Carbohydrate Research, 161 (1987) 97–112
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF OLIGOSACCHARIDE FRAGMENTS OF THE REPEATING UNIT OF *Salmonella kentucky* O-SPECIFIC POLYSACCHARIDE AND CONVERSION OF THE OLIGOSACCHARIDES INTO THE GLYCOSYL PHOSPHATES*

VLADIMIR I. TORGOV, CAMELIA A. PANOSSIAN, AND VLADIMIR N. SHIBAEV
N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)
(Received April 30th, 1986; accepted for publication, October 2nd, 1986)

ABSTRACT

α-D-Man-(1→2)-α-D-Man-(1→3)-D-Gal, a structural fragment of the main chain of *Salmonella* serogroups C_2 and C_3 O-specific polysaccharides, and the isomer with the central residue β have been synthesised, as have some oligosaccharides related to the structure of the O-specific polysaccharide of *S. kentucky* (serogroup C_3), namely, α-D-Glc-(1→4)-D-Gal, α-D-Man-(1→3)-[α-D-Glc-(1→4)]-D-Gal, and α-D-Man-(1→2)-α-D-Man-(1→3)-[α-D-Glc-(1→4)]-D-Gal, and the isomers with the D-Glc unit β. Each oligosaccharide was converted into the α-glycosyl phosphate.

INTRODUCTION

Synthetic polyprenyl pyrophosphate sugars, first prepared by Warren and Jeanloz[1], are useful for investigating the mechanism of the biosynthesis of bacterial polysaccharides and studying the specificity of the enzymes involved in this process[2]. The phosphoimidazolidate method for the synthesis of these compounds[3] allows the preparation of a wide range of polyprenyl pyrophosphate oligosaccharides, precursors of the O-specific polysaccharides of *Salmonella* serogroups B and E, and their derivatives[3,4]. These derivatives enable a chemical–enzymic synthesis of the polysaccharides[5,6], demonstration of the incorporation of modified monosaccharide residues into the polymers[6–8], the preparation of polysaccharides with glucose residues in the side chains[9], and the demonstration of the sensitivity of glycosyl transferases towards the configuration of glycosidic bonds in the oligosaccharide chain of precursors[10].

The present work is part of a programme on the synthesis and study of biosynthetic precursors of *Salmonella* serogroups C_2 and C_3 O-specific polysaccharides. The main chain of these polysaccharides comprises the tetrasaccharide repeating-unit **1**. In the serogroup C_3 bacterium *S. kentucky*, the main polysaccharide

*Dedicated to Roger W. Jeanloz.

0008-6215/87/$ 03.50

chain is substituted with residues of α-abequose (at HO-3 of rhamnose) and α-D-glucose (at HO-4 of galactose)[11].

β-L-Rha-(1→2)-α-D-Man^{II}-(1→2)-α-D-Man^{I}-(1→3)-D-Gal
1

During an investigation of the biosynthesis of these polymers[12], it was shown that assembly of the repeating unit starts with the formation of polyprenyl pyrophosphate galactose and includes the sequential transfer of mannose and rhamnose residues. The resulting tetrasaccharide derivative can be enzymically polymerised without incorporation of side chains. Syntheses of the polyprenyl pyrophosphate derivatives of α-D-galactose[1,3] and D-mannosyl-D-galactose, a disaccharide fragment of **1**[13], have been reported. We now report syntheses of α-D-Man-(1→2)-α-D-Man-(1→3)-D-Gal (**2**), the trisaccharide fragment of the main polysaccharide chain, and its isomer α-D-Man-(1→2)-β-D-Man-(1→3)-D-Gal (**3**).

In order to study the enzymic glycosylation during the assembly of the polymer repeating-unit, the oligosaccharide precursors with the glycosyl side-chain incorporated have been synthesised. For this purpose, the disaccharide **4** and oligosaccharides **5** and **6** derived therefrom were synthesised, as well as the analogous compounds **7–9** containing a β-D-Glc residue.

α-D-Glc-(1→4)-D-Gal
3
↑
R

4 R = H
5 R = α-D-Man-(1→
6 R = α-D-Man^{II}-(1→2)-α-D-Man^{I}-(1→

β-D-Glc-(1→4)-D-Gal
3
↑
R

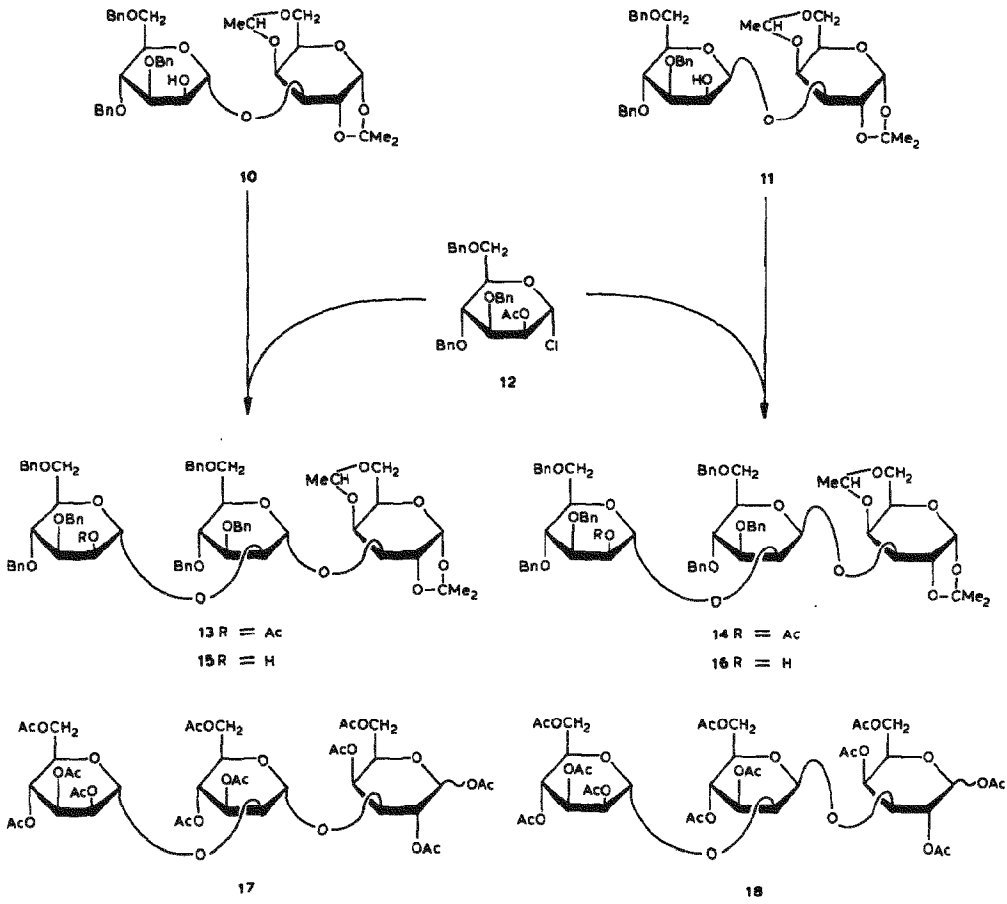
7 R = H
8 R = α-D-Man-(1→
9 R = α-D-Man^{II}-(1→2)-α-D-Man^{I}-(1→

The synthetic oligosaccharides were converted into the corresponding α-glycosyl phosphates which may be used as starting materials for the synthesis of polyprenyl pyrophosphate oligosaccharides.

