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Convergent synthesis of the hexasaccharide related to the repeating unit of the *O*-antigen from *E. coli* O120[†]

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Chemical synthesis of the hexasaccharide, α_{-L} -Rhap- $(1 \rightarrow 4)$ - β_{-D} -GlcAp- $(1 \rightarrow 2)$ - α_{-L} -Rhap- $(1 \rightarrow 3)$ - β_{-D} -GalNAcp, is reported by following a [2 + 4] convergent strategy. A disaccharide α_{-D} -Galp- $(1 \rightarrow 3)$ - β_{-D} -GalNAcp and a tetrasaccharide α_{-L} -Rhap- $(1 \rightarrow 4)$ - β_{-D} -GlcAp- $(1 \rightarrow 2)$ - α_{-L} -Rhap- $(1 \rightarrow 2)$ - α_{-L} -Rhap were synthesized from commercially available monosaccharides through rational protecting group manipulations and stereoselective glycosylation involving activation of thioglycoside using *N*-iodosuccinimide and H₂SO₄-silica. Final glycosylation between the tetrasaccharide donor and the disaccharide acceptor was achieved by the activation of trichloroacetimidate using H₂SO₄-silica alone. A late stage TEMPO-mediated oxidation installed the required uronic acid moiety. Finally, global deprotection furnished the target molecule. Successful chemical synthesis of the repeating unit of *E. coli* O120 will help to design suitable vaccine candidates against this deadly pathogen belonging to the STEC family.

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Introduction

The O-antigen (O-polysaccharide) is an important part of the lipopolysaccharide present on the surface of the Gram-negative bacteria. The O-antigen consists of many repeats of an oligosaccharide called O-units and this is one of the most variable constituents of the cell. The commensal and pathogenic clones of E. coli are normally identified by the combination of their somatic (O), flagellar (H) and capsular (K) antigens.¹ A class of E. coli, called as Shiga toxin producing E. coli (STEC), is the causative agent for numerous food-borne disease, haemorrhagic colitis and haemolytic-uremic syndrome in human and cattle.2 Strains of E. coli O120 isolated from swine feces or beef products are identified as STEC.3,4 Recently, Knirel et al. elucidated the structure of the repeating unit of the O-antigen from E. coli O120.5 Herein, we report the concise synthesis of the hexasaccharide repeating unit in the form of its 4-methoxyphenyl glycoside (1, Fig. 1). The concise strategy will give the scope for the preparation of this important oligosaccharide structure in the pure form and in quantity that will pave the path for understanding its role in the pathogenic cycle. Moreover, the selective oxidative removal of the 4-methoxyphenyl group using ceric ammonium nitrate (CAN) followed by formation of corresponding trichloroacetimidate derivative will HO OHHO O

Fig. 1 Structure of the target hexasaccharide 1.

allow the formation of glycoconjugate with suitable aglycons targeting potential vaccine candidates against this deadly pathogen.

Results and discussion

Retrosynthetic analysis of the hexasaccharide revealed that a convergent [2 + 4] approach will suit best for the synthesis of the linear hexasaccharide. Owing to this, it was first disconnected to a disaccharide, α -D-Galp-(1 \rightarrow 3)- β -D-GalNAcp and a tetrasaccharide, α -L-Rhap-(1 \rightarrow 4)- α -D-GlcAp-(1 \rightarrow 2)- α -L-Rhap-(1 \rightarrow 2)- α -L-Rhap. The disaccharide was further disconnected to two monosaccharides that can be derived from commercially



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available D-GalNH₂ and D-Gal. On the otherhand, the tetrasaccharide can be further disconnected to four monosaccharide units, which could be prepared from commercially available D-Glc and three L-Rha moieties through sequential glycosylations. All the monosaccharide derivatives may be obtained from their respective commercially available sugars through rational protecting group manipulations (Fig. 2).

Synthesis of the disaccharide acceptor 7 commenced with the known galactose derivative, 4-tolyl-3,4-O-isopropylidene-1thio-β-D-galactopyranoside (2).6 Selective alkylation through a phase transfer reaction of compound 2 with 4-methoxybenzyl chloride in presence of TBAB7 incorporated a 4-methoxy benzyl group at the 2-OH position to furnish compound 3. The remaining primary hydroxyl group was further acetylated in the presence of Ac₂O in pyridine⁸ to form the desired galactosyl donor 4 in 94% yield. Glycosylation of the donor 4 with the known galactose acceptor, 4-methoxyphenyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside (5)⁹ through activation of the thioglycoside using NIS in the presence of H₂SO₄silica^{10,11} at -45 °C furnished the disaccharide 6 in 83% isolated yield. Only the desired 1,2-cis disaccharide was formed as evident by the ¹H signal at δ 5.0 (d, 1H, $J_{1',2'}$ 3.5 Hz) and the ¹³C signal at δ 95.4 assigned to the newly formed linkage. The



Fig. 2 Retrosynthetic analysis for the synthesis of the target hexasaccharide 1.

exclusive formation of the 1,2-*cis* linked disaccharide may be explained by the choice of the donor 4 having non-participating 4-methoxybenzyl group at 2-*O*-position and a α -directive acetyl group at 6-*O*-position. The 3,4-*O*-isopropylidene group further provides the required rigidity for α -glycosylation. Finally, oxidative cleavage of the 4-methoxybenzyl group using DDQ¹² gave the desired disaccharide acceptor 7 in 81% yield (Scheme 1).

Synthesis of the tetrasaccharide donor 16 commenced with the known disaccharide acceptor 8 (ref. 13) prepared from suitably protected L-rhamnose derivatives following the reported literature procedure. The structure of the disaccharide acceptor was satisfactorily characterized by NMR and mass spectrometry and matched satisfactorily with the data available in the literature. The disaccharide acceptor 8 was then coupled with a known glucose donor, 4,6-O-benzylidene-2,3-di-O-benzyl- β -D-glucopyranoside (9)¹⁴ using NIS in the presence of H₂SO₄silica at -55 °C to afford the protected trisaccharide **10** in 88% yield. The newly formed 1,2-cis glycosidic linkage was confirmed by the ¹H signal at δ 4.83 (d, 1H, $J_{1'',2''}$ 4.5 Hz) and the ¹³C signal at δ 96.8. The presence of non-participating 2-O-benzyl group and the structural rigidity imposed by 4,6-O-benzylidene group in donor 9 may be attributed for the exclusive formation of the cis-glycoside. Further, the benzylidene ring of the trisaccharide 10 was hydrolyzed using 80% AcOH at 80 °C (ref. 15) to form the diol 11 in 91% yield. Selective chloroacetylation of the primary hydroxyl group was achieved using chloroacetic anhydride and Et_3N^{16} at -5 °C to form the desired trisaccharide acceptor 12 in 89% yield. The trisaccharide acceptor was finally coupled with a known rhamnose donor, 4-tolyl 2,3,4-tri-O-acetyl-1-thio-a-Lrhamnopyranoside $(13)^{17}$ using NIS in the presence of H₂SO₄silica at 0 °C to furnish the protected tetrasaccharide derivative 14 in 85% isolated yield. Only the desired 1,2-trans glycoside was formed as evident by the ¹H signal at δ 4.94 and the ¹³C signal at δ 97.6 assigned to the newly formed linkage. CAN-mediated¹⁸ oxidative cleavage of the 4-methoxyphenyl group resulted the corresponding hemiacetal 15 in 78% yield, which was subsequently treated with trichloroacetonitrile in the presence of DBU19 to furnish the tetrasaccharide



Scheme 1 Synthesis of the disaccharide acceptor 7. Reagents and conditions: (a) 4-methoxybenzylchloride, TBAB, 87%; (b) Ac₂O, Py, 94%; (c) NIS, H_2SO_4 -silica, -45 °C, 83%; (d) DDQ, 81%.



