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SYNTHESIS OF RNA ANALOGUES

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SYNTHESIS OF RNA ANALOGUES

by

WIESLAW ADAM MAZUR

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.

1984

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

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II

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A. ABSTRACT

The synthesis of nucleoside and nucleotide analogues in which the 3'-oxygen is replaced by a methylene group has been the object of this research program.

Various approaches to these substances have been evaluated and a general strategy has been developed which can be applied to the synthesis of 3'-modified nucleosides as well as isosteric nucleic acid analogues.

Two routes to these substances were used. The first began with specific protection of nucleosides at the 2'- and 5'-hydroxyls using silyl based reagents, thus exposing the 3'-carbon to selective transformations. Despite successful oxidations of the 3'-carbon in uridine and guanosine, all attempts to generate a new carbon-carbon bond in these positions failed.

The second strategy, which led to a development of synthetic routes to an isosteric trinucleotide (16 steps) and isomers of 3'-hydroxymethyluridine (9 steps), utilized 3-deoxy-1,2:5,6- diisopropylidene-3-C-methylene-α-D-glucofuranose (1) as a common substrate.

In the preparation of trinucleotide, the methylene group in (1) was converted stereospecifically to a 3-hydroxymethyl function and then to a 3-bromomethyl derivative. The bromination method involving bromine,

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triphenylphosphine and NBS was improved and now can be applied for substitution of hydroxyls with bromine in substances very sensitive to acids. Displacement of bromine by phosphorus was achieved by means of Arbuzov reaction with triisopropyl phosphite, following a conversion of the D-glucofuranose structure into an D-ribofuranose one. Further cleavage of the isopropyl functions, after introduction of the uracil ring at C-1, produced 3'-dihydroxyphosphonomethyl derivative of 3'-deoxyuridine. A required differentiation of the properties of the 2'- and 5'- hydroxyls in this nucleotide was achieved by protection of the 5'-hydroxyl, prior to the Arbuzov reaction, with the 3-benzoylpropionyl group while the 2'-hydroxyl was acetylated. This system proved to be stable in a wide range of reactions involving the 1- and 3- positions of the ribose ring. The 3-benzoylpropionyl group was selectively cleaved in the final steps giving access to isosteric trinucleotide analogue.

The same pattern of the hydroxyl protection was applied to the synthesis of 3-deoxy-3-methyleneuridine. Despite extensive efforts to epoxidize the 3'-olefinic bond in the latter, none were successful. However, this olefin reacted readily with osmium tetroxide giving a mixture of isomeric at C-3' 3'-hydroxymethyluridines.

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ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to the members of the Queens College Chemistry Department for their help throughout the course of this work.

In particular, I thank Professor Robert Engel for his guidance and encouragement through the years.

I also thank Professor George Axelrad and Professor David Locke for their frendship and advice throughout my doctoral education.

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B. INTRODUCTION

B.1. General

The present work is concerned with the synthesis of nucleotides in which an esteric oxygen is replaced by a carbon atom.

Naturally occurring nucleic acids contain the phosphodiester linkage joining nucleosides, as shown in (1). Isosteric phosphonic acid analogues of nucleotides and nucleic acids have been of interest for some time [1]. Such species have a carbon function linking the phosphorus and the nucleoside, as in (2), rather than oxygen. The central task in the synthesis of such isosteric compounds is the search for efficient ways to introduce the phosphonic acid linkage.

Among the methods for the synthesis of phosphorus-carbon bonds, two are the most important for the present work. The first one is the Arbuzov reaction which involves reaction of a trivalent phosphorus ester and alkyl halide, as shown in figure 1. The second approach involves the Wittig-type reaction between a carbonyl group and phosphorus ylide, generally followed by reduction of the carbon-carbon double bond, as shown in Figure 2. Cleavage of the phosphonic esters (9) give phosphonic acids (10). Paths to isosteric analogues of nucleotide species would

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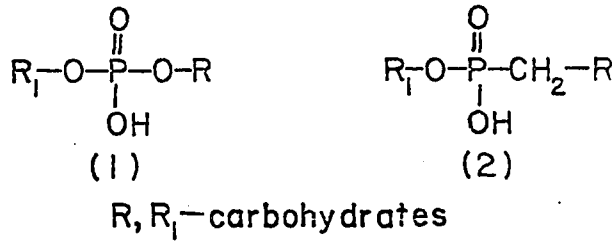

Figure 1

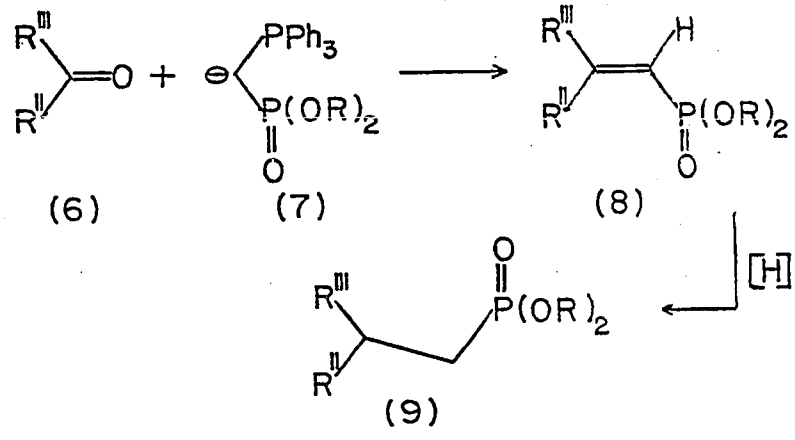

Figure 2

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involve as precursors or intermediates, carbohydrates or nucleosides of the types (11), (12), (13), or (14), that can subsequently undergo Arbuzov reactions. Two general strategies have been developed for the approach to these materials and their analogues.

The first of these utilizes nucleosides themselves as the starting materials, while the second begins with carbohydrates. The synthetic sequence that starts from nucleosides involves a specific protection of the hydroxyl groups that should remain unchanged during the synthesis. The protected nucleoside is then subjected to the required transformations. Pyrimidine or purine components retain their positions (at C-1') through all the steps. Obviously, any functional group on the base that can react in the course of the synthesis must be protected as well. The presence of the base also imposes limitations on the extent of modifications which may be made on the ribose ring. These problems have been circumvented in the second strategy.

The synthesis from carbohydrates may begin with 1,2:5,6-diisopropylidene -α-D-glucofuranose (15), which is commercially available. Proper functionalization of the hydroxyl group at C-3 and the cleavage of the C-5, C-6 diol system give access to 3' and 5' substituted analogs of nucleosides. The base is introduced at C-1 in the final

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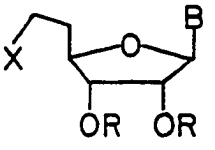

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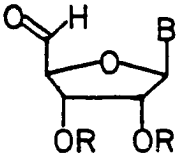

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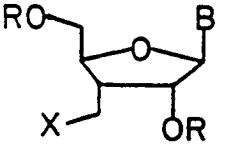

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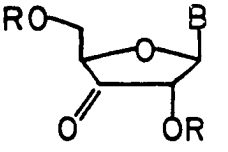
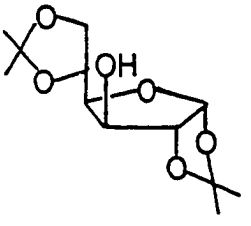


(14)

B= base, OCOR, OMe X= halogen



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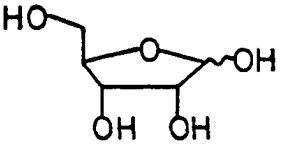

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steps of the synthesis. Therefore, pyrimidine and purine components do not interfere with transformations of the sugar portion. The second candidate for a carbohydrate starting material is ribose itself (16), but its utility is generally limited to 5' modified nucleosides since the selective protections that could give access to the C-3 position exclusively, are not efficient.

B.2. Specific Protection of Hydroxyl Groups in Nucleosides.

Discussion of specific protection here will be limited to the conversion of 2', 3' (18) and 2', 5' hydroxyl groups (17). Such protection affords possibilities for further transformations of the 5' and 3' hydroxyl groups, respectively, that lead to the analogs of naturally occurring nucleotides and ribonucleic acid species.

B.2.1. 2', 3' Protection.

This protection is based on the properties of cis-diol systems and has been provided by reactions of nucleosides with carbonyl compounds and orthoesters[3,4], as shown in Figure 3. The most common reagents for this reaction are acetone or its ketal,

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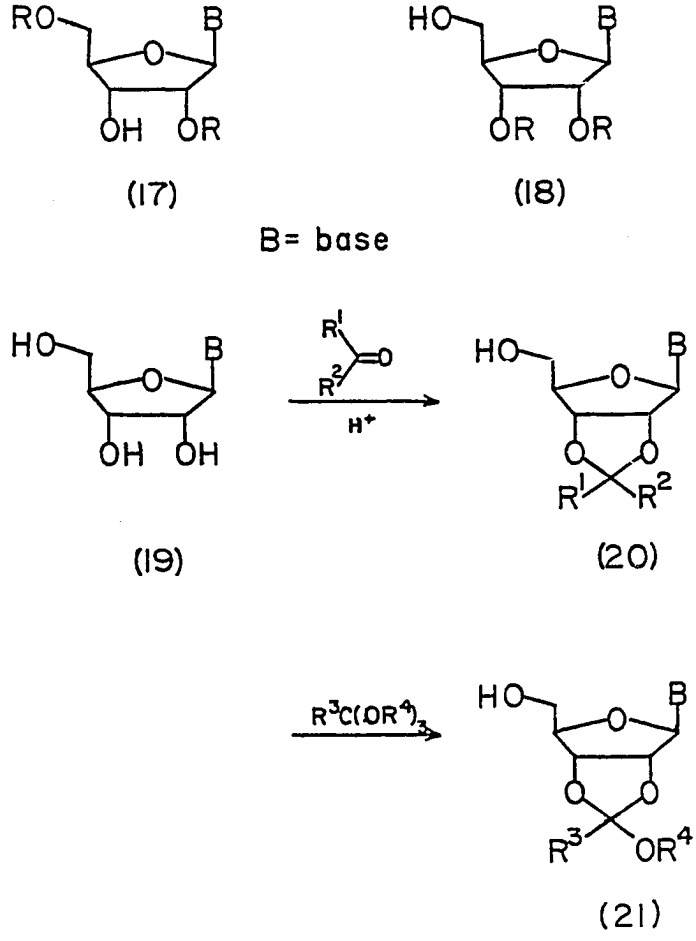

Figure 3

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2,2-dimethoxypropane[5], and cyclohexanone[6]. The alternate approach utilizes orthomethylesters of formic[3], acetic and benzoic acids[4]. Development of orthoesters as the protecting reagents has been prompted by the need for more acid-labile protection than that derived from simple carbonyl compounds. Both carbonyl and orthoester derivatives are stable toward bases. Deprotection in either case is accomplished with acids. The acyl groups from orthoester derivatives may be removed in two steps, initially giving 3'-O-acyl nucleosides (22). Such partial hydrolysis should not be construed as meaning the nucleoside could be protected selectively at 3'-hydroxyl group, since migration of R^3CO between 3' and 2' positions (23) is well known[7].

Finally, the dibutylstannylene group has also been examined for 2',3' protection[8], as shown in Figure 5 .

B.2.2. 2',5' Protection

Selective substitution of the 2',5' hydroxyl groups has been provided by bulky substituents. Triphenylmethyl[9,10,11] and trisubstituted silyl groups have been introduced for this purpose. Their steric interactions decrease the rate of simultaneous substitution at any two neighbouring hydroxyls, thus exposing the 3' OH

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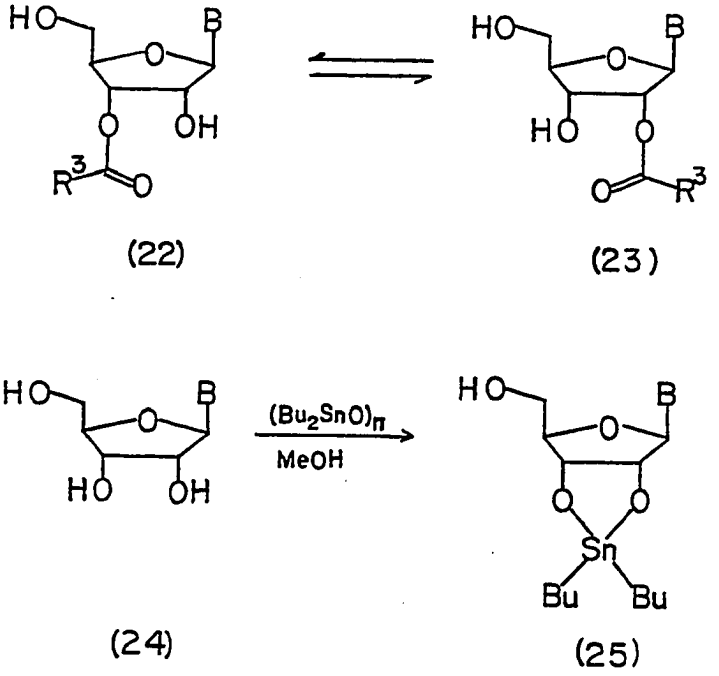
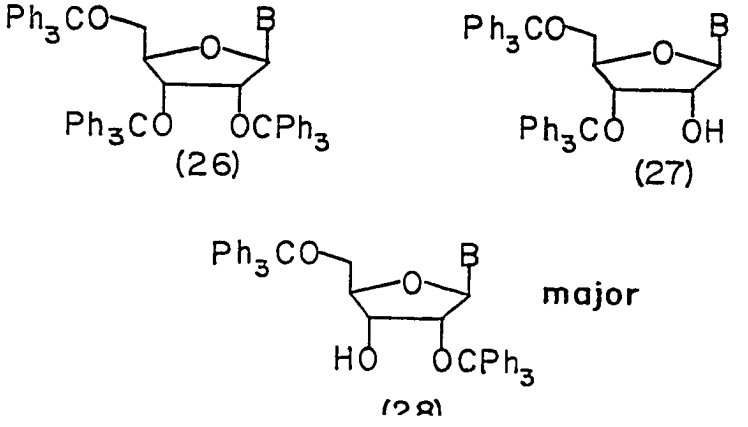


Figure 5



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group. Protection involving triphenylmethyl groups and their methoxy derivatives is obtained by treatment of the nucleoside with an appropriate triarylmethyl chloride in pyridine. The resulting reaction mixture contains 2',3',5' trisubstituted (26), 3',5' disubstituted (27) and 2',5' disubstituted (28) nucleosides. The last compound is the major product that can be isolated and purified by fractional crystallization.

Since removal of triphenylmethyl (trityl) group requires either hydrogenolysis or strong acids, which can be damaging for some nucleosides, mono, di, and trimethoxytrityl groups have been introduced. Each methoxy substituent increases by an order of magnitude the rate of hydrolysis[10]. Examples of various syntheses employing (4-methoxyphenyl)diphenylmethyl, bis(4-methoxyphenyl)phenylmethyl and tris(4-methoxyphenyl)methyl groups are available[12,13,14].

Among silyl-based protecting groups, the most popular are tert-butyldimethylsilyl (TBDMSi), triisopropylsilyl (TISi)[15], and tert-butyldiphenylsilyl[16]. Protection is accomplished by treatment of the nucleoside with the substituted silyl chloride in DMF in the presence of imidazole. The reaction also gives trisubstituted and 3',5' disubstituted nucleosides, and their amounts vary with the

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reaction conditions[17,18]. Sometimes, there are additional complications with selectivity of the process due to the fact that silyl groups have a tendency to migrate between 2' and 3' positions. This has been investigated with the tert-butyldimethylsilyl group[18,19], as shown in Figure 6. This isomerization is found to occur on silica gel surfaces as well as in aqueous or protic solvents. Migration was found to be much more rapid in methanol than in ethanol. The ratio of 2' to 3' isomers at equilibrium is virtually the same (approximately 6:4) regardless of the nucleoside and whether the 5' position is substituted or free, although uracil and adenine nucleosides are found to isomerize much faster than the correspondig cytidine and guanine compounds[20]. The silyl protection has an advantage over the trityl one in the case of $-NH_2$ bearing nucleosides (eg. guanosine) since tritylation also gives substitution at

$-NH_2$. Under the conditions of silylation (imidazole, DMF and work up with water) amino groups are not affected.

Protections with two different kinds of groups in 5' and 2' positions, p-methoxytrityl and tert-butyldimethylsilyl respectively , have also been reported[17,18]. Such a pattern allows removal of one group at a time.

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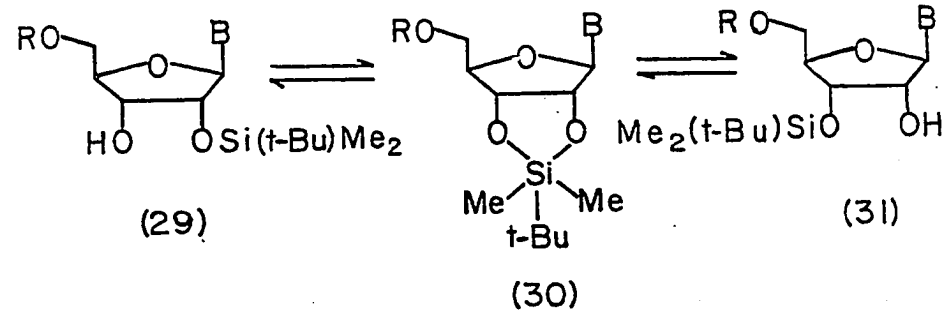
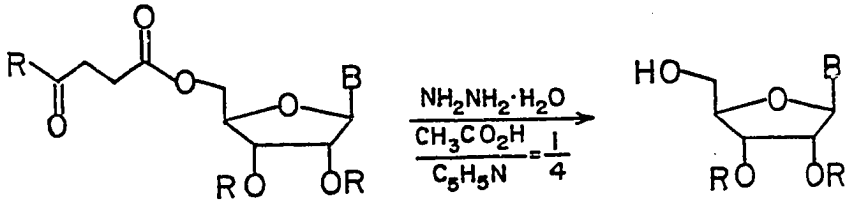


(29) (30) (31)

Figure 6



(32) (33) + (34)

Figure 7

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Both kinds of protecting groups, trityl as well as silyl, can be removed with methanolic HCl[16,19]. Another effective reagent is 80% acetic acid, of use except with the tert-butyldiphenylsilyl group[16]. Some silyl substituents can also be removed by ethanolic ammonia[18] or sodium hydroxide[16]. The most universal reagent for cleavage of the silicon-oxygen bond is tetra-n-butylammonium fluoride. However, it also causes partial cleavage of the internucleotide linkage[17,21]. Generally, 5' substituents are more labile under the cleavage conditions than 2' groups[18], a fact that may be utilized for partial deprotection.

Since selective removal of substituents may be envisaged as a complementary method to achieve selective substitution, it is important to mention two more protecting functions, the 3-benzoylpropionyl- and the 1,3-dioxopentyl- [22,23,24]. These can be cleaved with hydrazine hydrate, as shown in Figure 7. The rate of removal for the 1,3-dioxopentyl group is 100 times greater than that for the 3-benzoylpropionyl[22].

B.3. Preparation of Carbonyl Derivatives.

Among various methods used for oxidation of carbohydrates and nucleosides[25-30], those with the

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widest application are based on dimethylsulfoxide (DMSO)[25,29,30]. A general mechanism of DMSO-DCC oxidation involves formation of the intermediate (A) by nucleophilic attack of DMSO on the electrodeficient center of dicyclohexylcarbodiimide . The intermediate (A) reacts further with a hydroxyl group to give (B) which decomposes producing the carbonyl compound.

Yields are often highest with DCC, although other reagents may be used. However, this procedure sometimes fails with carbohydrates having a sterically hindered hydroxyl group. Such substances may be oxidized with acetic anhydride or P_2O_5 but these two reagents also lead to the formation of methylthiomethyl esters as byproducts. With acetic anhydride some acetylation may also occur. An advantage of using DMSO with acetic anhydride is that the reaction product can be isolated by lyophilization of the reaction mixture[31].

A recent modification of the DMSO-based method was introduced by Swern[28] who used oxalyl chloride as the activating agent, as shown in Figure 9.

Binkley described a method in which the hydroxyl was first converted to the pyruvate ester followed by photochemical decomposition to the carbonyl compound.

The chromium trioxide-pyridine method is also frequently used in the oxidation of carbohydrates[26].

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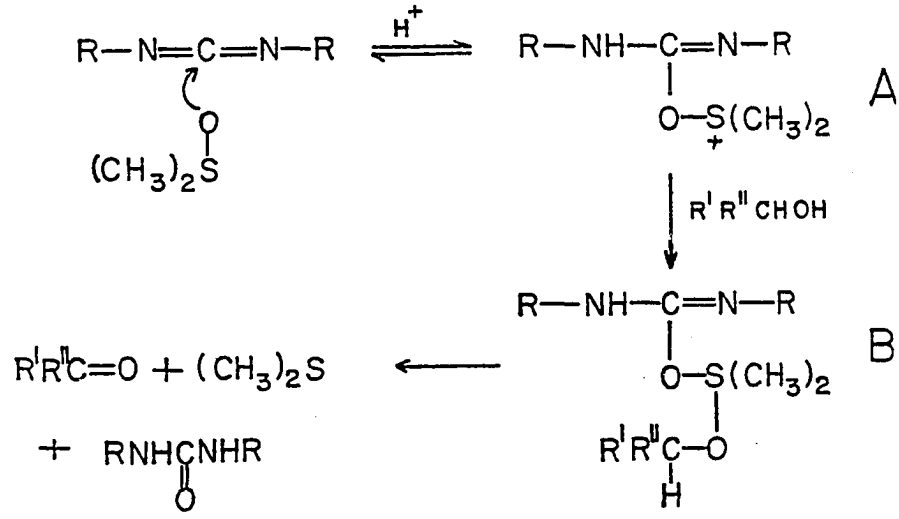

Figure 8

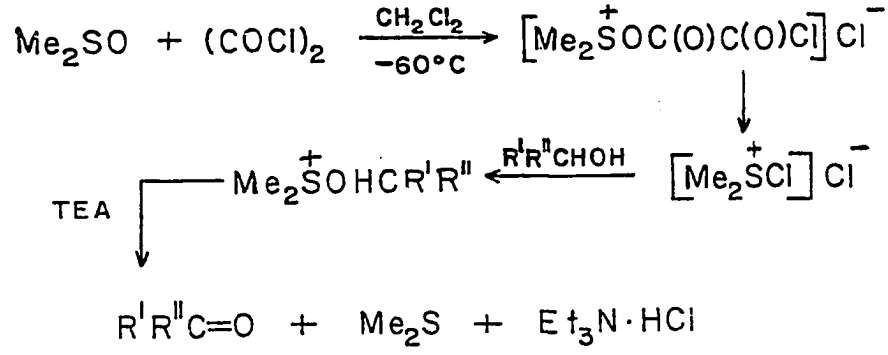

Figure 9

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Ruthenium tetroxide oxidizes 1,2:5,6-diisopropylidene-α-D-glucofuranose to the 3-keto form, but fails with 2,3-O-isopropylidene-α-D-glucofuranoside. It should also be pointed out that this last reagent attacks olefinic linkages[32].

The preparation and handling of 5-aldehydes of ribose (or 5'-aldehydes of nucleosides) is complicated by epimerization at C-4 (or 4') which occurs with bases or on silica gel[29]. Furthermore, the aldehyde may even eliminate acetal or ester from C-3 giving the unsaturated aldehyde (39)[33]. These problems have been circumvented by application of N,N'-diphenylethylenediamine (Wanzlick's reagent)[34] as a protecting group for the aldehyde (37), as shown in Figure 10. Regeneration of the aldehyde function is accomplished by controlled acidic hydrolysis of (37). Thus, p-toluenesulfonic acid produces aldehydes (35) with amorphous characteristics whereas hydrolysis with Dowex 50 resin results in crystalline hydrates (38).

There is substantially less information concerning oxidation of 3' OH in nucleosides. To date, there have been reported examples of succesful oxidations of the 2',5'-dityrityl derivatives of uridine[35] and cytidine[27] to their 3'-keto forms using DMSO-based methods.

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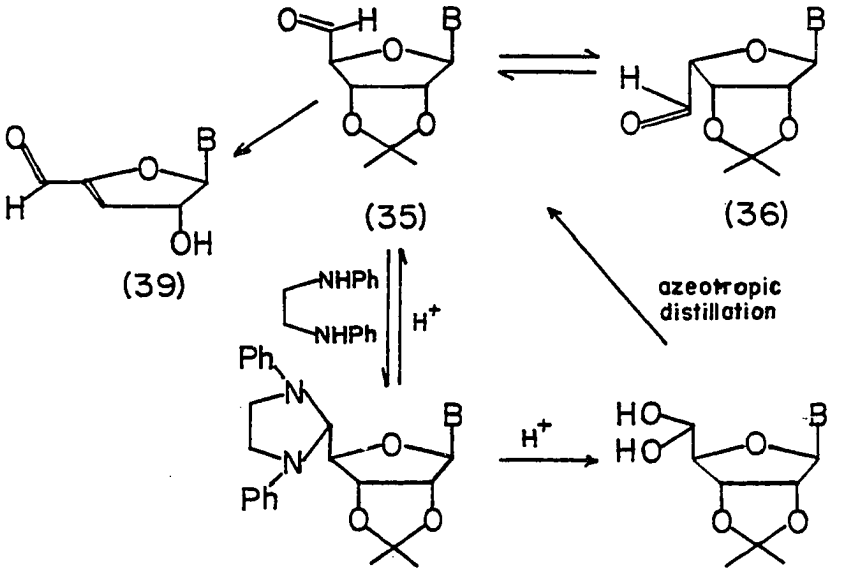
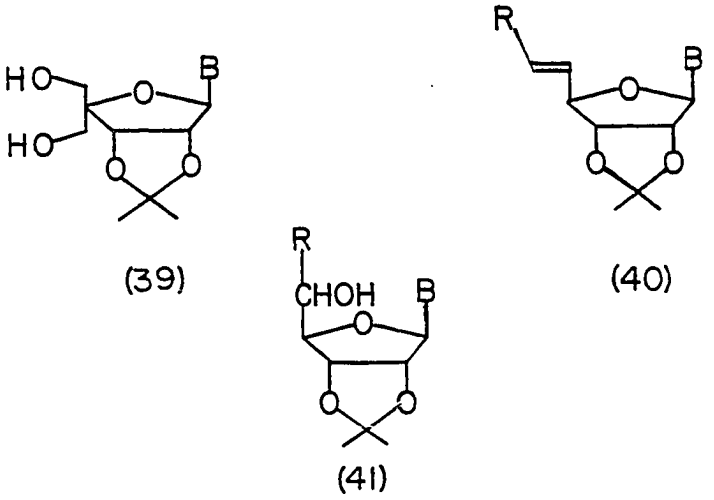


Figure 10



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B.4. Properties of carbonyl derivatives.

The 5-aldehydes of ribose and 5'-aldehydes of nucleosides have emerged as versatile intermediates for diverse reactions. The most extensive application of these compounds has been found for the generation of new carbon-carbon bonds at C-4 (44)[36,37] and at C-5 (40), (41)[29,38,39]. The hydroxymethyl compounds (44) were synthesized by means of an aldol condensation between 5-aldehydes and formaldehyde[36,33] in the presence of sodium hydroxide, as shown in Figure 11. A change of the base to potassium carbonate resulted in formation of the unsaturated aldehyde (39) instead of the aldol product (44).

The 5- or 5'-hydroxy derivatives (41) were obtained in the reaction of aldehydes with Grignard reagents[40], cyanide ion[29], and nitromethane[41]. These reactions are stereoselective. Additions of nitromethane and methylmagnesium chloride give two isomers, D-allo (45) and L-tallo (46), in the ratio 4 : 1 and 3 : 2, respectively. This stereoselectivity may be explained using the assumption that the reactions are kinetically controlled and the major product results from the attack on carbonyl group from the less hindered side, as shown in Figure 12.

