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THE SYNTHESIS OF TWO REPEATING UNITS OF *Haemophilus influenzae* TYPE *a* CAPSULAR ANTIGEN*

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ABSTRACT

The repeating units 2-*O*-β-D-glucopyranosyl-L-ribitol 4′- and 1-phosphate of *Haemophilus influenzae* type *a* capsular antigen have been synthesised by condensation of an α-D-glucopyranosyl bromide derivative with 5-*O*-allyl-1,2,3-tri-*O*-benzyl-D-ribitol followed by selective deprotection of HO-4′ or HO-1, phosphorylation, and removal of the blocking groups.

INTRODUCTION

The structures of the capsular antigens of the six known types *a–f* of *Haemophilus influenzae* have been determined in the last decade[1], but little work on the synthesis of the repeating units of these substances has been reported. Garegg and his co-workers[2,3] have described the syntheses of two repeating units of the type *b* antigen.

The structure of the type *a* antigen had been determined by Lindberg and co-workers[4] as 2-*O*-β-D-glucopyranosyl-L-ribitol linked at O-1 of L-ribitol and O-4 of D-glucose by phosphodiester groupings (**1**). Thus, three repeating units of the antigen can be represented by the formulae **2**–**4**. Whereas **2** and **4** are specific only for *H. influenzae* type *a* antigen, **3** may be regarded also as a repeating unit of *Bacillus subtilis* teichoic acid[5,6] (**5**). We now report syntheses of **2** and **3**.

RESULTS

5-*O*-Allyl-1,2,3-tri-*O*-benzyl-D-ribitol (**12**), needed for the synthesis of **2** and **3**, was prepared from methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside[7,8] (**6**) in six steps as follows. Treatment of **6** with allyl bromide in the presence of aqueous 50% sodium hydroxide and tetrabutylammonium bromide gave 87% of the 5-*O*-allyl derivative **7**. Hydrolysis of **7** with trifluoroacetic acid in aqueous methanol furnished the diol **8** in quantitative yield and with partial anomerisation. The 2,3-di-*O*-benzyl derivative **9** was obtained from **8** under standard conditions. Hydrolysis of the

*Dedicated to Professor Rezső Bognár in the year of his 75th birthday.

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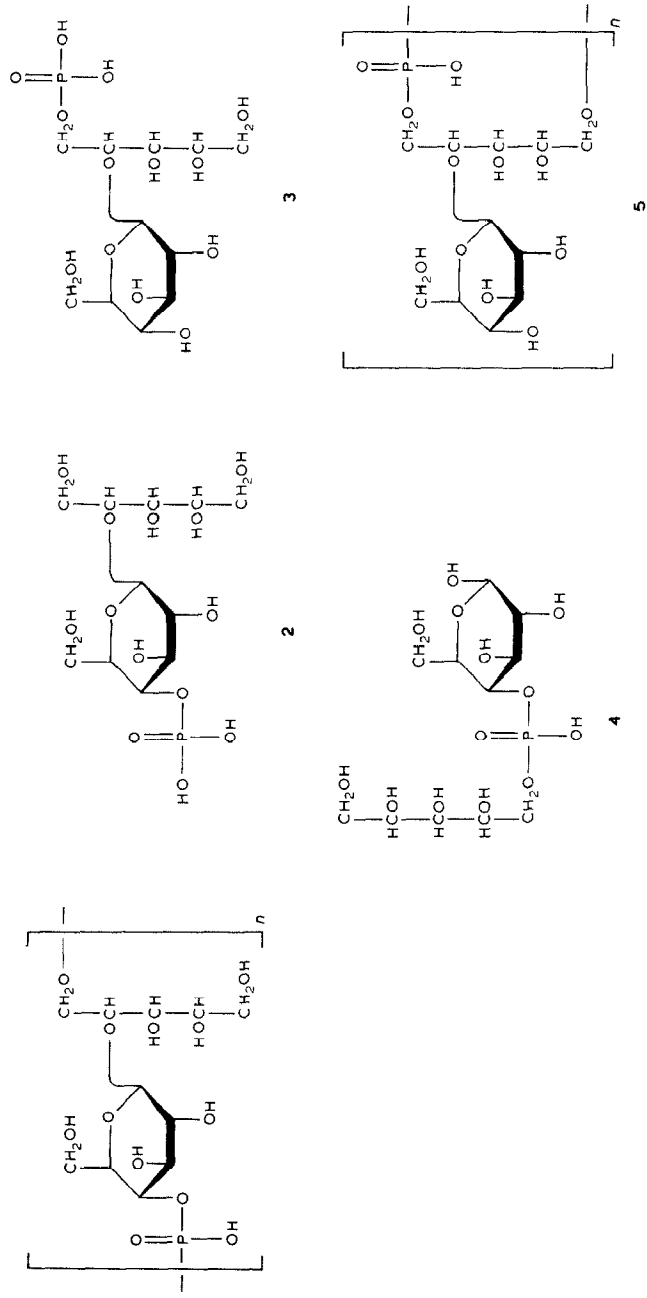
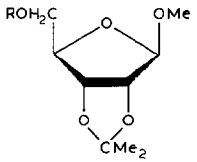
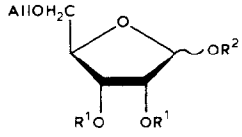


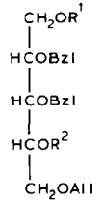
anomeric methyl group in **9** with hydrochloric acid in aqueous 1,4-dioxane gave 86% of **10**, which was reduced with sodium borohydride to give the D-ribitol derivative **11** (95%). Phase-transfer benzylation of **11** gave 75% of a 3:1 mixture of the monobenzylated products **12** and **13** from which **12** was isolated by chromatography.