STol BnÖ AcC BnÓ 13 BnO OŚn AcO AcC ÓΑc 14 CCL BnO BnÓ Bn O BnO BnC OBr AcC 16

Τo

. OBn

g

OPME

BnO

BnC

BnÓ

OBn 10

OPMP

trichloroacetimidate **16** in 84% yield. Considering the reactivity of the trichloroacetimidate derivative, it was directly used for glycosylation without further characterization (Scheme 2).

Glycosylation of the disaccharide acceptor 7 with the tetrasaccharide donor 16 through the activation of the trichloroacetimidate by H2SO4-silica20 afforded the protected hexasaccharide 17 in 81% isolated yield. The desired 1,2-trans glycoside was formed solely, as evident by the 1 H signal at δ 5.05 and the ¹³C signal at δ 100.6 assigned to the newly formed linkage. Once the protected hexasaccharide was obtained, the chloroacetate group was selectively deprotected using thiourea and 2,4,6-collidine²¹ to form the hexasaccharide 18. The primary OH group thus obtained was oxidized to the corresponding uronic acid using TEMPO in the presence of iodosobenzene diacetate.²² It is worth noting that the 4-methoxyphenyl group at the reducing end of the protected hexasaccharide remained unaffected by the TEMPO mediated oxidation. Next, the benzylidene and isopropylidene rings were hydrolyzed by 80% AcOH at 80 °C. Further, the phthalimido functionality was converted to the required acetamido moiety by the treatment of ethylene diamine in presence of n-butanol23 followed by acetylation using Ac2O and pyridine. Subsequently, hydrogenolysis using 10% Pd-C cartridge in a continuous flow hydrogenation assembly followed by



Scheme 3 Synthesis of the target hexasaccharide 1. Reagents and conditions: (a) H_2SO_4 -silica, 81%; (b) thiourea, 89%; (c) TEMPO, IBDA; (d) 80% AcOH, 80 °C; (e) ethylene diamine, *n*-butanol; (f) H_2 , Pd-C; (g) NaOMe, MeOH, 54% overall yield.

Zemplén de-O-acetylation using NaOMe in MeOH²⁴ furnished the target hexasaccharide 1 in 54% overall yield (Scheme 3).

Conclusions

In conclusion, we have developed a concise strategy for the synthesis of the hexasaccharide repeating unit of the *O*-antigen from *E. coli* O120 in the form of its 4-methoxyphenyl glycoside (1). The synthesis of the target compound is achieved through protecting group manipulations on commercially available mono-saccharides and stereo-selective glycosylations. The vital glycosylations were achieved either by the activation of thioglyco-sides using NIS in the presence of H_2SO_4 -silica or by the activation of trichloroacetimidate using H_2SO_4 -silica alone. H_2SO_4 -silica was found to be an efficient alternative to the toxic and hygroscopic acid promoters such as TfOH or TMSOTf. The presence of the 4-methoxyphenyl glycoside would allow selective oxidative deprotection using CAN and further trichloroacetimidate formation to the corresponding hemiacetal. This may open up the path for further glycoconjugate formation with suitable aglycon.

Experimental section

General methods

All solvents and reagents were dried prior to use according to standardized methods.²⁵ The commercially purchased reagents were used without any further purification unless mentioned otherwise. Dichloromethane was 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-F₂₅₄ 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-line at ambient temperature. ¹H and ¹³C NMR were recorded on Bruker 500 MHz spectrometer. ¹H NMR values were denoted as H for the reducing end galactosamine unit, H' for the galactose unit, H" for the rhamnose unit connected to the 2-*O*-position of the galactose unit, H" for the fourth rhamnose unit from the reducing end, H"" for the glucoronic acid moiety linked to the 2-*O*-position of the rhamnose unit and H"" for the rhamnose unit present in the non-reducing end.

Preparation of H₂SO₄-silica

To slurry of silica gel (10 g, 230–400 mesh) in dry diethyl ether (50 mL) was added commercially available concentrated H_2SO_4 -silica (1 mL), and the slurry was shaken for 5 min. The solvent was evaporated under reduced pressure, resulting in free flowing H_2SO_4 -silica, which was dried at 110 °C for 3 h and then used for the reactions.

4-Tolyl 2-O-(4-methoxybenzyl)-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside (3)

Compound 2, 4-tolyl 3,4-O-isopropylidene-1-thio-β-D-galactopyranoside (2 g, 6.1 mmol) was dissolved in CH₂Cl₂ (40 mL). To it, 10% NaOH solution (5 mL) followed by TBAB (2.2 g, 6.7 mmol) and 4-methoxybenzyl chloride (1.2 mL, 8.6 mmol) added. Reaction continued at room temperature for overnight. The organic layer was washed with water (2 \times 50 mL). The organic layer was collected, dried over Na₂SO₄, evaporated in vacuo and finally purified by flash chromatography using *n*-hexane–EtOAc (2:1) to afford the pure product 3 (2.4 g, 87%). $[\alpha]_{D}^{25}$ +106 (*c* 1.0, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ : 7.45, 7.13 (2d, 4H, J 8.0 Hz, CH₂C₆H₄OCH₃), 7.38, 6.91 (2d, 4H, J 9.0 Hz, SC₆H₄CH₃), 4.78, 4.65 (2d, 2H, J 11.0 Hz, CH₂C₆H₄OCH₃), 4.59 (d, 1H, J_{1,2} 9.5 Hz, H-1), 4.28 (t, 1H, J_{2,3}, J_{3,4} 6.0 Hz, H-3), 4.19 (dd, 1H, J_{3,4} 6.0 Hz, J_{4,5} 2.0 Hz, H-4), 3.95 (q, 1H, J_{5,6a} 8.5 Hz, J_{6a,6b} 15.0 Hz, H-6a), 3.83 (s, 3H, CH₂C₆H₄OCH₃), 3.79 (m, 2H, H-5, H-6b), 3.53 (dd, 1H, J_{1,2} 9.5 Hz, J_{2,3} 6.0 Hz, H-2), 2.35 (s, 3H, $SC_6H_4CH_3$, 1.44, 1.37 (2s, 6H, 2 × isopropylidene-CH₃). ¹³C NMR (CDCl₃, 125 MHz) δ: 159.3, 137.7, 132.5(2), 129.9, 129.8(2), 129.6(2), 129.5, 113.6(2) (ArC), 110.2 [C(CH₃)₂], 86.2 (C-1), 79.8, 77.9, 76.6, 73.8, 73, 62.5, 55.2 ($CH_2C_6H_4OCH_3$), 27.7, 26.3 (2 × isopropylidene- CH_3), 21 (SC₆H₄ CH_3). HRMS calcd for $C_{24}H_{30}O_6SNa (M + Na)^+$: 469.1661, found: 469.1659.

