THIO SUGARS: STEREOCHEMICAL QUESTIONS AND SYNTHESIS OF ANTIMETABOLITES

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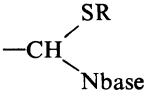
ABSTRACT

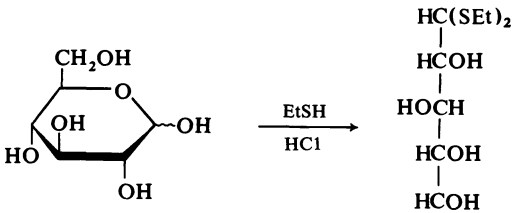
Dithioacetals of sugars provide useful starting materials for synthesis of various deoxy sugar derivatives by way of polythio intermediates, and also afford convenient access to acyclic aldose derivatives having two different substituents at C-1. The conformations of aldose diethyl and diphenyl dithioacetals in solution, as determined by p.m.r. spectroscopy with the aid of lanthanide salts, follow the general principle of avoidance of parallel 1,3-interactions earlier advanced for peracylated derivatives. The introduction of additional alkylthio groups into the chain, as observed by Brigl, offers a useful route to thio sugars and deoxy sugars, but configurational assignment of the thio derivatives has proved difficult; the extreme readiness whereby 2-alkyl-2-thioaldoses undergo epimerization is a contributing factor that has been studied by use of deuterium incorporation and other procedures. The diethyl dithioacetal of 2-*S*-ethyl-2-thio-D-mannose, a compound whose conformation has been studied comparatively by p.m.r. spectroscopy in solution and by x-ray crystallography in the solid state, is readily converted in high yield into ethyl 2-*S*-ethyl-2-thio-α-D-mannofuranoside. The latter compound, also obtainable by nitrous acid deamination of the diethyl dithioacetal of 2-amino-2-deoxy-D-glucose, constitutes a convenient starting material for synthesis of 2′-thio and 2′-deoxyfuranosyl nucleosides, certain of which display *in vivo* tumor-inhibitory activity. Other tumor-inhibitory nucleoside analogues, having an acyclic sugar chain, are obtained from protected aldose dithioacetals by halogenation and subsequent coupling with a derivative of a heterocyclic base, followed by removal of protecting groups. The biological activities of the products show variation according to the stereochemistry of the acyclic chain. These acyclic sugar nucleosides, which have the



functionality at C-1, are frequently obtained as pairs of C-1 epimers, and the assignment of stereochemistry at this position is discussed, together with determination of the favored conformation of the acyclic chain.

1. INTRODUCTION

The reactions of sulfur-containing sugar derivatives have intrigued synthetic carbohydrate chemists ever since Emil Fischer[1] treated D-glucose with ethanethiol in the presence of aqueous hydrochloric acid and obtained the beautifully crystalline diethyl dithioacetal in high yield.



Fischer 1894

This reaction opens up the aldopyranose ring and gives an acyclic sugar derivative. The dithioacetals have proved to be exceptionally versatile as starting points for the synthetic manipulation of sugars in their acyclic form, and this field has been studied extensively by the late M. L. Wolfrom and his associates[2]. The kinetically controlled ring-closure of dithioacetals by the action of heavy-metal ions is a valuable route to furanoid sugar derivatives, and the reductive cleavage of sulfur provides a useful method for obtaining deoxy sugars.

Together with the many attractive synthetic possibilities presented by sugars containing bivalent sulfur, there are interesting potentialities in the field of carbohydrate biochemistry for analogues of carbohydrate metabolites in which one or more of the oxygen atoms are replaced by sulfur. For example, certain 1-thioglycosides are effective inducers, although not substrates, for the enzymes that catalyze hydrolysis of the corresponding oxygen glycosides. In other instances, thio sugars act as antimetabolites by inhibiting certain enzymes that require the normal sugar derivative in an essential metabolic step; such compounds are of interest both as biochemical probes for elucidating metabolic pathways and as potential therapeutic agents.

At the same time, the chemistry of thio sugars poses some particular synthetic problems and structural questions. Introduction of the dithioacetal function in aldoses is readily accomplished, but there is no general procedure for replacement of oxygen by sulfur at the secondary positions of the sugar. An individual investigation is usually necessary to establish a route for each particular example, and the position of substitution by sulfur in the product also needs to be proved. Another question of long standing concerns the stereochemical disposition of sulfur substituents at the secondary positions. A related stereochemical problem that has remained unsolved for many years is the assignment of configuration at C-1 in mixed aldose dithioacetals and their analogues; in other words, in acyclic sugar derivatives having two different substituents at C-1. Furthermore, there is the question of conformation: what is the static and dynamic conformational behavior of acyclic sugars in the form of their dithiocetals and how does this behavior relate to the properties and reactivity of acyclic sugars?

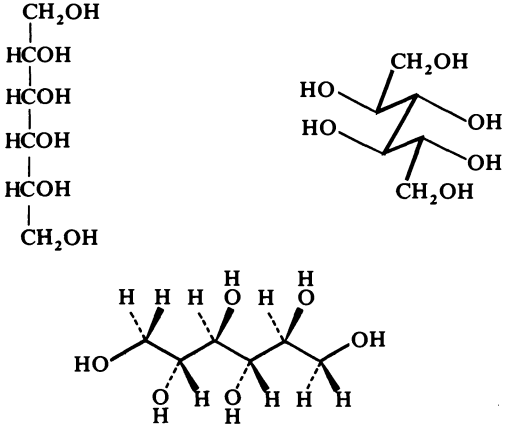
Questions such as these have prompted us to focus on several related areas of research during the past few years. This report is concerned with (a) the conformations of acyclic sugars. as exemplified by the dithioacetals, (b) assignment of stereochemistry in thio sugar derivatives having sulfur at a secondary position, (c) the use of thio sugars in deoxynucleoside syn-

thesis, (d) the synthesis of nucleoside analogues having the sugar chain acyclic, and (e) the assignment of chirality at C-1 in acyclic aldose derivatives having two different substituents at C-1.

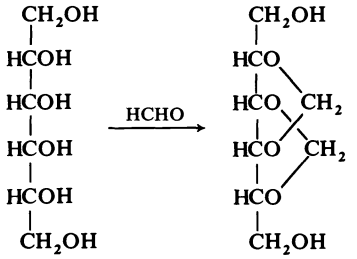
2. CONFORMATIONS OF ALDOSE DITHIOACETALS

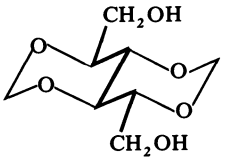
Several years ago we initiated a systematic study of the conformations of cyclic and acyclic sugars in solution, as revealed from proton–proton spin-couplings determined by nuclear magnetic resonance spectroscopy[3,4]. Particular emphasis was placed on securing data for complete configurational series so that all possible stereochemical variants could be compared. For acyclic sugar systems, the dithioacetals have been an important feature of these investigations. The C-1 substituent alters the magnetic environment of the protons along the chain and by dispersion of proton n.m.r. signals facilitates experimental observation of the individual signals. In contrast, such acyclic compounds as the alditols give a complex envelope of signals from which the various spin-coupling values are not easy to extract. For the alditols, it is thus difficult to make direct and detailed comparison of vicinal proton–proton couplings with the detailed crystallographic data determined by Jeffrey's group[5].

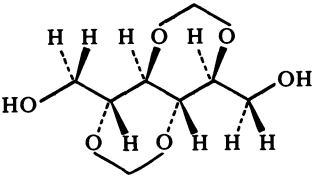
When this work was started, it was rather generally assumed that acyclic sugar molecules adopt fully extended conformations having an essentially planar, zigzag backbone of carbon atoms, as illustrated here for allitol from two different perspectives. This conformation results from application of the principle of maximum staggering of large groups along each carbon–carbon bond. This is an entirely reasonable postulate for a derivative such



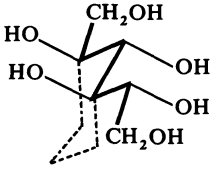
as 2,4:3,6-di-*O*-methyleneallitol[6], as the resultant bicyclic structure could adopt a *trans*-decalin type of ring system, as depicted on the following page by the same projections as in the preceding example. However, it should be borne in mind that, in the absence of the acetal bridges, there exists a parallel 1,3-interaction between two pairs of hydroxyl groups that is tantamount to having a pair of *syn*-diaxial substituents on a six-membered ring, as shown by the dotted, imaginary ring depicted on the following page.

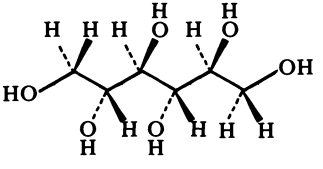






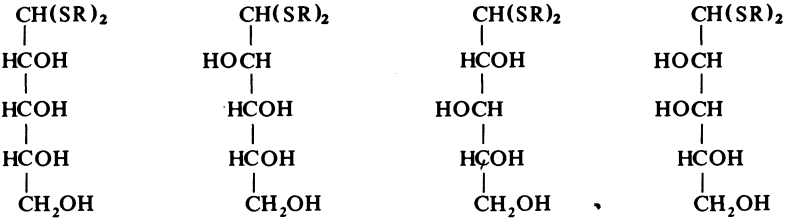
As such 1,3-interactions are normally regarded as highly unfavorable in the absence of restraint by a bridging group, the molecule might be considerably more stable in some other conformation in which this interaction is alleviated. This could be achieved by causing rotation along certain





carbon–carbon bonds to place carbon atoms in gauche, rather than anti-parallel, disposition so that the chain is bent into a so-called 'sickle' conformation instead of being extended as a planar, zigzag form. It was to test these hypotheses systematically that we examined the conformations of aldose dithioacetals and other acyclic sugar derivatives.

