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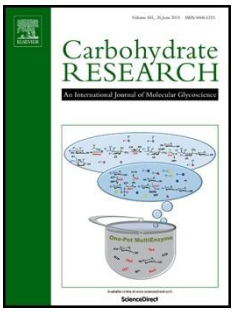

Title: Synthesis of a trisaccharide repeating unit of the o-antigen from *burkholderia anthina* and its dimer

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Synthesis of a Trisaccharide Repeating Unit of the O-Antigen from *Burkholderia anthina* and Its Dimer

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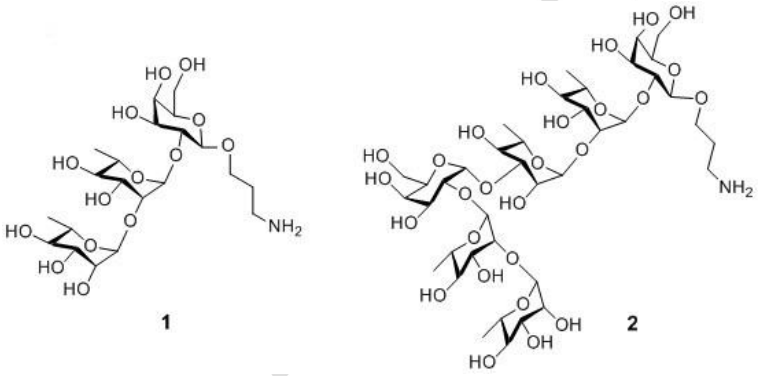
Highlights:

- A trisaccharide repeating unit of the O-antigen from *Burkholderia anthina* and its dimer-hexasaccharide were synthesized via a highly convergent and efficient assembly strategy.
- The α-1,2-linked disaccharide was successfully achieved by the armed-disarmed glycosylation strategy.
- The synthetic oligosaccharide fragments are useful antigen targets for the development of O-antigen-based vaccines against *B. anthina* infectious disease.

Graphical Abstract



Abstract: A trisaccharide repeating unit of the O-antigen from *Burkholderia anthina*, α-L-Rha-(1→2)-α-L-Rha-(1→2)-β-D-Gal-O$(CH_2)_3NH_2$ (**1**), and its dimer, α-L-Rha-(1→2)-α-L-Rha-(1→2)-α-D-Gal-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→2)-β-D-Gal-O$(CH_2)_3NH_2$ (**2**), were synthesized via a highly convergent and efficient assembly strategy. Sequential glycosylation of galactosyl acceptor **6** with rhamnosyl thioglycoside **7**, followed by

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condensation of the resulting disaccharide acceptor **9** with rhamnosyl imidate donor **10**, gave the title molecule **1** after global deprotection. The title hexasaccharide **2** was assembled in a convergent [2+2+2] manner, in which α-1,2-linked disaccharide **12** was initially obtained by the coupling reaction of disarmed thiorhamnoside acceptor **15** with armed thiogalactoside donor **14**. Sequential glycosylation of disaccharide acceptor **9** with thioglycoside donors **12** and **13** afforded the target compound **2** after global deprotection.

Keywords: *Burkholderia anthina*; Lipopolysaccharide; O-Antigen; Carbohydrate vaccine; Synthesis

1. Introduction

Lipopolysaccharides (LPSs) on the bacterial cell surface, which are the major components of bacterial cell glycocalyx of Gram-negative bacteria, are important virulence factors for bacterial pathogenicity.[1,2] O-Polysaccharides (OPSs), or O-antigens, are prominent part of LPSs exposed on the bacterial cell surface, which have exhibited important roles in bacterial immunology, such as protecting bacteria against host immune recognition, complement attack, and other related responses.[2,3] Thereby, they are considered as the attractive candidates for the development of novel protective and therapeutic carbohydrate-based antibacterial vaccines. [4]

The *Burkholderia cepacia complex* (Bcc), a group of phenotypically similar but genotypically distinct Gram-negative bacteria,[5,6] is recognized as an important opportunistic human pathogen that can cause fatal lung infections in cystic fibrosis patients, resulting in rapid and clinically uncontrollable "cepacia syndrome" such as necrotizing pneumonia and septicemia that usually lead to high mortality.[7-11] Therefore, the development of safe and effective therapeutic protocols for prevention and treatment of Bcc infections are in urgent need. It has been clearly revealed that the LPS O-antigens of Bcc were associated with bacterial invasion and virulence.[12,13] For example, the O-antigen of *B. cenocepacia* K56-2 was conductive to inflammatory cytokine IL-1β production.[12] Moreover, it also modulated phagocytosis of macrophages and interfered with bacterial adhesion to bronchial epithelial cells.[13] Thus, the LPS O-antigens from Bcc bacteria are useful antigen targets in developing carbohydrate-based vaccines against Bcc infection.[14,15]

Many O-antigens from Bcc have been structurally elucidated.[16] For example, Molinaro and coworkers have recently isolated and identified the O-antigen from *B. anthina* strain LMG20983

with the following trisaccharide repeat unit: α-L-Rha-(1→2)-α-D-Rha-(1→2)-α-D-Gal-(1→3)[17] (Figure 1). Subsequently, Vogel and coworkers reported the chemical synthesis of one form of this trisaccharide repeating unit and its dimer in which the L-rhamnosyl residue was the reducing end.[18,19] Using NMR technology and molecular dynamics (MD) simulation approaches, Silipo and coworkers investigated the binding between these synthetic oligosaccharide ligands and the lipopolysaccharide-specific monoclonal antibody (5D8).[19] Saturation transfer difference (STD) NMR experiments revealed that the D-galactosyl residue might play an important role in the intermolecular interactions because it gave rise to significant STD signals compared to that of the rhamnosyl residues. In view of the important role played by galactosyl residue, we present herein a chemical synthesis of the trisaccharide repeat unit of O-antigen from *B. anthina* and its dimer **1-2** containing terminal D-galactosyl residue (Figure 1). Moreover, these synthetic targets were designed to contain a free amino group linked to their reducing end, which would enable their regioselective conjugation with carrier molecules to generate glycoconjugate vaccines.

2. Results and Discussion

The synthesis of compound **1** is outlined in Scheme 1. First, 1-thio-β-D-galactopyranoside (IPTG) **3** was converted into **4** in a good yield of 78% via tin complex-directed[20] regioselective benzylation. Thereafter, the 4,6-*O*-positions in **4** were protected with α,α-dimethoxytoluene using a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) in CH_3CN[21] to afford **5** in a 95% yield. As previously reported,[22] the 2-*O*-unprotected thioglycoside **5** was directly used as a glycosyl donor for the glycosylation of 3-azido-1-propanol[23] under the influence of *N*-iodosuccinimide (NIS) and trimethylsilyl triflate (TMSOTf) at -40 ^{o}C to generated β-linked **6**[24] selectively in a high yield (80%). The coupling constant of H-1 signal ($J_{1,2}$ = 7.8 Hz) at δ 4.30 ppm in the ^{1}H NMR spectrum of **6** confirmed the β-configuration of the new glycosidic bond. Glycosylation of **6** with thiorhamnoside **7**[25] under the promotion of NIS and TMSOTf furnished disaccharide **8** that was deacetylated with sodium methoxide in methanol to afford **9** as a glycosyl acceptor in an overall yield of 82% for 2 steps. Then, **9** was glycosylated with trichloroacetimidate **10**[26] in the presence of TMSOTf in CH_2Cl_2 at 0 ^{o}C smoothly to produce the fully protected trisaccharide **11** in an 85% yield. Finally, global deprotection of **11** in 2 steps, including debenzoylation with sodium methoxide in methanol and debenzylation under hydrogenation conditions in methanol and H_2O (9:1) with 10% Pd/C as the catalyst, afforded

trisaccharide **1** in a good 75% overall yield, after purification by size-exclusion chromatography on a Bio-Gel P-2 column. The ^{1}H, ^{13}C NMR and MS data of the product were in full agreement with the desired structure of **1**.

For the synthesis of compound **2**, the dimer of the above repeating unit, the least straightforward step was constructing the α-galactopyranosyl linkage. Accordingly, our plan was to accomplish the α-galactopyranosylation first, and the whole molecule could be assembled via a convergent [2+2+2] strategy with **9**, **12** and **13** as the key disaccharide blocks (Scheme 2). Consequently, the α-1,2-linked disaccharide **12** could be prepared from glycosylation of disarmed thiorhamnoside **15** with armed thiogalactoside **14**,[27] whereas **13** could be obtained from the coupling reaction of rhamnosyl acceptor **16**[25] with trichloroacetimidate **10**.

