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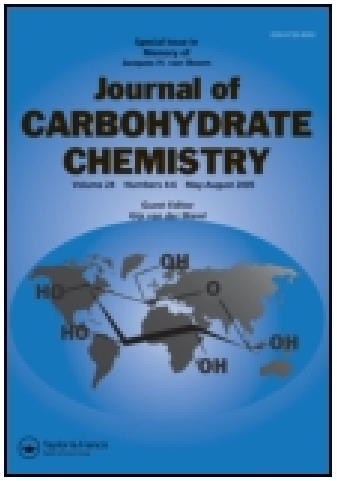

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A Facile Synthesis of the Tetrasaccharide Repeating Unit of the O-Antigen from Cronobacter turicensis

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A Facile Synthesis of the Tetrasaccharide Repeating Unit of the O-Antigen from *Cronobacter turicensis*

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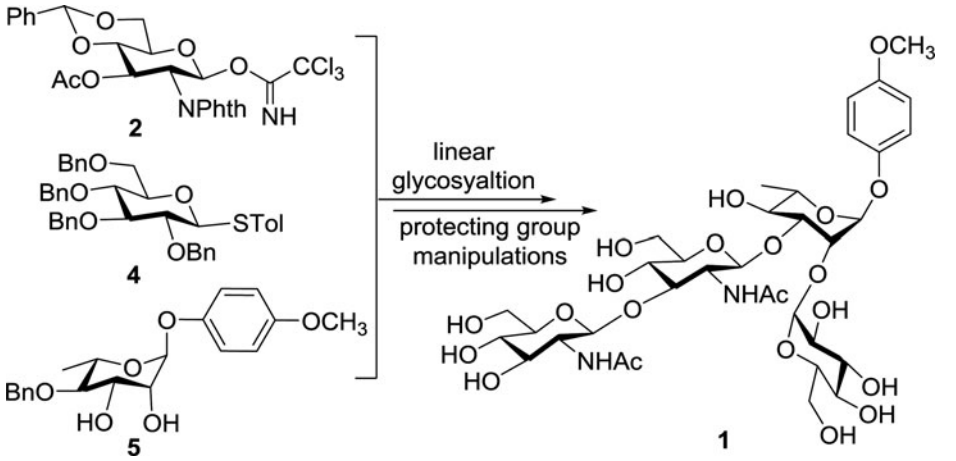
GRAPHICAL ABSTRACT



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The *para*-methoxyphenyl glycoside of a tetrasaccharide repeating unit, β-D-GlcNAc-(1→3)-β-D-GlcNAc-(1→3)-[α-D-Glc-(1→2)]-α-L-Rha-O-(C_6H_4-*p*-OMe), of the polysaccharide O-antigen from *Cronobacter turicensis* was synthesized by an efficiently and linear assembly strategy. Consecutive glycosylation of the 3-*O*- and 2-*O*-positions of an L-rhamnopyranoside derivative with free 2,3-hydroxyl groups using a D-glucosaminyl and a D-glucosyl donor, respectively, accomplished a branched trisaccharide rapidly. Selective 3′-*O*-deacetylation of the trisaccharide followed by glucosaminylation afforded the fully protected tetrasaccharide. Multistep protecting group manipulations of the fully protected tetrasaccharide eventually completed the synthesis of the target molecule.

Keywords *Cronobacter turicensis*; O-Antigen; Synthesis; Regioselective glycosylation

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INTRODUCTION

Cronobacter spp., previously classified as *Enterobacter sakazakii*, is a genus of gram-negative and opportunistic bacterial pathogens that can lead to various life-threatening human diseases, including meningitis, necrotizing enterocolitis, and septicemia.[1] *Cronobacter* infections have received considerable attention on account of its high risk for neonates and infants and cause of fatalities.[2] More recently, powdered infant formula has been identified as a source of contamination of *Cronobacter* organisms in some infant infection cases.[3] Consequently, sensitive technologies for the detection and control of *Cronobacter* bacterium in powdered infant formula and safe and effective therapeutic protocols for the prevention and treatment of *Cronobacter* infections are needed.

Bacterial cell surface lipopolysaccharides (LPSs) are the major components of gram-negative bacterial cell glycocalyx and play an important role in bacterium–host interactions and regulation of the host immune response,[4–6] thereby making them attractive antigen candidates for the development of carbohydrate-based antibacterial vaccines.[6,7] O-polysaccharide (OPS), also known as O-antigen, as the main component of LPS is exposed on the bacterial cell surface and also protects bacteria against host immune recognition, complement attack, and other related responses.[8] Recently, Czerwicka and coworkers isolated and characterized the OPSs from three *C. turicensis* sequence type 5 strains, and these OPSs have the same repeat unit: β-D-Glc*p*NAc-(1→3)-β-D-Glc*p*NAc-(1→3)-[α-D-Glc*p*-(1→2)]-β-L-Rha*p*-(1→4).[9] We present here the first chemical synthesis of this tetrasaccharide unit as a *para*-methoxyphenyl (MP) glycoside **1** (Fig. 1). In the synthetic target, we designed to have the MP group at the reducing end, because this group can be readily selectively removed in the presence of other protecting groups to facilitate further elaborations of the fully protected oligosaccharides at the reducing end, such as its activation for coupling with other molecules, constructing oligomers of this repeating unit, and so on.

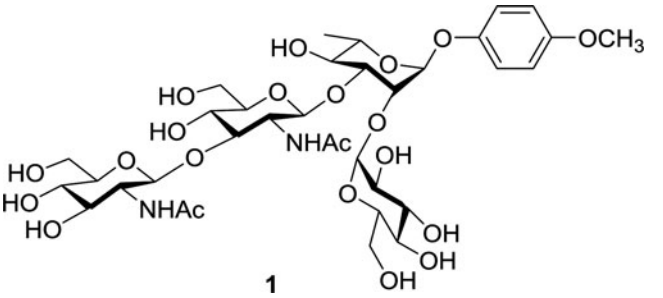

Figure 1: The chemical structure of target compound (**1**).

