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Synthesis of oligosaccharides related to the repeating unit of the capsular polysaccharide from *Streptococcus pneumoniae* type 37

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Abstract—A tetra- and a pentasaccharide were synthesized as analogues to the structure of the *Streptococcus pneumoniae* type 37 capsular polysaccharide, a homopolymer with a disaccharide-repeating unit of →3)[β-D-Glc*p*-(1→2)]-β-D-Glc*p*-(1→. Synthesis of the tetrasaccharide employed a β-(1→2)-diglycosylation of a β-(1→3)-linked disaccharide. Subsequently, the pentasaccharide was synthesized from a suitably protected tetrasaccharide derivative by a β-(1→3)-extension at O-3′. Steric crowding was found to be an important factor in the formation of the pentasaccharide.

Keywords: Bacterial polysaccharide; Glycosylation; Branched structure; Steric

1. Introduction

Carbohydrate polymers constitute part of the cell wall of bacteria as well as the coating at the bacterial cell surface as lipopolysaccharides (LPS) and/or capsular polysaccharides (CPS).[1] These polysaccharides are important for the bacteria since they can facilitate protection against phagocytosis and increase survival in a foreign host, for example, man. Parts of a polysaccharide form epitopes, that is, three-dimensional structures recognized by other molecules, in particular antibodies and lectins. These carbohydrate polymers may be similar to endogenous carbohydrate structures on cells, thereby evading the protective immune system of the host. The CPS is commonly also a most important virulence factor, meaning that without it the pathogen's capability to infect is severely reduced. In addition, the polysaccharides confer the bacterium with suitable rheological properties for its survival in different environments. It must be noted that, although a great many bacteria are pathogenic, most are not a threat to man and some are even beneficial. Thus, there are several reasons for obtaining more information on these complex biopolymers, their structures and their properties.

Streptococcus pneumoniae, however, is a major cause of acute respiratory infections worldwide.[2] Recent developments using synthetic oligosaccharide–protein conjugates are promising, since protective antibodies against specific pneumococci serotypes can be induced upon immunization.[3,4] Thus, the approach may lead to effective vaccines against pneumococci.

2. Results and discussion

The CPS from the bacterium *S. pneumoniae* type 37 (S37) is the only homopolymer reported for the pneumococcus species and it consists of repeating units with two glucosyl residues having the structure: →3)[β-D-Glc*p*-(1→2)]-β-D-Glc*p*-(1→.[5] It is anticipated that this branched type of polymer will form a crowded three-dimensional structure. Interestingly, it has been shown that only one glycosyl transferase is needed in the biosynthesis of this CPS, even though two residues in a different setting are to be synthesized.[6] The smallest oligosaccharide possibly being a relevant model for the polymer would be a tetrasaccharide. Whether this is

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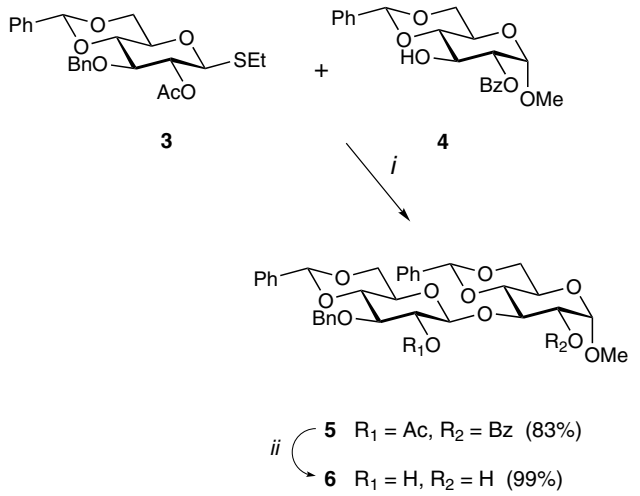
sufficient or if higher analogues such as hexa- or octasaccharides are necessary to describe the three-dimensional structure is of fundamental interest and it should be possible by ^{1}H NMR analysis to ascertain this issue, prior to analysis of the conformational dynamics of an appropriate oligosaccharide. Since also short oligomers of polysaccharides can generate a sufficient antibody response,[7] an efficient synthesis of the *S. pneumoniae* type 37 CPS analogues could be used to prepare oligosaccharides for conjugation to proteins, and thus to be used as vaccines. We have previously synthesized trisaccharides with similar structures,[8,9] and therefore set out to synthesize oligosaccharides related to the repeating unit of the CPS of S37.