RESULTS AND DISCUSSION

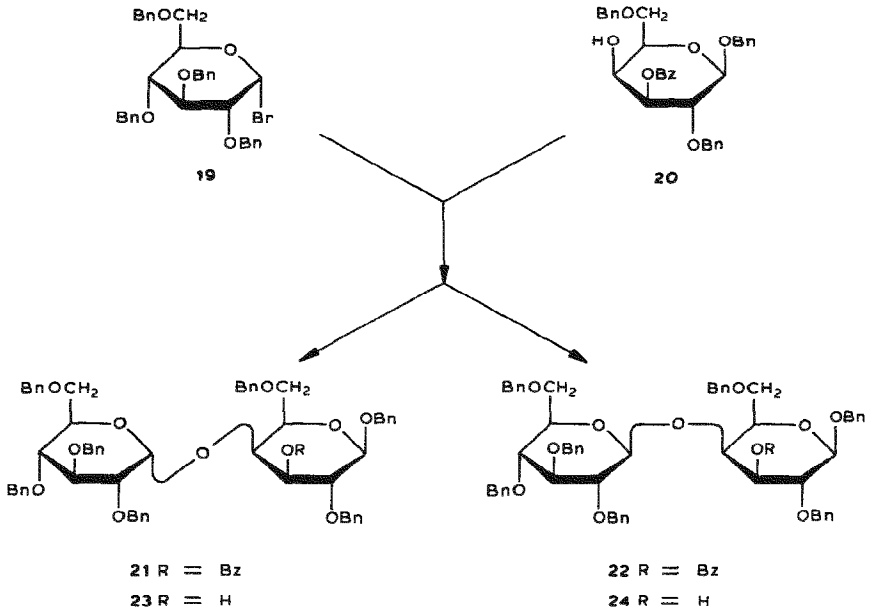
The disaccharide synthons[13] **10** and **11** were used for the synthesis of trisaccharide **2** and its β-isomer **3**.

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[14] (**12**) reacted slowly under Helferich conditions [acetonitrile, $Hg(CN)_2$] with **10** and **11** to give the trisaccharide derivatives **13** (62%) and **14** (65%), respectively. Dry reagents and solvents were essential for good results. The structures of **13** and **14** were confirmed by their ^{1}H- and ^{13}C-n.m.r. spectra (see Experimental). Zemplén



deacetylation of **13** and **14** gave **15** and **16**, respectively. Catalytic hydrogenolysis of **15** and **16**, acetylation, and then mild acetolysis gave the undeca-acetates (**17** and **18**) of **2** and **3**, respectively.

A partially protected derivative of the disaccharide **4** has been described[15], but the combination of protecting groups used (6-*p*-nitrobenzoate in the Glc residue, 3-benzoate in the Gal residue, with all the other hydroxyl groups benzylated) was not satisfactory for the planned synthesis of **5** and **6**. A strategy using BzO-3 as a temporary protecting-group in the Gal residue with the other hydroxyl groups benzylated was explored. Thus, glycosylation of benzyl 3-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranoside[15] (**20**) with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide, under the conditions described by Lemieux *et al.*[16], gave only traces of the disaccharide derivative, whereas the use of $AgSO_3CF_3$ as a promotor and acceptor of HBr gave a large amount of by-products. However, when the glycosylation was performed in CH_2Cl_2 in the presence of $Hg(CN)_2$, 86% of a 1:2 mixture of the α- (**21**) and β-disaccharide derivatives (**22**) was obtained.



Compounds **21** and **22** were isolated by h.p.l.c., and the ^{1}H-n.m.r. signals for H-3 of the Gal residues were at low field, indicating benzoylation at C-3. In the ^{13}C-n.m.r. spectra of **21**, the signal at 99.2 p.p.m. was characteristic of α-Glc and that at 101.95 p.p.m. of β-Glc for **22**.

Zemplén debenzoylation of **21** and **22** gave the respective monohydroxy derivatives **23** and **24**, and catalytic hydrogenolysis then gave the disaccharides **4** and **7**, the structures of which were evident from their ^{13}C-n.m.r. spectra (Table I). Thus, for **4**, the signal of C-1 at δ 101.3 proved the Glc moiety to be α. The series of signals corresponding to α- and β-Gal were compatible only with its glycosylation at C-4. Likewise, for **7**, the signal at δ 104.9 showed the Glc unit to be β and substitution at C-4 was proved by the signals of the reducing Gal unit.

The trisaccharide derivatives **25** (50%) and **26** (60%) were obtained *via* mannosylation of **23** and **24** with **12** in the presence of $Hg(CN)_2$.

The ^{13}C-n.m.r. spectra of **25** contained easily identified signals for C-1 of β-Gal*p* (δ 103.2), α-Glc*p* (δ 99.3), and α-Man*p* (δ 94.3) residues, and of the glycosylated carbon atoms of the β-Gal*p* residue (δ 81.7 and 81.2). The spectrum of **26** contained signals for C-1 of β-Gal*p* (δ 103.9), β-Glc*p* (δ 102.9), and α-Man*p* (δ 97.4), and two signals of glycosylated atoms of the galactose residue (δ 84.7 and 82.8).

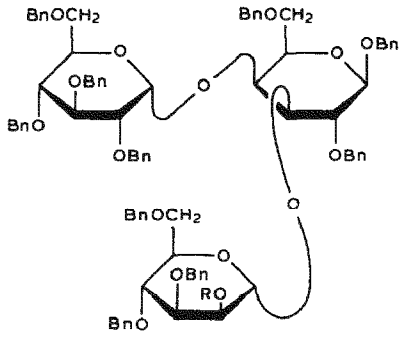
Saponification of **25** and **26** gave the hydroxy derivatives **27** and **28**, and catalytic hydrogenolysis then afforded the trisaccharides **5** and **8** in high yield. The ^{13}C-n.m.r. spectra of **5** contained signals at δ 101.3 and 101.4 (due to the influence of the anomeric configuration of the reducing unit) which were assigned to C-1 of

