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Chemical synthesis of the tetrasaccharide repeating unit of the O-antigenic polysaccharide from *Plesiomonas shigelloides* strain AM36565

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ABSTRACT

Chemical synthesis of the tetrasaccharide repeating unit of the O-antigenic polysaccharide from *Plesiomonas shigelloides* strain AM36565 is reported. Glycosylations between suitably protected monosaccharide synthons were achieved by the activation of thioglycosides in the presence of H_2SO_4–silica in conjunction with *N*-iodosuccinimide. The glycosylations accomplished were highly stereoselective and afforded the desired products in good to excellent yields.

1. Introduction

The O-polysaccharide constituents of many pathogenic and non-pathogenic bacterial species play diverse roles in determining and regulating the biology of the organisms.[1] The bacterial O-antigens, constituents of the lipopolysaccharides of the bacterial cell wall, consist of many repeats of the oligosaccharide units. Taking advantage of the presence of a variety of sugar residues, these O-antigens are considered to be one of the most diverse classes of molecules present on the bacterial cell wall. They play a key-role as an elicitor of innate immune responses.[2] They also contribute to the pathogenicity by virtue of their protecting ability of the infecting bacteria by killing serum complement and phagocytosis.[2] Due to this antigenic character of bacterial O-antigens, they are widely studied as potential vaccine-targets. However, isolation of these oligosaccharides from natural sources in adequate quantity and purity is almost impossible. Thus, synthetic methods are employed to explore their potential for future carbohydrate-based vaccines.

Plesiomonas shigelloides is a Gram-negative, facultative anaerobe that causes enteric disease in human, especially following the consumption of raw seafood. They are generally found in fresh or estuarine waters as saline environment restricts their growth. Hence, it has been found to be responsible for various outbreaks of diarrheal disease associated with contaminated water. To date, only a few of the O-antigenic polysaccharide structures of *P. shigelloides* have been determined and elucidated.[3–6] Recently, Sawen et al.[7] elucidated the structure of the tetrasaccharide repeating unit O-antigenic polysaccharide from *P. shigelloides* strain AM36565. Structural analysis of the oligosaccharide showed that it consists of a glucosamine, a galactosamine and two rhamnose moieties. Herein, we report the total synthesis of the tetrasaccharide repeating unit in the form of its *p*-methoxyphenyl glycoside (Fig. 1) through suitable protecting group manipulations on the commercially available monosaccharides followed by stereoselective chemical glycosylations.

2. Results and discussion

With the aim of designing the synthetic strategy for the effective synthesis of the target tetrasaccharide, *p*-methoxyphenyl group was selected at the reducing end as it leaves the possibility of further glycoconjugate formation by selective removal of the glycoside from the per-O-acetylated form of the target tetrasaccharide **1**. Retrosynthetic analysis of **1** suggests a sequential glycosylation strategy with suitably protected monosaccharide synthons (Fig. 2).

Thus, the known *p*-methoxyphenyl 2-azido-2-deoxy-α-D-glucopyranoside **2**[8] was selectively protected at the C-4 and C-6 positions by benzaldehyde dimethyl acetal[9] in the presence of 10-camphorsulfonic acid (CSA) to form the required acceptor **3** in 83% yield. The selection of azido group as the acetamido precursor is essential to have 1,2-*cis*-glycoside at the reducing end. Then the acceptor **3** was coupled to known thioglycoside donor **4**[10] having

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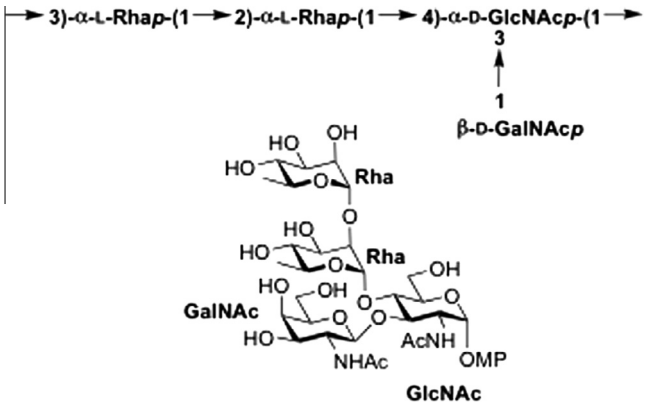


Figure 1. Structure of the tetrasaccharide repeating unit and the target tetrasaccharide as its *p*-methoxyphenyl glycoside.

phthalimido as the acetamido precursor to facilitate required 1,2-*trans* glycosylation through neighboring group participation. Activation of the thioglycoside using NIS in the presence of H_2SO_4–silica[11–15] afforded the desired disaccharide **5** in 85% yield stereospecifically as evident from NMR spectra. Hydrolysis of the benzylidene acetal using 80% AcOH[16] at 80 °C furnished the diol **6** in 94% yield. Owing to the greater reactivity of the primary OH group, it was regioselectively benzoylated using BzCN in the presence of Et_3N[17] to afford the required disaccharide acceptor **7** in 88% yield. It is worth noting that the obvious choice of reductive opening of the benzylidene acetal using $NaCNBH_3$ and HCl–ether[18] or triethylsilane and $BF_3{\cdot}Et_2O$[19] to form 4-OH acceptor failed to afford the desired product in reasonable yield. Further, 2-OH position of the known *p*-tolyl 3,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**8**)[20] was protected with chloroacetyl group using chloroacetic anhydride in the presence of pyridine[21] to yield *p*-tolyl 3,4-di-*O*-benzyl-2-*O*-chloroacetyl-1-thio-α-L-rhamnopyranoside (**9**) in 85% isolated yield. Presence of the chloroacetate group at C-2 is justified as it will ensure the 1,2-*trans* glycosylation through neighboring group participation and it is orthogonal to the acetates present in the disaccharide acceptor.

