Chemical synthesis of the hexasaccharide related to the repeating unit of the capsular polysaccharide from carbapenem resistant *Klebsiella pneumoniae* 2796 and 3264†

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The total synthesis of the hexasaccharide repeating unit of the CPS from carbapenem resistant *K. pneumoniae* 2796 and 3264 is reported using a sequential glycosylations approach. The total synthesis has been accomplished by glycosylation of rationally protected monosaccharide synthons derived from the commercially available sugars. The required uronic acid on the galactose moiety was successfully installed by a TEMPO-mediated late stage oxidation. The glycosylations were performed by the NIS-mediated activation of thioglycosides using H$_2$SO$_4$-silica as the promoter. Chloroacetate group was extensively used as a temporary protecting group to facilitate stereoselective glycosylations.

Introduction

Bacterial capsular polysaccharides (CPS) and lipopolysaccharides (LPS) are responsible for their virulence factor. CPS and LPS are made up of oligosaccharide repeating units with varied sugar residues. As they remain exposed on the outer surface of the bacterial cell wall, they play pivotal role in infection. Due to the presence of different sugar residues in the oligosaccharide repeats, CPS and LPS demonstrate diverse character and act as the elicitor of innate immune responses. Literature reports suggest that there are potential scope with these bacterial O-antigens as vital candidates for anti-microbial agents and vaccine-targets.1–3 However, it require tedious isolation and purification processes to harness these complex oligosaccharides in adequate quantity. Thus the chemical synthesis of the required oligosaccharides remains the only way to explore their vivid biological roles and potential as vaccine targets.

*Klebsiella pneumoniae* (*K. pneumoniae*) is an opportunistic pathogen that is responsible for community or hospital acquired infections. It mostly affects the urinary and respiratory tracts. Both CPS and LPS of the *K. pneumonia* are found to be responsible for the virulence. The CPS antigens are used for K-typing of *K. pneumoniae* whereas LPS antigens are used for O-typing.4 The capsular antigens are protective against capsular pathogens such as *H. influenza* type b, meningococci and pneumococci.5 Among large number of *K. pneumoniae* K-antigens only a few are associated with human disease.6 This is a limiting factor for the development of suitable vaccine against this pathogen. Particularly the carbapenem resistant K-antigens (CRKP) are rarely known in the literature. Only recently, Kubler-Kielb has reported the structures of the CRKP CPS and LPS from clinical isolates collected from the infected patients of a CRKP outbreak in the US.7 Herein, we report the total synthesis of the hexasaccharide repeating unit of the CPS from *K. pneumoniae* 2796 and 3264 in the form of its p-methoxyphenyl glycoside (Fig. 1). The particular aglycon in the reducing end will enable us to form further glycoconjugates.
after the selective removal of the same from the per-O-acetylated derivative of the target oligosaccharide.

Results and discussion

Judicious retro-synthetic analysis indicated that a sequential glycosylations strategy would be the best fit for the successful synthesis of the target hexasaccharide 1. Thus, three rhamnose moieties were planned to be stitched by using the same synthon 5 with the reducing end galactose moiety 6. The chloroacetate group was thought to be the perfect choice as a temporary protecting group as it can ensure the required 1,2-trans glycosylations as well as can be de-protected selectively to pave the path for introduction of the next sugar unit. Next, a suitably protected galactose synthon 14 having a non-participating group at 2-position thought to be ideal for the required 1,2-cis linkage. A per-O-acetylated rhamnose derivative 17 will complete the target hexasaccharide in its protected form. Finally, a TEMPO mediated oxidation of the primary hydroxyl group of the non-reducing end galactose moiety followed by global de-protection would furnish the required molecule (Fig. 2).

Therefore, the we started our synthesis with known p-tolyl 4-O-benzoyl-2,3-O-isopropylidene-1-thio-a-L-rhamnopyranoside (2).8 Hydrolysis of the isopropylidene group using 80% AcOH at 80 °C (ref. 9) gave the diol 3 in 91% yield. Next, selective benzylation at the equatorial 3-OH was accomplished by following stannylene chemistry10 to give the derivative 4 in 85% yield. Finally, protection of the sole 2-OH with chloroacetate11 group afforded the required donor 5 in 89% yield. Donor 5 was coupled with the known acceptor 6 (ref. 12) by the activation of the thioglycosyl using NIS in the presence of H2SO4-silica13 at 0 °C to afford the disaccharide 7 in 91% yield. It is worth noting that the use of H2SO4-silica as the promoter for NIS-mediated activation of the thioglycoside donor found to be beneficial compared to the use of toxic, fuming and hygroscopic TfOH or TMSOTf. Further, selective de-protection of the chloroacetate group using thiourea14 gave the disaccharide acceptor 8 in 87% isolated yield. Subsequently, glycosylations with the same donor 5 followed by de-protection of the chloroacetate group using the same reagent combination and condition was iterated twice to obtain the tetrasaccharide acceptor 12 (Scheme 1). The yields of the individual steps involved are mentioned in the Scheme 1.

