Tetrahedron: *Asymmetry* 21 (2010) 725–730

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Concise synthesis of a hexasaccharide present in the cell wall lipopolysaccharide of *Azospirillum lipoferum* Sp59b

Samir Ghosh, Anup Kumar Misra *

Bose Institute, Division of Molecular Medicine, P-1/12, C.I.T. Scheme VII-M, Kolkata 700 054, India

ARTICLE INFO

Article history:
Received 22 March 2010
Accepted 31 March 2010
Available online 3 May 2010

ABSTRACT

Concise chemical synthesis of a hexasaccharide repeating unit found in the cell wall lipopolysaccharide of *Azospirillum lipoferum* Sp59b was achieved in excellent yield. A [3+3] block synthetic strategy has been applied for the construction of the target hexasaccharide. During the synthesis, the orthogonal property of thioglycosides has been successfully exploited. Yields were high in all intermediate steps.

1. Introduction

Among several growth-promoting rhizobacteria, *Azospirilla* play a significant role in plant growth and development by fixing atmospheric nitrogen and making it available to the plants in an asymbiotic manner.[1] *Azospirilla* are Gram-negative proteobacteria widely present in soil and enhance the growth and yield of many important crops through the production of several phytohormones, vitamins and bioactive substances.[2] They establish conjugate relationships with the roots of leguminous and non-leguminous crops.[3] Spores of *Azospirillum* species are commonly used as biofertilizer. Several macromolecules such as exopolysaccharides (EPS), lipopolysaccharides (LPS) and capsular polysaccharides (CPS) present in the cell wall of *Azospirillum* have significant roles in establishing the *Azospirillum*-plant interactions.[4] As LPS is the major antigen present in the bacterial outer cell wall, the role of the LPS together with other polysaccharides is considered to be essential for the plant-bacterial interactions. Despite their usefulness only a few structures of LPS have been reported from *Azospirillum* species.[5] Fedonenko et al. reported the structure of a hexasaccharide repeating unit found in the LPS of *Azospirillum lipoferum* Sp59b (Fig. 1).[6]

Development of plant growth-promoting agents for the enhancement of crop production is currently a major thrust. For a detailed understanding of the role of the LPS in the plant-bacterial interactions it is essential to carry out different biological experiments with this particular hexasaccharide. For this purpose, sufficient quantities of the hexasaccharide repeating unit are required, which is not possible from the natural source. A concise chemical synthetic strategy could provide the required quantity of the hexasaccharide repeating unit as well as its analogues. As a part of our ongoing program on the synthesis of complex oligosaccharides,[7] we report herein a concise chemical synthesis of the hexasaccharide as its 4-methoxyphenyl glycoside found in the cell wall lipopolysaccharide of *A. lipoferum* Sp59b (Fig. 2). The 4-methoxyphenyl group has been chosen as a temporary protecting group at the reducing end due to its easy removal under oxidative conditions to furnish a hexasaccharide hemiacetal derivative to prepare glycoconjugate derivatives.

2. Results and discussion

In order to achieve the synthesis of the target hexasaccharide as its 4-methoxyphenyl glycoside (Fig. 2) containing a β-D-mannose unit linked to a terminal tri-L-rhamnoside moiety **1**, a [3+3] block synthetic strategy has been undertaken. Due to the difficulty in the preparation of the β-D-mannopyranosidic linkage directly from D-mannosyl derivatives, a suitably protected β-D-Glucose derivative has been used as the precursor of β-D-mannose moiety. A number of noteworthy features are present in the synthetic strategy, which are (a) conversion of the β-D-glucose unit to the β-D-man-

α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-β-D-Man*p*
1
↓
4
→3)-α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→

Figure 1. Hexasaccharide repeating unit of the lipopolysaccharide found in the cell wall of *Azospirillum lipoferum* Sp59b.

* Corresponding author. Tel.: +91 33 25693240; fax: +91 33 2355 3886.
E-mail address: akmisra69@gmail.com (A.K. Misra).

0957-4166/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2010.03.042

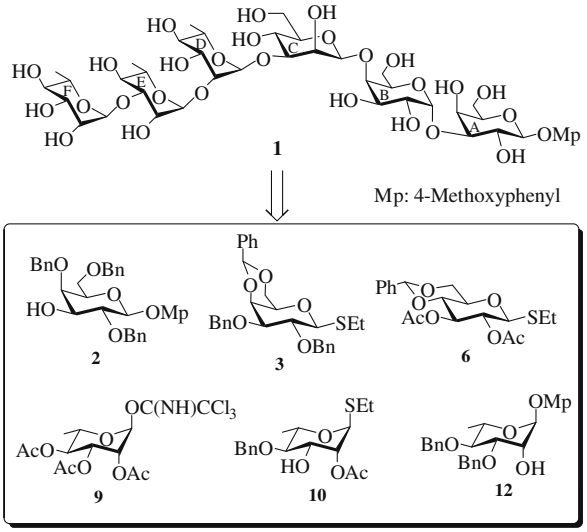
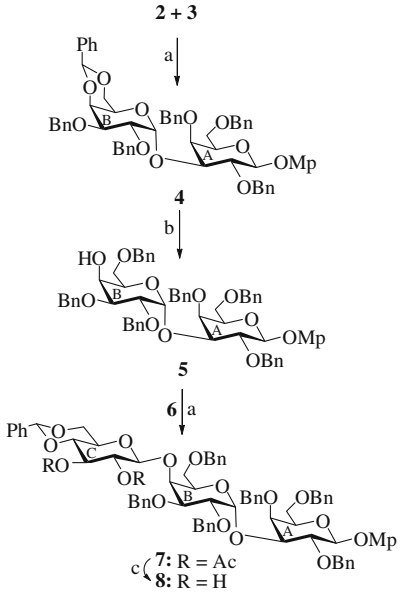


Figure 2. Structure of the synthesized hexasaccharide as its 4-methoxyphenyl glycoside corresponding to the lipopolysaccharide of *Azospirillum lipoferum* Sp59b.



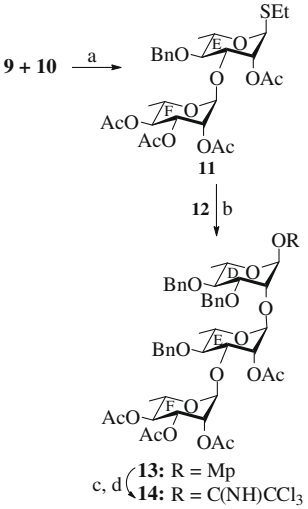
Scheme 1. Reagents and conditions: (a) *N*-iodosuccinimide (NIS), TfOH, CH_2Cl_2, MS 4 Å, −40 °C, 30 min, 80% for **4** and 81% for **7**; (b) Et_3SiH, I_2, CH_2Cl_2, 0–5 °C, 15 min, 75%; (c) CH_3ONa, CH_3OH, room temperature, 3 h, quantitative.

nose unit after completion of the glycosylation steps, (b) preparation of two trisaccharide derivatives as their 4-methoxyphenyl (OMp) glycosides **8** and **13** and conversion of one OMp glycoside **13** to trichloroacetimidate derivative **14** for its use as glycosyl donor, (c) exclusively regio- and stereoselective glycosylation of an L-rhamnosyl trisaccharide trichloroacetimidate donor **14** using a trisaccharide acceptor **8** having 2,3-dihydroxylated D-glucose moiety under Schmidt glycosylation condition and (d) use of a L-rhamnosyl thioglycoside derivative **10** as a glycosyl acceptor in the presence of L-rhamnosyl trichloroacetimidate derivative **9** as the glycosyl acceptor.