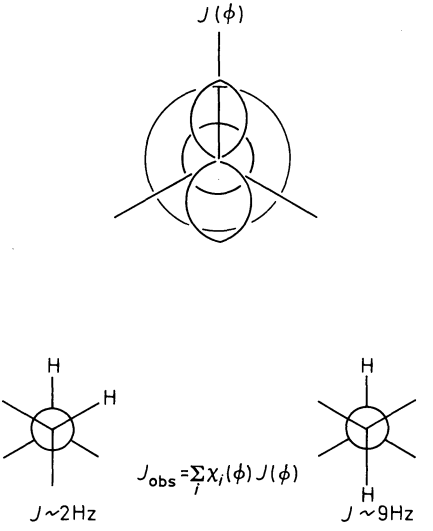
The early results have already been described in the literature[7], but some examples with the D-aldopentose dithioacetals serve to illustrate the principal conclusions. The four diethyl dithioacetals were obtained by the



R = Et
R = Ph

classic procedure, and the corresponding diphenyl analogues were also prepared without difficulty, notwithstanding the remark by Emil Fischer that he was unable to prepare diphenyl dithioacetals of sugars. For procedural reasons the peracetates were the first products to be examined, as they give n.m.r. spectra that are better-dispersed than those of the free hydroxyl analogues.

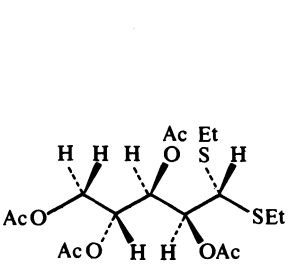
At the heart of the entire treatment is the assumption, based on a qualitative interpretation of the Karplus relationship, that small couplings (<3 Hz) between vicinal protons denote a gauche (~60°) disposition of these protons, whereas large couplings (> 8 Hz) signify vicinal protons that are oriented antiparallel (~180°) to each other. Energy barriers to rotation about single bonds are low, so that the observed couplings actually



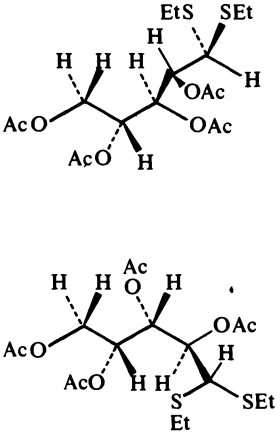
represent a population-weighted time-average of various conformational states in a mobile equilibrium. Vicinal coupling values of intermediate magnitude (between 3 and 8 Hz) reflect appreciable population of conformers having the protons in question in both the antiparallel and one or both gauche orientations. The method requires only the qualitative information that couplings are either 'small' or 'large' to assign the protons in question as 'gauche-disposed' or 'antiparallel', and no attempt is made to interpret

couplings in terms of exact dihedral angles. The soundness of this qualitative approach is verified independently by comparisons made with conformations determined by x-ray crystallography, as will be described later. The potential for misleading interpretations made by attempted quantitative treatment will also be brought out by these comparisons.

For the acetylated D-aldopentose diethyl dithioacetals and, indeed, for all of the numerous acyclic sugar systems examined, it is found that the molecule adopts a planar, zigzag conformation unless this would lead to parallel 1,3-interactions of substituent groups. When such an interaction would be present, the molecule actually assumes one or more 'sickle' conformations generated from the extended form by rotation about one or more carbon–carbon bonds to a different, staggered rotamer state. The first situation is exemplified by the D-*arabino* derivative, for which the spin-couplings observed ($J_{1,2}$ 8.3, $J_{2,3}$ 2.8 and $J_{3,4}$ 7.9 Hz) are in clear accord with the planar, zigzag conformation illustrated[7]. In contrast, for the



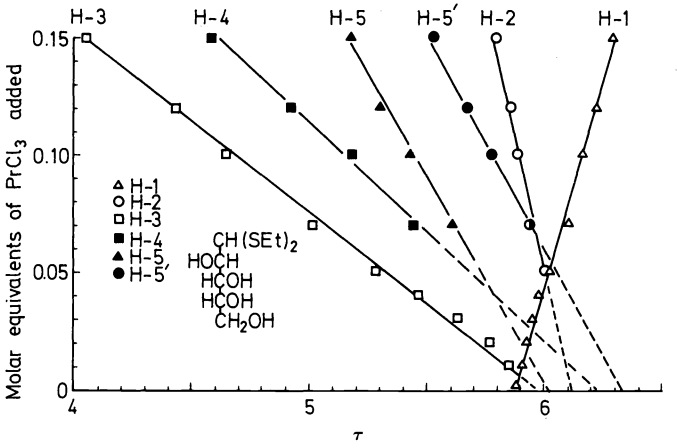
D-*arabino*
Planar zigzag



D-*xylo*
Sickle

D-*xylo* derivative, the couplings of $J_{1,2}$ 5.9, $J_{2,3}$ 5.9, and $J_{3,4}$ 4.2 Hz indicate a mixture of rotameric states; these are interpreted in terms of an interconverting, equilibrium mixture that includes substantial proportions of the two sickle forms illustrated. The major one of these is derived from the extended form by rotation about the C-2–C-3 bond, thus giving large $J_{1,2}$ and $J_{2,3}$ couplings. Some contribution from the one arising by rotation about C-3–C-4 is also evident.

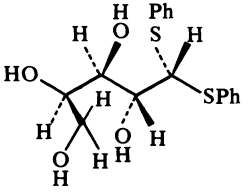
In extending this study to the non-acylated analogues, difficulties were encountered in obtaining spectra well enough resolved to permit extraction of all necessary coupling data, even with the assistance of computer-simulated spectra. Improved spectral dispersion was achieved, however, by adding such salts as praseodymium chloride to solutions of the dithioacetals in methanol-d_4 and mixtures of pyridine and methanol[8]. These results are illustrated diagrammatically for the diethyl dithioacetal of D-arabinose, and it can be seen that the individual proton signals, closely grouped together when no shift reagent is present, become progressively more spread out as



the reagent is added in proportionately greater concentration. The assignment of signals and the extraction of coupling constants is greatly facilitated by this procedure. It is noteworthy that signals of all protons except that of H-1 are shifted to lower field by the reagent.

It could be argued that the shift reagent may itself affect the conformation of the molecule, but the facts that the shift gradients are linear, and no changes in magnitudes of coupling constants were observed with progressive addition of shift reagent, suggest that this is not a significant factor, at least at the low relative concentrations of shift reagent used in these studies.

The conclusions reached for the non-acylated derivatives accord closely with those drawn for the acylated analogues. The destabilization by parallel, 1,3-substituents again emerges as a significant factor, causing molecules having such an interaction in their extended form to adopt sickle conformations in which this interaction is alleviated. Thus the two D-arabinose derivatives show couplings in clear agreement with the extended, zigzag conformation, whereas the couplings observed for D-ribose diphenyl dithioacetal (which would have a parallel 1,3-interaction between O-2 and O-4 in the extended conformation) are $J_{1,2}$ 1.6, $J_{2,3}$ 7.9, $J_{3,4}$ 3.6, and $J_{4,5}$ 2.6 Hz. These values are in agreement with preponderance of the conformation



D-Ribose Diphenyl Dithioacetal: Conformation in CD_3OD—C_5D_5N

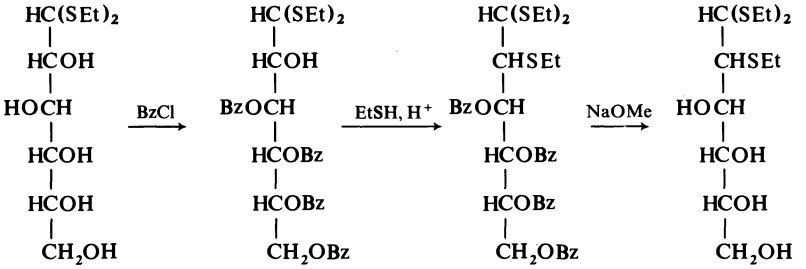
shown, derived from the planar, zigzag form by counterclockwise rotation about the C-3–C-4 bond to place O-4 in the position formerly occupied by C-5.

The correspondence between the conformational behavior of the free hydroxylated acyclic sugar derivatives and their acetylated counterparts

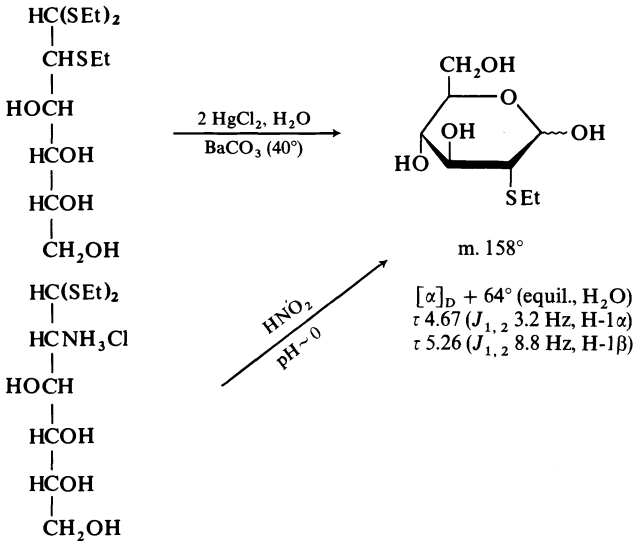
suggests that the principle of destabilization by parallel 1,3-interaction of non-hydrogen substituents should apply quite generally. Thus it may be a significant factor in influencing such behavior as kinetically controlled ring-closure in various chemical reactions, and interconversions in biological systems involving acyclic-sugar intermediates.