6 R = H
7 R = All



8 $R^1 = H, R^2 = Me$
9 $R^1 = Bzl, R^2 = Me$
10 $R^1 = Bzl, R^2 = H$



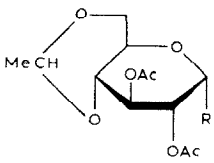
11 $R^1 = R^2 = H$
12 $R^1 = Bzl, R^2 = H$
13 $R^1 = H, R^2 = Bzl$

The glycosyl bromide **15**, prepared from 1,2,3-tri-*O*-acetyl-4,6-*O*-ethylidene-α-D-glucopyranose[9] (**14**) in the usual way, was coupled with **15** in the presence of 1:1 mercuric bromide–mercuric cyanide and molecular sieves (3 Å), to yield 86% of the disaccharide derivative **16** together with 3-*O*-acetyl-1,2-*O*-cyanoethylidene-4,6-*O*-ethylidene-α-D-glucopyranose (**17**). 1,2-*O*-Cyanoethylidene derivatives are formed sometimes when heavy metal cyanides are employed in the Koenigs–Knorr condensation[10,11].

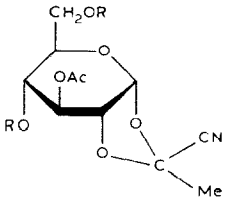
Attempts at acid-catalysed hydrolysis of the ethylidene grouping in **16** failed. Therefore, both the acetyl groups of **16** were replaced directly by benzyl groups to furnish **18**. Hydrolysis of **18** with Dowex 50 (H^+) resin in methanol then yielded 80% of **19** and subsequent phase-transfer benzylation gave 50% of the 6-*O*-benzyl derivative **20**. Remarkably, ~40% of the 4-*O*-benzyl derivative **21** was also obtained. The phosphorotriazolidate method was selected for the phosphorylation of **20** since it involves mild reaction conditions and usually gives high yields of products[12]. Thus, when **20** was treated with an excess of diphenyl phosphoro(1,2,4-triazolidate) and 4-dimethylaminopyridine as catalyst, 85% of the phosphate **22** was obtained. Likewise, **21** was phosphorylated to give 77% of **23**.

Deprotection of **22** was achieved by first removing the allyl group, using palladium chloride–sodium acetate in acetic acid[13], to give 74% of **24**, and then hydrogenolysis of the benzyl and phenyl groups to afford **2** in a quantitative yield.

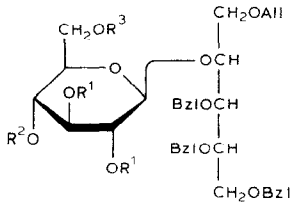
The repeating unit **3** was obtained in an essentially similar way. Glycosylation of **12** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of mercuric cyanide and molecular sieves (4 Å) gave 76% of the β-D-glucopyranosyl-D-ribitol derivative **25** together with ~25% of 3,4,6-tri-*O*-acetyl-1,2-*O*-cyanoethylidene-α-D-glucopyranose (**26**). Deallylation of **25**, as described above, gave 86% of **27**, Zemplén deacetylation of which followed by hydrogenolysis afforded 77% of the known 2-*O*-β-D-glucopyranosyl-L-ribitol[14,15] (**28**).



14 R = OAc
15 R = Br



17 R, R = >CHMe
26 R = Me



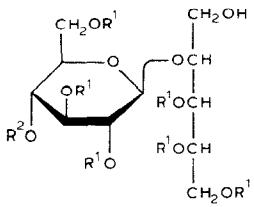
16 R^1 = Ac, R^2, R^3 = >CHMe
18 R^1 = Bzl, R^2, R^3 = >CHMe
19 R^1 = Bzl, R^2 = R^3 = H
20 R^1 = R^3 = Bzl, R^2 = H
21 R^1 = R^2 = Bzl, R^3 = H
22 R^1 = R^3 = Bzl, R^2 = $PO(OPh)_2$
23 R^1 = R^2 = Bzl, R^3 = $PO(OPh)_2$

Phosphorylation of **27** yielded **29**, which was deacetylated and then debenzylated to furnish ~70% of **3**.

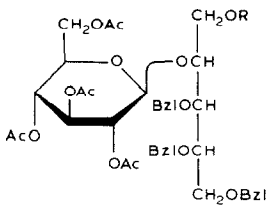
DISCUSSION

Condensation of the glycosyl bromide **15** with **12** in the presence of mercuric cyanide gave 14% of **16**. A similar yield (16%) was achieved when silver perchlorate–silver carbonate was the catalyst. Earlier work, aimed at similar 2-*O*-β-D-glucopyranosyl-L-ribitol derivatives, gave even lower yields of products[14,15]. High yields of **16** and **25** were subsequently obtained, which demonstrated once again the critical importance of the proportions the glycosylating agent, the alcohol, and the catalyst[16] in the Koenigs–Knorr reaction.

Both reaction sequences leading to **2** and **3** were high-yielding, with overall



24 R^1 = Bzl, R^2 = $PO(OPh)_2$
28 R^1 = R^2 = H



25 R = All
27 R = H
29 R = $PO(OPh)_2$

yields of 22 and 23%, respectively, based on **12**, and are suitable for large-scale syntheses.