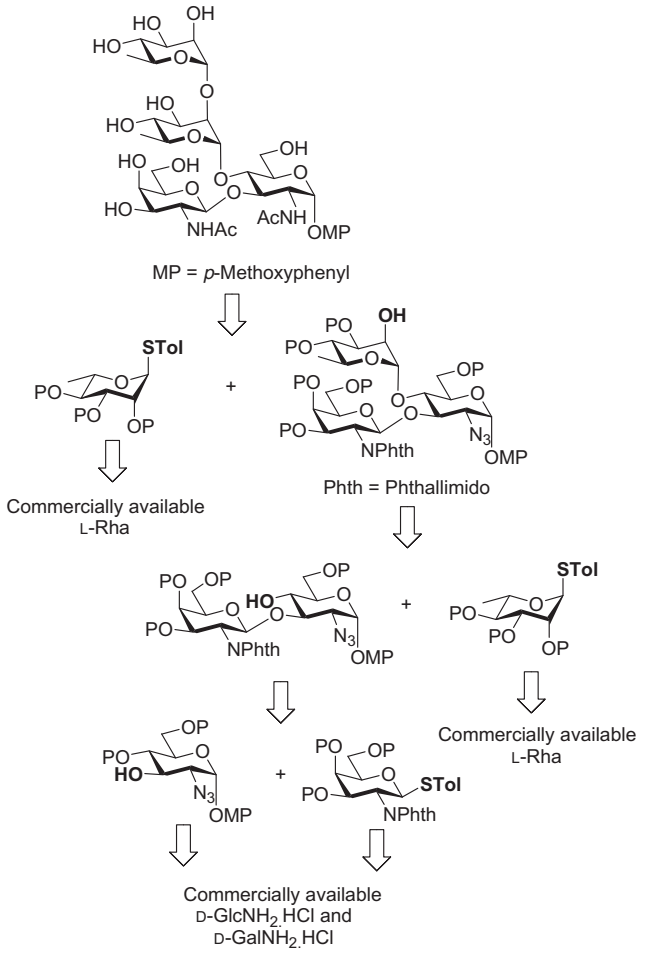


Figure 2. Retrosynthetic analysis for the synthesis of the target tetrasaccharide **1**.

Glycosylation between the disaccharide acceptor **7** and rhamnosyl donor **9** was accomplished by activating the thioglycoside with NIS in the presence of H_2SO_4–silica furnishing the trisaccharide **10**. Purification of the trisaccharide **10** was unsuccessful as traces of the unreacted acceptor contamination remained. Selective removal of the chloroacetate group using thiourea[22] afforded the trisaccharide acceptor **11** in 78% yield. Final glycosylation of the trisaccharide acceptor **11** with the known *p*-tolyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**12**)[23] using the similar thioglycoside activation furnished the protected tetrasaccharide **13** in 80% yield.

Once the tetrasaccharide framework is established, the next challenge was to convert the acetamido precursors into the desired acetamido forms. First, the azido group was converted into acetamido using thioacetic acid by a slow reaction for 72 h at ambient temperature in the dark[24] to give the corresponding acetamido derivative 14 in 72% yield. Next, the phthalimido group was removed by ethylenediamine[25] and the free amine thus formed, was acetylated by using Ac_2O and pyridine. It is worth mentioning that the benzoyl group along with other acetate groups was removed by the action of ethylenediamine and subsequently acetylated during acetylation of the free amine. Next, the removal of the benzyl groups was accomplished by catalytic hydrogenation using Pd–C in the presence of H_2. Finally, the acetates were removed by Zemplén de-O-acetylation[26] using NaOMe in methanol to afford the target tetrasaccharide 1 in 74% yield.

