STUDIES DIRECTED TOWARD THE DEVELOPMENT OF NEW C-NUCLEOSIDE SYNTHESES: SILVER (I) INDUCED EXTRUSION-REARRANGEMENT IN SULFUR BRIDGED NUCLEOSIDES.

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by

Michael Vernon Pickering, B.S., M.S.
Beaverton, Oregon
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CERTIFICATION OF APPROVAL

This thesis has been examined and approved.

Dr. G. D. Dayes, Jr., Thesis Advisor

Dr. E. J. Baum, Chairman, Exam Committee

Dr. T. M. Loehr

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ABSTRACT

A general discussion of the syntheses of C-nucleosides is presented. A proposed new reaction type which employs silver (I) in a sulfur extrusion-rearrangement is discussed.

Precedent for the new reaction type sought, one of metal induced sulfur extrusion with concomitant carbon-carbon bond formation between the termini of the sulfur bridge, may be found in the ease of complex formation between metals (e.g. Ag^{+1}, Hg^{+2}) and sulfide sulfur and also in the effective use, in cyclic systems, of Ni^{+2} to extrude sulfur with bond formation between the termini of the sulfur bridge.

The nature of this new type reaction for the synthesis of C-nucleosides is explored employing 3-(2'-tetrahydrofuranylthio)indole (8b) in reactions with anhydrous silver perchlorate. This study provided 2-(2'-tetrahydrofuranyl)indole (10b), a model C-nucleoside. A general mechanism for the silver (I) induced reactions of 3-(2'-tetrahydropyranylthio)indole (8a) and 3-(2'-tetrahydrofuranylthio)indole (8b) is proposed. It was concluded on the basis of this study that a second "holding" linkage should be introduced prior to reaction with Ag^{+1}.

Nucleophilic displacement reactions on derivatives of 3-bromopyrazolo[4,3-d]pyrimidin-7-one (21) and 3-(5-)-bromo-5-(3-)-methyl-4-nitropyrazole (26) employing sulfur nucleophiles are discussed. This

study provided 1,6-dibenzyl-3-(phenylthio)pyrazolo[4,3-d]pyrimidin-7-one (40) and 3-methyl-4-nitro-5-(2'-tetrahydropyranylthio)pyrazole (36) a model sulfur bridged nucleoside.

The reaction of S-(β-D-ribofuranosyl)-5-mercaptouracil (41) with aqueous silver nitrate provided a quantitative yield of the silver salt of 5-mercaptouracil (43). An attempt to synthesize the 2'-0-p-toluenesulfonyl derivative of S-[5'-0-(t-butyldimethylsilyl)-β-D-ribofuranosyl]-5-mercaptouracil (49) provided the rearrangement, elimination product S-{3'-[5'-(t-butyldimethylsilyloxymethyl)furanyl]}-5-mercaptouracil (50). From this study it was noted that labilizing a ribosyl hydroxyl in the presence of the sulfide sulfur creates the probability of sulfur migration to the carbon bearing the activated hydroxyl.

The photolysis of derivatives of D-mannopyrano[cis-1,2-b]dihydrobenzothiazine (42) in trimethylphosphite as solvent and thiophile are presented. The nature of the interaction between silver (I) and derivatives of D-mannopyrano[cis-1,2-b]dihydrobenzothiazine (42), especially 6'-0-(t-butyldimethylsilyl)-D-mannopyrano[cis-1,2-b]dihydrobenzothiazine (51), is discussed. From this study it was noted that an N-anhydro "holding" linkage places the nucleophilic nitrogen bridge in proximity for capture of the reactive sugar terminus of the sulfur bridge after lysis by Ag^{+1} or photolysis.

TABLE OF CONTENTS

PREFACE

The biological importance[1-4] of the C-nucleosides is well established. This physiological activity of the C-nucleosides creates for the medicinal chemist what might be termed a problem of variations; needed are derivatives and analogues of the basic molecules which exhibit activity *in vivo* or *in vitro*. This need for variation arises for several reasons, e.g., to alter specificity, to attenuate harmful side effects or to enhance useful effects or for studies of the mode of action of a chemical or class of chemicals.

Considerable activity has been directed toward the synthesis[5] of the C-nucleosides as a consequence of their biological activity. Consideration of these available methods[5] focused attention on the need for a new, general synthetic approach comparable in utility to the methods[6] long employed in the syntheses of N-nucleosides. This thesis describes work directed toward the development of a proposed new reaction type for the synthesis of C-nucleosides.

INTRODUCTION

Formalizing the various possible approaches to the synthesis of C-nucleosides provides four alternatives in two classes (see Table 1).

CLASS A 1) Preformed sugar from which base is generated. 2) Preformed base from which sugar is generated.
CLASS B 1) Preformed base coupled with preformed sugar. 2) Preformed base and preformed sugar linked via bridging group which is extruded.

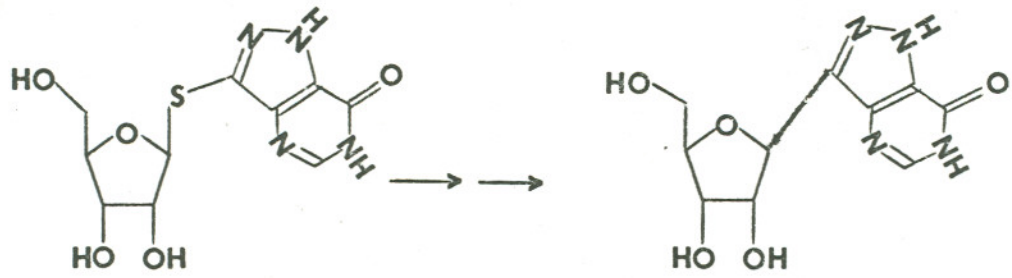
TABLE 1. Four diverse approaches to the synthesis of C-nucleosides are presented.

Class A represents methods wherein the essential C-C bond between the heterocyclic base and the sugar is formed early in the sequence and is followed by extensive synthetic modification. Although no example of method A-2 exists, a truly elegant example of method A-1 may be found in the synthesis of formycin-B by Acton, et al.[5] The obvious disadvantage of Class A methods for general applicability is the requirement for synthesis of a base (or sugar) which may be otherwise readily available. Further, a unique C-1 substituted sugar (i.e., a diazomethyl[5]) restricts one to a specific (i.e., a pyrazolo-) nucleoside. Finally, by joining the sugar and the base early, one may either disallow or complicate subsequent modification of the base by methods not compatible with sugar functionality.

Class B represents methods wherein the essential C-C bond between the heterocyclic base and the sugar is formed late in the sequence and is followed by only minor synthetic manipulations. An excellent example of method B-1, the coupling of a lithiopyrimidine with an aldehydo sugar, is provided by the synthesis of pseudouridine.[5] Class B methods offer the most generality in that both diverse bases and diverse sugars may, prior to coupling, be optimally obtained.

Method B-2 represents methods which have proven effective in the synthesis of cyclic systems[7-9] but as yet have not been applied to the synthesis of C-nucleosides. We have chosen a method B-2 approach which envisages the initial linkage of a base with a sugar derivative through a bridging sulfur atom followed by extrusion of the sulfur bridge with

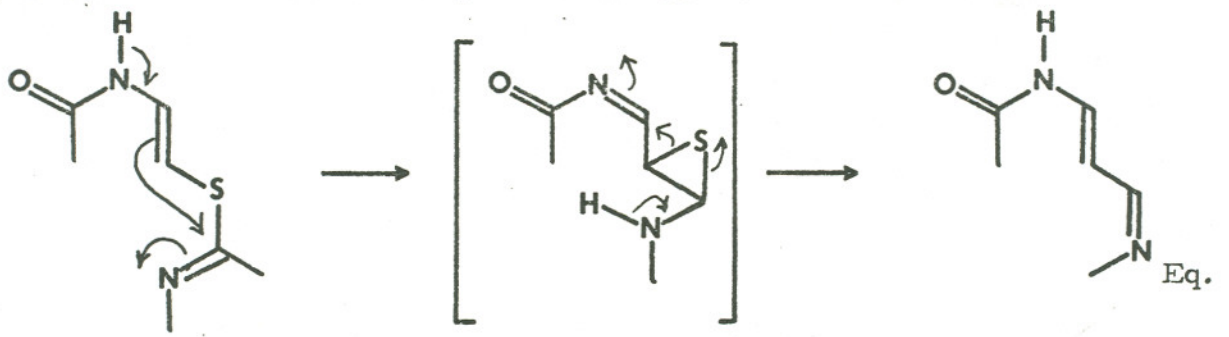
concomitant formation of the C-nucleoside linkage. (See Equation 1)



Eq. 1

Eschenmoser[10,11] has described several efficacious extrusion reactions employed in studies directed toward the synthesis of vitamin B_{12}. These extrusion reactions are analogous to those we contemplate using in a C-nucleoside synthesis.

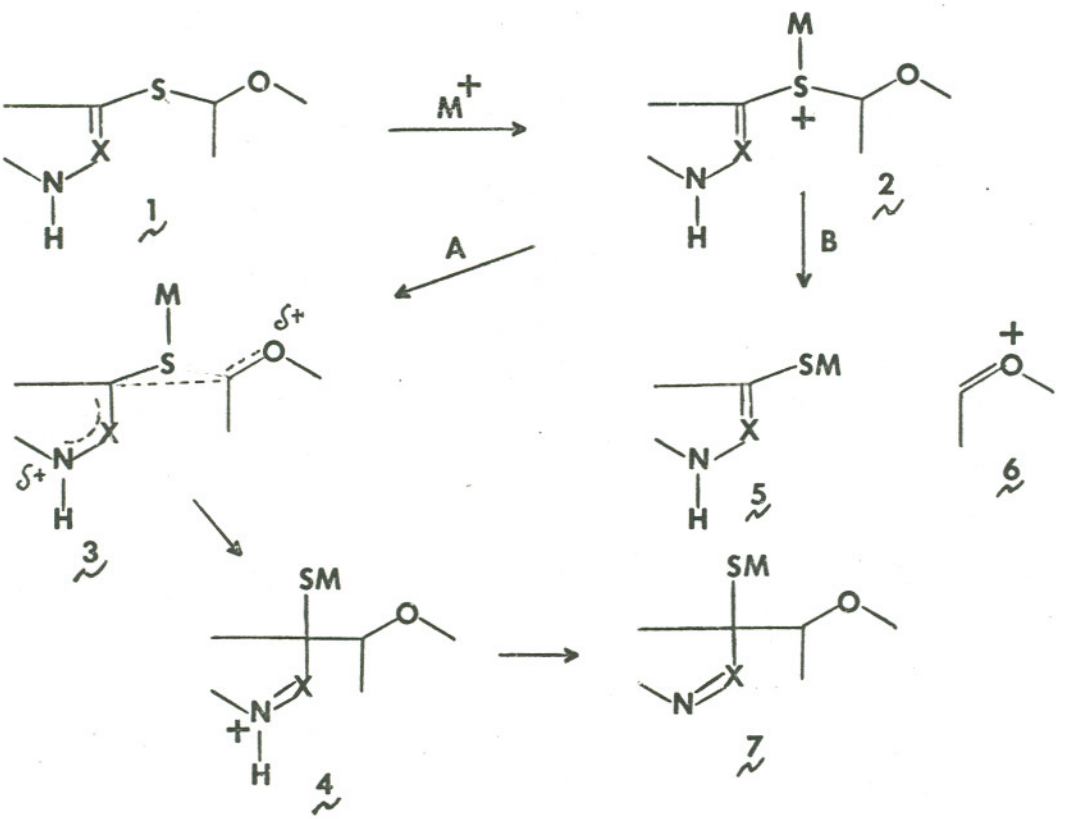
Examination of the sulfur bridged intermediate employed by Eschenmoser[10,11] (see Equation 2) suggests that methods may be



Eq. 2

developed for the synthesis of 3-pyrrolo- or 3-pyrazolo-C-nucleosides; i.e., those which possess similar "eneamine" character. The investigation of this potential reaction is illustrated in Scheme 1.

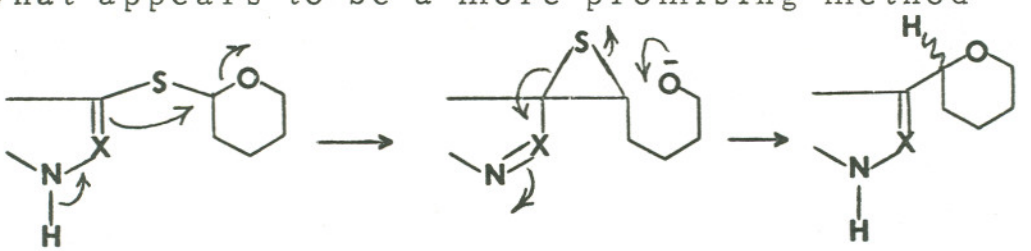
Although Eschenmoser[10,11], in one instance, used trivalent phosphorus as a thiophile, this approach requires that the oxygen heterocycle (sugar or pyran) open and reclose, thus creating the danger of loss of stereochemical control (see Equation 3). However, an observation by Holland and Cohen[12] suggests



X = C or N

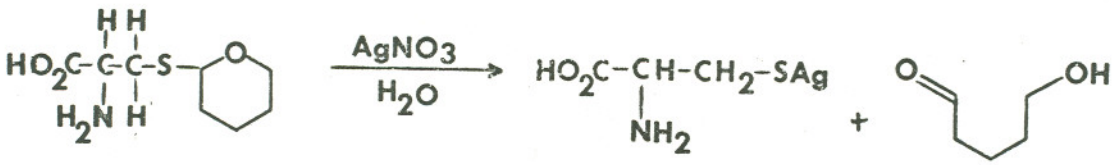
Scheme 1. A schematic representation of the hypothetical new reaction type.

what appears to be a more promising method



Eq. 3

for extrusion of the sulfur atom from 1. It was discovered that treatment of the 2-tetrahydropyranyl derivative of cysteine with aqueous silver nitrate at 0° resulted in the immediate precipitation of the silver mercaptide of cysteine with formation of 5-hydroxyvaleraldehyde (see Equation 4).



Eq. 4

If the compounds, 1, were treated with a metal ion of high affinity for sulfur (Cu^{+2}, Ni^{+2}, Pb^{+2}, Ag^{+1}), in the absence of an external nucleophile, reaction via path A (Scheme 1) may follow. It is pertinent in this regard that Eschenmoser[10, 11] has used nickel perchlorate to extrude sulfur with intramolecular carbon-carbon bond formation.

There is every reason to suppose that the sulfur-hemiacetal linkage in species of type 1 would be labile toward metal cations with high sulfur affinity. This expectation was borne out with silver ion in protic and aprotic media[13]. Other observations of sulfur-metal complexes (of type 2) in less labile sulfides (i.e., Ar_2S, ArSR and RSR) with silver[14] and mercury[15] support the intermediacy of species 2 in the metal induced reactions of 1.

The overall reaction of 1 to 7 is formally analogous to the Stevens rearrangement[16] of sulfonium ylids. However, as current thought[17] supports a radical pair mechanism for the Stevens and the metal induced reaction of 1 is expected to be a polar process the contrast is clear. Comparison of reactivity between a radical pair and a molecule-ion pair can only be speculative.

SECTION I

The study, initiated by Lee[18], of the nature of the effect of silver ion on the sulfur-hemiacetal linkage of 3-(2'-tetrahydropyranylthio)indole (8a) has been completed employing the analogous substance 3-(2'-tetrahydrofuranylthio)indole (8b)[13]. In aprotic media, Lee[18] obtained supportive evidence for the presence of a complex of type 2a as a reaction intermediate (see Scheme 1). However, no carbon alkylated products were observed to result from the silver ion mediated decomposition of 8a. This circumstance prompted further study of the reaction in order to determine whether any evidence (i.e., a carbon alkylated product) could be obtained which would substantiate reaction via Path A (see Scheme 1). In the hope of enhancing conditions favoring carbon alkylation (Path A, Scheme 1), the analogue, 8b, was selected for this work. This choice follows from the greater ease of nucleophilic displacement at a 5- as compared to a 6-membered ring[19]. In this instance, it was hoped that the more stable transition state[20] possible in a 5-membered ring would enhance the reaction via Path A (Scheme 1).

