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Synthesis of glycosyl phosphates and azides

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ABSTRACT

Anomerically enriched diphenyl hexopyranosyl phosphate triesters have been prepared from *O*-alkyl and -acylated hexopyranoses, using diphenyl chlorophosphate and 4-*N*,*N*-dimethylaminopyridine. Glycosyl phosphate triesters of D-gluco-, D-galacto-, D-manno, 2-acetamido-2-deoxy-D-gluco-, L-fuco-, and L-rhamno-pyranosyl derivatives have been obtained by this procedure. At temperatures 0° and above, and under thermodynamic control, diphenyl glycosyl phosphates *cis* to the pyranosyl C-2 substituent are formed predominantly, whereas at low temperatures and under kinetic control, glycosyl phosphate triesters having 1,2-*trans* stereochemistry are obtained. The β-glycosyl phosphate triesters of D-glucose and D-galactose derivatives are unstable and undergo anomerization to the α-glycosyl phosphate triesters, in contrast to the stable β-phosphate derivatives of L-rhamnose and D-mannose. These phosphate triesters have been deprotected to glycosyl phosphate triethylammonium salts, suitable for the preparation of other key biological derivatives, such as nucleotide sugars. In addition, the diphenyl phosphate groups at the anomeric center have been displaced by azide to give the glycosyl azides, key intermediates in the synthesis of glycosyl amino acids.

INTRODUCTION

Glycosyl phosphates are key intermediates in biological synthesis of the nucleotide sugars that are involved in the assembly of oligosaccharide chains of glycoproteins and glycolipids[1]. In the presence of nucleoside triphosphates, glycosyl phosphates are converted into nucleotide sugars by the nucleotide sugar synthetase enzymes. Once formed, these nucleotide sugars function as the donor substrates for glycosyltransferases, which transfer an α- or β-glycosyl residue to a growing oligosaccharide acceptor substrate[2]. Both nucleotide sugars and glycosyltransferases are key tools for enzymic modification of cell-surface oligosaccharide structures and such modifications are very useful for probing the biological roles of carbohydrates on glycoproteins and glycolipids[3]. As several purified glycosyltransferases are available commercially, it is important to prepare structurally diverse nucleotide sugar substrates for these enzymes to carry out structural modifications of the cell-surface oligosaccharides. Several literature reports describe the enzymic preparation of nucleotide sugars[4–6], and some of these, particularly the natural sugar nucleotides, are also commercially available at a very high cost. Nevertheless, the structural diversity in the glycosyl residues available from these

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sources is limited, as the enzymic preparation of nucleotide sugars is dependent on the substrate specificity of the nucleotide-sugar synthetase enzymes[4].

Chemical coupling of glycosyl phosphates to activated nucleoside mono- or di-phosphates constitutes an attractive alternative for preparing structurally diverse nucleotide sugar derivatives[7,8], and thus the availability of glycosyl phosphates is an important requirement for the success of this process. Hashimoto and coworkers have recently demonstrated the use of glycosyl phosphate triesters in the preparation of glycosides[9]. We report now a convenient procedure for preparing anomerically enriched α- and β-glycosyl phosphate diphenyl esters, starting with the readily available hexopyranoses and the commercially available diphenyl chlorophosphate and 4-*N*,*N*-dimethylaminopyridine (DMAP). The glycosyl phosphates were prepared as their triesters so that the products could be purified by conventional chromatography. These glycosyl phosphate triesters were then deprotected to the natural glycosyl monophosphates in forms suitable for reaction with activated nucleoside mono- or di-phosphates. Finally, it is shown that the phosphate triesters may be used to prepare glycosyl azides, key intermediates in the chemical synthesis of glycopeptides[10]. The sugars examined are acetylated pyranose derivatives of glucose, galactose, 2-acetamido-2-deoxyglucose, mannose, and benzylated and benzoylated pyranose derivatives of glucose and galactose as representatives of the D sugars. Acetylated pyranose derivatives of rhamnose and fucose constitute 6-deoxy-L-sugars.

Several reports describe the preparation of glycosyl phosphates of specific sugars using *O*-phenylene phosphorochloridate[11], dibenzylchlorophosphate and butyllithium[12], dibenzylphosphorofluoridate and cesium fluoride[13], hexopyranosyl imidates and dibenzylphosphates[14], diphenyl chlorophosphate and thallium ethoxide or butyllithium[9,15], hexopyranosyl acetates and phosphoric acid[16], and glycosyl orthoesters and dibenzyl hydrogenphosphate[17].

RESULTS

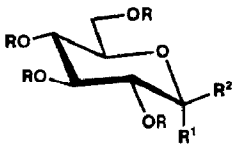
Preparation of protected hexopyranoses. — Acetylated and benzoylated hexopyranoses **1a,b, 6a, 11, 16, 21,** and **26** (Scheme 1) were prepared by the silver carbonate-catalyzed hydrolysis of the acetylated or benzoylated glycosyl halides[18] in aqueous acetone. Compound **11** was extremely soluble in water and special processing of the reaction mixture had to be followed (see Experimental). The anomeric composition of all compounds in chloroform was determined by ^{1}H-n.m.r. Crystallization of **1a** and **6a** from benzene gave products enriched in the β anomers, whereas the the mother liquor contained predominantly the α anomer. The β-enriched product could be converted into the α anomer by reaction with DMAP in dichloromethane. Tetra-*O*-benzyl-gluco- and galacto-pyranoses were prepared by hydrolysis of the corresponding methyl β-glycosides[16] to afford a mixture enriched in the α anomer. Hydrolysis of the acetylated manno- and rhamno-pyranosyl bromides gave a mixture that contained >90% of the α anomer. The acetylated fucopyranose **26** obtained from the bromide was a ~ 1:1 mixture of α and β anomers, from which >90% α-enriched anomer was obtained by crystalliza-

tion. The acetylated GlcNAc*p* derivative **11** obtained by hydrolysis of the corresponding glycosyl chloride contained >90% of the α anomer.

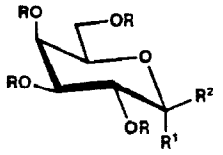
Preparation of α-glycosyl phosphate triesters. — The hexopyranoses underwent rapid phosphorylation with diphenyl chlorophosphate and DMAP to give glycosyl phosphate triesters, as shown in Scheme 2. To prepare the α-phosphates, the anomeric mixture of the hexopyranoses in dichloromethane was first treated with DMAP at 0–4° to obtain the α-enriched anomer. Subsequent addition of diphenyl chlorophosphate gave the α-phosphate triesters as predominant products for 2,3,4,6-tetra-*O*-acetyl-, benzoyl-, and benzyl derivative of glucose and galactose, and 2,3,4-tri-*O*-acetyl-L-fucopyranoses (Scheme 1, compounds **2a–cα**, **7aα**, **7bα**, and **27α**, respectively; see later for structural characterization). These compounds were stable and could be purified by chromatography on a column of silica gel. Phosphorylation of GlcNAc derivative **11** under similar conditions gave an anomeric mixture of glycosyl phosphate triesters, from which the β anomer decomposed during isolation, resulting in very low yield of the α-phosphate **12α**. However, by using a 10-fold excess of the phosphorylating reagent and by conducting the reaction at −30°, product **12α** was obtained in >67% yield.