However, addition of cyanide ion affords a 1 : 1

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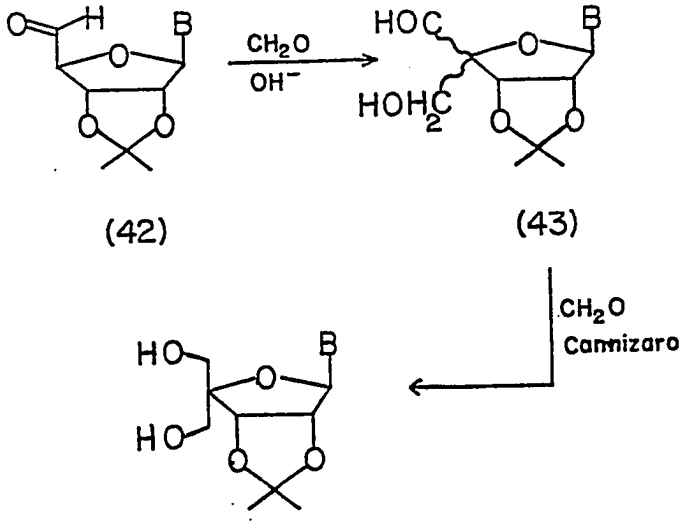
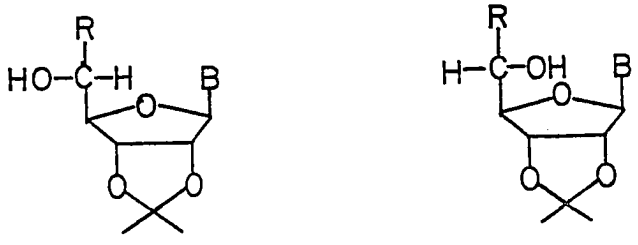


(42) (43) (44)

Figure II



(45) (46)

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epimeric mixture of cyanohydrins (45) and (46) (R=CN). This result may be understood as involving thermodynamic control which implies reversibility of the CN^- addition. Since both isomers, (45) and (46), have virtually the same thermodynamic stability, the mixture of these two compounds is produced.

Aldehydes (35) have also been subjected to the Wittig-type reactions[33,39,42,43,44,45]. Unsaturated derivatives (47) were synthesized using phosphoranes (48)[39,42]. Several isosteric 5'-phosphonate analogues of nucleotides (50) and ribose-5-phosphate (50) were also obtained[42,43,44,45] in the reactions of aldehydes (35) with diphenoxyphosphinylmethylenetriphenyl phosphorane (49).

The transformations of a carbonyl group at the 3' position of nucleosides are restricted due to the fact that 3'-keto nucleosides have the structure of β-ketoglucosylamines which makes them extremely sensitive to alkaline conditions. The 3'-ketouridine (45) and 3'-ketocytidine (51) are instantly cleaved in alkaline buffer (pH 10) to give the free pyrimidine bases and the unsaturated sugar fragment (52)[27,35]. However, the stability of 3'-ketonucleosides is significantly enhanced in the 2',5'-dityrityl derivatives. For example, 3'-ketouridine (51) undergoes reaction with hydroxylamine

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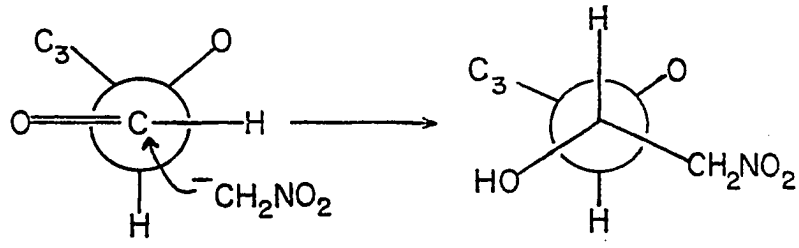
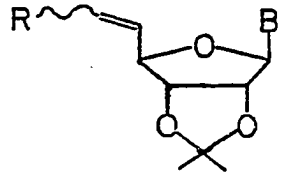
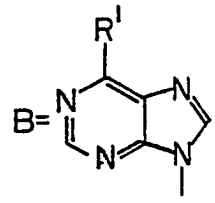


Figure 12



(47)

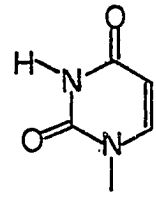
(47a) B=



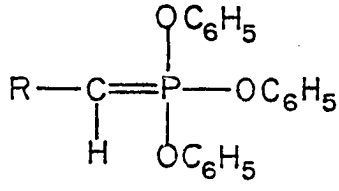
R= C_6H_5, CN, CO_2Et

(47b)

B =



R=CO_2Et



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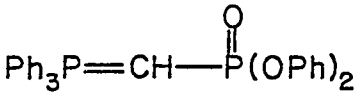

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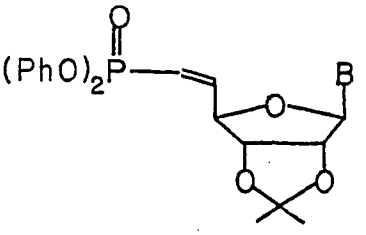

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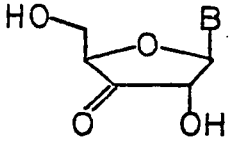

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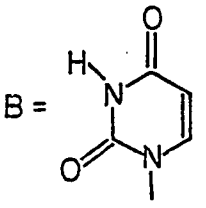

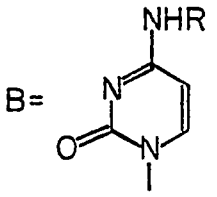

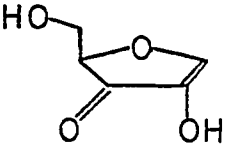

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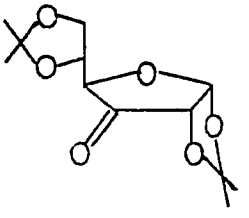

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to form the 3'-oxime, and reduction with sodium borohydride to afford a mixture of 3'-epimeric alcohols.

B.5. 3' Substituted Nucleosides From Carbohydrates.

The route to 3' substituted nucleosides begins with 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranose (15) or its 3-keto derivative (53). The latter is also commercially available in hydrated form.

Methodologically, one may envision three main segments that must be included in any synthetic scheme for nucleotide derivatives that starts from ketone (53).

1. Addition of a carbon nucleophile to the 3-keto function generates a new carbon-carbon bond. This is followed by transformations of the new substituent to secure the proper functionality at that position.
2. Synthesis of the D-ribose ring by degradation of 6-hydroxylmethylene group.
3. Introduction of a purine or pyrimidine base following the conversion of the C-1 center into an acetyloxy or halogen substituted site.

Additions of nucleophiles to (53) give access to the compounds with opposite configurations at C-3. The configuration in which the new carbon substituent is cis relative to the C-5:C-6 linkage results from reactions with

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organometallic reagents. The reverse configuration, trans, can be created indirectly after a condensation of the ylide or aldol-type, followed by reduction of the 3-C-methylene function.

Reactions of (53) with Grignard reagents have been the subject of extensive investigations[46,47,48,49]. Reactions with alkylmagnesium halides were reported to give two major products (54) and (55)[46], as shown in Figure 13. The compound (55) was the main product in the reaction with cyclohexylmagnesium bromide. Furthermore, this reagent causes partial removal of the 5,6-isopropylidene group. High yields of D-allofuranoses (54) were obtained with phenylmagnesium halides. The relative amounts of these products vary with changes of solvent (THF, ethyl ether) and temperature.

Reaction with methylene dimagnesium bromide in ether : benzene (1 : 1) affords an important compound, the 3-C-methylene derivative (57) of glucofuranose[47]. An unexpected product (58) was obtained in 86% when this reaction was carried out in pure benzene.

Ethynylation of (53) with ethynylmagnesium bromide to give (54) was the first step in the synthesis of agarose by Horton and coworkers[50].

Several investigators examined the utility of organolithium compounds, (LiR') to introduce various functionalities into

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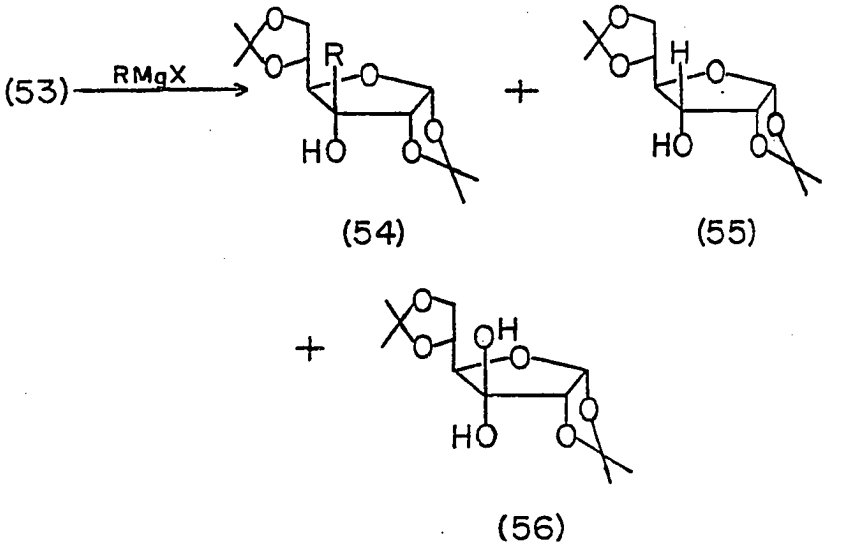
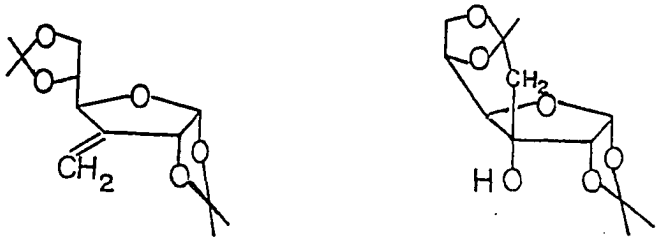


Figure 13



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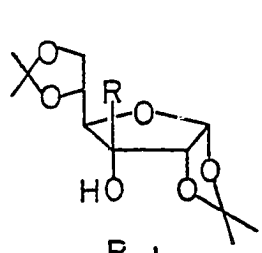

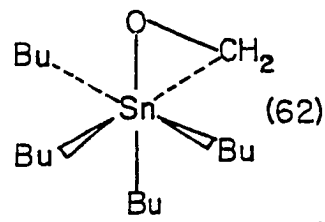

(62)

R	
R'	(59a)—(61a)
$-CH_2OH$	(62a)
$EtO_2CCH(NO_2)-$	(63)

R'	
R'' S, S	(59)
$-CH_2P(=O)(OCH_3)_2$	(60)
OCH_3	(61)

Li.R'

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(53). These lithium reagents include 2-alkyl-2-dithianyl lithium (59)[51], lithio ethanephosphonic acid ester (60)[57], as well as species that represent "umpolung" functional groups 1-methoxyvinyl lithium (61)[53,54] and unconventional, hydroxymethylating tin compounds (62)[55]. However, the last reagent produced only 11% of the hydroxymethyl derivative (62a).

The same configuration that results from the reaction of (53) with organometallics can be obtained in the condensation of this compound with methyl nitroacetate forming the 3-C-nitro(methoxycarbonyl)methyl derivative (63)[56].

The Wittig reaction has been so far the most fruitful method for the generation of the 3-C-methylene compounds (122) and (123). Although the reaction has been known for a long time, its mechanism has not been entirely clear. The stereochemical outcome of the Wittig reaction in the case of 1,2:5,6-di-O-isopropylidene-α- D-hexofuranos-3-ulose (53) can support a mechanism proposed by Bestman.[57]

The results of various experiments are collected in table I. The variation of cis/trans ratio may be explained through a consideration of dipolar, anionic intermediate (121).

As it was illustrated in the addition of organometallic compounds to (53), the approach of an incoming anion to

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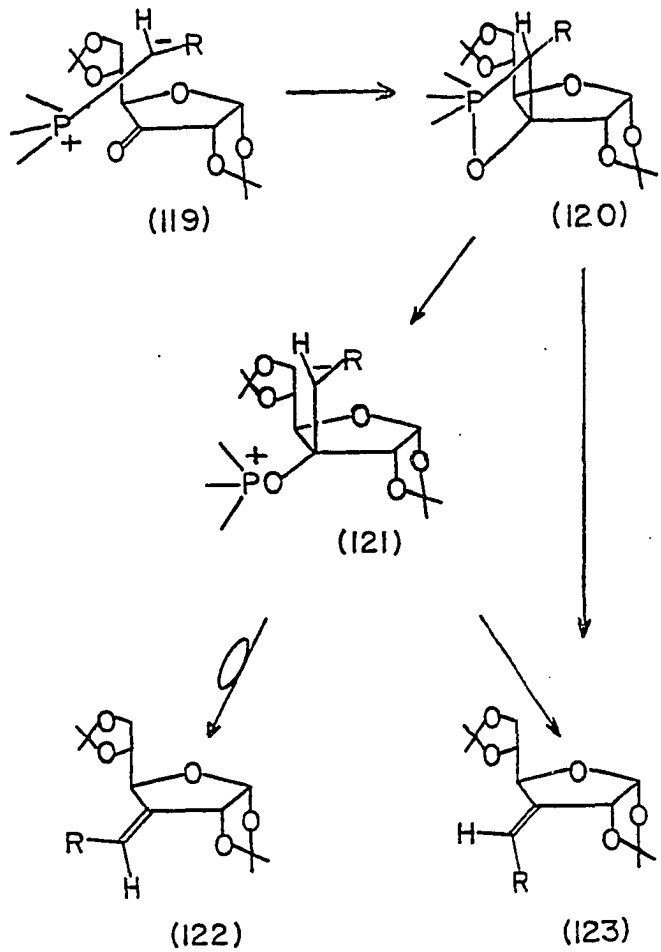

Figure 14

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(53) occurred from the direction cis to C-5:C-6 (-side). An attaching ylid should also follow this approach, the largest group, substituted phosphorus, being directed away from the C-5:C-6, (119), as shown in Figure 14. Also, the C-5:C-6 carbons are located between the second largest substitutent of the ylid (R) and hydrogen, as shown in the structure (119). Such addition gives betaine (120) which, if it eliminates $Ph_3P=O$ directly, produces the olefin (123) with H-3' trans to C-2.

However, if oxaphosphetane (120) opens C-P bond to give betaine (121), the latter can either eliminate phosphine oxide without any further structural reorganization and produce trans-olefin (123) or reorganize an anionic center before evolution of the double bond is complete and produces less strained cis-olefin (122). Substituents R that are particularly large are expected to remain in the same configuration in the anion (121), since their rotation would be required to overcome steric interactions with the phosphine substituents as well as with C-5, C-6 acetonide ring. As a result of this, the isomer (123) will be preferred.

From table I, it may be noted that well-established ylids with relatively small groups (CN, Ph, entries 4 and 5) gave predominantly cis olefins (66), whereas ylids bearing larger groups entris (1, 2, 3, 10) produced mainly

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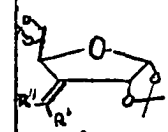
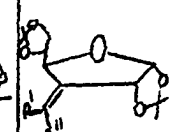
$R_3P=C(R')(R'')$			Solvent	 cis	 trans	Ref.	Ent. No
R	R'	R''					
Ph	H	CO_2Et	C_6H_6	16	84	58	1a
			$CHCl_3$	21	79		1b
			DMF	20	73		1c
		COMe	C_6H_6	23	69		2a
			$CHCl_3$	32	62		2b
			DMF	26	39		2c
		COPh	C_6H_6	traces	80		3a
			$CHCl_3$	17	75		3b
			DMF	35	50		3c
		CN	C_6H_6	59	41	61	4a
			Et_2O	62	38		4b
			DMF	74	26		4c
			MeOH	55	45		4d
		Ph	Et_2O-DMSO	63	37	59	5
		Cl	THF	77	123	60	6
		Br	THF	21	29		7
		I	THF	19	31		8
	F	Cl	Hexane	16	31	62	9
$(CH_3O)_2\overset{O}{P}-CH_2CO_2CH_3$			DMF	6	39	89	10

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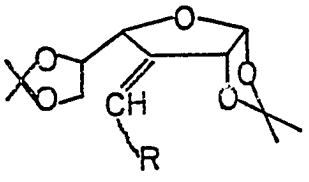
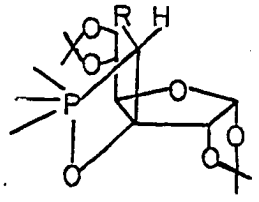
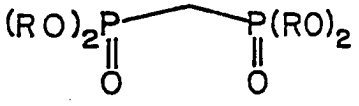


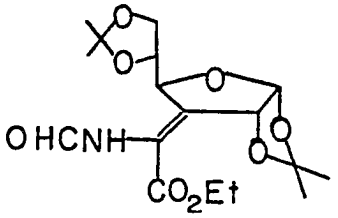
(70)



(120a)



R= C_2H_5 (71)
R= i-C_3H_7 (72)



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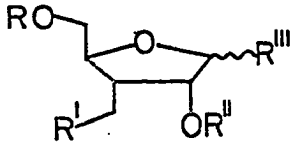
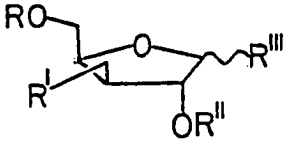


(74)



(75)

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trans olefins (65).

A change in solvent from less to more polar resulted in increase in the amount of cis isomer that was expected since a polar environment stabilizes dipolar intermediate (121). Strongly polar N,N-dimethylformamide was used as a solvent (entries 1c, 2c, 3c), the reactions were complicated by formation of lyxo isomers (67). Even in chloroform small amounts of lyxo isomers were detected[54]. All ylids with halogens (entries 6, 7, 8, 9) also gave more of the trans isomers.

These would not be expected to stabilize efficiently the anion (121) and the ratio of cis/trans would be fixed predominantly by relative populations of (120) and (120a). Due to the small size of halogens, the population of oxaphosphetane (120a) may be significant.

In addition to the reactions collected in Table I, two other reagents are important for this work. Tetraethyl methylenediphosphonate (71)[53] and tetraisopropyl methylenediphosphonate (72)[64] in reactions with (53) give vinyl phosphonates (124) opening routes to isosteric phosphonates of carbohydrates and nucleoside 3'-phosphates.

One further method of carbon-carbon bond formation in (53) involves condensation of it with ethyl isocyanoacetate affording derivative (73)[65].

After introduction of a required group in the

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3-position the compound may be hydrolyzed selectively to remove the 5,6-isopropylidene group, and the resultant 5,6-diol system may undergo cleavage with sodium meta-periodate followed by reduction with sodium borohydride to establish the 5-hydroxy group. Finally, protection of this group and hydrolysis of the 1,2-isopropylidene group with subsequent esterification gives the ribose structure (74) or (75).

Having established the appropriate functionalities in the ribose ring the synthesis of nucleoside derivatives requires the introduction of a base on C-1. A compendious description of this process can be made by combining three available procedures (Koenings-Knorr, Hilbert-Johnson, fusion)[63] into one scheme, as depicted in Figure 15. It must be noted that in most situations, also in this work, only β-nucleosides are desired and conditions that lead to this isomer are important.

Dissociation of the ribose derivative (76) is facilitated by the neighbouring acyl group and the Lewis catalyst. This generates the cation (77) which eventually produces the β-nucleoside. Formation of the α-anomer (78) is not facilitated by the 2-acyloxy substituents. It gives cation (79) which may rearrange to (77) if conditions permit, or react with a base affording a mixture of α and β-nucleosides (81), (80).

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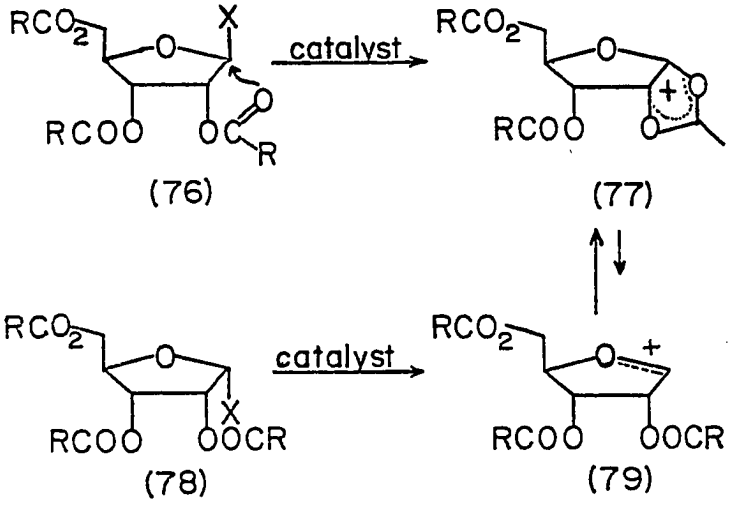
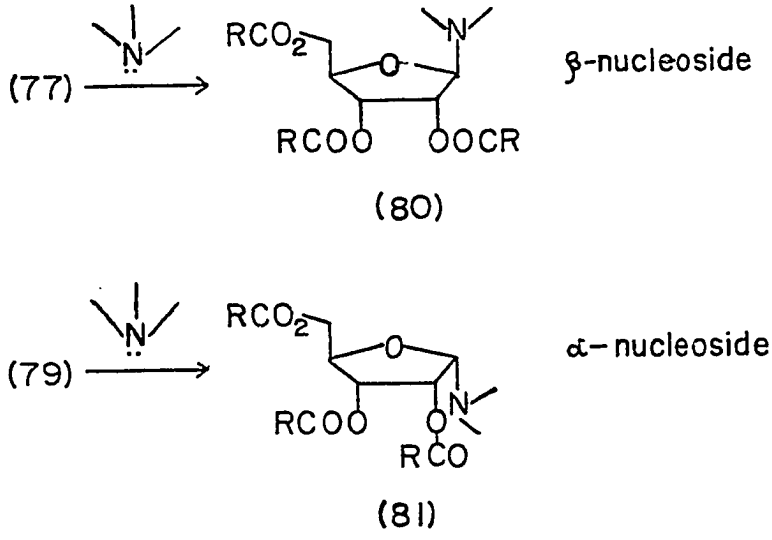


Figure 15



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Catalysts make formation of the 1,2-acyloxonium cation easier and prolong the lives of cationic species. They, therefore, create conditions favoring the rearrangement of (79) to (77) which results in predominant formation of - nucleosides[67].

The main differences betwen the various procedures lie in the reactions of cations (77) and (79) with pyrimidine and purine bases. The pyrimidines can be used in the forms of 2,4-pyrimidinediones (32) or protected 2,4-dihydroxypyrimidines (83). All available data suggest[66] that pyrimidinediones (82) produce first glycoside intermediates (85) that subsequently undergo intermolecular transformations to nucleosides, as shown in Figure 15. This reaction requires Lewis acids, usually mercury or silver salts, as catalysts which influence not only first step, glycoside formation, but also catalyze the transformation of glycoside to nucleoside, (85) to (86). The entire process is reported to be stereospecific in both steps, that is, only -glycosides and -nucleosides are obtained regardless of whether the -isomer or a mixture of - and -halogenoses were used. The method described above is known as the heavy metal of Koenings-Knorr procedure.

If protected 2,4-dihydroxypyrimidines are used, the reaction is known as the Hilbert-Johnson procedure[69]. Unlike the Koening-Knorr reaction, hydroxypyrimidines do not give glycosides with ribose. Thus, a direct alkylation

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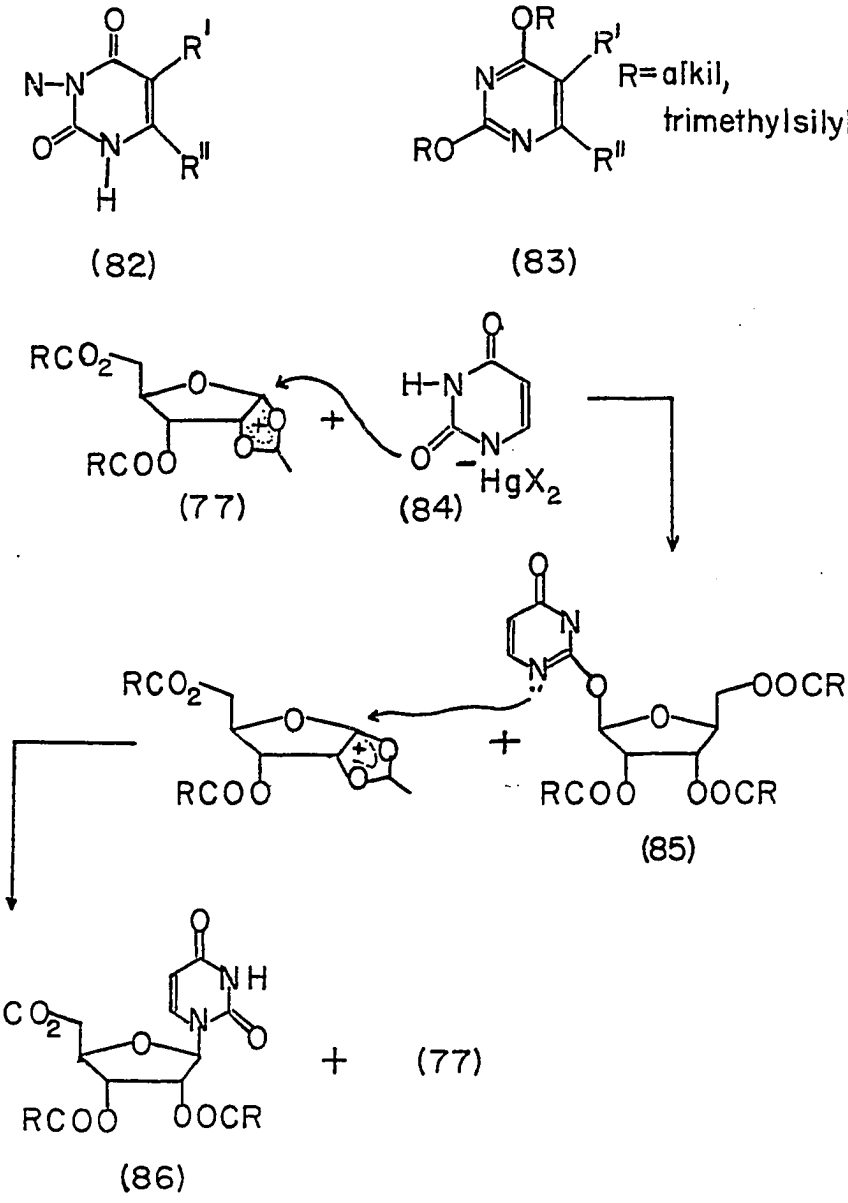

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of the nitrogen atom was postulated in this procedure[66].

A choice of the protecting groups R in 2,4-dihydroxypyrimidines (83) is very important. Silyl groups are better than alkyl because the latter are sometimes difficult to remove at the end of the synthesis[70,70a], usually give lower yields, and can not be applied to the synthesis of 6-substituted nucleosides[71]. When the reaction is performed without catalyst, both anomers, However, catalysts also give σ -complexes with bases at N-1 (88) decreasing the ability of the base to react with cation (77) and changing the reaction site on the base from N-1 to N-3.

The effects of catalysts were investigated in the reaction of silylated 2,4-dihydroxypyrimidines with 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (90). Since only the free base (87) can react to give the N-1 nucleoside, the stronger the σ-complex, the more of undesired N-3 nucleoside is produced[72].

