UNIVERSITY OF MANITOBA

ALKYLSILYL BLOCKING GROUPS AND ANHYDRONUCLEOSIDES IN SYNTHETIC NUCLEOSIDE CHEMISTRY AND PHOTOCHEMISTRY

by

Elaine Adeline Thompson

A Thesis

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Department of Chemistry
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ELAINE ADELINE THOMPSON

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

To my husband, Ed.

ACKNOWLEDGEMENTS

It has been my privilege over the past four years to have been associated with a number of helpful persons and organizations. I would like, at this time, to acknowledge their contributions to this thesis.

My appreciation is extended

to the members of my Ph.D. Advisory Committee - Dr. K. K. Ogilvie, Dr. J. L. Charlton, Dr. D. M. McKinnon, Dr. G. E. Dunn and Dr. J. F. Templeton - for their guidance throughout the course of this study,

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to Marvin Arneson for his mass spectral analyses,

to Krishan Sadana for his gift of 2'3'-O-isopropylidine uridine, and,

to Joyce Nicholson for typing this manuscript.

I am grateful for their valued assistance.

ABSTRACT

The general purpose of this study was to investigate possible means of improving or simplifying nucleic acid synthesis.

One approach involved the use of alkylsilyl chlorides as specific and stable blocking groups which could be readily removed under neutral conditions without affecting any other functional groups. Six different alkylsilyl chlorides were prepared by the reaction of silyl chlorides with alkyl lithium reagents. Their reactions with thymidine were studied to determine their specificity. The stabilities of various alkylsilyl thymidine derivatives to acidic and basic conditions were tested. One long chain alkylsilyl group (t-butylmethyloctadecylsilyl) was found to be stable enough to be potentially useful as a lipophilic handle for nucleic acid synthesis. Two other groups (triisopropyl silyl and tetramethylene-t-butylsilyl) exhibited an appreciable specificity for the primary OH group of thymidine, possessed high stability to both acidic and basic conditions, and were readily removed by treatment with tetra-n-butylammonium fluoride in tetrahydrofuran.

Another approach involved a series of unsuccessful nucleophilic displacement and oxidation reactions on the sulfide linkage of 8,2'-thioanhydroadenosine.

The photochemical preparation of dihydroanhydrouridine, a possible precursor for dihydrouridine in RNA synthesis, was

accomplished by irradiation of O^2,2'-anhydrouridine in aqueous ethanol. Other photoproducts formed in the photoreaction included isomeric ethanol adducts which were identified by n.m.r. and mass spectrometry.

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ABBREVIATIONS

A	adenosine
Ac	acetyl
Ac_2AnU	3'5'-diacetyl anhydrouridine
Ac_2H_2AnU	3'5'-diacetyl dihydroanhydrouridine
Ac_2O	acetic anhydride
AcOH	acetic acid
$Ac_2$8,2'SAnA	3'5'diacetyl 8,2'-thioanhydroadenosine
An	anisoyl
AnU	anhydrouridine
B	base (pyrimidine or purine)
Bz	benzoyl
C	cytidine
β-CEP	β-cyanoethylphosphate
DCC	dicyclohexylcarbodiimide
DEAE	diethylaminoethyl
DMF	N,N-dimethylformamide
DNA	deoxyribonucleic acid
EtOAc	ethyl acetate
EtOH	ethyl alcohol
G	guanosine
GC	gas chromatography
I	inosine
iB	isobutyryl
iPrOH	isopropyl alcohol
i.r.	infrared
max	maximum
MDIPSi	methyldiisopropylsilyl
Me	methyl
m/e	mass to charge ratio
MMTr	monomethoxytrityl
MODIPSi	methyloctadecylisopropylsilyl
MS	mass spectrometry
MS	mesitylene sulfonyl chloride
Nu	nucleophile
p	phosphate
Rm	relative mobility on electrophoresis
RNA	ribonucleic acid
RT	room temperature
8,2'SAnA	8,2'-thioanhydroadenosine
T	thymidine
TBDMSi	t-butyldimethylsilyl
TBMODSi	t-butylmethyloctadecylsilyl
Th	thymine
THF	tetrahydrofuran
TLC or t.l.c.	thin layer chromatography
TMIPSi	tetramethyleneisopropylsilyl
TMTBSi	tetramethylene-t-butylsilyl
Tp	thymidine-3'-phosphate
TPS	triisopropylbenzenesulfonyl chloride

ABBREVIATIONS (continued)

$tRNA^{ala}$	alanine transfer ribonucleic acid
Tr	trityl (triphenylmethyl)
UV	ultraviolet

SYMBOLS

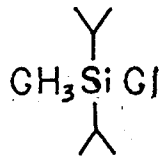
ϕ	phenyl
	isopropyl
	t-butyl
	thymidine
λ	wavelength

Nomenclature in Organosilicon Chemistry

According to an "Editorial Report on Nomenclature" in the Journal of the Chemical Society[95] when two or more different substituents are attached to silicon, the rule of alphabetical preferences applies in naming the compounds. An example of this, given by Eaborn[96] is the molecule $C_2H_5(CH_3)_2SiC_6H_5$-ethyldimethylphenylsilane. (This rule is not applied when it leads to ambiguity.)

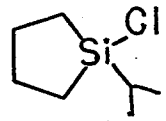
The radical H_3Si- is the silyl radical.[96]

The molecules prepared in this study were named in the following manner on the basis of the two preceding rules:



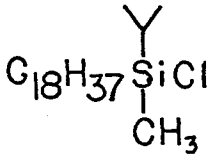
methyldiisopropylsilyl chloride

MDIPSiCl



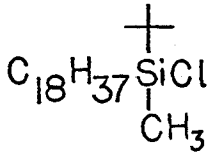
tetramethyleneisopropylsilyl chloride

TMIPSiCl



methyloctadecylisopropylsilyl chloride

MODIPSiCl

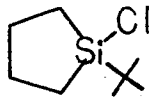


t-butylmethyloctadecylsilyl chloride

TBMODSiCl

Only one alkylsilyl chloride was not named according to these

rules -



tetramethylene-t-butylsilyl chloride

TMTBSiCl

There were two reasons for this divergence.

To name it t-butyltetramethylenesilyl chloride (according to the rules) would have given the molecule the initials TBTMSi, which, when spelled out in conversation, is easily confused with the TBDMSi group (t-butyldimethylsilyl) introduced by Corey[75] and used by Ogilvie in his work.[79,80]

Secondly, it was felt that since the tetramethylene structure is a distinctive feature of both the molecule under discussion and tetramethylene isopropylsilyl chloride, naming it first would point out this similarity between the two molecules. Attention can be brought more clearly to the fact that TMIPSiCl and TMTBSiCl are homologous molecules when the same order of naming is maintained in both molecules - "tetramethyleneisopropylsilyl" and "tetramethylene-t-butylsilyl" instead of "t-butyltetramethylenesilyl". Maintaining the same order clarifies any discussion comparing properties of the two groups as well.

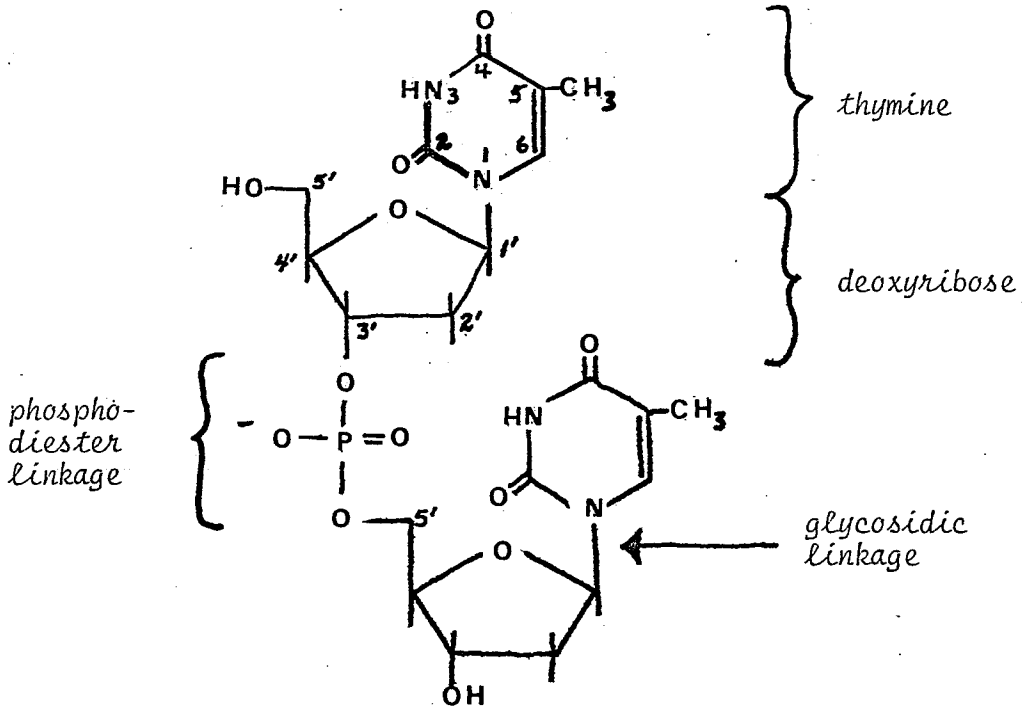
ALKYLSILYL BLOCKING GROUPS AND ANHYDRONUCLEOSIDES IN SYNTHETIC NUCLEOSIDE CHEMISTRY AND PHOTOCHEMISTRY

INTRODUCTION

Following elucidation of the nature of the internucleotidic bond (Figure I) in 1955[1], nucleic acid chemistry entered a new era in which synthesis and sequence determination of polynucleotides became the major thrusts of research. Most of the early work in the chemical synthesis was done by Khorana while he was in British Columbia and later at the University of Wisconsin.

FIGURE I

STRUCTURE OF THYMIDYLYL (3'→5') THYMIDINE (TpT)

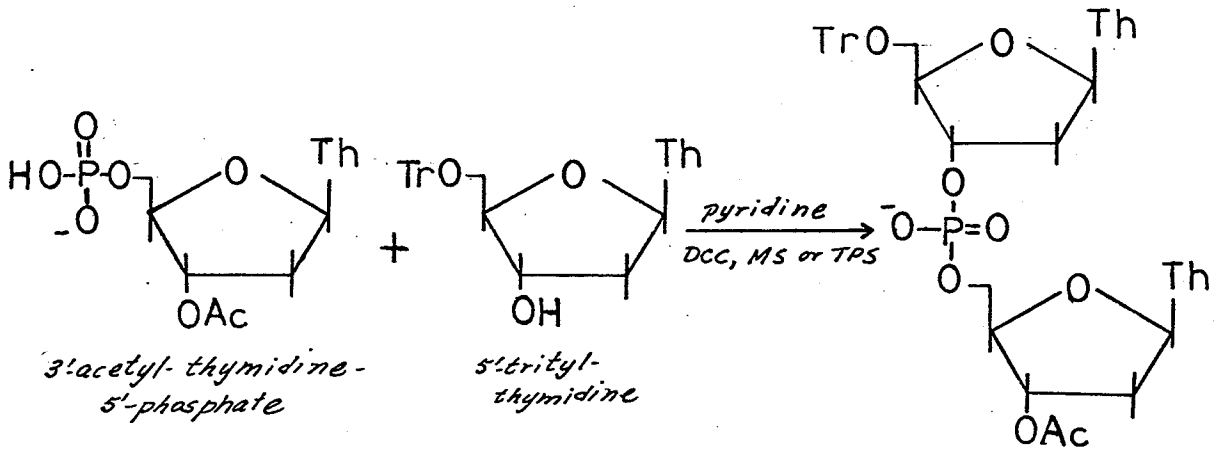


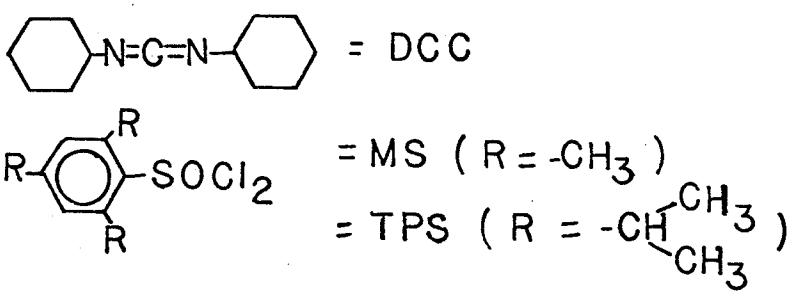
The Phosphodiester Method of Deoxyribonucleic Acid Synthesis

The approach taken by Khorana for deoxyribonucleic acid synthesis involved the joining of a 5'-trityl (or monomethoxytrityl) nucleoside to a 3'-acetyl nucleoside-5'-phosphate through a phosphodiester linkage. This was brought about in the presence of a condensing agent such as dicyclohexylcarbodiimide or an arylsulfonyl chloride[3,4] in anhydrous pyridine (Scheme 1). Subsequent removal of the 3'-acetyl group of the dinucleoside monophosphate left the molecule open for further extension through a condensation reaction with a second 3'-acetyl-5'-nucleotide.

SCHEME 1

DIESTER INTERNUCLEOTIDE BOND FORMATION





There are a number of problems associated with the phosphodiester approach to nucleic acid synthesis. Removal of the trityl group required acid conditions drastic enough to effect hydrolysis of the glycosidic linkage of purine nucleosides (i.e. depurination occurs).[23,24] This was largely overcome by switching to the more labile monomethoxytrityl group[25] and by adding some pyridine to the acetic acid solution used in the de-tritylation reactions.[30,34]

Secondly, condensations in the phosphodiester method required increasingly larger excesses of the incoming nucleotide as the oligonucleotide chain increased in length in order to maintain a reasonable yield at each condensation step.[26]

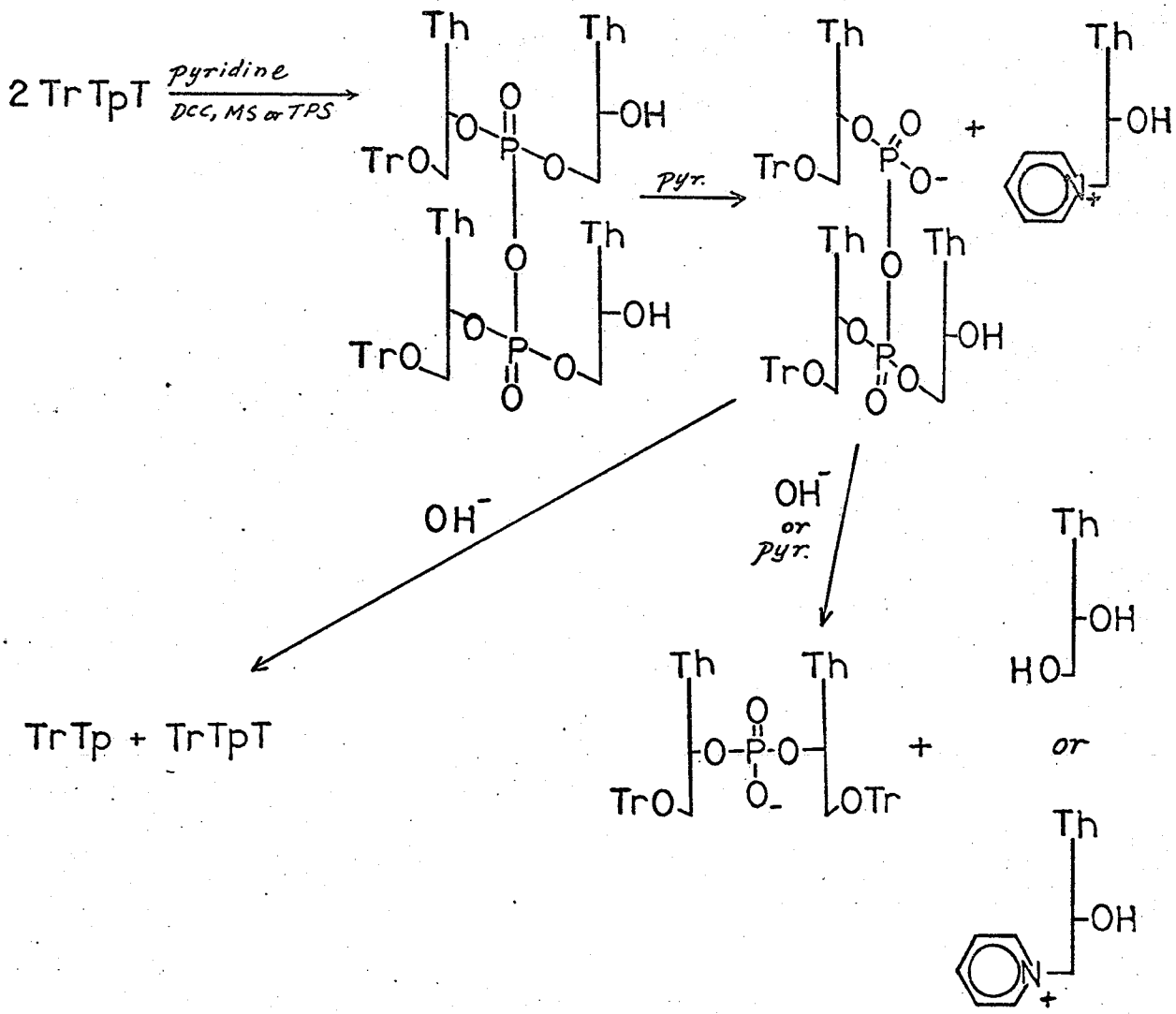
Furthermore, because each phosphodiester linkage is capable of being activated at each condensation step, side reactions tend to reduce yields of the products and of the reclaimed starting materials. For example, in a reaction between a thymidine dodecanucleotide and a thymidine tetranucleotide, the desired hexadecanucleotide was obtained in 20% yield, but only 23% of the unreacted starting material was recovered.[32] By varying the excess of nucleotidic material being added to the chain and by adjusting the amount of condensing agent used, Khorana was able to attain an optimal point where a high yield of condensation product was accompanied by minimal breakdown of polynucleotide chains.[29,33]

The observed cleavage of the internucleotide bond[31] and formation of unnatural C3'→C3' phosphodiester linkages[32] have

been postulated to arise from the formation of pyrophosphate linkages between phosphomonoesters and phosphodiesters[31] (Scheme 2) or by the formation of a neutral triester resulting from the reaction between the phosphodiester bond and a nucleoside or a nucleotide containing a free 3'-OH group.[32] (Scheme 3).

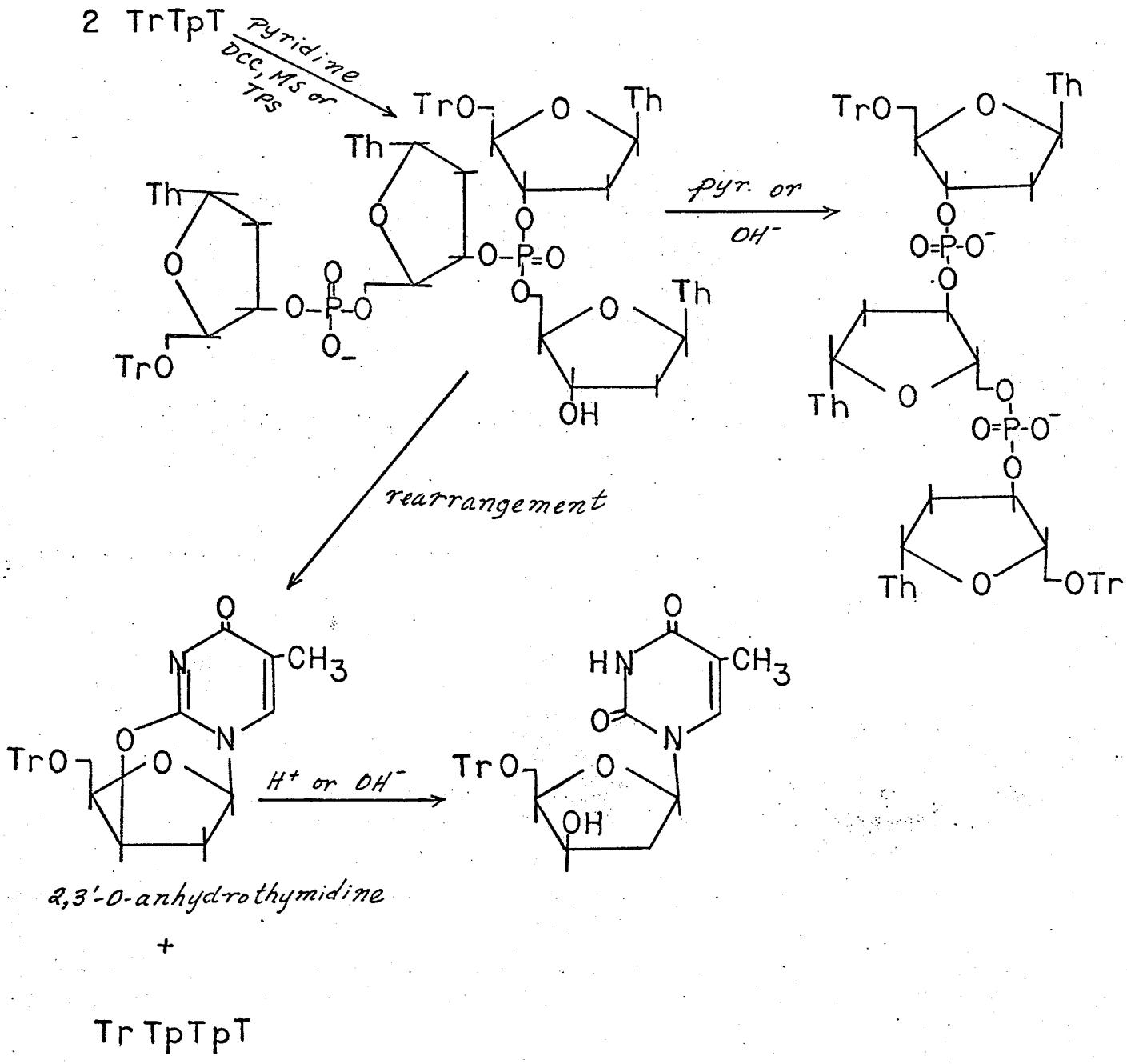
SCHEME 2

PYROPHOSPHATE FORMATION AND ITS POSSIBLE REACTIONS



SCHEME 3

NEUTRAL TRIESTER FORMATION AND ITS POSSIBLE REACTIONS



Another difficulty with the phosphodiester approach to DNA synthesis is the formidable task of separation and purification of the desired polynucleotide in the presence of so many possible side products. The system used was DEAE-cellulose anion exchange column chromatography, a low capacity technique which is extremely time consuming. DEAE-cellulose chromatography of the reaction mixture in the preparation of the dodecanucleotide d-MMTrG^{iB}pC^{An}pT(pC^{An})$_3$(pT)$_2$pA^{Bz}pG^{iB}pC^{An}pA^{Bz} required 125 hours (5 days) for completion.[29]

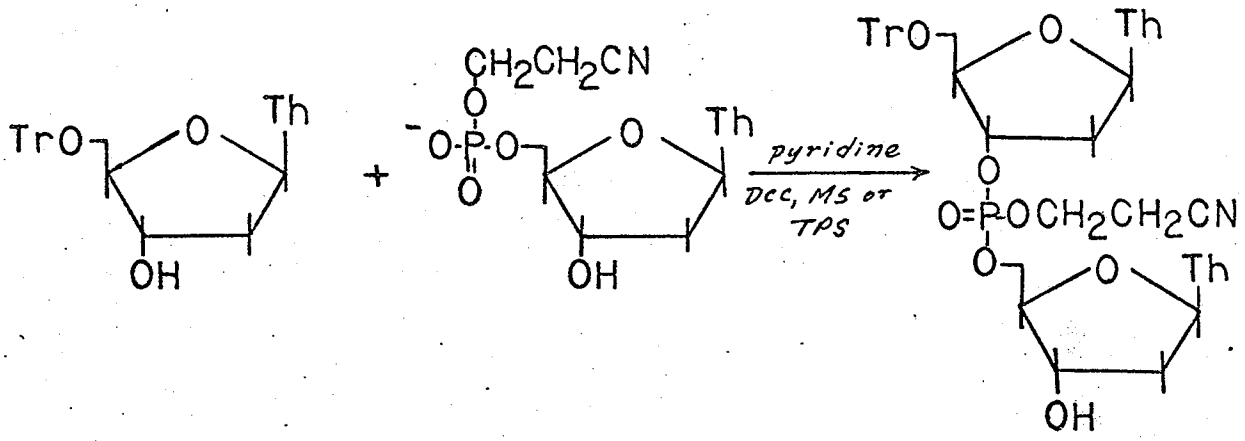
The Phosphotriester Approach to Deoxyribonucleic Acid Synthesis

The triester approach to DNA synthesis was developed as a means of avoiding many of the problems associated with the diester approach. This new method, developed concurrently by Letsinger[35] and Eckstein[19], involved the condensation of a nucleoside (or its 3'-acetyl derivative) with a 5'-trityl-nucleotide whose 3'-phosphate group was protected either by a β-cyanoethyl group[2,35] or a trichloroethyl group.[19] (See Schemes 4 and 5.)

The resulting phosphotriester is completely neutral and can be handled by silica gel chromatography, a technique with much higher capacity and flow rate than DEAE-cellulose column chromatography. This approach eliminates side reactions on the internucleotide linkages and increases the solubility of the oligonucleotides in anhydrous solvents (which was another problem in the diester synthesis[23]). These factors have the effect of maintaining reasonably high yields in the condensation reactions

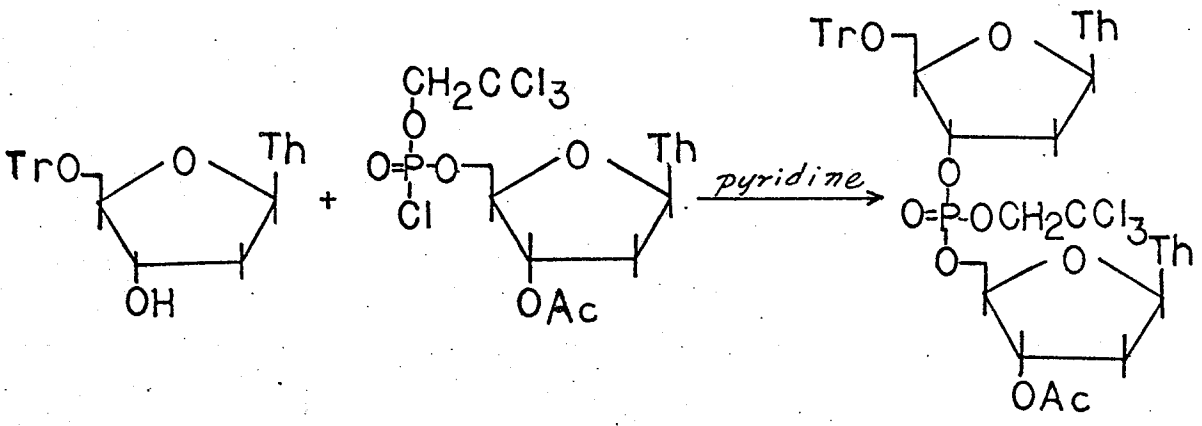
SCHEME 4

PHOSPHOTRIESTER SYNTHESIS WITH THE CYANOETHYL GROUP



SCHEME 5

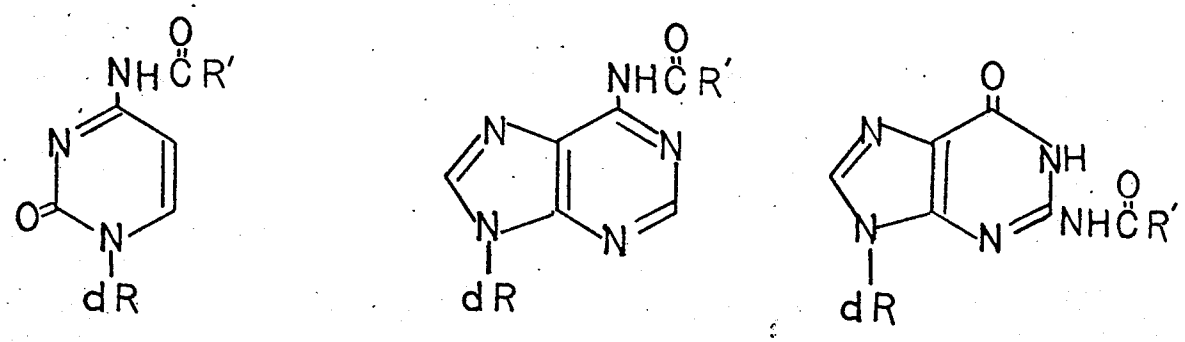
PHOSPHOTRIESTER APPROACH WITH THE TRICHLOROETHYL GROUP



without requiring the use of increasingly larger excesses of nucleoside as stepwise synthesis progresses. Contrary to Khorana's assertion that the yields at each phosphotriester condensation would have to be close to 100% in order to separate reaction components on silica gel, no difficulties were met in the triester synthesis of TpTpTpT;[19,35] TpTpTpTpT[36], d-ApApApX (X = A, G, T, C, or I)[37] or the nonaribonucleotide GpCmpUpCpApUpApApC[38].

Blocking Groups in Diester DNA Synthesis

The blocking groups associated with phosphodiester synthesis were an acid-labile group (trityl, monomethoxytrityl) for the 5'-hydroxyl group of the initial nucleoside plus a base-labile group (acetyl) on the 3'-OH position of the incoming nucleotide. Depurinations which accompanied de-tritylation reactions in acidic conditions were largely overcome by use of the more labile methoxytrityl group[25] and by the addition of pyridine to the acetic acid solution.[30] Alkaline conditions which were used to remove acetyl groups prior to each nucleotide addition had no effect on the N-acyl protecting groups on adenosine, guanosine and cytidine.[26] Such N-acyl groups could be cleaved by treatment with concentrated ammonia.[26]



d-cytidine (N-acyl) d-adenosine (N-acyl) d-guanosine (N-acyl)

FIGURE 2 - THE STRUCTURES OF SOME N-ACYL NUCLEOSIDES

Hydroxyl Blocking Groups in Triester DNA Synthesis

In the triester approach to DNA synthesis, the acid-labile methoxytrityl group for the 5'-OH position of the initial nucleoside is compatible with a base labile 3'-acetyl group and both the trichloroethyl and β-cyanoethyl groups. The trichloroethyl group requires reductive cleavage brought about either by Zn dust in 80% acetic acid[19] or by Zn/Cu in dimethylformamide[7] while the cyanoethyl group is cleaved by ammonium hydroxide.[35] The disadvantage of the trityl group is that it is cleaved by adsorption on silica gel (an acidic medium) for several hours,[21] and silica gel chromatography is one of the advantageous aspects of the triester approach to DNA synthesis.[35] Thus a desirable blocking group for the 5'-hydroxyl position of the initial nucleoside would be a selective (bulky) group which would be stable to both acidic and basic conditions.

The alkali-labile 3'-acetyl group is compatible with an acid-labile 5'-blocking group, with N-acyl blocking groups (which are removed by ammonia at the end of polynucleotide synthesis) and with the trichloroethylphosphotriester, but not with the β-cyanoethylphosphotriester, which is cleaved by basic conditions.[22,35]

The β-benzoylpropionyl group ($PhCOCH_2CH_2CO$) was suggested as a possible blocking group for the 3'-position since it can be removed under neutral conditions - hydrazine hydrate in pyridine buffered with acetic acid.[28] It was found, however, that these conditons were also effective in cleavage of N-acyl

groups.[64] A suitable blocking group for the 3'-position of a nucleoside-5'-β-cyanoethylphosphate would be one which could be removed under neutral conditions without affecting the acid labile (or acid- and base-stable) 5'-blocking group, the N-acyl blocking groups, the β-cyanoethyl group or the glycosidic linkage of N-acyl purine nucleosides.

Another possible alternative would be to use a bulky base-labile group for the 5'-position such as the pivaloyl group - $(CH_3)_3CCO$ - together with a group on the 3'-position which could be removed under mild enough acidic or neutral conditions which would not affect any other part of the oligonucleotide. Such an arrangement would be compatible with N-acyl groups since they could be removed all at once (along with the β-cyanoethyl groups if they were used) at the end of the polynucleotide synthesis.

Hydroxyl Blocking Groups in Phosphodiester RNA Synthesis

Ribonucleic acid synthesis is complicated by the presence of a secondary 2'-hydroxyl group. The diester approach to RNA synthesis involves condensation of a 2',5'-blocked ribonucleotide with a 2',3'-blocked ribonucleoside.[182-185] Extension of the polyribonucleotide chain was effected by removal of the 5'-blocking group of the resulting dinucleoside monophosphate followed by condensation with another 2',5'-blocked ribonucleotide.[27,184-186] Since repeated removal of the 5'-blocking group is required for this approach to RNA synthesis, it must be one

whose removal does not affect either the terminal 2',3'-blocking groups or the 2'-blocking group.

SCHEME 6

PHOSPHODIESTER POLYRIBONUCLEOTIDE SYNTHESIS

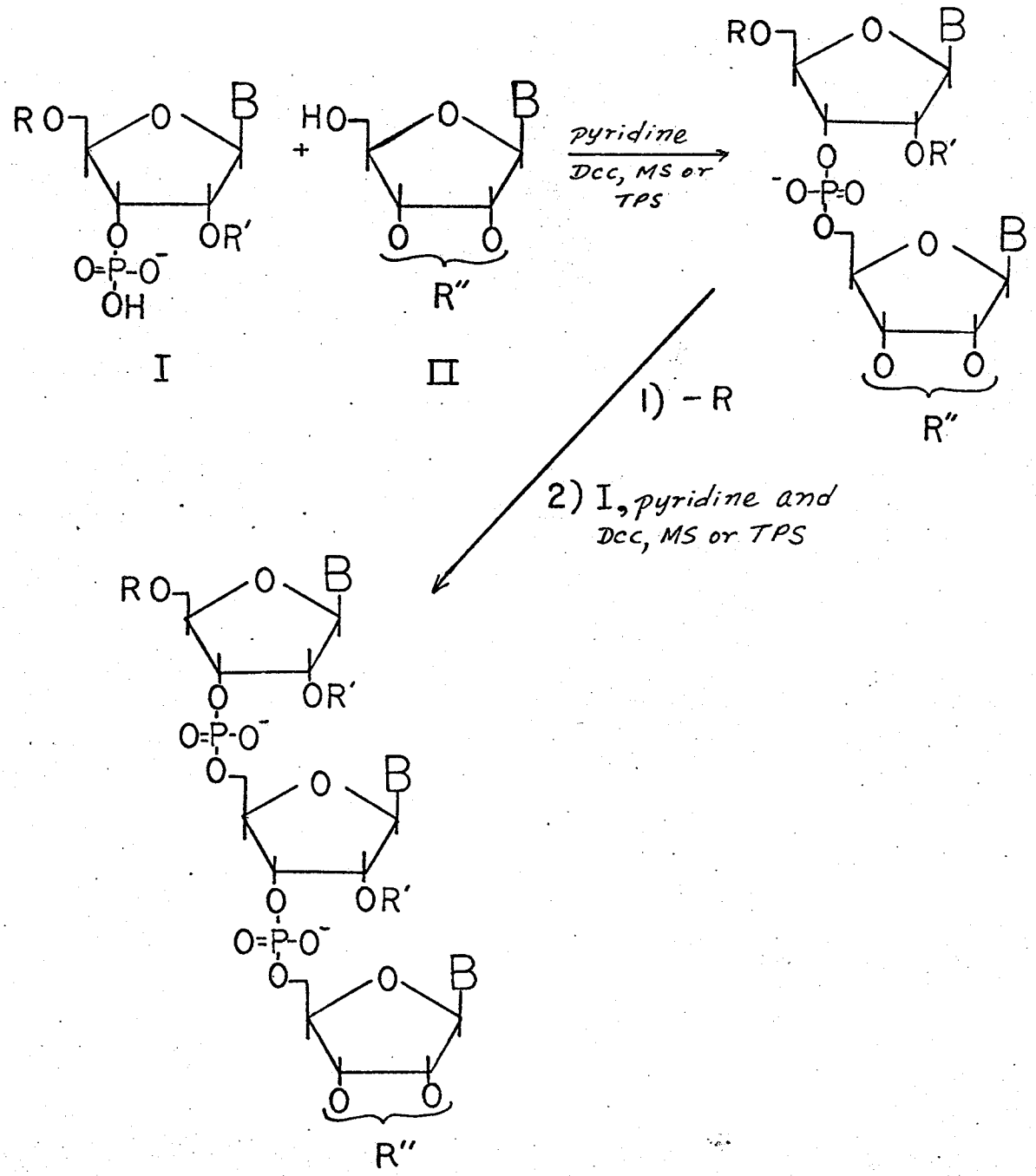
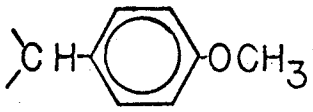


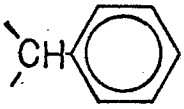
FIGURE 3

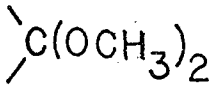
BLOCKING GROUPS USED IN NUCLEIC ACID SYNTHESIS

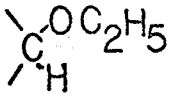
FOR HYDROXYL GROUPS

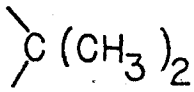
ACID LABILE

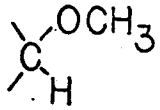
p-anisylidene 

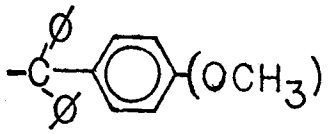
benzylidene 

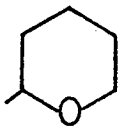
dimethoxy-methylene 

ethoxy-methylene 

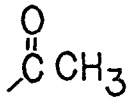
isopropylidene 

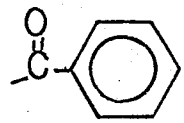
methoxy-methylene 

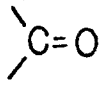
(monomethoxy) trityl 

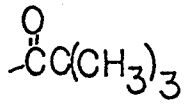
tetrahydro-pyranyl 

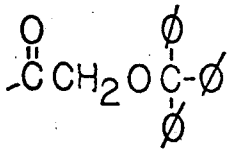
BASE LABILE

acetyl 

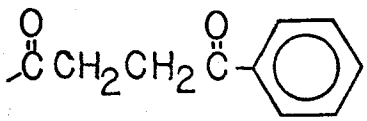
benzoyl 

cyclic carbonate 

pivaloyl 

trityloxyacetyl 

REMOVED UNDER NEUTRAL CONDITIONS

β-benzoylpropionyl 

An acid-labile 5'-blocking group such as the methoxytrityl group would preclude use of such acid-labile 2',3'-blocking groups as the p-anisylidene,[65,183] methoxymethylene,[184,187] ethoxymethylene,[188,189] dimethoxymethylene,[190] and perhaps even the more stable benzylidene[191] and isopropylidene[192] groups. Acid-labile 2'-tetrahydropyranyl[183,193] and methoxy-tetrahydropyranyl groups would also be incompatible with the methoxytrityl group.[195] Base-labile groups such as the 2',3'-dibenzoyl,[27] 2',3'-diacetyl,[196] and 2',3'-cyclic carbonate[50,197] could be used instead at the terminal nucleoside while the base-labile groups like the acetyl would occupy the 2'-positions along the oligoribonucleotide chain. The difficulty with the acetyl group is that it is quite labile under normal conditions of DEAE-celluose chromatography,[198,199] and any other acyl groups might provide steric interference for phosphorylation and condensation reactions involving the 3'-phosphate group. Hence, an acid- and base-stable blocking group for the 2'-position would be desirable for this approach to RNA synthesis.

Using a base-labile 5'-blocking group such as the acetyl group which could be removed with dilute alkali without affecting N-acyl groups [26] would allow use of most of the above-mentioned acid-labile 2'- and 2',3'-blocking groups except for those (like isopropylidene and benzylidene) whose acid removal would effect simultaneous 2'-blocking group removal of the other nucleotides in the molecule, leading to phosphoryl migration to form unnatural 2'→5'-phosphodiester linkages. The danger of phosphoryl

migration is always present when the 2'-protecting group must be removed under acidic conditions. Reese[128] claimed that the tetrahydropyranyl group can be removed using acid conditions mild enough such that phosphoryl migration does not take place.

Other possible 5'-acyl blocking groups include the pivaloyl[200] and triphenylmethoxyacetyl (trityloxyacetyl)[176] groups, but these suffer from the disadvantage that their removal in the approach described in Scheme 6 (page 11) would result in the cleavage of N-acyl groups as well.[177,186]

Taking these factors into consideration, one can see that it would be an advantage to have a 2'-blocking group and a 2',3'-blocking group which were stable in acid and base, but which could be removed under neutral conditions without adversely affecting any other parts of the molecule.

All the disadvantages for diester synthesis of DNA apply equally as well for the diester synthesis of RNA. Significant recent progress has been made in the triester approach to polyribonucleic acid synthesis.

Triester Approach to Polyribonucleotide Synthesis

The foregoing discussion on blocking groups in the diester synthesis of polyribonucleic acids applies to the triester approach using the trichloroethyl phosphotriester. The trichloroethyl group is not affected by acidic or basic conditions and its removal at the end of a synthetic series by Zn/Cu in DMF

at 50°C would have no effect on the other blocking groups. Its removal by Zn dust in 80% acetic acid might bring about partial removal of any trityl or tetrahydropyranyl groups present in the molecule. Since acetic acid catalyzes phosphoryl migration[198] a small amount of 2'→5'linked product may be formed in the event that the 2'-position is deblocked prior to TCE removal.

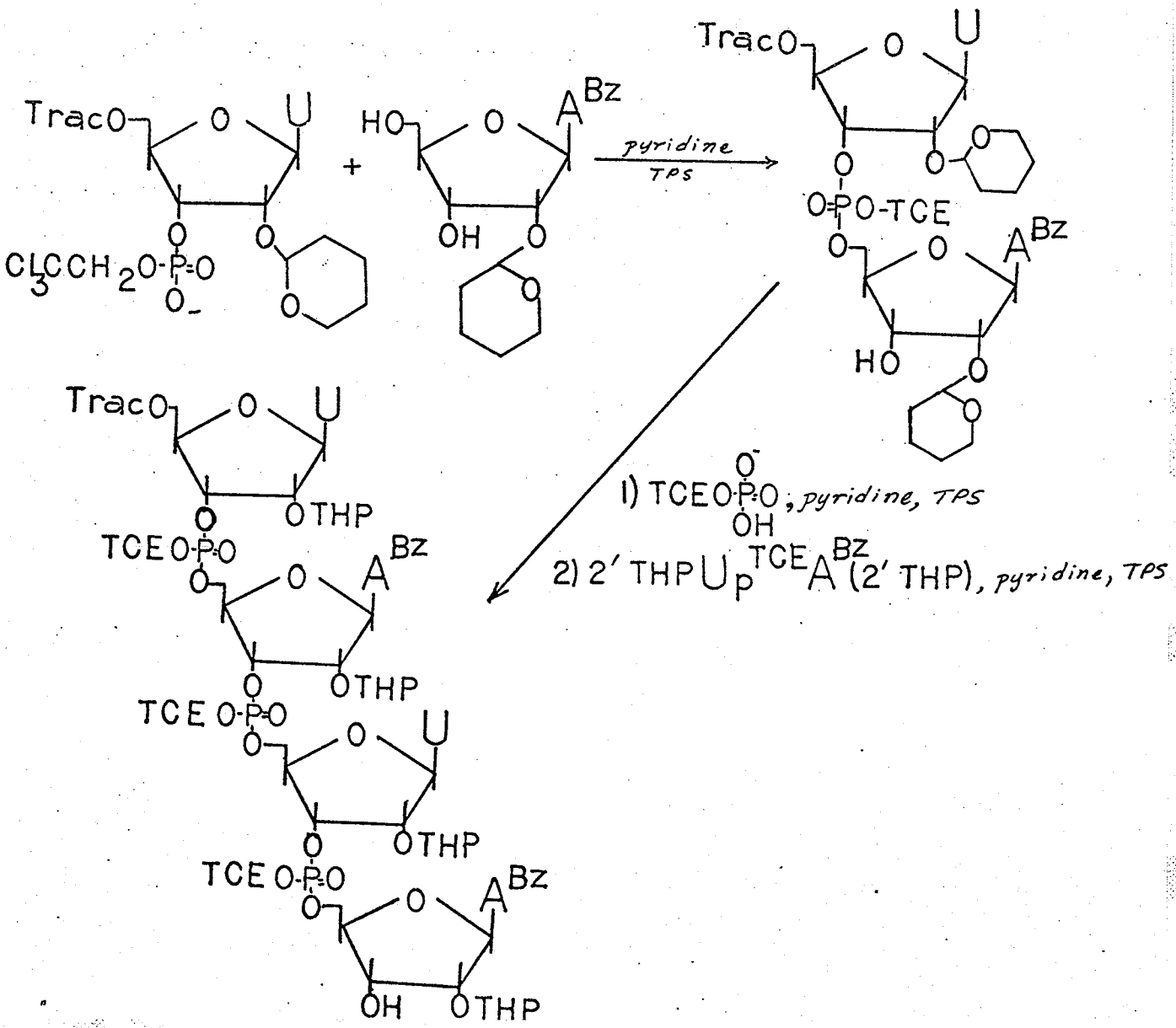
The use of the β-cyanoethyl group, however, introduces the additional limitation that the 5'-protecting group (if it is to be removed to permit elongation of the polyribonucleotide) must not be removed under basic conditions, as these would also remove the cyanoethyl group. With the β-cyanoethyl group, then, one is limited to groups for the 5'-position which are labile to mild acid or which can be removed under neutral conditions (preferrably the latter, which would avoid complications arising from blocking group removal by silica gel). Since the only selective acid-labile group, the trityl group (or its methoxy derivative) is ruled out for the triester synthesis because of its lability in silica gel,[21] one would do well to find a selective blocking group which could be readily removed under neutral conditions which would not affect any other part of the polyribonucleotide.

Werstiuk and Nielson have taken a slightly different approach to the triester synthesis of polyribonucleotides.[38] Instead of a 2',3'-blocked ribonucleoside, they used a 2'-blocked ribonucleoside in a reaction with a 2',5'-blocked nucleotide, relying on the steric interference of the 2'-group of the former

and the bulkiness of the activated phosphate group of the latter to ensure specific 3'→5' condensation.[195] Thus, 5'-trityloxyacetyl-2'-tetrahydropyranyl-uridine-3'-phosphate (trichloroethyl) was allowed to react with 2'-tetrahydropyranyl-N^6-benzoyladenosine in pyridine in the presence of triisopropypbenzenesulfonyl chloride (TPS) to give the desired coupled product 5'-Trac-2'-THP-$Up^{TCE}A^{Bz}$(2'-THP). (Scheme 7) Phosphorylation of the

SCHEME 7

NIELSON'S APPROACH TO PHOSPHOTRIESTER POLYRIBONUCLEOTIDE SYNTHESIS



3'-hydroxyl of the product using trichloroethylphosphate and TPS in pyridine followed by coupling with a dinucleotide with free 5'- and 3'- hydroxyl groups, a tetranucleotide with the desired 3'→5'- linkages was obtained in 93% yield.[195] This method differs fron the conventional approach to RNA synthesis in that condensations proceed in the same direction as in DNA synthesis. The difference is that a polyribonucleotide-3'-phosphate condenses with a free 5'hydroxyl group while a polydeoxyribonucleotide-3'-hydroxyl group condenses with a deoxyribonucleoside-5'-phosphate.