Phosphorylation of tetra-*O*-acetyl-D-mannopyranose (**16**) and tri-*O*-acetyl-L-rhamnopyranose (**21**) by procedures similar to that described for glucose and galactose gave, besides the α-phosphate triesters (**17α** and **22α**, respectively) significant amounts of the β-phosphates (**17β** and **22β**, respectively). However, by reversing the addition of DMAP and diphenyl chlorophosphate (see Experimental) and by lowering the reaction temperature to −30°, >90% of α-phosphates **17α** and **22α**, respectively were obtained. As in the case of glucose and galactose series, these phosphates were stable and could be readily purified.

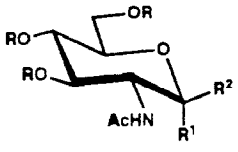
Preparation of β-glycosyl phosphate triesters. — As the equatorial anomeric hydroxyl group was expected to be more reactive[19] than the axial one, β-enriched glycosyl phosphate triesters could be selectively prepared at low temperatures. In the β-enriched acetylated glucose and galactose derivatives (**1a** and **6a**, respectively), the β-anomeric hydroxyl group was selectively phosphorylated at −25°. The α-anomeric hydroxyl group did not react appreciably at this temperature. As a result of the dynamic equilibrium between the two anomers, selective consumption of the β anomer via phosphorylation shifted the equilibrium toward the β anomer, eventually giving >90% of the β-phosphate triesters **2aβ** and **7aβ**, respectively. The β-phosphate triesters of tetra-*O*-acetyl-D-gluco- and galacto-pyranose (Scheme 2, **2aβ**, **7aβ**, respectively) could be isolated from the mixture and characterized by n.m.r. (Table I). However, at room temperature, these products anomerized to the more stable α-phosphates. Nevertheless, compounds **2aβ** and **7aβ** could be separated by chromatography (see Table I for ^{1}H-n.m.r.) and immediately hydrogenated to obtain the more stable β-phosphate ammonium salts (compounds **4β** and **8aβ**, respectively). The β-phosphate triesters were found to be more stable when the protecting groups were changed from acetate to benzoate (Scheme 1, **2bβ**). However, the selectivity in phosphorylation (**2bβ** *vs.* **2bα**) decreased, even though these could be readily separated by conventional chromatography. For 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose (**11**), the forma-



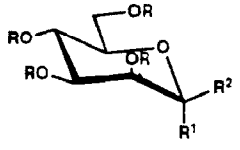
1a, R = Ac, R^1= H, R^2= OH or R^1 = OH, R^2= H
1b, R = Bz, R^1= H, R^2= OH or R^1 = OH, R^2= H
1c, R = Bn, R^1= H, R^2= OH or R^1 = OH, R^2= H
2aα, R = Ac, R^1= $PO(OPh)_2$, R^2= H
2aβ, R = Ac, R^1= H, R^2= $PO(OPh)_2$
2bα, R = Bz, R^1= $PO(OPh)_2$, R^2= H
2bß, R = Bz, R^1= H, R^2= $PO(OPh)_2$
2cα, R = Bn, R^1= $PO(OPh)_2$, R^2= H
3aα, R = Ac, R^1= $PO(OH)_2NEt_3$, R^2= H
4α, R = H, R^1= $PO(OH)_2NEt_3$, R^2= H
5ß, R = OAc, R^1= H, R^2= N_3



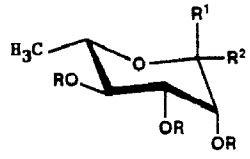
6a, R = Ac, R^1= H, R^2= OH or R^1 = OH, R^2= H
6b, R = Bn, R^1= H, R^2= OH or R^1 = OH, R^2= H
7aα, R = Ac, R^1= $PO(OPh)_2$, R^2= H
7aβ, R = Ac, R^1= H, R^2= $PO(OPh)_2$
7bα, R = Bn, R^1= $PO(OPh)_2$, R^2= H
8aα, R = Ac, R^1= $PO(OH)_2NEt_3$, R^2= H
9α, R = H, R^1= $PO(OH)_2NEt_3$, R^2= H
10ß, R = OAc, R^1= H, R^2= N_3



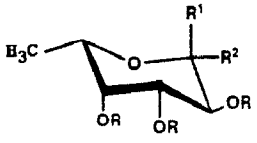
11, R = Ac, R^1= H, R^2= OH or R^1 = OH, R^2= H
12α, R = Ac, R^1= $PO(OPh)_2$, R^2= H
13α, R = Ac, R^1= $PO(OH)_2NEt_3$, R^2= H
14α, R = H, R^1= $PO(OH)_2NEt_3$, R^2= H
15ß, R = OAc, R^1= H, R^2= N_3



16a, R = Ac, R^1= H, R^2= OH or R^1 = OH, R^2= H
17α, R = Ac, R^1= $PO(OPh)_2$, R^2= H
17ß, R = Ac, R^1= H, R^2= $PO(OPh)_2$
18α, R = Ac, R^1= $PO(OH)_2NEt_3$, R^2= H
18β, R = Ac, R^1= H, R^2= $PO(OH)_2NEt_3$
19α, R = H, R^1= $PO(OH)_2NEt_3$, R^2= H
19β, R = H, R^1= H, R^2= $PO(OH)_2NEt_3$
20α, R = OAc, R^1= N_3, R^2= H
20β, R = OAc, R^1= H, R^2= N_3

