

Facile synthesis of a tetrasaccharide corresponding to the capsular polysaccharide of *Streptococcus pneumoniae* type 15B

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Abstract

A convergent synthetic approach is presented for the straightforward synthesis of the tetrasaccharide fragment of the capsular polysaccharide of *Streptococcus pneumoniae* type 15B. Coupling of a disaccharide thioglycoside donor with a disaccharide acceptor furnished the required tetrasaccharide in excellent yield.

Keywords: Oligosaccharides, glycosylation, antigen, vaccine, *Streptococcus pneumoniae*

Introduction

Infections due to *Streptococcus pneumoniae* remain one of the major concerns in the developed and developing countries.¹ Pneumonia and meningitis are still a major cause of death worldwide. Because of the increasing antibiotic resistance to the bacterial infections, there has been renewed interest in vaccination as an effective way of controlling the infections. In order to induce a protective immune response in the host preparation of glycoconjugates, vaccines has gained considerable interest.² A capsular polysaccharide (CPS) based vaccine against streptococcal infection has been used for a long time.³ Although, these capsular polysaccharides (CPSs) have been isolated from the natural sources, earlier studies showed that synthetic neoglycoconjugates derived from smaller oligosaccharide fragments of the capsular polysaccharide can generate sufficient protective immune response after conjugation with proteins.⁴ Several syntheses of oligosaccharides corresponding to the CPSs of *S. pneumoniae* have appeared in the literature.⁵ One of the components of the commercially available 23-valent pneumococcal polysaccharide vaccine is the CPS of *S. pneumoniae* type 15B. This particular serotype is the causative agent for the invasive childhood infections in Bangladesh.⁶ A revised structure of the pentasaccharide repeating unit of the CPS has been reported by Jones *et. al.*⁷ In order to estimate the antigenicity of the capsular polysaccharide fragments or its smaller fragments, it is essential to develop concise synthetic strategies for their preparation. In this communication we describe herein a straightforward synthesis of a tetrasaccharide fragment corresponding to the CPS of *S. pneumoniae* type 15B as its 4-methoxyphenyl glycoside. The 4-methoxyphenyl group acts as a

temporary protecting group of the anomeric position at the reducing end, which can be removed for coupling the tetrasaccharide with a protein through a spacer linker to generate a glycoconjugate.

