UNIVERSITY OF MANITOBA

2,2'-ANHYDRO -4-THIOURIDINE NUCLEOSIDES

presented by

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ABSTRACT

This work was designed to determine if various blocked 2,2'-anhydro-4-thiouridine nucleosides could be converted into dinucleotides using standard phosphorylating techniques.

The main problem occured in obtaining a 5'-blocked 2,2'-anhydro-4-thiouridine. Attempts to convert 4-thiouridine derivatives into 2,2'-anhydro-4-thiouridine derivatives, proved unsuccessful.

2,2'-Anhydro-4-thiouridine was then obtained by a new procedure but was found to be unstable to attempts to block the molecule in the 5'-position.

2,2'-Anhydro-4-thiouridines were obtained by thiating fully protected 2,2'-anhydrouridines. After thiation, attempts were made to remove the blocking group on the 3'-position without effecting the anhydro-linkage or the blocking group on the 5'-position. The blocking groups used in this work were the methoxytrityl, trityl, acetyl, isobutyl carbonate, 1-ethoxyethyl and the pivalyl groups.

When finally a 5'-blocked 2,2'-anhydro-4-thiouridine was obtained, the thione function was found to be unstable to standard phosphorylation conditions.

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INTRODUCTION

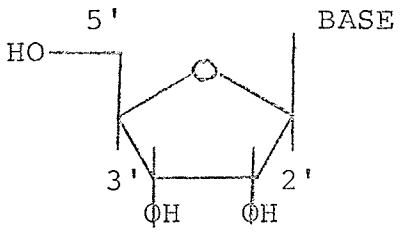
Biological and biochemical research has recently increased in interest, especially in the area dealing with the transfer of genetic information from generation to generation. This information transfering material is comprised mainly of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). The building blocks or nucleosides comprising these large DNA and RNA molecules have been isolated and their structures elucidated.

It is extremely important that fast, efficient synthetic techniques be developed for coupling these building blocks or nucleosides together to form nucleotides. The synthesis of nucleotides of predetermined sequence in high yields is absolutely essential. Such synthetic nucleotides have already been used to break the genetic code and will provide the tools for investigations which will further unravel the secrets of the genetic process.

Good synthetic techniques for producing modified nucleosides and nucleotides are also important. It is known (24) that halogenation with iodine on the 5-position of pyrimidine nucleosides such as uridine or cytosine result in powerful therapeutic agents. In view of these results, producing modified nucleosides and nucleotides and studying them may hold the key to many of man's health problems today.

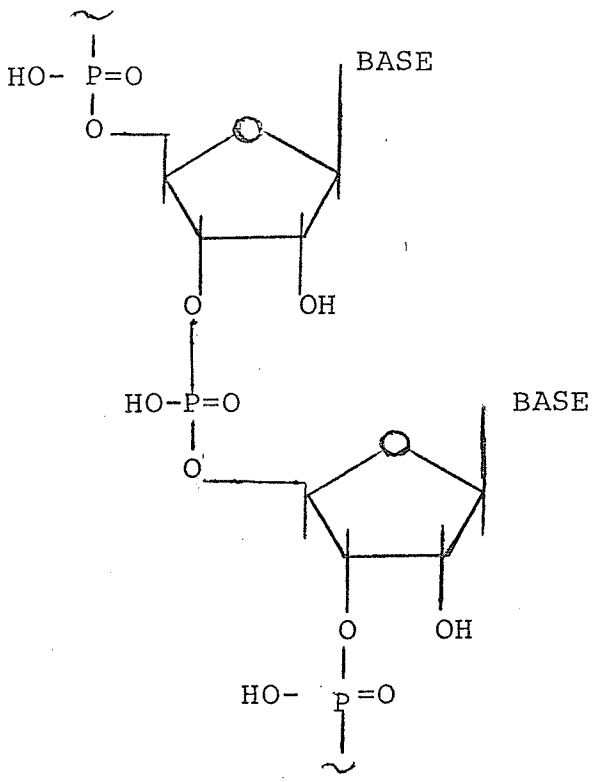
It should be obvious that development of efficient synthetic procedures in this area is of primary importance.

RNA, a biologically important compound, is comprised of mainly four bases, uridine, cytosine, adenosine and guanosine. The problem of incorporating these four bases into an RNA chain lies with the pentose sugar (β-D-ribose) portion of the nucleoside. The sugar portion of a nucleoside is illustrated below.



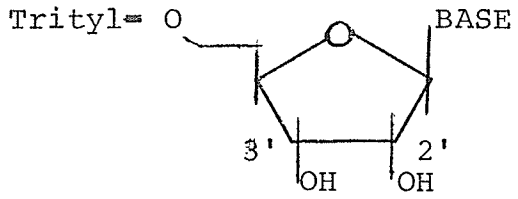
Since the hydroxyl on the 5'-position is much less sterically hindered than the hydroxyls on the 2' and 3'-carbons, a large blocking group such as a triphenylmethyl (trityl) will preferentially attack the 5'-position (1). This leaves the 2' and 3'-hydroxyl positions open for phosphorylation.

A naturally occuring RNA molecule, in which the sugar units are linked by phosphate bridges between the 3'-position of one molecule and the 5'-position of the next molecule is illustrated on the following page.



A.

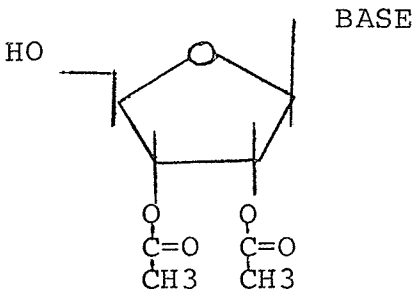
The problem in synthesizing a diribonucleotide from two nucleosides lies in the equivalence of the 2' and 3'-hydroxyl positions on the sugar. If a phosphorylating agent is attacking a 5'-blocked nucleoside shown below,



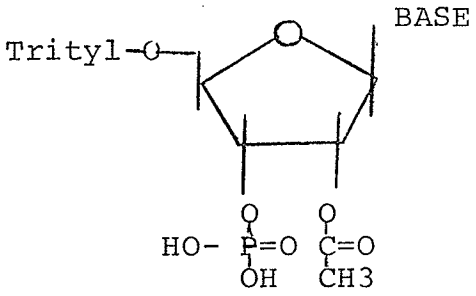
the phosphorylating agent can not differentiate between the 2' and 3'-hydroxyls and therefore attack will occur at both positions. However, in naturally occuring molecules, as in diagram A the phosphate moiety is only found on the 3'-

hydroxyl position. Therefore an attempt must be made to block the 2'-hydroxyl, so phosphorylation will only occur on the 3'-position, as is the case in natural molecules.

The most general methods for the synthesis of oligoribonucleotides was developed by Khorana (25). The procedure he developed involved condensing nucleosides of type I and type II together to form nucleotides.



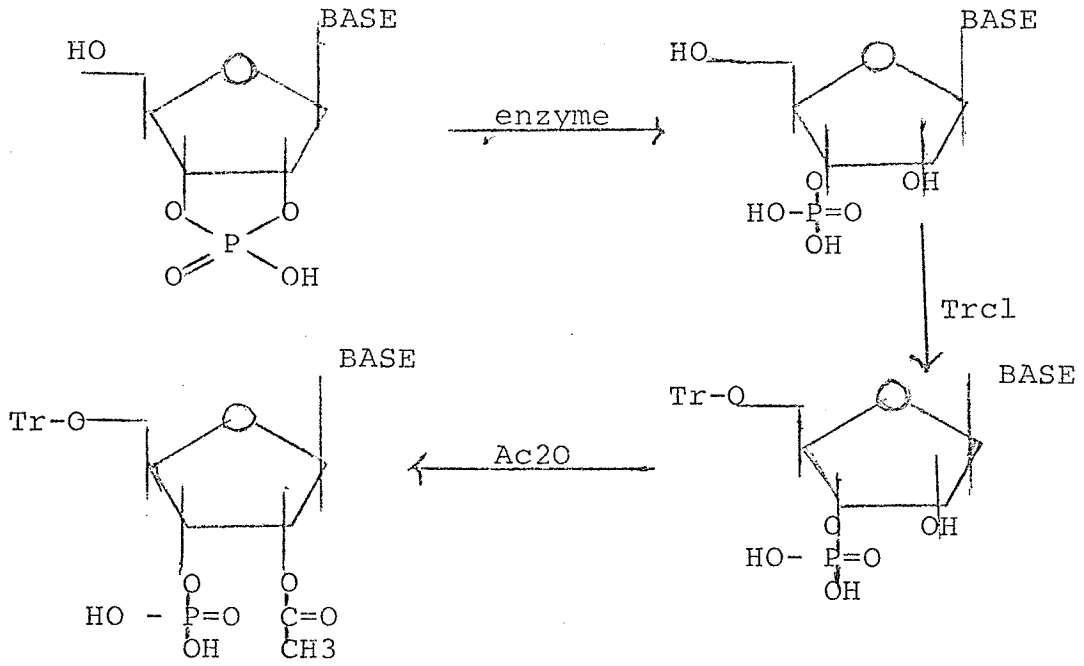
I



II

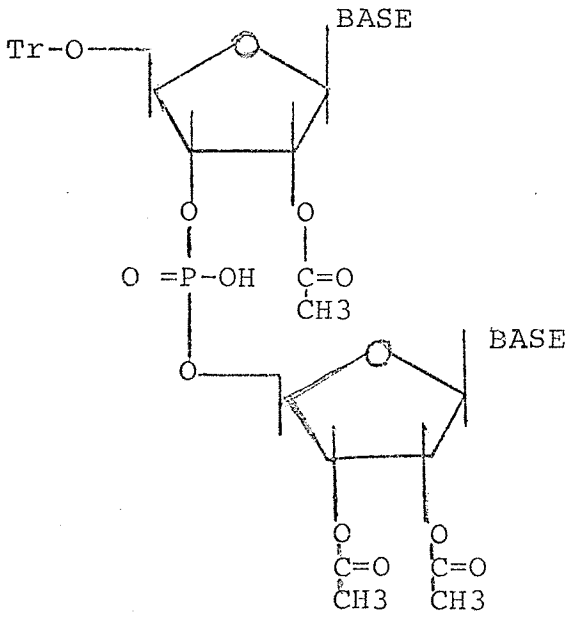
The main problem in this procedure lies in obtaining the type II compounds.

The procedure that Khorana followed was to obtain the 2', 3' -cyclic phosphate of a nucleoside and enzymatically convert this molecule to the 3'-phosphate of that nucleoside. This molecule was then treated with trityl chloride which blocks only the 5'-position with the acid labile trityl group. The 2'-position of this nucleoside is then blocked by a base labile acetyl group.



II

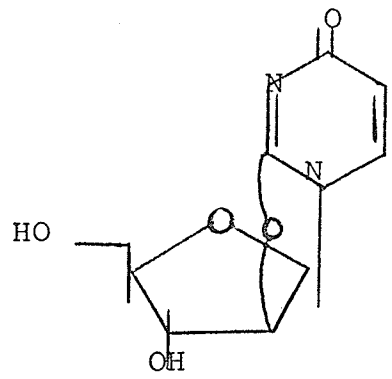
This nucleoside (type II) can then be condensed with a type (I) nucleoside to yield a dinucleotide.



Treatment of this molecule with acid will remove the trityl group which then will allow further condensation with another type II nucleoside. In this manner the chain length can be increased. The acetyl groups are removed by treatment with base, as a final step.

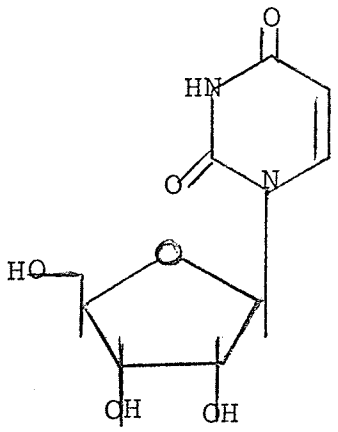
The difficulties with this method are as follows. The starting material, the 3'- phosphate, is not obtained completely chemically, but is obtained by treatment with enzymes and often only in millimolar quantities. The procedures used to make these molecules are long and tedious. The length of a nucleotide chain obtained by chemical condensations is limited and enzymes must then be used to further lengthen the chain. Therefore, development of a completely chemical, less tedious procedure is still a necessity in this field.

A method that could be used for blocking the 2'- hydroxyl for phosphorylation is the formation of an ether linkage, between the 2'- hydroxyl on the sugar and the 2-Keto group on the base. For uridine this 2,2'- anhydrouridine (2,2'- anhydro-1-β-D-arabinofuranosyl uracil) has been made (2) and is illustrated on the following page.

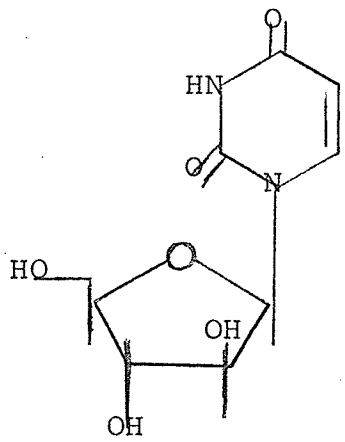


The sugar portion now resembles a deoxyribose sugar for which chemical methods have been developed to produce deoxyribonucleotides (13) in any predetermined sequence.

It has been shown (3) for anhydrouridine molecules that the hydroxyl on the 5'- carbon can be selectively blocked and the hydroxyl on the 3'- carbon can be phosphorylated under normal conditions, using β - cyanoethylphosphate and dicyclohexylcarbodiimide. This 3'- phosphorylated molecule can be condensed with the 5'- position of another nucleoside, to give a dinucleotide. Conditions have been developed to break the anhydrolinkage to give the naturally occuring ribose form (10) or its derivative the arabino form (3).



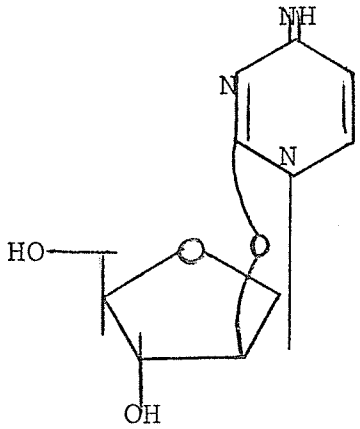
ribose



arabino

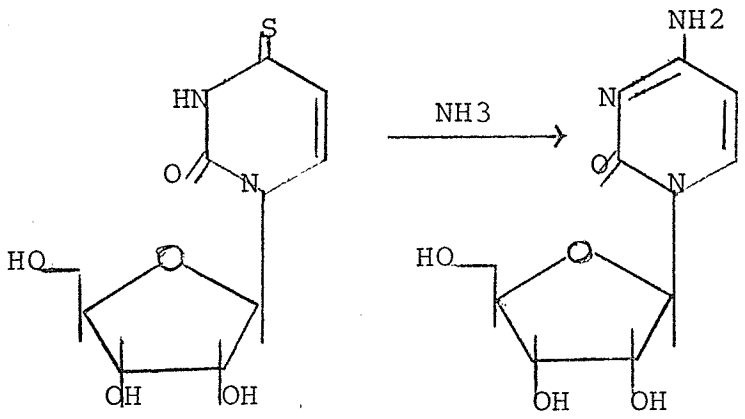
In fact this approach has already been applied to anhydrouridine (3) and guanosine and adenosine derivatives are being adapted to this approach (26).