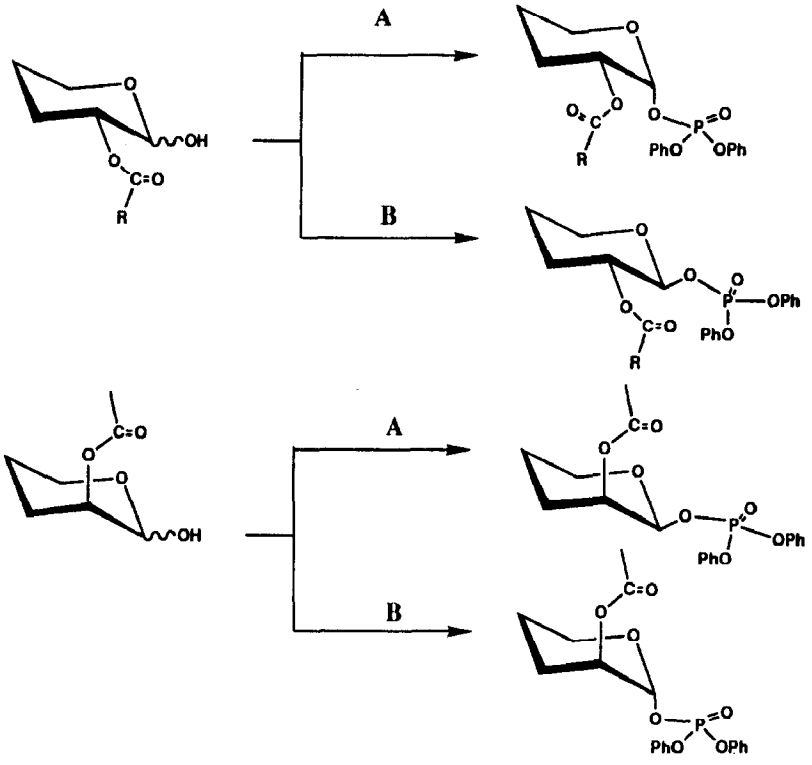


21, R = Ac, R^1= H, R^2= OH or R^1 = OH, R^2= H
22α, R = Ac, R^1= $PO(OPh)_2$, R^2= H
22ß, R = Ac, R^1= H, R^2= $PO(OPh)_2$
23α, R = Ac, R^1= $PO(OH)_2NEt_3$, R^2= H
23β, R = Ac, R^1= H, R^2= $PO(OH)_2NEt_3$
24α, R = H, R^1= $PO(OH)_2NEt_3$, R^2= H
24β, R = H, R^1= H, R^2= $PO(OH)_2NEt_3$
25α, R = OAc, R^1= N_3, R^2= H
25β, R = OAc, R^1= H, R^2= N_3



26, R = Ac, R^1= H, R^2= OH or R^1 = OH, R^2= H
27α, R = Ac, R^1= $PO(OPh)_2$, R^2= H
28α, R = Ac, R^1= $PO(OH)_2NEt_3$, R^2= H
29α, R = H, R^1= $PO(OH)_2NEt_3$, R^2= H
30ß, R = OAc, R^1= H, R^2= N_3

Scheme 1. The various hexopyranosyl derivatives prepared in this report.



Scheme 2. Phosphorylations of hexopyranoses under conditions: A, DMAP followed by the addition of diphenylphosphorochloridate at 0°; B, the reverse addition at −25°.

tion of a u.v.-active product (presumably the β-phosphate) could be evidenced only on t.l.c. plates. Warming the mixture to 0° resulted in the formation of an oxazolidine derivative which appeared to react further. The fucose derivative **26** did not give β-phosphate triesters even at low temperatures and all attempts resulted in the exclusive formation of α-phosphate triester **27**α.

For acetylated D-manno and L-rhamno-pyranoses (**16** and **21**, Scheme 2), which have the β-anomeric hydroxyl group synclinal to the 2-acetoxy substituent, the β-anomeric hydroxyl group was less reactive as compared to that of glucose and galactose derivatives **1a** and **6a**, respectively. Furthermore, the starting hexopyranose was composed of >85% of the α anomer. However, by performing the reaction at room temperature in the presence of excess DMAP and by limiting the amount of the phosphorylating agent in the mixture, their β-phosphate triesters **17**β and **22**β could be obtained predominantly, along with minor proportions of α-phosphate (β:α was ~4:1). These were separated by chromatography. In contrast to the β-phosphate triesters of the gluco and galacto series, the mannose and rhamnose β-phosphate triesters were very stable at room temperatures.

Synthesis of glycosyl phosphate triethylammonium salts and glycosyl azides. — The phenyl groups present in the phosphate triesters were removed by hydrogenation over

TABLE I

^1H- and ^{13}C chemical shifts of derivatives of hexopyranosyl phosphates and hexopyranosyl azides. All of the phosphate deprotected compounds were characterized as their mono(triethylammonium)salts.

Compd.	*Nucleus*	*Atoms*								
		1	*2*	*3*	*4*	*5*	*6*	*6B*	^{31}P	$[\alpha]_D^{25}$ *and i.r. data*
2aα	$^1H^a$	6.06	5.02	5.52	5.13	4.07	4.18	3.88		+78.8 ±2.2° (*c* 0.89, $CHCl_3$),
	$^{13}C^a$	95.0	69.35	69.81	67.6	76.6	61.1		−13.48	
2aβ	$^1H^a$	5.45	——5.22–5.07——			3.81	4.23	4.05		
2bα	$^1H^a$	6.42	5.58	6.28	5.83	4.59	4.51	4.39		+71.5 ±2° (*c* 1.03, $CHCl_3$)
	$^{13}C^a$	95.4	71.0	69.8	68.7	70.4	62.3		−13.44	
2bβ	$^1H^a$	5.81	5.67	5.73	5.87	4.27	4.55	4.39		
2cα	$^1H^a$	6.06	3.63	3.88	3.75	3.75	3.63	3.35		+61.9 ±0.7° (*c* 2.80, $CHCl_3$), lit.[15] +68° (*c* 3, $CHCl_3$)
	$^{13}C^a$	96.9	78.9	80.9	76.4	75.1	67.4		−14.63	
3aα	$^1H^b$	5.71	5.07	5.51	5.17	4.42	4.42	4.20		+77.7 ±2° (*c* 1.08, MeOH), lit.[16c] +110° (*c* 0.70, MeOH–H_2O)
	$^{13}C^b$	91.3	70.5	68.1	67.6	70.4	61.7			(potassium salt)
4α	$^1H^b$	5.45	3.51	3.8	3.42	3.8——	——	——3.7		+73.2 ±2.2° (*c* 0.92, H_2O)
	$^{13}C^b$	94.8	71.7	72.6	69.6	72.9	60.7		0.939	see ref. 22
5β	$^1H^a$	4.65	4.96	5.23	5.12	3.80	4.29	4.18		−21.8 ±2° (*c* 1.04, $CHCl_3$)
	$^{13}C^a$	87.9	70.7	72.7	68.0	74.1	61.7			ν_{max}^{film} 2118 cm^{-1} (N_3)
7aα	$^1H^a$	6.12	5.26	5.38	5.48	4.35	4.08	3.92		+79.6 ±2° (*c* 0.99, $CHCl_3$)
	$^{13}C^a$	95.7	66.9	68.8	67.4	77.2	61.0		−13.3	
7aβ	$^1H^a$	5.45	5.35	5.04	5.43	4.13	4.13	4.06		
7bα	$^1H^a$	6.07	4.12	3.83	3.98	3.98	3.51	3.28		
8aα	$^1H^b$	5.80	5.29	5.48	5.63	——	4.0–4.2	——		68.6 ±2° (*c* 1.05, MeOH), lit.[11d] +105° (*c* 1, H_2O)
	$^{13}C^b$	91.8	67.9	67.8	66.8	68.6	61.8			(monoammonium salt)
8aβ	$^1H^b$	5.28	5.16	5.31	5.51	——	——	4.3——		
9α	$^1H^b$	5.52	3.78	3.91	4.01	4.16	——	——3.7——	1.05	+66.5 ±2.3° (*c* 0.85, H_2O), lit.[11d] +71° (*c* 1.47, H_2O)
	$^{13}C^b$	94.7	68.8	69.6	69.5	71.5	61.4			see ref. 22, (dicyclohexylammonium salt)

