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SYNTHESES OF A BRANCHED HEPTASACCHARIDE HAVING PHYTOALEXIN-ELICITOR ACTIVITY*

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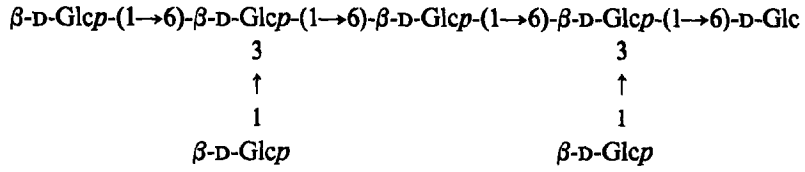
ABSTRACT

Syntheses are described of a D-glucose heptasaccharide, **1**, corresponding to a glucan structure recognised by the soybean when infected by the fungus *Phytophthora megasperma* f. sp. *glycinea* and which stimulates the formation of phytoalexins. The synthetic strategy is based upon 1,2-*trans*-glycoside formation assisted by participating benzoate groups in the 2-position, with silver triflate as promoter and glycosyl bromides as donors for making the smaller fragments, and with methyl triflate as promoter with thioglycosides as donors for making the larger ones. Regioselective reductive openings of 4,6-benzylidene acetals play a key role in obtaining free 6-OH groups with benzyl protection at O-4.



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INTRODUCTION

We have previously described the synthesis of a branched heptasaccharide **1**, which corresponds to the glucan fragment responsible for triggering the defense of the soybean to infections by the mould *Phytophthora megasperma*[1,2]. All the glycosidic links in that oligosaccharide have the β-D-configuration. The classical synthesis of such 1,2-*trans*-glycosidic linkages is the Koenigs–Knorr reaction, in which an acylated glycosyl halide is allowed to react with a hydroxylic compound in the presence of an insoluble silver catalyst[3]. The use of soluble mercury catalysts such as

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mercury (II) bromide and cyanide, usually in toluene, nitromethane or mixtures of these, was later introduced and represented a considerable advance over the original procedure. This method, often referred to as the Helferich modification of the Koenigs–Knorr reaction[4,5], still enjoys considerable popularity as a reliable method for making di- and oligo-saccharides joined by 1,2-*trans*-glycosidic linkages. A more recent modification involves the use of silver triflate as promoter[6], and this subject has been extensively reviewed[7]. Higher yields are obtained with a participating *O*-benzoyl rather than an *O*-acetyl group in the 2-position of the glycosyl halide[8].

In our previous synthesis of **1** we used the block condensation method. One advantage of block synthesis of oligosaccharides is that manipulations of protecting group on large fragments are kept to a minimum[7]. A distinct disadvantage is the need (usually) for the conversion of an oligosaccharide into a glycosyl halide. This is apt to produce an unsatisfactory yield[1]. Several solutions to this problem have been suggested[9–12].

Efficient syntheses have now been developed in this laboratory, based on thioglycosides as potential glycosyl donors, and the use of thiophilic activators[10,11]. In the present communication we describe a most efficient route to the heptasaccharide **1**. The synthesis employs the following key methods: (1) the use of glucosyl bromides carrying participating benzoyl groups in the 2-position[8] as donors in silver triflate-promoted glycosylations[6] for making the smaller oligosaccharide fragments; (2) the use of regioselective reductive cleavage of benzylidene acetals[13]; and (3) the use of thioglycosides activated by methyl trifluoromethanesulfonate ("triflate") in glycosidations involving large blocks[10].

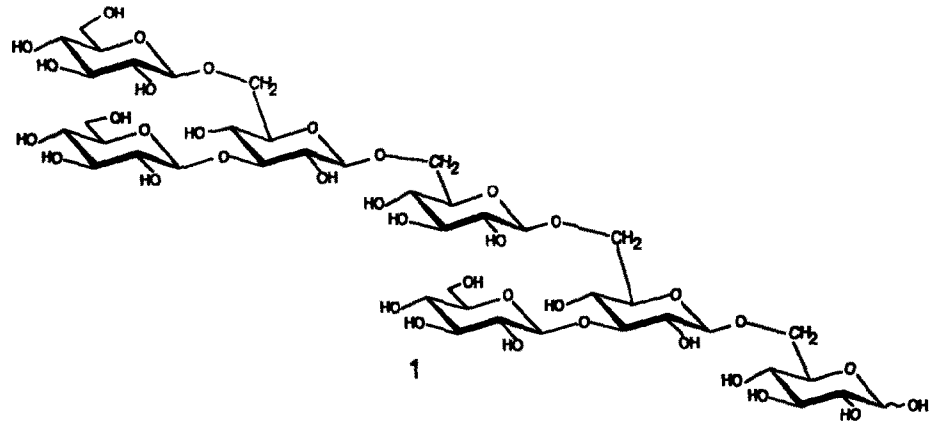
RESULTS

Two routes were explored to the disaccharide derivative **5**. In the first of these, methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside[14] (**2**) was deacetylated and then converted into the 2-*O*-benzoyl-4,6-*O*-benzylidene derivative **4**, by treatment with benzaldehyde and *p*-toluenesulfonic acid to give **3** followed by solid–liquid partial phase-transfer benzoylation[15]. Silver triflate-promoted glycosylation[6,8] of **4** with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[16] afforded the disaccharide derivative **5** in 35% overall yield.

In the second, more direct route the 4,6-*O*-benzylidene derivative **3** was subjected to silver triflate-promoted partial glycosylation using the same glycosyl bromide, and the product was then benzoylated in the 2-position to give **5** in 38% overall yield.

Treatment of **5** with borane–trimethylamine and aluminium chloride afforded **6**, which has a free hydroxyl group in the 6-position, in 90% yield. Silver triflate-promoted condensation of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide with **6** afforded the trisaccharide **7**, later to be used as a glycosyl donor, in 86% yield.

The tetrasaccharide **12**, later to be used as a glycosyl acceptor, was synthesised as follows. Benzyl β-D-glucopyranoside[17,18] was tritylated in the 6-position, ben-



