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Synthesis of two phosphate-containing "heptasaccharide" fragments of the capsular polysaccharides of *Streptococcus pneumoniae* types 6A and 6B

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ABSTRACT

The "heptasaccharides" *O*-α-D-galactopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→3)-α,β-L-rhamnopyranose 2''-[*O*-α-D-galactopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→3)-D-ribit-5-yl sodium phosphate] (**25**) and *O*-α-D-galactopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→3)-α,β-L-rhamnopyranose 2''-[*O*-α-D-galactopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→4)-D-ribit-5-yl sodium phosphate] (**27**), which are structural elements of the capsular polysaccharides of *Streptococcus pneumoniae* types 6A and 6B {[→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→X)-D-RibOH-(5-P→]$_n$; 6A X = 3, 6B X = 4}, respectively, have been synthesised. 2,4-Di-*O*-acetyl-3-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranosyl trichloroacetimidate (**13**) was coupled with 5-*O*-allyloxycarbonyl-1,2,4-tri-*O*-benzyl-D-ribitol (**10**), using trimethylsilyl triflate as a promotor (→**14**), and deallyloxycarbonylation (→**15**) and conversion into the corresponding triethylammonium phosphonate then gave **16**. Condensation of **16** with 4-methoxybenzyl 2,4-di-*O*-benzyl-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranoside (**22**) followed by oxidation and deprotection afforded **25**. 5-*O*-Allyl-1-*O*-allyloxycarbonyl-2,3-di-*O*-benzyl-D-ribitol (**12**) was coupled with **13**, using trimethylsilyl triflate as a promoter, the resulting tetrasaccharide-alditol derivative **17** was deallyloxycarbonylated (→**18**), acetylated (→**19**), and deallylated (→**20**), and the product was converted into the triethylammonium phosphonate derivative **21**. Condensation of **21** with **22** followed by oxidation and deprotection afforded **27**.

INTRODUCTION

In studies of the development of synthetic vaccines, based on oligosaccharide conjugates, against infections by *Streptococcus pneumoniae* serotypes, attention has been focused on the preparation of structural elements of the capsular polysaccharides of the serotypes 6A and 6B {[→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→X)-D-RibOH-(5-P→]$_n$; 6A X = 3, 6B X = 4}. Recently, the building block, 4-methoxybenzyl 2,4-di-*O*-benzyl-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranoside[1] (**22**) has been synthesised, which is a starting point suitable for the preparation of higher oligomers. Furthermore, syntheses of the non-phosphorylated structural elements[2], α-D-Glc*p*-

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(1→3)-L-Rha*p*, α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-L-Rha*p*, α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-RibOH, and α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-RibOH have been described.

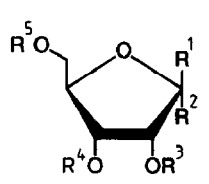
We now report the synthesis of two phosphate-containing "heptasaccharide" fragments of the capsular polysaccharides of the serotypes 6A and 6B, respectively, namely, α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-RibOH-(5-P→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-L-Rha*p* (**25**) and α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-RibOH-(5-P→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-L-Rha*p* (**27**).

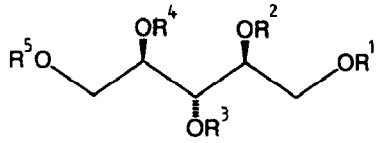
RESULTS AND DISCUSSION

The synthesis of **25** and **27** involved the tetrasaccharide derivatives **15** and **20**, respectively, and the trisaccharide derivative **22** (ref. 1) as key intermediates. Both **15** and **20** have the same precursor, namely, the trisaccharide imidate **13** (ref. 2). For the introduction of the phosphodiester bridge between **15** and **22**, and **20** and **22**, the phosphonate approach[3-5] was selected. Phosphonic monoesters are generated easily through a base-induced reaction between salicylchlorophosphite and a hydroxyl function, followed by hydrolysis[4-9]. In order to synthesise phosphonic diesters, the monoesters can be activated with pivaloyl chloride[7] in the presence of an aglycon and a mild base such as pyridine. The intermediates in the phosphonate method are stable enough to be purified by column chromatography before oxidation to the corresponding phosphoric diesters.

For the synthesis of **15** and **20**, the ribitol synthons **10** and **12** were needed and were prepared as follows. Methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside[10,11] (**1**) was crotylated (→**2**, 98%), de-isopropylidenated by methanolysis (→**3**, 73%), and selectively benzylated using a phase-transfer catalyst[1,2,12] (→**4**, 61%). After crotylation[13] of **4** (→**5**, 95%), removal of MeO-1 (→**6**, 85%), and reduction with sodium borohydride (→**7**, 84%), the product was benzylated to give **8** (97%). Decrotylation (15 min) of **8** was performed with KO^{t}Bu in *N*,*N*-dimethylformamide at 80° (→**9**, 58%) and the primary hydroxyl group was selectively protected with the allyloxycarbonyl group[14] to yield 5-*O*-allyloxycarbonyl-1,2,4-tri-*O*-benzyl-D-ribitol (**10**, 78%). In a similar way, the primary hydroxyl function of 5-*O*-allyl-2,3-di-*O*-benzyl-D-ribitol[15] (**11**) was protected with the allyloxycarbonyl group to afford 5-*O*-allyl-1-*O*-allyloxycarbonyl-2,3-di-*O*-benzyl-D-ribitol (**12**, 52%).

Coupling of 2,4-di-*O*-acetyl-3-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranosyl trichloroacetimidate[2] (**13**) with the ribitol synthon **10** in dichloromethane, using trimethylsilyl triflate as a promoter, gave **14** (75%), and removal of the allyloxycarbonyl group[14] then gave **15** (77%). Conversion of **15** into its triethylammonium phosphonate derivative, using salicylchlorophosphite[6], followed by the addition of water and pyridine yielded **16** (58%). The ribitol synthon **12** was also coupled with **13** in dichloromethane, using trimethylsilyl triflate as a promoter, to give **17** (88%). Subsequent removal of the allyloxycarbonyl group[14] (→**18**, 73%), acetylation (→**19**, 82%), and de-allylation with





1	R^1 = OMe, R^2 = R^5 = H, R^3,R^4 = C(Me)$_2$	**7**	R^1 = R^4 = H, R^2 = Bn, R^3 = R^5 = crotyl
2	R^1 = OMe, R^2 = H, R^3,R^4 = C(Me)$_2$, R^5 = crotyl	**8**	R^1 = R^2 = R^4 = Bn, R^3 = R^5 = crotyl
3	R^1,R^2 = H,OMe, R^3 = R^4 = H, R^5 = crotyl	**9**	R^1 = R^2 = R^4 = Bn, R^3 = R^5 = H
4	R^1 = OMe, R^2 = R^4 = H, R^3 = Bn, R^5 = crotyl	**10**	R^1 = R^2 = R^4 = Bn, R^3 = H, R^5 = AOC
5	R^1 = OMe, R^2 = H, R^3 = Bn, R^4 = R^5 = crotyl	**11**	R^1 = R^4 = H, R^2 = R^3 = Bn, R^5 = allyl
6	R^1,R^2 = H,OH, R^3 = Bn, R^4 = R^5 = crotyl	**12**	R^1 = AOC, R^2 = R^3 = Bn, R^4 = H, R^5 = allyl

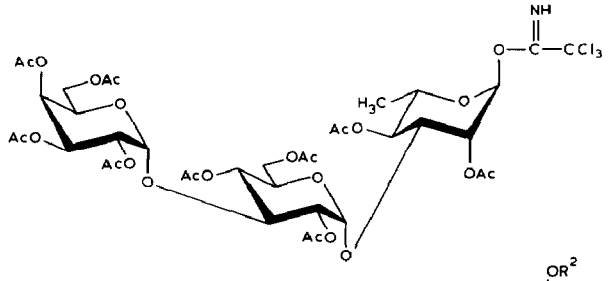
AOC = allyloxycarbonyl

palladium(II) chloride[16] afforded **20** (70%). Compound **20** was converted, as described for **15**, into the triethylammonium phosphonate derivative **21** (55%). The phosphonate derivative **23** (70%) of **22** was synthesised also by the above route.

