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SYNTHESIS AND STEREOCHEMICAL CHARACTERIZATION OF A SERIES OF FIVE-CARBON, ACYCLIC-SUGAR DERIVATIVES OF 1,6-DIHYDRO-6-THIOXOPURINE (6-MERCAPTOPURINE)*†

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ABSTRACT

The four acetylated D-aldose diethyl dithioacetals (**1a–d**) were treated with bromine to give the corresponding, unstable 1-bromides (**2a–d**), which were immediately condensed with 6-chloro-9-(chloromercuri)purine (**3**) to furnish the respective, protected nucleosides (**4a–d**). Subsequent treatment with thiourea gave the crystalline 6-mercaptopurine analogs (**5a–d**) which, upon deacetylation in butylamine–tetrahydrofuran, gave the free, acyclic-sugar nucleosides (**6a–d**). With the exception of the *arabino* derivative **4b**, the 6-chloropurine derivatives were mixtures of 1′-epimers. Crystallization of the acylated 6-mercaptopurine derivatives afforded single 1′-epimers for **5a–c**; **5d** remained an epimeric mixture. A positive Cotton effect for both the *ribo* and *arabino* analogs **5a** and **5b**, and a negative Cotton effect for the *xylo* derivative **5c**, suggested the (1′*R*) configuration for **5a** and **5b**, and the (1′*S*) configuration for **5c**. Proof of the stereochemistry at C-1 (as well as proof of derivatization at N-9 of the purine ring) was afforded by X-ray crystallographic analysis of the *arabino* derivative **5b**, which was demonstrated to have the (1′*R*) configuration. Use of the Generalized Heterocycle Rule also supported the stereochemical attributions at C-1′ for the remaining compounds. The *arabino* derivative **5b** adopts an extended, planar, zigzag conformation of the side chain in solution, as well as in the crystalline state, whereas the *ribo* (**5a**) and *xylo* (**5c**) derivatives exist in solution as non-extended, sickle conformations; the *lyxo* analog (**5a**) was found to be a mixture of 1′-epimers, both of which adopt in solution an extended, planar, zigzag arrangement of the sugar chain.

INTRODUCTION

Acyclic-sugar nucleoside analogs of the type polyhydroxyalkyl-CH(XR)-heterocyclic base (where XR = alkyloxy or alkylthio) having various purine and pyrimidine

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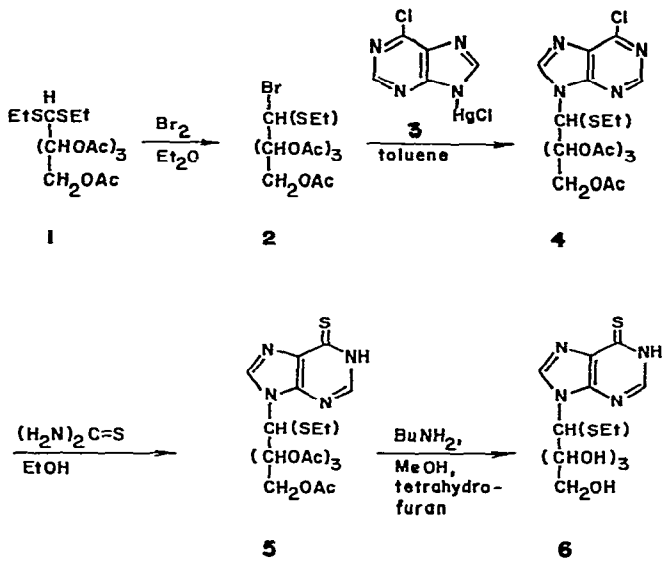
bases as the heterocyclic portion have been synthesized in this laboratory. Specifically, such bases as uracil[3], thymine[4, 5], cytosine[3], 5-fluorouracil[6], adenine[3, 4, 7–10], 6-chloropurine[6, 11], and 6-mercaptopurine[11, 12] have been coupled with acyclic sugar-chains derived from various D-aldohexoses and D-aldopentoses[13]. The resultant nucleosides are frequently obtained as single 1-epimers at the newly created, asymmetric center at C-1′, as shown by n.m.r. spectroscopy, but the specific C-1′ chiral assignment is not immediately evident. In general, where isomeric pairs have been isolated and separated, one isomer has been found strongly dextrorotatory at the sodium D-line, and the other strongly levorotatory. The Generalized Heterocycle Rule[14a] proposed by El Khadem *et al.*, and its modification[14b], were not considered fully secure in these instances for the reliable attribution of chiralities at asymmetrically substituted, hydroxyl-containing centers attached to heterocyclic bases, where -XR is an alkylthio group, particularly in view of the complexities encountered in interpreting chiroptical data for conventional, cyclic-sugar nucleosides[14a,15].

Aside from their chemical aspects, acyclic-sugar nucleosides are of potential interest in chemotherapy, as the hydrophilic side-chain could possibly serve to aid in transporting a biologically active, heterocyclic base in living systems. It is noteworthy that a pair of 9-substituted 6-mercaptopurine derivatives[11], one bearing the D-*gluco* and the other the D-*galacto* chain, has shown interesting differences; the D-*gluco* derivative was markedly more active against L-1210 leukemia than the *galacto* isomer, pointing to a possible link of biological activity with chain configuration and the resulting conformational disposition.

The synthesis of a series of acyclic-sugar nucleoside derivatives was proposed with a view to establishing, by reliable structural correlations, the complete structures of such a series of compounds. The heterocycle 6-mercaptopurine[16], whose 9-β-D-ribosyl nucleoside[17] and numerous, simple 9-alkyl derivatives[18] are known to be potent carcinostatic agents, was chosen as the base. A series of acyclic-sugar nucleosides of this base and in the pentose series was then synthesized, with the aim of resolving the outstanding structural problems: (*1*) the position of glycosylation on the heterocyclic base, (*2*) the stereochemistry at C-1′, (*3*) the conformation of the acyclic, polyhydroxylated chain, and (*4*) the tautomeric form (either thiol, -N=C-SH, or thione, -NHC=S) that is adopted by the sulfur-containing heterocycle.

