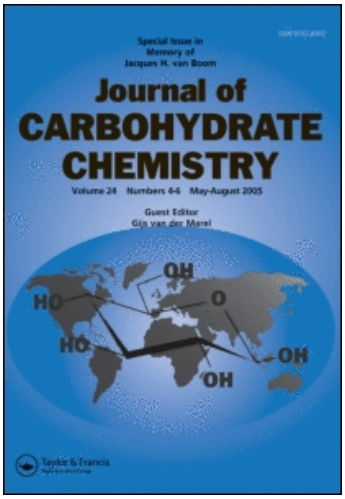

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SYNTHESES OF A HEPTASACCHARIDE β-LINKED TO AN 8-METHOXYCARBONYL-OCT-1-YL LINKING ARM AND OF A DECASACCHARIDE WITH STRUCTURES CORRESPONDING TO THE PHYTOELICITOR ACTIVE GLUCAN OF *PHYTOPHTHORA MEGASPERMA* F. *SP. GLYCINEA*

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ABSTRACT

Syntheses are described of the 8-methoxycarbonyloct-1-yl β-D-glycoside of $3^2,3^4$-di-β-D-glucopyranosylgentiopentaose and also of $3^2,3^4,3^6$-tri-β-D-glucopyranosylgentioheptaose, both of which were required for phytochemical studies of the defense mechanism of the soybean plant to infection by the mould *Phytophthora megasperma* f.sp. *glycinea*. Block synthesis strategies were used, relying on promotion by methyl triflate and the use of thioglycosides as glycosyl donors in the condensation of the oligosaccharide fragments. Highly stereoselective β-D-glycosylation was ensured by the presence of benzoyl groups in the 2-positions of the glycosyl donors.

INTRODUCTION

When the soybean plant is invaded by the mould *Phytophthora megasperma*, a fragment of a glucan in the latter elicits a defense mechanism in which the phytoalexin glyceollin is produced.[1-5] The structural fragment in the mould responsible for initiating this process was shown by chemical degradation and structural studies in combination with synthesis to have the carbohydrate part of structure I depicted above. Syntheses of this heptasaccharide, $3^2,3^4$-di-β-D-glucopyranosylgentiopentaose,[4,6] and of the isomeric $3^3,3^4$-di-β-D-glucopyranosylgentiopentaose[7] (III) have been described. We now describe syntheses of the heptasaccharide glycoside I, containing a linking arm, which makes it possible to join it to a protein, thereby obtaining an artificial immunogen, and also to join I to a suitable polymer such as aminated BioGel, to obtain an affinity column. Also described is the synthesis of the decasaccharide $3^2,3^4,3^6$-tri-β-D-glucopyranosylgentioheptaose (II).

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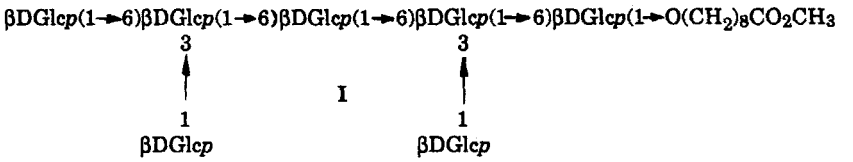
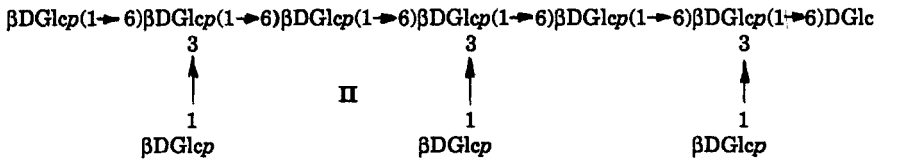
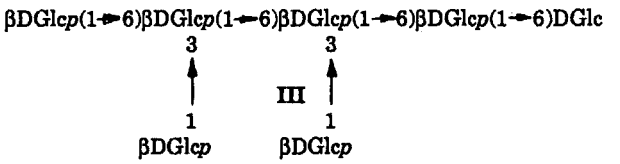