Prior to the synthesis of **25** and **27**, the conjugation of the trisaccharide phosphonate derivative **23** with **15** and **20** was studied. Coupling of **15** with **23** in the presence of pivaloyl chloride[7] in acetonitrile and pyridine or with 3,3'-(chlorophosphonylidene)-bis(2-oxo-1,3-oxazolidene)[8,17] in pyridine gave the intermediate phosphonic diester, oxidation of which with iodine in pyridine–water gave **24** (12%). In a similar way, **20** was condensed with **23** to give, after oxidation, **26** (14%). The rather low yields of **24** and **26** were due to degradation of the phosphonate derivative **23** into **22** during the coupling. The tetrasaccharide-alditol derivatives **15** and **20** were recovered easily after the oxidation of the intermediate phosphonic diesters. The activation of **23** was possible only with the addition of pivaloyl chloride in amounts less than 1 equiv., otherwise the hydroxyl functions of **15** and **20** reacted with pivaloyl chloride.

The disappointing results using **23** prompted a change in strategy in which the tetrasaccharide phosphonate derivatives **16** and **21** were used to generate the final phosphodiester bridges. Coupling of **16** with 4-methoxybenzyl 2,4-di-*O*-benzyl-3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranoside[1] (**22**) in the presence of pivaloyl chloride in acetonitrile and pyridine gave the phosphonic diester, oxidation of which with iodine in pyridine–water afforded the phosphoric diester **24** (71%). Deprotection of **24** yielded the required "heptasaccharide" **25** (59%). In a similar way, **21** was coupled with **22** to give, after oxidation, the phosphoric diester **26** (67%), and deprotection then gave the required "heptasaccharide" **27** (60%).

The relevant ^{1}H-n.m.r. data for **25** and **27** are summarised in Table I. The assignment of the signals was guided by the n.m.r. data of the capsular polysaccharides of serotypes 6A and 6B, and the oligosaccharide α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-RibOH-(5-P→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-RibOH (**HF**) obtained[18] by depolymerisation of the polysaccharide



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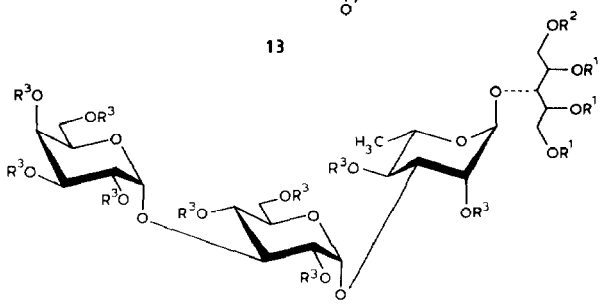
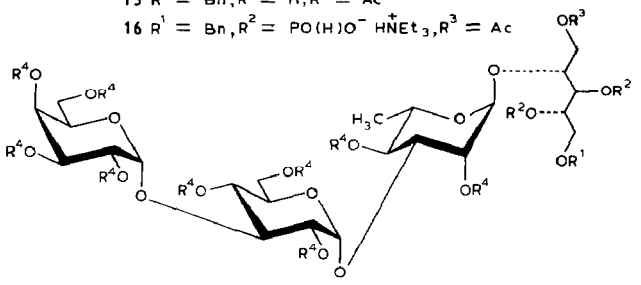
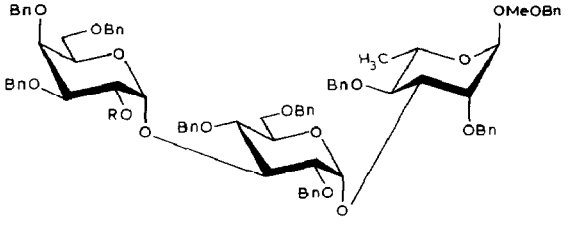


14 R^1 = Bn, R^2 = AOC, R^3 = Ac
15 R^1 = Bn, R^2 = H, R^3 = Ac
16 R^1 = Bn, R^2 = $PO(H)O^- \overset{+}{H}NEt_3$, R^3 = Ac

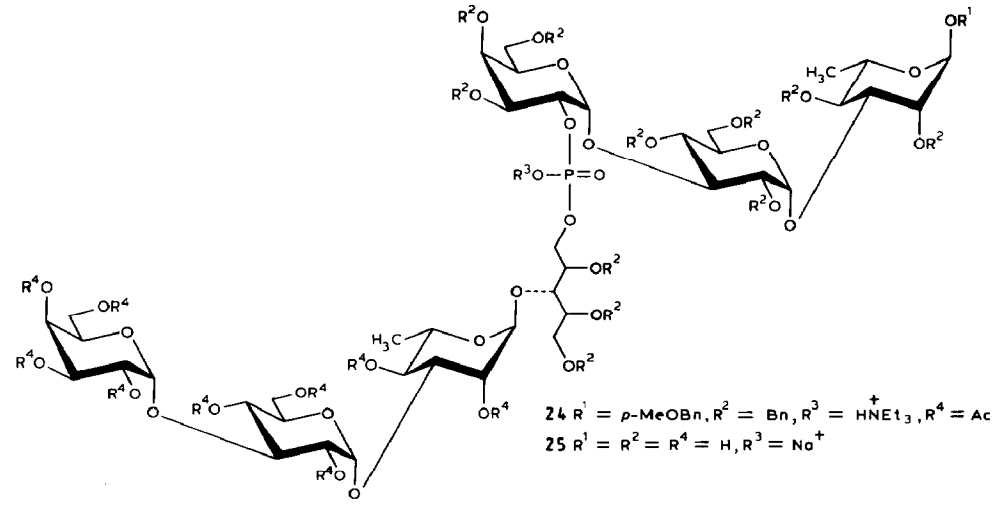


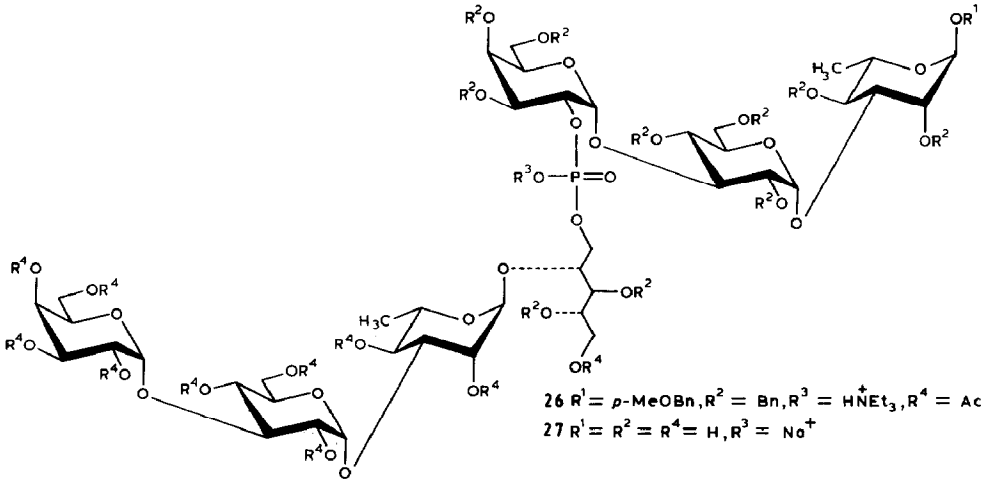
17 R^1 = AOC, R^2 = Bn, R^3 = allyl, R^4 = Ac
18 R^1 = H, R^2 = Bn, R^3 = allyl, R^4 = Ac
19 R^1 = R^4 = Ac, R^2 = Bn, R^3 = allyl
20 R^1 = R^4 = Ac, R^2 = Bn, R^3 = H
21 R^1 = R^4 = Ac, R^2 = Bn, R^3 = $PO(H)O^- \overset{+}{H}NEt_3$



22 R = H
23 R = $PO(H)O^- \overset{+}{H}NEt_3$

AOC = allyloxycarbonyl





6B with hydrogen fluoride, as well as by applying $^1H\{^{31}P\}$ relayed spin-echo difference spectroscopy (RESED)[19] to **25** and **27**.