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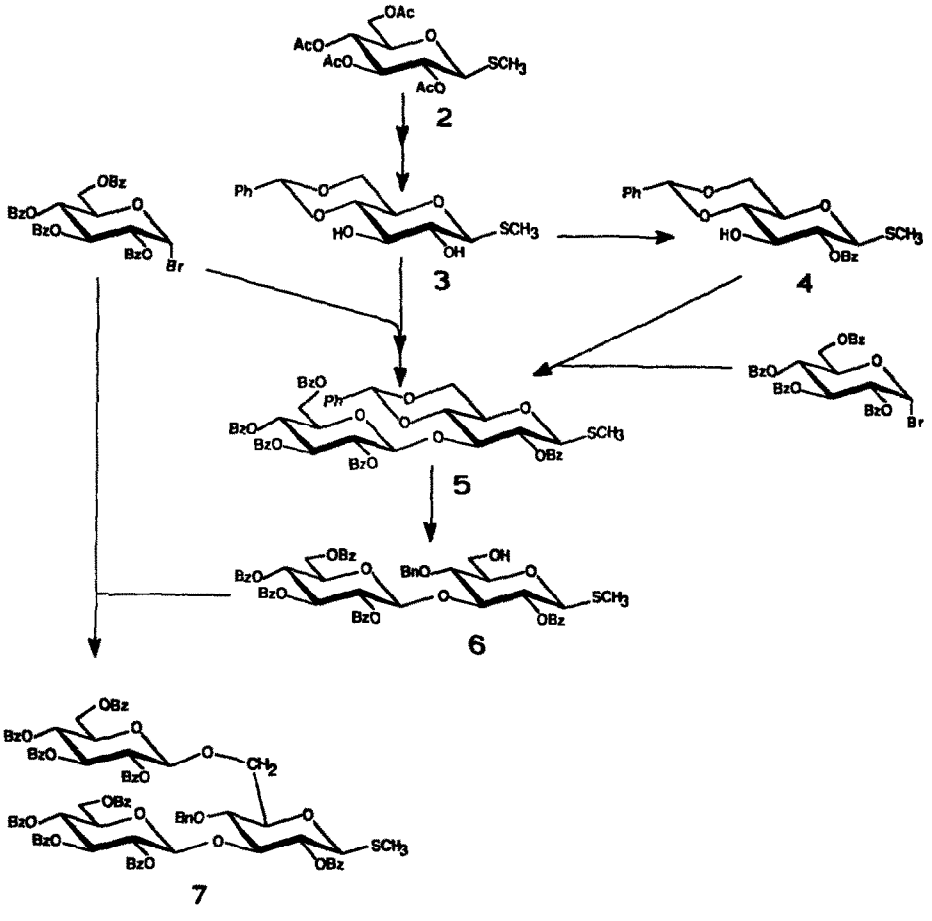

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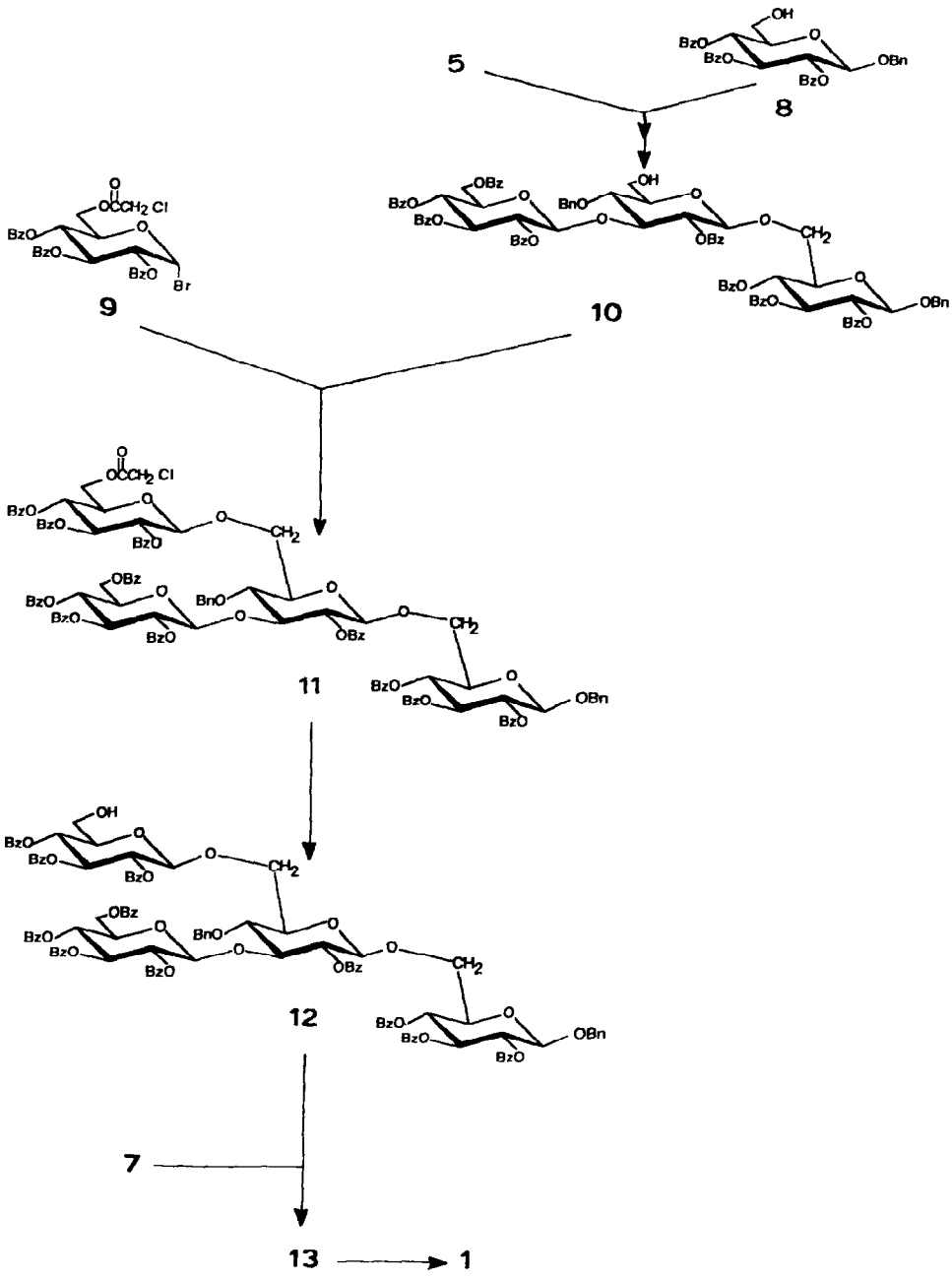
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zoylated, and detritylated to yield the 2,3,4-tribenzoate **8**. This was glycosylated in the 6-position by a methyl triflate-promoted condensation[10] with **5** to give the expected trisaccharide, which then was treated with trimethylamine–borane and aluminium chloride[13], thereby opening the 4′, 6′-benzylidene acetal ring to give the trisaccharide derivative **10** having a free 6′-OH group, in 84% yield.

D-Glucose was treated first with chlorotriphenylmethane and then with benzoyl chloride in pyridine to give 1,2,3,4-tetra-*O*-benzoyl-6-*O*-triphenylmethyl-D-glucopyranose as an anomeric mixture. This was then detritylated, and converted into the 6-*O*-chloroacetyl derivative. The latter upon treatment with hydrogen bromide in acetic acid gave 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-glucopyranosyl bromide **9** which was used directly. Silver triflate-promoted condensation of **9** with the glycosyl acceptor **10** gave the tetrasaccharide **11** in 77% yield, from which the chloroacetyl group was removed by treatment with hydrazine dithiocarbonate[19] to give a 78% yield of **12**.