4-Tolyl 6-O-acetyl-2-O-(4-methoxybenzyl)-3,4-Oisopropylidene-1-thio-β-D-galactopyranoside (4)

Compound 3 (2.4 g, 5.4 mmol) was acetylated using acetic anhydride (10 mL) in presence of pyridine (10 mL). The reaction was completed after 2 h as judged by TLC (*n*-hexane–EtOAc; 3 : 2). The solvents were evaporated using toluene as co-solvent. The crude residue was then purified by flash chromatography using *n*-hexane–EtOAc (4 : 1) to afford the monosaccharide donor 4 (2.5 g, 94%). $[\alpha]_D^{25}$ +121 (*c* 1.0, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ : 7.45, 7.09 (2d, 4H, *J* 8.0 Hz, CH₂C₆H₄OCH₃), 7.35, 6.88 (2d, 4H, *J* 8.5 Hz, SC₆H₄CH₃), 4.75, 4.63 (2d, 2H, *J* 11.0 Hz, $CH_2C_6H_4OCH_3$), 4.54 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 4.32 (m, 2H, H-6), 4.25 (t, 1H, $J_{2,3}$, $J_{3,4}$ 6.0 Hz, H-3), 4.16 (dd, 1H, $J_{3,4}$ 6.0 Hz, $J_{4,5}$ 2.0 Hz, H-4), 3.89 (m, 1H, H-5), 3.8 (s, 3H, $CH_2C_6-H_4OCH_3$), 3.51 (dd, 1H, $J_{1,2}$ 9.5 Hz, $J_{2,3}$ 6.5 Hz, H-2), 2.32 (s, 3H, $COCH_3$), 2.06 (s, 3H, SC₆H₄CH₃), 1.42, 1.34 (2s, 6H, 2 × isopropylidene-CH₃). ¹³C NMR (CDCl₃, 125 MHz) δ : 170.7 (COCH₃), 159.3, 137.6, 132.6(2), 129.9(4), 129.4(2), 113.7(2) (ArC), 110.3(2) [$C(CH_3)_2$], 86.5 (C-1), 79.5, 77.8, 74, 73.5, 73.1, 63.7, 55.2 (CH₂-C₆H₄OCH₃), 27.7, 26.3 (2 × isopropylidene-CH₃), 21.1 (SC₆H₄CH₃), 20.8 (COCH₃). HRMS calcd for C₂₆H₃₂O₇SNa (M + Na)⁺: 511.1766, found: 511.1763.

4-Methoxyphenyl 6-O-acetyl-2-O-(4-methoxybenzyl)-3,4-Oisopropylidene- β -D-galactopyranosyl-(1 \rightarrow 3)-4,6-Obenzylidene-2-deoxy-2-phthalimido- β -D-galactopyranoside (6)

A mixture of galactose donor 4 (1.3 g, 2.6 mmol), known monosaccharide acceptor, 4-methoxyphenyl 2-phthalimido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside 5 (1.0 g, 2 mmol) and MS 4 Å (2.0 g) in dry CH_2Cl_2 (20 mL) was stirred under nitrogen for 1 h. NIS (750 mg, 3.4 mmol) was added to it and the mixture was cooled to -45 °C followed by addition of H₂SO₄silica (50 mg). The mixture was allowed to stir at the same temperature for 1 h when TLC (n-hexane-EtOAc; 1:1) revealed that entire acceptor 5 was consumed. Et₃N was added to neutralize the reaction. The reaction mixture was immediately filtered through a pad of Celite. The filtrate was successively washed with Na₂S₂O₃ (2 \times 50 mL), NaHCO₃ (2 \times 50 mL) and brine (50 mL). The organic layer was collected, dried (Na_2SO_4) and evaporated in vacuo. The crude product thus obtained was purified by flash chromatography using *n*-hexane-EtOAc (3:2)to give the pure disaccharide 6 (1.3 g, 83%) as a white foam. $[\alpha]_{D}^{25}$ +86 (*c* 1.0, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ : 7.85–7.06 (m, 13H, ArH), 6.88, 6.71 (2d, 4H, J 9.0 Hz, C₆H₄OCH₃), 5.73 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.5 (s, 1H, CHPh), 5.0 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.97 (dd, 1H, *J*_{1,2} 8.5 Hz, *J*_{2,3} 11.5 Hz, H-2), 4.78 (dd, 1H, *J*_{2,3} 11.0 Hz, J_{3,4} 3.5 Hz, H-3), 4.52 (bs, 2H, CH₂C₆H₄OCH₃), 4.41 (d, 1H, J_{3,4} 3.5 Hz, H-4), 4.37 (dd, 1H, J_{5,6a'} 1.5 Hz, J_{6a',6b'} 12.0 Hz, H-6a'), 4.18 (t, J_{2',3'}, J_{3',4'} 6.0 Hz, H-3'), 4.06 (dd, 1H, J_{5,6b'} 1.5 Hz, $J_{6a',6b'}$ 12.0 Hz, H-6b'), 3.92 (m, 3H, H-4', H-6), 3.76 (s, 3H, CH₂C₆H₄OCH₃), 3.71 (m, 4H, H-5, C₆H₄OCH₃), 3.62 (bs, 1H, H-5'), 3.56 (dd, 1H, $J_{1',2'}$ 3.5 Hz, $J_{2',3'}$ 6.0 Hz, H-2'), 2.73 (s, 6H, 2 × isopropylidene-CH₃), 2.02 (s, 3H, COCH₃). ¹³C NMR (CDCl₃, 125 MHz) δ: 170.4 (COCH₃), 168.8, 167.5 (C=O of NPhth), 159.1, 155.4, 150.3, 137.7, 134.1, 134.0, 131.6, 131.5, 130.2, 129.5(2), 128.9, 128.1(2), 128(2), 126.4(2), 123.6, 123.2, 119.2(2), 114.3(2), 113.6(2) (ArC), 109.5 [C(CH₃)₂], 100.9 (CHPh), 98.2 (C-1), 95.4 (C-1'), 74.7, 73.6, 72.6, 72.5, 72.4, 72.2, 69.2, 67.3, 66.8, 62.6, 55.5 $(C_6H_4OCH_3)$, 55.2 $(CH_2C_6H_4OCH_3)$, 52.2 (C-2), 27.2, 25.6 (2×10^{-6}) isopropylidene- CH_3 , 20.8 (CO CH_3). HRMS calcd for $C_{47}H_{49}O_{15}NNa (M + Na)^+$: 890.3000, found: 890.2997.

4-Methoxyphenyl 6-O-acetyl-3,4-O-isopropylidene- β -D-galactopyranosyl- $(1 \rightarrow 3)$ -4,6-O-benzylidene-2-deoxy-2-phthalimido- β -D-galactopyranoside (7)

Disaccharide 6 (1.3 g, 1.5 mmol) was dissolved in $CH_2Cl_2-H_2O$ (20:5) mixture. DDQ (660 mg, 2.9 mmol) was added to the

reaction mixture and it was kept stirring for 2 h, as monitored by TLC (*n*-hexane–EtOAc; 1 : 1). The reaction mixture was then successively washed with H_2O (2 \times 50 mL). The organic layer was collected, dried over Na₂SO₄, filtered and evaporated in vacuo. The product, thus obtained, was purified using flash chromatography using *n*-hexane–EtOAc (1:1) as eluent to furnish the disaccharide acceptor 7 (910 mg, 81%) in pure form. $[\alpha]_{D}^{25}$ +108 (c 1.0, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ : 7.9-7.38 (m, 9H, ArH), 6.9, 6.73 (2d, 4H, J 9.5 Hz, C₆H₄OCH₃), 5.83 (d, 1H, J_{1.2} 8.0 Hz, H-1), 5.62 (s, 1H, CHPh), 5.04 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 4.86 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 11.0 Hz, H-2), 4.79 (dd, 1H, J_{2.3} 11.0 Hz, J_{3.4} 4.0 Hz, H-3), 4.44 (d, 1H, J_{3.4} 3.5 Hz, H-4), 4.41 (dd, 1H, J_{5,6a'} 1.0 Hz, J_{6a',6b'} 12.5 Hz, H-6a'), 4.13 (dd, 1H, *J*_{5,6b'} 1.5 Hz, *J*_{6a',6b'} 12.0 Hz, H-6b'), 4.07 (t, *J*_{2',3'}, $J_{3',4'}$ 6.0 Hz, H-3'), 3.84 (m, 2H, H-6), 3.82 (dd, 1H, $J_{3',4'}$ 6.0 Hz, J_{4',5'} 2.5 Hz, H-4'), 3.71 (m, 2H, H-2, C₆H₄OCH₃), 3.67 (m, 1H, H-5), 3.65 (m, 1H, H-5'), 2.0 (s, 3H, COCH₃), 1.41, 1.22 (2s, 6H, $2 \times \text{isopropylidene-CH}_3$). ¹³C NMR (CDCl₃, 125 MHz) δ : 170.2 (COCH₃), 168.4, 167.7 (C=O of NPhth), 155.6, 150.7, 137.2, 134.3, 131.4, 131.3, 129.2, 128.4(2), 128.3, 126.3(2), 123.7, 123.4, 119.3(3), 114.4(2) (ArC), 109.7 [C-(CH₃)₂], 101.3 (CHPh), 97.9 (C-1), 94.5 (C-1'), 75.4, 72.3, 71.8, 71.6, 69.2, 68.6, 66.9, 66.6, 62.7, 55.5 ($C_6H_4OCH_3$), 52.1 (C-2), 27.4, 25.7 (2 × isopropylidene-CH₃), 20.8 (COCH₃). HRMS calcd for $C_{39}H_{41}O_{14}NNa (M + Na)^+$: 770.2425, found: 770.2422.

