{19}H_{18}Cl_2N_4O_4S$	A	30	62-67	−57.2
12	$C_{22}H_{26}N_4O_7S$	B	45	93	−53.7
13	$C_{20}H_{22}N_4O_5S \cdot 1.1H_2O$ $^3/_{25}C_{20}H_{24}N_4O_5S$	B	10	47-48	
14	$C_{19}H_{20}N_4O_4S$	C	5	96-98	*f*
15	$C_{19}H_{18}N_4O_4S$	D	28	(softens 134) 137	−66.8
16	$C_{21}H_{22}N_4O_4S$	A	10	145-149	−62.4
17	$C_{25}H_{26}N_4O_7S$[g]	*b*	40	*h*	−35.4
18	$C_{19}H_{21}N_4O_7PS$	*a*	60	*i*	*f*

[a] See Experimental Section. [b] Commercial halide. [c] Calcd: C, 54.80; H, 4.84; N, 13.46; S, 7.70. Found: C, 54.76; H, 4.92; N, 13.22; S, 7.60. NMR indicated the presence of ethanol. (See Experimental Section.) [d] c 0.5, DMF. [e] Mixture of 88% unsaturated and 12% saturated substituent by NMR. [f] Limited sample available, rotation not determined. [g] Calcd: C, 57.02; H, 4.98; N, 10.64; S, 6.09. Found: C, 57.28; H, 4.92; N, 10.22; S, 5.85. [h] Pale yellow glass. [i] Salt, melting point not taken. Purity was assessed by HPLC and base/ribose/phosphate ratios (see Experimental Section).

was no evidence (TLC) of thio ester formation during the reaction. The NMR spectral data obtained for this intermediate agreed with those in the literature.[3a] The yields and some physical properties of the compounds are given in Table II. The UV and NMR spectra are provided only for those compounds described in detail.

Biology. Comparison of the values for the minimum inhibitory concentration (MIC[12]) of the nucleosides against *Eimeria tenella* in vitro (Table I) indicates greater than a 1500-fold variation in potency for the compounds in the series and greater than a 250-fold variation in activities among the cinnamylthio derivatives (2 to 13). The least effective compound in vitro was the (*Z*)-4-cinnamylthio derivative (14). A comparison of the MIC values for the active *E* isomer (2) and the inactive *Z* isomer (14) shows that a dramatic decrease in activity (>250-fold) resulted from changing the geometry about the double bond in the cinnamyl moiety.

The nature of the host cell used in tissue culture systems was reported to affect the assessment of some known anticoccidial agents.[13] Some compounds can be metabolized to active agents in embryonic chick liver cell systems but not in adult chick kidney cell systems. In the present study, the sensitivity of *E. tenella* to the (*E*)-4-cinnamylthio derivative (2) was similar, whether the parasites were grown in embryonic chick liver cells or adult chick kidney cells. (Only the results for the embryonic liver cells have been quoted in Table I.) It can be concluded that 2 has inherent anticoccidial activity; however, the results in vitro do not preclude the formation of active metabolites in vivo.

Of the compounds in this series, only the 4-(3,4-dichlorocinnamyl)thio derivative (11) was noticeably toxic to chick embryo liver host cells in vitro. Toxicity with 11 was observed at 20 and 5 mg/L; however the MIC for this compound was 0.31 mg/L or 16-fold lower than the level where host cell toxicity was apparent. The apparent lack of toxicity in vitro in this series was substantiated in vivo. No toxic effects due to the compounds were seen in chicks when 4 and 12 were administered at the high level of 400 ppm in the diet. Indeed, medication of chicks with the (*E*)-4-cinnamylthio derivative (2) at levels up to 800 ppm did not cause any major adverse effects.