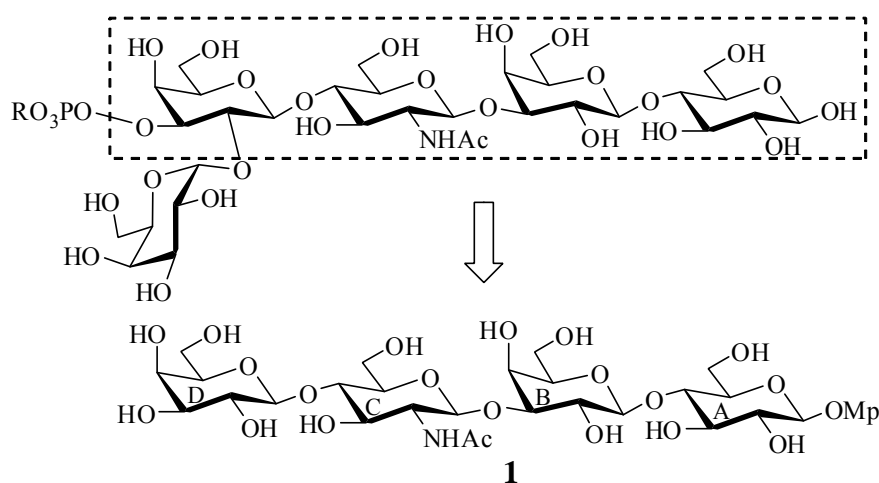
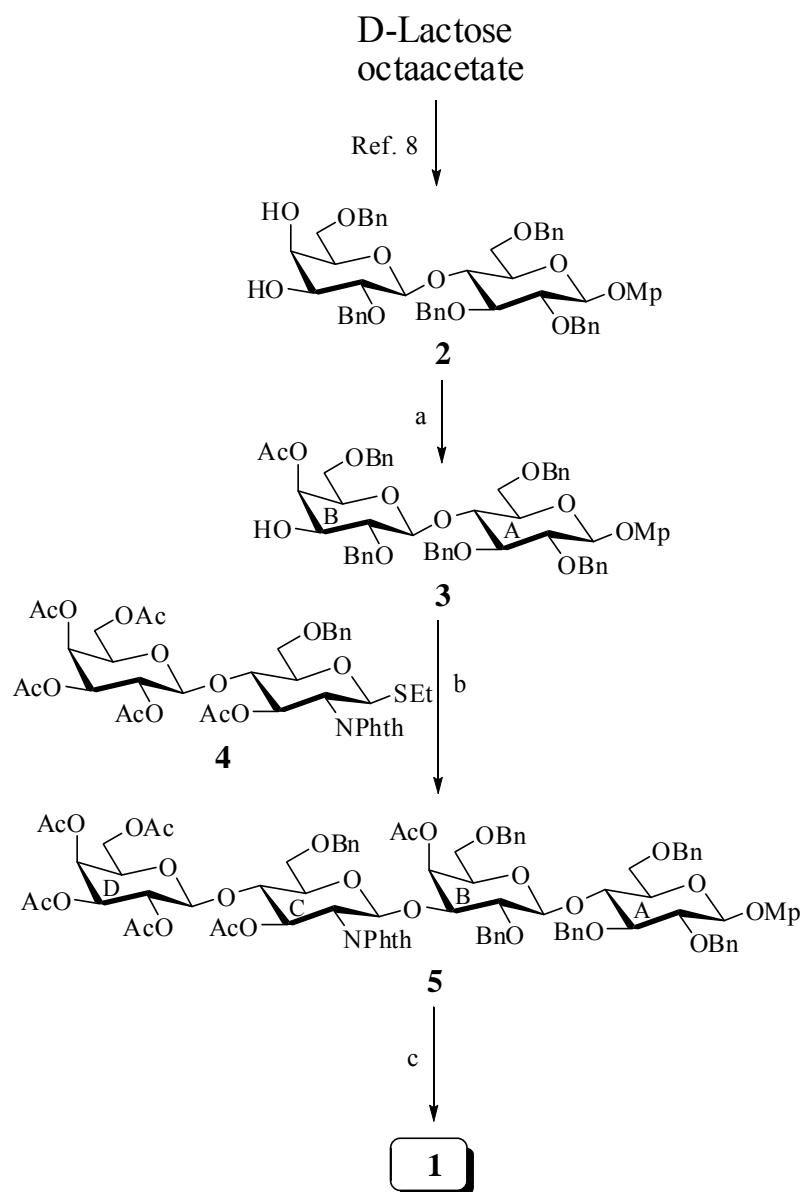


Figure 1. Structure of the pentasaccharide repeating unit of *Streptococcus pneumoniae* type 15B and synthesized tetrasaccharide fragment as its 4-methoxyphenyl glycoside (**1**).