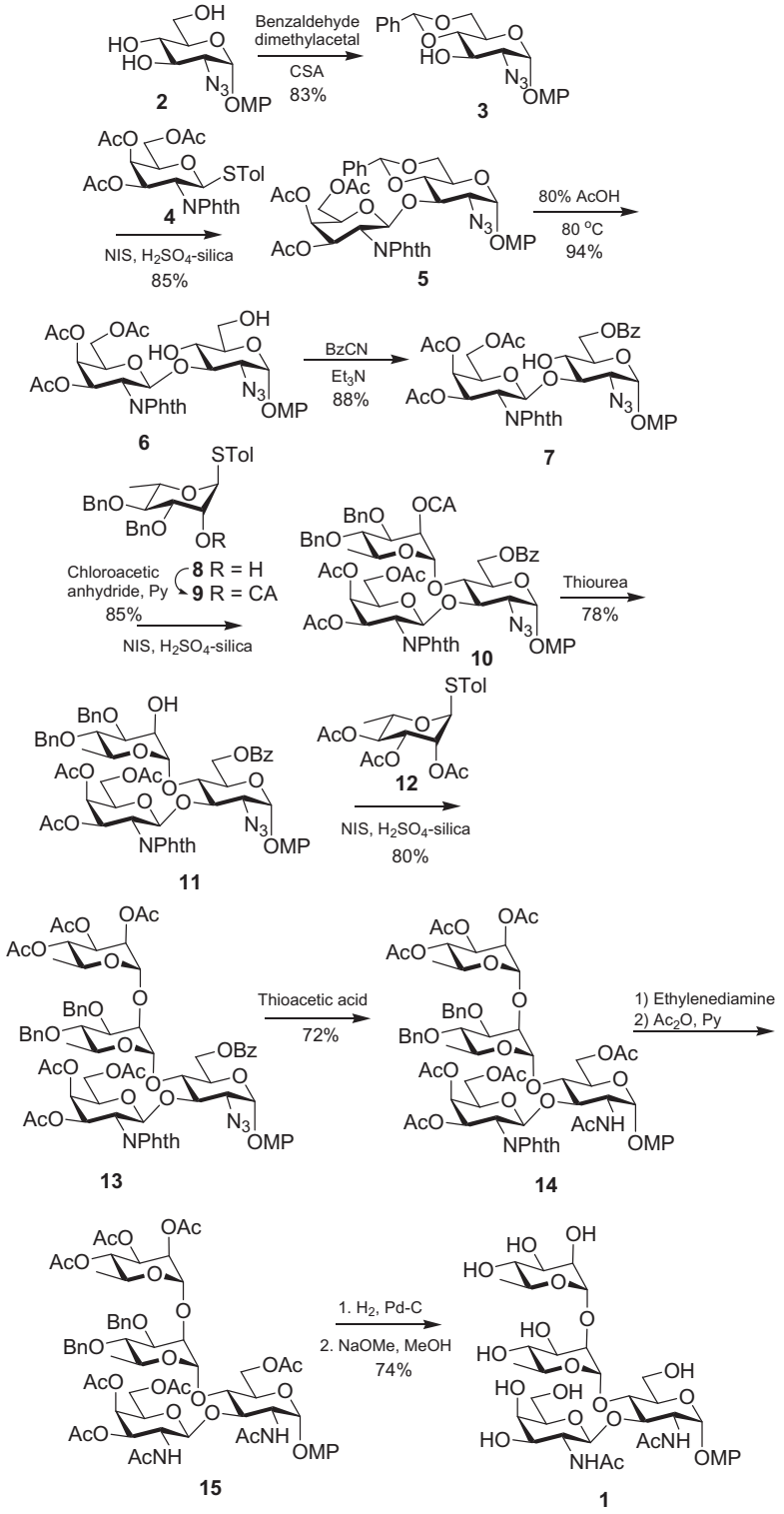
3. Conclusion

In summary, we have achieved the chemical synthesis of the tetrasaccharide repeating unit of the O-antigenic polysaccharide from *Plesiomonas shigelloides* strain AM36565 in the form of its *p*-methoxyphenyl derivative through rational protecting group manipulations and stereoselective glycosylations using thioglycosides activated by H_2SO_4–silica in conjunction with NIS. Further, the *p*-methoxyphenyl group can be cleaved selectively from the per-O-acetylated tetrasaccharide and conjugated with specific aglycon using trichloroacetimidate chemistry (Scheme 1).

4. Experimental section

4.1. General methods

All solvents and reagents were dried prior to use according to literature methods.[27] The commercially purchased reagents were used without any further purification unless mentioned otherwise. Dichloromethane wad dried and distilled over P_2O_5 to make it anhydrous and 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. Flash chromatography was performed with Silica Gel 230–400 mesh. Optical rotations were measured on sodium D-line at ambient temperature. ^1H and ^{13}C NMRs were recorded on Bruker 500 MHz spectrometer. In the case of the tetrasaccharide, ^1H NMR values are denoted as H for the reducing end



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

glucosamine unit, H′ for the galactosamine unit, H″ for the rhamnose unit attached to the glucosamine unit, and H‴ for the remaining rhamnose unit.

4.2. Preparation of H_2SO_4–silica

To slurry of silica gel 230–400 mesh (10 g) in dry Et_2O (20 mL) was added concd. H_2SO_4 (1 mL) and the mixture was hand shaken for couple of minutes. Then the solvents were evaporated in vacuo and the residue was dried at 100 °C for 3 h. The dried material was kept in an airtight bottle for further use.

4.3. *p*-Methoxyphenyl 2-azido-4,6-*O*-benzylidiene-2-deoxy-α-D-glucopyranoside (3)

To a mixture of known *p*-methoxyphenyl 2-azido-2-deoxy-α-D-glucopyranoside (**2**)[8] (3.0 g, 9.6 mmol) in dry acetonitrile (30 mL) was added benzaldehyde dimethyl acetal (2.2 mL, 14.5 mmol) fol-