There was a greater degree of correlation between in vitro and in vivo activities in this series (Table I) than was previously observed in the 4-alkylthio[1] and 4-alkylamino[2] series. The novel compounds in this series with MIC values in vitro of 0.31 mg/L or less (2, 6, 11, 12, 15, 16, and 18) were highly active in vivo, clearing five out of five chicks of *Eimeria tenella* at 200 ppm in the diet, except in the case of 11 where two out of five chicks were cleared. Compounds 3, 9, and 17 were more active in vivo than predicted from the MIC values in vitro. The converse was true for 11. In the case of the 2,3,5-tri-*O*-acetyl derivative (17), the high in vivo activity might be accounted for by the possibility that 17 is a prodrug[14] of the (*E*)-4-cinnamylthio derivative (2). These two compounds were both active in vivo between the levels of 200 and 50 ppm, with five out of five chicks cleared of parasitic lesions. At 25 ppm, 2 still cleared five chicks of lesions, whereas 17 cleared one out of five chicks. At this low level of medication, the effect of deacylation may have become apparent and, as a result, the parent compound was more effective than the prodrug. The 5′-monophosphate (18) was also as effective as the parent compound 2 in vivo and was similar to the parent (2) in vitro (Table I). Based on reports in the literature[15] which indicate that only small amounts of intact nucleotides can penetrate cell walls, it is probable that 18 was dephosphorylated and that the active entity was 2.

The (*Z*)-4-cinnamylthio derivative (14) did not clear chicks of *E. tenella* at a dose level of 100 ppm. This was four times higher than the lowest level at which the *E*

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derivative (2) cleared five out of five chicks of the infection. Substitution in the phenyl ring with 3-bromo (3), 4-chloro (6), 4-benzyloxy (9), or 2,3,4-trimethoxy (12) substituents resulted in compounds that were as active as the unsubstituted parent compound (2) at 200 ppm in the diet; however, none was as active as 2 at the lower dose levels. The 4-methoxy (8), 2,4-dichloro (10), 3,4-dichloro (11), and 2-methoxy (13) derivatives were only slightly active in vivo. In contrast to the 3-bromo derivative (3), the 3-hydroxy derivative (4) was inactive even at 400 ppm. The 3-trifluoromethyl derivative (5) cleared only one out of five chicks at 200 ppm, and of the remaining infected chicks, one died.

Two related compounds with unsaturation in the arylalkyl moiety were active in vivo. The 4-(3-phenylpropynyl)thio and 4-(5-phenyl-2,4-pentadienyl)thio derivatives (15 and 16) cleared all five chicks of *E. tenella* at 200 and 50 ppm, respectively. Thus, other unsaturated moieties were tolerated, although the linear alkyne derivative 15 was less active than either the alkene or diene derivatives (2 or 16). The in vitro and in vivo activities of the 4-allylthio and 4-crotylthio derivatives have been reported previously.[1] They are repeated in Table I as entries 19 and 20 to illustrate that an approximately 4-fold greater activity in vivo resulted from changing the hydrogen atom in 19 to the phenyl group in 2 and that an 8-fold greater activity in vivo resulted from replacing the methyl group in 20 with the phenyl group in 2. It is conceivable that the lipophilic affinity of the phenyl group enhanced the activity of 2 in vivo.

No compound showed activity against *E. acervulina* in the in vivo screen at the concentrations tested in Table I.

The efficacy of the (*E*)-cinnamylthio derivative (2) was studied further against five species of chicken coccidia: *E. tenella* and *E. necatrix*, which cause hemorrhage and death, and *E. maxima*, *E. brunetti*, and *E. acervulina*, which erode the intestinal tract, causing reduced weight gains and poor food conversion.[16] Groups of chickens were infected singly with each species at both high and low levels. These experimental conditions were chosen to simulate an acute outbreak of coccidiosis in a flock or a lighter chronic coccidial infection. Both of these conditions may occur in poultry houses where large numbers of chickens are crowded together.[17] The studies indicated that overall, clinical disease and oocyst production due to *E. tenella*, *E. necatrix*, *E. brunetti*, and *E. maxima* were controlled by 2 at levels in the range of 25 to 100 ppm in the diet. A level of about 800 ppm of 2 was required for control of *E. acervulina* oocyst production (unpublished results of R.B.W.).

The present study evaluated the anticoccidial activity of a series of pyrazolo[3,4-*d*]pyrimidine ribonucleosides that contained unsaturated (arylalkyl)thio substituents in the 4-position of the aglycon. The broad spectrum of anticoccidial activity shown by 2, a product of a rational drug design, represents a significant improvement over the activities reported for related compounds.[1,2] Future reports will deal with ribonucleosides of other pyrazolo[3,4-*d*]pyrimidines that demonstrate anticoccidial activity and our attempts to maximize the activity of the compounds to all five species of *Eimeria*.