Condensation of the thioglycoside **7** with **12** in the presence of methyl triflate as promoter afforded the protected heptasaccharide **13** in 91% yield, the deprotection of **13** gave **1**.

In an alternative route to **1**, **6** was condensed with the glycosyl bromide **9** using silver triflate as promoter. The key compound **14** was the glycosyl donor in the final condensation. It was also the precursor for the glycosyl acceptor, obtained as follows. Condensation of **14** with 1,2,3,4-tetra-*O*-benzoyl-β-D-glucopyranose using methyl triflate as promoter afforded tetrasaccharide **15** in 83% yield. The chloroacetyl group in **15** was removed by treatment with hydrazine dithiocarbonate[19] to give **16** in 88% yield.

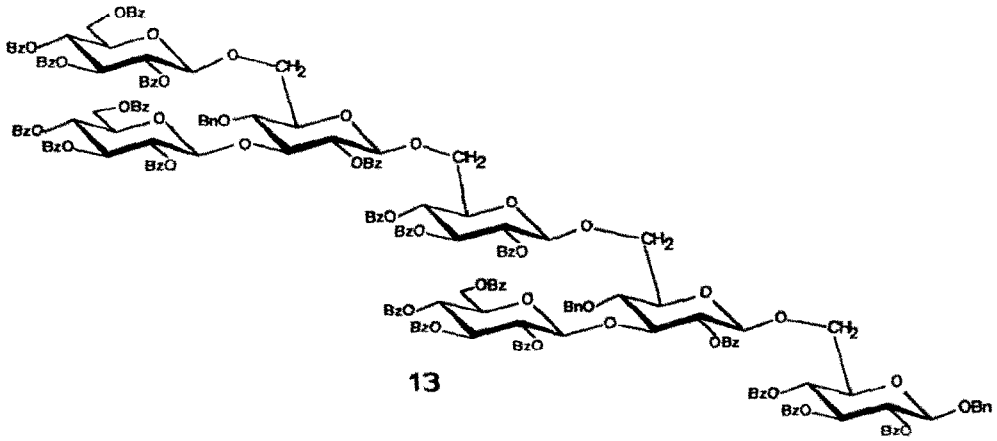
The final condensation of the glycosyl donor **14** with the acceptor **16** in dichloromethane, again using methyl triflate as promoter, gave the protected heptasaccharide **17** in 93% yield. This was deblocked by debenzoylation and hydrogenolysis to give the heptasaccharide **1**, which had optical rotation, n.m.r. spectra, l.c. retention time, and phytoalexin-elicitor activity identical to the material previously synthesised.

Of the two syntheses, the latter one was preferred for large-scale work (gram quantities).

EXPERIMENTAL

General methods. — These were the same as previously described[1]. N.m.r. spectra were recorded using Jeol FX 100 and GX 400 instruments. All spectra were in agreement with postulated structures and are obtainable upon request. Only selected, particularly significant n.m.r. data are given below. The purity of syrupy intermediates, for which elemental analyses were not performed, was carefully ascertained by t.l.c. and n.m.r.

*Methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside* (**2**). — Triethylamine (7.0 mL, 50 mmol) and iodomethane (6.3 mL, 101 mmol) were added at room



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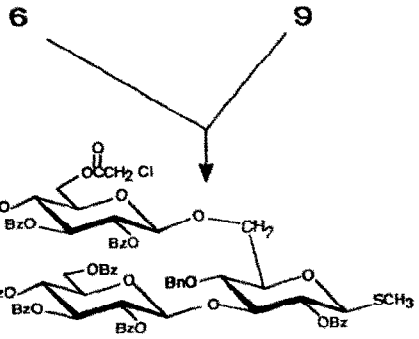

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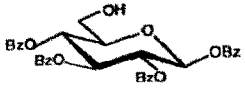

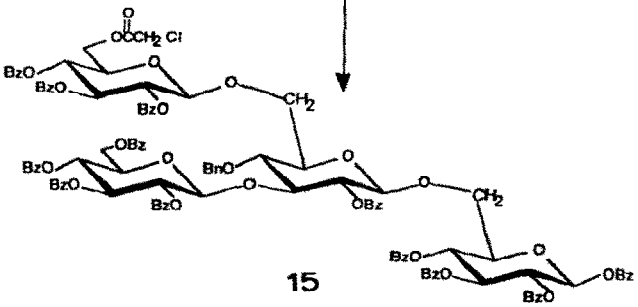

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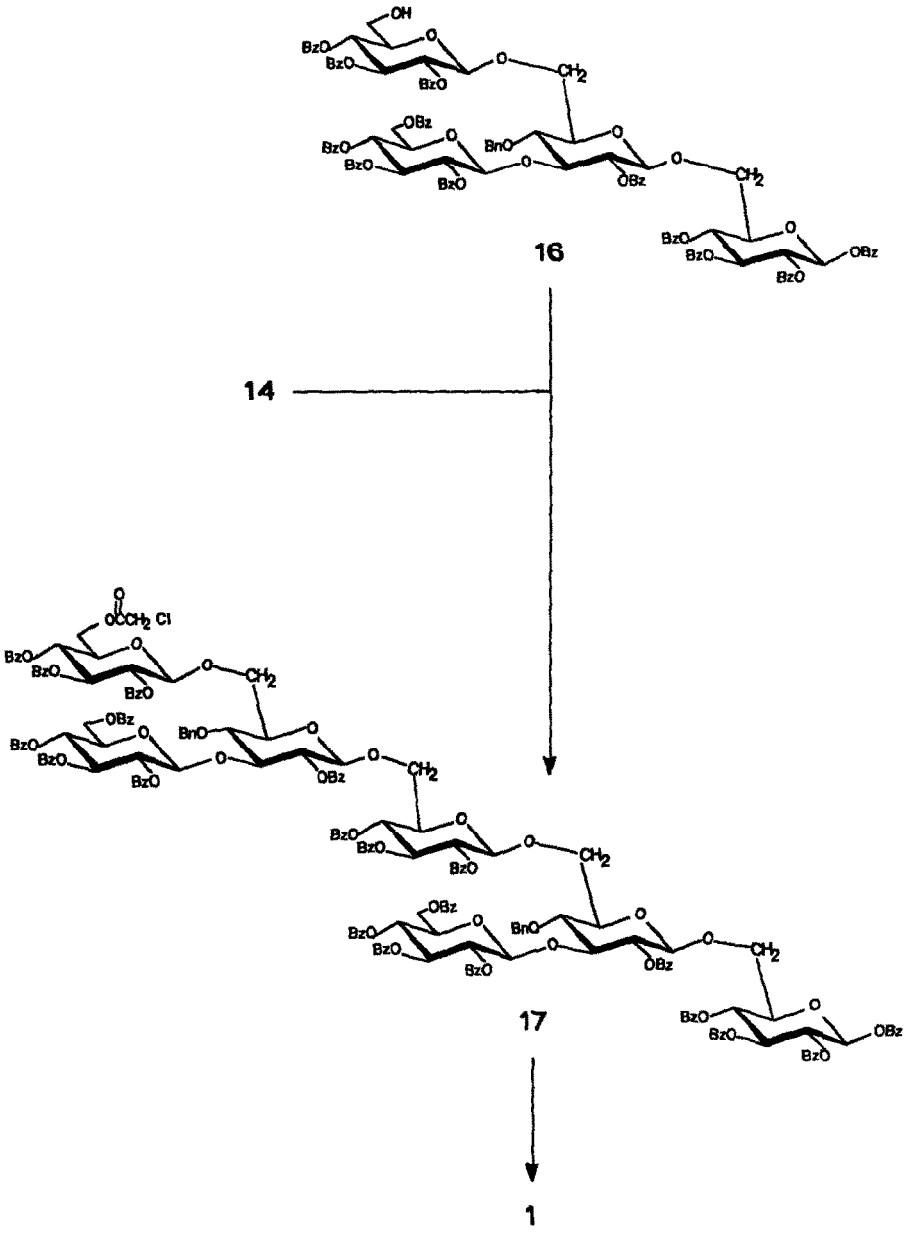

