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Synthesis of carbon nucleosides : potential antibiotics

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SYNTHESIS OF CARBON NUCLEOSIDES:

POTENTIAL ANTIBIOTICS

by

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Abstract

This paper deals with two synthetic routes designed to yield carbon nucleosides. The C-nucleosides are compounds of potential antibiotic utility. The first approach, designed to yield 4-(2'-deoxyribofuranosyl)-1,2,3-triazole (1), consisted of silylation of methyl vinyl ketone (4) to give 2-trimethylsilylosy-1,3-butadiene (6), followed by a crossed aldol condensation with a heteroaromatic aldehyde to yield the aldol 3. Epoxidation and cyclization of 3 was expected to yield the target molecule 1.

The initial step, silylation of methyl vinyl ketone (4), was successful. Using model compounds the second step of the synthesis was attempted. Numerous attempts to condense (6) with 2-pyridinecarboxaldehyde (12) using a $TiCl_4$ catalyst proved to be unsuccessful with only unreacted 12 being recovered. The unreactivity of this system suggested a stable bidentate chelate between $TiCl_4$ and 2-pyridinecarboxaldehyde. Benzaldehyde (15) was used as a simpler model system. $TiCl_4$ promoted reaction between 15 and 6 resulted in a mixture of products, which proved very difficult to identify and separate. Fluoride ion was then exchanged for the $TiCl_4$ catalyst. Using phase transfer catalysts such as crown ethers and quartenary ammonium fluorides complex mixtures of reaction products were again obtained. Some of the isolated products indicated that 6 had undergone Diels-Alder reactions.

The second approach towards synthesis of C-nucleosides involved elaboration of the heterocyclic unit from a protected sugar moiety. D-ribose was isopropylidated to di-2,3,O-isopropylidene-D-ribofuranose (19). Tritylation of 19 yielded 2,3-di-O-isopropylidene-5-O-trityl-D-ribofuranose (20). The chloro sugar 2,3-di-O-isopropylidene-5-O-trityl-β-D-ribofuranosyl chloride (21)

was prepared from 20. We then planned to make the acid chloride of 21 which was expected to react with an aziridine to yield the 1-acylaziridine (26). The 1-acylaziridine was expected to undergo iodide catalyzed isomerization to give an oxazoline C-nucleoside. Attempts to form the acid chloride 23 were unsuccessful due to our inability to form the Grignard of 21. Present strategies towards production of the 23 involve making the nitrile of 21 and converting this to the carboxylic acid which should then give the acid chloride 23.

To Grampa

With appreciation to Dr. Thomas Newton, whose patience and experience often calmed me, whose guidance often directed me, and whose energy and enthusiasm always motivated me.

J.D.

Acknowledgements

Many thanks to:

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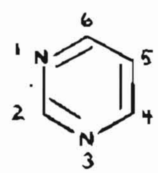
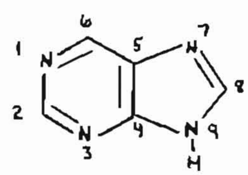
I - Introduction:

The ability to reproduce is unique to living organisms. Molecules known as nucleic acids play a central role in the reproduction process. An understanding of the chemistry of life, of heredity, and often of disease ultimately requires an understanding of the chemistry of nucleic acids. Nucleic acids are composed of three primary chemical constituents: nitrogenous bases, pentose sugars, and phosphate groups. This introduction presents the basic chemical information required to understand the composition and importance of nucleic acids, and to discuss the structure and synthesis of carbon nucleosides, whose relationship to nucleic acids, make them compounds of potential medical significance.

The nucleic acids have two types of heterocyclic bases as constituents: the pyrimidines and the purines. Structures and numbering schemes of the ring positions for these nitrogenous bases are shown below:

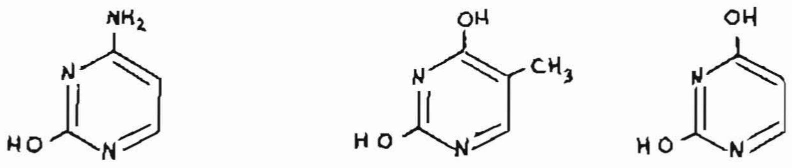


pyrimidine



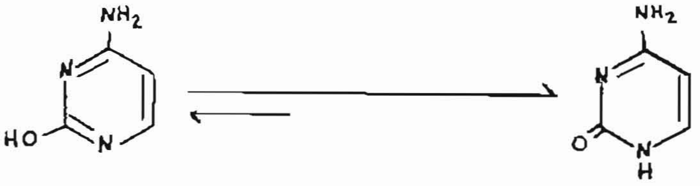
purine

The pyrimidines and purines that occur in living organisms have substituents at various ring positions, usually at C-2 and C-6. The three most common pyrimidines are cytosine (6-amino-2-hydroxypyrimidine), thymine (2,6-dihydroxy-5-methylpyrimidine) and uracil (2,6-dihydroxypyrimidine):



cytosine thymine uracil

Though depicted in their enol form pyrimidines undergo a facile keto-enol tautomerization in solution:



enol keto

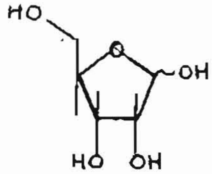
The importance of the keto forms will be examined later in the introduction.

A purine is basically a pyrimidine with an imidazole ring fused to it. Two essential purines occur most commonly in nature - adenine (6-amino-purine) and guanine (2-amino-6-hydroxypurine):

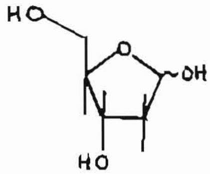
adenine guanine

In addition to these five primary nitrogenous bases, a number of other pyrimidines and purines occur as less common constituents of nucleic acids.

When incorporated into nucleic acids these heterocyclic molecules are always attached to a 5-carbon sugar, either ribose or 2-deoxyribose:

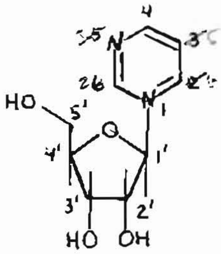


ribose

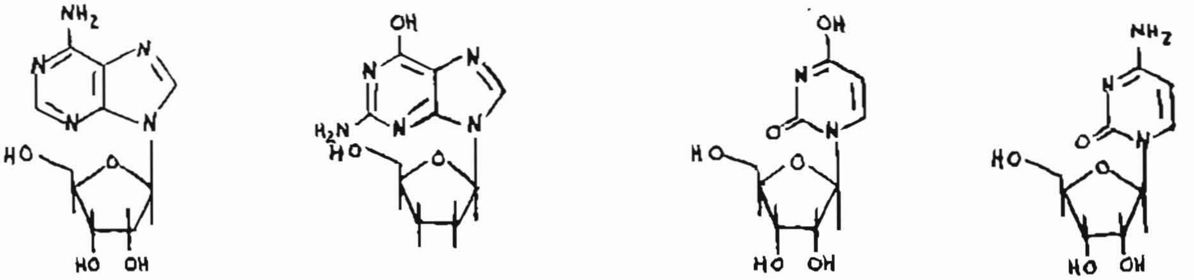


2-deoxyribose

A heterocyclic-sugar derivative of this sort is called a nucleoside or glycoside. The ring positions of the carbohydrate unit in a nucleoside are numbered with primes while the positions on the heterocyclic are without primes:

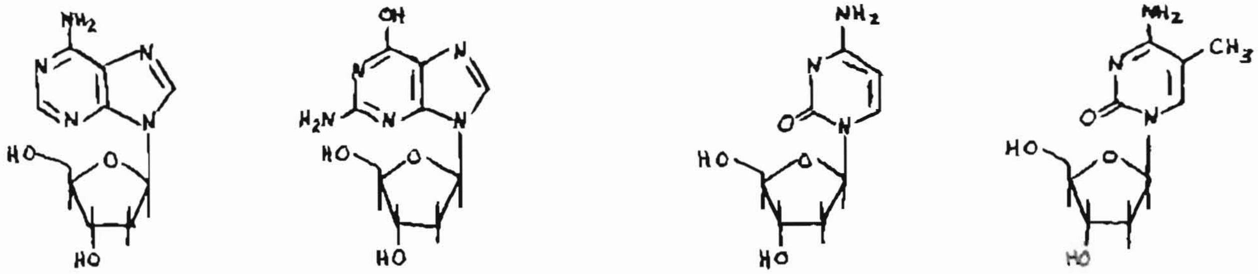


The heterocycle is always attached to C-1' of the sugar. This bond between the sugar and the heterocycle is often referred to as a glycosidic bond. The structures of the sugar may differ in their configuration at C-1'. They are known as anomers. The nucleosides are termed β if the orientation of the glycosidic bond is such that the $-CH_2OH$ on C-4' and the heterocycle are on the same side of the plane of the pentose ring. If the heterocycle is on the opposite side of the plane, the glycoside is in an α-configuration. The anomeric carbon, C-1', may be bonded to any atom of the heterocycle. The type of glycosidic bond is indicated by a prefix N- or C- depending on the atom that is attached to C-1'. Thus, a N-glycoside (or N-nucleoside) indicates that C-1' is bonded to a heterocyclic nitrogen atom. There are eight important N- nucleosides in nature, four ribonucleosides and four deoxyribonucleosides. These essential biomolecules are shown in Figures 1 and 2, respectively.



adenosine	guanosine	uridine	cytidine
9-(β-D-ribofuranosyl) adenine	9-(β-D-ribofuranosyl) guanine	1-(β-D-ribofuranosyl) uracil	1-(β-D-ribofuranosyl) cytosine

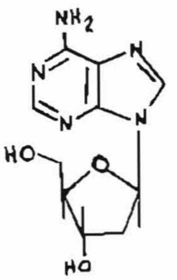
Fig. 1 - The Essential Ribonucleosides



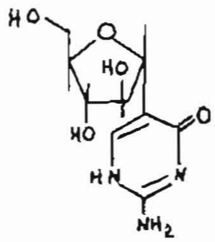
2-deoxyadenosine	2-deoxyguanosine	2-deoxycytidine	thymidine
9-(β-2'-deoxy-D-ribofuranosyl) adenine	9-(β-2'-deoxy-D-ribofuranosyl) guanine	1-(β-2'-deoxy-D ribofuranosyl) cytosine	1-(β-2'-deoxy-D ribofuranosyl) thymine

Fig. 2 - The Essential Deoxyribonucleosides

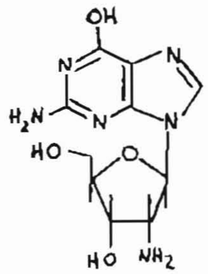
Historically only pyrimidine and purine N-glycosides of ribose and 2-deoxyribose were defined as nucleosides. Now the term applies to all carbohydrate derivatives of heterocyclic compounds. The heterocycle may be natural or synthetic and the glycosidic bond may be through a nitrogen or a carbon atom. The complete description of a nucleoside depends on the structures of the heterocycle and the sugar, and the configuration of the glycosidic bond. Thus, the following compounds are all classified as nucleosides: 2'-deoxyadenosine, a nucleoside obtained from DNA; α-Ara-ψ-Isocytosine, a synthetic nucleoside with a C-C glycosidic bond in the α-configuration and with arabinose as its sugar; 2'-aminoguanosine, a nucleoside with an amino sugar moiety. These nucleosides are shown in Figure 3.



2-deoxyadenosine
9-(β-2'-deoxy-D-ribofuranosyl)adenine



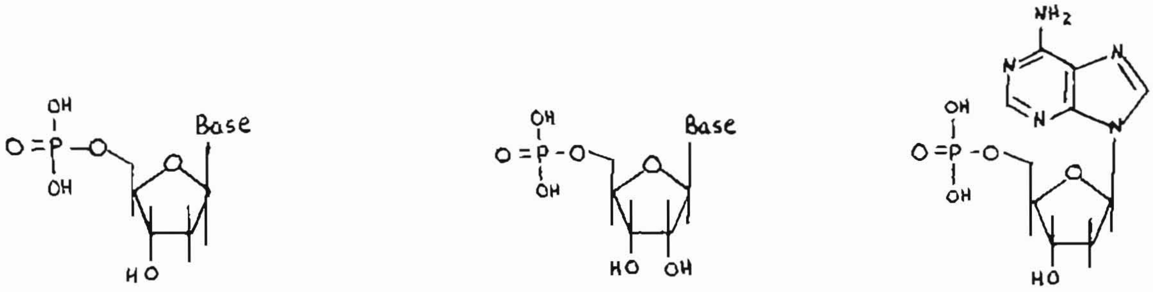
Ara-ψ-Isocytosine
5-(α-D-arabinofuranosyl)isocytosine



2'-aminoguanosine

Fig. 3 - Structures of Three Nucleosides

The 5'-O-phosphate esters of nucleosides are termed nucleotides. The general formulas for 5'-nucleotides and the formula for a specific nucleotide, adenosine-5'-monophosphate are shown in Figure 4.



5'-deoxyribonucleotides   5'-ribonucleotides   adenosine-5'-monophosphate

Fig. 4 - Structures of Nucleotides

Nucleic acid polymers result from combination of nucleotide monomers through sugar-phosphate linkages. Such a polymer is illustrated in Figure 5. There are two major nucleic acids, distinguished by the pentose sugar, found in each type. Ribonucleic acid (RNA) is made up of ribonucleotides while deoxyribonucleic acid (DNA) is a polymer of deoxyribonucleotides. DNA always contains a phosphate group linking a C-5' of one deoxyribose to C-3' of the next sugar group. Such a series of linkages yields a polymer with a sugar and phosphate backbone and a network of nitrogenous bases extending out from this backbone as shown in Figure 5.

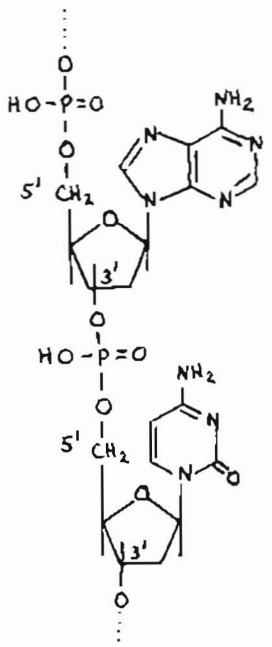


Fig. 5 - A Partial Structure of a DNA Polymer

Typical DNA molecules contain four nitrogenous bases: adenine, cytosine, guanine and thymine. RNA contains adenine, cytosine, guanine and uracil. An important characteristic of DNA is that it contains two separate nucleotide chains held together through its nitrogenous bases. Watson and Crick[1] showed that the two chains form a helix. Hydrogen bonding between pyrimidine and purine bases is responsible for the helical structure. It is the keto forms of pyrimidines, previously mentioned, that enable them to hydrogen bond to the purines as diagrammed in Figure 6. The keto forms of the pyrimidines permit configurations that allow thymine and adenine to form two hydrogen bonds, while cytosine and guanine bond exclusively together forming three hydrogen bonds.

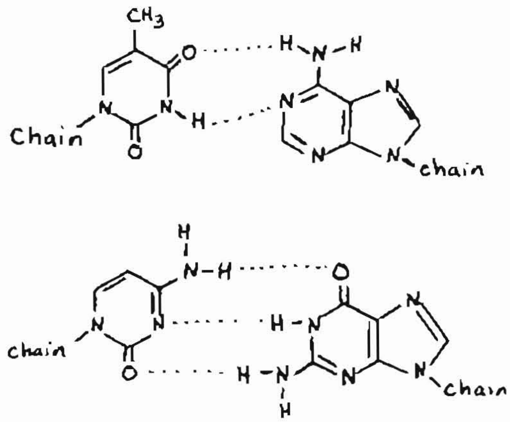
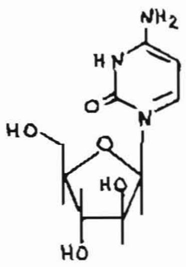


Fig. 6 - Hydrogen Bonding Between Pyrimidines and Purines

The primary function of DNA is to transmit a cell's genetic information to future generations. It is the base sequence of DNA that ultimately controls production of the cell's proteins. Each series of three nucleotides in DNA specifies for one of the 20 basic amino acids essential for protein synthesis. This triplet code is transcribed onto an RNA template. Amino acids, which polymerize to form proteins, are then attached to this template according to the sequence of transcribed triplets. Linkage of these amino acids as they arrive at the template results in protein production. A more detailed discussion of the role of nucleic acids in protein synthesis is available in any contemporary biochemistry text.

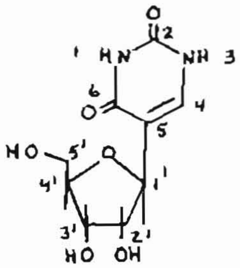
Any factor that disrupts the structure and/or function of DNA will alter the chemical information received by the cell and result in failure of the cell to function properly. Chemotherapy, the treatment of disease with chemicals, is designed to utilize the ability of certain compounds to disrupt cellular metabolism. One approach to such disruption is to inhibit the normal biosynthesis of the nucleic acids. Many nucleosides have been found to be successful in inhibition of DNA synthesis, and subsequently they exhibit anti-biotic effects. One such antibiotic is cytosine arabinoside[2]. This nucleoside analogue differs from cytidine only in the orientation of the -OH group at C-2'.



Cytosine Arabinoside

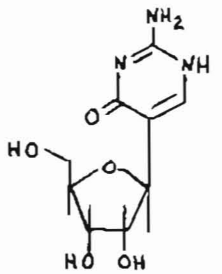
Since medical research is constantly searching to discover drugs with the ability to inhibit the growth of diseased cells, one can understand the present eagerness to study the cytotoxcity of various nucleosides.

A group of nucleosides recently receiving attention regarding their antibiotic and anticancer properties are the carbon nucleosides, termed C-nucleosides. C-nucleosides are unique in that the glycosidic bond is a carbon-carbon linkage instead of the carbon-nitrogen bond found in most naturally occuring nucleosides. The C-1' of the sugar moiety is bonded to another carbon in the heterocycle. A number of C-nucleosides have been isolated from natural sources. The first C-nucleoside isolated from nature was pseudouridine, 5-(β-ribofuranosyl)uracil, which was obtained from bacterial RNA[3]:



Pseudouridine

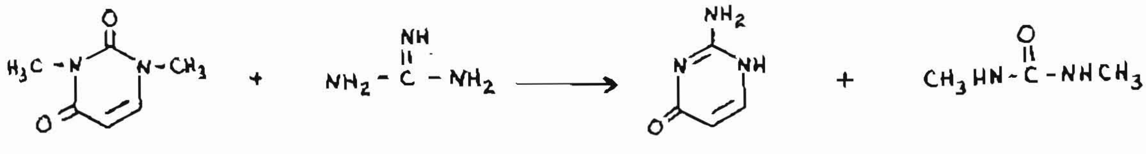
Although pseudouridine itself has not displayed antibiotic properties, many naturally occuring C-nucleosides, some of them similar to pseudouridine, do. These naturally occuring C-nucleosides, reviewed extensively by Suhadolnik[2,4], have become targets for the synthetic organic chemist. Simultaneously, chemists have begun to search for synthetic analogues of these natural C-nucleosides that may display chemotherapeutic activity. ψ-Isocytidine, 5-(β-D-ribofuranosyl) isocytosine, the first synthetic pyrimidine C-nucleoside antibiotic, has been synthesized by J.J. Fox et al[5]:



Ψ -Isocytidine

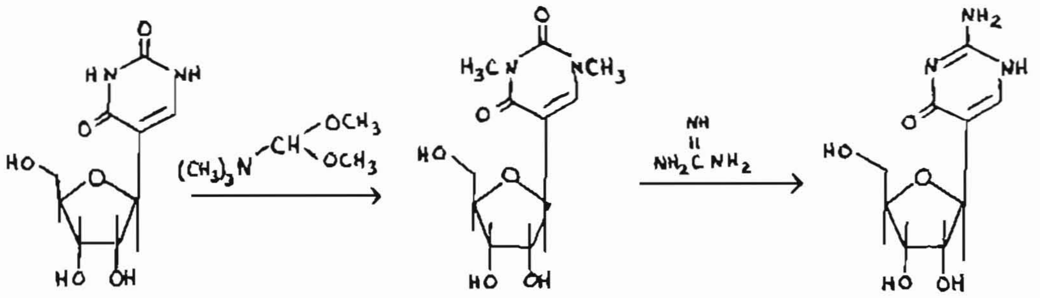
ψ-Isocytidine, closely related to pseudouridine, has been shown to be phosphorylated and subsequently incorporated directly into the nucleic acids when administered to animals. Presumably this C-nucleoside interferes with normal cell metabolism resulting in destruction of the cell. Considering their potential value in medicine, it is natural that organic chemists should be intent upon developing methods of synthesis of C-nucleosides. Presently there are three general strategies for C-nucleoside synthesis.