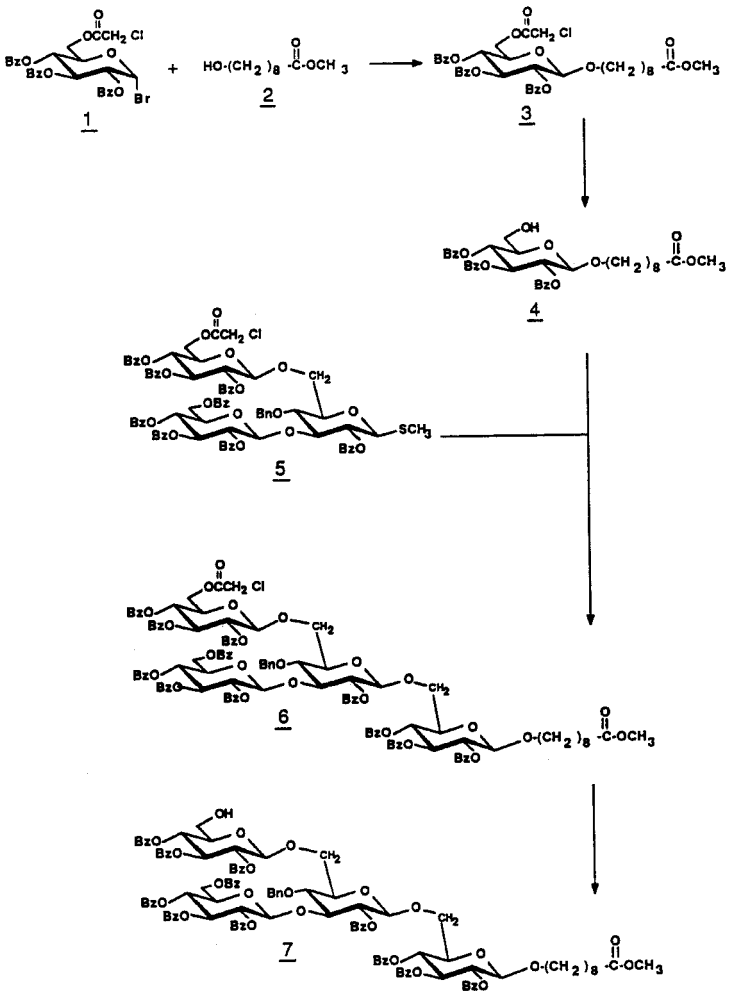


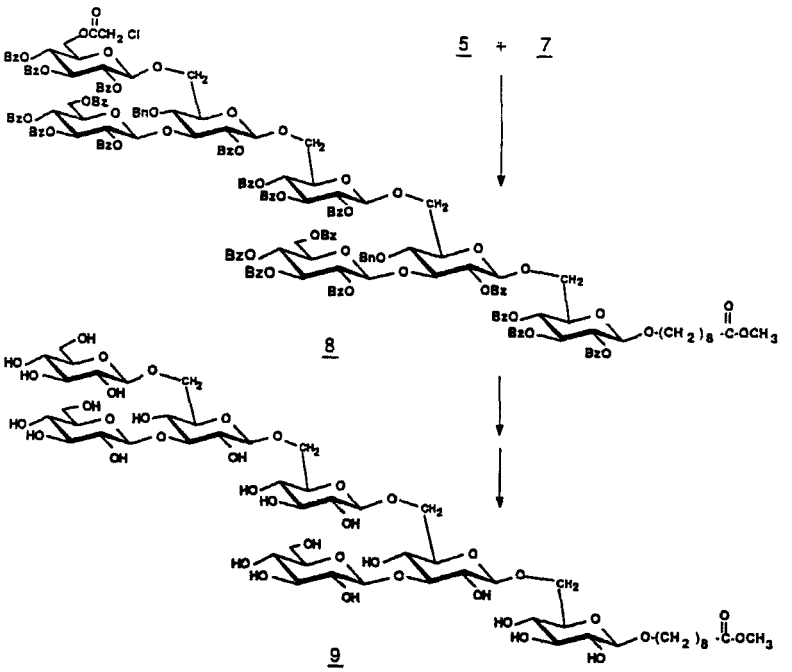


RESULTS AND DISCUSSION

Several unsuccessful attempts were made to convert the fully protected heptasaccharide, carrying a β-linked benzoate group at C-1, described previously,[6] into the corresponding 8-methyloxycarbonyloct-1-yl glycoside. The attempts included its direct condensation with 8-methyloxycarbonyloctan-1-ol in the presence of trimethylsilyl triflate and its attempted conversions into a thioglycoside, followed by methyl triflate[8] or dimethyl(thiomethyl)sulfonium triflate[9] promoted reaction of the resulting heptasaccharide thioglycoside with the alcohol. Recourse was therefore taken to building the heptasaccharide from a glucosyl monomer, carrying the required linking arm.