4-Methoxyphenyl 4,6-O-benzylidene-2,3-di-O-benzyl- β -D-glucopyranosyl (1 \rightarrow 2)-3,4-di-O-benzyl- α -L-rhamnopyranosyl (1 \rightarrow 2)-3,4-di-O-benzyl- α -L-rhamnopyranoside (10)

To a mixture of disaccharide acceptor 8 (2.0 g, 2.6 mmol), known donor 4-tolyl 4,6-O-benzylidene-2,3-di-O-benzyl-1-thio- β -D-glucopyranoside (9) (1.9 g, 3.4 mmol) in dry CH₂Cl₂ (30 mL) and 4 Å powdered molecular sieves (3.0 g) was added and the mixture was stirred under nitrogen atmosphere for 1 h. NIS (1.0 g, 4.4 mmol) was added to the reaction mixture and it was cooled to -55 °C. H₂SO₄-silica (50 mg) was added to the reaction mixture and it was stirred for 25 min until TLC (hexane-EtOAc; 2:1) indicated complete consumption of acceptor 8. The reaction mixture was diluted with CH₂Cl₂ (30 mL) and filtered through a pad of Celite. The filtrate was then successively washed with aq. $Na_2S_2O_3$ (2 \times 50 mL), saturated aq. NaHCO₃ (2 \times 50 mL) and brine (50 mL). The organic layer was separated, dried (Na₂SO₄) and evaporated in vacuo. The crude trisaccharide thus obtained, was purified by flash chromatography using *n*-hexane–EtOAc (3:1) as eluent to afford pure trisaccharide **10** (2.7 g, 88%). $[\alpha]_{D}^{25}$ +78 (c 0.9, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ: 7.57-7.28 (m, 35H, ArH), 6.99, 6.86 (2d, 4H, *J* 9.0 Hz, C₆*H*₄OCH₃), 5.61 (s, 1H, CHPh), 5.38 (d, 1H, *J*_{1,2} 1.5 Hz, H-1), 5.11 (d, 1H, J_{1',2'} 1.5 Hz, H-1'), 4.96 (m, 3H, CH₂Ph), 4.84 (m, 2H, CH₂Ph), 4.83 (d, 1H, J_{1",2"} 4.5 Hz, H-1"), 4.77, 4.64 (2d, 2H, J 12.0 Hz, CH₂Ph), 4.76, 4.59, 4.44 (3d, 3H, J 11.5 Hz, CH₂Ph), 4.7, 4.68 (2d, 2H, *J* 11.0 Hz, CH₂Ph), 4.32 (m, 1H, H-5"), 4.2 (t, 1H, J_{1,2}, J_{2,3} 2.5 Hz, H-2), 4.18 (t, 1H, J_{1',2'}, J_{2',3'} 2.5 Hz, H-2'), 4.13 (m, 2H, H-3", H-4"), 4.06 (dd, 1H, $J_{2',3'}$ 3.0 Hz, $J_{3',4'}$ 9.5 Hz, H-3'), 3.99 (dd, 1H, *J*_{2,3} 3.0 Hz, *J*_{3,4} 9.5 Hz, H-3), 3.85 (m, 2H, H-5, H-5'), 3.81 (s, 3H, C₆H₄OCH₃), 3.66 (m, 3H, H-4, H-6"), 3.49

(m, 2H, H-2", H-4'), 1.35 (d, 3H, $J_{5,6}$ 6.0 Hz, C–C H_3), 1.3 (d, 3H, $J_{5',6'}$ 6.5 Hz, C–C H_3). ¹³C NMR (CDCl₃, 125 MHz) δ : 154.8, 150.2, 138.9, 138.6, 138.4, 138.3, 138.2, 137.6, 128.8, 128.4(3), 128.3(3), 128.2(3), 128.1(3), 128.0(3), 127.9(3), 127.8(3), 127.7(3), 127.6(2), 127.5(3), 127.4, 127.3, 127.2(2), 126.1(2), 117.4(2), 114.6(2) (ArC), 101.2 (CHPh), 98.8 (C-1'), 97.7 (C-1), 96.8 (C-1"), 82.4, 80.4, 80.1, 79.6, 79.3, 78.1, 78, 75.3, 75, 74.3, 74.2, 72.3, 72.2(2), 69.1, 68.9, 68.5, 62.5, 55.6 (C₆H₄OCH₃), 18.0(2) (2 × C–CH₃). HRMS calcd for C₇₄H₇₈O₁₅Na (M + Na)⁺: 1229.5238, found: 1229.5235.

4-Methoxyphenyl 2,3-di-O-benzyl- β -D-glucopyranosyl $(1 \rightarrow 2)$ -3,4-di-O-benzyl- α -L-rhamnopyranosyl $(1 \rightarrow 2)$ -3,4-di-O-benzyl- α -L-rhamnopyranoside (11)

The trisaccharide 10 (2.7 g, 2.2 mmol) was treated with 80% AcOH (20 mL) at 80 °C for 2 h resulting in cleavage of benzylidene ring. The solvents were then evaporated using toluene as co-solvent and the reaction mixture was dried in vacuum. The product was then purified by flash chromatography using *n*-hexane–EtOAc (1:1) as eluent to furnish pure product 11 (2.3 g, 91%). $[\alpha]_{D}^{25} + 131 (c 1.0, \text{CHCl}_3)$. ¹H NMR (500 MHz, CDCl₃) δ: 7.47-7.22 (m, 30H, ArH), 6.99, 6.86 (2d, 4H, J 9.0 Hz, $C_6H_4OCH_3$, 5.39 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 5.16 (s, 1H, H-1'), 4.97 (m, 3H, CH_2Ph), 4.87 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1''), 4.81, 4.76 (2d, 2H, J 12.0 Hz, CH₂Ph), 4.77 (d, 2H, J 11.5 Hz, CH₂Ph), 4.71, 4.69 (2d, 2H, J 11.0 Hz, CH₂Ph), 4.66 (d, 1H, J 11.5 Hz, CH₂Ph), 4.45 (bs, 2H, CH₂Ph), 4.21 (t, 1H, J_{1.2}, J_{2.3} 2.5 Hz, H-2), 4.2 (t, 1H, J_{1'.2'}, J_{2',3'} 2.5 Hz, H-2'), 4.07 (dd, 1H, J_{2,3} 3.0 Hz, J_{3,4} 9.0 Hz, H-3), 4.04 (m, 1H, H-5''), 4.0 (dd, 1H, $J_{2',3'}$ 3.0 Hz, $J_{3',4'}$ 9.5 Hz, H-3'), 3.9 (d, 1H, J_{2",3"} 9.0 Hz, H-3"), 3.85 (m, 2H, H-5, H-5'), 3.8 (s, 3H, C₆H₄OCH₃), 3.65 (m, 2H, H-4', H-6a"), 3.56 (m, 2H, H-4", H-6b"), 3.51 (t, 1H, J_{3,4}, J_{4,5} 9.5 Hz, H-4), 3.44 (dd, 1H, J_{1",2"} 3.5 Hz, J_{2",3"} 9.5 Hz, H-2"), 2.56, 2 (2bs, 2H, 2 \times CH₂OH), 1.38 (d, 3H, $J_{5,6}$ 6.0 Hz, C-CH₃), 1.31 (d, 3H, $J_{5',6'}$ 6.0 Hz, C-CH₃). ¹³C NMR (CDCl₃, 125 MHz) δ : 154.8, 150.1, 138.7, 138.4, 138.3, 138.1, 128.5(3), 128.4(3), 128.3(3), 128.2(3), 128.1(3), 128.0(3), 127.8(3), 127.7(3), 127.6(3), 127.5(2), 127.4(3), 117.3(2), 114.6(2) (ArC), 98.4 (C-1'), 97.6 (C-1), 95.0 (C-1"), 80.7, 80.4, 80.3, 79.7, 79.6, 78.1, 75.3, 75, 74, 73.4, 72.4, 72.1, 71.7, 71, 70.5, 68.9, 68.5, 62.4, 60.3, 55.6 ($C_6H_4OCH_3$). 18.1, 18.0 (2 × C-CH₃). HRMS calcd for $C_{67}H_{74}O_{15}Na (M + Na)^+$: 1141.4925, found: 1141.4923.

