Some β-1,3- and β-1,6-linked D-Glucose Di- and Trisaccharide L-Serine Derivatives for Glycopeptide Synthesis

Per J. Garegg,[a] Stefan Oscarson,[a] Ingemar Kvarnström,[b] Annika Niklasson,[b] Gunilla Niklasson,[b] Stefan C. T. Svensson[b] and Judson V. Edwards[c]

[a]Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden, [b]Department of Chemistry, University of Linköping, S-581 83 Linköping, Sweden and [c]Merrell Dow Research Institute, Merrell Dow Pharmaceuticals Inc., PO Box 156300, Cincinatti, Ohio 45215-6300, USA

Garegg, P. J., Oscarson, S., Kvarnström, I., Niklasson, A., Niklasson, G., Svensson, C. T. and Edwards, J. V., 1990. Some β-1,3- and β-1,6-linked D-Glucose Di- and Trisaccharide L-Serine Derivatives for Glycopeptide Synthesis. – Acta Chem. Scand. 44: 625–629.

Syntheses are described of protected mono-, di- and tri-saccharides, *O*-linked to *N*-fluorenylmethoxycarbonyl-L-serine *p*-nitrophenyl ester, suitable for solid-supported or liquid-phase synthesis of glycopeptides. The saccharide moieties are fully benzylated β-glucopyranosyl and β-laminarotriosyl and also benzoylated β-glucopyranosyl, β-gentiobiosyl and 3-*O*-β-D-glucopyranosyl-β-gentiobiosyl residues.

In connection with studies on the biochemistry of plant protection against fungal infection, we have synthesised several oligosaccharides.[1-4] These contain a β-1,6-linked chain of D-glucopyranose units, with β-1,3-linked branches of single D-glucopyranosyl residues. The smallest oligosaccharide with phytoelicitor activity was a heptasaccharide.[5-7] It subsequently became of interest to see if smaller fragments corresponding to these structures, linked to peptides also might have phytoelicitor activity.

Results and discussion

For peptide synthesis we chose the activated ester method,[8] using protection of the amino group in serine by the Fmoc group, and protection of the carboxy group as a *p*-nitrophenyl ester. In order to find the best protection groups for the saccharide portion of the glycopeptides to be made, *O*-protection as benzyl ethers as well as benzoates was carried out.