The testing of **25** and **27** in immunological inhibition experiments is being investigated and may help to identify the antigenic determinant of serotypes 6A and 6B.

EXPERIMENTAL

General methods. — The ^1H- (360 and 500 MHz), ^{13}C- (50 MHz), and ^{31}P- (80 and 202 MHz) n.m.r. spectra were recorded variously with Bruker WP 200, WM 200, HX 360, and AM 500 spectrometers at 25°. Chemical shifts (δ) are given in p.p.m. relative to the signal for internal Me_4Si ($CDCl_3$) or internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (D_2O; indirectly to internal acetone, δ 2.225) for ^1H, relative to the signal for internal Me_4Si ($CDCl_3$; indirectly to $CDCl_3$, δ 76.9) and external Me_4Si (D_2O; indirectly to internal acetone, δ 31.55) for ^{13}C, and relative to the signal for external 85% H_3PO_4

TABLE I

^{1}H-N.m.r. chemical shift data[a] (δ) for **25** and **27**, the polysaccharides **6A** and **6B** from *Streptococcus pneumoniae* serotypes 6A and 6B, respectively, and the oligosaccharide **HF** prepared from polysaccharide 6B[18]

Proton	*Compound*[b]							
	6A	**25**		**6B**[c]	**HF**		**27**	
		Gly'	*Gly*		*Gly'*	*Gly*	*Gly'*	*Gly*
Gal								
H-1	5.606(4.2)	5.395(4.0)	5.630(4.0)	5.607	5.399(4.0)	5.623(4.0)	5.399(4.0)	5.625(4.1)
H-2			4.32[d]			4.295		4.30[d]
H-3			4.01[d]			4.007		4.01[d]
Glc								
H-1	5.124(4.0)	5.136(3.8)	5.096(3.9)[e]	5.146	5.153(3.6)	5.118(3.9)	5.152(3.9)	5.095(3.8)[f]
			5.123(3.9)[e]					5.122(3.9)[f]
Rha								
H-1	5.039(bd)	5.042(1.5)	5.154(1.9)α	5.146	5.153(bd)	5.077(1.8)	5.156(bd)	5.156(bd)α
			4.868(s) β					4.868(s) β
CH_3	1.312(6.2)	1.311(6.2)	1.297(6.2)α	1.319	1.310(6.3)	1.310(6.3)	1.319(6.2)	1.297(6.2)α
			1.311(6.2)β					1.314(5.9)β
RibOH								
H-5		n.d.			4.233		4.24[d]	
H-5'		4.12[d]			4.122		4.12[d]	

[a] Chemical shifts are relative to the signal of sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone at δ 2.225 p.p.m.) in D_2O. Coupling constants (Hz) for $J_{1,2}$ and $J_{5,6}$ are given in brackets. [b] The primes in the structures **25**, **27** and **HF** have been placed to distinguish the "reducing-end" monosaccharides (Gly) from the "non-reducing" ones (Gly'). Note that this coding system differs from the usual coding system applied in the Experimental. [c] Recorded at 50°, poorly resolved spectrum. [d] Determined from $^1H\{^{31}P\}$ relayed spin-echo difference spectroscopy experiments[19]. [e,f] Because of the anomerisation effect of the reducing Rha unit, two doublets are observed for H-1 of the adjacent Glc residue; the lowest δ value corresponds to the α anomer.

for ^{31}P data. $^1H\{^{31}P\}$ relayed spin-echo difference spectroscopy based on MLEV-17 (RESED) was carried out as described[19].

Column chromatography was performed on Kieselgel 60 (Merck, <230 mesh) and fractions were monitored by t.l.c. on Kieselgel 60 F_{254} (Merck). Detection was effected by charring with sulfuric acid after examination under u.v. light. Optical rotations were measured at 20° with a Perkin–Elmer 241 polarimeter, using a 10-cm microcell. Evaporations were conducted *in vacuo* at 40° (bath). All solvents were distilled from appropriate drying agents.

Methyl 5-O-crotyl-2,3-O-isopropylidene-β-D-ribofuranoside (**2**). — To a stirred suspension of sodium hydride (7.2 g) in dry *N*,*N*-dimethylformamide (40 mL) at 0° was added a mixture of methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside[10,11] (**1**; 16.5 g, 80.8 mmol) and crotyl bromide (11.4 mL) in *N*,*N*-dimethylformamide (30 mL). After stirring

the mixture for 2 h at room temperature, the reaction was complete (t.l.c., R_F 0.43, 9:1 dichloromethane–ethyl acetate). The excess of sodium hydride was decomposed with methanol, the mixture was poured onto crushed ice and extracted with ether (3 × 150 mL), and the combined extracts were dried ($MgSO_4$), filtered, and concentrated. Column chromatography (99:1 dichloromethane–ethyl acetate) of the residue gave **2**, isolated as a syrup (20.5 g, 98%), $[\alpha]_D$ −55° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^1H, δ 1.320 and 1.484 (2 s, each 3 H, CMe_2), 1.715 (d, 3 H, $J_{CH_3,CH}$ 6.0 Hz, $OCH_2CH=CHCH_3$), 3.322 (s, 3 H, OMe), 3.943 (d, 2 H, $OCH_2CH=CHCH_3$), 4.961 (s, 1 H, H-1), 5.641 (m, 2 H, $OCH_2CH=CHCH_3$); ^{13}C, δ 17.5 ($OCH_2CH=CHCH_3$), 24.8 and 26.3 [$C(CH_3)_2$], 54.5 (OMe), 70.5 and 71.7 ($CH_2CH=CHCH_3$, C-5), 82.0 and 85.0 (2 C) (C-2,3,4), 109.0 (C-1), 110.5 [$C(CH_3)_2$], 127.2 and 129.4 ($OCH_2CH=CHCH_3$).

Anal. Calc. for $C_{13}H_{22}O_5$: C, 60.45; H, 8.58. Found: C, 60.30; H, 8.57.

Methyl 5-O-crotyl-α,β-D-ribofuranoside (**3**). — A solution of **2** (20.4 g, 79.0 mmol) in methanol (220 mL) and 2M sulfuric acid (22 mL) was boiled under reflux until t.l.c. showed complete conversion into **3** (R_F 0.30, 1:1 dichloromethane–ethyl acetate). The mixture was neutralised with sodium hydrogencarbonate, concentrated, diluted with 1:1 dichloromethane–methanol (400 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (1:1 dichloromethane–ethyl acetate) of the residue gave **3**, isolated as a syrup (12.6 g, 73%), $[\alpha]_D$ −15° (*c* 1, dichloromethane), (α,β-ratio 1:4). N.m.r. data ($CDCl_3$): ^1H, δ 1.717 (d, 3 H, $J_{CH_3,CH}$ 6.4 Hz, $OCH_2CH=CHCH_3$), 3.360 and 3.485 (2 s, together 3 H, OMe), 4.843 (s, 0.8 H, H-1β), 4.947 (d, 0.2 H, $J_{1,2}$ 4.5 Hz, H-1α), 5.655 (m, 2 H, $OCH_2CH=CHCH_3$); ^{13}C, δ 17.6 ($OCH_2CH=CHCH_3$), 54.9 and 55.4 (OMe), 102.7 (C-1α), 108.2 (C-1β), 127.1 and 129.8 ($OCH_2CH=CHCH_3$).

Anal. Calc. for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 54.70; H, 8.33.