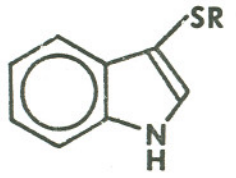
At -78° in tetrahydrofuran, no evidence for the reaction of 8a with silver perchlorate was obtained.[18] At higher temperature (-15 to 25°) 8a underwent reaction presumably via the intermediacy of a complex of type 2a, which, unlike its behavior in benzene and toluene, appears to be soluble in tetrahydrofuran. In contrast, 3-(2'-tetrahydrofuranylthio)-indole

(8b) reacted in the presence of silver ion even at -78°. Except for these differences in affinity for coordination and reaction rate (which parallel the behaviors of 2-O-alkylacetals of tetrahydropyran and tetrahydrofuran toward acid-catalyzed hydrolysis)[21,22] the reactions of 8a and 8b with silver perchlorate in tetrahydrofuran were similar.

Silver ion mediated decomposition of 8b in tetrahydrofuran produced heterogeneous precipitates which, in favorable instances, accounted for essentially all of the added reactants (8b and silver perchlorate). Removal of silver ion from a crude reaction product mixture (suspended in methanol) was accomplished by treatment with hydrogen sulfide. The resulting product mixture was then subjected to chromatographic separation following desulfurization with Raney nickel. In all instances, yields of characterized products were low, accompanied by highly colored, polar material[23] which was not characterized.

The precipitate obtained by reaction of 8b with silver perchlorate in tetrahydrofuran at -15° was treated with hydrogen sulfide to remove silver followed by Raney nickel desulfurization. From the resulting product mixture, 1- and 2-(2'-tetrahydrofuranyl)indoles (9b and 10b) were isolated. When 8a was treated similarly and the reaction mixture was fractionated following removal of silver but without desulfurization, the disulfide (11a) of 1-(2'-tetrahydropyranyl)-3-thioindole and 1-(2'-tetrahydropyranyl)-3-(2'-tetrahydropyranylthio)indole (12a) were isolated.[18]

In a reaction[18] of 8a with silver ion, when silver was removed after 30 minutes at 25° (during which time a precipitate accounting for 25% of the reactants had formed), 8a was recovered unchanged from the filtrate. When the filtrate was treated with excess methyl iodide (to methylate all silver coordination sites), 3-methylthioindole (13)[24] and 3-methylthio-1-(2'-tetrahydropyranyl)indole (14a) were produced.[18]



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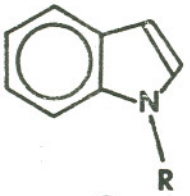

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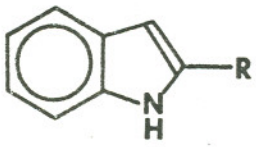

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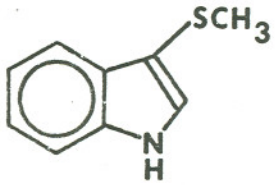

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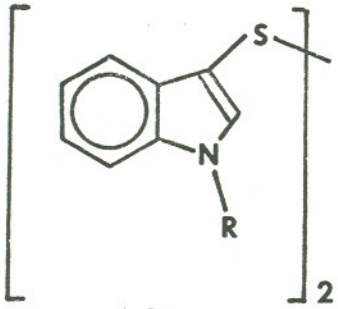

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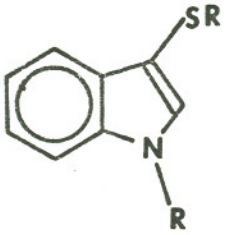

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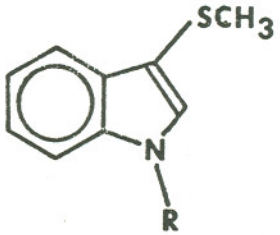
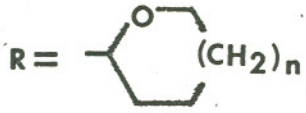


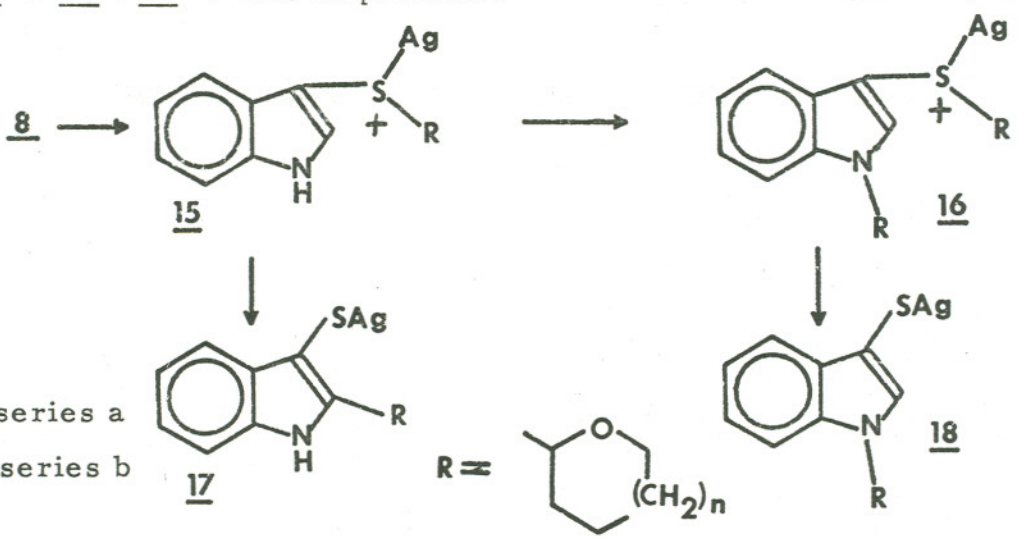
14

n = 1, series a
n = 0, series b



The results of this study allow the construction of Scheme 2 which indicates the general features of the silver ion-catalyzed reactions of 8. Products isolated irrespective of reaction conditions (i.e., 9, 11, 12, 14) all possess a 2'-tetrahydropyranyl(furanyl) substituent on the

indole nitrogen. The formation of such "rearrangement" products clearly must occur via an intermolecular pathway. The probable presence in solution of the secondary coordination compound 16, as evidenced by the isolation[18] of 12a (and of 14a from reactions in which methyl iodide was used), and the insolubility of 3-thioindole silver salt indicate that this "migration" of a cyclic ether moiety from sulfur to nitrogen is likely to occur exclusively via the path 15 → 16 → 18. Interestingly, this sequence does not require formation of significant amounts of 3-thioindole silver salt since, once some 16 is available, the reaction 15 + 16 → 18 + 16 → etc. is possible.



Scheme 2. The general features of the silver ion induced reactions of 8.

While tne pathway to the 1-substituted compound 18 is clearly intermolecular, the origin of the 2-substituted product 17 is less certain. It is possible that a 2-substituted coordination compound analogous to 16 is an intermediate; however, as no evidence for such was found, the

possibility that 17 arises via an intramolecular process must be considered. It is well known[25] that electrophilic substitution of 3-substituted indoles occurs readily at carbon-2, probably via a mechanism involving rearrangement of the adduct formed by initial attack at C-3.[26,27] In this light, although no direct evidence for the reaction process 1 → 3 (see Scheme 1) was found, the possibility that 17 is formed via a pathway of the type (2 → 3 → 4) → 17 is an attractive one.

The spectral properties of the variously substituted indoles comprising this study are highly characteristic and facilitated structural assignments. Among the isolated products from reactions of 8 with silver ion are compounds in which cyclic ether moieties are bonded variously to carbon, sulfur or nitrogen in the 3-thioindole nucleus. The nuclear magnetic resonance (nmr) chemical shift exhibited by the proton on C-2' of the cyclic ether moiety was, in every case, clearly identifiable and led readily to the correct assignment of the indole bonding site. Thus, the C-2' proton of 8b (C-2' bonded to S) appears at δ 5.34, the corresponding resonance for 1-(2'-tetrahydrofuranyl)indole (9b, C-2' bonded to N) appears at δ 5.92 and the signal in the spectrum of 10b (C-2' bonded to C) appears at δ 5.12. The compounds in the 2'-tetrahydropyranyl series[18] exhibited analogous resonances, although shifted toward higher field, e.g. for 8a (C-2' bonded to S) the signal appears at δ 4.84 and for 9a (C-2' bonded to N) the signal is at δ 5.35.

Definitive assignment of structure 10b (as opposed to the isomeric structure 3-(2'-tetrahydrofuranyl)indole) was made by consideration of the nmr chemical shifts observed for the 2- and 3-protons of various indole derivatives. Thus, in the nmr spectrum[28] of 3-methylindole, the hydrogen at C-2 gives rise to a signal at δ 6.78 whereas the C-3 hydrogen in 2,5-dimethylindole appears at δ 6.10.[28,29] In the spectrum of 1-(2'tetrahydrofuranyl)indole (9b), the C-2 hydrogen is observed at δ 6.9 and C-3 hydrogen appears at δ 6.30. The resonance at δ 6.27 in the spectrum of 10b clearly is that of the C-3 hydrogen and establishes the attachment of the tetrahydrofuranyl moiety as occurring at C-2.

These assignments are corroborated by examination of the mass spectra of the variously substituted compounds. The fragmentation of 1-(2'-tetrahydrofuranyl)indole (9b, Fig. 1A) is dominated by cleavage of the tetrahydrofuranyl moiety from the indole nucleus (i.e., m/e 71 and 117). The fragmentations of the various S-tetrahydrofuranyl and S-tetrahydropyranyl compounds are similar in this respect (see Experimental). In contrast, the electron bombardment-induced fragmentation of 2-(2'-tetrahydrofuranyl)indole (10b, see Fig. 1B), in which the cyclic ether is linked to the indole nucleus via a carbon-carbon bond, is considerably more complex. Whereas the indole$^+$ rearrangement ion (m/e 117), formed by loss of dihydrofuran, is the most abundant ion (Σ_{43} = 46%) in the spectrum of 9b (Fig. 1A), this is a low abundance ion (Σ_{39} = 3%) in the spectrum of 10b (Fig. 1B). Although the molecular

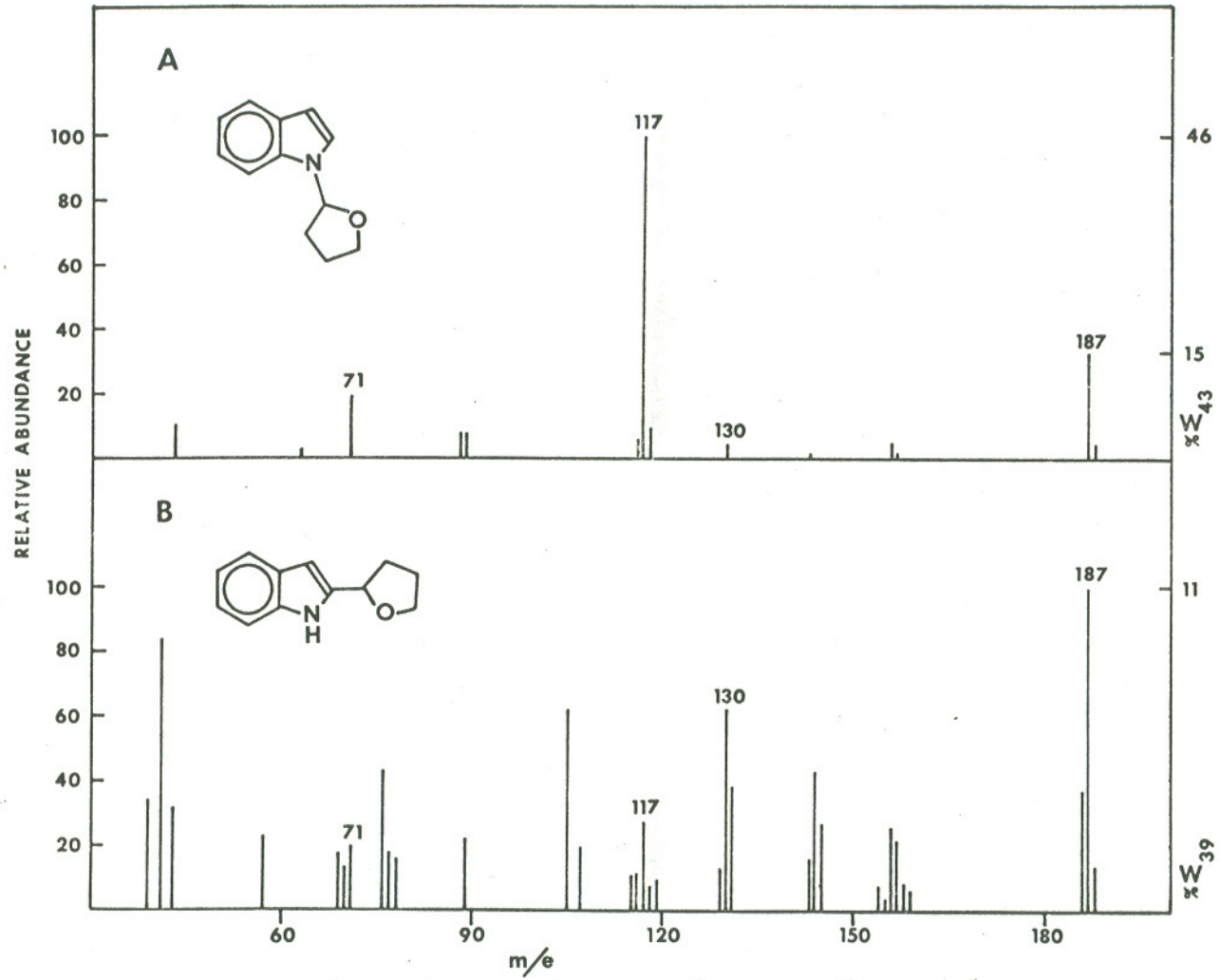
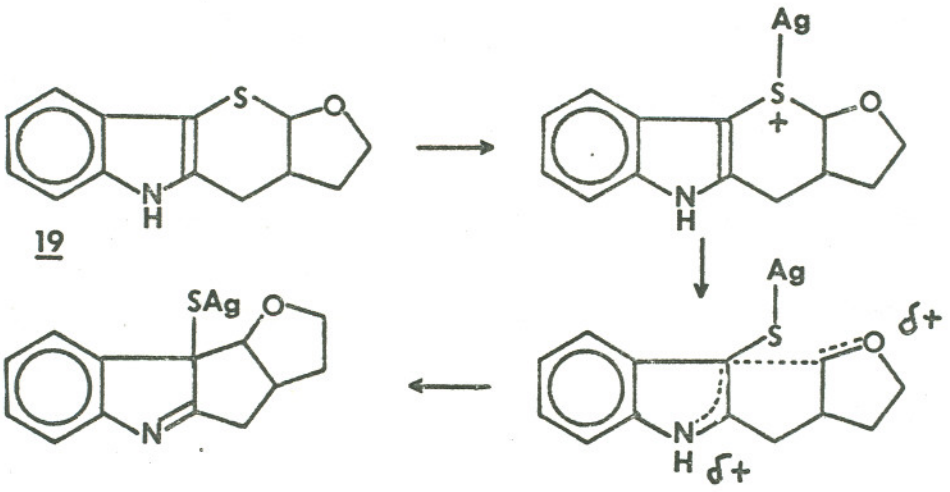


Figure 1. Mass Spectra of 1-(2'-Tetrahydrofuranyl)indole (9b), A, and 2-(2'-Tetrahydrofuranyl)indole (10b), B.

ions represent comparable percentages of total ionization (i.e., 15% for 9b and 11% for 10b), this ion is the base peak in the spectrum of 10b (Fig. 1B). These differences are strikingly similar to those observed in the spectra of N- and C-nucleosides.[30]

Interpretation of these results (see Scheme 2) in terms of the desired rearrangement via Path A (Scheme 1) is admittedly complicated by the ease of oxidation[23] of 8. Serious losses of material result from such oxidative decomposition. The yield data do, however, show that ten times more N-alkylated product (9b) than C-alkylated product (10b) was formed. Assuming that 10b results from an intramolecular process as suggested above, and further noting that perchlorate ion is not significantly nucleophilic, there follows the conclusion that appreciable 3-indolyl capture of a carbonium ion requires a "fixing" linkage elsewhere in the molecule. Such a linkage for system 1 (or 8) could be thought of as completing a six-membered ring, which upon reaction would hold the reacting termini in geometrically well disposed proximity for collapse to a 5-membered ring. (see Scheme 3)



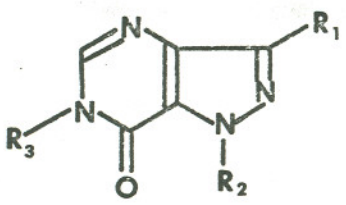
Scheme 3. A possible solution to the entropically disfavored intramolecular capture of a released carbonium ion.

