Chemical Synthesis of β-L-Fucopyranosyl Phosphate and β-L-Rhamnopyranosyl Phosphate†

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ABSTRACT: Solvolysis in wet ether in the presence of silver carbonate converts 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl chloride to 2,3,4-tri-*O*-acetyl-β-L-fucopyranose which slowly anomerizes to 2,3,4-tri-*O*-acetyl-α-L-fucopyranose in aqueous ethanol or ether. *o*-Phenylene phosphorochloridate reacts rapidly with 2,3,4-tri-*O*-acetyl-β-L-fucopyranose and with 2,3,4-tri-*O*-acetyl-β-L-rhamnopyranose to yield the corresponding phosphodiesters which, upon oxidation with bromine and treatment with base, give the title compounds isolated as their dicyclohexylammonium salts. The α anomers of these sugar phosphates were prepared by the reaction of *o*-phenylene phosphorochloridate with the corresponding α triacetates. β-D-Glucopyranosyl phosphate was also prepared by this method. Paper electrophoresis at pH 6.2–6.5 appears to be a general method for the separation of the anomeric pairs of glycosyl phosphates.

The β-glycosyl nucleotides, β-L-rhamnopyranosyl (thymidine 5′-pyrophosphate), β-L-rhamnopyranosyl (uridine 5′-pyrophosphate), and β-L-fucopyranosyl (guanosine 5′-pyrophosphate) are widely occurring cellular constituents involved in the glycosylation of the endotoxins of *Salmonella*, blood group substances and other glycoproteins, glycosides, and polysaccharides (Osborn, 1969; Spiro, 1970; Nikaido and Hassid, 1971; Heath, 1971). Small quantities of these compounds have been made enzymatically, but chemical synthesis has not been achieved to date because of the lack in each case of the key intermediate, the β-glycosyl phosphate. We report here the syntheses of both β-L-fucopyranosyl phosphate and β-L-rhamnopyranosyl phosphate. The general approach involving phosphorylation of the hemiacetal hydroxyl group by *o*-phenylene phosphorochloridate has already been described in our paper on the synthesis of β-D-mannopyranosyl phosphate (Prihar and Behrman, 1972). This new method is in principle suitable for the synthesis of any glycosyl phosphate and makes available compounds unobtainable by the older procedures (MacDonald, 1972).

Results and Discussion

Synthesis of Fucose and Rhamnose 2,3,4-Triacetates. The starting material for the synthesis of β-L-fucopyranosyl phosphate was 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl chloride (Leaback *et al.*, 1969). Reaction of this compound with one molar equivalent of water in ether in the presence of silver carbonate yielded a syrupy mixture of fucose triacetates. Fractional crystallization from anhydrous ether yielded isomer A, mp 102°, $[\alpha]_D^{25}$ −5°, and isomer B, mp 117°, $[\alpha]_D^{25}$ −118°. Both isomers gave analytical data in accord with the formula $C_{12}H_{18}O_8$. Isomer A mutarotated in aqueous ethanol to a final value of −119° corresponding to isomer B. Isomer A was converted by acetylation into the known compound, 1,2,3,4-tetra-*O*-acetyl-β-L-fucopyranose (Westphal and Feier, 1956). These data and the nmr spectra (see later) are thus consistent with the assignments of the structures 2,3,4-tri-*O*-acetyl-β-L-fucopyranose to isomer A and 2,3,4-tri-*O*-acetyl-α-L-fucopyranose to isomer B.

Fischer *et al.* (1920) had already described the preparation of the anomeric L-rhamnose triacetates. In our hands, however, 2,3,4-tri-*O*-acetyl-β-L-rhamnopyranose failed to crystallize and was used as a syrup immediately after its preparation. The α anomer was easily prepared as a crystalline material by anomerization of the β isomer.

Rates of Anomerization. It is critical for this synthetic approach that the rate of phosphorylation be rapid as compared with the rate of anomerization. The half-time for anomerization of 2,3,4-tri-*O*-acetyl-β-L-fucopyranose at 25° is about 45 hr in absolute ethanol and about 55 hr in tetrahydrofuran containing 10% *sym*-collidine. The corresponding data for 2,3,4-tri-*O*-acetyl-β-L-rhamnopyranose are 16 hr in ethanol and an estimate of 20 hr in the phosphorylation solvent (tetrahydrofuran + collidine). We have not measured the rate of phosphorylation but since the reactions are complete within 30 min, the minimum rate of phosphorylation is of the order of 100 times the rate of anomerization.