Two structures related to the CPS were synthesized (Fig. 1). At the reducing end of the oligosaccharides to be synthesized we chose an α-D-Glc-OMe residue since it provides a handle when analyzed by NMR spectroscopy. Our synthetic route to tetrasaccharide **2** first involved formation of the β-(1→3)-linked disaccharide corresponding to the backbone of the polymer. This would allow us to introduce the two β-(1→2)-linked residues in one step through a diglycosylation reaction with the disaccharide acceptor. Moreover, it also seemed a plausible strategy for the synthesis of larger analogues as well. As glycosyl donor for the first glycosylation we selected ethyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside **3**.[10] Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside **4**[11] was used as the common reducing end acceptor for all derivatives synthesized.

NIS/TfOH-promoted[12] glycosylation of donor **3** in CH_2Cl_2 at room temperature with acceptor **4** gave disaccharide **5** in 83% yield (Scheme 1). The β-(1→3) glycosidic linkage can also be formed with the same donor and promotor system but with methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[13] as acceptor in a regioselective manner in 73% yield. However, solubility problems hampered the purification and rendered this shortcut less practical when scaled up.

Treatment of disaccharide **5** with methanolic sodium methoxide removed the ester protecting groups yielding



Scheme 1. Formation of disaccharide acceptor **6**. Reagents and conditions: (i) NIS/TfOH, CH_2Cl_2, 25 °C, 0.5 h; (ii) NaOMe, MeOH/CH_2Cl_2 2/1, 25 °C, 2 h.

the disaccharide diol acceptor **6** in 99% yield. Diglycosylation of **6** with thioglycosyl donors and NIS/TfOH or DMTST[14] activation resulted in poor yields due to incomplete glycosylation forming a mixture of tri- and tetrasaccharides. However, tetrasaccharide **8** could be produced in 84% yield by a AgOTf-promoted[15] glycosylation with 4.5 equiv of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucosyl bromide **7** in the presence of *sym*-collidine at −55 °C. The benzoyl groups were removed by treatment of **8** with sodium methoxide in methanol. Catalytic hydrogenolysis (5% Pd–C) in a mixture of methanol, water and acetic acid removed the remaining protecting groups yielding the deprotected tetrasaccharide **2** in 79% yield (Scheme 2).

The ^{1}H NMR spectrum of **2** showed extensive overlap in the anomeric region for the two β-(1→2)-linked glucosyl residues. However, if the temperature was raised to 70 °C the anomeric signals could be resolved. In spite of this, these conditions are not particularly suitable for future investigations. In addition, the tetrasaccharide ^{1}H and ^{13}C NMR chemical shifts also deviates considerably