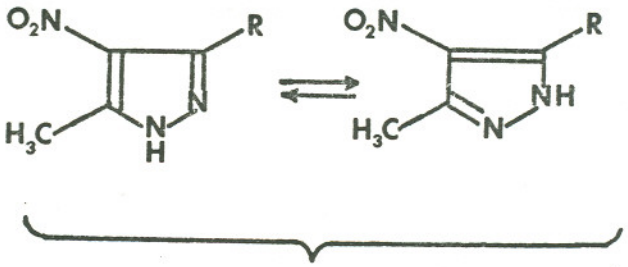
Visualizing the tetrahydrofuranyl ring in 19 as a furanose leads readily to the consideration of the analogous (oxygen or nitrogen replacing the methylene bridge) anhydro-cyclonucleosides as a promising system for further study. Work conducted in such systems will be discussed in Section III.

SECTION II

Selected reactions of pyrazolo [4, 3-d] pyrimidin-7-one(20)[31] and 3-methyl-4-nitropyrazole (25).

Since continuation of the study of the new silver induced carbon-carbon bond forming reaction required the synthesis of an anhydro-cyclonucleoside, or comparable system, it seemed most useful to select, rather than a model, a system that would provide an actual C-nucleoside product. Thus, the aglycone of formycin-B, pyrazolo-[4, 3-d] pyrimidin-7-one (20)[31] was selected for synthetic elaboration.





	R
25	H
26	Br

	R_1	R_2	R_3
20	H	H	H
21	Br	H	H
22	Br	Bz	H
23	Br	Bz	Bz
24	β-ribosyl	H	H

Bz = Benzyl

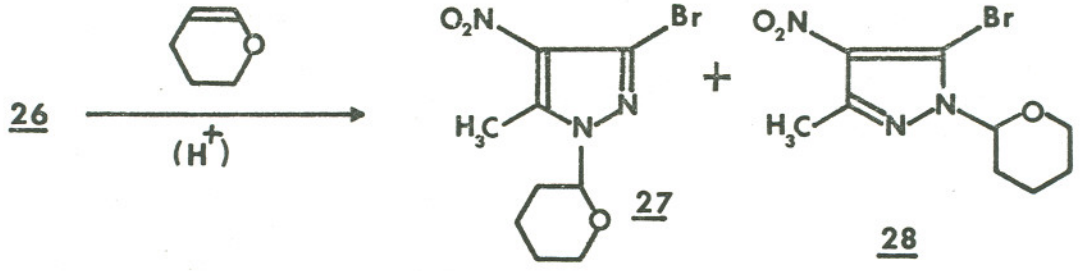
Pyrazolo [4, 3-d] pyrimidin-7-one (20) was synthesized by a modification of the 8-step procedure of Robins, et al,[31] with an improved overall yield of greater than 10%. A synthetic intermediate provided by this procedure is 3-methyl-4-nitropyrazole (25) which was used in a study of the displacement of a 3-bromo substituent in that nucleus (see 26). This study was conducted as a model for the expected method of introducing thiol functionality in the pyrazolo [4, 3-d] pyrimidin-7-one[31] system via nucleophilic displacement in the known 3-bromopyrazolo [4, 3-d] pyrimidin-7-one (21).[32]

The requisite 3-bromo-5-methyl-4-nitropyrazole (26)[33] was synthesized from 25 employing the conditions of Grandberg, et al.[34]

Attempts to displace the bromo substituent of pyrazole 26 with sodium hydrosulfide in refluxing water proved fruitless. As in other systems,[35] where an acidic N-H was present in the heterocycle, abstraction of the acidic hydrogen by the nucleophile deactivates the nucleus toward nucleophilic displacement. Fortunately this difficulty is readily overcome by alkylation of the ring nitrogen(s). The tetrahydropyranyl blocking group was selected because of its base stability and ease of removal under mildly acidic conditions. Thus pyrazole 26 reacted (see Equation 5)with dihydropyran under anhydrous acid catalysis to provide the isomeric mixture of 3-(5)-bromo-5-(3)-methyl-4-nitro-1-(2'-tetrahydropyranyl)pyrazole (27, 28). Crystallization of the mixture (27, 28) from 95% ethanol provided colorless crystals with a broad melting point range. Evaporation of the mother liquors resulted in a viscous oil which was vacuum distilled.

Examination of the two fractions by nmr (see Experimental) demonstrated that both the crystals and the oil were mixtures; each had two methyl singlets at δ 2.56 and δ 2.72 and two doublets of doublets for 1'-protons at δ 5.42 ($J_1 \cong$ 3 cps, $J_2 \cong$ 8 cps) and δ 5.58 ($J_1 \cong$ 3 cps, $J_2 \cong$ 9 cps). Relative intensities indicated that the methyl singlet at δ 2.56 was associated with the doublet of doublets at δ 5.58.

It can be noted that the 4-nitro substituent in 28 is better disposed than the 4-nitro substituent of 27 to activate the molecule for nucleophilic displacement of the 5-(3)-bromo substituent. However, the conditions under which the displacement reactions were run (see Experimental) did not provide information regarding the relative rates of displacement in the two isomers (27 and 28). Nor was there any evidence of N-dealkylation as has recently been found[36] in reactions of an N-alkylated heterocycle with nucleophiles.

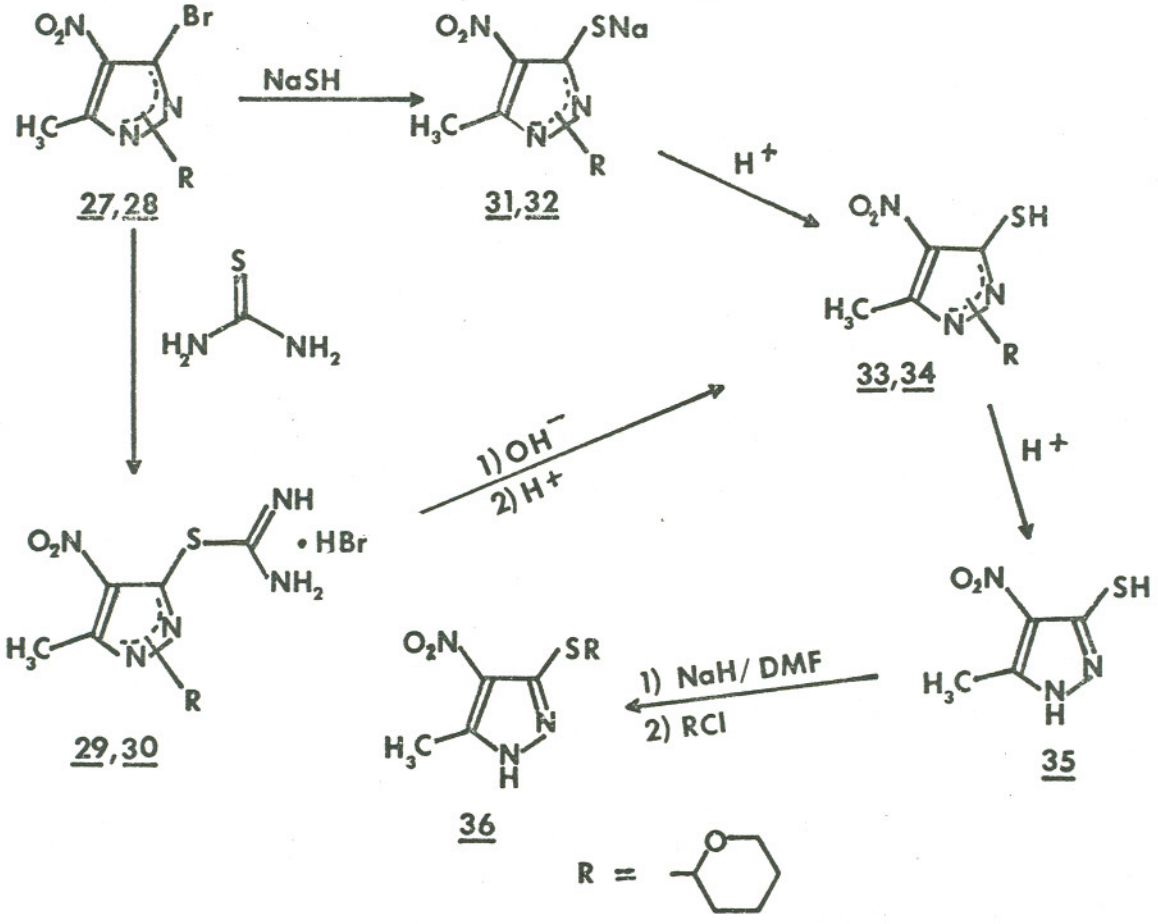


Eq. 5

Refluxing the isomeric bromides (27, 28) with thiourea in 50% aqueous ethanol provided a mixture of thiouronium salts (29, 30), while treatment, at 85° in the same solvent, with sodium hydrosulfide provided a mixture of sodium salts (31, 32). Base hydrolysis (and neutralization)

of the thiouronium salts (29, 30) and neutralization of the sodium salts (31, 32) each provided the same oily mixture of isomeric mercaptans (33, 34) (see Scheme 4).

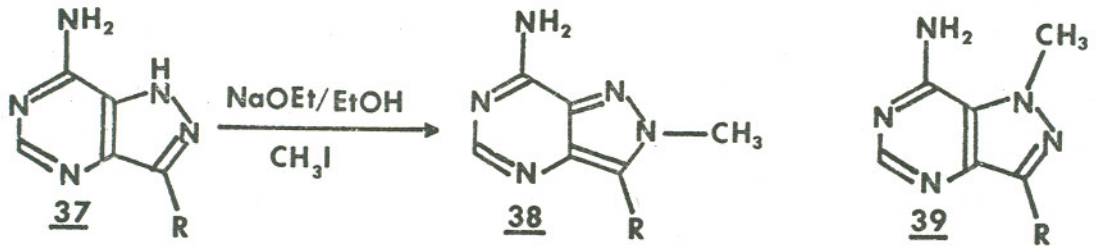
Removal of the tetrahydropyranyl blocking group was accomplished with aqueous acetic acid. The resultant mercaptan, 3-methyl-4-nitro-5-thiopyrazole (35), as well as the N-blocked mercaptans 33 and 34 were not readily purified. However, the crystalline derivative, 3-methyl-4-nitro-5-(2'-tetrahydropyranylthio) pyrazole (36), analogous to a sulfur bridged nucleoside, was easily synthesized and characterized.



Scheme 4. Transformations of the pyrazolo nucleus.

This facile displacement of the 3-bromo substituent of 27 and 28 was an encouraging result. However, 27 and 28 possess an activating 4-nitro substituent. As no such obvious activation is present in 21, some attenuation of this ease of displacement was anticipated on changing to the pyrazolopyrimidine system.

Treatment of an aqueous suspension of 20 with bromine readily provided 3-bromopyrazolo [4, 3-d] pyrimidin-7-one (21).[32] Dissolved in aqueous ammonia at 25°, 21 did not react with benzylchloride. However, as a solution in excess (>3 fold) aqueous sodium hydroxide, 21 reacted with excess benzylchloride to provide a good yield of 1-benzyl-3-bromo-pyrazolo [4, 3-d] pyrimidin-7-one (22). This result was surprising in view of the observation made by Long[37] that methylation of formycin (37) provided the 2-methyl isomer (38) as the major product with only minor amounts of 1-methyl product (39) (see Equation 6). That 27 does not



37 R = β-ribosyl

38 R 24.4%

39 R 3.8%

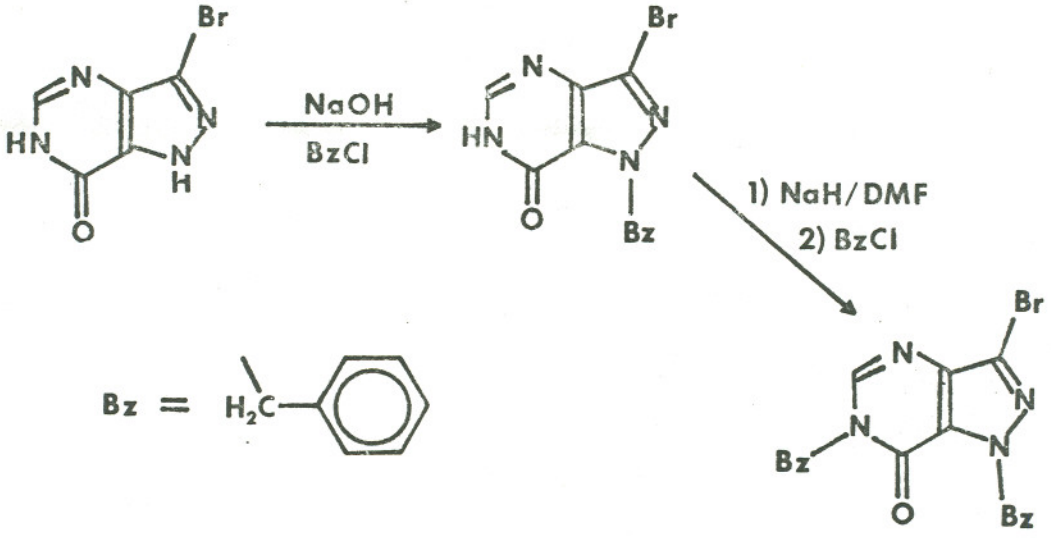
Eq. 6

benzylate in aqueous sodium hydroxide (i.e., only a trace of doubly benzylated material was formed) is reminiscent of the behavior of 21 in ammonia water. Once again a stronger base, sodium hydride in dimethylformamide, was necessary to effect the desired alkylation

(see Scheme 5).

The assignment of structures 22 and 23 followed readily from spectroscopic properties, notably their ultraviolet spectra which do not differ significantly from that of 21. This is consistent with the observation made by Long[37] that the ultraviolet spectra of 37 and 39 are similar while the ultraviolet spectrum of 38 is significantly different.

As for pyrazole 26, the unblocked pyrazolopyrimidine 21 proved unreactive to nucleophiles under the conditions employed.