Thus, with strongly basic compounds (87) yields of N-1 nucleosides are higher when weaker Lewis acids are used. For example, compound (87), R=H, R_1=CH_3 afforded 71% of N-1 nucleoside with ribose (90) in acetonitrile when reaction was catalyzed by $(CH_3)_3SiO\text{-}SO_2CF_3$, compared to $SnCl_4$. Polarity of the solvent plays

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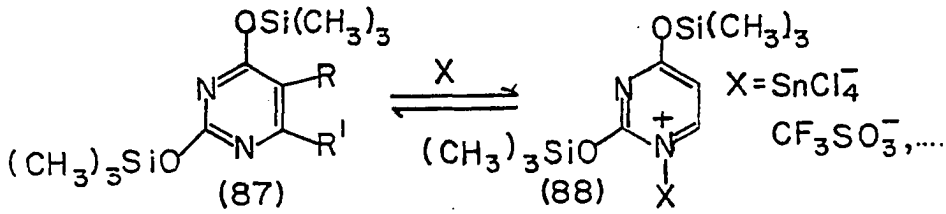
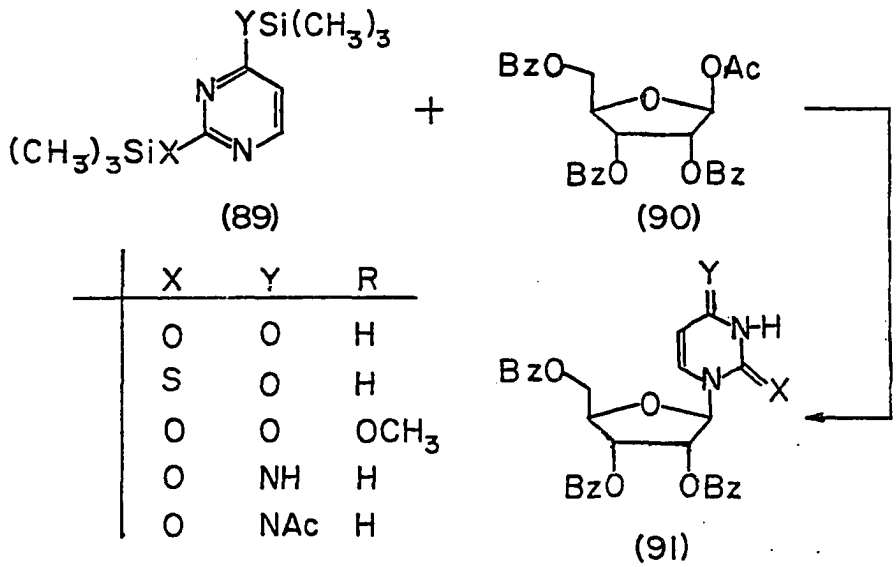
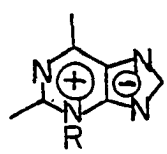




X	Y	R
O	O	H
S	O	H
O	O	OCH_3
O	NH	H
O	NAc	H

catalysts: CF_3SO_3H, $C_4F_9SO_3K$, $SnCl_4$, NH_4ClO_4, $NaBF_4$



(92)

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crucial directing role. More polar solvents compete with Lewis acids in binding to the base making the σ-complex (88) weaker. It facilitates formation of N-1 nucleosides. Acetonitrile has emerged as the most convenient solvent.

The latest achievement in the synthesis of pyrimidine nucleosides belongs to Vorbruggen and coworkers[72,73,74] who worked out a one-step/one pot reaction in which the bases (91) were mixed in acetonitrile with catalysts, protected ribose (90), hexamethyldisilazane and trimethylchlorosilane. Yields of β-nucleosides (91) ranged from 40% to 80%. The reaction is shown in Figure 16.

Purine bases[66] (92) attack the sugar cation (77) with the N-3 atom to produce initially N-3 nucleosides followed by rearrangement on the sugar portion to N-9[72,75a,b,c]. The driving force for the rearrangement is electronic imbalance in the purine ring resulting from N-3 glycosylation[76]. The pyrimidine portion becomes π-deficient while the imidazole portion is π-excessive (92).

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C. RESULTS AND DISCUSSION.

The work presented in this chapter is concerned with the synthesis of two classes of compounds. One is in the nucleotide series, oligonucleotide analogue and the other the nucleoside category the methylene derivative, related to uridine (94).

Both compounds have a methylene group in the 3'-position instead of an oxygen atom. In naturally occurring nucleic acids this oxygen is a part of a phosphodiester linkage between two nucleosides.

Attempts to synthesize the compounds of the structures (93) and (94) involved examination of two potential approaches, as they were scrutinized in the first part of this Thesis. The two synthetic routes started from available nucleosides, and diisopropylidene-α-D-glucose, respectively.

The first part of this research was devoted to the synthesis of nucleosides with selectively protected 2',5'-hydroxyl groups, and their oxidation to 3'-ketonucleoside derivatives. For the selective protection of the 2',5'-hydroxyl groups in guanosine and uridine, tert-butyldiphenylsilyl chloride was chosen. A silyl-based reagent was not expected to give a isolable compound involving reaction with the amino group of the

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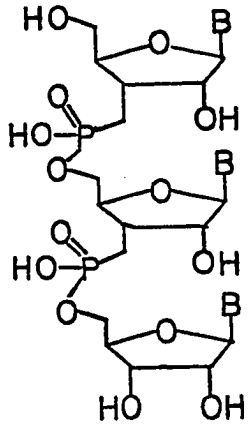
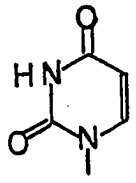
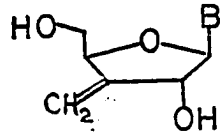


(93)

B=





(94)

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purine ring in guanosine since the silyl-nitrogen bonds are usually very susceptible to attack by water. The work up of the reaction mixture in an aqueous solution would cleave any possible silyl-nitrogen connection. The tert-butyldiphenylsilyl substituent on oxygen was reported to have an enhanced stability towards cleavage with bases and acids[16] as compared to other silyl-based reagents. This stability would facilitate further transformations of 2',5'-disubstituted nucleosides.

A reaction of guanosine with excess of tert-butyldiphenylsilyl chloride and imidazole in dimethylformamide at 100-117°C for 4 hours gave 2',5'-bis(tert-butyldiphenylsilyl)guanosine (125), B=guanine, as a major product with a small amount of 3',5'-isomer. Uridine, under the same conditions afforded a mixture of 2',5' (125), B=uracil, and 3',5'-bis(tert-butyldiphenyl)silyl uridine (126) in approximately equal amounts. When the reaction with guanosine was performed at room temperature for 20-24 hours, only 5'-(tert-butyldiphenyl)silylguanosine was isolated.

Since 2',5' protected uridine is known to undergo oxidation to its 3'-keto derivative by DMSO based methods, these oxidations were examined with 2',5'-bis(tert-butyldiphenyl)guanosine (125). However, no

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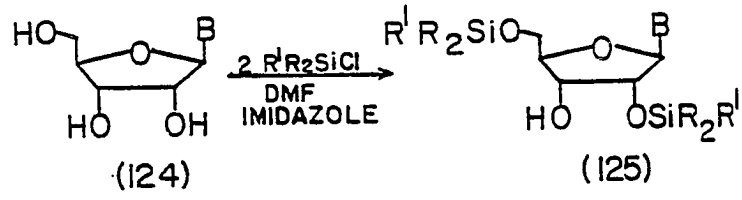
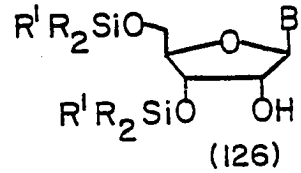


(124) (125)

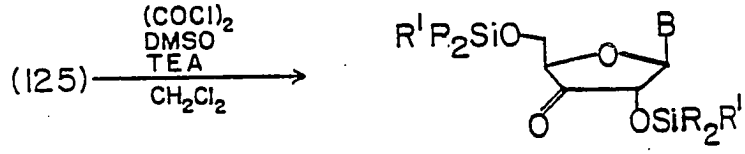
R=Ph

R'=t-Bu

B=uracil, guanine



(126)



B= guanine

(127)

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conversion of the compound (125), B=guanine, was detected using TLC and IR spectroscopy when acetic anhydride or DCC or P_2O_5 in mixtures with DMSO were used. Only the Swern oxidation produced a new compound for which the IR spectrum exhibited a strong carbonyl band. Attempts to purify this product by column chromatography on silica gel were unsuccessful and resulted in decomposition.

Using the assumption that the stability of 2',5'-bis(tert-butyldiphenyl)silyl-3'-ketoguanosine might be improved by a protection of its amino group, the product of the Swern oxidation was allowed to react with benzoyl chloride and triethylamine, but a complex mixture of new substances was produced. Due to these results, further examinations of the transformations at 3' carbon in nucleosides were discontinued.

Another approach to the synthesis of trinucleotide (93) and the epoxy derivative (94) of uridine shared a common starting material. This was 1,2:5,6-diisopropylidene-3-deoxy-3-C-methylene-α-D-glucofuranose (57). This substrate was prepared in two steps from 1,2:5,6-diisopropylidene-α-D-glucofuranose (15).

In the first step, oxidation of (15) was performed with acetic anhydride in dimethylsulfoxide (DMSO). It was noted that the best yields of (53) were obtained when the mixtures of acetic anhydride and DMSO were allowed to stand

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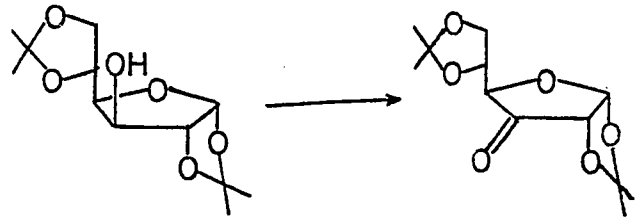
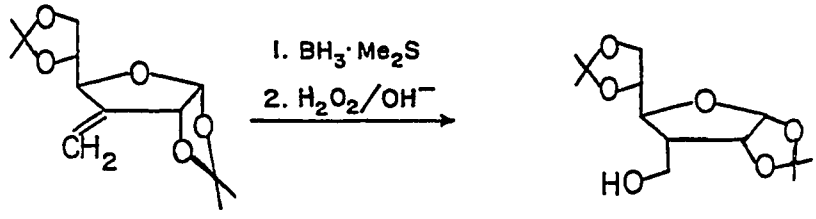
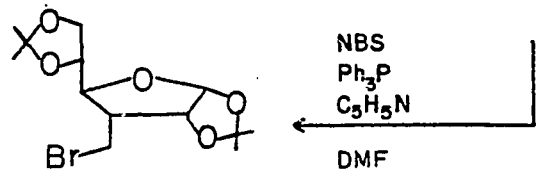


(15) (53)



(57) (95)



(96)

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for 45-60 min at room temperature before the addition of the glucose derivative (15). If the carbohydrate were added immediately to the oxidizing mixture, large amounts of an acetylated compound were obtained and the yield of the 3-keto form decreased. This result sugested that the rate of a formation of the oxidizing complex from DMSO and acetic anhydride was comparable to the rate of acetylation of (15).

The ketone (53) was transformed in the second step into 1,2:5,6-diisopropylidene-3-deoxy-3-C-methylene-α-D-glucofuranose (57) by means of the Wittig reaction using triphenylphosphonium methylide in DMSO followed by usual work up of the reaction mixture with water.

C.1. Preparation of the Isosteric Oligonucleotide.

The central problem in the preparation of isosteric oligonucleotide was the synthesis of the 3'-deoxy-3'-phosphonic acid (106) analogue of uridine. The basic framework of the furanose ring was retained through all the transformations. One asymmetry center was established on C-3' and inversion of the symmetry was performed on C-1'.

In the first step the compound (57) was hydrated to 3-deoxy- 3 -hydroxymethyl-α-D-glucofuranose (95) by

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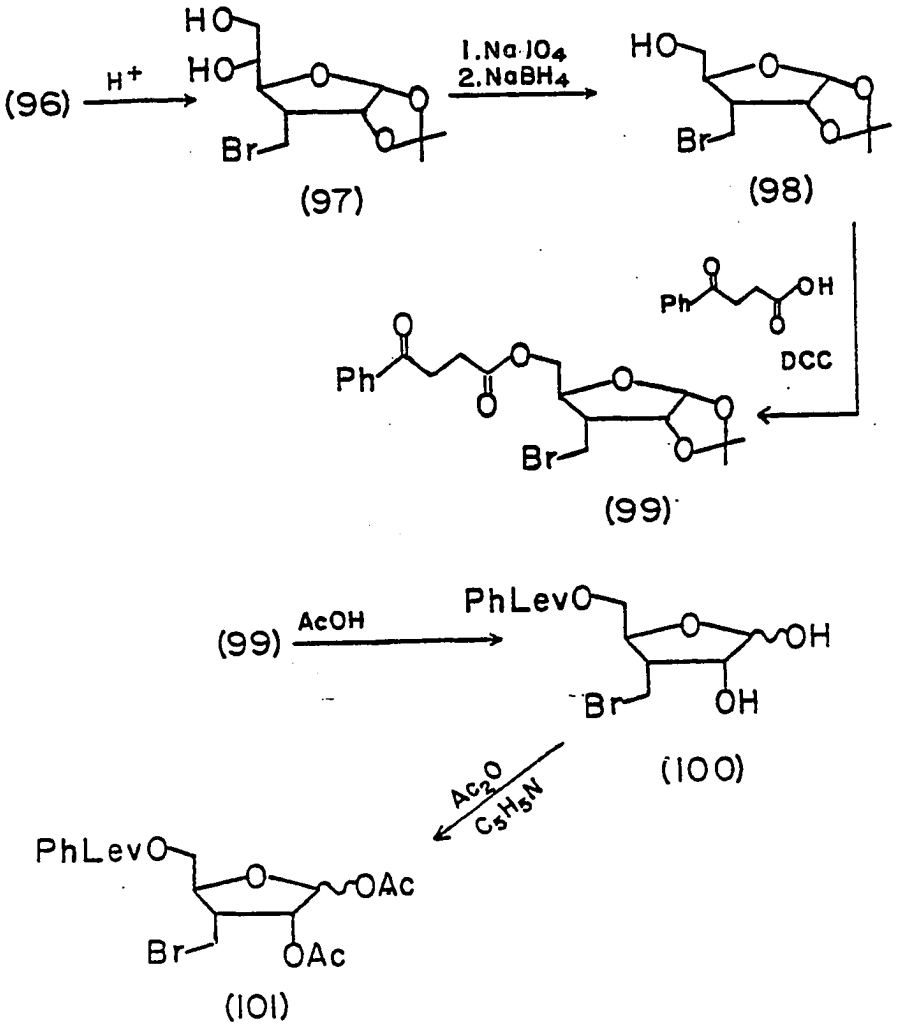

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hydroboration with borane- dimethylsulfide complex followed by oxidation of the borane adduct with hydrogen peroxide. Several attempts to convert the compound (95) into its 3-bromomethyl derivative (96) according to the procedure of Ariatti and Zemlicka with N-bromosuccinimide and triphenylphosphine in DMF, gave only 10-30% yield although the original author reported yields for this reactions of over 90%.[77]

However, the same reaction gave 75% yield of the product (96) when there was added to the reaction mixture a molar equivalent of dry pyridine. Apparently, pyridine was needed as an efficient scavenger of hydrogen bromide which, if not neutralized, cleaved the 5,6-O-isopropylidene group in (95) or (96).

3-Deoxy-3-bromomethyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (96) was next hydrolyzed in a homogeneous solution of chloroform, methanol and 1.6% sulfuric acid to 3-deoxy-3-bromomethyl-1,2-O-isopropylidene-α-D-glucofuranose (97). The latter compound was subjected to a cleavage of its 5,6-diol system with sodium meta-periodate followed by a reduction of the cleavage product with sodium borohydride. This sequence gave 3-deoxy-3-bromomethyl-1,2-O-isopropylidene-α-D-ribofuranose (98).

At this point it was necessary to introduce proper

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protecting groups at the 1,2 and 5 oxygens of the compound (98). Thus, (98) was treated with 3-benzoylpropionic acid and dicyclohexylcarbodiimide to give 3-benzoylpropionyl-3-bromomethyl-3-deoxy-1,2-O-isopropylidene-α-D-ribofuranose (99). This latter compound was converted to 3-benzoylpropionyl-3-bromomethyl-3-deoxy-1,2- diacetyl--D-ribofuranose (101) in two steps which involved hydrolysis of (99) to (100) with 80% acetic acid followed by acetylation of this product with acetic anhydride in pyridine. The compound (101) contains a pattern of hydroxyl protection which allows selective transformations leading to the introduction of the uracil base at C-1 as well as the dihydroxyphosphinyl isosteric system at C-3.

An attempt to introduce the nucleoside base by the reaction of (101) with 1,2-bis(trimethylsilyl) pyrimidine and stannous chloride in acetonitrile, according to the method of Vorbruggen[72,73,74], gave a product (97) of which the NMR spectrum was difficult to interpret. The elementary analysis was consistent with the assumption that the product (97) existed in the form of its dihydrate. However, the results of elementary analysis repeated after several days of drying of (97) under vacuum over P_2O_5 remained unchanged. Since one should not expect any exceptionally strong bonding between the water

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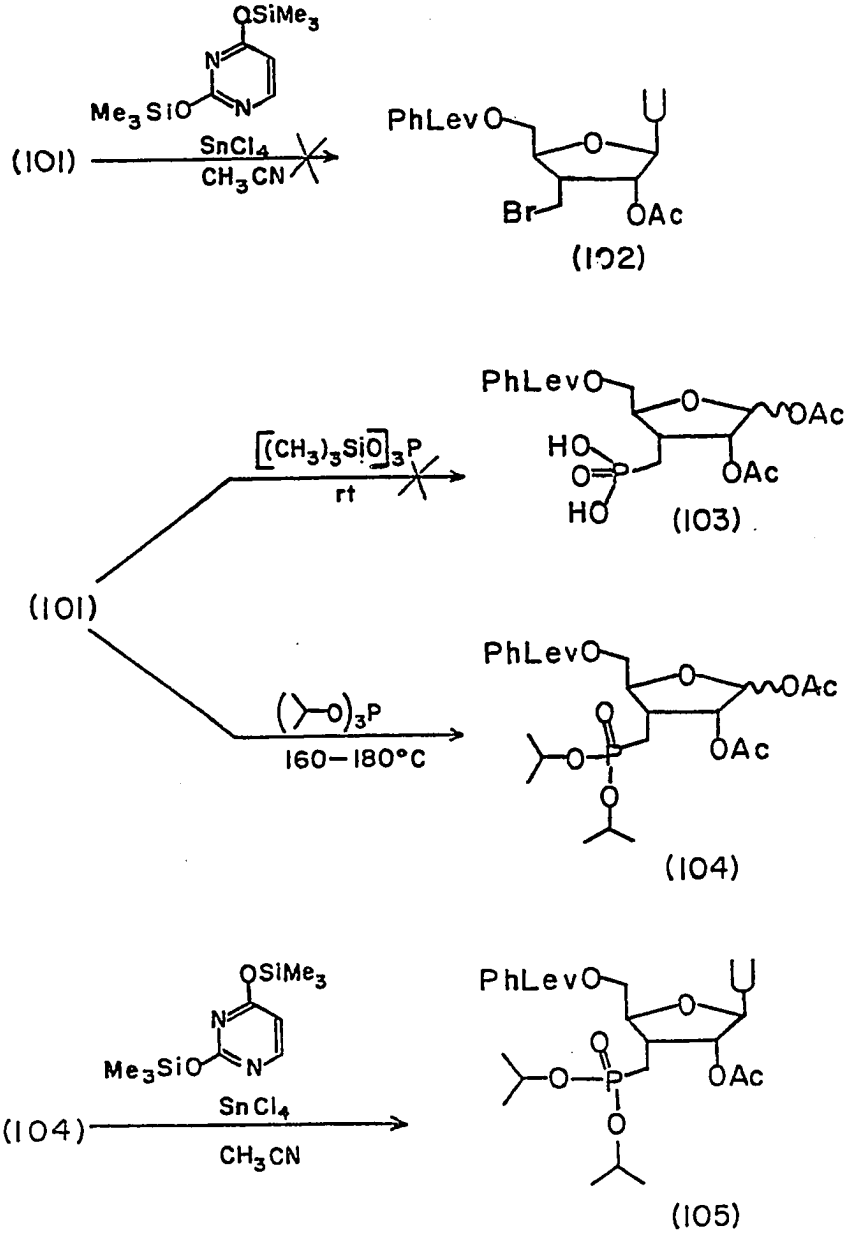

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molecules and nucleoside (97), the hypothesis about the existence of (97) in the dihydrate form could not be accepted. Therefore, it was necessary to examine the possibility of the introduction of the hihydroxyphosphinyl group in the compound (101) directly.

A reaction between (101) and tri(trimethylsilyl)phosphite in dry tetrahydrofuran at room temperature resulted in cleavage of the 3-benzoylpropionyl protection. Finally, a successful substitution of bromine by phosphorus was achieved upon treatment of (101) with triisopropylphosphite at 160-180. This approach produced 5-(3-benzoylpropionyl)-3-deoxy-1,2-diacetyl-3-C-(diisopropoxyphosphinylmethyl)-(+)-D-ribofuranose (104). This compound was treated with 2,4-bis (trimethylsilyloxy)pyrimidine and stannous chloride in dry acetonitrile at room temperature to give 5'-(3-benzoylpropionyl) -3'-deoxy-3'-C-(diisopropoxyphosphinylmethyl)uridine (105). Cleavage of isopropyl groups in (105) was done by means of trimethylsilyl bromide followed by treatment with a water-pyridine mixture that produced a pyridinium salt of 5'-(3-benzoylpropionyl)- 3'-deoxy-3'-C-dihydroxyphosphinylmethyluridine (106) Condensation of the latter compound with 2',3'-isopropylideneuridine and dicyclohexylcarbodiimide in

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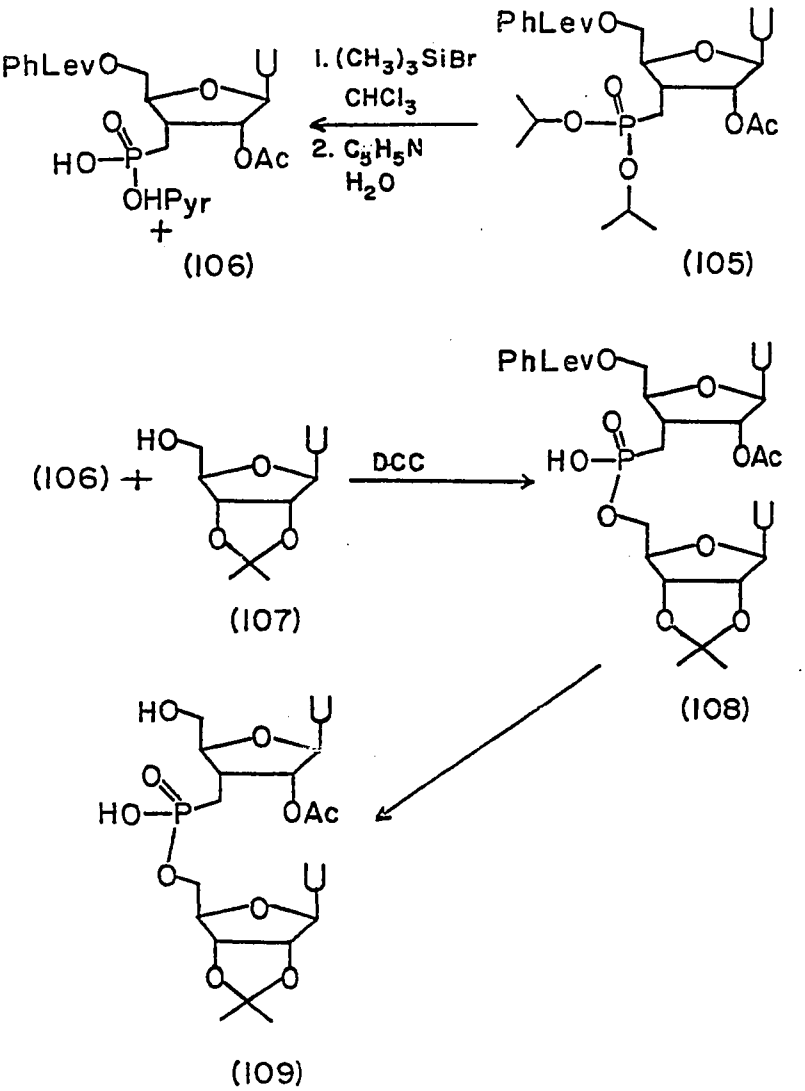

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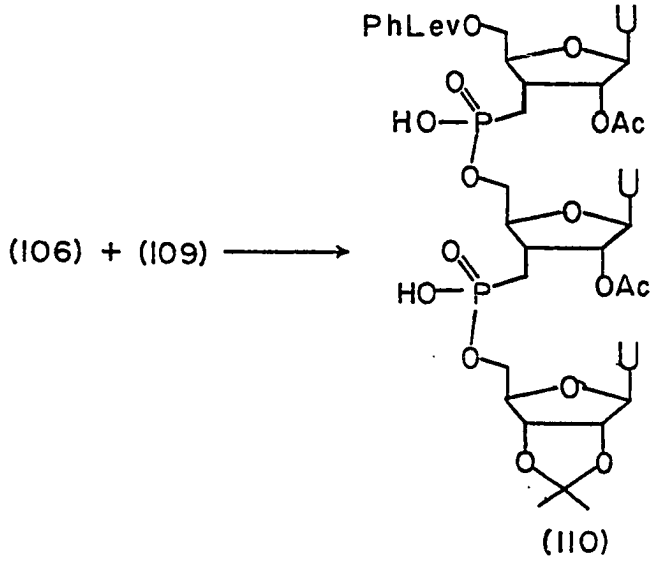

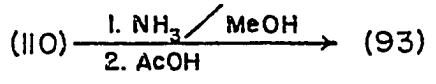

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dry pyridine in the presence of Dowex 50 (pyridinium form) resulted in formation of dinucleotide analogue (108). A selective removal of the 5'-(3-benzoylpropionyl) group with hydrazine in an acetic acid-pyridine buffer transformed the analogue (108) into (109). The condensation of (109) with one more molecule of the nucleotide (106) gave oligonucleotide analogue (110). The latter was deprotected in two steps, fist being treated with ammonia in methanol to cleave 3-benzoylpropionyl and acetyl groups, and finally acetic acid removed isopropylidene protection affording oligonucleotide (93).

C.2. Methylene Derivative of Uridine.

Removal of the 5,6-isopropylidene group in 1,2:5,6-diisopropylidene-3-deoxy-3-C-methylene-α-D-glucofuranose (57) was achieved in a solution of acetic acid, water and ethanol. Unlike the hydrolysis of the 5,6-isopropylidene group in 1,2:5,6-diisopropylidene-3-deoxy-3-C-bromomethyl-α-D-glucofuranose (96), which was performed in a solution of dilute sulfuric acid, methanol and chloroform, the compound (57) underwent complete destruction under the same conditions. Furthermore, when the reaction was attempted according to the procedure of Brimacombe, et al[78] in

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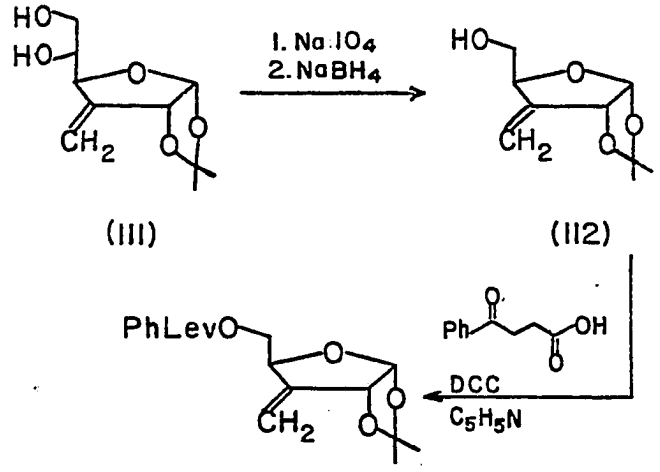

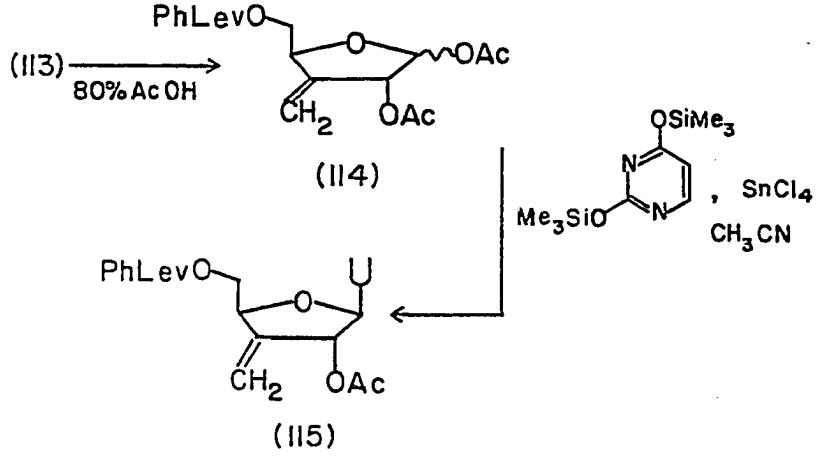

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70% acetic acid, thin-layer chromatography revealed a faster moving compound in approximately equal amount with the expected product.