A dihydroxyl groups containing trisaccharide acceptor **8** has been synthesized from three suitably derivatized monosaccharide intermediates **2**,[8] **3**[9] and **6**.[10] Stereoselective 1,2-*cis* glycosylation of D-galactose-derived compound **2** with the D-galactosyl thioglycoside derivative **3** in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)[11] furnished disaccharide derivative **4** in 80% yield. Exclusive formation of the 1,2-*cis* glycosyl linkage was achieved using a glycosyl donor having a non-participating benzyloxy group at the C-2 position of the glycosyl donor **3** which was confirmed from its NMR spectral data [δ 5.21 (d, J = 3.3 Hz, H-1_B), 4.86 (d, J = 7.6 Hz, H-1_A) in the ^{1}H NMR and at δ 103.8 (C-1_A), 101.3 (PhCH), 96.4 (C-1_B) in the ^{13}C NMR spectra]. Regioselective ring opening of the benzylidene acetal of compound **4** using a combination of triethylsilane and molecular iodine[12] produced disaccharide acceptor **5** in 75% yield. Stereoselective 1,2-*trans* glycosylation of compound **5** with thioglycoside derivative **6** in the presence of NIS-TfOH[11] afforded the trisaccharide derivative **7** in 81% yield, which on saponification quantitatively furnished trisaccharide diol acceptor **8**. Exclusive formation of the 1,2-*trans* glycosylation product **7** was achieved due to the presence of participating acetoxy group at the C-2 position of glycosyl donor **6**, which was further confirmed from the spectral analysis of compound **7** [δ 5.20 (br s, H-1_B), 4.74 (d, J = 7.9 Hz, H-1_A), 4.71 (d, J = 9.8 Hz, H-1_C) in ^{1}H NMR and δ 103.6 (C-1_C), 102.5 (C-1_A), 101.9 (PhCH), 95.7 (C-1_B) in the ^{13}C NMR] (Scheme 1).

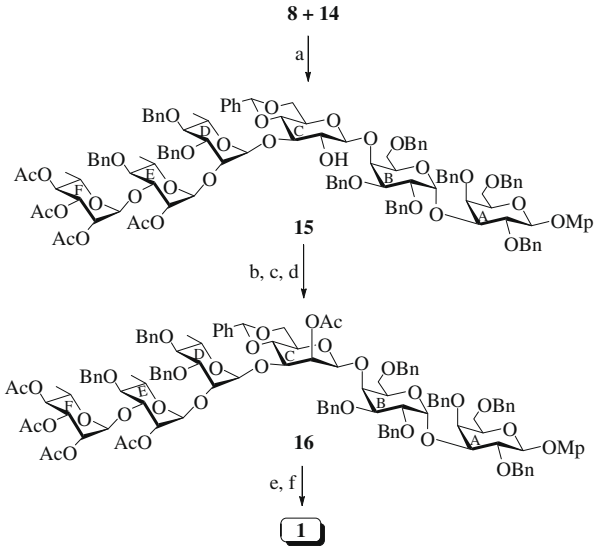
In another experiment, a di-L-rhamnopyranosyl thioglycoside derivative **11** was synthesized in 83% yield by stereoselective condensation of L-rhamnosyl trichloroacetimidate donor **9**[13] and L-rhamnosyl thioglycoside derivative **10**[14] as acceptor in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as glycosylation activator under Schmidt's glycosylation condition.[15]

The presence of signals at δ 5.07 (br s, H-1_E), 4.92 (br s, H-1_F) in the ^{1}H NMR and δ 99.6 (C-1_F), 82.4 (C-1_E) in the ^{13}C NMR spectra confirmed its formation. In this case thioglycoside derivative **10** acts as an orthogonal glycosyl acceptor. Further glycosylation of the disaccharide derivative **11** with compound **12**[16] in the presence of NIS-TfOH furnished trisaccharide derivative **13** in 78% yield, which was confirmed from its NMR spectral analysis [signals at δ 5.34 (br s, H-1_D), 5.05 (br s, H-1_E), 4.97 (br s, H-1_F) in the ^{1}H NMR and at δ 99.6 (C-1_E), 99.5 (C-1_F), 97.9 (C-1_D) in the ^{13}C NMR spectra confirmed the structure of **13**]. Removal of the anomeric 4-methoxyphenyl group of trisaccharide derivative **13** using ammonium cerium nitrate (CAN)[17] followed by treatment of the hemiacetal derivative with trichloroacetonitrile in the presence of DBU[15a] resulted in the formation of trisaccharide trichloroacetimidate derivative **14** in 77% yield (Scheme 2).



Scheme 2. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −20 °C, 45 min, 83%; (b) NIS, TfOH, CH_2Cl_2, MS 4 Å, −40 °C, 30 min, 78%; (c) CAN, CH_3CN–H_2O (9:1), 0–5 °C, 2 h; (d) CCl_3CN, DBU, CH_2Cl_2, −10 °C, 1 h, 77% in two steps.

Regioselective coupling of the trisaccharide donor **14** with the trisaccharide diol acceptor **8** in the presence of TMSOTf[15] resulted in the stereoselective formation of the hexasaccharide derivative **15** in 76% yield. Compound **15** was characterized by the presence of signals at δ 5.24 (br s, H-1_E), 5.19 (d, J = 3.1 Hz, H-1_B), 5.00 (br s, H-1_F), 4.97 (br s, H-1_D), 4.76 (d, J = 7.4 Hz, H-1_A), 4.31 (d, J = 7.8 Hz, H-1_C) in the ^{1}H NMR and at δ 106.5 (C-1_C), 103.6 (C-1_A), 101.9 (PhCH), 99.5 (3C, C-1_D, C-1_E, C-1_F), 96.1 (C-1_B) in the ^{13}C NMR spectra. Exclusive formation of the (1→3)-glycosylation product was confirmed from the 1D and 2D NMR spectra analysis of the acetylated product of compound **15** (data not included). Oxidation of the free hydroxyl group at C-2_C position in compound **15** using Dess–Martin periodinane[18] followed by sodium borohydride reduction[19] of the resulting ketone afforded the hexasaccharide derivative containing a β-D-mannosidic moiety. Due to the removal of *O*-acetyl groups present in compound **15** under sodium borohydride reduction conditions, the resulting epimerized hexasaccharide derivative was acetylated to give compound **16** in 74% yield and characterized by NMR spectroscopic analysis. Appearance of signals at δ 5.47 (d, J = 2.6 Hz, H-2_C), 5.06 (d, J = 2.9 Hz, H-1_B), 4.99 (br s, H-1_F), 4.97 (br s, H-1_E), 4.77 (br s, H-1_D), 4.74 (br s, H-1_C), 4.66 (d, J = 7.7 Hz, H-1_A) in the ^{1}H NMR and δ 103.7 (C-1_A), 101.6 (PhCH), 100.0 (C-1_C), 99.5 (C-1_F), 99.0 (C-1_E), 95.9 (C-1_D), 95.6 (C-1_B) in the ^{13}C NMR spectra confirmed the formation of compound **16**. Finally, complete deprotection of the hexasaccharide derivative under saponification followed by hydrogenolysis over Pd(OH)$_2$–C[20] furnished the target hexasaccharide **1** as its 4-methoxyphenyl glycoside in 71% yield. Signals at δ 4.99 (br s, H-1_E), 4.97 (d, J = 3.7 Hz, H-1_B), 4.95 (br s, H-1_D), 4.85 (br s, H-1_F), 4.71 (d, J = 7.9 Hz, H-1_A), 4.69 (br s, H-1_A) in the ^{1}H NMR and at δ 103.1 (C-1_F), 102.9 (C-1_E), 102.8 (C-1_C), 101.5 (C-1_A), 97.0 (C-1_B), 95.4 (C-1_D) in the ^{13}C NMR spectra confirmed the structure of compound **1** (Scheme 3).