3. ASSIGNMENT OF STEREOCHEMISTRY IN THIO SUGAR DERIVATIVES HAVING SULPHUR AT A SECONDARY POSITION

In 1930, Brigl and Mühlschlegel[9] found that controlled benzoylation of D-glucose diethyl dithioacetal gave a tetrabenzoate unsubstituted at O-2. When this product was treated with excess ethanethiol and hydrogen cloride, the 2-OH group was replaced by an ethylthio group to give a tris(ethylthio) derivative that Brigl and colleagues[10] formulated as the diethyl dithioacetal of 2-*S*-ethyl-2-thio-D-glucose, although they advanced no firm evidence for the stereochemical assignment at C-2.



A year later, Brigl and Schinle[11] treated the saponified tris(ethylthio) derivative with aqueous mercuric chloride at 40°C in the presence of barium

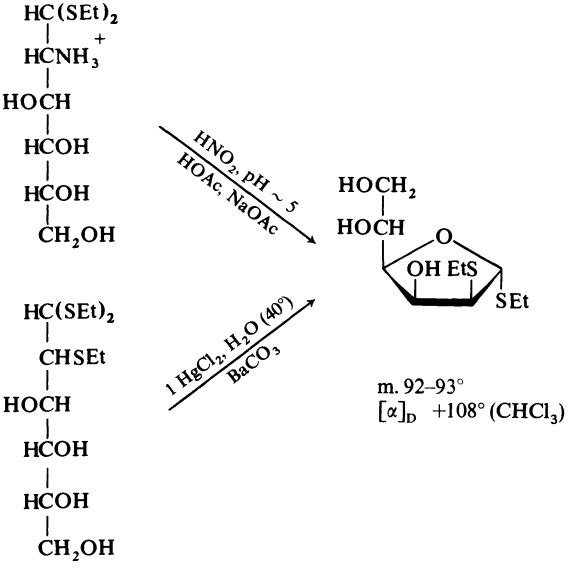


carbonate and obtained small prisms of a product melting at 158°C. They formulated this product, without evidence for the stereochemistry at C-2, as 2-*S*-ethyl-2-thio-D-glucose.

In a report[12] published in 1968 we showed that the nitrous acid deamination of the diethyl dithioacetal of 2-amino-2-deoxy-D-glucose in acid media gave rise to 2-*S*-ethyl-2-thio-D-glucose, whose stereochemistry was conclusively established by n.m.r. spectroscopy of the pyranose anomers and the β-tetraacetate. Our product was crystalline and identical with material obtained by repetition of Brigl's preparative conditions.

This result suggested that Brigl's trithio derivative was indeed the D-*gluco* product, even though mechanistic arguments involving participation of one of the 1-ethylthio groups in a 1,2-episulfonium ion type of intermediate, leading to the D-*manno* structure in both reactions, seem simpler than processes involving double inversions.

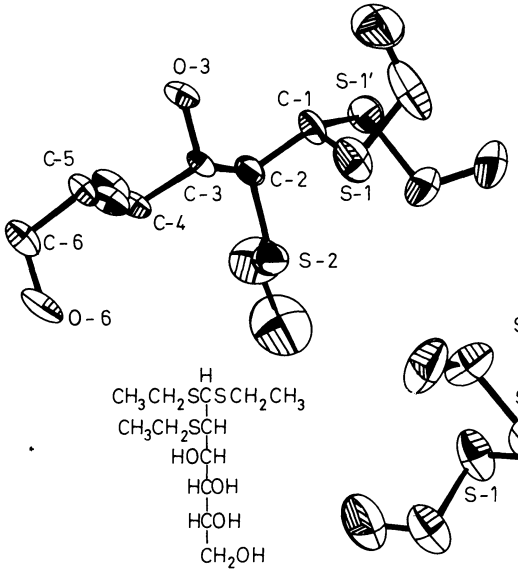
When the deamination reaction is conducted in acetic acid solution buffered with sodium acetate, the ethylthio sugar is not produced and there results instead a dithio derivative thought initially[13] to be the diethyl dithioacetal of 2,5-anhydro-D-glucose but later shown unequivocally by x-ray crystallography[14] and by chemical transformations[15] to be ethyl 2-*S*-ethyl-1,2-dithio-α-D-mannofuranoside. The identical product was obtained when Brigl's trithio derivative was treated with *one* mole of mercuric

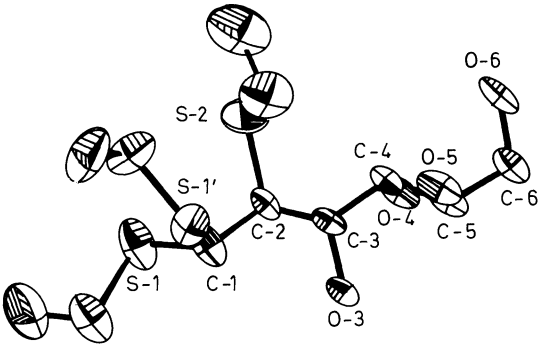


chloride[16]. This result clearly accords better with the idea that Brigl's compound was the D-*manno* derivative. In the deamination reaction, a mechanism involving a 1,2-episulfonium ion having the D-*manno* configuration, attacked at C-1 by O-4 to give the dithioglycoside, appeared the most plausible, but a similar ion suffering attack by solvent (water) in the aqueous, acidic medium should give 2-*S*-ethyl-2-thio-D-mannose and not the observed D-glucose derivative.

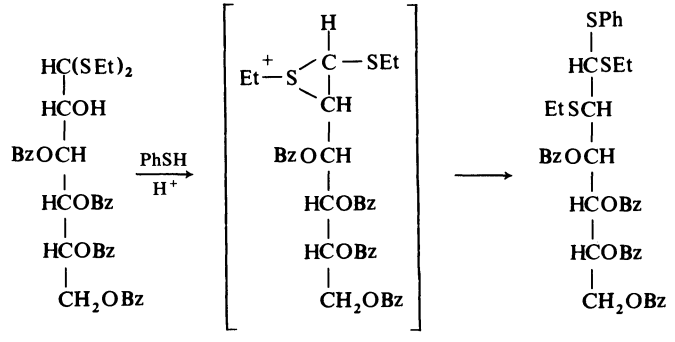
This apparent contradiction was resolved by studies on the desulfurization of the trithio compound under strictly controlled conditions, by studies on the formation of the trithio product with use of a different thiol and by x-ray crystallographic studies on Brigl's trithio derivative.

The crystallographic investigation decisively established[17] that Brigl's compound was the D-*manno* derivative, and the hypothesis of a *manno* 1,2-episulfonium ion intermediate leading to its formation thus appeared





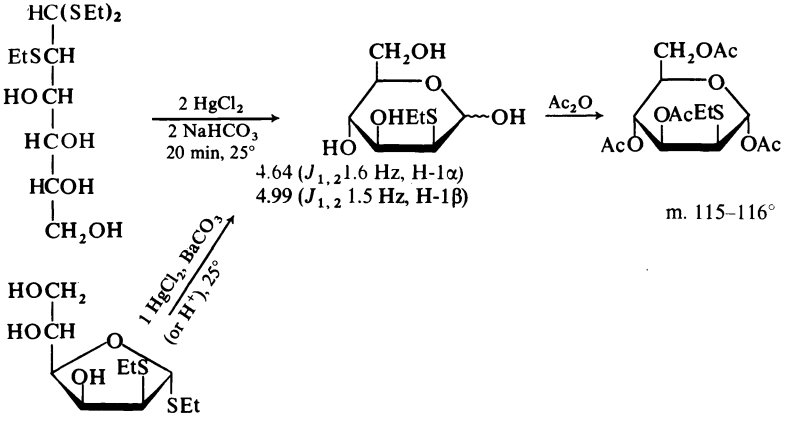
more credible. An experiment in which benzenethiol was used to introduce the third RS group showed that 1 → 2 migration of one ethylthio group undoubtedly occurs. An alternative mechanism involving direct intermolecular displacement of the protonated 2-hydroxyl group could be rejected. Possibly the 3-benzoyloxy group assists in the reaction through a 2,3-cyclic ion.



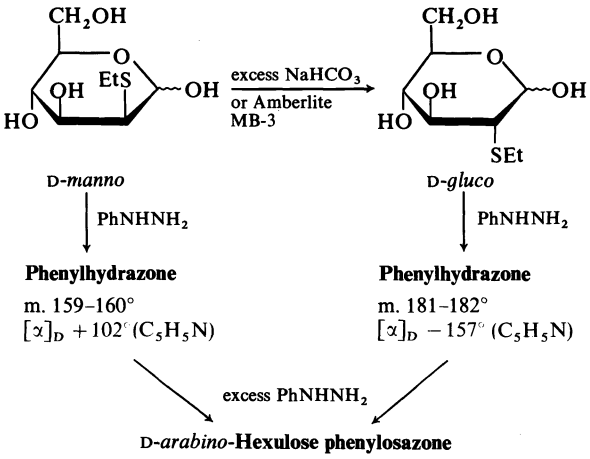
Detailed investigations of the desulfurization of the trithio derivative with two moles of mercuric chloride, or the dithioglycoside with one mole

of mercuric chloride, showed that the initial product was, in each instance, 2-*S*-ethyl-2-thio-D-mannose. However, this product was remarkably readily epimerized, under very mild basic conditions, to give the *gluco* analogue. This factor, together with the fact that 2-*S*-ethyl-2-thio-D-mannose has not thus far been obtained crystalline, explains why Brigl and co-workers obtained the crystalline product later identified as 2-*S*-ethyl-2-thio-D-glucose by desulfurization of the trithio compound. Likewise, the observed formation of the D-*gluco* product from the nitrous acid deamination of 2-amino-2-deoxy-D-glucose diethyl dithioacetal might result from C-2 epimerization during isolation of the initial product having the D-*manno* configuration.