The fourth major natural occuring nucleoside is cytidine. To incorporate cytidine into a dinucleotide using this method is not as easy; for it has been found that anhydrocytosine (2,2'- anhydro-1-β-D-arabinofuranosyl cytosine) as illustrated below is not stable (4).

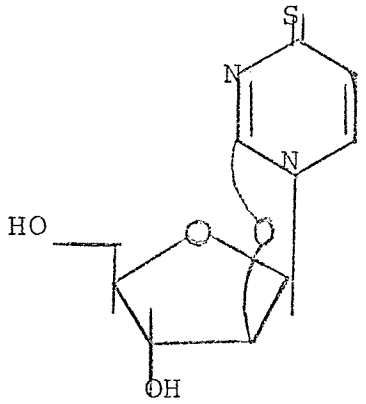


Therefore a slightly different approach had to be tried to incorporate cytosine into a dinucleotide using the anhydrolinkage method. The basis for attempting this method is as follows.

It has been shown (5) that 4-thiouridine on treatment with ammonia give cytosine.



It is also known that 4-thiouridine derivatives can be phosphorylated under the usual conditions, using β - cyanoethylphosphate (7) (8) and dicyclohexylcarbodiimide without destruction of the thione group. Compounds containing a 4-thio group and a 2,2'- anhydrolinkage have also been made (4) (5). Therefore, it was intended to synthesize a nucleotide by the usual methods, but containing a 4-thioanhydro moiety (2,2'- anhydro - 1 - β - D - arabinofuranosyl - 4 - thiouracil), shown on the following page.



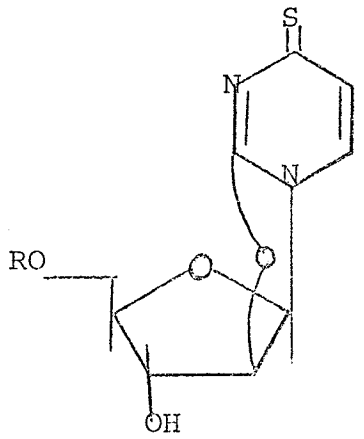
A dinucleotide containing a 4- thioanhydro moiety could be treated with ammonia which would displace the sulphur and give nucleotides containing cytosine derivatives.

At first sight all the possibilities of this method, may not be appreciated. 4- thiouridine molecules are known to be present in t-RNA to a small extent (9). This method would incorporate 4- thiouridine derivatives into an RNA chain. This procedure could also incorporate cytosine derivatives into an RNA chain. This method would also facilitate the study of anhydronucleotides themselves, which are of interest because they are resistant to enzymatic degradation (12). Lastly it has been shown that 4-thiouridine can be oxidized to uridine (6). Therefore, oxidation of the dinucleotide containing the 4-thioanhydrouridine moiety would hopefully give uridine derivative containing dinucleotides.

It is obvious that an efficient synthetic procedure for incorporating 4- thioanhydrouridine into a nucleotide, could be extremely useful for the chemical synthesis of these biologically important molecules. Therefore, the purpose of this research was to investigate the methods for incorporating 4- thioanhydrouridine into a nucleotide chain.

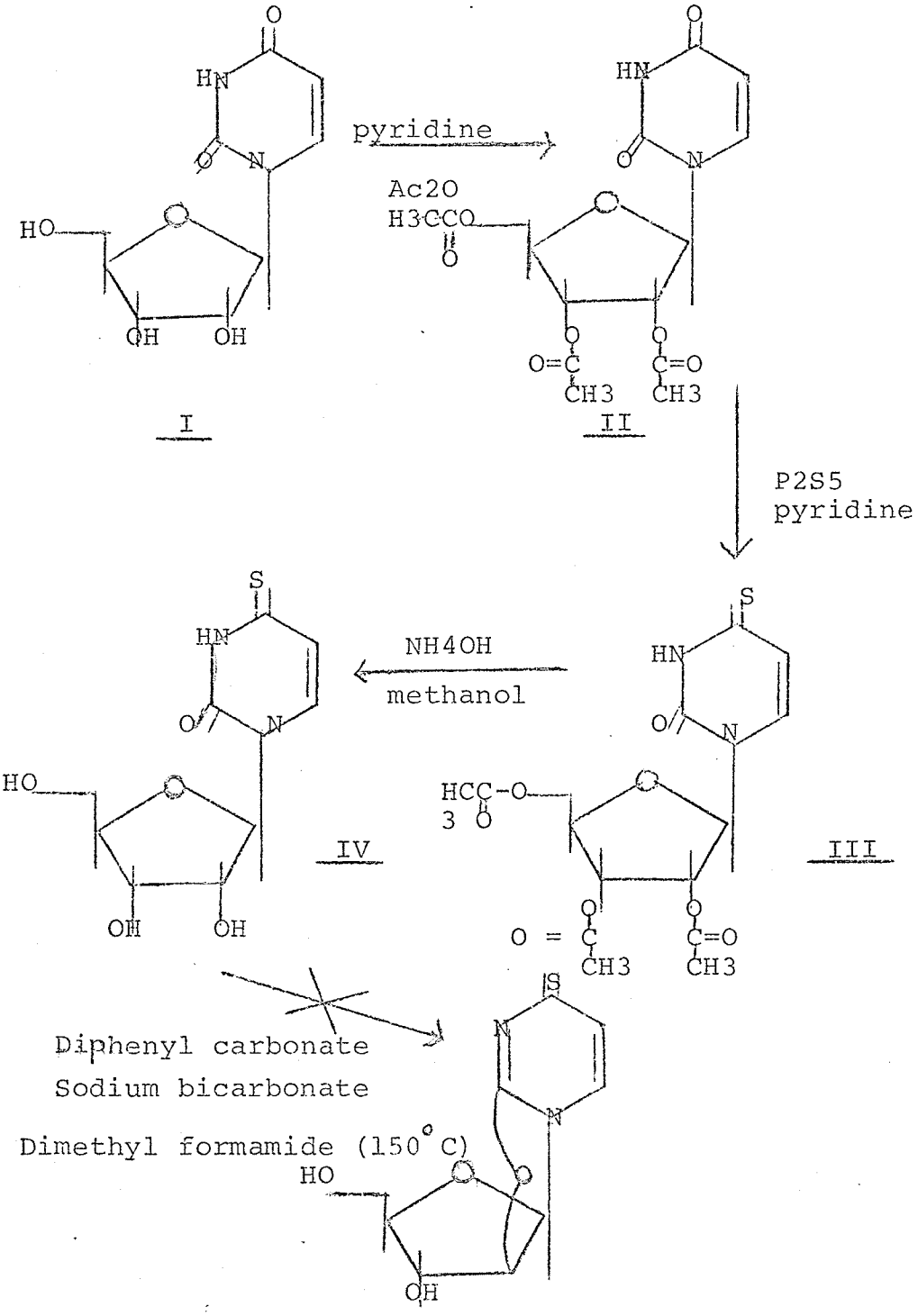
DISCUSSION AND RESULTS

The initial aim of this investigation was to incorporate a 5'-blocked 4- thioanhydrouridine type molecule as shown below into a dinucleotide.



The first approach attempting to make 2,2-anhydro -4- thiouridine followed the procedure developed by Hampton and Nichol (2) for the conversion of uridine to 2,2'-anhydrouridine. D. Iwacha (14) has shown that this procedure will also convert 5'-0- trityluridine to 5'-0-trityl-2,2'-anhydrouridine. Therefore, an attempt was made to convert the analogous 4- thiouridine type molecules into the 2,2'-anhydro -4-thiouridine type molecules, using similar conditions. The scheme attempted is outlined below.

APPROACH I

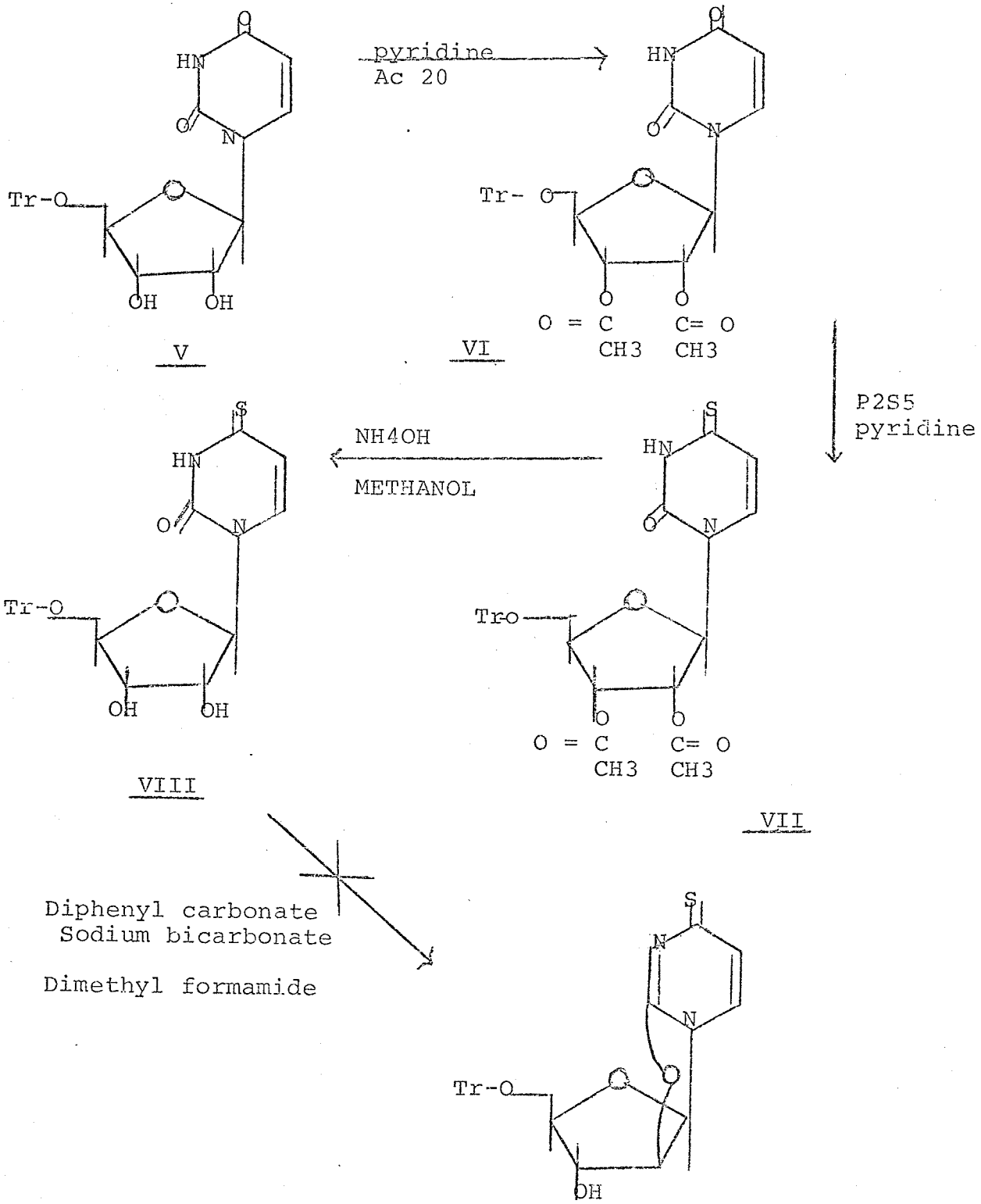

pyridine
Ac2O
H3CCO
I
II
P2S5
pyridine
NH4OH
methanol
IV
III
Diphenyl carbonate
Sodium bicarbonate
Dimethyl formamide (150° C)

2',3',5' -tri-o-acetyluridine was obtained by the slight modification of an established procedure (15) and was converted to 2',3',5' -tri-o-acetyl-4-thiouridine (compound III) by phosphorus pentasulphide in refluxing pyridine. Compound IV, 4-thiouridine was obtained by deacetylating compound III and found to have properties identical to those given by Scheit (16) for this molecule.

This 4-thiouridine was treated with sodium bicarbonate, diphenyl carbonate in dimethyl formamide (150° C) for 0.5 hours. The reaction mixture was then poured into a large volume of ether and the precipitate that formed was collected. This precipitate was dissolved in ethanol and an ultraviolet spectrum was taken. Thiones have a characteristic absorption in the ultraviolet at approximately 330nm with an extinction coefficient of about 20,000 (4). This absorption was absent in our spectrum. Therefore, the reaction proved to be a failure; for if there is no thione peak present in the ultraviolet, there can not possibly be any 4-thioanhydrouridine present.

The Hampton and Nichol conditions were also tried on 5'-0-trityl-4-thiouridine which was made by the scheme outlined below.

APPROACH II


pyridine
Ac 20
V
VI
P2S5
pyridine
NH4OH
METHANOL
VIII
VII
Diphenyl carbonate
Sodium bicarbonate
Dimethyl formamide

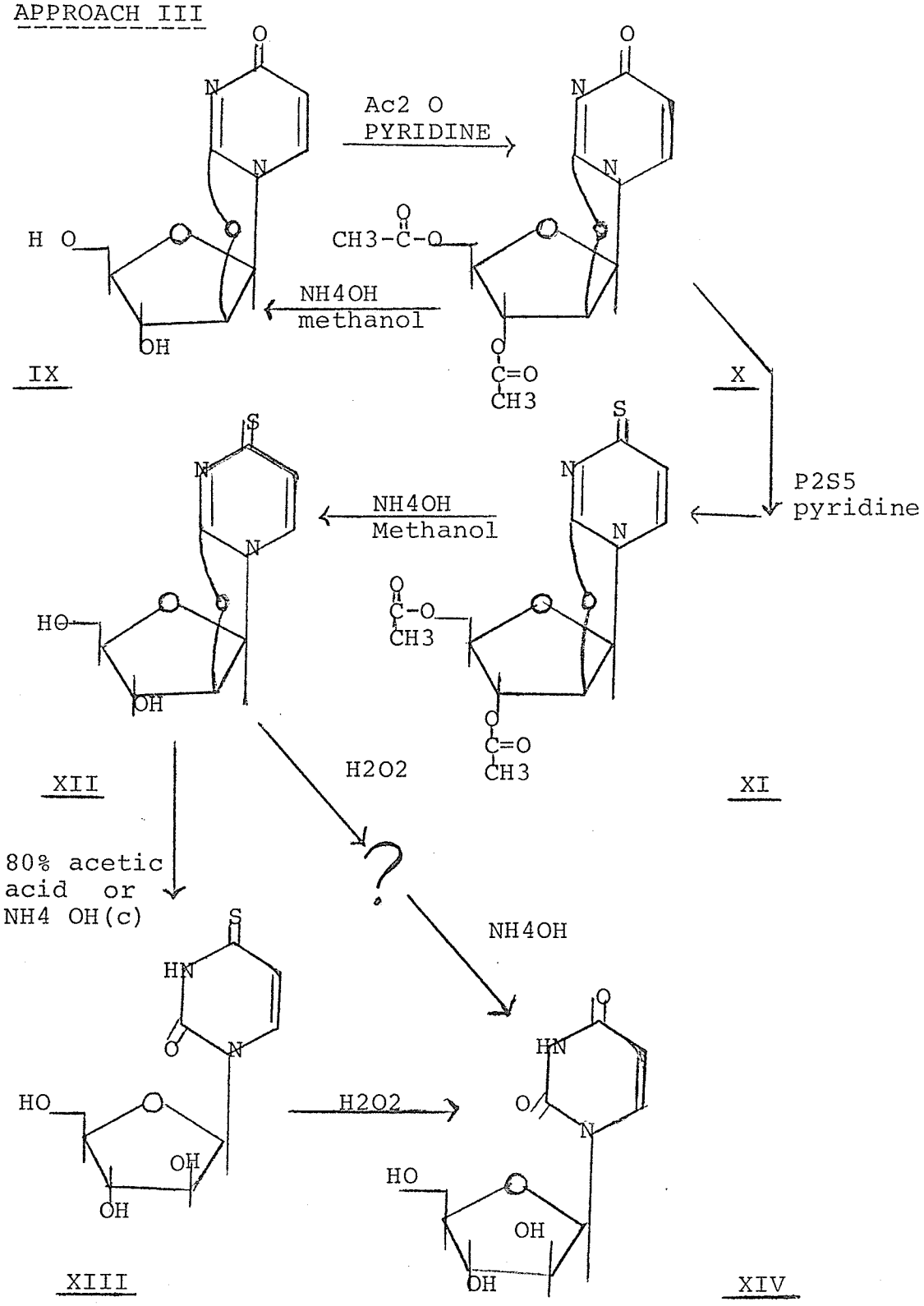
Compound V, 5' - 0 - trityluridine (17) was converted to 5'-o-trityl-2',3'-di-o-acetyluridine (compound VI). using pyridine and acetic anhydride (1). 5'-0-trityl - 2',3'-di-o-acetyl-4-thiouridine was obtained by treating compound VI with phosphorus pentasulphide (8) in refluxing pyridine. This compound was deacetylated to yield 5'-0-trityl-4-thiouridine which was treated with sodium bicarbonate, and diphenyl carbonate in dimethylformamide (150° C) for 0.5 hours. The reaction mixture was poured into a large volume of ether and the precipitate that formed was collected.Again the ultraviolet spectrum of the products dissolved in ethanol showed no thione absorption at 330nm, therefore this reaction proved to be a failure. Clearly the 4-thio group is being transformed under these reaction conditions.