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Experimental Section

Anticoccidial Evaluation. Activity against *E. tenella* (Weybridge strain) growing in embryonic chick liver cells or adult chick kidney cells in vitro was determined as previously described.[13] Activity against this organism in vivo with a simultaneous infection of *E. acervulina* (Ongar strain) was evaluated in week-old chicks as previously described.[1]

In secondary in vivo tests, severe or mild infections of *E. tenella*, *E. acervulina*, *E. maxima*, *E. necatrix*, or *E. brunetti* were administered to replicate groups of week-old chicks, 24 h after commencement of the medication, which was administered throughout the test, lasting up to 1 week. Clinical signs were recorded throughout, and overall weight gains, food conversion ratios, oocyst production, and postmortem pathognomonic lesions were also assessed as appropriate. Weight gains, food conversion ratios, and mortalities of uninfected, medicated chicks were recorded to assess toxic effects.

Physical Characterizations of Compounds. All compounds listed in Table II gave elemental analyses within ±0.4% of calculated values, except as noted for 17. 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 with a Varian Super-Scan 3 or Pye-Unicam SP1800 spectrophotometer. A Varian XL-100 or CFT-20 provided the NMR spectra in Me_2SO-d_6. Optical rotations were obtained with a Perkin-Elmer Model 141 polarimeter. Table II lists some physical constants for the pyrazolo[3,4-*d*]pyrimidine ribonuclosides.

Syntheses. Silica gel (0.02–0.6 μm) for chromatography was purchased from Merck. Preparative reversed-phase chromatography was performed at 80–100 psi on Merck RP-18 silica gel, 25–40 μm, packed in a glass column, 1.5 × 50 cm (Laboratory Data Control) with water or aqueous methanol as the eluant. The styrene–divinylbenzene copolymer, Amberlite XAD-2, was from Rohm and Haas. The samples were applied in water and eluted with mixtures of water and ethanol. Analytical HPLC was conducted on a Whatman ODS-2 column, 4.2 mm × 25 cm, at 1200 to 1500 psi. Compounds were eluted with aqueous methanol. Polyacrylamide gel, P-2, was supplied by Bio-Rad Laboratories. Column dimensions are given as diameter times height. Evaporations in vacuo were performed at or below 40 °C. TLC was conducted on Eastman Chromagram silica gel or cellulose sheets with fluorescent indicator.

The preparation of compounds 3, 4, and 14 are given below and are designated as methods A–C to refer to the procedures used in the preparation of the halides. These methods are also designated for each compound in Table II, along with some physical constants.

The purification procedures for compounds that were not exemplified in detail below are summarized as follows. Compounds 5–7, 10–12, and 16 were purified by chromatography on silica gel with mixtures of chloroform–methanol and crystallized as follows: 5, from ether–hexane; 6 and 16, from methanol; 7, from ethanol; 10, from chloroform–hexane; 12, from dichloromethane–petroleum ether. Compound 11 was obtained by extraction of the solid obtained from the chromatographic separation into ether and evaporation of the solvent in vacuo. Crystallization from ethanol gave 9 and 15. Crystallization from methanol gave 8. Chromatography on silica gel with mixtures of dichloromethane–methanol, followed by reversed-phase chromatography and crystallization from ethanol–water, gave 13.

Preparation of (*E*)-4-(Cinnamylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (2). A suspension of 4-mercapto-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine[1] (1; 2.0 g, 7.74 mmol) and K_2CO_3 (1.07 g, 7.74 mmol) in 15 mL of DMF was stirred for 10 min at 40 °C, and cinnamyl bromide (1.53 g, 7.04 mmol) was added. After 3 h at room temperature, the mixture was added to 200 mL of water. The solid that formed was collected and crystallized from methanol to give 2 (1.36 g 48%): mp 165–167 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 253 (20.5), 285 (sh) (17.8), 295 nm (20.4); at pH 13, 253 (20.7), 285 (sh) (18.3), 294 nm (20.8); NMR δ 8.86 (s, 1 H, H_6), 8.44 (d, 1 H, J = 0.6 Hz,

H_3), 7.35 (m, 5 H, Ar), 6.77 (d, 1 H, J = 16.0 Hz, =C*H*Ar), 6.45 (dt, 1 H, CH=), 6.23 (d, 1 H, J = 4.4 Hz, $H_{1'}$), 4.32 (m, 4 H, SCH_2 and 5'-CH_2). Anal. ($C_{19}H_{20}N_4O_4S$) C, H, N, S.