10β	$^1H^a$	4.59	5.16	5.03	5.42	4.01	4.19	4.14		$-7.1 \pm 2.3°$ (c 0.87, $CHCl_3$)
	$^{13}C^a$	88.4	68.4	70.9	67.1	73.1	61.3			ν_{max}^{film} 2118 cm^{-1} (N_3)
12α	$^1H^a$	5.95	4.41	5.25	5.18	4.05	4.16	3.89		$+57.1 \pm 2.2°$ (c 0.94, $CHCl_3$)
	$^{13}C^a$	97.4	52.3	70.1	67.5	70.3	61.3		−13.85	
13α	$^1H^b$	5.45	4.27	5.31	5.10	4.4	4.4	4.17		$+49.9° \pm 2°$ (c 1.04, EtOH)
	$^{13}C^b$	92.0	51.9	68.7	67.9	71.9	62.1			
14α	$^1H^b$	5.37	3.91	3.79	3.49	3.91	3.86	3.77		$+44.6 \pm 2°$ (c 1.08, H_2O), lit.[12b] $+61°$ (c 1.0, H_2O)
	$^{13}C^b$	94.7	55.4	73.2	72.1	74.0	62.6		1.608	(dipotassium salt)
15β	$^1H^a$	4.75	3.92	5.25	5.11	3.79	4.29	4.16		$-43.2 \pm 2.3°$ (c 0.86, $CHCl_3$), lit.[25] $-43°$
	$^{13}C^a$	88.5	54.5	72.4	68.6	74.2	62.1			ν_{max}^{film} 2120 cm^{-1} (N_3)
17α	$^1H^a$	5.87	———	5.3 to 5.4	———	4.08	4.20	3.93		$+32.5 \pm 2.3°$ (c 0.87, $CHCl_3$)
	$^{13}C^a$	96.1	68.7	68.2	65.4	70.8	61.8		−13.96	
17β	$^1H^a$	5.59	5.49	5.07	5.25	3.78	4.27	4.12		$-6.7 \pm 2.4°$ (c 0.84, $CHCl_3$)
	$^{13}C^a$	94.8	68.1	70.1	65.7	73.2	62.1		−13.64	
18α	$^1H^b$	5.54	5.41	5.50	5.37	4.46	4.54	4.24		$+30.5$ (c 1.03, MeOH)
	$^{13}C^b$	92.7	69.9	68.5	65.5	69.4	61.8			
18β	$^1H^b$	5.47	5.54	5.37	5.23	4.1	4.46	4.42		$-15.1 \pm 2.3°$ (c 0.85, MeOH)
	$^{13}C^b$	93.2	70.0	71.1	65.4	71.6	61.8			
19α	$^1H^b$	5.35	3.94	3.87	3.61	3.94	3.85	3.72		$+19.7°$ (c 0.98, H_2O)
	$^{13}C^b$	95.4	70.9	70.2	67.0	73.1	61.2		0.31	
19β	$^1H^b$	5.10	3.99	3.91	3.51	3.41	3.70	3.67		$-3.3 \pm 2.3°$ (c 0.87, H_2O) lit.[11c] -6.5 (c 1.0, H_2O)
	$^{13}C^b$	95.1	71.2	72.8	66.8	76.6	61.3		0.67	(dicyclohexylammonium salt)
20α	$^1H^a$	5.39	5.15	5.26	5.29	4.10	4.30	4.1		$+92.7 \pm 3.1°$ (c 0.64, $CHCl_3$)
	$^{13}C^a$	87.6	68.4	69.4	66.0	70.9	62.3			ν_{max}^{film} 2120 cm^{-1} (N_3)
20β	$^1H^a$	4.73	5.44	5.04	5.26	3.76	4.29	4.20		$-67.3 \pm 2.2°$ (c 0.89, $CHCl_3$)
	$^{13}C^a$	85.3	69.4	71.1	65.8	74.9	62.5			ν_{max}^{film} 2120 cm^{-1} (N_3)
22α	$^1H^a$	5.80	5.31	5.31	5.09	3.95	1.11			$-41.3 \pm 2°$ (c 0.97, $CHCl_3$)
	$^{13}C^a$	96.4	69.2	68.9	68.3	70.4	17.2		−13.83	
22β	$^1H^a$	5.55	5.48	5.00	5.07	3.62	1.24			$+10.9 \pm 2°$ (c 0.99, $CHCl_3$)
	$^{13}C^a$	94.8	68.6	70.4	70.1	71.5	20.5		−13.55	

(continued)

TABLE I (continued)

Compd.	*Nucleus*	*Atoms*								
		1	*2*	*3*	*4*	*5*	*6*	*6B*	^{31}P	$[\alpha]_D^{25}$ *and i.r. data*
24α	$^1H^b$	5.27	3.94	3.85	3.39	3.87	1.26			$-18.9 \pm 2°$ (*c* 1.08, H_2O), lit.[11a] $-21.5°$ (*c* 1.0, H_2O)
	$^{13}C^b$	95.2	71.0	70.0	69.0	72.4	16.9		0.451	(dicyclohexylammonium salt)
24β	$^1H^b$	5.06	4.00	3.63	3.34	3.39	1.29			$+13.8 \pm 2.4°$ (*c* 0.84, H_2O), lit.[11a] $+11.9°$ (*c* 1.0, H_2O)
	$^{13}C^b$	95.0	71.3	72.5	71.9	72.5	16.8		0.711	(dicyclohexylammonium salt)
25α	$^1H^a$	5.30	5.13	5.19	5.07	4.02	1.27			$-141.2 \pm 2°$ (*c* 1.04, $CHCl_3$)
	$^{13}C^a$	87.7	68.5	69.7	68.8	70.7	17.5			ν_{max}^{film} 2118 cm^{-1} (N_3)
27α	$^1H^a$	6.07	5.21	5.35	5.27	4.17	1.0			$-88.2 \pm 2°$ (*c* 0.99, $CHCl_3$)
	$^{13}C^a$	96.3	67.1	67.4	67.3	70.6	20.4		−13.29	
29α	$^1H^b$	5.44	3.74	3.88	3.81	4.21	1.19			$-65.8 \pm 0.2°$ (*c* 0.97, H_2O), lit.[11a] -77.8 (*c* 1.0, H_2O)
	$^{13}C^b$	95.1	68.2	69.5	67.5	71.9	15.5		0.417	(dicyclohexylammonium salt)
30β	$^1H^a$	4.58	5.14	5.03	5.27	3.90	1.25			$+25 \pm 2°$ (*c* 1.01, $CHCl_3$)
	$^{13}C^a$	88.3	70.2	71.3	68.6	71.7	16.0			ν_{max}^{film} 2118 cm^{-1} (N_3)