RESULTS AND DISCUSSION

Synthesis. — Following an adaptation[19] of the procedure of Weygand and associates[20], the four 2,3,4,5-tetra-*O*-acetyl-D-pentose diethyl dithioacetals (**1a–d**)[21–24] were treated with bromine in dry ether, to give the syrupy, highly reactive 2,3,4,5-tetra-*O*-acetyl-1-bromo-1-*S*-ethyl-1-thio-D-pentitols (**2a–d**). These compounds, of undetermined stereochemistry at C-1′, were isolated as thick syrups and were used directly in reaction with a suspension of 6-chloro-9-(chloromercuri)purine[25] (**3**) in



1 2 4

5 6

a D-*ribo*
b D-*arabino*
c D-*xylo*
d D-*lyxo*

boiling toluene to give the nucleoside adducts as viscous syrups that were purified by a combination of column and loose-layer[26,27] chromatography. The products, with the exception of the D-*arabino* derivative **4b**, were shown by n.m.r. spectroscopy (see Table I) to consist of mixtures of C-1′ epimers, as evidenced by the doubling of nearly every resonance in their spectra. In each example, the H-1′ resonances were clearly distinguishable in the δ 5.97–6.42 region, permitting quantitative determination of the isomer ratio, which ranged from as high as 5.2 : 1.0 for **4a** to 2.5 : 1.0 for **4d** (see Table II). The *arabino* derivative **4b** exhibited a single, H-1′ resonance at δ 6.05, indicating a C-1′-epimerically pure compound. Although no definitive structural assignments could be made at this point, the u.v. data (see Table II) suggested that the adducts are indeed the desired 9-substituted 6-chloropurines; their absorption maxima (264 nm, log ε = 4.0) are in close agreement with values in the literature for 6-chloro-9-methylpurine[28]. This evidence, coupled with the n.m.r. data, would tend to indicate that the derivatives **4a**, **4c**, and **4d** are, in fact, mixtures of C-1′ epimers that were not resolved by adsorption chromatography on silica gel.

The crude products **4a–d** were treated directly with an excess of thiourea in boiling ethanol, to give the 6-thiopurine derivatives **5a–d**, isolated crystalline in yields of 44–73% (see Table III). Examination of the H-1′ signals in the n.m.r. spectra revealed that, from the mixed, 1′-epimeric precursors **4a**, **4c**, and **4d**, the respective crystalline products **5a** and **5c** constituted pure C-1′ epimers; only **5d**

TABLE I

100-MHz, ^{1}H-N.M.R.-SPECTRAL DATA

Compd. No. Stereochemistry[a]	*Solvent*[b]	*Chemical shifts (δ[c]) and first-order couplings (Hz)*							
		H-1′	*H-2′*	*H-3′*	*H-4′*	*H-5′,5′a*	*H-2 and H-8*	*Ethylthio*	*Acetate*
4a (1′*R*) *ribo* major epimer	$(CD_3)_2CO$	6.12d ($J_{1',2'}$ 3.8)	5.67dd ($J_{2',3'}$ 7.0)	5.46dd ($J_{3',4'}$ 4.0)	5.25m (width, 14 Hz)	4.07, 4.32m[d] ($J_{5',5'a}$ 12.0 $J_{4',5'}$ 3.9 $J_{4',5'a}$ 7.0)	8.71s, 8.78s	1.14t, 2.54q	1.89s, 2.00s 2.10s, 2.13s
4a (1′*S*) *ribo* minor epimer	$(CD_3)_2CO$	6.42d ($J_{1',2'}$ 4.1)				4.05, 4.30[d] ($J_{5',5'a}$ 12.4 $J_{4',5'}$ 6.8 $J_{4',5'a}$ 5.1)	8.71s, 8.78s	1.15t, 2.55q	1.76s, 1.96s 2.08s, 2.12s
4b (1′*R*) *arabino*	$(CD_3)_2CO$	6.05s		5.14m		3.85, 4.32[d] ($J_{3',5'a} \sim 12.5$)	8.77s, 8.78s	1.14t, 2.57q	1.91s, 2.02s 2.08s, 2.15s
4c (1′*R*) *xylo*, major epimer	$(CD_3)_2CO$	5.97d ($J_{1',2'}$ 5.0)	5.81t ($J_{2',3'} \sim 5$)	5.33–5.61m		3.80, 5.65[d] ($J_{5',5'a}$ 12.0 $J_{4',5'a}$ 4.6 $J_{4',5'a}$ 6.3)	8.75s, 8.77s	1.12t, 2.64q	1.98s, 2.01s 2.06s, 2.18s
4c (1′*S*) *xylo*, minor epimer	$(CD_3)_2CO$	6.27d ($J_{1',2'}$ 5.0)					8.73s, 8.75s	1.21t, 2.66q	
4d (1′*R*) *lyxo*, major epimer	$(CD_3)_2CO$	6.06d ($J_{1',2'}$ 2.8)	5.50dd ($J_{2',3'}$ 9.3)	5.68dd ($J_{3',4'}$ 3.0)	5.36m (width, 15.5)	3.94, 4.30[d] ($J_{5',5'a}$ 11.7 $J_{4',5'}$ 5.0 $J_{4',5'a}$ 7.1)	8.68s, 8.88s	1.14t, 2.54q	1.93s, 1.96s 2.03s, 2.28s
4d (1′*S*) *lyxo*, minor epimer	$(CD_3)_2CO$	6.30d ($J_{1',2'}$ 3.0)	5.82dd ($J_{2',3'}$ 9.2)	4.86dd ($J_{3',4'}$ 3.0)			8.85s	1.13t, 2.55q	1.91s, 1.94s 2.08s, 2.20s