Thus, 2,3,4-tri-*O*-benzoyl-6-chloroacetyl-α-D-glucopyranosyl bromide (1)[6] was condensed with 8-methyloxycarbonyloctan-1-ol (2) in the presence of silver imidazolate,[10] which proved superior to other silver salt promoters for this glycosidation. The glycoside 3, which was obtained in 68% yield was then deblocked at C-6 by treatment with hydrazine dithiocarbonate.[11] The product 4 was glycosylated with the thioglycoside trisaccharide 5[6] under promotion by methyl triflate. The product 6, obtained from 4 in 89% yield, was treated with hydrazine dithiocarbonate and the glycosyl acceptor 7 thus obtained was glycosylated with the above thioglycoside trisaccharide 5 to yield the fully protected heptasaccharide glycoside 8 in 76% yield from 7. The ^{13}C NMR spectrum of 8, shown in FIG. 1, shows the presence of seven anomeric β-D-linked glycosidic carbons in the molecule. A small number of signals in the CH_2 region, due to impurities, were absent in the ^{13}C NMR spectrum of 9. The heptasaccharide glycoside 8 was deblocked by treat-





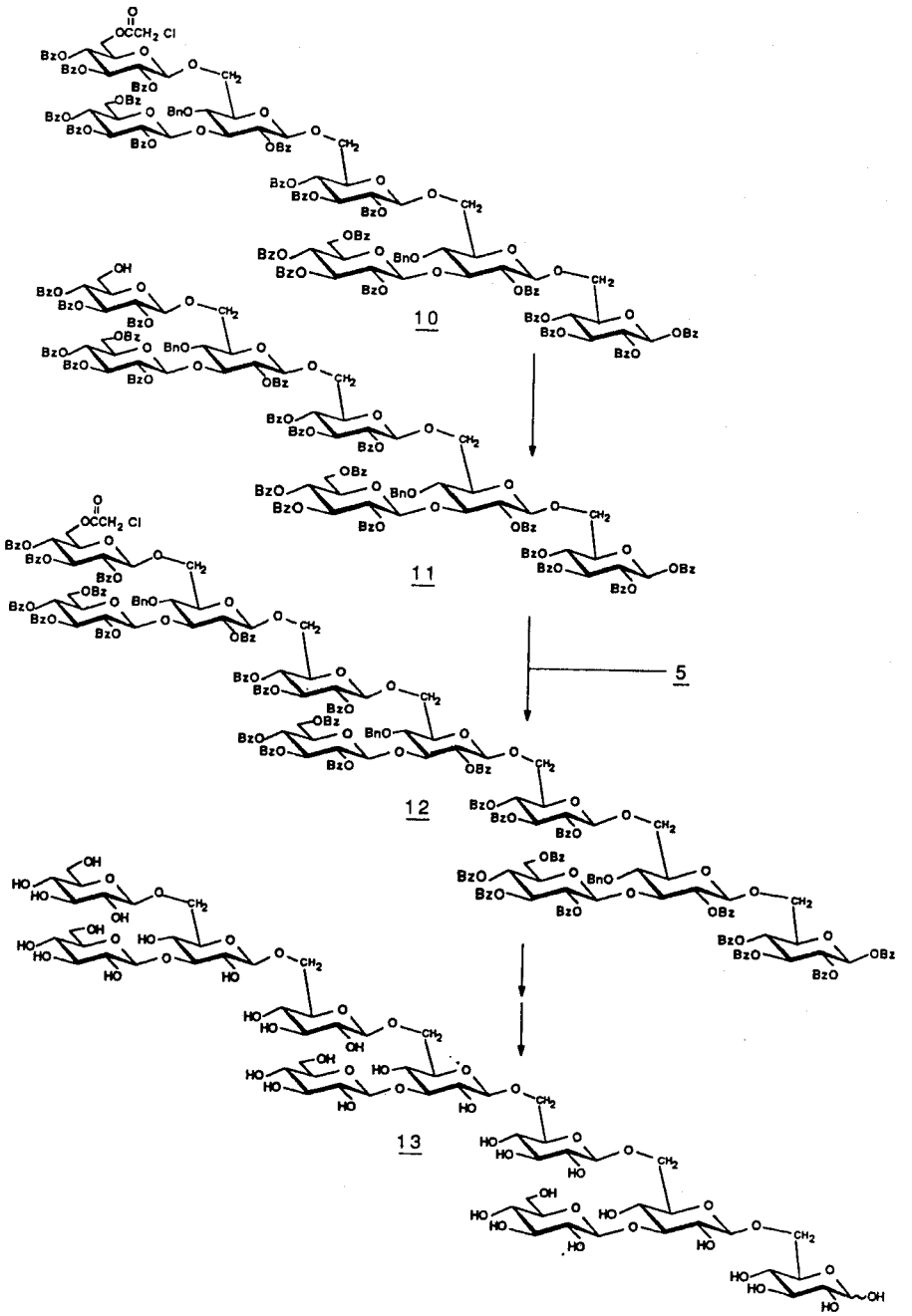
ment with sodium methoxide in methanol and hydrogen in the presence of Pd-C to give the target glycoside **9** in nearly quantitative yield from **8**. Negative ion FAB-MS gave the expected [M-1] ion.