Scheme 3. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −20 °C, 45 min, 76%; (b) Dess–Martin periodinane, CH_2Cl_2, room temperature, 2 h; (c) $NaBH_4$, CH_3OH, 0 °C–room temperature, 12 h; (d) acetic anhydride, pyridine, room temperature, 5 h, 74% in three steps; (e) CH_3ONa, CH_3OH, room temperature, 3 h; (f) H_2, 20% Pd(OH)$_2$–C, CH_3OH, room temperature, 24 h, 71%.

3. Conclusion

In summary, a concise synthetic strategy for the preparation of the hexasaccharide repeating unit of the lipopolysaccharide found in the cell wall of *A. lipoferum* Sp59b has been developed. Regio- and stereoselective [3+3] glycosylation allowed achieving the target hexasaccharide in minimum number of steps. Conversion of a β-D-glucosyl moiety into β-D-mannosyl moiety in the later stage avoided the difficulties for the formation of β-D-mannosyl linkage. All intermediate steps were high yielding and highly reproducible for a scale-up preparation.

4. Experimental

4.1. General methods

All the reactions were monitored by thin layer chromatography over silica gel-coated TLC plates. The spots on TLC were visualized by warming ceric sulfate (2% Ce(SO$_4$)$_2$ in 2 N H_2SO_4)-sprayed plates in hot plate. Silica gel 230–400 mesh was used for column chromatography. ^{1}H and ^{13}C NMR, 2D COSY, HSQC and NOESY spectra were recorded on a Brucker Advance DPX 300 MHz using $CDCl_3$ and D_2O as solvents and TMS as internal reference unless stated otherwise. Chemical shift values are expressed in δ ppm. ESI-MS were recorded on a Micromass Quttro II mass spectrometer. Elementary analysis was carried out on a Carlo Erba-1108 analyzer. Optical rotations were measured at 25 °C on a Perkin Elmer 341 polarimeter. Commercially available grades of organic solvents of adequate purity are used in many reactions.

4.1.1. 4-Methoxyphenyl (2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 4

To a solution of compound **2** (1.5 g, 2.58 mmol) and compound **3** (1.5 g, 3.05 mmol) in anhydrous CH_2Cl_2 (15 mL) was added MS 4 Å (2 g) and the reaction mixture was allowed to stir at room temperature for 30 min under argon. The reaction mixture was cooled to −40 °C and *N*-iodosuccinimide (NIS; 825 mg, 3.66 mmol) followed by TfOH (8 μL) were added to it. After stirring at the same temperature for 30 min the reaction was quenched with Et_3N (50 μL), filtered through a Celite® bed and washed with CH_2Cl_2 (50 mL). The combined organic layer was washed with 5% $Na_2S_2O_3$, satd $NaHCO_3$, water in succession, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (8:1) as an eluant to give pure **4** (2.1 g, 80%). Colourless oil; $[\alpha]_D^{25} = +5.4$ (*c* 1.0, $CHCl_3$); IR (neat): 3062, 3032, 2920, 2902, 2854, 1605, 1508, 1453, 1402, 1366, 1249, 1154, 1102, 1079, 1027, 994, 825, 797, 696 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48–7.12 (m, 30H, Ar-H), 7.00 (d, J = 9.1 Hz, 2H, Ar-H), 6.78 (d, J = 9.1 Hz, 2H, Ar-H), 5.27 (s, 1H, PhC*H*), 5.21 (d, J = 3.3 Hz, 1H, H-1_B), 5.09 (d, J = 11.6 Hz, 1H, PhCH_{2a}), 5.06 (d, J = 10.6 Hz, 1H, PhCH_{2a}), 4.90 (d, J = 11.6 Hz, 1H, PhCH_{2b}), 4.86 (d, J = 7.6 Hz, 1H, H-1_A), 4.78 (d, J = 12.2 Hz, 1H, PhCH_{2a}), 4.73 (d, J = 12.2 Hz, 1H, PhCH_{2b}), 4.70 (d, J = 11.4 Hz, 1H, PhCH_{2a}), 4.51 (d, J = 10.6 Hz, 1H, PhCH_{2b}), 4.45 (d, J = 11.7 Hz, 1H, PhCH_{2a}), 4.40 (d, J = 11.4 Hz, 1H, PhCH_{2b}), 4.38 (d, J = 11.7 Hz, 1H, PhCH_{2b}), 4.18–4.15 (dd, J = 10.1, 3.3 Hz, 1H, H-3_A), 4.05–4.01 (m, 2H, H-2_A, H-3_B), 3.91 (d, J = 2.4 Hz, 1H, H-4_B), 3.86–3.80 (m, 3H, H-2_B, H-5_B, H-6_{aB}), 3.78 (d, J = 3.2 Hz, 1H, H-4_A), 3.75 (s, 3H, OCH_3), 3.65–3.56 (m, 3H, H-5_A, H-6_{abA}), 3.27–3.24 (m, 1H, H-6_{bB}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.6–114.9 (Ar-C), 103.8 (C-1_A), 101.3 (PhCH), 96.4 (C-1_B), 78.6 (C-2_A), 77.9 (C-3_B), 76.5 (C-2_B), 75.9 (C-3_A), 75.8 (PhCH_2), 75.2 (PhCH_2), 75.0 (PhCH_2), 74.8 (C-5_A), 74.2 (C-4_A), 73.9 (PhCH_2), 72.9 (C-4_B), 72.1 (PhCH_2), 69.5 (C-6_A), 69.3 (C-6_B), 62.8 (C-5_B), 56.1 (OCH_3); ESI-MS: *m/z* 1033.4 [M+Na]$^+$; Anal. Calcd for $C_{55}H_{62}O_{18}$ (1010.39): C, 65.34; H, 6.18. Found: C, 65.15; H, 6.40.

4.1.2. 4-Methoxyphenyl (2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 5

To a solution of compound **4** (2.0 g, 1.98 mmol) in CH_2Cl_2 (10 mL) were added Et_3SiH (650 μL, 4.07 mmol) and iodine (150 mg, 0.59 mmol) at 0 °C. After stirring at the same temperature

for 15 min the reaction mixture was diluted with CH_2Cl_2 (50 mL) and successively washed with 5% $Na_2S_2O_3$, satd $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (6:1) as an eluant to give pure **5** (1.5 g, 75%). Colourless oil; $[\alpha]_D^{25} = +3.6$ (*c* 1.0, $CHCl_3$); IR (neat): 3462, 3062, 3030, 2919, 2852, 1725, 1606, 1506, 1454, 1363, 1221, 1098, 1028, 909, 827, 749, 734, 696 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.38–7.15 (m, 30H, Ar-H), 6.98 (d, J = 8.9 Hz, 2H, Ar-H), 6.77 (d, J = 8.9 Hz, 2H, Ar-H), 5.21 (d, J = 3.3 Hz, 1H, H-1_B), 5.06 (d, J = 11.5 Hz, 1H, PhCH_2), 5.02 (d, J = 10.7 Hz, 1H, PhCH_2), 4.85 (d, J = 11.6 Hz, 1H, PhCH_2), 4.78 (d, J = 7.6 Hz, 1H, H-1_A), 4.72–4.68 (m, 2H, PhCH_2), 4.64 (d, J = 11.2 Hz, 1H, PhCH_2), 4.45–4.36 (m, 5H, PhCH_2), 4.31 (d, J = 11.2 Hz, 1H, PhCH_2), 4.25–4.23 (m, 1H, H-5_B), 4.07–4.03 (dd, J = 7.8, 7.8 Hz, 1H, H-2_A), 4.02–3.99 (dd, J = 9.8, 3.2 Hz, 1H, H-2_B), 3.93 (d, J = 2.3 Hz, 1H, H-4_A), 3.89–3.87 (dd, J = 9.8, 3.0 Hz, 1H, H-3_B), 3.86–3.84 (dd, J = 10.0, 2.3 Hz, 1H, H-3_A), 3.77–3.76 (m, 1H, H-4_B), 3.75 (s, 3H, OCH_3), 3.61–3.56 (m, 3H, H-5_A, H-6_{abA}), 3.42–3.40 (m, 2H, H-6_{abB}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.1–114.9 (Ar-C), 103.8 (C-1_A), 95.6 (C-1_B), 78.5 (C-2_A), 78.2 (C-3_B), 77.0 (C-3_A), 76.1 (C-2_B), 75.8 (PhCH$_2$), 75.0 (PhCH$_2$), 74.9 (PhCH$_2$), 74.1 (C-5_A), 73.9 (PhCH$_2$), 73.5 (PhCH$_2$), 72.8 (C-4_A), 72.4 (PhCH$_2$), 70.5 (C-6_B), 69.3 (C-6_A), 68.8 (C-4_B), 68.3 (C-5_B), 56.1 (OCH_3); ESI-MS: *m/z* 1035.4 $[M+Na]^+$; Anal. Calcd for $C_{55}H_{64}O_{18}$ (1012.40): C, 65.21; H, 6.37. Found: C, 65.0; H, 6.55.

