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ACYCLIC-SUGAR 6-CHLOROPURINE NUCLEOSIDE ANALOGS DERIVED FROM D-ALDOHEXOSES HAVING THE D-*erythro* STEREOCHEMISTRY AT C-4 AND C-5*

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ABSTRACT

1-(6-Chloropurin-9-yl)-1-*S*-ethyl-1-thio-D-mannitol, 1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-D-altritol, and 1-(6-chloropurin-9-yl)-3-deoxy-1-*S*-ethyl-1-thio-D-*arabino*-hexitol were prepared by a new method of direct coupling of an acyclic-sugar derivative to the base. The coupling products were obtained as pairs of 1′-epimers. The epimeric mixture of D-*manno* derivatives was resolved by liquid chromatography, and the products were fully characterized. Glycosylation at N-9 of the purine ring was established by u.v. and ^{13}C-n.m.r. spectroscopy, and the chirality at C-1′ by circular dichroism measurements. The conformations of the acyclic-sugar chains in solution were also examined.

INTRODUCTION

The synthesis, characterization, and biological properties of acyclic-sugar nucleosides has been pursued in this laboratory as part of a long-standing, general interest in acyclic, carbohydrate derivatives[3]. The synthetic procedure developed has involved the conversion of acylated sugar dithioacetals into 1-bromo derivatives, and subsequent condensation of these reactive α-halo thioethers with suitably activated purines or pyrimidines, to afford 1′-epimeric mixtures of acyclic-sugar nucleoside derivatives. Although technical aspects of this procedure have been greatly improved, difficulties were encountered in scaling up the reported[1] preparation of (*1S*)-2,3,4,5,6-penta-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-D-glucitol (**5S**). Furthermore, the bromination reaction is not effective with 2-deoxy sugar derivatives[2], presumably because of changes in the electronic environment at C-1 caused by the absence of an electron-withdrawing acyloxy group at C-2. Therefore, it was considered desirable to develop a procedure that would give more-consistent results and be of broader applicability. We now describe a new, simplified method of coupling to purines in

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For previous, related papers, see refs. 1 and 2.

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which the activation of neither the sugar nor the purine base (by bromination and chloromercuration, respectively) is required.

Complete, structural identification of the products requires firm proof of the position of attachment of the sugar chain to the heterocycle, as well as the stereochemistry at C-1′ of the acyclic-sugar chain. Earlier work[4] left undefined the C-1′ chirality, and the site of glycosylation was not always unambiguously established. These questions have been considered in detail[3] and more-recent work has utilized as examples[1,5,6] several compounds whose structures have been established by X-ray crystallographic analysis, in order to provide key reference points for correlation of the structural framework and stereochemistry at C-1′ in various series of related derivatives.

For characterization of conventional nucleosides, ^{13}C-n.m.r. spectroscopy has been found a very useful tool [7–9], and we now report its application in determination of the site of glycosylation in acyclic-sugar nucleosides.

The purpose of the preparation of acyclic-sugar nucleosides reported here was to provide compounds for the study of their cyclization by microbiological oxidation. The structural requirements[10] for a substrate to be oxidized by *Acetobacter suboxydans* may be expressed as $HOH_2C—\underset{HO}{\overset{H}{C}}—\underset{HO}{\overset{H}{C}}—$. D-Glucose, D-mannose, D-altrose, and 3-deoxy-D-*arabino*-hexose were chosen for use, because they all possess configurations that contain this structural feature, and thus should meet the requirements for functioning as substrates for the micro-organism.

RESULTS AND DISCUSSION

Synthesis. — Evidence that nucleoside coupling can be effected without prior formation of a metal salt of the purine is abundant in the literature. One important example is the work of Yamaoka *et al.*[11], in which they successfully coupled purine bases with per-*O*-acylglycosyl halides in the presence of mercuric cyanide. Furthermore, there are also reports[2,12] of the preparation of nucleosides by direct condensation of dithioacetals with the mercury salt of a purine. In view of these results, it was considered that a protected aldose dithioacetal might react directly with a purine base to afford an acyclic-sugar nucleoside.

Direct condensation of equimolar amounts of 6-chloropurine with each of the acetylated dithioacetals (**1**–**4**) of D-glucose, D-mannose, D-altrose, and 3-deoxy-D-*arabino*-hexose took place in boiling nitromethane, in the presence of mercuric cyanide and yellow mercuric oxide plus a drying agent (calcium sulfate), to give the nucleoside adducts in yields ranging from 50 to 70%. This reaction was studied under a variety of conditions (see Table I), and it was found that, in boiling nitromethane, it is complete within 4 h. Prolonged heating did not improve the yield. Acetonitrile could also be used as the solvent in this reaction, but the reaction proceeded much more slowly than with nitromethane, and the yield was lower. As shown in Table I,

TABLE I

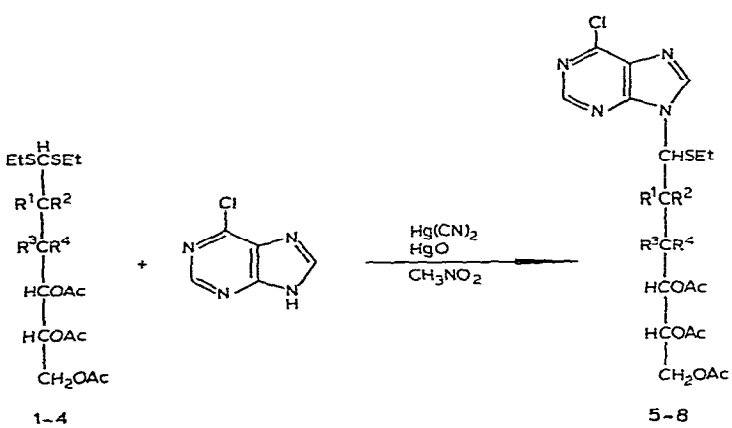
PREPARATION OF PERACETYLATED 1-(6-CHLOROPURIN-9-YL)-1-*S*-ETHYL-1-THIO-D-HEXITOLS OF THE *gluco* (5), *manno* (6), *altro* (7), AND 3-DEOXY-*arabino* (8) STRUCTURES