lowed by a catalytic amount of CSA at room temperature. The pH was checked till optimum acidity (pH ~3 to 4) was attained. The reaction mixture was allowed to stir for 2 h until the TLC (*n*-hexane/EtOAc; 1:1) showed complete conversion of the starting material. Then the solution was neutralized with Et_3N, the solvent was evaporated in vacuo and the crude mixture thus obtained was purified by flash chromatography using *n*-hexane/EtOAc (2:1) as the eluent to afford pure compound **3** (3.2 g, 83%) as white amorphous mass. $[\alpha]_D^{25}$ +103° (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 7.51–6.85 (m, 9H, ArH), 5.56 (s, 1H, C*H*Ph), 5.45 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.41(t, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 4.26 (dd, 1H, $J_{5,6b}$ 5.0 Hz, $J_{6a,\ 6b}$ 10.0 Hz, H-6b), 4.06 (m, 1H, H-5), 3.79 (s, 3H, $C_6H_4OCH_3$), 3.76 (m, 1H, H-6a), 3.6 (t, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4), 3.4 (dd, 1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 9.5 Hz, H-2), 2.95 (br s, 1H, O*H*). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 129.4, 128.4(2), 126.3(3), 118.2(2), 114.7, 114.6 (Ar*C*), 102.2 (*C*HPh), 98.3 (C-1), 81.7 (C-4), 68.7 (C-3), 68.6 (C-6), 63.0 (C-5), 62.9 (C-2), 55.6 ($C_6H_4OCH_3$). HRMS calcd for $C_{20}H_{21}N_3O_6$ $(M+Na)^+$: 399.3972, found: 399.3976.

4.4. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(13)-2-azido-4,6-*O*-benzylidiene-2-deoxy-α-D-glucopyranoside (5)

A mixture of acceptor **3** (1.1 g, 2.75 mmol) and donor **4**[10] (1.8 g, 3.30 mmol) and MS 4Å (2.5 g) in dry CH_2Cl_2 (25 mL) was stirred under nitrogen atmosphere for 30 min. Then NIS (970 mg, 4.3 mmol) was added followed by the addition of H_2SO_4–silica (100 mg) and the mixture was stirred at 0 °C for 25 min till the TLC (*n*-hexane/EtOAc; 1:1) showed complete conversion of the acceptor **3**. The mixture was immediately filtered through a bed of Celite and 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 over anhydrous Na_2SO_4, and filtered. The solvents were evaporated in vacuo. The crude residue was purified by flash chromatography using *n*-hexane/EtOAc (1:1.2) to afford pure disaccharide **5** (1.9 g, 85%) as white foam. $[\alpha]_D^{25}$ +97° (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 7.87–6.78 (m, 13H, ArH), 5.86 (dd, 1H, $J_{2',3'}$ 11.5 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 5.57 (s, 1H, C*H*Ph), 5.54 (d, 1H, $J_{1',2'}$ 8.5 Hz, H-1′), 5.44 (d, 1H, $J_{3',4'}$ 3.5 Hz, H-4′), 5.32 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.62 (dd, $J_{1',2'}$ 8.5 Hz, $J_{2',3'}$ 11.5 Hz, 1H, H-2′), 4.22 (m, 2H, H-3, H-6a′), 4.12 (dd, 1H, $J_{5,6a}$ 4.0 Hz, $J_{6a,6b}$ 11.0 Hz, H-6a), 4.0 (m, 1H, H-5), 3.93 (m, 1H, H-5′), 3.84 (dd, 1H, $J_{5,6a}$ 5.5 Hz, $J_{6a,6b}$ 11.0 Hz, H-6b), 3.74 (s, 3H, $C_6H_4OCH_3$), 3.71 (m, 2H, H-4, H-6b′), 3.27 (dd, 1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 9.5 Hz, H-2), 2.17, 1.96, 1.85 (3s, 9H, 3 × $COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.1, 170.0, 169.6 (3 × $COCH_3$), 168.3, 167.5 (2 × phthalimido-*C*O), 136.8, 134.1, 134.0(2), 131.6, 131.5, 131.3, 129.0, 28.1(2), 128.0, 126.1, 126.0(2), 125.8(2), 123.4, 123.3, 118.1, 117.9(2) (Ar*C*), 101.3 (*C*HPh), 99.2 (C-1′), 97.7 (C-1), 55.4 ($C_6H_4OCH_3$), 20.6, 20.5, 20.4, (3 × $COCH_3$). HRMS calcd for $C_{40}H_{40}N_4O_{15}$ $(M+Na)^+$: 816.2490, found: 816.2493.

4.5. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(13)-2-azido-2-deoxy-α-D-glucopyranoside (6)

Compound **5** (1.9 g, 2.3 mmol) was dissolved in AcOH/H_2O (9:1, 20 mL) and the solution was stirred at 80 °C for 2 h until the starting material was completely converted into a more polar compound as observed by TLC (*n*-hexane/EtOAc; 1:1). After evaporating the solvents and co-evaporating with toluene, the crude product was purified by flash chromatography using *n*-hexane/EtOAc (1:1) as the eluent to afford pure disaccharide diol **6** (1.6 g, 94%) as white foam. $[\alpha]_D^{25}$ +103° (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 7.87–6.76 (m, 8H, ArH), 5.92 (dd, 1H, $J_{2',3'}$ 11.5 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 5.51 (d, 1H, $J_{3',4'}$, 3.5 Hz, H-4′), 5.47 (d, 1H, $J_{1',2'}$ 8.5 Hz, H-1′), 5.31 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.62 (dd, 1H, $J_{1',2'}$ 8.5 Hz, $J_{2',3'}$ 11.5 Hz, H-2′), 4.25 (m, 3H, H-6a′, H-5′, H-6a), 3.9 (dd, 1H, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 9.0 Hz, H-3), 3.8 (m, 3H, H-5, H-6b′, H-6a), 3.73 (s, 3H, $C_6H_4OCH_3$), 3.64 (t, 1H, $J_{3,4}$ $J_{4,5}$ 9.0 Hz, H-4), 3.12 (dd, 1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 11.0 Hz, H-2), 2.21, 2.07, 1.87 (3s, 9H, 3 × $COCH_3$), 1.25 (s, 2H, OH). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.4, 170.1, 169.6 (3 × $COCH_3$), 168.3, 167.6 (2 × phthalimido *C*O), 134.3, 134.2, 131.6, 131.3, 123.6, 123.5, 118.1(3), 114.7(3) (Ar*C*), 99.8 (C-1′), 97.4 (C-1), 55.6 ($C_6H_4OCH_3$), 20.6, 20.5, 20.4, (3 x $COCH_3$). HRMS calcd for $C_{33}H_{36}N_4O_{15}$ $(M+Na)^+$: 728.2177, found: 728.2181.