One method of synthesizing C-nucleosides depends on the conversion of available C-nucleosides to new ones. By modification of the heterocycle on a pre-formed C-nucleoside a new C-nucleoside may be obtained. There are numerous reactions of the nitrogenous bases that may act to transform C-nucleosides. A novel reaction of pyrimidines, utilized by Watanbe and Fox[6], serves as a prime example of the strategy involved in C-nucleoside conversions. These authors found that 1,3-dipolarophiles containing N-C-N fragments could displace the N_1-C_2-N_3 portion of 1,3-dialkyluracils. Treatment of 1,3-dimethyluracil with the dipolarophile guanidine afforded 2-amino-4-hydroxypyrimidine with liberation of 1,3-dimethylurea:



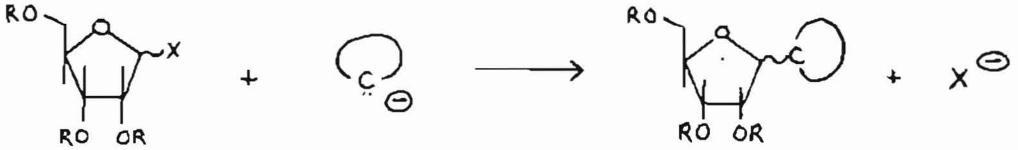
This pyrimidine-pyrimidine transformation has been applied to C-nucleoside conversions by Fox et al[6]. His group obtained the antibiotic ψ-isocytidine from the naturally occuring pseudouridine by use of a pyrimidine-pyrimidine conversion. The transformation required just two steps. The first step was methylation of pseudouridine to 1,3-dimethylpseudouridine.

This was followed by the reaction of the dialkylpseudouridine with guanidine:

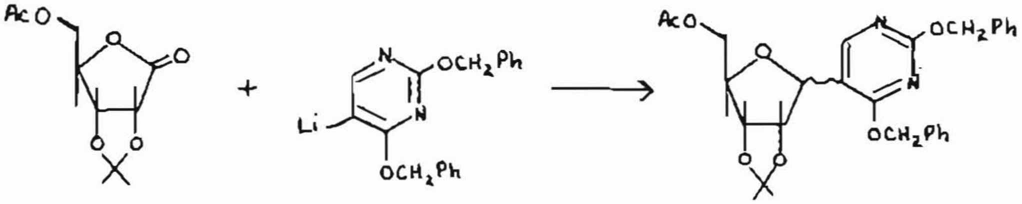


The scope of pyrimidine-pyrimidine transformations is being studied to determine their utility in other C-nucleoside conversions[7].

The second major approach towards C-nucleoside synthesis involves the direct condensation of a suitably protected sugar derivative with a carbanion derived from an appropriate heterocycle. Such a condensation, schematically diagrammed below, results in the formation of the requisite C-glycosidic bond:



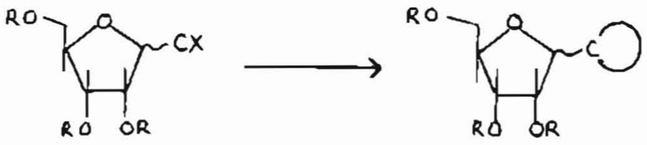
Asbun and Binkley[8] used this type of approach to synthesize pseudouridine. They obtained a pseudouridine derivative by condensing the protected sugar 5-O-acetyl-2,3-di-O-isopropylidene-D-ribofuranose with the substitued pyrimidine 2,4-dibenzyloxy-5-lithiopyrimidine. The derivative was then reduced and acidified to obtain pseudouridine:



The key to this synthesis, the condensation step, depends on the lithium atom inducing nucleophilic character on C-5 of the pyrimidine. The carbanion center at C-5 can then undergo addition to the carbonyl carbon, C-1', on the protected carbohydrate to form the essential C-C bond.

The condensation approach has its drawbacks. The reactions are difficult to perform. The yields are low and the technique is unsuitable for large-scale preparations. More importantly the condensations are specific for each C-nucleoside. For the synthesis of a C-nucleoside with a particular heterocyclic unit a specific 5-lithio pyrimidine derivative is required.

A more flexible route to C-nucleosides, which does not suffer from the restrictions of the condensation method, entails the elaboration of the desired heterocycle from a sugar derivative suitably functionalized at C-1':



There are numerous functional groups which may be used to synthesize C-nucleosides[9]. An example of this "elaboration" method and its generality is seen in the synthesis of both pseudouridine and Ψ-isocytidine by Fox et al[5] shown in Figure 12.

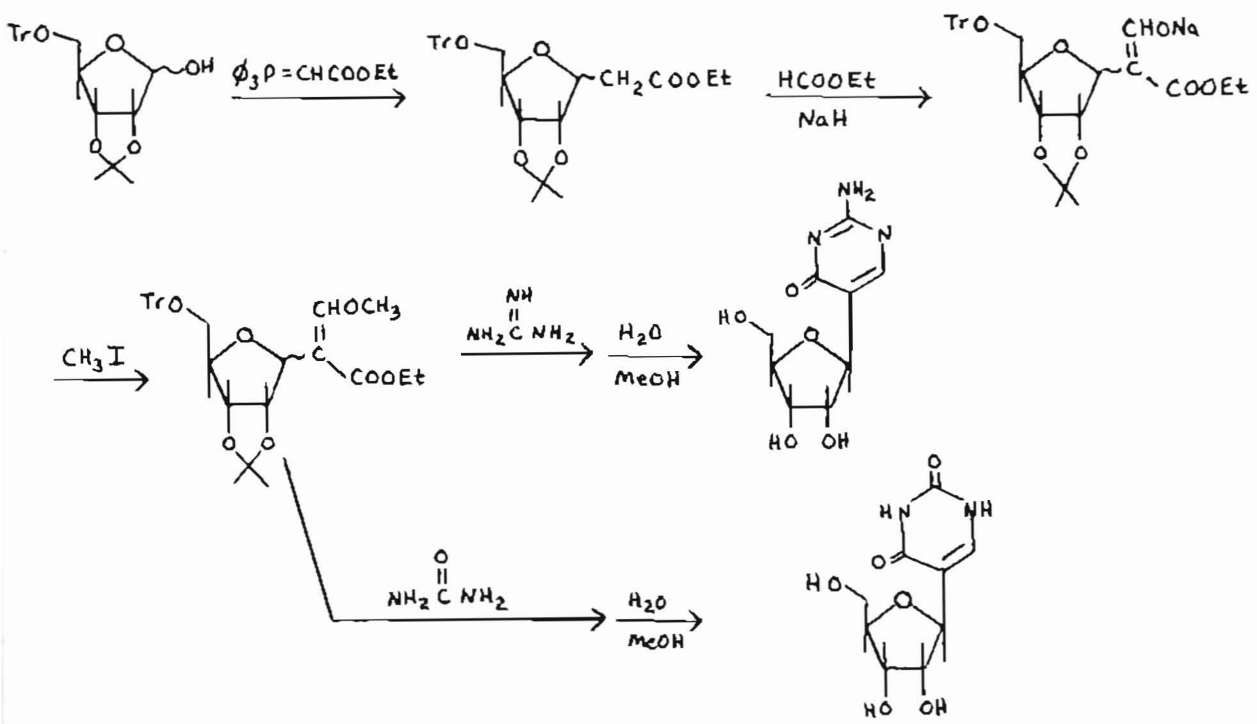
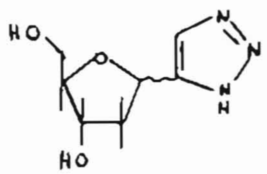


Fig. 12 - Elaboration of Pseudouridine and ψ-isocytidine

The starting material, 2,3-di-O-isopropylidene-5-O-trityl-D-ribofuranose, was reacted with the modified Wittig reagent ethoxycarbonylmethylenetriphenylphosphorane. Formylation of the resulting product with ethyl formate is the key step in this synthesis. It forms an intermediate which upon methylation readily undergoes cycloaddition with a variety of 1,3-dipolarophiles. Thus the generality of the elaboration is exemplified by the reaction of the methylated intermediate with urea and guanidine to give blocked pseudouridine and blocked Ψ-isocytidine respectively. Subsequent removal of the sugars' protecting groups yields the desired C-nucleosides. One can postulate the synthesis of a variety of pyrimidine C-nucleosides by utilizing the functional group in reacting different 1,3-dipolarophiles with the methylated intermediate. Since C-1' may be substituted with various functional groups containing a C-C linkage the flexibility of the "elaboration" approach makes it the most fruitful of the three synthetic methods at present.

The potential of C-nucleosides as antibiotics prompted us to propose a novel synthetic route to this class of compounds. We hoped this proposed route would complement existing methods of C-nucleoside synthesis, while offering advantages that none of the other three methods have. Our approach differed from the others in that we sought to elaborate the furanose ring from an appropriately functionalized heterocycle. Choosing 4-(2'-deoxyribofuranosyl)-1,2,3-triazole (1) as a target we investigated our novel approach in hopes of developing the methodology required to efficiently synthesize C-nucleosides.



4-(2'-deoxyribofuranosyl)-1,2,3-triazole (1)

II - Results and Discussion

The approach to our target molecule, 4-(2'-deoxyribofuranosyl)-1,2,3-triazole (1), follows from the retrosynthetic analysis outlined in Figure 13.

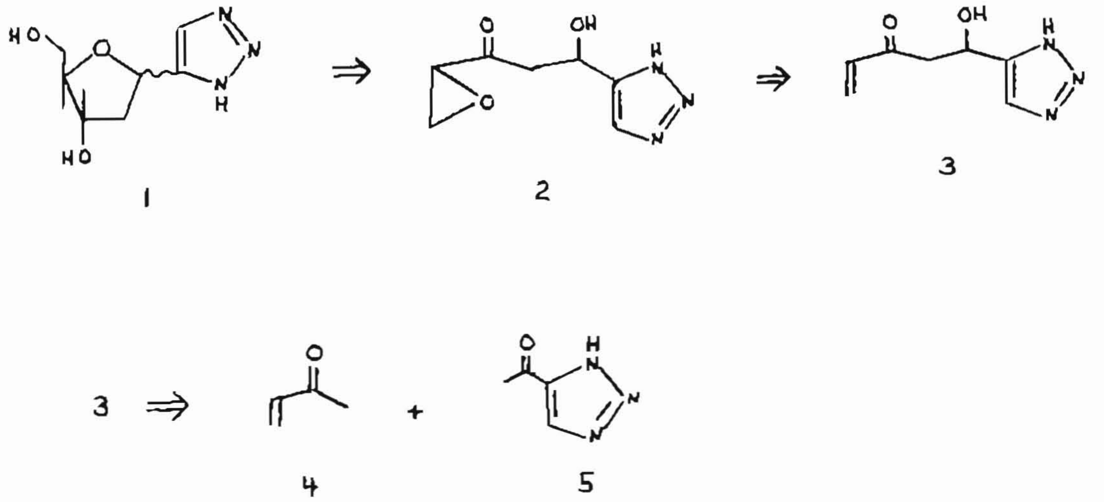
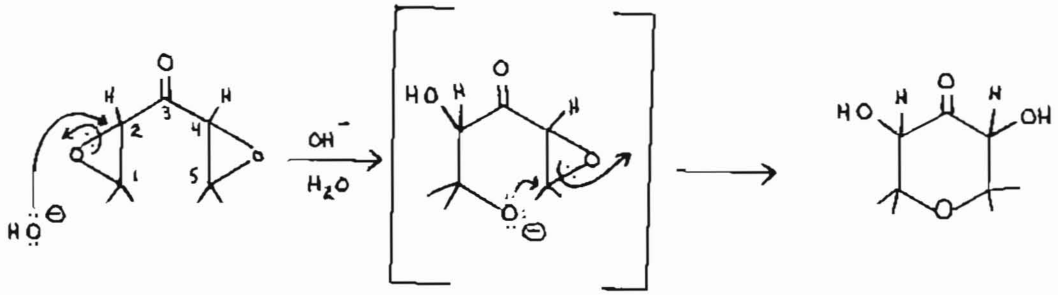
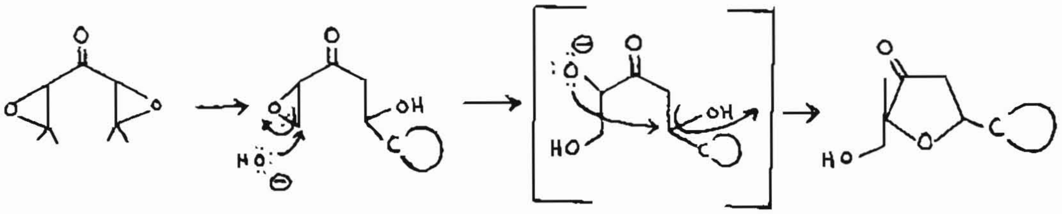


Fig. 13 - A Retrosynthetic Analysis of 4-(2'-deoxyribofuranosyl)-1,2,3-triazole

We chose 1 as a target because of its structural similarity to 1-N-(2'-deoxyribofuranosyl)-1,2,3-triazole-4-carboxamide, a nucleoside of known antibiotic activity. As previously mentioned the novelty of our approach consists of the elaboration of the C-nucleoside's sugar moiety from a heterocyclic aldehyde. All other nucleoside syntheses entail the use of a preformed sugar. The important step in the formation of the pentose ring was patterned after chemistry developed by Tischenko[10]. His group discovered that cyclic ethers with ketone functionalities could be formed from certain epoxides:



The $OH^{\ominus}$ acts as a nucleophile, attacking the electrophilic C-2 and opening the epoxide ring. The resultant intermediate, with formal charge on the oxygen, then cyclizes through the oxygen to C-5. This results in opening of the second epoxide ring and formation of a 6-membered pyranose. Our epoxide ring-opening reaction differed in that we hoped the nucleophile, OH^-, would attack C-1, thereby initiating the formation of a furanose ring:



Even if the nucleophile added to C-2, resulting in formation of a 6-membered ring, we could have utilized a pyranose to furanose transformation to obtain the desired 5-membered sugar.

These epoxides are prepared from vinyl ketones such as 3. It was our hope that a heterocyclic base attached to a carbon skeleton containing the appropriate vinyl ketone functionality could be transformed into the C-nucleoside precursor 2.

Linkage of the heterocycle to the vinyl ketone is the other major requirement of the synthesis. Our important intermediate 3 can be made by formation of a C-C bond via an aldol condensation between methyl vinyl ketone (4) and a heterocycle equipped with an aldehydic function such as 5. This is a key reaction in that the C-C bond formed will eventually become the glycosidic bond of the nucleoside. Having determined the essential requirements of the synthesis from the retrosynthetic analysis, we proposed the synthetic plan outlined in Figure 14.

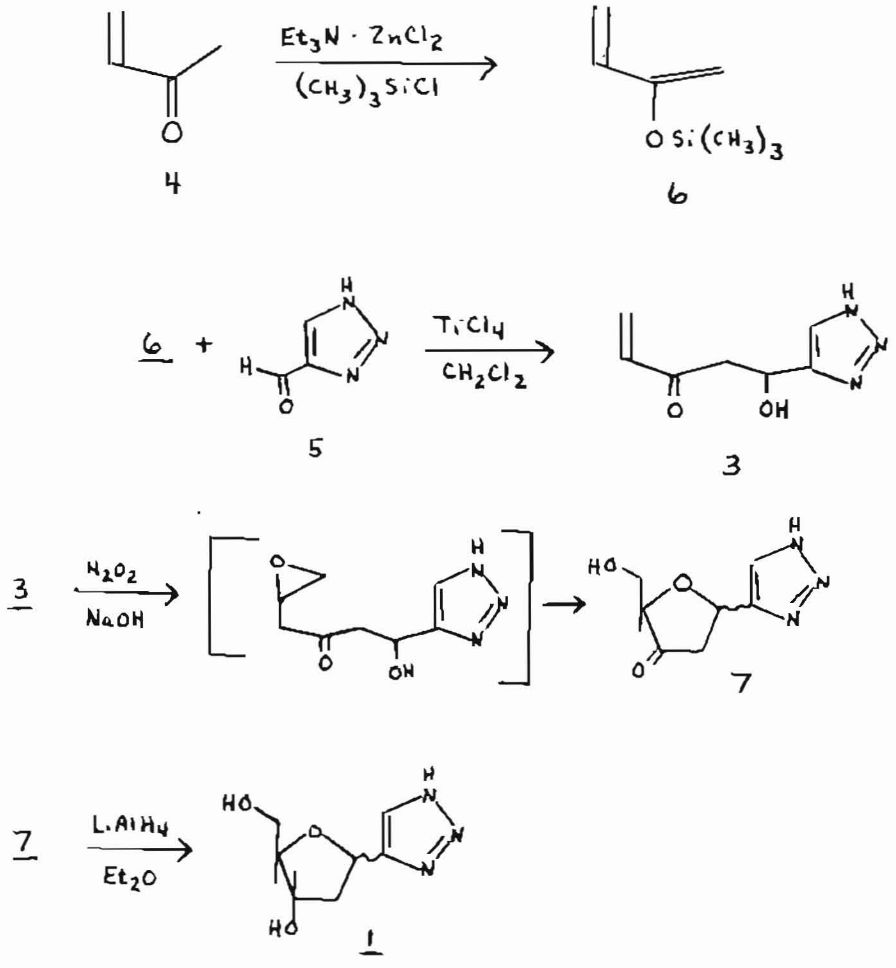
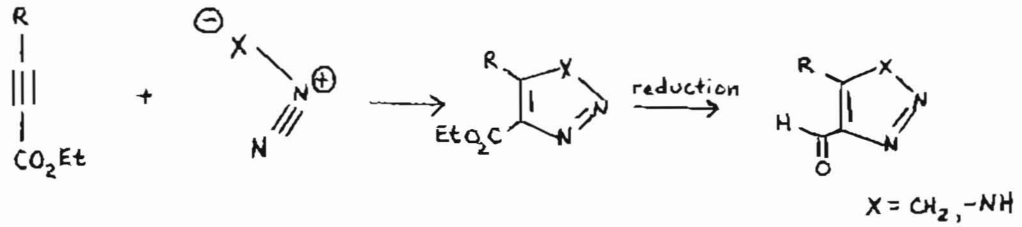


Fig. 14 - A Proposed Synthetic Plan for 4-(2'-Deoxyribofuranosyl)-1,2,3-triazole

This synthetic plan began with the silylation of methyl vinyl ketone (4) to its trimethylsilylenol ether (6), a reaction developed by House[11]. A crossed aldol condensation of 6 with 1,2,3-triazole-4-carboxaldehyde (5) using a $TiCl_4$ catalyst was designed to yield 3[12]. Epoxidation of 3 using basic hydrogen peroxide was expected to give 2 which should cyclize directly to the 3-furanone 7[10]. Reduction of 7 should have completed the synthesis.

