Carbohydrate Research, 80 (1980) 241–262

ACYCLIC-SUGAR PURINE NUCLEOSIDES DERIVED FROM D-GLUCOSE: STEREOCHEMICAL CORRELATIONS IN ACYCLIC-SUGAR DERIVATIVES UNEQUALLY SUBSTITUTED AT C-1*†

KATHLEEN C. BLIESZNER, DEREK HORTON, AND ROBERT A. MARKOVS
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U.S.A.)
(Received August 18th, 1979; accepted for publication, October 24th, 1979)

ABSTRACT

Condensation of 2,3,4,5,6-penta-*O*-acetyl-1-bromo-1-*S*-methyl-1-thio-D-glucitol (**1**) with 6-chloro-9-(chloromercuri)purine gave 49% of crystalline, levorotatory (1*S*)-2,3,4,5,6-penta-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-methyl-1-thio-D-glucitol (**3**), together with a smaller proportion of the syrupy, dextrorotatory (1*R*) isomer. Thiourea converted **3** into its 6-mercaptopurine analog, whose *O*-deacetylated derivative could be *S*-methylated to the corresponding 6-(methylthio)purin-9-yl analog; all compounds in this sequence were crystalline and were the pure (1*S*) isomers, as were the corresponding 1′-*S*-ethyl derivatives prepared by a similar route. Crystal-structure analysis of the *O*-deacetylated derivative of the 1′-*S*-ethyl analog of **3** established the relative stereochemistry of the ethylthio group, permitting assignment of the (1*S*) absolute stereochemistry to this compound and thus to all compounds in the sequence starting from **1**, including the previously described, crystalline, levorotatory 1-(1,6-dihydro-6-thioxopurin-9-yl)-1-*S*-ethyl-1-thio-D-glucitol, whose chirality at C-1 had not hitherto been established. The close similarity of the chiroptical properties of the crystalline 1′-*S*-methyl derivatives to those of their 1′-*S*-ethyl counterparts permitted firm attribution of (1*S*) chirality to the former series also. Conformational studies showed that all of the derivatives have the sugar chain in a non-extended (sickle) conformation.

INTRODUCTION

Acyclic-sugar derivatives asymmetrically substituted at the position of the original carbonyl group are relatively uncommon[4], although a substantial number of such compounds has been prepared in this laboratory from aldose dithioacetal precursors by replacement of one alkylthio group by a nucleic acid base or analog

*Supported, in part, by Grant No. CA-15675 from the National Cancer Institute (The Ohio State University Research Foundation Project 3980).
†For preliminary accounts, see refs. 1–3.

thereof; references to such work were detailed in earlier papers[5]. These compounds are of potential interest in chemotherapy and as metabolic probes[6]. Total, covalent characterization of such compounds is complicated by the fact that the epimeric identity (1*R* or 1*S*) at C-1 of such acyclic products cannot readily be assigned by any generally established method. This situation contrasts with that for the cyclic derivatives of sugars, where the polarimetrically based, Hudson rules for α,β nomenclature[7] permit, with very rare exceptions[8], the correct assignment of stereochemistry at the carbon atom rendered asymmetrically substituted by virtue of ring-closure.

An earlier report[9] described the synthesis from D-glucose diethyl dithioacetal of a crystalline, levorotatory derivative (**10**, NSC 115963) in which one ethylthio group had been replaced by a 1,6-dihydro-6-thioxopurin-9-yl substituent; the compound showed *in vivo* activity[6] (T/C 147 at 400 mg/kg) in the murine L-1210 leukemia screen. Although **10** was evidently a single epimer at C-1 of the sugar chain, the chirality at C-1 was not established, nor was the other possible C-1 epimer isolated. Even had the latter been obtained, the attribution of absolute chirality at C-1 on the basis of chiroptical data could not have been made with certainty, because *a priori* assumptions would not be justified regarding (*a*) the magnitude or the sign of the rotatory contribution arising from "atomic asymmetry" at C-1, and (*b*) contributions from "conformational asymmetry" (ref. 10) of the chiral centers at C-2, 3, 4, and 5, although it might reasonably be estimated that the high polarizability of the C-1 heterocycle would confer a large magnitude on factor (*a*), and substantial cancellations of partial rotatory contributions through the chain's being acyclic would render factor (*b*) relatively small, so that the net rotatory power would be dominated by factor (*a*).

This paper describes the stepwise synthesis of **10** by way of a 6-chloropurin-9-yl precursor, with subsequent conversion into the 6-(methylthio) analog, together with synthesis of the corresponding series having a methylthio group at C-1 of the sugar chain; all products were isolated crystalline, and have now been firmly assigned as the (1*S*) isomers* by reference to one structure in the sequence for which relative stereochemistry determined by X-ray crystallography has allowed absolute stereochemistry to be attributed throughout the series. Careful application of separation methods has permitted the isolation and characterization of the minor, syrupy, (1*R*) isomer of one of the compounds in this study.

These structural correlations should be of value as a base of reference for reliable, stereochemical assignment at C-1, by use of chiroptical methods of comparison, of similarly constituted, acyclic aldose derivatives having unequal substitution at C-1.

*By strict application of standard carbohydrate nomenclature[11], the five-center, chiral prefix (D-*glycero*-D-*ido*) is applied, but the general difficulties in establishing chirality at C-1 (numerous acyclic-sugar nucleosides still await such characterization[5,6]) give justification for retaining an alternative terminology that employs a separate, chiral locant at C-1 in these acyclic derivatives, assigned by use of the Sequence Rule designators[12].

RESULTS AND DISCUSSION

Synthesis. — Treatment of penta-*O*-acetyl-D-glucose dimethyl dithioacetal[13] in ether with 1 equiv. of bromine at room temperature gave the syrupy, unstable bromide **1**; evaporation of the solvent at ~25° served to remove the methylsulfenyl bromide formed. The previously reported[14] *S*-ethyl analog (**2**) was prepared similarly. Both products were obtained quantitatively as syrups, but it was not established whether they were single or mixed epimers at C-1. Each was subjected without delay to coupling with 6-chloro-9-(chloromercuri)purine in hot toluene in the presence of cadmium carbonate (as acid acceptor) and Celite. A range of different conditions and solvents was evaluated[3], and the conditions recorded in the Experimental section gave the best yields of the corresponding nucleoside-coupled products in which the bromine atom had been replaced by the 6-chloropurin-9-yl group. Long reaction-times were not essential; formation of the coupled product from **1** appeared to be largely complete after 1.5 h at ~25°, and that from **2** reached completion after 1.5 h at 50°.

Inspection of the chromatographically homogeneous, syrupy adducts from **1** and from **2** by ^{1}H-n.m.r. spectroscopy in chloroform-*d* revealed that two compounds were present in each, as indicated especially by two, individual doublets for H-1′ in each product. The C-1′ epimeric products from **1** showed a major component whose H-1′ resonance appeared at δ 5.87 ($J_{1',2'}$ = 3.5 Hz), and a minor one whose H-1′ signal appeared at δ 6.34 ($J_{1',2'}$ = 3.5 Hz); the two isomers were present in the ratio of ~6:1. The major product was later shown to be the (1*S*) isomer (**3**). Likewise, the condensation product from **2** showed the major product [later established to be the (1*S*) isomer **4**], giving the H-1′ resonance at δ 5.98 ($J_{1',2'}$ = 3.5 Hz), preponderating by ~4:1 over the minor product, which gave δ 6.20 for H-1′ and $J_{1',2'}$ = 3.0 Hz.

The syrupy products obtained by base-coupling with **1** and with **2** crystallized from abs. ethanol upon refrigeration (use of 1,2-dichloroethane or nitromethane as the condensation solvents gave products that required prior column-chromatographic purification before crystallization could be achieved), and the purified, crystalline products obtained were the homogeneous (1*S*) derivatives (**3** and **4**, respectively), as shown by their n.m.r. spectra (see Tables I and II for details of chemical shifts and first-order couplings). A crystalline form of **4** obtained from benzene was found to be a benzene solvate; recrystallization from ethanol gave the non-solvated form.

Compounds **3** and **4** were strongly levorotatory (−77 and −105°, respectively, in chloroform) and their u.v. spectra (266 nm, ε_{mM} 34.00 for **3**, and 264 nm, ε_{mM} 34.00 for **4**) were in close accord with literature values[15] for 9-alkyl-6-chloropurines, and at variance with values[15] for 3- or 7-alkyl analogs.