Realizing that 4-thiouridine could not easily be converted to 4-thioanhydrouridine, a new approach was tried based on the fact that 2,2'-anhydrouridine type molecules can be thiated with phosphorus pentasulphide (5) in refluxing pyridine without breaking the anhydrolinkage. In all the literature studied thiations that have been done on nucleosides have the hydroxyls on the sugar portion of the molecule blocked.

The reason for this is that phosphorus pentasulphide will probably attack the hydroxyls on the sugar, as well as the 4-keto position on the pyrimidine ring. Just the same, an attempt was made to thiate 2,2'-anhydrouridine under the conditions shown not to break an anhydrolinkage (5), hopefully to obtain 2,2'-anhydro -4-thiouridine. 2,2'-Anhydrouridine was treated with phosphorus pentasulphide and refluxing pyridine and the reaction worked up by silica gel chromatography. The large number of products obtained and the lack of predominance of any of them was convincing evidence that this method was useless as a synthetic tool. So as all previous workers,a return to blocked nucleosides was made.

The blocking group that one uses is very important. The blocking group must be stable during the thiation conditions and removable once thiation is complete. However, the conditions for removal of blocking groups must not break the anhydrolinkage. The acetyl group as a blocking group fits these requirements perfectly. The scheme developed is outlined on the following page.

APPROACH III



Compound X, 3'5'-di-o-acetyl-2,2'-anhydrouridine was made in 90% yield by treating 2,2'-anhydrouridine with acetic anhydride and pyridine. This compound was treated with 15% ammonium hydroxide-methanol to deacetylate and found to be completely converted to 2,2'-anhydrouridine. Therefore, it has been shown that acetyl groups can be removed without breaking the anhydrolinkage, a necessary qualification for a blocking group in this scheme.

Compound Xl, 3'5'-di-o-acetyl-2,2'anhydro-4-thiouridine was prepared in 73% yield by treating compound X with phosphorus pentasulphide in refluxing pyridine and isolated by silica gel chromatography. It was found, however, to be impossible to purify the compound by chromatography. The compound was always slightly contaminated by the 4-keto anhydro compound.

Compound Xll, 2,2'-anhydro-4-thiouridine was made in 83% yield by deacetylating compound Xl with ammonium hydroxide and methanol and crystallizing the product from methanol.

At this point a problem occured which was to reoccur from this time forth in this work. It seemed peculiar that workers who have done research on 4-thio-2,2'-anhydro compounds (4) (5) have never purified these compounds by silica gel chromatography. The reason for this started to become apparent at this stage of research. The mother liquor from the crystallized 4-thioanhydrouridine was applied to silica gel plates which were developed in tetrahydrofuran and ethanol. No thione containing material could be isolated from the plates. Then pure crystallized 4-thioanhydrouridine was dissolved in ethanol and applied to silica gel plates which were developed in tetrahydrofuran and ethanol. Again no thione containing material could be isolated. This suggests that maybe it was not coincidence that 3',5'-di-o-acetyl-4-thioanhydrouridine could not be isolated pure on silica gel plates and perhaps this lack of stability on silica gel is characteristic of most 4-thioanhydrouridine type molecules. This conclusion will be further verified by later experiments.

The crystallized 4-thioanhydrouridine obtained had its structure assigned on the basis of a thione peak at 325nm in the ultraviolet, no acetyl carbonyl peaks present in the

infra red and a parent peak of 242 in the mass spectrum of the compound. But this compound had a slightly different ultraviolet spectrum and a different sintering point from that which is given in the literature (4) for 4-thioanhydrouridine made by a different procedure. The molecule isolated was definitely 4-thioanhydrouridine but it was decided to do a few simple chemical experiments to prove its structure further.

Compound XIII, 4-thioarabinouridine, can be made by treating compound XII with concentrated ammonium hydroxide or by treating with 80% glacial acetic acid. This structure was assigned to this compound by the presence of a thione peak in the ultraviolet, by the chromatographic data and the fact that hydrogen peroxide readily converted the molecule into arabinouridine (compound XIV).

Compound XII, was also treated with hydrogen peroxide to yield an intermediate product that moved slightly slower than 2,2'-anhydrouridine on paper chromatography and which had an ultraviolet spectrum in water containing a shoulder at 255nm and a minimum at 250nm. This compound was not further identified but was treated with concentrated ammonium hydroxide and found to yield arabinouridine (XIV).

Since it has been definitely proved that the isolated molecule is 4-thioanhydrouridine, the next step required is the blocking of the 5'-hydroxyl position so that the 3'-hydroxyl can be phosphorylated. D. Iwacha (3) has shown that 2,2'-anhydrouridine can be treated with triphenylmethyl chloride (TrCl) or paramethoxy triphenylmethyl chloride (MTrCl) to yield the blocked 5'-o-(Tr or MTr)-2,2'-anhydrouridine in good yield. A similar procedure was attempted on 4-thioanhydrouridine.

The 4-thioanhydrouridine was treated with pyridine and paramethoxy triphenylmethyl chloride and thin layers were run on the reaction mixture daily, for a period of four days. The result of these thin layers were not as expected. Only one spot moving faster than starting material (R_f 0.8 ethyl acetate) could be detected (blocked nucleosides move faster on thin layer than non blocked nucleosides). This spot was very weak in intensity and the relative intensity of this spot did not increase after day one. The reaction was worked up on silica gel plates and the compound at R_f 0.8 (ethyl acetate) was isolated. Its ultraviolet spectrum was very peculiar with maxima at 355nm and 312nm and minima at 340nm and 292nm.

The mass spectrum of this compound showed a parent peak of 524, ten units higher than the desired product 5'-0-methoxytrityl-4-thioanhydrouridine. The reaction was repeated using trityl chloride instead of methoxytrityl chloride but a similar type of compound in the same poor yield was obtained. Since this is the only compound moving faster than starting material on chromatography and is present in a yield of less than 10%, it was decided that the 5'-position of 4-thioanhydrouridine could not easily be blocked with a methoxytrityl or a trityl group.

It was now thought that perhaps the isobutyloxycarbonyl group could be used as a blocking group for the 5'-position in a manner similar to that used by Ogilvie and Letsinger (19) for thymidine.

The 4-thioanhydrouridine was dissolved in dry pyridine and treated with a 1:1 equivelance of isobutylchloroformate. The reaction was worked up on silica gel plates that were developed in hexane, then ether.Three main yellow bands were seen after development in ether and by analogy to Ogilvie and Letsinger's work (19) the slowest moving band was assumed to be the isobutyl 4-thioanhydrouridine 5'-carbonate. This material was eluted from the silica gel using ether. A thin layer chromatogram showed only one spot.

However, on concentrating the ether to two or three milliliters using reduced pressure and then running thin layers in ether,it was found that the compound was decomposing to a slower moving material. It was found that by replating the material two or three times and isolating each time, nearly all the product can be converted to this slower moving decomposition material. The ultra-violet spectrum of this material in 95% ethanol showed maxima at 286nm and 235nm and a minima at 265nm. This decomposition material has no thione present in it.

Some of the assumed product was isolated, even though knowing that on isolation it became impure with the slower moving decomposition material. This product was treated with 80% glacial acetic acid on a steam bath for three hours to break the anhydrolinkage (3). The starting material and the resultant material after treatment with the acid moved identically on thin layer chromatography in ether.This created doubts to whether the anhydrolinkage was still present in the compound that was isolated. In fact isobutyl3'-0-acetyl -4-thioanhydrouridine 5'-carbonate was made by another procedure (approach IV) and found not to move in ether on thin layer chromatography.

This compound is blocked in the 3' - position, which the desired compound from this last reaction is not. Blocking the molecule in the 3'-position would cause it to move faster on thin layer chromatography than the molecule that is not blocked in the 3'- position. The only way the isolated compound would move that fast in ether on chromatography is if the anhydro-linkage is no longer present.

At this stage it was decided that a blocking group could not be put on 4-thioanhydrouridine in the 5'-position and keep the molecule together. In fact D. Lin (11) has found in attempting to silylate (11) 4-thio-anhydrouridine for work in mass spectroscopy, that even under the mild silylating conditions the 4-thioanhydro-uridine moiety falls apart.

Realizing that a blocking group can not easily be placed on the 5'-position of 4-thioanhydrouridine, another approach was attempted. The intention was to put an acid labile blocking group (trityl) on the 5'-position of 2,2'-anhydrouridine and a base labile blocking group (acetyl) on the 3'-position. This molecule 5'-0-trityl-3'-0-acetyl-2,2'-anhydrouridine was to be thiated. After the thiation the acetyl group was to be removed without breaking the anhydrolinkage to yield hopefully, 5'-0-trityl-2,2'-anhydro-4-thiouridine.

Therefore, 5'-O-trityl-3'-O-acetyl-2,2'-anhydro-uridine was made (14) and thiated by treatment with phosphorus pentasulphide in refluxing pyridine. This reaction was worked up on silica gel plates. A yellow band moving on silica gel plates as the product would be expected to move was isolated, but found to be impure on thin layer chromatography. After several more purification attempts on silica gel chromatography, thin layers still showed the compound to be chromatographically impure. Attempts to purify the compound using column chromatography failed. Several attempts to crystallize the product from different solvents also had no success. When the methoxytrityl group was substituted for the trityl group as a blocking agent for the 5'-position, the same results were obtained, ie. no purification possible.

Therefore, the reaction was worked up on paper chromatography using Solvent L. The desired yellow product moving very close to the solvent front was eluted with ethanol. Thin layer, however, still showed the material to be impure. This material, isolated from paper chromatography, was deacetylated and without any purification was treated under the usual phosphorylation conditions. The ultraviolet

spectrum of the deacetylated material before treatment under the phosphorylation conditions contained a strong thione absorption at 330nm. The two phosphorylation conditions the material was treated under were:

(a) β-cyanoethylphosphate, dicyclohexylcarbodiimide and dry pyridine.
(b) mesitylene sulphonyl chloride and dry pyridine.

At the end of the usual reaction times, the pyridine was removed under reduced pressure, the residue dissolved in ethanol and an ultraviolet spectrum taken. The ultraviolet spectrum of both tests (a) and (b) had no longer a thione absorption present. At this point it became necessary to find out if 4-thioanhydrouridine type molecules were stable to the known phosphorylation conditions, for in this instance the molecule was obviously not stable to these conditions.

The 4-thioanhydro compound that had been found to be the most stable in the research up to this point was 3',5'-di-o-acetyl-4-thioanhydrouridine, the only thioanhydro compound that could be isolated on silica gel chromatography reasonably pure. If this compound is stable, phosphorylation conditions should have no effect on this molecule, for there is no position open for phosphorylation (ie) all the hydroxyls on the sugar are blocked. This compound was treated with

four different phosphorylating and condensing systems. These systems are listed below:

(a) Mesitylene sulphonyl chloride and dry pyridine (20).

(b) Triisopropyl benzene sulphonyl chloride and dry pyridine (20).

(c) β-cyanoethylphosphate, dicyclohexylcarbodiimide and dry pyridine (8).

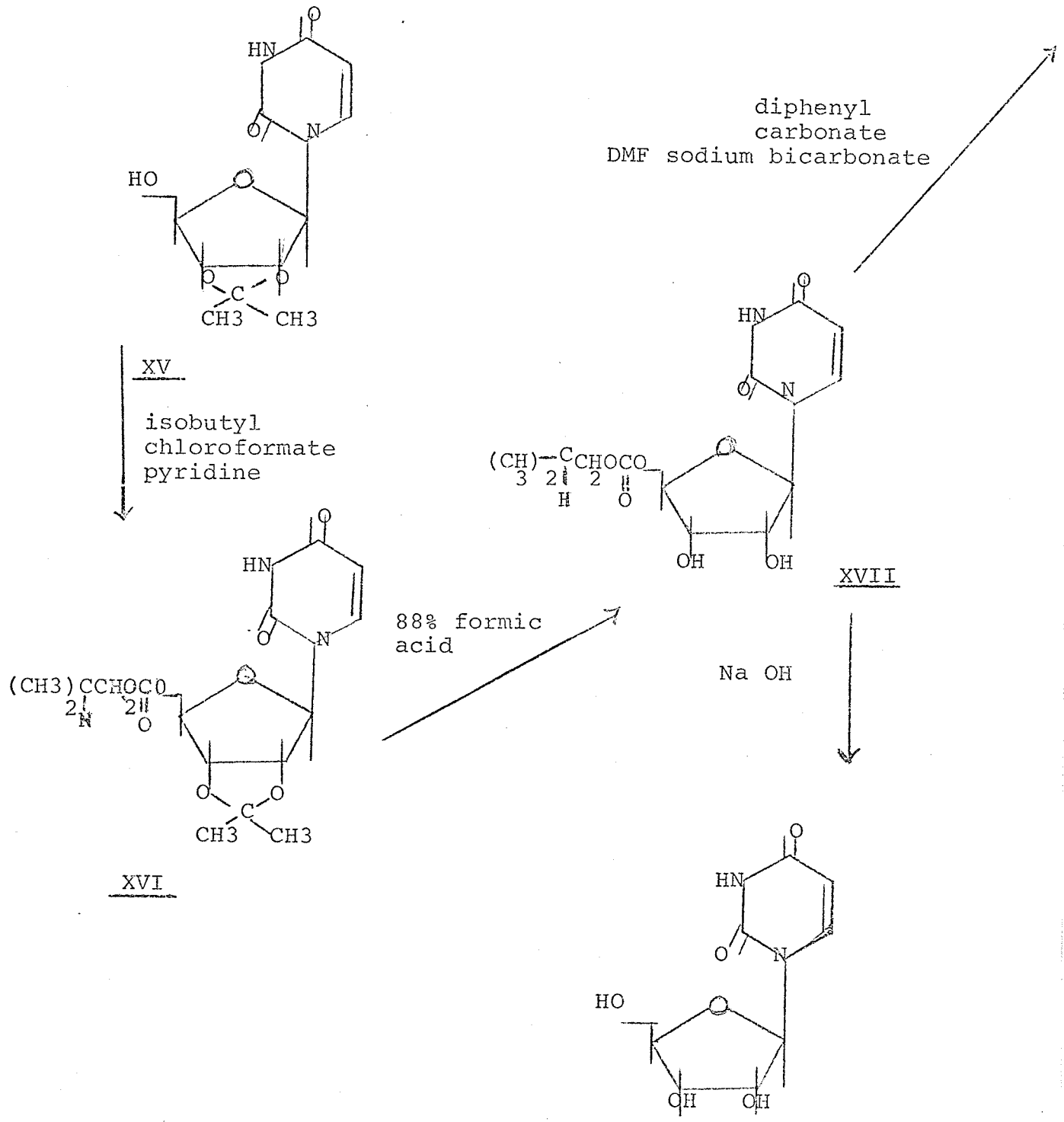
(d) Phosphoric acid methyl ester dichloride and dry pyridine (21).

