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A NEW PROCEDURE FOR THE SYNTHESIS OF GLYCO SYL PHOSPHATES

DISSERTATION

Presented in Partial Fulfillment of the Requirement for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By

Harbhajan Singh Prihar, B.Sc. M. Sc.

The Ohio State University
1972

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ACKNOWLEDGMENTS

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PUBLICATIONS


ABSTRACTS

1. Incorporation of D-galactose and N-Acetyl-D-Glucosamine into Gamma-Globulins in Lymph Node Extracts.

2. The Biosynthesis of the phosphomannan of Hensenula-Capsulata.


FIELDS OF STUDY

Major Field: Undergraduate: Chemistry, Physics and Mathematics - Graduate: Chemistry and Biochemistry

Studies in Carbohydrate Chemistry. Professor Derek Horton

Studies in Carbohydrate Metabolism. Professor Joseph Mednicino

Studies in Biosynthesis of Phosphomannan. Professor Robert M. Mayer
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INTRODUCTION

Sugar phosphates are known to be intermediates in a large number of biological processes. Robison and Morgan\textsuperscript{1} wrote in 1928, "Since the original work of Harden and Young on the function of phosphates in alcoholic fermentation, the idea that the formation of phosphoric esters may be an essential stage not only in the biochemical degradation of hexoses but also in the condensation of these sugars to the polysaccharides has taken firm hold of biochemical imagination". Thereafter, many new facts have been discovered on the subject and many new sugar phosphates have been obtained by chemical synthesis because it cannot be predicted which of the purely synthetic substances will be found in nature as research proceeds. The general subject of phosphorylation has been reviewed\textsuperscript{2-7} and, more specifically, excellent reviews have appeared on sugar phosphates.\textsuperscript{8-12}

Phosphate esters may be obtained by isolation from natural sources or by enzymic or chemical synthesis. Except in a few instances, such as the preparation of D-fructose 1,6-diphosphate from yeast,\textsuperscript{13,14} isolation from natural sources is not the method of choice. For preparation of certain glycosyl phosphates enzymic methods
have been found the simplest e.g. α-D-glucopyranosyl phosphate is readily prepared by the phosphorolysis of starch or glycogen\textsuperscript{15,16} and α-D-ribofuranosyl phosphate\textsuperscript{17} and 2-deoxy-α-D-erythro-pentofuranosyl phosphate\textsuperscript{18} can be prepared from the appropriate nucleosides. In many instances chemical synthesis is to be preferred over other preparative methods.

Synthesis may involve two distinctly different approaches. The first is concerned with electrophilic attack of an activated phosphoryl group on a hydroxyl group\textsuperscript{9}. To activate the phosphoryl group the phosphorus atom must acquire an electropositive character. This was done by taking various derivatives of phosphoric acid and experimentally testing to determine whether the electropositive character acquired was sufficient to activate the phosphoryl group. Thus, various phosphorylating reagents were developed. Phosphoryloxychloride was the first such reagent (10) but was soon replaced by several (I-LV) monofunctional derivatives of phosphoric acid. A monofunctional reagent should be (a) easy to prepare on a large scale, (b) stable for long periods, (c) very reactive,
and (d) such that the protected intermediates could be converted into the desired products under very mild conditions.

\[
\begin{align*}
\text{I} & \quad \text{II} \\
\begin{array}{c}
\text{RO} - \text{P} - \text{Cl} \\
\text{OR}
\end{array} & \quad \begin{array}{c}
\text{O} \\
\text{N} - \text{P} - \text{N} \\
\text{Br}
\end{array}
\end{align*}
\]

\[
\begin{align*}
\text{III} & \quad \text{IV} \\
\begin{array}{c}
\text{RO} \quad \text{P} - \text{O} - \text{P} - \text{OR} \\
\text{RO} \quad \text{P} - \text{O} - \text{P} - \text{OR}
\end{array} & \quad \begin{array}{c}
\text{O} \\
\text{P} - \text{Cl}
\end{array}
\end{align*}
\]

Diphenyl phosphorochloridate\textsuperscript{19} (I; R=Ph) satisfies both criteria (a) and (b). It has been used with aldoses\textsuperscript{20}, ketoses\textsuperscript{21}, sugar acids\textsuperscript{22}, reduced sugars\textsuperscript{23}, N-acylated amino sugars\textsuperscript{24}, and deoxy sugars\textsuperscript{25}; the number of carbons in the substrate sugar varying from 2 (glycolaldehyde\textsuperscript{26}) to 12 (trehalose\textsuperscript{27}). Overall yields of the purified sugar phosphates vary from 50 to 80% of the theoretical. Using pyridine as solvent and diphenyl phosphorochloridate as the phosphorylating reagent Posternak\textsuperscript{28} obtained a 10% yield of \(\alpha\)-D-glucose-1-phosphate. Dibenzylphosphorochloridate\textsuperscript{29} (I; R-CH\textsubscript{2}Ph)
and dimorpholinophosphorobromidate\textsuperscript{30} (II) satisfy criterion (a) but not criterion (b) whereas the opposite is true for bis-p-nitrobenzyl phosphorochloridate\textsuperscript{31} (I; R=p-nitrobenzyl) Tetrakis-p-nitrophenylpyrophosphate\textsuperscript{32} (III; R=p-nitrophenyl) is not easy to prepare on a large scale and has not been isolated in a pure state, Witzel \textit{et al.}\textsuperscript{33} have described bis-2-cyanoethyl phosphorochloridate (I; R = NC CH\textsubscript{2} CH\textsubscript{2}), but its use as a phosphorylating reagent needs further investigation. o-Phenylene phosphorochloridate\textsuperscript{34} (IV) was first employed by Reich\textsuperscript{35} as a phosphorylating agent. Recently, its use in the conversion of alcohols into their monophosphate esters\textsuperscript{36} and in the phosphorylation of nucleosides\textsuperscript{37} has been described with experimental details. It appears to be a versatile phosphorylating reagent and certainly meets all the criteria (a), (b), (c) and (d) (see before).

Hata \textit{et al.}\textsuperscript{38} reported the use of 2-chloromethyl-4-nitrophenyl phosphorochloridate for the preparation of alkyl dihydrogen phosphates.

The second type of phosphorylation involves the nucleophilic attack of a phosphate anion on the carbon of an alkyl halide or a related compound\textsuperscript{9}. This method of phosphorylation has been most commonly used in the synthesis of glycosyl phosphates.
The glycosyl phosphates are readily synthesized from the fully acetylated aldopyranosyl bromides by a halogen replacement reaction employing silver salts. In the original procedure by Cori and co-workers\textsuperscript{15} treatment with trisilver phosphate results in the formation of triglycosyl phosphate ester which on partial hydrolysis yields a monoester of the same anomeric configuration as the starting bromo derivative. Posternak\textsuperscript{28} introduced the use of silver diphenyl phosphate. The condensation product was hydrogenated to remove the phenyl groups and subsequent basic hydrolysis afforded the desired phosphate monoester in much higher yields.

If, however, silver dibenzyl phosphate is employed, according to Wolfrom and co-workers\textsuperscript{29,39} condensation occurs with a Walden inversion at C-1, provided the acetylated hydroxyl at C-2 has the same configuration as the bromine at C-1.

Khorana, \textit{et al.}\textsuperscript{40,41} showed that both the phosphorylating agent and the neighboring C-2 group effect may control the steric course of phosphorylation reaction at C-1. Antia and Watson\textsuperscript{42} have concluded that, with silver dibenzyl phosphate, the steric course of phosphorylation at C-1 is controlled not only by C-2 neighboring group effect but also by a second effect from the substituent at C-5.
In 1962, MacDonald\(^4\) published a new method for the preparation of \(\alpha\)-D-glucose-1-phosphate and \(\alpha\)-D-galactose-1-phosphate. This method has been applied to the synthesis of N-acetyl \(\alpha\)-D-glucosamine 1-phosphate and N-acetyl \(\alpha\)-D-galactosamine 1-phosphate\(^4\) and appears to be applicable to aldoses in general. In all cases cited, the \(\beta\)-anomer of the fully acetylated aldose, when heated for 2 hours at 50\(^\circ\) in vacuo with crystalline anhydrous phosphoric acid, has given rise to the \(\alpha\)-glycosylphosphate. A base-catalyzed hydrolysis takes off the remaining acetyl groups. Soon after the original communications\(^3,4\) it was demonstrated\(^5\) that a mixture of \(\alpha\) and \(\beta\) anomers of the acetylated-1-phosphates can be obtained if the reaction is run for a shorter period.

A recent report\(^6\) discussed the possible mechanism of the fusion method as suggested by Jeanloz and co-workers\(^7\). In the D-mannose series, neighboring group participation leads invariably to the formation of the stable anomer in good yield.\(^4,7-5\) By analogy with the reaction for D-mannose, a syrupy mixture of \(L\)-rhamnose tetraacetates also leads to the formation of the more stable \(\alpha\)-L-rhamnopyranosyl phosphate\(^5,1,2\). However, the beta form has actually been isolated\(^5\) only in limited quantities and the two forms separated chromatographically in an acid solvent.
A similar situation prevails with anomeric \( \text{L}^- \)-fucopyranosyl phosphates. Condensation of \( \alpha - \text{L}^- \)-chloro 2,3,4 tri-O-acetyl fucopyranose\(^{53}\) with silver dibenzyl phosphate or disilver hydrogen phosphate gave only the alpha anomer. The MacDonald procedure involving the condensation of the crystalline \( \alpha \)-tetraacetate with anhydrous phosphoric acid also gave the \( \alpha \)-phosphate\(^{53}\). However, the \( \beta \)-phosphate has been synthesized enzymatically\(^{54}\) with an animal kinase and ATP but only in limited quantities.

Two other approaches have been reported recently. The first one involves the trans addition of hydrogen dibenzyl phosphate to the 1,2-epoxy sugar\(^{55}\) and the second is the interesting observation of Orgel et al\(^{56}\) that a system containing D-ribose, cyanogen and orthophosphate gives modest yields of \( \beta - \text{D}^- \)-ribofuranosyl phosphate as the only isomer.