The generality of this approach appeared to be limited by the availability of heteroaromatic aldehydes such as 5. These compounds are prepared easily. Propiolate esters act as dipolarophiles toward a variety of 1,3-dipolar reagents to produce a wide array of heteroaromatic esters. These, in turn, may be reduced to heteroaromatic aldehydes:



It is the linkage of 5 to methyl vinyl ketone (MVK) that produces the essential intermediate 3. The C-C bond is formed by addition of the enolate ion of 4, or its synthetic equivalent, to the carbonyl carbon of the aldehyde. The mechanism of this reaction, a crossed-aldol reaction, is shown in Figure 15.

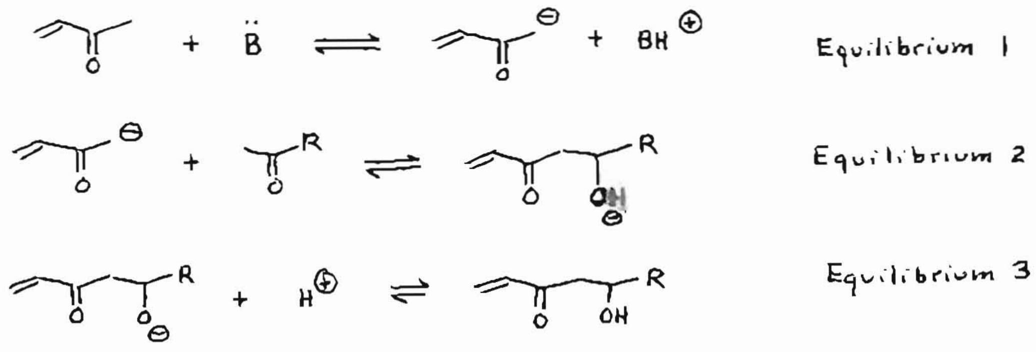


Fig. 15 - Mechanism of Crossed-Aldol Reaction Between 4 and an Aldehyde

The first equilibrium of the aldol condensation utilizes a base of sufficient strength to convert 4 to its enolate anion. The enolate is formed by removal of one of the acidic methyl protons α to the carbonyl. This enolate then attacks the electrophilic carbonyl of an aldehyde to form the alkoxide salt in equilibrium 2. The desired β-hydroxyketone is produced upon protonation of the alkoxide.

The crossed aldol condensation, reviewed by Nielson[13], is not as simple as portrayed. There are severe limitations to the reaction. Self-condensation of the reactants is one such limitation. MVK is especially notorious for self-condensation and polymerization. The enolate of 4 can undergo Michael addition to the β carbon of another molecule of 4 to produce a dimer:

The MVK dimer is susceptible to further attack resulting in MVK polymerization. It is the production of self-condensation products that diminishes the utility of the aldol condensation. Thus chemists interested in utilizing the crossed-aldol condensation have developed procedures to circumvent these difficulties. The use of metal enolates, for example, has been popular. Wittig used lithio imines to effect crossed aldol condensations[14] as pictured in Figure 16.

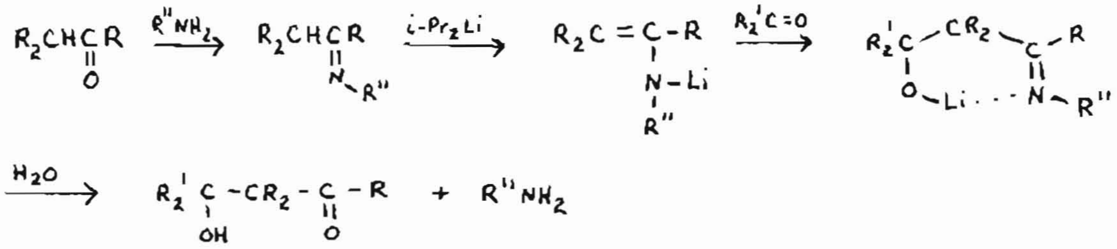
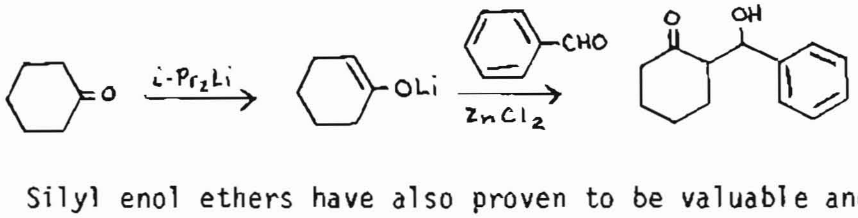
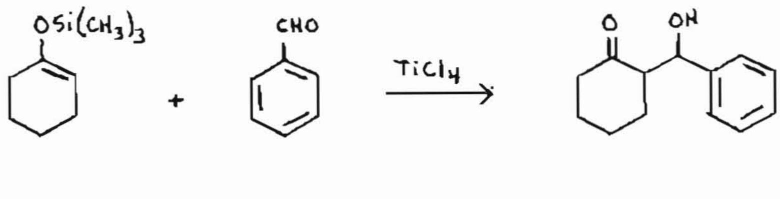


Fig. 16 - Use of Lithio Imines to Effect Crossed Aldol Condensation

House has utilized lithio enolates in combination with Lewis acid metal salts for the crossed-aldol reactions:[15]

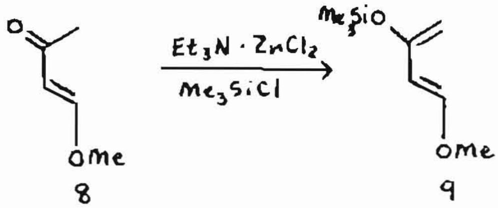


Silyl enol ethers have also proven to be valuable and versatile precursors in the production of specific enolates or their equivalents:[16]



Knowing that these silyl enol ethers undergo Lewis acid promoted aldol condensations with aldehydes[12] we began our synthetic work by forming 2-trimethylsilyloxy-1,3-butadiene (6), the silyl enol ether of 4.

Our initial attempts to prepare 6 were patterned after the chemistry of Danishefsky[17]. His group reported the silylation of trans-4-methoxy butene-2-one (3), a compound structurally similar to 4:



Using Danishefsky's exact procedure we attempted the silylation of 4. Although there was evidence of reaction, we failed to isolate any of the desired product 6. Upon addition of a benzene solution of 4 to the $ZnCl_2$ suspension in triethylamine a blue color formed. We believe that this color is an indication of a $ZnCl_2$-enolate complex as shown in Figure 17.

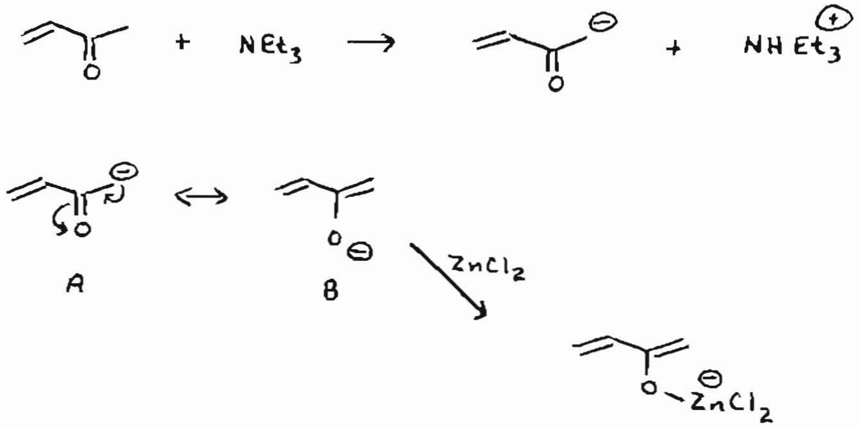


Fig. 17 - Proposed $ZnCl_2$-enolate Complexation

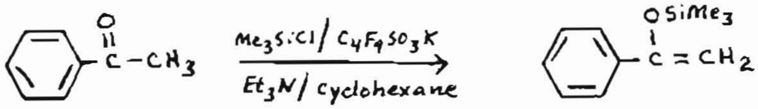
Although particular canonical forms of the enolate do not actually exist independently, we may consider the complexation of $ZnCl_2$ to be through the oxygen of canonical from B. Since it is the canonical form A that is responsible for attack of another molecule of 4, the use of $ZnCl_2$ in "trapping" the enolate in canonical form B, inhibits self-condensation and polymerization of methyl vinyl ketone. Evidence of reaction was also indicated by disappearance of the blue color upon addition of the silylating agent, $(CH_3)_3SiCl$, to the $ZnCl_2$-enolate complex. Precipitation of a copious amount of triethylamine hydrochloride and formation of a brown color also were indicative of a reaction. That no silyl enol ether was isolated can be ascribed to difficulties with the aqueous work-up and not necessarily in a failure to silylate 4. Silyl enol ethers are sensitive to aqueous solutions, being hydrolyzed back to the original ketone.[18]

The successful silylation of 4 has been reported by Conia[19] in 50% yield and by Jung[20] in 36-45% yield. Both utilized a procedure developed by House.[11] The House method, like Danishefsky's, utilizes triethylamine as a base and $(CH_3)_3SiCl$ as a silylating agent. The differences between Danishefsky's procedure and the House method consist in use of dimethylformamide as a solvent instead of benzene, and in the absence of $ZnCl_2$ as a Lewis acid complexing agent.

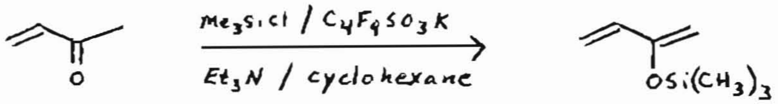
By repeating exactly the directions of Jung[20] a clear liquid characterized as 6 was obtained in 33% yield from 4. NMR data of 6 agreed with Jung's literature values (see Experimental section). We also obtained a fair amount of a higher boiling mixture which was yellow. Vpc analysis of this liquid indicated at least five components. The major component comprised 60-70% of this high boiling liquid. This higher boiling mixture is believed to contain condensation products of 4 due to the Michael addition reactions, as previously discussed.

A Michael addition results in the formation of a compound with two types of carbonyl groups, a conjugated one and a normal unconjugated carbonyl. The IR data of the high boiling fraction offers evidence for a Michael addition. Two carbonyl stretching frequencies existed, one at 1705 cm^{-1} and the second at 1670 cm^{-1}. These two carbonyl bands are separated by 35 cm^{-1}, exactly the separation expected between a normal ketone and a α-β unsaturated ketone. Separation and characterization of the high-boiling components from this reaction offers a challenge for furthur study since no mention of such products have been found in the literature.

Although successful in providing us with a supply of 10-15 grams of 6, the low yields obtained using the House method, coupled with the difficult and tedious workup of the reaction mixture prompted us to investigate two other modern silylation procedures. A method utilized by Vorbruggen[21] was tried with a hint of success. Vorbruggen was able to convert enolizable ketones into silyl enol ethers by the use of a reactive silylating agent, trimethylsilyl nonafluorobutanesulfonate (trimethysilyl nonaflate). The sensitive silylating agent was generated in situ by addition of chlorotrimethylsilane to a suspension of potassium nonaflate in cyclohexane. Using triethylamine as a base Vorbruggen silylated acetophenone in 71% yield:



Using commercial potassium nonaflate[22] we followed Vorbruggen's procedure in an attempt to silylate 4:



4 6

A single attempt to obtain an improved yield of 6 failed due to a mechanical malfunction. During the requisite reflux period the condensor water shut off. The resultant complete evaporation of solvent left a congealed mass in the reaction flask. Fresh cyclohexane was added in hopes of extracting any products present. Work-up and fractional distillation yielded primarily high-boiling fractions, similar to those obtained by the House method. A scant amount of clear liquid was obtained in the expected boiling range of 6 (50-55° at 50 mm). NMR analysis of this liquid indicated that it was 6. Despite the logistical problems encountered during this reaction run this method merits furthur attention. It appears to be a clean reaction, with minimal discoloration and little triethylamine hydrochloride salt being produced.

Another attractive silylation method was devised by Kuwajima.[23] His method circumvents the aqueous work-up of silyl enol ether products, which is a problem in the House procedure. The Kuwajima method is also useful in that it requires no solvent and results in no inorganic salt formation. Kuwajima's group obtained these desirable effects by use of a new silylating reagent, ethyltrimethylsilylacetate-tetra-n-butylammonium fluoride (ETSA-TBAF). By reacting methyl isopropyl ketone (10) with an equimolar amount of ETSA and a catalytic amount of TBAF Kuwajima formed the silyl enol ether (11) in 84% yield:

10

While the above authors make no mention of a mechanism, we propose the mechanism outlined in Figure 19.

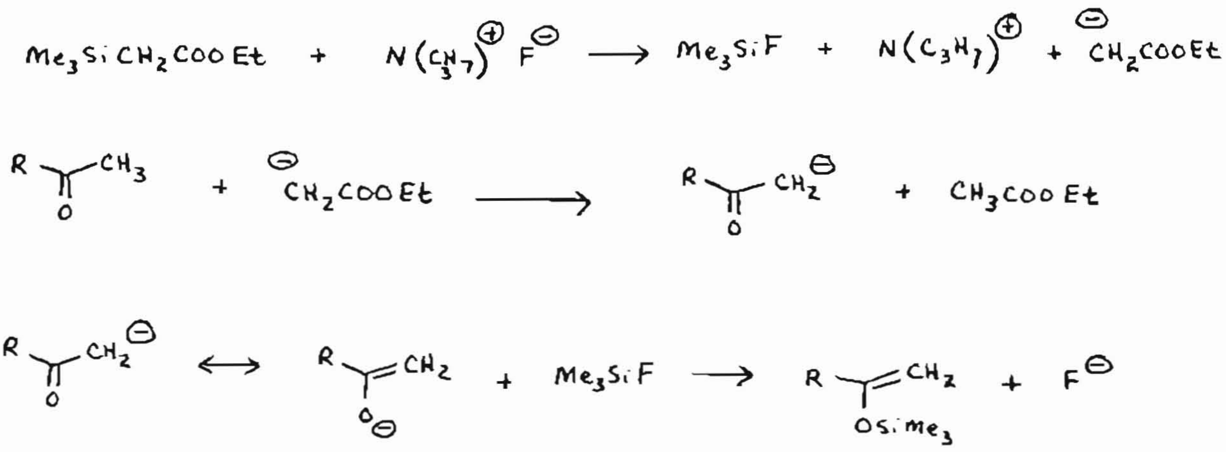


Fig. 19 - Proposed Mechanism for Silylation of Ketone Using ETSA/TBAF

TBAF acts as a catalyst by generating, in situ, fluorotrimethylsilane, the actual silylating agent. The strongly basic $^{\ominus}CH_2COOEt$ forms the enolate of (10), which is silylated by Me_3SiF. The result of this reaction pathway is the formation of (11) and ethylacetate along with the regeneration of the catalytic TBAF. We attempted to apply Kuwajima's procedure, replacing TBAF with trimethylbenzylammonium fluoride (TMBAF) which we prepared by neutralization of 10% aqueous trimethylbenzylammonium hydroxide with dilute HF:

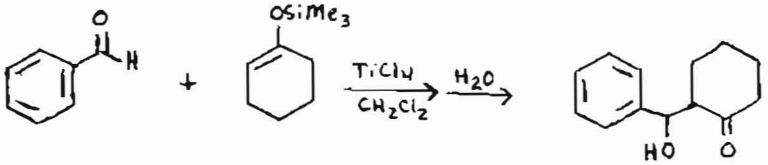
$$C_6H_5CH_2\,N^+(CH_3)_3OH^{\ominus} + HF \longrightarrow C_6H_5CH_2\,\overset{+}{N}\,(CH_3)_3\,\overset{\ominus}{F} + H_2O$$

We tested the reaction of TMBAF-ETSA with 4 at 20° and 0° under a N_2 atmosphere:

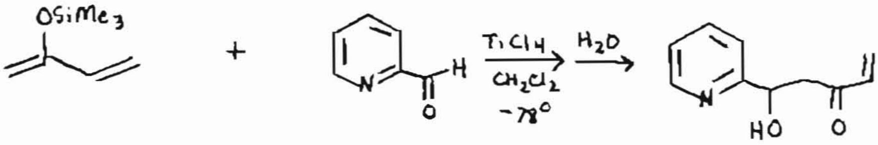
4 6

The reaction at 20° was violent. Such an exothermic reaction suggested lowering the reaction temperature. Reaction of 0°C resulted immediately in a bright yellow color. This was followed by a gradual darkening as the reaction progressed until a dark brown persisted after 30 minutes. Though time did not allow work-up of the reaction the apparent reactivity of the TMBAF-ETSA system suggests future study of this novel silylating method. This reagent is made more appealing by the discovery that tetraalkylammonium fluorides catalyze aldol condensations between silyl enol ethers and carbonyl compounds.[24] The potential for a "one-pot" synthesis involving both silylation and aldol condensation of the resultant silyl enol ether with an aldehyde is presented by TMBAF.

House's method provided sufficient quantities of pure 6 to allow us to investigate its reactivity in a crossed-aldol condensation. It was the crossed-aldol condensation method developed by Mukaiyama[12] that we initially investigated. His group's discovery that silyl enol ethers react with carbonyl compounds, including aromatic aldehydes, to give aldols was of considerable interest to us. The mild reaction conditions and the high product yields added to the attractiveness of this $TiCl_4$ promoted method. For instance, reaction at -78°C of the cyclohexanone sily enol ether with benzaldehyde produced 2-(1'-hydroxybenzyl)-1-cyclohexanone in 92% yield:



Formation of our key intermediate 3, as discussed previously, necessitated a crossed-aldol condensation between 6 and 5. Before synthesizing the triazole aldehyde 5 we decided to investigate the use of an aldehyde with a simpler heteroaromatic system. We chose pyridine-2-carboxaldehyde (12), expecting to obtain the aldol product 14:



6 12 14

Considering the mechanism proposed by Mukaiyama we postulated that the $TiCl_4$, using its empty 3d orbitals, would accept electron density from the oxygen of 12 as shown in Figure 20. This Lewis acid-base complexation should result in a weakening of the carbonyl double bond and thus impart electrophilic character on the carbonyl carbon. Such a carbon is activated for nucleophilic attack by the silyl enol ether resulting in C-C bond formation. The dissociation of the intermediate 13 should be inhibited by formation of a stable 6-membered titanium chelate. Hydrolysis of 13 should then afford the desired aldol.