In all cases after the usual reaction times, the dry pyridine was removed under reduced pressure, and an ultraviolet spectrum was taken of the residue dissolved in ethanol. The ultraviolet spectrum now showed no thione absorption at 330nm in cases (a), (b) and (c). The ultraviolet spectrum of case (d) still showed a thione absorption at 330nm, but thin layer showed two new compounds as well as starting material to be present in the residue.

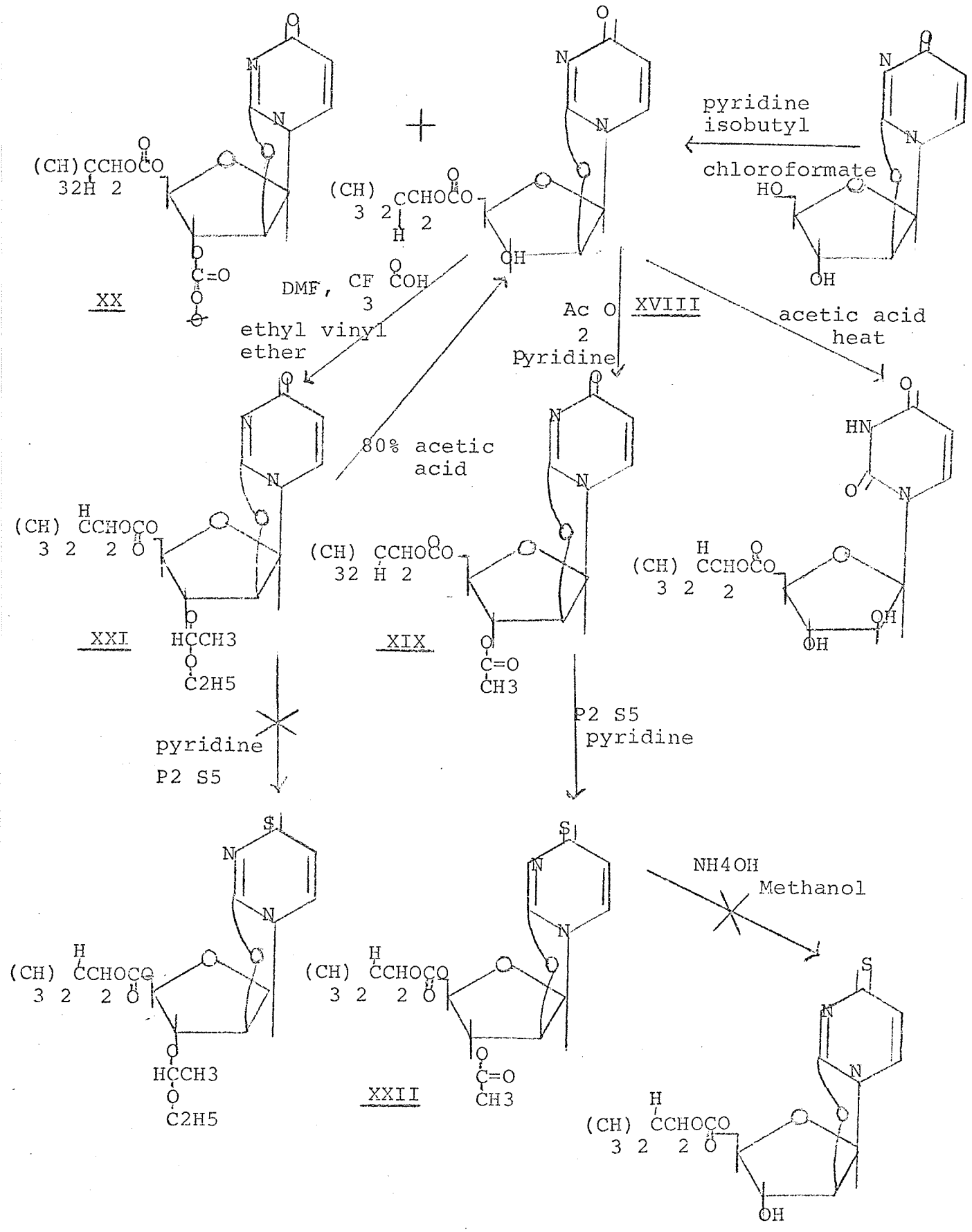
It was also noticed that 4-thioanhydro type compounds were found to be decomposing on storage. Often one of the decomposition products found to be formed had an oxygen instead of the sulphur on the 4-position of the pyrimidine ring. It was thought that the sulphur was being displaced in air, so an experiment was devised to see if this was true. 3', 5'-Di-o-acetyl-4-thioanhydrouridine was prepared and found to be 95% pure by relative intensity on thin layer. For nineteen days, half of this material was stored under a nitrogen atmosphere, and the other half stored in air. After this period of time thin layers were run on the separately stored materials and compared. The thin layers of the separately stored materials were found to be identical. In both cases two new compounds were found to have formed, that were not present in the original starting material. After nineteen days of storage the starting material was determined to be 80% pure by relative intensity of the spots on the thin layer.

It still was necessary, at this time, to produce a pure 5'-blocked 4-thioanhydro compound for treatment with the phosphoric acid methyl ester dichloride to see if a dinucleotide could be formed. The group that was chosen to block the 5'-position was the isobutyl-oxycarbonyl and the sequence developed is outlined below.

APPROACH IV


HO
HN
O
N
O
O
C
CH3
CH3
XV
isobutyl
chloroformate
pyridine
(CH3)2CHCO
NH
O
XVI
88% formic
acid
diphenyl
carbonate
DMF sodium bicarbonate
(CH3)2CHCO
H
O
OH
OH
XVII
Na OH
HO
HN
O
N
OH
OH

APPROACH IV -continued



Isopropylideneuridine (22) (compound XV) was treated with pyridine and isobutylchloroformate to yield isobutyl 2',3'-isopropylideneuridine 5'-carbonate (compound XVI) in a 75% yield. Treatment of this compound with formic acid to remove the isopropylidene group yielded isobutyl uridine 5'-carbonate (compound XVII). This compound could be converted to uridine by treatment with sodium hydroxide and dioxane (19).

The isobutyl uridine 5'-carbonate was treated with diphenyl carbonate, sodium bicarbonate and dimethylformamide (2) to yield two products in poor yield. The first product isolated was isobutyl 2,2'-anhydrouridine 5'-carbonate-3'-phenyl carbonate and the second, the desired product (compound XVIII) isobutyl 2,2'-anhydrouridine 5'-carbonate. This compound, also could be obtained in good yields by treating anhydrouridine with pyridine and isobutyl chloroformate.

At the compound XVIII stage, two approaches were attempted for the blocking of the 3'-position for thiation. Method (a) used a base labile blocking group and method (b) used an acid labile blocking group.

(a) The base labile acetyl group was used to block the 3'-position. It was hoped that it could be removed after thiation by mild basic conditions without affecting the isobutyl carbonate group in the 5'-position which is also base labile.

Compound XIX, isobutyl 3'-acetyl-2,2'-anhydro-uridine 5'-carbonate was obtained in 80% yield by treating compound XVIII with acetic anhydride and pyridine. This compound was treated with phosphorus pentasulphide in refluxing pyridine to thiate and by the isolation procedure used, the compound was estimated to be 95% pure by thin layer chromatography. Attempts to purify the compound completely on silica gel chromatography failed. On silica gel chromatography the product was found to be decomposing to starting material (compound XIX). Attempts to crystallize the material from various solvents also failed. Therefore, the compound was used without further purification for the next reaction, the deacetylation step.

It was found however, after thiation that one is not able to deacetylate the molecule without also removing the isobutyl carbonate from the 5'-position.

(b) Therefore, it was decided to use an acid labile blocking group for the 3'-position.The blocking group chosen was the 1-ethoxyethyl group (23).

Compound XVIII was treated with dimethylformamide, ethyl vinyl ether and trifloroacetic acid to obtain in a yield of 75% isobutyl 3'-O-(1-ethoxyethyl)-2,2'-anhydrouridine 5'-carbonate (compound XXI). It was found that treatment with acetic acid could remove the 1-ethoxyethyl group to yield compound XVlll without breaking the anhydrolinkage. This is a necessary requirement for

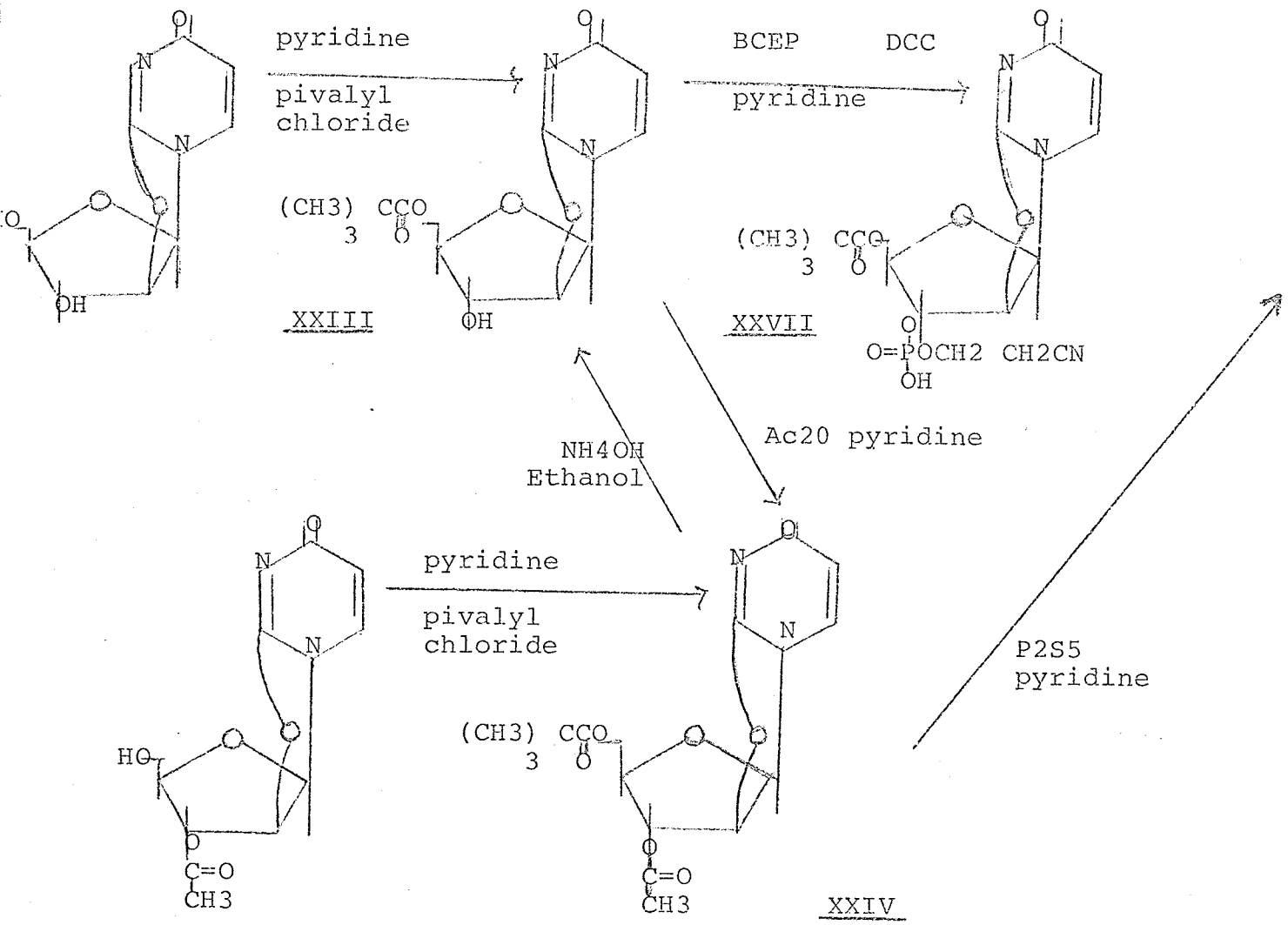
a 3'-hydroxyl blocking group.

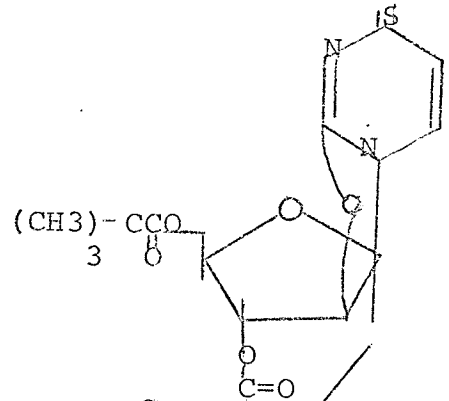
The next step involved thiating this blocked nucleoside. A series of thiating conditions were tried on this molecule and all were unsuccessful. After each thiation attempt, thin layers showed no material moving faster than starting material (compounds with the sulphur on the 4-position on the pyrimidine ring move approximately 0.3 R_f units faster on thin layer than a compound with an oxygen on the 4-position). It was still possible that thiation had occured, but the blocking group on the 3'-position had fallen off making the compound move slower on thin layer chromatography.To check this possibility the reaction was worked up on silica gel plates. No thione containing material in a yield greater than 8% could be isolated. Attempts to crystallize a product out of this reaction also failed. It was decided that the 1-ethoxy-ethyl blocking group was not stable to thiation conditions.

It was realised at this time that no success at all had been obtained in isolating a blocked 4-thioanhydro compound pure, when one of the blocking groups present was acid labile. Therefore, it was decided to work with only base labile blocking groups from this time forth.

The procedure decided on was to block the 5'-position with a very stable base labile blocking group such as pivalyl (18) (2,2-dimethylpropanoyl). The 3'-position was to be blocked with an acetyl group which has been shown to be removable after a thiation. The scheme developed is outlined below.