The Phosphorylation Procedure. Khwaja and Reese have described the procedures for the phosphorylation of alcohols using *o*-phenylene phosphorochloridate (Khwaja and Reese, 1966; Khwaja *et al.*, 1970; Khwaja and Reese, 1971). Phosphorylation of the hemiacetal group was carried out by reaction with *o*-phenylene phosphorochloridate in anhydrous tetrahydrofuran containing one molar equivalent of *sym*-collidine as previously described (Prihar and Behrman, 1972). Reactions were complete within 30 min as judged by the absence of the hemiacetal upon subsequent electrophoresis. If 2,6-di-*tert*-butylpyridine was used in place of collidine, no reaction was detected. In the presence of triethylamine, the phosphodiester was converted back to the starting hemiacetal. The intermediate phosphodiesters were not isolated but instead were oxidized directly with bromine and deacetylated to yield the desired glycosyl phosphates. These were purified by precipitation as their barium salts and finally crystallized as their dicyclohexylammonium derivatives. Scheme I outlines the synthetic scheme for the case of β-L-fucopyranosyl phosphate.

Nuclear Magnetic Resonance (Nmr) Spectra. The nmr

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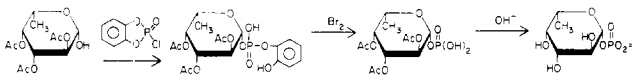
TABLE I: Comparison of the Molecular Rotations of the Glycosyl Phosphates with the Related Methyl Aldopyranosides.

Aldose Component	Obsd Mol Rotation		Probable Conformation	C1 Substituent
	Phosphate Ester	Methyl Pyranoside		
α-D-Xylopyranose	+24,800[a]	+25,300[a]	C1(D)	Axial
β-D-Xylopyranose	+300[a]	−10,800[a]	C1(D)	Equatorial
β-L-Arabinopyranose	+39,000[a]	+40,000[a]	C1(L)	Axial
α-L-Arabinopyranose	+13,200[a]	+2,900[a]	C1(L)	Equatorial
α-L-Rhamnopyranose	−9,200[b]	−11,100[c]	1C(L)	Axial
β-L-Rhamnopyranose	+5,300[b]	+17,000[c]	1C(L)	Equatorial
α-L-Fucopyranose	−34,400[d]	−35,600[d]	1C(L)	Axial
β-L-Fucopyranose	−9,000[b]	+2,530[d]	1C(L)	Equatorial
α-D-Mannopyranose	+13,200[e]	+15,400[f]	C1(D)	Axial
β-D-Mannopyranose	−3,200[b]	−13,500[f]	C1(D)	Equatorial
α-D-Glucopyranose	+29,300[g]	+30,900[a]	C1(D)	Axial
β-D-Glucopyranose	+3,800[g]	−6,700[a]	C1(D)	Equatorial
α-D-Galactopyranose	+36,000[a]	+38,000[a]	C1(D)	Axial
β-D-Galactopyranose	+10,000[a]	0[a]	C1(D)	Equatorial

[a] Putman and Hassid (1957). [b] This paper. [c] Lemieux and Martin (1970), from the rotation of the enantiomer. [d] Leaback *et al.* (1969). [e] Hill and Ballou (1966). [f] Bates (1942). [g] MacDonald (1966).

SCHEME I



spectra were taken in D_2O with Me_4Si as external standard. α-L-Rhamnopyranosyl phosphate showed splitting of the C-1 hydrogen signal by H-2 and by phosphorus to give a quartet at τ 4.30 with $J_{1,2}$ = 1.5 Hz and $J_{H\text{-}1,P}$ = 10 Hz. These values are in agreement with those reported by Chatterjee and MacDonald (1968) and indicate equatorial–equatorial coupling of the protons on C-1 and C-2. β-L-Rhamnopyranosyl phosphate gave a quartet for H-1 at τ 4.48 with $J_{1,2}$ = 1.0 Hz and $J_{H\text{-}1,P}$ = 10 Hz, indicating axial–equatorial coupling of the C-1 and C-2 protons. These values are consistent with those reported by Horton and Turner (1965) for the β-D-mannose pentaacetates and suggest that both of the rhamnose phosphates adopt the 1C(L) conformation in D_2O. The methyl group protons in the spectrum of the α anomer appeared as a clean doublet at τ 8.34 with $J_{5,6}$ = 6.5 Hz, whereas in the β anomer a complex multiplet at τ 8.0–8.35 was observed. We attribute this to a similar chemical shift of the H-4 and H-5 protons (Anet, 1961).