temperature to a stirred solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose[20], prepared from 2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiouronium bromide (24.5 g, 50 mmol)[20] and used directly, in dichloromethane (200 mL). After 10 min the solution was washed with water, dried ($MgSO_4$), filtered, and concentrated to give **2** (14.7 g, 77%), m.p. 92–93°, $[\alpha]_D^{20}$ −19° (*c* 2.6, chloroform); lit.[14] m.p.

94–95°, $[\alpha]_D^{20}$ −18° (tetrachlorethane); ^{13}C-n.m.r. ($CDCl_3$): δ 170.5, 170.0, 169.3 (2C) (4 *C*=O), 82.9 (C-1), 20.5 (4 *C*H_3CO), and 11.2 (S*C*H_3).

*Methyl 4,6-O-benzylidene-1-thio-β-*D*-glucopyranoside* (**3**). — Methanolic sodium methoxide (1M, 2 mL) was added at room temperature to a stirred mixture of **2** (56.8 g, 150 mmol) in methanol (200 mL). After standing overnight the reaction mixture was neutralised with Dowex 50 (H^+ form), filtered, concentrated, and dried in a vacuum over phosphorus pentaoxide. The product was treated with benzaldehyde (200 mL, 1.98 mol) and *p*-toluenesulphonic acid monohydrate (0.5 g) for 2.5 h at room temperature. Light petroleum (40–60°, 500 mL) and 10% aqueous sodium hydrogencarbonate were added with vigorous stirring. The solid was filtered off, washed first with water then light petroleum, and recyrstallised from ethanol to yield **3** (32.6 g, 73%), m.p. 184–185°, $[\alpha]_D$ −49° (*c* 0.55, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 102.1 (PhC), 86.7 (C-1), 12.4 (S*C*H_3).

Anal. Calc. for $C_{14}H_{18}O_5S$: C, 56.4; H, 6.1; O, 26.8. Found: C, 56.4; H, 6.1; O, 27.1.

*Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-*D*-glucopyranoside* (**4**). — Tetrabutylammonium iodide (2.77 g, 7.5 mmol) and potassium carbonate (dried at ~250°, 3.46 g, 25 mmol) were added to a stirred solution of **3** (1.49 g, 5 mmol) in dry chloroform (30 mL), followed by the addition of benzoyl chloride (0.7 mL, 6.0 mmol). After 2 days at room temperature the mixture was diluted with chloroform (100 mL) and filtered. The combined filtrate and washings (2 × 50 mL, chloroform) was washed with water (5 × 50 mL), dried ($MgSO_4$), filtered and concentrated. Separation by silica gel column chromatography (7:3 light petroleum–ethyl acetate) yielded, in order of elution, the dibenzoate of **3** contaminated with benzoyl chloride; **4** (1.20 g, 60%), m.p. 187–188°, $[\alpha]_D^{20}$ −26° (*c* 1.1, chloroform); methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (0.535 g, 27%), m.p. 158–159°, $[\alpha]_D$ −117° (*c* 1.1, chloroform); and starting material (0.07 g, 5%). ^{13}C-N.m.r. data ($CDCl_3$) for **4** were: δ 165.6 (*C*=O), 102.0 (PhCH), 83.8 (C-1), 11.7 (S*C*H_3); ^{1}H-n.m.r. ($CDCl_3$): δ 5.24 (dd, 1 H, $J_{1,2}$ 9.8, $J_{2,3}$ 8.8 Hz, H-2), 4.69 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1).

Anal. Calc. for $C_{21}H_{22}O_6S$: C, 62.7; H, 5.5; O, 23.8. Found: C, 62.6; H, 5.5; O, 24.0.

Methyl O*-(2,3,4,6-tetra-*O*-benzoyl-β-*D*-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-*D*-glucopyranoside* (**5**). — *Method A*. A solution of silver triflate (1.61 g, 6.25 mmol) in dry toluene (20 mL) was added to a stirred mixture of **4** (2.01 g, 5.0 mmol), 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[16] (4.11 g, 6.25 mmol) and 4Å molecular sieves (5 g) in dry dichloromethane (30 mL) under nitrogen at −40°, followed by the addition of 2,4,6-trimethylpyridine (0.40 mL, 3.0 mmol). Stirring was continued for 2 h at −40°. Then silver triflate (0.64 g, 2.5 mmol) in dry toluene (20 mL) and 2,4,6-trimethylpyridine (0.20 mL, 1.5 mmol) were again added. After another 6 h, pyridine was added, the mixture was filtered through Celite, the solids were washed with dichloromethane, and the combined filtrate and washings was with washed aqueous sodium thiosulfate, water, M

sulfuric acid, sodium hydrogencarbonate, and water, then dried ($MgSO_4$), filtered, and concentrated. Purification by silica gel column chromatography (95:5 toluene–ethyl acetate) and recrystallisation from ethyl acetate–light petroleum gave **5** (3.95 g, 81%), m.p. 228–229°, $[\alpha]_D$ +16° (*c* 2.1, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 165.8, 165.1, 164.9, 164.7, 164.7, (5 *C*=O), 101.6 (Ph*C*), 100.9 (C-1'), 83.7 (C-1), and 11.3 (S*C*H_3).

Anal. Calc. for $C_{55}H_{48}O_{15}S$: C, 67.3; H, 4.9; O, 24.5. Found: C, 67.5; H, 5.0; O, 24.3.