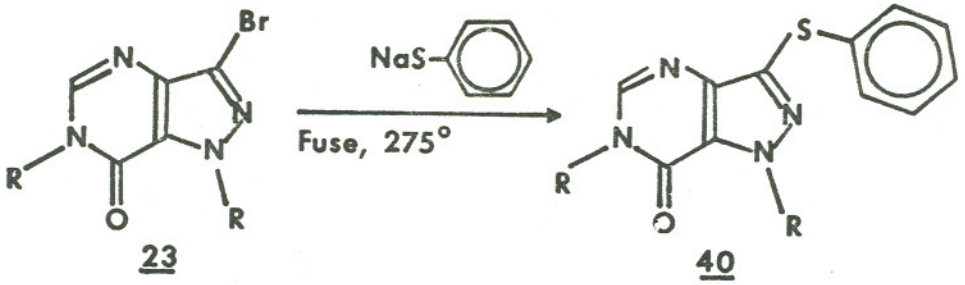
Scheme 5. The two step benzylation of 26 is illustrated.

Thus treatment of 21 with thiourea under reflux in ethanol, water, 2N sulfuric acid and dimethylformamide did not effect the desired dis-

placement. Nor was fusion with sodium hydrosulfide at 270°-310° effective in this regard.

The observation of unreactivity for 21 was not unexpected. However, the same conditions also proved insufficient to cause displacement of the bromo substituent of 22. Although it seemed unlikely that ionization of the 6-hydrogen of 22 was the source of deactivation (since 22 does not react with benzylchloride in aqueous sodium hydroxide) this last ionizable site was alkylated to form 23.

Treatment of 23 with thiourea or sodium hydrosulfide in refluxing ethanol, dimethylformamide and ethylene glycol or fusion with sodium hydrosulfide at 360° failed to effect the desired displacement. Thus ionization of the 6-hydrogen was not the cause of deactivation. In only a single instance was any evidence for displacement obtained. When 23 was fused as an intimate mixture with excess sodium thiophenoxide at 275°, a trace of displacement product, 1, 6-dibenzyl-3-(phenyl thio)pyrazolo [4, 3-d]pyrimidin-7-one (40), was isolated (see Equation 7).



23 40

R = Benzyl

Eq. 7

In order to circumvent the displacement route to the desired 3-thiopyrazolo [4, 3-d] pyrimidin-7-one (41) several electrophilic

reactions with 20 were explored in a preliminary way. They are 1) diazo coupling with benzene diazonium chloride,[38] 2) nitration,[39] 3) direct dimethylsulfuranylidene ylid generation with dicyclohexylcarbodiimide (DCC)-dimethylsulfoxide(DMSO),[40] 4) electrophilic addition of thiourea,[41] and 5) electrophilic chlorosulfonation.[42] In no instance was the desired product obtained.

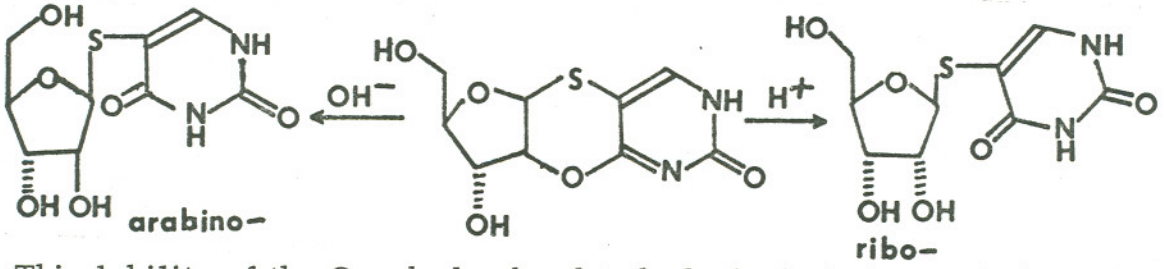
The results with the electrophilic reagents and the difficulty experienced in the displacement reactions with sulfur nucleophiles prompted consideration of alternate substances for the exploration of the new reaction type sought (see Scheme 1). Two such available systems will be discussed in Section III.

SECTION III

Selected Reactions of S-(β-D-Ribofuranosyl)-5-mercaptouracil (41)[43] and D-Mannopyrano[cis-1,2-b]dihydrobenzothiazine (42).[44]

The title substances 41[43] and 42[44] are available through literature syntheses. S-(β-D-Ribofuranosyl)-5-mercaptouracil (41)[43] is a sulfur nucleoside of the type required for the study of the new reaction (see Introduction). However, synthetic modification of 41 was required to produce a molecule which possessed a second, "holding" linkage (i.e., the formation of an anhydro-cyclonucleoside). D-Mannopyrano [cis-1,2-b]-dihydrobenzothiazine (42)[44] possesses the desired sulfur hemiacetal linkage as well as an N-anhydrocyclonucleoside bond.

The O-anhydro linkage offers utility in varying the stereochemistry of the sugar. Thus, not only may the linkage be formed at several sites in the sugar[45] but the lability of the O-anhydro bond to both acid and base allows reversal of stereochemistry at the site of bonding in the sugar moiety:

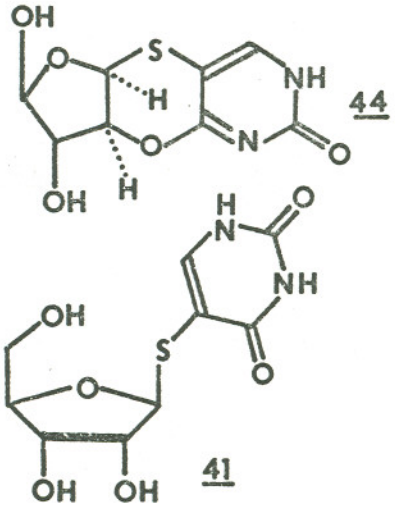


This lability of the O-anhydro bond to hydrolysis is in marked contrast to the stability of the N-anhydro bond to acid and base.[46]

Nucleoside 41, when treated with aqueous silver nitrate, produced a quantitative yield of the silver salt of 5-mercaptouracil (43). The only significant difference, in reaction with aqueous silver ion, between 41

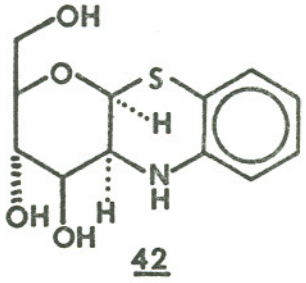
and 8 was the sluggishness of reaction of 41. This rate difference is consistent with observed[21,22] rates in acid catalyzed hydrolyses of 1-0-alkylacetals of sugars as compared to the corresponding cyclic ethers.

The formation of S-(4,2'-0-anhydro-β-D-arabinofuranosyl)-5-mercaptouracil (44) not only fixes the incipient carbonium ion but also should control the stereochemistry of the rearrangement to produce the required β-glycosidic linkage. An alternate

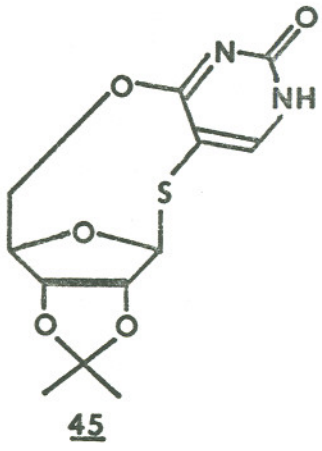


44

41

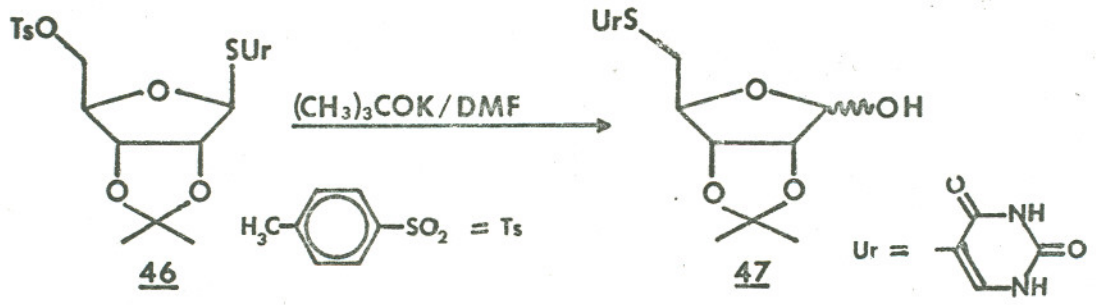


42



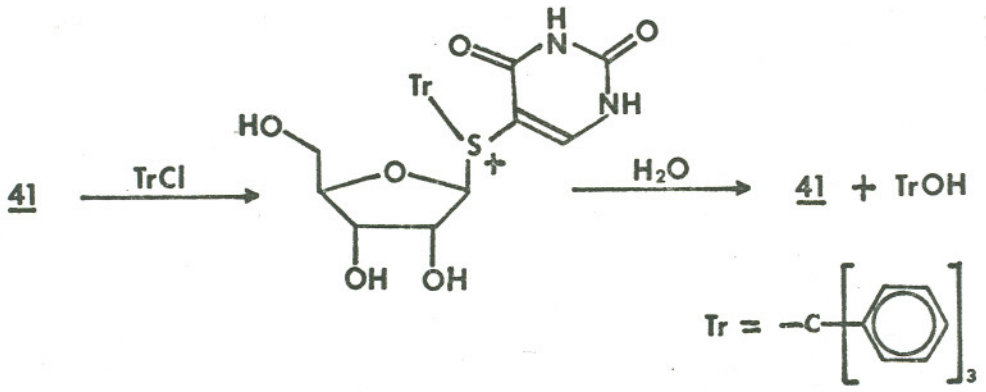
45

anhydro-cyclonucleoside, S-[4,5'-0-anhydro-(2',3'-0-isopropylidene)-β-D-ribofuranosyl]-5-mercaptouracil (45)[47] would serve the same purpose. However, attempts to synthesize 45 from the corresponding 5'-0-tosylate (46) resulted only in the isolation of the rearrangement product S-[5'-(2',3'-0-isopropylidene)-D-ribofuranosyl]-5-mercaptouracil (47)[48] (see Equation 8).



Eq. 8

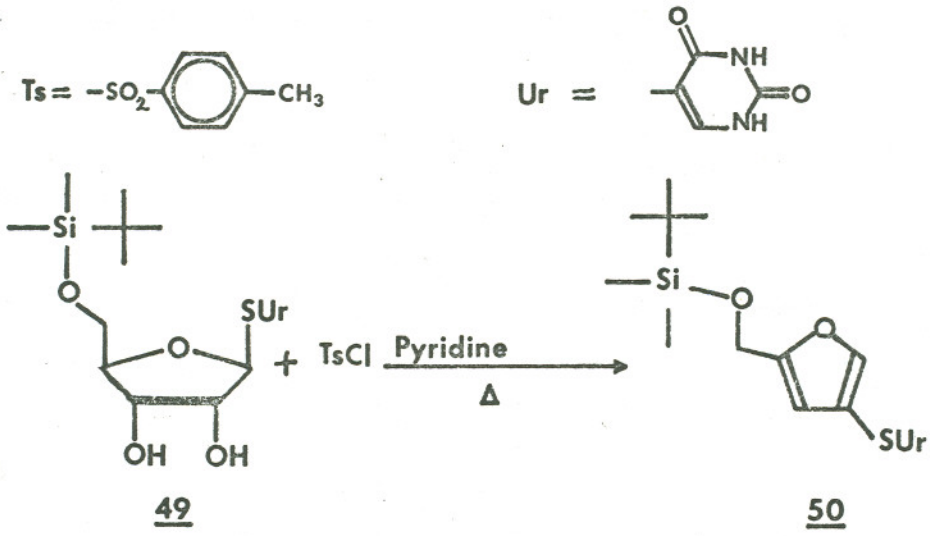
This observation of the nucleophilicity of the sulfur atom of 46[48] has proven to be prophetic (see below). It may also account for the repeated recovery of starting material in the attempted tritylation of 41 by standard methods. Also interesting in this regard is the extended time required and the low yields obtained by Ogilvie and Slotin in the synthesis of 5'-O-monomethoxytrityl-8,2'-thioanhydroinosine.[49] Perhaps the sulfur in 41 is sufficiently nucleophilic to form the sulfonium salt (48) which collapses on work-up (see Equation 9).



Eq. 9

Yet another example of the nucleophilicity and mobility of the sulfur was observed when S-[5'-O-(t-butyldimethylsilyl)-β-D-ribofuranosyl]-5-mercaptouracil (49), formed from 41 and t-butyldimethylsilylchloride,[50] was treated with p-toluenesulfonyl chloride. Thus a solution of 49 and

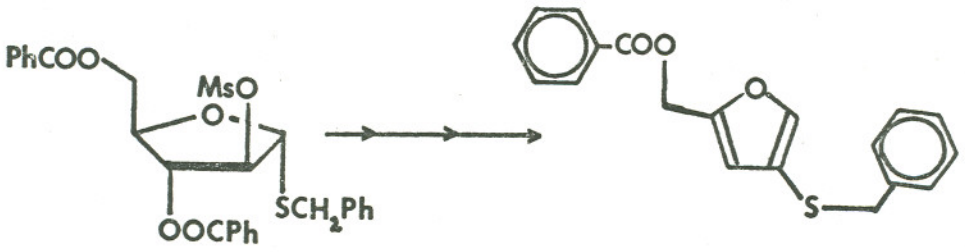
p-toluenesulfonyl chloride in pyridine--kept for several days at room temperature--resulted upon work-up in the recovery of starting material, 49. However, if this same mixture was warmed (60-70°), in several hours all of the nucleoside, 49, was consumed producing a small yield of S- 3'-[5'-(t-butyldimethylsilyloxymethyl)furanyl] -5-mercaptouracil (50) (see Equation 10). This result is analogous to



49 50

Eq. 10

the results obtained by Ryan and coworkers[51] with 1-thiosugars:



In large scale fusions the furan was the major product.[51]

The structure of 50 was established on the basis of its nmr and mass spectra. In the nmr of 50 the t-butyl moiety appeared at δ 0.86

(lit.[52] δ 0.92), the 4-furanyl proton appeared at δ6.40 (lit.[51] δ6.35), the 2-furanyl proton appeared at δ7.58 (lit.[51] δ7.49), the exocyclic methylene appeared at δ4.57 (lit.[51] δ5.21 for O-benzoyl) and the 6-uracil proton appeared at δ7.70. In the mass spectrum of 50, no molecular ion (m/e 354) was evident; the ion at highest mass, produced by loss of a methyl radical, was at m/e 339 (3%). The base peak, m/e 297, was formed by loss of a butyl radical while loss of a t-butyldimethylsilyloxy radical resulted in a peak at m/e 223 (20%). The peak at m/e 223 is characteristic in that electron bombardment induced fragmentations of 5'-O-t-butyldimethysilyl derivatives of nucleosides do not undergo this cleavage.[53] Loss of a silyloxy radical in the mass spectral fragmentation of 50 can be taken to result from the benzylic character of the 5'-methylene and therefore may be a useful fragmentation for such furans.

An alternative route for the synthesis of 44 was explored employing 49. Greenberg and Moffatt[54] elaborated a method which employed 2-acetoxyisobutrylchloride to synthesize 2'-chloro-2'-anhydro-ribonucleosides. The proposed mechanism[54] invoked an anhydrocyclonucleoside as a key intermediate and in fact, under certain conditions, the cyclonucleoside could be isolated. However, repeated attempts employing 2-acetoxyisobutrylchloride and 49 under the specified conditions[54] produced only complex mixtures which were not characterized. At least partly responsible for this difficulty was the ready lability[49,52] of the silyl ether

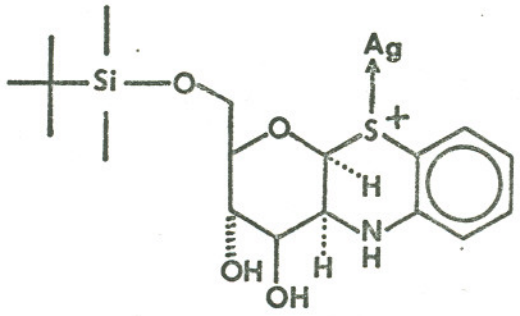
to the acid generated during the reaction. This lability disallowed, as well, the use of comparable reagents (i.e., halomethylenedimethylammoniumhalide[55]) which are acidic in nature.

