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Chemical synthesis of a tetrasaccharide related to the repeating unit of the *O*-polysaccharide isolated from *Salmonella enterica* O59

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Dedicated to Professor Ulf J. Nilsson, Lund University

ABSTRACT

The chemical synthesis of the tetrasachharide repeating unit of the *O*-polysaccharide of *Salmonella enterica* O59 in the form of its *p*-methoxyphenyl glycoside is reported. The total synthesis of the tetrasaccharide has been achieved through concise protecting group manipulations and stereoselective glycosylations via thioglycoside activation using $La(OTf)_3$ as a Lewis acid promoter in conjunction with *N*-iodosuccinimide. $La(OTf)_3$ was found to be a better alternative to the more traditional Lewis acid promoters such as TfOH or TMSOTf.

1. Introduction

Bacterial *O*-antigens (*O*-polysaccharides), constituents of the lipopolysaccharides on the cell walls of Gram-negative bacteria, are important for the pathogenicity of the bacteria and responsible for interactions with the host immune response.[1] *O*-Antigens are one of the most diverse classes of molecules present in the bacterial cell wall due to genetic variations in the *O*-antigen gene clusters. Due to the antigenic character, bacterial *O*-antigens in the form of glyco-conjugates are potential targets for the development of anti-bacterial agents or vaccines. There are already reports of such kind in the literature.[2] However, it is impossible to collect adequate amounts of material for making suitable glyco-conjugates and detailed biological studies to evaluate their potential use. Therefore, a synthetic procedure is required for the large-scale production of the *O*-antigen structure from commercially available sugars.

Salmonella enterica, the causative agent for salmonellosis, is a major pathogen of humans and animals. It is responsible for millions of cases of food-related diseases and an alarming number of deaths in the US per annum.[3] On the basis of the diverse *O*-antigens present in the lipopolysaccharide, strains of *S. enterica* are classified into 46 *O*-serogroups. The structures of the *O*-antigens of many of them have been elucidated.[4] Recently, Perepelov et. al.[5] reported the structure of the tetrasachharide repeating unit of the *O*-antigen isolated from the lipopolysaccharide of *S. enterica* O59 containing a galactose, a rhamnose and two glucosamine moieties (Fig. 1). Herein, we report the total synthesis of the tetrasaccharide repeating unit of the *O*-antigen from *S. enterica* in the form of its *p*-methoxyphenyl glycoside.

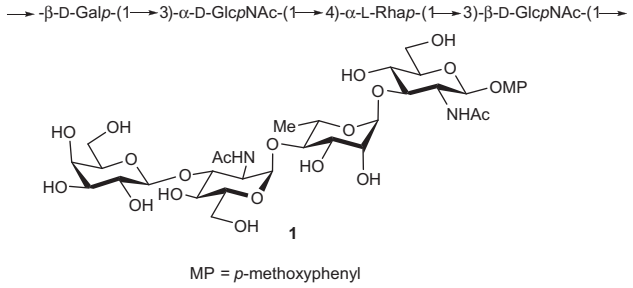

Figure 1. Structure of the repeating unit of the *O*-antigen from *S. enterica* and the synthetic target.

2. Results and discussion

The *p*-methoxyphenyl group was preferred as the reducing end glycoside due to its chemical stability during various protecting group manipulations and glycosylation reactions. Moreover, it can be removed selectively from the acylated/arylated target structure and allow trichloroacetimidate chemistry for further conjugation with suitable aglycons. A sequential glycosylation strategy using acetals as permanent protecting groups and acyls as temporary protecting groups was assumed to be suitable for the synthesis of the target tetrasaccharide **1**. Therefore, four suitably protected monosaccharide synthons **2**–**5** were prepared following literature procedures (Fig. 2).

Glycosylation between acceptor **2**[6] and donor **3**[7] was achieved by activation of the thioglycoside with *N*-iodosuccinimide (NIS) in the presence of $La(OTf)_3$[8] giving the disaccharide **6** in 86% yield. Solid, moisture tolerant $La(OTf)_3$ was proven to be a better alternative to the more conventional Lewis acids such as TfOH or TMSOTf.