Method B. A solution of silver triflate (3.85 g, 15.0 mmol) in dry toluene (50 mL) was added to a stirred mixture of **3** (2.98 g, 10.0 mmol), 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[16] (8.24 g, 12.5 mmol) and 1Å molecular sieves (10 g) in dry nitromethane (150 mL) and dichloromethane (100 mL) at −25°. After 1.5 h at −25° more silver triflate (1.28 g, 5.0 mmol) in dry toluene (20 mL) was added. After a further 2 h, pyridine was added. The reaction mixture was worked up as described above for **5**, and the product was purified by silica gel column chromatography (9:1 → 4:1 toluene–ethyl acetate) to yield *methyl* O-*(2,3,4,6-tetra*-O-*benzoyl-β-*D-*glucopyranosyl)-(1→3)-4,6*-O-*benzylidene-1-thio-β-*D-*glucopyranoside* (4.80 g, 55%), $[\alpha]_D$ +6° (*c* 1.1, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 166.1, 165.8, 165.1, 164.9, 164.7 (5 *C*=O), 101.6 (Ph*C*), 100.9 (C-1'), 83.7 (C-1), and 11.3 (S*C*H_3).

Anal. Calc. for $C_{48}H_{44}O_4S$: C, 65.7; H, 5.1; O, 25.5. Found: C, 65.7; H, 5.1; O, 25.4.

The product (4.38 g, 5.0 mmol) in pyridine (30 mL) was treated with benzoyl chloride (1.16 mL, 10 mmol) for 1 day at room temperature. Water was added, and after 10 min, dichloromethane (500 mL). The solution was washed with M sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Silica gel column chromatography (9:1 toluene–ethyl acetate) gave crystalline **5** (4.63 g, 94%) after recrystallisation from ethyl acetate–light petroleum.

Methyl O-*(2,3,4,6-tetra*-O-*benzoyl-β-*D-*glucopyranosyl)-(1→6)*-O-[*(2,3,4,6-tetra*-O-*benzoyl-β-*D-*glucopyranosyl)-(1→3)*]-*2*-O-*benzoyl-4*-O-*benzyl-1-thio-β-*D-*glucopyranoside* (**7**). — A solution of aluminium chloride (2.67 g, 20.0 mmol) in diethyl ether (30 mL) was added during 15 min to a stirred mixture of **5** (4.90 g, 5.0 mmol), borane–trimethylamine[13] (14.59 g, 200 mmol) and 4Å molecular sieves (5.0 g) in dichloromethane (100 mL) and diethyl ether (20 mL) at 0°. After 30 min the mixture was filtered through Celite and the solids were washed with dichloromethane (100 mL). The combined filtrate and washings was stirred with M sulfuric acid (250 mL) for 30 min. The organic layer was washed with aqueous sodium hydrogencarbonate, water, dried ($MgSO_4$), filtered, and concentrated. Purification by silica gel column chromatography (85:15 toluene–ethyl acetate) gave *methyl* O-*(2,3,4,6-tetra*-O-*benzoyl-β-*D-*glucopyranosyl)-(1→3)*-2-O-*benzoyl-4*-O-*benzyl-1-thio-β-*D-*glucopyranoside* (**6**), (4.47 g, 91%), $[\alpha]_D$ +5° (*c* 1.2, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 166.1, 165.7, 165.4, 165.2, 164.7 (5 *C*=O), 138.2 (aromatic C-1 of $PhCH_2$ at O-4), 100.9 (C-1'), 83.1 (C-1), 63.4 (C-6'), 62.3 (C-6), and 11.6 (S*C*H_3).

A solution of silver triflate (0.039 g, 0.15 mmol) in dry toluene (3 mL) was

added to a stirred mixture of **6** (0.098 g, 0.10 mmol), 2,3,4,6-tetra-*O*-benzoyl-α-D-glucosyl bromide[16] (0.099 g, 0.15 mmol), and 4Å molecular sieves (0.2 g) in dry dichloromethane (5 mL) under nitrogen at −20°. After 45 min more silver triflate (0.039 g, 0.15 mmol) was added and the temperature was allowed to rise to 0° during 1 h. The reaction mixture was worked up as described above for **5**. Purification by silica gel column chromatography (9:1 toluene–ethyl acetate) gave **7** (0.133 g, 86%), $[\alpha]_D$ +4° (*c* 0.5, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 166.2–164.7 (9 partially overlapping *C*=O), 138.3 (aromatic C-1 of $PhCH_2$ at O-4), 101.4, 100.9 (C-1′,1″), and 82.9 (C-1).

Anal. Calc. for $C_{89}H_{76}O_{24}S$: C, 68.4; H, 4.9; O, 24.6. Found: C, 68.5; H, 5.1; O, 24.6.

Benzyl 2,3,4-tri-O-benzoyl-β-D-glucopyranoside (**8**). — Chlorotriphenylmethane (8.4 g, 30 mmol) was added to a solution of benzyl β-D-glucopyranoside[17,18] (5.40 g, 20 mmol) in pyridine (50 mL). After 20 h at room temperature, benzoyl chloride (10.5 mL, 90 mmol) was added at 0°. After 4 h at room temperature ice was added, and then dichloromethane (500 mL). The organic phase was washed with M sulfuric acid, aqueous sodium hydrogencarbonate, and water, then dried ($MgSO_4$), filtered, and concentrated. The product was treated with 80% aqueous acetic acid (250 mL) for 2 h at 90°, concentrated, and several times treated with toluene and reconcentrated. The product was purified by silica gel column chromatography (9:1 → 8:2 → 7:3 toluene–ethyl acetate, stepwise) to give **8** (9.24 g, 79%), $[\alpha]_D$ −21° (*c* 1.9, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 165.8, 165.8, 165.0 (3 *C*=O), 136.7 (aromatic C-1 of $PhCH_2$ at O-1), 99.5 (C-1), and 61.3 (C-6).

Anal. Calc. for $C_{34}H_{30}O_9$: C, 70.1; H, 5.2. Found: C, 70.0; H, 5.2.

2,3,4-Tri-O-benzoyl-6-O-chloroacetyl-α-D-glucopyranosyl bromide (**9**). — Chlorotriphenylmethane (41.8 g, 150 mmol) was added to D-glucose (18.0 g, 100 mmol) dissolved in dry pyridine (100 mL), and the mixture was stirred overnight at ambient temperature. Benzoyl chloride (60 mL, 516 mmol) in dry pyridine (100 mL) was added at a rate that kept the reaction temperature below 60°.

The reaction mixture was stirred for 1 h at 60° and then overnight at ambient temperature. After the addition of ice and stirring for 30 min the reaction mixture was diluted with dichloromethane (1 L), and the organic phase was washed with 0.5M sulfuric acid (4 × 500 mL), aqueous sodium hydrogencarbonate (2 × 500 ml), water (500 mL), dried ($MgSO_4$), and concentrated. The brown residue was heated in 80% aqueous acetic acid (1 L) for 1 h at 100°, after which thin layer chromatography (9:1 toluene–ethyl acetate) indicated complete detritylation. The reaction mixture was evaporated, then subjected to three successive additions of toluene and evaporation, and the resulting 1,2,3,4-tetra-*O*-benzoyl-α/β-D-glucopyranose (39.37 g, 66%) was separated by column chromatography (95:5 → 4:1 toluene–ethyl acetate); ^{13} C-n.m.r. ($CDCl_3$): δ 165.8–164.9 (4 *C*=O), 92.9 (C-1β), 90.2 (C-1α), and 61.0 (C-6).