In Nielson's method, preparation of dinucleoside monophospahate blocks requires removal of the trityloxyacetyl groups and this has been found to effect N-acyl cleavage.[177] An effective blocking group for this approach to triester RNA synthesis would be a bulky, selective moiety which could be readily removed under neutral conditions without affecting any other part of the oligonucleotide.

Blocking Group Needs in Nucleic Acid Synthesis

To summarize the blocking group needs for nucleic acid synthesis as suggested in the preceding discussion, then, the following would be helpful:

1) A selective blocking group which is stable to both acidic and basic conditions for the 5'-position of the initial nucleoside in triester deoxyribonucleic acid synthesis.

2) A blocking group for the 3'-position of deoxyribonucleosides which could be removed under mildly acidic or neutral conditions in triester DNA synthesis without affecting any other part of the molecule.

3) An acid- and base-stable blocking group for the 2'-position of ribonucleosides in the diester and triester approach to RNA synthesis.

4) A 2',3'-blocking group which is stable in acid and base but which can be removed under neutral conditions.

5) A selective 5'-blocking group which could be readily removed under neutral conditions without affecting any other part of the molecule in the triester synthesis of polyribonucleotides.

This thesis describes research directed at developing such blocking groups.

Nucleic Acid Synthesis on Polymer Supports

Because of the potential that the method holds for eliminating time-consuming purification steps, the polymer support approach to nucleic acid synthesis has been explored by a number of researchers. Based om Merrifield's polymer support method for polypeptide synthesis,[39,40] the approach immeasurably simplifies separation of products from reactants - one merely collects the insolulbe polymer from the reaction mixture by simple filtration or first precipitates out the soluble polymer (there are the two types) by pouring the reaction mixture into water. Polymers that have been studied for use in polynucleotide synthesis include a styrene-divinylbenzene popcorn polymer,[41] polystyrene,[42] silica,[43] sephadex LH 20,[44] polyethylene glycol,[45] polyuridylic acid for preparation of polyadenylic acid,[46] poly-L-lysine hydrobromide,[47] and a copolymer of vinyl acetate and N-vinyl pyrrolidone.[48]

None of these polymers have found extensive application

in oligonucleotide synthesis. This is principally because the entire success of the polymer support synthesis depends on obtaining complete reaction at each and every step. Although many different phosphorylating agents have appeared in the literature,[2,5-20] nucleotide condensation reactions as developed at this time are not 100% efficient. For the present, this effectively precludes a totally efficient synthesis of nucleic acids on conventional polymer supports.

Lipohilic Handles for Nucleic Acid Synthesis

There is, however, another approach possible which may have some of the advantages of polymer support synthesis and which may, at the same time, compensate for the present inefficiency of internucleotide bond formation by rendering the oligonucleotide sufficiently soluble for purification by silica gel chromatography. By building up a polynucleotide on a molecule which contained a long alkyl side chain, one should be able to separate the lipophilic polynucleotide (triester) molecules from the rest of the reaction mixture by precipitation in an aqueous medium and/or simple filtration. The desired molecule containing N+1 nucleotides might then be separated from the unreacted starting material containing N nucleotides by silica gel chromatography. This doctoral thesis addresses itself in part to development of such a lipophilic 5'-blocking group which could be used for polydeoxyribonucleotide synthesis.

A Novel Approach to Ribonucleic Acid Synthesis

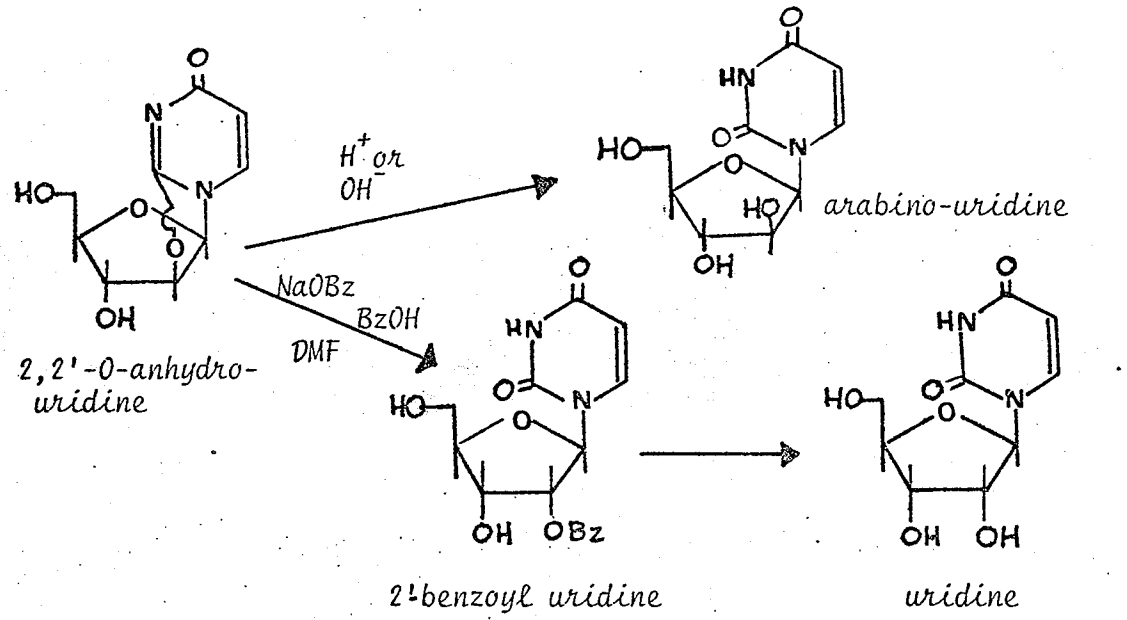
As mentioned previously, ribonucleic acid synthesis is complicated by the necessity to block the 2'-hydroxyl group in order to ensure the specific linkage of a 3'-hydroxyl group to a 5'-hydroxyl group through a phosphodiester bond. A novel approach to RNA synthesis could circumvent the interference from the 2'-hydroxyl group by removing it temporarily from the molecule. Anhydronucleosides whose 2'-positions are taken up with an extra covalent linkage via a bridging atom to the purine or pyrimidine base could act as such molecules of convenience. Using methods developed in DNA synthesis, a polyanhydronucleotide of the desired sequence might be synthesized, followed by reaction(s) to regenerate the 2'-hydroxyl group to produce a polyribonucleic acid. A number of such 2'-anhydronucleosides with O, S, or N bridging atoms have been synthesized: 2,2'-S-anhydrothymidine,[49] 2,2'-O-anhydrouridine,[50] 2,2'-O-anhydrocytidine,[51,52] 2,2'-O-anhydro-5,6-dihydrouridine,[53] 8,2'-O-anhydroadenosine,[54] 8,2'-N-anhydroadenosine,[55] 8,2'-S-anhydroadenosine,[57] 8,2'-S-anhydroxanthosine,[57] and 8,2'-S-anhydroinosine.[58]

Although an arabinonucleoside results from acid or base hydrolysis of anhydropyrimidine nucleosides,[51,59] treatment with sodium benzoate and benzoic acid in refluxing dimethylformamide generates a ribonucleoside.[60] (Scheme 8). Thus it appeared that at least the anhydropyrimidine nucleosides could be used for this novel approach to ribonucleic acid synthesis.

Information as to similar reactions with anhydropurine

SCHEME 8

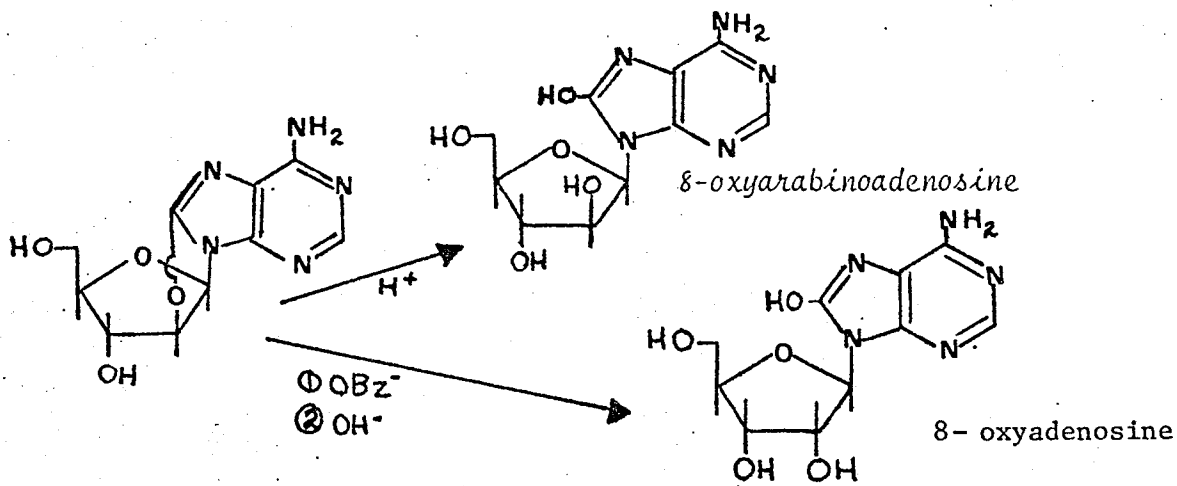
DISPLACEMENTS ON ANHDRYOPYRIMIDINE NUCLEOSIDES



nucleosides is scarce. Treatment of 8,2'-O-anhydroadenosine with acid gives 8-oxyarabinoadenosine while reaction with the benzoate ion leads to the formation of 8-oxyadenosine.[54] (Scheme 9) While removal of the oxygen atom of the latter product to give adenosine might be possible by heating the molecule with zinc dust,[61] it is unlikely that a polynucleotide

SCHEME 9

DISPLACEMENT REACTIONS ON 8,2'-O-ANHYDROADENOSINE

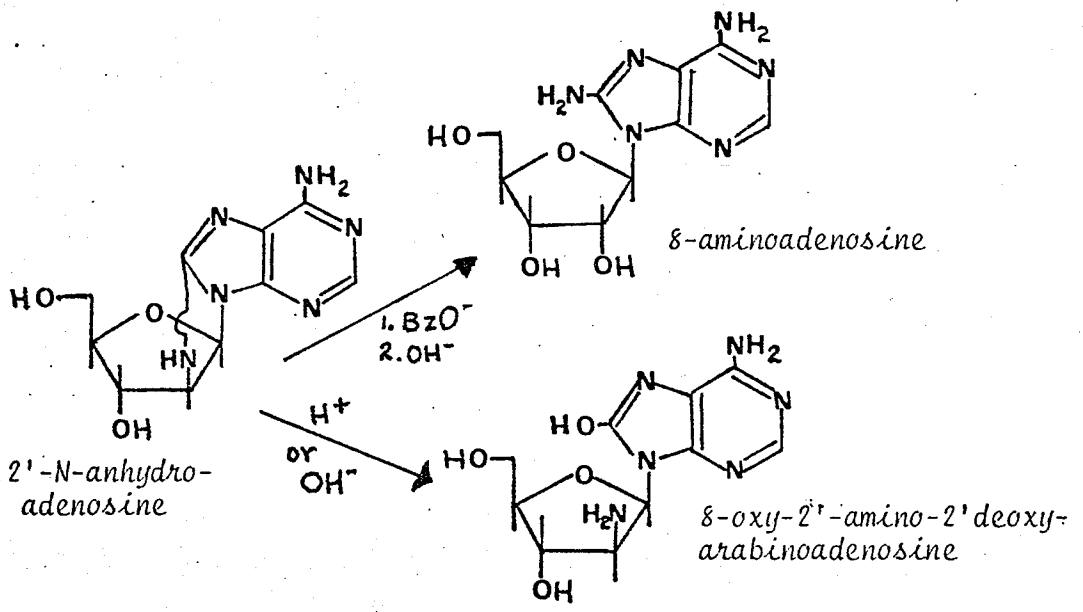


containing 8-oxyadenosine could withstand such treatment. The use of 8,2'-O-anhydroadenosine as a "molecule of convenience" for RNA synthesis is thus severely limited by the difficulties involved with the removal of the 8-oxy group.

The nucleophilic displacement reactions on 8,2'-N-anhydropurines, by analogy to the 8,2'-O-anhydropurines, would be expected to lead to the formation of either 8-aminopurine nucleoside or 8-oxy-2'-amino-2'-deoxyarabino purine nucleoside (Scheme 10). Subsequent reactions to remove the amino group of either molecule would also adversely affect the amino function of any adenine, cytosine or guanine ring present in the polynucleotide. This then severely limits the use of 8,2'-N-anhydropurines in the novel approach to ribonucleic acid synthesis.

SCHEME 10

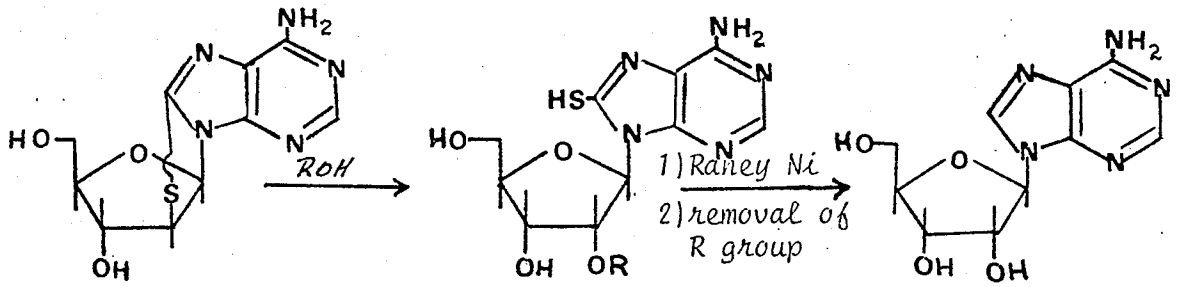
HYPOTHETICAL DISPLACEMENT REACTIONS ON 8,2'-N-ANHYDROADENOSINE



Nucleophilic displacement reactions on 8,2'-S-anhydro adenosine with acid and base had been attempted without much success.[62] However, it was felt that it would be useful to explore the possibilities of displacements with other nucleophilic reagents on 8,2'-S-anhydroadenosine and other 8,2'-S-anhydropurine nucleosides. If conditions for such a displacement were found, one of the possible products of such a reaction would be 8-thioadenosine. The sulfur atom of this molecule could be readily removed by Raney Nickel treatment to give adenosine (Scheme 11). Consequently, the object of one of the studies of this thesis was to subject 8,2'-S-anhydroadenosine to a variety of nucleophilic and oxidation reactions in an endeavour to convert that purine anhydronucleoside into a ribonucleoside.

SCHEME 11

HYPOTHETICAL DISPLACEMENT REACTION ON 8,2'-S-ANHYDROADENOSINE



8,2'-S-anhydroadenosine *adenosine*

A New Photoreduction System?

During the course of an investigation on the photodimerization of 3',5'-diacetyl-2,2'-O-anhydrouridine,[63] it was found that

irradiation of this molecule dissolved in an aqueous solution saturated with chloroform led to the appearance of the dihydro-derivative of this modified nucleoside. Nucleophilic displacement on dihydroanhydrouridine (or its diacetylated derivative) with the benzoate ion might be expected to lead to the formation of dihydrouridine. Dihydrouridine is one of several modified nucleosides which is found in naturally-occurring RNA species. Acid or base hydrolysis of dihydro-anhydrouridine might lead to the synthesis of dihydroarabino-uridine, a molecule which had not been previously prepared. Curious as to the role that chloroform played in the photo-reduction and encouraged by the potential usefulness of the resulting photoproducts, a more extensive study of this photoreduction was attempted.

In summary then, research for this thesis has consisted of three different, but interrelated projects:-

(1) The synthesis and investigation into the use of various alkyl silyl chlorides as
    (a) lipophilic handles for nucleic acid synthesis and as
    (b) acid and base stable blocking groups, removable in neutral conditions.
(2) Nucleophilic displacement reactions and oxidations on 8,2'-S-anhydroadenosine
(3) The investigation into the photoreduction of anhydrouridine in aqueous solution saturated with chloroform.

RESULTS AND DISCUSSION

PART A - ALKYLSILYL BLOCKING GROUPS IN NUCLEOSIDE CHEMISTRY

In the preceding discussion on blocking groups involved in nucleic acid synthesis (pages 8 - 18), a need was noted for a selective 5'-blocking group which would be stable to acid and base but which could be removed under neutral conditions which would have no adverse effects on the rest of the molecule. Blocking groups for the secondary hydroxyl groups in nucleosides (3'-OH in deoxyribonucleosides, 2'- and 2',3'-OH groups in ribonucleosides) which would posses similar stability and deblocking properties were needed as well.

The ideal properties of a 5'-blocking group in nucleic acid synthesis would include the following:

1) It can be easily introduced into the molecule.

2) Under certain conditions, it can be made to react selectively with the 5'-hydroxyl group of nucleosides.

3) It should be stable under the reaction conditions to which the blocked nucleoside would be subjected (e.g. phosphorylation, acidic or basic medium)

4) It should render the nucleoside soluble in aprotic solvents (e.g. dry pyridine), thereby facilitating the reactions and purification of oligonucleotides.

5) It should be easily removed without affecting any other parts of the molecule.

It was felt that such a blocking group might be found

among a class of compounds known as alkylsilyl chlorides, for the following reasons:

1) Corey[75,76] and Ogilvie[79,80] had shown that the t-butyldimethylsilyl (TBDMSi) group could be easily introduced into a molecule by reaction of the alcohol and t-butyldimethylsilyl chloride in dimethylformamide in the presence of imidazole.

2) Ogilvie[79] found that a specificity of 82% for the 5'-OH group of thymidine could be obtained by using thymidine, silyl chloride and imidazole in the proportions 1:1.1:2.2 in the reaction mixture. Increasing the bulkiness of the alkyl substituents attached to the silicon atom might lead to higher selectivity for the primary 5'-OH group.

3) The TBDMSi group was found to be stable to various reaction conditions[79] which are found in polynucleotide synthesis, but was found to be removed by treatment with 80% acetic acid on the steam bath for 15 minutes. It was felt that higher stability to acidic conditions would be found in bulkier alkylsilyl groups.

4) Monosilylation of thymidine had rendered the nucleoside sufficientlysoluble in aprotic solvents that its silyl ethers could be isolated by silica gel chromatography in ether.[79]

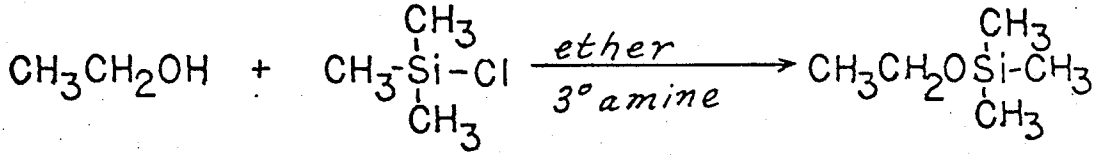
5) It had been shown that removal of the TBDMSi group could be effected by treatment of the silyl ether with tetra-n-butylammonium fluoride in tetrahydrofuran.[76] Ogilvie reported that these conditions had no adverse effects on the

β-benzoylpropionyl group or on acid- or base-labile groups in general.

In the search for a selective 5'-blocking group in the field of alkylsilyl chlorides, it was found that some of the compounds prepared could be used as well to block secondary hydroxyl groups and that others provided examples of blocking groups which might be used as lipophilic handles for polynucleotide synthesis. A blocking group for the 2',3'-cis glycol system of ribonucleosides was also sought among the class of compounds known as alkylsilyl chlorides.

Development of Trialkylsilylation Reactions on Alcohols

The earliest example of trialkylsilylation is that of Sauer[66] in 1944, who described the reaction of trimethylsilyl chloride with methanol and ethanol in ether. The trimethylsilylation of alcohols, amines, amides, acids and phenols has since been frequently performed in the preparation of volatile silyl ethers for gas chromatography and mass spectrometry.[67]

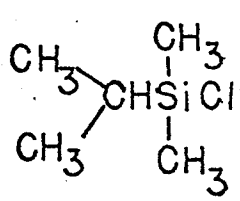


Other trisubstituted silyl chlorides prepared previously include triisopropylsilyl chloride,[68] triphenylsilyl chloride,[69] tricyclohexylsilyl chloride[70] and tri-1-naphthylsilyl chloride.[71] Their reactions with alcohols and with water were studied in various solvents[72,73] and it was found that rates varied greatly, being in the order methanol>ethanol>>2-propanol. The addition of small amounts of pyridine drove the silylation reactions to completion, as pyridine reacts with the hydrochloric acid formed in the reaction, thereby preventing acid hydrolysis of the silyl ethers being formed.

Corey has described the use of dimethylisopropylsilyl chloride[74] and the more stable dimethyl t-butylsilyl chloride[75] (Figure 4) in prostaglandin synthesis. Using imidazole as a catalyst and dimethylformamide as a solvent, Corey obtained t-butyldimethylsilyl (TBDMSi) ethers in high yields under mild conditions.[76] (Scheme 12)

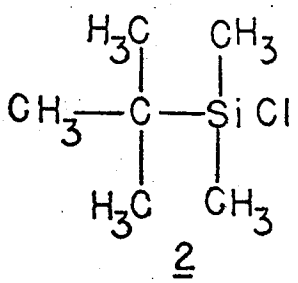
FIGURE 4

STRUCTURES OF COREY'S ALKYL SILYL CHLORIDES



1

dimethylisopropylsilyl chloride (DMIPSiCl)

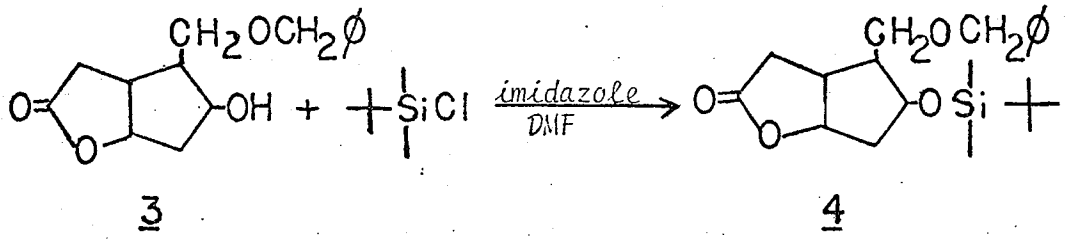


2

t-butyldimethylsilyl chloride (TBDMSiCl)

SCHEME 12

SILYLATION OF PROSTAGLANDIN SUBSTRATES

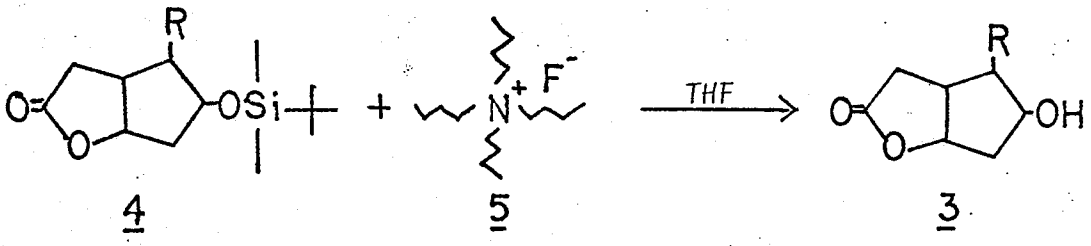


3 4

Corey also discovered that the silyl ethers are cleaved rapidly to alcohols by treatment with tetra-n-butyl ammonium fluoride in tetrahydrofuran at 25°C. (Scheme 13) A mechanism

SCHEME 13

CLEAVAGE OF Si-O BOND BY TETRA-N-BUTYL AMMONIUM FLUORIDE (5)

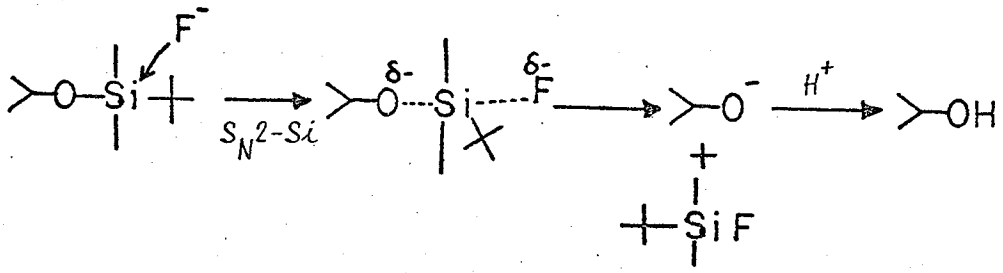


4 5 3

for this reaction was not given, but it would presumably involve nucleophilic attack (S_N2-Si) on the silicon atom by the fluoride ion to form a Si-F bond, as the silicon atom forms its strongest bonds with fluorine.[77] Although not mentioned in any of Corey's papers, a proton source is required to convert the tetra-n-butyl ammonium salt of the alcohol back to the alcohol. Silica gel used for chromatography could act as a source of such protons. Participation of silicon's 3d orbitals could

SCHEME 14

SUGGESTED MECHANISM FOR Si-O CLEAVAGE WITH $(\underline{n}Bu)_4$ NF (5)



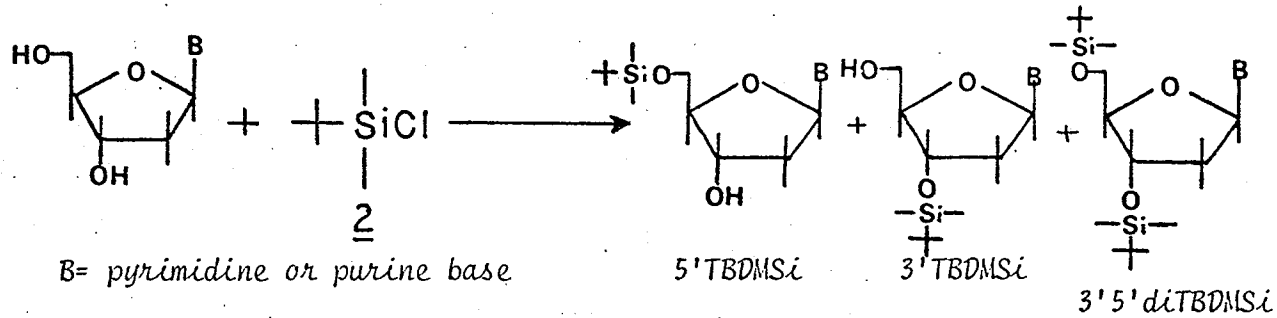
lower the free energy of the S_N2-Si transition state and thereby contribute to the speed of the reaction,[78] which takes place in 40 minutes or less.[76]

The TBDMSi ethers were stable to aqueous or alcoholic base, to hydrogenolysis and to mild chemical reduction.[76] Furthermore, the TBDMSi derivatives were found to be crystalline and suitable for gas chromatography and mass spectral measurements.

Shortly following Corey's publications, the TBDMSi group was used for the protection of hydroxyl functions of nucleosides.[79,80]

SCHEME 15

REACTION OF T-BUTYLDIMETHYLSILYL CHLORIDE WITH DEOXYNUCLEOSIDES



Ogilvie found[79] that the TBDMSi group on nucleosides was stable

to normal conditions of phosphorylation, O-acyl cleavage (9 M ammonium hydroxide or 15% ammonium hydroxide in ethanol) and N-acyl cleavage (hydrazine in acetic acid and pyridine). It could, however, be removed by strong base (0.5 N sodium hydroxide), by mild acid (80% acetic acid) and by tetra-n-butyl ammonium fluoride in tetrahydrofuran. Removal by tetra-n-butyl ammonium fluoride did not affect any of the other protecting groups on the nucleoside. However, removal of a trityl group with 80% acetic acid would also result in removal of the TBDMSi group. The TBDMSi chloride was found to react preferentially with hydroxyl groups and not with amino groups, and the specificity the reagent for the 5'-hydroxyl function was 82%. This was not as high as one would ideally require for an excellent blocking group. Consequently, the synthesis of a series of trialkyl silyl chlorides and the testing of their specificity and stability by reactions with thymidine were initiated in order to find the ideal blocking group for nucleoside chemistry.

Lipophilic Handles for Polynucleotide Synthesis

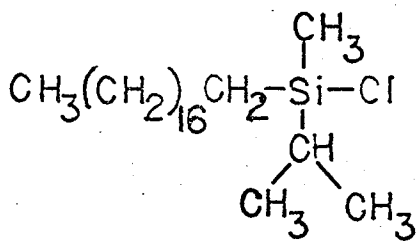
The Methyloctadecylisopropylsilyl Group

The first molecule to be prepared was one which was designed to be used as a lipophilic handle for nucleic acid synthesis - methyloctadecylisopropylsilyl chloride 6 (MODIPSiCl - Figure 5). It was prepared by the dropwise addition of isopropyl lithium (8) in pentane to a hexane solution of octadecyl-

methylsilyl dichloride (7). Both reagents were commercially

FIGURE 5

STRUCTURE OF METHYLOCTADECYLISOPROPYLSILYL CHLORIDE (MODIPSiCl)



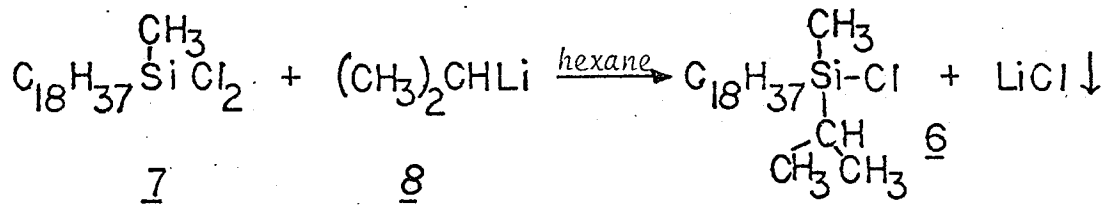
6

available at that time, but later developments required that the isopropyl lithium be prepared in the laboratory. The progress of the reaction was followed by the technique of gas chromatography-mass spectrometry (GC/MS). Isopropyl lithium was added in portions, the reaction mixture allowed to stir for a few hours and then a sample was removed for analysis by gas chromatography-mass spectrometry. This was continued until GC/MS indicated that all the starting material had been converted to products. (The peak corresponding to starting material retention time had completely disappeared.) Filtration of the solution removed the lithium salts. High vacuum distillation (2-5 mm) at elevated temperature (195-200°C) of a portion of the filtered solution yielded a viscous clear liquid which solidified at approximately 15°C to form a white waxy amorphous material identified as methyloctadecylisopropylsilyl chloride (6).

The reaction of thymidine with 1.1 equivalents of the non-distilled methyloctadecylisopropylsilyl chloride (6) and two equivalents of imidazole in dimethylformamide for two hours at

SCHEME 16

PREPARATION OF METHYLOCTADECYLISOPROPYLSILYL CHLORIDE(MODIPSiCl)



7 8 6

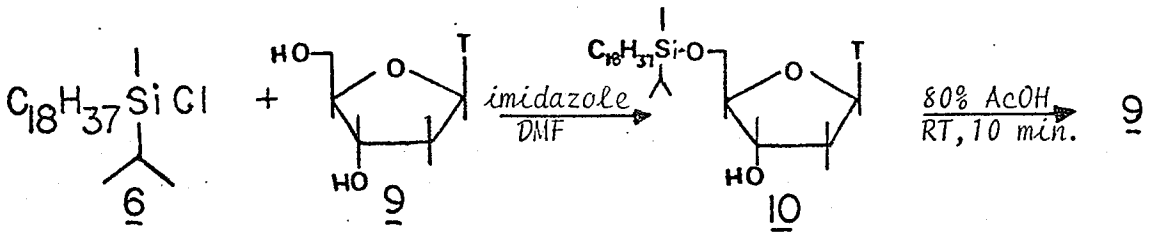
room temperature gave a 26% yield of 5'-MODIPSi thymidine and a 1% yield of 3'-MODIPSi thymidine. The yield of disilyl thymidine was not determined, as it co-chromatographed with unreacted silyl chloride on silica gel plates developed in ether. The 5'-MODIPSi thymidine was recrystallized from ethanol-water to give a white crystalline material with a sharp melting point at 74.5-75.5°C. This molecule, as all subsequent silyl derivatives of thymidine, was characterized by infrared spectroscopy, mass spectrometry and melting point . Elemental analyses were performed on just the 5'- silyl derivatives of thymidine.

Hydrolysis of the MODIPSi group on the primary hydroxyl of thymidine by 80% acetic acid was complete after 10 minutes at room temperature. This lability to acid parallels that of the dimethyl-isopropylsilyl group, of which the MODIPSi group is a homologue. Corey found that the dimethylisopropylsilyl group was removed from a secondary hydroxyl of protaglandin E_2 by treatment with 75% acetic acid at 35°C for 10 minutes.

In order to see whether the octadecyl side-chain might be effective in bringing the nucleoside out of solution in a

SCHEME 17

PREPARATION OF 5'MODIPSi THYMIDINE AND ITS ACID HYDROLYSIS



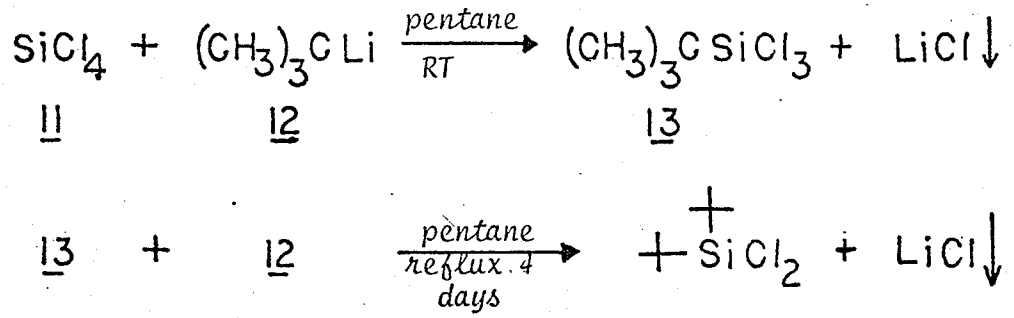
predominantly aqueous solution, some 5'MODIPSi thymidine was dissolved in pyridine and the solution poured into ice water. A precipiate was formed immediately and a 92% recovery of the 5'MODIPSi thymidine was effected. Thus it was shown that a nucleoside attached to a long chain alkyl silyl group could be chromatographed on silica gel and/or precipitated out of an aqueous solution. Although it was stable to mild basic conditions, the acid lability of the MODIPSi group was a disappointment. It was somewhat surprising that replacing one of the methyl groups of the dimethylisopropylsilyl ether with an octadecyl group did so little to increase its stability to acid. The effect of the octadecyl group seemed to be very similar to that of a methyl group. It was decided to take advantage of this similarity by preparing several different alkyl silyl groups containing one methyl group, and testing the acid stability of each such "model compound". Finding a group that was stable, one would then synthesize a long chain alkyl homologue of it using dodecysilyl trichloride as the starting material. Two such models were the di-t-butylmethylsilyl and the methyldiisopropylsilyl chlorides.

The Di-t-Butylmethylsilyl Group - a Model

It was felt that di-t-butylmethyl(or octadecyl)silyl chloride would provide the most stable alkyl silyl ether possible because of t-butyl group bulkiness. $(t\text{-Bu})_2SiCl_2$ had been made previously[81] and it seemed a simple matter to treat it with methyl lithium to yield the desired di-t-butylmethylsilyl chloride. The original preparation of di-t-butylsilyl dichloride[81] had involved the addition of silicon tetrachloride 11 to t-butyl lithium 12 in pentane to form t-butylsilyl trichloride 13 at ambient temperature. The t-butylsilyl trichloride formed in the reaction was isolated and purified, then added to a slight excess of t-butyl lithium under nitrogen. Since no reaction was found to occur at room temperature, the mixture was heated to reflux. Over a period of four days the reaction temperature was slowly raised to 70°C by intermittent removal of pentane through the fractionating column. After four days, a 59% yield of di-t-butylsilyl dichloride 14 had been obtained. (Scheme 18)

SCHEME 18

REPORTED PREPARATION OF Di-t-BUTYLSILYL DICHLORIDE[81]

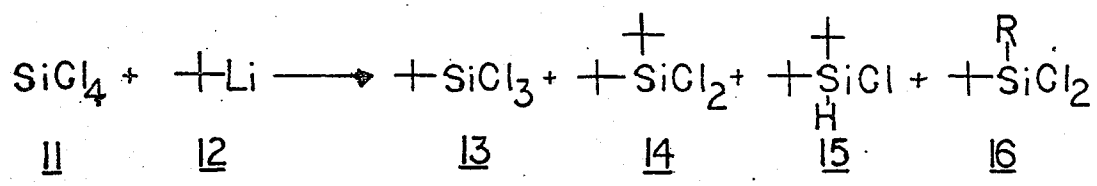


Rather than add silicon tetrachloride to t-butyl lithium,[81] it was decided to modify the approach to this reaction by adding t-butyl lithium to silicon tetrachloride. In the first attempt to prepare di-t-butylsilyl dichloride, the alkyl lithium 12 was added to undiluted silicon tetrachloride. In the second experiment, the silicon tetrachloride was diluted with some dry tetrahydrofuran. The third experiment was silicon tetrachloride diluted with pentane including a catalytic amount of tetrahydrofuran. (It had been discovered that tetrahydrofuran greatly increased the rate of alkylation reactions on silicon.) In all three experiments the t-butyl lithium was added dropwise to a stirred, cooled solution of silicon tetrachloride. In the first experiment, the reaction was heated to reflux for four days after addition of t-butyl lithium had been completed. In the next two experiments the reactions were maintained at room temperature. The progress of each reaction was monitored by gas chromatography-mass spectrometry (GC/MS). In none of the cases could the reaction be driven to completion by the presence of an excess of t-butyl lithium - some t-butylsilyl trichloride was always present. Also formed in the reaction were products resulting from hydride formation and rearrangement reactions (15 and 16), both of which co-distilled with di-t-butylsilyl dichloride. On the basis of these results, it is strongly suspected that the di-t-butylsilyl dichloride obtained by Tyler, Sommer and Whitmore in 1948[81] was not as

pure as the results of their elemental analysis would lead one to believe.

SCHEME 19

OBSERVED PRODUCTS OF REACTION BETWEEN $SiCl_4$ AND t-BULi



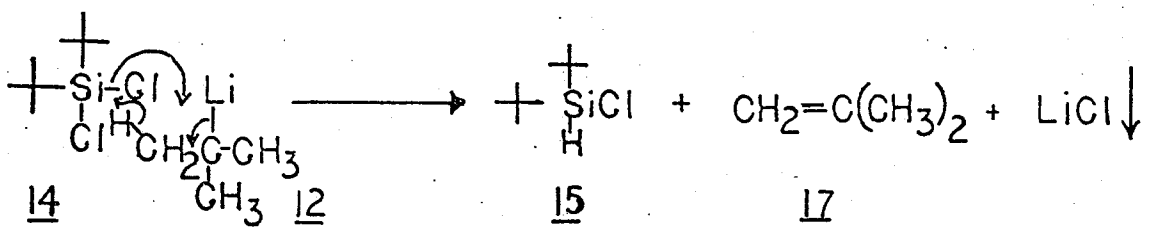
*R, a saturated 4-carbon group, was not fully identified.

Possible Explanation for Observed Side Products (15 and 16)

The hydride formed in the t-butyl alkylation reaction of silicon tetrachloride could be the result of a broadside attack of the t-butyl lithium on the di-t-butylsilyl dichloride (Scheme 20). This would be analogous to the reaction that is observed between phenylsilyl trichloride and sterically hindered Grignard reagents.[82,83,84] In those reactions a phenyl dialkyl

SCHEME 20

PROPOSED MECHANISM FOR HYDRIDE FORMATION

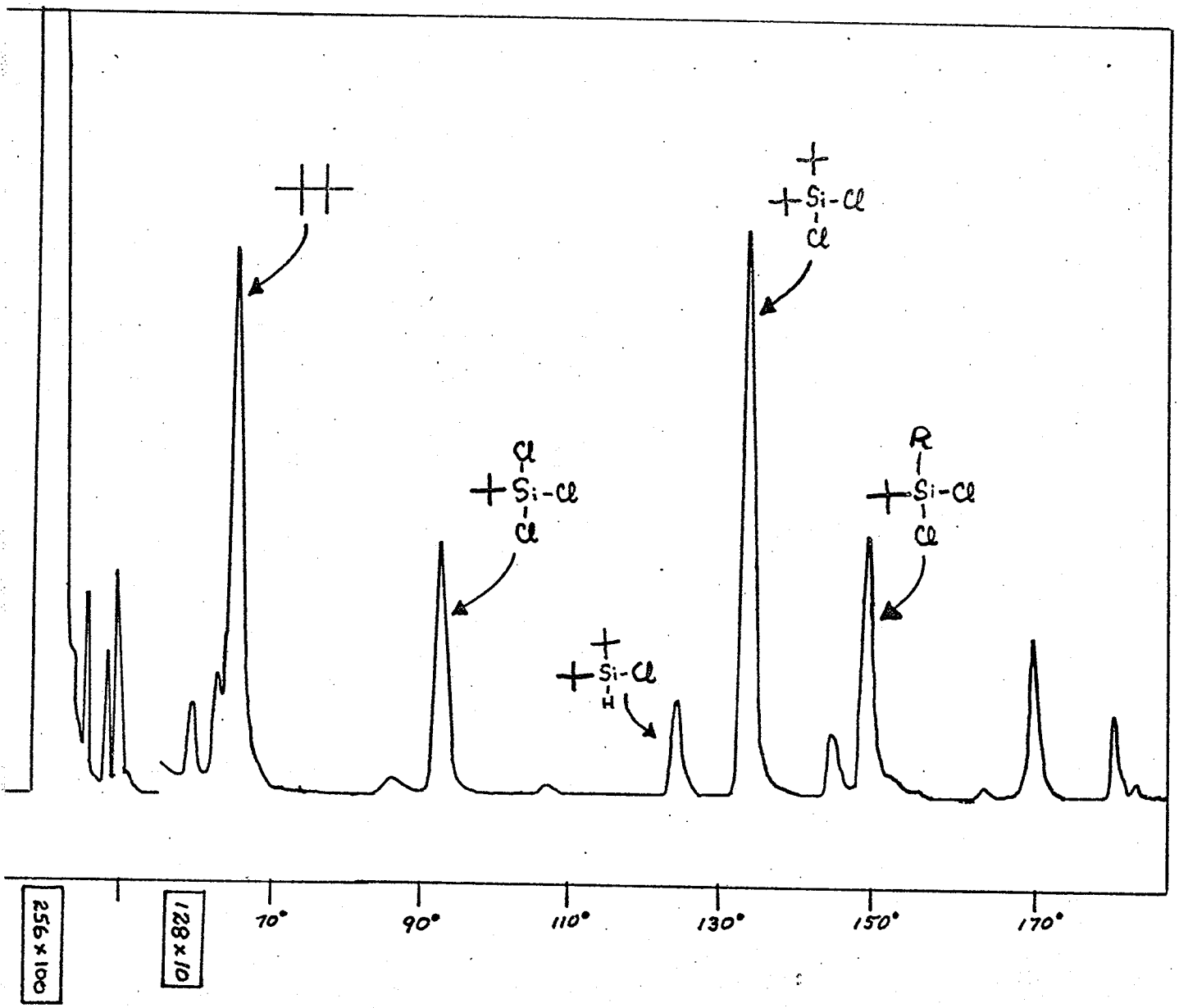


silane is produced whereas in the reaction in Scheme 20 the

FIGURE 6

GAS CHROMATOGRAM OF REACTION BETWEEN $SiCl_4$ AND t-BuLi

The gas chromatogram was performed on a Varian 1700 GC. The column was 10% UCW-98 on 80/100 mesh acid-washed-DMCS-Chromosorb W, 20 inches long with a 2 mm inner diameter, made of stainless steel. Helium carrier flow-rate was 25 ml/min. Column temperature programmed at 10°/min.

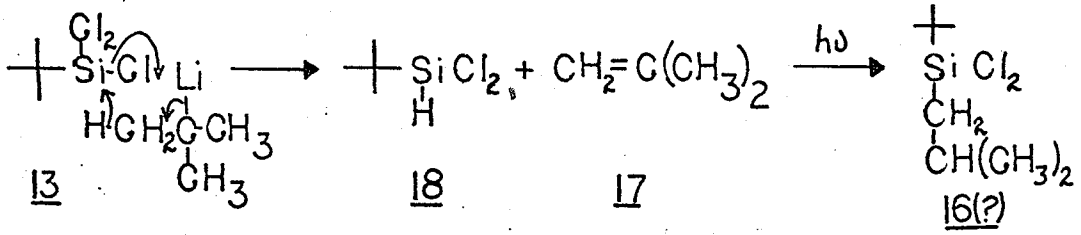


Temperatures of injector and FID were at 200° and 250° C resp.

bulkiness of the t-butyl group forces hydride formation before a trisubstituted silyl chloride is formed. In fact, it is impossible to prepare tri-t-butylsilyl chloride.[81] This is due to the serious hindrance to the approach of the α-C atom of t-butyl lithium to the silicon atom. There is, however, relatively little hindrance to the approach of a β-hydrogen atom (as shown in Scheme 20) to produce a hydride of silicon. It is somewhat surprising that t-butyl lithium would react with di-t-butylsilyl dichloride in this manner while there is still a considerable amount of t-butylsilyl trichloride available for alkylation. It is possible, however, that even the t-butylsilyl trichloride undergoes a reaction similar to that shown in Scheme 19 to form t-butyldichlorosilane (18). The reaction of t-butylsilyl trichloride 13 with t-butyl lithium might lead to the formation of t-butyldichlorosilane 18 (Scheme 21), a molecule whose retention time would be expected to be slightly shorter than that of t-butylsilyl trichloride due to its smaller mass. Although such a compound was not identified in the reaction mixture, the

SCHEME 21

PROPOSED MECHANISM FOR REARRANGEMENT PRODUCT (16) FORMATION



small peak with slightly shorter retention time may have been caused by a small amount of t-butyldichlorosilane. (Figure 6)

The hydrides of chlorosilanes have been reported to react with olefins to form alkylsilyl chlorides by a free radical mechanism initiated by the presence of light or peroxide.[85] A similar reaction between the hydride and olefin formed in Scheme 20 (t-butyldichlorosilane 18 and isobutylene 17) could result in the formation of t-butylisobutylsilyl dichloride 16, as light had not been excluded from any of the alkylation reactions. The assignment of an isobutyl structure to the R group was in agreement with the observed results. Mass spectrometry showed that 16 had the same fragmentation pattern as di-(t-butyl)silyl dichloride. This meant that the R group was a saturated 4-carbon entity. The fact that its retention time was longer than that observed for di-(t-butyl) silyl dichloride indicated that the R group was not as highly branched a 4-carbon structure as was the t-butyl group.[86] This meant then that R could have any one of n-butyl, iso-butyl or sec-butyl structures. The observed hydride formation accompanied by presumed isobutylene production immediately suggests rapid recombination between the two molecules to form t-butylisobutylsilyl chloride. However, since the reaction does proceed by a free radical mechanism, migration of a methyl group to help stabilize the radical by forming a sec-butyl group cannot

be ruled out.