Results and Discussion

The synthesis of the tetrasaccharide **1** as its 4-methoxyphenyl glycoside was achieved using a 2+2 convergent synthetic approach by coupling a disaccharide acceptor **3** with a disaccharide donor **4**. D-lactose derived disaccharide acceptor **3** was prepared from disaccharide diol derivative **2**⁸ by regioselective acetylation via orthoesterification⁹ using triethylorthoacetate followed by acidic hydrolysis of the orthoester in 90% yield. The disaccharide thioglycoside donor (**4**) was prepared following our earlier reported methodology.¹⁰ *N*-Iodosuccinimide (NIS)/trimethylsilyl trifluoromethanesulfonate (TMSOTf) mediated glycosylation¹¹ of compound **3** with compound **4** furnished tetrasaccharide derivative **5** in 88% yield. Signals at δ 102.5 (C-1_A), 101.8 (C-1_B), 100.4 (C-1_D), 98.5 (C-1_C) in the ¹³C NMR spectra confirmed its formation. Complete deprotection of the tetrasaccharide derivative **5** involving the conversion of the *N*-phthaloyl group to *N*-acetyl group by hydrazinolysis¹² followed by *N*-acetylation, saponification and hydrogenolysis over Pearlmans catalyst¹³ afforded target tetrasaccharide as its 4-methoxyphenyl glycoside (**1**) in 73% yield. Presence of signals at δ 5.02 (d, $J = 7.8$ Hz, H-1_A), 4.71 (d, $J = 7.9$ Hz, H-1_C), 4.46 (2 d, $J = 8.0$ Hz each, H-1_B, H-1_D) in the ¹H NMR and at δ 101.6 (C-1_D), 101.5 (C-1_B), 101.4 (C-1_C), 99.7 (C-1_A) in the ¹³C NMR spectra confirmed the formation of compound **1**. Additional support for the assignments of proton and carbon resonances of the protected tetrasaccharide **5** and the deblocked tetrasaccharide (**1**) was obtained by analysis of their 1D and 2D NMR spectra.



Scheme 1. Reagents: (a) (i) Triethylorthoacetate, *p*-TsOH, DMF, rt, 1 h; (ii) 80% aq. AcOH, rt, 30 min, 90%; (b) *N*-iodosuccinimide, TMSOTf, CH₂Cl₂, MS-4Å, -30 °C, 45 min, 88%; (c) (i) NH₂NH₂·H₂O, C₂H₅OH, 80 °C, 6 h; (ii) acetic anhydride, pyridine, rt, 2 h; (iii) 0.1 M CH₃ONa, CH₃OH, 3 h; (iv) H₂, 20% Pd(OH)₂-C, CH₃OH, rt, 24 h, 73% in four steps.

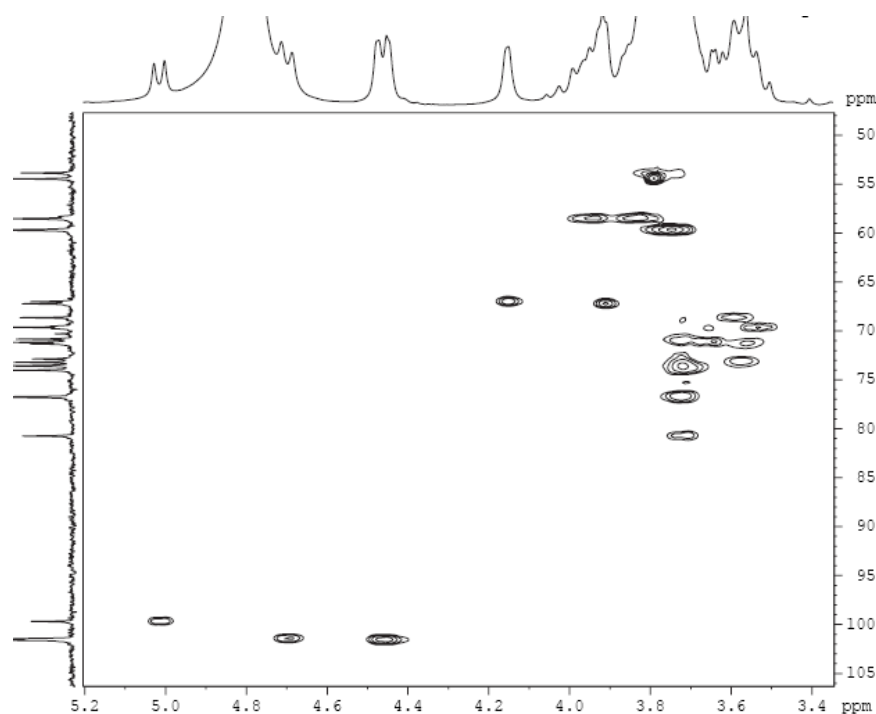


Figure 2. 2D HSQC spectrum of compound **1** (D₂O, 75 MHz) (expanded region).