TABLE I

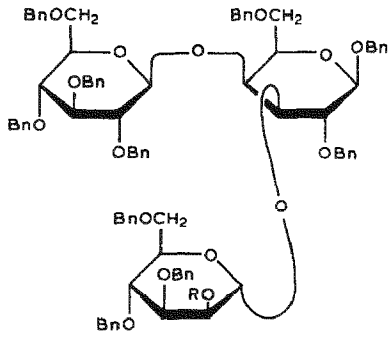
ASSIGNMENTS[a] OF THE ^{13}C-N.M.R. RESONANCES FOR THE OLIGOSACCHARIDES **4–9**

Compound	*Residue*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*Compound*	*Residue*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
4	α-D-Glc	101.3 101.4	73.1	74.0	70.6	73.1	61.4	**7**	β-D-Glc	104.9	74.9	77.0	70.9	77.0	62.1
	α-D-Gal	93.6	70.0 (69.75)	69.75 (70.0)	79.95	72.1	61.4		α-D-Gal	93.5	70.1	70.8 (71.0)	79.5	71.0 (70.8)	62.1
	β-D-Gal	97.4	73.1 (73.6)	73.6 (73.1)	78.7	76.3	61.4		β-D-Gal	97.6	73.6	74.4	78.6	75.5	61.9
5	α-D-Man	97.6	71.5	71.7	67.85 67.9	73.65	62.1	**8**	α-D-Man	98.2 98.5	71.4	71.6	67.8 67.9	73.9	62.0
	α-D-Glc	101.3 101.5	73.1 (73.3)	74.0	70.5	73.3 (73.1)	61.4		β-D-Glc	104.4	74.6	77.0 (76.8)	71.0	76.8 (77.0)	62.0
	α-D-Gal	93.4	68.1	73.8 (75.5)	75.5 (73.8)	72.4	61.8		α-D-Gal	93.6	68.3	75.4 (74.2)	74.2 (75.4)	71.2	62.0
	β-D-Gal	97.8	71.5	77.1	74.1	76.4	61.4		β-D-Gal	97.6	71.7	78.7	73.4	75.5	62.0
6	α-D-Man^{II}	103.65	71.2	71.55	68.3	73.8	62.1	**9**	α-D-Man^{II}	104.3 (104.6)	71.1	71.6	68.0 (68.3)	73.8	62.1 (62.7)
	α-D-Man^{I}	96.7 97.0	80.3	71.2	68.3	74.5	62.5		α-D-Man^{I}	96.05 96.3	81.2	70.9	68.3 (68.0)	74.3	62.7 (62.1)
	α-D-Glc	102.1 102.3	73.3 (73.4)	73.8	70.4	73.4 (73.3)	61.3		β-D-Glc	104.6 (104.3)	74.5	77.2 (76.8)	71.05	76.8 (77.2)	62.1
	α-D-Gal	93.5	68.2	74.7	77.9	72.1	62.1		α-D-Gal	93.5	68.3	75.4 (74.75)	74.75 (75.4)	71.1	62.1
	β-D-Gal	97.75	71.7	78.4	76.1	76.4	61.9		β-D-Gal	97.5	71.7	78.1	74.5	75.4	62.1

[a]Assignments may be interchanged with those in brackets.



25 R = Ac
27 R = H

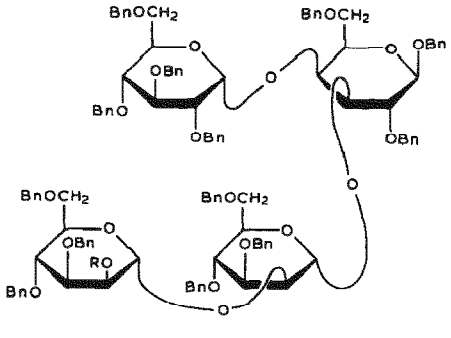


26 R = Ac
28 R = H

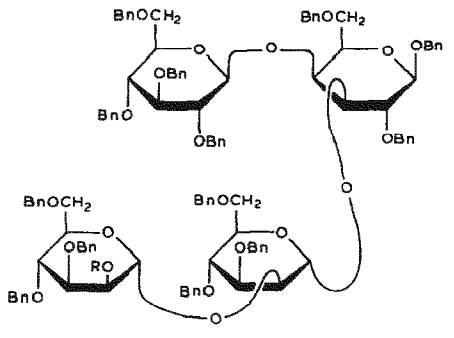
α-Glc*p*, and the signal at δ 97.6 was characteristic for C-1 of α-D-Man*p* linked to C-3 of D-Gal[17]. Low-field signals at δ 73.8 and 75.5 (α-series) or 77.1 and 74.1 (β-series) indicated 3,4-disubstitution of the Gal*p* residue. Similar signals were present in the spectrum of **8**, except that for C-1 of α-D-Glc*p* which was replaced by one at δ 104.4 characteristic of C-1 of β-D-Glc*p*.

Mannosylation of the trisaccharide derivatives **27** and **28**, using **12** under the conditions of the trisaccharide synthesis, gave the tetrasaccharide derivatives **29** (80%) and **30** (53%), respectively. The ^{13}C-n.m.r. spectra of **29** and **30** contained signals for C-1 of Glc*p* (α for **29** and β for **30**), β-Gal*p*, α-Man*p*^{I}, α-Man*p*^{II}, C-2 substituted Man^{I}, and 3,4-disubstituted Gal (see Experimental).

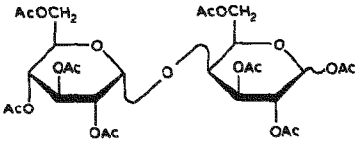
Zemplén saponification of **29** and **30** gave monohydroxy derivatives **31** and **32**, respectively, catalytic hydrogenolysis of which gave high yields of the tetrasaccharides **6** and **9**. The structures of **6** and **9** were proved by the ^{13}C-n.m.r. data. Comparison of the spectra of **6** and **9** clearly showed the attachment in **6** of an additional α-D-Man*p* residue to C-2 of an α-D-Man*p* residue with characteristic signals at δ 102.6 (C-1 of Man^{II}) and 80.3 (C-2 of Man^{I}). An analogous conclusion may be drawn from comparison of the spectra of **9** and **8**.



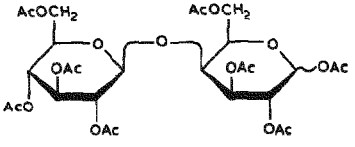
29 R = Ac
31 R = OH



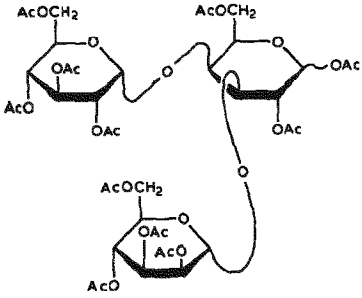
30 R = Ac
32 R = H



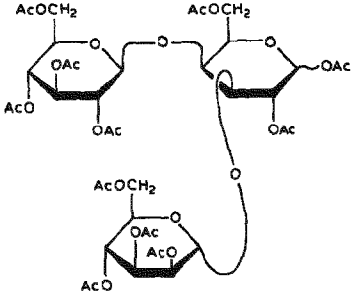
33



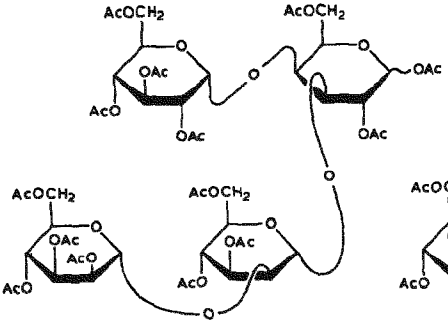
34



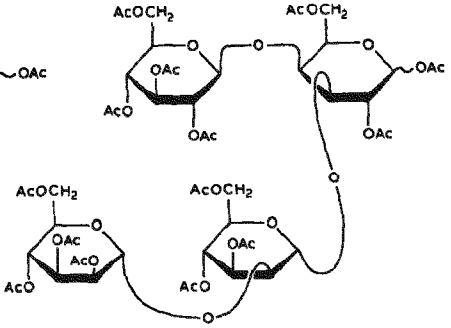
35



36



37



38

Oligosaccharides **4–9** were converted into the fully acetylated derivatives by treatment with acetic anhydride in pyridine.

Treatment of the undeca-acetates (**17** and **18**) of the linear trisaccharides **2** and **3** with anhydrous H_3PO_4 under conditions similar to MacDonald procedure[18,19], followed by deacetylation with LiOH and ion-exchange chromatography of the products, yielded the glycosyl phosphates **39** (65%) and **40** (51%), respectively.