As shown in Scheme 3, treatment of **5** with *para*-methoxybenzyl chloride and sodium hydride in DMF at 0 ^{o}C afforded thiogalactoside **14** (87%) smoothly, which was used to successfully glycosylate the disarmed thiorhamnoside **15** under the promotion of NIS and AgOTf in Et_2O[28] at -30 ^{o}C to furnish the desired disaccharide **12** in a 73% yield. The α-linkage in **12** was assigned based on the coupling constant of the galactosyl H-1' signal ($J_{1',2'}$ = 3.6 Hz) at δ 5.27 ppm of its ^{1}H NMR spectrum. On the other hand, glycosylation of **16** with trichloroacetimidate **10** in the presence of a catalytic amount of TMSOTf in anhydrous CH_2Cl_2 at 0 ^{o}C afforded disaccharide **13** in an excellent yield (85%).

With disaccharides **9**, **12**, and **13** in hand, compound **2** was assembled in a [2+2+2] manner (Scheme 4). Glycosylation of **9** with **12** at -78 ^{o}C in CH_2Cl_2 using NIS and AgOTf as promoters gave tetrasaccharide **17**, which was then treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[29] in CH_2Cl_2 and H_2O (v/v 14:1) to selectively cleavage the PMB group at the galactosyl residue 2-O-postison, furnishing tetrasaccharide **18** in a 57% overall yield. Condensation reaction between **18** and **13** in CH_2Cl_2 was promoted with NIS and AgOTf at -78 ^{o}C to afford the fully protected hexasaccharide **19** in a high yield (85%). Subsequently, the target compound **2** was obtained upon global deprotection in two steps, as described for **1**, in a 90% overall yield. The $^{1}J_{CH}$ coupling constants of all rhamnosyl residues in the ^{1}H-coupled gHSQC spectrum of **2** were over 172 Hz, which confirmed the α-configuration of all rhamnosidic bonds. The synthetic

target and all of the synthetic intermediates involved were fully characterized with 1D, 2D ^{1}H and ^{13}C NMR spectrometry and MS.

In conclusion, a highly efficient and convergent strategy was developed for the synthesis of the trisaccharide repeating unit of *B. anthina* O-antigen and its dimer, in which the D-galactosyl residue is at the reducing end. The trisaccharide was assembled via stepwise glycosylation using monosaccharyl building blocks **6, 7** and **8**. The dimer-hexasaccharide was prepared via [2+2+2] glycosylation reaction, in which the α-1,2-linked disaccharide **12** was successfully achieved by the armed-disarmed glycosylation strategy. Moreover, there was a 3-aminopropanyl linker at the reducing end of both target molecules **1** and **2**, which would enable them convenient attachment to functionalized biomolecules, such as immunological carrier molecules, for the exploration of *B. anthina* immunology and the development of O-antigen-based vaccines against *B. anthina*.

3. Experimental Section

3.1 General methods: Optical rotations were determined at 25 ^{o}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 the DHO signal as reference when 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. MALDI-TOF mass spectra were recorded on an AXIMA Confidence spectrometer using 2,5-dihydroxybenzoic acid (DHB) as matrix. Thin layer chromatography (TLC) was performed on silica gel HF_{254} plates, detected by charring using 30% (v/v) H_2SO_4 in MeOH or by means of a UV detector. Silica gel column chromatography was conducted with mixtures of ethyl acetate and petroleum ether (b. p. 60–90 ^{o}C), hexane, or toluene as the eluents. Solution concentration was performed at <60 ^{o}C under diminished pressure.

3.2 Isopropyl 3-*O*-Benzyl-1-thio-β-D-galactopyranoside (4)

A mixture of **3** (IPTG, 3.0 g, 12.6 mmol) and Bu_2SnO (4.72 g, 18.9 mmol) in dry MeOH and toluene (v/v 1:1, 60 mL) was refluxed for 4 h. After the solvent was removed under reduced pressure, the resulting residue was dissolved in toluene (60 mL), and TBAB (0.81 g, 2.52 mmol)

and BnBr (2.3 mL, 18.9 mmol) were added. The mixture was stirred at 70 ^{o}C for 5 h, and then concentrated under reduced pressure. The above residue was diluted with CH_2Cl_2 (100 mL) and washed with water (2×50 mL). The organic phase was dried over Na_2SO_4, and then concentrated. Purification of the residue by column chromatography ($CHCl_3$/MeOH 15:1) yielded **4** (3.2 g, 78%) as a white solid. $[\alpha]_D^{25}$ -7^{o} (*c* 0.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.41-7.27 (m, 5H, Ph), 4.78 (d, 1H, *J* = 11.8 Hz, C*H*$_2$Ph), 4.76 (d, 1H, *J* = 11.8 Hz, C*H*$_2$Ph), 4.37 (d, 1H, *J* = 9.6 Hz, H-1), 4.04 (br s, 1H, H-4), 3.94 (ddd, 1H, *J* = 11.4, 6.8, 4.2 Hz, H-6a), 3.82-3.73 (m, 2H, H-2, H-6b), 3.52 (m, 1H, H-5), 3.45 (dd, 1H, *J* = 9.0, 3.6 Hz, H-3), 3.28-3.16 (m, 1H, -SC*H*-), 2.63 (s, 1H, -O*H*), 2.49 (s, 1H, -O*H*), 2.20 (m, 1H, -O*H*), 1.34 (d, 3H, *J* = 6.6 Hz, -SCH(C*H*$_3$)$_2$), 1.33 (d, 3H, *J* = 6.6 Hz, -SCH(C*H*$_3$)$_2$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 137.62, 128.62, 128.12, 127.92, 86.09, 81.14, 78.27, 72.20, 69.63, 67.43, 62.70, 35.56, 24.20, 24.09; MALDI-TOF MS: calcd for ($C_{16}H_{24}O_5S$+Na^+) *m/z*, 351.12; found, 351.74.

3.3 Isopropyl 3-*O*-Benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (5)

To a solution of **4** (3.0 g, 9.14mmol) in dry CH_3CN (20 mL) was added benzaldehyde dimethyl acetal (1.9 mL, 13.72 mmol) and TsOH (120 mg, 0.7 mmol) at rt. The reaction mixture was stirred at 50 ^{o}C for 5 h, at which time TLC indicated the completion of reaction. The mixture was neutralized with Et_3N and concentrated. The residue was purified by flush silica gel column chromatography with petroleum ether and ethyl acetate (3:1) as the eluents to afford **5** (3.61 g, 95%) as a white foamy solid. $[\alpha]_D^{25}$ -4^{o} (c 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.52-7.48 (m, 2H, Ph), 7.41-7.38 (m, 2H, Ph), 7.37-7.26 (m, 6H, Ph), 5.44 (s, 1H, C*H*Ph), 4.77 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.74 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.41 (d, 1H, *J* = 9.6 Hz, H-1), 4.27 (dd, 1H, *J* = 12.6, 1.2 Hz, H-6a), 4.17 (d, 1H, *J* = 3.6 Hz, H-4), 4.01 (td, 1H, *J* = 9.6, 1.2 Hz, H-2), 3.96 (dd, 1H, *J* = 12.6, 1.8 Hz, H-6b), 3.50 (dd, 1H, *J* = 9.6, 3.6 Hz, H-3), 3.36 (s, 1H, H-5), 3.32-3.23 (m, 1H, -SC*H*-), 2.68 (d, 1H, *J* = 1.2 Hz, -O*H*), 1.37 (d, 3H, *J* = 6.6 Hz, -SCH(C*H*$_3$)$_2$), 1.32 (d, 3H, *J* = 6.6 Hz, -SCH(C*H*$_3$)$_2$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 138.13, 137.86, 128.94, 128.77, 128.44, 128.12, 127.87, 127.83, 126.42, 125.89, 101.19, 85.48, 80.34, 73.51, 71.48, 70.00, 69.44, 68.46, 34.57, 24.49, 23.99; MALDI-TOF MS: calcd for ($C_{23}H_{28}O_5S$+Na^+) m/z, 439.15; found, 439.77.