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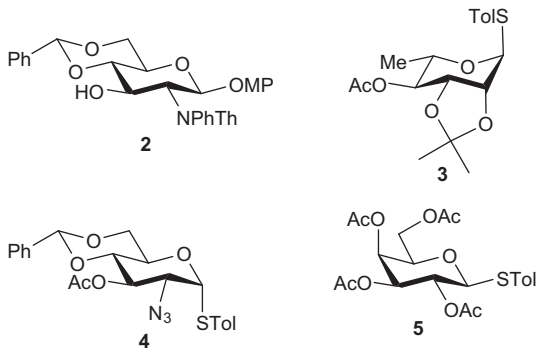
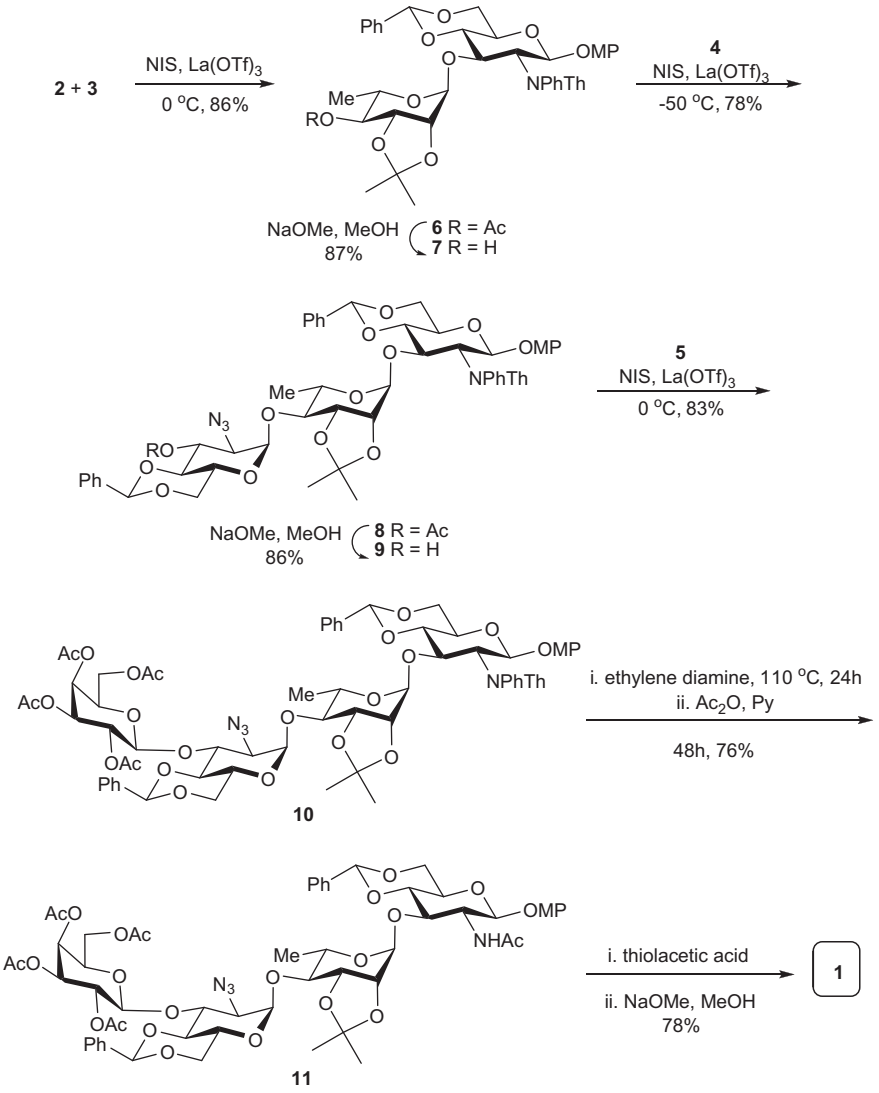


Figure 2. Suitably protected monosaccharide synthons.