Anal. Calc. for $C_{34}H_{28}O_{10}$: C, 68.4; H, 4.7. Found: C, 68.5; H, 4.7.

The first fraction was recrystallised from ethyl acetate–hexane to give 1,2,3,4-

tetra-*O*-benzoyl-β-D-glucopyranose (24.8 g, 42%), m.p. 183–184°, $[\alpha]_D$ −15° (*c* 1.7, chloroform). R_F-Values in 4:1 toluene–ethyl acetate were: α-anomer, 0.38; β-anomer, 0.47.

Chloroacetyl chloride (2.5 mL, 25 mmol) was added dropwise under stirring to 1,2,3,4-tetra-*O*-benzoyl-α/β-D-glucopyranose (4.93 g, 8.3 mmol) in dry pyridine (40 mL). After workup[21] the mixture was separated by silica gel column chromatography (9:1 toluene–ethyl acetate) to yield 1,2,3,4-tetra-*O*-benzoyl-6-*O*-chloroacetyl-α/β-D-glucopyranose (3.85 g, 69%). The β-anomer was obtained by further silica gel column chromatography (9:1 toluene–ethyl acetate) and recrystallisation from ethanol; m.p. 180–181°, $[\alpha]_D$ −3° (*c* 0.5, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 167.1 ($COCH_2Cl$), 165.7, 165.3, 165.2, 164.7 (4 *C*=O), 92.8 (C-1), and 40.8 ($COCH_2Cl$).

Anal. Calc. for $C_{36}H_{29}ClO_{11}$: C, 64.2; H, 4.3. Found: C, 64.1; H, 4.3; O, 26.3.

A saturated solution of hydrogen bromide in acetic acid (30 mL) was added to 1,2,3,4-tetra-*O*-benzoyl-6-*O*-chloroacetyl-α/β-D-glucopyranose (3.2 g, 4.8 mmol) in dichloromethane (25 mL) at room temperature. After 2 h the mixture was diluted with dichloromethane and washed with ice-water, aqueous sodium hydrogencarbonate, and water. The dried ($MgSO_4$) and filtered solution was concentrated to give syrupy **9** (2.72 g, 91%). This compound was used directly in the next step, but could be purified by silica gel column chromatography (12:1 toluene–ethyl acetate). ^{13}C-n.m.r. ($CDCl_3$) were: δ 167.0 ($COCH_2Cl$), 165.6, 165,3, 165.3 (3 *C*=O), 86.8 (C-1), and 40.7 ($COCH_2Cl$).

Benzyl O-*(2,3,4,-tri-*O-*benzoyl-β-*D-*glucopyranosyl)-(1→6)-*O-[*(2,3,4,6-tetra-*O-*benzoyl-β-*D-*glucopyranosyl)-(1→3)*]-O-*(2-*O-*benzoyl-4-*O-*benzyl-β-*D-*glucopyranosyl)-(1→6)-2,3,4-tri-*O-*benzoyl-β-*D-*glucopyranoside* (**12**). — A mixture of **5** (1.96 g, 2.00 mmol), **8** (1.16 g, 2.00 mmol) and 4Å molecular sieves (5 g) in dichloromethane (20 mL) was stirred under dry nitrogen for 30 min at room temperature. Methyl triflate (1.10 mL, 10.0 mmol) was injected through a rubber septum and the mixture was stirred for 18 h at room temperature. Triethylamine (5.0 mL) was added and the mixture was diluted with dichloromethane (200 mL) and filtered through Celite. The solids were washed with dichloromethane and the combined filtrate and washings was then washed with M sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Silica gel column chromatography (9:1 toluene–ethyl acetate) gave *benzyl* O-*(2,3,4,6,-tetra-*O-*benzoyl-β-*D-*glucopyranosyl)-(1→3)-*O-(*2-*O-*benzoyl-4,6-*O-*benzylidene-β-*D-* glucopyranosyl) - (1→6) - 2,3,4 - tri -* O - *benzoyl -* β-D - *glucopyranoside* (2.56 g, 85%), ^{13}C-n.m.r. ($CDCl_3$): δ 166.0–164.5 (8 partially overlapping *C*=O), 137.0 (aromatic C-1 of $PhCH_2$ at O-1), 101.4 (Ph*C*), 101.1, 100.6 (C-1′,1″), 98.8 (C-1), and 63.0 (C-6). A portion (1.52 g, 1.0 mmol) of this product in dry dichloromethane (20 mL) containing trimethylamine–borane (2.92 g, 40 mmol) was treated with aluminium chloride (0.53 g, 4.0 mmol) in dry diethyl ether (10 mL) for 10 min at room temperature, then M sulfuric acid (50 mL) was added slowly and the mixture was stirred for 30 min. Dichloromethane (100 mL) was added and the water layer was extracted with di-

chloromethane. The combined organic phase was washed with aqueous sodium hydrogencarbonate, water, dried ($MgSO_4$), filtered, and concentrated. Purification by means of silica gel column chromatography (4:1 toluene–ethyl acetate) gave *benzyl* O-*(2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl)-(1→3)*-O-*(2*-O-*benzoyl*-4-O-*benzyl*-β-D-*glucopyranosyl)-(1→6)-2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranoside* (**10**); 1.27 g, 84%), $[\alpha]_D$ −17° (*c* 0.5, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 166.0–164.5 (8 partially overlapping *C*=O), 138.1 (aromatic C-1 of $PhCH_2$ at O-4'), 136.6 (aromatic C-1 of $PhCH_2$ at O-1), 100.6, 100.2 (C-1',1''), 99.0 (C-1), and 61.9 (C-6).

A mixture of crude **9** (0.66 g, 1.05 mmol), **10** (1.06 g, 0.70 mmol), and 4Å molecular sieves (5.0 g) in dichloromethane (20 mL) was stirred under dry nitrogen and cooled to −40°. A solution of silver triflate (0.270 g, 1.05 mmol) in dry toluene (10 mL) was added. After 30 min, t.l.c. (9:1 toluene–ethyl acetate) showed the presence of a total of about 30% of **9** and **10**. Silver triflate (0.270 g, 1.05 mmol) was again added at −40°. After 1 h only traces of starting materials remained (t.l.c.). Pyridine was added, the mixture was diluted with dichloromethane (100 mL) and filtered, the solids were washed with dichloromethane, and the combined filtrate and washings was worked up as described above for **5**. Purification of the product by silica gel column chromatography (9:1 toluene–ethyl acetate) gave **11** (1.12 g, 77%), $[\alpha]_D$ −20° (*c* 0.5, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 167.2 ($COCH_2Cl$), 166.1–164.6 (11 partially overlapping *C*=O), 138.2, 136.9 (aromatic C-1 of $PhCH_2$ at O-4' and O-1, respectively), 101.6, 100.4, 100.4 (C-1',1'',1'''), 99.2 (C-1), and 40.9 ($COCH_2Cl$).