4.6. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(13)-6-*O*-benzoyl-2-azido-2-deoxy-α-D-glucopyranoside (7)

To a solution of compound **6** (1.6 g, 2.2 mmol) in CH_3CN (15 mL), BzCN (260 μL, 2.2 mmol) was added followed by Et_3N (50 μL) and the mixture was stirred at 0 °C for 10 min until TLC (*n*-hexane/EtOAc; 1:1) showed complete consumption of the starting material. After quenching the reaction by adding MeOH (1 mL), the solvents were evaporated in vacuo and the crude product was purified by flash chromatography using *n*-hexane/EtOAc (1:1) to afford pure disaccharide acceptor **7** (1.6 g, 88%) as white foam. $[\alpha]_D^{25}$ +98° (*c* 1.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 7.95–6.78 (m, 13H, ArH), 5.95 (dd, 1H, $J_{2',3'}$ 11.5 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 5.53 (d, 1H, $J_{3',4'}$, 3.5 Hz, H-4′), 5.49 (d, 1H, $J_{1',2'}$ 8.5 Hz, H-1′), 5.33 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.65 (dd, 1H, $J_{1',2'}$ 8.5 Hz, $J_{2',3'}$ 11.5 Hz, H-2′), 4.28 (dd, 1H, $J_{5,\ 6a'}$ 6.5 Hz, $J_{6a',\ 6b'}$ 12.0 Hz, H-6a′), 4.22(m, 3H, H-5′, H-6a, H-6b), 4.09 (m, 1H, H-5), 3.93 (dd, 1H, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 8.5 Hz, H-3), 3.7 (s, 3H, $C_6H_4OCH_3$), 3.66 (t, 1H, $J_{3,4}$ $J_{4,5}$ 9.0 Hz, H-4), 3.17 (dd, 1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 11.0 Hz, H-2), 2.21, 2.07, 1.88 (3s, 9H, 3 × $COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5, 170.1, 169.6 (3 × $COCH_3$), 168.3, 167.6 (2 × phthalimido *C*O), 166.2 (*C*OPh), 134.3, 134.2, 133.0(2), 131.5, 131.3, 130.1, 129.8, 129.7(3), 128.4, 128.3(3), 123.6, 123.5, 118.1(3), 114.5(3) (Ar*C*), 99.9 (C-1′), 97.0 (C-1), 55.5 ($C_6H_4OCH_3$), 20.6, 20.5, 20.4, (3 x $COCH_3$). HRMS calcd for $C_{40}H_{40}N_4O_{16}$ $(M+Na)^+$: 832.2439, found: 832.2442.

4.7. *p*-Tolyl 3,4-di-*O*-benzyl-2-*O*-chloroacetyl-1-thio-α-L-rhamnopyranoside (9)

To a mixture of *p*-thio-3,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**8**) (3.5 g, 7.8 mmol) in dry CH_2Cl_2 (20 mL) was added pyridine (5 mL) followed by the addition of choloroacetic anhydride (2.0 g, 11.7 mmol) keeping the temperature at 0 °C. The reaction mixture was allowed to stir at the same temperature for 1 h until complete conversion of the starting material as evident from TLC (*n*-hexane/EtOAc; 1:1). Then pyridine was co-evaporated with toluene and the crude product obtained was purified by flash chromatography using *n*-hexane/EtOAc (2:1) as the eluent to afford pure **9** (3.5 g, 6.65 mmol, 85%) as colorless syrup. $[\alpha]_D^{25}$ +81° (*c* 1.1, $CHCl_3$). IR (neat): 3438, 2853, 1721, 1519, 1389, 1267, 1081, 990, 710 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.40–7.15 (m, 14H, ArH), 5.68 (dd, 1H, $J_{1,2}$ 2.0 Hz, $J_{2,3}$ 3.5 Hz, H-2), 5.37 (d, $J_{1,2}$ 2.0 Hz, H-1), 4.93 (d, 1H, *J* 10.5 Hz, CH_2Ph), 4.74 (d, 1H, *J* 11.5 Hz, CH_2Ph), 4.65 (d, 1H, *J* 11.5 Hz, CH_2Ph), 4.59 (d, 1H, *J* 10.5 Hz, CH_2Ph), 4.26 (m, 1H, H-5), 4.16 (d, 2H, *J* 2.5 Hz, COCH_2Cl), 3.96 (dd, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.5 Hz, H-3), 3.49 (t, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4), 2.35 (s, 3H, S-$C_6H_4CH_3$), 1.36(d, 3H, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 138.1(2), 137.4, 132.4(2), 129.9(2), 129.6, 128.6(2), 128.4(2), 128.2(2), 127.9(3), 127.8 (Ar*C*), 86.1 (C-1), 79.9 (C-4), 78.1 (C-3), 72.5 (C-2), 69.0 (C-5), 40.8 (COCH_2Cl), 21.1 (S-$C_6H_4CH_3$), 17.8 (C–CH_3). HRMS calcd for $C_{29}H_{31}ClO_5S$ $(M+Na)^+$: 526.1581, found: 526.1584.