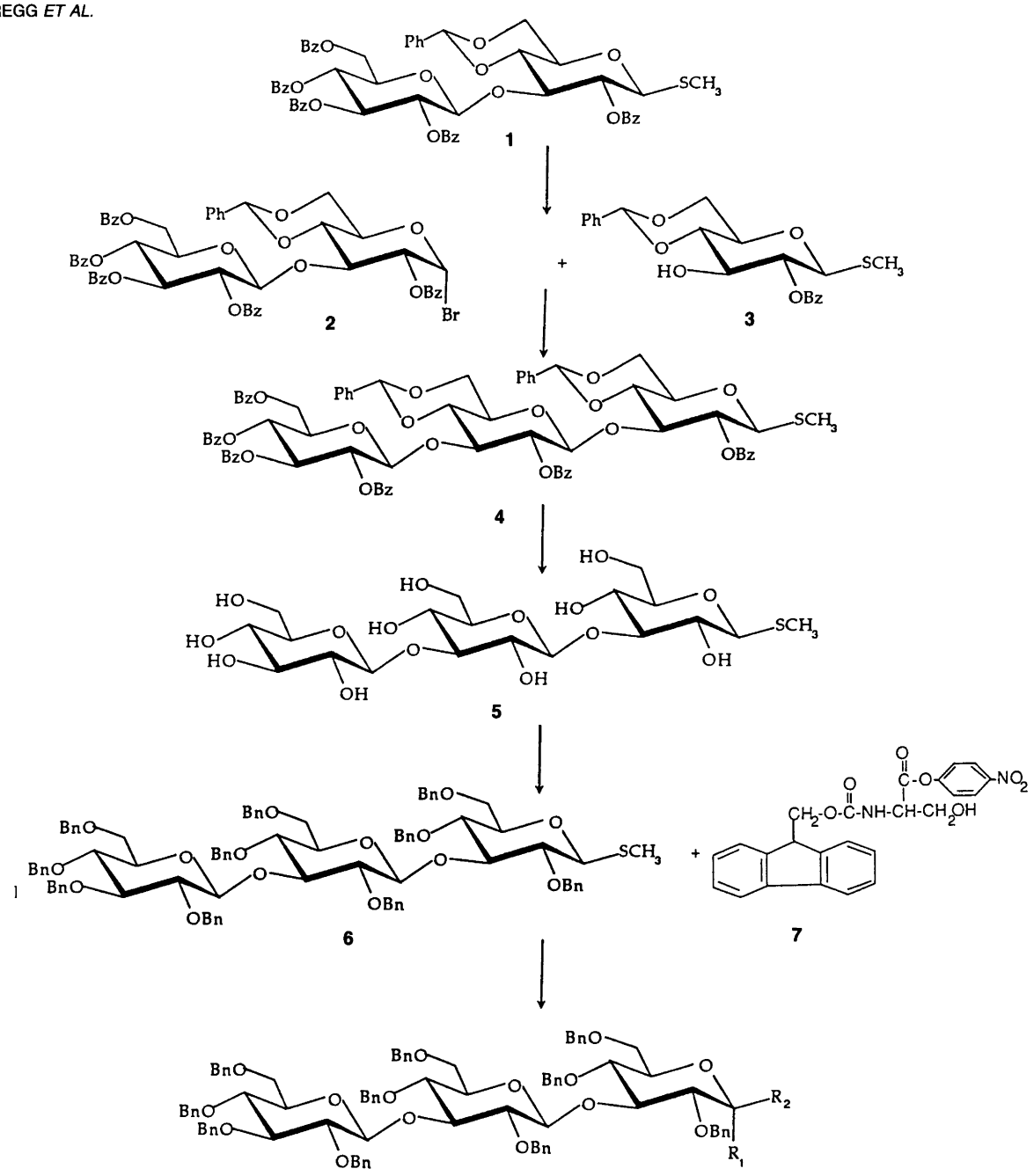
The disaccharide thioglycoside **1**[2] was converted into the corresponding glycosyl bromide **2**, which under silver triflate promotion,[9] and by neighbouring-group participation of the 2-*O*-benzoyl group,[10] was reacted with the monosaccharide glycosyl acceptor **3**[2] to give the trisaccharide derivative **4** in 89 % yield (Scheme 1). Deprotection led to the thioglycoside trisaccharide **5**. This was fully benzylated and the product **6** was used in a methyl triflate[11] promoted condensation with the protected serine derivative **7**.[8,12] Acetonitrile was used as the solvent to give predominately the β-anomer **8b** (α/β ratio = 2:3). If dichloromethane was used instead, the total yield of serine glycosides was the same (78 %) but the α/β-ratio was higher (3:2). Separation of the two anomers, **8a** and **8b**, was found to be troublesome, due to the lability of the derivatives during silica gel chromatography. The losses could be minimized using 0.25 % acetic acid in the eluent,[12] to give the desired compound **8b** in 30 % yield. This route was found to give better yield of **8b** than one proceeding from **6** via the corresponding α-trichloroimidate.[13]

The glycosyl serine β-glucoside derivative **10b** was similarly obtained in 40 % yield by treating methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**9**)[14] with the serine derivative **7** with promotion by methyl triflate.[11]

As anticipated, the yields of the required β-anomer in the final glycosylation reactions were better in the syntheses of the remaining three glycosylserine derivatives, due to stereocontrol by 2-*O*-benzoyl groups. The thioglycoside disaccharide **11** was made by silver triflate promoted condensation of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[15] with methyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside. The product was then treated with the serine derivative **7** with methyl triflate promotion[11] to yield the target compound **12** in 83 % yield. Similarly, the previously described[2] trisaccharide thioglycoside **13** was condensed with **7**, again with methyl triflate promotion[11] to yield the target trisaccharide serine derivative **14** in 88 % yield. Also made was the glucosyl serine compound **15**, by a silver triflate promoted reaction between 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide and the serine derivative **7**.