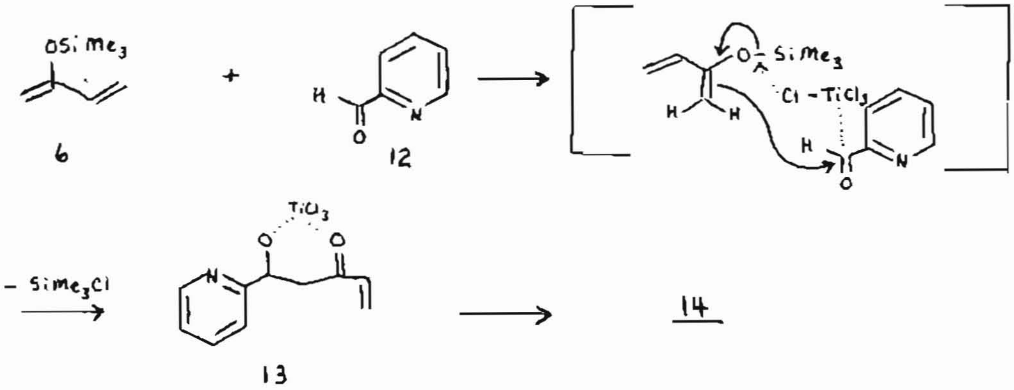
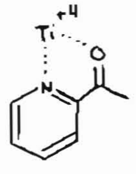


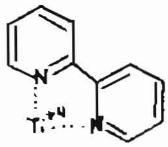
Fig. 20 - A Proposed Mechanism for $TiCl_4$ Promoted Aldol Condensation

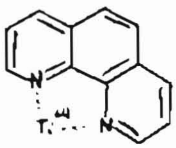
The crossed-aldol reaction was run according to the procedure of Mukaiyama. Upon addition of $TiCl_4$ to a CH_2Cl_2 solution of 12 at -78°C a dark brown solid formed. Addition of 6 resulted in no apparent change. After an hour the suspension was hydrolyzed, dissolving the solid, work-up of the solution yielded a red liquid which tlc and nmr analysis indicated to be unreacted 12. This procedure gave the same results when repeated several times.

We believe that the failure of the reaction was due to the formation of a five-members chelate ring between 12 and $TiCl_4$:



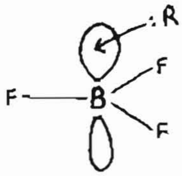
The solid formed upon addition of $TiCl_4$ to a solution of 12 was believed to be the coordination complex pictured above. Once 12 was removed from the CH_2Cl_2 solution due to chelation, 6 could not attack the carbonyl center of 12. The pyridine carboxaldehyde - $TiCl_4$ chelate is plausible since $TiCl_4$ forms adducts with a variety of bidentate organic donor molecules. There are reports of strong chelates formed by $TiCl_4$ with bipyridine and o-phenanthroline[25,26], two compounds quite similar to 12. These adducts are believed to form stable 5-membered rings:





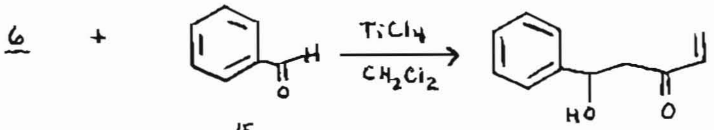
The proposed structures for the above two adducts are based primarily on infra-red spectroscopy studies. The use of the IR would be helpful in determining the nature of coordination complexes between $TiCl_4$ and 12. Coordination of the oxygen by Ti^{+4} should weaken the C=O bond of 12 resulting in a lowering of the C=O stretching frequency. Clark[25,26] observed that coordination of $TiCl_4$ to the nitrogen atoms of bipyridine shifted and split the out of plane C-H deformations. A similar situation should exist with 12. Thus if a bidentate chelate is indeed formed, both C=O stretches and C-H deformations should be shifted in comparism to free 12. We did not investigate this possibility.

If the failure of this reaction is due to the stable bidentate adduct formed between $TiCl_4$ and 12 a change of catalysts might produce better results. The Lewis acid BF_3 is unable to accept more than one electron pair from any donor molecule. Having no d orbitals, BF_3 may only utilize its one empty p orbital to accept electron density:



This negates the chance of a bidentate chelate being formed with 12. Thus BF_3, a crossed-aldol promoter[12], may prove more fruitful than $TiCl_4$ for the crossed aldol condensation of 12 and 6.

In order to test our idea about the formation of a bidentate complex and to develop confidence in working with 6 we examined the aldol reaction in a simpler system. We turned our attention to the condensation of 6 with benzaldehyde (15):

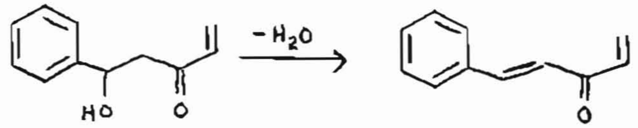


15

16

Entirely different results were obtained when benzaldehyde was substituted for 12. With no nitrogen atom in 15 to complex with the $TiCl_4$ the reaction proceeded more smoothly. Addition of $TiCl_4$ to a CH_2Cl_2 solution of 15 resulted in a yellow color with formation of a fine yellowish-orange precipitate. Addition of the enol ether 6 to the $TiCl_4$-benzaldehyde mixture turned it brown. Hydrolysis of the reaction was accompanied by the dissolution of the solid and regeneration of the yellow color. Work-up of the reaction mixture gave a reddish oil in roughly 80% yield. After washing with $NaHSO_4$ to remove unreacted 15, the oil was vigorously triturated to give a yellow powder.

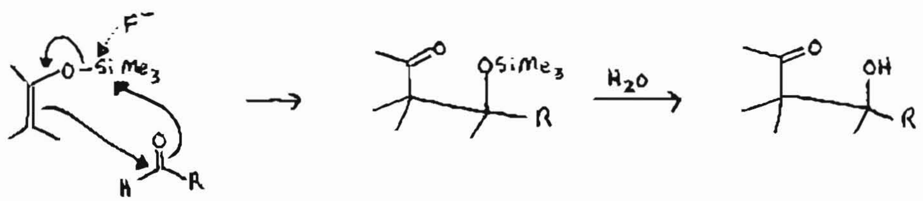
The IR spectrum of this yellow powder showed a broad band of medium intensity at 3480 cm^{-1}; indicative of an alcohol O-H stretch. Qualitative tests, both Jones oxidation and acetyl chloride tests, proved positive for alcohol functionality. The IR also indicated a band at 1700 cm^{-1} corresponding to a C=O stretch. This band was not sharp but was a broad band clearly containing two or three overlapping C=O bands. The nmr spectrum of this powder was not very conclusive. There was only the slightest evidence for the expected vinyl protons. Tlc analysis showed two large spots at R_f=0.70 and R_f=0.57. The data seemed to indicate the presence of a mixture of products. Some of the desired product 16 is probably present along with a number of other side products, among them the dehydration product of 16:



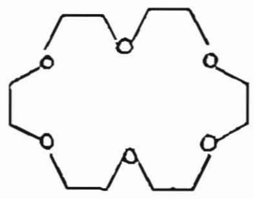
16

Various Michael addition products are probably produced; $TiCl_4$ has in-fact been shown to be a Michael addition catalyst between silyl enol ethers and α-β unsaturated ketones[27]. Also, 6 may react as a diene to form Diefs-Alder adducts as will be discussed later. Silyl enol ethers prepared from saturated ketones have been found to undergo $TiCl_4$ catalyzed crossed-aldol reactions with carbonyl compounds. However, our results have led us to question the success of this reaction when using silyl enol ethers of α-β unsaturated ketones, such as 6.

The difficulties encountered with the $TiCl_4$ promoted reactions prompted us to consider alternative reaction catalysts. We postulated that the nucleophilic fluoride anion might attack the electropositive silicon atom of the silyl enol ether of 4. This would generate a carbanion that should attack the carbonyl center of the aldehyde. The mechanism we envisioned was also proposed by Corriu[28]:

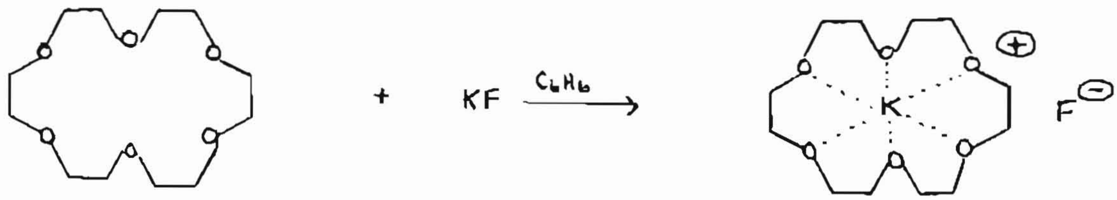


The key step in this reaction pathway is generation of the inorganic fluoride anion in an organic solvent. We anticipated that the use of crown ethers could produce the desired results. Crown ethers are macro-cyclic polyethers that have the ability to form stable molecular complexes with various metal salts. The application of crown ethers to organic syntheses has been extensively reviewed by Gokel[29]. A useful crown ether, 18-crown-6 (1,4,7,10,13,16-Hexaoxacyclooactadecane) is shown below:

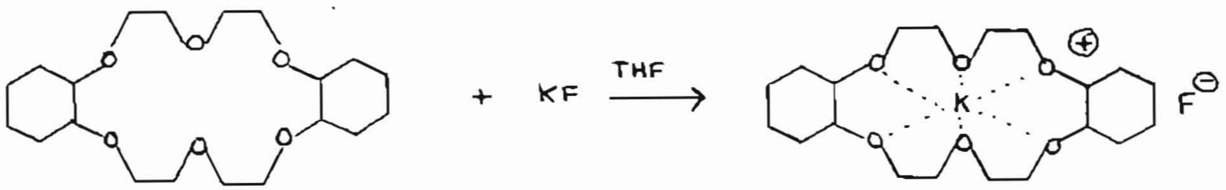


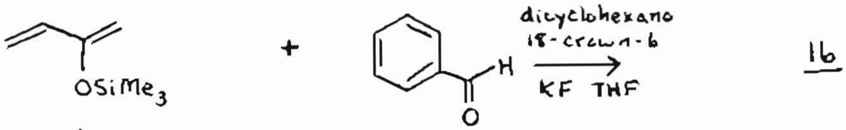
18-Crown-6

The complexation is essentially a Lewis acid-base phenomenon, the basic heteroatoms of the crown ether coordinating with a cation of the inorganic salt. The cation fits into the cavity of the crown ether. The better it fits in the cavity the more stable is the complex. The complexes are soluble in organic solvents, with the anion being relatively unsolvated and subsequently very reactive. The diameter of K^+ is almost identical to the cavity diameter of 18-crown-6. Therefore the complex of KF with 18-crown-6 results is an extremely powerful nucleophile due to the "naked" fluoride ion that is the counter-ion for the crown ether-potassium complex:



We made use of this chemistry by trying to catalyze the aldol reaction of 6 and 15 with dicyclohexano-18-crown-6 and KF:

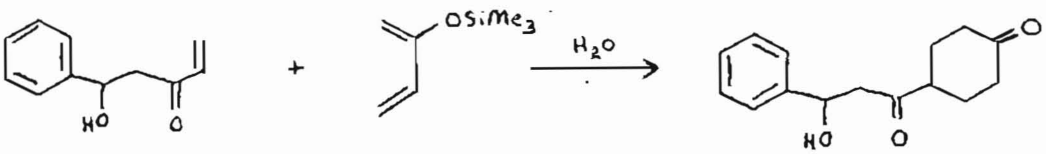




6 15

Equivalent amounts of 6, 16, and KF, along with a catalytic amount of crown ethers were refluxed for a week. The reaction, monitored by both tlc and nmr, indicated no reaction during this time. Reaction of this identical system in refluxing toluene gave similar results.

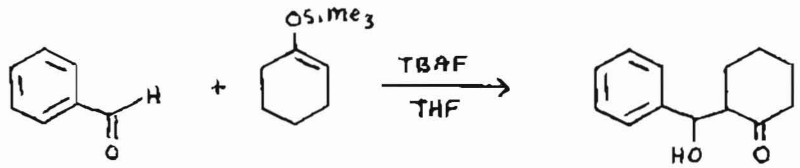
A control reaction, without the crown ether, was run in toluene. After an acidic aqueous work-up an oil was isolated, from which a scant amount of white crystals were separated. Nmr analysis of these crystals showed two types of aromatic protons in a 3:2 ratio, indicative of a mono-substituted benzene derivative. The nmr also showed evidence for the existence of cyclohexyl protons, while the ir indicated that two types of carbonyl groups and some alkene character existed. We have hypothesized that these crystals might have resulted from a Diels-Alder reaction between a molecule of the desired product 16 and 6:



16 6

Jung has found 6 to be a reactive diene in Diels-Alder reactions[30]. It is very useful in construction of substituted cyclohexanones. Conjugated ketones, such as MVK, will react with 6 to give good yields of Diels-Alder adducts. While the spectral data is fairly inconclusive, we can postulate that the reactions of 6 as a diene might conceivably result in undesired side products, further complicating the crossed-aldol condensation of 6 and 15.

Finding the crown ether catalyst unproductive in the crossed-aldol reaction, we turned our attention to another type of fluoride catalyst designed by Kuwajima[24]. His group obtained 2-(1'-hydroxybenzyl)-cyclohexanone in greater than 90% yield when tetrabutylammonium fluoride (TBAF) was used as a reaction catalyst:



TBAF, previously mentioned as a silylating agent, is also a phase-transfer catalyst and therefore soluble in organic solvents. This solubility allows the fluoride anion to attack the silicon of the silyl enol ether and initiate the proposed reaction. We used trimethylbenzylammonium fluoride (TMBAF) as an aldol catalyst instead of TBAF. Following the procedure of Kuwajima we reacted 6 and 15 in the presence of a catalytic amount of TMBAF. Although our solution turned yellow tlc monitoring did not indicate much reaction had occurred. The reaction was allowed to run overnight at room temperature before being subjected to an aqueous work-up.

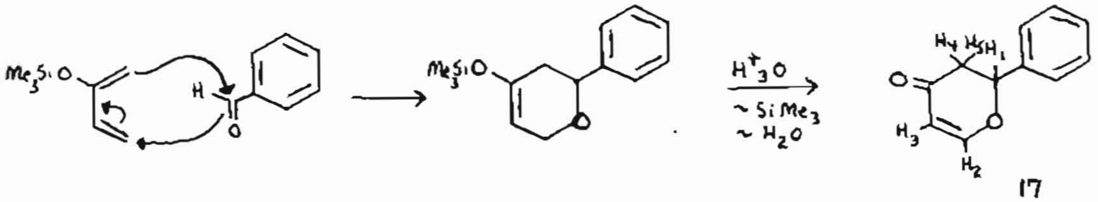
Again an oil, thought to be a mixture of aldol products, was obtained from which a small amount of white crystals were separated.

This solid had some similar spectral data to the crystals obtained from the crown ether control reaction. However the spectra were more clearly defined.while we recognize that 6 might have reacted in a Diels-Alder reaction with an alkene functionality, the spectral data has prompted us to postulate an alternative cycloaddition. Although not strictly pertinent to our study, a discussion of this proposed cycloaddition and the spectral data that supports it is of some interest. An ir of this solid indicated on α-β unsaturated ketone, aromatic ring, and the C-O group of an ether. The nmr data for this solid is summarized in Table 1.

δ(ppm)	signal	integration	#H
1.27	singlet	30	2
1.47	singlet	15	1
7.13-7.57	multiplet	83	5
8.00-8.16	doublet of doublets	32	2

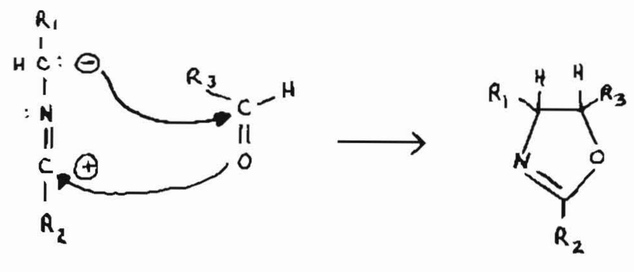
Table 1 - NMR Data for Crystals Obtained from TMBAF Catalyzed Reaction Between 6 and 15

To explain the structure of these crystals we postulated that 6 acted as a dipolarophile, initiating a cycloaddition to the C=O double bond of 15:



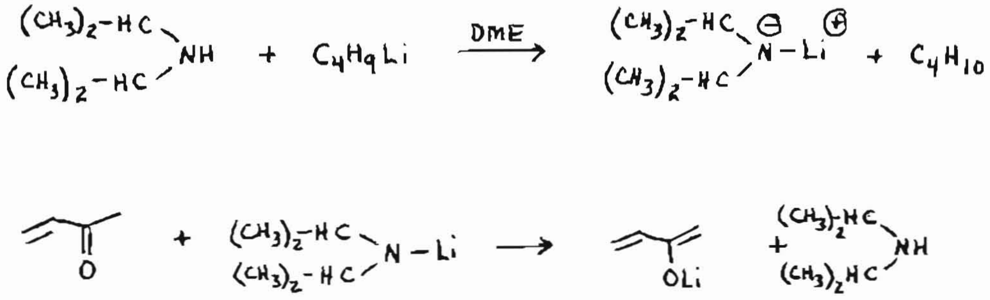
17

The proposed structure for 17 accounts for the α-β unsaturated ketone and ether functionalities indicated by the ir. The nmr signal at 7.13-7.57 account for the five aromatic protons. The signal at 8.00-8.16 ppm corresponds to the two vinyl protons, H_4 and H_5, which are shifted downfield due to conjugation with the carbonyl and proximity to the ether function. The methylene group (H_4 and H_5) and H_1 resonate at 1.27 ppm and 1.47 ppm respectively. This proposed Diels-Alder cycloaddition is certainly plausible from literature precedent. 2-Trimethylsilyloxy-1,3-butadiene has been found to attack a variety of C=C bonds. The idea that it might attack a C=O bond in a similar manner is prompted by the reactions of various dipolarophiles with carbonyls to form cyclic ethers. Huisgen for example described the addition of nitrile ylides to the C=O multiple bond of benzaldehyde to give a 4^3-oxazoline[31]:

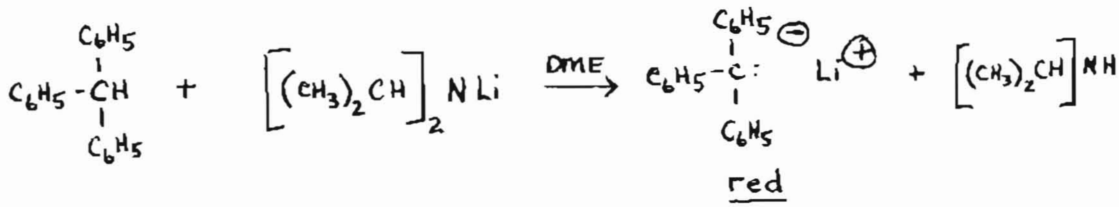


This is a reaction with some similarities to our proposed cycloaddition. Future study of the cycloaddition of 6 would constitute an interesting and possibly fruitful project.

