Order Number 8820865

Synthesis of oligonucleotide analogues

Gandhi, Rajendrakumar Chimanlal, Ph.D.

City University of New York, 1988

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs or pages _____
2. Colored illustrations, paper or print _____
3. Photographs with dark background _____
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy ✓
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Dissertation contains pages with print at a slant, filmed as received _____
16. Other _____

_____

_____

SYNTHESIS OF OLIGONUCLEOTIDE ANALOGUES

by

RAJENDRAKUMAR CHIMANLAL GANDHI

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1988

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirements for the degree of Doctor of Philosophy.

1/15/88
date

Robert Engel
Chairman of Examining Committee

1/19/88
date

A. M.
Executive Officer

Arthur D. Baker

Thomas C. Stekas

Herman E. Zieger
Supervisory Committee

The City University of New York

ABSTRACT

SYNTHESIS OF OLIGONUCLEOTIDE ANALOGUES

by

Rajendrakumar Chimanlal Gandhi

Adviser: Professor Robert Engel

The research project concerns with the synthesis of the isosteric analogues of uridine nucleotides in which a 5'-Oxygen atom has been replaced by a methylene group. In the pursuit of this goal, various uridine nucleosides were synthesized. Among these, the synthesis of 2',3'-O-isopropylideneuridine-6'-phosphonic acid by several routes, was investigated.

The first strategy involved the simultaneous protection of the 2'- and 3'-hydroxyl groups in the form of an isopropylidene group, allowing further derivatization at the 5'-position. This derivatization included oxidation followed by Horner-Emmons reaction to afford the 6'-diphenyl-2',3'-O-isopropylideneuridine phosphonate. Hydrogenation of this ester with Adam's catalyst resulted in the simultaneous reduction of the double bond and the diphenyl phosphonate ester to yield the desired aforementioned phosphonic acid. Hydroboration followed by reduction of the aforementioned diphenyl phosphonate ester yielded the corresponding ∝-hydroxyphosphonic acid.

The second strategy involved the conversion of the 5'-hydroxyl group of 2',3'-O-isopropylideneuridine into the corresponding 5'-iodo compound via the tosylate derivative. The nucleophilic substitution of the 5'-iodo group by the preformed dimethyl ∝-lithiomethylphosphonate afforded the 6'-dimethyl-2',3'-O-isopropylideneuridine phosphonate and a cyclic compound as a minor product. Dealkylation attempts on this dimethyl phosphonate with trimethylsilyl bromide- which, if successful, would have produced the aforementioned 6'-phosphonic acid- remained unsuccessful.

Model studies involving the ∝-lithiation of bis-trimehthylsilyl chloromethyl phosphonic acid and its ∝-alkylation using simple halides remained unsuccessful.

Several 2',5'-O-protected uridine derivatives were synthesized for the coupling experiments. In particular, the 2',5'-di-O-trityluridine by tritylation of uridine in pyridine and 2'-tetrahydropyranyl- 5'-dimethoxytrityluridine in three steps from uridine, were synthesized and employed in the coupling experiments with the 2',3'-isopropylideneuridine-6'-phosphonic acid. After several unsuccessful coupling attempts the DCC coupling between the aforementioned phosphonic acid and the 2',5'-di-O-trityluridine followed by the acetic acid treatment afforded the isosteric dinucleotide $UCH_{2p}U$.

ACKNOWLEDGEMENTS

I am grateful to my wife, Mangal, for being understanding and, providing encouragement and support through the years.

I wish to express my sincere gratitude to Dr. Robert Engel for his guidance, understanding, patience, and support.

I thank Dr. William Berkowitz for his excellent teaching of Organic Reactions, Synthesis, and Mechanisms through the coursework and the problem sessions.

Finally, I am thankful to the members of the Queens College Chemistry Department for the help throughout the course of this work.

TABLE OF CONTENTS

INTRODUCTION

1. General

Chemical reagents or drugs, when introduced to an organism for the treatment of genetic disease, may be recognized as foreign and degraded by the organism. This may result in greater demand of the drug by the organism for an effective treatment. Such demand will be dependent on the rate of the degradation process.

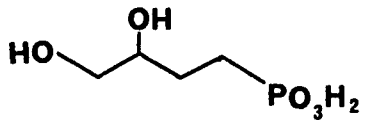
Among the available modes of degradation for phosphate species is the hydrolytic cleavage of the phosphate ester linkages. Even when natural agents such as ordinary nucleic acid m-RNA or comparatively longer-lived species are introduced, cleavage occurs readily. This points to a possible role for non-degradable analogues of nucleic acid species as chemical agents for genetic engineering or other applications.

It has been postulated that the phosphonic acid analogues of natural phosphates in which the oxygen atom of the phosphate ester is substituted by a methylene group might be suitable substitutes for the related natural phosphates in most biochemical processes, except when the cleavage of the phosphate ester linkage is involved.[1] For example, 3,4-dihydroxybutyl-1-phosphonic acid (1) was predicted to be a metabolic regulator, and was found to be one for certain of the enzymes in E.

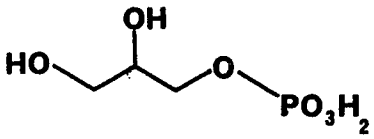
coli which catalyze reactions normally involving glycerol-3-phosphate (2).[2,3] Structurally the only difference between (1) and (2) is that the phosphate ester oxygen in (2) is replaced by a methylene group in (1). As (1) and (2) bear the same size and shape, they are referred to as isosteric analogues. The term isosteric is used here although it strictly refers to compounds of identical size and shape, and crystallographic data for a series of phosphates and their corresponding phosphonic acids indicate small differences.[4-10]

It was also found that the isosteric analogues and their natural phosphate counterparts exhibit a parallel behavior with respect to their transport into or exclusion from cells.[11-15] A prediction was made and realized in these laboratories that 1-deoxy-1-dihydroxyphosphinylmethylfructose (4), the isosteric analogue of fructose-1-phosphate (3), would be an in vivo inhibitor of growth E. Coli which are constitutive for the hexose phosphate transport system.[16,17]

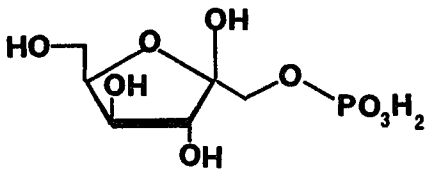
Similarly, 5-carboxy-4-hydroxy-4-methylpentyl-1-phosphonic acid (5),[18] an isosteric analogue of 5-phosphomevalonic acid (6),[19] was found to be a most potent in vitro inhibitor of sqalene biosynthesis at the pre-isoprene stage of metabolism.


HO
OH
PO3H2

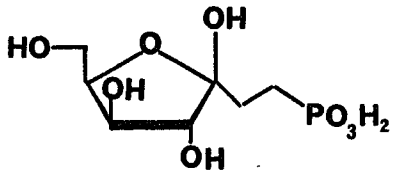
1


HO
OH
O
PO3H2

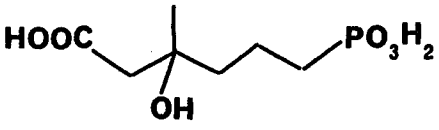
2


HO
O
OH
OH
O
PO3H2
OH

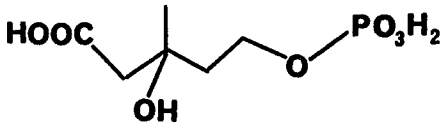
3


HO
O
OH
OH
PO3H2
OH

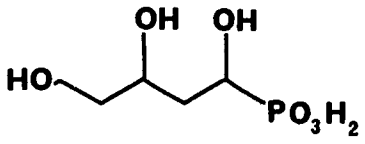
4


HOOC
PO3H2
OH

5


HOOC
O
PO3H2
OH

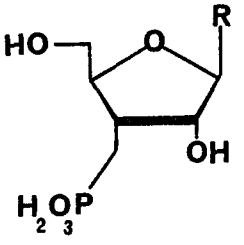
6


HO
OH
OH
PO3H2

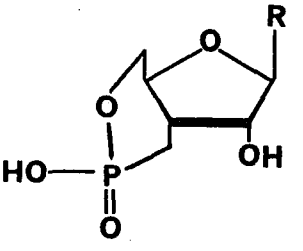
7

In Vitro studies with (1) revealed its utility as a substrate for CDP-diglyceride:*sn*-glycerol-3-phosphate phosphatidyltransferase (*E. Coli*)[20,21] and also found it to be an inhibitor of the anaerobic *sn*-glycerol-3-phosphate:NAD(P) oxireductase of *E. Coli*. These studies found, however, a lack of interaction of (1) with acyl coenzyme A:*sn*-glycerol-3-phosphate acyltransferase. It was postulated that the loss of binding capability resulted from the substitution of a methylene group for the ester oxygen in glycerol-3-phosphate (2). Cooperman and Chiu,[22] in studying the effect of electrophilic reagents on the enzymatic activity of inorganic pyrophosphatase, observed that a simple substitution of a hydrogen by a hydroxyl group at the methylene group of methane(bis)phosphonate led to a marked increase in the affinity for the enzyme and hence decreased the enzymatic rate of pyrophosphate hydrolysis. An attempt was made to test the hypothesis that the aforementioned inactivity of (1) resulted from the loss of a binding function. 1,3,4-Trihydroxybutyl-1-phosphonic acid (7) was synthesized from L-malic acid with chirality at the 3-position corresponding to that of *sn*-glycerol-3-phosphate[23] and was found to serve as a substrate when investigated with acyl coenzyme A:*sn*-glycerol-3-phosphate acyltransferase, thus supporting the hypothesis.[24,25]

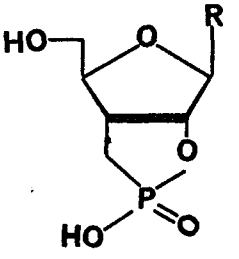
Moffatt et al.[26] have reported the synthesis of a series of phosphonic acids (8), which are isosteric analogues of 3'-nucleoside phosphates. It was reported that the 3'-nucleotide analogue (8) and the cyclic nucleotide analogues (9) and (10) exhibit activity in controlling metabolic processes and in producing metabolic deficiencies.[27-29]



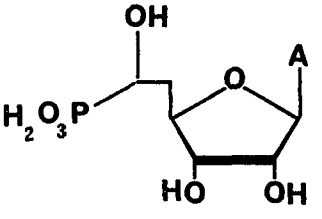
8



10



9

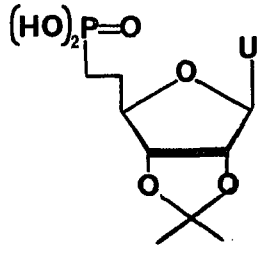


11

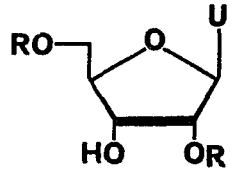
Hampton et al.[30] have reported that the compound (11), "isosteric" with AMP, served as inhibitor of snake venom 5'-nucleotidase.

STATEMENT OF THE PROBLEM

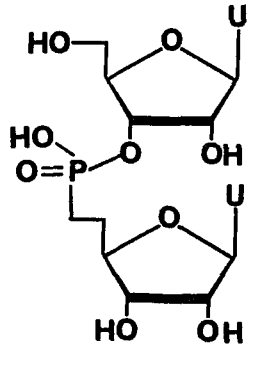
The present work is concerned with the studies toward the synthesis of isosteric analogues of ribonucleotides. These studies involve the synthesis of the uridine dinucleotide U U (12) in which one of the 5'oxygen atoms has been replaced by a methylene group, and of the two key precursors, the 6'-homouridine phosphonic acid (13) and the 2',5'-O-protected uridine (14).



13



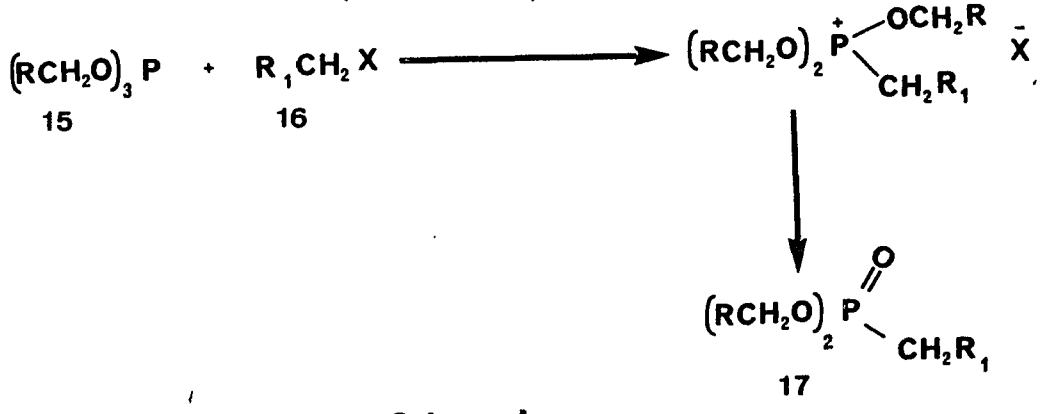
14



12

2. Carbon-Phosphorous Bond Formation

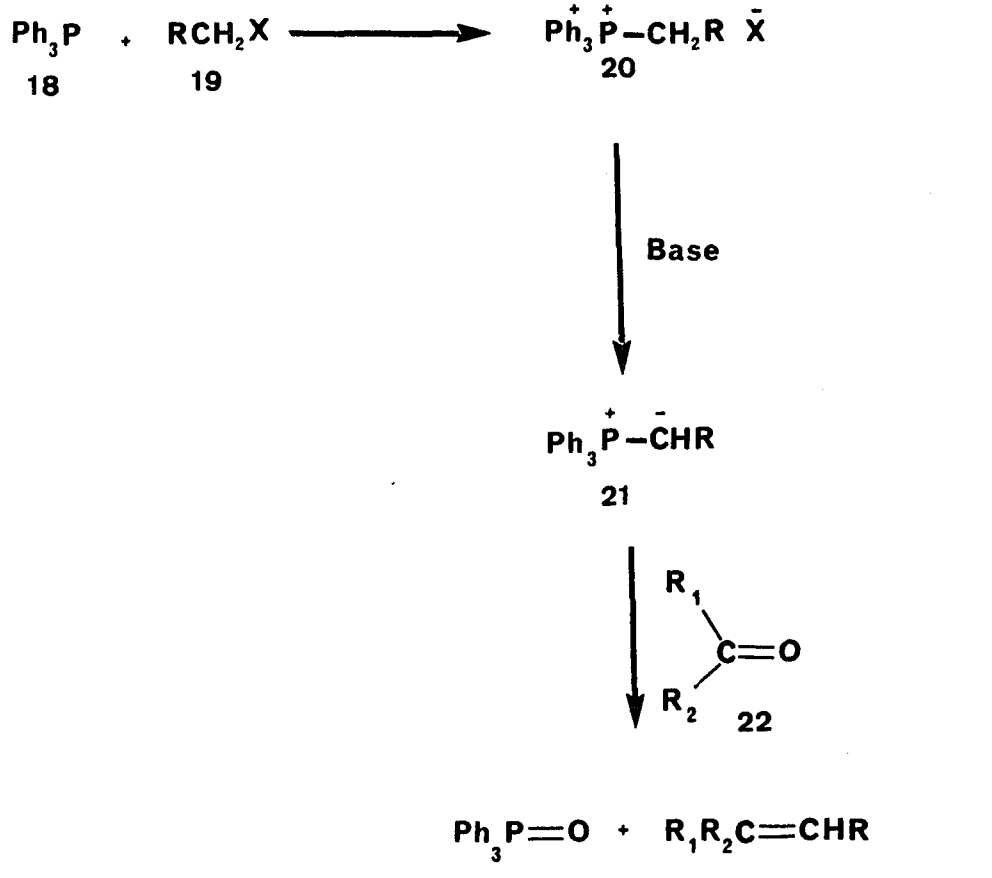
One of the key steps involved in the generation of such isosteric analogues is the formation of the carbon-phosphorous bond. Two of the more commonly employed methods, in relation to the present work and in general, involve the Arbuzov and the Menshutkin-type reactions. The former involves a reaction between a trialkyl phosphite (15) and an alkyl halide (16) to give a dialkyl alkylphosphonate (17) (Scheme I). The latter involves a reaction between triphenyl phosphine (18) and an alkyl halide (19) or alkyl-X where X is a suitable leaving group, to give a stable quarternary phosphonium salt (20). In the Wittig reaction, treatment of (20) with a suitable base results in formation of an ylide (21), reaction of which may then be performed with a carbonyl compound (22). This leads to carbon-carbon double bond formation (Scheme II).



Scheme I

If the R group in (21) is diphenyl phosphonate, the product obtained is a diphenyl vinylphosphonate (23)

which may ultimately be converted to its corresponding phosphonic acid (24).[31]



Scheme II

3. Protection of Hydroxyl Groups

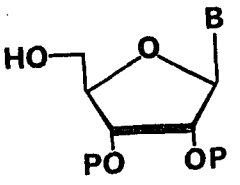
Another critical aspect of the present synthetic effort is the temporary protection of reactive functionalities while reactions are performed at another

site of the intermediate species. In this regard, hydroxyl groups present particular challenges. There is the inevitable possibility for the hydroxyl groups to undergo a variety of undesired reactions such as oxidation, acylation, alkylation, dehydration, halogenation, or others involving neighboring group participation. In order to preclude these undesired side reactions and to effect a chemical change at the intended site in the molecule, the protection of the appropriate hydroxyl groups is essential.

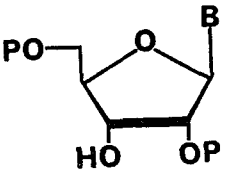
The protection of alcoholic hydroxyl groups has received widespread attention in organic synthetic studies and there is continued interest in the quest for discovering new protecting groups. While there are a variety of protecting groups available, many of these are often not suitable for the desired application. This problem arises due to the required conditions to put them in place, and later in the synthetic scheme to remove them.

Hydroxyl(s) protected ribonucleosides are key precursors required for the synthesis of the isosteric analogues of oligoribonucleotides. The protection of hydroxyl groups at 2' and 3' positions in (25) and 2' and 5' positions in (26) affords the possibilities for a) synthetic transformations to be performed at the

unprotected hydroxyl group, and b) further modification in the protected hydroxyl groups.

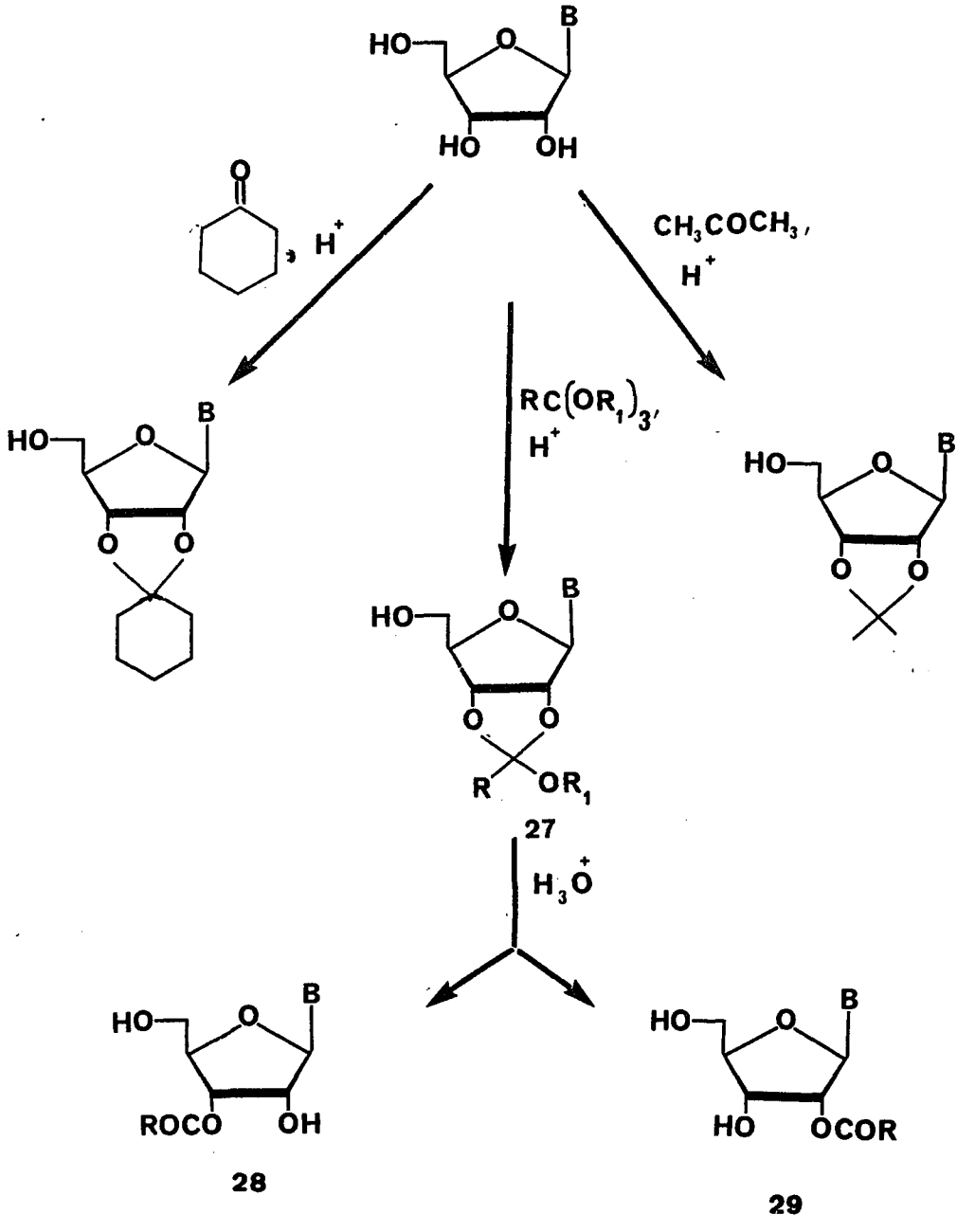


25



26

Acid catalyzed protection of the 2',3'-hydroxyl groups has been obtained by reactions with certain carbonyl compounds and ortho esters. These include acetone,[32] 2,2-dimethoxy propane,[33] cyclohexanone[34] and orthoesters of formic, acetic and benzoic acids.[35,36] While both, the carbonyl and orthoester derivatives are stable under basic conditions, the latter are more acid-labile and readily hydrolyzed thus allowing deprotection under milder acidic conditions (Scheme III). It was shown that upon hydrolysis, the orthoester (27) yielded 2'- and 3'-O-acyl derivatives (28 and 29),[37] resulting from equilibration between the two derivatives. Interestingly it was observed that the mixture of these two derivatives in acid-free solvents often deposited the 3'-O-acyl derivative in a pure crystalline state.

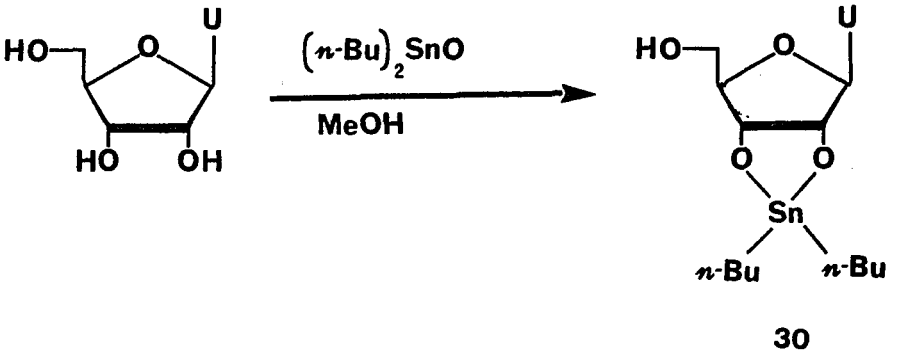


Scheme III

The cyclohexylidene and the isopropylidene protecting groups can be removed under acidic conditions. The cyclohexylidene group was found to be

three times more stable to acidic hydrolysis than the isopropylidene group, and thus offered an advantage over it.[34]

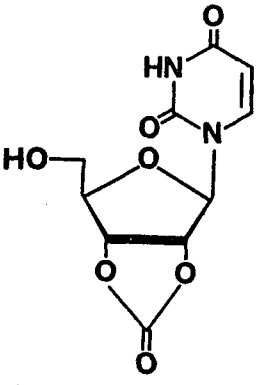
Recently, use of di-n-butyl tin oxide for the protection of hydroxyl groups leading to the di-n-butyl stannylene derivative (30) has been explored by Moffatt et al.[38] (Scheme IV).