The decasaccharide **13** was also synthesised. Thus, the 6-chloroacetyl group in the heptasaccharide derivative **10**[6] was removed by treatment with hydrazine dithiocarbonate, and the glycosyl acceptor **11** thus obtained was condensed with the above thioglycoside trisaccharide **5** to yield the fully protected decasaccharide derivative **12** in 32% yield from **11** and with 62% recovery of unreacted **11**. A ^{13}C NMR spectrum of **12** is shown in FIG.2. Deblocking of **12** in two steps, consisting of deacylation with sodium methoxide in methanol and then hydrogenolysis, gave the free decasaccharide **13** in quantitative yield from **12**. The final compound **13** gave a FAB-MS with the expected [M-1] ion.

EXPERIMENTAL

General methods. - These were the same as those described before.[4,6] The purity of compounds (generally hygroscopic) below, for which elemental analyses were not performed, were carefully ascertained by TLC in solvent systems which gave R_F values close to 0.5 and their identities were verified by NMR, and for **9** and **13** by FAB-MS as well as bioassay.[12]

8-Methoxycarbonyloct-1-yl 2,3,4-tri-O-benzoyl-β-D-glucopyranoside (**4**). - A mixture of **1** (0.225 g, 0.356 mmol), **2** (0.101 g, 0.537 mmol), and 3Å molecular sieves (0.5 g) in dry dichloro-



methane (20 mL) was stirred under dry nitrogen at room temperature for 30 min. Silver imidazolate (47 mg, 0.269 mmol) and zinc chloride (293 mg, 2.150 mmol) were added and the reaction mixture was kept in the dark at 40 °C for two days during which time it was monitored by TLC (toluene-ethyl acetate, 9:1). After dilution with dichloromethane, the reaction mixture was filtered through Celite. The solid residue was washed with dichloromethane and the combined filtrate and washings were stirred with 10% sodium carbonate for 30 min. The organic layer was separated from the water layer and then washed with aqueous sodium hydrogencarbonate, water, dried ($MgSO_4$), filtered, and concentrated. Silica gel column chromatography (toluene-ethyl acetate, 9:1) gave **3** (0.179 g, 68%). ^{13}C NMR data ($CDCl_3$, 25 MHz): δ 174.1 (C=O, 8-methoxycarbonyloct-1-yl), 167.0 ($\underline{C}OCH_2Cl$), 165.7, 165.2, 165.0 (3C=O), 101.3 (C-1), 64.0 (C-6), 51.3-24.9 ($7CH_2$ and $1CH_3$, 8-methoxycarbonyloct-1-yl), and 40.7 ($CO\underline{C}H_2Cl$).

A stock solution of hydrazine dithiocarbonate[11] was added dropwise at room temperature to a solution of **3** (0.2034 g, 0.275 mmol) in dioxane (5 mL). To prevent the product **4** from precipitating, dry *N,N*-dimethylformamide (3 mL) was added. After 15 min, TLC (toluene-ethyl acetate, 4:1) indicated complete reaction. The solution was concentrated, the residue was dissolved in dichloromethane and the solution was washed with M sulfuric acid, sodium hydrogencarbonate, water, dried (Na_2SO_4), filtered, and concentrated. Silica gel column chromatography (isooctane-ethyl acetate, 1:1.5 and then toluene-ethyl acetate, 4:1) gave **4** (0.1108 g, 61%), $[\alpha]_D$ -10° (*c* 0.50, chloroform). ^{13}C NMR data ($CDCl_3$, 25 MHz): δ 174.1 (C=O, 8-methoxycarbonyloct-1-yl), 165.8 (2C=O), 164.9 (1C=O), 101.1 (C-1), 61.4 (C-6), and 51.3-24.8 ($7CH_2$ and $1CH_3$, 8-methoxycarbonyloct-1-yl).

Anal.: Calcd. for $C_{37}H_{42}O_{10}$: C, 68.7; H, 6.5. Found: C, 68.3; H, 6.4.

8-Methoxycarbonyloct-1-yl 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* (**7**). - A mixture of **4** (0.1004 g, 0.151 mmol), methyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-6-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (**5**)[6] (0.2386 g, 0.155 mmol) and 4Å molecular sieves (0.5 g) in dry dichloromethane (8 mL) were stirred at room temperature under dry nitrogen for 1 h. Methyl trifluoromethanesufonate ("methyl triflate", 84 μL, 0.762 mmol) was injected through a rubber septum, and the reaction mixture was stirred overnight (TLC, toluene-ethyl acetate, 4:1 and isooctane-ethyl acetate, 1:1.5). Triethylamine was added (to pH ~8), and the mixture was filtered through Celite. The solid residue was washed with dichloromethane and the filtrate was washed successively with M sulfuric acid, sodium hydrogencarbonate and water, dried (Na_2SO_4), filtered, and concentrated. Silica gel column chromatography (toluene-ethyl acetate, 4:1) gave the pure tetrasaccharide **6** (0.2897 g, 89%), $[\alpha]_D$ -16° (*c* 0.50, chloroform). ^{13}C NMR data ($CDCl_3$, 25 MHz): 174.1 (C=O, 8-methoxycarbonyloct-1-yl), 167.1 ($\underline{C}OCH_2Cl$), 165.9-164.3 (11 partially overlapping C=O), 138.0 (aromatic C-1 of $PhCH_2$ at O-4'), 101.4, 100.9, 100.3 (partially overlapping C-1, C-1', C-1", C-1"'), 51.4-24.9 (7 CH_2, and CH_3, 8-methoxylcarbonyloct-1-yl), and 40.7 ($CO\underline{C}H_2Cl$).