Preparation of (*E*)-4-[(3-Bromocinnamyl)thio]-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (3; Method A). A solution of 3-bromocinnamic acid (8.5 g, 37 mmol) in thionyl chloride (40 mL) and dry pyridine (3 mL) was stirred overnight at 40 °C. The excess thionyl chloride and pyridine were removed by vacuum distillation, and the crude acid chloride was dissolved in 30 mL of dry dioxane. The solution of the acyl chloride was added over 1 h to a suspension of sodium borohydride (8.5 g, 0.22 mol) in 30 mL of dry dioxane.[4] The mixture was stirred overnight at room temperature and cooled to 0 °C, and 40 mL of ice–water was added slowly to the stirred reaction. After the alkaline mixture was acidified with 40 mL of dilute HCl, it was extracted 3 times with chloroform. The combined extracts were dried ($MgSO_4$) and filtered, and the filtrate was evaporated in vacuo to yield the crude alcohol. Chlorination of the alcohol was conducted according to the method reported by Newman.[5] The alcohol was dissolved in 40 mL of benzene and added to thionyl chloride (40 mL) to which a few drops of pyridine had been added. After 3.5 days at room temperature, the solution was poured into ice–water and extracted with dichloromethane. The organic layer was separated, dried (Na_2SO_4), and filtered, and the solvent was removed in vacuo to give the crude 3-bromocinnamyl chloride. A portion of the chloride was chromatographed on silica gel with hexane as the eluant to give the desired compound as a pale yellow oil (1.1 g), which was used in the next step.

A suspension of 4-mercapto-1-β-D-ribofuranosyl-1*H*-pyrazolo-[3,4-*d*]pyrimidine (1; 1.0 g, 3.5 mmol) and K_2CO_3 (0.55 g, 3.97 mmol) in 10 mL of DMF was heated with stirring at 40 °C for 0.5 h. Next, 3-bromocinnamyl chloride (1.1 g, 4.75 mmol) was added. After an additional 0.5 h, the UV absorbance at 319 nm due to 1 had disappeared. The reaction mixture was poured into ice–water. The crude product that precipitated was collected, washed with hexane, and chromatographed on silica gel with mixtures of chloroform and methanol as the eluant. Combination of the appropriate fractions, removal of the solvent in vacuo and crystallization twice from water–ethanol gave 3 (0.6 g, 36%): mp (softens at 60 °C) 92 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 255 (18.6), 293 (19.9), 303 nm (sh) (17.1); at pH 13, decomposed; NMR δ 8.86 and 8.45 (2 s, 2 H, H_6 and H_3), 7.90–7.18 (m, 4 H, Ar), 6.75 (d, 1 H, J = 15.80 Hz, =C*H*Ar), 6.55 (dt, 1 H, CH=), 6.23 (d, 1 H, J = 4.50 Hz, $H_{1'}$), 4.29 (d, 2 H, J = 6.10 Hz, SCH_2). Anal. ($C_{19}H_{19}N_4O_4BrS \cdot 0.5H_2O$) C, H, N, S.

Preparation of (*E*)-4-[(3-Hydroxycinnamyl)thio]-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (4; Method B). The methyl ester of 3-hydroxycinnamic acid was prepared by a method in the literature.[6] The acid (10.0 g, 60.9 mmol) and boron trifluoride etherate (8.7 g, 60.9 mmol) were heated in refluxing methanol in a nitrogen atmosphere. After 20 h, 100 mL of a 5% Na_2CO_3 solution was added, and the solid that formed was collected and air-dried. (NMR showed a CO_2CH_3 singlet at δ 3.7.) The ester was dissolved in 100 mL of dry THF and added to a cold (−10 °C) suspension of lithium aluminum hydride (4.5 g, 0.12 mol) in 100 mL of dry THF. After 1 h,[18] some 10% NH_4Cl solution was added cautiously to the cold reaction to destroy the excess hydride. The inorganic salts were removed by filtration, and ether was added to the clear solution. The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was evaporated in vacuo. The crude alcohol was chromatographed on silica gel with mixtures of dichloromethane and ethyl acetate. Evaporation of the appropriate fractions in vacuo provided the alcohol, which was dissolved in 12 mL of pyridine and added to a cold (−10 °C) solution of 9.6 mL of thionyl chloride in petroleum ether (2 mL) and ether (2 mL).[7] The reaction was stirred at −10 °C until complete as shown by TLC.[19] Dilute HCl was added dropwise to the cold (−50 °C) reaction, and the acidic mixture was extracted with ether. The ether extract was washed with aqueous $NaHCO_3$, dried (Na_2SO_4), and filtered, and the filtrate was evaporated in vacuo to give 1.1 g of the crude chloride.

