UNIVERSITY OF MANITOBA

THE SYNTHESIS AND STUDY OF ANHYDROPURINE NUCLEOSIDES

by

LEWIS ALEXANDER SLOTIN

A Thesis
Submitted to
The Faculty of Graduate Studies and Research
of the University of Manitoba
in Partial Fulfillment
of the Requirements for the
Degree of Doctor of Philosophy

Winnipeg, Manitoba
September 1972

To my wife Linda

ACKNOWLEDGEMENTS

It has been my great fortune to be guided and inspired in this work by Dr. Kelvin Kenneth Ogilvie.

I would like to thank Dr. J.B. Westmore and Dr. D. Lin for their expert work on the mass spectral analyses of the anhydro nucleosides. I would also like to thank Mrs. Pat Rheault for the discussions we had concerning the mechanism of enzyme action and also her gifts of 5'-O-monomethoxytritylthymidine and 5'-O-monomethoxytritylthymidine 3'-phosphate.

I would like to thank Mr. Henry Chan and Mr. Don Iwacha for the gifts of 2',3'-O-isopropylideneuridine.

Financial assistance from the National Research Council of Canada and the University of Manitoba is gratefully acknowledged.

ABSTRACT

The aim of this investigation was a) the development of methods of synthesis of anhydropurine nucleosides, b) the conversion of these molecules to their corresponding nucleotides, c) the examination of the enzyme substrate activity of these modified nucleosides and nucleotides and d) the use of anhydro molecules in dinucleoside monophosphate synthesis.

Initially the first reported synthesis of 8,2'-thioanhydroguanosine is described(Scheme V). The synthesis involves the selective blocking of 8-bromoguanosine by first the N^2-amino group with dimethylaminomethylene followed by the blocking of the 5'-hydroxyl with the monomethoxytrityl group. The introduction of the mesitylenesulfonyl group to the 2'-position provides a leaving group for the 8,2'-cyclization. Following deblocking 8,2'-thioanhydroguanosine is obtained by treatment of 2'-O-mesitylenesulfonyl-8-bromoguanosine with sodium hydrogen sulfide. Structural identification was provided by Raney nickel reduction to the commercially available 2'-deoxyguanosine. 8,3'-Thioanhydroguanosine was synthesized in a similar manner(Scheme VI), this time employing the methanesulfonyl group on the 3'-position as leaving group. The product obtained was reduced to 3'-deoxyguanosine, an analog of Cordycepin.

A general method for synthesis of 8,2'-thioanhydro derivatives of adenosine, guanosine and inosine was accomplished in good yield by conversion of the corresponding 8-bromo derivative to its' 2',3'-carbonate. Treatment of these with thiourea or sodium hydrogen sulfide gives the 8,2'-thioanhydro molecules directly(Schemes VII and VIII). An alternative general method which provides either 8,2'-thio or 8,2'-aminoanhydropurine nucleosides from the 8-thio or 8-amino derivatives involves heating these derivatives in dimethylformamide in the presence of diphenyl carbonate and sodium bicarbonate(Schemes X and XI).

The 5'-monophosphates of 8,2'-thioanhydroadenosine, guanosine and inosine were obtained in excellent yield directly from the nucleoside by reaction with partially hydrolyzed phosphoryl chloride. From these nucleotides the 5'-diphosphates were synthesized by first conversion to the 5'-phosphoromorpholidates followed by phosphorylation using inorganic phosphoric acid and dicyclohexylcarbodiimide.

8,2'-Thio and aminoanhydroadenosine were substrates of adenosine deaminase and were quantitatively converted to the corresponding anhydroinosines. The 5'-monophosphates of the thioanhydro derivatives were not substrates of 5'-nucleotidase but were dephosphorylated with alkaline phosphatase. 8,2'-Thioanhydroadenosine 5'-mono and diphosphates were not affected by the action of adenylate kinase(myokinase).

For the synthesis of dinucleoside monophosphates containing the 8,2'-thioanhydro nucleosides 5'-O-monomethoxytrityl-N^6-benzoyl-8,2'-thioanhydroadenosine, $N^6,O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine, 5'-O-monomethoxytrityl-N^2-acetyl-8,2'-thioanhydroguanosine and 5'-O-monomethoxytrityl-8,2'-thioanhydroinosine were synthesized. While numerous dinucleoside monophosphate syntheses were attempted those successful were 1) the condensation of 5'-O-monomethoxytritylthymidine 3'-phosphate and $N^6,O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine using mesitylenesulfonyl chloride or DCC to give thymidylyl(3'-5')N^6-benzoyl-8,2'-thioanhydroadenosine, 2) the condensation of 5'-O-monomethoxytrityl-N^6-benzoyl-8,2'-thioanhydroadenosine and $N^6,O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine using DCC to give 8,2'-thioanhydroadenylyl(3'-5')8,2'-thioanhydroadenosine, 3) the condensation of 5'-O-monomethoxytrityl-N^2-acetyl-8,2'-thioanhydroguanosine and $N^6,O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine to give 8,2'-thioanhydroguanylyl(3'-5')8,2'-thioanhydroadenosine using DCC and 4) the condensation of 5'-O-monomethoxytrityl-8,2'-thioanhydroinosine and $N^6,O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine using DCC to give 8,2'-thioanhydroinosinyl(3'-5')8,2'-thioanhydroadenosine.

The latter three dinucleoside monophosphates were completely resistant to the action of spleen or snake venom phosphodiesterase. However thymidylyl(3'-5')N^6-benzoyl-8,2'-thioanhydroadenosine was degraded by spleen phosphodiesterase but unaffected by snake venom phosphodiesterase suggesting that the enzyme which must recognize the "anhydro" end of the dinucleoside monophosphate is inhibited by this mutation.

ABBREVIATIONS

DMAM	dimethylaminomethylene
MMTrCl	monomethoxytrityl chloride
TrCl	trityl chloride
MSCl	mesitylenesulfonyl chloride
MsCl	methanesulfonyl chloride
TsCl	toluenesulfonyl chloride
BzCl	benzoyl chloride
Ac_2O	acetic anhydride
Bz_2O	benzoic anhydride
cc	cyclic carbonate
pm	phosphoromorpholidate
p	phosphate
Isp	isopropylidene
Ac	acetyl
DMF	dimethylformamide
THF	tetrahydrofuran
EtOAc	ethyl acetate
HOAc	acetic acid
EtOH	ethanol
$POCl_3$	phosphoryl chloride
$(EtO)_3PO$	triethyl phosphate
DCC	dicyclohexylcarbodiimide
NaSH	sodium hydrogen sulfide
DPC	diphenyl carbonate
ATP	adenosine 5'-triphosphate
ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
Rm(Tp)	mobility on electrophoresis relative to thymidine 3'-phosphate
t.l.c.	thin-layer chromatography

Nucleosides

A	adenosine
G	guanosine
I	inosine
X	xanthosine
U	uridine
T	thymidine

Anhydropurine nucleosides and dinucleoside monophosphates(ex.)

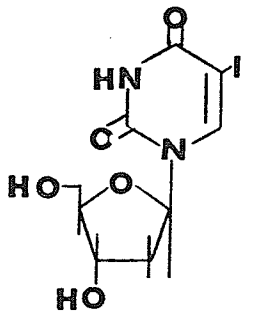
8,2'-SanhA	8,2'-thioanhydroadenosine
8,2'-NanhG	8,2'-aminoanhydroguanosine
8,2'-SanhI(3'-5')8,2'-SanhA	8,2'-thioanhydroinosinyl(3'-5')8,2'-thioanhydroadenosine

TABLE OF CONTENTS

INTRODUCTION

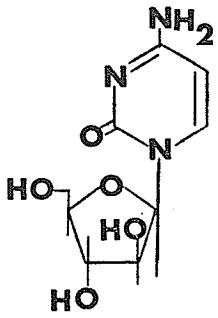
Within recent years a great deal of scientific interest has been directed towards the molecular biology of the cell and the genetic material which it contains. This material, comprised of DNA(deoxyribonucleic acid) and RNA(ribonucleic acid), is involved in all aspects of genetic expression. Both information storage and translational roles are carried out by the nucleic acids and hence these molecules have a fundamental role in the life process as we understand it. Since mutations or changes in the structure of the nucleoside or nucleotide units of the nucleic acids are believed to be directly connected to physical and mental diseases, it is therefore very important that these modified molecules be synthesized in order to facilitate an understanding of these biologically important mutagens.

It has been reported that modified nucleosides and nucleotides which do not occur naturally in DNA or RNA, may have significant biological importance. Specifically 5 -iodo-2'-deoxyuridine(I), a nucleoside modified in the 5-position of the pyrimidine

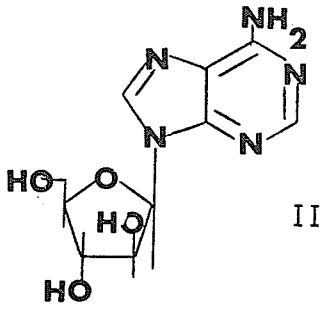


1

ring, has been used as a therapeutic agent in the treatment of an eye infection, herpes keratitis[1]. Moreover arabinocytidine[2](II) is effective as an anti-leukemic agent[3], and arabinoadenosine[4](III) has been described as a broad spectrum anti-DNA viral agent in vitro and in vivo[5]. These latter two molecules represent a growing list of



II

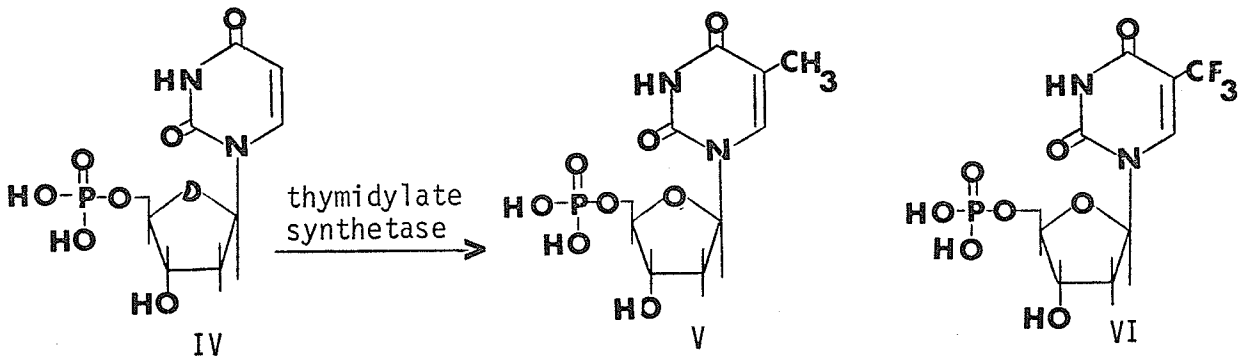


III

modified nucleic acid components which feature modification of the sugar moiety. This clearly illustrates that the chemical modification of naturally occurring nucleosides can lead to significant biological compounds. An example of where such a molecule is incorporated into a growing RNA molecule is the work of Fraenkel-Conrat et al.[6] which demonstrates the change in genetic properties of polycytidylic acid when the cytosine base is modified in the 5-position by bromine. When this molecule is then translated by RNA polymerase 5'-ATP is incorporated, in proportion to the bromine content, into the polyguanylic acid formed.

Another aspect of modified nucleosides and nucleotides is their use as substrates or inhibitors of various metabolizing enzymes. From this activity information may be obtained to aid in the elucidation of the enzyme mechanism. Thymidylate synthetase, the enzyme which catalyzes(Scheme I) the reductive methylation of

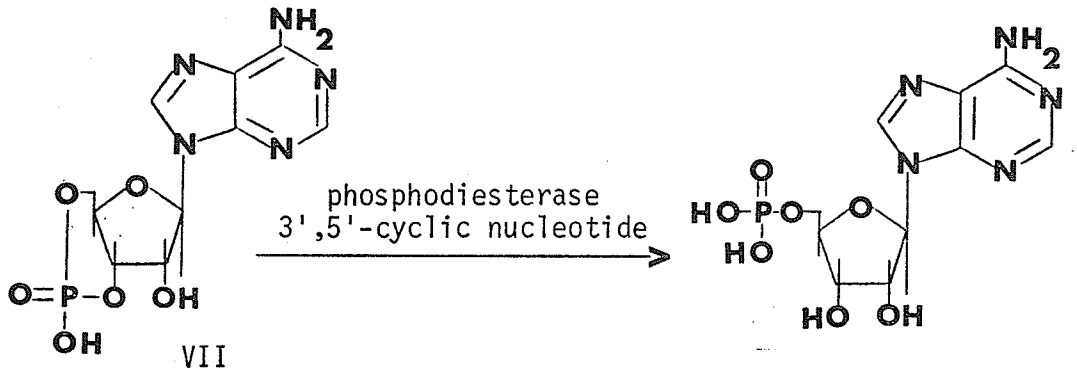
Scheme I The Action of Thymidylate Synthetase



IV V VI

2'-deoxyuridine 5'-monophosphate(IV) to thymidine 5'-monophosphate(V), is strongly and irreversibly inactivated by 5-trifluoromethyl-2'-deoxyuridylic acid(VI)[7]. The use of this substrate analog has provided considerable information concerning the functional groups involved at the active site of the enzyme[8]. Also the enzyme phosphodiesterase 3',5'-cyclic nucleotide is of considerable biological interest since it controls(Scheme II) the levels of adenosine 3',5'-

Scheme II Action of phosphodiesterase 3',5'-cyclic nucleotide



VII

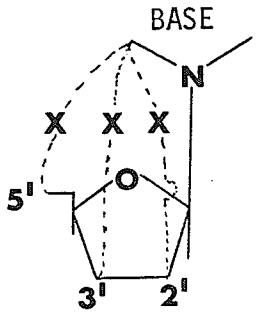
cyclic monophosphate(cAMP)(VII), a compound shown to stimulate the ATP dependent phosphorylation of a wide variety of proteins[9]. The synthesis of several 8-substituted adenosine 3',5'-cyclic monophosphate derivatives[10] has provided a number of potent inhibitors of this enzyme.

A recently recognized area of modified nucleosides which is attracting considerable attention is that of anhydronucleosides. These compounds feature, in addition to the N-glycoside linkage, a covalent linkage either directly[11-15] or via bridging atoms between the 2',3', or 5'-carbons of the sugar and a carbon or nitrogen atom(other than the nitrogen of the glycoside bond) of the purine or pyrimidine ring. Subsequently these anhydronucleosides contain both a modified base and modified sugar. While naturally occurring nucleosides rotate

freely about the N-glycoside bond[16] such rotation is prevented in the rigid anhydronucleosides. This characteristic permits the spectroscopic study of a particular nucleoside conformation[17-19] and moreover provides an excellent model for studying the effects of restricted rotation and conformation on enzyme activity.

Anhydronucleosides containing a thio ether, an ether or an amino group as its' bridging atoms(Figure 1) can usually undergo

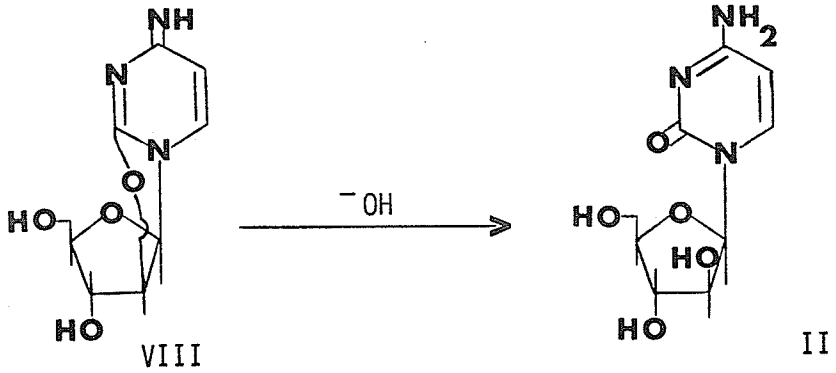
Figure 1 General structure of anhydronucleosides containing a hetero atom in the cyclo linkage



X = S, O or NH

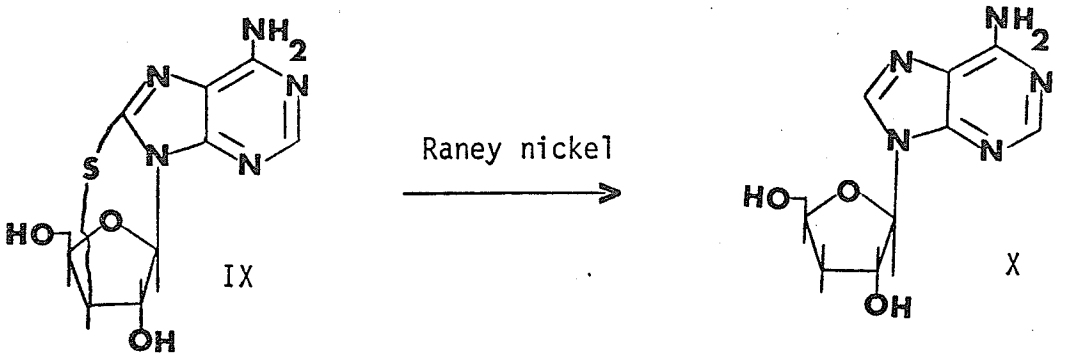
acid or base hydrolysis of the cyclo linkage to ribo, arabino or xylonucleosides[20]. Nucleophilic displacement of the cyclo linkage by benzoate, mercaptan, thiocyanate etc. leads to nucleosides modified on the sugar moiety[20-22] and occasionally on the base moiety[20, 23-25]. Arabinocytidine(II)(obtained by the basic hydrolysis of O^2,2'-anhydrocytidine[26](VIII, Scheme III) and cordycepin(X)

Scheme III Basic Hydrolysis of O^2,2'-anhydrocytidine



(obtained by the reduction of 8,3'-thioanhydroadenosine[25](IX, SchemeIV)

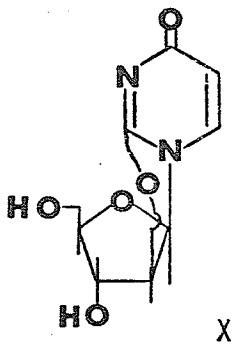
Scheme IV Reduction of 8,3'-thioanhydroadenosine



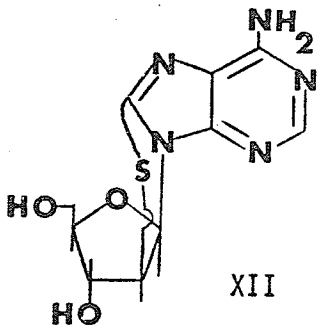
are but two of the biologically important compounds obtained from cyclonucleosides. Moreover anhydronucleosides have recently been detected as intermediates in proposed prebiotic syntheses[27,28].

Another valuable feature of anhydronucleosides is their potential for ribonucleotide synthesis. In ribonucleotide synthesis the problem of the specific preparation of the naturally occurring $C_{3'}$-$C_{5'}$ internucleotide linkages is complicated by the presence of the vicinal cis-hydroxyl groups in the ribofuranose moiety. It is this same factor which radically alters the hydrolytic and general chemical behaviour of the polyribonucleotides as compared to the polydeoxyribonucleotides. For synthetic work the requirement is one of preparing appropriately blocked ribonucleoside and ribonucleotide derivatives. The protecting groups must be so designed that they can be removed at the end without damage to the phosphodiester linkage and also avoid migration to the adjacent hydroxyl group.

The selective introduction of a blocking group to the 2'-position almost invariably results in a mixture of 2' and 3'-isomers[29-31]. This is further complicated by the fact that acyl blocking groups tend to migrate from the 2' to the 3'-position[32,33]. As a result the synthesis of RNA molecules is not nearly as extensively developed as that of DNA molecules[34]. However anhydronucleosides such as O^2,2'-anhydro-1-β-D-arabinofuranosyluracil(XI) or 8,2'-8-mercapto-9-β-D-arabinofuranosyladenine(XII) have the



XI

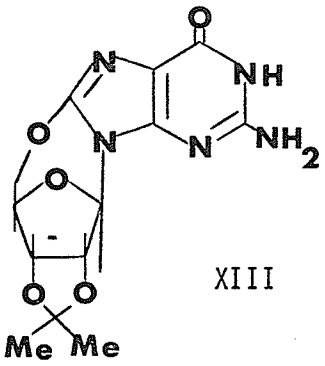


XII

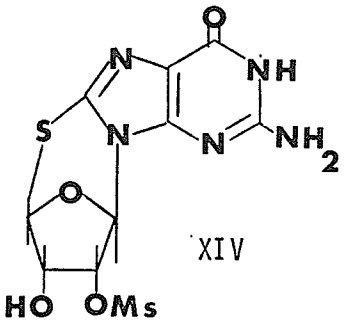
2'-position effectively blocked. The sugar portion now resembles a 2'-deoxyribose sugar for which several versatile methods have been developed to produce oligodeoxynucleotides[35-38]. Such methods should then be directly applicable to the synthesis of anhydroribonucleotides and in fact several recent reports[39-42] have confirmed this possibilty. If the anhydro linkage could then be displaced as mentioned earlier, to regenerate the ribonucleoside structure, this would then offer a convenient synthesis of RNA molecules where the anhydrolinkage is used as a 2'-hydroxyl blocking group.

It is therefore of great importance that methods of synthesis of anhydronucleosides be developed so that these molecules may be used as 1)models for enzyme action, 2)intermediates in the further chemical modification of nucleosides and 3)components of oligonucleotide synthesis. Because of these potentialities numerous syntheses of cyclonucleosides have been reported, particularly in the pyrimidine series. Hampton and Nichol[43] developed an exceedingly simple synthesis of O^2,2'-anhydrouridine(XI) and other procedures have been developed for the synthesis of 5' and 3'-cyclouridines[44-46] as well as cyclo derivatives of thymidine[47,48] and cytidine[26,27]. In the purine series adenosine is the only nucleoside for which 8,2'-,8,3'- and 8,5'-anhydro derivatives have been extensively developed [25]. Even so the syntheses of the S and O anhydro derivatives are often low yielding and occasionally irreproducible. Synthesis ofanhydronucleosidesof purines other than adenosine

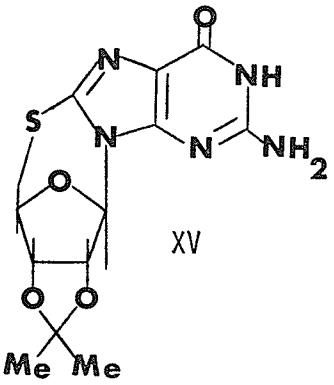
have been limited to 2',3'-O-isopropylidene-8,5'-anhydro-8-oxyguanosine[49](XIII), 2'-O-mesyl-8,5'-anhydro-8-mercapto-guanosine[50](XIV) and 2',3'-O-isopropylidene-8,5'-anhydro-8-mercaptoguanosine[51](XV). Thus the synthesis of the 8,2'-anhydropurine nucleosides has not been generally developed.



XIII



XIV



XV

It is then the object of this project to develop convenient methods of synthesis of the 8,2'-thioanhydropurine nucleosides and mononucleotides, to examine their behaviour towards various enzymes and to investigate the possibility of utilizing these molecules in oligoribonucleotide synthesis.

DISCUSSION AND RESULTS

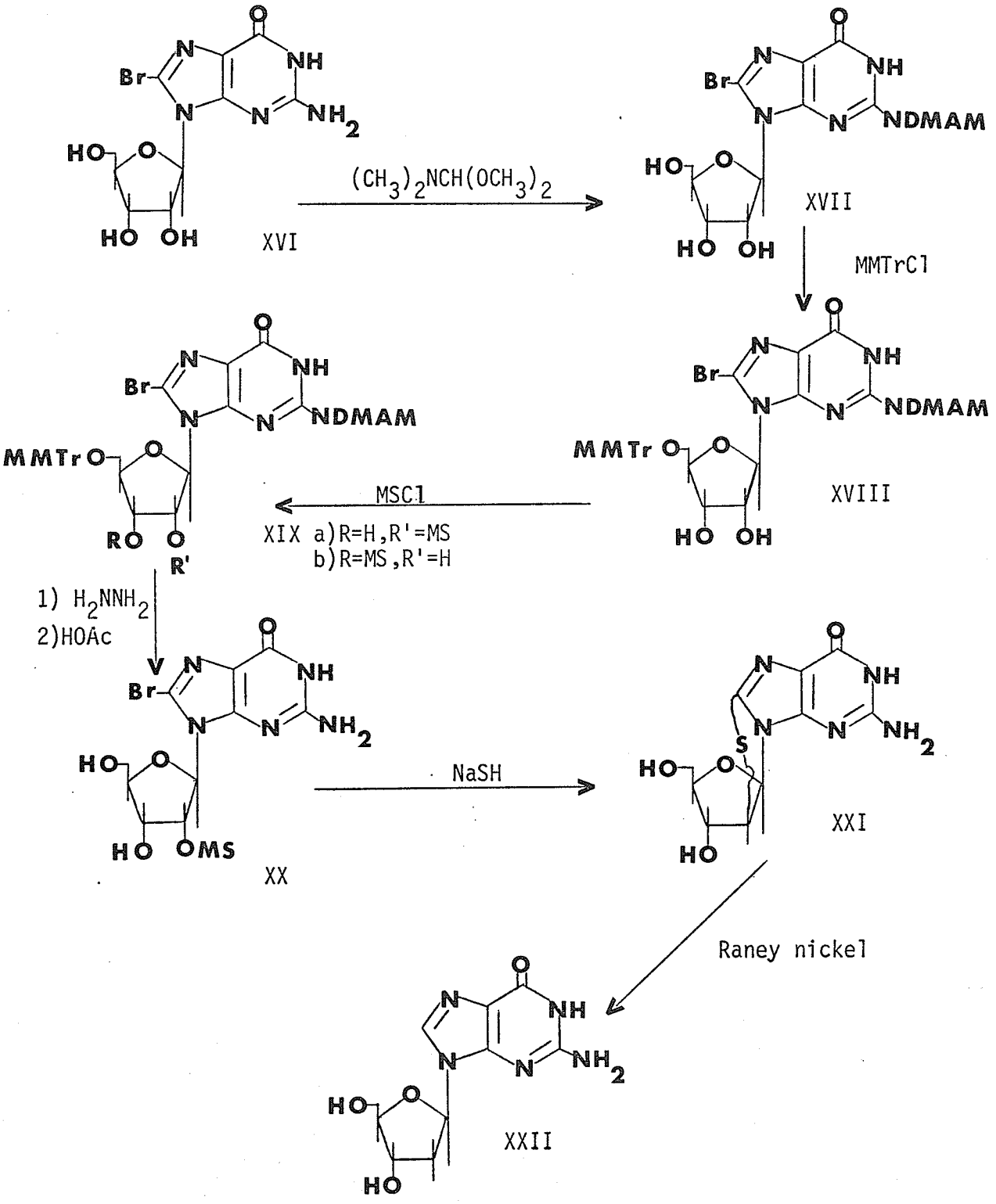
Synthesis of Anhydropurine Nucleosides

The most efficient syntheses of 8,2'-thioanhydropurine nucleosides have employed simultaneous displacement of a halogen from the 8-position of the base and a good leaving group(such as a sulfonate) from the 2'-position of the sugar of the parent nucleoside[52]. It was therefore felt that this procedure could be applied to the synthesis of 8,2'-thioanhydroguanosine, a new cyclonucleoside of guanosine. The synthesis of 8,2'-thioanhydroguanosine is outlined in Scheme V. The 2-NH_2 group of 8-bromoguanosine[53](XVI) was protected with the dimethylaminomethylene group[54]. The reaction between 8-bromoguanosine(XVI) and excess dimethylformamide dimethylacetal gives 8-bromo-N^2-dimethylaminomethyleneguanosine(XVII) in 95% yield. Compound XVII was quantitatively converted to the 5'-O-monomethoxytrityl derivative(XVIII).