Availability of \( \beta - \text{L}^- \)-fucose-1-phosphate and \( \beta - \text{L}^- \)-rhamnose-1-phosphate would aid in the synthesis of the naturally occurring \( \beta - \text{L}^- \)-fucopyranosyl (guanosine-5'-pyrophosphate), [GDP \( \beta - \text{L}^- \)-fucose] and \( \beta - \text{L}^- \)-rhamnopyranosyl (thymidine-5'-pyrophosphate),[dTDP-\( \beta - \text{L}^- \)-rhamnose]. These \( \beta \)-glycosyl nucleotides are widely occurring cellular constituents involved in the biosynthesis of the endotoxins of \text{Salmonella}, blood group substances and other glycoproteins and polysaccharides.
STATEMENT OF THE PROBLEM

The primary objective of this research was to develop a synthetic route to \( \beta-L\)-fucose-1-phosphate and \( \beta-L\)-rhamnose-1-phosphate. Among others, the use of o-phenylene phosphorochloridate, an excellent reagent for the phosphorylation of hindered alcohols, was, in particular, to be investigated for the stereo-specific synthesis of aldose-1-phosphates. Fully protected hexopyranoses having a free hemiacetal group in the desired configuration were selected. Wherever such a derivative was not readily available syntheses were aimed towards that goal. The protecting groups were selected with the expectation that they could be removed easily under mildly basic conditions.

Some aspects of the basic chemistry of the newly synthesized phosphates were to be studied. Agreement between the predicted and the observed optical rotation of the sugar phosphates made in this research was sought, especially in the D-manno series. The relative rates of hydrolysis of the anomeric pairs of the sugar phosphates were to be compared.
Another related objective of the present research was to develop a method for the detection of the anomeric impurities in the sugar phosphate.
A. SYNTHESIS OF GLYCOSYL PHOSPHATES

I. MacDonald's Short Term Fusion Procedure

It was pointed out by MacDonald in 1966, that the phosphorylation reaction by phosphoric acid is probably mechanistically similar to the halogenation by hydrochloric acid or hydrobromic acid in which case it is well established that the $\beta$-D-halide is the initial product formed. Therefore, the major product is expected to be the $\beta$-D-1-phosphate. Indeed, a mixture of $\alpha$ and $\beta$ anomers of D-glucopyranosyl phosphate was obtained when the fusion was carried out for a shorter time.

Following this suggestion a syrupy mixture of L-rhamnose tetraacetates was fused with anhydrous phosphoric acid at 50°C in vacuo for 5 min. The processing of the reaction mixture resulted in 60% yield of the $\alpha$-L-rhamnopyranosyl phosphate, isolated as the crystalline di(cyclohexylammonium) salt. The presence of the $\beta$-anomer could not be detected. A reaction time of ten minutes also gave only the $\alpha$-anomer. A possible explanation is suggested by the extreme acid lability of rhamnopysanosides; perhaps anomerization of the phosphate derivative to the more stable $\alpha$-anomer takes place under milder conditions than
occurs with ordinary hexoses.

II. Phosphorylation of the hemiacetal hydroxyl group.

In contrast to the fusion procedure in which an oxygen-carbon bond is formed, a new approach based on the phosphorylation of the hemiacetal hydroxyl group where a phosphorus oxygen bond is formed was tried in the present work. The hemiacetal is the nucleophile which attacks an electrophilic phosphorus. The critical step is illustrated in scheme 1.

The new procedure, then essentially consists of two steps: a) The synthesis of the protected sugar having a free hydroxyl group at C-1 in the desired configuration. b) Phosphorylation of the hemiacetal hydroxyl group. Theoretically both anomers of the hemiacetals can be obtained from the acetylated glycosyl halides by displacement. Invariably the thermodynamically less stable anomer anomerizes to the more stable one under favorable conditions. Provided that under the phosphorylating conditions this rate of mutarotation is much slower than the rate of phosphorylation, both anomers of any glycosyl phosphate should be obtainable.

(a) Synthesis of Hemiaceetals

The synthesis of the anomeric hemiacetals has been worked out for a number of acetylated sugars as shown in scheme 2.
Scheme 1: A general reaction of a phosphorylating reagent with a hemiacetal.
Scheme 2: The general scheme showing the formation of anomeric hemiacetal from the parent sugar.
The various sugars selected in the present research each having a free hydroxyl group at C-1 in the desired configuration, were the following: 2,3,4-tri-O-acetyl α-L-rhamnopyranose \(^4\) (I)* 2,3,4-tri-O-acetyl-β-D-rhamnopyranose \(^5\)** (II) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose \(^6\); (III) 2,3,4,6-tetra-O-acetyl-β-D-mannopyranose \(^7\); (IV) 2,3,4-tri-O-acetyl-β-L-fucopyranose (V) and 2,3,4-tri-O-acetyl-α-L-fucopyranose (VI). Compounds (II-IV) were readily available by displacement with inversion from the corresponding acetylated glycosyl halides. 2,3,4-Tri-O-acetyl-β-L-rhamnopyranose II failed to crystallize and thus was used as a syrup immediately after its preparation. Anomerization of II yielded the α-anomer (I) as a crystalline solid. The anomic fucosetriacetates

* The numbering of the compounds in the discussion and experimental sections is separate from the numbers in the introduction.

** In the original publication the two triacetyl rhamnoses were designated as α and β anomers in the order of their isolation. According to the recent carbohydrate nomenclature, the more levorotatory anomer in the L-series is designated as α-L. Consequently, Fischer's anomer which is in fact more dextrorotatory of the two anomers has been termed β in the present study.
(V & VI) have not been reported previously and are described in this work for the first time.

2,3,4-Tri-O-acetyl-α-L-fucopyranosyl bromide is obtained as an unstable oil whereas the corresponding chloride has been obtained recently as a white crystalline solid. Frequently the displacement of the bromine atom with a hydroxyl group at C-1 results, in addition to the two isomeric hemiacetals, in the formation of a third isomer in which the acetyl migration from C-2 to C-1 has taken place. However, in a similar displacement with a chloro derivative, acetyl migration does not occur. The action of stoichiometric amount of water on 2,3,4 tri-O-acetyl-α-L-fucopyranosyl chloride in dry ether in the presence of silver carbonate gave rise to a syrupy mixture of triacetyl fucoses (scheme 3) This mixture was fractionated by crystallization from anhydrous ether into isomer A, (Mp.102°) [α] D -5° and isomer B. (VI) [α] D 25°-117°. Both isomers gave elemental analytical data in accord with the formula C12H18O8 and showed characteristic n.m.r. spectra. Isomer A was observed to mutarotate slowly in absolute ethanol, changing from an initial value of -5° to a final value of -119°. The mutarotation product from isomer A showed an n.m.r. spectrum virtually identical to that of isomer B, thereby indicating that these two must be members of an anomeric pair. Isomer A was readily converted into the known 1,2,3,4-tetra-O-acetyl-β-L-fucopyranose by the action of acetic anhydride and pyridine at 0°. The available evidence thus indicates that the
Scheme 3: Reaction of 2,3,4-tri-O-acetyl-α-L-chlorofucopyranose with water in anhydrous ether.
isomer A must be $2,3,4$-tri-$O$-acetyl-$\beta$-$L$-fucopyranose (V).

(b) **Phosphorylation of the Hemiacetal Hydroxyl group**

Most phosphorylating reagents are insufficiently electrophilic to react with the hemiacetal hydroxyl group at an adequate rate. This is another statement of the fact that the hemiacetal hydroxyl group is approximately 1000 times more acidic than an alcoholic hydroxyl group\(^{64}\). Thus, Cori et al\(^{15}\) reported that phosphorus oxychloride did not react with $2,3,4,6$-tetra-$O$-acetyl glucopyranose. Likewise we were unsuccessful in inducing a reaction between phosphoryl chloride and $2,3,4$-tri-$O$-acetyl-$\alpha$-$L$-rhamnopyranose (I).

Hata's reagent\(^{38}\) 2-chloromethyl-4-nitrophenyl phosphorochloridate, a powerful reagent for the phosphorylation of alcohols was then tested for its ability to phosphorylate a hemiacetal hydroxyl group. Using $2,3,4$-tri-$O$-acetyl-$\alpha$-$\bar{L}$-rhamnopyranose (I) as the representative compound in dry tetrahydrofuran in the presence of pyridine at room temperature for 24 h., no phosphorylation resulted\(^{65}\).

The reagent described by Khwaja et al\(^{35}\) turned out to be the answer to the problem. This reagent is able to react with the hemiacetal hydroxyl group under very mild conditions. The reactions are shown in scheme 4.
Scheme 4: Reaction of a hemiacetal with o-phenylene phosphorochloridate at room temperature.
The average half time for the mutarotation of 2,3,4 tri-O-acetyl β-L-rhamnopyranose (II) in absolute ethanol calculated from the data reported by Rabe\textsuperscript{105} is 16 hours. In the case of tri-O-acetyl β-L-fucopyranose (V) the average half time for mutarotation in absolute ethanol is 35 hours. On the other hand, the initial sugar reading of V in dry tetrahydrofuran remained practically unchanged over a period of 23 hours. When the rotation of 2,3,4 tri-O-acetyl β-L-fucopyranose was followed in dry tetrahydrofuran containing 12.5% (v/v) sym collidine, the average half time for mutarotation was approximately sixty hours. The conditions employed in the latter case are similar to those used in the phosphorylation step in the present work. As phosphorylation is essentially complete within half an hour at room temperature, the mutarotation is roughly hundred times slower than the phosphorylation. Furthermore, during phosphorylation, the proportion of free base gradually decreases due to the neutralization by the halogen acid released, and the resulting hydronium ion of the base, presumably, is a poor catalyst for the mutarotation of the free hemiacetal group\textsuperscript{106}. Thus it appears that the reaction of o-phenylene phosphorochloridate with a hemiacetal offers an ideal situation for the preparation of anomerically pure glycosyl phosphates.
The protected sugars (I-VI) were each converted into their \( \text{o-o-phenylene phosphate esters} \) with equimolar amounts of \( \text{o-phenylene phosphorochloridate} \) in tetrahydrofuran containing stochiometric amounts of \( \text{sym-collidine} \) \( (2,4,6\text{-trimethyl pyridine}) \) at \( 15^\circ \text{C} \) in 30 min. The reaction is usually quantitative with respect to both the sugar and the phosphorylating reagent. On treatment with at least one molecular equivalent of base and then with an excess of water, the phosphate triesters (XIV) are quantitatively converted into the corresponding o-hydroxyphenyl phosphate esters (XV).