APPROACH V (a)



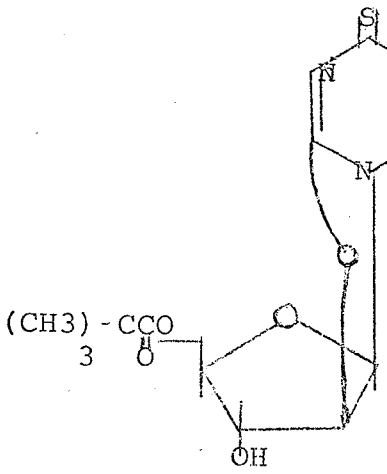


XXV

NH4OH
methanol

pyridine

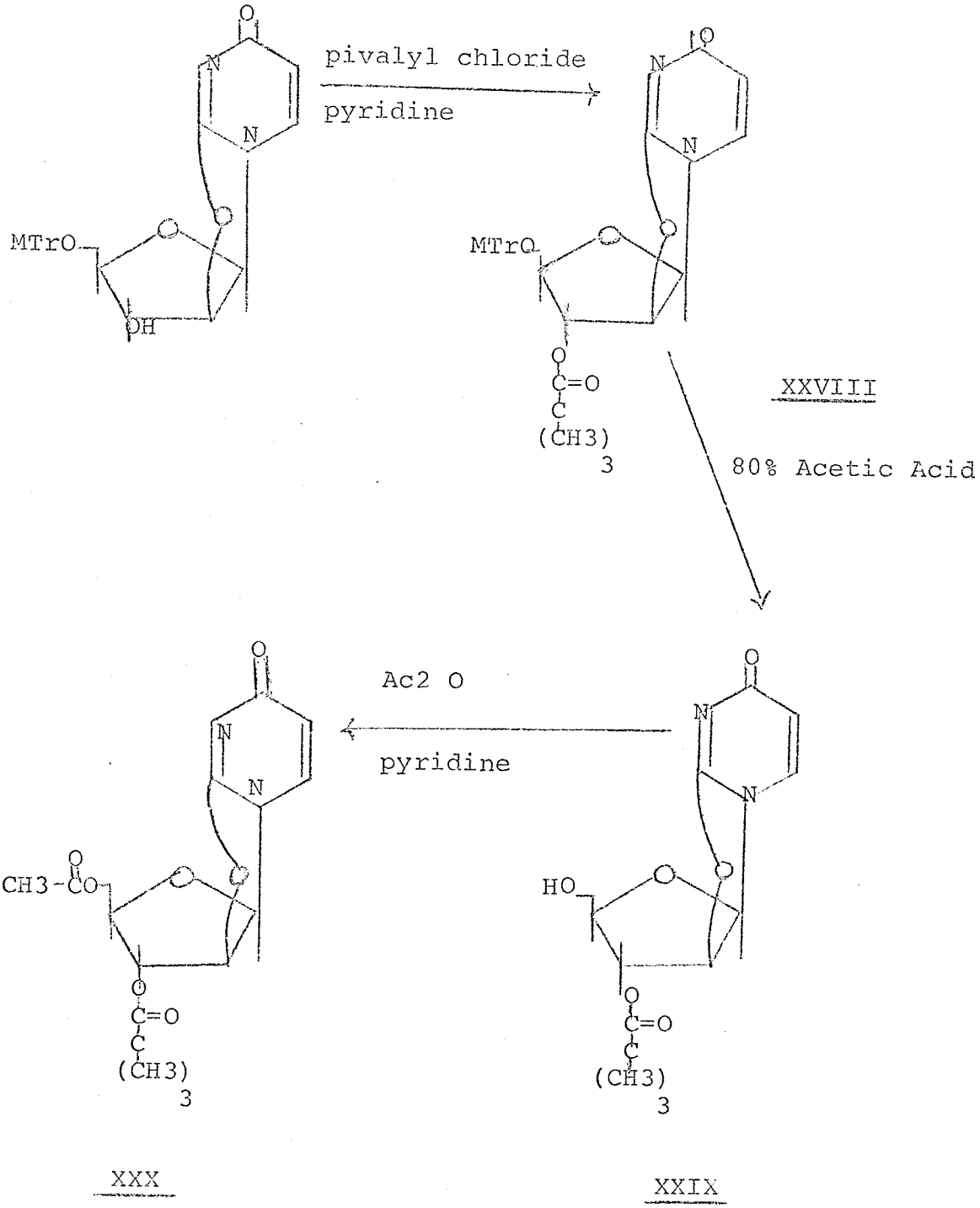
phosphoric acid methyl ester
dichloride
isopropylidene uridine



XXVI

No thione containing dinucleotide.

APPROACH V (b)


pivalyl chloride
pyridine
MTrO
OH
MTrO
O
C=O
C
(CH3)
3
XXVIII
80% Acetic Acid
Ac2 O
pyridine
CH3-CO
HO
XXX
XXIX

In attempting to synthesize 5'-O-pivalyl-2,2'-anhydrouridine (compound XXIII), anhydrouridine was treated with one to one ratio of pivalyl chloride using pyridine as a solvent. The reaction was worked up on silica gel plates using tetrahydrofuran as a solvent in one case and solvent B' in another case. In both of these work up procedures it was found that only two bands could be seen on the plates moving faster than starting material but at an R_f value where the product would approximately be expected to appear. The faster moving band was identified as 3',5'-di-O-pivalyl-2,2'-anhydrouridine. The material in the slower band was isolated and a melting range for this material of 136°C - 146°C was found. This material was then crystallized from hot ethanol to yield a compound that melted from 210°C - 212°C. The slower moving band on the silica gel plates obviously contains a mixture of 5'-O-pivalyl-2,2'-anhydrouridine and 3'-O-pivalyl-2,2'-anhydrouridine and one of these materials has crystallized out pure from the ethanol.

It now became necessary to synthesize both the 5' and 3'-pivalyl anhydrouridines unambiguously to determine which one the crystallized product was.

3'-O-Pivalyl-2,2'-anhydrouridine was obtained Approach V (b) by treating 5'-O-methoxytrityl -2,2'-anhydrouridine (14) with pivalyl chloride and pyridine to yield 5'-O-methoxytrityl - 3'-O-pivalyl-2,2'-anhydrouridine (compound XXVIII). This compound was detritylated using 80% glacial acetic acid to yield 3'-O-pivalyl -2,2'-anhydrouridine with a melting point of 240°C - 242°C. A mixed melting point with this material and the crystallized product brought about a melting point depression of 65°C to 175°C-180°C.

5'-O-Pivalyl-2,2'-anhydrouridine was obtained unambiguously (Approach V (a) by treating 3'-O-acetyl-2,2'-anhydrouridine (14) with pivalyl chloride and pyridine to yield 5'-O-pivalyl-3'-O-acetyl-2,2'-anhydrouridine (compound XXIV). This compound was deacetylated with ammonium hydroxide and ethanol to yield the desired product. 5'-O-Pivalyl-2,2'-anhydrouridine has the same melting point as the crystallized material and a mixed melting point showed no melting point depression. Therefore, the crystallized material was proved to be 5'-O-pivalyl-2,2'-anhydrouridine.

This compound can be converted to compound XXIV, 5'-O-pivalyl-3'-O-acetyl-2,2'-anhydrouridine by treatment with acetic anhydride and pyridine.

Compound XXlV was thiated using phosphorus pentasulphide and refluxing pyridine to yield 5'-o-pivalyl -3'-o-acetyl-4-thioanhydrouridine (compound XXV). This molecule was isolated and found to be impure by thin layer chromatography run in tetrahydrofuran.

However, if the thin layer slide was spotted with the product and the slide left in air overnight, then if the slide was run in tetrahydrofuran, different results were obtained. There were now three compounds present on the slide, one that was the desired product, one that moves identical to starting material (compound XXlV) and a faster moving unidentified compound. Just leaving the product on silica gel at room temperature, in air, causes the material to decompose rapidly.

It was found that on storing this material at 25°C in air for two days the compound partially decomposed. Therefore, the 5'-o-pivalyl-3'-o-acetyl-4-thioanhydrouridine was thought to be reasonably stable in air and only decomposing rapidly in conjunction with silica gel. Attempts to further purify the slightly impure product on silica gel plates and by crystallization failed. So the compound was used for the next step, the deacetylation without further purification.

Deacetylation was carried out using ammonium hydroxide and methanol. If 5'-O-pivalyl-3'-O-acetyl-2,2'-anhydrouridine is deacetylated, the reaction goes in a 95% yield. However, in deacetylating 5'-O-pivalyl-3'-O-acetyl -4-thioanhydrouridine under the same conditions, the yield drops to 25% with many other side products being formed. The 5'-O-pivalyl-4-thioanhydrouridine was isolated using silica gel chromatography with ethyl acetate as solvent. The compound was indicated to be reasonably pure by thin layer chromatography and mass spectroscopy.

The next step involved phosphorylation of the 3'-position. This material was treated under two different sets of phosphorylation conditions. Since it was shown that 5'-O-pivalyl-2,2'-anhydrouridine could be phosphorylated using dicyclohexylcarbodiimide, β - cyanoethylphosphate and pyridine, these same conditions were tried on the 5'-O-pivalyl-4-thioanhydrouridine. After the usual reaction time, the pyridine was removed under reduced pressure and an ultraviolet spectrum taken of the residue. No thione peak could be detected in the ultraviolet spectrum. The desired reaction did not go, but the reaction was worked up in the usual procedure to see what products could be isolated. One main compound was isolated from this reaction. This compound had a strange ultraviolet spectrum in water with maxima at 274nm and 220nm and a minima at 249nm.

This compound had R_f values of 0.55 in ethanol and 0.00 in tetrahydrofuran. The infra red spectrum showed no carbonyl (pivalyl) to be present. The mass spectrum of this compound had a parent peak at 242. However, in attempting to purify further this compound in Solvent A a new compound was obtained. This compound had an ultraviolet spectrum in water showing a shoulder at 265nm and a maximum at 249nm and a parent peak of 193 in the mass spectrum. Since the compounds have such low molecular weights and there is no pivalyl group present on the molecule, these compounds were thought not to be nucleosides and no further identification was carried out.

Instead an attempt was made to phosphorylate the 5'-O-pivalyl-4-thioanhydrouridine using the phosphoric acid methyl ester dichloride and condensing the product to isopropylideneuridine. This is the only phosphorylating system that did not completely decompose the blocked nucleoside, 3', 5'-di-O-acetyl-4-thioanhydrouridine in the phosphorylation tests. After the usual reaction time the pyridine was removed under reduced pressure and the residue was treated with 0.5 N sodium hydroxide for 0.5 hours to remove the pivalyl group, to remove the methyl group of the phosphate ester and to break the anhydro-linkage. The products were isolated by paper chromatography in Solvent A. The ultraviolet spectrum of all the compounds isolated showed no thione containing material to be present.

It has therefore been conclusively shown that 4-thioanhydrouridine type molecules are unstable to the three main types of phosphorylating conditions available at this time, ie (a) β - cyanoethylphosphate, dicyclohexylcarbodiimide and pyridine.

(b) mesitylene sulphonyl chloride, β -cyanoethylphosphate and pyridine.

(c) phosphoric acid methyl ester dichloride and pyridine.

These results were not to be expected. As was mentioned in the introduction, 4-thiouridines have been phosphorylated using the standard procedure of β - cyanoethylphosphate and dicyclohexylcarbodiimide (7,8). However, difficulties occured using other phosphorylating procedures on 4-thiouridines (8), where the mercapto group was found to be sensitive to acid hydrolysis or hydrogenolysis.

It would appear that the 2,2'-anhydro ring structure increases the lability of the sulphur in the 4-position under standard phosphorylation conditions.

Thus we have not been able to obtain a phosphorylated 4-thioanhydrouridine molecule by the phosphorylation of 4-thioanhydrouridines.

EXPERIMENTAL

General Methods

Thin layer chromatography was carried out by the ascending technique on Eastman Chromatogram Silica Gel Sheets 6060, containing fluorescent indicator, and cut to 10cm.X 2cm. Thick layer chromatography was carried out on glass plates (20cm.X 20cm.) coated with a 1mm. thick layer of silica gel DSF-5 (Mondray Chemicals Ltd.) Descending paper chromatograms were run on Whatman 3 MM papers using solvents which are listed below. The solvents were prepared on a volume basis.

Solvent A — isopropyl alchohol - concentrated ammonium hydroxide - water (7-1-2)

Solvent C — 1M ammonium acetate - ethanol (3-7)

Solvent L — ethanol - water (7-3)

Solvent E — 0.5M ammonium acetate - ethanol (3-7) adjusted to pH 3.5 with acetic acid.

Solvent B' — butanol - ethanol - water (4-1-5)- The organic layer used as eluting solvent while the bottom of the chromatography tank is covered, with the aqueous layer. Column chromatography was run using Fisher 60-200 mesh silica gel. Nucleosides and their derivatives were detected on paper chromatograms and on thick and thin layer chromatography using an ultraviolet light source (Mineralite - output $\sim$ 254nm.)

Trityl and monomethoxytrityl containing compounds were detected by means of a 10% perchloric acid spray in conjunction with warm air passing over the separating media. Developement of a yellow colour is indicative of a trityl group's presence while developement of an orange yellow colour is indicative of a monomethoxytrityl containing compound.

Infra-red spectrum were taken on a Perkin Elmer-337 instrument using KBr discs for sample preparation. Ultra-violet spectra were taken on a Cary 14. Mass spectra were run on a Hitachi RMU - 6D single focusing mass spectrometer. Melting points were determined on a Fisher-Johns melting point apparatus and are reported uncorrected.

Reagents

Reagent grade pyridine was distilled from p-toluene-sulphonyl chloride, redistilled from calcium hydride and stored over Linde Molecular Sieves. Reagent grade acetic anhydride was distilled from phthalic anhydride and stored in the dark.

Approach 1

2', 3',5'-tri-O-acetyluridine

Uridine (1.5g) was dissolved in dry pyridine (15ml)

and acetic anhydride (10ml) and stirred at room temperature in a closed flask for 16 hours. After this time the volume of the solution was concentrated to a thick syrup by evaporation of ethanol at reduced pressure. Thin layers in ethyl acetate at this time showed two compounds to be present in the reaction mixture having R_f values of 0.53 and 0.34. These two spots seen on chromatography were assumed to be tri-O-acetyluridine and a mixture of various di-O-acetyluridines. A water saturated sodium bicarbonate solution (50 ml) was added to the thick syrupy residue. The new solution formed was then extracted with two 50ml portions of chloroform. Thin layers in ethyl acetate showed that the di-O-acetyluridines had remained in the water solution but that the tri-O-acetyluridine had been extracted into the chloroform. The chloroform was then removed under reduced pressure and the residue dissolved in a small volume of tetrahydrofuran. Precipitation was caused by treatment of the tetrahydrofuran with hexane. The precipitated material was isolated as a gum. This gummy material was redissolved and reprecipitated from tetrahydrofuran three times. A type of gummy solid mixture was obtained which was placed in ether and left at 0°C for 24 hours. After this period of time the remaining gum was found to have turned solid. The solid

product was crushed with mortar and pestle and found to have been obtained in a yield of 61% (1.38g). The compound had a melting point of 129°C while the literature value (15) for the melting point of this compound is given as 128°C-130°C. The ultraviolet spectrum in 95% ethanol showed a maxima at 258nm and a minima at 228nm. The infra-red showed principal peaks at 1750 cm^{-1} , 1725 cm^{-1} and 1685 cm^{-1}.