TABLE I *(continued)*

Compd. No. Stereochemistry[a]	*Solvent*[b]	*Chemical shifts (δ[c]) and first-order couplings (Hz)*							
		H-1′	*H-2′*	*H-3′*	*H-4′*	*H-5′,5′a*	*H-2 and H-8*	*Ethylthio*	*Acetate*
5a (1′*R*) *ribo*	$(CD_3)_2SO$	6.08d ($J_{1',2'}$ 4.0)	5.64dd ($J_{2',3'}$ 6.0)	4.99t ($J_{3',4'}$ 6.0)	5.23m ($J_{4',5'}$ 3.5 $J_{4',5'a}$ 6.0)	4.06, 4.27[d] ($J_{5',5'a}$ 12.5)	8.30s, 8.49s	1.10t, 2.48q	1.84s, 1.97s 2.03s, 2.13s
5b (1′*R*) *arabino*	$(CD_3)_2SO$	5.75d ($J_{1',2'}$ 9.0)	6.05dd ($J_{2',3'}$ 2.4)	4.88dd ($J_{3',4'}$ 7.5)	5.05m (width 17.5, $J_{4',5'}$ 4.0 $J_{4',5'a}$ 5.8)	3.94, 4.16[d] ($J_{5',5'a}$ 12.4)	8.27s, 8.57s	1.07t, 2.50q	1.90s, 2.01s 2.07s, 2.13s
5c (1′*S*) *xylo*	$(CD_3)_2SO$	5.68d ($J_{1',2'}$ 7.0)	5.83dd ($J_{2',3'}$ 4.3)	5.10dd ($J_{3',4'}$ 5.7)	5.25m (width 15.4, $J_{4',5'}$ 3.8 $J_{4',5'a}$ 6.2)	3.98, 4.24[d]	8.26s, 8.50s	1.09t, 2.56q	1.98s, 2.04s 2.06s, 2.14s
5d (1′*R*) *lyxo*, major epimer	$(CD_3)_2SO$	5.82d ($J_{1',2'}$ 2.8)	5.44m		5.25m (width 15.9, $J_{3',4'} \sim 3.5$)	3.88, 4.20[d] ($J_{4',5'}$ 4.8 $J_{4',5'a}$ 6.9)	8.27s, 8.42s	1.05t, 2.49q	1.94s, 1.96s 2.02s, 2.18s
	C_5D_5N	6.18d ($J_{1',2'}$ 2.1)	5.96m		5.72m (width 18, $J_{3',4'} \sim 3$)	4.19, 4.60[d]	8.62s, 8.85s	1.08t, 2.48q	1.96s, 1.98s 2.08s, 2.22s
5d (1′*S*) *lyxo*, minor epimer	$(CD_3)_2SO$	6.00d ($J_{1',2'}$ 3.1)	5.62dd ($J_{2',3'}$ 8.7)	4.78dd ($J_{3',4'} \sim 3$)			8.27s, 8.44s	1.05t, 2.49q	2.01s, 2.09s 2.17s
	C_5D_5N	6.40d ($J_{1',2'}$ 3.1)			5.27m (width 12)		8.80s, 8.86s	1.08t, 2.47q	1.84s, 2.06s 2.18s
6a (1′*R*) *ribo*	C_5D_5N[e]	6.77d ($J_{1',2'}$ 2.1)	4.96dd ($J_{2',3'}$ 8.0)	3.88dd ($J_{3',4'}$ 4.8)	4.56m (width ~15)	4.28m	8.55s, 9.15s	1.12t, 2.48q	
6b (1′*R*) *arabino*	C_5D_5N[e]	6.56d ($J_{1',2'}$ 6.4)	5.05dd ($J_{2',3'}$ 3.4)		4.11–4.61m		8.54s, 9.00s	1.07t, 2.43q	
6c (1′*S*) *xylo*	C_5D_5N[e]	6.47d ($J_{1',2'}$ 4.0)	4.87t ($J_{2',3'} \sim 4$)		4.25–4.61m		8.61s, 9.11s	1.05t, 2.41q	
6d (1′*R*) *lyxo*	C_5D_5N[e]	6.81d ($J_{1',2'}$ 2.0)			4.20–4.87m		8.54s, 9.25s	1.13t, 2.48q	

[a]Stereochemistry at C-2′–C-4′ based on starting sugar; chirality at C-1′ by reference to chiroptical data and crystallographic reference-standard **5b**. [b]Containing tetramethylsilane as the internal standard. [c]Multiplicities: d = doublet, dd = doublet of doublets, m = multiplet, q = quartet, s = singlet, and t = triplet. [d]AB portion of an ABX system. [e]Exchangeable protons deuterated by repeated lyophilization from D_2O.

TABLE II

PHYSICAL CONSTANTS FOR THE TETRA-*O*-ACETYL-1-(6-CHLOROPURIN-9-YL)-1-*S*-ETHYL-1-THIO-D-PENTITOLS (4a–d)

Compound	*Yield*[a] *(%)*	*Epimer ratio*[b]	$[\alpha]_D^{22}$ *(degrees)*[c]	(c)	R_F[d]
4a	100	5.2:1	−5.3	0.7	0.34
4b	92	[e]	+81	2.0	0.35
4c	92	4.8:1	−57	0.8	0.45
4d	98	2.5:1	+39	1.2	0.40

[a]Products ~95% pure by t.l.c. [b]As shown by n.m.r. spectroscopy. [c]In chloroform at concentrations indicated. [d]On 0.25-mm plates of Silica Gel G, with 9:1 benzene–methanol as developer. [e]Obtained as a single epimer.

in the series remained as an intractable, ~3:1 isomeric mixture. The u.v. spectra showed maxima at 227 (sh) and 325 nm, in line with values reported[29] for 9-substituted 6-mercaptopurines. All compounds gave elemental analyses satisfactory for the proposed formulation as nucleoside adducts.

Deacetylation with butylamine, in a mixture of tetrahydrofuran and methanol, afforded the crystalline, nonacylated nucleosides. Recrystallization from either aqueous ethanol or methanol gave analytically pure samples of the resultant nucleoside analogs **6a–d**. U.v.-spectral measurements (see Table IV) conducted at pH values of 0, 5, and 12 matched most closely those reported[29] for 6-mercapto-9-methylpurine. At pH 5, where there is the clearest distinction[29] between 3-(λ_{max}^{pH5} 245, 338), 7-(λ_{max}^{pH5} 329), and 9-substituted derivatives (λ_{max}^{pH5} 229, 321) of 6-mercaptopurine, compounds **6a–d** showed λ_{max}^{pH5} 322 nm (log ε 4.41), with a shoulder at 227 nm (log ε 3.71), indicating probable 9-substitution.

X-Ray crystallographic characterization of the D-arabino *derivative* **5b**. — All of the crystalline compounds **5a–c** and **6a–c** were indicated by n.m.r. spectroscopy to be single compounds and not isomeric mixtures. The D-*arabino* derivative **5b** afforded crystals of sufficient quality and size for a detailed, single-crystal, X-ray analysis[2] kindly performed by Drs. A. Ducruix and C. Pascard-Billy. The crystals, obtained as thin plates (monoclinic) were of the space group $P2_1$, having the dimensions $a = 1.3192$, $b = 1.1429$, and $c = 1.7232$ nm, $\beta = 94.83°$, and $Z = 4$. The intensities were collected with a Philips, four-circle, automatic diffractometer using a graphite monochromator and CuKα radiation. A total of 3,955 reflections was recorded above background, and the structure was solved by direct methods through application of the phase function. The structure was refined by a full least-squares method to a final R index of 0.06.

Two molecules were found per asymmetric unit, each related by a pseudo-twofold axis located at $a/4$ and $c/4$. The bond angles and bond lengths of each unit structure are in agreement with generally observed values. The polyhydroxylated chain is attached (see Fig. 1) to N-9 of the heterocycle, and is found to adopt a linear,

TABLE III

PHYSICAL CONSTANTS FOR THE 2,3,4,5-TETRA-*O*-ACETYL-1-(1,6-DIHYDRO-6-THIOXOPURIN-9-YL)-1-*S*-ETHYL-1-THIO-D-PENTITOLS (5a–d)

Compound (C-1′ configuration)	*Amount[a] of* 4a–d		*Amount of thiourea*		*Yield of* 5a–d		R_F[b]	*M.p. (degrees)*	$[\alpha]_D^{22}$ *(degrees)*[c]	(c)	λ_{max}^{EtOH} *(nm)*	*log ε*
	g	*mmol*	*g*	*mmol*	*g*	%						
5a (*R*)	1.94	3.75	0.33	4.34	1.41	73	0.40	205–206	+185	0.4	325	4.4
											227	4.0
5b (*R*)	2.00	3.88	0.35	4.60	1.21	61	0.35	206–208[d]	+131	1.0	325	4.4
											227	4.0
5c (*S*)	5.18	10.0	0.85	11.2	2.26	44	0.58	177–179	−179	1.3	325	4.4
											227	4.0
5d (*RS*)	4.02	7.8	0.65	8.55	1.96	49	0.40	194–197	+47.2	1.0	325	4.4
											227	4.0

[a]Products were ~95% pure, as determined by t.l.c. and by n.m.r. spectroscopy. [b]T.l.c. on 0.25-mm layers of Silica Gel G, activated at 110°, with 3:1 ethyl acetate–chloroform as developer. Unreacted thiourea had R_F 0.20 in this system, and gave a u.v.-absorbing, noncharring zone. [c]Determined in chloroform at the concentrations indicated. [d]First melted at 179–180°, resolidified, and then melted at 206–208°.