The intermediate phosphate triesters (XIV) and phosphate diesters (XV) were not isolated and their tentative identification is based on electrophoretic data. The latter were oxidized with excess bromine in triethylammonium hydrogen carbonate buffer \( \text{pH} 7.2 \). Subsequent deacetylation with base yielded products (VII-XII). The phosphate esters were first precipitated as barium salts and then converted into the crystalline di(cyclohexylammonium) salts. Substances (VII-XII) all showed extreme lability to acid. Hydrolysis in 0.1M hydrochloric acid for 15 min. at \( 100^\circ \text{C} \) gave quantitative yield of both the sugar and inorganic phosphate in an equimolar ratio. Compounds (VII-XII) resembled the nonreducing sugars in reacting slowly with alkaline silver nitrate. The Benedict test was
negative in each case. Each of these compounds gave single spots in the following paper chromatographic systems: v/v 7:1:2 isopropyl alcohol ammonia (sp.gr. 0.88) water, v/v 7:3 ethanol-ammonium acetate (M.pH 7.5). Each of the products gave elemental analytical data in accord with the structures formed by the replacement of the anomeric hydroxyl group by an O-di(cyclohexylammonium) phosphoryl group. Therefore, the deacetylated products were formulated as α-\(-\)-rhamnopyranosyl di(cyclohexylammonium) phosphate (VII), β-\(-\)-rhamnopyranosyl di(cyclohexylammonium) phosphate (VIII), β-D-glucopyranosyl di(cyclohexylammonium) phosphate (IX), β-D-mannopyranosyl di(cyclohexylammonium) phosphate (X), β-L-fucopyranosyl di(cyclohexylammonium) phosphate (XI) and α-L-fucopyranosyl di(cyclohexylammonium) phosphate (XII).

The extraordinary reactivity of o-phenylene phosphorochloridate may be rationalized on the basis of the fact that the five membered cyclic aromatic phosphate, o-phenylene phosphate XVI, undergoes alkaline hydrolysis $6 \times 10^6$ times faster than does its open chain analog, diphenyl phosphate$^{66}$. 

![Chemical structures](image-url)
On this basis we can calculate that a reaction requiring half an hour with the cyclic reagent would require 345 years with the open chain analog. Therefore, while 2,3,4 triacetyl-\(\alpha\)-L-rhamnopyranose (I) did not react with either phosphoryloxyclyloride or 2-chloromethyl-4-nitrophenyl phosphorodichloridate (see before) it reacts smoothly with o-phenylene phosphorochloridate at room temperature.

Khwaja et al.\(^{36}\) devised three procedures for the removal of the o-hydroxy phenyl group from the intermediate phosphatedie ster: a) oxidation with bromine water in aqueous barium acetate or triethylammonium hydrogen carbonate (pH 7.5); b) oxidation with periodic acid; c) oxidation with lead tetraacetate in dioxan solution. In the present research procedure (b) could not be used because of the acid lability of the glycosyl phosphates and (c) was found unsatisfactory because any unreacted lead tetraacetate would attack the resulting glycosyl phosphates following deacetylation. Instead, procedure (a) was employed with fair success. Aqueous barium acetate buffer was found unsuitable for oxidation with bromine presumably because excess of barium ions in the reaction mixture retards deacetylation and renders the isolation of the barium salts of the phosphate esters through ethanol precipitation rather difficult. Furthermore, under these conditions unreacted hemiacetals are more easily oxidized to aldonic
acids which interfere with the isolation of the phosphate esters in pure form. Triethylammonium hydrogen carbonate introduced by Porath was found to be the buffer of choice. The base used in the present work is consistently sym-collidine. The use of a highly hindered base such as 2,6-diter-butyl pyridine or a less hindered base such as triethylamine was found unsatisfactory for phosphorylation. Using 2,6-diter-butyl pyridine as the base, the starting hemiacetal was recovered unchanged; no precipitation of the base hydrochloride was observed during the addition of the phosphorylating reagent. Triethylamine was also an unsuitable base probably because the intermediate phosphate triester or phosphate diester are likely to be base-labile. Triethylamine is often a suitable base for the phosphorylation of simple alcohols. However, in the nucleoside field 2,6 lutidine was found to be a better choice.
B. Molecular rotation data:

The optical rotations of the compounds (VII-XII) are presented in Table 1 along with available literature values. The data clearly show that the molecular rotation for compounds VII, IX and XII agree closely with the reported values.

The di(cyclohexylammonium) salt of X showed \( [\alpha]^D_D^{-6.5^\circ} \) as compared with the corresponding salt of the \( \alpha \)-anomer, which had \( [\alpha]^D_D^{+28^\circ} \). Although Hudson's rules of isorotation have played an important role in classifying anomeric structures, it may be noted that these rules are based on the assumption that the A-contribution to rotation is derived from the anomeric center only. However, it is now apparent\(^68\) that the presence or absence of an asymmetric conformational unit defined by the aglycon and a substituent at position 2 enters into the calculation of the A-value. Lemieux and Martin\(^69\) have thus pointed out that the 2A value for an anomeric pair of D-mannopyranosides is that of D-gluco derivative minus twice the contribution of an asymmetric unit defined by the 1 and 2-substituents in a gauche relationship. Following Whiffen\(^70\), Lemieux and Martin used a value of 4500 for this interaction. On the basis of a 2A value of 25,400 for the phosphate group in the gluco series, as determined by Putman and Hassid\(^71\), the corresponding value for 2A in the manno series is 16,400. Since the cyclohexylammonium salt of \( \alpha \)-D-mannopyranosyl phosphate has \( [M]_D^{+13200} \), the \( \beta \)-anomer should have \( [M]_D^{(13,200-} \).
<table>
<thead>
<tr>
<th>Aldose Component</th>
<th>Molecular Weight</th>
<th>$[\alpha]_{D}^{25}$ In Water at pH 7.8</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-D-Glucopyranose</td>
<td>458</td>
<td>+64.0°</td>
<td>71</td>
</tr>
<tr>
<td>$\beta$-D-Glucopyranose $\text{C}_2\text{H}_5\text{OH}.\text{H}_2\text{O}$</td>
<td>522</td>
<td>+7.3°</td>
<td>71</td>
</tr>
<tr>
<td>$\beta$-D-Glucopyranose .3H$_2$O</td>
<td>512</td>
<td>+4.1°</td>
<td>p.</td>
</tr>
<tr>
<td>$\beta$-D-Glucopyranose</td>
<td>458</td>
<td>+5.9°</td>
<td>104</td>
</tr>
<tr>
<td>$\alpha$-D-Mannopyranose</td>
<td>458</td>
<td>+28.7°</td>
<td>49</td>
</tr>
<tr>
<td>$\beta$-D-Mannopyranose H$_2$O</td>
<td>476</td>
<td>-6.5°</td>
<td>p.</td>
</tr>
<tr>
<td>$\alpha$-L-Rhamnopyranose 0.5H$_2$O</td>
<td>451</td>
<td>-21.5°</td>
<td>52</td>
</tr>
<tr>
<td>$\alpha$-L-Rhamnopyranose 0.5H$_2$O (VII)</td>
<td>451</td>
<td>-21.5°</td>
<td>p.</td>
</tr>
<tr>
<td>$\beta$-L-Rhamnopyranose (VIII)</td>
<td>442</td>
<td>+11.9°</td>
<td>p.</td>
</tr>
<tr>
<td>$\alpha$-L-Fucopyranose</td>
<td>442</td>
<td>-77.8°</td>
<td>53</td>
</tr>
<tr>
<td>$\alpha$-L-Fucopyranose (XII)</td>
<td>442</td>
<td>-77.0°</td>
<td>p.</td>
</tr>
<tr>
<td>$\beta$-L-Fucopyranose (XI)</td>
<td>442</td>
<td>-20.5°</td>
<td>p.</td>
</tr>
</tbody>
</table>

* p. indicates present work
Comparison of the Molecular Rotations of the Glycosyl Phosphates with the related methyl Aldopyranosides

<table>
<thead>
<tr>
<th>Aldose Component</th>
<th>Obs. Molecular Rotation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphate Ester</td>
<td>Methyl Pyranoside</td>
</tr>
<tr>
<td>α -D-Xylopyranose</td>
<td>24,800</td>
<td>25,300</td>
</tr>
<tr>
<td>β -D-Xylopyranose</td>
<td>300</td>
<td>-10,800</td>
</tr>
<tr>
<td>β -L-Arabinopyranose</td>
<td>39,000</td>
<td>40,000</td>
</tr>
<tr>
<td>α -L-Arabinopyranose</td>
<td>13,200</td>
<td>2,900</td>
</tr>
<tr>
<td>α -L-Rhamnopyranose</td>
<td>-9,200</td>
<td>-11,100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>β -L-Rhamnopyranose</td>
<td>+5,300</td>
<td>+17,000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>α -L-Fucopyranose</td>
<td>-34,400</td>
<td>-35,600</td>
</tr>
<tr>
<td>β -L-Fucopyranose</td>
<td>-9,000</td>
<td>+2,530</td>
</tr>
<tr>
<td>α -D-Mannopyranose</td>
<td>+13,200</td>
<td>+15,400</td>
</tr>
<tr>
<td>β -D-Mannopyranose</td>
<td>-3,200</td>
<td>-13,500</td>
</tr>
<tr>
<td>α -D-Glucopyranose</td>
<td>29,300</td>
<td>30,900</td>
</tr>
<tr>
<td>β -D-Glucopyranose</td>
<td>3,800</td>
<td>-6,700</td>
</tr>
<tr>
<td>α -D-Galactopyranose</td>
<td>36,000</td>
<td>38,000</td>
</tr>
<tr>
<td>β -D-Galactopyranose</td>
<td>10,000</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>p indicates present work

* From the rotation of the enantiomer.
16,400) = -3200. The observed value for \([\alpha]_D^{28}\) yields \([M]_D^{25}\) = -3100.