Product	*Acetylated sugar dithioacetal*		*6-Chloropurine*		*Reaction conditions*[a]	*Yield*		*Epimer ratio*[b]
	(g)	*(mmol)*	*(g)*	*(mmol)*		*(g)*	*(%)*	
5	5	10.08	1.38	8.92	5 h at 70° and 24 h at 100°	2.58	49.5	5:1
	5	10.08	1.56	10.08	6 h at 120°	3.51	59	
	0.5	1.01	0.08	0.51	4 h at 110°	0.42	70	
	0.25	0.504	0.156	1.01	6 h at 120°	0.22	75	5.2:1
	0.25	0.504	0.156	1.01	72 h at 100° (in CH_3CN)	0.18	61	
6	5	10.08	1.56	10.08	4 d at 120°	2.9	50	
	5	10.08	1.56	10.08	3 h at 80° and 3 d at 120°	3.33	56	2.7:1
	5	10.08	2	13.07	42 h at 120°	3.76	63	2.7:1
	0.25	0.504	0.156	1.01	5 h at 110°	0.13	45[c]	2.8:1
	0.25	0.504	0.156	1.01	5 h at 110°	0.20	68	
7	5	10.08	1.56	10.08	40 h at 120°	3.43	58	
	2.5	5.04	0.78	5.04	6 h at 120°	1.8	60	
	0.5	1.01	0.16	1.01	4 h at 110°	0.47	79	
8	5	11.42	1.56	10.08	6 h at 120°	2.9	54	4:1
	2.5	5.71	0.78	5.04	72 h at 120°	1.46	55	4.6:1

[a]In nitromethane, unless specified otherwise. [b]Determined by ^{1}H-n.m.r. spectroscopy. [c]Charring of the reactant was responsible for the low yield.

use of either reactant in large or slight excess did not give better yields than when equimolar amounts of reactants were used.

The products from the foregoing coupling-reactions were obtained as mixtures of two isomers differing only in configuration at C-1′. The ^{1}H-n.m.r. spectra of these



1,5: $R^1 = R^4 = H$, $R^2 = R^3 =$ OAc — D-*gluco*
2,6: $R^2 = R^4 = H$, $R^1 = R^3 =$ OAc — D-*manno*
3,7: $R^2 = R^3 = H$, $R^1 = R^4 =$ OAc — D-*altro*
4,8: $R^2 = R^3 = R^4 = H$, $R^1 =$ OAc — 3-deoxy-D-*arabino*

TABLE II

90-MHz, ^{1}H-N.M.R., CHEMICAL-SHIFT DATA FOR COMPOUNDS **5S**, **6R**, **6S**, **7R**, AND **8S**

Compound	*Solvent*	*Chemical shifts (δ)*							*Acetate*	*Ethyl-thio*	*H-2 H-8*
		H-1'	*H-2'*	*H-3'*	*H-4'*	*H-5'*	*H-6'*	*H-6"*			
5S[a]	$(CD_3)_2SO$–5% $CDCl_3$	5.93d	5.75t	5.38dd	5.56dd	5.05m	4.26dd	4.08dd	2.24s, 2.08s 2.01s, 1.98s 1.96s	2.63q 1.21t	8.88s, 8.82s
6R	$CDCl_3$	5.92d	5.31dd	5.63dd	5.42dd	4.98m	4.20dd	3.99dd	2.26s, 2.07s 2.03s, 2.01s	2.43q 1.14t	8.74s, 8.57s
6S	$CDCl_3$	6.13d	5.74dd	5.12dd	5.42dd	4.93m	4.20dd	3.98dd	2.12s, 2.09s 1.97s, 1.87s 1.84s	2.38q 1.26t	8.72s, 8.49s
7R	$CDCl_3$	6.05d	5.48–5.04m	5.72dd	5.48–5.04m		4.38–4.05m		2.12s, 2.09s 1.87s, 1.84s	2.47q 1.16t	8.69s, 8.45s
	$(CD_3)_2SO$	5.85d	6.03dd	4.94–4.87m	5.10dd	4.94–4.78m	4.18–3.85m		2.07s, 2.02s 1.99s, 1.85s 1.83s	2.44q 1.00t	8.91s, 8.82s
8S	$CDCl_3$	5.89d	5.17–4.87m	1.74–1.43m	5.57o	5.17–4.87m	4.19dd	4.01dd	2.04s, 2.02s 1.99s, 1.93s	2.39q 1.17t	8.76s, 8.50s

[a]From ref. 1.

chromatographically pure, syrupy, 1′-epimeric mixtures in chloroform-*d* were not fully interpretable, but did reveal the presence of two products, as shown by the doubling of the H-2, H-8 signals, of the ethylthio proton signals, and, especially, of the doublet for H-1′. For the D-*gluco* pair of 1′-epimers (**5**), the H-1′ signals are observed at δ 6.20 (minor) and 5.93 (major), with $J_{1',2'}$ 3.0 and 3.5 Hz, respectively. The corresponding H-1′ signals of the D-*manno* products (**6**) resonate at δ 5.92 ($J_{1',2'}$ 2.6 Hz, major) and 6.13 ($J_{1',2'}$ 4.5 Hz, minor), whereas those of the 3′-deoxy-D-*arabino* products (**8**) fell at δ 5.98 ($J_{1',2'}$ 3.8 Hz, major) and 5.89 ($J_{1',2'}$ 4.3 Hz, minor). The ratios of the 1′-epimers were determined from integration of these two signals (see Table I). The two H-1′ signals of the D-*altro* products (**7**) fell at δ 5.96 ($J_{1',2'}$ 3.0 Hz, minor) and 6.05 ($J_{1',2'}$ 5.7 Hz, major). These two sets of doublets were partially overlapped, making accurate measurements of the ratio impossible. The ^{1}H-n.m.r. data are listed in Table II.

Conventional t.l.c. and column chromatography are generally not effective for resolving the 1′-epimers, although there are instances[13] where 1′-epimeric pairs have been separated by loose-layer chromatography or fractional recrystallization, or both. However, crystallization of such a mixture from a suitable solvent has frequently given the major epimer pure. The major C-1′ epimer of **5**, in a syrupy mixture, crystallized from benzene or absolute ethanol[1]. This crystalline compound (**5S**) was unequivocally established as having the (1′*S*) configuration by X-ray crystallographic analysis of its deprotected analog. Similarly, in the present work, one epimer was successfully isolated crystalline from the syrupy, epimeric mixtures of **6**, and of **7**, by dissolution of the syrups in abs. ethanol and keeping the solutions at room temperature. These crystalline products (**6R** and **7R**) were the major epimers formed, as shown by their ^{1}H-n.m.r. spectra, which displayed only a single H-1′ signal, that of the major component in the original mixture. In contrast, all attempts to crystallize the syrupy product **8**, having the 3-deoxy-D-*arabino*-hexose sugar chain, failed. Interestingly, all coupling products of 2-deoxy-D-*erythro*-pentose and 2-deoxy-D-*arabino*-hexose with 6-chloropurine and 5-fluorouracil likewise gave[2] syrupy, inseparable mixtures of 1′*S* and 1′*R* epimers.