In a separate experiment, known p-tolyl 2,3-di-O-benzyl-6-O-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (13)15 was treated with chloroacetic anhydride in the presence of dry pyridine to give the completely protected donor (14) in 88% yield. Next, glycosylations of the donor 14 with the tetrasaccharide acceptor 12 using NIS in the presence of H2SO4-silica at −50 °C gave the protected pentasaccharide 15 in 82% yield. Presence of the non-participating benzyl group at the 2-position of the galactosyl donor 14 and the reaction at very low temperature assured the formation of the desired 1,2-glycoside as the sole isolated product. Further, selective de-protection of

Fig. 2 Retro-synthetic analysis for the total synthesis of the target hexasaccharide 1.

Scheme 1 Synthesis of the tetrasaccharide acceptor 12.
the chloroacetate group using thiourea afforded the pentasaccharide acceptor 16 in 85% yield. Finally, glycosylations of 16 with the known donor 17 (ref. 16) using the same NIS/H2SO4-silica at 0 °C furnished the protected hexasaccharide 18 in 84% yield. At this stage, the strategically placed 4-methoxybenzyl group was selectively de-protected by oxidative cleavage using DDQ27 to afford the hexasaccharide derivative 19 in 78% isolated yield. Oxidation of the primary hydroxyl group using TEMPO in the presence of bis-acetoxy iodobenzene (BAIB)18 followed by catalytic hydrogenolysis and Zemplen de-O-acetylation19 gave the target hexasaccharide 1 in 64% yield over three steps (Scheme 2). The amorphous white powder of compound 1 was triturated with CH2Cl2 and filtered to remove aromatic impurities.

Conclusions

In conclusion, we have successfully accomplished the total synthesis of the hexasaccharide repeating unit of the CPS from K. pneumoniae 2796 and 3264 in the form of its p-methoxyphenyl glycoside. The practical synthetic strategy used the minimum protecting group manipulations and the chloroacetate group was used extensively as the temporary protecting group to ensure stereoselective glycosylations using rhamnose synths. A TEMPO-mediated late stage oxidation was used successfully to generate the desired uronic acid moiety. The synthetic will definitely enhance the scope for further biological evaluation of the target oligosaccharide related to the carbapenem resistant K. pneumoniae strains and pave the path for a potential vaccine target.

Experimental section

General methods

All solvents and reagents were dried prior to use according to standardized methods.20 The commercially purchased reagents were used without any further purification unless mentioned otherwise. 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 H2SO4. Flash chromatography was performed with Silica Gel 230–400 mesh.1H and 13C NMR spectra were recorded on Bruker Avance 500 MHz spectrometer (1H NMR at 500 MHz and 13C NMR at 125 MHz). HRMS analysis was performed with Micromass Q-TOF micro (Waters Corporation) instrument by +ve mode electro-spray ionization.

p-Tolyl-4-O-benzoyl-1-thio-α-D-rhamnopyranoside (3). Compound 2 (6 g, 14.5 mmol) was dissolved in AcOH–H2O (8 : 1, 36 mL) and the solution was stirred at 80 °C for 2 h until the starting material was completely converted to a slower moving spot as suggested by TLC (n-hexane–EtOAc; 3 : 1). The solvents were evaporated and co-evaporated twice with toluene followed by purification of the crude product by flash chromatography using n-hexane–EtOAc (3 : 5 : 1) to afford pure compound 3 (4.9 g, 91%) as colourless syrup. [α]D25 +103° (c 1.0, CHCl3).1H NMR (CDCl3, 500 MHz) δ: 8.09–7.15 (m, 9H, ArH), 5.51 (d, 1H, J1,2 1.5 Hz, H-1), 5.09 (t, 1H, J1,3 1.0 Hz, J1,2 3.0 Hz, J2,3 3.0 Hz, H-2), 4.07 (dd, 1H, J2,3 3.0 Hz, J3,4 9.5 Hz, H-3), 3.31 (bs, 1H, OCH3). HRMS calcd for C20H22O5SNa (M + Na) + : 397.1086, found: 397.1084.