The nmr spectra of the fucose phosphates were in agreement with those already reported by Leaback *et al.* (1969) for the chemically synthesized α anomer and the biosynthetic β anomer. The spectra of the fucose 2,3,4-triacetates in chloroform showed several features obscured in the spectra of the phosphates by the HDO signal. The β-triacetate showed the H-1 resonance at τ 5.80 with $J_{1,2}$ = 7.0 Hz (axial–axial). The α-triacetate gave a corresponding resonance at τ 4.96 with $J_{1,2}$ = 3.6 Hz (equatorial–axial). The H-5 quartet is centered at τ 6.0 for the β anomer, but at τ 5.58 in the case of the α anomer.

Molecular Rotations. We have previously shown (Prihar and Behrman, 1972) that our observed value for the rotation of β-D-mannopyranosyl phosphate corresponds well with the value expected on the basis of a 2*A* value for the phosphate group in the gluco series (25,400) corrected by 9000 for the interaction of the 1 and 2 substituents. This yields a 2*A* value of 16,400 for the manno series. $[M]_D$ for the dicyclohexylammonium salt of α-D-mannopyranosyl phosphate is +13,200. The predicted value for the β anomer is thus (+13,200 − 16,400) = −3100. We observe −3200. We can use this value together with the expected change (*ca.* +3400) for the conversion of the 6-hydroxymethyl group to a methyl group from the data collected by Lemieux and Martin (1970) to predict a value of about +6500 for β-L-rhamnopyranosyl phosphate. We observe +5300 which is in fair agreement if one takes into account possible variation based on the nature of the 1 substituent. It should be pointed out that Chatterjee and MacDonald (1968) calculated the expected rotation for this compound without taking into account the contribution due to the 1,2-gauche interaction.

The expected rotation for β-L-fucopyranosyl phosphate has also been miscalculated. Leaback *et al.* (1969) predicted $[M]_D$ +2500 expecting the rotation to be the same as that of methyl β-L-fucopyranoside. We have calculated the rotation of β-L-fucopyranosyl phosphate on the basis of a 2*A* value of 26,000 for the phosphate group in the D-galacto series (Putman and Hassid, 1957). Since α-L-fucopyranosyl phosphate has $[M]_D$ −34,400, the β anomer should have $[M]_D$ (−34,400 + 26,000) = −8400. We observe −9000. Table I gives the molecular rotation data for the newly synthesized glycosyl phosphates and compares these with literature data for the corresponding methyl pyranosides. The data for a number of previously known compounds are presented as well. It can be seen that the molecular rotation is dependent upon the nature of the C1 substituent only when the substituent is equatorial.

Reaction of Glycosyl Phosphates with N,N'-Dicyclohexylcarbodiimide (DCC). Khorana *et al.* (1957) have shown that DCC-promoted cyclization of a glycosyl phosphate is possible under specified conditions when the relationship of the phosphate group to the adjacent hydroxyl is equatorial–equatorial (trans) or axial–equatorial (cis) but not when the relationship is axial–axial (trans). This latter situation is present in the α anomers of mannose and rhamnose 1-phosphate in the C1(D)

and 1C(L) conformations, respectively. That these compounds failed to cyclize and that their β anomers underwent complete reaction to give a mixture of the cyclic phosphates and *N*-phosphorylureas served as confirmatory evidence of the conformation of the anomers. Both the α and β anomers of fucose 1-phosphate in the 1C(L) conformation would thus be expected to undergo cyclization with DCC and both were observed to do so.

Periodate Oxidations. The oxidation of the newly synthesized glycosyl phosphates with periodate provided further support for the structures assigned to these compounds. β-L-Rhamnopyranosyl phosphate and β-L-fucopyranosyl phosphate each consumed 2 mol of sodium metaperiodate/mol of sugar phosphate. One mole of formic acid was formed in each case.