Methyl 2-O-benzyl-5-O-crotyl-β-D-ribofuranoside (**4**). — To a stirred solution of **3** (1.00 g, 4.58 mmol) in dichloromethane (46 mL) was added tetrabutylammonium bromide (370 mg), benzyl bromide (6.2 mL), and aqueous 10% sodium hydroxide (4.6 mL). After stirring the mixture overnight, t.l.c. (5:1 toluene–ethyl acetate) revealed the absence of **3**. The mixture was diluted with dichloromethane (100 mL), washed with water (3 × 30 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (R_F 0.42, 5:1 toluene–ethyl acetate) of the residue gave **4**, isolated as a syrup (870 mg, 61%), $[\alpha]_D$ +3° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^1H, δ 1.702 (d, 3 H, $J_{CH_3,CH}$ 6.4 Hz, $OCH_2CH=CHCH_3$), 3.352 (s, 3 H, OMe), 3.481 (dd, 1 H, $J_{4,5b}$ 6.5, $J_{5a,5b}$ 10.4 Hz, H-5b), 3.598 (dd, 1 H, $J_{4,5a}$ 3.9 Hz, H-5a), 3.977 (d, 2 H, $OCH_2CH=CHCH_3$), 4.629 and 4.736 (2 d, 2 H, $PhCH_2$), 4.897 (s, 1 H, H-1), 7.316–7.367 (m, 5 H, Ph); ^{13}C, δ 17.7 ($OCH_2CH=CHCH_3$), 55.1 (OMe), 71.4, 72.0, and 72.7 ($OCH_2CH=CHCH_3$, $PhCH_2$, and C-5), 71.8 (C-3), 81.8 and 83.0 (C-2,4), 105.7 (C-1), 127.4–129.4 and 137.1 ($C_6H_5CH_2$ and $OCH_2CH=CHCH_3$).

Anal. Calc. for $C_{17}H_{24}O_5$: C, 66.21; H, 8.31. Found: C, 66.30; H, 7.91.

Methyl 2-O-benzyl-3,5-di-O-crotyl-β-D-ribofuranoside (**5**). — To a stirred suspension of sodium hydride (210 mg) in *N,N*-dimethylformamide (10 mL) was added a mixture of **4** (850 mg, 2.75 mmol) and crotyl bromide (0.5 mL) in *N,N*-dimethylformamide (15 mL) at 0°. After stirring the mixture for 2 h at room temperature, the

crotylation was complete (t.l.c., R_F 0.60, 5:1 toluene–ethyl acetate). The excess of sodium hydride was decomposed with methanol, the mixture was poured onto crushed ice and extracted with ether (4 × 20 mL), and the combined extracts were dried ($MgSO_4$), filtered, and concentrated. Column chromatography (5:1 toluene–ethyl acetate) of the residue gave **5**, isolated as a syrup (950 mg, 95%), $[\alpha]_D$ +23° (*c* 1, dichloromethane). ^{13}C-N.m.r. data ($CDCl_3$): δ 17.7 (2 $OCH_2CH=CHCH_3$), 55.0 (OMe), 71.1, 71.2, 71.8, and 72.2 (2 $OCH_2CH=CHCH_3$, $PhCH_2$, and C-5), 78.1, 79.6, and 80.4 (C-2,3,4), 106.3 (C-1), 127.3–129.7 and 137.8 ($OCH_2CH=CHCH_3$ and $C_6H_5CH_2$).

Anal. Calc. for $C_{23}H_{30}O_5$: C, 69.59; H, 8.34. Found: C, 69.19; H, 8.00.

2-O-Benzyl-3,5-di-O-crotyl-α,β-D-ribofuranose (**6**). — To a solution of **5** (868 mg, 2.39 mmol) in 1,4-dioxane (52 mL) was added 2M hydrochloric acid (13 mL), and the mixture was boiled under reflux until t.l.c. showed complete conversion into **6** (45 min; R_F 0.41, 8:2 dichloromethane–ethyl acetate). The mixture was neutralised with sodium hydrogencarbonate, concentrated, diluted with 1:1 methanol–dichloromethane (100 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (8:2 dichloromethane–ethyl acetate) of the residue gave **6**, isolated as a syrup (709 mg, 85%), $[\alpha]_D$ +54° (*c* 1, dichloromethane), (α,β-ratio 1:1). ^{13}C-N.m.r. data ($CDCl_3$): δ 17.3 (2 $OCH_2CH=CHCH_3$), 95.7 (C-1α), 99.7 (C-1β), 126.7–129.5, 137.1, and 137.5 ($C_6H_5CH_2$ and 2 $OCH_2CH=CHCH_3$).

2-O-Benzyl-3,5-di-O-crotyl-D-ribitol (**7**). — To a solution of **6** (597 mg, 1.71 mmol) in ethanol (8.5 mL) was added sodium borohydride (110 mg), and the mixture was stirred overnight at room temperature, when t.l.c. showed complete conversion into **7** (R_F 0.41, 7:3 dichloromethane–ethyl acetate). The pH of the mixture was adjusted to 5 with aqueous 96% acetic acid, and the mixture was concentrated, diluted with dichloromethane (60 mL), washed with M hydrochloric acid (30 mL) and water (30 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (7:3 dichloromethane–ethyl acetate) of the residue gave **7**, isolated as a syrup (505 mg, 84%), $[\alpha]_D$ +11° (*c* 1, dichloromethane). ^{13}C-N.m.r. data ($CDCl_3$): δ 17.4 (2 $OCH_2CH=CHCH_3$), 60.7 (C-1), 70.1 (C-4), 70.5, 71.5, 71.6, and 72.2 (2 $OCH_2CH=CHCH_3$, $PhCH_2$, and C-5), 78.6 and 78.9 (C-2,3), 126.4–129.3 and 137.8 (2 $OCH_2CH=CHCH_3$ and $C_6H_5CH_2$).

Anal. Calc. for $C_{20}H_{30}O_5$: C, 68.53; H, 8.63. Found: C, 68.05; H, 8.76.

1,2,4-Tri-O-benzyl-3,5-di-O-crotyl-D-ribitol (**8**). — To a stirred suspension of sodium hydride (350 mg) in *N,N*-dimethylformamide (5 mL) was added a mixture of **7** (488 mg, 1.39 mmol) and benzyl bromide (1 mL) in *N,N*-dimethylformamide (5 mL) at 0°. After stirring the mixture for 2 h at room temperature, benzylation was complete (t.l.c., R_F 0.78, 19:1 toluene–acetone). The excess of sodium hydride was decomposed with methanol, the mixture was poured onto crushed ice and extracted with ether (3 × 50 mL), and the combined extracts were dried ($MgSO_4$), filtered, and concentrated. Column chromatography (99:1 dichloromethane–ethyl acetate) of the residue gave **8**, isolated as a syrup (715 mg, 97%), $[\alpha]_D$ −1° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^{1}H, δ 1.667 and 1.694 (2 d, each 3 H, 2 $OCH_2CH=CHCH_3$), 4.505 (s), 4.572 (d), 4.615 (d), 4.691 (d), and 4.706 (d) (6 H, 3 $PhCH_2$), 5.597 (m, 4 H, 2

$OCH_2CH=CHCH_3$), 7.242–7.364 (m, 15 H, 3 Ph); ^{13}C, δ 17.6 (2 $OCH_2CH=CHCH_3$), 69.5, 70.0, 71.1, 72.1 (2 C), 72.3, and 73.0 (3 $PhCH_2$, 2 $OCH_2CH=CHCH_3$, and C-1,5), 78.2 and 78.3 (2 C) (C-2,3,4), 127.1–128.9, 138.3, and 138.5 (2 C) (3 $C_6H_5CH_2$ and 2 $OCH_2CH=CHCH_3$).

Anal. Calc. for $C_{34}H_{42}O_5$: C, 76.95; H, 7.98. Found: C, 77.02; H, 8.00.

1,2,4-Tri-O-benzyl-D-ribitol (**9**). — To a solution of **8** (265 mg, 0.50 mmol) in *N,N*-dimethylformamide (5 mL) was added KO^{t}Bu (200 mg) at 80°. After 15 min, the decrotylation was complete (t.l.c., R_F 0.29, 8:2 toluene–acetone). The mixture was cooled, diluted with dichloromethane (50 mL), washed with aqueous 5% sodium chloride, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 dichloromethane–acetone) of the residue gave **9**, isolated as a syrup (122 mg, 58%), $[\alpha]_D$ +1° (*c* 1, dichloromethane). ^{13}C-N.m.r. data ($CDCl_3$): δ 61.0 (C-5), 69.6, 71.4, 72.0, and 73.3 (3 $PhCH_2$ and C-1), 71.7 (C-3), 77.8 and 78.3 (C-2,4), 127.6–128.2, 137.8 (2 C), and 137.9 (3 $C_6H_5CH_2$).