[a] $CDCl_3$. [b] D_2O.

platinum oxide catalyst[20]. The hydrogenation was facile when acetate protecting groups were present. Subsequently, the acetate groups were removed by treatment with aqueous methanolic triethylamine solution. The n.m.r. data for all the parent glycosyl phosphates are presented in Table I. For benzoate derivatives **2bα** and **2bβ**, the hydrogenation caused further reduction of the benzoate groups to cyclohexylcarbonyl groups, along with removal of the phenyl groups of the phosphate triesters. These cyclohexylcarbonyl groups could not be readily removed without loss of the phosphate groups. In the phosphate triesters having benzyl protecting groups (**2cα** and **7bα**), the hydrogenation was incomplete and afforded a number of intermediates.

The glycosyl phosphate triesters underwent displacement reactions when treated with sodium azide or a combination of trimethylsilyl triflate and trimethylsilyl azide. When the anomeric phosphate triester group was synclinal to the adjacent C-2 substituent, displacement of the phosphate group by azide was facile. For example, in the gluco-, galacto-, 2-acetamido-2-deoxy-gluco- and fuco-pyranosyl α-phosphate triesters (**2a-cα**, **7aα**, **7bα**, **12α**, and **27α**, respectively), treatment with sodium azide in DMF at 50–70° provided exclusively the corresponding β-azides (**5**, **10**, **15**, and **30**, respectively). Higher reaction temperatures (70°) and longer reaction times were needed when benzoate protecting groups were present, whereas the displacement reaction was faster when benzyl protecting groups were present. Similarly, the β-phosphate triesters of manno- and rhamno-pyranoses (**17β** and **22β**) afforded the corresponding α azides (**20α** and **25α**, respectively) under similar conditions.

Displacement of the diphenylphosphate groups by sodium azide was difficult when they were *trans* to C-2 substituent (compounds **2bβ**, **7aβ**, **17α** and **22α**). This may be due to steric hindrance to the incoming nucleophile from the adjacent acetoxy or benzoyloxy groups. On the other hand, in the presence of Lewis acid catalysts such as trimethylsilyl triflate, the foregoing phosphate triesters reacted instantly with trimethylsilyl azide to afford an anomeric mixtures of glycosyl azides.

N.m.r. characterization of diphenyl-glycosyl phosphate triesters. — The n.m.r. data for glycosyl phosphate derivatives and glycosyl azides are presented in Table I. Typically, for the glycosyl phosphates of acetylated or benzylated derivatives of α-gluco-, galacto- and fuco-pyranosyl derivatives (**2aα**, **2cα**, **7aα**, **7bα**, **12α**, **27α**), the anomeric hydrogen signals appear as a doublet of doublets around 6.1 p.p.m., with a $^{1}H–^{31}P$ coupling constant of ~6.8 Hz. The coupling constant of H-1 to H-2 (3.8 Hz) confirmed their synclinal relationship and consequently established the α configuration of the phosphate residue. Besides, in all of the α-phosphates, four-bond coupling between H-2 and phosphorus was observed[21], whereas it was not seen in the β anomer. It is to be noted that in all of the α-glycosyl phosphates, both the H-3 and H-5, which are 1,3-*syn* diaxial to the diphenylphosphate group, are deshielded by 0.2 p.p.m. as compared to the β-phosphates (Table I). Thus, these hydrogens could be used as reporter groups for the unequivocal assignment of anomeric configuration for phosphate derivatives, especially for compounds where the coupling between H-1 and H-2 was not useful for assigning the anomeric configuration (for example the mannose and rhamnose derivatives **17** and **22**, respectively).

For mannose and rhamnose α-phosphate derivatives (**17**α and **22**α), the anomeric hydrogen resonances appeared around 5.8 p.p.m. (^1H–^{31}P coupling constant of ~6.7 Hz), whereas for the β anomers, these were around 5.5 p.p.m. For the mannose derivative **17**α, the signals for H-2, H-3 and H-4 overlapped extensively too with each other to allow definitive assignments. In contrast, these were well resolved in the β anomer, allowing extensive comparison. As may be seen for gluco and galacto phosphate derivatives (Table I), H-5 in **17**α was deshielded by ~ 0.2 p.p.m. as compared to that in **17**β, thereby confirming the anomeric configuration. For rhamnose, complete assignments were made by inspection of the spectra and once again, the chemical shifts of H-3 and H-5 were used as reporter groups for assigning the anomeric configuration (Table I).

The ^{13}C and ^{31}P chemical shifts for most of the glycosyl phosphate derivatives are presented in Table I. The identification of signals for carbons C-1 and C-2 in all these derivatives was straightforward, as these appeared as doublets through coupling with phosphorus. The assignments for other atoms are tentative and are based on the literature data reported for a number of similar glycosyl derivatives[22].

DISCUSSION

A number of biologically important glycosyl phosphates have been prepared by using diphenyl chlorophosphate–DMAP-mediated phosphorylation of hexopyranoses. The advantage of this procedure is that the starting material, namely the sugar having a free anomeric hydroxyl group may be readily obtained either from the glycosyl halides or by the deprotection of glycosides having methoxy, allyloxy, or benzyloxy group at the anomeric center. The use of a mild base DMAP as compared to such strong bases as butyllithium[12], allows the presence of such base-labile protecting groups as acetates.