30

Scheme IV

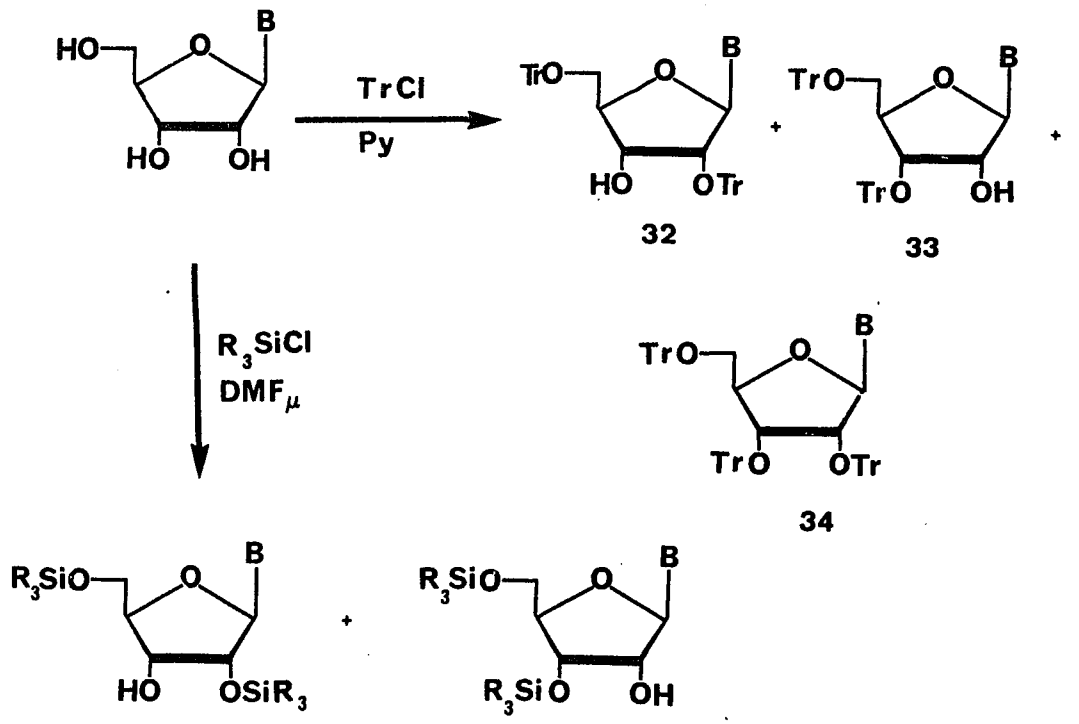
Cyclic carbonate esters used as hydroxyl protecting functions have found limited use in nucleoside chemistry. Derived from the reaction of 2',3'-hydroxyl groups of a ribonucleoside with phosgene or suitable chloroformate derivatives in pyridine, the cyclic carbonate (31)[39] withstood the typical conditions required to remove the isopropylidene group. It has been cleaved under mild basic conditions.[40]



31

The attempts to protect 2',5'-hydroxyl groups often have led to concurrent 3',5'-hydroxyl protection as well. Following the protection of 5'-hydroxyl group, the steric factors favor the protection of the less sterically hindered 2'-hydroxyl over the 3'-hydroxyl group, thus yielding more of the 2',5'isomer. This result has been supported by the use of bulky protecting groups such as triphenylmethyl (trityl)[41-43] and trisubstitued silyl chlorides.[44] The trityl derivatives have been prepared by the reaction of a ribonucleoside with trityl chloride and pyridine. Although the protection led to formation of three isomers (32, 33 and 34), the 2',5' isomer was found to be the major product (Scheme V). Its isolation and purification by fractional

crystallization has been achieved.

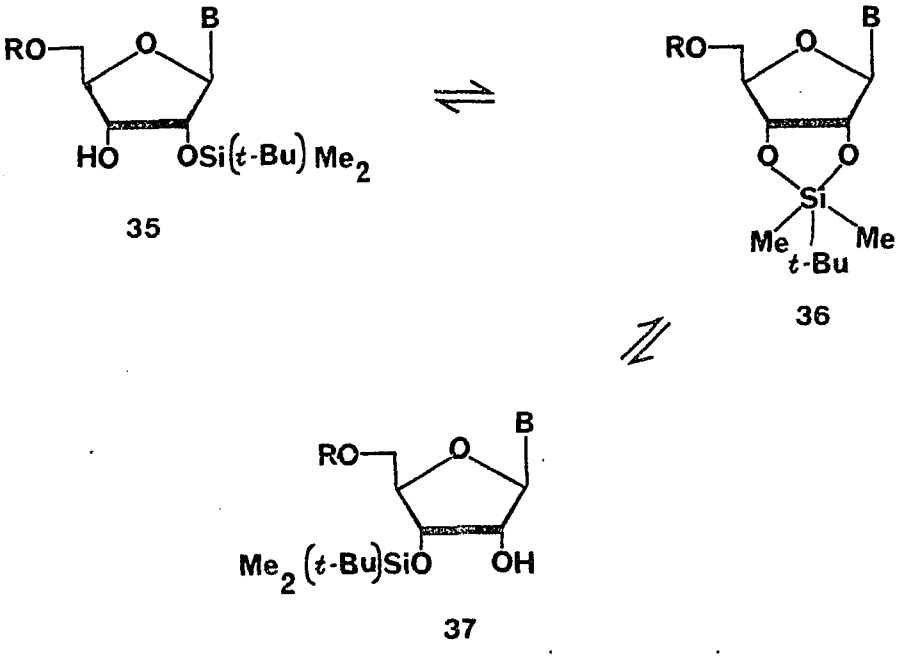


Scheme V

The deprotection of trityl groups has been achieved by either catalytic hydrogenation or strong acidic

hydrolysis. The latter method limits the usefulness of the trityl group as a protecting function in the synthesis of oligoribonucleotides. Khorana et al.[42] overcame this difficulty by using modified trityl groups containing one or two p-methoxy substituents in pyridine solution. Modified trityl groups, when compared with the unsubstituted system, undergo the acidic hydrolysis at a rate that increases by approximately one order of magnitude for each p-methoxy substituent. Khorana et al.[44] have widely used mono- and di-methoxytrityl protecting groups in nucleotide synthesis.

Protection with silyl groups has been carried out by reaction of the ribonucleoside with an appropriate trisubstited silyl chloride in the presence of imidazole in DMF solution. Among the silyl groups employed thus far, the most common are tert-butyldimethylsilyl (TBDMSi),[45] triisopropylsilyl,[46] and tert-butyldiphenylsilyl.[47] It was observed that this protection, in addition to yielding 2',5'-, 3',5'-, and 2',3',5'- protected derivatives, presents a complication in terms of isolation. Studies with the tert-butyldimethylsilyl group showed that the silyl group has a tendency to migrate between 2'- and 3'- positions (Scheme VI) on silica gel surface and in protic or aqueous conditions.[48,49]

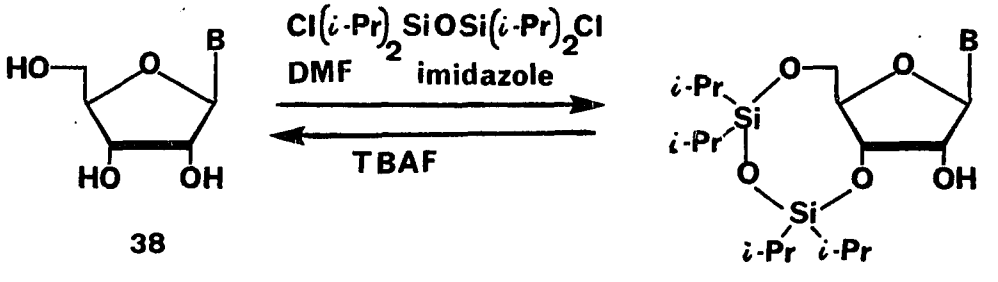


Scheme VI

Proper synthetic manipulation of the hydroxyl groups afforded the protection of 2',5'-hydroxyl groups with two different kinds of protecting groups (e.g. tert-butyldimethylsilyl at the 2'-position and p-methoxytrityl at the 5'-position).[48]

Under standard conditions, the amino group of a nucleoside (e.g. guanosine) does not undergo the silylation reaction in contrast to tritylation. In this regard the silyl protecting groups offer a further advantage over trityl protecting groups.[50]

Recently a novel bifunctional silyl protecting group has been discovered.[51] Tetraisopropyl disiloxane-1,3-diyl (TIPDSi) afforded simultaneous protection of 3'- and 5'-hydroxyl groups of a ribonucleoside. The function is applied in the presence of imidazole in DMF solution. This functionality has opened up new routes for the functionalization of the 2'-hydroxyl group. The reagent has found wide application in nucleic acid and phospholipid chemistry. It has been found to be stable under conditions used for introduction of other hydroxyl protecting groups, and can be removed quantitatively using acidic or basic conditions. Among the reagents used for deprotection, the more popular ones are tetra-n-butylammonium fluoride (TBAF), and tri-n-butylammonium fluoride (TBAHF) (Scheme VII).[52-54]



38 39

Scheme VII

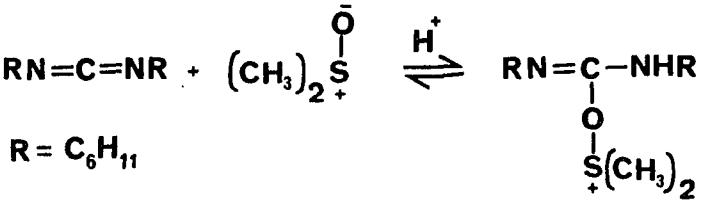
Other aforementioned silyl protecting groups can generally be removed with methanolic HCl,[49] 80% acetic acid,[47] ethanolic ammonia,[48] sodium hydroxide,[47] and TBAF,[55] which causes partial cleavage of internucleotidic linkages as well. The deprotection of less sterically hindered 5'-substituents would lead to selective deprotection under suitable conditions.[48]

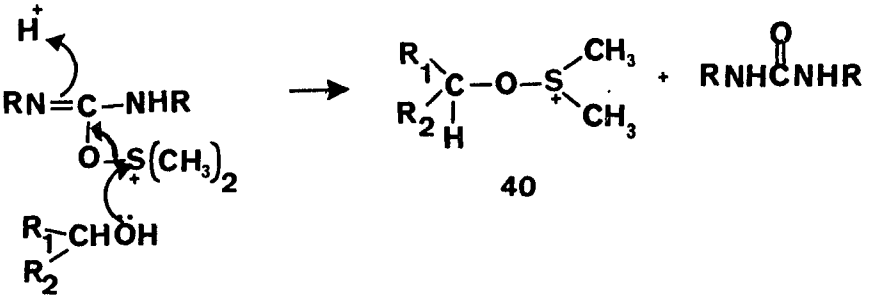
4. Preparation of Carbonyl Derivatives

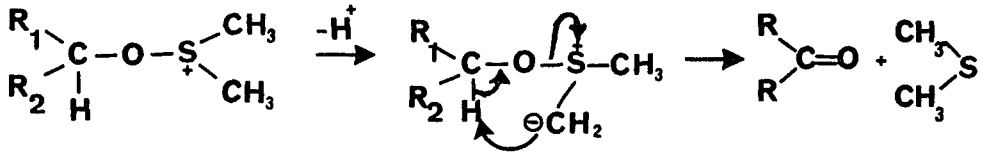
A variety of oxidation methods for the conversion of a hydroxyl group in carbohydrates and nucleosides into the carbonyl functionality have been employed.[56-60] Among these, the use of dimethyl sulphoxide (DMSO) with electrophilic "activators" under mild conditions has proven highly useful. Among the "activators" used are dicyclohexyl carbodiimide (DCC), acetic anhydride, sulphur trioxide, phosphorous pentoxide, oxalyl chloride and trifluoroacetic anhydride. The method employing DMSO-DCC system offers a few advantages: a) the oxidation often proceeds in high yields; b) it allows the use of a mild proton source, pyridinium trifluoroacetate, thus providing essentially neutral conditions; and c) it minimizes serious side reactions. Proposed by Moffatt et al.,[61] a general mechanism for the oxidation of a primary alcohol into aldehyde employing DMSO-DCC system is presented in Scheme VIII.

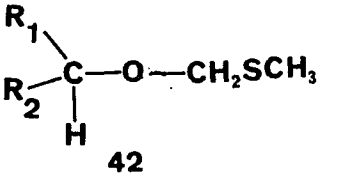
Activation of DMSO by DCC in the presence of a

proton donor, leads to the formation of the sulfoxonium intermediate which then undergoes nucleophilic attack by the hydroxyl function resulting in the









Scheme VIII

formation of alkoxysulfonium derivative (40). The

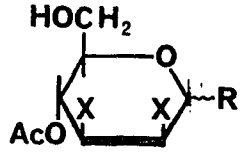
formation of dicyclohexylurea (DCU) renders this reaction irreversible and provides a powerful driving force. The sulfur ylide (41), formed from (40) by the subsequent abstraction of a proton by a weak base, collapses *via* a five-membered cyclic transition state to aldehyde and dimethyl sulfide. No traces of carboxylic acid were detected.

The DMSO-acetic anhydride system is best suited to the oxidation of rather hindered alcohols. It is not as widely applicable as the DMSO-DCC system. The former offers an advantage when dealing with alcohols appreciably soluble in water. Removal of most of the reaction by- products can be achieved by lyophilization,[62] thus avoiding the necessity of extraction. However, the formation of thiomethoxymethyl ether (42) *via* an intramolecular rearrangement of (41), a key step in the Pummerer rearrangement, can become a serious side reaction during oxidations using the DMSO-acetic anhydride system and is significant when phosphoric acid is used as the proton source. In addition this oxidation leads to formation of acetate esters.

It has been observed that the oxidation using DMSO-phosphorous pentoxide system has not proved satisfactory for the preparation of 2',3'-O-protected nucleoside 5'-aldehydes although 2'- and 3'-ketonucleosides can be

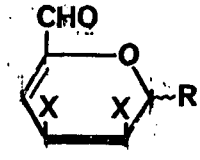
prepared readily from suitable intermediates.

The use of the DMSO-SO_3.pyridine complex system in the presence of triethylamine offers an advantage in that there is no formation of thiomethoxymethyl ethers mentioned above, but is somewhat limited due to the possibility of elimination brought about by the strongly basic conditions. For example, 2,3,4-tri-O-acetyl-D-mannopyranoside (43), on oxidation, yielded the unsaturated aldehyde (44) in 75% yield.[63]



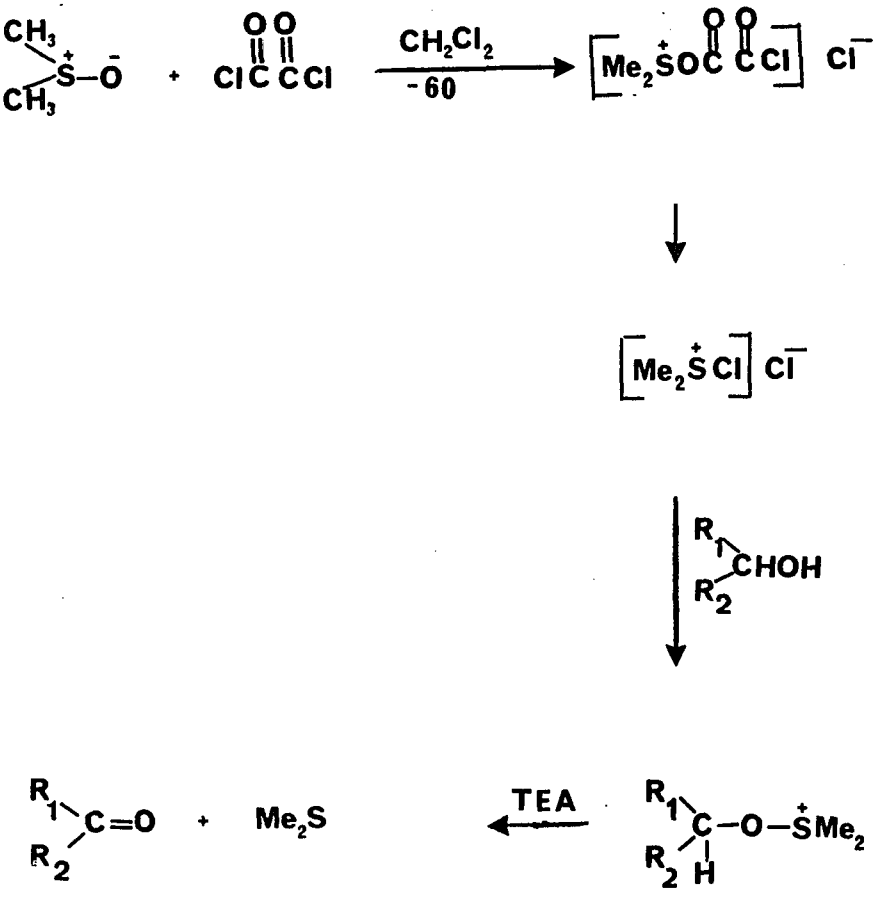
43

X = OAc



44

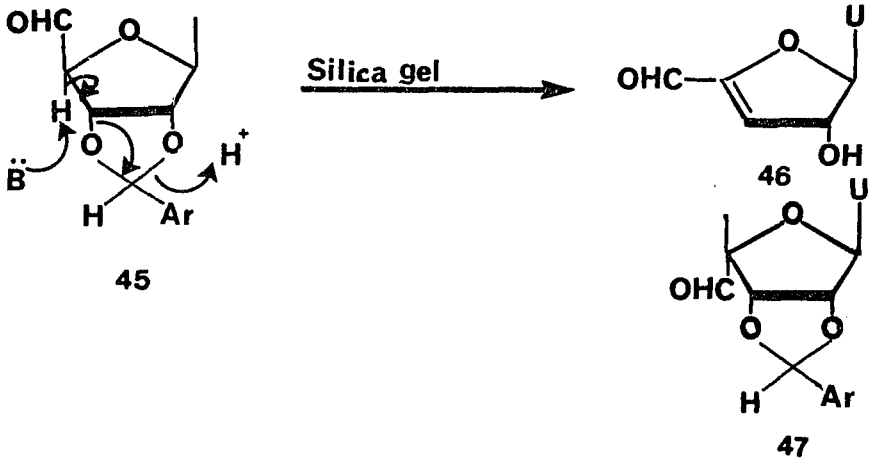
Swern oxidation,[58] a recent modification of the Moffatt oxidation, employs trifluoroacetic anhydride and oxalyl chloride as the DMSO activators. The mechanism of the oxidation using the DMSO-oxalyl chloride system is presented in Scheme IX.



Scheme IX

Binkley [64] has reported the preparation of carbonyl compounds from the photochemical decomposition of a pyruvate ester derived from an alcohol.

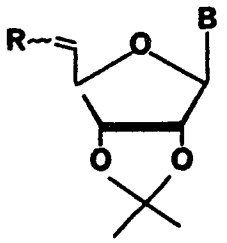
Attempts at isolation and purification of 2',3'-O-protected nucleoside 5'-aldehydes have been met with considerable difficulties. These difficulties arise due to epimerization at C-4' and elimination of the 2',3' O-acetal following the abstraction of C-4' proton, both of which are likely to occur during chromatography and in the presence of a variety of bases. For example, attempted chromatography of 2',3'-O-benzylideneuridine-5'-aldehyde (45) on silicic acid, alumina, or florisil, led to facile elimination yielding the 3',4'-unsaturated aldehyde (46) and some accompanied epimerization to the α-L-lyxosyluridine 5'-aldehyde (47)[65] (Scheme X). However, pure nucleoside-5'-aldehydes can be isolated as crystalline N,N'-diphenylimidazolidine derivatives by reaction with Wanzlick's reagent, N,N'-diphenylethylenediamine.[66] In subsequent reactions, the 5'-carbonyl is regenerated under mild controlled acidic hydrolysis.[67]



5. Carbonyl Derivatives in Synthesis

The nucleoside 5'-aldehydes have been employed in Wittig-type reactions, among others, for new carbon-carbon bond formation. Several[68-72] α,β-unsaturated derivatives (48) from nucleoside 5'-aldehydes have been prepared by reaction of 2',3'-O-protected nucleoside 5'-aldehyde with a variety of phosphoranes (49). Moffatt et al.[31] have reported the syntheses of several isosteric analogues (50) of nucleotides by reaction of the 5'-aldehydes with a highly stabilized Wittig reagent, diphenyl triphenylphosphoranylidenemethylphosphonate (51).[71] Montgomery et al.[72] have reported a similar reaction of 3'-O-acetyl-2'-deoxy-5-fluorouridine-5'-aldehyde to yield the corresponding vinyl phosphonate (52) [Scheme XI].

As shown in Scheme XI, condensation[73] of the nucleoside 5'-aldehyde (53) with formaldehyde yielded the β-hydroxy aldehyde (54) which underwent a Cannizzaro reaction with formaldehyde to form the 1,3-diol derivative (55). When potassium carbonate was used as a base in the condensation, the product obtained was the α,β-unsaturated aldehyde (56).



B = U, G

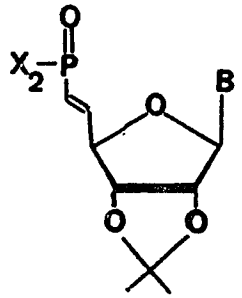
48

$RCH=PX_3$

R = Ph, CN, COOEt

X = OPh

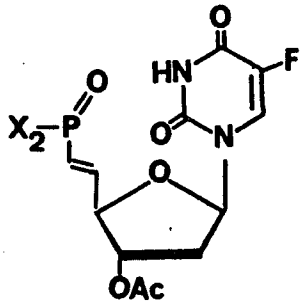
49



50

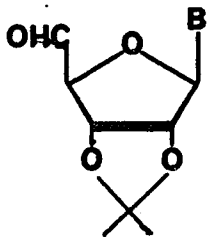
$Ph_3P=CHP(=O)-X_2$

51



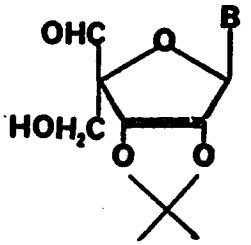
52

Scheme X



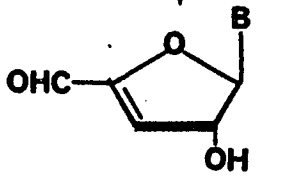
53

HCHO, OH^-

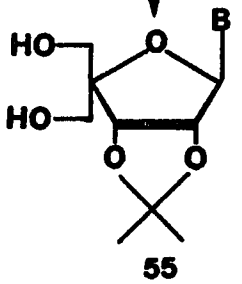


54

HCHO
K_2CO_3



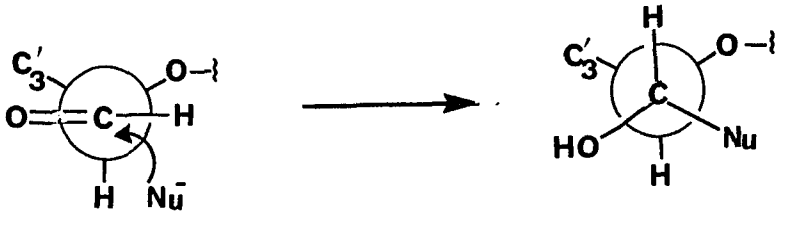
56



55

Scheme XI

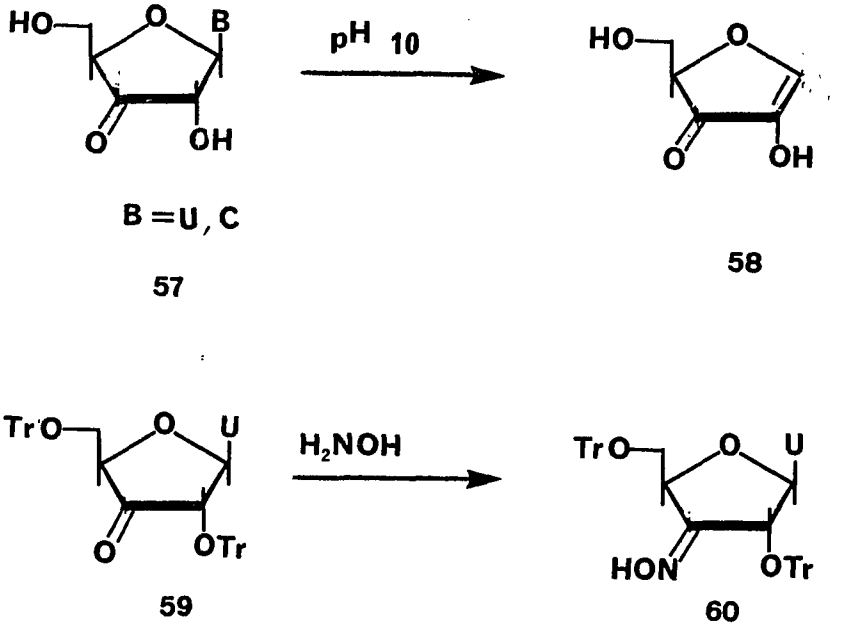
Stereoselective reactions have been performed on the 5'-aldehydes using nucleophiles such as Grignard reagents,[74] cyanide ion,[59] and nitromethylate anion[75]. These reactions are thought to be kinetically controlled and the formation of the major product is explained as resulting from attack by the nucleophile at the carbonyl carbon approaching from the less hindered side of a favored conformation (Scheme XII).