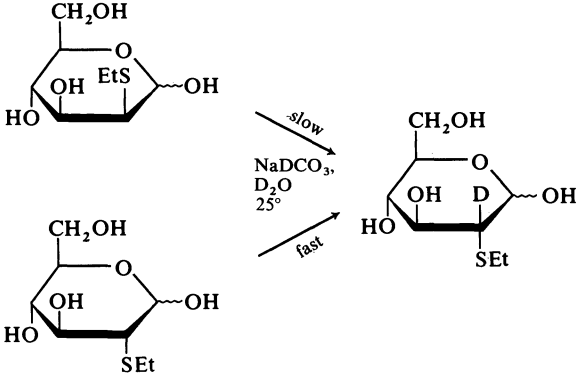
We re-examined the desulfurization of Brigl's trithio derivative under controlled conditions with an exact stoichiometric proportion of acid acceptor and were able to isolate the pure, syrupy 2-*S*-ethyl-2-thio-D-mannose in 92 per cent yield as an α,β mixture of pyranose anomers. Acetylation gave the crystalline α-tetraacetate. The n.m.r. spectra of these two pyranose products leaves no doubt about the D-*manno* configuration. The same syrupy 2-*S*-ethyl-2-thio-D-mannose was also obtained when the



dithioglycoside was carefully desulfurized with one mole of mercuric chloride or hydrolyzed by acid at room temperature. The use of elevated temperatures, extended reaction times, an excess of sodium hydrogen carbonate or anion-exchange resins in the hydroxide form (as present in mixed-bed resins used for demineralization) in every instance led to epimerization of the D-*manno* derivative into the D-*gluco* product. This transformation could be monitored by n.m.r. spectroscopy, by direct isolation of the crystalline D-*gluco* product, or by isolation of the distinctively different, crystalline phenylhydrazones of the two products in admixture. An aqueous solution of pure 2-*S*-ethyl-2-thio-D-mannose with one-quarter of its weight of sodium hydrogen carbonate, evaporated at 35° and the residue kept for 35 h at 25°C, gave 88 per cent of the crystalline 2-*S*-ethyl-2-thio-D-glucose. When the reaction was conducted in deuterium oxide with $NaDCO_3$, the D-*gluco* product was fully deuterated at C-2. The rate of deuterium incorporation paralleled the rate of epimerization of the D-*manno* derivative, and no substantial proportion of deuterated D-*manno* derivative was observed



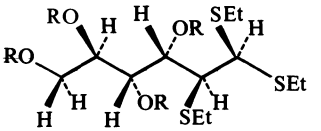
during the progress of the reaction. After 5 h, the mixture contained about 50 per cent of unlabelled starting material and 50 per cent of deuterated product. Starting from 2-*S*-ethyl-2-thio-D-glucose, deuterium incorporation was more rapid and was 50 per cent complete after one hour.



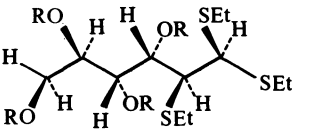
The composition of the final epimerization mixtures appears to depend on the particular reaction conditions, but general indications are that the D-*manno* product constitutes only about ten per cent of the equilibrated product mixture. Clearly, this ready epimerization under very mildly basic conditions, evidently resulting from increased acidity of H-2 as a result of the sulfur atom, needs to be kept in mind in interpreting the stereochemical behaviour of related thio sugar derivatives.

The n.m.r. parameters for solutions of the acyclic trithio derivative, 2-*S*-ethyl-2-thio-D-mannose diethyl dithioacetal, and its tetraacetate, interpreted by the same general methods as those earlier described, showed that H-1 and H-2 are in gauche-disposition, as are H-3 and H-4, whereas H-2 and H-3, as well as H-4 and H-5, are antiparallel. With these assignments, the favored conformation can be depicted as having a planar, zigzag backbone of carbon atoms. Of the three possible rotamers at C-1, that having H-1 and H-2 antiparallel can be ruled out because $J_{1,2}$ is small. One of the

two H-1–H-2 gauche rotamers can be excluded because it would place O-3 and one S-1 atom in a 1,3-parallel disposition. The assigned conformation, as depicted, lacks this destabilizing factor, although, as can be seen, it does have potential van der Waals destabilization from the fact that S-2 bisects



1a R = H
2a R = Ac



1b R = H
2b R = Ac

Table 1. PMR and crystallographic data for **1** and its tetraacetate (**2**)

Vicinal protons	PMR data (solution)[a] First-order couplings (Hz) 2	1	Couplings calculated for 1 by Karplus equation from x-ray data I[b]	II[c]	Dihedral angles (crystal) for 1 Molecule A (degrees)	Molecule B (degrees)
1,2	3.6	2.1	3.1	5.4	+64	+58
2,3	9.5	10.5	7.9	9.5	−171	−165
3,4	1.9	<1	2.2	3.2	−56	−58
4,5	7.6	6.6	8.2	9.2	−172	+172
5,6[d]	3.2	[e]	1.2	2.5	−61	−70
5,6′	5.3′	[e]	2.1	3.3	+63	+53

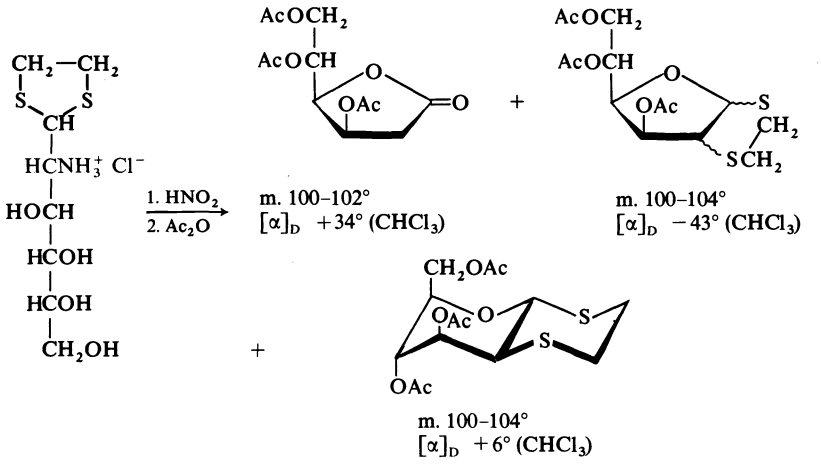
[a]220 MHz. [b]Uncorrected for electronegativity; cf. ref. 17. [c]Corrected for electronegativity; cf. ref. 17. [d]The proton on C-6 resonating at higher field is designated by a prime. [e]Unavailable, because of incomplete signal separation. For other vicinal protons, $\Delta\delta > 25$ Hz.

the angle of the two C—S bonds at C-1, bringing the sulfur atoms within their van der Waals contact distance. Nevertheless, the validity of the conformational assignments from the n.m.r. data for this molecule (and, by extension, for the other acyclic-sugar molecules studied) is securely confirmed by the crystallographic data for the non-acetylated compound in the solid state. The x-ray data show an essentially zigzag backbone chain and verify in particular the rotameric assignment at C-1, where the distances between S-2 and the two sulfur atoms at C-1 are indeed exceedingly short.

This direct correlation between crystallographic data and n.m.r. data for the same acyclic compound is particularly valuable, as it affirms the soundness of the general assumption that small vicinal couplings (< 3 Hz) indicate gauche-disposed protons and large (> 8 Hz) couplings signify vicinal protons that are preponderantly antiparallel. At the same time as the validity of n.m.r. data for *qualitative* conformational assignments to acyclic sugar derivatives is affirmed, the previous cautions we have expressed against using coupling data to assign precise dihedral angles are strongly reinforced. The use of Karplus-type relationships to determine angles purported to correspond to actual proton–proton dihedral angles can be dangerously misleading. *Table 1* shows the vicinal spin-couplings that could be calculated by the Karplus relationship from crystallographically determined dihedral angles. Comparison of these with the actual couplings measured in solution shows that there is reasonable *qualitative* agreement, enough to engender confidence in the general types of conformational assign-

ments made, but it is clear that attempts to specify vicinal dihedral angles to the nearest few degrees from n.m.r. data are quite unjustified. This point was earlier emphasized in work on a pyranoid sugar derivative[18] and recently on a furanoid system[19].