The Methyldiisopropylsilyl Group - a Model

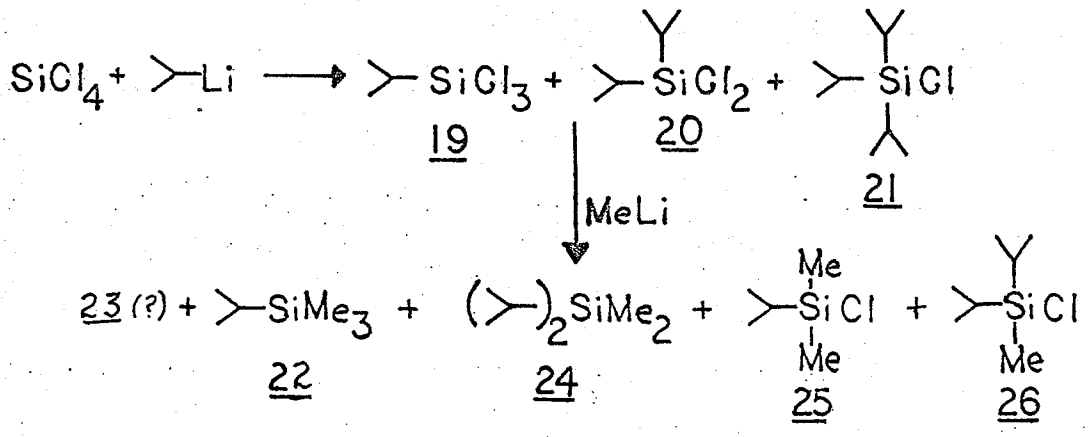
Since both the hydride 15 and the rearranged silyl dichloride 16 could not be separated from the desired product di(t-butyl)silyldichloride by fractional distillation, it seemed that the preparation of pure di(t-butyl)methylsilyl chloride would be a formidable task indeed. Consequently, it was decided to forego the synthesis of that very sterically hindered molecule and to prepare and test methyldiisopropylsilyl chloride instead.

The synthesis of methyldiisopropylsilyl chloride was accomplished by the addition of two equivalents of freshly prepared isopropyl lithium followed by the addition of one equivalent of methyl lithium to a stirred, cooled solution of silicon tetrachloride in pentane and tetrahydrofuran. Since the alkylation of silyl chlorides does not proceed in a neat stepwise manner, the addition of the isopropyl lithium resulted in the formation of isopropylsilyl trichloride, diisopropylsilyl dichloride and triisopropylsilyl chloride. Following the addition of methyl lithium, the reaction mixture contained the desired methyldiisopropylsilyl chloride 26, chlorine containing impurities dimethylisopropylsilyl chloride 25 and triisopropyl-silyl chloride 21, as well as fully alkylated molecules trimethylisopropylsilane 22, dimethyldiisopropyl silane 24 and an unknown molecule 23. (Scheme 22) Fractional distillation

was effective in removing only the triisopropylsilyl chloride from the reaction mixture; a small amount of dimethylisopropylsilyl chloride remained as an impurity along with the fully alkylated silanes. A much better approach

SCHEME 22

PREPARATION OF METHYLDIISOPROPYLSILYL CHLORIDE (MDIPSiCl) 26



to the synthesis of methyldiisopropylsilyl chloride would have been the addition of two equivalents of isopropyl lithium to commercially available methylsilyl trichloride.

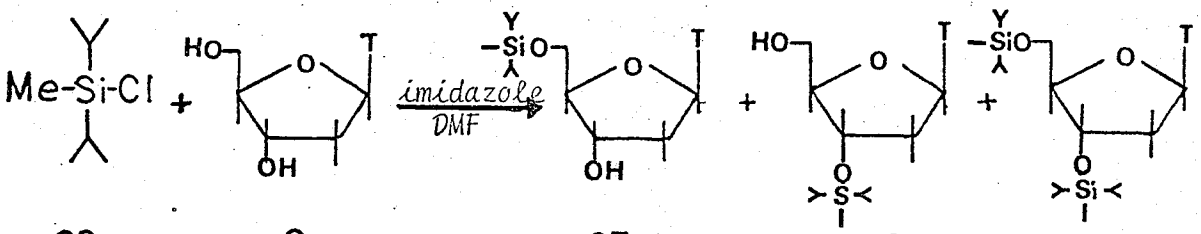
The reaction of this impure MDIPSi chloride with thymidine gave a 59% isolated yield of 5'-alkylsilyl thymidine 27 which was equivalent to a specificity of 64% (8% thymidine remained unreacted). The 5'MDIPSi thymidine 27 was purified by crystallization from ethanol-water and characterized.

The 3'-isomer 28 contained 15% 3'-dimethylisopropylsilyl thymidine as an impurity, as indicated by GC analysis.

Consequently a good melting point of the compound 28 could not be obtained. The 3'5'-disilyl thymidine 29 could not be obtained as a solid at all because of its impurities as well.

SCHEME 23

REACTION OF MDIPSiCl(26) WITH THYMIDINE



26 9 27 28 29

That the 5'-MDIPSi thymidine could be obtained as pure as it was may be due to the instability of the 5'-dimethylisopropylsilyl thymidine to the conditions of recrystallization (heating in aqueous ethanol). The hydrolysis of 5'DMIPSi thymidine would give thymidine which would remain in solution.

Regarding its stability to acid, it was found that the 5'-MDIPSi group was indeed more stable than the methyloctadecylisopropylsilyl group (which is the long chain homologue of the dimethylisopropylsilyl group). The time required for complete hydrolysis in 80% acetic acid at room temperature increased from 10 minutes for MODIPSi to 90 minutes for the MDIPSi group. Even so, this methyldiisopropylsilyl substituent was less stable to acid hydrolysis than Corey's t-butyldimethylsilyl group which was found to require 10 hours to effect its removal under the same conditions. Translating this information

to the case of dodecylsilyl trichloride, it can be deduced that replacing two of the chlorine atoms with a t-butyl and a methyl group would produce a more stable blocking group than would their replacement with two isopropyl groups.

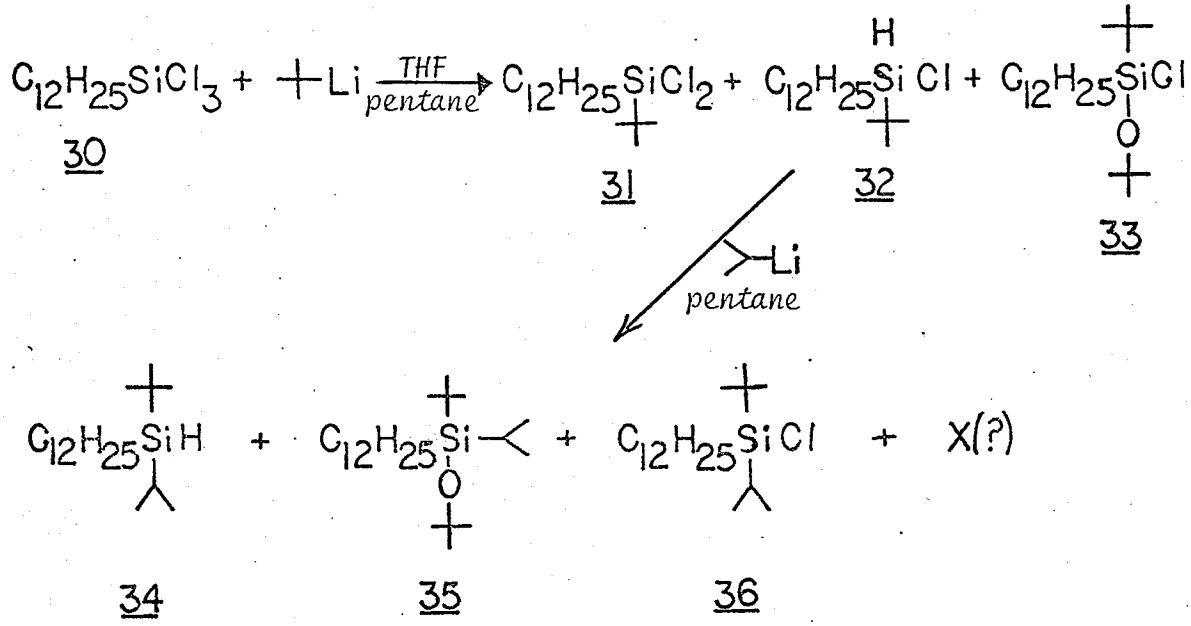
The t-Butyldodecylisopropylsilyl Group

The presence of a tertiary butyl entity on the long chain alkyl silyl chloride appeared essential. Since it had been seen that the formation of inseparable side products made the synthesis of a silyl chloride containing two t-butyl groups a practical impossibility (Scheme 18), it was decided to attempt synthesis of a long chain alkylsilyl chloride containing one t-butyl and one isopropyl group.

The reaction of dodecylsilyl trichloride (30) with t-butyl lithium in pentane and tetrahydrofuran gave the desired t-butyldodecylsilyl dichloride (31) along with side products t-butyldodecylchlorosilane (32) and t-butyl-t-butoxydodecylsilyl chloride (33). Isopropyl lithium was added to the reaction mixture, but absolutely none of the desired t-butyldodecylisopropyl-silyl chloride 36 was formed. Instead, three new products were formed, none of which contained any chlorine atoms. One was determined to be t-butyl-t-butoxydodecylisopropylsilane 35, another was identified as t-butyldodecylisopropylsilane 34, while the third, which had the longest retention time, could not be identified. (Scheme 24)

SCHEME 24

ATTEMPTED PREPARATION OF t-BUTYLDODECYLISOPROPYLSILYL CHLORIDE



30 31 32 33 34 35 36

It was then decided to isolate the t-butyldodecylsilyl dichloride in order to use it in microscale reactions for optimizing conditions for the attachment of the isopropyl group. Unfortunately, fractional distillation was not effective in separating the t-butyldodecylsilyl dichloride from its impurities.

In another experiment, pentane was replaced by heptane and the reaction mixture was heated to the reflux temperature of heptane (98°C) for seven hours in an attempt to effect the addition of the isopropyl group to t-butyldodecylsilyl dichloride Although some t-butyldodecylisopropylsilyl chloride had been formed, the hydride 34 was the major product and many other unidentified side products had appeared, complicating the

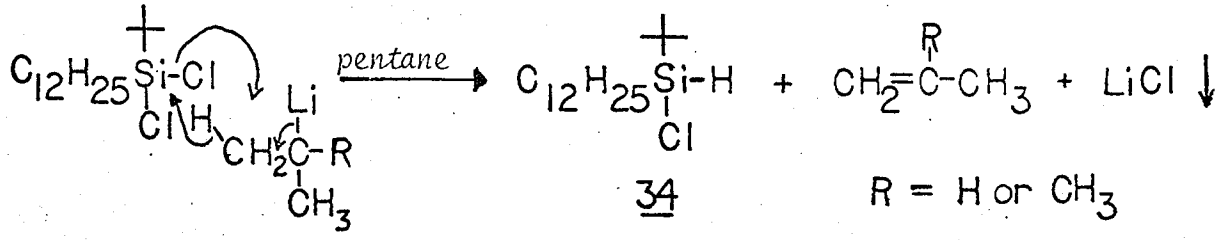
purification of the desired product. When fractional distillation of the reaction mixture was attempted (after filtration to remove lithium chloride), it was found that none of the material would distill over (even under high vacuum) once the heptane had been removed. The whole idea of preparing t-butyldodecylisopropylsilyl chloride was abandoned as being impractical.

Explanations for the Formation of Side Products

It is interesting to speculate on how the hydride 32 and the t-butoxy compound 33 may have been formed in the reaction shown in Scheme 24. The hydride could have arisen by the same manner proposed to explain the formation of di(t-butyl)chlorosilane in Scheme 20. This involves the broadside attack on t-butyldodecylsilyldichloride by t-butyl lithium as shown in Scheme 25 below.

SCHEME 25

PROPOSED MECHANISM FOR T-BUTYLDODECYLCHLOROSILANE (34) FORMATION



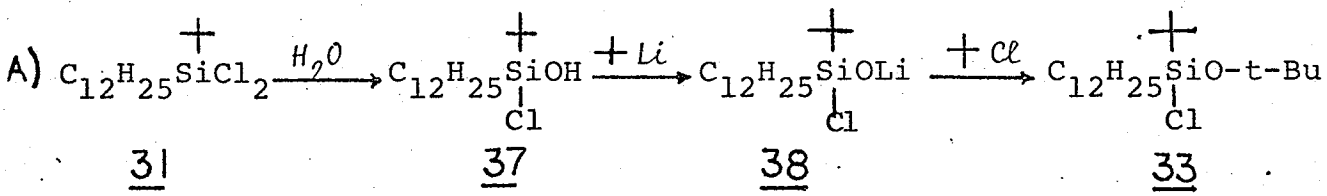
34 R = H or CH_3

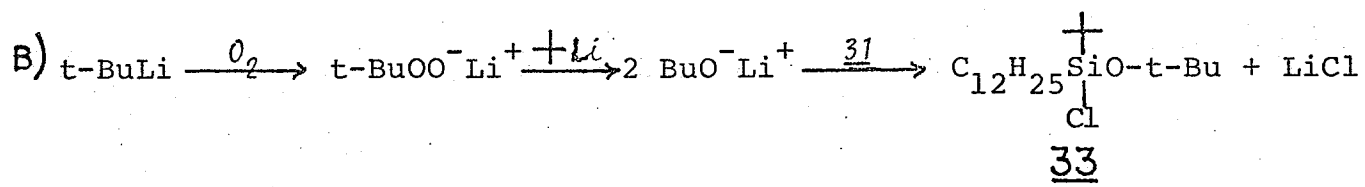
Formation of the t-butoxy compound 33 might involve either water (Mechanism A) or oxygen (Mechanism B) in one of the

following series of reactions. (Scheme 26)

SCHEME 26

TWO POSSIBLE MECHANISMS FOR THE FORMATION OF T-BUTYL-T-BUTOXY-DODECYLSILYL CHLORIDE





Mechanism A requires the presence of small amounts of water which may have been introduced as an impurity in the pentane or the tetrahydrofuran if either had not been completely dry. (Tetrahydrofuran was used in the alkylation reactions of silyl chlorides because of the accelerating effect it has on coupling reactions in which lithium halide is eliminated[87a].) The source of the t-butyl chloride in A would be the t-BuLi. With the preparation of t-butyl lithium from a reaction between lithium metal and t-butyl chloride, it is difficult to avoid the presence of unreacted t-butyl chloride in the product [87a].

Mechanism B is the more likely mechanism, as the storage of the alkyl lithium and its being tranferred into the dropping

funnel could easily lead to at least a brief exposure to the oxygen in the atmosphere. A study into the oxidation of alkyl lithiums conducted in 1939 [87b] revealed that oxygen reacted very vigorously with n-BuLi to give n-butanol in 75% yield. It was presumed that the reaction with oxygen goes through a peroxide intermediate which then oxidizes a second molecule of alkyl lithium to the carbinol and is itself reduced to the carbinol. In mechanism B, the lithium t-butoxide formed could readily react with the silyl dichloride 31 to form the t-butoxy compound 33.

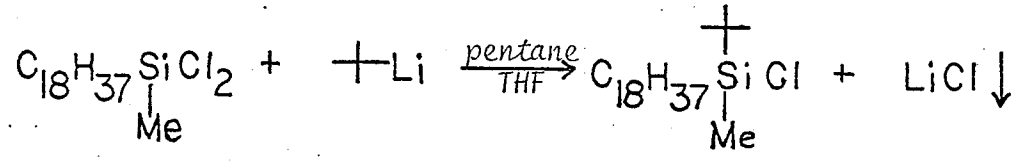
The t-Butylmethyloctadecylsilyl Group

Faced with the failure to prepare in reasonable amounts and in reasonable purity any alkylsilyl chloride containing two t-butyl groups or one t-butyl and one isopropyl group, an expedient alternative was sought. It was felt that the most stable long chain alkylsilyl chloride which could be most practically prepared would be one which contained one t-butyl and one methyl group. Consequently, methyloctadecylsilyl dichloride was treated with t-butyl lithium to yield t-butyl-methyloctadecylsilyl chloride, TBMODSiCl (Scheme 27). One relatively volatile minor side-product (presumably 2,2,3,3-tetramethylbutane from a Wurtz reaction between t-butyl lithium and t-butyl chloride, also observed in Figure 6) and

another unidentified side product with a retention time longer than that of the desired product accounted for approximately 11% of the reaction mixture. The reaction mixture was filtered to remove lithium chloride and the pentane removed on a rotary evaporator. The semi-solid TBMODSi chloride was used as isolated without further purification, as it was found that a sample of the TBMODSiCl could not be distilled even under relatively high vacuum (~5 mm) and at elevated temperatures (~200°) due to the size of the molecule $C_{23}H_{49}SiCl$. As a comparison in carbon chemistry, the molecule tetracosane $C_{24}H_{50}$ which is slightly smaller and considerably less polar than TBMODSiCl ($C_{23}H_{49}SiCl$) has a boiling point of 231°C at 10 mm pressure.[88]

SCHEME 27

PREPARATION OF t-BUTYLMETHYLOCTADECYLSILYL CHLORIDE



7 39

The reaction of TBMODSi chloride with thymidine was complicated by the fact that this silyl chloride was insoluble in dimethylformamide, the solvent used for all previous silylation reactions. The addition of dry tetrahydrofuran to the silyl-chloride-imidazole-thymidine-dimethylformamide mixture eventually

led to homogeneity, but absolutely no reaction occurred between the silyl chloride and thymidine under those conditions. Using only dry tetrahydrofuran as the solvent (i.e., no dimethylformamide) also resulted in no reaction. This was somewhat surprising since Corey had used dry tetrahydrofuran as a solvent for the silylation of prostaglandin E_2. There are two possible explanations for the failure of thymidine and t-butylmethyloctadecylsilyl chloride to react in a solvent containing tetrahydrofuran. One is that the tetrahydrofuran may not have been as dry as it had been thought to be. Another is that a reaction between the silyl chloride and tetrahydrofuran had occurred. Ethers have been reported to undergo cleavage to the corresponding organohalides by halosilanes in the presence of pyridine hydrohalide as a catalyst.[89] In this case, the formation of a small amount of imidazole hydrochloride (from the condensation of several thymidine molecules with the silyl chloride) could have provided the catalysis needed for the ether cleavage reactions which would have destroyed the silyl chloride.

A two-phase reaction system was then devised wherein the thymidine and imidazole were dissolved in dimethylformamide, the t-butylmethyloctadecylsilyl chloride was dissolved in pentane and the two interdispered by rapid stirring of the resulting mixture. Although much of the thymidine remained unchanged, some reaction did occur to yield 5'TBMODSi thymidine in yields ranging from 19 to 25%.

By replacing both imidazole and dimethylformamide by dry pyridine in the previous sytem, a homogeneous system was attained when the silyl chloride dissolved in pentane was added to the reaction mixture. Pyridine acted as the solvent, as the scavenger for hydrochloric acid and as the activator of the silyl chloride. The yield of 5'-TBMODSi thymidine increased slightly to 30%. This was when the ratio of silyl chloride reagent to thymidine was 1.1 to 1. However, it was found that by increasing that ratio to approximately 3 to 1, nearly all the thymidine could be forced to react, resulting in a 76% yield of 5'-TBMODSi thymidine.

It had been suggested that the reason for the low yields of 5'-TBMODSi thymidine might be that the TBMODSi chloride was only 25-35% pure. Such a suggestion has some merit, particularly in view of the fact that 1.1 "equivalents" of the reagent gave 5'-TBMODSi thymidine in 19-30% yields while 3 "equivalents" gave 5'-TBMODSi thymidine in 76% yield, along with a 10% yield of 3'5-di (TBMODSi) thymidine, which together used up a total of 0.96 equivalents of TBMODSi chloride. A re-examination of this reaction with the aid of gas-chromatography-mass spectrometry revealed two reasons for the low yields. First, the reduced polarity of the reaction medium by the addition of pentane (which was necessary to effect solution of the silyl chloride) greatly retarded the reaction rate. Confirmation of this was obtained by observing the very slow reaction rate between

t-butyldimethylsilyl chloride with thymidine under similar solvent conditions. Secondly, examination of the reaction mixture after 24 hours revealed that nearly all the unreacted silyl chloride had been converted to the corresponding silanol. The quality of the "dry" pyridine used was suspect. Prepared by distillation from toluenesulfonyl chloride and from calcium hydride and stored over molecular sieves, the pyridine appeared to have none-the-less absorbed enough water to seriously affect this reaction. By using freshly prepared dry pyridine, the yield of 5'-TBMODSi thymidine was increased to 41 % when 1.1 equivalents of TBMODSiCl were added to thymidine.

Both 5'-t-butylmethyloctadecylsilyl thymidine and 3'5'-di-(butylmethyloctadecylsilyl) thymidine were isolated in the reaction between thymidine and TBMODSi chloride. Both compounds were characterized by melting points, infrared spectrum and elemental analysis. The stability of the 5'-TBMODSi derivatives to acidic and basic conditions was tested. The t-butylmethyl-octadecylsilyl group was found to be somewhat more stable to 80% acetic acid than its homolog, the t-butyldimethylsilyl group and much more stable than methyloctadecylisopropyl group. Thus, when heated on the steam bath in 80% acetic acid, the 5'-MODIPSi group of thymidine was completely removed in 3 minutes and the 5'-TBDMSi group was removed in 15 minutes, but the 5'-TBMODSi group required 60 minutes to effect its complete removal. The 5'-TBMODSi group was unaffected by treatment with 50% ammonium

hydroxide in ethanol.

In summary then, a long chain alkylsilyl chloride has been developed which could be used as a lipophilic handle for nucleic acid synthesis. The t-butylmethyloctadecylsilyl group is reasonably stable to acid and is completely stable in the basic conditions used to remove acetyl groups. The t-butylmethyl-octadecylsilyl chloride exhibits a high specificity for the 5'-hydroxyl groups of nucleosides, renders nucleosides soluble in solvents like ether and causes its nucleoside derivatives to precipitate out in aqueous solutions. Whether the TBMODSi group will act as a soluble or insoluble handle will depend on the reaction conditions. Since 5'-TBMODSi thymidine chromatographs well on silica gel using ether as a solvent (R_f = 0.55), one would expect that this blocking group could maintain desirable lipophilic character and chromatographic mobility on silica gel for oligonucleotides of a considerable chain length. It is being left for others in the future to ascertain if this is so.

Specific Stable Alkylsilyl Blocking Groups

Research was then directed to the development of a specific acid and base stable alkylsilyl blocking group to replace the acid labile trityl or monomethoxy trityl groups in nucleoside chemistry.

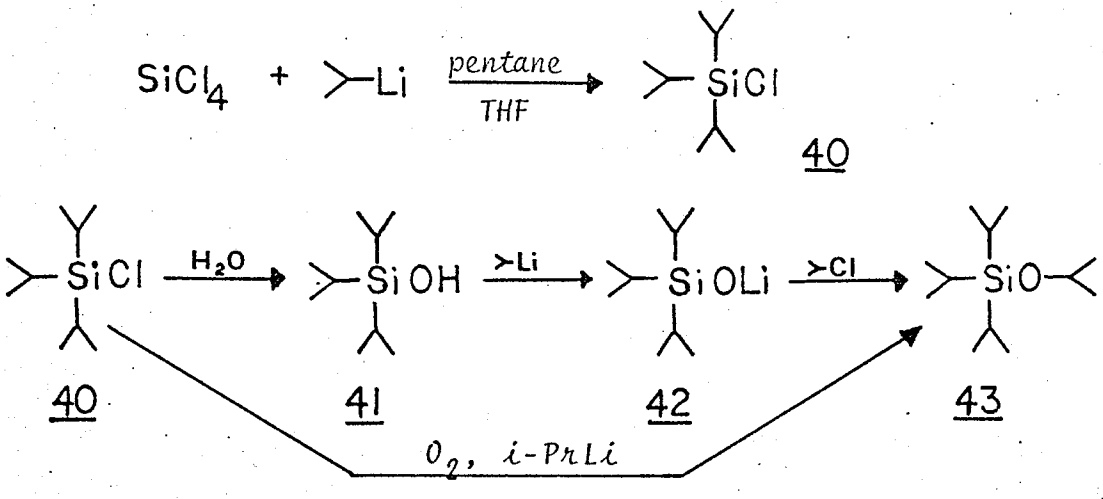
The Triisopropylsilyl Group

After discovering how surprisingly labile to acid the

methyldiisopropylsilyl thymidine had proved to be, it was curious to see how much difference the replacement of the methyl group by another isopropyl group would make. It was found that this change made a large difference in the acid stability and specificity of the group. The synthesis of triisopropylsilyl chloride described in this thesis was a slight modification of the original preparation.[68] Freshly prepared isopropyl lithium was added dropwise into a stirred cooled solution of silicon tetrachloride in pentane and tetrahydrofuran under nitrogen. The progress of the reaction was monitored by gas-chromatography-mass spectrometry. When all the diisopropylsilyl dichloride had reacted with isopropyl lithium, triisopropylsilyl chloride (40) was found to be virtually pure, containing only triisopropylisopropoxysilane (43) as a non-reactive harmless impurity. Fractional distillation yielded triisopropylsilyl chloride whose purity was estimated to be 89.5%. The impurity 43 could have arisen from

SCHEME 28

PREPARATION OF TRIISOPROPYLSILYL CHLORIDE



the reaction of triisopropylsilyl chloride with water to yield triisopropylsilanol (41). This molecule, in turn, would react with any excess isopropyl lithium present to give lithium triisopropylsilanolate 42 which could, in turn, react with any isopropyl chloride present as an impurity in the isopropyl lithium preparation to produce triisopropylisopropoxysilane. Alternatively, reaction between triisopropylsilyl chloride and $i\text{-PrO}^-Li^+$ formed from a reaction between i-PrLi and O_2 could give 43.

Reaction of triisopropylsilyl chloride with thymidine gave 5'-triisopropylsilyl thymidine in an isolated yield of 82%. The specificity of TIPSi chloride for the 5'-position of thymidine was 93%. (The reason for the difference between specificity and isolated yield is the fact that some thymidine remained unreacted in the reaction mixture.)

5'-triisopropylsilyl thymidine was not affected by 50% ammonium hydroxide in ethanol. Complete removal of the 5'-TIPSi group with 80% acetic acid required 2½ hours of heating on the steam bath. Thus it was found that by replacing the methyl group of methyldiisopropylsilyl thmidine with an isopropyl group has the effect of increasing hydrolysis time on the steam bath 50-fold from 3 minutes to 2½ hours.

The Tetramethyleneisopropylsilyl Group

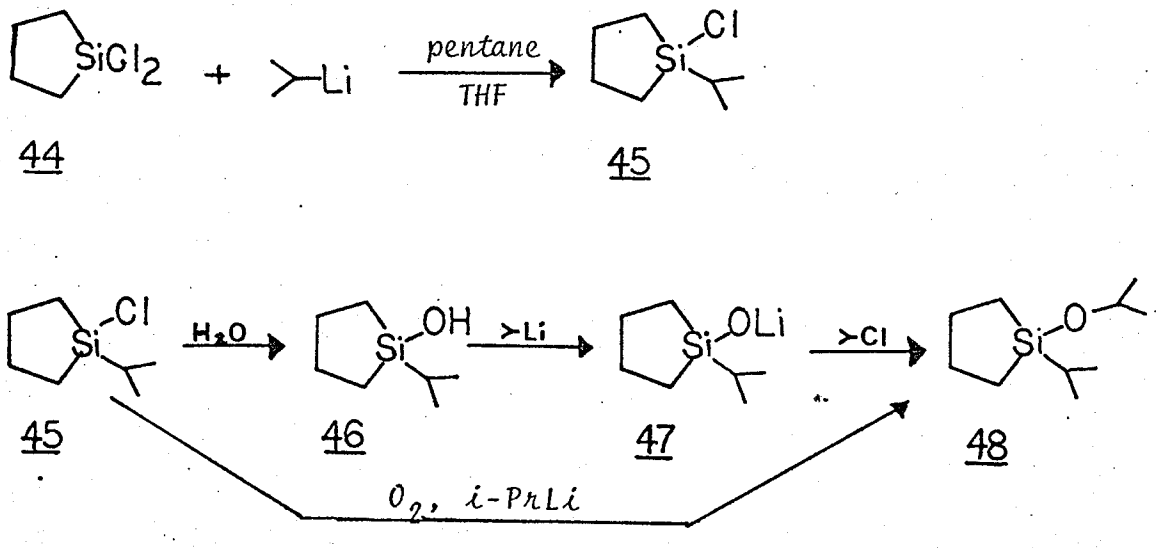
It was felt that the space-filling properties of a cyclic silyl chloride might provide the maximum degree of specificity

and stability for a minimal increase in molecule weight. Such properties are desirable since in general the smaller the silylating agent used in derivatization of a compound for mass spectrometry, the more volatile is the resulting derivative. Higher volatility increases ease of handling in mass spectrometry and maximizes the chance of obtaining the parent ion in the mass spectrum. This in turn simplifies identification of compounds. Thus in the field of nucleoside chemistry, it would be advantageous to find the smallest stable silyl chloride that could act as an specific blocking group and at the same time provide a fairly volatile derivative of the nucleoside.

For those reasons then, tetramethylenesilyl dichloride 44 (or silacyclopentyl dichloride) was used as the starting material for the reaction to replace one of the chlorine atoms with an isopropyl group. (Scheme 29) Freshly prepared isopropyllithium

SCHEME 29

PREPARATION OF TETRAMETHYLENEISOPROPYLSILYL CHLORIDE (TMIPSiCl)



was added dropwise to a stirred, cooled solution of tetramethylenesilyl dichloride in pentane and tetrahydrofuran. The reaction was monitored by gas chromatography-mass spectrometry. Examination of the reaction mixture after all the starting material had been converted to products showed that the desired tetramethyleneisopropylsilyl chloride was accompanied by several sideproducts, of which four were tentatively identified. One was tetramethyleneisopropylisopropoxysilane 48 which could arise by the series of reactions indicated in Scheme 29. The remaining side products included tetramethylene-n-propylsilyl chloride, tetramethylenediisopropylsilane, tetramethyleneisopropylisopropoxysilane, tetramethylenehexylsilyl chloride (due to impure i-PrLi) and several unidentified compounds. (Chlorine-containing compounds were identified by examination of the mass spectrum. As the natural abundance of Cl^{35} and Cl^{37} are 75.5% and 24.5% respectively,[90] the peak of a fragment containing a chlorine is always accompanied by a second peak two mass units higher with approximately one-third the intensity of the first.) Repeated fractional distillation afforded tetramethylenesilyl chloride (TMIPSiCl) which was 89% pure.

The reaction of tetramethyleneisopropylsilyl chloride reagent with thymidine gave 5' - TMIPSi thymidine in 35% yield; the 5'-isomer constituted 40% of the isolated products (i.e.,

specificity ~ 40%).

The acid stability of 5'-tetramethyleneisopropylsilyl thymidine was only slightly higher than that of 5'-methyldiisopropylsilyl thymidine. At room temperature, the 5'-MDIPSi group was completely hydrolyzed by 80% acetic acid in 1½ hours, while the 5'-TMIPSi group required 2 hours for its complete removal. In the steam bath in 80% acetic acid, the 5'-MDIPSi group was off in 3 minutes, while the 5'-TMIPSi group needed 5 minutes for complete hydrolysis. However, neither the stability nor the specificity of the tetramethyleneisopropylsilyl group were anything near that which had been observed for the triisopropylsilyl group.

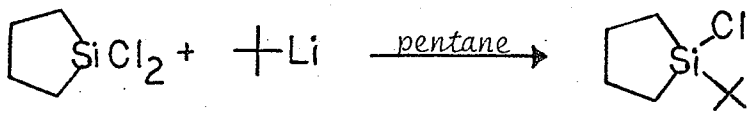
The Tetramethylene-t-Butylsilyl Group

The next step was to replace the isopropyl group of the previous silyl chloride with a tertiary butyl group. T-butyl lithium was added dropwise to neat tetramethylenesilyl dichloride (44) which was cooled and stirred rapidly. Gas chromatography-mass spectral analysis showed the reaction to be very clean, with virtually no side products being formed. Fractional distillation yielded pure tetramethylene-t-butylsilyl chloride (49) at 182-184°C.

Reaction between tetramethylene-t-butylsilyl chloride and thymidine gave 5'-TMTBSi thymidine in an isolated yield of 82%. The specificity of the TMTBSi group for the 5'-hydroxyl function

SCHEME 30

PREPARATION OF TETRAMETHYLENE-t-BUTYLSILYL CHLORIDE (TMTBSiCl)



44 49

of thymidine was found to be 95%. (5'-TMTBSiT made up 95% of the reaction products while the 3'-isomer and the 3'5'-disilyl derivative constituted 1% and 4% of the products respectively.)

Hydrolysis by 80% acetic acid on the steam bath of 5'-TMTBSi thymidine took so long that considerable amounts of acetylated thymidine derivatives were formed in the process. This tended to confuse the monitoring of the disappearance of the silyl thymidine on thin layer chromatography, since 5'-TMTBSi thymidine and some thymidine acetates were found to have similar R_f values. Nevertheless, the time required for complete removal of the 5'-TMTBSi group by 80% acetic acid at 100°C was found to be 37½ hours, considerably longer than that observed for any other silylating agent tested in this study. The tetramethylene-t-butylsilyl group was by far the most acid stable alkyl silyl group prepared.

A much cleaner acid hydrolysis was done using 0.01 N hydrochloric acid on the steam bath. Hydrolysis of 5'-TMTBSi

thymidine under those conditions was complete in 35 minutes. The next most stable group prepared in this series, the triisopropylsilyl group, required only 15 minutes for its complete removal under similar conditions. Although both the TIPSi and the TMTBSi groups have similar specificities for a primary hydroxyl group (93% and 95% respectively), the latter is considerably more stable to acid.

Suggestion for the Stability of the TMTBSi Group

In going from the TMIPSi to the TMTBSi group, a hydrogen atom is replaced by a methyl group. The large change in acid stability resulting from such a small change in the total mass of the blocking group is truly remarkable. This is probably due to steric rather than inductive effects, based on the following discussion.

Steric effect is strongly influenced by β-substitution. Evidence for this is provided in a comparison the relative rate constants for S_N2 halide exchange in acetone for the following series of β-substituted alkyl halides[91] - n-propyl bromide, isobutyl bromide and neopentyl bromide (Figure 7). The large steric effect observed is due to the interaction between the H-atoms of a β-methyl group and the incoming halide ion. With only one methyl group, the interference can be avoided by rotating the C_α-C_β bond (Figure 8), thereby pushing the β-methyl group out of the way of the bromide ion.

FIGURE 7

RELATIVE RATE CONSTANTS FOR S_N2 HALIDE EXCHANGE IN ACETONE (25°C)

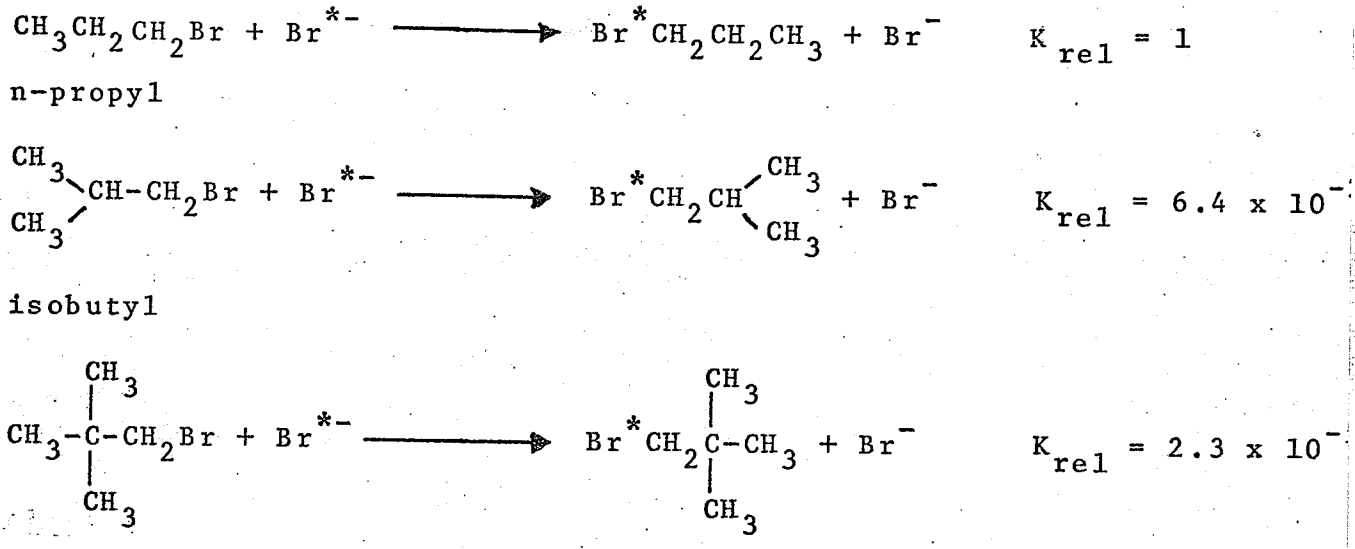
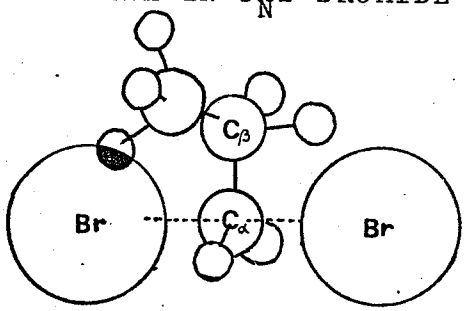


FIGURE 8

ACTIVATED COMPLEX IN S_N2 BROMIDE EXCHANGE



With two β-methyl groups, as in isobutyl bromide, both will try to avoid interaction with the bromide ion, resulting in somewhat restricted rotations of the C_α-C_β bond. This raises the free energy of the transition state. As the free energy of

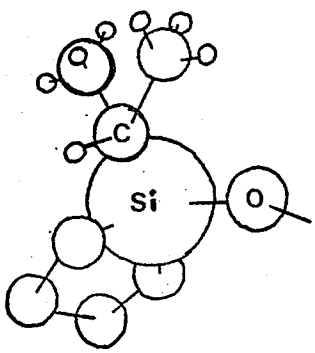
activation for the reaction increases, the rate of S_N2 displacement decreases.

With three β-methyl groups as in the neopentyl situation, there is no way that steric interference can be avoided, and the reaction rate is severely retarded.[91]

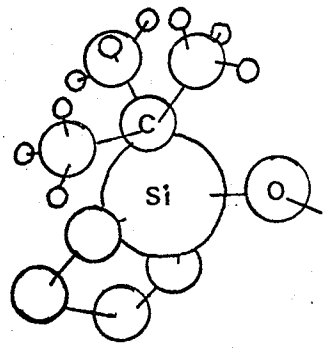
Since the S_N2-Si mechanism is the most common mechanism for polar reactions of molecules of the type R_3SiX,[78] the preceding discussion on the effect of β-methyl groups could apply also to displacement reactions of alkylsilyl compounds. In going from the TMIPSi to the TMTBSi group (Figure 9) the substitution is at the position β to the reaction site and would have the same effects of interference with incoming nucleophiles and restriction of rotation about the Si-C atom as are observed in alkyl halide substitution reactions.

FIGURE 9

COMPARISON OF THE TMIPSi AND TMTBSi ETHERS



TMIPSi-O



TMTBSi-O

The inductive effect of the extra methyl group in the

TMTBSi group would result in a slight increase in the electron releasing ability of the carbon atom next to the silicon atom. This would tend to increase slightly the electron density on the silicon atom, making it less susceptible to nucleophilic attack. However, because the + I effect of alkyl groups is very small and its influence falls off rapidly with increasing distance of the substituent from the reactive site, it is highly unlikely that inductive effects could account for more than a tiny fraction of the increase in acid stability of the TMTBSi group over the TMIPSi group.

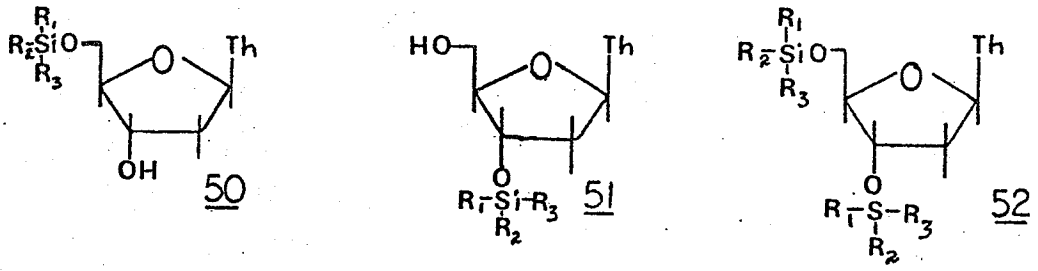
3'- and 3'5'- Di-Silyl Ethers of Thymidine

Previous discussion limited itself to the synthesis, purification and characterization of the 5'- alkylsilyl thymidines prepared in the search for a stable, specific blocking group and for a lipophilic handle for nucleic acid synthesis. This study did, however, include the synthesis of the 3'- isomers and the fully blocked 3'5'- disilyl isomers of thymidine as well. Elemental analysis on these compounds were not performed, but rather, characterization was based on mass spectral fragmentation patterns. The long chain alkyl silyl ethers of thymidine are an exception to the last two statements, as their 3'-isomers had not been prepared at all and in only one case was the 3'5'- disilyl ether isolated and characterized (3'5'- di-TBMODSiT). Since the molecular weight of the latter was too high for characteri-

zation by the departmental mass spectrometer, the structure of 3'5'-di-TBMODSi thymidine was verified by elemental analysis.

FIGURE 10

STRUCTURES OF 3', 5' AND 3'5'-DI-SILYL ETHERS OF THYMIDINE



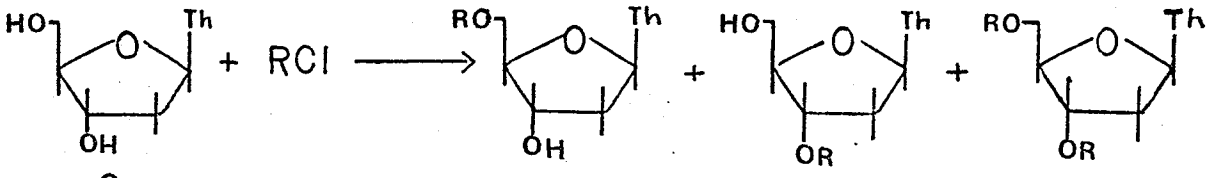
Two different approaches were taken towards the synthesis of the 3' and 3'5' isomers. In the first case, these isomers were isolated in the reactions to prepare 5'-alkylsilyl thymidine by treating thymidine with 1.1 equivalents of alkylsilyl chlorides. (Table 1) Alternatively, the 3'-isomers were obtained by specific synthesis from 5'-trityl thymidine and the 3'5'-disilyl thymidines were prepared by the addition of excess alkylsilyl chloride to thymidine in the presence of imidazole in dimethylformamide.

Hydrolysis of Alkylsilyl Ethers of Thymidine

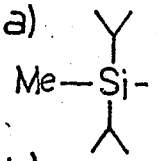
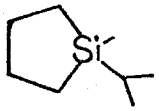
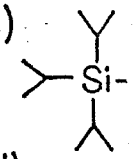
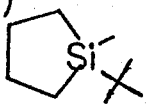
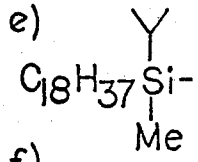
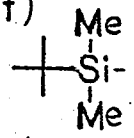
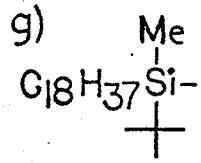
The 5'-alkylsilyl thymidines were tested for their stability under the following conditions: in 80% acetic acid at room temperature and at steam bath temperature (100°C); in 15% and in 50% ammonium hydroxide in ethanol at room temperature for 24 hours and at 60°C for one hour. In addition, the more stable groups

TABLE 1

REACTION OF ALKYLSILYL CHLORIDES (1.1 EQUIV) WITH THYMIDINE



9 50 51 52

Structure	Abbreviation	Isolated Percent Yields 50	51	52
a) 	MDIPSi	59	5	28
b) 	TMIPSi	35 ** (44)*	6** (12)*	46** (14)*
c) 	TIPSi	82 (69)*	2 (3)*	4 (3)*
d) 	TMTBSi	82 (75)*	1 (4)*	3 (3)*
e) 	MODIPSi	26	1	-
f) 	TBDMSi (done for comparison)	73	1	15
g) 	TBMODSi	41	0	2

* Values obtained when the alkylsilyl chloride was added last to the reaction mixture.

** 1.5 equivalents of TMIPSi chloride used.

TABLE 2

HYDROLYSIS OF 5'-SILYL ETHERS OF THYMIDINE

NUCLEOSIDE	REAGENT USED	TEMP	TIME[1]	% HYDROLYZED TO THYMIDINE
5'-MDIPSiT	80% AcOH	RT	1.5 hr	100
		100°	3 min	100
	15% NH_4OH	RT	24 hr	0
		60°	1 hr	0
	50% NH_4OH	RT	24 hr	2
		60°	1 hr	0
5'-TMIPSiT	80% AcOH	RT	2 hr	100
		100°	5 min	100
	15% NH_4OH	RT	24 hr	7
	50% NH_4OH	RT	24 hr	49
5'-TIPSiT	80% AcOH	RT	24 hr	37
		100°	2.5 hr	100
	0.01 N HCl	100°	15 min	100
	15% NH_4OH	RT	24 hr	0
		60°	1 hr	0
	50% NH_4OH	RT	24 hr	0
		60°	1 hr	0
5'-TMTBSiT	80% AcOH	RT	24 hr	0
		100°	37.5 hr	100
	0.01 N HCl	100°	35 min	100
	15% NH_4OH	RT	24 hr	0
		60°	1 hr	0
	50% NH_4OH	RT	24 hr	0
		60°	1 hr	2
5'-MODIPSiT	80% AcOH	RT	10 min	100
	15% NH_4OH	60°	1 hr	4
	50% NH_4OH	RT	24 hr	0
	50% NH_4OH	60°	1 hr	8
	0.5 N NaOH	RT	24 hr	100
5'-TBMODSiT	80% AcOH	100°	1 hr	100
	15% or 50% NH_4OH	RT	24 hr	0
	15% or 50% NH_4OH	60°	1 hr	0
5'-TBDMSiT[2]	80% AcOH	RT	10 hr	100
		100°	15 min	100

[1] Progress of the hydrolysis was monitored carefully by thin layer chromatography.