EXPERIMENTAL

General methods. — ^{1}H-N.m.r. spectra were recorded for solutions in $CDCl_3$ with Jeol JNM-4H-100 (100 MHz), Bruker WM-100 and 400 (^{1}H and ^{13}C), and Jeol PST 100 (^{31}P) spectrometers. Optical rotations were measured with a Perkin–Elmer 141 automatic polarimeter.

Solvents were dried by refluxing over calcium hydride and distillation, and then stored over molecular sieves (4 Å). Pyridine was distilled from toluene-*p*-sulfonyl chloride. Condensation reactions were carried out under argon.

T.l.c. was performed on Silica Gel 60 F_{254} (Merck), and silica gel (230–400 mesh, Merck) was used for column chromatography.

*Methyl 5-O-allyl-2,3-O-isopropylidene-β-*D*-ribofuranoside* (**7**). — To a vigorously stirred suspension of **6**[7,8] (54 g, 0.265 mol) in allyl bromide (38.1 mL, 0.45 mol) was added aqueous 50% sodium hydroxide (40 mL) and tetrabutylammonium bromide (1.21 g). Stirring was continued for 2 h and more (10 mL) aqueous 50% NaOH was then added. After completion of the reaction (4 h; t.l.c., light petroleum–ether, 1:1), the organic phase was separated, dried, and concentrated to dryness. Column chromatography (light petroleum–ether, 4:1) of the residue gave **7** (56 g, 87%), isolated as a colorless syrup, $[\alpha]_D^{20}$ −70° (*c* 2.2, chloroform); ν_{max} 1640 cm^{-1}. ^{1}H-N.m.r. data: δ 5.91 (m, 1 H, allyl CH=), 5.43–5.05 (m, 2 H, allyl CH_2=), 4.96 (s, 1 H, H-1), 4.58, 4.68 (ABq, 2 H, $J_{2,3}$ 6.0 Hz, H-2,3), 4.33 (bt, 1 H, Σ*J* 15 Hz, H-4), 4.09–3.94 (m, 2 H, allyl CH_2O), 3.53–3.36 (m, 2 H, H-5,5′), 3.30 (s, 3 H, OMe), 1.31, 1.47 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{12}H_{20}O_5$: C, 59.0; H, 8.3. Found: C, 58.8; H, 8.2.

*Methyl 5-O-allyl-α,β-*D*-ribofuranoside* (**8**). — To a solution of **7** (56 g, 0.23 mol) in methanol (383 mL) was added aqueous 50% trifluoroacetic acid (148 mL), and the mixture was boiled under reflux. After 8 h, the solution was neutralised with Amberlite IR-410 (HO^-) resin, filtered, and concentrated to give **8** (46.6 g, 100%) as a slightly yellow syrup. A sample of **8** was acetylated under standard conditions. T.l.c. (light petroleum–ethyl acetate, 2:1) showed two products. Column chromatography (above solvent system) gave methyl 2,3-di-*O*-acetyl-5-*O*-allyl-β-D-ribofuranoside, b.p. 116° (bath)/0.1 Torr, $[\alpha]_D^{20}$ −21° (*c* 2.1, chloroform). ^{1}H-N.m.r. data: *inter alia,* δ 5.41–5.07 (m, 4 H, allyl CH_2=, H-2,3), 4.90 (s, 1 H, H-1), 4.26 (q, 1 H, Σ*J* 16.2 Hz, H-4), 3.62–3.48 (m, 2 H, H-5,5′), 3.38 (s, 3 H, OMe), 2.05, 2.10 (2 s, 6 H, 2 OAc).

Anal. Calc. for $C_{13}H_{20}O_7$: C, 54.2; H, 7.0. Found: C, 54.1; H, 7.2.

Eluted second was the α anomer, b.p. 148° (bath)/0.2 Torr, $[\alpha]_D^{12}$ +93° (*c* 1.6, chloroform). ^{1}H-N.m.r. data: *inter alia,* δ 5.37–4.86 (m, 5 H, $J_{1,2}$ 4.5 Hz, H-

1,2,3, allyl CH_2=), 4.21 (q, 1 H, ΣJ 9 Hz, H-4), 3.72–3.62 (m, 2 H, H-5,5'), 3.45 (s, 3 H, OMe), 2.12 (s, 6 H, 2 OAc).

Anal. Found: C, 53.7; H, 7.0.

The $\alpha\beta$-ratio was 1:3.6.

Methyl 5-O-allyl-2,3-di-O-benzyl-α,β-D-ribofuranoside (**9**). — To a suspension of pulverized potassium hydroxide (8.4 g) in methyl sulfoxide (60 mL) at 5° was added a solution of **8** (3.08 g, 15 mmol) in methyl sulfoxide (20 mL) followed, dropwise, by a solution of benzyl bromide (6 mL) in methyl sulfoxide (10 mL). The mixture was stirred at room temperature until t.l.c. (light petroleum–ethyl acetate, 4:1) indicated complete reaction of **8**. The mixture was then diluted with ice–water and extracted with chloroform, and the extract was dried and concentrated. Column chromatography (light petroleum–ethyl acetate, 9:1) of the syrupy residue gave **β-9** (3.38 g, 58%), b.p. 185°/0.4 Torr, $[\alpha]_D^{20}$ +25° (*c* 3.6, chloroform). ^{1}H-N.m.r. data: *inter alia,* δ 4.89 (s, 1 H, H-1), 4.27 (m, 1 H, H-4), 4.10–3.89 (m, 3 H, H-3, allyl CH_2O), 3.80 (d, 1 H, $J_{2,3}$ 4.5 Hz, H-2), 3.66–3.39 (m, 2 H, H-5,5'), 3.31 (s, 3 H, OMe).