Scheme XII

β-Ketoglucosylamines render themselves extremely sensitive to strongly basic conditions. Upon treatment with base there results the elimination of the β-amino functionality. The 3'-carbonyl derivatives of ribonucleosides (57) structurally resembling β-ketoglucosylamines undergo such elimination at pH 10 to

release the pyrimidine base and yield the unsaturated sugar derivative (58).[74] This limits the application of certain synthetic transformations at the 3'-position. However, such sensitivity is alleviated with the protection of 2'- and 5'- hydroxyl groups. For example, 2',5'-O-protected 3'-ketouridine (59)[57] on reaction with hydroxyl amine, afforded the corresponding oxime (60) without the loss of the pyrimidine ring (Scheme XIII).

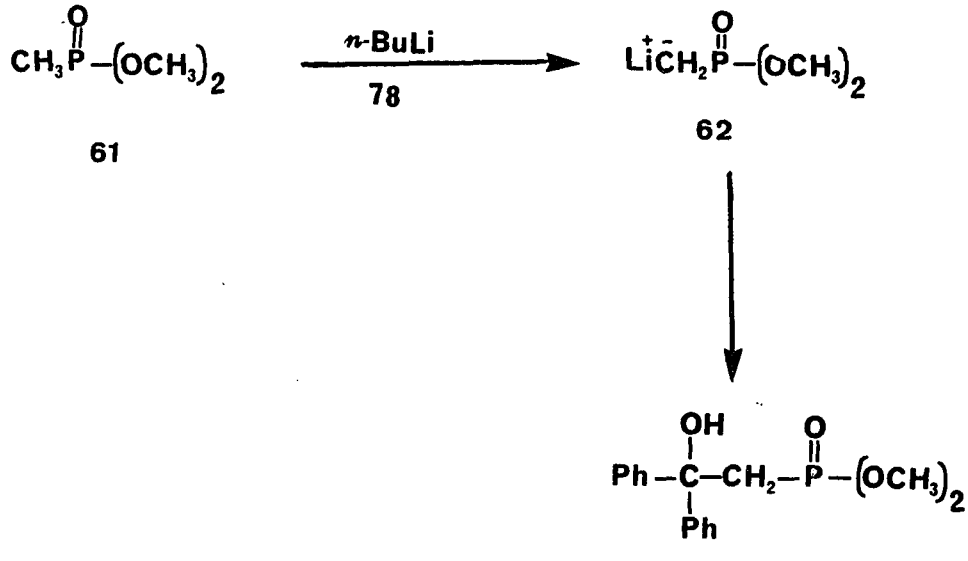


Scheme XIII

6. ∝-Lithiophosphonates in Syntheses

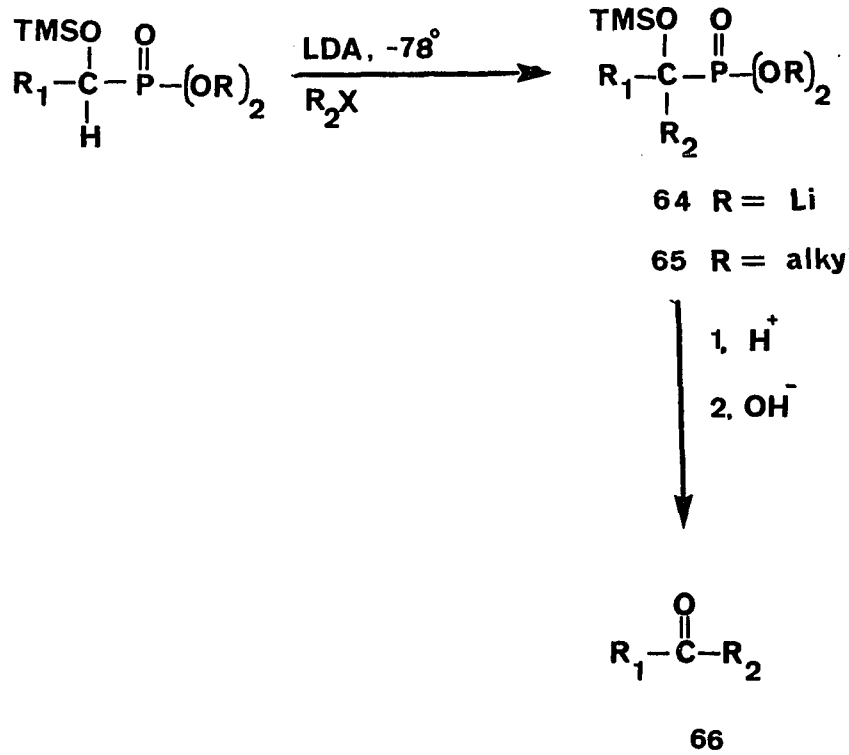
The facile generation of the carbanions in the ∝-position relative to a phosphonate functionality and their high reactivity toward certain electrophiles has been used in organic synthetic applications. In particular, dialkyl ∝-lithioalkylphosphonates have found wide application in recent years for natural product syntheses.

Corey et al.[75] reported the formation of the dimethyl ∝-lithiomethylphosphonate (62) from the reaction of the parent phosphonate (61) with one equivalent of n-butyl lithium at -78°C in 5 minutes, and its subsequent reaction in situ with benzophenone leading to the formation of the β-hydroxy phosphonate (63) [Scheme XIV].



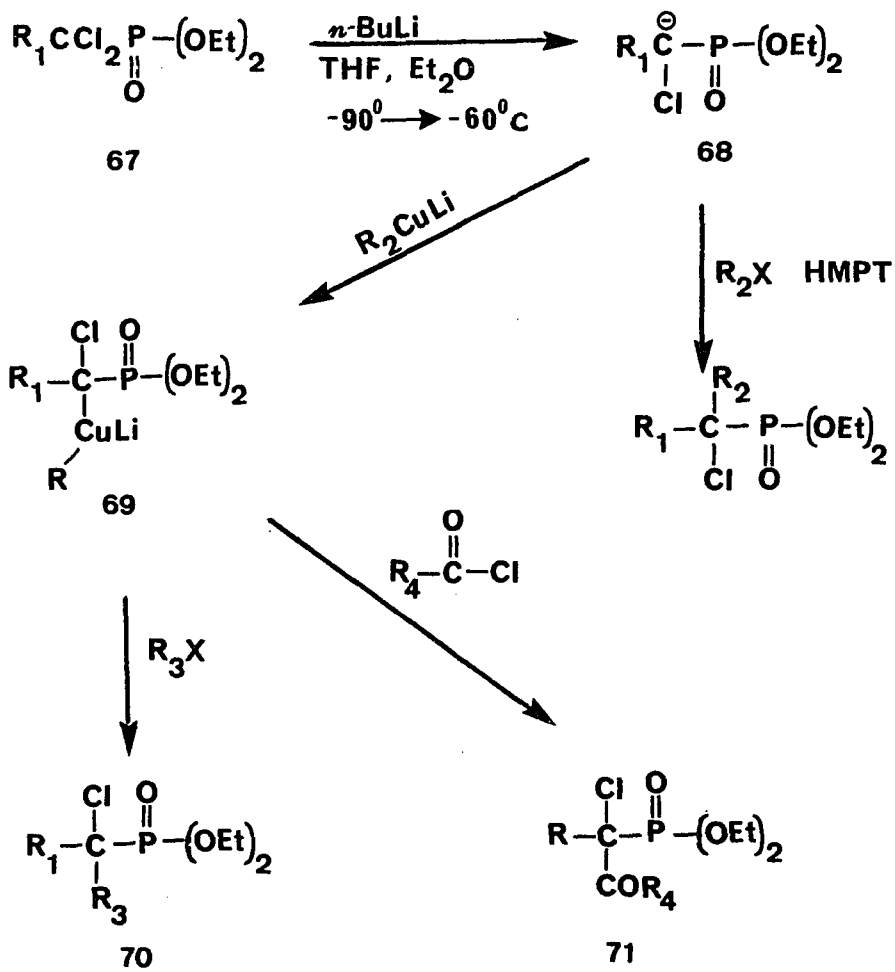
Scheme XIV

T. Hata et al.,[76] among others, have reported the generation of a series of ∝-lithio ∝-trimethylsilyloxy phosphonates (64) using lithium diisopropylamide at -78 C and their subsequent alkylation with a suitable alkyl halide, affording ∝-alkyl derivatives (65). These ∝, ∝-dialkyl phosphonates (65) were ultimately converted to their corresponding ketones (66) [Scheme XV].



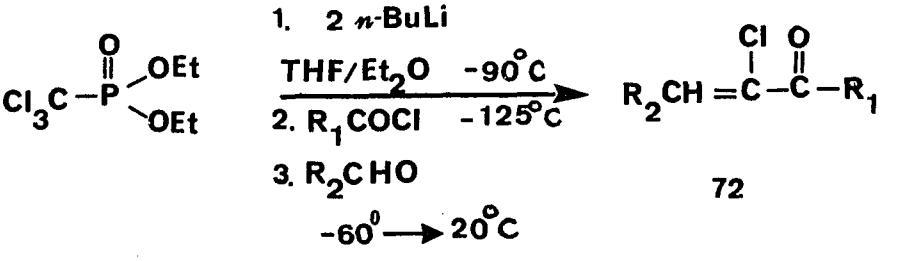
Scheme XV

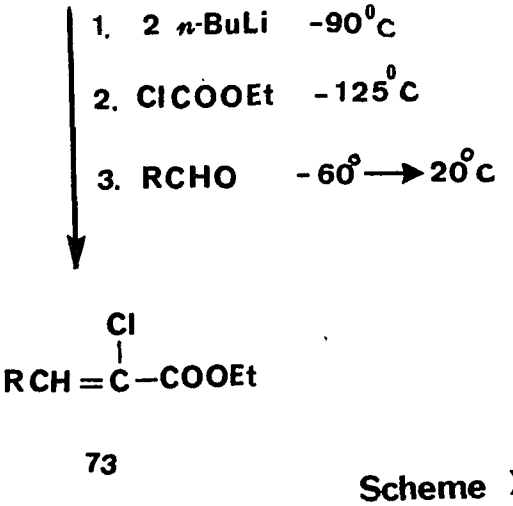
Villieras et al.[77] have described the lithiation of 1,1-dichloroalkanephosphonates (67) using n-BuLi in THF/ether. The ∝-lithiodialkylalkanephosphonate (68) thus derived was either alkylated in the presence of HMPT or converted to the corresponding ∝-lithiocuprate derivative (69) to increase the electrophilic character of the organometallic reagent (68). A variety of electrophiles including certain alkyl halides and acyl halides were employed in these reactions to produce ∝-alkyl (70) or acyl (71) derivatives [Scheme XVI].



Scheme XVI

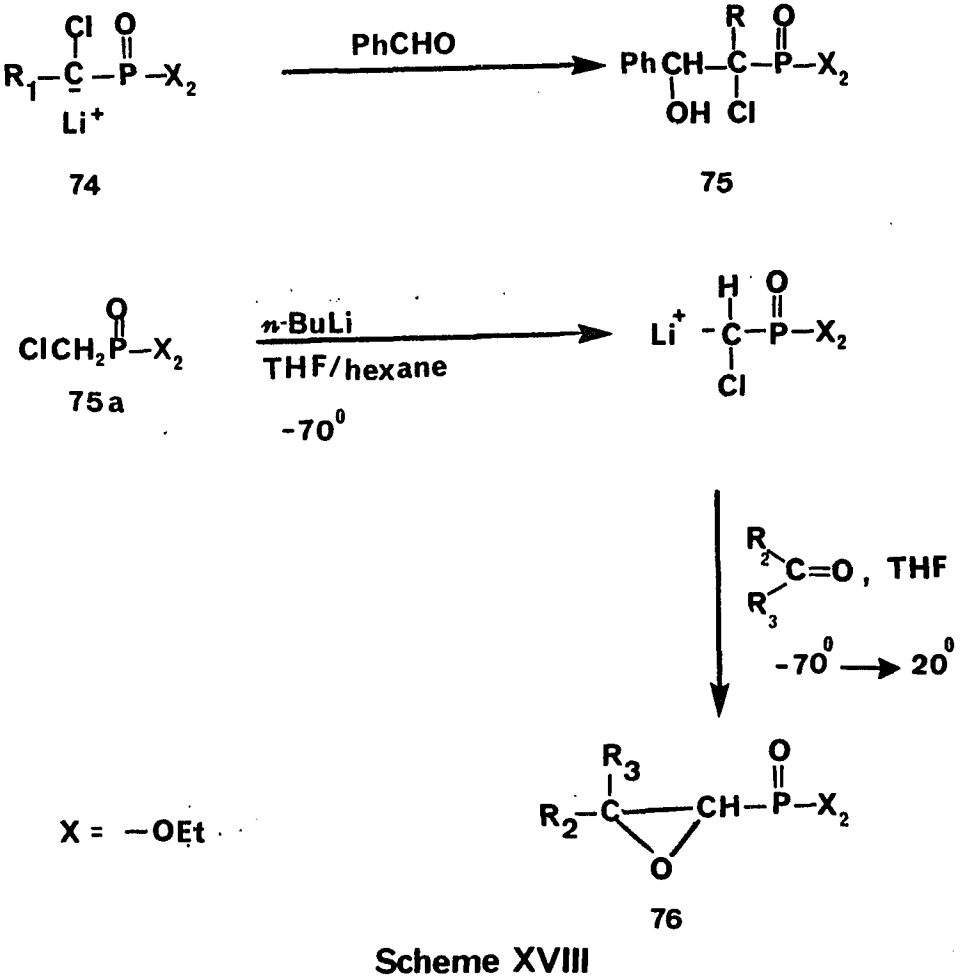
Villieras et al.[78] have further reported the generation of the ∝-lithio ∝-acyldialkylphosphonates directly from trichloromethyldiethylphosphonate and the subsequent Wittig-Horner reaction in situ with reactive aldehydes to form ∝-chloro- ∝,β-unsaturated ketones (72) having the (E) configuration. A similar reaction sequence employing ethylchloroformate yielded a mixture of (E) and (Z) isomers of the corresponding ∝-chloro- ∝,β-unsaturated ester (73) [Scheme XVII].





Scheme XVII

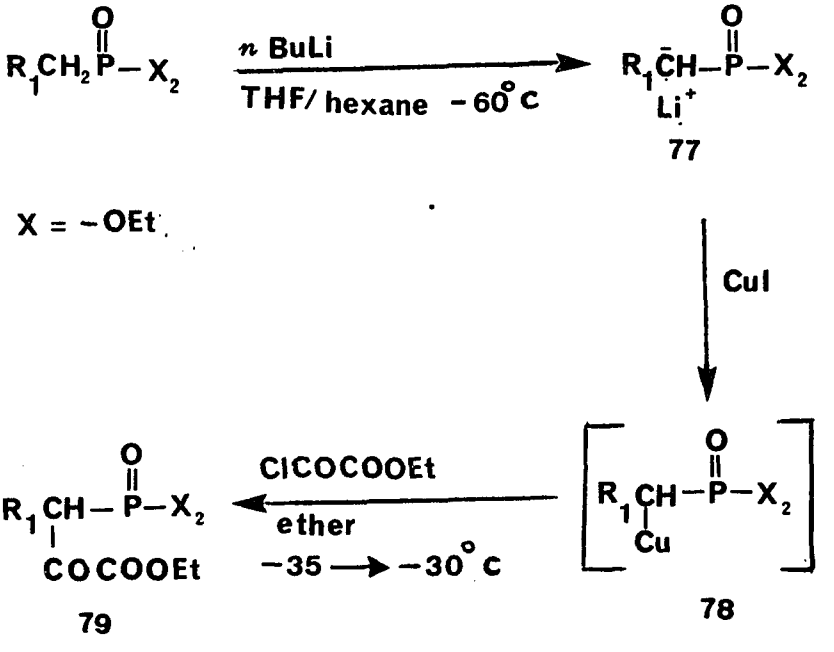
The chlorohydrins (75) have been prepared by the reaction of α-lithio-α chlorodiethylphosphonate (74) with benzaldehyde. Elimination of lithium diethyl phosphate was not observed. However, in the presence of one equivalent of HMPT the reaction afforded the α,β-epoxyphosphonate. Savignac et al.[79] have reported a one-step synthesis of diethyl 1,2-epoxyalkanephosphonates (76). Lithiation of chloromethyl diethylphosphonate (75a) followed by the reaction with a series of ketones led to the formation of (76) [Scheme XVIII].



Scheme XVIII

The epoxyphosphonates (76) derived from the unsymmetrical ketones were found to have the (E) configuration which is favored by the presence of the bulky substituents in the ketone. However the epoxyphosphonates derived from aldehydes afforded both (E) and (Z) isomers with the (E) form being preferred.

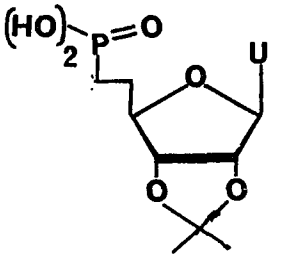
Savignac et al.[80] have converted the ∝-lithio-∝-alkyldiethylphosphonate (77) into its corresponding cupro derivative by reaction with one equivalent of cuprous iodide. This cupro derivative (78) has been shown to undergo reaction with ethylchloroformate to yield the β-ketoester derivative (79) [Scheme XIX].



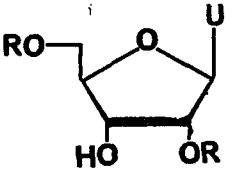
Scheme XIX

RESULTS AND DISCUSSION

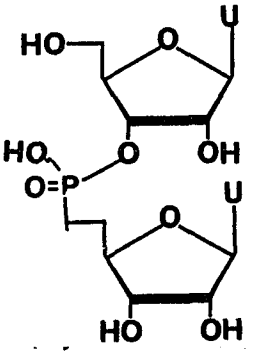
The work presented here is concerned with the synthesis of the key precursors (13) and (14) which were employed in the synthesis of the desired dinucleotide system (12).



13

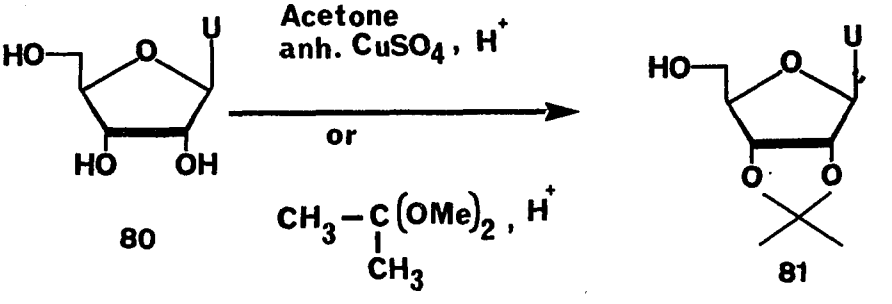


14

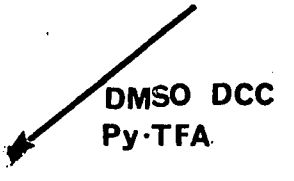


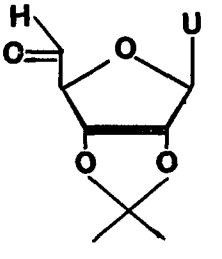
12

The commercially available uridine (80) was converted to 2',3'-O-isopropylidene uridine (81) by two different methods. The method employing Acetone/H^+/anh. $CuSO_4$ system was found to be more convenient and productive in terms of handling and yield. Use of the alternate method involving 2,2-dimethoxypropane makes that method a bit more expensive and the yields were consistently moderate in our hands.



80 81



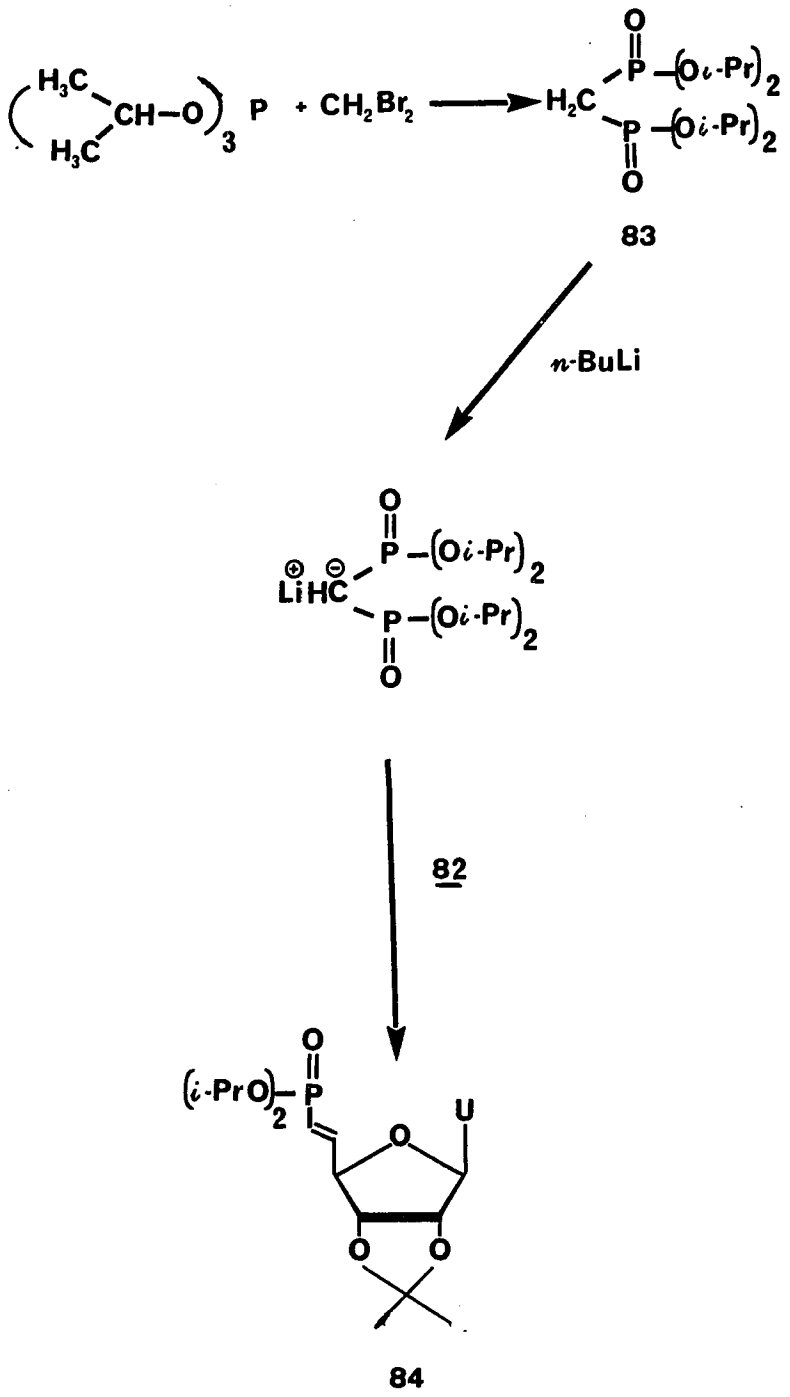


82

Further derivatization of (81) requires oxidation of the primary alcohol function. The more common oxidation methods for the conversion of a primary alcohol into its corresponding aldehyde have remained unsatisfactory in the area of carbohydrate/nucleoside chemistry. Moffatt et al.[61] have investigated the DMSO/DCC/H^+ oxidation in great detail and found it to be a superior method. We attempted the Swern ($COCl_2$ /Et_3N) and pyridinium chlorochromate oxidation methods on the 2',3'-O- isopropylidene uridine but met with no success. However, when compound (81) was subjected to the Moffatt oxidation employing DCC/DMSO/pyridinium trifluoroacetatate system, the corresponding 5'-aldehyde (82) was obtained in fair yields based on its NMR spectrum.