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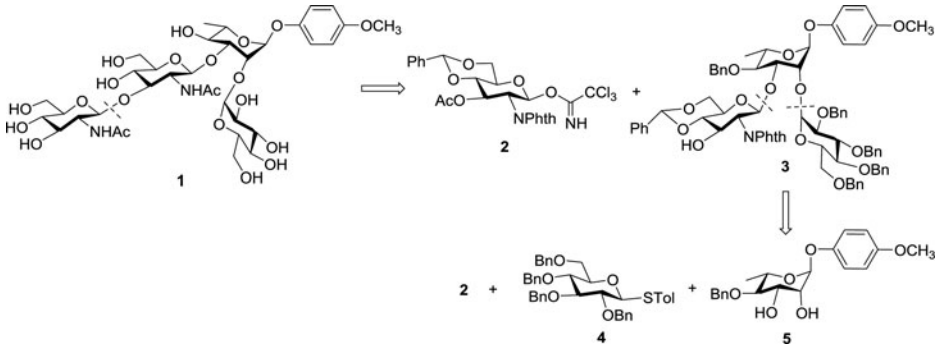
RESULTS AND DISCUSSION

Retrosynthetic disconnection of the synthetic target **1** resulted in three key monosaccharide building blocks, namely, D-glucosaminyl trichloroacetimidate **2**[10] and D-glucosyl thiosglycoside **4**[11,12] as glycosyl donors and a 2,3-diol derivative **5** of L-rhamnose[13,14] as a glycosyl acceptor (Sch. 1). In this design, we intended to take advantage of the demonstrated higher reactivity of the rhamnosyl 3-OH group than its 2-OH group[15,16] to accomplish consecutive 3-*O*- and 2-*O*-glycosylation, which would significantly simplify the monosaccharide building block preparation and the protection tactics, as well as the target molecule assembly.



Scheme 1: Retrosynthetic plan for the synthetic target **1**.

The thioglucoside donor **4** and the diol acceptor **5** were synthesized according to the literature procedures.[11,13] The trichloroacetimidate donor **2**,[10] predominantly in the β-form, was prepared from compound **6**[17,18] in a 52% overall yield in two steps, including removal of the MP group at the anomeric position with cerium ammonium nitrate (CAN)[19] and trichloroacetimidation[20] of the resulting hemiacetal with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Sch. 2). The β-configuration of **2** was

confirmed by the large coupling constant of the H-1 signal (δ 6.72 ppm, $J_{1,2}$ = 9.0 Hz) in its ^{1}H NMR spectrum. It was then used to regioselectively glycosylate rhamnosyl diol **5** in the presence of a catalytic amount of trimethylsilyl triflate (TMSOTf) at –15°C to afford the desired β-(1→3)-linked disaccharide **7** in a good isolated yield (58%). The 1→3 linkage in **7** was confirmed by an acetylation experiment, and we found that the H-2^{Rha} signal (δ 5.38 ppm) in the ^{1}H NMR spectrum of the acetylated product **8** shifted downfield by more than 1 ppm, as compared to that of **7** (δ 4.30 ppm). The newly formed glycosidic linkage was proved β, judging from the coupling constant of its H-1^{GlcN} signal (δ 5.81 ppm, $J_{1,2}$ = 8.4 Hz). Coupling of **7** with thioglucopyranoside donor **4** (1.5 eq.) in the presence of *N*-iodosuccinimide (NIS) and a catalytic amount of TMSOTf in anhydrous diethyl ether[21] at –30°C successfully furnished the trisaccharide as an α,β-mixture (3:1 ratio) in an 83% overall yield. The two isomers were further separated by column chromatography, and the desired α-isomer **9** was obtained in a 62% isolated yield. The coupling constant ($J_{1,2}$ = 3.0 Hz) of the H-1^{Glc} signal of **9** at δ 5.12 ppm in its ^{1}H NMR spectrum confirmed the α-configuration of the new glucosidic linkage. Selective deacetylation of **9** using sodium methoxide in methanol produced the trisaccharide acceptor **3** in a 93% yield. Subsequently, compound **3** was glycosylated again with trichloroacetimidate **2**, which was catalyzed with TMSOTf (0.1 eq.) to afford the fully protected tetrasaccharide **10** in a good yield (73%). The newly introduced glucosaminyl residue in **10** was confirmed being β-linkage based on the coupling constant ($J_{1,2}$ = 7.8 Hz) of the H-1 signal at δ 5.51 ppm in its ^{1}H NMR spectrum. It was interesting to observe that some of the proton and carbon signals of the rhamnosyl and glucosaminyl residues in the ^{1}H and ^{13}C spectra of compounds **8**–**10** were significantly broadened, in some cases too broad to be identifiable, as compared to signals of other nuclei in the same structure or even the same sugar residue. Similar results were observed for NMR spectra obtained in different solvents. However, the MS data of these compounds were in perfect agreement with the expected structures, and furthermore, all of the proton and carbon signals became sharp and clear after deprotection. A potential explanation for this phenomenon was that these fully protected and branched structures possessed enormous steric hindrance and thereby limited their conformational freedom. As a result, the NMR signals of these compounds were broadened. Finally, global deprotection of **10**, including replacement of the Phth groups with Ac groups on the amino group of glucosaminyl residues, was achieved in three separate steps to cope with the different solubility of involved intermediates. First, **10** was treated with hydrazine hydrate in refluxing ethanol to concomitantly remove the Ac and Phth groups.[22] Then, the exposed amino groups were selectively acetylated with acetic anhydride in methanol, which was followed by hydrogenolytic debenzylation in 50% aqueous acetic acid with 10% Pd/C as the catalyst to afford the desired product **1** in a 67% overall yield after purification by size-exclusion chromatography on a Bio-Gel P-2 column.