Anal. Calc. for $C_{23}H_{28}O_5$: C, 71.8; H, 7.3. Found: C, 72.0; H, 7.4.

Eluted second was **α-9** (0.99 g, 17%), isolated as a syrup, $[\alpha]_D^{14}$ +79.5° (*c* 1.9, chloroform). ^{1}H-N.m.r.: *inter alia,* δ 4.90 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.20 (m, 1 H, H-4), 3.95–3.65 (m, 4 H, H-2,3, and allyl CH_2O), 3.44 (s, 3 H, OMe), 3.39–3.28 (m, 2 H, H-5,5').

Anal. Calc. for $C_{23}H_{28}O_5 \cdot H_2O$: C, 68.6; H, 7.5. Found: C, 68.6; H, 7.3.

5-O-Allyl-2,3-di-O-benzyl-β-D-ribofuranose (**10**). — A solution of **β-9** (37.75 g, 0.098 mol) in 1,4-dioxane (317 mL) was boiled under reflux with 2M hydrochloric acid (317 mL) until **β-9** disappeared (8 h; t.l.c., light petroleum–ether–methanol, 25:25:1). The mixture was then neutralised with sodium hydrogencarbonate and concentrated, and a solution of the residue in chloroform was dried and concentrated. Column chromatography (light petroleum–ethyl acetate, 9:1) of the residue gave **10** (26.3 g, 86%), m.p. 41.5–42.5° (from light petroleum–ethyl acetate, 4:1), $[\alpha]_D^{20}$ +62° (*c* 2, chloroform). ^{1}H-N.m.r. data: *inter alia,* δ 5.31 (s, 1 H, H-1), 4.42–3.27 (m, 6 H, H-2,3,4, OH, allyl CH_2O), 3.68–3.32 (m, 2 H, H-5,5').

Anal. Calc. for $C_{22}H_{26}O_5$: C, 71.3; H, 7.1. Found: C, 70.9; H, 7.1.

5-O-Allyl-2,3-di-O-benzyl-D-ribitol (**11**). — To a solution of sodium borohydride (3.34 g, 0.088 mol) in dry ethanol (100 mL) at 5° was added dropwise a solution of **10** (19.1 g, 0.052 mol) in ethanol (200 mL), and the mixture was stirred for 2 h at room temperature. Acetic acid was added to pH 5, followed by chloroform (150 mL) and M hydrochloric acid (150 mL). The organic layer was washed with water, dried, and concentrated. Column chromatography (light petroleum–ethyl acetate, 4:1) of the residue gave **11** (18.35 g, 95%), b.p. 205°/0.3 Torr, $[\alpha]_D^{20}$ +15° (*c* 2.3, chloroform). ^{1}H-N.m.r. data: *inter alia,* δ 4.08–3.28 (m, 10 H, H-1a,1b,2,3,4,5a,5b, OH and allyl CH_2O).

Anal. Calc. for $C_{22}H_{28}O_5$: C, 70.9; H, 7.6. Found: C, 70.3; H, 7.6.

5-O-Allyl-1,2,3- (**12**) *and -2,3,4-tri-O-benzyl-D-ribitol* (**13**). — To a solution

of **11** (24.7 g, 0.066 mol) in dichloromethane (500 mL) was added aqueous 5% sodium hydroxide (150 mL), tetrabutylammonium bromide (3.75 g), and benzyl bromide (10.2 mL), and the mixture was stirred vigorously and boiled under reflux. After 16 h, the organic layer was separated, dried, and concentrated to dryness. Column chromatography (light petroleum–ethyl acetate, 9:1) of the oily residue yielded **12** (15.58 g, 56%), **13** (5.29 g, 19%), and **11** (2.3 g).

Compound **12** had b.p. 220°/0.4 Torr, $[\alpha]_D^{20}$ +6° (*c* 2.1, chloroform). N.m.r. data: ^{1}H, *inter alia*, δ 4.05–3.46 (m, 10 H, H-1a,1b,2,3,4,5a,5b, OH, and allyl CH_2O); ^{13}C, δ 134.6, 117.1, 71.2 (allyl C), 72.4, 73.4, 73.7 (3 Ph*C*H$_2$), 78.9, 79.3 (C-2,3), 70.9 (C-4), 72.2 (C-5), 69.7 (C-1).

Anal. Calc. for $C_{29}H_{34}O_5$: C, 75.3; H, 7.4. Found: C, 75.2; H, 7.5.

Compound **13** was isolated as a syrup, $[\alpha]_D^{16}$ −15° (*c* 2, chloroform). N.m.r. data: ^{1}H, *inter alia*, δ 3.98–3.54 (m, 9 H, H-1a,1b,2,3,4,5a,5b, allyl CH_2O), 2.69–2.36 (bs, 1 H, OH); ^{13}C, δ 134.7, 116.8, 72.2 (allyl C), 71.9, 72.4, 73.9 (3 Ph*C*H$_2$), 78.1, 78.8, 79.0 (C-2,3,4), 69.7 (C-5), 61.3 (C-1).

Anal. Found: C, 74.8; H, 7.7.