Hudson\(^{72}\) has pointed out that the conversion of methyl \(\alpha\)-D-mannopyranoside into methyl \(\alpha\)-D-rhamnopyranoside results in a diminution of about 4,000 in the molecular rotation. As the \(\text{di(cyclohexylammonium)}\) salt of \(\beta\)-D-mannopyranosyl phosphate has \([M]_D = -3100\), \(\beta\)-D-rhamnopyranosyl phosphate should have \([M]_D = -7100\) and its enantiomorph +7100. The di(cyclohexylammonium) salt of VIII (mol wt 442) should show \([\alpha]_D +16^0\). The value actually observed for VIII is +12^0. This discrepancy may be partly due to the fact that the conversion of methyl \(\beta\)-D-mannopyranoside into methyl \(\beta\)-D-rhamnopyranoside results in diminution of less than 4000 in the molecular rotation (see Table 2). Thus it appears that the nature of the equatorial substituent at C-1 in the \(\beta\)-D-(C1) conformation is a contributory factor to the rotation of the molecule, presumably, due to its interaction either with C-6 hydroxyl group or the ring oxygen atom. It may be pointed out that Chatterjee and MacDonald\(^{52}\) have miscalculated the molecular rotation for the \(\beta\)-L-anomer. Using a 2A value of 25,400 for the phosphate group, thus ignoring the contribution of the gauche interaction in the rhamno series, they expected a molecular rotation of +15,700 for a hydrated cyclohexylammonium salt of \(\beta\)-L-rhamnopyranosyl phosphate.

By analogy with the optical rotation of methyl \(\beta\)-L-fucopyranoside, Roseman \textit{et al} expected an \([M]_D\) of +2500 from \(\beta\)-L-fucopyranosyl phosphate (Table 3). While this corres-
pondance is observed in certain cases reported by Carlson et al., it is by no means a rule as is quite evident from the data reported on phosphate esters and methyl pyranosides in Table 2. It is interesting to note that in all cases where the nature of aglycon has a bearing on the molecular rotation, the substituent at the anomeric carbon occupies an equatorial position. At this point this observation cannot be explained with certainty. However, it may be pointed out that unlike 1-axial methyl glycopyranosides, the molecular rotations of 1-equatorial methyl glyco-
pyranosides cannot be predicted accurately from the application of the empirical rules. Lemieux and Martin have suggested that conformers involving rotation about the C-1-0CH₃ bond, contributing to the rotation are less clearly predictable for the 1-equatorial methyl glycosides than the corresponding rotamers for their anomers. It was predicted on the basis of the n.m.r. evidence that for both methyl β-D-glucopyranoside and its 2-deoxy derivative the conformations having the glycosidic methyl flanked by the ring oxygen and C-2 was the most favored. However, these conformations would be much less stable for 1-axial methyl-glycopyranosides on steric grounds and this indeed was confirmed by ¹³C n.m.r. spectroscopy.

The di(cyclohexylammonium) salt of α-L-fucopyranosyl phosphate (XII) (Mol. wt. 442) has [α]D -77.8 and [M]D -34,400. On the basis of a 2A value of 26,000 for the phosphate group in the D-galacto series as reported by
The predicted and the observed optical rotation of the di(cyclohexylammonium) salts of some glycopyranosyl phosphates.

<table>
<thead>
<tr>
<th>Phosphate</th>
<th>Molecular Rotation</th>
<th>Predicted</th>
<th>Present Work</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>β -L-Mannopyranose H₂O (X)</td>
<td>−3200</td>
<td></td>
<td>−3100</td>
<td></td>
</tr>
<tr>
<td>β -L-Rhamnopyranose (VIII)</td>
<td>+15700</td>
<td>+7100</td>
<td>+5300</td>
<td></td>
</tr>
<tr>
<td>β -L-Fucopyranose (XII)</td>
<td>+2500</td>
<td>−8400</td>
<td>−9000</td>
<td></td>
</tr>
</tbody>
</table>

Molecular rotation = Sp. rotation \times \text{mol. wt.}
Putman and Hassid\textsuperscript{71}, the molecular rotation of the $\beta$-L-anomer should be -8400, giving $\left[\alpha\right]_D -19^\circ$ for anhydrous cyclohexylammonium salt of $\beta$-L-fucopyranosyl phosphate. The value actually observed for the phosphate reported herein is $-20.5^\circ$, suggesting that it is the $\beta$-L-anomer.

C. Reaction of glycosyl phosphates with N,N'-dicyclohexyl carbodiimide.

Khorana\textsuperscript{74, 75} et al have shown that ribonucleoside 2'(3') phosphate esters which possess an adjacent cis-hydroxyl function react with N,N'-dicyclohexylcarbodiimide in aqueous pyridine at room temperature to form the five-membered ribonucleoside 2',3'-cyclic phosphates and that the latter react further to form N-phosphoryl-ureas (partial formulas XVIII - XIX - XX). The reaction sequence was followed by paper chromatography, using the solvent system: v/v 7:1:2 isopropyl alcohol-ammonia-water, the $R_f$'s of the products following, invariably the order XX $> XIX > XVIII$. Extending their observations on cyclic phosphates Khorana et al\textsuperscript{76} have also shown that cyclization of a glycosyl phosphate is possible both when the relationship of the phosphate group to the adjacent hydroxyl is axial to equatorial (cis) and when this relationship is equatorial to equatorial (trans). Apparently, in the latter situation, the large size of the phosphorous atom, as compared with that of the carbon atom, enables the cyclization reaction
to occur since neither acyl group\textsuperscript{77} migration nor isopropylidene derivative\textsuperscript{78,79} formation has ordinarily been observed when the hydroxyls are trans (equatorial to equatorial). On the other hand cyclization does not occur when the phosphate and hydroxyl group on the adjacent
carbon atom are both axial (trans). This situation is present in \( \alpha\)-D-mannopyranosyl phosphate and \( \alpha\)-L-rhamnopyranosyl phosphate and these substances did not form a cyclic phosphate on treatment with \( N,N' \)-dicyclohexyl carbodiimide. However, under identical conditions \( \beta\)-D-mannopyranosyl phosphate and \( \beta\)-L-rhamnopyranosyl phosphate VIII) gave first the cyclic phosphates and then the N-phosphorylureas. Thus, the specification of the reaction served as a diagnostic test for establishing the configuration of anomic pair of the glycosyl phosphates.

However, it should be noted that this test serves only a limited value in ascertaining conformation. While inertness of the \( \alpha\)-D-mannopyranosyl phosphate confirms the \( C_1(D) \) conformation (XXII), the participation of the \( \beta\)-anomer in the cyclization reaction fails to distinguish between the two chair forms \( C_1(D) \) (XXIII) and \( 1C(D) \) (XIV).

As expected, both anomers of \( L\)-fucopyranosyl phosphate (XI & XII) formed cyclic phosphate on treatment with \( N,N' \) dicyclohexyl carbodiimide, thus proving that \( \beta\)-L-fucopyranosyl phosphate does attain \( 1C(L) \) conformation (XV) rather than the \( C_1(L) \) conformation (XVI) which would not be expected to form a cyclic phosphate (trans axial, axial).

D. Separation of the anomers of glycosyl phosphates.

A related objective of this research was the separation of one anomer of a glycosyl phosphate from the other, which
would allow the detection of trace amounts of anomeric impurities in a given sample of glycosyl phosphates. This separation is made very difficult by close similarity in the physical and chemical behavior of the members of any pair of anomers. Up-to-date, no uniformly successful method has been reported for such a separation.

A two-dimensional system of paper chromatography has been developed for the separation from each other of a wide variety of phosphorylated carbohydrates, however, this system is not suitable for good separation of anomeric pairs. Recently, the anomers of D-glucopyranosyl phosphate and L-rhamnopyranosyl phosphate have been separated from each other on paper in an acid solvent. This system failed to separate anomeric pairs of D-mannopyranosyl phosphate and L-fucopyranosyl phosphate in the present work. The anomers of 2-deoxy-D-erythro pentofuranosyl phosphate have been separated on paper in an ammoniacal solvent employing excessively long development times. Gas liquid chromatography is not ideally suited to the separation of glycosyl phosphates presumably because the phosphates are too labile.

Ion exchange chromatography has been used occasionally for certain separations with fair success. The α-and β-anomers of 2-acetamido 2-deoxy-D-glucopyranosyl phosphate were separated with an ion exchange resin by use of dilute
hydrochloric acid as the eluent. Ray and Roscelli have separated the anomers of D-glucopyranose 1,6 diphosphate on a column of Dowex-1-(HCOO) by the use of a linear pyridine-formic acid gradient at pH3. Other successful separations under neutral conditions that have been reported include (i) the anomers of D-galactopyranosyl phosphate by use of Dowex-1-(Cl)- and elution with ammonium chloride (ii) the anomers of 2-acetamido 2-deoxy-D-glucopyranosyl phosphate by use of gradients of lithium chloride or ammonium chloride or ammonium chloride on Dowex-1 (Cl) and (iii) the anomers of D-glucopyranosyl phosphate and D-galactopyranosyl phosphate by use of triethylammonium hydrogen carbonate gradient at pH7.2 on Dowex-1-X8 (HCO3)86.

Thin layer chromatography has been used to separate certain pairs of glycosyl phosphates. Using silica Gel G (Merck) and 5:3:1 ethanol-conc. ammonium hydroxide-water, a good separation was achieved with 2-deoxy-D-erythropento-furanosyl phosphates, and only a moderate resolution was obtained with the anomers of D-ribopyranosyl. D-Ribofuranosyl and L-arabinopyranosyl were not resolved.