Experimental

General procedures. Optical rotations were determined using a Perkin-Elmer 141 polarimeter. NMR spectra were recorded using either a JEOL JNM FX-100 or a GX-270 instrument. Chemical shifts are given in ppm relative to internal tetramethylsilane, unless otherwise stated. Assign-

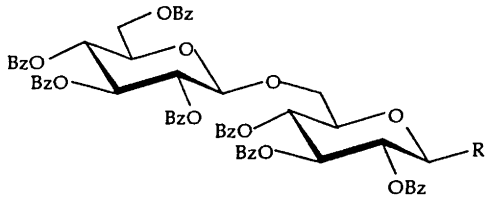


8a R_1= O-serine derivative, R_2= H
8b R_1= H, R_2= O-serine derivative

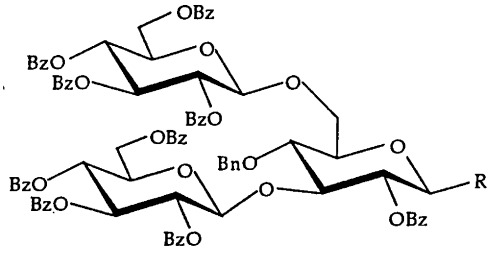
Scheme 1.

ment of shifts for ring carbons in the glucopyranose moieties in compounds **10a** and **10b** and for benzyl methylene carbon are based on published results.[16] TLC was performed using silica gel plates (F_{254}, Merck) and the spots were detected with UV light and/or by charring with sulfuric acid/ethanol (1:1). Column chromatography was performed on silica gel 60 (0.040–0.063 mm, Merck).

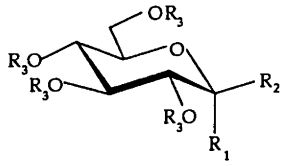
Methyl O-*(2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-*β*-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-*β*-D-glucopyranoside* (**4**). A bromide solution (17.9 ml of a solution of 0.17 ml bromide in 28 ml dry dichloromethane) was added to a stirred mixture of methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside[11] (**1**, 1.89 g, 1.93 mmol) and 4 Å molecular sieves (0.8 g) in dry dichloromethane (18 ml) at room temperature under nitrogen. After 20 min, tetraethylammonium bromide (0.94 g, 4.47 mmol) was added. Conversion of the thioglycoside into the glycosyl bromide **2** was complete within 3 h (TLC, toluene–ethyl acetate 7:1, R_f 0.48). The mixture was diluted with



11 R= SCH_3
12 R= O-serine derivative



13 R= SCH_3
14 R= O-serine derivative



9 R_1= H, R_2= SCH_3, R_3= Bn
10a R_1= O-serine derivative, R_2= H, R_3= Bn
10b R_1= H, R_2= O-serine derivative, R_3= Bn
15 R_1= H, R_2= O-serine derivative, R_3= Bz

dichloromethane, filtered through Celite and the filtrate was washed successively with water, aqueous sodium hydrogencarbonate and then water, dried ($MgSO_4$), filtered and concentrated to yield **2** as a foam (1.87 g, 96 %). ^{13}C NMR data ($CDCl_3$, 25 MHz): δ 63.0, 67.2, 67.6, 69.5, 71.8, 71.8, 72.7, 72.7, 75.9, 78.1 (C-2 to C-6, C-2′ to C-6′), 87.9 (C-1), 101.0, 101.3 (PhCH, C-1′), 125.7–129.4, 132.6–133.3., 136.4 (aromatic C), 164.4, 164.5, 164.7, 165.3, 165.7 (C=O).