Anal. Calc. for $C_{26}H_{30}O_5$: C, 73.91; H, 7.16. Found: C, 73.55; H, 7.14.

5-O-Allyloxycarbonyl-1,2,4-tri-O-benzyl-D-ribitol (**10**). — To a solution of **9** (150 mg, 0.36 mmol) in dichloromethane (2 mL) and pyridine (2.1 mL) at −35° was added allyl chloroformate (2 × 14 μL). After stirring the mixture for 1 h, the reaction was complete (t.l.c., R_F 0.47, 95:5 dichloromethane–acetone). The mixture was concentrated, diluted with ether (15 mL), thrice washed with saturated aqueous sodium chloride (adjusted with hydrochloric acid to pH 2, 10 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (chloroform) of the residue gave **10**, isolated as a syrup (140 mg, 78%), $[\alpha]_D$ +1° (*c* 1, dichloromethane). ^{13}C-N.m.r. data ($CDCl_3$): δ 66.7, 68.5, 69.6, 72.0, 72.1, and 73.5 (3 $PhCH_2$, $OCOOCH_2CH=CH_2$, and C-1,5), 70.9 (C-3), 77.3 (2 C) (C-2,4), 118.9 ($OCOOCH_2CH=CH_2$), 127.7–128.4, 137.8 (2 C), and 138.1 (3 $C_6H_5CH_2$), 131.5 ($OCOOCH_2CH=CH_2$), 155.0 ($OCOOCH_2CH=CH_2$).

Anal. Calc. for $C_{30}H_{34}O_7$: C, 71.13; H, 6.76. Found: C, 70.95; H, 6.85.

5-O-Allyl-1-O-allyloxycarbonyl-2,3-di-O-benzyl-D-ribitol (**12**). — To a stirred solution of 5-*O*-allyl-2,3-di-*O*-benzyl-D-ribitol[15] (**11**; 512 mg, 1.37 mmol) in dichloromethane (5 mL) and pyridine (5 mL) at −35° was added allyl chloroformate (2 × 50 μL). After 1 h, the reaction was complete (t.l.c., R_F 0.77, 9:1 dichloromethane–acetone). The mixture was concentrated, diluted with ether (50 mL), thrice washed with saturated aqueous sodium chloride (adjusted with hydrochloric acid to pH 2, 25 mL) and then water (20 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (dichloromethane) of the residue gave **12**, isolated as a syrup (323 mg, 52%), $[\alpha]_D$ +1° (*c* 1, dichloromethane). ^{13}C-N.m.r. data ($CDCl_3$): δ 67.0, 68.3, 70.8, 72.1, 72.3, and 73.6 (2 $PhCH_2$, $OCH_2CH=CH_2$, $OCOOCH_2CH=CH_2$, and C-1,5), 70.3 (C-4), 77.5 and 78.6 (C-2,3), 117.2 and 118.7 ($OCH_2CH=CH_2$ and $OCOOCH_2CH=CH_2$), 127.6–128.2 and 138.0–138.1 (2 $C_6H_5CH_2$), 131.4 ($OCOOCH_2CH=CH_2$), 134.3 ($OCH_2CH=CH_2$), 154.6 ($OCOOCH_2CH=CH_2$).

Anal. Calc. for $C_{26}H_{32}O_7$: C, 68.40; H, 7.06. Found: C, 67.95; H, 7.03.

5-O-Allyloxycarbonyl-1,2,4-tri-O-benzyl-3-O-{2,4-di-O-acetyl-3-O-[2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-

rhamnopyranosyl}-D-*ribitol* (**14**). — A suspension of 2,4-di-*O*-acetyl-3-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranosyl trichloroacetimidate[2] (**13**; 200 mg, 0.20 mmol), **10** (112 mg, 0.22 mmol), and molecular sieves 4 Å (2 g) in dichloromethane (7 mL) was stirred for 2 h at room temperature. Then, at −30°, trimethylsilyl triflate (18 μL) was added and, after 20 min, t.l.c. showed the reaction to be complete (R_F 0.43, 8:2 dichloromethane–ethyl acetate). Pyridine was added (0.5 mL), the mixture filtered through Celite, and co-concentrated thrice each with toluene (20 mL), ethanol (20 mL), and dichloromethane (20 mL). Column chromatography (95:5 dichloromethane–acetone) of the residue gave **14**, isolated as a syrup (200 mg, 75%), $[\alpha]_D$ +10° (*c* 0.33, dichloromethane). ^{13}C-N.m.r. data ($CDCl_3$): δ 17.1 (C-6'), 20.3–20.6 (CH_3CO), 60.6 and 61.4 (C-6'',6'''), 65.9, 68.3, 68.9, 71.9 (2 C), and 73.0 (3 PhCH_2, OCOOCH_2CH=CH_2, and C-1,5), 93.2, 95.8, and 97.7 (C-1',1'',1'''), 118.6 (OCOOCH$_2$CH=CH_2), 127.3–128.1, 137.3, and 137.7 (2 C) (3 $C_6H_5CH_2$), 131.3 (OCOOCH$_2$CH=CH_2), 154.5 (OCOOCH$_2$CH=CH_2), 169.2–170.4 (CH_3CO).

Anal. Calc. for $C_{63}H_{82}O_{30}$: C, 58.11; H, 6.16. Found: C, 58.20; H, 6.09.

1,2,4-Tri-O-benzyl-3-O-{*2,4-di-O-acetyl-3-O-*[*2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl*]*-α-L-rhamnopyranosyl*}*-D-ribitol* (**15**). — A mixture of **14** (152 mg, 0.11 mmol) and tetrakis(triphenylphosphine)-palladium (25 mg) in tetrahydrofuran (2.5 mL) and water (0.25 mL) was boiled under reflux until t.l.c. showed complete conversion into **15** (45 min; R_F 0.56, 9:1 dichloromethane–acetone). The mixture was concentrated, diluted with dichloromethane (20 mL), thrice washed with aqueous 10% sodium chloride (20 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 dichloromethane–acetone) of the residue gave **15**, isolated as a syrup (107 mg, 77%), $[\alpha]_D$ +45° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^{1}H, δ 1.048 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 1.963, 2.045, 2.050, 2.064, 2.067, 2.078, 2.088, 2.119, and 2.123 (9 s, each 3 H, 9 Ac), 3.775 (m, 1 H, H-5''), 4.443 (d), 4.507 (d), 4.516 (s), 4.612 (d), and 4.668 (6 H, 3 PhCH_2), 4.841 (dd, 1H, $J_{1'',2''}$ 3.4, $J_{2'',3''}$ 10.1 Hz, H-2''), 5.056 (bs 1 H, H-1'), 5.271 (d, 1 H, $J_{1''',2'''}$ 3.6 Hz, H-1'''), 5.398 (bd, 1 H, $J_{3''',4'''}$ 3.3 Hz, H-4'''), 7.215–7.349 (m, 15 H, 3 Ph); ^{13}C, δ 17.2 (C-6'), 20.4–20.7 (CH_3CO), 93.3, 95.9, and 97.6 (C-1',1'',1'''), 127.3–128.5, 137.5, and 137.7 (2 C) (3 $C_6H_5CH_2$), 169.2–170.5 (CH_3CO).

Anal. Calc. for $C_{62}H_{78}O_{28}$: C, 58.47; H, 6.19. Found: C, 58.44; H, 6.43.