Similar glycosylation reactions with TfOH[9] and TMSOTf afforded the desired disaccharide in 56% and 65% yield, respectively, and partial hydrolysis of the benzylidene acetal was observed in both cases. De-O-acetylation of disaccharide **6** in the presence of NaOMe in MeOH[10] afforded disaccharide acceptor **7** in 87% yield. Further glycosylation of acceptor **7** with monosaccharide donor **4**[11] was accomplished by similar NIS-La(OTf)$_3$ mediated activation at −50 °C. The reaction proceeded smoothly to afford trisaccharide **8** in 78% yield, exclusively as the α-anomer. De-O-acetylation of the trisaccharide **8** gave the trisaccharide acceptor **9** in 86% yield. Final glycosylation between trisaccharide acceptor **9** and known monosaccharide donor **5**[12] using NIS-La(OTf)$_3$ mediated thioglycoside activation afforded the protected tetrasaccharide **10** in 83% yield. The N-phthalimido group was converted to an *N*-acetyl derivative using ethylene diamine in *n*-butanol[13] followed by acetylation using Ac_2O in pyridine to afford tetrasaccharide **11** in 76% yield. Next, tetrasaccharide **11** was treated with thiolacetic acid[14] to convert the azido functionality to desired *N*-acetyl. Global deprotection was achieved by hydrolysis of the acetals using 80% acetic acid at 80 °C[15] followed by de-O-acetylation with NaOMe in MeOH to give the target tetrasaccharide **1** in 78% overall yield (Scheme 1).

3. Conclusion

The chemical synthesis of the tetrasaccharide repeating unit of the *O*-antigen from *S. enterica* in the form of its *p*-methoxyphenyl glycoside has been accomplished by rational protecting group manipulations and stereoselective glycosylations. The final product is suitable for further conjugation with the desired aglycon to form glyco-conjugates. Further studies in this direction are currently ongoing and the results will be reported in due course.



Scheme 1. Synthesis of the tetrasaccharide **1**.

4. Experimental

4.1. General methods

All glycosylation reactions were carried out in septum-capped, oven dried round bottom flasks. N_2 gas was purged to make the reaction conditions free of moisture. Dichloromethane was dried and distilled over P_2O_5 to make it moisture free. All reactions were monitored by Thin layer chromatography (TLC) on Silica gel 60-F_{254} with detection by fluorescence followed by charring after immersion in 10% ethanolic solution of H_2SO_4. All reagents and solvents were dried prior to use according to standard methods.[16] Commercial reagents were used without further purification unless otherwise stated. Flash chromatography was performed with Silica Gel 230–400 mesh. Optical rotations were measured on sodium D-line at ambient temperature. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz Spectrometer. In case of tetrasaccharide, ^{1}H NMR values are denoted as H for reducing end Glucosamine, H′ for Rhamnose unit and H″ for 2nd Glucosamine unit and H‴ for the Galactose unit.

4.1.1. *p*-Tolyl 3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside 4

The known *p*-tolyl 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-glucopyranoside (4.80 g, 12.0 mmol) was dissolved in pyridine (5 mL) followed by the addition of Ac_2O (5 mL) and the solution was stirred at room temperature for 2 h until the completion of the reaction was evident from TLC (*n*-hexane–EtOAc; 3:1). Solvents were evaporated using co-evaporation with toluene under reduced pressure. This residue was dissolved in CH_2Cl_2 (20 mL) and washed successively with H_2O (20 mL) and brine (20 mL). The organic layer was collected, dried (Na_2SO_4) and filtered. The solvents were evaporated under reduced pressure and the crude material was purified by flash chromatography to give compound **4** (5.10 g, 97%) as a colourless gel. $[\alpha]_D^{25} = +112$ (*c* 1.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.49–7.36 (m, 7H, ArH), 7.15 (d, 2H, $C_6H_4CH_3$), 5.57 (d, 1H, $J_{1,2}$ 5.5 Hz, H-1), 5.53–5.49 (m, 2H, H-3, C*H*Ph), 4.55 (m, 1H, H-5), 4.25 (d, 1H, $J_{5,6a}$ 10.0 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 4.06 (dd, 1H, $J_{1,2}$ 5.5 Hz, $J_{2,3}$ 10.5 Hz, H-2), 3.79 (t, 1H, $J_{5,6b}$ 10.0 Hz, $J_{6a,6b}$ 10.5 Hz, H-6b), 3.69 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4), 2.35 (s, 3H, $C_6H_4SCH_3$), 2.16 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 169.33 ($COCH_3$), 138.35, 136.71, 133.05 (2), 129.90 (2), 128.98, 128.68, 128.11 (2), 126.01 (2), 101.49 (*C*HPh), 87.87 (C-1), 79.40, 70.35, 68.32, 63.66, 62.69, 21.01 ($C_6H_4SCH_3$), 20.66 ($COCH_3$). HRMS calcd for $C_{22}H_{23}N_3O_5SNa$ (M+Na)$^+$: 464.1256, found: 464.1251