*5-O-Allyl-1,2,3-tri-O-benzyl-4-O-(2,3-di-O-acetyl-4,6-O-ethylidene-β-*D*-glucopyranosyl)-*D*-ribitol* (**16**). — A solution of **14**[9] (4 g, 12 mmol) in dichloromethane was saturated with hydrogen bromide for 2 h at −20° (t.l.c.; toluene–acetone, 9:1). The solvent was evaporated, the residue was three times dissolved in toluene, and the solution was concentrated to dryness to give homogeneous syrupy 2,3-di-*O*-acetyl-4,6-*O*-ethylidene-α-D-glucopyranosyl bromide (**15**), which was used directly. To a solution of **12** (0.94 g, 2.04 mmol) in dry benzene (24 mL) were added mercuric bromide (3.66 g, 10.16 mmol), mercuric cyanide (2.56 g, 10.16 mmol), and pulverised molecular sieves (3 Å, 0.94 g). The suspension was stirred for 2 h at room temperature and then cooled to 0°, a solution of **15** (4 g, 12 mmol) in dry nitromethane (24 mL) was added dropwise, and stirring was continued for 1 h until room temperature was attained. The mixture was filtered through a short column of Celite and concentrated, and a solution of the residue in chloroform was washed successively with aqueous 5% potassium iodide and water, dried, and concentrated. Column chromatography (light petroleum–ether, 7:2) of the residue gave **17** (0.585 g, 17%), m.p. 167–168° (from light petroleum–ether, 4:1), $[\alpha]_D^{14}$ +18° (*c* 2.1, chloroform). ^{1}H-n.m.r. data: δ 6.36 (s, 1 H, H-1), 4.73 (q, 1 H, *J* 4.9 Hz, CH_3CH=), 4.47 (dd, 1 H, $J_{3,2}$ 6.1 Hz, $J_{3,4}$ 7.8 Hz, H-3), 4.23 (d, 1 H, H-2), 4.14 (dd, 1 H, $J_{6e,6a}$ 10.3 Hz, $J_{6e,5}$ 5.3 Hz, H-6*e*), 3.66 (dt, 1 H, $J_{5,4}$ 10.2 Hz, $J_{5,6a}$ 10.2 Hz, H-5), 3.48 (t, 1 H, H-6*a*), 3.37 (dd, 1 H, H-4).

Anal. Calc. for $C_{13}H_{17}NO_7$: C, 52.2; H, 5.7; N, 4.7. Found: C, 52.4; H, 5.7; N, 5.2.

Eluted second was **16** (1.31 g, 86%), isolated as a syrup, $[\alpha]_D^{16}$ −60° (*c* 2.1, chloroform). N.m.r. data: ^{1}H, *inter alia*, δ 4.96 (dd, 1 H, $J_{2,3}$ 9.2 Hz, $J_{2,1}$ 7.8 Hz, H-2), 4.87 (d, 1 H, H-1), 4.25–3.40 (m, 12 H, H-1a,1b,2,3,4,5a,5b,4′,6′*a*,6′*e*, allyl CH_2O), 3.31 (dt, 1 H, $J_{5,4} \approx J_{5,6a} \approx 9.3$ Hz, $J_{5,6e}$ 4.7 Hz, H-5′); ^{13}C, δ 134.6, 116.9, 72.0 (allyl C), 72.2, 72.7, 73.3 (3 Ph*C*H$_2$), 100.9 (C-1′), 99.9 (CH_3CH=), 78.0,

78.6, 79.1 (C-2,3,4), 77.9 (C-4′), 71.8 (C-2′), 71.5 (C-3′), 70.8 (C-5), 69.7 (C-1), 68.0 (C-6′), 66.1 (C-5′).

Anal. Calc. for $C_{41}H_{50}O_{12}$: C, 67.0; H, 6.9. Found: C, 66.6; H, 7.0.

5-O-Allyl-1,2,3-tri-O-benzyl-4-O-(2,3-di-O-benzyl-4,6-O-ethylidene-β-D-glucopyranosyl)-D-ribitol (**18**). — To a stirred solution of **16** (1.11 g, 1.5 mmol) in dry *N,N*-dimethylformamide (20 mL) was added sodium hydride (2.22 g, 50% dispersion in oil) at 0°, and the solution was allowed to attain room temperature (2 h, 20°). A solution of benzyl bromide (0.72 mL, 6.04 mmol) in *N,N*-dimethylformamide (1 mL) was added, and the mixture was stirred for 1.5 h at room temperature and then worked-up conventionally. Column chromatography (light petroleum–ether, 4:1) of the product gave **18** (0.87 g, 70%), isolated as a syrup, $[\alpha]_D^{10}$ −10° (*c* 2.1, chloroform). N.m.r. data: ^{1}H, *inter alia,* δ 4.23–3.31 (m, 12 H, H-1a,1b,2,3,4,5a,5b,4′,6′*a*,6′*e*, allyl CH_2O), 3.19 (dt, 1 H, $J_{5',4} \approx J_{5'6'a} \approx 9.4$ Hz, $J_{5',6'e}$ 5.0 Hz, H-5′); ^{13}C, δ 134.7, 116.8, 72.2 (allyl C), 72.2, 73.3, 74.0, 74.9 (2) (5 Ph*C*H_2), 99.4 (*C*H_3*C*H=), 103.2 (C-1′), 82.3 (C-3′), 81.0 (C-2′,4), 79.4 (C-3), 78.0 (C-2,4′), 70.1 (C-1,5), 68.2 (C-6′,1), 65.8 (C-5′).

Anal. Calc. for $C_{51}H_{58}O_{10}$: C, 73.7; H, 7.0. Found: C, 73.6; H, 7.0.