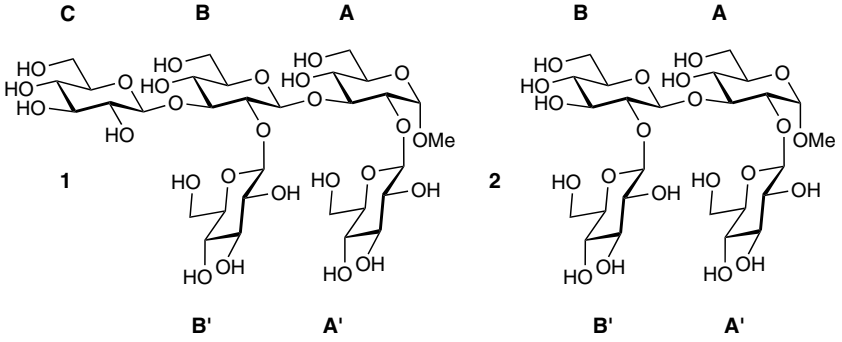
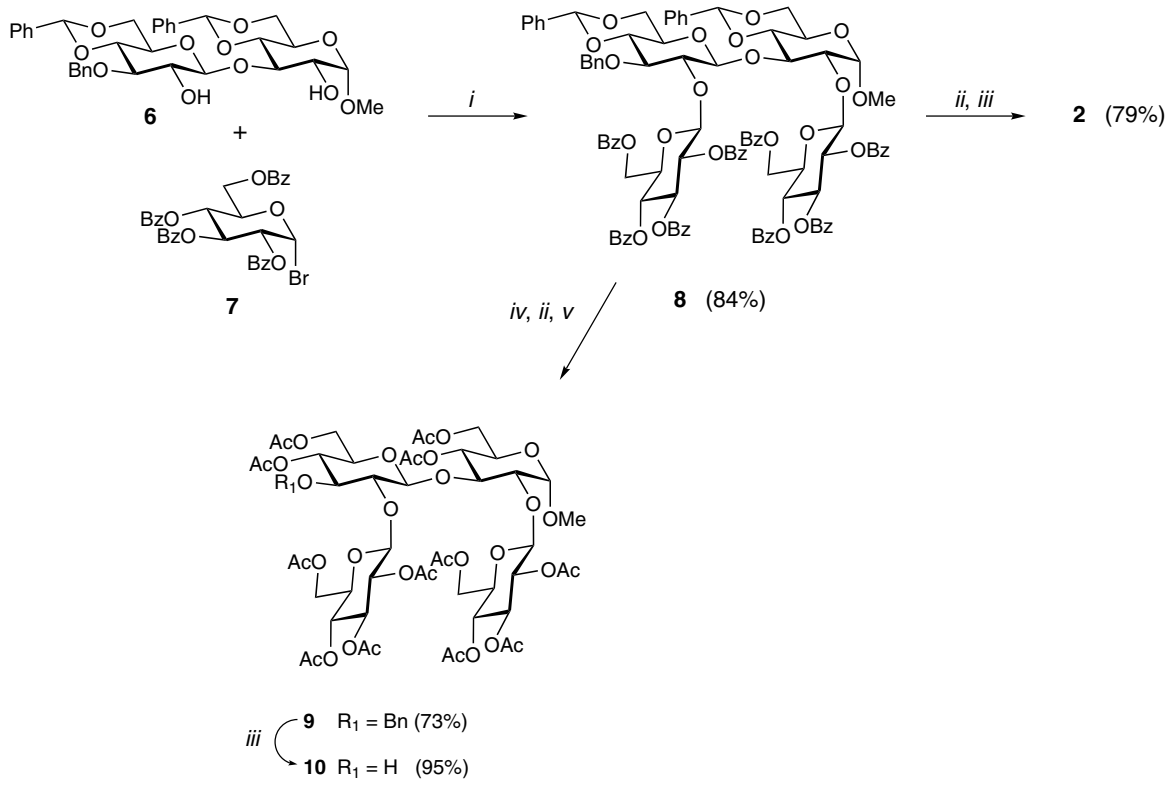


Figure 1. Pentasaccharide **1** and tetrasaccharide **2**.