The noncrystalline, mother liquors from the crystallization of **3** were observed (^{1}H-n.m.r.) to be enriched in the minor, C-1′ epimer, but isolation of the latter proved impossible by conventional, column chromatography. Use of high-performance, liquid chromatography (l.c.) permitted isolation of the pure, syrupy, (1*R*) isomer (**15**), which, in l.c., migrated slightly faster than **3**, and was strongly dextrorotatory

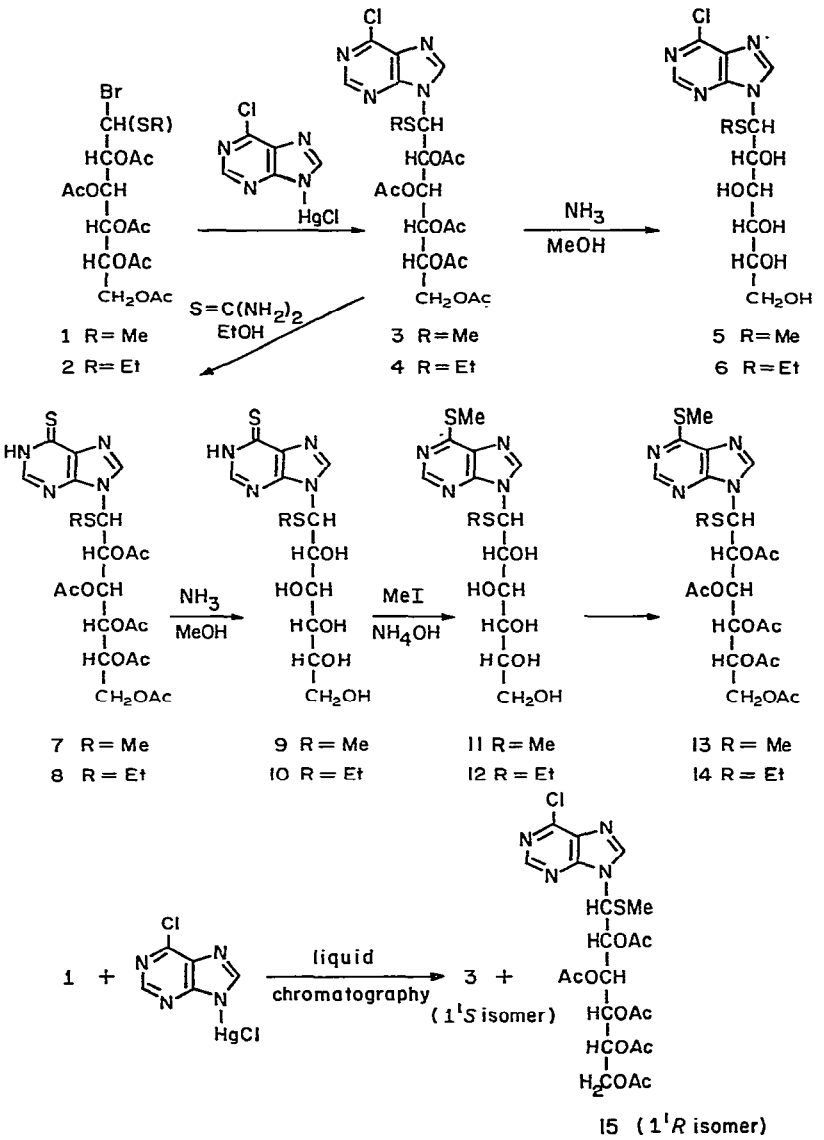
TABLE I

100-MHz, ^{1}H-N.M.R., CHEMICAL-SHIFT DATA FOR COMPOUNDS 3-9 AND 11-15

Compound	*Base*[a]	*SR*[b]	*Solvent*[c]	*Chemical shifts*[d] *(δ)*							*Acetate, methylthio*	*Ethyl-thio*	*H-2, H-8*
				H-1′	*H-2′*	*H-3′*	*H-4′*	*H-5′*	*H-6′*	*H-6″*			
3	6-Cl	SMe	$CDCl_3$	5.87d	←	5.76–5.48m	→	5.12m	4.30q	4.09q	2.33s, 2.90s 2.19s, 2.01s		8.71s, 8.56s
			C_6D_6	←		6.04–5.70m	→	5.32m	4.34q	4.04q	1,89s, 1.78s 1.73s, 1.67s 1.61s		8.47s
			$(CD_3)_2CO$	5.90d	5.78dd	5.52dd	5.66dd	5.12o	4.31q	4.10q	2.30s, 2.24s 2.08s, 2.04s 2.02s, 1.98s		8.79s, 8.72s
4	6-Cl	SEt	$CDCl_3$	5.98d	←	5.76–5.58m	→	5.12m	4.30q	4.10q	2.32s, 2.06s 1.97s, 1.94s	2.58q, 1.28t	8.70s, 8.54s
			C_6D_6		←	6.10–5.84m	→	5.38m	4.37q	4.09q	1.94s, 1.83s 1.78s, 1.72s 1.59s	2.15q, 0.94q	8.50s, 8.48s
			$(CD_3)_2CO$	6.03d	←	5.78–5.55m	→	5.12m	4.32q	4.12q	2.35s, 2.04s 2.02s, 1.98s 1.97s	2.72q, 1.30t	8.79s, 8.74s
			$(CD_3)_2SO$–5% $CDCl_3$	5.93d	5.75t	5.38dd	5.56dd	5.05m	4.26q	4.08q	2.24s, 2.08s 2.01s, 1.98s 1.96s	2.63q, 1.21t	8.88, 8.82
5	6-Cl	SMe	$(CD_3)_2SO$–5% $CDCl_3$	5.92d	←	5.60m, 4.6–4.1m	→	←	3.8–3.2m	→	1.98s		8.87s, 8.78s
6	6-Cl	SEt	C_5H_5N	6.64d	←	6.20–5.40m	→	←	4.68–4.36m	→		2.43q, 1.02t	8.80s, 8.69s
7	6-MP	SMe	$CDCl_3$		←	5.80–5.50m	→	5.18m	4.31q	4.10q	2.32s, 2.21s 2.08s, 2.04s 2.00s		8.87s, 8.82s

			$(CD_3)_2SO$–5% $CDCl_3$	5.66d	5.83t	5.23t	5.50t	5.02m	4.22q	4.10q	2.13s, 2.10s 2.08s, 2.02s 1.98s		8.43s, 8.22s
8	6-MP	SEt	$(CD_3)_2SO$–5% $CDCl_3$	6.17d	←	5.78–5.24m	→	5.02m	←4.38–3.94m→		2.04s, 2.02s 2.01s, 1.98s	2.56q, 1.16t	8.47s, 8.27s
			$CDCl_3$	6.39bs	←	6.99–5.43m	→	5.17m	←4.56–4.02m→		2.23s, 2.11s 2.09s, 2.01s	2.57q, 1.36t	8.97s, 8.91s
9	6-MP	SMe	$(CD_3)_2SO$–5% $CDCl_3$	5.80d	←	5.0–4.0m	→	← 3.70–3.2m		→	1.94s		8.53s, 8.19s
11	6-SMe	SMe	$(CD_3)_2SO$–5% $CDCl_3$	5.92d	←	5.0–4.2m	→	← 3.8–3.2m		→	2.72s, 2.00s		8.75s, 8.71s
12	6-SMe	SEt	D_2O	6.45d	←	4.91m, 4.64–4.34m				→	2.96s	2.94q, 1.57t	9.26s, 8.96s
13	6-SMe	SMe	$CDCl_3$	5.80d	5.68dd	5.51dd	5.67dd	5.14m	4.31q	4.09q	2.13s, 2.12s 2.06s, 2.04s 1.98s, 1.96s		8.66s, 8.37s
14	6-SMe	SEt	$(CD_3)_2CO$	5.96s	5.73dd	5.57dd	5.70dd	5.12o	4.31q	4.11q	1.98s, 1.96s 2.72s	2.66q, 1.25t	8.74s, 8.50s
			$CDCl_3$	5.92bs	←	5.76–5.56m	→	5.14m	4.30q	4.10q	2.35s, 2.07s 1.99s, 1.97s	2.54q, 1.15t	8.66s, 8.36s
			$(CD_3)_2SO$–5% $CDCl_3$	← 5.81m →		5.32dd	5.53dd	5.03m	4.23q	4.07q	2.68s, 2.20s 2.02s, 2.01s 1.97s, 1.95s	2.54q, 1.15t	8.74s, 8.62s
15	6-Cl	SMe	$CDCl_3$	6.34d	5.54dd	4.76dd	5.75dd	4.85m	←4.04–4.35m→		2.22s, 2.16s 2.11s, 2.07s 1.98s, 1.60s		8.80s, 8.53s
			$(CD_3)_2CO$	6.38d	5.59dd	4.83m	5.75dd	4.83m	←4.17–4.20m→		2.20s, 2.16s 2.10s, 1.94s 1.54s		8.82s, 8.74s

[a]6-Cl, 6-chloropurine; 6-MP, 6-mercaptopurine; 6-SMe, 6-(methylthio)purine. [b]Group at C-1′. [c]Containing tetramethylsilane as the internal standard. [d]Signal multiplicities; b, broad; d, doublet; dd, doublet of doublets; m, multiplet; o, octet; q, quartet; s, singlet; and t, triplet.



($[\alpha]_D^{24}$ +81° in methanol); the two isomers were virtually indistinguishable by t.l.c. That **15** was a C-1′ configurational isomer of **3**, rather than a positional isomer on the purine ring, was supported by its u.v. spectrum (λ_{max} 266 nm).

Crystallizability of the products from these coupling-reactions was essential for the conduct of detailed, sequential reactions, as the large-scale use of l.c. at the outset would have been impractical. Careful attention to specific, procedural details was necessary for reproducible isolation of crystalline **3** and **4**. These single, C-1′ epimers having the (1*S*) configuration served as the basis for preparation of all 1′-epimerically pure products described in the following transformations.