The nitrous acid deamination of the diethyl dithioacetal of 2-amino-2-deoxy-D-glucose provides, as we have already seen, a route for the preparation of either 2-*S*-ethyl-2-thio-D-glucose or ethyl 2-*S*-ethyl-1,2-dithio-α-D-mannofuranoside, according to the reaction conditions used. An interesting related reaction is that of the corresponding ethylene dithioacetal[20]. At pH 5.6, the reaction leads to three products in approximately equal proportions; these are 2-deoxy-D-*arabino*-hexonic acid (isolated as its acetylated 1,4-lactone) and two dithio derivatives. One dithio derivative is most probably 1,2-*S*-ethylene-1,2-dithio-α-D-mannopyranose, as determined by 250 MHz n.m.r. spectroscopy on its triacetate, and the other is a furanoid analogue,

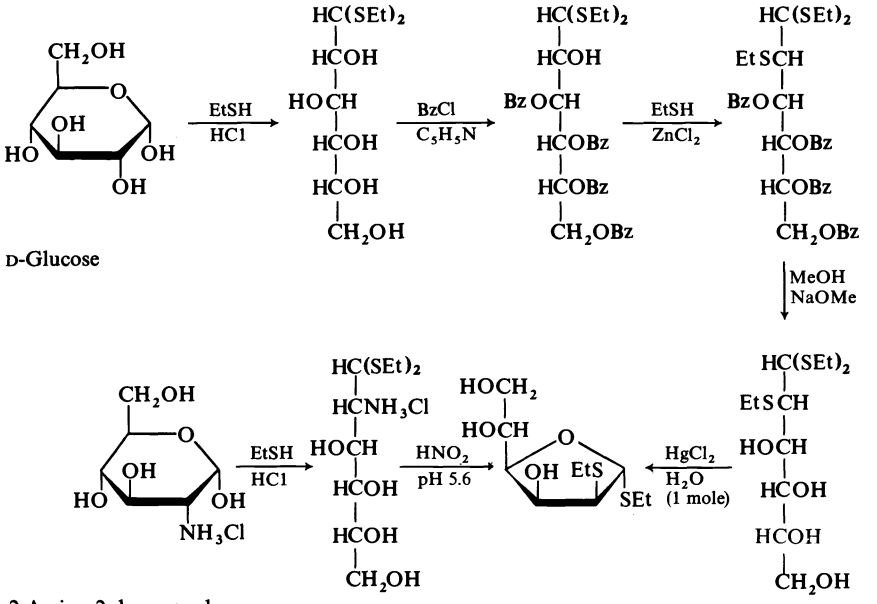


probably also having the α-D-*manno* stereochemistry. These products all probably arise from a common intermediate derived from the diazotization reaction; a 1,2-hydride shift followed by attack of water at C-1 and subsequent hydrolysis would lead to the deoxy acid or its 1,4-lactone, whereas a competing 1 → 2 migration of sulfur with attack at C-1 intramolecularly by either O-4 or O-5 would lead to the observed glycosides. At strongly acid pH, the deamination gives mostly the deoxy acid derivative and little of the glycosides, as might be expected from the decreased nucleophilicity of sulfur in acidic media.

4. USE OF 1,2-DITHIOGLYCOSIDES IN SYNTHESIS

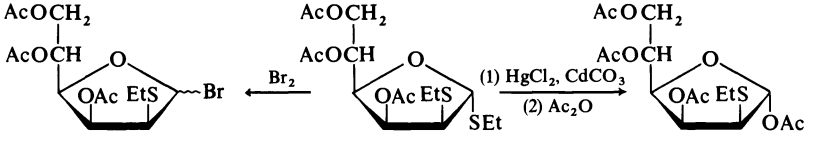
The 1,2-dithiofuranosides are attractive starting materials for synthesis, as the 1-alkylthio group can be selectively replaced as a route for attaching various substituents at C-1, and the C-2 position is then available for further modification. We have used this general procedure as a route to prepare nucleoside and 2′-deoxynucleoside analogues. For large-scale work we have found it most convenient to prepare 2-*S*-ethyl-2-thio-D-mannose

Synthetic Routes to Ethyl 2-*S*-Ethyl-1,2-dithio-α-D-mannofuranoside



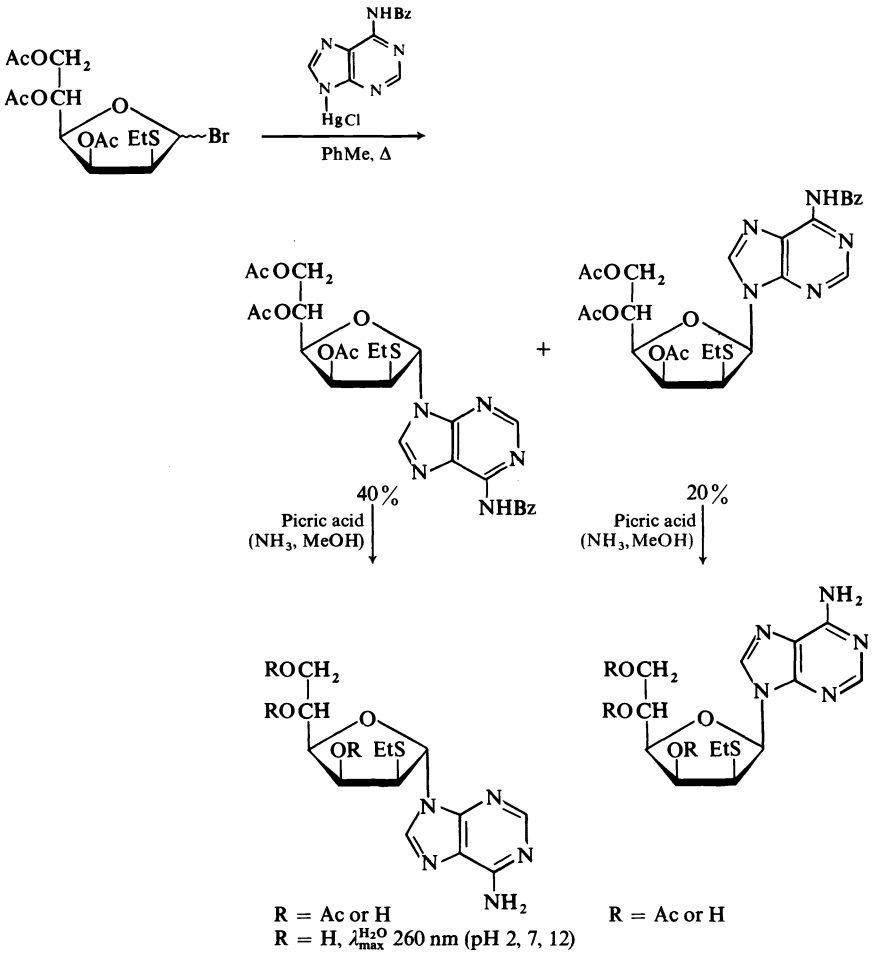
diethyl dithioacetal on a 5–10 molar scale essentially by Brigl's procedure, and then convert it by the action of one mole of mercuric chloride into ethyl 2-*S*-ethyl-1,2-dithio-α-D-mannofuranoside.

Two general types of approach to nucleoside structures were found useful[21]. In one of these, the acetylated dithiofuranoside was treated with

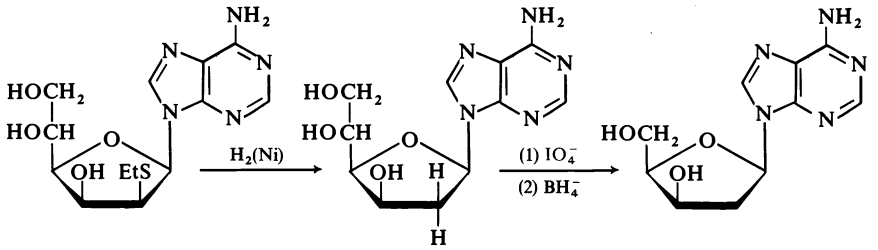


bromine in an inert solvent, a reaction that replaces the glycosidic ethylthio group by bromine but does not affect the 2-ethylthio group. The resultant glycosyl bromide was then used directly in a coupling reaction with the appropriate heterocyclic base. The other route involves the two-step replacement of the 1-ethylthio group by acetoxyl to give the crystalline α-acetate in good yield.

Condensation of the bromide with 6-benzamido-9-chloromercuripurine in boiling toluene gave an anomeric mixture of protected nucleosides from which 40 per cent of the pure α-anomer and 20 per cent of the pure β-anomer were separated, and these were converted into the free nucleosides by *N*-debenzoylation and subseqent deacetylation. The u.v.-spectral data for both nucleosides indicated that they were glycosylated at the 9-position, but their anomeric configurations were not readily assigned from n.m.r.-spectral

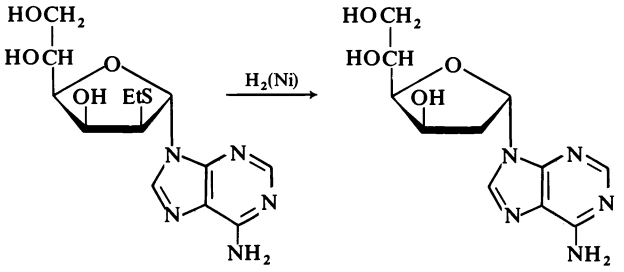


data, as both gave $J_{1,2}$ couplings of about 9 Hz, although the H-1 chemical shifts were different. Assignments of anomeric configuration from optical rotatory data are not always secure with nucleosides. However, the anomeric assignments were firmly established by degradative experiments. Desulfurization of the levorotatory anomer with hydrogen-saturated Raney nickel gave the corresponding deoxynucleoside, which showed unequal couplings of H-1 with the two protons at C-2. The other anomer gave a product in which the two $J_{1,2}$ couplings were equal. A sequence of periodate oxidation–borohydride reduction on the first anomer, to effect C-5′–C-6′ chain degradation of the molecule, gave the known[22] D-*threo* analogue of 2′-deoxyadenosine, thereby providing solid evidence for the structure of the precursors. The same steps could be effected in the reverse sequence, by initial C-5′–C-6′ degradation followed by desulfurization to give the deoxynucleosides, with the same known nucleoside resulting in the β-series. Yields in the desulfurization reaction, the most difficult step in the sequence,



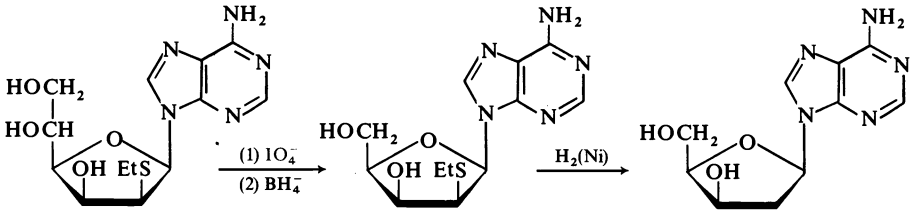
$J_{1,2} = 2.5$ Hz
$J_{1,2'} = 8.5$ Hz

m. 191–192°
(lit. m. 191–192°)



$J_{1,2} = J_{1,2'} = 5.0$ Hz

were 40–55 per cent and were achieved by using Raney nickel in refluxing *N*,*N*-dimethylformamide containing ten per cent of water.