Cleavage of 5,6-diol in (111) was performed in a manner analogous to the conversion of (97), using sodium meta-periodate followed by reduction with sodium borohydride. The resulting 5-hydroxyl group in 3-deoxy-3-C-methylene-1,2-O-isopropulidene-α-D-ribofuranose (112) was protected by means of esterification with 3-benzoylpropionic acid. This protected derivative (113) was then subjected to hydrolysis by 80% acetic acid at 80% during 24 hrs. After the conversion of (113) was complete, the reaction mixture was evaporated to dryness, and treated (without further purification) with acetic anhyride in pyridine at room temperature to give 3-benzoylpropionyl-1,2-diacetyl-3-deoxy-3-C-methylene--D-ribofuranose (114). Preparation of the uridine derivative (115) from (114) was accomplished by reaction of (114) with 2,4-bis(trimethylsilyloxy)uridine and stannous chloride in dry acetonitrile. Deprotection of the uridine derivative (115) was accomplished by treatment with 3M solution of ammonia in methanol to give 3-deoxy-3-C-methyleneuridine (116).

Despite extensive efforts to epoxidize the compound (116), the epoxy derivative of (116) could not be obtained

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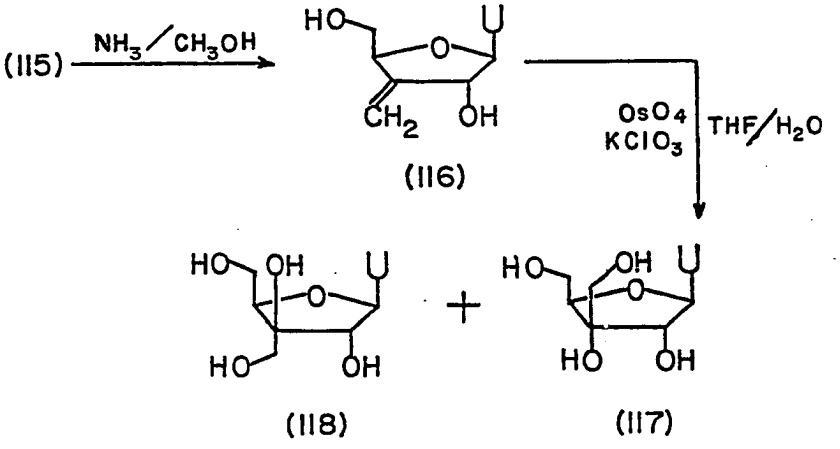

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in a direct, one step reaction. No conversion of the nucleoside was detected upon the treatment of (116) with the following reagents: m-chloroperbenzoic acid in tetrahydrofuran [79,80,88], Na_2WO_4 and H_2O_2 in a methanol/water mixture, iodine/KIO_3 in a dioxane/water mixture[81], acetonitrile/H_2O_2 in methanol[81]. An attempt using (116) with tert-butylperoxide and Triton B in tetrahydrofuran[83] led to a decomposition of the nucleoside, whereas an effort to use vanadyl acetylacetonate ($VO(acac)_2$)[84,85] instead of triton B in the same process resulted in the immediate reaction between the nucleoside (116) and vanadyl acetylacetone. Due to these adversities, another approach was applied. This consisted of hydroxylation of the 3'-methylene group in the nucleoside (116) to a diol system which might be converted to the epoxide. The hydroxylation was accomplished using osmium tetroxide and potassium chlorite in a tetrahydrofuran/water[86,87] mixture at room temperature during 3 hours. This reaction gave a mixture of two isomeric compounds (117) and (118), established by inspection of the ^{13}C and ^{1}H NMR spectra of the reaction product.

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D. EXPERIMENTAL

1. Purification of solvents and reagents.

Dimethylsulfoxide was distilled in vacuum from calcium hydride Tetrahydrofurane and Pyridine were refluxed for 2 hours with calcium hydride and then distilled. Dimethylformamide was mixed with benzene (about 5% by volume) and distilled until boiling point reached 90-100°C. The remaining solution was cooled to room temperature and shaken with activated alumina which had been freshly dried at the temperature 160-180°C, over P_2O_5 for 24 hours. The decanted solution of dimethylformamide was next distilled under reduced pressure. Acetonitrile was refluxed with calcium hydride for 2 hours and distilled. The distillate was distilled again from P_2O_5. Chloroform was distilled from P_2O_5. 2,4-Bis(trimethylsilyloxy)pyrimidine was prepared by refluxing dry uracil (10g) with hexamethyldisilazane (80 mL) until uracil dissolved. Vacuum distillation of the resulting solution gave the product which was collected in the temperature 123°C (18 Torr). Yield 20.88g (91%). TLC was performed on the plates KODAK 13179 using the same developing solutions as for column chromatography (see below). NMR were measured with EM-360 (60 MHz), Perkin-Elmer, and WP-200SY (200 MHz), Brucker, instruments.

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D.1. 1,2 :5,6-di-isopropylidene-D-glucofuranos-3ulose (53).

A solution of dimethylsulfoxide (620 mL) and acetic anhydride (410 mL) was allowed to stand at room temperature for 1 hour. In this solution, 1,2:5,6-diisopropylidene-D-glucofuranose (52,06 g, 0.20 mole) was dissolved and the mixture was kept overnight at room temperature. The solvents were removed by distillation under reduced pressure (1.0-0.5 Torr) until boiling point reached 70C. Final distillation of the product was done under the pressure of 0.3 Torr. The product was collected at temperatures 110-115 C. The yield was 41.8g (80.9%). Elementary analysis and IR, NMR spectra were compatible with those known for the product.

D.2. 1,2:5,6-Di-O-isopropylidene-3-deoxy-3-C-methylene-D-glucofuranose (57).

Sodium hydride, in 50% oil suspension (10.78 g, .225 mole), and dry dimethylsulfoxide (415 mL) under nitrogen were stirred at 75°C until all of the sodium hydride dissolved (about 45 min.). The resulting solution was treated at 20°C, with external cooling, with methyltriphenylphosphonium bromide (88.46 g, 0.225 mole).

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After 30 min of stirring at this temperature, a solution of 1,2:5,6-di-O-isopropylidene- -D-ribofuranos-3-ulose (19.35 g, 0,075 mole) in dimethylsulfoxide (180 mL) was added while the temperature was maintained in the range 18-20°C. The reaction mixture was stirred for 3 hr. at room temperature and added to 2,5 liters of cold water. The resulting mixture was extracted with ethyl ether (5 x 400 mL). The etheral solution was washed with water (2 x 150 mL), dried with anhydrous sodium sulfate, evaporated to a small volume and cooled. Crystalline triphenylphosphine oxide was filtered, and the remaining ether was evaporated. The residue was chromatographed on a silica gel column (60-200 mesh, 45 x 3,5 cm) using first toluene (500 ml), then toluene : ethyl acetate = 3:1 as the eluants. Evaporation of fractions containing the pure substance gave 13.4 g (70 %) of the product. Calculated: C 60.92%, H 7.86%. Found: 60.78%, H 7.84%.

NMR H 4.00-4.80 (4H,d,C_4H,C_5H,C_6H_2),
4.90 (1H,d,$J_{1,2}$=3Hz,C_2H), 5.80
(1H,d,$J_{1,2}$=3Hz,D_1H), 5.35-5.60
(2H,m,$C_{3}H_2$), 1.30-1.70 (12H,$4CH_3$)

IR (cm^{-1}): 2985, 2930, 1450, 1380, 1360, 1210, 1157.

D.3. 3-Deoxy-3-C-(hydroxymethyl)-1,2:5,6-diisopropylidene-α -D-allofuranose (95).

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To a solution of 1,2:5,6-di-O-isopropylidene-3-deoxy-3-C-methylene-α-D-ribofuranose (11 g, 0.043 mole) in dry tetrahydrofuran (95 mL) at 0-4°C under nitrogen, was added a 10.0 M solution of BH_3 in Me_2S (13 mL) and the resulting solution was stored for 24 hr. at this temperature. With very effective external cooling which maintained the temperature at -5 to +5°C, the reaction mixture was treated successively with a solution of tetrahydrofuran and water 1 : 1 (45 mL), 2 N sodium hydroxide (132 mL) and 30 % aqueous hydrogen peroxide (75 mL). The external cooling was then discontinued and the mixture was allowed to warm to 23-27°C at which point it was stirred for 2 hrs. Evaporation in vacuo afforded a white solid which was dissolved in water (500 mL) and extracted with ethyl ether (5 x 100 mL). The ethyl ether extract was dried with anhydrous magnesium sulfate and evaporated to an oil which was chromatographed on a silica gel column (60-200 mesh, 45 x 3.5 cm) with toluene : ethyl acetate = 3 : 2 to give 9.14 g (78 %) of the product. Calculated: C 56.92%, H 5.83%. Found: C 57.18%, H 5.83%

NMR H 5.75 (1H,d,$J_{1,2}$=3Hz,C_1H), 4.75 (1H,t,$J_{1,2}$=$J_{2,3}$=3Hz,C_2H), 3.60-4.20 (6H,m,C_3H_2,C_4H,C_5H,C_6H_2), 3.25 (1H,m,C_4H), 1.40 (12H,m,4H)

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D.4. 3-Deoxy-3-C-(bromomethyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranos (96).

To a solution of N-bromosuccinimide (3.94 g, 0.022 mole) in dry dimethylformamide (55 mL) was added triphenylphosphine (6.36 g, 0.022 mole) with stirring. A strongly exothermic reaction occurred and the temperature rose to 60-65°C. The solution was cooled to room temperature with cold water and was added dropwise to a solution of 3-deoxy-3-C-(hydroxymethyl)-1,2:5,6-diisopropylidene-α-D-allofuranose (5.48 g, 0.020 mole) and pyridine (1.62 mL, 0.020 mole) in dry dimethylformamide (27 mL) while the temperature of the reaction mixture was maintained at 0°C with external cooling. The brown solution was then allowed to warm to room temperature and was stirred for 1 hour. Finally, the reaction mixture was stirred at 50-60° for 3hrs., the solvent was evaporated under vacuum, and the residue was chromatographed on a silica gel column (60-200 mesh, 45 x 3.5 cm) with toluene : ethyl acetate= 8 : 1. Evaporation of the solvents gave 5.12 g (76 %) of the oily product. Calculated: C 46.30%, H 6.28%. Found: C 46.18%, H 6.31%.

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?MR H 5.72 (1H,d,$J_{1,2}$=3Hz,C_1H), 4.75
(1H,t,$J_{1,2}$=$J_{2,3}$=3Hz,C_2H), 3.30-4.30
(7H,m,C_3H,C_3,H_2,C_4H,C_{5H},
C_6H_2), 1.40 (12H,m,$4CH_3$)

D.5. 3-Deoxy-3-C-(bromomethyl)-1,2-O-isopropylidene-α-D-allofuranose (97).

A solution of 3-deoxy-3-C-(bromomethyl)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (3.37 g, 0.01 mole), methanol (49 mL), chloroform (16mL) and 1.6 % H_2SO_4 (7.8 mL) was kept at room temperature for 3 days. The progress of the reaction was monitored on TLC plates (Kodak 13179) with developing solution of toluene : ethyl acetate = 10 : 1. When the hydrolysis was completed, the solution was treated with 2 g of sodium bicarbonate and evaporated to dryness. The residue was partitioned between water (20 mL) and chloroform (5 x 20 mL). The chloroform solution was dried with anhydrous sodium sulfate and evaporated to oily residue. Yield 2.72 g (95 %). Calculated: C 40.42%, H 5.77%. Found: C 40.15%. H 5.60%

$[\alpha]_D^{22}$=99.5

NMR H 5.80 (1H,d,$J_{1,2}$=3Hz,C_1H), 4.80
(1H,t,$J_{1,2}$=2,3=3Hz), 4.00-2.90

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(6H,m,C_3,H_2,C_4H,C_5H,C_6H_2), 2.50

(1H,m,C_3H), 1.47 (6H,d,2CH_3)

IR (cm^{-1}): 3500, 2990, 2940, 1455, 1385, 1375, 1240, 1167.

D.6. 3-Deoxy-3-C-(bromomethyl)-1,2-O-isopropylidene-α-D-ribofuranose (98).

A flask with a solution of 3-deoxy-3-C-(bromomethyl)-1,2-isopropylidene-α-D-allofuranose (2.86 g, 10.0 mmole), ethanol (130 mL) and water (80 mL) was covered with a light-reflecting foil and treated with a solution of sodium meta-periodate (2.22 g, 10.3 mmole) in water (42 mL) followed by addition of sodium bicarbonate (1.0 g). The reaction mixture was stirred at room temperature for 3 hrs.. Sodium borohydride (1.93 g, 82.4 mmole) was added in small portions while temperature was maintained below 25°C with external cooling. The mixture was stirred overnight, the excess of sodium borohydride was decomposed by addition of 50 % acetic acid until the pH reached 5. The solution was decolorized by titration with 10 % aqueous solution of sodium bisulfite followed by extraction with chloroform (5 x 50 mL). The organic phase was dried with anhydrous sodium sulfate and the residue, after evaporation of the solvent was

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chromatographed on a silica gel column (60-200 mesh, 48 x 2.7 cm) with toluene : ethyl acetate = 55 : 45. Evaporation of the eluate gave 1.93 g (72 %) of the product.

$[\alpha]_D^{22}$=106.8

NMR H 5.81 (1H,d,$J_{1,2}$=3Hz,C_1), 4.80 (1H,t,$J_{1,2}$=$J_{2,3}$=3Hz, C_2H), 4.10-2.80 (7H,m,OH,C_3,H,C_4H,C_5H), 2.50 (1H,m, C_3H) 1.53 (3H,s,CH_3), 1.37 (3H,s,CH_3)

IR (cm^{-1}): 3600, 3500, 3000, 2950, 2890, 1450, 1385, 1375, 1260, 1170.

D.7. 5-(3-Benzoylpropionyl)-3-deoxy-3-C-(bromomethyl)-1,2-O-isopropylidene-D-ribofuranose (99).

A solution of 3-deoxy-3-C-(bromomethyl)-1,2-O-isopropylidene-α-D-ribofuranose (2.86 g, 10.0 mmol), 3-benzoylpropionic acid (7.13 g, 40.0 mmol) and dicyclohexylcarbodiimide (DCC) (10.83 g, 52.0 mmole) in dry pyridine (100 mL) was stored at room temperature for 24 hrs.. The excess of DCC was destroyed by stirring the mixture with water (20 mL) for 3 hrs.. The precipitate was filtered, the solvent removed by repeated evaporation with toluene, the residue was purified on a silica gel column (60-200 mesh, 48 x 2.7 cm) with hexane : ethyl acetate = 3 : 1. The yield 2.29 g (53.5 %).

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Calculated: C 51.48%, H 5.23%. Found: C 51.79%, H 5,19%.

$[\alpha]_D^{22}$=49.0

NMR H 8.20-7.80 (5H,m,C_6H_5), 5.80 (1H,d,$J_{1,2}$=4Hz, C_1H),
4.72 (1H,t,$J_{1,2}=J_{2,3}$=4Hz, C_2H), 4.40-3.80
(3H,m,C_4H_2) 3.10-3.40
(4H,m,$J_{7',8'}$=6Hz,$C_{8'},C_3,H_2$), 2.80
(2H,t,$J_{7',8'}$=6Hz,$C_{7'}H_2$), 2.40
(1H,m,C_3H), 1.5 (3H,s,CH_3), 1.3 (3H,s,CH_3)

IR (cm^{-1}): 2980, 2930, 2850, 1735, 1685, 1595, 1450,
1385, 1375, 1230, 1165.

D.8.

5-(3-Benzoylpropionyl)-3-deoxy-3-C-(bromomethyl)-1,2-diacetyl-(α+β)-D-ribofuranose (101).

A solution of 5-(3-benzoylpropionyl)-3-deoxy-3-C-(bromomethyl)-1,2-O-isopropylidene-α-D-ribofuranose (2.14 g, 0.01 mol) in 80% acetic acid (100 mL) was stirred at 80°C for 24 hrs. The solvent was evaporated in vacuo, the residue was repeatedly evaporated with toluene and dried under vacuum over P_2O_5 for 24 hrs. This glassy material was dissolved in a mixture of dry pyridine (12 mL) and acetic anhydride (6 mL). The solution was stirred overnight at room temperature, the solvents were removed by repeated

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evaporation with toluene and the residue was purified on a silica gel column (60-200 mesh, 45 x 3.5cm) with hexane : ethyl acetate = 7 : 3. The yield 3.56 g (76%). Calculated: C 50.97%, H 4.92%. Found: C 50.71%, H 4.85%.

$[\alpha]_D^{22}$=8.47

NMR H 8.00 (5H,m,PhH_5), 6.00 (1H,s,C_1), 5.23 (1H,d,$J_{2,3}$=4Hz, C_2H), 4.31 (3H,s,C_4H,C_5H_2), 3.70-3.10 (4H,m, $J_{7'',8''}$=6Hz,$C_{8''}H_2$,$C_{3'}H_2$), 2.80 (3H,t,$J_{7'',8''}$=6Hz, $C_{7''}H_2$,C_3H), 2.10 (3H,s,CH_3), 2.05 (3H,s,CH_3)

IR (cm^{-1}): 2980, 2955, 2855, 1745, 1690, 1595, 1450, 1375, 1235, 1165.

D.10.. 5-(3-Benzoylpropionyl)-3-deoxy-1,2-diacetyl-3-C-(diisopropoxyphosphinylmethyl)-(α+β)-D-ribofuranose (104).

A solution of 5-(3-benzoylpropionyl)-3-deoxy-1,2-diacetyl-3-C-(bromomethyl)-(α+β)-D-ribofuranose (2.35 g, 0.005 mole) in triisopropylphosphite (15 mL) was heated to 160-180°C with exclusion of moisture for 3 days. The solvent was evaporated in vacuo and the residue was purified on a silica gel column (60-200 mesh, 48 x 2.7 cm) with chloroform : ethyl acetate = 1 : 1. The yield 1.73 g

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(68%). Calculated: C 56.11%, H 6.70. Found: C 56.45%, H 6.60%.

$[\alpha]_D^{22}$=19.6

NMR H 8.00-7.30 (5H,m,PhH_5), 6.01 (1H,s,C_1H), 5.16 (1H,d,$J_{2,3}$=4Hz,C_2H), 5.00-4.00 (5H,m,esterC'H,C''H, C_5H_2,C_4), 3.30 (2H,t,$J_{7'',8''}$=6Hz,$C_{8''}H_2$), 3.00-2.20 (m,$C_{7''}H_2$"U,C_3H), also C_3,H_2 is in the region 2.2-3.4, 2.1 (6H,2xs,acetylCH_3), 1.37 and 1.23 (6H,2xs,isopropylCH_3)

IR (cm^{-1}): 2985, 2830, 1745, 1690, 1595, 1450, 1390, 1375, 1235, 1170.

D.11.

2'-Acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-deoxy-3-C-(diisopropoxyphosphinylmethyl)uridine (105).

To a solution of 5-(3-benzoylpropionyl)-3-deoxy-1,2-diacetyl-3-C-(diisopropoxyphosphinylmethyl)-(α+β)-D-ribofuranose (2.50 g, 0.0045 mole) in dry acetonitrile (90 mL) at 0°C with external cooling was added bis(trmethylsilyl)uracil (1.25 g, 4.9 mmol) and $SnCl_4$ (0.62 mL, 5.3 mmol). The solution was stirred at room temperature for 24 hrs., diluted with methylene chloride (125 mL) and extracted with a saturated solution of sodium

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bicarbonate (100 mL). The organic layer was dried with anhydrous sodium sulfate. Evaporation in vacuo gave 2.2 g (80%) of the product which was sufficiently pure for further transformations. The product can be crystallized from ethyl ether. Calculated: C 55.26%, H 6.13%, N 4.60%. Found: C 54.91%, H 5.98%, N4.43%.

$[\alpha]_D^{22}$=55.16

NMR H 9.58 (1H,s,N_3H), 7.00-8.00 (6H,m,PhH_5,C_6H),
5.64 (2H,m,C_5H,C_1,H), 5.98 (1H,q,C_3,H),
4.80-3.80 (5H,m,C_5,H,C_4,H,esterC'H,C''H) 3.23
(2H,m,C_8,,,H_2), 2.90-2.50
(3H,m,C_7,,,H_2, C_3,H), 2.01
(3H,s,acetylCH_3), 2.00-1.58 (2H,m,C_3,H_2),
1.18 (12H,2xs,isopropyl4xCH_3)

D.12.. 2'-Acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-C-(dihydroxyphosphinylmethyl)uridine (106).

To a solution of 2'-acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-C-(diisopropoxyphosphinylmethyl)uridine (2.10 g, 3.45 mmol) in dry chloroform (25 mL) was added trimethylsilylbromide (5.2 mL). The progress of the reaction was monitored with NMR (change in the chemical shift of isopropyl peaks) and

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after 7 hrs. the mixture was evaporated and dried under vacuum. The residue was stirred with a solution of water (35 mL) and pyridine (14 mL) for 1 hrs., followed by extraction of the resulting solution with ether (10 mL). The water layer was evaporated to dryness and crystallized from an ethanol/ether solution. Yield 1.85 (80%). Calculated: C 52.73%, H 5.01%, N 6.96%. Found: C 52.95%, H 4.97%, N 6.75%.

$[\ ]_D^{22} = 44.5$

NMR H 7-9, protons from pyridine, PhH_5, C_6H,

5.50 (2H,m,C_5H,C_1,H), 5.22 (1H,m,C_2,H),

3.17 (2H,d,C_8 ,,,H_2), 2.46

(3H,s,C_7,,,H_2,C_3'H), 2.25-1.30

(5H,m,acetylCH_3,C_3,,$_2$)

D.13. -Acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-dihydroxyphos=
=phinylmethyluridylyl-3(3'-5')-2',3'-isopropylideneuridine (108).

A mixture of 2'-acetyl-5'-(3-benzoylpropionyl)-3-deoxy-3-C-(dihydroxyphosphinylmethyl)uridine, pyridinium salt (0.4 g 0.664 mmole), 2',3'-isopropylideneuridine (0.53 g, 1.87 mmol), Dowex 50, pyridinium form (1.52 g) was rendered anhydrous by repeated evaporation wiith anhydrous prydine

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and dried under vacuum over P_2O_5 . The residue was dissolved in dry pyridine (8 mL), the flask was covered with a light-reflecting foil, dicyclohexylcarbodiimide (DCC) (1.8 g, 8.74 mmol) was added and the mixture was kept at room temperature for a period of 6 days. The excess of dicyclohexylcarbodiimide was destroyed by diluting the reaction mixture with water (8 mL), resulting precipitate was filtered, and the filtrate was extracted with ethyl ether (10 mL). The water layer was evaporated to dryness and the residue was purified on a silica gel column (60-200 mesh,) with chloroform : methanol = 20 : 1. Evaporation of the eluate gave 0.45 g (71%) of the product. Calculated: C 51.64%, H 4.98%. Found: C 51.95%, H 5.01%.

$[\alpha]_D^{22}$ =36.9

NMR H 10.1 (s, N-H), 7-8 (C_6H, Ph), 5.2-6 (C_5H C_1,H), 4.98 (s, C_2,H), 4.7 (s, C_2,H), 3.2 and 2.75 (2xCH_2 from benzoylpropionyl), 2.0 (acetyl), 1-1.6 (isopropyl).

D.14. 2'-Acetyl-3'-deoxy-3'-dihydroxyphosphinylmethyluridylyl-(3'-5')-2',3'-isopropylideneuridine (109).

A mixture of dinucleotide D.13. (0.6 g, 0.82 mmole) in pyridine/acetic acid buffer (4 : 1), (2.5 mL) and 98%

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hydrazine hydrate (0.041 mL, 0.84 mmole) was kept at room temperature for 24 hours. The solution was evaporated several times with fresh 95% ethanol and the residue was chromatographed on a silica gel column with $CHCl_3$/MeOH = 7 : 1. Evaporation of the eluate gave 0.25 g (50%) of the product. Calculated: C 45.74%, H 4.92%. Found: C 45.48%, H 4.90%.

$[\alpha]_D^{22}$=54.1

NMR H 8-7.5 (m, C_6H), 5-6 (m, $C_1'H$, $C_2'H$), 1.2-1.7 (isopropyl).

D.15. 2'-Acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-dihydroxyphos=
=phinylmethyluridylyl-(3'-5')-2'-acetyl-3'-deoxy-3'-dihydro=
=xyphosphinylmethyl-(3'-5')-2',3'-isopropylideneuridine (110).

A mixture of dinucleotide (D.14.) (0.18 g, 0.286 mmole), 2'-acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-C-(dihydroxyphosphinylmethyl) uridine (0.215 g, 0.36 mmole), Dowex 50-pyridine form (0.57 g) was rendered anhydrous by repeated evaporation with anhydrous pyridine under vacuum over P_2O_5 for 24 hours. To this mixture, pyridine (2.5 mL) and dicyclocarbodiimide (0.6 g) were added, the flask was wrapped in a light-reflecting foil and kept 6 days at room temperature. The reaction mixture was treated with water (2.5 mL) and kept for 2 hours at room

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temperature. A precipitate was filtered, the filtrate was extracted with chloroform (5 mL). The aqueous layer was evaporated to dryness and chromatographed on a silica gel column with $CHCl_3$/MeOH = 10 : 1 as a eluant.
Calculated: C 48.60%, H 4.79%, N 7.39%. Found: C 48.14%, H 4.69%.
$[\alpha]_D^{22}$ =44.5
NMR H 7-9, N-H, C-6H, Ph, 5-6, C-1'H, C-2'H, 2.1, (acetyls), 1.2-1.6 (isopropyl).

D.16.

3'-Deoxy-3'-dihydroxyphosphinylmethyluridylyl-(3'-5')-3'-deoxy-3'-dihydroxyphosphinylmethyluridylyl-(3'-5')-uridine (93).

A solution of protected trinucleotide (D.15.) (0.3 g) and methanol saturated with ammonia (10 mL) was kept at room temperature for 24 hours. The solvent was evaporated and the residue was dissolved in water (10 mL) and extracted with portions (5 mL) of ethyl acetate until the organic layer became colorless. The water layer was evaporated. The residue was dissolved in 80% acetic acid (10 mL) and stirred at the temperature 80-90^{o}C for 24 hours. The solvents were evaporated and the residue crystallized from absolute ethanol. TLC (two-dimensional):

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methanol : chloroform=4 : 20, followed by isopropanol : ammonia : water=6 : 3 : 1. Calculated: C 44.94%, H 4.49%. Found: C 33.10%, H 5.78% (for hydrate with 11 molecules of water).

NMR H 8.2-7.5 (C_6H), 6.0-5.5 (C_1,H, C_2,H),
2.5-1.5 (C_3,H, C_3,,H_2).

D.17. 1,2-O-isopropylidene-3-deoxy-3-C-methylene--D-glucofuranose (111).