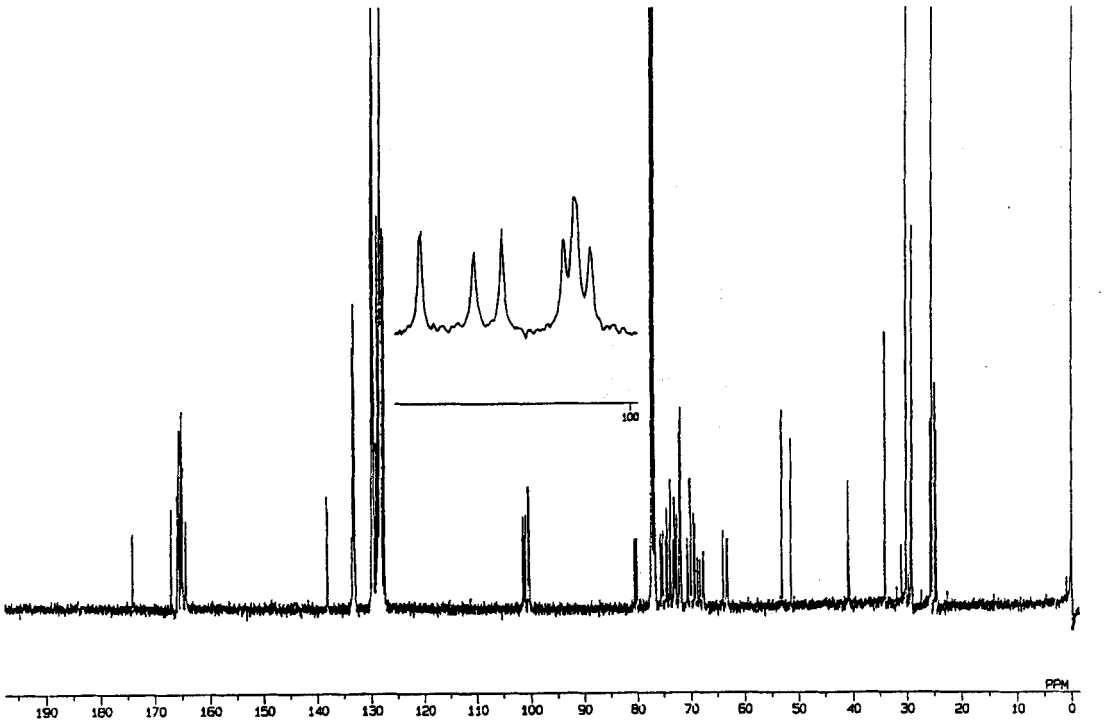


FIG. 1. 100 MHz ^{13}C NMR spectrum of 8.

Hydrazine dithiocarbonate (1.53 mL, 0.573 mmol) was added dropwise at room temperature to a solution of **8** (0.2797 g, 0.130 mmol) in dioxane (6 mL). To prevent the product **7** from precipitating, dry *N,N*-dimethylformamide (4 mL) was added. After 45 min (TLC, toluene-ethyl acetate, 4:1) the reaction mixture was worked up as described for **4** above. Silica gel column chromatography (isooctane-ethyl acetate 1:1.5 and then dichloromethane-ethyl acetate-methanol 14:5:1) gave **7** (0.1900 g, 70%), $[\alpha]_D$ -17° (*c* 0.50, chloroform). ^{13}C NMR data ($CDCl_3$, 25 MHz): δ 174.1 (C=O, 8-methoxycarbonyloct-1-yl), 165.9-164.5 (11 partially overlapping C=O), 138.1 (aromatic C-1 of $PhCH_2$ at O-4'), 101.4, 100.8, 100.3, and 100.0 (C-1, C-1', C-1", C-1"'), and 51.4-24.9 (7 CH_2, and 1 CH_3, 8-methoxylcarbonyloct-1-yl).