2',3',5'-Tri-O-acetyl-4-thiouridine

Phosphorus pentasulphide (2g) was added to a solution of tri-O-acetyluridine (1.3g) dissolved in dry pyridine (25ml) and the resulting mixture was refluxed for a period of 0.3 hours. Water (0.3ml) was then added to the solution and reflux was then carried out for a further 3.5 hours. On cooling the solution a yellow material precipitated out and was filtered off. This precipitate was extracted with pyridine and this pyridine added to the original solution. The solution was concentrated in volume to a residue by evaporation of ethanol at reduced pressure. The residue was treated with water (200ml) and the precipitate that was formed was collected. The precipitate was extracted with chloroform and the chloroform was reduced in volume in vacuo and then applied to silica gel plates. The silica

gel plates were developed in ether and the yellow band found at R_f 0.4 was eluted with ethyl acetate. The ethyl acetate was reduced to a small volume in vacuo and the product was obtained in 71% (.96g) yield by treatment of the ethyl acetate with hexane to cause precipitation. The melting point of the compound was 57°C - 63°C. The ultraviolet spectrum in 95% ethanol showed maxima at 328nm and 248nm and minima at 275nm and 222nm. The infrared spectrum showed no hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1750 cm^{-1}, 1725 cm^{-1} and 1625 cm^{-1}.

4-thiouridine

Tri-O-acetyl-4-thiouridine (500mg) was dissolved in a 15% ammonium hydroxide-methanol solution (50ml) and was stirred at room temperature in a closed container for 16 hours. The solution was then reduced to a small volume in vacuo and applied to silica gel plates. The plates were developed in ether, then ethyl acetate and the yellow band at R_f 0.2 (ethyl acetate) was eluted with tetrahydrofuran. The tetrahydrofuran was reduced to a small volume in vacuo and the product obtained by treatment of the tetrahydrofuran with hexane to cause precipitation. A gum was obtained which was dissolved in water and freeze dried to yield a solid product in 83% (280mg) yield. The compound had a melting point of 139°C while

the literature value (16) for the melting point of this compound is given as 139°C - 140°C. The ultraviolet spectrum in 95% ethanol showed maxima at 332nm and 248 nm and minima at 278nm and 227nm. (Lit. (16) λmax. 330nm 246nm, λmin. 275nm 222nm in methanol). The infra-red spectrum showed a large hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and principal peaks at 1700 cm^{-1} and 1625 cm^{-1}.

Attempted synthesis of 2,2'-anhydro-4-thiouridine from 4-thiouridine

Diphenyl carbonate (0.51g) and sodium bicarbonate (0.01g) were added to a solution of 4-thiouridine (0.47g) dissolved in dimethylformamide (1ml). The resulting mixture was heated at 150°C for 0.5 hours, cooled, and then poured into ether (200ml). The precipitate that formed was collected and dissolved in 95% ethanol. An ultraviolet spectrum of this solution showed no absorption from 350nm to 300nm ie (no thione peak).

APPROACH II

5'-O-trityluridine

This compound with melting point 114°C - 117°C was obtained by Khorana's procedure (17).

2',3'-Di-O-acetyl-5'-O-trityluridine

This compound with melting point 100°C was obtained by Levene's procedure (1).

2',3'-Di-O-acetyl-5'-O-trityl-4-thiouridine

Phosphorus pentasulphide (550mg) was added to a solution containing 2',3'-di-O-acetyl-5'-O-trityluridine (356mg) dissolved in dry pyridine (5ml) and the resulting mixture was refluxed for 3 hours. After refluxing, the pyridine was removed by evaporation of ethanol at reduced pressure. The residue was treated with water saturated with sodium chloride (150ml) and the precipitate that formed was collected and extracted with chloroform. The chloroform was dried with sodium sulphate for 0.5 hours and then reduced to a small volume in vacuo. The residual chloroform was applied to silica gel plates which were developed in hexane and then ether. The yellow band found at R_f 0.75 (ether) was eluted with tetrahydrofuran. The tetrahydrofuran was reduced to a small volume in vacuo and the product obtained in a 60% (218mg) yield by treatment of the tetrahydrofuran with hexane to cause precipitation. The melting point of this compound was found to be 126°C while the melting point given in the literature (8) for this compound is given as 126°C - 128°C.

The ultraviolet spectra in 95% ethanol showed maxima at 329nm and 242nm and a minima at 278nm. (Lit. (8) λmax. 329 nm, 242nm, in ethanol) The infra-red spectra shows prominent peaks at 1750 cm^{-1} 1700 cm^{-1} and 700 cm^{-1} .

5'-O-trityl-4-thiouridine

2',3'-Di-O-acetyl-5'-O-trityl-4-thiouiridne (120mg) was dissolved in a 15% ammonium hydroxide -methanol solution (20ml) and stirred for 2.5 hours in a closed container at 25°C. The solution was then evaporated in vacuo and the residue dissolved in ethyl acetate (2ml). The product was obtained in a 90% (78mg) yield by treating the ethyl acetate with hexane to cause precipitation. The ultra-violet spectra in 95% ethanol showed maxima at 325nm (thione) and 225nm and a minima at 279nm. The infra-red spectrum showed no carbonyl peak (acetyl) at 1750 cm^{-1} but showed prominent peaks at 1700 cm^{-1} , 1625 cm^{-1} and 700 cm^{-1}(trityl).

Attempted synthesis of 5'-O-trityl-2,2'-anhydro-4-thiouridine from 5'-O-trityl-4-thiouridine

Diphenyl carbonate (0.40g) and sodium bicarbonate (7mg) were added to a solution of 5'-O-trityl-4-thiouridine (0.75g) dissolved in dimethylformamide (0.7ml).

The resulting mixture was heated at 150°C for 0.5 hours, cooled, and then poured into ether (200ml). The precipitate that formed was collected and dissolved in 95% ethanol. An ultraviolet spectra of this solution showed no absorption from 350nm to 300nm ie (no thione peak) proving the reaction had failed.

APPROACH III

Attempted synthesis of 4-thioanhydrouridine from anhydrouridine

Anhydrouridine (215mg) was added to a clear refluxing solution of phosphorus pentasulphide (500mg) in dry pyridine (10ml). The solution was refluxed for 0.5 hours and then reduced in volume using ethanol in vacuo. The residue was applied to silica gel plates which were developed in ether, then tetrahydrofuran and finally ethanol. Nine bands were isolated and none were found to be in a yield of greater than 10%.

3',5'-Di-O-acetyl-2,2'-anhydrouridine

Acetic anhydride (4.5ml) was added to a solution of anhydrouridine (0.78g) in dry pyridine (7.5ml) and this new solution was stirred at room temperature, in the dark, in a closed container for 16 hours. The solution was then reduced to a volume of 2ml by evaporation of ethanol at reduced pressure. These 2ml were treated with cold ether

(250ml) and vigourous stirring. After 5 minutes a fluffy precipitate emerged, was collected and found to be the desired product in 90% (.96g) yield. The product was recrystallized from hot ethanol and found to have a melting point of 180°C - 183°C. The ultraviolet spectra in water showed maxima at 250nm and 223nm and showed minima at 235nm and 211nm. The mass spectra of the compound showed a parent peak at 310. The infra-red spectrum showed no hydroxyl absorption from 3500cm^{-1} to 3000 cm^{-1} and had prominent peaks at 1740 cm^{-1} and 1625 cm^{-1} .

2,2'-anhydrouridine from 3',5'-di-O-acetyl-2,2'-anhydrouridine

3',5'-Di-O-acetyl-2,2'-anhydrouridine (5mg) was dissolved in a 15% ammonium hydroxide-methanol solution (2.5ml) and stirred at room temperature in a closed container for 2.5 hours. Thin layers run in ethanol and tetrahydrofuran and papers run in solvents A at the end of this time, showed complete conversion to anhydrouridine.

3',5'-Di-O-acetyl-2,2'-anhydro-4-thiouridine

3',5'-Di-O-acetyl-2,2'-anhydrouridine (300mg) was added to a clear refluxing solution of phosphorus penta-sulphide (700mg) in dry pyridine (10ml) and this new solution was refluxed for a further 10 minutes. The solution was then reduced in volume to a thick syrup by evaporation

of ethanol at reduced pressure. This residual syrup was treated with an ice water mixture (200ml) and the precipitate that formed was filtered as quickly as possible. The water during filtration was kept at approximately pH7 by addition of sodium bicarbonate. The filtered precipitate was then extracted with chloroform (100ml) and this chloroform solution was then dried over sodium sulphate for 0.5 hours. The chloroform was then removed by evaporation under reduced pressure and the residual few milliliters were applied to silica gel thick layer plates. The plates were developed first in ether, then in ethyl acetate. The yellow band on the plates at R_f 0.4 (ethyl acetate) was eluted with tetrahydrofuran. The tetrahydrofuran was reduced to a few milliliters in volume in vacuo and the product was obtained in a yield of 73% (230mg) by treatment of the tetrahydrofuran with hexane to cause precipitation. This compound had a melting point of 160°C - 163°C. The mass spectra of this compound showed a parent peak of 326. The ultraviolet spectrum in 95% ethanol showed maxima at 332nm and 241nm and minima at 255nm and 223nm. The infra-red spectrum showed no hydroxyl absorption at 3500 cm^{-1} to 3000 cm^{-1} and showed prominent peaks at 1740 cm^{-1}, 1725 cm^{-1} and 1630 cm^{-1}.

2,2'-anhydro-4-thiouridine

3',5'-Di-O-acetyl-2,2'-anhydro-4-thiouridine (700mg) was dissolved in a 15% ammonium hydroxide-methanol solution (15ml) and this new solution was stirred in a closed container at room temperature for 2.5 hours. At the conclusion of this time the volume of the solution was reduced to approximately 4 milliliters by evaporation under reduced pressure and the product left to crystallize out at 0°C. Crystallization of the product occured in a period of 16 hours at 0°C in a 83% (420mg) yield. (If the reaction is worked up on silica gel plates developed in tetrahydrofuran and ethanol, no product containing a thione peak in the ultraviolet can be isolated). A sintering point range was obtained for this compound of 150°C - 170°C while the sintering point range given in the literature (4) is 226°C -251°C. The product had a parent peak in the mass spectra of 242. The infra-red spectrum showed a large hydroxyl absorption from 3500 cm^{-1} to 3000 cm-1 and had a prominent peak at 1640 cm^{-1} . The ultraviolet spectrum obtained in water showed maxima at 322nm, 254nm and 217nm and a minima at 268nm and 237nm.
(Lit. (4) λmax. 327nm, 266nm, 242nm λmin. 275nm, 250nm, 226nm, pH 1-7).

This product was further identified by the following conversion to: 4-thioarabinouridine

(a) 4-thioanhydrouridine (5mg) was dissolved in 3ml of 80% glacial acetic acid and this solution was heated on a steam bath for 3 hours.

(b) 4-thioanhydrouridine (5mg) was dissolved in 3ml of concentrated ammonium hydroxide and this solution was heated on a steam bath for 1 hour.

In both cases (a) and (b), the same product was formed which had an ultraviolet spectrum taken in water showing maxima at 332nm and 260nm and a minima at 280nm.

Arabinouridine

(a) This compound was obtained (3) by treating 2,2'-anhydrouridine with glacial acetic acid on a steam bath for 3 hours. This compound had an ultraviolet spectrum taken in water which showed a maxima at 262nm and a minima at 235nm. This compound had R_f values of 0.56 in Solvent A and 0.75 in Solvent L.

(b) 15 µl of 30% hydrogen peroxide were added to a solution of 4-thioarabinouridine (2mg) dissolved in pH8 tris buffer (2ml). It was found that complete conversion of 4-thioarabinouridine to arabinouridine occured at room temperature in 0.5 hours.

(c) 15 µl of 30% hydrogen peroxide were added to a solution containing 4-thioanhydrouridine (2mg) dissolved in pH8 tris buffer (3ml). This solution was left to stand for 0.5 hours at room temperature. The product formed from this reaction had an ultraviolet spectrum in water which showed a shoulder at 255nm and a minima at 250nm. On paper chromatography the product moves slightly slower than 2,2'-anhydrouridine. The R_f values of the product are (0.43) in solvent A and (0.64) in solvent L. The R_f values of 2,2'-anhydrouridine are in solvent (A) (0.50) and in solvent L (0.69). Without further identification the product was treated with concentrated ammonium hydroxide on a steam bath for 1 hour, to yield quantitatively arabinouridine.

Attempted synthesis of 5'-O-methoxytrityl-2,2'-anhydro -4-thiouridine from 2,2'-anhydro-4-thiouridine

4-thioanhydrouridine (176mg) was added to a solution containing paramethoxytrityl chloride (417mg) dissolved in dry pyridine (5ml) and this solution was stirred in a closed container at room temperature for 4 days. At the conclusion of this time the pyridine was removed by evaporation of ethanol at reduced pressure and the residue applied to silica gel plates. The plates were developed

in ether, then ethyl acetate and finally tetrahydrofuran. The band found at R_f 0.8 (ethyl acetate) was eluted with tetrahydrofuran which was then reduced to a small volume in vacuo. A product (40mg) was obtained by treatment of the tetrahydrofuran with hexane to cause precipitation. This product has a mass spectra parent peak of 524 and gives a positive perchloric acid test for methoxytrityl groups. The ultraviolet spectrum of this unknown compound in 95% ethanol shows maxima at 355nm and 312nm and minima at 340nm and 292nm. This compound was not further identified but was proven not to be the desired product by it's ultraviolet spectra and it's mass spectra.

Attempted synthesis of isobutyl 2,2'-anhydro-4-thiouridine 5'-carbonate from 2,2'-anhydro-4-thiouridine

Isobutyl chloroformate (180 µl) was added to a solution containing 4-thioanhydrouridine (329mg) dissolved in dry pyridine (8ml) and this new solution was stirred in a closed container at room temperature for 16 hours. At the conclusion of this time the pyridine was removed by evaporation of ethanol at reduced pressure. The residual syrup was applied to silica gel plates which were developed in hexane and then in ether. After development in ether three yellow bands were seen, the slowest

moving expected to be the product. This band was eluted with ether and the ether was then removed in vacuo. The isolated material was found to be decomposing and neither the original material or the decomposition product was the desired product (discussion and results).

5'-O-trityl-3'-O-acetyl-2,2'-anhydrouridine

This compound was obtained by the procedure developed by D. Iwacha (14).

5'-O-trityl-3'-O-acetyl-2,2'-anhydro-4-thiouridine

To a refluxing solution of phosphorus pentasulphide (500mg) in dry pyridine was added 5'-O-trityl-3'-O-acetyl-2,2'-anhydrouridine (300mg) and this new solution was refluxed for a further 10 minutes. At the conclusion of this time the pyridine was removed by evaporation of ethanol at reduced pressure and the residue was treated with water (200ml) saturated with sodium chloride. The precipitate that formed was collected and extracted with chloroform (50ml). The chloroform solution was then dried under sodium sulphate for 0.5 hours. At this stage three procedures were attempted to isolate a pure product.

(a) The chloroform was applied to silica gel plates which were developed in ether, then ethyl acetate. A yellow band found at R_f0.4 (ethyl acetate) was eluted with ethyl acetate and thin layers were run on the eluant. Thin layers showed the compound to be impure.

(b) The reaction was worked up on column chromatography with the column being run in ether and ethyl acetate. Once again, a pure product could not be isolated as determined by thin layer chromatography using ethyl acetate and tetrahydrofuran as solvents.

(c) The reaction was worked up on paper chromatography using Solvent L. The yellow band found at the solvent front was eluted with ethanol which was then reduced to a small volume in vacuo. A product was obtained in 98% yield by treating the small volume of ethanol with water (100ml) to cause precipitation. Thin layer however, showed the isolated material to be impure. The ultraviolet spectrum of this material in 95% ethanol showed maxima at 330nm, 270nm and 230nm and minima at 275nm and 248nm. The infrared was not sharp but showed prominent peaks at 1750 cm^{-1}, 1625 cm^{-1} and 700 cm^{-1}. Attempts to crystallize out a pure product from ethyl acetate, ethanol, benzene, ether and chloroform failed. So the product was used without further purification for the deacetylation step.