The parent nucleoside 41 was also treated with 2-acetoxyisobutrylchloride under the specified conditions.[54] When a solution of 41 and 2-acetoxyisobutrylchloride in dimethylformamide was allowed to react at ambient temperature for three hours there resulted a complex mixture of products from which no characterized products were isolated.

The repeated difficulties encountered in attempted syntheses of 44 and the availability of 42[44] encouraged studies with the latter molecule. The ease of synthesis of 42, however, was offset by its stability in the presence of aqueous silver nitrate. It was anticipated[21, 22] that, as a gluconucleoside, 42 would react more slowly than 8 or 41 with silver ion. Indeed, the reaction of 42 with aqueous silver ion at room temperature is so slow that a solution of 42 and one equivalent of silver nitrate showed no signs of reaction after several days. This inactivity can be overcome, albeit in an unproductive fashion, by heating the solution; so that heating an aqueous solution of 42 and an equimolar portion of silver nitrate on a steam bath produced an intractable tar.

In order to obtain some insight about the nature of the interaction of the silver ion with the sulfur atom of 42, solutions of 6'-O-(t-butyldimethylsilyl)-D-mannopyrano[cis-1,2-b]-dihydrobenzothiazine (51) (chosen because of its ready solubility) and excess silver perchlorate in various organic

solvents were mixed and examined. The solvents chosen were acetonitrile, benzene, ether, methanol and tetrahydrofuran. In every solvent but acetonitrile, when solutions of silver perchlorate and of 51 were mixed and examined by thin layer chromatography (tlc), pronounced streaking was observed. That the streaking was the direct result of association of the silver and the sulfur of 51 is substantiated in the following way. Addition of pyridine (which like acetonitrile strongly coordinates silver ion) to a solution containing the presumed complex, caused 51 to chromatograph as a well defined spot without any indication of streaking. It is also significant that the solution in ether (which is the poorest coordinating solvent in the study) formed an almost quantitative (87%) yield of white, amorphous solid (52). This white solid is hygroscopic (see ref. 14) and its nmr spectrum showed no significant shifts as compared to the spectrum of 51. Of particular importance is the fact that the aromatic region showed no upfield shifts or changes in symmetry which would be expected[56] if the silver ion were associated with the aromatic ring as a π-complex. Addition of a strong base (sodium hydride or potassium-t-butoxide) to any of the solutions causes an immediate black precipitate which is characteristic of a silver-nitrogen complex.[57] It therefore seems likely that the white solid (52), formed in ether, is a silver-sulfur complex of type 2 (see Scheme 1).

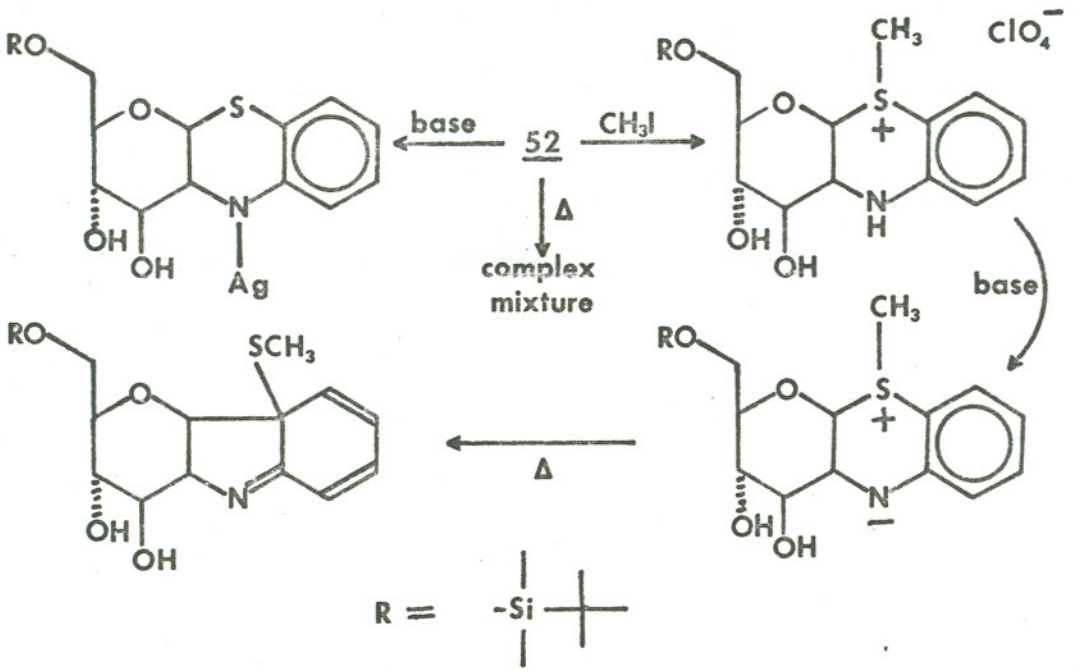


52

The formation of this silver-sulfur complex was however only an apparent advance toward the desired reaction via Path A (see Scheme 1) because warming the complex produced an uncharacterized mixture. Also, on standing at room temperature, 52 slowly darkens over several days.

The stability of the silver complex 52 and its marginal solubility in benzene suggested the possibility that a methyl sulfonium salt might be formed which could be induced to undergo a Stevens rearrangement.[16] This alternative to a polar, metal induced rearrangement (see Scheme 6) was necessitated by the non-selective thermal decomposition of 52.

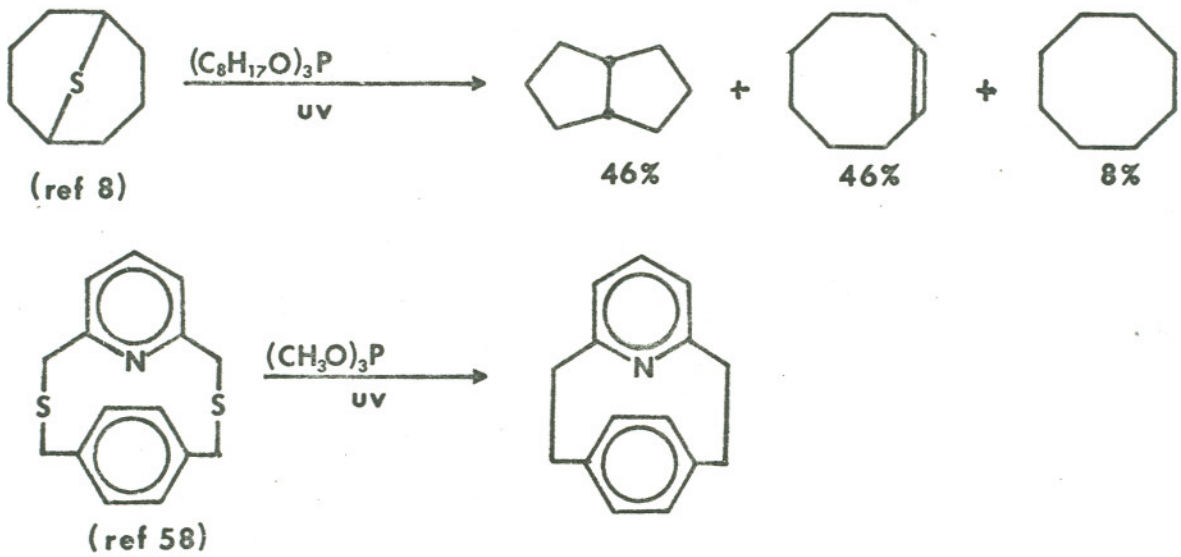
When a benzene solution of 52 was treated with excess methyl iodide, a solid coprecipitated with the insoluble silver iodide. The precipitate was demonstrated to contain all the added reactants as no residue remained upon evaporation of the separated benzene layer. The solid could be readily separated from the silver iodide by extraction with



Scheme 6. A hypothetical Stevens[16] rearrangement.

methanol. Evaporation of this methanol solution under reduced pressure resulted in a residue which exhibited an nmr spectrum (dmso-d_6) with several features: it appeared to be a mixture, it contained no evidence of methyl singlets and the silyl blocking group was absent. The absence of the silyl group complicated the interpretation of the results as there was no easy way to determine at what stage in the reaction it was lost. Therefore no judgement could be made concerning the cause of the cleavage of the silyl group nor what affect its loss had on the overall reaction.

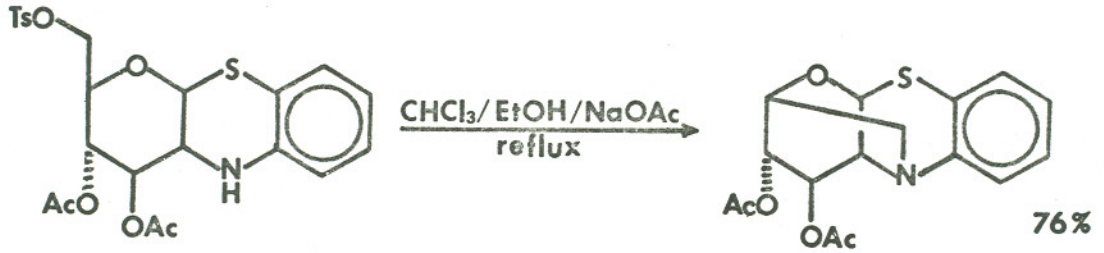
Corey and Block[8] and Boekelheide[58] and coworkers have effectively used photochemical sulfur extrusions in cyclic, sulfur bridged hydrocarbons (see Scheme 7). A photochemical sulfur extrusion



Scheme 7. Photochemical extrusion of sulfur in sulfur bridged hydrocarbons.

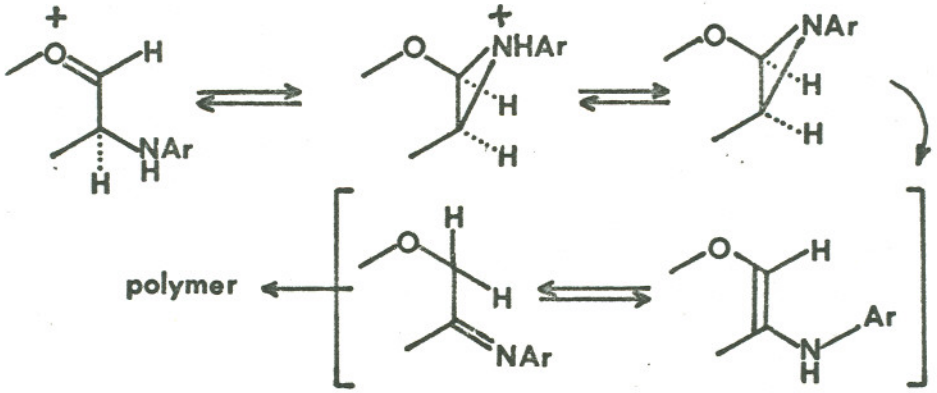
route to the desired carbon-carbon bond formation was also sought in the photolysis of 42 and its 3,4,6-tri-O-acetyl derivative (53). However, irradiation of 42 or 53 in trimethylphosphite as solvent (and thiophile) with a medium pressure Hanovia source filtered through quartz consumed starting material and produced an uncharacterized mixture of products. When 42 was irradiated in trimethylphosphite at selected wave lengths (i.e., λ max 309, 265, 225, which correspond to the three λmax in the ultra violet spectrum of 42) no evidence of reaction was observed.

The difficulties encountered with the derivatives of 42 upon lysis of the sulfur-hemiacetal bond may result indirectly from the nucleophilicity which has been observed[59] for the nitrogen atom. Sekiya and Ishiguro[59] have prepared 3,4-di-O-acetyl-6,N-anhydro[cis-1,2-b]-dihydrobenzothiazine (55) from 3,4-di-O-acetyl-6-O-tosyl-D-mannopyrano-[cis-1,2-b] dihydrobenzothiazine (54) in an isolated yield of 76% (see Equation 11).



54 55 Eq. 11

Perhaps the generation of a carbonium ion or radical at the 1-position of the sugar of 42 results in the formation of an aziridine which under the conditions of the reaction may open forming a reactive eneamine (see Scheme 8). An analogous pathway can be envisioned for a radical process.



Scheme 8. Hypothetical mechanism for polymerization of 42 and its derivatives.

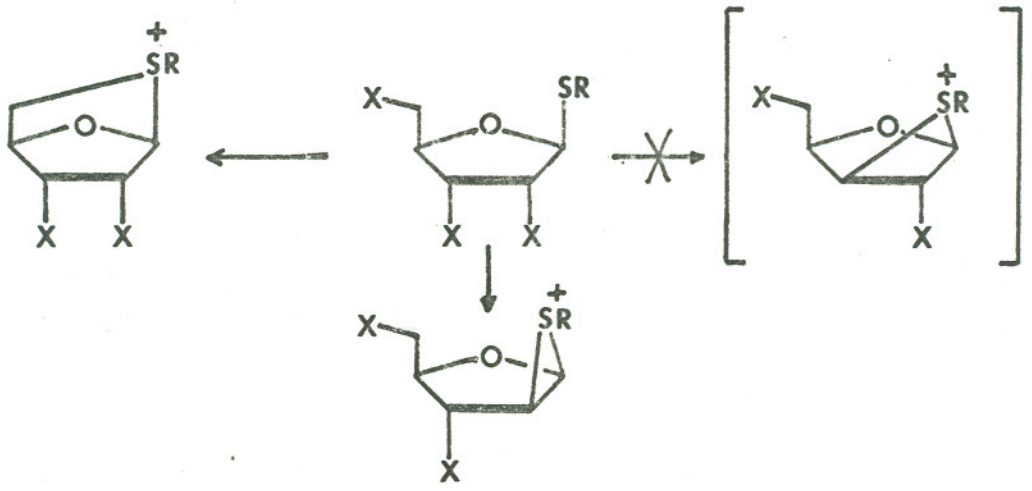
It might also be noted that reaction via Path A (Scheme 1) requires the interruption of aromaticity in the aromatic ring:

It thus appears that the intramolecular nucleophilicity of the nitrogen atom is more readily expressed through space (aziridine formation) rather than by interruption of aromaticity in the aromatic portion of the molecule.

CONCLUSIONS AND RECOMMENDATIONS

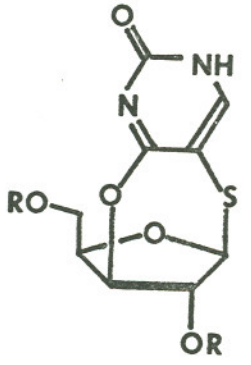
From the results of the study with 3-[2'-tetrahydropyranyl-(furanyl)thio]indole (8) it is clear that to achieve the desired rearrangement will require that the sugar and nitrogen heterocyclic moieties be held together by a second linkage in addition to the sulfur-hemiacetal bond. Studies with 42 and its derivatives strongly suggest that the intra-molecular nucleophilicity of the nitrogen atom rules out the use of an N-anhydro holding linkage. The efficacy of the nitrogen in capturing a carbonium ion or radical at the sugar 1-position is reminicent of the displacement-rearrangement observed with sulfur.[51]

The formation of furan 50 in an attempt to synthesize the 2'-O-tosyl derivative of 49 presents a problem which may be difficult to avoid. Sulfide sulfur is a powerful intramolecular nucleophile[60] when a 3 or a 5 membered sulfonium ion can be formed by internal displacement (see Scheme 9). However such pronounced anchimeric assistance is not expected for reaction at the sugar 3-position since a disfavored 4 membered ring would result. This "immunity" of the sugar 3-position might provide a means of avoiding the rearrangement observed with 49. Thus a suitably



Scheme 9. Anchimeric assistance by sulfide sulfur in a ribofuranose is illustrated.