4-Methoxyphenyl 2,3-di-O-benzyl-6-O-chloroacetyl- β -D-glucopyranosyl (1 \rightarrow 2)-3,4-di-O-benzyl- α -L-rhamnopyranosyl (1 \rightarrow 2)-3,4-di-O-benzyl- α -L-rhamnopyranoside (12)

Trisaccharide **11** (2.3 g, 2.1 mmol) was dissolved in dry CH₃CN (20 mL) and Et₃N (0.3 mL, 2.1 mmol) and stirred at -5 °C for 10 minutes. Chloroacetic anhydride (390 mg, 2.3 mmol) was added and the solution was stirred at -5 °C for 1 hour when TLC (*n*-hexane–EtOAc; 3 : 2) revealed complete conversion of the starting material. The solvents were evaporated and coevaporated with toluene for complete removal of pyridine. The crude residue thus obtained was purified by flash chromatography using *n*-hexane–EtOAc (3 : 1) as eluent to afford the desired trisaccharide acceptor **12** (2.3 g, 91%). [α]_D²⁵+84 (*c* 1.0, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ : 7.46–7.30 (m, 30H, Ar*H*),

7.02, 6.89 (2d, 4H, J 9.0 Hz, C₆H₄OCH₃), 5.43 (d, 1H, J_{1,2} 1.5 Hz, H-1), 5.14 (d, 1H, J_{1',2'} 1.0 Hz, H-1'), 5.01 (m, 3H, CH₂Ph), 4.92 (d, 1H, J_{1",2"} 3.0 Hz, H-1"), 4.86-4.68 (m, 7H, CH₂Ph), 4.43 (s, 2H, CH₂Ph), 4.29 (m, 3H, H-2', H-5", H-6a"), 4.24 (t, 1H, J_{1,2}, J_{2,3} 2.0 Hz, H-2), 4.15 (m, 1H, H-6b"), 4.11 (dd, 1H, J_{2,3} 2.5 Hz, J_{3,4} 9.0 Hz, H-3), 4.04 (m, 3H, H-3', COCH₂Cl), 3.89 (m, 3H, H-3", H-5, H-5'), 3.82 (s, 3H, C₆H₄OCH₃), 3.69 (t, 1H, J_{3',4'}, J_{4',5'} 9.5 Hz, H-4'), 3.55 (m, 2H, H-4, H-4"), 3.47 (dd, 1H, J_{1",2"} 3.5 Hz, J_{3",4"} 9.5 Hz, H-2"), 1.42 (d, 3H, J_{5,6} 6.5 Hz, C-CH₃), 1.36 (d, 3H, J_{5',6'} 6.5 Hz, C-CH₃). ¹³C NMR (CDCl₃, 125 MHz) δ: 167.3 (COCH₂Cl), 154.7, 150.1, 138.5, 138.3(3), 138.2(2), 138.1, 128.4(5), 128.3(5), 128.2(2), 128.1(2), 128(2), 127.8(2), 127.7(4), 127.6, 127.5(2), 127.3(2), 127.1(2), 117.3(2), 114.5(2) (ArC), 98.4 (C-1'), 97.6 (C-1), 95 (C-1"), 80.5, 80.3(2), 79.6, 79.5, 78, 75.4, 75.3, 75.1, 74, 73.5, 72.4, 72.3, 71.7, 69.5, 69, 68.8, 68.5, 64.5, 55.5 (C₆H₄OCH₃), 40.6 $(COCH_2Cl)$, 18.0(2) $(2 \times C-CH_3)$. HRMS calcd for $C_{69}H_{75}O_{16}$ - $ClNa (M + Na)^{+}$: 1217.4641, found: 1217.4638.

4-Methoxyphenyl 2,3,4-tri-O-acetyl- α -L-rhamnopyranosyl- $(1 \rightarrow 4)$ -2,3-di-O-benzyl-6-O-chloroacetyl- β -D-glucopyranosyl- $(1 \rightarrow 2)$ -3,4-di-O-benzyl- α -L-rhamnopyranosyl- $(1 \rightarrow 2)$ -3,4-di-O-benzyl- α -L-rhamnopyranoside (14)

A suspension of trisaccharide acceptor 12 (2.3 g, 1.95 mmol), the known rhamnose donor, 4-tolyl 2,3,4-tri-O-acetyl-1-thio-α-Lrhamnopyranoside 13 (1.0 g, 2.5 mmol) and MS 4 Å (2 g) in dry CH₂Cl₂ (20 mL) was stirred under nitrogen for 30 minutes. After adding NIS (740 mg, 3.3 mmol), the mixture was cooled to 0 °C. After 10 minutes, H₂SO₄-silica (50 mg) was added and the mixture was stirred at 0 °C for 50 minutes when TLC (n-hexane-EtOAc; 2:1) revealed complete consumption of the donor. The reaction mixture was then immediately filtered through a pad of Celite and washed with CH₂Cl₂. The combined filtrate obtained was washed successively with $Na_2S_2O_3$ (2 \times 50 mL), NaHCO₃ $(2 \times 50 \text{ mL})$ and finally with brine (50 mL). The organic layer was separated, dried (Na₂SO₄), filtered and evaporated in vacuo. The residue was then purified by flash chromatography using *n*-hexane-EtOAc (3:1) as eluent to give the disaccharide 14 (2.3 g, 85%) as white foam. $[\alpha]_{D}^{25}$ +148 (c 0.9, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ: 7.5-7.23 (m, 30H, ArH), 7.02, 6.88 (2d, 4H, J 9.0 Hz, C₆H₄OCH₃), 5.42 (d, 1H, J_{1.2} 1.5 Hz, H-1), 5.3 (dd, 1H, $J_{2''',3'''}$ 3.5 Hz, $J_{3''',4'''}$ 10.0 Hz, H-3'''), 5.16 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 5.06 (m, 5H, H-4", CH₂Ph), 4.94 (m, 2H, H-1", CH₂Ph), 4.8 (m, 5H, H-1", H-2", CH₂Ph), 4.67 (d, 1H, J 12.0 Hz, CH₂Ph), 4.38 (dd, 1H, $J_{5'',6a''}$ 2.0 Hz, $J_{6a'',6b''}$ 12.5 Hz, H-6a''), 4.31 (m, 3H, CH_2Ph), 4.24 (m, 2H, H-2, H-2'), 4.16 (m, 2H, H-5', H-5'''), 4.1 (m, 3H, COCH₂Cl, H-3'), 4.05 (dd, 1H, J_{2,3} 3.5 Hz, J_{3,4} 9.5 Hz, H-3), 3.94 (t, 1H, *J*_{2",3"}, *J*_{3",4"} 9.0 Hz, H-3"), 3.88 (m, 2H, H-5, H-5"), 3.8 (s, 3H, C₆H₄OCH₃), 3.74 (m, 3H, H-4', H-4", H-6b"), 3.59 (t, 1H, J_{3,4}, J_{4,5} 9.5 Hz, H-4), 3.46 (dd, 1H, *J*_{1",2"} 3.5 Hz, *J*_{2",3"} 9.0 Hz, H-2"), 2.16 (s, 3H, COCH₃), 2.08 (bs, 6H, 2 \times COCH₃), 1.43 (d, 3H, $J_{5,6}$ 6.0 Hz, C-CH₃), 1.37 (d, 3H, J_{5',6'} 6.5 Hz, C-CH₃), 0.98 (d, 3H, J_{5^{'''},6^{'''}} 6.5 Hz, C–CH₃). ¹³C NMR (CDCl₃, 125 MHz) δ: 170, 169.9, 169.7 (3 \times COCH₃), 166.6 (COCH₂Cl), 154.7, 150, 138.4, 138.3(2), 138.2(4), 138.1(2), 138.0(2), 128.4, 128.3(4), 128.2(2), 128.1, 128(4), 127.9(2), 127.8, 127.7(3), 127.6(2), 127.5, 127.4, 127.3, 127.2, 126.9, 117.2(2), 114.5(2) (ArC), 98.1 (C-1'), 97.6(2)