The aldehyde (82) decomposed on standing and generally was used immediately in the subsequent reactions. Its isolation has proved to be difficult and was used in situ in subsequent reactions. In the preparation of this aldehyde it was found necessary a) to render the 5'-hydroxy compound (81) anhydrous by repeated evaporations (2-3 times) with anhydrous pyridine and complete removal of pyridine on the vacuum pump, and b) to use the freshly distilled, dry DMSO.

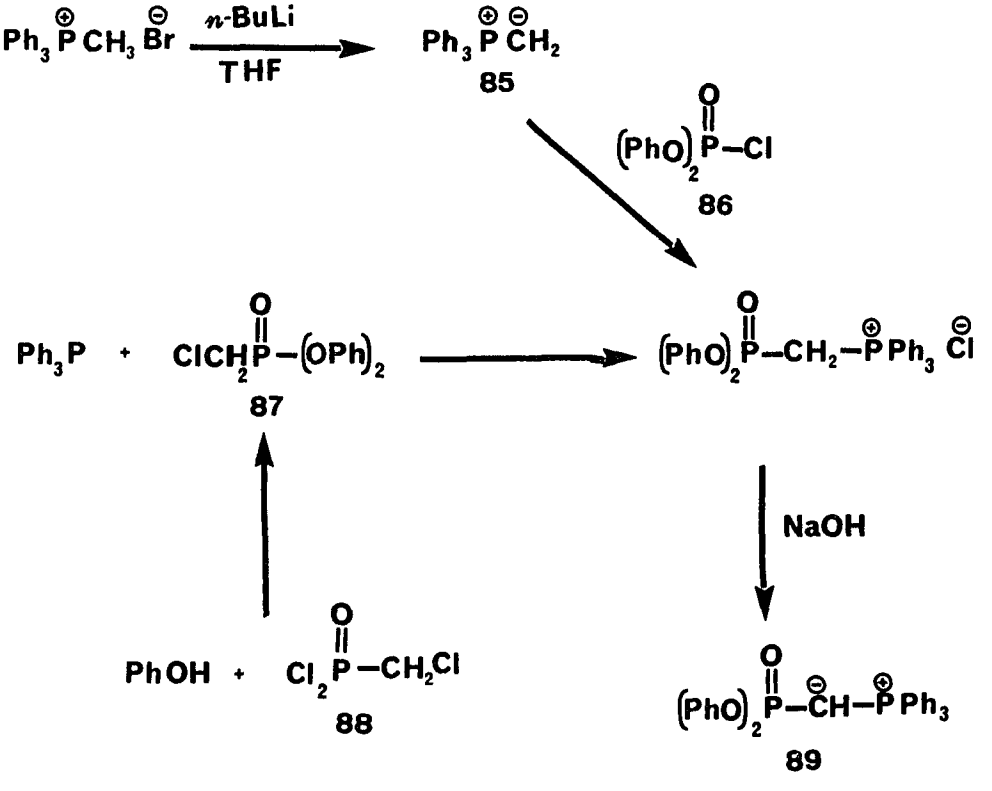
Tetraisopropylmethylenebisphosphonate (TIMBP)[81] (83)


H3C
CH–O
H3C
3
P + CH2Br2
H2C
P –(Oi-Pr)2
P –(Oi-Pr)2
O
83
n-BuLi
LiHC
82
(i-PrO)2
U
84

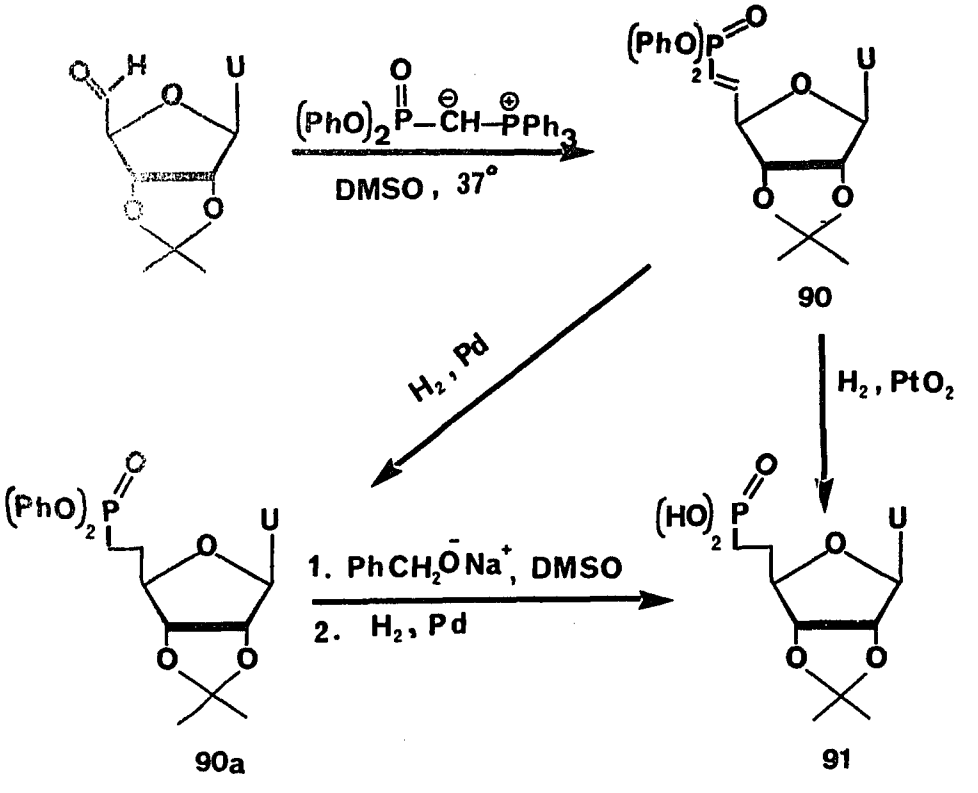
has been found to be a very good phophonylating reagent in this laboratory,[82] and afforded good yields of the corresponding vinylic phosphonates when allowed to react with a variety of carbonyl compounds. This reagent was prepared by the reaction of triisopropyl phosphite with dibromomethane and can be stored at room temperature. When the aldehyde (82) reacted with TIMBP in the presence of n-BuLi and solvents such as THF, DME and heptane, the desired α,β—unsaturated product (84) was not obtained. This result could be attributed to the presence of DMSO from the synthesis of aldehyde (82) in the reaction medium. Highly polar solvents have previously been found unsatisfactory for similar transformations in this laboratory. Partial insolubility of the (82) was also observed when using heptane and DME as the solvents in this reaction. Thus, attempts to synthesize the α,β-unsaturated diisopropyl phosphonate (84) remained unsuccessful.

Another approach to synthesize the α,β-unsaturated phosphonate derivative was undertaken. Moffatt et al.[71] have reported the synthesis of a stable Wittig reagent, diphenyl triphenylphosphoranylidenemethylphosphonate (89). This reagent was prepared by two different methods: a) addition of diphenyl chlorophosphate (86) to two equivalents of tripheylmethylphosphorane (85) and b)

reaction of diphenyl chloromethylphosphonate (87) prepared from phenol and chloromethyl phosphonic dichloride (88) with triphenyl phosphine (18). The former method afforded poor yields and isolation of the product was very difficult; the latter method gave much better results and was the preferred method.



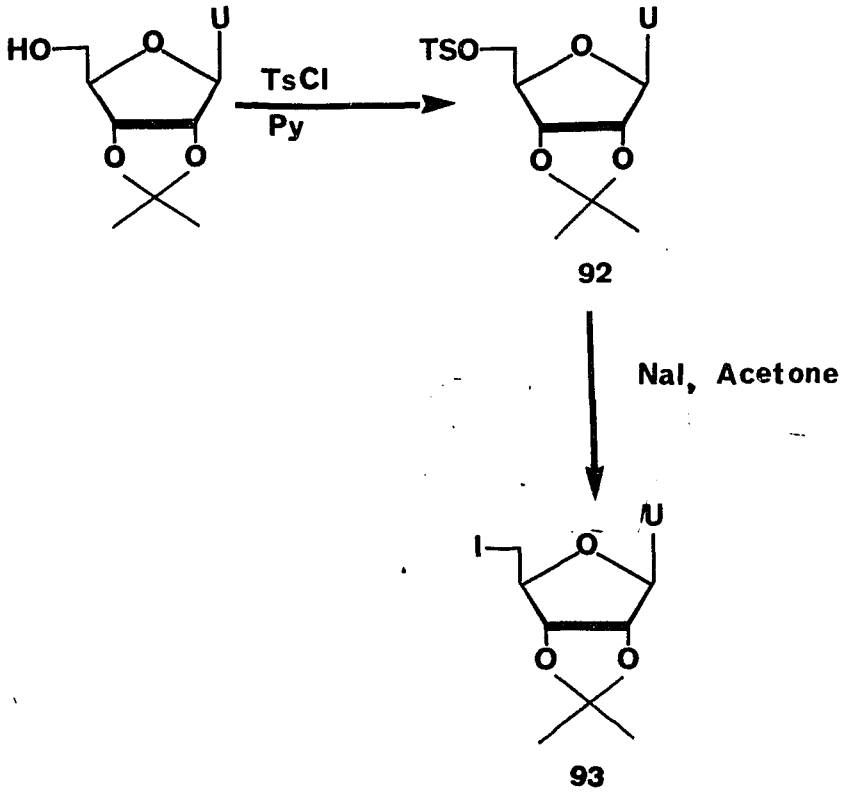
Based on Moffatt's oxidation studies, the Moffatt oxidation was assumed to have formed the aldehyde (82) in quantitative yield. Prior to the subsequent reaction, the precipitates of the side product, dicyclohexylurea, were filtered off. The aldehyde thus obtained <u>in situ</u> was allowed to react directly with two equivalents of the Wittig reagent (89) in anhydrous DMSO at 37 C for 20 hours. The temperature of the reaction was found to be critical as the aldehyde (82) decomposed readily at higher temperatures (greater than 40-45°C). Evaporation of DMSO under reduced pressure followed by column chromatography afforded the α,β-unsaturated diphenylphosphonate (90) in moderate yields.



Conversion of the α,β-unsaturated diphenyl phosphonate (90) into its corresponding saturated 6'-phosphonic acid (91) was achieved by two different routes: a) the phosphonate ester (90) was hydrogenated (H_2 /Pd), converted into its corresponding dibenzyl ester by reaction with 4 equivalents of sodium benzoxide in DMSO, and hydrogenated again; b) the phosphonate ester (90) was converted directly into the desired phosphonic acid (91) by hydrogenation over Adam's catalyst in abs. ethyl alcohol, a simpler approach. The NMR (D_2O) of the product indicated the disappearence of the phenyl groups and the presence of the HOD peak at 5 ppm. A side product was observed and presumed by NMR evidence to be a product in which one phenyl group was still uncleaved.

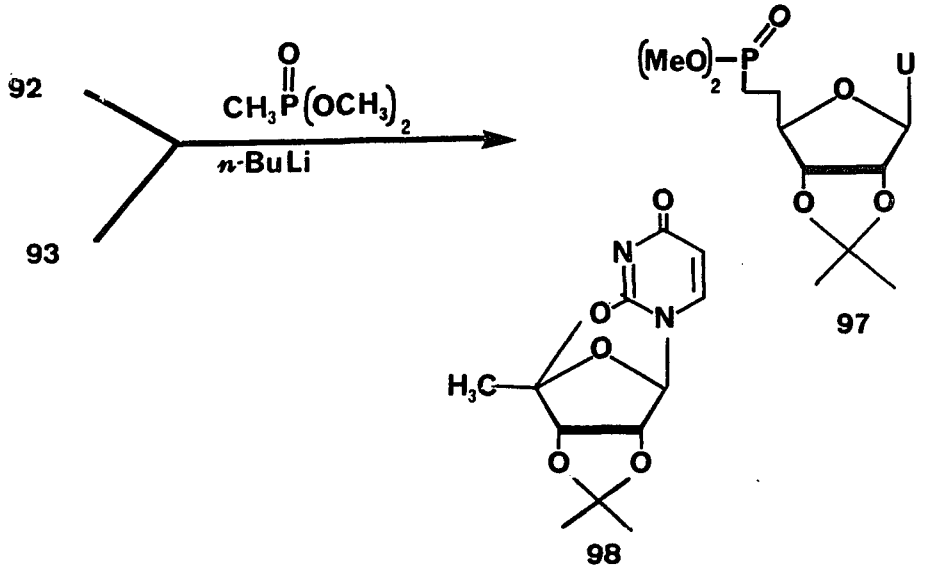
Prior to the observation above, other efforts to synthesize the 6'-phosphonic acid (91) were undertaken. Unsatisfactory yields in many of the aforementioned transformations prompted us to look into other synthetic methodology for the synthesis of 6'-phosphonic acid (91). It was envisioned that if the 5'-hydroxyl group in 2',3'-O-isopropylidine uridine (81) could be substituted by a group which then in turn can participate in a subsequent S_N2 type reaction with a suitable α-lithio phosphonate, the desired 6'-phosphonic acid (91) could be formed. To our knowledge, we found no precedent for

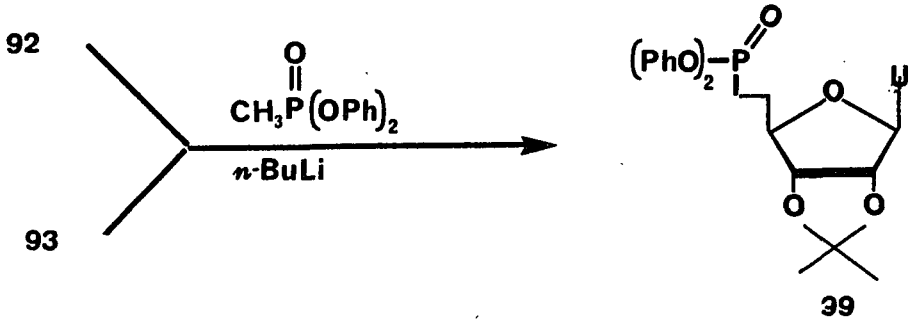
such transformation in the literature.

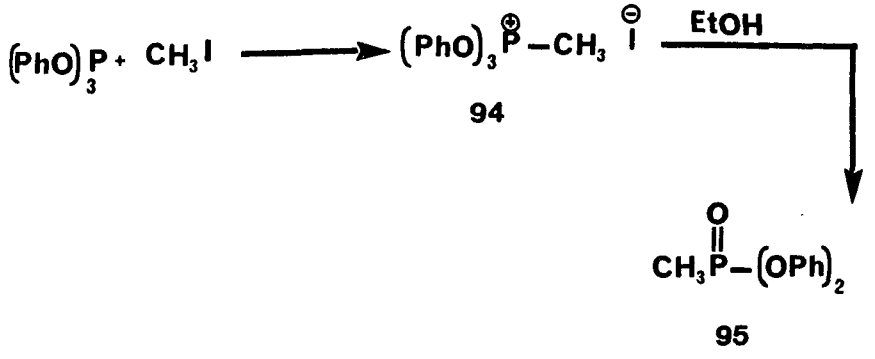


In order to study this approach, the 2',3'-O-isopropylidine uridine was converted into its 5'-tosylate dervative (92) which underwent the Finklestein reaction (NaI/acetone) to afford the corresponding 5'-iodo derivative (93). This compound was alternatively synthesized in one step by reaction of 2',3'-O-isopropylidine uridine with triphenyl phosphite methiodide (94) which was prepared by heating triphenyl phosphite with methyl iodide. Triphenyl phosphite methiodide[88] was converted into diphenyl methyl phosphonate (95) by treatment with ethyl alcohol which

yielded ethyl iodide as the side product and the main product was obtained by vacuum distillation.



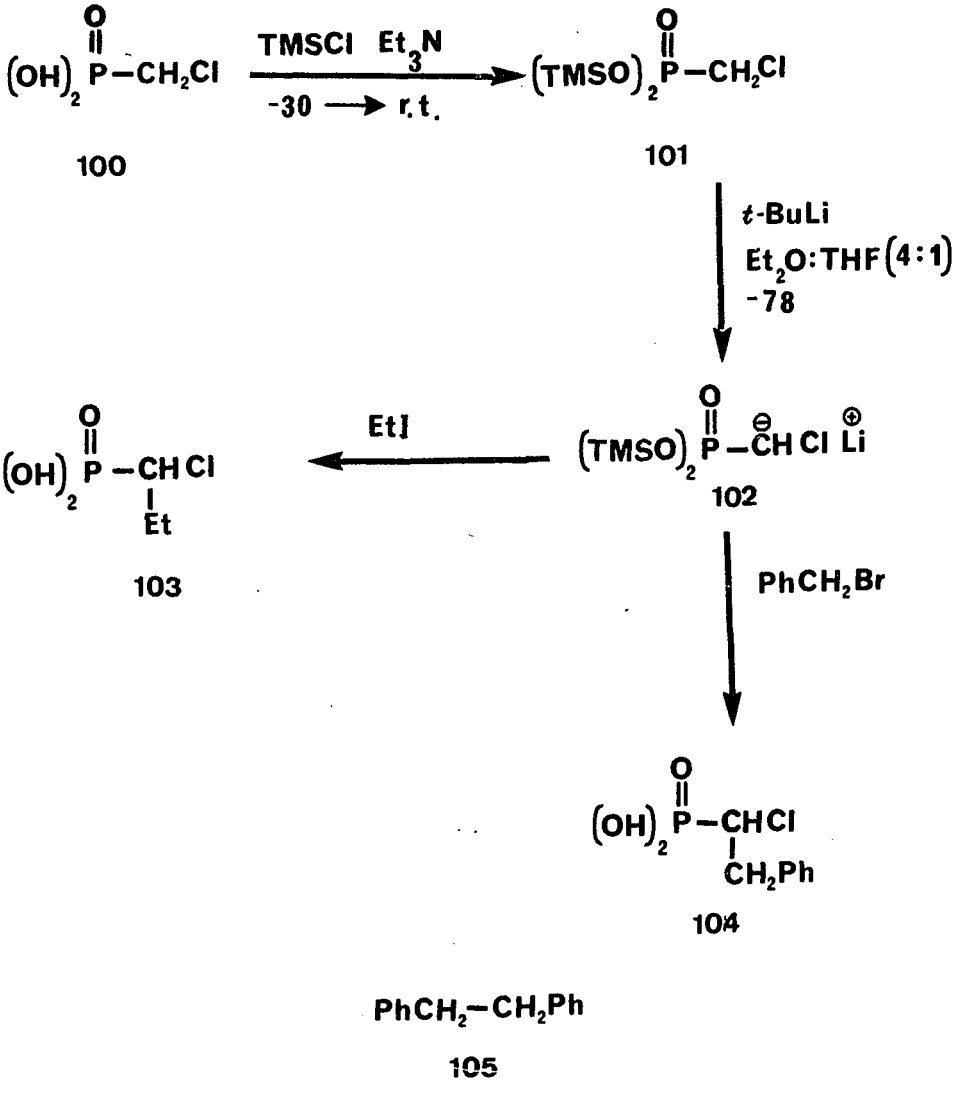




The 5'-tosyl (92) and 5'-iodo (93) derivatives were then subjected to reaction with the preformed ∝-lithio anions derived from dimethyl methylphosphonate (96) and diphenyl methylphosphonate (95) separately at -78°C. The reaction employing dimethyl methylphosphonate was successful affording the corresponding 6'-dimethyl phosphonate derivative (97) in low yields. The cyclic derivative (98) in the reaction involving iodouridine (93), was obtained as the side product. However, diphenyl methylphosphonate did not afford the desired diphenyl phosphonate (99). Due to the encouraging studies with dimethyl methylphosphonate reaction, we sought to do model studies involving simple halides such as ethyl iodide and benzyl bromide with ∝-lithio derivative of a commercially available material, chloromethyl phosphonic acid. It was hoped that successful carbon-phosphorous bond formation using this approach would lead to a new improved and shorter synthetic route to the desired phosphonic acid (91).

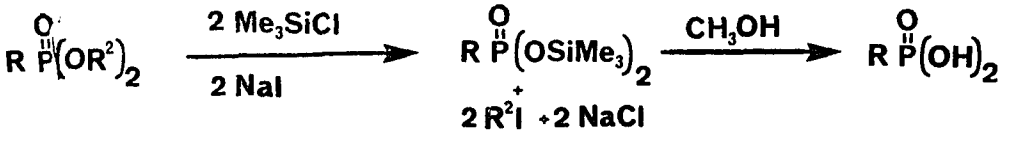
Chloromethylphosphonic acid (100) was silylated in the presence of triethyl amine to afford the bis-trimethylsilyl chloromethyl phosphonic acid (101). This material was distilled and obtained as a light brown oil. The NMR data supported the structure of this compound. ∝-Lithiation of compound (101) was done with two equivalents of n-BuLi in ether:THF (4:1) at -78°C and

the presumably formed α-lithio derivative (102) was then subjected to reaction with ethyl iodide and benzyl bromide respectively. These reactions did not afford the expected alkylated products (103) and (104). In the instance of benzyl bromide significant amount of a byproduct, bibenzyl (105) was isolated.

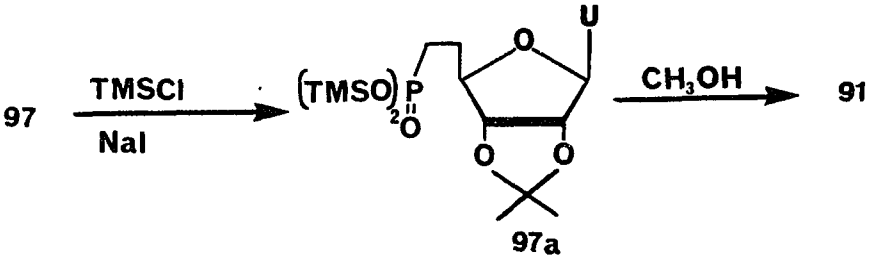


100 101 102 103 104 105

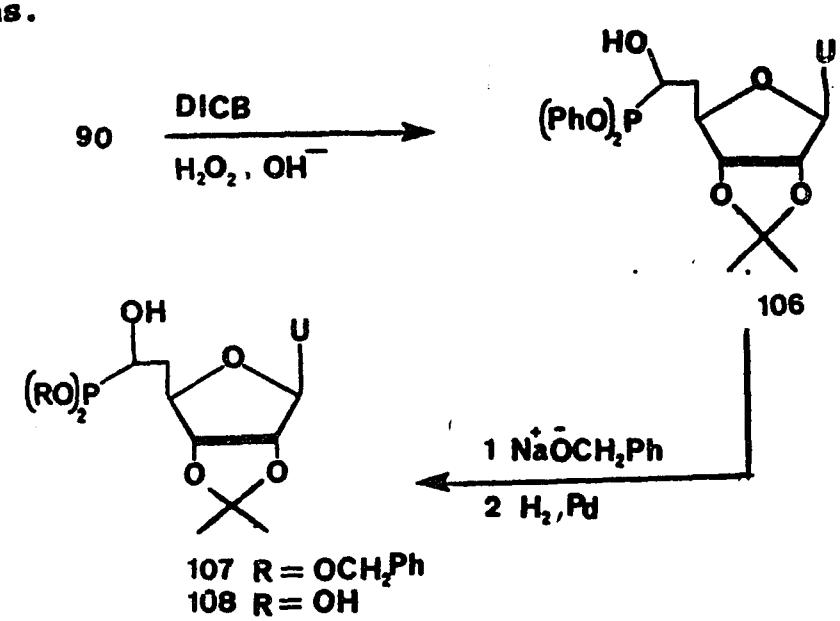
McKenna et al.[84] have reported the use of bromotrimethylsilane for the conversion of dialkyl phosphonates into the corresponding bis(trimethylsilyl) esters under mild conditions. A few years later, T. Morita et al.[85] described the scope and limitation of dealkylation of dialkyl phosphonates and acetals with chlorotrimethylsilane/sodium iodide. The dealkylation shown below, monitored by proton NMR, proceeded to completion within 15 minutes at room temperature.