A solution of 1,2:5,6-di-O-isopropylidene-3-deoxy-3-C-methylene--D-ribofuranose (5.12 g, 20 mmol), acetic acid (43 mL), water (27 mL) and ethanol (15 mL) was stirred for 24 hrs at room temperature. The acid was neutralized with sodium hydroxide (conc. 30%) with external cooling at 10-20°C. The solution was extracted several times with ethyl acetate (20 mL) until the TLC indicated no further product in a successive portion of the extract. The organic extract was dried over anhydrous sodium sulfate, evaporated, and the residue was purified on a silica gel column (60-200 mesh, 45 x 3.5 cm) with chloroform : ethyl acetate = 3 : 7. Yield 3.0 g (69%). Calculated: C 55.55%, H 7.46%. Found: C 55.65%, H 7.44.

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$[\alpha]_D^{22}$=136.9

NMR H 5.85 (1H,d,$J_{1,2}$=4Hz,C_1), 5.48 (2H,s,C_3,H_2),
4.95 (1H,d,$J_{2,3}$=4Hz,C_2H), 4.97 (1H,s,C_4H),
3.75 (5H,C_5H,C_6H_2,2OH), 1.40 and 1.50
(6H,2xs,2xCH_3)

IR (cm^{-1}): 3450, 2990, 2930, 2880, 1382, 1372, 1205, 1158.

D.18. 3-Deoxy-3-C-methylene-1,2-O-isopropylidene-α-D-ribofuranose (112).

A solution of 3-deoxy-3-C-methylene-1,2-O-isopropylidene-α-D-allofuranose (6.20 g, 28.7 mmol) in ethanol (387 mL) and water (248 mL) was covered with a light reflecting foil and treated with a solution of sodium m-periodate (6.90 g, 32.2 mmol) in water (139 mL) and sodium bicarbonate (2.70 g) at room temperature and stirred for 3 hr. The mixture was cooled to 10-15° what was followed by addition of sodium borohydride (4.96 g). The reaction mixture was stirred overnight, excess of sodium borohydride was then removed by acidification of the mixture to pH 5 with 50% acetic acid. The resulting brown solution was titrated with 10% sodium bisulfite to remove iodine (decolorization).

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The product was extracted several times with chloroform until TLC indicated no further product in a successive portion of the extract. The chloroform solution was dried with anhydrous sodium sulfate, the solvent was evaporated and the residue was purified on a silica gel column (60-200 mesh, 45 x 3.5 cm) with chloroform : ethyl acetate = 3 : 2. Evaporation of the eluate gave a colorless oil, 5.08 g (95%). Calculated: C 58.05%, H 7.58%. Found: C 58.22%, H 7.56%.

$[\alpha]_D^{22}$=18.8

NMR H 5.80 (1H,m,$J_{1,2}$=4Hz,C_1H), 5.36 (1H,s,C_3,H_b),
5.11 (1H,s,C_3,H_a), 4.95 (2H,m,C_4H,C_nH),
4.95-4.31 (2H,m,C_4H,C_2H), 4.00-3.11
(3H,m,C_5H_2, OH), 1.45 and 1.30
(6H,2xs,2xCH_3)

IR (cm^{-1}): 3580, 3460, 2975, 2920, 2870, 1445, 1367, 1220, 1152.

D.19. 5-(3-Benzoylpropionyl)-3-deoxy-1,2-O-isopropylidene-3-C-methylene-D-ribofuranose (113).

A solution of 3-deoxy-1,2-isopropylidene-3-C-methylene--D- ribofuranose (2.88 g, 15.5 mmol), 3-benzoylpropionic acid (11.1 g, 66.9 mmol), dicyclohexylcarbodiimide (DCC)

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(17.1 g, 83.0 mmol) in anhydrous pyridine (175 mL) was kept at room temperature for 24 hrs. The excess of DCC was destroyed with water (15 mL) and resulting precipitate was filtered. The filtrate was evaporated under vacuum to dryness repeatedly with fresh toluene until a smell of pyridine disappear and the residue was chromatographed on a silica gel column (60-200 mesh, 45 x 3.5 cm) with hexane : ethyl acetate = 3 : 2. Evaporation of the eluate gave a yellow viscous liquid, 3.81 g (65%). Calculated: C 65.88%, H 6.40%. Found: C 66.07%, H 6.36.

$[\alpha]_D^{22}$=-64.74

NMR H 8.10-7.10 (5H,m,PhH_5), 5.90 (1H,d,$J_{1,2}$=4Hz,C_1H),
5.48 (1H,m,C_3,H_b), 5.20 (1H,m,C_3,H_a),
4.90 (2H,m,C_4H,C_2H), 4.45-3.80
(2H,m,C_5H_2), 3.38
(2H,t,$J_{7'',8''}$=6Hz,$C_{8''}H_2$) 2.83
(2H,t,$J_{7'',8''}$=6Hz,$C_{7''}H_2$), 1,53 and 1.40
(6H,2xs,2xCH_3)

IR (cm^{-1}): 2980, 2920, 2840, 1750, 1650, 1590, 1442, 1155

D.20. 5-(3-Benzoylpropionyl)-3-deoxy-1,2-diacetyl-3-C-methylene-D-ribofuranose (114).

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A solution of 5-(3-benzoylpropionyl)-3-deoxy-1.2-isopropylidene--C-methylene-α-D-ribofuranose (2g) in 80% (v/v) acetic acid (100 mL) was stirred at 80°C for 24 hrs. The solvent was removed by repeated evaporation with toluene and the residue was dried under vacuum over P_2O_5. The oily product was dissolved in dry pyridine (30 mL) and acetic anhydride (15 mL). The solution was kept overnight at room temperature, evaporated repeatedly with toluene and chromatographed on a silica gel column (60-200 mesh, 48 x 2.7 cm) with a solution of hexane : ethyl acetate = 3 : 2. Evaporation of the solvents gave yellow, viscous oil, 1.68 g, (74%). Calculated: C 61.53%, H 5.68%. Found: C 61.25%, H 5.63%.

D.21.

2-Acetyl-5-(3-benzoylpropionyl)-3-deoxy-3-C-methyleneuridine (115).

A solution of 5-(3-benzoylpropionyl)-3-deoxy-1,2-diacetyl-3- C-methylene--D-ribofuranose (6.55 g, 16.8 mmol) in dry acetonitrile was treated at 0°C with

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2,4-bis(trimethylsilyloxy)pyrymidine (4.63 g, 18.0 mmol), $SnCl_4$ (2.34 mL) and was stirred at room temperature for 24 hrs. The reaction mixture was partitioned between a saturated solution of sodium bicarbonate (390 mL) and methylene chloride (390 mL), the organic layer was dried with anhydrous sodium sulfate. Evaporation of the solvents followed by chromatography on a silica gel column (60-200 mesh, 45 x 3.5 cm) with chloroform : ethyl acetate = 3 : 2 gave a viscous oil, 5.13 g (69%). Calculated: C 59.72%, H 5.01%. Found: C 59.97%, H 5.08%.

$[\alpha]_D^{22}$ =104.7

NMR H 8.10-7.10 (6H,m,PhH_5,C_6H), 6.10-5.50 (2H,m,C_5H,C_1,H)
5.42 (1H,s,C_3,,H_b), 5.30 (1H,s,C_3,,H_a),
4.90 (1H,s,C_2,H), 4,39
(2H,d,$J_{4',5'}$=4Hz,C_5H_2), 3.36
(2H,t,$J_{7''',8'''}$=6Hz,$C_{8'''}H_2$), 2.79
(2H,t,$J_{7''',8'''}$=6Hz,$C_{7'''}H_2$), 2.18
(3H,s,acetyl)

IR (cm^{-1}): 1735, 1687, 1448, 1370, 1275, 1230, 1155.

D.22. 3-Deoxy-3-C-methyleneuridine (116).

A solution of 2-acetyl-5-(3-benzoylpropionyl)-3-deoxy--3-C-methyleneuridine (2.21 g, 10.0 mmol) in methanol

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saturated with ammonia was kept at ambient temperature for 18 hrs. The volatile components were evaporated under vacuum, the residue was chromatographed on a silica gel column (60-200 mesh, 48 x 2.7 cm) with ethyl acetate = ethanol = 5 : 1, what was followed by liquid chromatography using the LC-500 apparatus and a solution of hexane : acetone = 1 : 1. Calculated: C 50.00%, H 5.03%. Found: C 49.88%, H 4.99%

$[\alpha]_D^{22}$=45.33

NMR H 7.92 (1H,d,$J_{5,6}$=8.2Hz,C_6H), 5.81 (1H,d,$J_{1',2'}$=6.7Hz,C_1,H) 5.73 (1H,d,$J_{5,6}$=8.2Hz,C_5H), 5.35 (1H,t,$C_{3'}$,H_b), 5.23 (1H,t,$C_{3'}$,H_a), 4.64 (2H,m,C_4,H,C_2,H), 3.75 (2H,m,C_5H_2)

D.23. 3'-Hydroxymethyluridines (117), (118).

To a solution of 3'-deoxy-3'-C-methyleneuridine (0.48 g, 2.0 mmole) and potassium chlorate (0.32 g, 2.6 mmole) in tetrahydrofurane (2 mL) and water (4 mL) was added osmium tetroxide (0.1 g, 0.39 mmole). After 4 hours of stirring at room temperature, TLC showed complete conversion of 3'-deoxy-3'-C-methyleneuridine. The solution was treated with $Na_2S.9H_2O$ (0.38 g, 1.57 mmole), the precipitate was filtered, the filtrate was evaporated to

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dryness and the residue was chromatographed on a silica gel column with ethyl acetate : ethanol : water = 10 : 10 : 2. Evaporation of eluates gave 0.4 g (75%) of the product. Calculated: C 43.80%, H 5.14%. Found: C 43.66%, H 5.10%. NMR H 8.8-8.1 (1H, 2xd, $J_{5,6}$=8Hz), 6.10-5.80 (3H, m, C_5H, $C_1'H$, $C_2'H$), 3.7-4.4 (8H, m).

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E. SUMMARY

The intent of this thesis project was the synthesis of an analogue of an oligonucleotide which would incorporate a reduced-lability phosphorus linkage (phosphonate or phosphoramide) while remaining isosteric with a natural oligonucleotide. Such an analoge should be capable of testing for stimulation of ribosomal binding of t-RNA, i.e. it should incorporate three nucleotide units.

The synthesis of such an analogue has been accomplished with UCH_2pUCH_2pU as described herein. Such phosphonate linkages have been noted[1] to be virtually isosteric with natural phosphate esters. To date this material has not been tested for biological actitvity.

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SPECTRA

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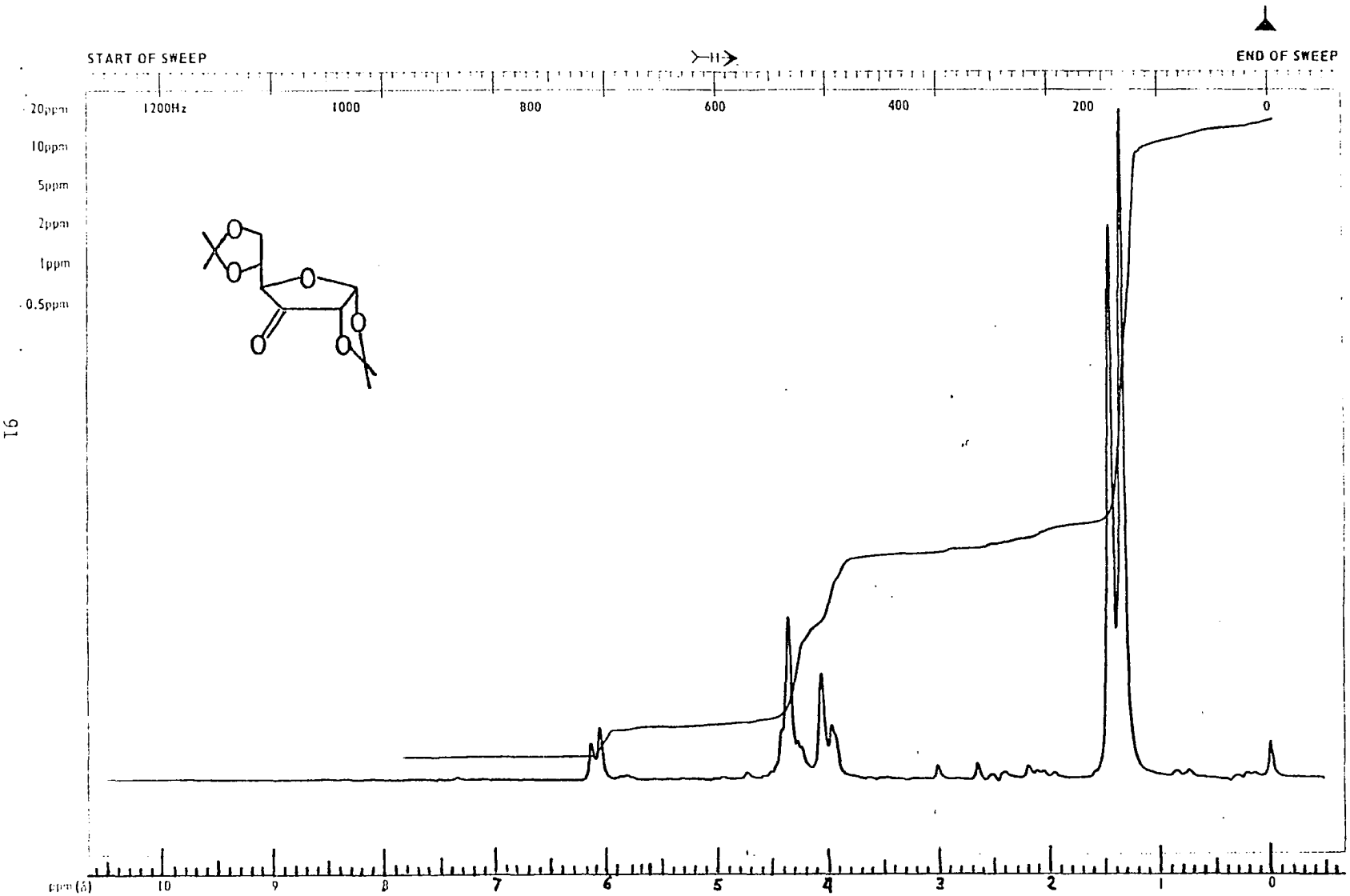

D.l. 1,2 :5,6-Di-isopropylidene-D-glucofuranos-3-ulose (53).

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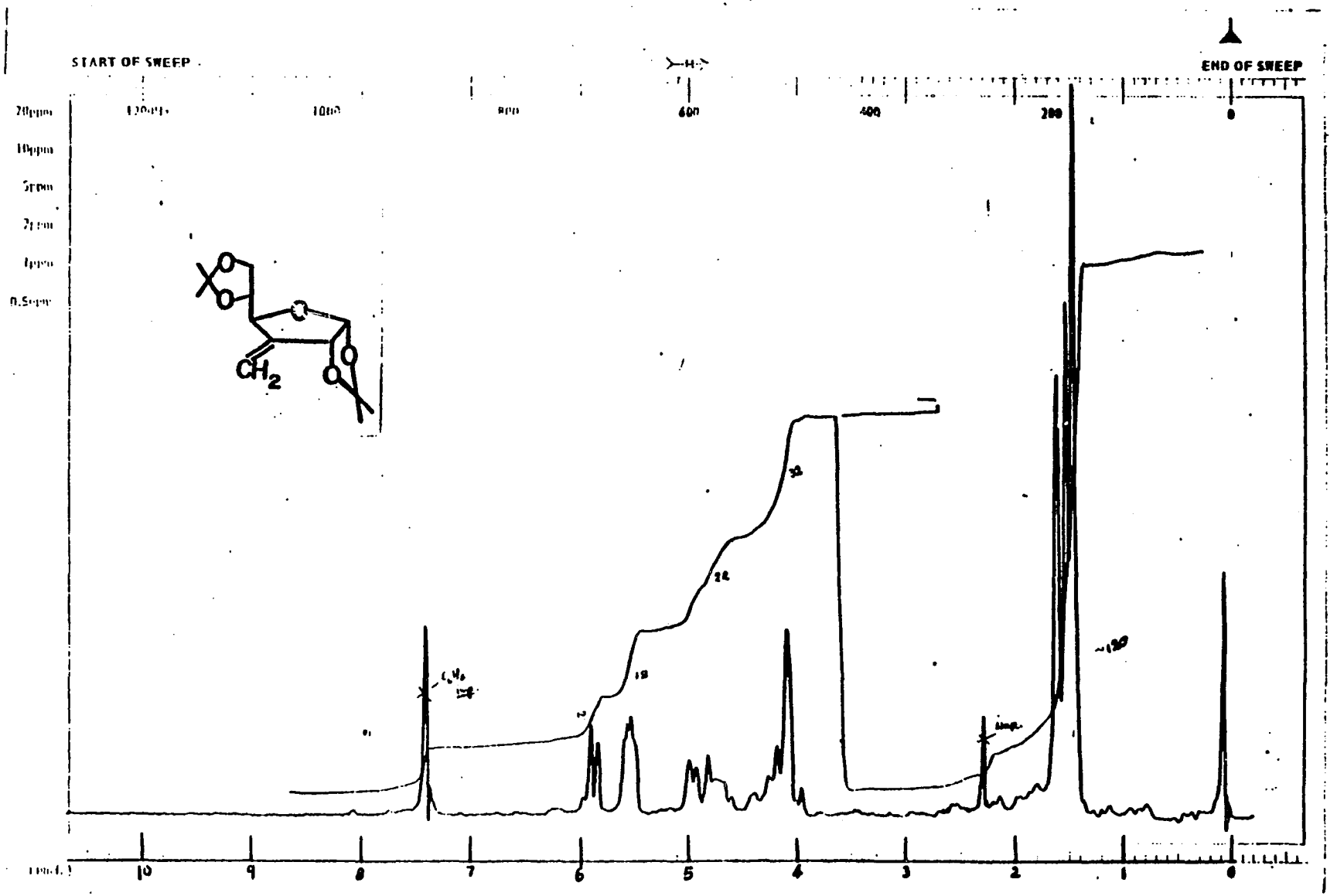
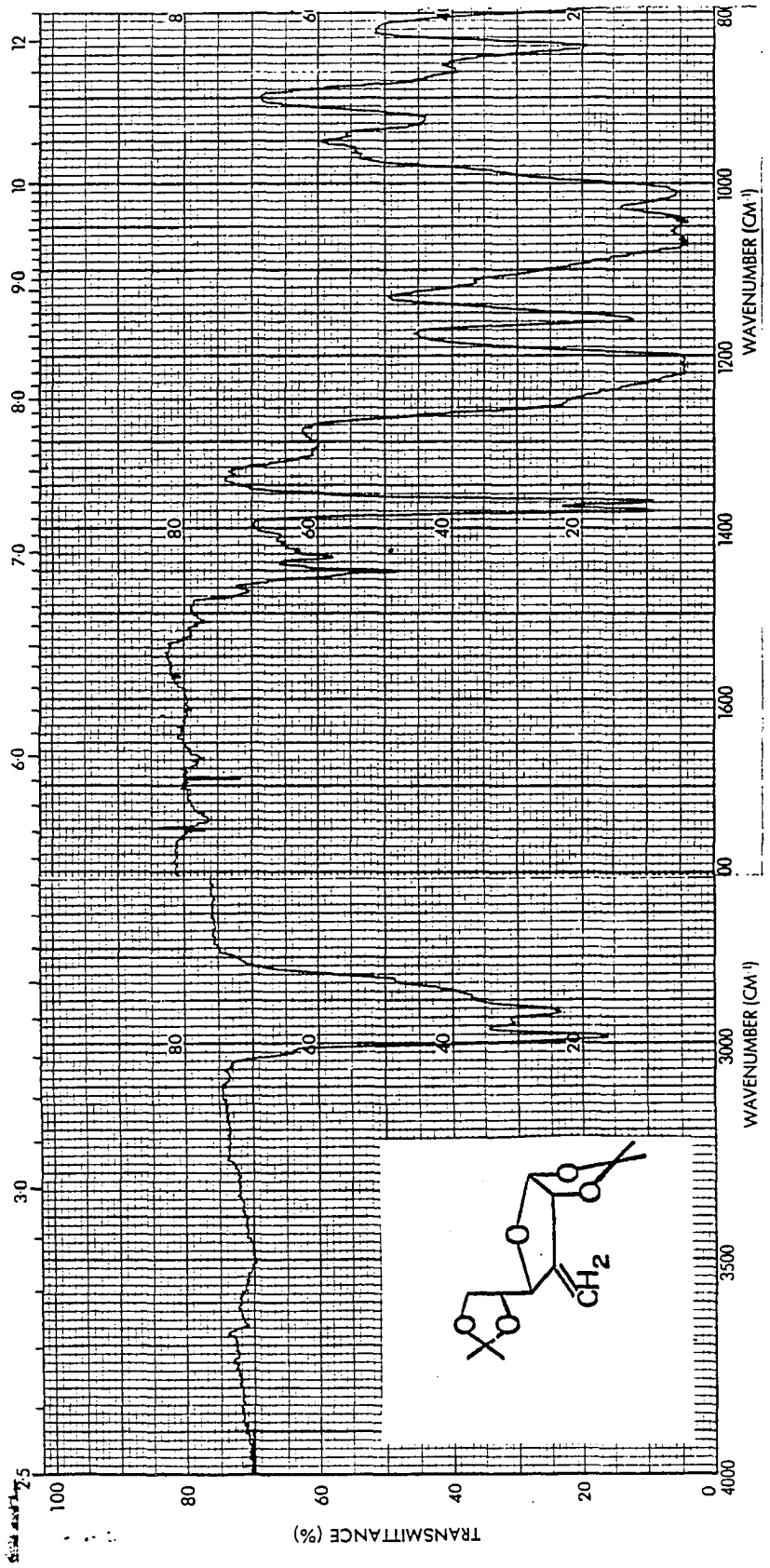


D.2. 1,2:5,6-Di-O-isopropylidene-3-deoxy-3-C-methylene-D-glucofuranose (57).



D.2. 1,2:5,6-Di-O-isopropylidene-3-deoxy-3-C-methylene-D-glucofuranose (57).

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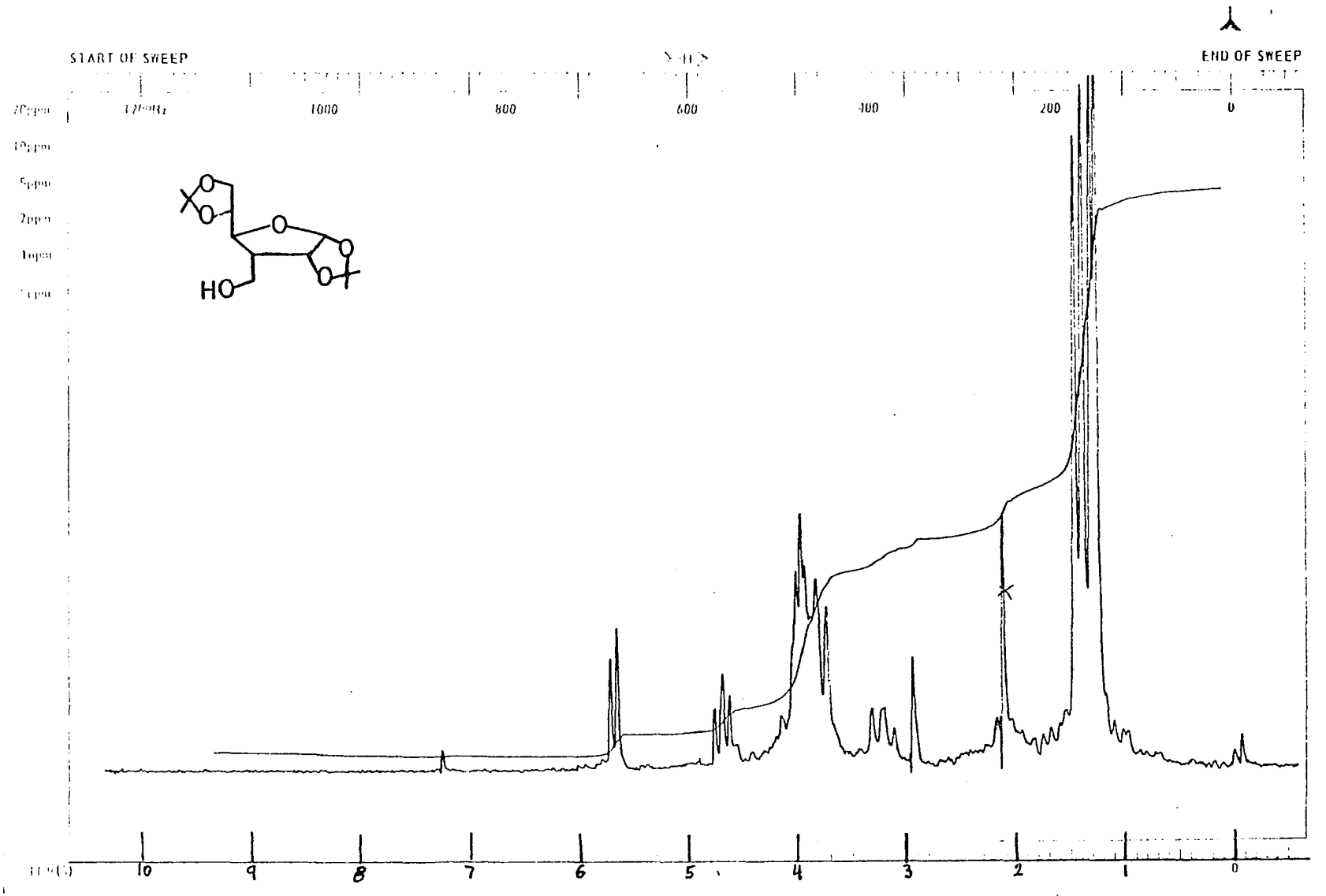

D.3. 3-Deoxy-3-C-(hydroxymethyl)-1,2:5,6-diisopropylidene-
-D-allofuranose (95).

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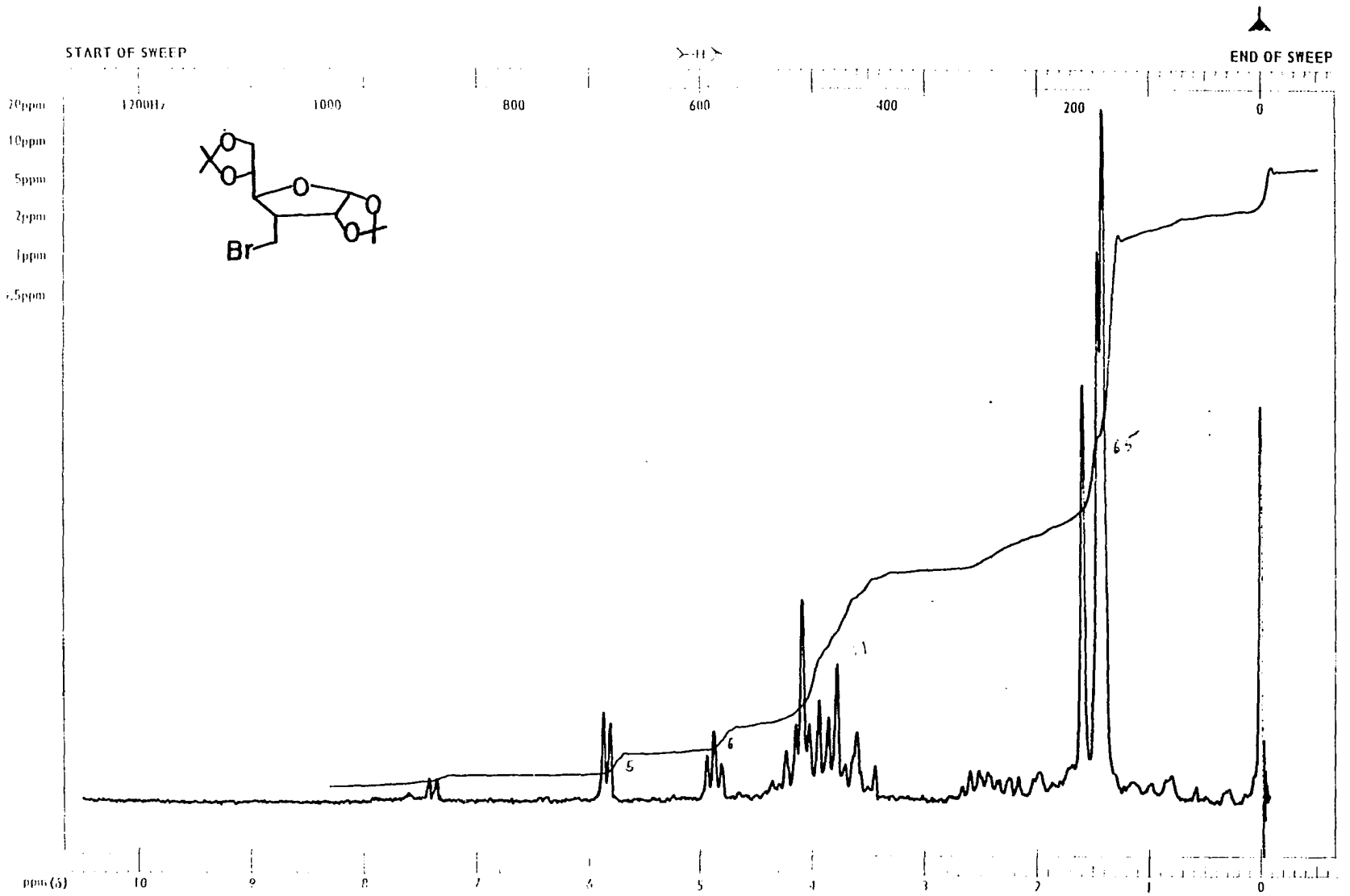

D.4.
3-Deoxy-3-C-(bromomethyl)-1,2:5,6-di-O-isopropylidene-
-D-allofuranose (93).