TABLE IV

PHYSICAL CONSTANTS FOR THE 1-(1,6-DIHYDRO-6-THIOXOPURIN-9-YL)-1-*S*-ETHYL-1-THIO-D-PENTITOLS (6a–d)

Compound (C-1' configuration)	*Amount of* 5a–d *mg*	*mmol*	*Reaction time for deacetylation (h)*	*Yield[a] of* 6a–d *mg*	%	R_F[b]	*M.p.*[c] *(degrees)*	$[\alpha]_D^{22}$ *(degrees)*[c,d]	(c)	$\lambda_{max}^{H_2O}$ *(nm)*	*log ε*
6a (*R*)	300	0.58	11[e]	170	49	0.36	178–180	+86	0.9	324 (pH 0)[f]	4.34
										322 (pH 5)[f]	4.41
										231, 312 (pH 12)	4.12, 4.36
6b (*R*)	550	1.07	8[g]	254	69	0.29	194–195.5	+60	1.6	324 (pH 0)[f]	4.34
										322 (pH 5)[f]	4.41
										231, 312 (pH 12)	4.12, 4.36
6c (*S*)	400	0.78	4[h]	207	77	0.38	167–168	−84.5	1.0	324 (pH 0)[f]	4.34
										322 (pH 5)[f]	4.41
										231, 312 (pH 12)	4.12, 4.36
6d (*RS*)	300	0.58	6.5[h]	176	88[i]	0.42	192–194[j]	+53	0.8	324 (pH 0)[f]	4.34
										322 (pH 5)[f]	4.41
										231, 312 (pH 12)	4.12, 4.36

[a]Based on products determined to be >98% pure by paper chromatography and n.m.r. spectroscopy. [b]Paper chromatography was performed on Whatman No. 1 paper with butanol saturated with water as solvent. [c]Determined on recrystallized, analytically pure samples. [d]Determined in pyridine at the concentration indicated. [e]In 15 ml of 1:1 methanol–tetrahydrofuran plus 0.3 ml of butylamine. [f]An apparent shoulder of a peak appears at 227 nm (log ε = 3.71). [g]In 25 ml of 1:1 methanol–tetrahydrofuran plus 1.0 ml of butylamine. [h]In 40 ml of 1:1 methanol–tetrahydrofuran plus 1.0 ml of butylamine. [i]This non-recrystallized sample (see Experimental) had m.p. 188–191° and $[\alpha]_D^{22}$ +46° (*c* 1, pyridine), indicating a (1'*RS*) epimeric mixture. [j]Recrystallized from 6 ml of methanol containing a trace of water; optical rotation and n.m.r. data (see Table I) indicated pure, (1'*R*) epimer.

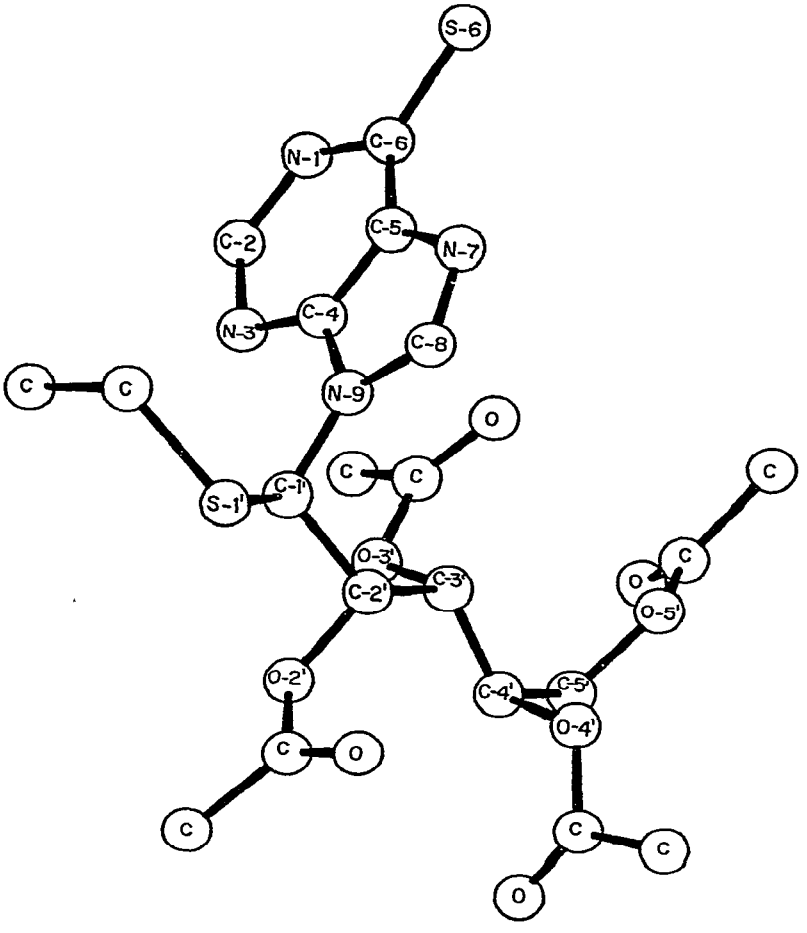


Fig. 1. The structure of (1*R*)-2,3,4,5-tetra-*O*-acetyl-1-(1,6-dihydro-6-thioxopurin-9-yl)-1-*S*-ethyl-1-thio-D-arabinitol (**5b**) as determined by X-ray crystallography. (For further, detailed parameters, see ref. 30.)

planar, zigzag conformation, having C-1′ slightly out-of-plane (by 50 pm). The ethylthio group is fully extended, and antiparallel to C-3′, with the heterocycle assuming a *gauche* orientation. By comparison with the known, absolute stereochemistry of the chiral centers in D-arabinose (namely, C-2–C-5), the configuration at C-1′ is established definitively as (1′*R*). The values found for the carbon–sulfur bond-length indicate[2,30] a $\rangle C{=}S$ function, by comparison with values for other heterocycles known to be thiones.