When the compound (97) was subjected to Morita's dealkylation conditions, an exothermic reaction took place which was consistent with the dealkylations of dialkyl phosphonates. However, following the treatment of the presumably formed bis(trimethylsilyl) phosphonate (97A) with methanol, the material obtained was extremely hygroscopic. While the NMR of the crude material was indicative of the dealkylation, purification attempts led to the decomposition of the material.



∝-Hydroxyphosphonates/nucleotides have also been of interest in this laboratory. Hence we sought to explore the hydroboration reaction of the ∝,β-unsaturated diphenyl phosphonate ester derivative (90). In our initial attempt, the ester (90) on hydroboration with diisopinocamphenylborane[96] afforded the diphenyl ∝-hydroxyphosphonate derivative (106) in very low yields. Steric factors at 5' and 6' positions may have played some role in this reaction and the position of the hydroxyl group would presumably depend on these factors as well. Conversion of the hydroxy ester (106) into dibenzyl ester derivative (107) by reaction with sodium benzoxide in DMSO proceeded in fair yield and hydrogenation(H_2 /Pd) yielded the ∝-hydroxyphosphonic acid (108). Reproducibility of the hydroboration reaction has presented great difficulties which we realized when we sought to employ it in future coupling reactions.



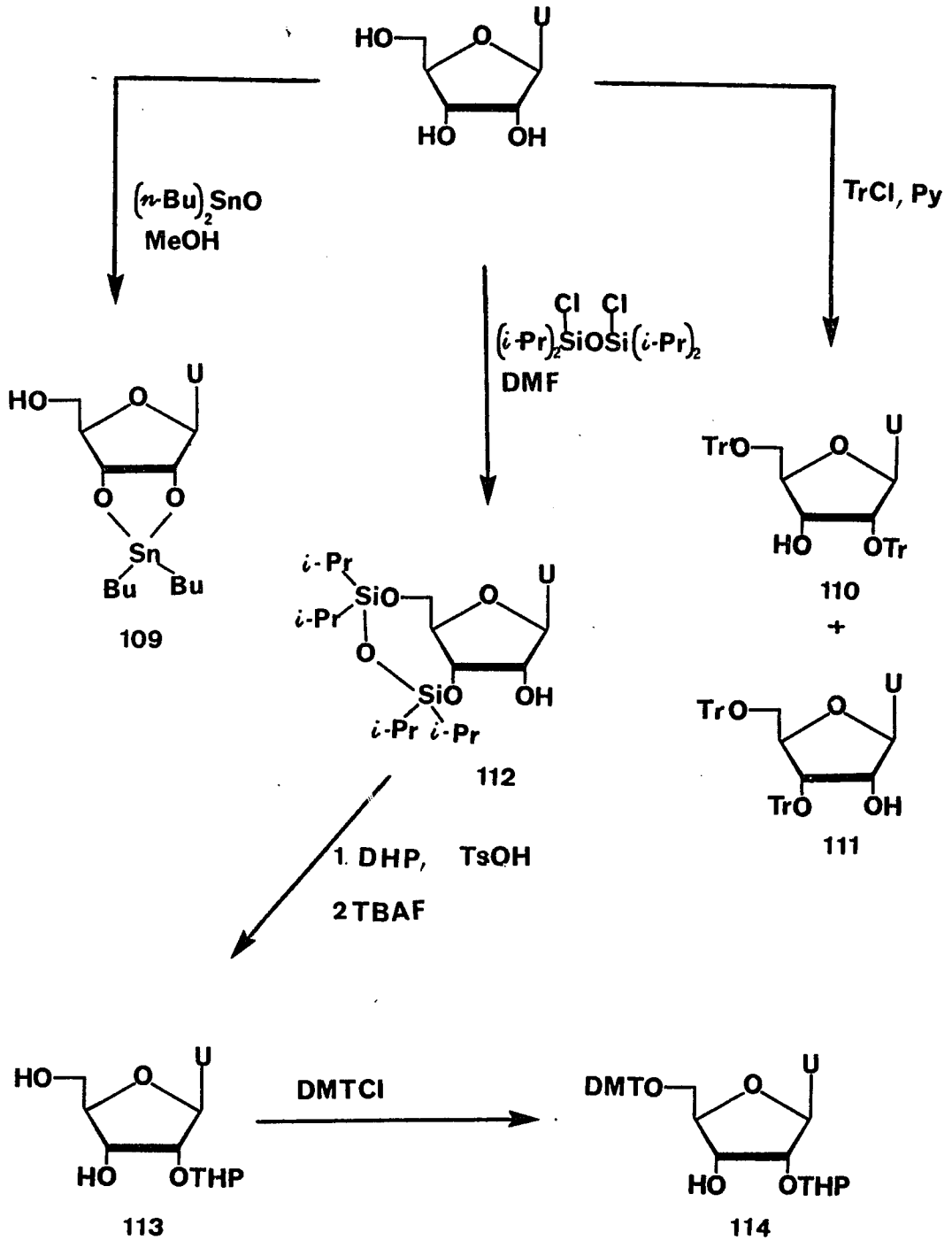
107 R = OCH_2Ph
108 R = OH

The synthesis of the key precursor (14) was undertaken with different approaches at different stages in the course of the synthetic work. Uridine was tritylated in pyridine solution to yield the 2',5'-di-O-trityluridine (110) and 3',5'-di-O-trityluridine (111).[43] The compound (110) was selectively crystallized out from benzene-ether solvent system and was the major product.

Uridine was treated with the bifunctional protecting group, 1,1,3,3,- tetraisopropyldisiloxane to yield the 3',5'-bis-O-silylated derivative (112) which was subsequently treated with dihydropyran in the presence of p-toluenesulphonic acid to protect the 2' hydroxyl group. This was followed by the use of tetra n-butylammonium fluoride resulting in the formation of a diastereoisomeric mixture of (113). Silica gel chromatography did not afford a complete separation of the diastereoisomeric mixture as some contamination was invariably detected after repeated chromatography. Tritylation of (113) afforded the 2',5'-O-protected uridine in excellent yield.[54]

Finally, uridine was converted to its di-n-butylstannylene derivative (109) which was not explored for further derivatization.[38]

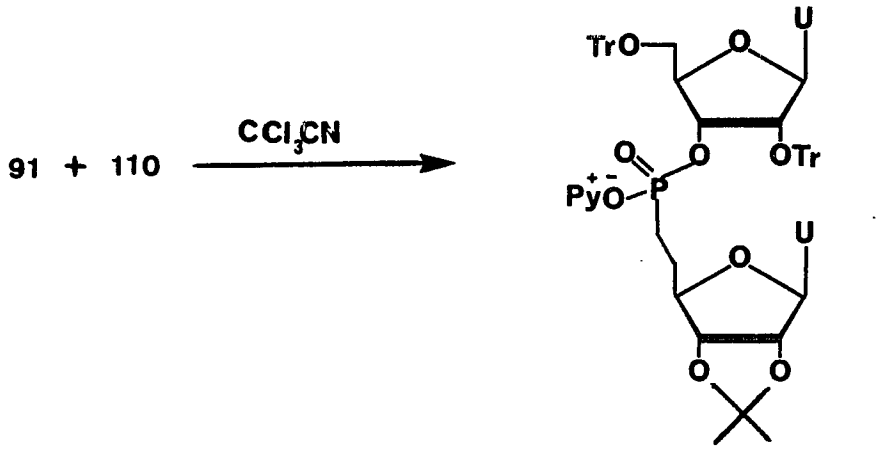
Our initial attempts to achieve the coupling between (91) and (110) to produce the dinucleotide (115)


HO
O
U
HO
OH
(n-Bu)2SnO
MeOH
TrCl, Py
Cl Cl
(i-Pr)2SiOSi(i-Pr)2
DMF
HO
O
U
O
O
Sn
Bu
Bu
109
i-Pr
SiO
i-Pr
O
SiO
OH
i-Pr
i-Pr
112
TrO
O
U
HO
OTr
110
+
TrO
O
U
TrO
OH
111
1. DHP, TsOH
2 TBAF
HO
O
U
HO
OTHP
113
DMTCl
DMTO
O
U
HO
OTHP
114

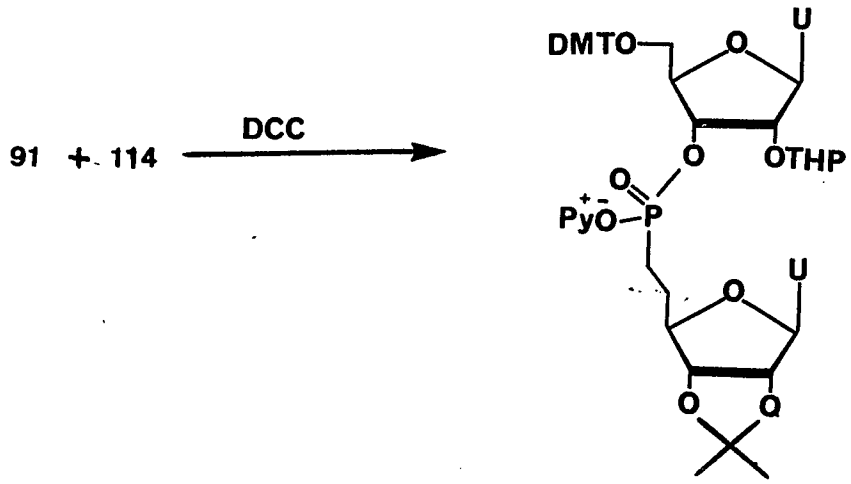
using trichloroacetonitrile as the coupling reagent, remained unsuccessful. The HPLC examination of the crude mixture revealed several peaks. Column chromatographic separation proved difficult to overcome the contamination problem, due to the close R_f values.

Similarly, the coupling attempts between the pyridinium salt of (91) and (114) using dicyclohexylcarbodiimide[87] as the coupling reagent were met without success. The difficulty in separation of the product mixture could concievably be attributed to the stereoisomeric mixture of (114) and a partial loss of the dimethoxytrityl group.

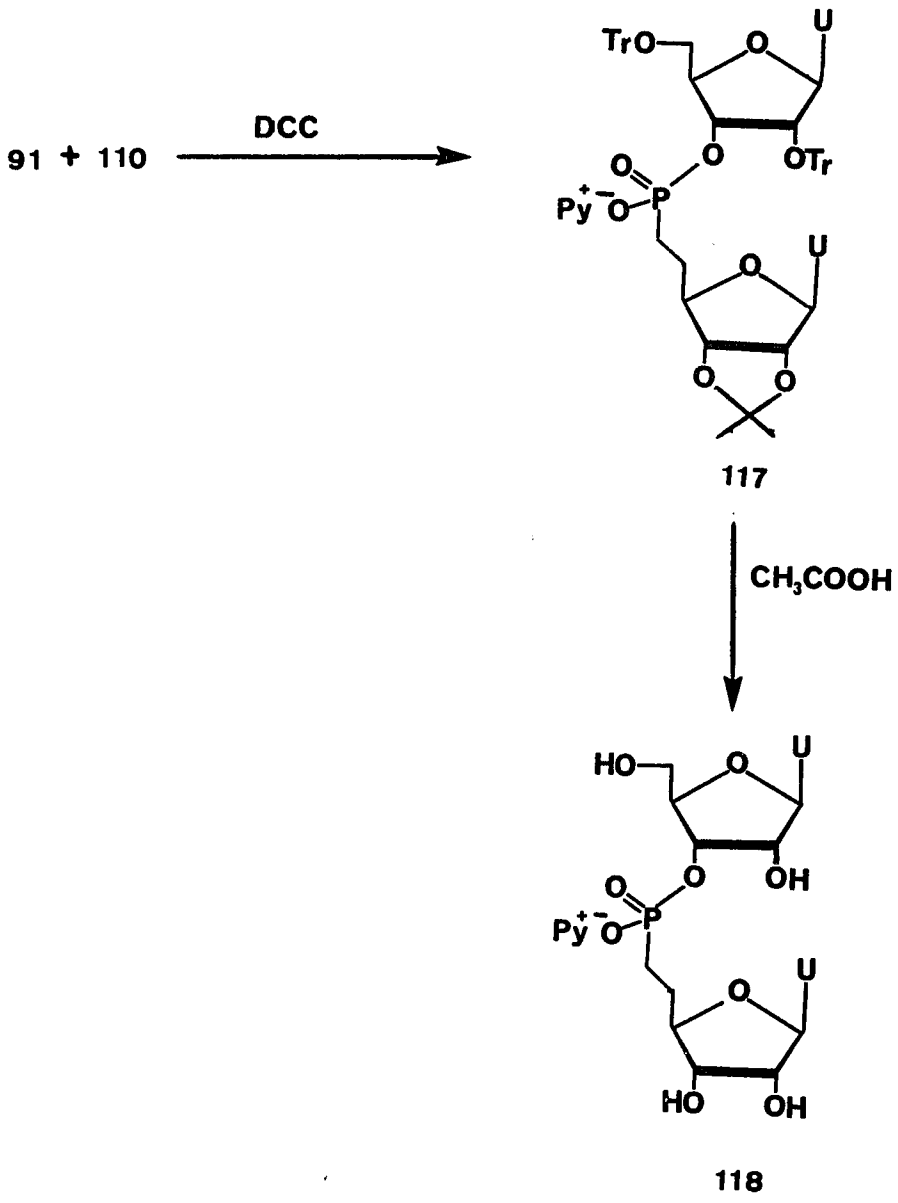
A different approach was taken with the DCC coupling between the pyridinium salt of (91) and (110). The reaction was thought to have formed the protected dinucleotide (117) which was not isolated. Following the workup, the treatment of (117) with 80% acetic acid removed the protecting groups and yielded the deprotected dinucleotide (118).



115



116


91 + 110
DCC
TrO
U
O
OTr
Py+ O
P
117
CH3COOH
HO
OH
118

EXPERIMENTAL

Dimethyl sulfoxide was stirred for 6 hours over calcium hydride and then vacuum distilled. Tetrahydrofuran was refluxed over lithium aluminium hydride for 2 hours and freshly distilled prior to use. Pyridine was refluxed over calcium hydride and was distilled prior to use. Acetone was refluxed over potassium permanganate and distilled; the distillate was shaken with anhydrous calcium sulfate, filtered and redistilled. Dimethyl formamide was heated over barium oxide at 160 C for 2 hours and then distilled under vacuo. Benzene and dioxan were dried over sodium wire. Acetonitrile was stirred over calcium hydride for 4 hours and distilled using the fractionating column. Trimethylchlorosilane was distilled from calcium hydride prior to use. p-Toluenesulfonyl chloride was dissolved in chloroform (about 2.5 mL/g) and diluted with 5 volumes of light petroleum ether (b.p. 40-60); the solution was filtered and treated with decolorizing charcoal, filtered and concentrated to afford the crystals. TLC was performed on KODAK 13179 plates with fluorescence indicator or MACHERY NAGEL Sil-N-HR plates. IR spectra were obtained on Perkin-Elmer and the NMR spectra were obtained on Varian EM-360 (60 MHz) and Brucker WP-200SY (200 MHz). Column chromatography was performed generally with Silica Gel (60-80 mesh).

Preparation of 2',3'-O-Isopropylideneuridine (81): Method A. Uridine (10 g, 0.04 mol) and toluenesulphonic acid monohydrate (1 g, 0.13 mol) were stirred with anhydrous 2,2-dimethoxypropane (27.5 g, 0.26mol) and acetone (150 mL) at 20 C for 16 hours. Freshly prepared saturated solution of methanolic sodium methoxide (100 mL) was added to the mixture to neutralize the acid and the products were filtered through a sintered glass funnel. The filtrate was evaporated under the reduced pressure which gave a viscous glass. This material was mixed with 25 mL of water and the water was evaporated on a rotary evaporator. This was repeated twice more and and then the residual solid was recrystallized from water (charcoal treatment) to yield 5.3 g (46%) of the compound (81), m.p. 161-163 C.

Method B.[32] To a 500 mL Pyrex bottle containing 250 mL of dry acetone were added 10 g (0.04 mol) of dry uridine, 20 g of anhydrous copper sulfate and 0.25 mL of concentrated sulfuric acid. The bottle was tightly corked with a rubber stopper and the suspension was shaken on a shaker at room temperature for 48 hours. The mixture was suction filtered through a sintered glass funnel and the bottle, the cork and the copper sulfate were washed with five 25 mL portions of pure dry acetone. The clear filtrate and the washings were combined and transferred to a 500 mL Pyrex bottle

containing 10 g of dry calcium hydroxide powder, the bottle was tightly corked and the suspension was shaken at room teperature for 1 hour to neutralize the sulfuric acid. The mixture was filtered through a Celite bed (2g in 20 mL acetone) and again the bottle was washed well with five 25 mL portions of pure acetone. The combined filtrate and the washings were evaporated under reduced pressure yielded a solid white mass which on recrystallization from absolute methyl alcohol yielded 9.5 g (82%) of the compound (81), m.p. 160-162 C. ^{1}H-n.m.r. (dimethylsulfoxide-d_6): 1.34 (s, 3, CMe_2), 1.55 (s, 3, CMe_2), 3.35-3.6 (m, 2, C_5' H), 4.0 (hex, 1, C_4' H), 4.6 (q, 1, C_3' H), 4.7 (q, 1, C_2' H), 5.7 (d, 1, J=7 Hz, C_5 H), 5.85 (d, 1, J=3 Hz, C_1' H), 7.8 (d, 1, J=7 Hz, C_6H)

Preparation of 2',3'-O-isopropylideneuridine-5'-aldehyde (82): 2',3'-Isopropylideneuridine (0.85 g, 3 mmol) was dissolved in 9 mL of anhydrous DMSO at room temperature under nitrogen atmosphere. To this mixture were added DCC (1.85 g, 9 mmol) followed by pyridinium trifluoroacetate (0.3 g, 1.5 mmol). The stoppered reaction mixture was stirred at room temperature for 20 hours and then filtered to remove the precipitates of dicyclohexylurea. The precipitates were washed once with 5 mL of anhydrous DMSO and the filtrates combined. This solution was used subsequently without further purification. NMR of this crude mixture indicated the

presence of the aldehyde group in 9-10 ppm region.

In another variation of this oxidation, the reaction was carried out in the mixture of equal amounts of DMSO and benzene to avoid coagulation of the precipitates of dicyclohexylurea. After 20 hours of stirring the mixture was diluted with 10 mL of anhydrous ether. Anhydrous oxalic acid (0.8 g, 9mmol) was added to the reaction mixture and the gas (dimethyl sulfide) began to evolve quickly through the nitrogen bubbler. After the cessation of the gas evolution 15 mL of water were added and the dicyclohexylurea precipitates were removed by suction filtration. The reaction flask was washed once with 5 mL of water and the solution filtered. The filtrate and the washing were combined and transferred to a separatory funnel. The lower aqueous layer and any traces of ether were evaporated under reduced pressure. Then removal of most of the water and DMSO was carried out under vacuo with a warm water-bath providing a temperature not exceeding 45 C. After each run the presence of the aldehyde group was confirmed by NMR spectroscopy. ^{1}H-n.m.r. (crude): 1.3 (s,3, CMe_2) 1.45 (s, 3, CMe_2), 9.3 (s, 1, CHO)

Preparation of Tetraisopropyl-methylene-bisphosphonate (83): Triisopropyl phosphite (312.4 g, 1.5 mol) and dibromomethane (78 g, 0.5 mol) were combined in a 500 mL flask fitted with a thermometer, a

magnetic stirrer and a 24" long fractionating column for separating the isopropyl bromide by-product from the reaction mixture. The temperature inside the fractionating column was maintained in 65-70 C range by circulating hot water through it. This temperature was sufficient to retain the unreacted starting material in the reaction mixture and the isopropyl bromide to be distilled off. A Dewar condenser was attached to the fractionating column by means of a bent tube which was wrapped with a heating tape hooked up to a thermostat. The purpose of the tube was to keep the tube hot (65 C) in order to facilitate the collection of isopropyl bromide in a round bottom flask attached to the Dewar condenser. The Dewar condenser was cooled with dry-ice/isopropyl alcohol mixture. The system was protected from the atmospheric moisture by a drying tube attached to the Dewar condenser. The mixture was gently heated to 145 C in an oil-bath. At this time, the temperature of the oil-bath was maintained at 180 C. The reaction took place at 145 C and the mixture began to reflux. The temperature of the reaction mixture stayed at 145 C for 3-4 hours and gently rose. It was further raised to 185 C over a 3 hour period and kept constant for additional 2 hours. The mixture was allowed to cool down to room temperature. The excess triisopropyl phosphite was removed from the reaction mixture by vacuum distillation, bp 35-45/.01 mm. Further distillation gave

127 g (74%) of bp 95-100 /0.01 mm.

Preparation of Triphenylphosphoranylidenemethylphosphonate (89): Method A. To a solution of n-BuLi (31 mL of 1.6 M solution in hexane, 50 mmol) in 150 mL dry THF were added additional 200 mL of dry THF at room temperature under nitrogen atmosphere, followed by triphenylphosphonium bromide (17.7 g, 50 mmol) over a period of 10-15 minutes. The color of the reaction mixture quickly changed from the initial reddish to yellow. The mixture was stirred for 4 hours at room temperature. Diphenyl chlorophosphate (6.6 g, 25 mmol) in 25 mL dry THF was added dropwise to the reaction mixture which was then stirred for 2 hours and filterd. The filtrate was extracted with three 75 mL portions of 3N HCl. The extracts were combined and neutralized with 3N NaOH. The solution was transferred to a separotory funnel and the upper layer was separated. The solvent was removed under reduced pressure and the residue was taken up in 15 mL of ethyl acetate. Ethyl acetate was carefully removed on a vacuum pump. This procedure was repeated several times, yielding a solid residue. Recrystallization from ethyl acetate gave 2 g of 89 (16%), mp 146-148 C, lit.[71] m.p. 149-150 C.

Method B. Chloromethyl phosphonyldichloride (46.8 g, 0.28 mol) was added dropwise to Phenol (53 g, 0.56

mol) with stirring under a nitrogen atmosphere at room temperature. The resulting pale yellowish solution was vacuum dried on pump for 2 h. Then the flask was attached to a condenser and the mixture heated at 155 C in an oil bath for 14 hours. The mixture was cooled and heated again at 150 C for 4 hours at 15 mm pressure to remove the HCl formed, using a solid NaOH trap cooled in an ice bath. The reaction mixture was cooled to room temperature. Vacuum distillation afforded 75.5 g (95%) of diphenyl chloromethylphosphonate (87), b.p. 145-155 C/ 0.1 mm.

87 (28.3 g, 0.1 mol) was injected into a three-necked 100 mL round bottom flask under a nitrogen atmosphere and triphenyl phosphine (26.2 g, 0.1 mol) was added to it in three portions. The mixture was stirred for 7 minutes and the slurry was vacuum dried for 45 minutes on the pump. The mixture was heated at 180 C under nitrogen atmosphere for 4 hours. The yellow opaque liquid was cooled to room temperature and 40 mL of anhydrous ether were added. The solid hard mass was scraped off, transferred to a sintered glass funnel and washed several times with 40 mL portions of anhydrous ether. This gave 36 g of the salt 88. 31.5 g of this material was stirred with 175 mL of water for 10-15 minutes. The solution was filtered and the filtrate neutralized with 6N NaOH solution. The filtration

followed by drying of the solid on a vacuum pump gave 27 g of the white compound which on recrystallization from ethyl acetate yielded 20 g (45%) of 89, m.p. 147-150 C. ^{1}H-n.m.r. (chloroform-d_1): 1.5 (s, 1, CH), 7.15 (s, 15, aromatic $\overset{+}{P}$), 7.35 (s, 10, phenoxy). Anal. calculated C:73.22, H: 5.15; found C: 73.02, H: 5.37.