p-Tolyl-4-O-benzoyl-3-O-benzyl-1-thio-α-D-rhamnopyranoside (4). A mixture of compound 3 (4.8 g, 13 mmol) and Bu2SnO (4.3 g, 17 mmol) was refluxed for 3 h at 80 °C in dry MeOH (30 mL). The resulting solution was concentrated in vacuo. The crude residue was dried under vacuum for 1 h. The residue was then dissolved in dry DMF (20 mL) followed by addition of Bu2N(I) (5.3 g, 14.5 mmol) and stirred at room temperature for 10 min. BnBr (2.0 mL, 17 mmol) was added to the mixture and the solution was stirred for 10 h. After evaporating the solvents in vacuo the residue was dissolved in CH2Cl2 (40 mL) and washed successively with H2O (50 mL), Na2SO3 (50 mL) and brine (50 mL). The organic layer was collected, dried (Na2SO4) and evaporated in vacuo. The crude product was purified by flash chromatography using n-hexane–EtOAc (6 : 1) as eluent to give the pure compound 4 (5.0 g, 85%). [α]D25 +96° (c 1.0, CHCl3).1H NMR (CDCl3, 500 MHz) δ: 8.03–7.10 (m, 14H, ArH), 5.45 (d, 1H, J1,2 < 1.0 Hz, H-1), 5.42 (t, 1H, J1,3 9.5 Hz, J3,4 9.5 Hz, H-4), 4.65, 4.51 (ABq, 2H, J3,4 12.0 Hz, CH2Ph), 4.37 (m, 1H, J4,5 9.5 Hz, H-3).
3.08 (bs, 1H, OH), 2.31 (s, 3H, S–C6H4–CH3), 1.24 (d, 3H, J5,6 6.5 Hz). 13C NMR (CDCl3, 125 MHz): δ: 165.9 (COCH3), 137.9, 137.3, 133.3, 132.1 (2), 130.0 (3), 129.9 (2), 128.6 (2), 128.5 (2), 128.1 (4) (Arc), 87.4 (C–1), 77.5, 71.1, 69.8, 67.7, 21.2 (SC6H4CH3), 17.5 (C–CH3). HRMS calec for C22H16O4Sn (M + Na)+: 487.1555, found: 487.1553.

p-Tolyl-4-O-benzyl-3-O-benzyl-2-O-chloroacetyl-1-thio-alpha-L-rhamnopyranoside (5). Compound 4 (5.0 g, 11 mmol) and chloroacetic acid anhydride (4.3 g, 25 mmol) were taken in 20 mL dry CH2Cl2 and cooled to 0 °C. Pyridine (3.6 mL, 44 mmol) was then added and after 2 h TLC (n-hexane–EtOAc; 7:1) showed complete conversion of the reactant to a faster moving spot, the mixture was evaporated in vacuo and co-evaporated twice with toluene to obtain a syrupy residue, which was then purified by flash chromatography using n-hexane–EtOAc (8:1) as eluent to give the pure compound 5 (5.2 g, 89%) as light yellow syrup. [α]D25 +113° (c 0.8, CHCl3). 1H NMR (CDCl3, 500 MHz): δ: 8.04–7.15 (m, 14H, ArH), 5.73 (dd, 1H, J3,3 3.0 Hz, H-2), 5.45 (d, 1H, J1a,1.5 1.5, H-1), 5.36 (t, 1H, J1a,1.5 10.0 Hz, H-4), 4.66, 4.45 (ABq, 2H, Jα,β 12.5 Hz, CH2Ph), 4.36 (m, 1H, H-5), 4.25, 4.17 (ABq, 2H, Jα,β 15.5 Hz, COCH2Cl3), 3.98 (dd, 1H, J2,3 3.0 Hz, Jα,β 10.0 Hz, H-3), 2.35 (s, 3H, S–C6H4–CH3), 1.29 (d, 3H, J5,6 6.5 Hz). 13C NMR (CDCl3, 125 MHz): δ: 166.7 (COCH3Cl), 165.5 (COCH3), 138.3, 136.8, 133.2, 132.4 (2), 129.9 (2), 129.8 (2), 129.5, 129.1, 128.3 (2), 128.2 (2), 128.0 (2), 127.8 (Arc), 86.1 (C-1), 74.2, 72.6, 71.8, 71.3, 67.9, 74.2, 72.6, 71.8, 71.3, 67.9, 40.8 (COCH2Cl3), 21.0 (SC6H4CH3), 17.2 (C–CH3). HRMS calec for C29H29ClO6SNa (M + Na)+: 563.1271, found: 563.1269.