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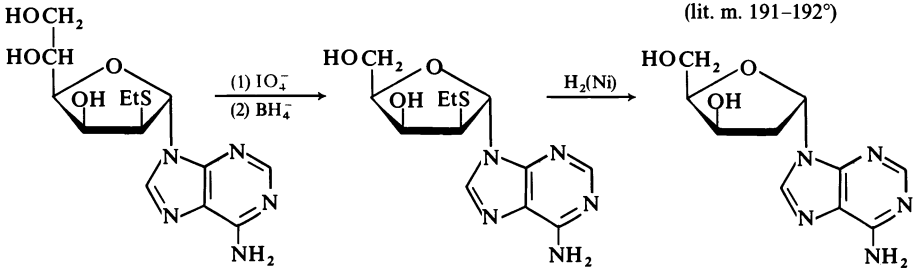
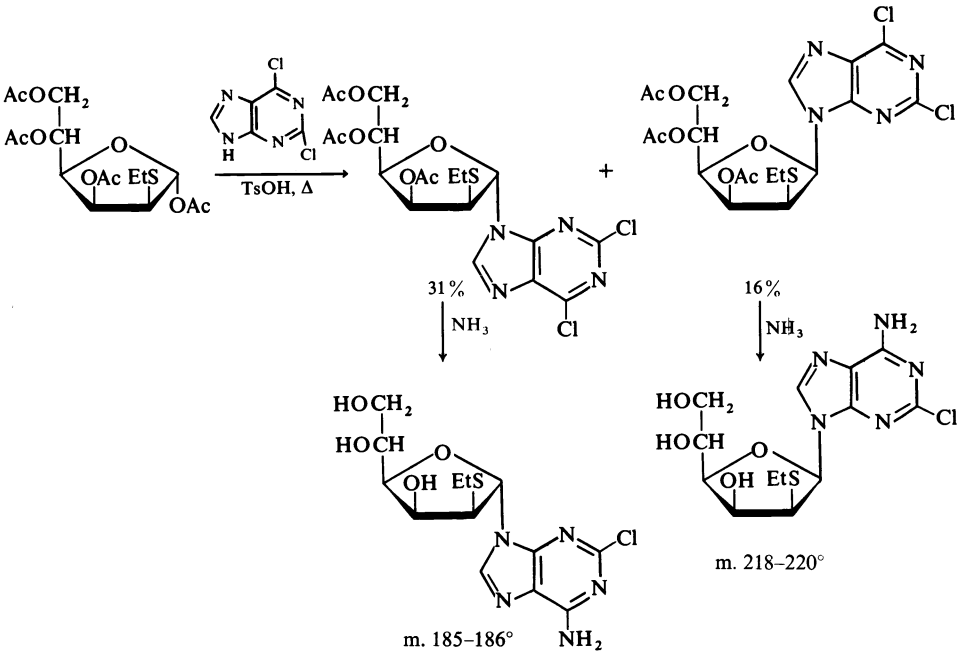
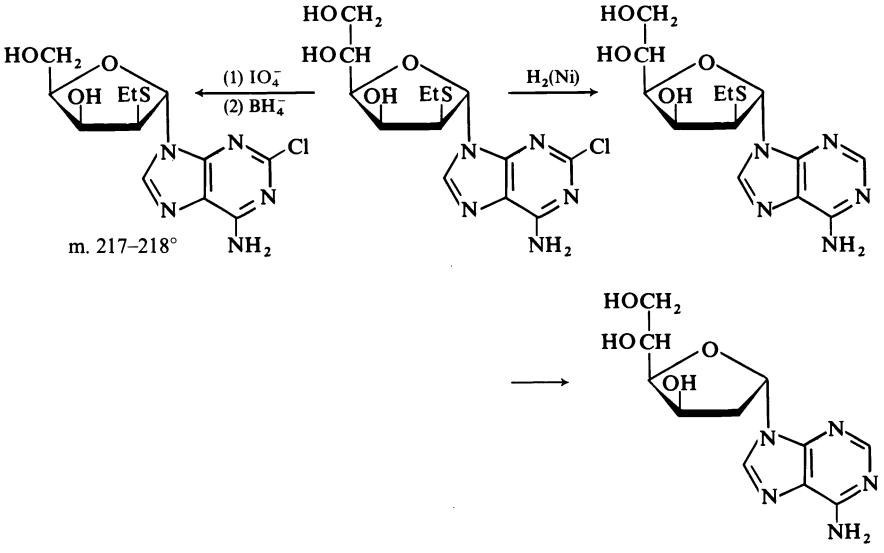


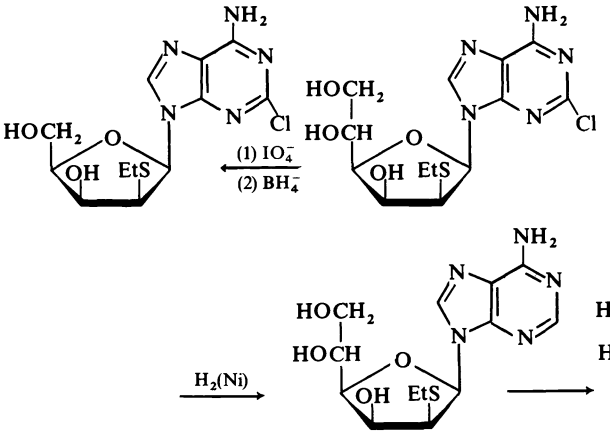
In the second approach, the 1-acetate already mentioned was subjected to condensation under fusion conditions with 2,6-dichloropurine. The α-nucleoside was obtained in 31 per cent yield and the yield of β-nucleoside was 16 per cent. Saponification with methanolic ammonia led to simultaneous amination at the 6-position. The structures assigned were again confirmed by degradation studies. Raney nickel reacts with both nucleosides



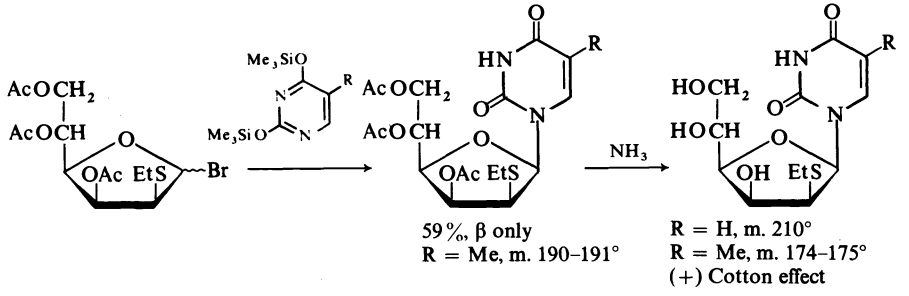
to effect initial dechlorination of the purine. This is followed, under more forcing conditions, by reductive cleavage of the ethylthio group. Again, the structure and configuration of the compound in the β-series could be assigned by direct comparison with the product obtained by way of the bromide.

These syntheses of purine nucleosides give both anomers, although the α-anomer is favored. In contrast, preparation of pyrimidine nucleosides

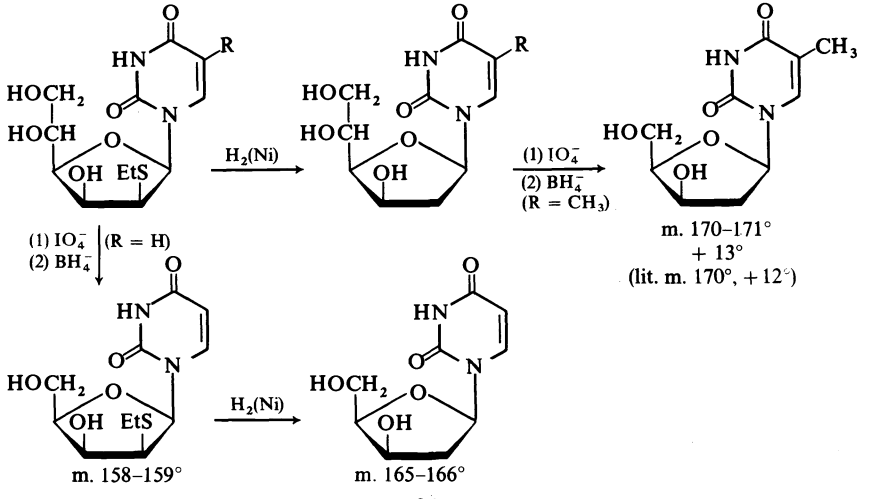




by using 2,4-bis(trimethylsilyloxy)pyrimidine or its 5-methyl analogue in reaction with the bromide gives only the β-nucleoside, isolated in about 60 per cent yield. Ammonolysis removed the protecting groups, and the

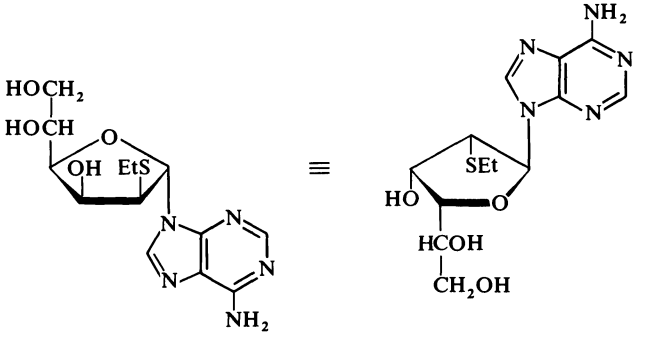


product showed a positive Cotton effect in its optical rotatory dispersion spectrum. The β-configuration assigned was confirmed by desulfurizing



at C-2′ and then degrading the C-5′–C-6′ side chain to the pentose analogue, to give (in the thymine series) a known compound[23].