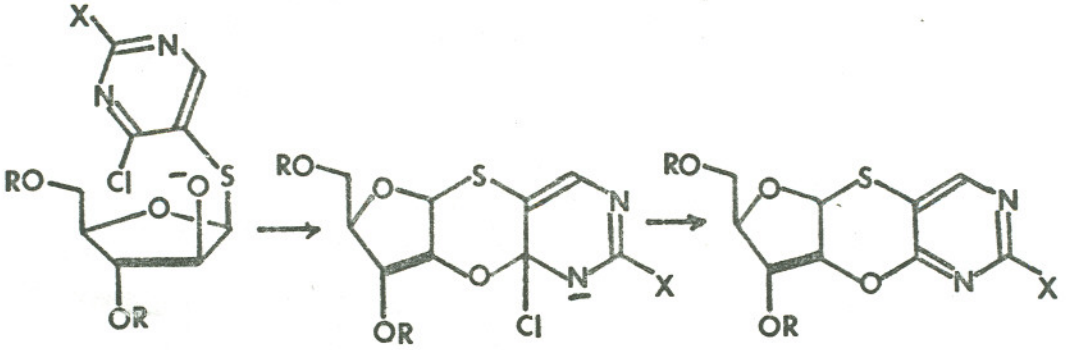
derivatized nucleoside, with a leaving group in the sugar 3-position, might provide a 4, 3'-O-anhydrocyclonucleoside:



While this latter alternative may overcome the rearrangement of the sulfur, it requires the formation of a 7-membered ring.

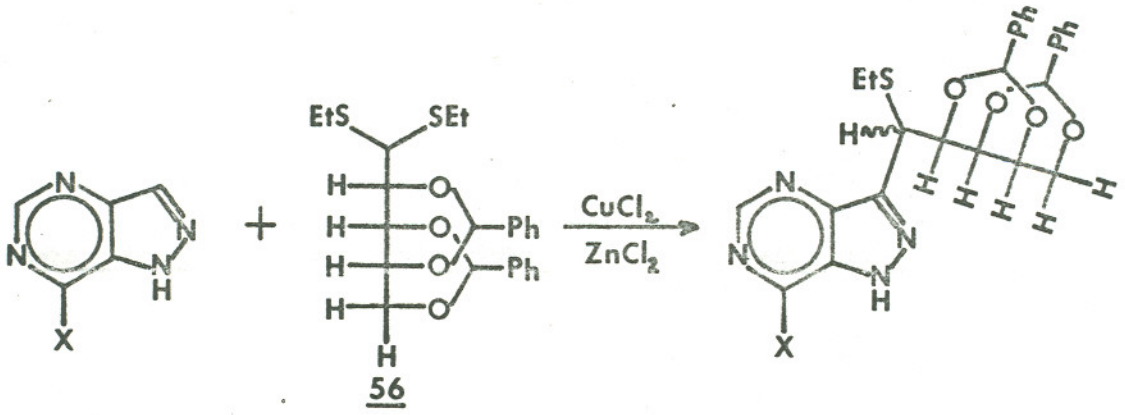
It might be well to explore the possibility of forming the O-anhydro linkage via nucleophilic displacement on the nitrogen heterocycle by a

2'-hydroxyl of an arabino-nucleoside:

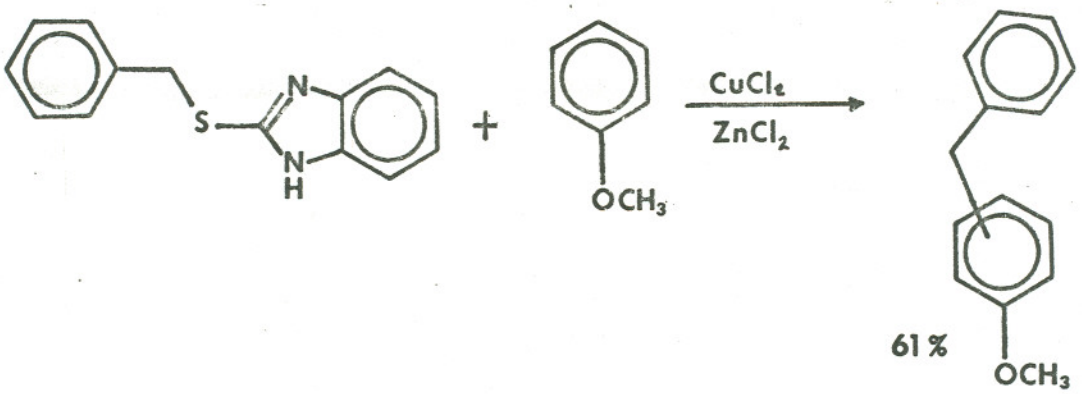


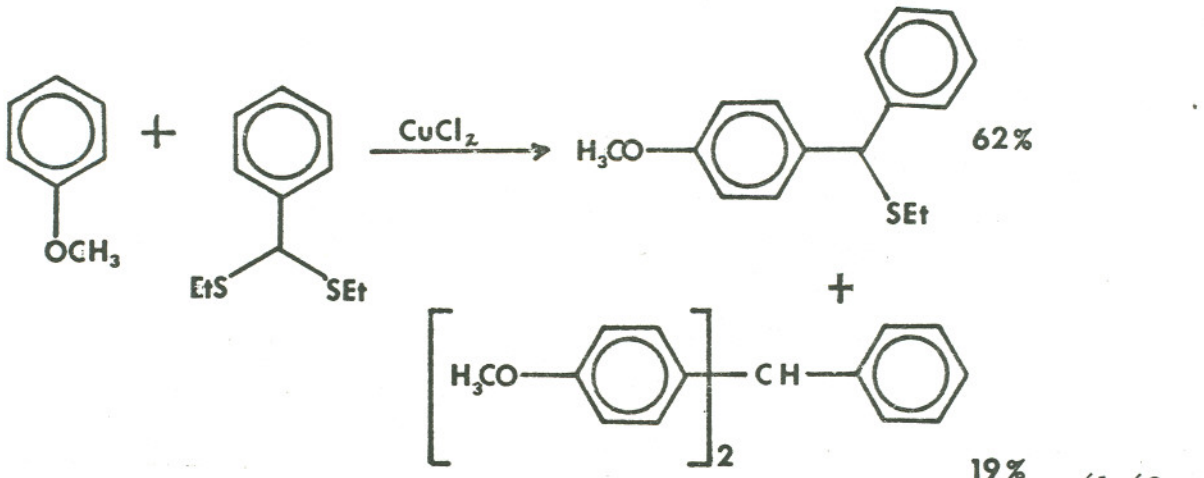
However this avenue is not devoid of difficulty. The nature of substituent X is of critical importance; it must be amenable to the regeneration of the "hydroxyl" (i.e., amide) function under conditions which do not hydrolyze the O-anhydro bond. This latter requirement is imposed since an "eneamine" is needed in the desired rearrangement (see Scheme 1).

As a final note, an intriguing variation on the use of sulfur in the synthesis of C-nucleosides may be inferred from the work of T. Mukaiyama and coworkers[61, 62] (see Scheme 10). It is of particular importance to note two features of Scheme 10: 1) the reaction results in carbon alkylation even in the presence of an active N-H (i.e., indazole) and 2) the analogy between the (alkyl and aryl) dithioacetals and the dithio-acetal of a 2,4-3,5-di-O-benzylidine derivative of ribose (56)[63] is apparent. One could anticipate a comparable reaction between a suitable N-heterocyclic base and 56 in the presence of copper chloride-zinc chloride:



Such a reaction may in fact be enhanced by the formation of an N-copper salt. Thus the N-Grignards of imines[64] show exclusive C-alkylation and





Scheme 10. Metal-induced lability of a sulfur-benzyl (or alkyl) bond.[61, 62]

the N-Grignards of indoles may be induced to form predominantly C-alkylated products by the judicious choice of solvent and alkylating agent.[65,66]

EXPERIMENTAL

Infrared spectra were determined on a Perkin-Elmer 337 grating spectrophotometer. Nmr spectra were determined with a Varian HA-100 spectrometer; chemical shifts are expressed as parts per million (δ) down field from an internal standard of tetramethylsilane. Mass spectra were obtained with a CEC DuPont Model 21-110B spectrometer operated at 70eV. Thin layer chromatography (tlc) was done on silica gel G. Melting points were determined on a microscope hot stage and are uncorrected. Elemental analyses were by Heterocyclic Chemical Corp., Harrisonville, Missouri. Tetrahydrofuran (THF)[67] was prepared immediately before use by refluxing over and distilling from lithium aluminum hydride.[68] N, N-Dimethylformamide (DMF)[67] was dried by vacuum distillation from phosphorus pentoxide (10% by weight) and the distillate was stored over molecular sieves.

3-(2'-Tetrahydrofuranylthio)indole (8b).[13a]---To 100 ml of anhydrous dimethylformamide maintained under nitrogen was added 18 g (0.12 m) of 3-thioindole[41] followed by 3.2 g (0.134 m) of sodium hydride. After 40 minutes, 14.2 g (0.134 m) of 2-chlorotetrahydrofuran[69] was added dropwise and after 20 minutes reaction the mixture was poured into 500 g of crushed ice. The gummy product which separated was extracted with

methylene chloride (4 x 100 ml), the combined extracts were washed with water, dried over anhydrous sodium sulfate and the solvent was removed to provide the crude product as a viscous red oil. This crude product in 50 ml of methylene chloride was filtered through a short (5 cm) column of silica gel. After removal of solvent from the eluate, the product was crystallized from a mixture of ethyl acetate and hexane to yield 9 gm (34%) of colorless crystals, mp 90-94°. Recrystallization from 95% ethanol afforded colorless crystals: mp 98-100°, nmr (CCl_4) 1.8-2.4 m (3',4'-H), 3.8-4.15 m (5'-H), 5.3-5.42 m (2'-H), 7.02 d (J=2cps; 2-H), 7.1-7.2 m (5-7-H), 7.7-7.86 m (4-H), 8.48 br (N-H).

Anal. Calcd. for $C_{12}H_{13}NOS$: C, 65.8; H, 5.94; N, 6.39. Found: C, 65.8; H, 6.16; N, 6.18.

1-(2'-Tetrahydropyranyl)indole (9a).[13]---To a stirred solution of 2 g (0.017 m) of indole in 50 ml of freshly distilled tetrahydrofuran was added 7.7 ml of a hexane solution of n-butyllithium[70] (0.017 m). After five minutes, 2.04 g (0.017 m) of 2-chlorotetrahydropyran[71] was added. After 30 minutes the solvent was evaporated and was replaced with 50 ml of methylene chloride. The methylene chloride solution was washed with water (2 x 50 ml), dried and removed. The residue was chromatographed on a column of silicic acid (3 x 30 cm). 1-(2'-Tetrahydropyranyl)indole (9a), 1.44 g (42%), was eluted with 25% petroleum ether in benzene as a colorless

oil: bp 118° (0.2 mmHg); nmr (CCl_4) 1.4-2.1 m (3', 5'-H), 4.58 m (6'-H), 4.96 m (6'-H), 5.35 dd (J_1=8cps, J_2=4cps; 2'-H), 6.36 d (J=3.5cps; 3-H), 6.9-7.5 m (2,4-7-H); Mass spectrum, m/e (rel intensity), 201 (44), 144 (7), 130 (13), 117 (100), 85 (16).

Anal. Calcd. for $C_{13}H_{15}NO$: C, 77.6; H, 7.46; N, 6.96. Found: C, 77.3; H, 7.44; N, 6.87.

Reaction of 3-(2'-tetrahydrofuranylthio)indole (8b) with silver perchlorate in tetrahydrofuran. ---To a stirred, cooled (-15°) solution of 2.19 g (0.01 m) of 3-(2'-tetrahydrofuranylthio)indole (8b) in 75 ml of dry tetrahydrofuran was added a cooled (-15°) solution of 2.07 g (0.01 m) of silver perchlorate in 10 ml of dry tetrahydrofuran whereupon the solution immediately became yellow. After 30 minutes the precipitate which had formed was collected and resuspended in 50 ml of methanol and treated with hydrogen sulfide, the excess hydrogen sulfide being discharged with a nitrogen jet. The precipitated silver sulfide was removed with the filtrate passing directly into a suspension of ca 10 g of Raney nickel in 150 ml of 7N ammonium hydroxide. This mixture was heated under reflux on a steam bath for two hours. The hot mixture was then filtered to remove the Raney nickel which was washed with 95% ethanol. The combined filtrate was evaporated and the resulting residue was triturated with methylene chloride (3 x 25 ml). The methylene chloride soluble portion was sub-

jected to preparative chromatography on silica gel to yield two products: 1-(2'-tetrahydrofuranyl)indole (9b), (50 mg) and 2-(2'-tetrahydrofuranyl)-indole (10b), (5 mg). 1-(2'-Tetrahydrofuranyl)indole (9b): nmr (CCl_4) 1.62-2.16 m (3', 4'-H), 3.6-4.0 m (5'-H), 5.92 t (J=4cps; 2'-H) 6.30 d (J=3.5cps; 3-H), 6.8-7.3 m (2, 5-7-H), 7.4 m (4-H); mass spectrum, m/e (rel intensity), 187 (33), 130 (5), 117 (100), 71 (20), (see Fig.1); ir, no NH. 2-(2'-Tetrahydrofuranyl)indole (10b): nmr (CCl_4) 1.86-2.44 m (3', 4'-H), 3.84-4.02 m (5'-H), 5.12 dd (J_1=8cps, J_2=1cps; 2'-H), 6.2 m (3-H), 6.9-7.3 m (5-7-H), 7.44 m (4-H), 8.36 br (NH); mass spectrum, m/e (rel intensity), 187 (100), 154 (80), 144 (43), 130 (63), 117 (28), 71 (20), (see Fig. 1); ir (KBr) 3420 and 3310 cm^{-1}, NH.

The reactions[31] in the synthetic sequence leading to pyrazolo-[4, 3-d]pyrimidin-7-one (20) have been modified and improved and are included here.

Sodium ethyl acetopyruvate.[72]---In a 5 l round bottom flask, fitted with a reflux condenser and a mechanical stirrer and containing 2800 ml of anhydrous ethanol was added 125 g (5.4 m) of sodium metal during 1-2 hr. Stirring was continued as the solution cooled to room temperature. A mixture of diethyl oxalate (673 ml, 5m) and reagent grade acetone (366 ml, 5 m) was added over a period of ca. 8 hr. Stirring was continued for one hr. after the addition was completed; the sodium salt was then collected by vacuum filtration on two 40 cm Buchner funnels. The

collected salt was washed with ether and dried to yield 790 g (4.4 m, 88%).

Ethyl-3-methylpyrazole-5-carboxylate. ---To a stirred solution of 180 g (1 m) of sodium ethyl acetopyruvate in 1.2 l of water was added 84 ml (1 equiv.) of 12N hydrochloric acid. The reaction vessel was then cooled in an ice bath and a mixture of 72 g (1.2 m) of 85% hydrazine hydrate and 75 ml of water was added during 1-2 hr. The mixture was stirred for 1/2 hr. after addition was complete. When the product did not spontaneously crystallize the volume was reduced and the concentrated solution was cooled to yield almost colorless crystals, 82 g (53%) mp 82-83° ex ether.

3-Methylpyrazole-5-carboxylic acid.---A warm (85°) solution of 154 g (1 m) of ethyl 3-methylpyrazole-5-carboxylate in 1 ℓ of 1N sodium hydroxide (1 equiv) was stirred for 2 hr. The solution was treated with charcoal, acidified (pH3) using hydrochloric acid and allowed to cool. The product which crystallized was collected, 102 g (88%), mp 236-238° recrystallized from water.