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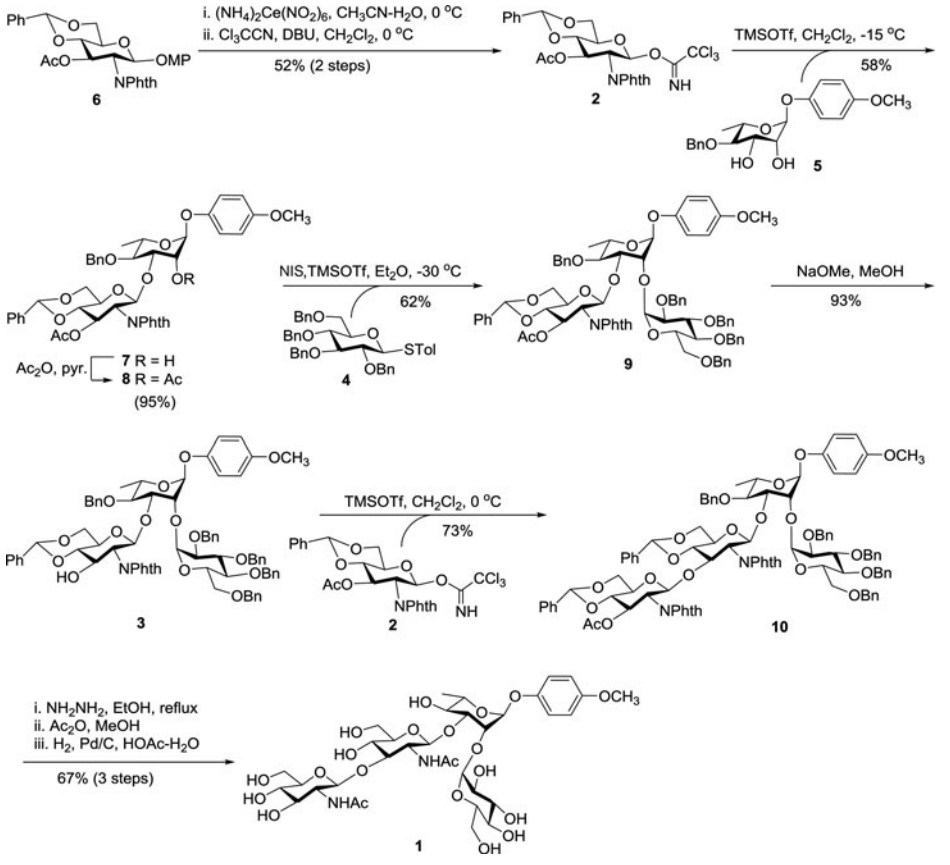
The structures of **1** and all synthetic intermediates were fully characterized by ^{1}H NMR, ^{13}C NMR, and ESI-HRMS spectra.



Scheme 2: Synthesis of the target molecule **1**.

CONCLUSION

In summary, we have described herein a concise and efficient synthesis of the tetrasaccharide repeating unit of the O-antigen from *C. turicensis* LPS. This synthesis is highlighted with the regioselective 3-O-glycosyltion of a 2,3-diol glycosyl acceptor **5**, which was followed by 2-O-glycosyltion, based on the observation that in rhamnose derivatives carrying free 2,3-OH groups, the 3-OH group is more nucleophilic than the 2-OH group. This has significantly simplified the target synthesis in terms of both monosaccharide building block preparation and protecting group design. It is also worth pointing out that the MP group in the structure of fully protected tetrasaccharide **10** can be selectively removed to facilitate further elaborations at the reducing end, which should

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enable its application to conjugating with other biomolecules useful for studies such as vaccine development. We are currently pursuing these directions.

EXPERIMENTAL SECTION

General Methods

Optical rotations were determined at 20°C with a Rudolph Autopol I automatic polarimeter. ^{1}H and ^{13}C NMR spectra were recorded with an Agilent 600 MHz spectrometer for solutions in $CDCl_3$ or D_2O. Chemical shifts (δ) are given in ppm downfield from internal Me_4Si or with DHO signal as a reference, where D_2O was used as the solvent. Positive-mode electrospray ionization (ESI) high-resolution mass spectroscopy (HRMS) was recorded on a JEOL JMS-DX-303HF spectrometer. Thin layer chromatography (TLC) was performed on silica gel HF_{254} plates with detection by charring using 30% (v/v) H_2SO_4 in MeOH or by a UV detector. Silica gel column chromatography was conducted with mixtures of ethyl acetate and petroleum ether (b.p. 60–90°C) as the eluents. Solution concentrations were performed at <60°C under diminished pressure.

3-O-*Acetyl-4,6*-O-*benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (2)*[10]