Structurally modified nucleosides are of high interest as potential antitumor agents, acting as antimetabolites through interference with a necessary step in metabolism, the desired goal being a selective antimetabolite that inhibits more effectively a step in the metabolism of a cancer cell than in that of a normal cell. A number of nucleosides having significant antitumor activity have been found, and among these are several 2′-deoxynucleosides having the unnatural (α-D) anomeric configuration. The foregoing syntheses in the purine series favor the α-nucleosides and increase their availability for biological studies. Although the biological evaluation of these products is not yet complete, it is noteworthy that the adenine α-nucleoside still containing the 2′-ethylthio group shows *in vivo* activity in the mouse L-1210 leukemia assay[24]. The compound also shows *in vitro* growth inhibition against L-1210 cells in cell culture, and against some bacterial cell lines[25]. Although the α-nucleosides appear at first sight

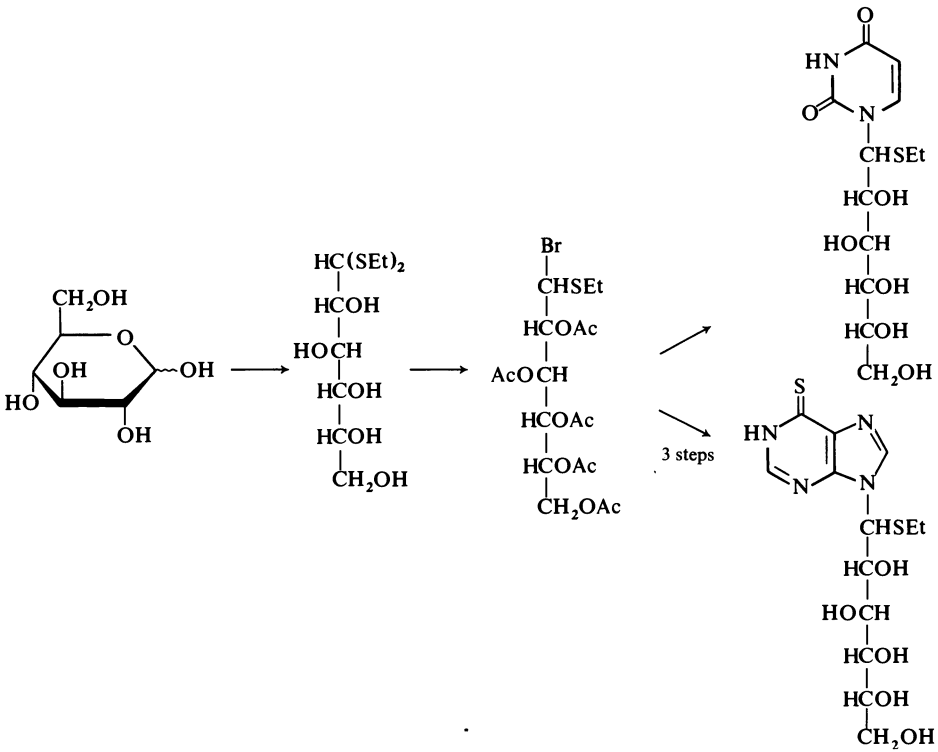


in vivo activity (mouse, L-1210 leukemia)	T/C 132 (400 mg/kg)
in vitro growth inhibition:	
L-1210 cell culture	40% at 10^{-4}M
Streptococcus faecalis	50% at 10^{-3}M
Escherichia coli K_{12}	50% at 5×10^{-4}M

to be sterically very dissimilar to their β-anomers, examination of molecular models from a different viewpoint reveals that the α-nucleoside can, in fact, sterically mimic a β-nucleoside quite closely.

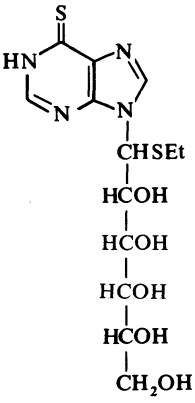
5. SYNTHESIS OF ACYCLIC SUGAR NUCLEOSIDES

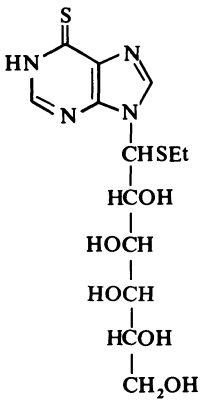
The last phase of this presentation is concerned with our work on the use of dithioacetals in the synthesis of nucleoside analogues in which the sugar chain is acyclic. The general approach, as illustrated in a synthesis starting from D-glucose, involves bromination of the acetylated dithioacetal to give the unstable monobromo derivative, which is at once condensed with the appropriate nucleoside-base derivative such as 6-chloro-9-(chloromercuri)purine or 2,4-bis(trimethylsilyloxy)pyrimidine. Removal of the protecting groups gives the acyclic sugar nucleoside derivatives; in the purine series the 6-chloro group is replaced by sulfur in this example to



give the corresponding 6-mercaptopurine derivative[26]. These syntheses have been conducted systematically to afford complete stereochemical series of products with the D-pentose precursors together with several D-hexose precursors, coupled with the bases 6-mercaptopurine[27], uracil[28], cytosine[29], adenine[30] and 5-fluorouracil[31]. By appropriate variations of the synthesis it is also possible to obtain the 1-alkoxy analogues of the 1-alkylthio compounds illustrated. Two stereoisomers, differing in configuration at C-1, are possible; some of the syntheses afford both products, whereas others lead to only a single isomer. For the total structural identification of the products it is necessary to establish definitively the position of attachment of the sugar chain to the heterocycle, to identify the chirality at C-1, to determine the tautomeric form of the heterocycle, and to establish the favored conformation of the molecule. Knowing that acyclic chains may favor non-extended conformations as a result of parallel 1,3-interactions, we speculated that some of these products could adopt folded conformations that might mimic part of the topology of a normal β-D-pentofuranosyl nucleoside. If this hypothesis is valid, there should be significant differences in biological activity between stereoisomers[32].

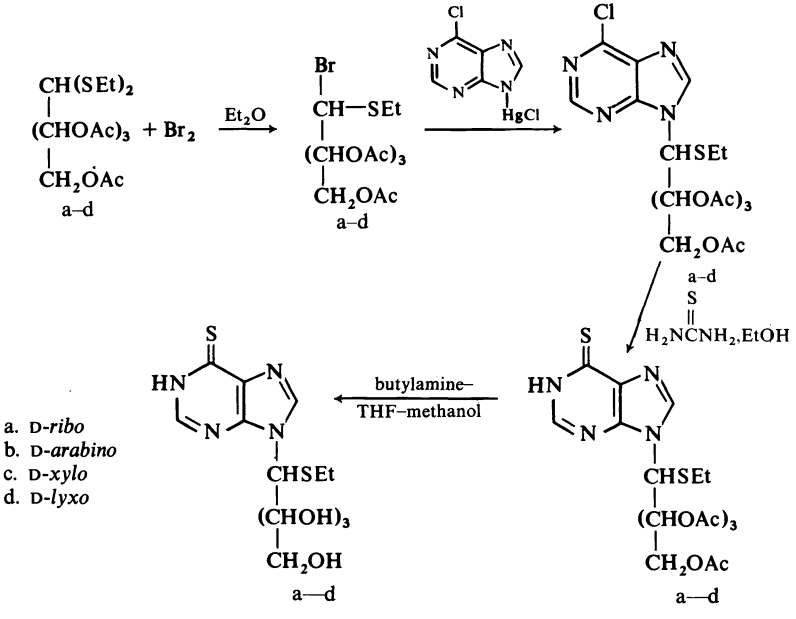
Comparing the biological activity of the acyclic 6-mercaptopurine nucleosides from the diethyl dithioacetals of D-glucose and D-galactose lends support to this hypothesis. The D-*gluco* compound shows significant *in vivo* activity in the L-1210 screen[24], whereas the D-*galacto* compound is inactive. Bacterial-inhibition studies[25] show that the D-*gluco* compound is





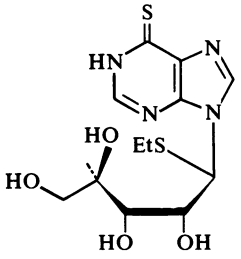
Organism	Molarity for 50% inhibition	
	gluco	*galacto*
Streptococcus faecalis	4×10^{-4}	10^{-4}
Escherichia coli K-12	4×10^{-5}	10^{-3}
Leukemia L-1210 cells (*in vitro*)	10^{-4}	10^{-4}
Leukemia L-1210 (mouse) (*in vivo*)	T/C 147 (400 mg/kg)	T/C 100 (200 mg/kg)

a more potent inhibitor of *Escherichia coli* K-12 than the D-*galacto* compound. If the sugar chain served merely as a water-solubilizing carrier for 6-mercaptopurine, a known antimetabolite, the activities should be essentially the same[32].