p-Methoxyphenyl 4-O-benzyl-3-O-benzyl-2-O-chloroacetyl-alpha-L-rhamnopyranosyl-(1→3)-4-O-acetyl-2,6-di-O-benzyl-beta-D-galactopyranoside (7). A mixture of donor 5 (1.3 g, 2.5 mmol) and 4 (4 Å) (1.5 g) in dry CH2Cl2 (15 mL) was stirred under nitrogen atmosphere for 30 min. NIS (1 g, 4.4 mmol) was added and the mixture was cooled in ice-water bath (~5 °C) before adding H2SO4-silica (75 mg). The mixture was stirred at the same temperature for 30 min when TLC (n-hexane–EtOAc; 3:1) indicated complete consumption of the donor. The reaction mixture was neutralized with Et3N and the mixture was filtered through a pad of Celite. The filtrate was washed successively withaq. Na2SO4 (2 × 30 mL),aq. NaHCO3 (2 × 30 mL) and brine (30 mL). Organic layer was separated, dried over Na2SO4 and evaporated in vacuo. The syrupy crude product thus obtained was purified by flash chromatography using n-hexane–EtOAc (4:1) as eluent to afford pure disaccharide 7 (2.3 g, 91%) as white foam. [α]D25 +86° (c 0.9, CHCl3). 1H NMR (CDCl3, 500 MHz): δ: 8.07–7.12 (m, 20H, ArH), 7.05, 6.82 (2d, 4H, C6H4OCH3), 5.52 (dd, 1H, J3,3 1.5 Hz, Jα,β 0.9 Hz, H-2), 5.39 (dd, 1H, J3,3 1.5 Hz, H-2), 5.23 (d, 1H, J3,3 1.0 Hz, H-1), 5.22 (t, 1H, J1a,1.5 10.0 Hz, H-4), 5.06, 4.75 (ABq, 2H, Jα,β 11.0 Hz, CH2Ph), 4.92 (d, 1H, J1a,1.5 7.0 Hz, H-1), 4.61, 4.42 (ABq, 2H, Jα,β 12.5 Hz, CH2Ph), 4.55, 4.48 (ABq, 2H, Jα,β 10.0 Hz, H-4), 5.06, 4.75 (ABq, 2H, Jα,β 11.0 Hz, CH2Ph), 4.92 (d, 1H, J1a,1.5 7.0 Hz, H-1), 4.61, 4.42 (ABq, 2H, Jα,β 12.5 Hz, CH2Ph), 4.55, 4.48 (ABq, 2H, Jα,β 10.0 Hz, H-4), 5.06, 4.75 (ABq, 2H, Jα,β 11.0 Hz, CH2Ph), 4.92 (d, 1H, J1a,1.5 7.0 Hz, H-1), 4.61, 4.42 (ABq, 2H, Jα,β 12.5 Hz, CH2Ph), 4.55, 4.48 (ABq, 2H, Jα,β 10.0 Hz, H-4). HRMS calec for C49H52O13Na (M + Na)+: 927.3106, found: 927.3104.
pyranosyl-(1→4), 127.6, 127.5 (2), 118.2 (2), 114.5 (2) (Ar
155.3, 151.1, 137.7 (3), 137.3, 133.0, 132.9, 130.0, 129.9, 129.8 (2), 128.5 (4), 128.3 (5), 128.2 (2), 128.1 (2), 127.8 (4), 127.7 (3), 118.3 3), 114.6 (3) (Ar), 103.0 (C-3), 100.4 (C-1′), 99.4 (C-1″), 78.3, 76.5, 76.0, 75.5, 74.6, 74.7, 73.5, 73.2, 72.9, 72.5, 71.0, 72.3, 69.6, 68.4, 67.6, 67.4, 55.6 (OC6H4OC2H5), 40.9 (COCH3), 20.7 CH3), 17.8, 17.4 (2 × C-CH3). HRMS calced for C17H21
C1O5O14Na (M + Na+): 1287.4332, found: 1287.4330.

p-Methoxyphenyl 4-O-benzoyl-3-O-benzyl-α-L-rhamnopyranosyl
(1→2)-4-O-benzyl-3-O-benzyl-α-L-rhamnopyranosyl (1→3)-4-O-
acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (10). Pure trisaccha-
ride 9 (2 g), thioeurea (600 mg, 7.9 mmol) and collidine (1 mL, 7.9 mmol) were dissolved in 20 mL CH2Cl2–MeOH (2 : 3). The mixture was refluxed for 10 h when the complete conversion of the starting material to a slower moving spot was judged by the TLC (n-hexane–EtOAc; 2 : 1). The mixture was evaporated in vacuo. The solid residue thus obtained was dissolved in CH2Cl2 and washed with 1 (N) HCl (2 × 30 mL) and brine (2 × 30 mL). The organic layer was separated, dried (Na2SO4) and evaporated in vacuo to get the crude product. It was purified by flash chromatography using n-hexane–EtOAc (5 : 2) as the eluent to get the pure compound 10 (1.7 g, 88%) as white powder.