Silver triflate (0.475 g, 1.85 mmol) in dry toluene (5.8 ml) was added to a stirred mixture of methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[1] (**3**, 0.58 g, 1.44 mmol), compound **2** (1.85 g, 1.82 mmol) and powdered molecular sieves 4 Å (1.5 g) in dry dichloromethane (9 ml) at −40 °C under nitrogen, followed by the addition of 2,4,6-trimethylpyridine (0.12 ml). After 2 h at −40 °C, additional silver triflate (0.188 g, 0.73 mmol) in dry toluene (5.8 ml) and 2,4,6-trimethylpyridine (60 µl) were added. After 5 h at −40 °C, pyridine was added and the mixture was filtered through Celite and the filtrate was washed with aqueous sodium thiosulfate, water, 1M sulfuric acid, aqueous sodium hydrogencarbonate and water, dried ($MgSO_4$), filtered and concentrated. The crude product was purified by silica gel column chromatography (toluene–ethyl acetate 6:1, R_f 0.48) to give compound **4** (1.71 g, 89 %). $[\alpha]_D$ +6.3° (*c* 0.5, chloroform). ^{13}C NMR data ($CDCl_3$, 68 MHz): δ 11.9 (SCH_3), 63.0, 65.3, 68.5, 68.9, 70.0, 71.1, 71.8, 72.0, 72.3, 72.8, 73.1, 74.9, 78.8, (C-2 to C-6, C-2′ to C-6′, C-2″ to C-6″), 84.1 (C-1), 97.7, 98.3 (C-1′, C-1″), 100.4, 101.9 (PhCH), 126.0–133.5, 137.1, 137.3 (aromatic C), 164.7, 164.8, 165.1, 165.2, 165.7, 166.1 (C=O). Anal. $C_{75}H_{66}O_{21}S$: C, H.

Methyl O-*(2,3,4,6-tetra*-O-*benzyl*-β-D-*glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl*-β-D-*glucopyranosyl)-(1→3)-2,4,6-tri*-O-*benzyl-1-thio*-β-D-*glucopyranoside* (**6**). A solution of compound **4** (1.33 g, 1.15 mmol) in 80 % acetic acid (25 ml) was heated at 100 °C for 1 h. The solvent was evaporated and the product was codistilled several times with toluene to yield the debenzylidenated product (1.06 g, 92 %, TLC toluene–ethyl acetate 1:5, R_f 0.05). A solution of the crude product (1.00 g, 0.86 mmol) in methanol (120 ml) was treated with methanolic sodium methoxide (12 ml, 1 M) and the mixture was stirred for 2 h at room temperature. The solution was neutralized with cation exchange resin (Dowex 50 WX8), filtered and concentrated. The crude product was purified by flash chromatography (acetonitrile–water 9:1, R_f 0.13) to give compound **5** (0.44 g, 89 %). ^{13}C NMR data [D_2O with dioxane (δ 67.40) as internal standard, 25 MHz]: δ 12.4 (SCH_3), 61.5 (C-6, C-6′, C-6″, overlapping), 68.8, 68.8, 70.4, 72.2, 74.1, 74.3, 76.4, 76.4, 76.8, 80.4, 85.1, 86.2, 86.2 (C-1 to C-5, C-2′ to C-5′, C-2″ to C-5″), 103.2, 103.6 (C-1′, C-1″).

A solution of compound **5** (0.44 g, 0.82 mmol) in dry *N,N*-dimethylformamide (23 ml) was stirred with sodium hydride (previously washed with light petroleum and dried under N_2, 0.40 g, 16.5 mmol) for 1 h at room temperaure. benzyl bromide (freshly distilled, 1.96 ml, 16.5 mmol) was added dropwise and the mixture was stirred for 3.5 h at room temperature. Methanol was added in order to decompose the excess of hydride. Most of the solvent was evaporated and a solution of the residue in dichloromethane was washed with water, dried ($MgSO_4$), filtered and concentrated. Purification by silica gel column chromatography (toluene–ethyl acetate 9:1, R_f 0.55) gave compound **6** (0.71 g, 60 %). $[\alpha]_D$ +26.3° (*c* 1.48, chloroform). ^{13}C NMR data (68 MHz, $CDCl_3$): δ 13.1 (SCH_3), 69.1, 69.2, 69.2 (C-6, C-6′, C-6″), 73.4, 74.5, 74.7, 75.1, 75.7, 76.1, 78.3, 78.9, 80.6, 81.7, 82.0, 83.1, 83.9, 84.9, 85.3 (C-1 to C-5, C-2′ to C-5′, C-2″ to C-5″, benzyl CH_2), 102.2, 102.9 (C-1′, C-1″), 127.3–128.5, 137.7–138.7 (aromatic C). Anal. $C_{89}H_{94}O_{15}S$: C, H.