4.8. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(13)-4-*O*-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2-azido-6-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (11)

A mixture of acceptor **7** (1.6 g, 1.9 mmol) and donor **9** (1.3 g, 2.5 mmol) and MS 4Å (2 g) in dry CH_2Cl_2 (20 mL) was stirred under nitrogen atmosphere for 30 min. Then NIS (730 mg, 3.25 mmol) was added followed by the addition of H_2SO_4–silica (75 mg) and the mixture was stirred at 0 °C for 20 min till the TLC showed complete consumption of the donor **9**. The mixture was immediately filtered through a bed of Celite and 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 over anhydrous Na_2SO_4, and filtered. The solvents were evaporated in vacuo. The crude product was purified by flash chromatography using *n*-hexane/EtOAc (1.5:1) to give the required trisaccharide **10** with traces of unreacted acceptor **7**. The mixture was subjected to the next reaction without further purification. To a solution of the mixture (1.3 g, 1.05 mmol) in CH_2Cl_2/MeOH (2:3, 50 mL) was added thiourea (400 mg, 5.3 mmol) followed by the addition of 2,4,6-collidine (700 μL, 5.3 mmol) and the mixture was allowed to stir under reflux for 20 h until complete conversion of the starting material into a more polar compound as evident from TLC (*n*-hexane/EtOAc; 1:1). After cooling to room temperature, the solution was diluted with CH_2Cl_2 (20 mL) and washed successively with 1 M HCl followed by saturated aq. $NaHCO_3$ (2 × 30 mL) and brine (25 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, and the solvents were evaporated in vacuo. The crude product was purified by flash chromatography using *n*-hexane/EtOAc (1:1) as the eluent to give pure trisaccharide **11** as colorless foam (3.3 g, 78%). $[\alpha]_D^{25}$ +121° (*c* 0.9, $CHCl_3$). IR (neat): 3441, 2847, 1716, 1513, 1394, 1263, 1088, 995, 703 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 8.05–6.63 (m, 24H, ArH), 6.07 (dd, 1H, $J_{2',3'}$ 11.5 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 5.49 (d, 1H, $J_{3',4'}$, 3.5 Hz, H-4′), 5.46 (d, 1H, $J_{1',2'}$ 8.5 Hz, H-1′), 5.27 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.98 (d, 1H, *J* 11.5 Hz, CH_2Ph), 4.93 (s, 1H, H-1″), 4.75 (d, 1H, *J* 11.5 Hz, CH_2Ph), 4.67 (m, 3H, CH_2Ph, H-6a′), 4.53 (m, 2H, H-2′, H-5″), 4.42 (dd, 1H, $J_{5,\ 6a}$ 6.0 Hz, $J_{6a,\ 6b}$ 11.0 Hz, H-6a), 4.26 (m, 3H, H-3, H-6b, H-6b′), 4.16 (m, 2H, H-5, H-5′), 4.12 (m, 1H, H-2″), 3.84 (dd, 1H, $J_{2'',3''}$ 3.0 Hz $J_{3'',4''}$ 9.0 Hz, H-3″), 3.81 (t, 1H, $J_{3,4}$, $J_{4,5}$ 8.5 Hz, H-4), 3.72 (s, 3H, $C_6H_4OCH_3$), 3.52 (t, 1H, $J_{3'',4''}$, $J_{4'',5''}$ 9.0 Hz, H-4″), 2.95 (dd, 1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 10.0 Hz, H-2), 2.63 (br s, 1H, O*H*), 2.1, 1.83, 1.71 (3s, 9H, 3 × COCH_3), 1.48 (d, 3H, *J* 6.5 Hz, C–CH_3) ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5, 170.3, 169.5 (3 × *C*OCH$_3$), 168.1, 167.6 (2 × phthalimido *C*O), 165.9 (*C*OPh), 134.3, 134.0, 133.2, 131.8, 131.2, 129.8(3), 129.5, 128.5(3), 128.4(2), 128.2(3), 127.8(3), 123.6(2), 123.5, 123.4, 118.1(2), 114.6(3),114.5(3) (Ar*C*), 99.5 (C-1″), 98.9 (C-1′), 97.0 (C-1), 55.5 ($C_6H_4OCH_3$), 21.0, 20.7, 20.4, (3 × *C*OCH$_3$), 17.6 (C–*C*H$_3$). HRMS calcd for $C_{60}H_{62}N_4O_{20}$ $(M+Na)^+$: 1158.3957, found: 1158.3959.