3.4 3-Azidopropyl 3-*O*-Benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (6)

To a mixture of **5** (1.0 g, 2.4 mmol), 3-azido-1-propanol (364 mg, 3.6 mmol), and 4 Å MS (1.0 g) in anhydrous CH_2Cl_2 (15 mL) were added NIS (648 mg, 2.88 mmol) and AgOTf (10.2 mg, 0.04 mmol) at -20 ^oC under a N_2 atmosphere. The mixture was stirred under these conditions for 30 min, and then neutralized with Et_3N and concentrated. The residue was purified by column chromatography (petroleum ether/ethyl acetate 8:1) to give **6** (847 mg, 80%) as syrup. $[\alpha]_D^{25}$ 6^o (*c* 0.2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.53-7.49 (m, 2H, Ph), 7.40-7.37 (m, 2H, Ph), 7.37-7.26 (m, 6H, Ph), 5.45 (s, 1H, C*H*Ph), 4.74 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.72 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.30 (d, 1H, *J* = 7.8 Hz, H-1), 4.29 (dd, 1H, *J* = 12.6, 1.2 Hz, H-6a), 4.12 (d, 1H, *J* = 3.0 Hz, H-4), 4.04-3.96 (m, 3H, H-2, H-6b, -OC*H*$_2$-), 3.66-3.60 (m, 1H, -OC*H*$_2$-), 3.48 (dd, 1H, *J* = 9.6, 3.6 Hz, H-3), 3.47-3.40 (m, 2H,-C*H*$_2$N$_3$), 3.36 (br s, 1H, H-5), 2.46 (d, 1H, *J* = 1.8 Hz, -O*H*), 1.96-1.85 (m, 2H, -OCH$_2$C*H*$_2$CH$_2$N$_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 137.97, 137.67, 128.91, 128.46, 128.11, 127.89, 127.84, 126.31, 103.00, 101.07, 79.14, 72.98, 71.48, 69.91, 69.25, 66.67, 66.48, 48.42, 29.09; ESI-HRMS: calcd for ($C_{16}H_{24}O_5S$+NH_4^+) *m/z*, 459.2244; found, 459.2233.

3.5 3-Azidopropyl 2-*O*-Acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (8)

To a solution of **6** (300 mg, 0.678 mmol) and **7** (401 mg, 0.816 mmol) in anhydrous CH_2Cl_2 (10 mL) was added NIS (184 mg, 0.813 mmol) and AgOTf (15 mg, 0.038 mmol) at -78 ^oC under a N_2 atmosphere. The reaction mixture was stirred for 2 h under these conditions, when TLC indicated the disappearance of **6**. The mixture was neutralized with Et_3N and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 3:1) to yield **8** (468 mg, 85%) as a white foamy solid. $[\alpha]_D^{25}$ 4^o (*c* 0.2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.61-7.18 (m, 20H, Ph), 5.52 (dd, 1H, *J* = 3.6, 1.8 Hz, H-2′), 5.46 (s, 1H, C*H*Ph), 5.29 (d,1H, *J* = 1.8 Hz, H-1′), 4.92 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.73 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.69 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.66 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.63 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.51 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.30 (dd, 1H, *J* = 12.0, 1.2 Hz, H-6a), 4.29 (d, 1H, *J* = 7.8 Hz, H-1), 4.19-4.13 (m, 1H, H-5′), 4.13-4.09 (br d, 1H, *J* = 3.6 Hz, H-4), 4.03 (t, 1H, *J* = 9.6 Hz, H-2), 4.02 (dd, 1H, *J* = 12.0, 1.2 Hz, H-6b), 4.00-3.94 (m, 1H, -OC*H*$_2$-), 3.89 (dd, 1H, *J* = 9.6, 3.6 Hz, H-3′), 3.56 (dd, 1H, *J* = 9.6, 3.6 Hz, H-3), 3.55-3.50 (m, 1H, -OC*H*$_2$-), 3.42 (t, 1H, *J* = 9.6 Hz, H-4′),

3.41-3.28 (m, 3H, H-5, -C*H*$_2$N$_3$), 2.12 (s, 3H, -COC*H*$_3$), 1.89-1.80 (m, 2H, -OCH$_2$C*H*$_2$CH$_2$N$_3$), 1.30 (d, 3H, *J* = 6.0 Hz, H-6′); ^{13}C NMR (150 MHz, CDCl$_3$): δ 170.22 (-*C*OCH$_3$), 138.77, 138.23, 137.81, 137.67, 128.89, 128.43, 128.33, 128.26, 128.13, 128.07, 128.02, 127.88, 127.79, 127.60, 127.52, 126.29, 101.82 (C-1), 100.96 (*C*HPh), 98.20 (C-1′), 80.39 (C-3), 80.11 (C-4′), 78.23 (C-3′), 75.24 (*C*H$_2$Ph), 73.08 (C-2), 72.64 (C-4), 71.62 (*C*H$_2$Ph), 71.08 (*C*H$_2$Ph), 69.24 (C-6), 68.68 (C-2′), 67.63 (C-5′), 66.42 (C-5), 66.17 (-O*C*H$_2$CH$_2$-), 48.43 (-*C*H$_2$N$_3$), 29.19 (-OCH$_2$*C*H$_2$CH$_2$N$_3$), 21.10 (-CO*C*H$_3$), 17.85 (C-6′); ESI-HRMS: calcd for (C$_{45}$H$_{51}$O$_3$S$_{11}$+NH$_4^+$) *m/z*, 827.3867 found, 827.3883.

3.6 3-Azidopropyl 3,4-di-*O*-Benzyl-α-D-rhamnopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (9)

To a solution of **8** (468 mg, 0.58 mmol) in CH$_2$Cl$_2$ and MeOH (v/v 1:1, 10 mL) was added NaOMe (0.5 M) in MeOH (1 mL) at rt. After the reaction mixture was stirred for 2 h, it was neutralized with Amberlite IR-120 (H$^+$), filtered, and concentrated. Purification of the residue by flash column chromatography with hexane and ethyl acetate (7:3) as the eluents gave **3** (430 mg, 97%) as a white foamy solid. $[\alpha]_D^{25}$ -7^o (*c* 0.2, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$): δ 7.69-7.15 (m, 20H, Ph), 5.46 (s, 1H, C*H*Ph), 5.33 (s, 1H, H-1′), 4.89 (d, 1H, *J* = 11.4 Hz, C*H*$_2$Ph), 4.70 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.66 (d, 1H, *J* = 11.4 Hz, C*H*$_2$Ph), 4.65-4.60 (m, 3H, C*H*$_2$Ph), 4.35-4.25 (m, 2H, H-1, H-6a), 4.17-4.07 (m, 2H, H-4, H-5′), 4.05-3.96 (m, 4H, H-2, H-2', H-6b, -OC*H*$_2$-), 3.80 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3′), 3.60-3.51 (m, 2H, H-3, -OC*H*$_2$-), 3.46 (t, 1H, *J* = 9.6 Hz, H-4'), 3.45-3.34 (m, 2H, -C*H*$_2$N$_3$), 3.32 (br s, 1H, H-5), 2.37 (d, 1H, *J* = 1.8 Hz, -O*H*), 1.91-1.81 (m, 2H, -OCH$_2$C*H*$_2$CH$_2$N$_3$), 1.30 (d, 3H, *J* = 6.0 Hz, H-6′); ^{13}C NMR (150 MHz, CDCl$_3$): δ 138.65, 138.17, 137.96, 137.69, 128.95, 128.47, 128.46, 128.32, 128.17, 127.89, 127.87, 127.80, 127.72, 127.61, 126.38, 102.12 (C-1), 101.10 (*C*HPh), 99.74 (C-1′), 80.34 (C-3), 80.11 (C-4′), 80.09 (C-3′), 75.25 (*C*H$_2$Ph), 73.67 (C-2), 72.73 (C-4), 71.76 (*C*H$_2$Ph), 71.06 (*C*H$_2$Ph), 69.24 (C-6), 68.37 (C-2′), 67.44 (C-5′), 66.40 (C-5), 66.19 (-O*C*H$_2$-), 48.43 (-*C*H$_2$N$_3$), 29.22 (-OCH$_2$*C*H$_2$CH$_2$N$_3$), 17.88 (C-6′); ESI-HRMS: calcd for (C$_{43}$H$_{49}$O$_3$S$_{10}$+NH$_4^+$) *m/z*, 785.3762; found, 785.3768.

3.7 3-Azidopropyl 2,3,4-tri-*O*-Benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (11)