Experimental Section

General Procedures. All the reactions were monitored by thin layer chromatography over silica gel coated TLC plates. The spots on TLC were visualized by warming ceric sulphate (2% Ce(SO₄)₂ in 2N H₂SO₄) sprayed plates on a hot plate. Silica gel 230-400 mesh was used for column chromatography. FAB mass spectra were recorded on JEOL SX 102/DA-6000 mass using Argon /Xenon (6 KV, 10 MA) as the ionising gas. ¹H and ¹³C NMR was recorded on Bruker Advance DPX 300 MHz using TMS as internal reference. Chemical shift value is expressed in δ ppm. Elementary analysis was carried out on Carlo ERBA-1108 analyzer. Optical rotations were measured at 25 °C on a Rudolf Autopol III polarimeter. Commercially available grades of organic solvents of adequate purity are used in many reactions.

4-Methoxyphenyl (4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3). To a solution of compound **2** (9 g, 10 mmol) in anhydrous DMF (50 mL) were added triethyl orthoacetate (9 mL, 49 mmol) and *p*-TsOH (0.5 g) and the reaction mixture was allowed to stir at room temperature for 3 h. The solvents were removed under reduced pressure and a solution of the crude reaction mixture in 80% AcOH (100 mL) was allowed to stir at room temperature for 1 h. The reaction mixture was evaporated to dryness and the crude product was purified over SiO₂ using hexane-EtOAc (6:1) as eluant to give pure **3** (8 g, 85%) as a syrup; [α]_D²⁵ + 23 (*c* 1.5, CHCl₃); IR (neat): 2922, 2858, 2361, 1738, 1503, 1454, 1369, 1225, 1064, 752, 699 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 7.42-7.21 (m, 25 H, Ar-H), 7.03 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.83 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.33 (d, *J* = 3.0 Hz, 1 H, H-4_B), 5.07-5.01 (m, 2 H, PhCH₂), 4.88-4.85 (m, 2 H, PhCH₂), 4.86 (d, *J* = 8.7 Hz, 1 H, H-1_A), 4.84-

4.73 (m, 2 H, PhCH₂), 4.58 (d, *J* = 12 Hz, 1 H, PhCH₂), 4.53 (d, *J* = 7.8 Hz, 1 H, H-1_B), 4.50-4.43 (m, 2 H, PhCH₂), 4.30 (d, *J* = 12.1 Hz, 1 H, PhCH₂), 4.05 (t, *J* = 8.7 Hz, 1 H, H-3_A), 3.76-3.79 (m, 2 H, H-6_{a,bA}), 3.80 (s, 3 H, OCH₃), 3.70-3.64 (m, 3 H, H-3_B and H-6_{a,bB}), 3.61-3.44 (m, 3 H, H-4_A, H-5_A and H-5_B), 3.41-3.35 (m, 2 H, H-2_A and H-2_B), 2.06 (s, 3 H, COCH₃); ¹³C NMR (75 MHz, CDCl₃): δ 170.7 (COCH₃), 155.3-114.5 (Ar-C), 102.9 (C-1_A), 102.5 (C-1_B), 82.6 (C-2_A), 81.5 (C-5_A), 80.2 (C-2_B), 76.5 (C-5_B), 75.3 (2 C, 2 PhCH₂), 75.1 (2 C, C-4_B and PhCH₂), 73.4, 73.2 (2 PhCH₂), 72.5 (C-3_B), 72.1 (C-4_A), 69.8 (C-3_A), 68.2 (C-6_B), 67.3 (C-6_A), 55.4 (OCH₃), 20.7 (COCH₃); ESI-MS: *m/z* 963.5 [M+Na]⁺; Anal. Calcd. for C₅₆H₆₀O₁₃ (940.40): C, 71.47; H, 6.43; found: C, 71.30; H, 6.65.