A stock solution of hydrazine dithiocarbonate[19] was added dropwise at room temperature to a solution of **11** (1.04 g, 0.50 mmol) in dioxane (25 mL). To prevent the product **12** from precipitating, dry *N*,*N*-dimethylformamide (10 mL) was added. After 5 min, t.l.c. (4:1 toluene–ethyl acetate) indicated complete reaction. The solution was concentrated, the residue was dissolved in dichloromethane (200 mL), and the solution was washed with M sulfuric acid, sodium hydrogencarbonate, and water, dried ($MgSO_4$), and concentrated. Silica gel column chromatography (9:1, then 4:1 toluene–ethyl acetate) gave **12** (0.776 g, 78%), $[\alpha]_D$ −23° (*c* 0.5, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 166.0–164.7 (11 partially overlapping *C*=O), 138.7, 136.7 (aromatic C-1 of $PhCH_2$ at O-4' and O-1, respectively), 101.5, 100.2, 100.2 (C-1',1'',1'''), 99.0 (C-1), and 61.6 (C-6'').

Anal. Calc. for $C_{115}H_{98}O_{32}$: C, 69.3; H, 5.0. Found: C, 69.5; H, 5.0.

Benzyl O-*(2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl)-(1→6)*-O-*[(2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl)-(1→3)]*-O-*(2*-O-*benzoyl*-4-O-*benzyl*-β-D-*glucopyranosyl*-*(1→6)*-O-*(2,3,4*-*tri*-O-*benzoyl*-β-D-*glucopyranosyl)-(1→6)*-O-*[(2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl)-(1→3)]*-O-*(2*-O-*benzoyl*-4-O-*benzyl*-β-D-*glucopyranosyl)-(1→6)-2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranoside* (**13**). — A mixture of **7** (0.47 g, 0.30 mmol), **12** (0.60 g, 0.30 mmol) and 4Å molecular sieves (5 g) in dichloromethane (20 mL) was stirred under nitrogen at room temperature. Methyl triflate (0.16 mL, 1.50 mmol) was injected through a rubber septum. After

16 h, t.l.c. (9:1 toluene–ethyl acetate) indicated the presence of about 10% of **12**, but no **7**, in addition to the presumed **13**. More **7** (0.094 g, 0.06 mmol) was added. After another 4 h the mixture was worked up as described above for the reaction of **5** with **8**. Silica gel column chromatography (9:1 toluene–ethyl acetate) gave **13** (0.95 g, 91%), $[\alpha]_D$ −22° (*c* 0.5, chloroform); ^{13}C-n.m.r. (100 MHz, $CDCl_3$): δ 166.1–164.4 (20 partially overlapping *C*=O), 138.4, 138.2 (aromatic C-1 of $PhCH_2$ at O-4′ and O-4‴), 137.0 (aromatic C-1 of $PhCH_2$ at O-1), 101.5, 101.3, 100.5, 100.5, 100.3, 100.3 (6 anomeric C), and 99.2 (C-1).

Anal. Calc. for $C_{203}H_{170}O_{56}$: C, 69.6; H, 4.9. Found: C, 69.5; H, 4.9.

Methyl O-*(2,3,4-tri*-O-*benzoyl-6*-O-*chloroacetyl-β*-D-*glucopyranosyl)-(1→6)*-O-[*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→3)*]-*2*-O-*benzoyl-4*-O-*benzyl-1-thio-β*-D-*glucopyranoside* (**14**). — Acceptor **6** (1.9 g, 2.0 mmol) and donor **9** (1.58 g, 2.5 mmol) were dissolved in dry dichloromethane (50 mL). Molecular sieves (4A, 5.0 g) was added and the mixture was stirred under dry nitrogen for 30 min at −25°. A solution of silver triflate (0.77 g, 3.0 mmol) in dry toluene (20 mL) was added during 30 min, and the reaction mixture was kept at −25° for another 30 min (t.l.c. 4:1 toluene–ethyl acetate). After the addition of pyridine and dilution with dichloromethane (100 mL) the reaction mixture was filtered through Celite. The solid residue was washed with dichloromethane and the filtrate was washed successively with sodium thiosulfate, M sulfuric acid, sodium hydrogencarbonate, and water, dried ($MgSO_4$), and concentrated. Silica gel column chromatography (9:1 toluene–ethyl acetate) gave the pure trisaccharide **14** (2.56 g, 83%), $[\alpha]_D$ −6° (*c* 1.1, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 167.1 ($COCH_2Cl$), 166.0, 165.8, 165.7, 165.4, 165.3, 165.3, 165.1, 164.6 (8 *C*=O), 138.3 (aromatic C-1 of $PhCH_2$ at O-4), 101.4, 100.9 (C-1′,1″), 82.7 (C-1), 40.8 ($COCH_2Cl$), and 11.3 (SCH_3).

Anal. Calc. for $C_{84}H_{73}ClO_{24}S$: C, 65.8; H, 4.8; Cl, 2.3; O, 25.0. Found: C, 66.0; H, 4.8; Cl, 2.4; O, 25.2.

O-*(2,3,4-Tri*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→6)*-O-[*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→3)*]-O-*(2*-O-*benzoyl-4*-O-*benzyl-β*-D-*glucopyranosyl)-(1→6)-1,2,3,4-tetra*-O-*benzoyl-β*-D-*glucopyranose* (**16**). — A mixture of **14** (6.28 g, 4.1 mmol), 1,2,3,4-tetra-*O*-benzoyl-β-D-glucopyranose (2.39 g, 4.0 mmol), and 4Å molecular sieves (10 g) in dry dichloromethane (50 mL) was stirred under nitrogen for 30 min at room temperature. Methyl triflate (2.19 mL, 20 mmol) was injected through a rubber septum, and the reaction mixture was stirred for 8 h (t.l.c. 9:1 toluene–ethyl acetate). Triethylamine (5.0 mL) was added and the mixture was worked up as described above for compound **14**. Column chromatography (9:1 toluene–ethyl acetate) gave O-*(2,3,4-tri*-O-*benzoyl-6*-O-*chloroacetyl-β*-D-*glucopyranosyl)-(1→6)*-O-[*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→3)*]-O-*(2*-O-*benzoyl-4*-O-*benzyl-β*-D-*glucopyranosyl)-(1→6)-1,2,3,4-tetra*-O-*benzoyl-β*-D-*glucopyranose* (**15**; 6.85 g, 83%), $[\alpha]_D$ −18° (*c* 1.0, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 167.1 ($COCH_2Cl$), 166.0–164.7 (12 partially overlapping *C*=O), 138.0 (aromatic C-1 of $PhCH_2$ at O-4′), 101.4, 100.1, 99.7 (C-1′,1″,1‴), 93.0 (C-1), and 40.8 ($COCH_2Cl$).