To a solution of *para*-methoxyphenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido- β-D-glucopyranoside **6**[17,18] (5.45 g, 10.0 mmol) in CH_3CN and H_2O (60 mL, v/v 4:1) was added CAN (8.22 g, 15.0 mmol) at 0°C. The reaction mixture was stirred for 40 min at 0°C, at which time TLC indicated the disappearance of the starting material. The reaction mixture was then diluted with EtOAc (200 mL) and washed with saturated aq. $NaHCO_3$ solution and water. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was crystallized from hexane-EtOAc (2:1) to yield the crude hemiacetal (3.2 g) as a pale yellow solid. This above compound was dissolved in CH_2Cl_2 (20 mL), and trichloroacetonitrile (3.6 mL, 36.5 mmol) was added. The reaction mixture was stirred with a catalytic amount of DBU (0.4 mL, 2.67 mmol) at 0°C for 2 h. After concentration, the residue was purified by silica gel flash column chromatography with 2:1 petroleum ether-ethyl acetate as the eluent to afford **2** (3.03 g, 52%) as a white foamy solid. $[\alpha]_D^{20}$ 32.2 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.65 (s, 1H, NH), 7.87–7.55 (m, 9H, *Ph*), 6.72 (d, 1H, $J = 9.0$ Hz, H-1), 6.00 (dd, 1H, $J = 10.2$, 9.6 Hz, H-3), 5.56 (s, 1H, PhC*H*), 4.60 (dd, 1H, $J = 10.2$, 9.0 Hz, H-2), 4.50 (dd, 1H, $J = 10.2$, 4.8 Hz, H-6a), 3.96 (m, 1H, H-5), 3.89 (t, 1H, $J = 10.2$ Hz, H-6b), 3.88 (t, 1H, $J = 9.6$ Hz, H-4); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.1, 160.6, 136.7, 134.4, 131.2, 129.3, 128.3, 126.3, 123.7, 101.8, 94.0, 90.1, 78.9, 69.4, 68.4, 67.0, 54.3, 20.6.

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para-*Methoxyphenyl 3*-O-*Acetyl-4,6*-O-*benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-4*-O-*benzyl-α-L-rhamnopyranoside (7)*

To a solution of **5** (450 mg, 1.25 mmol) and **2** (760 mg, 1.30 mmol) in anhydrous CH_2Cl_2 (10 mL) was added TMSOTf (24 μL, 0.13 mmol) at –15°C under a N_2 atmosphere. After the reaction mixture was stirred for 1 h, it was neutralized with Et_3N and then concentrated. The resulting residue was purified by flash column chromatography (2:1 petroleum ether-ethyl acetate) to yield **7** (566 mg, 58%) as a white foamy solid. $[\alpha]_D^{20}$ –44.6 (*c* 1.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75–7.35 (m, 9H, *Ph*), 7.15–7.06 (m, 3H, *Ph*), 6.98 (d, 2H, J = 9.0 Hz, *Ph*), 6.89 (d, 2H, J = 6.6 Hz, *Ph*), 6.81 (d, 2H, J = 9.0 Hz, *Ph*), 5.87 (dd, 1H, J = 10.2, 9.0 Hz, H-3^{GlcN}), 5.81 (d, 1H, J = 8.4 Hz, H-1^{GlcN}), 5.56 (s, 1H, PhC*H*), 5.42 (d, 1H, J = 1.2 Hz, H-1^{Rha}), 4.45 (dd, 1H, J = 10.2, 8.4 Hz, H-2^{GlcN}), 4.43 (dd, 1H, J = 10.2, 4.2 Hz, H-6a^{GlcN}), 4.40 (d, 1H, J = 11.4 Hz, PhCH_2), 4.30–4.24 (m, 2H, H-2^{Rha} and PhCH_2), 4.10 (dd, 1H, J = 9.6, 3.6 Hz, H-3^{Rha}), 3.89–3.80 (m, 3H, H-4,5,6b^{GlcN}), 3.76 (s, 3H, -OCH_3), 3.74 (m, 1H, H-5^{Rha}), 3.41 (t, 1H, J = 9.6 Hz, H-4^{Rha}), 2.83 (d, 1H, J = 1.8 Hz, -O*H*), 1.87 (s, 3H, Ac), 1.07 (d, 3H, J = 6.6 Hz, H-6^{Rha}); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.1, 154.8, 150.0, 138.0, 136.7, 117.5, 114.6, 101.8 (Ph*C*H), 99.2 (C-1^{GlcN}), 97.6 (C-1^{Rha}), 82.9 (C-3^{Rha}), 78.8 (C-4^{GlcN}), 78.6 (C-4^{Rha}), 74.6 (Ph*C*H$_2$), 70.0 (C-2^{Rha}), 69.7 (C-3^{GlcN}), 68.5 (C-6^{GlcN}), 67.7 (C-5^{Rha}), 66.4 (C-5^{GlcN}), 55.6 (-O*C*H$_3$), 55.4 (C-2^{GlcN}), 20.5 (-CO*C*H$_3$), 17.6 (C-6^{Rha}); ESI-HRMS (positive ion): calcd for ($C_{43}H_{43}NO_{13}$+NH_4^+): 799.3073; found m/z: 799.3079.

para-*Methoxyphenyl 3*-O-*Acetyl-4,6*-O-*benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2*-O-*acetyl-4*-O-*benzyl-α-L-rhamnopyranoside (8)*