A mixture of 1 (1.5 g, 5.28 mmol) and Na_2CO_3 (0.62 g, 5.8 mmol) in DMF (20 mL) was heated with stirring at 40 °C for 0.5 h, and 3-hydroxycinnamyl chloride (1.1 g, 6.0 mmol) was added. The reaction was conducted at 40 °C overnight, cooled, and poured into water. The mixture was extracted several times with ethyl acetate. After removal of the solvent, the crude product was chromatographed on silica gel with mixtures of dichloromethane and ethyl acetate as the eluant. The appropriate fractions were combined, and the solvent was removed in vacuo. The solid was crystallized from ethanol to give 4 (0.65 g, 30%): mp 176–178 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 257 (17.8), 297 nm (21.7); at pH 13, 295 nm (20.3); NMR δ 9.36 (s, 1 H, C_6H_4-O*H*), 8.86 and 8.45 (2 s, 2 H, H_6 and H_3), 7.19–6.62 (m, 4 H, Ar), 6.68 (d, 1 H, J = 16.0 Hz, =C*H*Ar), 6.36 (dt, 1 H, CH=), 6.24 (d, 1 H, J = 4.7 Hz, $H_{1'}$), 4.27 (d, 2 H, J = 6.2 Hz, SCH_2), 1.06 (t, CH_3CH_2OH). Anal. ($C_{19}H_{20}N_4O_5S \cdot 0.1C_2H_6O$) C, H, N, S.

Preparation of (*Z*)-4-(Cinnamylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (14; Method C). Phenylpropargyl aldehyde (10 g, 0.077 mol) was added dropwise to a suspension of sodium borohydride (15 g, 0.388 mol) in 50 mL of ethanol which was cooled to 10 °C. After the reaction was stirred at ambient temperature for 2 h, 25 mL of distilled water was added slowly to the reaction while it was cooled in an ice bath. The cold solution was acidified with dilute HCl, and the aqueous solution was extracted twice with chloroform. The chloroform solution was dried ($MgSO_4$) and filtered, and the filtrate was evaporated in vacuo to give 6.0 g of alcohol.

The phenylpropargyl alcohol (2.5 g, 19 mmol) was dissolved in 18 mL of benzene containing 0.3 mL of quinoline and hydrogenated at 30 psi in the presence of 0.12 g of Lindlar catalyst[8] for 0.5 h. The catalyst was removed by filtration, and the benzene filtrate was washed successively with dilite HCl, saturated aqueous $NaHCO_3$, and water. Removal of the dried ($MgSO_4$) solvent in vacuo gave the crude (*Z*)-cinnamyl alcohol (2.0 g, 15 mmol) in 77% yield.

The alcohol was chlorinated[9] in a reaction with methanesulfonyl chloride (1.2 mL, 15.5 mmol), *s*-collidine (2.0 g, 16.5 mmol), and lithium chloride (0.62 g, 14.5 mmol) in DMF at 0 °C for 2 h. The reaction was allowed to come to room temperature, and ice–water was added. The aqueous reaction mixture was extracted with ether, and the ether solution was washed with a saturated aqueous solution of $CuSO_4$. The ether extract was dried (Na_2SO_4) and filtered, and the filtrate was evaporated in vacuo to give the crude (*Z*)-cinnamyl chloride (2.0 g, 13 mmol) in 50% yield.