5'-O-Trityl-2,2'-anhydro-4-thiouridine

A solution of 5'-O-trityl-3'-O-acetyl-2,2'-anhydro-4-thiouridine (250mg) dissolved in a 15% ammonium hydroxide-methanol solution (30ml) was stirred in a closed container at room temperature for 2 hours. At the conclusion of this time the methanol was reduced to a small volume in vacuo. A product was isolated by treatment of the methanol with water (100ml) to cause precipitation. The product isolated was impure on thin layer chromatography. The material had an ultraviolet spectrum in 95% ethanol that showed maxima at 326nm, 270nm and 229nm and minima at 278nm and 248nm. The infra-red spectrum showed a hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1625 cm^{-1} and 700 cm^{-1} . This material was used without further purification for the phosphorylation tests.

Phosphorylation tests on 5'-O-trityl-2,2'-anhydro-4-thiouridine

(a) 5'-O-trityl-2,2'-anhydro-4-thiouridine (35mg), 0.25ml of β-cyanoethylphosphate (0.5mmole/ml), dicyclohexylcarbodiimide (1.0g) and dry pyridine (0.5ml) were stirred together at room temperature in the absence of water for 3 days. At the conclusion of this time the pyridine was removed in vacuo and an ultraviolet spectrum was taken of the residue dissolved in 95% ethanol.

There was no longer a thione absorption at 330nm present in the ultraviolet spectra.

(b) 5'-O-trityl-2,2'-anhydro-4-thiouridine (2mg), mesitylene sulphonyl chloride (4mg) and dry pyridine (0.2ml) were stirred at room temperature in the absence of water for 6 hours. At the conclusion of this time the pyridine was removed in vacuo and an ultraviolet spectrum was taken of the residue dissolved in 95% ethanol. There was no longer a thione absorption at 330nm present in the ultraviolet spectra.

Phosphorylation tests on 3',5'-di-O-acetyl-2,2'-anhydro-4-thiouridine

(a) 3',5'-Di-O-acetyl-2,2'-anhydro-4-thiouridine (2mg), mesitylene sulphonyl chloride (4mg) and dry pyridine (0.2ml) were stirred at room temperature in the absence of water for 6 hours.

(b) 3',5'-Di-O-acetyl-2,2'-anhydro-4-thiouridine (2mg), triisopropyl benzene sulphonyl chloride (4mg) and dry pyridine (0.2ml) were stirred at room temperature in the absence of water for 6 hours.

(c) 3',5'-Di-O-acetyl-2,2'-anhydro-4-thiouridine (65mg), 1.6ml of β-cyanoethylphosphate (0.5mmole/ml), dicyclohexylcarbodiimide (650mg) and dry pyridine (1ml) were stirred together in the absence of water at room temperature for 3 days.

(d) 3',5'-Di-O-acetyl-2,2'-anhydro-4-thiouridine (36mg), 0.02ml of phosphoric acid methyl ester dichloride and dry pyridine (1ml) were stirred together in the absence of water for 17.5 hours.

At the conclusion of the given times in the above cases the pyridine was removed in vacuo and an ultraviolet spectrum was taken of the residue dissolved in 95% ethanol. The ultraviolet spectrum of cases (a), (b) and (c) no longer showed a thione absorption at 330nm. Case (d) still showed a thione absorption at 330nm in the ultraviolet.

APPROACH IV

Isobutyl 2',3'-isopropylidineuridine 5'-carbonate

Isobutyl chloroformate (140 µl) was added to a solution of isopropylideneuridine (22) (281 mg) in dry pyridine (5ml) and this new solution was stirred at

room temperature in a closed container for 16 hours. The solution was then concentrated to a small volume by evaporation with ethanol at reduced pressure and applied to silica gel plates. The plates were developed in hexane, then ether and the material found at R_f 0.58 (ether) was eluted with ethyl acetate. The product (melting point 130°C) was obtained in a 75% (255mg) yield by treatment of the ethyl acetate (concentrated) with hexane to cause precipitation. Isopropylidineuridine (30mg of starting material) was also recovered from the silica gel plates. The ultraviolet spectrum of the product in 95% ethanol showed a maxima at 259nm and a minima at 230nm. The infrared spectrum showed no hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1770 cm^{-1}, 1710 cm^{-1} and 1685 cm^{-1}. The mass spectra showed a parent peak of 384.

Isobutyl uridine 5'-carbonate

Isobutyl 2',3'-isopropylidineuridine 5'-carbonate (255mg) was dissolved in 88% formic acid (20ml) and the solution was stirred at room temperature for 4 hours. The solution was then concentrated by evaporation with ethanol at reduced pressure and applied to silica gel plates. The plates were developed in ether, then ethyl acetate and the band found at R_f 0.33 (ethyl acetate) was eluted with tetrahydrofuran. The product (melting point 115°C) was obtained in 93% (211mg) yield by treatment of the tetrahydrofuran (concentrated) with hexane to cause precipitation. The ultraviolet

spectrum in 95% ethanol showed a maxima at 261nm and a minima at 230nm. The infra-red spectrum showed a hydroxyl absorption at 3500 cm^{-1} - 3000 cm^{-1} and prominent peaks at 1770 cm^{-1}, 1700 cm^{-1} and 1685 cm^{-1}. The mass spectra showed a parent peak of 344.

Uridine

Isobutyl uridine 5'-carbonate (2mg) was added to a solution of 1M sodium hydroxide (0.3ml) and dioxane (0.7ml) and the resulting solution stirred at room temperature for 0.5 hours. Thin layer chromatography showed complete conversion to uridine.

Isobutyl 2,2'-anhydrouridine 5'-carbonate and isobutyl 2,2'-anhydrouridine 5'-carbonate -3'-phenyl carbonate

To a solution of isobutyl uridine 5'-carbonate (200mg) in dimethylformamide (0.4ml) was added diphenyl carbonate (214mg) and sodium bicarbonate (1mg). The resulting mixture was heated at 150°C for 15 minutes, cooled, and applied to silica gel plates. The plates were developed in ether, ethyl acetate and then tetrahydrofuran. The band at R_f 0.48 (tetrahydrofuran) was eluted with tetrahydrofuran and the product, isobutyl 2,2'-anhydrouridine 5'-carbonate-3'-phenyl carbonate (melting point 204°C - 208°C) was obtained in 22% (57mg) yield by treatment of the tetrahydrofuran (concentra-

ted) with hexane to cause precipitation. The ultraviolet spectrum in 95% ethanol showed maxima at 248nm and 223nm and a minima at 238nm. The infra-red spectrum showed prominent peaks at 1770 cm^{-1} , 1750 cm^{-1} and 1630 cm^{-1} . The mass spectra showed a parent peak of 446.

The band at R_f 0.38 (tetrahydrofuran) was eluted with ethanol and the product, isobutyl 2,2'-anhydrouridine 5'-carbonate (melting point 122°C - 127°C) was isolated in 11.5% (22mg) yield by evaporating off the ethanol and precipitating the product from tetrahydrofuran with hexane. The ultraviolet spectrum in 95% ethanol showed maxima at 248nm and 223nm and a minima at 238nm. The infra-red spectrum showed a hydroxyl absorption from 3500 cm^{-1} - 3000 cm^{-1} and prominent peaks at 1750 cm^{-1} and 1650 cm^{-1} . The mass spectra showed a parent peak of 326.

Isobutyl arabinouridine 5'-carbonate

Isobutyl 2,2'-anhydrouridine 5'-carbonate (2mg) was heated on a steam bath in 80% glacial acetic acid (0.5ml) for 3 hours. Thin layer chromatography showed the product to move identical to isobutyl uridine 5'-carbonate.

Isobutyl 2,2'-anhydrouridine 5'-carbonate (Method II) from 2,2'-anhydrouridine

2,2'-anhydrouridine (218mg) was placed in dry pyridine

(25ml) and the pyridine was heated at 100°C until all the anhydrouridine had dissolved. The solution was then cooled to room temperature and isobutyl chloroformate (190 µl) was added. The resulting solution was stirred in a closed container at room temperature for 18 hours. The solution was then concentrated using ethanol and reduced pressure and applied to silica gel plates. The plates were developed in ether, ethyl acetate and then tetrahydrofuran. Three main bands were seen and the slowest moving (R_f 0.38 tetrahydrofuran) was eluted with ethanol. The product, isobutyl 2,2'-anhydrouridine 5'-carbonate was isolated in 76% (240mg) yield by evaporating the ethanol and precipitating the product from tetrahydrofuran using hexane. This compound was found to be identical in properties to the sample prepared previously.

This reaction did not work, however, if the isobutyl chloroformate was added to the pyridine without having the anhydrouridine dissolved. This reaction also did not work if mixtures of dimethylformamide and pyridine were used as solvent, instead of pure pyridine.

Isobutyl 3'-O-acetyl-2,2'-anhydrouridine 5'-carbonate

Isobutyl 2,2'-anhydrouridine 5'-carbonate (150mg) was dissolved in a solution of dry pyridine (3ml) and acetic anhydride (1ml) and the resulting solution was stirred in a closed container, at room temperature, in the absence of

light for 16 hours. The solution was then concentrated by evaporation with ethanol at reduced pressure, and applied to silica gel plates. The plates were developed in ether, ethyl acetate and then tetrahydrofuran. The band found at R_f 0.38 (tetrahydrofuran) was eluted with ethanol. The product, isobutyl 3'-O-acetyl-2,2'-anhydro-uridine 5'-carbonate (melting point 172°C - 175°C) was isolated in an 80% (135 mg) yield by evaporating off the ethanol and precipitating the product from tetrahydrofuran using hexane. The ultraviolet spectrum in 95% ethanol showed maxima at 248nm and 225nm and a minima at 241nm. The infra-red spectrum showed no hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1750 cm^{-1} and 1650 cm^{-1} . The mass spectra showed a parent peak of 368.

Isobutyl 3'-O-acetyl-2,2'-anhydro-4-thiouridine 5'-carbonate

Isobutyl 3'-O-acetyl-2,2'-anhydrouridine 5'-carbonate (126mg) was added to a clear refluxing solution of phosphorus pentasulphide (300mg) in dry pyridine (4ml) and this new solution was refluxed for a further 0.25 hours. The solution was then concentrated to a syrup by evaporation with ethanol at reduced pressure and this syrup was treated with cold water, saturated with sodium chloride (100ml). The yellow

precipitate that formed was collected and extracted with chloroform (50ml). The chloroform was then dried over sodium sulphate for 0.5 hours. The product was obtained in a 67% (88mg) yield by treatment of the chloroform (concentrated) with hexane to cause precipitation. Thin layer showed the product to be approximately 95% pure by relative intensity of spots. The ultraviolet spectrum in 95% ethanol showed maxima at 332nm and 242nm and minima at 256nm and 225nm. The infra-red showed no hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and showed prominent peaks at 1750 cm^{-1} and 1635 cm^{-1} . Attempts to purify the compound completely on silica gel chromatography using ether, ethyl acetate and tetrahydrofuran as solvents failed. Attempts to crystallize the compound from ethanol, ether, benzene and ethyl acetate also failed.

Attempted synthesis of isobutyl 2,2'-anhydro-4-thiouridine 5'-carbonate

Isobutyl 3'-O-acetyl-2,2'-anhydro-4-thiouridine 5'-carbonate (50mg) was dissolved in a 15% ammonium hydroxide -methanol solution (10ml) and this solution was stirred in a closed container at room temperature for 1.5 hours. Thin layer chromatography showed most of the material to be converted to 2,2'-anhydro-4-thiouridine.

Isobutyl 3'-O-(1-ethoxyethyl)-2,2'-anhydrouridine 5'-carbonate

Isobutyl 2,2'-anhydrouridine 5'-carbonate (326mg) was added to a solution of dry dimethylformamide (1.5ml) and ethyl vinyl ether (1.3ml). This new solution was cooled to -70°C and then treated dropwise with trifloro-acetic acid (0.5ml). The solution was then stirred at 0°C for 3 hours. Benzene (3ml) was then added and this new solution was extracted three times with 10ml portions of a water-sodium bicarbonate solution. The water solution was freeze dried and the residue extracted with chloroform. The product, isobutyl 3'-O-(1-ethoxyethyl)-2,2'-anhydro-uridine 5'-carbonate (melting point 110°C) was obtained in a 75% (300mg) yield by treatment of the chloroform (concentrated) with hexane to cause precipitation. The ultraviolet spectrum in 95% ethanol showed maxima at 248nm and 226nm and a minima at 238nm. The infra-red spectrum showed no hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1750 cm^{-1} and 1640 cm^{-1}. The mass spectrum showed a parent peak of 398.

Isobutyl 2,2'-anhydrouridine 5'-carbonate

Isobutyl 3'-O-(1-ethoxyethyl)-2,2'-anhydrouridine 5'-carbonate is converted to isobutyl 2,2'-anhydrouridine 5'-carbonate by treatment with 80% glacial acetic acid at room temperature for 1.5 hours.

Attempted synthesis of isobutyl 3'-O-(1-ethoxyethyl)-2,2'-anhydro-4-thiouridine 5'-carbonate

Various thiation attempts all unsuccessful, were tried on isobutyl 3'-O-(1-ethoxyethyl)-2,2'-anhydro-uridine 5'-carbonate using phosphorus pentasulphide in benzene or carbon disulphide for periods of time varying from 10 minutes to 60 minutes. When using pyridine as a solvent, the same results were obtained. The general procedure used is outlined below.

To a refluxing solution of phosphorus pentasulphide (250mg) in dry pyridine (4ml) was added isobutyl 3'-O-(1-ethoxyethyl)-2,2'-anhydrouridine 5'-carbonate (100mg) and this new solution was refluxed a further 0.25 hours. The solution was then concentrated to a small volume by evaporation with ethanol at reduced pressure. At this point two different procedures were tried to isolate a product.

(a) The reaction was worked up on silica gel plates developed in ether and ethyl acetate. No thione containing material could be found in a yield greater than 8%.

(b) Attempts to crystallize a product from benzene, ethyl acetate or ethanol failed.

Approach V

5'-O-methoxytrityl-3'-O-pivalyl-2,2'-anhydrouridine

Pivalyl chloride (150 µl) was added to a solution of

5'-O-methoxytrityl-2,2'-anhydrouridine (3) (75mg) in dry pyridine (1ml) and the resulting solution was stirred at room temperature in a closed container for 20 hours. The solution was then concentrated by evaporation with ethanol at reduced pressure and applied to silica gel plates. The plates were developed first in ethyl acetate, and then tetrahydrofuran and the band found at R_f 0.57 (tetrahydrofuran) was eluted with tetrahydrofuran. The product (melting point 94°C - 99°C) was obtained in a 75% (65mg) yield by treatment of the tetrahydrofuran (concentrated) with hexane to cause precipitation.The ultraviolet spectrum in 95% ethanol showed a maxima at 228nm and a minima at 223nm. The infra-red spectrum showed prominent peaks at 1740 cm^{-1} , 1640 cm-1 and 700 cm^{-1} . The mass spectra showed a parent peak of 578.