The stereochemical outcome in these phosphorylation reactions may be rationalized on grounds similar to those proposed by Schmidt and Michel[19] for the base-catalyzed preparation of anomerically enriched glycosyl imidates from hexopyranoses. For the glucose and galactose derivatives **1**, **11**, and **6**, the β-anomeric hydroxyl group is expected to be more reactive than the α anomer. In fact, phosphorylation at low temperature and in the absence of free base DMAP led to the predominant formation (>90% as evidenced by ^1H-n.m.r.) of the thermodynamically less-stable β-phosphates under kinetic conditions. This is exemplified by the exclusive formation of β-phosphates **2a**β and **7a**β from the β-enriched hexopyranoses **1a** and **6a**. As noted by Schmidt and Michel for glycosyl imidates[19], these β-phosphates also anomerized at room temperature to the more stable α-phosphates. In contrast to the glucose and galactose derivatives, for the mannose and rhamnose derivatives, selective preparation of their β-phosphates **17**β and **22**β required the use of excess free base and limited phosphorylating reagent and higher reaction temperature (Scheme 2). As the mannose and rhamnose derivatives **16** and **21** exist predominantly in the α-pyranose form, we suggest that the excess base is required to catalyze the anomerization of the α to the less stable β form,

which then reacts at room temperature to give predominantly the β-phosphates **17**β and **22**β, respectively (β:α was ~4:1). We attribute the lower reactivity of the β-anomeric hydroxyl group of mannose and rhamnose derivatives **16** and **17** as compared to **1** and **6**, to the presence of an adjacent acetoxy group in synclinal orientation.

For glucose and galactose derivatives, formation of the thermodynamically more stable α-phosphates was favored by the presence of excess base and higher reaction temperatures. We could observe by ^{1}H-n.m.r. that the addition of DMAP to **1** and **6** (see Experimental for the preparation) catalyzed the conversion of β-enriched hexopyranoses overwhelmingly to the α-pyranose form. Phosphorylation of this mixture above 0° caused predominant formation of the α-phosphates **2a**α and **7a**α, respectively. Minor β-phosphates that were formed anomerized readily under these conditions, giving eventually the α-enriched phosphates. For mannose and rhamnose derivatives **16** and **21**, respectively, the α-anomeric hydroxyl group was more reactive as compared to that of the glucose and galactose derivatives **1** and **6** respectively and underwent selective phosphorylation at −30° (the α-anomeric hydroxyl groups of **1** and **6**, did not react appreciably at this temperature) to give the corresponding α-phosphates **17**α and **22**α, respectively.

The stability of the β-glycosyl phosphates depends on the nature and the stereochemistry of the protecting groups next to the anomeric center. The presence of a *trans* 2-acetamido group destabilized the phosphate the most, followed by the acetoxy group. The benzoyloxy group, on the other hand, stabilized the β-phosphates, as seen for compound **2b**β. When the acetoxy group was synclinal to the β-phosphate (for example **17**β and **22**β), the product was very stable. Also, when the phosphate triesters **2a**β and **7a**β were converted into their triethylammonium salts of the parent glycosyl phosphate **4**β and **7**β, these were found to be stable.

The phosphorylation procedure described in this report should constitute a general methodology for the preparation of a variety of glycosyl phosphates triesters (Scheme 1). The phenyl protecting groups in the phosphate triesters may be conveniently removed by hydrogenation over platinum oxide catalyst[20] and the resulting glycosylphosphoric acid may be quenched with cyclohexylamine. These salts are key intermediates in the preparation of nucleotide sugars[11b], and phosphate-linked oligosaccharide determinants[21,23]. We find acetate protecting groups the more useful, as they may be readily removed to afford the parent glycosyl phosphates.

Finally, it may be mentioned that the ready availability of glycosyl phosphate triesters having benzoate and acetate protecting groups should attract their use as glycosyl donors, as already demonstrated by Hashimoto and coworkers[9]. The ease of displacement of the diphenylphosphate group by azide in the glycosyl phosphate triesters should make available a number of glycosyl azides, which after reduction may be coupled to amino acids[10]. Glycosyl azides are conventionally prepared by the displacement from the glycosyl halides. In view of the instability of glycosyl halides, especially those having an acetamido group next to the anomeric center, preparation of such glycosyl azides as **15** via the phosphate intermediate may be considered. As the commonly available glycosyl chloride and bromides place the anomeric halogen axial to

the pyranose ring because of the anomeric effect, the preparation of glycosyl azides via the halide always gives products having the azido group contained in the equatorial orientation. We show here that, by utilizing the stable β-phosphates of mannose and rhamnose (**17**β and **22**β), the corresponding axial α-azides **25**α and **30**α may be prepared by displacement with sodium azide. Thus, the preparation of glycosyl azides by the S_N2 displacement of a glycosyl phosphate may provide a complementary synthetic methodology for the preparation of various anomeric glycosyl azides. The preparation of anomerically enriched phosphates and azides by the method described here should be applicable to suitably protected disaccharides or oligosaccharides having a free anomeric hydroxyl group. The preparation of such derivatives should facilitate the block incorporation of oligosaccharides in to glycopeptides.

EXPERIMENTAL

General methods. — All reagents were purchased from Aldrich Chemical Co. T.l.c. was performed on precoated plates of silica gel 60 F_{254} (EM Science), and the spots were visualized with a spray containing 5% H_2SO_4 in EtOH followed by heating. Column chromatography was done on silica gel 60 (230–400 mesh, EM Science). Optical rotations were measured with a Perkin–Elmer 241 polarimeter at ambient temperatures. The structural identity and purity (C, H analyses are included for key phosphate and azide derivatives) were established by n.m.r. methods. ^{1}H-N.m.r. spectra were recorded at 300 MHz (GE NMR QE-300) and the ^{13}C- and ^{31}P-n.m.r. spectra were recorded at 75.48 and 121.71 MHz with the same instrument. The hydrogen and carbon chemical shifts (Table I) in organic solvents are expressed relative to Me_4Si. For solutions of compounds in D_2O or deuterated MeOH, the hydrogen chemical shifts are expressed relative to the HOD signal (4.80 p.p.m. at 296°K), and the carbon chemical shifts are expressed relative to external Me_4Si using the deuterium lock of the spectrometer, which sets the chemical shifts of 1,4-dioxane at 66.9 p.p.m. The ^{31}P chemical shifts are expressed relative to external H_3PO_4.

Tetra-O-*acetyl*-D-*glucopyranose* (**1a**). — A modified procedure of that reported by McCloskey and Coleman[18a] was used. A solution of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (75.0 g) in acetone (150 mL) was added to a vigorously stirred suspension of Ag_2CO_3 (35.0 g) in 50% aqueous acetone (340 mL) over a period of 90 min. After 30 min, the mixture was filtered over a pad of diatomaceous earth and the filtrate was evaporated to near dryness. The residue was then dissolved in CH_2Cl_2 and the organic layer successively washed with water, ice-cold 0.5M HCl, and saturated $NaHCO_3$ solution. After drying ($MgSO_4$), the solution was evaporated to a dry residue, which was recrystallized (33.1 g) from benzene. The mother liquor upon evaporation afforded an amorphous material (29.7 g, 99% combined yield). ^{1}H-N.m.r. in $CDCl_3$ indicated the crystals were a 5:2 mixture of β and α anomers and the foam from the mother liquor was a 1:1 anomeric mixture.