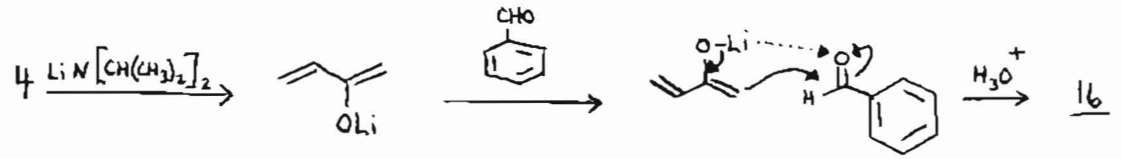
Having failed to produce 16 from the silyl enol ether 6 we decided to investigate another method for producing the desired aldol. We attempted to condense MVK directly with benzaldehyde by using the very strong base lithium diisopropylamide. The reagent, formed from n-butyllithium and diisopropylamine, was designed to permit quantitative enolate formation and prevention of side reactions[32]:



This base has its advantages in that it is soluble in THF, DME and ethers and its presence may be detected by the red color of the triphenyl methide anion formed by proton abstraction from the triphenylmethane indicator used in these reactions:

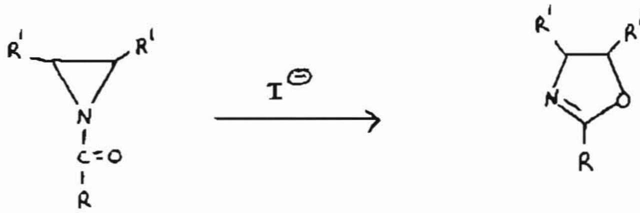


We reacted diisopropylamide with MVK in THF to form the enolate which we postulated should then add to benzaldehyde:

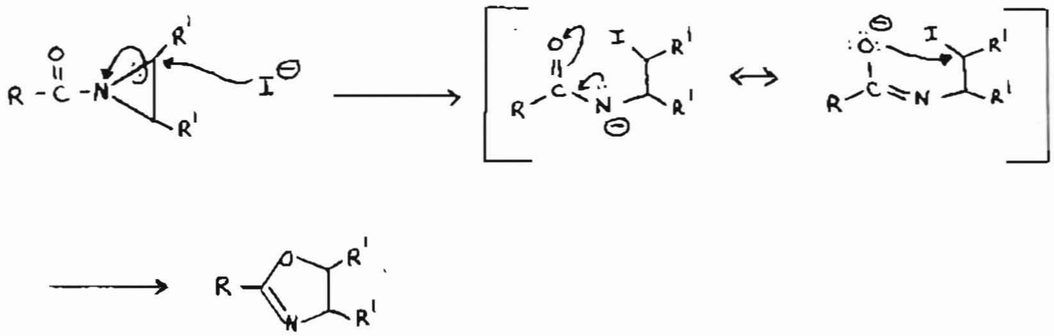


This reaction gave a golden oil in about 80% yield. Tlc, ir, and nmr analysis of this oil indicated it to very similar to the yellow powder from the $TiCl_4$ promoted reaction. In an effort to separate some of the number of reaction products we used high-pressure liquid chromatography (hplc) techniques. Hplc indicated that this oil was a mixture of at least four compounds. Preparative hplc allowed us to obtain a UV spectrum of the first, and major, component of the mixture. The UV spectrum was sufficiently informative to suggest that hplc techniques are of potential utility in separating components from the oil obtained using lithium diisopropylamide (see Appendix). The difficulties in separating the many apparent reaction products from our crossed-aldol condensation prompted us to discard our initial synthetic route and adopt a new approach to the synthesis of C-nucleosides.

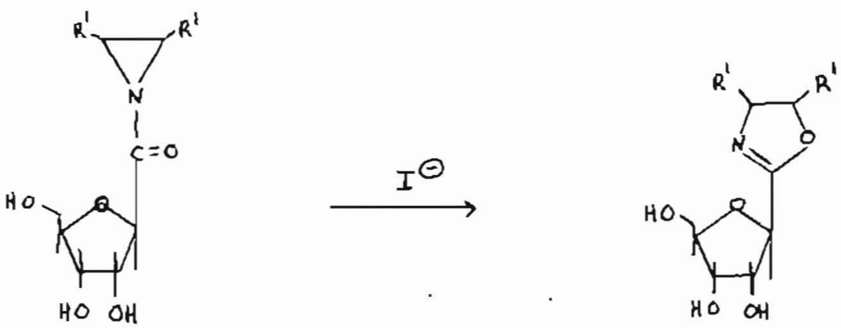
Our second approach to the synthesis of C-nucleosides differed from our initial route in that we planned to elaborate the nucleoside's heterocyclic unit from a pre-formed sugar instead of elaborating the sugar moiety from a pre-formed heterocycle. We patterned our approach on chemistry developed by Heine-Heine found that iodide ion catalyzes the isomerization of 1-acylaziridenes to oxazolines[33]:



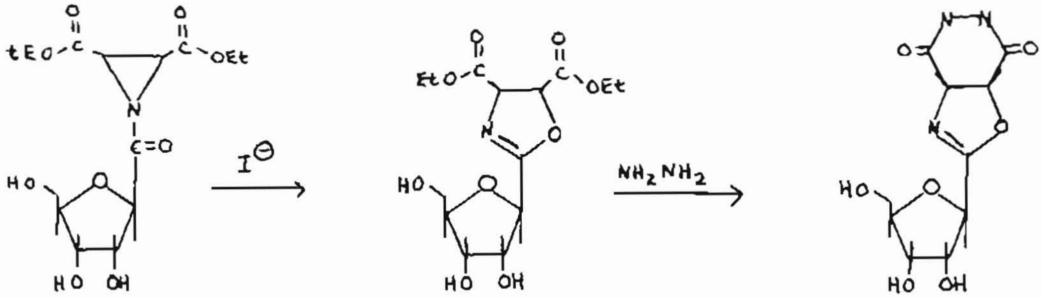
He proposed that the iodide ion attacks one of the carbon atoms of the aziridine ring to generate an ambident anion. This ion undergoes an intramolecular substitution reaction at the carbon initially attacked by iodide ion to produce the oxazoline:



If the R substituent on the carbonyl group of the aziridine is a sugar, then a C-nucleoside analog should be produced from this isomerization:

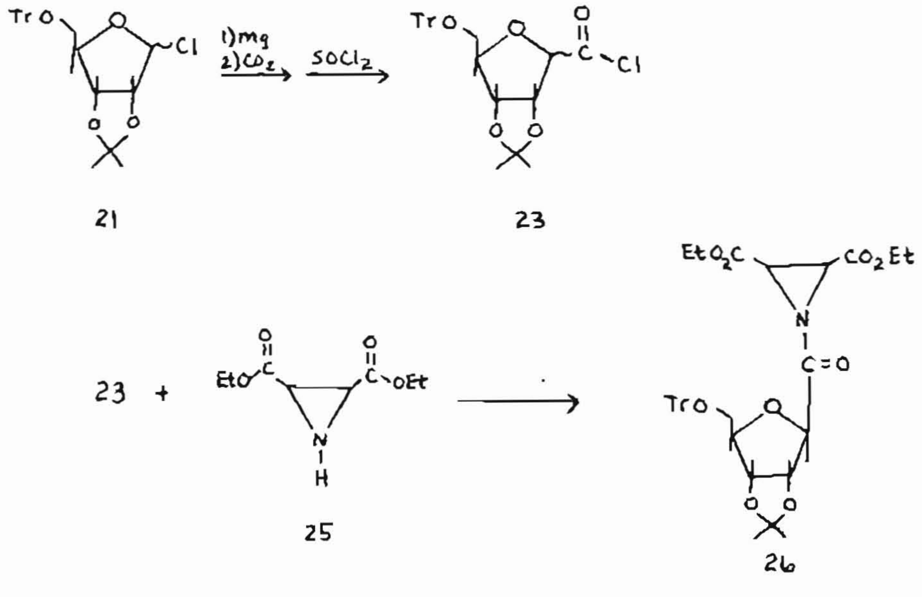


An attractive aspect of this proposed route is that the substituents on the aziridine ring allow the construction of a second heterocyclic system. A specific example is shown below:



Generation of this second heterocycle yields analogs of purine based C-nucleosides. While there are many strategies to pyrimidine C-nucleosides, Fox has noted that the routes to purine C-nucleosides are presently scarce[7]. We anticipated that our proposed route might act to fill the present void that exists in the methodology of purine C-nucleoside synthesis.

The key step in this proposed route is the preparation of the substituted 1-acylaziridine. These compounds are easily made from aziridines and acid chlorides[34]. We envisioned the synthetic route to the C-nucleosides shown in Figure 21:



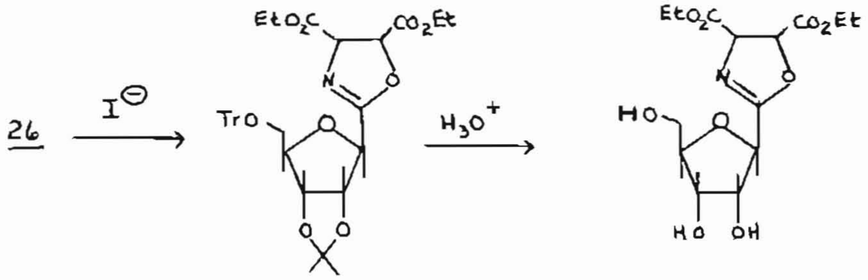
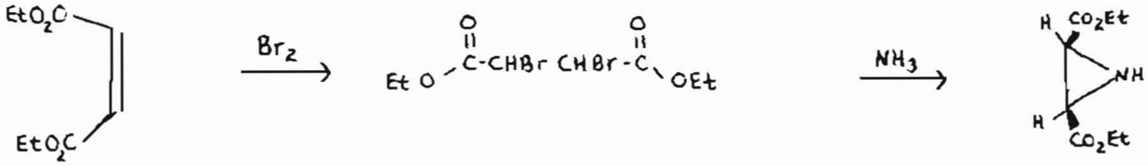


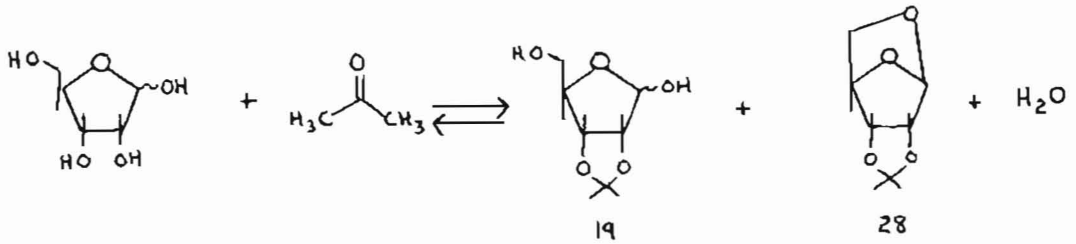
Fig. 21 - An Alternative Route to C-Nucleosides

We expected that the easily prepared, protected chloro sugar 21[35], could form a Grignard which would then subsequently be used to make the carboxylic acid. Using standard chemistry, this acid could be derivatized to its acid chloride[36]. It was this sequence of reactions that we were primarily concerned with, for once we had formed 23 from 21 we expected it to react smoothly with 25 to give the requisite 1-acylaziridine. The preparation of the substituted aziridine 25 is straightforward[37]:



The formation of the appropriate protected chloro sugar, with the chlorine at C-1', necessitated a series of three synthetic steps. The first step involved the isopropylidenation of ribose by the acid catalyzed addition of acetone to the hydroxyl groups on C-2 and C-3. We utilized the basic procedure of Levene[38] to effect this reaction in about 75% yield. We did however make a few minor but important experimental modifications that improve the efficiency of this reaction.

Following the suggestion of Klein[39] we substituted p-toluenesulfonic acid for Levene's $CuSO_4$-H_2SO_4 mixture as a reaction catalyst. Klein noted that use of p-toluenesulfonic acid avoids the formation of a major side product, 1,5-anhydro-2,3-di-O-isopropylidene ribofuranose (28):

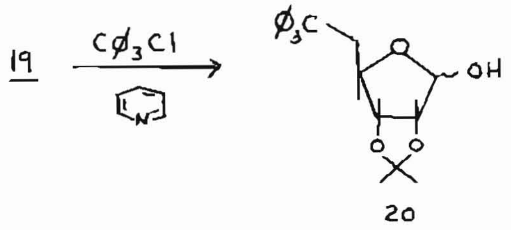


Marrs and Newton[40] had indeed found that 28 contaminated the yield of di-2,3-O-isopropylidene-D-ribofuranose (19) when $CuSO_4$ was used. Tlc analysis indicated that we were successful in suppressing formation of 28 by using p-toluenesulfonic acid.

Another modification of the reaction procedure involved the use of molecular sieves as a reaction dessicant. We added a few scoops of 4A° molecular sieves to the reaction mixture of ribose and acetone. The sieves, by capturing any water present, should drive the reaction to the right, towards production of 19. Apparently the molecular sieves had the desired effect since tlc indicated that the reaction was complete in 1.5 hours, as compared to the six hours required by Saulnier[41] when using the identical procedure except without the molecular sieves.

The third modification involved the neutralization of the reaction mixture with solid $NaHCO_3$. This modification, suggested by Saulnier[41], was designed to avoid the formation of a yellow syrup when the usual 10% $NaHCO_3$-90% NaOH system of Klein was used. Neutralization with solid $NaHCO_3$ produced a colorless syrup. The syrup contained the desired product 19 and a residual amount of acetone. NMR analysis indicated that even prolonged rotary evaporation at reduced pressure could not decrease the residual acetone below 14%. Tlc analysis of the syrup showed two spots. One spot, the faster moving and larger spot, had an R_f of 0.57 while the smaller spot had an R_f of 0.35. Fox[42] has suggested that these two spots correspond to the two anomers of 19, with the β-anomer corresponding to the larger spot while the smaller, slower spot was due to the α-anomer.

Crude 19 was used directly for the subsequent tritylation reaction; a reaction designed to protect the hydroxyl group on C-5 of 19:



The mechanism postulated for the tritylation is pictured in Figure 22.

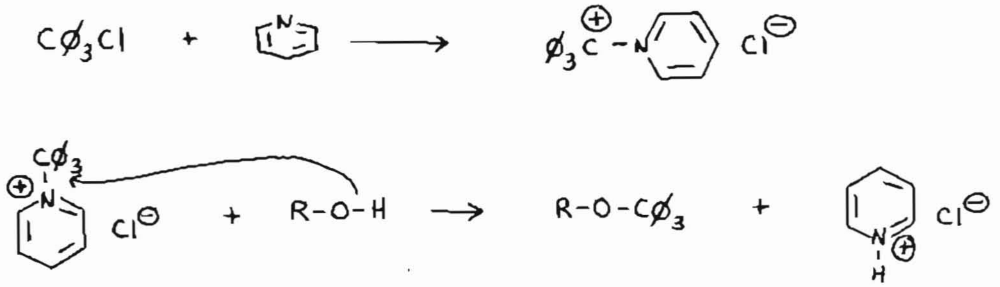
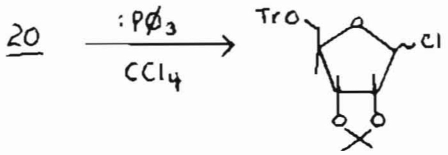


Fig. 22 - A Proposed Mechanism for the Tritylation Reaction

The pyridine, which must be scrupulously dry, is thought to complex with the trityl chloride. The oxygen on an alcohol may then react with the electropositive carbon of the trityl group to form the tritylated product and pyridine hydrochloride.

We followed the exact procedure of Klein[35] in synthesizing 20. After the work-up we obtained a syrup from which some solid crystallized during rotary evaporation. Both the oil and the solid were determined by nmr analysis to be 20. The preparation of 20 in crystalline form has not been reported previously.

Production of the chloro sugar, 21, from 20 was then undertaken:



The reaction, using triphenylphosphine and anhydrous CCl_4, has been postulated to have the following mechanism[45]:

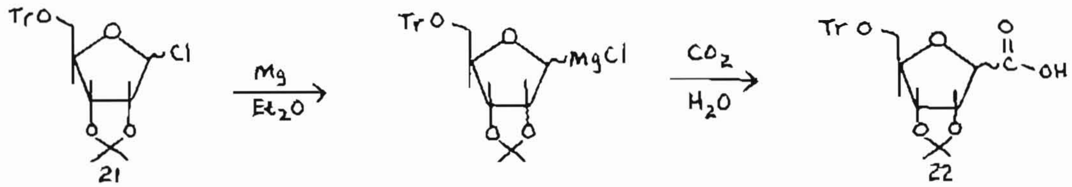
$$\phi_3P + CCl_4 \longrightarrow [\phi_3P\text{-}CCl_3]^{\oplus} Cl^{\ominus} \xrightarrow{ROH} [\phi_3P{=}OR]^{\oplus} Cl^{\ominus}$$

$$\longrightarrow \phi_3P{=}O + RCl + CHCl_3$$

The generation of triphenylphosphine oxide along with the desired chloro compound is a complicating factor in the reaction. The crude syrup obtained from the reaction contained both 21 and the undesired oxide. By dissolving the syrup in a pet ether - ether mixture and passing the solution through a chromatography column filled with silica gel the triphenylphosphine oxide was efficiently removed. Our only modification of the published procedure involved the use of acetonitrile (CH_3CN) as a solvent in lieu of DMF. This was suggested by Klein[39] because he found CH_3CN easier to use. Unlike DMF acetonitrile does not require aqueous work-up; a work-up which results in unavoidable loss of material. Instead CH_3CN may be simply evaporated to yield 21 directly.

Using this modified procedure we obtained 15 g of crystalline 21, a sufficient quantity for further work. This chloro sugar decomposes to 28 quite readily. Thus it is necessary to keep the chloro sugar in cold, dark and dry conditions, especially when stored for extended periods of time.

Having made an ample supply of the protected chloro sugar we attempted to make the carboxylic acid 22. From 22 we expected to obtain the acid chloride 23, from which our acylaziridine would be generated. We felt that if we could form a Grignard of 21 addition of CO_2 would then yield 22.

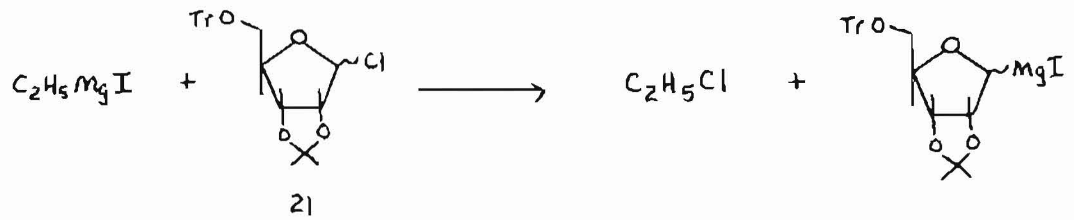


The essential requirement for our synthetic approach then became the preparation of the Grignard of 21. Our major concern in this reaction was whether the Grignard, if formed, would react to open either the ribose ring or the isopropylidene ring. Since ethers are very stable towards Grignards we hoped that ring opening side reactions would not cause problems.

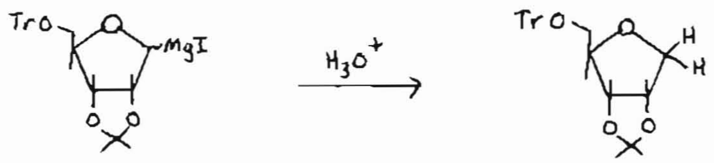
Our initial attempts in forming the Grignard involved preparing a solution of 21 in ether and adding it dropwise to an ethereal suspension of magnesium metal. We observed no apparent reaction. Various attempts to initiate this reaction failed. We tried crushing the magnesium, adding I_2 as a catalyst, adding CH_3I in hopes of prompting some organometallic reaction, refluxing and stirring the solution with vigor. All of these attempts to initiate reaction proved fruitless as the magnesium remained unconsumed in the reaction flask and tlc indicated the chloro sugar had not reacted.

We then attempted the Grignard formation with two major modifications. We substituted tetrahydrofuran for ether as a solvent and we used Baeyer activated magnesium[43]. Using these modifications we again failed to form the Grignard from 21. The magnesium remained unreacted and tlc indicated no reaction of the chlorosugar.