3-Methyl-4-nitropyrazole-5-carboxylic acid.[33] --- To a warm, stirred mixture of fuming nitric acid (248 ml) and fuming sulfuric acid (378 g) was added 3-methylpyrazole-5-carboxylic acid (252 g, 2 m). The reaction was maintained at 85° until evolution of nitrogen dioxide ceased (1-6 hr. depending on the freshness of the nitric acid). The mixture was

then poured carefully onto 3 kg of crushed ice. The white product which separated was collected and washed with water to yield 360 g (2m, 100%, calculated as the monohydrate), mp 193° dec. ex water.

3-Methyl-4-nitropyrazole (25).[73]---3-Methyl-4-nitropyrazole-5-carboxylic acid (360 g, 2 m) was placed in an oil-jacketed vacuum flask and the flask was evacuated by a water aspirator. The flask was then heated at 140-160° until bubbling due to released carbon dioxide ceased. The melt was then carefully poured into 1 ℓ of hot water. When necessary the water was reheated to effect solution. The crystalline product which separated was collected, 196 g (1.5 m, 77%), mp 134°.

4-Nitropyrazole-3-carboxylic acid.[73]---To a round bottom flask containing a solution of 50 g (.38 m) of 3-methyl-4-nitropyrazole (25)[73] in 500 ml of water was affixed an extractor containing 187 g (3 equiv) of potassium permanganate. The solution was heated under reflux until all the potassium permanganate had been consumed. The manganese dioxide was then removed by filtration and the filtrate was acidified (pH3) with hydrochloric acid and cooled to yield pale yellow needles, 35 g (.22 m, 57%), mp 250°.

Ethyl-4-nitropyrazole-3-carboxylate.---Anhydrous hydrogen chloride was passed through a suspension of 20 g (.125 m) of 4-nitropyrazole-3-carboxylic acid in 150 ml of absolute ethanol heated under reflux. The reaction was continued until all the nitro acid was dissolved and

precipitation of an uncharacterized side product commenced (ca. 1.5-2 hr.). The solid by-product was removed and the filtrate was evaporated. The resulting crude ester was recrystallized from benzene to yield 19 g (.1 m, 75%), mp 126°.

Pyrazolo[4,3-d]pyrimidin-7-one (20).[31]---Ten grams (.054 m) of ethyl 4-nitro-3-pyrazolecarboxylate in 120 ml of methanol was hydrogenated over .3 g of platinum oxide under 30 psi of hydrogen pressure for a period of 2 hr. The catalyst was removed by filtration into 50 ml of formamide and most of the methanol was removed under reduced pressure. The formamide solution was heated at boiling temperature for 1.5-2 hr. during which time the volume was reduced to half. The solution was cooled, 50 ml of water was added and the mixture was treated with charcoal. After refrigeration overnight the precipitated product was collected to yield 4.7 g (.035 m, 65%), mp>300°, u.v., λmax (pH 1) 274 nm (ϵ=6800), λmax (pH 11) 283 nm (ϵ=6400).

3-Bromo-5-methyl-4-nitropyrazole (26).[33]---A solution of 2.52 g of bromine in 20 ml of methanol saturated with potassium bromide was added dropwise to a solution formed by dissolving 4 g of 3-methyl-4-nitropyrazole (25)[73] and 2.58 g of sodium acetate in 60 ml of methanol saturated with potassium bromide. The resulting solution was stored at room temperature overnight. The excess bromine was then discharged with sodium dithionite and the solvent was removed under reduced

pressure. The resulting residue was triturated with water and the product was collected by filtration, washed with water and dried to provide 4.52 g (70%) of colorless crystals, mp 145°, lit.[33] 145°.

3-(5-)-Bromo-5-(3-)-methyl-4-nitro-1-(2'-tetrahydropyranyl)-pyrazole (27,28). --- To 50 ml of ethyl acetate heated to 50° was added 2.05 g (10 mmole) of 3-bromo-5-methyl-4-nitropyrazole.[33] The solution was vigorously stirred and 3 mg of anhydrous p-toluenesulfonic acid was added. To this mixture was added dropwise a solution of 0.15 ml of 1,2-dihydropyran dissolved in 4 ml of ethyl acetate. This addition required 10 minutes. Heating was then discontinued and the solution was allowed to cool to room temperature. The cooled solution was treated with 40 ml of ice water and the aqueous phase was neutralized with aqueous ammonia. The ethyl acetate solution was washed twice more with water and finally dried over sodium sulfate. The ethyl acetate was removed under reduced pressure to provide 2.3 g (80%) of a thick syrup consisting of a mixture of 27 and 28 which was used in subsequent reactions without further purification.

An analytical sample, consisting of predominantly one isomer, was prepared by crystallization from 95% ethanol. The colorless, crystalline product had mp 143-148°, nmr ($CDCl_3$) 1.6-2.5 m (4',5'-H), 2.56 s (CH_3), 2.72 s (CH_3), 3.6-3.9 m (6'-H), 3.9-4.2 m (6'-H), 5.42 dd (J_1=3cps, J_2=8cps; 2'-H), 5.58 dd (J_1=3cps, J_2=9cps; 2'-H). Integration indicates that

the methyl singlet at δ 2.72 is associated with the doublet of doublets at δ 5.42 and that this isomer represents ca 67% of the mixture.

Anal. Calcd. for $C_9H_{12}BrN_3O_3$: C, 37.3; H, 4.17; N, 14.5. Found: C, 37.4; H, 4.44; N, 14.4.

3-(5-)-Methyl-4-nitro-1-(2'-tetrahydropyranyl)-5-(3-)-thiopyrazole(33, 34). Method A.---To a solution of 7 g of (27, 28) in 50 ml of 50% aqueous ethanol was added 2.3 g of thiourea and the resulting solution was refluxed for 36 hours. The ethanol was then removed at reduced pressure and the resulting aqueous suspension was adjusted to pH 9 with aqueous sodium hydroxide and was heated at 85° for 1.5 hours. The red solution was then cooled to room temperature and acidified to pH 1 with concentrated hydrochloric acid. The oily product which separated was extracted (4 x 20 ml) with ethyl acetate. The combined ethyl acetate extracts were washed with water, dried over sodium sulfate and solvent was removed to yield 4.6 g (80%) of 3-(5-)-methyl-4-nitro-1-(2'-tetrahydropyranyl)-5-(3-)-thiopyrazole (33, 34) as a viscous oil, ir (KBr), 2450 cm^{-1} (SH), 1570 cm^{-1} (N=N).

Method B.---To a solution of 10 g of (27, 28) in 150 ml of ethanol contained in a 500 ml round bottom flask was added a solution of 3.5 g of sodium hydrosulfide in 100 ml of water. The flask was tightly stoppered and heated in a steam cone for 5 hours. After standing overnight at room temperature a solid had deposited which was collected and discarded.

Solvent was removed from the filtrate and the resulting residue, as an aqueous suspension, was adjusted to pH 9 with aqueous sodium hydroxide and was treated with decolorizing charcoal. After removal of the charcoal, the filtrate was acidified to pH 1 with concentrated hydrochloric acid. The acidified suspension was extracted (3 x 100 ml) with ethyl acetate. The combined ethyl acetate extracts were dried with sodium sulfate and solvent was removed to provide 4.55 g (55%) of a mixture of 33 and 34 as a viscous oil. The material thus obtained was identical to the material prepared by method A. This material, as well as that prepared by method A, was used without further purification.

3-Methyl-4-nitro-5-(2'-tetrahydropyranylthio)pyrazole (36). --- A mixture of 1.82 gm (7.49 mmole) of a mixture of 33 and 34 and 50 ml of 40% acetic acid was stirred at room temperature overnight. The mixture was then evaporated to dryness under vacuum. The dry residue, dissolved in 5 ml of anhydrous dimethylformamide and maintained at ca. 10°, was treated with 0.18 g (1 eq; 0.35 gm as a 52% suspension in nujol) of sodium hydride. After 30 minutes, 0.90 gm (1 eq.) of 2-chlorotetrahydropyran[71] was added in one portion and the resulting solution was stirred for an additional 1.5 hours. After removal of solvent at reduced pressure, 20 ml of water was added and the resulting suspension was extracted (3 x 20 ml) with ethyl acetate. The combined ethyl acetate extracts were washed with water, dried over sodium sulfate and the solvent was removed to provide

the product as a yellow residue. Crystallization was affected with a mixture of ether and methanol to provide 0.64 g (35%) of pale yellow crystals, mp 156-159°. A colorless, crystalline sample, obtained by recrystallization from ethyl acetate, had mp 160-161°. Nmr (dmso-d_6) 1.40-2.20 m (4',5'-H), 2.50 s (CH_3), 3.40-3.72 m (6'-H), 3.72-4.04 m (6'-H), 5.86 m (2'-H), 13.6 br (NH).

Anal. Calcd. for $C_9H_{13}N_3O_3S$: C, 44.4; H, 5.38; N, 17.3. Found: C, 44.3; H, 5.43; N, 17.0.

1-Benzyl-3-bromopyrazolo[4,3-d]pyrimidin-7-one (22). ---To a solution of 1.84 g of 3-bromopyrazolo[4,3-d]pyrimidin-7-one (21)[32] in 50 ml of water containing an excess (>3 fold) of sodium hydroxide was added 1.6 g (1.5 eq.) of benzyl chloride. The resulting mixture was stirred overnight at room temperature; the basic solution was then extracted with ether (3 x 20 ml) and subsequently acidified with acetic acid to pH 5. The precipitated product was collected and dried at 85° to yield 2 g (73%) of colorless crystals. Recrystallization from 95% ethanol provided an analytical sample, mp > 250°, dec.; nmr (dmso-d_6, external TMS reference) 4.7 br (N-H), 5.72 s (CH_2), 7.26 s (Ph), 7.96 d (J=6cps; 5-H).

Anal. Calcd. for $C_{12}H_9BrN_4O$: C, 47.3; H, 2.95. Found: C, 47.7; H, 3.12.

3-Bromo-1, 6-dibenzylpyrazolo[4, 3-d]pyrimidin-7-one(23). --- To 0.5 g of 22 in 15 ml of anhydrous dimethylformamide was added 0.039 g (1 eq; 0.076 g as a 52% suspension in nujol) of sodium hydride. After 30 minutes 0.26 g (1 eq.) of benzyl chloride was added to the stirred suspension and stirring was continued for 30 minutes. The solvent was then removed under reduced pressure and water (30 ml) was added to the residue. The product was extracted with ethyl acetate (3 x 20 ml). The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and the solvent was removed. The residue was crystallized from hexane-ethyl acetate to yield 0.5 gm (77%) of colorless crystals, mp 107-109°. Recrystallization of a small sample from 95% ethanol raised the melting point to 110-111°. Nmr ($CDCl_3$) 5.14 s (6-CH_2), 5.72 s (1-CH_2), 7.28 s (Ph), 7.88 s (5-H); uv (methanol) λmax 232 (ϵ10300), λmax 295 (ϵ6400); ir (KBr) 1680 cm^{-1} (C=O); mass spectrum, m/e (rel intensity) 396 (17), 394 (17), 315 (82), 91 (100).

Anal. Calcd. for $C_{19}H_{15}BrN_4O$: C, 57.8; H, 3.79; N, 14.2. Found: C, 57.8; H, 3.85; N, 14.2.

Treatment of 3-bromopyrazolo[4, 3-d]pyrimidin-7-one (21)[32] with thiourea in 2N sulfuric acid (after Cullen and Harrison[74]). --- To 25 ml of 2N sulfuric acid was added 2.15 g of 21 and 1.9 g (2.5 eq.) of thiourea. This mixture was heated under reflux for three hours during which time no change was apparent, and after cooling, 21 was recovered by filtration.

Treatment of 3-bromopyrazolo[4, 3-d]pyrimidin-7-one (21) with thiourea in ethanol and dimethyl formamide. --- To a solution of 0.14 g (1 eq.) of thiourea in 30 ml of ethanol was added 0.4 g of 21 and the resulting suspension was heated under reflux for 72 hours during which time no change was apparent. Solvent was then removed and was replaced with 5 ml of dimethylformamide. This latter solution was heated under reflux for three hours resulting in a slight darkening of the solution. Water (20 ml) was added to the warm solution and cooling resulted in an amorphous precipitate of 21.

Treatment of 3-bromopyrazolo[4, 3-d]pyrimidin-7-one (21)[32] with sodium hydrosulfide in water. ---A solution of ca. 0.1 g of sodium hydrosulfide and 0.15 g of 21 in 50 ml of water was heated under reflux for 24 hours. The volume was then reduced and resulted in the precipitation of 21 contaminated with S_8 (as determined by mass spectroscopy).

Treatment of 3-bromopyrazolo[4, 3-d]pyrimidin-7-one (21) with sodium hydrosulfide as an intimate fusion. ---A slurry of ca. 1 g of sodium hydrosulfide and 1.3 g of 21 was formed in a small volume of water. The water was distilled and, by means of an air bath heated with a microburner, the mix was fused to a black pellet at 270-310°C. The black, glassy mass was dissolved in 5 ml of 1N sodium hydroxide. The basic solution was treated on the steam bath with charcoal and subsequently acidified to pH 5 with acetic acid which caused 21 to separate as an amorphous

precipitate.

Treatment of 1-Benzyl-3-bromopyrazolo[4, 3-d]pyrimidin-7-one (22) with sodium hydrosulfide in water. ---A solution of ca. 200 mg of sodium hydrosulfide and 200 mg of 22 in 7 ml of water was heated under reflux for three hours. The solution was then cooled and acidified with acetic acid to pH 5 which produced a precipitate of 22 contaminated with S_8.

Treatment of 1-Benzyl-3-bromopyrazolo[4, 3-d]pyrimidin-7-one (22) with sodium hydrosulfide in ethylene glycol. ---A solution of 1 gm of 22 in 25 ml of ethylene glycol was heated to 155-165°. As this solution was stirred, a solution of ca. 1 g of sodium hydrosulfide in 5 ml of ethylene glycol was added during 20 minutes. The solution was maintained at 155-165° for a total of two hours after which it was cooled to room temperature. Water (50 ml) was then added and the basic (pH≥11) solution was acidified to pH 5 with acetic acid. The amorphous solid which resulted was 22 contaminated with S_8.

Treatment of 3-Bromo-1, 6-dibenzylpyrazolo[4, 3-d]pyrimidin-7-one (23) with thiourea in ethanol and ethylene glycol. ---A solution of 270 mg (2 eq.) of thiourea and 700 mg of 23 in 10 ml of water was heated under reflux for 2 hours. No change was apparent on tlc (silica gel, ether), hence the sample was sealed in a glass tube and heated at 120° overnight. Again tlc showed only 23 and thiourea. The ethanol was then removed and

was replaced with 5 ml of ethylene glycol. The ethylene glycol solution was heated at 180° for one hour. Cooling produced crystalline 23.

Treatment of 3-Bromo-1, 6-dibenzylpyrazolo[4, 3-d]pyrimidin--7-one (23) with sodium hydrosulfide as an intimate fusion. ---A mixture of 0.5 g each of 23 and sodium hydrosulfide was finely ground together in a mortar. The mixture was placed in a tube in contact with a thermometer. The tube was evacuated to ca. 1 mm Hg. By means of an air bath heated with a micro-burner, the sample was quickly taken to 360° and held at that temperature for five minutes. The cooled pellet was removed and completely dissolved in water (10 ml) resulting in a solution of pH≥11. This solution was extracted with ether (1 x 10 ml). Tlc of the ether phase (silicic acid, benzene:ether 1:1) showed the absence of starting bromide, 23. The aqueous solution was acidified with acetic acid to pH 5 which resulted in the precipitation of S_8.