4.1.2. *p*-Methoxyphenyl 4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-*N*-phthalimido-β-D-glucopyranoside 6

A mixture of acceptor **2** (1.0 g, 2.0 mmol), donor **3** (840 mg, 2.4 mmol) and MS 4 Å (1.5 g) in dry CH_2Cl_2 (15 mL) was stirred under nitrogen for 30 min. Then NIS (710 mg, 3.1 mmol) was added followed by La(OTf)$_3$ (50 mg) and the mixture was stirred at room temperature for 45 min when TLC (*n*-hexane-EtOAc; 2:1) showed complete consumption of the donor **3**. The mixture was filtered through a pad of Celite, washed with CH_2Cl_2 and the combined filtrate was washed successively with aq $Na_2S_2O_3$ (2 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL) and brine (25 mL). The organic layer was collected, dried (Na_2SO_4) and filtered, and the solvents were evaporated in vacuo. The crude residue was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to afford pure disaccharide **6** (1.25 g, 86%) as a white foam. $[\alpha]_D^{25} = +127$ (c 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.34–7.89 (m, 9H, ArH), 6.84, 6.82 (2dd, 4H, $C_6H_4OCH_3$), 5.82 (d, 1H, H-1), 5.57 (s, 1H, C*H*Ph), 4.75 (s, 1H, H-1′), 4.74 (m, 1H, H-3), 4.63 (dd, 1H, H-4′), 4.55 (dd, 1H, $J_{5,6a}$ 10 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 4.03 (dd, 1H, $J_{1',2'}$ 1.9 Hz, $J_{2',3'}$5.5 Hz, H-2′), 3.88–3.75 (m, 5H, H-6b, H-5′, H-3′, H-4, H-5), 3.72 (s, 3H, $C_6H_4OCH_3$), 2.00 (s, 3H, $COCH_3$), 1.3 (s, 3H, C-CH_3), 1.03 (s, 3H, C-CH_3), 0.57 (d, 3H, $J_{5',6'}$ 6.0 Hz, H-6′). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 169.92 ($COCH_3$), 155.69, 150.45, 136.94, 134.53, (2), 129.23, 129.01, 128.58, 128.94, 128.20 (2), 128.13, 126.96 (2), 123.78, 118.72 (2), 114.5 (2), 109.46 (isopropylidene-*C*), 102.15 (*C*HPh), 98.05 (C-1), 97.49 (C-1′), 80.39, 75.76, 75.46, 74.04, 73.84, 68.69, 66.57, 64.59, 56.61, 55.56 ($C_6H_4OCH_3$), 27.36, 26.05 (2 × C–CH_3), 20.93 ($COCH_3$), 16.03 (C-6′). HRMS cald for $C_{39}H_{41}NO_{13}Na$ (M+Na)$^+$: 754.2476, found: 754.2469.

4.1.3. *p*-Methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-*N*-phthalimido-β-D-glucopyranoside 7

To a solution of disaccharide **6** (1.25 g, 1.7 mmol) in MeOH (10 mL) was added NaOMe in MeOH (0.5 M, 1.0 mL) and the solution was stirred at room temperature for 30 min. After completion of the reaction, it was neutralized with DOWEX 50 W H$^+$ resin and filtered through a cotton plug. The solvent was evaporated in vacuo and the crude material was purified by flash chromatography using *n*-hexane–EtOAc (1:1) as eluent to afford pure disaccharide acceptor **7** (1.0 g, 87%) as a white foam. $[\alpha]_D^{25} = +132$ (*c* 1.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.87–7.33 (m, 9H, ArH), 6.8, 6.7 (2d, 4H, $C_6H_4OCH_3$), 5.82 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.55 (s, 1H, C*H*Ph), 4.71 (s, 1H, H-1′), 4.70 (m, 1H, H-3), 4.55 (dd, 1H, $J_{1,2}$ 9.0 Hz, $J_{2,3}$ 10.0 Hz, H-2), 4.42 (dd, 1H, $J_{5,6a}$ 10.0 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.92 (t, 1H, H-6b), 3.87–3.72 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-4), 3.70 (s, 3H, $C_6H_4OCH_3$), 3.14 (m, 1H, H-5), 2.52 (bs, 1H, OH), 1.26 (s, 3H, C–CH_3), 0.71 (d, 3H, $J_{5'6'}$ 6.5 Hz, H-6′). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 155.56, 150.37, 136.83, 134.39 (2), 131.13, 129.11, 128.11 (2), 126.35 (2), 123.64 (2), 118.63 (2), 114.41 (2), 108.98 (isopropylidene-*C*), 102.02 (*C*HPh), 97.95 (C-1), 97.83 (C-1′), 80.24, 78.05, 75.66, 74.21, 74.20, 68.53, 66.55, 66.23, 56.57 (C-6), 55.45 ($C_6H_4OCH_3$), 27.60, 25.69 (2 × C–CH_3), 16.44 (C-6′). HRMS cald for $C_{37}H_{39}NO_{12}Na$ (M+Na)$^+$: 712.2370, found: 712.2362