TABLE II

PROTON–PROTON, SPIN-COUPLING DATA FOR COMPOUNDS 3–9 AND 11–15

Compound	*Base*[a]	*SR*[b]	*Solvent*	*First-order couplings*[c] *(Hz)*						
				$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
3	6-Cl	SMe	$CDCl_3$	3.5			7.5	3.0	5.5	13.5
			$(CD_3)_2CO$	4.5	6.0	3.5	7.0	3.5	5.5	12.5
			C_6D_6				8.0	3.0	5.0	12.5
4	6-Cl	SEt	$CDCl_3$	3.5			8.0	3.0	5.0	12.5
			C_6D_6							
			$(CD_3)_2CO$	4.0			7.5	3.0	5.0	12.0
			$(CD_3)_2SO$–5% $CDCl_3$	4.75	6.1	3.5	7.0	3.0	5.3	12.5
5	6-Cl	SMe	$(CD_3)_2SO$–5% $CDCl_3$	4.5						
6	6-Cl	SEt	C_5H_5N	4.0						
7	6-MP	SMe	$CDCl_3$					3.0	5.5	13.0
			$(CD_3)_2SO$–5% $CDCl_3$	6.0	5.0	5.0	6.0	3.5	6.0	12.5
8	6-MP	SEt	$(CD_3)_2SO$–5% $CDCl_3$	3.0						
			$CDCl_3$	4.0						
9	6-MP	SMe	$(CD_3)_2SO$–5% $CDCl_3$	4.5						
11	6-SMe	SMe	$(CD_3)_2SO$–$CDCl_3$	4.5						
12	6-SMe	SEt	D_2O	4.2						
13	6-SMe	SMe	$CDCl_3$	4.0	7.0	3.0	7.7	3.0	5.5	12.5
14	6-SMe	SEt	$CDCl_3$				7.0	3.0	5.0	12.5
			$(CD_3)_2CO$	4.0	7.25	3.0	7.5	3.25	5.5	12.5
			$(CD_3)_2SO$–5% $CDCl_3$		5.0	3.5	6.8	3.0	5.5	12.5
15	6-Cl	SMe	$CDCl_3$	3.5	8.5	2.1	9.0			
			$(CD_3)_2CO$	3.2	8.3	2.2	8.5			

[a]6-Cl, 6-chloropurine; 6-MP, 6-mercaptopurine; 6-SMe, 6-(methylthio)purine. [b]Group at C-1′. [c]Couplings refer to protons on the sugar chain. Spacings were measured on an expanded, 100-MHz spectrum (sweep width, 250 Hz).

Compounds **3** and **4** were routinely *O*-deacetylated with methanolic ammonia, to afford the crystalline, strongly levorotatory, deprotected products **5** and **6**, respectively; their corresponding u.v. absorption-maxima were 266 and 264 nm, as expected[15] for 9-substituted derivatives. The *S*-ethyl derivative (**6**) was isolated by slow evaporation from ethanol, as monoclinic crystals suitable for single-crystal structure-analysis, which was performed with a four-circle diffractometer and MoKα radiation. The space group was $P2_1$ and the cell parameters were $a = 553.7$, $b = 901.0$, and $c = 1684.8$ pm, $\beta = 94.63°$, and $Z = 2$; the final R index was 0.06. As indicated in a preliminary report[3] and later described in detail[16] (the synthetic origin of compound **4** was inadvertently not cited in the latter report), the structure analysis established the relative stereochemistry of the chiral centers and, as the absolute stereochemistry at C-2, 3, 4, and 5 is known, it was possible to assign as (1*S*) the stereochemistry at C-1′. The structure analysis also affirmed beyond doubt that the sugar chain was attached to N-9 of the purine ring.

The close similarity between **5** and **6** leaves no doubt that the former is also the (1*S*) isomer, as are the crystalline pentaacetate precursors **3** and **4**. Treatment of **3**

with 1.15 mol of thiourea in boiling ethanol caused replacement of the chlorine atom by SH, and the product (**7**) was isolated crystalline in 72% yield; it was formulated as the thione tautomer by analogy with previous work[5], and u.v. data[3] provided further, independent evidence for attachment of the sugar chain to N-9. Deesterification with methanolic ammonia gave the crystalline (1*R*)-pentol **9**.

The 1′-ethylthio analog (**8**) of **7** was likewise prepared from **4** by the action of thiourea; synthesis of this crystalline compound (and its *O*-deacetylated analog **10**) was reported in an earlier paper[9], and it is now possible to specify that these products have the (1*S*) chirality at C-1′. Although some of the 100-MHz, ^{1}H-n.m.r. spectra of these 6-mercaptopurine derivatives were not completely analyzable, the fact that singlets were observed for H-2 and H-8 of the purine component, and a sole doublet appeared for H-1′, indicated that all of these products were exclusively the (1*S*) epimers.

The 6-*S*-methylation of compounds **9** and **10** was accomplished routinely with iodomethane in dilute, aqueous ammonia, in an adaptation of a standard procedure[17] for *S*-methylation of nucleosides of 6-mercaptopurine, and the corresponding 6-(methylthio) derivatives (**11** and **12**) were isolated crystalline. Again, these products could be assigned the (1*S*) stereochemistry at C-1′, and were shown by ^{1}H-n.m.r. spectroscopy to be single isomers; u.v. data[3] again supported the anticipated, N-9 substitution-pattern.

TABLE III

m/e VALUES OF SELECTED, ELECTRON-IMPACT FRAGMENTS[a] FROM COMPOUNDS **3**, **4**, **7**, **8**, **13**, AND **14**

Fragment	**3**	**4**	**7**	**8**	**13**	**14**
$M^{\pm}$	574(9.0)	588(1.3)	572(0.6)	586(1.3)	586(9.0)	600(5.1)
$M^{\pm} + 1$	575(2.0)	589(0.5)	573(0.3)	587(0.3)	587(2.6)	601(1.5)
$M^{\pm} + 2$	576(4.5)	590(0.6)	574(0.2)	—	588(1.5)	602(0.2)
$M^{\pm} - SR$	527(0.2)	527(1.6)	525(0.3)	525(4.0)	539(1.0)	539(1.0)
A_1	485(1.1)	485(1.2)	483(0.4)	483(8.0)	497(2.0)	497(3.5)
B_1	331(2.5)	331(2.8)	331(0.8)	331(3.0)	331(1.6)	331(1.5)
C_1	441(0.2)	441(0.2)	439(0.1)	439(0.5)	—	—
D_1	421(0.4)	435(0.5)	421(0.7)	435(5.0)	421(2.8)	—
E_1	259(2.0)	259(1.0)	259(1.0)	259(1.0)	259(1.3)	259(0.9)
F_1	434(0.3)	—	—	434(4.0)	—	—
G_1	514(0.2)	528(1.9)	—	526(9.0)	526(0.3)	540(6.0)
Base	153(0.6)	153(1.8)	—	151(2.3)	165(4.0)	165(3.5)
Base + 1	154(1.5)	154(2.0)	152(5.0)	152(3.0)	166(8.5)	166(11.5)
Base + 2	155(14.0)	155(27.0)	153(3.0)	153(5.0)	167(15.0)	167(22.0)
$B\text{-}CH{=}S^{+}H$	199(2.0)	199(6.5)	—	197(0.8)	211(3.0)	211(4.0)
$B\text{-}CH{=}S^{+}R$	213(9.5)	—	211(3.0)	225(2.9)	225(5.0)	239(7.5)
$B\text{-}CH{=}CHOH^{+}$	196(0.8)	196(1.0)	—	194(1.0)	208(2.4)	208(2.5)
RS^{+}	47(1.5)	61(1.6)	47(4.5)	61(4.0)	47(6.0)	61(1.0)
Ac_2OH^{+}	103(2.5)	103(4.0)	103(3.0)	103(15.0)	103(2.5)	103(2.5)
Ac_3O^{+}	145(2.0)	145(5.0)	145(2.0)	145(4.0)	145(0.6)	145(4.0)
Ac^{+}	43(100)	43(100)	43(100)	43(100)	43(100)	43(100)

[a]Intensities in parentheses.

Finally, the 6-(methylthio)purin-9-yl nucleosides (**11** and **12**) were acetylated with acetic anhydride–pyridine, to give the corresponding, crystalline, levorotatory pentaacetates (**13** and **14**, respectively); both were homogeneous, pure (1*S*) epimers.

The mass spectra of the peracetylated compounds **3**, **4**, **7**, **8**, **13**, and **14** have been analyzed and tabulated in detail[3], and Table III summarizes the most significant features of these spectra. The fragmentation patterns combine characteristics of the mass spectra observed for nucleosides, for peracetylated aldose dialkyl dithioacetals, and for peracetylated hexoses; several reviews[18–21] on the mass spectra of carbohydrates and of nucleosides served as valuable guidelines for the analysis. The nucleosides generally display $M^{+\cdot}$, $M^{+\cdot} + 1$, and $M^{+\cdot} + 2$ ions, together with an $M^{+\cdot} - \cdot SR$ ion (compare ref. 22), and seven families of ions (A–F) could be discerned; the first member of each family is listed in Table III, and sequential loss of molecules of acetic acid and ketene following classic patterns lead to daughter-ions in each series[3]. Ion A is formed by loss of the alkylthio radical followed by loss of ketene, and ion B evidently arises from loss of the base and of alkylthio acetate (compare ref. 23). Ions of the C series, formed by loss of alkyl acetate and an acetoxyl radical, may be formulated as cyclic structures having a sulfur atom in the ring and the base at C-1. Loss of the base, followed by elimination of ketene and acetic acid, yields the D series of ions, which may be cyclic structures having an oxygen atom in the ring and the alkylthio group at C-1. Cleavage of C-1–C-2 followed by elimination of acetic anhydride gives a series of ions, presumably cyclic, designated E. Ions of the F and G series are considered to be acyclic, F arising from elimination of the protonated base, and G by elimination of acetic acid (presumably from C-1 and C-2) of the molecule-ion. Fragments involving elimination of such neutral molecules as ketene and acetic acid were confirmed by the presence of metastable peaks.

Other, self-evident ions are listed at the end of Table III.

Under chemical ionization with ammonia as the reagent gas, the hydroxylated derivative **6** gave a weak, protonated molecule-ion (*m/e* 379) and $M \cdot NH_4^+$ capture-ion (*m/e* 397), and a strong peak (*m/e* 242, 100%) for loss of the base from the latter, with subsequent moderate peaks corresponding to dehydration of *m/e* 242; strong peaks were also observed at *m/e* 155 (65%) and 172 (38%) for loss of the sugar component from *m/e* 379 and 397, respectively.