N-*(Fluoren-9-ylmethoxycarbonyl)*-O-*(2,3,4,6-tetra*-O-*benzyl*-β-D-*glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl*-β-D-*glucopyranosyl)-(1→3)-(2,4,6-tri*-O-*benzyl*-α- (**8a**) *and* -β-D-*glucopyranosyl)-(1→0)*-L-*serine* p-*nitrophenyl ester* (**8b**). A mixture of **6** (0.27 g, 0.19 mmol) and *N*-(fluoren-9-ylmethoxycarbonyl)-L-serine *p*-nitrophenyl ester[2,3] (**7**, 85 mg, 0.19 mmol) in dry acetonitrile (6.0 ml) containing

powdered molecular sieves 3 Å (1.2 g) was stirred at room temperature under nitrogen for 0.5 h. Methyl triflate (105 µl, 0.96 mmol) in dry dichloromethane (0.5 ml) was added in small portions over 3 h. After 5 h methanol was added and the mixture was put on top of a silica gel column (toluene–ethyl acetate 6:1, containing 0.25 % acetic acid: and eluted to give an α/β mixture (0.27 g, 78 %) of serine glycosides **8a** and **8b** (R_f 0.46 for the α-anomer and R_f 0.41 for the β-anomer). The glycosides were separately by column chromatography on silica gel (toluene–ethyl acetate 6:1, containing 0.25 % acetic acid) to give compound **8a** (80 mg, 23 %) and its β-anomer **8b** (104 mg, 30 %) and a mixed fraction containing both anomers (23 mg, 6 %).

When glycosylation to the serine glycosides **8a** and **8b** was performed using dichloromethane as the solvent instead of acetonitrile according to the above procedure, compounds **8a** and **8b** were obtained in a total yield of 79 %. Compound **8a** was isolated in 30 % yield and the β-anomer **8b** in 22 % yield. Compound **8a** has $[\alpha]_D$ +27.3° (*c* 1.02, chloroform). ^{13}C NMR data ($CDCl_3$, 68 MHz): δ 47.0 (C-9-Fmoc), 54.8 (C^{α}), 67.4, 68.5, 69.1, 69.2, 69.9 (C-6, C-6′, C-6″, C-β, CH_2-Fmoc), 70.5, 73.3, 73.4, 73.5, 74.5, 74.6, 74.8, 75.1, 75.7, 75.9, 76.4, 78.3, 80.7, 81.2, 83.1, 83.9, 84.9 (C-2 to C-5, C-2′ to C-5′, C-2″ to C-5″, benzyl CH_2), 97.9 (C-1), 102.4, 102.8 (C-1′, C-1″), 119.9, 122.4, 125.1–128.8, 137.7–138.7, 141.2, 143.7, 145.5 (aromatic C), 154.9, 156.2 (OCON, C-1-*p*-nitrophenyl), 168.2 (COO). Anal. $C_{112}H_{110}N_2O_{22}$: C, H, N. Compound **8b** had $[\alpha]_D$ +15.8° (*c* 1.28, chloroform). ^{13}C NMR data ($CDCl_3$, 68 MHz): δ 47.0 (C-9-Fmoc), 54.9 (C-α), 67.4, 69.0, 69.1, 69.3, 69.3 (C-6, C-6′, C-6″, C-β, CH_2-Fmoc), 73.4, 73.5, 74.6, 74.8, 75.1, 75.7, 75.9, 78.3, 80.2, 80.6, 83.0, 83.1, 83.9, 84.9, (C-2 to C-5, C-2′ to C-5′, C-2″ to C-5″, benzyl CH_2), 102.4, 102.8 (C-1′, C-1″), 103.2 (C-1), 120.0, 122.5, 125.0–128.5, 137.8–138.7, 141.3, 143.6, 145.6 (aromatic C), 155.1, 156.0 (OCON, C-1-*p*-nitrophenyl), 168.0 (COO). Anal. $C_{112}H_{110}N_2O_{22}$: C, H, N.