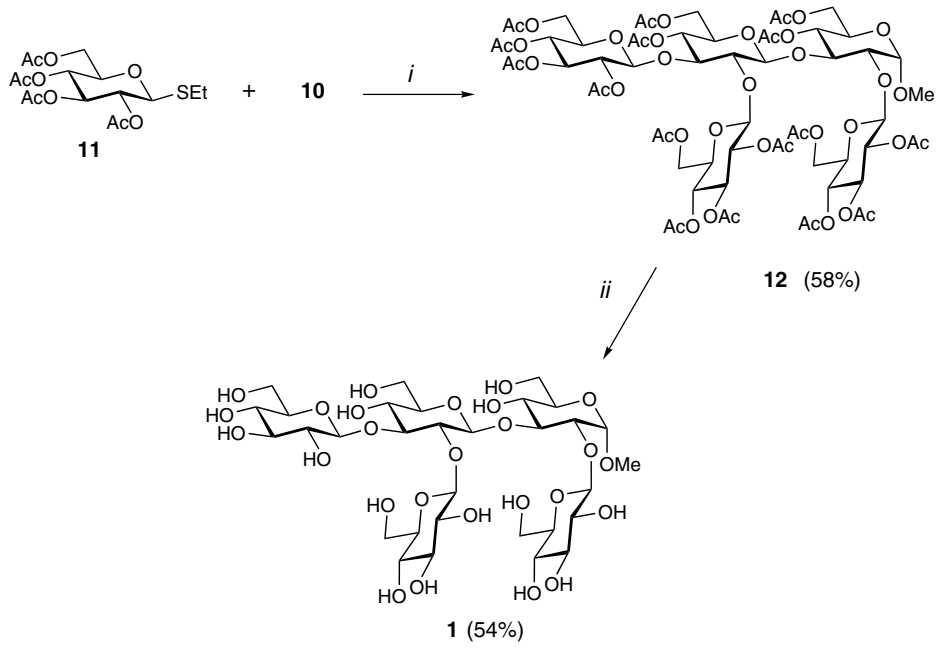
Scheme 2. Formation, modification and deprotection of the tetrasaccharide. Reagents and conditions: (i) AgOTf, CH_2Cl_2, −55 °C, 5 h; (ii) NaOMe, MeOH; (iii) H_2/Pd–C; (iv) 90% aq TFA; (v) Ac_2O, pyridine, DMAP, CH_2Cl_2.

from those observed in the native polymer, indicating that a larger analogue is needed if a polymer-like tertiary structure is to be present.

Given the fact that the diglycosylation was successful, a triglycosylation appeared to be an efficient route to a hexasaccharide representing a trimer of the repeating unit in the polymer. Hence, a trisaccharide triol acceptor was successfully constructed by elongation at O-3′, based on an acceptor produced via the 3′-*O*-*p*-methoxybenzyl analogue of **5** followed by the removal of the ester functionalities. The glycosylation of this substance with **7** was sluggish, producing mainly pentasaccharides. Only a trace amount of the hexasaccharide was formed according to MALDI-TOF MS. In addition, an *O*-benzylated trisaccharide triol acceptor lacking all the acid labile protecting groups was also produced, albeit without improving the outcome of the glycosylation. The results of the glycosylations employing trisaccharide acceptors and applying a strategy similar to that used to form the tetrasaccharide were not encouraging. An alternative route with a different acceptor was therefore needed.

With this in mind, it seemed reasonable to believe that if we produced a tetrasaccharide carrying an orthogonal protecting group at the O-3′-position further elongation should be possible. Neither 3′-*O*-allyl nor *p*-methoxybenzyl protected analogues of **6** were to any greater extent compatible with the conditions necessary to form the corresponding tetrasaccharide. Instead, we decided to exploit the protecting group pattern already present in tetrasaccharide **8** (Scheme 2), that is, to use the orthogonality of the benzyl ether.

The 4,6-*O*-benzylidene acetal and benzoyl groups of **8** were removed using 90% aq TFA and sodium methoxide in methanol, respectively. Subsequent *O*-acetylation was performed by Ac_2O in pyridine to give **9** in 73% over three steps. Initially, considering the superior stability of benzoyl esters as compared to acetyl groups we synthesized the *O*-benzoylated analogue of **9**. However, removal of the benzyl group turned out to be problematic. Treatment with H_2/Pd–C at 110 psi produced only small amounts of the *O*-benzoylated acceptor. Reactions involving heterogeneous catalysts are sensitive to steric hindrance. A method that should be more compatible with steric hindrance for removal of the *O*-benzyl group by reaction with sodium bromate/sodium dithionite in ethyl acetate/water,[16] only partially solved the problem since the yields were moderate. Even worse, the formed acceptor did not work well in glycosylations, presumably as a consequence of steric hindrance. To reduce the amount of possible steric interactions we substituted the benzoyl groups at the 4- and 6-positions on the backbone glucosyl residues with *O*-acetyl groups, yet the results were analogous to the *O*-benzoylated