[α]D25 +78° (c 0.7, CHCl3). 1H NMR (CDCl3, 500 MHz) δ: 8.08–7.10 (m, 30H, ArH), 6.96, 6.74 (2d, 4H, 4'CeH4OC2H5), 3.53 (m, 1H, H-4), 3.53 (t, 1H, Jf′,x′,Jf′,x 10.0 Hz, H-4′), 5.30 (t, 1H, Jf′,x′,Jf′,x 10.0 Hz, H-4′), 5.19 (d, 1H, Jf′,x′,Jf′,x 10.0 Hz, H-4′), 5.09 (d, 1H, Jf′,x′,Jf′,x 2.0 Hz, H-1′), 4.91, 4.82 (ABq, 2H,Jα,β 12.5 Hz, CH2Ph), 4.86 (d, 1H, Jf′,x′,Jf′,x 7.5 Hz, H-1′), 4.64, 4.52 (ABq, 2H,Jα,β 12.0 Hz, CH2Ph), 4.56–4.42 (m, 4H, 2 × CH2Ph), 4.31 (d, 1H, Jf′,x′,Jf′,x 2.0 Hz, H-3′, Jf′,x′,Jf′,x 3.0 Hz, H-3′,Jf′,x′,Jf′,x 10.0 Hz, H-3′), 3.85–3.82 (m, 1H, H-5′), 3.89 (d, 1H, Jf′,x′,Jf′,x 3.0 Hz, Jf′,x′,Jf′,x 10.0 Hz, H-3′), 3.75 (d, 2H, Jf′,x′,Jf′,x 10.0 Hz, H-3′), 3.69 (s, 3H, OC6H4OC2H5), 3.32 (m, 2H, H-6a, H-6b), 2.62 (bs, 1H, OH), 1.93 (s, 3H, COCH3), 1.23 (d, 3H, Jf′,x′,Jf′,x 6.5 Hz, C-CH3), 1.03 (d, 3H, Jf′,x′,Jf′,x 6.5 Hz, C-CH3). 13C NMR (CDCl3, 125 MHz) δ: 169.9 (COCH3), 165.8, 165.7, 165.6 (3 × CO2H), 155.3, 151.5, 137.8, 137.7, 137.4, 133.2, 131.2 (3), 130.0 (2), 129.9 (2), 129.8 (2), 128.4 (14), 128.3 (8), 128.2 (2), 128.1 (2), 127.8 (6), 127.7 (2), 127.5 (2), 118.3 (2) (Ar), 101.1 (C-1‴), 100.7 (C-1″), 78.2, 76.1, 75.7, 75.4, 74.6, 73.9, 73.7 (2), 73.3, 73.1, 72.9, 72.7, 71.8 (2), 71.3, 70.2, 69.6, 68.4, 67.7, 67.6, 67.5, 53.6, 40.9 (COCH3), 20.6 (COCH3), 17.8, 17.7, 17.5 (3 × C-CH3). HRMS calced for C58H49Cl2O24Na (M + Na+: 1627.5643, found: 1627.5641.

p-Methoxyphenyl 4-O-benzoyl-3-O-benzyl-α-L-rhamnopyranosyl
(1→2)-4-O-benzoyl-3-O-benzyl-α-L-rhamnopyranosyl (1→3)-4-O-
benzoyl-3-O-benzyl-α-L-rhamnopyranosyl (1→3)-4-O-
acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (12). To a solution of the tetr saccharide 11 (1.7 g, 1.1 mmol) and thioeurea (400 mg, 5.3 mmol) in 20 mL CH2Cl2–MeOH (2 : 3), collidine (0.7 mL, 5.3 mmol) was added and the mixture was refluxed for 12 h till TLC (n-hexane–EtOAc; 2 : 1) indicated the complete conversion of the starting material to a slower moving spot. This mixture was evaporated in vacuo and the residue was dissolved in CH2Cl2. It was further washed with 1 (N) HCl (2 × 30 mL) and brine (2 × 30 mL). Resulting organic layer was collected, dried (Na2SO4) and evaporated in vacuo to obtain the crude product. It was further purified by flash chromatography using n-hexane–EtOAc (2 : 1) as the eluent to obtain the pure tetr saccharide acceptor 12 (1.5 g, 90%) as white foam.