The essential step in the synthesis is the introduction of a good leaving group to the 2'-position. Once this is achieved the 8,2'-thio bridge can be introduced in a single step[52]. The monomethoxytrityl group on the 5'-position screens the 3'-hydroxyl much more than it does the 2'-hydroxyl. As a result large sulfonating agents or triphenylmethyl chlorides react preferentially or exclusively at the 2'-position[45,52].

For these reasons XVIII was reacted with mesitylenesulfonyl chloride(MSCl). MSCl is a bulky sulfonating agent but one that does react with hydroxyl groups at a reasonable rate[55,56]. MSCl reacted with XVIII in dry pyridine at room temperature for two days to produce XIXa in 42% yield. Starting material XVIII was also recovered(34%) from the reaction

Scheme V Synthesis of 8,2'-thioanhydroguanosine via 2'-O-mesitylenesulfonyl-8-bromoguanosine



mixture. While others[52] have detected small amounts of 3'-isomers from similar reactions, here there was no detectable XIXb. Generally a 3'-substituted nucleoside will have a slower chromatographic mobility than the 2'-isomer on silica gel t.l.c.[52,57]. The structure of XIXa was confirmed by the subsequent steps which converted it into the 8,2'-thioanhydro-nucleoside XXI and 2'-deoxyguanosineXXII.

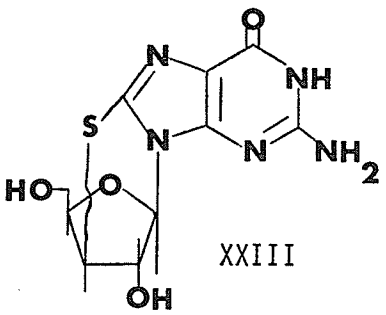
The N^2 and $O^{5'}$ protecting groups were first removed since it was anticipated that they would be at least partially hydrolyzed durring the step to form the anhydro linkage. The dimethylaminomethylene group was quantitatively removed from XIXa using either a hydrazine hydrate solution(hydrazine hydrate:acetic acid:pyridine, 1:6:24) or ammonium hydroxide solution at room temperature. The monomethoxytrityl group was then removed using 80% acetic acid at room temperature for three hours to produce 2'-mesitylenesulfonyl-8-bromo-guanosine(XX) in 77% overall yield from XIXa.

To form the 8,2'-thioanhydro linkage, XX was treated with sodium hydrogen sulfide in N,N-dimethylformamide. Using a six-fold excess of the sulfide and a temperature of 70°C. for twenty hours, a 16% yield of XXI was obtained. By increasing the temperature to 100°C., and the sulfide to an eleven fold excess, the yield was increased to 40%. Both the elemental analysis and the mass spectrum of the tetrasilyl derivative (m/e=585) agreed with the assigned structure as that of 8,2'-thioanhydroguanosine(XXI). Reduction of XXI with Raney nickel gave 2'-deoxyguanosine(XXII) which was identical to an authentic sample[58]. Further proof of the structure of XXII was obtained by acidic hydrolysis[59] to 2-deoxyribose which gave positive tests with Dische reagent[60] and cysteine-sulfuric acid reagent[61].

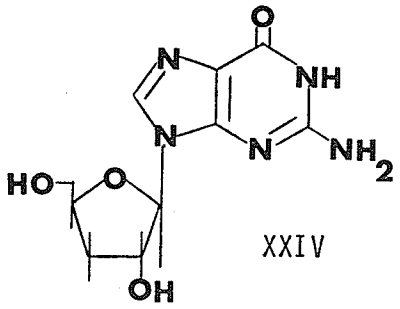
This then represents the first reported synthesis of

8,2'-thioanhydroguanosine(XXI) and its' reduction to 2'-deoxyguanosine(XXII) provides an alternate route to the synthesis of the 2'-deoxy nucleoside.

An interesting isomer of 8,2'-thioanhydroguanosine is 8,3'-thioanhydroguanosine(XXIII). Aside from its' structural characteristics, XXIII is a potential intermediate



XXIII



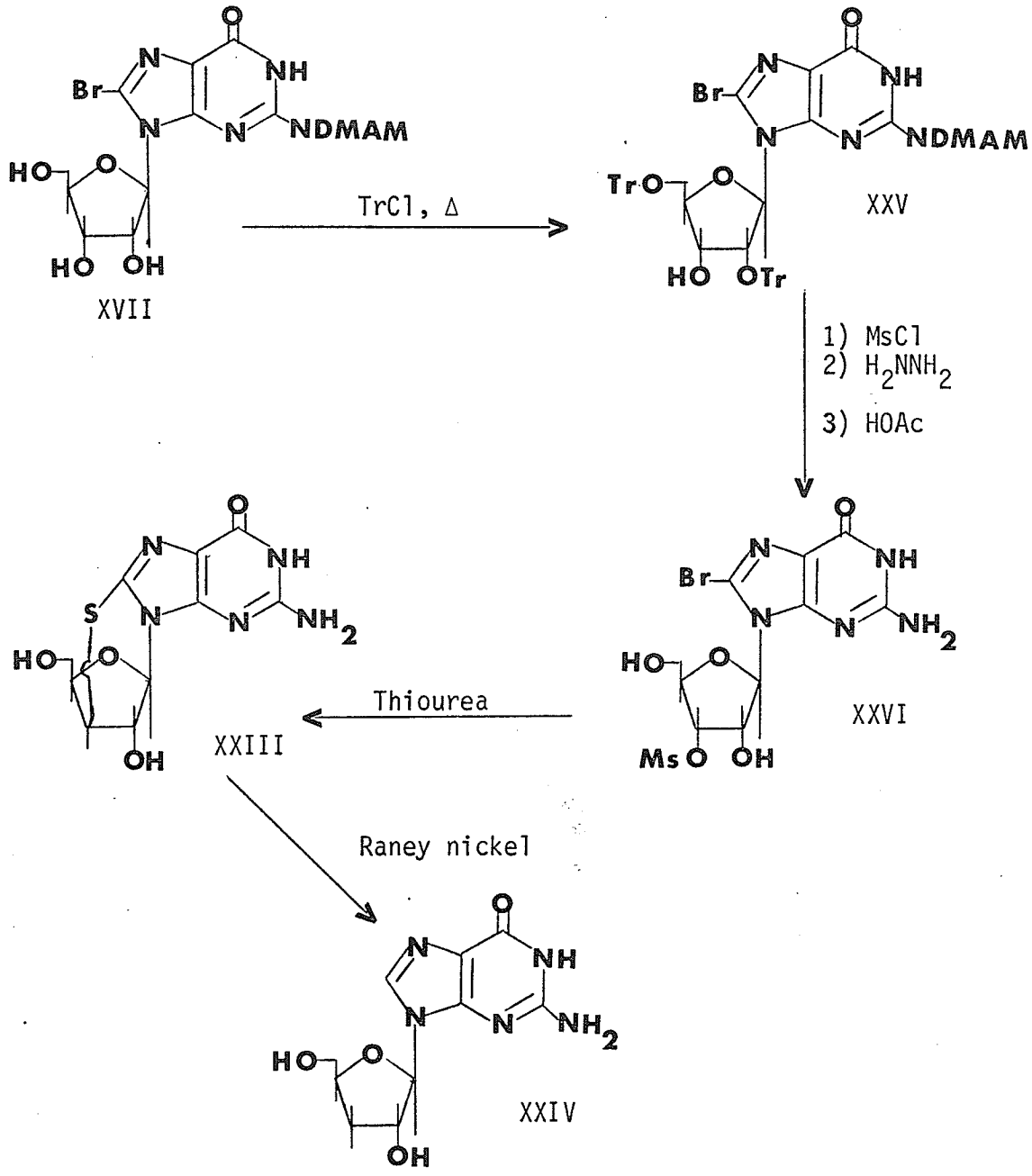
XXIV

in the synthesis of 3'-deoxyguanosine(XXIV) which is an analog of Cordycepin(X). The synthesis of this anhydro molecule was then performed in a manner similar to the above synthesis of 8,2'-thioanhydroguanosine(Scheme VI).

N^2-Dimethylaminomethylene-8-bromoguanosine(XVII) was treated with excess trityl chloride at elevated temperatures[62] to produce 2',5'-di-O-trityl-N^2-dimethylaminomethylene-8-bromoguanosine(XXV) in 74% yield. This product was treated with methanesulfonyl chloride in pyridine to introduce the sulfonate into the 3'-position. Prior to the formation of the anhydro linkage the N^2 and $O^{2',5'}$ protecting groups were removed using hydrazine hydrate solution followed by acetic acid to give 3'-O-methanesulfonyl-8-bromoguanosine(XXVI) in 55.5% overall yield from XXV.

The anhydro linkage was formed by treating XXVI with thiourea in refluxing n-butanol producing 8,3'-thioanhydroguanosine(XXIII) in 37% yield. Compound XXIII was identified

Scheme VI Synthesis of 8,3'-thioanhydroguanosine



by mass spectral analysis[63] including a high resolution mass spectrum of its' tetrasilylated derivative. Conversion of XXIII to 3'-deoxyguanosine(XXIV)[64] was accomplished using Raney nickel.

Characterization of XXIV also included acidic hydrolysis[59] to 3-deoxyribose which showed a negative Dische test[60] but positive cysteine[61] and aniline[65] tests. 3'-Deoxyguanosine (XXIV) has very similar properties($\lambda_{max}^{H_2O}$ 254nm, $\lambda_{sh}^{H_2O}$ 270nm, m/e of the tetrasilyl derivative = 555) to 2'-deoxyguanosine(XXII) except for paper chromatography in Solvent A where XXIV has R_f 0.24 while XXII has R_f 0.32. The synthesis of 8,3'-thioanhydroguanosine establishes unequivocally the identity of 8,2'-thioanhydroguanosine prepared above.

The synthesis of 8,2'-thioanhydroguanosine suffered from the same disadvantage as the reported syntheses of 8,2'-O- and 8,2'-S-anhydroadenosines, namely the multistep aspect of the procedures. The difficulty has arisen in the selective introduction of a good leaving group at the 2'-position of the ribose sugar. Unless the 5'-hydroxyl possesses a bulky substituent, sulfonating agents show only a slight preference for the 2'-hydroxyl over the 3'-hydroxyl. However placing a bulky substituent at the 5'-hydroxyl usually introduces several additional steps into the synthesis. Ikehara[66] partially overcame this problem with some adenosine 5'-phosphates by using aqueous sodium hydroxide along with a sulfonating agent. Under these conditions sulfonation appeared to occur exclusively at the 2'-position. However this still necessitated blocking of the 5'-position with phosphate.

It has been reported that a 2',3'-carbonate on a pyrimidine nucleoside is readily displaced by nucleophilic groups on the base ring[67]. Further it has been found that there is no need to block either the 5'-hydroxyl or amine groups on the base ring in forming 2',3'-carbonates of purine nucleosides[11,68]. This suggested the possibilty of introducing a good leaving

group(CO_2) to the 2'-position of any 8-halopurine nucleoside in a single step. Simultaneous displacement of the 8-halogen and the carbonate from the 2'-position should lead to the 8,2'-cyclo nucleoside. On this basis a general procedure(Scheme VII and Scheme VIII) has been developed for the synthesis of 8,2'-thioanhydropurine nucleosides.

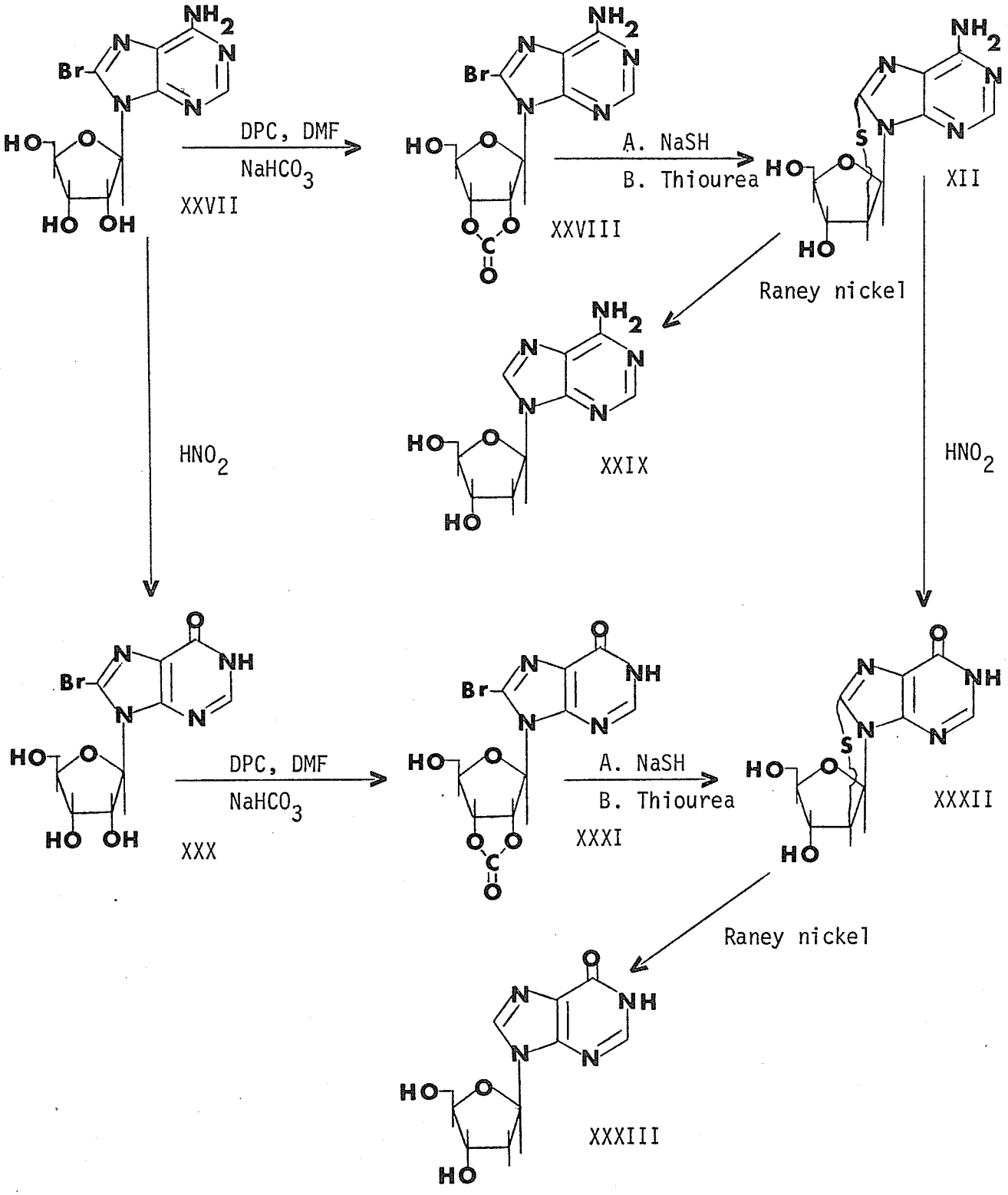
8-Bromopurine nucleosides are readily available by standard procedures. Treatment of adenosine with bromine-water gives a 71% yield of 8-bromoadenosine[52](XXVII). Deamination[69] of XXVII gives a 73% yield of 8-bromoinosine[70](XXX). 8-Bromoguanosine(XVI) was prepared as reported earlier.

Formation of cyclic carbonates from the 8-bromo nucleosides requires a 0.5hr. treatment of the 8-bromo nucleoside with a slight excess of diphenyl carbonate in dimethylformamide at 150°C. in the presence of a catalytic amount of sodium bicarbonate. Yields ranged from slightly over 70% for 8-bromoadenosine 2',3'-carbonate(XXVIII) and 8-bromoinosine 2',3'-carbonate(XXXI) to 98% for 8-bromoguanosine 2',3'-carbonate(XXXIV). Compounds XXVIII, XXXI and XXXIV all possessed a characteristic absorption in the i.r. at 5.5μ indicative of a cyclic, 5-membered carbonate grouping.

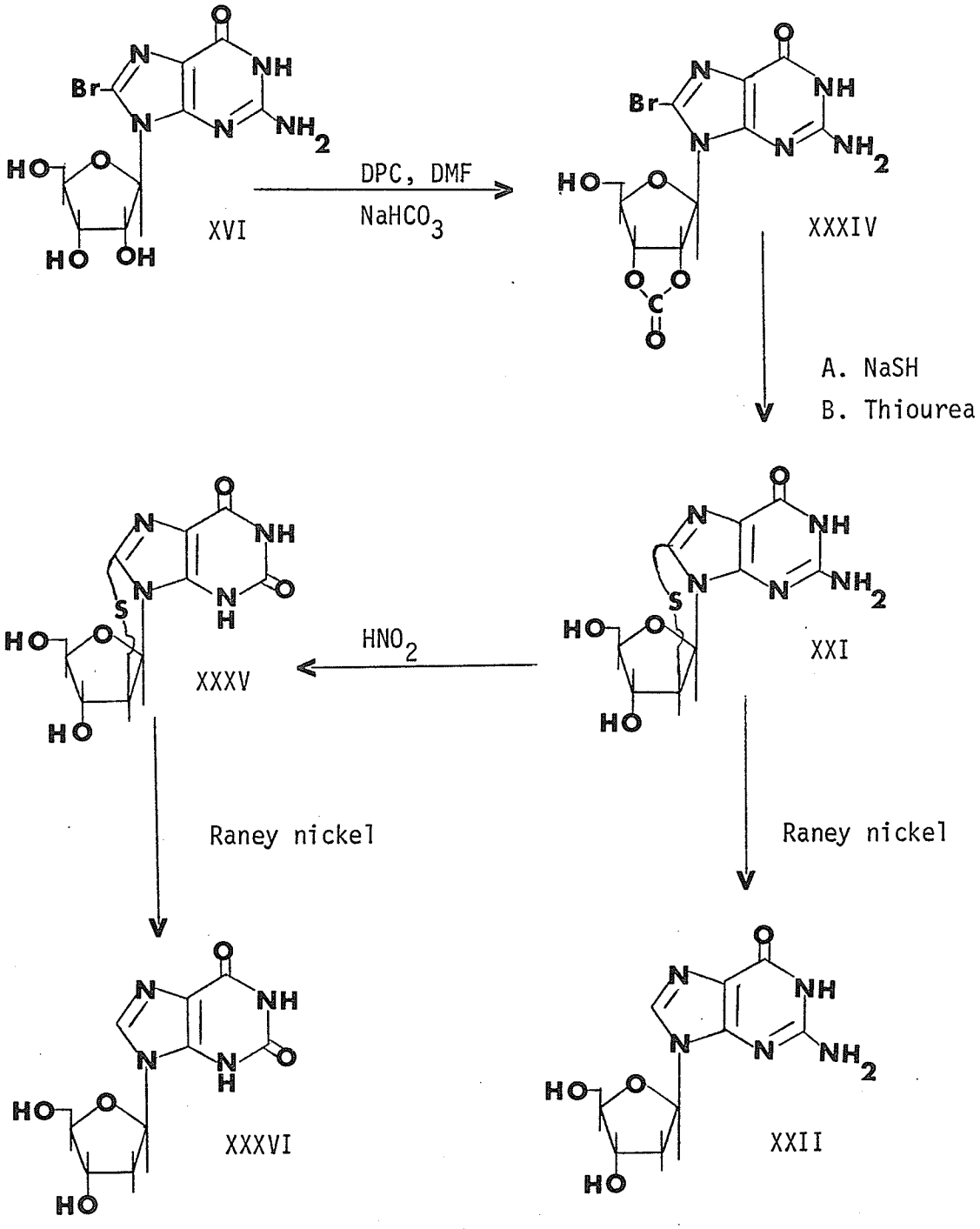
The formation of the anhydro linkage involves simultaneous displacement of the bromine from the 8-position and the carbonate from the 2'-position. Two methods were used to accomplish this: Method A using sodium hydrogen sulfide in hot dimethylformamide and Method B using thiourea in refluxing n-butanol. 8,2'-Thioanhydroadenosine(XII) was obtained in 79% yield from XXVIII using Method A but using Method B only 66% yield was realized. Compound XII was identical in all respects to 8,2'-thioanhydroadenosine prepared by the method of Ikehara[59].

8,2'-Thioanhydroinosine(XXXII) was obtained in several ways. From XXXI, XXXII was prepared in 73% yield by Method A and in 69% yield by Method B. Further 8,2'-thioanhydroadenosine(XII)

Scheme VII General synthesis of 8,2'-thioanhydropurine nucleosides via 2',3'-carbonates



Scheme VIII General synthesis of 8,2'-thioanhydropurine nucleosides via 2',3'-carbonates



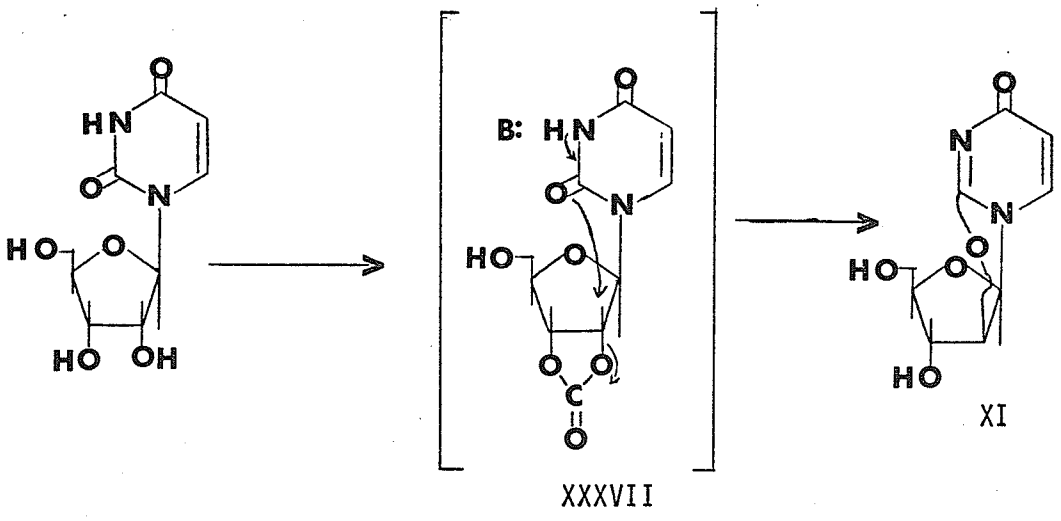
was readily deaminated by nitrous acid treatment to give XXXII quantitatively. These results represent the first reported chemical synthesis of 8,2'-thioanhydroinosine which was further characterized by Raney nickel reduction to 2'-deoxyinosine[58] (XXXIII).

8-Bromoguanosine 2',3'-carbonate(XXXIV) was converted to 8,2'-thioanhydroguanosine(XXI) in 56% yield by Method A while the yield using Method B was 41%. This was identical in all respects to the 8,2'-thioanhydroguanosine synthesized earlier. Chemical deamination of XXI by nitrous acid treatment yields another new anhydronucleoside, 8,2'-thioanhydroxanthosine(XXXV). The structure of XXXV was confirmed by mass spectral analysis[63] and its' reduction to 2'-deoxyxanthosine[71](XXXVI).

This approach then is the first general route to the 8,2'-thioanhydropurine nucleosides providing an efficient means of obtaining these compounds.

In the reported synthesis of $O^{2'}$,2'-anhydrouridine[43] from uridine using diphenyl carbonate and sodium bicarbonate in dimethylformamide, the mechanism(Scheme IX) undoubtedly involves

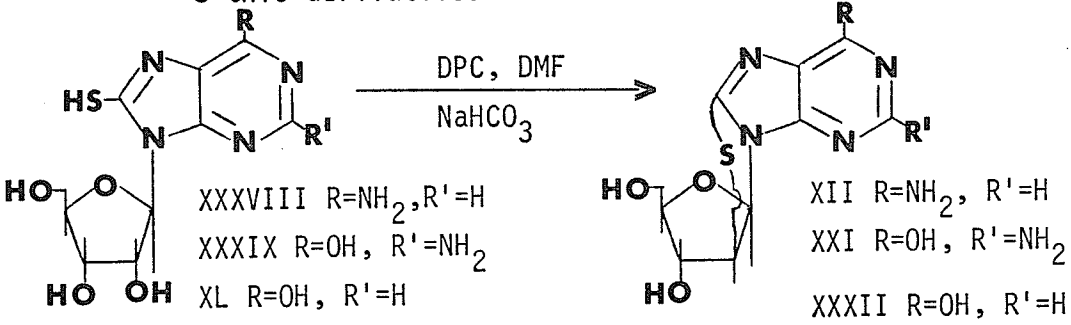
Scheme IX Mechanism of $O^{2'}$,2'-anhydrouridine formation



the formation of uridine 2',3'-carbonate(XXXVI) via an acid-catalyzed ester exchange reaction followed by base-catalyzed cyclization[67] to XI. In view of this it was thought that the 2',3'-carbonates of 8-thioadenosine(XXXVIII), 8-thioguanosine (XXXIX) and 8-thioinosine(XL) might closely resemble uridine 2',3'-carbonate(XXXVII) and might therefore be easily converted to the corresponding 8,2'-thioanhydro nucleosides.

Taking 8-thioadenosine[70](XXXVIII), 8-thioguanosine[70] (XXXIX) and 8-thioinosine[64](XL) and treating them with sodium bicarbonate and an excess of diphenyl carbonate in dimethylformamide at 150°C. for 30 minutes(Scheme X) results in very

Scheme X Synthesis of 8,2'-thioanhydropurine nucleosides via 8-thio derivatives



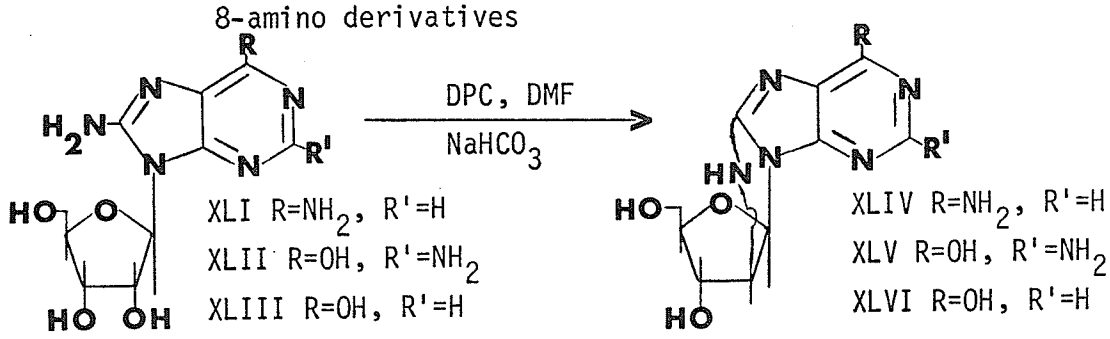
XXXVIII R=NH_2, R'=H
XXXIX R=OH, R'=NH_2
XL R=OH, R'=H

XII R=NH_2, R'=H
XXI R=OH, R'=NH_2
XXXII R=OH, R'=H

good yields of the corresponding 8,2'-thioanhydropurine nucleosides XII, XXI and XXXII. After completion of this work it was reported that 8,2'-thioanhydroadenosine[73] and 8,2'-thioanhydroguanosine[74] had been synthesized using this same method.