Separation of the Anomeric Glycosyl Phosphates by Paper Electrophoresis. No general method has thus far been published for the separation of the anomeric pairs of glycosyl phosphates. We have found a simple and apparently general method which consists of paper electrophoresis in the vicinity of the second pK_a of the glycosyl phosphate. Our results are tabulated in Table II. Electrophoresis at pH 3.6 or 7.2 did not result in separation. The anomeric pairs must differ slightly in their pK_a values although these values have not been determined. These differences in acidities lead to differences in the fraction ionized which are maximized in the region of the pK_a. This is most easily seen in the expression (Albert and Serjeant, 1971)

$$\% \text{ ionized} = \frac{100}{1 + \text{antilog}\,(pK_a - pH)}$$

We note that in all cases the β anomer migrated more rapidly than the α anomer implying a higher acidity for the β anomer.

Acid-Catalyzed Hydrolyses. The rates of hydrolysis were measured at 28° in 0.25 N HCl. The pseudo-first-order rate constants were (±5%): 2.2×10^{-2} min^{-1} (α-L-fucopyranosyl phosphate); 2.5×10^{-2} min^{-1} (β-L-fucopyranosyl phosphate); 3.5×10^{-3} (α-L-rhamnopyranosyl phosphate); 10.3×10^{-3} min^{-1} (β-L-rhamnopyranosyl phosphate). These may be compared with data for other glycosyl phosphates (MacDonald, 1972) and the discussion of the factors influencing the rates of hydrolysis of glycopyranosides (Capon, 1969; Tables XX–XXII).

Experimental Section

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

Hexoses and 6-deoxyhexoses were determined by the phenolsulfuric (Hodge and Hofreiter, 1962) and cysteine-sulfuric acid (Dische and Shettles, 1948) methods, respectively. Reducing sugars, total phosphate, and inorganic phosphate, were estimated by the methods of Park and Johnson (1949), Ames and Dubin (1960), and Lowry and Lopez (1946), respectively. We note that cyclohexylamine interferes in the latter determination. Analysis of sugars and sugar phosphates was carried out in the following solvent systems by descending paper chromatography: (a) ethanol–ammonium acetate (v/v 7:3) (1 M, pH 7.0); (b) isopropyl alcohol–ammonium hydroxide (sp gr 0.88)–water (7:1:2); (c) *n*-butyl alcohol–acetic acid–water (5:2:3); (d) methanol–formic acid–water (80:15:5). Sugars and sugar phosphates on paper chromatograms were revealed by the silver nitrate dip procedure (Trevelyan *et al.*, 1950) and the Hanes–Isherwood spray reagent (Bandurski and Axelrod, 1951).

TABLE II: Relative Paper Electrophoretic Mobilities of the Glycosyl Phosphates in 0.05 M Sodium Maleate Buffer.[a]

Aldose Component	Relative Mobility $R_{picrate}$ pH 6.5	pH 6.2
α-D-Glucopyranose[b]	1.49	1.46
β-D-Glucopyranose[c]	1.63	1.57
α-D-Mannopyranose[b]	1.45	1.31
β-D-Mannopyranose[d]	1.55	1.36
α-D-Galactopyranose[b]	1.45	1.29
β-D-Galactopyranose[c]	1.47	1.34
α-L-Rhamnopyranose[e]	1.45	1.16
β-L-Rhamnopyranose[e]	1.59	1.29
α-L-Fucopyranose[e]	1.45	1.37
β-L-Fucopyranose[e]	1.54	1.49

[a] Electrophoresis was carried out in an apparatus like that described by Crestfield and Allen (1955) at 25 V/cm. The relative mobilities, $R_{picrate}$, are given by cm (phosphate)/cm (picrate). The samples were treated with Dowex-50, H^+ prior to spotting. [b] Sigma Chemical Co. [c] Synthesized by G. A. Barber by the procedure of MacDonald (1968). [d] Prihar and Behrman (1972). [e] This paper.