To a solution of **9** (50 mg, 0.065 mmol) and **10** (68 mg, 0.097 mmol) in anhydrous CH_2Cl_2 (10 mL) was added TMSOTf (7 μL, 0.038 mmol) at -78 ^oC under a N_2 atmosphere. After the reaction mixture was stirred for 2 h, it was neutralized with Et_3N and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 3:1) to yield **11** (68 mg, 85%) as a white foamy solid. $[\alpha]_D^{25}$ 124^o (*c* 0.6, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 8.08 (d, 2H, *J* = 7.8 Hz, Ph), 7.97 (d, 2H, *J* = 7.8 Hz, Ph), 7.82 (d, 2H, *J* = 7.8 Hz, Ph), 7.61 (t, 1H, *J* = 7.8 Hz, Ph), 7.55-7.46 (m, 5H, Ph), 7.43-7.30 (m, 13H, Ph), 7.29-7.22 (m, 5H, Ph), 7.20-7.13 (m, 3H, Ph), 7.11 (t, 2H, *J* = 7.8 Hz, Ph), 5.87 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2′′), 5.85 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3′′), 5.60 (t, 1H, *J* = 9.6 Hz, H-4′′), 5.40 (s, 1H, C*H*Ph), 5.38 (d, 1H, *J* = 1.8 Hz, H-1′), 5.18 (d, 1H, *J* = 1.8 Hz, H-1′′), 4.95 (d, 1H, *J* = 11.4 Hz, CH_2Ph), 4.74 (d, 1H, *J* = 11.4 Hz, CH_2Ph), 4.71 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.68 (d, 1H, *J* = 13.2 Hz, CH_2Ph), 4.65 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.64 (d, 1H, *J* = 13.2 Hz, CH_2Ph), 4.29 (d, 1H, J = 7.8 Hz, H-1), 4.28-4.23 (m, 2H, H-6a, H-5′′), 4.18-4.11 (m, 1H, H-5'), 4.10 (t, 1H, *J* = 2.4 Hz, H-2'), 4.07-4.03 (m, 2H, H-2, H-4), 4.01-3.96 (m, 2H, H-6b, -OCH_2-), 3.87 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3'), 3.68 (t, 1H, *J* = 9.5 Hz, H-4'), 3.58-3.53 (m, 1H,-OCH_2-), 3.51 (dd, 1H, *J* = 9.6, 3.6 Hz, H-3), 3.44-3.33 (m, 2H, -CH_2N_3), 3.30 (s, 1H, H-5), 1.90-1.84 (m, 2H, -$CH_2CH_2CH_2N_3$), 1.37 (d, 3H, *J* = 6.0 Hz, H-6′), 1.19 (d, 3H, *J* = 6.6 Hz, H-6′′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.84, 165.35, 165.17, 138.80, 138.52, 137.91, 137.66, 133.27, 133.22, 132.90, 129.91, 129.80, 129.66, 129.41, 129.28, 129.02, 128.90, 128.49, 128.45, 128.37, 128.26, 128.19, 128.12, 128.10, 127.83, 127.64, 127.47, 127.32, 126.31, 101.98 (C-1), 100.97 (*C*HPh), 99.54 (C-1′), 99.38 (C-1′′), 80.23 (C-4′), 80.17 (C-3), 79.81 (C-3′), 76.14 (C-2′), 75.38 (*C*H_2Ph), 73.56 (C-2), 72.68 (C-4), 72.24(*C*H_2Ph), 71.90 (C-4′′), 70.91(*C*H_2Ph), 70.64 (C-2′′), 69.99 (C-3′′), 69.20 (C-6), 68.17 (C-5′), 67.02 (C-5′′), 66.36 (C-5), 66.20 (-O*C*H_2-), 48.45 (-*C*H_2N_3), 29.20 (-$CH_2CH_2CH_2N_3$), 17.89 (C-6′), 17.53 (C-6′′); ESI-HRMS: calcd for ($C_{70}H_{71}N_3O_{17}$+NH_4^+) *m/z*, 1243.5122; found, 1243.5146.

3.8 3-Aminopropyl α-L-Rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside (1)

To a solution of **11** (50 mg, 0.041mmol) in MeOH (5 mL) was added NaOMe (1.0 M) in MeOH dropwise until the pH value reached 10. The mixture was stirred at rt for 2 h, and then neutralized with Amberlite IR 120 (H^+). The solution was filtered and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 1:1). This product was dissolved in MeOH and H_2O (v/v 1:9, 2 mL), to which was added 10% Pd/C (5 mg). The suspension was stirred under a H_2 atmosphere at rt for 12 h. The solid materials were filtrated off, and the solution was concentrated. The residue was purified by size exclusion chromatography on a Bio-Gel P-2 column with distilled water as the eluent, which was followed by lyophilization to give **1** (16.3 mg, 75% for 2 steps) as a white solid. $[\alpha]_D^{25}$ -97° (*c* 0.1, MeOH); ^{1}H NMR (600 MHz, D_2O): δ 5.06 (s, 1H, H-1′), 4.78 (s, 1H, H-1′′), 4.32 (d, 1H, *J* = 7.8 Hz, H-1), 3.89 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2′′), 3.85 (br s, 1H, H-2′), 3.80 (m, 1H, -OC*H*$_2$-), 3.76 (m, 1H, H-5′), 3.71 (d, 1H, *J* = 3.0 Hz, H-4), 3.70-3.63 (m, 2H, H-3′, -OC*H*$_2$-), 3.63-3.56 (m, 4H, H-3, H-3′′, H-6a,b), 3.56-3.47 (m, H-5, H-5′′), 3.39 (dd, 1H, *J* = 9.0, 7.8 Hz, H-2), 3.31 (t, 1H, *J* = 9.6 Hz, H-4′), 3.26 (t, 1H, *J* = 9.6 Hz, H-4′′), 2.97-2.85 (m, 2H, -C*H*$_2$NH$_2$), 1.85-1.76 (m, 2H, -OCH$_2$C*H*$_2$CH$_2$NH$_2$), 1.11 (d, 3H, *J* = 6.0 Hz, H-6′), 1.09 (d, 3H, *J* = 6.0 Hz, H-6′′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 102.06 (C-1′′), 101.44 (C-1), 99.44 (C-1′), 77.87 (C-2′), 76.71 (C-2), 74.94 (C-5), 73.35 (C-3), 71.85 (2C, C-4′, C-4′′), 69.89 (C-3′′), 69.86 (C-2′′), 69.81 (C-3′), 69.01 (C-5′′), 68.87 (C-4), 68.79 (C-5′), 67.30 (-OCH$_2$-), 60.85 (C-6), 37.12 (-*C*H$_2$NH$_2$), 27.41 (-OCH$_2$*C*H$_2$CH$_2$NH$_2$), 16.54 (C-6′), 16.50 (C-6′′); ESI-HRMS: calcd for ($C_{21}H_{39}NO_{14}$+H^+) *m/z*, 530.2443; found, 530.2441.

3.9 Isopropyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-*para*-methoxybenzyl-β-D-galactopyranoside (14)

To an ice-bath cooled solution of **5** (2.5 g, 6.0 mmol) in dry DMF (15 mL) was added NaH (318 mg, 60% in kerosene, 8.0 mmol) and PMBCl (1.32 mL, 9.7 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, diluted with EtOAc (100 mL), and washed successively with cooled water, 1 M aq. HCl, and brine. The organic phase was dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 3:1) to afford **7** (2.8 g, 87%) as a white foamy solid. $[\alpha]_D^{25}$ 89° (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.54 (d, 2H, *J* = 7.8 Hz, Ph), 7.41-7.26 (m, 10H, Ph), 6.85 (d, 2H, *J* = 9.0 Hz, Ph), 5.46 (s, 1H, C*H*Ph), 4.81 (d, 1H, *J* = 10.2 Hz, C*H*$_2$Ph), 4.77 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.74 (d,

1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.73 (d, 1H, *J* = 10.2 Hz, C*H*$_2$Ph), 4.47 (d, 1H, *J* = 9.6 Hz, H-1), 4.28 (dd, 1H, *J* = 12.0, 1.2 Hz, H-6a), 4.13 (d, 1H, *J* = 3.0 Hz, H-4), 3.95 (dd, 1H, *J* = 12.0, 1.2 Hz, H-6b), 3.84 (t, 1H, *J*= 9.6 Hz, H-2), 3.79 (s, 3H, -OC*H*$_3$), 3.57 (dd, 1H, *J* = 9.0, 3.6 Hz, H-3), 3.32 (s, 1H, H-5), 3.31-3.25 (m, 1H, -SC*H*-), 1.37 (dd, 3H, *J* = 6.6 Hz, -SCH(C*H*$_3$)$_2$), 1.33 (dd, 3H, *J* = 6.8 Hz, -SCH(C*H*$_3$)$_2$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 159.23, 138.35, 137.88, 130.66, 130.01, 128.97, 128.36, 128.14, 127.73, 127.70, 126.57, 113.69, 101.48, 84.39, 81.11, 75.41, 74.05, 71.82, 69.69, 69.42, 55.28, 34.62, 24.37, 23.81; ESI-HRMS: calcd for ($C_{31}H_{36}O_6S$+NH_4^+) *m/z*, 554.2576; found, 554.2571.