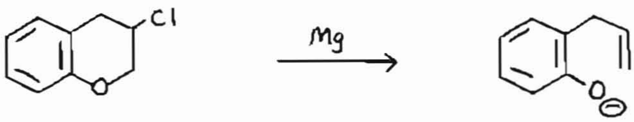
Having failed to initiate formation of the Grignard by conventional means we then attempted to form the Grignard of 21 via a process known as entrainment[44]. Entrainment consists of treating a halide that does not yield a Grignard directly with an equivalent of a preformed Grignard reagent from a reactive halide. We hoped that the Grignard of ethyl iodide would react with 21 as shown below:



We made the Grignard of ethyl iodide and added it to an equivalent of 21. There was a visible reaction as the reaction mixture turned a bright yellow, with a white precipitate and gas bubbles also forming. We then subjected this mixture to an aqueous work-up, expecting to protonate the Grignard reagent of 21 at C-1:

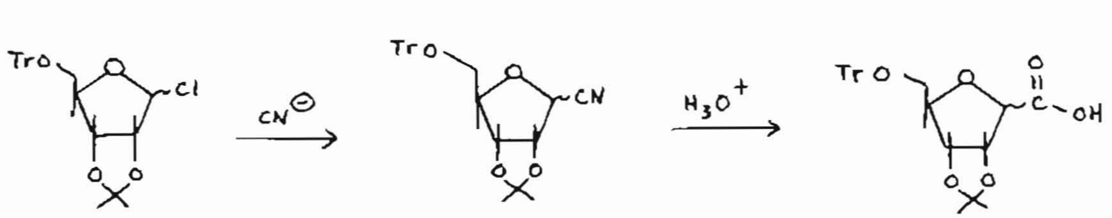


NMR studies of the products showed them to lack the anomeric hydrogen at C-1, indicating that there might indeed be two equivalent protons attached to C-1. However nmr data also indicated that a ring opening reaction had occurred. There are numerous examples of Grignard reagents acting to cleave cyclic ethers[45]:



Tlc analysis indicated that three major products had been formed. None of these were characterized.

Having decided that 21 was not a suitable chloride for preparation of Grignards we envisioned an alternative method for formation of the carboxylic acid. This involved the introduction of a nitrile group to C-1 in place of the chloride and the subsequent hydrolysis of the cyano compound to the carboxylic acid:



21 22

Similar chemistry, utilized by Bobek and Farkas[46], has proven successful in preparing carboxylic acid derivatives of sugars for nucleoside synthesis.

Presently it is this reaction that merits attention as we search for new synthetic routes to C-nucleosides.

II. Experimental

A. General Procedures

Infra-red spectra were obtained with a Perkin-Elmer 137 infrared spectrophotometer using a polystyrene reference. Liquids were examined neat between salt plates. Solids were run in KBr pellets or in CCl_4 solution. Nuclear magnetic resonance spectra were obtained using a 60 MHz Varian EM 360 L spectrophotometer. Liquids were examined neat with an internal tetramethylsilane reference. Solids and oils were dissolved in NMR grade CCl_4 or $CDCl_3$ with tetramethylsilane as an internal reference. Ultraviolet spectra were recorded in 1 cm quartz cells using a Perkin-Elmer Hitachi 200 spectrophotometer with a deuterium lamp. High pressure liquid chromatography was done on a Beckman Model 110A chromatograph using a C-18 reversed phase column and a 254 nm ultra-violet detector.

All work requiring dry conditions was done in either nitrogen or helium swept reaction vessels. These reaction vessels were either flame-dried under a stream of nitrogen and flushed with nitrogen until cool, or dried overnight at 110° and assembled hot under a positive pressure of nitrogen and allowed to cool while flushing with nitrogen. Transfer of liquid reagents was done using gas-tight syringes equipped with Luer tips and stainless steel cannulae. Transfer of air sensitive reagents such as n-butyl lithium was done with 5 ft. 18 or 20 gauge double-ended needles. Transfer techniques were done using procedures described by Lane and Kramer[47].

Methylene chloride was dried overnight with $CaCl_2$, filtered and distilled. Trimethylchlorosilane was distilled prior to use, and stored over 4A° molecular sieves. Dimethoxyethane and diisopropylamine were both dried overnight with NaH, distilled from the NaH, and stored over 4A° molecular sieves. Pyridine was dried overnight with KOH, refluxed for two hours over fresh KOH, and then distilled. Acetonitrile was dried overnight with 4A° molecular sieves, stirred with NaH for 24 hours, distilled from NaH, and stored over 4A° molecular sieves. Tetrahydrofuran was dried over $MgSO_4$ for 72 hours, distilled, refluxed over lithium aluminum hydride for two hours, distilled from lithium aluminum hydride and stored over 4A° molecular sieves. Titanium chloride, anhydrous ether and dimethyl formamide were used as received.

2-Trimethylsilyoxy-1,3-butadiene and 2,3-di-O-isopropylidene-5-O-trityl-β-D-ribofuranosyl chloride were both stored in serum stoppered bottles in a refrigerated dessicator so as to minimize decomposition.

B. Synthetic Procedures

1. Preparation of 2-Trimethylsilyoxy-1,3-Butadiene (6)[20]

An oven dried 500 ml 3-necked flask was equipped with two addition funnels, a reflux condenser, a thermometer, a nitrogen inlet and a magnetic stirrer. Under an atmosphere of nitrogen was added 100 ml of dry dimethyformamide and 43.36 g (400 mmol) of freshly distilled triethylamine, followed by a solution of 25.10 g (350 mmol) of methyl vinyl ketone in 25 ml DMF. A solution of 43.36 g (400 mmol) of dry chlorotrimethylsilane in 25 ml of ? was then added dropwise over a 30 minute period. During this time the temperature of the reaction mixture rose from 20°C to 44°C, and the mixture turned brown. After one hour of stirring at room temperature the reaction was refluxed for 17 hours.

After cooling, the reaction mixture was filtered directly into a two liter seperatory funnel. A 300 ml portion of pentane was added to the seperatory funnel. The resulting DMF-pentane mixture was shaken vigorously. One liter of cold 5% $NaHCO_3$ solution was added to the seperatory funnel. The resulting mixture was gently swirled for 10 seconds to allow the DMF to be extracted into the aqueous phase. The bottom, darker, aqueous phase was drawn off and extracted twice with 150 ml portions of pentane. The pentane extracts were combined, washed briefly with 100 ml of cold H_2O, dried with $MgSO_4$, filtered, and concentrated by rotary evaporation. Distillation at reduced pressure yielded 16.89 g (33%) of 6 as a colorless or slightly yellow liquid, b.p.=47-54°C (50 mm).

NMR (Spectrum #1)

δ(ppm)=0.19 (9H,S,CH_3), 4.28 (2H,broads,Ha), 5.00 (1H,doublet of m,Hc), 5.42 (1H,doublet of d,Hd,J_{B-D}=16 Hz,J_{D-C}=3 Hz), 6.20 (1H,doublet of d,H_B, J_{B-C}=16 H_z)

ir (neat) = 3080 cm^{-1} (alkene C-H stretch, 1586 cm^{-1} (C=C stretch), 1300 cm^{-1} (C-O stretch), 1250 cm^{-1} (C-Si strethc), 1050^{-1} (Si-O stretch)

A higher boiling fraction, obtained over a boiling point range of 100-180°C (50 mm), yielded 20.5 g of a yellow liquid, as yet uncharacterized.

2. Attempted Crossed Aldol Reaction Between 6 and Benzaldehyde[12]

A dry 50 ml round bottomed flask was fitted with an addition funnel and a nitrogen inlet. Under an atmosphere of N_2 was added a dry methylene chloride solution (20 ml) of 0.70 g (0.63 mmol) of benzaldehyde. To this solution at -78°C was added 0.60 ml (0.63 mmol) of $TiCl_4$ via syringe. The mixture turned bright yellow with formation of a fine yellow precipitate. A methylene chloride solution (10 ml) of 0.95 g (0.63 mmol) of 6 was added dropwise. The reaction mixture, which changed from yellow to orange-brown, was stirred for one hour at -78°C.

After the requisite reaction time the mixture was hydrolyzed by addition of 5% $NaHCO_3$ solution until the solution appeared neutral to pH paper. During the neutralization a white solid, presumably TiO_2, precipitated from the solution. After neutralization the organic phase was extracted with 30 ml of ether. The yellow ethereal solution was washed with 15 ml of H_2O, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to yield 1.44 g of a yellow oil.

Tlc analysis (ethyl acetate) of this oil indicated two major spots with R_f= 0.70 and R_f = 0.57. Unreacted benzaldehyde, which could be detected by nmr, was removed by washing an ethereal solution of the oil with a saturated aqueous solution of $NaHSO_4$. The solution was dried over $MgSO_4$, filtered, and concentrated to give an oil which after extensive and vigorous trituration yielded 0.50 g of a yellow powder (m.p. 50-60°C). This yellow powder tested positively for both the Jones oxidation and the acetyl chloride tests for alcohol functions.

ir (CCl_4) = 3400 cm^{-1} (O-H stretch), 1700 cm^{-1} (C=O stretch of a ketone)

nmr - The yellow powder gave poor spectra. Aromatics predominate at δ(ppm) = 7.25, other prominant peaks were a multiplet between 2.7-3.1 ppm and a singlet at 0.70 ppm.

3. Preparation of Trimethylbenzylammonium Fluroide[23]

In a plastic container 500 ml of 10% aqueous trimethylbenzylammonium hydroxide was neutralized by slow addition of dilute HF solution. The resultant cloudy solution was rotary evaporated on the steam bath to give a copious amount of a white, hygroscopic solid, trimethylbenzylammonium hydroxide. This mass dried by rotary evaporation at 7 mm for 24 hours, pulverized into a fine powder, and then rotary evaporated for 48 hours at 1 mm. The dry salt was stored in a stoppered, nitrogen evacuated flask. This flask was fitted with a $CaCl_2$ drying tube.

4. Preparation of 2,3-O- Isopropylidene-D-ribofuranose (19)[38]

To a 500 ml round bottomed flask containing 400 ml of freshly opened spectroquality acetone was added a few scoops of 4A° molecular sieves and 20.0 g (130 mmol) of D-ribose. This suspension was treated with 2.5 (13 mmol) of p-toluenesulfonic acid monohydrate with rapid mechanical stirring. After 1.5 hours the original thick suspension had become a slightly yellow homogeneous solution. A tlc of the solution (1:9, CH_3OH-$CHCl_3$) indicated two spots with R_f=0.57 and R_f=0.35. As noted in the discussion the larger and faster moving spot has been attributed to the β-anomer of the isopropylidene-D-ribofuranose, while the α-anomer corresponds to the smaller spot. The tlc indicated no ribose (R_f=0.11) remained after 1.5 hours.

The solution was made neutral to pH paper by the addition of solid $NaHCO_3$. The mixture was stirred vigourously during the neutralization. The neutral solution was dried with $MgSO_4$, filtered thru a glass wool plug, and concentrated to a thick colorless syrup on the rotary evaporator at a temperature below 40°C. After 24 hours at 1 mm Mg 18.0 g of syrup, containing 14.3% acetone, was obtained.

NMR (Spectrum #3)

δ(ppm) = 1.27 (3H,S,CH_3), 1.43 (3H,S,CH_3), 3.65 (2H,d,2H_5), 4.30 (1H,S,OH), 4.47-4.87 (4H,m,H_4,H_3,H_2,OH), 5.41 (1H,S,H_1)

5. Preparation of 2,3-O-Isopropylidene-5-O-trityl-D-ribofuranose (20)[44]

The crude 19 (18.0 g, 90 mmol) was dissolved in 40 ml of dry pyridine in a 100 ml round bottomed flask fitted with a $CaCl_2$ drying tube and a magnetic stirring bar. Addition of 28.0 g (100 mmol) of triphenylchloromethane to the stirred mixture caused the colorless solution to become orange-brown. After 5 minutes at room temperature tlc analysis (5:1, ethyl acetate-benzene) indicated two spots with R_f=0.51 and R_f=0.06. The slower moving spot was due to unreacted starting material. The reaction mixture was stirred overnight at room temperature.

After 24 hours the reaction was quenched by pouring the suspension into 500 ml of vigorously stirred cold distilled H_2O in a 500 ml round bottomed flask. The aqueous phase was decanted from the viscous yellow syrup. The syrup was washed with 100 ml of H_2O and dissolved in 300 ml of CH_2Cl_2. The CH_2Cl_2 solution was shaken with a solution of 50.0 g of $CdCl_2 \cdot 2H_2O$ in 400 ml of H_2O in a 1-liter separatory funnel. An emulsion formed. The bottom organic layer was filtered into a 500 ml Erlenmeyer flask. The organic phase was dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to yield 37.12 g of crude yellow syrup: the desired product contaminated with CH_2Cl_2 and pyridine. NMR data for syrup (Spectrum #4):

δ(ppm) = 1.31 (3H,S,CH_3), 1.50 (3H,S,CH_3), 3.35 (2H,d,2H_5), 3.72 (1H, broads, OH), 4.10-4.40 (1H,m,H_4), 4.65 (2H,m,H_2,H_3), 5.34 (1H,d,H_1), 5.67 (d,H,α-isomer), 7.30 (15H,m,aromatic)

During rotary evaporation of the syrup about 0.50 g of material crystallized in the neck of the evaporator. This white powder had a melting point of 40-45°. Tlc analysis (5:1, ethyl acetate-benzene) of the solid indicated a single spot of R_f=0.64.

NMR (Spectrum #4)

δ(ppm) = 1.33 (3H,S,CH_3), 1.50 (3H,S,CH_3), 3.35 (2H,d,$2H_5$), 3.80-4.40 (2H,m,H_4,OH), 4.65 (2H,m,H_2,H_3), 5.34 (1H,d,H_1, β-isomer), 5.67 (d,H_1, α-isomer), 7.30 (15H,m,aromatic). NMR analysis indicated that α/β ratio was 1.8/6.5 = 0.25

IR (Spectrum #)

λ(microns) = 2.93, 3.30, 3.41, 5.02, 5.17, 6.67, 6.88, 7.20, 8.20, 8.70, 10.00, 11.18, 11.55

6. Preparation of 2,3-O-Isopropylidene-5-O-trityl-β-D-ribofuranosyl chloride (21)[44]

A 250 ml round bottomed flask was charged with 37.0 g (84.4 mmol) of crude (20), 65 ml of freshly distilled dry acetonitrile, and 40 ml (402 mmol) of spectroquality CCl_4. This solution was magnetically stirred while 22.10 g (84 mmol) of triphenylphosphine was slowly added. After about 10 minutes at room temperature the reaction mixture had become deep yellow and the reaction became quite warm. A tlc (20:1, pet ether-ether) indicated two spots with R_f=0.57 and R_f=0.20. After 2 hours only one spot appeared with R_f=0.31.

The acetonitrile was removed by vacumn distillation at room temperature to give a yellow syrup which was dissolved in a mixture of 70 ml of diethyl ether and 70 ml of 30-60° petroleum ether. The resultant solution was allowed to sit overnight and then filtered from the insoluble triphenylphosphine oxide. The filtrate was concentrated to 150 ml. During concentration 11.00 g of a white crystalline solid (m.p.=105-107°) precipitated. The solution was decanted from the solid and filtered by gravity through a column of 60-200 mesh Davison silica gel. The chromatographed solution was concentrated to give 7.23 g of a yellow syrup which was dissolved in 10 ml of pet ether. After sitting overnight 4.01 g of white solid (m.p.=96-106°C) crystallized. The total yield of (21) was 15.01 g (34.2 mmol), 26.3% from D-ribose.

NMR (Spectrum #5)

(ppm) = 1.27 (3H,S,CH_3), 1.40 (3H,S,CH_3), 3.40 (2H,m,$2H_5$), 4.63 (2H,m,H_3,H_4), 4.80 (1H,d,$J_{2,3}$=6.0 Hz,H_2), 6.03 (1H,S,H_1), 7.30 (15H, m,aromatic)

Appendix

NMR Spectra

1. 2-trimethylsilyloxy-1,3-butadiene
2. Reaction Product from TMBAF catalyzed reaction between 2-trimethyl-silyoxy-1,3-butadiene and benzaldehyde
3. 2,3-di-O-isopropylidene-D-ribofuranose
4. 2,3-di-O-isopropylidene-5-O-trityl-D-ribofuranose
5. 2,3-di-O-isopropylidene-5-O-trityl-D-ribofuranose chloride

IR Spectra

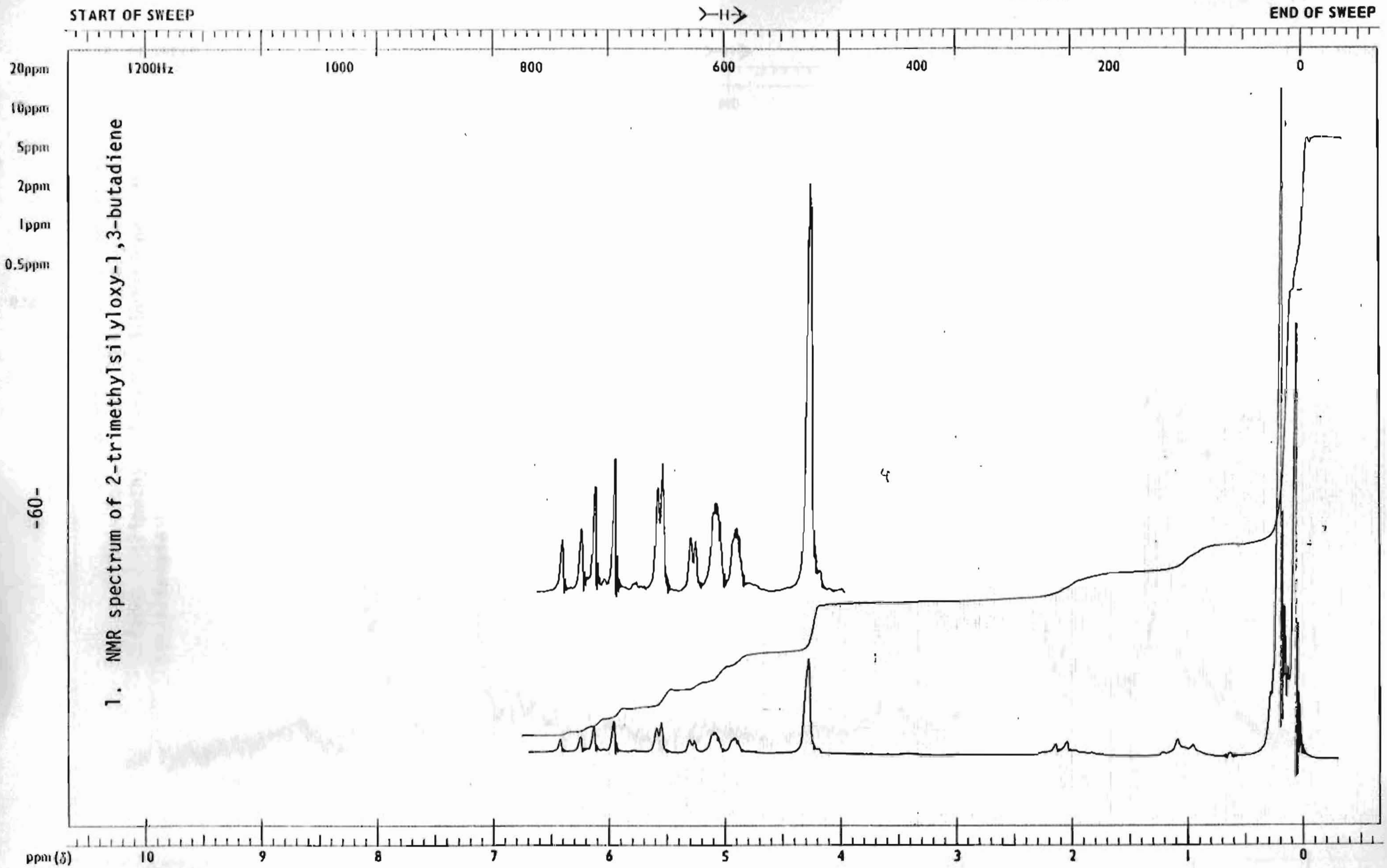
6. 2-trimethylsilyloxy 1,3-butadiene
7. TMBAF catalyzed reaction product between (6) and (15)

UV Spectra

8. Fraction 1 - Reaction between (4) and (15), $LiN(CH(CH_2)_2)_2$ as base

HPLC Chromatogram

9. Product Mixture - Reaction between (4) and (15), $LiN(CH(CH_3)_2)_2$ as base



1. NMR spectrum of 2-trimethylsilyloxy-1,3-butadiene

SPECTRUM AMPL. 100/2

FILTER .05 sec

RF POWER .02 mG

SWEEP TIME 5 min.