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(m, 4H, H-3", H-3", H-5', H-5'"), 4.01-3.96 (m, 2H, H-2', H-5")", 3.88-3.84 (m, 3H, H-2, H-3, H-5), 3.79 (dd, 1H, Jα,β = 3.5 Hz, Jβ,γ = 6.5 Hz, H-3"), 3.77 (s, 3H, OC6H5OCH3), 3.58 (m, 2H, H-6a, H-6b), 2.57 (bs, 1H, OH) 1.96 (s, 3H, COCH3), 1.28 (d, 3H, Jα,γ = 6.0 Hz, C-CH3), 1.16 (d, 3H, Jα,β = 6.0 Hz, C-CH3), 1.11 (d, 3H, Jα,γ = 6.0 Hz, C-CH3). 13C NMR (CDCl3, 125 MHz) δ: 165.0 (COCH3), 165.9, 165.8, 165.6 (3 × COOCH3), 155.4, 155.2, 127.8, 137.7 (4), 137.5, 133.1 (2), 133.0 (2), 130.6 (6), 129.8 (2), 128.4 (6), 128.3 (4), 128.2 (2), 128.0 (4), 127.8 (6), 127.7 (4), 127.6 (4), 118.3 (2), 114.5 (2) (Arc), 103.0 (C-1), 101.2 (C-1"), 100.9 (C-1"'), 100.6 (C-1"'), 78.1, 76.4, 76.2, 76.0, 75.4 (2), 74.8, 74.6, 73.7, 73.2, 73.1, 73.0, 72.9, 71.7, 71.6, 71.5, 69.6, 68.5, 68.2, 67.2 (6), 66.9, 55.6, 20.6 (COCH3), 17.8, 17.7, 17.4 (3 × CH3). HRMS calecd for C20H15O2Na (M + Na)1: 3515.5927, found: 3515.5925.

4-Toly-2,3-di-o-benzyl-4-o-chloroacetyl-6-O-(4-methoxybenzyl)-1-thio-D-galactopyranoside (14). The compound 15 (1.3 g, 2.2 mmol) was dissolved in dry CH2Cl2 (20 mL) in presence of chloroform anhydride (770 mg, 4.5 mmol) and kept at 0 °C for 10 min. Pyridine (0.7 mL, 9.0 mmol) was then added to react the reaction mixture and stirred for 2 hours when TLC (n-hexane-ETOA Oc: 4 : 1) showed complete evolution of the starting material to a faster moving spot. The reaction mixture was evaporated in vacuo and co-evaporated with toluene. The crude product thus obtained was further subjected to purification by flash chromatography using n-hexane-ETOAc (6 : 1) as eluent to get the pure compound 14 (1.3 g, 89%). δα′β+139° (c 1.0, CHCl3). 1H NMR (CDCl3, 500 MHz) δ: 7.54–6.96 (m, 18H, ArH), 5.74 (dd, 1H, Jα,β = 3.0 Hz, Jα,γ = 1.5 Hz, H-4), 4.83–4.53 (m, 4H, 2 × CH2Ph), 4.67 (d, 1H, Jα,γ = 9.0 Hz, H-1), 4.55, 4.42 (Abq, 2H, Jα,β = 12.5 Hz, CH2PhOme), 4.10, 4.03 (Abq, 2H, Jα,β = 15.5 Hz, CH2Cl), 3.85 (s, 3H, OC6H5OCH3), 3.79 (dd, 1H, Jα,β = 6.5 Hz, Jα,γ = 9.5 Hz, H-6a), 3.71 (dd, 1H, Jα,β = 9 Hz, Jα,γ = 3 Hz, H-3), 3.65 (t, 1H, Jα,β = 9.0 Hz, H-2), 3.64 (m, 1H, H-5), 3.56 (dd, 1H, Jα,β = 7.5 Hz, Jα,γ = 9.5 Hz, H-6b), 2.37 (3, 3H, S-C6H5-CH3). 13C NMR (CDCl3, 125 MHz) δ: 166.9 (COCH3), 159.3, 138.0, 137.6, 137.3, 132.6 (2), 129.7 (2), 129.5 (2), 129.4, 129.3, 128.3 (2), 128.2 (4), 128.0 (2), 127.8, 127.7, 113.7 (2) (Arc), 87.8 (C-1), 80.9, 76.5, 75.6, 75.2, 73.1, 72.0, 68.7, 67.0, 55.1, 40.7 (COCH3), 21.0 (SC6H5-H3). HRMS calecd for C20H17O2Na (M + Na)1: 385.1201, found: 385.1200.