α-D-Man-(1→2)-α-D-Man-(1→3)-α-D-Gal-P **39**
α-D-Man-(1→2)-β-D-Man-(1→3)-α-D-Gal-P **40**

The structures of **39** and **40** were confirmed by sugar and acid-labile-phosphate analyses, mobilities in paper electrophoresis, and the ^{13}C-n.m.r. data (Table II). The ^{13}C signals at δ 95.0 and 68.15 for **39** and δ 95.5 and 69.25 for **40**, which

TABLE II

ASSIGNMENTS[a] OF ^{13}C-N.M.R. RESONANCES FOR THE GLYCOSYL PHOSPHATES **39–46**

Compound	*Residue*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	$J_{1,P}$ $J_{2,P}$
39	α-D-Man^{II}	103.2	70.9 (71.2) (70.8)	71.2 (70.9) (70.8)	67.9	73.55	62.1	
	α-D-Man^{I}	95.4	80.05	70.8 (70.9) (71.2)	67.9	74.2	61.9	
	α-D-Gal	95.0	68.15	74.5	66.2	71.9	62.2	5.0 7.0
40	α-D-Man	102.6 (102.1)	71.25	72.3	67.9	73.5	62.1	
	β-D-Man	102.1 (102.6)	76.8 (77.7)	74.9	68.2	77.7 (76.8)	62.2	
	α-D-Gal	95.5	69.25	80.7	70.35	71.55	62.3	5.0 7.0
41	α-D-Glc	101.3	73.3 (73.0)	74.0	70.8	73.0 (73.3)	61.7	
	α-D-Gal	95.5	70.1	70.35	80.0	72.9	61.7	5.5 7.0
42	β-D-Glc	104.6	74.7	76.8	70.7 (70.8)	76.8	61.9 (61.8)	
	α-D-Gal	95.1	70.1	70.8 (70.7)	79.3	71.8	61.8 (61.9)	5.5 7.0
43	α-D-Man	97.9	71.45	71.7	68.0	73.7	62.1	
	α-D-Glc	101.4	73.2 (73.1)	73.9	70.5	73.1 (73.2)	61.6	
	α-D-Gal	95.7	68.4	74.3 (75.5)	75.5 (74.3)	73.1	61.4	5.0 7.0
44	α-D-Man	98.1	71.3	71.7	67.7	73.6	61.8	
	β-D-Glc	104.8	74.2	76.7	70.7	76.6	61.8	
	α-D-Gal	95.2	68.45	75.5 (74.3)	74.3 (75.5)	71.15	61.8	4.9 7.3
45	α-D-Man^{II}	103.2	71.2	71.5	68.1	73.8	62.1	
	α-D-Man^{I}	97.1	79.2	70.6	68.3	74.4	62.2	
	α-D-Glc	101.7	73.2 (73.3)	73.8	71.0	73.3 (73.2)	61.8	
	α-D-Gal	95.1	68.75	75.6	77.2	72.8	61.5	5.0 7.0
46	α-D-Man^{II}	104.2 (104.6)	71.1	71.6	68.2	73.8	62.2	
	α-D-Man^{I}	96.8	81.0	71.0	68.2	74.5	62.5	
	β-D-Glc	104.6 (104.2)	74.5	77.2 (76.8)	71.0	76.8 (77.2)	62.0	
	α-D-Gal	95.4	68.7	75.3 (74.5)	74.5 (75.3)	71.8	62.2	5.5 7.0

[a]Assignments may be interchanged with those in brackets.

were split by coupling with ^{31}P (J 5.0 and 7.0 Hz, respectively), are characteristic for C-1 and C-2 of α-D-Gal-P fragment[13,20]. Comparison of the spectra with the data for the corresponding disaccharide phosphates[13], methyl α- and β-D-manno-pyranoside, and methyl 2-*O*-α-D-mannopyranosyl-D-mannopyranoside[14] shows that the observed pattern is compatible only with an α-(1→2) linkage between Man^{II} and Man^{I} residues and an α-(1→3) linkage between Man^{I} and Gal (*cf.* ref. 17 for a discussion of glycosylation effects). For **39**, the characteristic signals were at δ 103.2 (C-1 of Man^{II} linked to C-2 of α-D-Man^{I}), 95.4 (C-1 of Man^{I} linked to C-3 of D-Gal), 80.05 (C-2 of D-Man^{I}), and 74.5 (C-3 of D-Gal substituted with α-D-Man); signals near δ 77 (characteristic of C-5 of β-D-Man*p*) were absent. For **40**, the signals at δ 102.6 and 102.1 clearly showed the presence of α-D-Man^{II}-(1→2)-D-Man^{I} and β-D-Man^{I}-(1→3)-D-Gal linkages[17]. The downfield shift of the C-3 signal (80.7 p.p.m.) for D-Gal is characteristic for substitution with a β-D-Man*p* residue[17], the signals at δ 77.7 and 76.8 correspond to C-2 and C-5 of a 2-substituted β-D-Man^{I} residue, and the absence of additional signals in this region shows the Man^{II} residue to be α. Treatment of the octa-acetates **33** and **34**, the undeca-acetates **35** and **36**, and the tetradeca-acetates **37** and **38** with H_3PO_4, as described for **17** and **18**, gave 50–74% of the glycosyl phosphates **41–46**.

R→3-α-D-Gal-P
4
↑
1-α-D-Glc

41 R = H
43 R = α-D-Man-(1→
45 R = α-D-Man^{II}-(1→2)-α-D-Man^{I}-(1→

R→3-α-D-Gal-P
4
↑
1-β-D-Glc

42 R = H
44 R = α-D-Man-(1→
46 R = α-D-Man^{II}-(1→2)-α-D-Man^{I}-(1→

The structures of glycosyl phosphates **41–46** were confirmed as for **39** and **40**. The presence in each of the ^{13}C-n.m.r. spectra of signals for C-1 (δ 95.1–95.7) and C-2 (δ 70.1 for **41** and **42**, and 68.3–68.7 for **43–46**) of Gal split by coupling with ^{31}P clearly showed[13,20] the glycosyl phosphates to be α. The other signals in the spectra of **41–46** were similar to those for oligosaccharides having the α configuration at the reducing end (*cf.* Tables I and II), thus confirming the absence of any changes in the oligosaccharide moieties.

EXPERIMENTAL

Optical rotations were determined with a Perkin–Elmer 141 polarimeter at 20 ±2°. N.m.r. spectra were recorded with a Bruker WM-250 spectrometer for solutions in $CDCl_3$ (internal Me_4Si) and D_2O (internal MeOH). T.l.c. was performed on Kieselgel G-60 (Merck), using *A*, benzene–ethyl acetate (9:1); *B*, benzene–ether (8:2); *C*, benzene–ethyl acetate (2:3); *D*, benzene–ether (7:3); *E*,

ethyl acetate–methanol (7:3), *F*, chloroform–methanol–water (60:25:4); and detection by charring with sulfuric acid. Column chromatography was performed on Silpearl (Chemapol. C.S.S.R.). Preparative h.p.l.c. was performed on a column (250 × 25 mm) of Silasorb 600, 10 μ (Chemapol, C.S.S.R.), using benzene–ethyl acetate (97:3) at 10 mL/min and detection with a Knauer (F.R.G.) refractometer. Ion-exchange chromatography was performed on a column (100 × 3 mm) of DA-X8 11F resin, using 0.5M sodium borate buffer (pH 8.8) at 18 mL/h and 70°. The eluate was monitored with the orcinol–sulfuric acid reagent. Monosaccharide analysis was performed for oligosaccharides and glycosyl phosphates after hydrolysis in M HCl for 16 h at 100°. Ion-exchange chromatography of glycosyl phosphates was performed on a column (250 × 9 mm) with AG-X8 resin (−400 mesh) (Bio-Rad) at 5 mL/min, using a linear gradient of water →0.1M NH_4HCO_3 (250 mL in each vessel). The eluate was monitored with the orcinol–sulfuric acid reagent, acid-labile phosphate was determined[21], and paper electrophoresis was performed[4]. Solutions were concentrated *in vacuo* at 40°. Acetonitrile and dichloromethane were distilled twice over CaH_2. For polarimetry, the concentration of glycosyl phosphates was measured on the basis of the content of acid-labile phosphate.