Scheme 3. Formation and deprotection of the pentasaccharide. Reagents and conditions: (i) NIS/TfOH, MS 4 Å, CH_2Cl_2, 25 °C; (ii) NaOMe, MeOH.

derivative. However, if also the benzoyl groups at the terminal glucosyl residues were exchanged for *O*-acetyl groups, removal of the *O*-benzyl group from **9** by catalytic hydrogenation succeed in giving tetrasaccharide acceptor **10** (Scheme 2). More importantly, NIS/TfOH-promoted glycosylation of **10** at room temperature with the per-*O*-acetylated thioglucosyl donor **11**[17] produced the per-*O*-acetylated pentasaccharide **12** in 58% yield (Scheme 3). Final deprotection by sodium methoxide in methanol followed by purification via gel permeation chromatography yielded 54% of pentasaccharide **1**. One may note that in this pentasaccharide the anomeric ^1H chemical shift of residue **B** is closely similar to the corresponding one in the backbone of the CPS from S37 and that δ_{H1} of **B′** has changed compared to tetrasaccharide **2** towards that observed for the side-chain residue in the polymer. Recently, the EPS of *P. freudenreichii* ssp. *shermanii* JS was shown to produce a glucan with an identical disaccharide-repeating unit to that of the CPS from S37.[18] The reported ^1H and ^{13}C NMR chemical shifts of the EPS will be useful in comparison to the synthesized oligosaccharides.

We have shown that reducing the size of the protecting groups is essential for this system, also for the glucosyl units not directly associated with the site of glycosylation. With the successful introduction of the fifth glucosyl residue, this approach might allow the synthesis of a hexasaccharide corresponding to a trimer of the repeating unit of the CPS from S37. However, as the oligosaccharide structure approaches that of the native polymer, an increasing steric demand is to be expected.

3. Experimental

3.1. General procedures

TLC was performed on Precoated Merck 60 F_{254} plates and visualized with UV-light or 8% H_2SO_4. For column chromatography Amicron silica gel 0.040–0.063 mm was used. NMR spectroscopy was performed on Varian 300, 400 and 600 MHz spectrometers at 25 °C. Chemical shifts are referenced to internal TMS (δ_H 0.00) and to the solvent in $CDCl_3$ (δ_C 77.23), to internal Sodium 3-trimethylsilyl-propanoate-2,2,3,3-d_4, (TSP) (δ_H 0.00) and external dioxane in D_2O (δ_C 67.4) for D_2O solutions.

For assignments of ^1H and ^{13}C resonances, the following experiments were used: ^{13}C APT, $^1H,^1H$ COSY, $^1H,^1H$ TOCSY (mixing time 30 ms), $^1H,^{13}C$ HSQC, $^1H,^{13}C$ HMBC (mixing time 50 ms). Backbone residues are referred to as **A**, **B** and **C**, where **A** corresponds to the reducing end and side chains are primed and denoted by **A′** and **B′** in relation to their backbone residues.

MALDI-TOF MS was performed on a Bruker Biflex III spectrometer in reflex mode with 2′,4′,6′-trihydroxyacetophenone (THAP) as a matrix. A typical sample preparation from organic solvents employed 0.5 μL of a very dilute solution of the sample applied onto a crystal layer of THAP, whereas from water a 0.5 μL sample was first premixed with 0.5 μL of a 1/1 solution of H_2O and a saturated solution of THAP in acetone, followed by application to a crystal layer of THAP.