Synthesis of the 1-*S*-ethyl-1-[purin-9-yl-6(1*H*)-thione]-1-thio-D-pentitols

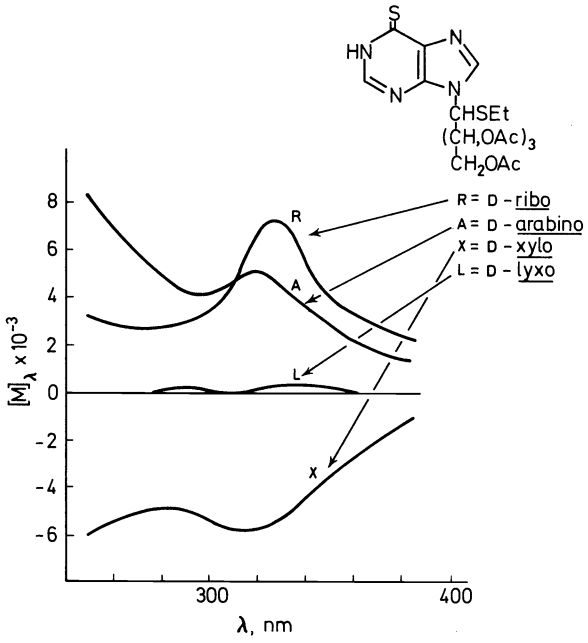
The D-pentose analogues of this structure were synthesized[27] by the same general method and obtained crystalline. The dextrorotatory D-*ribo* and D-*arabino* derivatives, and the levorotatory D-*xylo* analogue, were single epimers, as indicated by a single H-1 doublet in their n.m.r. spectra, whereas the D-*lyxo* derivative was a mixture of the two C-1 epimers. In the assay with *E. coli* K-12, the D-*ribo* derivative showed 50 per cent growth inhibition at 8×10^{-5} molar, whereas the other isomers were considerably less active[25]. It could be speculated that the D-*ribo* derivative might readily adopt



Possible folded conformation in the D-*ribo* derivative

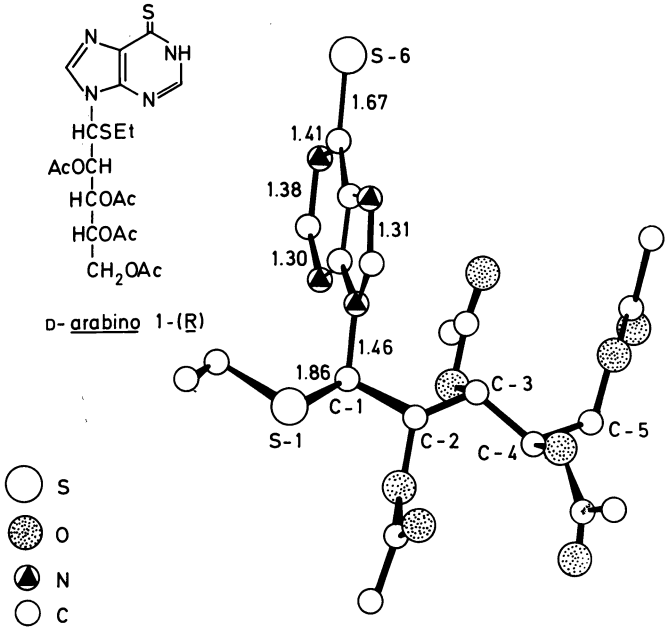
a folded type of conformation resembling the normal nucleoside structure. Data from n.m.r. spectroscopy on the acetates do confirm, for example, that the D-*arabino* derivative has the anticipated extended conformation, whereas the D-*ribo* derivative adopts a non-extended conformation.

The question of assigning chirality at C-1 is not readily resolved by chemical transformations. Optical rotatory methods based on the Generalized Heterocycle Rule[33] could be of value, provided that a suitable reference



compound of firmly established stereochemistry can be used to serve as a basis of reference. The optical rotatory dispersion spectra of the four pentose derivatives show that the D-*ribo* and D-*arabino* derivatives exhibit positive Cotton effect curves. The Heterocycle Rule would indicate these to have the 1-(*R*) configuration; similarly the 1-(*S*) configuration is indicated for the D-*xylo* compound from its negative Cotton effect.

A definitive assignment, which establishes a base of reference for the entire series, is provided by an x-ray crystallographic structure analysis of the acetylated D-*arabino* derivative[34]. The structure demonstrates unambiguously that the sugar chain is attached to N-9 of the heterocycle,



proves that the chirality at C-1 is 1-(*R*), shows from the C-6—S bond distance that the heterocycle is in the thiono tautomeric form and indicates an extended-chain conformation for the sugar chain, in good agreement with the n.m.r. spectral data. This structure determination permits correlations throughout the series, by chemical manipulation of the side chain and by optical rotatory methods, for the secure assignment of chirality at C-1 and the position of substitution on the heterocycle. At the same time, the conformations of the various series, including sets where all of the 1-(*R*) and 1-(*S*) isomers can be examined separately, can be delineated by n.m.r. spectral methods[3].

6. SUMMARY

1. Long-standing questions of stereochemical assignment in some 2-thio sugar derivatives have been resolved and the exceptional stereochemical lability of 2-thioaldoses in basic media has been demonstrated.

2. Acyclic sugar derivatives containing sulfur have featured prominently in a detailed analysis of the conformational behavior of acyclic sugars and their derivatives.

3. Thio sugar precursors have been used in syntheses of nucleoside antimetabolites of varied stereochemistry and conformation.

4. The chirality at C-1 has been established for various acyclic sugar derivatives having two different substituents at C-1.

REFERENCES

[1] E. Fischer, *Ber. Dtsch. Chem. Ges.* **27**, 673 (1894).
[2] M. L. Wolfrom, in W. Pigman and D. Horton (eds.), *The Carbohydrates*, Vol. IA, Chapter 10. Academic Press: New York (1972); See also J. D. Wander and D. Horton, *Advan. Carbohyd. Chem. Biochem.*, **32**, in press (1975).
[3] D. Horton, P. L. Durette, and J. D. Wander, *Ann. NY Acad. Sci.* **222**, 884 (1973).
[4] P. L. Durette and D. Horton, *Advan. Carbohyd. Chem. Biochem.* **26**, 49 (1971).
[5] G. A. Jeffrey and H. S. Kim, *Carbohyd. Res.* **14**, 207 (1970).
[6] R. M. Hann and C. S. Hudson, *J. Amer. Chem. Soc.* **66**, 1909 (1944).
[7] D. Horton and J. D. Wander, *Carbohyd. Res.* **10**, 279 (1969); **13**, 33 (1970).
[8] D. Horton and J. D. Wander, *J. Org. Chem.* **39**, 1859 (1974).
[9] P. Brigl and H. Mühlschlegel, *Ber. Dtsch. Chem. Ges.* **63**, 1551 (1930).
[10] P. Brigl, H. Mühlschlegel and R. Schinle, *Ber. Dtsch. Chem. Ges.* **64**, 2921 (1931).
[11] P. Brigl and R. Schinle, *Ber. Dtsch. Chem. Ges.* **65**, 1890 (1932).
[12] A. E. El Ashmawy, D. Horton, L. G. Magbanua, and J. M. J. Tronchet, *Carbohyd. Res.* **6**, 229 (1968).
[13] J. Defaye, *Bull. Soc. Chim. Fr.* 1101 (1967).
[14] J. Defaye, A. Ducruix, and C. Pascard-Billy, *Bull. Soc. Chim. Fr.* 4514 (1970).
[15] J. Defaye, T. Nakamura, D. Horton, and K. D. Philips, *Carbohyd. Res.* **16**, 133 (1971).
[16] B. Berrang and D. Horton, *Chem. Commun.* 1038 (1970); paper to be published.
[17] A. Ducruix, C. Pascard-Billy, D. Horton, and J. D. Wander, *Carbohyd. Res.* **29**, 276 (1973).
[18] P. W. R. Corfield, J. D. Mokren, P. L. Durette, and D. Horton, *Carbohyd. Res.* **23**, 158 (1972).
[19] W. Depmeier, O. Jarchow, P. Stadler, V. Sinnwell, and H. Paulsen, *Carbohyd. Res.* **34**, 219 (1974).
[20] P. Angibeaud, C. Bosso, J. Defaye, and D. Horton, *Abstr. Papers Amer. Chem. Soc. Meeting*, **168**, CARB-6 (1974); paper to be published.
[21] D. Horton and M. Sakata, *Carbohyd. Res.*, **39**, 67 (1975); *Abstr. Papers Amer. Chem. Soc. Meeting*, **165**, CARB-19 (1973); paper to be published.
[22] M. Ikehara, Y. Nakahara, and S. Yamada, *Chem. Pharm. Bull. (Japan)*, **19**, 538 (1971).
[23] K. V. Bhat and W. W. Zorbach, *Carbohyd. Res.* **6**, 63 (1968).
[24] Results courtesy of Dr H. B. Wood Jr and the Cancer Chemotherapy National Service Center, US Public Health Service.
[25] A. Bloch, personal communication.
[26] M. L. Wolfrom, H. B. Bhat, P. McWain, and D. Horton, *Carbohyd. Res.* **23**, 296 (1972).
[27] D. C. Baker and D. Horton, *Abstr. Papers Amer. Chem. Soc. Meeting*, **168**, CARB-64 (1974); paper to be published.
[28] D. Horton and S. S. Kokrady, *Carbohyd. Res.* **24**, 333 (1972).
[29] D. Horton, S. S. Kokrady, and J. D. Wander, to be published.
[30] D. Horton, S. S. Kokrady, and J. Defaye, *Abstr. Papers Amer. Chem. Soc. Meeting*, **163**, CARB-11 (1972).
[31] D. Horton and R. A. Markovs, to be published.
[32] A. Bloch in E. A. Ariens (ed.), *Drug Design*, p 353. Academic Press: New York (1973).
[33] H. El Khadem and Z. M. El-Shafei, *Tetrahedron Letters*, 1887 (1963).
[34] D. C. Baker, A. Ducruix, D. Horton, and C. Pascard-Billy, *Chem. Commun.*, 729 (1974).