4.9. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(12)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(14)-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-2-azido-6-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (13)

A mixture of acceptor **11** (1.7 g, 1.5 mmol) and donor **12** (750 mg, 1.9 mmol) and MS 4Å (2 g) in dry CH_2Cl_2 (20 mL) was stirred under nitrogen atmosphere for 30 min. Then NIS (555 mg, 2.5 mmol) was added followed by H_2SO_4–silica (60 mg) and the mixture was stirred at 0 °C for 20 min till the TLC (*n*-hexane/EtOAc; 1:1) showed complete consumption of the donor **12**. The mixture was immediately filtered through a bed of Celite and 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 over anhydrous Na_2SO_4, and filtered. The solvents were evaporated in vacuo. The crude residue was purified by flash chromatography using *n*-hexane/EtOAc (1:1) to afford pure tetrasaccharide **13** as white foam (1.7 g, 80%). $[\alpha]_D^{25}$ +88° (*c* 0.9, $CHCl_3$). IR (neat): 2940, 1762, 1724, 1515, 1387, 1363, 1239, 1229, 1059, 751, 695 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.97–6.7 (m, 25H, ArH), 6.07 (dd, 1H, $J_{2',3'}$ 11.5 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 5.49 (d, 1H, $J_{3',4'}$, 3.5 Hz, H-4′), 5.46 (d, 1H, $J_{1',2'}$ 8.5 Hz, H-1′), 5.4 (m, 1H, H-2‴), 5.28 (dd, 1H, $J_{3''',4'''}$ 3.5 Hz, $J_{4''',5'''}$ 10.0 Hz, H-4‴), 5.25 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.98 (m, 2H, H-3‴, CH_2Ph), 4.94 (s, 1H, H-1‴), 4.91 (s, 1H, H-1″), 4.8 (m, 2H, H-6a′, CH_2Ph), 4.67 (dd, 2H, *J* 11.5 Hz, CH_2Ph), 4.57 (m, 1H, H-5″), 4.51 (dd, 1H, $J_{1',2'}$ 8.5 Hz, $J_{2',3'}$ 11.5 Hz, H-2′), 4.45 (dd, 1H, $J_{5,\ 6a}$ 5.5 Hz, $J_{6a,\ 6b}$ 10.5 Hz, H-6a), 4.27 (dd, 1H, $J_{5,\ 6b}$ 8.0 Hz, $J_{6a,\ 6b}$ 10.5 Hz, H-6b), 4.15 (m, 4H, H-3, H-5, H-5′, H-6b′), 4.06 (m, 1H, H-2″), 3.84 (m, 2H, H-3″, H-5″), 3.75 (t, 1H, $J_{3,4}$, $J_{4,5}$ 8.5 Hz, H-4), 3.72 (s, 3H, $C_6H_4OCH_3$), 3.61 (t, 1H, $J_{3'',4''}$, $J_{4'',5''}$ 9.0 Hz, H-4″), 2.95 (dd, 1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 10.0 Hz, H-2), 2.1, 2.05, 1.97, 1.86, 1.82, 1.71 (6s, 8H, 6 × COCH_3), 1.51 (d, 3H, *J* 6.0 Hz, C–CH_3), 1.02 (d, 3H, *J* 6.5 Hz, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5, 170.3, 169.9(2), 169.7, 169.5 (6 × *C*OCH$_3$), 168.1, 167.6 (2 × phthalimido *C*O), 165.7 (*C*OPh), 138.7, 138.2, 134.3, 134.0, 133.2, 131.9, 129.7, 129.8(2), 129.4, 129.0, 128.5(2), 128.3(3), 128.2(3), 128.1(3), 127.5, 127.3, 127.2(3), 123.5, 123.4, 118.0, 114.7(Ar*C*), 99.7 (C-1″), 99.0 (C-1‴), 98.9 (C-1′), 96.8(C-1), 55.5 ($C_6H_4OCH_3$), 22.6, 20.8, 20.7, 20.5, 20.4, 20.0 (6 × *C*OCH$_3$), 17.6, 17.4 (2 × C–*C*H$_3$). HRMS calcd for $C_{72}H_{78}N_4O_{27}$ $(M+Na)^+$: 1430.4854, found: 1430.4858.

4.10. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(12)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(14)-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-2-acetamido-6-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (14)