3.2. Experimental

3.2.1. Methyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (5). A solution of methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (1.32 g, 3.41 mmol) and ethyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (1.67 g, 3.75 mmol) dissolved in 12 mL of CH_2Cl_2 under Argon atmosphere was pre-dried for 25 min with crushed 4 Å molecular sieves. NIS (0.915 g, 4.09 mmol) was added to the reaction mixture in one portion. The reaction was initiated by the addition of a catalytic amount of TfOH. The reaction was followed by TLC (T/E: 3/1), which after 40 min indicated that the starting materials had been consumed. The reaction mixture was filtered directly through a short plug of silica into a separating funnel containing 100 mL of aq $Na_2S_2O_3$ and $NaHCO_3$. The organic phase was washed and separated, dried with Na_2SO_4 and evaporated. The crude product was purified by silica column chromatography (T/E: 6/1) to give **3** (2.18 g, 2.84 mmol) as a white solid in 83% yield. MALDI-MS: $[M+Na]^+$ *m/z* 791.7, expected 791.3. ^{13}C NMR: 20.4 (Me), 55.6 (OMe), 62.9, 66.4, 68.9, 69.1, 72.8, 73.9, 74.3, 75.9, 78.8, 79.3, 81.3 (10 × C-2–C-6, O*C*H_2Ph), 97.8, 101.3, 101.4, 101.6 (4 × acetal C), 126.2–133.7 (aromatic C), 166.0, 169.4 (2 × C═O).

3.2.2. Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-α-D-glucopyranoside (6). Disaccharide **5** (1.80 g, 2.34 mmol) was dissolved in 200 mL of CH_2Cl_2/MeOH 2/1 and 1 M NaOMe in MeOH was added until pH was >12. After 2 h, MALDI-MS showed that the reaction was complete. The reaction mixture was neutralized with Dowex-(H^+), filtered and evaporated to dryness. The crude product was recrystallized from CH_2Cl_2/toluene giving **6** (1.44 g, 2.31 mmol) in 99%. MALDI-MS: $[M+Na]^+$ *m/z* 645.6, expected 645.2. ^{13}C NMR: 55.6 (OMe), 63.1–82.1 (10 × C-2–C-6, 1 × O*C*H_2Ph), 99.9, 101.3, 101.5, 105.4 (4 × acetal C), 126.2–138.4 (aromatic C).

3.2.3. Methyl [2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→2)]-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→2)]-4,6-*O*-benzylidene-α-D-glucopyranoside (8). Acceptor **6** (100 mg, 0.160 mmol) and 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl bromide **7** (317 mg, 0.480 mmol) were dissolved in 8 mL of CH_2Cl_2 and pre-dried with crushed 4 Å molecular sieves under argon at −55 °C for 30 min. *sym*-Collidine (57 mg, 0.464 mmol) and AgOTf (164 mg, 0.640 mmol) were then added. After 2 h, an additional amount of donor (159 mg, 0.242 mmol) and AgOTf (90 mg, 0.350 mmol) was added and the temperature was allowed to rise to 0 °C. After 3 h, the reaction was quenched by the addition of 3 mL Et_3N. The solution was then filtered through a pad of Celite and evaporated. The crude product was purified by silica column chromatography (gradient, T→T/E: 2/1) to give an 84% yield of tetrasaccharide **8** (240 mg, 0.135 mmol). MALDI-MS: $[M+Na]^+$ *m/z* 1802.4, expected 1802.6. ^{13}C NMR: 55.7 (OMe), 61.8–81.4 (C-2–C-6, O*C*H_2Ph), 99.4, 100.6, 100.7, 100.8, 101.3, 101.4 (6 × acetal C), 126.1–138.6 (aromatic C), 165.2–166.2 (C═O).