The (*Z*)-cinnamyl chloride was reacted with 1 (3.0 g, 10.6 mmol) and Na_2CO_3 (3.0 g, 28 mmol) at 40 °C as in the preparation of 4. The crude product was purified by medium-pressure reversed-phase chromatography. Evaporation of the solvent in vacuo gave 14 (0.2 g, 5%);[20] mp 96–98 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 245 (sh) (14.4), 297 nm (18.4); at pH 13, 245 (sh) (14.8), 295 nm (17.5); NMR δ 8.77 and 8.46 (2 s, 2 H, H_6 and H_3), 7.40 (m, 5 H, Ar), 6.66 (d, 1 H, J = 11.5 Hz, =C*H*Ar), 6.23 (d, 1 H, J = 4.4 Hz, $H_{1'}$), 5.92 (dt, 1 H, CH=), 4.31 (m, 4 H, SCH_2 and 5'-CH_2). Anal. ($C_{19}H_{20}N_4O_4S$) C, H, N, S.

Preparation of (*E*)-4-(Cinnamylthio)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (17). A mixture of 1 (1.0 g, 3.5 mmol) and K_2CO_3 (0.48 g, 3.5 mmol) in 5 mL of acetic anhydride was stirred at ambient temperature overnight and then heated at 70 °C for 1.5 h. Methanol was added, and the whole was taken to dryness in vacuo. Water was added to the residue, and it was filtered in vacuo. The aqueous filtrate was extracted 3 times with chloroform. The chloroform extracts were dried ($MgSO_4$) and filtered, and the filtrate was taken to dryness in vacuo. The 1-(2,3,5-tri-*O*-acetyl-1-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4-thione hemihydrate was obtained as a yellow foam (1.0 g, 70%): $[\alpha]^{20}_D$ −76° (*c* 0.75, $CHCl_3$) [lit.[3a] $[\alpha]^{20}_D$ −52.7° (*c* 1.01, $CHCl_3$)]. This was mixed with K_2CO_3 (0.37 g, 2.68 mmol) in 10 mL of DMF and heated with stirring at 40 °C for 10 min, and then cinnamyl bromide (0.53 g, 2.68 mmol) was added. After stirring for 3 h at 40 °C, the solution was filtered, and 100 mL of chloroform was added. The

(18) These reductions were conducted as quickly as possible. Disappearance of starting material was followed by TLC on silica gel with CH_2Cl_2/EtOAc (1:1, v/v).

(19) TLC on silica gel with EtOAc/Et_2O (1:1, v/v).

(20) An additional 1 g of 14 was obtained, which was slightly impure.

solution was extracted with water. The chloroform layer was dried ($MgSO_4$) and filtered, and the filtrate was taken to dryness in vacuo. The residue was dissolved in chloroform and filtered through a Millipore filter in vacuo, and the filtrate was evaporated in vacuo to give 17 (0.5 g) as a glass: yield 25% based on 1: UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, decomposed; at pH 13, 257 (21.0), 287 (sh) (16.8), 294 nm (17.9); NMR δ 8.88 and 8.55 (2 s, 2 H, H_6 and H_3), 7.32 (m, 5 H, Ar), 6.77 (d, 1 H, J = 16 Hz, =C*H*Ar), 6.45 (dt, 1 H, CH=), 6.51 (d, 1 H, J = 2.6 Hz, $H_{1'}$), 4.33 (m, 4 H, SCH_2 and 5'-CH_2), 2.11, 2.07, and 1.98 (3 s, 9 H, $COCH_3$). Anal. ($C_{25}H_{26}N_4O_7S$) C, H, S.; N: calcd, 10.64; found, 10.22.

Preparation of (*E*)-4-(Cinnamylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine 5'-Monophosphate Disodium Salt (18). Compound 2 was phosphorylated according to the method of Yoshikawa et al.[11] A mixture of 2 (0.5 g, 1.25 mmol) in 4 mL of triethyl phosphate was stirred and cooled in a stoppered flask (−10 °C bath). Phosphorus oxychloride (0.48 mL, 5.0 mmol) was added, and the reaction was stirred at −10 °C for 10 min, at 0 °C for 45 min, and at 0 to +5 °C for 25 min. The solution was poured onto ice, and 2 N NaOH was added until the pH value of the solution was 7. The solution was washed with chloroform and then ether. The aqueous phase was readjusted with 2 N NaOH to give a pH value of 7.58.