4.1.4. *p*-Methoxyphenyl 3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-*N*-phthalimido-β-D-glucopyranoside 8

A mixture of disaccharide acceptor **7** (1.0 g, 1.5 mmol), donor **4** (775 mg, 1.75 mmol) and MS 4 Å (1.5 g) in dry CH_2Cl_2 (15 mL) was stirred under nitrogen for 30 min. Then NIS (525 mg, 2.3 mmol) was added followed by La(OTf)$_3$ (50 mg) and the mixture was stirred at −50 °C for 45 min when TLC (*n*-hexane–EtOAc, 2:1) showed complete conversion of the starting materials to form a new compound. The mixture was filtered through a pad of Celite, washed with CH_2Cl_2 and the combined filtrate was washed successively with aq. $Na_2S_2O_3$ (2 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL) and brine (25 mL). The organic layer was collected, dried (Na_2SO_4) and filtered, and the solvents were evaporated in vacuo. The crude residue was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to afford pure trisaccharide **8** (1.15 g, 78%) as a white foam. $[\alpha]_D^{25} = +142$ (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.87–7.30 (m, 14H, ArH), 6.87–6.75 (2dd, 4H, $C_6H_4OCH_3$), 5.84 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.56 (s, 1H, C*H*Ph), 5.54 (t, 1H, $J_{2'',3''}$ 10.0 Hz, $J_{3'',4''}$ 10.0 Hz, H-3″), 5.46 (s, 1H, C*H*Ph), 4.86 (d,1H, $J_{1'',2''}$ 4.0 Hz, H-1″), 4.68 (s, 1H, H-1′), 4.64 (dd, 1H, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 10.5 Hz, H-2), 4.42 (m, 1H, H-6b), 4.19 (dd, 1H, $J_{5'',6a''}$ 10.0 Hz, $J_{6a'',6b''}$ 10.0 Hz, H-6a″), 4.11 (m, 1H, H-4′), 3.99 (m, 1H, H-5′), 3.86–3.76 (m, 5H, H-6a, H-5′, H-2′, H-3′, H-4), 3.72 (s, 3H, $C_6H_4OCH_3$), 3.66 (t, 1H, $J_{5'',6b''}$ 9.5 Hz, $J_{6a''6b''}$ 10.0 Hz, H-6b″), 3.58 (t, 1H, $J_{3'',4''}$ 10.0 Hz, $J_{4'',5''}$ 9.5 Hz, H-4″), 3.16–3.09 (m, 2H, H-2″, H-5), 2.14 (s, 3H, $COCH_3$), 1.25,1.04 (s, 3H, 2 × C-CH_3), 0.70 (d, 3H, $J_{5',6'}$ 6.0 Hz,

H-6′). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 169.81, (*C*OCH$_3$), 155.68, 150.49, 136.96, 138.7, 134.53 (2), 131.25, 129.28, 129.08, 128.24 (3), 128.20 (2), 126.67 (2), 126.17 (2), 123.74 (2), 118.79 (2), 114.50 (2), 108.79 (isopropylidene-*C*), 102.49, 101.56 (2 × *C*HPh), 99.04 (C-1″), 98.04 (C-1), 97.69 (C-1′), 81.09, 80.35, 79.29, 76.16, 75.75, 74.59, 68.84, 68.70, 66.73, 65.31, 62.50, 61.86, 56.58, 55.57 ($C_6H_4OCH_3$), 53.41, 27.80, 25.94 (2 × C–*C*H$_3$), 20.86 (CO*C*H$_3$), 16.60 (C-6′). HRMS cald for $C_{52}H_{54}N_4O_{17}Na$ (M+Na)$^+$: 1029.3382, found: 1029.3389.