N-*(Fluoren-9-ylmethoxycarbonyl)-(2,3,4,6-tetra*-O-*benzyl*-α- (**10a**) *and* -β-D-*glucopyranosyl)-(1→*O)-L-*serine* p-*nitrophenyl ester* (**10b**). A mixture of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside[14] (**9**, 63 mg, 0.11 mmol) and **7** (51 mg, 0.11 mmol) in dry acetonitrile (1.0 ml) containing powdered molecular sieves 3 Å (0.1 g) was stirred under nitrogen for 30 min and then cooled to −30 °C. Methyl triflate (61 µl, 0.56 mmol) was added and the temperature was gradually raised to room temperature over 4 h. After 7 h, the mixture was put on top of a silica gel column (toluene–ethyl acetate 6:1, containing 0.25 % acetic acid) and eluted to give a 2:3 α/β mixture of **10a** (R_f 0.35) and **10b** (R_f 0.29) (0.105 g, 98 %). Separation on silica gel (chloroform:methanol 60:1, containing 0.25 % acetic acid) gave **10a** (26 mg, 24 %), and **10b** (43 mg, 40 %) and a mixed fraction containing both anomers (15 mg, 14 %). When glycosylation to the serine glycosides **10a** and **10b** was performed at room temperature using dichloromethane as the solvent instead of acetonitrile according to the above described procedure, compound **10a** and **10b** was obtained in a total yield of 93 %. An anomeric ration (calculated from ^{13}C NMR) of α/β 3:2 was observed. The serine glycosides **10a** and **10b** were, however, not separated. Compound **10a** had $[\alpha]_D$ +14.40 (*c* 2.1, chloroform). ^{13}C NMR data ($CDCl_3$, 25 MHz): δ 46.9 (C-9-Fmoc), 54.7 (C-α), 67.3, 68.1, 69.6 (C-6, C-β, CH_2-Fmoc), 71.0 (C-5), 77.4 (C-4), 73.3, 73.5, 75.1 (benzyl CH_2, partly overlapping), 79.9 (C-2), 81.5 (C-3), 98.2 (C-1), 119.7, 122.3, 124.7, 124.9, 126.8–128.1, 137.3, 137.5, 138.2, 140.9, 143.3, 145.2 (aromatic C), 154.6, 155.7 (OCON, C-1-*p*-nitrophenyl), 167.8 (COO). Anal. $C_{58}H_{54}N_2O_{12}$: C, H, N. Compound **10b** had $[\alpha]_D$ +7.8° (*c* 1.6, chloroform). ^{13}C NMR data ($CDCl_3$, 25 MHz): δ (C-9-Fmoc), 54.8 (C-α), 67.2, 68.5, 69.3 (C-6, C-β, CH_2-Fmoc), 73.3, 74.7, 74.9 (C-5, benzyl CH_2, partly overlapping), 77.4 (C-4), 81.7 (C-2), 84.4 (C-3), 103.2 (C-1), 119.7, 122.2, 124.7, 124.9, 126.8–128.1, 137.4, 137.6, 138.0, 140.9, 143.3, 145.3 (aromatic C), 154.7, 155.7 (OCON, C-1-*p*-nitrophenyl), 167.7 (COO). Anal. $C_{58}H_{54}N_2O_{12}$: C, H, N.