1,2,4-Tri-O-benzyl-3-O-{*2,4-di-O-acetyl-3-O-*[*2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl*]*-α-L-rhamnopyranosyl*}*-D-ribitol 5-(triethylammonium phosphonate)* (**16**). — To a stirred solution of **15** (83 mg, 65 μmol) in dry acetonitrile (0.4 mL) and dry pyridine (0.2 mL) was added 0.4M salicylchlorophosphite[6] in dry acetonitrile (80 μmol). The mixture was stirred for 2 h, when additional salicylchlorophosphite (50 μmol) was added. T.l.c. (8:2 dichloromethane–acetone) then showed no change, and 1:1 pyridine–water (0.5 mL) was added. The mixture was diluted with dichloromethane (15 mL), washed with water (2 × 10 mL) and M triethylammonium hydrogencarbonate (2 × 10 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography of the residue on silica gel (8:2 dichloro-

methane–acetone containing 1% of triethylamine, followed by 8:2 dichloromethane–methanol containing 1% of triethylamine) and then on Sephadex LH-20 (2:1 dichloromethane–methanol containing 1% of triethylamine) gave **16**, isolated as a syrup (54 mg, 58%), $[\alpha]_D$ +37° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^1H, δ 1.004 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 1.232 [t, 9 H, $N(CH_2CH_3)_3$], 1.960, 2.049, 2.055, 2.064, 2.078, 2.113, and 2.117 (7 s, 3, 6, 3, 3, 6, 3, and 3 H, 9 Ac), 2.944 [q, 6 H, $N(CH_2CH_3)_3$], 4.423 (d), 4.472 (d), 4.487 (d), 4.638 (s), and 4.728 (d) (6 H, 3 $PhCH_2$), 4.863 (dd, 1 H, $J_{1'',2''}$ 3.4, $J_{2'',3''}$ 10.1 Hz, H-2''), 5.023 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1'), 5.147 (d, 1 H, H-1''), 5.262 (d, 1 H, $J_{1''',2'''}$ 3.6 Hz, H-1'''), 5.389 (bd, 1 H, $J_{3''',4'''}$ 3.3 Hz, H-4'''), 6.853 (d, 1 H, $J_{H,P}$ 620 Hz, phosphonate), 7.229–7.332 (m, 15 H, 3 Ph); ^{31}P, δ 5.313 (dt, $^1J_{P,H}$ 619, $^3J_{P,H}$ 8.0 Hz).

5-O-Allyl-1-O-allyloxycarbonyl-2,3-di-O-benzyl-4-O-{2,4-di-O-acetyl-3-O-[2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranosyl}-D-ribitol (**17**). — A suspension of **12** (156 mg, 0.34 mmol), **13** (ref. 2) (320 mg, 0.32 mmol), and molecular sieves 4 Å (4 g) in dichloromethane (10 mL) was stirred for 2 h at room temperature. Then, at −30°, trimethylsilyl triflate (30 μL) was added and, after 30 min, the reaction was complete (t.l.c., R_F 0.34, 8:2 dichloromethane–ethyl acetate). Pyridine (1 mL) was added, and the mixture was filtered through Celite and thrice co-concentrated each with toluene, ethanol, and dichloromethane. Column chromatography (9:1 dichloromethane–acetone) of the residue gave **17**, isolated as a syrup (362 mg, 88%), $[\alpha]_D$ +55°(*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^1H, δ 1.041 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 1.962, 2.027, 2.048, 2.066, 2.075, 2.083, 2.119, and 2.150 (8 s, 3, 3, 6, 3, 3, 3, 3, and 3 H, 9 Ac), 7.253–7.333 (m, 10 H, 2 Ph); ^{13}C, δ 17.2 (C-6'), 20.4–20.7 (CH_3CO), 60.7 and 61.5 (C-6'',6'''), 66.5, 68.4, 69.9, 72.0, 72.3, and 73.4 (2 $PhCH_2$, $OCOOCH_2CH=CH_2$, $OCH_2CH=CH_2$, and C-1,5), 93.2, 95.9, and 96.9 (C-1', 1'', 1'''), 117.0 ($OCH_2CH=CH_2$), 118.7 ($OCOOCH_2CH=CH_2$), 127.7–128.3 and 137.5 (2 $C_6H_5CH_2$), 131.4 ($OCOOCH_2CH=CH_2$), 134.2 ($OCH_2CH=CH_2$), 169.2–170.5 (CH_3CO).

Anal. Calc. for $C_{62}H_{80}O_{30}$: C, 57.05; H, 6.18. Found: C, 56.66; H, 6.26.

5-O-Allyl-2,3-di-O-benzyl-4-O-{2,4-di-O-acetyl-3-O-[2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranosyl}-D-ribitol (**18**). — To a solution of **17** (193 mg, 0.15 mmol) in tetrahydrofuran (2.5 mL) and water (0.25 mL) was added tetrakis(triphenylphosphine)palladium (30 mg), and the mixture was boiled under reflux until t.l.c. (19:1 dichloromethane–methanol) showed the disappearance of **17**. The mixture was diluted with dichloromethane (20 mL) and 1,4-dioxane (20 mL), and co-concentrated thrice with 1,4-dioxane (20 mL). Column chromatography (92:8 dichloromethane–acetone) of the residue gave **18**, isolated as a syrup (131 mg, 73%), $[\alpha]_D$ +44° (*c* 1, dichloromethane), R_F 0.26 (19:1 dichloromethane–methanol). N.m.r. data ($CDCl_3$): ^1H, δ 1.063 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 1.962, 2.029, 2.051, 2.067, 2.076, 2.090, 2.119, and 2.156 (8 s, 3, 3, 6, 3, 3, 3, 3, and 3 H, 9 Ac), 5.828 (m, 1 H, $OCH_2CH=CH_2$), 7.273–7.358 (m, 10 H, 2 Ph); ^{13}C, δ 17.2 (C-6'), 20.4–20.7 (CH_3CO), 60.7, 61.0, and 61.4 (C-1,6'',6'''), 71.1, 71.7 (2 C), and 73.6 (2 $PhCH_2$, $OCH_2CH=CH_2$, and C-5), 93.0, 95.9, and 96.9 (C-1',1'',1'''), 117.0 ($OCH_2CH=CH_2$), 127.8–128.3 and 137.5 (2 $C_6H_5CH_2$), 134.2 ($OCH_2CH=CH_2$), 169.2–170.5 (CH_3CO).

Anal. Calc. for $C_{58}H_{76}O_{28}$: C, 57.03; H, 6.27. Found: C, 56.34; H, 6.53.

1-O-Acetyl-2,3-di-O-benzyl-4-O-{2,4-di-O-acetyl-3-O-[2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranosyl}-D-ribitol (**20**). — A solution of **18** (131 mg, 0.11 mmol) in 1:1 pyridine–acetic anhydride (2 mL) was stirred overnight, when t.l.c. showed complete conversion into **19** (R_F 0.43, 9:1 dichloromethane–acetone). The mixture was co-concentrated thrice each with toluene, ethanol, and dichloromethane. Column chromatography (9:1 dichloromethane–acetone) of the residue gave **19**, isolated as a syrup (117 mg, 82%), $[\alpha]_D$ +48° (*c* 1, dichloromethane). ^{13}C-N.m.r. data ($CDCl_3$): δ 17.2 (C-6′), 20.4–20.7 (CH_3CO), 60.6, 61.4, and 63.2 (C-1,6″,6‴), 70.1, 72.0, and 72.3 (2 C) (2 PhCH_2, OCH_2CH=CH_2, and C-5), 93.3, 95.9, and 97.0 (C-1′,1″,1‴), 117.0 (OCH$_2$CH=CH_2), 127.7–128.3 and 137.5 (2 $C_6H_5CH_2$), 134.2 (OCH$_2$CH=CH$_2$), 169.2–170.6 (CH_3CO).

To a solution of **19** (80 mg, 63 μmol) in acetic acid (1 mL) was added sodium acetate (43 mg) and palladium(II) chloride (56 mg), and the mixture was sonicated overnight, when t.l.c. showed the absence of **19** (9:1 dichloromethane–acetone). The mixture was diluted with dichloromethane (50 mL), filtered through Celite, washed with water (20 mL), saturated aqueous sodium hydrogencarbonate (20 mL), and water (20 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (85:15 dichloromethane–acetone) of the residue gave **20**, isolated as a syrup (64 mg, 70%), $[\alpha]_D$ +57° (*c* 1, dichloromethane), R_F 0.10 (9:1 dichloromethane–acetone). N.m.r. data ($CDCl_3$): ^{1}H, δ 1.078 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6′), 1.966, 2.034, 2.044, 2.049, 2.080, 2.123, and 2.154 (7 s, 3, 3, 3, 6, 9, 3, and 3 H, 10 Ac), 7.264–7.347 (m, 10 H, 2 Ph); ^{13}C, δ 17.2 (C-6′), 20.5–20.8 (CH_3CO), 60.6, 61.2, 61.5, and 62.9 (C-1,5,6″,6‴), 71.9 and 73.9 (2 PhCH_2), 93.8, 95.9, and 96.5 (C-1′,1″,1‴), 128.0, 128.4, and 137.3 (2 $C_6H_5CH_2$), 169.2–170.6 (CH_3CO).