A solution of **7** (15 mg, 0.019 mmol) in pyridine (3 mL) and acetic anhydride (2 mL) was stirred at rt overnight. The reaction mixture was then coevaporated with toluene (2 × 10 mL), and the resulting residue was purified by flash column chromatography (2:1 petroleum ether-ethyl acetate) to yield **8** (15 mg, 95%) as a white foamy solid. $[\alpha]_D^{20}$ –18.8 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.70–7.09 (m, 12H, *Ph*), 6.96 (d, 2H, J = 9.0 Hz, *Ph*), 6.90 (d, 2H, J = 6.6 Hz, *Ph*), 6.80 (d, 2H, J = 9.0 Hz, *Ph*), 5.85 (dd, 1H, J = 10.2, 9.0 Hz, H-3^{GlcN}), 5.74 (d, 1H, J = 8.4 Hz, H-1^{GlcN}), 5.53 (s, 1H, PhC*H*), 5.38 (dd, 1H, J = 3.0, 1.8 Hz, H-2^{Rha}), 5.28 (d, 1H, J = 1.8 Hz, H-1^{Rha}), 4.44 (d, 1H, J = 12.0 Hz, PhCH_2), 4.41 (dd, 1H, J = 9.0, 3.0 Hz, H-6a^{GlcN}), 4.39 (dd, 1H, J = 10.2, 8.4 Hz, H-2^{GlcN}), 4.27 (d, 1H, J = 12.0 Hz, PhCH_2), 4.24 (dd, 1H, J = 9.6, 3.6 Hz, H-3^{Rha}), 3.80–3.72 (m, 7H, H-4,5,6b^{GlcN}, H-5^{Rha}, and -OCH_3), 3.40 (t, 1H, J = 9.6 Hz, H-4^{Rha}), 2.18 (s, 3H, Ac), 1.86 (s, 3H, Ac), 1.10 (d, 3H, J = 6.0 Hz, H-6^{Rha}); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.2, 170.1, 155.0, 149.9, 137.9, 136.9, 117.7, 114.5, 101.7, 99.0, 96.0, 79.2, 79.1, 79.0, 74.7, 71.3, 69.9, 68.5, 67.9, 66.0, 55.6, 55.5, 21.1, 20.5, 17.7; ESI-HRMS (positive ion): calcd for ($C_{45}H_{45}NO_{14}$+NH_4^+): 841.3178; found m/z: 841.3189.

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para-*Methoxyphenyl 3*-O-*Acetyl-4,6*-O-*benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*benzyl-α-D-glucopyranosyl-(1→2)]-4*-O-*benzyl-α-L-rhamnopyranoside (9)*

To a solution of **7** (482 mg, 0.616 mmol) and **4** (600 mg, 0.927 mmol) in anhydrous Et_2O (20 mL) was added NIS (312 mg, 1.39 mmol) and TMSOTf (17 μL, 0.093 mmol) at –30°C under a N_2 atmosphere. The reaction mixture was stirred for 30 min, and TLC indicated the complete disappearance of **7**. The reaction mixture was then neutralized with Et_3N and concentrated, and the resulting residue was purified by flash column chromatography (2:1 hexane-ethyl acetate) to furnish the trisaccharide product as an α,β-mixture (665 mg). Then, the above mixture was further purified by flash column chromatography with 15:1 toluene-ethyl acetate as the eluents to yield **9** (388 mg, 62%) as a white foamy solid. $[\alpha]_D^{20}$ –5.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.65–7.23 (m, 29H, *Ph*), 7.14–7.08 (m, 3H, *Ph*), 6.90 (d, 2H, J = 9.0 Hz, *Ph*), 6.89 (br s, 2H, *Ph*), 6.78 (d, 2H, J = 9.0 Hz, *Ph*), 5.85 (t, 1H, J = 10.2 Hz, H-3^{GlcN}), 5.81 (br d, 1H, J = 7.2 Hz, H-1^{GlcN}), 5.42 (d, 1H, J = 1.8 Hz, H-1^{Rha}), 5.28 (s, 1H, PhC*H*), 5.12 (d, 1H, J = 3.0 Hz, H-1^{Glc}), 5.00, 4.92 (2 d, 2 × 1H, J = 11.2 Hz, PhCH_2), 4.97, 4.64 (2 d, 2 × 1H, J = 11.4 Hz, PhCH_2), 4.72, 4.63 (2 d, 2 × 1H, J = 10.8 Hz, PhCH_2), 4.71, 4.60 (2 d, 2 × 1H, J = 11.4 Hz, PhCH_2), 4.42 (dd, 1H, J = 10.2, 8.4 Hz, H-2^{GlcN}), 4.27 (dd, 1H, J = 10.2, 4.8 Hz, H-6a^{GlcN}), 4.23 (d, 1H, J = 10.8 Hz, PhCH_2), 4.21 (dd, 1H, J = 3.0, 1.8 Hz, H-2^{Rha}), 4.18 (dd, 1H, J = 8.4, 3.0 Hz, H-3^{Rha}), 4.17–4.13 (m, 2H, H-5^{Glc}, PhCH_2), 4.12 (t, 1H, J = 9.6 Hz, H-3^{Glc}), 3.90, 3.84 (br d, 2 × 1H, J = 10.2 Hz, H-6a,b^{Glc}), 3.80 (t, 1H, J = 9.6 Hz, H-4^{Glc}), 3.76 (s, 3H, -OCH_3), 3.75–3.70 (m, 2H, H-5^{Rha}, H-5^{GlcN}), 3.64 (t, 1H, J = 9.0 Hz, H-4^{GlcN}), 3.62 (dd, 1H, J = 9.6, 3.6 Hz, H-2^{Glc}), 3.58 (t, 1H, J = 10.2 Hz, H-6b^{GlcN}), 3.38 (br s, 1H, H-4^{Rha}), 1.86 (s, 3H, Ac), 0.98 (br s, 3H, H-6^{Rha}); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.1, 154.8, 150.3, 139.0, 138.8, 138.4, 138.1, 137.9, 136.9, 117.8, 114.5, 101.4 (Ph*C*H), 100.0 (C-1^{GlcN}), 96.4 (C-1^{Glc}), 96.2 (C-1^{Rha}), 81.7 (C-3^{Glc}), 80.4 (C-2^{Glc}), 80.1 (C-3^{Rha}), 79.1 (C-4^{Rha}), 78.8 (C-4^{GlcN}), 77.7 (C-4^{Glc}), 76.6 (C-2^{Rha}), 75.6, 74.6, 73.6, 72.7 (5C, 5 × Ph*C*H$_2$), 70.8 (C-5^{Glc}), 69.9 (C-3^{GlcN}), 68.6 (C-6^{Glc}), 68.5 (2C, C-5^{Rha} and C-6^{GlcN}), 55.9 (C-5^{GlcN}), 55.6 (2C, C-2^{GlcN} and -O*C*H$_3$), 20.6 (-OC*C*H$_3$), 17.9 (C-6^{Rha}); ESI-HRMS (positive ion): calcd for ($C_{77}H_{77}NO_{18}$+NH_4^+): 1321.5479; found m/z: 1321.5493.