Compound **13** (1.7 g, 1.2 mmol) was dissolved in thioacetic acid (20 mL) and the mixture was stirred at room temperature for 3 days in the dark, until the TLC (*n*-hexane/EtOAc; 1:1) showed complete conversion of the starting material. The solvent was evaporated and co-evaporated with toluene and the crude residue thus obtained, was purified by flash chromatography to yield the desired tetrasaccharide **14** (1.2 g, 72%) as white foam. $[\alpha]_D^{25}$ +94° (*c* 0.8, $CHCl_3$). IR (neat): 2935, 1760, 1721, 1511, 1383, 1361, 1249, 1225, 1056, 758, 691 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.97–6.69 (m, 26H, ArH), 5.95 (dd, 1H, $J_{2',3'}$ 12.0 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 5.78 (d, 1H, $J_{3',4'}$, 3.5 Hz, H-4′), 5.46 (d, 1H, $J_{1',2'}$ 8.5 Hz, H-1′), 5.4 (m, 1H, H-2‴), 5.27 (dd, 1H, $J_{3''',4'''}$ 3.5 Hz, $J_{4''',5'''}$ 10.0 Hz, H-4‴), 5.06 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.95 (m, 3H, H-3‴, CH_2Ph, H-1‴), 4.92 (s, 1H, H-1″), 4.71 (m, 4H, H-6a′, CH_2Ph(3)), 4.48 (m, 2H, H-5″, H-2′), 4.18 (m, 3H, H-6a, H-6b, H-5′), 4.09 (m, 3H, H-3, H-5,, H-6b′), 4.03 (m, 1H, H-2″), 3.87 (m, 2H, H-3″, H-5″), 3.78 (t, 1H, $J_{3,4}$, $J_{4,5}$ 8.5 Hz, H-4), 3.71 (s, 3H, $C_6H_4OCH_3$), 3.62 (m, 2H, H-4″, H-2), 2.11, 2.06, 2.05, 1.97, 1.90, 1.78, 1.68 (7s, 21H, 7 × COCH_3), 1.56 (d, 3H, *J* 6.0 Hz, C–CH_3), 1.27 (m, 3H, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.8, 170.7, 170.6, 170.0, 169.8, 169.7, 169.6 (7 × *C*OCH$_3$), 168.3, 167.4 (2 × phthalimido *C*O), 165.8 (*C*OPh), 134.1, 134.0, 133.11, 131.8, 131.2, 129.8(2), 129.4, 128.4(2), 128.3(3), 128.1(3), 127.9(2), 127.4, 127.3, 127.2(2), 123.5, 123.4, 117.8(2), 114.7(2) (Ar*C*), 99.6 (C-1″), 99.0 (C-1‴), 98.6 (C-1′), 96.6(C-1), 55.5 ($C_6H_4OCH_3$), 22.6, 20.8, 20.7(2), 20.6, 20.4, 19.9 (7 × *C*OCH$_3$), 17.9, 17.4 (2 × C–*C*H$_3$). HRMS calcd for $C_{69}H_{80}N_2O_{28}$ $(M+Na)^+$: 1384.4898. found: 1384.4899.

4.11. *p*-Methoxyphenyl α-L-rhamnopyranosyl-(12)-α-L-rhamnopyranosyl-(14)-3-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-2-acetamido-2-deoxy-α-D-glucopyranoside (1)

To the solution of compound **14** (1.1 g, 0.8 mmol) in *n*-butanol (20 mL), ethylenediamine (1.2 mL) was added and the reaction mixture was allowed to stir for 24 h at 110 °C. Then the solvents were evaporated and co-evaporated with toluene and the crude product thus obtained is dissolved in pyridine (5 mL) followed by the addition of Ac_2O (5 mL). The reaction mixture was allowed to stir at room temperature for 10 h when the TLC (*n*-hexane/EtOAc; 1:1) showed complete conversion of the starting material. The reaction mixture was then co-evaporated with toluene to give the required tetrasaccharide, *p*-methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(12)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(14)-3-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-galactopyranosyl)-2-acetamido-6-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**15**). A dilute solution of compound **15** in MeOH (50 mL) and AcOH (1 mL) was passed through the flow hydrogenation assembly fitted with a Pd–C cartridge at 50 °C at normal atmospheric pressure of hydrogen. TLC (CH_2Cl_2/MeOH; 4:1) showed complete conversion of the starting material after three cycles. The solvents were evaporated in vacuo and the residue was dissolved in MeOH (5 mL) followed by the addition of NaOMe in MeOH (1 mL, 0.5 M) and stirred at 50 °C for 14 h. Then the solution was neutralized by DOWEX 50 W H^+ resin. The mixture was filtered through a cotton plug to remove DOWEX and the filtrate was evaporated in vacuo to afford pure target tetrasaccharide **1** (330 mg, 80%) as white sticky compound. $[\alpha]_D^{25}$ +57° (*c* 0.7, MeOH). IR (neat): 3438, 3019, 1708, 1467, 1233, 773, 672 cm^{-1}. ^1H NMR (500 MHz, D_2O) δ: 7.04–6.82 (m, 4H, ArH), 5.17 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.06 (s, 1H, H-1‴), 4.92 (d, 1H, $J_{1'',2''}$ 1.0 Hz, H-1″), 4.79 (d, 1H, $J_{1',2'}$ 8.5 Hz, H-1′), 4.55 (m, 1H, H-2″), 4.16 (m, 2H, H-2, H-2‴), 3.97 (m, 1H, H-4″), 3.82 (m, 6H, H-3, H-3′, H-4′, H-4‴, H-6a, H-6b), 3.72 (s, 3H, $C_6H_4OCH_3$), 3.62 (m, 8H, H-4, H-5, H-5′, H-5″, H-2′, H-5‴, H-6a′, H-6b′), 3.29 (m, 3H, H-5, H-3″, H-3‴), 2.07, 1.97 (2s, 6H, 2 × $COCH_3$), 1.33 (d, 3H, *J* 6.0 Hz, C–CH_3), 1.25 (d, 30H, *J* 6.5 Hz, C–CH_3). ^{13}C NMR (125 MHz, D_2O) δ: 174.4, 173.6 (2 × $COCH_3$), 119.4 (3), 115.6 (2) (Ar*C*), 99.0 (C-1), 99.1 (C-1‴), 102.4 (C-1′), 104.0 (C-1″), 23.3, 23.2 (2 × $COCH_3$), 18.3, 18.0 (2 × C–CH_3). HRMS calcd for $C_{35}H_{54}N_2O_{20}$ $(M+Na)^+$: 822.3270, found: 822.3273.

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