Anal.: Calcd. for $C_{118}H_{110}O_{33}$: C, 68.9; H, 5.4. Found: C, 68.8; H, 5.5.

8-Methoxycarbonyloct-1-yl 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)*-β-D-*glucospyranoside* (**9**). - The tetrasaccharide **7** (185 mg, 89.3 μmol) was coupled to trisaccharide (**5**) (140.5 mg, 91.6 μmol) as described for compound **6** above. Silica gel column chromatography (toluene-ethyl acetate, 4:1 and then isooctane-ethyl acetate, 1:1.5) gave the fully protected heptasaccharide **8** (0.2404 g, 76%), $[\alpha]_D$ -24° (*c* 0.50, chloroform). ^{13}C NMR

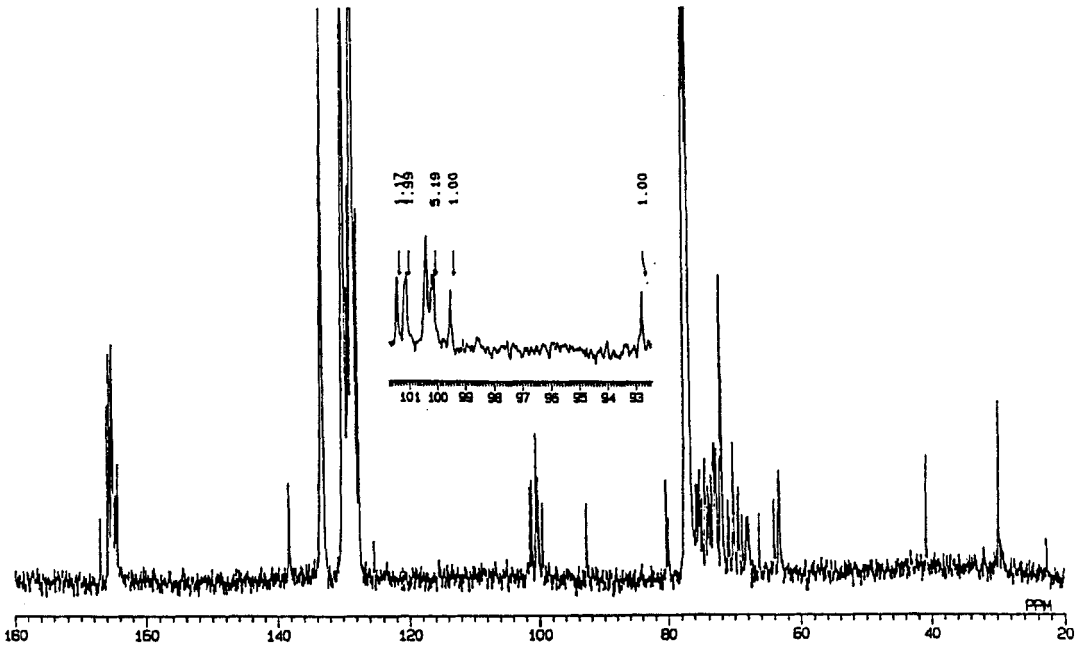


FIG. 2. 100 MHz ^{13}C NMR spectrum of 12.

data ($CDCl_3$, 100 MHz): δ 174.2 (C=O, 8-methoxycarbonyloct-1-yl), 138.3, 138.1 (aromatic C-1 of $PhCH_2$ at O-4' and O-4'''), 101.4, 101.0, 100.9, 100.5, 100.4, 100.3 (partially overlapping C-1, C-1', C-1'', C-1''', C-1'''', C-1'''''), 80.6, 80.2 (C-3', C-3'''), and 51.4-24.9 (7 CH_2, and 1 CH_3, 8-methoxylcarbonyloct-1-yl). (FIG. 1).

A solution of the protected heptasaccharide 8 (0.2614 g, 73.5 μmol) dissolved in dry tetrahydrofuran (5 mL) and methanol (15 mL) was treated with M sodium methoxide (0.7 mL) at room temperature overnight. After neutralisation with methanol-washed Dowex 50 (H+), filtration and washing of the resin with methanol, the combined filtrate and washings were concentrated to yield the partly deblocked heptasaccharide. The residue was dissolved in 10% aqueous ethanol (10 mL) and 99.5% ethanol (3 mL) and hydrogenolysed at 54 psi overnight in the presence of 10% Pd-C (0.2 g). After filtration through Celite, and washings of the Celite with distilled water and 50% aqueous ethanol, the solution was concentrated and purified on Bio-Gel P-2. The heptasaccharide 9 was eluted with 20% aqueous ethanol and the final solution was freeze-dried to give 9 in 95% yield from 8, $[\alpha]_D$ -28° (*c* 0.50, DMSO), $[\alpha]_D$ -36° (*c* 0.25, H_2O). ^{13}C NMR data (DMSO-d_6 70 °C, 125 MHz, DMSO C set at 39.25 p.p.m.): δ 173.4 (C=O, 8-methoxycarbonyloct-1-yl), 104.04, 104.02, 103.51, 103.44, 102.85, 102.82, 102.78 (C-1, C-1', C-1'', C-1''', C-1'''', C-1''''', C-1'''''), 86.96, 86.93 (C-3', C-3''').(FIG. 3).