para-*Methoxyphenyl 4,6*-O-*Benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*benzyl-α-D-glucopyranosyl-(1→2)]-4*-O-*benzyl-α-L-rhamnopyranoside (3)*

To a solution of **9** (326 mg, 0.25 mmol) in methanol (10 mL) was added 1 M NaOMe in MeOH solution dropwise until pH = 10 was reached. The reaction mixture was stirred at rt for 2 h and then neutralized with Amberlite IR 120 (H^+). The reaction solution was filtered and concentrated. The resulting

residue was purified by flash column chromatography (3:2 petroleum ether-ethyl acetate) to afford **3** (294 mg, 93%) as a white foamy solid. $[\alpha]_D^{20}$ 1.6 (*c* 1.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.55–7.20 (m, 29H, *Ph*), 7.16–7.08 (m, 3H, *Ph*), 6.90 (d, 2H, J = 9.0 Hz, *Ph*), 6.87 (br d, 2H, J = 5.4 Hz, *Ph*), 6.78 (d, 2H, J = 9.0 Hz, *Ph*), 5.58 (br d, 1H, J = 8.4 Hz, H-1^{GlcN}), 5.41 (d, 1H, J = 1.8 Hz, H-1^{Rha}), 5.28 (s, 1H, PhC*H*), 5.10 (d, 1H, J = 3.6 Hz, H-1^{Glc}), 5.02, 4.97 (2 d, 2 × 1H, J = 10.8 Hz, PhCH_2), 4.98, 4.62 (2 d, 2 × 1H, J = 10.8 Hz, PhCH_2), 4.74, 4.65 (2 d, 2 × 1H, J = 11.4 Hz, PhCH_2), 4.71, 4.60 (2 d, 2 × 1H, J = 10.8 Hz, PhCH_2), 4.59 (ddd, 1H, J = 10.2, 9.0, 4.0 Hz, H-3^{GlcN}), 4.32 (dd, 1H, J = 10.2, 8.4 Hz, H-2^{GlcN}), 4.26 (dd, 1H, J = 10.2, 4.8 Hz, H-6a^{GlcN}), 4.24–4.14 (m, 6H, H-2,3^{Rha}, H-3,5^{Glc}, PhCH_2), 3.93, 3.84 (br d, 2 × 1H, J = 9.0 Hz, H-6a,b^{Glc}), 3.83 (t, 1H, J = 9.0 Hz, H-4^{Glc}), 3.77 (s, 3H, -OCH_3), 3.74 (m, 1H, H-5^{Rha}), 3.63 (m, 1H, H-5^{GlcN}), 3.62 (dd, 1H, J = 9.0, 3.0 Hz, H-2^{Glc}), 3.57 (t, 1H, J = 10.2 Hz, H-6b^{GlcN}), 3.40 (br t, 1H, J = 9.0 Hz, H-4^{Rha}), 3.37 (t, 1H, J = 9.0 Hz, H-4^{GlcN}), 2.01 (br d, 1H, J = 4.0 Hz, -O*H*), 1.00 (br d, 3H, J = 6.0 Hz, H-6^{Rha}); ^{13}C NMR (150 MHz, $CDCl_3$): δ 154.8, 150.3, 139.1, 138.9, 138.5, 138.1, 137.9, 137.0, 117.8, 114.5, 101.7 (Ph*C*H), 100.5 (C-1^{GlcN}), 96.1 (2C, C-1^{Glc}, C-1^{Rha}), 81.72 (C-4^{GlcN}), 81.67 (C-3^{Glc}), 80.5 (C-2^{Glc}), 79.8 (C-3^{Rha}), 79.2 (C-4^{Rha}), 77.7 (C-4^{Glc}), 76.2 (C-2^{Rha}), 75.5, 74.8, 74.0, 73.6, 72.6 (5C, 5 × Ph*C*H$_2$), 70.8 (C-5^{Glc}), 68.63 (C-6^{Glc}), 68.55 (C-5^{Rha}), 68.5 (2C, C-3,6^{GlcN}), 55.9 (C-5^{GlcN}), 55.9 (C-2^{GlcN}), 55.6 (-O*C*H$_3$), 18.0 (C-6^{Rha}); ESI-HRMS (positive ion): calcd for ($C_{75}H_{75}NO_{17}$+NH_4^+): 1279.5373; found m/z: 1279.5372.

para-*Methoxyphenyl 3*-O-*Acetyl-4′,6′*-O-*benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-4,6*-O-*benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[2,3,4, 6-tetra*-O-*benzyl-α-D-glucopyranosyl-(1→2)]-4*-O-*benzyl-α-L-rhamnopyranoside (10)*