In the present study, isolation by crystallization generally afforded one 1′-epimer pure, and the remaining syrup was enriched in the other. Attempts to separate the epimeric pairs by liquid chromatography (l.c.) met with some success, as previously reported[1] with the 1′-*S*-methyl analog of **5**. The epimeric pair **6** (D-*manno*) was separated in a semipreparative, reverse-phase column (μBondapak C_{18}) eluted with 3:2 water–acetonitrile. Separation was achieved by recycling and "shaving" (see Fig. 1). The slower-eluting component (**6R**) was identical with the crystalline compound obtained directly from the mixture. The faster-eluting component (**6S**), obtained by recycling, was shown (^{1}H-n.m.r.) to be epimerically pure also.

Deacetylation of compounds **5S**, **6R**, and **7R** with methanolic ammonia afford the nonacylated nucleosides **9S**, **10R**, and **11R**, suitable for the cyclization study. When the 1′-epimeric mixture **8** was similarly deprotected, one of the resultant 1′-epimers crystallized out. This epimerically pure compound (**12S**) was then re-

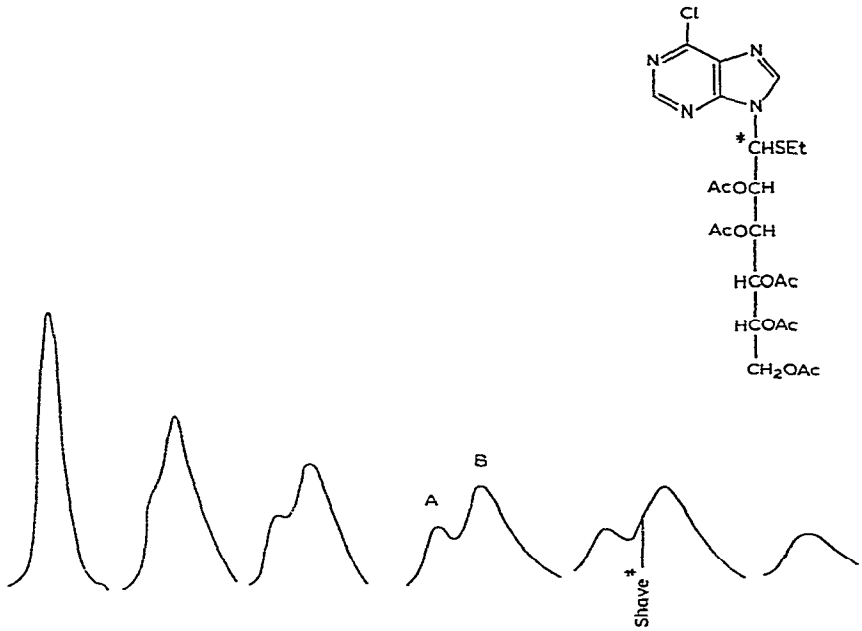
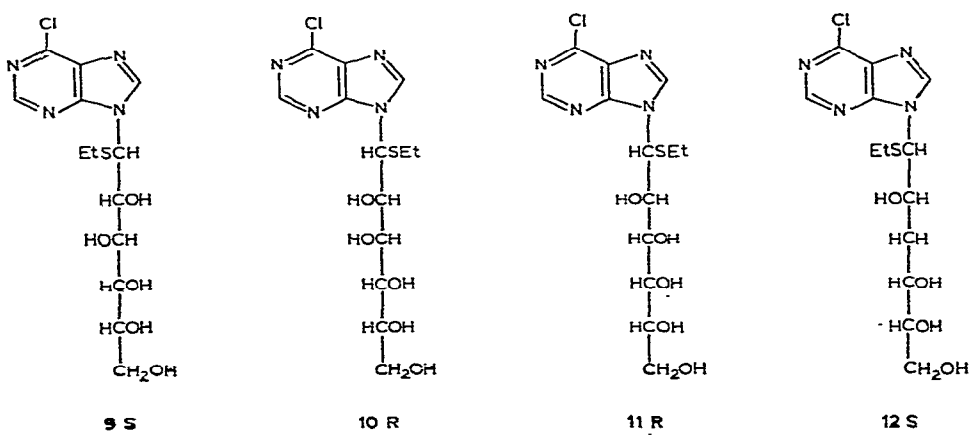


Fig. 1. Liquid chromatogram of the epimeric mixture of nucleosides 6: A = 6S, B = 6R. [Column μBondapak C_{18} (7.8 mm i.d. × 30 cm); 2:3 acetonitrile–water; 6 recycles; flow rate 1 mL/min; r.i. detector 32 × (—), u.v. 254 nm.]



acetylated, to give the 1′-epimerically pure tetraacetate **8S** for the purpose of physical characterization.

Position of attachment of the sugar chain to the purine base. — The u.v.-absorption maxima of **6**, **7**, and **8** (264.5, 265, and 266 nm, respectively) correspond closely to the literature values[14,15] for 9-alkyl-6-chloropurines. The u.v. spectra of the corresponding, deprotected nucleosides **10R**, **11R**, and **12S** were measured at three different pH values, namely 1, 7, and 12. The positions of the absorption maxima,

and their relative insensitivity to pH, are quite consistent with those observed[1] for (1*S*)-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-D-glucitol (**9S**) and 9-alkyl-6-chloropurines, and are at variance with those of 7-alkyl-6-chloropurines[16]. These data tend to support the assignment that the sugar chain is attached to the purine at N-9.

A ^{13}C-n.m.r.-spectral method for assignment of the site of glycosylation of nitrogen heterocycles had been developed[7], the methodology being based on reports by Pugmire and coworkers[8,9] that, in a nitrogen-heterocycle system, when the free pair of electrons on the nitrogen atom in the anion is protonated, an upfield shift for the carbon atom α to the protonated nitrogen, and a downfield shift for the β and γ carbon atoms, was observed. The large, upfield, α shifts and small, downfield, β shifts, termed the "protonation parameters", are also observed[9] in various heterocyclic base-systems when compared with their base anion.

To determine whether the effect of *N*-glycosylation in these acyclic-sugar systems is similar to that in the previously reported[7] five- and six-membered, and other, fused-ring systems, the ^{13}C chemical-shifts of **9S** were examined, and compared with those of the anion of 6-chloropurine, formed by treatment of 6-chloropurine with lithium hydroxide in Me_2SO-d_6. The site of glycosylation in **9S** has been firmly established by X-ray analysis[1]. The deacetylated nucleosides **10R**, **11R**, and **12S** were also studied. The results are summarized in Table III.

