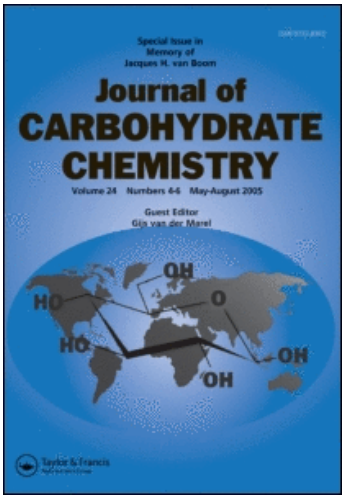

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Synthesis of a Heptasaccharide, Structurally Related to the Phytoelicitor Active Glucan of Phytophthora Megasperma F.SP. Glycinea

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SYNTHESIS OF A HEPTASACCHARIDE, STRUCTURALLY RELATED TO THE PHYTOELICITOR ACTIVE GLUCAN OF *PHYTOPHTHORA MEGASPERMA* F.SP. *GLYCINEA*

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ABSTRACT

A synthesis is described of the heptasaccharide **1**, which may form part of the phytoelicitor-active glucan of *Phytophthora megasperma* f.sp. *glycinea*. Silver triflate was used as the promoter in Koenigs-Knorr type condensations using glycosyl bromides, each with a participating benzoyl group in the 2-position, for the synthesis of the smaller oligosaccharide fragments. For joining the larger ones, methyl triflate was used as the promoter and an oligosaccharide thioglycoside carrying a participating benzoyl group in the 2-position was used as the glycosyl donor.

ßDGlc*p*(1→6)ßDGlc*p*(1→6)ßDGlc*p*(1→6)ßDGlc*p*(1→6)DGlc

3 3

↑ ↑

1 1

ßDGlc*p* ßDGlc*p*

1

INTRODUCTION

We have previously described the synthesis of the heptasaccharide 3^2,3^4-di-ß-D-glucopyranosylgentiopentaose corresponding to the phytoelicitor active fragment[1,2] of a minor glucan in the mould *Phytophthora megasperma* f.sp. *glycinea*.[3] We now describe a synthesis of the isomeric 3^3,3^4-di-ß-D-glucopyranosylgentiopentaose (**1**) which was required for further phytochemical studies, using the same methodology as that described before.[2]

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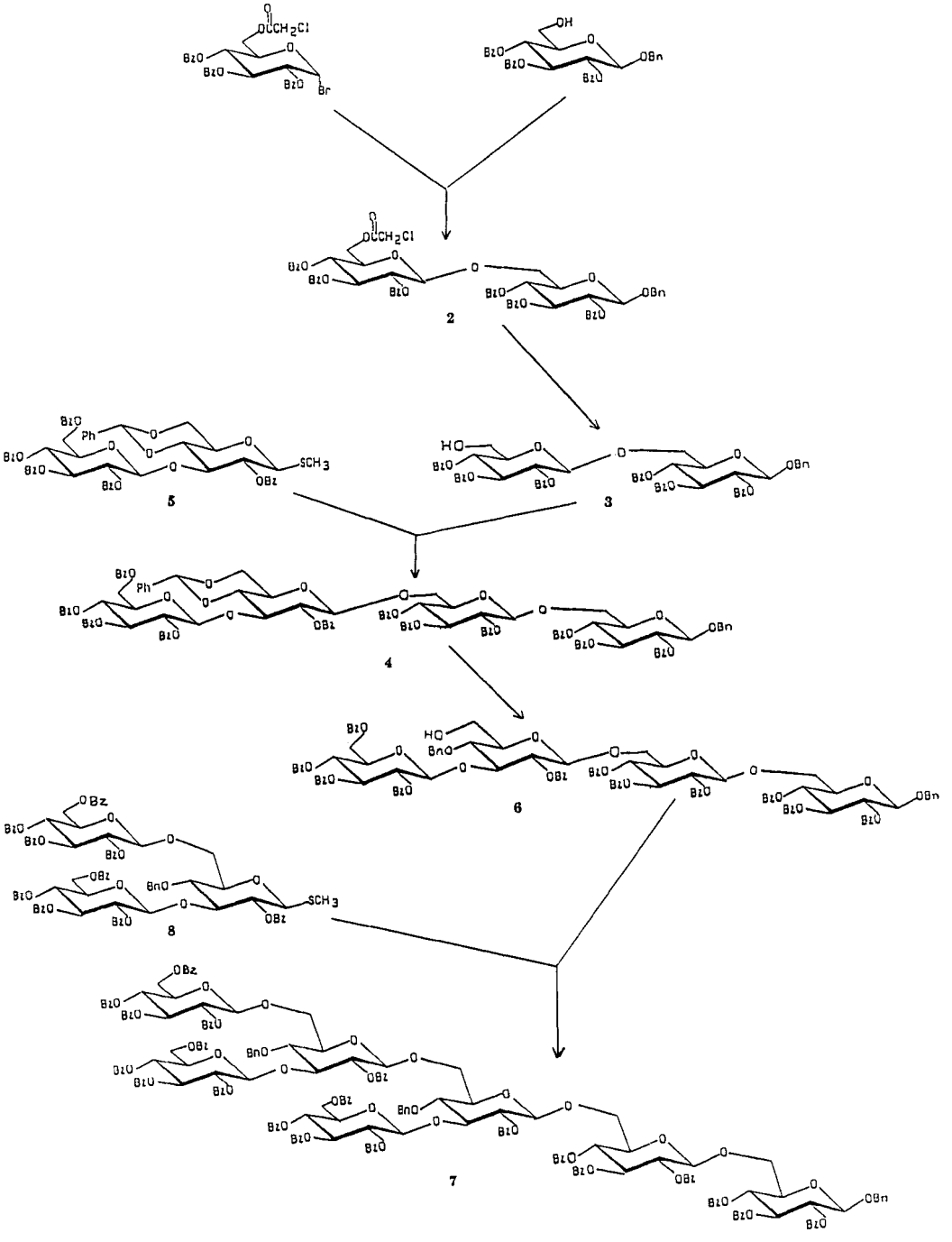