In the present work anomeric pairs of D-glycopyranosyl phosphate could neither be differentiated at pH3.6 (.037 M ammonium formate) nor at pH7.2 (.05 M triethylammonium hydrogen carbonate) by electrophoresis. In the former the pH of the buffer is about 3 pH units above the pK1, of
a glycopyranosyl phosphate whereas in the latter the buffer employed is approximately 1 pH unit higher than the pK' of the phosphate ester. In both cases one would expect essentially no difference in the ionizable forms of a given anomeric pair of glycosyl phosphates and hence no separation in electrophoresis. However, if one uses a buffer having a pH close to the pK' of the phosphate ester one would expect a significant difference in the dissociation of the anionic glycopyranosyl phosphate for a given anomeric pair, even if there are very small differences in pK' of the phosphoryl group, of the order of one tenth of a pH unit. This is evident from the following relationship for anionic dissociation.

\[
\text{% Ionized} = \frac{100}{1 + \text{antilog} (\text{pK}_a - \text{pH})}
\]

The difference in dissociation between members of an anomeric pair of glycosyl phosphate should permit their resolution in electrophoresis. This indeed was found to be the case. Anomeric pairs of D-glucopyranosyl, D-mannopyranosyl, D-galactopyranosyl, L-rhamnopyranosyl and L-fucopyranosyl phosphates were separable at pH 6.2 (see Table 4). Invariably the -\(\beta\)-anomer was the faster of a given pair, thus implying that the -\(\beta\)-anomer is more acidic than the -\(\alpha\)-isomer.

The spots were visualized by spraying with ammonium molybdate and exposing to ultra violet light. Thus, a
TABLE 4
Relative paper electrophoretic mobilities of the glycosyl phosphates in .05M sodium maleate buffer (pH6.5 or pH6.2)

<table>
<thead>
<tr>
<th>Aldose Component</th>
<th>Relative Mobility Rp&lt;sub&gt;p&lt;/sub&gt;icrate pH6.5</th>
<th>Relative Mobility Rp&lt;sub&gt;p&lt;/sub&gt;icrate pH6.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-D-Glucopyranose</td>
<td>1.49</td>
<td>1.46</td>
</tr>
<tr>
<td>β-D-Glucopyranose</td>
<td>1.63</td>
<td>1.57</td>
</tr>
<tr>
<td>α-D-Mannopyranose</td>
<td>1.45</td>
<td>1.31</td>
</tr>
<tr>
<td>β-D-Mannopyranose</td>
<td>1.55</td>
<td>1.36</td>
</tr>
<tr>
<td>α-D-Galactopyranose</td>
<td>1.45</td>
<td>1.29</td>
</tr>
<tr>
<td>β-D-Galactopyranose</td>
<td>1.47</td>
<td>1.34</td>
</tr>
<tr>
<td>α-L-Rhamnopyranose</td>
<td>1.45</td>
<td>1.16</td>
</tr>
<tr>
<td>β-L-Rhamnopyranose</td>
<td>1.59</td>
<td>1.29</td>
</tr>
<tr>
<td>α-L-Fucopyranose</td>
<td>1.45</td>
<td>1.37</td>
</tr>
<tr>
<td>β-L-Fucopyranose</td>
<td>1.54</td>
<td>1.49</td>
</tr>
</tbody>
</table>
method was established for checking the anomeric impurities in a given sample of glycosyl phosphates. Substances VII - XII synthesized in this work were found to be analytically pure by this method.

**Periodate Oxidation of the glycosyl phosphates.**

The oxidation of the newly synthesized glycosyl phosphates with periodate provide further support for the structures assigned to these compounds. Electrophoretically pure $\alpha$-D-mannopyranosyl phosphate (X), $\alpha$-L-rhamnopyranosyl phosphate (VIII) and $\beta$-L-fucopyranosyl phosphate (XI), each as expected, consumed two moles of sodium metaperiodate per mole of sugar phosphate in sodium acetate buffer pH 4.3. One mole of formic acid was formed per mole of the phosphate ester in each case. The periodate consumed was measured spectrophotometrically and the formic acid produced was distilled at low temperature under vacuum.
E. **Nuclear magnetic resonance spectra**

The n.m.r. spectra of substances (I-XII) showed features of some interest. A comparison of the anomeric pairs of hemiacetals (I-II & V-VI) and their monophosphate esters (VII-VIII & XI-XII) was therefore made. Studies were made at 35° centigrade. Interpretation of n.m.r. spectra is basically concerned with chemical shifts and spin couplings. The observed spin couplings in (I-XII) were unexceptional and were analogous to related compounds. It was noted, however, that the C-5 methyl group in the α-L-rhamnopyranose derivatives gives complex signals whereas simple patterns were obtained in the β-L-fucopyranose derivatives.

In the following discussion, the first order interpretation of the spectra of compounds I-VI in deuterochloroform and compounds VII-XII in deuterated water is presented along with the comparison of the data.

The spectrum of (I) shows the methyl protons as a sharp doublet at 8.80 with J =6.5Hz. The three acetyl groups appeared as three 3-proton singlets at 7.82, 7.95 and 8.04. The rest of the spectrum was unresolved. The corresponding β-anomer (II) could not be crystallized in the present work...
n.m.r. spectrum of the syrup (II) showed the methyl protons as a pair of doublets at τ 8.75 and 8.80. This could have resulted from anomerization of the less stable β-anomer into the α-form in the n.m.r. tube. The acetyl protons appeared in the region 7.80-8.00. No definitive assignments could be made to the ring protons. However, the compound was assumed to be the β-anomer based on its optical rotation.

The methyl group in the n.m.r. spectrum of (V) gave rise to a sharp doublet at τ 8.75 and the three proton ring-lets at τ 7.78, 7.85 and 7.98 were assigned to three acetyl groups. A quartet at τ 6.0 with $J_{5,6} = 6.5$Hz was assigned to H-5 and H-1 appeared as a doublet at τ 5.80 with $J_{1,2} = 7.0$Hz. (see Table 5)

The n.m.r spectrum of VI showed a three proton doublet centered at τ 8.85 with $J = 6.5$Hz. A hydroxyl group signal at τ 6.40 disappeared on deuteration in deuterated ethanol. The H-5signal appeared as a quartet at τ 5.58 with $J_{5,6} = 6.5$Hz and a doublet at τ 4.96 having $J = 3.6$Hz was assigned to H-1.

These spectral assignments are in accord with those reported by Leaback et al\textsuperscript{53} for several anomeric pairs of fucopyranosides. The β-substituent has a small deshielding effect on the C-6 hydrogens whereas the H-5 is strongly deshielded by an axial substituent at the anomeric carbon. The spectral data indicate that (V) & VI are the β- and the α—anomers respectively and they adopt $\perp C$ conformation
<table>
<thead>
<tr>
<th>L-Fucopyranose Derivatives</th>
<th>C-1 Proton (Doublet)</th>
<th>C-5 Proton (Quartet)</th>
<th>C-6 Protons (3 Doublets)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,4-Tri-O-acetyl α-L-Fucopyranosyl chloride (XIII)</td>
<td>3.65  3.5</td>
<td>5.60  6.5</td>
<td>8.84  6.5</td>
<td>p*</td>
</tr>
<tr>
<td>2,3,4-Tri-O-acetyl α-L-Fucopyranose (VI)</td>
<td>4.96  3.6</td>
<td>5.58  6.5</td>
<td>8.85  6.5</td>
<td>p</td>
</tr>
<tr>
<td>2,3,4-Tri-O-acetyl β-L-Fucopyranose (V)</td>
<td>5.80  7.0</td>
<td>6.00  6.5</td>
<td>8.75  6.5</td>
<td>p</td>
</tr>
<tr>
<td>1,2,3,4-Tetra-O-acetyl α-L-Fucopyranose</td>
<td>3.70  3.0</td>
<td>5.70  6.5</td>
<td>8.86  6.5</td>
<td>53</td>
</tr>
<tr>
<td>1,2,3,4-Tetra-O-acetyl β-L-Fucopyranose</td>
<td>4.27  7.0</td>
<td>5.95  6.5</td>
<td>8.77  6.5</td>
<td>53</td>
</tr>
<tr>
<td>2,3,4-Tri-O-acetyl α-L-Methylfucopyranoside</td>
<td>5.08  3.5</td>
<td>5.85  6.5</td>
<td>8.87  6.5</td>
<td>53</td>
</tr>
<tr>
<td>2,3,4-Tri-O-acetyl β-L-Methylfucopyranoside</td>
<td>5.62  7</td>
<td>6.1     6.5</td>
<td>8.77  6.5</td>
<td>53</td>
</tr>
</tbody>
</table>

*p indicates present work
Examination of the potassium salts of the anomeric \(\text{L-}\)rhamnopyranosyl phosphates (VII & VIII) in \(D_2O\) by nuclear magnetic resonance spectroscopy against tetramethylsilane as external standard showed the following:

(a) Splitting of C-1 hydrogen signal by H-2 and P-31 gave a quartet at \(\tau 4.30\) with \(J_{1,2}=1.5\text{Hz}\) and \(J_{H1,P}=10\text{Hz}\) in the spectrum of VII, indicating an equatorial-equatorial coupling of the protons on C-1 and C-2, H-2 being in an antiperiplanar relationship with the ring oxygen atom \(88-90\). These values are in agreement with those reported by MacDonald.

In the n.m.r. spectrum of VIII, H-1 appeared as a quartet at \(\tau 4.48\) having \(J_{1,2}=1.0\text{Hz}\), \(J_{H1,P}=10\text{Hz}\) indicating an axial-equatorial coupling of the protons on C-1 and C-2. This is again in general agreement with the values reported by Horton and Turner for \(\alpha-D-\)mannose pentaacetates. The data indicate that VII
and VIII adopt the \(1C(L)\) conformation in \(D_2O\).

(b) The methyl group protons in the spectrum of VIII appeared at \(\tau 8.34\) as a clean doublet with \(J_{5,6}=6.5\,\text{Hz}\) whereas in VIII a 3 proton complex multiplet at \(\tau 8.0-8.35\) was observed instead. Since the sample used was analytically as well as anomerically pure the splitting of the methyl group might have been the result of a similar chemical shift of H-4 and H-5\(^91\). However, in the present study no effort has been made to resolve the H-4 and H-5 signal.

The n.m.r. spectrum of X showed the H-1 signal as a quartet at \(\tau 4.30\) having \(J_{1,2}=1.0\,\text{Hz}\) and \(J_{H1,P}=10\,\text{Hz}\). This indicates an axial equatorial relationship of the protons on C-1 and C-2 in \(D\)-mannopyranose derivative\(^90\). Under similar conditions for \(\alpha\)-\(D\)-mannopyranosyl phosphate a \(J_{1,2}\) value of 1.5Hz has been reported for the H-1 signal which appeared at \(\tau 4.1\).\(^47\) Thus, it appears that (X) assumes the \(1C(D)\) conformation in \(D_2O\).