4.1.3. 4-Methoxyphenyl (2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 7

To a solution of compound **5** (1.4 g, 1.38 mmol) and compound **6** (660 mg, 1.66 mmol) in anhydrous CH_2Cl_2 (15 mL) was added MS 4 Å (2 g) and the reaction mixture was allowed to stir at room temperature for 30 min under argon. The reaction mixture was cooled to −40 °C and *N*-iodosuccinimide (NIS; 450 mg, 2.0 mmol) followed by TfOH (5 μL) were added to it. After stirring at the same temperature for 30 min the reaction was quenched with Et_3N (50 μL), filtered through a Celite® bed and washed with CH_2Cl_2 (50 mL). The organic layer was washed with 5% $Na_2S_2O_3$, satd $NaHCO_3$, water in succession, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (6:1) as an eluant to give pure **7** (1.5 g, 81%). Colourless oil; $[\alpha]_D^{25} = +2.2$ (*c* 1.0, $CHCl_3$); IR (neat): 3458, 3031, 2923, 2863, 1752, 1507, 1454, 1370, 1242, 1219, 1097, 1067, 1029, 906, 750, 735, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.37–7.18 (m, 35H, Ar-H), 6.94 (d, J = 9.1 Hz, 2H, Ar-H) 6.75 (d, J = 9.1 Hz, 2H, Ar-H), 5.47 (s, 1H, PhCH), 5.31 (t, J = 9.4 Hz each, 1H, H-3_C), 5.20 (br s, 1H, H-1_B), 5.03–4.99 (m, 2H, 2PhCH_2), 4.98 (t, J = 10.0 Hz each, 1H, H-2_C), 4.78–4.69 (m, 3H, 3PhCH$_2$), 4.74 (d, J = 7.9 Hz, 1H, H-1_A), 4.71 (d, J = 9.8 Hz, 1H, H-1_C), 4.61–4.59 (m, 2H, PhCH_2), 4.44–4.33 (m, 5H, PhCH_2), 4.31–4.29 (m, 1H, H-5_B), 4.15–4.12 (m, 1H, H-6_{aC}), 4.06–4.02 (dd, J = 7.9, 7.9 Hz, 1H, H-2_A), 3.91–3.88 (m, 3H, H-2_B, H-3_B, H-4_A) 3.85–3.82 (dd, J = 2.5, 9.9 Hz, 1H, H-3_A), 3.75–3.74 (m, 1H, H-4_B), 3.74 (s, 3H, OCH_3), 3.68–3.64 (m, 1H, H-6_{bC}), 3.64 (t, J = 9.4 Hz each, 1H, H-4_C), 3.58–3.50 (m, 4H, H-5_A, H-6_{abA}, H-6_{aB}), 3.42–3.35 (m, 2H, H-5_C, H-6_{bB}), 2.04, 1.83 (2 s, 6H, 2COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5 (*C*OCH$_3$), 170.2 (*C*OCH$_3$), 155.5–114.9 (Ar-C), 103.6 (C-1_C), 102.5 (C-1_A), 101.9 (PhCH), 95.7 (C-1_B), 78.8 (C-4_C), 78.6 (C-2_B), 78.5 (C-2_A), 77.7 (C-3_A), 77.3 (C-3_B), 76.7 (C-4_B), 75.5 (PhCH$_2$), 75.0 (PhCH$_2$), 74.9 (PhCH$_2$), 74.1 (C-5_A), 73.8 (PhCH$_2$), 73.5 (PhCH$_2$), 73.0 (C-4_A), 72.9 (PhCH$_2$), 72,9 (C-2_C), 72.2 (C-3_C), 69.9 (C-6_B), 69.3 (C-6_A), 69.1 (C-5_B), 68.9 (C-6_C), 66.4 (C-5_C), 56.1 (OCH$_3$), 21.2 (COCH$_3$), 21.1 (COCH$_3$); ESI-MS: *m/z* 1369.5 $[M+Na]^+$; Anal. Calcd for $C_{72}H_{82}O_{25}$ (1346.51): C, 64.18; H, 6.13. Found: C, 64.0; H, 6.35.

4.1.4. 4-Methoxyphenyl (4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 8

A solution of compound **7** (1.4 g, 1.04 mmol) in 0.1 M CH_3ONa in methanol (30 mL) was allowed to stir at room temperature for 3 h. The reaction mixture was neutralized with Dowex 50W X8 (H^+) resin, filtered and concentrated under reduced pressure. The crude product was passed through a short pad of SiO_2 to give pure **8** (1.3 g, quantitative). Colourless oil; $[\alpha]_D^{25} = +2.6$ (*c* 1.0, $CHCl_3$); IR (neat): 3431, 3030, 2922, 2854, 1507, 1454, 1369, 1221, 1099, 1074, 1028, 912, 750, 735, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.33–6.92 (m, 35H, Ar-H), 6.91 (d, J = 9.1 Hz, 2H, Ar-H) 6.73 (d, J = 9.1 Hz, 2H, Ar-H), 5.47 (s, 1H, PhCH), 5.15 (d, J = 3.2 Hz, 1H, H-1_B), 5.03 (d, J = 11.3 Hz, 1H, PhCH_2), 4.97 (d, J = 11.3 Hz, 1H, PhCH_2), 4.77 (2 d, J = 12.2 Hz, 2H, PhCH_2), 4.76 (d, J = 7.6 Hz, 1H, H-1_A), 4.69–4.64 (m, 3H, PhCH_2), 4.45–4.35 (m, 3H, PhCH_2), 4.33 (d, J = 7.7 Hz, 1H, H-1_C), 4.30–4.26 (m, 2H, PhCH_2), 4.24–4.22 (m, 1H, H-5_B), 4.09–4.06 (m, 1H, H-6_{aC}), 4.07–4.00 (m, 2H, H-2_A, H-2_B), 3.96–3.94 (m, 1H, H-3_B), 3.91 (d, J = 2.5 Hz, 1H, H-4_B), 3.78–3.66 (m, 7H, H-3_A, H-4_A, H-5_A, H-6_{abA}, H-6_{aB}, H-6_{bC}), 3.58 (s, 3H, OCH_3), 3.52–3.40 (m, 4H, H-2_C, H-3_C, H-4_C, H-6_{bB}), 3.25–3.20 (m, 1H, H-5_C); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.6–114.8 (Ar-C), 106.5 (C-1_C), 103.5 (C-1_A), 102.2 (PhCH) 95.9 (C-1_B), 80.6 (C-3_A), 80.4 (C-2_B), 78.3 (C-2_A), 78.2 (C-4_C), 78.0 (C-4_B), 76.9 (C-3_B), 76.1 (C-5_A), 75.6 (PhCH$_2$), 75.0 (PhCH$_2$), 74.9 (PhCH$_2$), 74.4 (PhCH$_2$), 73.9 (2C, C-2_C, C-4_A), 73.1 (C-3_C), 73.0 (PhCH$_2$), 69.1 (C-6_B), 68.9 (C-6_A), 68.7 (C-6_C), 68.5 (C-5_B), 67.1 (C-5_C), 55.9 (OCH$_3$); ESI-MS: *m/z* 1285.5 $[M+Na]^+$; Anal. Calcd for $C_{68}H_{78}O_{23}$ (1262.49): C, 64.65; H, 6.22. Found: C, 64.48; H, 6.48.