Methyl 6-O-*(2,3,4,6-tetra*-O-*benzoyl*-β-D-*glycopyranosyl)-2,3,4-tri*-O-*benzoyl-1-thio*-β-D-*glucopyranoside* (**11**). A solution of silver triflate (0.19 g) in toluene (3 ml) was added to a cooled (0 °C) and stirred mixture of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[15] (0.35 g) and methyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (m.p. 123–124 °C, $[\alpha]_D$ −4.1° (*c* 0.6, chloroform), derived by tritylation, benzoylation and detritylation from methyl 1-thio-β-D-glucopyranoside[2]) (0.22 g) in dichloromethane (5 ml) containing crushed molecular sieves (4 Å). The mixture was allowed to attain room temperature and then put on top of a column of silica gel and eluted (toluene–ethyl acetate 20:1) to give **11** (0.40 g, 86 %), which crystallized from ethanol–acetone, m.p. 216–217 °C, $[\alpha]_D$ +18° (*c* 1.2, chloroform). ^{13}C NMR data ($CDCl_3$, 68 MHz): δ 11.5 (SCH_3), 63.0 (C-6′), 68.5, 69.7 (2 C), 70.0, 71.9, 72.3, 72.9, 74.0, 78.1 (C-2 to C-6, C-2′ to C-5′), 83.2 (C-1), 101.5 (C-1′), 128.3–133.5 (aromatic C), 165.2, 165.4, 165.7, 165.8 (carbonyl C). Anal. $C_{62}H_{52}O_{17}S$: C, H.

N-*(Fluoren-9-ylmethoxycarbonyl)*-O-*(2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl)-(1→6)*-O-*(2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranosyl)-(1→*O)-L-*serine* p-*nitrophenyl ester* (**12**). Methyl triflate (50 µl) was added at room temperature to a stirred mixture of **11** (0.14 g) and **7** (50 mg) in dichloromethane (4 ml) containing crushed molecular sieves (4 Å). The mixture was left overnight and then put on top of a column of silica gel and eluted (toluene–ethyl acetate 15:1) to give **12** (0.14 g, 83 %), $[\alpha]_D$ −24° (*c* 1.7, chloroform). ^{13}C NMR data ($CDCl_3$, 68 MHz): δ 47.2 (C-9-Fmoc), 54.5 (C-α), 62.9 (C-6′), 67.2, 68.7, 69.1, 69.7, 71.9, 72.0, 72.4, 72.5, 72.7, 74.0, 79.1 (C-2 to C-6, C-2′ to C-5′, C-β, CH_2-Fmoc), 101.0, 101.6 (C-1, C-1′), 120.1, 122.6, 125.1–133.6, 141.4, 143.6, 143.8, 145.6 (aromatic C), 155.1, 155.9 (OCON, C-1-*p*-nitrophenyl), 165.2, 165.4, 165.6, 165.8, 166.1, 167.6 (carbonyl C). Anal. $C_{85}H_{68}N_2O_{24}$: C, H, N.

N-*(Fluoren-9-ylmethoxycarbonyl)*-O-*(2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl)-(1→3)*-O-*[(2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl)-(1→6)]*-*(2*-O-*benzoyl-4*-O-benzyl-β-D-*glucopyranosyl)-(1→*O)-L-*serine* p-*nitrophenyl ester* (**14**). Methyl triflate (50 μl) was added at room temperature to a stirred mixture of methyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)]-2-*O*-benzoyl-4-*O*-benzyl-1-thio-β-D-glucopyranoside[2] (**13**) (0.10 g) and **7** (50 mg) in dichloromethane (4 ml) containing crushed molecular sieves (4 Å). The mixture was left overnight and then put on top of a column of silica gel and eluted (toluene–ethyl acetate 13:1) to give **14** (0.11 g, 88 %), $[\alpha]_D$ −17° (*c* 0.6, chloroform). ^{13}C NMR data ($CDCl_3$, 68 MHz): δ 47.0 (C-9-Fmoc), 54.3 (C-α), 63.0, 63.2 (C-6′, C-6″), 67.2, 68.2, 68.6, 69.5, 70.0, 71.9, 72.0, 72.6, 72.9, 73.5, 74.8, 75.7, 80.7 (C-2 to C-6, C-2′ to C-5′, C-2″ to C-5″, C-β, CH_2-Fmoc, benzyl CH_2, overlap), 100.3, 101.0, 101.5 (C-1, C-1′, C-1″), 120.0, 122.6, 125.1–133.6, 138.0, 141.3, 143.8, 145.5 (aromatic C), 155.1, 155.9 (OCON, C-1-*p*-nitrophenyl), 164.5, 165.2, 165.6, 165.8, 166.0, 167.7 (carbonyl C). Anal. $C_{112}H_{92}N_2O_{31}$: C, H, N.