RESULTS AND DISCUSSION

In order to minimize protecting group manipulations on large oligosaccharide intermediates, a convergent block synthesis strategy was devised for making the heptasaccharide **1**. In this, benzoate was used as a participating *O*-2 substituent[4] for 1,2-*trans*-glycoside synthesis.[4] Silver triflate[5] was used as a promoter for glycosidation with glycosyl bromides as donors for the synthesis of the smaller oligosaccharides. Methyl triflate[6] was the promoter, with thioglycosides as donors, for the synthesis of the larger ones. By contradistinction to the previous synthesis of the phytoelicitor-active 3^2,3^4-di-ß-D-glucopyranosylgentiopentaose,[2] benzyl rather than benzoyl was used at the ultimate C-1 position. This was because the protecting group scheme involved a reductive 4,6-*O*-benzylidene ring opening on the intermediate tetrasaccharide **4** and a 1-*O*-benzoyl group is not stable under these conditions.[7]

Thus, 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-glucopyranosyl bromide[2] was condensed with benzyl 2,3,4-tri-*O*-benzoyl-ß-D-glucopyranoside[2,8] to give the disaccharide **2** (80%). Removal of the chloroacetyl group from **2** gave **3** (91%). Compound **3** was then glycosylated with the thioglycoside donor **5**[2] to give the tetrasaccharide **4** (92%). The 4,6-*O*-benzylidene group in **4** was reductively ring-opened by treatment with aluminium chloride and borane-trimethylamine[2,9] to yield the 4"-*O*-benzyl derivative **6** with the 6"-hydroxyl group free (77%). The regioselectivity in this reaction has previously been shown to be solvent-dependent. Thus, with tetrahydrofuran as the solvent, an unprotected hydroxyl group at carbon 4, and a benzyloxy group at carbon 6 are obtained. In toluene, however, the reverse regioselectivity is obtained.[9] The latter regioselectivity, but with increased yields was later obtained using dichloromethane containing small amounts of diethyl ether as the solvent[2] and this was the method used in the present synthesis. The presence in **6** of a free 6-hydroxyl group was demonstrated by the ^{13}C NMR spectrum, in which the C-6" signal of **6** was at δ 61.5. The heptasaccharide **7** was then obtained in 95% yield, by condensation of **6** with the thioglycoside trisaccharide **8**.[2] Deprotection of **7** as described before[2] finally gave **1** (80%).

EXPERIMENTAL

General procedures. These were the same as those previously described.[2] Melting-points are uncorrected. NMR spectra were recorded using Jeol FX-100 and GX-400 machines. All spectra were in agreement with postulated structures and are obtainable upon request. Only selected, particularly significant data are given below.