3.2.4. Methyl [β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-D-glucopyranoside (2). To a solution of **8** (53 mg, 0.030 mmol) in 2 mL MeOH/toluene: 3/1, 1 M NaOMe was added until the pH > 12. After 3 h MALDI-MS showed only the *O*-deacylated tetrasaccharide ($[M+Na]^+$ *m/z* 969.4, expected 969.3). The reaction mixture was neutralized with Dowex-(H^+), filtered and subsequently evaporated to dryness. The crude product was then directly dissolved in 4 mL of MeOH/HOAc/H_2O: 6/1/1. A catalytic amount of 5% Pd–C was added and the reaction vessel transferred to a Parr apparatus and subjected to a 111 psi of H_2 for 20 h. MALDI-MS confirmed complete disappearance of the starting material. The reaction mixture was filtered through a pad of Celite, concentrated and passed through a column of Bio-Gel P-2. Relevant fractions were freeze dried to give **2** in 79% yield (16 mg, 0.024 mmol). MALDI-MS: $[M+Na]^+$ *m/z* 703.4 expected 703.2. Selected $^1H/^{13}C$ NMR data, D_2O, 70 °C, $J_{H\text{-}1,\ H\text{-}2}$ in brackets: **B**: 5.08 [7.9] (H-1)/100.4 (C-1), 3.62 (H-2), 3.75 (H-3); **A**: 5.01 [3.5] (H-1)/100.0 (C-1), 3.99 (H-2), 4.10 (H-3); **A′**: 4.836 [7.6] (H-1)/103.6 (C-1), 3.38 (H-2), 3.54 (H-3); **B′**: 4.832 [7.9] (H-1)/104.3 (C-1), 3.42 (H-2), 3.58 (H-3). Additional ^{13}C data: 82.4 (C-2**B**), 80.0 (C-2**A**), 79.1 (C-3**A**), 77.0–68.7 (13 × C-2–C-5), 62.0–61.6 (4 × C-6), 55.7 (OMe).

3.2.5. Methyl [2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-α-D-glucopyranoside (9). To a flask containing tetrasaccharide **8** (118 mg, 0.066 mmol), 3 mL of TFA (90%) was added. The flask was immediately connected to a rotoevaporator and the solution evaporated. TLC and MALDI-MS confirmed the complete removal of the two benzylidene groups. The residue was dissolved in CH_2Cl_2 and washed with aq $NaHCO_3$ and with water. The organic phase was dried, filtered and subsequently evaporated. The residue was dissolved in 2 mL of dry MeOH and the pH was adjusted to >12 with 1 M NaOMe in MeOH. When TLC and MALDI-MS indicated the complete removal of the benzoyl groups the solution was neutralized with Dowex-(H^+), filtered and evaporated. To the dry residue, 6 mL of pyridine/CH_2Cl_2 3/1 was added. The solution was cooled to 0 °C with an ice bath and an excess of

Ac_2O (1 mL) was then added to the stirred solution. The reaction was monitored by MALDI-MS and TLC. After 20 h the reaction was complete and the reaction mixture was poured onto a slurry of ice and water. When the ice melted, the phases were separated and the organic phase was subsequently washed with 1 M HCl, aq $NaHCO_3$ and water and finally dried, filtered and evaporated. The crude product was purified by silica column chromatography (gradient, T→T/E: 1/1) to give **9** in 73% yield (61 mg, 0.048 mmol). MALDI-MS; $[M+Na]^+$ *m/z* 1298.8, expected 1297.4. ^{13}C NMR: 20.7–22.0 (12 × Me), 55.3 (OMe), 61.8–73.6 (17 × C-2–C-6), 76.1 (OCH_2Ph), 81.4, 81.9, 82.4 (3 × ring C), 99.1, 99.9, 101.2, 101.5 (4 × C-1), 127.5–138.3 (aromatic C), 169.6–171.3 (12 × C=O).