Paper electrophoresis was conducted on Schleicher and Schuell No. 589 orange ribbon paper strips (4 × 22.5 in.) in the following buffers: (A) 0.05 M ammonium formate (pH 3.6); (B) 0.05 M triethylammonium hydrogen carbonate (pH 7.2); and (C) 0.05 M sodium maleate (pH 6.2 and 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; dioxane and tetrahydrofuran were purified by distillation from lithium aluminum hydride. The chloroform used was AR grade and contained approximately 0.75% of ethanol. Before use it was shaken with anhydrous calcium chloride and filtered. Anhydrous ether was obtained by distillation and storage over sodium.

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

The nmr spectra of the acetylated glycopyranoses were determined at 60 MHz with a Varian T-60 nmr spectrometer at 35° on 10% solutions in chloroform-*d* containing 2% of tetramethylsilane (τ 10.00) as internal indicator. The nmr spectra of glycopyranosyl phosphates (lithium or potassium salts, lyophilized 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 midpoints 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 mμ (Rammler and Rabinowitz, 1962). We emphasize the fact pointed out by these authors that cyclohexyl-

amine interferes with this determination. The determination of formic acid produced was carried out by low temperature vacuum distillation and titration (Grant, 1946).

o-Phenylene phosphorochloridate was purchased from the Aldrich Chemical Co., Inc., Milwaukee, Wis.

2,3,4-Tri-O-acetyl-α-L-rhamnopyranose and 2,3,4-Tri-O-acetyl-β-L-rhamnopyranose. Mixed L-rhamnose tetraacetates were obtained as a syrup by the action of acetic anhydride and pyridine on α-L-rhamnose (Chatterjee and MacDonald, 1968). The syrup was dissolved in hydrobromic acid–acetic acid solution (Eastman Kodak Co., 30–32% hydrobromic acid–acetic acid) and the resulting mixture was maintained at room temperature for 2.5 hr. The resulting product, 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide, was isolated by the procedure of Haworth *et al.* (1929) and gave physical constants in agreement with the reported values. The following procedure was adapted from Fischer *et al.* (1920). A solution of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (5 g) in dry acetone (50 ml) was cooled to 0° and treated with water (0.25 ml) and freshly prepared dry silver carbonate (4 g). 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 (25 ml). The combined washings and the filtrate were concentrated to a syrup: yield 3.2 g (80%); $[\alpha]_D^{25}$ +21° (*c* 1, absolute ethanol, 5 min after dissolution.) This corresponds to a mixture containing 85% of the β anomer.

The syrup failed to crystallize from anhydrous ether. On keeping the ethereal solution at 0° overnight a solid was obtained which gave an $[\alpha]_D$ +3°. This was presumably a mixture of the anomers. When crystallized once from ether it afforded the pure α anomer, $[\alpha]_D$ −18° (*c* 1, absolute ethanol) [lit. values ($[\alpha]_D$) for α and β anomers are −19 and +28°, respectively] (Fischer *et al.*, 1920): nmr for the α anomer, τ 4.80–5.10 (four-proton complex multiplet), 5.70–6.2 (two-proton complex multiplet), 7.82, 7.95, and 8.04 (three three-proton singlets, OAc), and 8.80 (three-proton doublet, J = 6.5 Hz).

Syrups containing predominantly the β anomer showed mutarotation in absolute ethanol. In a typical experiment the initial specific rotation of +11.8° changed to +7.36° in 3 hr and to −13.25° in 7 days at room temperature.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranose. Commercial β-D-glucose pentaacetate (Pfanstiehl Laboratories, Inc., Waukegan Ill.) was allowed to react with hydrobromic acid–acetic acid solution (Eastman Kodak Co., 30–32% hydrobromic acid–acetic acid) at room temperature. The tetra-*O*-acetylglucopyranosyl bromide was isolated by the method of Jeremias *et al.* (1948). It was then converted into 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose by the action of 1 equiv of water in dry acetone in the presence of freshly prepared silver carbonate. (McCloskey and Coleman, 1955). The resulting product showed $[\alpha]_D^{25}$ +0.4° (*c* 3, absolute ethanol) [lit. $[\alpha]_D^{25}$ −4.2° (EtOH)] (Hendricks *et al.*, 1936); nmr data: τ 4.85–5.3 (four-proton multiplet H-4, -3, -2, and -1), 5.85 (three-proton multiplet assigned to H-5, -6, -6′), 6.3 (one-proton broadened singlet, 1-OH) 7.85, 7.88, 7.90, and 8.00 (four three-proton singlets, four acetyl groups).