Benzyl O-*(2,3,4-Tri*-O-*benzoyl*-6-O-*chloroacetyl*-ß-D-*glucopyranosyl)-(1→6)-2,3,4-tri*-O-*benzoyl*-ß-D-*glucopyranoside* (**2**). Silver triflate (1.16 g, 4.5 mol) in dry toluene (20 mL) was added to a stirred mixture of benzyl 2,3,4-tri-*O*-benzoyl-ß-D-glucopyranoside[2,8] (1.75 g, 3.00 mmol), 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-glucopyranosyl bromide[2] (2.37 g, 3.75 mmol) and 4Å molecular sieves (5.0 g) in dichloromethane (40 mL) under nitrogen at -40 °C. After 1 h, when t.l.c. (toluene - ethyl acetate 5:1) indicated the presence of about 20% unchanged aglycon, more silver triflate (128 mg, 0.5 mmol) in dry toluene (10 mL) was added. After 1 h at -40 °C, pyridine was added, the mixture was filtered through Celite, the solids were washed with dichloromethane, and the combined filtrate and washings was washed with aqueous sodium thiosulfate, water, aqueous M sulfuric acid, aqueous sodium hydrogencarbonate, and water, then dried ($MgSO_4$), filtered and concentrated. Purification by silica gel column chromatography (toluene - ethyl acetate 9:1) gave **2** (2.73 g, 80%), mp 187-188 °C (from ethyl acetate - light petroleum), $[\alpha]_D$ -29° (*c* 0.9, chloroform). ^{13}C NMR ($CDCl_3$): δ 40.5 ($COCH_2Cl$), 68.5 (C-6), 63.6 (C-6'), 98.5 (C-1), 100.8 (C-1').

Anal. Calcd for $C_{63}H_{53}ClO_{18}$: C, 66.8; H, 4.7. Found: C, 66.7; H, 4.7.

Benzyl O-*(2,3,4-Tri*-O-*benzoyl*-ß-D-*glucopyranosyl)-(1⟶6)-2,3,4-tri*-O-*benzoyl*-ß-D-*glucopyranoside* (**3**). Compound **2** (2.0 g) in 1:7 ethyl acetate - methanol (7 mL), was treated with thiourea (0.5 g) and 2,4,6-trimethylpyridine (0.15 mL) at 40 °C for 24 h. The solution was diluted with dichloromethane and then extracted with a saturated aqueous solution of sodium chloride and then water. The organic phase was concentrated and the residue was purified by silica gel column chromatography (toluene - ethyl acetate 7:1) to give **3** (1.7 g, 91 %),

mp 184-185 °C (from ethyl acetate - light petroleum), $[\alpha]_D$ −41° (*c* 1.6, chloroform). ^{13}C NMR ($CDCl_3$): δ 60.8 (C-6'), 68.2 (C-6), 98.6 (C-1), 100.5 (C-1').

Anal. Calcd for $C_{61}H_{52}O_{17}$: C, 69.3; H, 5.0. Found: C, 69.3; H, 4.9.

Benzyl O-*(2,3,4,6-Tetra*-O-*benzoyl*-ß-D-*glucopyranosyl)-(1*⟶*3)*-O-*(2*-O-*benzoyl-4,6*-O-*benzylidene*-ß-D-*glucopyranosyl)-(1*⟶*6)*-O-*(2,3,4-tri*-O-*benzoyl*-ß-D-*glucopyranosyl)-(1*⟶*6)-2,3,4-tri*-O-*benzoyl*-ß-D-*glucopyranoside* (**4**). A mixture of compound **3** (1.32 g, 1.25 mmol), methyl *O*-(2,3,4,6-tetra-*O*-benzoyl-ß-D-glucopyranosyl)-(1-3)-2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-ß-D-glucopyranoside[2] (**5**, 1.34 g, 1.37 mmol), and 4Å molecular sieves (5 g) in dichloromethane (20 mL) was stirred under nitrogen at room temperature for 30 min. Methyl triflate (0.65 mL, 6.3 mmol) was added and the mixture was stirred at room temperature for 24 h. Triethylamine was added and the mixture was diluted with dichloromethane, filtered through Celite and the combined filtrate and washing was extracted with aqueous M sulfuric acid, aqueous sodium hydrogencarbonate, water, dried ($MgSO_4$), filtered and concentrated. Purification of the residue by silica gel column chromatography (toluene - ethyl acetate 9:1) gave **4** (2.29 g, 92 %), $[\alpha]_D$ − 24° (*c* 1.0, chloroform). ^{13}C NMR ($CDCl_3$): δ 62.5 (C-6'''), 79.0 (C-3''), 99.0 (C-1), 100.6 (C-1', C-1'', C-1''', partly overlapping), 100.9 (Ph*C*H).