Comparative chiroptical behavior. — Epimeric homogeneity of the foregoing acyclic-sugar nucleosides was readily established by n.m.r. spectroscopy. However, as pointed out in the Introduction, monochromatic polarimetry alone cannot be regarded as a reliable tool for directly assigning stereochemistry at C-1 in asymmetrically 1-substituted, acyclic aldose derivatives, because there is insufficient theoretical understanding of the factors contributing to the net rotation in such systems. Even in the restricted situation of these acyclic-sugar nucleosides, major changes in the heterocyclic base or RX group at C-1 might affect the sign and magnitude of the rotation, especially when configurational or conformational variations in the sugar chain, or both, may be involved. Nevertheless, the fact that all of the C-1′ configurationally correlated (1*S*) derivatives (**3**–**14**) showed strong levorotation at the sodium D

line, whereas the (1*R*) example (**15**) exhibited high dextrorotation at this wavelength, suggested that polarimetric data might be of value for configurational assignment within a limited series of related compounds, once a firm point of reference had been established, as was achieved here with compound **6**. To secure a fuller understanding of chiroptical behavior in such compounds, a detailed, comparative evaluation of the optical rotatory dispersion (o.r.d.) and circular dichroism (c.d.) spectra of compounds **3**–**15** in a common solvent (methanol) was made.

At the outset, a direct comparison of (1*S*)-1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-D-glucitol (**6**), having $[\alpha]_D$ −83° in methanol (−112° in water) and whose chirality at C-1′ is established, with its 1′-*S*-methyl analog **5** ($[\alpha]_D$ −70° in methanol) was performed (see Fig. 1). The essential identity of both the o.r.d. and c.d. spectra of **5** and **6** leave no doubt that both compounds have the same chirality at C-1′, and thus **5** may be securely attributed as the (1*S*) isomer. All 1′-epimerically pure compounds chemically related to **6** (namely, **4**, **8**, **10**, **12**, and **14**) and to **5** (namely, **3**, **7**, **9**, **11**, and **13**) by reactions not involving transformations at C-1′ may thus, likewise, be firmly assigned the (1*S*) configuration. The o.r.d. spectra of **5** and **6** show negative Cotton-effects, progressing to a negative extremum (trough) at ~277 nm and changing sign at 265 nm; moreover, the c.d. curves show a maximum of negative sign centered at 265 nm, and this wavelength also corresponds to the u.v.-absorption maximum (~265 nm) of the two compounds. Not directly evident in the

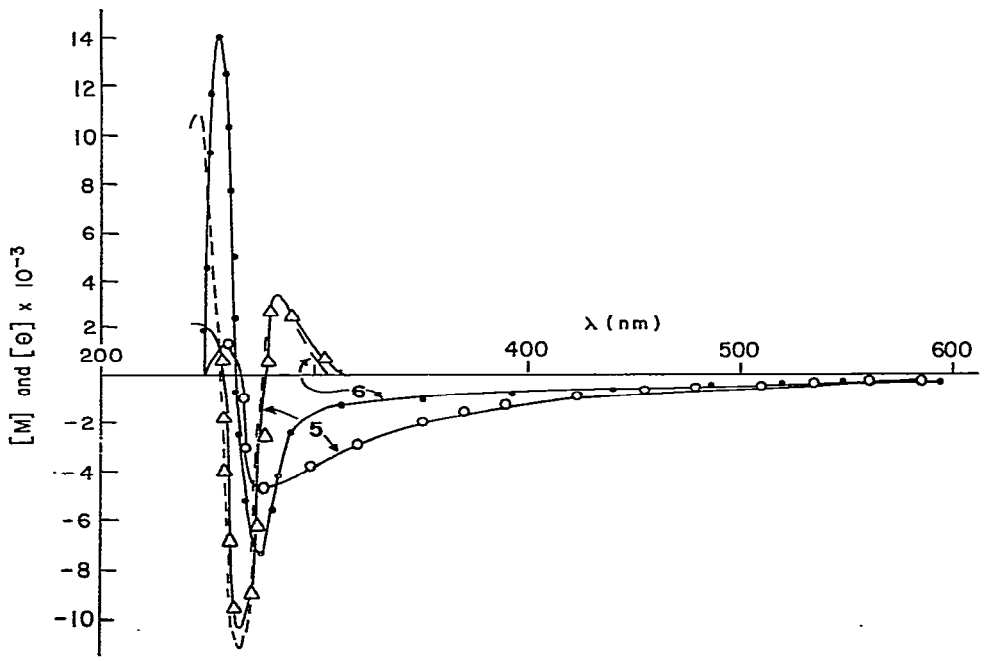


Fig. 1. The o.r.d. (○——○) and c.d. (△——△) spectra of (1*S*)-1-(6-chloropurin-9-yl)-1-*S*-methyl-1-thio-D-glucitol (**5**), and the o.r.d. (•——•) and c.d. (————) spectra of the 1′-*S*-ethyl analog **6**, all in methanol.

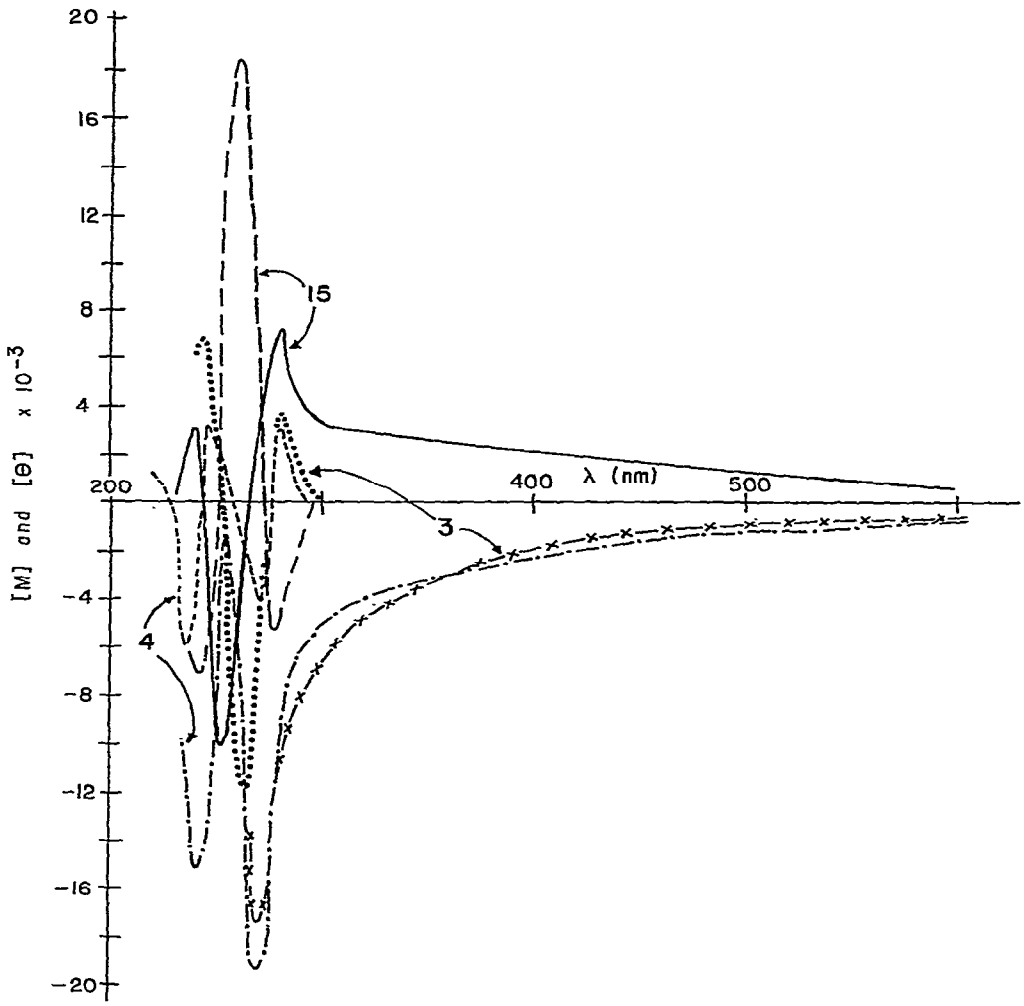


Fig. 2. The o.r.d. (—×—×—) and c.d. (·····) spectra of (1*S*)-2,3,4,5,6-penta-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-methyl-1-thio-D-glucitol (**3**), and the o.r.d. (———) and c.d. (— — — —) spectra of its (1*R*) isomer **15**; also included are the o.r.d. (—·—·—·—) and c.d. (------) spectra of the 1′-*S*-ethyl analog **4** of **3**, all in methanol.

o.r.d. spectrum is the consequence of a small, positive, optically active absorption centered at 283 nm in the c.d. spectrum. The principal u.v. absorption of compounds **3** and **4** (~265 nm) thus corresponds to the optically active band that determines the sign of the c.d. absorption and the Cotton effect observed.

Fig. 2 shows the o.r.d. and c.d. spectra of the (1*S*) (**3**) and (1*R*) (**15**) isomers of 2,3,4,5,6-penta-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-methyl-1-thio-D-glucitol. Compound **3** has $[\alpha]_D$ −83° in methanol, and its C-1′ epimer has $[\alpha]_D$ +81° in the same solvent. Comparison with Fig. 1 shows that acetylation of the chain has a negligible effect on chiroptical behavior for compounds having the same chirality at C-1′;

the spectra of **3** and **5** are essentially identical, except for minor, quantitative differences in rotatory magnitudes. The same may be said for the pentaacetate **4** (also included in Fig. 2) of **6** (see Fig. 1). The (1*S*) acetates (**3** and **4**) display negative Cotton-effects in their o.r.d. spectra, and the curves show somewhat greater complexity; likewise, the c.d. spectra demonstrate, in addition to a major, negative peak at ~262 nm (corresponding to the principal u.v. absorption), two small, positive peaks at 280 and 245 nm. Totally the reverse behavior is exhibited by the (1*R*) isomer (**15**) of **3**; its o.r.d. spectrum shows a positive Cotton-effect, and its c.d. spectrum, a principal, positive peak centered at ~262 nm; the curves observed for **15** are essentially the opposite of those recorded for **3**, and indicate that the chiroptical behavior of **3** and **15** is dominated by the effect of atomic asymmetry at C-1′, where two strongly polarizable substituents (SR and the heterocycle) are present. In this instance, the stereochemistry at the other chiral centers remains the same, so that the rotatory contribution of the rest of the chain (C-2,3,4,5) may be a constant factor of small, relative magnitude. However, it may be significant that, for another acyclic sugar–purine nucleoside example[5], a positive Cotton effect and c.d. maximum have been correlated with (1*R*) stereochemistry, and the reverse behavior with the (1*S*) configuration, even when the relative stereochemistry of the pendant sugar-chains differed.