To examine the generality of this method the synthesis of the 8,2'-aminoanhydro nucleosides was attempted, beginning with the 8-amino substituted derivatives of adenosine, guanosine and inosine(Scheme XI). 8-Aminoadenosine[75](XLI), 8-amino-

Scheme XI Synthesis of 8,2'-aminoanhydropurine nucleosides via 8-amino derivatives



XLI R=NH_2, R'=H
XLII R=OH, R'=NH_2
XLIII R=OH, R'=H

XLIV R=NH_2, R'=H
XLV R=OH, R'=NH_2
XLVI R=OH, R'=H

guanosine[75](XLII) and 8-aminoinosine[76](XLIII) were prepared according to the literature and the yields were comparable with the reported values. 8-Aminoadenosine(XLI) was cyclized in good yield using diphenyl carbonate to 8,2'-aminoanhydroadenosine (XLIV). The product was identical in all respects with an authentic sample prepared in the literature by cyclization of 2'-O-triisopropylbenzenesulfonyl-8-aminoadenosine[77]. Thus the diphenyl carbonate route provides a much simpler method of XLIV synthesis.

Both 8,2'-aminoanhydroguanosine(XLV) and 8,2'-aminoanhydroinosine(XLVI) were prepared by this method except that the anhydroguanosine synthesis required a mixture of pyridine and dimethylformamide as solvent. The identities of XLV and XLVI were confirmed by mass spectral analysis[63] including high resolution mass spectra of their silylated derivatives. 8,2'-Aminoanhydroinosine(XLVI) could also be obtained by nitrous acid deamination of XLIV in a yield of 84%. Thus the diphenyl carbonate procedure represents a novel, convenient method for the general synthesis of 8,2'-thio and aminoanhydropurine nucleosides.

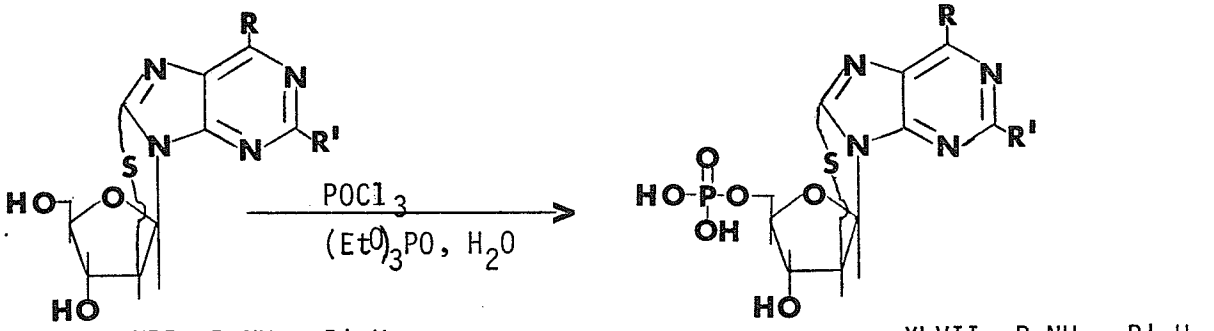
It is interesting to note that while the 8-thio and 8-aminopurine nucleosides will cyclise to the corresponding 8,2'-anhydro nucleosides using diphenyl carbonate(the aminos giving lower yields), we have found that 8-hydroxyguanosine and 8-hydroxyadenosine do not cyclise at all[68] while others report that 8-hydroxyadenosine cyclises in a yield of only 2.5%[73]. This result reflects a difference in the ability of the 8-hydroxy function to interact with the 2'-position of the ribose moiety as compared to the 8-thio or 8-amino groups. This order of reactivity(S>N>O) would therefore appear to follow from both the size of the hetero atom(S>N>O) and its' nucleophilicity(S>N>O)[78].

8,2'-Thioanhydropurine Nucleoside 5'-Mono and Diphosphates

In choosing a method for the synthesis of 5'-mononucleotides it is important to choose one which is efficient in terms of yield and minimizes the number of side products. In 1969 Yoshikawa et al.[79] reported the selective 5'-phosphorylation of unprotected nucleosides using partially hydrolyzed phosphoryl chloride in a trialkyl phosphate solvent. Such a method eliminated the necessity of prior protection of the amino groups of the base and the secondary hydroxyls of the sugar. Also eliminated was the requirement of absolute dryness generally required for phosphorylations[80].

8,2'-Thioanhydroadenosine(XII), 8,2'-thioanhydroguanosine(XXI) and 8,2'-thioanhydroinosine(XXXII) were successfully converted(Scheme XII) to their corresponding 5'-phosphates

Scheme XII Synthesis of 8,2'-thioanhydropurine nucleoside 5'-monophosphates



XII R=NH_2, R'=H
XXI R=OH, R'=NH_2
XXXII R=OH, R'=H

XLVII R=NH_2, R'=H
XLVIII R=OH, R'=NH_2
XLIX R=OH, R'=H

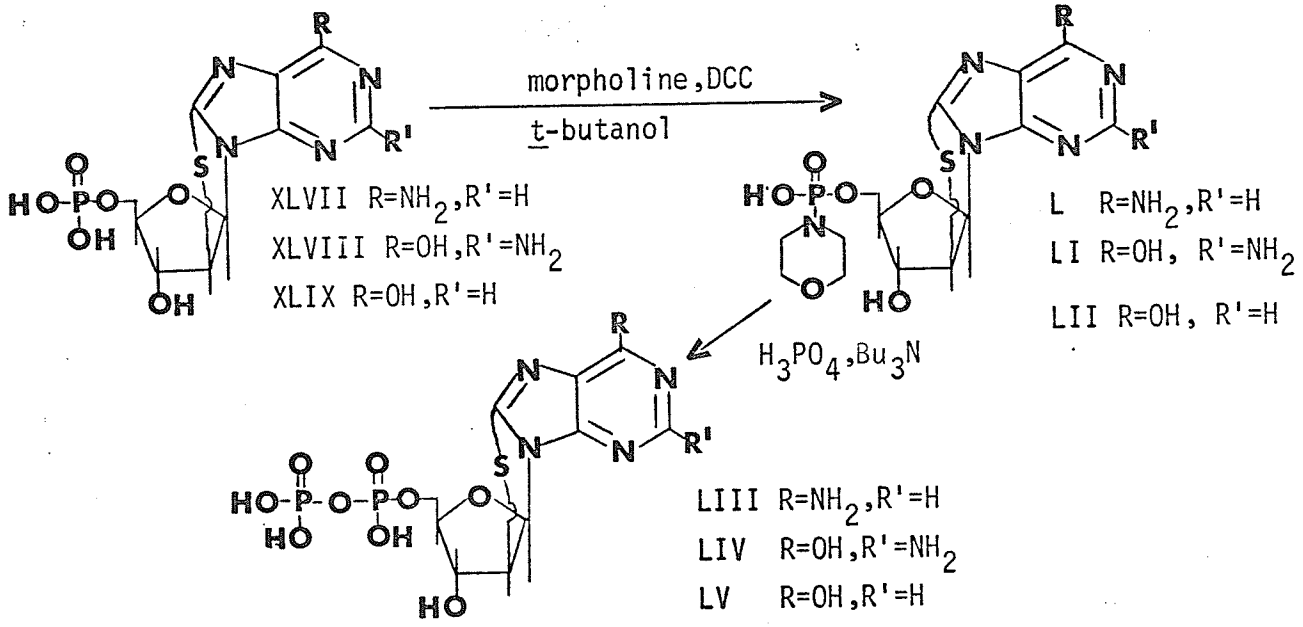
using a large excess of phosphoryl chloride in triethyl phosphate in the presence of a limited amount of water. 8,2'-Thioanhydroadenosine 5'-phosphate(XLVII) was obtained in 95% yield and was identical to an authentic sample prepared by cyclization of 2'-O-tosyl-8-bromoadenosine 5'-phosphate with sodium hydrogen sulfide[66]. The structure was further confirmed by Raney nickel reduction to 2'-deoxyadenosine 5'-phosphate followed by treatment with 5'-nucleotidase to give 2'-deoxyadenosine[58]. 5'-Nucleotidase is specific for a 5'-phosphate and thus establishes the position of phosphorylation.

8,2'-Thioanhydroguanosine(XXI) and 8,2'-thioanhydroinosine(XXXII) were phosphorylated in a similar manner in yields of 43% for 8,2'-thioanhydroguanosine 5'-phosphate(XLVIII) and 74% for 8,2'-thioanhydroinosine 5'-phosphate(XLIX). The structures of XLVIII and XLIX were confirmed by reduction to the corresponding 2'-deoxynucleoside 5'-phosphates and then 5'-nucleotidase action to 2'-deoxyguanosine and 2'-deoxyinosine respectively[58].

Having obtained the mononucleotides described above it remained to investigate the possibility of converting them to the corresponding 5'-di and triphosphates. To date the best reported method for the synthesis of nucleoside 5'-di and triphosphates is that of Moffatt and Khorana[81]. The method involves the conversion of the mononucleotide to the phosphoromorpholidate using morpholine and dicyclohexylcarbodiimide(DCC) followed by treatment with a suitable phosphorylating reagent.

The reaction of XLVIII and XLIX(Scheme XIII) with morpholine and DCC in refluxing aqueous t-butanol resulted in quantitative conversion to the corresponding 5'-phosphoromorpholidates LI and LII. The reactions were monitored on electrophoresis at pH 7.5 until only one compound was detectable

Scheme XIII Synthesis of 8,2'-thioanhydropurine nucleoside 5'-diphosphates



with a mobility approximately one-half that of the starting nucleotide. In the case of 8,2'-thioanhydroadenosine 5'-phosphate(XLVII) the reaction was not quantitative(78%). This necessitated purification of the morpholidate L by paper chromatography in Solvent F prior to phosphorylation.

Initial attempts to synthesize both the 5'-di and triphosphates from L, LI and LII by using bis-(tri-n-butylammonium)pyrophosphate in pyridine[81] as phosphorylating reagent were unsuccessfull as no pyrophosphate was detectable. However treating the morpholidates with inorganic phosphoric acid and tri-n-butylamine in pyridine[82](Scheme XIII) resulted in the synthesis of 8,2'-thioanhydroadenosine 5'-diphosphate(LIII,46%), 8,2'-thioanhydroguanosine 5'-diphosphate(LIV,37.9%) and 8,2'-thioanhydroinosine 5'-diphosphate(LV,43%). Electrophoretic and paper chromatographic mobility as well as phosphate analysis[83] confirmed the structures as the 5'-diphosphates.

Finally the synthesis of the triphosphates was attempted beginning with the 5'-monophosphates of 8,2'-thioanhydroadenosine, guanosine and inosine. Using 85% orthophosphoric acid, tri-n-butylamine and the mononucleotide in pyridine together with a large excess of DCC[84] the only change in the starting material was the production of a small amount of 5'-diphosphate.

While the attempts to synthesize the triphosphates have been unsuccessful , the synthesis of the diphosphates provides analogs of the naturally occurring adenosine, guanosine and inosine diphosphates. These compounds should thus provide interesting models for enzymes which utilize nucleoside 5'-diphosphates as substrates.

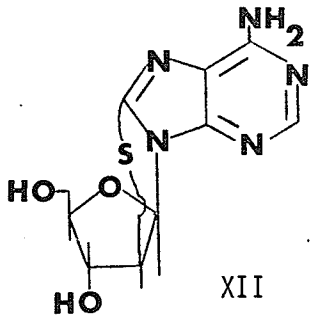
Enzyme Studies

Adenosine Deaminase

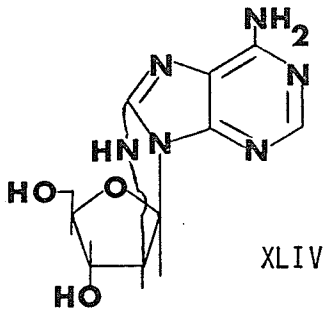
The study of enzymes responsible for the deamination of purine and pyrimidine nucleosides has attracted considerable interest. A great number of potentially useful chemotherapeutic agents have been synthesized which possess the nucleoside structure only to find that while the compounds were effective antimetabolites *in vitro* or in tissue culture, they were rapidly inactivated by deamination *in vivo*. While arabinoadenosine(III) has exhibited antiviral activity, it is rapidly deaminated to arabinoinosine by adenosine deaminase[85]. Therefore a thorough understanding of the effect of substrate modification on enzyme activity could afford the knowledge necessary to design effective chemotherapeutic agents that would either be resistant to, or inhibitors of the mammalian deaminases.

Previous studies with adenosine deaminase have shown that various modifications can be made in the carbohydrate moiety of the adenosine molecule to produce analogs of adenosine that still retain all or part of their ability to be bound to the enzyme[85,86]. It is known that 2'-and 3'-hydroxyl groups of adenosine do not play a critical role in substrate activity but that a 5'-hydroxyl or other hydroxyl capable of acting in place of the 5'-hydroxyl is essential for substrate activity[87-89]. Simon et al.[90] have recently found that 2-substituted purine nucleosides bind to the enzyme while bulky substituents in the 8-position prevent these molecules from being substrates. It may well be that molecules such as 8-bromoadenosine are not substrates because of a number of factors one of which being that they exist in the syn conformation[91]. Other derivatives such as 8-aminoadenosine, where the substituent is small enough to permit the purine ring to remain in an anti conformation, act as substrates.

Since the anhydro molecules XII and XLIV feature an



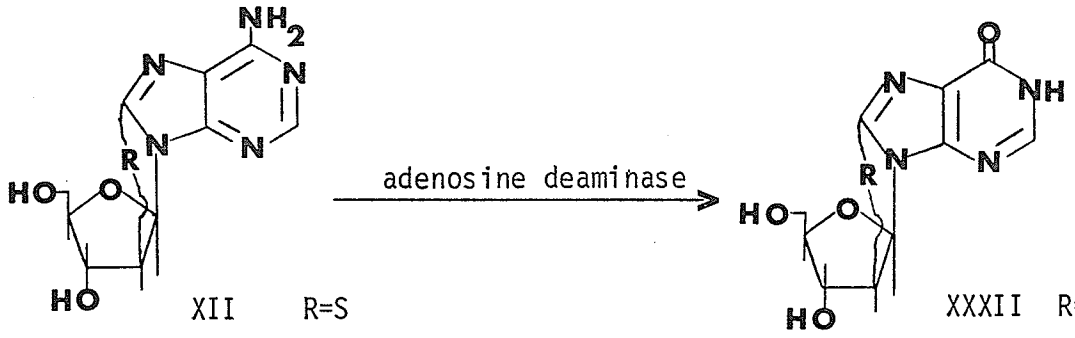
XII



XLIV

adenosine molecule which is fixed in the anti conformation, they should provide excellent models as to the specificity of the enzyme towards nucleoside conformation. When these two compounds were treated with adenosine deaminase(SchemeIV) both were quantitatively converted to the corresponding 8,2'-anhydroinosines XXXII and XLVI.

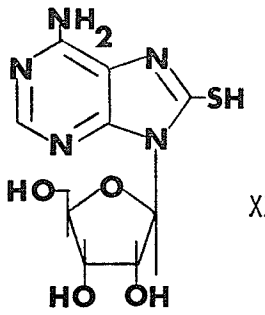
Scheme XIV Action of adenosine deaminase on 8,2'-anhydropurine nucleosides



XII R=S
XLIV R=NH

XXXII R=S
XLVI R=NH

It has been shown that 8-thioadenosine[90](XXXVIII) is



XXXVIII

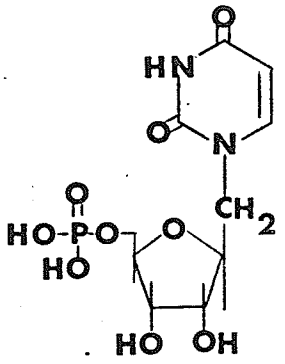
not a substrate for the enzyme. The results with 8,2'-thioanhydroadenosine would therefore indicate that XXXVIII probably exists in the syn conformation since if only the bulk tolerance of the 8-substituent was the determining factor both XII and XXXVIII might be expected to have similar activity. However it is possible that the non-substrate activity of XXXVIII may be attributed to its' interacting with a non-active site area of the enzyme. Recent results[92] with nucleoside analogs lend further support to the view that substrate conformation is a necessary factor in

determining activity.

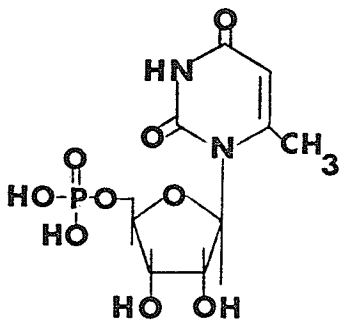
As an additional consequence of this study the quantitative conversion to the anhydroinosines XXXII and XLVI offers an extremely efficient synthesis of these molecules using adenosine deaminase as a synthetic tool. This represents the first reported synthesis of an anhydropurine nucleoside enzymatically.

5'-Nucleotidase

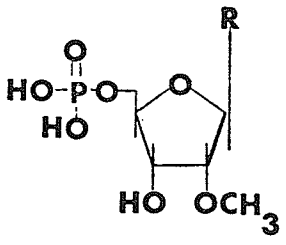
5'-Nucleotidase is an enzyme which catalyzes the selective removal of a monophosphate from the 5'-position of naturally occurring nucleotides. While the mechanism of the enzyme has not been studied in any detail, work with modified nucleotides has given a small degree of insight into its' action. Specifically the work with 1'-homouridine 5'-phosphate[93](LVI), 6-methyluridine 5'-phosphate[94](LVII) and the 2'-O-methylribonucleoside 5'-phosphates[95](LVIII), all



LVI



LVII

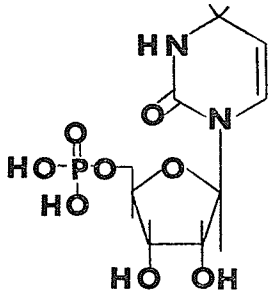


LVIII

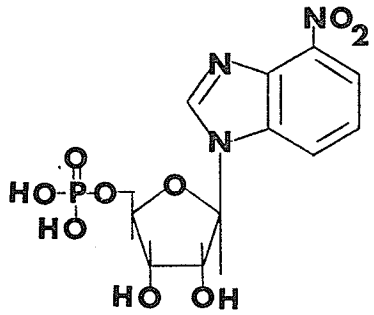
R= purine or pyrimidine base

of which are not substrates of the enzyme, might suggest that the ability of the substrates to adopt a required conformation at the active site is a requirement for activity. However the

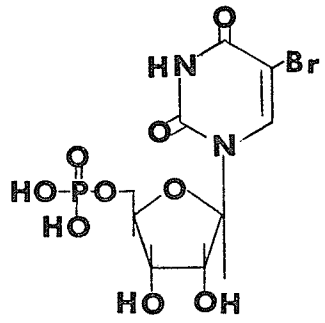
report that molecules LIX and LX are non-substrates while LXI and LXII are substrates[96] suggests that the character of the heterocyclic base is an important factor.



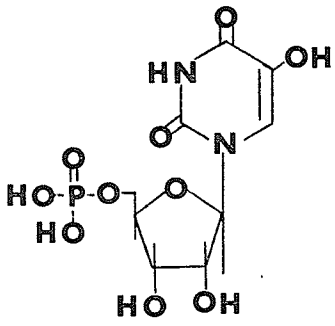
LIX



LX



LXI



LXII

Upon testing the 8,2'-thioanhydropurine nucleoside 5'-phosphates XLVII, XLVIII and XLIX with the enzyme all were found to be inactive. Further investigation into the mechanism of enzyme action is required however, before any conclusion can be drawn as to the reason for the anhydro nucleotides' lack of activity.

Another enzyme which catalyzes the dephosphorylation of nucleotides is alkaline phosphatase. With this enzyme the anhydro nucleotides were completely converted to their corresponding nucleosides reaffirming the conclusion that this enzyme action is completely independent of the nature of the base.[94]

Adenylate kinase(Myokinase)

Adenylate kinase catalyzes the reaction (1) a phos-

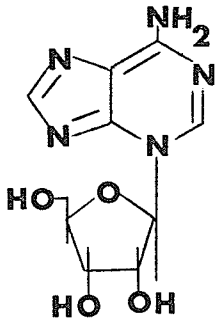
$$AMP \xrightarrow{ATP} ADP \quad (1)$$

phorylation involving added ATP, as well as the reverse (2),

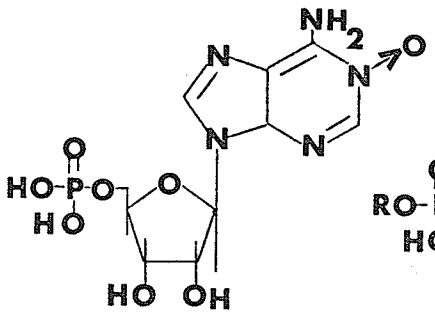
$$ADP \longrightarrow AMP + ATP \quad (2)$$

a disproportionation reaction. Incubation of 8,2'-thioanhydro-adenosine 5'-monophosphate(XLVII) in tris-buffer, pH 7.6, with the enzyme and added ATP gave no detectable diphosphate formation as monitored by electrophoresis. A similar lack of activity was found when 8,2'-thioanhydroadenosine 5'-diphos-phate(LIII) was incubated with the enzyme.

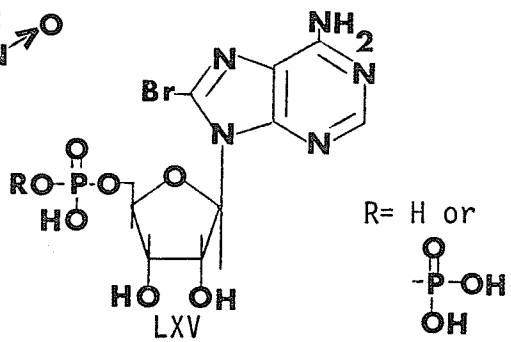
Since Leonard and Laursen[97] found that the 5'-mono, di and triphosphates of isoadenosine(LXIII) are substrates of adenylate kinase while M^{c}Cormick has reported that LXIV[98] and LXV[99] are not, it would appear only certain changes in the electronic structure of the base and/or conformation decrease substrate activity. The work with the thioanhydroadenosine nucleotides would seem to support this conclusion.



LXIII



LXIV



LXV

Studies on Dinucleoside Monophosphate Synthesis

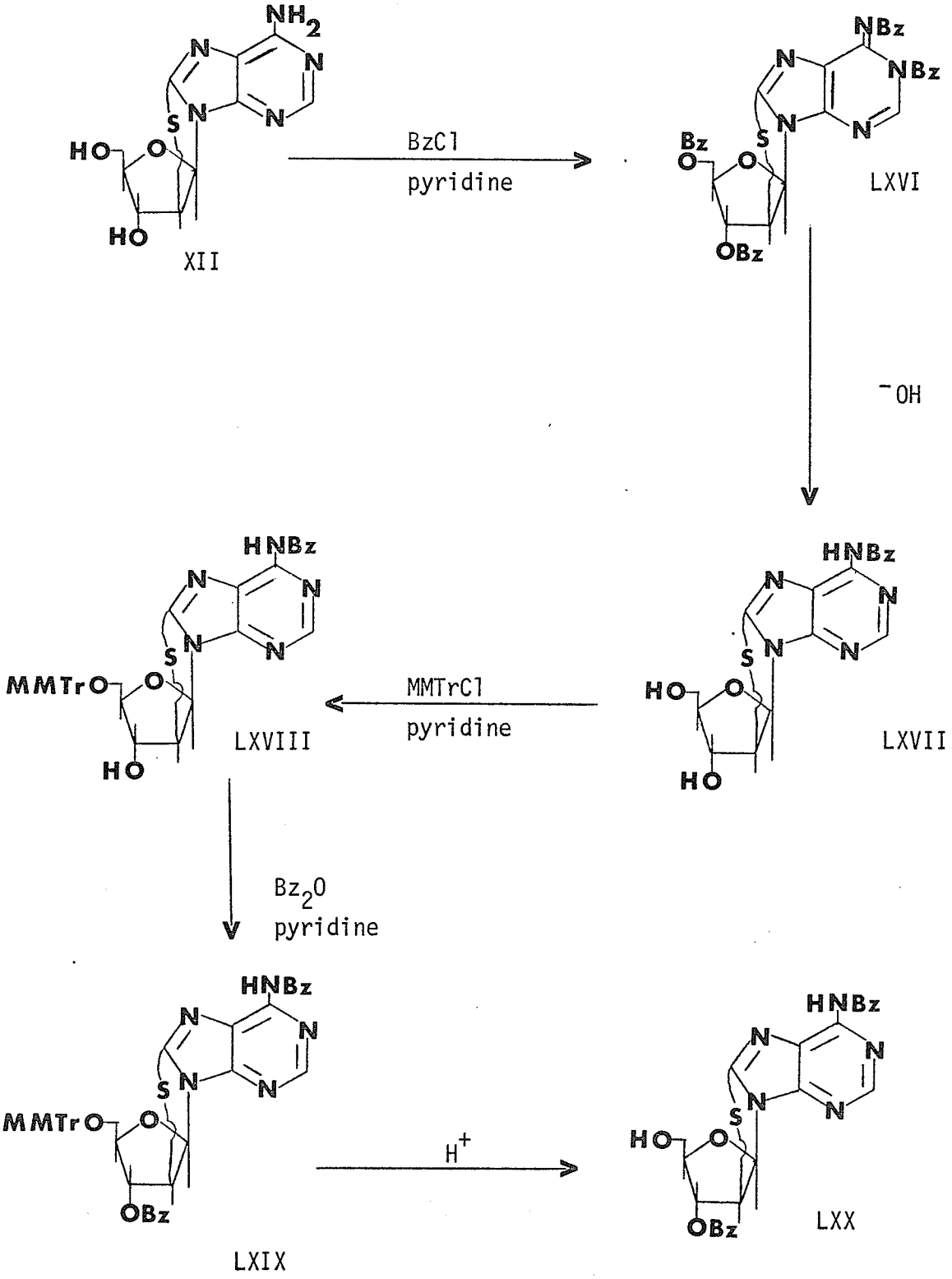
The two major problems which must be overcome for the synthesis of ribooligonucleotides are 1)the preparation of suitably protected nucleosides or nucleotides so as to leave only the desired 3' or 5'-hydroxyl function for a phosphorylation reaction and 2)the use of efficient phosphorylation techniques for the formation of phosphodiesters. As outlined earlier, the anhydro linkage of the 8,2'-anhydropurine nucleosides provides an effective blocking group for the 2'-hydroxyl. It remains to block either the 5' or 3'-hydroxyl and in the case of adenosine or guanosine derivatives, the amino group on the purine ring.

The synthesis of $N^6,O^{3'}$ and $N^6,O^{5'}$-blocked 8,2'-thioanhydroadenosine compounds is outlined in Scheme XV. 8,2'-Thioanhydroadenosine(XII) was treated with excess benzoyl chloride in pyridine[100] to give N,N,$O^{3'}$,$O^{5'}$-tetrabenzoyl-8,2'-thioanhydroadenosine(LXVI) quantitatively. Controlled basic hydrolysis of LXVI gave N^6-benzoyl-8,2'-thioanhydroadenosine(LXVII) which is characterized by a bathochromic shift in the u.v. absorption maximum to 300.5nm from 275.5nm in XII.

Blocking the 5'-hydroxyl was then accomplished by treatment of LXVII with monomethoxytrityl chloride to give LXVIII in 87% yield. Thus LXVII has the N^6-amino, 2' and 5'-hydroxyls effectively blocked leaving only the 3'-position available for phosphorylation.