Anal. Calcd for $C_{115}H_{96}O_{32}$: C, 69.4; H, 4.9. Found: C, 69.2; H, 4.9.

Benzyl O-*(2,3,4,6-Tetra*-O-*benzoyl*-ß-D-*glucopyranosyl)-(1*⟶*3)*-O-*(2*-O-*benzoyl-4*-O-*benzyl*-ß-D-*glucopyranosyl)-(1*⟶*6)*-O-*(2,3,4-tri*-O-*benzoyl*-ß-D-*glucopyranosyl)-(1*⟶*6)-2,3,4-tri*-O-*benzoyl*-ß-D-*glucopyranoside* (**6**). A solution of aluminium chloride (0.53 g, 4.0 mmol) in diethyl ether (20 mL) was added dropwise with stirring at room temperature to a solution of compound **4** (1.99 g, 1.0 mmol) and borane-trimethylamine[9] (2.92 g, 40 mmol) in a mixture of dichloromethane (30 mL) and diethyl ether (10 mL). After 30 min at room temperature when t.l.c. (toluene - ethyl acetate 4:1) indicated total conversion of starting material, M aqueous sulfuric acid was added and the mixture was stirred for 1 h. Aqueous 35% hydrogen peroxide (4.0 mL) was added. The organic layer was separated, washed with aqueous sodium hydrogencarbonate, water,