In compound **9S**, C-4 and C-8 are α to the glycosylated nitrogen atom (N-9), and C-5 is β-, and C-6 γ-, disposed. An associated, large, upfield shift of 8.44 p.p.m. for C-4, and of 6.15 p.p.m. for C-8, were observed. An upfield shift of 1.02 p.p.m.,

TABLE III

COMPARISON OF ^{13}C-N.M.R. CHEMICAL SHIFTS FOR THE ACYCLIC-SUGAR NUCLEOSIDES **9S**, **10R**, **11R**, AND **12S**

Compound	*Chemical shifts*[a]				
	C-2	*C-4*	*C-5*	*C-6*	*C-8*
6-Chloropurine anion (**I**)	149.03	159.80	131.35	145.87	152.67
(1*S*)-1-(6-Chloropurin-9-yl)-1-*S*-ethyl-1-thio-D-glucitol (**9S**)	151.36	151.36	130.33	148.94	146.52
(1*R*)-1-(6-Chloropurin-9-yl)-1-*S*-ethyl-1-thio-D-altritol (**11R**)	151.94	151.94	130.92	149.49	147.04
(1*R*)-1-(6-Chloropurin-9-yl)-1-*S*-ethyl-1-thio-D-mannitol (**10R**)	151.84	151.60	130.73	149.33	146.89
(1*S*)-1-(6-Chloropurin-9-yl)-3-deoxy-1-*S*-ethyl-1-thio-D-*arabino*-hexitol (**12S**)	151.26	151.86	130.20	148.76	146.39
$^{\Delta\delta}$**I** − **9S**	−2.33	+8.44	+1.02	−3.07	+6.15
$^{\Delta\delta}$**I** − **11R**	−2.91	+7.86	+0.43	−3.62	+5.63
$^{\Delta\delta}$**I** − **10R**	−2.81	+8.20	+0.62	−3.46	+5.78
$^{\Delta\delta}$**I** − **12S**	−2.23	+7.94	+1.15	−2.89	+6.28

[a]Shifts given in p.p.m. downfield from Me_4Si, in Me_2SO-d_6.

which contradicts the predicted[8] downfield β shift, was observed for C-5. This reversal in trend was expected[9] for bridgehead carbon atoms. Values for compound **9S** confirmed that the protonation parameters in this system are no exception to those already observed. The chemical shifts for **10R**, **11R**, and **12S** were also compared with that of the 6-chloropurine anion (**I**), and the resulting shifts were found very similar to that in the spectrum of **9S** (see Table III), thus establishing that the position of glycosylation is N-9 of 6-chloropurine, as in **9S**.

Optical properties and chirality at C-1'. — As the absolute stereochemistry at the C-1' chiral center of **9S** and its acetylated analog **5S** had been established[1] as (*S*), examination of the circular dichroism (c.d.) spectra should delineate the absolute configurations of the nucleosides prepared in this study, because of the close structural similarities in this series of compounds. The c.d. spectra were measured in the neighborhood of the u.v.-absorption maxima (~265 nm), from 350 to 220 nm, in methanol at room temperature. By simple comparison of these spectra, it is clear that the overall shapes of the spectra of **6R** and **7R**, although closely similar to each other, are roughly

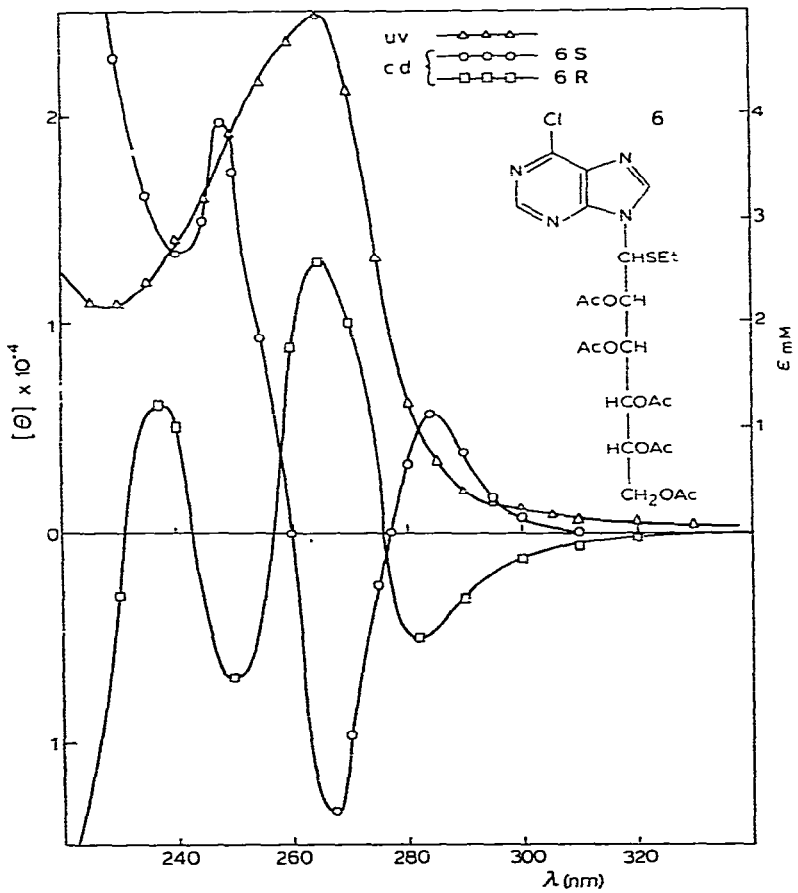
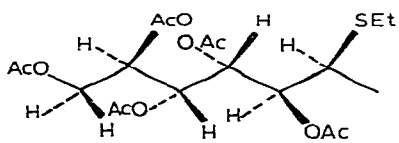


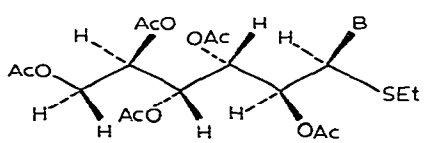
Fig. 2. U.v. absorption and c.d. spectra of **6R** and **6S**.

mirror images of that of **5S**. On the other hand, the spectra of **6S** and **8S** are essentially identical with that of **5S**. It may be concluded that **6S** and **8S** have the same relative configuration as **5S**, and are therefore assigned the absolute configuration (*S*) at C-1′, whereas **6R** and **7R** have the opposite, relative configuration, and are assigned as (1′*R*). As the free-hydroxyl acyclic-sugar nucleosidés give c.d. spectra similar to those of their fully acetylated counterparts, the same assignments are also made for compounds **10R**, **11R**, and **12S** as for their acetylated precursors. Fig. 2 shows the c.d. spectra of the epimeric pair **6R** and **6S**. Compound **6S** has $[\alpha]_D$ −94°, and **6R** has $[\alpha]_D$ +62° in chloroform. Among the compounds considered here, those having (1′*R*) chirality showed strong dextrorotation at the sodium D-line, whereas those having (1′*S*) chirality exhibited high levorotation at the same wavelength.