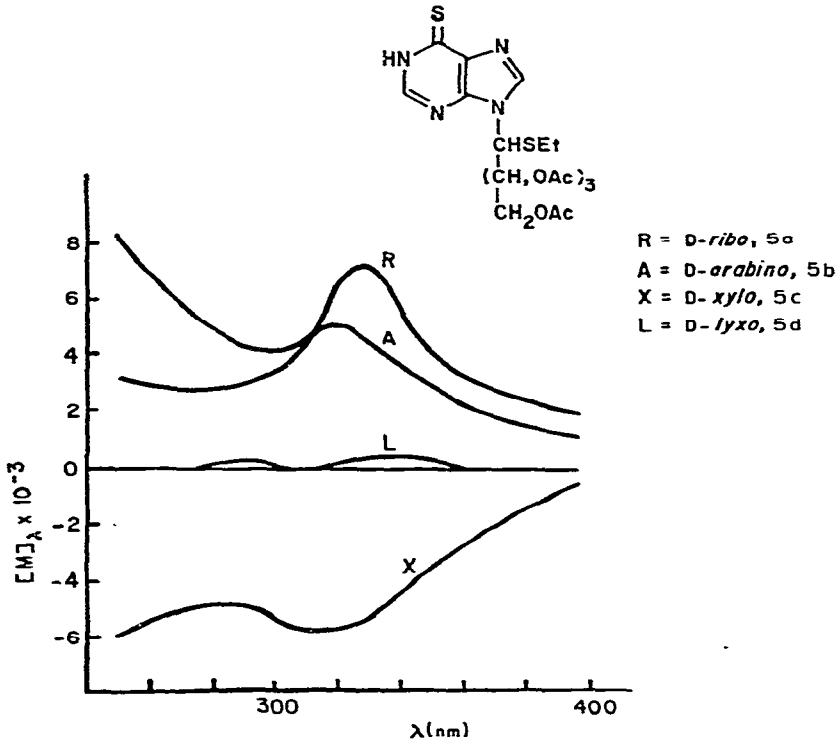


Fig. 2. The optical rotatory dispersion spectra of (1*R*)-2,3,4,5-tetra-*O*-acetyl-1-(1,6-dihydro-6-thioxopurin-9-yl)-1-*S*-ethyl-1-thio-D-ribitol (**5a**), its (1*R*)-D-*arabino* analog (**5b**), its (1*S*)-D-*xylo* analog (**5c**), and its (1*R*, 1*S*)-D-*lyxo* analog (**5d**).

Stereochemical assignments of **5a**, **5c**, *and* **6a–d** *based on optical rotation data.* — Having established the absolute chirality at C-1′ for the D-*arabino* derivative **5b**, the

N
|
chiral center H-C-SR was shown to behave analogously with the multitude of exam-
|
C

ples used to establish the Generalized Heterocycle Rule[14]; that is, the (1′*R*) derivative **5b** had a specific rotation that was large and positive ($[\alpha]_D$ +131°, see Table III), as would be predicted by the rule for the corresponding oxygen analogs. As expected on the basis of the fundamental principles[15,31] that have been shown to govern such systems, the highly polarized, C-1′ asymmetric center contributes[15] the dominant component of the magnitude of the rotation, whereas the centers at C-2′–C-4′ do not alter the sign of the rotation, as each center more remote from the chromophore contributes little to the overall value. These correlations are better illustrated by the o.r.d. data (see Fig. 2), where **5b** may be seen to exhibit a large, positive curve.

Examination of the optical rotatory data for the D-*ribo* compound **5a** and the D-*xylo* isomer **5c** showed that the rotations at the sodium D-line were large and

positive, and large and negative, respectively (see Table III), indicating, on the basis of the logic developed in the foregoing paragraph, that **5a** has the (1′*R*), and **5c** the (1′*S*), configuration. These assignments are more clearly indicated by the o.r.d. data (see Fig. 2), where **5a** shows a large, positive curve, and **5c** exhibits a curve that is large and negative. That the curves for **5a**, **5b**, and **5c** differ in magnitude might well be accounted for on the basis of the conformational disposition of their respective side-chains (see later, for details). The D-*lyxo* derivative **5d** shows a low rotation ($[\alpha]_D$ +47.2°), and its o.r.d. curve is essentially without structure, suggesting a mixture of (1′*R*) and 1′*S*) isomers; this supposition is further substantiated by the n.m.r. spectrum in methyl sulfoxide-d_6, which shows two, distinct doublets for H-1′, at δ 5.82 and 6.00 (see Table I). The deacetylated nucleosides **6a–c** exhibited optical rotations of the same sign as that of their respective precursors; thus, **6a** and **6b** showed $[\alpha]_D$ values of +86° and +60°, respectively, whereas **6c** had $[\alpha]_D$ −84.5°; manifestly, no epimerization to give products inverted at C-1′ had taken place. Thus, the assignments of (1′*R*) are considered firm for both the D-*ribo* (**6a**) and the D-*arabino* (**6b**) nucleosides; the (1′*S*) configuration is indicated for the D-*xylo* derivative **6c**. The D-*lyxo* analog, derived from an ~3:1 mixture of (1′*R*) and (1′*S*) epimers (**5d**, $[\alpha]_D$ +47.2°) in a yield of 88%, undoubtedly contains both epimers, as evidenced by its even lower specific rotation, namely $[\alpha]_D$ +46°. Recrystallization of the sample from methanol–water gave a product of higher specific rotation ($[\alpha]_D$ +53°) that consisted of the single epimer (1′*R*); this conclusion is further substantiated by its n.m.r. spectrum (see Table I), where a single resonance is shown for H-1′.

Conformation in solution. — In order to investigate the relationship of conformational preference in the crystalline state to that in solution, the n.m.r. spectrum of the D-*arabino* derivative **5b** in methyl sulfoxide-d_6 was recorded, and interpreted, essentially on a largely first-order basis. Examination of the spin–spin coupling-data (see Table I and Fig. 3) shows the H-1′ signal at δ 5.75 as a wide doublet ($J_{1',2'}$ 9 Hz), indicating an antiparallel arrangement of H-1′ and H-2′, whereas the values for $J_{2',3'}$ (2.4 Hz) and $J_{3',4'}$ (7.5 Hz) show, respectively, a *gauche* orientation between H-2′ and H-3′, and an antiparallel disposition between H-3′ and H-4′. Such data establish an extended, planar, zigzag arrangement of the C-1′–C-4′ chain. Couplings between H-4′ and the two methylene protons on C-5′ suggest that the terminal acetoxyl group interconverts in a rotameric mixture of those forms (*a*) having the -OAc group in an extended orientation antiparallel to C-3, and (*b*) having the -OAc group antiparallel to H-4. The large value of $J_{1',2'}$ (and consequent, antiparallel disposition of H-1′ and H-2′), taken together with the established (1′*R*) chirality, dictate that the sulfur atom of the ethylthio group is disposed antiparallel to C-1′–C-2′, and that the ethylthio group is maintained in a fully extended state; the nitrogenous heterocycle is evidently spatially less demanding than the ethylthio group, and is forced into a *gauche* disposition. The data thus accord with a fully extended, planar, zigzag structure that extends from the CH_3- of the ethylthio group, along the backbone of the acyclic sugar structure, and terminates with the primary acetoxyl group. There are no 1,3-interactions of bulky groups in this conformation.