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)*-O-β-D-*gluco-*

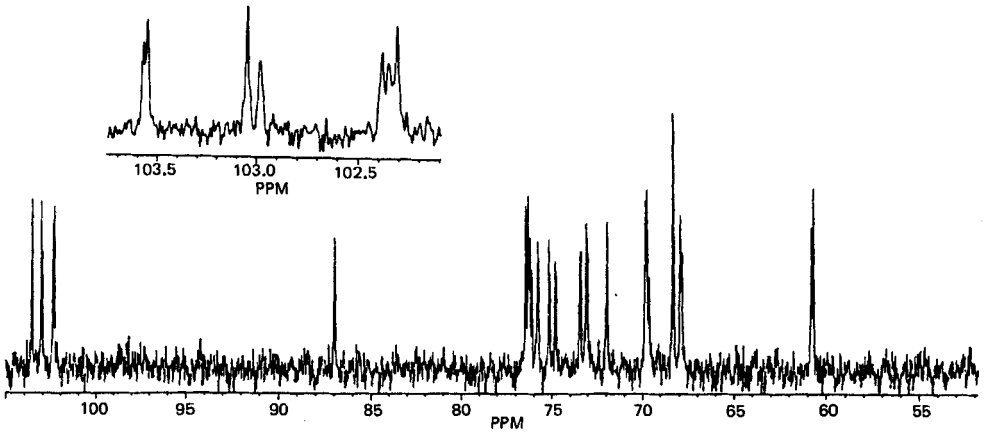


FIG. 3. 125 MHz ^{13}C NMR spectrum of 9.

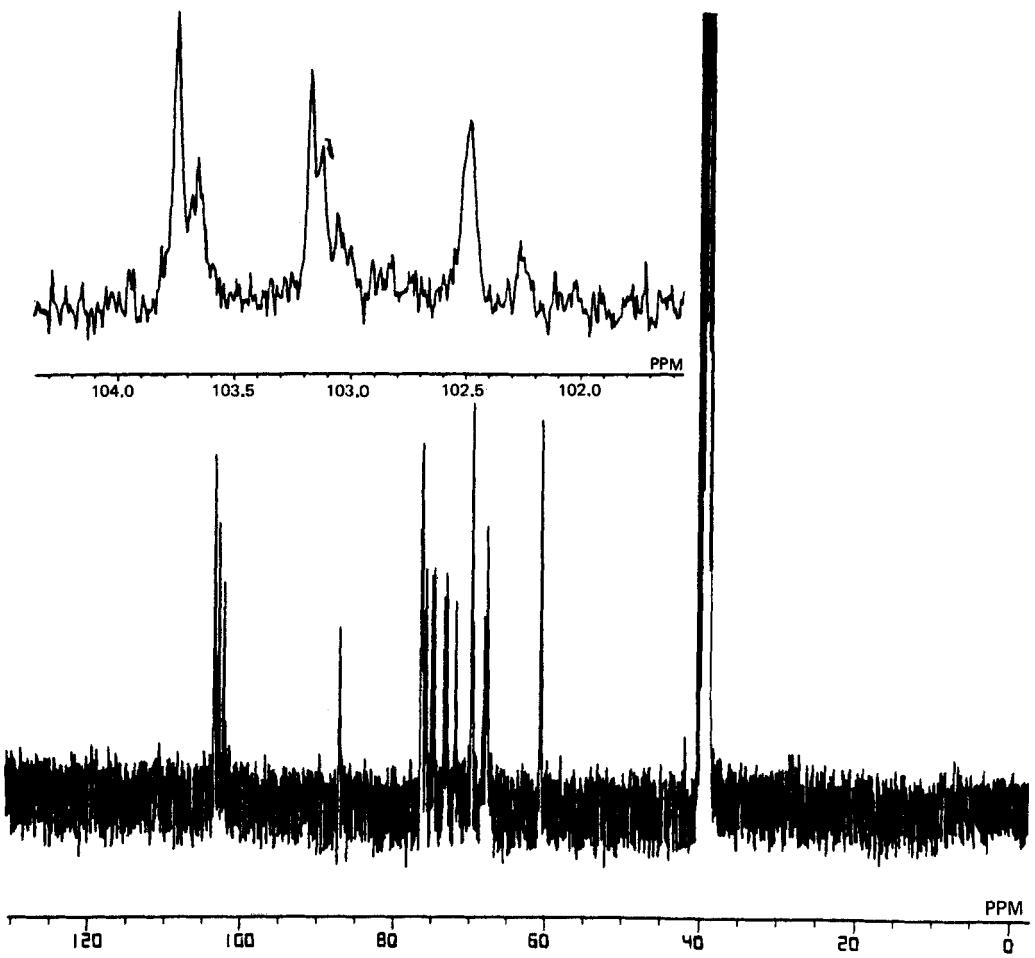


FIG. 4. 100 MHz ^{13}C NMR spectrum of 13.