Hydrazine dithiocarbonate (1.40 g, 10 mmol) was added to a solution of **15** (6.25 g, 3.0 mmol) in acetonitrile (50 mL) followed by dropwise addition of water (5.0 mL). To ensure a homogeneous reaction, *N*,*N*-dimethylformamide (50 mL) and enough water to dissolve the reagent were added. The reaction mixture was stirred for 30 min at ambient temperature (t.l.c. 4:1 toluene–ethyl acetate) and then worked up as described for **14**, to give **16** (5.32 g, 88%), $[\alpha]_D$ −16.0° (*c* 0.80, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 166.1–164.8 (11 partially overlapping *C*=O), 138.1 (aromatic C-1 of $PhCH_2$ at O-4′), 101.4, 99.9, 99.7 (C-1′,1″,1‴), 92.9 (C-1), and 61.8 (C-6″).

Anal. Calc. for $C_{115}H_{96}O_{33}$: C, 68.9; H, 4.8. Found: C, 68.7; H, 5.0.

O-*(2,3,4-Tri*-O-*benzoyl-6*-O-*chloroacetyl-β*-D-*glucopyranosyl)-(1→6)*-O-[*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→3)*]-O-*(2*-O-*benzoyl-4*-O-*benzyl-β*-D-*glucopyranosyl)-(1→6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→6)*-O-

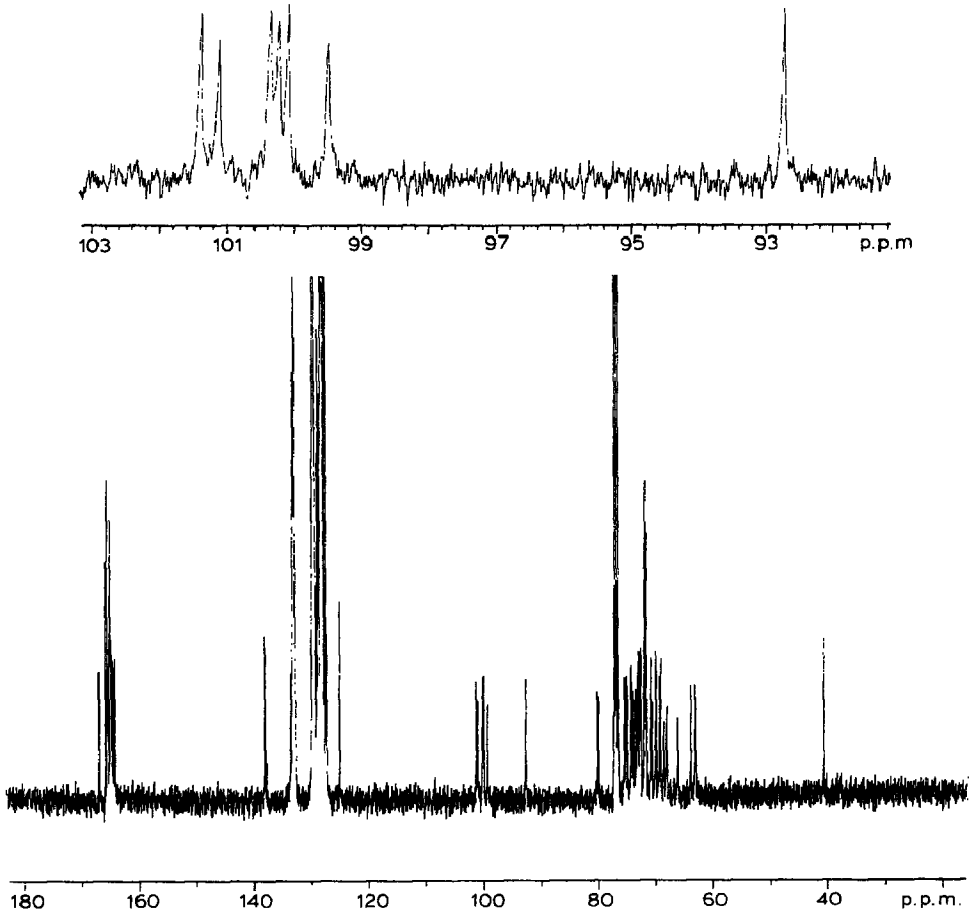

Fig. 1. 100 MHz ^{13}C-n.m.r. spectrum of **17**.

[(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-(1→3)]-O-(2-O-benzoyl-4-O-benzyl-β-D-glucopyranosyl)-(1→6)-1,2,3,4-tetra-O-benzoyl-β-D-glucopyranose (**17**). — Tetrasaccharide **16** (3.01 g, 1.5 mmol) was coupled to trisaccharide **14** (2.53 g, 1.65 mmol) as described for compound **15**, above. Silica gel column chromatography (9:1 toluene–ethyl acetate) gave the fully protected heptasaccharide **17** (4.90 g, 94%), $[\alpha]_D$ −20° (*c* 1.2, chloroform); ^{13}C-n.m.r. (100 MHz, $CDCl_3$): δ 167.2 ($COCH_2Cl$), 166.1–164.4 (20 partially overlapping (C=O), 138.4, 138.2 (aromatic C-1 of $PhCH_2$ at O-4′ and O-4‴), 101.5, 101.2, 100.5, 100.4, 100.2, 99.6 (6 anomeric C), 92.8 (C-1), 80.4, 80.2 (C-3′,3‴), and 40.8 ($COCH_2Cl$) (Fig. 1).

Anal. Calc. for $C_{198}H_{165}ClO_{57}$: C, 68.1; H, 4.8; O, 26.1. Found: C, 67.9; H, 4.8; O, 26.0.

O-β-D-*Glucopyranosyl-(1→6)*-O-[β-D-*glucopyranosyl-(1→3)*]-O-β-D-*glucopyranosyl-(1→6)*-O-β-D-*glucopyranosyl-(1→6)*-O-[β-D-*glucopyranosyl-(1→3)*]-O-β-D-*glucopyranosyl-(1→6)*-D-*glucose (3^2,3^4-di-β-D-glucopyranosylgentiopentaose)* (**1**). — *From* **13**. The protected heptasaccharide (350 mg, 0.1 mmol) was dissolved in a mixture of dry tetrahydrofuran (10 mL) and methanol (20 mL). To this solution was added M sodium methoxide (0.5 mL). The reaction mixture was allowed to stand at room temperature overnight. After neutralisation with methanol-washed Dowex-50 (H^+), filtration, and washing of the resin with methanol, the filtrate was concentrated to yield the partly deblocked heptasaccharide.

The residue was dissolved in 10% aqueous ethanol (50 mL) and treated with hydrogen at 330 kPa overnight in the presence of 10% Pd–C (0.2 g). After filtration through Celite the solution was concentrated and purified on Bio-Gel P-2. The final solution was freeze-dried to give the free heptasaccharide **1** (98 mg, 85%).

From **17**: Precursor **17** (350 mg, 0.1 mmol) was deblocked as described for **13** to give **1** (104 mg, 90%). The product **1** was indistinguishable (n.m.r., h.p.l.c., and bioassay) from that previously made[1].

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