Conformations in solution. — The 90-MHz, ^{1}H-n.m.r. spectra of the D-*manno* compounds **6R** and **6S** in chloroform-*d* were nearly first-order. For **8R**, the observed $J_{2',3'}$ and $J_{4',5'}$ values (9.5 and 9.8 Hz, respectively) demonstrated the preponderance of the conformation having H-2′ and H-3′, and H-4′ and H-5′, antiparallel. The small values of $J_{1',2'}$ and $J_{3',4'}$ (2.6 and 1.7 Hz) indicate a gauche relationship between H-1′ and H-2′, and between H-3′ and H-4′. The conformation consistent with these vicinal coupling-data is a planar, zigzag arrangement of carbon atoms 1–6. The symbolism[17] **P** is assigned, in order to indicate that this conformer is virtually the exclusive form. No unfavorable, parallel 1,3-interaction[18] of acetoxyl groups is present in this arrangement.



D-*manno*-(1′/*R*)(6R-P)



D-*manno*-(1′/*S*)(6S-P)

In the spectrum of **6S**, the coupling data are also entirely consistent with the **P** backbone, *i.e.*, the extended, planar, zigzag arrangement advanced for **6R**. The large $J_{2',3'}$ and $J_{4',5'}$ couplings (8.3 and 8.6 Hz, respectively) and small $J_{3',4'}$ value (2.5 Hz) are similar to those for **6R** (see Table IV). The value of $J_{1',2'}$ (4.5 Hz) is, however, intermediate between that expected for the antiparallel and the gauche arrangements of H-1′ and H-2′. The former arrangement would have a parallel 1,3-interaction between the acetoxyl group on C-3′ and the ethylthio group on C-1′. For **6S**, there may be substantial, simultaneous population of both the H-1′–H-2′ antiparallel and the one gauche conformer illustrated, or, alternatively, the H-1′–H-2′ dihedral angle may be substantially altered from the nominal, 60°, gauche relationship, to relieve the gauche interaction between the base and C-3′.

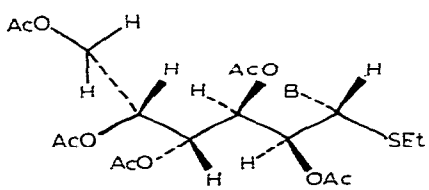
TABLE IV

PROTON–PROTON SPIN-COUPLING DATA FOR COMPOUNDS 5S, 6R, 6S, 7R, AND 8S

Compound	*Solvent*	*First-order couplings (Hz)*							
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{3',4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
5S[a]	$(CD_3)_2SO$–5% $CDCl_3$	4.7	6.1	3.5		7.0	3.0	5.3	12.5
6R	$CDCl_3$	2.6	9.5	1.7		9.8	2.9	4.4	12.3
6S	$CDCl_3$	4.5	8.3	2.5		8.6	3.5	4.9	12.6
7R	$CDCl_3$	5.7	3.5	6.0					
	$(CD_3)_2SO$	8.5	2.1	7.3		4.4			
8S	$CDCl_3$	4.3		3.2	5.3	10.6	3.8	7.2	11.6

[a]From ref. 1.

For the D-*altro* derivative **7R**, the coupling data measured (Me_2SO-d_6) were $J_{1',2'}$ (8.5), $J_{2',3'}$ (2.1), $J_{3',4'}$ (7.3), and $J_{4',5'}$ (4.4 Hz). Although no information was secured on the couplings between H-5′ and H-6′,6″, the data are sufficient to indicate the conformation between C-1′ and C-5′. The two large couplings ($J_{1',2'}$ and $J_{3',4'}$) establish the antiparallel relationship between H-1′ and H-2′ and H-3′ and H-4′, whereas the small $J_{2',3'}$ value (2.1 Hz) indicates the gauche relationship between H-2′ and H-3′. The intermediate value of $J_{4',5'}$ (4.4 Hz) indicates that there is rotation about the C-4′–C-5′ bond, as expected because of the presence of an unfavorable 1,3-interaction between O-3′ and O-5′ in the planar, zigzag conformation. This ${}_4G^{\mp}$ conformation, which is free from parallel 1,3-interaction, may be regarded as virtually the exclusive form. The approximate conformation of **7R** is depicted as follows.



D-*altro*-(1′/*R*) (7*R*–${}_4G^{+}$)

When the conformations, as determined by ^{1}H-n.m.r. spectroscopy, of **5S**, **6R**, **6S**, and **7R** are compared with those of the corresponding, peracetylated aldose diethyl dithioacetals[19], striking similarities between them are observed. Both **5S** and D-glucose diethyl dithioacetal pentaacetate adopt a sickle conformation (${}_2G^{-}$) having C-1 exoplanar; **6R**, **6S**, and D-mannose diethyl dithioacetal pentaacetate assume the same, extended, zigzag conformation (**P**). The same applies to **7R** and D-altrose diethyl dithioacetal pentaacetate, as evidenced by the conformational identity of their C-1–C-5 fragment (${}_4G^{+}$). Furthermore, the same conformations are also adopted by their corresponding alditols, both in the solid state[20] and in solution[21]. These observed similarities in conformation may prove useful in predicting

the solution conformation of acyclic-sugar nucleosides, and in future designs to improve the biological activity; the possible link of biological activity with the chain configuration, and the resulting, conformational disposition, has been demonstrated[1,3,5].

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover "Unimelt" apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 recording polarimeter. U.v. spectra were recorded with a Cary 15 u.v. spectrophotometer, and i.r. spectra with a Perkin–Elmer Infracord spectrophotometer. X-Ray powder diffraction data give interplanar spacings in Å for CuKα radiation (camera diameter = 114.59 mm). Relative intensities were estimated visually; m, moderate; s, strong; v, very; w, weak. ^{1}H-N.m.r. spectra were recorded at 100 or 90 MHz with a Varian HA-100 or Bruker HX-90 spectrometer, respectively, at ~25°. All samples were dissolved in chloroform-*d*, with tetramethylsilane (Me_4Si) as the lock signal, unless noted otherwise. ^{13}C-N.m.r. spectra were recorded by Dr. C. Cottrell with a Bruker WP-80 spectrometer operating at 20 MHz in the Fourier-transform mode at ~25°. Chemical shifts are reported in p.p.m. relative to Me_4Si. C.d. spectra were recorded with a Durrum–Jasco, o.r.d.–c.d. spectrometer at room temperature for solutions in a 1-cm, optical cell. T.l.c. was conducted on silica gel 60-F-254 (E. Merck). Preparative t.l.c. was performed on chromatoplates (200 × 200 × 2.5 mm) of silica gel 60 PF-254 (E. Merck) containing 1% of Lumilux Green 25. Silica gel 60 (E. Merck) was used for liquid-column chromatography, in a Waters Associates ALC-244 system equipped with a U6K injector, M-6000 pump, 440 u.v. detector, and R 401 refractive-index detector. Microanalyses were mainly made by W. N. Rond, with some analyses by Galbraith Analytical Laboratories, Knoxville, Tenn.