Traces of ether were removed in vacuo from the above neutralized solution. One-half of this solution was applied to a column containing Amberlite XAD resin (200 mL) which had been equilibrated with water. The column was washed with 3 column volumes of water to elute sodium phosphate. The nucleotide was eluted with 8 column volumes of 50% aqueous ethanol.

The remaining half of the neutalized solution was treated similarly with a 100-mL column of resin.

Both batches of nucleotide were combined and lyophilized. The lyophilized powder was dissolved in water (15 mL) and applied to a (5 × 100 cm) column containing polyacrylamide gel (P-2). The nucleotide was eluted with water. Fractions containing the nucleotide were combined and lyophilized. The powder was dissolved in 5 mL of water and precipitated by adding 50 mL of 1-propanol. This step was repeated, and the final precipitate was dried by lyophilization. The overall yield of 4-(cinnamylthio)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine 5'-monophosphate disodium salt (18) was 60% (0.36 g). Purity was estimated by high-performance liquid chromatography to be 99%.[21] The base/ribose/phosphate ratios were 1.00:0.96:0.97.[22] Hydrolysis by 5'-nucleotidase (EC 3.1.3.5) gave compound 2 as shown by TLC.[23]

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Registry No. 1, 54524-71-9; 2, 86687-41-4; 3, 86687-42-5; 4, 86610-57-3; 5, 86687-43-6; 6, 86687-44-7; 7, 86687-45-8; 8, 86610-58-4; 9, 86610-59-5; 10, 86687-46-9; 11, 86687-47-0; 12, 86610-60-8; 13, 86610-61-9; 14, 86687-48-1; 15, 77975-42-9; 16, 86687-49-2; 17, 86687-50-5; 18, 86687-51-6; (*E*)-cinnamyl bromide, 26146-77-0; (*E*)-3-bromocinnamic acid, 14473-91-7; (*E*)-3-bromocinnamic acid chloride, 59114-87-3; (*E*)-3-bromocinnamyl alcohol, 86610-62-0; (*E*)-3-bromocinnamyl chloride, 86610-63-1; methyl (*E*)-3-hydroxycinnamate, 66417-46-7; (*E*)-3-hydroxycinnamyl alcohol, 51765-22-1; (*E*)-3-hydroxycinnamyl chloride, 86610-64-2; phenylpropargyl aldehyde, 2579-22-8; phenylpropargyl alcohol, 1504-58-1; (*Z*)-cinnamyl alcohol, 4510-34-3; (*Z*)-cinnamyl chloride, 39199-93-4; 1-(2,3,5-tri-*O*-acetyl-1-β-D-ribofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4-thione, 64372-70-9.

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(23) TLC on cellulose with 1-PrOH/NH_4OH/H_2O (6:3:1, v/v): R_f of 2, 0.97; of 18, 0.46.

Nitrogen Bridgehead Compounds. 38.[1] New Antiallergic 4*H*-Pyrido[1,2-*a*]pyrimidin-4-ones. 3[2]

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The weak antiallergic activity of 6-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid (4) on the rat reaginic passive cutaneous anaphylaxis test was enhanced by the introduction of appropriate functional groups into position 9 of the pyridopyrimidine ring. The most active 9-substituted pyridopyrimidinecarboxylic acids contained an oxime, a phenylamino, or a (phenylamino)thioxomethyl group in position 9. The 9-phenylcarboxamido and 9-phenylhydrazono moieties may be regarded as bioisosteric groups in the pyridopyrimidinone series. In the series of 9-(arylamino)dihydropyridopyrimidines, the structure–activity relationship study revealed similar relationships as found for the 9-(arylhydrazono)tetrahydropyridopyrimidines. The biological activity was due to the 6*S* enantiomers. A monosubstituted arylamino moiety in position 9 was necessary for the intravenous activity. The most active compound, 9-[(3-acetylphenyl)amino]-6-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid (40) was three times as active as the reference sodium chromoglycate (DSCG) in the passive cutaneous anaphylaxis (PCA) test.

We recently described a series of 9-hydrazono-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acids (1) with potent intravenous antiallergic activity in the rat.[2,3] The most active derivatives contain a methyl group in position 6. They are more potent than sodium

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