4.1.5. Ethyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside 11

A solution of compound **9** (1.8 g, 4.14 mmol) and compound **10** (1.0 g, 2.94 mmol) in anhydrous CH_2Cl_2 (15 mL) was allowed to stir at −20 °C for 15 min under argon. To the cold reaction mixture was added TMSOTf (50 μL) and the reaction mixture was allowed to stir at same temperature for 45 min. The reaction was quenched with Et_3N (100 μL) and diluted with CH_2Cl_2 (50 mL). The organic layer was washed with satd $NaHCO_3$ and water in succession, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (7:1) as an eluant to give pure **11** (1.5 g, 83%). Colourless oil; $[\alpha]_D^{25} = -8.8$ (*c* 1.0, $CHCl_3$); IR (neat): 3476, 3033, 2962, 2926, 2854, 1749, 1627, 1498, 1456, 1375, 1260, 1226, 1138, 1040, 982, 933, 911, 803, 749, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.24–7.19 (m, 5H, Ar-H), 5.22–5.21 (m, 1H, H-2_F), 5.16–5.15 (m, 1H, H-2_E), 5.14–5.11 (dd, J = 10.0, 3.3 Hz, 1H, H-3_F), 5.07 (br s, 1H, H-1_E), 4.95 (t, J = 9.9 Hz, 1H, H-4_F), 4.92 (br s, 1H, H-1_F), 4.71 (d, J = 10.9 Hz, 1H, PhCH_2), 4.58 (d, J = 10.9 Hz, 1H, PhCH_2), 4.01–3.97 (m, 2H, H-3_E, H-5_E), 3.82–3.79 (m, 1H, H-5_F), 3.44 (t, J = 9.5 Hz, 1H, H-4_E), 2.57–2.51 (m, 2H, SCH_2CH$_3$), 2.15 (s, 3H, COCH_3), 2.00 (s, 3H, COCH_3), 1.99 (s, 3H, COCH_3), 1.91 (s, 3H, COCH_3), 1.26 (d, J = 6.3 Hz, 3H, CCH_3), 1.21 (t, J = 7.4 Hz, SCH$_2$CH_3), 1.13 (d, J = 6.3 Hz, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7 (*C*OCH$_3$), 170.2 (2C; 2*C*OCH$_3$), 170.0 (*C*OCH$_3$), 138.2–128.1 (Ar-C), 99.6 (C-1_F), 82.4 (C-1_E), 81.3 (C-4_E), 77.2 (C-3_E), 75.9 (PhCH$_2$), 74.2 (C-2_E), 71.0 (C-4_F), 70.1 (C-2_F), 69.4 (C-3_F), 68.9 (C-5_E), 67.7 (C-5_F), 25.9 (SCH$_2$CH$_3$), 21.4 (COCH$_3$), 21.2 (COCH$_3$), 21.1 (COCH$_3$), 21.0 (COCH$_3$), 18.3 (SCH$_2$CH$_3$), 17.7 (CCH$_3$), 15.4 (CCH$_3$); ESI-MS: *m/z* 635.2 $[M+Na]^+$; Anal. Calcd for $C_{29}H_{40}O_{12}S$ (612.22): C, 56.85; H, 6.58. Found: C, 56.67; H, 6.80.

4.1.6. 4-Methoxyphenyl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 13

To a solution of compound **11** (1.5 g, 2.45 mmol) and compound **12** (1.0 g, 2.29 mmol) in anhydrous CH_2Cl_2 (15 mL) was added MS

4 Å (2 g) and the reaction mixture was allowed to stir at room temperature for 30 min under argon. The reaction mixture was cooled to −40 °C and *N*-iodosuccinimide (NIS; 660 mg, 2.93 mmol) followed by TfOH (5 μL) were added to it. After stirring at the same temperature for 30 min the reaction was quenched with Et_3N (50 μL), filtered through a Celite® bed and washed with CH_2Cl_2 (50 mL). The organic layer was washed with 5% $Na_2S_2O_3$, satd $NaHCO_3$, water in succession, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (10:1) as an eluant to give pure **13** (1.8 g, 78%). Colourless oil; $[\alpha]_D^{25} = -6$ (*c* 1.0, $CHCl_3$); IR (neat): 3478, 3032, 2979, 2935, 1750, 1595, 1508, 1487, 1371, 1245, 1223, 1139, 1078, 1052, 983, 926, 830, 752, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.35–7.25 (m, 19H, Ar-H), 6.89 (d, *J* = 9.1 Hz, 2H, Ar-H), 6.77 (d, *J* = 9.1 Hz, 2H, Ar-H) 5.34 (br s, 1H, H-1_D), 5.33–5.30 (m, 2H, H-2_E, H-2_F), 5.24–5.21 (dd, *J* = 10.2, 3.3 Hz, 1H, H-3_F), 5.05 (br s, 1H, H-1_E), 5.03 (t, *J* = 10.2 Hz each, H-4_F), 4.97 (br s, 1H, H-1_F), 4.85 (d, *J* = 10.9 Hz, 1H, PhC*H*$_2$), 4.78 (d, *J* = 10.9 Hz, 1H, PhC*H*$_2$), 4.71–4.70 (m, 2H, PhC*H*$_2$), 4.64 (d, *J* = 10.9 Hz, 1H, PhC*H*$_2$), 4.60 (d, *J* = 10.9 Hz, 1H, PhC*H*$_2$), 4.20–4.18 (dd, *J* = 7.1, 3.4 Hz, 1H, H-3_E), 4.07–4.03 (m, 1H, H-2_D), 4.02–3.96 (m, 2H, H-3_D, H-5_D), 3.86–3.81 (m, 2H, H-5_E, H-5_F), 3.75 (s, 3H, OC*H*$_3$), 3.51–3.45 (m, 2H, H-4_D, H-4_E), 2.21 (s, 3H, COC*H*$_3$), 2.08 (s, 6H, 2COC*H*$_3$), 1.99 (s, 3H, COC*H*$_3$), 1.31 (d, *J* = 6.2 Hz, 3H, CC*H*$_3$), 1.24 (d, *J* = 6.2 Hz, 3H, CC*H*$_3$), 1.18 (d, *J* = 6.2 Hz, 3H, CC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.4 (*C*OCH$_3$), 170.2 (*C*OCH$_3$), 170.1 (*C*OCH$_3$), 170.0 (*C*OCH$_3$), 155.3–114.9 (Ar-C), 99.6 (C-1_E), 99.5 (C-1_F), 97.9 (C-1_D), 81.1 (C-4_D), 80.5 (C-4_E), 79.7 (C-3_D), 76.3 (C-3_E), 76.1 (PhCH$_2$), 75.8 (C-2_D), 75.7 (PhCH$_2$), 72.6 (PhCH$_2$), 72.2 (C-2_E), 71.1 (C-4_F), 70.1 (C-2_F), 69.5 (C-3_F), 68.9 (2C, C-5_E, C-5_F), 67.7 (C-5_D), 55.8 (OCH$_3$), 21.4 (COCH$_3$), 21.2 (COCH$_3$), 21.1 (COCH$_3$), 21.0 (COCH$_3$), 18.5 (CCH$_3$), 18.4 (CCH$_3$), 17.6 (CCH$_3$); ESI-MS: *m/z* 1023.4 $[M+Na]^+$; Anal. Calcd for $C_{54}H_{64}O_{18}$ (1000.4): C, 64.79; H, 6.44. Found: C, 64.60; H, 6.27.