Preparation of diphenyl 2',3'-O-isopropylidine-6'-phosphonate (90): The aldehyde 82 (0.56 g, 2 mmol) prepared in situ, was directly added to a stirring solution of the Wittig reagent 89 (2.4 g, 4 mmol) in 15 mL anhydrous DMSO and the mixture was stirred for 20 hours at 37 C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The solvent was removed under reduced pressure (pump), however traces of the solvent still remained. Column chromatography (silica gel) using a gradient of ethyl acetate in benzene yielded 0.4 g (40%) of (90), R_f= 0.2 in ethyl acetate:benzene (1:2), m.p. 142-143 C. ^{1}H-n.m.r. (chloroform-d): 1.34 (s, 3, CMe_2), 1.54 (s, 3, CMe_2), 4.7 (m, 1, $C_{4'}$ H), 4.9 (q, 1, $C_{3'}$ H), 5.1 (2d, 1, $C_{2'}$ H), 5.7-5.9 (m, 2, C_1H, $J_{1',2'}$= 2 Hz and C_5H, $J_{5,6}$= 8 Hz), 6.3 (m, 1, $C_{6'}$ H, $J_{6',5'}$= 17 Hz, $J_{H,P}$= 22 Hz), 7.25 (2m, 14, phenyl, $C_{5'}$ H and C_6H), 9.5 (br s, 1, NH). Anal. calculated C: 57.59, H: 5.02; found C: 57.67, H: 4.92.

Preparation of 5'-deoxy-2',3'-O-isopropylidenehomouridine-6'-phosphonic acid (91): The

compound 90 (500 mg, 1 mmol) was dissolved in absolute ethanol (50 mL) and hydrogenated over platinum dioxide (100 mg) at 60 lb/in for 4 hours on a Parr hydrogenator at room temperature. The reaction was monitored by TLC in ethyl acetate:hexane (3:1) which showed that the starting material (R_f =0.2) had completely disappeared and the product appeared at R_f =0. The catalyst was filtered off and washed with absolute ethanol (2x10 mL). The solvent was evaporated and the white residue was taken up in anhydrous ether (20 mL). The mixture was filtered and the precipitates were dried to afford 300 mg (89%) of the compound 91. It was further purified by crystallization from absolute ethanol to afford an analytical sample.

Alternatively, the compound (91) was prepared using a similar procedure to that described for the preparation of the compound (108) from (106). The proton n.m.r. (D_2O) showed the presence of the isopropylidene group, the vinylic protons of the uracil ring and the HOD peak. Calculated C: 41.55, H: 5.49; Found C: 41.61, H: 4.90.

Preparation of 5'-tosyl 2',3'-O-isopropylidene uridine (92): Under a nitrogen atmosphere in a 3-necked flask equipped with a septum and a nitrogen inlet were placed 2',3'-isopropylidene uridine (6.0 g, 21 mmol), 35 mL of anhydrous pyridine, and p-toluene sulphonyl

chloride (4.2 g, 22 mmol) and the mixture was stirred overnight at room temperature. Water (3 mL) was added and the mixture was left standing for 30 minutes. Additional water (75 mL) was added and the mixture was stirred for 15 minutes. The mixture was extracted with chloroform (3 x 150 mL). The combined organic extracts were washed with chilled sulfuric acid (5% conc. sulfuric acid in 150 mL water) followed by chilled sodium bicarbonate solution (5 % in 150 mL water). The organic layer was dried over anhydrous sodium sulfate and the solvent evaporated on a rotary evaporator. Pyridine from thus obtained yellow syrup was removed by repeated evaporations from absolute ethanol on a vacuum pump. The crude product was scraped off, transferred into another flask and dried on the pump at 45 C for 1 hour. The product (92) thus obtained (7.5 g, 82 %), was homgeneous on TLC (ethyl acetate:hexane 3:1, R_f= 0.24) and was used subsequently without further purification. ^{1}H-n.m.r. (chloroform-d): 1.34 (s, 3, CMe_2), 1.55 (s, 3, CMe_2), 2.52 (s, 3, Ar-CH_3), 4.37 (m, 3, C_5' H and C_4' H), 4.94 (m, 2, C_2' and C_3' H), 5.65 (d, 1, C_1' H), 5.75 (d, 1, C_5H), 7.35 (d, 1, C_6H), 7.75 (d, 2, Ar-H), 7.45 (d, 2, Ar-H), 10.0 (br s, 1, NH).

Preparation of 5'-iodo- 2',3'-O-isopropylidene uridine (93): Under a nitrogen atmosphere 92 (6.0 g, 14 mmol) was dissolved in dry acetone (60 mL) and sodium

iodide (6.0 g, 40 mmol) was added. The reaction mixture was refluxed for 90 minutes. During this period the solution turned brown and the precipitated of the product were deposited on the wall of flask. The mixture was cooled to room temperature and the solvent removed to dryness on a rotary evaporator. The pale yellow mass was taken up in absolute ethanol (20 mL) and the solvent evaporated off under vacuum. The procedure was repeated three times and then the dry product was dissolved in 60 mL of chloroform:water (5:1) solvent mixture. The mixture was transferred to a separatory funnel; aqueous solution of sodium thiosulfite was added dropwise until the yellow color of the top layer faded and the two layers were separated. The aqueous layer was washed with chloroform (3x20 mL) ; the combined organic extracts were washed once with water (10 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was evaporated on a rotary evaporator. The product thus obtained was taken up in ethanol (20 mL) and the solvent evaporated under a vacuum pump. This procedure was repeated three times. The product 93 (4.9 g, 90 %) thus obtained was chromatographically pure, however it may be recrystallized from acetone for improved color purity, m.p. 182-183 C, R_f = 0.29 in ethyl acetate:hexane (3:1). ^{1}H-n.m.r. (chloroform-d): 1.35 (s, 3, CMe_2), 1.64 (s, 3, CMe_2), 3.4-3.9 (m, 2, C_5' H), 4.34 (hex, 1, C_4'), 4.94 (q, 1, C_3'), 5.15 (q, 1, C_2' H), 5.75 (d, 1, C_1' H,

$J_{1',2'}$= 2 Hz), 5.9 (d, 1, C_5H, $J_{5,6}$= 8 Hz), 7.45 (d, 1, C_6 H, $J_{5,6}$= 8 Hz), 10.2 (br s, 1, NH).

Preparation of triphenyl phosphite methiodide (94): Under a nitrogen atmosphere, tripheyl phosphite (31 g, 0.1 mol) and methyl iodide (21 g, 0.15 mol) were heated together to reflux in an oil bath. The temperature of the reaction mixture initially rose to 90 C and then gradually to 115 C during the 36 hour period. The reaction mixture was cooled to room temperature and washed with anhydrous ether (10x100 mL). The solvent was decanted after each washing carefully, avoiding the exposure to the moisture. The yellow mass was dried on the pump and stored under vacuum. The product (94) thus obtained (27 g, 60%) was used directly without further purification.

Preparation of diphenyl methyl phosphonate (95): Compound 94 (27.0 g, 60 mmol) was dissolved in absolute ethanol (45 mL) and the reaction mixture stirred for 30 minutes. The side product ethyl iodide was removed under reduced pressure. The mixture was then subjected to vacuum distillation. After the removal of phenol at 30-40 C/ .01 mm, the remainder of the mixture was treated with 20 mL of 1% sodium hydroxide solution and the aqueous layer separated. The organic layer consisting mainly of the product, yielded 14.4 g, (97%) of 95 on vacuum distillation at 94-100 C/ 0.15 mm.

Preparation of 6'-dimethyl 2',3'-O-isopropylidene uridine phosphonate(97): Under a nitrogen atmosphere was added dropwise a solution of n-BuLi (1.77 mmol of 1.7M solution in hexane) to a stirred solution of 93 (1.5 mmol 0.2 g) in 10 mL anhydrous THF at -78 C. The reaction mixture was stirred for 30 minutes at -78 C and then allowed to warm up to the room temperature. It was stirred for additional 30 minutes. The solvent was removed on a rotary evaporator. The crude yellow mass was purified either a) directly by silica gel column chromatography using ethanol gradient in ethyl acetate yielding 270 mg (47%) of (97) or b) the crude product was dissolved in 40 mL of chloroform:water (3:1), the organic layer was separated. The aqueous layer was extracted with chloroform (2x20 mL), all the organic extracts were combined and dried over anhydrous sodium sulfate, filtered and the solvent evaporated. The column chromatography as described above yielded 220 mg (37%) of the product (97) and 40 mg (%) of the side product (98) which had a higher R value. The compound (98) was futher purified on HPLC using ethyl acetate:hexane (2:1) as the eluent. Calculated for (97), C: 39.21, H: 5.26; Found C:39.16, H:5.45. Calculated for (98), C: 53.2, H: 5.39; Found C: 53.10, H: 5.45. ^{1}H-n.m.r. (chloroform-d_1) for (97): 1.4 (s, 3, CMe_2), 1.58 (s, 3, CMe_2), 1.5 (d, 2, $C_{6'}H$, J_{P-H} = 17 Hz), 3.3-3.5 (m, 2, $C_{5'}H$, $J_{5',6'}$ = 2 Hz), 3.7 (d, 6, OMe, J_{P-H} = 8 Hz), 4.1-4.4 (m, 2, $C_{4'}H$ and $C_{6'}$

H), 4.9 (q, 1, $C_{3'}$ H), 5.0 (q, 1, $C_{2'}$ H), 5.6-5.9 (2d, 2, $C_{1'}$ H and C_5H, $J_{1',2'}$ = 2 Hz, $J_{5,6}$ = 8 Hz), 7.45 (d, 1, C_6 H, $J_{5,6}$ = 8 Hz), 8.8 (br s, 1, NH).

Attempted dealkylation of 6'-dimethyl 2',3'-O-isopropylideneuridine (97): under a nitrogen atmosphere, chlorotrimethylsilane (260 mg, 2.4 mmol) was added to a solution of sodium iodide (360 mg, 2mmol) and the compound 97 (500 mg, 2.4 mmol) in 5 mL of dry acetonitrile. The mixture stirred at room temperature for 30 minutes. An instant exothermic reaction took place resulting in the formation of the sodium chloride precipitates. The mixture was quickly filtered and the solvent evaporated. The residue was treated with absolute methanol (2 mL) and the solvent evaporated. The column purification of the crude brown material which was very hygroscopic, resulted in decomposition of the material.

Preparation of bis (trimethylsilyl) chloromethyl phosphonic acid (100): Under a nitrogen atmosphere, a solution of chloromethyl phosphonic acid (3.8 g, 35 mmol) in dry tetrahydrofuran (60 mL) was cooled to -25 C and triethyl amine (10 mL) was added dropwise. Chlorotrimethylsilane (7.87 g, 72 mmol) was added dropwise over a period of 30 minutes resulting in the substantial formation of white precipitates of triethyl ammonium hydrochloride. Additional tetrahydrofuran (10

mL) and anhydrous ether (20 mL) were added. The mixture was allowed to warm up to the room temperature and stirred for 2 hours. It was then left overnight in the refrigerator, filtered and the precipitates washed with 20 mL of dry tetrahydrofuran. The filtrate was evaporated on a rotary evaporator to afford light brown oil which on vaccum distillation yielded 7.6g (86.5%) of the product (100), bp 54/.12 mm. NMR (neat): 0.3 (s, 18), 3.4 (d, 2).

Preparation of 6'-hydroxy 6'-dibenzyl 2',3'-O-isopropylideneuridine (107): In a 50 mL three necked flask, equipped with a condenser, a nitrogen bubbler and a rubber septum, were placed sodium borohydride (30 mg, 0.8 mmol), diglyme (10 mL), and (-) ∝-pinene (300 mg, 2 mmol) diluted with diglyme (5 mL), under a nitrogen atmopsphere. The flask was placed in a water-bath (20 C) and boron trifluoride etherate (.15 mL diluted in 5 mL of diglyme) was added to the stirring reaction mixture over a period of 10 minutes. The (+)-diisopinocampheylborane precipitated out as a white solid. The flask was cooled to -10 C. The solution of compound 90 (0.5 g, 1 mmol) in dry THF (5 mL) was added to the mixture and the mixture was stirred for 3 hours at 0 C. The mixture was allowed to warm up to the room temperature. Sodium hydroxide (1 mL of 3M solution) was rapidly added followed by a slow addition of hydrogen

peroxide (1 mL of 30 % solution) while maintaining the the temperature below 30 C. After stirring for an additional 1 hour the product (106) was extracted with chloroform (15 mL) from the reaction mixture. The extract was washed with saturated sodium chloride solution (5 mL) , dried over anhydrous sodium sulfate and evaporated to afford 150 mg (31%) of crude 106. The compound 106 (200 mg, 0.4 mmol) was dissolved in anhydrous DMSO (3 mL) and sodium benzoxide (200 mg, 1.6 mmol) was added. The reaction mixture was stirred for 2 hours. The solvent was removed under vacuo and the residue was placed in 50 mL of chloroform:water (1:1). The organic layer was separated and the aqueous layer was extracted again with chloroform (25 mL). The combined extracts were dried over anhydrous sodium sulfate, filtered and the filtrate was evaporated to a brown gum. Silica gel chromatography using methanol ingredient in chloroform yielded 50 mg (25%) of 107. Calculated C: 56.24, H: 5.76; found C: 56.12, H: 5.68.

Preparation of 6'-hydroxy 2',3'-O-isopropylideneuridine- 6'-phosphonic acid (108): The hydrogenolysis of the compound 107 (100 mg, 0.1 mmol) was carried out over 5% palladium on charcoal (500 mg) in absolute methanol (20 mL) overnight at room temperature and atmospheric pressure. Filtration of the solution followed by evaporation of the solvents

afforded 30 mg (86%) of 108. The product was homogeneous on the TLC in Chloroform:methanol (4:1), R_f = 0.10. Calculated C: 40.79, H: 4.43, N: 6.47; found C: 40.89, H: 4.21, N: 6.47.

Preparation of 2',3'-O-(dibutylstannylene) uridine (109): Uridine (2.5 g, 10 mmol) and di-n-butyl tin oxide (2.5 g, 10 mmol) were suspended in anhydrous methanol (50 mL). The mixture was heated to reflux until a clear solution was obtained. The solution was cooled to room temperature. The solvent was evaporated and the product (109) dried on a vacuum pump. The yield was 4 g (89%); The product was recrystallized from ethanol, m.p. 232-234 . Calculated C: 42.97, H: 5.94; Found C: 42.91, H: 5.91

Preparation of 2',5'-di-O-trityluridine (110): Uridine (7.5 g, 31 mmol) and triphenylmethyl chloride (25.7 g, 92 mmol) were stirred overnight in anhydrous pyridine (75 mL) and then the mixture was heated at 100 C for 4 hours. The mixture, a light brown solution, was poured into vigorously stirred ice-water (1 L). The gummy precipitate was dissolved in chloroform (75 mL). The organic layer was separated, washed with 5% aqueous cadmium chloride solution (25 mL) followed by water (25 mL), dried over anhydrous sodium sulfate, and evaporated to a yellow syrup. Crystallization of the syrup from benzene:ether gave 6.5 g (29%) of the product (110), mp

216-219 C.

Preparation of 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)uridine (112): Under a nitrogen atmosphere, imidazole (3 g, 44 mmol) was added to a solution of uridine (2.45 g, 10 mmol) in DMF (10 mL). The mixture turned pale yellow. Tetraisopropyldisiloxane-1,3-diyl (3.5 g, 11 mmol) was added over a period of 15 minutes. The mixture was stirred for 2 hours and left standing overnight. The solvent was removed under vacuo and the residue partitioned between chloroform (30 mL) and water (15 mL). The two layers were separated and the aqueous layer was washed with chloroform (2x15 mL). The organic extracts were combined and dried over anhydrous sodium sulfate. Evaporation of the solvent followed by silica gel chromatography of the residue (ethyl acetate: toluene 5:1, then methanol) yielded 2.4 g (50%) of the compound 112. Calculated C: 50.42, H: 7.95; found C: 50.51, H: 8.27.

Preparation of 2'-O-(tetrahydropyranyl)uridine (113): To a solution of 112 (800 mg, 1.6 mmol) in dry dioxane (5 mL) cooled to 0 C, were added p-toluenesulfonic acid (14 mg, 0.07 mmol) and dihydropyran (500 mg, 5.28 mmol). The mixture was warmed to room temperature over a period of 20 minutes and stirred for 2 hours. The mixture was again cooled to 0 C and neutralized with 1M methanolic solution. The mixture was

filtered and the filtrate was evaporated, first on rotary evaporator and then vacuum pump. The residue was was dissolved in dry THF (8 mL) and 1M tetra-n-butylammonium in dry THF (8 mL) was added. The solution was stirred for 10 hours and then diluted with 40 mL of pyridine:water:methanol (3:1:1) and treated with Dowex 50W-X2 (pyridinium form) for 3 hours. The resin was removed removed by filtration and the filtrate was evaporated in vacuo. The silica gel chromatography of the residue (methylene chloride:ether 1:1 and then methanol) yielded 400 mg (75%) of the diastreoisomeric mixture of the compound 113, R = 0.43 in methylene chloride:methanol (9:1).

Preparation of 2'-O-(tetrahydropyranyl)-5'-O-(dimethoxytrityl)uridine (114): A solution of 113 (165 mg, 0.05 mmol) and dimthoxytrityl chloride (190 mg, 0.055 mmol) in anhydrous pyridine (3 mL) was kept under nitrogen for about 12 hours. The mixture was quenched with ethanol (3 mL) and poured into water (10 mL). The mixture was extracted with methylene chloride (3x20 mL) and the combined organic extracts were washed once with water (10 mL). The organic layer was dried over anhydrous sodium sulfate. Evaporation of the solvent and the silica gel chromatography of the residue (methylene chloride in 0 to 2% methanol) yielded 300 mg (95%) of the product 114, R = 0.62 in methylene

chloride:methanol (9:1).

Preparation of 5'-deoxy-5'-dihydroxyphosphinylmethyluridyl-(3'-5')-uridine (118): The compound 91 (100 mg, 0.28 mmol) was converted to its pyridinium salt as follows: Pyridine (2 mL) and acetic anhydride (0.5 mL) were added to (91) and the mixture was kept in dark overnight at room temperature. Water (3 mL) was added. The solvents were evaporated under vacuo. Pyridine (2 mL) was added to the residue and evaporated under vacuo. This procedure was repeated 6 times.

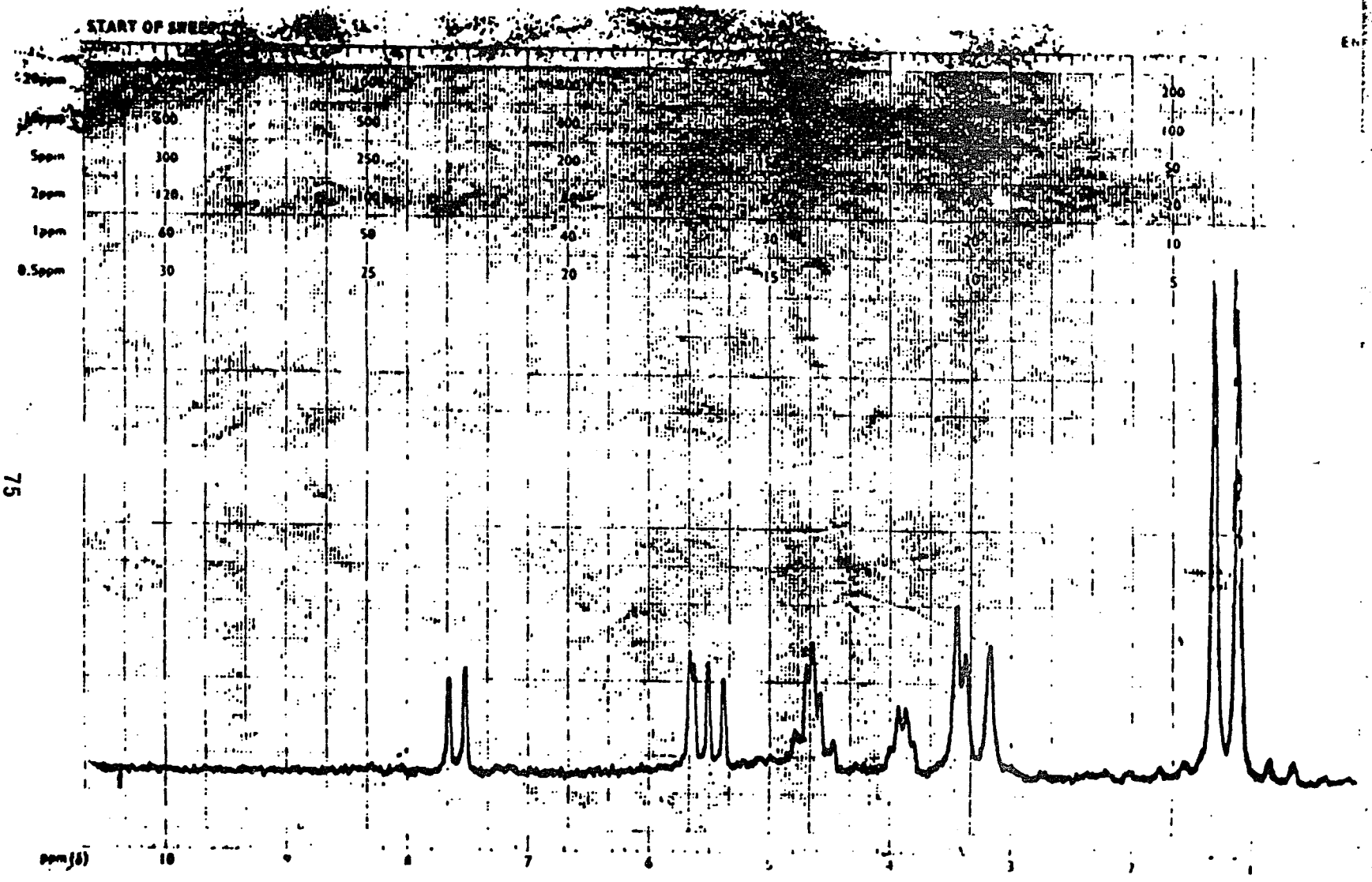
Compound 110 (204 mg, 0.28 mmol) was rendered anhydrous by repeated evaporations (3 times) with anhydrous pyridine (3 mL each time) prior to the coupling reaction.