3.10 *p*-Tolyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-*para*-methoxybenzyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (12)

To a solution of **14** (254 mg, 0.047 mmol), **16** (200 mg, 0.43 mmol), and 4 Å MS (600 mg) in anhydrous Et_2O (15 mL) was added NIS (117 mg, 0.52 mmol) and AgOTf (5.2 mg, 0.02mmol) at -30 ^{o}C under a N_2 atmosphere. The reaction mixture was stirred for 30 min, at which time TLC indicated the disappearance of **14**. The reaction mixture was neutralized with Et_3N, filtered and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 3:1) to yield **12** (290 mg, 73%) as a white foamy solid. $[\alpha]_D^{25}$ 28^{o} (*c* 0.45, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.04 (d, 2H, *J* = 7.8 Hz, Ph), 7.58 (t, 1H, *J* = 7.8 Hz, Ph), 7.50-7.40 (m, 4H, Ph), 7.40-7.22 (m, 13H, Ph), 7.21-7.15 (m, 2H, Ph), 7.12 (d, 2H, *J* = 7.8 Hz, Ph)), 7.02 (d, 2H, *J* = 8.4 Hz, Ph), 6.64 (d, 2H, *J* = 8.4 Hz, Ph), 5.83 (dd, 1H, *J* = 3.0, 1.2 Hz, H-2), 5.49 (d, 1H, *J* = 1.2 Hz, H-1), 5.34 (s, 1H, C*H*Ph), 5.27 (d, 1H, *J* = 3.6 Hz, H-1′), 4.77 (d, 1H, *J* = 11.4 Hz, C*H*$_2$Ph), 4.71 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.67 (d, 1H, *J* = 11.4 Hz, C*H*$_2$Ph), 4.65 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.50 (d, 1H, *J* = 11.4 Hz, C*H*$_2$Ph), 4.47 (d, 1H, *J* = 11.4 Hz, C*H*$_2$Ph), 4.39-4.31 (m, 1H, H-5), 4.27 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3), 4.12 (br d, 1H, *J* = 12.6, 1.2 H, H-6a′), 4.07 (dd, 1H, *J* = 10.2, 3.6 Hz, H-2′), 3.92 (dd, 1H, *J* = 10.2, 3.6 Hz, H-3′), 3.82 (br d, 1H, *J* = 3.0 Hz, H-4′), 3.76 (s, 3H, -OC*H*$_3$), 3.71 (t, 1H, *J* = 9.6 Hz, H-4), 3.66 (br s, 1H, H-5′), 3.57 (br d, 1H, *J* = 12.6 Hz, H-6b′), 2.33 (s, 3H, -SPhC*H*$_3$), 1.42 (d, 3H, *J* = 6.0 Hz, H-6); ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.79, 158.79, 138.77, 138.04, 137.91, 137.86, 133.15, 132.35, 130.47, 130.09, 129.98, 129.91, 129.88, 129.23, 129.04, 128.88, 128.47, 128.35, 128.27, 128.23, 128.10, 127.83, 127.78, 127.57, 127.37, 126.40, 125.30, 113.41, 101.05 (*C*HPh), 93.94 (C-1′), 86.50

(C-1), 79.91(C-4), 75.33 (*C*H_2Ph), 75.13 (C-3′), 74.67 (C-4′), 74.31 (C-2′), 72.75 (C-3), 72.49 (*C*H_2Ph), 71.81 (*C*H_2Ph), 70.22 (C-2), 69.20 (C-5), 69.17 (C-6′), 62.53 (C-5′), 55.18 (-O*C*H_3), 21.14 (-SPh*C*H_3), 18.05 (C-6); ESI-HRMS: calcd for ($C_{55}H_{56}O_{11}S+NH_4^+$) *m/z*, 942.3887; found, 942.3902.

3.11 *p*-Tolyl 2,3,4-tri-*O*-Benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyide (13)

To a solution of **16** (184 mg, 0.41mmol) and **10** (280 mg, 0.45mmol) in anhydrous CH_2Cl_2 (10 mL) was added TMSOTf (15 μL, 0.08 mmol) at -78 ^oC under a N_2 atmosphere. The reaction mixture was stirred under these conditions for 2 h, when TLC indicated the disappearance of **10**. The mixture was neutralized with Et_3N and then concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 6:1) to yield **13** (317 mg, 85%) as a white foamy solid. $[\alpha]_D^{25}$ 22^o (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.09 (d, 2H, *J* = 7.2 Hz, Ph), 7.97 (d, 2H, *J* = 7.2 Hz, Ph), 7.82 (d, 2H, *J* = 7.2 Hz, Ph), 7.63 (t, 1H, *J* = 7.2 Hz, Ph), 7.52-7.48 (m, 3H, Ph), 7.43 (t, 1H, *J* = 7.2 Hz, Ph), 7.40-7.32 (m, 10H, Ph), 7.30 (t, 1H, *J* = 7.2 Hz, Ph), 7.28-7.21 (m, 4H, Ph), 7.17 (t, 1H, *J* = 7.2 Hz, Ph), 7.12 (d, 2H, *J* = 7.8 Hz, Ph), 5.88-5.83 (m, 2H, H-2,3′), 5.63 (t, 1H, *J* = 9.6 Hz, H-4′), 5.44 (d, 1H, *J* = 1.2 Hz, H-1), 5.16 (s, 1H, H-1′), 4.99 (d, 1H, *J* = 11.0 Hz, C*H*$_2$Ph), 4.79 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.75 (d, 1H, *J* = 11.0 Hz, C*H*$_2$Ph), 4.70 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.29-4.16 (m, 3H, H-2,5, H-5′), 3.92 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3), 3.74 (dd, 1H, *J* = 9.6 Hz, H-4), 2.32 (s, 3H, -SPhC*H*$_3$), 1.41 (d, 3H, *J* = 6.0 Hz, H-6), 1.25 (d, 3H, *J* = 6.0 Hz, H-6′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.78, 165.44, 165.26, 138.45, 138.02, 137.83, 133.39, 133.29, 133.03, 132.23, 130.48, 129.91, 129.77, 129.67, 129.50, 129.28, 129.22, 128.54, 128.47, 128.40, 128.39, 128.25, 128.21, 127.85, 127.72, 127.68, 99.59 (C-1′), 87.94 (C-1), 80.20 (C-4), 79.83 (C-3), 78.04 (C-2), 75.60 (*C*H_2Ph), 72.67 (*C*H_2Ph), 71.77 (C-4′), 70.60 (C-2′), 69.90 (C-3′), 69.54 (C-5), 67.21 (C-5′), 21.11 (-SPhC*H*$_3$), 17.88 (C-6), 17.56 (C-6′); ESI-HRMS: calcd for ($C_{54}H_{52}SO_{11}+NH_4^+$) *m/z*, 926.3574; found, 926.3584.

3.12 3-Azidopropyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-*para*-methoxybenzyl-α-D-galactopyranosyl-(1→3)-2-*O*-

benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (17)

To a solution of **9** (75 mg, 0.098 mmol), **12** (100 mg, 0.1082 mmol), and 4Å MS (300 mg) in anhydrous CH_2Cl_2 (4 mL) were added NIS (30 mg, 0.14 mmol) and AgOTf (13 mg, 0.05mmol) at -78 ^oC under a N_2 atmosphere. After the reaction mixture was stirred under these conditions for 2 h, it was neutralized with Et_3N, filtered, and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 3:1) to yield **17** (133 mg, 85%) as a white foamy solid. $[\alpha]_D^{25}$ 124^o (*c* 0.6, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 8.05 (d, 2H, *J* = 7.2 Hz, Ph), 7.58 (t, 1H, *J* = 7.2 Hz, Ph), 7.55-7.50 (m, 2H, Ph), 7.47-7.41 (m, 4H, Ph), 7.41-7.10 (m, 31H, Ph), 7.02 (d, 2H, *J* = 8.4 Hz, Ph), 6.60 (d, 2H, *J* = 8.4 Hz, Ph), 5.77 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2′′), 5.37 (s, 1H, C*H*Ph), 5.35 (d, 1H, *J* = 3.6 Hz, H-1′′′), 5.30 (s, 1H, C*H*Ph), 5.28 (d, 1H, *J* = 1.8 Hz, H-1′), 5.15 (d, 1H, *J* = 1.8 Hz, H-1′′), 4.92 (d, 1H, *J* = 10.8 Hz, CH_2Ph), 4.66 (m, 9H, CH_2Ph), 4.51 (d, 1H, *J* = 11.4 Hz, CH_2Ph), 4.42 (d, 1H, *J* = 11.4 Hz, CH_2Ph), 4.32 (dd, 1H, *J* = 10.2, 3.0 Hz, H-3′′), 4.26 (br d, 1H, *J* = 11.4 Hz, H-6a), 4.25 (d, 1H, *J* = 7.8 Hz, H-1), 4.12-4.06 (m, 3H, H-6a′′′, H-2′, H-5′′), 4.03 (dd, 1H, *J* = 10.2, 3.6 Hz, H-2′′′), 4.01 (dd, 1H, *J* = 9.6, 7.8 Hz, H-2), 3.99-3.92 (m, 4H, H-4, H-5′, H-6b, -OCH_2-), 3.88 (dd, 1H, *J* = 10.2, 3.6 Hz, H-3′′′), 3.82 (dd,1H, *J* = 9.6, 3.0 Hz, H-3′), 3.75 (d, 1H, *J* = 3.0 Hz, H-4′′′), 3.72 (s, 3H, -OCH_3), 3.65 (br s, 1H, H-5′′′), 3.60-3.49 (m, 4H, H-4′, H-4′′, H-6b′′′, -OCH_2-), 3.47 (dd, 1H, *J* = 9.6, 3.6 Hz, H-3), 3.41-3.30 (m, 2H, -CH_2N_3), 3.26 (br s, 1H, H-5), 1.88-1.80 (m, 2H, -OCH$_2$CH_2CH$_2$N$_3$), 1.28 (d, 3H, *J* = 6.0 Hz, H-6′′), 1.24 (d, 3H, *J* = 6.6 Hz, H-6′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.43, 158.67, 138.86, 138.84, 138.63, 138.27, 138.01, 137.93, 137.69, 132.93, 130.64, 130.15, 130.03, 129.18, 129.03, 128.92, 128.78, 128.46, 128.33, 128.25, 128.23, 128.21, 128.14, 128.03, 127.94, 127.84, 127.72, 127.70, 127.57, 127.46, 127.41, 127.31, 127.24, 127.15, 126.41, 126.37, 113.31, 102.00 (C-1), 101.04 (*C*HPh), 100.96 (*C*HPh), 99.51 (C-1′), 99.21 (C-1′′), 93.20 (C-1′′′), 80.45 (C-4′), 80.06 (C-3), 79.74 (2C, C-4′′ and C-3′), 75.39 (*C*H_2Ph), 75.08 (C-3′′′), 75.07 (*C*H_2Ph), 74.88 (C-2′), 74.61 (C-4′′′), 74.43 (C-2′′′), 73.41 (C-2), 72.86 (C-4), 72.26 (*C*H_2Ph), 72.08 (*C*H_2Ph), 71.62 (2C, C-3′′ and *C*H_2Ph), 71.09 (*C*H_2Ph), 69.30 (C-6′′′), 69.19 (C-6), 68.31 (C-2′′), 68.12 (C-5′), 68.00 (C-5′′), 66.31 (C-5), 66.17 (-O*C*H_2CH_2-), 62.21 (C-5′′′), 55.14 (-O*C*H_3), 48.43 (-*C*H_2N_3), 29.16 (-OCH$_2$*C*H$_2$CH$_2$N$_3$), 18.10 (C-6′), 17.92 (C-6′′); ESI-HRMS: calcd for ($C_{91}H_{97}N_3O_{21}$+NH_4^+) *m/z*, 1585.6958; found, 1585.6962.