4.1.7. 4-Methoxyphenyl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-αα-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 15

To a solution of compound **13** (1.50 g, 1.50 mmol) in CH_3CN–H_2O (15 mL; 4:1, v/v) was added ammonium cerium nitrate (CAN; 1.25 g, 2.28 mmol) and the reaction mixture was allowed to stir at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (80 mL) and the organic layer was washed with satd $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated to dryness to give disaccharide hemiacetal. To a solution of the hemiacetal in anhydrous CH_2Cl_2 (15 mL) was added trichloroacetonitrile (1.2 mL, 12 mmol) and the reaction mixture was cooled to −10 °C. To the cooled reaction mixture was added DBU (0.1 mL, 0.65 mmol) and it was allowed to stir at −10 °C for 1 h. The reaction mixture was evaporated to dryness and the crude product was passed through a short pad of SiO_2 to furnish (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl trichloroacetimidate (**14**; 1.2 g, 77%), which was used immediately for the next step. A solution of compound **8** (1.2 g, 0.95 mmol) and compound **14** (1.1 g, 1.06 mmol) in anhydrous CH_2Cl_2 (20 mL) was cooled to −20 °C. To the cooled reaction mixture was added TMSOTf (30 μL) and it was allowed to stir at −20 °C for 45 min. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and the organic layer was washed with satd $NaHCO_3$ and water in succession, dried (Na_2SO_4) and evaporated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (5:1) as an eluant to give pure **15** (1.6 g, 76%). Colourless oil; $[\alpha]_D^{25} = -10$ (*c* 1.0, $CHCl_3$); IR (neat): 3435, 3031, 2929, 2872, 1749, 1508, 1455, 1371, 1244, 1226, 1138, 1082, 1057, 1028, 912, 750, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.32–7.21 (m, 50H, Ar-H), 6.94 (d, *J* = 9.1 Hz, 2H, Ar-H), 6.75 (d, *J* = 9.1 Hz, 2H, Ar-H), 5.46 (s, 1H, PhC*H*), 5.34–5.33 (m, 1H, H-2_F), 5.32–5.31 (m, 1H, H-2_E), 5.24 (br s, 1H, H-1_E), 5.23–5.21 (m, 1H, H-3_F), 5.19 (d, *J* = 3.1 Hz, 1H, H-1_B), 5.00 (br s, 1H, H-1_F), 5.04 (t, *J* = 9.4 Hz each, 1H, H-4_F), 4.99 (d, *J* = 11.4 Hz, 1H, PhC*H*$_2$), 4.97 (br s, 1H, H-1_D), 4.95 (d, *J* = 11.3 Hz, 1H, PhC*H*$_2$), 4.80 (d, *J* = 11.2 Hz, 1H, PhC*H*$_2$), 4.76 (d, *J* = 7.4 Hz, 1H, H-1_A), 4.75–4.71 (m, 3H, PhC*H*$_2$), 4.67–4.73 (m, 5H, PhC*H*$_2$), 4.57 (d, *J* = 10.9 Hz, 1H, PhC*H*$_2$), 4.55 (d, *J* = 10.9 Hz, 1H, PhC*H*$_2$), 4.46–4.37 (m, 3H, PhC*H*$_2$), 4.31 (d, *J* = 7.8 Hz, 1H, H-1_C), 4.28 (d, *J* = 11.9 Hz, 1H, PhC*H*$_2$), 4.25–4.18 (m, 3H, H-3_C, H-3_D, PhC*H*$_2$), 4.11–4.08 (m, 2H, H-2_D, H-3_E), 4.04–3.99 (m, 4H, H-2_A, H-2_B, H-5_E, H-6_{aC}), 3.98–3.95 (m, 1H, H-3_B), 3.91 (d, *J* = 2.5 Hz, 1H, H-4_B), 3.89–3.85 (m, 2H, H-3_A, H-6_{bC}), 3.81–3.76 (m, 2H, H-5_D, H-5_F), 3.75 (s, 3H, OC*H*$_3$), 3.72 (br s, 1H, H-4_A), 3.68 (t, *J* = 10.3 Hz, 1H, H-4_E), 3.60–3.56 (m, 4H, H-5_A, H-5_B, H-6_{abB}), 3.47–3.41 (m, 4H, H-2_C, H-4_C, H-6_{abA}), 3.40–3.36 (t, *J* = 9.5 Hz, 1H, H-4_D), 3.28–3.23 (m, 1H, H-5_C), 2.16 (s, 3H, COC*H*$_3$), 2.07 (s, 3H, COC*H*$_3$), 2.05 (s, 3H, COC*H*$_3$), 1.97 (s, 3H, COC*H*$_3$), 1.22 (d, *J* = 6.2 Hz, 3H, CC*H*$_3$), 1.17 (d, *J* = 6.2 Hz, 3H, CC*H*$_3$), 1.01 (d, *J* = 6.2 Hz, 3H, CC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.6 (*C*OCH$_3$), 170.5 (2C, 2*C*OCH$_3$), 170.2 (*C*OCH$_3$), 155.3–114.9 (Ar-C), 155.5–114.8 (Ar-C), 106.5 (C-1_C), 103.6 (C-1_A), 101.9 (PhCH), 99.5 (3C, C-1_D, C-1_E, C-1_F), 96.1 (C-1_B), 81.3 (C-4_D), 81.0 (C-3_A), 80.6 (C-4_E), 79.8 (C-3_D), 79.2 (C-2_B), 78.5 (C-4_C), 78.4 (C-4_B), 77.7 (C-3_B), 77.5 (2C, C-2_A, C-3_E), 77.3 (C-2_D), 76.1 (C-5_A), 75.9 (PhCH$_2$), 75.6 (PhCH$_2$), 75.5 (C-2_C), 75.4 (PhCH$_2$), 75.0 (PhCH$_2$), 74.9 (PhCH$_2$), 74.1 (PhCH$_2$),74.0 (C-4_A), 73.9 (PhCH$_2$), 73.3 (C-3_C), 73.0 (PhCH$_2$), 72.3 (C-2_F), 72.2 (PhCH$_2$), 71.1 (C-4_F), 70.1 (C-2_E), 69.6 (C-3_F), 69.2 (C-6_B), 68.9 (C-6_A), 68.8 (C-6_C), 68.7 (C-5_B), 68.5 (C-5_F), 68.0 (C-5_E), 67.6 (C-5_D), 67.4 (C-5_C), 56.0 (OCH$_3$), 21.4 (COCH$_3$), 21.3 (COCH$_3$), 21.2 (COCH$_3$), 21.1 (COCH$_3$), 18.0 (CCH$_3$), 17.9 (CCH$_3$), 17.6 (CCH$_3$); ESI-MS: *m/z* 2234.0 $[M+Na]^+$; Anal. Calcd for $C_{131}H_{142}O_{31}$ (2210.95): C, 71.11; H, 6.47. Found: C, 70.92; H, 6.73.