5-O-Allyl-1,2,3-tri-O-benzyl-4-O-(2,3-di-O-benzyl-β-D-glucopyranosyl)-D-ribitol (**19**). — To a solution of **18** (0.096 g, 0.12 mmol) in methanol (5 mL) was added Dowex 50W-X4 (H^+) resin (0.5 g), and the mixture was stirred and boiled under reflux until t.l.c. (light petroleum–ethyl acetate, 1:1) showed the disappearance of **18** (6 h). The mixture was then filtered and concentrated. Column chromatography (light petroleum–ethyl acetate, 4:1) of the syrupy residue yielded **19** (0.051 g, 80%), isolated as a syrup, $[\alpha]_D^{18}$ −14° (*c* 1.2, chloroform). N.m.r. data: ^{1}H, δ 4.11–3.15 (m, 13 H, H-1a,1b,2,3,4,5a,5b,4′,5′,6′a,6′b, allyl CH_2O); ^{13}C, δ 134.7, 116.8, 72.0 (allyl C), 72.2, 73.7, 74.1, 74.2, 75.2 (5 Ph*C*H_2), 103.3 (C-1′), 84.0 (C-3′), 81.9 (C-2′), 77.7, 78.6, 78.9 (C-2,3,4), 75.2 (C-5′), 70.2 (C-4′,5), 70.0 (C-1), 62.0 (C-6′).

Anal. Calc. for $C_{49}H_{56}O_{10}$: C, 73.1; H, 7.0. Found: C, 73.0; H, 7.0.

5-O-Allyl-1,2,3-tri-O-benzyl-4-O-(2,3,6-tri-O-benzyl-β-D-glucopyranosyl)-D-ribitol (**20**) *and 5-O-allyl-1,2,3-tri-O-benzyl-4-O-(2,3,4-tri-O-benzyl-β-D-glucopyranosyl)-D-ribitol* (**21**). — To a solution of **19** (1.49 g, 1.85 mmol) in dichloromethane (10 mL) were added aqueous 5% sodium hydroxide (2.5 mL), tetrabutylammonium bromide (0.042 g), and benzyl bromide (0.26 mL, 2.2 mmol), and the mixture was stirred vigorously and boiled under reflux. After 19 h, the organic layer was separated, dried, and concentrated. Column chromatography (light petroleum–ether, 4:1) of the residue gave, first, **21** (0.62 g, 46%), isolated as a syrup, $[\alpha]_D^{18}$ +2° (*c* 1.1, chloroform). N.m.r. data: ^{1}H, δ 4.14–3.38 (m, 11 H, H-1a,1b,2,3,4,5a,5b,6′a,6′b, allyl CH_2O), 3.39 [t, 1 H, *J* 9.5 Hz, H-3′(4′)], 3.30 [t, 1 H, *J* 9.2 Hz, H-4′(3′)], 3.23 (dt, 1 H, $J_{5',6'a} \approx J_{5',4'} \approx 9.2$, $J_{5',6'b}$ 5 Hz, H-5′); ^{13}C, δ 134.7, 116.6, 72.0 (allyl C), 72.2, 73.0, 73.9, 74.2, 75.1, 75.4 (6 Ph*C*H_2), 103.1 (C-1′), 84.5 (C-3′), 82.3 (C-2′), 77.8, 78.6, 78.9 (C-2,3,4), 77.5 (C-4′), 74.8 (C-5′), 70.3 (C-1,5), 61.7 (C-6′).

TABLE I

^{13}C-N.M.R. DATA FOR 2-*O*-β-D-GLUCOPYRANOSYL-L-RIBITOL (**28**) AND ITS 4′- (**2**) AND 1-PHOSPHATE (**3**)

	C-1′	*C-2′*	*C-3′*	*C-4′*	*C-5′*	*C-6′*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*
28	103.2	74.1	76.4	70.3	76.4	61.4	63.4	72.6	72.2	81.7	61.1
2	103.4	73.1	76.4	74.4	76.3[a]	61.8	63.9	73.1	72.8	82.1	61.6
3	103.5	74.5	76.9[b]	70.8	77.0[b]	61.9	63.8	73.0	72.6	80.2[c]	66.2[d]

[a]$^{3}J_{C,P}$ 6.3 Hz. [b]The assignments may be reversed. [c]$^{3}J_{C,P}$ 7.3 Hz. [d]$^{2}J_{C,P}$ 5.3 Hz.

Anal. Calc. for $C_{56}H_{62}O_{10}$: C, 75.1; H, 7.0. Found: C, 75.4; H, 7.0.

Eluted second was **20** (0.66 g, 49%), isolated as a syrup, $[\alpha]_D^{18}$ −16° (*c* 2.5, chloroform). N.m.r. data: ^{1}H, δ 4.25–3.32 (m, 13 H, H-1a,1b,2,3,4,5a,5b,4′,5′, 6′a,6′b, allyl CH_2O); ^{13}C, δ 134.8, 116.7, 72.1 (allyl C), 72.1, 73.3, 73.6, 73.9, 74.0, 75.1 (6 PhCH_2), 103.1 (C-1′), 84.2 (C-3′), 81.9 (C-2′), 78.2, 78.4, 79.6 (C-2,3,4), 74.4 (C-5′), 71.9 (C-4′), 70.5 (C-5), 70.3 (C-1), 70.1 (C-6′).

Anal. Found: C, 75.3; H, 7.0.

Eluted third was **19** (0.29 g).