[2] Done for comparison.

were treated with 0.01 N hydrochloric acid on the steam bath. The results of these experiments are shown in Table 2. Other experiments showed that complete removal of each 5'-alkylsilyl group could be effected in 30 minutes by treatment with tetra-n-butylammonium fluoride in dry tetrahydrofuran.

The 3'-alkylsilylthymidines were tested for their stability under acidic conditions only, since work on the 5'-isomers showed that the alkylsilyl ethers were generally inert to conditions of removal of acetyl groups - namely ethanolic ammonium hydroxide. The results of these experiments are found in Table 3. By comparing the results of Tables 2 and 3, it can be seen that the 3'-isomers are more stable to acid hydrolysis than are the 5'-alkylsilyl thymidines.

TABLE 3

ACID HYDROLYSIS OF 3'-ALKYLSILYL ETHERS OF THYMIDINE

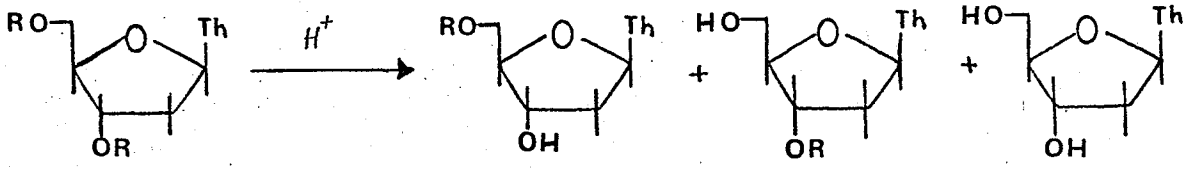
NUCLEOSIDE	REAGENT USED	TEMP	TIME	% HYDROLYZED
3'-MDIPSiT	80% AcOH	RT	6 hr	100
	80% AcOH	100°	1.5 hr	100
	0.01 N HCl	100°	3 min	100
3'-TMIPSiT	80% AcOH	RT	6 hr	100
	80% AcOH	100°	23 min	100
3'-TIPSiT	80% AcOH	RT	24 hr	3.5
	80% AcOH	100°	31 hr	100
	0.01 N HCl	100°	80 min	100
3'-TMTBSiT	80% AcOH	RT	24 hr	0
	0.01 N HCl	100°	2 hr	100
3'-TBDMSiT*	80% AcOH	100°	7.5 hr	100
	0.01 N HCl	100°	6 min	100

*The 3'-t-butyldimethylsilyl thymidine hydrolysis was done for comparison.

Since the alkylsilyl group on the 5'-position of thymidine was more labile under acidic conditions than on the 3'-position, it was expected that acid hydrolysis of the 3'5'-di(alkylsilyl)thymidines would lead to the predominant formation of 3'-alkylsilyl derivatives. The results of such hydrolysis reactions in Table 4 indicate that while more 3'-isomer than 5'-isomer was formed, the relative amounts of 3':5' was not as large as one might expect on the basis of their relative stabilities to acid. The reason for this is that the presence of 3'-substitution increases the stability of the 5'-alkylsilyl group. As an example, it can be seen that although the

TABLE 4

ACID HYDROLYSIS OF 3',5'-DI-ALKYLSILYL ETHERS OF THYMIDINE



52 50 51 9

R	REAGENT USED	TEMP	TIME	% st. mat. and products isolated 52	50	51	9
MDIPSi	80% AcOH	100°	10 min	28	0	6	66
TMIPSi	80% AcOH	RT	7.5 hr	5	13	25	49
TIPSi	0.01 N HCl	100°	2 hr	90	2	5	1
TIPSi	0.10 N HCl	100°	1.5 hr	9	5	25	61
TMTBSi	0.05 N HCl	100°	3 hr	50	6	28	16
TMTBSi	0.10 N HCl	100°	4 hr	6	0	37	57

5'-MDIPSi group is completely removed from monosubstituted thymidine by heating for 3 minutes in 80% acetic acid, 28% of the 5'-MDIPSi group survives when 3'5'-di(MDIPSi)thymidine is heated in 80%

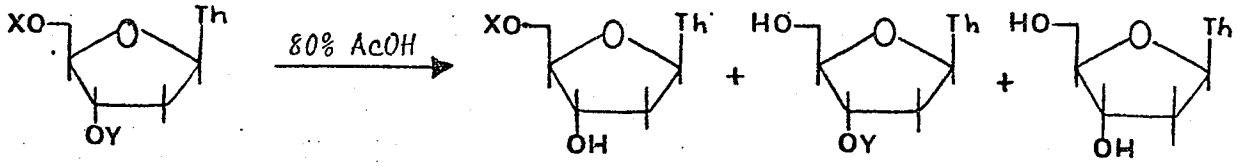
acetic acid for more than 3 times as long a period (10 minutes). Similarly, Khorana[23,25] had found an increase in the stability of the 5'-trityl group when the 3'-position was taken up in a phosphomonoester or phosphodiester bond. It was this difficulty which had led to his development of the more acid labile mono- and di-methoxytrityl blocking groups for nucleotide chemistry.

Using Two Different Alkylsilyl Blocking Groups on the Same Molecule

From Table 2 it can be seen that the alkylsilyl blocking groups prepared in this study showed a wide range of stability to acid conditions. Several molecules containing one labile and one relatively stable alkylsilyl group were prepared. In order to check the compatibility of the two groups, acid hydrolyses on the molecules were done in order to see whether one alkylsilyl substituent could be selectively removed without affecting the other alkylsilyl group on the same molecule. Carefully monitored by thin layer chromatography, each hydrolysis reaction was allowed to proceed just until no more disilyl thymidine was detected (with the exception of one experiment involving 5'-TBDMSi-3'-TMTBSi thymidine which was stopped before hydrolysis was complete). The results of these experiments are in Table 5. Several conclusions can be drawn from the results in Table 5. Firstly, of all the combinations attempted, the TMIPSi and TMTBSi groups are the most compatible. That is, acid removal of the TMIPSi group from the 3'- or 5'-position can be brought about with very little or no effect on the TMTBSi group. Secondly, acid hydrolysis at lowered temperature reduces the

TABLE 5

CID HYDROLYSIS OF THYMIDINE BLOCKED BY TWO DIFFERENT ALKYLSILYL GROUPS



	X	Y	TEMP	TIME	RELATIVE % YIELDS OF THE PRODUCTS 3'Y5'XT	5'XT	3'YT	T
53	TBDMSi	TMTBSi	100°	30 min	25	0	70	5
54	TMTBSi	TBDMSi	100°	2.5 hr	0	79	0	21
55	TMIPSi	TMTBSi	100°	3 min	0	0	100	0
56	TMTBSi	TMIPSi	100°	30 min	0	88	0	12
56	TMTBSi	TMIPSi	RT	18 hr	0	95	0	5
57	TMIPSi	TIPSi	RT	5 hr	0	0	98	2
58	TIPSi	TMIPSi	100°	50 min	0	74	0	26
58	TIPSi	TMIPSi	RT	18 hr	0	83	0	17

amount of thymidine formed in the time required to remove the more labile group. That is, higher temperatures appear to increase the rate of hydrolysis of the less labile groups proportionately more than that of the more labile substituents. Thirdly, the presence of a substituent on the 5'-position increases the stability of the 3'-alkylsilyl group, just as substitution on the 3'-position had generally increased the stability of the 5'-substituent. For example, removal of the 3'-TMIPSi group of the monosubstituted thymidine required 6 hours of 80% acetic acid treatment at room temperature (Table 3). Complete removal of the same group from 5'-TMTBSi-3'-TMIPSi thymidine 56 required 18 hours of reaction time under the same conditions. A rather curious observation involving this same molecule (5'-TMTBSi-3'-TMIPSi thymidine) is that after 18 hours in 80% acetic acid at room

temperature 5% of the 5'-TMTBSi group has been hydrolyzed (Table 5) while treatment of 5'-TMTBSi thymidine 50d with 80% acetic acid for 24 hours at room temperature had shown no hydrolysis of the 5'-TMTBSi group whatsoever (Table 2).

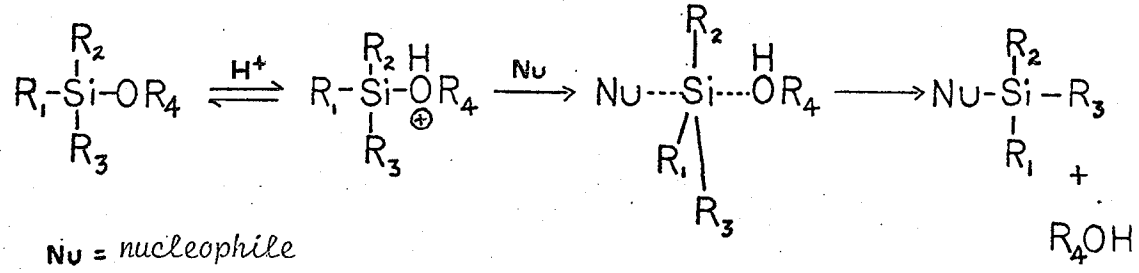
Mechanism of Si-O Bond Cleavage

Although experiments designed to elucidate the mechanism of alkylsilyl group removal by acetic acid and by tetra-n-butylammonium fluoride were not included in this study, some discussion on the matter is still in order.

Acid hydrolysis of alkylsilyl ethers would be expected to involve initial protonation of the oxygen atom of the Si-O bond. Subsequent cleavage of the Si-O bond might then follow any one of the following mechanisms - S_N1-Si, involving the formation of a siliconium ion; S_Ni-Si - which results in the formation of a quasi-cyclic transition state; S_N2-Si, which, in the case of acyclic compounds proceeds via a backside attack on the reacting silicon atom to give a trigonal bipyramid transition state similar to that observed in the S_N2 reactions of carbon; and S_N2^*-Si which involves the formation of a pentacovalent silicon intermediate.[78] Since the S_N2-Si mechanism is most commonly observed mechanism for polar reactions of molecules of the type R_3SiX,[78] it is also the most likely mechanism involved in the acid hydrolysis of alkylsilyl ethers, the oxonium ion acting as an effective leaving group (Scheme 31). The nature of the

SCHEME 31

SUGGESTED MECHANISM FOR ACID CATALYZED Si-O BOND CLEAVAGE

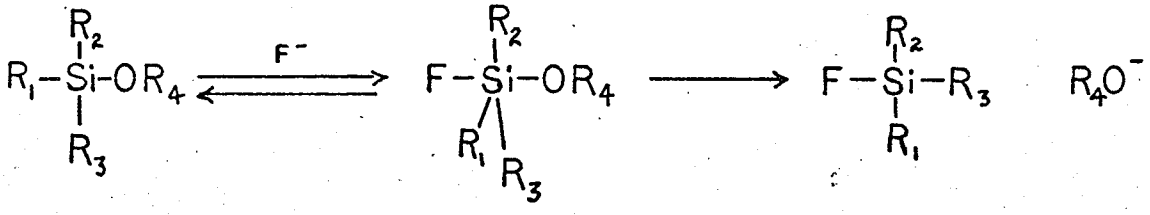


nucleophile involved in the reaction in Scheme 31 has not been determined.

As for the cleavage of the Si-O bond by tetra-n-butylammonium fluoride in tetrahydrofuran, an $S_N i$-Si or an $S_N 2^*$-Si mechanism involving a pentacovalent intermediate cannot be ruled out. Kinetically, the two mechanisms cannot be differentiated.[78] Silicon forms its strongest bonds with fluoride atoms[77] and its next strongest bond is with oxygen. Consequently, R_4O^- constitutes a poor leaving group, while the fluoride ion acts as a very powerful nucleophile. The end result of this might well be the formation of a pentacovalent intermediate as shown in Scheme 32.

SCHEME 32

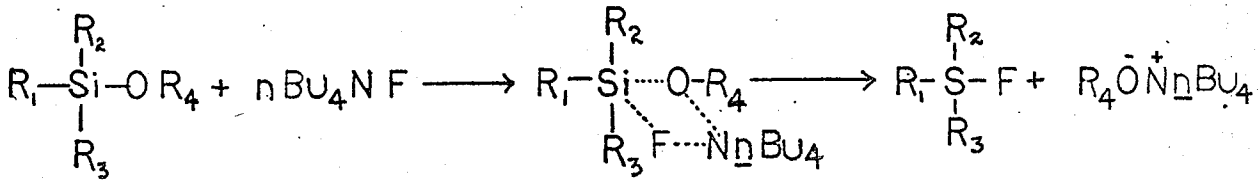
AN $S_N 2^*$-Si MECHANISM FOR Si-O BOND CLEAVAGE BY FLUORIDE ION



Alternatively, the departure of the poor leaving group may be facilitated through the formation of a four-centred transition state with the tetra-n-butylammonium ion providing extra pull for cleavage of the Si-O bond. (Scheme 33)

SCHEME 33

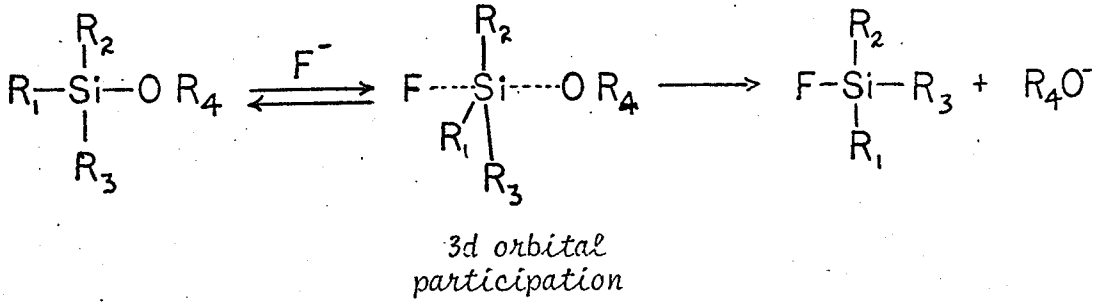
S_Ni-Si MECHANISM FOR Si-O BOND CLEAVAGE BY FLUORIDE ION



However, the most likely explanation for the rapid cleavage of the Si-O bond by tetra-n-butylammonium fluoride might be the lowering of the free energy of activation by 3d orbital participation in an S_N2-Si mechanism. (Scheme 34)

SCHEME 34

S_N2-Si MECHANISM FOR Si-O BOND CLEAVAGE BY FLUORIDE ION



One could differentiate mechanism S_Ni-Si from S_N2-Si and S_N2^*-Si by an examination of the resulting alkylsilyl fluoride. If the silicon atom in Schemes 32 to 34 were a chiral centre, the S_Ni-Si scheme would lead to retention while the S_N2-Si and S_N2^*-Si mechanisms would give inversion of configuration.

Differentiation Between 3'- and 5'- Alkylsilyl Thymidines

Thin layer chromatography of the various reactions between thymidine and alkylsilyl chlorides has shown that, without exception, the 3'- alkylsilyl derivatives of thymidine move faster on silica gel TLC developed in ether than do the 5'- alkylsilyl isomers. That these faster moving compounds were indeed the 3' isomers was established first by their unequivocal preparation from 5'- trityl thymidine and secondly by their mass spectral fragmentation patterns. It had been previously observed that the 3'- and the 5'-t-butyldimethylsilyl derivatives of thymidine had characteristically different mass spectra.[92] Examination of various 3'- and 5'- alkylsilyl thymidines prepared in this study revealed that the characteristic fragmentation patterns observed in TBDMSi derivatives are also found in other alkylsilyl derivatives. In all spectra, the molecular ion is not of significant abundance and is often completely missing. The highest mass peak of appreciable intensity occurs at $[M-57]^+$ for molecules containing a t-butyl group (tetramethylene-t-butyl silyl and t-butylmethyloctadecylsilyl groups) and at $[M-43]^+$ for entities possessing an

isopropyl-containing group like the methyldiisopropylsilyl, triisopropylsilyl, tetramethyleneisopropylsilyl and methyloctadecylisopropylsilyl groups. The most obvious ions used to differentiate 5'-isomers from 3'-isomers are those resulting from two successive losses of H_2O from the $[M-57]^+$ or the $[M-43]^+$ fragments, to give $[M-75]^+$ and $[M-93]^+$ ions or $[M-61]^+$ and $[M-79]^+$ ions respectively. These are much more prominent in the mass spectra of 5'-isomers than in the spectra of 3'-isomers. (Table 6, Figures 11 and 12, Scheme 35)

TABLE 6

Partial Mass Spectra of 5'- and 3'-Alkylsilyl Thymidines Showing Relative Intensities of some Diagnostically Important Ions

		NORMALIZED INTENSITY OF IONS						BASE
UCLEOSIDE	M.WT.	$[M-43]^+$	$[M-61]^+$	$[M-79]^+$	$[M-57]^+$	$[M-75]^+$	$[M-93]^+$	PEAK *m/e*
;' MDIPSiT	370	6	36	13				81
3' MDIPSiT	370	9	0.7	0				129
;' TIPSiT	398	4	3.0	13				81
3' TIPSiT	398	3	0.1	0				173
;' TMIPSiT	368	7	25	4				157
3' TMIPSiT	368	7	4	0				99
;' TMTBSiT	382				6	31	8	81
3' TMTBSiT	382				15	0	0	153
;' TBDMSiT[92]	356				7	30	6	81
3' TBDMSiT[92]	356				10	0.5	0	75

Experiments using deuterium labelled derivatives in order to determine the nature of these and other possibly diagnostic fragments are presently under way in the laboratory of J. B. Westmore at the University of Manitoba.

FIGURE 11

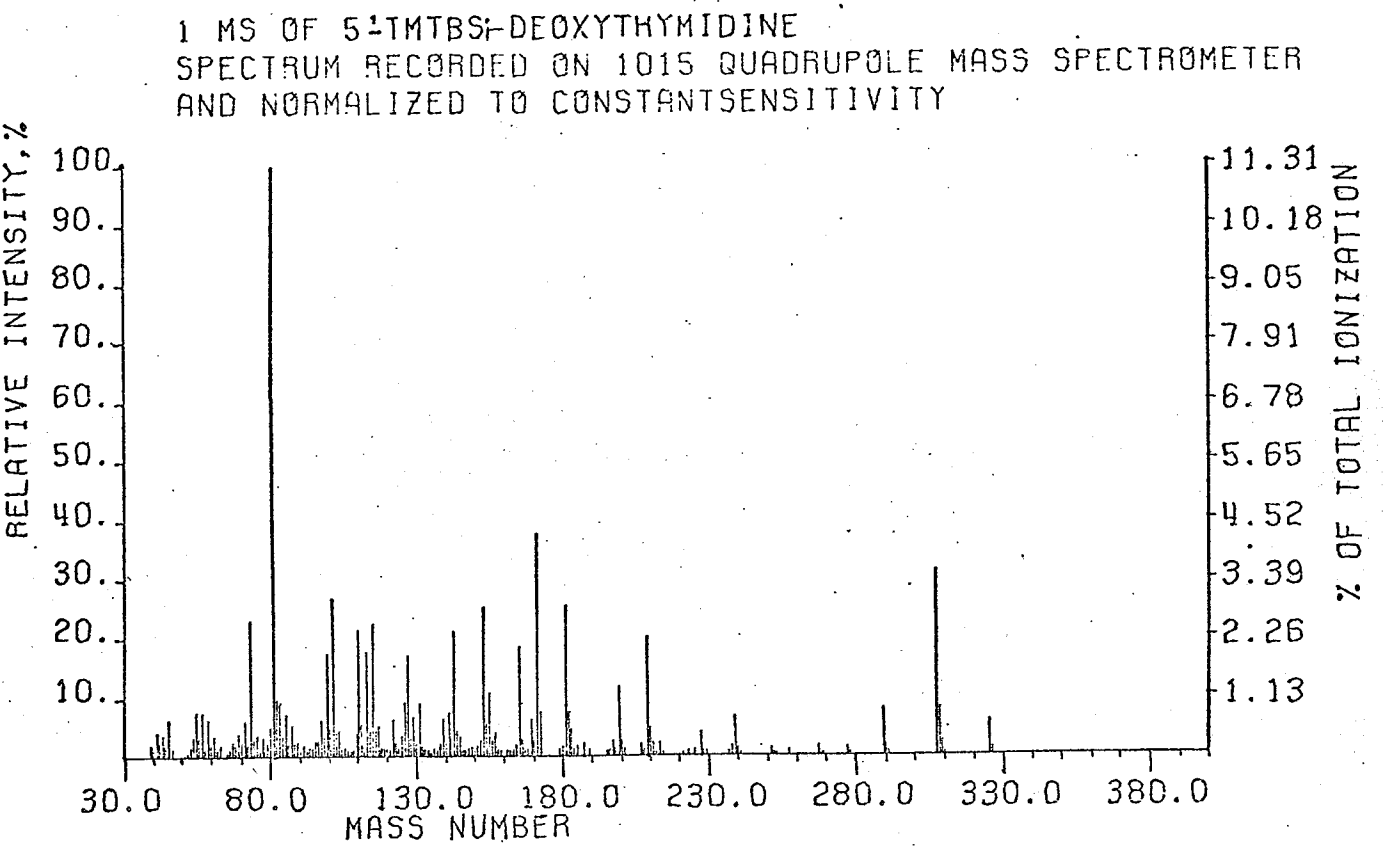
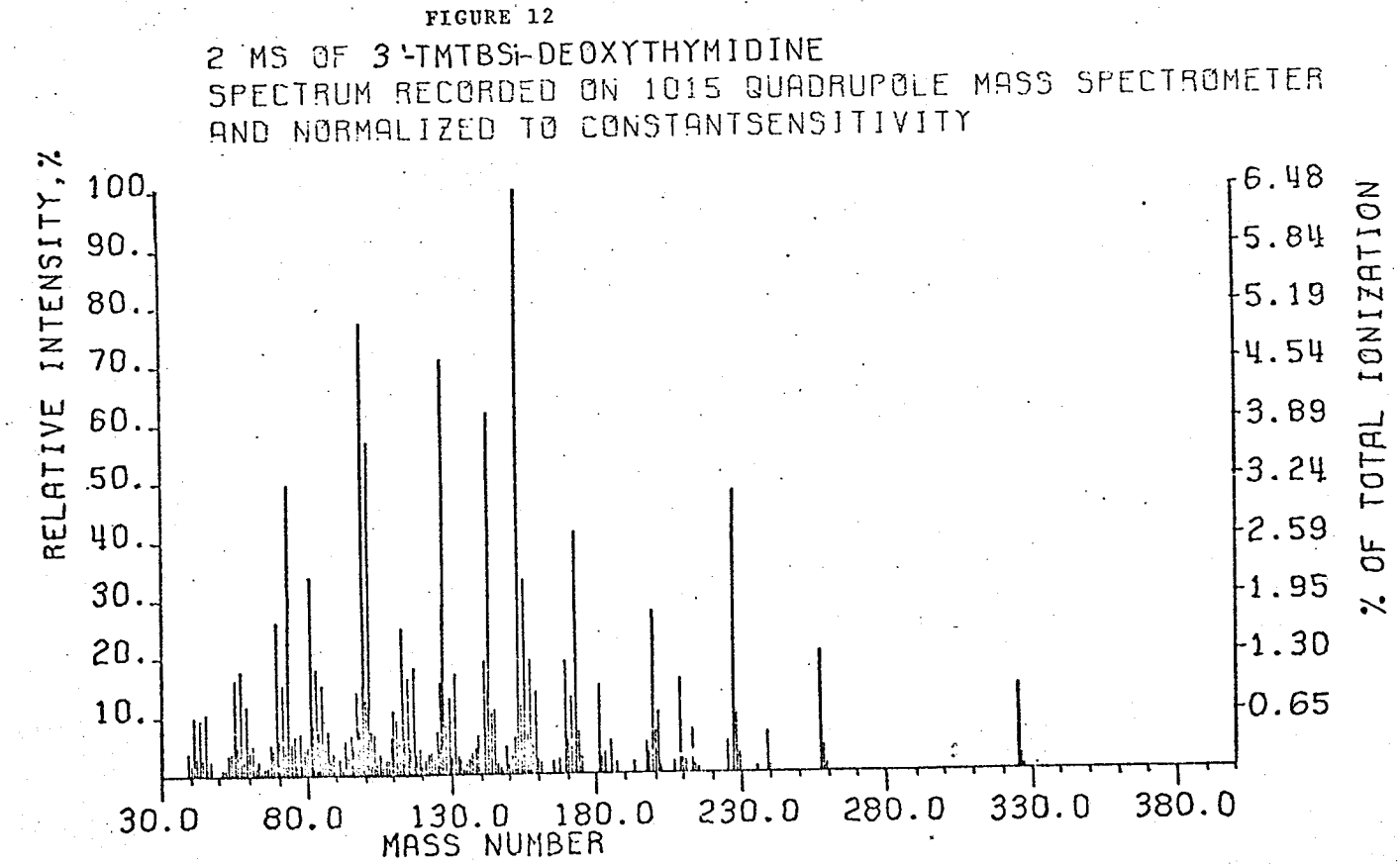
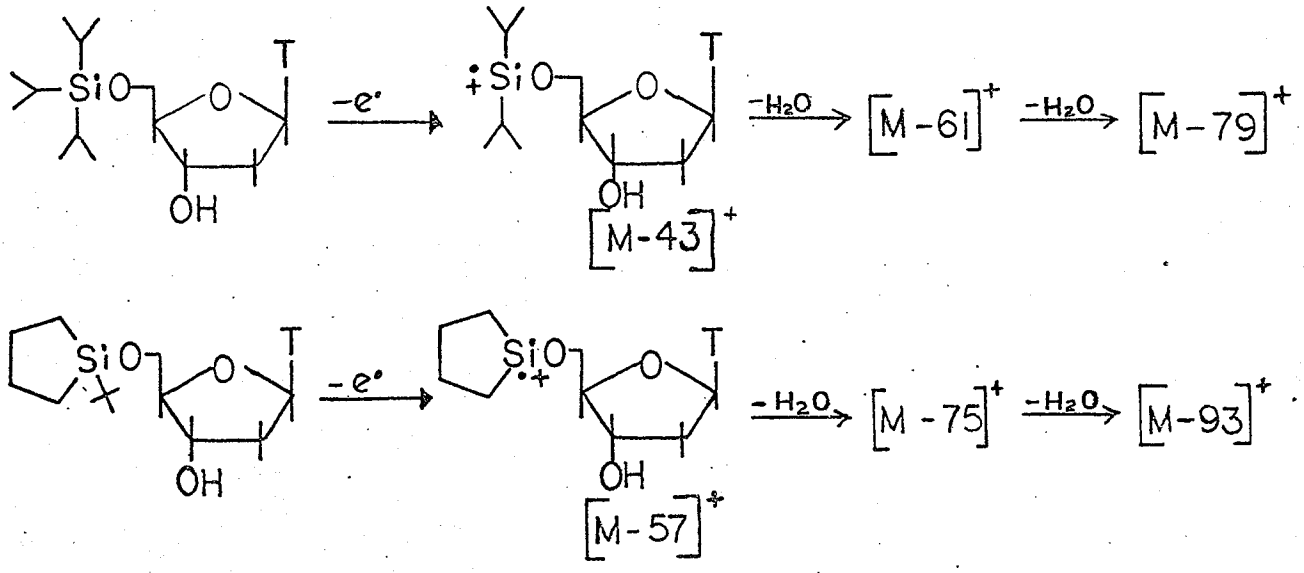


FIGURE 12



SCHEME 35

MASS SPECTRAL FRAGMENTATION OF A 5'-ALKYLSILYL DERIVATIVE OF THYMIDINE



Alkylsilyl Dichlorides as Cis-Glycol Blocking Groups

Encouraged by the stability of some of the 5'-alkylsilyl blocking groups, it was felt that alkylsilyl dichlorides might provides chemists who are involved in polyribonucleotide synthesis with an acid- and base-stable protecting group for the 2',3'-cis-glycol system of ribonucleosides. The removal of such a blocking group, like the removal of the trialkylsilyl groups, would be effected by tetra-n-butylammonium fluoride -

neutral conditions which would not affect any other blocking groups on the molecule.[79]

Di-t-Butylsilyl Dichloride

It would be expected that the most stable silyl dichloride that could be used for cis-glycol protection would be di-t-butylsilyl dichloride. Although this reagent had reportedly been prepared in 1948,[81] it was found that attempts to repeat that work led to the formation of side products (compound 15 and 16 in Scheme 19), which would not be separated from the desired product by fractional distillation.

Tetramethylenesilyl Dichloride

Uridine and 5'-trityl uridine were treated with commercially available tetramethylenesilyl dichloride under two different solvent conditions -- ether in the presence of triethylamine or dimethylformamide in the presence of imidazole. Thin layer chromatography of the reaction mixture in each case indicated the presence of a complex mixture of products.

Diisopropylsilyl Dichloride

Diisopropylsilyl dichloride, an intermediate in the preparation of triisopropylsilyl chloride, was obtained in 80% purity by a series of fractional distillations from the reaction mixture. Impurities included triisopropyl silyl chloride (2.5%), hexylisopropylsilyl dichloride (1.5%), hexylsilyltrichloride (4%) and

three unidentified compounds adding up to 12%. (The hexyl group appeared in the products following the addition of commercially obtained isopropyl lithium after the supply of freshly prepared isopropyl lithium had run out.)

The reaction of trityl uridine with diisopropylsilyl dichloride in ether and triethylamine appeared to result in the formation of a new compound whose R_f value was approximately twice that of trityl uridine. This compound was separated from unreacted trityl uridine by thin layer chromatography and purified by crystallization from ethanol-water to give a white solid of melting point 247-248.5°C. Examination by mass spectrometry indicated that fragmentation of the trityl group dominated the spectrum. Elemental analysis of the product showed it to be a monohydrate of 2',5'-ditrityluridine. That its melting point is different from that reported in the literature[93] (223-4°C) can be accounted for by the fact that a different solvent was used for recrystallization. Levene and Tipson had recrystallized their ditrityl uridine from benzene-ether whereas the higher melting ditrityl uridine had been recrystallized from aqueous ethanol. Reexamination of the trityl uridine which had been used in the reaction with diisopropylsilyl dichloride revealed that some ditrityl uridine was present as an impurity. It had been reported by Levene and Tipson that a small amount of ditrityl uridine is always found in the preparation of trityl uridine.[93] Clearly then, when diisopropylsilyl dichloride had been added to the trityl

uridine - triethylamine - ether suspension, it must have been destroyed by some impurity in the reaction mixture. Gas chromatographic examination of commercially obtained triethylamine, done some time after these experiments, revealed that the triethylamine contained many unidentified impurities, some of which caused a precipitate to form in the presence of an alkylsilyl chloride. Repeated fractional distillations were necessary in order to remove the interfering impurities.[94]

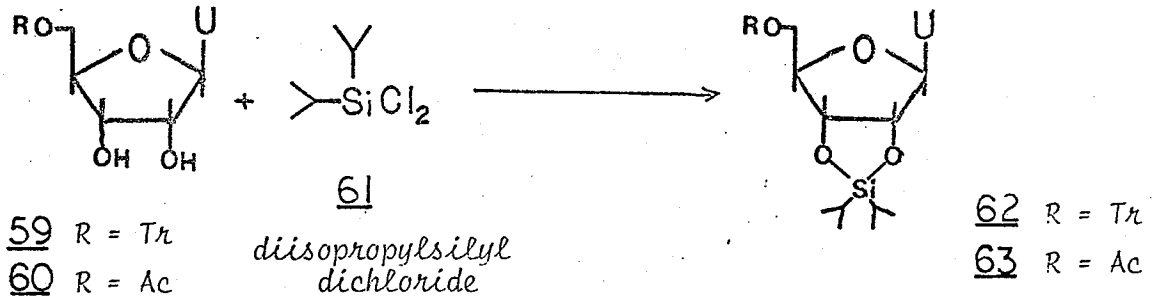
Another reaction between trityl uridine and diisopropylsilyl dichloride performed in dimethylformamide in the presence of imidazole gave a number of products, but one fast moving compound (on TLC in ether) was the major product. It was separated from the rest of the products by silica gel thin layer chromatography. An attempt to crystallize this compound from aqueous ethanol led to its decomposition into seven different products. Thus, if the major compound obtained in this reaction was indeed the desired 5'-trityl-2',3'-O-diisopropylsilyl uridine, then it appears that this compound is unstable.

Following this, 5'-acetyl uridine (prepared from 2',3'-O-isopropylidine uridine) was treated with the diisopropylsilyl dichloride in the presence of imidazole in dimethylformamide. Because of the higher volatility of 5'-acetyl uridine as compared with 5'-trityl uridine, this reaction mixture could be analyzed by gas chromatography and mass spectrometry. The major product isolated when the reaction mixture was applied to silica gel plates

developed in ether was identified by gas chromatography-mass spectrometry as 5'-acetyl-2',3'-O-diisopropylsilyl uridine 63. However, attempts to purify the compound by crystallization from ether-hexane or from aqueous ethanol led to its decomposition to four different compounds (unidentified).

SCHEME 36

REACTION BETWEEN DIISOPROPYLSILYL DICHLORIDE AND 5' SUBST. URIDINE



59 R = Tr
60 R = Ac

62 R = Tr
63 R = Ac

This instability of the 5'-trityl and 5'-acetyl derivatives of 2',3'-O-diisopropylsilyl uridine is rather unfortunate, as it provides a serious limitation to the usefulness of this symmetrical silyl dichloride as a cis-glycol blocking group. In view of previous observations regarding isopropyl and t-butyl groups, one could guess that a more stable silyl dichloride might be t-butylmethylsilyl dichloride. It has the disadvantage, however, of having a chiral silicon atom which might lead to difficulties in crystallization of a mixture of diastereoisomers.

RESULTS AND DISCUSSION

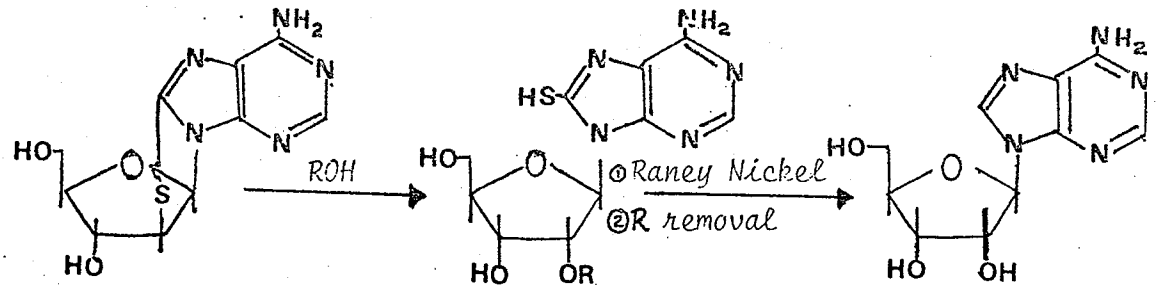
PART B - REACTIONS OF 8,2'-THIOANHYDROADENOSINE (8,2'SAnA)

Preamble

Nucleophilic displacement reactions on 8,2'SAnA (64) were attempted in order to effect transformation of the anhydronucleoside into adenosine (66) through an intermediate such as 8-thioadenosine (65), for example.

SCHEME 37

CONVERSION OF 8,2'-S-ANHYDROADENOSINE TO ADENOSINE



64 65 66

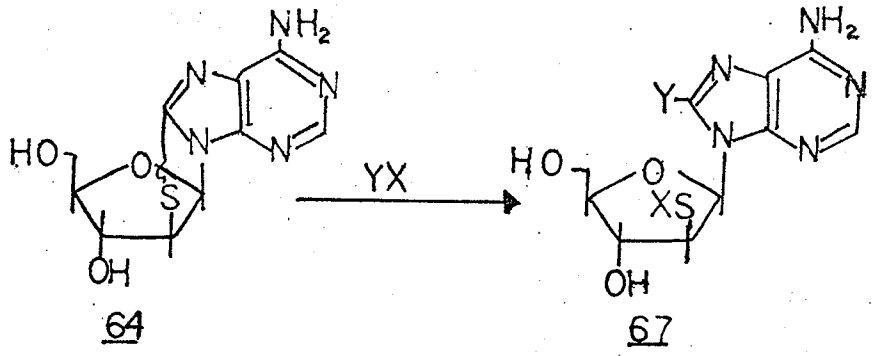
The reason behind this work was the hope that by using thioanhydropurines which lack the complicating 2'-hydroxyl group of ribonucleosides, one could synthesize an RNA precursor using methods developed for DNA synthesis. Following this, ribonucleic acid (RNA) might be generated by nucleophilic displacement of the thioanhydrolinkage followed by Raney nickel desulfurization of the resulting molecule. 8,2'-thioanhydroadenosine was used as the test system for this novel approach to RNA synthesis.

Outline of the Problem

It must be kept in mind that the reaction in Scheme 36 represents the ideal, desired nucleophilic attack at C-2'. It is also quite possible that the nucleophile might attack at the C-8 position (Scheme 37) to give an 8-substituted-2'-thioderivative of deoxyadenosine (67) or at the sulfur atom (Scheme 39) to give molecules of the type 68 or 67. If Y was a reasonably good leaving group, then molecule 68 would be

SCHEME 38

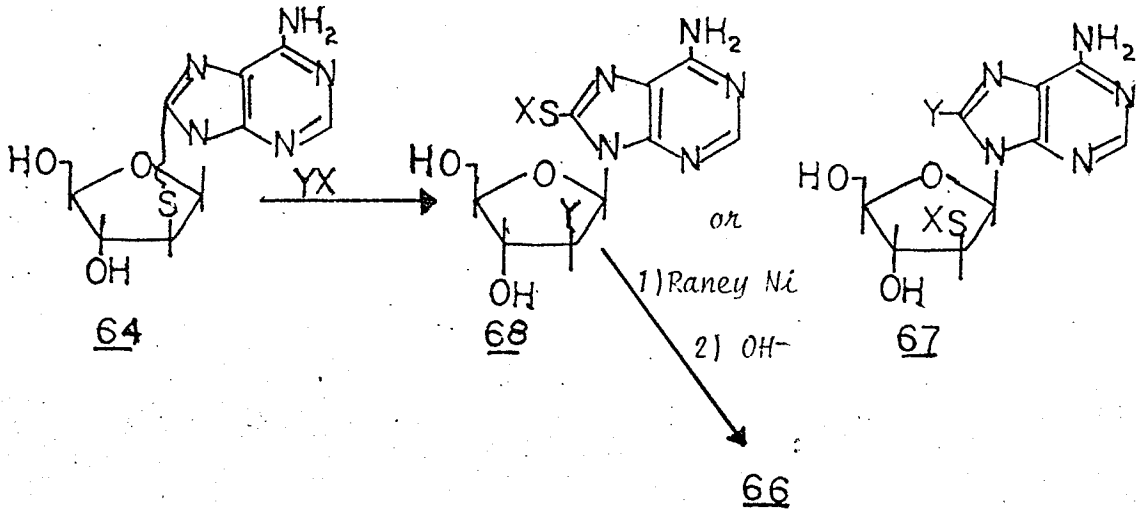
NUCLEOPHILIC ATTACK AT C-8 POSITION OF 8,2'SAnA



64 67

SCHEME 39

NUCLEOPHILIC ATTACK ON SULFUR ATOM OF 8,2'SAnA



64 68 67

66

treated with Raney nickel to give 2'-Y-arabinoadenosine which might be subjected to nucleophilic attack by base for example to give the desired adenosine molecule 66.

Using UV Spectrometry to Examine Reaction Products

When the products of a nucleophilic displacement reaction on 8,2'-thioanhydroadenosine were examined by UV spectrometry, compounds of the type 65 with an 8-thioadenosine-type chromophore and of the type 68 with an 8-SX adenosine chromophore were sought. In water, 8-thioadenosine has UV maxima at 298 and 229 nm and a shoulder at 304 nm[120] in the UV spectrum. It is somewhat more difficult to predict what the UV maximum of an 8-SX adenosine chromophore would be. Generally, the addition of an alkyl, acyl, alkenyl or a nitrile group to a sulfide attached to a UV-absorbing system raises the wavelength of the UV maximum (Table 7). However, in the case of 8-thioadenosine, methylation or replacement of hydrogen with a cyano group appears to lower the UV maximum of the molecule. (Table 8). Thus, whereas one might expect that replacing a methylthio group by a thiocyanate group in the 8-position of adenosine would tend to increase the wavelength of the UV maximum of the molecule, there is, in fact, virtually no change observed. Thus molecules of the type 68 (Scheme 39) might be expected to have a UV maximum around 280 nm (where X is other than H).

TABLE 7

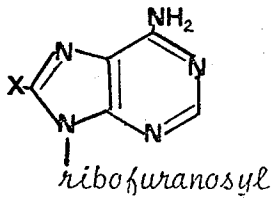
ULTRAVIOLET ABSORPTION CHARACTERTERISTICS OF SULFUR-CONTAINING MOLECULES[98]

COMPOUND	SOLVENT	λ max *
Alkyl Sulfides		
C_2H_5SH	H_2O	193.5, 225
$C_2H_5SCH_3$	EtOH	210 (229)
CH_3SCH_3	EtOH	210 (229)
$(n\text{-}C_4H_9)_2S$	EtOH	210 (229)
$CH_3SCH=CH_2$	EtOH	230, 240
$C_4H_9SCCH_3$ (O)	MeOH	231
Aryl Sulfides		
C_6H_5SH	benzene	236
	EtOH	237[99]
$C_6H_5SCH_3$	EtOH	254 (275)
$C_6H_5SCH=CH_2$	EtOH	247,266
$C_6H_5SC=N$	H_2O	230,260
Alkenyl Sulfides		
$CH_2=CHSCH_3$	EtOH	230, 240
$CH_2=CHSCH=CH_2$	dioxane	240, 255

*values in parenthesis are inflexions rather than clear maxima

TABLE 8

ULTRAVIOLET ABSORPTION CHARACTERISTICS OF 8-SUBSTITUTED ADENOSINE



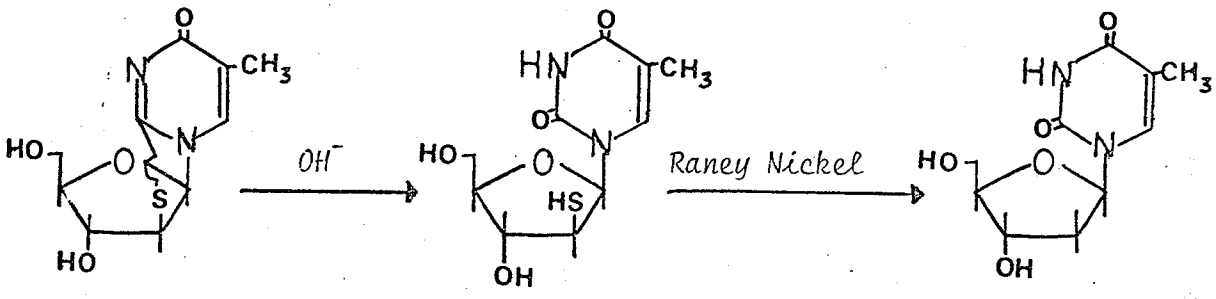
	X	SOLVENT	λ max (nm)	ref
66	H	EtOH	260	88c
70	SH	pH 1 pH 11	308,240,222 297,230	} 99
71	SCH_3	pH 1 pH 11	291 279	} 99
72	SCN	pH 1 pH 7	279 281	} 100
73	OH	pH 1 pH 11	284,264 280	} 101
74	OCH_3	pH 1 pH 11	261 259	} 101

Previous Reactions on the Thioanhydro Linkage

Very little work had previously been done on the displacement of a thioanhydrolinkage in nucleosides. Pyrimidine derivatives that had been investigated included 2,2'-thioanhydrothymidine[49] (75), 2,3'-thioanhydrothymidine[102] and 6,5'-thioanhydrouridine.[103] In each case, displacement of the thio-anhydro linkage under alkali conditions was from the pyrimidine side of the C-S bond, leaving the sulfur atom on the sugar portion of the nucleoside, as in Scheme 40. Raney nickel desulfurization of these molecules leads to the formation of deoxynucleosides.

SCHEME 40

NUCLEOPHILIC DISPLACEMENT ON 2,2'-THIOANHYDROTHYMIDINE BY BASE



75 76 77

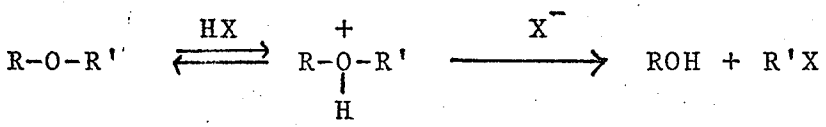
A number of purine 8,2'-, 8,3'- and 8,5'-thioanhydronucleosides have been synthesized and their properties investigated,[57,104-109] but they were found in general to be resistant toward mildly acidic or alkaline hydrolysis.[109] Treatment with strong acid or base led to the degradation of the nucleoside. The only reaction which could be readily performed on thioanhydropurine is the desulfurization by Raney nickel to the corresponding deoxynucleosides[57, 104-109]. There were two reported examples of oxidation with N-bromosuccinimide to form the sulfoxide of 8,3'- and 8,5'-thioanhydroadenosine.[110,111]

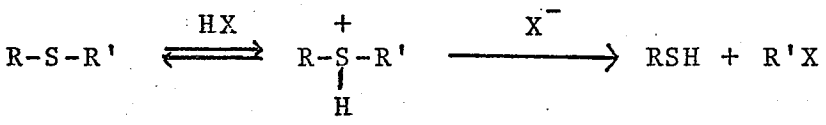
Nucleophilic Displacements on Thioethers

In general, thioethers are cleaved much less readily than ethers both with acidic and with basic reagents. Also, sulfides are much less basic than the corresponding ethers and form the conjugate acid less easily in polar acids. Consequently, the S - R bond is less readily cleaved by nucleophilic

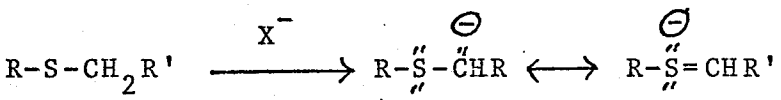
SCHEME 41

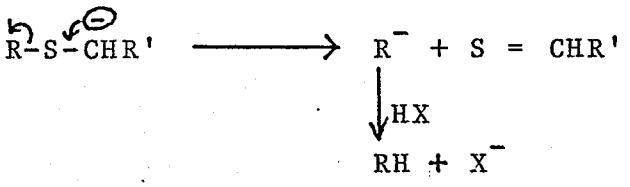
MECHANISM FOR ACID CATALYZED ETHER AND THIOETHER CLEAVAGE





displacement.[112] However, under forcing conditions, a base can remove a proton α to the sulfur atom.[113]





This proton abstraction is facilitated by the resonance stabilization provided by sulfur d-orbitals of the resulting carbanion.[98b] The carbanion then undergoes R-S cleavage.[114]

Alternatively, C-S cleavage may be effected by the attack of a base on the sulfur atom, particularly if the sulfur atom is rendered electron deficient by its proximity to a carbonyl or other

electron withdrawing group.[115] The ability of causing C-S

$$R\text{-}S\text{-}Z \xrightarrow{X^-} R^{\ominus} + ZSX \xrightarrow{HX} RH + ZSX + X^-$$

fission in this manner depends not on the base strength, but rather on the thiophilicity of the attacking base.[116] Thus hydroxide and ethoxide ions are ineffective in the cleavage of the C-S bond of β-carbonyl sulfides; only highly polar nucleophiles such as RS^-, ϕS^-, ϕ_3P, CN^-, $(NH_2)_2C{=}S$, SCN^- and I^- were found to be effective.[116]

Although the ideal nucleophilic attack would be at the 2'-carbon atom adjacent to the sulfur, several nucleophilic displacements were attempted using highly polar molecules which might be expected to attack the sulfur atom.