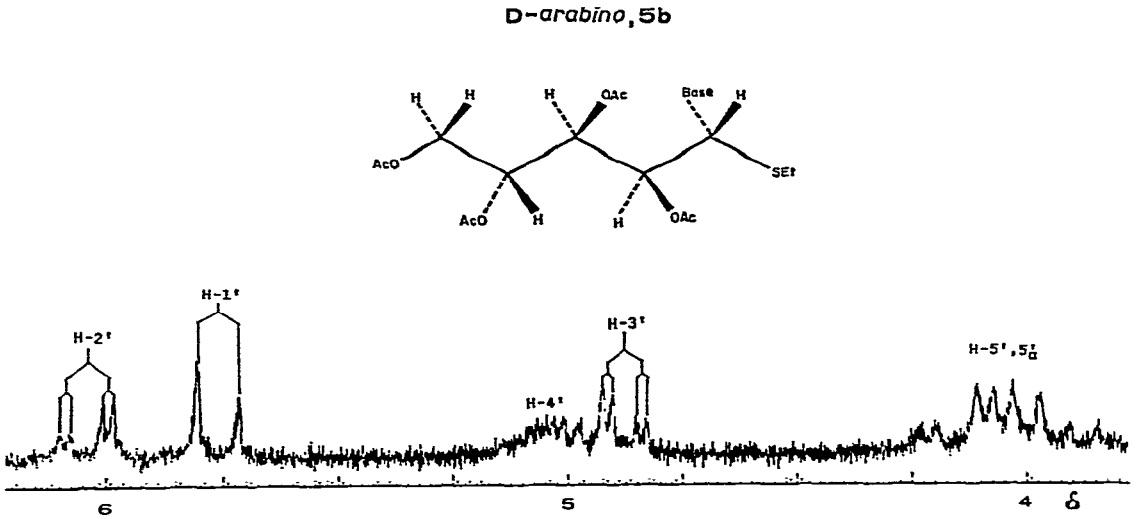


Fig. 3. The 100-MHz, ^{1}H-n.m.r. spectrum of (1*R*)-2,3,4,5-tetra-*O*-acetyl-1-(1,6-dihydro-6-thioxopurin-9-yl)-1-*S*-ethyl-1-thio-D-arabinitol (**5b**) in dimethyl sulfoxide-d_6.

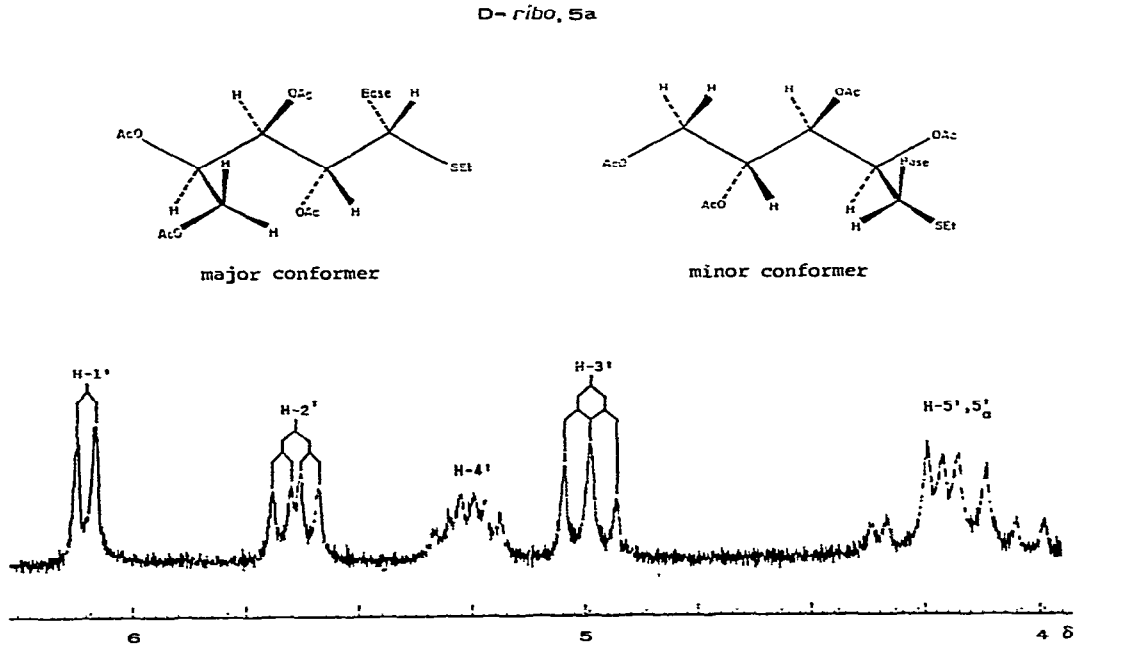
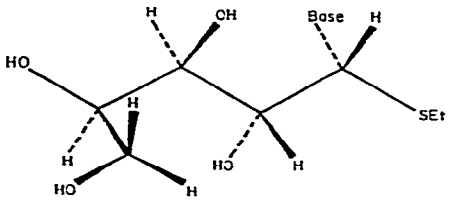


Fig. 4. The 100-MHz, ^{1}H-n.m.r. spectrum of (1*R*)-2,3,4,5-tetra-*O*-acetyl-1-(1,6-dihydro-6-thioxopurin-9-yl-)-1-*S*-ethyl-1-thio-D-ribitol (**5a**) in dimethyl sulfoxide-d_6.

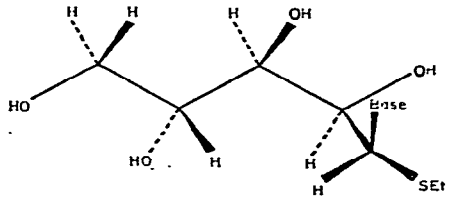
This semi-quantitative, conformational assignment in solution accords closely with that rigorously established[2,30] by X-ray methods for compound **5b** in the crystalline state (see Figs. 1 and 3).