Similar o.r.d. and c.d. studies have been conducted on the 6-mercaptopurine derivatives (**7**–**10**) and the 6-(methylthio)purine derivatives (**11**–**14**), and full details

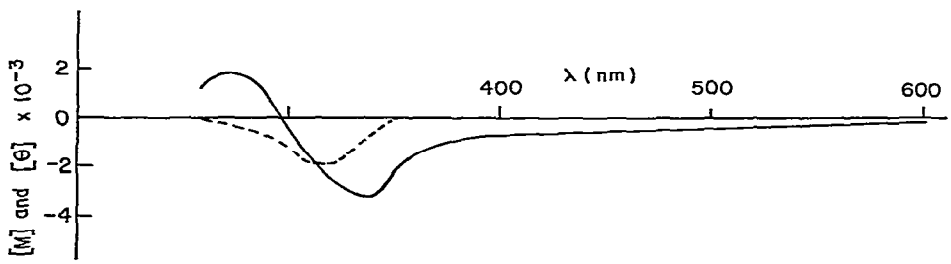


Fig. 3. The o.r.d. (———) and c.d. (— — — —) spectra of (1*S*)-1-(1,6-dihydro-6-thioxopurin-9-yl)-1-*S*-methyl-1-thio-D-glucitol (**9**) in methanol.

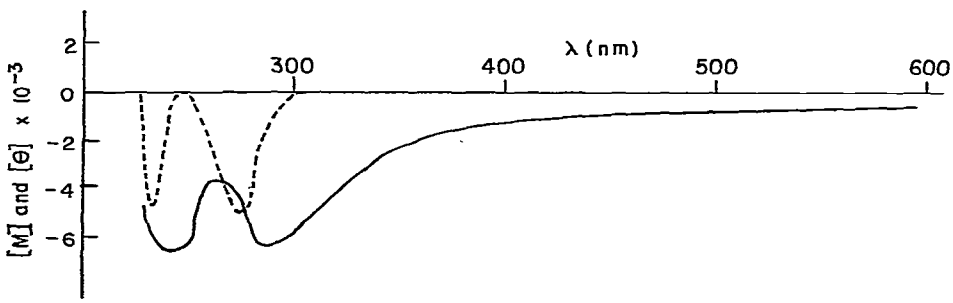


Fig. 4. The o.r.d. (———) and c.d. (— — — —) spectra of (1*S*)-1-*S*-methyl-1-(6-methylthiopurin-9-yl)-1-thio-D-glucitol (**11**) in methanol.

are recorded in ref. 3. Representative spectra are presented in Fig. 3, for compound **9**, and Fig. 4, for compound **11**. Compound **9**, whose $[\alpha]_D$ value in methanol is $-82°$, displays (see Fig. 3) a negative Cotton-effect having a trough at ~338 nm, and a single, negative, c.d. peak centered at 321 nm, close to the longer-wavelength, u.v. absorption (324 nm) for **9**. The o.r.d. and c.d. spectra of the 1-*S*-ethyl analog (**10**), and of the corresponding pentaacetates (**7** and **8**) of **9** and **10**, are very similar in all respects, except for small variations in the quantitative amplitudes of the curves.

The 6-(methylthio)purine derivatives (**11–14**) are all strongly levorotatory in methanol at the sodium D line, exhibit two negative, c.d. maxima, and give o.r.d. spectra showing a negative Cotton-effect and two troughs that correlate with the c.d. absorptions. The illustrated example (**11**, Fig. 4) typifies the four compounds; the longer-wavelength, negative, c.d. absorption is observed at 278 nm (close to the u.v. maximum at 284 nm and the first o.r.d. inflection), and a second, negative, c.d. peak falls at 230 nm, close to the short-wavelength, u.v. absorption (226 nm) of compound **11**, and clearly associated with the second, shorter-wavelength trough in the o.r.d. spectrum.

Within the limited series of compounds examined here, several generalizations may be drawn. Variation of the *S*-substituent from methyl to ethyl exerts an insignificant effect. Acetylation of the sugar chain tends to increase the magnitudes of molecular rotations and ellipticities, but causes no gross, qualitative change. Variation of the pattern of purine-ring substitution shifts the positions of the u.v. maxima, and the associated c.d. bands and o.r.d. inflections, and changes the values of the molecular rotations and ellipticities, but does not alter the qualitative signs of the chiroptical data.

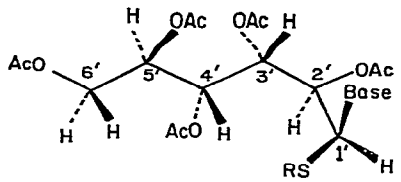
Taking into account previous data[5] on related derivatives having the four different D-aldopentose configurations, it may be concluded that opposite stereochemistry at C-1′ produces c.d. curves of opposite sign, regardless of the configuration of the rest of the carbohydrate chain (compare, ref. 24).

The o.r.d. and c.d. curves thus far examined indicate that, in the purine–acyclic-sugar nucleoside series, those compounds having the (*R*) stereochemistry at C-1′ will display a positive sodium D-line rotation and a positive Cotton-effect at the longest-wavelength, optically active absorption-maximum, whereas a negative sodium D-line rotation and a negative Cotton-effect at the longest-wavelength, optically active absorption-maximum is characteristic of those acyclic-sugar–purine derivatives having the *S* stereochemistry at C-1′.

Conformations of the acyclic-sugar nucleosides. — Detailed, 100-MHz, ^{1}H-n.m.r.-spectral analyses were conducted on the compounds recorded in this study (see Tables I and II). Second-order effects were in evidence for most of the hydroxylated compounds, but solvents or solvent-combinations were found that afforded essentially first-order spectra for the acetylated derivatives; signal assignments were verified, as necessary, by spin decoupling. Even those spectra that were not fully resolved gave partial information[3] sufficient to establish that the products were single epimers.

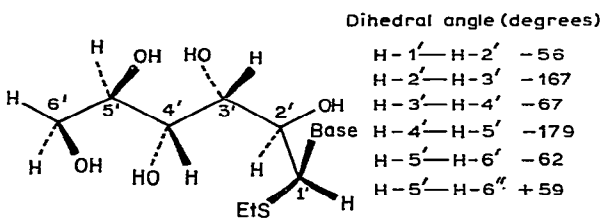
The acetylated 6-chloropurine derivatives **3** and **4** gave interpretable spectra

in acetone-d_6 and 19:1 dimethyl sulfoxide-d_6–chloroform-d, respectively, and the vicinal spin-coupling data extracted from the spectra indicated close conformational similarities between the two compounds, despite the differences in solvent. The observed couplings ($J_{1',2'}$ ~4, $J_{2',3'}$ 6, $J_{3',4'}$ 3.5, $J_{4',5'}$ 7, $J_{5',6'}$ ~3, and $J_{5',6''}$ ~5.5 Hz) indicated that, as expected[25], the molecules did not favor the planar, zigzag conformation in solution (this arrangement would generate an unfavorable interaction between O-2 and O-4); such a conformation would require small values (~3.5 Hz) for both $J_{2',3'}$ and $J_{3',4'}$, and a large value (~9 Hz) for $J_{4',5'}$. The relatively large $J_{2',3'}$ value observed indicates that there is rotation about C-2–C-3 to populate significantly (but not exclusively) the $_2G^-$ rotamer that would alleviate the O-2–O-4 interaction without generating a similar interaction between C-1 and O-3.



$_2G^-$ Conformation of 3 and 4

It is noteworthy that, despite the evident differences between the situation existing with compounds **3** and **4** in solution on the one hand, and of the crystalline, deacetylated product **6** on the other, there exists, nevertheless, a close correspondence between the conformation of **6** in the crystal and of **3** and **4** in solution. Thus, the crystal-structure analysis[3,16] of **6** reveals that carbon atoms 2, 3, 4, 5, and 6 are approximately coplanar, whereas C-1 deviates from this plane by ~100 pm, as shown in the accompanying formula, which also gives proton–proton, dihedral angles estimated from the crystallographic data[16].



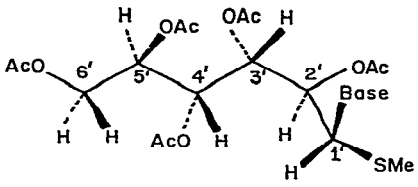
Approximate conformation of 6 from crystallographic data

The non-extended disposition of O-6 in crystalline **6** is undoubtedly promoted by hydrogen-bonding considerations, and the data for **3** and **4** indicate (from the low value of $J_{5',6''}$) that, in solution, there is also a substantial population of this rotamer, derived by rotation about C-5–C-6.

The spin-coupling data for the 6-(methylthio)purine derivatives **13** and **14** (in chloroform-d and acetone-d_6, respectively), closely resemble those for **3** and **4**, and therefore, by similar arguments, the $_2G^-$ conformation may be assigned as a principal, although not exclusive, contributor to the conformational population in

solution. Analysis of the spectrum of the 6-mercaptopurine derivative 7 (in 19:1 dimethyl sulfoxide-d_6–chloroform-d) gives apparent couplings whose intermediate values suggest that the compound may exist as a mixture of several rotamers, with no single one clearly favored; however, the spectral analysis is at the limit of reliability in assigning coupling constants by the first-order approximation.