4-Methoxyphenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→3)-(4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5). To a solution of compound **3** (1.2 g, 1.27 mmol) and disaccharide thioglycoside donor **4** (1.3 g, 1.6 mmol) in anhydrous CH₂Cl₂ (25 mL) was added powdered MS-4Å (3 g) and the reaction mixture was stirred at room temperature under argon for 1 h. After cooling the reaction mixture to -30 °C, *N*-iodosuccinimide (430 mg, 1.91 mmol) was added to it followed by TMSOTf (10 μL) and allowed to stir at same temperature for 45 min. The reaction mixture was quenched by adding 5% aq. Na₂S₂O₃, diluted with CH₂Cl₂ (100 mL) and filtered through a Celite[®] bed. The organic layer was washed successively with aq. NaHCO₃ and water, dried (Na₂SO₄) and concentrated under reduced pressure. The crude product was purified over SiO₂ using hexane-EtOAc (4:1) to afford pure tetrasaccharide derivative **5** (1.9 g, 88%) as colorless solid; m.p. 98-100 °C; [α]_D + 6.3 (*c* 1.2, CHCl₃); IR (KBr): 3456, 2924, 2379, 2129, 1750, 1654, 1378, 1228, 1061, 730 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 7.51-6.88 (M, 38 H, Ar-H), 5.67 (t, *J* = 9.0 Hz, 1 H, H-3_C), 5.50 (d, *J* = 8.3 Hz, 1 H, H-1_C), 5.43 (d, *J* = 2.8 Hz, 1 H, H-4_B), 5.26 (d, *J* = 2.8 Hz, 1 H, H-4_D), 5.01 (dd, *J* = 7.9 Hz each, 1 H, H-2_D), 4.95-4.80 (m, 4 H, H-3_D, PhCH₂), 4.77-4.64 (m, 2 H, PhCH₂), 4.62 (d, *J* = 7.5 Hz, 1 H, H-1_A), 4.57-4.49 (m, 2 H, H-1_D, PhCH₂), 4.48-4.38 (m, 2 H, PhCH₂), 4.30-4.13 (m, 5 H, H-1_B, H-2_C, PhCH₂), 4.10-3.98 (m, 4 H, H-4_A, H-6_{a,bC}, PhCH₂), 3.90 (t, *J* = 9.0 Hz, H-4_C), 3.83-3.80 (m, 2 H, H-6_{a,bD}), 3.78 (s, 3 H, OCH₃), 3.71-3.62 (m, 2 H, H-6_{a,bB}), 3.59-3.25 (m, 9 H, H-2_A, H-2_B, H-3_A, H-3_B, H-5_A, H-5_B, H-5_D, H-6_{a,bA}), 3.08-3.0 (m, 1 H, H-5_C), 2.10, 2.05, 1.95, 1.84 (4 s, 18 H, 6 COCH₃); ¹³C NMR (CDCl₃, 75 MHz): δ 170.0, 169.9, 169.8, 169.7 (2 C), 168.6, 167.4 (2 C), (6 COCH₃, COPht), 154.0-114.4 (Ar-C), 102.5 (C-1_A), 101.8 (C-1_B), 100.4 (C-1_D), 98.5 (C-1_C), 82.4 (C-2_A), 81.2 (C-5_A), 78.7 (C-2_B), 75.3 (2 C, C-3_A, C-4_C), 75.2 (C-5_C), 75.1 (C-4_A), 74.8 (2 C, C-3_B, C-5_B), 74.6 (2 C, PhCH₂), 73.6 (2 C, PhCH₂), 73.4 (PhCH₂), 73.0 (PhCH₂), 72.5 (C-5_D), 70.9 (C-3_C), 70.8 (C-3_D), 70.3 (C-4_B), 69.7 (C-6_B), 69.0 (C-2_D), 68.1 (C-6_A), 67.1 (C-6_D), 66.6 (C-4_D), 60.7 (C-6_C), 56.6 (OCH₃), 55.1 (C-2_C), 20.7, 20.6 (2 C), 20.5 (2 C), 20.4 (6 COCH₃); ESI-MS (1693.63): *m/z* 1716.7 [M+Na]⁺; Anal. Calcd. for C₉₃H₉₉NO₂₉: C, 65.91; H, 5.89; found: C, 65.70; H, 6.12.