Tetra-O-*acetyl*-D-*galactopyranose* (**6a**), *tetra*-O-*acetyl*-D-*mannopyranose* (**16**), *tri*-O-*acetyl*-L-*rhamnopyranose* (**21**), *and tri*-O-*acetyl*-L-*fucopyranose*[11b] (**26**) were pre-

pared by the hydrolysis of the corresponding bromides by procedures similar to that described for **1a**.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*D*-glucopyranose* (**11**). — A solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (31.0 g; prepared according to Horton[18c,*], the crystallized product contained ~ 5–10% of 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose) in MeCN (125 mL) was added to a suspension of Ag_2CO_3 (30.0 g) in 50% aqueous MeCN (220 mL) over a period of 15 min and the reaction was continued for 16 h at room temperature. The mixture was filtered over a pad of Celite and the solution was evaporated to a volume of 150 mL. The solution was filtered again over a pad of Celite and the residue washed with water (50 mL). The filtrate was extracted with CH_2Cl_2 (3 × 75 mL, most of the desired product remained in the aqueous layer). The organic layer was repeatedly extracted with water (6 × 100 mL) and all the aqueous solutions were combined. T.l.c. examination of the aqueous layer showed the presence of a homogeneous product, whereas the organic layer contained traces of the title compound and the peracetylated material that was present in the starting material. The aqueous layer was evaporated to dryness, the residue redissolved in CH_2Cl_2, and the solution was then dried ($MgSO_4$) and evaporated to obtain an amorphous product (24.0 g, 82% yield). ^{1}H-N.m.r. confirmed the structure of the product **11** containing >90% of the α anomer.

*Diphenyl (tetra-*O*-acetyl-α-D-glucopyranosyl) phosphate* (**2aα**). — A solution of tetra-*O*-acetyl-D-glucopyranose (**1**, 2.0 g) in CH_2Cl_2 (40 mL) containing DMAP (1.64 g) was stirred at room temperature for 15 min and then cooled to −10°. Diphenylchlorophosphate (2.8 mL) was added dropwise and the solution was stirred for 2 h between −10° to 0° and for 1 h at 4°. The mixture was then diluted with CH_2Cl_2 and the organic layer was washed with ice-cold water, ice cold 0.5M HCl acid and saturated $NaHCO_3$. Chromatographic purification using 2:3 EtOAc–hexane afforded the title compound as a syrup, 2.6 g (79% yield), whose structure was confirmed by ^{1}H- and ^{13}C-n.m.r. (Table I).

Anal. Calc. for $C_{26}H_{29}O_{13}P$: C, 53.7; H, 4.99, Found: C, 52.64; H, 5.22.

*Diphenyl (tetra-*O*-benzoyl-α-D-glucopyranosyl) phosphate* (**2bα**). — A solution of tetra-*O*-benzoyl-D-glucopyranose (3.0 g) in CH_2Cl_2 (40 mL) was cooled to −15°, and DMAP (2.4 g) and diphenyl chlorophosphate (4.2 mL) were added. The solution was stirred for 2 h between −15° and −10°. The reaction could not be monitored by t.l.c. as the α-phosphate triester product had nearly the same mobility as the starting material. Work up of the mixture, followed by chromatographic purification (3:8 EtOAc–hexane = 3:8), gave pure α-phosphate (2.5 g, 60% yield) along with some impure product (971 mg). The ^{1}H- and ^{13}C-n.m.r. data (Table I) were consistent with the structure **2bα**.

*Diphenyl (tetra-*O*-benzyl-α-D-glucopyranosyl) phosphate* (**2cα**). — The title compound was prepared as described for **2aα**. The ^{1}H- and ^{13}C-n.m.r. (Table I) indicated the

* A one-fold excess of AcCl than recommended was added. After 24 h, an equal volume of CH_2Cl_2 was added and the solution was stirred for another 48 h. This procedure resulted in lesser contamination of the 1-chloride with the corresponding 1-acetate.

crude product to be essentially the desired α-phosphate and in accordance with the published results[15].

*Diphenyl (tetra-*O*-acetyl-α-*D*-galactopyranosyl) phosphate* (**7aα**). — Tetra-*O*-acetyl-D-galactopyranose (**6a**, 3.0 g, recrystallized from benzene) was converted into the title compound as described for **2aα**. The yield of **7aα** was 3.9 g (78%); ^{1}H- and ^{13}C-n.m.r. ($CDCl_3$) see Table I.

Anal. Calc. for $C_{26}H_{29}O_{13}P$: C, 53.7; H, 4.99, Found: C, 53.05; H, 4.86.

*Diphenyl (tetra-*O*-acetyl-β-*D*-galactopyranosyl) phosphate* (**7aβ**). — A solution of tetra-*O*-acetyl-D-galactopyranose (2.0 g, recrystallized from benzene) in CH_2Cl_2 (40 mL) was cooled to −20°. Diphenylchlorophosphate (2.8 mL) was added followed by the dropwise addition of a solution of DMAP (1.64 g) in CH_2Cl_2 (10 mL). The reaction was conducted for 60 min between −20° and −10° and the product was worked up. ^{1}H-n.m.r. (see Table I) of the crude product showed it to be predominant in β-phosphate. Rapid chromatography afforded the title compound; yield 1.65 g (50%). The product underwent anomerization and hydrolysis upon being kept at room temperature.

*Diphenyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl) phosphate* (**12α**). — To a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose (**11**, 5.0 g) in CH_2Cl_2 (400 mL) at −30° containing DMAP (15.0 g), diphenyl chlorophosphate (20 mL) was added and the mixture was stirred for 2 h between −30 and −25°. Examination of the mixture showed a single major product (the α-phosphate **12α**) along with traces of a minor product. The mixture was worked up as already described and purified by chromatography using 3:2 EtOAc–hexane as eluant; yield of amorphous material 5.2 g (62%); ^{1}H- and ^{13}C-n.m.r. see Table I.

Anal. Calc. for $C_{26}H_{30}NO_{12}P$: C, 53.79; H, 5.17, Found: C, 52.88; H, 5.57.

*Diphenyl (tetra-*O*-acetyl-α-*D*-mannopyranosyl) phosphate* (**17α**). — To a solution of tetra-*O*-acetyl-D-mannopyranose (**16**, 3.0 g) in CH_2Cl_2 (50 mL) at −30°, a solution of DMAP (2.4 g) and diphenyl chlorophosphate (4.2 mL) in CH_2Cl_2 (20 mL) was added during 30 min. After 1 h, the mixture was warmed to −10° and then maintained for 2 h between 0 and −10°. The mixture was worked up and purified by chromatography using 3:8 EtOAc–hexane as eluant; yield of α-phosphate 4.7 g (94%); ^{1}H- and ^{13}C-n.m.r. see Table I.