Anal. Calc. for $C_{57}H_{74}O_{29}$: C, 55.96; H, 6.12. Found: C, 55.68; H, 6.20.

1-O-Acetyl-2,3-di-O-benzyl-4-O-{2,4-di-O-acetyl-3-O-[2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranosyl}-D-ribitol 5-(triethylammonium phosphonate) (**21**). — To a stirred solution of **20** (38 mg, 31 μmol) in dry acetonitrile (1.0 mL) and dry pyridine (0.2 mL) was added 0.12M salicylchlorophosphite[6] in dry acetonitrile (40 μmol). The mixture was stirred for 2 h and additional salicylchlorophosphite (22 μmol) was added. T.l.c. (8:2 dichloromethane–acetone) then showed no change, and 1:1 pyridine–water (0.5 mL) was added. The mixture was diluted with dichloromethane (15 mL), washed with water (2 × 10 mL) and M triethylammonium hydrogencarbonate (2 × 10 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography of the residue on silica gel (8:2 dichloromethane–acetone containing 1% of triethylamine, followed by 8:2 dichloromethane–methanol containing 1% of triethylamine) and then on Sephadex LH-20 (2:1 dichloromethane–methanol containing 1% of triethylamine) gave **21**, isolated as a syrup (24 mg, 55%), $[\alpha]_D$ +37° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^{1}H, δ 0.994 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6′), 1.212 [t, 9 H, N(CH$_2$CH_3)$_3$], 1.959, 1.989, 2.025, 2.044, 2.051, 2.059, 2.072, 2.078, 2.116, and 2.133 (10 s, each 3 H, 10 Ac), 2.878 [q, 6 H, N(CH_2CH$_3$)$_3$], 4.600 (d), 4.625 (s), and 4.702 (d) (4 H, 2 PhCH_2), 4.868 (dd, 1 H, $J_{1'',2''}$ 3.4, $J_{2'',3''}$ 10.2 Hz, H-2″),

5.130 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1'), 5.165 (d, 1 H, H-1''), 5.260 (d, 1 H, $J_{1''',2'''}$ 3.6 Hz, H-1'''), 5.390 (bd, 1 H, $J_{3''',4'''}$ 3.3 Hz, H-4'''), 6.837 (d, 1 H, $J_{H,P}$ 615 Hz, phosphonate), 7.255–7.327 (m, 10 H, 2 Ph); ^{31}P, δ 5.196 (dt, $^1J_{P,H}$ 614, $^3J_{P,H}$ 7.8 Hz).

*4-Methoxybenzyl 2,4-di-*O*-benzyl-3-*O*-{2,4,6-tri-*O*-benzyl-3-*O*-[3,4,6-tri-*O*-benzyl-α-*D*-ga-lactopyranosyl 2-(triethylammonium phosphonate)]-α-*D*-glucopyranosyl}-α-*L*-rhamnopyranoside* (**23**). — To a stirred solution of **22** (ref. 1) (312 mg, 0.26 mmol) in dry acetonitrile (8.5 mL) was added salicylchlorophosphite[6] (62.8 mg), and the mixture was stirred until t.l.c. (R_F 0.60, 15:1 toluene–acetone) showed no change. 1:1 Pyridine–water (1 mL) was added, and the mixture was diluted with dichloromethane (30 mL), washed with M triethylammonium hydrogencarbonate (2 × 25 mL) and water (20 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography of the residue on silica gel (9:1 dichloromethane–methanol containing 1% of triethylamine) and then on Sephadex LH-20 (2:1 dichloromethane–methanol containing 1% of triethylamine) gave **23**, isolated as a syrup (273 mg, 70%), $[\alpha]_D$ +38° (*c* 1, dichloromethane). N.m.r. data ($CDCl_3$): ^1H, δ 1.155 [t, 9 H, $N(CH_2CH_3)_3$], 1.294 (d, 3 H, $J_{5,6}$ 6.2 Hz, 3 H-6), 2.816 [q, 6 H, $N(CH_2CH_3)_3$], 3.770 (s, 3 H, OMe), 4.814 (bs, 1 H, H-1), 5.184 (d, 1 H, $J_{1',2'}$ 3.4 Hz, H-1'), 5.665 (d, 1 H, $J_{1'',2''}$ 3.7 Hz, H-1''), 6.806 and 6.985–7.401 (m, 44 H, 8 Ph and MeOPh), 7.050 (d, 1 H, $J_{H,P}$ 626 Hz, phosphonate); ^{13}C, δ 8.1 [$N(CH_2CH_3)_3$], 17.6 (C-6), 45.0 [$N(CH_2CH_3)_3$], 54.9 (OMe), 93.1 and 96.4 (C-1,1'), 97.4 (d, $^3J_{1'',P}$ 3.0 Hz, C-1''), 113.4, 127.2–129.1, 137.4–138.7, and 158.9 (9 $C_6H_5CH_2$ and $MeOC_6H_4CH_2$); ^{31}P, δ 3.47 (dd, $^1J_{P,H}$ 626, $^3J_{P,H}$ 12.0 Hz).

O-α-*D*-*Galactopyranosyl-(1→3)*-O-α-*D*-*glucopyranosyl-(1→3)*-α,β-*L*-*rhamnopyranose 2''*-[O-α-*D*-*galactopyranosyl-(1→3)*-O-α-*D*-*glucopyranosyl-(1→3)*-O-α-*L*-*rhamnopyranosyl-(1→3)*-*D*-*ribit-5-yl sodium phosphate*] (**25**). — A mixture of **16** (34 mg, 24 μmol) and **22** (ref. 1) (37 mg, 28 μmol) in dry pyridine (1.0 mL) was concentrated under argon. A solution of the residue in dry acetonitrile (0.5 mL) and dry pyridine (0.1 mL) was treated with pivaloyl chloride (2 × 3.0 μL, 24 μmol) at intervals of 20 min and at room temperature. After stirring the mixture for 70 min, t.l.c. (8:2 toluene–acetone) showed no further change (intermediate phosphonic diester, R_F 0.42), and it was quenched with water (0.5 mL), diluted with dichloromethane (10 mL), washed with water (3 × 8 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 dichloromethane–acetone) of the residue gave **22** and the phosphonic diester, isolated as a syrup, a solution of which in dry acetonitrile (1.2 mL) was treated with 0.5M iodine in 95:5 pyridine–water (100 μL). After 15 min, the oxidation was complete as shown by t.l.c. (9:1 dichloromethane–acetone), R_F ~0 for **24**, and the mixture was diluted with dichloromethane (15 mL), washed with 0.5M sodium thiosulfate (2 × 10 mL), and M triethylammonium hydrogencarbonate (2 × 10 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 dichloromethane–acetone containing 1% of triethylamine, followed by 9:1 dichloromethane–methanol containing 1% of triethylamine) of the residue gave **24**, isolated as a syrup (48 mg, 71%), $[\alpha]_D$ +18° (*c* 0.5, dichloromethane). ^1H-N.m.r. data ($CDCl_3$): δ 0.929 (d, 3 H, $J_{5'''',6''''}$ 6.2 Hz, 3 H-6''''), 1.241 [t, 9 H, $N(CH_2CH_3)_3$], 1.284 (d, 3 H, $J_{5,6}$ 6.2 Hz, 3 H-6), 1.958, 2.023, 2.029, 2.042, 2.071, and 2.114 (6 s, 3, 6, 9, 3, 3, and 3 H, 9 Ac), 2.898 [q, 6 H, $N(CH_2CH_3)_3$],

3.776 (s, 3 H, OMe), 4.660 (m, 1 H, $J_{1'',2''}$ 3.4, $J_{2'',3''} = {}^3J_P = 10.1$ Hz, H-2''), 4.863 (dd, 1 H, $J_{1'''',2''''}$ 3.4, $J_{2'''',3''''}$ 10.2 Hz, H-2''''), 4.953 (t, 1 H, $J_{3'''',4''''} = J_{4'''',5''''} = 9.9$ Hz, H-4''''), 5.246 (d, 1 H, $J_{1''''',2''''}$ 3.6 Hz, H-1'''''), 5.380 (dd, 1 H, $J_{3''''',4''''}$ 4.4 Hz, H-4'''''), 5.799 (d, 1 H, H-1''), 6.808 and 6.974–7.329 (m, 59 H, 11 Ph and MeOPh).