 $\begin{array}{l} (C\text{-1}, C\text{-1}'''), \ 94.1 \ (C\text{-1}''), \ 80.4, \ 80.3, \ 80.2, \ 79.6, \ 79.2, \ 77.9, \ 75.3, \\ 75.2, \ 74.8, \ 73.7, \ 73.1, \ 72.5, \ 72.2, \ 71.7, \ 70.8, \ 69.6, \ 68.8, \ 68.7, \ 68.5, \\ 67.9, \ 66.9, \ 63.6, \ 60.2, \ 55.4 \ (C_6H_4OCH_3), \ 40.6 \ (COCH_2Cl), \ 20.7, \\ 50.6, \ 20.5(3 \ \times \ COCH_3), \ 17.9(2) \ (2 \ \times \ C\text{-}CH_3), \ 17.0 \ (C\text{-}CH_3). \\ HRMS \ calcd \ for \ C_{81}H_{91}O_{23}ClNa \ (M \ + \ Na)^+: \ 1489.5537, \ found: \\ 1489.5533. \end{array}$

4-Methoxyphenyl 2,3,4-tri-O-acetyl- α -1-rhamnopyranosyl (1 \rightarrow 4)-2,3-di-O-benzyl-6-O-chloroacetyl- β -D-glucopyranosyl (1 \rightarrow 2)-3,4-di-O-benzyl- α -1-rhamnopyranosyl (1 \rightarrow 2)-3,4-di-O-benzyl- α -1-rhamnopyranosyl (1 \rightarrow 2)-6-O-acetyl-3,4-O-isopropylidene- β -D-galactopyranosyl-(1 \rightarrow 3)-4,6-O-benzylidene-2-deoxy-2-phthalimido- β -D-galactopyranoside (17)

A mixture of disaccharide acceptor 7 (500 mg, 0.7 mmol), trichloroacetamidate donor 16 (1.3 g, 0.87 mmol) and freshly activated MS 4 Å (1.0 g) in dry CH_2Cl_2 (10 mL) was stirred under nitrogen for 1 hour at room temperature. After cooling the reaction mixture to 0 °C, H₂SO₄-silica (50 mg) was added and the mixture was allowed to stir at the same temperature for 30 min when TLC (n-hexane-EtOAc; 1:1) showed complete consumption of acceptor 7. The reaction mixture was neutralized with Et₃N and filtered through a pad of Celite. The filtrate was diluted with CH2Cl2 and was successively washed with NaHCO₃ (2 \times 50 mL) and brine (50 mL). The organic layer was separated, dried (Na_2SO_4) and evaporated *in vacuo*. The crude product thus obtained was purified by flash chromatography using *n*-hexane–EtOAc (1.2:1) to give the pure hexasaccharide 17 (1.0 g, 81%). $[\alpha]_{D}^{25}$ +124 (c 0.8, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ: 7.89-7.05 (m, 39H, ArH), 6.82, 6.69 (2d, 4H, J 9.0 Hz, C₆H₄OCH₃), 5.64 (d, 1H, J_{1,2} 8.5 Hz, H-1), 5.6 (s, 1H, CHPh), 5.2 (dd, 1H, J₂"", 3"" 3.5 Hz, J₃"", 4"" 10.0 Hz, H-3""), 5.05 (m, 2H, H-1", H-4""), 4.92 (m, 9H, H-1', H-1"", H-1"", H-2, H-2""", CH₂Ph), 4.71 (m, 5H, H-3, CH₂Ph), 4.59 (d, 1H, J_{1"",2""} 3.5 Hz, H-1""), 4.27 (m, 3H, H-4, H-6a"", CH₂Ph), 4.11 (m, 8H, H-2", H-2", H-3', H-5"", H-5""), 4.03 (bs, 2H, COCH2Cl), 3.91 (m, 2H, H-4', H-5), 3.83 (m, 3H, H-3"", H-5", H-6a'), 3.77-3.59 (m, 12H, H-2', H-3", H-3"', H-4"", H-5', H-5"', H-6a, H-6b', H-6b"", C₆H₄OCH₃), 3.49 (bs, 1H, H-6b), 3.44 (m, 2H, H-4", H-4"), 3.3 (dd, 1H, *J*₁^{""}, 2^{""} 3.5 Hz, *J*_{2,3}^{""} 9.0 Hz, H-2^{""}), 2.11, 2.03, 2.02, 1.99 (4s, 12H, $4 \times COCH_3$), 1.39, 1.19 (2s, 6H, $2 \times isopropylidene-$ *CH*₃), 1.37 (d, 3H, *J*_{5",6"} 6.5 Hz, C-*CH*₃), 1.35 (d, 3H, *J*_{5",6"} 6.5 Hz, C-CH₃), 0.89 (d, 3H, J_{5"",6""} 5.5 Hz, C-CH₃). ¹³C NMR (CDCl₃, 125 MHz) δ : 170.2, 170.1, 170, 169.8 (4 \times COCH₃), 168.9 (COCH₂Cl), 167.5, 166.7 (2 × CO of NPhth), 155.5, 150.8, 138.5, 138.4, 138.3, 138.2, 138.1, 137.8, 134.4, 134.3, 131.5, 128.8, 128.5(2), 128.4(3), 128.3(3), 128.2(4), 128.1(5), 128(2), 127.9(2), 127.8, 127.7, 127.6, 127.5, 127.4, 127.3(2), 126.3, 126.2(2), 124.4, 124.3, 124, 123.6, 123.5, 123.4, 119.2(2), 114.3(2) (ArC), 109.1 [C-(CH₃)₂], 100.6 (C-1"), 100.2 (CHPh), 99.6 (C-1"), 98.1 (C-1), 97.9 (C-1'), 97.6 (C-1""), 93.8 (C-1""), 80.5, 80.4, 80.2, 79.9, 79.3, 78, 76, 75.9, 75.6, 75.3, 75.2, 74.9, 74.8, 73.5, 72.8, 72.7, 72.6, 72.5, 71.6, 71(2), 69.7, 68.9, 68.8, 68.7, 68.3, 67.8, 67.0, 66.8, 66.4, 63.7, 62.2, 55.5 (C₆H₄OCH₃), 52.3 (C-2), 40.8 (COCH₂Cl), 28.2, 26.2 (2 \times isopropylidene-*C*H₃), 20.9, 20.8(2), 20.7 (4 \times $COCH_3$), 18.1, 17.9, 17.1 (3 × C-CH₃). HRMS calcd for $C_{113}H_{124}O_{35}CINNa (M + Na)^+: 2112.7540$, found: 2112.7536.