4.1.5. *p*-Methoxyphenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2-deoxy-*N*-phthalimido-4,6-*O*-benzylidene-β-D-glucopyranoside 9

To a solution of the trisaccharide **8** (1.15 g, 1.14 mmol) in MeOH (10 mL) was added NaOMe in MeOH (0.5 M, 1.0 mL) and the reaction was stirred at room temperature for 30 min. After completion of the reaction, it was neutralized with DOWEX 50 W H$^+$ resin and filtered through the cotton plug. The solvents were evaporated in vacuo and the crude material was purified by flash chromatography using *n*-hexane–EtOAc (1:1) as eluent to afford pure compound **9** (950 mg, 86%) as a white foam. $[\alpha]_D^{25} = +148$ (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.88–7.16 (m, 14H, ArH), 6.85–6.75 (2dd, 4H, $C_6H_4OCH_3$), 5.84 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.56 (s, 1H, C*H*Ph), 5.51 (s, 1H, C*H*Ph), 4.81 (d, 1H, $J_{1'',2''}$ 4.0 Hz, H-1″), 4.67 (s, 1H, H-1′), 4.66 (m, 1H, H-3), 4.55 (dd, 1H, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 10.0 Hz, H-2), 4.43 (dd, 1H, $J_{5,6a}$ 10.5 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 4.19–4.14 (m, 2H, H-3″, H-6a″), 4.04–3.99 (m, 2H, H-4′, H-5″), 3.86 (m, 1H, H-6b), 3.80–3.76 (m, 4H, H-3′, H-2′, H-4, H-5′), 3.7 (s, 3H, $C_6H_4OCH_3$), 3.66 (t, 1H, $J_{5'',6b''}$ 10.0 Hz, $J_{6a''6b''}$ 10.5 Hz, H-6b″), 3.49 (t, 1H, $J_{3'',4''}$ 9.5 Hz, $J_{4'',5''}$ 9.5 Hz, H-4″), 3.20 (dd, 1H, $J_{1'',2''}$ 4.0 Hz, $J_{2'',3''}$ 10.0 Hz, H-2″), 3.13 (dd, 1H, H-5), 2.68 (bs, 1H, OH), 1.27, 1.08 (s, 3H, 2 × C-C*H*$_3$), 0.69 (d, 3H, $J_{5',6'}$ 6.0 Hz, H-6′). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 155.69, 150.48, 137.85, 136.94, 136.87, 134.49 (2), 131.25, 129.34, 129.02, 128.37 (2), 128.22 (2), 126.62 (2), 126.26 (2), 125.28, 123.74, 118.77 (2), 114.51 (2), 108.80 (isopropylidene-*C*), 102.49, 102.02 (2 × *C*HPh), 98.82, (C-1), 98.03 (C-1″), 97.75 (C-1′), 81.71, 80.89, 80.34, 76.29, 75.89, 74.73, 68.75, 68.71, 68.64, 66.71, 65.42, 63.20, 62.13, 56.66, 55.57 ($C_6H_4OCH_3$), 27.82, 26.00 (2 × C–*C*H$_3$), 16.62 (C-6′). HRMS cald for $C_{50}H_{52}N_4O_{16}$-Na (M+Na)$^+$: 987.3276, found: 987.3271.

4.1.6. *p*-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-*N*-phthalimido-β-D-glucopyranoside 10