From LXVIII it is then easy to obtain an $N^6,O^{3'}$-blocked nucleoside. Benzoylation of LXVIII with benzoic anhydride gives a good yield of 5'-O-monomethoxytrityl-$N^6,O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine(LXIX). Acetic acid treatment of LXIX on a steam bath for ten minutes affords the required $N^6,O^{3'}$-blocked nucleoside LXX[101]. Thus LXVIII and LXX represent the necessary blocked nucleosides for synthesis of dianhydro nucleotides incorporating 8,2'-thioanhydroadenosine into a specific 3'-5' internucleotide bond.

Scheme XV Synthesis of 8,2'-thioanhydroadenosine derivatives

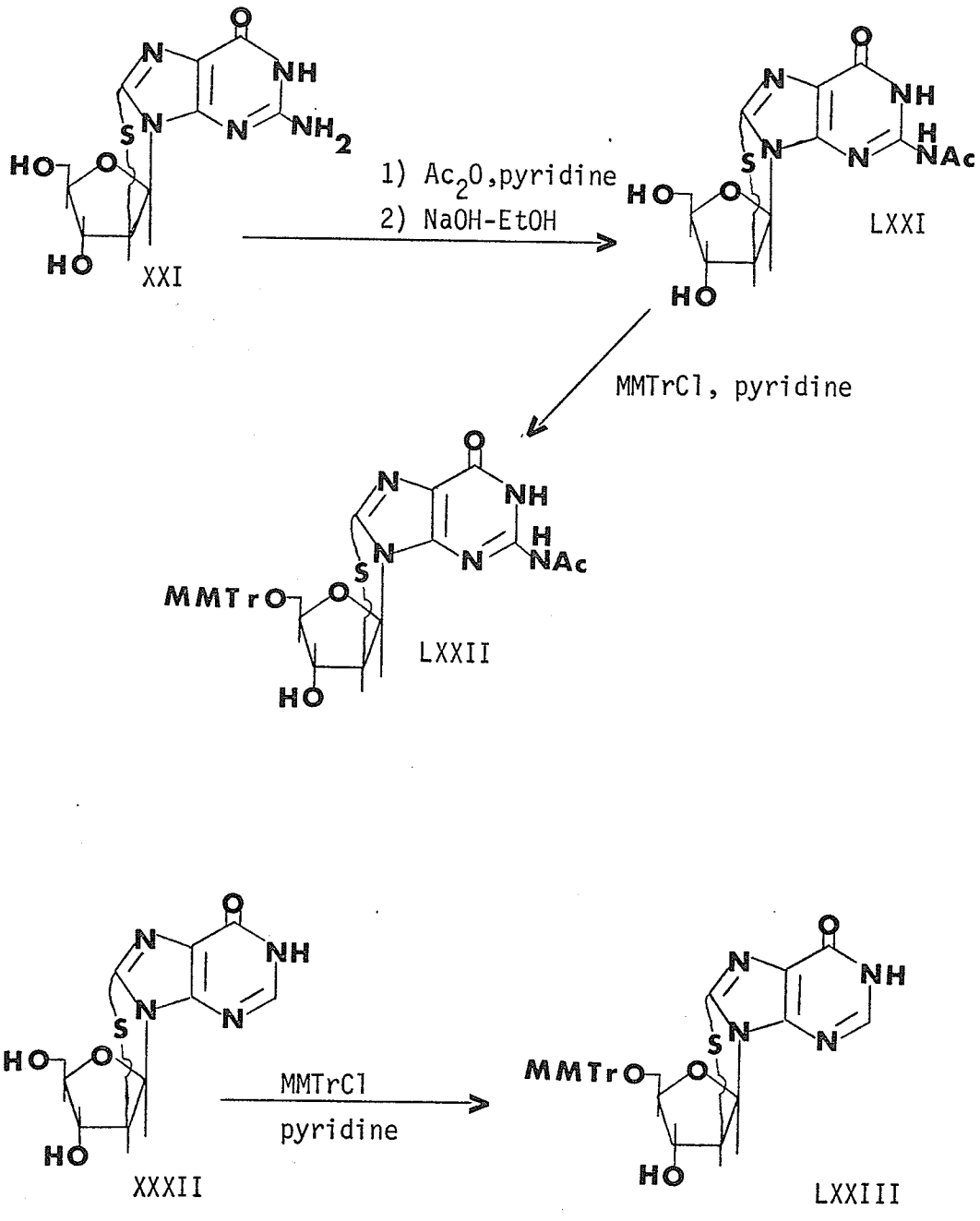


In order to incorporate 8,2'-thioanhydroguanosine and 8,2'-thioanhydroinosine the following blocked derivatives were synthesized(Scheme XVI). 8,2'-Thioanhydroguanosine(XXI) was reacted with an excess of acetic anhydride and subsequent hydrolysis in 2N sodium hydroxide-ethanol gave a 61% yield of N^2-acetyl-8,2'-thioanhydroguanosine(LXXI). Acetylation of the 2-amino function was confirmed by the bathochromic shift in the u.v. absorption of the shoulder at 283nm of XXI to a maximum at 295nm for LXXI. From LXXI an N^2,$O^{5'}$-blocked nucleoside was prepared by treatment with monomethoxytrityl chloride. Upon work-up a 50% yield of 5'-O-monomethoxytrityl-N^2-acetyl-8,2'-thioanhydroguanosine(LXXII) was obtained. The preparation of the $O^{5'}$ blocked 8,2'-thioanhydroinosine molecule LXXIII was accomplished by the reaction of XXXII with excess monomethoxytrityl chloride[102]. Thus all three 8,2'-thioanhydronucleosides are available for dinucleoside monophosphate synthesis.

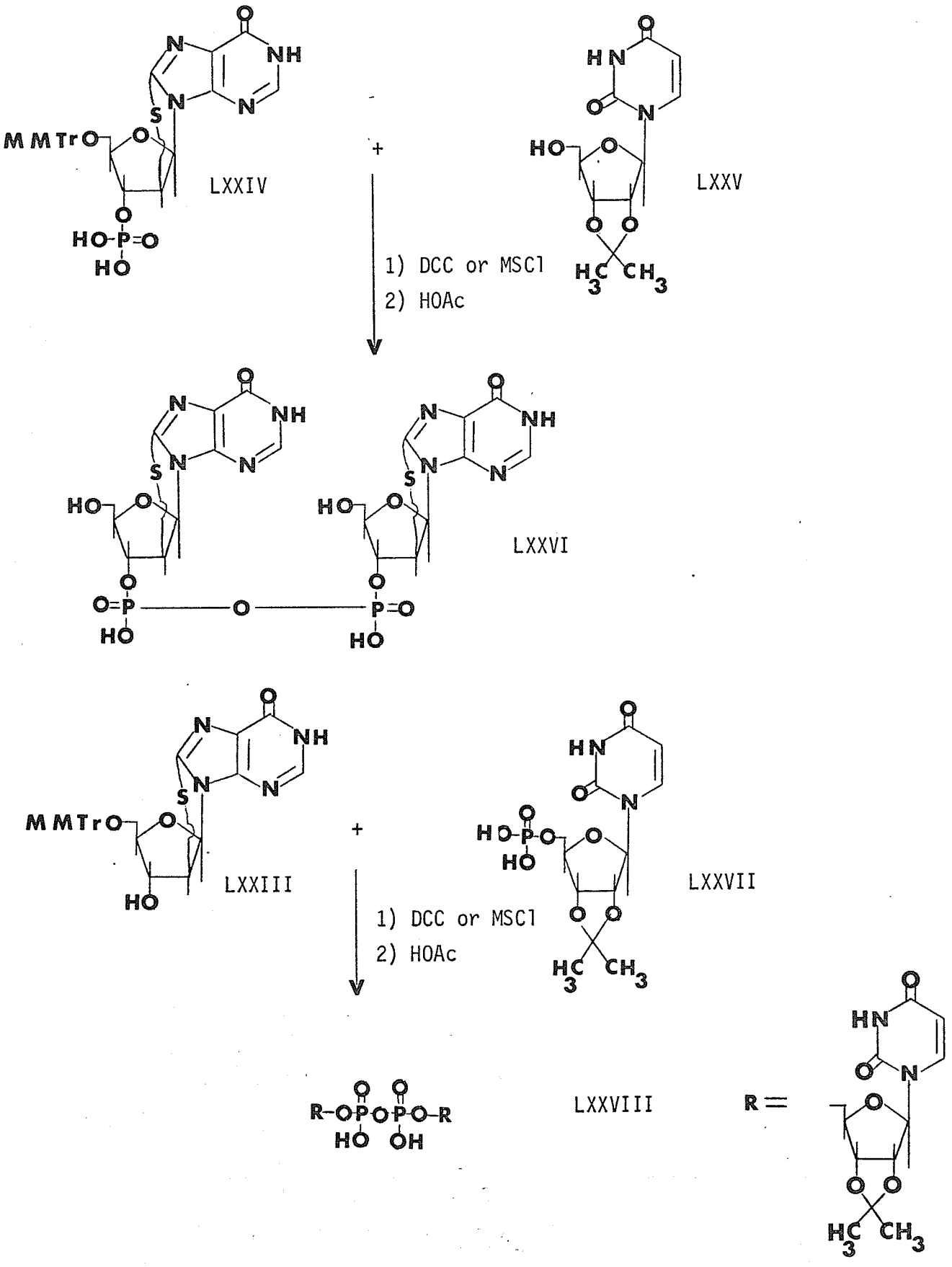
The general synthesis of the internucleotide bond consists in activation of the phosphoryl group of one component in the presence of a suitably protected second component, bearing a free hydroxylic function. The activation is performed with N,N'-dicyclohexylcarbodiimide[103] or aromatic sulfonyl chlorides[55,104]. This path usually affords a high yield of the dinucleoside phosphate both in the deoxyribo and the ribo series. These methods were then applied to the synthesis of dinucleoside monophosphates containing 8,2'-thioanhydropurine nucleosides.

Initially the synthesis of 8,2'-thioanhydroinosinyl (3'-5')2',3'-O-isopropylideneurinine was attempted by two approaches(Scheme XVII). 5'-O-Monomethoxytrityl-8,2'-thioanhydroinosine(LXXIII) was phosphorylated in the 3'-position using β-cyanoethyl phosphate and mesitylenesulfonyl chloride according to the method of Tener[105]. The dicharged phosphate LXXIV was obtained in 87.6% yield from LXXIII by subsequent

Scheme XVI Synthesis of derivatives of 8,2'-thioanhydroguanosine and 8,2'-thioanhydroinosine



Scheme XVII Attempted synthesis of 8,2'-thioanhydroinosinyl (3'-5') 2',3'-O-isopropylideneuridine

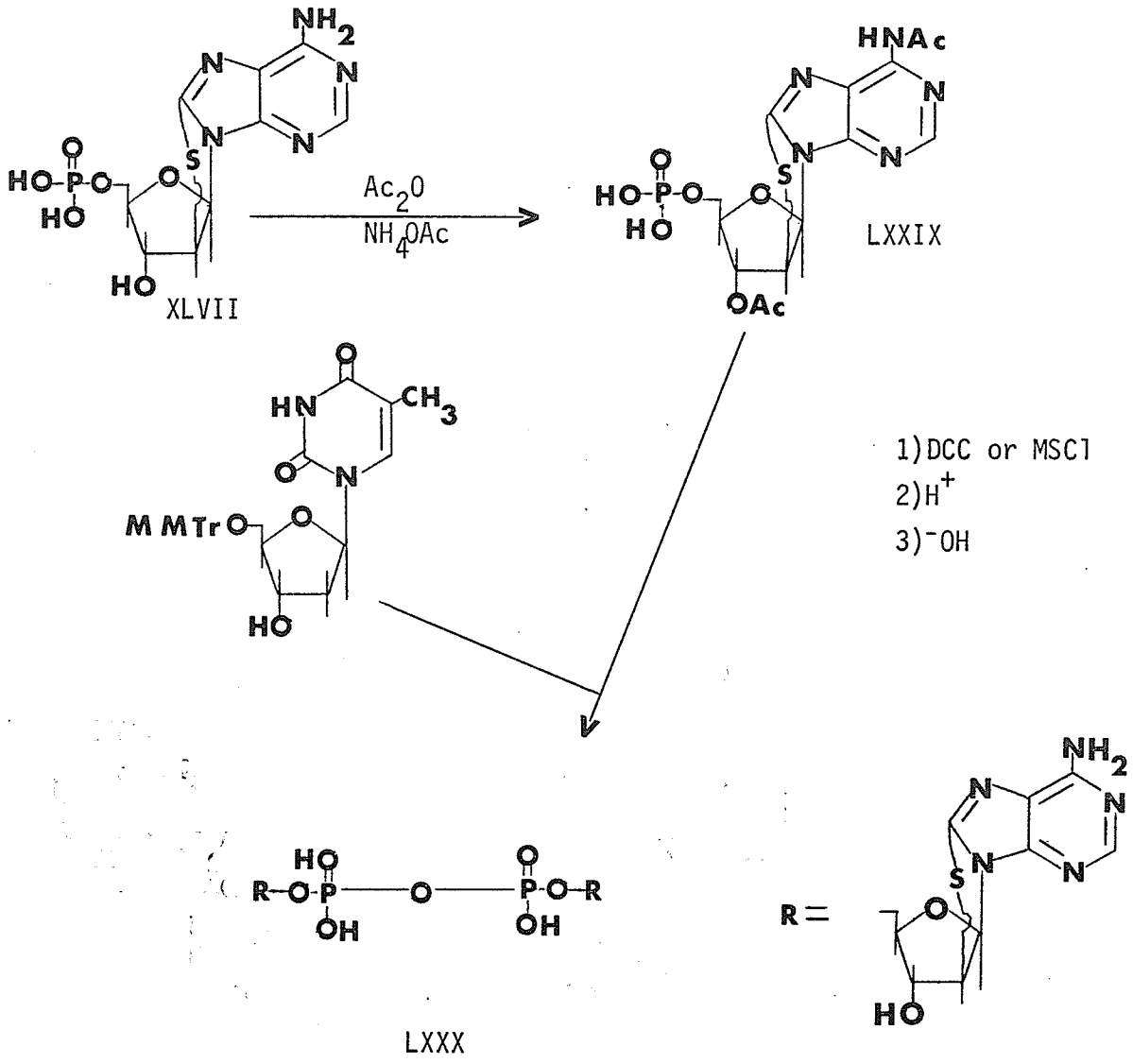


basic hydrolysis of the cyanoethyl blocking group. Treatment of LXXIV with excess 2',3'-O-isopropylideneuridine(LXXV) and either dicyclohexylcarbodiimide or mesitylenesulfonyl chloride in pyridine under standard condensation reaction conditions[106] led not to the expected dinucleoside monophosphate, but rather LXXIV was quantitatively converted to the corresponding 3'-3' pyrophosphate LXXVI. The structure of LXXVI was confirmed by its' electrophoretic mobility as well as complete hydrolysis to 8,2'-thioanhydroinosine 3'-phosphate by reaction with acetic anhydride in pyridine[107].

In an alternative approach to this synthesis the thioanhydro molecule LXXIII was used as the nucleosidic component while 2',3'-O-isopropylideneuridine 5'-phosphate(LXXVII) was the starting nucleotide. Synthesis of LXXVII was accomplished by treating LXXV with β-cyanoethyl phosphate and mesitylenesulfonyl chloride in pyridine followed by basic hydrolysis. When the condensation reaction between LXXIII and LXXVII was attempted, the only new product in the reaction mixture was the 5'-5' pyrophosphate LXXVIII.

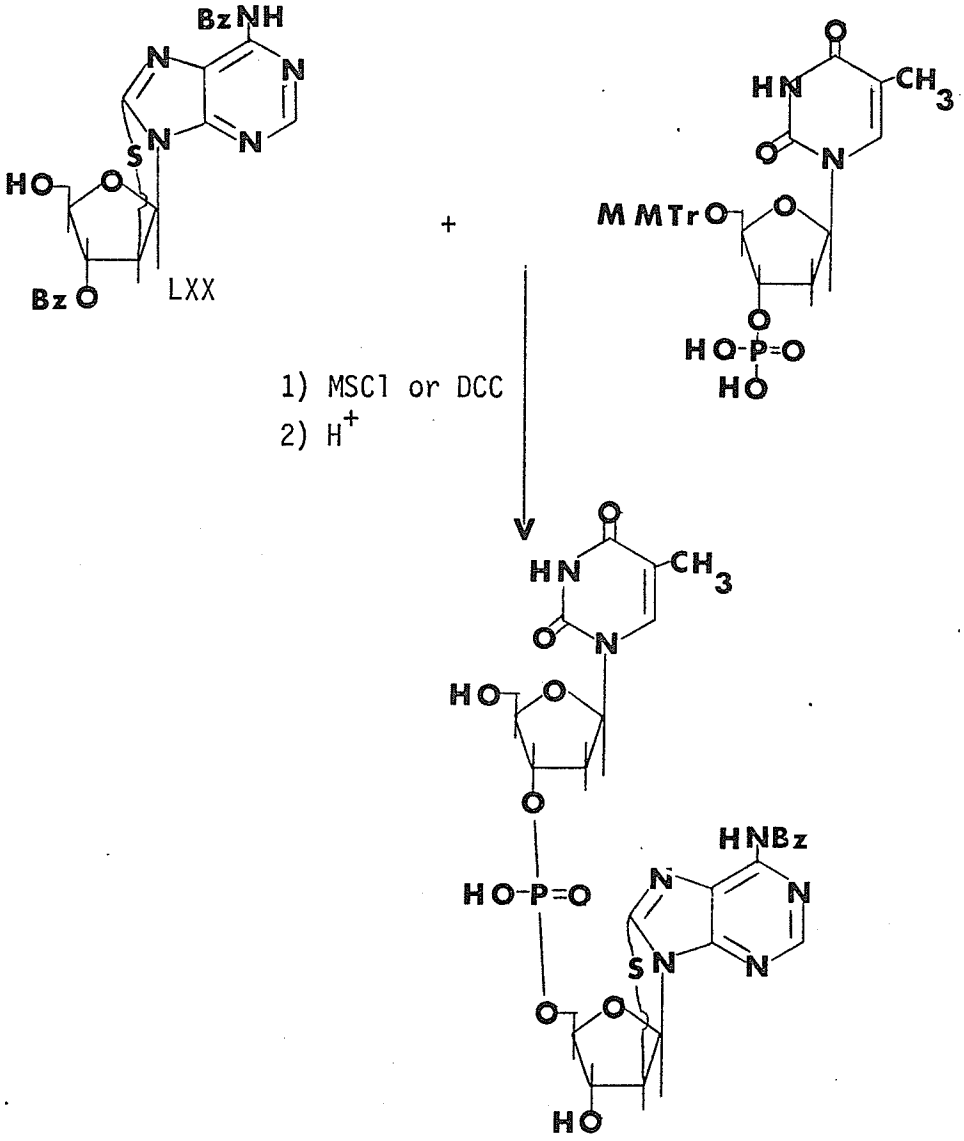
In both the methods of Scheme XVII the thioanhydro nucleoside was designed for the 5'-end of the proposed dinucleoside monophosphate. To examine the possibility of using such a molecule in the 3'-positon of a dinucleoside monophosphate it was necessary to synthesize a blocked thioanhydro nucleoside 5'-phosphate. For this purpose $N^6,O^{3'}$-diacetyl-8,2'-thioanhydroadenosine 5'-phosphate(LXXIX) was prepared(Scheme XVIII). 8,2'-Thioanhydro adenosine 5'-phosphate(XLVII) was acetylated with excess acetic anhydride in the presence of ammonium acetate and pyridine to give LXXIX in 79% yield. However attempted condensation (Scheme XVIII) with 5'-O-monomethoxytritylthymidine[102] using either DCC or MSCl led, after deblocking, to the 5'-5' pyrophosphate(LXXX) almost quantitatively.

Scheme XVIII Attempted synthesis of thymidylyl(3'-5') 8,2'-thioanhydroadenosine



The only other approach along these lines was to attempt the condensation of an N,$O^{3'}$-blocked anhydro nucleoside with a suitably blocked "normal" 3'-nucleotide. This method is outlined in Scheme XIX.

Scheme XIX Synthesis of thymidylyl(3'-5') 8,2'-thioanhydro-adenosine



LXXXI

Compound LXX and a slight excess of 5'-O-monomethoxytritylthymidine 3'-phosphate[102] were treated with two equivalents of mesitylenesulfonyl chloride in pyridine at room temperature for 24 hours. Following acetic acid treatment to remove the monomethoxytrityl group and preparative paper chromatography in Solvent A(which removes the 3'-O-benzoyl), a band at R_f0.42 was obtained which was subsequently eluted with water and lyophilized. This material had electrophoretic mobility of Rm(Tp) 0.25 and the u.v. spectrum in water showed a maximum at 267.5nm and a shoulder at 300nm. To establish the identity of this compound, it was subjected to the action of spleen phosphodiesterase, an enzyme reported to act specifically on oligonucleotides possessing a free terminal 5'-hydroxyl group[108]. The material was completely degraded to thymidine 3'-phosphate and N^6-benzoyl-8,2'-thioanhydroadenosine(nucleotide/nucleoside =1.12). Thus the product LXXXI isolated in 19% yield was the desired dinucleotide. The yield was only 14.6% when DCC was used instead of MSCl as condensing agent.

Compound LXXXI when subjected to the action of snake venom phosphodiesterase was unaffected. This enzyme is reported[109] to act specifically on oligonucleotides having a free 3'-hydroxyl. It is apparent from these enzyme studies that the phosphodiesterase being used will not hydrolyze the phosphodiester bond if the end which it must recognize is an anhydro nucleoside. This observation has been substantiated by other unpublished results[102,110] on anhydronucleotides.

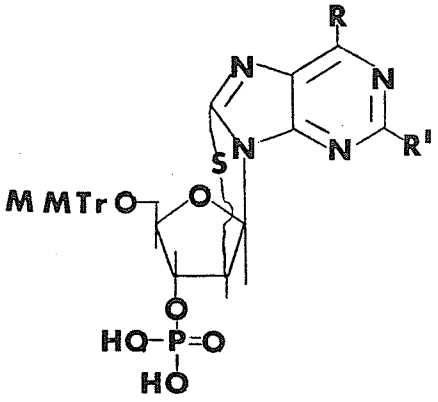
Recently Ikehara[101] has reported the synthesis of 8,2'-thioanhydroadenylyl(3'-5') 8,2'-thioanhydroadenosine by condensation of 5'-O-trityl-N^6-dimethylaminomethylene-9-(β-D-arabinofuranosyl)adenine 3'-phosphate with N^6,$O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine using twelve equivalents of DCC and allowing the reaction to continue for eleven days.

The significant difference between this procedure and those previously mentioned is in the preparation of the mononucleotide. Before the condensation reaction this mononucleotide is passed through a Dowex ion-exchange column(pyridinium form). The resulting nucleotide is then added to the condensation reaction along with more ion-exchange resin. This method was then employed for the synthesis of dinucleotides containing 8,2'-thioanhydroadenosine, guanosine and inosine(Scheme XX).

5'-O-Monomethoxytrityl-N^6-benzoyl-8,2'-thioanhydroadenosine(LXVIII) was phosphorylated in the 3'-position using β-cyanoethyl phosphate(3 equivalents) and DCC in pyridine.[101] The cyanoethyl group was removed using methanol-concentrated ammonia(1:1) at 45°C. for ten hours.[101] However under these conditions the benzoyl group on the N^6-position was also removed. It was then necessary to reblock the amino group and this was accomplished by using dimethylformamide dimethylacetal to introduce the dimethylaminomethylene to this position. Once isolated LXXXIII was dissolved in 50% aqueous pyridine and passed through a column of Dowex 50W-X8(pyridinium form). The resulting nucleotide was then dried and combined with DCC(12 equivalents), ion-exchange resin, LXX and stirred in pyridine at room temperature for 11 days.[101] Following deblocking first with methanolic ammonia and then 80% acetic acid,[101] LXXXV was isolated in approximately 30% yield on paper chromatography in Solvent A. The product was identical in all respects to an authentic sample[101] and found to be completely resistant to both snake venom and spleen phosphodiesterases as had been reported.

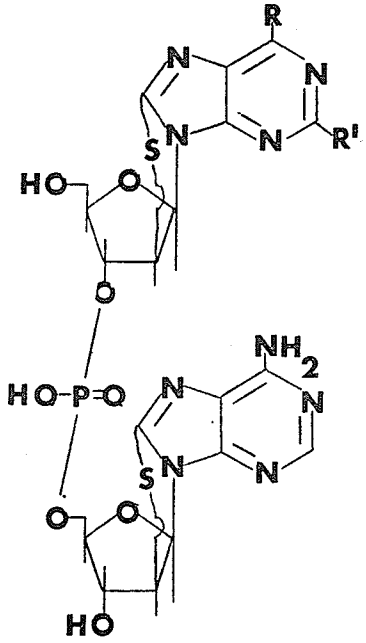
The synthesis of 8,2'-thioanhydroguanylyl(3'-5')8,2'-thioanhydroadenosine(LXXXVI) was accomplished in a similar manner. 5'-O-Monomethoxytrityl-N^2-acetyl-8,2'-thioanhydroguanosine(LXXII) was converted to the

Scheme XX General synthesis of di-thioanhydropurine nucleoside monophosphates

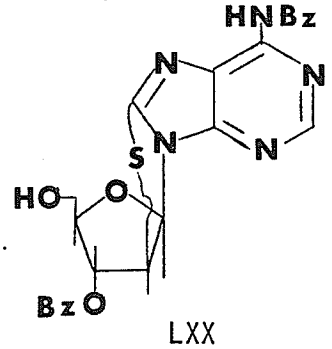


LXXXIII R=NDMAM
R'=H
LXXXIV R=OH
R'=NDMAM
LXXIV R=OH
R'=H

1)DCC,pyridine
Dowex 50W-X8
(pyridinium)
2) OH
3) H^+



+



LXX

LXXXV R=NH_2, R'=H
LXXXVI R=OH, R'=NH_2
LXXXVII R=OH, R'=H

corresponding N^2-dimethylaminomethylene 3'-phosphate(LXXXIV) by the same procedure outlined above for the synthesis of LXXXIII. In both nucleotide syntheses the introduction of the dimethylaminomethylene was observed by the appearance of a maximum in the u.v. spectrum in the range 310 - 320nm. Following treatment of the nucleotide (LXXXIV) with the ion-exchange column the condensation was accomplished in 21.4% yield to LXXXVI using excess DCC, ion-exchange resin and pyridine at room temperature for 11 days. The dinucleoside monophosphate had Rm(pA) 0.24 and the u.v. spectrum in water showed a maximum at 268nm with shoulders at 276nm and 220nm. Compound LXXXVI was totally resistant to snake venom and spleen phosphodiesterases and structural confirmation was provided by Raney nickel reduction to 2'-deoxyguanylyl(3'-5') 2'-deoxyadenosine, identical to an authentic sample[111].

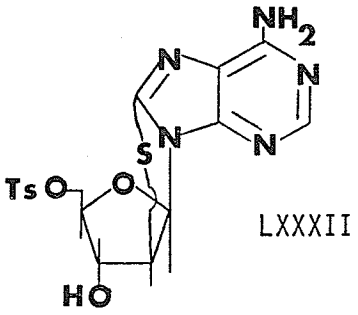
Finally the synthesis of 8,2'-thioanhydroinosinyl (3'-5') 8,2'-thioanhydroadenosine(LXXXVII) was accomplished from LXXIV and LXX in 8.1% yield using the same procedure. The structure of LXXXVII was confirmed by Raney nickel reduction followed by snake venom phosphodiesterase action to give 2'-deoxyinosine[58] and 2'-deoxyadenosine 5'-phosphate which was available from the earlier reduction of 8,2'-thioanhydroadenosine 5'-phosphate(nucleotide/nucleoside =1.09). The starting dinucleoside monophosphate LXXXVII was not degraded at all by snake venom or spleen phosphodiesterase.