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To a solution of the hexasaccharide 17 (1.0 g, 0.5 mmol) in CH₂Cl₂-CH₃OH (2 : 3, 20 mL), thiourea (180 mg, 2.4 mmol) and collidine (0.3 mL, 2.4 mmol) were added and it was refluxed at 40 °C for 10 hours when TLC (n-hexane-EtOAc; 1:1) showed complete conversion of the starting material to a slower moving spot. After evaporating the solvents in vacuo, the residue was dissolved in CH₂Cl₂ (50 mL) and washed with 1 M HCl (50 mL) and H₂O (50 mL). The organic layer was collected, dried (Na₂SO₄), filtered and evaporated. The crude product thus obtained was purified by flash chromatography using *n*-hexane–EtOAc (1:1) as eluent to afford the pure hexasaccharide acceptor 18 (850 mg, 89%) as colourless foam. $\left[\alpha\right]_{D}^{25}$ +101 (c 0.7, CHCl₃). ¹H NMR (500 MHz, CDCl₃) δ: 7.89-7.06 (m, 39H, ArH), 6.82, 6.68 (2d, 4H, J 9.0 Hz, C₆H₄OCH₃), 5.64 (d, 1H, J_{1,2} 9.0 Hz, H-1), 5.59 (s, 1H, CHPh), 5.25 (dd, 1H, *J*_{2"", 3""} 3.5 Hz, *J*_{3"", 4""} 10.0 Hz, H-3"""), 5.12 (dd, 1H, J_{1""" 2}"" 1.5 Hz, J_{2""", 3"}" 3.5 Hz, H-2"""), 5.05 (d, 1H, J_{1",2"} 1.5 Hz, H-1"), 4.95 (m, 6H, H-1", H-2, H-4"", CH₂Ph), 4.87 (s, 1H, H-1""), 4.85 (d, 1H, *J*_{1',2'} 3.5 Hz, H-1'), 4.78 (d, 1H, *J* 12.0 Hz, *CH*₂Ph), 4.67 (m, 7H, H-1^{""}, H-3, CH₂Ph), 4.29 (d, 1H, J_{3.4} 3.0 Hz, H-4), 4.23 (m, 2H, CH₂Ph), 4.15 (m, 2H, H-3', CH₂Ph), 4.06 (m, 3H, H-2", H-2", H-5"""), 3.91 (m, 2H, H-4', H-5""), 3.82 (m, 4H, H-3", H-3"", H-3"", H-6a'), 3.75 (m, 1H, H-5"'), 3.65 (m, 10H, H-2', H-4"", H-5, H-5', H-5", H-6"", C₆H₄OCH₃), 3.49 (bs, 1H, H-6a), 3.42 (m, 3H, H-4", H-4^{'''}, H-6b'), 3.35 (dd, 1H, J₁^{''''}, 2^{''''} 3.5 Hz, J₂^{''''}, 3^{''''} 9.0 Hz, H-2^{''''}), 3.24 (d, 1H, $J_{6a,6b}$ 12 Hz, H-6b), 2.11, 2.02, 2.00, 1.99 (4s, 12H, 4 \times COCH₃), 1.39, 1.19 (2s, 6H, 2 × isopropylidene-CH₃), 1.34 (m, 6H, $2 \times C-CH_3$, 0.79 (d, 3H, $J_{5'''',6'''}$ 6.0 Hz, C-CH₃). ¹³C NMR (CDCl₃, 125 MHz) δ: 170.2, 170.1, 170, 169.9 (4 × COCH₃), 168.9, 167.5 (2 × CO of NPhth), 157.1, 155.5, 150.8, 147.9, 138.7, 138.5, 138.4, 138.3, 138.2, 138.1, 137.8, 134.4, 134.3, 131.5, 128.8, 128.4(3), 128.3(2), 128.2(4), 128.1(3), 128.0(4), 127.9(4), 127.8, 127.6, 127.5(2), 127.4, 127.3(2), 127.2, 127.0, 126.5(2), 126.2(2), 123.6, 123.4, 119.2(2), 114.3(2) (ArC), 109.1 [C-(CH₃)₂], 100.5 (C-1"), 100.2 (CHPh), 99.5 (C-1""), 98.1 (C-1), 97.9 (C-1"""), 97.1 (C-1'), 94.1 (C-1""), 80.4, 80.3, 79.9, 79.1, 78, 75.9, 75.8, 75.5, 75.3, 75.2, 74.8, 73.8, 73.5, 72.8, 72.7, 72.6, 72.3(2), 71.7, 71.1, 71, 70.5, 69.8, 69.2, 68.8, 68.6, 68.3, 66.7, 66.6, 66.3, 62.2, 60.8, 55.5 (C₆H₄OCH₃), 52.3 (C-2), 28.2, 26.2 (2 \times isopropylidene-CH₃), 20.9, 20.8(2), 20.7 (4 \times $COCH_3$, 18.1, 17.8, 16.9 (3 × C-CH₃). HRMS calcd for $C_{111}H_{123}O_{34}NNa (M + Na)^+$: 2036.7824, found: 2036.7821.

4-Methoxyphenyl α -L-rhamnopyranosyl $(1 \rightarrow 4)$ - β -Dglucopyranosyluronic acid $(1 \rightarrow 2)$ - α -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - α -L-rhamnopyranosyl- $(1 \rightarrow 2)$ - β -D-galactopyranosyl- $(1 \rightarrow 3)$ -2-acetamido-2-deoxy- β -D-galactopyranoside (1)

To a solution of the hexasaccharide acceptor **18** (850 mg, 0.4 mmol) in $CH_2Cl_2-H_2O$ (2 : 1, 20 mL), TEMPO (12 mg, 0.07 mmol) was added followed by iodosobenzene diacetate (IBDA) (310 mg, 1 mmol) and the mixture was vigorously stirred at room temperature for 2 hours till TLC (EtOAc) indicated