pyranosyl-(1→6)-O-*[β-D-glucopyranosyl-(1→3)]*-O-β-D-*glucopyranosyl*-O-*(1→6)*-D-*glucose (32,34,36-tri*-β-D-*glucopyranosylgentioheptaose)* (13). - *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*-[(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 10[6] was dechloroacetylated as described above for 3. After 30 min, TLC (toluene-ethyl acetate, 4:1) indicated complete reaction. Work-up, as described above for 3 followed by silica gel column chromatography (isooctane-ethyl acetate, 1:1.5 and then toluene-ethyl acetate, 4:1) gave 11 (117.4 mg, 60%, after some losses during the chromatographic purification) with a free 6''''-OH group, $[\alpha]_D$ -20° (*c* 0.55, chloroform). ^{13}C NMR data ($CDCl_3$, 67.8 MHz): δ 166.0-164.7 (partially overlapping C=O), 138.2, 138.1 (aromatic C-1 of $PhCH_2$ at O-4' and O-4'''), 101.3, 101.0, 100.1, 99.4 (6 partially overlapping anomeric C), 92.7 (C-1), 80.3, 79.8 (C-3', C-3''').

A mixture of 11 (0.080 g, 23.4 μmol), 5[6] (0.0368 g, 24 μmol) and 4Å molecular sieves (0.2 g) in dry dichloromethane (5 mL) were stirred at room temperature under dry nitrogen for 1 h. Methyl triflate (13 μl, 117 μmol) was injected through a rubber septum, and the reaction mixture was stirred at room temperature overnight (TLC, toluene-ethyl acetate, 4:1 and isooctane-ethyl acetate, 1:1.5). Triethylamine was added (to pH ~8), and the mixture was filtered through Celite. The residue was washed with dichloromethane and the filtrate was washed successively with M sulfuric acid, sodium hydrogencarbonate and water, dried (Na_2SO_4), filtered, and concentrated. Silica gel column chromatography (toluene-ethyl acetate, 4:1) gave the pure protected decasaccharide 12 (0.0372 g, 32%) $[\alpha]_D$ -21° (*c* 0.50, chloroform). ^{13}C NMR data ($CDCl_3$, 100 MHz): δ 167.1 ($\underline{C}OCH_2Cl$), 166.0-164.3 (partially overlapping C=O), 138.4, 138.3, 138.2 (aromatic C-1 of $PhCH_2$ at O-4', O-4''' and O-4''''''), 101.4, 101.2, 101.1, 100.4, 100.2, 100.1, 99.5 (9 partially overlapping anomeric C), 92.8 (C-1), 80.3, 80.0 (partially overlapping C-3', C-3''' and C-3''''''), and 40.7 ($CO\underline{C}H_2Cl$) (FIG. 2). Unreacted heptasaccharide 11 (0.050 g) was recovered.

A solution of 12 (0.035 g, 7.1 μmol) dissolved in dry tetrahydrofuran (1 mL) and methanol (2 mL) was treated with M sodium methoxide (0.1 mL) at room temperature overnight. After neutralisation with methanol-washed Dowex 50 (H^+), filtration and washings of the resin with methanol, the filtrate was concentrated to yield the partly deblocked decasaccharide. The residue was dissolved in 10% aqueous ethanol (2.5 mL) and 99.5% ethanol (0.5 mL) and hydrogenolysed at 52 psi overnight in the presence of 10% Pd-C (0.1 g). After filtration through Celite, and washings of the Celite with distilled water and 50% aqueous ethanol, the solution was freeze-dried. The decasaccharide 13, (11.7 mg, 100%) had $[\alpha]_D$ -17° (*c* 0.17, H_2O), $[\alpha]_D$ -20° (*c* 0.6, DMSO). ^{13}C NMR data (DMSO-d_6 70 °C, 100 MHz, DMSO C set at 39.50 p.p.m.): δ 103.74, 103.68, 103.66, 103.64, 103.17, 103.12, 103.06, 102.49 (10 partially overlapping anomeric C), 87.26, 87.24, 87.23 (C-3', C-3''' and C-3''''') (FIG. 4).

ACKNOWLEDGEMENTS

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