Compounds XI & XII showed n.m.r. spectra that were in complete agreement with those already reported by Leaback et al. Thus, it is indicated that the \(\beta\)-\(L\)-fucopyranosyl phosphate synthesized in the present study is identical with the biosynthetic material reported by Leaback et al.\(^53\).
EXPERIMENTAL

General - Evaporations were performed under reduced pressure on a rotatory evaporator, with the bath temperature below 45°. Capillary tubes were used to obtain the melting point, uncorrected. Specific rotations were measured with a 2-dm. tube, at the wave length of the sodium D line.

Hexoses and 6-deoxy hexoses were determined by the phenolsulfuric and cysteine-sulphuric acid methods respectively. Reducing sugars, total phosphate and inorganic phosphates, were estimated by the methods of Park-Johnson, Ames and Dubin and Lowry-Lopez respectively. Analysis of sugars and sugar phosphates was carried out in the following solvent systems by descending paper chromatography.

(a) v/v 7:3 ethanol: ammonium acetate (1M.pH7.0). (b) 7:1:2 isopropanol-ammonium hydroxide (sp.gr. 0.88)-water. (c) 5:2:3 n-butanol-acetic acid-water (d) 80:15:5 methanol-formic acid-water. Sugars and sugar phosphates on paper chromatograms were revealed by the silver nitrate dip procedure and the Hanes-Isherwood spray reagent.

Paper electrophoresis was conducted on Schleicher and Schuell No. 589 orange ribbon paper strips (4x22.5 in.) in the following buffers. (A) .05M ammonium formate
(pH 3.6). (B) .05M triethylammonium hydrogen carbonate (pH 7.2) and (C) .05M sodium maleate (pH 6.2 and/or 6.5). The electrophoretograms were air dried before revelation of the spots.

Sym.-collidine and triethylamine were heated with calcium hydride under reflux and redistilled before use; dioxan and tetrahydrofuran were purified by distillation from lithium aluminum hydride. The chloroform used was A.R grade and contained approximately 0.75% of ethanol. Before use it was shaken with anhydrous calcium chloride and filtered to obtain dry chloroform. Anhydrous ether was obtained by distilling and keeping it over sodium.

The microanalyses were performed by Galbraith Analytical Laboratories, Knoxville, Tennessee.

The n.m.r. spectra of the acetylated glycopyranoses were determined at 60 MHz with a Varian T-60 n.m.r. spectrometer, on 10% solutions in chloroform-d containing 2% of tetramethylsilane (τ 10.00) as internal indicator. The n.m.r. spectra of glycopyranosyl phosphates (lithium or potassium salts, lyopholyzed powders) were determined on 15-20% solutions in deuterium oxide, using tetramethylsilane as the external standard. Spectra were analyzed on a first order basis and the coupling constants recorded are the measured line-spacings. Chemical shift values are given on the τ scale, and correspond to the mid-
points of each singlet or multiplet.

The utilization of periodate during the oxidation of various glycosyl phosphates was measured spectrophotometrically by following the decrease in light absorption due to periodate ion at 300 μm. The simultaneous determination of formic acid produced was carried out by low temperature vacuum distillation.

β-Phenyleneposphorochloridate was purchased from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin.
Preparation of 2,3,4-tri-O-acetylα-L-rhamnopyranose (I)
and 2,3,4-tri-O-acetylβ-L-rhamnopyranose (II)

L-Rhamnose tetraacetates were obtained as a syrup by the action of acetic anhydride and pyridine on α-L-rhamnose. The method used was essentially that reported by Chatterjee and MacDonald. The syrup was dissolved in hydrobromic acid-acetic acid solution (Eastman Kodak Co. 30-32% hydrobromic acid-acetic acid) and the resulting mixture maintained at room temperature for 2.5 h. The resulting product 2,3,4 tri-O-acetyl-α-L-rhamnopyranosyl bromide was isolated by the procedure of Haworth et al and gave physical constants in agreement with the reported values.

The following procedure was adapted from Fischer et al for the preparation of (I) and (II).

A solution of 2,3,4-tri-O-acetylα-L-bromo-rhamnopyranose (5g.) in dry acetone (50ml.) was cooled to 0° and treated with water (0.25ml) and freshly prepared dry silver carbonate (4g.). The mixture was vigorously agitated for 5 min. and then stirred at 0° for 30 min. The silver salts were filtered off and the residue washed with cold acetone (25ml). The combined washings and the filtrate were concentrated to a syrup; yield 3.2g (80%) [α]D^25 = +21°
The syrup failed to crystallize from anhydrous ether. On keeping the ethereal solution at 0°C overnight a solid was obtained which gave an $[\alpha]_D +3^\circ$. This was presumably a mixture of I and II. When crystallized once from ether it afforded pure (I), $[\alpha]_D =-18^\circ$ (cl, absolute ethanol) [lit$^5$ values $[\alpha]_D$ for $\alpha$- and $\beta$- anomers are $-19^\circ$ and $+28^\circ$ respectively].

N.m.r. data: For (I) $\tau$ 4.80-5.10 (4 proton complex multiplet), $\tau$ 5.70-6.2 (2-proton complex multiplet), $\tau$ 7.82, 7.95, 8.04 (Three 3-proton singlets - OAC) and $\tau$ 8.80 (3-proton doublet $J=6.5$ Hz).

Compound (II) showed mutarotation in absolute ethanol In a typical experiment the initial specific rotation of $+11.8^\circ$ of the syrup (presumably (I) + (II) changed to $+7.36^\circ$ in 3h. and to $-13.25^\circ$ in seven days at room temperature.

Preparation of 2,3,4,6-tetra-O-acetyl $\beta$-D-glucopyranose (III)

Commercial $\beta$-D-glucose pentaacetate (Pfanstiehl Laboratories, Inc., Waukegan, Illinois) reacts with hydrobromic acid-acetic acid solution (Eastman Kodak Co. 30-32% hydorbromic acid-acetic acid) at room temperature. The tetra-O-acetyl glucopyranosyl bromide was isolated by the method of Jeremias et al.$^{102}$. It was then converted
into 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (III) by the action of one equivalent of water in dry acetone in the presence of freshly prepared silver carbonate. The method used was essentially the one employed by McClosky et al. The resulting product showed $[\alpha]_D^{25} + 0.4^\circ$ (c3, abs ethanol) [lit$^{103}$ $[\alpha]_D^{25} - 4.2^\circ$ (EtOH)].

N.m.r. data: $\tau 4.85-5.3$ (4-proton multiplet H-4,3,2 and H-1), $\tau 5.85$ (3 proton multiplet assigned to H-5,6,6'), $\tau 6.3$ (1-proton broadened singlet 1-OH) $\tau 7.85, 7.88, 7.90, 8.00$ (4-3 proton singlets, 4 acetyl groups).

Preparation of 2,3,4,6-tetra-O-acetyl-α-D-mannopyranose (IV).

The method used for the preparation of this compound was essentially that adapted by Bonner$^{59}$. D-mannose (Pfanstiehl Laboratories, Inc., Waukegan, Illinois) was converted into syrupy pentaacetates which were directly used for reaction with titanium tetrachloride to yield 2,3,4,6-tetra-O-acetyl-α-D-mannopyanosyl chloride in 80% yield $[\alpha]_D^{25} + 82^\circ$ (c2, CHCl$_3$) [lit$^{59}$ $[\alpha]_D + 89^\circ$ (CHCl$_3$)]. The acetylated mannopyanosyl chloride was then converted into 2,3,4,6-tetra-O-acetyl-β-D-mannopyranose in a yield of 70%; m.p. 122$^\circ$, $[\alpha]_D^{25} -14^\circ$ (c1.0, chloroform), [lit$^{59}$ m.p. 124$^\circ$, $[\alpha]_D^{25} -15.5^\circ$ (c2.0, chloroform).

N.m.r. data: $\tau 4.50-5.0$ (4 proton multiplet, H-2,3,4 and H-1), $\tau 5.80$ (3-proton multiplet, presumably
H-5, 6, 6'), T 6.2 (1-proton broadened singlet - OH), T 7.78
7.85, 7.90, 7.95 (4 three proton singlets, OAc).

Preparation of 2,3,4-tri-O-acetyl-α-L-fucopyranosyl Chloride
(XIII).

Dry L-fucose (Pfanstiehl Laboratories, Inc., Waukegan, Illinois) was converted into the crystalline 1,2,3,4-tetra-O-acetyl-L-fucopyranose by the method described by Leaback et al.\textsuperscript{53} with the difference that the tetraacetate readily crystallized on pouring the reaction mixture onto ice-water. Therefore, the chloroform extraction step was omitted. The crystalline tetraacetates were used directly for reaction with titanium tetrachloride after the method of Leaback et al.\textsuperscript{53}. The reaction mixture was refluxed over a steam bath with the exclusion of moisture. The overall yield of the recrystallized product varied from 60-65% in different preparations. The product XIII gave the melting point the specific rotation and the n.m.r. spectrum in accord with those reported by Leaback et al.\textsuperscript{53}.

2,3,4-Tri-O-acetyl-β-L-fucopyranose (V)

A solution of crystalline 2,3,4-tri-O-acetyl-α-L-fucopyranosyl chloride (XIII, 5g.) in anhydrous ether (50 ml.) was treated with freshly prepared dry silver carbonate (4g.) To the mixture was added 0.15 ml water with vigorous stirring over a 5-minute period at room
temperature. The mixture was stirred for 40 minutes in dim light, filtered, the residue washed with anhydrous ether (30 ml). The combined washings and the filtrate were free of chloride ion and were concentrated to a syrup under diminished pressure. This was dissolved in anhydrous ether (15 ml), whereupon crystallization proceeded rapidly. After one hour at 0°, the crystals were filtered, washed with a small volume of cold ether and dried under vacuum, giving 3.0 g. of isomer A, 2,3,4-tri-O-acetyl-ß-L-fucopyranose, m.p. 102°-103°, [α]D25 -5.19° → -77°, (in 8 days) (c 1, absolute ethanol).