Discussion of Experimental Results

Reaction with Cyanide Ion

No reaction took place between 8,2'SAnA and potassium cyanide in water at 100°C. Changing the solvent to dimethylformamide and heating the solution for 1¼ hours at 150°C gave mainly starting material along with decomposition products (fluorescent bands on the paper chromatograms) and a minor product with a UV maximum at 275 nm. (Starting material, 8,2'SAnA has UV maxima at 276.5 and and 222 nm.) Increasing reaction time to 39 hours and maintaining the temperature at 110-160°C in an attempt to drive the reaction to completion gave only decomposition products. Thus it appeared that there was no reaction between the cyanide ion and 8,2'SAnA in

aqueous solution at 100°C and that subjecting the two reactants to more strenuous conditions leads to the decomposition of the nucleoside.

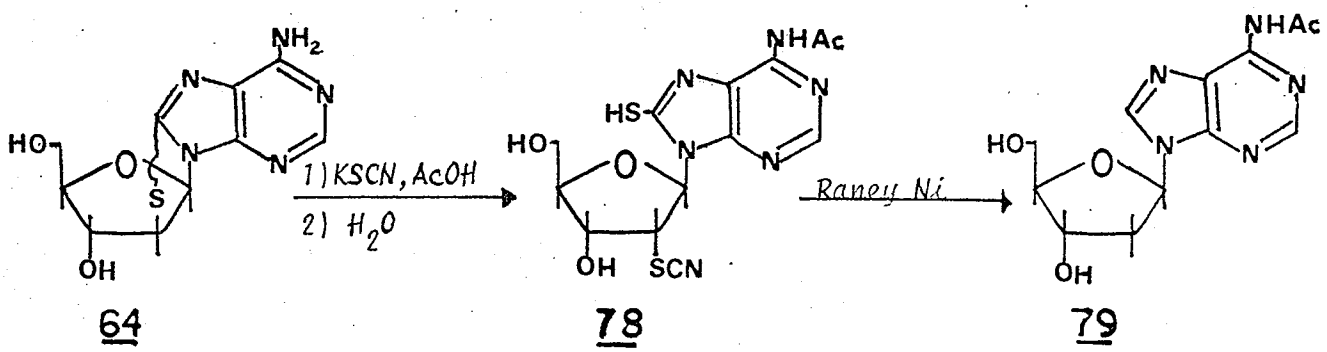
Reaction with Thiocyanate Ion

The reaction of 8,2'SAnA with potassium thiocyanate at 100°C in acetic acid, gave, after four hours, mainly unchanged starting material along with nine minor products. One of these nine products possessed UV maxima at 319 and 250 nm, suggesting an 8-thioadenosine chromophore whose conjugation had been extended further by an auxochrome. Raney nickel treatment of this compound gave a product with a UV maximum at 271.5 nm, which is very similar to that of the N^6-acetyladenosine chromophore (λ max = 272 nm).[117] It is possible that nucleophilic attack at the C2'-position to form 8-thioadenosine had been accompanied by N^6-acetylation of adenosine in the heated acetic acid solution. Although acetic acid is not normally used for N-acetylation without a condensing agent,[118] conditions prevailing in this reaction could perhaps have led to N-acetylation. It is unlikely that acetylation of the 8-thio group would have extended the conjugation of the molecule to 319 nm, since we have seen that adding a nitrile group to the 8-thio position results in a decrease in wavelength of the UV maxima in going from 8-thioadenosine 70 to 8-thiocyanato-adenosine (72) in Table 8. Since N^6-acetylation of adenosine moved its UV maximum up by 12 nm (from 260 to 272 nm), one would

expect that N^6-acetylation of 8-thioadenosine would move its UV maximum from 308 to 320 nm. The observed maximum at 319 nm is quite close to the predicted value for the N^6-acetyl-8-thioadenosine chromophore. Thus it appeared that reaction between 8,2'SAnA and thiocyanate had led to nucleophilic attack at C2' to give 2'-thiocyanate-8-thioadenosine. N^6-acetylation of this compound could have given the observed minor product with UV maxima at 319 and 250 nm. (78) The Raney nickel reduction would have produced, then, N^6-acetyl-2'-deoxyadenosine. (79) An attempt was made to drive the thiocyanate reaction with 8,2'SAnA towards completion by extending reaction time to fifteen hours.

SCHEME 42

ONE POSSIBLE REACTION OF THIOCYANATE WITH 8,2'S-ANHYDROADENOSINE



64 78 79

This resulted in a reduction in the number of products formed to four. The most intense band on papers was still that of starting material. None of the three other products had a chromophore corresponding to 8-thioadenosine or N^6-acetyl-8-thioadenosine. Thus it appeared that by attempting to obtain more of the product

tentatively assigned the structure 78, that particular molecule and at least five other intermediates had undergone structural changes to form entirely new compounds. At this point, investigations into the reaction of potassium thiocyanate on 8,2'-thioanhydroadenosine were terminated.

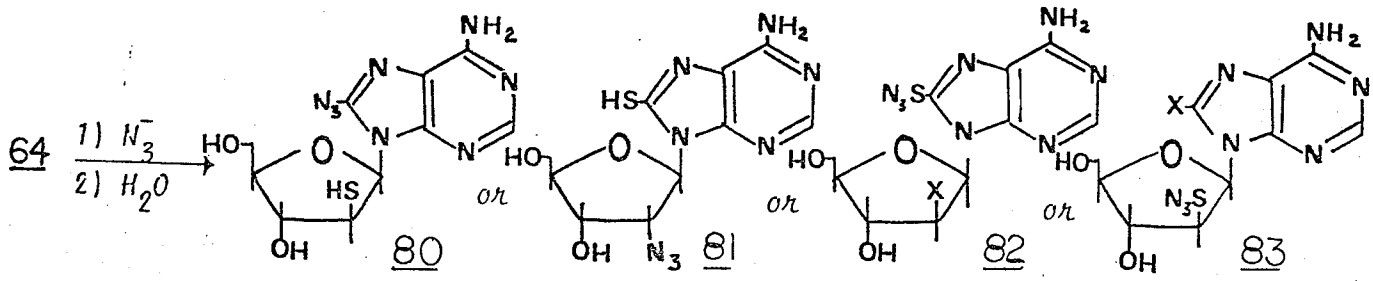
Reaction with Azide Ion

A reaction between 8,2'S-anhydroadenosine and sodium azide was also attempted. After maintaining the two reactants at 70°C in dimethylformamide for 6½ days, it was observed that only a small amount of a new product had been formed, while most of the starting material remained unchanged. This new compound had a UV maximum at 273 nm. It could not have been an 8-azido adenosine chromophore, as 8-azidoadenosine has a UV maximum at 281 nm.[101] Nor could it have been the product of azido attack on the C2'-position as the expected product of such a displacement would have contained the 8-thioadenosine chromophore with λ max at 308 nm.[97] Attack at the sulfur atom could produce an unprecedented 8-thioazidoadenosine chromophore whose UV maximum would be expected to be about that of 8-methylthio or 8-thiocyanate adenosine (~ 280 nm) or slightly higher. The observed maximum at 273 nm did not correspond to what one might expect from nucleophilic attack of C2' at C8 or at the sulfur atom.

Increasing the reaction temperature to 150°C and heating the reactants in dimethylformamide for three hours led to some

SCHEME 43

EXPECTED PRODUCTS OF AZIDO ATTACK ON 8,2'SAnA



decomposition. The major product of this reaction was still unreacted starting material - four other products formed in low yields were also isolated. One of these four products exhibited UV maxima at 314 and 275 nm - possibly some derivative of an 8-thioadenosine system. The mass spectrum of this molecule contained peaks as high as 352 mass units and could not be readily interpreted. Since this product and three others were obtained in such low yields, and since the mass spectra were quite inconclusive, this reaction and its products were not studied any further.

Complexing with Mercury to Assist Displacements

Since nucleophilic displacements with polar nucleophiles had not occurred readily and cleanly, it was decided to attempt another approach. It is known that mercuric ions form stable complexes with sulfides.[119] Complex formation would lead to utilization of either the 3p or 3-d orbitals of the sulfur atom. If 3p orbitals were used, the sulfur atom would become positively charged,

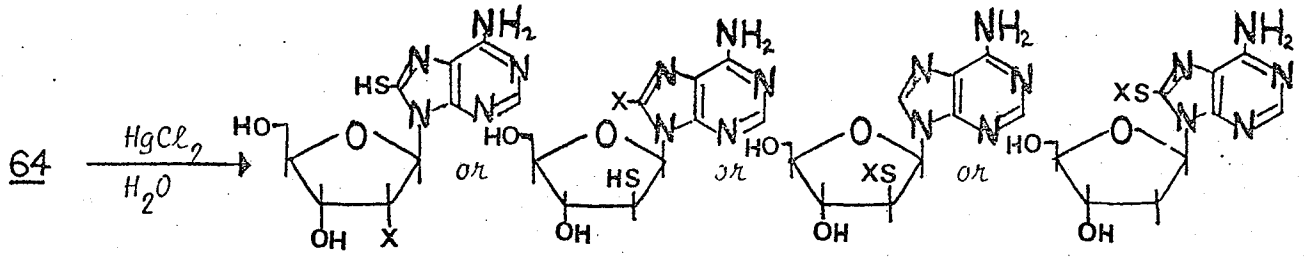
thereby making the molecule more susceptible to nucleophilic attack than the corresponding non-complexed sulfide. If the 3d orbitals were utilized, the S-R bond would take on a certain measure of d-orbital character which would result in a weakening of the S-R bond, making it perhaps slightly more susceptible to nucleophilic attack. Thus it was hoped that nucleophilic attack at either C8 or C2' might be assisted by the presence of mercuric chloride. Several experiments were conducted to see if this were so.

It was found that there was no reaction between water and 8,2'SAnA in the presence of mercuric chloride when the reaction mixture was heated at 100°C for half an hour. Adding acetone to the reaction mixture in order to help dissolve the mercuric chloride and allowing the reaction to proceed at room temperature for three days resulted in the formation of two compounds in low yields. Most of the starting material remained unchanged. Only one of the two new compounds possessed a maximum peak in the UV. Its peak at 272 nm could not belong to a molecule resulting from attack at C2' (to give 84 with its 8-thioadenosine chromophore, λ max 308 nm) or at C8 to give 85(a) with the 8-oxyadenosine chromophore (λ max ~ 280 nm) or 85(b) with the 8-chloroadenosine chromophore (λ max ~ 265 nm since 8-bromoadenosine has $\lambda_{max}^{H_2O}$ 265.5 nm[120] and 8-fluoroadenosine has $\lambda_{max}^{pH\ 7}$ 263 nm[121]) or attack at the sulfur atom to give 86 (adenosine chromophore λ max ~ 260 nm).

The only product which might be capable of giving a UV maximum at

SCHEME 44

EXPECTED PRODUCTS OF MERCURIC ION ASSISTED DISPLACEMENT BY WATER



84 85 86 87

a) X = OH, b) X = Cl

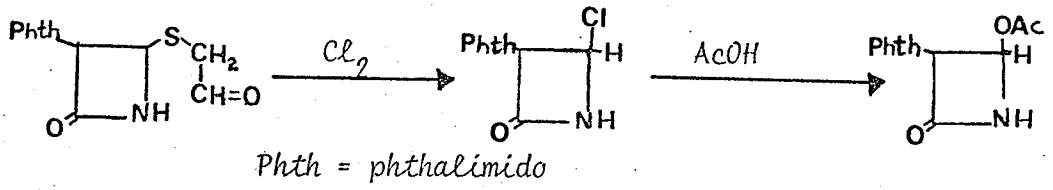
272 would be 87, an unknown molecule containing an 8-SOH group (87a) or an 8-SCl group (87b). In either case, the deoxy nature of the 2' position would make the whole displacement useless since the object of this work was to prepare a ribonucleoside derivative. Consequently the reaction was not explored further.

An attempt was then made to effect displacement by the benzoate ion in the presence of mercuric chloride. There was no reaction between 8,2'SAnA and sodium benzoate, benzoic acid and mercuric chloride when the mixture was heated at 150°C in dimethylformamide for one hour.

Attempted Cleavage by Chlorine

Sheehan had found that selective carbon-sulfur cleavage in penicillin derivatives could be effected by passing an excess of chlorine through a suspension of the sulfide in carbon tetrachloride.[12]

This had left a reactive chlorine atom on the carbon atom of the C-S bond which could be readily displaced by methoxide, acetate and thiophenoxide at room temperature. A similar reaction on



Phth = phthalimido

8,2'SAnA would at best leave a chlorine atom on the 2'- position of the nucleoside, the nucleophilic displacement of which might lead to the formation of arabinoadenosine after desulfurization. However, it was found that treatment of 8,2'SAnA with chlorine gave no reaction.

Attempts at Preparing and Using A Sulfoxide of 8,2'SAnA

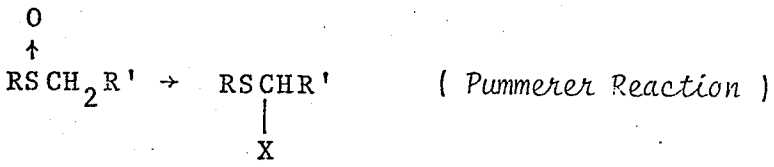
It became apparent that nucleophilic displacement of the C-S linkage of 8,2'SAnA was not feasible. The investigation then turned to the chemistry of sulfoxides for a solution to the problem.

Reactions leading to the formation of α-halogeno-sulfoxides were ruled out since these products were reported to be generally unreactive towards nucleophiles.[123]

Scission of the C-S bond of a sulfoxide with N-bromo- or N-chlorosuccinimide[124] would also have been a futile reaction since it is essentially an S_N1 displacement which would lead to a mixture of ribo- and arabinoadenosine derivatives.

Only one sulfoxide reaction - the Pummerer reaction[125,126] - had the potential of producing the desired results. This involved

the acid catalyzed conversion of sulfoxides possessing at least one α-hydrogen atom into α-substituted sulfides. An α-acetoxy sulfide is obtained when the sulfoxide is treated with acetic

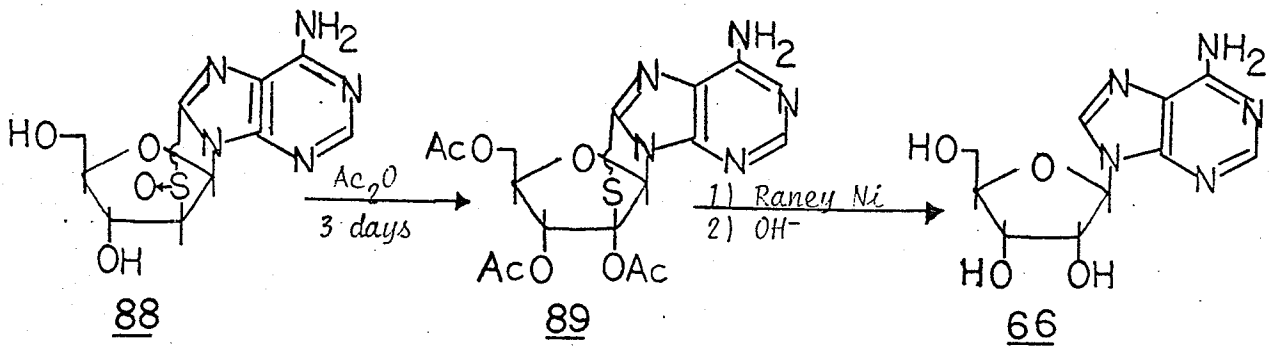


(*Pummerer Reaction*)

anhydride for three days. A similar reaction on the sulfoxide of 8,2'SAnA would result in the synthesis of the 2'-acetoxy derivative of N^6,3',5'-triacetyl-8,2'SAnA which could be converted to adenosine by desulfurization and base hydrolysis of the acetyl groups.

SCHEME 45

PROPOSED PUMMERER REACTION ON 8,2'SAnA SULFOXIDE



88 89 66

The first step, then, would be to convert 8,2'SAnA to its sulfoxide. A number of different reagents have been used in the past to convert a sulfide to a sulfoxide. However, oxidizing agents such as peracetic and m-chloroperbenzoic acid were immediately ruled out since they had been reported to react with adenosine to form 1-N-oxides.[127,128] Subsequent reduction of the

N-oxide by catalytic hydrogenation,[129] reaction with phosphorus trichloride[129] or reaction with sodium in liquid ammonia[130] could adversely affect any oligonucleotide of which such a sulfoxide of 8,2'SAnA might be a part.

Use of N-Bromosuccinimide as an Oxidizing Agent

N-bromosuccinimide had been used to convert 8,3'-thioanhydroadenosine ($\lambda_{max}^{H_2O}$ 283,275,287 sh) to the corresponding sulfoxide ($\lambda_{max}^{H_2O}$ 284).[131] Based on this observation, it was felt that a sulfoxide of 8,2'SAnA would have a UV maximum at or slightly above that observed for 8,2'SAnA (276.5 nm). The reaction of N-bromosuccinimide with 8,2'SAnA produced nine different compounds, only one of which had a UV maximum above 276.5 nm. The mass spectrum of the material showed the highest mass peak at 149, the significance of which could not be interpreted.

Use Of Iodobenzene Dichloride

Iodobenzene dichloride had been reported to oxidize sulfides specifically in the presence of N-functional groups.[132] Both aqueous acetonitrile[132] and aqueous pyridine[133] were used in the reactions of this reagent with 8,2'SAnA. A large number of products were obtained in each of these reactions and no consistent results could be obtained. A complicating factor in these reactions may have been the iodobenzene dichloride oxidation of the nucleoside secondary hydroxyl group.[97]

Some consistency in observed results was attained when the nucleoside used was the 3',5'-diacetylated derivative of 8,2'SAnA. Using aqueous pyridine at 0°C in the absence of light, the reactions yielded three spots on t.l.c. developed in EtOAc - a yellow coloured material at R_f 0.08, unreacted $Ac_2$8,2'SAnA at R_f .17 and a faster moving, unstable material at R_f .40. On standing in solution or on TLC exposed to air, the R_f .40 material decomposed to a yellow compound (R_f 0.08) and to starting material.

The sulfoxide, being more polar than the sulfide, would be expected to have the lower R_f value on TLC developed in ethyl acetate. A closer examination of the yellow material at R_f 0.08 on TLC revealed either that it decomposed rapidly on silica gel or that it was not a pure compound in the first place - five bands were observed when it was chromatographed in EtOAc-iPrOH-H_2O (75:16:9). Only one of these five compounds contained a UV maximum higher than that of starting material, but its infrared spectrum did not have strong peaks at 1040-1060 cm^{-1} which would have indicated the presence of a sulfoxide group. In the mass spectrum, the highest mass peak was at 365, which corresponded to the molecular weight of starting material $Ac_2$8,2'SAnA.

In order to avoid the possibility that decomposition of the sulfoxide had been occurring on the silica gel, the reaction mixture was diluted with water, then extracted with chloroform which was dried, then concentrated under vacuum. The reaction

mixture was then subjected to the Pummerer reaction by the addition of dry acetic anhydride, followed by three days of stirring. The compound sought was N^6,3',5'-triacetyl-2'-acetoxy-8,2'-thioanhydroadenosine, a molecule of molecular weight of 465 mass units and an expected UV maximum at 292.5 nm (by analogy with N^6,3'-$Ac_2$8,2'SAnA-5'-phosphate[120]). The major product, purified by TLC and paper chromatography exhibited UV maximua at 290 and 224.5 nm and inflections at 295.5, 257 and 229 nm, but the mass spectrum of the material indicated a mass of 407 which would correspond to N^6,3',5'-triacetyl-8,2'SAnA. The significance of a small anomalous peak at m/e 446 could not be interpreted.

Like the N-bromosuccinimide oxidations, the reaction of iodobenzene dichloride with 8,2'SAnA or its diacetylated derivative led to the formation of a number of compounds, except that here the starting material was the major product. Iodobenzene dichloride oxidation of 8,2'SAnA did not appear to lead to sulfoxide synthesis.

Sodium Metaperiodate Oxidations

Narang had used a 5 molar excess of sodium metaperiodate to convert the phenylmercaptoethyl group of a trinucleotide to a sulfoxide.[134] Kingsbury had performed periodate oxidations of sulfides in methanol[135] while Johnson used water or aqueous alcohol solutions with a 5% excess of $NaIO_4$ to convert sulfides to sulfoxides.[136] In each case, temperature control was very important in preventing over-oxidation. These reactions, maintained in ice-bath temperatures, were complete in less than twelve hours,

often with better than 90% yields.

However, when 3',5'-diacetyl-8,2'SAnA was stirred with an equivalent of sodium metaperiodate for periods of two to seven days, only a minute amount of a new material with R_f lower than that of starting material was observed on TLC. Since its UV spectrum was similar to that of starting material, it was thought to be the product resulting from the loss of an acetyl group after such prolonged exposure to an aqueous ethanol solution of sodium metaperiodate.

The reaction of 8,2'SAnA with a 5M excess of $NaIO_4$ for an hour at room temperature produced two new products (both of which were more polar than starting material) in very low yields. The UV spectra of these two products (262 and 260 nm respectively) suggested that the C(8)-S bond had been cleaved, leaving an adenosine and/or an 8-OR adenosine-type chromophore (Table 8).

When an identical reaction mixture was heated in 25° steps from room temperature to 100°C and the progress of the reaction was monitored by frequently placing a spot of the reaction mixture on papers, the gradual increase in amounts of two new more polar products relative to starting material was observed. The R_f values of these two products were similar to those observed in the preceding one hour room temperature reaction. However, although the UV maximum of the most polar compound was the same (262 nm), the other product now had a UV maximum at 286 nm, which is close to that observed for 8-hydroxyadenosine at pH 1, but much higher than what would be expected of a sulfoxide of 8,2'SAnA.

When the above reaction mixture was allowed to stand at room temperature overnight, white crystals with UV maximum at 260.5 nm were formed. Paper chromatography indicated that the R_f value of the crystalline material corresponded to that observed for the more polar of the two reaction products. Electrophoretic mobility of this compound (R_m = 0.47, relative to Tp) indicated that it was a monocharged species. Electrophoretic and UV results suggested that the crystals were either the 2'-sulfonic acid of 2'-deoxyadenosine or the 2'-sulfonic acid of 2'-deoxy-8,5'-O-anhydroadenosine. Ikehara had observed similar properties in a molecule identified as the 3'-sulfonic acid of 3'-deoxy-8,5'-O-anhydroadenosine.[131] Elemental analysis gave values which were lower than the expected percentages for C, H and N, suggesting the presence of an impurity like $NaIO_4$ in the sample.

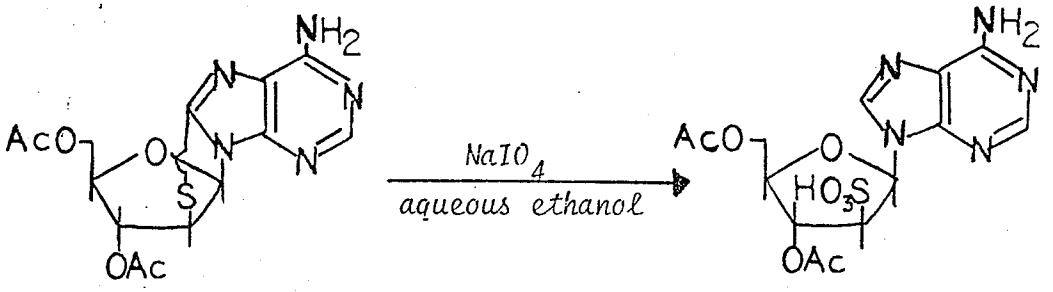
Reducing the amount of sodium metaperiodate to about one equivalent in the heated reaction did not alter the nature of the products formed in the reactions with 8,2'SAnA.

Switching to the more volatile molecule 3',5'-diacetyl 8,2'SAnA (90) in the $NaIO_4$ oxidations permitted the more effective use of the mass spectrometer in identification of the reaction products. The compound formed in the reaction between $Ac_2$8,2'SAnA and $NaIO_4$ had a molecular ion peak at m/e 415. This corresponded to the molecular weight of the 2'-sulfonic acid of 3',5'-diacetyl-2'-deoxyadenosine (91).

Compared with the sodium metaperiodate oxidations of normal

SCHEME 46

METAPERIODATE OXIDATION OF 3,5'-DIACETYL-8,2'-S-ANHYDROADENOSINE



90 91

sulfides which are complete in less than twelve hours,[136] the oxidation of 8,2'SAnA proceeds extremely slowly (if at all) when exposed to an equivalent of the oxidizing agent at or below room temperature. Increasing the amount of sodium metaperiodate present and/or raising the reaction temperature has the effect of increasing the rate of oxidation of 8,2'SAnA. The sulfoxide does not appear to be formed in these reactions at all. Over-oxidation products seem to predominate whether the reaction is done at room temperature or at elevated temperatures.

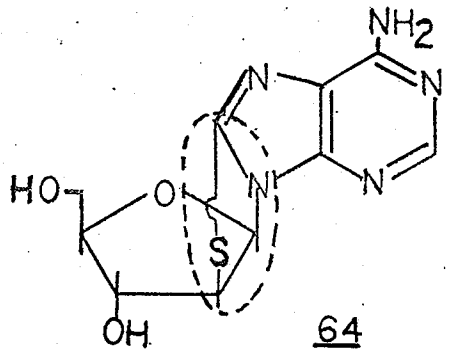
Singlet Oxygen Oxidation

An attempt at a photochemical oxidation of 8,2'SAnA by bubbling oxygen through a solution of the nucleoside and the singlet oxygen sensitizer, rose bengal, proved to be futile. Most of the starting material remained unchanged after irradiations lasting up to fourteen hours. Two products with UV maxima at 270 and 260 nm respectively were isolated in low yields after the fourteen hour irradiation. However, since their UV absorbances were at lower wavelength than was expected of the desired sulfoxide, these compounds were not characterized further.

The inability to prepare, isolate and identify the sulfoxide of 8,2'-thioanhydroadenosine has been a most frustrating experience. The extreme stability of the molecule has been attributed to the strain-free thiazolidine ring system of which the thioanhydro linkage is a part.[137] (Figure 13)

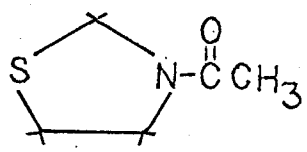
FIGURE 13

THE THIAZOLIDINE RING SYSTEM



64

8,2'-thioanhydroadenosine



92

N-acetyl thiazolidine

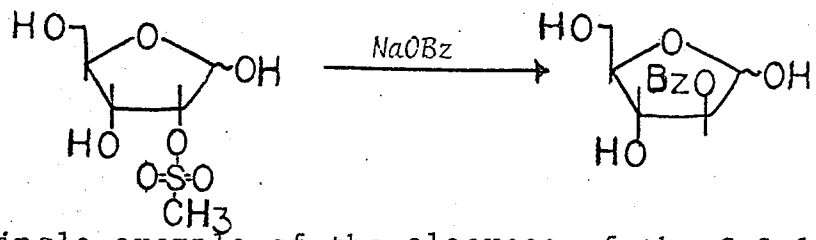
Earlier workers had found that they could not isolate the sulfoxide of N-acetyl thiazolidine either, presumably because of its instability, although the sulfone could be readily prepared and isolated when two equivalents of oxidizing agent had been used.[138]

Further, the oxidation of 8,3'-thioanhydroadenosine with two equivalents of N-bromosuccinimide had produced only one of two possible isomeric sulfoxides and no sulfone.[131] Steric shielding by the 5'-hydroxyl group was cited as a possible explanation for the results observed in that experiment. Such interference may

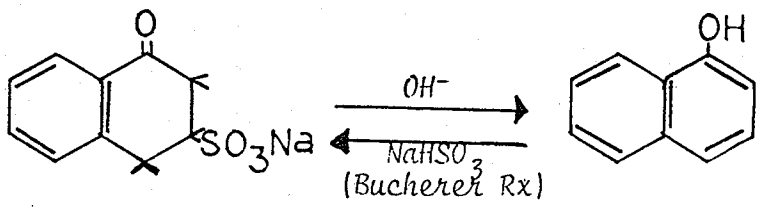
also provide at least a partial rationalization for the failure of 8,2'-thioanhydroadenosine to undergo oxidation to a sulfoxide.

Displacement of a Sulfonic Acid

Since oxidation of Ac_2 8,2'SAnA by sodium metaperiodate had led to the formation of a sulfonic acid, a check of the literature was made into the possibility of nucleophilic displacement of a sulfonic acid. It was found that although numerous nucleophilic displacements of carbohydrate sulfonates had been reported,[139-141] they all involved the cleavage of the C-O rather than the C-S bond.

e.g. 

A single example of the cleavage of the C-S linkage of a sulfonate is the reverse Bucherer reaction,[142] which is limited to phenols which have a tendency to ketonize.[143]



Thus it did not appear that nucleophilic displacement of a 2'-sulfonate to produce adenosine was a feasible reaction.

Further studies on this project were terminated, as the results of the attempted nucleophilic displacement reactions and monoxidation reactions on 8,2'-thioanhydroadenosine had not

held out much promise for this method.

One final approach to this problem might involve the possibility of an intramolecular displacement of the thioanhydro linkage by a nucleophilic substituent situated in the 3'-position.

RESULTS AND DISCUSSION

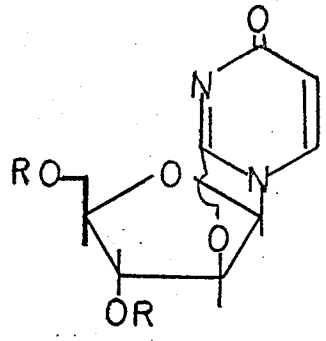
PART C - PHOTOCHEMISTRY OF ANHYDROURIDINE

Preamble

O^2,2'-anhydrouridine (93) was first prepared in 1956 by Todd.[144] Research into the insertion of this molecule into

FIGURE 14

STRUCTURE OF O^2,2'-ANHYDROURIDINE



93 R = H
94 R = Ac

oligonucleotides[145] and studies into the photochemistry of its diacetylated derivative 94 had been carried out in the laboratories of K. K. Ogilvie between 1970 and 1973.[63,146] In the course of one of the photochemistry studies,[63] it was found that irradiation of a chloroform-saturated aqueous solution containing diacetyl-anhydrouridine led to the formation of diacetyl-5,6-dihydroanhydro-uridine. Since this photoreduction provided a simple means of obtaining a potentially useful molecule, (as an antimetabolite or as a source of information regarding the role of dihydrouridine in t-RNA), it was decided to investigate this reaction more fully.

The photoreduction of anhydrouridine had not been previously reported, although similar reactions on uracil and uridine have. Uracil has been converted to dihydrouracil by UV irradiation in isopropanol,[147] aqueous isopropanol,[148] aqueous acetone [148,149] as well as in aqueous solutions of methionine,[147] ethylenediaminetetraacetic acid[147] and cysteine.[148,150] Uridine had been reduced by irradiation in formic acid[151] and in an aqueous solution containing an excess of sodium borohydride.[152,153] There had been no reports of using chloroform as a catalyst or a proton source in photoreductions.

Roles of Ethanol and Chloroform

Attempts to effect photoreduction of diacetylanhydrouridine in chloroform-saturated aqueous solutions as the previous researcher had done[63] were unsuccessful. Discussions with Dr. J. L. Charlton raised the possibility that ethanol, present in chloroform as a preservative, may be having some effect on the reaction. Indeed it was! Addition of a small amount of ethanol to the chloroform-aqueous solution of diacetylanhydrouridine which was then irradiated led to the formation of a single UV peak at 238 nm. This corresponded to the UV maximum previously observed[63] for the photoreduction product of Ac_2AnU. Up to a point, the addition of more ethanol increased the rate of the photoreduction reaction. Beyond a certain point, compounds having different UV maxima began to appear. Removal of the chloroform seemed to have no effect on the

photoreduction. Apparently, the chloroform that had been used a year earlier had contained sufficient ethanol to bring about the observed photoreduction.

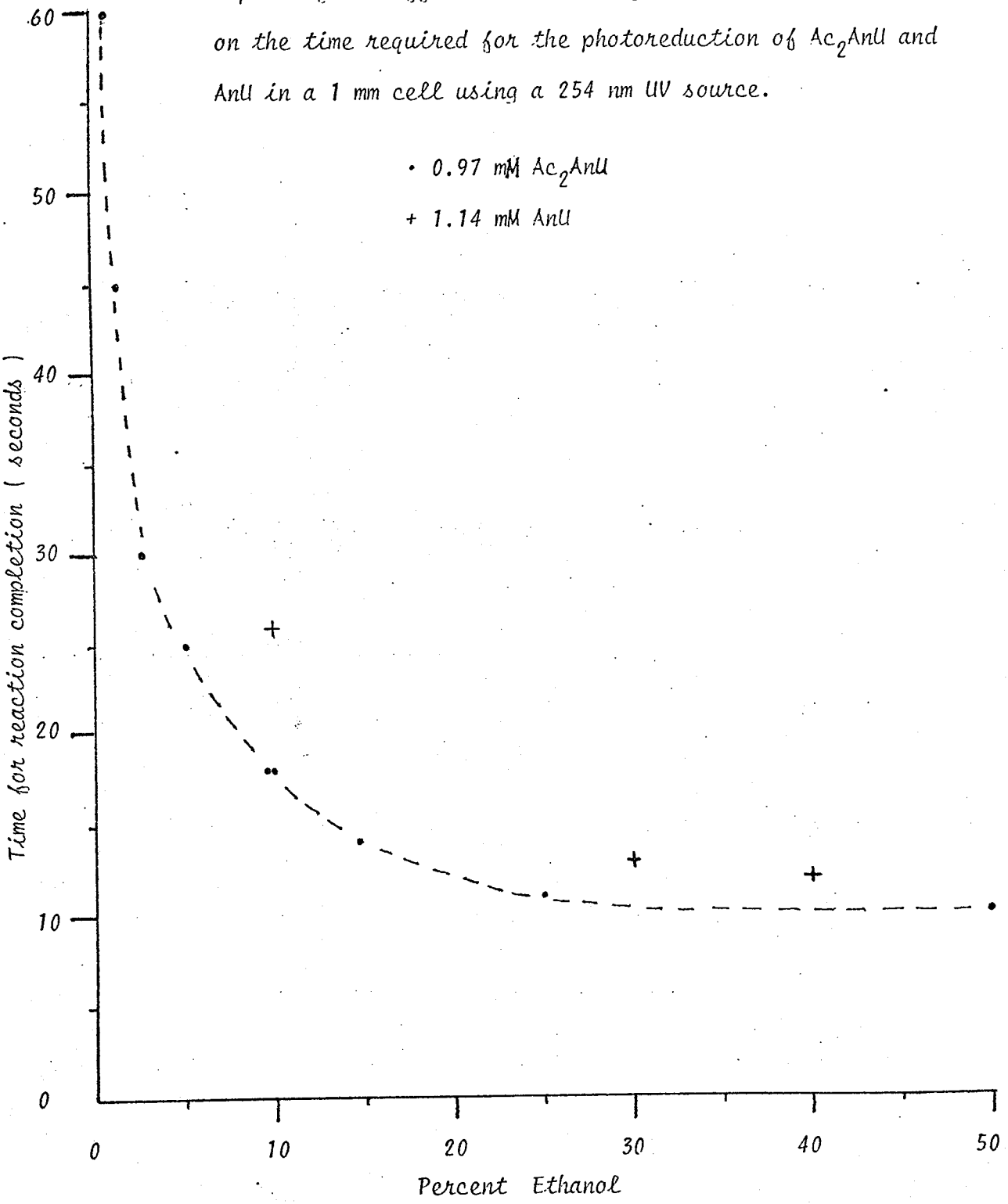
Irradiation of Diacetyl Anhydrouridine

Initial experiments used diacetylanhydrouridine, the higher volatility of which facilitated the use of the mass spectrometer as an investigative tool. The progress of the photoreduction was monitored by taking the UV spectrum of the irradiated solution at regular intervals. As photoreduction occurred, the collapse of the two maxima of the starting material (at 250 and 224 nm) into a single maximum at 238 nm was observed. This peak at 238 nm grew to a maximum absorbance, then began to drop in intensity if irradiation were continued. The photoreduction was assumed to be over when maximum peak height at 238 nm was attained. The effects of increasing ethanol and solute concentrations on reaction rate and product-type were determined by following the progress of the reaction by UV spectrometry.

It was observed that as the amount of ethanol was increased from 0.7% to 50%, irradiation time decreased from 60 seconds to 10 seconds using a 1 mM solution in a 1 millimeter UV cell (Figure 15). Increasing solute concentration in 50% ethanol solution to 5 mM resulted in the formation of a new UV peak in addition to that observed for the original photoreduction product. It was the 25% ethanol solution which proved to the most versatile,

FIGURE 15

A plot of the effect of increasing ethanol concentration on the time required for the photoreduction of Ac_2AnU and AnU in a 1 mm cell using a 254 nm UV source.



as the solute concentration in this solvent could be raised as high as 33 mM without apparently altering the type of product(s) formed in the photochemical reaction; i.e., a single maximum at 238 nm was still observed at that concentration.

Isolation of the Photoproducts

After irradiation had been completed, the reaction mixture was concentrated to a syrup and applied to paper chromatograms which were developed in solvent B (organic layer of 1-butanol-ethanol-water, 4:1:5). Several faint bands were observed at the lower R_f values, along with two intense almost overlapping bands, moving close to one another at R_f .52 and .62 respectively. The highest mass peak for the R_f .52 material was at 312 mass units (the molecular weight of diacetyldihydroanhydrouridine) while that of the R_f .62 material came at 313 mass units. This peak at an odd numbered mass to charge (m/e) value was believed to represent a fragment of the actual compound rather than its molecular ion. An attempt was made to crystallize the Ac_2H_2AnU-containing material from the R_f .52 band. When this failed, the R_f .52 material was rechromatographed on papers in solvent B. Instead of the expected single band at R_f .52, three bands were observed as a result of the material being rechromatographed several days after it had been isolated. It was disturbing that the photoproducts would be undergoing changes as they were being isolated and purified. The exact nature of these changes was not determined. However, it was found that re-examination of a chromatographically pure sample

of chemically prepared diacetyldihydroanhydrouridine which had been allowed to stand in ethanol solution for a few days revealed the appearance of a second spot on TLC.

Irradiation of Anhydrouridine

Consequently it was decided to use the parent compound, anhydrouridine, instead of its diacetylated derivative for subsequent photochemical studies. Purification of the photoproducts was greatly simplified as a result of this change. Thin layer chromatography of the reaction mixture revealed five faint bands at R_f .36, .41, .54, .70 and .88, and three dark bands at R_f .00, .15 and .24.

Identification of the Photoproducts of Anhydrouridine

The material at the origin could not be identified as paper and thin layer chromatography in various solvents produced only a long streak containing at least three darker areas, but no distinct bands. This material may have contained products resulting from the secondary photochemical reactions of the photoadducts which have been found to occur more efficiently than the photoaddition.[154]

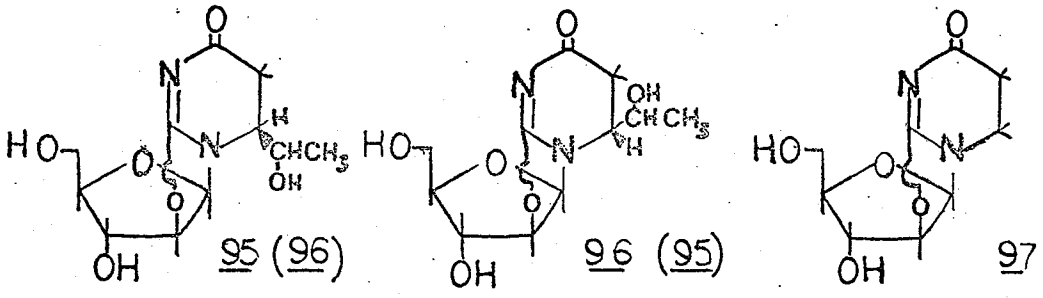
Rechromatographing the R_f .15 material on papers in solvent B gave two bands which moved close together. In order to effect their separation the papers had to be developed for periods of forty hours or more. The slower material was identified as unreacted anhydrouridine (18%) while the faster one turned out to

be 6(α-hydroxyethyl)-5,6-dihydroanhydrouridine 95.

Prolonged paper chromatographic development of the R_f .24 material in solvent B also gave two bands which moved close together. The slower band was found to contain dihydroanhydrouridine 97 while the faster moving compound was identified as another isomer of 6-(α-hydroxyethyl)-5,6-dihydroanhydrouridine 96.

FIGURE 16

PHOTOPRODUCTS IDENTIFIED



The ethanol adducts 95 and 96 were identified by mass spectrometry and by an analysis of the n.m.r. spectrum of their respective triacetylated derivatives. The actual symmetry at C-6 of each adduct was not determined. Since both have an additional chiral centre at the α-carbon of the hydroxy-ethyl side chain, each isomer, 95 and 96, would be expected to contain two epimers. However, only one of the adducts showed evidence of a second epimer in the n.m.r. The approximate ratio of the two epimers in that case was approximately 3:1.

Dihydroanhydrouridine was identified by comparison with an authentic sample.[53]

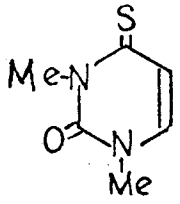
The minor bands were also isolated and their weights and UV spectra obtained. However, because of their low yields, these

products were not identified.

Was Addition at C-6 an Expected Reaction?

There have been quite a number of examples of photoadditions across the 5,6 double bond of uracil, uridine and uracil derivatives to give the 6-substituted (rather than the 5-substituted) 5,6-dihydro compound. Photohydrations, for example, have led to the formation of 6-hydroxy-5,6-dihydro uracil and uridine.[149,155-159] In one of these experiments, two isomers of the hydrate were found in the ratio of 3:2.[156] (The ratio of the two ethanol adducts of anhydrouridine was 3:1.)

The photoaddition of nucleopiles such as hydrazine, methylamine, HCN, HSO_3^- and BH_4^- to uracil also gave 6-substitution on 5,6-dihydrouracil.[160] Irradiation of 1,3-dimethyl uracil in methanol[161] or in frozen aqueous 2% methanol[162] resulted in the formation of 6-methoxy-1,3-dimethyl-5,6-dihydrouracil. Ethanol and 2-propanol have been reported to undergo photoaddition to the ene-thione system of 1,3-dimethyl-4-thiouracil (98) [163] to form the C-6 hydroxy-alkylated 5,6-dihydro derivative as the major photoproduct. The mechanism of



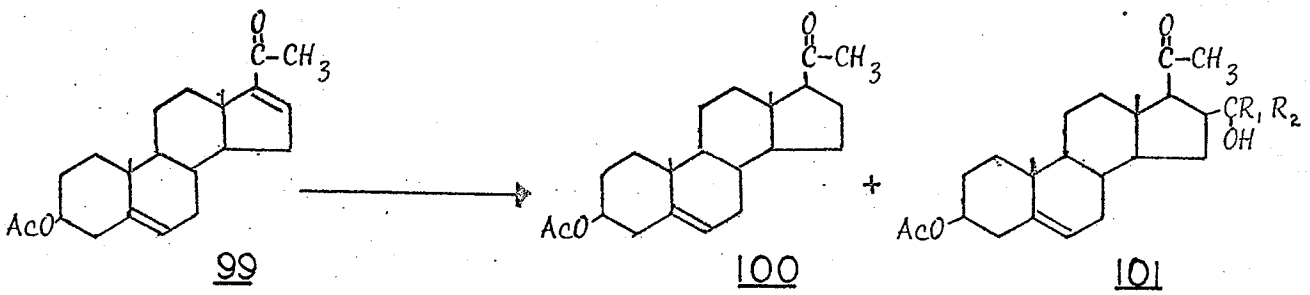
98

this last reaction might be expected to be similar to that involved in the photoaddition of ethanol to anhydrouridine.

Photoreduction and Photoaddition Reactions of α,β-Unsaturated Ketones

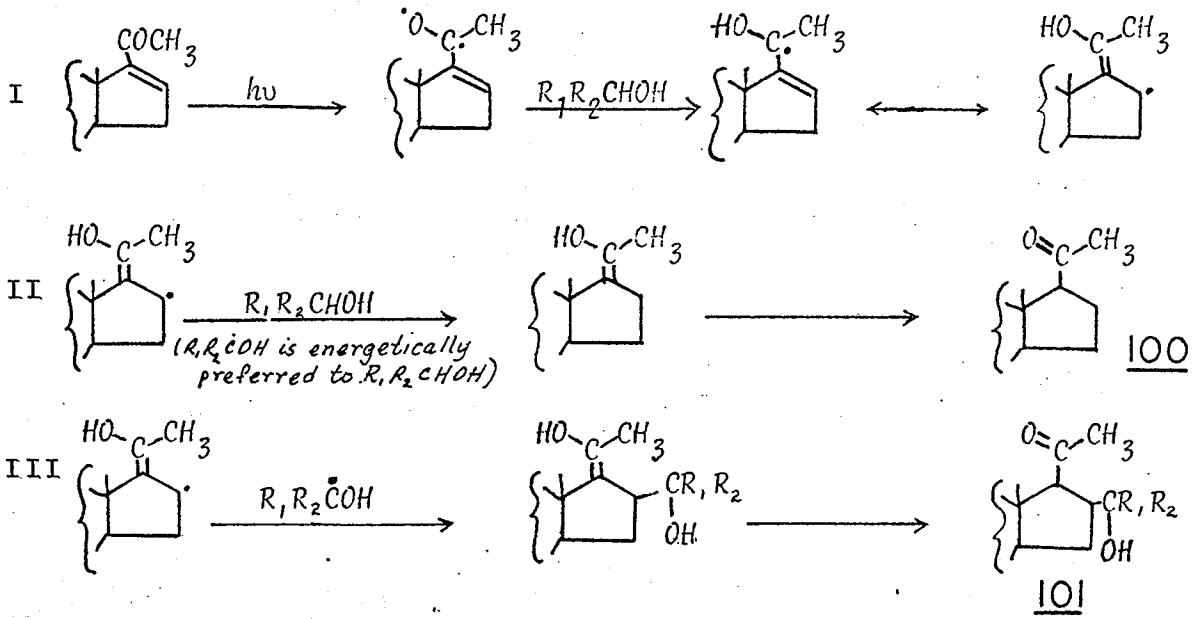
The structure of anhydrouridine 93 bears a resemblance to that

of an α,β-unsaturated ketone. This resemblance is parallelled by a similarity in the types of photoproducts formed when each is irradiated in the presence of an alcohol. Williams and Bladon[164] had found that when 3-β-acetoxypregna-5,16-diene-20-one (99) was irradiated in ethanol, 2-propanol or cyclohexanol, two products were formed - one resulting from the reduction of the double bond to give 100; the other from the addition of the alcohol across the double bond to give 101.