5-O-Allyl-1,2,3-tri-O-benzyl-4-O-(2,3,6-tri-O-benzyl-4-O-diphenoxyphosphoryl-β-D-glucopyranosyl)-D-ribitol (**22**). — To a solution of 1,2,4-triazole (0.021 g, 0.39 mmol) and triethylamine (0.043 mL, 0.31 mmol) in oxolane (0.5 mL) were added triethylamine (0.04 mL) and diphenyl phosphorochloridate (0.06 mL) at 10° under argon, and the mixture was stirred for 10 min at 10° and then for 45 min at room temperature. Insoluble material was removed, the filtrate was concentrated to ~0.5 mL, and a solution of **20** (0.092 g, 0.103 mmol) in pyridine (0.5 mL) and a crystal of 4-dimethylaminopyridine were added. The mixture was heated to 40°. After 1.5 h (t.l.c.; light petroleum–ethyl acetate, 4:1), two drops of water were added, and the mixture was stirred for 0.5 h and concentrated. A solution of the residue in dichloromethane was filtered through a short column of silica gel and then subjected to flash chromatography (light petroleum–ethyl acetate, 4:1) to yield **22** (0.099 g, 85%), isolated as a syrup, $[\alpha]_D$ +4.5° (*c* 1.8, chloroform). N.m.r. data: ^{13}C, δ 134.9, 116.8, 72.2 (allyl C), 72.3, 73.4, 73.5, 74.1, 74.5, 74.9 (6 PhCH_2), 103.1 (C-1′), 82.4 (d, $^{3}J_{C,P}$ 2.7 Hz, C-3′), 82.3 (C-2′), 78.4, 78.7, 79.8 (C-2,3,4), 77.0 (d, $^{2}J_{C,P}$ 7.4 Hz, C-4′), 74.0 (d, $^{3}J_{C,P}$ 5.6 Hz, C-5′), 70.3, 70.4 (C-1,5), 69.0 (C-6′); ^{31}P, δ 13.5.

Anal. Calc. for $C_{68}H_{71}O_{13}P$: C, 72.4; H, 6.3. Found: C, 72.4; H, 6.4.

5-O-Allyl-1,2,3-tri-O-benzyl-4-O-(2,3,4-tri-O-benzyl-6-O-diphenoxyphosphoryl-β-D-glucopyranosyl)-D-ribitol (**23**). — Treatment of **21** (0.3 g, 0.335 mmol), as described for **22**, with a reagent prepared from 1,2,4-triazole (69 mg) and diphenyl phosphorochloridate (0.208 mL) gave **23** (0.26 g, 77%), isolated as a colorless syrup, $[\alpha]_D^{18}$ −2.3° (*c* 1.9, chloroform). N.m.r. data: ^{13}C, δ 134.7, 116.7, 72.2 (allyl C), 72.1, 73.2, 73.9, 74.4, 74.8, 75.5 (6 PhCH_2), 103.1 (C-1′), 84.5 (C-3′), 82.2 (C-2′),

78.4, 78.6, 79.7 (C-2,3,4), 76.9 (C-4'), 73.6 (C-5'), 70.2, 70.5 (C-1,5), 67.6 (d, $^2J_{C,P}$ 4.9 Hz, C-6'); ^{31}P δ 12.2.

Anal. Calc. for $C_{68}H_{71}O_{13}P$: C, 72.4; H, 6.3; P, 2.7. Found: C, 72.4; H, 6.6; P, 3.1.

2-O-β-D-Glucopyranosyl-L-ribitol 4'-phosphate (**2**). — To a solution of **22** (0.215 g, 0.19 mmol) in acetic acid–water (2:1, 2.25 mL) were aded palladium dichloride (0.038 g) and sodium acetate (0.038 g), and the mixture was stirred overnight at 40° (t.l.c.; light petroleum–ether, 1:1), then neutralised with sodium hydrogencarbonate, diluted with acetone, filtered, and concentrated to dryness. A solution of the residue in ether was filtered through Celite and concentrated. Column chromatography (light petroleum–ethyl acetate, 7:3) of the syrupy residue gave **24** (0.153 g, 74%), isolated as a syrup, $[\alpha]_D^{19}$ +8.5° (*c* 1.9, chloroform).

To a solution of **24** (0.14 g, 0.13 mmol) in ethanol–ethyl acetate (1:1, 20 mL) were added 10% Pd/C (0.14 g) and platinum oxide (0.14 g), and the mixture was hydrogenated for 48 h until **24** had disappeared (t.l.c.; chloroform–methanol–water–ammonium hydroxide, 30:30:1:0.1). The solution was then filtered through Celite and concentrated to give syrupy **2** (0.051 g, 100%), $[\alpha]_D^{13}$ −2° (*c* 1.1, methanol). The ^{13}C-n.m.r. data are given in Table I.

5-O-Allyl-1,2,3-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-D-ribitol (**25**). — To dry mercuric cyanide (2.46 g, 9.5 mmol) and pulverised molecular sieves (4 Å, 1.5 g) was added a solution of **12** (1.5 G, 3.25 mmol) in benzene (36 mL). The suspension was stirred under argon for 1 h and cooled to 0°, and a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (4.023 g, 9.8 mmol) in nitromethane (36 mL) was added. The mixture was stirred for 3 h at 50° under argon. After completion of the reaction (t.l.c.; light petroleum–acetone, 7:3), the mixture was filtered through Celite and concentrated. A solution of the oily residue in chloroform was washed with aqueous 5% potassium iodide and water, dried, and concentrated. Column chromatography (light petroleum–acetone, 9:1) of the residue gave **25** (1.945 g, 75.7%), isolated as an oil, $[\alpha]_D^{14}$ −14.5° (*c* 1.2, chloroform); ν_{max} 1755, 1070, 1040 cm^{-1}. ^{1}H-N.m.r. data: *inter alia*, δ 4.35–3.54 (m, 12 H, H-1a,1b,2,3,4,5a,5b,5',6'a,6'b, allyl CH_2O), 1.95, 1.99, 2.00, 2.02 (4 s, 12 H, 4 OAc).