p-Methoxyphenyl 2,3-di-o-benzyl-4-o-chloroacetyl-6-O-p-methoxybenzyl-α-D-galactopyranosyl(1→2)-4-o-benzoyl-3-O-benzyl-α-D-xylo-o-ramnopopyanosyl(1→2)-4-O-benzoyl-3-O-benzyl-α-D-xylo-o-ramnopopyanosyl(1→3)-4-O-acetyl-2,6-di-o-benzyl-β-D-galactopyranoside (15). A mixture of tetrasaccharide acceptor 12 (1.3 g, 0.85 mmol), donor 14 (740 mg, 1.1 mmol) and MS 4 Å (2.0 g) in dry CH2Cl2 (20 mL) was stirred under nitrogen atmosphere for 30 min. NIS (322 mg, 1.4 mmol) was added and the mixture was cooled to ~50 °C followed by the addition of H2SO4-silica (50 mg) and the mixture was allowed to stir at the same temperature for 15 minutes. As TLC (n-hexane-ETOAc; 5 : 2) suggested the full consumption of the donor, the reaction was quenched by Et3N. It was then followed by filtration of the mixture through a Celite pad. Resulting solution was then successively washed withaq. Na2SO3 (2 × 30 mL),aq. NaHCO3 (2 × 30 mL) and brine (30 mL). It was then dried (Na2SO4) and evaporated in vacuo. The crude product was then purified by flash chromatography using n-hexane-η-EtOAc (3 : 1) as the eluent. Thus the pure pentasaccharide 15 (1.4 g, 82%) was furnished as white foam. δα′β+108° (c 0.7, CHCl3). 1H NMR (CDCl3, 500 MHz) δ: 8.07–6.98 (m, 55H, ArH), 6.87, 6.76 (2d, 4H, CH2CH2OCH3), 5.44–5.34 (m, 4H, H-4", H-4", H-4"'), 5.21 (d, 1H, Jα,β = 1.0 Hz, H-1"'), 5.15 (d, 1H, Jα,β = 2.0 Hz, H-1"), 5.11 (d, 1H, Jα,γ = 1.0 Hz, H-1"'), 4.95, 4.81 (Abq, 2H, Jα,β = 11.5 Hz, CH2Ph), 4.87 (d, 1H, Jα,γ = 7.0 Hz, H-1), 4.82 (d, 1H, Jα,β = 3.0 Hz, H-1"'), 4.70–4.40 (m, 14H, 7 × CH2Ph), 4.37–4.36 (m, 3H, H-2", H-2", H-4"'), 4.13 (m, 1H, H-5"'), 4.06–3.90 (m, 4H, H-2', H-3', H-3', H-5', H-5"), 3.87–3.79 (m, 4H, H-2, H-3, H-3, H-5), 3.77 (s, 3H, OC6H5OCH3), 3.70 (s, 3H, OCH2CH2OCH3), 3.58 (m, 3H, H-6a, H-6b), 3.64 (m, 1H, H-3"'), 2.82 (brs s, 1H, OH), 1.94 (s, 3H, COCH3), 1.27 (d, 3H, Jα,γ = 6.0 Hz, C-CH3), 1.16 (d, 3H, Jα,β = 6.0 Hz, C-CH3), 1.09

**p-Methoxyphenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-O-benzyl-α-D-galactopyranosylsodium(1→2)-4-O-benzoyl-3-O-benzyl-α-L-rhamnopyranosyl(1→2)-4-O-benzoyl-3-O-benzyl-α-L-rhamnopyranosyl(1→2)-4-O-benzoyl-3-O-benzyl-α-L-rhamnopyranosyl(1→3)-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (19).** To a solution of the pure hexasaccharide 18 (950 mg, 0.4 mmol) in CH2Cl2 (24 mL) water (5 mL) and DDQ (190 mg, 0.8 mmol) were consecutively added and vigorously stirred for 3 h when the TLC (n-hexane-EtOAc; 2 : 1) suggested complete conversion of the starting material to a slower moving spot. The reaction mixture was washed successively with H2O and brine. Organic layer was collected, dried (Na2SO4) and evaporated in vacuo. The crude product thus obtained was purified by flash chromatography using n-hexane-EtOAc (3 : 1) to afford the pure compound 19 (702 mg, 78%) as foam. [α]D20 +172 (c 0.7, CHCl3).

1H NMR (CDCl3, 500 MHz): δ 8.11–7.11 (m, 50H, ArH), 6.99, 6.79 (2d, 4H, C6H5OCH3), 5.46–5.38 (m, 6H, H-2’′, H-4’, H-4’′, H-5′, H-5’′), 5.28 (dd, 1H, JH-3′, H-3′′ = 3.5 Hz, JH-4′, H-4′′ = 6.5 Hz, H-3′′), 5.24 (d, 1H, JH-1′, H-1′′ = 1.0 Hz, H-1′′), 5.16 (d, 1H, JH-1′, H-1′′ = 1.0 Hz, H-1′), 5.11 (d, 1H, JH-1′, H-1′′ = 1.0 Hz, H-1′), 5.02–4.42 (m, 12H, 6 × CH2Ph), 4.99 (m, 1H, H-4′, H-4′′), 4.90 (d, 1H, JH-1′, H-1′′ = 6.5 Hz, H-1′), 4.43 (m, 1H, H-4’, H-4′′), 4.37 (s, 1H, H-2’), 4.18 (t, 1H, JH-3′, H-3′′ = 6.0 Hz, H-4′′), 4.05–3.95 (m, 8H, H-2’, H-3’, H-3′′, H-5’, H-5’′, H-6′, H-6′′), 3.91–3.73 (m, 3H, H-2’, H-3’, H-5’, H-5′′), 3.81 (dd, 1H, JH-4′, H-4′′ = 7.5 Hz, JH-5′, H-5′′ = 2.5 Hz, H-3′′), 3.77 (s, 3H, OCH3CHOH3), 3.71–3.58 (m, 3H, H-6′, H-6′′), 3.47 (m, 1H, H-3′′, H-3′′′), 2.06, 1.99, 198 (3s, 12H, 4 × COCH3), 1.29 (d, 3H, JH-3′′, H-3′′′ = 6.0 Hz, C-CH3), 1.21 (d, 3H, JH-3′′, H-3′′′ = 6.0 Hz, C-CH3), 1.17 (d, 3H, JH-3′′, H-3′′′ = 6.0 Hz, C-CH3), 1.09 (d, 3H, JH-3′′, H-3′′′ = 6.5 Hz, C-CH3).