3'-O-pivalyl-2,2'-anhydrouridine

5'-O-methoxytrityl-3'-O-pivalyl-2,2'-anhydrouridine (60mg) was treated with 80% glacial acetic acid (3ml) at room temperature for 2.5 hours. The solution was then applied to silica gel plates which were developed first, in ethyl acetate and then in a 1:1 ratio of ethanol-tetrahydrofuran. The band at R_f 0.35 (tetrahydrofuran-ethanol) was eluted with ethanol and the product (melting point 240°C - 242°C) was obtained in an 80% (25mg) yield by evaporation of the ethanol and trituration of the residue with ether. The ultraviolet spectrum in 95% ethanol showed maxima at 246nm and 224nm and a minima at 236nm. The mass spectra

showed a parent peak of 310. The infra-red spectrum shows a hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1740 cm^{-1} and 1630 cm^{-1} .

3'-O-Pivalyl-5'-O-acetyl-2,2'-anhydrouridine

Acetic anhydride (0.5ml) was added to a solution of 3'-O-pivalyl-2,2'-anhydrouridine (83mg) in dry pyridine (1.5ml) and the resulting solution was stirred in a closed container at room temperature, in the absence of light for 20 hours. The solution was then concentrated by evaporation with ethanol at reduced pressure and the product (melting point 170°C - 173°C) was obtained in a 90% (85mg) yield by precipitation with ether. The ultraviolet spectrum in 95% ethanol showed a maxima at 248nm and 225nm and a minima at 236nm. The mass spectra showed a parent peak of 352. The infra-red spectra showed no hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1740 cm^{-1} and 1640 cm^{-1} .

5'-O-Pivalyl-3'-O-acetyl-2,2'-anhydrouridine

Method (a) Pivalyl chloride (0.4ml) was added to a solution of 3'-O-acetyl-2,2'-anhydrouridine (14) (300mg) in dry pyridine (20ml) and the resulting solution was stirred at room temperature in a closed container for 20 hours. The solution was then concentrated by evaporation

with ethanol at reduced pressure and applied to silica gel plates. The plates were developed in ethyl acetate and then tetrahydrofuran and the band found at R_f 0.39 (tetrahydrofuran) was eluted with tetrahydrofuran. The product (melting point 198°C - 202°C) was obtained in a 95% (320mg) yield by treatment of the tetrahydrofuran (concentrated) with hexane to cause precipitation. The ultraviolet spectrum in 95% ethanol showed maxima at 246 nm and 225nm and a minima at 236nm. The mass spectra showed a parent peak of 352. The infra-red spectrum showed no hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1740 cm^{-1} and 1630 cm^{-1} .

Method (b) Acetic anhydride (0.5ml) was added to a solution of 5'-O-pivalyl-2,2'-anhydrouridine (32mg) in dry Pyridine (1ml) and the resulting solution was stirred at room temperature, in a closed container, in the absence of light for 20 hours. The solution was then concentrated by evaporation with ethanol at reduced pressure and applied to silica gel plates. The product was isolated as in Method (a)in a 72% (26mg) yield. This compound had properties identical to the sample isolated by Method (a).

5'-O-pivalyl-2,2'-anhydrouridine

Method (a) 5'-O-pivalyl-3'-O-acetyl-2,2'-anhydrouridine (30mg) was dissolved in a 15% ammonium hydroxide-ethanol solution and this new solution was stirred at room temperature, in a closed container for 1.5 hours. The solution was then concentrated using reduced pressure an applied to silica gel plates. The plates were developed in ethyl acetate and then a 1:1 ratio of ethanol-tetrahydrofuran. The band found at R_f 0.35 (ethanol-tetrahydrofuran) was eluted with ethanol. The product (melting point 210°C - 215°C) was obtained in a 90% (24mg) yield by treatment of the ethanol (concentrated) with ether to cause precipitation. The mass spectra showed a parent peak of 310. The ultraviolet spectrum in 95% ethanol showed maxima at 248nm and 225nm and a minima at 239nm. The infra-red spectra showed a hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1740 cm^{-1} and 1625 cm^{-1}.

Method (b) 2,2'-anhydrouridine (400mg) was placed in dry pyridine (50ml) and the pyridine was heated at 100°C until all the anhydrouridine had dissolved. The solution was then cooled to room temperature and pivalyl chloride (400 µl) was added. The resulting solution was stirred in a closed container at room temperature for 20 hours.

The solution was then concentrated to a syrup by evaporation with ethanol at reduced pressure. The syrup was treated with a 1:1 chloroform-water mixture (100ml) and the two solvents were then separated. Thin layer chromatography showed the desired product to be present in the water layer. The water was removed by freeze drying and the residue was dissolved in hot ethanol. The desired product crystallized from the ethanol in a 50% (275mg) yield. A mixed melting point with this compound and the sample isolated by Method A showed no melting point depression.

3',5'-Di-O-pivalyl-2,2'-anhydrouridine (melting point 227°C - 231°C)

This product is a by-product of the above procedure and is obtained by applying the chloroform layer (concentrated) from the extraction of the syrup, to silica gel plates. The plates were developed in ethyl acetate and then tetrahydrofuran and the band found at R_f 0.47 (tetrahydrofuran) was eluted with tetrahydrofuran. The product was obtained by treatment of the tetrahydrofuran (concentrated) with hexane to cause precipitation. The ultraviolet spectrum in 95% ethanol showed maxima at 248nm and 226nm and a minima at 238nm. The infra-red spectra showed no hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1740 cm^{-1} 1625 cm^{-1} . The mass spectra showed a parent peak of 394.

5'-O-Pivalyl-2,2'-anhydrouridine-3' β-cyano-ethylphosphate

5'-O-Pivalyl-2,2'-anhydrouridine (12mg) was stirred at room temperature in a closed container for 4 days with 0.3ml of β-cyanoethylphosphate (0.5mmole/ml) and dicyclohexyl-carbodiimide (130mg). Water (0.3ml) was then added and the solution was stirred for a further 16 hours. The solution was filtered and the filtrate was applied to papers for chromatography. The papers were run in Solvent B'. The band at R_f0.25 was eluted with water and the product obtained by freeze drying the water. The ultraviolet spectrum in water showed maxima at 248nm and a minima at 238nm. The product moved similar to thymidylyl - (3'-5')-thymidine on electrophoresis (pH7.5). In infra-red spectra showed a hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1730 cm^{-1} and 1625 cm^{-1} .

5'-O-Pivalyl-3'-O-acetyl-2,2'-anhydro-4-thiouridine

5'-O-Pivalyl-3'-O-acetyl-2,2'-anhydrouridine (150mg) was added to a solution of phosphorus pentasulphide (200mg) in refluxing pyridine (1.5ml) and the resulting solution was refluxed for a further 10 minutes. The solution was then concentrated to a residue by evaporation with ethanol at reduced pressure. The residue was treated with a water saturated sodium chloride solution (100ml) and the yellow precipitate that formed was collected and extracted with chloroform (75ml). The chloroform was dried over sodium

sulphate for 0.25 hours. The product was obtained in a 80% (125mg) yield by treatment of the chloroform (concentrated) with hexane to cause precipitation. This compound could not be isolated pure on thick layer silica gel chromatography. The ultraviolet spectrum in 95% ethanol showed maxima at 330nm and 239nm and a minima at 256nm and 225nm. The mass spectra showed a parent peak of 368. The infra-red spectra showed prominent peaks at 1740 cm^{-1} ,1725 cm^{-1} and 1625 cm^{-1} .

5'-O-Pivalyl-2,2'-anhydro-4-thiouridine

5'-O-Pivalyl-3'-O-acetyl-2,2'-anhydro-4-thiouridine (100mg) was dissolved in a 15% ammonium hydroxide-methanol solution (25ml) and the resulting solution was stirred at room temperature in a closed container for 2.5 hours. The solution was the concentrated under reduced pressure and applied to silica gel plates. The plates were developed in only ethyl acetate and the yellow band found at R_f 0.2 (ethyl acetate) was eluted with ethanol. The ethanol was then removed under reduced pressure. The product was obtained in a 25% (23mg) yield by precipitating the product dissolved in chloroform with hexane. Thin layer chromatography showed the product to be reasonably pure. The mass spectra showed a parent peak of 326. The ultraviolet spectra in 95% ethanol showed maxima at 331nm and 238nm and minima

at 258nm and 225nm. The infra-red spectrum showed a hydroxyl absorption from 3500 cm^{-1} to 3000 cm^{-1} and prominent peaks at 1740 cm^{-1} and 1625 cm^{-1} .

Phosphorylation Attempts on 5'-O-pivalyl-2,2'-anhydro-4-thiouridine

(a) 5'-O-Pivalyl-2,2'-anhydro-4-thiouridine (122mg) β-cyanoethylphosphate (1.5mmole) and dicyclohexylcarbodiimide (1.24g) were stirred in dry pyridine (1ml) in a closed container for 4 days at room temperature. Water (0.5ml) was then added and the solution was stirred for a further 16 hours. The solution was then filtered and the filtrate applied to a silica gel plate. The plate was developed in ethanol. 122mg Of a product (Discussion and Results) was eluted from the plate with ethanol.

(b) 5'-O-Pivalyl-2,2'-anhydro-4-thiouridine (47mg) dissolved in dry pyridine (2ml) was added drop-wise with the exclusion of water to a solution of the phosphoric acid methyl ester dichloride (26µl) in dry pyridine (2.2ml). The resulting solution was stirred at room temperature in a closed container for 6 hours. Then isopropylidineuridine (30mg) dissolved in dry pyridine (1ml) was added to the solution and the resulting mixture was stirred for a further 17 hours. The pyridine was then removed under

reduced pressure and the residue treated with 0.5N sodium hydroxide for 0.5 hours. The solution was neutralized with dowex 50W-X8 (pyridinium) resin and applied to paper chromatography which was run in Solvent A. The bands on the paper were eluted with water and ultraviolet spectra were taken. None of the bands eluted contained a thione absorption at 330nm in the ultraviolet.

TABLE 1. Thin Layer Chromatographic Data - R_f values

APPROACH1,	SOLVENT			
Compound	Ethanol	Tetrahydrofuran	Ethyl Acetate	Ether
Uridine	0.60	0.55	0.03	0.00
2',3',5'-Tri-O-acetyluridine	0.82	0.80	0.53	0.09
2',3',5'-Tri-O-acetyl-4-thio-uridine	0.85	0.80	0.80	0.40
4-Thiouridine	0.75	0.80	0.20	0.00
APPROACH II,				
5'-O-Trityluridine	0.75	0.84	0.40	0.07
5'-O-Trityl-2',3'-di-O-acetyluridine	0.80	----	0.68	0.50
5'-O-Trityl-2',3'-di-O-acetyl-4-thio-uridine	----	----	0.75	0.75
5'-O-Trityl-4-thio-uridine	----	----	0.57	0.46

TABLE II. Thin Layer Chromatographic Data - R_f values Mass Spectra U.V.

APPROACH III.	SOLVENT				Parent Peak	
Compound	Ethanol	Tetrahydrofuran	Ethyl Acetate	Ether		
2,2'-Anhydro-uridine	0.55	0.05	0.02	0.00		
3'5'-Di-O-acetyl-2,2'-anhydrouridine	0.67	0.21	0.02	0.00	310	λmax.250,223 λmin.235,211 (H2O)
3',5'-Di-O-acetyl-2,2'-anhydro-4-thiouridine	0.88	0.83	0.37	0.00	326	λmax.332,241 λmin.255,233 (ETOH)
2,2'-anhydro-4-thiouridine	0.70	0.10	0.00	0.00	242	λmax.332,254 λmin.268,237 (H2O)
4-Thioarabino-uridine	----	0.80	0.20	----	----	λmax.332,260 λmin.280(H2O)
Arabino-uridine	0.60	0.55	0.02	0.00	----	λmax.262 λmin.235(H2O)
Unknown from 2,2'-anhydro-4-thiouridine with H2O2	0.55	0.01	0.00	----	----	λmax.255, λmin.250(H2O)

TABLE III. Thin Layer Chromatographic Data - R_f values Mass Spectra U.V.
Parent Peak

APPROACH IV.	SOLVENT					
Compound	Ethanol	Tetrahydrofuran	Ethyl Acetate	Ether		
2',3'-Isopropylidineuridine	0.83	0.72	0.27	0.14		
Isobutyl 2',3'-isopropylidine-uridine 5'-carbonate	----	----	----	0.58	384	λmax.259 λmin.230(EtOH)
Isobutyl uridine 5'-carbonate	----	0.64	0.33	0.07	344	λmax.261 λmin.230(EtOH)
Isobutyl 2,2'-anhydrouridine 5'-carbonate-3'-phenyl carbonate	----	0.48	0.04	0.00	446	λmax.248,223 λmin.238(EtOH)
Isobutyl 2,2'-anhydrouridine 5'-carbonate	0.65	0.38	0.00	0.00	326	λmax.248,223 λmin.238(EtOH)

APPROACH IV continued:

Compound	Ethanol	Tetrahydrofuran	Ethyl Acetate	Ether	Mass Spectra Parent Peak	U.V.
Isobutyl arabinouridine 5'-carbonate	----	0.64	0.33	----	----	λmax.258 λmin.230(EtOH)
Isobutyl 3'-O-acetyl-2,2'-anhydrouridine 5'-carbonate	0.69	0.38	0.07	----	368	λmax.248,225 λmin.241(EtOH)
Isobutyl 3'-O-acetyl-2,2'-anhydro-4-thiouridine 5'-carbonate	----	0.67	0.36	----	----	λmax.332,242 λmin.256,225 (EtOH)
Isobutyl 3'-O-(1-ethoxyethyl)-2,2'-anhydrouridine 5'-carbonate	----	0.62	0.23	0.00	398	λmax.248,226 λmin.238(EtOH)

TABLE IV. Thin Layer Chromatographic Data R_f values Mass Spectra Parent Peak U.V.

APPROACH V.	SOLVENT					
Compound	Ethanol	Tetrahydrofuran	Ethyl Acetate	Ether		
3'-O-acetyl-2,2'-anhydro-uridine	0.61	0.13	0.00	0.00		
5'-O-pivalyl-3'-O-acetyl-2,2'-anhydro-uridine	0.70	0.39	0.03	0.00	352	λmax.246,225 λmin.236(EtOH)
5'-O-pivalyl-2,2'-anhydro-uridine	0.60	0.23	0.00	0.00	310	λmax.248,225 λmin.239(EtOH)
3',5'-O-di-pivalyl-2,2'-anhydrouridine	0.70	0.47	0.02	0.00	394	λmax.248,226 λmin.238(EtOH)
5'-O-methoxy-trityl-3'-O-pivalyl-2,2'-anhydrouridine	0.67	0.57	0.07	0.00	578	λmax.228 λmin.223(EtOH)

APPROACH V continued:

Compound	Ethanol	Tetrahydrofuran	Ethyl Acetate	Ether	Mass Spectra Parent Peak	U.V.
3'-O-pivalyl-2,2'-anhydrouridine	0.60	0.23	0.00	0.00	310	λmax.246,224 λmin.236(EtOH)
3'-O-pivalyl-5'-O-acetyl-2,2'-anhydrouridine	0.70	0.39	0.05	0.00	352	λmax.248,225 λmin.236(EtOH)
5'-O-pivalyl-3'-O-acetyl-2,2'-anhydro-4-thiouridine	----	0.74	0.43	----	368	λmax.330,239 λmin.256,225 (EtOH)
5'-O-pivalyl-2,2'-anhydro-4-thiouridine	0.90	0.70	0.40	----	326	λmax.331,238 λmin.258,225 (EtOH)

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