Anal. Calcd., for C12H18O8: C, 49.63; H, 6.25. Found
C, 49.58; H, 6.16.

N.m.r. data: \(\tau \) 4.60-4.98 (3-proton multiplet, H-2,3,4), \(\tau \) 5.3 (1-proton broadened singlet, 1-OH), \(\tau \) 5.8-6.2 (2 proton multiplet, H-1,5), \(\tau \) 7.78, 7.85, 7.98 (9 protons 3 singlets, 3-OAc) and \(\tau \) 8.78 (3-proton doublet \(J=6.5\), 6-CH₃).

Acetylation of 2,3,4-tri-O-acetyl ß-L-fucopyranose (V)

A solution of V (0.37 g) in 1:1 pyridine-acetic anhydride mixture (10 ml) was allowed to stand at r.t. for 90 min. It was then poured onto ice-water (40 ml). Stirred for 45 min. at 0°, extracted with chloroform (3x25 ml). The chloroform extract was washed with dilute hydrochloric
acid, water, saturated sodium bicarbonate and then twice with water, dried over sodium sulfate. The solvent was removed under reduced pressure. There resulted an 85-95% yield of the crude syrup which crystallized from cold water, \([\alpha]_D^{25} = 34.16^\circ\) (c 1.19, CHCl₃) [lit\(^{53}\)[\(\alpha\)]_D^-39^\circ chloroform.]

2,3,4-Tri-0-acetyl-\(\alpha\)-L-fucopyranose (VI)

The mother liquors from the preparation of (V) described above were allowed to evaporate to a thick syrup in the presence of trace amounts of water. The syrup was washed with small amounts of petroleum ether and kept at r.t. for 2 days. The solid was dissolved in anhydrous ether by warming. On cooling and scratching, crystallization set in. After 4 h. at 0° the crystals (isomer B) were separated and dried over P₂O₅ m.p. 117° [\(\alpha\)]_D^-118° [c 1, absolute ethanol].

Isomer B did not show mutarotation in absolute ethanol however, in aqueous pyridine (5:1 pyridine-water) the specific rotation changed from an initial value of -117° to a final value of -83° in 2 hr. Anal. Calcd. for C\(_{12}\)H\(_{18}\)O₈: C, 49.63; H, 6.25. Found C, 49.55, H, 6.35.

N.m.r. data: \(\tau\) 4.40-4.80 (3-proton complex multiplet H-2,3,4), \(\tau\) 4.95 (1-proton doublet J=3.0 Hz, H1) \(\tau\) 5.58 (1-proton quartet J=7.0Hz, H-5), \(\tau\) 6.38 (1-proton broadened...
singlet, disappears on deuteration 1-OH), \( \tau 7.80, 7.90, 
8.0 \) (9-proton, 3 singlets, three acetyl groups) and \( \tau 8.85 
\) (3-proton doublet, \( J=6.5 \text{ Hz.} \) 6-CH\(_3\)).

\[ \alpha-L-\text{Rhamnopyranosyl di(cyclohexylammonium) phosphate (VII)} \]

A solution of o-phenylene phosphorochloridate
(0.77g, 4 mmoles) in anhydrous tetrahydrofuran (5 ml)
was added dropwise with stirring at room temperature with
occasional cooling to a solution of I (1.15g, 4 m moles) in
anhydrous tetrahydrofuran (10 ml) containing sym-collidine
(0.56 ml, 4 m moles).

Following the final addition, the mixture was stirred
for 25 min. The precipitated salts were filtered off and
washed with tetrahydrofuran (10 ml). The combined filtrate
and washings were treated with sym collidine (0.56,4 m moles)
and then with water (0.12 ml, 6.6 m moles). After 30 min.
the products were concentrated under reduced pressure to
a gum. Paper electrophoresis (system A) of an aliquot
showed a u.v.-absorbing major component (\( \sim 80\% \)) having
R picrate 0.76. It reacted slowly with alkaline silver
nitrate and is presumably the phosphatediester (XV). Bromine
(1 ml, 24 mmole) was added dropwise to a vigorously
stirred solution of the gum in aqueous triethylammonium
hydrogen carbonate buffer, pH 7.5 (150 ml, 0.2 M). After
10 min. at room temperature the orange precipitate was
filtered off and the filtrate extracted with benzene (2x50 ml). During this period the pH dropped from 7 to 2-3. The aqueous layer was readjusted to pH 8.0 with a freshly prepared solution of lithium hydroxide. The products were centrifuged to remove the precipitate, and the supernatant liquid was decanted, concentrated to about 40 ml, adjusted to pH 11.0 with lithium hydroxide and kept overnight at room temperature. The pH of the solution was lowered to 8.0 with Dowex-50-H^+, the solution was filtered and the filtrate treated with Norit A. The colorless filtrate was then treated with 1.0 g barium acetate, centrifuged and the supernatent material treated with ethanol until a faint turbidity persisted. After several hours at 4°, the precipitate was collected by centrifugation, washed with ether and dried in vacuo over phosphorus pentaoxide. The barium salt was dissolved in water, centrifuged to remove traces of insoluble matter and reprecipitated by the addition of three volumes of ethanol. This process was repeated three times; yield 0.65 g (45%). Anal. Calc, for C_{6}H_{9}O_{8}P Ba_{3}H_{2}O: C, 16.66; H, 3.47, P, 7.17. Found: C, 16.86; H, 3.61; P, 6.31.

The presence of traces of inorganic phosphate was shown by chromatography on Whatman No. 1 paper with solvent (b) and by colorimetric analysis. The barium salt was converted into the
di(cyclohexylammonium) salt by passing the aqueous solution through a pre-cooled column of Dowex-50W-x8, 200-400 mesh, in the hydrogen form. The effluent was collected in 100 ml cold water containing freshly distilled cyclohexylamine (1 ml). The column was washed with water (150 ml). The combined percolate was concentrated to 25 ml. The product (VII) crystallized on gradual addition of four volumes of acetone. After 2 days at 4°, the colorless crystalline salt was separated and dried over phosphorus pentaoxide at 56°; yield 0.59g. (33%), [α]D^25 -21.5°, (c1, water).

lit^52 [α]D^25 -21.5° (c1, water). No trace of inorganic phosphate or reducing sugar could be detected by colorimetric analysis^96,94.

8-L-Rhamnopyranosyl di(cyclohexylammonium) phosphate (VIII).

A solution of o-phenylene phosphorochloridate (0.77g, 4 mmoles) in anhydrous tetrahydrofuran (8 ml) was added dropwise with stirring at room temperature with occasional cooling to a solution of II [1.16g syrupy, 4 mmoles, [α]D^25 +20° (c1.1 absolute ethanol)] in anhydrous tetrahydrofuran (12 ml) containing anhydrous sym-collidine (0.56 ml, 4 mmoles).

The reaction mixture was processed by the procedure described earlier for the isolation of the α- L-
rhamnopyranosyl phosphate. The barium salt was purified through ethanol precipitation, converted into the di(cyclohexylammonium) salt by passing through Dowex-50W-X8, 200-400 mesh, in the cyclohexylammonium form. The colorless crystalline salt was separated and dried over phosphorous pentoxide at 56°, yield 0.45g. (25%). 

\[ [\alpha]_D^{25} + 11.9^\circ \text{ (c1, water).} \]

Anal. Calc. for C_{18}H_{39}N_{2}O_{8}P:

- C, 48.87; H, 8.82; N, 6.33; P, 7.01. Found: C, 48.68; H, 8.80
- N, 6.04; P, 6.89.

The salt VIII was non-reducing. Hydrolysis in 0.1 N hydrochloric acid at 100°C released the reducing power and inorganic phosphate in equimolar ratio. The released sugar was identified as L-rhamnose in chromatographic solvents (a) and (c).

Electrophoresis in system (C) at pH 6.5 showed a single spot having \( R_{picrate} = 1.59 \) thus indicating that VIII was anomerically pure.

**Reaction of \( \alpha-L \)-rhamnopyranosyl phosphate (VIII) with N,N'-dicyclohexylcarbodiimide.**

The barium salt of VIII (10 mg) was converted into the pyridinium salt by treating with Dowex-50W-X8, 200-400 mesh, in the pyridinium form, at room temperature for 6 h. The resin was removed by filtration and the filtrate was freeze-dried. The residue was dissolved in 2.4 mL of
pyridine containing 0.4 ml water. After adding 40 mg. of N,N'-dicyclohexylcarbodiimide, the homogenous reaction mixture was kept at 37° for 16 h. The urea derivative crystallized out on adding water (5 ml) and was filtered off. The filtrate was extracted twice (2x10 ml) with ether. The aqueous layer was concentrated to 0.2 ml. Paper chromatography in solvent (b) showed, in addition to traces of inorganic phosphate (R_f 0.08), complete conversion of VIII (R_f 0.12) into the product having R_f 0.42. This corresponds to the hexopyranosyl 1,2-cyclic phosphate.

Reaction of α-L-rhamnopyranosyl phosphate (VII) with N,N'-dicyclohexylcarbodiimide.

The above reaction between the glycosyl phosphate and N,N'-dicyclohexylcarbodiimide was repeated using VII. However, the latter remained unaffected and was recovered unchanged (R_f 0.12 solvent (b)).

β-D-Glucopyranosyl di(cyclohexylammonium) phosphate (IX)

A solution of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose III (1.39 g, 4 mmoles) in anhydrous tetrahydrofuran (10 ml) containing sym. collidine (0.56 ml, 4 mmoles) was treated with a solution of o-phenylene phosphorochloridate (0.77 g 4 mmoles) in dry tetrahydrofuran by the procedure used for the preparation of VII. The gum obtained after aqueous
treatment and evaporation was found to contain a major component having $R_p$ picrate 0.69 (electrophoresis system A) and was oxidized with bromine in 0.2M triethylammonium hydrogen carbonate. Subsequent deacetylation with aqueous barium hydroxide afforded the barium salt of the phosphate monester (IX) which was precipitated with four volumes of ethanol. Electrophoresis in system A showed that the barium salt contained trace amounts of a nonphosphorylated product having $R_p$ picrate 0.63 as indicated by silver nitrate spray reagent. The barium salt was converted into the di(cyclohexylammonium) salt by the procedure described under preparation of VIII. After repeated evaporation with ethanol, the product crystallized from absolute ethanol and dried over phosphorus pentaoxide at r.t., yield, 0.35g (17%), $[\alpha]_D^{25} +4.1^\circ$ (c 1.14, H$_2$O) [lit-$[\alpha]_D^{25} +5.4^\circ$ (H$_2$O)]. Anal. Calc. for C$_{18}$H$_{39}$N$_2$O$_9$P.$3$H$_2$O: C, 43.68; H, 8.69; N, 5.66; P, 6.27. Found: C, 43.28; H, 8.91; N, 5.53; P, 6.08.