Glycosylation reactions were performed using a high-vacuum line for drying reagents and transfer of solvent essentially as described by Byramova *et al.*[22], except that smaller reaction vessels were used.

3-O-[2-O-(2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl]-4,6-O-ethylidene-1,2-O-isopropylidene-α-D-galacto-pyranose (**13**). — A solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride[14] (**12**; 190 mg, 0.37 mmol) in acetonitrile (1 mL) was added to a stirred mixture of 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-galactopyranose[13] (**10**; 180 mg, 0.265 mmol) and $Hg(CN)_2$ (130 mg, 0.52 mmol) in acetonitrile (2 mL). The mixture was kept at 20° for 60 h, then diluted with $CHCl_3$ (15 mL), washed with water (2 × 15 mL), and concentrated. Column chromatography (solvent *B*) of the residue gave **13** (190 mg, 62.1%), $[\alpha]_D^{20}$ +42° (*c* 1, chloroform), R_F 0.3 (solvent *B*). N.m.r. data: ^{1}H, δ 7.18–7.39 (30 H, 6 Ph), 5.78 (d, 1 H, $J_{1,2}$ 4 Hz, H-1 of Gal), 5.50 (dd, 1 H, $J_{1,2}$ 1.75, $J_{2,3}$ 3 Hz, H-2 of Man^{II}), 2.12 (s, 3 H, Ac), 1.34 and 1.46 (2 s, 6 H, CMe_2), 1.29 (d, 3 H, *J* 5 Hz, CH*Me*); ^{13}C, δ 109.4 [*C*$(CH_3)_2$], 99.3 (C-1 of Man^{II}), 98.7 (C-1 of Gal), 98.4 (HCMe), 95.4 (C-1 of Man^{I}), 79.8 (C-3 of Gal), 74.0 (C-2 of Man^{I}).

Anal. Calc. for $C_{65}H_{76}O_{17}$: C, 69.15; H, 6.40. Found: C, 69.13; H, 6.71.

3-O-[2-O-(2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-O-benzyl-β-D-mannopyranosyl]-4,6-O-ethylidene-1,2-O-isopropylidene-α-D-galacto-pyranose (**14**). — Treatment of 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-3-*O*-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-α-D-galactopyranose[13] (**11**; 200 mg, 0.3 mmol) with $Hg(CN)_2$ (148 mg, 0.59 mmol) and **12** (160 mg, 0.31 mmol), as described for **13**, gave **14** (220 mg, 64.7%), $[\alpha]_D^{20}$ −2° (*c* 1, chloroform), R_F 0.44 (solvent *B*). N.m.r. data: ^{1}H, δ 7.0–7.2 (30 H, 6 Ph), 5.63 (d, 1 H, $J_{1,2}$ 4 Hz, H-1 of Gal), 5.48 (dd, 1 H, $J_{1,2}$ 1.75, $J_{2,3}$ 3 Hz, H-2 of Man^{II}), 1.95 (s, 3 H, Ac), 1.14 and 1.25 (2 s, 6

H, CMe_2), 0.85 (d, 3 H, *J* 5 Hz, CHMe); ^{13}C, δ 110.0 (CMe_2), 99.8 (C-1 of Man^{II}), 98.9 (C-1 of Man^{I}), 98.8 (C-1 of Gal), 98.3 ($HCCH_3$), 82.8 (C-2 of Man^{I}), 80.3 (C-3 of Gal).

Anal. Calc. for $C_{65}H_{76}O_{17}$: C, 69.15; H, 6.40. Found: C, 69.10; H, 6.68.

4,6-O-Ethylidene-1,2-O-isopropylidene-3-O-[3,4,6-tri-O-benzyl-2-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-galactopyranose (**15**). — A mixture of **13** (90 mg, 0.08 mmol) in MeOH (5 mL) and 2M MeONa in MeOH (0.06 mL) was kept for 16 h at 20°, then deionised with KU-2 (H^+) resin, filtered, and concentrated *in vacuo*. Column chromatography (solvent *B*) of the residue gave **15** (65 mg, 74.7%), $[\alpha]_D^{20}$ +54° (*c* 1, chloroform), R_F 0.14 (solvent *B*). ^{1}H-N.m.r. data: δ 7.15–7.36 (30 H, 6 Ph), 5.8 (d, 1 H, $J_{1,2}$ 4 Hz, H-1 of Gal), 1.28–1.48 (9 H, 3 Me).

Anal. Calc. for $C_{63}H_{74}O_{16}$: C, 69.61; H, 6.81. Found: C, 68.85; H, 6.80.

4,6-O-Ethylidene-1,2-O-isopropylidene-3-O-[3,4,6-tri-O-benzyl-2-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-β-D-mannopyranosyl]-α-D-galactopyranose (**16**). — Treatment of **14** (220 mg, 0.19 mmol) as described for **13** gave **16** (197 mg, 93.2%), $[\alpha]_D^{20}$ +8° (*c* 1, chloroform), R_F 0.14 (solvent *B*). ^{1}H-N.m.r. data: δ 7.0–7.2 (30 H, 6 Ph), 5.5 (d, 1 H, $J_{1,2}$ 4 Hz, H-1 of Gal), 0.8–1.2 (9 H, 3 Me).

Anal. Calc. for $C_{63}H_{76}O_{16}$: C, 69.61; H, 6.81. Found: C, 69.52; H, 6.75.

1,2,4,6-Tetra-O-acetyl-3-O-[3,4,6-tri-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-D-galactopyranose (**17**). — A solution of **15** (106.6 mg, 0.095 mmol) in ethanol (10 mL) was hydrogenolysed over 10% Pd/C at 36°. The reaction was monitored by t.l.c. (solvent *E*). The mixture was filtered and concentrated, and the residue was treated with acetic anhydride in pyridine and then acetolysed[23] to give **17** (68 mg, 92%), $[\alpha]_D^{20}$ +61.5° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 2.0–2.4 (33 H, 11 Ac).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.59. Found: C, 49.69; H, 5.59.

1,2,4,6-Tetra-O-acetyl-3-O-[3,4,6-tri-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl]-D-galactopyranose (**18**). — Treatment of **16** (175 mg, 0.156 mmol) as described for **15** gave **18** (64 mg, 68.8%), $[\alpha]_D^{20}$ +38° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 1.98–2.15 (33 H, 11 Ac).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.59. Found: C, 49.54; H, 5.55.

Benzyl 3-O-benzoyl-2,6-di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-β-D-galactopyranoside (**21**) *and benzyl 3-O-benzoyl-2,6-di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-β-D-galactopyranoside* (**22**). — A solution of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide[24] (**24**; 900 mg, 1.5 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a stirred mixture of benzyl 3-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranoside[15] (**20**; 500 mg, 1 mmol), $Hg(CN)_2$ (500 mg, 2 mmol), molecular sieve 3 Å (1 g), and CH_2Cl_2 (3 mL). The suspension was stirred for 2 h at 20° and then filtered, and insoluble material was washed with $CHCl_3$ (25 mL). The combined filtrate and washings were washed with water (2 × 30 mL) and then concentrated *in vacuo*. H.p.l.c. of the residue gave **21** (300 mg, 28.7%) and **22** (580 mg, 57.3%). Compound **21** had $[\alpha]_D^{20}$ +75° (*c* 1, chloroform),

R_F 0.63 (solvent *A*). N.m.r. data: ^{1}H, δ 7.0–7.7 (40 H, 8 Ph), 5.12 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3 Hz, H-3 of Gal); ^{13}C, δ 103.15 (C-1 of Gal), 99.2 (C-1 of Glc), 81.75, 80.7 (C-3,4 of Gal).