A mixture of trisaccharide acceptor **9** (950 mg, 0.984 mmol), donor **5** (540 mg, 1.2 mmol) and MS 4 Å (1.5 g) in dry CH_2Cl_2 (15 mL) was stirred under nitrogen for 30 min. Then NIS (360 mg, 1.6 mmol) was added followed by La(OTf)$_3$ (50 mg) and the mixture was stirred at room temperature for 45 min when TLC (*n*-hexane–EtOAc, 2:1) showed complete conversion of the starting materials to form a new compound. The mixture was filtered through a pad of Celite, washed with CH_2Cl_2 and the combined filtrate was washed successively with aq $Na_2S_2O_3$ (2 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL) and brine (25 mL). The organic layer was collected, dried (Na_2SO_4) and filtered, and the solvents were evaporated *in vacuo*. The crude residue was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to afford pure tetrasaccharide **10** (1.05 g, 83%) as a white foam. $[\alpha]_D^{25} = +103$ (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.87–7.35 (m, 14H, ArH), 6.85–6.75 (2d, 4H, $C_6H_4OCH_3$), 5.84 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.58, 5.56 (2s, 2H, 2 × C*H*Ph), 5.37–5.24 (m, 2H, H-4‴,H-2‴), 4.82 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1″), 4.75 (d, 1H, $J_{1''',2'''}$ 8.0 Hz, H-1‴), 4.67 (m, 2H, H-1′, H-3), 4.54 (t, 1H, $J_{1,2}$ 8.5 Hz, $J_{2,3}$ 9.5 Hz, H-2), 4.42 (m, 1H, H-6a), 4.20–3.97 (m, 6H, H-3″, H-4′, H-5″, H-6a‴, H-6b‴, H-6a″), 3.86 (m, 6H, H-2′, H-3′, H-6b, H-4″, H-4, H-5′), 3.70 (s, 3H, $C_6H_4OCH_3$), 3.65–3.63 (m, 1H, H-6b″), 3.23 (m, 1H, H-2″), 3.10 (m, 1H, H-5′), 2.15–1.91 (3s, 12H, 4 × CO*C*H$_3$), 1.25, 1.01 (2s, 6H, 2 × C-C*H*$_3$), 0.69 (d, 3H, $J_{5',6'}$ 6.0 Hz, H-6′). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.24, 170.20, 170.07, 169.49 (4 × *C*OCH$_3$), 155.72, 150.46, 134.51 (2), 131.24, 130.86, 129.31, 129.26, 129.18, 129.01, 128.78, 128.33 (2), 128.30 (2), 126.64 (2), 126.62 (2), 126.52 (2), 123.74, 118.78 (2), 114.51 (20, 108.85 (isopropylidene-*C*), 102.44, 101.42 (2 × *C*HPh), 101.58 (C-1″), 98.79 (C-1‴), 98.04 (C-1), 97.79 (C-1′), 80.54, 80.38, 76.22, 75.88, 74.85, 71.11, 70.64, 69.35, 68.82, 68.71, 66.72, 66.69, 65.38, 65.34, 62.98, 62.58, 56.63, 55.57 ($C_6H_4OCH_3$), 27.82, 25.98 (2 × C–*C*H$_3$), 20.81, 20.75, 20.63, 20.56 (4 × CO*C*H$_3$), 16.68 (C-6′). HRMS cald for $C_{64}H_{70}N_4O_{25}Na$ (M+Na)$^+$: 1317.4227, found: 1317.4222.

4.1.7. *p*-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside 11

To a solution of compound **10** (1.05 g, 0.8 mmol) in *n*-butanol (10 mL) was added ethylene diamine (0.2 mL) and the reaction mixture was allowed to stir at 110 °C for 24 h. The solvents were removed under reduced pressure and a solution of the crude product in pyridine (5 mL) was treated with acetic anhydride (5 mL) at room temperature for 3 h. The reaction mixture was evaporated and co-evaporated with toluene and passed through a short pad of SiO_2 using *n*-hexane–EtOAc (1:1) as eluent to give the pure compound **11** (740 mg, 76%) as colourless syrup. $[\alpha]_D^{25} = +117$ (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.49–7.32 (m, 10H, ArH), 6.88–6.74 (2d, 4H, $C_6H_4OCH_3$), 6.52 (bd, 1H, N*H*Ac), 5.52, 5.42 (2s, 2H, 2 × C*H*Ph), 5.36–5.23 (m, 2H, H-4‴, H-2‴), 5.15 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.06 (s, 1H, H-1′), 4.99 (m, 1H, H-3‴), 4.85 (d, 1H, $J_{1'',2''}$ 3.0 Hz, H-1″), 4.73, (d, 1H, $J_{1''',2'''}$ 8.0 Hz, H-1‴), 4.32 (m, 1H, H-6a), 4.20–4.03 (m, 8H, H-6b‴, H-6a‴, H-6a″, H-4′, H-5″, H-3″, H-2, H-3), 3.84 (m, 3H, H-6b, H-4″, H-5′), 3.70 (s, 3H, $C_6H_4OCH_3$), 3.67 (m, 4H, H-4, H-6b″, H-2′, H-3′), 3.23 (m, 2H, H-2″, H-5), 2.16–1.90 (s, 15H, 5 × CO*C*H$_3$), 1.40, 1.24 (s, 6H, 2 × C–CH$_3$), 0.75 (d, 3H, $J_{5',6'}$ 5.5 Hz, H-6′). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.66, 170.25, 170.18, 170.09, 169.39, 155.45, 150.90, 137.02, 129.15, 129.09, 128.23, 128.20, 128.14, 128.02, 126.46, 126.26, 126.03, 125.90, 1125.80, 118.59 (2), 114.59, 114.46, 108.94 (isopropylidene-*C*), 102.03 (*C*HPh), 101.44 (C-1‴), 101.35 (*C*HPh), 100.09 (C-1), 98.63 (C-1″), 97.79 (C-1′), 80.94, 80.25, 79.55, 76.07, 76.03, 71.00, 70.85, 70.49, 69.25 69.12, 68.73, 68.63, 68.53, 66.63, 66.34, 65.06, 63.94, 62.87, 62.69, 62.52, 62.38, 60.60, 60.32, 57.30, 57.15, 55.46 ($C_6H_4OCH_3$), 28.02, 26.26, (2 × C–*C*H$_3$), 23.19 (NHCO*C*H$_3$), 20.53, 20.47, 20.44, 20.39 (4 × CO*C*H$_3$), 14.07 (C-6′). HRMS cald for $C_{58}H_{70}N_4O_{24}Na$ (M+Na)$^+$: 1229.4278, found: 1229.4272.