3.13 3-Azidopropyl 3-*O*-Benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (18)

To solution of **17** (100 mg, 0.069 mmol) in CH_2Cl_2 and H_2O (v/v 14:1, 15 mL) was added DDQ (19 mg, 0.083 mmol) at rt. The reaction mixture was stirred for 2 h, at which time TLC indicated the complete consumption of **17**. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with 10% aq. $NaHSO_3$ (30 mL). The organic phase was dried over Na_2SO_4, and concentrated. The residue was subjected to silica gel column chromatograph using toluene and ethyl acetate (3:1) as the eluents to give **18** (67 mg, 67%) as a white foamy solid. $[\alpha]_D^{25}$ 61° (*c* 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.09 (d, 2H, *J* = 7.8 Hz, Ph), 7.64 (t, 1H, *J* = 7.8 Hz, Ph), 8.11-8.07 (m, 4H, Ph), 7.47-7.43 (m, 2H, Ph), 7.41-7.05 (m, 31H, Ph), 5.67 (dd, 1H, *J* = 3.0, 1.2 Hz, H-2″), 5.37 (s, 1H, C*H*Ph), 5.36 (d, 1H, *J* = 3.6 Hz, H-1‴), 5.28 (d, 1H, *J* = 1.8 Hz, H-1′), 5.21 (s, 1H, C*H*Ph), 5.06 (d, 1H, *J* = 1.2 Hz, H-1″), 4.89 (d, 1H, *J* = 10.8 Hz, CH_2Ph), 4.76-4.57 (m, 8H, CH_2Ph), 4.42 (d, 1H, *J* = 11.4 Hz, CH_2Ph), 4.32 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3″), 4.26 (dd, 1H, *J* = 12.6, 1.2 Hz, H-6a), 4.25 (d, 1H, *J* = 7.8 Hz, H-1), 4.22 (ddd, 1H, *J* = 10.2, 6.6, 3.6 Hz, H-2‴), 4.12-4.06 (m, 2H, H-2′, H-5″), 4.04-3.93 (m, 6H, H-2, H-4, H-5′, H-6b, H-6a‴, -OCH_2-), 3.81 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3′), 3.68 (d, 1H, *J* = 3.6 Hz, H-4‴), 3.60 (dd, 1H, *J* = 10.2, 3.6 Hz, H-3‴), 3.56-3.51 (m, 2H, H-5‴, -OCH_2-), 3.51-3.45 (m, 2H, H-3, H-4′), 3.42 (t, 1H, *J* = 9.6 Hz, H-4″), 3.40-3.31 (m, 3H, H-6b‴, -CH_2N_3), 3.27 (s, 1H, H-5), 2.23 (d, 1H, *J* = 6.6 Hz, -O*H*), 1.89-1.80 (m, 2H, -OCH$_2$CH_2CH$_2N_3$), 1.29 (d, 3H, *J* = 6.0 Hz, H-6″), 1.25 (d, 3H, *J* = 6.0 Hz, H-6′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 166.26, 138.75, 138.65, 138.45, 138.01, 137.85, 137.67, 133.42, 130.02, 129.75, 129.02, 128.92, 128.77, 128.47, 128.38, 128.25, 128.13, 128.04, 127.91, 127.86, 127.69, 127.55, 127.45, 127.30, 127.17, 126.98, 126.35, 126.28, 125.28, 102.00 (C-1), 101.02 (*C*HPh), 100.88 (*C*HPh), 99.52 (C-1′), 99.34 (C-1″), 95.74 (C-1‴), 80.34 (C-4′), 80.06 (C-3), 79.94 (C-4″), 79.88 (C-3′), 75.68 (C-3‴), 75.37 (*C*H_2Ph), 75.08 (2C, C-2′ and *C*H_2Ph), 74.10 (C-4‴), 73.48 (C-2), 73.00 (C-3″), 72.82 (C-4), 72.22 (*C*H_2Ph), 71.38 (*C*H_2Ph), 71.05 (*C*H_2Ph), 69.19 (C-6‴), 69.16 (C-6), 69.10 (C-2″), 68.46 (C-5′), 68.05 (C-5″), 67.85 (C-2‴), 66.32 (C-5), 66.18 (-OCH_2-), 62.58 (C-5‴), 48.42 (-*C*H_2N_3), 29.16 (-OCH$_2$*C*H$_2$CH$_2N_3$), 18.10

(C-6′), 17.82 (C-6″); ESI-HRMS: calcd for ($C_{83}H_{89}N_3O_{20}$+NH_4^+) *m/z*, 1465.6383; found, 1465.6401.

3.14 3-Azidopropyl 2,3,4-tri-*O*-Benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (19)