Anal. Calc. for $C_{68}H_{68}O_{12}$: C, 75.84; H, 6.31; Found: C, 75.74; H, 6.28.

Compound **22** had $[\alpha]_D^{20}$ +50° (*c* 1, chloroform), R_F 0.55 (solvent *A*). N.m.r. data: ^{1}H, δ 7.0–7.6 (40 H, 8 Ph), 5.34 (dd, $J_{2,3}$ 10, $J_{3,4}$ 3 Hz, H-3 of Gal); ^{13}C, δ 103.4 (C-1 of Gal), 102.95 (C-1 of Glc), 84.8, 82.45 (C-3,4 of Gal).

Anal. Found: C, 75.80; H, 6.32.

Benzyl 2,6-di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-β-D-galactopyranoside (**23**). — A solution of **21** (180 mg, 0.167 mmol) in MeOH (7 mL) and pyridine (1 mL) was mixed with 2M MeONa in MeOH (0.09 mL) and kept for 16 h at 20°. The mixture was deionised with KU-2 (H^+) resin, filtered, and concentrated. Column chromatography (solvent *A*) of the residue gave **23** (98 mg, 57%), $[\alpha]_D^{20}$ +30° (*c* 1, chloroform), R_F 0.29 (solvent *A*). ^{1}H-N.m.r. data: δ 7.18–7.34 (35 H, 7 Ph), 2.72 (bs, 1 H, OH).

Anal. Calc. for $C_{61}H_{64}O_{11}$: C, 69.58; H, 6.08. Found: C, 69.55; H, 6.0.

Benzyl 2,6-di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**24**). — Treatment of **22** (300 mg, 0.28 mmol) as described for **21** gave **24** (230 mg, 84.9%), $[\alpha]_D^{20}$ −4° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.1–7.37 (35 H, 7 Ph), 2.64 (bs, OH).

Anal. Calc. for $C_{61}H_{64}O_{11}$: C, 69.58; H, 6.08. Found: C, 69.48; H, 6.09.

4-O-α-D-Glucopyranosyl-D-galactose (**4**). — Compound **23** (90 mg, 0.093 mmol) was hydrogenolysed over 10% Pd/C in ethanol (10 mL) and ethyl acetate (1 mL) at 36°. The reaction was monitored by t.l.c. (solvent *E*). The solution was filtered, the insoluble material was washed with ethanol, and the combined filtrate and washings were concentrated to give **4** (30 mg, 94.9%), $[\alpha]_D^{20}$ +118° (*c* 1, water); Glc:Gal ratio, 1:1. For the ^{13}C-n.m.r. data, see Table I.

4-O-β-D-Glucopyranosyl-D-galactose (**7**). — Treatment of **24** (120 mg, 0.123 mmol) as described for **23** gave **7** (40 mg, 95.2%), $[\alpha]_D^{20}$ +28.5° (*c* 1, water); Glc:Gal ratio, 1:1. For the ^{13}C-n.m.r. data, see Table I.

Benzyl 3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,6-di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-β-D-galactopyranoside (**25**). — Reaction of **23** (97 mg, 0.1 mmol), **12** (50 mg, 0.1 mmol), and $Hg(CN)_2$ (25 mg, 0.1 mmol), under conditions described for the synthesis of **13**, gave, after column chromatography (solvent *B*), **25** (80 mg, 56.1%), $[\alpha]_D^{20}$ +46° (*c* 1, chloroform), R_F 0.48 (solvent *B*). N.m.r. data: ^{1}H, δ 7.1–7.4 (50 H, 10 Ph), 2.13 (s, 3 H, Ac); ^{13}C, δ 103.2 (C-1 of Gal), 99.3 (C-1 of Glc), 94.3 (C-1 of Man), 81.7 and 81.2 (C-3,4 of Gal).

Anal. Calc. for $C_{90}H_{94}O_{17}$: C, 74.68; H, 6.50. Found: C, 74.53; H, 6.51.

Benzyl 3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,6-di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**26**). — Reaction of **24** (230 mg, 0.24 mmol), **12** (126 mg, 0.25 mmol), and $Hg(CN)_2$ (110 mg, 0.43 mmol), as described above, gave, after column chromatography

(solvent *B*), **26** (205 mg, 60%), $[\alpha]_D^{20}$ +15° (*c* 1, chloroform), R_F 0.4 (solvent *B*). N.m.r. data: ^{1}H, δ 7.1–7.45 (50 H, 10 Ph), 2.0 (s, 3 H, Ac): ^{13}C, δ 103.9 (C-1 of Gal), 102.95 (C-1 of Glc), 94.4 (C-1 of Man), 84.7 and 82.8 (C-3,4 of Gal).

Anal. Calc. for $C_{90}H_{94}O_{17}$: C, 74.68; H, 6.5. Found: C, 74.50; H, 6.49.

*Benzyl 2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl)-3-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-β-*D*-galactopyranoside* (**27**). — Deacetylation of **25** (430 mg, 0.3 mmol), as described for **23**, gave **27** (320 mg, 76.7%), $[\alpha]_D^{20}$ +59° (*c* 1, chloroform), R_F 0.24 (solvent *D*). ^{1}H-N.m.r. data: δ 7.16–7.42 (50 H, 10 Ph), 2.59 (bs, 1 H, OH).

Anal. Calc. for $C_{88}H_{92}O_{16}$: C, 75.21; H, 6.55. Found: C, 75.00; H, 6.60.

*Benzyl 2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-3-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-β-*D*-galactopyranoside* (**28**). — Deacetylation of **26** (375 mg, 0.26 mmol) produced **28** (300 mg, 82.4%), $[\alpha]_D^{20}$ +18° (*c* 1, chloroform), R_F 0.22 (solvent *D*). ^{1}H-N.m.r. data: δ 7.0–7.5 (50 H, 10 Ph), 2.76 (bs, 1 H, OH).

Anal. Calc. for $C_{88}H_{92}O_{16}$: C, 75.21; H, 6.55. Found: C, 75.14; H, 6.45.

*4-*O*-α-*D*-Glucopyranosyl-3-*O*-α-*D*-mannopyranosyl-*D*-galactose* (**5**). — Debenzylation of **27** (108 mg, 0.08 mmol), as described above for **23**, gave **5** (36 mg, 93%), $[\alpha]_D^{20}$ +149° (*c* 1, water); Man:Gal:Glc ratio, 1:1:1. For the ^{13}C-n.m.r. data, see Table I.

*4-*O*-β-*D*-Glucopyranosyl-3-*O*-α-*D*-mannopyranosyl-*D*-galactose* (**8**). — Debenzylation of **28** (150 mg, 0.104 mmol), as described for **23**, gave **8** (53 mg, 98.5%), $[\alpha]_D^{20}$ +71° (*c* 1, water); Man:Gal:Glc ratio, 1:1:1. For the ^{13}C-n.m.r. data, see Table I.