To a solution of **3** (185 mg, 0.146 mmol) and **2** (130 mg, 0.22 mmol) in anhydrous CH_2Cl_2 (5 mL) was added TMSOTf (6 μL, 0.03 mmol) at 0°C under a N_2 atmosphere. After the mixture was stirred for 50 min, it was neutralized with Et_3N and concentrated. The resulting residue was purified by flash column chromatography (3:2 petroleum ether-ethyl acetate) to yield **10** (180 mg, 73%) as a white foamy solid. $[\alpha]_D^{20}$ –22.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, CD_3COCD_3): δ 7.80–7.10 (m, 43H, *Ph*), 6.90 (br d, 2H, J = 9.0 Hz, *Ph*), 6.80 (d, 2H, J = 9.0 Hz, *Ph*), 5.64 (d, 1H, J = 8.4 Hz, H-1$^{GlcN'}$), 5.57 (s, 1H, PhC*H*), 5.52 (t, 1H, J = 9.6 Hz, H-3$^{GlcN'}$), 5.43 (s, 1H, PhC*H*), 5.40–5.32 (br s, 1H), 5.30 (d, 1H, J = 3.6 Hz, H-1^{Glc}), 4.96 (d, 1H, J = 11.4 Hz, PhCH_2), 4.93 (d, 1H, J = 10.8 Hz, PhCH_2), 4.86 (d, 1H, J = 11.4 Hz, PhCH_2), 4.82 (dd, 1H, J = 10.2, 8.4 Hz), 4.79–4.60 (m, 5H, PhCH_2), 4.38–4.29 (m, 1H), 4.28–4.20 (m, 3H), 4.17 (dd, 1H, J = 6.6, 3.0 Hz), 4.08–4.01 (m, 3H), 3.96–3.88 (m, 1H, H-6), 3.87–3.80 (m, 1H, H-6), 3.79–3.63 (m, 6H), 3.71 (s, 3H, -OCH_3), 3.61–3.55 (m, 1H), 3.54 (dd, 1H, J = 9.6, 3.0 Hz, H-2^{Glc}), 3.38–3.30 (m, 1H), 1.64 (s, 3H, Ac), 0.90–0.60 (br s, 3H, H-6^{Rha}); ^{13}C NMR (150 MHz, CD_3COCD_3): δ 154.8, 150.3,

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140.6, 138.9, 138.8, 138.6, 135.4 (3C), 119.0, 115.3, 102.2, 101.9, 99.6, 82.4, 81.4 (2C), 79.5, 78.8, 77.0, 76.4, 75.8, 75.2, 74.0, 72.7, 71.8, 70,7, 70.3, 69.25, 69.17, 67.3 (2C), 67.2, 56.6, 56.5, 55.9, 19.3, 18.6; ESI-HRMS (positive ion): calcd for ($C_{98}H_{94}N_2O_{24}$+NH_4^+): 1700.6535; found *m*/*z*: 1700.6553.

para-*Methoxyphenyl 2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→2)]-α-L-rhamnopyranoside (1)*

A solution of **10** (50 mg, 0.03 mmol) in ethanol (10 mL) and 80% hydrazine hydrate (1 mL) was refluxed under an argon atmosphere for 20 h. The reaction mixture was then concentrated under dismissed pressure and purified by flash column chromatography (85:10:5 EtOAc-MeOH-H_2O) to get a white solid. The product was dissolved in methanol (10 mL) containing acetic anhydride (4 mL). The reaction mixture was stirred at rt overnight and then concentrated to give a pale yellow solid, which was then dissolved in 50% aqueous acetic acid (5 mL) and vigorously stirred with 10% Pd/C (20 mg) under a hydrogen atmosphere at rt for 20 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated. The resulting residue was purified by size-exclusion chromatography on a Bio-Gel P-2 column with distilled water as the eluent and then lyophilized to give **1** (16.8 mg, 67% for 3 steps) as a white solid. $[\alpha]_D^{20}$ –6.7 (*c* 0.2, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 6.93 (d, 2H, J = 9.0 Hz, *Ph*), 6.80 (d, 2H, J = 9.0 Hz, *Ph*), 5.33 (s, 1H, H-1^{Rha}), 4.89 (d, 1H, J = 3.0 Hz, H-1^{Glc}), 4.52 (d, 1H, J = 9.0 Hz, H-1^{GlcN}), 4.40 (d, 1H, J = 8.4 Hz, H-1^{GlcN}), 4.17 (br s, 1H, H-2^{Rha}), 4.04 (br d, 1H, J = 11.2 Hz, H-5), 3.90 (dd, 1H, J = 9.6, 3.0 Hz, H-3^{Rha}), 3.77–3.63 (m, 5H, 4 × H-6, H-5^{Rha}), 3.62 (s, 3H, -OCH_3), 3.61–3.51 (m, 5H, H-2^{GlcN}, 2 × H-3, 2 × H-6), 3.48 (t, 1H, J = 9.6 Hz, H-2$^{GlcN'}$), 3.44 (t, 1H, J = 9.6 Hz, H-4^{Rha}), 3.40 (m, 1H, H-5), 3.36–3.24 (m, 6H, H-2^{Glc}, H-3, 3 × H-4, H-5), 1.87, 1.83 (2 s, 2 × 3H, 2Ac), 1.06 (d, 3H, J = 6.0 Hz, H-6^{Rha}); ^{13}C NMR (150 MHz, D_2O): δ 174.2, 173.5, 154.6, 149.1, 118.6, 114.9, 103.1 (C-1^{GlcN}), 100.9 (C-1$^{GlcN'}$), 97.2 (C-1^{Glc}), 96.4 (C-1^{Rha}), 81.1, 77.8, 75.6, 75.2, 74.9, 73.0, 72.6, 71.31, 71.25, 71.18, 69.9, 69.5, 69.2, 68.5, 60.6, 60.3, 59.8, 55.55, 55.52, 54.7, 22.2, 22.1, 16.2; ESI-HRMS (positive ion): Calcd for ($C_{35}H_{54}N_2O_{21}$+H^+): 839.3292; found *m*/*z*: 839.3302; calcd for ($C_{35}H_{54}N_2O_{21}$+Na^+): 861.3117; found *m*/*z*: 861.3109.

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SUPPLEMENTARY DATA

Supplementary data including ^{1}H NMR, ^{13}C NMR, and ESI-HRMS spectra of **2–3**, **7–10**, and **1** are available from the corresponding authors.

REFERENCES

1. Lai, K.K. *Enterobacter sakazakii* infections among neonates, infants, children, and adults: case reports and a review of the literature. *Medicine* **2001**, *80*, 113–122.