To a solution of **18** (60 mg, 0.041 mmol) and **13** (56 mg, 0.062 mmol) in anhydrous CH_2Cl_2 (4 mL) were added NIS(17 mg, 0.074 mmol) and AgOTf (13 mg, 0.05mmol) at -78 ^oC under a N_2 atmosphere. The reaction mixture was stirred for 2 h, neutralized with Et_3N, and concentrated. The residue was purified by flash column chromatography (toluene/ethyl acetate 6:1) to yield **19** (78 mg, 85%) as a white foamy solid. $[\alpha]_D^{25}$ 46^o (*c* 0.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.12 (d, 2H, *J* = 8.4 Hz, Ph), 8.00 (d, 2H, *J* = 8.4 Hz, Ph), 7.93 (d, 2H, *J* = 8.4 Hz, Ph), 7.76 (d, 2H, *J* = 8.4 Hz, Ph), 7.60-7.00 (m, 57H, Ph), 5.80-5.75 (m, 2H, H-2,4′′′′′), 5.71 (t, 1H, *J* = 2.4 Hz, H-2′′), 5.48 (t, 1H, *J* = 9.6 Hz, H-3′′′′′), 5.41 (d, 1H, *J* = 3.0 Hz, H-1′′′), 5.35 (s, 1H, C*H*Ph), 5.27 (d, 1H, *J* = 1.2 Hz, H-1′), 5.26 (d, 1H, *J* = 1.2 Hz, H-1′′′′), 5.20 (s, 1H, C*H*Ph), 5.06 (d, 1H, *J* = 1.8 Hz, H-1′′), 4.97 (br s, 1H, H-1′′′′′), 4.91 (d, 1H, *J* = 10.8 Hz, CH_2Ph), 4.84 (d, 1H, *J* = 11.4 Hz, CH_2Ph), 4.76 (d, 1H, *J* = 11.4 Hz, CH_2Ph), 4.74-4.59 (m, 7H, CH_2Ph), 4.58 (d, 1H, *J* = 13.2 Hz, CH_2Ph), 4.45 (d, 1H, *J* = 13.2 Hz, CH_2Ph), 4.42 (dd, 1H, *J* = 9.6, 3.0 Hz, H-2′′′), 4.35 (dd, 1H, *J* = 9.6, 2.4 Hz, H-3′′), 4.25 (dd, 1H, *J* = 12.0, 1.2 Hz, H-6a), 4.23 (d, 1H, *J* = 7.8 Hz, H-1), 4.18 (m, 1H, H-5′′′′′), 4.12 (br d, 1H, *J* = 12.0 Hz, H-6a′′′), 4.09-3.99 (m, 5H, H-2,5′, H-2,5′′′′, CH_2Ph), 3.98 (dd, 1H, *J* = 9.6, 7.8 Hz, H-2), 3.96-3.90 (m, 6H, H-3′′′, H-5′′, H-4,6b, CH_2Ph, -OCH_2-), 3.80 (br s, 1H, H-5′′′), 3.78 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3′′′′), 3.66 (d, 1H, *J* = 3.0 Hz, H-4′′′), 3.61 (t, 1H, *J* = 9.6 Hz, H-4′′), 3.59 (t, 1H, *J* = 9.6 Hz, H-4′), 3.57 (br d, 1H, *J* = 12.0 Hz, H-6b′′′), 3.51 (m, 1H, -OCH_2-), 3.50 (t, 1H, *J* = 9.6 Hz, H-4′′′′), 3.45 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3), 3.43 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3′), 3.40-3.28 (m, 2H, -C$H_2$$N_3$), 3.25 (s, 1H, H-5), 1.86-1.79 (m, 2H, -OCH$_2$CH_2C$H_2$$N_3$), 1.39 (d, 3H, *J* = 6.0 Hz, H-6′), 1.27 (d, 3H, *J* = 6.0 Hz, H-6′′′′), 1.23 (d, 3H, *J* = 6.0 Hz, H-6′′), 1.06 (d, 3H, *J* = 6.0 Hz, H-6′′′′′); ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.84, 165.22, 164.99, 164.65, 139.07, 138.86, 138.70, 138.63, 138.56, 138.18,

137.98, 137.81, 137.68, 133.16, 132.87, 132.81, 130.69, 129.87, 129.80, 129.64, 129.43, 129.28, 128.93, 128.70, 128.44, 128.41, 128.38, 128.33, 128.28, 128.25, 128.23, 128.19, 128.14, 128.08, 128.02, 127.94, 127.84, 127.70, 127.62, 127.44, 127.40, 127.38, 127.23, 127.21, 127.04, 126.97, 126.90, 126.81, 126.38, 126.21, 101.99 (C-1), 101.09 (*C*HPh), 100.78 (C-1′), 100.62 (*C*HPh), 99.49 (C-1′′′′), 99.21 (2C, C-1′′, C-1′′′′′), 94.21 (C-1′′′), 80.41 (C-4′′′′), 80.33 (C-3), 80.08 (C-4′), 79.77 (C-3′), 79.70 (C-4′′), 79.51 (C-3′′′′), 76.46 (C-3′′′), 76.33 (C-2′), 75.38 (CH_2Ph), 75.31 (CH_2Ph), 75.05 (CH_2Ph), 74.94 (C-2′′′′), 73.65 (C-4′′′), 73.46 (C-2), 72.92 (C-4), 72.18 (C-2′′′), 71.91 (2C, C-4′′′′′, CH_2Ph), 71.74 (CH_2Ph), 71.16 (CH_2Ph), 70.99 (C-3′′), 70.85 (CH_2Ph), 70.46 (C-2′′′′′), 69.95 (C-3′′′′′), 69.31 (C-6′′′), 69.18 (C-6), 68.66 (C-5′′), 68.60 (C-5′), 67.97 (C-5′′′′), 67.82 (C-2′′), 66.87 (C-5′′′′′), 66.29 (C-5), 66.19 (-OCH_2-), 62.02 (C-5′′′), 48.42 (-CH_2N_3), 29.15 (-OCH$_2$$CH_2CH_2N_3$), 18.18 (C-6′′), 18.02 (2C, C-6′, C-6′′′′), 17.34 (C-6′′′′′); ESI-HRMS: calcd for ($C_{130}H_{133}N_3O_{31}$+2NH_4^+) *m/z*, 1134.4822; found, 1134.4834.

3.15 3-Aminopropyl α-L-Rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-D-galactopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside (2)

To a solution of **19** (30 mg, 0.0134 mmol) in MeOH (3 mL) was added NaOMe (1.0 M) in MeOH dropwise until the pH value reached 10. The mixture was stirred at rt for 5 h, and then neutralized with Amberlite IR 120 (H^+). The solution was filtered and concentrated. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate 4:1). The product was dissolved in MeOH and H_2O (v/v 1:9, 2 mL), and then 10% Pd/C (5 mg) was added. The suspension was stirred under a H_2 atmosphere at rt for 24 h. The solid materials were filtrated off, and the solution was concentrated. The residue was purified by size exclusion chromatography on a Bio-Gel P-2 column with distilled water as the eluent, which was followed by lyophilization to give **2** (11 mg, 90% for 2 steps) as a white solid. $[\alpha]_D^{25}$ -10^o (*c* 0.1, MeOH); ^{1}H NMR (600 MHz, D_2O): δ 5.09 (s, 1H, H-1), 5.03 (s, 1H, H-1), 5.01 (d, 1H, *J* = 2.4 Hz, H-1), 4.80 (br s, 2H, 2×H-1), 4.33 (d, 1H, *J* = 7.8 Hz, H-1), 4.11 (s, 1H, H-2), 4.03 (t, 1H, *J* = 6.0 Hz), 3.94-3.84 (m, 5H), 3.84-3.65 (m, 8H), 3.64-3.47 (m, 10H), 3.46-3.37 (m, 2H), 3.36-3.23 (m, 3H), 2.99-2.89 (m, 2H, -CH_2NH_2), 1.87-1.77 (m, 2H, -OCH$_2$CH_2CH$_2$NH$_2$), 1.16-1.08 (m, 4×3H, 4×H-6); ^{13}C NMR (150 MHz, D_2O): δ 102.07 (2C, 2×C-1), 101.46 (C-1), 100.24 (C-1), 99.42 (C-1), 94.15 (C-1),

78.22, 77.75, 76.80, 74.96, 74.28, 74.03, 73.34, 71.85, 71.84, 71.77, 70.66, 70.10, 69.90, 69.87, 69.79, 69.74, 69.38, 69.24, 69.11, 69.08, 68.98, 68.87, 68.80, 67.26 (-O*C*H_2-), 66.23, 60.86 (C-6), 60.69 (C-6), 37.12 (-*C*H_2NH_2), 27.06 (-OCH_2*C*$H_2CH_2NH_2$), 16.65 (C-6), 16.61 (C-6), 16.57 (C-6), 16.49 (C-6); ESI HRMS: calcd for ($C_{39}H_{69}NO_{27}+H^+$) *m/z*, 984.4135; found, 984.4135.

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Supplementary data

Supplementary data including ^{1}H NMR, ^{13}C NMR, and ESI-HRMS spectra of all final products and intermediates are available from the corresponding authors.