SWEEP WIDTH 10 ppm or Hz

END OF SWEEP 0 ppm or Hz

SAMPLE: 2-Trimethylsilyloyy-1,3-butadiene

SOLVENT: Neat

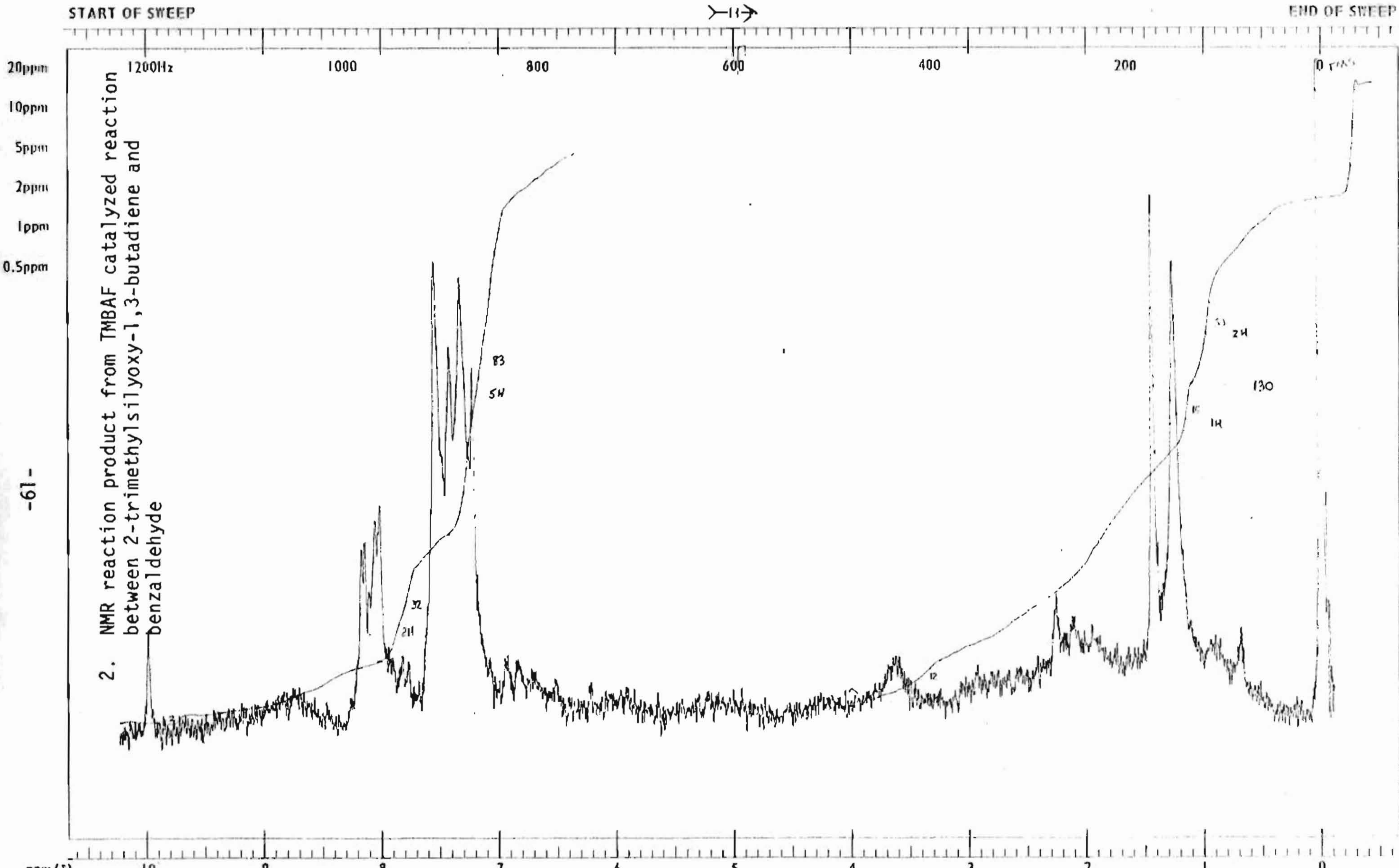
REMARKS: Peaks at 2.0 and 1.0 are side bands. Peak at 0.1 is TMS Internal reference

OPERATOR Jeff Davis

DATE 1/15/81

SPECTRUM NO.

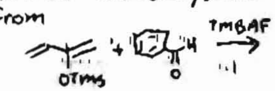
2. NMR reaction product from TMBAF catalyzed reaction between 2-trimethylsilyoxy-1,3-butadiene and benzaldehyde



SPECTRUM AMPL. 1000/10

SWEEP TIME 5 min.

SAMPLE: White crystals from



REMARKS:

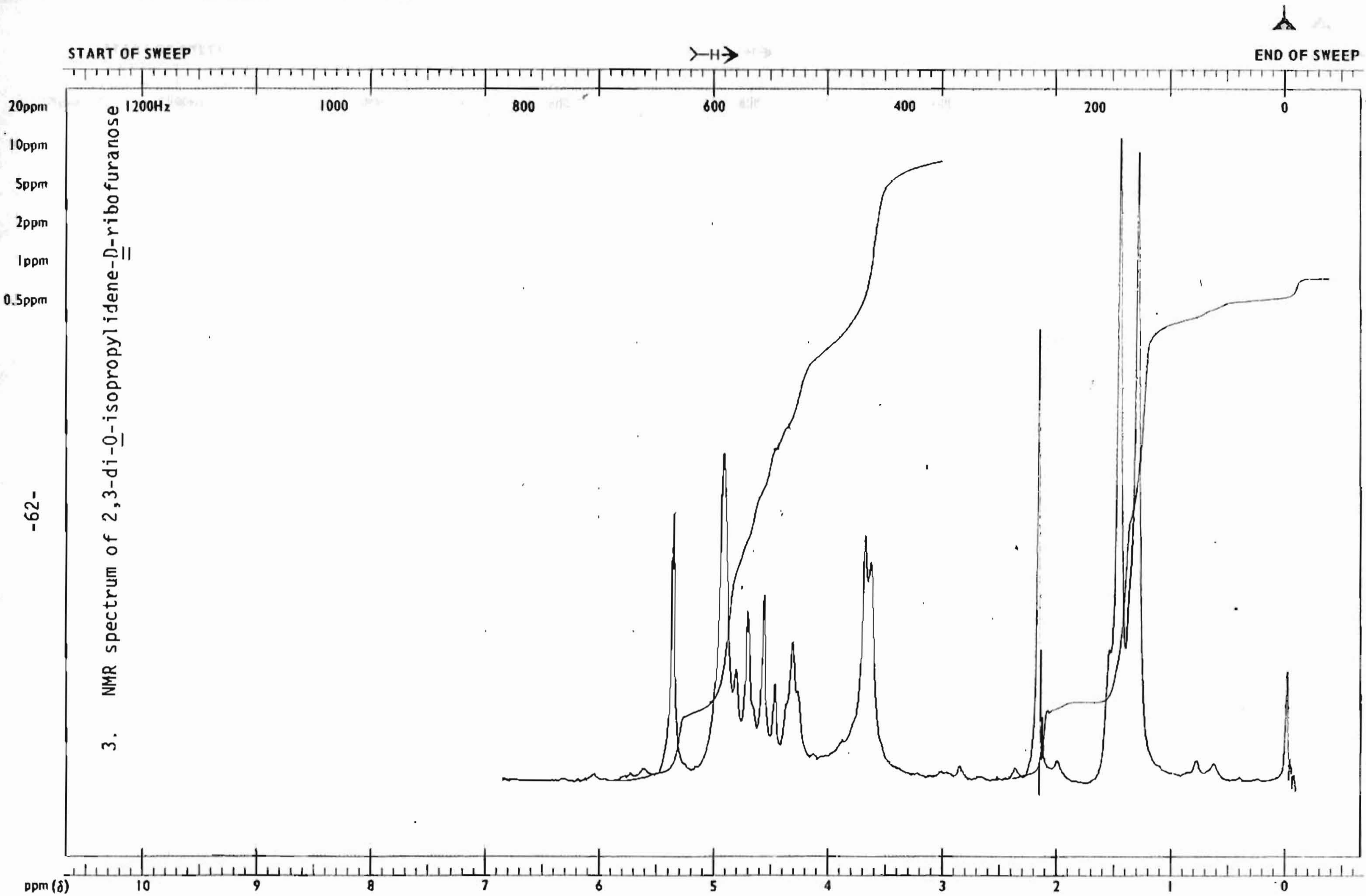
OPERATOR J. Davis

FILTER .05 sec

SWEEP WIDTH 10 ppm or Hz

DATE 2/5/81

Phone: (609) 687-0020

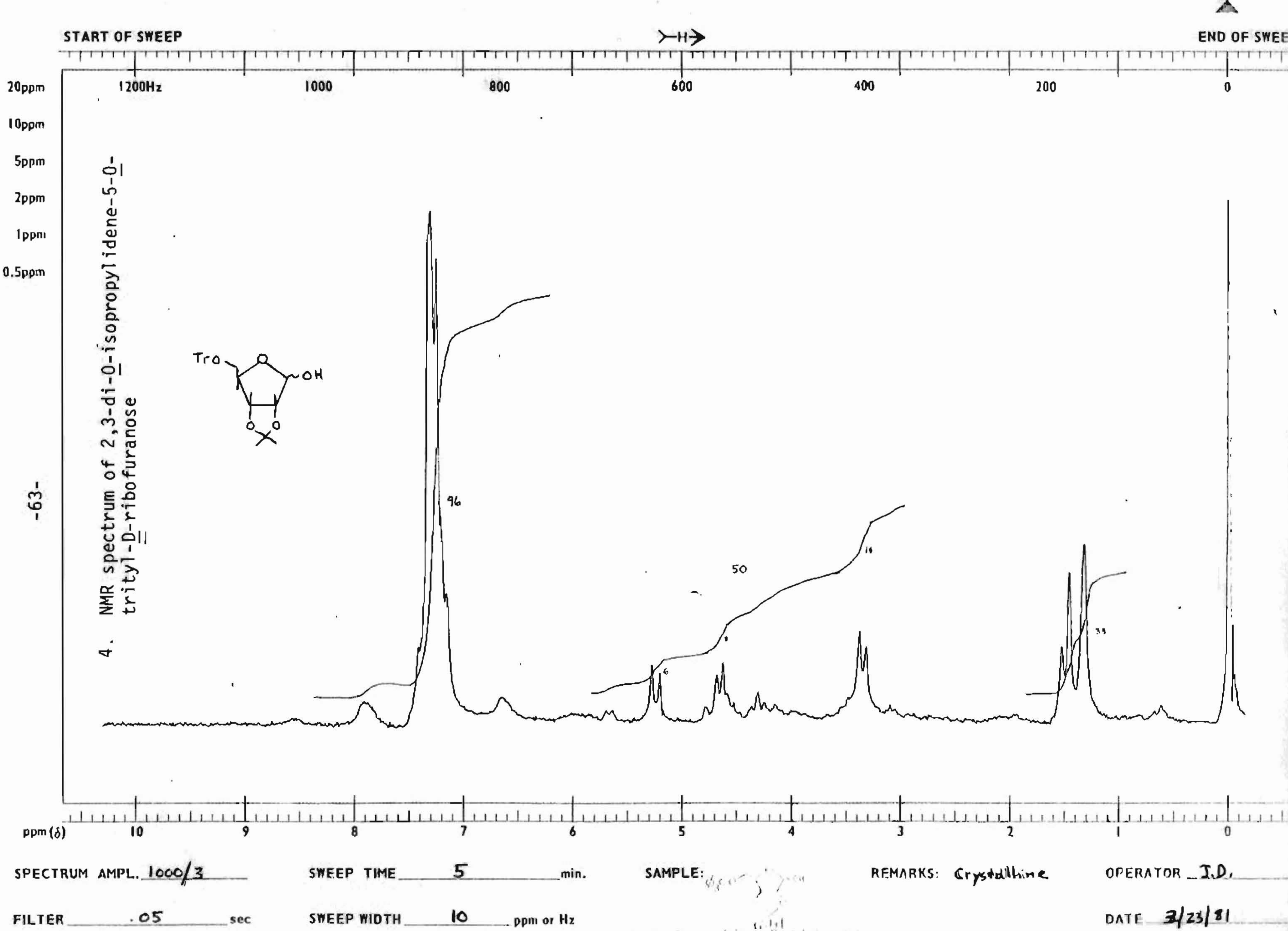


3. NMR spectrum of 2,3-di-O-isopropylidene-D-ribofuranose

SPECTRUM AMPL. 1000/2 SWEEP TIME 5 min. SAMPLE: REMARKS: OPERATOR

FILTER 0.5 sec SWEEP WIDTH 10 ppm or Hz DATE

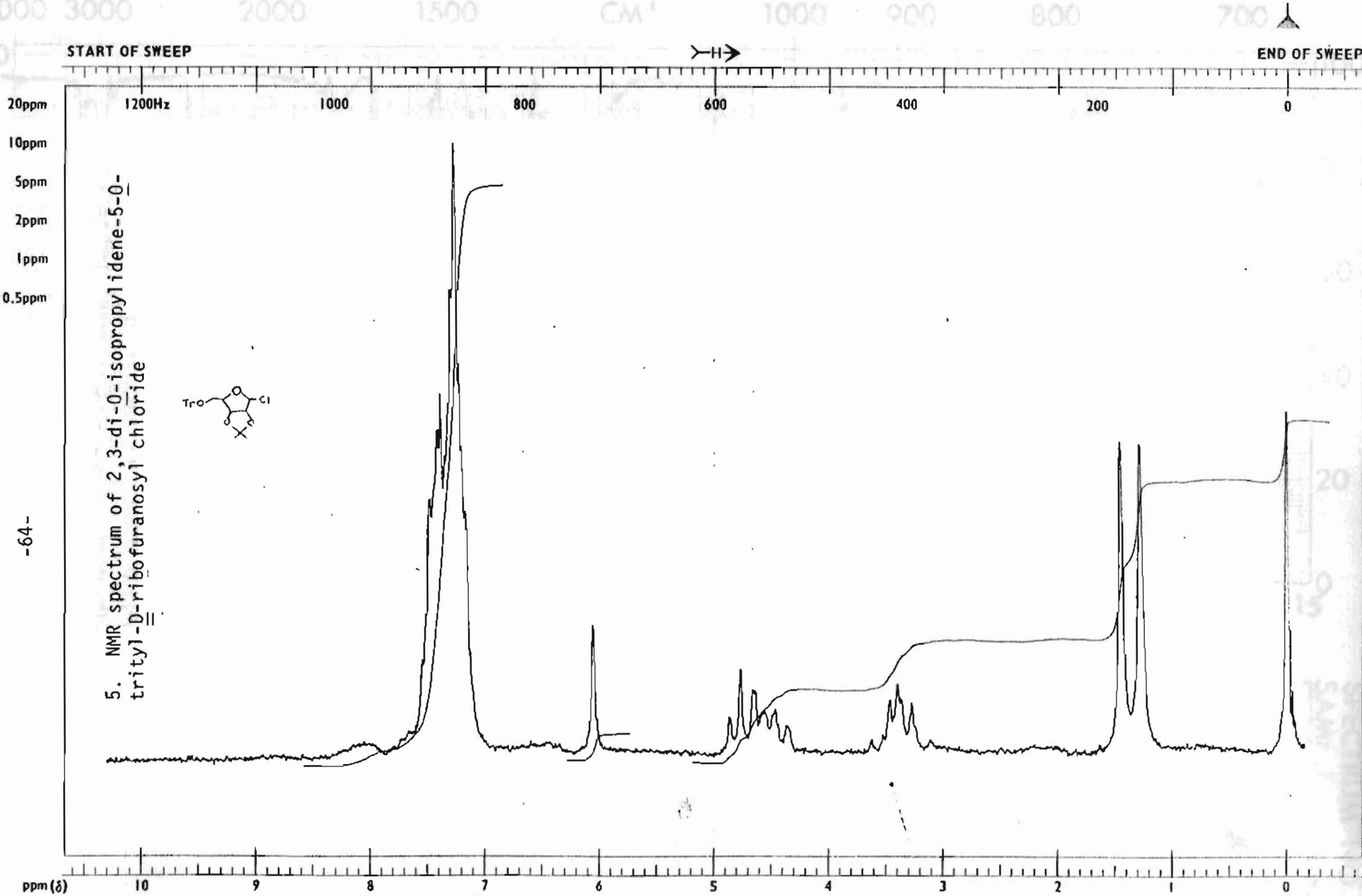
H 27% acetone in this syrup



4. NMR spectrum of 2,3-di-O-isopropylidene-5-O-trityl-D-ribofuranose

314 ARBOR AVENUE
LANDISVILLE, N.J. 08326
Phone: (609) 697-0020

5. NMR spectrum of 2,3-di-O-isopropylidene-5-O-trityl-D-ribofuranosyl chloride



SPECTRUM AMPL. 1000/3

SWEEP TIME 5 min.