4-Methoxyphenyl (β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (1). To a solution of compound **5** (1 g, 0.59 mmol) in C₂H₅OH (50 mL) was added hydrazine monohydrate (0.5 mL) and the reaction mixture was allowed to stir at 80 °C for 6 h. The solvents were removed under reduced pressure and the crude mass in acetic anhydride-pyridine (1:1 v/v; 20 mL) was kept at room temperature for 2 h. The solvents were removed under reduced pressure and co-evaporated

with toluene (3x20 mL). A solution of the crude mass in 0.1 M CH₃ONa in CH₃OH (30 mL) was allowed to stir at room temperature for 3 h and neutralized with Amberlite IR-120 (H⁺) cation exchange resin. The reaction mixture was filtered and evaporated to dryness. To a solution of the crude product in CH₃OH (20 mL) was added 20% Pd(OH)₂-C (200 mg) and the reaction mixture was stirred at room temperature under a positive pressure of hydrogen for 24 h. The reaction mixture was filtered through a celite bed and concentrated to a white powder, which was further purified through a Sephadex LH-20 using CH₃OH-H₂O (4:1) as eluent to furnish pure tetrasaccharide **1** as an amorphous powder (350 mg, 73%); m.p. 130-32 °C; [α]_D -6.5 (c 1.0, H₂O); IR (KBr): 3514, 3442, 2925, 2381, 1679, 1518, 1462, 1218, 1023, 771 cm⁻¹; ¹H NMR (D₂O, 300 MHz): δ 7.10 (d, *J* = 8.8 Hz, 2 H, Ar-H), 6.96 (d, *J* = 8.8 Hz, 2 H, Ar-H), 5.02 (d, *J* = 7.8 Hz, 1 H, H-1_A), 4.71 (d, *J* = 7.9 Hz, 1 H, H-1_C), 4.46 (2 d, *J* = 8.0 Hz each, 2 H, H-1_B, H-1_D), 4.15 (br s, 1 H, H-4_B), 4.02-3.90 (m, 4 H, H-4_C, H-4_D, H-6_{a,bA}), 3.88-3.83 (m, 3 H, H-2_C, H-6_{a,bC}), 3.80 (s, 3 H, OCH₃), 3.78-3.67 (m, 11 H, H-3_A, H-3_B, H-3_C, H-3_D, H-4_A, H-5_C, H-5_D, H-6_{a,bB}, H-6_{a,bD}), 3.62-3.50 (m, 5 H, H-2_A, H-2_B, H-2_D, H-5_A, H-5_B), 2.0 (s, 3 H, NHCOCH₃); ¹³C NMR (D₂O, 75 MHz): δ 173.6 (NHCOCH₃), 153.4-113.7 (Ar-C), 101.6 (C-1_D), 101.5 (C-1_B), 101.4 (C-1_C), 99.7 (C-1_A), 80.7 (C-3_C), 76.8 (C-3_D), 76.7 (C-3_B), 74.0 (C-4_C), 73.6 (2 C, C-3_A, C-5_D), 73.2 (C-4_A), 72.8 (C-2_D), 71.3 (C-5_C), 71.2 (C-2_A), 70.8 (C-5_A), 69.6 (C-5_B), 68.6 (C-2_B), 67.2 (C-4_D), 67.0 (C-4_B), 59.7 (2 C, C-6_B, C-6_D), 58.5 (2 C, C-6_A, C-6_C), 54.5 (OCH₃), 53.8 (C-2_C), 20.8 (NHCOCH₃); ESI-MS: *m/z* 836.2 [M+Na]⁺; Anal. Calcd. for C₃₃H₅₁NO₂₂ (813.29): C, 48.71; H, 6.32; found: C, 48.50; H, 6.60.

Conclusions

In summary, the synthesis of a tetrasaccharide fragment as its 4-methoxyphenyl glycoside corresponding to the capsular polysaccharide of *Streptococcus pneumoniae* type 15B has been successfully achieved in a straightforward manner. The glycosylation protocol is robust and can be used for larger scale reactions.

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