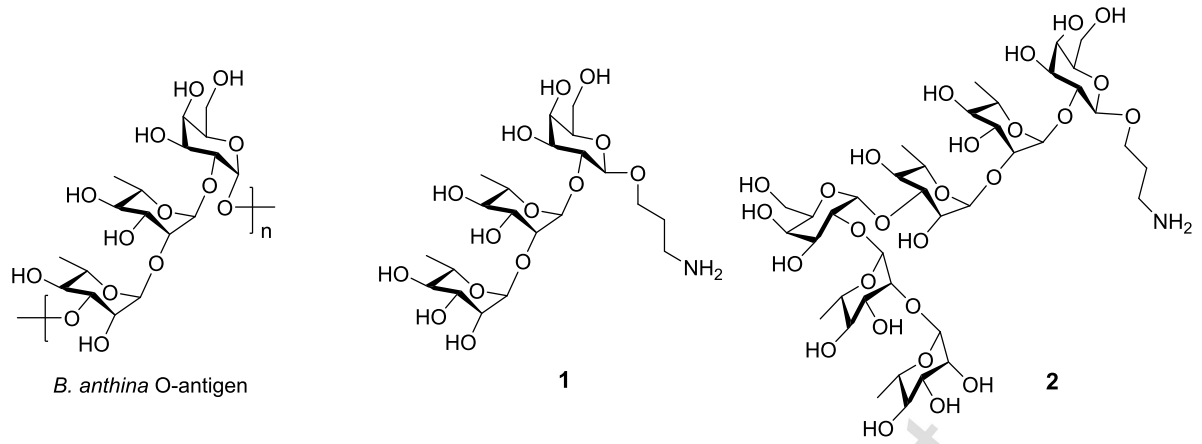
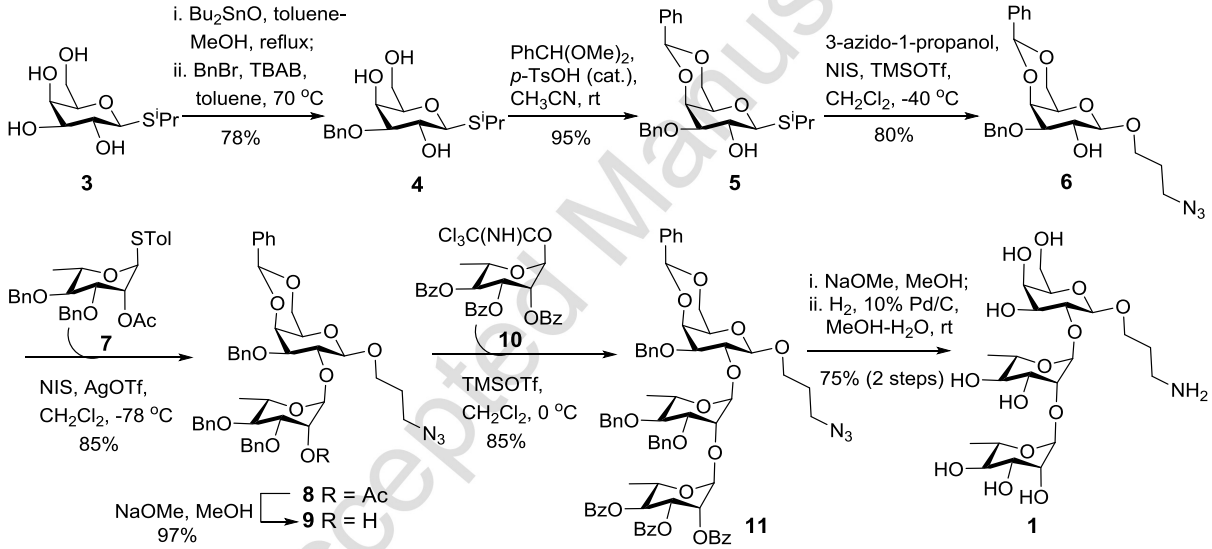
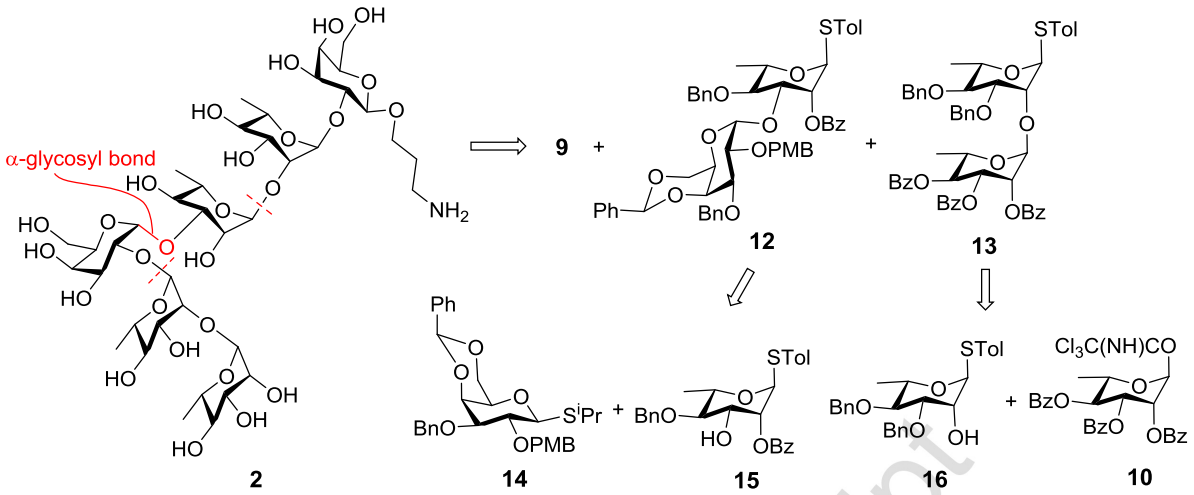


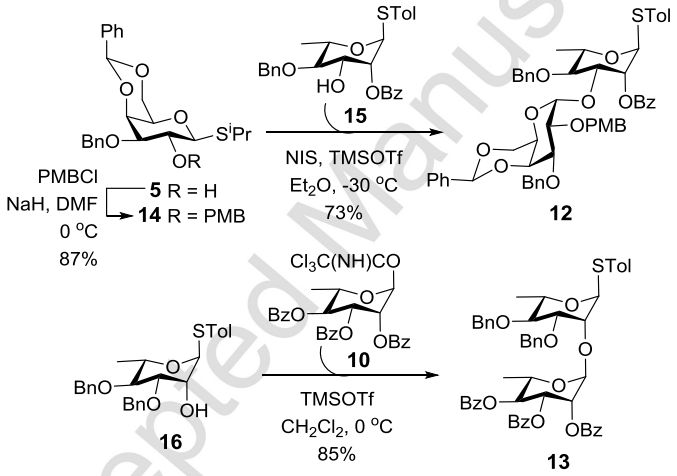
Figure 1. Structures of the O-antigen from *B. anthina* strain LMG20983 and the synthetic targets **1** and **2**.



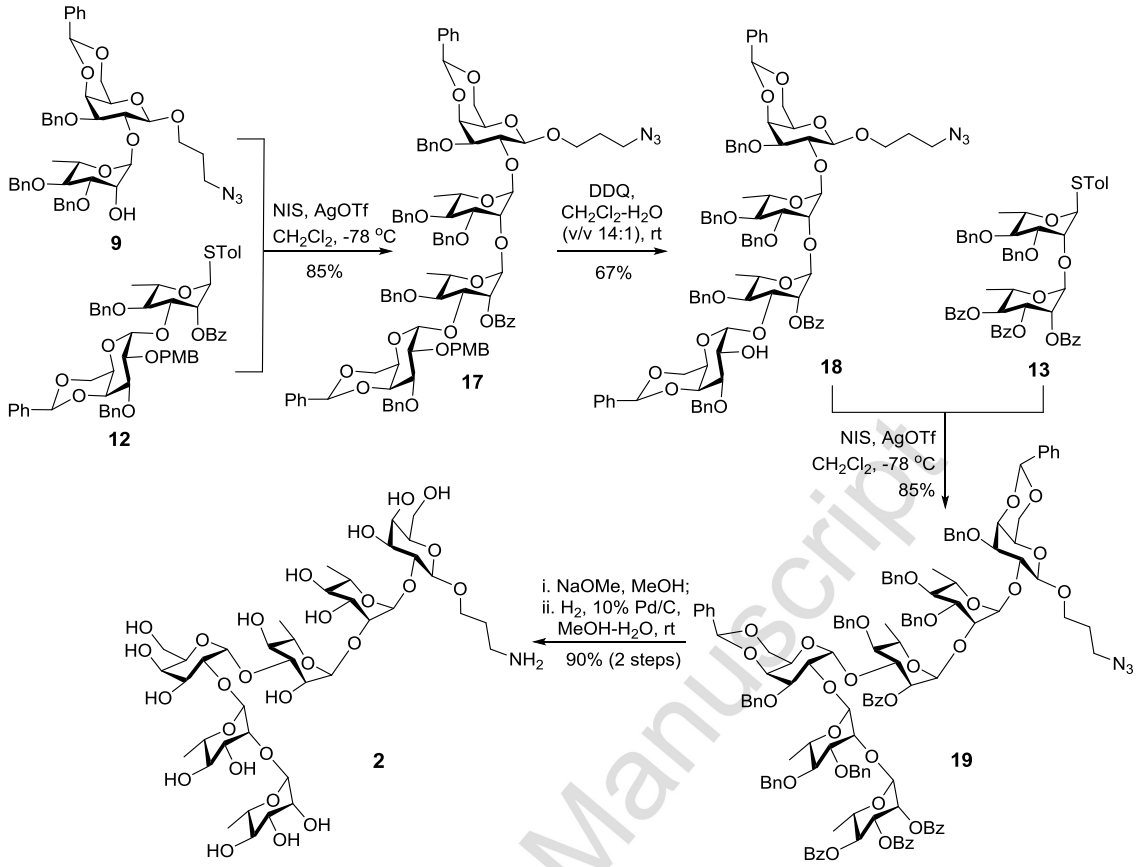
Scheme 1. Synthesis of target molecule **1**



Scheme 2. Retro-synthetic analysis of target molecule **2**



Scheme 3. Synthesis of key disaccharide blocks **12** and **13**



Scheme 4. Synthesis of target molecule **2**