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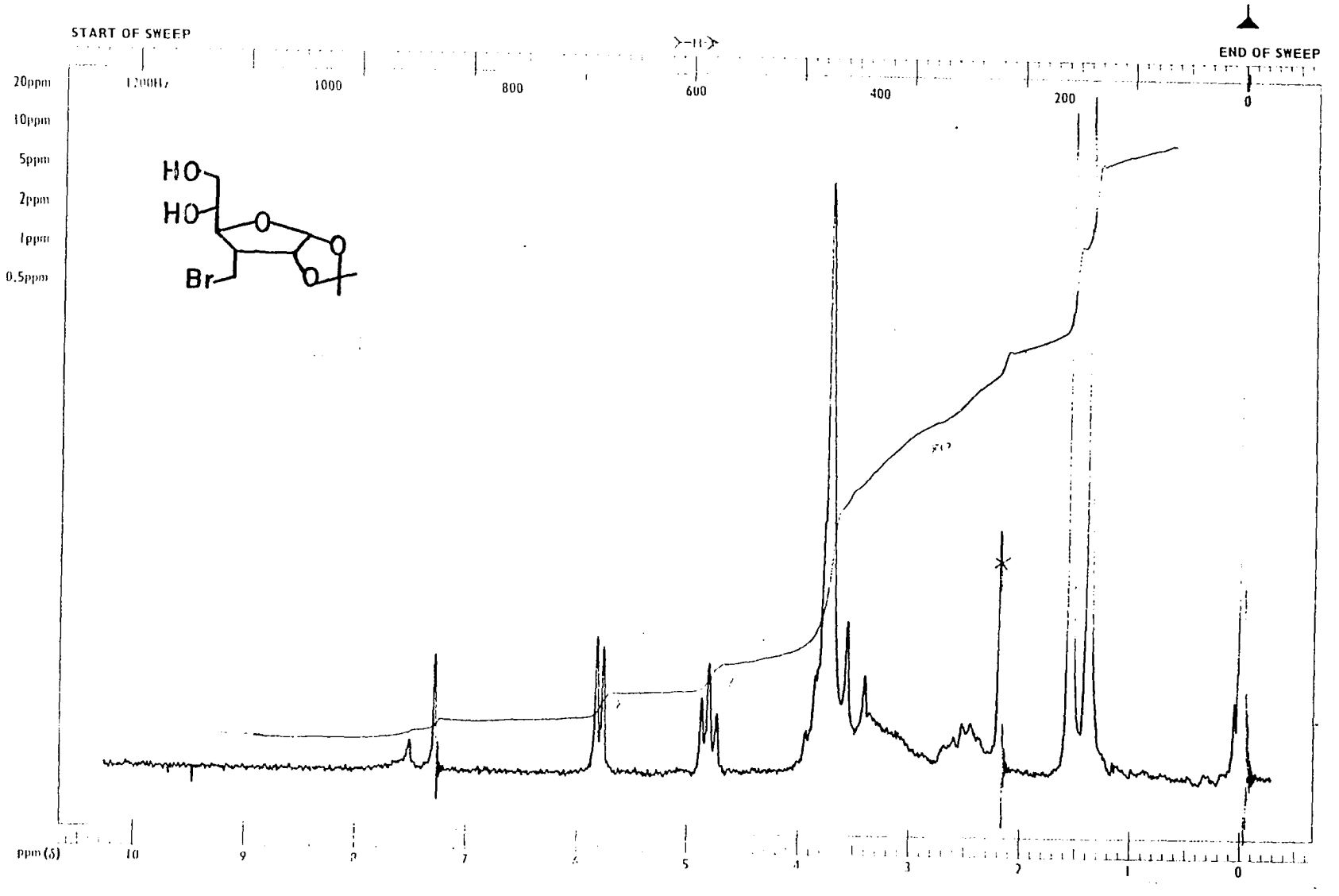
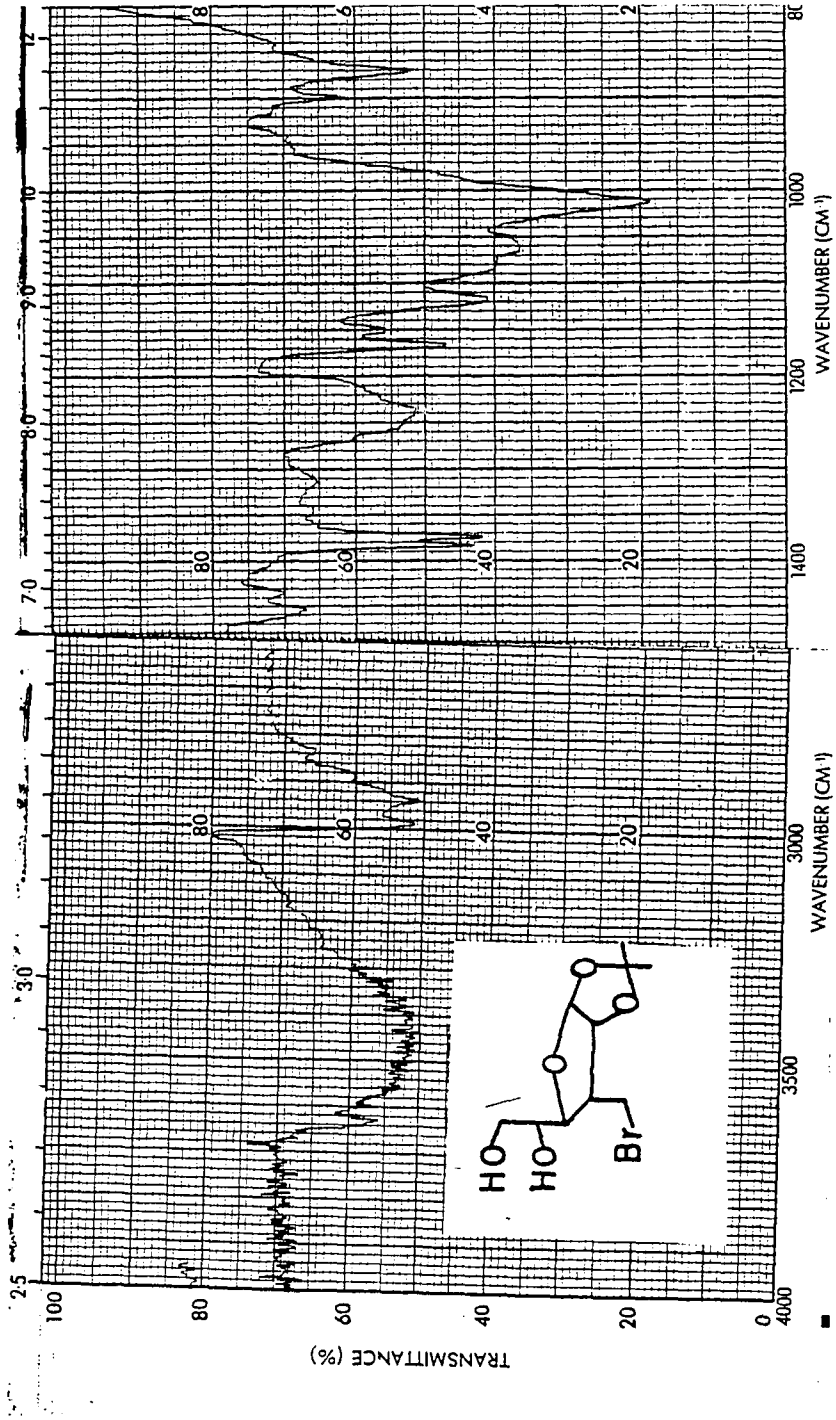


D.5. 3-Deoxy-3-C-(bromomethyl)-1,2-O-isopropylidene-α-D-allofuranose (27).



D.5. 3-Deoxy-3-C-(bromomethyl)-1,2-O-isopropylidene-
-D-allofuranose (97).

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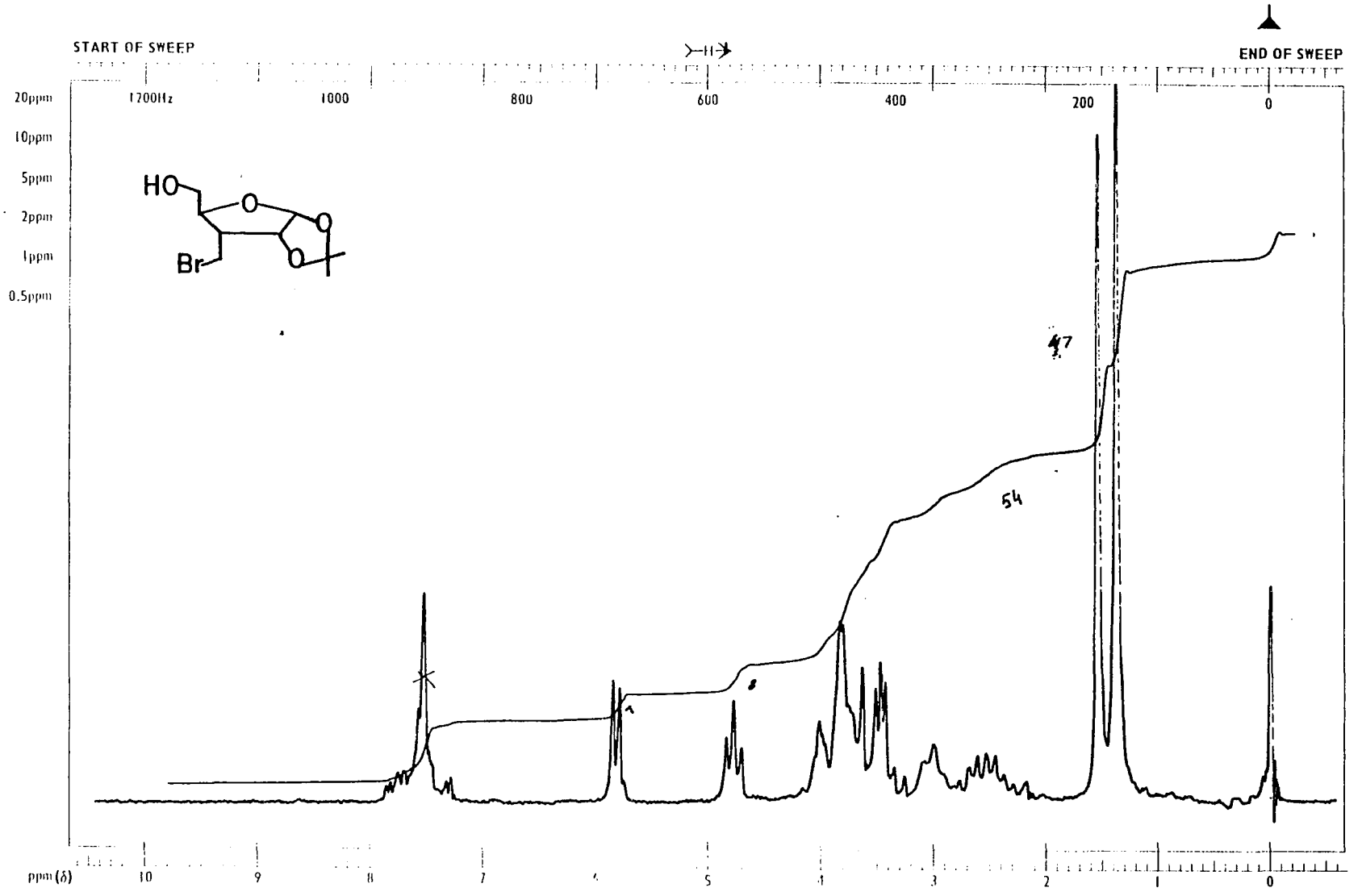

2.6. 3-Deoxy-3-C-(bromomethyl)-1,2-O-isopropylidene-
-D-ribofuranose (93).

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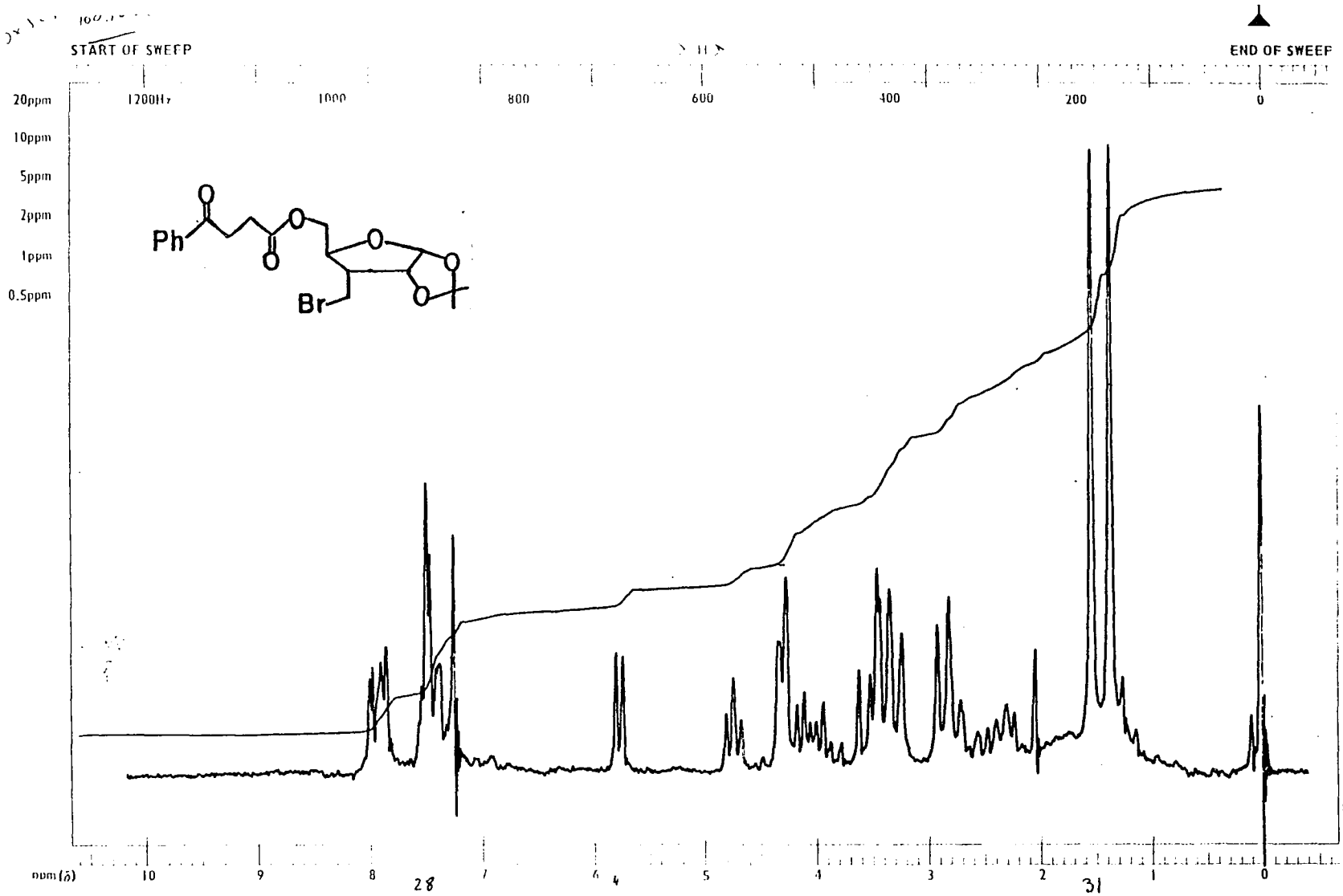

D.7. 5-(3-Benzoylpropionyl)-3-deoxy-3-C-(bromomethyl)-1,2-O-isopropylidene-D-ribofuranose (39).

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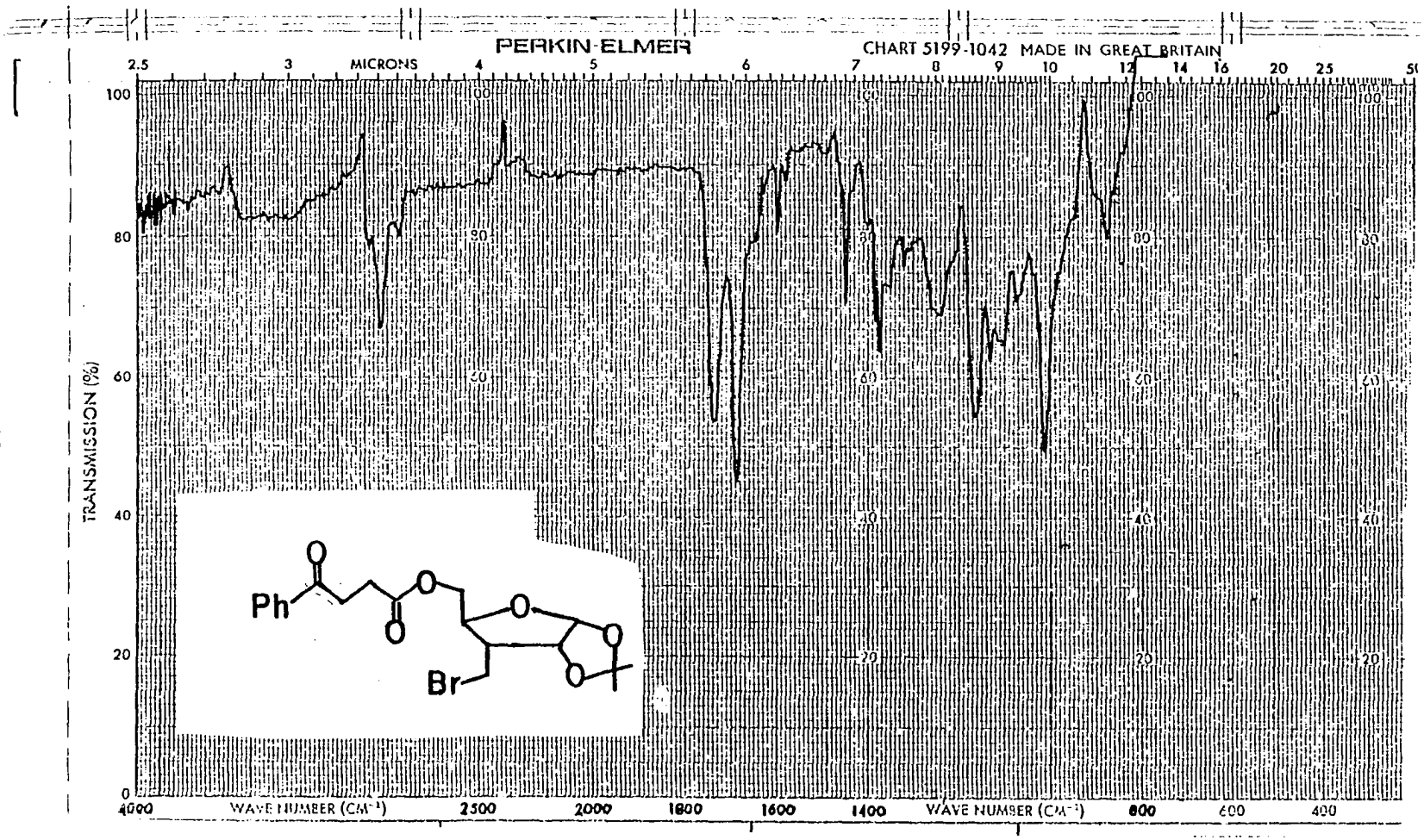

D.7. 5-(3-Benzoylpropionyl)-3-deoxy-3-C-(bromomethyl)-1,2-O-isopropylidene-D-ribofuranose (99).

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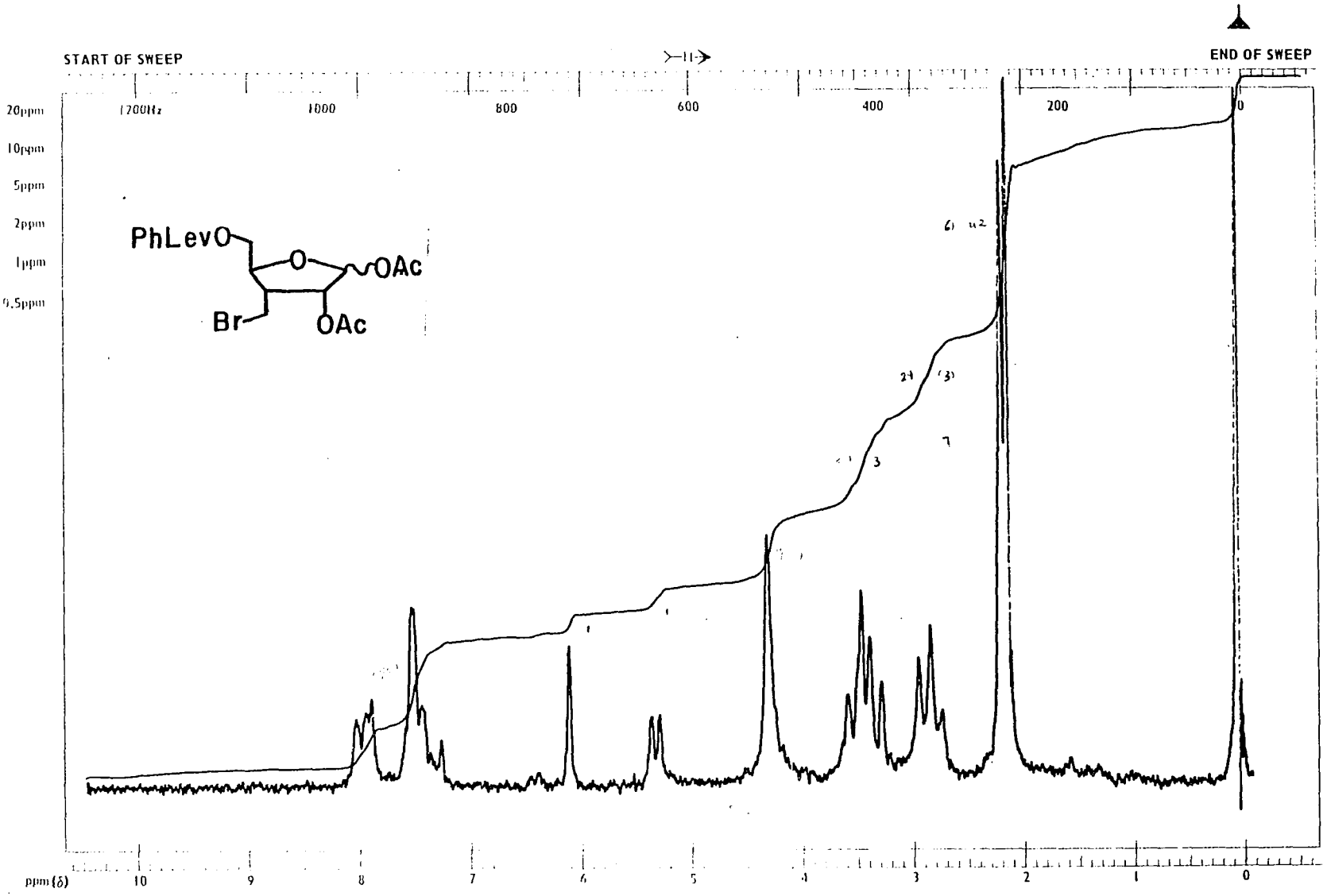

D.3.
5-(3-Benzoylpropionyl)-3-deoxy-3-C-(bromomethyl)-1,2-diacetyl-(+)-D-ribofuranose (101).

D.3.

5-(3-Benzoyloxymethyl)-3-benzyl-3-C-(bromomethyl)-1,2-diacetyl-(
+)-D-glucofuranose (101).

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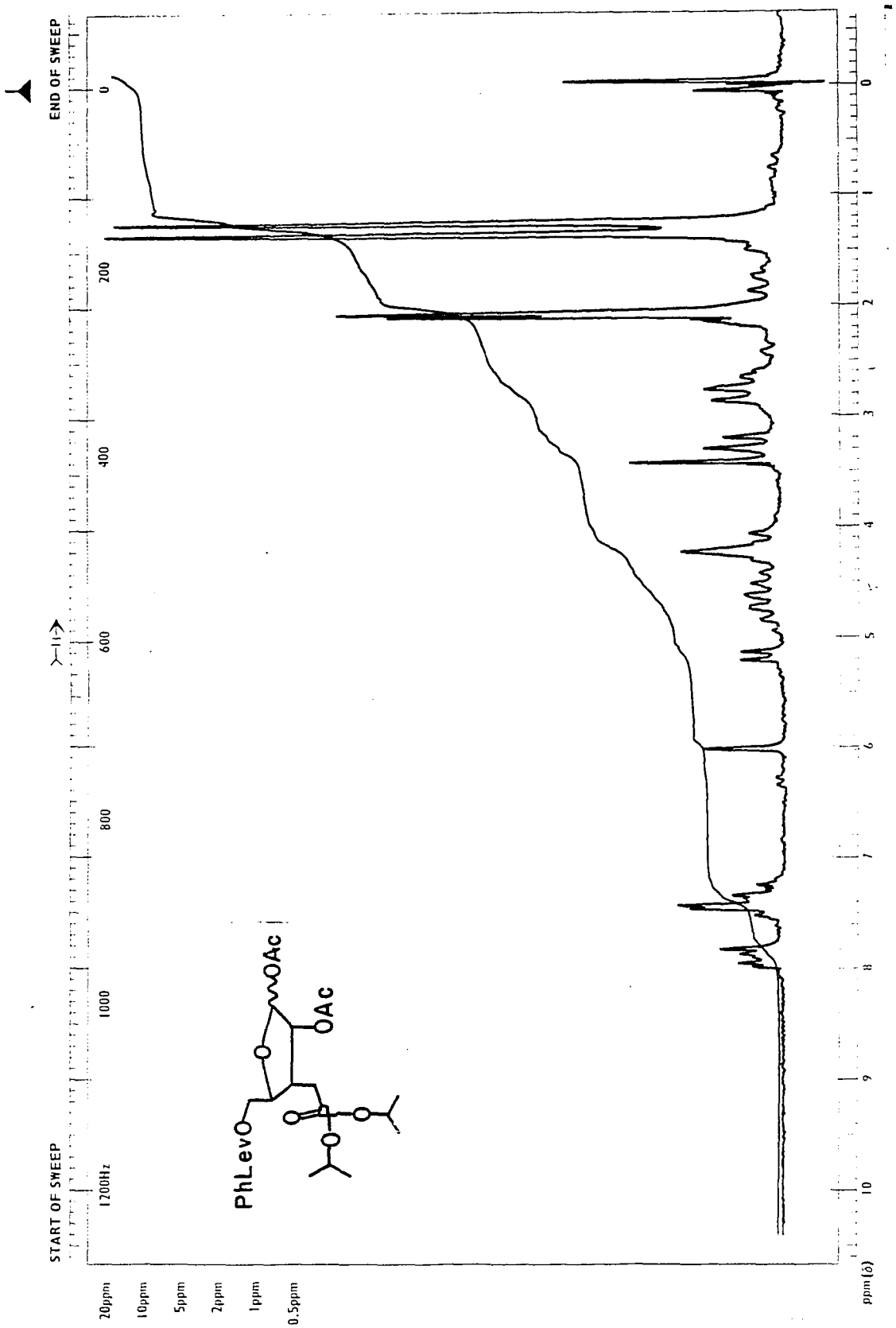

Fig. 10. 5-(3-Benzoylpropionyl)-3-deoxy-1,2-diacetyl-3-C-(Allopyranosylthiomethyl)-(+)-D-ribofuranose (104).