SAMPLE: 2,3-O-isopropylidene-5-O-trityl-β-D-ribofuranosyl chloride

REMARKS: Integration indicates some contamination of aromatics, probably triphenylphosphine oxide

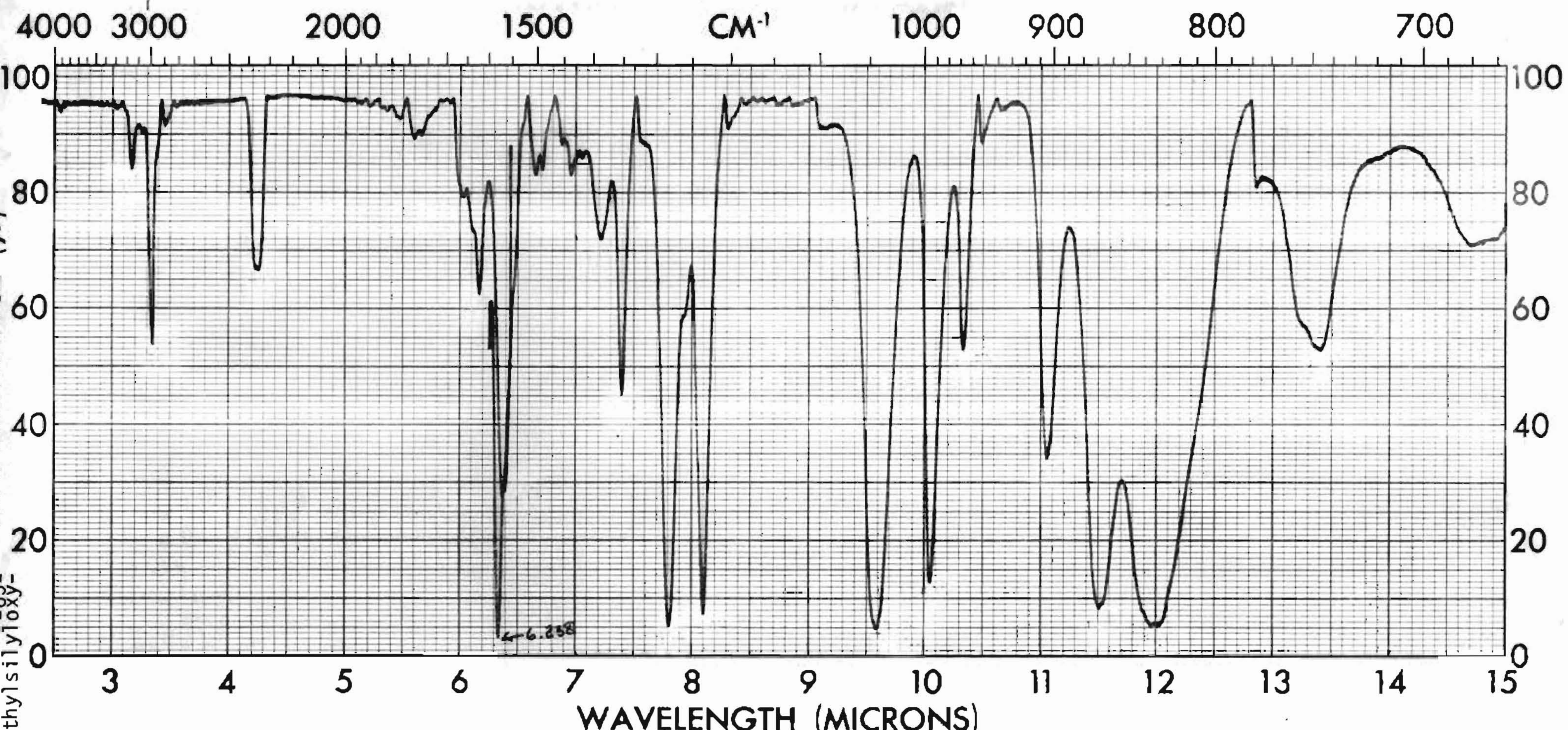
OPERATOR J D

FILTER .05 sec

SWEEP WIDTH 10 ppm or Hz

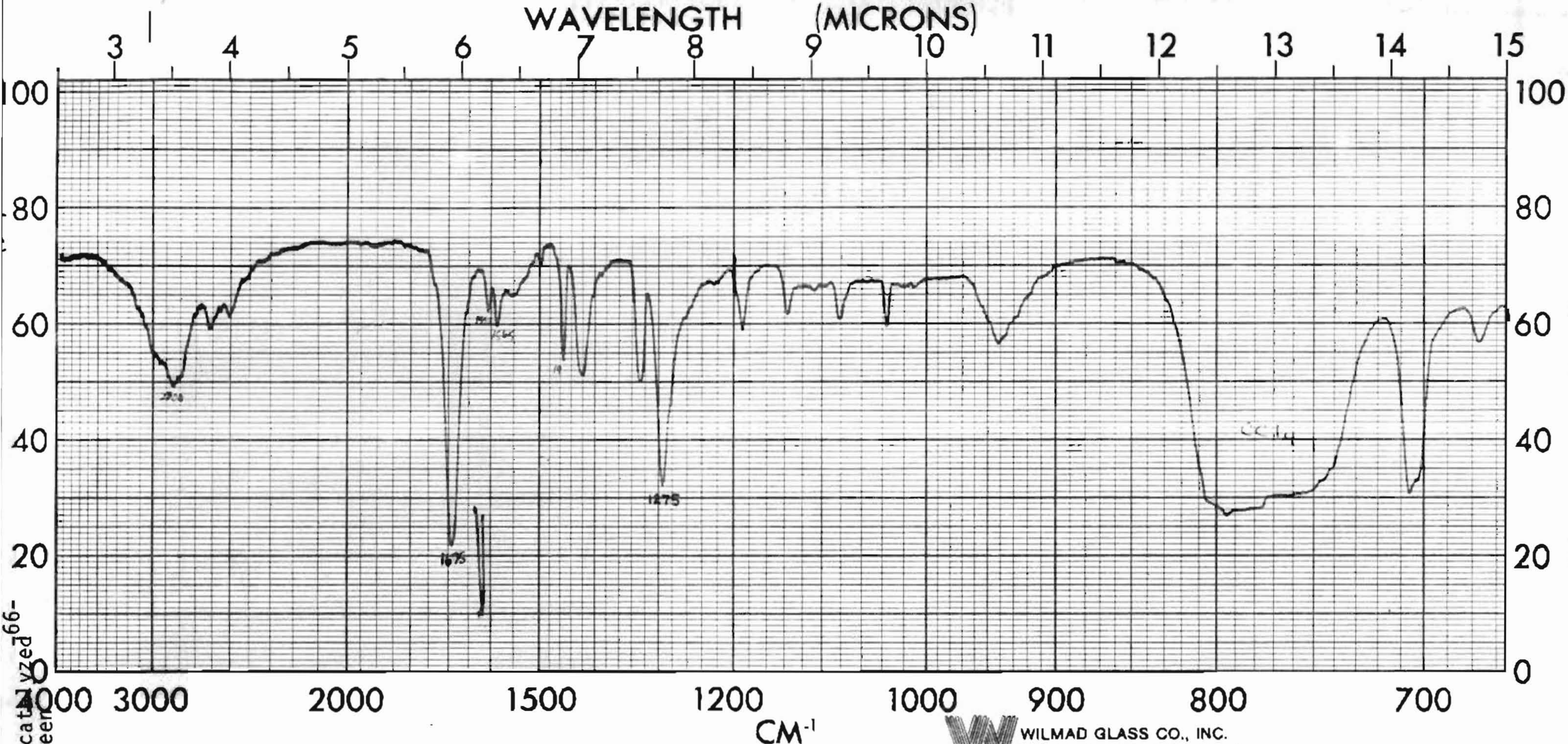
DATE 2/26/81

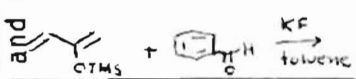
IR spectrum of 2-trimethylsilyloxy-
,3-butadiene



SPECTRUM NO.	ORIGIN Reaction of MVK	LEGEND	REMARKS 6.07 - =C-O
SAMPLE Silyl Enol Ethers	with $(CH_3)_3SiCl$ House's Methods	1. 3.25 - H-C= vinyl proton	7.70 - -C(=O)-O 8.00: CH_3Si
60° (100mm) Fraction	PURITY Trace $(CH_3)_3SiCl$	2. 6.30 - C=C-C=C-O (conjugated double bond)	9.50 - Si-O 9.95 - CH=CH_2
			CH=CH_2

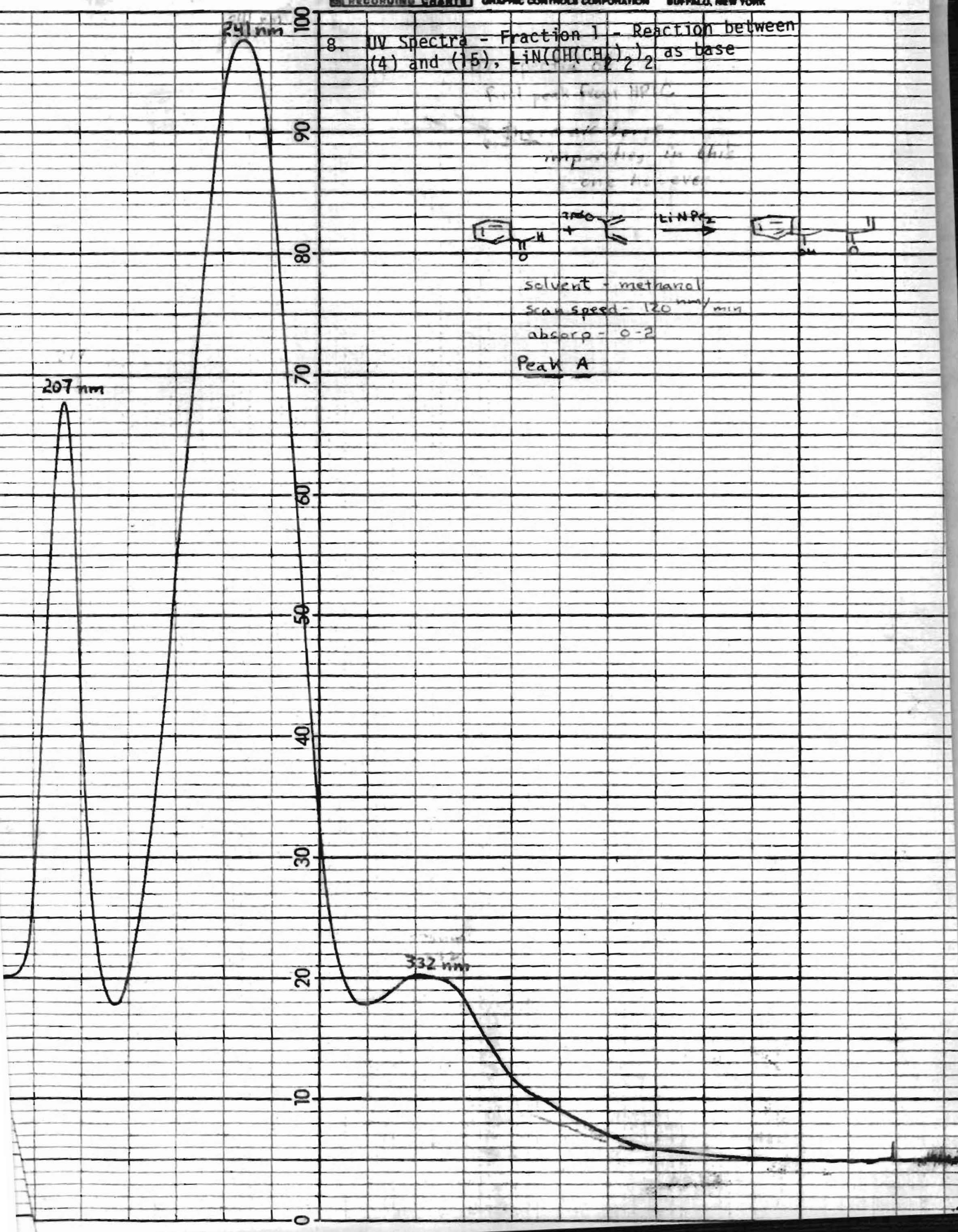
SPECTRUM NO.
SAMPLE

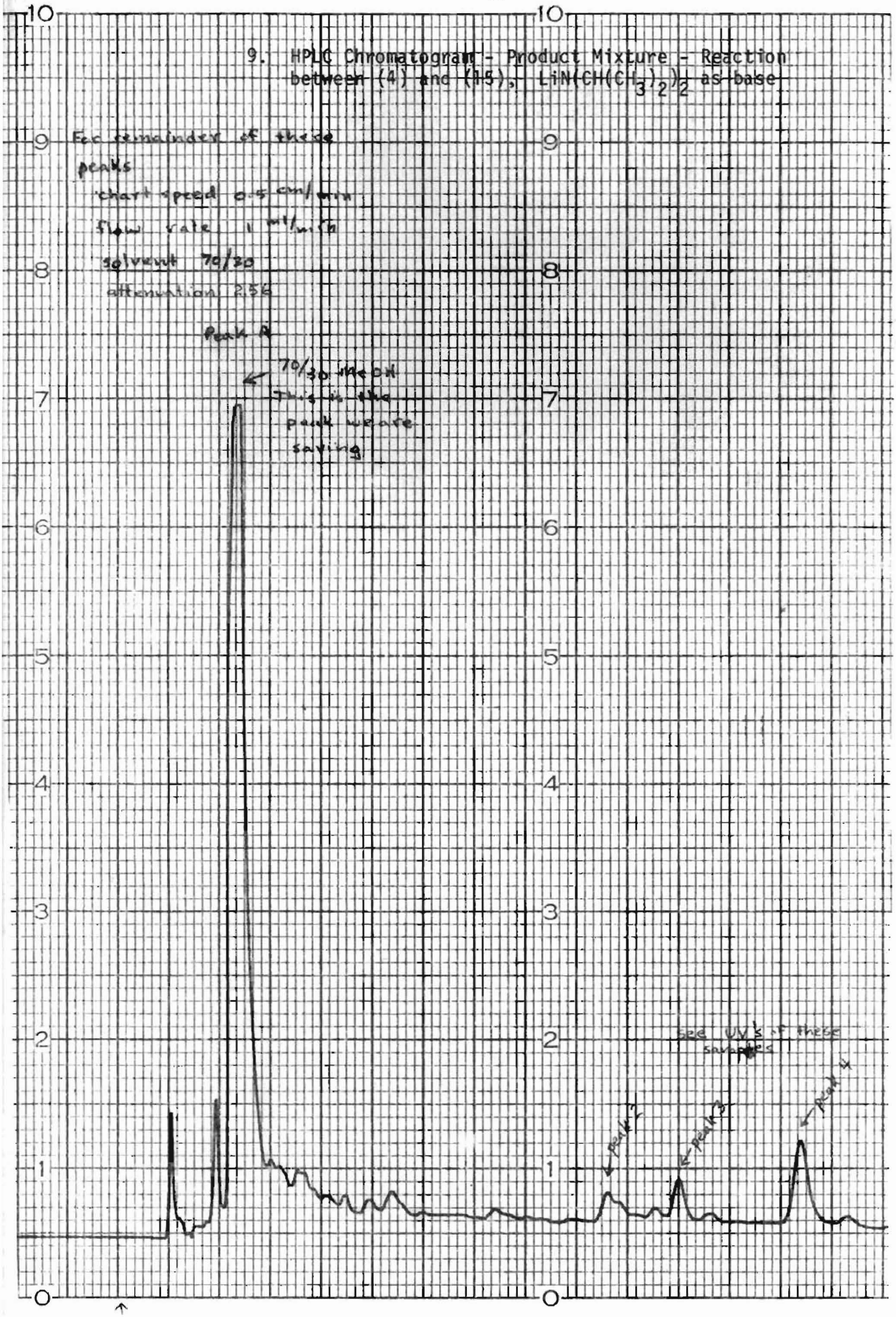


SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE white crystals		1.	
	PURITY	2.	

IR spectrum of TMBAF catalyzed reaction product between (6) and (45)

8. UV Spectra - Fraction 1 - Reaction between (4) and (15), $LiN(CH(CH_3)_2)_2$ as base





9. HPLC Chromatogram - Product Mixture - Reaction between (4) and (15), $LiN(CH(CH_3)_2)_2$ as base

References

1. R.D. Watson and R.H.C. Crick, Nature, 1953, 171, 737, 964.

2. R.J. Suhadolnik "Nucleosides as Biological Probes", Wiley-Interscience, New York, N.Y., 1979.

3. F.F. Davis and F.W. Allen, J. Biol. Chem, 1957, 227, 907.

4. R.J. Suhadolnik "Nucleoside Antibiotics", Wiley-Interscience, New York, N.Y., 1970.

5. C.K. Chu, I. Wempen, K.A. Watanbe and J.J. Fox, J. Org. Chem., 1976, 41, 2793.

6. K.A. Watanbe and J.J. Fox, J. Org. Chem, 1978, 43, 1193.

7. J. J. Fox et al, Nucleosides, Nucleotides, 1978, 81, 241.

8. W.A. Asbun and S. B. Binkley, J. Org. Chem., 1968, 33, 148.

9. J.G. Moffat, H.P. Albrecht, G.H. Jones, D.B. Repke, G. Trummlitz, H. Ohrui, C. Gupta, Chemistry and Biology of Nucleosides and Nucleotides, 1978, 359.

10. N. Nazarov, A.A. Akhrem, I.G. Tischenko, J. Gen. Chem. U.S.S.R. (Eng. trans.), 1955, 25, 677.

11. H.O. House, L.J. Czuba, M. Gall, H.D. Olmstead, J. Org. Chem., 1969, 34, 2324.

12. T. Mukaiyama, K. Banno, and K. Marasaka, J. Amer. Chem. Soc., 1974, 96, 7503.

13. A.T. Nielsen and J.W. Houlihan, Org. React., 1968, 16, 1.

14. G. Wittig and A. Hesse, Org. Syn., 1970, 50, 66.

15. H. O. House, O.S. Crumrine, A.Y. Teranshi, and H.D. Olmstead, J. Amer. Chem. Soc., 1973, 95, 3310.

16. H. Rasmussen, Synthesis, 1977, 91.

17. S. Danishefsky and T. Kitahara, J. Am. Chem. Soc., 1974, 96, 7807.

18. G. Stork and P.F. Hurdlik, J. Am. Chem. Soc., 1968, 90, 4462.

19. C. Girard, P. Amice, J.P. Barnier and J.M. Conia, Tet. Letters, 1974, 37, 3329.

20. M.E. Jung and C.A. McCombs, Organic Syntheses, 1978, 58, 163.

21. H. Vorbruggen and K. Krolikiewicz, Synthesis, 1979, 34.

22. We thank Dr. H. Niederprum, Inorganic Chem. Division, Bayer A-G, D-5090 Leverkusen, for a supply of potassium nonaflate.

23. E. Nakamura, T. Murofushi, M. Shimizu, I. Kuwajima, J. Am. Chem. Soc., 1976, 98, 2346.

24. R. Noyori, K. Yokoyama, J. Sakata, I. Kuwajima, E. Nakamura, Mishimizu, J. Am. Chem. Soc., 1977, 99, 1265.

25. R.J.H. Clark, J. Chem. Soc., 1963, 1377.

26. R.J.H. Clark & W. Errington, J. Chem. Soc., 1967, 258.

27. K. Narasaka, K. Soai, Y. Aikawa and T. Mukaiyama, Bull. Chem. Soc. Jpn., 1976, 49, 779 (Eng.).

28. R.J.P. Corriu, J. Boyer, R. Perz and C. Reye, J. of Organometallic Chem., 1980, 184, 157.

29. G. Gokel, Synthesis, 1976, 168.

30. M.E. Jung and C.A. McCombs, Tetrahedron Lett., 1976, 34, 2935.

31. R. Huisgen, Angew. Chem. Internat. Edit., 1963, 2, 565.

32. H.O. House, M. Gall, H.D. Olmstead, J. Org. Chem., 1971, 36, 2361.

33. H. W. Heine and M.S. Kaplan, J. Org. Chem., 1967, 32, 3069.

34. H.W. Heine, M. Fetler, and E.M. Nicholson, J. Am. Chem. Soc., 1959, 81, 2202.

35. R.S. Klein, H. Ohrui, and J.J. Fox, J. Carbohy. Nucleosides Nucleotides, 1974, 1, 265.

36. L.F. Fieser and K.L. Williamson "Organic Experiments", D.C. Heath and Co., Lexington, Mass., 1975.

37. J.B. Lee and T.J. Nolan, Tetrahedron, 1967, 23, 2798.

38. P.A. Levene and E.T. Stiller, J. Biol. Chem., 1933, 102, 187.

39. T.A. Newton, personal communication.

40. D. Marrs and T.A. Newton, unpublished results.

41. M.G. Saulnier, unpublished results.

42. T.A. Newton, personal communication.

43. M.S. Kharasch and O. Reinmuth "Grignard Reactions of Noumetallic Substances", Prentice-Hall Inc., New York, N.Y., 1954.

44. Ibid, p. 38.

45. Ibid, p. 1013.

46. M. Bobek and J. Farkas, Coll. Czech. Chem. Commun., 1969, 34, 247.

47. C. Lane and G. Kramer, Aldrichimica Acta, 1977, 10, 11.