*Diphenyl (tetra-*O*-acetyl-β-*D*-mannopyranosyl) phosphate* (**17β**).— To a solution of tetra-*O*-acetyl-D-mannopyranose (3.0 g) in CH_2Cl_2 (50 mL) at room temperature containing DMAP (2.4 g), a solution of diphenyl chlorophosphate (4.2 mL) in CH_2Cl_2 (20 mL) was added during 60 min. After 2 h, the reaction was worked up and the products were isolated by chromatography using 3:8 EtOAc–hexane as eluant. After elution of the less polar α-phosphate (937 mg), the eluant was changed to 2:3 EtOAc–hexane to afford the major β-phosphate (3.85 g, 77%). The structures of **17α** and **17β** were confirmed by ^{1}H- and ^{13}C-n.m.r. (see Table I).

Anal. Calc. for $C_{26}H_{29}O_{13}P$: C, 53.7; H, 4.99, Found: C, 53.99; H, 4.78.

*Diphenyl (tri-*O*-acetyl-α-*L*-rhamnopyranosyl) phosphate* (**22α**). — Tri-*O*-acetyl-L-rhamnopyranose (**21**, 3.0 g) was converted into the phosphate **22α** according to the

procedure described for **17α**. The yield of the purified product was 3.5 g (65%). The structure was confirmed by ^{1}H- and ^{13}C-n.m.r. (Table I).

Anal. Calc. for $C_{24}H_{27}O_{11}P$: C, 55.06; H, 5.16, Found: C, 54.51; H, 5.35.

*Diphenyl (tri-*O*-acetyl-β-*L*-rhamnopyranosyl) phosphate* (**22β**). — Tri-O-acetyl-L-rhamnopyranose (**21**, 1.0 g) was converted into the phosphate **22β** according to the procedure described for **17β**. The yield of the β-phosphate **22β** was 1.1 g (61%) and that of **22α** was 380 mg (21%). The structures were confirmed by ^{1}H- and ^{13}C-n.m.r. (Table I).

Anal. Calc. for $C_{24}H_{27}O_{11}P$: C, 55.06; H, 5.16, Found: C, 54.94; H, 5.34.

*Diphenyl (tri-*O*-acetyl-α-*L*-fucopyranosyl) phosphate* (**27α**). — Tri-*O*-acetyl-L-fucopyranose (**26**, 2.0 g) was converted into the phosphate **27α** according to the procedure described for **7α**. The yield was 2.0 g (56%) and the structure was confirmed by ^{1}H- and ^{13}C-n.m.r. (Table I).

Anal. Calc. for $C_{24}H_{27}O_{11}P$: C, 55.06; H, 5.16, Found: C, 55.16; H, 5.36.

Deprotection of glycosyl phosphate triesters. — The phenyl protecting group at the phosphate and the acetate groups on the pyranosyl residue for the relevant compounds were removed by the procedure illustrated for the preparation of **4α**.

*Triethylammonium α-*D*-glucopyranosyl phosphate* (**4α**). — A solution of compound **2aα** (500 mg) in 1:1 EtOAc–EtOH (10 mL) was hydrogenated (55 lb.in^{-2}) in the presence of PtO_2 catalyst (10 mg) for 16 h. The completion of the reaction was evidenced by the disappearance of the u.v.-active starting and intermediate phosphate diesters. The catalyst was filtered and the solution was neutralized with Et_3N. Evaporation of the solvent afforded a syrup. The structure of this product as mono(triethylammonium) tri-*O*-acetyl-α-D-glucopyranosyl) phosphate (**3aα**) was confirmed by ^{1}H-n.m.r. (see Table I).

The foregoing product was dissolved in 2:1:1 MeOH–Et_3N–H_2O (20 mL) and then kept for 5 days at 0°. The solution was then evaporated to dryness and the residue redissolved in water. This solution was then lyophilized and the procedure was repeated once to afford a colorless solid (350 mg, 73% yield). The structure of the title compound **4α** was evident from its ^{1}H- and ^{13}C-n.m.r. (Table I).

Preparation of glycosyl azides from glycosyl phosphate triesters. — The following procedure was used for the preparation of glycosyl azides **5, 10, 15, 20α, 25α**, and **30**.

Tetra O*-acetyl-β-*D*-glucopyranosyl azide* (**5β**). — A solution of compound **2aα** (2.3 g) in anhydrous dimethylformamide (100 mL) containing NaN_3 (2.0 g) was heated for 2 h to 50° and for 1 h to 70°. All the starting material disappeared and only one product was formed. The mixture was evaporated to dryness and the residue was extracted with CH_2Cl_2. This was then washed with water and then with saturated $NaHCO_3$ solution. Evaporation of the solvent afforded a colorless solid (1.5 g, quantitative yield), m.p. 127°. As evidenced from the ^{1}H-n.m.r. spectrum (see Table I for chemical-shift assignments), the crude product was sufficiently pure to require no chromatographic separation; ^{1}H- and ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{14}H_{19}N_3O_9$: C, 45.03; H, 5.09, Found: C, 45.38; H, 5.20.

*Tetra-*O*-acetyl-β-*D*-galactopyranosyl azide* (**10β**). — This was prepared from **7α** in 80% yield as described for **5β**; m.p. 79°; ^{1}H- and ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{14}H_{19}N_3O_9$: C, 45.03; H, 5.09, Found: C, 45.54; H, 5.10.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (**15β**). — This was prepared from **12α** in quantitative yield as described for **5β**; m.p. 163° (dec.); ^{1}H- and ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{14}H_{20}N_4O_8$: C, 45.16; H, 5.38, Found: C, 45.17; H, 5.52.

Tetra-O-acetyl-α-D-mannopyranosyl azide (**20α**). — This was prepared from **17β** in 86.2% yield as described for **5β**; ^{1}H- and ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{14}H_{19}N_3O_9$: C, 45.03; H, 5.09. Found: C, 45.41; H, 5.33.

Tri-O-acetyl-α-L-rhamnopyranosyl azide (**25α**). — Compound **22β** (540 mg) was converted into **25α** as described already for **5β**. Purification by chromatography using EtOAc–hexane gave the title compound (310 mg, 95% yield); see Table I for ^{1}H- and ^{13}C-n.m.r. chemical shifts.

Tri-O-acetyl-β-L-fucopyranosyl azide (**10β**). — This was prepared from **27α** in 76% yield as described for **5β**; m.p. 127°; ^{1}H- and ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{12}H_{17}N_3O_7$: C, 45.71; H, 5.40, Found: C, 45.75; H, 5.47.

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