In contrast, the D-*ribo* analog **5a** shows couplings that, even superficially

D-*ribo*, 6a



Conformer A



Conformer B

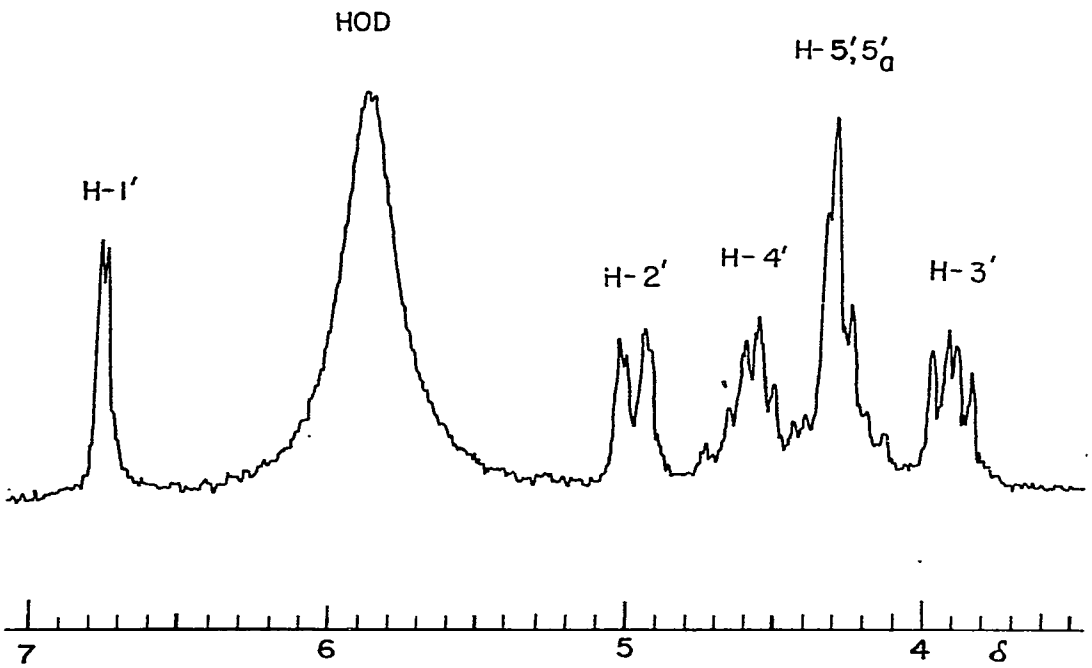


Fig. 5. The 100-MHz, ^{1}H-n.m.r. spectrum of (1*R*)-1-(1,6-dihydro-6-thioxopurin-9-yl)-1-*S*-ethyl-1-thio-D-ribitol (6a) in pyridine-d_5.

(see Fig. 4), appear quite different. The signal at δ 6.08 for H-1′ is a narrow doublet ($J_{1',2'}$ 4.0 Hz), indicating a *gauche* arrangement for H-1′–H-2′. The signals for H-2′ and H-3′ respectively appear as a doublet of doublets (δ 5.64, $J_{2',3'}$ 6.0 Hz) and an apparent triplet (δ 4.99, $J_{3',4'}$ 6.0 Hz); these moderately large values of $J_{2',3'}$ and $J_{3',4'}$ indicate a largely antiparallel arrangement for H-2′, H-3′, and H-4′. These data are not in accord with an extended form, but are best accommodated by a pair

of sickle conformers (see Fig. 4), one obtained by rotation along C-3–C-4 of the extended form, and another, apparently minor, conformer generated from the extended form by rotation along C-2′–C-3′. The values of 6.0 Hz for the 2′,3′ and 3′,4′ couplings are somewhat depressed from values (~9 Hz) expected for exclusively antiparallel protons, and indicate contributions from other conformers having appreciable population at equilibrium. No signals were present that could be attributed to a second, isomeric compound.

Similar, n.m.r. analysis of the D-*xylo* isomer **5c** suggests that the acyclic-sugar chain exists as an equilibrium mixture of two sickle conformations, formed *via* appropriate rotations about C-2′–C-3′ and C-3′–C-4′ from the hypothetical, extended form. For the D-*lyxo* derivatives **5d**, treatment of the n.m.r. data obtained for the mixture of C-1′ epimers was less satisfactory, as the spectral lines of the major epimer (1′*R*) could not be resolved, because of overlapping resonances. However, analysis of the minor (1′*S*) epimer of **5d** showed that its chain exists in a fully extended, planar, zigzag form that allows for maximum relief of the 1,3-interactions in a way similar to that of the D-*arabino* analog **5b**; the heterocycle is again constrained into the *gauche* disposition, and the ethylthio group is fully extended.

Of the nucleoside derivatives **6a–d**, only the D-*ribo* product (**6a**) gave an n.m.r. spectrum whose resonances were sufficiently separated to allow a largely first-order analysis (see Fig. 5). Such examples are relatively rare[32], and a major proportion of earlier conformational work on acyclic-sugar derivatives had been conducted on acetylated compounds. The H-1′ signal of **6a** appeared as a narrow doublet ($J_{1',2'}$ 2.1 Hz), with H-2′ and H-3′ resonating as separate doublets of doublets ($J_{2',3'}$ 8.0 Hz and $J_{3',4'}$ 4.8 Hz). The H-4′ signal appeared as a multiplet (X-portion of an ABX system). These data indicate a *gauche* disposition for H-1′ and H-2′, and an antiparallel arrangement between H-2′ and H-3′. The intermediate magnitude of the coupling between H-3′ and H-4′ indicates substantial population by more than one rotamer, with the preponderant conformer having a *gauche*, H-3′–H-4′ disposition. Conformer A in Fig. 5 is a structure that accommodates these data for the major conformational species; the second, less-populated species, is indicated by conformer B in Fig. 5. This contributing conformer has the antiparallel H-3′–H-4′ arrangement, and its contribution would account for the somewhat enlarged value of $J_{3',4'}$ (4.8 Hz) that is observed. Again, the evidence indicates that the ethylthio group is oriented antiparallel to C-3, and that the base is *gauche*-disposed.

Biological evaluation. — The four (free hydroxy) nucleosides **6a–d** and their tetraacetates **5a–d** were screened for activity *in vitro* against certain selected bacteria. Of these, the free hydroxy D-*ribo* compound **6a** consistently showed activities that were 20–80 times those of its stereoisomeric analogs. The concentrations required for 50% inhibition after growth are as follows: for *Escherichia coli B* and *E. coli* K_{12} 80 μM, and for *Streptococcus faecalis*, 60 μM. The latter is of interest, as this organism has been found particularly sensitive to antimetabolites in mammalian systems[33]. In L-1210 cell culture, **6a** was inhibitory at 80 μM.