Compound **15**, the (1*R*) isomer of **3**, displays coupling data (in chloroform-d or acetone-d_6) whose magnitudes, close to extreme values observed for gauche or antiparallel protons, indicate a high degree of conformational homogeneity in the $_2G^-$ conformation.



$_2G^-$ Conformation of 15

These conformational data are of value in guiding the design of (*a*) biochemical probes having structural components isosteric with natural nucleoside metabolites, and (*b*) analogs having antimetabolite activity; several purine nucleoside analogs having acyclic, hydroxylated, alkyl chains at N-9 are of demonstrated interest[26] as inhibitors of adenosine deaminase, and the biological activity has been shown[27] to depend markedly upon stereochemistry (and conformation).

EXPERIMENTAL

General methods. — Solvents were evaporated in a rotary evaporator (aspirator vacuum) at −40°. Optical rotations were determined in a 1-dm tube with a Perkin–Elmer Model 141 spectropolarimeter; o.r.d. and c.d. determinations were made by using a Durrum–Jasco ORD/UV-5 spectropolarimeter equipped with a Sproul Scientific SS-20 CD modification. Melting points were determined in open, glass capillaries by using a Thomas–Hoover "Unimelt" apparatus and are uncorrected. U.v. spectra were recorded with a Spectronic 505 instrument. I.r. spectra were recorded with a Perkin–Elmer Model 137 "Infracord" i.r. spectrometer; those of liquids were measured on thin films between sodium chloride plates, and solids were ground with potassium bromide and measured as compressed discs. Fuller details are recorded in ref. 3. N.m.r. spectra were recorded at 100 MHz with a Varian HA-100 spectrometer; chemical shifts (see Table I) refer to an internal standard of tetramethylsilane (δ = 0.00). Spin-coupling data are given in Table II. Sample concentrations were approximately 15%, with 5% (v/v) of tetramethylsilane added as the internal reference and lock. T.l.c. was performed on precoated plates of Silica Gel 60 (E. Merck, Darmstadt); zones were detected by u.v. light, and by spraying with 10% aqueous ammonium sulfate and subsequent heating. Column chromatography was

performed with Silica Gel No. 7734 (0.05–0.2 mm, 70–325 mesh, E. Merck, Darmstadt). Solvent volumes are v/v. Mass spectra (Table III gives principal peaks and assignments; fuller details are given in ref. 3) were recorded by C. R. Weisenberger with an AEI MS-9 double-focusing, high-resolution, mass spectrometer operating at an ionizing potential of 70 eV and an accelerating potential of 8 kV; the source temperature (direct-inlet system) was 150–250°. Mass numbers were calibrated by the addition of tris(nonafluorobutyl)amine ("heptacosa") as an internal standard after the analytical spectrum had been recorded. Microanalyses were performed by W. N. Rond. X-Ray powder diffraction data are expressed as interplanar spacings in Å, and were determined in a camera of 114.59-mm diameter with CuKα radiation. Relative intensities were estimated visually: m, moderate; s, strong; v, very; w, weak. The strongest lines are numbered in order of decreasing intensity (1, strongest); double numbers indicate approximately equal intensities.

2,3,4,5,6-Penta-O-*acetyl-1-bromo-1*-S-*methyl-1-thio*-D-*glucitol* (**1**). — To a solution of 2,3,4,5,6-penta-*O*-acetyl-D-glucose dimethyl dithioacetal[13] (4.69 g; 10 mmol) in anhydrous ethyl ether (45 mL) at 0° was added bromine (0.5 mL, 1.6 g; 10 mmol) in anhydrous ethyl ether (20 mL) dropwise, during 10 min. The mixture was stirred for 20 min at ~25°, the solvent was evaporated off at ~25°, fresh portions of ether (2 × 20 mL) were added to, and evaporated from, the solute, and the crude, syrupy bromide was used immediately for conversion into **3**.

The reaction was also conducted at room temperature with identical results.

2,3,4,5,6-Penta-O-*acetyl-1-bromo-1*-S-*ethyl-1-thio*-D-*glucitol* (**2**). — The following is a modification of the original procedure of Weygand *et al.*[14]. To a solution of penta-*O*-acetyl-D-glucose diethyl dithioacetal[28] (5.5 g, 11.09 mmol) in anhydrous ether (60 mL) was added, with stirring, a solution of bromine (1.87 g, 11.68 mmol) in ether (18 mL). The mixture was then processed as in the preceding experiment, to give **2** as a light-yellow syrup; yield 5.65 g (98%). The syrup was unstable, and was used within the next hour for conversion into **4**.

2,3,4,5,6-Penta-O-*acetyl-1-(6-chloropurin-9-yl)-1*-S-*methyl-1-thio*-D-glycero-D-ido-*hexitol* [(*1S*)-*2,3,4,5,6-penta*-O-*acetyl-1-(6-chloropurin-9-yl)-1*-S-*methyl-1-thio*-D-*glucitol*] (**3**). — A well-stirred suspension of 6-chloro-9-(chloromercuri)purine[29] (3.90 g, 10 mmol), Celite (1 g), and cadmium carbonate (4 g, 0.02 mol) in toluene (150 mL) was dried by distillation of 25 mL of the solvent. The mixture was cooled to 40–45°, and a solution of the bromide **1** in toluene (15 mL) was added with stirring. The mixture was boiled for 2 h under reflux, filtered hot, and the filter cake washed with warm chloroform; the filtrate and washings were evaporated, to yield a yellow syrup to which chloroform (150 mL) was added. The insoluble, yellow precipitate was filtered off, and the filtrate evaporated. Three fresh portions of abs. ethanol were added to, and evaporated from, the residue to remove all traces of chloroform and toluene, and the resulting, yellow foam was dissolved in the minimum volume of abs. ethanol, and refrigerated to induce crystallization. The resulting, white crystals were recrystallized from abs. ethanol, to yield 2.33 g (41%) of **3**. The mother liquors were combined and applied to a column (4 × 60 cm) of silica gel, which was

eluted first with benzene (100 mL) and then with 1 : 1 benzene–ethyl acetate. Fractions containing the product were combined and evaporated. Crystallization of the residue from abs. ethanol yielded an additional 0.51 g of **3**; total yield 49%. An analytical sample was obtained after several recrystallizations from abs. ethanol; m.p. 132–133° (clear melt), $[\alpha]_D^{24}$ −77° (*c* 2.0 chloroform), $[\alpha]_D^{24}$ −83° (*c* 1.1, methanol); R_F 0.35 (1 : 1 benzene–ethyl acetate), 0.9 (3 : 17 methanol–chloroform); ν_{max}^{KBr} 1755 (C=O) and 1590 cm^{-1} (purine ring); λ_{max}^{MeOH} 266 nm (ε_{mM} 34.10); X-ray powder diffraction data: 10.80 w, 9.18 s (3), 7.93 s (2), 6.66 m, 6.28 s (1), 5.57 w, 5.33 w, 5.02 m, 4.54 w, 4.37 w, 4.24 m, 4.08 w, and 3.82 w.

Anal. Calc. for $C_{22}H_{27}ClN_4O_{10}S$ (575.0): C, 45.95; H, 4.73; Cl, 6.17; N, 9.74; S, 5.98. Found: C, 45.90; H, 4.73; Cl, 6.37; N, 9.47; S, 5.87.

*2,3,4,5,6-Penta-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-methyl-1-thio-*D*-glycero-*D*-gulo-hexitol* [(*1*R)-*2,3,4,5,6-penta-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-methyl-1-thio-*D*-glucitol*] (**15**). — The mother liquor from the preceding experiment was passed through a short column of silica gel, with 1 : 1 hexane–ethyl acetate as eluant, to remove non-migrating contaminants. The syrupy material was indistinguishable from **3** by conventional t.l.c., but high-performance, liquid chromatography (l.c.) showed it to be a mixture of a faster-migrating component (**15**) and the slower-migrating **3** in ~3 : 1 ratio. The syrup was resolved preparatively on a column (7.8 × 300 mm) of μPorasil (Waters Associates) in a Waters Associates Liquid Chromatograph, Model No. ALC 244. Elution was conducted at 2 mL/min with 1 : 1 hexane–ethyl acetate, and fractions were monitored by u.v. absorbance and refractive index. Several minor components were first eluted, followed by **15** (T_R 17 min) and then **3** (T_R 19 min). Evaporation of solvent from the fraction containing **15** gave the compound as a syrup*, homogeneous by l.c., $[\alpha]_D^{24}$ +81° (*c* 1, methanol); ν_{max}^{KBr} 1755 (C=O) and 1590 cm^{-1} (purine); λ_{max}^{MeOH} 266 nm (ε_{mM} 34.10).

Anal. Calc. for $C_{22}H_{27}ClN_4O_{10}S$ (574.1136 for ^{35}Cl isotope); C, 45.95; H, 4.73; Cl, 6.17; N, 9.74; S, 5.98. Found: C, 46.07; H, 4.75; Cl, 5.88; N, 9.49; S, 5.62; *m/e* 374.1144 (M^{+}).