Thus it has been demonstrated that 8,2'-thioanhydro nucleosides may be utilized for dinucleoside monophosphate synthesis and that the anhydro linkage acts as a useful 2'-hydroxyl blocking group. The resistance of dinucleoside monophosphates LXXXV, LXXXVI and LXXXVII to the phosphodiesterase enzymes may have significant biological importance with respect to the use of these dinucleoside monophosphates in <u>in vivo</u> or <u>in vitro</u> studies.

In exploring alternate routes to dinucleoside monophosphates it was noted that Todd and co-workers[112,113] had synthesized uridine dinucleoside monophosphates by nucleophilic displacement

of a 5'-halo substituent on a uridine molecule with 3'-uridylic acid. It was felt that if the corresponding 5'-halogenated-8,2'-thioanhydropurine nucleosides could be reacted in a similar manner with the available 3'-phosphates of anhydro nucleosides, this may provide an alternate method of synthesis of di-thioanhydropurine nucleoside monophosphates.

The initial approach was the conversion of 8,2'-thioanhydroadenosine(XII) to the corresponding 5'-O-tosyl derivative (LXXXII). Stirring XII in pyridine with excess p-toluene-



LXXXII

sulfonyl chloride gave a 63% yield of LXXXII. 5'-O-Tosyl-8,2'-thioanhydroadenosine (LXXXII) was characterized by a covalent sulfonate absorption at 8.55μ in the i.r. However when sodium iodide displacement[114] of the tosylate was attempted no reaction occurred and LXXXII was recovered quantitatively. In an attempt to synthesize a dinucleotide directly from LXXXII, 5'-O-monomethoxytrityl-8,2'-thioanhydroinosine 3'-phosphate(LXXIV) and LXXXII were stirred together for 24 hours. No detectable condensation occurred after this time and even after heating at 60°C. for one hour the only apparent change in the starting materials was a small amount of dephosphorylation of LXXIV. This lack of reactivity of the 5'-O-tosylate is not all that

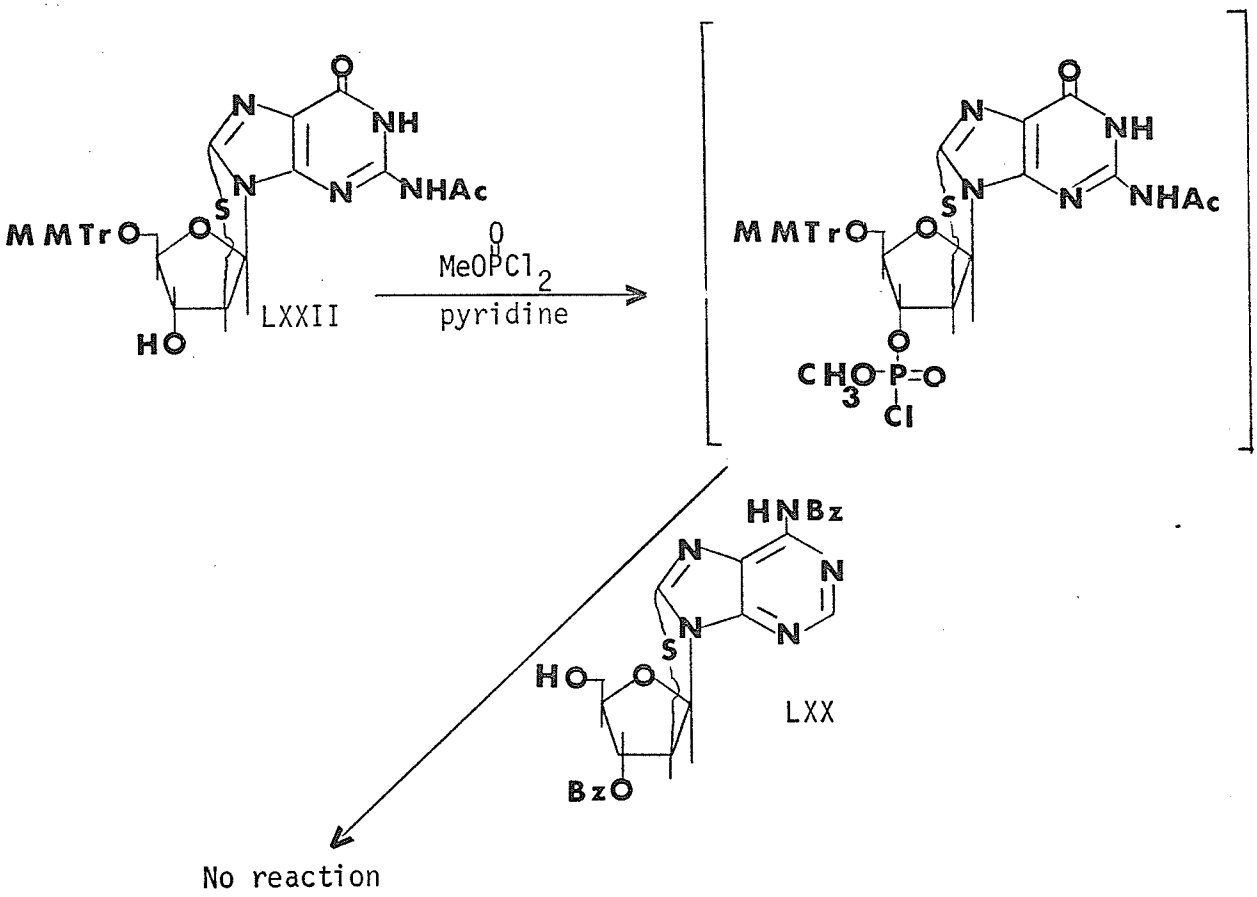
surprising since it has been shown[113] that even slight modifications of the sugar conformation drastically alters the leaving ability of the sulfonate in the presence of nucleophiles.

In 1971 Kikugawa and Ichino[115] reported the direct halogenation of the 5'-hydroxyl of ribonucleosides using thionyl chloride in hexamethylphosphoramide. Attempts to utilize this procedure to produce the 5'-chloro derivatives of the 8,2'-thioanhydropurine nucleosides were unsuccessful as only starting material was detectable in the reaction mixture.

The final approach to be explored was dinucleoside monophosphate synthesis via 5'-phosphorofluoridates or 3'-phosphorochloridates. It had been reported that thymidine 5'-phosphate could be converted to the corresponding 5'-phosphorofluoridate by reaction with 2,4-dinitrofluorobenzene and triethylamine in dry dimethylformamide[116]. Reaction of this product with 3'-O-monomethoxytritylthymidine in anhydrous potassium t-butoxide - hexamethylphosphoramide[117] gave an excellent yield of the dithymidine monophosphate. When 8,2'-thioanhydroadenosine 5'-phosphate(XLVII) was treated with 2,4-dinitrofluorobenzene and triethylamine in dimethylformamide there was complete loss of material with u.v. maximum at 275.5nm(8,2'-thioanhydroadenosine) and no detectable monocharged material on electrophoresis. While the identity of the products was not established it was clear that the desired nucleoside 5'-phosphorofluoridate was not present.

Finally the use of methylphosphorodichloridate[118] was explored(Scheme XXI). 5'-O-Monomethoxytrityl-N^2-acetyl-8,2'-thioanhydroguanosine(LXXII) was treated with an equivalent amount of methylphosphorodichloridate in pyridine for three hours at room temperature. Thin-layer chromatography indicated complete conversion to the phosphorochloridate. One

Scheme XXI Attempted dinucleoside monophosphate synthesis via phosphorochloridate



equivalent of $N^6,O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine(LXX) was added and the reaction continued for 24 hours. Following work-up and deblocking the only nucleotide material present was identified as N^2-acetyl-8,2'-thioanhydroguanosine 3'-phosphate on the basis of the u.v. spectrum in water and its' electrophoretic mobility.

Attempted Synthesis of Polymeric Anhydronucleic Acids

Since the 8,2'-thioanhydropurine nucleosides are rigid molecules locked in a particular conformation, it would be of interest to synthesize polymers of these molecules in order to examine their helical properties and ability to interact with "normal" polynucleotides. Khorana[119,120] has chemically polymerized nucleoside 5'-phosphates using DCC or aromatic sulfonyl chlorides as activating agents. Using either of these conditions as applied to 8,2'-thioanhydroadenosine 5'-phosphate(XLVII) the only change in the starting material was its conversion to the corresponding pyrophosphate LXXX. When the reaction using DCC was heated at 60°C. for one hour, the remaining starting material was completely dephosphorylated. When the mesitylenesulfonyl chloride reaction was similarly heated, dephosphorylation and sulfonation occurred quite rapidly.

Recently Pongs and Ts'O[121] reported the polymerization of unprotected thymidine 5'-phosphate when refluxed for 0.5 hour in a solution of dry dimethylformamide, triethylamine and dioxane saturated with dry hydrochloric acid. When this procedure was attempted using 8,2'-thioanhydroadenosine 5'-phosphate(XLVII), electrophoretic and paper chromatographic analysis of the reaction mixture revealed almost complete dephosphorylation of the starting material.

Although the attempted chemical polymerizations of anhydro nucleotides were unsuccessful this does not rule out the possibility of an enzymatic polymerization. The enzyme polynucleotide phosphorylase(nucleoside diphosphate: polynucleotide nucleotidyltransferase) has been shown to polymerize numerous modified nucleoside 5'-diphosphates[122]. Since the 5'-diphosphates of 8,2'-thioanhydroadenosine, guanosine and inosine have now been synthesized, a proposal for future research would entail an examination of their substrate activity with polynucleotide phosphorylase.

EXPERIMENTAL

General Methods

Descending paper chromatography was carried out using Whatman 3MM paper. The solvent systems employed were: Solvent A, isopropyl alcohol-concentrated ammonium hydroxide-water(7:1:2); Solvent B, ethanol-water(7:3); Solvent C, n-butanol-ethanol-water(4:1:5, organic phase); Solvent D, 1M ammonium acetate-ethanol(3:7); Solvent E, 0.5M ammonium acetate-ethanol(3:7, adjusted to pH 3.5 with acetic acid); Solvent F, n-propanol-concentrated ammonium hydroxide-water(55:10:35); Solvent G, ethanol-water(7:3, saturated with sodium chloride). The solvents were prepared on a volume basis. Thin-layer chromatography was carried out employing the ascending technique in closed jars which were not coated with absorbent paper. All thin-layer chromatography was run on Eastman Chromagram Sheets 6060, silica gel with fluorescent indicator, on strips 10cm. x 2cm. Thick-layer chromatography was carried out on glass plates(20cm. x 20cm.) coated with a 2mm. thick layer of silica gel DSF-5(Mondray Chemicals Ltd.). Paper electrophoresis was performed using Whatman 3MM paper in a Savant Flat Plate electrophoretic chamber with a Savant Model HV power supply operated at 2000V for 1 hour; the solution was a triethylammonium bicarbonate buffer(0.05M, pH7.5), prepared by making 15.15g triethylamine up to 3l volume with water and then bubbling 20g carbon dioxide through the solution. Nucleosides and their derivatives were detected on paper chromatograms, thin and thick-layer sheets using an ultraviolet light source(Mineralite, output ∿254nm). Compounds containing trityl or p-monomethoxytrityl groups were detected on chromatography by spraying with 10% perchloric acid solution and drying them in a stream of warm air.

Infrared spectra were obtained on a Perkin-Elmer 337 recording instrument using KBr disks for sample preparation. Ultraviolet spectra were obtained on a Cary Model 14 recording spectrophotometer. Water or 95% ethanol was used for neutral solutions whereas for pH1 a buffer of 27ml of 0.2M KCl and 73ml of 0.2M HCl was employed. Mass spectra were obtained on a Hitachi RMU-6D instrument. High resolution mass spectra were obtained on an AEI MS-9 high resolution mass spectrometer at the University of Alberta.

Melting points were determined on a Fisher-Johns melting point apparatus and are reported uncorrected. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville Tennessee. Samples submitted to them were prepared by crystallization, lyophilization or precipitation from tetrahydrofuran with hexane followed by heating in a drying apparatus over P_2O_5. In some cases the molecular formula includes fractional moles of solvent as a correction for the analysis. These corrections are added only when a corresponding weight loss was detected on extensive drying of the sample. Phosphate analyses were performed by the method of Lowry and Lopez[83] using ammonium molybdate and ascorbic acid. After release of the phosphate from the nucleotide following alkaline phosphatase action, the phosphate concentration was measured on a Zeiss PMQ 9 absorption spectrometer at 700nm as the phosphomolybdate. This value is reported relative to the same reaction on an equal concentration of adenosine 5'-monophosphate.

Reagents and Chemicals

Reagent grade pyridine was distilled from p-toluenesulfonyl chloride, redistilled from calcium hydride, and stored over Linde Molecular Sieves. Reagent grade N,N-dimethylformamide was dried by distillation from calcium hydride and

stored over Linde Molecular Sieves. Reagent grade acetic anhydride was distilled from phthalic anhydride and stored in the dark. n-Butanol and phosphoryl chloride were freshly distilled before use. Reagent grade mesitylenesulfonyl chloride purchased from Aldrich was recrystallized from pentane and stored under pentane in the dark. Pyridinium Dowex was prepared by forming a column of Dowex 50W-X8(H^+ form) and washing first with 0.1N HCl(1l), then water(1l), then 10% aqueous pyridine(1l) and finally water(1l). Pyridinium mono-β-cyanoethyl phosphate was prepared from the barium salt by passage over a column of Dowex 50W-X8 resin(pyridinium form). The clear solution containing the pyridinium salt was first concentrated to a small volume and then lyophilized to a gum. The gum was dissolved in pyridine and diluted to a known volume and stored in a sealed flask under refrigeration. Sodium hydrogen sulfide solution(40%) was prepared by dissolving 18g sodium sulfide in 50ml of water and adding 6.0 g of sodium bicarbonate in small amounts until complete dissolution occurs. Methanol(50ml) was added with stirring and cooling below 20°C. Following filtration and washing with methanol the filtrate is concentrated to one-tenth the volume and stored under refrigeration.

Nucleosides were purchased from Sigma Chemical company. 8-Aminoadenosine(219°C. dec.,77%), 8-aminoguanosine (220°C. dec.,60%) and 8-aminoinosine(179°C. dec.,58%) were synthesized according to the literature[75,76]. 8-Bromoadenosine (195°C. dec.,71%), 8-bromoguanosine(191-194°C. 89%) and 8-bromoinosine(218°C. dec.,73%) were prepared according to the literature[52,53,70].

Enzyme Assays

a) Spleen phosphodiesterase

Lyophilized spleen phosphodiesterase(10-15 units) obtained from Nutritional Biochemicals Corp. was dissolved

in 1ml of 0.01M sodium pyrophosphate buffer adjusted to pH6.5 with phosphoric acid. An aliquot(0.1ml) of this solution, 0.2ml of 0.05M ammonium acetate(adjusted to pH6.5 with acetic acid) and 0.1 - 1mg of the nucleotide substrate were incubated at 37°C. The solution was then applied to Whatman 3MM paper as a band and developed with Solvent A. Nucleoside and nucleotide bands were cut out, eluted with water and diluted to a volume of 10ml. Absorbances were adjusted by using blanks cut from papers treated the same as the sample material. The term OD unit refers to the extinction of the absorbing material in 1ml of neutral solution in a quartz cell with a 1-cm. path length.

b) Snake venom phosphodiesterase

Two hundred units of snake venom phosphodiesterase obtained from Calbiochem was dissolved in 1ml of tris(hydroxymethyl)aminomethane buffer(adjusted to pH 9.2 with 0.1N HCl). An aliquot(0.2ml) of the enzyme solution was added to the nucleotide material(∿1mg) and incubated a 37°C. The same procedure as in a) was then followed.

c) Adenosine deaminase

Adenosine deaminase from Sigma Chemical Co. was dissolved in a ph7.5 buffer(50ml potassium dihydrogen phosphate (0.1M) and 41.1ml sodium hydroxide(0.1M)) and added to the nucleoside material at 25°C.

d) 5'-Nucleotidase

5'-Nucleotidase(0.5mg) from Sigma Chemical Co. was dissolved in a pH9.0 buffer(50ml 0.025M borax and 4.6ml 0.1M HCl) along with 1mg nucleotidic materialand the solution incubated at 37°C. for one hour.

e) Alkaline phosphatase

Nucleotidic material(1mg) was treated with 0.1ml of a suspension of alkaline phosphatase in ammonium sulfate (Sigma Chemical Co.) along with 1ml pH10.4 buffer(50ml 0.05M sodium bicarbonate and 16.5ml of 0.1M sodium hydroxide) and incubated at 37°C. for five minutes.

f) Adenylate kinase

i) 2 Units of adenylate kinase(Sigma Chemical Co.), nucleotidic material(1mg) and 0.1 ml pH7.6 buffer(50ml of tris(hydroxymethyl)aminomethane and 40.3ml 0.1M HCl) and the solution incubated at 37°C. for four hours.

ii) The conditions were the same as i) except 2mg ATP and 3.4mg magnesium sulfate were also added.

General Procedures

All reactions of more than 0.2mmole of the limiting reagent were carried out in tightly stoppered ground glass joint flasks. Reactions of less than 0.2mmole of the limiting reagent were carried out in Pyrex test tubes(10ml, screw cap type). Reactions requiring drying by solvent evaporation were performed by evaporation under reduced pressure and air was readmitted through a column(30cm. x 2cm.) of anhydrous magnesium perchlorate.

Synthetic Methods

N^2-Dimethylaminomethylene-8-bromoguanosine (XVII)

8-Bromoguanosine(XVI, 3.63g, 10mmole) and N,N-dimethylformamide dimethylacetal(5ml, 50mmole) were dissolved in DMF(50ml) and stirred at room temperature for 12 hours. The solvents were removed at reduced pressure and the residue was crystallized from water to yield 3.99g(96%) of XVII(m.p. 216°-218°C.). Chromatographic data is listed in Tables I and II while spectral properties(u.v. and i.r.) are recorded in Table III.

Anal. Calcd. for $C_{13}H_{17}N_6O_5Br$: C, 37.42; H, 4.11; Br, 19.15
Found: C, 37.69; H, 4.20; Br, 19.08

5'-O-Monomethoxytrityl-N^2-dimethylaminomethylene-8-bromoguanosine (XVIII)

XVII(3.78g, 9.1mmole) and monomethoxytrityl chloride (3.16g, 10.2mmole) were dissolved in pyridine(15ml) and the solution stirred at room temperature for 24 hours. The solution was then poured into ice water with vigorous stirring. A precipitate formed which was collected by filtration, washed with water and reprecipitated from tetrahydrofuran using hexane. The resulting white solid(m.p. 158° dec.) was collected by filtration(6.17g, 99%) and identified as XVIII. Chromatographic data is listed in Tables I and II while spectral properties(u.v. and i.r.) are recorded in Table III.

Anal. Calcd. for $C_{33}H_{33}N_6O_6Br$: C, 57.48; H, 4.82; Br, 11.59
Found: C, 57.60; H, 4.95; Br, 11.84

5'-O-Monomethoxytrityl-2'-O-mesitylenesulfonyl-N^2-dimethylaminomethylene-8-bromoguanosine (XIXa)

XVIII(1.0g, 1.45mmole) and mesitylenesulfonyl chloride (0.63g, 2.9mmole) were dissolved in pyridine(5ml) and the solution was stirred at room temperature for 48 hours. The solution was poured into ice water and the precipitate was

TABLE I Paper Chromatographic* Data of Guanosine Derivatives

	SOLVENT+					
COMPOUND	A	B	C	D	E	F
8-BrG(XVI)	0.28	0.58	0.40	0.65	0.69	0.56
N^2-DMAM-8-BrG(XVII)	0.29	0.64	----	0.59	----	----
5'-O-MMTr-N^2-DMAM-8-BrG(XVIII)	0.79	0.88	----	----	0.93	----
5'-O-MMTr-2'-O-MS-N^2-DMAM-8-BrG(XIXa)	0.88	0.92	----	----	0.95	----
2'-O-MS-8-BrG(XX)	0.73	0.82	----	----	0.91	----
8,2'-SanhG(XXI)	0.20	0.58	0.22	0.58	0.69	0.43
2'-dG(XXII)	0.32	0.62	0.24	0.59	0.67	0.59

* Whatman 3MM - Descending technique

+ Described in General Methods

TABLE II Thin-layer* Chromatographic Data of Guanosine Derivatives

	SOLVENT			
COMPOUND	EtOH	$CHCl_3$-EtOH(7:3)	THF	EtOAc
8-BrG(XVI)	0.60	0.30	0.00	0.00
N^2-DMAM-8-BrG(XVII)	0.45	0.51	0.19	0.00
5'-O-MMTr-N^2-DMAM-8-BrG(XVIII)	----	----	0.41	0.05
5'-O-MMTr-2'-O-MS-N^2-DMAM-8-BrG(XIXa)	----	----	0.68	0.19
2'-O-MS-8-BrG(XX)	0.70	0.76	0.41	0.00
8,2'-SanhG(XXI)	0.43	0.31	0.00	0.00
2'-dG(XXII)	0.58	0.25	0.00	0.00

* Eastman Chromagram Sheets 6060, silica gel, with fluorescent indicator, strips 10cm. x 2cm.

TABLE III I.R. and U.V. Spectral Properties of Guanosine Derivatives

	U.V. Spectra		I.R. Spectra*
COMPOUND	SOLVENT	max,nm(ε)	μ
8-BrG(XVI)	water	262(15550),269(14100)sh	5.90,6.14, 6.60, 10.20
N^2-DMAM-8-BrG(XVII)	water	302(24400),233(12600)	5.85,8.95, 11.95, 12.85
5'-O-MMTr-N^2-DMAM-8-BrG(XVIII)	95% EtOH	309(24900),284(17200)sh 233(32800)	5.90,8.55, 14.30
5'-O-MMTr-2'-O-MS-N^2-DMAM-8-BrG(XIXa)	95% EtOH	309(24800),288(20300) 234(30000)	5.90,8.48,8.55,14.2
2'-O-MS-8-BrG(XX)	95% EtOH	275(12200)sh,265.5(14100),237(13100)	5.85,8.40,8.50
8,2'-SanhG(XXI)	water	283(13590)sh,268(16630)	5.90,6.55,12.00
2'-dG(XXII)	water	270(8600)sh,253(12800)	-------

* Principal bands

collected by filtration and dissolved in chloroform(100ml). The chloroform solution was dried over sodium sulfate, concentrated to a small volume and applied to five thick-layer plates. These plates were developed twice in ether followed by one development in tetrahydrofuran. Three nucleoside bands were present at R_f 0.72, 0.48 and 0.20. The band at R_f 0.20 was eluted from the plates with ethanol and upon evaporation of the ethanol 55mg of XVII were obtained. The band at R_f 0.48 was eluted with tetrahydrofuran and upon evaporation of the solvent 340mg of XVIII was obtained. The band at R_f0.72 was also eluted with tetrahydrofuran and yielded 530mg(42%) of XIXa(m.p. 148°-150°C.) upon precipitation with hexane. Chromatographic data is listed in Tables I and II while spectral properties(u.v. and i.r.) are recorded in Table III.

Anal. Calcd. for $C_{42}H_{43}N_6O_8BrS$: C, 57.86; H, 4.97
Found: C, 58.50; H, 5.04

5'-O-Monomethoxytrityl-2'-O-mesitylenesulfonyl-8-bromoguanosine

Method A Compound XIXa(200mg, 0.23mmole) was dissolved in 3ml of a solution of 85% hydrazine hydrate-acetic acid-pyridine(1:6:24) and the solution was stirred at room temperature for 24 hours. The solution was poured into ice water and the precipitate was collected by filtration to yield after drying 181mg(96%) of 5'-O-monomethoxytrityl-2'-O-mesitylenesulfonyl-8-bromoguanosine(m.p. 162°C. dec.). Thin-layer chromatography showed R_f values of 0.18(ethyl acetate) and 0.60(tetrahydrofuran). Principal bands in the i.r. spectrum occurred at 5.85, 8.40, 8.50 and 14.20μ. The u.v. spectrum in 95% ethanol showed maxima at 276, 267 and 233.5nm with a shoulder at 269nm.

Anal. Calcd. for $C_{39}H_{38}N_5O_8BrS$: C,57.35; H, 4.69; Br, 9.78
Found: C,57.73; H, 4.62; Br, 11.11

Method B The dimethylaminomethylene group could be removed in 98% yield by stirring XIXa in concentrated ammonium hydroxide for 24 hours at room temperature.

2'-O-Mesitylenesulfonyl-8-bromoguanosine (XX)

5'-O-Monomethoxytrityl-2'-O-mesitylenesulfonyl-8-bromoguanosine(500mg, 0.61mmole) was stirred in 80% acetic acid(10ml) for three hours at room temperature. The solvent was removed at reduced pressure and the residue was dissolved in tetrahydrofuran(3ml) and applied to three thick-layer plates which were developed in tetrahydrofuran. A nucleoside band appeared at R_f 0.40 and was eluted with tetrahydrofuran. The product was collected by precipitation with hexane followed by filtration to yield 274mg(80%) of XX(m.p. 183°C. dec.). Chromatographic data is listed in Tables I and II while spectral properties(u.v. and i.r.) are recorded in Table III.

Anal. Calcd. for $C_{19}H_{22}N_5O_7BrS$: C, 41.92; H, 4.07; Br, 14.68
Found: C, 42.20; H, 4.27; Br, 14.59

8,2'-Thioanhydroguanosine (XXI)

Method A Compound XX(50mg, 0.09mmole) and 0.2ml of a 40% solution of sodium hydrogen sulfide(1.25mmole) and water were added to DMF(3ml) and the solution heated at 100°C. for 18 hours. The solution was cooled and applied to Whatman 3MM paper developed in Solvent A. Nucleoside bands appeared at R_f 0.73 and 0.19. The band at 0.73 was eluted with water and lyophilized to yield 24mg of XX. The band at R_f 0.19 was also eluted with water and the aqueous solution was lyophilized to yield 11mg(39%) of XXI(m.p. 268°C. dec.). The mass spectrum of the tetrasilyl derivative showed a parent peak at m/e 585. Chromatographic data is listed in Tables I and II while spectral properties(u.v. and i.r.) are recorded in Table III.

Anal. Calcd for $C_{10}H_{11}N_5O_4S$: C, 40.40; H, 3.73; N, 23.56
Found: C, 40.34; H, 3.99; N, 23.05

Method B The above procedure was repeated using a six-fold excess of sodium hydrogen sulfide and a temperature of 70°C. for 18 hours. The yield of XXI was 16%.

2'-Deoxyguanosine XXII

Compound XXI(10mg, 0.03mmole) was dissolved in water(1ml) and the solution refluxed with a spoonfull of Raney nickel for three hours. The product, 2'-deoxyguanosine(XXII, m.p. 215°C. dec.) was isolated by paper chromatography in Solvent A and was identical in all respects to an authentic sample[58].

The product was hydrolyzed[59] to 2-deoxyribose which gave positive tests with Dischereagent[60] and cysteine-sulfuric acid reagent[61].