EXPERIMENTAL

General methods. — All solutions were evaporated at ~40° (aspirator vacuum). N.m.r. measurements at 100 MHz were performed on ~10% solutions by using a Varian Associates HA-100 instrument; chemical shifts were measured downfield (δ) from tetramethylsilane, used as the internal standard. Optical rotations were determined in a 1-dm tube with a Perkin–Elmer model 141 spectropolarimeter; o.r.d. determinations were extended to 240 nm by using a JASCO-5 instrument. I.r. (potassium bromide discs) and u.v. spectra were recorded with Perkin–Elmer 141 and Cary-14 instruments, respectively. All melting points are uncorrected values taken on a Thomas–Hoover apparatus. Elemental analyses were performed in this laboratory by W. N. Rond. Thin-layer chromatography was effected on plates (0.25 mm) of Silica Gel G (E. Merck), previously activated at 110°. Column and loose-layer chromatography was performed with Silica Gel-60 (particle size, 63–200 μm; E. Merck No. 7734).

*2,3,4,5-Tetra-*O*-acetyl-1-bromo-1-*S*-ethyl-1-thio-*D*-pentitols* (**2a–d**). — To a solution of 5.09 g (12 mmol) of the appropriate pentose diethyl dithioacetal tetraacetate[21–24] (**1a–d**) in dry ether (60 ml) was added dropwise, with stirring at 0°, bromine (0.85 ml; 1 equiv.). After an additional 20 min at 0–5°, the ether was evaporated *in vacuo*, and three, 30-ml portions of ether were added to and evaporated from the residue of syrupy **2a–d**; this was then used in the next step. The products (except **2c**) could be stored for up to 1 h *in vacuo* at 23°. Product **2c** had to be used within 15 min of synthesis.

*2,3,4,5-Tetra-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D*-pentitols* (**4a–d**). — A well-stirred suspension of 4.68 g (12 mmol) of 6-chloro-9-(chloromercuri)purine[25] (**3**), Celite® (1.5 g), and cadmium carbonate (0.5 g) in toluene (100 ml) was boiled under reflux, and ~20 ml of solvent was distilled off. The mixture was cooled to 45°, and a toluene solution (~15 ml) of the crude bromo derivative (**2a–d**), prepared in the foregoing step, was added with stirring. The mixture was again boiled under reflux, an additional volume (~20 ml) of solvent being distilled off; on boiling under reflux for 3.5–4.0 h, t.l.c. (see Table II for details) indicated the formation of a major, u.v.-active zone that charred upon heating after treatment with sulfuric acid spray-reagent.

The hot suspension was filtered through Celite, and the filter was washed with hot chloroform. The filtrate was successively washed with three, 100-ml portions of 30% aqueous potassium iodide, and water (100 ml), dried (magnesium sulfate), and evaporated, to give a yellow to brown syrup that contained the protected nucleoside **4a–d** as the major component (by t.l.c.).

The crude syrup, dried *in vacuo* at 25°, was applied to a column (4 × 60 cm) of silica gel, and successively eluted with chloroform (~200 ml) and 1:99 methanol–chloroform. The fractions that contained the desired product (as determined by t.l.c., and u.v. absorption at 254 nm) were combined and evaporated, to give **4a–d** as

light-yellow syrups that were ~95% pure by n.m.r. spectroscopy (see Table I) and satisfactory for use in the next step.

Additional purification to give products suitable for determination of physical constants was performed by applying the foregoing preparations to loose-layer plates[26,27] (0.2 × 20 × 20 cm) of silica gel, which were developed with 9:1 benzene–methanol. The appropriate zones were excised, and eluted with chloroform to give, upon evaporation of the solvent and drying at 23° *in vacuo*, homogeneous, gummy products (see Table II for physical data). The products, all having λ_{max}^{EtOH} 264 nm (log ε 4.0), invariably retained a small proportion (<5%) of chloroform, as determined by n.m.r. spectroscopy and elemental analyses.

2,3,4,5-Tetra-O-acetyl-1-(1,6-dihydro-6-thioxopurin-9-yl)-1-S-ethyl-1-thio-D-pentitols (**5a–d**). — To a solution of 1.94–5.18 g (3.75–10 mmol) of the appropriate derivative **4a–d** (see Table III for details) in abs. ethanol (50–100 ml) was added thiourea (0.33–0.65 g; 4.34–11.2 mmol), and the mixture was boiled for 3 h under reflux, at which time t.l.c. (see Table III) indicated conversion of **4a–d** into **5a–d**, respectively. The hot, yellow solution was decolorized with bone charcoal (~100 mg), and the product was allowed to crystallize by slow cooling to room temperature. The product was filtered off, the mother liquors were evaporated, and the residue was extracted with three, 30-ml portions of hot ethyl acetate. Evaporation of the extract, and crystallization of the residue from abs. ethanol, gave additional product. Recrystallization of the combined products from abs. ethanol gave 1.21–2.26 g (44–73%) of analytically pure **5a–d** (see Table I for n.m.r.-spectral data, and Table III for other physical constants).

Anal. Calc. for $C_{20}H_{26}N_4O_8S_2$: C, 46.68; H, 5.09; N, 10.88; S, 12.46. Found: for **5a**, C, 46.65; H, 5.02; N, 10.59; S, 12.56; for **5b**, C, 47.00; H, 5.28; N, 11.04; S, 12.60; for **5c**, C, 46.39; H, 5.26; N, 10.37; S, 12.51; for **5d**, C, 46.39; H, 5.01; N, 10.59; S, 12.53.

1-(1,6-Dihydro-6-thioxopurin-9-yl)-1-S-ethyl-1-thio-D-pentitols (**6a–d**). — A solution of 300–550 mg (0.58–1.07 mmol) of the appropriate compound **5a–d** (see Table IV for details) in 1:1 methanol–tetrahydrofuran (15–40 ml) containing butylamine (0.3–1.0 ml) was boiled under reflux in an atmosphere of nitrogen for 4–11 h, at which time t.l.c. revealed almost complete conversion into **6a–d**. The solvents were evaporated off, and the glassy residue was triturated with three, 5-ml portions of hot chloroform to remove (insoluble) residual starting-material. The extract was filtered, and the filtrate evaporated, to give 170–254 mg (49–88%) of **6a–d** as a solid that, by i.r. spectroscopy, showed negligible absorption at 5.78 μm (acetate C=O). Paper chromatography (see Table IV) revealed a major zone for **6a–d**, together with traces of minor, faster-moving components. Analytical samples were prepared by 1 or 2 recrystallizations from the minimal volume of 10:1 ethanol–water (see Table I for n.m.r.-spectral data, and Table IV for other physical constants).

Anal. Calc. for $C_{12}H_{18}N_4O_4S_2$: C, 41.60; H, 5.24; N, 16.18; S, 18.51. Found: for **6a**, C, 41.12; H, 5.34; N, 15.97; S, 18.84; for **6b**, C, 41.79; H, 5.38; N, 15.99; S,

18.30; for **6c**, C, 41.32; H, 5.35; N, 16.27; S, 18.29; for **6d**, C, 42.04; H, 5.56; N, 16.25; S, 18.23.

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