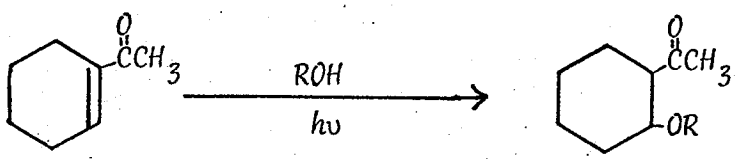
99 100 101

The photoreaction was thought to have proceeded in the following steps:[164]



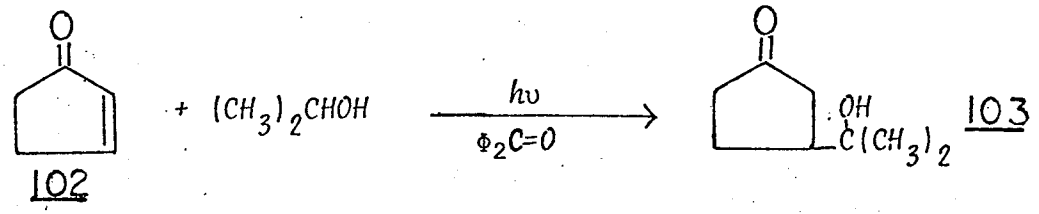
Thus steps I and II led to the formation of reduction product 100, while steps I and III gave the addition product. The primary photochemical process was thought to involve the absorption of light in the $n\rightarrow\pi^*$ absorption band of the unsaturated ketone.[165]

A study into the mechanism of the photochemical alcohol addition to a simple α,β-unsaturated ketone was also done,[154] using 1-acetylcyclohexene as the model compound. Ethers were formed in this



case. The same products were obtained whether a broad spectrum source with pyrex filters or a 254 A^o source with quartz vessels were used. This suggested that the initial $n\rightarrow\pi^*$ or $\pi\rightarrow\pi^*$ excitation led to a common excited state. In this work, the $\pi\rightarrow\pi^*$ triplet was assumed to be the reactive state. Attempts to quench the photoreaction with piperylene or oxygen had failed, suggesting that the alcohol addition was a singlet state process or else that it proceeded via a short-lived triplet state.[154]

An example of the photoaddition of an alcohol to a cyclic α,β-unsaturated ketone was the irradiation of cyclopentene-2-one 107 in isopropanol in the presence of benzophenone.[166] This reaction resulted in the addition of the dimethylhydroxymethyl radical $(CH_3)_2\dot{C}OH$ at the β position to give 3-(α-hydroxyisopropyl) cyclopentanone, 103. Sensitization by benzophenone would be expected to form the cyclopentenone $n\rightarrow\pi^*$ triplet. This could



102 103

subsequently react with the alcohol by proton abstraction and radical addition to give 103.

Suggested Mechanism for Anhydrouridine Photoreduction and Photoaddition Reactions

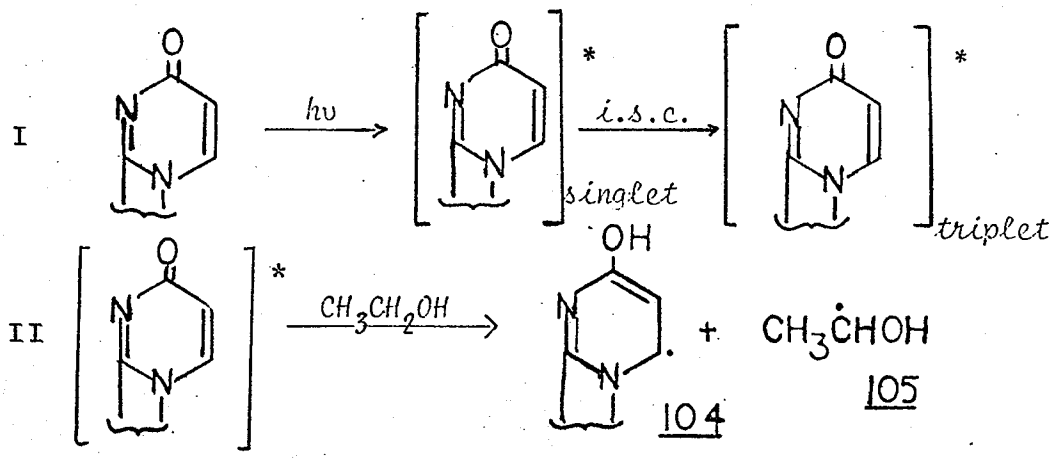
The $n\rightarrow\pi^*$ band of anhydrouridine is not visible in the UV spectrum. It is possible that the $n\rightarrow\pi^*$ band (normally at 300-350 nm in α,β-unsaturated ketones)[169] may be blue-shifted sufficiently by the presence of the nitrogen atoms in the molecule to be buried beneath the $\pi\rightarrow\pi^*$ band of anhydrouridine at 250 nm. (The highly electron-negative nitrogen atom at N^1 and perhaps N^3 would cause electron withdrawal from the α,β-unsaturated ketone system, thereby widening the energy gap between the ground state and the π^* excited state.[170]) Although the exact character of the excited state of anhydrouridine is unknown, an $n\rightarrow\pi^*$ state is not unlikely, particularly since it has been demonstrated that hydrogen abstraction involves the $n\rightarrow\pi^*$ state.[171] The reason for this is that $n\rightarrow\pi^*$ excitation results in a decrease in negative charge on the oxygen atom, making the radical more electrophilic and thereby facilitating hydrogen abstractions.[171b] Excitation to the $\pi\rightarrow\pi^*$ state, on the other hand, gives less free radical character

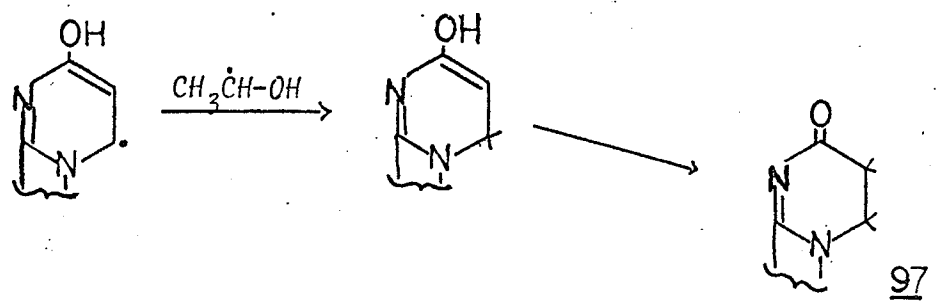
to any particular atom than does the $n\rightarrow\pi^*$ state and would require extensive electron reorganization for hydrogen abstraction.[171a]

Thus, the first step of the anhydrouridine photoreduction in aqueous ethanol might be the formation of either an $n\rightarrow\pi^*$ or a $\pi\rightarrow\pi^*$ singlet. The $n\rightarrow\pi^*$ singlet may photoreact or it may undergo inter-system crossing to form an $n\rightarrow\pi^*$ triplet. The $\pi\rightarrow\pi^*$ singlet may undergo a transition to form either the $n\rightarrow\pi^*$ singlet or the $n\rightarrow\pi^*$ triplet. Either of the two $n\rightarrow\pi^*$ states might be involved in hydrogen abstraction, although the longer life of a triplet state makes it the more likely reactive species. This intermediate 104 would abstract a hydrogen atom from the ketyl radical 105 to give dihydroanhydrouridine 97 (in other words, a disproportionation reaction (III)with radical 105 to produce dihydroanhydrouridine and acetaldehyde.) Alternatively, the radical 104 can combine with 105, yielding the observed ethanol adducts of anhydrouridine, 95 and 96.

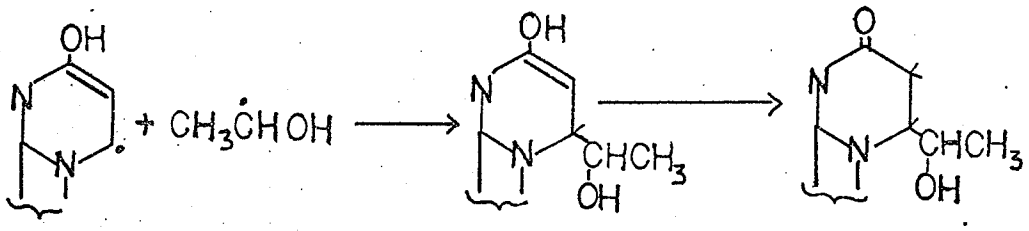
SCHEME 47

SUGGESTED MECHANISM FOR THE FORMATION OF ANHYDROURIDINE PHOTOPRODUCTS



III 

97

IV 

95 96

Singlet or Triplet?

Two experiments were done in an attempt to determine whether the reactive species in the photoreaction was the singlet or triplet state. In the first experiment, irradiations were carried out using two 100 milligram samples of anhydrouridine. One was degassed with nitrogen, while the other sample was not. In both cases, the UV maximum of the irradiated solution was at 238 nm after 22 minutes of irradiation. In the second experiment, two 200 mg samples were prepared. One was dissolved in 25% ethanol in water, the other was dissolved in 1 M potassium bromide in 25% ethanol in water. After 47 minutes of irradiation, the UV maximum of both solutions appeared at 238.5 nm. Since oxygen is known to be an effective quencher of triplet states[172,174] and the bromide ion has reportedly the same effect [173] and neither appeared to have any effect on the photoreaction of anhydrouridine, one is led to two possible conclusions. The first is that the singlet state is the reactive species. The second is that a

short-lived triplet state undergoes photoreduction and photoaddition reactions at a rate much faster than the quenching rate. A similar short-lived triplet has been suggested in the photochemical alcohol addition to α,β-unsaturated ketones[154] and in photodimer production in DNA or TpT.[175]

SUMMARY AND CONCLUSIONS

The general purpose of this research has been the development of methods to improve nucleic acid synthesis. Chronologically, the first approach undertaken in the laboratories of K. K. Ogilvie involved the use of anhydronucleosides for polyribonucleic acid synthesis. One graduate student had studied the feasibility of using O^2,2'-anhydropyrimidine nucleosides for this purpose[145] while this author undertook to investigate the suitability of 8,2'-thioanhydropurine nucleosides for RNA synthesis. In the former case, it was found that nucleophilic displacement reactions on a dinucleotide containing anhydrouridine led to internucleotidic cleavage. In the latter case, it was found that no straightforward nucleophilic displacement of the thioanhydro linkage took place when a number of different nucleophiles were used under several different conditions. As a thioether forming part of a thiazolidine ring system, the thioanhydro linkage was very stable to nucleophilic displacement reactions. Attempts to convert the sulfide to a sulfoxide which might then undergo a Pummerer rearrangement were also unsuccessful. However, in the presence of excess oxidizing agent and at elevated temperature, the thioanhydro linkage was finally oxidized to give the 2'-sulfonic acid of 2'-deoxyadenosine. Since nucleophilic displacement of the C-S linkage of alkyl sulfonic acids was unknown, and because of the problems encountered in the displacement and oxidation reactions of the sulfide, the project was terminated. The inherent stability of the thiazolidine ring system and steric interference by the 5'-

hydroxyl group of the 8,2'-thioanhydroadenosine might play a role in the extreme non-reactivity of 8,2'SAnA under various conditions.

Dihydroanhydrouridine synthesis by the photoreduction of anhydrouridine was studied more because of its potential use as a precursor of dihydrouridine and arabino dihydrouridine than as a molecule of convenience for RNA synthesis. Once the roles of chloroform and ethanol were clarified, experiments were conducted to determine the optimal conditions for the photoreduction. It was found that 25% ethanol in water allowed use of the widest range of solute concentration and required a reasonably short irradiation time without apparently altering the nature of the photoproducts. The three major photoproducts were identified as dihydroanhydrouridine resulting from the photoreduction of the 5,6-double bond of anhydrouridine and as two isomers of 6-(α-hydroxyethyl)-5,6-dihydroanhydrouridine resulting from the photoaddition of ethanol to anhydrouridine. These photoreactions bear a striking resemblance to the results of irradiations of α,β-unsaturated ketones in the presence of alcohols.

Since the novel approach of using anhydronucleosides for the synthesis of polyribonucleotides had not been as successful as had been hoped, a more conventional approach was taken. This involved the development of stable, specific and/or highly lipophilic alkylsilyl blocking groups which could be removed using specific neutral conditions. An attempt by Nielsen to do the same[176] by

using the tritylacetoxy group had met with disappointment.[177]

Six different trialkylsilyl chlorides in varying degrees of purity were prepared and the specificity of each for the 5'-hydroxyl group of thymidine was determined. Methyloctadecylisopropylsilyl chloride and t-butylmethyloctadecylsilyl chloride were tested for suitability as a lipophilic handle for nucleic acid synthesis. Only the latter provided a blocking group which was sufficiently stable to acid to be useful towards this end.

In a concurrent study, methyldiisopropylsilyl chloride, triisopropylsilyl chloride, tetramethyleneisopropylsilyl chloride and tetramethylene-t-butylsilyl chloride were investigated for their usefulness as specific, stable blocking groups in nucleoside chemistry. Two of these four reagents, triisopropylsilyl chloride and tetramethylene-t-butylsilyl chloride, were found to exhibit high specificity for the primary 5'-hydroxyl function of thymidine and considerable stability in acidic conditions. The latter reagent, easily obtained in a pure state, provided the most stable blocking group of all the alkylsilyl chlorides prepared. Each was readily removed by treatment with tetra-n-butylammonium fluoride in tetrahydrofuran, neutral conditions with no adverse effects on other blocking groups.

In the course of this investigation, the 5'-, 3'- and 3',5'-di-silyl thymidine derivatives were prepared, isolated and characterized by melting point, infrared spectroscopy and mass spectrometry. The 5'-alkylsilyl derivatives of thymidine were further identified by elemental analysis.

One other aspect of silyl chlorides was investigated - the use of alkylsilyl dichlorides as blocking groups for the cis-glycol system of ribonucleosides. That diisopropylsilyl dichloride reacted with 5'-acetyl uridine to give the desired 5'-acetyl-2',3'-O-diisopropylsilyl uridine was verified by gas chromatography and mass spectrometry. However, attempts to purify this compound by crystallization led to its decomposition, suggesting that the molecule was somewhat unstable.

A further word about the combined techniques of gas chromatography and mass spectrometry (GC/MS) is in order. In the capable hands of fellow graduate student, Michael A. Quilliam, this technique has proven to be indispensable for the optimization of reactions to prepare alkylsilyl chlorides, for the identification of side products formed in these preparations, and for the characterization of most of the alkylsilyl derivatives of thymidine and uridine that had been prepared. In the absence of this technique, the alkylsilyl chlorides project would have taken considerably longer than the five months it required for its completion and the quality of the results reported here might have been lowered considerably.

EXPERIMENTAL

GENERAL METHODS

Reagents

Dry pyridine was obtained in the following manner: Technical grade pyridine (Fisher Scientific) was distilled from p-toluenesulfonyl chloride (after a period of refluxing) into a round-bottom flask containing calcium hydride. Following this, the pyridine was refluxed over calcium hydride, distilled and stored over Linde Molecular Sieves.

Dry N,N-dimethyl formamide was prepared by refluxing reagent grade DMF over calcium hydride, followed by distillation and storage over Linde Molecular Sieves.

Dry, unsaturate free pentane was prepared in the following manner: Practical grade pentane (Eastman Organic Chemicals) was vigourously stirred with concentrated sulfuric acid for three days. The pentane was then separated from the sulfuric acid, shaken with sodium carbonate solution, washed twice with water and dried over anhydrous sodium sulfate. The pentane was then fractionally distilled and the fraction boiling at 36.0-36.5^{o}C was collected.

Dry tetrahydrofuran was prepared by passing technical grade tetrahydrofuran through a column of activated silica and collected over Linde Molecular Sieves.

Commercially prepared isopropyl lithium and t-butyl lithium were obtained from Alfa Products in Montreal. Isopropyl lithium was also obtained from Research Organic Chemical Company in Belleville, New Jersey.

Dodecylsilyl trichloride, methyloctadecylsilyl dichloride and tetramethylenesilyl dichloride were ordered from PCR Incorporated in Gainesville, Florida.

Chromatography

Descending paper chromatography used Whatman 3MM papers. Solvents used were : Solvent A, 2-propanol-conc. ammonium hydroxide-water (7:1:2) and Solvent B, 1-butanol-ethanol-water (4:1:5, organic phase).

Thin layer chromatography was carried out on Eastman Chromagram Sheets 6060, silica gel with fluorescent indicator, using strips 10 cm x 2 cm. Thick layer silica gel chromatography used glass plates (20 cm x 20 cm) coated with a 2mm thick layer of silica gel DSF-5 (Mondray Chemicals Ltd.).

Paper electrophoresis was performed using Whatman 3MM paper in a Savant Flat Plate electrophoretic chamber with a Savant Model HV power supply operated for one hour in a bicarbonate buffer at pH 7.5.

Nucleosides, their derivatives and their photoproducts were detected on paper and silica gel chromatograms using an ultraviolet source (Mineralite, output about 254 nm) .

Equipment

Infrared spectra were recorded on a Perkin Elmer 337 spectrometer. KBr discs were used.

Ultraviolet spectra were recorded on a Cary 14 recording spectrometer.

Gas chromatography and mass spectrometry were performed on a combination Varian 1700 gas chromatograph (GC)- Finnigan 1015 Quadropole mass spectrometer (MS) with a Watson-Biemann separator interface. The column was 10% UCW-98 on 80/100 mesh acid washed-DMCS-Chromosorb W, 20 inches long with a 2mm inner diameter, stainless steel, with a 25 ml/minute helium carrier flow. The column temperature was linearly programmed from 60^{o} to 220^{o}C. at 10^{o}/minute. The temperatures of the injector and of the flame ionization detector were at 200^{o} and 250^{o}C., respectively.

Ultraviolet irradiations were carried out in a quartz 250 ml tube using a low pressure mercury lamp (wavelength, 254nm) from Ultra Violet Products, Inc., San Gabriel, California, Model No. PCQX1.

Melting points were determined on a Fisher-Johns melting point apparatus and are reported uncorrected.

The microdistillation apparatus consisted of a 20 ml pear-shaped flask connected to a 6 inch Vigreux column which was in turn joined to a 6 inch condenser. It was used for the fractional distillation of alkylsilyl chlorides that had been prepared.

Analyses

Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

GC/MS analyses of the reaction mixtures formed in the reactions to prepare alkylsilyl chlorides was made up of the following procedures:

a) observation of the gas chromatographic peaks, the elution order of which is generally in order of increasing mass[94].

b) obtaining the mass spectrum of the material at each major peak in the gas chromatogram

c) examining the mass spectrum for the molecular ion and other characteristic fragments

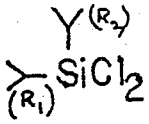
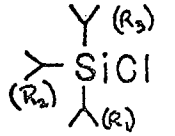
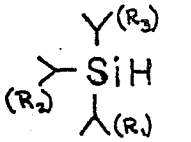
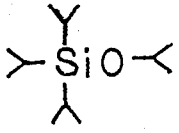
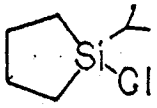
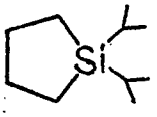
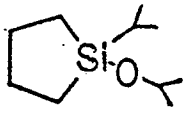
d) calculating the approximate amount of each component in the reaction mixture by measuring areas under the peaks and correcting roughly for variations in the response of the flame ionization detector by dividing the areas obtained by the number of carbon atoms in the molecule.

e) determining the number of chlorine atoms in the molecule by examining the isotope peaks associated with the molecular ion or with some of the fragments in the MS.

Characteristic fragments in the mass spectrum were the molecular ion M^+ (except for the dodecyl and octadecyl-containing molecules) and fragments corresponding to $[M-R]^+$ where R is any of the alkyl groups attached to the silicon atom. Tetramethylene alkylsilyl compounds contained additional characteristic fragments such as $[M-R-HCl]^+$ and $[M-R-C_2H_4]^+$.

Characteristic fragments of alkylsilyl thymidines were the $[M-R]^+$ fragments; no M^+ fragments were observed.

TYPICAL MASS SPECTRAL PEAKS USED TO IDENTIFY ALKYLSILYL CHLORIDES AND ASSOCIATED SIDEPRODUCTS

Structure assigned	Fragments observed			
	M^+	$[M - R_1]^+$	$[M - R_2]^+$	$[M - R_3]^+$
	176/178 (3 Cl)	133/135	NA	NA
	184/186 (2 Cl)	---------141/143----------		NA
	192/194 (1 Cl)	---------- 149/151 ----------		
	158	---------- 115 ----------		
	216	---------- 173 ----------		
	M^+	$[M - R]^+$	$[M-R-HCl]^+$	$[M-R-C_2H_4]^+$
	162/164 (1 Cl)	119/121	83	91/93
	170	127	NA	99
	186	143	NA	

NA = Not Applicable

(More detailed analysis of the mass spectra of these types of compounds are in progress in the laboratory of Dr. J.B. Westmore.)

PROCEDURES

Alkylsilyl Blocking Groups

Isopropyl Lithium - Flattened lithium wire (42 g, 6 moles) was cut into small pieces under ether and transferred to a one-liter 3-neck flask containing dry unsaturate-free pentane (400 ml) using a paper cone. Nitrogen gas applied from a second inlet was passed over the pentane and the incoming lithium.

Freshly distilled isopropyl chloride (225.5 g, 262 ml, 3 moles) was dissolved in dry pentane (200 ml) and placed in a dropping funnel. About 40 ml of this solution was added to the lithium wire in pentane. The reaction mixture was kept under a slight positive pressure of nitrogen by means of a balloon of nitrogen fixed at the end of the condenser. A second balloon of N_2 was attached to the dropping funnel containing the isopropyl chloride solution in order to equalize the pressure.

The reaction mixture was heated just enough to maintain reflux while the solution was stirred. After 5-10 minutes, the heat source was removed and the solution continued to reflux as the exothermic reaction had been initiated. Refluxing was maintained by the dropwise addition of the remaining isopropyl chloride solution. Stirring of the solution was continued overnight after all the isopropyl chloride had been added. The bluish to purple coloured suspension was then allowed to settle, leaving a light yellow pentane solution of isopropyl lithium. The solution was

carefully decanted, as needed, into a graduated dropping funnel under nitrogen. Alternatively, nitrogen pressure was used to force the solution through glass tubing into the graduated dropping funnel.

Methyloctadecylisopropylsilyl Chloride - To a cooled, stirred solution of methyloctadecylsilyl dichloride (36.5 g, 0.1 mole) in hexane (90 ml), isopropyl lithium solution (85 ml of commercially obtained solution labelled as 1.2 M) was added dropwise over a period of 30 minutes. GC/MS analysis of the mixture after 4 hours of stirring showed that approximately 50% of the starting material remained unchanged. Reexamination 24 hours later by GC/MS revealed that the reaction had not proceeded any further. A further 85 ml of isopropyl lithium was added. Three hours later, GC/MS analysis showed only a small peak at the retention time corresponding to that of starting material. After addition of 10 ml more of isopropyl lithium solution, followed by stirring for 3½ hours, the solution was filtered to remove lithium chloride. Filtration through fluted filter paper was a very slow process due to the thick liquid-wax nature of the product. Distillation of the material at 2-5 mm pressure at temperatures of 195-200°C gave a clear viscous liquid which solidified around 15°C to form a white waxy amorphous solid. On the basis of GC retention time and mass spectral fragmentation (m/e 331/333, 1 Cl, and 121/123, corresponding to $[M-43(\underline{i}\text{-Pr})]^+$ and $[M-253(C_{18}H_{37})]^+$ of $C_{22}H_{47}SiCl$), the compound was determined to have a structure corresponding to that of methyloctadecylisopropylsilyl chloride (31.0 g, 83%)

Attempted synthesis of di-t-butylsilyl dichloride

(A) A pentane solution of t-butyl lithium (95.3 ml of 2.1 M solution, 0.2 mole) was added dropwise to freshly distilled silicon tetrachloride (17.0 g, 0.1 mole) over a period of 30 minutes. The solution was maintained at reflux temperature for four days. The reaction mixture was then diluted with pentane and the resulting solution filtered to remove lithium salts. GC/MS revealed the presence of four major products. In order of elution these were:

1) t-butylsilyl trichloride 13, about 49% of the reaction mixture, M^+= 190/192 (3 Cl); $[M-57(t\text{-Bu})]^+$= 133/135

2) di-t-butylchlorosilane 15, about 40%, M^+= 178/180 (1 Cl); $[M-57]^+$= 121/123

3) di-t-butylsilyl dichloride 14, about 9% of reaction mixture, M^+= 212/214 (2 Cl); $[M-57]^+$= 155/157

4) a molecule whose fragmentation pattern in the MS was identical with that of di-t-butylsilyl dichloride 14 but which had a longer retention time in the GC than 14 (suggesting that it was not as highly branched a molecule as 14[94]) and which was tentatively assigned as t-butylisobutyl dichloride 16 (about 1 %).

An attempt was made to isolate the di-t-butylchlorosilane by two fractional distillations. However, analysis of the fractions by GC/MS revealed the presence of all the above-mentioned impurities as well as several unidentified peaks. The materials appeared to co-distill.

(B) A second synthesis was attempted in the presence of tetahydrofuran, with no heating. Silicon tetrachloride (17.0 g, 0.1 mole) was mixed with THF (5 ml). A pentane solution of t-butyl

lithium (95.3 ml, 2.1 M, 0.2 mole) was added dropwise with stirring and cooling over a period of 1½ hours. GC/MS analysis of the reaction mixture revealed the following roughly calculated composition: t-butylsilyl trichloride 13 (about 70-80%), di-t-butylsilyl dichloride 14 (10 to 20%) and di-t-butylchlorosilane 15 (around 5-10%). The assignments of structure were made on the same evidence as was obtained in the previous preparation (A).

More tetrahydrofuran (10 ml) and t-butyl lithium (38 ml, 2.1 M, 0.08 mole) were added to the reaction mixture, the latter being added over a period of one hour. Examination of the reaction mixture by gas chromatography and mass spectrometry revealed that t-butylsilyl trichloride still constituted approximately 20% of the reaction mixture, that the amounts of di-t-butylsilyl dichloride and di-t-butylchlorosilane had increased and that two new side-products (tentatively t-butylisobutylsilyl dichloride plus an unidentified compound) had begun to be formed. (Assignments made as before).

The addition of a further 20 ml of t-butyl lithium solution did not succeed in the complete conversion of t-butylsilyl trichloride to products; instead, the amount of Wurtz reaction product, 2,2,3,3-tetramethylbutane (early eluting compound, $M^+ = 144$, see Figure 6, page 36), was substantially increased. The compounds once again co-distilled when fractional distillation was attempted.

(C) A third attempted in which the silicon tetrachloride (0.05 mole) and THF (5 ml) were diluted with pentane (75 ml) prior to the addition of t-butyl lithium solution (0.97M, 103 ml, 0.1 mole) did not succeed in the elimination of sideproduct formation.

Methyldiisopropylsilyl chloride - Silicon tetrachloride (5.7 g, 0.0335 mole) was dissolved in pentane (30 ml) and tetrahydrofuran (3 ml). To this stirred, cooled solution, freshly prepared isopropyl lithium (125 ml, estimated concentration 0.56M, 0.069 mole) was added dropwise over a period of 65 minutes. The reaction mixture was allowed to stir for a further 10 minutes, then methyl lithium in ether (20 ml, 1.82M, 0.034 mole) was added dropwise. As GC/MS analysis indicated that the peak at retention time corresponding to that of diisopropylsilyl dichloride (M^+=184/186 (2 Cl); $[M-43(i\text{-}Pr)]^+$= 141/143) was still present, a further 10 ml of the above methyl lithium solution was added. GC re-examination of the reaction mixture indicated the complete removal of the diisopropylsilyl dichloride peak.

The reaction mixture was filtered, pentane was largely removed on the rotary evaporator, and the resulting solution distilled into 8 different fractions. The fractions with boiling point ranges 134-147°C and 148-154°C were found to contain the desired methyldiisopropylsilyl chloride (M^+= 164/166 (1 Cl); $[M-43]^+$= 121/123) along with small amounts of impurities, some of which were tentatively identified as the following: (in order of elution)

1) trimethylisopropylsilane (highest mass peak, determined by examining the oscilloscope of the mass spectrometer, found to be around 118; molecular weight of $C_6H_{16}Si$ is 116 mass units),

2) an unidentified compound with mass spectral fragments of m/e values of 133, 118 and 91,

3) another unidentified compound with mass spectral fragments with m/e values of 146, 118 and 75,

4) dimethyldiisopropylsilane, containing mass spectral fragments

identified on the oscilloscope of the mass spectrometer as m/e 145, 102 and 75. The molecular weight of $C_8H_{20}Si$ is 144 mass units.

The relative amounts of the products in each fraction could not be determined from the gas chromatogram due to overloading of the GC column. (Yield of mixture was 2.3 g, 42% approximately.)

Attempted Synthesis of t-Butyldodecylisopropylsilyl Chloride

(A) Dodecylsilyl trichloride (15.18 g, 0.05 mole) was dissolved in pentane (50 ml) and tetrahydrofuran (5 ml) then cooled in an ice bath. A pentane solution of t-butyl lithium (24 ml, 2.1M, 0.05 mole) was added dropwise to the above solution which was stirred and cooled. GC/MS analysis of the reaction mixture revealed that the peak with retention time corresponding to that of starting material (whose GC retention time had been determined before this experiment was begun) was still present in the gas chromatogram. Addition of more t-butyl lithium (37 ml) resulted in the disappearance of the peak corresponding to starting material. Formed in the reaction were the desired t-butyldodecylsilyl dichloride 31 (m/e 267/269 (2 Cl) and 155/157 (2 Cl) corresponding to the $[M-57(t\text{-}Bu)]^+$ and $[M-169(C_{12}H_{25})]^+$ fragments respectively of $C_{16}H_{34}SiCl_2$) as well as several sideproducts, one of which was recognized as t-butyldodecylchlorosilane 32 (m/e 233/235 (1 Cl) and 121/123 (1 Cl) corresponding to the $[M-57]^+$ and $[M-169]^+$ fragments of $C_{16}H_{35}SiCl$). The retention time of this molecule was slightly less than that of 31.

Following this, commercial isopropyl lithium solution (25 ml, 2M in pentane, 0.05 mole) was added. GC/MS analysis revealed that the peak determined earlier to correspond to t-butyldodecyl-

chlorosilane 32 had disappeared, and in its place, at somewhat longer retention time, a new peak thought to be that of t-butyldodecylisopropylsilane 34 (m/e 255, 241 and 129 corresponding to $[M-43(i\text{-}Pr)]^+$, $[M-57(t\text{-}Bu)]^+$, and $[M-169(C_{12}H_{25})]^+$ resp., of the molecule $C_{19}H_{42}Si$). At longer retention times, two other new compounds were discerned. One was not identified while the other was believed to represent the compound t-butyl-t-butoxydodecylisopropylsilane 35 (m/e 313, corresponding to $[M-57]^+$ of $C_{23}H_{50}SiO$). The unidentified peak was found to contain no isotope peaks in the mass spectrum which would indicate the presence of chlorine atoms. The major product in the reaction mixture was still t-butyldodecylsilyl dichloride (m/e 267/269 and 155/157) and there was no evidence of a peak which might correspond to the desired t-butyldodecylisopropylsilyl chloride (expected fragments would be $[M-43]^+$= 289/291, $[M-57]^+$= 275/277 and $[M-169]^+$= 163/165). The reaction mixture was filtered then subjected to fractional distillation at 2 - 5 mm pressure to give three fractions: (1) 134-139°C, (2) 141-143°C and (3) 143-145°C. Gas chromatographic analysis of the fractions showed a number of peaks, indicating that the materials present in the reaction mixture had co-distilled.

(B) t-Butyl lithium (45 ml, 0.97M, 0.043 mole) was added dropwise into a stirred, cooled solution of dodecylsilyl trichloride (9.3 g, 0.037 mole), pentane (37 ml) and dry tetrahydrofuran (3.7 ml). GC/MS analysis of the reaction mixture showed peaks corresponding to t-butyldodecylsilyl dichloride (about 90% of the reaction mixture, identified as in method A above) as well as t-butyl-t-butoxydodecylsilyl chloride 33 (m/e 305/307 (1 Cl) and 193/195

(1 Cl) corresponding to the $[M-57(\underline{t}\text{-Bu})]^+$ and $[M-169(C_{12}H_{25})]^+$ fragments of $C_{20}H_{43}SiOCl$) and other unidentified impurities.

Isopropyl lithium (10 ml, estimated concentration 2.86M) was added slowly to the reaction mixture. Two new products were formed in low yields, one of which was tentatively identified as the desired t-butyldodecylisopropylsilyl chloride 36 (m/e 289/291, 1 chlorine atom, corresponding to $[M-43]^+$ of $C_{19}H_{41}SiCl$). Allowing the reaction mixture to stir at room temperature for a week did not bring about an increase in the relative amount of 36, as observed from analysis of the gas chromatogram taken of the reaction mixture after that period of time.

Part of the pentane was removed by distillation and replaced with heptane (10 ml). Heating the reaction mixture for two hours at 75-80°C produced and increase of about 17% in the amount of side-products formed, a slight increase (about 1%) in the amount of t-butyldodecylisopropylsilyl chloride 36 formed and an 18% drop in the amount of t-butyldodecylsilyl dichloride 31. Addition of a further 15 ml of isopropyl lithium solution, followed by refluxing at 100°C, led to the complete removal of 31. The desired product 36 constituted 16% of the reaction mixture. The solution was then filtered and the heptane removed by fractional distillation. Following the removal of heptane, no other product could be forced to distill over, even though the liquid temperature exceeded 200°C and a pressure of about 1 to 5 mm of mercury was maintained.

t-Butylmethyloctadecylsilyl Chloride - Methyloctadecylsilyl dichloride (36.7 g, 0.1 mole) was dissolved in pentane (100 ml)

and dry tetrahydrofuran (10 ml). To this solution, t-butyl lithium (105 ml, 0.97M, 0.1 mole) was added dropwise with stirring and cooling. Gas chromatographic results indicated that the peak corresponding to the starting material (whose retention time had been determined before the start of this reaction) had completely disappeared. Three products appeared on the gas chromatogram. In order of elution, they were:
1) 2,2,3,3-tetramethylbutane (M^+= 144), which accounted for about 5% of the reaction mixture,
2) t-butylmethyloctadecylsilyl chloride (m/e 331/333 (1 Cl) and 135/137 (1 Cl), corresponding to the $\left[M-57(t\text{-}Bu)\right]^+$ and the $\left[M-253(C_{18}H_{37})\right]^+$ fragments of $C_{23}H_{49}SiCl$) which made up 89% of the reaction mixture and
3) an unidentified compound whose retention time was longer than that of t-butylmethyloctadecylsilyl chloride.

The reaction mixture was passed through filter paper to remove the lithium salts to leave 36.9 grams of a reagent determined by GC to be 89% pure t-butylmethyloctadecylsilyl chloride. This was a yield of 84.5%. Subsequent handling of this reagent reavealed that it contained some lithium salts as an impurity as well, which would effectively have reduced the yield of this reaction. The reagent, found to be a semi-solid at room temperature, could not be distilled under low pressure (1 to 5 mm) and at elevated temperatures (around 200°C).

Triisopropylsilyl Chloride - Silicon tetrachloride (8.5 g, 0.05 mole) was dissolved in pentane (50 ml) and THF (5 ml) and the resulting solution cooled. Freshly prepared isopropyl lihtium (unknown concentration) was added in portions, dropwise, with stirring and cooling under nitrogen. The reaction mixture was examined after addition of each portion. (From the changes observed in the GC/Ms results following the addition of a measured portion of isopropyl lithium solution, it was possible to calculate the approximate concentration of the isopropyl lithium.) A total of 276 ml of an estimated 0.56M solution was needed to prepare triisopropylsilyl chloride. The major impurity (about 8% of the reaction mixture) was found to be triisopropylisopropoxysilane (m/e 216 and 173 corresponding to the molecular ion and the $[M-43]^+$ fragment of $C_{12}H_{28}SiO$, respectively). Minor impurities included triisopropylsilane (m/e 158 and 115, corresponding to the molecular ion and $[M-43]^+$ fragment of $C_9H_{22}Si$, respectively) and four or more unidentified compounds with retention time considerably longer than that observed for the other products in the mixture. The order of elution for the compounds in this reaction were - triisopropylsilane, triisopropylsilyl chloride (m/e 192/194 (1 Cl), and 149/151, corresponding to the molecular ion and the $[M-43]^+$ ion of $C_9H_{21}SiCl$), triisopropylisopropoxylsilane, then the unidentified minor side-products. Fractional distillation of the reaction mixture after filtration and removal of pentane gave triisopropylsilyl chloride

in about 90% purity in the fraction with boiling range 199-203°C (5.23 g, 54% yield) Impurities included triisopropylisopropoxysilane (6%), triisopropylsilane (1%) and several unidentified compounds which constituted 3% of the triisopropylsilyl chloride reagent. Other fractions contained triisopropylsilyl chloride in varying degrees of purity as well - e.g. 170-195°C - 75% (0.49 g); 195-199°C - 80% (0.62 g); and 203-209°C - 86% (1.64 g).

Tetramethyleneisopropylsilyl Chloride - Tetramethylenesilyl dichloride (silacyclopentyldichloride) 44, freshly distilled, (b.p. 138-139°C, 15.5g, 0.1 mole) was dissolved in pentane (100 ml) and tetrahydrofuran (10 ml). Freshly prepared isopropyl lithium (undetermined concentration, 50 ml) was added dropwise with stirring and cooling under a nitrogen atmosphere. GC/MS analysis revealed that the peak corresponding to starting material (whose retention time had been determined previously) constituted 53% of the reaction mixture. Addition of 67 ml more of the isopropyl lithium solution in two portions (each addition being followed by GC/MS analysis) effected the complete conversion of staring material 44 to tetramethyleneisopropylsilyl chloride (m/e 162/164 (1 Cl), 119/121, 83 and 91/93 corresponding to the fragments M^+, $[M-43(i\text{-}Pr)]^+$, $[M-43-36(HCl)]^+$ and $[M-43-28(C_2H_4)]^+$ of $C_7H_{15}SiCl$). The reaction mixture was filtered and distilled under nitrogen into six fractions. The fractions 127-131°C and 132-190°C were combined and redistilled into two fractions, 132-164°C and 165-167.5°C. The latter solution was fractionated once more and the material in the boiling point range 165-175°C was collected (2.60 g, 16%) and was found to contain tetramethyl-

eneisopropylsilyl chloride in 89% purity. In order of elution from the GC column, impurities included:

1) three or more unidentified compounds, about 4% of the reagent;

2) a compound whose mass spectrum was identical with that of TMIPSi chloride, but whose GC retention time was slightly longer, and which was tentatively assigned as tetramethylene-n-propylsilyl chloride, about 3% of the reagent;

3) another unidentified compound (less than 1% of the reagent);

4) tetramethyleneisopropylisopropoxysilane (about 2% of the reagent) (m/e 186 and 143 corresponding to the molecular ion and the $[M-43]^+$ fragment of $C_{10}H_{22}SiO$);

4) tetramethylenediisopropylsilane (m/e 170 and 127 corresponding to the molecular ion and $[M-43]^+$ fragment of $C_{10}H_{22}Si$); and

5) a compound identified as tetramethylenehexylsilyl chloride (m/e 204/206 (1 Cl), 119/121, 83, and 91/93, corresponding to the fragments M^+, $[M-85(C_6H_{13})]^+$, $[M-85-36(HCl)]^+$ and $[M-85-28(C_2H_4)]^+$ of the molecule $C_{10}H_{21}SiCl$), arising perhaps from a small amount of commercial isopropyl lithium which may have been added to drive the reaction to completion. Hexyl side-chains were found to be formed in the preparation of diisopropylsilyl dichloride (page 144) where the commercial i-PrLi was noted to have been used.

Tetramethylene-t-butylsilyl Chloride - Tetramethylenesilyl dichloride (15.5 g, 0.1 mole) was stirred and cooled in an ice-bath while a solution of t-butyl lithium (49 ml, 2.1M, 0.1 mole) was added dropwise over a period of one hour. GC/MS analysis indicated

that the desired compound tetramethylene-t-butylsilyl chloride (m/e 176/178 (1 Cl), 119/121, 83 and 91/93 corresponding to the fragments M^+, $[M-57(t\text{-}Bu)]^+$, $[M-57-36(HCl)]^+$, and $[M-57-28(C_2H_4)]^+$ of the molecule $C_8H_{17}SiCl$) was obtained almost pure. The actual amounts of the impurities could not be determined from the gas chromatogram due to accidental overloading of the column. The reaction mixture was filtered, pentane was distilled and the resulting liquid fractionally distilled to give pure tetramethylene-t-butylsilyl chloride (b.p. 182-184°C, 9.41 g, 53% yield).

Diisopropylsilyl dichloride - Silicon tetrachloride (85 g, 0.5 mole) was dissolved in pentane (500 ml) and tetrahydrofuran (50 ml). Freshly prepared isopropyl lithium (900 ml of undetermined concentration in pentane) was added with stirring and cooling to the silicon tetrachloride solution under nitrogen.

This had begun as a preparation of triisopropylsilyl chloride and GC/MS analysis indicated that significant amounts of diisopropylsilyl dichloride (M^+= 184/186 (2 Cl), $[M-43(i\text{-}Pr)]^+$= 141/143, $[M-43-36(HCl)]^+$= 105 (a trace)) and isopropylsilyl trichloride (M^+= 176/178 (3 Cl), $[M-43]^+$= 133/135) were still present in the reaction mixture. Since the supply of freshly prepared i-PrLi had been exhausted, commercially obtained isopropyl lithium was then added (335 ml, 1M). GC/MS analysis following this last addition showed the sudden appearance of many side-products, some of which seemed to contain hexyl groups. No more of this inferior quality reagent was added to the reaction mixture.

Instead, the reaction mixture was filtered and distilled

into five fractions. The portion with boiling point 161-177°C was fractionated again into six samples. The fraction with b.p. 162-170°C was redistilled into three fractions. Diisopropylsilyl dichloride (determined as before, on the previous page) was found to comprise 80% of the fraction with boiling range 165.5-167.0°C. Impurities included triisopropylsilyl chloride (3%, M^+= 192/194 (1 Cl), $[M-43]^+$= 149/151) and a number of hexyl-containing compounds making up 17% of the reaction mixture. It was this solution which was used in the diisopropylsilyl dichloride reactions with 5'-trityl and 5'-acetyl uridine on pages 164 and 165 respectively. (Yield - 0.78 g, 0.8%)

SYNTHESIS OF ALKYLSILYL DERIVATIVES OF THYMIDINE

General Procedure

The general procedure used was as follows:

Thymidine (242 mg, 1 mmole), imidazole (136 mg, 2 mmole) and the alkylsilyl chloride (1.1 mmole) were dissolved in dry dimethylformamide (1 ml) and stirred at room temperature until TLC developed in ether indicated that the reaction was complete (usually within one-half hour). The reaction mixture was applied to two silica gel plates , which, when developed in ether, revealed (usually) the presence of four compounds - unreacted thymidine (R_f .04), 5'-alkylsilyl thymidine (R_f .35), 3'-alkylsilyl thymidine (R_f .53) and 3,5'-di(alkylsilyl)thymidine (R_f .85).

The general equation and the yields obtained for reactions

carried out in this manner are shown for reagents a, b, c, d, e and f in Table 1 on page 63.

In an attempt to increase the specificity of a reagent, the following modification was used with three of the reagents - b, c, and d:

Thymidine (242 mg, 1 m mole) and imidazole (136 mg, 2 m mole) were dissolved in dry DMF (1 ml). The alkylsilyl chloride (1.1 m mole) was added dropwise to the rapidly stirred DMF solution. The same work-up as that described in the general procedure was used. The yields resulting from this modification are shown in brackets in Table 1 on page 63.

5'-t-Butylmethyloctadecylsilyl Thymidine

Because TBMODSi chloride was found to be insoluble in DMF, different solvents had to be used in its reaction with thymidine.

(A) Thymidine (242 mg, 1 mmole) was dissolved in dry pyridine (5 ml). t-Butylmethyloctadecylsilyl chloride (428 mg, 1.1 mmole) was dissolved in pentane (1.5 ml) and added to the stirred pyridine solution. The reaction mixture was allowed to stir at room temperature for 1½ days. GC/MS examination of the reaction mixture revealed that the peak representing TBMODSi chloride had been replaced by a peak corresponding to t-butylmethyloctadecylsilanol. The solvents were removed by a stream of air directed across the surface of the reaction mixture in a bell jar, the residue dissolved in tetrahydrofuran and the reaction mixture applied to three silica gel plates which were developed in ether-hexane (2:1) to give

unreacted thymidine at the origin and 5'-TBMODSi thymidine as the only other nucleosidic material (175 mg, 29.5% yield, identified on the basis of the results shown in Tables 9 and 10 on pages 150 and 151 respectively).

Using freshly prepared dry pyridine in the above reaction raised the yield of 5'-TBMODSi thymidine to 245 mg (41%) and gave a 2% yield of 3',5'-di(TBMODSi)thymidine (see also Tables 9 and 10).

(B) Thymidine (242 mg, 1 mmole), TBMODSi chloride (428 mg, 1.1 mmole), (apparently not very) dry pyridine (5 ml) and pentane (1.5 ml) were stirred at room temperature for 22 hours. As TLC in ether indicated that a large amount of unreacted thymidine at R_f .04 was still present, more TBMODSi chloride (200 mg) was added to the reaction mixture. Over the next five days, for the same reason as stated above, another 600 mg of the TBMODSi chloride reagent was added to the reaction mixture. Solvents were removed, the residue dissolved in ether and applied to six silica gel plates which were developed in ether-hexane (2:1). Three bands were observed - material at the origin, two products intermingling at R_f .35 (poor quality silica gel plates) and a faintly absorbing material at the solvent front, presumed to be the t-butylmethyloctadecysilanol or the unreacted silyl chloride. Both the material at the origin and that at R_f .35 were rechromatographed on silica gel plates (developed twice) in ether-hexane (2:1) to give thymidine at the origin, a compound at R_f .50 identified as 5'-TBMODSi thymidine (451 g, 76%, mp 80-80.5°C, 95% ethanol)

or 81.5 - 82.0°C (aqueous ethanol), highest mass peak at 537, M-43, satisfactory elemental analysis in Table 10, page 151 , and a compound at R_f .67 identified as 3'5'-di(TBMODSi) thymidine (60 mg, 10%, mp 35.7 - 38.5°C from 95% ethanol, elemental analysis in Table 10, page 151).