2,3,4-Tri-O-acetyl-α-L-fucopyranosyl Chloride. Dry L-fucose (Pfanstiehl Laboratories, Inc., Waukegan, Ill.) was converted into the crystalline 1,2,3,4-tetra-*O*-acetyl-L-fucopyranose by the method described by Leaback *et al.* (1969) with the difference that the tetraacetate readily crystallized on pouring the reaction mixture into 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.* (1969). The reaction mixture was refluxed over a steam bath with the exclusion of moisture. The overall yield of the recrystallized product varied from 60 to 65% in different preparations. The product gave the melting point, the specific rotation, and the nmr spectrum in accord with those reported by Leaback *et al.* (1969).

2,3,4-Tri-O-acetyl-β-L-fucopyranose. A solution of crystalline 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl chloride (5 g) in anhydrous ether (50 ml) was treated with freshly prepared dry silver carbonate (4 g). To the mixture was added 0.15 ml of water with vigorous stirring over a 5-min period at room temperature. The mixture was stirred for 40 min in dim light, filtered, and 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 1 hr 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-β-L-fucopyranose, mp 102–103°, $[\alpha]_D^{25}$ −5.19° → −77° (in 8 days) (*c* 1, absolute ethanol).

Anal. Calcd for $C_{12}H_{18}O_8$: C, 49.63; H, 6.25. Found: C, 49.58; H, 6.16.

Nmr data for isomer A are: τ 4.60–4.98 (three-proton multiplet, H-2, -3, -4), 5.3 (one-proton broadened singlet, 1-OH), 5.8–6.2 (two-proton multiplet, H-1, -5), 7.78, 7.85, 7.98 (nine protons, three singlets, 3-OAc) and 8.78 (three-proton doublet, J = 6.5 Hz, 6-CH_3).

Acetylation of 2,3,4-Tri-O-acetyl-β-L-fucopyranose. A solution of isomer A (0.37 g) in 1:1 pyridine–acetic anhydride mixture (10 ml) was allowed to stand at room temperature for 90 min. It was then poured onto ice–water (40 ml), stirred for 45 min at 0°, and extracted with chloroform (3 × 25 ml). The chloroform extract was washed with dilute hydrochloric acid, water, saturated sodium bicarbonate, and then twice with water, and 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° (*c* 1.19, $CHCl_3$) [lit. $[\alpha]_D^{25}$ −39° chloroform] (Westphal and Feier, 1956).

2,3,4-Tri-O-acetyl-α-L-fucopyranose. The mother liquors from the preparation of isomer A 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 room temperature for 2 days after which it crystallized. The solid was dissolved in anhydrous ether by warming. On cooling and scratching, crystallization set in. After 4 hr at 0° the crystals (isomer B) were separated and dried over P_2O_5; mp 117°; $[\alpha]_D^{25}$ −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 $C_{12}H_{18}O_8$: C, 49.63; H, 6.25. Found: C, 49.55; H, 6.35.

Nmr data for isomer B are: τ 4.40–4.80 (three-proton complex multiplet, H-2, -3, -4), 4.95 (one-proton doublet, J = 3.0 Hz, H-1), 5.58 (one-proton quartet, J = 7.0 Hz, H-5), 6.38 (one-proton broadened singlet, disappears on deuteration, 1-OH), 7.80, 7.90, 8.0 (nine protons, three singlets, three acetyl groups), and 8.85 (three-proton doublet, J = 6.5 Hz, 6-CH_3).

α-L-Rhamnopyranosyl Dicyclohexylammonium Phosphate. A solution of *o*-phenylene phosphorochloridate (0.77 g, 4 mmol) in anhydrous tetrahydrofuran (5 ml) was added dropwise with stirring at room temperature with occasional cooling to a solution of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranose (1.15 g, 4 mmol) in anhydrous tetrahydrofuran (10 ml) containing *sym*-collidine (0.56 ml, 4 mmol). The reaction mixture was processed as previously described (Prihar and Behrman, 1972) for the isolation of the crude barium salt of β-D-mannopyranosyl phosphate. The presumed phosphodiester intermediate was obtained as a gum and had $R_{picrate}$ = 0.76 upon electrophoresis in solvent A. The crude dry barium salt was dissolved in water, centrifuged to remove traces of insoluble matter, and reprecipitated by the addition of 3 vol of ethanol. This process was repeated three times; yield 0.65 g (45%). *Anal.* Calcd for $C_6H_9O_8PBa \cdot 3H_2O$: 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 (Lowry and Lopez, 1946).