2,3,4,5,6-Penta-O-*acetyl-1-(6-chloropurin-9-yl)-1*-S-*ethyl-1-thio*-D-glycero-D-ido-*hexitol* [(*1S*)-*2,3,4,5,6-penta*-O-*acetyl-1-(6-chloropurin-9-yl)-1*-S-*ethyl-1-thio*-D-*glucitol*] (**5S**). — A mixture of penta-*O*-acetyl-D-glucose diethyl dithioacetal[22] (**1**; 5.0 g, 10.08 mmol), 6-chloropurine (1.56 g, 10.09 mmol), mercuric cyanide (2.55 g, 10.12 mmol), mercuric oxide (1.37 g, 6.34 mmol), calcium sulfate (1 g), and nitromethane (120 mL) was boiled under reflux for 4 h at 110°. The mixture was filtered hot, and the filtrate was evaporated under diminished pressure at 50°. The resulting syrup was extracted with hot chloroform, and the insoluble, yellow precipitate was filtered off. The filtrate was washed successively with 30% aqueous potassium iodide (three times) and water (three times), dried (sodium sulfate), and evaporated to a syrup. Elution with 1:1 benzene–ethyl acetate from a column (3.5 × 60 cm) of silica gel yielded syrupy **5** (3.6 g, 59%) as a 5:1 mixture of the (1′*S*) and (1′*R*) epimers.

The (1′*S*) epimer (**5S**) was isolated crystalline upon refrigeration of a solution of the foregoing syrup in abs. ethanol; yield 1.73 g (29%), m.p. 138°, $[\alpha]_D^{25}$ −105° (*c* 1.0, chloroform); {lit.[1] m.p. 137–138°, $[\alpha]_D^{25}$ −105° (*c* 1.0, chloroform)}; ^{13}C-

n.m.r. (chloroform): δ 14.14 (SCH_2CH_3), 25.54 (SCH_2CH_3), 59.10 (C-1′), 68.17 (C-5′), 61.62 (C-6′), 67.61, 69.43, 71.76 (C-2′,3′,4′), 131.78 (C-5), 143.62 (C-8), 150.99 (C-6), 151.18 (C-4), and 151.67 (C-2).

2,3,4,5,6-Penta-O-*acetyl-1-(6-chloropurin-9-yl)-1*-S-*ethyl-1-thio*-D-glycero-D-galacto-*hexitol* [*(1*R)-*2,3,4,5,6-penta*-O-*acetyl-1-(6-chloropurin-9-yl)-1*-S-*ethyl-1-thio*-D-*mannitol*] (**6R**). — A mixture of penta-*O*-acetyl-D-mannose diethyl dithioacetal[23] (**2**; 5.0 g, 10.08 mmol), 6-chloropurine (1.56 g, 10.09 mmol), mercuric cyanide (1.58 g, 6.27 mmol), mercuric oxide (1.33 g, 6.16 mmol), and nitromethane (100 mL) was boiled for 4 h at 110° under reflux. The mixture was then treated by the general isolation procedure described for the preparation of derivative **5**. The resulting syrup was eluted with 1:1 benzene–ethyl acetate through a column (2.5 × 200 cm) of silica gel, and the desired product **6** was obtained as a chromatographically homogeneous, pure syrup (3.76 g, 63%), whose ^{1}H-n.m.r. spectrum showed it to be a 2.7:1 epimeric mixture.

A solution of the light-yellow syrup in the minimum amount of abs. ethanol was kept at room temperature. Granular crystals formed after one day; yield 0.44 g (20%), m.p. 107–108°, $[\alpha]_D^{23}$ +61.2° (*c* 1.04, chloroform); R_F 0.38 (1:1 benzene–ethyl acetate); ν_{max}^{KBr} 2980 (C-H), 1750 (C=O of acetate), 1590, 1570 (purine ring), 1375, 1215, and 1050 cm^{-1} (C-O-C); ^{13}C-n.m.r. (chloroform): δ 13.97 (SCH_2CH_3), 26.23 (SCH_2CH_3), 60.08 (C-1′), 71.04 (C-2′), 68.18 (C-3′), 67.45 (C-4′), 67.79 (C-5′), 61.68 (C-6′), 131.70 (C-5) 144.35 (C-8), 151.39 (C-6), and 152.01 (C-4 and C-2); X-ray powder diffraction data: 3.82 w, 3.89 w, 4.11 m, 4.26 m, 4.43 s (1), 6.08 vw, 6.40 w, 6.60 w, 7.76 m (3), 8.88 s (2), and 11.21 w.

Anal. Calc. for $C_{23}H_{29}ClN_4O_{10}S$: C, 46.93; H, 4.97; N, 9.52; S, 5.44. Found: C, 46.96; H, 5.11; N, 9.49; S, 5.62.

2,3,4,5,6-Penta-O-*acetyl-1-(6-chloropurin-9-yl)-1*-S-*ethyl-1-thio*-D-glycero-D-talo-*hexitol* [*(1*S)-*2,3,4,5,6-penta*-O-*acetyl-1-(6-chloropurin-9-yl)-1*-S-*ethyl-1-thio*-D-*mannitol*] (**6S**). — The mother liquor from the preceding crystallization was further purified by liquid chromatography, to separate the (1′*S*) from the (1′*R*) epimer. The syrup (20-mg portions) was injected into a μ-Bondapak C_{18} column (30 cm × 7.8 mm i.d., Waters Associates) and eluted with 2:3 acetonitrile–water at a flow rate of 1 mL/min and at a pressure of 1500–2000 lb.in.$^{-2}$. The eluate was monitored both by u.v. absorbance (254 nm) and refractive index. Separation was achieved by recycling and "shaving". After six or seven recycles, the faster-eluting peak was collected. Removal of solvent by evaporation *in vacuo*, followed by freeze-drying, gave a syrup that was further purified by applying it to a preparative-t.l.c. plate (200 × 200 × 2.5 mm) of silica gel, which was developed twice with 2:1 hexane–ethyl acetate. The major zone was excised, extracted with chloroform, and the extract evaporated, to give pure **6S** as a homogenous syrup; yield 8 mg, $[\alpha]_D^{20}$ −93.9° (*c* 0.3, chloroform); R_F 0.38 (1:1 benzene–ethyl acetate); λ_{max}^{MeOH} 264.5 nm (ε_{mM} 5.00); ν_{max}^{KBr} 2980 (C-H), 1750 (C=O of acetate), 1590, 1570 (purine ring), 1375, 1215, and 1050 cm^{-1} (C-O-C); ^{13}C-n.m.r. (chloroform): δ 14.17 (SCH_2CH_3), 25.63 (SCH_2CH_3), 57.42 (C-1′), 70.40 (C-2′), 67.98 (C-3′), 67.42 (C-4′), 67.73 (C-5′), 61.67 (C-6′), 131.47 (C-5),

143.42 (C-8), 151.33 (C-6), 151.89 (C-4), and 152.08 (C-2). The mass spectrum of **6S** displayed a fragmentation pattern identical with that of **6R**.