*Benzyl 3-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl]-2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl)-β-*D*-galactopyranoside* (**29**). — The coupling of **27** (220 mg, 0.153 mmol) and **12** (85 mg, 0.17 mmol) in the presence of $Hg(CN)_2$ (75 mg, 0.3 mmol), under the conditions described for **13**, gave, after column chromatography (solvent *B*), **29** (250 mg, 81.3%), $[\alpha]_D^{20}$ +42° (*c* 1, chloroform), R_F 0.83 (solvent *B*). N.m.r. data: ^{1}H, δ 7.1–7.43 (65 H, 13 Ph), 2.1 (s, 3 H, Ac); ^{13}C, δ 103.0 (C-1 of Gal), 100.3 (C-1 of Man^{II}), 99.75 (C-1 of Glc), 95.2 (C-1 of Man^{I}), 81.3 and 81.75 (C-3,4 of Gal), 79.6 (C-2 of Man^{I}).

Anal. Calc. for $C_{117}H_{122}O_{22}$: C, 74.22; H, 6.55. Found: C, 73.85; H, 6.53.

*Benzyl 3-*O*-[2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl]-2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-β-*D*-galactopyranoside* (**30**). — Reaction of **28** (70 mg, 0.049 mmol), **12** (25 mg, 0.05 mmol), and $Hg(CN)_2$ (25 mg, 0.09 mmol), as described above, gave **30** (52 mg, 53.4%), $[\alpha]_D^{20}$ +27° (*c* 1, chloroform), R_F 0.85 (solvent *B*). N.m.r. data: ^{1}H, δ 7.0–7.4 (65 H, 13 Ph), 2.1 (s, 3 H, Ac); ^{13}C, δ 104.2 (C-1 of Gal), 102.65 (C-1 of Glc), 101.0 (C-1 of Man^{II}), 95.0 (C-1 of Man^{I}), 85.2 and 82.5 (C-3,4 of Gal), 79.5 (C-2 of Man^{I}).

Anal. Calc. for $C_{117}H_{122}O_{22}$: C, 74.22; H, 6.55. Found: C, 73.91; H, 6.48.

Benzyl 2,6-di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-3-O-[3,4,6-tri-O-benzyl-2-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-β-D-galactopyranoside (**31**). — Deacetylation of **29** (250 mg, 0.133 mmol), as described for the preparation of **23**, gave, after column chromatography (solvent *B*), **31** (180 mg, 73.6%), $[\alpha]_D^{20}$ +53° (*c* 1, chloroform), R_F 0.38 (solvent *B*). ^{1}H-N.m.r. data: δ 6.99–7.39 (65 H, 13 Ph), 2.39 (bs, 1 H, OH).

Anal. Calc. for $C_{115}H_{120}O_{21}$: C, 75.16; H, 6.54. Found: C, 74.85; H, 6.49.

Benzyl 2,6-di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-3-O-[3,4,6-tri-O-benzyl-2-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-β-D-galactopyranoside (**32**). — Deacetylation of **30** (120 mg, 0.064 mmol), as described above, gave **32** (105 mg, 89.5%), $[\alpha]_D^{20}$ +28° (*c* 1, chloroform), R_F 0.61 (solvent *B*). ^{1}H-N.m.r. data: δ 7.0–7.41 (65 H, 13 Ph), 2.41 (bs, 1 H, OH).

Anal. Calc. for $C_{115}H_{120}O_{21}$: C, 75.15; H, 6.54. Found: C, 74.90; H, 6.48.

4-O-α-D-Glucopyranosyl-3-O-(2-O-α-D-mannopyranosyl-α-D-mannopyranosyl)-D-galactose (**6**). — Catalytic hydrogenolysis of **31** (180 mg, 0.1 mmol), as described above, gave **6** (59 mg, 90.5%), $[\alpha]_D^{20}$ +127° (*c* 1, water); Man:Gal:Glc ratio, 2:1:1. For the ^{13}C-n.m.r. data, see Table I.

4-O-β-D-Glucopyranosyl-3-O-(2-O-α-D-mannopyranosyl-α-D-mannopyranosyl)-D-galactose (**9**). — Catalytic hydrogenolysis of **32** (105 mg, 0.06 mmol), as described above, gave **9** (37 mg, 97.2%), $[\alpha]_D^{20}$ +70° (*c* 1, water); Man:Gal:Glc ratio, 2:1:1. For the ^{13}C-n.m.r. data, see Table I.

Acetylation of oligosaccharides. — Acetic anhydride (1 mL) was added to a solution of the oligosaccharide (30–50 mg) in pyridine (1 mL), and the mixture was kept at 20° for 16 h and then concentrated. Column chromatography of the residue then gave the product. The following acetates were prepared in this manner.

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-D-galactopyranose (**33**; 55 mg, 92%), $[\alpha]_D^{20}$ +128° (*c* 1, chloroform), R_F 0.49 (solvent *C*). ^{1}H-N.m.r. data: δ 1.98–2.09 (24 H, 8 Ac).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.26; H, 5.60. Found: C, 49.24; H, 5.62.

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-galactopyranose (**34**; 127 mg, 87%), $[\alpha]_D^{20}$ +49° (*c* 1, chloroform), R_F 0.4 (solvent *C*). ^{1}H-N.m.r. data: δ 1.92–2.1 (24 H, 8 Ac).

Anal. Found: C, 49.21; H, 5.50.

1,2,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-D-galactopyranose (**35**; 52 mg, 90.7%), $[\alpha]_D^{20}$ +74° (*c* 1, chloroform), R_F 0.33 (solvent *C*). ^{1}H-n.m.r. data: δ 2.0–2.19 (33 H, 11 Ac).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.59. Found: C, 49.43; H, 5.47.

1,2,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-D-galactopyranose (**36**; 80 mg, 84%),

$[\alpha]_D^{20}$ +37° (*c* 1, chloroform), R_F 0.33 (solvent *C*). ^{1}H-n.m.r. data: δ 1.98–2.20 (33 H, 11 Ac).

Anal. Found: C, 49.52; H, 5.50.

1,2,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-3-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-D-galactopyranose (**37**; 80 mg, 74.6%), $[\alpha]_D^{20}$ +70° (*c* 1, chloroform), R_F 0.24 (solvent *C*). ^{1}H-N.m.r. data: δ 2.1–2.17 (42 H, 14 Ac).

Anal. Calc. for $C_{52}H_{70}O_{35}$: C, 41.79; H, 5.58. Found: C, 41.53; H, 5.60.

1,2,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-D-galactopyranose (**38**; 84 mg, 94.5%), $[\alpha]_D^{20}$ +25° (*c* 1, chloroform), R_F 0.24 (solvent *C*). ^{1}H-N.m.r. data: δ 2.0–2.15 (42 H, 14 Ac).

Anal. Found: C, 41.49; H, 5.55.

Preparation of glycosyl phosphates. — The acetylated oligosaccharide was melted *in vacuo* at 56° with 10 mol of H_3PO_4 for 2 h. The mixture was then diluted with M LiOH to pH >11 and stirred overnight. The Li_3PO_4 was removed by centrifugation, and the supernatant solution was neutralised with KU-2 (Py^+) resin to pH 7 and then subjected to ion-exchange chromatography (see above). The following glycosyl phosphates were prepared in this way.

3-*O*-(2-*O*-α-D-Mannopyranosyl-α-D-mannopyranosyl)-α-D-galactopyranosyl phosphate (**39**; 46 μmol, 65.5%), $[M]_D^{20}$ +528° (water), $[\alpha]_D^{20}$ +85° (*c* 10mM, water), $E_{Glc\text{-}1\text{-}P}$ 0.68; Man:Gal:phosphate ratio, 2:1:1.