The barium salt was converted into the dicyclohexylammonium salt by passing the aqueous solution through a precooled column of Dowex 50W-X8, 200–400 mesh, in the hydrogen form. The effluent was collected in 100 ml of 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 crystallized on the gradual addition of 4 vol of acetone. After 2 days at 4°, the colorless crystalline salt was separated and dried over phosphorus pentaoxide at 56°: yield 0.59 g (33%); $[\alpha]_D^{25}$ −21.5° (*c* 1, water) [lit. $[\alpha]_D^{25}$ −21.5° (*c* 1, water)] (Chatterjee and MacDonald, 1968). No trace of inorganic phosphate or reducing sugar could be detected by colorimetric analysis (Lowry and Lopez, 1946; Trevelyan *et al.*, 1950).

β-L-Rhamnopyranosyl Dicyclohexylammonium Phosphate. A solution of *o*-phenylene phosphorochloridate (0.77 g, 4 mmol) in anhydrous tetrahydrofuran (8 ml) was added dropwise with stirring at room temperature with occasional cooling to a solution of 2,3,4-tri-*O*-acetyl-β-L-rhamnopyranose [1.16 g, syrup, 4 mmol, $[\alpha]_D^{25}$ +20° (*c* 1, absolute ethanol)] in anhydrous tetrahydrofuran (12 ml) containing anhydrous *sym*-collidine (0.56 ml, 4 mmol).

The reaction mixture was processed by the procedure described for the isolation of α-L-rhamnopyranosyl phosphate. The barium salt was purified through ethanol precipitation and converted into the dicyclohexylammonium salt by passing through Dowex 50W-X8, 200–400 mesh, in the cyclohexylammonium form. The colorless crystalline salt was separated and dried over phosphorus pentaoxide at 56°: yield 0.45 g (25%); $[\alpha]_D^{25}$ +11.9° (*c* 1, water). *Anal.* Calcd for $C_{18}H_{39}N_2O_8P$: 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 was nonreducing (Park and Johnson, 1949). Hydrolysis in 0.1 N hydrochloric acid at 100° released the reducing power and inorganic phosphate in equimolar ratio (Lowry and Lopez, 1946; Park and Johnson, 1949). 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 $R_{picrate}$ of 1.59, indicating anomeric purity.

Reaction of β-L-Rhamnopyranosyl Phosphate with N,N′-Dicyclohexylcarbodiimide. The barium salt of β-L-rhamnopyranosyl phosphate (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 hr. 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 of water. After adding 40 mg of *N,N′*-dicyclohexylcarbodiimide, the homogeneous reaction mixture was kept at 37° for 16 hr. The urea derivative crystallized out on adding water (5 ml) and was filtered off. The filtrate was extracted twice (2 × 10 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 the starting material (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 with N,N′-Dicyclohexylcarbodiimide. The above reaction between the glycosyl phosphate and *N,N′*-dicyclohexylcarbodiimide was repeated using α-L-rhamnopyranosyl phosphate. This anomer was recovered unchanged (R_F 0.12, solvent b).