Anal. Calc. for $C_{23}H_{29}ClN_4O_{10}S$ (588.1293). Found: 588.1309 (exact mass).

*2,3,4,5,6-Penta-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D*-glycero-*D*-gluco-hexitol* [*(1*R*)-2,3,4,5,6-penta-*O*-acetyl-1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D*-altritol*] (**7R**). — A mixture of penta-*O*-acetyl-D-altrose diethyl dithioacetal[19] (**3**; 5 g, 10.08 mmol), 6-chloropurine (1.56 g, 10.09 mmol), mercuric cyanide (1.58 g, 6.27 mmol), mercuric oxide (1.34 g, 6.21 mmol), calcium sulfate (1 g), and nitromethane (100 mL) was boiled under reflux for 5 h at 120°. By following the previously described procedure, a crude syrup was obtained that was applied to a column (4.5 × 80 cm) of silica gel, and eluted with 1:1 benzene–ethyl acetate. Fractions containing the desired product were pooled, and evaporated, to give **7** as a pale-yellow syrup (3.43 g, 58%). A solution of the syrup in the minimal amount of abs. ethanol was kept overnight at room temperature, inducing crystallization. The suspension was then refrigerated, and the crystals were filtered off, and dried, to give **7R** as a white solid; yield, 1.01 g (17%); m.p. 111.5–112.5°, $[\alpha]_D^{23}$ +104.2° (*c* 1.0, chloroform); R_F 0.36 (1:1 benzene–ethyl acetate); λ_{max}^{MeOH} 265 nm (ε_{mM} 7.50); ν_{max}^{KBr} 2980 (C-H), 1750 (C=O of acetate), 1580, 1540 (purine), 1375, 1220, and 1050 cm^{-1} (C-O-C); ^{13}C-n.m.r. (chloroform-*d*): δ 14.00 (SCH_2CH_3), 26.05 (SCH_2CH_3), 59.97 (C-1′), 61.40 (C-6′), 68.88, 69.05, 69.56, 70.51 (C-2′,3′,4′,5′), 131.59 (C-5), 143.75 (C-8), 151.36 (C-6), 151.53 (C-4), and 151.94 (C-2); X-ray powder diffraction data: 3.69 w, 3.90 s (3), 4.10 vw, 4.33 m, 4.65 s (2), 5.02 w, 5.51 vw, 5.79 w, 6.22 m, 7.03 w, 7.98 m, and 9.22 s (1).

Anal. Calc. for $C_{23}H_{29}ClN_4O_{10}S$: C, 46.93; H, 4.97; N, 9.52; S, 5.44. Found: C, 47.03; H, 5.06; N, 9.31; S, 5.34.

*3-Deoxy-*D-arabino-*hexose diethyl dithioacetal.* —This compound was prepared from methyl 3-deoxy-α-D-*arabino*-hexopyranoside[24] (9.04 g, 50.8 mmol) by shaking with ethanethiol (10 mL) and conc. hydrochloric acid (10 mL). The solution was diluted with methanol, made neutral with lead carbonate, the mixture filtered, the solid washed with methanol, and the filtrate evaporated to a syrup. A portion (3.5 g) thereof was applied to a column (2.5 × 100 cm) of silica gel. Elution with 4:1 chloroform–methanol gave the desired product, which immediately followed a yellow band. Both the pure syrup and the remaining crude syrup solidified to fine needles on standing; yield, 11.7 g (85%), m.p. 70–71° (lit.[24] reported a syrup), $[\alpha]_D^{24}$ −35.0° (*c* 1.1, methanol) {lit.[24] $[\alpha]_D^{18}$ −38.0° (*c* 2.1, methanol)}.

This compound was then acetylated by the conventional, acetic anhydride–pyridine method, to give the tetraacetate[24] **4**.

*2,4,5,6-Tetra-*O*-acetyl-1-(6-chloropurin-9-yl)-3-deoxy-1-*S*-ethyl-1-thio-*D*-arabino-hexitol* (**8**) (*C-1′ epimeric mixture*). — A mixture of 2,4,5,6-tetra-*O*-acetyl-3-deoxy-D-*arabino*-hexose diethyl dithioacetal[24] (**4**; 5.0 g, 11.42 mmol), 6-chloropurine (1.56 g, 10.09 mmol), mercuric cyanide (1.58 g, 6.27 mmol), mercuric oxide (1.33 g, 6.16 mmol), and nitromethane (120 mL) was boiled for 6 h under reflux with vigorous stirring at 110°. The hot mixture was filtered, and treated as before. The crude syrup

was applied to a column (3.5 × 80 cm) of silica gel, and eluted with 1:1 benzene–ethyl acetate. The fractions containing the product were pooled and evaporated, to afford an orange syrup; yield 2.90 g (54%). ^{1}H-N.m.r. spectroscopy showed that the syrup was an epimeric mixture in the ratio of 4.6:1; $[\alpha]_D^{29}$ +26° (*c* 0.6, chloroform); R_F 0.32 (1:1 benzene–ethyl acetate); λ_{max}^{MeOH} 266 nm (ε_{mM} 1.38); ν_{max}^{film} 2980, 2918 (C-H), 1750 (C=O of acetate), 1580, 1540 (purine), 1374, 1220, and 1050 cm^{-1} (C-O-C).

Attempts to separate the epimers by the l.c. conditions used for the separation of the epimeric pair **6** were unsuccessful, the epimers being only slightly resolved after several recycles.