2. Caubilla-Barron, J.; Hurrell, E.; Townsend, S.; Cheetham, P.; Loc-Carrillo, C.; Fayet, O.; Prère, M.-F.; Forsythe, S.J. Genotypic and phenotypic analysis of *Enterobacter sakazakii* strains from an outbreak resulting in fatalities in a neonatal intensive care unit in France. *J. Clin. Microbiol.* **2007**, *45*, 3979–3985.

3. FAO/WHO. *Enterobacter sakazakii (*Cronobacter *spp.)* in powdered follow-up formulae. Microbiological Risk Assessment Series No. 15. **2008**, Rome.

4. Robbins, J.B.; Schneerson, R.; Egan, W.B.; Vann, W.; Liu, D.T. Virulence properties of bacterial capsular polysaccharides-unanswered questions. *Life Sci. Res. Rep.* **1980**, *16*, 115–132.

5. Moxon, E.R.; Kroll, J.S. The role of bacterial polysaccharide capsules as virulence factors. *Curr. Top. Microbiol. Immunol.* **1990**, *150*, 65–85.

6. Weintraub, A. Immunology of bacterial polysaccharide antigens. *Carbohydr. Res.* **2003**, *338*, 2539–2547.

7. Plotkin, S.A. Vaccines: past, present and future. *Nat. Med.* **2005**, *11*, S5–S11.

8. Raetz, C.R.H.; Whitfield, C. Lipopolysaccharide endotoxins. *Annu. Rev. Biochem.* **2002**, *71*, 635–700.

9. Czerwicka, M.; Marszewska, K.; Forsythe, S.J.; Bychowska, A.; Mazgajczyk, A.; Dziadziuszko, H.; Ossowska, K.; Stepnowski, P.; Kaczyński, Z. Chemical structure of the O-polysaccharides isolated from *Cronobacter turicensis* sequence type 5 strains 57, 564, and 566. *Carbohydr. Res.* **2013**, *373*, 89–92.

10. Morando, M.; Yao, Y.; Martín-Santamaría, S.; Zhu, Z.; Xu, T.; Cañada, F.J.; Zhang, Y.; Jiménez-Barbero, J. Mimicking chitin: chemical synthesis, conformational analysis, and molecular recognition of the $\beta(1\rightarrow3)$ *N*-acetylchitopentaose analogue. *Chem. Eur. J.* **2010**, *16*, 4239–4249.

11. Veleti, S.K.; Lindenberger, J.J.; Thanna, S.; Ronning, D.R.; Sucheck, S.J. Synthesis of a poly-hydroxypyrolidine-based inhibitor of *Mycobacterium tuberculosi*s GlgE. *J. Org. Chem.* **2014**, *79*, 9444–9450.

12. France, R.R.; Rees, N.V.; Wadhawan, J.D.; Fairbanks, A.J.; Compton, R.G. Selective activation of glycosyl donors utilising electrochemical techniques: a study of the thermodynamic oxidation potentials of a range of chalcoglycosides. *Org. Biomol. Chem.* **2004**, *2*, 2188–2194.

13. Werz, D.B.; Seeberger, P.H. Total synthesis of antigen *Bacillus anthracis* tetrasaccharide-creation of an anthrax vaccine candidate. *Angew. Chem. Int. Ed.* **2005**, *44*, 6315–6318.

14. Roy, B.; Field, R.A.; Mukhopadhyay, B. Synthesis of a tetrasaccharide related to the repeating unit of the O-antigen from *Escherichia coli* K-12. *Carbohydr. Res.* **2009**, *344*, 2311–2316.

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15. Zhang, J.; Kong, F. Synthesis of β-D-Glc*p*-(1→2)-[β-D-Rib*f*-(1→3)]-α-L-Rha*p*-(1→3)- α-L-Rha*p*-(1→2)-α-L-Rha*p*, the repeating unit of the lipopolysaccharide of *Acetobacter diazotrophicus* PAL 5. *J. Carbohydr. Chem.* **2002**, *21*, 579–589.

16. Zhang, J.; Kong, F. Synthesis of a xylosylated rhamnose pentasaccharide-the repeating unit of the O-specific side chain of lipopolysaccharides from the reference strains for *Stenotrophomonas maltophilia* serogroup O18. *J. Carbohydr. Chem.* **2002**, *21*, 89–97.

17. Wang, P.; Wang, J.; Guo, T.T.; Li, Y. Synthesis and cytotoxic activity of the *N*-acetylglucosamine-bearing triterpenoid saponins. *Carbohydr. Res.* **2010**, *345*, 607–620.

18. Yang, Y.; Li, Y.; Yu, B. Total synthesis and structural revision of TMG-chitotriomycin, a specific inhibitor of insect and fungal β-*N*-acetylglucosaminidases. *J. Am. Chem. Soc.* **2009**, *131*, 12076–12077.

19. Fukuyama, T.; Laird, A.A.; Hotchkiss, L.M. *p*-Anisyl group: a versatile protecting group for primary alcohols. *Tetrahedron Lett.* **1985**, *26*, 6291–6292.

20. Schmidt, R.R.; Michel, J. Facile synthesis of α- and β-*O*-glycosyl imidates: preparation of glycosides and disaccharides. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 731–732.

21. Olsson, J.D.M.; Landström, J.; Rönnols, J.; Oscarson, S.; Widmalm, G. Synthesis of and molecular dynamics simulations on a tetrasaccharide corresponding to the repeating unit of the capsular polysaccharide from *Salmonella enteritidis*. *Org. Biomol. Chem.* **2009**, *7*, 1612–1618.

22. Maranduba, A.; Veyrières, A. Glycosylation of lactose: synthesis of branched oligosaccharides involved in the biosynthesis of glycolipids having blood-group I activity. *Carbohydr. Res.* **1986**, *151*, 105–119.

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