complete conversion of the starting material to a lower running spot. Na₂S₂O₃ solution (10% in H₂O, 10 mL) was added to quench the reaction. The mixture was then extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layer was dried over Na₂SO₄, filtered and evaporated in vacuo. The crude acid derivative was then treated with 80% AcOH (10 mL) at 80 °C for 4 hours resulting in the hydrolysis of both benzylidene and isopropylidene ring. The solvents were evaporated and co-evaporated with toluene. The diol formed was dissolved in n-butanol (15 mL) and ethylene diamine (1.5 mL) was added and the reaction mixture was allowed to stir for 24 h at 110 °C. The solvents were then evaporated and co-evaporated with toluene and the crude product thus obtained is dissolved in pyridine (10 mL) followed by the addition of Ac₂O (10 mL). The reaction mixture was allowed to stir at room temperature for 10 h when the TLC showed complete conversion of the starting material. The reaction mixture was then co-evaporated with toluene to give the required hexasaccharide, 4-methoxyphenyl-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl (1→4)-2,3-di-O-benzyl-β-D-glucopyranosyl- $(1 \rightarrow 2)$ -3,4-di-O-benzyl- α -L-rhamnopyranosyl- $(1 \rightarrow 2)$ -3,4-di-O-benzyl- α -L-rhamnopyranosyl- $(1 \rightarrow 2)$ -3,4,6-tri-Oacetyl- β -D-galactopyranosyl- $(1 \rightarrow 3)$ -4,6-di-O-acetyl-2-acetamido-2-deoxy-β-D-galactopyranoside in its crude state. The dried compound was dissolved in MeOH (100 mL) and it was passed through an H-cube flow hydrogenation assembly using a 10% Pd-C cartridge with a flow rate of 1 mL min⁻¹. Complete removal of the six benzyl groups was achieved after six cycles as confirmed by the mass spectra. MeOH was evaporated and the compound was dried well. The dried residue was re-dissolved in MeOH (10 mL) and NaOMe (1 mL, 0.5 M in MeOH) was added to it. The reaction mixture was stirred at room temperature for 10 hours. DOWEX 50W H⁺ resin was added to neutralize excess NaOMe. The solution was filtered, evaporated in vacuo and the residue was triturated with CH2Cl2 to remove the organic soluble side products/salts generated during TEMPO oxidation. The residue obtained thereafter was further purified by HPLC using C18 column and CH₃CN-H₂O mixture as eluent to afford the desired hexasaccharide 1 (230 mg, 54% overall) as white amorphous mass. $\left[\alpha\right]_{\rm D}^{25}$ +61 (c 1.0, MeOH). Partial ¹H NMR $(500 \text{ MHz}, \text{CDCl}_3) \delta$: 6.98, 6.81 (2d, 4H, J 9.0 Hz, C₆H₄OCH₃), 5.31 (d, 1H, J_{1,2} 8.5 Hz, H-1), 5.06 (d, 1H, J_{1",2"} 2.0 Hz, H-1"), 5.01 $(d, 1H, J_{1',2'} 3.5 Hz, H-1'), 4.93 (s, 1H, H-1'''), 4.92 (s, 1H, H-1''''),$ 4.84 (s, 1H, H-1""), 3.73 (s, 1H, C₆H₄OCH₃), 2.02 (s, 3H, NHCOCH₃), 1.26 (bd, 9H, J 6.0 Hz, C-CH₃). 13C NMR (CDCl₃, 125 MHz) & 173.4 (NHCOCH₃), 173.0 (COOH), 156.8, 153.2, 119.3(2), 115.5(2) (ArC), 102.3 (C-1"), 101.6 (C-1), 101.3 (C-1"), 101.2 (C-1""), 99.6 (C-1""), 97.9 (C-1'), 80, 79.4, 78.6, 76.6, 76.2, 76, 74.4, 74.1, 73.8(2), 73.7(2), 72.9, 72.5(2), 72.4, 72.2, 71.6, 71.2, 70.7, 70.6, 70.4, 66.1, 62.6, 62.1, 61.8, 56.1 (C₆H₄OCH₃), 20.9 $(NHCOCH_3)$, 18.3, 17.9, 17.8 $(3 \times C-CH_3)$. HRMS calcd for $C_{45}H_{69}O_{30}NNa (M + Na)^{+}$: 1126.3802, found: 1126.3798.

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Notes and references

- 1 P. P. Reeves and L. Wang, *Curr. Top. Microbiol. Immunol.*, 202, 264, 109–135.
- 2 J. L. Jaegar and D. W. Acheson, *Curr. Infect. Dis. Rep.*, 2000, 2, 61–67.
- 3 P. M. Fratamico, L. K. Bagi, E. J. Bush and B. T. Solow, *Appl. Environ. Microbiol.*, 2004, **70**, 7173–7178.
- 4 M. Blanco, N. L. Padola, A. Kruger, M. E. Sanz, J. E. Blanco, E. A. Gonzalez, G. Dahbi, A. Mora, M. I. Bernardez, A. I. Etcheverria, G. H. Arroyo, P. M. Lucchesi, A. E. Parma and J. Blanco, *Int. Microbiol.*, 2004, 7, 269–276.
- 5 A. V. Perepelov, Q. Wang, S. N. Senchenkova, Y. Gong, A. S. Shaskov, L. Wang and Y. A. Knirel, *Carbohydr. Res.*, 2012, **353**, 106–110.
- 6 A. K. Choudhury and N. Roy, *J. Carbohydr. Chem.*, 1997, **16**, 1363–1371.
- 7 D. Dubreuil, J. Cleophax and A. Loupy, *Carbohydr. Res.*, 1994, **252**, 149–157.
- 8 Z. Wang, M. Matin and S. Sheikh, *Molecules*, 2005, **10**, 1325–1334.
- 9 Z. Zhang and G. Magnusson, *Carbohydr. Res.*, 1996, **295**, 41–55.
- 10 (a) V. K. Rajput, B. Roy and B. Mukhopadhyay, *Tetrahedron Lett.*, 2006, 47, 6987–6991; (b) V. K. Rajput and B. Mukhopadhyay, *Tetrahedron Lett.*, 2006, 47, 5939–5941; (c) B. Mukhopadhyay, *Tetrahedron Lett.*, 2006, 47, 4337–4341; (d) B. Roy and B. Mukhopadhyay, *Tetrahedron Lett.*, 2007, 48, 3783–3787.
- 11 (a) S. Dasgupta, B. Roy and B. Mukhopadhyay, *Carbohydr. Res.*, 2006, **341**, 2708–2713; (b) K. B. Pal and

B. Mukhopadhyay, *Carbohydr. Res.*, 2013, **379**, 26–29; (*c*) P. R. Verma and B. Mukhopadhyay, *RSC Adv.*, 2013, **3**, 201– 207; (*d*) R. Das and B. Mukhopadhyay, *Carbohydr. Res.*, 2013, **376**, 1–6; (*e*) R. Das, M. Mahanti and B. Mukhopadhyay, *Carbohydr. Res.*, 2014, **399**, 15–20.

- 12 Y. Oikawa, T. Yoshioka and O. Yonemitsu, *Tetrahedron Lett.*, 1982, **23**, 885–888.
- 13 T. Ghosh and A. K. Misra, Carbohydr. Res., 2012, 362, 8-12.
- 14 T. Li, H. Ye, X. Cao, J. Wang, Y. Liu, L. Zhuo, Q. Liu, W. Wang, J. Shen, W. Zhao and P. Wang, *ChemMedChem*, 2014, 9, 1071–1080.
- 15 G. Medgyes, G. Jerkovich and J. Kuszmann, *Carbohydr. Res.*, 1989, **186**, 225–239.
- 16 M. Bertolini and C. P. J. Glaudemans, *Carbohydr. Res.*, 1970, 15, 263–270.
- 17 I. Cumpstey, A. J. Fairbanks and A. J. Redgrave, *Tetrahedron*, 2004, **60**, 9061–9074.
- 18 B. Classon, P. J. Garegg and B. Samuelson, Acta Chem. Scand., Ser. B, 1984, 38, 419–422.
- 19 J. Kerékgyártó, Z. Szurmai and A. Lipták, *Carbohydr. Res.*, 1993, **245**, 65–80.
- 20 V. K. Rajput and B. Mukhopadhyay, J. Org. Chem., 2008, 73, 6924–6927.
- 21 K. Takeo, K. Maki, Y. Wada and S. Kitamura, *Carbohydr. Res.*, 1993, **245**, 81–96.
- 22 L. J. van den Bos, J. D. C. Codeé, J. C. van der Toorn, T. J. Boltje, J. H. van Boom, H. S. Overkleeft and G. A. van der Marel, *Org. Lett.*, 2004, **6**, 2165–2168.
- 23 O. Kanie, S. C. Crawley, M. M. Palcic and O. Hindsgaul, *Carbohydr. Res.*, 1993, 243, 139–164.
- 24 G. Zemplén, Ber. Dtsch. Chem. Ges., 1926, 59, 1254-1266.
- 25 D. D. Perrin, W. L. Amarego and D. R. Perrin, *Purification of Laboratory Chemicals*, Pergamon, London, 1996.