β-D-Glucopyranosyl Dicyclohexylammonium Phosphate. A solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (1.39 g, 4 mmol) in anhydrous tetrahydrofuran (10 ml) containing *sym*-collidine (0.56 ml, 4 mmol) was treated with a solution of *o*-phenylene phosphorochloridate (0.77 g, 4 mmol) in dry tetrahydrofuran by the procedure used for the preparation of α-L-rhamnopyranosyl phosphate. The gum obtained after aqueous treatment and evaporation was found to contain a major component having $R_{picrate}$ = 0.69 (electrophoresis system A) and was oxidized with bromine in 0.2 M triethylammonium hydrogen carbonate. Subsequent deacetylation with aqueous barium hydroxide afforded the barium salt of the phosphate monoester which was precipitated with 4 vol of ethanol. Electrophoresis in system A showed that the barium salt contained trace amounts of a nonphosphorylated product having $R_{picrate}$ = 0.63 as indicated by the silver nitrate spray reagent. The barium salt was converted into the dicyclohexylammonium salt by the procedure described for the preparation of β-L-rhamnopyranosyl phosphate. After repeated addition and evaporation of ethanol, the product crystallized from absolute ethanol. It was dried over phosphorus pentaoxide at room temperature: yield 0.35 g (17%); $[\alpha]_D^{25}$ +4.1° (*c* 1.14, H_2O) [lit. $[\alpha]_D^{25}$ +5.4° (H_2O)] (MacDonald, 1966). *Anal.* Calcd for $C_{18}H_{39}N_2O_9P \cdot 3H_2O$: 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 hr at room temperature revealed that the product moved 33.2 cm from the origin in contrast to α-D-glucopyranosyl phosphate which moved 30 cm.

No trace of either inorganic phosphate or reducing sugar was present in the product. Hydrolysis in 0.1 N hydrochloric acid at 100° produced glucose, and inorganic phosphate in an equimolar ratio.

β-L-Fucopyranosyl Dicyclohexylammonium Phosphate. A solution of *o*-phenylene phosphorochloridate (0.77 g, 4 mmol) in anhydrous tetrahydrofuran (5 ml) was added dropwise with stirring at room temperature with occasional cooling to a solution of 2,3,4-tri-*O*-acetyl-β-L-fucopyranose (1.15 g, 4 mmol) in anhydrous tetrahydrofuran (10 ml) containing *sym*-collidine (0.56 ml, 4 mmol). Following the procedure used for the preparation of α-L-rhamnopyranosyl phosphate, a gum was obtained after aqueous treatment and evaporation. Electrophoresis (system A) of an aliquot showed an ultraviolet-absorbing, major component (~80%) having $R_{picrate}$ = 0.76. Following the procedure described for the preparation of α-L-rhamnopyranosyl phosphate, an aqueous solution

(25 ml) of the barium salt of the glycosyl phosphate was obtained ($R_{picrate}$ = 1.0, electrophoresis system A). Upon adding 8 vol 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 precipitate was collected by centrifugation, washed with ether, and dried over P_2O_5 *in vacuo* at room temperature. The barium salt of the fucopyranosyl phosphate was hygroscopic. It was converted into the dicyclohexylammonium salt by the procedure already described. The salt was crystallized from acetone and dried over phosphorus pentaoxide *in vacuo* at 56°: yield 0.20 g (11%); $[\alpha]_D^{25}$ −20.5° (*c* 1, water). *Anal.* Calcd for $C_{18}H_{39}N_2O_8P$: 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 Dicyclohexylammonium Phosphate. A solution of *o*-phenylene phosphorochloridate (0.77 g, 4 mmol) in anhydrous tetrahydrofuran (5 ml) was added dropwise with stirring at room temperature with occasional cooling to a solution of 2,3,4-tri-*O*-acetyl-α-L-fucopyranose (1.15 g, 4 mmol) in anhydrous tetrahydrofuran (10 ml) containing *sym*-collidine (0.56 ml, 4 mmol). Following the procedure used for the preparation of α-L-rhamnopyranosyl phosphate, a gum was obtained after aqueous treatment and evaporation. Electrophoresis (system A) of an aliquot showed a uv absorbing, major component (~80%) having $R_{picrate}$ = 0.76. Following the procedure described for the preparation of α-L-rhamnopyranosyl phosphate, an aqueous solution (25 ml) of the barium salt of the glycosyl phosphate was obtained ($R_{picrate}$ = 1.0, electrophoresis system A). Upon adding 4 vol of ethanol, a permanent turbidity was obtained. The solution was left at 4° for 2 days. The salts were separated by centrifugation, washed with cold acetone, and then dried over phosphorus pentaoxide; yield 0.60 g (40%).

The barium salt was converted into the dicyclohexylammonium salt: yield 0.39 g (21%); $[\alpha]_D^{25}$ −77° (*c* 1.05, water) [lit. $[\alpha]_D^{25}$ −77.8° (*c* 1, water)] (Leaback *et al.*, 1969).

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

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