4.1.8. 4-Methoxyphenyl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 16

To a solution of compound **15** (1.2 g, 0.54 mmol) in dry CH_2Cl_2 (10 mL) was added Dess–Martin periodinane (350 mg, 0.82 mmol) and the reaction mixture was allowed to stir at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and the organic layer was washed with 5% $Na_2S_2O_3$ and water in succession. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the crude ketone as a colourless oil, which was used in the next step without purification. To a solution of the crude ketone in CH_3OH (10 mL) was slowly added $NaBH_4$ (200 mg, 5.28 mmol) portionwise at 0 °C. The reaction mixture was allowed to stir at room temperature for 12 h and concentrated under reduced pressure. A solution of the crude product in acetic anhydride–pyridine (5 mL; 1:1 v/v) was allowed to stir at room temperature for 5 h and the solvents were removed under reduced pressure. The crude mass was dissolved in CH_2Cl_2 (50 mL) and the organic layer was washed with satd $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (5:1) as an eluant to give pure **16** (900 mg, 74%). Colourless oil; $[\alpha]_D^{25} = -7.1$ (*c* 1.0, $CHCl_3$); IR (neat): 3450, 3032, 2925, 2855, 1748, 1508, 1455, 1371, 1228, 1139, 1092, 1045, 1029, 984, 750, 737, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.23–7.08 (m, 50H, Ar-H), 6.86 (d, *J* = 9.1 Hz, 2H, Ar-H), 6.69 (d, *J* = 9.1 Hz, 2H, Ar-H), 5.47 (d, *J* = 2.6 Hz, 1H, H-2_C), 5.45 (s, 1H, PhC*H*), 5.28–5.25 (m, 2H, H-2_E, H-2_F), 5.18–5.15 (dd, *J* = 10.1,

3.4 Hz, 1H, H-3_F), 5.06 (d, J = 2.9 Hz, 1H, H-1_B), 4.99 (br s, 1H, H-1_F), 4.97 (br s, 1H, H-1_E), 4.95–4.93 (m, 3H, PhCH_2), 4.77 (br s, 1H, H-1_D), 4.74 (br s, 1H, H-1_C), 4.73–4.71 (m, 3H, PhCH_2), 4.66 (d, J = 7.7 Hz, 1H, H-1_A), 4.66–4.62 (m, 2H, PhCH_2), 4.58–4.55 (m, 3H, PhCH_2), 4.52–4.43 (m, 3H, PhCH_2), 4.36–4.20 (m, 6H, H-3_C, H-3_E, PhCH_2), 4.15–4.12 (dd, J = 9.6, 3.3 Hz, 1H, H-3_D), 4.10–4.07 (m, 1H, H-2_D), 3.99–3.91 (m, 3H, H-2_A, H-4_C, H-6_{aC}), 3.84–3.80 (m, 3H, H-3_B, H-4_B, H-4_F), 3.78–3.66 (m, 7H, H-4_A, H-4_E, H-5_D, H-5_F, H-6_{abB}, H-6_{bC}), 3.68 (s, 3H, OCH_3), 3.50–3.44 (m, 4H, H-5_A, H-5_B, H-6_{abA}), 3.40 (t, J = 9.6 Hz, 1H, H-4_D), 3.36–3.27 (m, 3H, H-3_A, H-2_B, H-5_E), 3.12–3.08 (m, 1H, H-5_C), 2.09 (s, 3H, COCH_3), 2.02 (s, 3H, COCH_3), 2.01 (s, 3H, COCH_3), 1.98 (s, 3H, COCH_3), 1.90 (s, 3H, COCH_3), 1.25 (d, J = 6.4 Hz, 3H, CCH_3), 1.11 (d, J = 6.2 Hz, 3H, CCH_3), 1.06 (d, J = 6.2 Hz, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.6 (*C*OCH$_3$), 170.5 (2C, 2*C*OCH$_3$), 170.4 (*C*OCH$_3$), 170.2 (*C*OCH$_3$), 155.3–114.8 (Ar-C), 155.5–114.8 (Ar-C), 103.7 (C-1_A), 101.6 (PhCH), 100.0 (C-1_C), 99.5 (C-1_F), 99.0 (C-1_E), 95.9 (C-1_D), 95.6 (C-1_B), 81.3 (C-4_D), 80.8 (C-3_A), 80.0 (C-3_D), 78.9 (C-4_F), 78.4 (C-3_B), 77.6 (C-2_B), 76.9 (C-4_C), 76.7 (C-4_B), 76.0 (C-2_A), 75.9 (PhCH), 75.6 (PhCH), 75.5 (C-3_E), 75.3 (PhCH), 75.2 (PhCH), 74.9 (PhCH), 74.5 (C-2_D), 74.1(C-5_A), 73.8 (PhCH), 73.7 (PhCH), 72.9 (PhCH), 72.6 (C-2_C), 72.3 (C-4_A), 72.2 (PhCH), 71.9 (C-3_C), 71.1 (C-2_F), 70.1 (C-4_F), 69.9 (C-6_B), 69.6 (C-2_E), 69.3 (C-6_A), 68.9 (C-3_F), 68.8 (C-5_B), 68.7 (C-6_C), 68.4 (C-5_F), 68.1 (C-5_E), 67.6 (2C, C-5_C, C-5_D), 56.1 (OCH$_3$), 21.4 (COCH$_3$), 21.3 (COCH$_3$), 21.2 (2C, 2COCH$_3$), 21.1 (COCH$_3$), 18.2 (CCH$_3$), 18.1 (CCH$_3$), 17.6 (CCH$_3$); ESI-MS: *m/z* 2276.0 [M+Na]$^+$; Anal. Calcd for $C_{133}H_{144}O_{32}$ (2252.96): C, 70.85; H, 6.44. Found: C, 70.64; H, 6.70.

4.1.9. 4-Methoxyphenyl (α-L-rhamnopyranosyl)-(1→3)-(α-L-rhamnopyranosyl)-(1→2)-(α-L-rhamnopyranosyl)-(1→3)-(β-D-mannopyranosyl)-(1→4)-(α-D-galactopyranosyl)-(1→3)-β-D-galactopyranoside 1

A solution of compound **16** (800 mg, 0.35 mmol) in 0.1 M CH$_3$O-Na in CH$_3$OH (25 mL) was allowed to stir at room temperature for 3 h. The reaction mixture was neutralized with Dowex 50W X8 (H^+) resin, filtered and concentrated. To a solution of the deacetylated product in CH$_3$OH (20 mL) was added 20% Pd(OH)$_2$–C (150 mg) and the reaction mixture was allowed to stir at room temperature under a positive pressure of hydrogen for 24 h. The reaction mixture was filtered through a Celite® bed and then washed with CH$_3$OH–H$_2$O (60 mL; 4:1 v/v). The combined filtrate was evaporated under reduced pressure to furnish compound **1**, which was purified through a Sephadex LH-20 column using CH$_3$OH–H$_2$O (5:1) as an eluant to give pure compound **1** (260 mg, 71%). White powder; $[\alpha]_D^{25} = +2.7$ (c 1.0, CH$_3$OH); IR (KBr): 3405, 2921, 2850, 2718, 1596, 1509, 1354, 1224, 1129, 1072, 985, 775 cm^{-1}; ^{1}H NMR (500 MHz, D$_2$O): δ 6.96 (d, J = 8.9 Hz, 2H, Ar-H), 6.86 (d, J = 8.9 Hz, 2H, Ar-H), 4.99 (br s, 1H, H-1_E), 4.97 (d, J = 3.7 Hz, 1H, H-1_B), 4.95 (br s, 1H, H-1_D), 4.85 (br s, 1H, H-1_F), 4.71 (d, J = 7.9 Hz, 1H, H-1_A), 4.69 (br s, 1H, H-1_C), 4.21–4.19 (m, 1H, H-2_C), 4.17–4.16 (m, 1H, H-4_B), 4.11 (br s, 1H, H-4_A), 4.03–3.98 (m, 2H, H-2_B, H-2_F), 3.91–3.87 (m, 3H, H-2_D, H-2_E, H-3_B), 3.86–3.72 (m, 5H, H-2_A, H-2_D, H-3_D, H-6_{aB}, H-6_{abC}), 3.71–3.67 (m, 5H, H-3_A, H-5_A, H-6_{abA}, H-6_{bB}), 3.65 (s, 3H, OCH_3), 3.64–3.55 (m, 5H, H-3_C, H-3_E, H-3_F, H-4_C, H-5_D), 3.53–3.37 (m, 3H, H-4_E, H-5_E, H-5_F), 3.34–3.29 (m, 2H, H-4_D, H-4_F), 3.26–3.21 (m, 2H, H-5_B, H-5_C), 1.20–1.14 (m, 9H, 3CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.6–114.5 (Ar-C), 103.1 (C-1_F), 102.9 (C-1_E), 102.8 (C-1_C), 101.5 (C-1_A), 97.0 (C-1_B), 95.4 (C-1_D), 79.1 (C-2_E), 79.0 (C-3_C), 78.4 (C-3_B), 77.5 (C-3_A), 77.1 (C-4_A), 76.5 (C-4_B), 75.4 (C-4_D), 73.3 (C-4_E), 73.0 (C-5_A), 72.2 (C-4_F), 71.2 (C-5_C), 71.1 (C-2_F), 70.9 (C-3_F), 70.8 (C-3_E), 70.7 (C-2_B), 70.4 (C-5_D), 69.7 (2C, C-5_E, C-5_F), 69.4 (C-6_C), 69.2 (C-2_A), 69.1 (C-5_B), 67.9 (C-2_D), 66.8 (C-3_D), 66.2 (C-4_C), 65.7 (C-2_C), 62.6 (C-6_B), 62.2 (C-6_A), 55.2 (OCH$_3$), 17.2 (CCH$_3$), 17.1 (CCH$_3$), 17.0 (CCH$_3$); ESI-MS: 1071.4 [M+Na]$^+$; Anal. Calcd for $C_{43}H_{68}O_{29}$ (1048.38): C, 49.23; H, 6.53. Found: C, 49.0; H, 6.80.