*2,4,5,6-Tetra-*O*-acetyl-1-(6-chloropurin-9-yl)-3-deoxy-1-*S*-ethyl-1-thio-*D*-mannitol* [*(1S)-2,4,5,6-tetra-*O*-acetyl-1-(6-chloropurin-9-yl)-3-deoxy-1-*S*-ethyl-1-thio-*D*-arabino-hexitol*] (**8S**). — A solution of compound **12S** (15 mg, 41 μmol) in dry pyridine (0.2 mL) was cooled in an acetone–Dry Ice bath, acetic anhydride (0.2 mL) was added, and the solution was kept overnight at 4°. Water was then added dropwise while the solution was kept in the acetone–Dry Ice bath, and the solution was evaporated, finally under high vacuum. The resulting syrup was chromatographed on a plate of silica gel developed with 1:1 benzene–ethyl acetate, and the major band was extracted with chloroform. Evaporation of the extract gave a light-yellow syrup, yield 19 mg (87%).

The product possessed the same physical properties as mixture **8**, except for the following: $[\alpha]_D^{20}$ −74.3° (*c* 0.9, chloroform); ^{13}C-n.m.r.: δ 14.19 (SCH_2CH_3), 25.65 (SCH_2CH_3), 61.12 (C-1′), 69.66, 71.67 (C-2′,4′), 32.50 (C-3′), 67.66 (C-5′), 61.78 (C-6′), 131.72 (C-5), 144.72 (C-8), 151.79 (C-6), and 152.12 (C-2 and C-4).

Anal. Calc. for $C_{21}H_{27}ClN_4O_8S$: C, 47.34; H, 5.13; Cl, 6.60; N, 10.56; S, 6.04. Found: C, 47.34; H, 5.45; Cl, 6.81; N, 10.29; S, 6.08.

*1-(6-Chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D*-glycero-*D*-galacto-hexitol* [*(1R)-1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D*-mannitol*] (**10R**).— Gaseous ammonia was bubbled for 30 min through a solution of compound **6R** (325 mg, 0.55 mmol) in methanol (20 mL) at 0°, and the solution was kept overnight at 4°, and then evaporated to dryness. The residual syrup was chromatographed on plates of silica gel, with 4:1 chloroform–methanol as the developer. The major zone was extracted with hot ethanol, and the extract evaporated, to afford a glass (180 mg, 86%); $[\alpha]_D^{23}$ +87° (*c* 0.4, water); R_F 0.54 (4:1 chloroform–methanol); $\lambda_{max}^{H_2O}$ (at pH 7) 263 (ε_{mM} 5.20), (at pH 1) 263.5 (5.20), and (at pH 12) 263 nm (5.20); ν_{max}^{KBr} 3340 (O-H), 1900 (C-H), 1595, 1560 (purine ring), 1390, 1340, 1190, and 1075 cm^{-1} (C-O-H); ^{13}C-n.m.r. (Me_2SO-d_6): δ 14.55 (SCH_2CH_3), 24.83 (SCH_2CH_3), 63.13 (C-1′), 72.95 (C-2′), 70.18 (C-3′), 69.28 (C-4′), 71.32 (C-5′), 63.91 (C-6′), 130.73 (C-5), 146.89 (C-8), 149.33 (C-6), 151.60 (C-4), and 151.84 (C-2).

Anal. Calc. for $C_{13}H_{19}ClN_4O_5S$: C, 41.26; H, 5.07; Cl, 9.25; N, 14.82; S, 8.46. Found: C, 41.37; H, 5.05; Cl, 9.28; N, 14.58; S, 8.38.

*1-(6-Chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D*-glycero-*D*-gluco-hexitol* [*(1R)-1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-*D*-altritol*] (**11R**). — A solution of compound **7R**

(400 mg, 0.68 mmol) in methanol (20 mL) was cooled to 0° in an ice bath, gaseous ammonia was bubbled through for 30 min, and then the flask was stoppered, and refrigerated overnight. The solution was now evaporated, and the residual syrup was chromatographed on two plates of silica gel, with 4:1 chloroform–methanol as the eluant. The major band was isolated as a colorless syrup; yield, 0.25 g (97%); $[\alpha]_D^{23}$ +89° (*c* 1.1, water); R_F 0.47 (4:1 chloroform–methanol); $\lambda_{max}^{H_2O}$ (at pH 7) 263 (ε_{mM} 5.90), (at pH 1) 262.5 (5.90), and (at pH 12) 263 nm (5.90); ν_{max}^{film} 3300 (O-H), 1590 (purine ring), 1400, 1340, and 1180 cm^{-1} (C-O-H); ^{13}C-n.m.r. (Me_2SO-d_6): δ 14.55 (SCH_2CH_3), 24.94 (SCH_2CH_3), 62.37 (C-1′), 63.00 (C-6′), 71.11 (C-2′), 71.54 (C-3′), 71.98 (C-4′), 73.34 (C-5′), 130.92 (C-5), 147.04 (C-8), 149.99 (C-6), and 151.94 (C-2,4).

Anal. Calc. for $C_{13}H_{19}ClN_4O_5S$: C, 41.26; H, 5.07; Cl, 9.25; N, 14.82; S, 8.46. Found: C, 41.41; H, 5.35; Cl, 9.13; N, 15.16; S, 8.53.

*1-(6-Chloropurin-9-yl)-3-deoxy-1-*S*-ethyl-1-thio-*D*-mannitol* [*(1S)-1-(6-chloropurin-9-yl)-3-deoxy-1-*S*-ethyl-1-thio-*D*-arabino-hexitol*] (**12S**). — A sample of **8** (epimeric mixture, 400 mg) was deacetylated by the procedure described for **10R**, and the resultant solution was evaporated to a thin syrup that was chromatographed on a preparative t.l.c. plate of silica gel, with 4:1 chloroform–methanol as the developer. The major zone was extracted with ethanol. Evaporation of the extract afforded a light-yellow syrup (200 mg, 74%) that crystallized from methanol, to yield **12S** (40 mg, 15%). Several recrystallizations from methanol gave a white solid; m.p. 138–139°, $[\alpha]_D^{29}$ −95° (*c* 0.3, methanol); R_F 0.61 (4:1 chloroform–methanol); λ_{max}^{MeOH} 265 nm (ε_{mM} 18.10;) ν_{max}^{KBr} 3310 (O-H), 2910 (C-H), 1580, 1500 (purine ring), 1390, 1340, 1190, and 1075 cm^{-1} (C-O-H); ^{13}C-n.m.r. (Me_2SO-d_6): δ 14.38 (SCH_2-CH_3), 24.69 (SCH_2CH_3), 63.05 (C-6′), 64.40 (C-1′), 67.67, 68.49 (C-2′,4′), C-3′ signal overlapped with that of the solvent, 74.81 (C-5′), 130.20 (C-5), 146.39 (C-8), 148.76 (C-6), 151.26 (C-2), and 151.86 (C-4).

This compound was fully characterized as its acetylated derivative **8S**.

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