The acidic solution was extracted with ethyl acetate (3 x 20 ml), the extracts were combined, dried over anhydrous sodium sulfate and the solvent was removed. The residue was dissolved in aqueous sodium hydroxide, excess methyl iodide was added and the solution was allowed to stand overnight. The excess methyl iodide was then discharged with a nitrogen jet and the aqueous phase was extracted with ethyl acetate (3 x 20 ml). The extracts were combined, dried over anhydrous sodium sulfate and the

solvent was removed. The residue was demonstrated by mass spectrometry not to contain the displacement product, 1,6-dibenzyl-3-thiopyrazolo[4,3-d]pyrimidin-7-one (m/e 348) nor its S-methyl ether (m/e 362) nor its disulfide (m/e 694) and was not further characterized.

Treatment of 3-Bromo-1,6-dibenzylpyrazolo[4,3-d]pyrimidin-7-one (23) with sodium thiophenoxide as an intimate fusion. ---An intimate mixture of 1.4 mg of 23 and 1.4 mg (3 eq.) of sodium thiophenoxide was formed by grinding the mixture in a mortar. The mixture was heated to 275° in a tube by means of an oil bath. Upon reaching 275° the tube was removed from the oil bath and although no obvious fusion had occurred, the sample had darkened considerably. Some diphenyldisulfide had volatilized and condensed above the oil bath level. The brown pellet was carefully removed to minimize contamination by the disulfide. Water (5 ml) was added and the resulting suspension was extracted with ether (3 x 10 ml). The combined ether extracts were dried over anhydrous sodium sulfate and solvent was removed. The residue was a complex mixture as demonstrated by tlc (silica gel, benzene). Ultra violet visualization showed at least thirteen fluorescing spots. The entire residue was chromatographed on a preparative silicic acid plate (8 in. x 8 in. x .5 mm) developed with benzene. A blue fluorescing band of Rf. 0.75 was selected and the material was eluted from the silicic acid with ether. The mass

spectrum of this trace product was consistent with the structure 1,6-dibenzyl-3-(phenylthio)pyrazolo[4,3-d]pyrimidin-7-one (40). Mass spectrum m/e (rel intensity) 424 (69), 392 (1), 347 (1), 333 (4), 315 (14), 225 (9), 110 (20), 109 (9), 91 (100), 78 (14), 65 (14).

Reaction of S-(β-D-ribofuranosyl)-5-mercaptouracil (41)[43] with aqueous silver nitrate. ---To a solution of 61.5 mg (.362 mmole) of silver nitrate in 10 ml of water was added 100 mg (.362 mmole) of S-(β-D-ribofuranosyl)-5-mercaptouracil.[43] A pale yellow solid formed over a period of 10-15 minutes. After standing overnight at room temperature the solid was collected by filtration and after drying in air 89 mg (98%) of 5-mercaptouracil silver salt (43) was obtained. An attempt to recover the ribose from the mother liquors as the 2,4-dinitrophenylhydrazone was unsuccessful.

Anal. Calcd. for $C_4H_3AgN_2O_2S$: C, 19.1; H, 1.20; N, 11.2. Found: C, 19.1; H, 1.32; N, 11.4.

Attempted tritylation of S-(β-D-ribofuranosyl)-5-mercaptouracil (41).[43] ---To a solution of 221 mg of S-(β-D-ribofuranosyl)-5-mercaptouracil (41)[43] in 6 ml of dry dimethylformamide was added 40 ml of pyridine, freshly distilled from barium oxide, followed by 0.1 g of freshly crystallized trityl chloride. The resulting solution was maintained at room temperature for 24 hours. The solution was concentrated under reduced pressure and the addition of water caused the precipitation of essentially all of the added nucleoside 41 and an almost quantitative yield of triphenylcarbinol which was

easily removed by extraction with chloroform. This is a representative result of several experiments that were conducted at various temperatures and for various durations.

S-[5'-O-(t-Butyldimethylsilyl)-β-D-ribofuranosyl]-5-mercapto-uracil (49) (after the procedure of Ogilvie and Iwacha.[52]). --- To 9 ml of anhydrous dimethylformamide was added 2.37 g (9.31 mmole) of S-(β-D-ribofuranosyl)-5-mercaptouracil (41),[43] 1.46 g (23.3 mmole) of imidazole and 1.42 g (10.2 mmole) of t-butyldimethylchlorosilane.[50] The mixture was tightly stoppered and left 48 hours at room temperature. The solution was then diluted with 110 ml of methylene chloride and the resulting cloudy suspension was stored at 0° overnight. The resulting semi-crystalline solid was collected by filtration. The yield of solid, which exhibited two spots on tlc (silica gel, ethyl acetate) with Rf. 0.5 and Rf. 0.0, was 3.5 g. These two materials were easily separated by the following procedure. The crude product was refluxed for 15 minutes in ethyl acetate (1 g/100 ml) and the soluble portion (fraction 1) was separated from the insoluble portion (fraction 2) by filtration. This process was repeated until no further material was extracted, leaving fraction 2 as a tan gum. Fraction 1 was concentrated to half the original volume and filtered through a short (4 cm) column of silica gel with ethyl acetate as eluent. Concentration of fraction 1 to a small volume provided 1.47 g (44%) of S-[5'-O-(t-butyldimethylsilyl)-β-D-ribofuranosyl]-5-mercaptouracil (49) as colorless crystals, mp 210-213°.

Anal. Calcd. for $C_{15}H_{26}N_2O_6SSi$: C, 46.1; H, 6.71; N, 7.17. Found: C, 46.2; H, 7.02; N, 7.28.

Reaction of S-[5'-0-(t-butyldimethylsilyl)-β-D-ribofuranosyl]-5-mercaptouracil (49) with p-toluenesulfonyl chloride. The formation of S-{3'-[5'-(t-butyldimethylsilyloxymethyl)furanyl]}-5-mercaptouracil (50). --- To 15 ml of dry pyridine was added 300 mg of S-[5'-0-(t-butyldimethylsilyl)-β-D-ribofuranosyl]-5-mercaptouracil (49) and 149 mg (1.02 equivalents) of p-toluenesulfonyl chloride. The resulting solution was heated (60-70°) for 3 hours and then poured into ice water. The resulting mixture was extracted with ethyl acetate, the combined ethyl acetate extracts were washed with a dilute solution of copper sulfate, and water, dried over sodium sulfate and evaporated under reduced pressure. The resulting residue was chromatographed on two silica gel plates (8 in. x 8 in. x .3 mm) eluted with ether. The product, a blue fluorescent band at Rf. 0.6, was isolated and extracted from the adsorbent with acetone. After evaporation of the acetone, a minor yellow impurity was removed by trituration with ethyl acetate leaving 8 mg (3%) of 50 as a colorless powder, mp 240-243°, dec.; nmr (dmso-d_6) 0.86 s (t-butyl), 6.40 s (4-H, furanyl), 7.58 s (2-H, furanyl), 4.57 s (CH_2), 7.70 s (6-H, uracil); mass spectrum m/e (rel intensity) 339 (3), 297 (100), 279 (7), 251 (5), 223 (20), 180 (10), 169 (5), 153 (13), 143 (4), 112 (3), 97 (6), 75 (21), 69 (24).

Anal. Calcd. for $C_{15}H_{22}N_2O_4SSi$: C, 50.8; H, 6.25; N, 7.90. Found: C, 51.0; H, 6.28; N, 8.03.

Reaction of S-[5'-0-(t-butyldimethylsilyl)-β-D-ribofuranosyl]-5-mercaptouracil (49) with 2-acetoxyisobutrylchloride[50] (after the procedure of Greenberg and Moffatt[54]). ---A solution of 164 mg of 2-acetoxyisobutrylchloride[50] in 3 ml of freshly distilled nitromethane was added to 390 mg of S-[5'-0-(t-butyldimethylsilyl)-β-D-ribofuranosyl]-5-mercaptouracil (49). The suspension was stirred at room temperature for 30 minutes which resulted in darkening. Filtration provided 221 mg of purple solid. Addition of absolute ethanol to this solid caused the color to fade. Tlc of the ethanol solution indicated the absence of 49 and that the material is a complex mixture. Nmr (dmso-d_6) of the solid shows that the t-butyldimethylsilyl group is absent.

Reaction of S-(β-D-ribofuranosyl)-5-mercaptouracil[43] with 2-acetoxyisobutrylchloride[50] (after the procedure of Greenberg and Moffatt[54]). ---To a cooled (0°) solution of 188 mg (0.68 mmole) of S-(β-D-ribofuranosyl)-5-mercaptouracil (41)[43] in 8 ml of dimethylformamide was added 67 mg (3 eq.) of 2-acetoxyisobutryl chloride.[50] The resulting solution was kept at room temperature for three hours. The solution was then treated with 25 ml of methylene chloride and 100 ml of ether. The resulting, cloudy suspension was kept at -15° until all the turbidity had settled. The solution phase was then decanted and the solid residue was dried with a

nitrogen jet. Nmr (dmso-d_6) of the solid indicated peaks between δ1.2-2.2 which were consistent with the signals expected[54] for the adducts. It was clear also that the solid was a mixture; this was confirmed by tlc. Attempts to separate the components from this mixture by either chromatography or by fractional crystallization were unsuccessful.

Reaction of D-mannopyrano[cis-1,2-b]dihydrobenzothiazine (42)[44] with aqueous silver nitrate. ---To a solution of 58 mg (0.216 mmole) of D-mannopyrano[cis-1,2-b]dihydrobenzothiazine (42)[44] in 30 ml of water-ethanol (3:1) was added 40 mg (0.235 mmole) of silver nitrate dissolved in 0.5 ml of water. The resulting solution was heated on a steam bath for 2.5 hours which caused considerable darkening and the deposition of solid. The mixture was then treated with 5 ml of a saturated sodium chloride solution which contained three drops of concentrated hydrochloric acid. This had no visible effect on the mixture. The dark insolubles were removed by Celite aided filtration and solvent was removed from the filtrate under reduced pressure. In an attempt to methylate all ionizable sites, the resulting residue was treated, according to Kuhn and Trischmann,[75] with 15 ml of dimethylformamide, 5 ml of water, 0.1 g of barium oxide, 0.1 g of barium hydroxide hexahydrate and .4 g of methyl sulfate. This mixture was stirred overnight at room temperature, treated with 1 ml of concentrated aqueous ammonia, stirred 0.5 hour and diluted with 30 ml of chloroform. The diluted mixture was poured into 100 ml of ice water, the

chloroform layer was separated, the aqueous phase was extracted with 20 ml of chloroform and the combined chloroform extracts were washed with water (3 x 100 ml), dried over sodium sulfate and evaporated under reduced pressure to provide 17 mg of a dark glass. Tlc of this residue indicated it to be a very complex mixture.

The original insoluble portion collected on Celite was extracted with dimethylsulfoxide and treated in the same fashion.[75] The results were similar.

6'-O-(t-Butyldimethylsilyl)-D-mannopyrano[cis-1,2-b]dihydrobenzothiazine (51) (after the procedure of Ogilvie and Iwacha[52]). ---To 1 ml of anhydrous dimethylformamide was added 0.269 g (1 mmole) of D-mannopyrano[cis-1,2-b]dihydrobenzothiazine (42),[44] 0.165 g (1.1 mmole) of t-butyldimethylchlorosilane[50] and 0.17 g (2.5 mmole) of imidazole. The tightly stoppered mixture was allowed to stand 24 hours at room temperature. The solution was then diluted with 20 ml of ether and poured into 30 ml of ice water. The ether layer was separated and the aqueous phase was extracted (2 x 10 ml) with fresh ether. The combined ether extracts were washed with fresh ice water (2 x 10 ml), dried over anhydrous sodium sulfate and evaporated under reduced pressure to an almost colorless gum. This gum was dissolved in a small volume of ether, pentane was added to the cloud point and the sample was stored overnight at -15°. The yield of

crude, although colorless crystals, was 0.228 g (60%), mp 70°. (The product had the distinct odor of t-butyldimethylchlorosilane.) The material recrystallized from carbon tetrachloride had mp 122-125°.

Anal. Calcd. for $C_{18}H_{29}NO_4SSi$: C, 56.4; H, 7.62; N, 3.65. Found: C, 56.02; H, 7.47; N, 3.51.

Reaction of 6'-0-(t-Butyldimethylsilyl)-D-mannopyrano[cis-1,2-b]-dihydrobenzothiazine (51) with silver perchlorate in various solvents. --- Several mg of 6'-0-(t-butyldimethylsilyl)-D-mannopyrano[cis-1,2-b]dihydrobenzothiazine (51) dissolved in acetonitrile, benzene, ether, methanol and tetrahydrofuran were treated with silver perchlorate as a solution in the corresponding solvent. Examination of the resulting mixtures by tlc indicated, in every case but acetonitrile, that 51 was streaking from the origin. The solution in acetonitrile showed 51 to chromatograph as a well defined spot with no streaking. Addition of pyridine to the mixtures caused the streaking to disappear; 51 chromatographed as a well defined spot. Addition of sodium hydride or potassium-t-butoxide to any of the mixtures caused an immediate black precipitate. The mixture in benzene became opalescent while that in ether deposited a white precipitate.

Reaction of 6'-0-(t-butyldimethylsilyl)-D-mannopyrano[cis-1,2-b]-dihydrobenzothiazine (51) with silver perchlorate in ether. --- To a solution of 31.4 mg of 6'-0-(t-butyldimethylsilyl)-D-mannopyrano[cis-1,2-b]dihydro-

benzothiazine (51) in 2 ml of ether was added a solution of 16.0 mg (1 eq.) of silver perchlorate as a solution in 3 ml of ether. Immediately a white solid formed. After 1.5 hours at room temperature and 3 hours at -15°, the solvent was decanted and the white solid was dried with a nitrogen jet. The yield of white solid, assigned structure 52, was 42 mg (87%).

Reaction of 6'-O-(t-butyldimethylsilyl)-D-mannopyrano[cis-1,2-b]-dihydrobenzothiazine (51) with silver perchlorate and methyl iodide in benzene. --- To a solution of 20.7 mg of 6'-O-(t-butyldimethylsilyl)-D-mannopyrano[cis-1,2-b]dihydrobenzothiazine (51) in 2 ml of benzene was added a solution of 11.1 mg (1 eq.) of silver perchlorate in 3 ml of benzene. Immediately a white precipitate formed which almost completely redissolved over a period of two hours at room temperature when excess methyl iodide was added. After stirring the resulting mixture for four hours at room temperature a dark reddish solid had deposited which was collected by decanting the colorless benzene layer. No solid residue remained when the benzene phase was evaporated. The dark solid was triturated with methanol which formed a colored solution and left a black solid which was removed by filtration. The methanol was evaporated from the filtrate to provide a brown residue. Nmr (dmso-d_6) of this brown, chloroform insoluble residue showed broad signals; of particular importance was the absence of the t-butyl group of the silyl substituent and methyl singlets. No assignment was possible.

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BIOGRAPHICAL NOTE

The author was born 3 August 1942, in Drumright, Oklahoma. In 1944 he moved to Los Angeles, California and there attended parochial grammar schools and graduated Cathedral High School in 1960. He then entered St. Mary's College of California where he received his Bachelor of Science degree in June 1964.

In September 1964 the author began a teaching position at Notre Dame High School, Sherman Oaks, California. For the period September 1965 to June 1968 he taught at Sacred Heart High School, San Francisco, California.

The author then began study at California State University at San Francisco which studies were completed in March 1970 with the degree Master of Science.

In the same month he began study at the Oregon Graduate Center where he completed the requirements for the degree Doctor of Philosophy in February 1974. In his last year of study at the Graduate Center the author held a Gottesman Foundation Fellowship.

The author has been married ten years to the former Geraldine Woods and they have two children, Damian, age 8, and Jennifer, age 5.

He is leaving the Graduate Center to accept a post doctoral position with ICN Pharmaceuticals--Nucleic Acid Research Institute, Irvine, California.