A solution of **24** (48 mg, 17 μmol) in methanolic 7M ammonia (1.2 mL) was heated for 48 h at 40°, then concentrated. A solution of the residue in methanol (3.0 mL), 2-propanol (3.0 mL), and acetic acid (0.7 mL) was hydrogenolysed overnight in the presence of 10% Pd–C (200 mg) at 4 atm., then filtered through Celite, which was washed with water (20 mL), methanol (20 mL), and water (20 mL). The combined filtrate and washings were concentrated. Chromatography (water) of the residue on Bio-Gel P-6 and lyophilisation gave a product, a solution of which in water (5 mL) was passed through a column of Dowex-50 (Na^+) resin to yield, after lyophilisation, **25** as a white powder (12 mg, 59%). N.m.r. data (D_2O): ^1H, see Table I; ^{31}P, δ 0.896 (m).

O-α-D-*Galactopyranosyl-(1→3)*-O-α-D-*glucopyranosyl-(1→3)*-α,β-L-*rhamnopyranose 2''-[O*-α-D-*galactopyranosyl-(1→3)*-O-α-D-*glucopyranosyl-(1→3)-O*-α-L-*rhamnopyranosyl-(1→4)*-D-*ribit-5-yl sodium phosphate]* (**27**). — A mixture of **21** (24 mg, 17 μmol) and **22**[1] (20 mg, 15 μmol) in dry pyridine (2.0 mL) was concentrated under argon. A solution of the residue in dry acetonitrile (0.5 mL) and dry pyridine (0.1 mL) was treated with pivaloyl chloride (2 × 2.1 μL, 17 μmol) at intervals of 20 min and room temperature. After stirring the mixture for 70 min, t.l.c. (8:2 toluene–acetone) showed no further change (intermediate phosphonic diester, R_F 0.42), and it was quenched with water (0.5 mL), diluted with dichloromethane (10 mL), washed with water (3 × 8 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 dichloromethane–acetone) of the residue gave **22** and the phosphonic diester, isolated as a syrup, a solution of which in dry acetonitrile (1.0 mL) was treated with 0.5M iodine in 95:5 pyridine–water (100 μL). After 15 min, the oxidation was complete as shown by t.l.c. (9:1 dichloromethane–acetone), $R_F \sim 0$ for **26**; the mixture was diluted with dichloromethane (10 mL), washed with 0.5M sodium thiosulfate (2 × 10 mL) and M triethylammonium hydrogencarbonate (2 × 10 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 dichloromethane–acetone containing 1% of triethylamine, followed by 9:1 dichloromethane–methanol containing 1% of triethylamine) of the residue gave **26**, isolated as a syrup (26 mg, 67%), $[\alpha]_D$ +3° (*c* 0.5, dichloromethane). ^1H-N.m.r. data ($CDCl_3$): δ 0.907 (d, 3 H, $J_{5'''',6''''}$ 6.2 Hz, 3 H-6''''), 1.014 [t, 9 H, $N(CH_2CH_3)_3$], 1.284 (d, 3 H, $J_{5,6}$ 6.2 Hz, 3 H-6), 1.896, 1.956, 1.987, 2.006, 2.024, 2.033, 2.042, 2.063, and 2.113 (9 s, 3, 3, 3, 3, 3, 6, 3, 3, and 3 H, 10 Ac), 2.583 [q, 6 H, $N(CH_2CH_3)_3$], 3.776 (s, 3 H, OMe), 4.628 (m, 1 H, $J_{1'',2''}$ 3.4, $J_{2'',3''} = {}^3J_P = 10.1$ Hz, H-2''), 4.868 (dd, 1 H, $J_{1'''',2''''}$ 3.3, $J_{2'''',3''''}$ 10.1 Hz, H-2''''), 4.973 (t, 1 H, $J_{3'''',4''''} = J_{4'''',5''''} = 9.9$ Hz, H-4''''), 5.380 (dd, 1 H, $J_{3''''',4''''}$ 4.3, $J_{4''''',5''''}$ 1.0 Hz, H-4'''''), 5.795 (d, 1 H, H-1''), 6.806 and 6.972–7.316 (m, 54 H, 10 Ph and MeOPh).

A solution of **26** (26 mg, 10 μmol) in methanolic 7M ammonia (1.0 mL) was heated for 48 h at 40°, then concentrated. A solution of the residue in methanol (2.1 mL), 2-propanol (2.1 mL), and acetic acid (0.5 mL) was hydrogenolysed overnight in the presence of 10% Pd–C (150 mg) at 4 atm., then filtered through Celite, which was

washed with water (20 mL), methanol (20 mL), and water (20 mL). The combined filtrate and washings were concentrated. Chromatography (water) of the residue on Bio-Gel P-6 and lyophilisation gave a product, a solution of which in water (5 mL) was passed through a column of Dowex-50 (Na^+) resin to yield, after lyophilisation, **27** as a white powder (7 mg, 60%). N.m.r. data (D_2O): ^1H, see Table I; ^{31}P, δ 0.720 (m).

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REFERENCES

1 T. M. Slaghek, M. J. van Vliet, A. A. M. Maas, J. P. Kamerling, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 195 (1989) 75–86.
2 T. M. Slaghek, A. H. van Oijen, A. A. M. Maas, J. P. Kamerling, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 207 (1990) 237–248.
3 R. H. Hall, A. Todd, and R. F. Webb, *J. Chem. Soc.*, (1957) 3291–3298.
4 P. J. Garegg, T. Regberg, J. Strawinski, and R. Strömberg, *Chem. Scr.*, 26 (1986) 59–62.
5 B. C. Froehler and M. C. Mateucci, *Tetrahedron Lett.*, 27 (1986) 469–472.
6 R. Anschütz and W. O. Emery, *Justus Liebigs Ann. Chem.*, 239 (1887) 301–313.
7 J. E. Marugg, M. Tromp, E. Kuyl-Yeheskiely, G. A. van der Marel, and J. H. van Boom, *Tetrahedron Lett.*, 27 (1986) 2661–2664.
8 B. Classon, P. J. Garegg, and I. Lindh, *Carbohydr. Res.*, 179 (1988) 31–35.
9 P. Westerduin, G.H. Veeneman, G. A. van der Marel, and J. H. van Boom, *Tetrahedron Lett.*, 27 (1986) 6271–6274.
10 N. J. Leonard and K. L. Carraway, *J. Heterocycl. Chem.*, 3 (1966) 485–489.
11 N. Berg and O. Kjølberg, *Carbohydr. Res.*, 57 (1977) 65–71.
12 V. Pozsgay, *Carbohydr. Res.*, 69 (1979) 284–286.
13 P. A. Gent, R. Gigg, and R. Conant, *J. Chem. Soc., Perkin Trans. 1*, (1972) 1535–1542.
14 P. Boullanger, P. Chatelard, G. Descotes, M. Kloosterman, and J. H. van Boom, *J. Carbohydr. Chem.*, 5 (1986) 541–559.
15 B. Grzeszczyk, A. Banaszek, and A. Zamojski, *Carbohydr. Res.*, 175 (1988) 215–226.
16 S. Sato, Y. Ito, T. Nukada, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, 167 (1987) 197–210.
17 S. B. Katti and K. L. Agarwal, *Tetrahedron Lett.*, 26 (1985) 2547–2550.
18 J. E. G. van Dam, J. Breg, R. Komen, J. P. Kamerling, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 187 (1989) 267–286.
19 P. de Waard and J. F. G. Vliegenthart, *J. Magn. Reson.*, 81 (1989) 173–177.