Paper chromatography in solvent (d) for 10 h. at room temperature revealed that IX moved 33.2 cm from the origin as opposed to $\alpha$-$D$-glucopyranosyl phosphate which moved 30 cm from the origin.

Traces of either inorganic phosphate or reducing sugar were absent in IX by colorimetric assays. Hydrolysis in 0.1N hydrochloric acid at 100$^\circ$C produced glucose, and
inorganic phosphate in equimolar ratio.

\[ \beta-D\text{-Mannopyranosyl di(cyclohexylammonium) phosphate (X)} \]

A solution 2,3,4,6-tetra-\(\text{O}\text{-acetyl}\)-\(\beta\)-D-mannopyranose, IV (1.39 g, 4 mmoles) in anhydrous tetrahydrofuran (8 ml) containing sym-collidine (0.56 ml, 4 mmoles) was treated with a solution of 0-phenylene phosphorochloridate (0.77 g 4 mmoles) in dry tetrahydrofuran (6 ml) by the procedure used for the preparation of VII. The gum obtained after aqueous treatment and evaporation was found to contain a major component having \(R_{\text{picrate}} \, 0.78\) (electrophoresis system A) and was oxidized with bromine by the procedure used for preparation of VII. Following deacetylation with aqueous lithium hydroxide the barium salt was precipitated with ethanol, yield 0.63 g (43\%) \([\alpha]^{25}_D\, -6^\circ\) (c 1.1, water).

The barium salt was converted into the di(cyclohexylammonium) salt as previously described for the preparation of VIII. The product was crystallized from acetone and dried over phosphorus pentaoxide in vacuo at 56\(^\circ\), yield 0.35 g (17\%) m.p. 180\(^\circ\), \([\alpha]^{25}_D\, -6.5^\circ\) (c 1, water).

Anal. Calc. for \(C_{18}H_{39}N_2O_9P\cdot H_2O\): C, 45.33; H, 8.60; N, 5.88; P 651. Found: C, 45.39; H, 8.60; N, 5.95; P, 6.46.

**Reaction of \(\beta\)-D-mannopyranosyl phosphate (X) with \(N,N'\)-dicyclohexylcarbodiimide.**
The pyridinium salt of X (10 mg) was dissolved in 2.5 ml of pyridine containing 0.4 ml water. After adding 40 mg of N,N'-dicyclohexylcarbodiimide, the homogenous reaction mixture was obtained by keeping at 37° for 20 min. The reaction mixture was then kept at r.t. for a further period of 3 h. and then processed according to the procedure described on page 58. Subsequent chromatography in solvent showed complete conversion of X (Rf 0.1) into major and minor products having Rf 0.48 and 0.82 respectively. These corresponded to the hexopyranosyl 1,2-cyclic phosphate and the N-phosphorylurea.

\[ \beta-L\text{-Fucopyranosyl di(cyclohexylammonium) phosphate (XI)} \]

A solution of o-phenylene phosphorochloridate (0.77g, 4 mmoles) in anhydrous tetrahydrofuran (5 ml) was added dropwise with stirring at room temperature with occasional cooling to a solution of V (1.15g, 4 mmoles) in anhydrous tetrahydrofuran (10 ml) containing sym-collidine (0.56 ml, 4 mmoles). Following the procedure used for the preparation of VII, a gum was obtained after aqueous treatment and evaporation. Electrophoresis (system A) of an aliquot showed a u.v. absorbing, major component (\( \sim 80\% \)) having R picrate 0.76. Following the procedure described under the preparation of VII, an aqueous solution (25 ml) of the barium salt of the glycosyl phosphate was obtained
(R-picrate 1.0, electrophoresis system A). Upon adding eight volumes of ethanol (200 ml), no turbidity was observed. The solvent was removed under reduced pressure and the concentrated solution was dissolved in absolute ethanol (50 ml). Cold acetone (100 ml) was then gradually added until a permanent turbidity was observed. The solution was allowed to stand at 4° for 2 days. The white ppt. was collected by centrifugation, washed with ether and dried over P₂O₅ in vacuo at r.t. The barium salt of the fucopyranosyl phosphate was hygroscopic. It was converted into the di(cyclohexylammonium) salt by the procedure described before. The salt was crystallized from acetone and dried over phosphorus pentaoxide under vacuo at 56° yield 0.20g. (11%), [α]D25 -20.5° (c1, water). Anal. for C₁₈H₃₉N₂O₈P: C, 48.87; H, 8.82; N, 6.33; P, 7.01. Found C, 49.09; H, 8.91; N, 6.30; P, 6.75.

α-L-Fucopyranosyl di(cyclohexylammonium) phosphate (XII)

A solution of o-phenylene phosphorochloridate (0.77g, 4 mmoles) in anhydrous tetrahydrofuran (5 ml) was added dropwise with stirring at room temperature with occasional cooling to a solution of VI (1.15g, 4 mmoles) in anhydrous tetrahydrofuran (10 ml) containing sym-collidine (0.56 ml, 4 mmoles). Following the procedure used for the preparation of VII, a gum was obtained after aqueous treatment and evaporation. Electrophoresis (system A) of an aliquot
showed a u.v. absorbing, major component (~80%) having R picrate 0.76. Following the procedure described under the preparation of VII, an aqueous solution (25 ml) of the barium salt of the glycosyl phosphate was obtained.

(R picrate 1.0, electrophoresis system A). Upon adding four volumes of ethanol, a permanent turbidity was obtained. The solution was left at 4°C for 2 days. The salts were separated by centrifugation, washed with cold acetone and then dried over phosphorus pentoxide. Yield 0.60 g. (40%)

The barium salt was converted into the di(cyclohexyl-hexylammonium) salt yield 0.39 g. (21%). [α]D^25 -77° (c 1.05 water).

Both XI and XII were found to cyclize on treatment with dicyclohexylcarbodiimide. Each produced L-fucose and inorganic phosphate in equimolar ratio on heating in 0.1N hydrochloric acid at 100°C for 15 min.
SUMMARY

A new method of general applicability for the synthesis of glycosyl phosphates is described. o-Phenylene phosphoro-
chloridate is shown to be an effective reagent for the phos-
phorylation of the hemiacetal hydroxyl group in a carbohy-
drate derivative in a suitable solvent such as tetrahydro-
furan in the presence of stochiometric amounts of sym
collidine. The intermediate phosphatediesters were easily
oxidized with bromine at neutral pH in triethylammonium
hydrogen carbonate buffer. Subsequent removal of the pro-
tecting groups yielded pure glycosyl phosphates which were
obtained as crystalline di(cyclohexylammonium) salts. The
rate of phosphorylation in this case is at least one hundred
times faster than the rate of anomerization of the starting
hemiacetal. Phosphorylation could not be induced either
between 2,3,4 tri-O-acetyl-α-L-rhamnopyranose (I) and
phosphoryloxychloride or between I and Hata's reagent, 2
chloromethyl-4-nitrophenyl phosphorochloridate.

Upon solvolysis in wet ether in the presence of silver
carbonate 2,3,4 tri-O-acetyl-α-L-fucopyranosyl chloride
(XIII) gave the 2,3,4 tri-O-acetyl-β-L-fucopyranose (V)
which slowly anomerized to 2,3,4-tri-O-acetyl-α-L-fuco-
pyranose (VI) on keeping in aqueous ethanol or ether. Both V
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and VI were characterized by n.m.r. spectroscopy, elemental analysis and the specific rotation data.

MacDonald's fusion procedure failed to yield $\beta$-D-mannopyranosyl phosphate (X), $\beta$-L-rhamnopyranosyl phosphate (VIII) and $\beta$-L-fucopyranosyl phosphate (XII). X, VIII and XII were, however, synthesized by the new procedure and characterized by the elemental analysis, n.m.r. data, specificity of the reaction with N,N'-dicyclohexylcarbodiimide periodate consumption, and the specific rotation data. A good agreement was attained between the predicted and observed optical rotation for the newly synthesized glycosyl phosphates. In addition $\alpha$-L-rhamnopyranosyl, $\alpha$-L-fucopyranosyl and $\beta$-D-glucopyranosyl phosphates were made by the new procedure.

Anomeric pairs of D-glucopyranosyl, D-mannopyranosyl, D-galactopyranosyl, L-fucopyranosyl and L-rhamnopyranosyl phosphates were separated by electrophoresis in sodium maleate buffer (pH 6.2 or pH 6.5).
Fig. 1 A 60 MHz n.m.r. spectrum of 2,3,4 tri-O-acetyl α-L-rhamnopyranose (I) in CDCl$_3$. 
Fig. 2 A 60 MHz n.m.r. spectrum of 2,3,4 tri-O-acetyl β-L-rhamnopyranose (II) in CDCl₃
Fig. 3 A 60 MHz n.m.r. spectrum of 2,3,4 tri-O-acetyl β-L-fucopyranose (V) in CDCl₃
Fig. 4 A 60 MHz n.m.r. spectrum of 2,3,4 tri-O-acetyl α-L-fucopyranose (VI) in CDCl₃
Fig. 5 A 60 MHz n.m.r. spectrum of $\alpha$-L-rhamnopyranosyl dipotassium phosphate (VII) in $D_2O$. 

The n.m.r. spectrum shows the characteristic peaks of the compound at different chemical shifts, with the HOD signal at 5.0 ppm.
Fig. 6 A 60 MHz n.m.r. spectrum of $\beta$-L-rhamnopyranosyl dipotassium phosphate VIII in $D_2Q_2$. 
Fig. 7 A 60 MHz n.m.r. spectrum of β-D-mannopyranosyl dilithium phosphate (X) in D$_2$O
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