13C NMR (CDCl3, 125 MHz): δ 170.0, 169.9, 169.7, 169.5 (4 × COCH3), 166.0, 165.8, 165.6 (3 × COCH3), 155.4, 151.2, 138.9, 138.5, 138.0, 137.8 (2), 137.7, 137.2, 133.3, 133.1, 132.9, 130.1 (2), 128.5 (2), 128.4 (3), 128.4 (3), 128.3 (2), 128.1 (4), 128.1 (3), 127.8, 127.7 (2), 127.6 (2), 127.5 (4), 127.2 (4), 127.1, 118.2 (2), 114.5 (2), 113.5 (2), 102.9 (C-1), 101.3 (C-1′′), 100.6 (C-1′′′), 99.2 (C-1′), 98.6 (C-1′′′), 96.6 (C-1′′′), 81.3, 78.1, 76.1, 76.0 (2), 75.4, 75.1, 74.7, 74.6, 74.2, 73.9 (2), 73.6, 73.2 (2), 73.1, 73.0, 72.9, 72.7, 72.5, 72.2, 71.6, 71.5, 71.1, 70.9, 69.7, 69.5, 69.4, 68.5, 68.1, 67.6, 67.5, 67.5, 66.7, 64.7, 55.5, 55.0, 20.8 (2), 20.7, 20.6 (2).
hydrogenolysis of the benzyl groups were complete after 3 such cycles as evident from mass spectroscopy. NaOMe in MeOH (0.5 M, 1 mL) was added to the solution and it was stirred at room temperature for 12 h. The solution was neutralized by DOWEX 50W H\(^+\) resin, filtered and evaporated in vacuo to afford the final hexaaccharide 1 (257 mg, 75%) as white amorphous mass. [\(\alpha\)]\(_D\)\(^{25}\) +54° (c 0.5, MeOH). HRMS calcld for C\(_{43}\)H\(_{56}\)O\(_{28}\)Na (M + Na): \(m/z\) 1069.3587, found: 1069.3585. \(^1\)H NMR (MeOD, 500 MHz) \(\delta\) 6.73, 6.70 (2d, 4H, C\(_6\)H\(_4\)OCH\(_3\)), 5.26 (d, 1H, J\(_\alpha-H^1\) 1.0 Hz, H-1), 5.24 (d, 1H, J\(_1H^2\cdotH_2\) 1.0 Hz, H-1\(^{\alpha}\)), 5.21 (d, 1H, J\(_1H^2\cdotH_2\) 1.0 Hz, H-1\(^{\alpha}\)), 5.11 (d, 1H, J\(_1H^2\cdotH_2\) 1.0 Hz, H-1\(^{\alpha}\)), 5.06 (d, 1H, J\(_1H^2\cdotH_2\) 1.0 Hz, H-1\(^{\alpha}\)), 4.77 (d, 1H, J\(_1J_2\) 8.0 Hz, H-1), 3.70 (s, 3H, C\(_6\)H\(_4\)OC\(_3\)H), 1.28–1.24 (m, 12H, 4 × CH\(_2\)). \(^13\)C NMR (125 MHz, MeOD) \(\delta\): 173.4 (COOH), 156.5, 152.1, 116.7 (2), 115.7 (2) (aromatic C), 103.3 (C-1), 102.7 (C-1\(^\alpha\)), 102.6 (C-1\(^{\alpha}\)), 102.4 (C-1\(^\alpha\)), 102.3 (C-1\(^{\alpha}\)), 101.2 (C-1\(^{\alpha}\)), 80.0, 79.8, 78.2, 77.7, 76.6, 76.4, 74.9, 74.4, 74.2, 73.9, 73.7, 73.5, 72.3, 72.2, 72.1, 72.0, 71.9, 71.8, 70.5, 70.4, 70.3, 70.2, 69.5, 63.9, 63.2, 56.2, 18.1, 18.0, 17.9 (2) (4 × C-CH\(_3\)).

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Notes and references