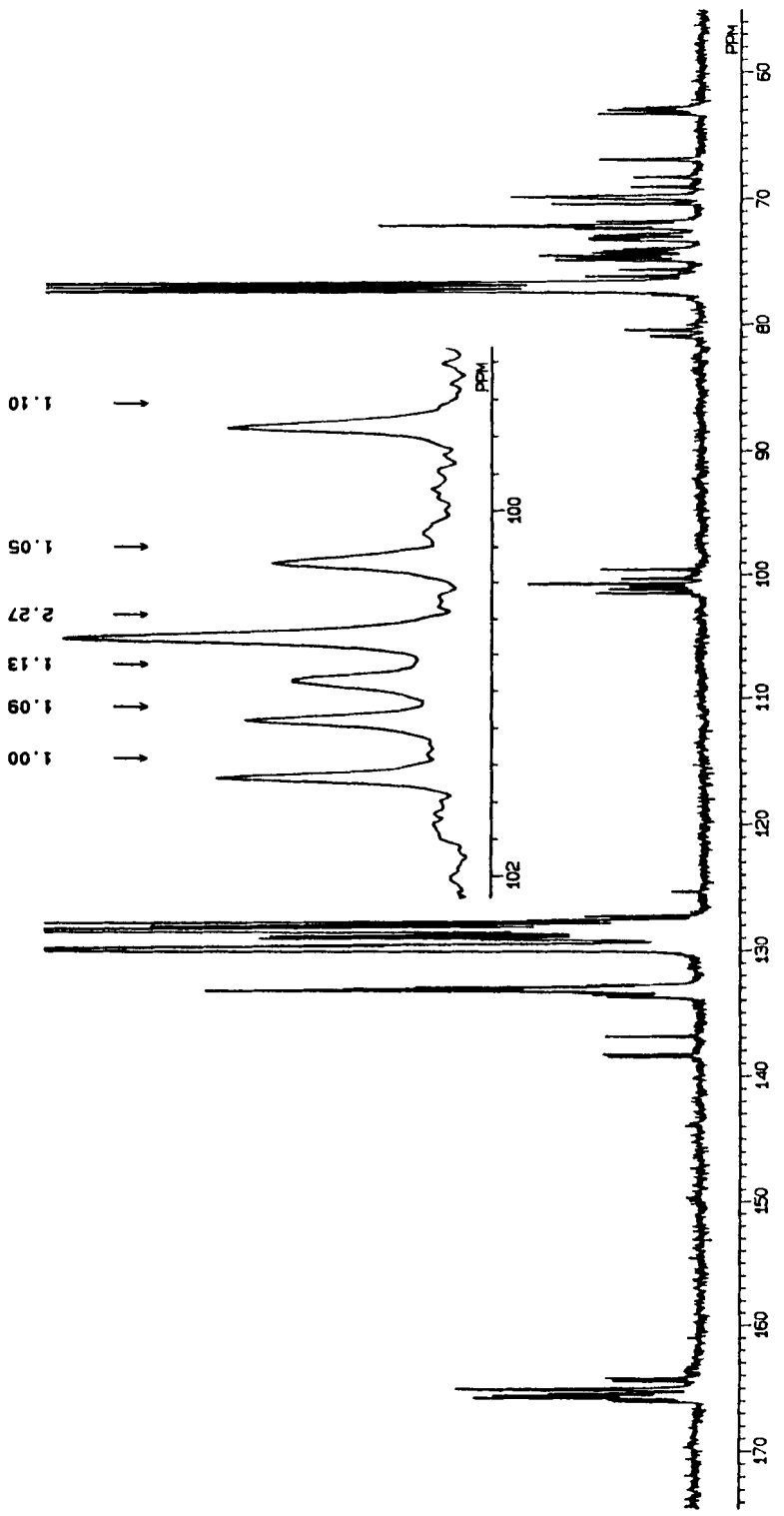

FIG. 1. 100 MHz ^{13}C NMR spectrum of protected heptasaccharide 7

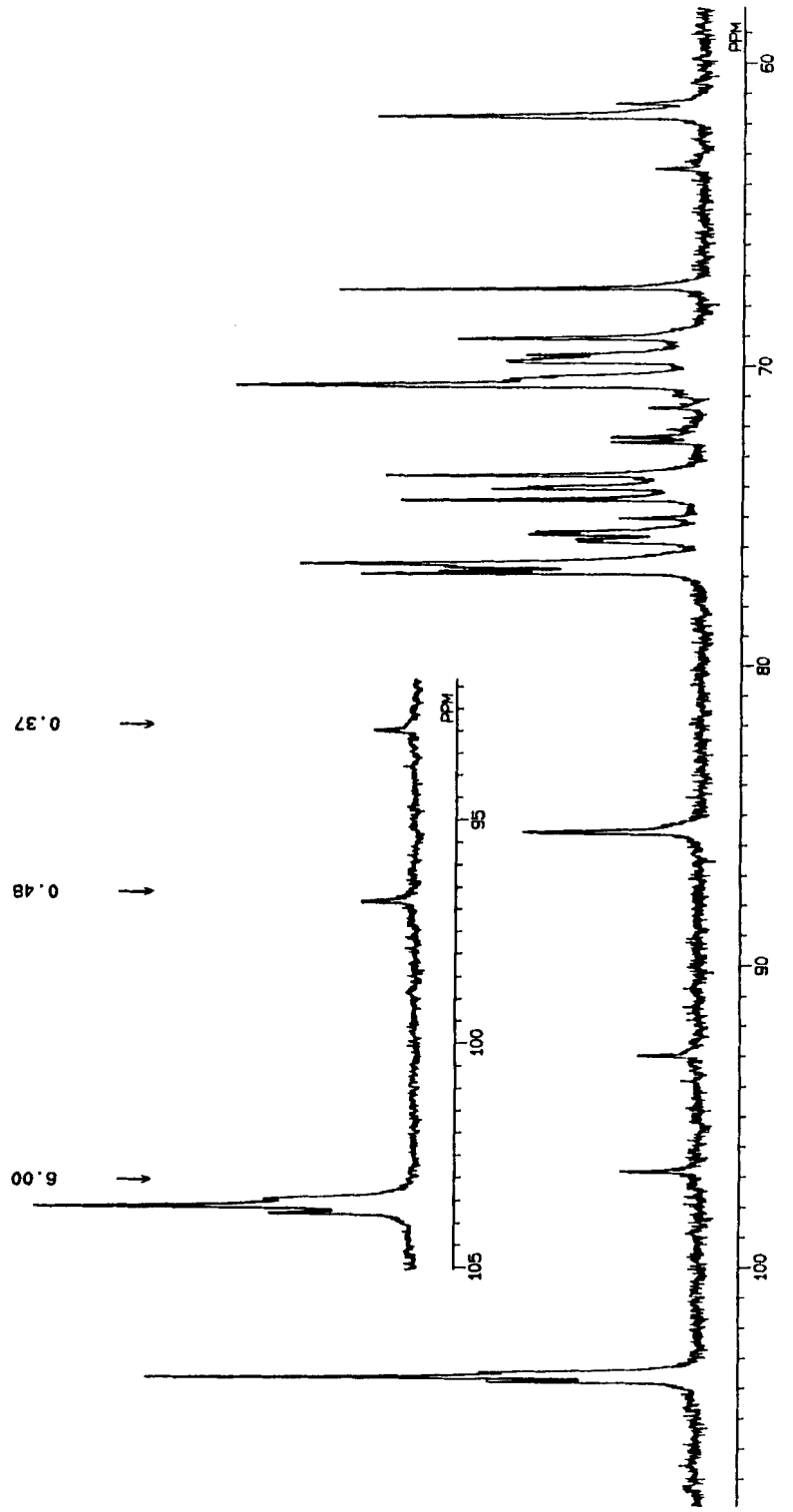

FIG. 2. 100 MHz ^{13}C NMR spectrum of heptasaccharide 1

dried ($MgSO_4$), filtered and concentrated. Purification of the residue by silica gel column chromatography (toluene - ethyl acetate 4:1) gave **6**, (1.53 g, 77%), $[\alpha]_D$ -35° (*c* 0.9, chloroform). ^{13}C NMR ($CDCl_3$): δ 61.5 (C-6"), 62.7 (C-6'"), 80.2 (C-3"), 98.9 (C-1), 100.6 (C-1', C-1", C-1'", partly overlapping).

Anal. Calcd for $C_{115}H_{98}O_{32}$: C, 69.3; H, 5.0. Found: C, 69.3, H, 4.9.

Benzyl O-*(2,3,4,6-Tetra*-O-*benzoyl*-ß-D-*glucopyranosyl)-(1⟶6)*-O-[*(2,3,4,6-tetra*-O-*benzoyl*-ß-D-*glucopyranosyl)*-(1⟶3)]-(2-O-*benzoyl-4*-O-*benzyl*-ß-D-*glucopyranosyl)-(1⟶6)*-O-[*2,3,4,6-tetra*-O-*benzoyl*-ß-D-*glucopyranosyl)*-(1⟶3)]-O-*(2*-O-*benzoyl-4*-O-*benzyl*-ß-D-*glucopyranosyl)-(1⟶6)*-O-*(2,3,4-tri*-O-*benzoyl*-ß-D-*glucopyranosyl)-(1⟶6)-2,3,4-tri*-O-*benzoyl-β*-D-*glucopyranoside* (**7**). A mixture of compound **6** (996 mg, 0.5 mmol), methyl *O*-(2,3,4,6-tetra-*O*-benzoyl-ß-D-glucopyranosyl)-(1→6)-*O*-[(2,3,4,6-tetra-*O*-benzoyl-ß-D-glucopyranosyl)-(1→3)]-2-*O*-benzoyl-4-*O*-benzyl-1-thio-ß-D-glucopyranoside[2] (**8**, 