3-*O*-(2-*O*-α-D-Mannopyranosyl-β-D-mannopyranosyl)-α-D-galactopyranosyl phosphate (**40**; 31.6 μmol, 51%), $[M]_D^{20}$ +332° (water), $[\alpha]_D^{20}$ +54° (*c* 32mM, water), $E_{Glc\text{-}1\text{-}P}$ 0.68; Man:Gal:phosphate ratio, 2:1:1.

4-*O*-α-D-Glucopyranosyl-α-D-galactopyranosyl phosphate (**41**; 33 μmol, 74.4%), $[M]_D^{20}$ +554° (water), $[\alpha]_D^{20}$ +121.5° (*c* 33mM, water), $E_{Glc\text{-}1\text{-}P}$ 0.87; Gal:Glc:phosphate ratio, 1:1:1.

4-*O*-β-D-Glucopyranosyl-α-D-galactopyranosyl phosphate (**42**; 43.9 μmol, 60%), $[M]_D^{20}$ +330° (water), $[\alpha]_D^{20}$ +72° (*c* 52mM, water), $E_{Glc\text{-}1\text{-}P}$ 0.87; Gal:Glc:phosphate ratio, 1:1:1.

4-*O*-α-D-Glucopyranosyl-3-*O*-α-D-mannopyranosyl-α-D-galactopyranosyl phosphate (**43**; 24.8 μmol, 55%), $[M]_D^{20}$ +709° (water), $[\alpha]_D^{20}$ +115° (*c* 12mM, water), $E_{Glc\text{-}1\text{-}P}$ 0.77; Man:Gal:Glc:phosphate ratio, 1:1:1:1.

4-*O*-β-D-Glucopyranosyl-3-*O*-α-D-mannopyranosyl-α-D-galactopyranosyl phosphate (**44**; 49.7 μmol, 64.8%), $[M]_D^{20}$ +432° (water), $[\alpha]_D^{20}$ +68° (*c* 48.5mM, water), $E_{Glc\text{-}1\text{-}P}$ 0.77; Man:Gal:Glc:phosphate ratio, 1:1:1:1.

4-*O*-α-D-Glucopyranosyl-3-*O*-(2-*O*-α-D-mannopyranosyl-α-D-mannopyranosyl)-α-D-galactopyranosyl phosphate (**45**; 35 μmol, 54.9%), $[M]_D^{20}$ +872° (water), $[\alpha]_D^{20}$ +111.5° (*c* 33mM, water), $E_{Glc\text{-}1\text{-}P}$ 0.69; Man:Gal:Glc:phosphate ratio, 2:1:1:1.

4-*O*-β-D-Glucopyranosyl-3-*O*-(2-*O*-α-D-mannopyranosyl-α-D-mannopyranosyl)-α-D-galactopyranosyl phosphate (**46**; 40 μmol, 60%), $[M]_D^{20}$ +508° (water),

$[\alpha]_D^{20}$ +66° (*c* 33mM, water), $E_{Glc\text{-}1\text{-}P}$ 0.69; Man:Gal:Glc:phosphate ratio, 2:1:1:1. The n.m.r. data for the glycosyl phosphates **39–46** are given in Table II.

ACKNOWLEDGMENTS

The authors thank Dr. A. S. Shashkov for measuring the n.m.r. spectra, Dr. Yu. A. Knirel for helpful discussion, and Professor N. K. Kochetkov for constant interest and encouragement.

REFERENCES

1 C. D. Warren and R. W. Jeanloz, *Biochemistry*, 11 (1972) 2565–2572.
2 V. N. Shibaev, *Adv. Carbohydr. Chem. Biochem.*, in press.
3 L. L. Danilov, S. D. Maltsev, V. N. Shibaev, and N. K. Kochetkov, *Carbohydr. Res.*, 88 (1981) 203–211.
4 L. L. Danilov, N. S. Utkina, V. N. Shibaev, and N. K. Kochetkov, *Bioorg. Khim.*, 7 (1981) 1718–1721.
5 V. N. Shibaev, T. N. Druzhinina, N. A. Kalinchuk, S. D. Maltsev, L. L. Danilov, V. I. Torgov, N. K. Kochetkov, S. Sh. Rozhnova, and V. A. Kilesso, *Bioorg. Khim.*, 9 (1983) 564–565.
6 T. N. Druzhinina, V. N. Shibaev, N. K. Kochetkov, S. Sh. Rozhnova, and V. A. Kilesso, *Bioorg. Khim.*, 10 (1984) 1548–1551.
7 N. K. Kochetkov, V. N. Shibaev, T. N. Druzhinina, L. M. Gogilashvili, L. L. Danilov, V. I. Torgov, S. D. Maltsev, and N. S. Utkina, *Dokl. Akad. Nauk SSSR*, 262 (1982) 1393–1397.
8 V. N. Shibaev, L. L. Danilov, T. N. Druzhinina, L. M. Gogilashvili, S. D. Maltsev, and N. K. Kochetkov,. *FEBS Lett.*, 139 (1982) 177–180.
9 V. N. Shibaev, T. N. Druzhinina, L. M. Gogilashvili, N. K. Kochetkov, S. Sh. Rozhnova, and V. A. Kilesso, *Dokl. Akad. Nauk SSSR*, 270 (1983) 897–899.
10 V. N. Shibaev, L. L. Danilov, T. N. Druzhinina, V. I. Torgov, L. M. Gogilashvili, and N. S. Utkina, *Bioorg. Khim.*, 8 (1982) 564–566.
11 C. G. Hellerqvist, J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 26 (1972) 3282–3286.
12 V. N. Shibaev, T. N. Druzhinina, A. N. Popova, S. Sh. Rozhnova, and V. A. Kilesso, *Eur. J. Biochem.*, 101 (1979) 309–316.
13 V. I. Torgov, C. A. Panossian, A. T. Smeljansky, and V. N. Shibaev, *Bioorg. Khim.*, 11 (1985) 83–90.
14 T. Ogawa and K. Sasajima, *Carbohydr. Res.*, 97 (1981) 205–227.
15 N. K. Kochetkov, V. I. Torgov, N. N. Malysheva, A. S. Shashkov, and E. M. Klimov, *Tetrahedron*, 36 (1980) 1227–1230.
16 R. U. Lemieux, K. B. Hendriks, R. V. Stick, and K. James, *J. Am. Chem. Soc.*, 97 (1975) 4056–4062.
17 N. K. Kochetkov, O. S. Chizhov, and A. S. Shashkov, *Carbohydr. Res.*, 133 (1984) 173–185.
18 D. L. MacDonald, *Methods Carbohydr. Chem.*, 6 (1972) 389–392.
19 L. L. Danilov, M. F. Troitsky, N. S. Utkina, V. N. Shibaev, and N. K. Kochetkov, *Carbohydr. Res.*, 87 (1980) 141–146.
20 J. W. O'Connor, H. A. Nunez, and R. Barker, *Biochemistry*, 18 (1979) 500–507.
21 L. L. Danilov, N. S. Utkina, and V. N. Shibaev, *Bioorg. Khim.*, 6 (1980) 780–782.
22 N. E. Byramova, L. V. Backinovsky, and N. K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1985) 1140–1145.
23 V. I. Betaneli, M. V. Ovchinnikov, L. V. Backinovsky, and N. K. Kochetkov, *Carbohydr. Res.*, 84 (1980) 211–224.
24 L. V. Backinovsky, A. R. Gomtzjan, N. E. Byramova, and N. K. Kochetkov, *Bioorg. Khim.*, 10 (1984) 79–87.