(C) Thymidine (242 mg) and imidazole (176 mg) were dissolved in 4.5 ml dry DMF-dry THF (1:1). A suspension of TBMODSi chloride (428 mg) in 3.4 ml of the same solvent was added to the thymidine solution. Addition of 7 ml more of dry THF was effective in finally dissolving the silyl chloride. Examination of the reaction mixture by TLC showed only starting material after several hours of stirring at room temperature.

(D) Thymidine (242 mg),imidazole (176 mg) and TBMODSi chloride (428 mg) were dissolved in dry THF (25 ml). TLC showed no product formation.

(E) Thymidine (242 mg) and imidazole (176 mg) were dissolved in dry DMF (2 ml). TBMODSi chloride (428 mg) was dissolved in pentane (3 ml) and added to the DMF solution. Rapid stirring for six days kept the two layers interspersed. Examination by TLC of the reaction mixture revealed two dark spots - thymidine at R_f .03 and 5'-TBMODSi thymidine at R_f .61 (in ether). Chromatography on silica gel plates in ether gave 5'-TBMODSi thymidine (142 mg, 24%).

Preparation of 5'-TBDMSi thymidine in pyridine and pentane

Thymidine (121 mg, 0.5 mmole) and TBDMSiCl (82.5 mg, 0.55 mmole) were dissolved in dry pyridine (2.5 ml) and pentane (1 ml). Monitored by TLC, progress of the reaction was observed to be very slow relative to reaction in dimethylformamide in the presence of imidazole. Two days later, the reaction mixture was concentrated to a solid, redissolved in ethanol and placed on a silica gel plate which was developed in ether. The major band was eluted with ether to give 5'-TBDMSi thymidine[79] (82.3 mg, 46.2% yield, highest mass peak at 299, which corresponds to the $[M-57(t\text{-}Bu)]^+$ fragment of 5'-TBDMSi thymidine)

3'-Tetramethylene-t-butylsilyl Thymidine - Specific Synthesis

5'-Trityl thymidine (484 mg, 1 mmole), imidazole (136 mg, 2 mmole), tetramethylene-t-butylsilyl chloride (194 mg, 1.1 mmole) and dry DMF (1 ml) were stirred at room temperature for 26 hours, then developed on silica gel plates in ether to give one band. This band was eluted with ether to give 593 mg of material presumed to be 5'-Tr-3'-TMTBSi thymidine (95% yield).

The above compound (226 mg, 0.366 mmole) was dissolved in 80% acetic acid and heated on the steam bath. Samples were removed from the reaction mixture in five minute intervals and chromatographed on TLC developed in ether. It was seen that, after 10 minutes, the spot at R_f .69 corresponding to the starting material had completely disappeared. In its place was a spot at R_f .53 corresponding to a 3'-R_3Si thymidine. The acetic acid was

removed, the residue dissolved in ether, and applied to two silica gel plates. The plates were developed first in hexane-ether (60:40) then in ether. The major band was eluted with ether to give a syrupy material which was crystallized from aqueous ethanol to give 3'-TMTBSi thymidine (100 mg, 72%, mp 72-73°C, highest mass peak at 325, M-57, see Table 9, page 150).

3'-Triisopropylsilyl Chloride - Specific Synthesis

5'-Trityl thymidine (242 mg, 0.5 mmole), imidazole (68 mg, 1 mmole), triisopropylsilyl chloride (144 mg, .75 mmole) and dry DMF (0.5 ml) were stirred at room temperature for 14 hours. The reaction mixture was applied to two silica gel plates which were developed in ether-hexane (2:1). The major band was eluted with ether to give 5'-trityl-3'-triisopropylsilyl thymidine (294 mg, 92%).

The 5'-Tr-3'-TIPSi thymidine was heated in 80% acetic acid (5 ml) on a steam bath for 35 minutes before complete removal of the TLC spot at R_f .76 (ether) corresponding to 5' Tr 3' TIPSi thymidine was effected. On TLC developed in ether, new spots appeared at R_f .04 (thymidine), at R_f .83 (tritanol) and at R_f .63 (3'-TIPSi thymidine). The reaction mixture was placed on two silica gel plates which were developed in ether-hexane (2:1) to give a major band at R_f .19. This band was eluted with ether to give 3'-triisopropylsilyl thymidine (130 mg, 65% overall, see Table 9, page 150). Considerable difficulty was encountered in crystalliaztion of 3'-TIPSi thymidine.

3'5'-DI(ALKYLSILYL)THYMIDINE DERIVATIVES

3'5'-Di(Methyldiisopropylsilyl) Thymidine

Thymidine (242 mg, 1 mmole), imidazole (272 mg, 4 mmole) and methyldiisopropylsilyl chloride reagent (500 mg of the 134-147°C and 148-154°C fractions) were dissolved in dry DMF (1 ml) and stirred at room temperature for four hours. TLC showed no apparent increase in the relative intensity of the spot corresponding to 3'5'-di(MDIPSi)T between t = ½ hour and t = 4 hours. A further 483 ml of the reagent were added. TLC examination of the reaction mixture five minutes after the addition showed that the spot corresponding to thymidine had completely disappeared and the spots representing 5'-MDIPSi thymidine and 3'-MDIPSiT had become quite faint in intensity. Silica gel chromatography in hexane-ether (60:40), followed by elution of the major band, gave impure 3'5'-di(methyldiisopropylsilyl) thymidine (362 mg ~72.5%). See Table 9, page 150. GC/MS analysis of this material showed it to contain several impurities resulting from the presence of dimethylisopropylsilyl chloride in the reagent used. The R_f values of the impurities and of the desired 3'5'-di(MDIPSi) thymidine were identical on TLC (R_f .73 in ether).

3'5'-Di(Tetramethyleneisopropylsilyl) Thymidine

Thymidine (242 mg, 1 mmole), imidazole (272 mg, 4 mmole) and dry DMF were stirred at room temperature while sufficient tetra-metyleneisopropylsilyl chloride was added dropwise to drive the reaction to completion. Progress of the reaction was monitored by

TABLE 9

PHYSICAL PROPERTIES OF ALKYLSILYL THYMIDINE DERIVATIVES

NUCLEOSIDE	MOL. WT.	(OBSERVED) HIGHEST MASS PEAK $[M-R]^+$	MELTING[1] POINT °C	INFRARED-Si-O[178] FREQUENCIES ± 5 cm^{-1}
5'-MODIPSiT	578	535	74.5- 75.5	1200,1090,940
5'-TBMODSiT	594	537	81.5- 82.0	1205,1095,940
5'-MDIPSiT	370	327	152.0-154.0	1201,1097,940
5'-TMIPSiT	368	325	139.0-140.0	1205,1098,940
5'-TIPSiT	398	355	157.0-158.5	1203,1092,940
5'-TMTBSiT	382	325	182.0-182.5	1203,1098,942
3'-MDIPSiT	370	327	59.0- 66.0[2]	1180(?),1110
3'-TMIPSiT	368	325	57.0- 64.0[3]	1192,1100,952
3'-TIPSiT	398	355	85.0- 88.0[3]	1200,1100,953
3'-TMTBSiT	382	325	72.0- 73.0	1180(?),1105,950
3'5'-di(TBMODSi)T	948	-[4]	37.5- 38.5	
3'5'-di(MDIPSi)T	498	455	-[5]	-
3'5'-di(TMIPSi)T	494	451	-[6]	1195,1095 or 1075,965
3'5'-di(TIPSi)T	554	511	125.0-126.5	1199,1098 or 1065,965
3'5'-di(TMTBSi)T	522	465	113.0-114.0	1197,1090 or 1075,965

[1] recrystallizations from aqueous ethanol

[2] contained a 15% impurity of 3'-dimethylisopropylsilyl thymidine

[3] difficult to crystallize

[4] beyond the range of the mass spectrometer used

[5] impossible to purify due to presence of dimethylisopropylsilyl group

[6] could not be crystallized

TABLE 10

ELEMENTAL ANALYSIS OF SOME ALKYLSILYL THYMIDINE DERIVATIVES

NUCLEOSIDE		%C	%H	%N
5'-MODIPSiT	calc	66.17	10.41	4.82
	obs	66.30	10.25	4.73
5'-MDIPSiT	calc	55.11	8.16	7.56
	obs	54.90	8.05	7.43
5'-TMIPSiT	calc	55.42	7.66	7.60
	obs	55.21	7.62	7.48
5'-TIPSiT	calc	57.27	8.60	7.03
	obs	57.27	8.76	6.96
5'-TMTBSiT	calc	56.53	7.91	7.33
	obs	56.34	7.89	7.40
5'-TBMODSiT	calc	66.63	10.51	4.71
	obs	66.80	10.47	4.75
3'5'-di(TBMODSi)T $\cdot H_2O$	calc	69.65	11.48	2.90
	obs	69.48	11.64	2.81

TLC. Separated from the reactants by chromatography on silica gel plates in ether-hexane (2:1), the desired 3'5'di(tetramethylene-isopropylsilyl) thymidine was obtained in almost quantitative yield (488 mg, 98.8%). See Table 9 , page 150 . An attempt to crystallize the compound from aqueous alcohol led to the formation of spots on TLC corresponding to small amounts of thymidine, 3'TMIPSi thymidine and 5'TMIPSi thymidine. 3'5'di(TMIPSi) thymidine could not be crystallized.

3'5'-Di(Triisopropylsilyl) Thymidine

Thymidine (242 mg, 1 mmole), imidazole (272 mg, 4 mmole), triisopropylsilyl chloride (580 mg, 3 mmole) and dry DMF (1 ml) were stirred at room temperature for 2½ days. After chromatography on silica gel plates in ether-hexane (2:1) and elution with ether, 3'5'di(triisopropylsilyl) thymidine was obtained (434 mg, 78%, mp 125-126.5°C from aqueous ethanol). See Table 9 , page 150 .

3'5'-Di(Tetramethylene-t-Butylsilyl) Thymidine

Thymidine (242 mg, 1 mmole), imidazole (272 mg, 4 mmole), tetramethylene-t-butylsilyl chloride (488 mg, 2.8 mmole) and dry DMF (1 ml) were stirred for 1½ days, at room temperature. The reaction mixture was chromatographed on silica gel plates in ether-hexane (2:1) to give one major compound. Eluted with ether, the major band yielded 3'5'di(tetramethylene-t-butylsilyl) thymidine (480 mg, 92%, mp 113-114°C from aqueous ethanol). See Table 9 , page 150 .

3'5'-Di(t-Butylmethyloctadecylsilyl) Thymidine

This compound was obtained as a side product in the preparation of 5'-TBMODSi thymidine. See page 146 and Tables 9 and 10 on pages 150 and 151 respectively for characterization data.

THYMIDINE BLOCKED BY TWO DIFFERENT ALKYLSILYL GROUPS

5'-t-Butyldimethylsilyl-3'-tetramethylene-t-butylsilyl Thymidine (5'-TBDMSi-3'-TMTBSiT)

5'-t-butyldimethylsilylthymidine[79] (200 mg, 0.56 mmole), imidazole (77 mg, 1.12 mmole), tetramethylene-t-butylsilyl chloride (110 mg, 0.62 mmole) and dry DMF (0.6 ml) were stirred at room temperature for 21½ hours. The reaction mixture was placed on two silica gel plates which were developed in ether. The major band was eluted with ether to give the desired 5'-TBDMSi-3'-TMTBSiT (263 mg, 94%, mp 119-120°C, highest mass peak at 439 or M-57).

5'-Tetramethylene-t-butylsilyl-3'-t-butyldimethylsilyl Thymidine

5'-tetramethylene-t-butylsilyl thymidine (100 mg, 0.26 mmole), imidazole (35 mg, .52 mmole), t-butyldimethylsilyl chloride (78 mg, .52 mmole) and dry DMF (0.3 ml) were stirred at room temperature for 2 hours. The reaction mixture was placed on a silica gel plate and developed in hexane-ether (60:40) to give one major band. Elution of this band with ether gave 5'-TMTBSi-3'-TBDMSiT (121 mg, 93%, mp 107-107.5°C, highest mass peak at 439 or M-57).

5'-Tetramethyleneisopropylsilyl-3'-Triisopropylsilyl Thymidine

3'-triisopropylsilyl thymidine (145 mg, .365 mmole) was mixed with imidazole (68 mg, 1 mmole), tetramethyleneisopropylsilyl chloride (90 mg, .545 mmole) and dry DMF (.4 ml). TLC indicated an almost instantaneous formation of a spot corresponding to a disubstituted thymidine molecule at R_f .85. Elution of the major band obtained from chromatography on silica gel plates developed in ether gave 5'-TMIPSi-3'-TIPSi thymidine (178 mg, 87.5%, highest mass peak at 524, molecular ion). An attempt to crystallize the material from aqueous ethanol led to the formation of an extra spot on TLC corresponding in R_f value to that of 3'-TIPSi thymidine. The material could not be made to crystallize.

5'-Triisopropylsilyl-3'-Tetramethyleneisopropylsilyl Thymidine

5'-triisopropylsilyl thymidine (100 mg, 0.25 mmole), imidazole (34 mg, 0.50 mmole), tetramethyleneisopropylsilyl chloride (61 mg, 0.38 mmole) and dry DMF (0.25 ml) were stirred at room temperature for twenty minutes, and chromatographed on plates in ether-hexane (2:1) to give only one product, 5'-TIPSi-3'-TMIPSi thymidine (131 mg, 99.5%, highest mass peak at 481, M-43). An attempt to crystallize the material was unsuccessful, as TLC in ether revealed the appearance of a spot corresponding to 5'-TIPSi thymidine at R_f .35 along with the 5'-TIPSi-3'TMIPSi thymidine at R_f .85.

5'-Tetramethylene-t-Butylsilyl-3'-Tetramethyleneisopropylsilyl Thymidine

5'-TMTBSi thymidine (100 mg, .262 mmole), imidazole (35.6 mg, .524 mmole), tetramethyleneisopropylsilyl chloride (47 mg, .29 mmole)

and dry DMF (0.3 ml) were stirred at room temperature until all the material had dissolved. The reaction mixture was then chromatographed on plates in ether to give 5'-TMTBSi-3'-TMIPSi thymidine (126 mg, 94.5%, highest mass peak at 451, M-57). An attempt to crystallize this compound from aqueous ethanol failed due to the formation of an impurity detected on TLC having an R_f value similar to that of 5'-TMTBSi thymidine.

5'-Tetramethyleneisopropylsilyl-3'-Tetramethylene-t-Butylsilyl Thymidine

3'-tetramethylene-t-butylsilyl thymidine (100 mg, .262 mmole), imidazole (35.6 mg, .524 mmole), tetramethyleneisopropylsilyl chloride (60 mg, .29 mmole) and dry DMF (0.3 ml) were stirred overnight at room temperature. The reaction mixture was chromatographed on silica gel plates in ether-hexane (1:1) to give the desired 5'-TMIPSi-3'-TMTBSi thymidine (87 mg, 65%, highest mass peak at 451, M-57). This compound failed to crystallize from an aqueous ethanol solution. Examination by TLC of the aqueous ethanol solution containing the once pure 5'-TMIPSi-3'-TMTBSiT revealed the presence of a significant spot at an R_f value corresponding to that of 3'-TMTBSi thymidine.

Lipophilic Nature of the MODIPSi Group

5'-methyloctadecylisopropylsilyl thymidine (100 mg) was dissolved in pyridine (1 ml), then poured onto ice (40 g). The thymidine derivative precipitated out and was collected (92.4 mg, 92% recovery).

HYDROLYSIS OF ALKYLSILYL THYMIDINE DERIVATIVES

Acid Hydrolysis of Monosubstituted Alkylsilyl Thymidines - General Procedures

(A) A small sample (~10 mg) of the 5'- or 3'-alkylsilyl thymidine was dissolved in 80% acetic acid (~0.5 ml).

(a) This solution was allowed to stir at room temperature. Progress of the hydrolysis was monitored by the removal of small samples (at regular intervals) which were analyzed by TLC. When the spot corresponding to starting material was no longer visible on TLC (i.e., when the only spot visible was that corresponding to thymidine), hydrolysis was considered complete and the time at which this occurred was noted.

(b) If complete hydrolysis were not achieved in 24 hours, the reaction was stopped at the 24 hour stage by applying the acid solution to a paper chromatogram which was then developed in solvent B to separate thymidine from its alkylsilyl derivative. Percent hydrolysis was calculated on the basis of the UV absorbance of the eluted bands made up to a known volume in ethanol and according to the following equation:

$$\% \text{ hydrolysis} = \frac{A_T V_T}{A_T V_T + A_S V_S} \times 100$$

where A_T = observed absorbance of the thymidine band

V_T = volume of the thymidine solution

A_S = absorbance of the silyl derivative of thymidine

V_T = volume of the silyl derivative solution

(c) The acetic acid solution was heated on the steam bath. TLC examination at regular intervals was the method used to closely monitor the progress of the hydrolysis. The time required for the complete removal of the spot corresponding to alkylsilyl thymidine was noted.

(B) A small sample (~10 mg) of the monosubstituted 5'- or 3'-alkylsilyl thymidine was dissolved in a solution of 0.01 N hydrochloric acid (~0.5 ml) which was then heated on the steam bath until TLC results (taken at regular intervals) indicated complete hydrolysis of the alkylsilyl thymidine to thymidine. The time required for complete hydrolysis to be effected was noted.

This latter approach (B) was the method of choice in the acid hydrolysis of the more stable derivatives like the 5'- and 3'-TIPSi and TMTBSi derivatives of thymidine. It was found that the prolonged heating in acetic acid (Method A(c)) required for the removal of these groups resulted in the acetylation of first the free hydroxyl group in the molecule, and secondly, of the thymidine formed once acid hydrolysis of the alkylsilyl group had taken place. This resulted in the formation of a number of products instead of just thymidine.

The results of the above acid hydrolysis reactions are shown in Tables 2 and 3 on pages 64 and 65 respectively.

Base Hydrolysis of 5'-Alkylsilyl Thymidines - General Procedures

(A) A small sample (~10 mg) of 5'-alkylsilyl thymidine was dissolved in 15% ammonium hydroxide in ethanol (~1 to 2 ml) and allowed to stir

at room temperature for 24 hours in a closed vial.

(B) 5'-alkylsilyl thymidine (~10 mg) was dissolved in ethanolic 15% ammonium hydroxide (~1 to 2 ml) in a closed vial and heated in a 60°C bath for one hour.

(C) 5'-alkylsilyl thymidine (~10 mg) was allowed to stir for 24 hours at room temperature in an ethanolic 50% ammonium hydroxide solution (~1 to 2 ml).

(D) A sample of 5'-alkylsilyl thymidine (~10 mg) in 50% ammonium hydroxide (~1 to 2 ml) was heated in a 60° bath for an hour.

Each reaction solution was then applied to a paper chromatogram which was developed in solvent B to separate thymidine from the alkylsilyl thymidine. The bands corresponding to the starting material (R_f ~0.90) and the product, thymidine (R_f ~0.55) were eluted with ethanol. The ethanol solution of each compound was quantitatively transferred to a volumetric flask (5, 10, 25 or 50 ml size) and made up to volume with ethanol. The UV absorbance at 267.5 nm of each solution relative to the base-line was noted and the percent of hydrolysis to thymidine calculated by the formula given on page 155 under the general procedures for acid hydrolysis of monosubstituted alkylsilyl thymidines.

The results of these base hydrolysis reactions on various 5'-alkylsilyl thymidines are shown in Table 2 on page 66.

Removal of Alkylsilyl Groups by Fluoride Ion

A small sample (~5 mg) of 5'-alkylsilyl thymidine (5'-TMIPSiT, 5'-MODIPSiT, 5'-TBMODSiT, 5'-MDIPSiT, 5'-TMTBSiT or 5'-TIPSiT) was dissolved in tetrahydrofuran (0.1 ml). To this solution was added tetra-n-butylammonium fluoride (0.4M in THF, 0.1 ml) and the reaction mixture was allowed to stand for several minutes at room temperature. After twenty minutes, 5'-TMIPSiT and 5'-MODIPSiT solutions were passed through 200-300 mg silica gel in a sintered glass funnel, being eluted with tetrahydrofuran. The same was done with 5'-TBMODSiT and 5'-MDIPSiT after twenty-five minutes and with 5'-TMTBSiT and 5'-TIPSiT after 35 minutes. Examination of the eluted THF solution from each sample by TLC in ether showed absence of a spot at R_f ~.44 corresponding to the 5'-alkylsilyl thymidine; only a spot at R_f .07 corresponding to thymidine was discernible. An exception to this observation was 5'-TBMODSiT, where a very faint spot corresponding to the thymidine derivative was seen. A thirty minute reaction time (instead of twenty-five) should have been used to effect complete removal of the t-butylmethyloctadecylsilyl group.

Acid Hydrolysis of 3'5'-Di(Alkylsilyl) Thymidines - General Procedures

3'5'-di(alkylsilyl) thymidine (0.5 mmole) was dissolved in acid solution (5 ml of 80% acetic acid, 0.01 N hydrochloric acid, 0.05 N hydrochloric acid or 0.10 N hydrochloric acid) and heated on the steam bath (or allowed to stir at room temperature). Monitored by TLC, the hydrolysis was allowed to proceed until most (or a

significant portion) of the disubstituted thymidine had been converted to thymidine and monosubstituted thymidine derivatives. The reaction mixture was then applied to a silica gel plate which was developed in ether. Each of the three or four resulting bands was eluted with ethanol or ether, these ethanol or ether solutions concentrated to a solid or semi-solid in a weighed vial and the amounts of each component calculated. The equation and the results of these experiments are shown in Table 4 on page 68.

Acid Hydrolysis of Thymidine Molecules Blocked by Two Different Alkylsilyl Groups - General Procedure

The disubstituted thymidine (~10 mg) was dissolved in 80% acetic acid (~0.5 ml) and stirred at room temperature (or heated on the steam bath) just until TLC monitoring indicated that all the starting material had been converted to products. The reaction mixture was then chromatographed on papers in solvent B and the resulting products were isolated. The relative yields of each product were determined by comparing UV absorbances of ethanol solutions of each made up to a known volume, using the following equation:

$$\% X = \frac{A_X V_X}{A_X V_X + A_Y V_Y} \times 100$$

where X = one of the products isolated from the acid hydrolysis reaction

Y = the other product isolated in the reaction

A_X, A_Y = observed absorbance of each compound, X and Y, in a known volume of solution

V_X, V_Y = volume to which each eluted band was diluted

Acid Hydrolysis of 5'-TBMDSi-3'-TMTBSi Thymidine

5'-TBDMSi-3'-TMTBSi thymidine (53.5 mg, 0.108 mmole) was dissolved in 80% acetic acid (0.5 ml) and heated on the steam bath for 30 minutes. The reaction mixture was chromatographed on a silica gel plate in ether to give three compounds - starting material (25%), 3'-TMTBSi thymidine (70%) and thymidine (5%).

The results of the above experiments are shown in Table 5 on page 70.

REACTIONS OF URIDINE DERIVATIVES WITH ALKYLSILYL DICHLORIDES

Uridine and Tetramethylenesilyl Dichloride

(A) Uridine (286 mg, 1.17 mmole), imidazole (159 mg, 2.34 mmole), tetramethylenesilyl dichloride (200 mg, 1.29 mmole) and dry DMF (1.2 ml) were stirred at room temperature for 26½ hours, at 60°C for 8½ hours and at 100°C for 23½ hours. Progress of the reaction was monitored by TLC developed in ether. Most of the uridine remained unchanged throughout the preceding treatment. However, some ill-resolved products appeared as a streak from the origin to R_f .61. No distinct spots were visible.

(B) Uridine (244 mg, 1 mmole) was suspended in anhydrous ether (75 ml) containing triethylamine (202 mg, 2 mmole).

Tetramethylenesilyl dichloride (155 mg, 1 mmole) was dissolved in anhydrous ether (75 ml) and added dropwise to the stirred nucleoside suspension. After addition was completed, the solution was filtered

and concentrated to dryness. This residue was dissolved in ethanol and applied to TLC which was developed in ether. Visible was a streak of UV-absorbing material (with no distinct bands) extending from the origin to R_f .38.

5'-Trityl Uridine and Diisopropylsilyl Dichloride

(A) 5'-trityl uridine (243 mg, 0.5 mmole), imidazole (136 mg, 2 mmole), diisopropylsilyl dichloride (116 mg of 80% reagent, 0.5 mmole) and dry DMF (0.5 ml) were stirred at room temperature for 43 hours. The reaction mixture was placed on three plates which were developed in ether. Streaking of UV-absorbing material from the origin to the solvent front was observed, with one distinct band discernible at R_f 0.80. This band was eluted with ether to give 190 mg of material. Attempted crystallization of this material from aqueous ethanol led to the appearance of seven bands on TLC developed in ether (R_f's .13, .27, .36, .45, .54, .64 and .72), indicating decomposition of the product.

(B) 5'-trityl uridine (243 mg, 0.5 mmole) was suspended in anhydrous ether (35 ml) which contained triethylamine (101 mg, 1 mmole).

Diisopropylsilyl dichloride (115 mg of 80% reagent, 0.6 mmole) was dissolved in anhydrous ether (35 ml) and added dropwise with stirring to the trityl uridine suspension. Examination of the reaction mixture seventeen hours later by TLC in ether showed that a small amount of a product appeared at R_f .58, (a spot that had also been noted about half an hour after addition of the silyl

dichloride) but that most of the starting material, 5' trityl uridine, remained unchanged at R_f .24. More diisopropylsilyl dichloride (168 ml of 80% reagent, 0.87 mmole) in ether (25 ml) and more triethylamine (2.4 ml) were added to the reaction mixture. Five days later, the reaction mixture was filtered, concentrated and applied to two silica gel plates which were developed in ether to give two bands - starting material plus the faster moving material observed on TLC five days earlier. The band with higher R_f value (.82) was eluted with ether, concentrated to a semi-solid and crystallized from aqueous ethanol to give white crystals (14 mg, mp 247-248.5°C, softening at 243°C). The mass spectrum was dominated by the trityl group fragmentation pattern, highest mass peak at 243 = ϕ_3C^+. Elemental analysis of this material gave the following results: C-75.67%, H-5.62% and N-3.72%, similar to that expected for the monohydrate of 2'5'-dityrityl uridine - $C_{47}H_{40}N_2O_6 \cdot H_2O$, expected analysis C-75.58%, H-5.67% and N-3.75%. 2'5' dityrityl uridine is an impurity frequently found in 5'-trityl uridine preparations.

5'-Acetyl Uridine and Diisopropylsilyl Dichloride

5'-acetyl uridine (323 mg, 1.13 mmole) and imidazole (306 mg, 4.5 mmole) were dissolved in dry DMF (25 ml). To this stirred solution, diisopropylsilyl dichloride (285 mg, 80% solution, 1 mmole) was added dropwise. Five more drops of the silyl dichloride solution were added when TLC examination of the reaction mixture showed that

some 5'-acetyl uridine (R_f .05 in ether) remained unreacted. Twenty-three hours later, GC/MS examination of the reaction mixture indicated that the major product formed in the reaction was the desired 5'-acetyl-2',3'-O-diisopropylsilyluridine (highest mass peak at m/e 355, M-43 $^+$). The reaction mixture was applied to two silica gel plates which were developed in ether. Four bands were distiguishable on the plates, which had UV-absorbing material smeared from the origin to the solvent front - at R_f .42, .58, .73, and .87. Each band was eluted with ether. GC/MS examination of the material from each band indicated that it was the R_f .58 band which contained the 5'-acetyl-2',3'-O-diisopropylsilyl uridine (55 mg, 13%, M-43 $^+$= 355). Attempted crystallization of this material in aqueous ethanol led to the formation of three spots on TLC developed in ether (R_f .05 - 5'AcU ?, .33 and .47), suggesting instability of the compound.

REACTIONS ON 8,2'-THIOANHYDROADENOSINE

Diacetyl-8,2'-Thioanhydroadenosine

8,2'-Thioanhydroadenosine[57,120] (50 mg) was dissolved in pyridine (0.6 ml) and acetic anhydride (0.2 ml). After 1½ hours, TLC in THF showed the complete disappearance of the 8,2'-SAnA at R_f .36 to form a new product at R_f.64. Removal of the solvents gave a solid (63 mg). Recrystallized fron aqueous ethanol, then from 95% ethanol, the material gave white crystals (mp 231.5-232.5°C, M^+= 365, λ_{max}^{EtOH} 276 nm (13,300) and 220 nm (11,100), R_f.19 in EtOAc, .69 in THF and .59 in EtOAc-i-PrOH-H_2O (75:16:9).

DISPLACEMENT REACTIONS AND OXIDATIONS ON 8,2'SAnA

(i) with KCN in water

8,2'-Thioanhydroadenosine (10 mg, 0.04 mmole), potassium cyanide (5.2 mg, 0.08 mmole) and water (1 ml) were heated on a steam bath for three hours. Examination of the reaction mixture by TLC in THF showed only starting material at R_f .44. Allowing the mixture to stand at room temperature for a week effected no change observed on TLC.

(ii) with KCN in dimethylformamide

8,2'SAnA (10 mg, 0.04 mmole) and potassium cyanide (5.2 mg, 0.08 mmole) were heated at 150°C in dry DMF (1 ml) for 1¼ hours. Paper chromatography in solvent B revealed a number of fluorescent bands to R_f .23, a low yield of a product at R_f .28 ($\lambda_{max}^{H_2O}$ 275 nm) and much unchanged starting material at R_f .36. Extending the reaction time to 39 hours led to the formation of decomposition products only as evidenced by the presence of a large number of poorly resolved fluorescent bands extending to R_f .34 on papers developed in solvent B. No UV-absorbing band corresponding to starting material was visible.

(iii) with KSCN in water

(A) 8,2'SAnA (20 mg, 0.07 mmole) and potassium thiocyanate (15.4 mg, 0.16 mmole) were heated in glacial acetic acid (0.5 ml) on a steam bath for four hours. Paper chromatography in solvent A revealed the presence of bands at the following R_f values (UV

maxima in water, nm) .00 (plateau at 262.5-271 nm), .09 (275), .11 (303.5, 276), .13 (276, 244 sh), .26 (272, 284 sh, 265 sh, 257.5 sh), .34 (275, 220 sh), .51 (276, 219), .65 (276.5), .82 (319,250). The R_f .51 band, corresponding to 8,2'SAnA, was by far the most intense band on the papers.

The R_f .82 material was refluxed with a spoonful of Raney nickel for 4 hours. The solution was filtered through Celite, concentrated and applied to a paper which was developed in solvent B. Three bands were eluted from the paper - a broad, faint band from R_f .00 - .13 (270.5 nm), a dark band at R_f .15 (271.5 nm) and a faint band at R_f .29 (272 nm).

(B) When 8,2'SAnA (10 mg, 0.04 mmole) was heated with KSCN (8 mg, 0.8 mmole) in glacial acetic acid (0.25 ml) for 15 hours on the steam bath, four different bands were observed on PC developed in solvent A: R_f ($\lambda_{max}^{H_2O}$ in nm) - .25 (289 sh, 285), .38 (271 sh, 261), .54 (276.5, 219) and .65 (276, 212.5 sh). Molecular ion peaks appeared at 293 for the R_f .38 material, at 281 for the R_f .54 material (starting material) and at 287 (?) for the R_f .65 band.

(iv) with sodium azide in DMF

(A) 8,2'SAnA (10 mg, 0.04 mmole) and sodium azide (10.4 mg, 0.16 mmole) were heated in dry DMF (0.8 ml) at 70-75°C for 6½ days. Examination of the reaction mixture by TLC in tetrahydrofuran showed only one spot - that corresponding to 8,2'SAnA at R_f .36. However, paper chromatography in solvent A revealed the presence of a faint band at R_f .30 as well as the dark band at R_f .56

corresponding to starting material. This new compound had R_f .30, had a UV maximum (H_2O) at 273 nm and an infrared absorbance at 2050 cm^{-1}.

(B) 8,2'SAnA (10 mg, 0.04 mmole) and sodium azide (10.4 mg, 0.16 mmole) were heated in dry DMF (0.8 ml) at 150°C for 3 hours. Applied to a paper and developed in solvent B, the reaction mixture produced a series of fluorescent bands from the origin to R_f .07 and five other bands: R_f ($UV_{max}^{H_2O}$ in nm) - .10 (272), .16 (276), .31 (276, 220), .43 (314, 275, 263 sh, 257 sh, 254 sh) and .51 (278.5, 267 sh, 259 sh, 253 sh). Mass spectrometry of the R_f .43 material gave highest mass peak at 352 and a fragment at 324 mass units.

(v) with mercuric chloride and water

8,2'SAnA (10 mg, 0.04 mmole) and mercuric chloride (20 mg, 0.07 mmole) were dissolved in two drops of water. The solution was observed to turn cloudy within 5 seconds of the addition of water. A further 0.6 ml water was added to the reaction mixture which was then heated on the steam bath for half an hour. Thin layer chromatography in THF showed a UV absorbing elongated "spot" at R_f .00 - R_f .20, a spot at R_f .38 corresponding to 8,2'SAnA and another spot at R_f .76 corresponding to $HgCl_2$. Paper chromatography in solvent A produced a streak from the origin to R_f .07 corresponding to $HgCl_2$ and a spot at R_f .52 (8,2'SAnA). Paper chromatography in solvent B revealed a UV-absorbing streak from the origin to R_f .80 including a darker region at R_f .38

(8,2'SAnA).

(vi) with mercuric chloride and aqueous acetone

8,2'SAnA (10 mg, 0.04 mmole), mercuric chloride (20 mg, 0.07 mmole) and approximately 20 mg calcium carbonate were stirred in aqueous acetone (1:1, 3.4 ml) at room temperature for 73 hours. Applied to Whatman paper and solvent B, the reaction mixture was found to be separated into four bands - R_f .14 ($\lambda_{max}^{H_2O}$ 272 nm), R_f .37 ($\lambda_{max}^{H_2O}$ 276, 220-s.m.), R_f .69 ($\lambda_{sh}^{H_2O}$ 260 nm) and R_f .82 ($\lambda_{sh}^{H_2O}$ 228-$HgCl_2$).

(vii) with mercuric chloride and sodium benzoate in DMF

8,2'SAnA (10 mg, 0.04 mmole) and mercuric chloride (20 mg, 0.07 mmole) were heated in DMF (3 ml) at 150°C for 30 minutes. Following this, sodium benzoate (30 mg, .21 mmole) and benzoic acid (10 mg, .08 mmole) were added to the reaction mixture which was heated at 150°C for an hour. Paper chromatography of samples of the reaction mixture versus all the reactants gave the following results in Solvent A - .05 ($HgCl_2$), .55 (8,2'SAnA) and .71 NaOBz and BzOH) and in solvent B - .34 (8,2'SAnA and NaOBz) and a streak extending to R_f .83 ($HgCl_2$ and BzOH).

(viii) with chlorine in chloroform

8,2'SAnA (10 mg, 0.04 mmole) was suspended in chloroform (5 ml) through which chlorine gas was then bubbled for ten minutes. The solvent was removed and the residue dissolved in water. UV spectrum of the solution gave λ_{max} 276, 222 nm, corresponding to that of unchanged starting material.

(ix) with N-bromosuccinimide

8,2'SAnA (10 mg, 0.04 mmole) and N-bromosuccinimide (14.2 mg, 0.08 mmole) were dissolved in 80% aqueous methanol (1.5 ml) and stirred at room temperature for 5½ hours. Applied to a paper which was developed in solvent B, the reaction mixture separated into nine bands: R_f ($UV_{max}^{H_2O}$ in nm) - .17 (291.5, 283.5, 276 sh), .21 (257, 268 sh, 262 sh, 253 sh), .30 (272), .35 (238 sh), .38 (240 sh), .44 (279.5 sh), .52 (268 sh, 235 sh), .62 (271, 276 sh) and .68 (276 sh). The highest mass peak of the R_f .17 material was at 149 mass units.

(x) with iodobenzene dichloride in aqueous acetonitrile

8,2'SAnA (28 mg, 0.1 mmole) was dissolved in water-acetonitrile (1:2, 1.5 ml). Iodobenzene dichloride^{180} (27.5 mg, 0.1 mmole) was dissolved in acetonitrile (1 ml) and added dropwise with stirring to the 8,2'SAnA solution over a period of 10-15 minutes. Stirred at room temperature for 30 minutes, the reaction mixture was then concentrated under vacuum and applied to papers which were developed in solvent B to give four bands - R_f .07 - $\lambda_{max}^{H_2O}$ 262.5, 282 sh, 265 sh - highest mass peak at 278; R_f .11 - a faint band which was not eluted; R_f .17 - $\lambda_{max}^{H_2O}$ 268, 280 sh, 264 sh, 260 sh, 225 sh, highest mass peak at 257 mass units; and R_f .26 (major product) - $\lambda_{max}^{H_2O}$ 274, 265 sh - highest mass peak at 241 mass units.

Infrared examination of the R_f .26 material did not indicate the presence of a sharp absorbance in the region 1040-1060 cm^{-1}.

(xi) with iodobenzene dichloride in pyridine

8,2'SAnA (28.1 mg, 0.1 mmole) was dissolved in 20% aqueous pyridine (1 ml). Iodobenzene dichloride (27.5 mg, 0.1 mmole) was dissolved in dry pyridine (1 ml) and added dropwise into the stirred nucleoside solution. After 30 minutes, the reaction mixture was placed on papers which were developed in solvent B for 38 hours. The following bands were observed (R_e relative to the edge of the paper): R_e - .10 (yellow), .15, .18 (yellow), .21, .29 (turquoise), .32 (bright blue), .34 (bright yellow under visible light), .37, .52 and .61. (The colours mentioned are those observed under UV light, except where noted.) The last four bands were eluted and their UV and mass spectra obtained - .34 - $\lambda_{max}^{H_2O}$ 263, 257 - highest mass peak at 151; .37 - $\lambda_{max}^{H_2O}$ 270.5, 265 sh, 258.5 sh - highest mass peak at 213; .52 - $\lambda_{max}^{H_2O}$ 275.5, 222 - M^+ at 281 mass units; and .61 - $\lambda_{max}^{H_2O}$ 275 - M^+ at 281.

(xii) with sodium metaperiodate in water

(A) 8,2'SAnA (20 mg, 0.07 mmole) was dissolved in water (3 ml). A five molar excess of sodium metaperiodate (3.58 ml, 0.1 M aqueous solution of $NaIO_4$) was added and the resulting solution stirred at room temperature for two hours. Samples of the reaction mixture were removed at regular intervals and spotted on a paper chromatogram. Developed in solvent B, the paper showed dark spots at R_f .03 and at .35 corresponding to $NaIO_4$ and 8,2'SAnA respectively as well as the slow appearance of two other spots at R_f's .07 and

.23. TLC of the reaction mixture(developed in THF) showed only the spots corresponding to the two starting materials (.00-.06 and .35).

The reaction mixture was placed in a 50° bath for half an hour, at 50-65°C for another 30 minutes, at 65-75°C for another half an hour and at 100°C for an hour. Samples were removed every half hour and spotted on papers. Developed in solvent B, the papers showed a gradual increase in intensity of the spots at R_f .07 and .23 and a decrease in intensity of the spot at R_f .35.

Allowing the reaction mixture to stand at room temperature for 20 hours resulted in the formation of white crystals ($UV_{max}^{H_2O}$ 260.5, highest mass peak at 243 mass units, electrophoretic mobility R_m 0.47 relative to Tp, R_f in solvent B at .07, observed elemental analysis - C - 33.85%, H - 3.28%,N - 18.91% - expected values for 2' deoxy 2' sulfonic acid of 8,5'-O-anhydroadenosine were C-36.5%, H - 3.34% and N - 21.3% and for the sulfoxide of 8,2'SAnA - C - 40.4%, H - 3.7% and N - 23.6%.

The solution from which the above white crystals had been filtered was applied to papers which were then developed in solvent B. Elution of the bands at R_f .07 and .23 gave material with UV maxima at 262 nm and at 286 nm respectively. The highest mass peak of the R_f .07 material came at 60 mass units, while that of the R_f .23 material came at 146 mass units.

(B) 8,2'SAnA (10.3 mg, 0.0366 mmole) and sodium metaperiodate (7.8 mg, 0.0366 mmole) were dissolved in water and stirred at room

temperature for 32 hours. The reaction was then stirred at 43°C for 15 hours and at 54°C for 28 hours. Developed in solvent B, the reaction mixture was separated into six bands R_f .02, ($NaIO_4$), .05 (261 nm), .07 (265 nm), .10 (269 nm), .21 (286.5 nm) and .35 (darkest band, 8,2'SAnA). Lyophilized, the material from R_f .05 gave a nice white solid which could not be volatilized for mass spectrometry (highest mass peak at 60 mass units). It possessed a strong i.r. absorbance in the 1040-1060 cm^{-1} region. The material at R_f .21 had its highest mass peak at 129 in the mass spectrum and contained no sharp absorbance in the region 1040-1060 cm^{-1}.

OXIDATIONS ON 3',5'-DIACETYL-8,2'-THIOANHYDROADENOSINE

(i) with iodobenzene dichloride

(A) 3',5'-$Ac_2$8,2'SAnA (23 mg, 0.06 mmole) was dissolved in 20% aqueous pyridine (0.4 ml). Iodobenzene dichloride (17.3 mg, 0.06 mmole) was dissolved in dry pyridine (0.1 ml) and added dropwise to the stirred nucleoside solution. TLC in EtOAc taken five minutes hence showed the reaction mixture to form a yellow streak from the origin to R_f .10, a spot at R_f .17 corresponding to $Ac_2$8,2'SAnA and another spot at R_f .40. Developing this same TLC in ethyl acetate two days later, the R_f .40 spot was observed to have separated into three materials – a yellow streak R_f .00-.12, a spot at R_f .20 (corresponding to $Ac_2$8,2'SAnA) and another spot at R_f .40 – just as the reaction mixture had been separated.

The reaction mixture was applied to a silica gel plate which

was developed in ethyl acetate. The yellow band (R_f .00-.20) was eluted with ethanol, concentrated and applied to a silica gel plate which was developed twice in the solvent ethyl acetate-isopropanol-water (75:16:9). Five bands were obtained and eluted to give the following: R_f (UV max in 95% ethanol, nm) - .31 (274, 287 sh), .39 (282 sh, 246 sh), .60 (276.5, 219), .75 (278) and .94 (270 sh, 249 sh). Examination of the infrared spectra taken of the R_f .39 and .75 materials did not reveal the presence of a strong absorbance in the 1040-1060 cm^{-1} region. The highest mass peak of the R_f .39 material was at 395, while that of the R_f .75 material came at 365 mass units.

(ii) with sodium metaperiodate

(A) Diacetyl-8,2'-thioanhydroadenosine (10 mg,.027 mmole) was combined with 0.5 M $NaIO_4$ (58 µl, .029 mmole) and methanol (~0.2-0.5 ml) and allowed to stir in an ice bath for 16 hours and at room temperature for seven days. Examination of the reaction mixture by TLC in methanol-ethylacetate (1:1) showed the presence of two compounds - starting material at R_f .76 and a low yield of a new material at R_f .69. The slower moving band was isolated by silica gel chromatography in ethyl acetate and was found to have a UV maxima at 275.5 and 222 nm.

(B) Diacetyl-8,2'-thioanhydroadenosine (36.5 mg, 0.1 mmole) was dissolved in aqueous ethanol (~1 ml). Excess sodium metaperiodate (~150 mg, 0.7 mmole) was added the the reaction mixture stirred at room temperature for two hours. TLC taken in ethyl acetate showed

only the starting materials at R_f .00-.04 ($NaIO_4$) and at .17 (Ac_2 8,2'SAnA) and a very faint spot at R_f .33.

The reaction mixture was then stirred at 60°C for seven hours. Monitoring by TLC in EtOAc showed an increase in the intensity of the spot at R_f .33 and the appearance of a faint spot at R_f .54. The reaction mixture was evaporated to dryness, then extracted with chloroform. The chloroform extract was applied to a silica gel plate which was developed in ethyl acetate to give an intense band at R_f .07 and two faint bands at R_f .21 and .49. The major product (R_f .07) possessed a UV maximum at 263.5 nm (in ethanol) and had a molecular ion peak at 415 mass units.

Attempted Photooxidation of 8,2'-Thioanhydroadenosine

8,2'-thioanhydroadenosine (40 mg) was dissolved in water to which a few crystals of rose bengal had been added. The solution was irradiated using a Hanovia Utility high pressure mercury vapour lamp (λ>285 nm, Model No. 30620) for two hours. The solution was concentrated and examined by TLC and paper chromatography. Developed in various solvents versus starting material on TLC and PC, the reaction mixture was found to contain only one UV-absorbing spot - that corresponding in R_f value to 8,2'SAnA.

The reaction mixture and a few more crystals of rose bengal were dissolved in aqueous ethanol (1:1, 60 ml), and irradiated for twelve hours while oxygen was bubbled into the solution. The reaction mixture was concentrated and applied to papers which were

developed in solvent B to give three bands - R_f .25 (medium intensity, highest mass peak at 281 mass units), $\lambda_{max}^{H_2O}$ 270 nm), R_f .35 (dark intensity, highest mass peak at 281, $\lambda_{max}^{H_2O}$ 220 nm) and R_f .42 (medium intensity, highest mass peak at 281 mass units, $\lambda_{max}^{H_2O}$ 260 nm).

Pummerer Reactions

(i) on the products of iodobenzene dichloride oxidation

3'5'-Diacetyl-8,2'-thioanhydroadenosine (50 mg, .137 mmole) was dissolved in 20% aqueous pyridine (0.9 ml) and stirred in an ice bath. Iodobenzene dichloride (37.8 mg, .137 mmole) was dissolved in 0.25 ml dry pyridine and chilled as well. The latter solution was then added dropwise to the nucleoside solution which was stirred in the dark in an ice-bath. Allowed to stir in the ice bath for ten minutes, the reaction mixture was then diluted with chloroform (13 ml), neutralized by shaking with aqueous sulfuric acid (10% solution), extracted three times with water, dried with anhydrous sodium sulfate, filtered and concentrated to approximately 3 ml of chloroform solution.

This chloroform solution was then subjected to the Pummerer reaction by allowing it to react with acetic anhydride (0.1 ml) for three days at room temperature. The reaction mixture was applied to a silica gel plate which was developed twice in ether and three times in ethyl acetate to give three bands - R_f .05 (yellow in

visible light), R_f .15 (λ_{max}^{EtOH} 277.5, 223.5, 221, 282 sh) and R_f .22 (major product, λ_{max}^{EtOH} 287, 230, 225, 292 sh, molecular ion at 407 mass units).

(ii) on the product of prolonged periodate oxidation

The R_f .69 material obtained from the seven day sodium meta-periodate oxidation of $Ac_2$8,2'SAnA (Method A, page 175 was dissolved in chloroform (1 ml) and allowed to react with acetic anhydride (0.1 ml) for six days at room temperature. The reaction mixture applied to papers developed in Solvent B gave a dark band at R_f .68. This material was eluted with ethanol to give a compound which had a molecular ion at m/e 407 and whose UV spectrum indicated a maximum at 285 nm and shoulders at 295 and 280 nm (in ethanol).