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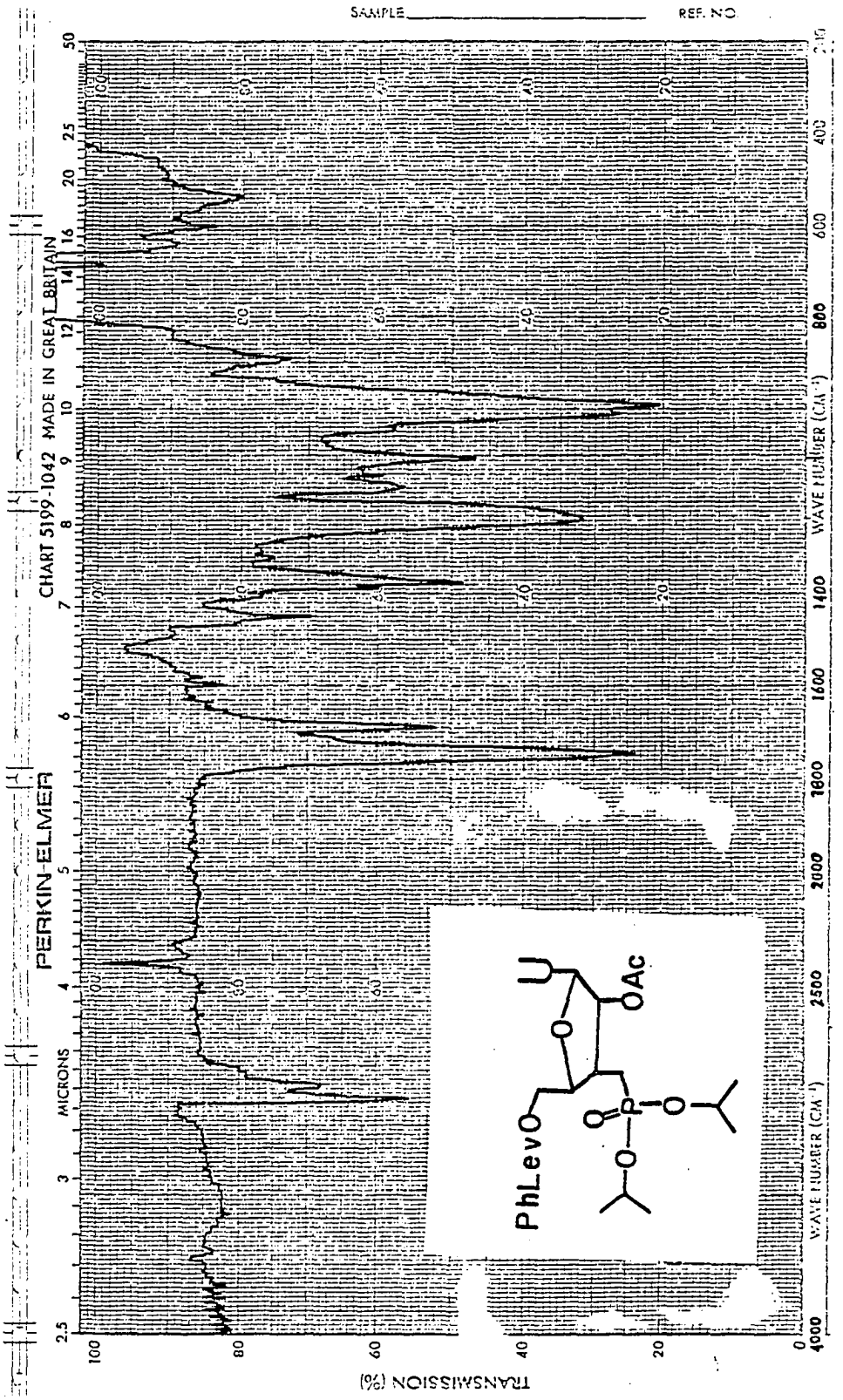

D.11. 2'-Acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-(diisopropoxyphosphinylmethyl)uridine (105).

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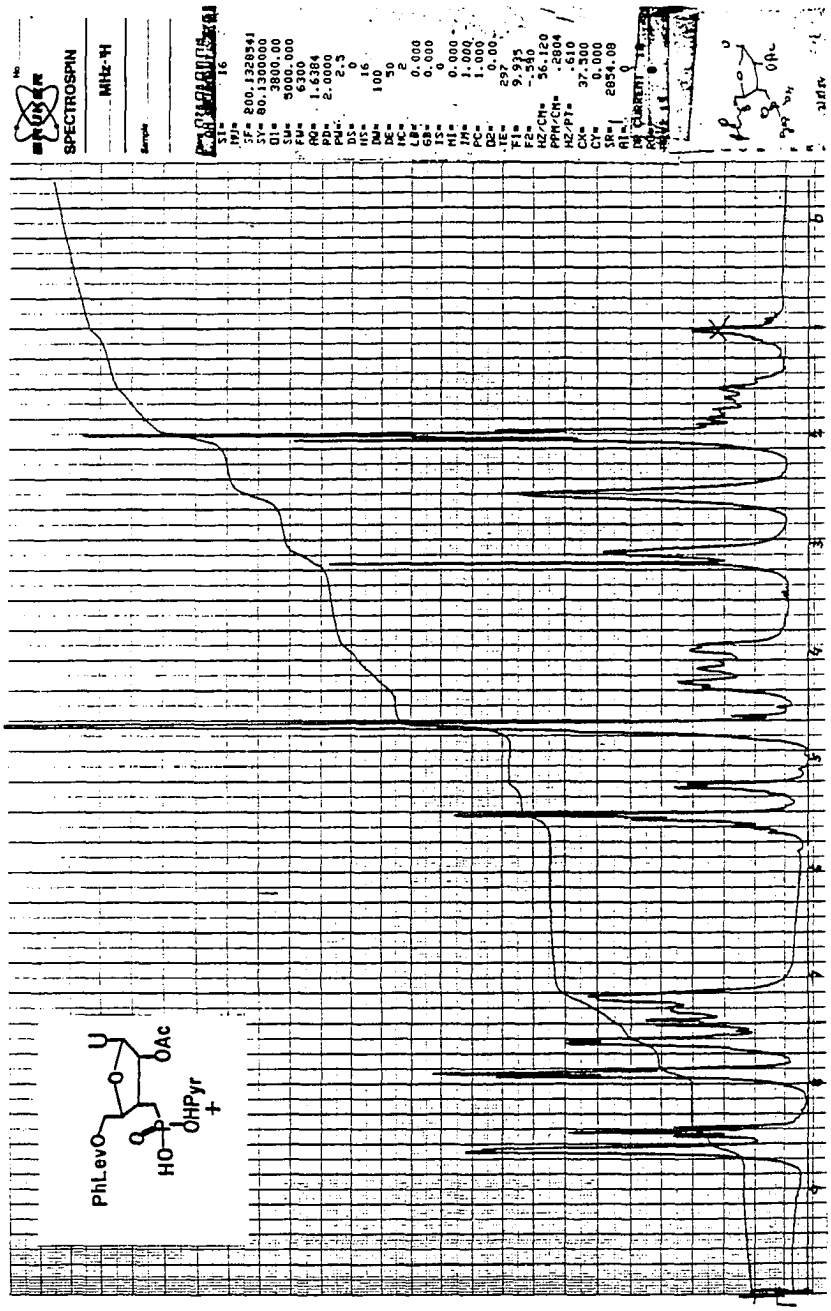

D.12. 2'-Acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-C-(dihydroxyphosphinylmethyl)uridine (105).

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D.13. 2'-Acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-dihydroxyphosphinylmethyluridylyl-(3'-5')-2',3'-isopropylidenecytidine (193).

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D.14. 2'-Acetyl-3'-deoxy-3'-dihydroxyphosphinylmethyluridylyl-(3'-5')-2',3'-isopropylideneuridine (109).

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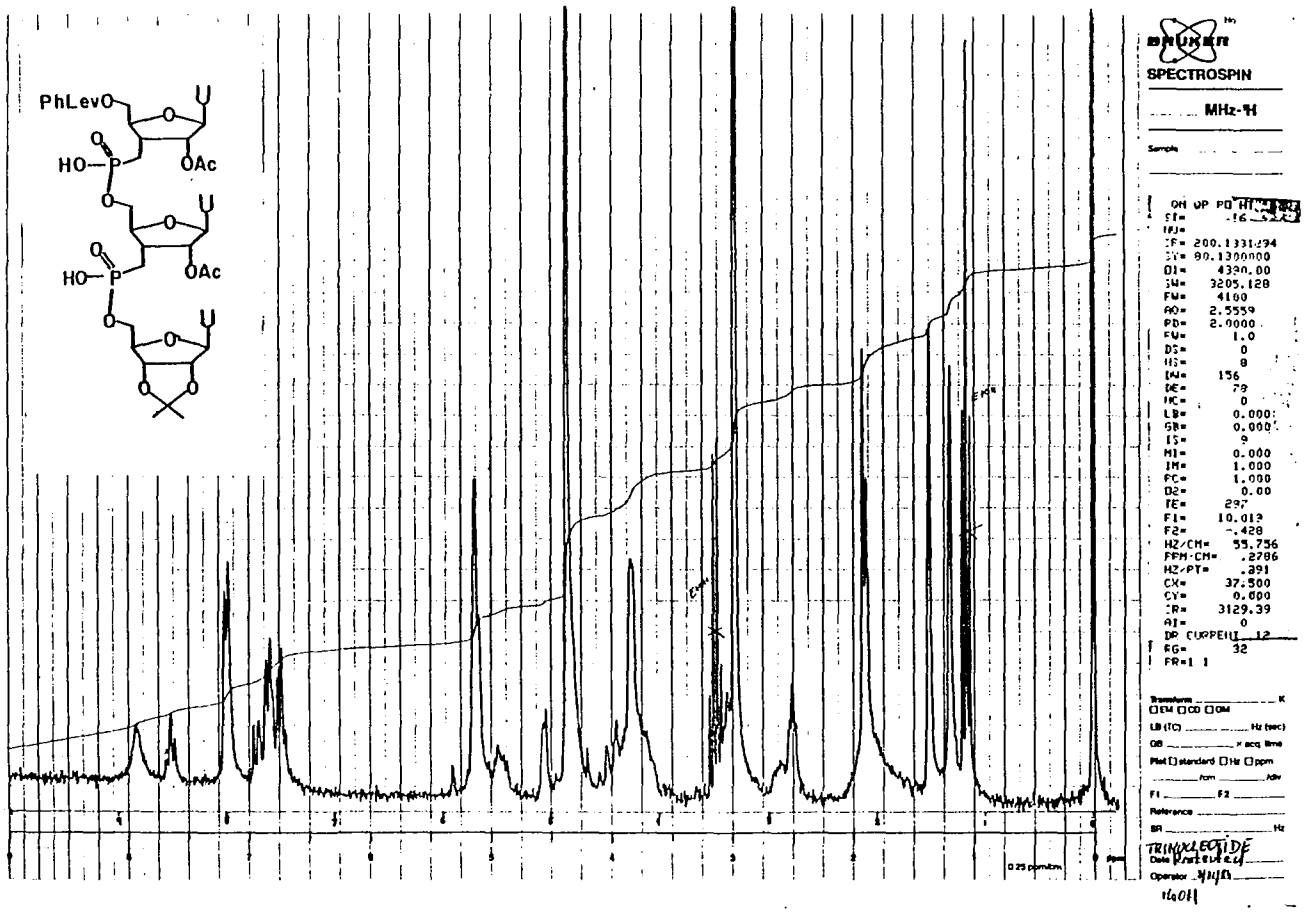

D.15. 2'-Acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-dihydroxyphos=
=phinylmethyluridylyl-(3'-5')-2'-acetyl-3'-deoxy-3'-dihydro=
=xyphosphinylmethyl-(3'-5')-2',3'-isopropylideneuridine (110).

D.15.
3'-deoxy-3'-dihydroxyphosphinylmethyluridylyl-(3'-5')-
3'-deoxy-3'-dihydroxyphosphinylmethyluridylyl-(3'-5')-uridine
(93).

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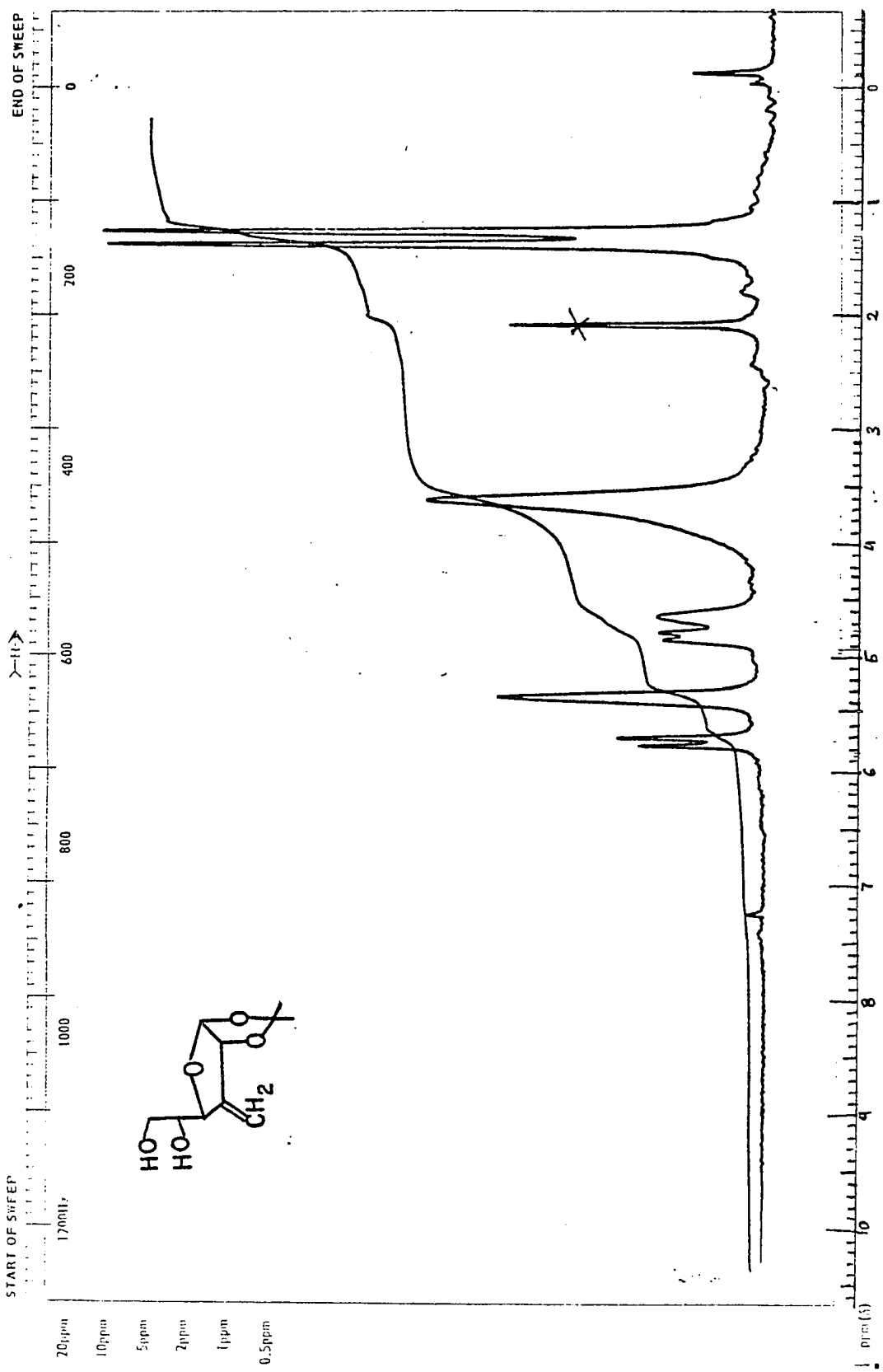

D. 17 . 1,2-O-isopropylidene-3-deoxy-3-C-methylene--D-glucofuranose (111).

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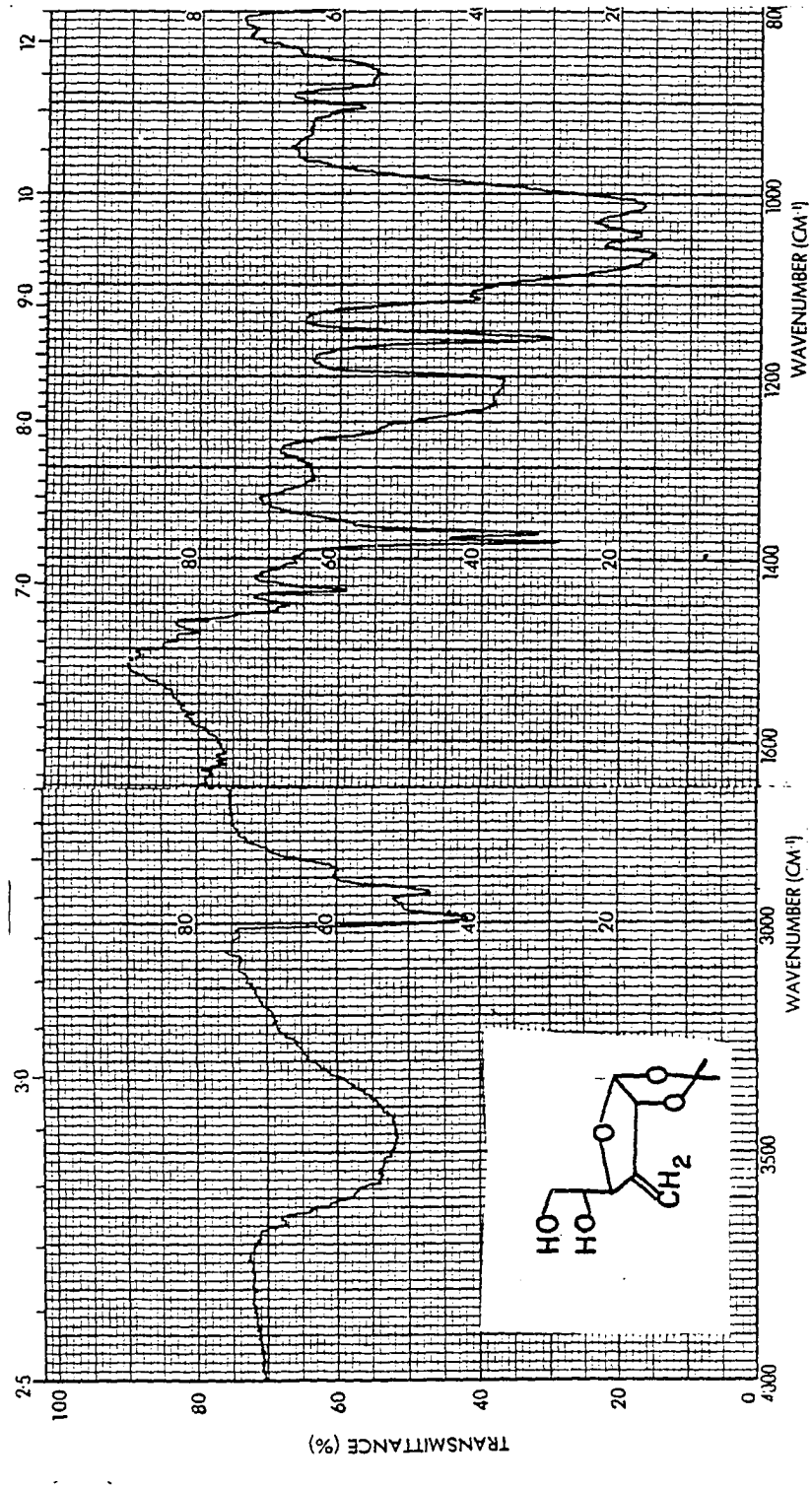

D.17. 1,2-O-isopropylidene-3-deoxy-3-C-methylene-
-D-glucofuranose (111).

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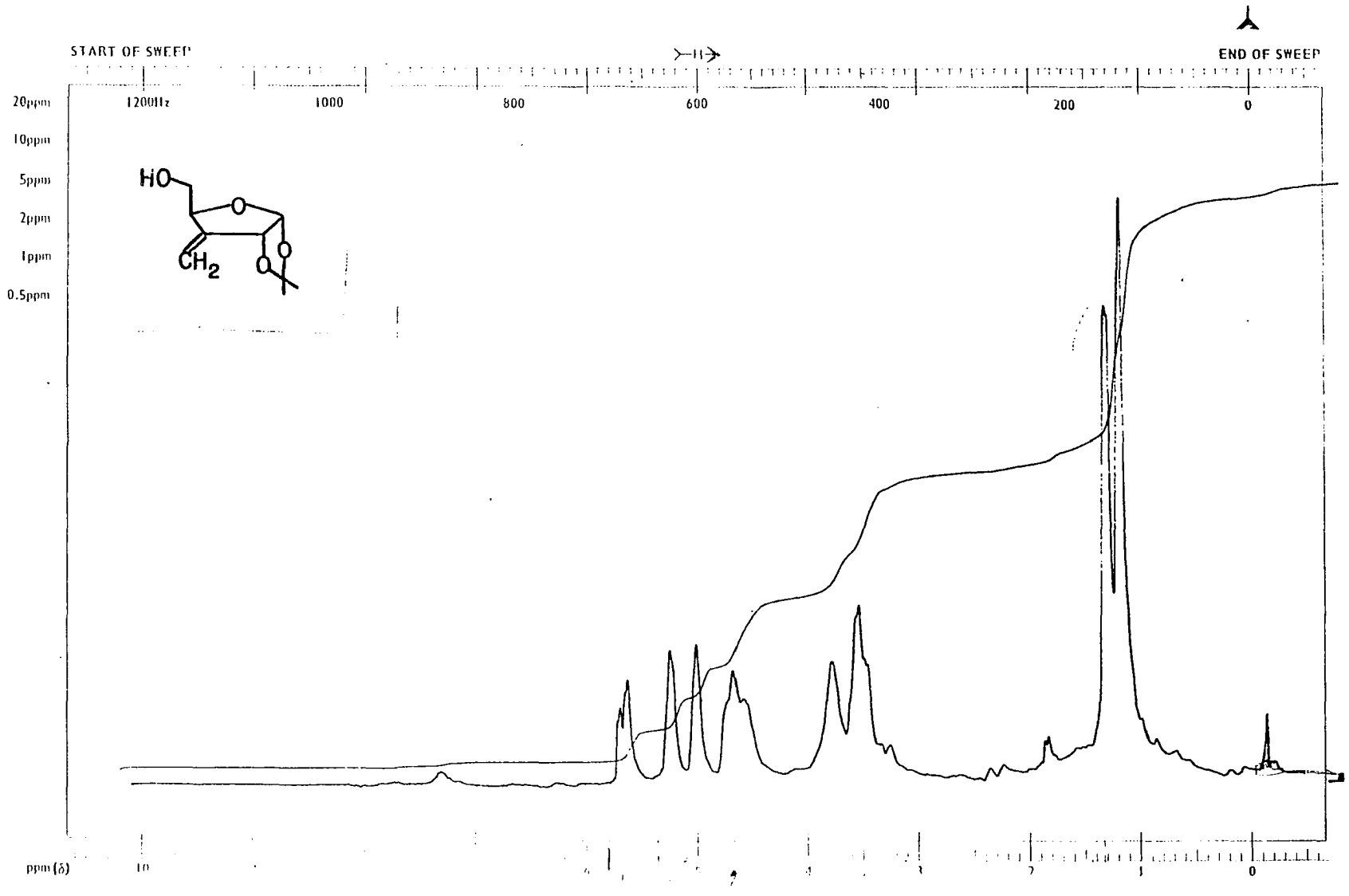
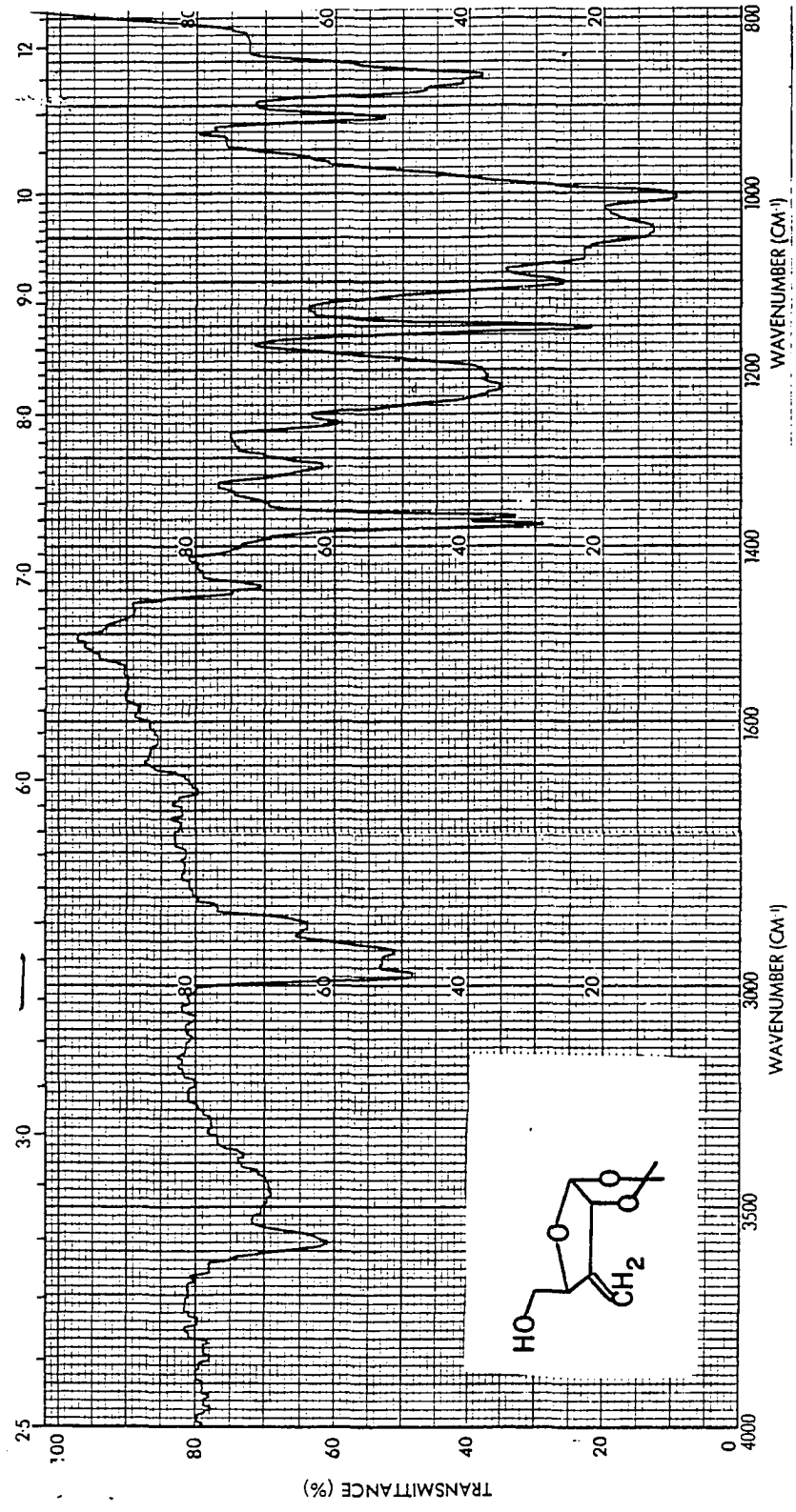


D.13. 3-Deoxy-3-C-methylene-1,2-O-isopropylidene-
-D-ribofuranose (112).