3.2.6. Methyl [2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-α-D-glucopyranoside (10). To a solution of **9** (60 mg, 0.047 mmol) in 3 mL of THF, a catalytic amount of 5% Pd/C was added. The reaction vessel was then put in a Parr apparatus and subjected to 100 psi of H_2 pressure for 30 h when MALDI-MS confirmed the complete formation of the *O*-debenzylated substance. The reaction mixture was filtered through a pad of Celite, concentrated and purified on silica column chromatography (gradient, T→T/E: 1/1) to give **10** (53 mg, 0.045 mmol) in 95% yield. MALDI-MS: $[M+Na]^+$ *m/z* 1207.0, expected 1207.4. ^{13}C NMR: 20.7–21.8 (12 × Me), 55.2 (OMe), 61.7–73.6 (18 × C-2–C-6), 82.0, 84.4 (2 × ring C), 98.9, 99.4, 101.2, 102.4 (4 × C-1), 169.6–171.3 (12 × C=O).

3.2.7. Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)]-4,6-di-*O*-acetyl-α-D-glucopyranoside (12). A solution of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **11** (26 mg, 0.066 mmol) and acceptor **10** (31 mg, 0.026 mmol) in 1.5 mL dry CH_2Cl_2 was pre-dried with 4 Å molecular sieves under argon for 30 min. NIS (18 mg, 0.080 mmol) and TfOH (0.2 μL) was added to initiate the reaction. After 40 min, TLC and MALDI-MS indicated that product formation had ceased. The reaction was then quenched by the addition of one drop of Et_3N, filtered through a pad of silica, which was thoroughly washed with EtOAc. The solution was transferred to a separating funnel and washed with aq $Na_2S_2O_3$ and water and subsequently dried and evaporated. The crude product was purified by column chromatography (T/E: 3/1→1/3) to give pentasaccharide **12** (23 mg, 0.015 mmol) in 58% yield. MALDI-MS: $[M+Na]^+$ *m/z* 1538.6, expected 1537.5. ^{13}C NMR: 20.7–21.6 (16 × Me), 55.6 (OMe), 62.0–62.8 (5 × C-6), 66.9–74.6 (17 × C-2–C-5), 78.8, 79.1, 80.5 (3 × ring C), 99.3, 99.4, 99.6, 99.7, 100.8 (5 × C-1), 169.1–170.9 (16 × C=O).

3.2.8. Methyl β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-D-glucopyranoside (1). Pentasaccharide **12**, (22 mg, 0.015 mmol) was dissolved in 2 mL of MeOH. NaOMe (1 M) was added until pH > 12. After 2 h MALDI-MS showed that the reaction was complete. The solution was neutralized with Dowex-(H^+), filtered and subsequently evaporated to dryness. The product was dissolved in H_2O and passed through a column of Bio-Gel P-2, freeze dried and purified on a column of Bio-Gel P-2 (2.6 × 68 cm) irrigated with water containing 1% *n*-butanol to give the target compound **1** (6.8 mg, 0.0081 mmol) after lyophilization in 54% yield. MALDI-MS: $[M+Na]^+$ *m/z* 865.5, expected 865.3. Selected $^1H/^{13}C$ NMR data, D_2O, 70 °C, $J_{H\text{-}1,\ H\text{-}2}$ in brackets: **B**: 5.14 [7.8] (H-1)/100.2 (C-1), 3.78 (H-2), 3.91 (H-3); **A**: 4.98 [3.7] (H-1)/99.9 (C-1), 4.00 (H-2), 4.08 (H-3); **B′**: 4.88 [8.0] (H-1)/103.6 (C-1), 3.33 (H-2), 3.51 (H-3); **C**: 4.78 [7.7] (H-1)/103.0 (C-1), 3.38 (H-2), 3.53 (H-3); **A′**: 4.77 [7.7] (H-1)/103.7 (C-1), 3.34 (H-2), 3.51 (H-3). Additional ^{13}C data: 85.6 (C-3**B**), 80.6 (C-2**B**), 80.1 (C-2**A**), 78.5 (C-3**A**), 77.1–68.8 (16 × C-2–C-5), 62.0–61.6 (5 × C-6), 55.6 (OMe).

Acknowledgements

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