Acknowledgements

S.G. thanks UGC, New Delhi for providing a Senior Research Fellowship. This work was partly supported by Ramanna Fellowship (A.K.M.), Department of Science and Technology, New Delhi (SR/S1/RFPC-06/2006).

References

1. (a) Bekri, M. A.; Desair, J.; Proost, P.; Searle-van Leeuwen, M.; Vanderleyden, J.; Vande Broek, A. *J. Bacteriol.* **1999**, *182*, 2440–2447; (b) Okon, Y.; Vanderleyden, J. *ASM News* **1997**, *63*, 366–370; (c) Okon, Y. *Trends Biotechnol.* **1985**, *3*, 223–228.
2. Tien, T. M.; Gaskins, M. H.; Hubbell, D. H. *Appl. Environ. Microbiol.* **1979**, *37*, 1016–1024.
3. Bashan, Y.; Holgium, G. *Can. J. Microbiol.* **1997**, *43*, 103–121.
4. (a) Jofre, E.; Lagares, A.; Mori, G. *FEMS Microbiol. Lett.* **2004**, *231*, 267–275; (b) Burdman, S.; Okon, Y.; Jurkevitch, E. *Crit. Rev. Microbiol.* **2000**, *26*, 91–110; (c) Bogatyrev, V. A.; Dykman, L. A.; Matora, L. Y.; Schwartsburd, B. I. *FEMS Microbiol. Lett.* **1992**, *75*, 115–118.
5. (a) Fedonenko, Y. P.; Zatonsky, G. V.; Konnova, S. A.; Zdorovenko, E. L.; Ignatov, V. V. *Carbohydr. Res.* **2002**, *337*, 869–872; (b) Fedonenko, Y. P.; Konnova, O. N.; Zatonsky, G. V.; Shashkov, A. S.; Konnova, S. A.; Zdorovenko, E. L.; Ignatov, V. V.; Knirel, Y. A. *Carbohydr. Res.* **2004**, *339*, 1813–1816; (c) Fedonenko, Y. P.; Konnova, O. N.; Zatonsky, G. V.; Konnova, S. A.; Kocharova, N. A.; Zdorovenko, E. L.; Ignatov, V. V. *Carbohydr. Res.* **2005**, *340*, 1259–1263; (d) Fedonenko, Y. P.; Konnova, O. N.; Zdorovenko, E. L.; Konnova, S. A.; Zatonsky, G. V.; Shashkov, A. S.; Ignatova, V. V.; Knirel, Y. A. *Carbohydr. Res.* **2008**, *343*, 810–816.
6. Fedonenko, Y. P.; Konnova, O. N.; Zatonsky, G. V.; Konnova, S. A.; Kocharova, N. A.; Zdorovenko, E. L.; Ignatova, V. V. *Carbohydr. Res.* **2005**, *340*, 1259–1263.
7. (a) Mandal, P. K.; Misra, A. K. *Bioorg. Chem.* **2010**, *38*, 56–61; (b) Guchhait, G.; Misra, A. K. *Tetrahedron: Asymmetry* **2009**, *20*, 1791–1797; (c) Ghosh, S.; Misra, A. K. *Tetrahedron: Asymmetry* **2009**, *20*, 2688–2693; (d) Pandey, S.; Ghosh, S.; Misra, A. K. *Synthesis* **2009**, 2584–2590; (e) Panchadhayee, R.; Misra, A. K. *Tetrahedron: Asymmetry* **2009**, *20*, 1550–1555; (f) Mandal, P. K.; Misra, A. K. *Synthesis* **2009**, 1348–1354; (g) Mukherjee, C.; Misra, A. K. *Tetrahedron: Asymmetry* **2009**, *20*, 473–477; (h) Mandal, P. K.; Misra, A. K. *Tetrahedron* **2008**, *64*, 8685–8691; (i) Mandal, P. K.; Misra, A. K. *Glycoconjugate J.* **2008**, *25*, 713–722; (j) Mukherjee, C.; Misra, A. K. *Tetrahedron: Asymmetry* **2008**, *19*, 2746–2751; (k) Panchadhayee, R.; Misra, A. K. *Glycoconjugate J.* **2008**, *25*, 817–826; (l) Kumar, R.; Maulik, P. R.; Misra, A. K. *Glycoconjugate J.* **2008**, *25*, 511–519; (m) Mukherjee, C.; Misra, A. K. *Glycoconjugate J.* **2008**, *25*, 611–624; (n) Tiwari, P.; Misra, A. K. *Glycoconjugate J.* **2008**, *25*, 85–99; (o) Mukherjee, C.; Misra, A. K. *Glycoconjugate J.* **2008**, *25*, 111–119; (p) Mandal, P. K.; Misra, A. K. *Synthesis* **2007**, 2660–2666; (q) Agnihotri, G.; Mandal, P. K.; Misra, A. K. *Tetrahedron* **2007**, *63*, 7240–7245.
8. Bazin, H. G.; Du, Y.; Polat, T.; Linhardt, R. J. *J. Org. Chem.* **1999**, *64*, 7254–7259.
9. Crich, D.; Yao, Q. *J. Am. Chem. Soc.* **2004**, *126*, 8232–8236.
10. Gridley, J. J.; Osborne, H. M. I.; Suthers, W. G. *Tetrahedron Lett.* **1999**, *40*, 6991–6994.
11. (a) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334; (b) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313–4316.
12. Panchadhayee, R.; Misra, A. K. *Synlett*, in press. doi:10.1055/s-0029-1219798.
13. van Steijn, A. M. P.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1991**, *211*, 261–277.
14. Zuurmond, H. M.; Veeneman, G. H.; van der Marel, G. A.; van Boom, J. H. *Carbohydr. Res.* **1993**, *241*, 153–164.
15. Schmidt, R. R.; Jung, K.-H. In *Preparative Carbohydrate Chemistry*; Hanessaian, S., Ed.; Marcel Dekker: New York, 1997; pp 283–312; (b) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235.
16. Tanikawa, T.; Fridman, M.; Zhu, W.; Faulk, B.; Joseph, I. C.; Kahne, D.; Wagner, B. K.; Clemons, P. A. *J. Am. Chem. Soc.* **2009**, *131*, 5075–5083.
17. Werz, D. B.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2005**, *44*, 6315–6318.
18. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.
19. Misra, A. K.; Roy, N. *Carbohydr. Res.* **1995**, *278*, 103–111.
20. Pearlman, W. M. *Tetrahedron Lett.* **1967**, *8*, 1663–1664.