Anal. Calc. for $C_{43}H_{52}O_{14}$: C, 65.2; H, 6.6. Found: C, 65.1; H, 6.7.

Eluted next was **26** (0.893 g, 25.6%), m.p. 75–77° (from ethanol), $[\alpha]_D^{21}$ +13.8° (*c* 1.3, chloroform); lit.[10] m.p. 77–78°, $[\alpha]_D^{20}$ +13.8° (chloroform).

1,2,3-Tri-O-benzyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-D-ribitol (**27**). — A solution of **25** (1.712 g, 2.16 mmol) in acetic acid (17.2 mL) and water (0.868 mL) was stirred with sodium acetate (0.434 g) and palladium chloride (0.434 g) at room temperature until **25** disappeared (48 h; t.l.c., light petroleum–ethyl acetate, 1:1). The mixture was neutralised with solid sodium hydrogencarbonate, diluted with acetone, filtered, and co-concentrated with toluene (removal of acetic acid). The residue was triturated with ether, and the solution was filtered through Celite and concentrated. Column chromatography (light petroleum–ethyl acetate,

85:15) of the residue gave **27** (1.404 g, 86.7%), isolated as a colorless oil, $[\alpha]_D^{14}$ −4° (*c* 1.5, chloroform); ν_{max} 1750, 1190 cm^{-1}. ^{1}H-N.m.r. data: *inter alia*, δ 4.35–3.50 (m, 10 H, H-1a,1b,2,3,4,5a,5b,5′,6′a,6′b), 1.97, 2.01, 2.03 (3 s, 12 H, 4 OAc).

Anal. Calc. for $C_{40}H_{48}O_{14}$: C, 63.8; H, 6.4. Found: C, 63.7; H, 6.5.

2-O-β-D-Glucopyranosyl-L-ribitol (**28**). — To a solution of **27** (0.166 g, 0.21 mmol) in methanol (4 mL) was added methanolic 0.1M sodium methoxide (0.3 mL). After completion of the reaction, the mixture was neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The residue was purified on a short column of silica gel with chloroform–methanol (3:1). The effluent was concentrated and a solution of the residue in ethanol was hydrogenated over 10% Pd/C (0.1 g). Filtration and concentration then gave amorphous **28** (50 mg, 100%), $[\alpha]_D^{15}$ −23° (*c* 1.5, water); lit.[14] m.p. 134–136°, $[\alpha]_D$ −22° (water). The ^{13}C-n.m.r. data are given in Table I.

1,2,3-Tri-O-benzyl-5-O-diphenoxyphosphoryl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-D-ribitol (**29**). — To a solution of 1,2,4-triazole (0.104 g, 1.5 mmol) in 1,4-dioxane was added triethylamine (0.209 mL, 1.5 mmol) at 10° under argon, followed by diphenyl phosphorochloridate (0.311 mL, 1.5 mmol) in 1,4-dioxane (0.5 mL). The mixture was stirred for 10 min at 10° and then for 45 min at room temperature.

A solution of **27** (0.376 g, 0.5 mmol) in dry pyridine (5 mL) was concentrated carefully to dryness under vacuum and to the residue was added a filtered solution of the phosphorotriazolide. The mixture was concentrated to ~1 mL, pyridine (1 mL) and a crystal of 4-dimethylaminopyridine were added, and the reaction was monitored by t.l.c. (light petroleum–ethyl acetate, 2:1). When the reaction was complete, 2 drops of water were added and the mixture was concentrated to dryness. Flash chromatography (light petroleum–ethyl acetate, 3:2) of the residue gave **29** (0.253 g, 51.4%), isolated as a colorless oil, $[\alpha]_D^{14}$ −13° (*c* 1.6, chloroform); ν_{max} 1750, 1200 cm^{-1}. ^{13}C-n.m.r. data: δ 101.2 (C-1′), 79.4 (d, $^3J_{C,P}$ 7.3 Hz, C-4), 79.0 (C-3), 77.8 (C-2), 72.3, 73.3, 73.9 (3 Ph*C*H_2), 72.7 (C-3′), 71.3, 71.7 (C-2′,5′), 69.3 (C-1), 69.2 (d, $^2J_{C,P}$ 6.5 Hz, C-5), 68.5 (C-4′), 61.8 (C-6′).

Anal. Calc. for $C_{52}H_{57}O_{17}P$: C, 63.4; H, 5.8. Found: C, 63.4; H, 5.9.

2-O-β-D-Glucopyranosyl-L-ribitol 1-phosphate (**3**). — A solution of **29** (0.24 g) in methanol (2 mL) was treated with methanolic 0.2M sodium methoxide (0.6 mL). After completion of the reaction, the mixture was neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated. Column chromatography (chloroform–methanol, 99:1) of the oily residue gave the amorphous deacetylated product (0.139 g, 70%), a solution of which in ethyl acetate–ethanol (11 mL, 1:10) was hydrogenated in the presence of 10% Pd/C (0.14 g) and PtO_2 (0.14 g). After completion of the reaction (t.l.c., chloroform-methanol, 1:1), the mixture was filtered and concentrated to yield amorphous **3** (0.67 g, 100%), $[\alpha]_D^{16}$ −14° (*c* 2.2, methanol). The ^{13}C-n.m.r. data are given in Table I.

Anal. Calc. for $C_{11}H_{23}O_{13}P \cdot H_2O$: C, 32.0; H, 6.1. Found: C, 32.1; H, 6.4.

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