2',5'-Di-O-trityl-N^2-dimethylaminomethylene-8-bromoguanosine (XXV)

N^2-Dimethylaminomethylene-8-bromoguanosine(XVII, 1.95g, 4.6mmole) and trityl chloride(3.96g, 13.95mmole) were stirred at room temperature in pyridine(25ml) for 72 hours followed by heating at 100°C. for 0.5 hour. The solution was cooled and poured into an ice water mixture(1l). The resulting precipitate was collected by filtration, dissolved in chloroform and dried over sodium sulfate. After filtration the solution was concentrated to a small volume in vacuo. Upon addition of hexane the product precipitated as a white solid(3.5g, 74%, m.p. 151°-153°C.). Chromatographic data is listed in Table IV while spectral properties(u.v. and i.r.) are recorded in Table V.

Anal. Calcd. for $C_{51}H_{45}N_6O_5Br$: C, 67.92; H, 5.03; Br, 8.86
Found: C, 67.78; H, 4.80; Br, 8.84

2',5'-Di-O-trityl-3'-O-methanesulfonyl-N^2-dimethylaminomethylene-8-bromoguanosine and 2',5'-Di-O-trityl-3'-O-methanesulfonyl-8-bromoguanosine

Compound XXV(3.5g, 3.88mmole) was dissolved in pyridine (20ml) and the solution was cooled to 0°C. Methanesulfonyl chloride

TABLE IV Paper* and Thin-layer+ Chromatographic Data of Guanosine Derivatives

	Paper SOLVENT°		Thin-layer SOLVENT			
COMPOUND	A	B	THF	EtOAc	EtOH	$CHCl_3$:EtOH(7:3)
2',5'-Di-O-Tr-N^2-DMAM-8-BrG (XXV)	----	0.94	0.60	0.20	----	----
2',5'-Di-O-Tr-3'-O-Ms-N^2-DMAM-8-BrG	----	0.94	0.72	0.35	----	----
2',5'-Di-O-Tr-3'-O-Ms-8-BrG	----	0.93	0.68	0.31	----	----
3'-O-Ms-8-BrG (XXVI)	0.47	0.75	0.14	0.00	0.83	----
8,3'-SanhG (XXIII)	0.32	0.58	0.00	0.00	0.45	0.31
3'-dG (XXIV)	0.24	0.61	0.00	0.00	0.58	0.25

* Whatman 3MM -Descending technique

\+ Eastman Chromagram Sheets 6060, silica gel with fluorescent indicator, strips 10cm. x 2cm.

o Described in General Methods

TABLE V I.R. and U.V. Spectral Properties of Guanosine Derivatives

	U.V. Spectra		I.R. Spectra*
COMPOUND	SOLVENT	max,nm(ε)	μ
2',5'-Di-O-Tr-N^2-DMAM-8-BrG (XXV)	95% EtOH	309(23800),233(25600) 284(23100)sh	14.20
2',5'-Di-O-Tr-3'-O-Ms-N^2-DMAM-8-BrG	95% EtOH	312(23700),284.5(22100) 232(25000)	8.5,14.20
2',5'-Di-O-Tr-3'-O-Ms-8-BrG	95% EtOH	266(15300),274(13180)sh 233(23600)sh	8.5,14.20
3'-O-Ms-8-BrG (XXVI)	95% EtOH	261.5(14100),270(12500)sh	8.5
8,3'-SanhG(XXIII)	water	272(14410),283(10730)sh	5.90, 13.00
3'-dG(XXIV)	water	253(13100),270(8900)sh	5.90, 10.30, 13.70

* Principal bands

(0.29ml, 4 mmole) was added and the solution was kept at 0°C. for 24 hours. An additional 4mmole of methanesulfonyl chloride was added and stirring continued for a further 24 hours at 0°C. The solution was poured into an ice water mixture, the precipitate was collected by filtration, dissolved in chloroform and the resulting solution was dried over sodium sulfate. The chloroform solution was collected by filtration, concentrated to a small volume and applied to 15 thick-layer plates which were developed three times in ether. The band at R_f 0.30 was eluted with tetrahydrofuran, the solution concentrated and on addition of hexane the product precipitated(3g, 79%, m.p. 144°-145°C.). Chromatographic data is listed in Table IV and spectral properties(i.r. and u.v.) in Table V.

Without further purification 2g(2.05mmole) of this product was dissolved in hydrazine hydrate-acetic acid-water (1:6:24, 10ml) and the solution was stirred at room temperature for 48 hours. The solution was poured into ice water and the resulting precipitate was collected and dried to yield 1.55g(85%, m.p. 169°C. dec.) of 2',5'-di-O-trityl-3'-O-methanesulfonyl-8-bromoguanosine. Chromatographic data is listed in Table IV while spectral properties(u.v and i.r.) are recorded in Table V.

Anal. Calcd. for $C_{49}H_{42}N_5O_7BrS$: C, 63.63; H, 4.58; Br, 8.64
Found: C, 63.62; H, 4.45; Br, 8.40

3'-O-Methanesulfonyl-8-bromoguanosine (XXVI)

2',5'-Di-O-trityl-3'-O-methanesulfonyl-8-bromoguanosine(1g, 1.08mmole) was dissolved in 80% acetic acid(7ml) and heated on a steam bath for 15 minutes. The solution was poured into ice water and the resulting precipitate gathered by filtration and recrystallized from 95% ethanol to give 390mg(83%, m.p. 187°C. dec.) of XXVI. Chromatographic data is listed in Table IV while spectral properties(u.v. and i.r.) are recorded in Table V.

Anal. Calcd. for $C_{11}H_{14}N_5O_7BrS$: C, 30.01; H, 3.21
Found: C, 30.11; H, 3.29

8,3'-Thioanhydroguanosine(XXIII)

Compound XXVI(51mg, 0.12mmole) and thiourea(18mg, 0.23mmole) were refluxed in freshly distilled n-butanol(4ml) for 5 hours. The solvent was removed at reduced pressure and the residue was dissolved in DMF-ammonium hydroxide(1:1, 2ml) and applied to Whatman 3MM paper which was developed in Solvent A. The band at R_f 0.32 was eluted with water and lyophilized to yield 13mg(37%, m.p. 183°C. dec.) of XXIII. Chromatographic data is listed in Table IV and spectral properties(u.v. and i.r.) in Table V. The mass spectrum of the tetrasilyl derivative showed a parent peak at m/e 585.

Mol. wt. Calcd. for $C_{22}H_{43}N_5O_4SSi_4$: 585.2113
Found: 585.2122

3'-Deoxyguanosine (XXIV)

Compound XXIII(25mg, 0.08mmole) was dissolved in hot water(2ml) and after addition of a spoonful of Raney nickel the mixture was refluxed for 3 hours. The mixture was filtered and the filtrate applied to Whatman 3MM paper which was developed in Solvent A. The band at R_f 0.24 was eluted with water and lyophilized to yield 4mg(18%, m.p. 198°C. dec.) of XXIV. Chromatographic data is listed in Table IV while spectral properties of the tetrasilyl derivative showed a parent peak at m/e 555.

3'-Deoxyguanosine was further identified by hydrolysis[59] to 3'-deoxyribose which gave a negative Dische test[53] but positive cysteine[61] and aniline[65] tests.

8-Bromoadenosine 2',3'-carbonate (XXVIII)

8-Bromoadenosine(XXVII, 1g, 2.9mmole), diphenyl carbonate(0.82g, 3.8mmole) and sodium bicarbonate(15mg) were heated in DMF(3ml) at 150°C. for 0.5 hour. The mixture was cooled to room temperature and applied to eight thick-layer plates which were developed first in ether and then in ethyl acetate. The band at R_f 0.35 was eluted with tetrahydrofuran and on precipitation with hexane yielded 0.75g (70%, m.p. 159°-162°C.) of XXVIII. Chromatographic data is listed in Table VI while spectral properties(u.v. and i.r.) are recorded in Table VII.

Anal. Calcd. for $C_{11}H_{10}N_5O_5Br \cdot 0.2THF$: C, 36.66; H, 3.02; Br, 20.67
Found: C, 36.91; H, 2.94; Br, 21.18

8-Bromoinosine 2',3'-carbonate (XXXI)

Compound XXXI was prepared from 8-bromoinosine(XXX) in the same manner as described for the preparation of XXVIII above. A 74% yield of XXXI(m.p. 218°C. dec.) was obtained. Chromatographic data is shown in Table VI while spectral properties(u.v. and i.r.) are recorded in Table VII. The compound was not further purified but was used directly to prepare 8,2'-thioanhydroinosine(XXXII).

8-Bromoguanosine 2',3'-carbonate (XXXIV)

8-Bromoguanosine(XVI, 1.0g, 2.76mmole), diphenyl carbonate(1.21g, 5.52mmole) and sodium bicarbonate(50mg) were heated in DMF(10ml) at 150°C. for 0.5 hour. The mixture was cooled to room temperature and poured into ether. A gummy precipitate was obtained which was washed with ether and then precipitated several times from DMF using ether. The solid was collected by filtration and dried to yield 1.03g(98%, m.p. 228°C. dec.) of XXXIV. Chromatographic data is shown in Table VIII and spectral properties(u.v. and i.r.) in Table IX.

Anal. Calcd. for $C_{11}H_{10}N_5O_6Br$: C, 34.04; H, 2.60
Found: C, 33.80; H, 2.59

TABLE VI Paper* and Thin-layer+ Chromatographic Properties of Purine Nucleosides

	Paper SOLVENT°				Thin-layer SOLVENT		
COMPOUND	A	B	C	F	THF	EtoAc	EtOH
8-BrA(XXVII)	0.61	0.64	0.49	0.75	0.65	0.11	----
8-BrA 2',3'-cc (XXVIII)	----	0.81	0.79	----	0.70	0.53	----
8,2'-SanhA(XII)	0.46	0.50	0.30	0.64	0.35	0.01	0.66
2'-dA(XXIX)	0.67	0.68	0.44	0.73	0.26	0.01	0.57
8-BrI(XXX)	0.61	0.63	0.37	----	0.34	0.00	0.56
8-BrI 2',3'-cc (XXXI)	----	0.65	0.48	----	0.17	0.60	----
8,2'-SanhI(XXXII)	0.34	0.69	0.28	0.59	0.06	0.00	0.58
2'-dI(XXXIII)	0.43	0.64	0.34	0.64	0.08	0.00	0.41

* Whatman 3MM - Descending technique

\+ Eastman Chromagram Sheets 6060, silica gel, with fluorescent indicator, strips 10cm. x 2cm.

o Described in General Methods

TABLE VII I.R. and U.V. Spectral Properties of Purine Nucleosides

	U.V. SPECTRA		I.R. SPECTRA*
COMPOUND	SOLVENT	max,nm(ε)	μ
8-BrA(XXVII)	water	265.5(18300)	6.10,8.90,9.20,11.65
8-BrA 2',3'-cc (XXVIII)	95% EtOH	263.5(14100)	5.55,6.80,8.85,9.25
8,2'-SanhA(XII)	water	276.5(17000),222(19100)	6.05,7.25,11.70
2'-dA(XXIX)	water	260(14000)	6.05,7.55,9.00,10.20
8-BrI(XXX)	water	254.5(15000)	5.85,7.15,8.05,10.20
8-BrI 2',3'-cc (XXXI)	95% EtOH	254(13800)	5.55,5.75,8.75,11.60
8,2'-SanhI(XXXII)	water	265(10080)	5.90,6.3,9.3,12.80
2'-dI(XXXIII)	water	249(12700)	5.85,7.00,9.20,10.65,14.20

*Principal bands

TABLE VIII Paper* and Thin-layer+ Chromatographic Properties of Purine Nucleosides

	Paper SOLVENT°			Thin-layer SOLVENT		
COMPOUND	A	B	C	THF	EtOAc:EtOH(2:1)	EtOH
8-BrG 2',3'-cc(XXXIV)	----	0.69	0.57	0.14	0.53	----
8,2'-SanhX(XXXV)	0.30	0.52	0.09	0.00	----	0.31
2'-dX(XXXVI)	0.26	0.46	0.12	0.00	----	0.39

* Whatman 3MM - Descending technique

\+ Eastman Chromagram Sheets 6060, silica gel with fluorescent indicator, strips 10cm. x 2cm.

o Described in General Methods

TABLE IX I.R. and U.V. Spectral Properties of Purine Nucleosides

	U.V. SPECTRA		I.R. SPECTRA*
COMPOUND	SOLVENT	max,nm(ε)	μ
8-BrG 2',3'-cc(XXXIV)	95% EtOH	263.5(14700),276(13600)sh	5.50,6.60,8.65,9.25
8,2'-SanhX(XXXV)	water	287.5(12620),258(14980)	5.60,5.85,6.30,7.10,10.85
2'-dX(XXXVI)	pH 1	268(9700),228.5(8400)	5.60,5.85,8.90,9.15

* Principal bands

8,2'-Thioanhydropurine nucleosides General Procedures(Schemes VII and VIII)

Method A In a typical experiment 1mmole of the cyclic carbonate dissolved in DMF(2ml) was treated with a 40% solution of sodium hydrogen sulfide(6 eqiv. of NaSH) and the solution was heated at 70°C. for 18 hours. The solution was then either applied directly to thick-layer plates or paper chromatograms.

Method B In a typical experiment 1mmole of the cyclic carbonate and two equivalents of thiourea were refluxed in freshly distilled n-butanol(15ml) for five hours. The solvent was then removed at reduced pressure and the residue dissolved in ethanol and applied to either thick-layer plates or paper chromatograms.

8,2'-Thioanhydroadenosine (XII)

The products from both Method A and Method B were worked up in the same way. The reaction products were applied to thick-layer plates which were developed first in ether, then ethyl acetate and finally tetrahydrofuran. The band at R_f 0.30 was eluted with ethanol, the solvent removed at reduced pressure and the residue crystallized from water to yield XII identical to authentic material[52]. The yield from Method A was 79% and 66% from Method B. Chromatographic data is shown in Table VI and spectral properties(u.v. and i.r.) in Table VII. The mass spectrum had a parent peak at m/e 281.

8,2'-Thioanhydroguanosine (XXI)

The reaction products from both Methods A and B were separated in the same manner. The reactionmixtures were applied to paper chromatograms which were developed in Solvent A. The band at R_f 0.24 was eluted with water which was lyophilized to yield XXI identical to 8,2'-thioanhydroguanosine prepared earlier. The yield was 56% from Method A and 41% from Method B. The mass spectrum of the tetrasilyl derivative had a parent peak at m/e 585.

8,2'-Thioanhydroxanthosine (XXXV)

Compound XXI(50mg, 0.17mmole) was suspended in acetic acid(3ml) and 0.3ml of sodium nitrite in water(3.9M) was added. The reaction mixture was stirred at room temperature for 5 hours, treated with ethanol(3ml) and concentrated to dryness. The residue was dissolved in DMF-concentrated ammonium hydroxide (1:1) and applied to eight paper chromatograms which were developed in Solvent C. The band at R_f 0.09 was eluted with water and lyophilized to yield 27mg XXXV(54%, m.p. 173°C. dec.). Chromatographic data is shown in Table VIII and spectral properties(u.v. and i.r.) in Table IX. The mass spectrum of the trimethylsilyl derivative had a parent peak at m/e 586.

8,2'-Thioanhydroinosine (XXXII)

Method A The reaction mixture was applied to paper chromatograms and developed in Solvent A. The band at R_f 0.34 was eluted with water and XXXII(73%, m.p. 217°C. dec.) collected by lyophilization. Chromatographic data is listed in Table VI while spectral properties(u.v. and i.r.) are recorded in Table VII. The mass spectrum of the trimethylsilyl derivative had a parent peak at m/e 498.

Anal. Calcd for $C_{10}H_{10}N_4O_4S \cdot 1.2H_2O$: C, 39.52; H, 4.11; S, 10.55
Found: C, 40.07; H, 4.63; S, 9.91

Method B The reaction mixture was applied to thick-layer plates which were developed in chloroform-ethanol(7:3). the band at R_f 0.68 was eluted with ethanol, the solvent removed at reduced pressure and residue crystallized from water to yield XXXII(69%).

Method C 8,2'-Thioanhydroadenosine(XII, 50mg, 0.18mmole) was treated with 2ml of acetic acid-water(4:15) and sodium nitrite(20mg) and the reaction stirred at room temperature for 3 hours. The reaction was applied to paper chromatograms in Solvent A. Following work-up as in Method A XXXII was obtained quantitatively.

Raney nickel reduction of the 8,2'-Thioanhydropurine nucleosides

The thioanhydronucleosides were dissolved in water and refluxed with Raney nickel for three hours. The products were isolated by paper chromatography in Solvent A. Compounds XII, XXXII and XXI led respectively to 2'-deoxyadenosine(XXIV), 2'-deoxyinosine(XXXIII) and 2'-deoxyguanosine(XXII) which were identical to authentic samples[58]. Compound XXXV led to 2'-deoxyxanthosine(XXXVI) which was identical in all respects to an authentic sample[71]. Chromatographic data are listed in Tables VI and VIII and spectral properties(u.v. and i.r.) in Tables VII and IX.

8,2'-Thio and aminoanhydropurine nucleosides from the corresponding 8-thio and 8-aminopurine nucleosides

8,2'-Thioanhydroadenosine (XII)

8-Thioadenosine(XXXVIII, 100mg, 0.34mmole), diphenyl carbonate(95mg, 0.44mmole) and sodium bicarbonate(5mg) were heated at 150°C. for 0.5 hour in DMF(2ml). The reaction mixture was cooled to room temperature, diluted with an equal volume of ammonium hydroxide and allowed to stand overnight at room temperature. The solution was applied to Whatman 3MM paper, developed in Solvent C and the product(R_f 0.27) eluted with water. On concentration XII (71.5mg, 75%) crystallized out and was identical in all respects to the 8,2'-thioanhydroadenosine synthesized earlier.

8,2'-Thioanhydroinosine (XXXII)

Compound XXXII was prepared from 8-thioinosine(XL) in the same manner as described for the above preparation of XII except Solvent A was used for the paper chromatography and the band at R_f 0.43 eluted with water. An 85% yield of XXXII was obtained and was identical in all respects to the 8,2'-thioanhydroinosine synthesized earlier.

8,2'-Thioanhydroguanosine (XXI)

Compound XXI was prepared from 8-thioguanosine(XXXIX) in the same manner as described for the above preparation of XXXII except 2 equivalents of diphenyl carbonate were used as well as a mixed (9:1) DMF-pyridine solvent. The band at R_f 0.18 (Solvent A) was eluted with water and a 70% yield of XXI was obtained upon crystallization. The product was identical in all respects to the 8,2'-thioanhydroguanosine synthesized earlier

8,2'-Aminoanhydroadenosine (XLIV)

8-Aminoadenosine(XLI, 25mg, 0.09mmole), diphenyl carbonate(25mg, 0.12mmole) and sodium bicarbonate(2mg) were heated at 150°C. for 0.5 hour in DMF(1ml). The reaction mixture was cooled to room temperature, diluted with an equal volume of ammonium hydroxide and allowed to stand overnight at room temperature. The solution was applied to Whatman 3mm paper, developed in Solvent B and the product(R_f 0.55) eluted with water and lyophilized to yield 20mg(85%, m.p. 260°C. dec.) of XLIV. The product was identical in all respects to an authentic sample[77]. Chromatographic data is listed in Table X while spectral properties (u.v. and i.r.) are recorded in Table XI. The mass spectrum of the tetrasilyl derivative had a parent peak at m/e 552.

8,2'-Aminoanhydroinosine(XLVI)

Method A Compound XLVI was prepared from 8-aminoinosine(XLIII) in the same manner as described for the above preparation of XLIV in a yield of 31%(m.p. 210°C. dec.). Chromatographic data is listed in Table X while spectral properties(u.v. and i.r.) are recorded in Table XI. The mass spectrum of the tetrasilyl derivative had a parent peak at m/e 553.

Mol. wt. Calcd. for $C_{22}H_{43}O_4N_5Si_4$: 553.2392
Found: 553.2406

TABLE X Paper* and Thin-layer+ Chromatographic Properties of Purine Nucleosides

	Paper SOLVENT°			Thin-layer SOLVENT	
COMPOUND	A	B	C	EtOH	$CHCl_3$:EtOH(7:3)
8-SHA(XXXVIII)	0.30	0.55	0.41	0.45	0.62
8-SHG(XXXIX)	0.15	0.51	0.29	0.40	0.29
8-SHI(XL)	0.41	0.43	0.26	0.17	----
8-NH_2A(XLI)	0.40	0.47	0.23	0.56	0.36
8,2'-NanhA(XLIV)	0.44	0.55	0.30	0.48	0.36
8-NH_2G(XLII)	0.23	0.49	0.14	0.28	0.05
8,2'-NanhG(XLV)	0.16	0.37	0.11	0.27	0.05
8-NH_2I(XLIII)	0.56	0.59	0.25	0.39	0.06
8,2'-NanhI(XLVI)	0.25	0.52	0.13	0.49	0.05

* Whatman 3MM - Descending technique

+ Eastman Chromagram Sheets 6060, silica gel with fluorescent indicator, strips 10cm. x 2cm.

o Described in General Methods

TABLE XI I.R. and U.V. Spectral Properties of Purine Nucleosides

	U.V. SPECTRA		I.R. SPECTRA*
COMPOUND	SOLVENT	max,nm(ε)	μ
8-SHA(XXXVIII)	water	298(25300),229(19800),304(24800)sh	6.05,6.90,11.50,12.85
8-SHG(XXXIX)	water	303(19000),285(19300),228(12100)	5.80,6.05,6.75,9.40
8-SHI(XL)	water	293(19630),235(6800)sh	5.90,7.20,8.90,9.30
8-NH_2A(XLI)	water	272(15130)	6.10,6.75,9.30,12.85
8,2'-NanhA(XLIV)	water	273(15100)	6.10,7.00,8.05,12.75
8-NH_2G(XLII)	water	293(9300),257(16100)	5.80,6.00,9.35,13.10
8,2'-NanhG(XLV)	water	258(15050),290(10100)sh	5.95,6.85,9.35
8-NH_2I(XLIII)	water	260(13700)	5.95,6.20,8.90,12.45
8,2'-NanhI(XLVI)	water	263(12800)	5.90,6.85,11.40,12.05

* Principal bands

Method B Compound XLVI was also prepared by treatment of 8,2'-aminoanhydroadenosine(XLIV, 10mg, 0.02mmole) in acetic acid-water(4:15, 1ml) with added sodium nitrite(2.5mg). The reaction was stirred at room temperature overnight and applied to paper chromatograms in Solvent A. The band at R_f 0.25 was eluted with water and lyophilized giving 8.4mg XLVI(84%). The product was identical with that prepared in Method A.

8,2'-Aminoanhydroguanosine (XLV)

8-Aminoguanosine(XLII, 50mg, 0.17mmole), diphenyl carbonate(0.36g, 1.68mmole) and sodium bicarbonate(10mg) were heated at 150°C. for 0.5 hour in DMF-pyridine(4:1, 3ml). The reaction mixture was applied to paper chromatograms, developed in Solvent C, the band at R_f 0.15 eluted with water and reapplied to papers in Solvent G. The band at R_f 0.35 was eluted with water and concentrated to dryness. The salt was extracted with hot DMF(5ml) and this solvent applied to papers in Solvent C. The band at R_f 0.15 was eluted with water and lyophilized to give 8.3mg XLV(17.6%, m.p. 203°C. dec.). Chromatographic data is listed in Table X and spectral properties(u.v. and i.r.) in Table XI. The mass spectrum of the pentasilyl derivative had a parent peak at m/e 640.

Mol. wt. Calcd. for $C_{25}H_{52}N_6O_4Si_5$: 640.2896
Found: 640.2879

8,2'-Thioanhydropurine nucleoside 5'-monophosphates General procedure(Scheme XII)

In a typical experiment 200mg of thioanhydro nucleoside was added to a cold (0°C.) solution of phosphoryl chloride (10 equivalents) and water(0.4 equivalents) in triethyl phosphate (5ml). Complete dissolution occurred after 0.5 hour and the reaction was continued at 0°C. for an additional 5.5 hours. Excess ether was added, the mixture centrifuged, decanted and the precipitate washed with ether. The resulting solid was dissolved in cold

water(2ml) and stirred at 0°C. for two hours. Both 8,2'-thioanhydroadenosine 5'-monophosphate(XLVII) and 8,2'-thioanhydroinosine 5'-monophosphate(XLIX) precipitated upon addition of excess ethanol. The yield of XLVII was 236mg(92%) and the product was identical to that reported in the literature[66]. The yield of XLIX was 188mg(74%). For the guanosine derivative, the aqueous solution was applied to paper chromatograms and developed in Solvent F. The band at R_f 0.19 was eluted with water and lyophilized to give 106mg of 8,2'-thioanhydroguanosine 5'-monophosphate(XLVIII, 43%). General properties of the monophosphates are listed in Table XII.

Structural identification of the 8,2'-thioanhydropurine nucleoside 5'-monophosphates

The monophosphates were dissolved in water and refluxed with Raney nickel for 3 hours. Following filtration through Celite powder the products were isolated on paper chromatography in Solvent F. Compounds XLVII, XLVIII and XLIX led respectively to 2'-deoxyadenosine 5'-monophosphate, 2'-deoxyguanosine 5'-monophosphate and 2'-deoxyinosine 5'-monophosphate. The properties of these are listed in Table XII. Each of the deoxynucleotides was then dissolved in a borate buffer(pH 9) and incubated at 37°C. with 5'-nucleotidase[58]. Analysis of the reaction mixtures on paper chromatography showed complete conversion to the corresponding 2'-deoxynucleosides which were identical with authentic samples[58].

8,2'-Thioanhydroadenosine 5'-phosphoromorpholidate (L)

8,2'-Thioanhydroadenosine 5'-monophosphate(XLVII, 50mg, 0.14mmole) was refluxed in a t-butanol-water mixture(1:1, 2ml) to which 0.05ml morpholine had been added. To this refluxing solution, a solution of DCC(115mg, 0.56mmole) in t-butanol was added dropwise over a period of 3 hours. After this time an electrophoresis showed 70% conversion to L(Rm(Tp)= 0.36). Further addition of 4 equivalents of morpholine and DCC did not increase the yield after an additional 3 hours. The reaction was then filtered and applied directly to paper chromatograms which were developed in Solvent F. The band at

TABLE XII Properties of 8,2'-Thioanhydro and 2'-deoxypurine nucleoside 5'-monophosphates

	Paper* SOLVENT^{o}	Electrophoresis	U.V. Spectra	
COMPOUND	F	Rm^{+}	SOLVENT	max,nm
8,2'-SanhA 5'-p(XLVII)	0.27	0.92	water	276, 219
8,2'-SanhG 5'-p(XLVIII)	0.19	0.79	water	263, 280sh
8,2'-SanhI 5'-p(XLIX)	0.33	0.83	water	265
2'-dA 5'-p	0.45	0.99	water	260
2'-dG 5'-p	0.26	0.88	water	255, 270sh
2'-dI 5'-p	0.41	0.92	water	249

* Whatman 3MM - Descending technique

o Described in General Methods

\+ Values relative to thymidine 3'-phosphate; triethylammonium bicarbonate buffer pH 7.5

R_f 0.62 was eluted with water and lyophilized to yield 47mg L (78.7%). Properties are listed in Table XIII. The product was not further purified but used directly for the synthesis of 8,2'-thioanhydroadenosine 5'-diphosphate(LIII).