*2,3,4,5,6-Penta-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D*-glycero-*D*-ido-hexitol* [(*1*S)-*2,3,4,5,6-penta-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D*-glucitol*] (**4**). — To an azeotropically dried mixture of 6-chloro-9-(chloromercuri)purine[29] (4.0 g, 10.28 mmol), cadmium carbonate (3.0 g, 17.44 mmol), Celite (1 g), and toluene (125 mL) was added compound **2** (5.5 g, 10.65 mmol), and the mixture was boiled for 4 h under reflux, with stirring. The hot mixture was filtered, and the filter cake washed with several portions of chloroform. The filtrate and washings were combined, successively washed with 30% aqueous potassium iodide (twice) and water (twice), dried (sodium sulfate), and evaporated to a syrup. A solution of the syrup in the minimum volume of benzene was kept at ~25°, whereupon it yielded a

*Since the time of submission of this manuscript, compound **15** crystallized on scratching, and was then recrystallized from ether–hexane; m.p. 114–115°; X-ray powder diffraction data: 9.13 m, 8.30 s (1), 6.64 w, 6.30 w, 5.93 w, 5.29 w, 4.93 vw, 4.79 w, 4.49 w, 4.47 m, 4.14 m (2), 3.79 w, 3.59 m (3,3), and 3.40 m (3,3).

crystalline mass. These crystals were separated, and determined to be a benzene solvate of the desired nucleoside **4**. The product was kept for 48 h under vacuum at ~25°, and was also dissolved in chloroform with subsequent removal of the chloroform (water aspirator), with no observed alteration of the crystals. The solvate had m.p. 72–75°, $[\alpha]_D^{25}$ −103° (*c* 1.0, chloroform); R_F 0.35 (2:1 benzene–ethyl acetate); the n.m.r. spectrum was the same as that of non-solvated material except for a 6-proton singlet at δ 7.2; X-ray powder diffraction data: 10.91 m (3), 7.64 m, 6.31 s (1), 4.55 s (2), and 3.15 w.

The benzene solvate was dissolved in a small volume of abs. ethanol. Refrigeration of the solution for 18 h afforded granular crystals, yield 3.5 g (58%), m.p. 137–138°, $[\alpha]_D^{25}$ −112° (*c* 1.0, methanol), $[\alpha]_D^{25}$ −105° (*c* 1.0, chloroform); R_F 0.35 (2:1 benzene–ethyl acetate); λ_{max}^{MeOH} 264 nm (ε_{mM} 34.00); ν_{max}^{KBr} 2940 (C-H), 1730 (C=O of acetate), 1580, and 1550 cm^{-1} (purine ring); X-ray powder diffraction data: 8.71 vs (1), 7.60 vs (2), 6.99 s (3), 6.00 w, 5.40 s, 4.68 m, 4.45 m, 4.29 s, 3.93 m, 3.67 s, 3.22 m, 3.02 w, 2.88 w, 2.66 w, and 2.39.

Anal. Calc. for $C_{23}H_{29}ClN_4O_{10}S$ (589.02): C, 46.89; H, 4.96; Cl, 6.02; N, 9.51; S, 5.64. Found: C, 47.12; H, 5.17; Cl, 6.31; N, 9.66; S, 5.64.

*1-(6-Chloropurin-9-yl)-1-*S*-methyl-1-thio-*D-glycero-D-ido-*hexitol* [(*1S*)-*1-(6-chloropurin-9-yl)-1-*S*-methyl-1-thio-*D*-glucitol*] (**5**). — A suspension of compound **3** (470 mg, 0.82 mmol) in abs. methanol (20 mL) was cooled to 0–5° in an ice bath, and ammonia was bubbled through it for 30 min. After 3 h at 0°, t.l.c. (17:3 chloroform–methanol) indicated disappearance of the starting material (R_F 0.9) and appearance of the product (R_F 0.13). The solvent was evaporated off, and the residue was crystallized from abs. methanol, to yield 213 mg (72%) of **5**. An analytical sample was obtained after several recrystallizations from abs. methanol; m.p. 171–172° (effervescence, dec.), $[\alpha]_D^{24}$ −70° (*c* 1.0, methanol); R_F 0.13 (3:17 methanol–chloroform); ν_{max}^{KBr} 3400, 3390 (OH), 2970, 2940, and 1595 cm^{-1} (purine ring); λ_{max}^{MeOH} 266 nm (ε_{mM} 13.00), $\lambda_{max}^{0.1M\ HCl}$ HCl 266 nm (ε_{mM} 12.90), $\lambda_{max}^{0.1M\ NaOH}$ 265 nm (ε_{mM} 13.00); X-ray powder diffraction data: 7.86 m (2), 5.98 w, 5.49 w, 5.06 vw, 4.59 s (1), 4.44 w, 4.23 vw, 3.96 w, 3.63 w, 3.47 m (3), and 3.41 w.

Anal. Calc. for $C_{12}H_{17}ClN_4O_5S$ (364.81): C, 39.51; H, 4.70; Cl, 9.72; N, 15.36; S, 8.79. Found: C, 39.74; H, 4.73; Cl, 9.89; N, 15.07; S, 9.04.

*1-(6-Chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D-glycero-D-ido-*hexitol* [(*1S*)-*1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D*-glucitol*] (**6**). — Gaseous ammonia was bubbled for 30 min through a solution of compound **4** (1.0 g, 1.70 mmol) in methanol (30 mL) cooled to 0°. The mixture was kept overnight at 0°, and then evaporated, to give a white solid; yield 0.57 g (89%), m.p. 178–179°, $[\alpha]_D^{25}$ −83° (*c* 0.5, methanol), $[\alpha]_D^{25}$ −112° (*c* 1.0, water); R_F 0.72 (4:1 chloroform–methanol); λ_{max}^{MeOH} 264 nm (ε_{mM} 13.00); ν_{max}^{KBr} 3330 (OH), 1600, 1450 (purine ring), 1090, 1040, and 1030 cm^{-1} (C-O-C); X-ray powder diffraction data: 7.93 s (2), 6.08 m, 5.55 s (3), 4.46 vs (1), 4.21 w, 3.94 s, 2.64 m, and 2.47 w.

Anal. Calc. for $C_{13}H_{19}ClN_4O_5S$ (378.84): C, 41.24; H, 5.02; Cl, 9.38; N, 14.79; S, 8.45. Found: C, 41.48; H, 5.30; Cl, 9.17; N, 14.96; S, 8.37.

2,3,4,5,6-Penta-O-*acetyl-1-(1,6-dihydro-6-thioxopurin-9-yl)-1*-S-*methyl-1-thio*-D-glycero-D-ido-*hexitol* [(*1S*)-*2,3,4,5,6-penta*-O-*acetyl-1-(1,6-dihydro-6-thioxopurin-9-yl)-1*-S-*methyl-1-thio*-D-*glucitol*] (**7**). — Compound **3** (81 mg, 1.4 mmol) and thiourea (123 mg, 1.62 mmol; 1.15-molar excess) were boiled in abs. ethanol (25 mL) for 3 h under reflux. The mixture was cooled to room temperature, and the resulting crystals were filtered off, to yield 574 mg (72%) of **7**. An analytical sample was obtained after several recrystallizations from abs. ethanol; m.p. 223–224°, $[\alpha]_D^{25}$ −82° (*c* 1.0, methanol), $[\alpha]_D^{24}$ −143° (*c* 1.05, chloroform); ν_{max}^{KBr} 1755 (C=O) and 1605 cm^{-1} (purine ring); λ_{max}^{MeOH} 324 (ε_{mM} 36.50) and 225 nm (22.00); X-ray powder diffraction data: 10.52 m, 7.82 s (1), 6.37 m, 5.81 w, 4.96 m, 4.61 m, 4.29 w, 4.15 w, 3.94 m (2), 3.73 w, 3.56 m, 3.43 vw, and 3.33 w.

Anal. Calc. for $C_{22}H_{28}N_4O_{10}S_2$ (572.6): C, 46.14; H, 4.93; N, 9.79; S, 11.20. Found: C, 46.24; H, 5.11; N, 9.88; S, 11.27.

Preparation of 2,3,4,5,6-penta-O-*acetyl-1-(1,6-dihydro-6-thioxopurin-9-yl)-1*-S-*ethyl-1-thio*-D-glycero-D-ido-*hexitol* [(*1S*)-*2,3,4,5,6-penta*-O-*acetyl-1-(1,6-dihydro-6-thioxopurin-9-yl)-1*-S-*ethyl-1-thio*-D-*glucitol*] (**8**). — This compound was prepared by the method described by Wolfrom *et al.*[9]; yield 85%, m.p. 223–224°, $[\alpha]_D^{26}$ −51° (*c* 1.0, chloroform) {lit.[9] m.p. 223–224°, $[\alpha]_D^{21}$ −53° (*c* 1.2, chloroform) for a compound of then-unassigned stereochemistry at C-1′}.

1-(1,6-Dihydro-6-thioxopurin-9-yl)-1-S-*methyl-1-thio*-D-glycero-D-ido-*hexitol* [(*1S*)-*1-(1,6-dihydro-6-thioxopurin-9-yl)-1*-S-*methyl-1-thio*-D-*glucitol*] (**9**). — Into a suspension of compound **7** (1.87 g, 3.27 mmol) in abs. methanol (60 mL), cooled to 0–5° in an ice bath, was bubbled ammonia for 30 min, and the solution was then refrigerated overnight. Evaporation gave a residue that crystallized from abs. methanol, to yield 778 mg (65%) of **9**. An analytical sample was obtained after several recrystallizations from abs. methanol; m.p. 164–165° (clear melt), $[\alpha]_D^{29}$ −82° (*c* 1.0, methanol); ν_{max}^{KBr} 3360 and 3050 cm^{-1} (OH); λ_{max}^{MeOH} 324 (ε_{mM} 25.00) and 220 nm (14.50), $\lambda_{max}^{0.1\,M\,HCl}$ 326 nm (ε_{mM} 22.70), $\lambda_{max}^{0.1\,M\,NaOH}$ 320 (ε_{mM} 26.00) and 237 nm (19.70); X-ray powder diffraction data: 10.73 m (1), 7.89 m (2), 6.79 vw, 5.45 m (3), 4.81 m, and 3.96 m.

Anal. Calc. for $C_{12}H_{18}N_4O_5S_2$ (362.42): C, 39.77; H, 5.01; N, 15.46. Found: C, 39.53; H, 4.97; N, 15.39.