D.18. 3-Deoxy-3-C-methylene-1,2-O-isopropylidene-
-D-ribofuranose (112).

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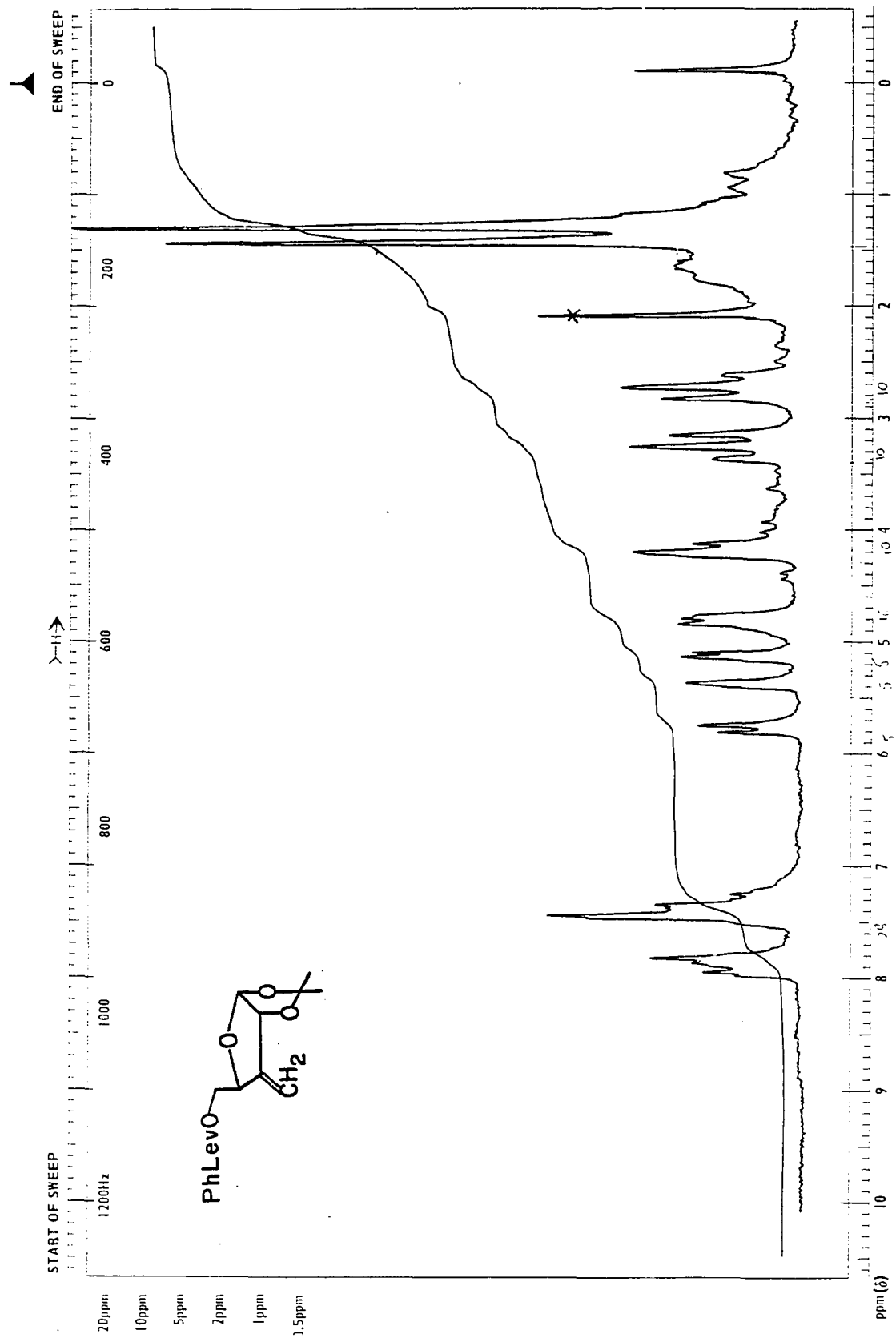

D.19. 5-(3-benzoylpropionyl)-3-oxy-1,2-O-isopropylidene-3-C-methyl-α-D-ribofuranose (113).

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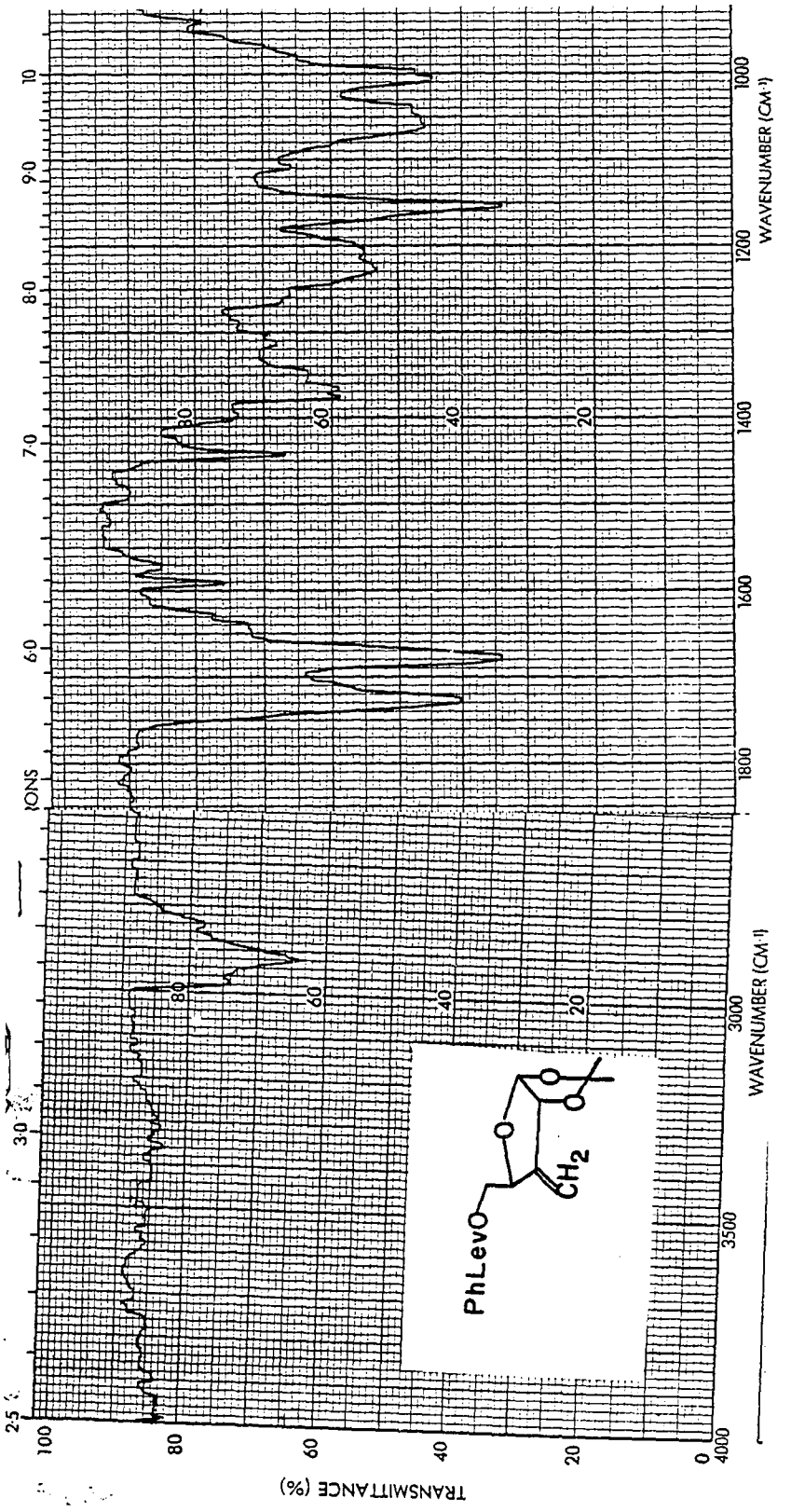

D.13. 5-(3-Benzoylpropionyl)-3-deoxy-1,2-O-isopropylidene-3-C-methylene-D-ribofuranose (113).

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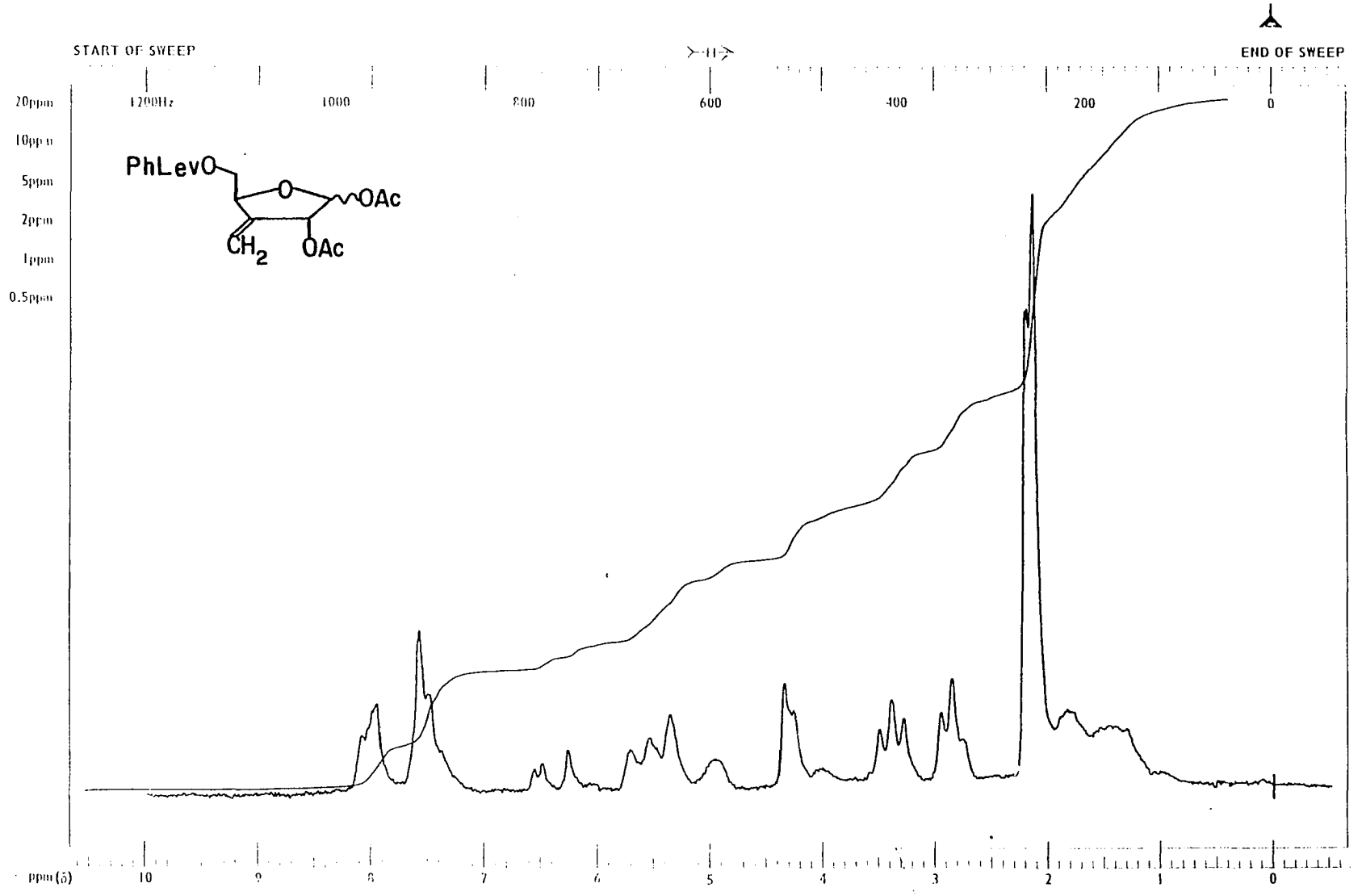

D.20. 5-(3-Benzoylpropionyl)-3-deoxy-1,2-diacetyl-3-C-methylene-D-ribofuranose (114).

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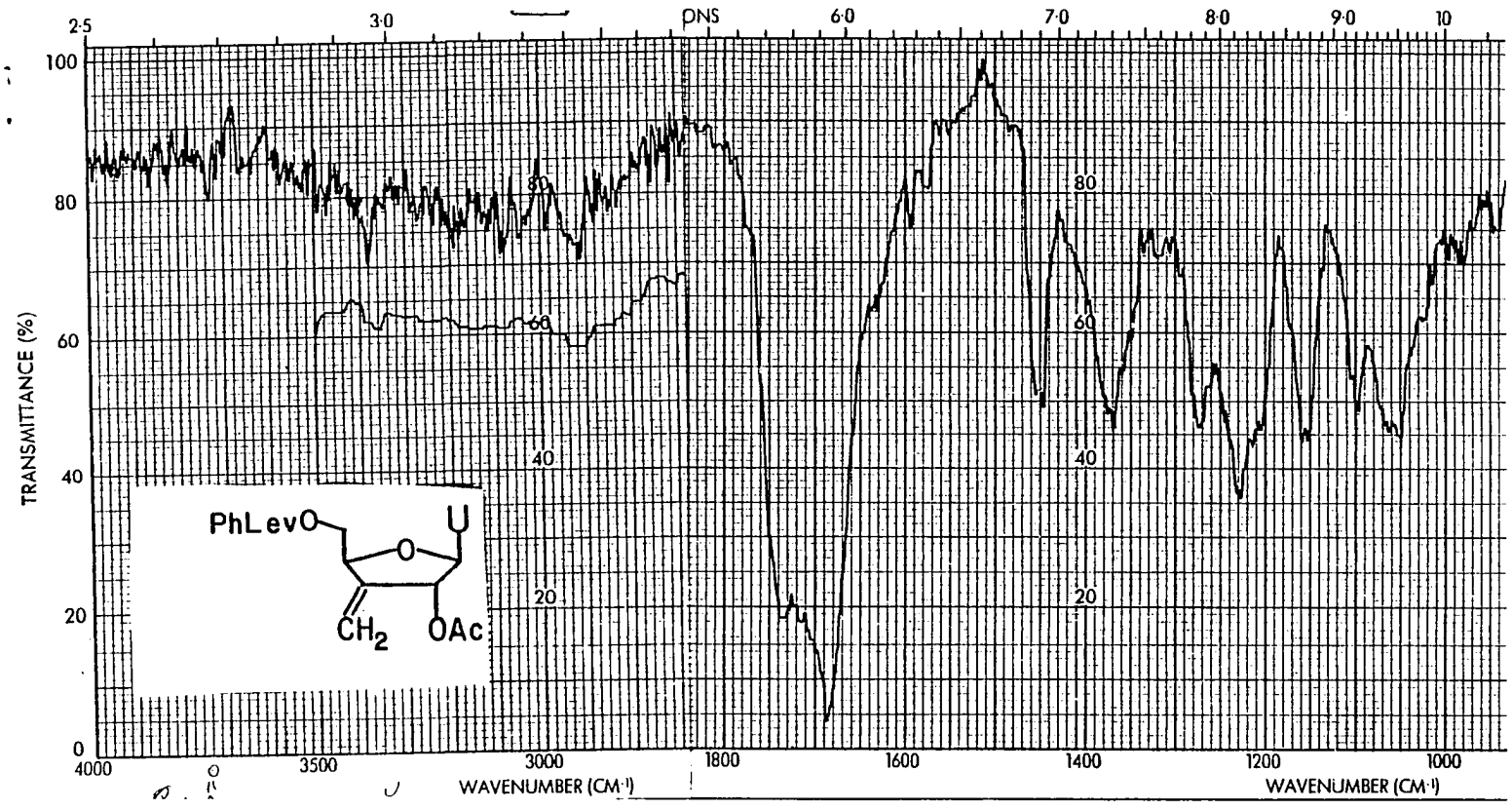

D.31.
2'-Acetyl-5'-(3-benzoylpropionyl)-3'-deoxy-3'-C-methyleneuridine (115).

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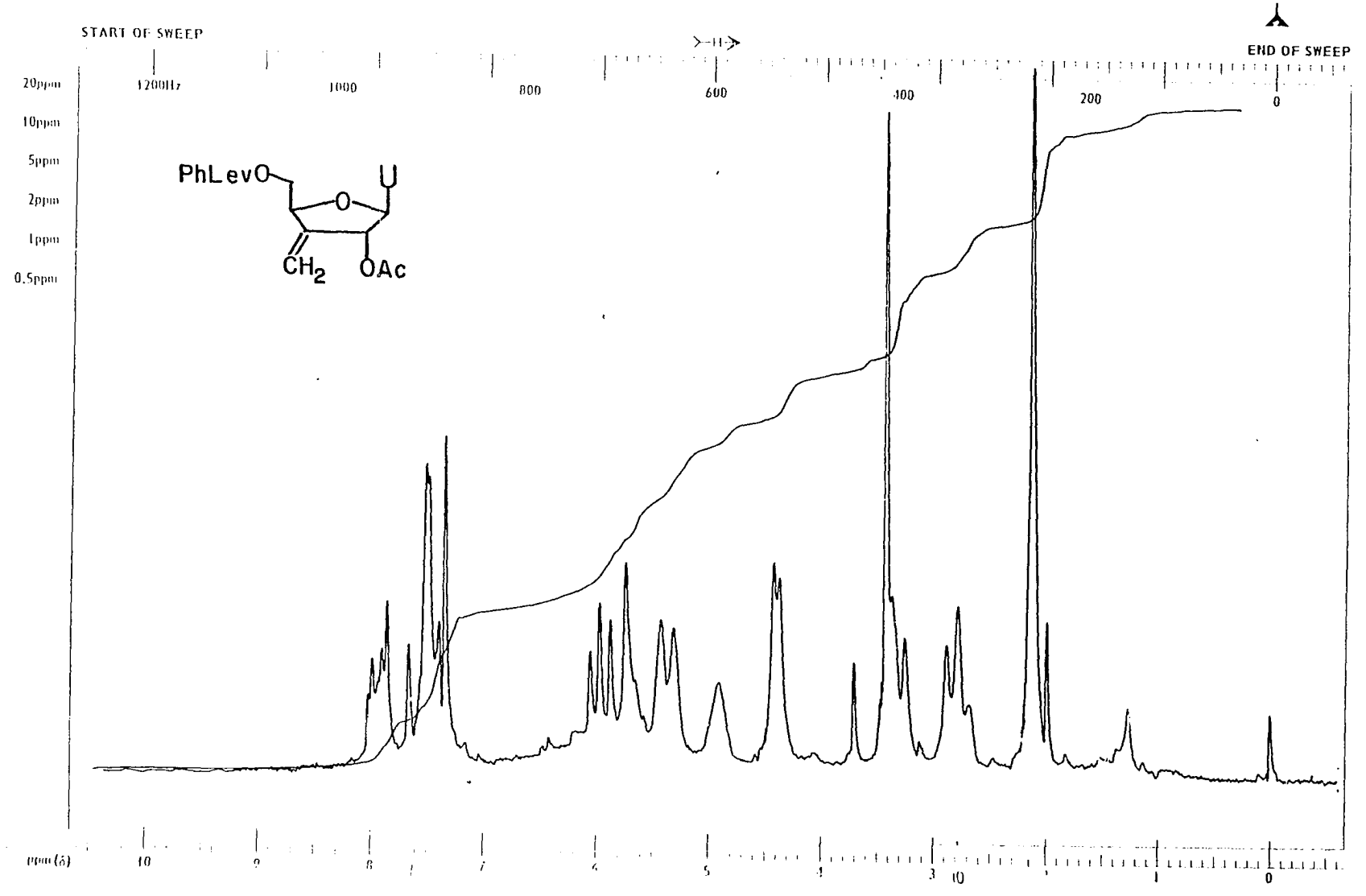
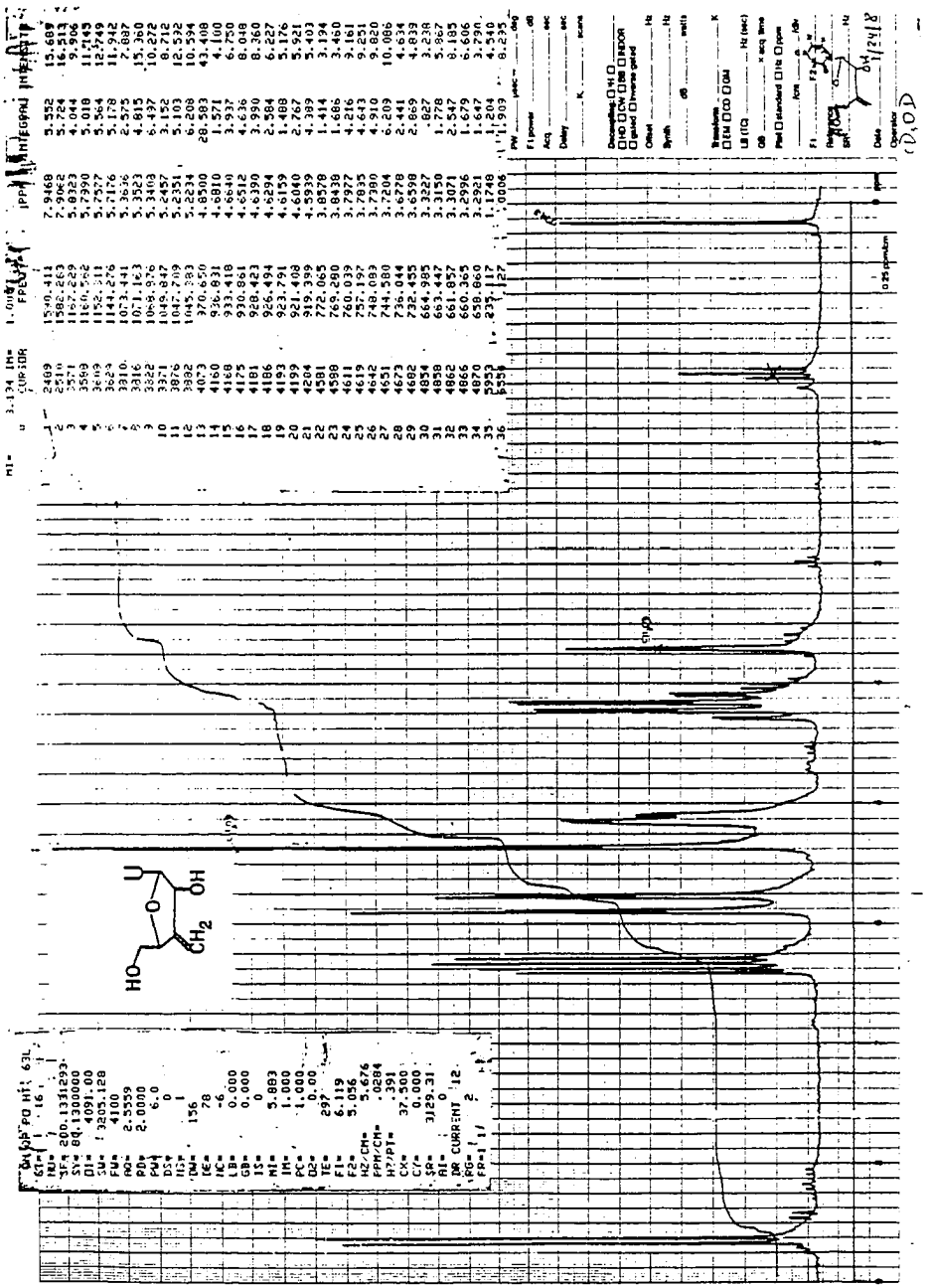


D.21.
2'-Acetyl-5'-(3-benzoylpropionyl)-2'-deoxy-3'-C-methyleneuridine (115).



D.22. 3'-Deoxy-3'-C-methylcarbocyclic (11G).

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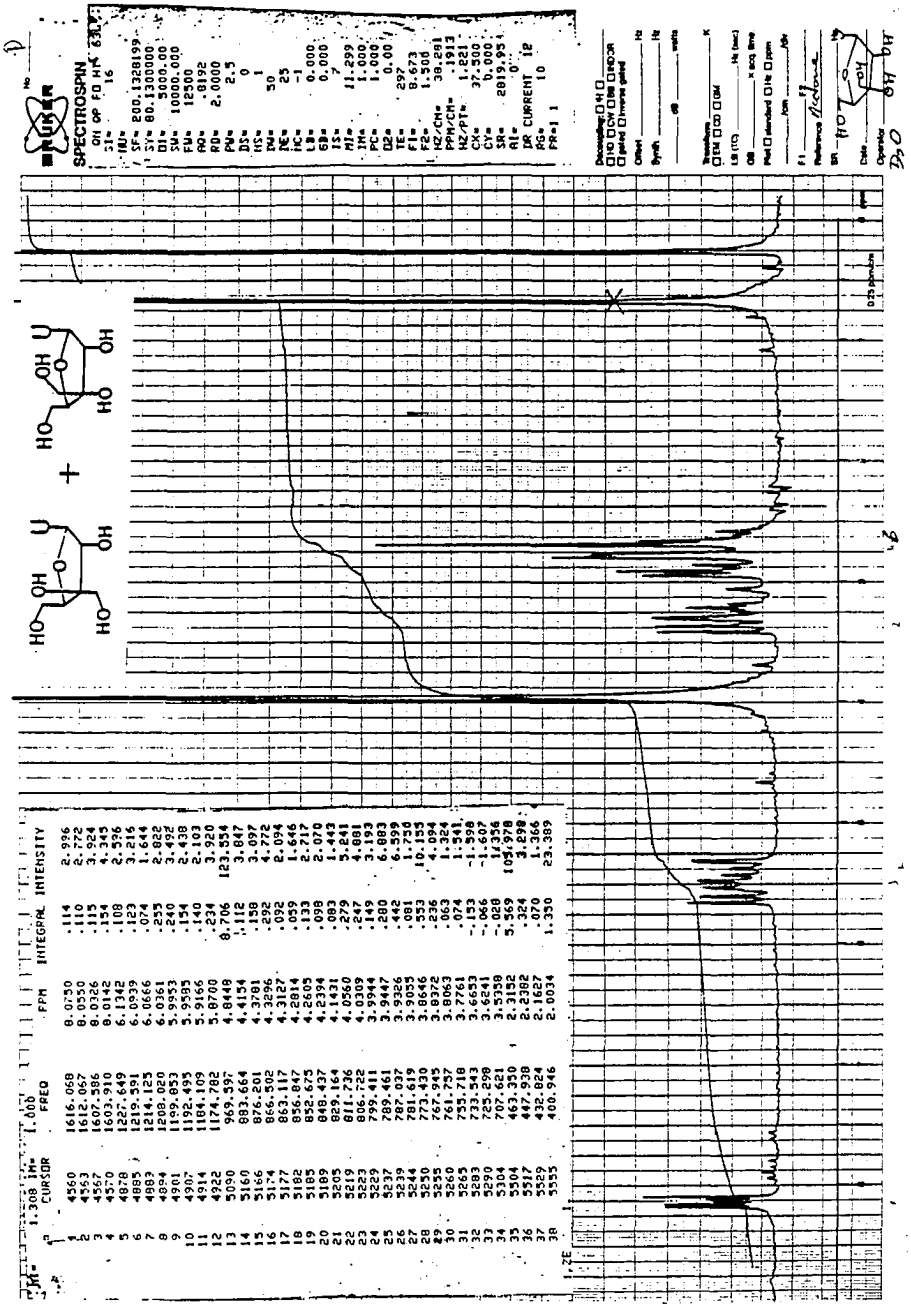

D.23. 3'-Hydroxymethylurilines (117), (118).

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