4.1.8. *p*-Methoxyphenyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside 1

Compound **11** (740 mg, 0.6 mmol) was dissolved in thiolacetic acid (5 mL) and this was stirred at room temperature for 48 h. After the completion of the reaction, the thiolacetic acid was co-evaporated with toluene and the residue was washed with $NaHCO_3$ (2 × 50 mL). The organic layer was separated, dried (Na_2SO_4) and filtered. The solvent was evaporated and the residue was dried. This was treated with 80% AcOH (10 mL) and stirred at 80 °C until the TLC showed complete conversion of the starting material. The solvent was co-evaporated with toluene and the residue was dried. The residue thus obtained was dissolved in MeOH (10 mL), followed by the addition of NaOMe in MeOH (0.5 M, 1 mL) and the solution was stirred at room temperature for 4 h. After completion

of the reaction, it was neutralized with DOWEX 50 W H^+ resin and filtered through the cotton plug. Solvents were evaporated in vacuo to afford pure target tetrasaccharide **1** (400 mg, 78%) as white solid. $[\alpha]_D^{25} = +81$ (*c* 0.8, H_2O). ^1H NMR (D_2O, 500 MHz) δ: 6.94, 6.85 (2d, 4H, $C_6H_4OCH_3$), 4.97 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 4.88 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1″), 4.76 (s, 1H, H-1′), 4.34 (d, 1H, $J_{1''',2'''}$ 8.0 Hz, H-1‴), 4.01–3.31 (m, 25H, H-2′, H-3′, H-4′, H-5′, H-2″, H-3″, H-4″, H-5″, H-6a″, H-6b″, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2‴, H-3‴, H-4‴, H-5‴, H-6a‴, H-6b‴, $C_6H_4OCH_3$), 1.93, 1.91 (2s, 6H, 2 × $COCH_3$), 1.13 (d, 3H, $J_{5'6'}$ 6.0 Hz, C-CH_3). ^{13}C NMR (D_2O, 125 MHz) δ: 174.58, 174.33 (2 × $COCH_3$), 154.93, 150.84, 118.46 (2), 115.08 (2), 103.37 (C-1‴), 101.13 (C-1′), 99.92 (C-1), 98.01 (C-1″), 81.42, 80.56, 76.17, 76.11, 75.27, 72.53, 71.52, 70.93, 70.71, 68.88, 68.57, 68.43, 68.28, 67.99, 61.02, 61.05, 60.09, 55.8 ($C_6H_4OCH_3$), 55.34, 52.76, 21.88, 21.84 (2 × $NHCOCH_3$) 16.80 (C-6′). HRMS calcd for $C_{35}H_{54}N_2O_{21}Na$ $(M+Na)^+$: 861.3117, found: 861.3123.

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