Preparation of 1-(1,6-dihydro-6-thioxopurin-9-yl)-1-S-*ethyl-1-thio*-D-glycero-D-ido-*hexitol* [(*1S*)-*1-(1,6-dihydro-6-thioxopurin-9-yl)-1*-S-*ethyl-1-thio*-D-*glucitol*] (**10**). — Deacetylation of **8** as already described[9] gave **10**; yield 89%, m.p. 189–190°, $[\alpha]_D^{26}$ −102° (*c* 1.0, water) {lit.[9] m.p. 189–190°, $[\alpha]_D$ −101° (*c* 0.7, water) for a compound of then-unassigned stereochemistry at C-1′}.

1-S-*Methyl-1-(6-methylthiopurin-9-yl)-1-thio*-D-glycero-D-ido-*hexitol* [(*1S*)-*1*-S-*methyl-1-(6-methylthiopurin-9-yl)-1-thio*-D-*glucitol*] (**11**). — To a suspension of compound **9** (78 mg, 2.15 mmol) in water (10 mL) was added concentrated ammonium hydroxide dropwise (10 drops) until complete dissolution occurred. Iodomethane (1.22 g, 0.55 mL; 8.6 mmol) was added, and the mixture was shaken for 1.5 h at room temperature, at which point the mixture solidified. The precipitate was filtered

off, and washed with cold water, to yield 54 mg (67%) of **11**. An analytical sample was obtained after several recrystallizations from 95% ethanol; m.p. 188–189°, $[\alpha]_D^{29}$ −66° (*c* 0.9, methanol); ν_{max}^{KBr} 3400 and 3300 cm^{-1} (OH); λ_{max}^{MeOH} 290 (ε_{mM} 22.70), 284 (23.00), and 226 nm (14.00); $\lambda_{max}^{0.1M\ HCl}$ 293 (ε_{mM} 21.00) and 229 nm (13.00); $\lambda_{max}^{0.1M\ NaOH}$ 290 (ε_{mM} 21.60), 284 (21.70), and 226 nm (13.70); X-ray powder diffraction data: 8.19 s (3,3), 6.06 w, 5.55 s (3,3), 5.14 m, 4.81 s (2), 4.57 s (1), 4.11 m, 3.95 w, 3.67 m, 3.56 m, 3.28 vw, 3.13 w, and 2.98 w.

Anal. Calc. for $C_{13}H_{20}N_4O_5S_2$ (376.45): C, 41.47; H, 5.36; N, 14.88; S, 17.04. Found: C, 41.70; H, 5.62; N, 14.76; S, 16.96.

1-S-*Ethyl-1-(6-methylthiopurin-9-yl)-1-thio*-D-glycero-D-ido-*hexitol* [(*1S*)-*1*-S-*ethyl-1-(6-methylthiopurin-9-yl)-1-thio*-D-*glucitol*] (**12**). — Crystalline compound **10** (1.8 g, 4.78 mmol) was suspended in water (25 mL) and enough aqueous ammonium hydroxide was added to effect dissolution. Iodomethane (0.81 g, 6.4 mmol) was added, and the mixture was stirred for 30 min at ~25°. The resultant solution was then freeze-dried, the residue was taken up in a small volume of water, and the solution was passed through a column (10 × 1.5 cm) of AG-1 X-2 (OH^-) ion-exchange resin (Bio-Rad Laboratories, Richmond, California). The effluent was collected, and freeze-dried, and the residue was crystallized from dry acetone–ethanol, to yield 1.65 g (89%) of **12**, m.p. 129–130°, $[\alpha]_D^{25}$ −89° (*c* 1.0, methanol); R_F 0.80 (4:1 chloroform–methanol); λ_{max}^{MeOH} 284 nm (ε_{mM} 16.40); ν_{max}^{KBr} 3330 (OH), 1575, and 1475 cm^{-1} (purine ring); X-ray powder diffraction data: 8.12 m (2), 5.45 s (1), 4.42 w (3), 3.48 w, and 2.65 w.

Anal. Calc. for $C_{14}H_{22}N_4O_5S_2$ (390.48): C, 43.08; H, 5.64; N, 14.36; S, 16.41. Found: C, 42.91; H, 5.52; N, 14.23; S, 16.46.

2,3,4,5,6-Penta-O-*acetyl-1*-S-*methyl-1-(6-methylthiopurin-9-yl)-1-thio*-D-glycero-D-ido-*hexitol* [(*1S*)-*2,3,4,5,6-penta*-O-*acetyl-1*-S-*methyl-1-(6-methylthiopurin-9-yl)-1-thio*-D-*glucitol*] (**13**). — To a solution of compound **11** (1.2 g, 3.2 mmol) in dry pyridine (14 mL) was added acetic anhydride (5 mL). After 18 h at ~25°, the mixture was poured over ice. Filtration of the resulting crystals, and recrystallization from abs. ethanol, yielded 1.32 g of **13**. An additional 390 mg was obtained by concentration of the mother liquors; total yield, 1.71 g (92%). An analytical sample was obtained after several recrystallizations from abs. ethanol; m.p. 124–125°, $[\alpha]_D^{29}$ −86° (*c* 0.9, methanol); ν_{max}^{KBr} 3120 (CH) and 1750 cm^{-1} (C=O); λ_{max}^{MeOH} 291 (ε_{mM} 37.00), 283 (37.90), and 230 nm (21.40); X-ray powder diffraction data: 7.82 s (1,1), 7.60 s (1,1), 6.64 s (1,1), 6.10 m, 5.59 w, 5.17 m (2), 4.70 m (3), 4.22 m, 3.95 w, 3.73 w, 3.51 w, 3.34 w, and 3.18 vw.

Anal. Calc. for $C_{23}H_{30}N_4O_{10}S_2$ (586.63): C, 47.09; H, 5.15; N, 9.55; S, 10.93. Found: C, 47.18; H, 5.27; N, 9.77; S, 10.99.

2,3,4,5,6-Penta-O-*acetyl-1*-S-*ethyl-1-(6-methylthiopurin-9-yl)-1-thio*-D-glycero-D-ido-*hexitol* [(*1S*)-*2,3,4,5,6-penta*-O-*acetyl-1*-S-*ethyl-1-(6-methylthiopurin-9-yl)-1-thio*-D-*glucitol*] (**14**). — To a solution of 1-*S*-ethyl-1-[6-(methylthio)purin-9-yl]-1-thio-D-glucitol (400 mg, 1.02 mmol) in pyridine (10 mL) was added acetic anhydride (10 mL) at ~25°. After 18 h, the mixture was poured over ice (125 mL). The resulting

precipitate was filtered off, and recrystallized several times from abs. methanol, to yield 263 mg (43%) of **14**; m.p. 125–126°, $[\alpha]_D^{29}$ −116° (*c* 1, methanol); ν_{max}^{KBr} 3130 (CH), 1710 (C=O), and 1580 cm^{-1} (purine ring); λ_{max}^{MeOH} 292 (ε_{mM} 23.00), 284 (23.00), and 228 nm (16.80); X-ray powder diffraction data: 8.64 s (1), 8.02 w, 7.29 m (2), 6.64 m, 6.13 w, 5.65 m, 5.14 w, 4.71 m, 4.41 w, 4.24 w, 4.01 m (3), 3.74 m, 3.56 m, 3.43 m, 3.32 w, 3.20 m, and 3.05 w.

Anal. Calc. for $C_{24}H_{32}N_4O_{10}S_2$ (600.66): C, 47.99; H, 5.37; N, 9.33; S, 10.68. Found: C, 47.85; H, 5.42; N, 9.42; S, 10.36.

Biological test-data. — Antitumor screening *in vivo* of compound **10** (NSC 115963) in the murine L-1210 assay showed T/C 147 at 400 mg/kg, and 150 at 600 mg/kg; it was essentially inactive in the P-388 screen (T/C 105 at 200 mg/kg); *in vitro* testing against human epidermoid carcinoma of the nasopharynx (KB), and against L-1210 and P-388 lymphocytic leukemia cells showed ED_{50} values of 3.0×10, 2.0×10, and 1×10^2, respectively. Compound **12** (NSC 234236) was neither active nor toxic in the *in vivo* L-1210 screen (T/C 99 at 200 mg/kg); *in vitro* tests in the KB and P-388 screens showed ED_{50} values of $> 1.0 \times 10^2$.

For several of the compounds, testing *in vitro* for growth inhibition was performed with L-1210 cells (A), HeLa cells (B), *Streptococcus faecalis* cells (C), and *Escherichia coli* K_{12} cells (D). The values given are molar concentrations for ID_{50}: **3**, A 5.2×10^{-5}, B 1.3×10^{-4}; **5**, A $> 10^{-4}$, B 10^{-4}; **6**, A $> 10^{-4}$, C $> 10^{-3}$, D $> 10^{-3}$; **7** A $> 10^{-4}$, B 10^{-4}; **8**, A $> 10^{-4}$, B 10^{-4}; **9**, A $> 10^{-4}$, B 10^{-4}; **10**, A $> 10^{-4}$, C 4×10^{-4}, D 4×10^{-5}; **11**, A $> 10^{-4}$, B 10^{-4}; **13**, A $> 10^{-4}$, B 10^{-4}; and **14**, A $> 10^{-4}$, and B 10^{-4}.

ACKNOWLEDGMENTS

The authors thank Dr. H. B. Wood, Jr., and the staff of the Division of Cancer Treatment, NCI, for the *in vivo* test-data, Dr. A. Bloch of Roswell Park Memorial Institute for the cell-culture and antibacterial determinations, Dr. T. D. Sokoloski of the Department of Pharmacy at The Ohio State University for use of the spectropolarimeter, and Drs. A. Ducruix and C. Pascard-Billy of the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, for performing the crystal-structure analysis of compound **6**.

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