N-*(Fluoren-9-ylmethoxycarbonyl)-(2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl)-(1→*O)-L-*serine* p-*nitrophenyl ester* (**15**). A solution of silver triflate (0.28 g) in toluene (4 ml) was added to a cooled (0 °C) and stirred mixture of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[15] (0.70 g) and **7** in dichloromethane (10 ml) containing crushed molecular sieves (4 Å). The mixture was allowed to attain room temperature and after an additional hour put on top of a column of silica gel and eluted (toluene–ethyl acetate 16:1) to give **15** (0.71 g, 89 %) which crystallized from ethyl acetate–*n*-hexane, m.p. 135–139 °C, $[\alpha]_D$ +7° (*c* 0.8, chloroform). ^{13}C NMR data ($CDCl_3$, 68 MHz): δ 47.1 (C-9-Fmoc), 54.5 (C-α), 62.7 (C-6), 67.1, 69.3, 69.4, 71.7, 72.4, 72.5 (C-2 to C-5, C-β, CH_2-Fmoc), 101.3 (C-1), 120.1, 122.3, 125.0–133.6, 141.3, 143.6 (aromatic C), 154.9, 155.8 (OCON, C-1-*p*-nitrophenyl), 165.1, 165.2, 165.7, 166.1, 167.5 (carbonyl C). Anal. $C_{58}H_{46}N_2O_{16}$: C, H, N.

Acknowledgements. We are indebted to the Swedish National Science Research Council and to the Swedish National Board for Technical Development for financial support.

References

1. Ossowski, P., Pilotti, Å., Garegg, P. J. and Lindberg, B. *J. Biol. Chem. 259* (1984) 1137.
2. Fügedi, P., Birberg, W., Garegg, P. J. and Pilotti, Å. *Carbohydr. Res. 164* (1987) 297.
3. Fügedi, P., Garegg, P. J., Kvarnström, I. and Svansson, L. *J. Carbohydr. Chem. 7* (1988) 389.
4. Birberg, W., Fügedi, P., Garegg, P. J. and Pilotti, Å. *J. Carbohydr. Chem. 8* (1989) 389.
5. Albersheim, P. and Valent, B. *J. Cell. Biol. 78* (1978) 627.
6. Sharp, J. K., McNeil, M. and Albertsheim, P. *J. Biol. Chem. 259* (1984) 11321.
7. Sharp, J. K., Albertsheim, P., Ossowski, P., Pilotti, Å., Garegg, P. J. and Lindberg, B. *J. Biol. Chem. 259* (1984) 11341.
8. Bodanszky, A., Bodanszky, M., Chandramouli, N., Kwei, J. Z., Martinez, J. and Tolle, J. C. *J. Org. Chem. 45* (1980) 72.
9. Hanessian, S. and Banoub, J. *Carbohydr. Res. 53* (1977) C13.
10. Garegg, P. J., Konradsson, P., Kvarnström, I., Norberg, T., Svensson, S. C. T. and Wigilius, B. *Acta Chem. Scand., Ser. B 39* (1985) 569.
11. Lönn, H. *Carbohydr. Res. 139* (1985) 105, 115.
12. Ferrari, B. and Pavia, A. A. *Tetrahedron 41* (1985) 1939.
13. Schmidt, R. R. *Angew. Chem., Int. Ed. Engl. 25* (1986) 212.
14. Norberg, T. and Ritzén, H. *Glycoconjugate J. 3* (1986) 135.
15. Ness, R. K., Fletscher, H. G., Jr. and Hudson, C. *J. Am. Chem. Soc. 72* (1950) 2200.
16. Dhawan, N., Chick, T. L. and Goux, W. J. *Carbohydr. Res. 172* (1988) 297.

Received November 24, 1989.