8,2'-Thioanhydroadenosine 5'-diphosphate (LIII)

Compound L(28mg, 0.065mmole) was dried by repeated evaporation of pyridine(5 x 0.5ml). To the residue was added tri-n-butylamine(0.095ml) as well as inorganic phosphoric acid(0.014ml) which had been previously dried by repeated evaporation of pyridine(5 x 0.5ml). The mixture was further dried by pyridine evaporation, pyridine added(1.5ml) and the reaction stirred at room temperature for 48 hours. The reaction mixture was then applied to paper chromatograms which were then developed in Solvent F. The band at R_f 0.35 was eluted with water and lyophilized giving LIII(12.9mg) in a yield of 46%. The band at R_f 0.62 was eluted with water and lyophilized yielding 10.2mg of unreacted L. Properties are listed in Table XIII. Phosphate analysis of LIII relative to an equal concentration of adenosine 5'-monophosphate was 2.11.

8,2'-Thioanhydroguanosine 5'-phosphoromorpholidate(LI) and 5'-diphosphate (LIV)

8,2'-Thioanhydroguanosine 5'-monophosphate(XLVIII, 50mg, 0.13mmole) was dissolved in t-butanol-water(1:1, 1ml) to which was added 0.13ml morpholine. The solution was refluxed for two hours during which time 90mg DCC in t-butanol(1ml) was added dropwise. Electrophoretic analysis revealed complete conversion to LI(Rm(Tp)=0.46). The solution was filtered and concentrated to dryness in vacuo. The residue was extracted twice with ether and then dried by repeated evaporation of pyridine(5 x 0.5ml). To this was added tri-n-butylamine(0.095ml) as well as inorganic phosphoric acid(0.014ml) which had been

TABLE XIII Properties of 8,2'-Thioanhydropurine Nucleoside 5'-Phosphoromorpholidates and 5'-Diphosphates

	Paper* SOLVENT°		Electrophoresis	U.V. Spectra	
COMPOUND	C	F	Rm+	SOLVENT	max,nm
8,2'-SanhA 5'-pm (L)	0.16	0.62	0.36	water	275
8,2'-SanhA 5'-dip (LIII)	0.00	0.35	1.13	water	275
8,2'-SanhG 5'-pm (LI)	0.12	0.54	0.46	water	265, 280sh
8,2'-SanhG 5'-dip (LIV)	0.00	0.23	0.97	water	267, 280sh, 263sh
8,2'-SanhI 5'-pm (LII)	0.12	0.62	0.45	water	265
8,2'-SanhI 5'-dip (LV)	0.00	0.15	1.01	water	265

* Whatman 3MM - Descending technique

o Described in General Methods

\+ Values relative to thymidine 3'-phosphate; triethylammonium bicarbonate buffer pH 7.5

previously dried by repeated evaporation of pyridine. The reaction was further dried by pyridine evaporation then dissolve in 1.0ml pyridine and stirred at room temperature for 2 days. Applying the reaction mixture to paper chromatograms and development in Solvent F gave two nucleotide bands R_f 0.54 and 0.23. The band at R_f 0.54 was eluted with water and lyophilized giving 17.3mg unreacted LI. The band at R_f 0.23 was eluted with water and and yielded 22.8mg LIV(37.9%) upon lyophilization. Properties of LI and LIV are recorded in Table XIII. Phosphate analysis of LIV relative to an equal concentration of adenosine 5'-monophosphate was 1.93.

8,2'-Thioanhydroinosine 5'-phosphoromorpholidate (LII) and 5'-diphosphate(LV)

8,2'-Thioanhydroinosine 5'-monophosphate(XLIX) was quantitatively converted to LII by the same method used above for the synthesis of LI from XLVIII. Electrophoresis showed Rm(Tp)=0.45. From LII the 5'-diphosphate derivative LV was synthesized using the same procedure outlined above. The paper chromatograms in Solvent F gave two bands R_f 0.15 and 0.62. The band at R_f 0.62 was eluted with water and lyophilized to recover 12.8mg of unreacted LII. The band at R_f 0.15 was also eluted with water and lyophilized to yield LV(26mg, 43%). Properties of LII and LV are listed in Table XIII. Phosphate analysis of LV relative to an equal concentration of adenosine 5'-monophosphate was 2.19.

Attempted syntheses of 8,2'-thioanhydropurine nucleoside 5'-triphosphates

Method A The 5'-phosphoromorpholidates L, LI, and LII(0.05mmole) were separately dried by repeated evaporation of pyridine. Aside from this three solutions of bis-(tri-*n*-butylammonium)pyrophosphate[72](0.5mmole) were dried by repeated pyridine evaporation. The solutions were mixed and evaporated once more. The residue was dissolved in 0.5ml pyridine and the reaction stirred at room temperature for 48hours. After this time there was no detectable change in the starting material either on paper chromatography or electrophoresis. Further

addition of pyrophosphate(0.5mmole) and an additional 48 hours of stirring at room temperature still gave no conversion.

Method B The 5'-phosphates XLVII, XLVIII and XLIX were separately treated with tri-n-butylamine(2 equivalents), 80% orthophosphoric acid(1 equivalent), DCC(433 equivalents and 3ml pyridine and the reaction stirred at room temperature for 48 hours. The mixture was then filtered and concentrated to a small volume in vacuo. Examination of these by paper chromatography and electrophoresis showed the only detectable change to be the appearance of a small amount of dinucleoside 5'-5' pyrophosphate.

Enzymatic synthesis of 8,2'-thio and aminoanhydroinosine(XXXII and XLVI) using adenosine deaminase

8,2'-Thioanhydroinosine (XXXII)

8,2'-Thioanhydroadenosine(XII, 250mg, 0.89mmole) was treated with adenosine deaminase(10mg) in 25ml of buffer solution(pH 7.5, 0.05M phosphate) at 25° for 1.25 hours. The solution was applied directly to Whatman 3MM paper and chromatographed in Solvent A. The product(R_f 0.34) was eluted with water and lyophilized giving a quantitative yield of XXXII which was identical to the 8,2'-thioanhydroinosine prepared earlier.

8,2'-Aminoanhydroinosine (XLVI)

8,2'-Aminoanhydroadenosine(XLIV, 5mg, 0.02mmole) was treated with adenosine deaminase(2mg) in 1ml buffer solution (pH 7.5, 0.05M phosphate) at 25°C. for 18 hours. The reaction solution was applied directly to paper chromatograms which were developed in Solvent A. The band at R_f 0.44 was eluted with water and lyophilized giving a quantitative yield of XLVI. This product was identical with the 8,2'-aminoanhydroinosine prepared earlier.

Enzymatic studies on 8,2'-thioanhydropurine nucleoside 5'-mono and diphosphates

5'-Nucleotidase

Separately 1mg of 8,2'-thioanhydroadenosine 5'-monophosphate(XLVII), 8,2'-thioanhydroguanosine 5'-monophosphate(XLVIII) and 8,2'-thioanhydroinosine 5'-monophosphate(XLIX) were dissolved in 1ml of buffer solution(pH 9.0, 0.025M borax), 0.5mg of 5'-nucleotidase added and the solution incubated at 37°C. for one hour. Examination of the reaction mixtures by paper chromatography and electrophoresis showed no dephosphorylation. However using the same concentration adenosine 5'-monophosphate was completely converted to adenosine in 2 minutes.

Treatment of the thioanhydro nucleotides with an alkaline phosphatase suspension in ammonium sulfate in the presence of 1ml of buffer solution(pH 10.5, 0.05M ammonium bicarbonate) and incubation at 37°C. gave rapid quantitative hydrolysis to the corresponding thioanhydro nucleosides.

Adenylate kinase (Myokinase)

8,2'-Thioanhydroadenosine 5'-diphosphate(LIII, 1mg,0.002mmole), adenylate kinase(1 µl, 2 units) and 0.1ml tris-buffer(pH 7.6) were incubated at 37°C. Continuous monitoring of the reaction mixture by paper chromatography and electrophoresis for 4 hours showed no detectable change in the starting material.

8,2'-Thioanhydroadenosine 5'-monophosphate(XLVII, 1mg, 0.003mmole), adenylate kinase(1 µl, 2 units), ATP(2mg, 0.003mmole), magnesium sulfate(3.4mg, 0.03mmole) and 0.1ml tris-buffer(pH 7.6) were incubated at 37°C. Continuous monitoring of the reaction mixture by paper chromatography and electrophoresis showed no detectable change in the starting material.

N,N,$O^{3'}$,$O^{5'}$-Tetrabenzoyl-8,2'-thioanhydroadenosine (LXVI) and N^6-Benzoyl-8,2'-thioanhydroadenosine (LXVII)

8,2'-Thioanhydroadenosine(XII, 0.5g, 1.78mmole) was dissolved in pyridine and benzoyl chloride(0.9ml, 7.5mmole) added. The reaction was stirred at room temperature for two hours, poured into ice water and the aqueous solution extracted with chloroform. After drying over sodium sulfate the chloroform solution was concentrated to a small volume and 1.18g LXVI(95%, m.p. 118°-119°C.) precipitated upon addition of excess hexane. Chromatographic data is shown in Table XIV and spectral properties(u.v. and i.r.) in Table XV.

Without further purification LXVI(515mg. 0.75mmole) was dissolved in ethanol-pyridine(3:2, 10ml), 6.0ml 2N sodium hydroxide-ethanol(1:1) added and the solution allowed to stand at room temperature for 5 minutes. An excess of Dowex 50W-X8 resin(H^+) was added, the solution filtered and the resin washed with warm DMF. The filtrate and washings were combined, concentrated to dryness and the residue washed with ether. Dissolution of the residue in DMF and addition of excess ether gave LXVII (187mg, 65%, m.p. 231°C. dec.) as a white powder. Chromatographic data is shown in Table XIV and spectral properties(u.v. and i.r.) in Table XV.

5'-O-Monomethoxytrityl-N^6-benzoyl-8,2'-thioanhydroadenosine (LXVIII)

Compound LXVII(1.03g, 2.67mmole), monomethoxytrityl chloride(0.99g, 3.2 mmole) and pyridine(40ml) were stirred at room temperature overnight. The solution was poured into ice water, extracted with chloroform and the chloroform solution dried over sodium sulfate. Following filtration of the chloroform and concentration to a small volume, LXVIII precipitated (1.55g, 87%, m.p. 134°-136°C.) upon addition of hexane. Chromatographic data is listed in Table XIV while spectral properties (u.v. and i.r.) are recorded in Table XV.

TABLE XIV Paper* and Thin-layer+ Chromatographic Properties of 8,2'-Thioanhydropurine Nucleoside Derivatives

	Paper SOLVENT°			Thin-layer SOLVENT	
COMPOUND	A	B	C	EtOAc	THF
N,N,$O^{3'}$,$O^{5'}$-tetraBz-8,2'-SanhA(LXVI)	0.89	0.90	0.93	0.69	0.73
N^6-Bz-8,2'-SanhA (LXVII)	0.77	0.80	0.62	0.04	0.47
5'-O-MMTr-N^6-Bz-8,2'-SanhA(LXVIII)	0.95	0.95	0.94	0.23	0.63
5'-O-MMTr-N^6,$O^{3'}$-di-Bz-8,2'-SanhA(LXIX)	0.95	0.98	0.97	0.58	0.88
N^6,O^3-diBz-8,2'-SanhA(LXX)	0.88	0.82	0.84	0.25	0.74
N^2-Ac-8,2'-SanhG (LXXI)	0.49	0.79	0.55	0.00	0.08
5'-O-MMTr-N^2-Ac-8,2'-SanhG(LXXII)	0.84	----	0.90	0.06	0.28
5'-O-MMtr-8,2'-SanhI (LXIII)	0.78	0.86	0.77	0.06	0.22

* Whatman 3MM- Descending technique

\+ Eastman Chromagram Sheets 6060, silica gel with fluorescent indicator, strips 10cm. x 2cm.

o Described in General Methods

TABLE XV I.R. AND U.V. Spectral Properties of 8,2'-Thioanhydropurine Nucleoside Derivatives

	U.V. Spectra		I.R. Spectra*
COMPOUND	SOLVENT	max,nm(ε)	μ
N,N,$O^{3'}$,$O^{5'}$-tetraBz-8,2'-SanhA(LXVI)	95% EtOH	298(18500),234(51200) 285(16800)sh	5.85,6.25,14.15
N^6-Bz-8,2'-SanhA (LXVII)	95% EtOH	300.5(20500),238(19150)	5.85,6.15,8.75,14.20
5'-O-MMTr-N^6-Bz-8,2'-SanhA(LXVIII)	95% EtOH	298(18500),235(29300) 285(14200)sh	5.85,6.20,8.05,14.20
5'-O-MMTr-N^6,$O^{3'}$-di-Bz-8,2'-SanhA(LXIX)	95% EtOH	297.5(19900),233(46000) 285(17000)sh	5.85,6.20,8.05,14.20
N^6,$O^{3'}$-diBz-8,2'-SanhA(LXX)	95% EtOH	297.5(12300),232(27800) 285.5(10600)sh	5.85,6.80,12.75,14.20
N^2-Ac-8,2'-SanhG (LXXI)	95% EtOH	295(14500),269(23600)	5.75,5.95,9.60,12.85
5'-O-MMTr-N^2-Ac-8,2'-SanhG(LXXII)	95% EtOH	270(21200),292.5(12800)sh 229(19500)sh	5.75,5.95,9.60,14.15
5'-O-MMTr-8,2'-SanhI (LXIII)	95% EtOH	265(13100),230(17500)	5.90,7.15,14.20

* Principal bands

5'-O-Monomethoxytrityl-N^6,$O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine (LXIX) and N^6,$O^{3'}$-Dibenzoyl-8,2'-thioanhydroadenosine(LXX)

Compound LXVII(0.9g, 1.35mmole) and benzoic anhydride (3.66g, 16.2mmole) were added to pyridine(8ml) and the resulting solution stirred in the dark at room temperature for 24 hours. The reaction was washed with hexane and the resulting oil was concentrated to a gum using ethanol to aid in the removal of the solvent. Thi residue was dissolved in tetrahydrofuran and LXIX(931mg, 89.4%, m.p. 128°-130°C.) precipitated as a white solid upon addition of hexane.

Without further purification LXIX(0.8g, 1.04mmole) was dissolved in 80% acetic acid and heated on a steam bath for 15 minutes. The solution was poured into ice water with vigorous stirring and the precipitate removed by filtration. The solid was washed with ether and dried to yield LXX as a white powder (0.5g, 96.5%, m.p. 173°-174°C.). Chromatographic data of LXIX and LXX are listed in Table XIV and spectral properties(u.v. and i.r.) in Table XV. LXX was identical in all respects to an authentic sample[101].

N^2-Acetyl-8,2'-thioanhydroguanosine (LXXI)

8,2'-Thioanhydroguanosine(XXI, 1.0g, 3.37mmole) and tetraethylammonium hydroxide(3.5ml) were rendered anhydrous by repeated evaporation of pyridine(5 x 20ml). Pyridine(20ml) and acetic anhydride(10ml) were added and the reaction was stirred at room temperature for 24 hours after which time a white precipitate formed. The mixture was heated on a steam bath for 3 minutes to dissolve the precipitate, cooled to room temperature, acetic anhydride added(5ml) and the solution stirred overnight at room temperature. The reaction was then concentrated to dryness using ethanol to aid in the removal of the solvents and the residue treated with ethanol-pyridine (3:2, 25ml) and 2N sodium hydroxide-ethanol(1:1, 15ml). Following

stirring of this solution for 10 minutes at room temperature an excess of Dowex 50W-X8 resin(H^+) was added. The solution was filtered and the resin washed with hot DMF. The filtrate and washings were combined, concentrated to a small volume and applied to thick-layer plates. One development in chloroform-ethanol(7:3) gave a band at R_f 0.25. This band was eluted with DMF, concentrated to a small volume and LXXI precipitated (700mg, 61%) as a white powder(m.p. 237°C. dec.) upon addition of ethanol. Chromatographic data is listed in Table XIV and spectral properties(u.v. and i.r.) in Table XV.

5'-O-Monomethoxytrityl-N^2-acetyl-8,2'-thioanhydroguanosine (LXXII)

Compound LXXI(680mg, 2.0mmole) and monomethoxytrityl chloride(1.92g, 6.21mmole) were dissolved in pyridine(10ml) and stirred at room temperature for 48 hours. The reaction was then added slowly to ice water with vigorous stirring. The resulting precipitate was removed by filtration, dissolved in chloroform, and dried over sodium sulfate. The chloroform solution was filtered, concentrated to a small volume and LXXII precipitated (598mg, 50%, m.p. 188°C. dec.) upon addition of excess ether. Chromatographic properties are listed in Table XIV while spectral properties (u.v. and i.r.) are recorded in Table XV.

5'-O-Monomethoxytrityl-8,2'-thioanhydroinosine(LXXIII)

8,2'-Thioanhydroinosine(XXXII, 0.5g, 1.78mmole) was dried by repeated evaporation of pyridine. DMF(2ml) and pyridine (25ml) were added along with monomethoxytrityl chloride(1.64g, 5.33mmole) and the solution stirred at room temperature for 4 days. The solution was then poured into ice water. The aqueous mixture was extracted with chloroform(3 x 100ml) and the chloroform dried over sodium sulfate. Following filtration the chloroform was concentrated to a small volume and LXXIII(330mg, 33.6%) precipitated as a white powder(m.p. 164°-166°C.) upon addition

of hexane. Chromatographic properties are listed in Table XIV while spectral properties are recorded in Table XV.

5'-O-Monomethoxytrityl-8,2'-thioanhydroinosine 3'-phosphate (LXXIV)

Compound LXXIII(50mg, 0.09mmole) and β-cyanoethyl phosphate(pyridinium form, 0.36ml, 0.18mmole) were dried by repeated pyridine evaporation(5 x 1ml). Mesitylenesulfonyl chloride (59mg, 0.27mmole) and pyridine(1ml) were added and the reaction stirred at room temperature for 7 hours. Cold distilled water (1ml) was added and the reaction stirred at room temperature for 16 hours. An additional 1ml of water was added followed by a saturated salt solution(5ml). Extraction of the aqueous solution with chloroform resulted in all the phosphorylated material being dissolved in the chloroform. The chloroform solution was concentrated to dryness, 9M ammonium hydroxide added(3ml) and the solution heated at 60°C. for one hour. The reaction mixture was applied to paper chromatograms which were developed in Solvent A. The band at R_f 0.23 was eluted with water and lyophilized yielding LXXIV(49mg, 87.6%). Properties are listed in Table XVI.

2',3'-O-Isopropylideneuridine 5'-phosphate (LXXVII)

2',3'-O-Isopropylideneuridine(LXXV, 100mg, 0.35mmole) and β-cyanoethyl phosphate(pyridinium form, 1.4ml, 0.70mmole) were dried by repeated evaporation of pyridine(5 x 2ml). Mesitylenesulfonyl chloride(229mg, 1.05mmole) and pyridine(2ml) were added and the reaction stirred at room temperature for 7 hours. Cold distilled water(2ml) was added and the reaction stirred at room temperature for 16 hours. The mixture was then concentrated to a syrup at reduced pressure, dissolved in 9M ammonium hydroxide(3ml) and heated at 60°C. for one hour. Upon cooling the solution was applied to paper chromatograms which were developed in Solvent A. The band at R_f 0.11 was eluted with

water and lyophilized yielding LXXVII(86mg, 67%). Properties are listed in Table XVI.

$N^6,O^{3'}$-Diacetyl-8,2'-thioanhydroadenosine 5'-phosphate (LXXIX)

Compound XLVII(14mg, 0.04mmole), Dowex 50W-X8(pyridinium form) and ammonium acetate(29.4mg, 0.38mmole) were dried by pyridine evaporation. Acetic anhydride(2ml) and pyridine(0.5ml) were added and the reaction stirred at room temperature for 14 hours. Complete dissolution occurred after 0.75 hour. Methanol (0.5ml) was added and the solution stirred for 0.5 hour at room temperature. The mixture was concentrated to dryness, the residue dissolved in 10% aqueous pyridine(2ml), and the solution stirred at room temperature for 3 hours. Application of the solution to paper chromatograms and subsequent development in Solvent B led to a nucleotide band at R_f 0.47. This band was eluted with water and lyophilized to give 17.3mg LXXIX(79%). Properties are listed in Table XVI.

Attempted synthesis of 8,2'-thioanhydroinosinyl(3'-5') 2',3'-O-isopropylideneuridine (Scheme XVII)

Method A Compound LXXIV(22.8mg, 0.036mmole) was dried by repeated evaporation of pyridine(5 x 0.5ml). Compound LXX (36mg, 0.072mmole), DCC(29.7mg, 0.14mmole) and pyridine(1ml) were added and the solution stirred at room temperature for 4 days. Water(2ml) was added and the mixture stirred at room temperature for 3 hours. The mixture was filtered, concentrated to dryness and heated on a steam bath for 15 minutes with added 80% acetic acid(1ml). The solution was cooled and applied to paper chromatograms which were then developed in Solvent A. The band at R_f 0.01 was eluted with water and lyophilized giving 27mg of LXXVI. The u.v. spectrum in water had a maximum at 265 and the compound showed electrophoretic mobility of Rm(Tp)=0.75.

The product was identified as the P^1,P^2-di-8,2'-thioanhydroinosine-3'-pyrophosphate by stirring with acetic

anhydride and pyridine in the dark for 3 days. Methanol(1ml) was added and stirring continued for 15 minutes. The solution was concentrated to dryness, the residue dissolved in concentrated ammonium hydroxide and the mixture stirred at room temperature for 10 hours. Examination of the reaction mixture by paper chromatography and electrophoresis showed the sole product to be identical to an authentic sample of 8,2'-thioanhydroinosine 3'-phosphate prepared by acid treatment of LXXIV.

Method B Compounds LXXIV(28mg, 0.044mmole) and LXX (14.5mg, 0.029mmole) were dried by repeated pyridine evaporation (5 x 0.5ml). Mesitylenesulfonyl chloride(12mg, 0.058mmole) and pyridine(0.5ml) were added and the reaction stirred at room temperature for 18 hours. Cold water(2ml) was added and the reaction stirred at $0^{\circ}C$. for 1 hour. The reaction was then worked up in the same manner as in Method A and the results were identical.

Method C Compound LXXVII(50mg, 0.14mmole), Dowex 50W-X8 (pyridinium form, 2ml) and compound LXXIII(150mg, 0.28mmole) were dried by repeated pyridine evaporation(7 x 1ml). Pyridine(3ml) and DCC(115mg, 0.56mmole) were added and the reaction stirred at room temperature for 2 days. Water(3ml) was added and the mixture stirred at room temperature overnight. Following filtration to remove the dicyclohexylurea the solution was concentrated to dryness, treated with 80% acetic acid and heated on a steam bath for 15 minutes. The solution was cooled and applied to paper chromatograms which were developed in Solvent C. Two nucleotide bands were obtained(R_f 0.17 and 0.02). The band at R_f 0.02 was eluted with water and lyophilized giving to yield 14mgLXXVII. The band at R_f 0.17 was eluted with water and lyophilized giving 64mg LXXVIII. The u.v. spectrum in water of LXXVIII showed a maximum at 261nm and the compound showed electrophoretic mobility of Rm(Tp)=0.75.

The product LXXVIII was identified as the P^1,P^2-di-2',3'-O-isopropylideneuridine 5'-pyrophosphate by acetic anhydride

treatment as described in Method A. Examination of the reaction mixture by paper chromatography and electrophoresis showed the sole product to be identical to 2',3'-O-isopropylideneuridine 5'-phosphate LXXVII.

Method D Compound LXXVII(25mg, 0.07mmole), Dowex 50W-X8 (pyridinium form, 1ml) and compound LXXIII(25mg, 0.047mmole) were dried by repeated evaporation of pyridine(5 x 0.5ml). Pyridine (1.5ml) and mesitylenesulfonyl chloride(20.8mg, 0.094mmole) were added and the solution stirred at room temperature for 24 hours. The reaction was then worked up in the same manner as Method B. The only nucleotide material isolated was LXXVIII(37mg). This 5'-5' pyrophosphate was identical in all respects to LXXVIII obtained by Method C.

Attempted synthesis of thymidylyl(3'-5')8,2'-thioanhydroadenosine (Scheme XVIII)

Method A 5'-O-Monomethoxytritylthymidine(20mg, 0.038mmole) and N^6,$O^{3'}$-diacetyl-8,2'-thioanhydroadenosine 5'-monophosphate (LXXIX, 31mg, 0.058mmole) were dried by repeated pyridine evaporation(5 x 1ml). Mesitylenesulfonyl chloride(11.8mg, 0.076mmole) and pyridine(1ml) were added and the reaction stirred at room temperature for 24 hours. Cold water(2ml) was added and the solution stirred at room temperature for an additional 24 hours. The solution was then concentrated to dryness, the residue dissolved in 80% acetic acid(2ml) and heated on a steam bath for 15 minutes. After cooling the solution was applied to paper chromatograms which were then developed in Solvent A. The band at R_f 0.01 was eluted with water and lyophilized yielding 51mg LXXX. This product had Rm(Tp)=0.77 and the u.v. spectrum in water showed a maximum at 275.5nm.

The structure of LXXX was confirmed as P^1,P^2-di-8,2'-thioanhydroadenosine 5'-pyrophosphate by the acetic anhydride treatment outlined earlier. Examination of this hydrolysis reaction by paper chromatography and electrophoresis showed the product to be 8,2'-thioanhydroadenosine 5'-phosphate(XLVII) identical to the material synthesized earlier.

Method B 5'-O-Monomethoxytritylthymidine(20mg, 0.038 mmole) and compound LXXIX(10mg, 0.019mmole) were rendered anhydrous in the usual manner. DCC(15.7mg, 0.076mmole) and pyridine (1ml) were added and the reaction stirred at room temperature for six days during which time an additional 3 equivalents of DCC were added. The reaction was then worked up in a similar manner to that outlined in Method A resulting in 14.8mg of LXXX being isolated.

Thymidylyl(3'-5')N^6-benzoyl-8,2'-thioanhydroadenosine (LXXXI) (Scheme XIX)

Compound LXX(17mg, 0.034mmole) and 5'-O-monomethoxytritylthymidine 3'-phosphate(30mg, 0.05mmole) were dried by repeated evaporation of pyridine(5 x 1ml). Mesitylenesulfonyl chloride(10.6mg, 0.068mmole) and pyridine(1ml) were added and the reaction stirred at room temperature for 24 hours. Cold water(2ml) was added and the solution continued stirring for an additional 24 hours. Following concentration to dryness, the residue was dissolved in 80% acetic acid(1ml) and heated on a steam bath for 15 minutes. After cooling the solution was applied to paper chromatograms and developed in Solvent A. The band at R_f 0.42 was eluted with water and lyophilized to give LXXXI(4.8mg, 19%). The product had Rm(Tp)=0.25 and the u.v. spectrum in water showed a maximum at 267.5nm and a shoulder at 300nm. Compound LXXXI could also be obtained using DCC as condensing agent(nucleotide:nucleoside;DCC 1:2:4, 96 hours) in 14.6% yield.

Approximatly 8.5 O.D. units LXXXI was treated with spleen phosphodiesterase for 24hours at 37°C. using standard procedures. The work-up yielded 4.7 O.D. units of Tp(R_f(A) 0.10) and 4.2 O.D. units of N^6-benzoyl-8,2'-thioanhydroadenosine (R_f(A) 0.88). The ratio nucleotide/nucleoside was 1.12. Treatment of 8.5 O.D. units of LXXXI with snake venom phosphodiesterase under standard conditions led to no detectable degradation.