The pyridinium salt of the compound 91 (100 mg, 0.28 mmol), the compound 110 (204 mg, 0.28 mmol) and Dowex 50-pyridinium form (200 mg) were rendered anhydrous by repeated evaporations (5 times) with anhydrous pyridine (3 mL each time). Under a nitrogen atmosphere pyridine (2 mL) and dicyclohexylcarbodiimide (316 mg) were added to the mixture. The mixture was left standing in the dark for 4 days at room temperature. Water (2 mL) was added. The mixture was carefully washed with ether (5x3 mL). The mixture was left standing for 3

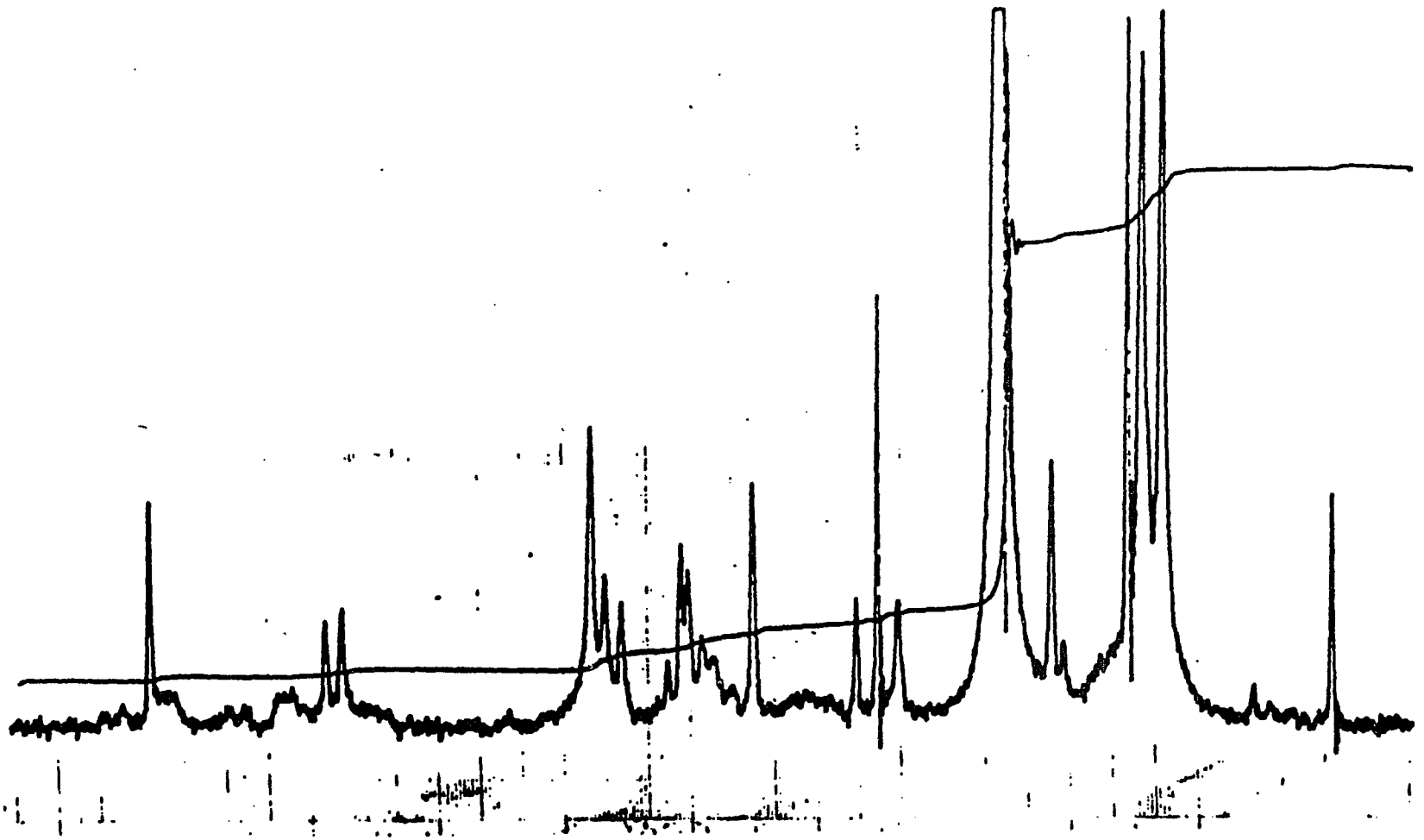
hours. The resin was filtered and washed with pyridine (3 mL). The solvents were removed under vacuo. The residue was treated with 80% acetic acid (5 mL) for 48 hours. The acetic acid was removed under vacuo. The residue was washed with anhydrous ether (5 mL). The residue was then purified by reverse phase HPLC using a gradient of 10-40% of acetonitrile in 0.1M triethylammonium acetate. The purified product(118) was taken up in anhydrous pyridine (5 mL) and pyridine was evaporated under vacuo. This procedure was repeated 5 times to afford 50 mg (28 %) of the dinucleotide 118. Calculated C: 45.65, H: 5.43; Found C: 45.31, H: 5.79.

SUMMARY

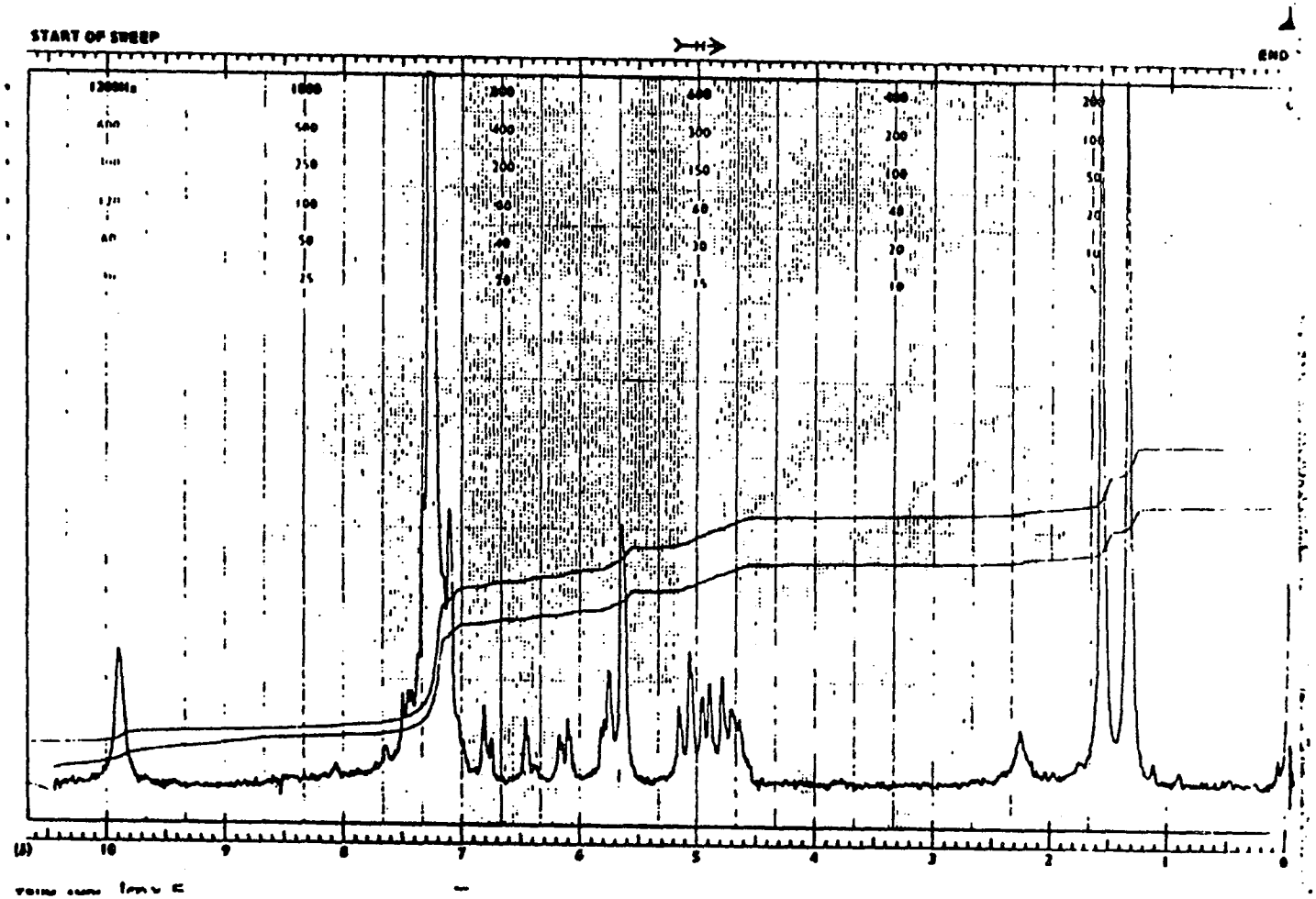
The synthesis of 2',3'-O-isopropylideneuridine-6'-phosphonic acid by several routes was investigated. In the process, the synthesis of 6'-dimethyl-2',3'-O-isopropylideneuridine which was considered a potential candidate for the synthesis of the acid (91), was achieved by a nucleophilic displacement reaction involving dimethyl ∝-lithiomethylphosphonate and 5'-iodo-2',3'-O-isopropylideneuridine (93). ∝-lithiation studies involving alkyl, aralkyl, and ∝-halomethylphosphonates and several simple halides were performed. Hydroboration reaction on ∝,β -unsaturated diphenylphosphonate provided a useful route to the synthesis of ∝-hydroxyphosphonic acids. Several candidates having a general structure of compound (14) were synthesized and studied in the coupling experiments with the acid (91). Finally, the synthesis of the dinucleotide $UCH_{2P}U$ (118), an isoteric analogue of the natural dinucleotide was achieved.



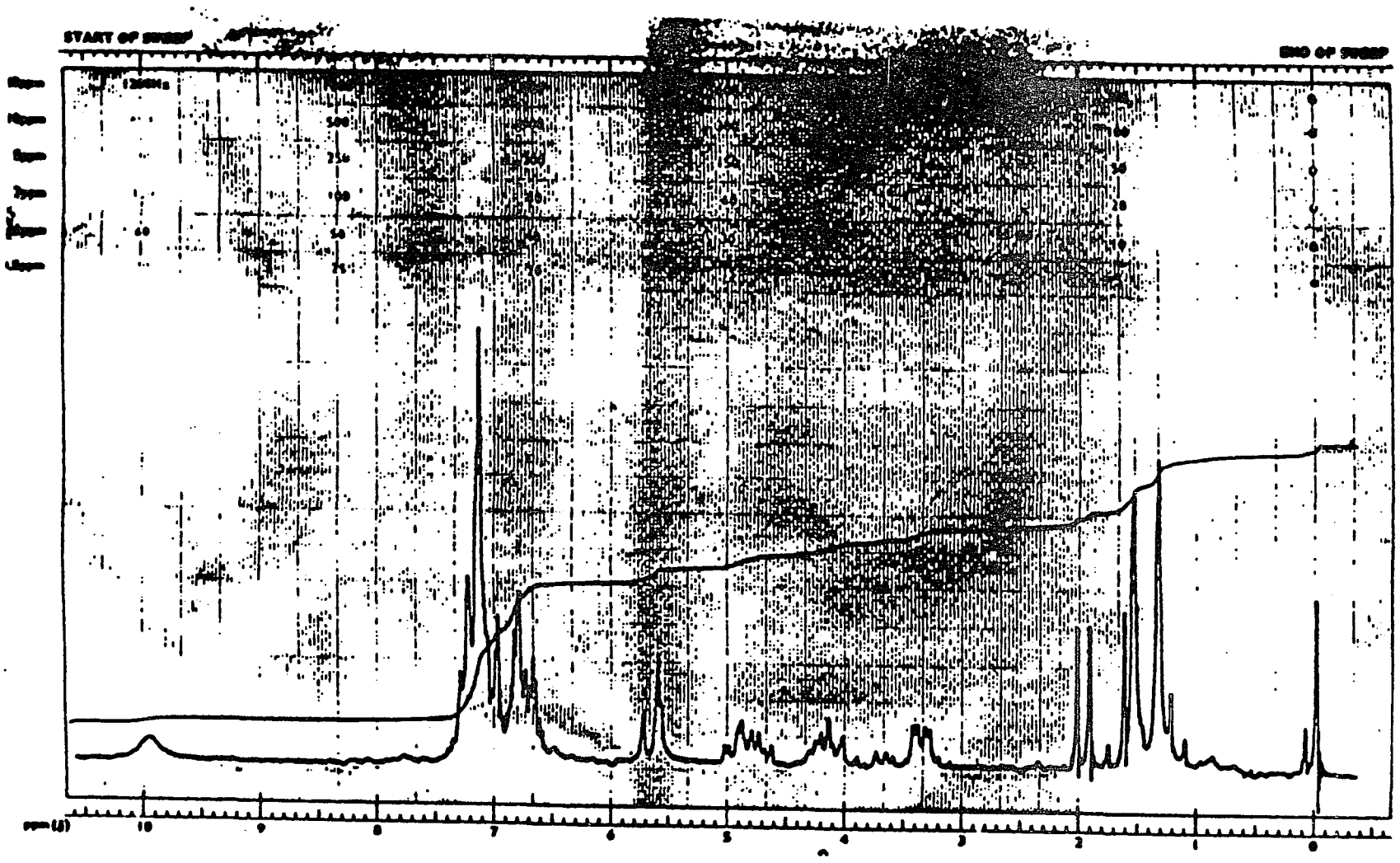
2',3'-O-isopropylideneuridine (81)



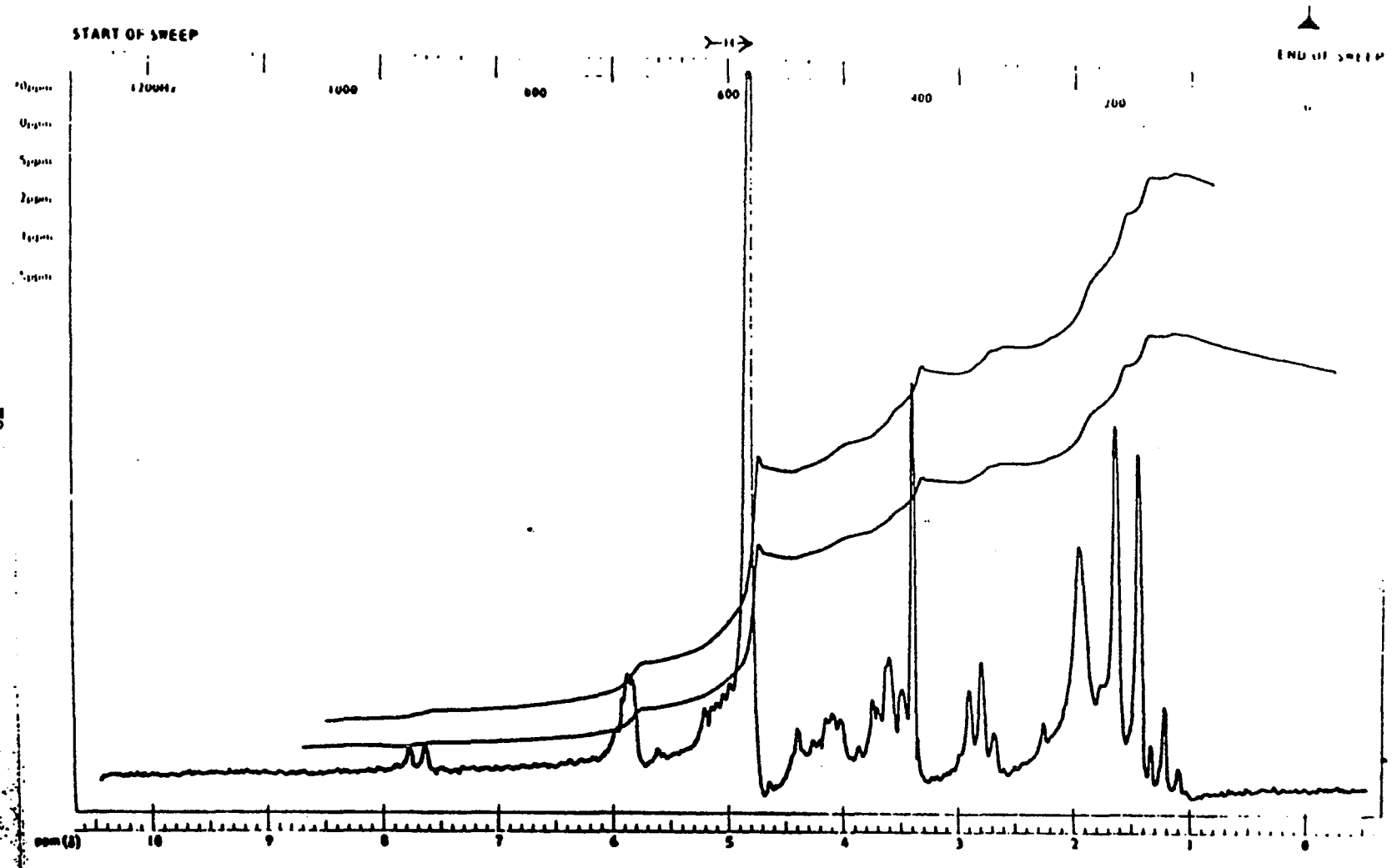
2',3'-O-isopropylideneuridine-5'-aldehyde (82)



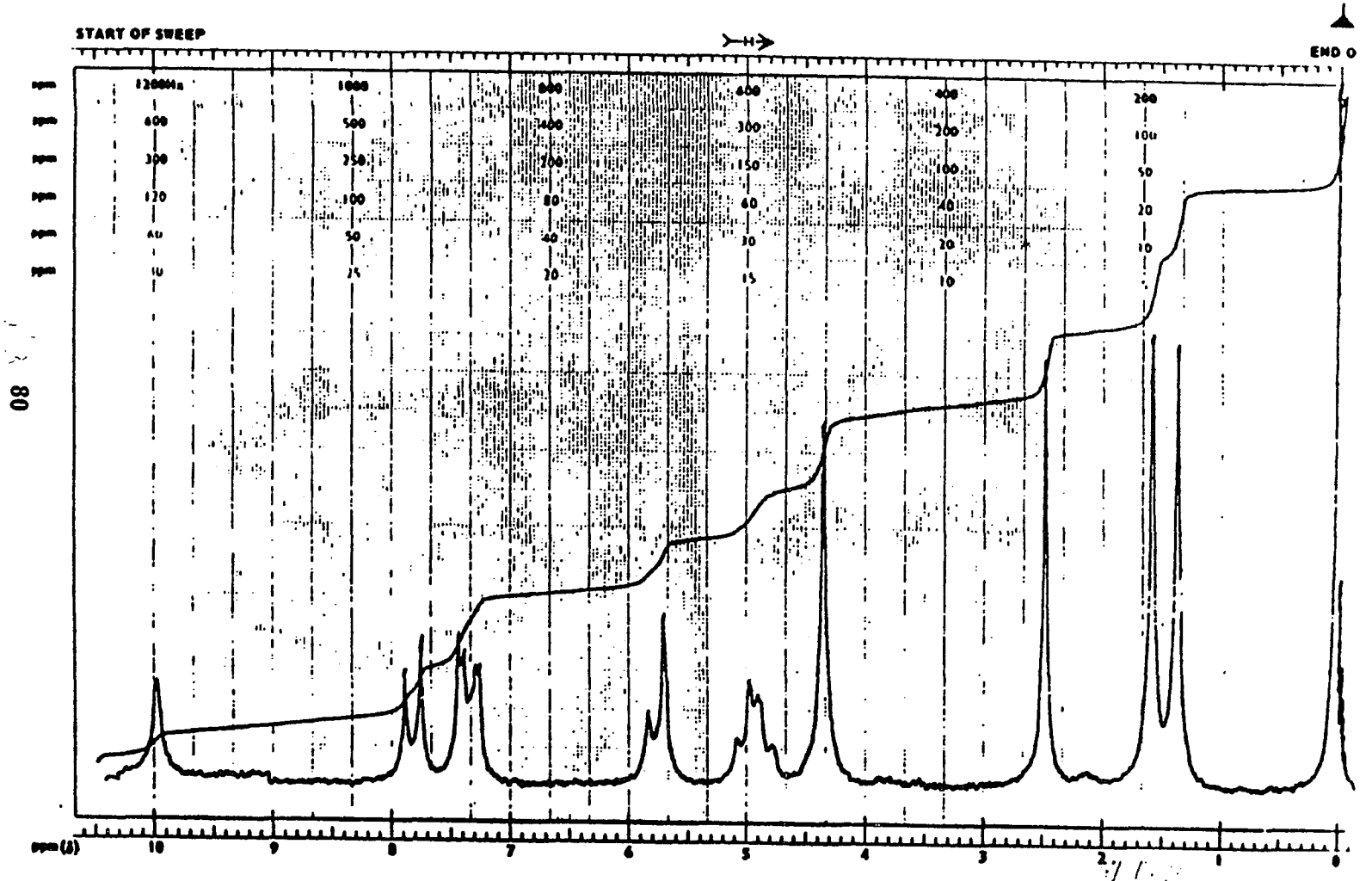
Diphenyl 2',3'-O-isopropylidene-6'-phosphonate (90)



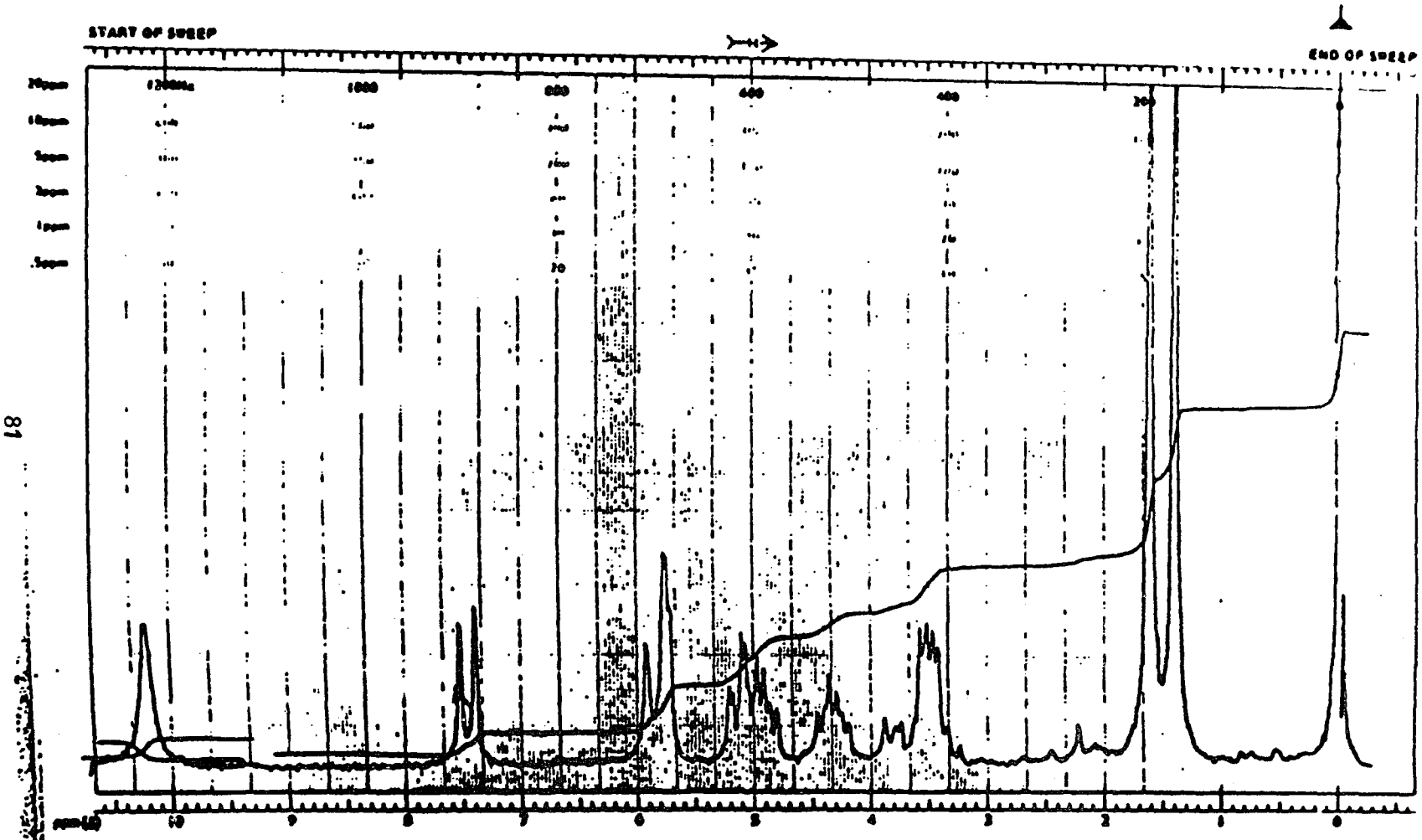
6'-Diphenyl-2',3'-O-isopropylideneuridine-6'-methylene phosphonate (90a)