IRRADIATION OF ANHYDROURIDINE

Initial Work Using Chloroform-Saturated Aqueous Solutions

Chloroform (100 ml) was vigorously stirred with water (400 ml) for three hours. The aqueous layer was separated from the chloroform layer and used in the irradiation.

3'5'-diacetyl anhydrouridine[63] (30 mg, .097 mmole, $UV_{max}^{H_2O}$ 249.5, 224, 269.5 sh) was dissolved in the above aqueous solution (100 ml), placed in a quartz tube 20 cm long and 3 cm in diameter and irradiated at 254 nm while the solution was stirred. After twenty minutes, an acrid-smelling gas was detected emanating from the photoreaction. After 50 minutes, the solution had UV maxima at 243 and 224 nm.

Irradiations in the Presence of Ethanol

(A) Chloroform (25 ml) and water (75 ml) were placed into each of seven Erlenmeyer flasks. To each flask, different amounts of ethanol were added - 0.5 ml, 1 ml, 2 ml, 4 ml, 8 ml, 12.5 ml and 25 ml. The solutions in each flask were vigorously stirred for half an hour, then allowed to stand at room temperature for several hours. The aqueous solution of each sample was then used as the solvent to dissolve 3',5'-diacetyl-2,2'-O-anhydro-uridine (3 mg in 10 ml, 0.97 mM solution)which was placed in a 1 mm quartz cell and irradiated until a single peak of maximum absorbance at 238 nm was obtained. (UV spectra of the irradiated solution were taken at regular intervals to monitor the progress of the irradiation.) The time required to effect this change was recorded in Table 11 and Figure 15, page 112.

(B) Samples of diacetyl anhydrouridine (3 mg) were dissolved in aqueous solutions containing 10, 50 and 75% ethanol and in 95% ethanol (10 ml, $\therefore$ c = 0.97 mM). The irradiation of these solutions in a 1 mm cell was followed as above, and the results are incorporated in Table 11.

(C) Solutions of varying concentrations of diacetyl anhydrouridine (2 mM, 5 mM, 6.7 mM, 16.7 mM and 33.3 mM) were irradiated in aqueous solutions containing 50% and 25% ethanol in a 1 mm quartz cell. The time required for the solution to attain a UV spectrum with a single maximum at 238 nm was recorded. Where other UV maxima were formed, the time recorded was the point at which irradiation was stopped

TABLE 11

IRRADIATION OF 0.97 mM DIACETYL ANHYDROURIDINE IN THE PRESENCE OF ETHANOL[+]

% ETHANOL	TIME (SEC) FOR REACTION "COMPLETION"	UV MAXIMA OF IRRADIATED SOLUTION (NM)
0.7*	60	238
1.3*	45	238
2.6*	30	238
5.1*	25	238
9.6*	18	238
10.0	18	238
14.6*	13	238
25.0*	11	238
50.0	10	238
75.0	13	236,255 sh
95.0	20	231,253 sh

+ All irradiations were conducted on solutions in a 1 mm quartz cell.

* These solutions contained chloroform as an impurity. The percentages of ethanol were calculated on the assumption that all the ethanol added to the chloroform-water mixture was dissolved in the aqueous layer.

when it became clear that a single maximum at 238 nm would not appear.

Optimal conditions for the formation of a single maximum at 238 nm were sought in this series of reactions. (Table 12)

TABLE 12

IRRADIATION OF INCREASING CONCENTRATIONS OF Ac_2AnU IN 50% AND 25% ETHANOL SOLUTIONS

CONC. OF Ac_2AnU (mM)	% ETHANOL	TIME (SEC)	UV OF SOLUTION (NM)
2	50	20	238
5*	50	480	235,255 sh
6.7	25	90	238
16.7	25	300	238
33.3	25	720	238

* irradiated in a 1 cm quartz cell, unlike the other solutions which were irradiated in a 1 mm quartz cell.

(D) Samples of anhydrouridine (2.4 mg, 0.0114 mmole) were dissolved in 10, 30, 40, 50 and 75% ethanol (10 ml, $\therefore$ c = 1.14 mM) and irradiated in a 1 mm quartz cell. The results of these irradiations are

shown in Table 13. The "time" indicates the point at which a single maximum at 238 nm had attained optimal peak height or the point at which irradiation was stopped when it became clear that a single maximum at 238 nm would not be formed.

TABLE 13

IRRADIATION OF 1.14 mM ANHYDROURIDINE IN THE PRESENCE OF ETHANOL

% ETHANOL	TIME (SEC)	UV MAX OF SOLUTION (NM)
10	26	238
30	13	238
40	12	238
50	10	237,255 sh
75	5	230,270 sh, 259 sh, 250 sh, 236 sh

Large Scale Reaction and Photoproduct Isolation

O^2,2'- Anhydrouridine (300 mg) was dissolved in 25% ethanol in water (v/v, 200 ml), placed in a quartz vessel and irradiated with stirring (without exclusion of air) for a period of sixty minutes. The reaction mixture was then concentrated on a rotary evaporator and applied to silica gel plates which were developed three times in the solvent 15% methanol in methylene chloride. The

following bands were observed and eluted with ethanol: R_f (UV $\lambda_{max}^{H_2O}$ in nm) -.00 (238.5), .15 (238), .24 (238), .36 (end absorption only), .41 (238, 269 sh), .54 (238, 272 sh), .70 (274) and .88 (end absorption only). Only the first three bands (R_f .00, .15 and .24) were of significant concentration.

The material at the origin (R_f .00, 85 mg, ~27%) could not be identified as paper and thin layer chromatography in various solvents produced only a streak of UV-absorbing material which contained at least three darker areas, but no distinct bands. Acetylation of the material was not effective in resolving the components on thin layer chromatography - a streak from the origin to R_f .82 containing six darker areas was observed when ethyl acetate -isopropanol - water (75:16:9) was used as the solvent.

The material from the R_f .15 band was separated into two bands moving close together by extended paper chromatography (40 hours or longer) in solvent B. The slower moving band was eluted to give a compound I identified as unreacted anhydrouridine (54 mg, 18%). The material II from the band with slightly higher R_f value was determined to be an ethanol adduct of anhydrouridine (75.3 mg, 21%). (See page 185 for information on identification of I and II.)

The material from the R_f .24 band was also found to contain two compounds with similar R_f values which could be separated from each other by extended development on papers in solvent B. The slower moving compound III in this case was found to be dihydroanhydrouridine 97 (31.5 mg, 11%). The component with slightly higher R_f

value (IV) was identified as another ethanol adduct of anhydrouridine (26.1 mg, 7%). (See page 193 for III and p. 194 for IV.)

SUMMARY OF PHOTOPRODUCT YIELDS

PRODUCT	ISOLATED WEIGHT (MG)	% YIELD
identified products:		
unreacted AnU	54.0	18
H_2AnU (97)	31.5	11
EtOH adduct II	75.3	21
EtOH adduct IV	26.1	7
unidentified products:		
R_f .00	85	~27
R_f .36 + .41	32	~10.5
R_f .54	7.7	~ 2.5
R_f .70	4.9	~ 1.5
R_f .88	3.2	~ 1.0

Alternative Work-Up Procedures

The reaction mixture was placed first on papers which were developed for a prolonged period of time in solvent B. Two intense bands were observed moving in close proximity to each other. Elution of the slower band followed by silica gel plate chromatography in 15% MeOH/CH_2Cl_2 gave anhydrouridine and dihydrouridine. Elution of the band with slightly higher R_f value on papers followed by silica gel plate chromatography in 15% MeOH/CH_2Cl_2 gave separation of the two ethanol adducts.

IDENTIFICATION OF THE MAJOR PHOTOPRODUCTS

PHOTOPRODUCT I

Compound I was identified as unreacted anhydrouridine on the basis of the following information:

UV - λ_{max}^{EtOH} 248.5, 224 λsh 270 nm

mass spectrum - parent ion peak, M^+ at 226
PC and TLC - compound I had the same R_f values on papers developed in B' and on TLC developed in 15% $MeOH/CH_2Cl_2$ (v/v) as did anhydrouridine.[50]

PHOTOPRODUCT II

Compound II had a UV maximum (in ethanol) at 238 nm. Mass spectral analysis gave the highest mass peak at m/e 242.

Compound II (40 mg) was acetylated by reaction with acetic anhydride (0.7 ml) in pyridine (1 ml) overnight at room temperature. The reaction mixture was concentrated and applied to silica gel plates which were developed twice in ethyl acetate to give the following bands:

R_f .03 (faint intensity, no UV max, just end absorption)

R_f .08 (medium intensity λ_{max}^{EtOH} 236.5 nm)

R_f .15 (faint, $\lambda_{shoulder}^{H_2O}$ 230 nm)

R_f .26 (dark band, major product, UV_{max}^{EtOH} 236.5 nm)

(The R_f .08 material was assumed to be the none fully acetylated derivative of the ethanol adduct II, the two minor side products presumably decomposition products of II.)

The mass spectrum of the R_f .26 derivative gave a highest mass peak at 399 mass units which corresponded to the M + 1 species

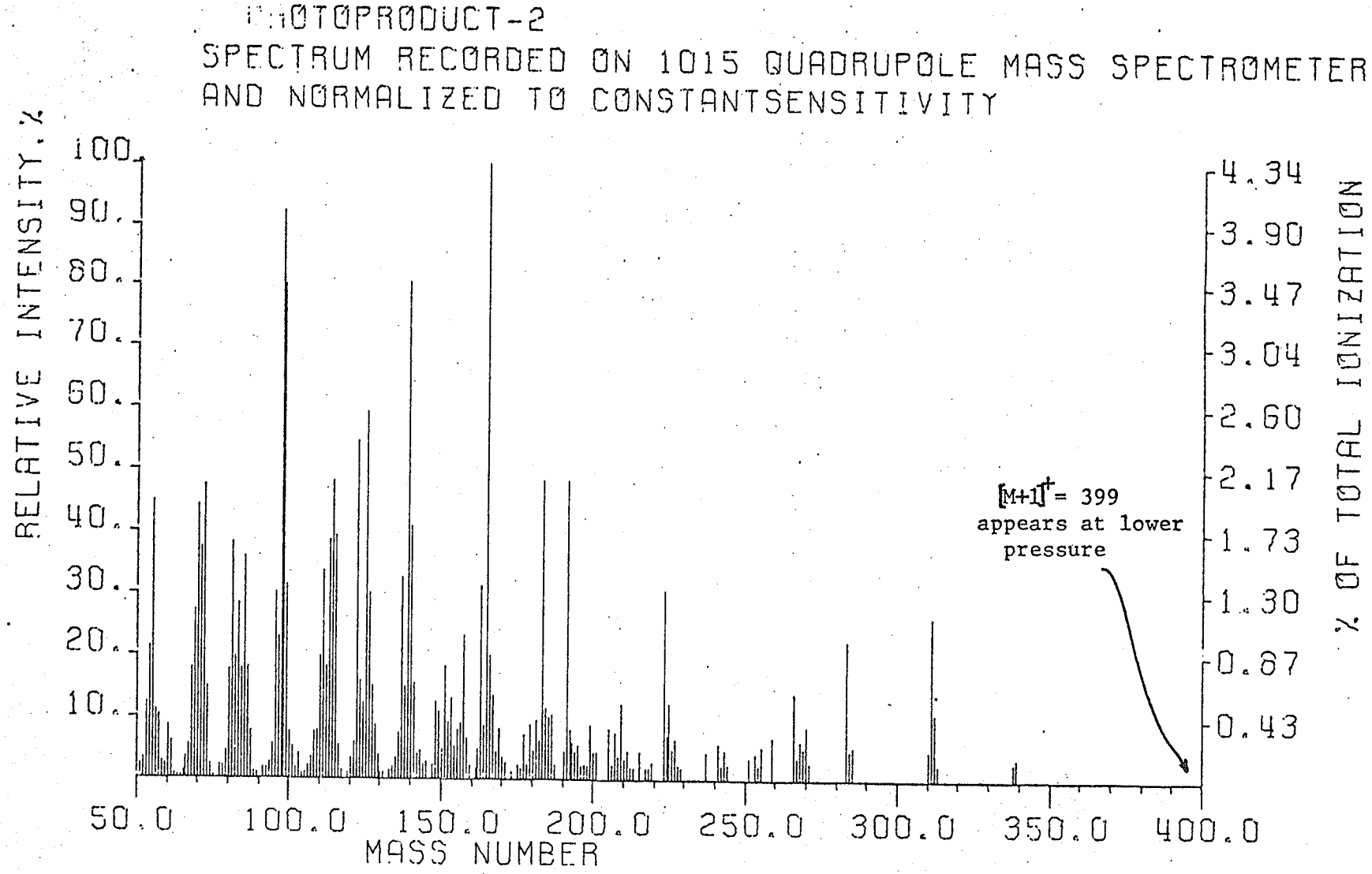
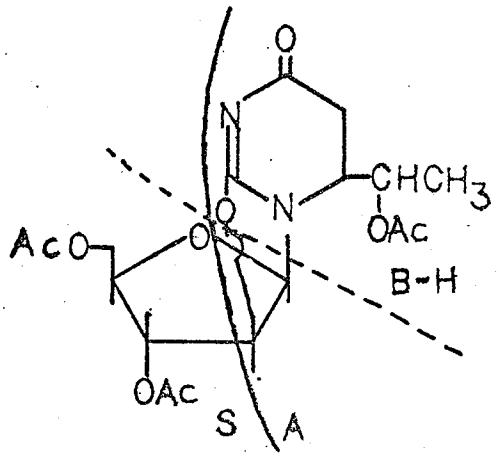


FIGURE 17. Mass spectrum of Photoproduct II (triacetylated)

FIGURE 18

MASS SPECTRAL FRAGMENTS OF TRIACETYLATED PHOTOPRODUCT II



$[M-CH_3CO_2H]^+ = 339$

$[B]^+ = 199$

$[B-H]^+ = 198$

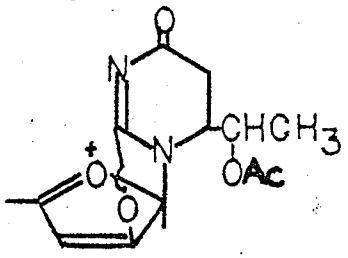
$[B-O]^+ = 183$

$[B-O-CH_3CO]^+ = 140$

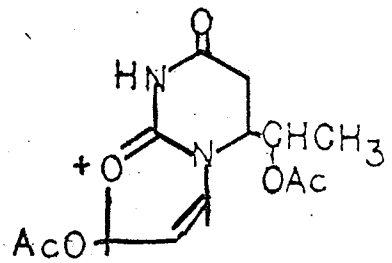
$[B-O-CH_3CO-H_2O]^+ = 122$

$[M-5'CH_2OAc]^+ = [M-73]^+ = 325$

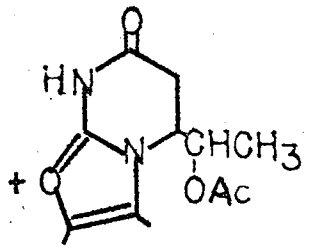
$[M-73-CH_3CO_2H]^+ = [C]^+ = 265$



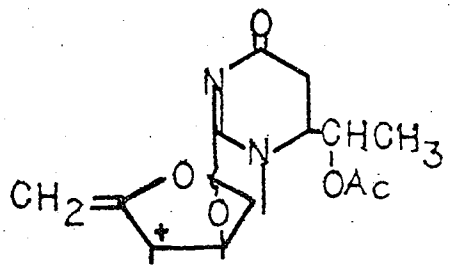
$[A + 73]^+ = 297$



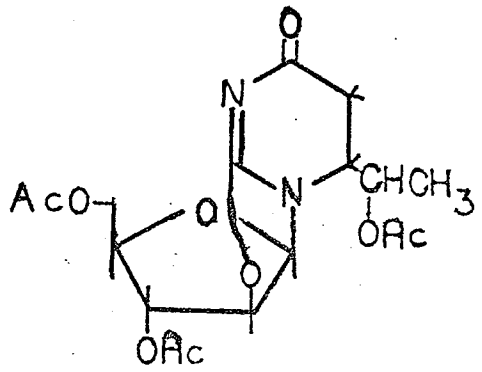
$[A + H]^+ = 225$



$[A + 55]^+ = 279$



of the following molecule:



The structure of several species observed in the fragmentation pattern of the triacetylated derivative of II was deduced by a comparison with the fragmentation pattern observed with 2,2' anhydronucleosides in general.[181] Some of the fragments observed are shown in Figure 17.

While mass spectrometry had identified compound II as the hydroxyethyl derivative of anhydrouridine, n.m.r. analysis was necessary in order to determine whether the hydroxyethyl group was position at C-5, C-6 or even at C-4 forming a hemiacetal. On the basis of analysis of n.m.r. spectra obtained on the 220 MHz N.M.R. Spectrometer of the Ontario Research Foundation, compound II was identified as a 76:24 mixture of two epimers of acetylated 6(α-hydroxyethyl)-5,6-dihydroanhydrouridine. (See Table 14 and Figure 18)

N.M.R. Assignments for acetylated Photoproduct II

When proton assignments for acetylated Photoproduct II were being made, reference was continually made to the assignments by Hall et al[53] for A) 5,6-dihydroanhydrouridine in d_6-DMSO, for B) 5-methyl-5,6-dihydroanhydrouridine in D_2O and for C) 6-methyl-carboxylate-5,6-dihydroanhydrouridine in d_6-DMSO. The stuctures of

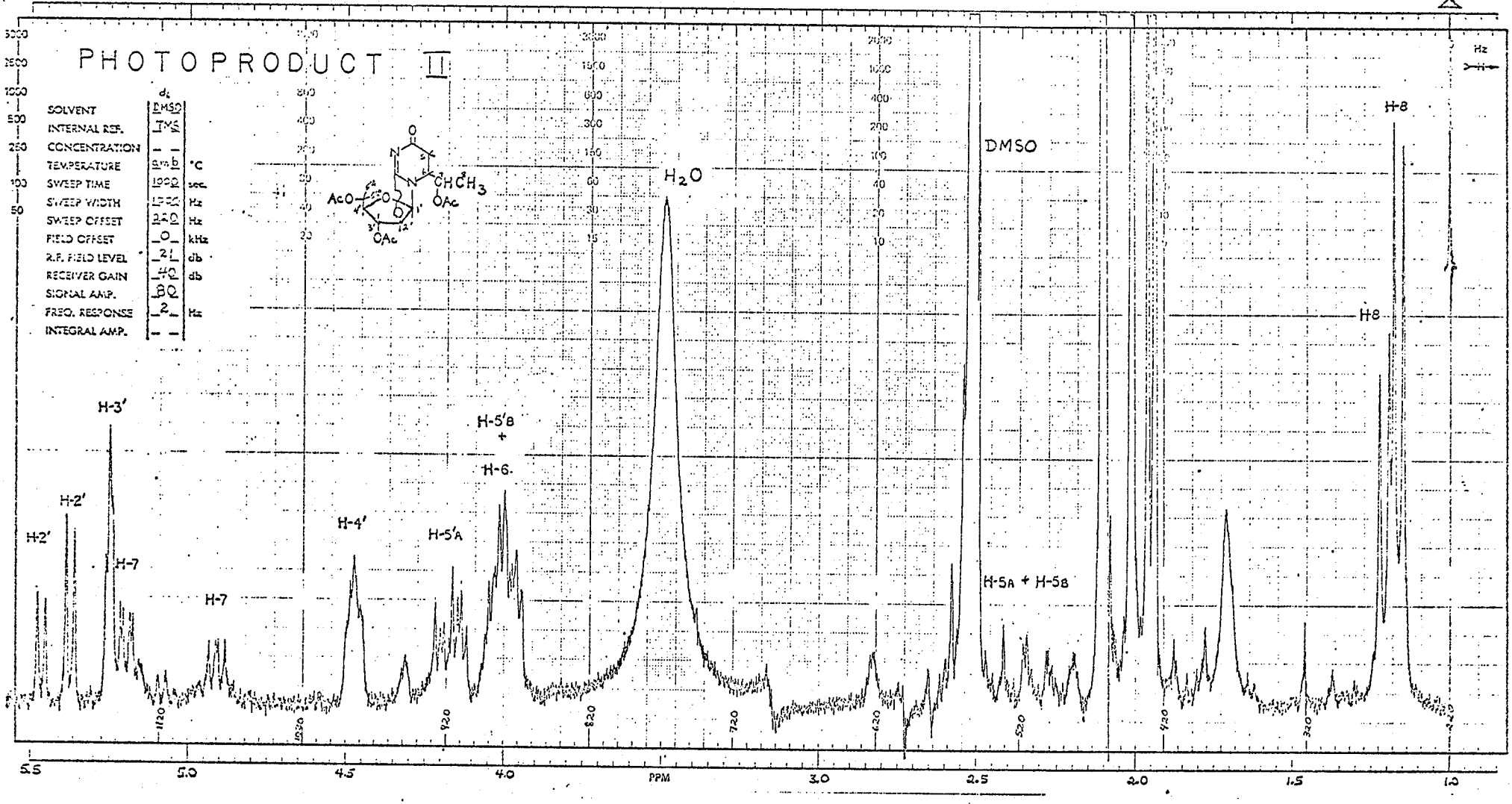
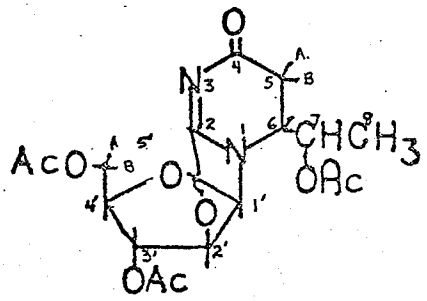


Figure 19. N.M.R. Spectrum of acetylated Photoproduct II

TABLE 14

N.M.R. ANALYSIS OF TRIACETYLATED PHOTOPRODUCT II IN d_6-DMSO

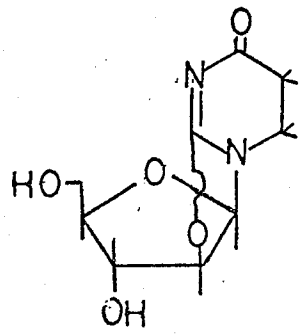


CHEMICAL SHIFT δ ppm			COUPLING CONSTANT, J cps		
PROTON	MAJOR	MINOR	PROTONS	MAJOR	MINOR
H_1'	6.10(d)	6.08(d)	$J_{1'2'}$	5.5	5.5
H_2'	5.38(d)	5.47(d)	$J_{2'3'}$	4	4
H_3'	5.25(d)	5.26(d)	$J_{3'4'}$	2.5	2.5
H_4'	4.48(u)	4.49(u)	$J_{4'5'A}$	6.2	6.2
			$J_{4'5'B}$	3.4	3.4
$H_{5'A}$	4.17(q)	4.16(q)			
$H_{5'B}$	3.98(q)	3.98(q)	$J_{5'A5'B}$	-12.3	-12.3
H_{5A}	2.31(q)	2.24(q)	$J_{5A,5B}$	-16	-16
H_{5B}	2.5 (q)	2.5 (q)	$J_{5A,6}$	2	-
H_6	4.0-4.2(u)	4.0-4.2(u)	$J_{5B,6}$	7.5	-
H_7	5.2(d,d)	4.91(d,d)	$J_{6,7}$	2	2
H_8	1.16(d)	1.21(d)	$J_{7,8}$	7	7

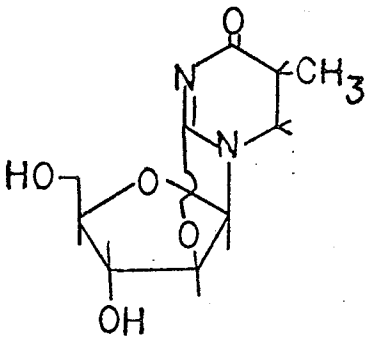
d - doublet, t - triplet, q - quartet, u - unresolved,

d,d - doubleton of doublets

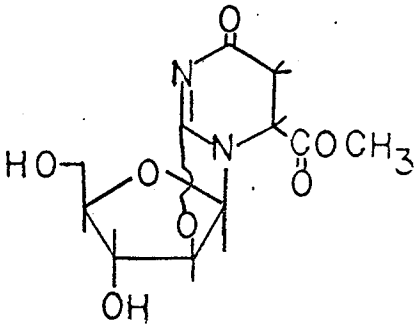
these molecules are as follows:



A



B



C

H-1' (A- 5.88, B- 6.07, C-6.04 ppm)

Two overlapping doublets with splittings of 5.5 cps centred at 6.10 and 6.08 ppm were assigned to the H-1' protons of the two epimers of II

H-2' (A-5.08, B-5.32, C-5.23 ppm)

Two doublets with splittings of 5.5 cps centred at 5.38 and 5.47 ppm were assigned to the H-2' protons of the two epimers of II

H-3' (A-4.32, B-4.57, C-4.38 ppm)

Acetylation of the 3'-OH group would be expected to move the H-3' chemical shift downfield somewhat when compared with the shift of the H-3' of non-acetylated molecules A,B, and C. Thus the H-3' of II might be expected to fall in the regions at 4.5, 4.9, 5.2 or 5.25 ppm.

H-4' (A-4.03, B-4.3, C-4.09 ppm)

H-4' would be expected to fall in the regions at 4.0, 4.2 or 4.5 ppm.

$H\text{-}5'_A$ (A-3.32, B-3.59, C-3.38 ppm)

$H\text{-}5'_B$ (A-3.32, B-3.59, C-3.49 ppm)

Acetylation of the 5'OH group of II would be expected to shift the peaks of the $5'_A$ and $5'_B$ protons downfield relative to the shift observed for A, B, and for C. Thus one might expect that these peaks may have been buried under the water peak at 3.5 ppm or that they may appear at the areas around 4.0 or 4.2 ppm.

$H\text{-}5_A$ (A-2.45, B-2.82, 2.79 (two isomers), C-2.65 ppm)

$H\text{-}5_B$ (A-2.45, --- , C-2.84 ppm, J= -16.6 cps)

A quartet with J=16 cps was found centred at 2.31 ppm, a second quartet with J=16 cps was centred at 2.24 ppm. These were assigned to the 5_A protons of the two epimers of II.

There was a suggestion of a quartet of similar coupling constant which was mostly buried under the DMSO peak at 2.5 ppm. This was presumed to be due to the 5_B protons of the two epimers of II.

H-6_A (A-3.57, B-3.40, 3.38 (2 isomers), C-4.80 ppm)

H-6_B (A-3.57, B-3.87, 3.83 ppm - two isomers)

Since the H-6 proton of compound II is situated next to a CH-O-Ac grouping, one would expect its peak to appear downfield of the H-6 proton of A, but not as far dowfield as the H-6 of C, which finds itself next to a CO_2 Me grouping. Thus one might expect to find H-6 of II at around 4.0 or 4.2 ppm.

H-7 Since this proton is part of a CHOAc system (similar to H-3'), it would be expected to resonate in approximately the same area, i.e. around 4.5 - 4.9, 5.2 or 5.25 ppm.

H-8 Three protons split into a doublet by the proton at C-7 would be expected to appear quite far upfield of the protons previously discussed here. Two doublets at 1.16 and at 1.21 ppm were assigned to the H-8 protons of the two epimers of II.

Spin Decoupling Experiments

Irradiation of H-8 at 1.21 ppm resulted in the collapse of the two doublets at 4.91 ppm to form a single doublet. Therefore the peaks at 4.91 ppm must correspond to the H-7 protons of the minor epimer of II.

Since the pair of doublets at 5.2 ppm had the same coupling constant as the pair of doublets at 4.91 ppm, they were assumed to correspond to the H-7 protons of the major epimer of II.

Irradiation at 5.2 ppm resulted in the collapse of the H-8 doublet at 1.16 ppm to a singlet, verifying the above assumption. Therefore, the peaks at 5.2 ppm belong to the H-7 protons of the major epimer of II.

Irradiation of H-7 protons at 4.91 and at 5.2 ppm did not alter in any way the quartets centred at 2.31 and 2.41 ppm, peaks corresponding to H-5 protons.

However, irradiation of H-7 protons at 5.2 ppm did alter the pattern of peaks in the region at 4.0 ppm. This is the region in which H-6 was expected to appear.

Therefore, H-7 is coupled to H-6 and not to H-5. The H-6 protons are situated in the region around 4.0 ppm.

However, since the area at 4.0 ppm integrated to show the presence of two protons, one other proton must resonate in the same region.

Further verification of the assignment of H-6 in the 4.0 ppm area was obtained from the following spin decoupling experiments:

Irradiation at 4.04 ppm reulted in the collapse of the H-5 quartet at 2.31 to a doublet.

Irradiation at 4.0 ppm led to the collapse of the two doublets at 5.2 ppm corresponding to H-7 to form a single doublet.

Therefore, H-6 is coupled to both H-7 and H-5 and the hydroxyethyl substitution must be at C-6 in Photoproduct II.

This left the peaks at 5.25, 4.48 and 4.18 unassigned, as well as an unassigned proton at 4.0 ppm (where H-6 is also found).

The region at 4.48 ppm was irradiated; —— this was found to have no effect on the H-2' protons at 5.38 and 5.47 ppm, but it did appear to have a slight effect on the two doublets at 5.25 ppm.

Therefore, H-4' is at 4.48 ppm.

This left only the peaks at 5.25, 4.18 and 4.0 ppm unassigned. Since H-3' was expected to appear at 4.5, 4.9, 5.2, or 5.25 ppm, the only possible assignment for H-3' was at 5.25 ppm. Since irradiation of H-4' had had an effect on the peaks at 5.25 ppm, the validity of the assignment was reinforced.

Therefore, H-3' is at 5.25 ppm.

This meant that $H\text{-}5'_A$ must be in the area at 4.18 ppm and $H\text{-}5'_B$ must be in the region at 4.0 ppm, together with H-6.

In the 4.18 ppm area two quartets with coupling constants of 3.4 cps were discerned. This would have corresponded to the $H\text{-}5'_A$ protons of the two epimers of II.

A quartet with coupling constant of 3.4 cps was at 3.98 ppm and was assigned to the $H\text{-}5'_B$ protons of both epimers of II.

Therefore, $H\text{-}5'_A$ is at 4.17 and 4.16 ppm for the two epimers of II and $H\text{-}5'_B$ is at 3.98 ppm for both epimers of II.

PHOTOPRODUCT III

The identity of Photproduct III was established by a comparison of its physical and spectral properties with those of an authentic sample of dihydroanhydrouridine prepared by the method of Hall et al.[53] Both the photproduct and the authentic sample had a UV maximum at 237.5 nm in ethanol, a melting point of 181 - 185 degrees C., and a molecular ion peak at 228 mass units in the mass spectrum.

PHOTOPRODUCT IV

The identification of photoproduct IV was achieved in a manner very similar to that used to determine the structure of compound II. The UV maximum in ethanol of IV was at 238 nm and its highest mass peak in the mass spectrum was at 242 mass units.

Photoproduct IV was treated with acetic anhydride in pyridine and the reaction mixture separated into five bands following silica gel plate chromatography in tetrahydrofuran. The products obtained were as follows: R_f .17 (major product, λ_{max}^{EtOH} 238.5, highest mass peak at 355, $[M-43]^+$), R_f .39 (faint in intensity, λ_{max}^{EtOH} 276, 239.5, 282 sh), R_f .64 (faint, just end absorption in UV), R_f .78 (medium intensity, λ_{max}^{EtOH} 238, 249.5 sh) and R_f .92 (medium intensity, λ_{sh}^{EtOH} 282, 270 nm). The major product, R_f .17 was identified using mass spectrometry and n.m.r. analysis.

The highest mass peak at m/e 355 could have corresponded to either the $[M-43]^+$ fragment of triacetylated IV or the $[M-1]^+$ fragment of diacetylated hydroxyethyl substituted anhydrouridine. However, since the fragments of photoproduct II identified in Figure 17 were present also in the mass spectrum of compound IV (except for the highest mass peak at 399), it was presumed that the R_f .17 material obtained from the acetylation of photoproduct IV was the triacetylated derivative.

Analysis by 220 MHz n.m.r. of this derivative revealed that photoproduct IV was the second isomer of 6(α-hydroxyethyl)-5,6-dihydroanhydrouridine. Unlike compound II, however, this isomer did not contain a mixture of epimers visible in the n.m.r.

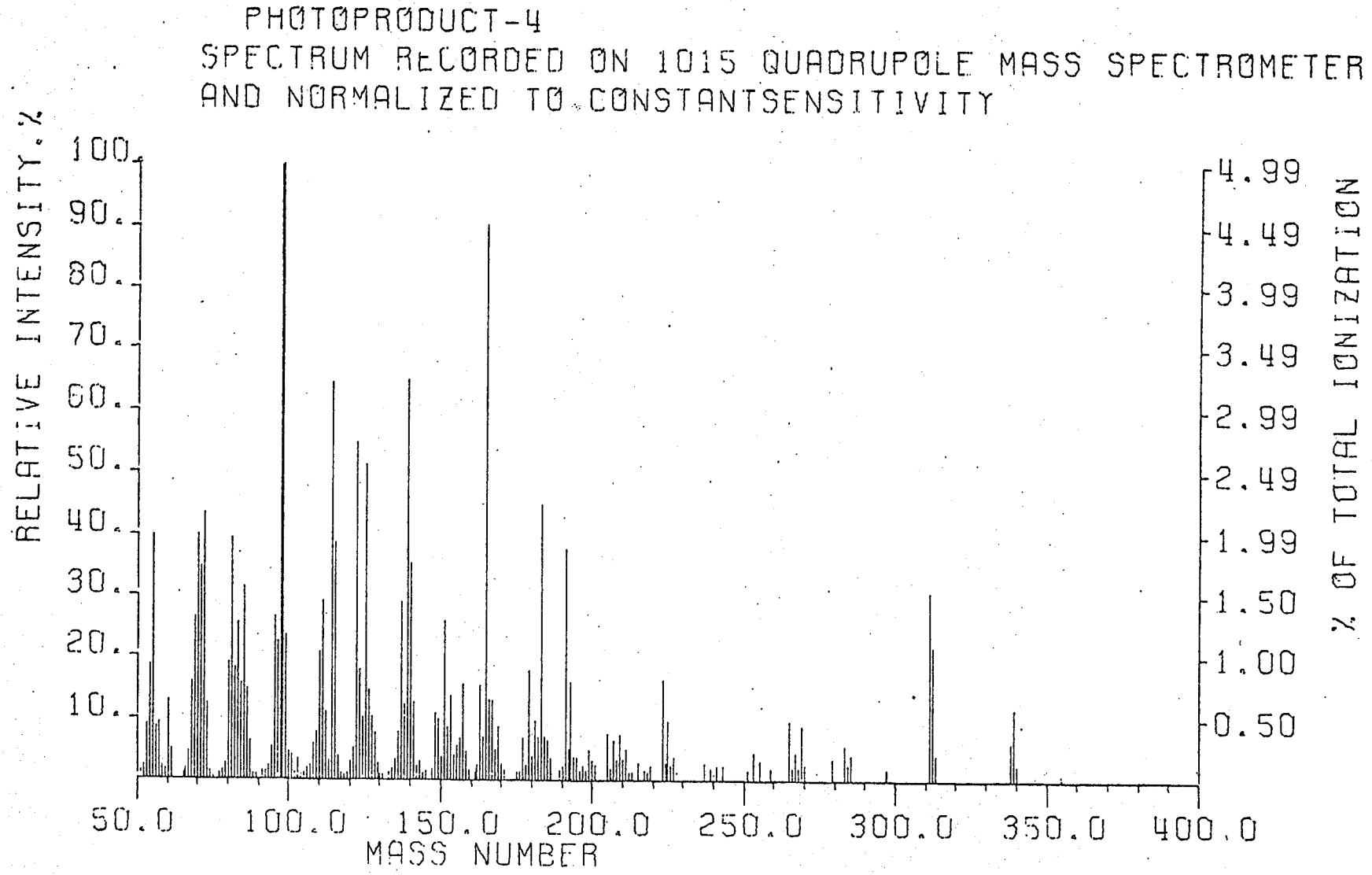


FIGURE 20. Mass spectrum of Photoproduct IV (

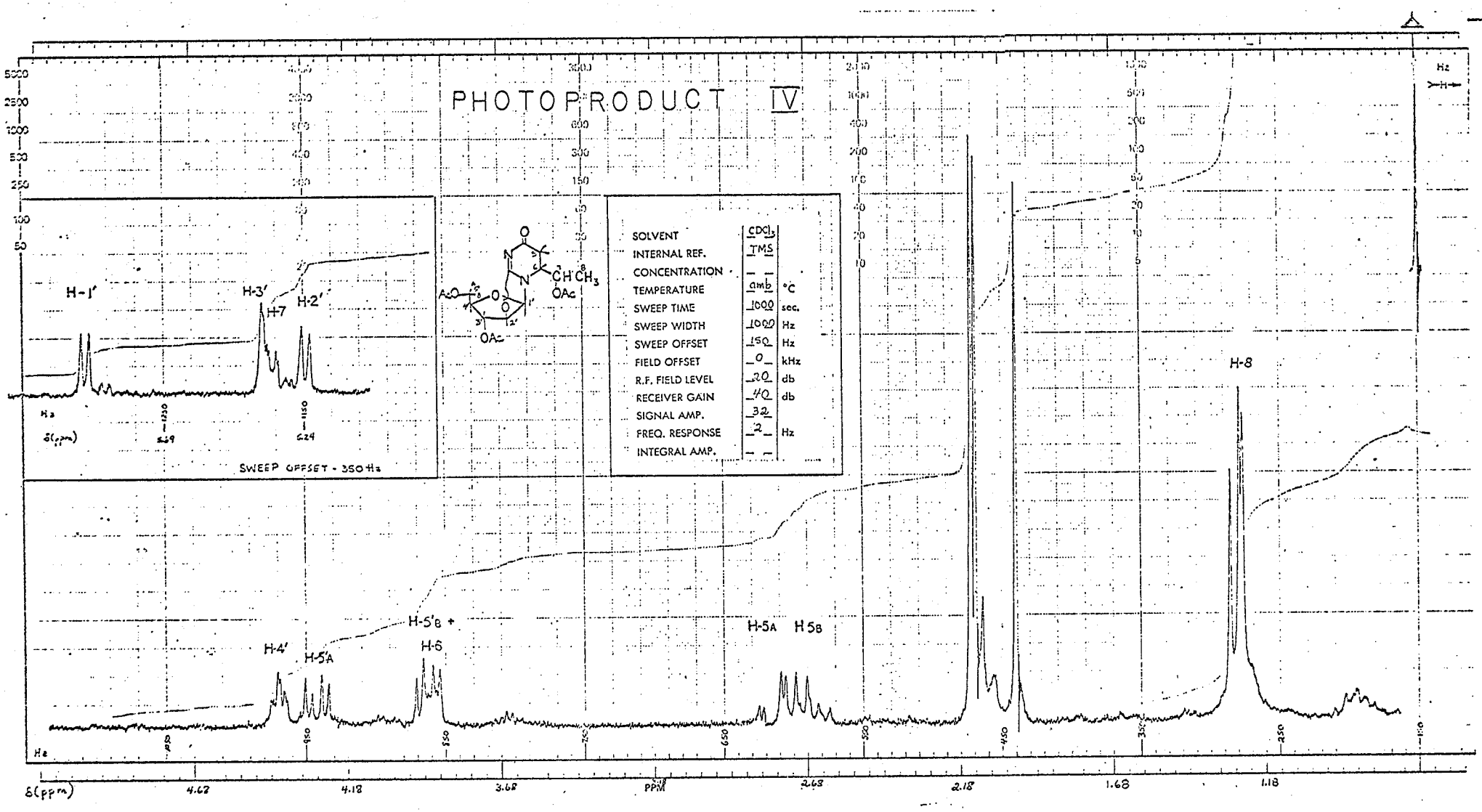
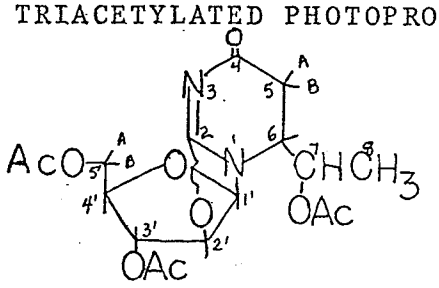


Figure 21 . N.M.R. spectrum of acetylated Photoproduct IV in $CDCl_3$

TABLE 15

N.M.R. ANALYSIS OF TRIACETYLATED PHOTOPRODUCT IV IN $CDCl_3$



PROTON	CHEMICAL SHIFT (δ ppm)	COUPLING CONSTANTS (J, cps)	
$H_{1'}$	5.93(d)	$J_{1'2'}$	5.5-6.0
$H_{2'}$	5.21(d)	$J_{2'3'}$	-
$H_{3'}$	5.36(u)	$J_{3'4'}$	2
$H_{4'}$	4.41(d,t)	$J_{4'5'A}$	5
		$J_{4'5'B}$	5
$H_{5'A}$	4.28(q)		
$H_{5'B}$	3.92(q)	$J_{5'A,5'B}$	-12
H_{5A}	2.80(q) or (d,d)		
H_{5B}	2.67(q)	$J_{5A,5B}$	-16
		$J_{5A,6}$	3.5
H_6	3.9 (d,q)	$J_{5B,6}$	8.0
H_7	5.34(d,q)	$J_{6,7}$	1.8
H_8	1.27(d)	$J_{7,8}$	6.5

d - doublet, t - triplet, q- quartet, u - unresolved,

d,d - doubleton of doublets, d,t - doubleton of triplets,

d,q - doubleton of quartets

N.M.R. Assignments for acetylated Photoproduct IV

When proton assignments for acetylated photoproduct IV were being made, close attention was paid to the assignments previously made for Photoproduct II. The solvent in this case was $CDCl_3$ instead of the d_6-DMSO used with II. The absence of DMSO and H_2O peaks and the absence of a second epimer greatly facilitated the assignment of the peaks in compound IV.

H-1' (II - 6.10 and 6.08 ppm)

The doublet at 5.93 ppm was assigned to H-1', J = 5.5-6.0 cps.

H-2' (II - 5.38 and 5.47 ppm)

The doublet at 5.21 ppm had J = 5.5-6.0 cps and was assigned to H-2'.

H-3' (II - 5.25 and 5.26 ppm)

The only peaks left in the desired area were a couple of doublets at 5.21 ppm and an unresolved peak at 5.36 ppm. Since these were very similar to peaks corresponding to H-7 and H-3' in compound II, the peak at 5.36 ppm was assigned to H-3'.

H-4' (II - 4.48 and 4.49 ppm)

A pair of overlapping triplets at 4.41 ppm were assigned to H-4'.

$H\text{-}5'_A$ (II - 4.17 and 4.16 ppm)

A quartet at 4.28 ppm was assigned to $H\text{-}5'_A$.

$H\text{-}5'_B$ (II - 3.98 ppm)

A quartet at 3.92 ppm was assigned to $H\text{-}5'_B$.

$H\text{-}5_A$ (II - 2.31 and 2.24 ppm)

$H\text{-}5_B$ (II - 2.5 ppm)

A pair of doublets at 2.8 ppm was assigned to $H\text{-}5_B$ and a quartet (or another pair of doublets) at 2.67 ppm was assigned to $H\text{-}5'_B$.

H-6 (II - 4.0 - 4.2 ppm)

Integration of the H-$5'_B$ area had, as in the case of compound II, indicated that more than one proton was present in the region around 3.9 ppm.

Buried in the H-$5'_B$ area, one can perceive a possible pair of quartets when the signal is amplified.

Therefore H-6 is located at 3.9 ppm.

H-7 (II - 5.2 and 4.91 ppm)

A pair of quartets at 5.34 ppm close by a tall peak at 5.36 ppm looked very similar to the H-3' and H-7 pattern observed in compound II, consequently, the pair of quartets at 5.34 ppm was ascribed to the H-7 proton.

H-8 (II - 1.16 and 1.21 ppm)

A doublet at 1.27 ppm was assigned to the three H-8 protons.

Spin Decoupling Experiments

Irradiation of H-8 at 1.27 ppm lead to the collapse of the pair of quartets at 5.21 ppm to form a very narrow doublet. Thus it was confirmed that H-7 of acetylated photoproduct IV is at 5.21 ppm.

Having established that H-7 is at 5.21 ppm, the area assumed to correspond to H-6 (3.92 ppm) was irradiated. The H-7 pair of quartets at 5.21 ppm were seen to collapse to a single quartet. At the same time the two quartets of H-5_A and H-5_B at 2.80 and at 2.67 respectively collapsed to a single quartet at 2.75 ppm with J = -15 cps.
Therefore, H-6 is indeed at 3.92 ppm and it is coupled to both H-7 and the H-5 protons.

Irradiation of H-4' at 4.41 ppm resulted in the sharpening up of the unresolved peaks at 5.36 ppm, thereby confirming that the assignment of H-3' to the peaks at 5.36 ppm was reasonable.

Diacetyl Dihydroanhydrouridine - Its Instability

Dihydroanhydrouridine[53] (10 mg) was dissolved in dry pyridine (0.4 ml) to which was added acetic anhydride (10 drops ~ 0.3 ml). Examination of the reaction mixture by TLC in tetrahydrofuran two hours later showed that the spot at R_f .05 corresponding to dihydroanhydrouridine had been completely replaced by a single spot at R_f .33. The reaction mixture was placed on a paper which was developed in solvent B to give a single band at R_f .54 (λ_{max}^{EtOH} 237.5 nm, molecular ion M^+ at 312 mass units). Re-examination of the ethanol solution three days later by TLC in tetrahydrofuran showed the presence of two spots - one at R_f .15 and the other at R_f .33, whereas previously there had only been one at R_f .33.

Triplet Quenchers

(i) effect of oxygen removal

Two samples of diacetyl anhydrouridine (100 mg each) were dissolved in 25% ethanol (100 ml) in a quartz vessel. Nitrogen was bubbled through one sample for five minutes prior to as well as during the irradiation. Both samples were irradiated for twenty-two minutes. At the end of this period, the UV spectrum of the sample that had been irradiated in the presence of oxygen showed a single maximum at 238 nm. The sample that had been de-oxygenated

by nitrogen also showed a single maximum at 238 nm in the UV spectrum. The presence of oxygen had no apparent effect on the progress of the photoreduction of diacetyl anhydrouridine.

Effect of Bromide Ion

Potassium bromide (11.9 g, 0.1 mole) was dissolved in 25% ethanol/H_2O (100 ml, $\therefore$ c = 1M). Anhydrouridine (200 mg) was dissolved in this solution which was then irradiated with stirring for 47 minutes. After this time, the solution exhibited a maximum at 238.5 nm in the UV spectrum.

Anhydrouridine (200 mg) was dissolved in 25% ethanol/H_2O and photolyzed for 47 minutes. Examination of the solution after this time showed a UV maximum at 238.5 nm.

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