8,2'-Thioanhydroadenylyl(3'-5') 8,2'-thioanhydroadenosine (LXXXV) (Scheme XX)

Compound LXVIII(167mg, 0.25mmole) and β-cyanoethyl phosphate(pyridinium form, 1.5ml, 0.75mmole) were dried by repeated evaporation of pyridine(5 x 2ml). This anhydrous mixture was then dissolved in pyridine(10ml) and DCC(1.2g, 5.9mmole) was added. After standing at 45°C. for 18 hours, pyridine-water (3:1, 30ml) was added and the solution filtered to remove dicyclohexylurea. The filtrate was extracted with three 40ml portions of hexane. The aqueous pyridine solution was kept at room temperature for 24 hours and then evaporated to dryness *in vacuo*. Methanol-concentrated ammonia(1:1, 25ml) was added to the residue and the resulting solution heated for 10 hours at 45° and then concentrated to dryness. DMF(2ml) and dimethylformamide dimethylacetal(0.2ml) were added, the solution stirred at room temperature overnight, and then evaporated to dryness. The residue was dissolved in 50% aqueous pyridine and the solution passed through a column of Dowex 50W-X8(pyridinium form) resin. The column was washed with 50% aqueous pyridine and the eluate concentrated and dried by repeated evaporation of pyridine. The resulting 5'-O-monomethoxytrityl-N^6-dimethylaminomethylene-8,2'-thioanhydroadenosine 3'-phosphate(LXXXIII) was not purified further but used directly in the condensation reaction.

Compound LXXXIII(∿ 0.09mmole), N^6,$O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine(LXX, 60mg, 0.1mmole) and Dowex 50W-X8 (pyridinium form, 0.5ml) resin were rendered anhydrous by pyridine evaporation. To the anhydrous residue was added pyridine(2ml) and DCC(246mg, 1.2mmole) and the mixture was stirred at room temperature for eleven days. 50% Aqueous pyridine(2ml) was added, the solution filtered and the filtrate extracted with three 20ml portions of hexane. The aqueous pyridine layer was kept at room temperature for 24 hours and then evaporated. The residue was dissolved in methanol saturated with ammonia at 0°C. (30ml), kept at 35°C. for 24 hours and then evaporated. The

residue was dissolved in 80% acetic acid(10ml) and heated on a steam bath for 12 minutes. The solution was cooled and applied to 8 paper chromatograms which were then developed in Solvent A. The band at R_f 0.20 was eluted with water and gave 16.5mg(LXXXV, 29.5%) upon lyophilization. The product was identical in all respects to an authentic sample[101] and properties are listed in Table XVI.

The product was completely resistant to both snake venom and spleen phosphodiesterases when incubated at 37°C. for 24 hours and then examined on paper chromatography in Solvent A.

8,2'-Thioanhydroguanylyl(3'-5') 8,2'-thioanhydroadenosine (LXXXVI) (Scheme XX)

5'-O-Monomethoxytrityl-N^2-dimethylaminomethylene-8,2'-thioanhydroguanosine 3'-phosphate(LXXXIV) was prepared from LXXII by the same method outlined above for the synthesis of LXXXIII. Following passage of an aqueous pyridine solution of LXXXIV through a Dowex 50W-X8(pyridinium) column and drying, the nucleotide was condensed with N^6,$O^{3'}$-dibenzoyl-8,2'-thioanhydroadenosine(LXX) using DCC and the procedure outlined earlier. Following basic and acidic deblocking procedures and work-up on paper chromatograms in Solvent A, the band at R_f 0.07 was eluted with water and lyophilized to give LXXXVI(11.6mg, 21.4%). Properties are listed in Table XVI. Incubation of the dinucleotide at 37°C. for 24 hours with snake venom and spleen phosphodiesterases showed no degradation.

Approximatly 5mg of the dinucleotide in water(1ml) was refluxed with 1 spoonful of Raney nickel for 6 hours. The solution was cooled, filtered with the aid of Celite powder and the filtrate applied to a paper chromatogram. After development in Solvent A the band at R_f 0.17 was eluted with water and the u.v. spectrum in water showed a maximum at 258nm. Electrophoretic

TABLE XVI Properties of Mononucleotides and Dianhydronucleoside Monophosphates

COMPOUND	Paper* SOLVENT° A	B	C	F	Electrophoresis+ Rm	U.V. Spectra(water) max, nm
5'-O-MMTr-SanhI 3'-p (LXXIV)	0.23	0.72	0.07	----	Rm(Tp) 0.65	267,230sh
2',3'-O-IspU 5'-p (LXXVII)	0.11	----	0.02	----	Rm(Tp) 1.00	260
N^6,$O^{3'}$-diAc-8,2'-SanhA5'-p(LXXIX)	0.09	0.47	0.06	----	Rm(Tp) 0.75	292.5,231,297sh
8,2'-SanhA-p-8,2'-SanhA (LXXXV)	0.20	0.42	0.08	0.43	Rm(pA) 0.28	271
8,2'-SanhG-p-8,2'-SanhA (LXXXVI)	0.07	0.40	0.04	0.40	Rm(pA) 0.24	268,276sh,220sh
8,2'-SanhI-p-8,2'-SanhA (LXXXVII)	0.10	0.49	0.04	0.45	Rm(pA) 0.27	267.5

* Whatman 3MM - Descending technique

o Described in General Methods

+ triethylammonium bicarbonate buffer pH 7.5

mobility relative to pA was 0.38 and chromatographic mobility in Solvent C R_f 0.10 and Solvent F R_f 0.49. This was identical to the reported[111] 2'-deoxyguanylyl(3'-5') 2'-deoxyadenosine.

8,2'-Thioanhydroinosinyl(3'-5') 8,2'-thioanhydroadenosine (LXXXVII) (Scheme XX)

5'-O-Monomethoxytrityl-8,2'-thioanhydroinosine 3'-phosphate(LXXXIV, 47mg, 0.09mmole) was dissolved in 50% aqueous pyridine(5ml) and passed through a column of Dowex 50W-X8 (pyridinium form) resin. The column was washed with 50% aqueous pyridine and the eluate concentrated to dryness and then rendered anhydrous by repeated pyridine evaporation(5 x 2ml). To the anhydrous residue was added LXX(60mg, 0.1mmole), DCC(246mg, 1.2mmole) and pyridine(2ml) and the reaction stirred at room temperature for eleven days. Following the same work-up as described above, 4.5mg LXXXVII(8.1%) was obtained from the band at R_f 0.10 in Solvent A. The product was completely resistant to spleen and snake venom phosphodiesterases and its' properties are listed in Table XVI.

Compound LXXXVII(∿ 3mg) was dissolved in 0.5ml water and refluxed with 0.5 spoonful of Raney nickel for 6 hours. The was cooled and applied to a paper chromatogram which was developed in Solvent A. The band at R_f 0.19 was eluted with water (u.v. maximum(water) 253nm) and lyophilized. This product(5.5 O.D. units) was incubated with snake venom phosphodiesterase for 7 hours at 37°C. Paper chromatographic examination of the reaction mixture in Solvents A, B, and F showed complete degradation to 2'-deoxyinosine(XXXIII, 2.79 O.D. units) and 2'-deoxyadenosine 5'-phosphate(3.02 O.D. units) both of which have been reported earlier. The ratio of nucleotide/nucleoside was 1.09.

5'-O-Toluenesulfonyl-8,2'-thioanhydroadenosine (LXXXII)

Compound XII(100mg, 0.36mmole) and p-toluenesulfonyl chloride(76mg, 0.40mmole) were stirred in pyridine(1ml) at room temperature for 24 hours. An additional 76mg of p-toluenesulfonyl chloride was added and the reaction continued for a further 24 hours. The solution was poured into ice water, filtered and the precipitate washed with ether. Upon drying over P_2O_5 LXXXII(98.7mg, 63%, m.p. 187°C. dec.) was obtained. Thin-layer chromatography in THF showed R_f 0.68 while paper chromatography in Solvent A showed R_f 0.74, Solvent C R_f 0.60 and Solvent F R_f 0.84. The u.v. spectrum in 95% ethanol showed maxima at 274nm(ε 17340) and 224.5nm(ε 32200) and a shoulder at 270nm(ε 17190). The i.r. spectrum showed a covalent sulfonate absorption at 8.55μ.

Attempted synthesis of 5'-halogeno-8,2'-thioanhydroadenosine

Method A Compound LXXXII(40mg, 0.09mmole) and sodium iodide(40mg, 0.27mmole) were dissolved in acetonylacetone(1ml) and heated on a steam bath for 3 hours. The reaction mixture was cooled, filtered and the filtrate concentrated to an oily residue. THF(1ml) was added along with an excess of hexane. The resulting precipitate was gathered by filtration and an i.r. showed that the solid(37mg) was unreacted LXXXII.

Method B Compound XII(100mg, 0.36mmole) was added to thionyl chloride(1ml) in hexamethylphosphoramide(5ml) and the resulting solution kept at room temperature for 15 hours. Water (9ml) was added and the solution applied to a Dowex 50W-X8(H^+) column and washed with water. The nucleosidic material was eluted with 1N ammonium hydroxide and the solution concentrated to dryness. The residue was crystallized from hot water to give almost quantitative recovery of the starting material.

Attempted dinucleoside monophosphate synthesis via 5'-tosylate displacement

5'-O-Monomethoxytrityl-8,2'-thioanhydroinosine 3'-phosphate(LXXIV, 30mg, 0.047mmole) was dried by repeated pyridine evaporation. Compound LXXXII(8.7mg, 0.02mmole) and pyridine(0.5ml) were added and the solution stirred at room temperature for 24 hours. Chromatographic and electrophoretic analysis of the reaction mixture at 60°C. for one hour showed partial dephophorylation of the starting nucleotide LXXIV and none of the desired dinucleoside monophosphate.

Attempted synthesis of 8,2'-thioanhydroadenosine 5'-phosphorofluoridate

Compound XLVII(20mg, 0.056mmole) was dried by repeated evaporation of DMF(5 x 0.5ml). The residue was dissolved in DMF(0.5ml), treated with 2,4-dinitrofluorobenzene(0.16ml, 0.17mmole) and triethylamine(0.016ml, 0.11mmole) and the orange-red solution stirred at room temperature for 48 hours. Electrophoresis showed no monocharged material present and while several unidentified compounds were isolated on paper chromatography in Solvent F none of these contained material with u.v. maximum(water) at 275.5nm which is characteristic of 8,2'-thioanhydroadenosine.

Attempted dinucleoside monophosphate synthesis via a nucleoside phosphorochloridate

5'-O-Monomethoxytrityl-N^2-acetyl-8,2'-thioanhydroguanosine(LXXII, 25mg,0.04mmole) was dried by repeated evaporation of pyridine(5 x 0.5ml). Pyridine(0.5ml) and methylphosphorodichloridate(6.5λl, 0.04mmole) were added and the reaction stirred at room temperature for 3 hours. Thin-layer chromatography in THF showed no starting material remaining and a deep spot containing monomethoxytrityl at the origin. N^6,$O^{3'}$-Dibenzoyl-8,2'-thioanhydroadenosine(LXX, 20mg, 0.04mmole) was dried separately by pyridine evaporation. The residue was dissolved in pyridine(0.1ml) and injected via syringe into the dichloridate reaction and the solution stirred at room temperature overnight. Water(1ml, 0°C.) was then added to the cooled(0°) reaction

mixture and the solution was concentrated to dryness. The residue was dissolved in 80% acetic acid(1ml) and heated on a steam bath for 15 minutes. Examination of the reaction mixture after acid treatment showed only two compounds present. The charged compound had Rm(Tp)= 0.79 which was identical to XLVIII. The u.v. spectrum in 95% ethanol showed this material to be identical to LXXI(Table XV). It was concluded that this product was N^2-acetyl-8,2'-thioanhydroguanosine 3'-phosphate. The other compound was unreacted LXX.

Attempted polymerization of 8,2'-thioanhydroadenosine 5'-monophosphate (XLVII)

Method A Compound XLVII(20mg, 0.055mmole) was dried by repeated evaporation of pyridine(5 x 0.5ml). DCC(22mg, 0.11mmole) and pyridine(0.5ml) were added and the reaction stirred in the dark at room temperature for 5 days. After this time approximatly 50% of the starting material was converted into P^1,P^2-di-8,2'-thioanhydroadenosine 5'-pyrophosphate(LXXX) as determined by chromatographic and electrophoretic analysis. Addition of 2 more equivalents had no effect. Warming of the reaction mixture at 60°C. for one hour resulted in complete dephosphorylation of the remaining starting material.

Method B Compound XLVII(20mg, 0.055mmole) was dried by repeated evaporation of pyridine or DMF. Mesitylenesulfonyl chloride(11.8mg, 0.076mmole) and pyridine or DMF(0.5ml) were added and the reaction mixture heated at 60°C. for one hour. After 15 minutes, electrophoretic and thin-layer chromatographic analysis of the reaction mixture showed that complete dephosphorylation of the nucleotide had occurred. Products which moved faster than 8,2'-thioanhydroadenosine on thin-layer in THF were probably sulfonated derivatives of XII.

Method C Compound XLVII(20mg, 0.055mmole) was dissolved in DMF(1ml). Triethylamine(0.1ml) and dioxane saturated with dry HCl(0.01ml) were added and the solution refluxed in the presence

of molecular sieves for 0.5 hour. Following cooling in ice water, the solution was concentrated to dryness and the residue dissolved in 0.1ml water. Upon examination of this solution on paper chromatography in Solvent F and electrophoresis almost complete dephosphorylation was observed. Only a small amount (<10%) of unreacted starting material was evident.

BIBLIOGRAPHY

1. H.E. Kaufman, E.L. Martola and C. Dohlman, Arch. Ophthalmol., 68 235(1962).
2. B. Shimizu and F. Shimizu, Chem. Pharm. Bull., 18 1060(1970).
3. S. Cohen, Progress in Nucleic Acid Research and Molecular Biology, 5 1(1966).
4. W.W. Lee, A. Benitez, L. Goodman and B.R. Baker, J. Am. Chem. Soc., 82 2648(1960).
5. F.M. Schabel, Chemotherapy, 13 321(1968).
6. G.E. Means and H. Fraenkel-Conrat, Biochim. Biophys. Acta, 247 441(1971).
7. P. Reyes and C. Heidelberger, J. Mol. Pharmacol., 1 14(1965).
8. D.V. Santi and T.T. Sakai, Biochemistry, 10 3598(1971).
9. D.A. Walsh, J.P. Perkins and E.G. Kreb, J. Biol. Chem., 243 3763(1968).
10. K. Muneyama, R.J. Bauer, D.A. Shuman, R.K. Robins and L.N. Simon Biochemistry, 10 2390(1971).
11. A. Hampton and A.W. Nichol, J. Org. Chem., 32 1688(1967).
12. D.J. Harper and A. Hampton, ibid., 37 795(1972).
13. A.W. Johnson, L. Mervyn, N. Shaw and E.L. Smith, J. Chem. Soc., 4146(1963).
14. T. Kunieda and B. Witkop, J. Am. Chem. Soc., 91 7752(1969).
15. J. Zemlicka, Coll. Chech. Chem. Comm., 33 3796(1968).
16. A.E.V. Haschemeyer and A. Rich, J. Mol. Biol., 27 369(1967).
17. D.L. Elder, E. Bunnenberg, C. Djerassi, M. Ikehara and W. Voelter, Tetrahedron Lett., 727(1970).
18. G.T. Rogers and T.L.V. Ulbricht, Eur. J. Biochem., 22 457(1971).

19. M. Ikehara, S. Uesugi and K. Yoshida, Biochemistry, 11 830(1972).

20. J.J. Fox, Pure and Appl. Chem., 18 223(1969).

21. M. Imazawa, T. Ueda and T. Ukita, Tetrahedron Lett., 4807(1970).

22. J.F. Codington, I.L. Doerr and J.J. Fox, J. Org. Chem., 29 558(1964).

23. M. Ikehara and K. Muneyama, Chem. Pharm. Bull., 18 1196(1970).

24. T. Ueda and S. Shibuya, ibid., 18 1076(1970).

25. M. Ikehara, Acts. Chem. Res., 2 47(1969).

26. T. Kanai, T. Kojima, O. Maruyama and M. Ichino, Chem. Pharm. Bull., 18 2569(1970).

27. C.M. Tapiero and J. Nagyvary, Nature, 231 42(1971).

28. R.A. Sanchez and L.A. Orgel, J. Mol. Biol., 47 531(1970).

29. H.P.M. Fromageot, B.E. Griffin, C.B. Reese and J.E. Sulston, Tetrahedron, 23 2315(1967).

30. J.H. van Boom, G.R. Owen, J. Preston, T. Ravindranathan and C.B. Reese, J. Chem. Soc.(C), 3230(1971).

31. H.P.M. Fromageot, B.E. Griffin, C.B. Reese and J.E. Sulston, Tetrahedron 22 705(1966).

32. G.A.R. Johnston, ibid., 24 6987(1968).

33. J.M. Sugihara, Adv. Carb. Chem., 8 1(1953).

34. H.G. Khorana, Pure and Appl. Chem., 25 91(1971).

35. G. Weimann and H.G. Khorana, J. Am. Chem. Soc., 84 419(1962).

36. F. Eckstein and I. Rizk, Chem. Ber., 102 2362(1969).

37. R.L. Letsinger and K.K. Ogilvie, J. Am. Chem. Soc., 91 3350(1969).

38. R.L. Letsinger, K.K. Ogilvie and P.S. Miller, ibid., 91 3360(1969).

39. K.K. Ogilvie and D. Iwacha, Can. J. Chem., 48 362(1970).

40. M. Ikehara, S. Uesugi and M. Vasumoto, J. Am. Chem. Soc., 92 4735(1970).

41. K.L. Nagpal and M.M. Dhar, Tetrahedron Lett., 47(1968).

42. J. Nagyvary and K.L. Nagpal, Science, 177 272(1972).

43. A. Hampton and A.W. Nichol, Biochemistry, 5 2076(1966).

44. D.M. Brown, A.R. Todd and S. Varadarajan, J. Chem. Soc.(C), 868(1957).

45. N.C. Yung and J.J. Fox, J. Am. Chem. Soc., 83 3060(1961).

46. S. Tang and J.S. Roth, Tetrahedron Lett., 2123(1968).

47. G. Kowollick, K. Gaertner and P. Langen, ibid., 3863(1969).

48. G. Kowollick, K. Gaertner, G. Etzold and P. Langen., Carbohyd. Res., 12 301(1970).

49. M. Ikehara and K. Muneyama, Chem. Pharm. Bull., 18 1196(1970).

50. M. Ikehara, H. Tada and K. Muneyama, ibid., 13 639(1965).

51. M. Ikehara and K. Muneyama, J. Org. Chem., 32 3042(1967).

52. M. Ikehara and M. Kaneko, Tetrahedron, 26 4251(1970).

53. R. Shapiro and S.C. Agarwal, Biochem. Biophys. Res. Comm., 24 401(1966).

54. S. Zemlicka and A. Holy, Coll. Czech. Chem. Comm., 32 3159(1967).

55. R. Lohrmann and H.G. Khorana, J. Am. Chem. Soc., 88 829(1966).

56. J.F. Bunnett and J.Y. Bassett Jr., ibid., 81 2104(1959).

57. K.K. Ogilvie and D. Iwacha, Can. J. Chem., 47 495(1969).

58. Sigma Chemical Co.

59. M. Ikehara and H. Tada, Chem. Pharm. Bull., 15 94(1967).

60. J. Buchanan, J. Chem. Soc.(C), 3162(1950).

61. J. Buchanan, Nature, 168 1091(1951).

62. J.J. Fox, J. Am. Chem. Soc., 83 3060(1961).

63. D. Lin, Doctoral Thesis U. of Manitoba

64. S.R. Jenkins, F.W. Holly and E. Walton, J. Org. Chem., 30 2851(1965).

65. S.M. Partridge, Nature, 164 443(1949).

66. M. Ikehara and S. Uesugi, Tetrahedron Lett., 713(1970).

67. K.K. Ogilvie and D. Iwacha, Can. J. Chem., 47 495(1969).

68. K.K. Ogilvie and L. Slotin, J. Org. Chem., 36 2556(1971).

69. M. Ikehara, Chem. Pharm. Bull., 8 367(1970).

70. R.E. Holmes and R.K. Robins, J. Am. Chem. Soc., 86 1242(1964).

71. H. Venner, Chem. Ber., 93 140(1960).

72. F. Ichino, Chem. Abstr., 72 P55843n

73. M. Ikehara and S. Tezuka, Tetrahedron Lett., 1169(1972).

74. M. Kaneko, M. Kimura, and B. Shimizu, Chem. Pharm. Bull., 20 635(1972).

75. R.E. Holmes and R.K. Robins, J. Am. Chem. Soc., 87 1772(1965).

76. R.A. Long, R.K. Robins and L.B. Townsend, J. Org. Chem., 32 2751(1967).

77. M. Kaneko, B. Shimizu and M. Ikehara, Tetrahedron Lett., 3113(1971).

78. J. March, Advanced Organic Chemistry : Reactions, Mechanisms and Structure, McGraw-Hill Inc., U.S.A., 1968, Chpt. 10.

79. M. Yoshikawa, T. Kato, and T. Takenishi, Bull. Chem. Soc., 42 3505(1969).

80. H.G. Khorana, Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest, John Wiley and Sons Inc., New York, 1961, Chpt. 2.

81. J.G. Moffatt and H.G. Khorana, J. Am. Chem. Soc., 83 649(1961).

82. M. Ikehara and M. Hattori, Biochim. Biophys. Acta, 269 27(1972).

83. O.H. Lowry and J.A. Lopez, J. Biol. Chem., 162 421(1946).

84. M. Smith and H.G. Khorana, J. Am. Chem. Soc., 80 1141(1958).

85. A. Bloch, M.J. Robins and J.R. McCarthy Jr., J. Med. Chem., 10 906(1967).

86. S. Frederikson, Arch. Biochem. Biophys., 113 383(1966).

87. H.J. Schaeffer, S. Gurwara, R. Vance and S. Bittner, J. Med. Chem., 14 367(1971).

88. R.H. Shaw, H.J. Schaeffer and D.H. Murray, J. Pharm. Sci., 54 15(1965).

89. J.L. York and G.A. Lepage, Can. J. Biochem., 44 331(1969).

90. L.N. Simon, R.J. Bauer, R.L. Tolman and R.K. Robins, Biochemistry, 9 573(1970).

91. S.S. Tavale and H.M. Sobell, J. Mol. Biol., 48 109(1970).

92. K.K. Ogilvie, unpublished results.

93. A. Holy, Coll. Czech. Chem. Comm., 35 81(1970).

94. A. Holy, R.W. Bald and F. Sorm, ibid., 37 592(1972).

95. M. Honjo, Y. Kanai, Y. Furukawa, Y. Mizuno and Y. Sanno, Biochim. Biophys. Acta, 87 696(1964).

96. Y. Mizuno, M. Ikehara, T. Ueda, A. Nomura, E. Ohtsuka, F. Ishikawa and Y. Kanai, Chem. Pharm. Bull., 9 338(1961).

97. N.J. Leonard and R.A. Laursen, Biochemistry, 4 365(1965).

98. D.B. McCormick, Biochemistry, 5 746(1966).

99. D.B. McCormick and G.E. Opar, J. Med. Chem., 12 333(1969).

100. H. Schaller, G. Weimann, B. Lerch and H.G. Khorana, J. Am. Chem. Soc., 85 3821(1963).

101. S. Uesugi, M. Yasumoto, M. Ikehara, K.N. Fang and P.O.P. Ts'O, J. Am. Chem. Soc., 94 5480(1972).

102. P. Rheault, MSc Thesis. U. of Manitoba

103. P.T. Gilham and H.G. Khorana, J. Am. Chem. Soc., 80 6212(1958).

104. T.M. Jacob and H.G. Khorana, ibid., 87 368(1965).

105. G.M. Tener, ibid., 83 159(1961).

106. G. Weimann and H.G. Khorana, ibid., 84 4329(1962).

107. H.G. Khorana and J.P. Vizsolyi, ibid., 81 4660(1959).

108. W.E. Razzell and H.G. Khorana, J. Biol. Chem., 236 1144(1961).

109. W.E. Razzell and H.G. Khorana, ibid., 234 2105(1959).

110. K.K. Ogilvie and D. Iwacha, unpublished results.

111. S.A. Narang, T.M. Jacob and H.G. Khorana, J. Am. Chem. Soc., 87 2988(1965).

112. D.T. Elmore and A.R. Todd, J. Chem. Soc.(C), 3681(1952).

113. N. Anand, U.M. Clark, R.H. Hall and A.R. Todd, ibid., 3665(1952).

114. A.R. Todd, C.B. Reese, D.B. Parihar and D.M. Brown, ibid., 3035(1958).

115. K. Kikugawa and M. Ichino, Tetrahedron Lett., 87(1971).

116. P.W. Johnson and M. Smith, Chem. Comm., 379(1971).

117. R. von Tigerstrom and M. Smith, Science, 167 1266(1970).

118. J. Smrt and J. Catlin, Tetrahedron Lett., 5081(1970).

119. R.K. Ralph and H.G. Khorana, J. Am. Chem. Soc., 83 2926(1961).

120. H.G. Khorana, J.P. Vizsolyi and R.K. Ralph, ibid., 84 414(1962).

121. O. Pongs and P.O.P. Ts'O, ibid., 93 5241(1971).

122. M. Grunberg-Manago, Progress in Nucleic Acid Research and Molecular Biology, 1 93(1963).

PUBLICATIONS

2',3'-Carbonates of 8-Hydroxypurine Nucleosides, K.K. Ogilvie and L. Slotin, J. Org. Chem. 36 2556(1971).

Novel Synthesis of 8,2'-Thioanhydropurine Nucleosides, K.K. Ogilvie and L. Slotin, Chem. Comm. 890(1971).

Novel Substrate of Adenosine Deaminase, Kelvin K. Ogilvie, Lewis Slotin and Pat Rheault, Biochem. Biophys. Res. Comm., 45 297(1971).

Synthesis of 8,2'-Thioanhydroguanosine, K.K. Ogilvie, L. Slotin, J.B. Westmore and D. Lin, Can. J. Chem., 50 1100(1972).

A General Synthesis of 8,2'-Thioanhydropurine Nucleosides, K.K. Ogilvie, L. Slotin, J.B. Westmore and D. Lin, Can. J. Chem., 50 2249(1972).

Synthesis of 8,3'-Thioanhydroguanosine, Kelvin K. Ogilvie, Lewis A. Slotin, Denis C.K. Lin and John B. Westmore, Can. J. Chem., in press.

Synthesis of Purine 8,2'-Cyclonucleosides, K.K. Ogilvie, L.A. Slotin, J.B. Westmore and D. Lin, J. Heterocyclic Chem., in press.

The Study and Characterization of Nucleosides by Mass Spectrometry III. Comparison between the Mass Spectra of Trimethylsilyl Derivatives of Purine 2'- and 3'-linked anhydro-, thioanhydro- and aminoanhydro nucleosides, Denis C.K. Lin, L. Slotin, K.K. Ogilvie and J.B. Westmore, submitted for publication.

ADDENDUM

8,2'-SanhA = 8,2'-thioanhydroadenosine = 8,2'-thioanhydro-8-mercapto-9-β-D-arabinofuranosyladenine
Similarly for the other anhydropurine nucleoside nomenclature used in this thesis.

In all cases where the molecular ion is indicated (m/e) the relative abundance was 100%.

All data in Tables of paper and thin-layer chromatographic data are R_f values where the value given represents the relative mobility of the compound to the solvent front.

$$\text{i.e. } R_f \text{ value} = \frac{\text{distance compound travels}}{\text{distance solvent front travels}}$$