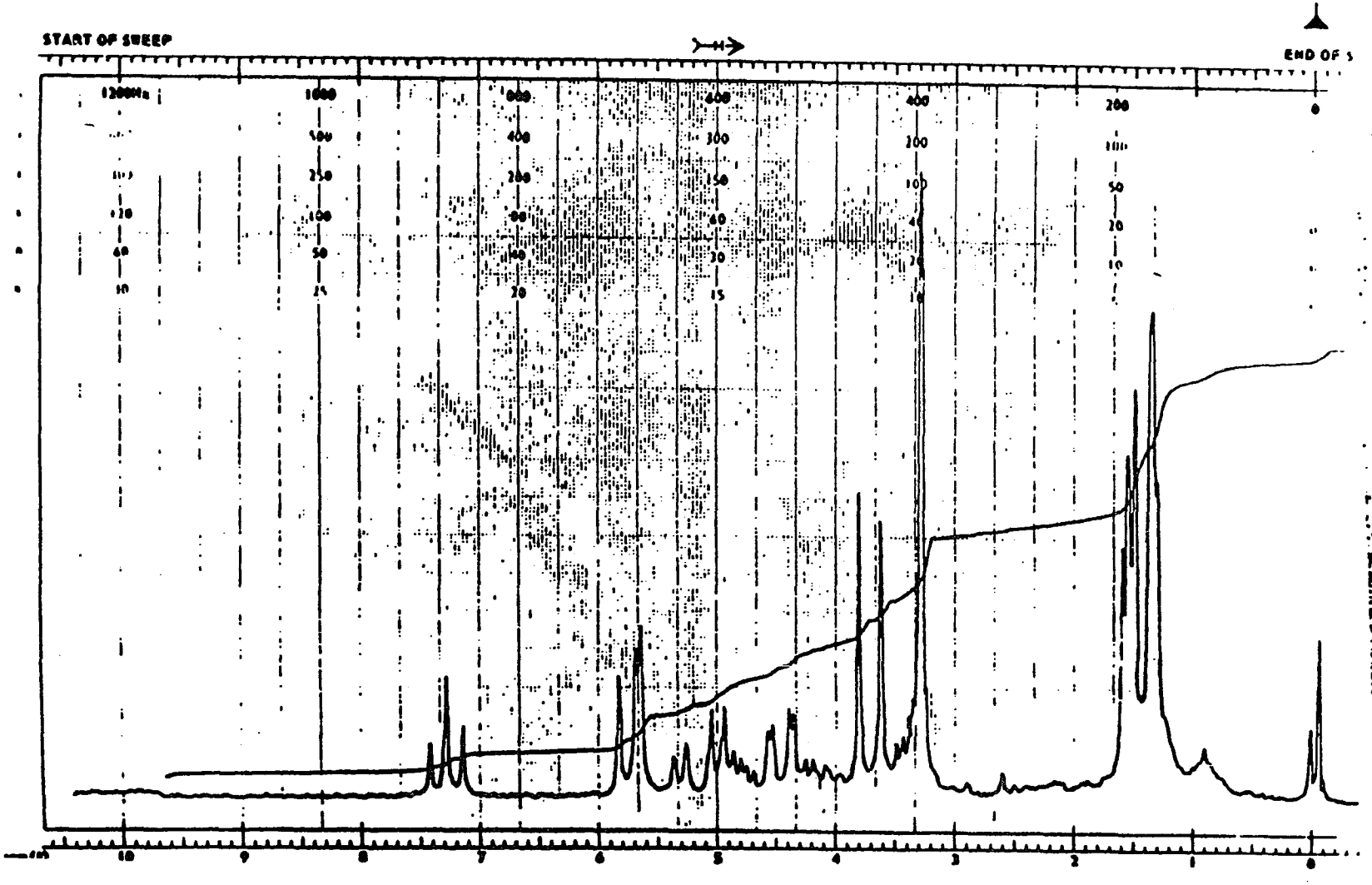
5'-Deoxy-2',3'-O-isopropylideneuridine-6'-phosphonic acid (91)



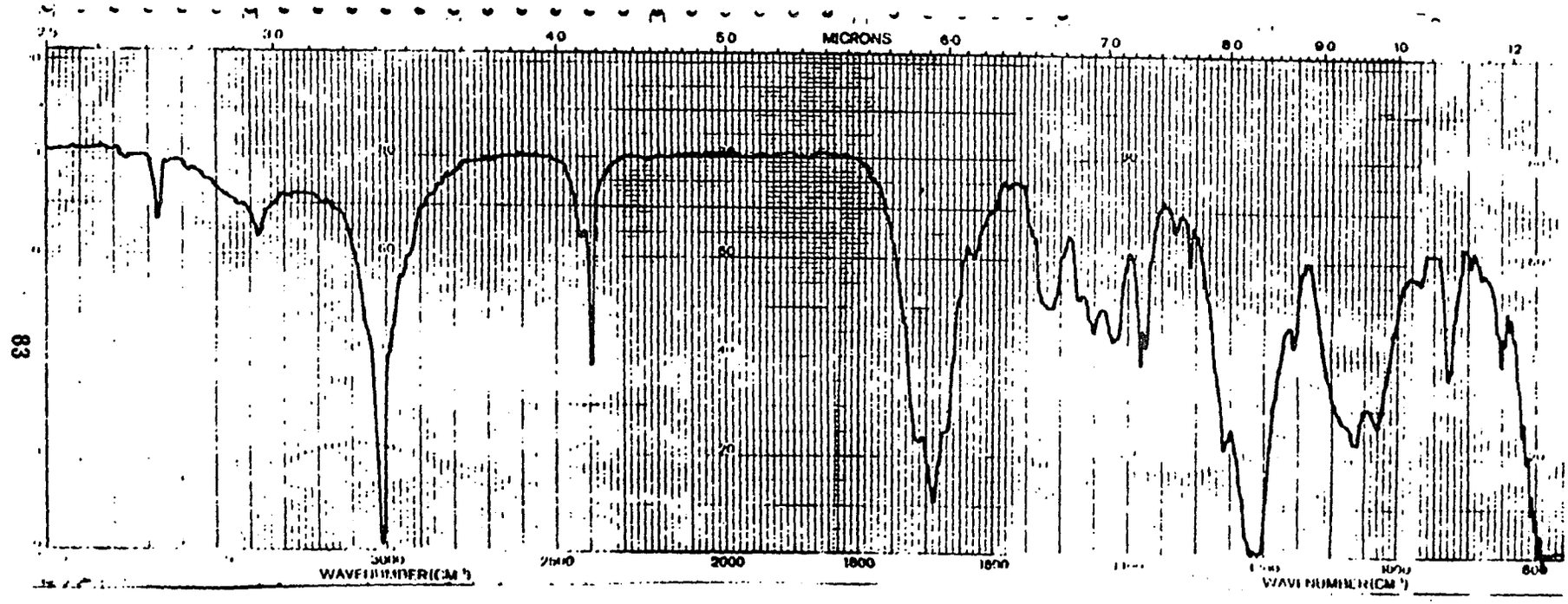
5'-Tosyl-2',3'-O-isopropylideneuridine (92)



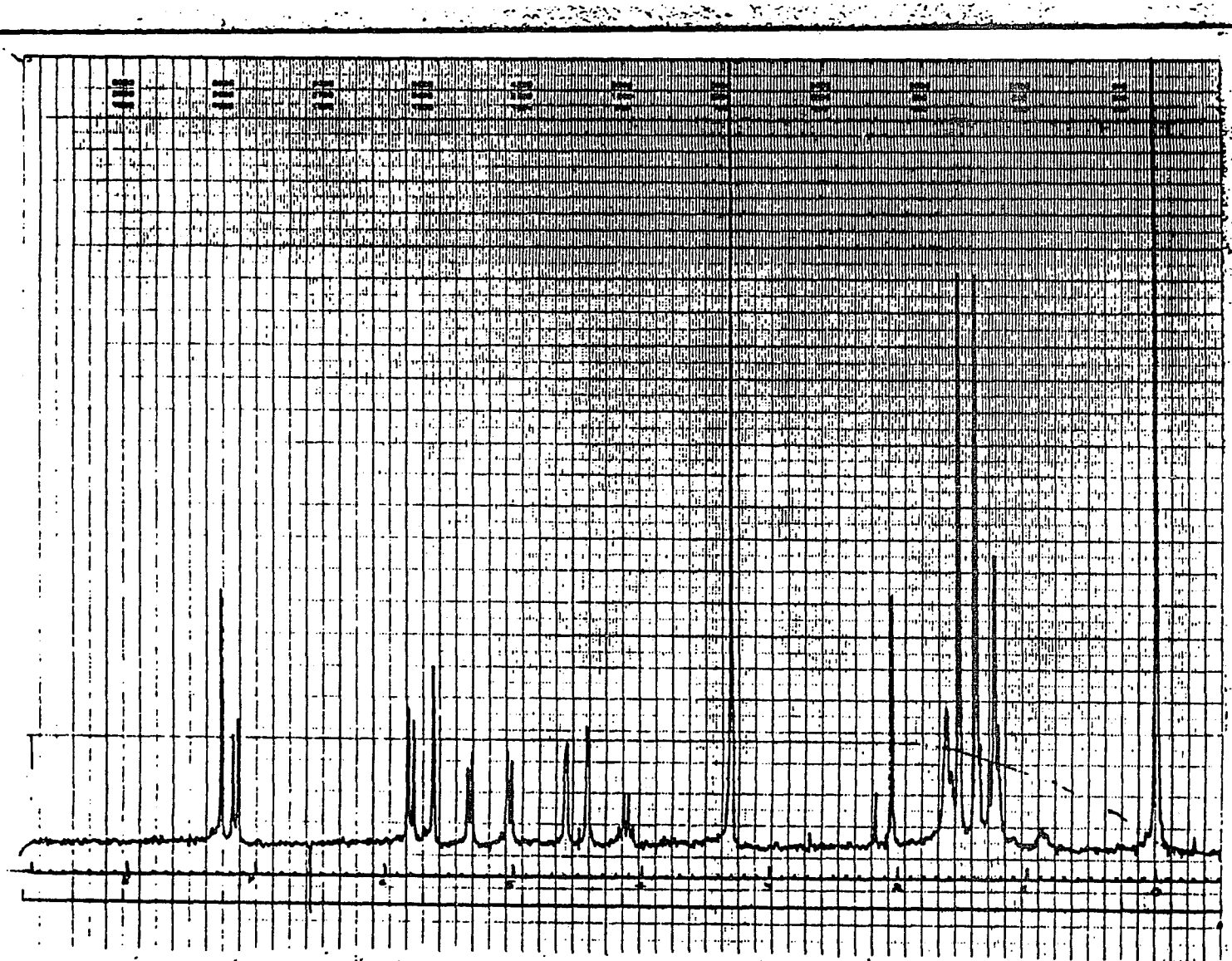
5'-Iodo-2',3'-O-isopropylideneuridine (93)



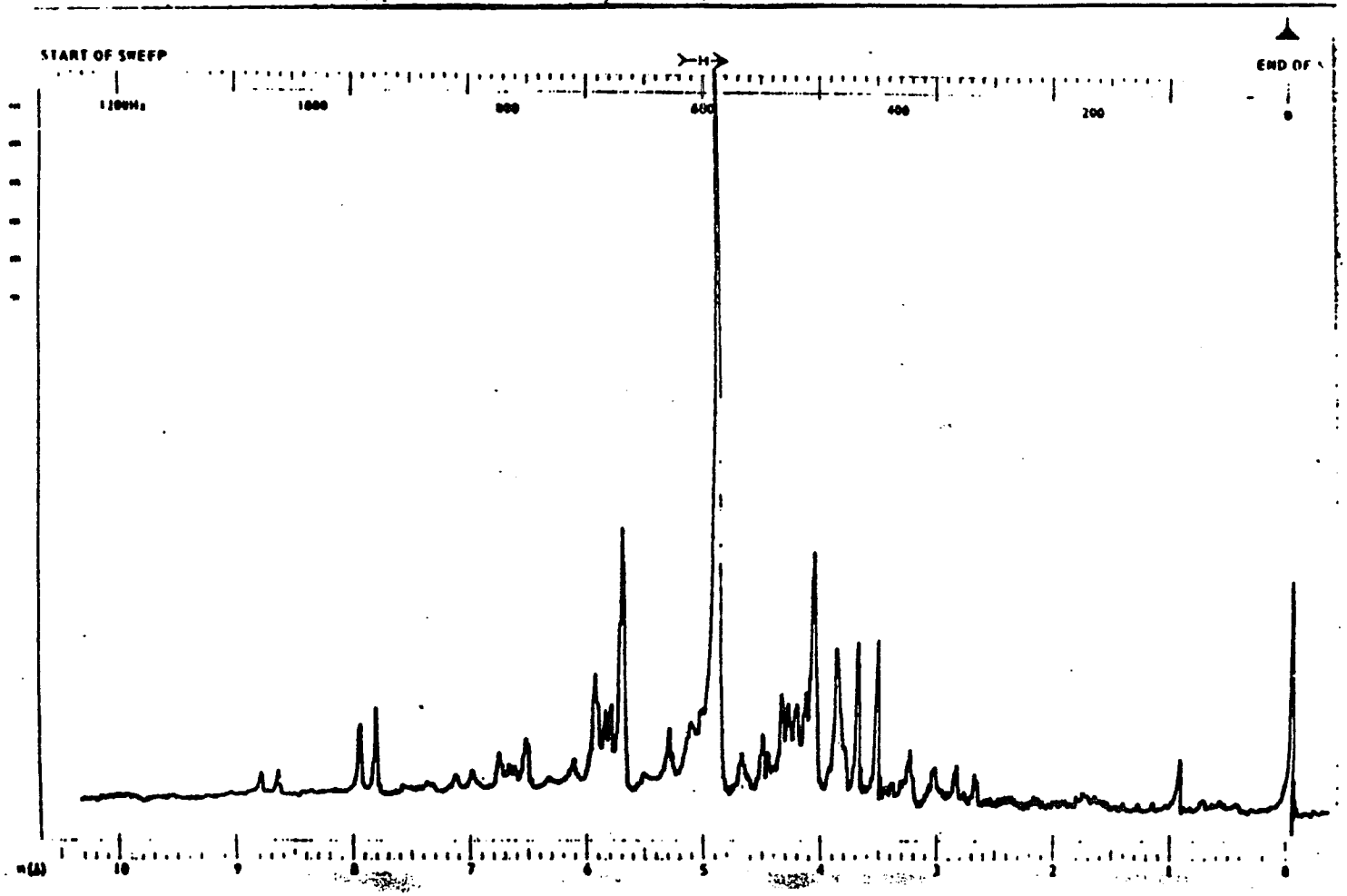
6'-Dimethyl-2',3'-O-isopropylideneuridine phosphonate (97)



6'-Dimethyl-2',3'-O-isopropylideneuridine phosphonate (97)



Compound 98



5'-Deoxy-5'-dihydroxyphosphinylmethyluridyl-(3'-5')-uridine (118)

REFERENCES

1. R. Engel, Chem. Rev., 77, 349 (1977).
2. C. Shopsis, R. Engel, and B. E. Tropp, J. Bacteriology, 112, 408 (1972).
3. C. S. Shopsis, W. D. Nunn, R. Engel, and B. E. Tropp, Antimicrobial Agents a,d Chemotherapy, 4, 467 (1973).
4. J. Kraut, Acta Crystallogr., 14, 1146, (1961).
5. W. G. Ferrier, A. R. Lindsay, and D. W. Young, Acta Crystallogr., 15, 616 (1962).
6. Y. Okaya, Acta Crystallogr., 20, 712 (1966).
7. S. Abrahamsson and I. Pascher, Acta Crystallogr., 21, 79 (1966).
8. S. T. Rao and M. Sundaralingam, J. Amer. Chem. Soc., 91, 1210 (1969).
9. M. A. Viswamitra, B. S. Reddy, G. H.-Y. Lin, and M. Sundaralingam, J. Amer. Chem. Soc., 93, 4565 (1971).
10. M. Larsen, R. Willett, and R. Yount, Science, 166, 1510 (1969).
11. J. Kabak, L. Defilippe, R. Engel, and B. Tropp, J. Med. Chem., 15, 1074 (1972).
12. S. L. Goldstein, D. Braksmayer, B. E. Tropp, and R. Engel, J. Med. Chem., 17, 363 (1974).
13. P.-J. Cheng, R. Hickey, R. Engel, and B. E. Tropp, Biochim. Biophys. Acta, 85, 341, (1974).
14. C.-T. Tang, R. Engel, and B. E. Tropp, Antimicrobial Agents and Chemotherapy, 11, 147 (1977).

15. Z. Leifer, R. Engel, and B. E. Tropp, J. Bacteriology, 130, 968 (1977).

16. J.-C. Tang, B. E. Tropp, and R. Engel, Tetrahedron Letters, 723 (1978).

17. C. Chen, R. Engel, and B. E. Tropp, unpublished results of this laboratory.

18. V. K. Sarin, B. E. Tropp, and R. Engel, Tetrahedron Letters, 351 (1977).

19. G. Popjak, T. Parker, V. K. Sarin, B. E. Tropp, and R. Engel, J. Amer. Chem. Soc., 100, 8014 (1978).

20. P. R. Adams, R. Harrison, and T. D. Inch, Biochem. J., 141 729 (1974).

21. P. -J. Cheng, W. D. Nunn, R. J. Tyhach, S. L. Goldstein, R. Engel, and B. E. Tropp, J. Biol. Chem., 250, 1633 (1975).

22. B. S. Cooperman and N. Y. Chiu, Biochem., 12, 1676 (1979).

23. K. -C. Tang, B. E. Tropp, and R. Engel, Tetrahedron, 34, 2873 (1978).

24. K. -C. Tang, B. Mildener, L. Eisikowitz, B. E. Tropp, and R. Engel, Paper #54, Division of Biological Chemistry, American Chemical Society and Chemical Society of Japan Joint Meeting, April 1979, Honolulu

25. B. Mildener, R. Engel, and B. E. Tropp, Unpublished data, 1981

26. H. P. Albrecht, G. H. Jones, and J. G. Moffatt, J. Amer. Chem. Soc., 92, 5511 (1970).

27. G. H. Jones, H. P. Albrecht, N. P. Damodaran, and J.G. Moffatt, J. Amer. Chem. Soc. 92, 5510 (1970).

28. G. H. Jones and J. G. Moffatt, U. S. Pat. 3,558,595, 26 January 1971; Chem. Abstr., 74, 112406W (1971).

29. G. H. Jones and J. G. Moffatt, Ger. Offen., 2,350,608, 25 April 1974; Chem. Abstr., 81, 13760V (1974).

30. A. Hampton, F. Perini, and P. J. Harpes, Biochemistry 12, 1780 (1973).

31. G. H. Jones and J. G. Moffatt, J. Amer. Chem. Soc., 90, 5337 (1968).

32. R. S. Tipson, Synthetic Procedures in Nucleic Acid Chemistry, Ed. W. Zorbach and R. S. Tipson, John Wiley and Sons, V. 1, P. 431, 1968.

33. B. E. Griffin and C. B. Reese, Tetrahedron, 23, 2315 (1967).

34. S. Chladek and J. Smrt, J. Coll. Czech. Chem. Comm., 28, 1301 (1963).

35. B. E. Griffin, C. B. Reese, and J. E. Sulton, Tetrahedron, 23, 2313 (1967).

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

37. C. B. Reese and D. R. Trentham, Tetrahedron Lett., 2467 (1965).

38. D. Wagner, J. P. H. Verheyden, and J. G. Moffatt, J. Org. Chem. 39, 24 (1974).

39. R. S. Wright, G. M. Tener, and H. G. Khorana, J. Amer. Chem. Soc., 80, 2004 (1958).

40. R. L. Letsinger and K. K. Ogilvie, J. Org. Chem., 32, 296 (1967).

41. H. G. Khorana and P. T. Gilham, J. Amer. Chem Soc., 80, 6216 (1958).

42. M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, J. Amer. Chem. Soc., 84, 430 (1962).

43. A. F. Cook and J. G. Moffatt, J. Amer. Chem. Soc., 89, 2968 (1967).

44. R. Lohrmann, D. Soll, H. Hayatsu, E. Ohtsuka, and H. G. Khorana, J. Amer. Chem. Soc., 88, 819 (1966); H. Kossel, H. Buchi, and H. G. Khorana, ibid., 89, 2185 (1967).

45. K. K. Ogilvie, K. K. Schifman, and C. L. Penny, Can. J. Chem., 57, 2230 (1979), and references therein.

46. K. K. Ogilvie, K. L. Sadna, E. A. Thompson, M. A. Quillian, and J. B. Westmore, Tetrahedron Lett., 33, 2861 (1974).

47. S. Hanessian and P Lavalle, Can. J. Chem., 53, 2975 (1975).

48. K. K. Ogilvie, S. L. Beacauge, A. L. Schifmann, N. Y. Theranli, and K. L. Sadna, Can. J. Chem., 56,

49. S. S. Jones and C. B. Reese, J. C. S. Perkin 1, 2762 (1979).

50. K. K. Ogilvie and D. W. Entwistle, Carbohydrate Res., 89, 203 (1981).

51. W. T. Markiewicz, J. Chem. Res. Synap., 24 (1979); W. T. Markiewicz and M. Wiewiorowski, Nucleic Acids Res. Special Publication, 4, 185 (1978).

52. C. Gioceli, M. Kwiatskowski, and J. B. Chatopadhyaya, Tetrahedron Lett. 22, 1741 (1981).

53. A. Matsuda, K. Pankiewicz, B. Marcus, K. A. Watanabe, and J. J. Fox, Carbohydrate Res., 100, 297 (1982).

54. H. Takaku, M. Yoshida, and T. Nomoto, J. Org. Chem., 48, 1399 (1983).

55. C. B. Reese, R. C. Titmas, and I. Yau, Tetrahedron, 2727 (1978).

56. R. E. Arrick, D. G. Baker, and D. Horton, Carbohydrate Res., 26, 441 (1973).

57. U. Brodbeck and J. G. Moffatt, J. Org. Chem., 35, 3552 (1970).

58. A. J. Mancuso, D. S. Brownfain, and D. Swern, J. Org. Chem., 44, 4148 (1979).

59. G. H. Jones et al., J. Carbohydrates, Nucleosides, Nucleotides, 6, 127 (1979).

60. K. E. Pfitzner and J. G. Moffatt, J. Amer. Chem. Soc., 87, 5661 (1965).

61. J .G. Moffatt in Oxidation, V. 2, P. 12, Ed. R. L.

Augustine and David J. Trecker, Marcel Dekker, Inc. 1971.

62. D. Horton and J. S. Jewell, Carbohydrate Res. 2, 251 (1966).

63. G. M. Cree, D. W. Mackie and A. S. Perlin, Can. J. Chem., 47, 511 (1969).

64. R. W. Binkley, Carbohydrate Res. 48,

65. G. H. Jones, J. P. H. Verheyden, and J. G. Moffatt, Abstract N-26 of the XXIst International Congress of Pure and Applied Chemistry, Prague 1967.

66. H. W. Wanzlick and W. Lochel, Chem. Ber., 86, 1463 (1953).

67. G. H. Jones and J. G. Moffatt, Abstracts, 158th National meeting of the American Chemical Society, New York, September 1969, CARB. 16

68. G. H. Jones, M. Taniguchi, D. Tegg, and J. G. Moffatt, J. Org. Chem., 44, 1309 (1979).

69. J. A. Montgomery, A. G. Laseter, and K. Hewson, J. Heterocycl. Chem., 11, 211 (1974).

70. M. Fueretes, J. J. Witkowski, D. G. Streeter, and R. K. Robins, J. Med. Chem., 17, 642 (1974).

71. G. H. Jones, E. K. Hamamura, and J. G. Moffatt, Tetrahedron Lett., 5731 (1968).

72. J. A. Montgomery, H. Jeanette Thomas, R. L. Kisliuk, and Y. Gaumont, J. Med. Chem., 22, 109 (1979).

73. R. D. Youssefyech, J. P. H. Verheyden, and J. G.

Moffatt, J. Org. Chem. 44, 1301 (1979).

74. A. F. Cook and J. G. Moffatt, J. Amer. Chem. Soc., 89, 2697 (1967).

75. E. J. Corey and G. T. Kwiatkowski, J. Amer. Chem. Soc., 88, 5654 (1966).

76. M. Sekine, M. Nakajima, and T. Hata, Bull. Chem. Soc. Jap., 55, 218 (1982).

77. P. Coutrot, C. Laurenco, J. F. Normant, P. Perriot, P. Savignac, and J. Villeras, Synthesis, 615 (1977).

78. J. Villeras, P. Perriot, and J. F. Normant, Synthesis, 29 (1978) and 31 (1978).

79. P. Coutrot and P. Savignac, Synthesis, 34 (1978).

80. P. Coutrot and P. Savignac, Synthesis, 36 (1978).

81. Clarence H. Roy, Chem. Abstr., 65, 3908d (1965).

82. Amar Gupta, Kenneth Sacks, Shaheed Khan, Burton E. Tropp, and Robert Engel, Syn. Comm., 10, 299 (1980).

83. S. R. Landauer and H. N. Rydon, J. Chem. Soc., C, 2224, (1953).

84. C. E. McKenna, M. T. Haga, N. H. Cheung, and M. McKenna, Tetrahedron Lett., 155 (1977).

85. T. Morita, Y. Okamoto, and H. Sakurai, Bull. Chem. Soc. Jap., 54, 267 (1981).

86. H. C. Brown in Organic Synthesis via Boranes, John Wiley and Sons, 1975.

87. A. Hampton, J. Amer. Chem. Soc., 83, 3640 (1961).

88. Y. Lapidot and H. G. Khorana, J. Amer. Chem. Soc., 85, 3857 (1963).