937 mg, 0.6 mmol), 4Å molecular sieves (3 g) in dichloromethane (30 mL) was stirred under nitrogen at room temperature for 1 h. Methyl triflate (0.33 mL, 3.0 mmol) was added and the mixture was stirred at room temperature for 20 h. Triethylamine was added and the product was worked up as described above for compound **4.** Purification of the residue by silica gel column chromatography (toluene - ethyl acetate 9:1) gave **7,** (1.67 g, 95%), $[\alpha]_D$ -28° (*c* 1.7, chloroform). ^{13}C NMR ($CDCl_3$): δ 62.8, 63.0, 63.3 (3 C-6 carrying *O*-benzoyl), 66.9, 68.2, 69.0, 69.9 (C-6, C-6', C-6", C-6'"), 80.4, 80.9 (C-3", C-3'"), 99.6 (C-1), 100.3, 100.7, 100.9, 101.1, 101.5 (6 anomeric carbons, 2 overlapping), see also FIG. 1.

33,34-Di-ß-D-*glucopyranosylgentiopentaose* (**1**). Compound **7** was deprotected as decribed before[2] for the synthesis of 32,34-di-ß-D-glucopyranosylgentiopentaose to yield compound **1** in 80% yield, $[\alpha]_D$ -12° (*c* 0.5, water), and with a 500 MHz ^1H n.m.r spectrum in agreement with the postulated structure; ^{13}C NMR (D_2O) see FIG. 2.

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